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Synthesis and stability of exocyclic triazine nucleosides

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Synthesis and stability studies of exocyclic amino triazine nucleosides were performed. Stability of the nucleosides was found to be dependent on triazine ring electron density, solvent, and pH. The nucleosides were found to be more stable when the triazine ring was electron deficient, in high pH aqueous solutions and in aprotic solvents.

Introduction

Exocyclic amino nucleosides (EANs) represent a class of biologically relevant nucleobases for which little stability and physical data are available (Fig. 1).[1,2] They result from oxidative damage to natural DNA, in the form of formamidopyrimidines that have been implicated in mutagenesis.[2] Secondary fungal metabolites, such as clitocine, fit the EAN motif and act as adenosine mimics that display anti-leukemic and insecticidal activities (Fig. 1).[1–3] Synthetic EANs like 4-substituted 8-(D-ribofuranosylamino) pyrimido[5,4-*d*]pyrimidines show antitumor and antiviral properties.[4] These selected examples would indicate that electron deficient heterocycles aid in forming stable EAN systems. s-Triazine represents such an electron deficient heterocycle, the synthesis and structure of which will follow.[5]

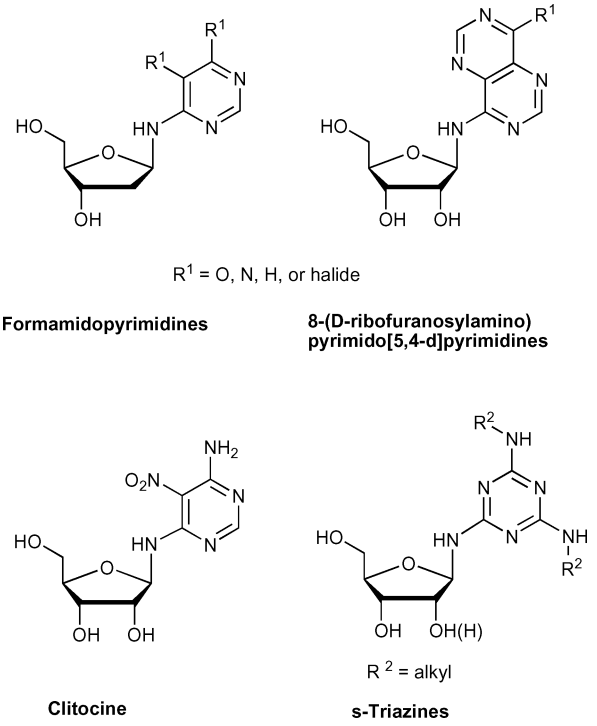

Fig. 1 Exocyclic amino nucleosides.

Another question which arises in the study of isosteric non-natural bases is to what extent they may have played a role in molecular evolution. Hydrogen cyanide, a likely component in a primordial soup, is known to form s-triazines.[6,7] Substituted triazines have been formed under conditions designed to simulate the prebiotic world. Melamine, ammelide, and cyanuric acid or precursors that hydrolyze to such triazines were found in the Murchison meteorite[6,8] (Fig. 2(a)). Given their possible presence at an early stage of the evolution of biomolecules, is it plausible that these triazine heterocycles could have contributed to the basis for an early coding alphabet?

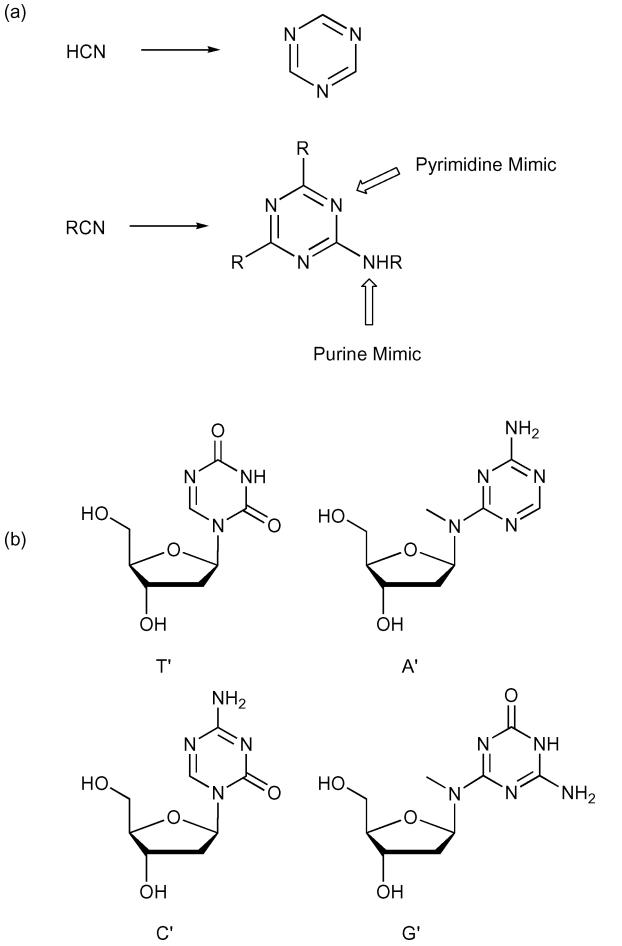

Fig. 2 Triazine based nucleosides. (a) Trimerization of hydrogen cyanide to give triazine derivatives. (b) Nucleosides based on a triazine core.

Functionalized triazines, derivatized through the ring nitrogens, mimic pyrimidine like C′ and T′ and as EANs they could mimic purines like A′ and G′ (Fig. 2(b)). Indeed, aminotriazines and their hydrolysis products could offer isosteres of both modern base families. Thus, in contrast to present day DNA, which requires pyrimidine and purine heterocyclic cores for a complete code, a triazine code would require only one. Whether this is an advantage, remains a philosophical issue.[9]

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Possible complications with EANs in aqueous media include epimerization, ring expansion, and hydrolysis.[10] The electron deficient triazines could conceivably decrease the incidence of isomerization.[11] Synthesis and study of the physical properties of exocyclic amino triazine nucleotides (EATNs) should provide insight into this potential prebiotic DNA coding system.[12,13] In aqueous solutions, studies on the isomerization and hydrolysis of EATNs show the dependence of such reactions on the solvent, pH, and the electron density of the heterocyclic base. From these studies, a general picture for what makes a stable EATN system thus develops.

Results and discussion

Synthesis of the desired EATNs began by selective TIPS protection of the primary hydroxyl in 2-deoxy-D-ribose to give aldehyde **1** in 56% yield (Scheme 1). Condensation of **1** with methylamine and subsequent nucleophilic aromatic substitution with cyanuric chloride afforded triazine nucleoside **2** in 58% yield as a mixture of anomers (1 : 1, α : β). The anomers were separated by chromatography and the anomer **2**-β was identified by an observed NOE between H-1′ and H-4′. From **2**-β, a diverse set of compounds can be prepared through nucleophilic aromatic substitutions.

