JOC The Journal of Organic Chemistry

Article

Alkynylated and Dendronized 5-Aza-7-deazaguanine Nucleosides: Cross-Coupling with Tripropargylamine and Linear Alkynes, Click Functionalization and Fluorescence of Pyrene Adducts

Dasharath Kondhare, Aigui Zhang, Peter Leonard, and Frank Seela

J. Org. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.joc.0c00926 • Publication Date (Web): 23 Jul 2020

Alkynylated and Dendronized 5-Aza-7-deazaguanine Nucleosides: Cross-Coupling with Tripropargylamine and Linear Alkynes, Click Functionalization and Fluorescence of Pyrene Adducts†

Dasharath Kondhare[a], Aigui Zhang[a], Peter Leonard[a] and Frank Seela[a,b]*

[a]*Laboratory of Bioorganic Chemistry and Chemical Biology, Center for Nanotechnology, Heisenbergstrasse 11, 48149 Münster, Germany and* [b]*Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie neuer Materialien, Universität Osnabrück, Barbarastrasse 7, 49069 Osnabrück, Germany*

Prof. Dr. Frank Seela

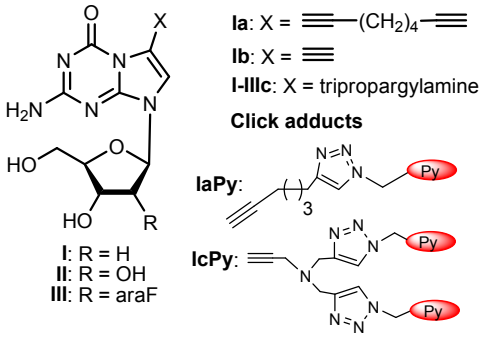
Phone: +49 (0)251-53406-500; Mobile: +49 (0)173-7250-297

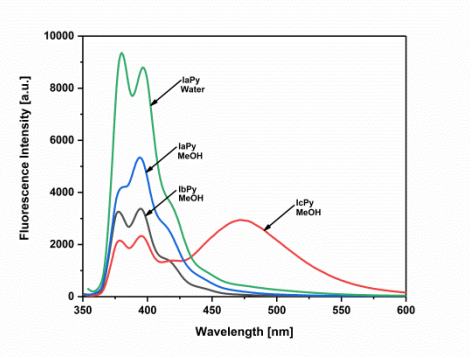
Fax: +49 (0)251-53406-587

E-mail: Frank.Seela@uni-osnabrueck.de

Homepage: www.seela.net

TABLE OF CONTENTS





Fluorescence of pyrene click adducts (Py)

ABSTRACT

The change of the recognition face of 5-aza-7-deazaguanine bridgehead nucleosides with respect to purine nucleosides permits the construction of new purine-purine or purine-pyrimidine base pairs in DNA and RNA. Clickable derivatives of 5-aza-7-deazaguanine were synthesized by introducing ethynyl, 1,7-octadiynyl and tripropargylamino side chains in the 7-position of the 5-aza-7-deazapurine moiety by *Sonogashira* cross-coupling. Click reactions were performed with 1-azidomethylpyrene by the copper-catalyzed azide-alkyne cycloaddition. The copper(I) catalyzed click reaction on the tripropargylamino nucleoside was significantly faster and higher yielding than that for nucleosides carrying linear alkynyl chains. Also, this reaction could be performed with copper(II) as catalyst. An autocatalyzed cycle was suggested in which the click product acts as a catalyst. Pyrene click adducts of linear alkynylated nucleosides showed pyrene monomer emission while tripropargylamino adducts showed monomer and excimer fluorescence. The fluorescence intensities of the 5-aza-7-deazaguanine nucleosides were higher than those of their 7-deazaguanine counterparts. The reported clickable nucleosides can be utilized to functionalize or to cross-link monomeric nucleosides or DNA for diagnostic or imaging purposes and other applications in nucleic acid chemistry and biotechnology.

INTRODUCTION

Bridgehead 5-aza-7-deazaguanine nucleosides contain a heterocyclic skeleton that differs from those of 7-deazapurine or 8-aza-7-deazapurine nucleosides (purine numbering is used throughout the results and discussion section). Due to the shift of nitrogen-7 to the bridgehead position-5, nitrogen-1 becomes the proton acceptor site and is no longer a donor site as existing in related guanine nucleosides. This change does not only affect the physical properties of this class of nucleosides but also changes base pairing.[1] As a consequence, 5-aza-7-deazaguanine forms base pairs with guanine and isoguanine.[1b] It produces stable "purine" DNA with parallel and antiparallel chain orientation (Figure 1).[1b] Base pairing with protonated dC has been reported[1a] and special dC analogs were used to expand the genetic alphabet by a six letter code.[1c] Also, programmable metal ion mediated base pairs with dC exist in the presence of silver ions.[2] Recently, 7-functionalized derivatives were synthesized in the series of ribo- and 2'-deoxyribonucleosides.[3] Earlier work on non-functionalized 5-aza-7-deazaguanine nucleosides has been reviewed.[4] Current work is cited in the manuscript.

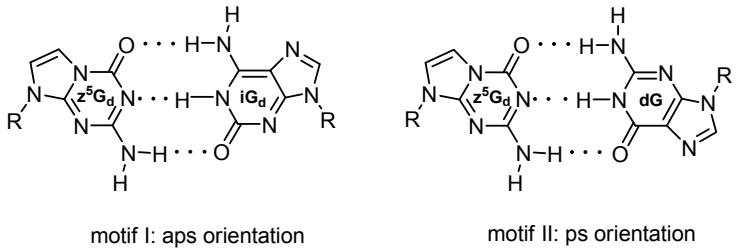


Figure 1. Base pair motifs for "purine" DNA with antiparallel (aps) and parallel (ps) chain orientation.[1b] z^5G_d corresponds to 5-aza-7-deaza-2'-deoxyguanosine, iG_d corresponds to 2'-deoxyisoguanosine and R corresponds to 2'-deoxy-D-ribofuranosyl.

Recently, fluorescent residues have been connected directly to the base moiety of 5-aza-7-deazaguanine nucleosides.[3] Nonetheless, with respect to oligonucleotide synthesis this requires conversion of each particular nucleoside conjugate to an individual phosphoramidite

building block which can be later employed in solid-phase synthesis. A more universal approach represents the use of click chemistry.[5] The method allows functionalization with a diversity of azido compounds and is therefore versatile for many applications.[6]

Now, clickable derivatives of 5-aza-7-deazaguanine were synthesized by introducing alkynyl side chains in the 7-position of the 5-aza-7-deazapurine moiety by *Sonogashira* cross-coupling. This position has steric freedom in canonical B-DNA and it is anticipated that this is also valid for "purine" nucleic acids.

In more detail, the synthesis of 5-aza-7-deazaguanine nucleosides with linear alkynyl chains or dendritic triprogargylamino residues is described in this work and various sugar residues were introduced at the nucleoside glycosylation site-9 (nucleosides **1**-**3**; Figure 2). This makes the molecules suitable for click reactions, expands the functionalities on the sugar moiety and provides 5-aza-7-deazapurine compounds with new properties as nucleosides or components of oligonucleotides. With the alkynylated and dendronized nucleosides **2a-c** and **3a-d** in hand click libraries can be produced. To demonstrate the utility of the compounds a pyrene azide was clicked to the nucleosides and steady-state fluorescence was investigated. During this work, it became apparent that tripropargylamine click cycloaddition can be induced by copper(II) ions in the absence of ascorbic acid as reducing agent. By serendipity, we also found that TBTA {tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine} acts as a catalyst in the *Sonogashira* cross-coupling reaction.

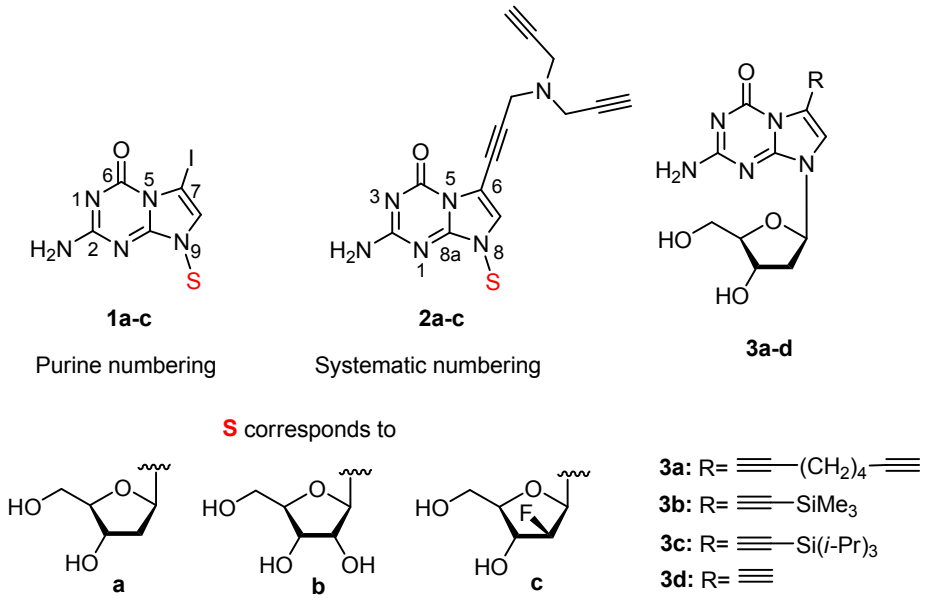


Figure 2. Structures of sugar-modified 5-aza-7-deazaguanine nucleosides used in this study.

RESULTS AND DISCUSSION

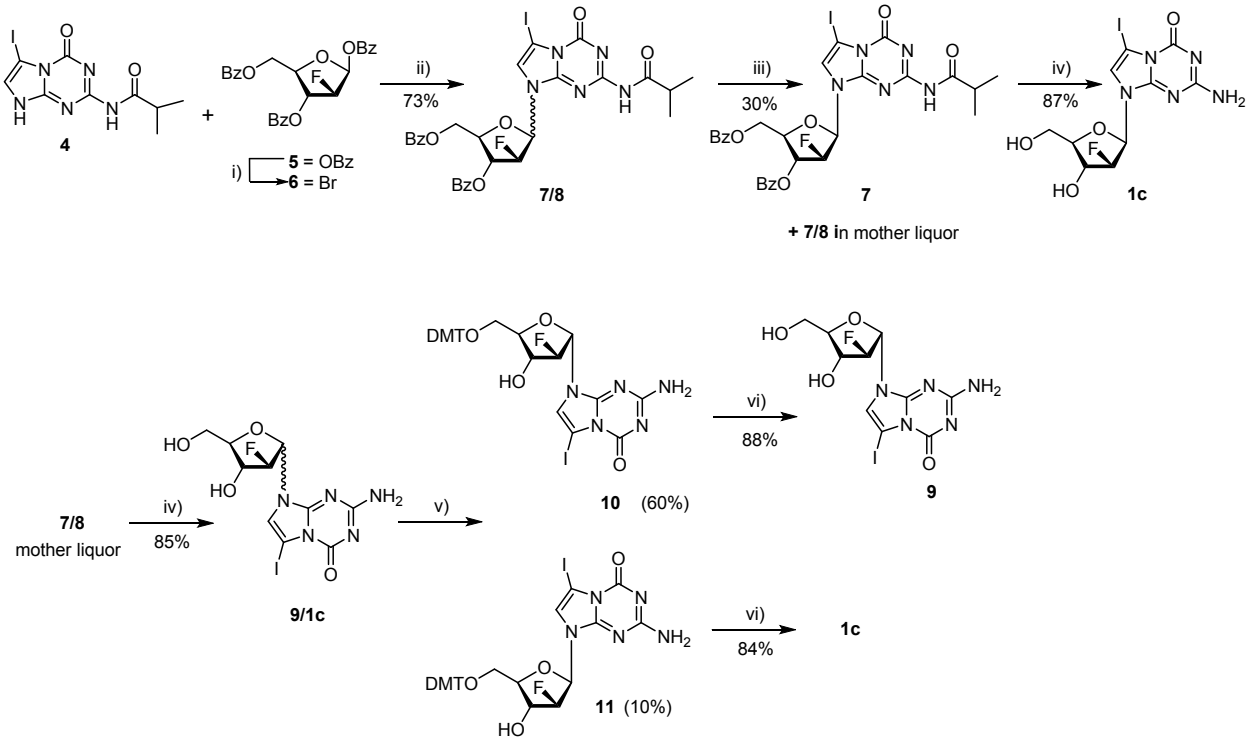
Synthesis of 7-Iodo-5-aza-7-deazaguanine Bridgehead Nucleosides. For the synthesis of the tripropargylamine and alkynyl nucleosides **2a-c** and **3a-d** the 7-iodo compounds **1a-c** were the precursors. The synthesis of the 7-iodonucleosides **1a** and **1b** has already been reported employing Vorbrüggen conditions or nucleobase anion glycosylation[3,7], whereas **1c** is unknown. Earlier, the unsubstituted 5-aza-7-deazaguanine 2'-arabinofluoro nucleoside was synthesized.[8a] As fluoro nucleosides play an important role in nucleoside and oligonucleotide chemistry[8b], the 7-iodo derivative **1c** was prepared by nucleobase anion glycosylation of a suitably protected nucleobase and the corresponding sugar derivative (Scheme 1).

