Antitumour imidazotetrazines. Part 33.[1] New syntheses of the antitumour drug temozolomide using 'masked' methyl isocyanates

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Ethyl 8-carbamoyl-4-oxo-3,4-dihydroimidazo[5,1-*d*]-1,2,3,5-tetrazin-3-ylacetate **6a** can be prepared by treating 5-diazoimidazole-4-carboxamide **3** with ethyl isocyanatoacetate or by diazotisation of *N*-(5-amino-4-carbamoylimidazol-1-ylcarbonyl)glycine ethyl ester **5**. Barton radical decarboxylation of the tetrazin-3-ylacetic acid **6b** affords temozolomide **1** (26%) whereas deprotection of the 3-trimethylsilylmethyl-imidazotetrazine **6g** with TBAF in acetonitrile–acetic acid yields **1** in 78% yield. 3-Benzylimidazotetrazinones **10a-c** are stable to hydrogenolytic or oxidative debenzylation reactions.

The original synthesis of the antitumour agent temozolomide **1** involved the conversion of 5-aminoimidazole-4-carboxamide hydrochloride **2** into the unstable diazoimidazole **3** which was subsequently treated with methyl isocyanate (MIC) (Scheme 1).[2] This synthesis gave temozolomide in high yield and of a clinical-grade quality without the necessity for further purification. With the requirement for larger supplies of the drug to sustain clinical trials, the use of single solvents (*e.g.* dichloromethane or ethyl acetate, in which the diazoimidazole was very sparingly soluble) was unsatisfactory and led to long reaction times, typically 2 months on a 100 g scale at 25 °C. Proposals to accelerate the reaction by ultrasonic or microwave methods were considered ill-advised because of the potentially explosive nature of the diazo compound **3**, although the latter technique has been used successfully in the small-scale synthesis of ^{11}C-labelled temozolomide.[3]

Following the Bhopal disaster MIC achieved a notoriety rivalled only by mustard gas on a chemical malevolency scale.[4] In view of the volatility of MIC (bp 37 °C) it was not considered prudent to increase the reaction temperature or constrain the reaction contents within a pressure vessel.[5] Thus, although the original synthesis was efficient in the respect that all atoms of the participating reactants in the key step **3** ⟶ **1** were incorporated into the product, it became a matter of urgency to explore alternative 'environmentally-friendly' routes to temozolomide obviating the use of MIC. In this paper we report on our work exploring several approaches to temozolomide which have met with varying degrees of success. Part of this work has been the subject of a preliminary communication.[6]

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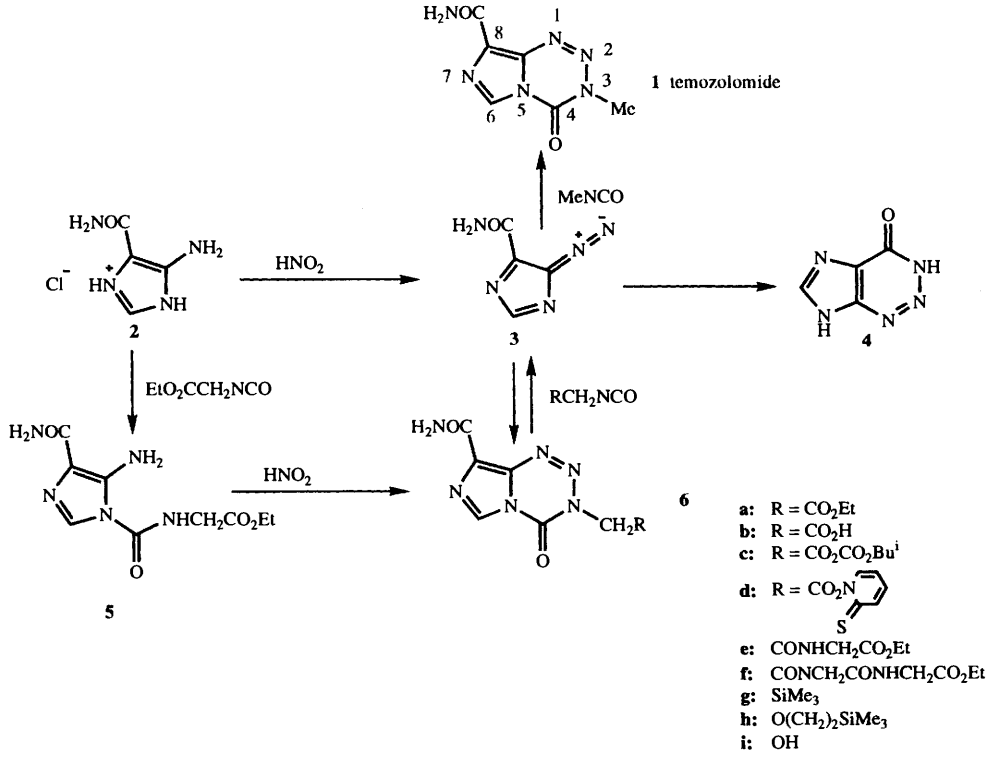