Dichlorotriazine **2**-β was treated with a saturated solution of NH_3 in tetrahydrofuran to give **3** as a mixture of equilibrating rotamers in 83% yield.[14,15] TBAF deprotection of **3** gave the desired electron poor derivative **4** in 79% yield. Further nucleophilic aromatic substitution on **3** using NaOMe in refluxing MeOH afforded **5** in 93% yield;[16] deprotection of **5** with TBAF gave **6a** in 66% yield. The electron rich adenosine mimic, **8**, was synthesized directly from **2** by double S_NAr with methylamine and subsequent TBAF deprotection (overall yield for 2 steps 86%). A crystal structure of **8** reaffirms the assignment of β to the anomeric position in the starting material **2** (Fig. 3).

A comparison of the crystal structures of 2′-deoxyadenosine and **8** showed that the two compounds are structurally similar. Each contains the base in the *anti* conformation. The heterocyclic moieties overlap well, maintaining the desired distances between the furanose ring and the hydrogen bonding faces allowing for Watson–Crick base pairing. The glycosidic nitrogen in **8** is sp^2 hybridized as indicated by bond angles (Fig. 3). It follows that the lone pair is fully conjugated with the triazine ring system. Additional support is seen with a short C5–N1 bond distance of 1.37 Å and torsional angles of 1° and 12° (Fig. 3).

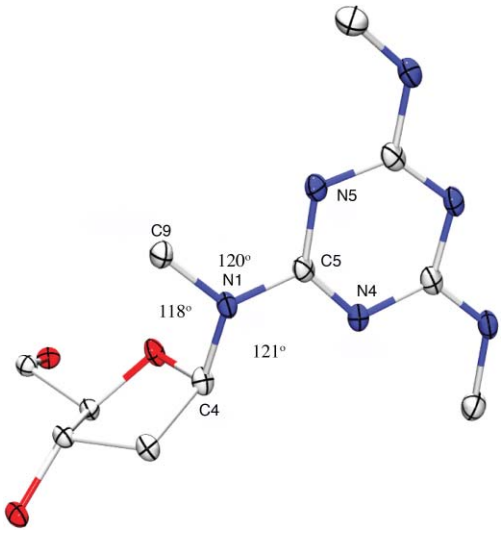
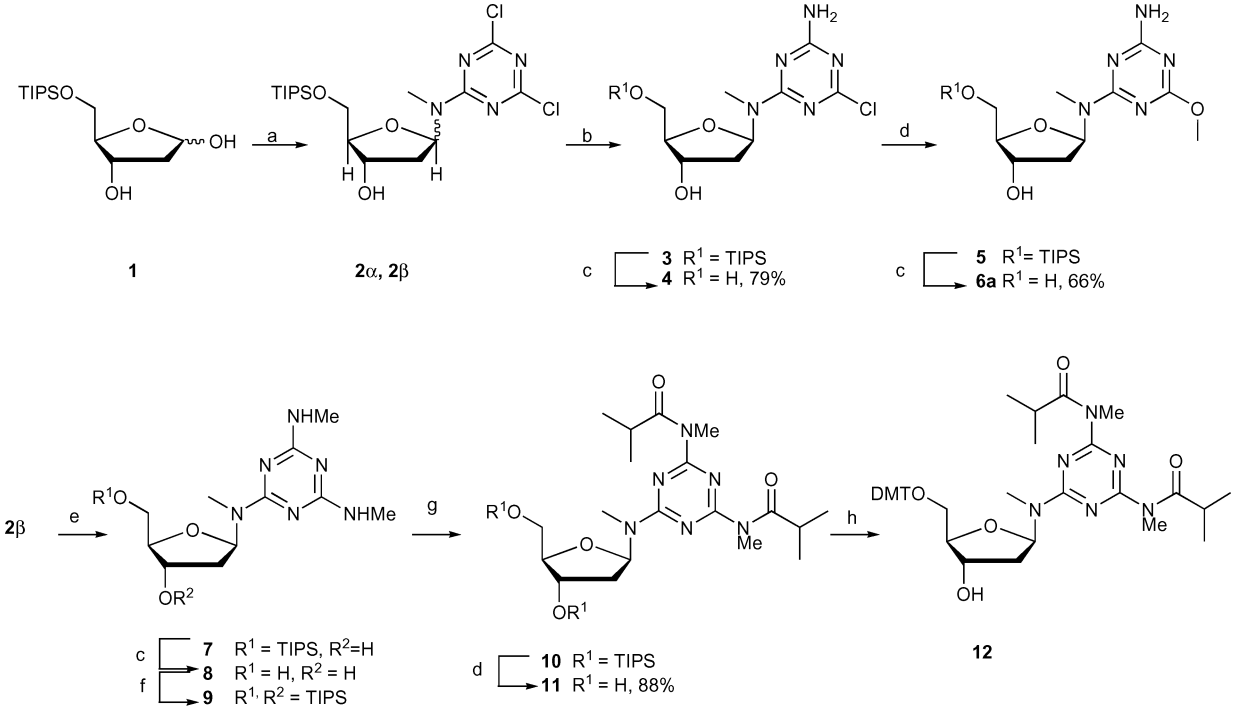


Fig. 3 Crystal structure of **8**. Carbon, oxygen and nitrogen are shown in white, red and blue, respectively. Hydrogens were omitted for clarity. Torsional angles C9–N1–C5–N4 and C4–N1–C5–N5 were found to be 1° and 12° respectively.

Although structural similarity between 2′-deoxyadenosine and **8** is important, its accommodation within a DNA duplex is ultimately the key test. To incorporate nucleoside **8** into DNA using standard solid phase oligonucleotide synthesis, the protected precursor **12** was required (Scheme 1). TIPS protection of **8** using TIPSTf in dichloromethane gave compound **9**, which



(a) NH_2CH_3 in MeOH; DIPEA, THF, -78 °C to -20 °C, cyanuric chloride, 58 % (b) NH_3, THF, 83% (c) TBAF, THF (d) NaOMe, MeOH, 93% (e) NH_2CH_3 in THF, 86% (f) TIPSTf, DIPEA, 100% (g) isobutyryl chloride, pyr, 60 °C, 63% (h) DMTrCl, CH_2Cl_2, Et_3N, 91%

Scheme 1 Synthesis of triazine nucleosides.

was then protected using isobutyryl chloride in pyridine at 60 °C to give **10** in 70% yield. TBAF deprotection and subsequent DMT protection of the primary hydroxyl gave compound **12** in 80% yield (2 steps).