In more detail, the fluoroarabino sugar **6** was prepared *in situ* from **5**.[9] Then, nucleobase anion glycosylation was carried out in MeCN with the protected nucleobase **4** and excess of **6**. DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) was used as base to generate the nucleobase anion. The reaction gave a mixture of the protected anomers **7**/**8** in 73% overall yield isolated after flash chromatography. The ratio of anomers **7**/**8** (1:1, β to α) was determined from the intensity of

anomeric ^{1}H NMR signals (Figure S10, Supporting Information). This mixture gave only one spot on TLC and was inseparable by column chromatography. However, the pure protected *β*-anomer **7** was obtained by crystallization from methanol in 30% yield. Deprotection of **7** in 7 N NH_3/MeOH afforded the *β*-D anomer **1c** in 87% yield. As the *α*-D anomer **8** could not be isolated from the mother liquor as clean material, another separation method was chosen. The mother liquor containing mainly the *α*-anomer was deprotected in 7 N NH_3/MeOH to yield an inseparable mixture of nucleosides **9** and **1c** in 85%. Then, the mixture of the 5-aza-7-deazaguanine 2'-deoxyribonucleoside *α*/*β*-anomers **9/1c** could be separated as 5'-*O*-DMT derivatives. Protection with DMT chloride in pyridine followed by flash chromatography afforded the pure *α*-nucleoside **10** (faster migrating) in 60% and the *β*-anomer **11** in 10% yield (slower migrating). Deprotection with trichloroacetic acid furnished the pure anomers **9** (*α*-D, 88%) and **1c** (*β*-D, 84%).

All compounds were characterized by mass spectra and high resolution ^{1}H, ^{13}C{^{1}H} and 2D NMR spectra (Figures S10-89, Supporting Information). ^{13}C{^{1}H}NMR chemical shifts are shown in Table S1, Supporting Information). The anomeric configuration of the fluoro nucleoside **1c** was assigned by long range couplings from H-7 and C-7 to C2'-F (Figure S21, Supporting Information) which indicate β-D configuration.[10] Also, the signals for H-1' (0.1 ppm), H-2' (0.24 ppm) and H-4' (0.4 ppm) as well as the carbon signals for C-1' (5 ppm), C-2' (3 ppm), C-3' (~1 ppm) and C-4' (3 ppm) are shifted when the anomeric configuration is altered from β-D to α-D. Furthermore, the coupling constants for ^{2}J(C1'-C2'-F) and (C2'-C2'-F) are different by 17 to 20 Hz. These observations are in line with reports on anomers of non-iodinated 2'-fluoro nucleosides.[8a]

Scheme 1. Synthesis and Separation of Anomeric 7-Iodo-5-aza-7-deaza-2'-deoxyfluoro arabinofuranosyl Nucleosides[a]

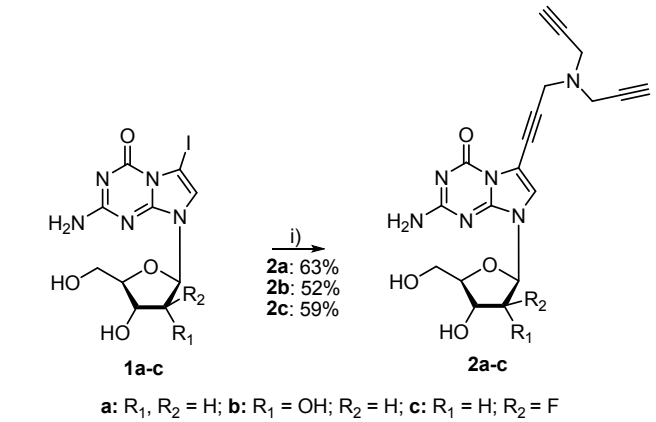


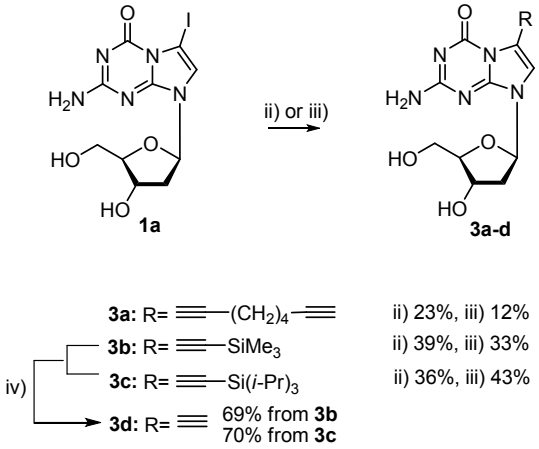
[a]Reagents and conditions: (i) HBr in glacial acetic acid, 16 h, rt; (ii) DBU, MeCN, 16 h, rt; (iii) Crystallization from MeOH; (iv) 7N NH_3/MeOH; (v) DMT-Cl, pyridine, 3.5 h, rt; (vi) 3% TCA in CH_2Cl_2, 16 h, rt.

Conversion of 7-Iodo Nucleosides to Alkynyl and Tripropargylamine Nucleosides. Next, alkynyl side chains (linear and dendronized) were introduced to nucleosides **1a-c**. For this, *Sonogashira* cross-coupling reactions were performed.[11] Compounds **1a-c** were treated with excess of alkyne in DMF in the presence of Pd(0) and copper(I) (Scheme 2, Table 1) (for details, see the Experimental Section). The reaction performed on **1a** with 1,7-octadiyne was slow and significant deiodination took place. To access the product, the reaction was monitored on TLC and was stopped before completion. Long reaction time resulted in decomposition. Due to deiodination and decomposition, the yield was only between 12-24%.

For the synthesis of the ethynyl nucleoside **3d** cross-coupling reactions were performed with TMS- and TIPS-acetylene. Compounds **3b** (TMS) and **3c** (TIPS) were obtained in moderate yields after a reaction time of 24 h (39% for **3b** and 36% for **3c**) but significant deiodination took place also in these cases. To solve this problem, *Sonogashira* reaction was performed on the nucleobase **4**. Unfortunately, deiodination occurred and an alkynylated product could not be isolated. The reaction time of *Sonogashira*-cross coupling reactions performed on nucleosides could be shortened with the help of TBTA {tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine} but led to lower or marginally higher yields (for details, see Table S2, Supporting Information). We anticipate that copper(I)ions are stabilized by TBTA as reported for click reactions.[12] Next, the silyl protecting groups were removed from **3b** and **3c** with 1 M TBAF/THF to afford nucleoside **3d** in 69% and 70% yield, respectively.

Scheme 2. Synthesis of 5-Aza-7-deazaguanine Ribo-, 2'-Deoxyribo, and 2'-Fluoroarabino Nucleosides with Tripropargyl Side Chains and 2'-Deoxyribonucleosides with Alkynyl Side Chains[a]





[a]Reagents and conditions: (i) Alkyne (10 eq.), $Pd(PPh_3)_4$, Et_3N, CuI, DMF, rt; (ii) alkyne, $Pd(PPh_3)_4$, Et_3N, CuI, DMF, rt; (iii) alkyne, $Pd(PPh_3)_4$, Et_3N, CuI, DMF, rt with TBTA (Table S2, Supporting Information). (iv) 1 M TBAF in THF.

Contrary to the linear alkynes, *Sonogashira* cross-coupling of **1a** with tripropargylamine was efficient and the dendronized nucleoside **2a** was obtained in 63% yield in 6 h. No deiodination was observed. With these results in hand, *Sonogashira* cross-coupling reactions with tripropargylamine were performed on nucleosides **1b** and **1c** yielding the ribonucleoside **2b** in 52% yield and the 2'-fluorinated nucleoside **2c** in 59%. The modification of the sugar residue causes a significant change on the hydrophobic character of the nucleosides. As demonstrated by the chromatographic mobilities on a reversed phase HPLC column (Figure

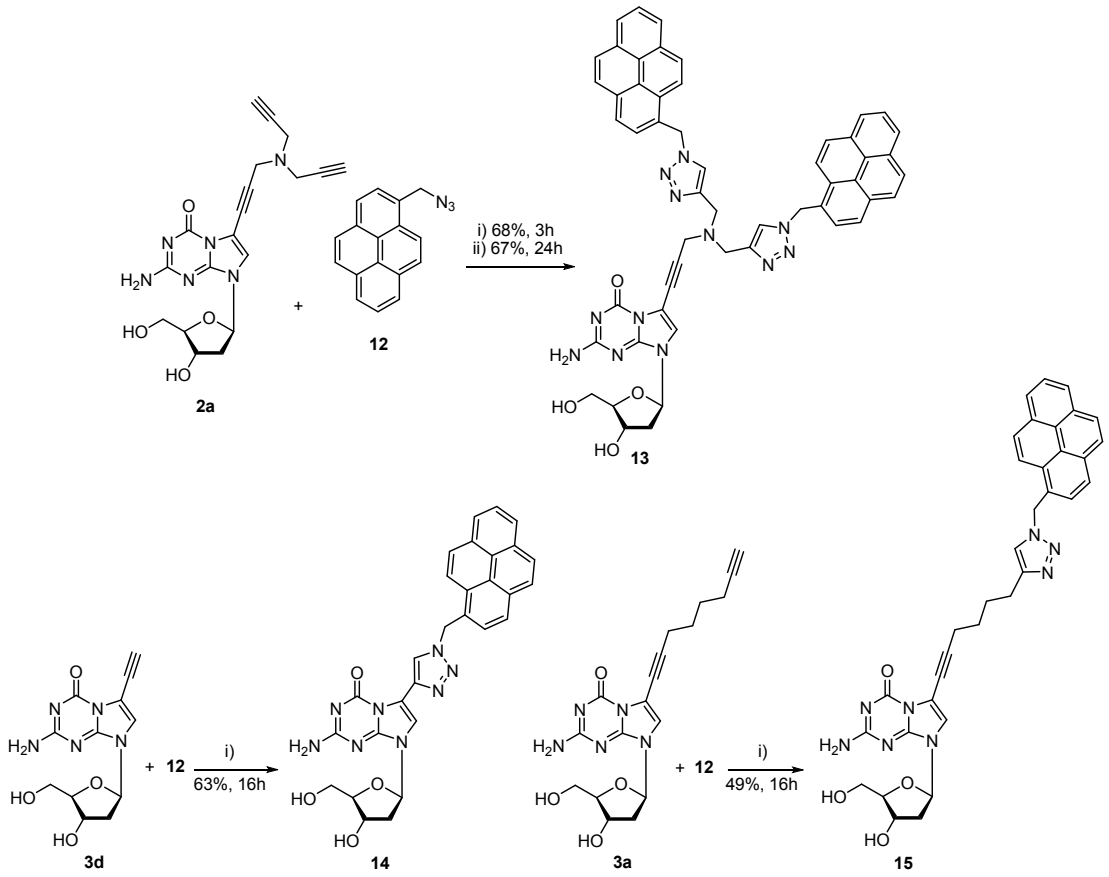
S6, Supporting Information) the fluoro nucleoside is the most lipophilic compound and the ribonucleoside the most hydrophilic.

Incorporation of the side chains was confirmed by strong shifts of the nucleobase carbon-7 in the $^{13}C\{^{1}H\}$ NMR spectra and the appearance of new signals for the side chains (Table S1, Supporting Information). From previous studies on 5-tripropargylamine-2'-deoxyuridine it is known that the introduction of side chains has an impact on the pK_a value of the nucleobase. As pK values are important for the recognition in base pairing they were determined from UV spectral changes for compounds **1c**, **2a**-**2c** and **3d**. They were found to be as follows **2a**-**2c** (pK_a = 3.4), tripropargylamino **3d** (pK_a = 3.5), 7-iodinated **1a**[7] and **1c** (pK_a = 3.6) and non-iodinated 5-aza-7-deaza-2'-deoxyguanosine (pK_a = 3.8)[13] (Figures S1-5, Supporting Information). The data reflect protonation of the nucleobases. As the pK values are all in the same range influence of the side chain on base protonation is small.

Click Reactions Performed On Linear and Dendronized Nucleosides with Pyrenemethylazide under Copper(I) and Copper(II) Catalysis. The Cu(I)-catalyzed *Huisgen-Sharpless-Meldal* cycloaddition[5] – "click chemistry" has become a key reaction to functionalize nucleosides and nucleic acids. The reaction has been performed in aqueous solution or mixtures of organic solvents and water.[6] To prove the applicability of the click reaction to nucleosides **2a**, **3a** and **3d**, the click functionalization was studied using 1-azidomethyl pyrene (**12**) as second component. First, the reactions were performed under standard conditions with $CuSO_4$•$5H_2O$ and sodium ascorbate as reducing agent in a THF/*t*-BuOH/H_2O (3:1:1) mixture at rt. The copper(I)-catalyzed reaction with the linear ethynyl and 1,7-octadiynyl derivatives **3a** and **3d** was complete after 16 h affording the pyrene adducts **14** and **15** (63% and 49% yield, respectively) (Scheme 3). In case of the tripropargylamine

nucleoside **2a** both terminal alkyne groups underwent click functionalization leading to the double click adduct **13** in 68% yield within 3 h.

Scheme 3. Synthesis of Pyrene Click Conjugates[a]



[a]Reagents and conditions: (i) $CuSO_4 \bullet 5H_2O$, sodium ascorbate, *t*-BuOH/THF/H_2O (3:1:1); (ii) $CuSO_4 \bullet 5H_2O$, *t*-BuOH/THF/H_2O (3:1:1).

