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7-Deaza-2′-deoxyguanosine: Selective Nucleobase Halogenation, Positional Impact of Space-Occupying Substituents, and Stability of DNA with Parallel and Antiparallel Strand Orientation

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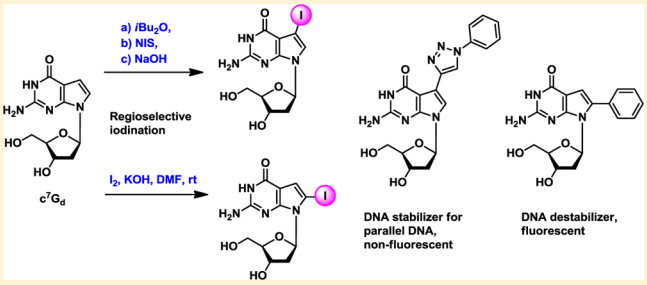
Supporting Information

ABSTRACT: The positional impact of phenyl or phenyltriazolyl residues on the properties of 7-deaza-2′-deoxyguanosine, such as fluorescence, sugar conformation, and stability in DNA and DNA-RNA double helixes was studied. To this end, regioselective iodination protocols were developed for 7-deaza-2′-deoxyguanosine affording the 7- and 8-iodo isomers. The aromatic side chains were introduced by Suzuki−Miyaura cross-coupling or click reaction. Only the 8-phenyl nucleoside shows strong fluorescence in polar aprotic solvents accompanied by solvatochromism. The conformation of the sugar moiety was shifted toward *S* due to the bulky 8-substituent. Phosphoramidite building blocks and oligonucleotides were synthesized. 8-Substituted 7-deaza-2′-deoxyguanosines destabilize canonical (aps) DNA as well as DNA with parallel strand (ps) orientation. The base pair stability was maintained when the space-occupying substitutes were located at the 7-position. Unexpectedly, the bulky phenyltriazolyl "click" residue is well-accommodated at the 7-position of ps DNA and even led to a stabilization of the parallel double helix. CD spectra indicate that functionalization leads only to local distortion of the double helix while the overall structure of aps and ps DNA is maintained.



INTRODUCTION

7-Deazapurine (pyrrolo[2,3-*d*]pyrimidine) nucleosides are widespread in nature as metabolites of microorganisms or constituents of nucleic acids.[1] They form stable Watson−Crick base pairs with pyrimidines similar to those of canonical DNA.[2] The triphosphates of 7-deazapurine nucleosides such as 7-deaza-2′-deoxyadenosine triphosphate (c^7A_dTP) and 7-deaza-2′-deoxyguanosine triphosphate (c^7G_dTP) are well-accepted by DNA polymerases, efficiently incorporated in the growing DNA chain, and have found application as dye terminators in Sanger dideoxy sequencing.[3] Even second generation sequencing (sequencing by synthesis) utilizes 7-deazapurine nucleosides for dye functionalization.[4] Overall, 7-deazapurine nucleosides, nucleotides, and oligonucleotides represent one of the most widely employed classes of nucleobase-modified nucleoside analogues for chemical and biotechnological application developed so far.[1−4] Our laboratory made significant contributions to the synthesis and properties of 7-deazapurine ribo- and 2′-deoxyribonucleosides as well as to oligonucleotides containing these modified nucleosides.[5] The favorable properties of 7-deazapurine nucleosides and oligonucleotides are maintained in canonical DNA when carbon-7 is decorated with halogens, short or long side chains, or bulky dye residues.[6,7] Furthermore, these nucleosides are stable with regard to glycosylic bond hydrolysis and do not show the acid sensitivity of purine nucleosides.[8] When small methyl or aryl groups were introduced at position 8 of 7-deazapurine or purine nucleosides, oligonucleotide duplex stability of canonical DNA decreases.[8,9] We anticipated that the situation is different in DNA with parallel strand (ps) orientation as the groove size is different in ps and aps DNA.[10−15] Ps DNA can be constructed from canonical DNA when 2′-deoxyguanosine (dG) is replaced by 2′-deoxyisoguanosine (isodG) and 2′-deoxycytidine (dC) by 2′-deoxyisocytidine (isodC).[12−14] The dA and dT building blocks can be kept unchanged as they can form base pairs by the Watson−Crick and reverse Watson−Crick mode.[9,10] Ps DNA is less stable than canonical DNA.[10−14]

Usually, the 7-deazaguanine moiety of a pyrrolo[2,3-*d*]pyrimidine nucleoside is nonfluorescent and often acts as a fluorescence quencher.[16] However, in a few cases, functionalization of the base leads to fluorescence.[17] This manuscript reports on the regioselective introduction of phenyl and phenyltriazolyl residues functionalizing 7-deaza-2′-deoxyguanosine at positions 7 and 8 (**1**−**4**, Figure 1). The positional impact of the space-occupying aromatic substituents on the fluo-

