Triazines and Related Products. Part 25.[1] Methods for the Attachment of Sugar Residues to Cytotoxic 1,3,5-Triazines

By **Richard J. Simmonds** and **Malcolm F. G. Stevens,*** Cancer Chemotherapy Research Group, Department of Pharmacy, University of Aston in Birmingham, Birmingham B4 7ET

The interaction of 2-chloro-4,6-bis(dimethylamino)-1,3,5-triazine (6) with amine nucleophiles gives high yields of substituted 2-amino-1,3,5-triazines. 2-Chloro- and 2-azido-4,6-bis(dimethylamino)-1,3,5-triazines do not afford carbohydrate-linked melamines (2,4,6-triamino-1,3,5-triazines) when treated with glucosamine or methyl 2-amino-2-deoxy-α-D-glucopyranoside.

1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (13) and the chlorotriazine (6) yielded the 3-*O*-substituted glucofuranose (14) when heated in *N,N*-dimethylformamide containing sodium hydride; selective removal of the 5,6- and 1,2-*O*-isopropylidene groups was achieved in acid media.

The quaternary salt (9) reacted with glucosamine in an alkaline medium to afford an *N*-triazinylglucosamine (18) and with D- and L-glucose to yield the β-D-glucopyranoside (20) and the enantiomeric β-L-glucoside, respectively.

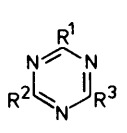
HEXAMETHYLMELAMINE (HMM) [2,4,6-bis(dimethylamino)-1,3,5-triazine] (1) is an antitumour drug with marked activity against certain solid human tumours.[2,3] In clinical use the agent suffers two major disadvantages: it elicits severe dose-limiting gastro-intestinal side effects[4] and is variably absorbed.[5] The drug is almost totally insoluble in water and has to be administered orally. Efforts to improve water solubility by molecular modification have, in general, produced dyschemotherapeutic effects.[6] HMM is extensively metabolised by oxidative de-methylation in animals and man, and structure-activity investigations have led to the conclusion[7] that a minimum of four methyl groups are required for antitumour activity in this series. Pentamethylmelamine (PMM) [2,4-bis(dimethylamino)-6-methylamino-1,3,5-triazine] (2) has a similar spectrum of antitumour activity to HMM but is appreciably more water-soluble.[8]

Our approach to the design of new water-soluble analogues of HMM has been to prepare melamine derivatives carrying the required minimum of four methyl groups but also bearing an attached carbohydrate moiety; we were encouraged to pursue this work because it has been demonstrated that many transformed malignant cells exhibit enhanced uptake of certain sugars in comparison to their untransformed counterparts.[9] As a prelude to this work we reinvestigated some nucleophilic substitution reactions of melamine derivatives.

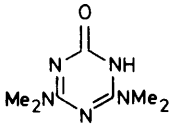
HMM is a chemically resilient molecule. It has pK_a 4.88 ± 0.08 (determined spectroscopically) and can be recovered quantitatively from hot 2N-hydrochloric acid or 2N-sodium hydroxide, or after exposure to sunlight for 14 d. The drug resists hydrazinolysis in boiling hydrazine hydrate and can be recovered unchanged after prolonged reaction with methyl iodide in boiling methanol, or dimethyl sulphate in boiling toluene, or hot 2N-sodium hydroxide.

HMM fails to afford an *N*-oxide, even when treated with 86% hydrogen peroxide in a hot acetic acid–sulphuric acid mixture. In fact, the only notable reaction of HMM discovered in the course of this work was its nitrosative de-methylation in a mixture of sodium nitrate and acetic acid at pH 4.2. The product (1%) was shown to be nitrosopentamethylmelamine (3) since it was identical with a sample independently prepared by nitrosation of PMM. No dimethylnitrosamine was detected, although this malevolent carcinogen is known to be formed by the action of nitrous acid on many compounds containing dimethylamino-groups.[10]

It is known that all three chloro-groups in cyanuric chloride (4) can be displaced by dimethylamine, with the second and third substitutions requiring progressively more forcing conditions.[11,12] However, 2,4-dichloro-6-dimethylamino-1,3,5-triazine (5) is more reactive than



	R^1	R^2	R^3
(1)	NMe_2	NMe_2	NMe_2
(2)	NMe_2	NMe_2	NHMe
(3)	NMe_2	NMe_2	N(NO)Me
(4)	Cl	Cl	Cl
(5)	NMe_2	Cl	Cl
(6)	NMe_2	NMe_2	Cl
(7)	NMe_2	N_3	N_3
(8)	NMe_2	NMe_2	N_3
(9)	NMe_2	NMe_2	$NMe_3^+Cl^-$
(10)	NMe_2	NMe_2	NH_2
(11)	NMe_2	NMe_2	$NHNH_2$



(12)

the mono-chloro analogue (6) towards azide ion. Thus, when equimolar quantities of compounds (5) and (6) competed for sodium azide (2 mol equiv.) in aqueous acetone only the diazide (7) was formed. The monoazide (8) was efficiently formed from the monochloro-precursor (6) and sodium azide in acetic acid. Both monoazido- and di-azido-triazines exist exclusively in their azido-forms in the solid phase and in a range of solvents.