Nucleoside incorporation into DNA using standard protocols requires the protected nucleoside be stable to oxidative, basic, and acidic conditions. Compound **11** was stable to the oxidative I_2–pyridine conditions. However when **11** was briefly treated with dichloroacetic acid in dichloromethane, simulating the DMT deprotection protocol, significant degradation took place. Although this acid instability is incompatible with standard oligonucleotide synthesis protocols, there are alternative acid-free routes for the synthesis of oligonucleotides.

Stability to aqueous environments is essential for testing duplex stability and designing EATNs that structurally mimic purines. Compound **11** was dissolved in D_2O–THF-d_8 and found to be stable. The stability of **11**, however, differed greatly from that of the electron rich deprotected form **8**, which was found to decompose rapidly in water within 15 min, presumably through the isomerization and hydrolysis path seen in the decomposition of formamidopyrimidines.[10] **6a** was then examined, as the triazine moiety is intermediate in electron density, which provides a slower, easier to study degradation. The ^{1}H NMR of **6a** after 30 h in D_2O showed significant degradation (Fig. 4(d)). A tentative mechanism for isomerization and hydrolysis of exocyclic amino triazine **6a** is shown in Fig. 5.

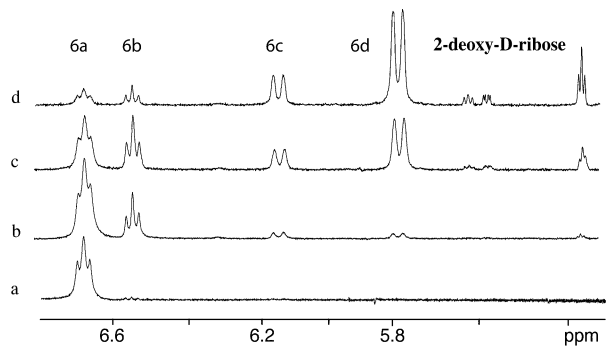


Fig. 4 Isomerization of **6a** in D_2O, pH = 6 followed by ^{1}H NMR of the anomeric protons at (a) $t = 0$, (b) $t = 1$ h, (c) $t = 9$ h, (d) $t = 30$ h.

To confirm that isomerization and hydrolysis were in fact the occurring degradation pathways, pyranose **6c** was independently synthesized (Scheme 2). Condensation of 2-deoxy-D-ribose with ammonia and subsequent nucleophilic aromatic substitution with cyanuric chloride afforded triazine nucleoside **14** which was treated with a saturated solution of NH_3 in acetonitrile to give **15** in 79% yield. Further nucleophilic aromatic substitution on **15** using NaOMe in refluxing MeOH afforded **6c** in 45% yield.

All protons and carbons of **6a** and **6c** were assigned using COSY, DEPT, and HMQC data. The relative ^{13}C NMR chemical shift of C-4′ distinguishes the difference between a pyranose and furanose. As expected, the crowded C-4′ in the furanose was shifted 16 ppm downfield from that in the pyranose ring. In addition, the large coupling constants seen in the ^{1}H NMR of pyranose **6c** are consistent with a six-membered ring and facilitated the assignment of **6c** as the β isomer.[17] The compound synthesized and confirmed to be **6c** was found to be identical to the isomerization product of **6a** seen in the ^{1}H NMR.

With the rearrangement established, the effect of pH on the triazine nucleosides was investigated. As suggested in Fig. 5, tetrahydrofuran protonation is necessary for isomerization. Proton NMR was used to study the stability of **6a** at pH 6, 7, 8, and 11. Epimerization of **6a** to **6b** occurred rapidly relative to isomerization as can be seen from the earlier appearance of **6b** relative to 2-deoxy-D-ribose and isomerization products **6c** and **6d** (Fig. 4). The pyranose forms **6c** and **6d** are the thermodynamically favored product as is the case with the formamidopyrimidines. A complete kinetic description of the decomposition is complex due to the existence of several competing reaction pathways: epimerization, ring expansion and hydrolysis (Fig. 4); however, the half-lives can be estimated by observing the decomposition to the point at which 50% of the starting material was left. Compound **6a** was found to have a half-live of 10 h, 5 d, and $\gg$10 d at pH 6, 7, and 8 respectively. As expected from the proposed mechanism, the rate of isomerization was found to increase at lower pH. In contrast to previously studied formamidopyrimidines, which have been shown to isomerize under basic conditions, **6a** was found to be completely stable at pH = 11. Unfortunately, **8** degraded within 15 min at pH 11. Thus, although pH can be used to control isomerization in some systems, it is ineffective with electron rich derivatives.

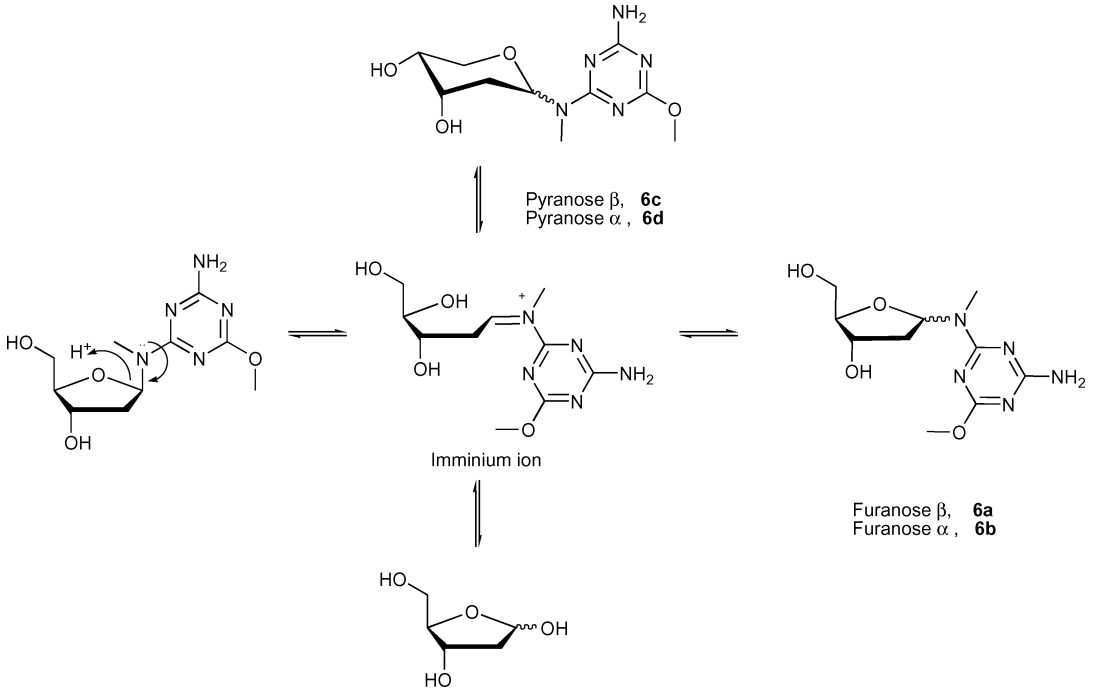
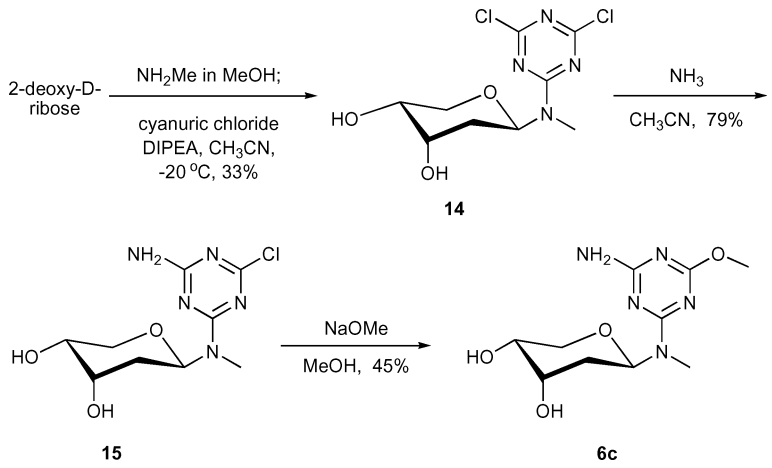