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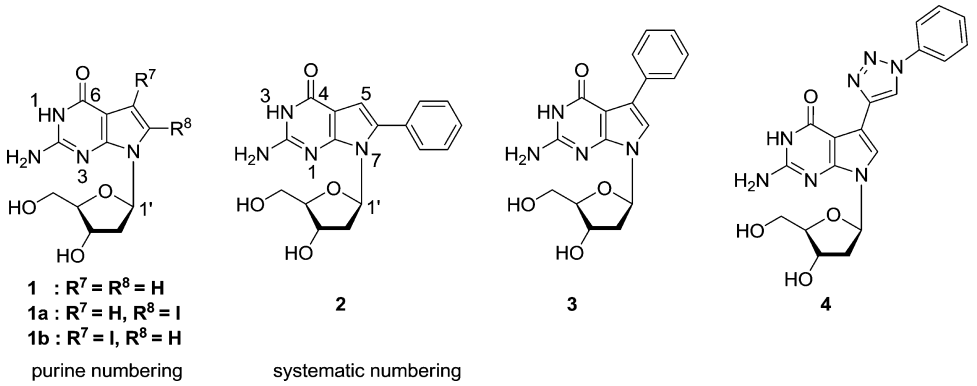


Figure 1. Structures of 7- and 8-substituted derivatives of 7-deaza-2′-deoxyguanosine.

rescence and its solvent dependence is studied. DNA double helices with parallel and antiparallel chain orientation are used to investigate the substituent influence on the DNA helix stability. To this end, the 8-iodo (**1a**) and 7-iodo (**1b**) derivatives of 7-deaza-2′-deoxyguanosine were synthesized as they are key precursors for the functionalization of nucleosides and oligonucleotides in particular by cross-coupling reactions.[6,7,18,19]

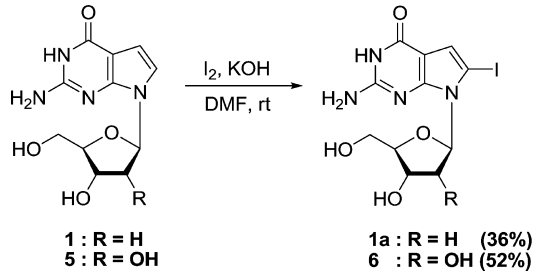
For solid-phase oligonucleotide synthesis, phosphoramidite building blocks of nucleosides **2**–**4** were synthesized and oligonucleotides were prepared. The impact of the space-occupying substituent at the 7- or 8-position of the 7-deazaguanine moiety was studied employing thermal DNA melting as well as CD spectroscopy on both types of DNA—with parallel and antiparallel chains. The impact of 7- and 8-phenyl groups on the fluorescence is investigated (**1**–**4**, Figure 1).

RESULTS AND DISCUSSION

1. Synthesis of Nucleosides and Phosphoramidites. The 7- and 8-iodinated nucleosides **1a** and **1b** are key precursors for the functionalization of the 7- and 8-positions of 7-deaza-2′-deoxyguanosine by Sonogashira, Stille, or Suzuki–Miyaura cross-coupling reactions.[6,7,18] To date, most of the synthetic procedures to access the 8- or 7-iodo compounds (**1a** or **1b**) make use of protected 7-deazaguanine nucleobases or nucleoside precursors.[19] All methods suffer from two major drawbacks: (i) multistep syntheses and (ii) low overall yields (<30%).[19]

Recently, Sasaki described the synthesis of the 8-iodo-7-deaza-2′-deoxyguanosine from 7-deaza-2′-deoxyguanosine (**1**).[19d] He followed a protocol that was reported earlier by Revankar for chlorination and bromination of the ribonucleoside 7-deazaguanosine.[20] In both cases, the sugar moieties were protected with acetyl groups, and *N*-halosuccinimides were used as halogenation reagents.[19d,20] This synthesis employing *N*-iodo succinimide (NIS) gave only 12% yield based on the starting material.[19d] We found it difficult to isolate a pure reaction product by this method. Consequently, we studied other iodination conditions. To this end, nucleoside **1** was treated with iodine in the presence of KOH in DMF at room temperature for 2 h (Scheme 1). Sonication was necessary to dissolve poorly soluble 2′-deoxyribonucleoside **1**. After workup and purification, 8-iodo nucleoside **1a** was isolated in 36% yield. For the utility of this procedure to be verified, the same reaction conditions were applied to the better soluble ribonucleoside **5**. In this case, 8-iodo-7-deazaguanosine (**6**) was isolated in 52% yield (Scheme 1). Although the yields of this protocol are moderate, the outcome of these reactions is superior to methods reported so far.[19d]

Scheme 1. Synthesis of 8-Iodo Nucleosides 1a and 6



7-Deaza-2′-deoxyguanosine was also used as starting material for iodination at position 7. Earlier, our laboratory showed that the electrophilic attack on 7-deazapurine nucleosides as well as on corresponding bases depends on the substituent pattern of the nucleobase, in particular on the substituent at position 2.[19a] Electrophilic attack on the 7-deazapurine system takes place at the 7-position when the 2-position is not functionalized or carries a methylthio group while the electrophilic substitution is shifted to position 8 in the presence of a 2-amino group.[5d,19] It is the result of sigma complex stabilization during the electrophilic attack at position 8 by the 2-amino group.[19a] This influence is circumvented when the 2-amino group is protected by a formyl, acetyl, or pivaloyl residue. Many synthetic protocols now make use of this observation.[5d,17b,19] However, none of them used 7-deaza-2′-deoxyguanosine for this purpose. In this work, the 2-amino group of nucleoside **1** was blocked with an isobutyryl residue. Isobutyrylated nucleoside **7** was prepared from **1** according to the literature.[21] Treatment of **7** with *N*-iodo succinimide in DMF afforded 7-iodo nucleoside **8** as the main product (44%) and a minor amount 7,8-di-iodonucleoside **9** (15%). It was not possible to avoid the formation of the bis-iodinated side product. Nevertheless, both isomers separate well on silica gel. Deprotection of **8** yielded 7-iodo nucleoside **1b** in 93% yield (Scheme 2).