Scheme 1

Our main strategy was to treat the diazoimidazole **3** with a less volatile 'protected' methyl isocyanate (RCH_2NCO) which would provide a series of novel imidazo[5,1-*d*]-1,2,3,5-tetrazinones **6** which might be converted into temozolomide by removal of the protecting group R (Scheme 1). Two possible complications could be expected at the outset. Because the diazoimidazole **3** readily undergoes intramolecular cyclisation to 2-azahypoxanthine (3,7-dihydro-4*H*-imidazo[4,5-*d*]-1,2,3-triazin-4-one) **4** within a wide pH range,[7] formation of this unwanted product might become dominant in cycloadditions with isocyanates less reactive than MIC; secondly, whereas temozolomide and other imidazotetrazinones are surprisingly stable at acid pH, they readily undergo ring-opening and destruction in basic media.[8] Thus, selection of solvent and conditions would be a critical determinant of the success, or otherwise, of the chosen route.

In pilot experiments we have shown that the diazoimidazole **3** can be crystallised to purity from hot THF but it cyclises to 2-azahypoxanthine **4** slowly in boiling acetone and ethyl acetate and more rapidly in hot water, methanol and ethanol. The best yield of **4** (93%) was obtained by warming **3** in aqueous ammonia and evaporating the mixture to dryness.

In view of the high solubility and reasonable stability of **3** in dry DMSO at 25 °C over several hours, this solvent was employed in the overnight reaction with ethyl isocyanatoacetate to give ester **6a** in 82% yield. The ester was stable in acetonitrile–acetic acid at 80 °C but afforded 2-azahypoxanthine **4** when boiled in acetic acid. Presumably, the ester undergoes a retro-cycloaddition regenerating the diazoimidazole **3** which cyclises under the reaction conditions.

Hydrolysis of the ester **6a** in hydrochloric acid (5 mol dm^{-3}) at 45 °C gave the carboxylic acid **6b** (80%) which analysed as a hemihydrate. Efforts to effect the direct decarboxylation of acid **6b** by solid-state thermolysis or in boiling acetic or trifluoroacetic acids were unsuccessful because of the instability of the imidazotetrazine ring-system. However, the Barton radical methodology[9] provided a new synthetic entry to temozolomide **1**. Thus, the acid **6b** was esterified with isobutyl chloroformate in DMF containing *N*-methylmorpholine at −15 °C to yield the active carbonate ester **6c**. The ester was then converted into the pyridine-2(1*H*)-thione **6d** with sulfanylpyridine 1-oxide. Reductive homolytic cleavage of the thione was accomplished by tributylstannane in DMF containing a catalytic amount of AIBN under irradiation with a 100 W tungsten lamp at 25 °C. This represented the first successful alternative (non-MIC) route to temozolomide although the overall yield for the 'one-pot' transformation **6b** ⟶ **6c** ⟶ **6d** ⟶ **1** was a modest 26%. The yield was only marginally improved when benzenethiol was employed instead of tributylstannane as reductant.‡

An additional pathway to temozolomide opened when 5-aminoimidazole-4-carboxamide hydrochloride **2** was converted (80%) into the urea **5** by reaction with ethyl isocyanatoacetate in DMSO–triethylamine. Nitrosation of the urea gave a near quantitative conversion into the aforementioned imidazotetrazine **6a**. This modification of the Barton route avoided both problematic precursors, the diazoimidazole **3** and MIC.

