Fluorescent Charge-Neutral Analogue of Xanthosine: Synthesis of a 2′-Deoxyribonucleoside Bearing a 5-Aza-7-deazaxanthine Base

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A concise route is described to prepare the 5-aza-7-deazapurine 2′-deoxyriboside (**4**), which presents the puADA hydrogen-bonding pattern, analogous to the hydrogen-bonding pattern presented by 2′-deoxyxanthosine (**2**). The route begins with the commercially available 1-α-chloro-2-deoxy-3-5-bistoluoyloxyribofuranose (**10**), which proves to be a versatile point of entry to β-2′-deoxyribofuranosides. In the first step, 2-nitroimidazole (**8**) is coupled with **10** to yield intermediate **11**. Reduction of the nitro group to an amino group yields **12**, which is treated with phenyl isocyanatoformate to complete the nucleobase to yield **13**. Removal of the toluoyloxy protecting groups of **13** yields the target nucleoside **4** in 40% overall yield in four steps. In an alternative strategy, convergent coupling of **14** with **10** under basic conditions was attempted but found to yield the heterocycle glycosylated at the undesired position. Compound **13** displays potentially useful fluorescence properties. After excitation at 250 nm, a solution of **13** in MeCN shows a fluorescence emission with a maximum at 410 nm. Furthermore, **13** is neutral at physiological pH, a property that it shares with natural nucleobases but not xanthosine itself, which is an acid with a pK_a of ca. 5.6. Furthermore, as part of the design, **4** is made capable of presenting an unshared pair of electrons to the DNA minor groove.

Introduction

The Watson−Crick nucleobase pair in DNA follows two rules of complementarity: size complementarity (large purines pair with small pyrimidines) and hydrogen-bonding complementarity (hydrogen-bond donors from one partner complement hydrogen-bond acceptors from the other partner). Ten years ago, we noted that 12 nucleobases forming six base pairs joined by mutually exclusive hydrogen-bonding patterns are possible within the geometry of the Watson−Crick base pair (Figure 1) and that these might be functionalized to enable a single biopolymer capable of both genetics and catalysis.[1] Expanded genetic alphabets have now been further explored in a variety of laboratories, and the possibility of a fully artificial genetic system has recently been advanced.[2−7]

Artificial genetic systems require their own set of manipulative tools, including polymerases to copy DNA analogues containing artificial nucleoside derivatives, kinases to phosphorylate them, and chemical methods to synthesize them. In addition, an artificial genetic system needs its own set of biophysical tools to permit us to explore their structure, conformation, and behavior. This includes functionalized derivatives and, most desirably, fluorescent derivatives, well-known to be useful in studies of natural DNA.[8]

To generate a fluorescent probe, we focused on the base pair joined by the pyDAD−puADA hydrogen-bonding scheme (Figure 1b).[1b,9] As originally implemented, this pair was formed between 2,4-diaminopyrimidine and xanthine, represented by the C-nucleoside (**1**) and deoxyxanthosine (**2**) or its 7-deaza analogue (**3**).[10] Neither of these components is fluorescent. Other heterocycles can

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(9) pyDAD: Pyrimidine analogue carrying a H-bond donor−acceptor−donor motif. Similarly, puADA: purine analogue carrying an acceptor−donor−acceptor motif.

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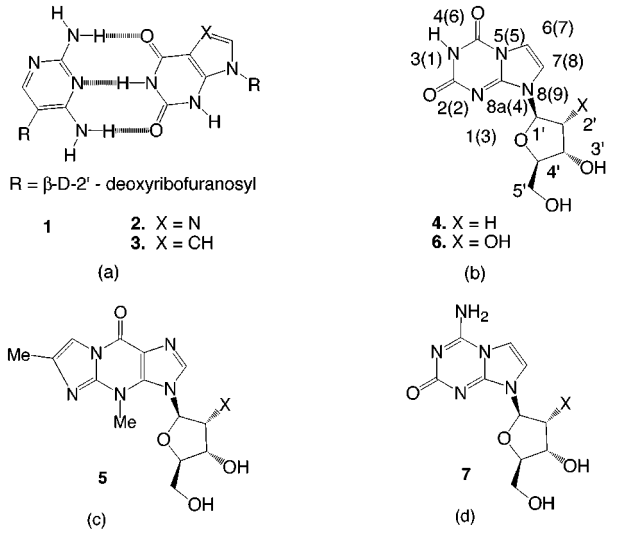