Fig. 5 Proposed mechanism of epimerization.



Scheme 2 Synthesis of pyranose **6c**.

To assess solvent effects on stability, **6a** was studied in water, methanol, DMSO, acetonitrile, and acetone. Significant isomerization occurred in water as discussed above. Isomerization was much slower in methanol (5% in 5 days). In the aprotic solvents DMSO, acetonitrile, and acetone, no isomerization was seen; thus, protic solvents seem to facilitate isomerization of **6a**. With the electron rich compound **8**, however, isomerization was also seen in aprotic polar solvents. Degradation of **7** is less pronounced suggesting that when the 5′ hydroxyl is present, an intramolecular proton transfer might catalyze the decomposition process.

In contrast to **8**, the isostructural diaminopurine is stable to aqueous conditions. Comparison of the structures showed that they are similar which implies that an electronic effect must be at the heart of the degradation path. For the molecule to degrade (Fig. 5), a Schiff base intermediate must form requiring a nucleophilic nitrogen. Whereas the nitrogen atom in position 7 of adenosine is sp^2 hybridized and part of a cyclic conjugated pi system, the exocyclic amine in **8** has a lone pair that is interacting to a greater or lesser extent with the heterocycle. In **8**, the presence of the two electron donating methyl amino substituents on the triazine makes the lone pair relatively free and available to form a Schiff base intermediate.

To maintain structural integrity, it is important to know the optimal electron density in the aromatic ring needed to prevent isomerization. Compounds **8**, **6a** and **4**, varying in electron density, were investigated. Both **8** and **6a** isomerize in water, although electron rich **8** isomerizes more than a 100 times faster than **6a** at neutral pH. Only slight isomerization was seen with the most electron poor nucleoside **4**, which was found to epimerize less than 10% in 7 d. One can imagine that the EATN from diamino triazine would have ample stability at neutral pH to survive for days in solution.

Conclusions

In conclusion, we have found that isomerization and hydrolysis are important degradation pathways for exocyclic amino triazine nucleosides, which has significant implications on the design and synthesis of future EATNs. Clearly, the EATNs, which contain electron rich aromatic rings can be expected to isomerize and hydrolyze over a wide range of pHs and in a variety of solvents resulting in conversion to the pyranose or 2-deoxy-D-ribose. In contrast, systems with intermediate electron density isomerize to the pyranose form only in protic solvents and can be maintained in aqueous conditions at high pH. Only the electron poor systems remain structurally intact at physiological pHs. This has implications on the homogeneity of libraries based on EATNs. For example, the 1152 triazine library synthesized at Ribafarm Inc., spans a wide range of electron richness in the rings and is therefore likely to present a range of structure types with varying degrees of stability, which may or may not parallel clitocine, the compound they seek to mimic. Nevertheless, they may have interesting biological activity, even if a direct structure activity relationship between the triazine library and clitocine is not indicated. Our results establish a clear caveat for the assumption of EAN structure and stability based on a few electron deficient model compounds. Indeed, all EANs synthesized should be carefully analyzed to confirm their structural identity until a better empirical base is in place.

The stability of these EATNs does not preclude the possibility for a prebiotic code containing EANs that mimic purines. The isomerization of the exocylic amino nucleosides could provide for a library of pyranoses and furanoses. Rapid hydrolysis remains an issue. To circumvent the isomerization and hydrolysis, the exocyclic amino nucleoside need only contain a relatively less nucleophilic exocyclic amine. Reducing the number of donor groups on the ring is one way to achieve this effect. In that regard, the more direct adenosine analog, the EATN from diamino triazine, should have ample stability at neutral pH to survive days in solution. The nucleophilicity also could be tuned through the use of an electron withdrawing group directly attached to the glycoside nitrogen. Alternatively, an electron deficient heterocycle, as was seen with **4**, could be used to control the isomerization event. From a completely different perspective, the triazine could have been part of a coding system containing an alternative backbone where none of the problems inherent to the glycoside bond would exist. In any event, these molecules provide a fruitful area of discussion, rich in research results.

Experimental

^{1}H- and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer. High resolution mass spectra were obtained by the Scripps Research Institute using either MALDI or ESI. All reactions were performed in oven-dried round bottom flasks under argon. Anhydrous pyridine and methanol were purchased from Fisher Scientific. Anhydrous tetrahydrofuran and dichloroethane were passed through a column of activated alumina. Commercial chemicals were used as supplied from Aldrich or Acros chemicals. Thin layer chromatography was performed using alumina glass-backed plates coated with 0.25 mm F254 silica gel. TLC plates were visualized using either UV light or charring after staining with CAM. For NMR experiments, buffered D_2O solutions were made using 0.1 mM phosphate buffers. The pH = 11 D_2O solution was made using K_2CO_3. All ^{1}H NMR experiments were performed using a 400 Varian MHz NMR with a delay time of 5 s. The following abbreviations were used: DIPEA, diisopropylamine; DMTrCl, 4,4′-dimethoxytritylchloride; DMAP, 4-(dimethylamino)pyridine;

pyr, pyridine; TBAF, tetrabutylammonium fluoride; TIPSTf, triisopropylsilyltriflate.