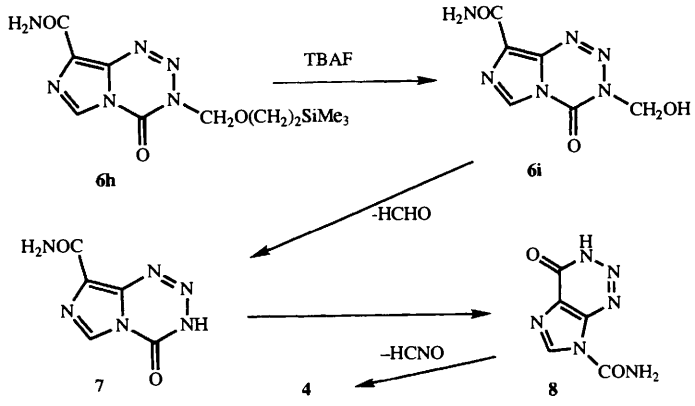
The reactive ester **6c**, generated *in situ* from the acid **6b**, is a potentially valuable intermediate for conjugating amino acids to the imidazotetrazine ring at the 3-position: reaction with glycine ethyl ester hydrochloride or glycylglycine ethyl ester hydrochloride in a DMF–triethylamine mixture afforded the modified peptides **6e** and **6f** in 62 and 65% yields, respectively.

‡ We thank Prof D. H. R. Barton for suggesting this modification to us.

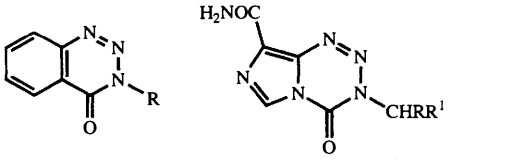
Silyl protecting groups have been used to mask methyl groups in many synthetic strategies.[10] Trimethylsilyl(TMS)-methyl isocyanate, which is not available commercially, was synthesised by treating iodomethyltrimethylsilane with potassium cyanate in dry DMSO at 120 °C under an argon atmosphere and distilling the isocyanate (ν_{max} 2291 cm^{-1}) through a short column. Interaction of the isocyanate with 5-diazoimidazole-4-carboxamide **3** in DMSO at 25 °C for 3 days gave the 3-(trimethylsilylmethyl)imidazotetrazine **6g**. The low yield (33%) presumably reflects the sterically hindered nature of the isocyanate group. In contrast, interaction of **3** with the less hindered (2-trimethylsilylethoxy)methyl isocyanate (SEM isocyanate), prepared from SEM chloride and silver cyanate in ether, gave the imidazotetrazine **6h** in 78% yield.

Compound **6g** retained the characteristic acid stability of 3-alkylimidazotetrazines[8] and was recovered unchanged from prolonged incubation with hydrochloric acid (1 mol dm^{-3}) or acetic acid at room temperature and an acetonitrile–hydrochloric acid mixture at 40 °C for 4 h. Of the range of conditions employed to remove the protecting TMS group [hydrofluoric acid in acetonitrile or pyridine; tetrabutylammonium fluoride (TBAF) at different stoichiometries in various solvents; or TBAF adsorbed onto silica] the optimum conditions required acetonitrile as solvent containing TBAF (1 mol equiv.) and acetic acid (3 mol equiv.) at room temperature. Pure temozolomide (78%) separated from the reaction mixture. In contrast, deprotection of the SEM-imidazotetrazine **6h** with TBAF under a range of conditions gave only a low isolated yield of 2-azahypoxanthine **4** (5–10%) and none of the expected 3-(hydroxymethyl)imidazotetrazine **6i**. Accounting for the formation of **4** in these reactions is problematical. A simple retro-cyclisation to regenerate the diazoimidazole **3** is unlikely to be involved given the mild deprotection conditions. A possible alternative mechanism is outlined (Scheme 2) and involves the intermediacy of an unstable hydroxymethyl derivative **6i** which could undergo elimination of formaldehyde to form the unsubstituted tetrazinone **7** possibly under the chromatographic work-up conditions. Then Dimroth-type rearrangement of **7** to the isomeric 9-carbamoyl-2-azahypoxanthine **8** followed by loss of cyanic acid would give 2-azahypoxanthine **4** as observed. Support for this proposal comes from the knowledge that *N*-hydroxymethyl compounds bearing electron-withdrawing groups attached to the nitrogen atom (as in **6i**) can be isolated[11] but normally decompose by formaldehyde elimination on chromatographic adsorbents. For example, the 3-methyl-1,2,3-benzotriazinone analogue of temozolomide **9a** undergoes *N*-demethylation by hepatic microsomes to the unstable 3-(hydroxymethyl) metabolite **9b**, which then readily eliminates formaldehyde to afford the unsubstituted 1,2,3-benzotriazin-4(3*H*)-one **9c**.[12]

