A new synthesis of temozolomide

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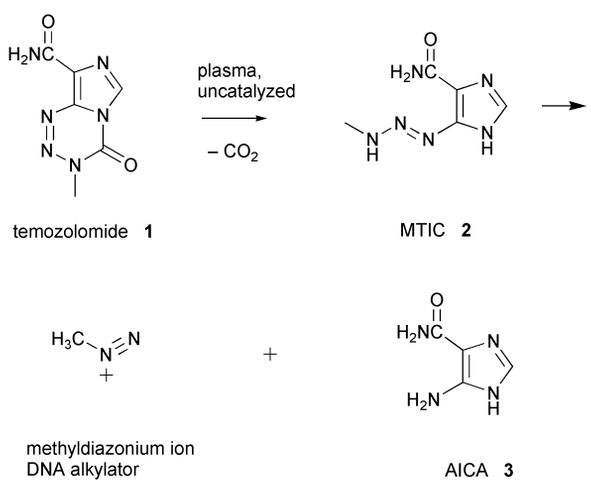
An efficient condensation reaction between nitrosoimidazoles and phenyl methylcarbazate forms the basis of a new synthetic route to phenyloxycarbonyl substituted triazenylimidazoles. Exposure of these triazenes to diffuse daylight is sufficient to induce (*E*) to (*Z*)-isomerization of the triazene –N=N– bond, resulting in spontaneous formation of temozolomide in high yield.

Introduction

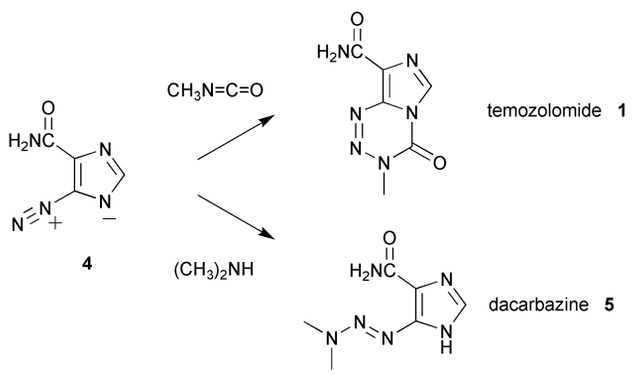
Temozolomide is one of the antitumour drugs that is capable of entering the central nervous system and is increasingly used to treat malignant brain tumours.[1–3] The mechanism of action of temozolomide has been extensively investigated by Stevens and co-workers.[4–6] In plasma the tetrazinone ring of temozolomide **1** spontaneously hydrolyses to methyltriazenylimidazole-4-carboxamide **2** (MTIC, Scheme 1), which falls apart to amino-



Scheme 1 Bioactivation of temozolomide.

imidazole-4-carboxamide AICA (**3**) and the methyldiazonium cation. This methylating agent, which is the protonated form of diazomethane, reacts at several positions in DNA and in particular methylation of the O-6 position of guanosine has a strong inhibitory effect with respect to cell replication. One of the earlier triazene antitumour drugs based on this mechanism is dacarbazine (DTIC, **5**, Scheme 2), a drug that has been used for many years against melanoma. Dacarbazine however requires hepatic activation by P450 oxidation before it enters the DNA-alkylation pathway *via* MTIC **2**. In contrast, the orally active and well-tolerated temozolomide is rapidly absorbed and shows linear plasma pharmacokinetics.[1]

The existing synthesis of temozolomide is straightforward and requires only a few steps from AICA (**3**) which is diazotized to **4** (Scheme 2).[7,8] Drawbacks from this synthesis are the instability of diazonium intermediate **4** and the requirement of excess methyl isocyanate. The development of improved and



Scheme 2 Literature synthesis.

safer synthetic routes to temozolomide and analogues is still under investigation.[9–12] We wish to report on a new, economical route to temozolomide that is based on condensation reactions between nitrosoimidazoles and hydrazides, applying the methodology we developed for 2-nitrosoadenosine.[13]

Results and discussion

4-Nitroimidazole **6** was chosen as starting material, and the carboxamide substituent present in temozolomide was introduced in the form of a nitrile (Scheme 3).

