Antitumor Imidazotetrazines. 35. New Synthetic Routes to the Antitumor Drug Temozolomide

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Three new pathways to the antitumor drug temozolomide (**4**) have been explored *via* intermediates **3**, **6**, and **7**. The key intermediate 5-amino-1-(*N*-methylcarbamoyl)imidazole-4-carboxamide (**6**) has been successfully converted to **4** in 45% yield by employing sodium nitrite in aqueous tartaric acid at 0−5 °C. Compound **6** is prepared from nitrophenyl carbamate **14a** and methylamine or directly from 5-aminoimidazole-4-carboxamide (**13**) and either methyl isocyanate or *N*-methylcarbamoyl chloride. Temozolomide (**4**) is also prepared from 8-cyano-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (**7**) by hydrolysis to the hydrochloride salt of **4** in 10 M hydrochloric acid. Compound **7** is prepared from either 5-diazoimidazole-4-carbonitrile (**28**) and methyl isocyanate or by diazotization of 5-amino-1-(*N*-methylcarbamoyl)imidazole-4-carbonitrile (**25**). Attempts to cyclize 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (**3**) with phosgene or phosgene equivalents were unsuccessful: only 2-azahypoxanthine (**11**) was isolated.

Introduction

The antitumor drug temozolomide (**4**) is the second example of the imidazo[5,1-*d*]-1,2,3,5-tetrazine ring system to enter clinical trials[1] and is currently undergoing clinical evaluation in patients suffering from brain tumors[2] and malignant melanoma.[3] The first synthesis of temozolomide, from the reaction of 5-diazoimidazole-4-carboxamide (**1**) with methyl isocyanate (MIC) in a DMSO−EtOAc mixed solvent system, probably proceeds *via* the dipolar intermediate **2** and affords material in both high purity and yield. The synthesis represents an excellent example of "atom economy" in that all atoms in the starting materials are incorporated into the product.[4] However, since our original work the catastrophic events at Bhopal in 1984 have given MIC an unsavory reputation.[5]

Our earlier efforts to develop alternative syntheses of temozolomide obviating the use of MIC centered on the formation of imidazotetrazines from less volatile "masked MICs" which could be deprotected to furnish the required 3-methyl functionality (Scheme 1). Whereas the acid **5a** was decarboxylated to temozolomide by Barton radical methodology in a poor overall yield of 26%,[6,7] the 3-(trimethylsilylmethyl)imidazotetrazine (**5b**) was efficiently deprotected (78%) with tetrabutylammonium fluoride in acetonitrile−acetic acid.[7] By employing acidic conditions in the aforementioned reactions, the integrity (to ring opening)[8] of the imidazotetrazine nucleus was retained. However, these alternatives still require the use of 5-diazoimidazole-4-carboxamide (**1**) to synthesize the precursor imidazotetrazines **5a**,**b**. Although solid aryldiazonium salts are notoriously unstable, the internally zwitterionic diazoimidazole **1** can be crystallized unchanged from hot THF and it can be used efficiently if due attention is given to its propensity to cyclize to 2-azahypoxanthine (**11**), especially in the presence of nucleophiles and protic solvents.[7]

In this paper we report on several synthetic strategies to temozolomide which avoid the use of MIC. These are cyclization of 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (**3**) employing phosgene (or phosgene equivalents); nitrosative cyclization of the (*N*-methylcarbamoyl)imidazole **6**, which has been the subject of a preliminary communication;[9] and hydrolysis of the 8-cyano-3-methylimidazotetrazine **7** ("cyanotemozolomide"). While these

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(4) Stevens, M. F. G.; Hickman, J. A.; Stone, R.; Gibson, N. W.; Baig, G. U.; Lunt, E.; Newton, C. G. *J. Med. Chem.* **1984**, *27*, 196−201.

(5) D'Silva, T. D. J.; Lopes, A.; Jones, R. L.; Singhawangcha, S.; Chan, J. K. *J. Org. Chem.* **1986**, *51*, 3781−3788.

(6) Wang, Y.; Stevens, M. F. G.; Thomson, W. T. *J. Chem. Soc., Chem. Commun.* **1994**, 1687−1688.

(7) Wang, Y.; Stevens, M. F. G.; Thomson, W. T.; Shutts, B. P. *J. Chem. Soc., Perkin Trans. 1.* **1995**, 2783−2787.

(8) Denny, B. J.; Wheelhouse, R. T.; Stevens, M. F. G.; Tsang, L. L. H.; Slack, J. A. *Biochemistry* **1994,** *33*, 9045−9051.

(9) Wang, Y.; Lowe, P. R.; Thomson, W. T.; Clark, J.; Stevens, M. F. G. *J. Chem. Soc., Chem. Commun.* **1997**, 363−364.

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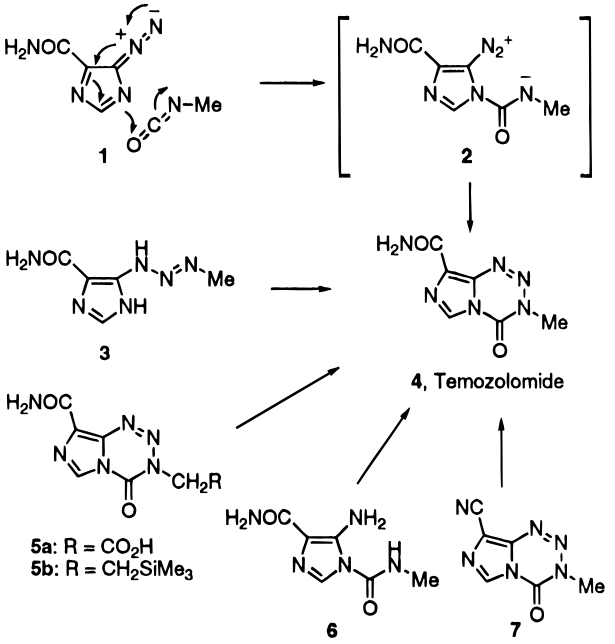
Scheme 1

