JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

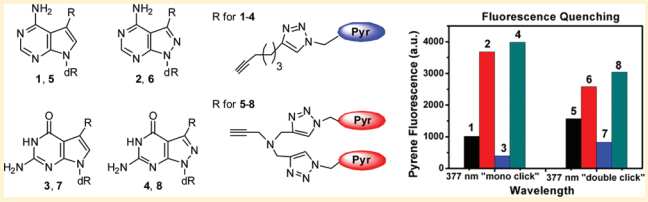
7-Deazapurine and 8-Aza-7-deazapurine Nucleoside and Oligonucleotide Pyrene "Click" Conjugates: Synthesis, Nucleobase Controlled Fluorescence Quenching, and Duplex Stability

Sachin A. Ingale, Suresh S. Pujari, Venkata Ramana Sirivolu, Ping Ding, Hai Xiong, Hui Mei, and Frank Seela*

Laboratory of Bioorganic Chemistry and Chemical Biology, Center for Nanotechnology, Heisenbergstraße 11, 48149 Münster, Germany and Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie, Universität Osnabrück, Barbarastraße 7, 49069 Osnabrück, Germany

Supporting Information

ABSTRACT: 7-Deazapurine and 8-aza-7-deazapurine nucleosides related to dA and dG bearing 7-octadiynyl or 7-tripropargylamine side chains as well as corresponding oligonucleotides were synthesized. "Click" conjugation with 1-azidomethyl pyrene (**10**) resulted in fluorescent derivatives. Octadiynyl conjugates show only monomer fluorescence, while the proximal alignment of pyrene residues in the tripropargylamine derivatives causes excimer emission. 8-Aza-7-deazapurine pyrene "click" conjugates exhibit fluorescence emission much higher than that of 7-deazapurine derivatives. They are quenched by intramolecular charge transfer between the nucleobase and the dye. Oligonucleotide single strands decorated with two "double clicked" pyrenes show weak or no excimer fluorescence. However, when duplexes carry proximal pyrenes in complementary strands, strong excimer fluorescence is observed. A single replacement of a canonical nucleoside by a pyrene conjugate stabilizes the duplex substantially, most likely by stacking interactions: 6−12 °C for duplexes with a modified "adenine" base and 2−6 °C for a modified "guanine" base. The favorable photophysical properties of 8-aza-7-deazapurine pyrene conjugates improve the utility of pyrene fluorescence reporters in oligonucleotide sensing as these nucleoside conjugates are not affected by nucleobase induced quenching.



INTRODUCTION

Fluorescence is a powerful tool for structural and functional studies of a diversity of molecules. Among the various fluorophores, pyrene derivatives are attractive fluorescent probes.[1] Their fluorescence properties are utilized for the investigation of water-soluble polymers[2a] or to detect toxic metals in water,[2b] glucose,[2c] and even explosives.[2d] Pyrene has several photophysical properties that make it extraordinarily suitable as fluorescent reporter, such as high fluorescence quantum yield, chemical stability, and long fluorescence lifetime.[2a,3,4] Moreover, the fluorescence of pyrene derivatives is sensitive to the polarity of the surrounding environment including solvent changes.[5]

In addition to monomer fluorescence, pyrene and its derivatives can show excimer emission. The excimer is formed when an excited pyrene monomer interacts in a specific manner with a proximal ground state (unexcited) pyrene.[6] However, pyrene-modified oligonucleotide probes based on monomer emission intensity are often affected by fluorescence quenching through an electron-migration process between the excited pyrene and nucleoside bases.[7] Pyrene excimer fluorescence is less sensitive to quenching than pyrene monomer emission.[8] For this reason, pyrene excimer emission has been explored for monitoring RNA and DNA hybridization[9] including studies on duplexes,[10] triplexes,[11] and quadruplexes,[12] as well as on artificial DNAs, e.g., those with parallel chain orientation.[13] For this, pyrene residues were linked to various positions of nucleic acids as well as to their constituents (nucleosides and nucleotides).[14−16]

The copper(I)-catalyzed Huisgen−Meldal−Sharpless azide−alkyne cycloaddition reaction (CuAAC)[17] has emerged as an ideal bioorthogonal protocol for the preparation of rich chemical diversity. Our laboratory has already reported on the functionalization of nucleosides and oligonucleotides using the CuAAC reaction.[18−21] This also includes the "double click" functionalization of oligonucleotides incorporating a branched side chain with fluorogenic 1-azidomethyl pyrene (**10**).[22] The "double click" reaction brings the new ligands (pyrene) of the branched side chain into a proximal position.

Earlier, it was observed that the fluorescence of nucleoside dye conjugates, such as coumarin, is sensitive to nucleobase modification.[18] The fluorescence is partially quenched when the dye is linked to a 7-deazapurine skeleton (purine numbering is used throughout the discussion). This prompted us to study the fluorescence behavior of nucleoside and oligonucleotide pyrene

Received: August 9, 2011
Published: November 30, 2011

   dx.doi.org/10.1021/jo202103q | J. Org. Chem. 2012, 77, 188−199

conjugates and to identify nucleobases related to dA and dG that form stable Watson−Crick base pairs but do not show unwanted nucleobase induced fluorescence quenching.

This manuscript reports on 2′-deoxyadenosine and 2′-deoxyguanosine derivatives with a 7-deazapurine (pyrrolo[2,3-*d*]pyrimidine) and an 8-aza-7-deazapurine (pyrazolo[3,4-*d*]pyrimidine) skeleton as nucleobase surrogates bearing octadiynyl or tripropargylamine side chains, which were clicked to 1-azidomethylpyrene (**10**) to form the "click" conjugates **1**−**8** (Figure 1). The monomer fluorescence of **1**−**8** was

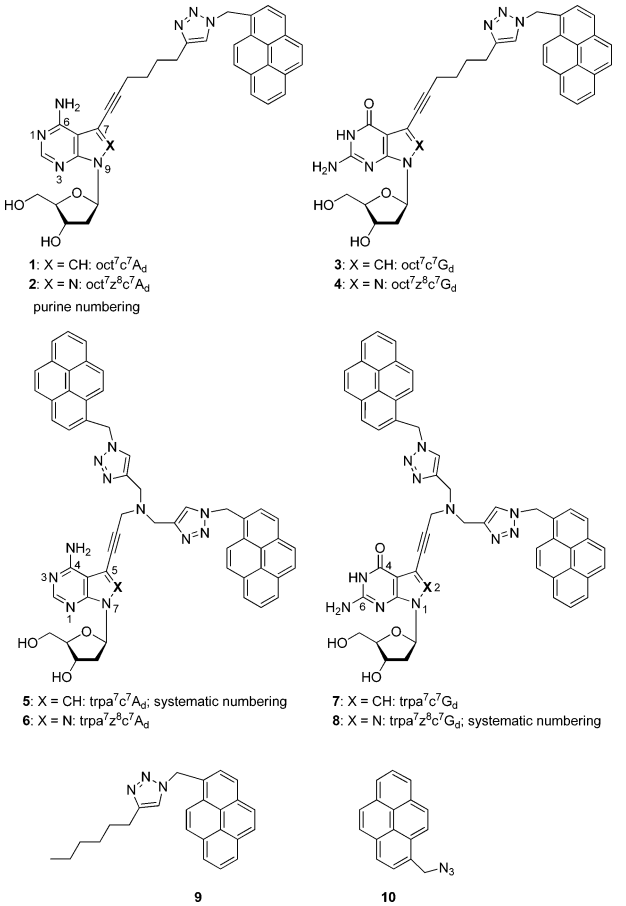


Figure 1. Pyrene "click" conjugates of 7-deazapurine and 8-aza-7-deazapurine nucleosides and abasic pyrene compound.

compared, and the influence of the nucleobase on fluorescence quenching was studied on nucleoside and oligonucleotide level. Furthermore, concentration-dependent fluorescence measurements on nucleoside conjugates with tripropargylamine side chains were performed, which verified that the excimer emission of tripropargylamine pyrene conjugates results from intramolecular pyrene contacts and not from intermolecular interactions. The influence of nucleobase pyrene conjugates **1**−**8** on the DNA duplex stability was studied with regard to base modification.

RESULTS AND DISCUSSION

1. Synthesis of Phosphoramidites 14 and 18. For our study, eight different phosphoramidites based on the 7-deazapurine system and the related 8-aza-7-deazapurine congeners bearing octadiynyl and tripropargylamine side chains were synthesized. The phosphoramidites of nucleosides **19**−**24** were prepared according to earlier reported literature from our group,[19−22] while the syntheses of phosphoramidites **14** and **18** are described below.

Phosphoramidite building block **14** was synthesized from nucleoside **11**.[19b] For this, compound **11** was protected at the 6-amino group with an isobutyryl residue affording the protected intermediate **12** in 86% yield. Then, compound **12** was converted to the 5′-*O*-DMT derivative **13** under standard conditions. Further phosphitylation yielded the phosphoramidite **14** (63%) (Scheme 1).

Next, phosphoramidite **18** was prepared from the alkynylated nucleoside **15**[20b] (Scheme 1). Amino group protection was performed on **15** with the *N*,*N*-dimethylaminomethylidene group to afford the intermediate **16** (73%). Compound **16** was converted to the respective 5′-*O*-DMT derivative under standard reaction conditions to yield **17** in 79%, and further phosphitylation gave the corresponding phosphoramidite **18** in 58%.

2. Synthesis and Characterization of Nucleoside Pyrene "Click" Conjugates with Octadiynyl and Tripropargylamine Side Chains. The Cu(I)-catalyzed "click" reaction was used for conjugation of the pyrene dye to the nucleobase. For this, 1-azidomethyl pyrene **10** was prepared from 1-pyrenemethanol following an already published procedure.[23] The pyrene "click" conjugates of 7-octadiynyl-7-deaza-2′-deoxyguanosine (**3**) and 7-tripropargylamino-7-deaza-2′-deoxyguanosine (**7**) have already been reported by our laboratory.[22] The 1,2,3-triazolyl pyrene nucleosides **1**, **2**, and **4** were synthesized from the 7-octadiynyl substituted nucleosides **19**, **20**, and **22** and 1-azidomethyl pyrene **10** in the presence of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate in a 3:1:1 mixture of THF/*t*-BuOH/H_2O (Scheme 2).

The two terminal triple bonds of nucleosides **11**, **15**, **23**, and **24** were functionalized with 1-azidomethyl pyrene (**10**), leading to the formation of the "double click" adducts **5**−**8** (Scheme 2). The reaction was performed by using the same procedure as that used for the synthesis of the octadiynyl pyrene "click" conjugates. In this reaction, a 2.7-fold excess of the pyrene azide was used to complete the "double click" conjugation. We found that both terminal triple bonds were functionalized simultaneously by the two pyrene reporters, even though they are space-demanding. "Double click" functionalization might result from the enhanced catalytic action of copper(I) being bound to the tripropargylamine side chain or to a monofunctionalized intermediate, thereby increasing the reaction rate for the second "click" reaction.[17e]

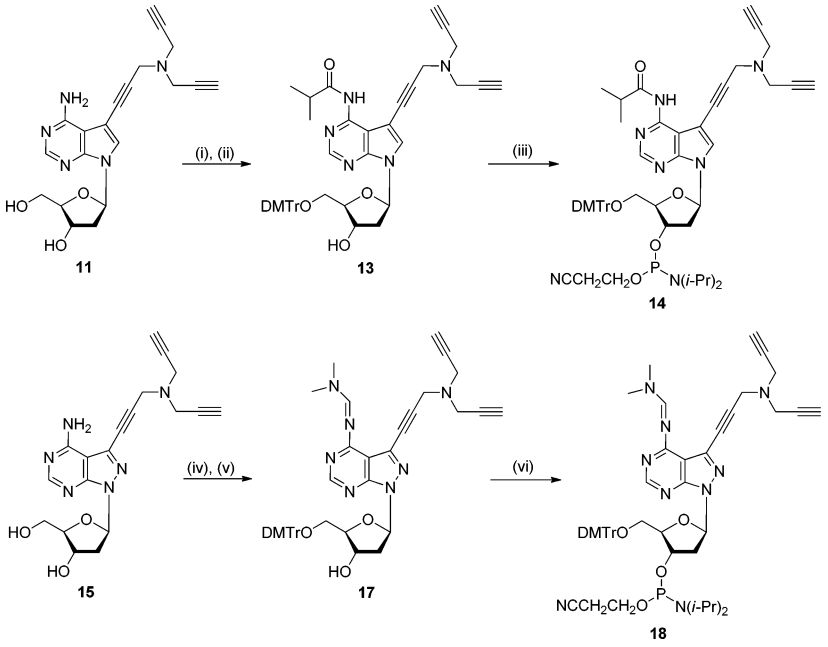
In order to determine the influence of the nucleobase on the pyrene fluorescence, the abasic pyrene conjugate **9**[22] was prepared for comparison (Figure 1). This compound contains all necessary elements of the system except the nucleobase.

