JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 479–484.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.574

Research Article

Labelled compounds of interest as antitumour agents – VIII. Synthesis of ^{2}H-isotopomers of pentamethylmelamine and of a potential prodrug thereof

Sandra Ferrer[1], Declan P. Naughton[2] and Michael D. Threadgill[2,*]
[1] *Department of Medical Sciences, University of Bath, Claverton Down, Bath BA2 7AY, UK*
[2] *Department of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK*

Summary

Treatment of 2-chloro-4,6-(dimethylamino)-1,3,5-triazine with trideuteromethylamine gave 4,6-(dimethylamino)-2-trideuteromethyl-1,3,5-triazine, an isotopomer of the experimental anticancer agent pentamethylmelamine (PMM). Mitsunobu coupling with 1,2-dimethyl-3-hydroxymethyl-5-methoxyindole-4,7-dione gave 1,2-dimethyl-3-(*N*-(4,6-bis(dimethylamino)-1,3,5-triazin-2-yl)-*N*-trideuteromethylaminomethyl)-5-methoxyindole-4,7-dione. This conjugate is a potential reductively triggered prodrug of PMM. Copyright © 2002 John Wiley & Sons, Ltd.

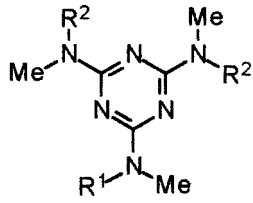
Key Words: deuterium; pentamethylmelamine; prodrug; indole-4,7-dione; Mitsunobu

Introduction

Hexamethylmelamine **1** (Figure 1) (altretamine, HMM, 2,4,6-tris(dimethylamino)-1,3,5-triazine) was originally developed as an insect

*Correspondence to: M. D. Threadgill, Department of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK. E-mail: m.d.threadgill@bath.ac.uk

Received 21 January 2002
Accepted 28 January 2002



1a: $R^1 = R^2 = CH_3$
1b: $R^1 = C^2H_3$, $R^2 = CH_3$
2: $R^1 = CH_2OH$, $R^2 = CH_3$
3a: $R^1 = H$, $R^2 = CH_3$
4: $R^1 = R^2 = CH_2OH$

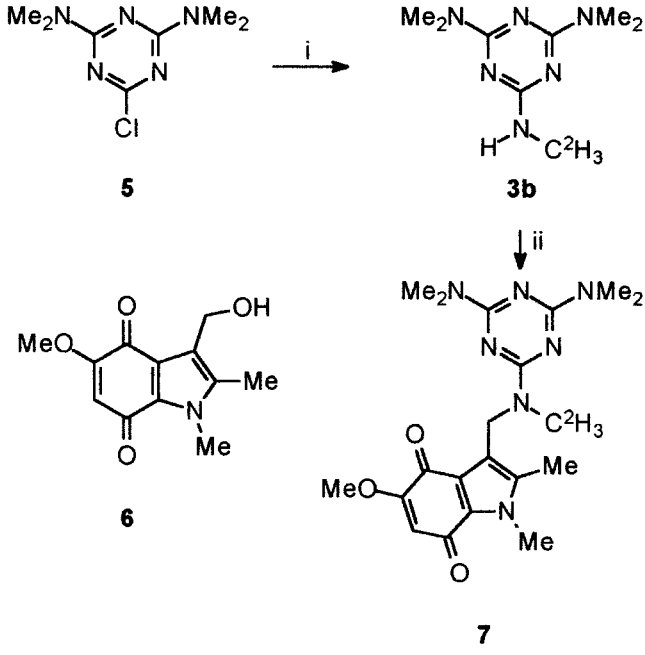
Figure 1. Structures of some therapeutically active melamines and their ^{2}H-isotopomers

chemosterilant[1] but it is as an antitumour agent that this compound has attracted much interest over the past 35 years.[2,3] In particular, it entered the clinic in the 1970s as a treatment for ovarian carcinoma but difficulties were encountered, as it is insoluble in water and is thus difficult to formulate. However, it has recently been recognised[4–6] as a second-line treatment for ovarian carcinoma. It is oxidatively metabolised[7,8] in the liver to the *N*-hydroxymethyl analogue **2**. This carbinolamine decomposes to give the *N*-desmethyl analogue, pentamethylmelamine **3a**, which also has antitumour activity[3] and is slightly soluble in water. Of considerably greater aqueous solubility is 2,4,6-tris(*N*-hydroxymethyl-*N*-methylamino)-1,3,5-triazine **4** (trimelamol), which also shows therapeutic activity,[9] however, the chemical instability of **4** precluded its routine use in the clinic. The hydroxymethylmelamines are electrophilic, reacting with DNA *in vivo* and with glutathione *in vitro*[10] *via* the corresponding iminium ions.