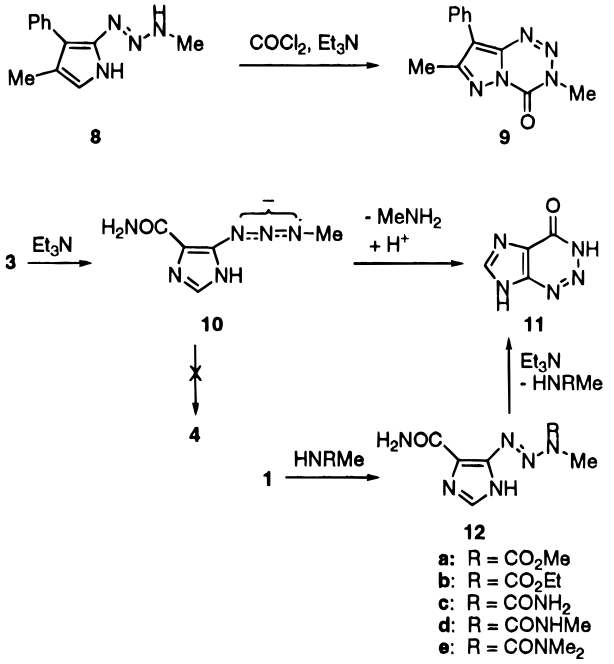


routes engendered unexpected complexities, these studies provided insight on the imidazotetrazine reactivity patterns of these potential temozolomide precursors.

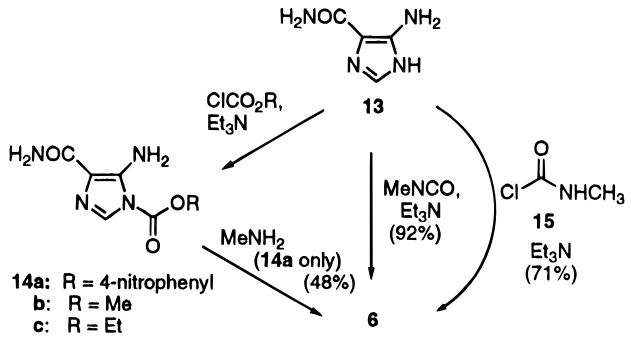
Results and Discussion

Cyclization of 5-(3-Methyltriazen-1-yl)imidazole-4-carboxamide (3). Ege and his colleagues have reported on the cyclization of the (methyltriazenyl)pyrazole **8** to the 3-methylpyrazolotetrazinone **9** (65%) with phosgene.[10] Application of this procedure using the methyltriazene **3**, triethylamine, and phosgene or the phosgene equivalents 1,1′-carbonyldiimidazole, 4-nitrophenyl chloroformate, or chloroformic acid trichloromethyl ester ("diphosgene") was unsuccessful, leading to the recovery of starting material or the formation of mixtures which did not contain temozolomide when monitored by ^{1}H NMR spectroscopy. An explanation for the latter outcome, which contrasts with the successful pyrazole cyclizations, is probably due to the instability of triazene **3** and the complexity of its chemistry under the reaction conditions. Thus, when a solution of **3** in $(CD_3)_2SO$ was maintained at 25 °C and monitored by ^{1}H NMR spectroscopy, after 5 days the methyl absorption of triazene **3** at δ 3.0 was replaced by a plethora of extra absorptions (>20) in the δ 2−4 range, probably attributable to methylated imidazoles. Also, the presence of the carboxamide group in the imidazole series can intercept the triazenyl anion **10** formed from the strongly acidic triazene **3** and triethylamine, leading to competitive cyclization to 2-azahypoxanthine (**11**). Further examples of such diversions were shown when attempts to couple the diazoimidazole **1** with methyl or ethyl *N*-methylcarbamates in acetonitrile, ethyl acetate, THF, or DMSO led only to the isolation of 2-azahypoxanthine (**11**). Similarly, efforts to couple the diazoimidazole **1** with *N*-methylurea, *N*,*N*′-dimethylurea, and *N*,*N*,*N*′-trimethylurea and to cyclize the intermediate triazenes **12a**−**e** *in situ* with triethylamine in refluxing THF led only to the isolation of a poor yield of 2-azahypoxanthine (**11**) (Scheme 2).