Having both iodo nucleosides in hand, they were employed in the Suzuki–Miyaura cross-coupling reaction to obtain the 7- and 8-phenyl nucleosides. For this, **1a** was treated with an excess of phenyl boronic acid in the presence of sodium carbonate and tetrakis(triphenylphosphine)palladium [Pd-(PPh_3)$_4$], affording nucleoside **2** in 67% yield (Scheme 3). For the synthesis of 7-phenylated nucleoside **3**, isobutyrylated

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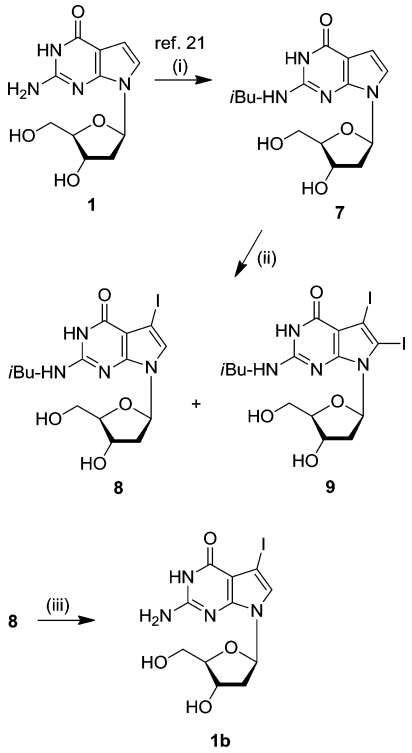
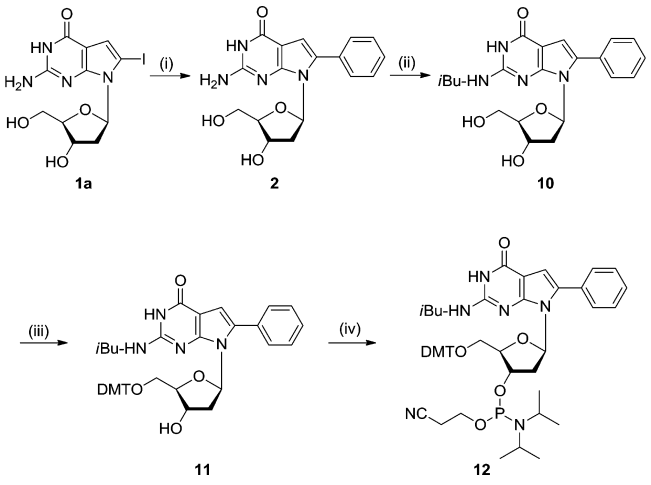
Scheme 2. Synthesis of 7-Iodo-7-deaza-2′-deoxyguanosine 1b[a]



[a]Reagents and conditions: (i) trimethylsilyl chloride, isobutyric anhydride, pyridine, rt, 4 h, 86%; (ii) *N*-iodo succinimide, DMF, rt, 24 h; **8** (44%) and **9** (15%); (iii) 2 N NaOH, reflux, 3 h, 93%.

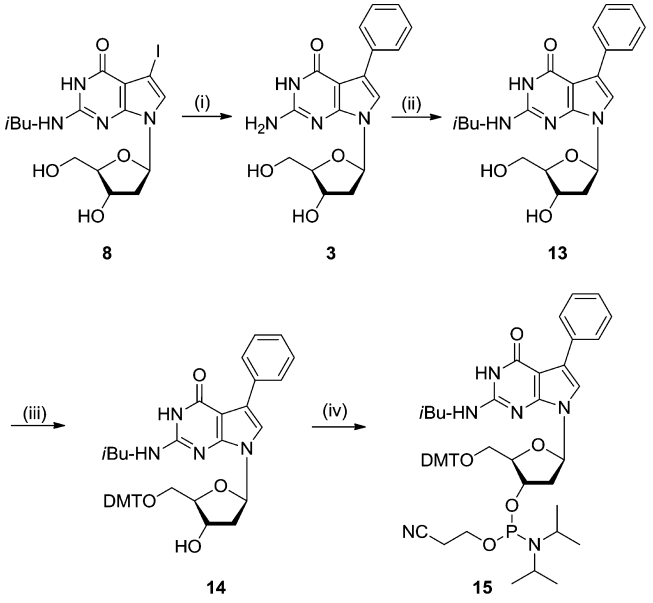
Scheme 3. Synthesis of Nucleoside 2 and Phosphoramidite Building Block 12[a]



[a]Reagents and conditions: (i) phenylboronic acid, $Pd(PPh_3)_4$, sodium carbonate, H_2O:CH_3CN, 105 °C, 2 h, 67%; (ii) trimethylsilyl chloride, isobutyric anhydride, pyridine, rt, 4 h, 73%; (iii) 4,4′-dimethoxytrityl (DMT) chloride, pyridine, rt, 20 h, 36%; (iv) $(i\text{-Pr})_2$NEt, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, DCM, rt, 25 min, 64%.