Figure 1. (a) Complementary Watson−Crick pairing between pyDAD (**1**) and puADA derivatives **2** and **3**. (b) Imidazo[1,2-*a*]triazine (purine in parentheses) and sugar numberings showed in **4**. (c) The rare tRNA constituent wyosine. (d) The puDAA analogue **7**.

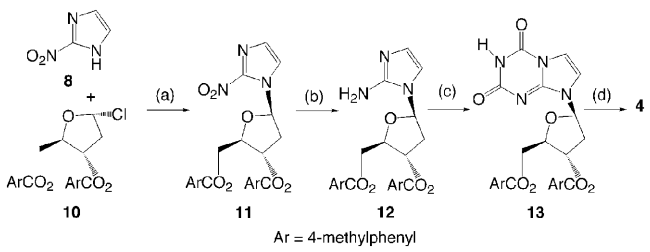
support a puADA hydrogen-bonding pattern, however. In particular, **4**, a 5-aza-7-deazapurine[imidazo[1,2-*a*]-pyrimidine] analogue, was attractive. We speculated that since the base moiety of the rare tRNA constituent wyosine (**5**), carrying a 5-aza-7-deazapurine substructure, is fluorescent, then **4** might also be fluorescent. The ribo analogue **6** of this molecule was prepared over 20 years ago by Moffat and his colleagues, but its photophysical properties were not mentioned.[11]

Synthetic approaches to the 5-aza-7-deazapurine 2′-deoxyriboside (**7**) were reported in these laboratories,[3b] but the synthetic routes used by both this laboratory and Moffat's for analogous compounds suggested that more efficient approaches were conceivable. This paper reports a simple and direct synthesis to prepare the 2′-deoxyribonucleoside (**4**) carrying imidazo[1,2-*a*]-1,3,5-triazine-2(8*H*),4(3*H*)-dione (5-aza-7-deazaxanthine).

Results and Discussion

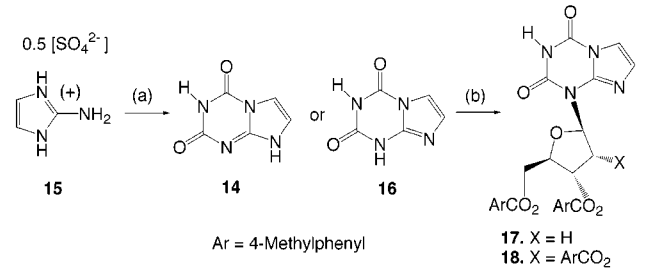
Previously, Vorbrueggen ribosylation of 2-nitroimidazole (**8**) or 2-aminoimidazole (**9**) had been used as the stereoselective step toward building the ADA (5-aza-7-deaza) backbone in the riboside **6**.[10] However, the deoxygenation at the 2′ position necessary to generate the 2′-deoxyribo derivative **4** makes this process lengthy and low-yielding. We decided to employ glycosylation of **8** as the key step, making use of the commercially available 1-α-chloro-2′-deoxyribose derivative (**10**) as the glycosyl donor. Reaction of **8** with **10** in the presence of K_2CO_3 and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) (phase-transfer conditions) yielded the nitrosugar (**11**) in 80% yield (Scheme 1).[12,13] K_2CO_3 was chosen because other bases (e.g., NaH and KOH) have been reported to yield mixtures of the 1′-α and the 1′-*β* epimers.

