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Nucleobase Functionalized 5-Aza-7-deazaguanine Ribo- and 2'-Deoxyribonucleosides: Glycosylation, Pd-Assisted Cross-Coupling and Photophysical Properties

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ABSTRACT

The special nucleobase recognition pattern of 5-aza-7-deazaguanine nucleosides makes them valuable for construction of homo purine DNA, silver-mediated base pairs and expansion of the four letter genetic coding system. To widen the utility of 5-aza-7-deazaguanine nucleosides, side chains were introduced at position-7 of the nucleobase. As key compounds 7-iodo nucleosides were synthesized. Nucleobase anion glycosylation of the iodo derivative of isobutyrylated 5-aza-7-deazaguanine with the bromo sugar of 2,3,5-tri-*O*-benzoyl-1-*O*-acetyl-D-ribofuranose gave the pure β-D anomeric N-9 glycosylation product (67%), whereas one-pot Vorbrüggen conditions gave only 42% of the iodinated nucleoside. The non-iodinated nucleoside was formed in 84%. For the synthesis of 2'-deoxyribonucleosides anion glycosylation performed with Hoffer's 2'-deoxyhalogenose yielded an anomeric mixture (α-D = 33% and β-D = 39%) of 2'-deoxyribonucleosides. Various side chain derivatives were prepared from non-protected nucleosides by Pd-assisted *Sonogashira* or *Suzuki-Miyaura* cross-coupling. Among the functionalized ribonucleosides and anomeric 2'-deoxyribonucleosides some of them showed strong fluorescence. Benzofuran and pyrene derivatives display high quantum yields in non-aqueous solvents and solvatochromism. A single-crystal X-ray analysis of 7-iodo-5-aza-7-deaza-2'-deoxyguanosine displayed intermolecular iodo-oxygen interactions in the crystal and channels filled with solvent molecules.

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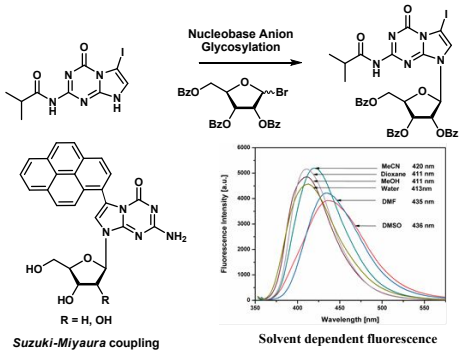


INTRODUCTION

7-Deazapurine nucleosides are common surrogates of purine nucleosides and serve as versatile building blocks for many purposes such as DNA detection, sequencing and imaging.[1,2] Functionalization at the 7-position (purine numbering is used throughout the manuscript) – a site which is not practical for purine nucleoside functionalization – provides derivatives with side chains that have sufficient space in DNA as they protrude to the major groove and are therefore well accommodated in the double helix.[3] Side chains can stabilize the DNA double helix[3a], represent clickable residues[3b], link fluorescent dyes or other reporter groups to the nucleobases.[3c] Basic work on nucleosides with a pyrrolo[2,3-*d*]pyrimidine and pyrazolo[3,4-*d*]pyrimidine nucleosides skeleton[4,5] was initiated by our laboratory and is ongoing.[6] Compared to this very little is known on nucleosides and oligonucleotides with imidazo[1,2-*a*]-*s*-triazines (5-aza-7-deazapurines) as base having the nitrogen in the bridgehead position. Only a few ribonucleosides, 2'-deoxyribonucleosides and fluoronucleosides are existing[7]. Side chain derivatives are totally unknown. Most of the existing work has been reviewed.[8]

5-Aza-7-deaza-2'-deoxyguanosine **1** and its α-D-anomer **3** form mismatches with dC. Stable base pairs are generated when complementary nucleosides provide hydrogen atoms for the formation of tridentated base pairs[9] which are also formed when the base becomes protonated.[10] Homo purine DNA base pairs are developed by **1** with 2'-deoxyguanosine and 2'-deoxyisoguanosine (motif I, Figure 1).[7c] Base pairs with "protonated" dC analogues are used to expand the genetic alphabet by a six letter code.[9] Also, programmable metal ion mediated base pairs with dC exist in the presence of silver ions (motif III, Figure 1).[11] The importance of 5-aza-7-deazapurine α-D anomeric nucleosides has been demonstrated by the formation of DNA with parallel strand orientation and of silver-mediated base pairs.[10-12] For

diagnostic and other purposes side chains are essential that carry reporter groups developing fluorescence or allow further functionalization.

In this work, the functionalization of 5-aza-7-deazapurine ribonucleosides and 2'-deoxyribonucleosides was investigated exactly at the same 7-position that has been used for the functionalization of the widely used pyrrolo[2,3-*d*]pyrimidine and pyrazolo[3,4-*d*]pyrimidine nucleosides (Figure 1).[3,4,13] Until now, the only reported 7-functionalized derivatives of the 5-aza-7-deazaguanine nucleosides are the 7-iodo derivative **4** and its α-D anomer **5** prepared by our laboratory some time ago.[7f] As 7-iodo nucleosides represent key compounds for the synthesis of side chain derivatives *via* cross-coupling reaction, the 7-iodo ribonucleoside **6** was synthesized and the access of pure anomers of the 2'-deoxyribonucleosides **4** and **5** was improved. A diversity of side chain derivatives of anomeric 2'-deoxyribonucleosides and ribonucleosides were synthesized by *Suzuki* and *Sonogashira* cross-coupling reactions performed on the iodo nucleosides **4**-**6** with a series of alkynes and boronic acid derivatives (Figure 1). Among them, the benzofuran and pyrene side chains were found to be particularly valuable as fluorescent reporter groups. Photophysical properties of various derivatives e.g.**7**-**9** were examined in different solvents and quantum yields were determined. A single-crystal X-ray analysis of 7-iodo-5-aza-7-deaza-2'-deoxyguanosine (**4**) was performed.

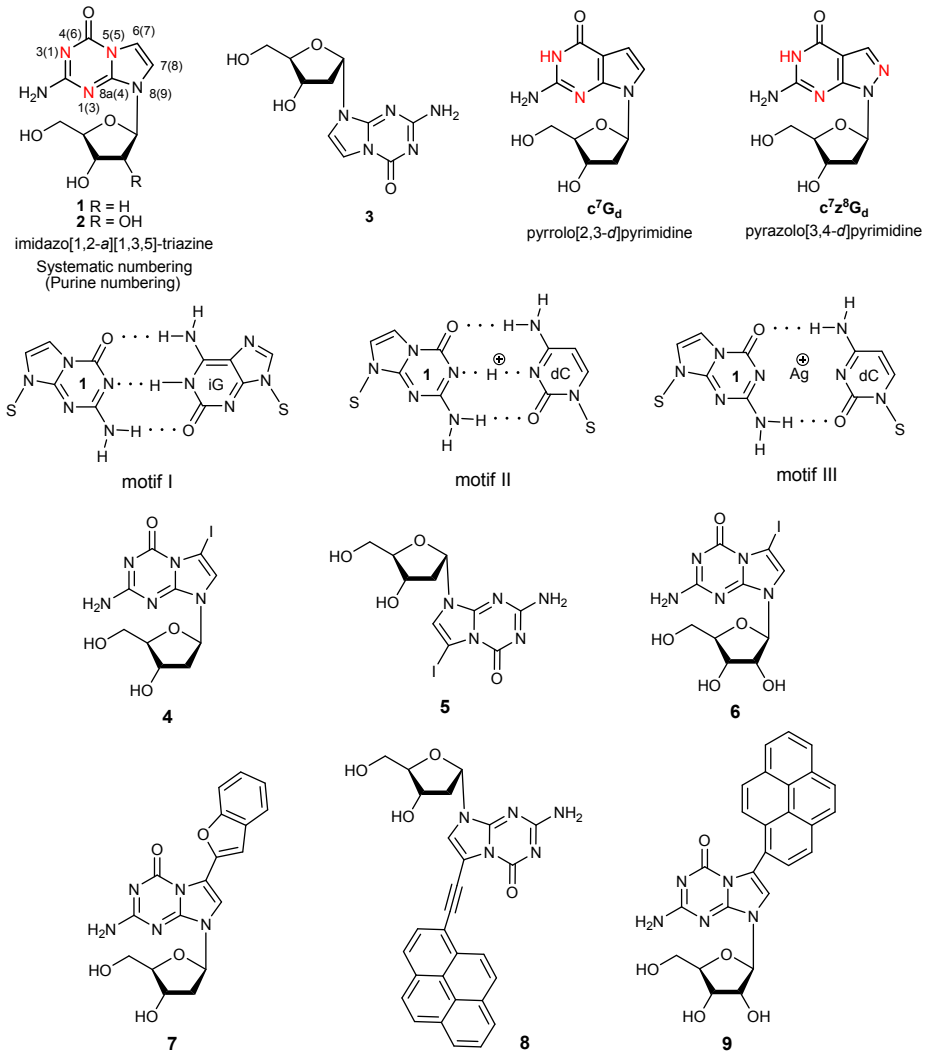


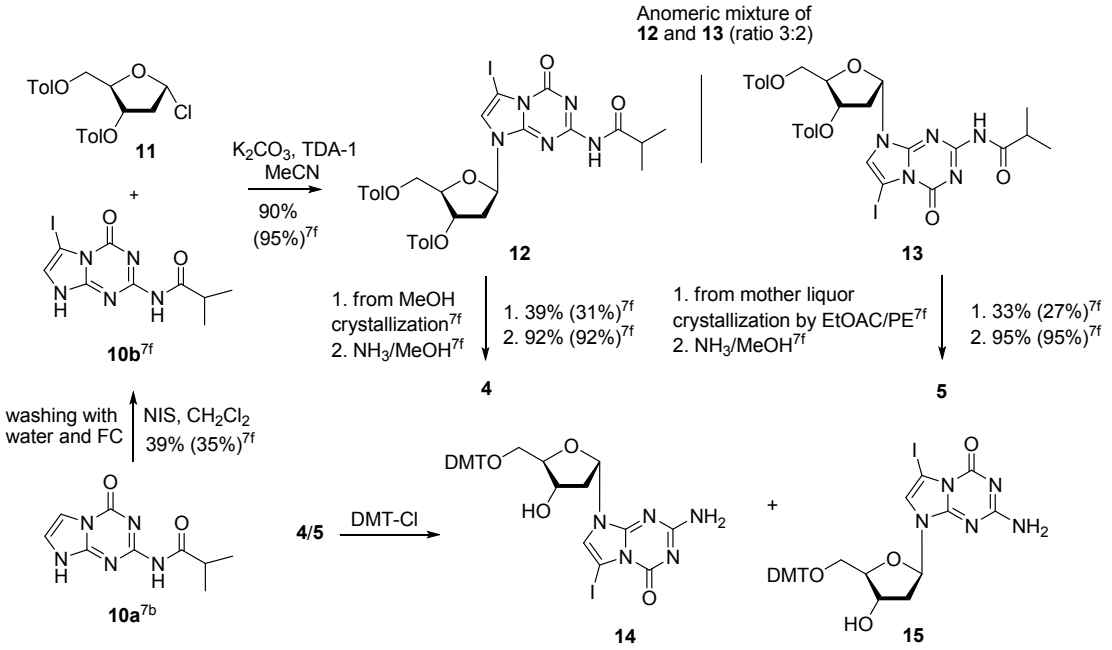
Figure 1. Various 7-deazaguanine 2'-deoxyribonucleosides and base pairs of **1** with iG_d, protonated dC and a silver-mediated construct. S corresponds to 2'-deoxyribose. iG corresponds to 2'-deoxyisoguanosine. Fluorescent side chain derivatives **7-9** obtained from 7-iodinated 5-aza-7-deazaguanine ribo- and 2'-deoxyribonucleosides and anomers **4-6** by cross-coupling reactions.

RESULTS AND DISCUSSION

Synthesis of the Anomeric 7-Iodo-5-aza-7-deazaguanine 2'-Deoxyribonucleosides 4 and 5 and the Ribonucleoside 6

Previously, the syntheses of 7-iodo-2'-deoxyribonucleoside **4** and its α-D anomer **5** have been described.[7f] To this end, the isobutyrylated iodobase **10b** was prepared by regioselective iodination of isobutyrylated 5-aza-7-deazaguanine **10a** with iodosuccinimide (→**10b**). Following the literature procedure for the preparation of **10b**, we were encountered with difficulties to obtain the clean iodinated base. Single column chromatography was not sufficient to remove the large amounts of succinimide formed during the reaction. To this end, a modified procedure was developed. Nucleobase **10a** was treated with *N*-iodosuccinimide as described.[7f] After solvent removal, the remaining residue was suspended in water, filtered and washed again. The residue was applied to column chromatography affording the pure base **10b** (39%) (for details see the Exp. Section). Then, nucleobase base **10b** was glycosylated as described resulting in a mixture of anomers **12** and **13** (90% yield).[7f,14] ^{1}H NMR spectra of the prepurified glycosylation mixture revealed a *β/α* ratio of 3:2. Both anomers were separated by crystallization and deprotected afterwards.[7f] Instead of crystallization of the *α*-anomer, the mother liquor from crystallization of the *β*-anomer **12** was deprotected with NH_3/MeOH to yield an inseparable mixture of nucleosides **4** and **5** (87% yield). For separation DMT residues were introduced to protect the 5'-OH groups followed by column chromatography. This resulted in the DMT derivative of the α-nucleoside **14** (40% yield) and its *β*-D counterpart **15** (12%). Compound **14** could be detritylated (trichloro acetic acid) to give **5** in 91% yield (for details, see the Exp. Section and Scheme S1, Supporting Information).