7-iodo-7-deaza-2′-deoxyguanosine **8** was employed in the Suzuki–Miyaura cross-coupling reaction owing to its good solubility. Similar reaction conditions as used for 8-substituted nucleoside **2** gave 7-phenyl-7-deazaguanosine **3**[22] in 49% yield (Scheme 4). Because of the alkaline reaction conditions, the isobutyryl residue was cleaved in situ.

Scheme 4. Synthesis of Nucleoside 3 and Phosphoramidite Building Block 15[a]



[a]Reagents and conditions: (i) phenylboronic acid, $Pd(PPh_3)_4$, sodium carbonate, H_2O:CH_3CN, 105 °C, 6 h, 49%; (ii) trimethylsilyl chloride, isobutyric anhydride, pyridine, rt, 4 h, 55%; (iii) 4,4′-dimethoxytrityl chloride, pyridine, rt, 20 h, 63%; (iv) $(i\text{-Pr})_2$NEt, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, DCM, rt, 40 min, 77%.

For oligonucleotides to be accessed, nucleosides **2** and **3** were converted into phosphoramidite building blocks **12** and **15** (Schemes 3 and 4). First, nucleosides **2** and **3** were protected at the 2-amino group with an isobutyryl residue, affording the protected intermediates **10** (73%) and **13** (55%). Then, compounds **10** and **13** were converted to 5′-*O*-DMT derivatives **11** (36%) and **14** (63%). The low yield resulting from the tritylation of **10** might be due to steric hindrance by the bulky 8-substituent. Finally, phosphitylation under standard conditions yielded phosphoramidites **12** (64%) and **15** (77%) (Schemes 3 and 4).

Recently, it was reported for pyrimidine nucleosides that consecutive phenyltriazolyl groups introduced at position 5 of 2′-deoxyuridine and 2′-deoxycytidine led to rather stable DNA-RNA hybrids, in particular when they are in a consecutive arrangement.[23] The stabilization most likely occurs through stacking of the phenyltriazolyl moieties.[23] To date, nothing is known on phenyltriazolyl moieties functionalizing 7-deazapurine nucleosides, in particular, to 7-deaza-2′-deoxyguanosine **1** in canonical aps or noncanonical ps DNA. To this end, a phenyltriazolyl residue was introduced in the 7-position of 7-deaza-2′-deoxyguanosine by using the copper(I) catalyzed Huisgen–Meldal–Sharpless azide–alkyne cycloaddition reaction (click reaction).[24] 7-Ethynyl-7-deaza-2′-deoxyguanosine (**16**)[25] was clicked to 1-azidobenzene (**17**)[26] in the presence of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate giving **4** in 51% yield. As the click reaction proceeded much faster on protected (isobutyryl and DMT) ethynylated nucleoside **18**[25] in comparison to that on **16**, compound **18** was used as starting material for the phosphoramidite synthesis (**18** to **20**). Click reaction of **18** with azide **17** in the presence of copper(I)

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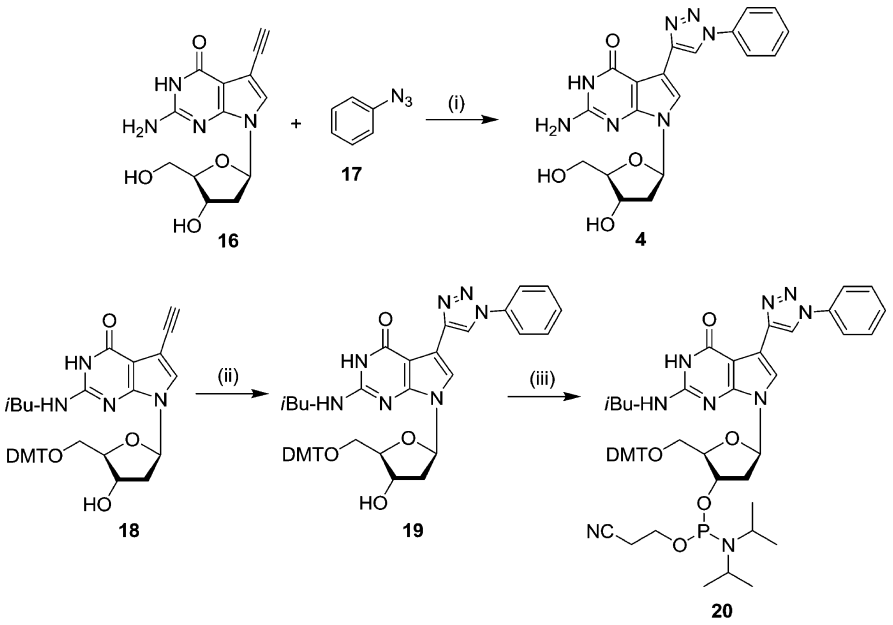
Scheme 5. Synthesis of Nucleoside 4 and Phosphoramidite 20[a]



[a]Reagents and conditions: (i) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, THF:H_2O, rt, 16 h, 51%; (ii) **17**, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, THF:H_2O:*t*-BuOH, rt, 3 h, 70%; (iii) $(i\text{-Pr})_2$NEt, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, DCM, rt, 30 min, 72%.