All of the synthesized compounds were characterized by elemental analyses, ^{1}H, ^{13}C, and ^{1}H−^{13}C-gated-decoupled as well as DEPT-135 NMR spectra (Supporting Information). ^{13}C NMR chemical shifts of "click" conjugates are summarized in Table 2 (Experimental Section). ^{13}C NMR chemical shifts were assigned by ^{1}H−^{13}C gated-decoupled spectra and DEPT-135 NMR spectra (Table S1, Supporting Information).

The ^{1}H NMR spectra of "click" conjugates show the disappearance of terminal C≡C hydrogens (singlet at $\delta \approx$ 3.25 ppm), whereas the new methylidene protons ($\delta \approx$ 6.32 ppm) and singlets appearing around δ = 7.9−8.5 ppm are attributed to the protons of the newly formed triazole rings. The intensity of the proton signals of two triazole rings clearly

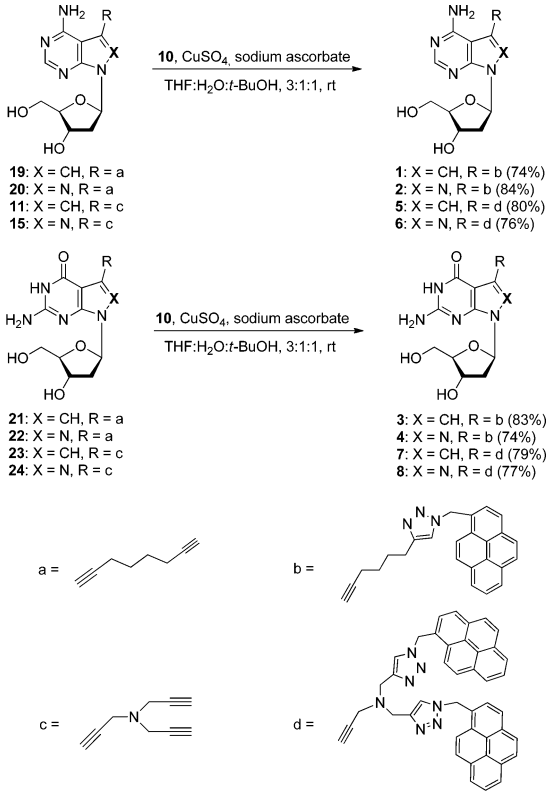
dx.doi.org/10.1021/jo202103q | J. Org. Chem. 2012, 77, 188−199

Scheme 1. Synthesis of Phosphoramidite Building Blocks 14 and 18[a]



[a]Reagents and conditions: (i) isobutyric anhydride, DMF, rt; **12**: 86%. (ii) DMTr-Cl, pyridine, rt; **13**: 80%. (iii) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $(i\text{-}Pr)_2EtN$, CH_2Cl_2, rt; **14**: 63%. (iv) DMF-DMA, MeOH, rt; **16**: 73%. (v) DMTr-Cl, pyridine, rt; **17**: 79%. (vi) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $(i\text{-}Pr)_2EtN$, CH_2Cl_2, rt; **18**: 58%.

Scheme 2. Cu(I)-Catalyzed "Double Click" Reaction Performed with Nucleosides 11, 15, and 19–24



demonstrate the formation of the bis-dye adducts. Furthermore, ^{13}C NMR spectra show the absence of the two terminal C≡C carbon atom signals, while new double bond carbon signals of the 1,2,3-triazole moiety appear. As indicated in Table 2, they are located around 143–147 ppm (quaternary C-atom) and around 122 ppm (triazole-C5).

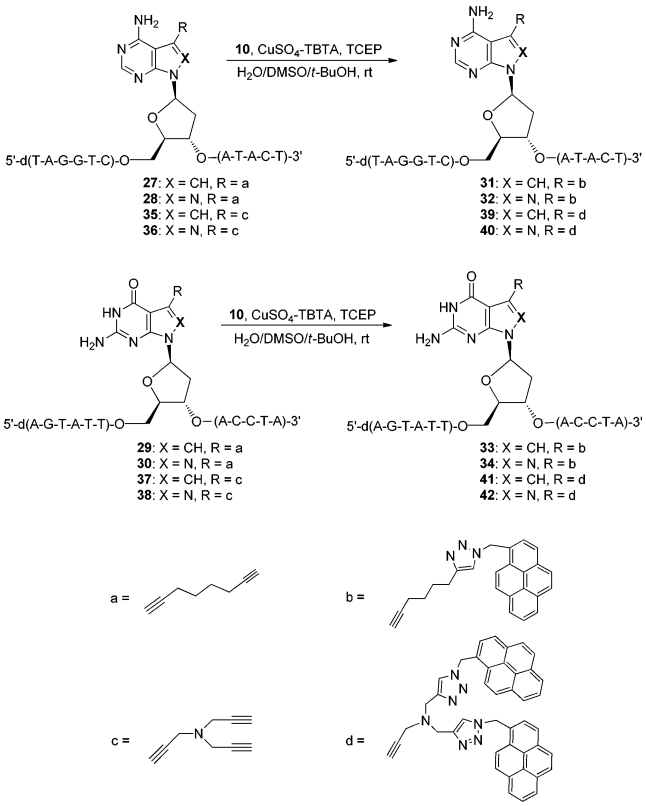
3. Synthesis and Duplex Stability of Oligonucleotide Pyrene "Click" Conjugates. To evaluate the potential of base modification on duplex stability and fluorescence behavior, oligonucleotides were prepared by solid-phase synthesis using standard phosphoramidites and the phosphoramidites of nucleosides **11**, **15**, and **19–24** as building blocks (for details see Exprimental Section).

A set of modified 12-mer oligonucleotides was synthesized and compared to the complementary reference oligonucleotides (ODNs) 5′-d(TAG GTC AAT ACT)-3′ (**25**) and 5′-d(AGT ATT GAC CTA)-3′ (**26**). Within the modified ODNs, a dA residue from a central position of **25** was replaced by nucleoside **11**, **15**, **19** or **20**, and the central dG residue of **26** was replaced by nucleoside **21–24**.

The "click" reaction was performed on oligonucleotides **27–30**, with 1-azidomethylpyrene **10** (Scheme 3). In order to use a more general protocol, "click" conjugation was carried out on oligonucleotide level instead of directly using phosphoramidites of the corresponding pyrene nucleoside "click" conjugates. The method of post-modification offers the possibility to utilize a broad range of azido reporter groups in the "click" conjugation of alkynylated oligonucleotides. The "click" reaction was carried out at room temperature in an aqueous solution containing *t*-BuOH and DMSO to yield ODNs **31–34**. At first the chelate ligand Cu·TBTA [tris(benzyltriazolylmethyl)amine] was added to the solvent. Excess and/or noncomplexed copper leads to cleavage of the oligonucleotide chain. Hence, it is necessary to add copper and azide in the specified amount. Then, the alkynyl oligonucleotide (5 A_{260} units) was introduced followed by the other components such as pyrene azide (**10**), TCEP [tris(carboxyethyl)phosphine], and $NaHCO_3$ (for oligonucleotides **30** and **38** benzoic acid was used instead), and the reaction mixture was stirred at rt for 12 h.

dx.doi.org/10.1021/jo202103q | J. Org. Chem. 2012, 77, 188–199

Scheme 3. Click Reaction Performed on Oligonucleotides Containing Tripropargylamine Nucleosides 11, 15, and 19–24



Next, the "double click" reaction was performed on oligonucleotides **35**–**38** with 1-azidomethylpyrene (**10**) (Scheme 3) using the same procedure as for the synthesis of the octadiynyl pyrene "click" conjugates to yield the ODNs **39**–**42**. For details, see the Experimental Section. All oligonucleotide pyrene "click" conjugates were purified by reversed-phase HPLC and characterized by MALDI-TOF mass spectroscopy (Table S2, Supporting Information). Oligonucleotide conjugates **33** and **41** were already described.[22]

Earlier, it was found that incorporation of a bulky tripropargylamine as well as an octadiynyl side chain does not perturb the DNA duplex structure.[18,20–22] In accordance to earlier observations,[21b,22] tripropargylamino nucleosides **11** (**35**·**26**) and **15** (**36**·**26**) stabilize duplex DNA (ΔT_m = +3 and +2 °C) as compared to the unmodified duplex **25**·**26** (Table 1 and Supporting Information, Figure S9). Consequently, the influence of dye modification with these linkers on the duplex stability was evaluated for the compounds described above. For this, the T_m values of fully matched duplexes containing a pyrene dye were measured. More stable duplexes are formed by the pyrene-modified oligonucleotides compared to the unmodified duplex **25**·**26**. A remarkable strong stabilization was observed for duplexes **31**·**26** and **32**·**26** (ΔT_m = +11 and +12 °C) containing an octadiynyl modified "dA" pyrene "click" conjugate as compared to duplexes **39**·**26** and **40**·**26** containing the corresponding tripropargylamino modified "dA" pyrene conjugate (ΔT_m = +6 and +10 °C). The stabilization for duplexes **25**·**33**, **25**·**34**, **25**·**41**, and **25**·**42** incorporating a "dG" pyrene "click" conjugate was still strong (ΔT_m = +2 to +6 °C) but much less pronounced than for duplexes containing a "dA" pyrene conjugate. The DNA duplexes having one pyrene reporter group in each strand (**31**·**33**, **31**·**34**, **32**·**33**, and **32**·**34**) are stable even to a greater extent (ΔT_m = +16 to +19 °C) than duplexes (**39**·**41**, **39**·**42**, **40**·**41**, and **40**·**42**) containing two pyrenes residue in each strand (ΔT_m = +8 to +13 °C).

So, duplexes containing 7-deaza-dA and 8-aza-7-deaza-dA pyrene "click" conjugates (**1**, **2**, **5**, and **6**) are much more stabilized than those incorporating 7-deaza-dG and 8-aza-7-deaza-dG pyrene "click" derivatives (**3**, **4**, **6**, and **8**). Earlier, duplex stabilization by pyrene residues has been exemplified in several cases for residues attached to terminal or internal positions of the oligonucleotide chain.[1e,5,24,25] However, to the best of our knowledge such a "dA" specific stabilization is unknown. This implies that probably the pyrene residue intercalates specifically with the "adenine" moiety (ΔT_m = +6 to +12 °C), but intercalation is less efficient with the "guanine" residue (ΔT_m = +2 to +6 °C). Such interactions are possible when the linker is long enough to form a fold back structure. Stabilization by pyrene intercalation seems to be most likely when compared to the T_m values of the pyrene-modified oligonucleotides with those of the parent alkynylated oligonucleotides. Although the alkynyl linkers are already stabilizing, the contribution of the pyrene residues to duplex stabilization is predominant. The assumption of pyrene stacking is further supported by the absorption spectra of ss and ds oligonucleotides (Figures S2–S3, Supporting Information). Upon duplex formation, a small bathochromic shift of the absorption maxima and a slightly reduced peak-to-valley ratio of the absorption bands are observed. Participation of the triazole moieties and/or nearest neighbor nucleobases cannot be excluded.

4. Photophysical Properties of Nucleoside Pyrene "Click" Conjugates. Photoexcitable dyes such as pyrene, anthracene, or coumarin suffer from fluorescence quenching, when conjugated to nucleobases such as purines, 7-deazapurines or pyrimidines,[18,20a,21a,22] caused by fluorescence resonance energy transfer (FRET) or charge separation (intramolecular electron transfer or hole transfer) between the dye and a base.[7] To evaluate fluorescence quenching properties of pyrene "click" conjugates linked to various nucleobases via octadiynyl or tripropargylamine linkers and 1,2,3-triazole residues, fluorescence data of pyrene "click" conjugates were compared with those of the abasic alkyne side chain conjugate **9**. It was anticipated that a simple octyne linker should have no significant influence on the fluorescence of pyrene, while the nucleobases might affect the fluorescence.

