Synthesis of *C*-ribosyl 1,2,4-triazolo[3,4-*f*][1,2,4]triazines as inhibitors of adenosine and AMP deaminases

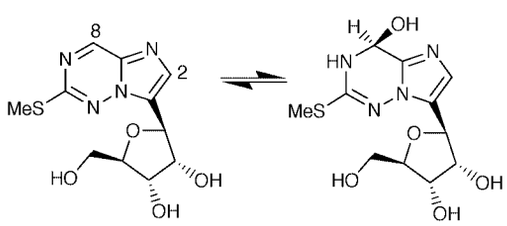
Philip J. Dudfield,[a] Van-Duc Le,[b] Stephen D. Lindell[a] and Charles W. Rees[b]

[a] *AgrEvo UK Limited, Chesterford Park, Saffron Walden, Essex, UK CB10 1XL*
[b] *Department of Chemistry, Imperial College of Science, Technology and Medicine, London, UK SW7 2AY*

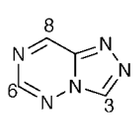
Received (in Cambridge, UK) 28th June 1999, Accepted 24th August 1999

Modified *C*-nucleosides and nucleotides with an enhanced tendency to undergo covalent hydration are of interest as potential inhibitors of adenosine deaminase (ADA) and AMP deaminase, respectively. In a search for such compounds we have synthesized 6-dimethylamino-3-(β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*][1,2,4]triazine **4** in four steps (42% overall yield) from the readily available allonic acid **6** and the hydrazine **7**. The hydrazide **16** derived from **6** and **7** (78%) is converted directly into the cyclised chloro compound **19** (62%) with phosphorus trichloride oxide, followed by dechlorination (96%) and deprotection (90%). Riboside **4** undergoes partial hydration in water to the covalent hydrate **22**, and is a modest inhibitor of mammalian ADA (IC_{50} 180 μM).

Modified *C*-nucleosides and nucleotides which are susceptible to covalent hydration in the aglycone ring system are potential inhibitors of adenosine deaminase (ADA) and AMP deaminase, respectively. We have recently described the design and synthesis of the *C*-nucleoside 6-methylsulfanyl-3-(β-D-ribofuranosyl)imidazo[2,1-*f*][1,2,4]triazine **1** as an inhibitor of adenosine deaminase (IC_{50} 40 μM) which we believe binds to the enzyme as its covalent hydrate **2**.[1] The tendency of **1** to undergo covalent hydration at C-8 was expected to be enhanced by the introduction of additional nitrogen heteroatoms into the aglycone. The calculated differences in the heats of formation for such heteroaromatic systems and their corresponding covalent hydrates support this proposal and indicate that 1,2,4-triazolo[3,4-*f*][1,2,4]triazine **3** should readily hydrate.[2] In an effort to produce more strongly inhibiting *C*-nucleosides we have now synthesized the *C*-riboside **4** of the ring system **3**, together with other related 1,2,4-triazolo[3,4-*f*][1,2,4]triazines.



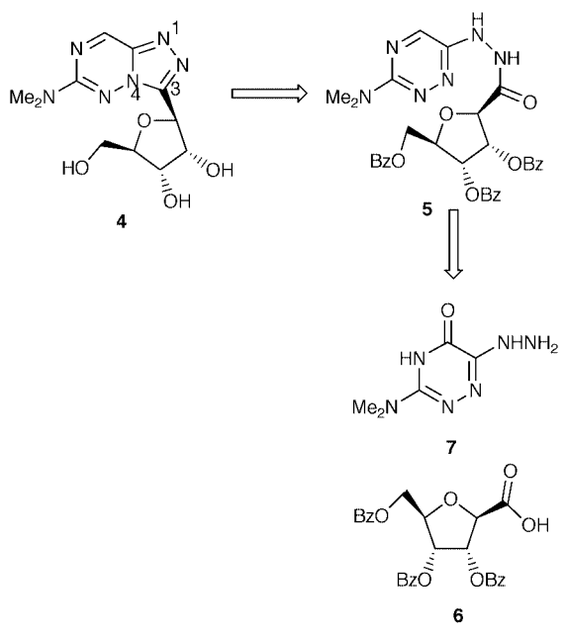
1 **2**



3

Results and discussion

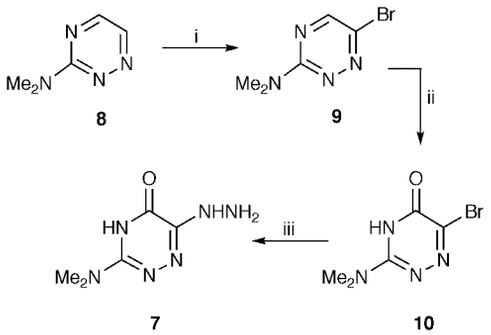
For reasons of synthetic convenience the 6-methylsulfanyl group of compound **1** was replaced by a 6-dimethylamino group in our present target nucleoside **4**. A convergent route to the target **4** based upon disconnection of the triazole C3–N4 bond is shown in Scheme 1. 2,5-Anhydro-3,4,6-tri-*O*-benzoyl-D-allonic acid **6** was available in substantial quantities (>10 g) as described by Chi and co-workers[3] and Kalvoda.[4] Commercial 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose was



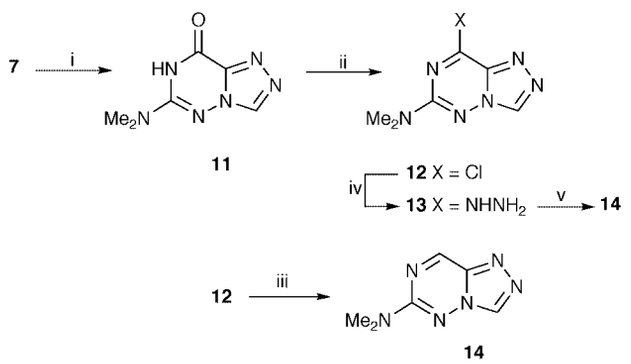
Scheme 1

treated with trimethylsilyl cyanide and anhydrous tin(IV) chloride followed by hydrolysis with conc. hydrochloric acid in acetic acid to give **6** in 65% overall yield.

3-Dimethylamino-6-hydrazino-1,2,4-triazin-5(4*H*)-one **7** was synthesized from 3-dimethylamino-1,2,4-triazine **8** (Scheme 2) which was made in three steps from thiosemicarbazide by *S*-methylation, condensation with glyoxal and treatment of the resulting 3-(methylthio)triazine with dimethylamine.[5] Bromine in tetrachloromethane converted **8** into the 6-bromo compound **9**,[6] but attempted nucleophilic displacement of the bromine with hydrazine was unsuccessful; the reaction was complex and a dark intractable tar was formed. 5-Unsubstituted 1,2,4-triazines are prone to oxidation and to nucleophilic attack at this position, and so **9** was oxidized with hydrogen peroxide and acetic acid to give the 5-oxo derivative **10**. Nucleophilic displacement of bromine from **10** with hydrazine monohydrate then proceeded smoothly in boiling water to give the hydrazine **7** in reasonable yield (Scheme 2).