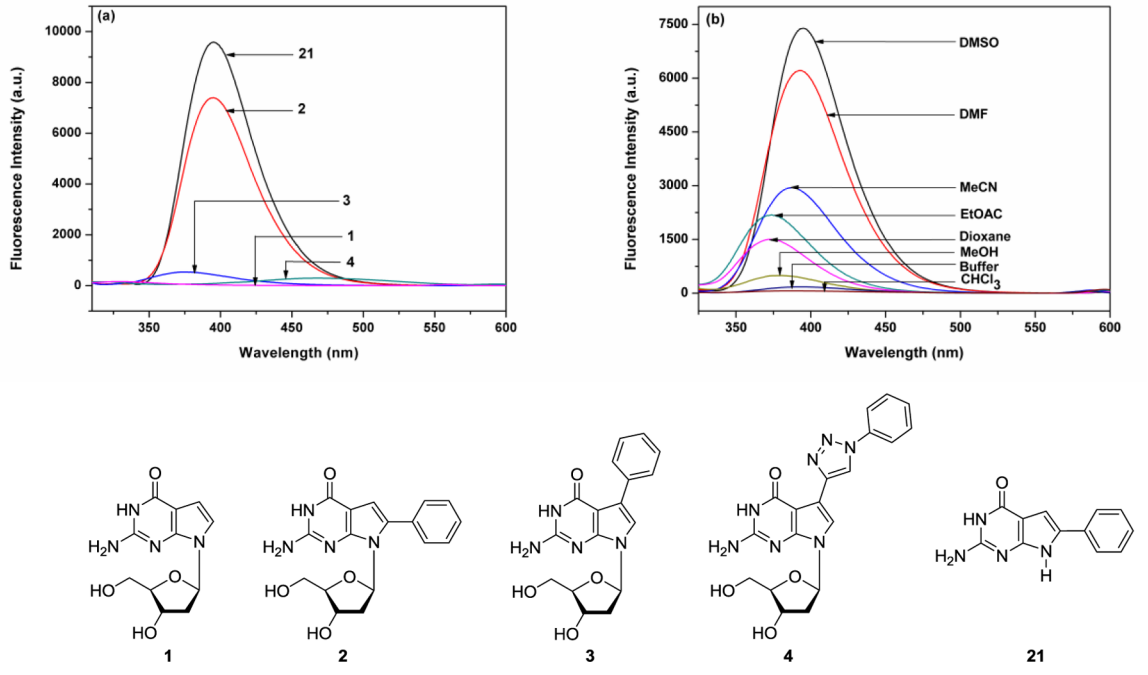


Figure 2. (a) Fluorescence emission spectra of nucleosides **1**–**4** and **21** (2 μM) in DMSO. Excitation wavelengths of nucleosides **1** (276 nm), **2** (298 nm), **3** (300 nm), **4** (294 nm), and **21** (328 nm); (b) fluorescence emission spectra of nucleoside **2** (2 μM concentration) in various solvents (for details, see Table 1).

afforded **19** (70% yield), and subsequent phosphitylation gave phosphoramidite **20** (72% yield, Scheme 5).

All synthesized compounds were characterized by ^{1}H and ^{13}C NMR spectra and ESI mass spectroscopy. DEPT-135 and ^{1}H–^{13}C gated-decoupled NMR spectra were used to assign the ^{13}C NMR signals (Tables S1 and S2). For details, see the Experimental Section (for spectra, see the Supporting Information). The differences in the chemical shifts of the ^{1}H and ^{13}C NMR signals of 7- and 8-substituted 7-deaza-2′-deoxyguanosine to those of 7-deaza-2′-deoxyguanosine are displayed in Table S3. A remarkably large upfield shift of the H2″ and a downfield shift of the H2′ signal is observed for 8-subtituted 7-deaza-2′-deoxyguanosine compared to those of nonfunctionalized or 7-substituted 7-deaza-2′-deoxyguanosines. In addition, an upfield shift of the C2′ signal is also detected for nucleoside **2** (Table S3). This chemical shift change observed for functionalization at position 8 is the result of a conformational change of the nucleobase moving the base in a *syn* conformation relative to the sugar moiety induced by the

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space-occupying 8-substituents. The data are in line with observations made on corresponding 8-substituted purine and 7-deazapurine nucleosides.[8b,19d,27]

It is documented that a change in the *syn*−*anti* conformation affects the conformation of the sugar residue.[27] Consequently, the influence of phenyl residues (at the 7- or 8-position) on the sugar puckering (*N* vs *S*, Figure S1) of 7-deaza-dG and derivatives were determined and compared. Data were calculated using the program PSEUROT (version 6.3).[28] The input used the following coupling constants: ^3J(H1′, H2′), ^3J(H1′, H2″), ^3J(H2′, H3′), ^3J(H2″, H3′), and ^3J(H3′, H4′). The coupling constants were taken from well-resolved ^{1}H NMR spectra measured in DMSO-d_6. All nucleosides (**1**−**4**) prefer the *S* conformation for the pentofuranose moiety. The degree of the *S* population is higher for 8-phenyl-7-deaza-dG (68%) compared to that for 7-phenyl-7-deaza-dG (59%; Figure S1). The *syn* sugar conformation of 8-phenyl-7-deaza-dG is in accordance with earlier observations made on related nucleosides existing preferentially in *syn* conformation.[27]

2. Photophysical Properties of Functionalized 7-Deaza-2′-deoxyguanosines. Recently, Dhimitruka et al. synthesized the base 8-phenyl-7-deazaguanine **21**, which shows fluorescence.[29] However, it was observed that compound **21** only develops strong fluorescence in aprotic polar solvents, for instance in DMSO (Φ = 0.96), whereas fluorescence decreases strongly in aqueous medium (Φ = 0.12).