Usually, copper(I) is used as catalyst to promote the azide alkyne click reaction and to control regioselectivity.[5,6] However, the reaction can be also performed with copper(II) when the copper ions are chelated.[14] Earlier, we reported on a stepwise click reaction employing a pyridine bis-azide.[14e] This click reaction was performed with copper(II) as catalyst which chelates to the azido group next to a pyrimidine nitrogen. Only this azido group formed a click product with an alkyne. Apparently, in an initial phase the chelated copper(II) is reduced

to copper(I), thereby forming the triazole click product. Only a mono click product was formed as the reaction is fast on this site compared to the site of the non-chelating azido group. Later, the second azido group could be functionalized by a second click reaction under classical copper(I) catalysis making the protocol to a stepwise process in which different alkynes can be used in each step.[14] In this stepwise process, the chelation of copper(II) is crucial for the reduction of copper(II) to copper(I). This shows that chelated copper(II) ions can be used to perform click reactions. The use of copper(II) in click reactions without using ascorbic acid has also been reported for other cases in which copper(II) ions were chelated by ligands.[14] As only copper(I) is able to catalyze the click reaction the reduction of copper(II) is always required.

Whitesides *et al*. described an autocatalytic cycle for the copper azide alkyne cycloaddition (CuAAC) reaction performed on tripropargylamine and 2-azidoethanol.[15] The reaction used copper(II) instead of copper(I). The reaction is promoted by (i) coordinating copper(II) and its reduction to copper(I) and (ii) by enhancing the catalytic reactivity of chelated copper(I) in the cycloaddition step. After an initiation phase in which copper(II) is reduced to copper(I), an autocatalytic cycle starts in which click products catalyze the click reaction. An autocatalytic cycle for a regular click reaction was already suggested by Fokin[16a], Devaraj for self-reproducing catalyst driving repeated phospholipid synthesis[16b] and Binder for multivalent poly(acrylate)s and poly(isobutylene)s.[16c] Single-crystal X-ray diffraction of the Cu(I) complex of TBTA revealed an unusual dinuclear dication with one triazole unit bridging two metal centers. The structure of the complex of TBTA with Cu(II) in the crystalline state is trigonal bipyramidal and can be reduced to the active 'click' catalyst by sodium ascorbate, copper metal, or other reducing agents.[17]

As the side chain of the tripropargylamine nucleoside **2a** shows structural similarities to the free tripropargylamine we anticipated a similar reaction sequence can occur when compound

2a is clicked to pyrene azide. Accordingly, the click reaction of **2a** with pyrene azide was carried out in the presence of Cu(II) in absence of ascorbic acid as reducing agent in a THF/*t*-BuOH/water (3:1:1) mixture at rt and were monitored by TLC. In the beginning, a very slow formation of click products took place compared to the click functionalization with Cu(I) reported above. After 8 hours the formation of traces of the double and mono click product along with a major amount of starting material was observed on TLC. In the next 12 h, prominent formation of the double click adduct **13** took place together with the mono click product and starting material. After additional 4 hours, the double click reaction was complete (total reaction time 24 h) and the conjugate **13** was isolated in 67% yield (Scheme 3).

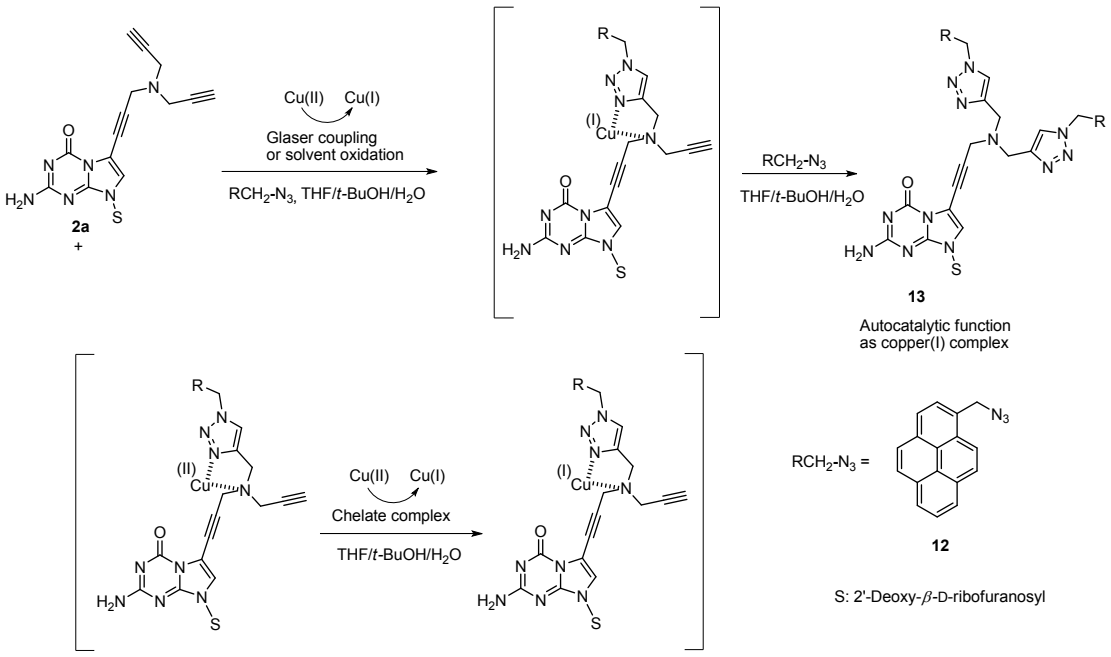


Figure 3. Schematic view on the reduction of copper(II) and the autocatalytic function of compound **13** copper(I) complex. Copper (I) and copper (II) complexes can display different structures as reported.[17]

We anticipate that in an initial phase copper(II) is reduced to copper(I) by oxidation of two alkynes (Glaser coupling) or solvent molecules as it has been reported by Whitesides.[15] The so-formed copper(I) catalyzes the click reaction to a monofunctional click product which was

not isolated but detected on TLC. Copper(II) can be reduced to copper(I) as free ion or chelated by the click adduct. The copper(II) complex might be more easily reduced as the non-chelated copper(II) ions. The copper(I) click product complex can catalyze the next click reaction. As soon as the first click products were formed an autocatalytic cycle starts. The autocatalytic cycle forms more and more of the active Cu(I) complex which accelerates the reaction in an autocatalytic way. So, the copper(I) click product catalyst is generated during product formation. Indeed, addition of the double click product **13** in an early stage of the reaction accelerates the reaction. Autocatalysis plays a major role in the processes of life. Here, it is illustrated by a copper catalyzed click reaction performed on a dendronized nucleoside. This process should not take place in the case of the 1,7-octadiyne nucleoside **3a** or the ethynyl nucleoside **3d**. In fact, click reactions performed on **3a** and **3d** using copper(II) without reducing agent are very sluggish.

The identity of click products was confirmed by ESI-TOF and ^{1}H, ^{13}C{^{1}H} and 2D NMR spectra. A signal for the triazole hydrogen H-C(5) (δ_H = 8.49-8.59 ppm) appeared, whereas the signals for the acetylenic protons for **2a** (δ_H 3.23 ppm), **3a** (δ_H 2.77 ppm) and **3d** (δ_H 4.58 ppm) disappeared. In the ^{13}C{^{1}H} NMR spectra the absence of the two terminal C≡C carbon signals and the appearance of two new double bond carbon signals for the 1,2,3-triazole moiety are observed (Table S1, Supporting Information).

Fluorescence of Pyrene Click Conjugates. Having the pyrene click conjugates **13**-**15** in hand their fluorescence properties were determined with respect to linker structure, solvent changes and related 7-deazapurine nucleosides. First, the fluorescence of the pyrene click conjugates **13**-**15** with ethynyl, octadiynyl and tripropargyl side chains was measured in methanol (Figure 4). In all three cases the excitation wavelength was 341 nm. The fluorescence monomer emission of the pyrene residues was the lowest for the tripropargyl compound **13** and the highest for the octadiynyl nucleoside **15**. Furthermore, conjugate **15**

with the octadiynyl linker shows high fluorescence in water (Figure 5a), whereas for **13** and **14** the fluorescence in water is low (Figures 5c,e). Apparently, a sufficient linker length is important for the solvation with water molecules or an intramolecular interaction between the nucleobase and the dye is controlled by the linker. The tripropargylamino nucleoside carrying two pyrene residues shows excimer fluorescence (Figure 5e).

Excimer fluorescence occurs when one pyrene molecule in the excited state forms a contact dimer with a second molecule in the ground state.[18] In the bis-click adduct of the tripropargylamine nucleoside **13** the two pyrene residues are linked to the nucleobase by short flexible chains. Thus, in solution the local concentration of the chromophores is greatly enhanced compared to monochromophoric systems and excimer emission can be detected at low concentration. According to observations on non-nucleoside systems, the extent of excimer emission is limited by the probability of the molecule to reach a conformation suitable for excimer emission. Conformational changes of the connecting chains have to be faster than the excimer forming step.[18b] Furthermore, it has been discussed that the pyrene residues have to be apart when light is absorbed and the excitation is localized in only one of the two pyrene residues forming the excimer.[18b] This is actual the case for the bis-click adduct **13** and thus the nucleoside is attractive to be used as environmentally sensitive reporter.

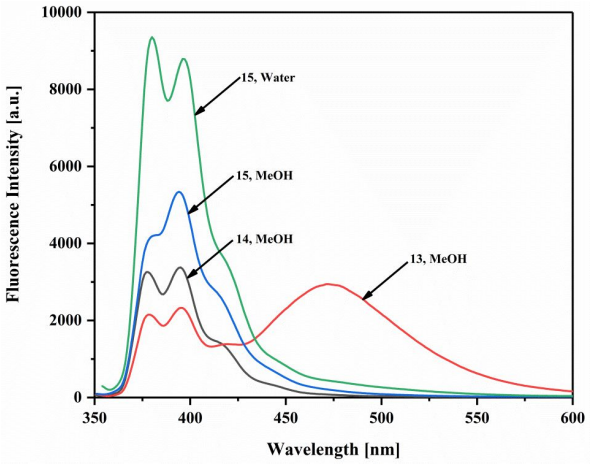
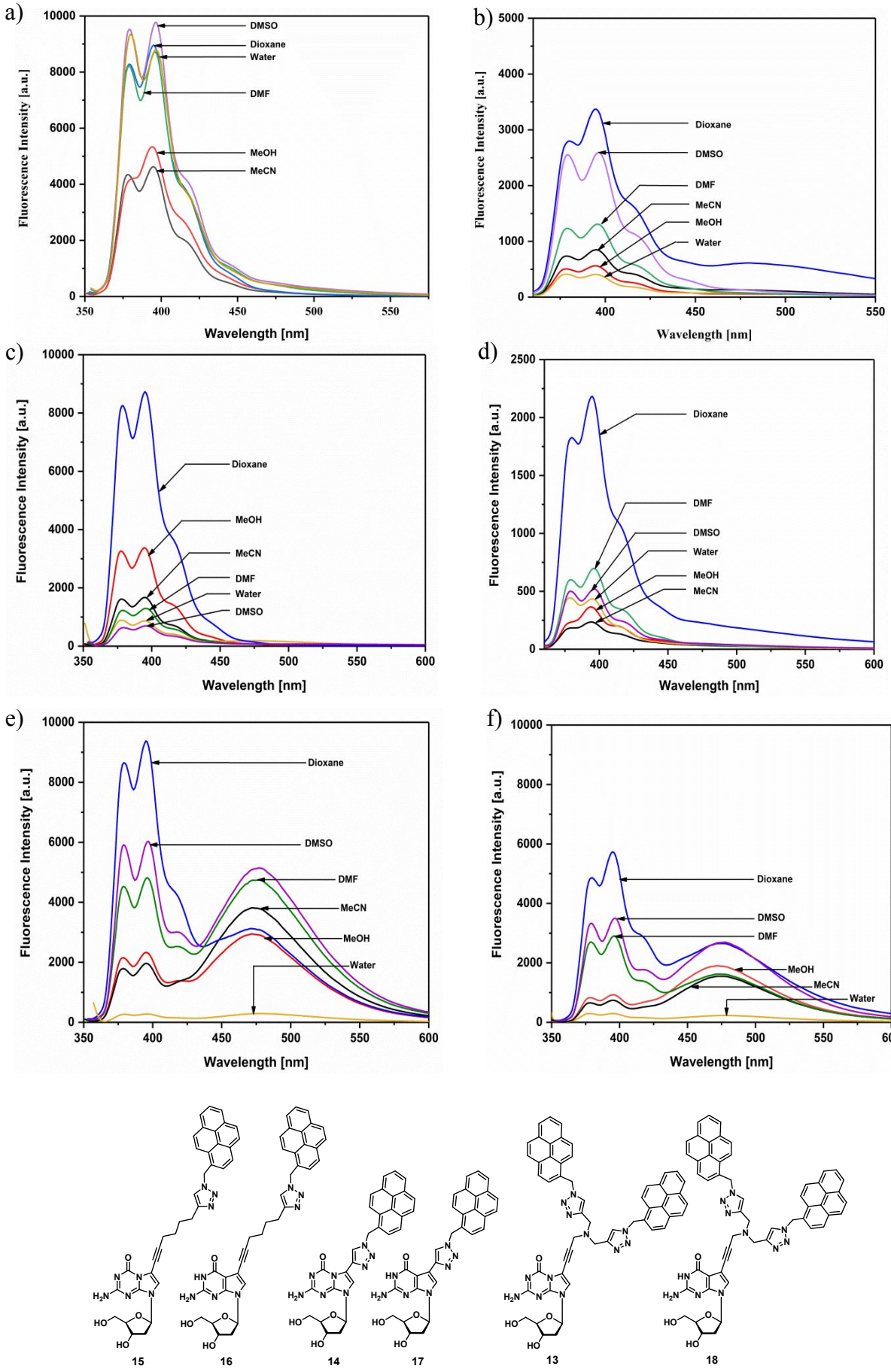


Figure 4. Fluorescence emission spectra of the 5-aza-7-deazaguanine pyrene click conjugates **13**-**15** measured in MeOH (10 μM) and for **15** in water (5 μM). Excitation wavelength was 341 nm for **13**-**15** in MeOH and 344 nm for **15** in water.