Based on the precedent of the 1,2,3-benzotriazinone series,[13] alkylation of the unsubstituted tetrazinone **7** should give a convenient route to temozolomide and its homologues. Sadly, efforts to synthesize this potentially valuable synthon were unsuccessful. Thus, conversion of 4-methoxybenzyl chloride and diphenylbromomethane into the corresponding isocyanates was accomplished with silver cyanate in refluxing ether. Then the diazoimidazole was successfully treated with benzyl isocyanate, 4-methoxybenzyl isocyanate and diphenylmethyl isocyanate, to yield the 3-benzylimidazotetrazinones **10a–c**, respectively, in high yields. All efforts to effect hydrogenolysis of the benzyl groups using 10–20% palladium or palladium hydroxide (Perlman's catalyst) on carbon at atmospheric or high pressure in acetic or trifluoroacetic acids led only to the recovery of starting materials. A similar negative outcome resulted from attempted oxidative cleavage of the substituted benzyl groups of the imidazotetrazinones **10b** and **10c** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).



Scheme 2



9 a: R = Me
b: R = CH_2OH
c: R = H

10 a: R = H, R^1 = Ph.
b: R = H, R^1 = C_6H_4OMe-*p*
c: R = R^1 = Ph

Experimental

Mps are uncorrected. Thin-layer chromatography (TLC) was performed on silica gel plates (Merck: 60 F254). Visualisation was achieved with either UV light or a spray made from cerium(IV) sulfate and ammonium molybdate in sulfuric acid (2 mol dm^{-3}). Flash chromatography was performed using silica gel (Sorbcil C30 40/60H). ^1H NMR and ^{13}C NMR spectra were recorded on either a Bruker AC-250 or a Bruker ARX-250 spectrometer at 250 and 63 MHz, respectively. δ Values are given using tetramethylsilane as internal standard; J values are given in Hz. IR spectra were obtained with a Mattson Galaxy 2020 spectrometer. Mass spectra were recorded at 70 eV with either a MS902 or a VG Quattro II spectrometer. FAB mass spectra were obtained with a VG AutoSpec spectrometer. Light petroleum refers to the fraction of bp 60–80 °C and ether refers to diethyl ether.

Ethyl 8-carbamoyl-3,4-dihydro-4-oxoimidazo[5,1-*d*]-1,2,3,5-tetrazin-3-ylacetate 6a

5-Diazoimidazole-4-carboxamide **3** (8.0 g), ethyl isocyanatoacetate (10 cm^3, 2 mol equiv.) and dry dimethyl sulfoxide (DMSO) (20 cm^3) were stirred at 25 °C for 24 h. The reaction mixture was diluted with ethyl acetate (50 cm^3) and the solid product was collected, washed with ethyl acetate (3 × 20 cm^3) and then dried under high vacuum to give the acetate **6a** (12.7 g, 82%), mp 162–164 °C (decomp.) (Found: C, 40.2; H, 3.5; N, 32.0. $C_9H_{10}N_6O_4$ requires C, 40.6; H, 3.8; N, 31.6%); ν_{max}(KBr)/cm^{-1} 3412, 3185, 3098, 2999, 1744, 1694, 1611 and 1260; $\delta_H[(CD_3)_2SO]$ 8.92 (1 H, s, 6-H), 7.92 (1 H, s, NH), 7.77 (1 H, s, NH), 5.23 (2 H, s, CH_2), 4.22 (2 H, q, J 7.1, CH_2) and 1.24 (3 H, t, J 7.1, CH_3); $\delta_C[(CD_3)_2SO]$ 168 (CO), 162 (CO), 140 (CO), 135 (s), 133 (s), 131 (d, C-6), 63 (t, CH_2), 51 (t, CH_2) and 15 (q, CH_3); m/z (FAB, NOBA) 296 [(M^+ + Na), 20%] and 267 [(M^+ + H), 100%].