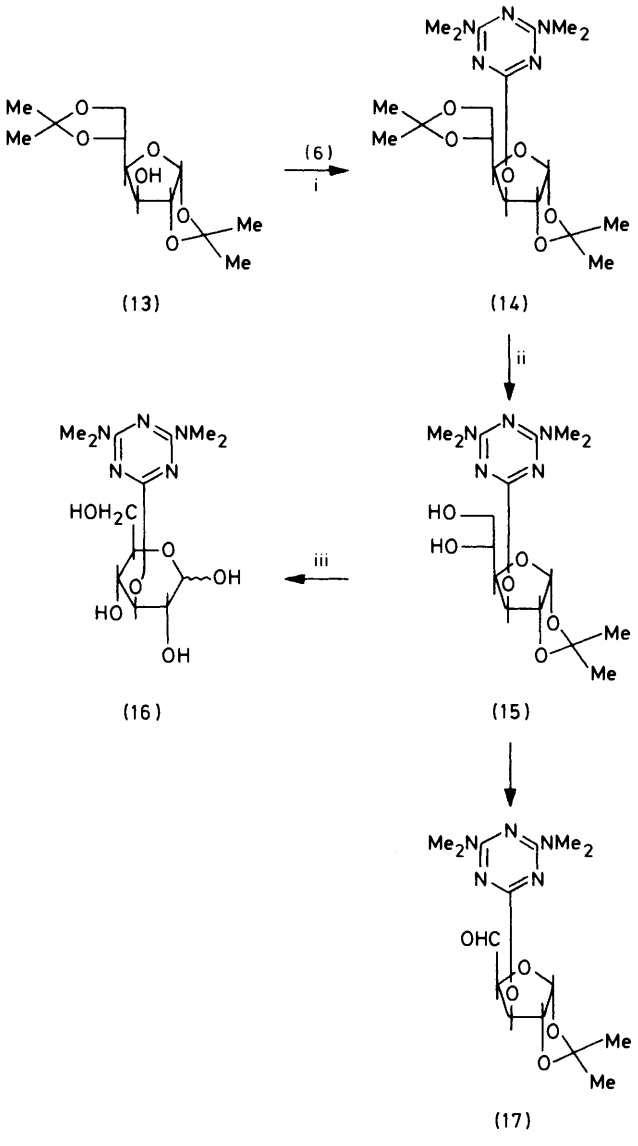
Apparently tetrazole-isomers of azido-1,3,5-triazines are not normally encountered.[13,14] Interaction of the monochlorotriazine (6) with trimethylamine in diethyl ether afforded the stable triazinyltrimethylammonium salt (9), this last compound proved to be the best substrate for carbohydrate-linking reactions (see later).

The monochlorotriazine (6) could not be converted into the corresponding amine (10) in boiling concentrated aqueous ammonia or molten urea; only starting material was recovered. However, the amine can be prepared by catalytic hydrogenation of the monoazide (8) over palladium–charcoal, or, more conveniently, by decomposing the azide in hot hydrazine hydrate. This behaviour of the azide (8) was surprising. It might have been expected to react in one of two possible ways; either to suffer displacement with hydrazine and yield the corresponding hydrazinotriazine (11), or to undergo reductive de-azidation in the manner of some arylazides.[15] Evidently, the azido-group attached to the π-deficient 1,3,5-triazine ring does not possess the leaving-group character of a 'pseudo-halogen' in its reaction with hydrazine, whereas the related chlorotriazine (6) is smoothly converted into the hydrazinotriazine (11) in refluxing hydrazine hydrate. In contrast, the chloro- and azido-triazines were both hydrolysed to the triazinone (12) at comparable rates in boiling 1N-hydrochloric acid.

In order to reduce the number of possible products formed from reactive melamine derivatives and carbohydrates, a partially blocked sugar was used in initial coupling experiments. Thus, prolonged reaction between 1,2:5,6-di-*O*-isopropylidene-α-D-gluco- furanose (13) and chlorotriazine (6) in *N*,*N*-dimethylformamide containing sodium hydride at 130 °C yielded the 3-*O*-substituted glucofuranose (14) in 75% yield (Scheme). Gentle hydrolysis in cold methanolic sulphuric acid led to the removal of the 5,6-*O*-isopropylidene group yielding the diol (15). Further hydrolysis of compound (15) to remove the 1,2-*O*-isopropylidene group was achieved in boiling 0.4N-hydrochloric acid; the highly water-soluble melamine-linked glucopyranose (16) was formed in moderate yield.