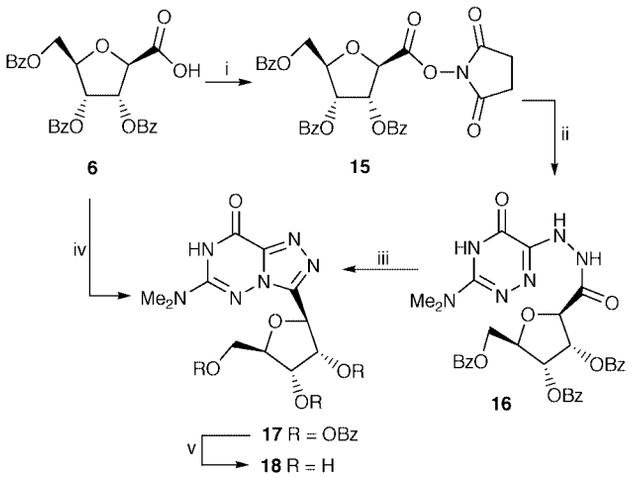
Scheme 2 *Reagents, conditions and yields*: (i) Br_2, CCl_4, Et_3N, RT, 12 h, 52%; (i.i) H_2O_2, AcOH, RT, 12 h, 68%; (iii) $N_2H_4{\cdot}H_2O$, H_2O, reflux, 4 h, 63%.



Scheme 3 *Reagents, conditions and yields*: (i) DMF, AcOH, reflux, 12 h, 55%; (ii) $POCl_3$, $PhNMe_2$, reflux, 40 min, 61%; (iii) H_2, Pd/C, MgO, EtOAc, RT, 4 d, 92%; (iv) $N_2H_4{\cdot}H_2O$, EtOH, RT, 5 min, 100%; (v) HgO, EtOH, reflux, 30 min, 54%.

Before proceeding with the synthesis of the *C*-nucleoside **4** we made the (unknown) aglycone, 6-dimethylamino-1,2,4-triazolo[3,4-*f*][1,2,4]triazine **14** (Scheme 3) as a model compound. The hydrazine **7** was heated under reflux in formic acid for 6 h, and evaporation and recrystallisation gave the desired triazolo-triazine **11**, but in low yield. A better yield was obtained with triethyl orthoformate in place of formic acid, but the best yield was obtained with a refluxing mixture of DMF in acetic acid which has been used for the cyclisation of similar hydrazines.[7] The oxo group of **11** was readily removed by conversion into the 8-chloro compound **12** with phosphorus trichloride oxide and *N*,*N*-dimethylaniline followed by hydrogenolysis with 5% Pd/C and magnesium oxide in ethyl acetate, or by displacement with hydrazine and oxidation of **13** with yellow mercury(II) oxide. Though more convenient and almost quantitative, the hydrogenolysis was much slower, requiring four days for completion. 6-Dimethylamino-1,2,4-triazolo[3,4-*f*][1,2,4]triazine **14** was obtained as pale yellow crystals which decomposed at their melting point (210 °C).

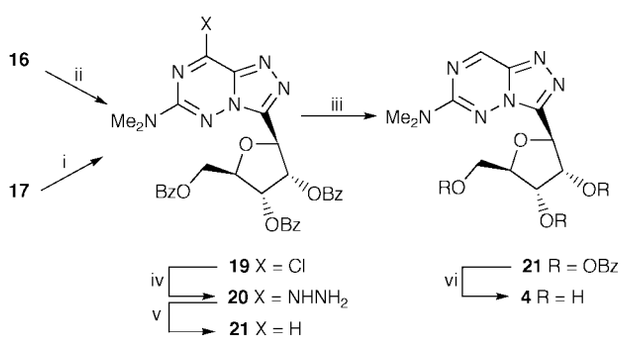
We now turned to the coupling of the hydrazine **7** and the allonic acid **6**; the formation of analogous hydrazides from this acid has been reported, using DCC alone[8] or activated by HOBT,[9] or with 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ).[10] We decided to use DCC and *N*-hydroxysuccinimide to activate the acid, expecting that the hydroxamic intermediate might be easier to handle. Exposure of acid **6** to *N*-hydroxysuccinimide and DCC in 1,2-dichloroethane at room temperature for 24 h gave the intermediate **15** in near-quantitative yield. This was isolated and, without purification, treated with the hydrazine **7** in DMF at 60 °C for 24 h to give hydrazide **16** as pale yellow crystals, which on refluxing in DMF for a further 24 h gave the protected *C*-nucleoside **17**. This *C*-nucleoside was also prepared, in better yield (65%), in a one-pot reaction by heating a mixture of the hydrazine **7**, acid **6**, DCC



Scheme 4 *Reagents, conditions and yields*: (i) DCC, *N*-hydroxysuccinimide, DCC, DCE, RT, 24 h, 99%; (ii) **7**, DMF, 60 °C, 24 h, 78%; (iii) DMF, reflux, 24 h, 58%; (iv) **7**, DCC, *N*-hydroxysuccinimide, DMF, reflux, 24 h, 65%; (v) NaOMe, MeOH, RT, 12 h, 66%.

and *N*-hydroxysuccinimide in dry DMF for 24 h (Scheme 4). The product was cleanly deprotected with sodium methoxide in methanol at room temperature to give *C*-nucleoside **18** as a colourless crystalline solid (66%). The β-configuration of **18** was assigned from its ^{1}H NMR spectrum in DMSO-d_6 which showed the H-1′ signal as a doublet at δ 4.99, J 5.78 Hz, similar, for example, to that of the closely related 3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-*f*][1,2,4]triazin-8(7*H*)-one.[10]

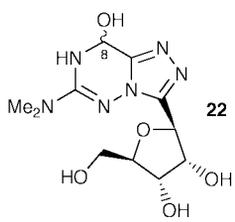
It remained to remove the lactam oxo group from the *C*-nucleoside **18**. Following the synthesis of the aglycone **14**, the tribenzoyl protected *C*-nucleoside **17** was chlorinated with phosphorus trichloride oxide and *N*,*N*-dimethylaniline (Scheme 5). After 40 min of gentle reflux, chloride **19** was isolated as a



Scheme 5 *Reagents, conditions and yields*: (i) $POCl_3$, $PhNMe_2$, reflux, 40 min, 60%; (ii) $POCl_3$, reflux, 40 min, 62%; (iii) H_2, Pd/C, MgO, EtOAc, RT, 4 days, 96%; (iv) $N_2H_4{\cdot}H_2O$, EtOAc, RT, 5 min, 88%; (v) HgO, EtOH, reflux, 1 h, 55%; (vi) MeOH–NH_3, RT, 2 days, 86% or NaOMe, MeOH, RT, 2 h, 90%.

yellow crystalline solid in 60% yield. A more convenient and higher yielding method of preparing **19** was to expose the uncyclised hydrazide **16** to phosphorus trichloride oxide alone, under gentle reflux, which gave the desired chloride in 62% yield. The chloro compound **19** was dechlorinated by the two methods described for the aglycone **14**: treatment with hydrazine monhydrate to give **20**, which was oxidised with mercury(II) oxide to **21** in moderate yield and, in much better yield, by hydrogenolysis of **19** with 5% palladium on carbon and magnesium oxide in ethyl acetate for 4 days. All the compounds in this sequence appeared to be homogeneous β-epimers by NMR examination. Deprotection of **21**, either in methanol saturated with ammonia gas at room temperature for 2 days or with sodium methoxide in methanol for 2 h, gave the desired *C*-nucleoside **4** in excellent yield (Scheme 5).