Recently a useful synthesis of the 5-nitroimidazole-4-carbonitrile **7** was described *via* a cine-substitution reaction of 1,4-dinitroimidazole with potassium cyanide.[14] The required 1,4-dinitroimidazole is the product of further nitration of 4-nitroimidazole **6** with HNO_3 and acetic anhydride. To improve the solubility of imidazole **7** the N–H was protected with dihydropyran to give THP-derivative **8** as a single regioisomer. The THP-substituent is most likely positioned on N-1, as was deduced from its azoxydimer **14** (*vide infra*). From our synthetic work on nitrosoadenosine[13] we noticed that partial reduction of this type of electron deficient heterocyclic nitro compound to the corresponding hydroxylamines proceeds readily with hydrogen and 10% Pd on carbon as a catalyst without the use of any additives. With nitroimidazole **8** however, considerable overreduction to the corresponding amine occurred. Changing the catalyst from Pd to Pt (10% on carbon) with EtOAc as a solvent was found to be an important improvement, giving almost quantitative formation of **9** after 1 hour at 1 atm of hydrogen. Oxidation of this hydroxylamine to the nitroso compound **10** proceeded efficiently with sodium periodate

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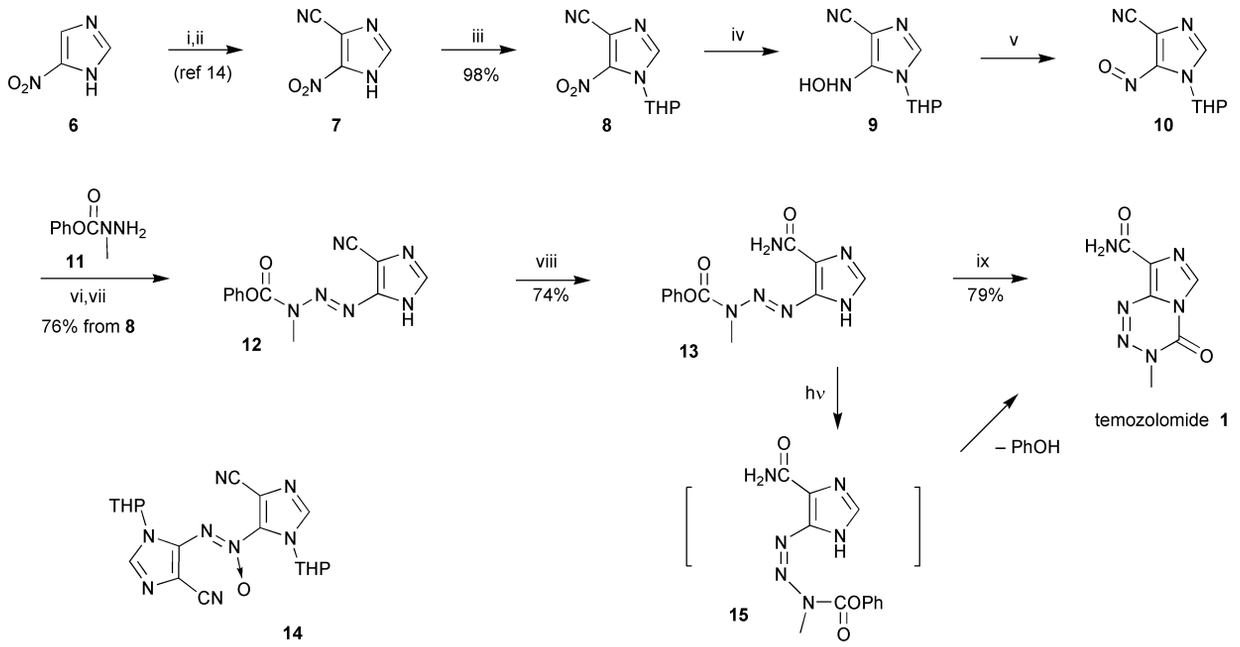
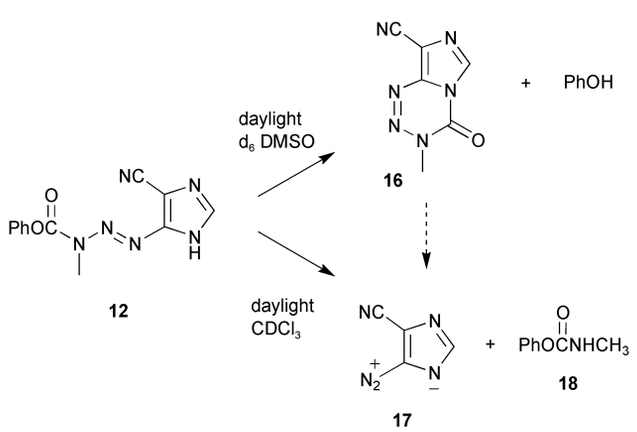