Scheme 1[a]



[a] Key: (a) K_2CO_3, TDA-1, MeCN, rt, 80%; (b) 10% Pd−C, H_2 (1 atm), THF, rt, quantitative; (c) PhOC(O)NCO, dioxane, rt, 55%; (d) MeOH−NH_3, rt, 60%.

Scheme 2[a]



[a] Key: (a) PhOC(O)NCO, NaH, DMF, rt, 60%; (b) K_2CO_3, TDA-1, MeCN, 10, rt, 24%.

Reduction of the nitro group to the amino group was carried out quantitatively by hydrogenation using 10% Pd−C as the catalyst. The amino derivative (**12**) was cyclized using phenyl isocyanatoformate in dioxane to yield **13**, which possesses the required 5-aza-7-deaza skeleton, in 50% yield. Deprotection of the toluoyloxy functionalities was performed in methanolic ammonia over 3 days to yield the nucleoside (**4**) in 80% yield.

In addition to the linear synthesis, a convergent protocol was also evaluated for the synthesis of **4** (Scheme 2). Here, the nucleobase imidazo[1,2-*a*]-1,3,5-triazine-2,4-(3*H*,8*H*)-dione (5-aza-7-deazaxanthine, **14**) was synthesized from **9** and obtained in situ from 2-aminoimidazolium sulfate (**15**) and phenyl isocyanatoformate. Conceivably, **14** may exist as the tautomer **16**. Interestingly, tautomer **16** was calculated to be favored by ca. 5 kcal/mol over **14** in the gas phase, following semiempirical AM1 calculations.[14] The base (**14**/**16**) was reacted with chlorosugar (**8**) under phase-transfer conditions. Resolution of the reaction mixture by silica gel column chromatography using 5−10% MeOH/CH_2Cl_2 yielded a single component (**17**). ^{1}H NMR showed that both the nucleobase and the toluoyloxy-protected 2′-deoxyribofuranose moiety were present. Nucleophilic substitutions by the 5-aza-7-deaza ring system may be preferred at the 1 (purine-3) position however.[3b] Comparison with the unambiguously synthesized nucleoside (**4**) suggested that the derivative (**17**) had its sugar joined at the 1′ position to the "1 position" of the nucleobase. The riboderivative (**18**) of **17** is known in the literature, and the convergent synthesis outlines another possible route to this class of nucleosides.[8a] The "1 vs 8" selectivity in alkylation of 5-aza-7-deazapurine analogues is influenced by the substituents at the 2 and 3 positions because it is reported that the imidazopyrimidine derivative (**19**) reacts with **10** in the presence of K_2CO_3 and TDA-1 to yield derivative

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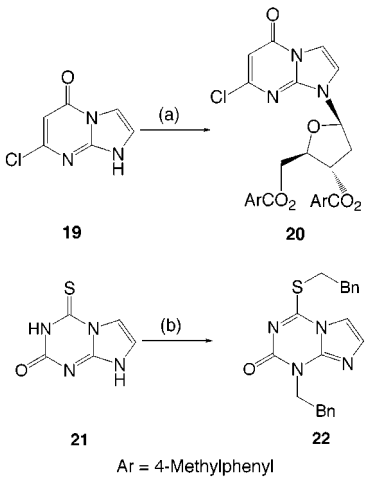
(13) The *β*-stereochemistry at the 1′ position was confirmed by a NOESY experiment following the observation of NOE between 1′-H and 4′-H and the absence of the same between 1′-H and 3′-H.

(14) Details of the MO calculations will be published elsewhere.

Scheme 3[a]



[a] Key: (a) K_2CO_3, TDA-1, MeCN, 10, rt (ref 11). (b) Na_2CO_3/DMF/BnBr, rt (ref 3b).