4.1. Monomer and Excimer Fluorescence of Nucleoside Pyrene "Click" Conjugates. At first, the monomeric pyrene "click" conjugates **1**–**4** were characterized by UV–vis spectra (Figure S1a, Supporting Information) measured in methanol. Based on the UV–vis spectra, the excitation wavelength for pyrene "click" conjugates **1**–**4** was chosen to be 340 nm. The tripropargylamine pyrene "click" conjugates **5**–**8** decorated with two pyrene residues show a UV absorbance (Figure S1b, Supporting Information) higher than that of the octadiynyl pyrene "click" conjugates **1**–**4** with no significant differences regarding the wavelength maxima and absorption pattern.

Then, fluorescence measurements were performed (Figure 2a). All "click" conjugates bearing one pyrene residue (**1**–**4**) show excitation maxima at 340 nm and monomeric pyrene emission maxima at 377 and 395 nm, while excimer emission was not observed. From Figure 2a, it is apparent that $c^7z^8G_d$ "click" conjugate **4** (Φ = 0.037) and the abasic pyrene

dx.doi.org/10.1021/jo202103q | J. Org. Chem. 2012, 77, 188–199

Table 1. T_m Values of Octadiynyl and Tripropargylamine Substituted Oligonucleotides and Pyrene "Click" Conjugates[a]

octadiynyl modified oligonucleotides			triporpargylamine modified oligonucleotides		
duplexes	T_m [°C]	ΔT_m [°C][b]	duplexes	T_m [°C]	ΔT_m [°C][b]
5′-d(TAG GTC AAT ACT) (**25**) 3′-d(ATC CAG TTA TGA) (**26**)	49	–	5′-d(TAG GTC AAT ACT) (**25**) 3′-d(ATC CAG TTA TGA) (**26**)	49	–
5′-d(TAG GTC **19**AT ACT) (**27**) 3′-d(ATC CAG TTA TGA) (**26**)	52[18c]	+3	5′-d(TAG GTC **11**AT ACT) (**35**) 3′-d(ATC CAG TTA TGA) (**26**)	52	+3
5′-d(TAG GTC **20**AT ACT) (**28**) 3′-d(ATC CAG TTA TGA) (**26**)	51[20a]	+2	5′-d(TAG GTC **15**AT ACT) (**36**) 3′-d(ATC CAG TTA TGA) (**26**)	51	+2
5′-d(TAG GTC AAT ACT) (**25**) 3′-d(ATC CA**21**TTA TGA) (**29**)	49[22]	0	5′-d(TAG GTC AAT ACT) (**25**) 3′-d(ATC CA**23**TTA TGA) (**37**)	50[22]	+1
5′-d(TAG GTC AAT ACT) (**25**) 3′-d(ATC CA**22**TTA TGA) (**30**)	49[21a]	0	5′-d(TAG GTC AAT ACT) (**25**) 3′-d(ATC CA**24**TTA TGA) (**38**)	49[21b]	0
5′-d(TAG GTC **1**AT ACT) (**31**) 3′-d(ATC CAG TTA TGA) (**26**)	60	+11	5′-d(TAG GTC **5**AT ACT) (**39**) 3′-d(ATC CAG TTA TGA) (**26**)	55	+6
5′-d(TAG GTC **2**AT ACT) (**32**) 3′-d(ATC CAG TTA TGA) (**26**)	61	+12	5′-d(TAG GTC **6**AT ACT) (**40**) 3′-d(ATC CAG TTA TGA) (**26**)	59	+10
5′-d(TAG GTC AAT ACT) (**25**) 3′-d(ATC CA**3** TTA TGA) (**33**)	53[22]	+4	5′-d(TAG GTC AAT ACT) (**25**) 3′-d(ATC CA**7** TTA TGA) (**41**)	51[22]	+2
5′-d(TAG GTC AAT ACT) (**25**) 3′-d(ATC CA**4** TTA TGA) (**34**)	54	+5	5′-d(TAG GTC AAT ACT) (**25**) 3′-d(ATC CA**8** TTA TGA) (**42**)	55	+6
5′-d(TAG GTC **1**AT ACT) (**31**) 3′-d(ATC CA**3** TTA TGA) (**33**)	65	+16	5′-d(TAG GTC **5**AT ACT) (**39**) 3′-d(ATC CA**7** TTA TGA) (**41**)	57	+8
5′-d(TAG GTC 1AT ACT) (**31**) 3′-d(ATC CA4 TTA TGA) (**34**)	68	+19	5′-d(TAG GTC **5**AT ACT) (**39**) 3′-d(ATC CA**8** TTA TGA) (**42**)	60	+11
5′-d(TAG GTC **2**AT ACT) (**32**) 3′-d(ATC CA**3** TTA TGA) (**33**)	66	+17	5′-d(TAG GTC **6**AT ACT) (**40**) 3′-d(ATC CA**7** TTA TGA) (**41**)	57	+8
5′-d(TAG GTC **2**AT ACT) (**32**) 3′-d(ATC CA**4** TTA TGA) (**34**)	67	+18	5′-d(TAG GTC **6**AT ACT) (**40**) 3′-d(ATC CA**8** TTA TGA) (**42**)	62	+13

[a]Measured at 260 nm in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) with 2 μM + 2 μM single-strand concentration. [b]Refers to the temperature difference of the modified duplex versus the unmodified reference duplex.

conjugate **9** (Φ = 0.034) show similar monomeric fluorescence intensity, whereas c^7G_d pyrene conjugate **3** (Φ = 0.004) shows significant lower monomer fluorescence. Monomer fluorescence for octadiynyl "click" conjugates increases in the following order: $oct^7c^7G_d$ (**3**) < $oct^7c^7A_d$ (**1**) < $oct^7z^8c^7A_d$ (**2**) < $oct^7z^8c^7G_d$ (**4**) < abasic conjugate (**9**), as illustrated by the bar diagram (Figure 2b).

In contrast to octadiynyl "click" conjugates, the tripropargylamine "double click" conjugates **5**–**8** with two proximal pyrenes show strong excimer fluorescence (Figure 3a,b) with $\lambda_{max} \approx$ 465 nm (band III) as well as monomer fluorescence at 377 nm (band I) and 395 nm (band II) upon excitation at 340 nm. From this, we conclude that the two pyrenes are stacking in all tripropargylamine nucleoside "double click" conjugates (**5**–**8**). The $c^7z^8G_d$ "click" conjugate **8** (excimer fluorescence, Φ = 0.059) shows the highest value, while the c^7G_d "click" conjugate **7** (excimer fluorescence, Φ = 0.020) shows low excimer emission. The monomer fluorescence intensity of tripropargylamine "click" conjugates **5**–**8** increases in the same order $trpa^7c^7G_d$ (**7**) < $trpa^7c^7A_d$ (**5**) < $trpa^7z^8c^7A_d$ (**6**) < $trpa^7z^8c^7G_d$ (**8**) (Figures 3a, 3b) as observed for the octadiynyl "click" conjugates **1**–**4** (Figure 2a,b). Interestingly, this order is changed for the "adenine click" conjugates **5** and **6**: $trpa^7c^7G_d$ (**7**) < $trpa^7z^8c^7A_d$ (**6**) < $trpa^7c^7A_d$ (**5**) < $trpa^7z^8c^7G_d$ (**8**) (Figure 3a,b). These different dependencies of excimer versus monomer fluorescence are in line with earlier observations.[8]

Excimer fluorescence of "double click" conjugates **5**–**8** could arise from either intramolecular or intermolecular interactions of two pyrene residues. To investigate this phenomenon concentration-dependent fluorescence measurements on the pyrene "double click" conjugates **7** and **8** were performed (Supporting Information, Figure S5). The $c^7z^8G_d$ "click" conjugate **8** develops the strongest eximer fluorescence, while the c^7G_d "double click" conjugate **7** shows low excimer

dx.doi.org/10.1021/jo202103q | J. Org. Chem. 2012, 77, 188–199

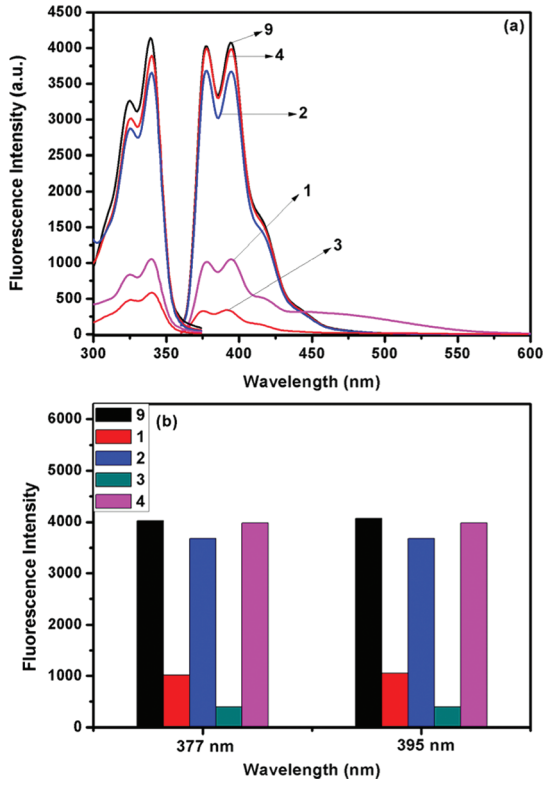


Figure 2. (a) Fluorescence spectra of octadiynyl nucleoside "click" conjugates **1**–**4** and abasic "click" conjugate **9** in methanol. (b) Bar diagram showing the monomer fluorescence intensity of "click" conjugates **1**–**4** and abasic "click" conjugate **9** in methanol. In all experiments the concentration of the dye conjugates was 6.8×10^{-6} M.

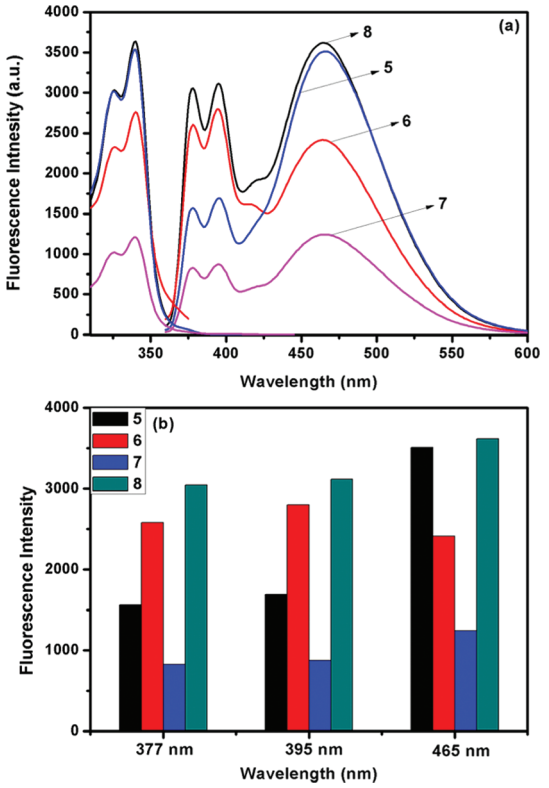


Figure 3. (a) Fluorescence spectra of tripropargylamine nucleoside "click" conjugates **5**–**8** in methanol. (b) Bar diagram showing the monomer emission and excimer fluorescence intensity of "click" conjugates **5**–**8** in methanol. The concentration of the dye conjugates was always 6.8×10^{-6} M.

emission (Figure 3a). Fluorescence intensity decreases almost linearly with decreasing concentration in both "click" conjugates (**7** and **8**). For "click" conjugate **8**, the Ex/M intensity ratio was unchanged, i.e., about 1.0, for all measured concentrations, indicating that intramolecular excimer formation takes place. In "double click" conjugate **7**, the excimer to monomer emission ratio is twice as high. In this case, the Ex/M ratio slightly decreases from 1.92 to 1.68 upon dilution (Supporting Information, Table S3). The observation that the monomer fluorescence is quenched to a greater extent than excimer fluorescence is in line with earlier findings of Yamana et al.[9c]

4.2. Fluorescence of 7-Deazapurine and 8-Aza-7-deazapurine Oligonucleotide Pyrene "Mono-Click" Conjugates. The quenching effects of 7-deazapurine and 8-aza-7-deazapurine nucleobases on the pyrene moiety were also investigated on single-stranded oligonucleotide (ss) pyrene "click" conjugates **31**–**34** and corresponding duplexes (ds). Absorption spectra varied only slightly, with a small red shift observed upon duplex formation (Figure S2, Supporting Information).