The ^{1}H NMR spectrum of **4** in DMSO-d_6 (containing ≈10% D_2O) confirmed that the configuration at C-1′ was β, and an aromatic singlet at δ 9.35, which integrated for one proton, showed there was no covalent hydration at the C-8 position. However, the ^{1}H NMR spectrum of **4** in D_2O alone was more complex and showed the presence of two components in a ratio of 55 : 45. In particular, the C8-H aromatic singlet and the C1′-H doublet which resonated at δ 9.32 and δ 5.42, respectively, now integrated for only 0.55 protons each and two new signals were observed at δ 6.33 (d) and δ 5.23 (dd), both integrating for 0.45 protons. These data indicate that in aqueous media compound **4** exists to the extent of 45% as the covalent hydrate **22**



22

(1 : 1 mixture of diastereoisomers at C-8). In contrast, the formation of a covalent hydrate was not detected in the ^{1}H NMR spectrum of compound **1** when run in D_2O or DMSO-d_6. This result experimentally supports our starting hypothesis that the 1,2,4-triazolotriazine ring would be more readily hydrated than the imidazotriazine. We had hoped to confirm the structure of **4** by X-ray crystallography and it was, therefore, converted into its tris-*p*-nitrobenzoate derivative but unfortunately the crystals proved to be unsuitable for diffraction analysis.

In summary, the synthesis of 6-dimethylamino-1,2,4-triazolo[3,4-*f*][1,2,4]triazine *C*-nucleoside **4** was accomplished employing a key cyclisation of the hydrazide **16** to the chloro compound **19** (Scheme 5). The route is convergent and efficient, requiring only four steps from the readily available 3-dimethylamino-6-hydrazinotriazine **7** and allonic acid **6** and proceeds in 42% overall yield. Preliminary biochemical studies show that compound **4** is a modest inhibitor of mammalian ADA (IC_{50} 180 μM). That it is a less potent inhibitor than riboside **1** is, we believe, attributable to the greater steric requirement of the NMe_2 group over the SMe group. In aqueous solution **4** exists to the extent of 45% as the hydrate **22** which, to the best of our knowledge, is the first time that such a covalent hydrate of a nucleoside has been observed by NMR. Further details of the biological activity will be reported elsewhere.

Experimental

For general points, see ref. 1. NMR assignments are tentative and coupling constants are in Hz. Ether refers to diethyl ether.

2,5-Anhydro-3,4,6-tri-*O*-benzoyl-D-allononitrile

To a stirred solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (Aldrich) (10.2 g, 20.3 mmol) in 1,2-dichloroethane (26 ml) was added cyanotrimethylsilane (5.4 ml, 40.5 mmol). The reaction mixture was treated in one portion *via* a syringe with anhydrous stannic [tin(IV)] chloride (2.37 ml, 20.3 mmol). The darkening solution was stirred for 2 min then poured into saturated aq. $NaHCO_3$ (200 ml) and stirred for 5 min (pH 7). DCM (250 ml) was added and the emulsion was filtered through a Celite pad. The DCM layer was separated, dried ($MgSO_4$) and evaporated *in vacuo* to afford light orange syrup, which was triturated with ethyl acetate–hexane (1 : 4). The solid was collected, and washed with hexane to give the title compound (7.54 g, 79%) as a white solid, mp 77–79 °C (lit.,[11] 77–78 °C) (from DCM–light petroleum); ν_{max}(NaCl/film)/cm^{-1} 2253 (CN), 1730 (C=O, ester), 1651, 1602, 1585, 1493, 1453, 1373, 1316, 1268, 1179, 1094, 1071, 1026; δ_H(270 MHz; $CDCl_3$) 7.92–8.16 (6H, m, Ar *H*), 7.33–7.89 (9H, m, Ar *H*), 6.03 (1H, dd, *J* 5.09, 4.39, *H*-3), 5.88 (1H, t, *J* 5.55, *H*-4), 5.00 (1H, d, *J* 4.39, *H*-2), 4.74–4.75 (1H, m, *H*-5), 4.62–4.75 (2H, m, H_2-6); δ_C(76 MHz; $CDCl_3$) [166.17, 165.13, 164.91] (*C*=O), [134.04, 133.87, 133.45, 129.88(×2), 129.82, 129.30, 128.67, 128.60(×2), 128.49, 128.26] (Ar *C*), 115.86 (*C*N), 80.96 (*C*-5), 74.53 (*C*-4), 71.96 (*C*-3), 69.52 (*C*-2), 63.26 (*C*-6); *m*/*z* (FAB, NBA) 472 (MH^+, 10%), 445 (MH^+ − CN, 6), 286 (30), 242 (4), 201 (4), 167 (15), 150 (25), 135 (18), 120 (32), 105 (PhCO, 100), 89 (64), 77 (72), 57 (38) (Found: MH^+, 472.1438. Calc. for $C_{27}H_{22}NO_7$: *M*H, 472.1396).

2,5-Anhydro-3,4,6-tri-*O*-benzoyl-D-allonic acid 6

A mixture of 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-allononitrile (2.91 g, 6.19 mmol), and conc. HCl (3 ml) in acetic acid (12 ml) was stirred at 100 °C for 15 min. The solution was cooled to 25 °C and poured into water (40 ml). The aqueous solution was extracted with ethyl acetate (40 ml) and the organic solution was washed with brine, dried ($MgSO_4$), and concentrated *in vacuo* to give a pale yellow oil, which was purified by flash chromatography on silica gel (gradient elution; 0–10% MeOH in DCM) to give the acid **6** (2.72 g, 82%) as a colourless foam; ν_{max}(NaCl/film)/cm^{-1} 3445, 3336 (OH), 1729 (C=O), 1697 (C=O), 1602, 1585, 1493, 1452, 1366, 1316, 1270, 1178, 1123, 1097, 1071, 1026; δ_H(270 MHz; $CDCl_3$) 7.89–8.10 (6H, m, Ar *H*), 7.31–7.62 (9H, m, Ar *H*), 5.98 (1H, dd, *J* 5.08, 3.93, *H*-3), 5.76 (1H, dd, *J* 6.01, 5.31, *H*-4), 4.87 (1H, d, *J* 4.16, *H*-2), 4.71–4.77 (3H, m, *H*-5 and H_2-6); δ_C(76 MHz; $CDCl_3$) 172.92 (CO_2H), [166.53, 165.29, 165.21] (*C*=O, ester), [133.71, 133.64, 133.35, 129.89, 129.82(×2), 129.45, 128.82, 128.73, 128.56, 128.49(×2)] (Ar *C*), 80.25 (*C*-5), 80.03 (*C*-4), 74.19 (*C*-3), 72.34 (*C*-2), 63.94 (*C*-6); *m*/*z* (FAB, NBA) 515 (MH^+ + Na, 4%), 491 (MH^+, 12), 445 (4), 369 (4), 341 (6), 201 (3), 167 (4), 150 (6), 135 (4), 105 (PhCO, 100), 89 (12), 77 (25), 69 (11), 43 (18) (Found: MH^+, 491.1369. Calc. for $C_{27}H_{23}O_9$: *M*H, 491.1342).

