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Letter: Synthesis of glycosides in which the aglycon is an *N*-(Hydroxymethyl)amino-1,3,5-triazine derivative

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The synthesis of analogues of the anti-tumour drug 2-[*N*-(hydroxymethyl)methylamino]-4,6-bis(dimethylamino)-1,3,5-triazine (HMPMM) in which the OH or a dimethylamino group is replaced by a carbohydrate has been explored. Triazinyl *β*-glycosides were readily prepared by reaction of sugars with trimethyl-triazinylammonium salts. These were made with one or two methylamino groups on the triazine for reaction with formaldehyde to give the cytotoxic $NMeCH_2OH$ group. However, reaction of the triazinyl glycosides with formaldehyde gave complex intractable mixtures. When the carbohydrate portion was changed to the fully protected 2,3,4,6-tetra-*O*-acetyl glucose a good yield of the 2-[*N*-(hydroxymethyl)methylamino]-4-(dimethylamino)-1,3,5-triazin-2-yl tetra-*O*-acetyl *β*-glucoside was obtained. However, de-acetylation using sodium methoxide also removed the $N–CH_2OH$ group. We are investigating protection of the base-sensitive $N–CH_2OH$ group as trialkylsilyl and benzyl ethers and are looking at de-acetylation methods that are more selective. We have prepared glycosides in which the sugar is joined through the oxygen of the $NMeCH_2OH$ group. Coupling of acetobromoglucose with HMPMM catalysed by silver salts was not successful. Although methyl and cyclohexyl derivatives of HMPMM may be produced in high yields by reaction of HMPMM with methyl and cyclohexyl alcohols under acidic catalysis, production of glycosides in this way gave poor yields. MNDO calculations on reactions of HMPMM helped us devise improved reaction conditions for the condensation of 2,3,4,6-tetra-*O*-acetyl glucose with HMPMM and its derivatives. The best procedure to generate one of the target glycosides is to react 2,3,4,6-tetra-*O*-acetyl glucose and formaldehyde with 2-methylamino-4,6-bis(dimethylamino)-1,3,5-triazine. The *β*-glycoside product was de-acetylated using potassium carbonate in dry methanol.

***Keywords*: Glycoside, synthesis, cancer, hexamethylmelamine, methylol, formaldehyde, modelling, deacetylation, triazine, *N*-hydroxymethyl**

***Abbreviations*: HMM, hexamethylmelamine (2) or 2,4,6-tris(dimethylamino)-1,3,5-triazine; HMPMM, hydroxymethylpentamethylmelamine or 2-[*N*-(hydroxymethyl)-methylamino]-4,6-bis(dimethylamino)-1,3,5-triazine; PMM, Pentamethylmelamine or 2-methylamino-4,6-bis(dimethylamino)-1,3,5-triazine; TBMS, *t*-Butyldimethylsilyl; *p*-TSA, *p*-Toluenesulphonic acid**

Hexamethylmelamine (HMM)–A drug for improvement

Hexamethylmelamine (HMM, **1**, Figure 1) is an anti-tumour drug, useful against ovarian cancer [1]. Since its solubility is low it is administered orally which causes clinical difficulties. It produces severe gastro-intestinal side-effects and is variably absorbed from the gut.

Metabolism (Figure 1) adds an OH to give HMPMM (**2**) which, unlike **1**, is active *in vitro*; the $N–CH_2OH$ group (methylol) appears to be the active anti-tumour moiety. HMPMM decomposes to formaldehyde and PMM (**3**, Figure 1) in aqueous solution.

Sugars are avidly transported into cancer cells but attachment of the triazine ring of tetramethylmelamine to monosaccharides as in **4** (Figure 2) produced [2] water soluble but inactive drugs.

This inactivity was because metabolism no longer gave $N–CH_2OH$ groups.

Anti-tumour mechanism of HMM and attachment to nucleophiles

The mechanism of anti-tumour action of HMM is unclear [3] but HMPMM reacts (Figure 3) with certain nucleophiles when protonated.

This may account for anti-cancer activity (by reaction with biomolecules) and might be useful in the synthesis of better drugs.

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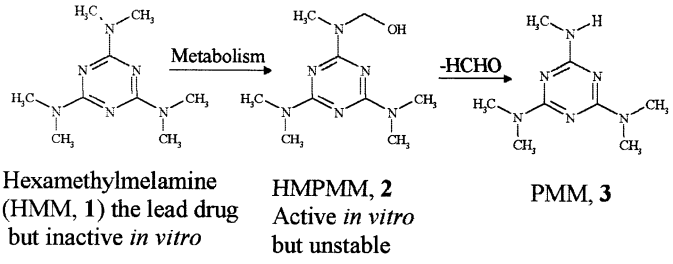


Figure 1.