Scheme 3 *Reagents and conditions*: (i) HNO_3, Ac_2O, 85%; (ii) KCN, $NaHCO_3$, MeOH, H_2O; rt, 62%; (iii) dihydropyran, *p*-TsOH, EtOAc; (iv) H_2, 10% Pt/C, EtOAc; rt, 60 min; (v) 2 eq. $NaIO_4$ in H_2O, EtOAc; 0 °C, 30 min; (vi) DCM–HOAc 5 : 2; rt, 2 h; (vii) TFA–HOAc–MeOH 3 : 6 : 10; rt, 5 h; (viii) conc. HCl–HOAc 2 : 1; 60–65 °C, 25 min; (ix) acetone–MeOH 2 : 1, 366 nm; rt, 1 h.

in water–ethyl acetate mixtures without the requirement of phase-transfer catalysts. Small amounts of unsymmetrical azoxy dimer **14** were formed as a result of a condensation reaction between the starting hydroxylamine **9** and the nitroso product **10**. Although compound **14** is of no synthetical use, it is helpful in elucidating the position of the THP-substituent. In the ^{1}H NMR spectrum of **14** the THP α-protons are shifted to 5.45 and 5.80 ppm, indicating the proximity of the azoxy substituent. If the THP was on the nitrogen atom next to the cyano substituent these chemical shifts would not be expected. Nitrosoimidazole **10**, which is present as its monomer according to ^{1}H NMR in $CDCl_3$-solution, did not crystallise and was used without purification in the next condensation reaction. In an initial attempt 1,1-dimethylhydrazine was used as nucleophile with the intention of preparing *N*,*N*-dimethyltriazene **5** (dacarbazine). However, extensive reduction of the nitroso substituent in **10** to hydroxylamine **9** occurred, due to the high reduction potential of alkyl- or aryl-substituted hydrazines. Acylated hydrazines show a more favourable reactivity pattern: the reducing properties are strongly decreased while their nucleophilicity towards electrophiles such as aldehydes or ketones (*e.g.* semicarbazone formation) is largely preserved. With phenyl methylcarbazate **11** and acetic acid as a catalyst[15] efficient triazene formation was observed. Upon addition of TFA and MeOH the THP-group was removed, providing unprotected imidazole **12** which directly precipitated from the reaction mixture in high overall yield.

Hydrolysis of a cyano group to a carboxamide requires rather drastic acidic conditions. Wang and Stevens have shown that the electronpoor tetrazinone ring system is sufficiently acid stable to allow hydrolysis of *e.g.* cyanotemozolomide to temozolomide with concentrated hydrochloric acid.[10] With aqueous hydrochloric acid and acetic acid as a co-solvent temozolomide precursor **13** could be prepared from the corresponding nitrile without appreciable hydrolysis of the carboxy-triazene.

Ring closure of **12** or **13** to the desired tetrazinone ring system was unsuccessful in spite of a variety of conditions such as base catalysis, mild or strong acid catalysis and thermal conditions. During one of these experiments however, a NMR tube containing cyanotriazene **12** in d_6-DMSO (2 mg in 0.6 mL) was kept in diffuse daylight (window-sill) to observe any (*E*) to (*Z*) isomerization of the triazene, which was expected to facilitate cyclization. The intermediate (*Z*)-triazene could not be detected with ^{1}H NMR, instead a clean conversion to cyanotemozolomide **16** (Scheme 4) took place with concomitant



Scheme 4

formation of phenol. After 2 days the conversion was complete. In an experiment to find a more suitable solvent, $CDCl_3$ and CD_2Cl_2 samples of **12** were exposed to daylight but this resulted in complete fragmentation of starting material and/or product to give phenyl methylcarbamate **18** and the instable diazoimidazole **17**. Probably the polarity of the DMSO stimulates the ring closing aminolysis and moreover DMSO may act as a filter for light of shorter wavelengths. The light-sensitivity of the tetrazinone ring system, both in the solid state and in aqueous solution has been noticed before by Stevens.[1] The photoisomerisation–ring closing process was scaled up with carboxamide **13** in a mixture of acetone and methanol as a suitable solvent system to replace DMSO, with irradiation at 366 nm in a Rayonet RS. This way temozolomide was obtained in 79% yield after trituration with acetone.