Scheme 2



Scheme 3

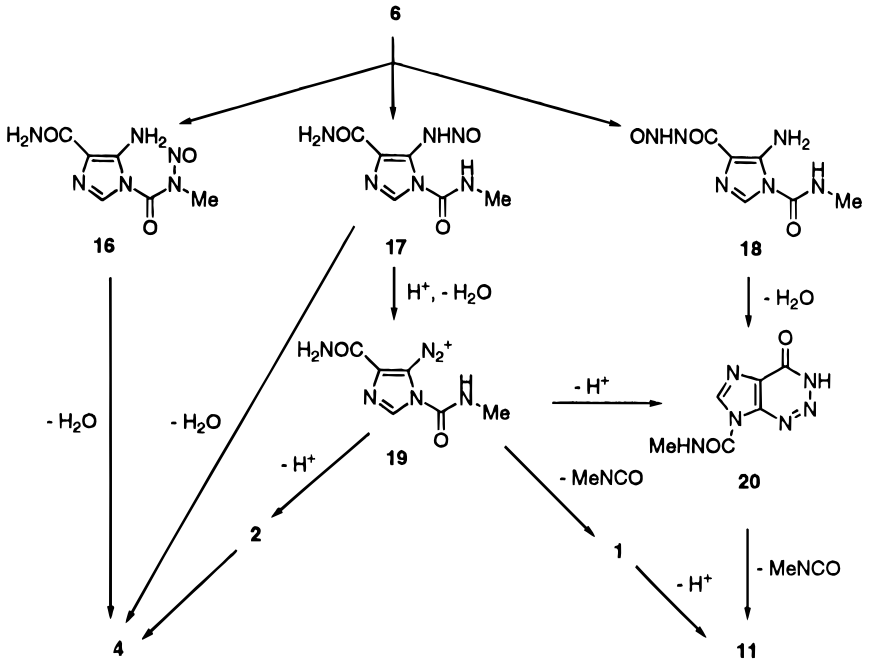


Cyclization of 5-Amino-1-(*N*-methylcarbamoyl)imidazole-4-carboxamide (6). Three routes to the required starting imidazole **6** have been devised (Scheme 3). 5-Aminoimidazole-4-carboxamide free base (**13**), generated from the hydrochloride salt with triethylamine, reacts regiospecifically with MIC in acetonitrile to afford **6** in 92% yield. (It is interesting to note that methyl isothiocyanate reacts with 5-aminoimidazole-4-carboxamide exclusively at the exocyclic amino group under the same conditions).[9] Two modifications which avoid the use of MIC were also developed. We have shown previously that **13** reacts at the imidazole N-1 position with methyl or ethyl chloroformate to yield the carbamates **14b,c**.[11] The first modification involves initial formation of the carbamate **14a** (80%) from **13** and 4-nitrophenyl chloroformate in dichloromethane. Reaction of carbamate **14a** with 1 equiv of methylamine furnished the required imidazole **6** (48%) together with 4-nitrophenol: use of excess methylamine led to cleavage of the carbamate with regeneration of the aminoimidazole **13**. The second modification, and more direct route, is carbamoylation of **13** using methylcarbamoyl chloride (**15**), in which case a 71% yield of **6** was obtained.

(10) Ege, G.; Gilbert, K.; Maurer, K. *Chem. Ber.* **1987**, *120*,1375−1395.

(11) Baig, G. U.; Stevens, M. F. G. *J. Chem. Soc., Perkin Trans. 1* **1987**, 665−670.

Scheme 4



Nitrosative cyclization of the urea **6** was first attempted under "normal" diazotization conditions—sodium nitrite in mineral acids (e.g. hydrochloric, sulfuric, nitric and boric acids, and potassium dihydrogen phosphate) at 0−5 °C. Always, an initial purple color developed which was usually discharged as the reaction proceeded. Yields of temozolomide (**4**) ranged from 0% (boric acid) to a maximum of 30% (2 M hydrochloric acid). These disappointing outcomes contrast with the quantitative yield of 3-methyl-1,2,3-benzotriazin-4(3*H*)-one from the nitrosative cyclization of *N*-methylanthranilamide with sodium nitrite in mineral acids.[12] In the imidazole cyclization the reaction pH had only a marginal bearing on the yield: using an aqueous sodium nitrite/sulfuric acid/pyridine mixture the yields of temozolomide ranged from 29% (pH 0.5) to 39% at pH 4.

A theoretical evaluation of likely mechanisms of cyclization gave some possible explanations into the reasons for the poor yields (Scheme 4). Nitrosation of the starting urea **6** could give the nitrosamine **17** or two isomers, the *N*-nitrosourea **16** and the nitrosocarboxamide **18**, the latter two species possibly accounting for the purple coloration. Conceivably, **16** and **17** might undergo dehydrative cyclization directly to temozolomide (**4**). A more likely pathway to temozolomide in the acidic medium would involve conversion of nitrosamine **17** to the diazonium species **19**. To cyclize efficiently to temozolomide the urea proton must ionize to generate the zwitterion **2**, the intermediate generated in the reaction between 5-diazoimidazole-4-carboxamide (**1**) and MIC which is known to cyclize quantitatively to temozolomide.[4] Unfortunately the electron-donating methyl group would suppress ionization and thereby inhibit cyclization. Consistent with this hypothesis other *N*-alkyl analogues of urea **6** with more electron-withdrawing groups (e.g. benzyl or CH_2CO_2Et) cyclize efficiently (>90% for CH_2CO_2Et) to imidazotetrazinones.[7,9] Sadly, basification of the reaction mixture after nitrosation to generate the required anion is not a practicable option because of the base-lability of temozolomide.[8]

To explore the influence of other nitrosation conditions on product yield, an extensive yield optimization study on the cyclization of urea **6** was conducted. Among the variables examined were the following: temperature range (−20 to 40 °C); different nitrosating reagents (potassium nitrite, nitrosonium tetrafluoroborate, organic nitrites); polar and nonpolar solvents (water, methanol, ethanol, acetone, ethyl acetate, acetonitrile, DMSO, formamide, DMF, THF, and DCM, plus mixtures of the aforementioned with water); a range of aliphatic acids (formic, acetic, chloroacetic, trifluoroacetic, propionic, (*S*)-2-chloropropionic, butyric, malonic, succinic, glutaric, adipic, lactic, citric, tartaric, tetrahydrofuran tetracarboxylic, glucuronic, and ascorbic); and the aromatic acids (benzoic, 3-chlorobenzoic, nicotinic, 2-chloronicotinic, *p*-toluenesulfonic acids, and 2-nitrophenol). In general, aqueous conditions were better than nonaqueous, inorganic nitrites superior to organic nitrites. The overall optimum conditions (45% solution yield) were using sodium nitrite (1 mol equiv), water, and tartaric acid (1 mol equiv) in a solvent-to-substrate ratio of 68:1 at 0−5 °C. Use of the phase transfer catalysts tetramethylammonium hydroxide, tetrabutylammonium hydroxide, tetrabutylammonium hydrogen sulfate, and hexadecyltrimethylammonium bromide did not improve the yields, and use of other potential cyclization modulators such as 15-crown-5, glyceraldehyde, succinimide, β-cyclodextrin, benzenesulfonamide, potassium thiocyanate, zinc or copper acetate, and lithium chlorate had no yield-enhancing effects.