Subsequently, fluorescence spectra of oligonucleotides **31**–**34** and their corresponding duplexes (Figure 4a,b) were performed. Complementary strands were always unmodified (**25** or **26**). All ss-oligonucleotides (**31**–**34**) as well as their duplexes show pyrene monomer fluorescence with $\lambda_{max} \approx 382$ nm (band I) and 398 nm (band II) upon excitation at 340 nm. The fluorescence emission of c^7G_d pyrene modified ss-oligonucleotide **33** is quenched strongly compared to that of the $c^7z^8G_d$ pyrene modified ss-oligonucleotide **34** (Figure 4b). These results correlate with the data found for the nucleoside conjugates **3** and **4**. Hybridization with complementary strands (→ duplexes **25**·**33** and **25**·**34**) leads to further fluorescence decrease.

The fluorescence intensity of pyrene modified 7-deazaadenine ss-oligonucleotide **31** is quenched (about 25%) compared to that of 8-aza-7-deazaadenine ss-oligonucleotide **32** (Figure 4a), which is in line with fluorescence data of the nucleosides. Surprisingly, the fluorescence intensity of the corresponding duplexes **31**·**26** and **32**·**26** containing **1** or **2** conjugates remains almost unchanged. This is different to the findings of the "dG" derivatives.

4.3. Fluorescence of 7-Deazapurine and 8-Aza-7-deazapurine Oligonucleotide "Double Click" Conjugates with Pyrene Modifications in One Strand of Duplexes. Fluorescence spectra of "dA" modified ss-oligonucleotides **39** and **40** show strong monomer fluorescence and weak excimer fluorescence. Monomer emission of ss-oligonucleotide **39** is higher than that of ss-oligonucleotide **40**, but excimer emission of **39** is slightly higher than that of **40** (Figure 5a). This result matches the fluorescence data obtained on nucleoside level (Figure 3a). When the corresponding duplexes are formed (**39**·**26** and **40**·**26**), monomer fluorescence intensity is increased strongly. Fluorescence spectra of "double clicked dG" ss-oligonucleotides **41** and **42** show only monomer fluorescence, and when duplexes are formed (**25**·**41** and **25**·**42**), the fluorescence intensity strongly decreases (Figure 5b). Similar to the series of octadiynyl derivatives, the fluorescence emission of the "double clicked" pyrene and 7-deazaguanine modified ss-oligonucleotide **41** is quenched

dx.doi.org/10.1021/jo202103q | J. Org. Chem. 2012, 77, 188–199

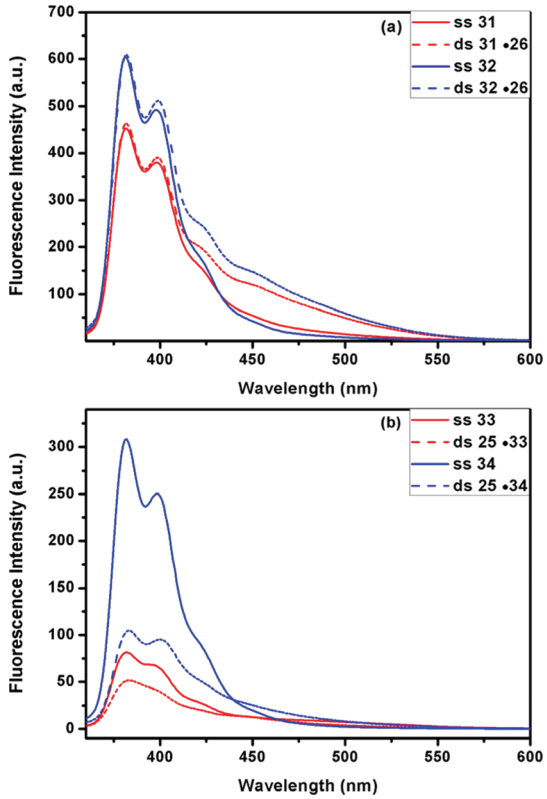


Figure 4. (a) Fluorescence emission spectra of the 2 μM ss-oligonucleotides **31**, **32** and duplexes **31·26**, **32·26** (2 μM of each strand). (b) Fluorescence emission spectra of the 2 μM ss-oligonucleotides **33**, **34** and duplexes **25·33**, **25·34** (2 μM of each strand). All spectra were measured in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0).

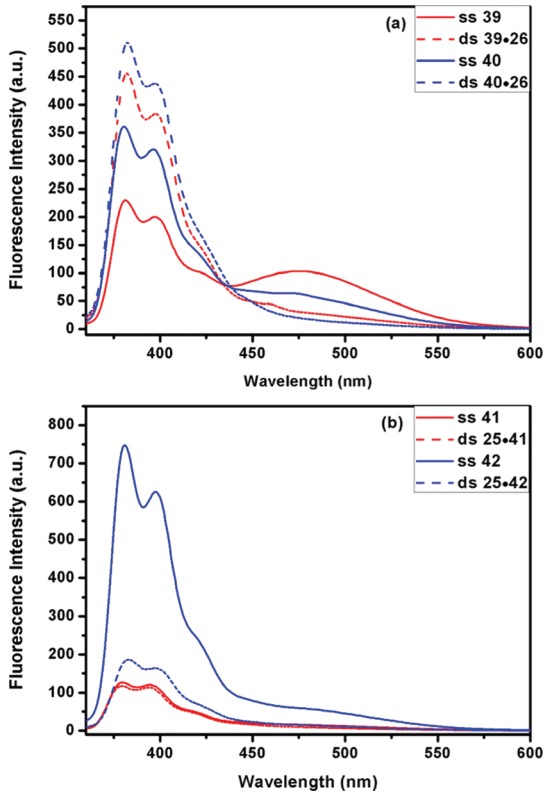


Figure 5. (a) Fluorescence emission spectra of the 2 μM ss-oligonucleotides **39** and **40** and duplexes **39·26**, **40·26** (2 μM of each strand). (b) Fluorescence emission spectra of the 2 μM ss-oligonucleotides **41** and **42** and duplexes **25·41**, **25·42** (2 μM of each strand). All spectra were measured in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0).

strongly compared to that of the corresponding 8-aza-7-deazaguanine ss-oligonucleotide **42**. Upon hybridization with complementary strands (→ duplexes **25·41** and **25·42**), the fluorescence intensity decreases further.

4.4. Fluorescence of 7-Deazapurine and 8-Aza-7-deaza-purine Oligonucleotide "Mono and Double Click" Conjugates with Pyrene Modifications in Both Strands of the Duplex. Tripropargylamine derivatives of 7-deaza-dG and 8-aza-7-deaza-dG develop excimer fluorescence neither in ss-oligonucleotides nor in ds DNA, when only one individual strand was decorated with two proximal pyrenes. Competing stacking interactions of one of the proximal pyrene residues with nucleobases accounted for this phenomenon. By that, one pyrene unit is not available for intramolecular pyrene interaction thus causing only monomer emission (Section 4.1 and Supporting Information).

Subsequently, UV−vis (Figure S4a, Supporting Information) and fluorescence emission spectra (Figure 6a) of duplexes **31·33**, **31·34**, **32·33**, and **32·34** were measured containing one pyrene modification in each strand. All duplex combinations studied herein show only monomer fluorescence and lack excimer fluorescence, demonstrating that the pyrene residues lie apart from each other. Fluorescence emission of duplex **32·34** with 8-aza-7-deaza-dA (**2**) and 8-aza-7-deaza-dG (**4**) was found to be highest, while duplex **31·33** with only 7-deazapurine modifications (**1** and **3**) showed the lowest fluorescence. The duplexes containing "mixed" 7-deazapurine and 8-aza-7-deazapurine modifications (**31·31** and **32·34**) exhibit fluorescence intensities in between.

Next, UV−vis (Figure S4b, Supporting Information) and fluorescence emission spectra (Figure 6b) of the duplexes **39·41**, **39·42**, **40·41**, and **40·42** containing one "double clicked" pyrene residue in each strand were investigated. All duplex combinations show strong excimer fluorescence and rather low monomer fluorescence (Figure 6b). Now, two pyrene residues lie in a close proximity, facilitating the $\pi-\pi$ interaction between electronic clouds, thus giving rise to excimer fluorescence. It is noteworthy to mention that each pyrene residue involved in excimer formation comes from the opposite strand[22,26] and that excimer fluorescence intensities are higher for the duplexes containing c^7A_d pyrene "click" conjugate **39** (**39·41** and **39·42**) than for those incorporating $c^7z^8A_d$ pyrene "click" conjugate **40** (**40·41** and **40·42**).

Throughout our studies on oligonucleotides, we found that the fluorescence intensity of ss-oligonucleotides **33** and **41** as well as their duplexes **25·33** and **25·41** containing the c^7G_d pyrene "click" conjugate **3** or **7** was quenched strongly.[7,27,28] In contrast, it was observed that the $c^7z^8G_d$ pyrene "click" conjugates **4** ($oct^7z^8c^7G_d$) and **8** ($trpa^7z^8c^7G_d$) do not develop significant fluorescence quenching. The 7-deazaguanine conjugates **3** ($oct^7c^7G_d$) and **7** ($trpa^7c^7G_d$) on the other hand show low monomer fluorescence compared to the abasic "click" conjugate **9**, pointing to the strong quenching of the pyrene fluorescence by the 7-deazaguanine moiety within both nucleoside "click" conjugates. Nucleobase-specific quenching by Förster resonance energy transfer (FRET) is ruled out as there is no overlap between excitation and emission spectra of the pyrene residue and nucleobases. So, quenching results from

dx.doi.org/10.1021/jo202103q | J. Org. Chem. 2012, 77, 188−199

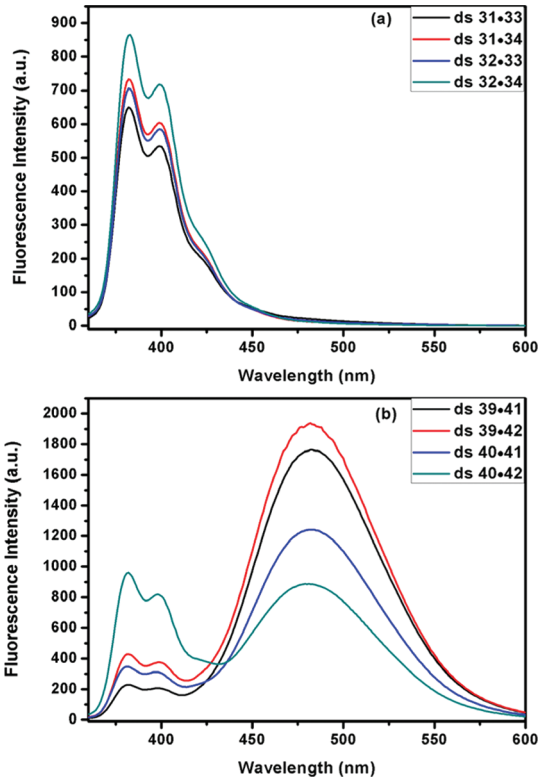


Figure 6. (a) Emission spectra of duplexes **31·33**, **31·34**, **32·33** and **32·34** (2 μM + 2 μM ss concentration) when excited at 340 nm. (b) Emission spectra of duplexes **39·41**, **39·42**, **40·41**, and **40·42** (2 μM + 2 μM ss concentration) when excited at 340 nm. All spectra were measured in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0).

an intramolecular charge transfer between the nucleobase and the dye and subsequent formation of radical cations and radical anions.[7,27]

Charge transfer between nucleobase and dye also depends upon the oxidation potential of the nucleobase. The 7-deazaguanine nucleoside has an oxidation potential lower than those of the other nucleosides.[29,31] Hence during charge separation, the 7-deazaguanine nucleobase can be easily oxidized, forming the radical cation, and the dye yields a radical anion ($Py^{\bullet -}$-$c^7G_d^{\bullet +}$).[30] This type of charge separation, intramolecular hole transfer, leads to the quenching of pyrene fluorescence in "click" conjugates. The 8-aza-7-deazaguanine moiety and the 8-aza-7-deazaadenine moiety are not able to quench the fluorescence of the dye significantly. As a possible explanation, we anticipate a higher oxidation potential for the pyrazolo[3,4-*d*]pyrimidine compared to the pyrrolo[2,3-*d*]-pyrimidine due to the additional ring nitrogen.