As nothing is known on the photophysical properties of 8-substituted 7-deaza-2′-deoxyguanosines, this subject was studied next, and the results were compared with the 7-functionalized compounds **1**, **3**, and **4** as well as with 8-substituted nucleobase **21**. At first, UV measurements were performed in DMSO. A significant difference exists in the UV maxima of 8-phenyl nucleoside **2** and nucleobase **21** (Figure S2). Different from nucleobase **21**, steric hindrance of the phenyl residue within the nucleoside can twist the phenyl residue out of plane with regard to the pyrrolo[2,3-*d*]-pyrimidine system, thereby reducing π-conjugation and shifting the UV maxima. Next, fluorescence measurements were performed in DMSO (Figure 2a). The fluorescence intensity of nucleoside **2** (Φ = 0.81) is slightly lower compared to that of nucleobase **21** (Φ = 0.96),[29] whereas other nucleosides (**1**, **3**, and **4**) are almost nonfluorescent.

As nucleobase **21** shows solvatochromism, the solvent dependence of UV and fluorescence spectra was studied on the nucleoside 8-phenyl-7-deaza-2′-deoxyguanosine (**2**). The changes on the spectroscopic properties of **2** are shown in Figure 2b and are summarized in Table 1. The UV maximum (289−298 nm) is red-shifted in aprotic polar solvents (DMSO, DMF) compared to that in protic polar solvents (water, methanol) (Figure S3). Similar to the UV spectra, the fluorescence intensity and emission wavelength (373−396 nm) is affected by the solvent polarity (Figure 2b and Table 1).

Regarding fluorescence, 7-deazapurine nucleoside **2** shows similarities but also differences to the corresponding purine nucleoside 8-phenyl-2′-deoxyguanosine. Almost identical emission maxima (395 nm for **2** and 393 nm for 8-phenyl-2′-deoxyguanosine) are observed, whereas UV absorption maxima are different (289 nm for **2** and 276 nm for 8-phenyl-2′-deoxyguanosine).[31] Furthermore, 8-phenyl nucleoside **2** has a significant lower quantum yield (Φ = 0.017) in aqueous buffer solution compared to that of 8-phenyl-2′-deoxyguanosine (Φ = 0.44). Additional quenching was observed for oligonucleotides containing nucleoside **2** (Figure S4). Similar behavior was also observed for oligonucleotides containing 8-phenyl-2′-deoxyguanosine.[31]

Table 1. Photophysical Data of 8-Phenyl-7-deaza-2′-deoxyguanosine Measured in Solvents of Different Polarities[a]

solvent	$\lambda_{abs,max}$ (nm)	ε [dm^3 mol^{-1} cm^{-1}]	λ_{em} (nm)	Stokes shift (Δν)[b] (cm^{-1})	Φ[c]	brightness[d]
DMSO	298	22500	396	8300	0.807	18200
DMF	295	21500	393	8400	0.634	13700
MeCN	291	21000	387	8500	0.310	6500
EtOAc	294	21000	374	7300	0.239	5000
dioxane	296	21000	373	7000	0.157	3300
MeOH	289	21000	381	8400	0.045	900
buffer[e]	289	20500	395	9300	0.017	400
$CHCl_3$	295	20500	388	8100	0.013	300

[a]All samples contained 1% DMSO, and concentration of nucleoside **2** was 2 μM. [b]Stokes shift is calculated as $(1/\lambda_{abs,max} - 1/\lambda_{em})$. [c]The fluorescence quantum yields (Φ) were calculated using quinine sulfate in 0.5 M H_2SO_4 (Φ_{fl} = 0.55).[30] [d]Brightness factors were calculated by ε × Φ. [e]Buffer = 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0).

3. Oligonucleotides and Impact of 7- and 8-Substituents on Double Helix Stability with Parallel and Antiparallel Chain Orientation. For evaluating the effect of phenyl residues at positions 7 or 8 of the 7-deazaguanine base on the stability of double helices with antiparallel and parallel strand orientation, oligonucleotides were prepared by solid-phase synthesis using the modified phosphoramidite building blocks **12**, **15**, and **20** together with phosphoramidites of nucleoside **1**,[32] 5-methyl-2′-deoxyisocytidine,[33] and 2′-deoxyisoguanosine[34] (Figure S5) as well as standard phosphoramidites. For solid-phase oligonucleotide synthesis, a standard protocol was used; 12-mer oligonucleotides (ODNs) were synthesized, and the dG residues (one or two) were replaced by nucleosides **1**−**4** at various positions (see Tables 2 and 3). Oligonuleotides were cleaved from solid support and deprotected in 28% aqueous ammonia at 55 °C for 16 h. The coupling yields were always higher than 95%. All synthesized oligonucleotides were purified before and after detritylation by using reversed-phase RP-18 HPLC, and the purity of the oligonucleotides was confirmed by HPLC (Figures