Via this route temozolomide was prepared in a reasonable overall yield of 44% starting from 5-nitroimidazole-4-carbonitrile **7**. Intermediate triazenes such as **13** are not only valuable photochemical precursors for temozolomide but can also be useful as hydrolysis sensitive prodrugs of MTIC (**2**) and the methyl diazonium ion formed thereof.

Experimental

General

All reagents and solvents were used as commercially available, unless indicated otherwise. Flash chromatography refers to purification using the indicated eluents and Janssen Chimica silica gel 60 (0.030–0.075 mm). Melting points were measured with a Leitz melting point microscope. Elemental analyses were performed by Kolbe, Mülheim an der Ruhr, Germany. Infrared (IR) spectra were obtained from $CHCl_3$ solutions unless indicated otherwise, using a Bruker IFS 28 FT-spectrophotometer and wavelengths are reported in cm^{-1}. Proton nuclear magnetic resonance (^1H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR; APT) spectra were determined in $CDCl_3$ at 300 K using a Bruker ARX 400 spectrometer, unless indicated otherwise.

5-Nitro-1-(tetrahydropyran-2-yl)imidazole-4-carbonitrile 8

A mixture of 5-nitroimidazole-4-carbonitrile **7**[14] (4.14 g, 30 mmol), 3,4-dihydro-2*H*-pyran (5.49 mL, 60 mmol) and *p*-TsOH (0.15 g) in EtOAc (60 mL) was stirred at room temperature during 2 h. Et_3N (0.105 mL) and light petroleum (90 mL) were added and the solution was slowly filtered over silica (15 g). The silica was washed with EtOAc (150 mL) and the solvents were removed *in vacuo*. The residue obtained after further drying (1 mbar, 45 °C) was stirred with a few mL light petroleum to induce crystallisation. Yield: 6.38 g (2.93 mmol, 97.5%), mp 87–88 °C (Found: C, 48.77; H, 4.50; N, 25.12. $C_9H_{10}N_4O_3$ requires C, 48.65; H, 4.54; N, 25.21%); ν_{max}/cm^{-1} (KBr) 3140, 2951, 2859, 2242, 1566, 1475; δ_H 7.82 (1H, s), 5.45 (1H, m), 4.22 (1H, m), 3.77 (1H, m), 1.7–2.3 (6H, m); δ_C (d_6-DMSO) 136.1, 107.9, 102.1, 86.0, 69.2, 32.1, 24.5, 22.3.

5-{3-Methyl-3-[(phenyloxy)carbonyl]triazen-1-yl}imidazole-4-carbonitrile 12

10% Pt on carbon (0.20 g) was added to a solution of **8** (2.18 g, 10.0 mmol) in EtOAc (50 mL) and the mixture was stirred vigorously under 1 atm of H_2 during 1 h. The reaction was followed with TLC (silica, eluent: EtOAc). The crystallised product was dissolved by adding some ethanol and the catalyst was removed by filtration over Celite. The solids were washed with EtOAc (50 mL), the filtrate was cooled in ice, and under vigorous stirring an ice-cold solution of $NaIO_4$ (4.28 g, 20.0 mmol) in water (80 mL) was added quickly. Vigorous stirring was continued for 30 min, the layers were separated and the green organic layer was washed once with water (25 mL) and dried over Na_2SO_4. Evaporation of the solvents gave nitrosoimidazole **10** as an oil which was used without purification in the next step, δ_H 7.87 (1H, s, 2-H), 5.52 (1H, dd, *J* 10.5 and 2.5, N–CH–O), 4.21 (1H, m), 3.77 (1H, m), 2.25 (1H, m) 2.13 (1H, m), 2.0 (1H, m), 1.7 (3H, m).