CONCLUSIONS AND OUTLOOK

Functionalization of 7-octadiynyl or 7-tripropargylamine derivatives of 7-deazapurine and 8-aza-7-deazapurine nucleosides (**19**–**22** and **11**, **15**, **23**–**24**) with 1-azidomethyl pyrene (**10**) by the copper(I) -catalyzed azide alkyne "click" reaction yielded fluorescent nucleoside dye conjugates (**1**–**8**). The octadiynyl conjugates show only monomer fluorescence, whereas the proximal arrangement of the pyrene residues in the tripropargylated nucleosides leads to monomer and excimer emission. Excimer fluorescence results from intramolecular contacts of the pyrene residues as verified by concentration-dependent fluorescence studies. Monomer as well as excimer fluorescence intensity differs among the various nucleobases. 8-Aza-7-deazapurine pyrene nucleoside (**2**, **4**, **6**, **8**) and oligonucleotide "click" conjugates (**32**, **34**, **40**, **42**) exhibit much higher fluorescence emission compared with that of 7-deazapurine derivatives (**1**, **3**, **5**, **7**, **31**, **33**, **39**, **41**). The observed fluorescence quenching is attributed to charge separation among the nucleobases and pyrene residues, which is strong for 7-deazapurine nucleoside conjugates due to their lower oxidation potential. Similar quenching is observed on nucleosides as well as for single and double stranded oligonucleotides. Henceforth, 8-aza-7-deazaadenine and 8-aza-

Table 2. ^{13}C NMR Chemical Shifts of Nucleoside Derivatives and Pyrene "Click" Conjugates[a]

	C(2),[b] C(6),[c] C(2)[d]	C(4),[b,c] C(6)[d]	C(4a),[b] C(3a),[c] C(5)[d]	C(5),[b] C(3),[c] C(7)[d]	C(6),[b] C(8)[d]	C(7a),[b,c] C(4)[d]	C≡C	CH_2/OMe	Trihazole	C1′	C2′	C3′	C4′	C5′
1	152.6	157.6	102.3	95.5	122.8	149.0	92.4, 73.7	50.8, 28.3, 27.8, 24.5, 18.6	147.0, 122.2	83.1	*f*	71.0	87.5	61.9
2	157.7	156.6	100.9	127.3[e]		153.6	96.5, 72.1	50.7, 28.3, 27.2, 24.4, 18.6	146.9, 122.2	84.0	37.8	70.9	87.7	62.3
4	155.5[e]	157.1[e]	100.0	130.2[e]		155.2[e]	93.4, 73.1	50.8, 28.1, 27.4, 24.4, 18.4	147.0, 122.2	83.1	37.7	71.0	87.5	62.4
5	152.7	157.5	102.3	94.7	123.7[e]	149.2	86.7, 78.6	50.8, 47.5	143.7, 122.7	83.2	42.0	71.0	87.5	61.9
6	157.7	156.7	101.0	126.5[e]		153.7	90.4, 76.8	50.9, 47.6	143.5, 122.7	84.1	37.9	70.9	87.7	62.3
8	155.5[e]	157.1[e]	100.4	129.2[e]		155.2[e]	88.1, 77.6	50.8, 47.5	143.7, 122.7	83.2	37.7	71.0	87.5	62.4
12	151.4	*g*	110.8	96.3	131.0	151.1	85.8, 78.9, 78.1, 76.1	42.4, 41.3		83.0	*f*	70.9	87.6	61.7
13	151.5	158.0	110.7	96.3	130.8	151.1	85.7, 78.9, 77.9, 76.0	42.3, 41.2/55.0		83.0	*f*	70.7	85.9	64.2
16	155.9	157.8	107.8	128.0		154.4	88.3, 78.7, 77.4, 76.2	41.9, 41.3		84.1	*f*	70.9	87.7	62.3
17	155.9	157.8	107.9	127.6		154.4	88.0, 78.7, 77.5, 76.1	41.9, 41.3/54.9		83.9	*f*	70.6	85.5	64.2

[a]Measured in DMSO-d_6 at 298 K. [b]Systematic numbering for 7-deazapurine derivatives. [c]Systematic numbering for 8-aza-7-deazapurine derivatives. [d]Purine numbering. [e]Tentative. [f]Superimposed by DMSO. [g]Not detected.

dx.doi.org/10.1021/jo202103q | *J. Org. Chem.* 2012, 77, 188–199

7-deazaguanine nucleoside and oligonucleotide pyrene conjugates are perfect purine surrogates for canonical nucleobases as pyrene is not quenched by those derivatives. Also, these mimics base pair as strongly and specifically as canonical DNA constituents, while their pyrene conjugates form even more stable duplexes. A single replacement of a canonical nucleoside by a pyrene conjugate stabilizes corresponding duplexes substantially: 6−12 °C for duplexes with a modified "adenine" base and 2−6 °C for modified "guanine" base.

8-Aza-7-deazapurine pyrene conjugates show photophysical advantages over corresponding 7-deazapurine pyrene conjugates as they do not suffer from nucleobase induced quenching. This improves the utility of pyrene fluorescence reporters for detection of oligonucleotides.

EXPERIMENTAL SECTION

General Methods. 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 aluminum 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: U-3000 spectrometer; λ_{max} in nm, ε in dm^3 mol^{-1} cm^{-1}. NMR spectra: measured at 300 MHz for ^{1}H, 75 MHz for ^{13}C and 121 MHz for ^{31}P. The J values are given in Hz and δ in ppm. For NMR spectra recorded in DMSO, the chemical shift of the solvent peak was set to 2.50 ppm for ^{1}H NMR and 39.50 ppm for ^{13}C NMR. Reversed-phase HPLC was carried out on a 250 mm × 4 mm RP-18 LiChrospher 100 column with a HPLC pump connected with a variable wavelength monitor, a controller and an integrator. Gradients used for purification of oligonucleotides by HPLC chromatography: A = MeCN; B = 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN, 95:5. Conditions: (I) 3 min 15% A in B, 12 min 15−50% A in B, and 5 min 50−10% A in B, flow rate 0.8 mL min^{-1}; (II) 0−25 min 0−20% A in B, flow rate 0.8 mL min^{-1}. Molecular mass of oligonucleotide "click" conjugates were determined by MALDI-TOF mass spectrometry in the linear negative mode with 3-hydroxypicolinic acid (3-HPA) as a matrix (Table S2, Supporting Information). Fluorescence spectra were recorded in the wavelength range between 300 and 600 nm using a fluorescence spectrophotometer. ^{13}C NMR chemical shifts of "click" conjugates are summarized in Table 2.

Fluorescence Measurements Performed on Nucleoside and Oligonucleotide Pyrene Conjugates. Fluorescence spectra of all nucleoside "click" conjugates (**1**−**8**) were measured in methanol (for solubility reasons all "click" conjugates were dissolved in 1 mL of DMSO and then diluted with 99 mL of methanol). All measurements were performed with identical concentrations of 6.8 μM. Fluorescence spectra of ss-oligonucleotide "click" conjugates (**31**−**34** and **39**−**42**) and their duplexes were measured in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate buffer (pH 7.0). All measurements were performed with identical concentrations, i.e., 2 μM for ss-oligonucleotides and 2 μM + 2 μM for ds-oligonucleotides.

The fluorescence quantum yields, Φ, of pyrene "click" conjugates were determined by using quinine sulfate in sulfuric acid (0.1 N) as a standard with a known Φ of 0.55.[32]

Synthesis, Purification, and Characterization of Oligonucleotides (27−30 and 35−38). The syntheses of oligonucleotides were performed on a DNA synthesizer at a 1 μmol scale (trityl-on mode) using the phosphoramidites of nucleosides **11**, **15**, and **19**−**24** and the standard phosphoramidite building blocks following the synthesis protocol for 3′-*O*-(2-cyanoethyl)phosphoramidites. After cleavage from the solid support, the oligonucleotides were deprotected in 25% aqueous ammonia solution for 12−16 h at 60 °C. The purification of the "trityl-on" oligonucleotides was carried out on reversed-phase HPLC (RP-18 column; gradient system I). The purified "trityl-on" oligonucleotides were treated with 2.5% of $Cl_2CHCOOH/CH_2Cl_2$ for 5 min at 0 °C to remove the 4,4′-dimethoxytrityl residues. The detritylated oligomers were purified by reversed-phase HPLC (gradient II). The oligomers were desalted on a short column (RP-18) using distilled water for elution of salt, while the oligonucleotides were eluted with H_2O/MeOH (2:3). Then, the solvent was evaporated using a SpeedVac evaporator to yield colorless solids which were frozen at −24 °C. The molecular masses of the oligonucleotides were determined by MALDI-TOF mass spectrometry in the linear negative mode. Extinction coefficients ε_{260} of nucleosides: **1**, 19400; **2**, 22600; **3**, 22900; **4**, 25600; **5**, 34300; **6**, 46000; **7**, 33700; and **8**, 39200.

General Procedure I for the Synthesis of Oligonucleotide Pyrene "Click" Conjugates. To a solution of the ss-oligonucleotide (**27**−**30** and **35**−**38**) (5 A_{260} units, 50 μM) in 20 μL of water were added a mixture of the $CuSO_4$·TBTA (1:1) ligand complex (50 μL of a 20 mM stock solution in *t*-BuOH/H_2O, 1:9), tris-(carboxyethyl)-phosphine (TCEP, 50 μL of a 20 mM stock solution in water), $NaHCO_3$ (50 μL, 200 mM stock solution in water), 1-azidomethylpyrene (**10**) (50 μL for **27**−**30** or 100 μL for **35**−**38** of a 20 mM stock solution in H_2O/dioxane/DMSO, 1:1:1), and DMSO (30 μL), and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated in a SpeedVac evaporator, and the residue was dissolved in 300 μL of bidistilled water and centrifuged for 20 min at 14000 rpm. The supernatant was collected and further purified by reversed-phase HPLC with the gradient [A: 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN 95:5; B: MeCN; gradient: 0−3 min 10−15% B in A, 3−15 min 15−50% B in A, 15−20 min 50−10% B in A, flow rate 0.8 cm^3 min^{-1}] to give the oligonucleotide pyrene conjugates in about 50−56% isolated yield. The molecular masses of the oligonucleotides were determined by MALDI-TOF spectra (Table S2, Supporting Information). Typical HPLC profiles of the oligonucleotides are shown in the Supporting Information (Figures S6 and S7).

Synthesis of Oligonucleotide Pyrene "Click" Conjugates 34 and 42. To a solution of the ss-oligonucleotide **30** or **38** (5 A_{260} units, 50 μM) in 20 μL of water were added a mixture of the $CuSO_4$·TBTA (1:1) ligand complex (50 μL of a 20 mM stock solution in H_2O/DMSO/*t*-BuOH, 4:3:1), tris(carboxyethyl)phosphine (TCEP, 50 μL of a 20 mM stock solution in water), benzoic acid (20 μL, 100 mM stock solution in DMSO), 1-azidomethylpyrene (**10**) (50 μL of a 20 mM stock solution in H_2O/dioxane/DMSO, 1:1:1), and DMSO (30 μL), and the reaction mixture was stirred at room temperature for 20 min. Then, $NaHCO_3$ (100 μL, 200 mM stock solution in water) was used for neutralization of the excess of benzoic acid. For workup, see the general procedure I.