6-Bromo-3-dimethylamino-1,2,4-triazine 9

A solution of bromine (3.20 g, 20 mmol) in tetrachloromethane (100 ml) was added in one portion to a stirred solution of the dimethylamine **8**[5] (1.24 g, 10 mmol) in tetrachloromethane (80 ml). Triethylamine (1.69 g, 16.7 mmol) was added to the reaction mixture, which was stirred at 25 °C for 12 h. Excess of solvent was removed *in vacuo* and the residue was purified by flash chromatography on silica gel (gradient elution; 10–15% ether in light petroleum) to furnish the bromo compound **9** (1.06 g, 52%) as a yellow solid, mp 65–66 °C (lit.,[6] 66–67.5 °C) (from DCM–light petroleum); ν_{max}(NaCl/film)/cm^{-1} 1578, 1556, 1471, 1412, 1346, 1203, 1076; δ_H(270 MHz; $CDCl_3$) 8.12 (1H, s, *H*-5), 3.22 (6H, s, NCH_3); δ_C(76 MHz; $CDCl_3$) 160.34 (*C*-3), 151.02 (*C*-5), 134.93 (*C*-6), 37.15 (N*C*H_3); *m*/*z* (EI) 204, 202 (M^+, 26%), 174 (5), 149 (15), 147 (15), 95 (5), 80 (6), 70 (100), 55 (5), 42 (16) (Found: M^+, 203.9821. Calc. for $C_5H_7{}^{81}BrN_4$: *M*, 203.9834. Found: M^+, 201.9838. Calc. for $C_5H_7{}^{79}BrN_4$: *M*, 201.9854).

6-Bromo-3-dimethylamino-1,2,4-triazin-5(4*H*)-one 10

Hydrogen peroxide (27 wt% solution in water; 0.53 ml, 4.56 mmol) was added to a solution of 6-bromo-3-dimethylamino-1,2,4-triazine **9** (500 mg, 2.46 mmol) in glacial acetic acid (4 ml) at 5 °C. The mixture was stirred at 25 °C for 12 h. The precipitate was collected, washed thoroughly with water, air dried, and crystallised from ethanol to furnish the *oxo compound* **10** (368 mg, 68%) as white crystals, mp 261–262 °C (from ethanol); ν_{max}(NaCl/Nujol)/cm^{-1} 3114, 1608, 1567, 1504, 1435, 1403, 1076, 1016; δ_H(270 MHz; DMSO-d_6) 3.72 (6H, s, NCH_3); δ_C(76 MHz; DMSO-d_6) 159.00 (*C*-5), 154.52 (*C*-3), 135.10 (*C*-6), 37.29 (NCH_3); *m*/*z* (EI) 220, 218 (M^+, 20%), 192 (4), 149 (6), 139 (M^+ − Br, 7), 129 (3), 113 (50), 105 (2), 71 (44), 44 (100) (Found: C, 27.6; H, 3.1; N, 25.5. $C_5H_7BrN_4O$ requires C, 27.4;

H, 3.2; N, 25.6%. Found: M^+, 220.9866. $C_5H_7{}^{81}BrN_4O$ requires *M*, 220.9861. Found: M^+, 218.9885. $C_5H_7{}^{79}BrN_4O$ requires *M*, 218.9881).

3-Dimethylamino-6-hydrazino-1,2,4-triazin-5(4*H*)-one 7

Hydrazine monohydrate (1.44 ml, 30 mmol) was added to a stirred solution of the bromo compound **10** (2.20 g, 10 mmol) in water (80 ml) at 25 °C. The reaction mixture was heated to reflux for 4 h, allowed to cool to room temperature, and was left unstirred overnight. The crystalline solid was collected, washed with water, and air dried to furnish the *hydrazine* **7** (1.08 g, 63%) as a white solid, mp 264–266 °C (from ethanol); ν_{max}(NaCl/Nujol)/cm^{-1} 3324, 3301 (NH), 1641 (C=O), 1575, 1516, 1397; δ_H(270 MHz; DMSO-d_6) 11.25 (2H, br s, NH_2), 7.18 (1H, br s, N*H*), 2.96 (6H, s, NCH_3); δ_C(76 MHz; DMSO-d_6) 158.59 (*C*-5), 154.22 (*C*-3), 147.02 (*C*-6), 37.02 (NCH_3); *m*/*z* (EI) 170 (M^+, 43%), 155 ($M^+ - CH_3$, 38), 140 ($M^+ - 2CH_3$, 22), 113 (43), 98 (15), 84 (7), 71 (27), 69 (32), 57 (6), 44 (100) (Found: M^+, 170.0933. $C_5H_{10}N_6O$ requires *M*, 170.0916).

6-Dimethylamino-1,2,4-triazolo[3,4-*f*][1,2,4]triazin-8(7*H*)-one 11

A mixture of the hydrazine **7** (500 mg, 2.94 mmol), glacial acetic acid (0.34 ml, 5.88 mmol) and DMF (50 ml) was refluxed for 12 h. After cooling to room temperature, crystals were induced to form by addition of ether. These were collected, and recrystallised from water to yield the *title compound* **11** (291 mg, 55%) as white crystals, mp >300 °C (decomp.) (from water); ν_{max}(NaCl/Nujol)/cm^{-1} 3151 (NH), 1719 (C=O), 1608, 1556, 1364, 1290; δ_H(270 MHz; DMSO-d_6) 9.06 (1H, s, *H*-3), 2.99 (6H, s, NCH_3); δ_C(76 MHz; DMSO-d_6) 153.71 (*C*-8), 152.77 (*C*-6), 139.82 (*C*-3), 139.42 (*C*-8a), 38.46 (NCH_3); *m*/*z* (EI) 180 (M^+, 35%), 138 (5), 110 (6), 96 (26), 82 (21), 71 (92), 57 (45), 43 (100) (Found: M^+, 180.0766. $C_6H_8N_6O$ requires *M*, 180.0760).

8-Chloro-6-dimethylamino-1,2,4-triazolo[3,4-*f*][1,2,4]triazine 12

A mixture of **11** (150 mg, 0.83 mmol), *N*,*N*-dimethylaniline (1 ml) and phosphorus trichloride oxide (4 ml) was heated to reflux for 40 min. After cooling to room temperature, excess of solvent was removed *in vacuo* and ice–water (2 ml) was added to the residue. The aqueous solution was extracted with ethyl acetate (3 × 2 ml), and the extract was dried ($MgSO_4$), and concentrated *in vacuo* to give a dark residue, which was purified by flash chromatography on silica gel (gradient elution; 10–25% ether in light petroleum) to afford the *title compound* **12** (101 mg, 61%) as yellow crystals, mp 188–190 °C (from DCM–light petroleum); ν_{max}(NaCl/Nujol)/cm^{-1} 1694, 1651, 1592, 1491, 1455, 1417, 1382; δ_H(270 MHz; $CDCl_3$) 8.81 (1H, s, *H*-3), 3.18 (6H, s, NCH_3); δ_C(76 MHz; $CDCl_3$) 155.94 (*C*-8), 152.36 (*C*-6), 138.93 (*C*-3), 138.67 (*C*-8a), 37.70 (NCH_3); *m*/*z* (EI) 200 (M^+, 32%), 198 (M^+, 100), 183 ($M^+ - CH_3$, 8), 168 ($M^+ - 2CH_3$, 8), 156 (8), 130 (25), 128 (62), 108 (8), 82 (28), 44 (45) (Found: C, 36.5; H, 3.5; N, 42.1. $C_6H_7ClN_6$ requires C, 36.3; H, 3.55; N, 42.3%. Found: M^+, 200.0398. $C_6H_7{}^{37}ClN_6$ requires *M*, 200.0391. Found: M^+, 198.0429. $C_6H_7{}^{35}ClN_6$ requires *M*, 198.0421).