Oxidation of the 1,2-blocked glucofuranose (15), to afford the aldehyde (17), with sodium metaperiodate proceeded much more slowly than is usual for such diols,[16] possibly because of steric interference by the 3-*O*-triazinyl moiety.

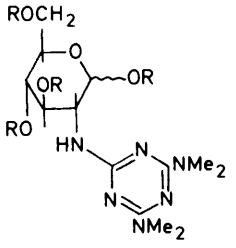
No suitable conditions could be found for the condensation of the chloro- or azido-triazines (6) and (8) with the amino-group of glucosamine or methyl 2-amino-2-deoxy-α-D-glucopyranose; decomposition of the amino-sugar was invariably encountered. However, success was achieved by employing the quaternary salt (9) as reactant. This reactive salt has an excellent leaving group, the volatile trimethylamine, and at 25 °C has a half-life of 4.2 and 210 min in 1N-sodium hydroxide and 1N-hydrochloric acid, respectively, hydrolysing to the triazinone (12) in a pseudo first-order reaction in both cases. The quaternary salt reacted with glucosamine at 0 °C in aqueous potassium hydroxide to afford an impure *N*-triazinylglucosamine (18) which was characterised by conversion into the tetra-*O*-acetate (19) in acetic anhydride–pyridine. The chemical shift of the anomeric proton in the ^{1}H n.m.r. 220 MHz spectrum of the tetra-acetate (δ 6.34 in $CDCl_3$) and the coupling constant (8.5 Hz) were indicative of the exclusive presence of the β-anomer.



SCHEME *Reagents:* i, NaH–DMF; ii, H_2SO_4–MeOH; iii, HCl

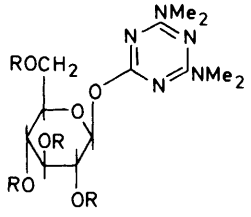
One of the acetyl groups in the tetra-acetate (19) absorbed at higher field (δ 1.91) than the remaining three acetate functions (δ 2.05). Inspection of molecular models of the tetra-acetate (19) suggested that the 3-acetate group would be the one most likely to be affected by the shielding influence of the melamine residue.

The synthetic usefulness of the quaternary salt (9) was further demonstrated in its reaction with the di-*O*-isopropylidene-α-D-glucofuranose (13) in cold aqueous sodium hydroxide to yield the *O*-substituted gluco-



(18) R = H
(19) R = Ac

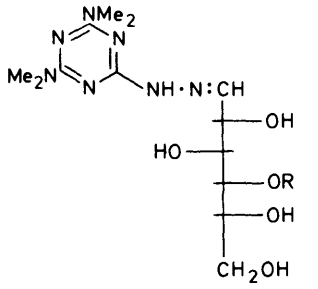
furanose (14). Similarly D-glucose reacted with the salt (9) under mildly basic conditions to yield the moderately water-soluble β-D-glucoside (20). Its structure was established by periodate oxidation. Under basic conditions, 2.11 ± 0.2 mol equiv. of periodate were consumed without production of formaldehyde; in acid media 4.9 ± 0.2 mol equiv. of periodate were consumed with the generation of *ca.* 1 mol equiv. of formaldehyde. Acetylation gave the tetra-*O*-acetate with an anomeric proton (δ 6.2; *J* 7.8 Hz), diagnostic of the β-configuration (21). Deacetylation with 0.2M-sodium methoxide furnished a near quantitative yield of pure β-D-glucopyranoside (20). A similar reaction between the quaternary salt (9) and L-glucose gave the enantiomeric β-L-glucoside, which readily crystallised from the reaction mixture although the presence of six minor impurities could be detected (t.l.c.).



(20) R = H
(21) R = Ac

Another convenient method for linking 1,3,5-triazinyl moieties to carbohydrates was found in the reactions of the hydrazinotriazine (11) with the reducing sugars glucose and lactose; the products were the monohydrazones (22) and (23), respectively. Although conditions were varied with respect to the nature of the acid catalyst, molar proportions of reactants, and pH, no osazones could be produced in these reactions when the hydrazinotriazine (11) alone was employed. However, qualitative experiments employing combinations of hydrazinotriazine (11) and phenylhydrazine indicated that further reaction to yield mixed osazones was practicable.