N-(5-Amino-4-carbamoylimidazol-1-ylcarbonyl)glycine ethyl ester 5

5-Aminoimidazole-4-carboxamide hydrochloride **2** (0.5 g), dry triethylamine (0.5 cm^3) and dry DMSO (3 cm^3) were mixed at 20 °C to form a homogeneous solution. Ethyl isocyanatoacetate (0.4 cm^3) was added and the mixture was stirred at 25 °C for 12 h. The reaction was quenched by the addition of crushed ice (20 g) and the product was collected, washed with ethyl acetate (2 × 10 cm^3) and acetone (10 cm^3) to give the glycine ester **5** (0.57 g, 80%), mp 183–185 °C (decomp.) (Found: C, 39.3; H, 5.5; N, 25.6%; M^+ + H, 256. $C_9H_{13}N_5O_4 \cdot H_2O$ requires C, 39.5; H, 5.4; N, 25.6%; M + H, 256); ν_{max}(KBr)/cm^{-1} 3499, 3426, 3334, 3236, 1727, 1658, 1555, 1505 and 1219; $\delta_H[(CD_3)_2SO]$ 9.07 (1 H, t, J 5.7, NH), 7.69 (1 H, s, 2-H), 6.95 (1 H, s, NH), 6.85 (1 H, s, NH), 6.41 (2 H, s, NH), 4.15 (2 H, q, J 7.1, CH_2), 4.00 (2 H, d, J 5.7, CH_2) and 1.21 (3 H, t, J 7.1, CH_3); $\delta_C[(CD_3)_2SO]$ 174.8 (s, CO), 171.7 (s, CO), 156.3 (s), 149.0 (s), 131.4, (d, C-2), 116.7 (s), 66.4 (t, CH_2), 47.2 (t, CH_2) and 19.6 (q, CH_3).

Diazotisation of the glycine ethyl ester **5** in hydrochloric acid (2 mol dm^{-3}) with sodium nitrite at 0 °C, followed by stirring for 1.5 h, gave the imidazotetrazine **6a** (93%), identical (TLC, IR and ^1H NMR) with the sample above.

8-Carbamoyl-3,4-dihydro-4-oxoimidazo[5,1-*d*]-1,2,3,5-tetrazin-3-ylacetic acid 6b

The ester **6a** (10.2 g) was suspended in hydrochloric acid (5 mol dm^{-3}; 50 cm^3). The suspension was stirred for 4 h at 40–45 °C until hydrolysis was complete (TLC). The mixture was concentrated under reduced pressure to 20 cm^3 and the solid product was collected and washed with acetone (3 × 20 cm^3). The product was dried under high vacuum to give the acetic acid **6b** (7.3 g, 80%), mp 165 °C (decomp.) (Found: C, 34.4; H, 2.7; N, 34.0. $C_7H_6N_6O_4 \cdot 0.5H_2O$ requires: C, 34.0; H, 2.80; N, 34.0%); ν_{max}(KBr)/cm^{-1} 3407, 3249, 3090, 3001, 1755, 1717, 1640 and 1588; $\delta_H[(CD_3)_2SO]$ 8.94 (1 H, s, 6-H), 7.92 (1 H, s, NH), 7.77 (1 H, s, NH) and 5.13 (2 H, s, CH_2); $\delta_C[(CD_3)_2SO]$ 173 (CO), 166 (CO), 144 (CO), 139, 137 (C-7, C-8), 134 (d, C-6) and 55 (t, CH_2).

8-Carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (temozolomide) 1

To a stirred mixture of **6b** (0.24 g) in dry dimethylformamide (DMF; 6 cm^3) at −10 to −15 °C was added isobutyl chloroformate (0.16 cm^3, 1.1 mol equiv.) and then *N*-methylmorpholine (0.12 cm^3, 1.1 mol equiv.) under nitrogen. After 10 min the reaction was complete and the mixture was evaporated to dryness. The residue was the crude carbonate ester **6c**, which can be purified by flash chromatography to yield a solid, mp 165–167 °C (decomp.); $\delta_H(CDCl_3)$ 0.91 (6 H, d, 2 × CH_3), 1.95 (1 H, m, CH), 3.99 (2 H, d, OCH_2), 5.14 (2 H, s, CH_2CO), 6.82 (1 H, br s, NH), 7.26 (1 H, br s, NH) and 8.43 (1 H, s, 6-H). The carbonate ester was dissolved in dry DMF (6 cm^3), 2-sulfanylpyridine 1-oxide (0.15 g, 1.2 mol equiv.) and

dry triethylamine (0.18 cm^3) were added and the mixture was stirred for a further 0.5 h at 10 to −15 °C. Solid morpholine hydrochloride was removed and the DMF solution of the pyridine-2(1*H*)-thione **6d** {δ_H[$(CD_3)_2SO$] 5.74 (2 H, s, CH_2), 7.02 (1 H, m, pyridyl CH), 7.56 (1 H, m, pyridyl CH), 7.65 (1 H, m, pyridyl CH), 7.89 (1 H, br s, NH), 8.03 (1 H, br s, NH), 9.07 (1 H, s, 6-H)} was added dropwise to a mixture of AIBN (catalytic amount) and tributylstannane (0.8 cm^3) in dry DMF (5 cm^3) under irradiation with a tungsten lamp (100 W) at room temperature under nitrogen. When reaction was complete (0.5 h), the solvent was removed from the mixture under reduced pressure and the residue was extracted with light petroleum (3 × 20 cm^3). The syrupy residue was purified by flash chromatography on silica gel to give temozolomide **1** (0.05 g, 26%) identical (^1H NMR) with an authentic sample.