Next, the fluorescence dependency of the click conjugates was evaluated in a series of different solvents. According to Figure 5, the fluorescence among the 5-aza-7-deazaguanine conjugates showed significant differences. Strong fluorescence was observed for the octadiynyl conjugate **15** in water, whereas the conjugates **13** and **14** show only weak fluorescence. For all three compounds the fluorescence is high in dioxane, but the order of fluorescence intensity changes in DMF, DMSO, MeOH and MeCN. Also, Stoke's shifts were measured and quantum yields were determined (Table 1).

For the tripropargylamino conjugate **13**, the intensity ratio of monomer to excimer emission is the highest in aprotic polar dioxane (I_M/I_E: ~2:1), whereas the ratio is almost 1:1 in polar protic solvents like MeOH and water and is altered in unipolar aprotic MeCN (I_M/I_E:1:2) The highest monomer emission is observed in dioxane and the highest excimer emission is found in DMSO. In water the system shows low monomer and excimer emission. Very little changes regarding wavelength are observed among the conjugates in the various solvents.


a)
Fluorescence Intensity [a.u.]
Wavelength [nm]
DMSO
Dioxane
Water
DMF
MeOH
MeCN
b)
Fluorescence Intensity [a.u.]
Wavelength [nm]
Dioxane
DMSO
DMF
MeCN
MeOH
Water
c)
Fluorescence Intensity [a.u.]
Wavelength [nm]
Dioxane
MeOH
MeCN
DMF
Water
DMSO
d)
Fluorescence Intensity [a.u.]
Wavelength [nm]
Dioxane
DMF
DMSO
Water
MeOH
MeCN
e)
Fluorescence Intensity [a.u.]
Wavelength [nm]
Dioxane
DMSO
DMF
MeCN
MeOH
Water
f)
Fluorescence Intensity [a.u.]
Wavelength [nm]
Dioxane
DMSO
DMF
MeOH
MeCN
Water

15
16
14
17
13
18

Figure 5. (a) Fluorescence emission spectra of 5-aza-7-deazaguanine conjugates **15** (a), **14** (c) and **13** (e) as well as 7-deazaguanine conjugates **16** (b), **17** (d) and **18** (f) measured in various solvents with a nucleoside concentration of 10 µM. For **15** in water the nucleoside concentration was 5 µM.

Table 1. Photophysical Data of Click Conjugates **13**-**18** 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	I_M/I_E[d]
15	Dioxane	342	395		3900	0.123	
	DMSO	344	396		3800	0.123	
	DMF	343	396		3900	0.101	
	MeCN	340	395		4100	0.060	
	MeOH	341	395		4000	0.043	
	Water	344	396		3800	0.337	
16	Dioxane	343	395		3800	0.026	
	DMSO	344	396		3800	0.011	
	DMF	343	396		3900	0.014	
	MeCN	341	395		4000	0.008	
	MeOH	340	395		4100	0.009	
	Water	341	395		4000	0.006	
14	Dioxane	343	395		3800	0.124	
	DMSO	344	396		3800	0.014	
	DMF	343	395		3800	0.011	
	MeCN	341	395		4000	0.028	
	MeOH	341	395		4000	0.052	
	Water	341	395		4000	0.174	
17	Dioxane	342	395		3900	0.021	
	DMSO	344	396		3800	0.017	
	DMF	343	396		3900	0.012	
	MeCN	341	394		3900	0.007	
	MeOH	341	394		3900	0.007	
	Water	341	395		4000	0.013	
			Monomer	Excimer			
13	Dioxane	343	395	471	7900	0.175	3:1
	DMSO	344	397	477	8100	0.105	1.2:1
	DMF	343	396	477	8200	0.111	1:1
	MeCN	341	395	474	8200	0.058	1:2
	MeOH	341	396	472	8100	0.080	1:1.3
	Water	347	396	478	7900	0.045	1:1
18	Dioxane	342	395	478	8300	0.069	2.2:1
	DMSO	343	396	477	8200	0.052	1.3:1
	DMF	344	396	477	8100	0.030	1.8:1
	MeCN	340	395	472	8200	0.034	1:2
	MeOH	340	395	471	8200	0.037	1:2
	Water	341	395	478	8400	0.018	1:1

[a] The concentration of nucleosides was 10 µM. For **15** in water the concentration was 5 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] I_M: monomer intensity at the monomer emission maximum. I_E: excimer intensity at the excimer emission maximum.

Then, the solvent dependent fluorescence of the 5-aza-7-deazaguanine nucleoside conjugates were compared with 7-deazaguanine conjugates **16-18**[19] having exactly the same structure but with nitrogen in position-7 and not in position-5 (Figure 5). It is apparent, that in all solvents the steady state fluorescence of the 5-aza-7-deazaguanine conjugates is significantly higher than the fluorescence of the 7-deazaguanine compounds (Figure 5). Also, the quantum yields shown in Table 1 differ significantly in both series with always higher quantum yields for the 5-aza-7-deazaguanine nucleosides.

Fluorescence quenching can be induced by fluorescence resonance energy transfer (FRET) or charge separation (intramolecular electron transfer or hole transfer) between the dye and the nucleobase.[20] The low oxidation potential of 7-deazaguanine nucleosides was made responsible for its strong fluorescence quenching caused by a charge transfer between base and the dye. Apparently, pyrene cannot be oxidized by the 5-aza-7-deazaguanine base resulting in higher fluorescence of its click products.

CONCLUSION

The synthesis of 5-aza-7-deazaguanine nucleosides with linear alkynyl chains or dendritic triprogargylamino residues was performed. Various sugar residues were introduced at the nucleoside glycosylation site-9. From 5-aza-7-deazaguanine nucleosides with ribo, 2'-deoxyribo and fluoroarabino sugar residues clickable derivatives were synthesized for the copper(I) promoted *Huisgen-Sharpless-Meldal* cycloaddition. Ethynyl, 1,7-octadiynyl and tripropargylamino side chains were introduced in the 7-position of the nucleobase by *Sonogashira* cross-coupling employing 5-aza-7-deaza-7-iodoguanine nucleosides and

corresponding alkynes. TBTA shortened the reaction times of *Sonogashira* cross-coupling reactions but led to lower or marginally higher yields.

Click reactions with tripropargylamino nucleosides and 1-azidomethylpyrene were significantly faster and higher yielding with respect to nucleosides carrying linear alkynyl side chains. Furthermore, the reaction could be performed with copper(II) as catalyst, whereas copper(I) is used for regular click reactions.

An autocatalyzed cycle is suggested in which copper(I) is chelated by the click product and the next click reaction is catalyzed by this copper(I) click complex. We observed that addition of click products at an early stage of the reaction accelerated product formation. This fulfills the criteria of autocatalysis.

Pyrene click adducts of 5-aza-7-deazaguanine nucleosides with linear and dendronized side chains show significantly higher fluorescence than previously reported 7-deazaguanine conjugates and similar intensities as 7-deaza-8-azaguanine compounds.[20d] Due to the absence of a hydrogen atom at nitrogen-1 the recognition face of 5-aza-7-deazaguanine differs from that of the other guanine nucleosides. Consequently, other base pairs are formed that can now be decorated with fluorescent tags.[1b] Furthermore, the clickable nucleosides reported in this work can be utilized to prepare libraries of antiviral active 5-aza-7-deazaguanine nucleosides[21] or being applicable for diagnostic or imaging purposes in DNA biotechnology.

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 $dm^3\ mol^{-1}\ cm^{-1}$. $^{13}C\{^1H\}$ NMR spectra were measured at 599.74 MHz or 399.89 MHz for ^1H and 150.82 MHz, 100.56 MHz or 75.47 MHz for ^{13}C. ^1H-^{13}C correlated (HMBC, HSQC) NMR spectra were used for the assignment of the ^{13}C signals (Table S1, 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.

Fluorescence Studies. Fluorescence measurements were performed with a F-2500 fluorescence spectrophotometer equipped with a cooling device or a F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). Fluorescence spectra of nucleoside 'click' conjugates were measured in different solvents. For solubility reasons, all click conjugates (2-4 mg) were dissolved in 0.5 mL of DMSO and then diluted with 9.5 mL of methanol. This solution was used as a stock solution (400 μmol). All measurements were performed with identical concentrations of 10 μM. Quantum yields *Φ* were determined by using quinine sulfate in 0.1 M sulfuric acid as a standard with a known *Φ* of 0.54.[22]

The quantum yield *Φ* (x) of the unknown compound can be calculated by the following equation:

$$\Phi\ (x) = \Phi\ (ST)(AST/AX)(FX/FST)(\eta^2X/\eta^2ST)$$

where *Φ* (ST) is the quantum yield of the standard, A is the absorbance at the excitation wavelength, F is the integrated area of the emission curve, the subscripts X and ST refer to unknown and standard and η is the refractive index of solvent.

3,5-Di-*O*-benzoyl-2-deoxy-2-fluoro-*α*-D-arabinofuranosyl Bromide (6).[9] Compound **6** was prepared according to a literature protocol.[9] To a solution of 1,3,5-tri-*O*-benzoyl-2-deoxy-2-

fluoro-α-D-arabinofuranose **5** (1.5 g, 3.21 mmol) in CH_2Cl_2 (15 mL) was added a 30% soln. of HBr in acetic acid (1.5 mL). The mixture was stirred at rt for 16 h and evaporated to dryness. The remaining syrup was dissolved in CH_2Cl_2 (20 mL), washed with sat. aq. $NaHCO_3$ soln. (10 mL), dried over Na_2SO_4, filtrated and concentrated to syrup. The syrup of compound **6** was used for the next step without any further purification.

2-[(N^2-Isobutyryl)amino]-8-(3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-6-iodo-imidazo[1,2-*a*]-*s*-triazin-4(8*H*)-one (7) and 2-[(N^2-Isobutyryl)amino]-8-(3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-6-iodo-imidazo[1,2-*a*]-*s*-triazin-4(8*H*)-one (8). The halogenose **6** was dissolved in CH_3CN (8 mL) and introduced into a suspension of compound **4** (500 mg, 1.44 mmol) and DBU (263 mg, 244 µL, 1.73 mmol) in MeCN (10 mL). Then, the reaction mixture was stirred at ambient temperature for 16 h and evaporated to dryness. After FC (silica gel, column 12 x 4 cm, CH_2Cl_2/MeOH 100:1→95:5) an anomeric mixture of **7** and **8** (723 mg, 73%) was obtained as colorless foam. ^{1}H NMR of the anomeric mixture showed a 1:1 β to α ratio.

2-[(N^2-Isobutyryl)amino]-8-(3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-6-iodo-imidazo[1,2-*a*]-*s*-triazin-4(8*H*)-one (7). The anomeric mixture of **7/8** (723 mg, 73%) was dissolved in MeOH (15 mL) under gentle warming for 3 min. After cooling to rt, the β-D anomer **7** precipitated as white powder. The precipitate was filtered-off and washed with MeOH (5 mL) affording the compound **7** (299 mg, 30%) as colorless solid. TLC (silica gel, CH_2Cl_2/MeOH, 95:5) R_f 0.4. λ_{max} (MeOH)/nm 283 (ε/dm^3 mol^{-1} cm^{-1} 11 700). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 1.04 (dd, J = 6.9, 1.6 Hz, 6H, $(CH_3)_2CH$), 2.89 (hept, J = 6.8 Hz, 1H, $CH(CH_3)_2$), 4.65 – 4.80 (m, 3H, H-4', H-5'), 5.72 (ddd, J = 50.5, 4.3, 2.7 Hz, 1H, H-2'), 5.90 (ddd, J = 18.7, 4.6, 2.6 Hz, 1H, H-3'), 6.45 (dd, J = 16.8, 4.2 Hz, 1H, H-1'), 7.50 – 7.54 (m, 2H, arom. H), 7.56 – 7.61 (m, 2H, arom. H), 7.65 – 7.69 (m, 2H, arom. H), 7.71 – 7.75

(m, 1H, H-8), 7.97 – 8.01 (m, 2H, arom. H), 8.05 – 8.08 (m, 2H, arom. H), 10.42 (s, 1H, NH). $^{13}C\{^{1}H\}$NMR (DMSO-d_6, 151 MHz): δ 19.0, 19.0, 34.6, 59.3, 63.7, 75.9, 76.1, 78.4, 78.5, 81.9, 82.1, 92.3, 93.5, 123.9, 123.9, 128.6, 128.8, 128.8, 129.2, 129.6, 133.5, 134.0, 150.1, 150.3, 160.0, 164.7, 165.4, 175.7. HRMS (ESI-TOF) m/z: [M + Na^+] calcd for $C_{28}H_{25}FIN_5O_7Na$, 712.0675; found, 712.0672.