The water-soluble carbohydrate-linked melamines (16), (18), and (20) showed no antitumour activity against the M5076 mouse-ovarian sarcoma, whereas hexa- and penta-methylmelamine have pronounced inhibitory effects. Preliminary experiments (by D. R. Ross) indicate that the sugar derivatives are poorly demethylated by mouse-liver preparations *in vitro* and that their inactivity may be a consequence of the low plasma concentrations of cytotoxic hydroxymethyl metabolites which can be achieved *in vivo*. These studies will be reported in full elsewhere.



(22) R = H
(23) R = β-D-galactopyranosyl

EXPERIMENTAL

Properties of 2,4,6-*Tris*(*dimethylamino*)-1,3,5-*triazine* (*Hexamethylmelamine*) (1).—(a) *p*K_a *Determination.* This was determined at 25 °C by a spectroscopic method [17] employing an analytical wavelength of 227 nm, the $\lambda_{max.}$ of the un-ionised species; the cation has $\lambda_{max.}$ 240 nm. The mean pK_a was 4.88 ± 0.08.

(b) *Attempted methylation.* (i) Hexamethylmelamine (2.0 g) was boiled in methanol (40 ml) containing methyl iodide (3 ml) for 2 h. The colourless solution deposited white needles (1.85 g), identical (i.r.) with the starting material. (ii) Unchanged starting material (95%) was recovered when hexamethylmelamine was boiled (4 h) in toluene containing an excess of dimethyl sulphate. (iii) Hexamethylmelamine (2.1 g) was dissolved in hot diglyme (20 ml) and the mixture was diluted with 1N-sodium hydroxide (20 ml). Dimethyl sulphate (2 ml) was added and the mixture was kept at 25 °C for 30 d. Basification of the mixture with concentrated aqueous ammonia afforded unchanged starting material (96%).

(c) *Attempted oxidation.* Hexamethylmelamine (3.4 g) was dissolved in a mixture of acetic acid (20 ml) and concentrated sulphuric acid (24 ml) at 0 °C. Hydrogen peroxide (86%; 3 ml) was added dropwise during 1 h at 25 °C and the mixture was then heated on a steam-bath for 8 h. Hexamethylmelamine (1.3 g) was precipitated on neutralisation of the mixture.

(d) *Otherr eactions.* (i) Hexamethylmelamine (1.0 g) was refluxed in hydrazine hydrate (5 ml) for 2 h. Dilution of the reaction mixture with water (20 ml) afforded unchanged hexamethylmelamine (0.95 g). (ii) Hexamethylmelamine was recovered unchanged when boiled (2 h) in either 2N-hydrochloric acid or 2N-sodium hydroxide. (iii) Exposure of solutions of hexamethylmelamine (1 mg per 100 ml) in 0.1N-, 1N-, and 2.5N-hydrochloric acid, and 0.1N-, 1N-, and 2.5N-sodium hydroxide to intermittent sunlight during 14 d did not induce any changes in the u.v. spectra of the solutions.

2,4-*Bis*(*dimethylamino*)-6-*methylnitrosoamino*-1,3,5-*triazine* [(N-*Nitrosopentamethylmelamine*) (3).—(a) 2,4-Bis-(dimethylamino)-6-methylamino-1,3,5-triazine(pentamethylmelamine) (0.59 g) [18] was dissolved in acetic acid

(10 ml) and treated with a solution of sodium nitrite (0.45 g) in water (5 ml) during 10 min at 25 °C. The pale yellow solution was stirred (3 h) until all the pentamethylmelamine was consumed (t.l.c. on silica gel plates using toluene–acetone, 7 : 3 as developing solvent). Dilution of the mixture with water (30 ml) precipitated a cream solid (0.18 g) which gave a positive Liebermann nitroso-test. Crystallisation of the solid from acetone afforded yellow needles of *nitrosopentamethylmelamine* (3), m.p. 91—93 °C (Found: C, 43.1; H, 6.9; N, 44.0%; M^+, 225. $C_8H_{15}N_7O$ requires C, 42.7; H, 6.7; N, 43.6%; *M*, 225); $\lambda_{max.}$ (EtOH) 228 nm; δ ($CDCl_3$) 3.2 (12 H, s, 2 × NMe_2) and 3.4 [3 H, s, N(NO)-Me]; $\nu_{max.}$ (KBr) 1 470 cm^{-1} (NO).