Table 2. Molecular Masses of Oligonucleotides Measured by MALDI-TOF Mass Spectrometry[a]

oligonucleotide	molecular weight calcd [M + 1]$^+$	molecular weight found [M + 1]$^+$
5′-d(TA**1** GTC AAT ACT) (**22**)	3644.4	3643.3
5′-d(AGT ATT **1**AC CTA) (**23**)	3644.4	3642.0
5′-d(TA**1** **1**TC AAT ACT) (**24**)	3643.4	3641.0
5′-d(TA**2** GTC AAT ACT) (**25**)	3720.5	3719.2
5′-d(AGT ATT **2**AC CTA) (**26**)	3720.5	3719.5
5′-d(TA**2** **2**TC AAT ACT) (**27**)	3795.6	3793.3
5′-d(TA**3** GTC AAT ACT) (**28**)	3720.5	3719.2
5′-d(AGT ATT **3**AC CTA) (**29**)	3720.5	3719.7
5′-d(TA**3** **3**TC AAT ACT) (**30**)	3795.6	3796.0
5′-d(TA**4** GTC AAT ACT) (**31**)	3787.6	3788.1
5′-d(AGT ATT **4**AC CTA) (**32**)	3787.6	3787.1
5′-d(TA**4** **4**TC AAT ACT) (**33**)	3929.7	3927.5

[a]Measured in positive linear mode.

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Table 3. T_m Values of Modified Duplexes[a,d]

antiparallel duplexes	T_m[b] (ΔT_m)[c] [°C]	parallel duplexes	T_m[b] (ΔT_m)[c] [°C]	antiparallel DNA-RNA hybrids	T_m[b] (ΔT_m)[c] [°C]
5'-d(TAG GTC AAT ACT) (**34**)	51	5'-d(TA G GT C AAT A CT) (**34**)	39	5'-d(TAG GTC AAT ACT) (**34**)	48
3'-d(ATC CAG TTA TGA) (**35**)	(-)	5'-d(AT^{Me}iC ^{Me}iCAiG TTA TiGA) (**36**)	(-)	3'- (AUC CAG UUA UGA) (**38**)	(-)
		5'-d(T^{Me}iCA TAA ^{Me}iCTiG iGAT) (**37**)	44	5'- (UAG GUC AAU ACU) (**39**)	51
		5'-d(A GT ATT GA C CTA) (**35**)	(-)	3'-d(ATC CAG TTA TGA) (**35**)	(-)
		7-Deaza-dG-Modified Duplexes			
5'-d(TA**1** GTC AAT ACT) (**22**)	50	5'-d(TA **1** GT C AAT A CT) (**22**)	38	5'-d(TA**1** GTC AAT ACT) (**22**)	47
3'-d(ATC CAG TTA TGA) (**35**)	(-1)	5'-d(AT^{Me}iC ^{Me}iCAiG TTA TiGA) (**36**)	(-1)	3'- (AUC CAG UUA UGA) (**38**)	(-1)
5'-d(TAG GTC AAT ACT) (**34**)	51	5'-d(T^{Me}iCA TAA ^{Me}iCTiG iGAT) (**37**)	45	5'- (UAG GUC AAU ACU) (**39**)	52 (+1)
3'-d(ATC CA**1** TTA TGA) (**23**)	(0)	5'-d(A GT ATT **1**A C CTA) (**23**)	(+1)	3'-d(ATC CA**1** TTA TGA) (**23**)	
5'-d(TA**1** **1**TC AAT ACT) (**24**)	49	5'-d(TA **1** **1**T C AAT A CT) (**24**)	37	5'-d(TA**1** **1**TC AAT ACT) (**24**)	46
3'-d(ATC CAG TTA TGA) (**35**)	(-2)	5'-d(AT^{Me}iC ^{Me}iCAiG TTA TiGA) (**36**)	(-2)	3'- (AUC CAG UUA UGA) (**38**)	(-2)
		8-Phenyl-7-deaza-dG-Modified Duplexes			
5'-d(TA**2** GTC AAT ACT) (**25**)	35	5'-d(TA **2** GT C AAT A CT) (**25**)	18	5'-d(TA**2** GTC AAT ACT) (**25**)	31
3'-d(ATC CAG TTA TGA) (**35**)	(-16)	5'-d(AT^{Me}iC ^{Me}iCAiG TTA TiGA) (**36**)	(-21)	3'- (AUC CAG UUA UGA) (**38**)	(-17)
5'-d(TAG GTC AAT ACT) (**34**)	33	5'-d(T^{Me}iCA TAA ^{Me}iCTiG iGAT) (**37**)	26	5'- (UAG GUC AAU ACU) (**39**)	35
3'-d(ATC CA**2** TTA TGA) (**26**)	(-18)	5'-d(A GT ATT **2**A C CTA) (**26**)	(-18)	3'-d(ATC CA**2** TTA TGA) (**26**)	(-16)
5'-d(TA**2** **2**TC AAT ACT) (**27**)	36	5'-d(TA **2** **2**T C AAT A CT) (**27**)	18	5'-d(TA**2** **2**TC AAT ACT) (**27**)	30
3'-d(ATC CAG TTA TGA) (**35**)	(-15)	5'-d(AT^{Me}iC ^{Me}iCAiG TTA TiGA) (**36**)	(-21)	3'- (AUC CAG UUA UGA) (**38**)	(-18)
		7-Phenyl-7-deaza-dG-Modified Duplexes			
5'-d(TA**3** GTC AAT ACT) (**28**)	49	5'-d(TA **3** GT C AAT A CT) (**28**)	36	5'-d(TA**3** GTC AAT ACT) (**28**)	44
3'-d(ATC CAG TTA TGA) (**35**)	(-2)	5'-d(AT^{Me}iC ^{Me}iCAiG TTA TiGA) (**36**)	(-3)	3'- (AUC CAG UUA UGA) (**38**)	(-4)
5'-d(TAG GTC AAT ACT) (**34**)	48	5'-d(T^{Me}iCA TAA ^{Me}iCTiG iGAT) (**37**)	43	5'- (UAG GUC AAU ACU) (**39**)	48
3'-d(ATC CA**3** TTA TGA) (**29**)	(-3)	5'-d(A GT ATT **3**A C CTA) (**29**)	(-1)	3'-d(ATC CA**3** TTA TGA) (**29**)	(-3)
5'-d(TA**3** **3**TC AAT ACT) (**30**)	49	5'-d(TA **3** **3**T C AAT A CT) (**30**)	36	5'-d(TA**3** **3**TC AAT ACT) (**30**)	45
3'-d(ATC CAG TTA TGA) (**35**)	(-2)	5'-d(AT^{Me}iC ^{Me}iCAiG TTA TiGA) (**36**)	(-3)	3'- (AUC CAG UUA UGA) (**38**)	(-3)
		7-Phenyltriazolyl-7-deaza-dG-Modified Duplexes			
5'-d(TA**4** GTC AAT ACT) (**31**)	49	5'-d(TA **4** GT C AAT A CT) (**31**)	40	5'-d(TA**4** GTC AAT ACT) (**31**)	45
3'-d(ATC CAG TTA TGA) (**35**)	(-2)	5'-d(AT^{Me}iC ^{Me}iCAiG TTA TiGA) (**36**)	(+1)	3'- (AUC CAG UUA UGA) (**38**)	(-3)
5'-d(TAG GTC AAT ACT) (**34**)	48	5'-d(T^{Me}iCA TAA ^{Me}iCTiG iGAT) (**37**)	47	5'- (UAG GUC AAU ACU) (**39**)	46
3'-d(ATC CA**4** TTA TGA) (**32**)	(-3)	5'-d(A GT ATT **4**A C CTA) (**32**)	(+3)	3'-d(ATC CA**4** TTA TGA) (**32**)	(-5)
5'-d(TA**4** **4**TC AAT ACT) (**33**)	50	5'-d(TA **4** **4**T C AAT A CT) (**33**)	41	5'-d(TA**4** **4**TC AAT ACT) (**33**)	49
3'-d(ATC CAG TTA TGA) (**35**)	(-1)	5'-d(AT^{Me}iC ^{Me}iCAiG TTA TiGA) (**36**)	(+2)	3'- (AUC CAG UUA UGA) (**38**)	(+1)