2-Amino-8-(2-deoxy-2-fluoro-*β*-D-arabinofuranosyl)-6-iodo-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (1c). From 7: Compound **7** (100 mg, 0.15 mmol) was suspended in 7N NH_3/MeOH (20 mL) and the mixture was stirred at rt 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→85:15). From the main zone compound **1c** (54 mg, 87%) was obtained as colorless solid. TLC (silica gel, CH_2Cl_2/MeOH, 90:10) R_f 0.4. λ_{max} (MeOH)/nm 266 (ε/dm^3 mol^{-1} cm^{-1} 13 500), 260 (12600). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 3.56 – 3.70 (m, 2H, H-5'), 3.80 (q, J = 4.7 Hz, 1H, H-4'), 4.33 (dt, J = 18.5, 4.7 Hz, 1H, H-3'), 5.08 – 5.21 (m, 2H, H-2', HO-5'), 5.96 (d, 1H, HO-3'), 6.14 (dd, J = 13.4, 4.5 Hz, 1H, H-1'), 7.01 and 7.05 (2s, 2H, NH_2), 7.49 (d, J = 1.8 Hz, 1H, H-8). $^{13}C\{^{1}H\}$ NMR (DMSO-d_6, 151 MHz): δ 57.6, 60.0, 71.8, 71.9, 81.1, 81.2, 83.5, 83.6, 94.5, 95.7, 121.9, 121.9, 150.0, 150.4, 150.4, 164.2. HRMS (ESI-TOF) m/z: [M + Na^+] calcd for $C_{10}H_{11}FIN_5O_4Na$, 433.9732; found, 433.9720.

From 11: Compound **11** (100 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (5 mL) and treated with trichloroacetic acid (1 mL of a 3% soln in CH_2Cl_2). The mixture was stirred for 16 h at rt. Then, the reaction mixture was neutralized with triethylamine (50 µL) and the solvent was evaporated. The remaining residue was applied to FC (silica gel, column 10 x 3 cm, CH_2Cl_2/ MeOH, 100:0→85:15). From the main zone, compound **1c** was obtained as a colorless solid (49 mg, 84%). Analytical data were identical to those described above.

Separation of the Anomeric Glycosylation Mixture of 7/8 by 4,4'-Dimethoxytritylation.

In a separate glycosylation experiment, the mother liquor obtained from crystallization of the *β*-D anomer **7** was evaporated to a colorless foam (424 mg, 0.62 mmol). Then, 7N NH_3/MeOH (100 mL) was added and the reaction mixture was stirred overnight at rt. 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→85:15). Evaporation of the main zone gave an anomeric mixture of **9** and **1c** as an off-white solid (216 mg, 85%). Then, 140 mg (0.34 mmol) of this mixture were dissolved in pyridine (5 mL), 4,4'-dimethoxytritylchloride (231 mg, 0.68 mmol) was added in three equal portions and the reaction mixture was stirred for 3.5 h at ambient temperature. Then, the solvent was evaporated and the remaining syrup was dissolved in CH_2Cl_2 (20 mL) washed with sat. aq. $NaHCO_3$ soln. (10 mL), dried over Na_2SO_4, filtrated and applied to FC (silica gel, column 10 x 2 cm, CH_2Cl_2/MeOH, 100:0→97:3).

2-Amino-8-[2-deoxy-2-fluoro-5-*O*-(4,4′-dimethoxytriphenylmethyl)-*α*-D-arabinofuranosyl]-6-iodo-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (10). From the faster migrating zone, compound **10** was obtained as colorless foam (145 mg, 60%). TLC (silica gel, CH_2Cl_2/MeOH, 95:5) R_f 0.4. λ_{max} (MeOH)/nm 270 (ε/dm^3 mol^{-1} cm^{-1} 15 600). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 3.09 – 3.15 (m, 2H, H-5'), 3.73 and 3.74 (2s, 6H, OCH_3) ; 4.34 (d, J = 19.5 Hz, 1H, H-4'), 4.47 (q, J = 5.0 Hz, 1H, H-3'), 5.39 (dt, J = 50.9, 2.7 Hz, 1H, H-2'), 6.03 (d, J = 3.8 Hz, 1H, 3'-OH), 6.07 (dd, J = 15.9, 2.4 Hz, 1H, H-1'), 6.78-6.83 (m, 4H, arom. H), 7.00 and 7.03 (2s, 2H, NH_2), 7.16-7.29 (m, 5H, arom. H), 7.32 (t, J = 7.7 Hz, 2H, arom. H), 7.38-7.44 (m, 2H, arom. H), 7.52 (s, 1H, H-8). ^{13}C{^{1}H} NMR (DMSO-d_6, 151 MHz): δ 54.9, 55.0, 57.7, 62.8, 73.7, 73.9, 85.3, 85.3, 85.4, 86.3, 86.6, 98.1, 99.9, 112.6, 113.2, 121.6, 126.7, 127.6, 127.8, 129.6, 129.6, 135.3, 135.4, 144.7, 150.0, 150.5, 158.1, 164.1. HRMS (ESI-TOF) m/z: [M + Na$^+$] calcd for $C_{31}H_{29}FIN_5O_6Na$, 736.1039; found, 736.1032.

2-Amino-8-[2-deoxy-2-fluoro-5-*O*-(4,4′-dimethoxytriphenylmethyl)-*β*-D-arabinofuranosyl]-6-iodo-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (11). From the slower migrating zone the *β*-D- anomer **11** was obtained (24 mg, 10%) as colorless foam. TLC (silica gel, CH_2Cl_2/MeOH, 95:5) R_f 0.3. λ_{max} (MeOH)/nm 267 (ε/dm^3 mol^{-1} cm^{-1} 16 000). ^{1}H NMR (DMSO-*d*$_6$, 600 MHz): *δ* 3.16 – 3.30 (2m, 2H, H-5'), 3.74 (s, 6H, OCH_3), 4.00 (d, 1H, H-4'), 4.33 (dq, *J* = 18.4, 4.6 Hz, 1H, H-3'), 5.13 (dt, *J* = 52.2, 3.9 Hz, 1H, H-2'), 6.00 (d, *J* = 4.7 Hz, 1H, OH-3'), 6.19 (dd, *J* = 15.2, 4.3 Hz, 1H, H-1'), 6.85 – 6.96 (m, 4H, arom. H), 7.03 and 7.07 (2s, 2H, NH_2), 7.16 (d, *J* = 2.0 Hz, 1H, H-8), 7.20 – 7.36 (m, 5H, arom. H), 7.32 (dd, *J* = 8.5, 7.1 Hz, 2H, arom. H), 7.37 – 7.46 (m, 2H, arom. H), 7.52 (s, 1H, H-8). ^{13}C{^{1}H} NMR (DMSO-*d*$_6$, 151 MHz) : *δ* 55.0, 55.1, 57.8, 63.1, 72.8, 73.0, 81.0, 81.2, 82.0, 82.0, 85.5, 94.0, 95.9, 113.2, 121.5, 126.7, 127.6, 127.8, 129.6, 129.6, 135.3, 135.4, 144.7, 149.9, 150.6, 158.1, 164.2. HRMS (ESI-TOF) m/z: [M + Na$^+$] calcd for $C_{31}H_{29}FIN_5O_6Na$, 736.1039; found, 736.1028.

2-Amino-8-(2-deoxy-2-fluoro-*α*-D-arabinofuranosyl)-6-iodo-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (9). Compound **10** (100 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (5 mL) and treated with trichloroacetic acid (1 mL of a 3% soln. in CH_2Cl_2) for 16 h at rt. The reaction mixture was neutralized with triethylamine (100 µL) and the solvent was evaporated. The remaining residue was applied to FC (silica gel, column 10 x 3 cm, CH_2Cl_2/ MeOH, 100:0→85:15). From the main zone, compound **9** was obtained as a colorless solid (51 mg, 88%). TLC (silica gel, CH_2Cl_2/MeOH, 9:1) R_f 0.3. λ_{max} (MeOH)/nm 266 (ε/dm^3 mol^{-1} cm^{-1} 13 200), 260 (12200). ^{1}H NMR (DMSO-*d*$_6$, 600 MHz): *δ* 3.45 – 3.56 (m, 2H, H-5'), 4.21 (q, *J* = 5.0 Hz, 1H, H-4'), 4.28 – 4.36 (m, 1H, H-3'), 5.01 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.39 (dt, *J* = 51.2, 3.1 Hz, 1H, H-2'), 5.96 – 5.99 (m, 1H, OH-3'), 6.03 (dd, *J* = 15.9, 2.8 Hz, 1H, H-1'), 7.01 and 7.03 (2s, 2H, NH_2), 7.51 (s, 1H, H-8). ^{13}C{^{1}H} NMR (DMSO-*d*$_6$, 151 MHz): *δ* 57.8, 60.5,

73.1, 73.2, 86.0, 86.2, 86.7, 86.7, 98.6, 99.8, 121.5, 150.0, 150.6, 164.1. HRMS (ESI-TOF) m/z: [M + Na^+] calcd for $C_{10}H_{11}FIN_5O_4Na$, 433.9732; found, 433.9726.

General Procedure for Sonogashira Cross-Coupling of Nucleosides 1a-c with Tripropargylamine without TBTA. To a suspension of the particular nucleoside (0.25 mmol, 1 eq.) in DMF (2 mL) in an oven dried round bottom flask $Pd(PPh_3)_4$ (30 mg, 0.025 mmol, 0.1 eq.), Et_3N (69 µL, 0.50 mmol, 2 eq.), CuI (10 mg, 0.050 mmol, 0.2 eq.), and tripropargyl amine (360 µL, 2.50 mmol, 10 eq.) were added. The reaction mixtures were stirred at rt for 6 h. Then, the solvent was evaporated and the remaining residues were purified by FC. From the main zones, compounds **2a-c** were obtained.

2-Amino-8-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-{3-[di(prop-2-yn-1-yl)amino]prop-1-yn-1-yl}-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (2a). As described above with compound **1a**. Purification by FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:13) and evaporation of the main zone gave compound **2a** (62 mg 63%) as pale yellow solid. TLC (silica gel, CH_2Cl_2/ MeOH, 9:1) R_f 0.4. λ_{max} (MeOH)/nm 273 (ε/dm^3 mol^{-1} cm^{-1} 16 200). ^1H NMR (DMSO-d_6, 600 MHz): δ 2.17 (ddd, J = 13.2, 6.1, 3.4 Hz, 1H, H-2'$_\alpha$), 2.36 (ddd, J = 13.1, 7.3, 5.8 Hz, 1H, H-2'$_\beta$), 3.23 (t, J = 2.4 Hz, 2H, 2 x C≡CH), 3.47 (d, J = 2.5 Hz, 4H, 2 x CH_2), 3.48 – 3.57 (m, 2H, H-5'), 3.63 (s, 2H, CH_2), 3.79 (td, J = 4.3, 2.8 Hz, 1H, H-4'), 4.30 (dq, J = 6.7, 3.5 Hz, 1H, H-3'), 4.98 (t, J = 5.4 Hz, 1H, OH-5'), 5.29 (d, J = 3.9 Hz, 1H, OH-3'), 6.14 (dd, J = 7.2, 6.1 Hz, 1H, H-1'), 7.00 and 7.02 (2s, 2H, NH_2), 7.78 (s, 1H, H-8). $^{13}C\{^1H\}$ NMR (DMSO-d_6, 151 MHz): δ 39.1, 41.0, 41.2, 42.0, 61.3, 70.2, 72.9, 76.0, 79.1, 82.9, 87.7, 90.9, 104.9, 119.5, 149.7, 150.0, 165.0. HRMS (ESI-TOF) m/z: [M + Na^+] calcd for $C_{19}H_{20}N_6O_4Na$, 419.1438; found, 419.1428.

Sonogashira Cross-Coupling of Compound 1a with Tripropargylamine and TBTA: To a suspension of compound **1a** in DMF (2 mL) in an oven dried round bottom flask $Pd(PPh_3)_4$ (30 mg, 0.025 mmol, 0.1 eq.), Et_3N (69 µL, 0.50 mmol, 2 eq.), CuI (10 mg, 0.050 mmol, 0.2 eq.), TBTA {tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amin)}(26.5 mg, 0.2 eq.) and tripropargylamine (360 µL, 2.50 mmol, 10 eq.) were added. The reaction time was 2.5 h until most of the staring material was consumed. The solvent was evaporated, and the remaining residue was applied to FC (silica gel, column 15 × 2 cm, CH_2Cl_2/MeOH, 87:13). From the main zone, compound **2a** was obtained as pale yellow solid (49 mg, 49%). Analytical data were identical to those described above.