Reductive cleavage of the pyridine-2(1*H*)-thione **6d** prepared from the tetrazinylacetic acid **6b** as above with benzenethiol (0.5 g) and 4-dimethylaminopyridine (DMAP, catalyst) in refluxing dry DMF (10 cm^3) also gave temozolomide **1** (31%).

N-{(8-Carbamoyl-3,4-dihydro-4-oxoimidazo[5,1-*d*]-1,2,3,5-tetrazin-3-yl)methylcarbonyl}glycine ethyl ester 6e

To a solution of the carbonate ester **6c** in dry DMF at −10 °C, was added glycine ethyl ester hydrochloride (0.24 g, 1.7 mol equiv.) and dry triethylamine (0.2 cm^3). The reaction mixture was stirred for 30 min at −10 °C. After evaporation of the mixture the residue was purified by flash chromatography on silica gel to give glycine ethyl ester **6e** (0.2 g, 62%), mp 145–146 °C (Found: C, 39.65; H, 4.10; M^+ + H, 324. $C_{11}H_{13}N_7O_5 \cdot 0.5H_2O$ requires C, 39.8; H, 4.2%; M^+ + H, 324); ν_{max}(KBr)/cm^{-1} 3455, 3323, 3151, 1749, 1691, 1656, 1457 and 1217; δ_H[$(CD_3)_2SO$] 8.92 (1 H, s, 6-H), 8.77 (1 H, t, *J* 5.8, NH), 7.90 (1 H, s, NH), 7.76 (1 H, s, NH), 5.02 (2 H, s, CH_2), 4.09 (2 H, q, *J* 7.1, CH_2), 3.89 (2 H, d, *J* 5.8, CH_2) and 1.18 (3 H, t, *J* 7.1, CH_3); δ_C[$(CD_3)_2SO$] 174.9 (s), 171.8 (s), 166.9 (s), 153.2 (s), 144.6 (s), 139.7 (s), 136.8 (s), 134.7 (d, C-6), 66.1 (t, CH_2), 56.3 (t, CH_2), 46.3 (t, CH_2) and 19.6 (q, CH_3).

N-{(8-Carbamoyl-3,4-dihydro-4-oxoimidazo[5,1-*d*]-1,2,3,5-tetrazin-3-yl)methylcarbonyl}glycylglycine ethyl ester 6f

This ester, similarly prepared from glycylglycine ethyl ester hydrochloride, was purified by flash chromatography (0.25 g, 65%), mp 136 °C (Found: C, 39.9; H, 4.2%; M^+ + H, 381. $C_{13}H_{16}N_8O_6 \cdot 0.5H_2O$ requires C, 40.1; H, 4.4%; M^+ + H, 381); ν_{max}(KBr)/cm^{-1} 3300, 3117, 2985, 1745, 1663, 1560, 1458, 1366 and 1216; δ_H[$(CD_3)_2SO$] 8.91 (1 H, s, 6-H), 8.70 (1 H, t, *J* 5.6, NH), 8.40 (1 H, t, *J* 5.7, NH), 7.89 (1 H, s, NH), 7.75 (1 H, s, NH), 5.03 (2 H, s, CH_2), 4.09 (2 H, q, *J* 7.1, CH_2), 3.84 (4 H, m, 2 × CH_2) and 1.88 (3 H, t, *J* 7.1, CH_3); δ_C[$(CD_3)_2SO$] 175.2, 174.9, 174.5, 171.5, 144.6, 139.7, 136.9, 134.6 (d, C-6), 66.0 (t, CH_2), 56.5 (t, CH_2), 47.2 (t, CH_2), 46.2 (t, CH_2) and 19.6 (q, CH_3).

Synthesis of isocyanates

(*i*) Iodomethyltrimethylsilane (48 g) and dry DMSO (120 cm^3) were stirred under argon and potassium cyanate (18.16 g) was added. The mixture was heated at 120 °C for 2 h and distilled through a 15 cm glass Vigreux column (bp 60–62 °C/58 mm of Hg) to give the yellow, oily, unstable trimethylsilylmethyl isocyanate (16.2 g, 56%); ν_{max}(neat)/cm^{-1} 2291 (N=C=O); δ_H($CDCl_3$) 0.13 (9 H, s, 3 × CH_3) and 2.72 (2 H, s, CH_2).