As part of a programme of design, synthesis and evaluation of prodrugs of anticancer and other drugs,[11–14] we required an isotopomer **3b** of pentamethylmelamine with deuterium located in the isolated methyl group. Further, we required the potential prodrug **7**, in which the labelled pentamethylmelamine is attached to an indole-4,7-dione moiety. The latter quinone unit can be bioreduced selectively in hypoxic solid tumours;[15] this reduction is designed to trigger the release of the drug selectively in these sites. The labelled prodrug **7** was required for NMR studies on the conformation of the prodrug and for mass spectrometric studies on release of the drug after reduction.

Results and discussion

Synthesis of a trideuteromethyl isotopomer **1b** of hexamethylmelamine by deprotonation of unlabelled pentamethylmelamine **3a** and alkylation of the anion with iodotrideuteromethane has been reported.[16] However, this process cannot be adapted to the preparation of the trideuteropentamethylmelamine isotopomer **3b** from 4,6-bis(dimethylamino)-1,3,5-triazin-2-amine, as over-alkylation to hexamethylmelamines can result from prototropic equilibration of the intermediate anions. However, treatment of the chlorotriazine **5**[17] with one equivalent of trideuteromethylamine in hot basic aqueous solution afforded the required pentamethylmelamine isotopomer **3b** in good yield (Scheme 1). The NMR spectra of **3b** confirmed the location of the deuterium atoms. The characteristic ^{1}H signal for the isolated methyl group in **3a** (δ 2.90) was missing from the spectrum of **3b**. In the ^{13}C NMR spectrum of **3b**, the CD_3 carbon resonated at δ 26.5 as a septet ($^{1}J_{C\text{-}D} = 20.8$ Hz).



Scheme 1. Synthesis of trideuteropentamethylmelamine 3b and its indole-4,7-dione conjugate 7. Reagents: i, CD_3NH_2.HCl, NaOH, H_2O; ii, 6, $EtO_2CN{=}NCO_2Et$, Ph_3P, THF.

Although the anion derived from pentamethylmelamine **3a** can be alkylated with simple electrophiles,[16] all attempts to alkylate the anions from **3a** and **3b** with 3-chloromethyl-1,2-dimethyl-5-methoxy indole-4,7-dione[15] failed. The indoledione unit was destroyed under the basic reaction conditions; the melamine anion is clearly much more basic than are the phenolate anions which are reported[15] to react smoothly with this reagent. However, we have very recently shown[12] that the corresponding alcohol **6** can act as an alkylating electrophile with isoquinolin-1-ones under Mitsunobu conditions. Mitsunobu reactions require the nucleophilic component (NH, OH, SH or CH) to be sufficiently acidic and simple amines fail to couple. However, treatment of the pentamethylmelamine isotopomer **3b** with **6** and 1.5 equivalents of triphenylphosphine and diethyl azodicarboxylate gave the required trideutero prodrug **7** in moderate yield. Again, the spectroscopic data confirmed the location of the deuterium atoms.

Experimental

Chemicals were purchased from Aldrich and were used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on JEOL GX270 or VARIAN EX400 spectrometers, using tetramethylsilane as internal standard. The FAB mass spectrum was recorded using 3-nitrobenzyl alcohol as the matrix.

4,6-Bis(dimethylamino)-2-trideuteromethylamino-1,3,5-triazine (**3b**): 2,4-Bis(dimethylamino)-6-chloro-1,3,5-triazine **5**[17] (2.00 g, 9.9 mmol) was boiled under reflux with $CD_3H_2 \cdot HCl$ (700 mg, 9.9 mmol) and NaOH (396 mg, 9.9 mmol) in water (15 ml) for 20 min. The solution was filtered while still hot. The evaporation residue was triturated with cold water (50 ml), filtered and dried. Recrystallization (aquous EtOH) gave **3b** (1.18 g, 60%) as a white crystalline solid: mp 94–95°C (literature[1] mp 98–103°C for **3b**); IR (KBr) ν_{max} 2073 cm^{-1}; NMR δ_H 3.07 (12 H, s, 4 × Me), 4.89 (1 H, s, NH); NMR δ_C 166.5, 165.4, 35.8, 26.5 (septet, $J = 20.8$ Hz, CD_3).