2-Amino-8-(*β*-D-ribofuranosyl)-6-{3-[di(prop-2-yn-1-yl)amino]prop-1-yn-1-yl}-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (2b). As described above without TBTA and compound **1b**. After purification by FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:13) compound **2b** (54 mg 52%) was obtained as pale yellow solid. TLC (silica gel, CH_2Cl_2/ MeOH, 85:15) R_f 0.4. λ_{max} (MeOH)/nm 273 (ε/dm^3 mol^{-1} cm^{-1} 15 900). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.23 (t, J = 2.4 Hz, 2H, 2 x C≡CH), 3.47 (d, J = 2.5 Hz, 4H, 2 x CH_2), 3.53 (ddd, J = 12.0, 5.4, 3.8 Hz, 1H, H-5'), 3.60 (dd, J = 5.3, 3.9 Hz, 1H, 2H-5'), 3.64 (s, 2H, CH_2), 3.87 (q, J = 3.8 Hz, 1H, H-4'), 4.06 (td, J = 4.8, 3.6 Hz, 1H, H-3'), 4.24 (q, J = 5.3 Hz, 1H, H-2'), 5.07 (t, J = 5.4 Hz, 1H, OH-5'), 5.15 (d, J = 4.7 Hz, 1H, OH-3'), 5.46 (d, J = 5.5 Hz, 1H, OH-2'), 5.76 (d, J = 5.5 Hz, 1H, H-1'), 7.02 and 7.04 (2s, 2H, NH_2), 7.82 (s, 1H, H-8). ^{13}C{^{1}H} NMR (DMSO-d_6, 101 MHz): δ 41.1, 42.0, 60.9, 70.0, 72.8, 73.6, 75.9, 79.0, 85.3, 86.4, 91.0, 105.0, 119.5, 149.7, 150.5, 165.0. HRMS (ESI-TOF) m/z: [M + Na$^+$] calcd for $C_{19}H_{20}N_6O_5Na$, 435.1387; found, 435.1384.

2-Amino-8-(2-deoxy-2-fluoro-*α*-D-arabinofuranosyl)-6-{3-[di(prop-2-yn-1-yl)amino]prop-1-yn-1-yl}-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (2c). As described above

without TBTA and compound **1c**. After purification by FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:13) compound **2b** (61 mg 59%) was obtained as pale yellow solid. TLC (silica gel, CH_2Cl_2/ MeOH, 85:15) R_f 0.4. λ_{max} (MeOH)/nm 272 (ε/dm^3 mol^{-1} cm^{-1} 15 200). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 3.23 (t, J = 2.4 Hz, 2H, 2 x C≡CH), 3.47 (d, J = 2.5 Hz, 4H, 2 x CH_2), 3.58 – 3.70 (m, 4H, CH_2 and 2 x H-5'), 3.82 (q, J = 4.8 Hz, 1H, H-3'), 4.34 (ddd, J = 18.1, 5.3, 4.1 Hz, 1H, H-4'), 5.16 (dt, J = 52.4, 4.3 Hz, 2H, H-2', OH-5'), 6.01 (m, 1H, OH-3'), 6.16 (dd, J = 13.0, 4.5 Hz, 1H, H-1'), 7.06 and 7.11 (2s, 2H, NH_2), 7.72 (s, 1H, H-8). ^{13}C{1H} NMR (DMSO-d_6, 151 MHz): δ 41.2, 42.0, 60.0, 71.7, 71.9, 72.6, 76.0, 79.1, 81.1, 81.2, 83.7, 83.7, 91.1, 94.3, 95.6, 95.7, 104.9, 120.4, 149.5, 150.0, 159.0, 162.0, 162.7, 164.9. HRMS (ESI-TOF) m/z: [M + Na$^+$] calcd for $C_{19}H_{19}FN_6O_4Na$, 437.1344; found, 437.1344.

General Procedure for Sonogashira Cross-Coupling of Nucleoside 1a with Linear Alkynes without TBTA (Method A). To a suspension of the particular nucleoside (0.25 mmol, 1 eq.) in DMF (2 mL) in an oven dried round bottom flask $Pd(PPh_3)_4$ (30 mg, 0.025 mmol, 0.1 eq.), Et_3N (69 µL, 0.50 mmol, 2 eq.), CuI (10 mg, 0.050 mmol, 0.2 eq.), and the linear alkyne (for eq., see the particular compound) were added. The reaction mixture was stirred at rt (for reaction time, see the particular compound). Then, the solvent was evaporated and the remaining residue was and purified by FC. From the main zone, compounds **3a-c** were obtained.

General Procedure for Sonogashira Cross-Coupling of Nucleosides 1a with Linear Alkynes with TBTA (Method B). To a suspension of the particular nucleoside (0.25 mmol, 1 eq.) in DMF (2 mL) in an oven dried round bottom flask $Pd(PPh_3)_4$ (30 mg, 0.025 mmol, 0.1 eq.), Et_3N (67 µL, 0.50 mmol, 2 eq.), CuI (10 mg, 0.050 mmol, 0.2 eq.), TBTA {tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amin)}(26.5 mg, 0.2 eq.) and the linear alkyne (for eq.,

see the particular compound) were added. The reaction mixture was stirred at rt (for reaction time, see the particular compound). Then, the solvent mixture was evaporated to dryness and purified by FC. From the main zone, compounds **3a-c** were obtained.

2-Amino-8-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-(1,7-octadiyn-1-yl)-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (3a). Method A: With compound **1a** and 1,7-octadiyne (250 μL, 1.88 mmol, 7.5 eq.). The reaction time was 5 h until most of the staring material was consumed. The solvent was evaporated, and the remaining residue was applied to FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:13). From the main zone, compound **3a** was obtained as pale yellow solid (22 mg, 23%). TLC (silica gel, CH_2Cl_2/MeOH, 9:1) R_f 0.4. λ_{max} (MeOH)/nm 274 (ε/dm^3 mol^{-1} cm^{-1} 15 900). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 1.61 (tdt, J = 6.9, 4.5, 2.4 Hz, 4H, 2 x CH_2), 2.16 (ddd, J = 13.2, 6.1, 3.3 Hz, 1H, H-2'$_\alpha$), 2.20 (td, J = 6.7, 2.6 Hz, 2H, CH_2), 2.36 (ddd, J = 13.2, 7.4, 5.7 Hz, 1H, H-2'$_\beta$), 2.43-2.48 (m, 2H, CH_2), 2.77 (t, J = 2.7 Hz, 1H, C≡CH), 3.46-3.58 (m, 2H, H-5'), 3.79 (td, J = 4.3, 2.8 Hz, 1H, H-4'), 4.29 (dq, J = 6.5, 3.3 Hz, 1H, H-3'), 4.97 (t, J = 5.4 Hz, 1H, OH-5'), 5.28 (d, J = 4.0 Hz, 1H, OH-3'), 6.14 (dd, J = 7.4, 6.1 Hz, 1H, H-1'), 6.96 (2s, 2H, NH_2), 7.67 (s, 1H, H-8). ^{13}C{^{1}H} NMR (DMSO-d_6, 151 MHz): δ 17.2, 18.4, 26.8, 27.0, 39.0, 61.3, 68.6, 70.3, 71.3, 82.7, 84.2, 87.7, 96.1, 105.8, 118.5, 149.7, 149.9, 164.9. HRMS (ESI-TOF) m/z: [M + Na$^+$] calcd for $C_{18}H_{21}N_5O_4Na$, 394.1486; found, 394.1487.

Method B: With compound **1a** and 1,7-octadiyne (338 μL, 2.54 mmol, 10 eq.). The reaction time was 3 h until most of the staring material was consumed. The solvent was evaporated, and the remaining residue was applied to FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:13). From the main zone, compound **3a** was obtained as pale yellow solid (11 mg, 12%). Analytical data were identical to those described above.

2-Amino-8-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-(trimethylsilylethynyl)-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (3b). Method A: With compound **1a** and trimethylsilylacetylene (310 μL, 2.2 mmol, 8.7 eq.). After 12 h $Pd(PPh_3)_4$ (30 mg, 0.025 mmol, 0.1 eq.), Et_3N (67 μL, 0.50 mmol, 2 eq.), CuI (10 mg, 0.050 mmol, 0.2 eq.) and trimethylsilylacetylene (310 μL, 2.2 mmol, 8.7 eq.) were added. The reaction mixture was stirred for additional 12 h until the starting material was almost consumed. The solvent was evaporated, and the remaining residue was applied to FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:13). From the main zone, compound **3b** was obtained as pale yellow solid (36 mg, 39%). TLC (silica gel, CH_2Cl_2/MeOH, 9:1) R_f 0.4. λ_{max} (MeOH)/nm 278 (ε/dm^3 mol^{-1} cm^{-1} 16 000). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 0.22 (s, 9H, 3 x CH_3), 2.17 (ddd, J = 13.2, 6.1, 3.4 Hz, 1H, H-2'$_\alpha$), 2.36 (ddd, J = 13.2, 7.3, 5.7 Hz, 1H, H-2'$_\beta$), 3.45-3.59 (m, 2H, H-5'), 3.79 (td, J = 4.3, 2.8 Hz, 1H, H-4'), 4.30 (dq, J = 6.5, 3.4 Hz, 1H, H-3'), 4.97 (t, J = 5.4 Hz, 1H, OH-5'), 5.28 (d, J = 3.9 Hz, 1H, OH-3'), 6.13 (dd, J = 7.3, 6.1 Hz, 1H, H-1'), 7.02 (2s, 2H, NH_2), 7.84 (s, 1H, H-8). ^{13}C{^{1}H} NMR (DMSO-d_6, 151 MHz): δ 0.3, 38.9, 61.3, 70.2, 82.9, 87.7, 92.4, 101.0, 105.2, 120.8, 149.6, 150.0, 165.0. HRMS (ESI-TOF) m/z: [M + Na^+] calcd for $C_{15}H_{21}N_5O_4SiNa$, 386.1255; found, 386.1258.

Method B: With compound **1a** and trimethylsilylacetylene (362 μL, 2.54 mmol, 10 eq.). The reaction time was 6 h until most of the staring material was consumed. The solvent was evaporated, and the remaining residue was applied to FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:13). From the main zone, compound **3b** was obtained as pale yellow solid (30 mg, 33%). Analytical data were identical to those described above.

2-Amino-8-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-(triisopropylsilylethynyl)-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (3c). Method A: With compound **1a** and triisopropylsilylacetylene (570 μL, 2.54 mmol, 10 eq.). The reaction time was 24 h until most of the staring material was consumed. The solvent was evaporated, and the remaining residue

was applied to FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:13). From the main zone, compound **3c** was obtained as pale yellow solid (40 mg, 36%). TLC (silica gel, CH_2Cl_2/MeOH, 9:1) R_f 0.3. λ_{max} (MeOH)/nm 279 (ε/dm^3 mol^{-1} cm^{-1} 16 700). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 1.09 (s, 21H, 6CH_3 and 3CH), 2.15 (ddd, J = 13.2, 6.1, 3.3 Hz, 1H, H-2'$_\alpha$), 2.39 (ddd, J = 13.2, 7.4, 5.7 Hz, 1H, H-2'$_\beta$), 3.53 (m, 2H, 2H-5'), 3.79 (td, J = 4.4, 2.9 Hz, 1H, H-4'), 4.30 (dq, J = 6.5, 3.3 Hz, 1H, H-3'), 4.98 (t, J = 5.5 Hz, 1H, OH-5'), 5.27 (d, J = 3.9 Hz, 1H, OH-3'), 6.13 (dd, J = 7.5, 6.0 Hz, 1H, H-1'), 6.98 and 7.02 (2s, 2H, NH_2), 7.84 (s, 1H, H-8). ^{13}C{^{1}H} NMR (DMSO-d_6, 151 MHz): δ 10.7, 18.4, 39.0, 45.7, 61.2, 70.2, 82.8, 87.70, 93.7, 97.4, 105.3, 120.7, 149.5, 150.0, 165.0. HRMS (ESI-TOF) m/z: [M + H$^+$] calcd for $C_{21}H_{34}N_5O_4Si^+$, 448.2375; found, 448.2405.

Method B: With compound **1a** and triisopropylsilylacetylene (570 µL, 2.5 mmol, 10 eq.). The reaction time was 16 h until most of the staring material was consumed. The solvent was evaporated, and the remaining residue was applied to FC (silica gel, column 15 x 2 cm, CH_2Cl_2/MeOH, 87:13). From the main zone, compound **3c** was obtained as pale yellow solid (48 mg, 43%). Analytical data were identical to those described above.

2-Amino-8-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-ethynyl-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (3d). From 3b: Compound **3b** (30 mg, 0.08 mmol) was dissolved in THF (2 mL) and treated with tetra-*n*-butylammonium fluoride (TBAF) (123 µL of a 1 M soln. in THF) for 5 min. at rt. The solvent was evaporated, and the remaining residue was applied to FC (silica gel, column 10 x 3 cm, CH_2Cl_2/ MeOH, 100:0→85:15). From the main zone, compound **3d** was obtained as a colorless solid (16 mg, 69%). TLC (silica gel, CH_2Cl_2/MeOH, 9:1) R_f 0.25. λ_{max} (MeOH)/nm 263 (ε/dm^3 mol^{-1} cm^{-1} 16 700). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.18 (ddd, J = 13.3, 6.1, 3.5 Hz, 1H, H-2'$_\alpha$), 2.37 (ddd, J = 13.1, 7.2, 5.7 Hz, 1H, H-2'$_\beta$), 3.54 (m, 2H, H-5'), 3.80 (td, J = 4.3, 2.9 Hz, 1H, H-4'), 4.31 (dq, J = 6.6, 3.5 Hz, 1H, H-3'), 4.58 (s, 1H, C≡H), 4.99 (t, J = 5.4 Hz, 1H, OH-5'), 5.29 (d, J = 4.0 Hz, 1H, OH-3'), 6.14 (dd, J = 7.2,

6.1 Hz, 1H, H-1'), 7.01 (s, 2H, NH_2), 7.85 (s, 1H, H-8). $^{13}C\{^1H\}$ NMR (DMSO-d_6, 151 MHz): δ 39.0, 61.2, 70.1, 71.5, 82.9, 87.1, 87.7, 104.7, 120.7, 149.6, 150.0, 165.0. HRMS (ESI-TOF) m/z: [M + H^+] calcd for $C_{12}H_{14}N_5O_4^+$, 292.1040; found, 292.1066.