(b) Hexamethylmelamine (5.25 g) was dissolved in 60% aqueous acetic acid (50 ml) containing sodium acetate trihydrate (21.7 g). The mixture (pH 4.2) was maintained at 80 °C while a solution of sodium nitrite (5.2 g) in water (100 ml) was added (30 min). The mixture was stirred at 80 °C (2 h), cooled, and extracted with diethyl ether (3 × 50 ml). The ethereal fractions were shaken with aqueous 10% potassium carbonate, dried over anhydrous sodium sulphate, and concentrated to 10 ml. The resulting crystal mass (3.4 g) was identified (i.r.) as unchanged hexamethylmelamine. The ethereal mother-liquor was evaporated to dryness and the residue redissolved in acetone. A further quantity of hexamethylmelamine (1.1 g) separated out. Chromatographic fractionation of the yellow acetone-soluble products was achieved on 1-mm silica gel plates using ethyl acetate–chloroform (1 : 9) as developing solvent. Two products were identified: hexamethylmelamine (R_F 0.76) and a yellow product (R_F 0.85). Removal of the zone at R_F 0.85, extraction, and crystallisation of the product from acetone afforded nitrosopentamethylmelamine (0.05 g) as yellow needles, identical (i.r.) with the sample described above. No dimethylnitrosamine (R_F 0.63) was detected in the aforementioned chromatographic separation.

2,4-*Diazido*-6-*dimethylamino*-1,3,5-*triazine* (7).—2,4-Dichloro-6-dimethylamino-1,3,5-triazine [19] (3.0 g) and sodium azide (2 mol equiv.) were boiled in acetic acid (10 ml) for 1 h. After being cooled, dilution of the solution with water gave colourless crystals of the diazidotriazine (7) (92%). The crude product crystallised from light petroleum (b.p. 60—80 °C) as white needles, m.p. 142—143 °C, $\nu_{max.}$ (KBr) 2 160 and 2 200 cm^{-1} (N_3); M^+, 206 ($C_5H_6N_{10}$ requires *M*, 206). The diazide was not subjected to elemental analysis because of the detonation risk. [**WARNING:** 2,4,6-triazido-1,3,5-triazine (cyanuric triazide) detonates violently when touched, and reactions involving cyanuric chloride (4) and an excess of sodium azide are *extremely* hazardous.]

2-*Azido*-4,6-*bis*(*dimethylamino*)-1,3,5-*triazine* (8).—This compound was prepared by the interaction of 2-chloro-4,6-bis(dimethylamino)-1,3,5-triazine [11] and sodium azide (1 mol equiv.) in boiling acetic acid (1 h). The azide (8), deposited on dilution with water, crystallised from aqueous ethanol, m.p. 104—106 °C (lit.,[20] m.p. 106—107 °C); $\nu_{max.}$ (KBr) 2 180 cm^{-1} (N_3).

2-*Amino*-4,6-*bis*(*dimethylamino*)-1,3,5-*triazine* (10).—(a) 2-Azido-4,6-bis(dimethylamino)-1,3,5-triazine (1.5 g) was boiled (45 min) in hydrazine hydrate (5 ml) and the solution was diluted with water (20 ml). The precipitated aminotriazine (10) (68%) crystallised from ethanol as colourless prisms, m.p. 220—222 °C (sinters above 200 °C) (lit.,[18] m.p. 227.5—228.5 °C).

(b) A solution of 2-azido-4,6-bis(dimethylamino)-1,3,5-triazine (3.0 g) in ethanol (100 ml) was hydrogenated over 5% palladium on charcoal (0.2 g) at atmospheric pressure (18 h). The heated, filtered solution yielded the same (i.r.) aminotriazine (2.6 g) on cooling, m.p. 228—230 °C.

No reaction occurred when 2-chloro-4,6-bis(dimethylamino)-1,3,5-triazine (6) was boiled in concentrated aqueous ammonia (4 h) or fused with an excess of urea at 180 °C (0.5 h).

2,4-*Bis*(*dimethylamino*)-6-*hydrazino*-1,3,5-*triazine* (11).—2-Chloro-4,6-bis(dimethylamino)-1,3,5-triazine (6.0 g) and hydrazine hydrate (7 ml) were boiled in ethanol (25 ml) for 1 h. Addition of water to the mixture afforded the hydrazinotriazine (11) (5.3 g) which crystallised from toluene–light petroleum (b.p. 60—80 °C) as colourless prisms, m.p. 150—152 °C (sinters at 145 °C) (lit.,[21] 148.5—152.5 °C).

4,6-*Bis*(*dimethylamino*)-1,3,5-*triazin*-2(1H)-*one* (12).—2-Chloro-4,6-bis(dimethylamino)-1,3,5-triazine (0.5 g) was boiled in 1N-hydrochloric acid (20 ml) for 3 h. An excess of sodium acetate trihydrate was added to the cooled mixture and the product was extracted into chloroform (3 × 25 ml). Removal of the solvent afforded the triazinone (12) (0.4 g), m.p. 285—288 °C (lit.,[22] m.p. 290—292 °C), $\nu_{max.}$ (KBr) 1 625 cm^{-1} (CO).