6-Dimethylamino-8-hydrazino-1,2,4-triazolo[3,4-*f*][1,2,4]-triazine 13

To a solution of chloro compound **12** (100 mg, 0.50 mmol) in ethanol (4 ml) was added hydrazine monohydrate (0.03 ml, 0.55 mmol) at 25 °C during 5 min. The precipitate which formed was collected, and washed with ethyl acetate. The crude product was recrystallised from ethanol to give the *title compound* **13** (108 mg, 100%) as colourless crystals, mp 255–260 °C (decomp.) (from ethanol); ν_{max}(NaCl/Nujol)/cm^{-1} 3319, 3255, 3151 (NH), 1565, 1549, 1416, 1354; δ_H(300 MHz; DMSO-d_6) 9.05 (1H, s, *H*-3), 7.00 (3H, br s, NHNH_2), 3.00 (6H, s, NCH_3); δ_C(76 MHz; DMSO-d_6) 158.31 (*C*-8), 148.55 (*C*-6), 139.00 (*C*-3), 133.66 (*C*-8a), 37.39 (NCH_3); *m*/*z* (EI) 194 (M^+, 100%), 179 ($M^+ - CH_3$, 5), 164 ($M^+ - 2CH_3$, 2), 151 (2), 122 (5), 110 (12), 95 (40), 82 (4), 69 (40), 57 (12), 44 (38) (Found: M^+, 194.1000. $C_6H_{10}N_8$ requires *M*, 194.1028).

6-Dimethylamino-1,2,4-triazolo[3,4-*f*][1,2,4]triazine 14

Method A. To a solution of the hydrazine **13** (80 mg, 0.41 mmol) in ethanol (4 ml) was added yellow mercury(II) oxide (268 mg, 1.24 mmol) at 25 °C. The reaction mixture was heated to reflux for 30 min, cooled and the inorganic residue was removed (Celite pad), washed with ethanol, and the combined organic layer evaporated *in vacuo* to yield a pale yellow solid. Purification of the solid by flash chromatography on silica gel (gradient elution; 30–40% ether in light petroleum) followed by recrystallisation from DCM–light petroleum gave the *title compound* **14** (37 mg, 54%) as pale yellow crystals, mp 210–215 °C (decomp.) (from DCM–light petroleum); ν_{max}(NaCl/Nujol)/cm^{-1} 1602, 1556, 1504, 1414; δ_H(270 MHz; $CDCl_3$) 9.16 (1H, d, *J* 0.7, *H*-8), 8.76 (1H, d, *J* 0.7, *H*-3), 3.19 (6H, s, NCH_3); δ_C(76 MHz; $CDCl_3$) 156.97 (*C*-6), 150.77 (*C*-8), 138.95 (*C*-8a), 137.67 (*C*-3), 37.58 (NCH_3); *m*/*z* (EI) 164 (M^+, 100%), 149 ($M^+ - CH_3$, 10), 135 (16), 108 ($M^+ - CNMe_2$, 5), 94 ($M^+ - NCNMe_2$, 70), 69 (28), 44 (29), 42 (46) (Found: M^+, 164.0803. $C_6H_8N_6$ requires *M*, 164.0810).

Method B. To a solution of chloro compound **12** (50 mg, 0.25 mmol) in dry ethyl acetate (4 ml) were added 5% palladium on carbon (28 mg) and magnesium oxide (26 mg, 0.66 mmol). The mixture was stirred at room temperature under a hydrogen balloon for 4 days. The inorganic material was removed by filtration through a short pad of Celite and was washed thoroughly with ethyl acetate. The filtrate was concentrated *in vacuo* and the residue was purified by flash chromatography on silica gel (gradient elution; 30–40% ether in light petroleum) to afford the title compound **14** (38 mg, 92%), identical to that described above.

N-(2,5-Anhydro-3,4,6-tri-*O*-benzoyl-D-allonoyl)-*N*′-(3-dimethylamino-4,5-dihydro-5-oxo-1,2,4-triazin-6-yl)hydrazine 16

To a solution of the acid **6** (1.85 g, 3.18 mmol) in dry 1,2-dichloroethane (32 ml) were added DCC (857 mg, 4.15 mmol) and *N*-hydroxysuccinimide (478 mg, 4.15 mmol). The reaction mixture was stirred at room temperature under N_2 for 24 h. The precipitate of dicyclohexyurea was filtered off and the filtrate was removed *in vacuo* to give the crude product **15** (99%) as a colourless foam, which was used without purification in the next step.

To a stirred solution of the hydrazine **7** (706 mg, 4.15 mmol) in dry DMF (120 ml) was added a solution of the activated acid **15** (2.20 g, 3.78 mmol) in dry DMF (20 ml) at room temperature. The reaction mixture was stirred at 60 °C under N_2 for 24 h. Excess of solvent was removed *in vacuo* and the residue was dissolved in DCM. The organic solution was washed successively with water and brine, dried ($MgSO_4$), and evaporated *in vacuo* to give the crude product as a yellow foam. Purification of this by flash chromatography on silica gel (gradient elution; 5–15% acetone in DCM) afforded the *hydrazide* **16** (1.88 g, 78%) as pale yellow crystals, mp 126–128 °C; ν_{max}(NaCl/film)/cm^{-1} 3253 (NH), 1730 (C=O, ester), 1715 (C=O, amide), 1651, 1644, 1634, 1602, 1587, 1515, 1505, 1454, 1271; δ_H(270 MHz; $CDCl_3$) (locants for carbohydrate moiety primed) 9.98 (1H, br s, N*H*), 8.96 (1H, d, *J* 3.46, N*H*), 7.83–7.96 (4H, m, Ar *H*), 7.80 (2H, d, *J* 1.38, Ar *H*), 7.28–7.58 (9H, m, Ar *H*), 6.16 (1H, dd, *J* 8.21, 4.62, *H*-2′), 6.00 (1H, dd, *J* 4.85, 2.08, *H*-3′), 4.94 (1H, d, *J* 2.08, *H*-1′), 4.81–4.86 (1H, m, *H*-4′), 4.67 (1H, s, N*H*), 4.62–4.71 (2H, m, H_2-5′), 3.04 (6H, s, NCH_3); δ_C(76 MHz; $CDCl_3$) (locants for carbohydrate moiety primed) 169.44 (*C*=O,

hydrazide), [166.52, 165.56, 165.26] (*C*=O, ester), 158.68 (*C*-5), 153.78 (*C*-3), 143.42 (*C*-6), [133.63, 133.56, 133.39, 129.78, 129.72, 129.55, 129.25, 129.02, 128.70, 128.49, 128.42, 128.29] (Ar *C*), 81.27 (*C*-4′), 79.22 (*C*-3′), 75.15 (*C*-2′), 71.52 (*C*-1′), 63.40 (*C*-5′), 36.76 (N*C*H$_3$); *m*/*z* (FAB, NBA) 643 (MH$^+$, 100%), 306 (6), 290 (10), 196 (10), 167 (11), 150 (20), 135 (18), 120 (24), 105 (74), 89 (40), 77 (46), 55 (22), 39 (39) (Found: C, 59.65; H, 4.4; N, 13.3. $C_{32}H_{30}N_6O_9$ requires C, 59.8; H, 4.7; N, 13.1%. Found: MH$^+$, 643.2222. $C_{32}H_{31}N_6O_9$ requires *M*H, 643.2153).