From 3c: Compound **3c** (30 mg, 0.07 mmol) was dissolved in THF (2 mL) and treated with tetra-*n*-butylammonium fluoride (TBAF) (100 µL of a 1 M soln. in THF) for 5 min. at rt. The solvent was evaporated, and the remaining residue was applied to FC (silica gel, column 10 x 3 cm, CH_2Cl_2/ MeOH, 100:0→85:15). From the main zone, compound **3d** was obtained as a colorless solid (14 mg, 70%). Analytical data were identical to those described above.

2-Amino-8-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-[3-{bis(1-(pyren-1-ylmethyl)-1H-(1,2,3-triazol-4-yl)methyl)amino}prop-1-yn-1-yl]-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (13).

Method A: Compound **2a** (100 mg, 0.25 mmol) and 1-azidomethylpyrene (**12**) (173 mg, 0.67 mmol) were dissolved in THF/H_2O/*t*-BuOH (3:1:1, v/v, 5 mL), then sodium ascorbate (100 µL, 0.10 mmol) of a freshly prepared 1 M solution in water was added, followed by the addition of copper(II)sulfate•$5H_2O$ (7.5% in water, 84 µL, 0.025 mmol). The reaction mixture was stirred for 3 h at rt. The solvent was evaporated, and the residue was applied to FC (silica gel, column 10 x 3 cm, CH_2Cl_2/ MeOH, 100:0→85:15). From the main zone, compound **13** was obtained as a yellowish solid (156 mg, 68%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.5. (λ_{max} (MeOH)/nm 265 (ε/dm^3 mol^{-1} cm^{-1} 22800), 276 (34900), 326 (16800), 342 (24600). ^1H NMR (DMSO-d_6, 600 MHz): δ 2.15 (ddd, J = 13.2, 6.1, 3.4 Hz, 1H, H-2'$_\alpha$), 2.33 (ddd, J = 13.1, 7.4, 5.7 Hz, 1H, H-2'$_\beta$), 3.37-3.44 (m, 2H, NCH_2), 3.46-3.56 (m, 2H, H-5'), 3.67-3.87 (m, 5H, $N(CH_2)_2$, H-4'), 4.28 (dq, J = 6.6, 3.4 Hz, 1H, H-3'), 4.96 (t, J = 5.4 Hz, 1H, OH-5'), 5.28 (d, J = 4.0 Hz, 1H, OH-3'), 6.13 (dd, J = 7.3, 6.1 Hz, 1H, H-1'), 6.33 (s, 4H, 2 x pyrene-CH_2), 7.01 and 7.03 (2s, 2H, NH_2), 7.72 (s, 1H, H-8), 7.96 (d, J = 7.9 Hz, 2H, pyrene-H), 8.08 (t, J = 7.6 Hz, 2H, pyrene-H), 8.13-8.23 (m, 6H, pyrene-H), 8.24-8.35 (m, 8H, pyrene-H), 8.49 and 8.52 (2s, 2H, triazole-H). $^{13}C\{^1H\}$ NMR (DMSO-d_6, 151 MHz): δ 39.0, 45.7, 47.5,

50.8, 61.3, 70.2, 73.2, 82.8, 87.7, 91.1, 105.1, 119.4, 122.7, 123.7, 123.9, 124.5, 125.0, 125.5, 125.6, 126.4, 127.2, 127.4, 127.7, 128.2, 128.3, 129.2, 130.1, 130.7, 130.9, 143.6, 149.8, 150.0, 165.0. HRMS (ESI-TOF) m/z: [M + Na^+] calcd for $C_{53}H_{42}N_{12}O_4Na$, 933.3344; found, 933.3345.

Method B: Compound **2a** (100 mg, 0.25 mmol) and 1-azidomethylpyrene (**12**) (173 mg, 0.67 mmol) were dissolved in THF/H_2O/*t*-BuOH (3:1:1, v/v, 5 mL), then copper(II)sulfate•$5H_2O$ (7.5% in water, 84 µL, 0.025 mmol) was added. The reaction mixture was stirred for 24 h at rt. The solvent was evaporated, and the residue was applied to FC (silica gel, column 10 x 3 cm, CH_2Cl_2/ MeOH, 100:0→85:15). From the main zone, compound **13** was obtained as yellowish solid (155 mg, 67%). Analytical data were identical to those described above.

2-Amino-8-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-[1-(pyrenmethyl)1*H*-1,2,3-triazol-4-yl]-*8H*-imidazo[1,2-*a*]-*s*-triazin-4-one (14). Compound **3d** (10 mg, 0.03 mmol) and 1-azidomethylpyrene (**12**) (24 mg, 0.09 mmol) were dissolved in THF/H_2O/*t*-BuOH (3:1:1, v/v, 1.5 mL), then sodium ascorbate (13 µL, 0.01 mmol) of a freshly prepared 1 M solution in water was added, followed by the addition of copper(II)sulfate•$5H_2O$ (7.5% in water, 3 µL, 0.003 mmol). The reaction mixture was stirred for 16 h at rt. The solvent was evaporated, and the residue was applied to FC (silica gel, column 6 x 2 cm, CH_2Cl_2/ MeOH, 100:0→85:15). From the main zone, compound **14** was obtained as a pale yellow solid (10 mg, 63%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.5. λ_{max} (MeOH)/nm 265 (ε/dm^3 mol^{-1} cm^{-1} 23200), 276 (34300), 326 (16700), 342 (24500). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.16 (ddd, J = 13.1, 6.0, 3.0 Hz, 1H, H-2'$_\alpha$), 2.42 (ddd, J = 13.2, 7.7, 5.7 Hz, 1H, H-2'$_\beta$), 3.47-3.59 (m, 2H, H-5'), 3.81 (td, J = 4.1, 2.5 Hz, 1H, H-4'), 4.31 (dt, J = 6.1, 3.1 Hz, 1H, H-3'), 4.98 (t, J = 5.2 Hz, 1H, OH-5'), 5.27 (d, J = 3.9 Hz, 1H, OH-3'), 6.22 (dd, J = 7.6, 6.0 Hz, 1H, H-1'), 6.46 (s, 2H, CH_2), 6.98 (s, 2H, NH_2), 7.81 (s, 1H, H-8), 8.04-8.15 (m, 2H, pyrene-H), 8.17-8.26 (m, 2H, pyrene-H), 8.28-8.39 (m, 4H, pyrene-H), 8.56 (m, 1H, pyrene-H), 8.59 (s, 1H, triazole).

$^{13}C\{^{1}H\}$ NMR (DMSO-d_6, 151 MHz): δ 38.8, 50.8, 61.3, 70.5, 82.8, 87.7, 112.4, 115.9, 122.7, 123.6, 124.0, 125.0, 125.5, 125.6, 125.7, 126.5, 127.2, 127.5, 127.8, 128.2, 128.4, 129.0, 130.1, 130.7, 131.0, 136.1, 150.4, 150.7, 164.6. HRMS (ESI-TOF) m/z: [M + H^+] calcd for $C_{29}H_{25}N_8O_4^+$, 549.1993; found, 549.1993.

2-Amino-8-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-{6-[1-(pyren-1-yl-methyl)1*H*-1,2,3-triazol-4-yl]hex-1-yn-1-yl}-8*H*-imidazo[1,2-*a*]-*s*-triazin-4-one (15). Compound **2a** (45 mg, 0.12 mmol) and 1-azidomethylpyrene (**12**) (50 mg, 0.19 mmol) were dissolved in THF/H_2O/*t*-BuOH (3:1:1, v/v, 3 mL), then sodium ascorbate (150 µL, 0.15 mmol) of a freshly prepared 1 M solution in water was added, followed by the addition of copper(II)sulfate•$5H_2O$ (7.5% in water, 130 µL, 0.04 mmol). The reaction mixture was stirred for 16 h at rt. The solvent was evaporated, and the residue was applied to FC (silica gel, column 10 x 3 cm, CH_2Cl_2/MeOH, 100:0→85:15). From the main zone, compound **15** was obtained as a yellowish solid (37 mg, 49%). TLC (silica gel, CH_2Cl_2/MeOH, 85:15) R_f 0.5. (λ_{max} (MeOH)/nm 265 (ε/dm^3 mol^{-1} cm^{-1} 26600), 276 (38000), 326 (17200), 342 (25200). ^1H NMR (DMSO-d_6, 600 MHz): δ 1.48-1.57 (m, 2H, CH_2), 1.71 (m, 2H, CH_2), 2.15 (ddd, J = 13.2, 6.1, 3.3 Hz, 1H, H-2'$_\alpha$), 2.34 (ddd, J = 13.2, 7.5, 5.7 Hz, 1H, H-2'$_\beta$), 2.42 (t, J = 7.0 Hz, 2H, CH_2), 1.58-2.66 (m, 2H, CH_2), 3.47-3.57 (m, 2H, H-5'), 3.79 (td, J = 4.3, 2.8 Hz, 1H, H-4'), 4.25-4.32 (m, 1H, H-3'), 4.97 (t, J = 5.4 Hz, 1H, OH-5'), 5.28 (d, J = 3.9 Hz, 1H, OH-3'), 6.13 (dd, J = 7.4, 6.1 Hz, 1H, H-1'), 6.32 (s, 2H, NCH_2), 6.98 (s, 2H, NH_2), 7.65 (s, 1H, H-8), 7.93-8.02 (m, 2H, pyrene-H), 8.10 (t, J = 7.6 Hz, 1H, pyrene-H), 8.16-8.23 (m, 2H, pyrene-H), 8.26-8.38 (m, 4H, pyrene-H), 8.51 (s, 1H, triazole).$^{13}C\{^{1}H\}$ NMR (DMSO-d_6, 151 MHz): δ 18.6, 24.4, 27.2, 27.9, 39.9, 48.6, 50.7, 54.9, 61.3, 68.5, 70.3, 82.7, 87.6, 96.3, 105.9, 118.5, 122.2, 122.7, 123.7, 124.0, 125.0, 125.5, 125.6, 126.4, 127.2, 127.5, 127.7, 128.2, 128.3, 129.3, 130.1, 130.7, 130.9, 147.0, 149.8, 149.9, 165.1. HRMS (ESI-TOF) m/z: [M + Na^+] calcd for $C_{35}H_{32}N_8O_4Na$, 651.2444; found, 651.2418.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPORTING INFORMATION

The Supporting Information is available free of charge on the ACS Publications website at DOI: xxx. ^{13}C NMR chemical shifts, reaction times and yields of *Sonogashira* reactions, pK_a determination, HPLC profile, UV spectra, photophysical data, ^{1}H, ^{13}C, ^{1}H-^{1}H-COSY, HSQC, and HMBC NMR spectra of all compounds.

ACKNOWLEDGEMENTS

We acknowledge Dr. Simone Budow-Busse for critical reading of the manuscript. We would like to thank Dr. Letzel, Organisch-Chemisches Institut, Universität Münster, Germany, for the measurement of the mass spectra and Prof. Dr. B. Wünsch, Institut für Pharmazeutische und Medizinische Chemie, Universität Münster, to provide us with 600 MHz NMR spectra and mass spectra. We appreciate technical assistance of Mrs Chaitali Kaldate. Funding by ChemBiotech, Münster, Germany is gratefully acknowledged.

DEDICATION

† Dedicated to Professor Helmut Vorbrüggen on occasion of his 90th birthday.

REFERENCES

(1) (a) Seela, F.; Melenewski, A. 5-Aza-7-deaza-2'-deoxyguanosine: oligonucleotide duplexes with novel base pairs, parallel chain orientation and protonation sites in the core of a double helix. *Eur. J. Org. Chem.* **1999**, 485-496. (b) Seela, F.; Amberg, S.; Melenewski, A.; Rosemeyer, H. 5-Aza-7-deazaguanine DNA: recognition and strand orientation of oligonucleotides incorporating anomeric imidazo[1,2-*a*]-1,3,5-triazine nucleosides. *Helv. Chim. Acta* **2001**, *84*, 1996-2014. (c) Singh, I.; Kim, M.-J.; Molt, R. W.; Hoshika, S.; Benner, S. A.; Georgiadis, M. M. Structure and biophysics for a six letter DNA alphabet that includes imidazo[1,2-*a*]-1,3,5-triazine-2(8*H*)-4(3*H*)-dione (X) and 2,4-diaminopyrimidine (K). *ACS Synth. Biol.* **2017**, *6*, 2118-2129. (d) Hoshika, S.; Leal, N. A.; Kim, M.-J.; Kim, M.-S.; Karalkar, N. B.; Kim, H.-J.; Bates, A. M.; Watkins, Jr., N. E.; SantaLucia, H. A.; Meyer, A. J.; DasGupta, S.; Piccirilli, J. A.; Ellington, A. D.; SantaLucia, Jr., J.; Georgiadis, M. M.; Benner, S. A. Hachimoji DNA and RNA: a genetic system with eight building blocks. *Science* **2019**, *363*, 884-887.