(*ii*) (2-Trimethylsilylethoxy)methyl chloride (SEM chloride; 9 g) in dry ether (100 cm^3) was refluxed with silver cyanate (10.52 g) under argon in the dark for 18 h. The ethereal solution was filtered to remove silver chloride and distilled to remove ether. (2-Trimethylsilylethoxy)methyl isocyanate (bp 82–84 °C/21 mm of Hg) was collected (2.7 g, 29%) (Found: C, 48.8; H, 8.8; N, 7.7. $C_7H_{15}NO_2Si$ requires C, 48.5; H, 8.7; N, 8.1%); ν_{max}(neat)/cm^{-1} 2280 (N=C=O); δ_H($CDCl_3$) 0.04 (9 H, s, 3 × CH_3), 0.96 (2 H, t, CH_2CH_2O), 3.67 (2 H, t, CH_2CH_2O) and 4.78 (2 H, s, CH_2NCO).

(*iii*) 4-Methoxybenzyl chloride (12 g) and silver cyanate were allowed to react in boiling ether as above (18 h) to give 4-methoxybenzyl isocyanate (7.9 g, 74%), bp 116–118 °C/6 mm of Hg; ν_{max}(neat)/cm^{-1} 2271 (N=C=O); δ_H($CDCl_3$) 3.79 (3 H, s, CH_3), 4.38 (2 H, s, CH_2), 6.88 (2 H, d, aryl 3-H, 5-H) and 7.22 (2 H, s, aryl 2-H, 6-H).

(*iv*) Bromodiphenylmethane and silver cyanate were allowed to react in refluxing ether (3 h) under argon to give diphenylmethyl isocyanate, bp 137–140 °C/2.3 mm of Hg; ν_{max}(neat)/cm^{-1} 2259 (N=C=O); δ_H($CDCl_3$) 5.79 (1 H, s, CH) and 7.28–7.32 (10 H, m, 2 × Ph).

8-Carbamoyl-3-trimethylsilylmethylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one 6g

Trimethylsilylmethyl isocyanate (1.85 g) and 5-diazoimidazole-4-carboxamide **3** (0.98 g) were stirred in dry DMSO (10 cm^3) for 70 h at 25 °C in the dark. The solution was diluted with water (10 cm^3) and extracted with chloroform (3 × 40 cm^3). The combined chloroform layers were washed with water (3 × 30 cm^3) and evaporated to give the imidazotetrazinone **6g** (0.62 g, 33%) as pink crystals, mp 190–192 °C (decomp.) (Found: C, 62.6; H, 4.0; N, 23.5%; M^+ + H, 267. $C_9H_{14}N_6O_2Si$ requires C, 62.4; H, 4.1; N, 23.5%; M^+ + H, 267); ν_{max}(KBr)/cm^{-1} 3435 (NH), 1721 (tetrazinone C=O) and 1687 ($CONH_2$); δ_H[$(CD_3)_2SO$] 0.16 (9 H, s, 3 × CH_3), 3.79 (2 H, s, CH_2), 7.67 (1 H, br s, NH), 7.81 (1 H, br s, NH) and 8.78 (1 H, s, 6-H).

The trimethylsilylmethylimidazotetrazine **6g** (0.1 g) in a mixture of dry acetonitrile (5 cm^3) and acetic acid (0.068 g, 3 mol equiv.) was treated with tetrabutylammonium fluoride (TBAF) (0.198 g, 1 mol equiv.) over 10 min. The mixture was stirred at 25 °C for 24 h and then evaporated to a small volume under reduced pressure. The white precipitate (0.058 g, 78%) was identical (TLC, IR, ^1H NMR, MS) with an authentic sample of temozolomide **1**.

8-Carbamoyl-3-(2-trimethylsilylethoxy)methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one 6h

This compound, prepared by a similar method to the above, from (2-trimethylsilylethoxy)methyl isocyanate and 5-diazoimidazole-4-carboxamide **3** in DMSO, to give imidazotetrazinone **6h** (78%), had mp 190–192 °C (decomp.) (Found: C, 42.9; H, 6.0; N, 26.95. $C_{11}H_{18}N_6O_2Si$ requires C, 42.6; H, 5.85; N, 27.1%); ν_{max}(KBr)/cm^{-1} 3380 and 3190 (NH), 1739 (tetrazinone C=O) and 1677 ($CONH_2$); δ_H[$(CD_3)_2SO$] −0.01 (9 H, s, 3 × CH_3), 0.91 (2 H, t, CH_2CH_2O), 3.71 (2 H, t, CH_2CH_2O), 5.64 (2 H, s, OCH_2N), 7.73 (1 H, br s, NH), 7.87 (1 H, br s, NH) and 8.88 (1 H, s, 6-H).