Certain aminoazoles afford isolatable nitrosamines when reacted with organic nitrites.[13] To more closely evaluate the possible role of nitrosamine **17** in the pathway to temozolomide, urea **6** was reacted with

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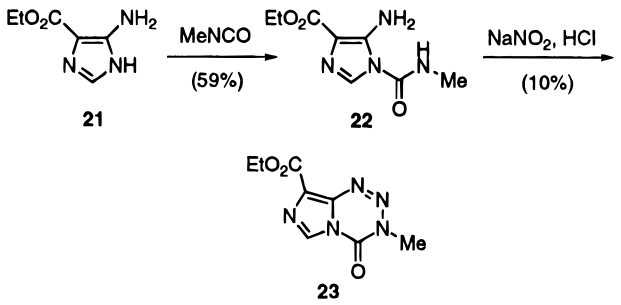
(13) (a) Butler, R. N.; Lambe, T.; Scott, F. L. *Chem. Ind. (London)* **1970**, 628−630. (b) Stollé, R.; Dietrich, W. *J. prakt. Chem.* **1934**, *139*, 193−210. (c) Hauser, M. U.S. Patent 3,431,251 (*Chem. Abstr.* **1969**, *70*, 96802*g*).

Scheme 5



isoamyl nitrite or *tert*-butyl nitrite in acetic, trifluoroacetic, and (*S*)-2-chloropropionic acids. Although a characteristic purple color was imparted to the reaction mixture, no nitrosamine **17** (or nitrosoamide **16**, or nitrosocarboxamide **18**) could be isolated, with temozolomide detected (HPLC) in only 5−15% yields.

In all the aforementioned nitrosative cyclizations, 2-azahypoxanthine (**11**) was a major byproduct. HPLC and NMR study of this reaction in D_2O showed that even at 0 °C (with acetic acid and $NaNO_2$) formation of two products [temozolomide (**4**) and the 9-(*N*-methylcarbamoyl)-2-azahypoxanthine (**20**)] set in at the commencement of the reaction in approximately equal amounts: this reaction is usually complete in ≈2 h. With time the hydrolysis of the 9-substituted azahypoxanthine **20** (i.e. formal elimination of MIC) proceeds to generate 2-azahypoxanthine (**11**). There are two other possible routes to 2-azahypoxanthine (**11**). Nitrosocarboxamide **18** could undergo dehydrative cyclization to the same 9-substituted azahypoxanthine **20** and thence 2-azahypoxanthine (**11**). Finally, loss of MIC directly from the diazonium intermediate **19** would lead to 5-diazoimidazole-4-carboxamide (**1**), which is known to cyclize readily to 2-azahypoxanthine (**11**) under acid conditions.[14]

These competitive pathways involving participation of the carboxamide group are not the full explanation for the poor yields of temozolomide, since the imidazole ester **22**, formed from ethyl 5-aminoimidazole-4-carboxylate (**21**) and MIC in acetonitrile−triethylamine (59%), on nitrosation with sodium nitrite in 50% aqueous acetic acid cyclized in only 10% yield to the imidazotetrazine **23** (Scheme 5). Clearly, in this example, the ester group cannot participate in a competing intramolecular coupling and this suggests that the weakly acidic nature of the urea proton in the diazonium intermediate **19** is probably the major impediment to its efficient cyclization to temozolomide, presumably allowing other degradation pathways to consume reactive intermediates such as **19**.

Hydrolysis of 8-Cyano-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (7). Because of the convenient acid stability of temozolomide,[8] an approach *via* hydrolysis of cyanotemozolomide **7** was expected to be feasible, provided a practical route to this precursor could be devised (Scheme 6).

5-Aminoimidazole-4-carboxamide (**13**) was first dehydrated to the corresponding 4-carbonitrile **24** (40%) with phosphorus oxychloride. Direct carbamoylation of **24** with MIC gave only a 5% yield of the urea **25**. A more efficient *de novo* synthesis of **25** (78%) was achieved by reacting aminomalononitrile **26** with *N*,*N*′-bis(*N*-methylcarbamoyl)formamidine (**27**) in acetic acid.[15] However, nitrosative cyclization of **25** with sodium nitrite in 2 M hydrochloric acid ("normal conditions") gave the required cyanotemozolomide **7** in only 9% yield. As with the cyclization of urea **6**, an extensive yield optimization study was similarly carried out for the conversion **25** to **7**. Variables examined included the following: temperature range (5 to ≈80 °C); different nitrosating reagents (potassium nitrite, nitrosonium tetrafluoroborate, nitrosylsulfuric acid, isoamyl nitrite, *tert*-butyl nitrite, *n*-butyl nitrite); polar and nonpolar solvents (water, acetonitrile, methylene chloride, DMF, and DMSO, plus mixtures of the aforementioned with water); pH range (concd HCl or H_2SO_4 to pH 5); acids [HCl, H_2SO_4, HOAc, KH_2PO_4, K_2HPO_4, $(n\text{-Bu})_4NHSO_4$]; concentration range (0.1−5%); and tuning of stoichioimetry and order of addition of reagents. As with urea **6,** only a marginal improvement in the yield for the cyclization of **25** was achieved. The optimal conditions for cyclization of **25** to **7** employed sodium nitrite (1.5 mol equiv) and acetic acid (68 mol equiv) in water at 25 °C. Spectroscopic analysis (^1H NMR and FT-IR) of the reaction mixture showed it to contain cyanotemozolomide **7** (23% solution yield, HPLC) and an unstable primary nitrosamine (or its diazohydroxide tautomer), possibly **30.** (The proposed assigned structure of **30** is consistent with the analytical results, including LC−MS of the reaction mixture.) The reaction monitoring indicated that diazotization is rapid to form the diazo intermediate **29**, which then forms **7** and **30** in approximately a 1:5 ratio throughout the course of the reaction. Attempts to either isolate **30** or to convert it to **7** were unsuccessful.