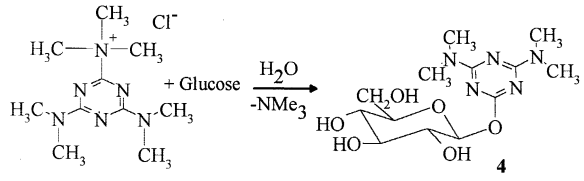
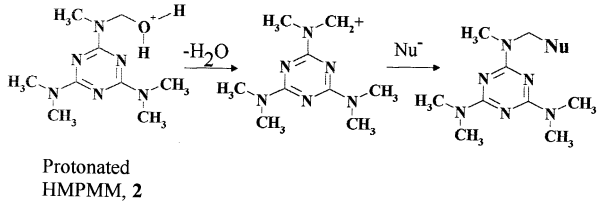


Figure 2.



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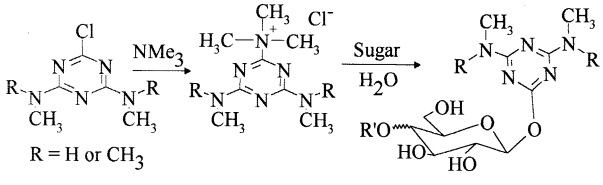

Figure 4.

Aims and strategy

To synthesise HMM derivatives bearing: (i) a monosaccharide (for solubility & selectivity) and an N–CH_2OH group (for activity without need for metabolism); or (ii) a monosaccharide and a latent N–CH_2OH group, such as N–CH_2O-Glu, that would be selectively hydrolysed to a N–CH_2OH group (by beta-glucosidases in this case) in cancer cells.

Synthesis of glycosides directly linked to the triazine ring

Displacement of Cl^- from triazinyltrimethylammonium salts (Figure 4) by free sugars in aqueous solution works well to give beta-glycosides. In addition to the previously reported [2] glycosides (R = Me, Figure 4) we have

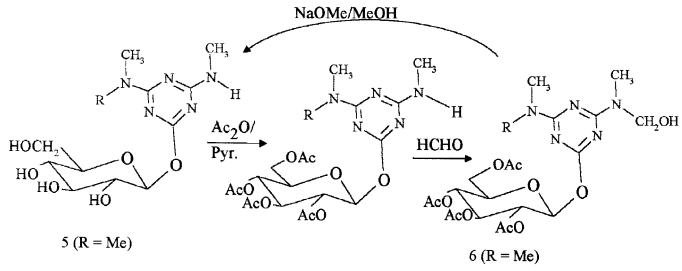

Figure 5.

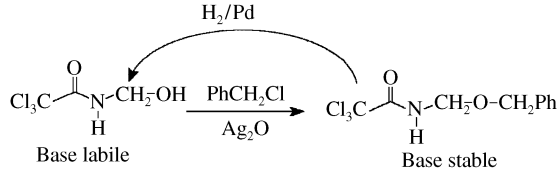

Figure 6.

prepared glucosides having free HNMe groups on the triazine ring, a beta-galactoside and a beta-lactoside. These were generally isolated as their acetylated derivatives in about 50% overall yield. When the unprotected glucosides (such as **5**, Figure 5) were reacted with formaldehyde in attempts to convert an NH into an N–CH_2OH group, intractable mixtures resulted apparently due to reactions involving carbohydrate OH groups.

An N–CH_2OH group was successfully introduced (Figure 5) when the sugar was protected by acetates but subsequent deprotection using MeONa/MeOH also removed the base-sensitive N–CH_2OH group.

Protection of the N–CH_2OH group prior to deblocking of the monosaccharide

We have examined the idea of protecting N–CH_2OH groups as benzyl ethers or *t*-butyltrimethylsilyl (TBMS) ethers so that deacetylation can be achieved and then the N–CH_2OH group deprotected to give the target glucose-HMPMM conjugate. *N*-(Hydroxymethyl)chloroacetamides, having similar stability to HMPMM, were readily protected as benzyl- (Figure 6) and TBMS- (Figure 7) derivatives that are stable to deacetylation conditions. HMPMM (**2**) proved more difficult to protect (neither TBMS chloride with imidazole, nor benzyl bromide with silver oxide gave the protected HMPMM) but we have obtained the HMPMM-TBMS ether (Figure 8) using TBMS chloride in pyridine. However, the low yield persuaded us that alternative strategies to the target compound should be investigated.