5-*O*-Triisopropylsilyl-2-deoxy-D-ribose 1

To a solution of 2-deoxy-D-ribose (25.0 g, 0.186 mol) in anhydrous pyridine (300 mL) was added triisopropylsilyl chloride (43.9 mL, 0.205 mol). The reaction was stirred at room temperature for 4 days. The solution was concentrated under reduced pressure and partitioned between ethyl acetate (600 mL) and water (200 mL). The organic layer was separated then washed with 0.05 M $HCl_{(aq)}$, $NaHCO_{3(aq)}$, and brine. The solution was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash column chromatography (33% to 50% hexanes in ethyl acetate) to afford the desired oil (30.5 g of α,β, 56%). IR film (υ_{max}/cm^{-1}) 3389, 2943, 2866, 1461; major isomer δ_H(400 MHz; $CDCl_3$; $CHCl_3$) 1.35–1.75 (21H, m), 1.87–2.30 (2H, m), 2.97 (1H, s, NMe), 3.55–3.82 (2H, m, H-5′), 3.90–3.99 (1H, m, H-4′), 4.40 (1H, br s, H-3′), 5.55 (1H, s, H-1′); δ_C(100 MHz; $CDCl_3$; $CHCl_3$) 11.8, 17.9, 41.4, 64.0, 73.4, 87.9, 99.4; HRMS (MALDI-FTMS) *m*/*z* calcd for $C_{14}H_{30}NaO_4Si$ $[M + Na]^+$ 313.1805, found 313.1807; R_f (50% hexanes in ethyl acetate) = 0.5.

4,6-Dichloro-N2-methyl-N2-(5′-*O*-triisopropylsilyl-2′-deoxy-α,β-D-erythro-pentofuranosyl)-[1,3,5]triazin-2-yl-amine, 2α and 2β

5′-*O*-Triisopropylsilyl-deoxy-D-ribose (25.8 g, 89 mmol) was dissolved in 2.0 M methylamine in methanol (800 mL) and stirred at room temperature for 16 h. The solution was concentrated under reduced pressure and residual methanol was removed by adding 3 × 150 mL of tetrahydrofuran and concentrating. The resulting oil was dissolved in tetrahydrofuran (800 mL) and cooled to −78 °C. To the solution was added DIPEA (17.1 mL, 98 mmol) and cyanuric chloride (12.63 g, 68.5 mmol). The reaction was stirred at −20 °C for 14 h. The tetrahydrofuran was removed under reduced pressure and the residue was partitioned between ethyl acetate and brine. The organic layer was separated and dried with Na_2SO_4, concentrated, and purified *via* flash column chromatography (20% hexanes in ethyl acetate) to afford an oil (23.3 g, 1 : 1 α : β, 58%). Ratio of anomers was determined by ^{1}H NMR. Separation of the epimers is accomplished using column chromatography (20% hexanes in ethyl acetate) resulting in pure fractions containing pure α or β and some mixed fractions of the two.

2β (R_f = 75% hexanes in ethyl acetate = 0.5). IR film (υ_{max}/cm^{-1}) 3414, 2943, 2867, 1612, 1565, 1484, 1417; δ_H(400 MHz; $CDCl_3$; $CHCl_3$) 1.04–1.08 (21H, m), 2.14 (1H, ddd, *J* 13.2, 6.6 and 3.8, H2′), 2.20–2.27 (1H, m, H2′), 3.10 (3H, s), 3.82 (1H, dd, *J* 10.4 and 4.8, H5′), 3.87–3.90 (1H, m, H5′), 3.95 (1H, dd, *J* 10.4 and 2.8, H4′), 4.50–4.53 (1H, m, H3′), 6.70 (1H, t, *J* 6.8, H1′); δ_C(100 MHz; $CDCl_3$; $CHCl_3$) 11.7, 17.8, 28.4, 37.2, 84.7, 85.7, 164.9, 169.4, 170.3; HRMS (MALDI-FTMS) *m*/*z* calcd for $C_{18}H_{32}Cl_2NaN_4O_3Si$ $[M + Na]^+$ 473.1513, found 473.1525. **2**α (R_f = 75% hexanes in ethyl acetate = 0.4). IR film (υ_{max}/cm^{-1}) 3423, 2951, 2864, 1571, 1475, 1414; δ_H(300 MHz; $CDCl_3$; $CHCl_3$) 1.04–1.08 (21H, m), 1.90 (1H, dt, *J* 13.5, 5.7), 2.62 (1H, dt, *J* 13.8, 6.9), 3.15 (3H, s), 3.71 (1H, dd, *J* 10.5 and 4.5), 3.79 (1H, dd, *J* 10.8 and 3.3), 4.08–4.12 (1H, m), 4.43–4.51 (1H, m), 6.54 (1H, dd, *J* 7.2 and 6.8); δ_C(100 MHz; $CDCl_3$; $CHCl_3$) 11.8, 17.9, 29.1, 38.7, 64.9, 72.3, 86.6, 87.0, 164.1, 169.1, 169.9; HRMS (MALDI-FTMS) *m*/*z* calcd for $C_{18}H_{33}Cl_2N_4O_3Si$ $[M + H]^+$ 451.1693, found 451.1704.

6-Chloro-N2-methyl-N2-(5′-*O*-triisopropylsilyl-2′-deoxy-β-D-erythro-pentofuranosyl)-[1,3,5]triazin-2,4-diamine 3

A solution of **2**β (1.90 g, 4.21 mmol) and DIPEA (0.808 mL, 4.63 mmol) in tetrahydrofuran (40 mL) was saturated with $NH_{3(g)}$ at 0 °C. The reaction was stirred at room temperature overnight, concentrated under vacuum and purified by flash column chromatography (66% ethyl acetate–hexanes) to afford **3** as a mixture of rotamers (1.50 g, 83%). R_f (50% hexanes in ethyl acetate) = 0.3; IR film (υ_{max}/cm^{-1}) 3327, 3222, 2943, 2873, 1641, 1571, 1501, 1492; δ_H(400 MHz; $CDCl_3$; $CHCl_3$) 1.04–1.09 (42H, m) 1.82–1.86 (2H, m), 2.16–2.23 (2H, m), 2.96 (3H, s), 3.02 (3H, s), 3.71–3.91 (6H, m), 4.48 (2H, s), 5.91 (4H, br s), 6.65–6.72 (2H, m); δ_C(100 MHz; $CDCl_3$; $CHCl_3$) 11.8, 17.9, 25.5, 27.9, 36.5, 36.8, 64.2, 67.8, 72.8, 77.2, 84.4, 85.2, 165.8, 166.4, 166.8, 169.1, 169.6; HRMS (MALDI-FTMS) *m*/*z* calcd for $C_{18}H_{35}ClN_5O_3Si$ $[M + H]^+$ 432.2192, found 432.2201.