A solution of nitrosoimidazole **10** (prepared from 10.0 mmol **8**) in a mixture of DCM (15 mL) and acetic acid (6 mL) was cooled in ice, phenyl methylcarbazate **11**[16] (1.83 g, 11 mmol) was added and the cooling bath was removed. After stirring the solution at room temperature for 2 h the DCM was removed *in vacuo* and the resulting acetic acid solution was diluted with methanol (10 mL) and trifluoroacetic acid (3 mL). Crystallization started after *ca*. 1 h, and after a reaction time of 5 h the mixture was cooled in ice and filtered. The yellow triazene **12** (2.05 g, 7.59 mmol, 76% from **8**) was washed 3 times with cold methanol and dried *in vacuo*, mp 105–110 °C (Found: C, 53.24; H, 3.85; N, 31.02. $C_{12}H_{10}N_6O_2$ requires C, 53.44; H, 3.73; N, 31.10%); ν_{max}/cm^{-1} (KBr) 3578, 3451, 3120, 2233, 1739, 1637; δ_H (d_6-DMSO) 13.84 (1H, br, NH), 8.00 (1H, s, 2-H), 7.50 (2H, m, ArH), 7.34 (3H, m, ArH), 3.52 (3H, s, CH_3); in ^{13}C NMR only 7 signals were observed; increasing the relaxation times or the temperature did not give improvement, δ_C (d_6-DMSO) 151.8, 150.7, 138.5, 129.6, 126.2, 121.6, 31.01.

5-{3-Methyl-3-[(phenyloxy)carbonyl]triazen-1-yl}imidazole-4-carboxamide 13

A suspension of nitrile **12** (1.35 g, 5.0 mmol) in a mixture of concentrated HCl (22 mL) and acetic acid (11 mL) was stirred vigorously at 60–65 °C (bath temperature) until the solids had dissolved (approx. 20 min). The solution was heated for an additional 5 min, cooled to room temperature and diluted with water (3 portions of 10 mL) which induced crystallisation of the product. After 16 h at 4 °C the mixture was filtered, the product was washed thoroughly with water and dried *in vacuo* to give 1.07 g (3.72 mmol, 74%) of carboxamide **13**, mp 170 °C (dec) (Found: C, 49.84; H, 4.28; N, 28.94. $C_{12}H_{12}N_6O_3$ requires C, 50.00; H, 4.20; N, 29.15%); ν_{max}/cm^{-1} (KBr) 3432, 1749, 1683, 1598; δ_H (d_6-DMSO) 13.4 (1H, br, NH), 8.00 and 7.34 (2 × 1H, br, NH_2), 7.92 (1H, s, 2-H), 7.51 (2H, m, ArH), 7.35 (3H, m, ArH), 3.54 (3H, s, CH_3); δ_C (d_6-DMSO) 158.9, 151.9, 150.6, 141.5, 136.2, 129.6, 126.3, 121.7, 121.2, 31.37.

Temozolomide 1

N_2 was bubbled through a suspension of **13** (0.100 g, 0.347 mmol) in a mixture of acetone (10 mL) and methanol (5 mL) in a normal 20 mL glass test tube. The mixture was irradiated at room temperature in a Rayonet RS at 366 nm during 1 h. Evaporation of the solvents and trituration with acetone produced temozolomide **1** (0.053 g, 0.273 mmol, 79%) as a slightly coloured crystalline solid, mp 175 °C (dec), literature values 212 °C (dec)[7] or 185 °C (dec);[8] ν_{max}/cm^{-1} (KBr) 3494, 3124, 1745, 1680; δ_H (d_6-DMSO) 8.83 (1H, s, 2-H), 7.80 and 7.68 (2 × 1H, br, NH_2), 3.89 (3H, s, CH_3); δ_C (d_6-DMSO) 161.5, 139.2, 134.6, 130.5, 128.4, 36.13.

Azoxy dimer 14

From an earlier synthesis of nitrosoimidazole **10** the azoxy dimer **14** was obtained, δ_H (selected signals) 7.94 (1H, s, 2-H), 7.88 (1H, s, 2′-H), 5.80 (1H, dd, *J* 10.5 and 2.1, N–CH–O), 5.45 (1H, dd, *J* 10.5 and 2.4, N–CH–O).