Selective deacetylation

Since the NaOMe/MeOH method of deacetylation causes base-catalyzed decomposition (Figure 5) of the N–CH_2OH

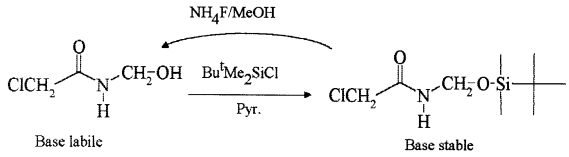

Figure 7.

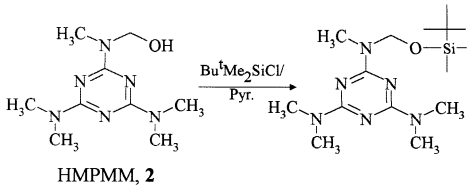

Figure 8.

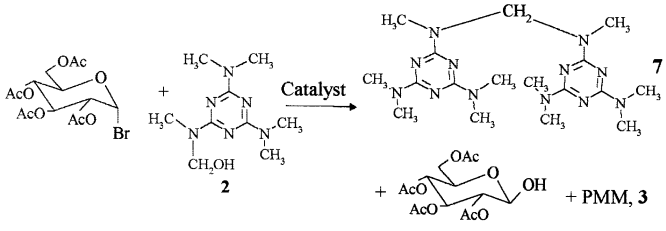

Figure 9.

group and protection of this group is difficult we have investigated less basic deacetylation methods.

HMPMM is stable to MgO in MeOH and so the glucose-linked-PMM derivative (**6**, Figure 5) has been subjected to this treatment which has been recommended [4] for deacetylation of base-sensitive carbohydrates. Some decomposition of the N–CH_2OH group occurs but isolated yields of the desired glycoside of up to 13% have been obtained and we expect to improve on this.

Synthesis of glycosides of HMPMM

The standard methods of glycosides synthesis were ineffective with HMPMM (**2**). Thus attempts to obtain the glucoside $TrNMeCH_{20}Glu$ (Tr is the triazine ring) by reaction of HMPMM with acetobromoglucose (Figure 9) and various catalysts (such as silver triflate with tetramethylurea) gave a dimerised form of HMPMM (**7**, Figure 9) with decomposition products.

HMPMM reacts with alcohols (Figure 10) such as methanol and cyclohexanol when heated under reflux with acidic catalysts to give [5] ethers, $TrNMeCH_2OR$, but reaction of HMPMM with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose gives poor yields of the glucoside. Molecular orbital calculations gave insight into the reaction mechanisms and revealed [3] that the activation barrier to product formation is lower than that for decomposition of HMPMM. This

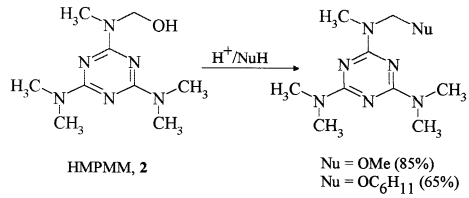

Figure 10.

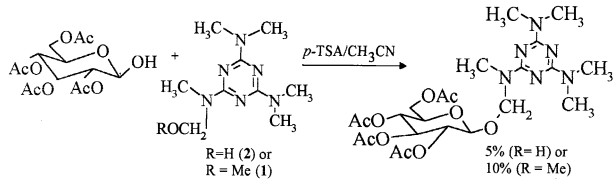

Figure 11.

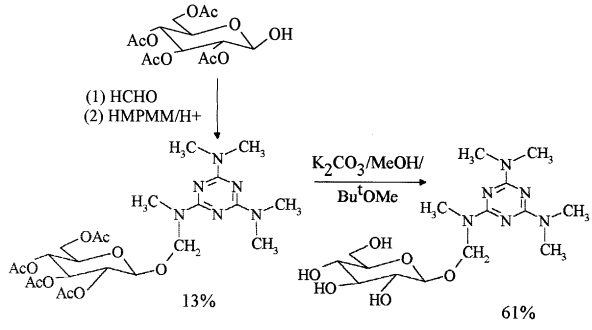

Figure 12.

suggested a lower reaction temperature and reaction (Figure 11) at 0 °C improved the yield but only to 5%.

Better yields were obtained using the methyl ether of HMPMM (Figure 11) with acid and tetraacetylglucose but the best yield (13%) of the glucoside (Figure 12) resulted from reaction of glucose with formaldehyde gas and then PMM. Deacetylation of this product to the desired glucoside (Figure 12) was achieved using K_2CO_3/MeOH/BuOMe. We are now increasing the scale of these syntheses to produce enough drug for anti-tumour testing.

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