Attempts to remove the silyl protecting group from **6h** with TBAF (6 mol equiv.) in acetonitrile, or TBAF adsorbed on silica gel (4 mol equiv.) in acetonitrile led, in both cases, to the isolation (flash chromatography) of low yields (10%) of 2-azahypoxanthine **4**.

3-Benzyl-8-carbamoylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one 10a

A mixture of 5-diazoimidazole-4-carboxamide **3** (2.5 g) and benzyl isocyanate (7 cm^3) was stirred in DMSO (10 cm^3) in the dark for 24 h. The white benzylimidazotetrazine (2.03 g, 36%) was collected after being washed with ethyl acetate, and had mp 188 °C (decomp.) (Found: C, 53.3; H, 3.7; N, 31.3; M^+ + H, 271. $C_{12}H_{10}N_6O_2$ requires C, 53.3; H, 3.7; N, 31.1%; M^+ + H, 271); ν_{max}(KBr)/cm^{-1} 3447, 3162, 3099, 1734, 1678, 1607, 1449 and 1366; δ_H[$(CD_3)_2SO$] 5.50 (2 H, s, CH_2), 7.29–7.45 (5 H, m, Ph), 7.71 (1 H, br s, NH), 7.84 (1 H, br s, NH) and 8.83 (1 H, s,

6-H); $\delta_C[(CD_3)_2SO]$ 162.37, 140.03, 136.46, 135.29, 131.85, 129.87, 129.40, 128.81 and 52.7.

Attempts to effect hydrogenolysis of **10a** using 10% palladium–charcoal catalyst in cyclohexene, ethyl acetate or acetic acid at 40 psi in a Parr hydrogenator, or employing 20% palladium hydroxide (Perlman's catalyst) in acetic acid at 40 psi led to the quantitative recovery of starting material in all cases.

8-Carbamoyl-3-(4-methoxybenzyl)imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one 10b

This compound, prepared using the method above, from 5-diazoimidazole-4-carboxamide **3** and 4-methoxybenzyl isocyanate, to give the methoxybenzylimidazotetrazine **10b** (46%), had mp 168–170 °C (decomp.) (Found: $M^+ + H$, 301. $C_{13}H_{12}N_6O_3$ requires $M^+ + H$, 301); ν_{max}(KBr)/cm^{-1} 3432 and 3132 (NH), 1741 (tetrazine C=O) and 1691 ($CONH_2$); $\delta_H[(CD_3)_2SO]$ 3.73 (3 H, s, CH_3), 5.42 (2 H, s, CH_2), 6.91 (2 H, d, aryl 3-H, 5-H), 7.37 (2 H, s, aryl 2-H, 6-H), 7.71 (1 H, br s, NH), 7.83 (1 H, s, NH) and 8.81 (1 H, s, 6-H).

Attempted removal of the 4-methoxybenzyl group by reduction (hydrogen over Perlman's catalyst at 40 psi in acetic acid–ethyl acetate) or oxidation (2,3-dichloro-5,6-dicyano-1,4-benzoquinone in dichloromethane) led to the quantitative recovery of the starting imidazotetrazine **10b**.

8-Carbamoyl-3-diphenylmethylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one 10c

Interaction of 5-diazoimidazole-4-carboxamide **3** and diphenylmethyl isocyanate in DMSO as above afforded the diphenylmethylimidazotetrazine **10c** (80%), mp 168–171 °C (decomp.) (Found: C, 62.0; H, 4.0; N, 23.5. $C_{18}H_{14}N_6O_2$ requires C, 62.4; H, 4.0; N, 24.3%); ν_{max}(KBr)/cm^{-1} 3438 and 3132 (NH), 1741 (tetrazinone C=O) and 1691 ($CONH_2$); $\delta_H[(CD_3)_2SO]$ 7.30 (1 H, s, Ph_2CH), 7.33–7.43 (10 H, br s, 2 × Ph), 7.69 (1 H, br s, NH), 7.83 (1 H, br s, NH) and 8.85 (1 H, s, 6-H). The imidazotetrazine was recovered unchanged following attempted hydrogenolysis or oxidation (see above).

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