Scheme 1. Synthesis and Separation of Anomeric 7-Iodinated 5-Aza-7-deazaguanine 2'-Deoxyribonucleosides[7f] and DMT Derivatives

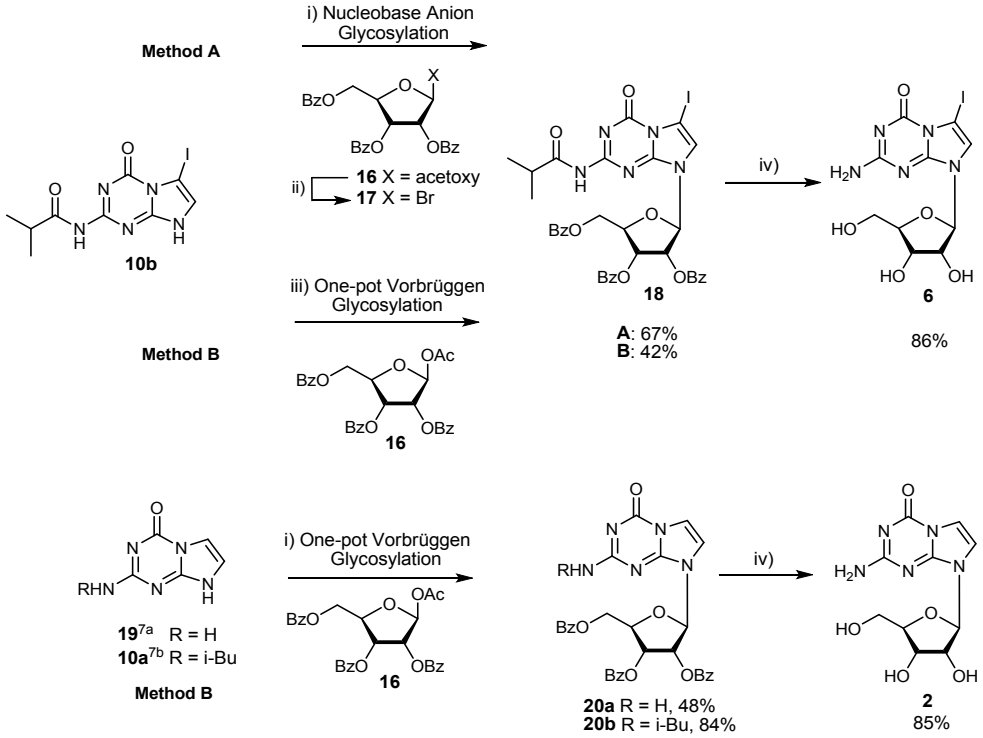


Next, the unknown 7-iodinated 5-aza-7-deazaguanine ribonucleoside **6** was prepared. Attempts to iodinate the non-functionalized ribonucleoside **2** with *N*-iodosuccinimide failed. Consequently, the ribonucleoside **6** was prepared by convergent synthesis utilizing the isobutyrylated iodo base **10b** and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-ribofuranose **16**. To this end, two different glycosylation protocols were employed: the nucleobase anion glycosylation (method A) developed in our laboratory[5a,b] and the so-called one-pot glycosylation (method B) previously used for 7-iodo-7-deaza-2'-deoxyguanosine[15]. In method B the base **10b** was silylated using *N*,*O*-bis(trimethylsilyl) acetamide (BSA). Then, glycosylation was performed with the protected ribose **16** using TMSOTf (Vorbrüggen conditions) in MeCN at 50°C for 16 h. After solvent evaporation and column chromatography compound **18** was isolated in 42% yield. As various other unidentified products were formed the isolation of pure material was time consuming. On the contrary, when the one-pot conditions were applied to the non-

iodinated protected and unprotected nucleobases **10a** and **19**, the protected nucleosides **20a** and **20b** were formed in 48% and 84% yield, respectively (Scheme 2).

In order to increase ribonucleoside formation nucleobase anion glycosylation was performed. The same glycosylation protocol was employed as described for the 2'-deoxyribonucleoside synthesis reported above. To this end, the bromo sugar **17** was prepared *in situ* from the 1-acetoxy compound **16** with a 30% solution of HBr in acetic acid overnight. This protocol does not require HBr gas and works efficiently as it was demostrated earlier.[16] Then, nucleobase anion glycosylation was performed at rt using MeCN as solvent with potassium carbonate and *tris*-[2-(2-methoxyethoxy)ethyl]amine (TDA-1) as catalyst. When the glycosylation was performed with the 5-aza-7-deazapurine base **10a** the reaction was sluggish and the yield of **20b** was only 10% (Scheme S1, Supporting information). This is in line with reactions performed on 7-deazapurines.[17] In contrary, when the iodinated base **10b** was employed the reaction was successful and the protected glycosylation product **18** was isolated in 67% yield (Scheme 2). Purification of the glycosylation products was easier than in case of the one-pot glycosylation. Then, the protecting groups were removed with NH_3/MeOH at rt yielding the iodinated ribonucleoside **6** in 86% yield. The results show that the nucleobase anion glycosylation works only efficiently when the pyrrole or imidazole ring carries electron-withdrawing substituents such as halogens, a phenomenon which was already observed in the synthesis of pyrrolo[2,3-*d*] pyrimidine nucleosides.[15,18] All new synthesized compounds were characterized by ^{1}H-, ^{13}C NMR spectra as well as ESI-TOF mass spectra (see Experimental section). The ^{1}H-^{13}C correlated (HMBC and HSQC) NMR spectra were used to assign the ^{13}C NMR signals. For details see the Experimental section (for spectra, see the Supporting Information).

Scheme 2. Synthesis of 5-Aza-7-deazaguanine Ribonucleosides[a]



[a]*Reagents and Conditions*: i) TDA-1, K_2CO_3, 4h, r.t.; ii) HBr in glacial acetic acid; iii) BSA, TMSOTf, CH_3CN, 16 h, 50°C; iv) NH_3/MeOH r.t, 24 h.

X-ray Analysis of 4, Iodo-Oxygen Interactions and Channel Formation

Earlier, X-ray studies on iodo nucleosides showed that some compounds show strong iodo-iodo interactions in the solid state. Also, an early report on 5-iodo-2'-deoxyuridine with iodo-oxygen contacts exists.[19] These observations prompted us to perform a single-crystal X-ray analysis of compound **4** (Figure 2). As a result, we obtained deeper insight into conformation, hydrogen bonding, nucleoside packing and the role of the iodo substituent of compound **4** in the crystalline state.

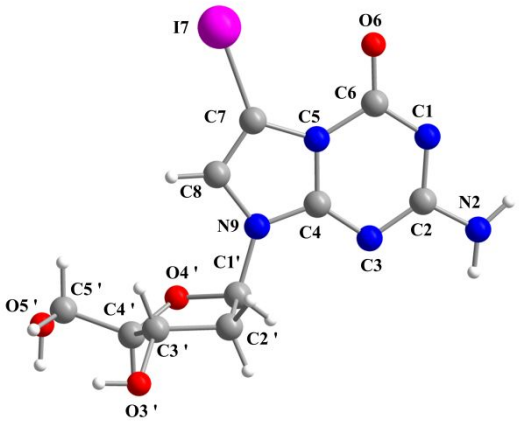


Figure 2. A perspective view of **4** showing the atomic numbering scheme. H-atoms are shown as small spheres of arbitrary size.

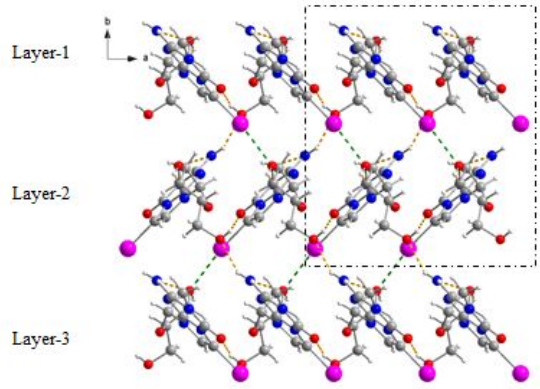
The three-dimensional structure of **4** is shown in Figure 2. For details of the X-ray crystallographic analysis see the Supporting Information. According to the Flack parameter, the anomeric centre shows R-configuration confirming the *β*-D anomer structure of **4**. The orientation of the nucleobase relative to the sugar residue[20] is *anti* with χ (O4'-C1'-N9-C4) = -139.9(6)°. The two principal puckering modes are C3'-*endo* (*N*) and C2'-*endo* (*S*) with preferred values of $P = 0 – 36°$ for C3'-*endo* and $P = 144 – 190°$ for C2'-*endo*.[21,22] The 2'-deoxyribosyl residue of **4** shows a C3'-*endo*-C4'-*exo* ($^3T_4$) sugar pucker with $P = 28.6°$ and $\tau_m = 34.3°$, referring to a *N*-type sugar conformation. This is different from the preferred sugar conformation of **4** observed in solution where the *S* conformation (62%) predominates.[7f] The torsion angle γ (O5'-C5'-C4'-C3') characterizes the orientation of the exocyclic 5'-hydroxy group relative to the sugar ring. For **4**, an antiperiplanar (*trans*) conformation is observed with γ = -172.7(4)°.

Figure 3 displays the crystal packing mode and hydrogen bonds of compound **4**. Hydrogen bonding occurs solely between the nucleobase and the sugar moieties but not between two

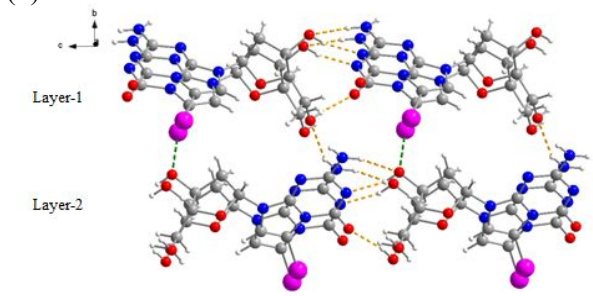
nucleobase moieties (Table S13, Supporting Information). The iodo substituent has a strong contact to oxygen O3' of the sugar residue (I7···O3' = 2.794(4) Å, C7-I7···O3' = 169.2(2)°) while iodo···iodo interactions are not observed. The crystal structure of **4** is composed of different layers and the molecules are ordered in a zig-zag-like arrangement. All intermolecular contacts, hydrogen bonds and the I···O interaction, occur solely between neighbouring layers.

Interestingly, Figure 3c shows formation of channels within the crystal structure. The channels are filled with solvent guest molecules, probably methanol molecules. However, due to the lack of specific interactions between the solvent molecules and compound **4**, the solvent molecules cannot be accurately located and were removed by applying the software tool PLATON/SQUEEZE.[23]

(a)



(b)



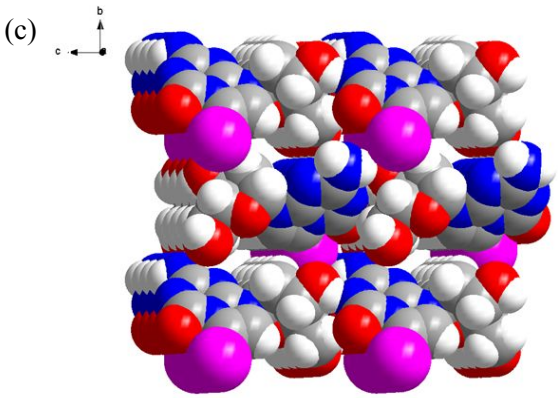


Figure 3. (a), (b) Crystal packing of **4** showing the hydrogen bonds and the iodine and O3' interaction between the different layers. (c) Space filling model of **4** showing channels within the crystal structure (viewed from the *a* direction). Solvent molecules within the channels were removed by applying the program PLATON/SQEEZE.[23]

Hirshfeld surface analysis and 2D fingerprint plots were used to visualize the intermolecular interactions of compound **4** in the solid state.[24] The Crystal Explorer 17 program[25] was used to carry out the Hirshfeld surface analysis mapped over a d_{norm} range of -0.5 to 1.5 Å, shape index (-1.0 to 1.0 Å) and curvedness (-4.0 to 0.4 Å) as well as a 2D fingerprint plot analysis. Figures 4a,b show the molecular Hirshfeld surfaces of **4** mapped over d_{norm}. For shape index and curvedness surfaces see the Supporting Information (Figures S6a-d, Supporting Information). The red areas indicate close contacts as these interactions are shorter than the sum of van-der-Waals radii and show negative d_{norm}. The results of the Hirshfeld analyses are consistent with the hydrogen bonding data (Table S13, Supporting Information). Most important, also the strong interaction between iodine and oxygen O3' is confirmed and appears as two distinct spikes in the fingerprint plots with a proportion of 3.5% (Figure S6e, Supporting Information).