20 (with an 8-1′ linkage, Scheme 3) while the thio analogue (**21**) of **14** reacts under similar conditions to yield **22** (with an 1-1′ linkage).[11,3b]

To learn about the fluorescence properties of the imidazo[1,2-*a*]-1,3,5-triazine-2(8*H*),4(3*H*)-dione moiety, the UV−visible absorption spectrum and the fluorescence emission spectrum of **13** (Figure 2panels a and b, respectively) were obtained. Compound **13** fluoresces with a blue color after excitation at 250 nm, with two emission maxima (λ_{max} = 410 and 580 nm).

Glycosylation of bases or base precursors is complicated due to the ambiguity of the stereochemical outcome (α/β) of the bond formed. The synthesis of **4** is further complicated by the regiochemistry of the newly formed bond (1−1′ or 8−1′). Employing the deoxyribose derivative **10** determines both the desired β-stereochemistry and the desired regiochemistry (1−8′) in the very first step.

Compound **4** turns out to have other advantages over **2/3** as a component of an expanded genetic alphabet. Xanthosine is an acid with a pK_a of ca. 5.6.[15,16] Therefore, the nucleobase is almost fully deprotonated at pH 7.4 and introduces a negative charge into the stacked nucleobases in the duplex. In contrast, **4** lacks a charge at physiological pH; an 80 mM solution of **4** in water shows a pH of 7, while a 15 mM aqueous solution of xanthosine, in contrast, shows (as expected) a pH of 4.

Furthermore, as part of the design, **4** is capable to present an unshared pair of electrons to the minor groove in DNA. We and others have suggested that this might be a recognition feature demanded by many polymerases.[17−20] Xanthosine and 7-deazaxanthosine derivatives

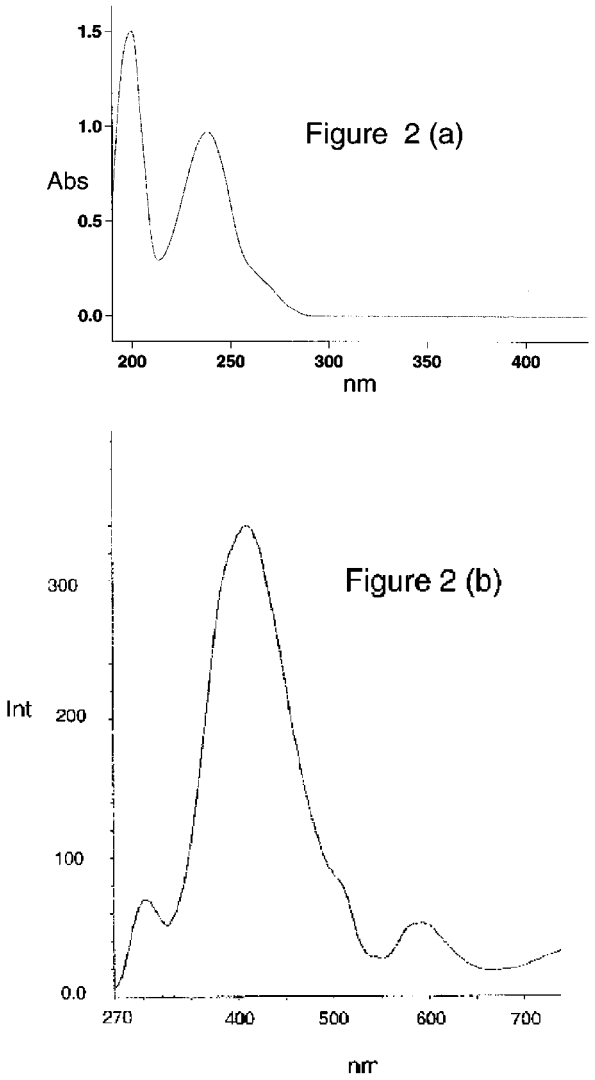


Figure 2. (a) UV−visible absorption spectrum of **13** in MeCN. (b) Emission spectrum of **13** in MeCN (λ_{max} = 410 nm).