(2) Guo, X.; Leonard, P.; Ingale, S. A.; Liu, J.; Mei, H.; Sieg, M.; Seela, F. 5-Aza-7-deaza-2'-deoxyguanosine and 2'-deoxycytidine form programmable silver-mediated base pairs with metal ions in the core of the DNA double helix. *Chem. Eur. J.* **2018**, *24*, 8883-8892.

(3) a) Leonard, P.; Kondhare, D.; Jentgens, X.; Daniliuc, C.; Seela, F. Nucleobase Functionalized 5-Aza-7-deazaguanine Ribo- and 2'-Deoxyribonucleosides: Glycosylation, Pd-Assisted Cross-Coupling and Photophysical Properties. *J. Org. Chem.* **2019**, *84*, 13313-13328.

(4) Seela, F.; Rosemeyer, H. 5-Aza-7-deazapurines: synthesis and properties of nucleosides and oligonucleotides. In *Recent Advances in Nucleosides: Chemistry and Chemotherapy*, Ed. C. K. Chu, Elsevier Science B. V., Amsterdam, 2002, 505-533.

(5) (a) Huisgen, R. Kinetics and reaction mechanisms: selected examples from the experience of forty years. *Pure Appl. Chem.* **1989**, *61*, 613-628. (b) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. Click chemistry: diverse chemical function from a few good reactions. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021. (c) Meldal, M.; Tornøe, C. W. Cu-catalyzed azide-alkyne cycloaddition. *Chem. Rev.* **2008**, *108*, 2952-3015.

(6) (a) Moses, J. E.; Moorhouse, A. D. The growing applications of click chemistry. *Chem. Soc. Rev.* **2007**, *36*, 1249-1262. (b) Gramlich, P. M. E.; Warncke, S.; Gierlich, J.; Carell, T. Click-click-click: single to triple modification of DNA. *Angew. Chem. Int. Ed.* **2008**, *47*, 3442-3444. (c) Mojibul, H. M.; Peng, X. DNA-associated click chemistry. *Sci. China Chem.* **2014**, *57*, 215-231. (d) Sirivolu, V. R.; Chittepu, P.; Seela, F. DNA with branched internal side chains: synthesis of 5-tripropargylamine-dU and conjugation by an azide-alkyne double click reaction. *ChemBioChem* **2008**, *9*, 2305-2316. (e) El-Sagheer, A. H.; Brown, T. Click chemistry with DNA. *Chem. Soc. Rev.* **2010**, *39*, 1388-1405. (f) Xiong, H.; Seela, F. Stepwise "click" chemistry for the template independent construction of a broad variety of cross-linked oligonucleotides: influence of linker length, position and linking number on DNA duplex stability. *J. Org. Chem.* **2011**, *76*, 5584-5597. (g) Kore, A. R.; Charles, I. Click chemistry based functionalizations of nucleoside, nucleotide and nucleic acids. *Curr. Org. Chem.* **2013**, *17*, 2164-2191. (h) Yang, H.; Seela, F. Circular DNA by "bis-click" ligation: template independent intramolecular circularization of oligonucleotides with terminal alkynyl groups utilizing bifunctional azides. *Chem. Eur. J.* **2016**, 22, 1435-1444. (i) Yang, H.; Seela, F. "Bis-click" ligation of DNA: template-controlled assembly, circularization and functionalization with bifunctional and trifunctional azides. *Chem. Eur. J.* **2017**, *23*, 3375-3385.

(7) (a) Lin, W.; Zhang, X.; Seela, F. 7-Iodo-5-aza-7-deazaguanine: syntheses of anomeric D- and L-configured 2-deoxyribonucleosides. *Helv. Chim. Acta* **2004**, *87*, 2235-2243. (b)

Niedballa, U.; Vorbrüggen, H. A general synthesis of pyrimidine nucleosides. *Angew. Chem. Int. Ed*., **1970**, *9*, 461-462.

(8) (a) Glaçon, V.; Seela, F. 2-Amino-8-(2-deoxy-2-fluoro-*β*-D-arabinofuranosyl)imidazo[1,2-*a*]-1,3,5-triazin-4(8*H*)-one: synthesis and conformation of a 5-aza-7-deazaguanine fluoronucleoside. *Helv. Chim. Acta* **2004**, *87*, 1239-1247. (b) Wilds, C. J.; Damha, M. J. 2'-Deoxy-2'-fluoro-β-D-arabinonucleosides and oligonucleotides (2'F-ANA): synthesis and physicochemical studies. *Nucleic Acids Res.* **2000**, *28*, 3625-3635.

(9) Tann, C. H.; Brodfuehrer, P. R.; Brundidge, S. P.; Sapino Jr., C.; Howell, H. G. *J. Org. Chem.* **1985**, *50*, 3644-3647.

(10) Tennilä, T.; Azhayeva, E.; Vepsäläinen, J.; Laatikainen, R.; Azhayev, A.; Mikhailopulo, I. A. Oligonucleotides containing 9-(2-deoxy-2-fluoro-*β*-D-arabinofuranosyl)-adenine and -guanine: synthesis, hybridization and antisense properties. *Nucleosides Nucleotides Nucleic Acids* **2000**, *19*, 1861-1884.

(11) (a) Agrofoglio, L. A.; Gillaizeau, I.; Saito, Y. Palladium-assisted routes to nucleosides. *Chem. Rev.* **2003**, *103*, 1875-1916. (b) Shaughnessy, K. H. Palladium-catalyzed modification of unprotected nucleosides, nucleotides, and oligonucleotides. *Molecules* **2015**, *20*, 9419-9454. (c) Li, Q.; Persoons, L.; Daelemans, D.; Herdewijn, P. Iron/copper co-catalyzed cross-coupling reaction for the synthesis of 6-substituted 7-deazapurines and the corresponding nucleosides. *J. Org. Chem.* **2020**, *85*, 403-418.

(12) (a) Thakur, K. G.; Jaseer, E. A.; Naidu, A. B.; Sekar, G. An efficient copper(I) complex catalyzed Sonogashira type cross-coupling of aryl halides with terminal alkynes. *Tetrahedron Lett.* **2009**, *50*, 2865-2869. (b) Gayakhe, V.; Sanghvi, Y. S.; Fairlamb, I. J. S.; Kapdi, A. R. Catalytic C-H bond functionalisation of purine and pyrimidine nucleosides: a synthetic and mechanistic perspective. *Chem. Commun.* **2015**, *51*, 11944-11960. (c) Chan, T. R.; Hilgraf,

R.; Sharpless, K. B.; Fokin, V. V. Polytriazoles as copper(I)-stabilizing ligands in catalysis. *Org. Lett*. **2004**, *6*, 2853-2855.

(13) Rosemeyer, H.; Seela, F. 5-Aza-7-deaza-2'-deoxyguanosine: studies on the glycosylation of weakly nucleophilic imidazo[1,2-*a*]-*s*-triazinyl anions. *J. Org. Chem.* **1987**, *52*, 5136-5143.

(14) (a) Brotherton, W. S.; Michaels, H. A.; Simmons, J. T.; Clark, R. J.; Dalal, N. S.; Zhu, L. Apparent copper(II)-accelerated azide-alkyne cycloaddition. *Org. Lett.* **2009**, *11*, 4954-4957. (b) Kuang, G.-C.; Michaels, H. A.; Simmons, J. T.; Clark, R. J.; Zhu, L. Chelation-assisted, copper(II) acetate-accelerated azide-alkyne cycloaddition. *J. Org. Chem.* **2010**, *75*, 6540-6548. (c) Uttamapinant, C.; Tangpeerachaikul, A.; Grecian, S.; Clarke, S.; Singh, U.; Slade, P.; Gee, K. R.; Ting, A. Y. Fast, cell-compatible click chemistry with copper-chelating azides for biomolecular labeling. *Angew. Chem. Int. Ed.* **2012**, *51*, 5852-5856. (d) Kuang, G.-C.; Guha, P. M.; Brotherton, W. S.; Simmons, J. T.; Stankee, L. A.; Nguyen, B. T.; Clark, R. J.; Zhu, L. Experimental investigation on the mechanism of chelation-assisted, copper(II) acetate-accelerated azide-alkyne cycloaddition. *J. Am. Chem. Soc.* **2011**, *133*, 13984-14001. (e) Ingale, S. A.; Seela, F. Stepwise click functionalization of DNA through a bifunctional azide with a chelating and a non-chelating azido group. *J. Org. Chem.* **2013**, *78*, 3394-3399.

(15) Semenov, S. N.; Belding, L.; Cafferty, B. J.; Mousavi, M. P. S.; Finogenova, A. M.; Cruz, R. S.; Skorb, E. V.; Whitesides, G. M. Autocatalytic cycles in a copper-catalyzed azide-alkyne cycloaddition reaction. *J. Am. Chem. Soc.* **2018**, *140*, 10221-10232.

(16) (a) Hein, J. E.; Fokin, V. V. Copper-catalyzed azide-alkyne cycloaddition (CuAAC) and beyond: new reactivity of copper(I) acetylides. *Chem. Soc. Rev.* **2010**, *39*, 1302-1315. (b) Hardy, M. D.; Yang, J.; Selimkhanov, J.; Cole, C. M.; Tsimring, L. S.; Devaraj, N. K. Self-reproducing catalyst drive repeated phospholipid synthesis and membrane growth. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 8187-8192. (c) Döhler, D.; Michael, P.; Binder, W. H.

Autocatalysis in the room temperature copper(I)-catalyzed alkyne azide "click" cycloaddition of multivalent poly(acrylate)s and poly(isobutylene)s. *Macromolecules* **2012**, *45*, 3335-3345.

(17) (a) Connell, T. U.; Schieber, C.; Proietti Silvestri, I.; White, J. M.; Williams, S. J.; Donnelly, P. S. Copper and silver complexes of tris(triazole)amine and tris(benzimidazole)amine ligands: evidence that catalysis of an azide-alkyne cycloaddition ("click") reaction by a silver tris(triazole)amine complex arises from copper impurities. *Inorg. Chem.* **2014**, *53*, 6503-6511. (b) Donnelly, P. S.; Zanatta, S. D.; Zammit, S. C.; White, J. M.; Williams, S. J. 'Click' cycloaddition catalysts: copper(I) and copper(II) tris(triazolylmethyl)amine complexes. *Chem. Commun.* **2008**, 2459-2461. (c) Jin, L.; Tolentino, D. R.; Melaimi, M.; Bertrand, G. Isolation of bis(copper) key intermediates in Cu-catalyzed azide-alkyne "click reaction". *Sci. Adv.* **2015**, e1500304.

(18) (a) Förster, T.; Kasper, K. Ein Konzentrationsumschlag der Fluoreszenz des Pyrens. *Zeitschrift für Elektrochemie* **1955**, *59*, 976-980. (b) Winnik, F. M. Photophysics of preassociated pyrenes in aqueous polymer solutions and in other organized media. *Chem. Rev.* **1993**, *93*, 587-614.

(19) Seela, F.; Ingale, S. "Double click" reaction on 7-deazaguanine DNA: synthesis and excimer fluorescence of nucleosides and oligonucleotides with branched side chains decorated with proximal pyrenes. *J. Org. Chem.* **2010**, *75*, 284-295.

(20) (a) O'Connor, D.; Shafirovich, V. Y.; Geacintov, N. E. Influence of adduct stereochemistry and hydrogen-bonding solvents on photoinduced charge transfer in a covalent benzo[a]pyrene diol epoxide-nucleoside adduct on picosecond time scales. *J. Phys. Chem.* **1994**, *98*, 9831-9839. (b) Netzel, T. L.; Zhao, M.; Nafisi, K.; Headrick, J.; Sigman, M. S.; Eaton, B. E. Photophysics of 2'-deoxyuridine (dU) nucleosides covalently substituted with either 1-pyrenyl or 1-pyrenoyl: observation of pyrene-to-nucleoside charge-transfer emission in 5-(1-pyrenyl)-dU. *J. Am. Chem. Soc.* **1995**, *117*, 9119-9128. (c) Kaden, P.; Mayer-Enthart,

E.; Trifonov, A.; Fiebig, T.; Wagenknecht, H.-A. Real-time spectroscopic and chemical probing of reductive electron transfer in DNA. *Angew. Chem. Int. Ed.* **2005**, *44*, 1636-1639.

(d) Ingale, S. A.; Pujari, S. S.; Sirivolu, V. R.; Ding, P.; Xiong, H.; Mei, H.; Seela, F. 7-Deazapurine and 8-aza-7-deazapurine nucleoside and oligonucleotide pyrene "click" conjugates: synthesis, nucleobase controlled fluorescence quenching, and duplex stability. *J. Org. Chem.* **2012**, *77*, 188-199.

(21) Dukhan, D.; Leroy, F.; Peyronnet, J.; Bosc, E.; Chaves, D.; Durka, M.; Storer, R.; La Colla, P.; Seela, F.; Gosselin, G. Synthesis of 5-aza-7-deazaguanine nucleoside derivatives as potential anti-flavirus agents. *Nucleosides, Nucleotides and Nucleic Acids* **2005**, *24*, 671-674.

(22) Melhuish, W. H.; Quantum efficiencies of fluorescence of organic substances: effect of solvent and concentration of the fluorescent solute. *J. Phys. Chem.* **1961**, *65*, 229-235.