3-O-[4,6-*Bis*(*dimethylamino*)-1,3,5-*triazin*-2-*yl*]-1,2:5,6-*di*-O-*isopropylidene*-α-D-*glucofuranose* (14).—To a solution of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose [23] (13) (11.7 g, 0.05 mol) and 6-chloro-2,4-bis(dimethylamino)-1,3,5-triazine (10.1 g, 0.05 mol) in dry *N*,*N*-dimethylformamide (50 ml) was added sodium hydride (50% in oil; 4 g, *ca.* 0.08 mol) and the mixture was heated at 130 °C for 18 h. The gum (15.3 g, 75%) which separated out when the mixture was poured into water was collected and crystallised from chloroform–light petroleum (b.p. 60—80 °C) to yield the 3-O-*triazinylglucofuranose* (14), m.p. 89—95 °C which could not be satisfactorily crystallised to analytical purity. The ^1H n.m.r. spectrum of the crude product showed δ ($CDCl_3$) 1.28 (6 H, s, 2 × Me), 1.40 (3 H, s, Me), 1.51 (3 H, s, Me), 3.08 (12 H, s, 2 × NMe 2), 4.2 (4 H, m), 4.6 (1 H, d), 5.56 (1 H, br s), and 5.9 (1 H, d).

The same product (48%) was precipitated when the glucofuranose (13) (2.6 g) and the quaternary salt (9) (2.6 g) were stirred in 0.1N-sodium hydroxide (100 ml) for 18 h at 25 °C.

3-O-[4,6-*Bis*(*dimethylamino*)-1,3,5-*triazin*-2-*yl*]-1,2-O-*isopropylidene*-α-D-*glucofuranose* (15).—A solution of compound (14) (3.0 g) in methanol (25 ml) and 0.3N-sulphuric acid (25 ml) was kept at 25 °C for 5 d. The solution was concentrated to 20 ml under reduced pressure and extracted with chloroform (3 × 20 ml). Addition of an excess of diethyl ether to the chloroform solution afforded a precipitate which was crystallised from chloroform–light petroleum to give the pure *triazinylglucofuranose* (15) (2.4 g, 90%), m.p. 189—190 °C (Found: C, 49.9; H, 7.0; N, 18.1. $C_{16}H_{26}N_5O_6$ requires C, 49.9; H, 7.1; N, 18.2%); $[\alpha]_D^{25}$ 123 ± 3° (*c* 1.6 in $CHCl_3$); δ ($CDCl_3$) 1.33 (3 H, s, Me), 1.55 (3 H, s, Me), 3.10 (12 H, s, 2 × NMe_2), 3.10—6.10 (9 H, br m).

3-O-[4,6-*Bis*(*dimethylamino*)-1,3,5-*triazin*-2-*yl*]-D-*glucopyranose* (16).—A solution of compound (15) (2.0 g) and 0.4N-hydrochloric acid (25 ml) was heated to boiling, cooled, and neutralised by stirring with IRA-400 (HCO_3^-) ion-exchange resin. The filtered solution was freeze-dried and the residue crystallised from ethyl acetate–ethanol to yield the 3-O-*triazinylglucopyranose* (16) (0.75 g, 44%), m.p. 179—181 °C (Found: C, 45.5; H, 7.2; N, 20.5. $C_{12}H_{19}N_5O_5$ requires C, 45.2; H, 6.7; N, 20.3%).

3-O-[4,6-*Bis*(*dimethylamino*)-1,3,5-*triazin*-2-*yl*]-1,2-O-*isopropylidene*-α-D-xylo-*pentodialdo*-1,4-*furanose* (17).—To a solution of compound (15) (1.3 g) in chloroform (15 ml) was added sodium hydrogencarbonate (0.2 g) in water (25 ml). The vigorously stirred emulsion was treated with sodium periodate (0.78 g) in portions. After 24 h a further quantity of sodium periodate (0.1 g) was added and the mixture was re-stirred (24 h). Evaporation of the chloroform layer gave a mixture of starting material and product (t.l.c.). Chromatographic fractionation of the mixture on a column of silica gel using diethyl ether as eluant gave, as the first fraction, the *triazinylpentodialdofuranose* (0.25 g), m.p. 109—118 °C (Found: C, 50.8; H, 6.4; N, 19.7. $C_{15}H_{23}N_5O_5$ requires C, 51.0; H, 6.6; N, 19.8%); *m/z* 353 (M^+), 338 (M^+ − Me), 266, 184, 183, 141, and 83.