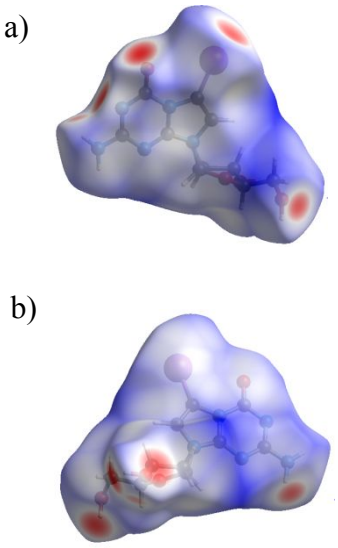


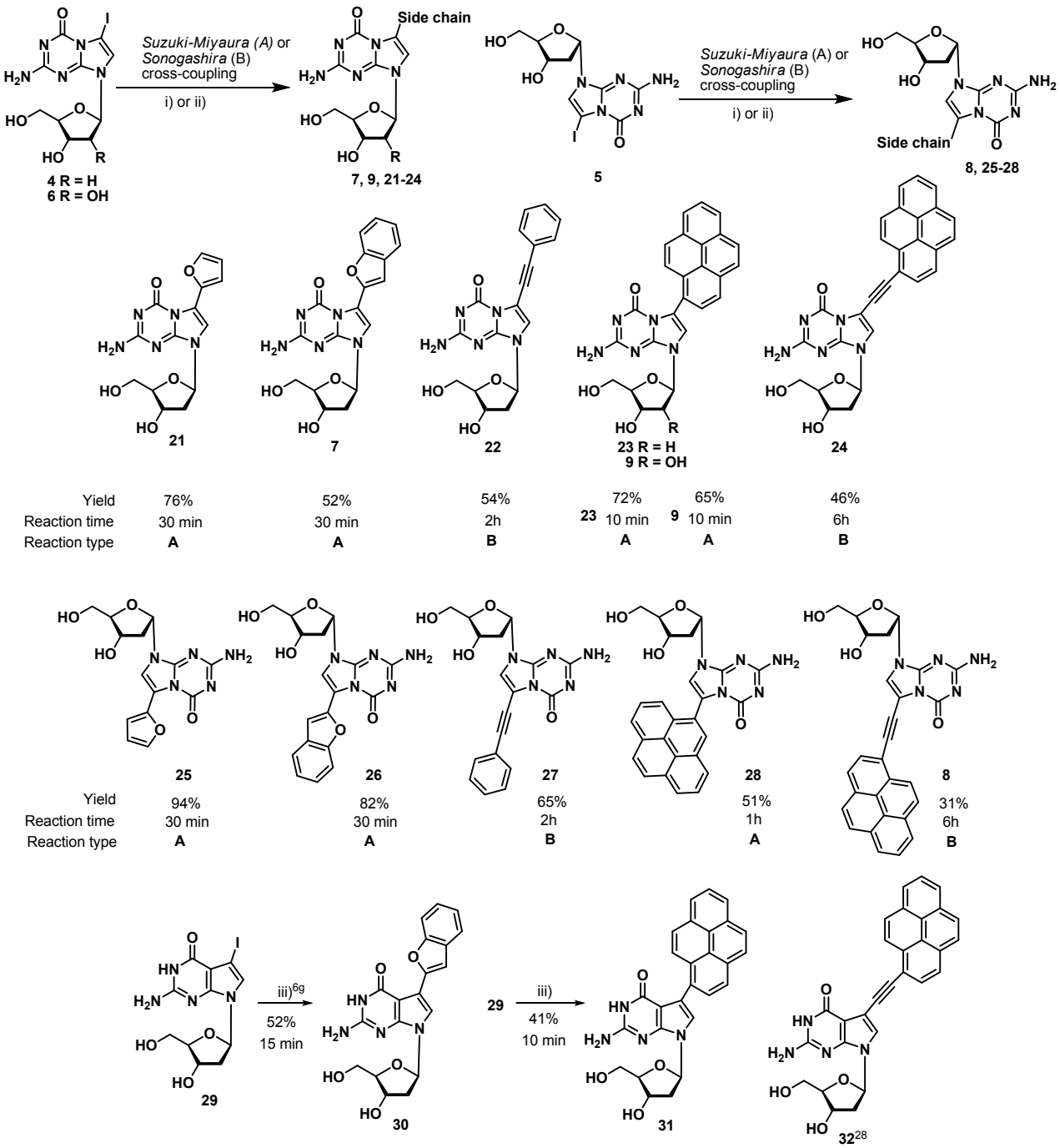
Figure 4. Hirshfeld surface of compound **4** mapped with d_{norm} (-0.5 to 1.5 Å). (a) Front view and (b) back view.

Synthesis of 7-Functionalized 5-Aza-7-Deazaguanine Nucleosides by Sonogashira and Suzuki-Miyaura Cross-coupling

The 7-iodo-5-aza-7-deaza-2'-deoxyguanosine **4**, its α-D anomer **5** as well as the iodo ribonucleoside **6** were then the starting materials for the synthesis of a diversity of side chain derivatives. To this end, alkynes were employed in *Sonogashira* cross-coupling or boronic acids derivatives were used in *Suzuki-Miyaura* reactions.[26] Among the various side chain derivatives, particular attention was given to compounds that show fluorescence as fluorescent 7-deazapurine nucleosides play a key role in the chemical manipulation of nucleic acids.[27] The *α*-anomeric coupling products were prepared as they are suitable for incorporation in DNA with parallel chain orientation or to be used in silver-mediated hybrid base pairs.[10-12]

Three types of 7-functionalized nucleoside derivatives were synthesized: (i) *β*-D 2'-deoxyribonucleosides carrying heterocyclic side chains and pyrene residues, (ii) *α*-D nucleosides that can be incorporated in DNA with parallel chain orientation, (iii) ribonucleosides as building blocks for RNA. Scheme 3 displays the various derivatives and shows reaction details.

Scheme 3. Synthesis of 7-Functionalized 5-Aza-7-deazaguanine *β*-D- and *α*-D-2'-Deoxyribonucleosides and *β*-D Ribonucleosides[a]



[a]*Reagents and Conditions*: i) *Suzuki-Miyaura* cross-coupling (A): $Na_2CO_3 \cdot 10H_2O$, tetrakistriphenylphosphin Pd(0), CH_3CN/H_2O 1:1 and the corresponding boronic acid or boronic acid pinacol ester at 105°C; ii) *Sonogashira* cross-coupling (B): tetrakistriphenylphosphin Pd(0), CuI, *N*-ethyldiisopropylamine, DMF and the corresponding

alkyne at r.t; iii) $CsCO_3$, $Pd(OAc)_2$, TPPTS, CH_3CN/H_2O 1:1 and the corresponding boronic acid. For boronic acids and alkynes used in these reactions, see the Exp. Section.

For *Suzuki-Miyaura* couplings the iodo nucleosides **4-6** were suspended in CH_3CN/H_2O 1:1. Then, Pd(0) (0.1 eq.), sodium carbonate (8 eq.) and the corresponding boronic acid (4 eq.) were added. In a few cases, the reaction proceeded in 10 min. For most compounds longer reaction times were required (see Scheme 3). However, only traces of deiodination were observed in all cases. Formation of the deiodinated nucleoside was confirmed by comparison with an authentic sample. For comparative fluorescence studies the 7-substituted 7-deazaguanine nucleosides **30-32** were prepared by *Suzuki-Miyaura* coupling. Compound **30** and **32** were already described in the literature[6d,28], whereas compound **31** is new. For **30** and **31** different reaction conditions using a combination of TPPTS (triphenylphosphine-3,3',3"-trisulfonic acid trisodium salt hydrate) and $Pd(OAc)_2$ were required. *Sonogashira* cross-coupling reactions of the 5-aza-7-deazaguanine nucleosides **4** and **5** were performed in DMF using tetrakistriphenylphosphin Pd(0) (0.1 eq.), CuI (0.2 eq.) and the corresponding alkyne (2-4 eq.) at r.t. Here, the reaction time for complete consumption of starting material had to be increased compared to *Suzuki* coupling leading to lower product yields as deiodination took place (for details, see the Exp. Section).

Photophysical Properties of 7-Functionalized 5-Aza-7-deazaguanine Nucleosides

As canonical nucleosides of DNA and RNA are virtually non-fluorescent, natural nucleosides were modified in various ways to generate fluorescence.[29] A number of reviews appeared reporting on this matter.[30] We anticipated that 5-aza-7-deazaguanine nucleosides become fluorescent when side chains are introduced that were already successfully employed in the series of other 7-deazapurine nucleosides. Furthermore, microenvironmental factors such as

polarity, viscosity, and base pairing influence the fluorescence of a particular nucleoside dye conjugate. However, we learned from nucleobase functionalized pyrrolo[2,3-*d*]pyrimidine (c^7G_d) or pyrazolo[3,4-*d*]pyrimidine ($c^7z^8G_d$, Figure 1) derivatives that the nucleobase structure has a significant impact on the fluorescence regarding emission maxima and quantum yield.[13,27c,28,29c,29f]

To this end, compounds shown in Scheme 3 were inspected for their fluorescence behaviour. It was found that the benzofuran derivatives **7** (β-D) and **26** (α-D) as well as pyrene derivatives show significant fluorescence, whereas others (phenylacetylen, furan) are only weakly fluorescent. Figure 5a displays UV and fluorescence excitation and emission spectra of **7** and **26** determined in methanol. Both anomers show almost idendical UV and fluorescence spectra with emission maxima at 352 nm when excited at 313 nm. Next, photophysical properties of **7** were determined in solvents of different polarity and the corresponding Stokes shift as well as quantum yields were determined (Table 1). The emission spectra for β-D benzofuran nucleoside **7** in various solvents are displayed in Figure 5b. It appears that fluorescence in aqueous solution is very low compared to other polar solvents. As it was not clear if the phenomenon is due to the benzofuran residue or the 5-aza-7-deazaguanine skeleton the related 7-deazaguanine benzofuran conjugate **30** was measured in the same solvents (Figure 5c and Table 1). Fluorescence quantum yields of the 5-aza-7-deazaguanine nucleoside **7** are almost 2-fold higher than those for the 7-deazaguanine nucleoside **30**. The order of solvent dependent fluorescence for **7** and **30** is very similar with the highest quantum yields in DMSO/DMF with emission maxima which are red-shifted compared to the other solvents. However, significant differences are observed among the two classes of molecules for MeOH (Figure 5b,c). Also, the quantum yields are significantly different (0.59 and 0.04, respectively). This indicates that the two molecules respond on

solvent polarity changes in a different way. Furthermore, they do not show a linear change of fluorescence when related to polarity.

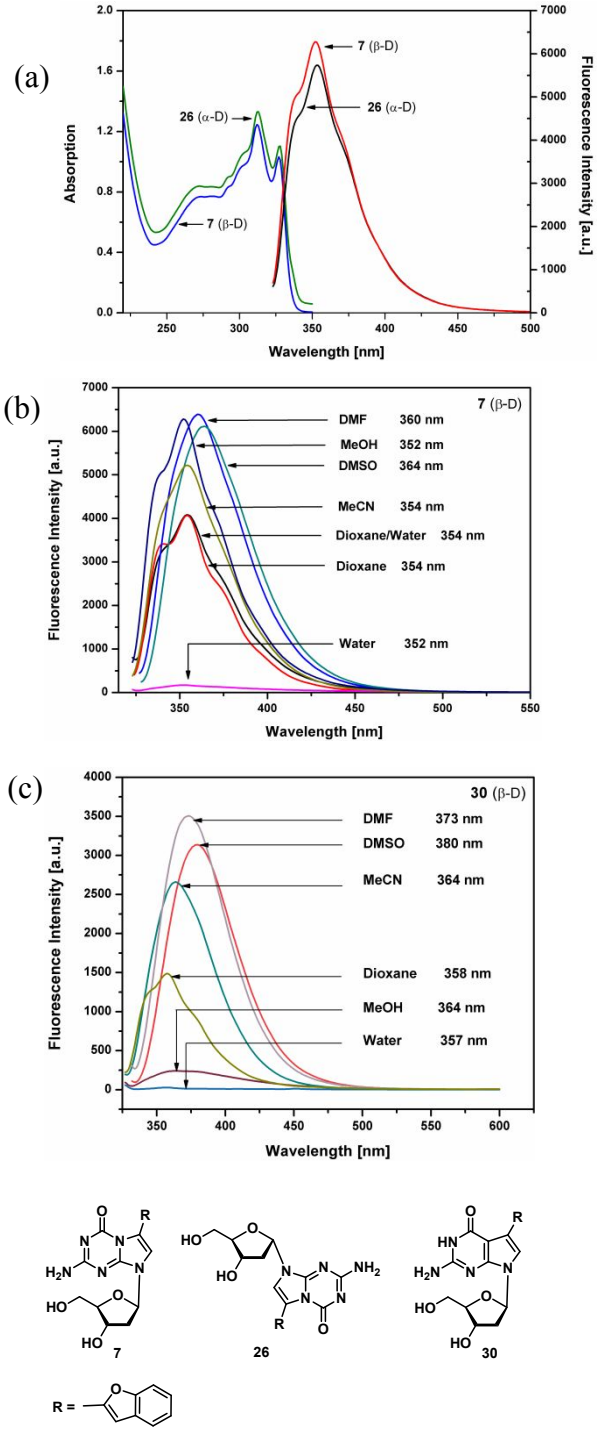
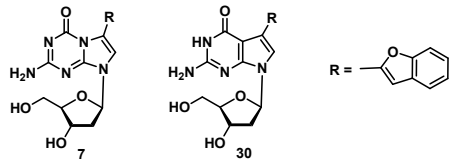


Figure 5. (a) UV and fluorescence emission spectra of **7** (β-D) and **26** (α-D) measured in methanol (50 μM for UV and 1 μM for fluorescence). Excitation wavelengths of nucleosides **7** (313 nm) and **26** (313 nm). Fluorescence emission spectra of nucleoside **7** (b) and **30** (c)

were measured in various solvents with a nucleoside concentration of 1 µM (for details see Table S1, Supporting Information).

Table 1. Photophysical Data of 7-Benzofuranosyl-5-aza-7-deaza-2'-deoxyguanosine (**7**) and 7-Benzofuranosyl-7-deaza-2'-deoxyguanosine (**30**) Measured in Solvents of Different Polarity[a]

	Solvent	$\lambda_{abs, max}$ Excitation (nm)	$\lambda_{max, em}$ Emission (nm)	Stokes shift ($\Delta\nu$)[b] (cm^{-1})	Φ[c]
7	DMSO	318	364	4000	0.69
	DMF	316	360	3900	0.68
	MeOH	313	352	3500	0.59
	MeCN	312	354	3800	0.47
	Dioxane	314	354	3700	0.26
	Water	311	352	3700	0.03
30	DMF	321	373	4300	0.38
	DMSO	322	380	4700	0.37
	MeCN	317	364	4100	0.25
	Dioxane	317	358	3600	0.13
	MeOH	317	364	4100	0.04
	Water	317	357	3500	<0.01



[a] The concentration of nucleosides **7** and **30** was 1 µM. [b] The Stokes shift was calculated from the equation $\Delta E_{photon} = hc(1/\lambda_{abs,max} - 1/\lambda_{max, em})$. [c] The fluorescence quantum yields (Φ) were calculated using quinine sulfate in 0.1 M H_2SO_4 ($\Phi_{St} = 0.54$).