(such as **2** and **3**) lack the sp^2 lone pair of electrons at the 3 position of the purine.

Experimental Section

General. Chlorosugar **10** was purchased from Berry & Associates. Measurement of pH was performed using color-pHast indicator strips purchased from EM Science. Anhyd MeCN, THF, DMF, and dioxane were purchased from Aldrich Chemical Co. Silica gel (200−450 mesh) was purchased from Fischer Scientific. NMR spectra: Varian XL-300 spectrometer at 300 MHz referenced to $SiMe_4$ (^{1}H), at 75.4 MHz referenced to $CDCl_3$, DMSO-d_6, or MeOD-d_4 (^{13}C). MS analyses: Finnigan MAT-95Q apparatus. UV−visible absorption spectra: Varian Cary 1-Bio UV−visible spectrophotometer. Emission spectra: Perkin-Elmer LS50B luminescence spectrophotometer. All reactions were performed under Ar. Volatiles were removed using a rotary evaporator and, finally, in vacuo. Chemical yields reported refer to the yields of pure isolated materials.

8-(β-D-2′-Deoxyribofuranosyl)imidazo[1,2-*a*]-1,3,5-triazine-2(8*H*),4(3*H*)-dione (4). Solid **13** (0.25 g, 0.5 mmol) was dissolved in methanolic ammonia (50 mL) and stirred at rt for 3 days. The volatiles were removed, and the crude material was purified by column chromatography on silica gel using 10−20% EtOH/CH_2Cl_2 as eluent to yield 0.11 g (80%) of **4** as a white solid. ^{1}H NMR (300 MHz, D_2O): δ 2.50−2.60 (m, 2H), 3.75 (dd, 1H, J = 5 Hz, J = 13 Hz), 3.85 (dd, 1H, J = 5 Hz, J = 13 Hz), 4.05−4.15 (m, 1H), 4.50−4.60 (m, 1H), 6.27 (t, 1H,

(15) Miles, H. Y.; Roy, K. B. *Nucleosides Nucleotides* **1983**, *2*, 231.
(16) The use of **2** appears to have some limitations due to its rather high acidity causing ionization, which seems to disrupt the base pairing between **1** and **2** in duplex DNA at neutral pH. Also, charged nucleobases may be undesirable polymerase substrates. Battersby, T.; Geyer, R.; Benner, S. A. Unpublished results.
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(19) Morales, J. C.; Kool, E. T. *J. Am. Chem. Soc.* **2000**, *122*, 1001.
(20) Evaluation of 3-deaza analogues of 2′-deoxyadenosine and 2′-deoxyguanosine derivatives are being pursued in our laboratory toward gaining more insight into this problem.

J = 9 Hz), 7.40 (1H, d, J = 2.5 Hz), 7.44 (1H, d, J = 2.5 Hz). ^{13}C NMR (75 MHz, D_2O): 39.1, 62.0, 71.5, 84.8, 88.0, 109.6, 117.3, 147.3, 150.8, 158.6. FAB (positive) MS (% relative abundance): 269 (M^+, 5), 221 (50), 207 (50), 149 (60), 147 (100). HRMS−FAB (m/z): [M^+] calcd for $C_{10}H_{13}N_4O_5$, 269.0886; found, 269.0878.