2-*Amino*-N-[4,6-*bis*(*dimethylamino*)-1,3,5-*triazin*-2-*yl*]-2-*deoxy*-D-*glucopyranose* (18).—To a solution of glucosamine hydrochloride (4.15 g) and potassium hydroxide (1.55 g) in water (5 ml) at 0 °C was added in portions a solution of the quaternary salt [24] (9) (5 g) in water (10 ml). The mixture was stirred at 0 °C for 4 d and the crude *amino*-D-*glucopyranose* (18) (3.5 g, 53%), m.p. 155—160 °C (with decomp.) was collected. The crude product (2.0 g) in pyridine (12 ml) was stirred at 25 °C with acetic anhydride (12 ml) for 66 h and poured into ice–water to afford the *tetra-acetate* (19) (1.8 g, 54%) which crystallised from dichloromethane–light petroleum-diethyl ether, m.p. 213—214 °C (Found: C, 48.9; H, 6.4; N, 16.5. $C_{21}H_{32}N_6O_9$ requires C, 49.2; H, 6.3; N, 16.4%); δ ($CDCl_3$) 1.91 (3 H, s, COMe), 2.05 (9 H, s, 3 × COMe), 3.12 (12 H, s, 2 × NMe_2), 3.5—4.3 (4 H, br m), and 6.34 (1 H, d, 1-H, d, 1-H, *J* 8.5 Hz).

2-O-[4,6-*Bis*(*dimethylamino*)-1,3,5-*triazin*-2-*yl*]-β-D-*glucopyranoside* (20; D-*enantiomer*).—D-Glucose (1.05 g) and potassium hydroxide (0.33 g) in water at 0 °C were treated with a solution of the quaternary salt (9) (1.5 g) in water (2 ml). The mixture was stirred at 0 °C (2 h) and allowed to warm to 25 °C (12 h). The crude β-D-*glucopyranoside* (20) (0.55 g) was collected, m.p. 197—198 °C. Further product was obtained by concentration of the mother-liquor (total yield 1.5 g, 52%). The crude product (0.25 g) in pyridine (5 ml) was stirred with acetic anhydride for 18 h at 25 °C and the mixture was poured into ice–water. The precipitate was collected, dried, and crystallised from chloroform–light petroleum to afford the 2-O-[4,6-*bis*(*dimethylamino*)-1,3,5-*triazin*-2-*yl*] 3,4:5,7-*di*-O-*tetra-acetyl*-β-D-*glucopyranoside* (21) (0.22 g), m.p. 194—195 °C (Found: C, 48.8; H 6.0; N, 13.55. $C_{21}H_{31}N_5O_{10}$ requires C, 49.1; H, 6.1; N, 13.6%); δ ($CDCl_3$) 2.03 (12 H, s, 4 × COMe), 3.13 (12 H, s, 2 × NMe_2), 4.86 (1 H, m), 4.20 (2 H, m), 5.25 (3 H, m), and 6.20 (1 H, d, 1-H, *J* 7.8 Hz).

De-acetylation of the tetra-acetate was achieved in a 0.02M-sodium methoxide solution at 25 °C (2 h); the product, m.p. 197—198 °C was identical (i.r. and t.l.c.) with the β-D-glucopyranoside (20).

2-O-[4,6-*Bis*(*dimethylamino*)-1,3,5-*triazin*-2-*yl*]-β-L-*glucopyranoside* (20; L-*Enantiomer*).—Interaction of L-glucose with the quaternary salt (9) according to the procedure described above yielded the β-L-*glucopyranoside* (27%), m.p. 196—197 °C (Found: C, 44.8; H, 6.65; N, 20.5. $C_{13}H_{23}N_5O_6$ requires C, 45.2; H, 6.7; N, 20.3%), with an i.r. spectrum indistinguishable from that of the D-enantiomer.

Reactions of 2,4-*Bis*(*dimethylamino*)-6-*hydrazino*-1,3,5-*triazine with Sugars*.—(a) A mixture of the hydrazinotriazine (11) (3.25 g) and anhydrous D-glucose (1.0 g) was refluxed in 0.5N-hydrochloric acid (30 ml) for 2 h. The solution was extracted with ethyl acetate (2 × 25 ml) to remove the excess of hydrazinotriazine and the aqueous layer was evaporated under reduced pressure to 15 ml. On cooling crude D-*glucose* 4,6-*bis*(*dimethylamino*)-1,3,5-*tetrazin*-2-*yl*-*hydrazone* (22) (1.25 g, 63%, based on D-glucose) was deposited, m.p. 200—212 °C (decomp.). Crystallisation (ethanol) afforded pure product, m.p. 205—206 °C (decomp.) (Found: C, 43.6; H, 7.2; N, 26.8. $C_{13}H_{25}N_7O_5$ requires C, 43.4; H, 7.0; N, 27.3%), δ [$(CD_3)_2SO$] 2.97 (12 H, s, 2 × $N(Me)_2$), 3.36 (5 H, s, exchanged with D_2O), 2.5—4.5 (3 H, m), and 5.83 (2 H, br s).