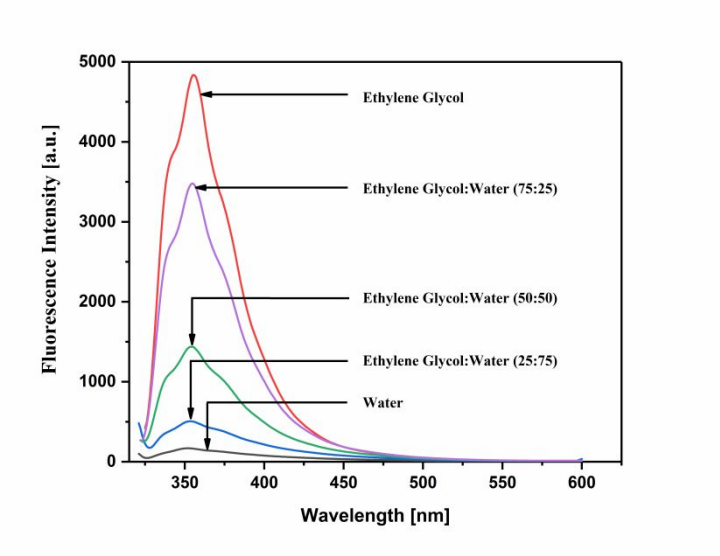
The conformation of the benzofuran moiety to the nucleobase forms a rotatable system, which is sensitive to molecular crowding and viscosity. High viscous media restrict rotation and enhance fluorescence.[31] This phenomenon was already reported for 2'-deoxyuridine nucleosides decorated with a benzofuran residue at position-5.[31b-e] Fluorescence was significantly increased in glycerol or ethylenglycol compared to water. Now, this matter was proven on the nucleoside benzofuran conjugate **7**.

Figure 6 displays the fluorescence emission spectra of **7** in glycerol, water and mixtures thereof. The nucleoside is low-emissive in water and also low-emissive in glycerol. However,

in glycerol/water mixtures the fluorescence increases with increasing glycerol content. Nevertheless, this is only valid for a glycerol content of about 50%. A higher proportion of glycerol leads to a fluorescence decrease. So, nucleoside **7** behaves different compared to 2'-deoxyuridine conjugates and shows the expected immobilization of the benzofuran moiety accompanied by fluorescence increase only when the glycerol content of the water/glycerol mixtures are below 50%. An explanation for this unusual behaviour cannot be given now, however it is apparent from differences in the shape of the fluorescence spectra that other factors account for this behaviour. In the ethylene glycol/water system the fluorescence depends on the viscosity in the expected way. It increases with increasing solvent viscosity as it was reported for pyrimidine benzofuran conjugates before (Figure 6b).[31b-e]

Normally, the low fluorescence intensity of nucleoside **7** in water is a disadvantage for application in bio-related systems. However, the phenomenon of fluorescence increase caused by immobilization of the benzofuran moiety is applicable to detect binding to biopolymers e.g. proteins or to study structural changes of oligonucleotide assemblies. As rotational motion is reduced during polymer binding the fluorescence increase is a valuable tool to detect interactions between molecules.[29c,31c,31e,31f]

a)



b)

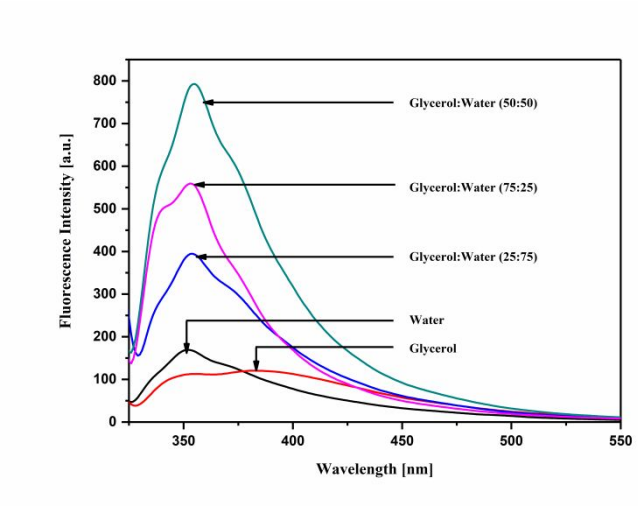


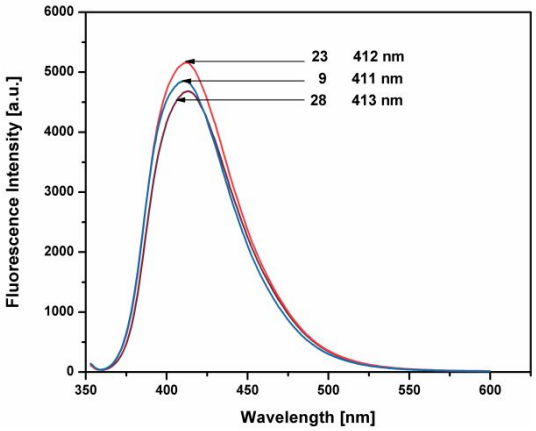
Figure 6. Fluorescence emission spectra of the 5-aza-7-deaza benzofuran conjugate **7** in (a) ethylenglycol and (b) glycerol. Excitation wavelength of nucleoside **7** was 312 nm in glycerol and 315 nm in ethylene glycol.

Next, the photophysical properties of the pyrene nucleoside conjugates were studied. Pyrene is a polycylcic aromatic molecule representing the class of photoexcitable dyes.[32] This behaviour makes it useful for studies on nucleic acids and other biopolymers. The molecule shows monomer and excimer fluorescence which is sensitive to the microenvionment.[33]

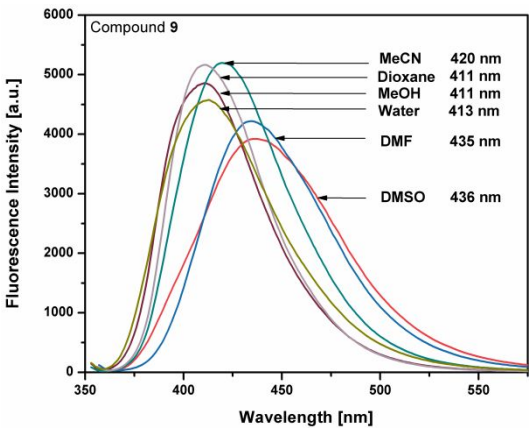
Excimer fluorescence is a useful tool for the detection of environmental changes and neighbouring effects in DNA duplexes.[27c]

The photophysical properties of 5-aza-7-deazaguanine pyrene conjugates **8**, **9**, **23**, **24** and **28** were determined in various solvents of different polarity and compared to the pyrene conjugates **31**, **32** of the related 7-deaza-2'-deoxyguanosine (Table 2 and Table S3, Supporting Information). The pyrene was either directly linked to position-7 of the nucleobase or connected *via* an alkynyl linker. Figure 7 shows fluorescence spectra and solvent dependencies.

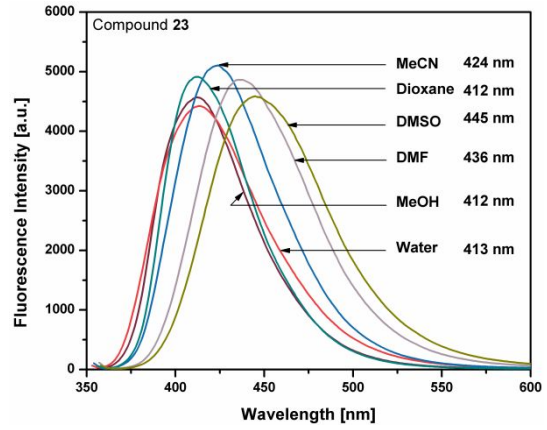
(a)



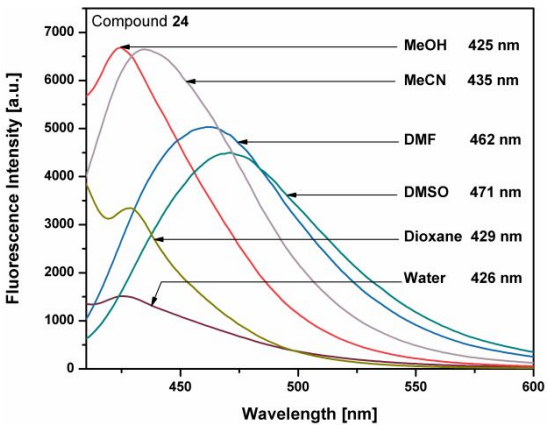
(b)



(c)



(d)



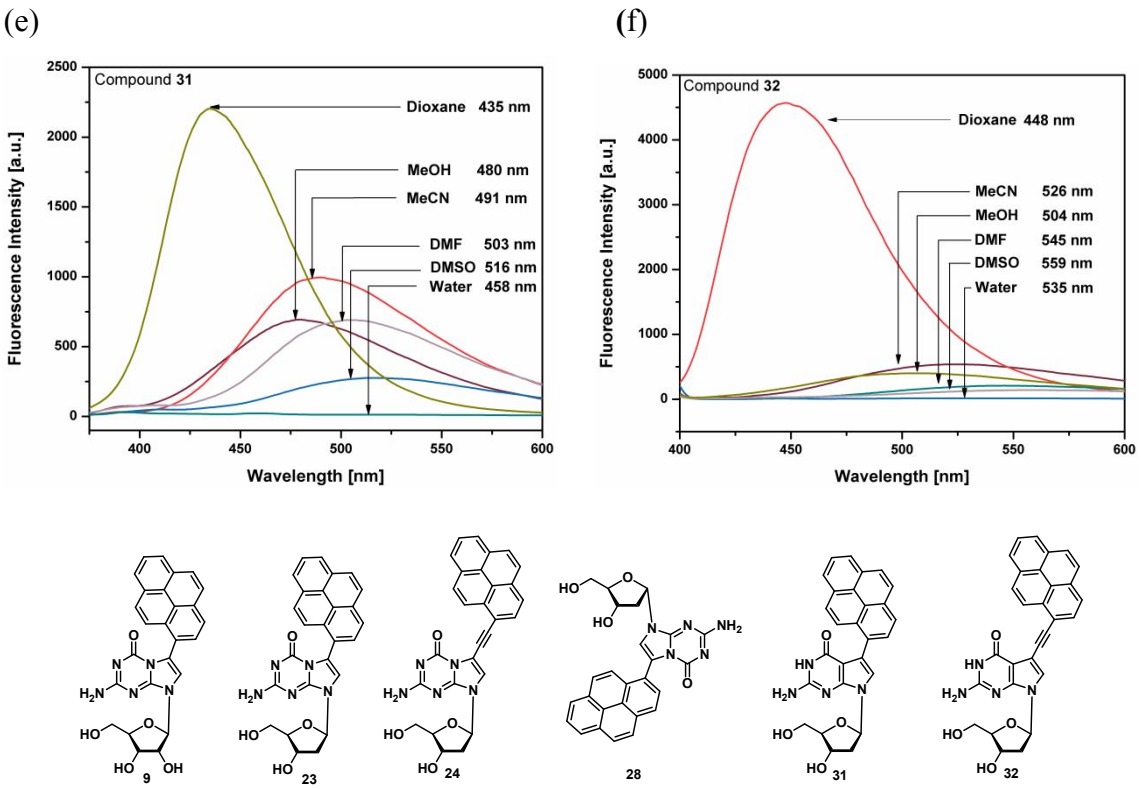
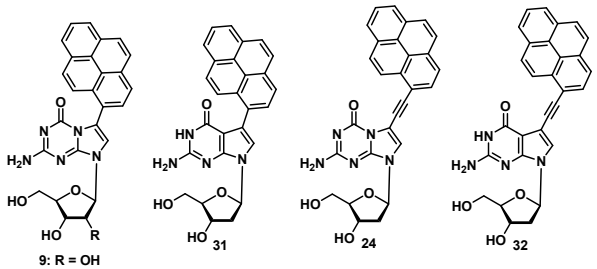


Figure 7. (a) Fluorescence emission spectra of pyrene nucleosides determined in MeOH. Fluorescence emission spectra of **9** (b), **23** (c), **24** (d), **31** (e) and **32** (f) measured in various solvents with a nucleoside concentration of 1 μM. For compound **24** the concentration in dioxane was 0.2 μM (for details see Table 2).

Table 2. Photophysical Data of 7-Pyrenyl Nucleosides 9, 23, 24, 31 and 32 Measured in Solvents of Different Polarity[a]

	Solvent	$\lambda_{abs,\ max}$ Ex [nm]	$\lambda_{max,\ em}$ Em [nm]	Stokes shift ($\Delta\nu$)[b] [cm^{-1}]	Φ[c]
9	DMSO	348	436	5800	0.68
	DMF	347	435	5800	0.59
	MeCN	343	420	5300	0.59
	Dioxane	346	411	4600	0.56
	Water	343	413	4900	0.52
	MeOH	343	411	4800	0.45
23	DMF	347	436	5900	0.71
	Dioxane	346	412	4600	0.50
	DMSO	348	445	6300	0.70
	MeOH	343	412	4900	0.40
	MeCN	344	424	5500	0.54
	Water	343	413	4900	0.53
31	Dioxane	350	435	5600	0.39
	MeCN	349	491	8300	0.24
	MeOH	346	480	8100	0.17
	DMF	356	503	8200	0.11
	DMSO	359	516	8500	0.11
	Water	345	458	7200	<0.01
24	DMF	397	462	3500	0.57
	MeCN	392	435	2500	0.57
	DMSO	400	471	3800	0.56
	Dioxane	396	429	1700	0.52[d]
	MeOH	391	425	2100	0.46
	Water	391	426	2300	0.24
32	Dioxane	389	448	3400	0.60
	MeCN	389	526	6700	0.08
	MeOH	389	504	5900	0.06
	DMF	389	545	7400	0.03
	DMSO	389	559	7800	0.03
	Water	389	535	7000	<0.01



[a] The concentration of nucleosides was 1 μM. [b] The Stokes shift was calculated from the equation $\Delta E_{photon} = hc(1/\lambda_{abs,max} - 1/\lambda_{max,em})$. [c] The fluorescence quantum yields (Φ) were calculated using quinine sulfate (1 μM) in 0.1 M H_2SO_4 ($\Phi_{St} = 0.54$). [d] Determined with a concentration of 0.2 μM.