6-Chloro-N2-methyl-N2-(2′-deoxy-β-D-erythro-pentofuranosyl)-[1,3,5]triazine-2,4-diamine 4

To a solution of **3** (512 mg, 1.18 mmol) in tetrahydrofuran (1 mL) was added 1.0 M TBAF in tetrahydrofuran (1.19 mL, 1.19 mmol). After 15 min the solution was concentrated and the crude product was purified by flash chromatography (25% acetonitrile in ethyl acetate) to afford a white solid (256 mg, 79%). δ_H(400 MHz; D_2O) 2.32 (1H, dt, *J* 14.0 and 7.0), 2.94 (3H, s), 3.75 (1H, dd, *J* 12.0 and 4.0), 3.85 (1H, dt, *J* 8.4 and 4.6), 4.36 (1H, dt, *J* 6.3 and 3.5), 6.57 (1H, br s); δ_C(100 MHz; D_2O) 28.3, 35.6, 61.8, 71.1, 84.9, 85.1, 165.3, 166.4, 166.5, 167.6, 167.7; (MALDI-FTMS) *m*/*z* calcd for $C_9H_{15}ClN_5O_4$ $[M + H]^+$ 276.0863, found 276.0863.

4-Chloro-6-methoxy-N2-methyl-N2-(5′-*O*-triisopropylsilyl-2′-deoxy-β-D-erythro-pentofuranosyl)-[1,3,5]triazin-2-yl-amine 5

A solution of **3** (1.20 g, 2.77 mmol) and NaOMe (643 mg, 11.1 mmol) in methanol (20 mL) was refluxed for 14 h. The solution was concentrated under vacuum and the crude product was purified *via* column chromatography (66% ethyl acetate in hexanes) to afford a solid (911 mg, 77%). IR film (υ_{max}/cm^{-1}) 3414, 2943, 2867, 1612, 1565, 1484, 1417; δ_H(400 MHz; $CDCl_3$; $CHCl_3$) 1.00–1.20 (21H, m), 2.06–2.08 (1H, m), 2.16–2.23 (1H, m), 2.99 (3H, s), 3.69–3.77 (3H, m), 3.91–3.94 (1H, m), 4.46 (1H, m), 5.38 (2H, br s), 6.77 (1H, br s, *J* 6.8); δ_C(100 MHz; $CDCl_3$; $CHCl_3$) 11.9, 18.0, 27.7, 36.5, 54.0, 64.3, 73.1, 84.2, 167.0, 167,6, 171.0; HRMS (MALDI-FTMS) *m*/*z* calcd for $C_{19}H_{38}N_5O_4Si$ $[M + H]^+$ 428.2687, found 428.2686.

4-Amino-6-methoxy-N2-methyl-N2-(2′-deoxy-β-D-erythro-pentofuranosyl)-[1,3,5]triazin-2-yl-amine 6a

To a solution of **5** (911 mg, 2.13 mmol) in tetrahydrofuran (2 mL) was added 1.0 M TBAF in tetrahydrofuran (2.10 mL, 2.10 mmol). After 15 min the solution was concentrated and purified *via* flash chromatography (50% acetonitrile in ethyl acetate) to afford a white solid (380 mg, 66%). UV $\lambda_{max}(H_2O)$/nm 214; δ_H(400 MHz; $CDCl_3$; $CHCl_3$) 2.09 (1H, ddd, *J* 14.0, 6.6 and 3.4, H2′), 2.35 (1H, dt, *J* 14.0 and 7.4, H2′), 2.97 (3H, s, NMe), 3.70 (1H, dd, *J* 12.2 and 5.4, H4′), 3.77 (1H, dd, *J* 12.2 and 4.2, H5′), 3.87–3.88 (1H, m, H4′), 3.90 (3H, s, OMe), 4.39 (1H, dt, *J* 6.9 and 3.4, H3′), 6.70 (1H, t, *J* 6.8, H1′); δ_C(100 MHz; $CDCl_3$; $CHCl_3$) 25.6 (NMe), 33.0 (C2′), 52.2 (OMe), 59.6 (C5′), 68.9 (C3′), 82.4 (C4′), 82.8 (C1′), 164.7, 165.6, 168.6; HRMS (MALDI-FTMS) *m*/*z* calcd for $C_{10}H_{18}N_5O_4$ $[M + H]^+$ 272.1353, found 272.1354; R_f (20% methanol in chloroform) = 0.1.

N4,N6-dimethyl-N2-methyl-N2-(5′-*O*-triisopropylsilyl-2′-deoxy-β-D-erythro-pentofuranosyl)-[1,3,5]triazine-2,4,6-triamine 7

To a solution of **2**β (4.16 g, 9.21 mmol) in tetrahydrofuran (18 mL) was added 2.0 M methylamine in tetrahydrofuran (55 mL). The solution was heated to 60 °C in a pressure tube for 4 h. The solution was concentrated under reduced pressure and the crude product was purified using flash column chromatography (33% hexanes in ethyl acetate) to give an oil (3.48 g, 86%). IR (υ_{max}/cm^{-1}) 3336, 3231, 2943, 2864, 1650, 1580, 1545; δ_H(400 MHz; $CDCl_3$; $CHCl_3$) 1.04–1.10 (21H, m), 2.08 (1H, m), 2.35 (1H, p_{app}, *J* 6.8 Hz), 2.91 (6H, br s), 2.98

(3H, s), 3.70–3.74 (1H, m), 3.77–3.80 (1H, m), 3.98 (1H, dd, *J* 9.6 and 3.2), 4.38 (1H, dt, *J* 6.4 and 4.8), 4.84 (2H, br s), 6.88 (1H, br s); δ_C(100 MHz; $CDCl_3$; $CHCl_3$) 12.0, 18.0, 27.4, 27.6, 36.4, 64.7, 73.6, 83.6, 84.0, 85.1, 165.6, 166.2; HRMS (MALDI-FTMS) *m/z* calcd for $C_{20}H_{40}N_6O_3Si$ $[M + H]^+$ 441.3004, found 441.3016.

N4,N6-dimethyl-N2-methyl-N2-(2′-deoxy-β-D-erythro-pentofuranosyl)-[1,3,5]triazine-2,4,6-triamine 8

To a solution of **7** (200 mg, 0.34 mmol) in tetrahydrofuran (0.5 mL) was added 1.0 M TBAF in tetrahydrofuran (0.369 mL, 0.369 mmol). The solution was stirred for 15 min at room temp, concentrated, and purified crude *via* flash chromatography (100% ethyl acetate) using basic alumina to afford **8** (71 mg, 76%). ^{1}H NMR shows some epimerization. Crystals were grown by slow evaporation of a solution in 5% methanol in chloroform. The crystal was dissolved in DMSO-d_6 right before the NMR was taken. δ_H(400 MHz; DMSO-d_6) 1.62–1.75 (1H, m), 1.84–2.18 (1H, m), 2.70 (6H, br s), 2.75–2.85 (3H, br s), 3.40 (1H, br s), 3.52 (1H, br s), 4.05–4.12 (1H, m), 4.71 (1H, t, *J* 7.4), 5.04 (1H, d, *J* 3.6), 5.74 (1H, s), 6.55 (1H, br s), 6.90 (2H, br s); HRMS (MALDI-FTMS) *m/z* calcd for $C_{11}H_{21}N_6O_3$ $[M + H]^+$ 285.1670, found 285.1667. Rapid degradation in solution prevented further characterization.