For completeness, another route to cyanotemozolomide **7** involved conversion of 5-aminoimidazole-4-carbonitrile (**24**) to the corresponding diazoimidazole **28** (83%), which was then reacted with MIC in DMSO to give the bicycle in 52% yield. Selective hydrolysis of the nitrile function of **7** was effected efficiently in 10 M hydrochloric acid at 60 °C. Removal of the acid gave a product with a ^1H NMR spectrum in $[(CD_3)_2SO]$ identical to that of temozolomide. However, elemental microanalysis confirmed that the new product was a hydrochloride salt of temozolomide (65% yield). This was unexpected since in all our previous, extensive studies on imidazotetrazines we had never observed such salts. As expected for a very weak base, the salt dissociated in water or in DMSO solution to give pure temozolomide base **4**. Structure **31** is assigned to this salt on the basis of our recent work on the synthesis of isotopically labeled temozolomide and a multinuclear (^1H, ^{13}C, ^{15}N) magnetic resonance study.[16] In the ^{15}N spectrum of temozolomide the absorption of the N(7) atom in DMSO at −105.6 ppm is shifted downfield to −168.1 ppm in TFA. Significantly, when the data were acquired with full ^1H NOE the signal became negative, confirming the attachment of a proton at N(7). Also, location of the proton at this site explains the unexpected acid stability of temozolomide, since protonation in the tetrazine ring would destabilize the bicyclic system.

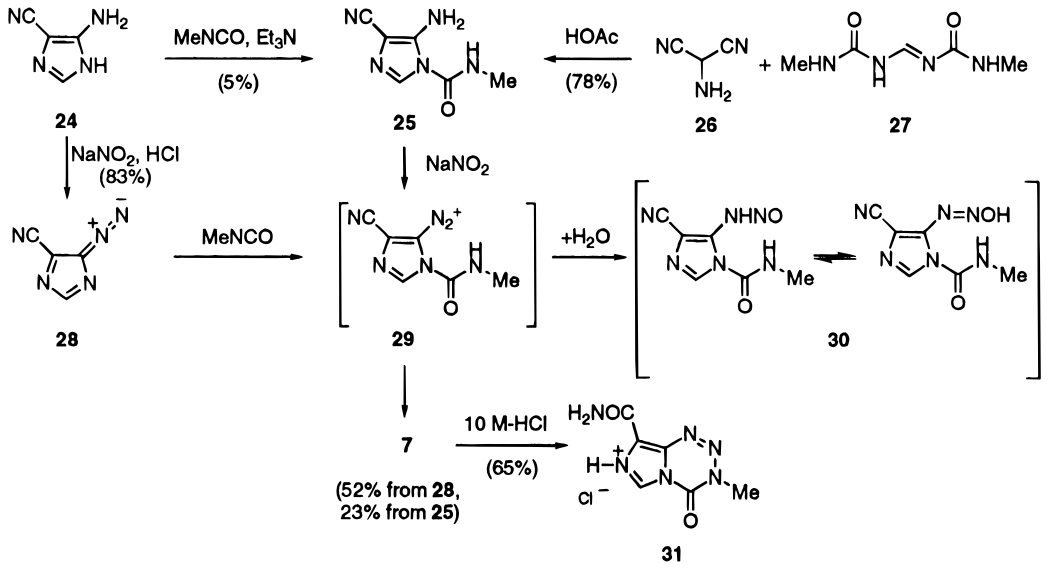
The disappointing results for the poor yields of imidazotetrazinones obtained in the cyclizations of **22** and **25** in comparison with **6** suggest that the 4-substituent in the imidazole ring plays a role in determining the

(14) Horton, J. K.; Stevens, M. F. G. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1433−1436.

(15) Details of this *de novo* synthesis of substituted imidazoles will be published elsewhere.

(16) Wheelhouse, R. T.; Wilman, D. E. V.; Thomson, W. T.; Stevens, M. F. G. *J. Chem. Soc., Perkin Trans. 1* **1995**, 249−252.

Scheme 6



cyclization outcome. (For example, **6** → **4** proceeds in 45% yield versus 10% and 23% respectively for **22** → **23** and **25** → **7**. Similarly, **1** → **4** proceeds in >90% yield versus 52% for **28** → **7**.) It may be that the CO_2Et and CN groups are too electron-withdrawing and inductively decrease the electron density of the urea nitrogen, whereas the $CONH_2$ group has minimal effect.

In conclusion, we report new syntheses of temozolomide that bypass the requirement for intermediates which might be problematic when considered for use on an industrial scale. Furthermore, subtle reactivity patterns were observed and point out the complexity in handling and constructing these unique ring systems, as exemplified by the differences in reactivity between **3** and **8** as well as between **6**, **22**, and **25**. The chemical reactions exploited to achieve the various syntheses of temozolomide are quite distinct from the chemistry of the drug *in vivo*, where the imidazotetrazine nucleus acts as a molecular device to deliver a methylating fragment to DNA.[8]

Experimental Section

General. Melting points are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or $(CD_3)_2SO$ solutions at 400 and 100 MHz, respectively, with Me_4Si as the internal standard, unless specified otherwise. The FT-IR reaction monitoring was carried out using an Applied Systems REACT-IR 1000 system. LC−mass spectra were acquired by electrospray ionization. Unless specified otherwise, all reagents and solvents were used as supplied by the manufacturer.