According to Figure 7 and Table 2 pyrene nucleosides derived either from α- or β-D 5-aza-7-deaza-2'-deoxyguanosine (**23, 28**) or the ribonucleoside 5-aza-7-deazaguanosine (**9**) show almost identical fluorescence spectra. The emission maxima of the pyrene conjugate **23** are significantly red-shifted (~20 nm) in polar aprotic solvents (DMF, DMSO) (Figure 7). Solvent dependent measurements of the directly conjugated pyrene 5-aza-7-deazaguanine ribonucleoside **23** gave high quantum yields in all cases (Φ = 0.4-0.7). The same is ~~true~~ valid for nucleosides **9** and **28**. Triple bond pyrene conjugates **8** and **24** show similar fluorescence properties as the directly attached pyrene conjugate **23**. Here, a red-shift of the excitation wavelengths (~50 nm) leads to a reduced Stokes shift in all solvents. Furthermore, the quantum yield in water is decreased (Figure 7 and Figure S1, Supporting Information). The related 7-deaza-2'-deoxyguanosine pyrene conjugate **31** (directly connected) shows fluorescence with much lower quantum yields and a stronger solvent dependency as observed for the 5-aza-7-deazaguanine conjugate **23**. The 7-deazaguanine pyrene nucleoside **32** connected *via* alkynyl linker is almost non-fluorescent. Only in dioxane the compound becomes fluorescent (Figure 7f).

Main differences exist between fluorescence of the benzofuran 5-aza-7-deazguanine residues **7** and **26** and the pyrene conjugates **24** and **31**. (i) The excitation and emission maxima of the pyrene conjugates are significantly red-shifted. (ii) The directly connected pyrene conjugate **23** and **9** shows minor solvent dependencies whereas the benzofuran conjugates **7** and **26** show strong solvent dependency.

The different quenching effects observed for the fluorescence of the 5-aza-7-deazaguanine and 7-deazaguanine pyrene conjugates most probably result from charge separation between the pyrene residues and the nucleobases (intramolecular electron transfer or hole transfer). It is reported that 7-deazapurine conjugates form a charge separated state with a nucleobase radical cation and a radical anion of pyrene.[27c,34] A similar behaviour is expected for 5-aza-7-

deazapurine pyrene conjugates. The higher quantum yields of the 5-aza-7-deazapurine pyrene conjugates might result from a higher oxidation potential of the 5-aza-7-deazaguanine to 7-deazaguanine. None of the pyrene compounds shows excimer fluorescence.

CONCLUSION

Nucleobase anion glycosylation of isobutyrylated 5-aza-7-deaza-7-iodoguanine (**10b**) with the bromo sugar of 2,3,5-tri-*O*-benzoyl-1-*O*-acetyl-D-ribofuranose **16** gave the pure β-D anomeric N-9 glycosylation product **18** in 67% yield. The corresponding one-pot glycosylation performed with the silylated base under Vorbrüggen condition (TMSOtriflate) resulted in lower yield and the glycosylation product was difficult to purify. When glycosylation of the non-iodinated base **19** was performed using the Vorbrüggen protocol, the yield was the same (84%) as that reported previously under $SnCl_4$ catalysis[7a]. All glycosylation products – ribo- and anomeric 2'-deoxyribo compounds – were deprotected to yield the free nucleosides **2**-**6**. The crystal structure of **4** showed intermolecular contacts with I···O interaction. Channels are formed in the crystal, that are filled with solvent guest molecules.

With the help of iodo nucleosides as starting materials a diversity of side chain derivatives were synthesized employing either *Sonogashira* or *Suzuki-Miyaura* cross-coupling. For the first time, functionalized 5-aza-7-deazaguanine nucleosides were accessible which were inspected for their photophysical properties. Among those, the benzofuran and pyrene derivatives show strong fluorescence and high quantum yields. Fluorescence spectra measured in various solvents show solvatochromism[35]. Higher quantum yields were obtained for 5-aza-7-deazaguanine nucleoside conjugates compared to those with a 7-deazaguanine

skeleton. The benzofuran conjugate **7** shows increase fluorescence in viscous media (ethylene glycol, glycerol) compared to water.

Functionalized 5-aza-7-deaza nucleosides reported in this work can be utilized as starting materials for chemical (phosphoramidites) or enzymatic synthesis (triphosphates) of modified DNA or RNA. According to the special recognition pattern of the 5-aza-7-deazaguanine base that lacks a hydrogen at position-1 base pairs with purines can be constructed[7c,10] and orthogonal base pairs with "protonated" dC analogues or silver ions can be formed to extent the 4-letter code of the genetic system.[9] The side chain derivatives with anomeric α-D configuration represent building blocks for duplex DNA with parallel chain orientation.[7c]

EXPERIMENTAL SECTION

General Methods and Materials. All chemicals and solvents were of laboratory grade as obtained from commercial suppliers and were used without further purification. Thin-layer chromatography (TLC) was performed on TLC aluminium sheets covered with silica gel 60 F254 (0.2 mm). Flash column chromatography (FC): silica gel 60 (40-60 μM) at 0.4 bar. UV-spectra were recorded on a UV-spectrophotometer: λ_{max} (ε) in nm, ε in $dm^3\ mol^{-1}\ cm^{-1}$. NMR spectra were measured at 599.74 MHz or 399.89 MHZ for ^1H and 150.82 MHz or 100.56 MHz for ^{13}C. ^1H-^{13}C correlated (HMBC, HSQC) NMR spectra were used for the assignment of the ^{13}C signals (Tables S1-2, Supporting Information). The *J* values are given in Hz; δ values in ppm relative to Me_4Si as internal standard. For NMR spectra recorded in DMSO-d_6, the chemical shift of the solvent peak was set to 2.50 ppm for ^1H NMR and 39.50 ppm for ^{13}C NMR. ESI-TOF mass spectra of nucleosides were recorded on a Micro-TOF spectrometer.

2-[(N^2-Isobutyryl)amino]-6-iodo-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (10b).[7f] To the stirred suspension of 2-[(N^2-Isobutyryl)amino]-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one[7b] (**10a**; 5.4 g, 17.53 mmol) in anh. CH_2Cl_2 (500 mL) was added *N*-iodosuccinimide (4.73 g, 21.03 mmol) in one portion at r.t. Stirring was continued for 30 min and the solvent was evaporated. The residue was stirred with water (25 mL) for 15 min. and then filtered. The residue was washed with another 25 mL water. The dry residue was applied to FC (silica gel, CH_2Cl_2/MeOH 100:2). Evaporation of the main zone gave compound **10b** (2.4 g, 39%) as yellowish powder. TLC (silica gel, CH_2Cl_2/MeOH 20:1) R_f 0.2. λ_{max} (MeOH)/nm 237 (ε/dm^3 mol^{-1} cm^{-1} 43300), 284 (10500). ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.98 (dd, *J* = 10.0, 6.9 Hz, 6H, CHCH_3), 2.87 (p, *J* = 6.8 Hz, 1H, CHCH$_3$), 4.70 (dd, *J* = 11.9, 5.9 Hz, 1H, H-5'), 4.77 (dd, *J* = 12.0, 4.2 Hz, 1H, H-5'), 4.86 (td, *J* = 5.9, 4.2 Hz, 1H, H-4'), 6.20 (dd, *J* = 6.0, 4.0 Hz, 1H, H-2'), 6.28 (t, *J* = 6.0 Hz, H-3'), 6.34 (d, *J* = 3.9 Hz, 1H, H-1'), 7.43-7.51 (m, 6H, arom. H), 7.63-7.67 (m, 3H, arom. H), 7.88-7.92 (m, 5H, H-7, arom. H), 7.95-7.98 (m, 2H, arom. H), 10.42 (s, 1H, NH). Analytical data were identical to those obtained earlier.[7f]

Glycosylation of 10b with 2-Deoxy-3,5-di-*O*-toluoyl-α-D-*erythro*-pentofuranosyl Chloride (11). As described in the literature[7f] with 2-[(N^2-Isobutyryl)amino]-6-iodo-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one **10b** (800 mg, 2.30 mmol), K_2CO_3 (1.01 g, 7.31 mmol), TDA-1 (0.1 mL, 0.29 mmol), 2-deoxy-3,5-di-*O*-toluoyl-α-D-*erythro*-pentofuranosyl chloride (1.43 g, 3.68 mmol) in CH_3CN (80 mL). After FC (silica gel, column, 12 x 4 cm, CH_2Cl_2/MeOH 100:1→95:5) an anomeric mixture of **12** and **13** (1.44 g, 90%) was obtained as colorless foam. ^{1}H NMR of the anomeric mixture showed a 3:2 *β* to *α* ratio. Crystallization of the residue from MeOH afforded the *β*-D anomer **12** (627 mg, 39%). Lit.[7f]: 31%. Crystallization from PE/EtOAC of the solid obtained after evaporation of the mother liquor from the first crystallization afforded

the α-D anomer **13** (521 mg, 33%). Lit.[7f]: 27%. Analytical data were identical to those reported in the literature.[7f]

Separation of the Anomeric Glycosylation Mixture by 4,4'-Dimethoxytritylation. In a separate experiment the mother liquor from glycosylation obtained after crystallization of the β-D anomer **12** was evaporated to a colorless foam (2.05 g, 2.93 mmol). Then, NH_3/MeOH (100 mL) was added and the reaction mixture was stirred overnight at r.t. The solvent was evaporated and the remaining residue was applied to FC (silica gel, column 12 x 3 cm, CH_2Cl_2/MeOH 100:1→9:1). Evaporation of the main zone gave the anomeric mixture of **4** and **5** as yellowish solid (1.0 g, 87%). Then, 200 mg (0.51 mmol) of this mixture were dissolved in pyridine (15 mL), 4,4'-dimethoxytritylchloride (237 mg, 0.61 mmol) was added and the reaction mixture was stirred for 5 h at ambient temperature. The solvent was evaporated and co-evaporated with toluene (2 x 10 mL). The remaining residue was applied to FC (silica gel, column 10 x 2 cm, CH_2Cl_2/acetone, 85 :15→70:30) and the anomers were separated.

2-Amino-8-[2-deoxy-5-*O*-(4,4'-dimethoxytriphenylmethyl)-*α*-D-*erythro*-pentofuranosyl)]-6-iodo-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (14). From the faster migrating zone compound **14** was obtained as colorless foam (143 mg, 40%). TLC (silica gel, CH_2Cl_2/MeOH, 95:5) R_f 0.4. λ_{max} (MeOH)/nm 266 (ε/dm^3 mol^{-1} cm^{-1} 19600). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.21 (dt, J = 14.2, 2.4 Hz, 1H, H-2'$_\alpha$), 2.67 (ddd, J = 14.2, 7.8, 6.3 Hz, 1H, H-2'$_\beta$), 2.97 (dd, J = 10.2, 4.6 Hz, 1H, H-5'), 3.09 (dd, J = 10.1, 4.0 Hz, 1H, H-5'), 3.739 (s, 3H, OCH_3), 3.740 (s, 3H, OCH_3), 4.25 (m, 2H, H-3', H-4'), 5.56 (d, J = 3.4 Hz, HO-3'), 6.21 (dd, J = 7.8, 2.6 Hz, 1H, H-1'), 6.86-7.00 (m, 6H, arom. H, NH_2), 7.18-7.27 (m, 5H, arom. H), 7.33 (dd, J = 8.6, 7.0 Hz, 2H, arom. H), 7.35-7.40 (m, 2H, arom. H), 7.63 (s, 1H, H-6). ^{13}C NMR (DMSO-d_6,

151 MHz): δ 39.3, 550, 56.9, 63.8, 68.5, 70.9, 83.9, 85.6, 87.2, 113.3, 122.1, 126.7, 127.7, 127.9, 129.7, 135.4,2, 135.49, 144.7, 150.2, 150.4, 158.1, 164.3. HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{31}H_{30}IN_5O_6H$ 696.1314; Found 696.1308.

2-Amino-8-[2-deoxy-5-*O*-(4,4'-dimethoxytriphenylmethyl)-*β*-D-*erythro*-pentofuranosyl)]-6-iodo-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (15). From the slower migrating zone compound **15** was obtained as colorless foam (41 mg, 12%). TLC (silica gel, CH_2Cl_2/MeOH, 95:5) R_f 0.25. λ_{max} (MeOH)/nm 266 (ε/dm^3 mol^{-1} cm^{-1} 18300). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.22 (ddd, J = 13.4, 6.5, 4.6 Hz, 1H, H-2'$_\alpha$), 2.47-2.52 (m, 1H, H-2'$_\beta$), 3.10 (dd, J = 10.4, 3.3 Hz, 1H, H-5'), 3.16 (dd, J = 10.4, 5.8 Hz, 1H, H-5'), 3.89 (dt, J = 5.6, 3.7 Hz, 1H, H-4'), 4.34 (dt, J = 8.8, 4.5 Hz, 1H, H-3'), 5.33 (d, J = 4.4 Hz, 1H, HO-3'), 6.14 (t, J = 6.4 Hz, 1H, H-1'), 6.85-6.88 (m, 4H, arom. H), 6.99 (s, 2H, NH_2), 7.19-7.26 (m, 4H, arom. H), 7.29 (dd, J = 8.5, 7.0 Hz, 2H, arom. H), 7.33-7.39 (m, 2H, arom. H), 7.42 (s, 1H, H-6). ^{13}C NMR (DMSO-d_6, 151 MHz): δ 38.7, 55.05, 57.7, 63.8, 70.1, 82,7, 85.6, 85.7, 113.2, 120.9, 126.7, 127.7, 127.9, 129.7, 135.4, 135.6, 144.8, 150.2, 150.6, 158.1, 164.3. HRMS (ESI-TOF) m/z: [M + Na$^+$] Calcd for $C_{31}H_{30}IN_5O_6Na$ 718.1133; Found 718.1114.