General Procedure II for the Synthesis of Nucleoside Pyrene "Click" Conjugates. To the solution of the individual compound (**11**, **15**, and **19**−**24**) and 1-azidomethyl pyrene (**10**) in a mixture of THF/H_2O/*t*-BuOH (3:1:1, 3 mL) was added a freshly prepared solution of 1 M sodium ascorbate in water followed by the addition of copper(II) sulfate pentahydrate 7.5% solution in water. The reaction mixture was stirred for 16 h at room temperature and was monitored by TLC. After completion of the reaction, the solvent was evaporated. The products were isolated by flash chromatography (silica gel, column 10 cm × 3 cm, CH_2Cl_2/MeOH, 90:10) and characterized by NMR and elemental analysis. All products were isolated in yields ranging from 74% to 84%. The details of the preparation and the characterization data are given in the particular procedures. The syntheses of compounds **3** and **7** were reported by our group earlier.[22]

4-Amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-[6-{1-(pyren-1-ylmethyl)-1*H*-1,2,3-triazol-4-yl}hex-1-yn-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (1). Compound **1** was prepared using the general procedure II with compound **19**[19a] (0.100 g, 0.28 mmol), 1-azidomethyl pyrene **10**[23] (0.102 g, 0.40 mmol), a freshly prepared solution of 1 M sodium ascorbate in water (113 μL, 0.11 mmol), and copper(II) sulfate pentahydrate 7.5% in water (89 μL, 0.03 mmol). Compound **1** was isolated as a light yellow solid (0.126 g, 74%): TLC (CH_2Cl_2/MeOH 90:10) R_f 0.42; UV λ_{max} (MeOH)/nm 264 (ε/dm^3 mol^{-1} cm^{-1} 30400), 275 (51400), 311 (12000), 325 (25500), 341 (38200). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 1.50−1.59 (m, 2H, CH_2), 1.64−1.73 (m, 2H, CH_2), 2.11−2.19 (m, 1H, H_α-2′), 2.41−2.46 (m, 2H, CH_2), 2.61 (t, J = 7.2 Hz, 2H, CH_2), 3.46−3.60 (m, 2H, H-5′), 3.80−3.83 (m, 1H, H-4′), 4.32−4.34 (m, 1H, H-3′), 5.09 (t, J =

dx.doi.org/10.1021/jo202103q | J. Org. Chem. 2012, 77, 188−199

5.7 Hz, 1H, HO-5′), 5.29 (d, J = 4.2 Hz, 1H, HO-3′), 6.32 (s, 2H, pyrene-CH_2), 6.46 (t, J = 6.7 Hz, 1H, H-1′), 7.63 (s, 1H, H-8), 7.91−8.52 (m, 11H, Ar-H, H-5-triazole, H-2). Anal. Calcd for $C_{36}H_{33}N_7O_3$ (611.69): C 70.69, H 5.44, N 16.03. Found: C 70.67, H 5.55, N 15.92.

4-Amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3-[6-{1-(pyren-1-ylmethyl)-1*H*-1,2,3-triazol-4-yl}hex-1-yn-1-yl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (2). Compound **2** was prepared using the general procedure II with compound **20**[20a] (0.100 g, 0.28 mmol), 1-azidomethyl pyrene **10**[23] (0.101 g, 0.39 mmol), a freshly prepared solution of 1 M sodium ascorbate in water (111 μL, 0.11 mmol), and copper(II) sulfate pentahydrate 7.5% in water (93 μL, 0.03 mmol). Compound **2** was isolated as a light yellow solid (0.144 g, 84%). TLC (CH_2Cl_2/MeOH 90:10) R_f 0.45; UV λ_{max} (MeOH)/nm 264 (ε/dm^3 mol^{-1} cm^{-1} 33300), 275 (52600), 311 (11200), 325 (25900), 341 (38700). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 1.57−1.72 (m, 4H, 2 × CH_2), 2.19−2.27 (m, 1H, H$_\alpha$-2′), 2.54−2.56 (m, 2H, CH_2), 2.60−2.65 (t, J = 6.9 Hz, 2H, CH_2), 2.72−2.80 (m, 1H, H$_\beta$-2′), 3.36−3.55 (m, 2H, H-5′), 3.79−3.84 (m, 1H, H-4′), 4.39−4.44 (m, 1H, H-3′), 4.80 (t, J = 6.0 Hz, 1H, HO-5′), 5.29 (d, J = 4.5 Hz, 1H, HO-3′), 6.32 (s, 2H, pyrene-CH_2), 6.53 (t, J = 6.6 Hz, 1H, H-1′), 7.92−8.52 (m, 11H, Ar-H, H-5-triazole, H-6). Anal. Calcd for $C_{35}H_{32}N_8O_3$ (612.68): C 68.61, H 5.26, N 18.29. Found: C 68.32, H 5.37, N 18.04.

6-Amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,5-dihydro-3-[6-{1-(pyren-1-ylmethyl)-1*H*-1,2,3-triazol-4-yl}hex-1-yn-1-yl]-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4). Compound **4** was prepared using the general procedure II with compound **22**[21a] (0.100 g, 0.27 mmol), 1-azidomethyl pyrene **10**[23] (0.097 g, 0.38 mmol), a freshly prepared solution of 1 M sodium ascorbate in water (108 μL, 0.11 mmol), and copper(II) sulfate pentahydrate 7.5% in water (89 μL, 0.03 mmol). Compound **4** was isolated as a colorless solid (0.125 g, 74%). TLC (CH_2Cl_2/MeOH 90:10) R_f 0.19; UV λ_{max} (MeOH)/nm 264 (ε/dm^3 mol^{-1} cm^{-1} 33100), 275 (47400), 311 (10900), 325 (25200), 341 (37900). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 1.54−1.56 (m, 2H, CH_2), 1.68−1.70 (m, 2H, CH_2), 2.11−2.15 (m, 1H, H$_\alpha$-2′), 2.41−2.45 (m, 2H, CH_2), 2.62−2.64 (m, 3H, H$_\beta$-2′, CH_2), 3.42−3.56 (m, 2H, H-5′), 3.72−3.80 (m, 1H, H-4′), 4.31−4.39 (m, 1H, H-3′), 4.74 (t, J = 4.5 Hz, 1H, HO-5′), 5.24 (d, J = 3.0 Hz, 1H, HO-3′), 6.25−6.32 (m, 3H, H-1′, pyrene-CH_2), 6.74 (br s, 2H, NH_2), 7.96−8.51 (m, 10H, Ar-H, H-5-triazole), 10.68 (s, 1H, NH). Anal. Calcd for $C_{35}H_{32}N_8O_4$ (628.68): C 66.87, H 5.13, N 17.82. Found: C 66.94, H 5.01, N 17.70.

4-Amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-[3-{bis-((1-(pyren-1-ylmethyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino}-prop-1-yn-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (5). Compound **5** was prepared using the general procedure II with compound **11**[19b] (0.100 g, 0.26 mmol), 1-azidomethyl pyrene **10**[23] (0.183 g, 0.71 mmol), a freshly prepared solution of 1 M sodium ascorbate in water (105 μL, 0.10 mmol), and copper(II) sulfate pentahydrate 7.5% in water (89 μL, 0.03 mmol). Compound **5** was isolated as a colorless solid (0.189 g, 80%). TLC (CH_2Cl_2/MeOH 90:10) R_f 0.50; UV λ_{max} (MeOH)/nm 264 (ε/dm^3 mol^{-1} cm^{-1} 60900), 275 (105000), 311 (24900), 325 (57300), 341 (84200). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 2.14−2.19 (m, 1H, H$_\alpha$-2′), 2.43−2.48 (m, 1H, H$_\beta$-2′), 3.43 (s, 2H, NCH_2), 3.48−3.61 (m, 2H, H-5′), 3.74 (s, 4H, 2 × NCH_2), 3.83 (s, 1H, H-4′), 4.31−4.37 (m, 1H, H-3′), 5.09 (t, J = 5.4 Hz, 1H, HO-5′), 5.29 (d, J = 3.9 Hz, 1H, HO-3′), 6.32−6.34 (br s, 4H, 2 × pyrene-CH_2), 6.49 (t, J = 7.1 Hz, 1H, H-1′), 6.87 (br s, 2H, NH_2), 7.74 (s, 1H, H-8), 7.93−8.50 (m, 21H, Ar-H, 2 × H-5-triazole, H-2). Anal. Calcd for $C_{54}H_{43}N_{11}O_3$ (893.99): C 72.55, H 4.85, N 17.23. Found: C 72.45, H 4.80, N 17.06.

4-Amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3-[3-{bis-((1-(pyren-1-ylmethyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino}-prop-1-yn-1-yl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (6). Compound **6** was prepared using the general procedure II with compound **15**[20b] (0.100 g, 0.26 mmol), 1-azidomethyl pyrene **10**[23] (0.183 g, 0.71 mmol), a freshly prepared solution of 1 M sodium ascorbate in water (106 μL, 0.10 mmol), and copper(II) sulfate pentahydrate 7.5% in water (89 μL, 0.03 mmol). Compound **6** was isolated as a colorless solid (0.178 g, 76%). TLC (CH_2Cl_2/MeOH 90:10) R_f 0.49; UV λ_{max} (MeOH)/nm 264 (ε/dm^3 mol^{-1} cm^{-1} 63000), 275 (95700), 311 (25000), 325 (45800), 341 (64100). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 2.19−2.27 (m, 1H, H$_\alpha$-2′), 2.74−2.82 (m, 1H, H$_\beta$-2′), 3.49−3.56 (m, 4H, NCH_2, H-5′), 3.76−3.82 (m, 5H, 2 × NCH_2, H-4′), 4.39−4.45 (m, 1H, H-3′), 4.80 (t, J = 5.7 Hz, 1H, HO-5′), 5.29 (d, J = 4.5 Hz, 1H, HO-3′), 6.33 (s, 4H, 2 × pyrene-CH_2), 6.54 (t, J = 6.3 Hz, 1H, H-1′), 7.94−8.50 (m, 21H, Ar-H, 2 × H-5-triazole, H-6). Anal. Calcd for $C_{53}H_{42}N_{12}O_3$ (894.98): C 71.13, H 4.73, N 18.78. Found: C 70.89, H 4.85, N 18.60.

6-Amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,5-dihydro-[3-{bis((1-(pyren-1-ylmethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-amino}prop-1-yn-1-yl]-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (8). Compound **8** was prepared using the general procedure II with compound **24**[21b] (0.100 g, 0.25 mmol), 1-azidomethyl pyrene **10**[23] (0.175 g, 0.68 mmol), a freshly prepared solution of 1 M sodium ascorbate in water (101 μL, 0.10 mmol), and copper(II) sulfate pentahydrate 7.5% in water (80 μL, 0.03 mmol). Compound **8** was isolated as a colorless solid (0.178 g, 77%). TLC (CH_2Cl_2/MeOH 90:10) R_f 0.3; UV λ_{max} (MeOH)/nm 264 (ε/dm^3 mol^{-1} cm^{-1} 53400), 275 (81700), 311 (20300), 325 (48100), 341 (71100). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 2.11−2.19 (m, 1H, H$_\alpha$-2′), 2.62−2.71 (m, 1H, H$_\beta$-2′), 3.43−3.52 (m, 2H, H-5′), 3.74−3.81 (m, 5H, 2 × NCH_2, H-4′), 4.33−4.40 (m, 1H, H-3′), 4.74 (t, J = 5.4 Hz, 1H, HO-5′), 5.24 (d, J = 4.2 Hz, 1H, HO-3′), 6.27−6.33 (m, 5H, H-1′, 2 × pyrene-CH_2), 6.78 (s, 2H, NH_2), 7.93−8.50 (m, 20H, Ar-H, 2 × H-5-triazole), 10.64 (s, 1H, NH). Anal. Calcd for $C_{53}H_{42}N_{12}O_4$ (910.98): C 69.88, H 4.65. Found: C 69.50, H 4.75.

7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-4-(isobutyrylamino)-5-{3-[di(prop-2-ynyl)amino]prop-1-ynyl}-7*H*-pyrrolo[2,3-*d*]pyrimidine (12). To a solution of compound **11**[19b] (0.430 g, 1.13 mmol) in anhydrous pyridine (6 mL) was added Me_3SiCl (1.5 mL, 11.6 mmol), and the solution was stirred at room temperature. After 45 min, isobutyric anhydride (1.25 g, 7.94 mmol) was introduced, and the solution was stirred for another 4 h. The mixture was cooled to 0 °C, diluted with H_2O (2 mL) and stirred for 10 min. After the addition of 12% aq NH_3 (4 mL), stirring was continued for 1 h at room temperature. The solution was evaporated and the residue was applied to FC (silica gel, column 10 × 3 cm, CH_2Cl_2/MeOH 96:4). Compound **12** was isolated as a colorless solid (0.440 g, 86%). TLC (CH_2Cl_2/MeOH 9:1) R_f 0.45; UV λ_{max} (MeOH)/nm 239 (ε/dm^3 mol^{-1} cm^{-1} 15000), 279 (13200). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 1.14, 1.17 (s, 6H, 2 × CH_3), 2.20−2.28 (m, 1H, H$_\alpha$-2′), 2.50−2.58 (m, 1H, H$_\beta$-2′), 2.76−2.83 (m, 1H, CH), 3.25 (s, 2H, 2 × C≡CH), 3.45 (s, 4H, 2 × CH_2), 3.52−3.63 (m, 3H, H-5′, CH_2), 3.82−3.85 (m, 1H, H-4′), 4.34−4.39 (m, 1H, H-3′), 5.01 (t, J = 5.4 Hz, 1H, OH-5′), 5.33 (d, J = 4.2 Hz, 1H, OH-3′), 6.63 (t, J = 6.6 Hz, 1H, H-1′), 8.12 (s, 1H, H-6), 8.61 (s, 1H, H-2), 10.14 (s, 1H, NH). Anal. Calcd for $C_{24}H_{27}N_5O_4$ (449.5): C 64.13, H 6.05, N 15.58. Found: C 64.05, H 6.01, N 15.50.