Crystal data. $C_{11}H_{22}N_6O_4$, $M = 302.35$, orthorhombic, $a = 12.9893(12)$, $b = 22.218(2)$, $c = 5.2165(5)$ Å, $U = 1505.5(2)$ Å^3, $T = 100$ K, space group $P2_12_12_1$, $Z = 4$, $\mu = 0.103$ mm^{-1}, 12910 reflections measured, 3420 unique ($R_{int} = 0.034$) which were used in all calculations. The final wR (F^2) was 0.136 [$I > 2\sigma(I)$].†

N4,N6-dimethyl-N2-methyl-N2-(3′,5′-di-*O*-triisopropylsilyl-2′-deoxy-β-D-erythro-pentofuranosyl)-[1,3,5]triazine-2,4,6-triamine 9

To a solution of **7** (2.53 g, 5.74 mmol) in dichloromethane (57 mL) in an ice bath was added DIPEA (1.10 mL, 6.31 mmol) and TIPSTf (1.37 mL, 5.10 mmol). After 15 min the solution was concentrated and the crude product was purified using flash chromatography (33% hexanes in ethyl acetate) to give an oil (3.42 g, 100%). IR film (υ_{max}/cm^{-1}) 3467, 3284, 2943, 2866, 1553, 1494, 1467, 1408; δ_H(400 MHz; $CDCl_3$; $CHCl_3$) 1.03–1.09 (42H, m), 1.88–2.06 (1H, ddd, *J* 12.8, 5.6 and 1.8), 2.09 (1H, ddd, *J* 14.8, 9.2 and 5.6,), 2.89 (3H, s), 2.90 (3H, s), 2.98 (3H, s), 3.73 (1H, dd, $J = 10.6$ and 5.0), 3.82 (1H, dd, *J* 10.6 and 3.0), 3.87 (1H, br s), 4.57 (1H, dt, *J* 3.6 and 1.6), 4.97 (2H, br s), 6.90 (1H, br s); δ_C(100 MHz; $CDCl_3$; $CHCl_3$) 12.1, 18.09, 18.13, 27.6, 37.2, 64.0, 73.0, 84.5, 86.0, 165.7; HRMS (MALDI-FTMS) *m/z* calcd for $C_{29}H_{61}N_6O_3Si_2$ $[M + H]^+$ 597.4344, found 597.4345.

N4,N6-diisobutyryl-N4,N6-dimethyl-N2-methyl-N2-(3′,5′-di-*O*-triisopropylsilyl-2′-deoxy-β-D-erythro-pentofuranosyl)-[1,3,5]triazine-2,4,6-triamine 10

To a solution of **9** (2.24 g, 3.75 mmol) in 1,2-dichloroethane (33 mL) was added DMAP (8.21 g, 67.2 mmol) and isobutyryl chloride (1.49 mL, 14.1 mmol). The solution was heated at 60 °C for 3 h then concentrated. The crude product was purified using flash chromatography (15% ethyl acetate in hexanes) to afford an oil (1.73 g, 63%). δ_H(400 MHz; $CDCl_3$; $CHCl_3$) 1.03–1.08 (44H, m,), 1.20 (1H, t, *J* 4.8), 1.94 (1H, dd, *J* 12.8 and 4.8), 2.14–2.20 (1H, m), 3.06 (3H, s), 3.37 (3H, s), 3.39 (3H, s), 3.70–3.90 (3H, m), 4.60 (1H, d, *J* 5.6), 6.74 (1H, dd, *J* 9.6, 5.6); δ_C(100 MHz; $CDCl_3$; $CHCl_3$) 12.0, 18.0, 20.3, 28.1, 33.1, 34.8, 37.7, 63.9, 72.9, 84.7, 86.8, 165.5, 166.2, 166.7, 180.9; HRMS (MALDI-FTMS) *m/z* calcd for $C_{37}H_{73}N_6O_5Si_2$ $[M + H]^+$ 737.5175, found 737.5145.

† CCDC reference number 266147. See http://dx.doi.org/10.1039/b503757c for crystallographic data in CIF or other electronic format.

N4,N6-diisobutyryl-N4,N6-dimethyl-N2-methyl-N2-(2′-deoxy-β-D-erythro-pentofuranosyl)-[1,3,5]triazine-2,4,6-triamine 11

To a solution of **10** (1.72 g, 2.33 mmol) in tetrahydrofuran (5 mL) was added 1.0 M TBAF in tetrahydrofuran (4.69 mL, 4.69 mmol). The reaction was stirred at room temp for 15 min, concentrated and the crude product was purified *via* flash chromatography (100% ethyl acetate) to afford an oil (873 mg, 88%). IR film (υ_{max}/cm^{-1}) 3441, 2960, 2871, 1684, 1572, 1529; δ_H(400 MHz; $CDCl_3$; $CHCl_3$) 1.14–1.26 (m, 14H), 1.67 (1H, br s), 1.90 (1H, br s), 2.14–2.20 (1H, m), 2.31–2.42 (1H, m), 3.06 (3H, s), 3.39 (6H, s), 3.71–3.87 (3H, m), 4.46 (1H, m), 6.76 (1H, t, *J* 7.0); HRMS (MALDI-FTMS) *m/z* calcd for $C_{19}H_{38}N_5O_4$ $[M + H]^+$ 425.2507, found 425.2500.

N4,N6-diisobutyryl-N4,N6-dimethyl-N2-methyl-N2-(5′-*O*-(4,4′-dimethoxytriphenylmethyl)-2′-deoxy-β-D-erythro-pentofuranosyl)-[1,3,5]triazine-2,4,6-triamine 12

To a 0 °C solution of **11** (25 mg, 0.059 mmol) in dichloromethane (0.3 mL) was added DIPEA (15.6 uL, 0.089 mmol) and DMTrCl (22 mg, 0.066 mmol). The solution was warmed to room temp and stirred for 2 h. The solution was concentrated and purified using flash column chromatography (0.5% Et_3N in 50% ethyl acetate–hexanes) to afford a white solid (39 mg, 91%). IR film (υ_{max}/cm^{-1}) 3463, 3058, 2965, 2872, 2836, 1735, 1685, 1607, 1562; δ_H(400 MHz; $CDCl_3$; $CHCl_3$) 1.16–1.22 (14H, m), 2.04 (1H, ddd, *J* 13.2, 6.0, and 3.2), 2.19–2.22 (1H, m), 3.02 (3H, s), 3.36 (3H, s), 3.72 (6H, s), 3.90 (2H, m), 3.95 (1H, dd, *J* 7.6 and 4.4), 4.40 (1H, m, *J* 3.2), 6.73 (1H, dd, *J* 8.0 and 6.0), 6.74–6.78 (4H, m), 7.15 (1H, d, $J = 7.2$), 7.19–7.24 (2H, m), 7.27–7.31 (4H m), 7.41 (2H, t, *J* 6.8); δ_C(100 MHz; $CDCl_3$; $CHCl_3$) 14.0, 20.1, 28.4, 33.1, 36.9, 55.0, 63.9, 72.3, 85.3, 85.6, 86.1, 126.5, 127.4, 127.7, 129.7, 135.4, 144.4, 158.1, 165.2, 166.2, 166.7, 180.94, 180.97.