2-Amino-8-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-iodo-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (4). Compound **4** was prepared according to a literature protocol[7f] with **12** (1.25 g, 1.78 mmol) and 7M NH_3/MeOH (60 mL). From FC compound **4** (646 mg, 92%) was obtained as colorless solid. Recrystallization from MeOH gave colorless crystals. CCDC No. 1917214. M.P.: 159-161 °C. Lit.[7f]: 92%. HRMS (ESI-TOF) m/z: Calcd for [M + Na$^+$] $C_{10}H_{12}IN_5O_4Na$ 415.9826; Found 415.9826. Analytical data were identical to those reported earlier.[7f]

2-Amino-8-(2-deoxy-*α*-D-*erythro*-pentofuranosyl)-6-iodo-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (5). Compound **14** (80 mg, 0.12 mmol) was dissolved in CH_2Cl_2 (5 mL) and treated with trichloroacetic acid (400 µL of a 3% soln. in CH_2Cl_2). The mixture was stirred for 16 h at r.t. Then, triethylamine (200 µL) was added to the solution and the solvent was evaporated. The remaining residue was applied to FC (silica gel, column 10 x 3 cm, CH_2Cl_2/MeOH, 100:1→9:1). From the main zone compound **5** was obtained as colorless solid (41 mg, 91%). TLC (silica gel, CH_2Cl_2/MeOH, 9:1) R_f 0.4. HRMS (ESI-TOF) m/z: Calcd for [M + Na^+] $C_{10}H_{12}IN_5O_4Na$ 415.9826; Found 415.9822. Analytical data were identical to those reported earlier.[7f]

2-[(*N*^{2}-Isobutyryl)amino]-8-[(2,3,5-tri-*O*-benzoyl)ribofuranosyl]-6-iodo-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (18). Method A: To a solution of 2,3,5-tri-*O*-benzoyl-1-*O*-acetyl-D-ribofuranose **16** (2.0 g, 3.96 mmol) in dichloromethane (12 mL) was added a 30% soln. of HBr in acetic acid (2.4 mL).[16] The mixture was stirred at r.t. for 16 h and evaporated to dryness. The syrup was dissolved in CH_2Cl_2 (20 mL) washed with sat. aq. $NaHCO_3$ soln. (10 mL), dried (Na_2SO_4) filtrated and concentrated to a syrup. The halogenose was dissolved in CH_3CN (8 mL) and introduced into a suspension of compound **10b** (761 mg, 2.2 mmol), K_2CO_3 (1.66 g, 12.06 mmol) and TDA-1 (0.1 mL, 0.32 mmol) in MeCN (100 mL). Then, the reaction mixture was stirred at ambient temperature for 5 h, filtered and the solvent of the filtrate was evaporated. The remaining residue was applied to FC (silica gel, column 12 x 3 cm, CH_2Cl_2/MeOH 100:1→3:1) to give **18** (1.16 g, 67%) as colorless foam. TLC (silica gel, CH_2Cl_2/MeOH, 95:5) R_f 0.7. λ_{max} (MeOH)/nm 237 (ε/dm^3 mol^{-1} cm^{-1} 43300), 284 (10500). ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.98 (dd, J = 10.0, 6.9 Hz, 6H, CH*CH$_3$*), 2.87 (p, J = 6.8 Hz, 1H, *CH*CH_3), 4.70 (dd, J = 11.9, 5.9 Hz, 1H, H-5'), 4.77 (dd, J = 12.0, 4.2 Hz, 1H, H-5'), 4.86 (td, J = 5.9, 4.2 Hz, 1H, H-4'), 6.20 (dd, J = 6.0, 4.0 Hz, 1H, H-2'), 6.28 (t, J = 6.0 Hz,

H-3'), 6.34 (d, J = 3.9 Hz, 1H, H-1'), 7.43-7.51 (m, 6H, arom. H), 7.63-7.67 (m, 3H, arom. H), 7.88-7.92 (m, 5H, H-7, arom. H), 7.95-7.98 (m, 2H, arom. H), 10.42 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 151 MHz): 18.90, 18.95, 34.6, 59.2, 63.8, 70.9, 73.7, 79.7, 87.2, 123.9, 128.37, 128.62, 128.72, 129.15, 129.20, 129.24, 129.26, 129.40, 129.47, 133.80, 133.96, 150.11, 150.25, 159.9, 164.42, 165.382, 165.389, 175.8. HRMS (ESI-TOF) m/z: [M + Na^+] Calcd for $C_{35}H_{30}IN_5O_9Na$ 814.0980; Found 814.0968.

Method B: To a stirred suspension of compound **10b** (200 mg, 0.58 mmol) in anhydrous MeCN (10 mL) was added BSA (176 mg, 0.2 mL, 0.86 mmol) at rt. After stirring for 30 min, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**16**) (436 mg, 0.86 mmol) was added, then TMSOTf (141 mg, 0.115 mL, 0.63 mmol) was introduced. The reaction mixture was stirred at 50°C for 16 h. The solution was cooled to room temperature and diluted with CH_2Cl_2 (50 mL). The organic phase was washed with sat. aq. $NaHCO_3$ and brine, dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by FC (silica gel, column 15 x 3 cm, CH_2Cl_2/MeOH, 100:1→3:1) gave **18** (190 mg, 42%) as a yellowish foam.

2-Amino-8-ribofuranosyl-6-iodo-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (6). Compound **18** (215 mg, 0.27 mmol) was suspended in methanolic ammonia (40 mL). The mixture was stirred at r.t. overnight. The solvent was evaporated and the residue was purified by FC (silica gel, column 10 x 2 cm, CH_2Cl_2/MeOH 100:1→3:1) to give **6** (95 mg, 86%) as a colorless solid. TLC (silica gel, CH_2Cl_2/MeOH, 4:1) R_f 0.2. λ_{max} (MeOH)/nm 266 (ε/dm^3 mol^{-1} cm^{-1} 11600). ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.52 (ddd, J = 12.0, 5.3, 3.8 Hz, 1H, H-5'), 3.60 (ddd, J = 11.9, 5.3, 3.9 Hz, 1H, H-5'), 3.85 (q, J = 3.7 Hz, 1H, H-4'), 4.04 (q, J = 4.0 Hz, 1H, H-3'), 4.24 (q, J = 5.4 Hz, 1H, H-2'), 5.07 (t, J = 5.3 Hz, 1H, OH-5'), 5.16 (d, J = 4.5 Hz, 1H, OH-3'), 5.44 (d, J = 5.6 Hz, 1H, OH-2'), 5.74 (d, J = 5.9 Hz, 1H, H-1'), 6.97 (s, 2H, NH_2), 7.61 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6, 151 MHz): 57.8, 61.1, 70.2, 73.5, 85.4, 86.2, 121.1,

150.2, 151.1, 164.3. HRMS (ESI-TOF) m/z: [M + Na^+] Calcd for $C_{10}H_{12}IN_5O_5Na$ 431.9775; Found 431.9774.

2-Amino-8-[(2,3,5-tri-*O*-benzoyl)ribofuranosyl]-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (20a).[7a] To a stirred suspension of compound **19** (100 mg, 0.66 mmol) in anhydrous MeCN (5 mL) was added BSA (148 mg, 0.18 mL, 0.73 mmol) at r.t. After stirring for 30 min, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**16**) (374 mg, 0.74 mmol) was added, then TMSOTf (159 mg, 0.13 mL, 0.71 mmol) was introduced. The reaction mixture was stirred at 50 °C for 16 h. The solution was cooled to room temperature and diluted with CH_2Cl_2 (50 mL). The organic phase was washed with sat. aq. $NaHCO_3$ and brine, dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by FC (silica gel, column 12 x 3 cm, CH_2Cl_2/MeOH, 100:1→95:5) to give **20a** (187 mg, 48%) as colorless foam. TLC (silica gel, CH_2Cl_2/MeOH, 95:5) R_f 0.5. λ_{max} (MeOH)/nm 238 (ε/dm^3 mol^{-1} cm^{-1} 32500), 259 (15300). ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.69 (dd, J = 12.0, 5.8 Hz, 1H, H-5'), 4.77 (dd, J = 12.0, 4.2 Hz, 1H, H-5'), 4.84 (td, J = 5.5, 4.2 Hz, 1H, H-4')), 6.03-6.07 (m, 1H, H-2'), 6.19 (dd, J = 6.2, 5.1 Hz, 1H, H-3'), 6.31 (d, J = 5.1 Hz, 1H, H-1'), 5.06 (t, J = 4.9 Hz, 1H, OH-5'), 6.98 and 7.09 (2s, 2H, NH_2), 7.43 (d, J = 2.8 Hz, 1H, H-6), 7.46 (dt, J = 16.0, 7.9 Hz, 4H, arom. H), 7.51 (t, J = 7.9 Hz, 2H, arom. H), 7.55 (d, J = 2.8 Hz, 1H, H-7), 7.63-7.69 (m, 3H, arom. H), 7.86-7.89 (m, 2H, arom. H), 7.90-7.94 (m, 2H, arom. H), 7.98-8.02 (m, 2H, arom. H). ^{13}C NMR (DMSO-d_6, 151 MHz): δ 63.8, 71.0, 72.9, 79.4, 85.8, 108.9, 114.9, 128.22, 128.23, 128.6, 128.77, 128.78, 129.18, 129.27, 129.30, 129.36, 133.6, 133.9, 134.0, 149.8, 150.6, 164.4, 164.6, 165.2, 165.4. HRMS (ESI-TOF) m/z: [M + Na^+] Calcd for $C_{31}H_{25}N_5O_8Na$ 618.1595; Found 618.1589.

2-[(N^2-Isobutyryl)amino]-8-[(2,3,5-tri-*O*-benzoyl)ribofuranosyl]-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (20b). Method A: As described for **18** with 2,3,5-tri-*O*-benzoyl-1-*O*-acetyl-D-ribofuranose **16** (500 mg, 0.99 mmol), dichloromethane (3 mL), 30% soln. of HBr in acetic acid[16] (0.6 mL), compound **10a** (185 mg, 0.6 mmol), K_2CO_3 (456 mg, 3.30 mmol) and TDA-1 (0.03 mL, 0.09 mmol) in MeCN (25 mL). FC (silica gel, column 12 x 3 cm, CH_2Cl_2/MeOH, 100:1→95:5) gave compound **20b** as colorless foam (40 mg, 10%). TLC (silica gel, CH_2Cl_2/MeOH, 95:5) R_f 0.6. λ_{max} (MeOH)/nm 238 (ε/dm^3 mol^{-1} cm^{-1} 30700), 281 (13000). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.00 (t, J = 7.0 Hz, 6H, CHCH_3), 2.89 (p, J = 6.8 Hz, 1H, CHCH$_3$), 4.72 (dd, J = 11.9, 6.2 Hz, 1H, H-5'), 4.79 (dd, J = 11.8, 4.4 Hz, 1H, H-5'), 4.88 (td, J = 6.0, 4.4 Hz, 1H, H-4'), 6.29 (dd, J = 6.1, 4.0 Hz, 1H, H-2'), 6.36 (t, J = 6.0 Hz, 1H, H-3'), 6.42 (d, J = 4.0 Hz, 1H, H-1'), 7.46 (dddd, J = 16.7, 8.3, 5.3, 1.6 Hz, 6H, arom. H), 7.62-7.68 (m, 4H, H-6, arom. H), 7.76 (d, J = 2.8 Hz, 1H, H-7), 7.89 (dq, J = 8.2, 1.4 Hz, 2H, arom. H), 7.91-7.94 (m, 2H, arom. H), 10.45 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 151 MHz): 18.93, 18.97, 34.6, 63.9, 71.2, 73.6, 79.7, 87.6, 109.0, 117.8, 128.3, 128.66, 128.74, 129.19, 129.21, 129.27, 129.38, 133.45, 133.81, 133.97, 149.47, 149.92, 160.7, 164.45, 164.47, 165.4, 175.8. HRMS (ESI-TOF) m/z: [M + Na$^+$] Calcd for $C_{35}H_{31}N_5O_9Na$ 688.2014; Found 688.2014.

Method B: As described for **18** with **10a** (100 mg, 0.32 mmol), MeCN (5 mL), BSA (73 mg, 0.08 mL, 0.36 mmol), TMSOTf (78 mg, 0.06 mL, 0.35 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**16**) (183 mg, 0.36 mmol). Purification by FC (silica gel, column 12 x 3 cm, CH_2Cl_2/MeOH, 100:1→95:5) gave **20b** (180 mg, 84%) as a colorless foam.

2-Amino-8-ribofuranosyl-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (2).[7a] Compound **20b** (100 mg, 0.15 mmol) was suspended in NH_3/MeOH (20 mL). The mixture was stirred at r.t. overnight. The solvent was evaporated and the residue was purified by FC (silica gel, column 10 x 2 cm, CH_2Cl_2/MeOH 3:1) to give **2** (36 mg, 85%) as a colorless foam. TLC (silica gel,

CH_2Cl_2/MeOH, 4:1) R_f 0.2. λ_{max} (MeOH)/nm 258 (ε/dm^3 mol^{-1} cm^{-1} 11600). ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.54 (ddd, J = 11.9, 5.0, 3.7 Hz, 1H, H-5'), 3.57-3.64 (m, 1H, H-5''), 3.88 (q, J = 3.7 Hz, 1H, H-4'), 4.06 (q, J = 4.0 Hz, 1H, H-3'), 4.28 (q, J = 5.3 Hz, 1H, H-2'), 5.06 (t, J = 5.3 Hz, 1H, HO-5'), 5.18 (d, J = 4.4 Hz, 1H, HO-3'), 5.47 (d, J = 5.3 Hz, 1H, HO-2'), 5.78 (d, J = 5.9 Hz, 1H, H-1'), 6.95 (s, 2H, NH_2), 7.39 (d, J = 2.8 Hz, 1H, H-6), 7.48 (d, J = 2.8 Hz, 1H, H-7). ^{13}C NMR (DMSO-d_6, 151 MHz): 61.1, 70.3, 73.6, 85.4, 86.3, 108.4, 114.3, 150.0, 150.7, 165.3. HRMS (ESI-TOF) m/z: [M + Na$^+$] Calcd for $C_{10}H_{13}N_5O_5Na$ 306.0809; Found 306.0811.