1,2-Dimethyl-3-(N-(4,6-bis(dimethylamino)-1,3,5-triazin-2-yl)-N-trideuteromethylaminomethyl)-5-methoxyindole-4,7-dione (**7**): Diethyl azodicarboxylate (78 mg, 450 μmol) was added dropwise to **3b** (89 mg, 450 μmol) and PPh_3 (119 mg, 450 μmol) in dry THF (15 ml) under dry Ar. The mixture was stirred for 15 min. 1,2-Dimethyl-3-hydroxymethyl-5-methoxyindole-4,7-dione **6**[15] (71 mg, 300 μmol) was added and the

mixture was stirred for 16 h. Evaporation and chromatography (silica gel, EtOAc) afforded **95** (35 mg, 20%) as an orange glass: IR (KBr) ν_{max} 3090, 2980, 2780, 1680, 1690, 1470 cm^{-1}; NMR δ_H 2.21 (3 H, s, indole 2-Me), 3.11 (12 H, s, $2 \times NMe_2$), 3.81 (3 H, s, indole 1-Me), 3.88 (3 H, s, OMe), 5.14 (2 H, m, CH_2), 5.61 (1 H, s, indole 6-H); NMR δ_C 10.1, 32.8, 36.1, 39.8, 56.6, 106.8, 120.3, 122.5, 138.1, 159.8, 165.2, 178.6 (the CD_3 signal was too weak to be observed); MS m/z 417.2440 (M + H) ($C_{20}{}^2H_3{}^1H_{24}N_7O_3$ requires 417.2441).

Conclusion

In this paper, we report the first synthesis of pentamethylmelamine labelled regiospecifically with a stable isotope. The ability of the sterically hindered secondary amine of this labelled melamine **3b** to react as the nucleophilic component in a Mitsunobu coupling is also demonstrated. Clearly, the secondary amine is sufficiently activated as an acid by the flanking endocyclic nitrogens to be able to be deprotonated by the Mitsunobu complex and to participate as a nucleophile. The deuterium-labelled 4,7-dioxoindol-3-ylmethyl prodrug **7** will facilitate studies *in vitro* and *in vivo* of reductively triggered release of pentamethylmelamine.

Acknowledgements

We thank Mr. D. J. Wood and Mr. R. R. Hartell for the NMR spectra, Mr. C. J. Cryer for the mass spectrum and Professor D. R. Blake for helpful discussions. SF held a University of Bath Research Studentship.

References

1. Borkovec AB, De Milo AB. *J Med Chem* 1967; **10**: 457–461.
2. Hahn DA. *Drug Intel Clin Pharm* 1983; **17**: 418–424.
3. Foster BJ, Clagett-Carr K. Hoth D, Leyland-Jones B. *Cancer Treatment Rep* 1986; **70**: 383–389.
4. Harries M, Kaye SB. *Expert Opinion Ther Drugs* 2001; **10**: 1715–1724.
5. Olver I, Davy M, Luftner D, Park SH, Egorin M, Ellis A, Webster L. *Cancer Chemother Pharmacol* 2001; **48**: 109–114.

6. Malik IA. *Jpn J Clin Oncol* 2001; **31**: 69–71.
7. Brindley C, Gescher A, Langdon SP, Broggini M, Colombo T, D'Incalci M. *Biochem Pharmacol* 1982; **31**: 625–631.
8. Damia G, D'Incalci M. *Clin Pharmacokinetics* 1995; **28**: 439–448.
9. Coley HM. *Gen Pharmacol* 1997; **28**: 177–182.
10. Addison SJ, Cunningham BDM, Gate EN, Shah PZ, Threadgill MD. *J Chem Soc, Perkin Trans 1* 1985; 75–79.
11. (Part 7) Parveen I, Threadgill MD. *J Label Compd Radiopharm* 2000; **43**: 883–889.
12. Ferrer S, Naughton DP, Parveen I, Threadgill MD. *J Chem Soc, Perkin Trans 1*, 2002; 335–340.
13. Parveen I, Naughton DP, Whish WJD, Threadgill MD. *Bioorg Med Chem Lett* 1999; **9**: 2031–2036.
14. Matthews SE, Pouton CW, Threadgill MD. *J Controlled Release* 2000; **67**: 129–139.
15. Naylor MA, Swann E, Everett SA, Jaffar M, Nolan J, Robertson N, Lockyer SD, Patel KB, Dennis MF, Stratford MRL, Wardman P, Adams GE, Moody CJ, Stratford IJ. *J Med Chem* 1998; **41**: 2720–2731.
16. Threadgill MD. *Isotopenpraxis* 1991; **27**: 143–144.
17. Pearlman WM, Banks CK. *J Am Chem Soc* 1948; **70**: 3727–3728.