7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-4-(isobutyrylamino)-5-{3-[di(prop-2-ynyl)amino]prop-1-ynyl}-7*H*-pyrrolo-[2,3-*d*]pyrimidine (13). Compound **12** (0.300 g 0.67 mmol) was dried by repeated co-evaporation with anhydrous pyridine (2 × 5 mL) before dissolving in anhydrous pyridine (8 mL). Then 4,4′-dimethoxytrityl chloride (0.300 g, 0.88 mmol) was added in three portions to the remaining solution at room temperature under stirring for 6 h. Thereupon, MeOH (2 mL) was added, and the mixture was stirred for another 30 min. The reaction mixture was evaporated to dryness under reduced pressure, and the remaining residue was dissolved in dichloromethane (50 mL) and washed with 5% aq $NaHCO_3$ solution (2 × 100 mL) and water (80 mL), dried over Na_2SO_4, and then concentrated. Purification by FC (silica gel, column 10 cm × 3 cm, CH_2Cl_2/acetone 7:3) gave a colorless foam of **13** (0.400 g, 80%). TLC (CH_2Cl_2/MeOH 94:6) R_f 0.65; UV λ_{max} (MeOH)/nm 236 (ε/dm^3 mol^{-1} cm^{-1} 34400), 277 (16000). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 1.10−1.17 (m, 6H, 2 × CH_3), 2.27−2.35 (m, 1H, H$_\alpha$-2′), 2.62−2.69 (m, 1H, H$_\beta$-2′), 2.80−2.84 (m, 1H, CH), 3.09−3.25 (m, 4H, 2 × C≡CH, H-5′), 3.43 (s, 4H, 2 × CH_2), 3.54 (s, 2H, CH_2), 3.73 (s, 6H, 2 × CH_3O), 3.95−3.98 (m, 1H, H−C4′), 4.34−4.41 (m, 1H, H-3′), 5.38 (d, J = 4.5 Hz, 1H, OH-3′), 6.63 (t, J = 6.3 Hz, 1H, H-1′), 6.81−6.85 (m, 4H, Ar-H), 7.17−7.38 (m, 9H, Ar-H), 7.96 (s, 1H, H-6), 8.60 (s, 1H, H-2), 10.11 (s, 1H,

dx.doi.org/10.1021/jo202103q | J. Org. Chem. 2012, 77, 188−199

NH). Anal. Calcd for $C_{45}H_{45}N_5O_6$ (751.87): C 71.89, H 6.03, N 9.31. Found: C 71.84, H 6.00, N 9.22.

7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-4-(isobutyrylamino)-5-{3-[di(prop-2-ynyl)amino]prop-1-ynyl}-7*H*-pyrrolo-[2,3-*d*]pyrimidine 3′-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (14). A stirred solution of **13** (0. 200 g, 0.27 mmol) in anhydrous CH_2Cl_2 (5 mL) was preflushed with argon and treated with (*i*-Pr)$_2$EtN (74 μL, 0.43 mmol) followed by 2-cyanoethyl-*N*,*N*-diisopropylphosphoramido-chloridite (122 μL, 0.56 mmol). After stirring for 45 min at rt, the solution was diluted with CH_2Cl_2 (30 mL) and extracted with 5% aq $NaHCO_3$ solution (20 mL). The organic layer was dried over Na_2SO_4 and concentrated. FC (silica gel, 10 cm × 2 cm, CH_2Cl_2/acetone 95:5) gave a colorless foam of **14** (0.160 g, 63%). TLC (CH_2Cl_2/acetone 90:10) R_f 0.71. ^{31}P NMR ($CDCl_3$, 121 MHz) (δ, ppm): 148.8, 148.6.

1-[2-Deoxy-β-D-*erythro*-pentofuranosyl]-3-[di(prop-2-ynyl)-amino]prop-1-ynyl]-4-{[(*N*,*N*-dimethylamino)methylidene)]-amino}-1*H*-pyrazolo[3,4-*d*]pyrimidine (16). To a solution of **15**[20b] (0.300 g, 0.78 mmol) in MeOH (40 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (2.32 g, 19.2 mmol), and the reaction mixture was stirred at rt for 30 min. Then, the solvent was evaporated, and the residue was subjected to FC (silica gel, column 10 cm × 4 cm, eluted with CH_2Cl_2/MeOH 97:3). Evaporation of fractions containing UV activity afforded compound **16** (0.251 g, 73%) as a colorless foam. TLC (CH_2Cl_2/MeOH 9:1) R_f 0.7; UV λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 7800), 324 (28900). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 2.22−2.30 (m, 1H, H$_\alpha$-2′), 2.76−2.88 (m, 1H, H$_\beta$-2′), 3.19, 3.23 (s, 6H, 2 × CH_3), 3.26−3.28 (m, 2H, 2 × C≡CH), 3.36−3.56 (m, 6H, H-5′, 2 × CH_2), 3.69 (s, 2H, CH_2), 3.79−3.84 (m, 1H, H-4′), 4.40−4.46 (m, 1H, H-3′), 4.78 (t, *J* = 5.7 Hz, 1H, OH-5′), 5.29 (d, *J* = 4.5 Hz, 1H, OH-3′), 6.59 (t, *J* = 6.3 Hz, 1H, H-1′), 8.45 (s, 1H, H-2), 8.92 (s, 1H, CH). Anal. Calcd for $C_{22}H_{25}N_7O_3$ (435.5): C 60.68, H 5.79, N 22.51. Found: C 60.81, H 5.66, N 22.32.

1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-4-{[(*N*,*N*-dimethyl-amino)methylidene]amino}-3-[di-(prop-2-ynyl)amino]prop-1-ynyl]-1*H*-pyrazolo[3,4-*d*]-pyrimidine (17). Compound **16** (0.240 g, 0.55 mmol) was dried by repeated co-evaporation with anhydrous pyridine (2 × 5 mL) before dissolving in anhydrous pyridine (8 mL). Then, 4,4′-dimethoxytrityl chloride (0.223 g, 0.66 mmol) was added in three portions to the remaining solution at rt under stirring for 6 h. Thereupon, MeOH (2 mL) was added, and the mixture was stirred for another 30 min. The reaction mixture was evaporated to dryness under reduced pressure, and the remaining residue was dissolved in dichloromethane (50 mL) and washed with 5% aq $NaHCO_3$ solution (2 × 100 mL) and water (80 mL), dried over Na_2SO_4, and then concentrated. Purification by FC (silica gel, column 10 × 3 cm, CH_2Cl_2/acetone 9:1) gave a colorless foam of **17** (0.321 g, 79%). TLC (CH_2Cl_2/MeOH 90:10) R_f 0.85; UV λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 34300), 251 (44200), 322 (33500). ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 2.27−2.36 (m, 1H, H$_\alpha$-2′), 2.77−2.83 (m, 1H, H$_\beta$-2′), 3.02−3.04 (d, *J* = 4.8 Hz, 2H, CH_2), 3.19, 3.23 (s, 6H, 2 × CH_3), 3.28−3.30 (m, 2H, 2 × C≡CH), 3.33−3.44 (m, 4H, 2 × CH_2), 3.68−3.70 (m, 8H, H-5′, 2 × OCH_3), 3.92−3.95 (m, 1H, H-4′), 4.50−4.56 (m, 1H, H-3′), 5.33 (d, *J* = 4.8 Hz, 1H, OH-3′), 6.61−6.75 (m, 5H, H-1′, Ar-H), 7.12−7.30 (m, 9H, Ar-H), 8.48 (s, 1H, H-2), 8.92 (s, 1H, CH). Anal. Calcd for $C_{43}H_{43}N_7O_5$ (737.8): C 70.00, H 5.87, N 13.29. Found: C 70.20, H 5.88, N 13.23.

1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-4-{[(*N*,*N*-dimethyl-amino)methylidene]amino}-3-[di-(prop-2-ynyl)amino]prop-1-ynyl]-1*H*-pyrazolo[3,4-*d*]-pyrimidine 3′-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (18). A stirred solution of **17** (0. 200 g, 0.27 mmol) in anhydrous CH_2Cl_2 (5 mL) was preflushed with argon and treated with (*i*-Pr)$_2$EtN (92 μL, 0.54 mmol) followed by 2-cyanoethyl-*N*,*N*-diisopropyl-phosphoramidochloridite (120 μL, 0.54 mmol). After stirring for 45 min at room temperature, the solution was diluted with CH_2Cl_2 (30 mL) and extracted with 5% aq $NaHCO_3$ solution (20 mL). The organic layer was dried over Na_2SO_4 and concentrated. FC (silica gel, 10 × 2 cm, CH_2Cl_2/acetone 90:10) gave a colorless foam of **18** (0.148 g, 58%). TLC (CH_2Cl_2/acetone 95:15) R_f 0.40. ^{31}P NMR ($CDCl_3$, 121 MHz) (δ, ppm): 148.4, 148.2.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H−^{13}C coupling constants of nucleoside derivatives and their pyrene "click" conjugates, molecular masses of modified oligonucleotides measured by MALDI-TOF mass spectrometry, concentration dependent UV and fluorescence spectra and Ex/M ratio of "click" conjugates **7** and **8**, HPLC profiles of oligonucleotides determined at 260 nm, melting curves of oligonucleotide duplexes, UV−vis spectra of pyrene modified nucleosides and oligonucleotides, ^{1}H NMR, ^{13}C NMR, Dept-135 NMR, and ^{1}H−^{13}C gated-decoupled NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

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

■ ACKNOWLEDGMENTS

We thank Mr. N. Q. Tran for the oligonucleotide synthesis and Dr. R. Thiele from Roche Diagnostics, Penzberg for the measurement of the MALDI spectra. We thank Dr. S. Budow for her continuous support throughout the preparation of the manuscript and appreciate critical reading of the manuscript by Dr. P. Leonard. Financial support by ChemBiotech, Münster, Germany is gratefully acknowledged.

■ REFERENCES

(1) (a) Yoshida, H.; Horita, K.; Todoroki, K.; Nohta, H.; Yamaguchi, M. *Bunseki Kagaku* **2003**, *52*, 1113−1119. (b) Drury, J.; Narayanaswami, V. *J. Biol. Chem.* **2005**, *280*, 14605−14610. (c) Li, Y.; Li, H.-W.; Ma, L.-J.; Dang, Y.-Q.; Wu, Y. *Chem. Commun.* **2010**, *46*, 3768−3770. (d) Ranasinghe, R. T.; Brown, T. *Chem. Commun.* **2005**, 5487−5502. (e) Østergaard, M. E.; Hrdlicka, P. J. *Chem. Soc. Rev.* **2011**, *40*, 5771−5788. (f) Wang, C.; Wu, C.; Chen, Y.; Song, Y.; Tan, W.; Yang, C. J. *Curr. Org. Chem.* **2011**, *15*, 465−476. (g) Sinkeldam, R. W.; Greco, N. J.; Tor, Y. *Chem. Rev.* **2010**, *110*, 2579−2619.

(2) (a) Winnik, F. M. *Chem. Rev.* **1993**, *93*, 587−614. (b) Kim, H. J.; Hong, J.; Hong, A.; Ham, S.; Lee, J. H.; Kim, J. S. *Org. Lett.* **2008**, *10*, 1963−1966. (c) Yu, C.; Yam, V. W.-W. *Chem. Commun.* **2009**, 1347−1349. (d) Lee, Y. H.; Liu, H.; Lee, J. Y.; Kim, S. H.; Kim, S. K.; Sessler, J. L.; Kim, Y.; Kim, J. S. *Chem.—Eur. J.* **2010**, *16*, 5895−5901.