Attempted Cyclization of 5-(3-Methyltriazen-1-yl)imidazole-4-carboxamide (3). A suspension of 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (0.1 g)[17] in dry THF (20 mL) containing Et_3N (1 mol equiv) was treated with phosgene (1.1 mol equiv) or the phosgene equivalents 1,1′-carbonyldiimidazole, 4-nitrophenyl chloroformate, or chloroformic acid trichloromethyl ester ("diphosgene"). The mixtures were stirred at 25 °C for 48 h and solids were collected and washed with dry THF. Analysis of the products by ^1H NMR spectroscopy confirmed that no temozolomide was formed in these reactions.

Coupling Reactions between 5-Diazoimidazole-4-carboxamide (1) and *N*-Methylcarbamates and *N*-Methylureas. (1) There was no reaction when the diazoimidazole **1** was stirred in excess methyl or ethyl *N*-methylcarbamates at 25 °C.

(2) When the diazoimidazole **1** was treated with methyl *N*-methylcarbamate (1 mol equiv) in either CH_3CN, EtOAc, or THF in the temperature range 25 °C to the boiling point of the solvent, or in DMSO at 25 °C, over the time range 4−72 h, 2-azahypoxanthine (**11**) was the only identified product: ^1H NMR $[(CD_3)_2SO]$ δ = 8.50 (s, 1 H), 14.25 (brs, 1 H), 15.07 (brs, 1 H), identical to the spectrum of an authentic sample.[7,14]

(3) To a solution of the diazoimidazole **1** (1.37 g) in dry THF (25 mL) was added *N*-methylurea, *N*,*N*′-dimethylurea, or *N*,*N*,*N*′-trimethylurea (1 mol equiv) and the solutions were stirred for 12 h. NEt_3 (1.01 g, 1 mol equiv) was added and the mixtures were refluxed for 24 h. Monitoring of the course of the reactions by ^1H NMR confirmed that no temozolomide (**4**) was formed.

5-Amino-1-[[(4-nitrophenyl)oxy]carbonyl]imidazole-4-carboxamide (14a). A suspension of 5-aminoimidazole-4-carboxamide hydrochloride (1.0 g, 6.15 mmol) in dry CH_2Cl_2 (30 mL) was treated with Et_3N (1.24 g) and stirred at 25 °C for 10 min. To the mixture at 0 °C was added (over 10 min) a solution of 4-nitrophenyl chloroformate (1.1 g, 7.0 mmol) in dry CH_2Cl_2 (20 mL). After 18 h a yellow precipitate was collected and washed with CH_2Cl_2. The product **14a** (1.6 g, 87%): mp 200 °C (dec); ^1H NMR $[(CD_3)_2SO]$ δ = 6.54 (brs, 2 H), 6.98 (brs, 1 H), 7.10 (brs, 1 H), 7.75 (d, J = 9.22, 2 H), 7.85 (s, 1 H), 8.39 (d, J = 9.22, 2 H); ^{13}C NMR $[(CD_3)_2SO]$ δ = 111.1, 123.3, 125.5, 127.7, 143.1, 145.7, 146.8, 154.0, 166.1; IR (KBr) 3482, 3450, 3320, 3295, 1765 (C=O), 1678 (C=O) cm^{-1}; MS (EI, 70 eV) m/z 291 (M^+). Anal. Calcd for $C_{11}H_9N_5O_5$: C, 45.37; H, 3.11; N, 24.05. Found: C, 45.12; H, 3.15; N, 23.76.

5-Amino-1-(*N*-methylcarbamoyl)imidazole-4-carboxamide (6). (1) A suspension of 5-aminoimidazole-4-carboxamide hydrochloride (1.0 g, 6.15 mmol), dry CH_3CN (20 mL), and Et_3N (1.24 g) was stirred at 25 °C for 10 min and then treated with methyl isocyanate (0.4 g, 7.0 mmol) over 5 min at −10 °C. The mixture was kept at 25 °C (18 h) and quenched with ice−water, and the white product (**6**) was collected and washed with EtOAc to yield 1.04 g (92%): mp 170 °C (dec); ^1H NMR $[(CD_3)_2SO]$ δ = 2.78 (d, 3 H), 6.38 (brs, 2 H), 6.83 (brs, 1 H), 6.93 (brs, 1 H), 7.62 (s, 1 H), 8.46 (q, 1 H); ^{13}C NMR $[(CD_3)_2SO]$ δ = 27.5, 112.1, 127.0, 144.3, 151.6, 167.2; IR (KBr) 3409, 1718, 1661, 1535, 1453, 1311, 1241, 947 cm^{-1}; MS (CI) m/z 184 ($M^+ + 1$). Anal. Calcd for $C_6H_9N_5O_2$: C, 39.34; H, 4.92; N, 38.25. Found: C, 39.06; H, 4.83; N, 38.10.

(2) Methylcarbamoyl chloride (**15**) was prepared following the procedure of Taylor and Pollard.[18] 5-Aminoimidazole-4-carboxamide hydrochloride (**13**) (1.82 g, 11.193 mmol) suspended in CH_3CN (20 mL) was cooled to 0 °C and then Et_3N (4.6 mL, 3.34

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(18) Taylor, R. W.; Pollard, M. D. European Patent Application No. 89312638.3, April 12, 1989.

g, 33.00 mmol) was added. After 15 min a solution of **15** (2.18 g, 23.313 mmol) in CH_3CN (5 mL) was added and the solution was stirred for 2 h. The reaction mixture was then concentrated under reduced pressure, the residue was cooled to 0 °C, and then ice water (25 mL) was added. The slurry was stirred for 30 min and filtered and the solid washed with ice water (2 × 3 mL), followed by CH_3CN−water (1:4 ratio) (8 mL), and dried under vacuum to afford 1.45 g of **6** (7.92 mmol, 71%).