(b) Interaction of lactose monohydrate with an excess of 2,4-bis(dimethylamino)-6-hydrazino-1,3,5-triazine (11) in boiling 0.5N-hydrochloric acid, according to the procedure described above, afforded 4-O-(β-D-*galactopyranosyl*)-D-*glucose* 4,6-*bis*(*dimethylamino*)-1,3,5-*tetrazin*-2-*ylhydrazone* (23) (60%) when the mixture was concentrated. The hydrazone crystallised from chloroform–methanol–light petroleum (b.p. 60—80 °C) as white crystals, m.p. 169—171 °C (decomp.) (Found: C, 39.1; H, 6.6; N, 17.4. $C_{19}H_{35}N_7O_{10}\cdot 3H_2O$ requires C, 39.5; H, 7.1; N, 17.0%).

We thank the M.R.C. for the award of a Research Fellowship (to R. J. S.).

[1/1713 *Received*, 3rd *November*, 1981]

REFERENCES

[1] Part 24, G. U. Baig, M. F. G. Stevens, R. Stone, and E. Lunt, preceding paper.
[2] R. H. Blum, R. B. Livingston, and S. K. Carter, *Eur. J. Cancer*, 1973, **9**, 195.
[3] S. S. Legha, M. Slavik, and S. K. Carter, *Cancer*, 1976, **38**, 27.
[4] B. L. Johnson, R. I. Fisher, R. A. Bender, V. T. DeVita, jun., B. A. Chabner, and R. C. Young, *Cancer*, 1978, **42**, 2157.
[5] M. D'Incalci, G. Bolis, C. Mangioni, L. Morasca, and S. Garattini, *Cancer Treatment. Rep.*, 1978, **62**, 2117.
[6] A. J. Cumber and W. C. J. Ross, *Chem.-Biol. Interact.*, 1977, **17**, 349.
[7] C. J. Rutty and T. A. Connors, *Biochem. Pharmacol.*, 1977, **26**, 2385.
[8] R. S. Goldberg, J. P. Griffin, J. W. McSherry, and I. H. Krakoff, *Cancer Treatment Rep.*, 1980, **64**, 1319.
[9] M. Hatanaka, *Biochim. Biophys. Acta*, 1974, **355**, 77.
[10] W. Lijinsky, E. Conard, and R. Van De Bogart, *Nature*, 1972, **239**, 165.
[11] J. T. Thurston, J. R. Dudley, D. W. Kaiser, I. Hechenbleikner, F. C. Schaefer, and D. Holm-Hansen, *J. Am. Chem. Soc.*, 1951, **73**, 2981.
[12] W. M. Pearlman and C. K. Banks, *J. Am. Chem. Soc.*, 1948, **70**, 3726.
[13] R. A. Henry, *J. Org. Chem.*, 1966, **31**, 1973.
[14] J. Kobe, B. Stanovnik, and M. Tišler, *Monatsh. Chem.*, 1970, **101**, 724.
[15] T. McC. Paterson, R. K. Smalley, and H. Suschitzky, *Tetrahedron Lett.*, 1977, 3973.
[16] R. Schaffer and H. S. Isbell, *J. Res. Nat. Bur. Stand.*, 1956, **56**, 191.
[17] A. Albert and E. P. Serjeant, 'Ionisation Constants of Acids and Bases,' Methuen, London, 1962.
[18] A. B. Bořkovec and A. B. DeMilo, *J. Med. Chem.*, 1967, **10**, 457.
[19] H. Koopman and J. Daams, *Recl. Trav. Chim. Pays-Bas*, 1958, **77**, 235.
[20] R. Kayama, S. Hasunuma, S. Sekiguchi, and K. Matsui, *Bull. Chem. Soc. Jpn.*, 1974, **47**, 2825.
[21] A. B. DeMilo, J. E. Oliver, and R. D. Gilardi, *J. Heterocycl. Chem.*, 1973, **10**, 231.
[22] J. J. Bishop, R. M. Thomas, and G. K. Weisse, U.S.P. 3,110,716 (*Chem. Abstr.*, 1964, **60**, 4163f).
[23] K. Freudenberg and K. Smeykal, *Ber.*, 1926, **59B**, 100.
[24] V. V. Dovlatyan, K. A. Elizyan, and L. G. Agadzhanyan, *Arm. Khim. Zh.*, 1977, **30**, 66 (*Chem. Abstr.*, 1977, **87**, 84954w).