6-Dimethylamino-3-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*][1,2,4]triazin-8(7*H*)-one 17

Method A. A solution of the hydrazide **16** (200 mg, 0.31 mmol) in dry DMF (40 ml) was heated to reflux under N_2 for 24 h. After the solution had cooled to room temperature, excess of solvent was removed *in vacuo* and the residue was dissolved in DCM. The organic solvent was washed successively with water and brine, dried ($MgSO_4$), and evaporated *in vacuo* to give the crude product as a yellow solid. Purification of the residue by flash chromatography on silica gel (gradient elution; 2–5% acetone in DCM) afford the title compound **17** (113 mg, 58%) as a white solid, mp 125–126 °C (from light petroleum–EtOAc); ν_{max}(NaCl/film)/cm^{-1} 3200 (NH), 1728 (C=O, ester), 1611, 1493, 1452, 1381, 1316, 1270, 1123; δ_H(270 MHz; $CDCl_3$) 7.92–8.11 (6H, m, Ar *H*), 7.31–7.57 (9H, m, Ar *H*), 6.44 (1H, dd, *J* 5.78, 4.39, *H*-2′), 6.12 (1H, t, *J* 6.24, *H*-3′), 5.82 (1H, d, *J* 4.39, *H*-1′), 4.64–4.80 (3H, m, *H*-4′ and H_2-5′), 3.19 (6H, s, NCH_3); δ_C(76 MHz; $CDCl_3$) [171.11, 166.29, 165.29] (*C*=O, ester), 153.32 (*C*-8), 149.93 (*C*-6), 147.09 (*C*-8a), 139.49 (*C*-3), [133.57(×2), 133.21, 130.14, 129.81(×2), 129.52, 128.92, 128.46(×2), 128.40(×2)] (Ar *C*), 79.59 (*C*-4′), 75.29 (*C*-3′), 73.12 (*C*-2′), 72.52 (*C*-1′), 63.92 (*C*-5′), 38.60 (N*C*H$_3$); *m*/*z* (FAB, NBA) 625 (MH$^+$, 100%), 503 (4), 259 (4), 209 (2), 178 (2), 165 (4), 135 (8), 123 (12), 105 (72), 81 (26), 69 (46), 55 (50) (Found: C, 60.7; H, 4.1; N, 13.1. $C_{32}H_{28}N_6O_8$ requires C, 61.5; H, 4.5; N, 13.5%. Found: MH$^+$, 625.2090. $C_{32}H_{29}N_6O_8$ requires *M*H, 625.2047).

Method B. A mixture of the hydrazine **7** (100 mg, 0.59 mmol), the acid **6** (318 mg, 0.65 mmol), DCC (134 mg, 0.65 mmol), and *N*-hydroxysuccinimide (74.5 mg, 0.65 mmol) in dry DMF (40 ml) was heated to reflux for 24 h. After allowing the mixture to cool to room temperature, excess of solvent was removed *in vacuo* and the residue was dissolved in EtOAc. The organic solvent was washed successively with water and brine, dried ($MgSO_4$), and evaporated *in vacuo* to give the crude product as a yellow solid. Purification of the residue by flash chromatography on silica gel (gradient elution; 2–5% acetone in DCM) gave the title compound **17** (63 mg, 65%), identical to that described above.

6-Dimethylamino-3-(β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*]-[1,2,4]triazin-8(7*H*)-one 18

To a stirred solution of the protected *C*-nucleoside **17** (100 mg, 0.16 mmol) in dry methanol (2 ml) was added sodium methoxide (52 mg, 0.96 mmol). The reaction mixture was stirred at room temperature for 12 h and was quenched with water (0.2 ml). The solution was evaporated to dryness and the residue was crystallised from ethanol to afford the *title compound* **18** (35 mg, 66%) as a white solid, mp 228–230 °C (from ethanol); ν_{max}(NaCl/Nujol)/cm^{-1} 3200 (OH, NH), 1723 (C=O, amide), 1608; δ_H(400 MHz; DMSO-d_6 + D_2O) 4.99 (1H, d, *J* 5.78, *H*-1′), 4.60 (1H, t, *J* 5.50, *H*-2′), 4.08 (1H, t, *J* 5.08, *H*-3′), 3.81 (1H, q, *J* 4.94, *H*-4′), 3.53–3.56 (1H, m, *H*-5a′), 3.39–3.43 (1H, m, *H*-5b′), 2.94 (6H, s, NCH_3); δ_C(76 MHz; DMSO-d_6) 159.30 (*C*-8), 159.07 (*C*-6), 147.18 (*C*-3), 141.43 (*C*-8a), 85.45 (*C*-4′), 76.04 (*C*-1′), 72.87 (*C*-2′), 72.15 (*C*-3′), 62.94 (*C*-5′), 38.09 (N*C*H$_3$); *m*/*z* (FAB, NBA) 335 (MH$^+$ + Na, 22%), 313 (MH$^+$, 85), 286 (15), 223 (16), 176 (38), 165 (20), 152 (34), 133 (25), 120 (45), 90 (65), 77 (100), 63 (42), 51 (46) (Found: MH$^+$, 313.1275. $C_{11}H_{17}N_6O_5$ requires *M*H, 313.1260).

8-Chloro-6-dimethylamino-3-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*][1,2,4]triazine 19

Method A. A mixture of the protected *C*-nucleoside **17** (150 mg, 0.24 mmol), *N*,*N*-dimethylaniline (1 ml) and phosphorus trichloride oxide (4 ml) was heated to reflux for 40 min. After cooling of the mixture to room temperature, excess of solvent was removed *in vacuo* and ice–water (4 ml) was added to the residue. The aqueous solution was extracted with ethyl acetate (3 × 6 ml), and the combined organic layer was dried ($MgSO_4$), and concentrated *in vacuo* to give a dark residue, which was purified by flash chromatography on silica gel (gradient elution; 30–40% ether in light petroleum) to afford the *chloro compound* **19** (93 mg, 60%) as yellow crystals, mp 88–90 °C (from DCM–light petroleum); ν_{max}(NaCl/film)/cm^{-1} 1729 (C=O), 1596, 1574, 1505, 1486, 1452, 1417, 1316, 1270, 1123, 1096; δ_H(270 MHz; $CDCl_3$) 7.91–8.00 (6H, m, Ar *H*), 7.49–7.56 (3H, m, Ar *H*), 7.31–7.38 (6H, m, Ar *H*), 6.48 (1H, dd, *J* 5.67, 4.39, *H*-2′), 6.18 (1H, t, *J* 6.24, *H*-3′), 5.88 (1H, d, *J* 4.39, *H*-1′), 4.63–4.82 (3H, m, *H*-4′ and H_2-5′), 3.21 (6H, s, NCH_3); δ_C(76 MHz; $CDCl_3$) [166.19, 165.21(×2)] (*C*=O), [155.87, 152.64, 146.41, 139.16] (Het *C*), [133.53, 133.48, 133.13, 129.76(×2), 129.70, 129.46, 128.88, 128.47, 128.41(×2), 128.32] (Ar *C*), 79.57 (*C*-4′), 75.38 (*C*-3′), 72.70 (*C*-2′), 72.51 (*C*-1′), 63.78 (*C*-5′), 37.93 (N*C*H$_3$); *m*/*z* (FAB, NBA) 643 (MH$^+$, 20%), 434 (M$^+$ − 2PhCO, 5), 393 (6), 336 (4), 322 (22), 282 (4), 250 (6), 165 (11), 133 (21), 105 (100), 91 (28), 81 (34), 69 (46), 55 (70) (Found: C, 59.5; H, 3.95; N, 12.9. $C_{32}H_{27}ClN_6O_7$ requires C, 59.8; H, 4.2; N, 13.1%. Found: MH$^+$, 643.1738. $C_{32}H_{28}ClN_6O_7$ requires *M*H, 643.1708).