6-Chloro-4-amino-N2-methyl-N2-(5′-*O*-triisopropylsilyl-2′-deoxy-α-D-erythro-pentofuranosyl)-[1,3,5]triazin-2,4-diamine (13 = 3α)[14]

A solution of **2**α (0.870 g, 1.93 mmol) and DIPEA (0.37 mL, 2.12 mmol) in tetrahydrofuran (19 mL) was saturated with $NH_{3(g)}$ at 0 °C. The reaction was stirred at room temperature overnight, concentrated under vacuum and purified by flash column chromatography (66% ethyl acetate–hexanes) to afford **13** as a mixture of rotamers (0.819 g, 98%). R_f (50% hexanes in ethyl acetate) = 0.3; IR film (υ_{max}/cm^{-1}) 3333, 3225, 2943, 2865, 1641, 1566, 1507, 1467; δ_H(400 MHz; $CDCl_3$; $CHCl_3$) 1.03–1.08 (21H, m), 1.97–2.05 (1H, m), 2.52–2.61 (1H, m), 3.10 (3H, s), 3.70 (1H, dd, *J* 13.6 and 8.4), 3.88 (1H, dd, *J* 13.6 and 4.0), 4.03–4.08 (1H, m), 4.42–4.48 (1H, m), 5.25–5.40 (2H, br s), 6.49 (1H, t, *J* 7); δ_C(100 MHz; $CDCl_3$; $CHCl_3$) 11.9, 17.8, 18.0, 29.9 (br), 37.8, 65.3, 73.7, 85.5, 86.0 (br), 165.0, 166.8 (br); HRMS (MALDI-FTMS) *m/z* calcd for $C_{18}H_{35}ClN_5O_3Si$ $[M + H]^+$ 432.2192, found 432.2197.

4,6-Dichloro-N2-methyl-N2-(2′-deoxy-α,β-D-erythro-pentopyranosyl)-[1,3,5]triazin-2-yl-amine 14

2-Deoxy-D-ribose (2.02 g, 15.1 mmol) was dissolved in 2.0 M methylamine in methanol (80 mL) and stirred at room temperature for 16 h. The solution was concentrated under reduced pressure. The resulting oil was dissolved in acetonitrile (80 mL) and cooled to −20 °C. To the solution was added DIPEA (2.9 mL, 16.6 mmol) and cyanuric chloride (3.06 g, 16.6 mmol). The reaction was stirred at −20 °C for 24 hours. The acetonitrile was removed and the crude was purified *via* flash column chromatography (20% CH_3CN in EtOAc) to afford an oil (1.47 g, 33%). IR (thin film) (υ_{max}/cm^{-1}) 3404, 2968, 2935, 2879, 1556, 1488, 1414; δ_H(400 MHz; DMSO-d_6) 6.02 (1H, d, *J* 11.2), 3.98 (1H, br s), 3.61–3.54 (3H, m), 2.98 (3H, s), 2.49 (2H, br s), 1.98 (1H, dt, *J* 13.6 and 2.4), 1.68 (1H, d, *J* 10.4); δ_C(100 MHz;

CD_3CN) 29.4, 34.3, 66.2, 66.8, 67.6, 78.9, 165.4, 169.9, 170.5; HRMS (MALDI-FTMS) *m/z* calcd for $C_9H_{12}Cl_2N_4O_3$ [M + Na]$^+$ 317.0179 found 317.0182.

N4-amino-6-chloro-N2-methyl-N2-(2′-deoxy-β-D-erythro-pentopyranosyl)-[1,3,5]triazin-2-yl-amine 15

A solution of **14** (0.900 g, 3.05 mmol) and DIPEA (0.604 mL, 3.35 mmol) in CH_3CN (30 mL) was saturated with $NH_{3(g)}$ at 0 °C. The reaction was stirred at room temperature overnight, concentrated under vacuum and purified by flash column chromatography (100% CH_3CN) to afford **15** (0.662 mg, 79%). δ_H(400 MHz; CD_3OD) 1.01 (1H, dd, *J* 3.6, 2.4), 1.77 (1H, dd, *J* 2.8, 2.4), 2.98 (3H, s), 3.65–3.70 (2H, m), 3.77–3.79 (1H, m), 4.14 (1H, d, *J* 6.0), 6.25 (1H, br s); δ_C(100 MHz; CD_3OD) 28.7, 35.2, 35.5, 66.5, 68.0, 68.5, 79.0, 166.6, 167.8, 168.2, 170.0, 170.3; HRMS (ESI-TOF) *m/z* [M + H]$^+$ calcd for $C_9H_{14}ClN_5O_3$ for 276.0863, found 276.0863.

N4-amino-6-methoxy-N2-methyl-N2-(2′-deoxy-β-D-erythro-pentopyranosyl)-[1,3,5]triazin-2-yl-amine 6c

A solution of **15** (0.662 g, 2.40 mmol) and NaOMe (1.30 g, 24.3 mmol) in methanol (20 mL) was refluxed for 24 hours. The solution was concentrated under vacuum and the crude product was purified *via* column chromatography (100% CH_3CN) to afford a solid (292 mg, 45%). NMR taken in D_2O, pH = 11. UV λ_{max} (H_2O)/nm 215; δ_H(400 MHz; D_2O) 1.92 (1H, d, *J* 14.0), 2.32 (1H, td, *J* 12.8, 2.4, H-2′), 3.00 (3H, s, NMe), 3.78–3.86 (3H, m, H-4′, H-5′), 3.91 (3H, s, OMe), 4.29 (br s, 1H, H-3′), 6.15 (1H, d, *J* 11.6, H-1′); δ_C(400 MHz; D_2O) 28.6, 33.9, 54.6, 64.8, 66.4, 67.2, 78.6, 166.7, 167.7, 171.8; HRMS (MALDI-FTMS) *m/z* calcd for $C_{10}H_{17}N_5O_4$[M + H]$^+$ 272.1353, found 272.1359.

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