1-(β-D-2′-Deoxy-3,5-bis(4-toluoyloxy)ribofuranosyl)-2-nitroimidazole (11). A slurry of **8** (1.0 g, 8.9 mmol) was stirred in anhyd MeCN (500 mL) at rt along with K_2CO_3 (4.0 g, 29 mmol) and TDA-1 (100 μL, 0.21 mmol) for 1 h, after which period of time sugar **10** (4.5 g, 11.6 mmol) was added, and stirring continued for 2 h. The mixture was filtered off, and the volatiles were removed. The crude material was purified by column chromatography on silica gel using 1−2% Me_2CO/CH_2Cl_2 as eluent to yield 3.3 g (80%) of **11** as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.40 (s, 3H), 2.44 (s, 3H), 2.40−2.45 (m, 1H), 3.05−3.20 (m, 1H), 4.60−4.80 (m, 3H), 5.60−5.67 (m, 1H), 6.80 (t, 1H, J = 6.5 Hz), 7.12 (d, 1H, J = 1.4 Hz), 7.23 (d, 2H, J = 8.0 Hz), 7.29 (d, 2H, J = 8.0 Hz), 7.59 (d, 1H, J = 1.4 Hz), 7.84 (d, 2H, J = 8.0 Hz), 7.96 (d, 2H, J = 8.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.0, 41.5, 65.0, 74.1, 85.5, 90.0, 121.5, 126.0, 126.5, 129.0, 129.4, 129.6, 130.1, 145.0, 145.5, 167.2, 168.0. FAB (positive) MS (% relative abundance): 353 (100), 112 (80).

1-(β-D-2′-Deoxy-3,5-bis(4-toluoyloxy)ribofuranosyl)-2-aminoimidazole (12). A solution of **11** (0.50 g,1.1 mmol) in THF (20 mL) was reduced in the presence of 10% Pd−C (0.42 g, 0.4 mmol) and H_2 (1 atm) for 8 h at rt. The mixture was filtered to remove the catalyst and washed with ethyl acetate (2 × 10 mL). The volatiles were removed to obtain 0.50 g of the amino sugar **12,** which was used for the cyclization without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.38 (s, 3H), 2.40 (s, 3H), 2.43−2.60 (m, 1H), 2.80−3.00 (m, 1H), 4.20−4.40 (bs, 2H), 4.65 (s, 1H), 4.66−4.70 (m, 2H), 5.60−5.62 (m, 1H), 5.90−6.00 (m, 1H), 6.60 (s, 2H), 6.63 (d, 1H, J = Hz), 7.22(d, 2H, J = 8.0 Hz), 7.27(d, 2H, J = 8.0 Hz), 7.86 (d, 2H, J = 8.0 Hz), 7.88 (d, 2H, J = 8.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.5, 35.9, 64.3, 75.0, 83.1, 85.2, 113.0, 125.4, 127.0, 127.1, 129.5, 129.6, 129.7, 130.3, 130.4, 144.8, 145.0, 149.0, 166.9, 167.0. FAB (positive) MS (% relative abundance): 436 (MH^+, 20), 353 (5), 281 (5), 223 (5), 207 (5), 147 (15), 119 (65), 91 (20), 84 (free base + $2H^+$, 35), 81 (100).

8-(β-D-2′-Deoxy-3′,5′-bis(4-toluoyloxy)ribofuranosyl)-imidazo[1,2-*a*]-1,3,5-triazine-2(8*H*),4(3*H*)-dione (13). To a solution of **12** (0.48 g, 1.1 mmol) in anhyd dioxane (2 mL) was added phenyl isocyanatoformate (225 μL, 1.7 mmol), and it was stirred for 8 h at rt. After addition of MeOH (5 mL), the volatiles were removed, and the crude material was purified by column chromatography on silica gel using 5−10% MeOH/CH_2Cl_2 as eluent to yield 0.30 g (55%) of **13** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H), 2.45 (s, 3H), 2.45−2.55 (m, 1H), 2.89−2.90 (m, 1H), 4.50−4.80 (m, 3H), 5.65 (dd, 1H, J = 6 Hz, J = 8.0 Hz), 6.96 (d, 1H, J = 3.2 Hz), 7.14 (d, 1H, J = 3.2 Hz), 7.23 (d, 2H, J = 8.0 Hz), 7.27 (d, 2H, J = 8.0 Hz), 7.88 (d, 2H, J = 8.0 Hz), 7.94 (d, 2H, J = 8.0 Hz). ^{1}H NMR (300 MHz, MeOD-d_4): δ 2.40 (s, 3H), 2.43 (s, 3H), 2.36−2.56 (m, 1H), 2.78−2.82 (m, 1H), 4.58−4.70 (m, 3H), 5.70−5.76 (m, 1H), 6.35 (t, 1H, J = 6.5 Hz), 7.22−7.34 (m, 6H), 7.90 (d, 2H, J = 8.0 Hz), 7.98 (d, 1H, J = 3.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.0, 38.1, 64.4, 74.8, 86.0, 87.1, 109.1, 114.8, 125.6, 126.7, 129.0, 129.3, 129.6, 130.0, 143.3, 144.0, 145.0, 150.0, 155.3, 166.2, 166.4. FAB (positive) MS (% relative abundance): 505 (MH^+, 5), 353 (M − free base $C_5H_3O_2N_4$, 20), 154 (10), 153, (10), 137 (10), 136 (10), 119 (70), 91 (20), 81 (100). HRMS−FAB (m/z): [MH^+] calcd for $C_{26}H_{25}O_7N_4$, 505.1723; found, 505.1738.