(3) Birks, J. B. *Org. Mol. Photophys.* **1975**, *2*, 409−613.

(4) (a) Yao, C.; Kraatz, H. B.; Steer, R. P. *Photochem. Photobiol. Sci.* **2005**, *4*, 191−199. (b) Crawford, A. G.; Dwyer, A. D.; Liu, Z.; Steffen, A.; Beeby, A.; Pålsson, L.-O.; Tozer, D. J.; Marder, T. B. *J. Am. Chem. Soc.* **2011**, *133*, 13349−13362.

(5) Bag, S. S.; Kundu, R.; Matsumoto, K.; Saito, Y.; Saito, I. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3227−3230.

(6) Birks, J. B. *Rep. Prog. Phys.* **1975**, *38*, 903−974.

(7) (a) Manoharan, M.; Tivel, K. L.; Zhao, M.; Nafisi, K.; Netzel, T. L. *J. Phys. Chem.* **1995**, *99*, 17461−17472. (b) Netzel, T. L. *Tetrahedron* **2007**, *63*, 3491−3514. (c) Mann, J. S.; Shibata, Y.; Meehan, T. *Bioconjugate Chem.* **1992**, *3*, 554−558. (d) Maie, K.; Nakamura, M.; Takada, T.; Yamana, K. *Bioorg. Med. Chem.* **2009**, *17*, 4996−5000. (e) Wagenknecht, H.-A. *Nat. Prod. Rep.* **2006**, *23*, 973−1006. (f) Grigorenko, N. A.; Leumann, C. J. *Chem. Commun.* **2008**, 5417−5419.

(8) Caldwell, R. A.; Creed, D.; DeMarco, D. C.; Melton, L. A.; Ohta, H.; Wine, P. H. *J. Am. Chem. Soc.* **1980**, *102*, 2369−2377.

(9) (a) Conlon, P.; Yang, C. J.; Wu, Y.; Chen, Y.; Martinez, K.; Kim, Y.; Stevens, N.; Marti, A. A.; Jockusch, S.; Turro, N. J.; Tan, W. *J. Am. Chem. Soc.* **2008**, *130*, 336−342. (b) Marti, A. A.; Li, X.; Jockusch, S.;

Li, Z.; Raveendra, B.; Kalachikov, S.; Russo, J. J.; Morozova, I.; Puthanveettil, S. V.; Ju, J.; Turro, N. J. *Nucleic Acids Res.* **2006**, *34*, 3161−3168. (c) Yamana, K.; Iwai, T.; Ohtani, Y.; Sato, S.; Nakamura, M.; Nakano, H. *Bioconjugate Chem.* **2002**, *13*, 1266−1273.
(10) (a) Skorobogatyi, M. V.; Malakhov, A. D.; Pchelintseva, A. A.; Turban, A. A.; Bondarev, S. L.; Korshun, V. A. *ChemBioChem* **2006**, *7*, 810−816. (b) Hwang, G. T.; Seo, Y. J.; Kim, S. J.; Kim, B. H. *Tetrahedron Lett.* **2004**, *45*, 3543−3546. (c) Østergaard, M. E.; Guenther, D. C.; Kumar, P.; Baral, B.; Deobald, L.; Paszczynski, A. J.; Sharma, P. K.; Hrdlicka, P. J. *Chem. Commun.* **2010**, *46*, 4929−4931.
(11) (a) Trkulja, I.; Biner, S. M.; Langenegger, S. M.; Häner, R. *ChemBioChem* **2007**, *8*, 25−27. (b) Mohammadi, S.; Slama-Schwok, A.; Léger, G.; El Manouni, D.; Shchyolkina, A.; Leroux, Y.; Taillandier, E. *Biochemistry* **1997**, *36*, 14836−14844. (c) Trkulja, I.; Häner, R. *J. Am. Chem. Soc.* **2007**, *129*, 7982−7989. (d) Trkulja, I.; Häner, R. *Bioconjugate Chem.* **2007**, *18*, 289−292. (e) Van Daele, I.; Bomholt, N.; Filichev, V. V.; Calenbergh, S. V.; Pedersen, E. B. *ChemBioChem* **2008**, *9*, 791−801.
(12) (a) Nagatoishi, S.; Nojima, T.; Juskowiak, B.; Takenaka, S. *Angew. Chem., Int. Ed.* **2005**, *44*, 5067−5070. (b) Zhu, H.; Lewis, F. D. *Bioconjugate Chem.* **2007**, *18*, 1213−1217.
(13) (a) Seela, F.; He, Y.; Wei, C. *Tetrahedron* **1999**, *55*, 9481−9500. (b) Rippe, K.; Fritsch, V.; Westhof, E.; Jovin, T. M. *EMBO J.* **1992**, *11*, 3777−3786.
(14) (a) Paris, P. L.; Langenhan, J. M.; Kool, E. T. *Nucleic Acids Res.* **1998**, *26*, 3789−3793. (b) Ren, R. X.-F.; Chaudhuri, N. C.; Paris, P. L.; Rumney, S. IV; Kool, E. T. *J. Am. Chem. Soc.* **1996**, *118*, 7671−7678. (c) Verhagen, C.; Bryld, T.; Raunkjær, M.; Vogel, S.; Buchalová, K.; Wengel, J. *Eur. J. Org. Chem.* **2006**, 2538−2548. (d) Prokhorenko, I. A.; Korshun, V. A.; Petrov, A. A.; Gontarev, S. V.; Berlin, Y. A. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2081−2084.
(15) (a) Wang, G.; Bobkov, G. V.; Mikhailov, S. N.; Schepers, G.; Van Aerschot, A.; Rozenski, J.; Van der Auweraer, M.; Herdewijn, P.; De Feyter, S. *ChemBioChem* **2009**, *10*, 1175−1185. (b) Kumar, T. S.; Madsen, A. S.; Østergaard, M. E.; Wengel, J.; Hrdlicka, P. J. *J. Org. Chem.* **2008**, *73*, 7060−7066.
(16) (a) Masuko, M.; Ohtani, H.; Ebata, K.; Shimadzu, A. *Nucleic Acids Res.* **1998**, *26*, 5409−5416. (b) Langenegger, S. M.; Häner, R. *Chem. Commun.* **2004**, 2792−2793. (c) Okamoto, A.; Kanatani, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 4820−4827. (d) Kerr, C. E.; Mitchell, C. D.; Ying, Y.-M.; Eaton, B. E.; Netzel, T. L. *J. Phys. Chem. B* **2000**, *104*, 2166−2175. (e) Mayer, E.; Valis, L.; Huber, R.; Amann, N.; Wagenknecht, H.-A. *Synthesis* **2003**, *15*, 2335−2340. (f) Jeong, H. S.; Kang, S.; Lee, J. Y.; Kim, B. H. *Org. Biomol. Chem.* **2009**, *7*, 921−925.
(17) (a) Huisgen, R.; Szeimies, G.; Möbius, L. *Chem. Ber.* **1967**, *100*, 2494−2507. (b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057−3064. (c) Meldal, M.; Tornøe, C. W. *Chem. Rev.* **2008**, *108*, 2952−3015. (d) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596−2599. (e) Rodionov, V. O.; Fokin, V. V.; Finn, M. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 2210−2215.
(18) (a) Seela, F.; Sirivolu, V. R. *Chem. Biodiversity* **2006**, *3*, 509−514. (b) Seela, F.; Sirivolu, V. R. *Helv. Chim. Acta* **2007**, *90*, 535−552. (c) Seela, F.; Sirivolu, V. R.; Chittepu, P. *Bioconjugate Chem.* **2008**, *19*, 211−224. (d) Sirivolu, V. R.; Chittepu, P.; Seela, F. *ChemBioChem* **2008**, *9*, 2305−2316. (e) Seela, F.; Sirivolu, V. R. *Org. Biomol. Chem.* **2008**, *6*, 1674−1687.
(19) (a) Seela, F.; Zulauf, M. *Synthesis* **1996**, 726−730. (b) Seela, F.; Zulauf, M.; Sauer, M.; Deimel, M. *Helv. Chim. Acta* **2000**, *83*, 910−927.
(20) (a) Seela, F.; Pujari, S. S. *Bioconjugate Chem.* **2010**, *21*, 1629−1641. (b) Seela, F.; Pujari, S. S.; Schäfer, A. H. *Tetrahedron* **2011**, *67*, 7418−7425.
(21) (a) Seela, F.; Xiong, H.; Leonard, P.; Budow, S. *Org. Biomol. Chem.* **2009**, *7*, 1374−1387. (b) Seela, F.; Xiong, H.; Budow, S. *Tetrahedron* **2010**, *66*, 3930−3943.
(22) Seela, F.; Ingale, S. A. *J. Org. Chem.* **2010**, *75*, 284−295.
(23) Park, S. Y.; Yoon, J. H.; Hong, C. S.; Souane, R.; Kim, J. S.; Matthews, S. E.; Vicens, J. *J. Org. Chem.* **2008**, *73*, 8212−8218.
(24) (a) Christensen, U. B.; Pedersen, E. B. *Nucleic Acids Res.* **2002**, *30*, 4918−4925. (b) Christensen, U. B.; Pedersen, E. B. *Helv. Chim. Acta* **2003**, *86*, 2090−2097. (c) Kottysch, T.; Ahlborn, C.; Brotzel, F.; Richert, C. *Chem.—Eur. J.* **2004**, *10*, 4017−4028.
(25) (a) Telser, J.; Cruickshank, K. A.; Morrison, L. E.; Netzel, T. L.; Chan, C.-K. *J. Am. Chem. Soc.* **1989**, *111*, 7226−7232. (b) Telser, J.; Cruickshank, K. A.; Morrison, L. E.; Netzel, T. L. *J. Am. Chem. Soc.* **1989**, *111*, 6966−6976. (c) Saito, Y.; Miyauchi, Y.; Okamoto, A.; Saito, I. *Chem. Commun.* **2004**, 1704−1705.
(26) (a) Marti, A. A.; Jockusch, S.; Stevens, N.; Ju, J.; Turro, N. J. *Acc. Chem. Res.* **2007**, *40*, 402−409. (b) Astakhova, I. V.; Malakhov, A. D.; Stepanova, I. A.; Ustinov, A. V.; Bondarev, S. L.; Paramonov, A. S.; Korshun, V. A. *Bioconjugate Chem.* **2007**, *18*, 1972−1980. (c) Okamoto, A.; Ochi, Y.; Saito, I. *Chem. Commun.* **2005**, 1128−1130. (d) Hrdlicka, P. J.; Babu, B. R.; Sørensen, M. D.; Wengel, J. *Chem. Commun.* **2004**, 1478−1479.
(27) (a) Amann, N.; Pandurski, E.; Fiebig, T.; Wagenknecht, H.-A. *Angew. Chem., Int. Ed.* **2002**, *41*, 2978−2980. (b) Wagenknecht, H.-A. *Angew. Chem., Int. Ed.* **2003**, *42*, 2454−2460. (c) Shafirovich, V. Y.; Courtney, S. H.; Ya, N.; Geacintov, N. E. *J. Am. Chem. Soc.* **1995**, *117*, 4920−4929.
(28) Wilson, J. N.; Cho, Y.; Tan, S.; Cuppoletti, A.; Kool, E. T. *ChemBioChem* **2008**, *9*, 279−285.
(29) (a) Seidel, C. A. M.; Schulz, A.; Sauer, M. H. M. *J. Phys. Chem.* **1996**, *100*, 5541−5553. (b) Nazarenko, I.; Pires, R.; Lowe, B.; Obaidy, M.; Rashtchian, A. *Nucleic Acids Res.* **2002**, *30*, 2089−2095.
(30) Wanninger-Weiß, C.; Valis, L.; Wagenknecht, H.-A. *Bioorg. Med. Chem.* **2008**, *16*, 100−106.
(31) (a) Kelley, S. O.; Barton, J. K. *Chem. Biol.* **1998**, *5*, 413−425. (b) Lewis, F. D.; Letsinger, R. L.; Wasielewski, M. R. *Acc. Chem. Res.* **2001**, *34*, 159−170.
(32) Demas, J. N.; Crosby, G. A. *J. Phys. Chem.* **1971**, *75*, 991−1024.