Irradiation of 12 in d_6-DMSO

A solution of **12** (4.0 mg) in d_6-DMSO (0.6 mL) was kept in diffuse daylight (window-sill) during 0.5 h. ^1H NMR showed starting material (54%), cyanotemozolomide (**16**) and phenol (each 46%). Cyanotemozolomide **16**: δ_H (d_6-DMSO) 9.07 (1H, s, 2-H), 3.93 (3H, s, CH_3); phenol: 9.33 (1H, s), 7.17 (2H, m), 6.76 (3H, m).

Irradiation of 12 in $CDCl_3$

A solution of **12** (4.0 mg) in $CDCl_3$ (0.6 mL) was kept in diffuse daylight (window-sill) during 4 h. In the ^1H NMR spectrum starting material (2-H at 7.63 ppm), an unidentified imidazole (2-H at 7.76 ppm) and phenyl methylcarbamate δ_H 7–7.5 (5H, m, ArH), 4.95 (1H, br, NH), 2.90 (3H, d, *J* 4.7, CH_3) were observed.

References

1 E. S. Newlands, M. F. G. Stevens, S. R. Wedge, R. T. Wheelhouse and C. Brock, *Cancer Treat. Rev.*, 1997, **23**, 35–61.
2 H. S. Friedman, T. Kerby and H. Calvert, *Clin. Cancer Res.*, 2000, **6**, 2585–2597.
3 R. Stupp, M. Gander, S. Leyvraz and E. Newlands, *Lancet Oncol.*, 2001, **2**, 552–560.
4 M. F. G. Stevens, J. A. Hickman, S. P. Langdon, D. Chubb, L. Vickers, R. Stone, G. Baig, C. Goddard, N. W. Gibson, J. A. Slack, C. G. Newton, E. Lunt, C. Fizames and F. Lavelle, *Cancer Res.*, 1987, **47**, 5846–5852.
5 B. J. Denny, R. T. Wheelhouse, M. F. G. Stevens, L. L. H. Tsang and J. A. Slack, *Biochemistry*, 1994, **33**, 9045–9051.
6 A. S. Clark, B. Deans, M. F. G. Stevens, M. J. Tisdale, R. T. Wheelhouse, B. J. Denny and J. A. Hartley, *J. Med. Chem.*, 1995, **38**, 1493–1504.

7 M. F. G. Stevens, J. A. Hickman, R. Stone, N. W. Gibson, G. U. Baig, E. Lunt and C. G. Newton, *J. Med. Chem.*, 1984, **27**, 196–201.
8 R. T. Wheelhouse, D. E. V. Wilman, W. Thomson and M. F. G. Stevens, *J. Chem. Soc., Perkin Trans. 1*, 1995, 249–252.
9 Y. Wang, M. F. G. Stevens, W. T. Thomson and B. P. Shutts, *J. Chem. Soc., Perkin Trans. 1*, 1995, 2783–2787.
10 Y. Wang, M. F. G. Stevens, T. Chan, D. DiBenedetto, Z. Ding, D. Gala, D. Hou, M. Kugelman, W. Leong, S. Kuo, J. L. Mas, D. P. Schumacher, B. P. Shutts, L. Smyth and Z.-Y. Zhan, *J. Org. Chem.*, 1997, **62**, 7288–7294.
11 J. Arrowsmith, S. A. Jennings, D. A. F. Langnel, R. T. Wheelhouse and M. F. G. Stevens, *J. Chem. Soc., Perkin Trans. 1*, 2000, 4432–4438.
12 Y. Wang and M. F. G. Stevens, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 185–188.
13 M. J. Wanner and G.-J. Koomen, *J. Chem. Soc., Perkin Trans. 1*, 2001, 1908–1915.
14 J. Suwinski and K. Swierczek, *Tetrahedron Lett.*, 1998, **39**, 3331–3332.
15 S. G. Zlotin, O. V. Prokshits and O. A. Lukyanov, *Bull. Acad. Sci. USSR, Div. Chem. Sci. (Engl. Transl.)*, 1990, **39**, 1078–1079.
16 C. T. Pedersen, *Acta Chem. Scand.*, 1964, **18**, 2199–2200.