Imidazo[1,2-*a*]-1,3,5-triazine-2,4(3*H*,8*H*)-dione (14). To a slurry of **15** (0.5 g, 1.9 mmol) in anhyd DMF (5 mL) was added a 60% dispersion of NaH in oil (0.46 g, 11.4 mmol), and it was stirred for 2 h at rt. Phenyl isocyanatoformate (1.0 mL, 7.6 mmol) was added, and stirring was continued for another 8 h. Water (1 mL) was added, the volatiles were removed, and the residue was crystallized from cold water to yield **14** as an off-white powder (0.5 g, 90% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.01 (d, 1H, J = 2 Hz), 7.32 (d, 1H, J = 2 Hz), 11. 4 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 110.0, 125.0, 146.0, 146.8, 152.5.

1-(β-D-2′-Deoxy-3′,5′-bis(4-toluoyloxy)ribofuranosyl)-imidazo[1,2-*a*]-1,3,5-triazine-2(1*H*),4(3*H*)-dione (17). A slurry of **14** (0.1 g, 0.7 mmol) in anhyd MeCN (50 mL) was stirred along with K_2CO_3 (0.5 g, 3.62 mmol) at rt and TDA-1 (10 μL, 0.02 mmol) for 1 h after which period of time the sugar **10** (0.54 g, 1.4 mmol) was added, and stirring was continued for 2 h. The mixture was filtered off, and the volatiles were removed. The crude material was purified by column chromatography on silica gel using 5−10% MeOH/CH_2Cl_2 as eluent to yield 0.08 g (24%) of **17** as a white solid. ^{1}H NMR (300 MHz, MeOD-d_4): δ 2.30 (s, 3H), 2.35 (s, 3H), 2.65 (d, 1H, J = 13.6 Hz), 2.80−3.00 (m, 1H), 4.40−4.50 (m, 2H), 4.90−4.95 (m, 1H), 5.58 (d, 1H, J = 6.5 Hz), 6.25 (d, 1H, J = 6.5 Hz), 7.15 (d, 2H, J = 8.0 Hz), 8.22 (d, 2H, J = 8.0 Hz), 7.30 (d, 1H, J = 3.3 Hz), 7.38 (d, 1H, J = 3.3 Hz), 7.70 (d, 2H, J = 8.0 Hz), 7.85 (d, H, J = 8.0 Hz). ^{13}C NMR (300 MHz, MeOD-d_4): δ 22.0, 38.0, 66.2, 76.6, 86.2, 88.5, 109.0, 118.5, 127.1, 127.6, 129.5, 130.0, 145.1, 145.6, 146.6, 158.5, 168.1, 168.5. FAB (positive) MS (% relative abundance) 505 (MH^+, 6), 353 (25).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectral data (in $CDCl_3$) for compounds **4**, **11**, **12**, and **13** and ^{1}H NMR spectral data (in MeOD-d_4) for **13** and **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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