(3) The same (*N*-methylcarbamoyl)imidazole **6** (0.15 g, 48%) was formed when the [[(4-nitrophenyl)oxy]carbonyl]imidazole **14a** (0.5 g, 1.72 mmol) was treated with a 33% ethanolic solution of $MeNH_2$ (0.21 mL, 1.72 mmol) at 25 °C. Examination of the reaction mixture (TLC) also confirmed the presence of 4-nitrophenol.

8-Carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazine-4(3*H*)-one (temozolomide) (4). 5-Amino-1-(*N*-methylcarbamoyl)imidazole-4-carboxamide **(6)** (0.1 g, 0.546 mmol) and H_2O (3 mL) were cooled to 0 °C. A solution of $NaNO_2$ (3.8 mL, 0.551 mmol, 0.145 M in H_2O), followed by tartaric acid (81 mg, 0.540 mmol) was then added. After 1 h the reaction mixture was transferred to a volumetric flask and diluted to 100 mL with DMSO. HPLC analysis indicated a 45% solution yield of temozolomide (**1**).

HPLC conditions: column, Zorbax ODS, 4.6 mm × 25 cm; mobile phase, 96:4 H_2O−MeOH and 0.5% HOAc (with 1.0 g/L hexanesulfonic acid sodium salt); flow rate, 1 mL/min; detector, 270 nm; injection volume, 15 μL; sample concentration, 1 mg/mL; retention time of temozolomide, 9.3 min.

^{1}H NMR monitoring of the reaction using the standard conditions was carried out using D_2O as the solvent (in place of water) and showed that the temozolomide and the 9-(*N*-methylcarbamoyl)-2-azahypoxanthine byproduct **20** are formed in approximately a 1:1 ratio throughout the course of the reaction. As the reaction progresses the (methylcarbamoyl)-2-azahypoxanthine **20** deacylates to give azahypoxanthine (**11**).

Ethyl 1-(*N*-methylcarbamoyl)imidazole-4-carboxylate (22). Ethyl 5-aminoimidazole-4-carboxylate[19] (0.88 g, 5.65 mmol) was suspended in a mixture of CH_3CN (10 mL) and Et_3N (0.57 g, 5.65 mmol) and treated with methyl isocyanate (0.40 g, 7.0 mmol) at 0 °C, and the mixture was allowed to react at 25 °C over 18 h to yield a white product (0.71 g, 59%): mp 146 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ = 1.24 (t, 3 H), 2.77 (d, 3 H), 4.17 (q, 2 H), 6.62 (brs, 2 H), 7.63 (s, 1 H), 8.51 (q, 1 H); ^{13}C NMR [$(CD_3)_2SO$] δ = 14.5, 26.6, 58.7, 108.3, 127.4, 146.6, 150.7, 163.5; MS (EI) *m*/*z* 212 (M^+). Anal. Calcd for $C_8H_{12}N_4O_3$: C, 45.28; H, 5.66; N, 26.41. Found: C, 45.00; H, 5.42; N, 26.28.

Ethyl 3,4-Dihydro-3-methyl-4-oxoimidazo[5,1-*d*]-1,2,3,5-tetrazine-8-carboxylate (23). Compound **22** (0.25 g, 1.61 mmol) in 50% aqueous acetic acid (25 mL) was treated with a solution of $NaNO_2$ (0.16 g) in H_2O (3 mL) at 0 °C. The mixture was maintained at 4 °C for 20 h, solvent was removed by vacuum distillation, and products were separated by flash column chromatography. The fraction with R_f = 0.5 was concentrated to yield the crude ester **23** (58 mg, 10%): ^{1}H NMR [$(CD_3)_2SO$] δ = 1.34 (t, 3 H), 3.89 (s, 3 H), 4.38 (q, 2 H), 8.84 (s, 1 H).

5-Amino-1-(*N*-methylcarbamoyl)imidazole-4-carbonitrile (25). 5-Aminoimidazole-4-carbonitrile (**24**) (1.08 g, 10.0 mmol), prepared from 5-aminoimidazole-4-carboxamide hydrochloride and phosphorus oxychloride,[20] was suspended in 30 mL of CH_3CN and treated with Et_3N (1.01 mL) followed by methyl isocyanate (0.68 g, 12 mmol) at 0 °C. After being kept at 25 °C for 18 h a gray solid was collected and washed with CH_3CN−Et_2O. Flash column chromatographic purification of the solid (eluting solvent: EtOAc−HOAc, 30:1) gave, on evaporation, a low yield (82.6 mg, 5%) of the (*N*-methylcarbamoyl)imidazole **25**: ^{1}H NMR [$(CD_3)_2SO$] δ = 2.77 (d, 3 H), 6.94 (brs, 2 H), 7.64 (s, 1 H), 8.56 (q, 1 H); IR (KBr) 2230 (CN) cm^{-1}.

5-Diazoimidazole-4-carbonitrile (28). This is an improvement of the method described by Shealy and O'Dell.[20] A solution of 5-amino-4-cyanoimidazole (7.0 g, 64.8 mmol) in 2 M hydrochloric acid (70 mL) at 0 °C was added dropwise to a solution of sodium nitrite (4.53 g, 65.6 mmol) in H_2O (70 mL) maintained at 0 °C. After 2 h an amber precipitate of the diazoimidazole **28** (6.38 g, 83%) was collected: mp 97−99 °C (lit.[20] mp 96−99 °C); ^{1}H NMR [$(CD_3)_2SO$] δ = 7.80 (s, 1 H); IR (KBr) 2238 (CN), 2185 (N_2) cm^{-1}.