2-Amino-8-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-(furan-2-yl)-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (21). A solution of compound **4** (100 mg, 0.25 mmol), furan-2-boronic acid (111 mg, 1 mmol), Na_2CO_3•10H_2O (360 mg, 1.26 mmol), and $Pd(PPh_3)_4$ (29 mg, 0.025 mmol) in CH_3CN/H_2O (1:1, 20 mL) was refluxed for 30 min. After cooling, the mixture was evaporated to dryness and purified by FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:13). From the main zone compound **21** was obtained as yellowish foam (63 mg, 76%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.5. λ_{max} (MeOH)/nm 272 (ε/dm^3 mol^{-1} cm^{-1} 10200). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.19 (ddd, J = 13.2, 6.1, 3.2 Hz, 1H, H-2''$_\alpha$), 2.43 (ddd, J = 13.2, 7.6, 5.7 Hz, 1H, H-2''$_\beta$), 3.49-3.62 (m, 2H, H-5''), 3.82 (td, J = 4.1, 2.7 Hz, 1H, H-4''), 4.33 (dq, J = 6.4, 3.3 Hz, 1H, H-3''), 5.01 (t, J = 5.3 Hz, 1H, HO-5''), 5.29 (d, J = 3.9 Hz, 1H, HO-3''), 6.23 (dd, J = 7.5, 6.0 Hz, 1H, H-1''), 6.55 (dd, J = 3.4, 1.8 Hz, 1H, furan), 7.01 (s, 2H, NH_2), 7.07 (dd, J = 3.4, 0.8 Hz, 1H, furan), 7.67 (s, 1H, H-7), 7.72 (dd, J = 1.9, 0.8 Hz, 1H, furan). ^{13}C NMR (DMSO-d_6, 151 MHz): δ 39.2, 61.3, 70.4, 82.9, 87.7, 111.5, 112.1, 112.5, 115.8, 142.3, 143.1, 149.9, 150.8, 164.7. HRMS (ESI-TOF) m/z: [M + Na$^+$] Calcd for $C_{14}H_{15}N_5O_5Na$ 356.0965; Found 356.0968.

2-Amino-8-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-(benzofuran-2-yl)-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (7). As described for **21** with **4** (100 mg, 0.25 mmol), $Pd(PPh_3)_4$ (29 mg, 0.025 mmol), $Na_2CO_3 \bullet 10H_2O$ (360 mg, 1.26 mmol), benzofuran-2-boronic acid (162 mg, 1 mmol) in CH_3CN/H_2O (1:1, 20 mL) under reflux for 30 min. The mixture was applied to FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:13). From the main zone compound **7** was obtained as colorless solid (50 mg, 52%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.5. λ_{max} (MeOH)/nm 312 (ε/dm^3 mol^{-1} cm^{-1} 22800), 327 (18800). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.23 (ddd, J = 13.2, 6.1, 3.2 Hz, 1H, H-2'$_\alpha$), 2.48-2.52 (m, 1H, H-2'$_\beta$), 3.56 (ddd, J = 11.8, 5.3, 4.1 Hz, 1H, H-5'), 3.61 (ddd, J = 11.7, 5.5, 4.3 Hz, 1H, H-5'), 3.85 (td, J = 4.1, 2.7 Hz, 1H, H-4'), 4.36 (dq, J = 6.5, 3.3 Hz, 1H, H-3'), 5.05 (t, J = 5.4 Hz, 1H, HO-5'), 5.32 (d, J = 3.9 Hz, 1H, HO-3'), 6.25 (dd, J = 7.4, 6.1 Hz, 1H, H-1'), 7.10 (2s, 2H, NH_2), 7.26 (td, J = 7.5, 1.0 Hz, 1H, benzofuran), 7.33 (ddd, J = 8.4, 7.3, 1.3 Hz, 1H, benzofuryl), 7.55 (dd, J = 8.2, 1.0 Hz, 1H, benzofuran), 7.67-7.71 (m, 1H, benzofuran), 7.95 (s, 1H, H-7). ^{13}C NMR (DMSO-d_6, 151 MHz): δ 39.1, 61.3, 70.4, 83.0, 87.8, 108.0, 110.7, 114.5, 115.6, 121.5, 123.2, 124.9, 144.7, 150.0, 151.3, 153.8, 164.8. HRMS (ESI-TOF) m/z: [M + Na$^+$] Calcd for $C_{18}H_{17}N_5O_5Na$ 406.1122; Found 406.1123.

2-Amino-8-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-[2-(phenyl)ethynyl]-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (22). A mixture of compound **4** (100 mg, 0.25 mmol), $Pd(PPh_3)_4$ (29 mg, 0.025 mmol) and CuI (10 mg, 0.05 mmol) was suspended in dry DMF (2 mL). Then, *N*-ethyldiisopropylamine (100 µL, 0.59 mmol) and phenylacetylene (110 µL, 1 mmol) were introduced and the reaction mixture stirred for 2 h. The solvent was evaporated and the remaining residue was applied to FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 88:12). From the main zone compound **22** was obtained as colorless foam (50 mg, 54%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.4. λ_{max} (MeOH)/nm 298 (ε/dm^3 mol^{-1} cm^{-1} 21700), 316

(20700). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.21 (ddd, J = 13.3, 6.1, 3.4 Hz, 1H, H-2'$_\alpha$), 2.40 (d, J = 13.1, 6.4 Hz, 1H, H-2'$_\beta$), 3.47-3.62 (m, 2H, H-5'), 3.82 (q, J = 4.3 Hz, 1H, H-4'), 4.33 (dq, J = 6.8, 5.5 Hz, 1H, H-3'), 4.99 (t, J = 5.5 Hz, 1H, HO-5'), 5.30 (d, J = 4.0 Hz, 1H, HO-3'), 6.18 (t, J = 6.7 Hz, 1H, H-1'), 7.03 (s, 2H, NH_2), 7.41-7.48 (m, 3H, phenyl), 7.48-7.53 (m, 2H, phenyl), 7.90 (s, 1H, H-7). ^{13}C NMR (DMSO-d_6, 151 MHz): δ 61.3, 70.2, 77.8, 82.9, 87.8, 94.4, 105.2.6, 119.7, 121.8, 128.8, 129.1, 131.0, 149.8, 150.2, 165.0. HRMS (ESI-TOF) m/z: [M + Na$^+$] Calcd for $C_{18}H_{17}N_5O_4Na$ 390.1173; Found 390.1169.

2-Amino-8-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-(1-pyrenyl)-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (23). As described for **21** with **4** (100 mg, 0.25 mmol), $Pd(PPh_3)_4$ (29 mg, 0.025 mmol), Na_2CO_3•$10H_2O$ (360 mg, 1.26 mmol), pyrenyl-1-boronic acid (246 mg, 1 mmol) in CH_3CN/H_2O (1:1, 20 mL) under reflux for 10 min. The mixture was applied to FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:13). From the main zone compound **23** was obtained as yellowish foam (85 mg, 72%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.5. λ_{max} (MeOH)/nm 241 (ε/dm^3 mol^{-1} cm^{-1} 42100), 266 (26600), 276 (32600), 329 (18400), 343 (25900). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.31 (ddd, J = 13.3, 6.0, 3.6 Hz, 1H, H-2'$_\alpha$), 2.49-2.52 (m, 1H, H-2'$_\beta$), 3.55 (qdd, J = 11.8, 5.5, 4.4 Hz, 2H, H-5'), 3.87 (td, J = 4.3, 2.7 Hz, 1H, H-4'), 4.37 (dq, J = 6.6, 3.3 Hz, 1H, H-3'), 4.93 (t, J = 5.4 Hz, 1H, HO-5'), 5.33 (d, J = 4.0 Hz, 1H, HO-3'), 6.38 (dd, J = 7.4, 6.1 Hz, 1H, H-1'), 6.97 (s, 2H, NH_2), 7.67 (s, 1H, H-6), 7.97-8.18 (m, 4H, pyrene), 8.18-8.39 (m, 5H, pyrene). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 39.0, 61.4, 70.4, 82.9, 87.7, 114.0, 121.6, 123.4, 123.6, 123.7, 124.1, 125.3, 125.5, 126.3, 127.2, 127.6, 127.8, 129.1, 130.3, 130.4, 130.7, 131.0, 150.1, 150.9, 164.9. HRMS (ESI-TOF) m/z: [M + Na$^+$] Calcd for $C_{26}H_{21}N_5O_4Na$ 490.1486; Found 490.1489.

2-Amino-8-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-[2-(1-pyrenyl)ethynyl]-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (24). As described for **22** with **4** (100 mg, 0.25 mmol), $Pd(PPh_3)_4$ (29 mg, 0.025 mmol), CuI (10 mg, 0.05 mmol), *N*-ethyldiisopropylamine (100 µL, 0.59 mmol), 1-ethynylpyrene (160 mg, 0.71 mmol) in dry DMF (2 mL) at r.t for 16 h. The mixture was applied to FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:13). From the main zone compound **24** was obtained as colorless foam (56 mg, 46%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.6. λ_{max} (MeOH)/nm 233 (ε/dm^3 mol^{-1} cm^{-1} 35300), 283 (22800), 303 (21500), 371 (33800), 394 (40700). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.24 (ddd, J = 13.4, 6.2, 3.4 Hz, 1H, H-2'$_\alpha$), 2.45-2.49 (m, 1H, H-2'$_\beta$), 3.51-3.69 (m, 2H, H-5'), 3.86 (q, J = 4.3 Hz, 1H, H-4'), 4.38 (dq, J = 6.7, 3.6 Hz, 1H, H-3'), 5.05 (t, J = 5.4 Hz, 1H, HO-5'), 5.57 (d, J = 3.9 Hz, 1H, HO-3'), 6.24 (t, J = 6.7 Hz, 1H, H-1'), 7.11 (2s, 2H, NH_2), 8.09 (s, 1H, H-7), 8.14 (t, J = 7.6 Hz, 1H, pyrene), 8.21 (dd, J = 13.2, 8.4 Hz, 2H, pyrene), 8.27 (d, J = 8.9 Hz, 1H, pyrene), 8.36 (m, 4H, pyrene), 8.89 (d, J = 9.1 Hz, 1H, pyrene). ^{13}C NMR (DMSO-d_6, 151 MHz): δ 39.0, 61.4, 70.3, 83.1, 83.4, 87.8, 94.1, 105.6, 116.3, 119. 9, 123.3, 123.6, 124.9, 125.4, 126.00, 126.02, 126.8, 127.2, 128.5, 128.7, 128.80, 128.84, 130.55, 130.75, 131.1, 131.5, 150.1, 150.4, 163.4. HRMS (ESI-TOF) m/z: Calcd for $C_{28}H_{21}N_5O_4H$ 492.1666; Found 492.1678.

2-Amino-8-(2-deoxy-*α*-D-*erythro*-pentofuranosyl)-6-(furan-2-yl)-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (25). As described for **21** with **5** (100 mg, 0.25 mmol), $Pd(PPh_3)_4$ (29 mg, 0.025 mmol), Na_2CO_3•$10H_2O$ (360 mg, 1.26 mmol), furan-2-boronic acid (111 mg, 1 mmol) in CH_3CN/H_2O (1:1, 20 mL) under reflux for 30 min. The mixture was applied to FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:13). From the main zone compound **25** was obtained as yellowish foam (78 mg, 94%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.5. λ_{max} (MeOH)/nm 272 (ε/dm^3 mol^{-1} cm^{-1} 10500). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.15 (dt, J =

14.5, 2.0 Hz, 1H, H-2'$_\alpha$), 2.29 (ddd, J = 14.4, 8.1, 6.4 Hz, 1H, H-2'$_\beta$), 3.41 (dd, J = 5.7, 4.6 Hz, 2H, H-5'), 4.14 (td, J = 4.5, 2.0 Hz, 1H, H-4'), 4.30 (ddt, J = 6.7, 3.6, 1.9 Hz, 1H, H-3'), 4.86 (t, J = 5.6 Hz, 1H, HO-5'), 5.54 (d, J = 3.3 Hz, 1H, HO-3'), 6.25 (dd, J = 8.1, 2.2 Hz, 1H, H-1'), 6.54 (dd, J = 3.4, 1.8 Hz, 1H, furan), 6.96 and 7.00 (2s, 2H, NH_2), 7.12 (dd, J = 3.4, 0.8 Hz, 1H, furan), 7.72 (dd, J = 1.9, 0.8 Hz, 1H, furan), 7.75 (s, 1H, H-7). ^{13}C NMR (DMSO-d_6, 151 MHz): δ 39.4, 61.7, 70.6, 83.8, 89.2, 111.5, 111.9, 113.4, 115.5, 142.5, 143.0, 150.1, 150.8, 164.7. HRMS (ESI-TOF) m/z: [M + Na$^+$] Calcd for $C_{14}H_{15}N_5O_5Na$ 356.0965; Found 356.0968.