Method B. A mixture of the hydrazide **16** (100 mg, 0.16 mmol) and phosphorus trichloride oxide (4 ml) was heated to reflux for 40 min. After cooling the mixture to room temperature, excess of solvent was removed *in vacuo* and ice–water (4 ml) was added to the residue. The aqueous solution was extracted with ethyl acetate (3 × 4 ml), dried ($MgSO_4$), and concentrated *in vacuo* to give a dark residue, which was purified by flash chromatography on silica gel (gradient elution; 30–40% ether in light petroleum) to afford the chloro compound **19** (78 mg, 62%), identical to that described above.

6-Dimethylamino-8-hydrazino-3-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*][1,2,4]triazine 20

To a solution of the chloro compound **19** (80 mg, 0.13 mmol) in ethyl acetate (2 ml) was added hydrazine monohydrate (0.007 ml, 0.14 mmol) at 25 °C during 5 min. A yellow precipitate was collected, and washed with ethyl acetate. The crude product was crystallised from ethyl acetate–light petroleum to give the *hydrazine* **20** (70 mg, 88%) as pale yellow crystals, mp 78–79 °C (from ethyl acetate–light petroleum); ν_{max}(NaCl/film)/cm^{-1} 3320, 3217 (NH), 1725 (C=O), 1602, 1584, 1548, 1537, 1452, 1316, 1270, 1124, 1098; δ_H(270 MHz; $CDCl_3$) 7.92–8.00 (6H, m, Ar *H*), 7.80 (1H, d, *J* 1.62, N*H*), 7.31–7.57 (9H, m, Ar *H*), 6.51 (1H, dd, *J* 5.66, 3.93, *H*-2′), 6.27 (1H, dd, *J* 6.93, 5.77, *H*-3′), 5.85 (1H, d, *J* 3.93, *H*-1′), 4.71–4.80 (2H, m, *H*-4′ and *H*-5a′), 4.64–4.68 (1H, m, *H*-5b′), 3.18 (6H, s, NCH_3); δ_C(68 MHz; $CDCl_3$) [166.21, 165.24, 165.18] (*C*=O), [158.08, 154.80, 149.77, 145.65] (Het *C*), [133.39, 133.35, 133.00, 131.86, 129.75, 129.56, 129.07, 129.01, 128.68, 128.37, 128.34, 128.24] (Ar *C*), 79.24 (*C*-4′), 75.58 (*C*-3′), 72.97 (*C*-2′), 72.57 (*C*-1′), 63.98 (*C*-5′), 37.62 (N*C*H$_3$); *m*/*z* (FAB, NBA) 639 (MH$^+$, 100), 461 (3), 433 (3), 391 (3), 371 (5), 309 (6), 290 (13), 273 (9), 221 (6), 166 (13), 152 (20), 135 (18), 120 (27), 105 (77), 89 (42), 77 (51), 69 (24), 55 (31) (Found: MH$^+$, 639.2366. $C_{32}H_{31}N_8O_7$ requires *M*H, 639.2316).

6-Dimethylamino-3-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*][1,2,4]triazine 21

Method A. To a solution of the hydrazine **20** (60 mg, 0.09 mmol) in ethanol (4 ml) was added yellow mercury(II) oxide (61 mg, 0.28 mmol) at 25 °C. The reaction mixture was heated to reflux for 1 h, cooled, and the inorganic residue was removed (Celite pad), washed with ethanol, and the combined organic layer evaporated *in vacuo* to yield a pale yellow solid. Purification of the solid by flash chromatography on silica gel (gradient elution; 10–20% ether in light petroleum) afforded the *title compound* **21** (31 mg, 55%) as pale yellow crystals, mp 79–80 °C (from DCM–light petroleum); ν_{max}(NaCl/film)/cm^{-1} 1726 (C=O), 1601, 1565, 1122, 1097, 1071, 1026; δ_H(270 MHz; $CDCl_3$) 9.19 (1H, s, *H*-8), 7.93–8.02 (6H, m, Ar *H*), 7.50–7.56 (3H, m, Ar *H*), 7.34–7.40 (6H, m, Ar *H*), 6.48 (1H, dd, *J* 5.78, 4.39, *H*-2′), 6.21 (1H, t, *J* 6.24, *H*-3′), 5.90 (1H, d, *J* 4.16, *H*-1′), 4.66–4.80 (3H, m, *H*-4′ and H_2-5′), 3.24 (6H, s, NCH_3); δ_C(76 MHz; $CDCl_3$) [166.20, 165.18(×2)] (*C*=O), [156.84, 151.06 (*C*-8), 145.00, 140.09] (Het *C*), [133.48, 133.42, 133.08, 129.93, 129.76(×2), 129.52, 128.93, 128.40(×2), 128.30(×2)] (Ar *C*), 79.44 (*C*-4′), 75.24 (*C*-3′), 72.84 (*C*-2′), 72.52 (*C*-1′), 63.93 (*C*-5′), 37.82 (N*C*H$_3$); *m*/*z* (FAB, NBA) 609 (MH$^+$, 100%), 487 (M$^+$ − $PhCO_2$, 5), 311 (4), 286 (6), 259 (4), 243 (6), 193 (4), 167 (8), 150 (12), 133 (16), 120 (12), 105 (84), 89 (22), 77 (34), 43 (22) (Found: C, 63.3; H, 4.6; N, 13.5. $C_{32}H_{28}N_6O_7$ requires C, 63.15; H, 4.6; N, 13.8%. Found: MH$^+$, 609.2113. $C_{32}H_{29}N_6O_7$ requires *M*H, 609.2098).

Method B. To a solution of the chloro compound **19** (100 mg, 0.16 mmol) in dry ethyl acetate (6 ml) were added 5% palladium on carbon (17 mg) and magnesium oxide (16 mg, 0.41 mmol). The reaction mixture was stirred at room temperature under a hydrogen atmosphere *via* a balloon for 4 days. The inorganic material was removed by filtration through a pad of Celite and was washed thoroughly with ethyl acetate. The filtrate was concentrated *in vacuo* and the residue was purified by flash chromatography on silica gel (gradient elution; 10–20% ether in light petroleum) to afford the title compound **21** (91.5 mg, 96%), identical to that described above.