3-Methylimidazo[5,1-*d*]-1,2,3,5-tetrazine-8-carbonitrile (cyanotemozolomide) (7). (1) 5-Diazoimidazole-4-carbonitrile (0.72 g, 6.15 mmol) in dry DMSO (3 mL) was stirred with methyl isocyanate (1.05 g, 18.45 mmol) at 25 °C in the dark for 24 h. Flash column chromatographic purification of the mixture (eluting solvent: EtOAc) afforded the imidazotetrazine **7** (0.56 g, 52%) as an amber solid, mp 185−187 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ = 3.91 (s, 3 H), 9.06 (s, 1 H); ^{13}C NMR [$(CD_3)_2SO$] δ = 36.8, 107.9, 112.6, 131.2, 138.4, 140.8; MS (EI) *m*/*z* 176 (M^+), 119 ($M^+ - CH_3NCO$). Anal. Calcd for $C_6H_4N_6O$: C, 40.91; H, 2.29; N, 47.71. Found: C, 40.98; H, 2.21; N, 47.84.

(2) 5-Amino-1-(*N*-methylcarbamoyl)imidazole-4-carbonitrile (**25**) (0.189 g, 1.146 mmol), HOAc (4.5 mL, 78.6 mmol, 68.6 equiv), and H_2O (4.5 mL) were cooled to 0 °C. $NaNO_2$ (0.121 g, 1.767 mmol, 1.54 equiv) was then added and the purple solution was stirred for 1.5 h, at which point it had turned orange. TLC analysis indicated complete disappearance of the starting material. The reaction mixture was then transferred to a volumetric flask and diluted to 100 mL with DMSO. HPLC analysis indicated 23% solution yield of cyanotemozolomide **7**.

HPLC conditions: column, Zorbax ODS, 4.6 mm × 25 cm; mobile phase, 90:10 H_2O−CH_3CN (with 0.94 g/L hexanesulfonic acid sodium salt); flow rate, 1 mL/min; detector, 254 nm; injection volume, 20 μL; sample concentration, 2 mg/mL; retention time of cyanotemozolomide, 15.3 min.

The ^{1}H NMR monitoring of the reaction was carried out as follows. 5-Amino-1-(*N*-methylcarbamoyl)imidazole-4-carbonitrile (**25**) (10.0 mg, 0.061 mmol) was dissolved in CD_3CO_2D (0.9 mL, 1.01 g, 15.76 mmol, 258 equiv). A solution of $NaNO_2$ dissolved in H_2O (0.809 M, 0.06 mL, 0.0485 mmol, 1.215 equiv) was then added, and NMR scans were obtained at 5 min intervals. The *N*-methyl peak of **25** at δ = 2.96 (d, J = 5.5 Hz; referenced to CD_3CO_2D at δ = 2.04) was consumed, and new *N*-methyl peaks at δ = 3.02 (d, J = 5.5 Hz), 2.80 (d, J = 5.5 Hz), and 4.02 (s, CH_3 of **7**) were observed. As the reaction progresses the peak at δ = 3.02 disappears. The ratio of the peaks at δ = 4.02 and 2.80 was approximately 1:5 throughout the course of the reaction. These results are consistent with the peak at δ = 2.96 being due to diazo intermediate **29**, which undergoes further reaction to form a mixture of **7** (δ = 4.02) and another compound, whose structure is proposed to be the primary nitrosamine **30** or its tautomer (δ = 2.80, d, J = 5.5 Hz, 3 H). Results from a LC−MS analysis of the NMR reaction mixture were consistent with the proposed structure **30**, as peaks at *m*/*z* 217 ($M + Na^+$), 195 ($M + H^+$), and 138 ($MH^+ - CONCH_3$) were observed.

The FT-IR monitoring of the reaction was carried out as follows. To the reaction vessel cell was charged **25** (0.997 g, 6.037 mmol), acetic acid (50 mL), and H_2O (5 mL). The mixture was stirred for 15 min and then $NaNO_2$ (1.049 g, 15.201 mmol) was added. The reaction was monitored using an AMT-IR sensor, and 50 coadded interferograms were measured every minute until the reaction was complete (typically 1 h). The overall IR reaction profile is consistent with the NMR reaction monitoring results and shows that the diazotization of **25** is rapid (disappearance of the NH band at 1629 cm^{-1}), with no significant buildup of the diazo intermediate and rapid transformation to **7** and **30**. Two new nitrile bands are observed at 2250 cm^{-1} (due to **7**) and 2192 cm^{-1} (consistent with the delocalized structure of **30**). In addition, several bands were also observed in the region 1300 − 1400 cm^{-1} (tentatively assigned to the N−OH stretch of **30**).

Attempts to isolate nitrosamine **30** by column chromatography or by extraction and concentration were unsuccessful (only decomposition occurred), as were attempts to convert **30** to **7** *in situ* by treating the reaction mixture with acid (*p*-TsCl, CF_3CO_2H, or H_2SO_4, either at 25 °C or with heating).

Hydrolysis of Cyanotemozolomide (7). The cyanoimidazotetrazine (1.07 g, 6.10 mmol) was heated with 10 M hydrochloric acid (3 mL) at 60 °C for 2.0 h. The mixture was cooled to 0 °C and a white precipitate (0.91 g, 65%) was collected and washed with cold THF. The product, temozolomide hydrochloride (**31**): mp 210 °C (dec). Anal. Calcd for $C_6H_6N_6O_2$·HCl: C, 31.24; H, 3.06; N, 36.43. Found: C, 31.46; H, 2.94; N, 36.44.

The hydrochloride salt dissociated to give the following spectral characteristics: ^{1}H NMR [$(CD_3)_2SO$] δ = 3.87 (s, 3 H),

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7.69 (brs, 1 H), 7.81 (brs, 1 H), 8.82 (s, 1 H), identical to the ^{1}H NMR spectrum of a reference sample of temozolomide free base.[4,7]

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Supporting Information Available: ^{1}H NMR spectrum of 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (**3**) in $(CD_3)_2SO$ after 5 days at 25 °C and comprehensive listings of the reaction conditions tried for the cyclization of **6** to temozolomide **4** and of **25** to cyanotemozolomide **7** (9 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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