2-Amino-8-(2-deoxy-*α*-D-*erythro*-pentofuranosyl)-6-(benzofuran-2-yl)-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (26). As described for **21** with **5** (100 mg, 0.25 mmol), $Pd(PPh_3)_4$ (29 mg, 0.025 mmol), Na_2CO_3•$10H_2O$ (360 mg, 1.26 mmol), benzofuran-2-boronic acid (162 mg, 1 mmol) in CH_3CN/H_2O (1:1, 20 mL) under reflux for 30 min. Compound **26** precipitated from the reaction mixture. The colorless precipitate was filtrated, washed with CH_3CN/H_2O (2:1, 5 mL) and dried (79 mg, 82%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.5. λ_{max} (MeOH)/nm 313 (ε/dm^3 mol^{-1} cm^{-1} 23000), 328 (19100). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.15 (dt, J = 14.5, 2.0 Hz, 1H, H-2'$_\alpha$), 2.29 (ddd, J = 14.4, 8.0, 6.3 Hz, 1H, H-2'$_\beta$), 3.43-3.44 (m, 2H, H-5'), 4.20 (td, J = 4.5, 1.9 Hz, 1H, H-4'), 4.34 (dt, J = 6.5, 1.8 Hz, 1H, H-3'), 4.89 (s, 1H, HO-5'), 5.60 (s, 1H, HO-3'), 6.28 (dd, J = 8.0, 2.2 Hz, 1H, H-1'), 7.05 and 7.10 (2s, 2H, NH_2), 7.25 (td, J = 7.5, 1.0 Hz, 1H, benzofuran), 7.32 (ddd, J = 8.4, 7.5, 1.4 Hz, 1H, benzofuran), 7.56 (dq, J = 8.3, 1.0 Hz, 1H, benzofuran), 7.68 (dt, J = 7.7, 1.0 Hz, 1H, benzofuran), 7.71 (d, J = 0.9 Hz, 1H, benzofuran), 8.00 (s, 1H, H-7). ^{13}C NMR (DMSO-d_6, 151 MHz): δ 39.2, 61.7, 70.7, 84.0, 89.4, 107.9, 110.7, 115.4, 121.5, 123.2, 124.9, 128.5, 144.8, 150.2, 151.2, 153.7, 164.8. HRMS (ESI-TOF) m/z: [M + Na$^+$] Calcd for $C_{18}H_{17}N_5O_5Na$ 406.1122; Found 406.1122.

2-Amino-8-(2-deoxy-*α*-D-*erythro*-pentofuranosyl)-6-[(2-phenyl)ethynyl]-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (27). As described for **22** with **5** (100 mg, 0.25 mmol), $Pd(PPh_3)_4$ (29 mg, 0.025 mmol), CuI (10 mg, 0.05 mmol), *N*-ethyldiisopropylamine (100 µL, 0.59 mmol), phenylacetylene (110 µL, 1 mmol) in dry DMF (2 mL) at r.t for 2h. The mixture was applied to FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 88:12). From the main zone compound **27** was obtained as colorless foam (60 mg, 65%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.4. λ_{max} (MeOH)/nm 299 (ε/dm^3 mol^{-1} cm^{-1} 21200), 317 (20100). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.19 (dt, J = 14.6, 2.1 Hz, 1H, H-2'$_\alpha$), 2.29 (ddd, J = 14.3, 7.9, 6.3 Hz, 1H, H-2'$_\beta$), 3.41 (t, J = 5.0 Hz, 2H, H-5'), 4.17 (td, J = 4.5, 2.0 Hz, 1H, H-4'), 4.30 (ddt, J = 5.5, 3.6, 2.1 Hz, 1H, H-3'), 4.87 (t, J = 5.6 Hz, 1H, HO-5'), 5.52 (d, J = 3.3 Hz, 1H, HO-3'), 6.19 (dd, J = 7.8, 2.2 Hz, 1H, H-1'), 7.00 and 7.07 (2s, 2H, NH_2), 7.41-7.46 (m, 3H, phenyl), 7.53 (d, J = 3.6 Hz, 2H, phenyl), 7.88 (s, 1H, H-7). ^{13}C NMR (DMSO-d_6, 151 MHz): δ 61.6, 70.6, 77.8, 84.1, 89.3, 94.4, 104.6, 120.8, 121.8, 128.8, 129.1, 131.0, 149.8, 149.9, 165.0. HRMS (ESI-TOF) m/z: [M + Na$^+$] Calcd for $C_{18}H_{17}N_5O_4Na$ 390.1173; Found 390.1172.

2-Amino-8-(2-deoxy-*α*-D-*erythro*-pentofuranosyl)-6-(1-pyrenyl)-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (28). As described for **21** with **5** (100 mg, 0.25 mmol), $Pd(PPh_3)_4$ (29 mg, 0.025 mmol), Na_2CO_3•$10H_2O$ (360 mg, 1.26 mmol), pyrenyl-1-boronic acid (246 mg, 1 mmol) in CH_3CN/H_2O (1:1, 20 mL) under reflux for 1 h. The mixture was applied to FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:13). From the main zone compound **28** was obtained as yellowish foam (60 mg, 51%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.6. λ_{max} (MeOH)/nm 241 (ε/dm^3 mol^{-1} cm^{-1} 43900), 266 (27700), 276 (34400), 329 (18600), 343 (26400). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.32 (d, J = 11.4 Hz, 1H, H-2'$_\alpha$), 2.78 (ddd, J = 14.4, 8.0, 6.5 Hz, 1H, H-2'$_\beta$), 3.42-3.50 (m, 2H, H-5'), 4.18 (td, J = 4.5, 2.3 Hz, 1H, H-4'), 4.33 (ddt, J = 6.1, 3.4, 2.3 Hz, 1H, H-3'), 4.89 (t, J = 5.6 Hz, 1H, HO-5'), 5.33 (d, J = 3.3 Hz,

1H, HO-3'), 6.39 (dd, *J* = 8.0, 2.6 Hz, 1H, H-1'), 6.95 (s, 2H, NH_2), 7.69 (s, 1H, H-6), 8.01-8.19 (m, 4H, pyrene), 8.21-8.38 (m, 5H, pyrene). ^{13}C NMR (DMSO-d_6, 151 MHz): δ 39.3, 61.7, 70.7, 83.7, 88.9, 115.2.0, 121.1, 123.4, 123.6, 123.8, 124.1, 125.4, 125.6, 126.4, 127.3, 127.6, 127.7, 127.9, 129.1, 130.3, 130.4, 130.7, 131.1, 150.2, 150.8, 164.9. HRMS (ESI-TOF) m/z: [M + Na^+] Calcd for $C_{26}H_{21}N_5O_4Na$ 490.1486; Found 490.1468.

2-Amino-8-(2-deoxy-*α*-D-*erythro*-pentofuranosyl)-6-[2-(1-pyrenyl)ethynyl]-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (8). As described for **22** with **5** (100 mg, 0.25 mmol), $Pd(PPh_3)_4$ (29 mg, 0.025 mmol), CuI (10 mg, 0.05 mmol), *N*-ethyldiisopropylamine (100 µL, 0.59 mmol), 1-ethynylpyrene (226 mg, 1 mmol) in dry DMF (2 mL) at r.t for 6 h. The mixture was applied to FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 88:13). From the main zone compound **8** was obtained as colorless foam (38 mg, 31%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.4. λ_{max} (MeOH)/nm 233 (ε/dm^3 mol^{-1} cm^{-1} 35800), 283 (23300), 303 (21200), 371 (33400), 394 (40300). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.24 (dt, *J* = 14.4, 2.1 Hz, 1H, H-2'$_\alpha$), 2.71 (ddd, *J* = 14.3, 7.9, 6.3 Hz, 1H, H-2'$_\beta$), 3.45 (dd, *J* = 3.4, 4.5 Hz, 2H, H-5'), 4.23 (td, *J* = 4.4, 2.1 Hz, 1H, H-4'), 4.32-4.38 (m, 1H, H-3'), 4.90 (t, *J* = 5.4 Hz, 1H, HO-5'), 5.57 (d, *J* = 3.4 Hz, 1H, HO-3'), 6.25 (dd, *J* = 7.8, 2.1 Hz, 1H, H-1'), 7.07 and 7.11 (2s, 2H, NH_2), 8.06 (s, 1H, H-7), 8.14 (t, *J* = 7.6 Hz, 1H, pyrene), 8.22 (d, *J* = 4.8 Hz, 1H, pyrene), 8.23 (d, *J* = 3.8 Hz, 1H, pyrene), 8.27 (d, *J* = 8.9 Hz, 1H, pyrene), 8.32 (d, *J* = 8.2 Hz, 1H, pyrene), 8.35 (d, *J* = 9.2 Hz, 1H, pyrene), 8.37 (dd, *J* = 7.7, 1.0 Hz, 1H, pyrene), 8.38-8.41 (m, 1H, pyrene), 8.90 (d, *J* = 9.1 Hz, 1H, pyrene). ^{13}C NMR (DMSO-d_6, 151 MHz): δ 61.6, 70.6, 83.5, 84.2, 89.4, 94.1, 104.9, 116.4, 121.0, 123.3, 123.6, 124.9, 125.4, 125.9, 126.0, 126.8, 127.2, 128.5, 128.7, 128.8, 128.9, 130.6, 130.7, 131.0, 131.4, 150.0, 150.1, 165.2. HRMS (ESI-TOF) m/z: [M + Na^+] Calcd for $C_{28}H_{21}N_5O_4Na$ 514.1486; Found 514.1480.

2-Amino-8-(*β*-D-ribofuranosyl)-6-(1-pyrenyl)-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (9). As described for **21** with **6** (100 mg, 0.24 mmol), $Pd(PPh_3)_4$ (29 mg, 0.025 mmol), $Na_2CO_3 \bullet 10H_2O$ (360 mg, 1.26 mmol), pyrenyl-1-boronic acid (246 mg, 1 mmol) in CH_3CN/H_2O (1:1, 20 mL) under reflux for 10 min. The mixture was applied to FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:12). From the main zone compound **9** was obtained as yellowish foam (75 mg, 65%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.5. λ_{max} (MeOH)/nm 241 (ε/dm^3 mol^{-1} cm^{-1} 50100), 266 (27800), 276 (35800), 329 (19800), 343 (28700). ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.55 (ddd, J = 11.9, 5.3, 3.8 Hz, 1H, H-5'), 3.63 (ddd, J = 11.9, 5.4, 3.9 Hz, 1H, H-5'), 3.95 (q, J = 3.7 Hz, 1H, H-4'), 4.14 (q, J = 4.4 Hz, 1H, H-3'), 4.39-4.50 (m, 1H, H-2'), 5.02 (t, J = 5.4 Hz, 1H, HO-5'), 5.21 (d, J = 4.6 Hz, 1H, HO-3'), 5.59 (d, J = 24.0 Hz, 1H, HO-2'), 6.00 (d, J = 5.7 Hz, 1H, H-1'), 7.00 (s, 2H, NH_2), 7.71 (s, 1H, H-6), 7.84-8.49 (m, 9H, pyrene). ^{13}C NMR (DMSO-d_6, 151 MHz): δ 61.1, 70.2, 73.8, 85.4, 86.5, 114.2, 121.7, 123.4, 123.6, 123.8, 124.1, 125.3, 125.4, 125.6, 126.4, 127.3, 127.7, 127.9, 128.9, 129.2, 130.3, 130.8, 131.1, 150.2, 151.5, 164.9. HRMS (ESI-TOF) m/z: [M + Na^+] Calcd for $C_{26}H_{21}N_5O_5Na$ 506.1435; Found 506.1448.

2-Amino-7-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-5-(benzofuran-2-yl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (30). Compound **30** was prepared according to a published procedure[6d] using compound **29** (100 mg, 0.26 mmol), $Pd(OAc)_2$ (5.7 mg, 0.025 mmol), $CsCO_3$ (415 mg, 1.27 mmol), TPPTS (73 mg, 0.13 mmol), benzofuran-2-boronic acid (413 mg, 2.55 mmol) in CH_3CN/H_2O (1:1, 20 mL) under reflux for 10 min. The mixture was applied to FC (silica gel, column 10 x 2 cm, CH_2Cl_2/MeOH, 89:11). From the main zone compound **30** was obtained as colorless solid (55 mg, 52%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.5. Lit.[6d]: 59%. λ_{max} (MeOH)/nm 264 (ε/dm^3 mol^{-1} cm^{-1} 17400), 317 (23800) 330

(20600). HRMS (ESI-TOF) m/z: [M + Na^+] Calcd for $C_{19}H_{18}N_4O_5Na$ 405.1169; Found 405.1178. Analytical data were identical to those reported in the literature.[6d]

2-Amino-7-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-5-(pyrenyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (31). As described for **30** with compound **29** (100 mg, 0.26 mmol), $Pd(OAc)_2$ (5.7 mg, 0.025 mmol), $CsCO_3$ (415 mg, 1.27 mmol), TPPTS (73 mg, 0.13 mmol), pyrenyl-1-boronic acid (123 mg, 0.5 mmol) in CH_3CN/H_2O (1:1, 20 mL) under reflux for 15 min. The mixture was applied to FC (silica gel, column 10 x 2 cm, CH_2Cl_2/MeOH, 89:11). From the main zone compound **31** was obtained as greenish foam (51 mg, 41%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.5. λ_{max} (MeOH)/nm 242 (ε/dm^3 mol^{-1} cm^{-1} 39400), 266 (21000), 276 (23700), 343 (17200). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.09 (ddd, *J* = 13.0, 5.9, 2.6 Hz, 1H, H-2'$_\alpha$), 2.52-2.56 (m, 1H, H-2'$_\beta$), 3.47-3.59 (m, 2H, H-5'), 3.83 (td, *J* = 4.6, 2.4 Hz, 1H, H-4'), 4.35 (dt, *J* = 6.2, 2.9 Hz, 1H, H-3'), 4.88 (t, *J* = 5.5 Hz, 1H, HO-5'), 5.25 (d, *J* = 3.7 Hz, 1H, HO-3'), 6.34 (s, 2H, NH_2), 6.50 (dd, *J* = 8.3, 5.9 Hz, 1H, H-1'), 7.22 (s, 1H, H-6), 8.00-8.12 (m, 3H, pyrene), 8.12-8.21 (m, 2H, pyrene), 8.21-8.31 (m, 4H, pyrene), 10.42 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 39.4, 61.9, 71.0, 82.4, 87.1, 99.5, 116.9, 117.8, 123.99, 124.06, 124.17, 124.5, 126.1, 126.6, 126.8, 127.4, 128.7, 128.9, 129.5, 130.6, 130.9, 151.2, 152.8, 158.4. HRMS (ESI-TOF) m/z: [M + Na^+] Calcd for $C_{27}H_{22}N_4O_4Na$ 489.1533; Found 489.1528.

SUPPORTING INFORMATION

The Supporting Information is available free of charge on the ACS Publications website at DOI: xxx. ^{13}C NMR chemical shifts, crystallographic data, UV spectra, shape index and curvedness surfaces, energy minimized structures, photophysical data, ^{1}H, ^{13}C, ^{1}H-^{1}H-COSY, HSQC, and HMBC NMR spectra of all compounds.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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