6-Dimethylamino-3-(β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*]-[1,2,4]triazine 4

Method A. A solution of the protected *C*-nucleoside **21** (90 mg, 0.15 mmol) in MeOH–NH_3 (5 ml, saturated at 0 °C) was stirred at room temperature for 2 days. Excess of solvent was removed *in vacuo* and the residue was purified by flash chromatography on silica gel (gradient elution; 10–15% MeOH in DCM) to give the *title compound* **4** (38 mg, 86%) as pale yellow crystals; mp 171–173 °C (from acetone); ν_{max}(NaCl/film)/cm^{-1} 3387, 3230 (OH), 1614, 1573, 1481, 1440, 1423, 1344, 1285, 1216, 1130, 1100, 1055, 1032; δ_H(270 MHz, DMSO-d_6 + D_2O) 9.35 (1H, s, *H*-8), 5.16 (1H, d, *J* 6.24, *H*-1′), 4.68 (1H, t, *J* 5.32, *H*-2′), 4.15 (1H, t, *J* 5.08, *H*-3′), 3.88–3.93 (1H, m, *H*-4′), 3.44–3.62 (2H, m, H_2-5′), 3.12 (6H, s, NCH_3); δ_H(270 MHz; D_2O) 9.32 (0.55H, s, *H*-8), 6.33 (0.45H, d, "*J*" 2.8, *H*-8), 5.42 (0.55H, d, *J* 6.2, *H*-1′), 5.23 (0.45H, dd, "*J*" 6.2 and 2.8, *H*-1′), 4.92 (0.55H, t, *J* 6.0, *H*-2′), 4.71 (0.45H, t, *J* 6.0, *H*-2′), 4.43 (0.55H, t, *J* 5.8, *H*-3′), 4.34 (0.45H, dt, "*J*" 5.8 and 9.4, *H*-3′), 4.17 (1H, m, *H*-4′), 3.68–3.92 (2H, m, H_2-5′), 3.23 (3.3H, s, NCH_3), 3.04 (2.7H, s, NCH_3); δ_C(76 MHz; DMSO-d_6) 156.90 (*C*-6), 152.52 (*C*-8), 147.11 (*C*-8a), 140.08 (*C*-3), 85.64 (*C*-4′), 74.87 (*C*-1′), 72.63 (*C*-2′), 71.91 (*C*-3′), 62.49 (*C*-5′), 37.74 (N*C*H$_3$); *m*/*z* (FAB, NBA) 319 (M + Na$^+$, 100%), 297 (MH$^+$, 53), 205 (31), 178 (9), 149 (24), 133 (21), 95 (34), 81 (59), 69 (100), 55 (95) (Found: C, 44.1; H, 5.25; N, 28.2. $C_{11}H_{16}N_6O_4$ requires C, 44.6; H, 5.4; N, 28.4%. Found: MH$^+$, 297.1330. $C_{11}H_{17}N_6O_4$ requires *M*H, 297.1311).

Method B. To a stirred solution of the protected *C*-nucleoside **21** (50 mg, 0.08 mmol) in dry MeOH (4 ml) was added sodium methoxide (14 mg, 0.26 mmol). The reaction mixture was stirred at room temperature for 2 h and was quenched with water (0.1 ml). Excess of solvent was removed *in vacuo* and the residue was purified by flash chromatography on silica gel (gradient elution; 10–15% MeOH in DCM) to give the *C*-nucleoside **4** (21.9 mg, 90%), which was identical to that described above.

6-Dimethylamino-3-(2′,3′,5′-tri-*O*-*p*-nitrobenzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*][1,2,4]triazine

To a stirred solution of the *C*-nucleoside **4** (10 mg, 0.03 mmol) in dry pyridine (2 ml) was added *p*-nitrobenzoyl chloride (37.6 mg, 0.20 mmol). The reaction mixture was stirred at room temperature for 2 h and excess of solvent was removed *in vacuo* to give a dark residue. The residue was dissolved in DCM, washed with brine, dried ($MgSO_4$), and concentrated *in vacuo* to give a crude product, which was purified by flash chromatography on silica gel (gradient elution; 0–5% ethyl acetate in ether) to furnish the *tris-p-nitrobenzoate* (23.3 mg, 93%) as yellow crystals, mp 114–115 °C (from DCM–hexane); ν_{max}(NaCl/film)/cm^{-1} 1734 (C=O), 1602, 1528, 1349, 1270; δ_H(270 MHz; $CDCl_3$) 9.21 (1H, s, *H*-8), 8.09–8.31 (12H, m, Ar *H*), 6.53 (1H, t, *J* 5.09, *H*-2′), 6.31 (1H, t, *J* 5.54, *H*-3′), 5.95 (1H, d, *J* 4.16, *H*-1′), 4.63–4.88 (3H, m, *H*-4′ and H_2-5′), 3.26 (6H, s, NCH_3); δ_C(68 MHz; $CDCl_3$) [164.44, 163.42(×2)] (*C*=O), [157.04, 151.21, 151.00, 150.76] (Het *C*), [144.71, 140.14, 134.71, 133.91, 131.04, 130.86(×2), 130.83, 123.84, 123.79(×2), 123.62] (Ar *C*), 79.45 (*C*-4′), 74.12 (*C*-3′), 73.99 (*C*-2′), 73.11 (*C*-1′), 64.25 (*C*-5′), 37.84 (N*C*H$_3$); *m*/*z* (FAB, NBA) 744 (MH$^+$, 8%), 669 (5), 515 (10), 485 (4), 461 (8), 369 (2), 273 (2), 135 (12), 109 (24), 91 (34), 69 (62), 55 (100) (Found: MH$^+$, 744.1684. $C_{32}H_{26}N_9O_{13}$ requires M*H*, 744.1650).

Acknowledgements

We thank AgrEvo UK Limited for a research studentship (to V.-D. Le), Ms T. Monk for performing the ADA assay, and the Wolfson Foundation for establishing the Wolfson Centre for Organic Chemistry in Medical Science at Imperial College.

References

1 P. J. Dudfield, V.-D. Le, S. D. Lindell and C. W. Rees, *J. Chem. Soc., Perkin Trans. 1*, 1999, 2929.
2 C. G. Earnshaw, AgrEvo UK Ltd, unpublished results. Further details of our calculations can be found in V.-D. Le, Ph.D. Thesis, University of London, 1997, p. 26 and in S. D. Lindell, B. A. Moloney, B. D. Hewitt, C. G. Earnshaw, P. J. Dudfield and J. E. Dancer, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 1985. See also M. D. Erion and M. R. Reddy, *J. Am. Chem. Soc.*, 1998, **120**, 3295.
3 H. K. Han, J. C. Lee, Y. H. Kang, J.-H. Kim and D. Y. Chi, *Synth. Commun.*, 1992, **22**, 2815.
4 L. Kalvoda, *J. Carbohydr. Nucleosides, Nucleotides*, 1976, **3**, 47.
5 W. W. Paudler and T.-K. Chen, *J. Heterocycl. Chem.*, 1970, **7**, 767; H. Neunhoeffer and B. Lehmann, *Chem. Ber.*, 1976, **109**, 1113.
6 B. T. Keen, R. J. Radel and W. W. Paudler, *J. Org. Chem.*, 1977, **42**, 3498; R. J. Radel, J. L. Atwood and W. W. Paudler, *J. Org. Chem.*, 1978, **43**, 2514.
7 C. A. Lovelette, *J. Heterocycl. Chem.*, 1979, **16**, 555.
8 Y. Kang, S. B. Larson, R. K. Robins and G. R. Revankar, *J. Med. Chem.*, 1989, **32**, 1547.
9 L. J. S. Knutsen, B. D. Judkins, W. L. Mitchell, R. F. Newton and D. I. C. Scopes, *J. Chem. Soc., Perkin Trans. 1*, 1984, 229.
10 K. Ramasamy, B. G. Ugarkar, P. A. McKernan, R. K. Robins and G. R. Revankar, *J. Med. Chem.*, 1986, **29**, 2231.
11 P. D. Cook and D. J. McNamara, *J. Heterocycl. Chem.*, 1986, **23**, 155.

Paper 9/05177E