[a]Measured at 260 nm in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) with 5 μM + 5 μM single-strand concentration. [b]T_m values were determined from the melting curves by using the software MELTWIN 3.0. [c]Refers to the temperature difference of the modified duplex vs the unmodified reference duplex. [d]For formulas, see Figure 3.

S6–S9). The composition of modified oligonucleotides was verified by MALDI-TOF mass spectrometry (Table 2).

Earlier, the duplex stability of 7-functionalized 7-deaza-2′-deoxyguanosine with substituents of moderate size replacing dG residues was studied.[6,7,19c] Previous studies were restricted to canonical DNA with antiparallel strand orientation. For ps and aps DNA, differences of the helix stability were expected as the groove size differs significantly.[15] Consequently, the stabilities of a series of 12-mer oligonucleotide duplexes containing 8-phenyl-7-deaza-2′-deoxyguanosine (**2**), 7-phenyl-7-deaza-2′-deoxyguanosine (**3**), and 7-phenyltriazolyl-7-deaza-2′-deoxyguanosine (**4**) as well as the parent nucleoside (**1**) were studied. Thermal stability data are summarized in Table 3 (for thermal denaturation curves see Figures S10–S21).

Duplexes with Antiparallel Strand Orientation. At first, oligonucleotides containing nucleosides **1**–**4** were hybridized with complementary strands of DNA or RNA to yield helices with aps orientation, and the melting temperatures (T_m) were determined (Table 3). According to Table 3, the following conclusions can be drawn: (i) duplexes containing non-functionalized 7-deaza-2′-deoxyguanosine (**1**) show similar duplex stability as that of the unmodified reference duplex;[6b] (ii) a single modification (phenyl or phenyltriazolyl residue) introduced at the 7-position of 7-deaza-2′-deoxyguanosine (**3**, **4**) induces a slightly negative effect on the duplex stability; (iii) on the contrary, isomeric 8-phenyl-7-deaza-2′-deoxyguanosine (**2**) strongly destabilizes the DNA double helix. A similar observation was made for duplex DNA incorporating 8-phenyl-2′-deoxyguanosine.[8b,31] (iv) The duplex stability of DNA-RNA

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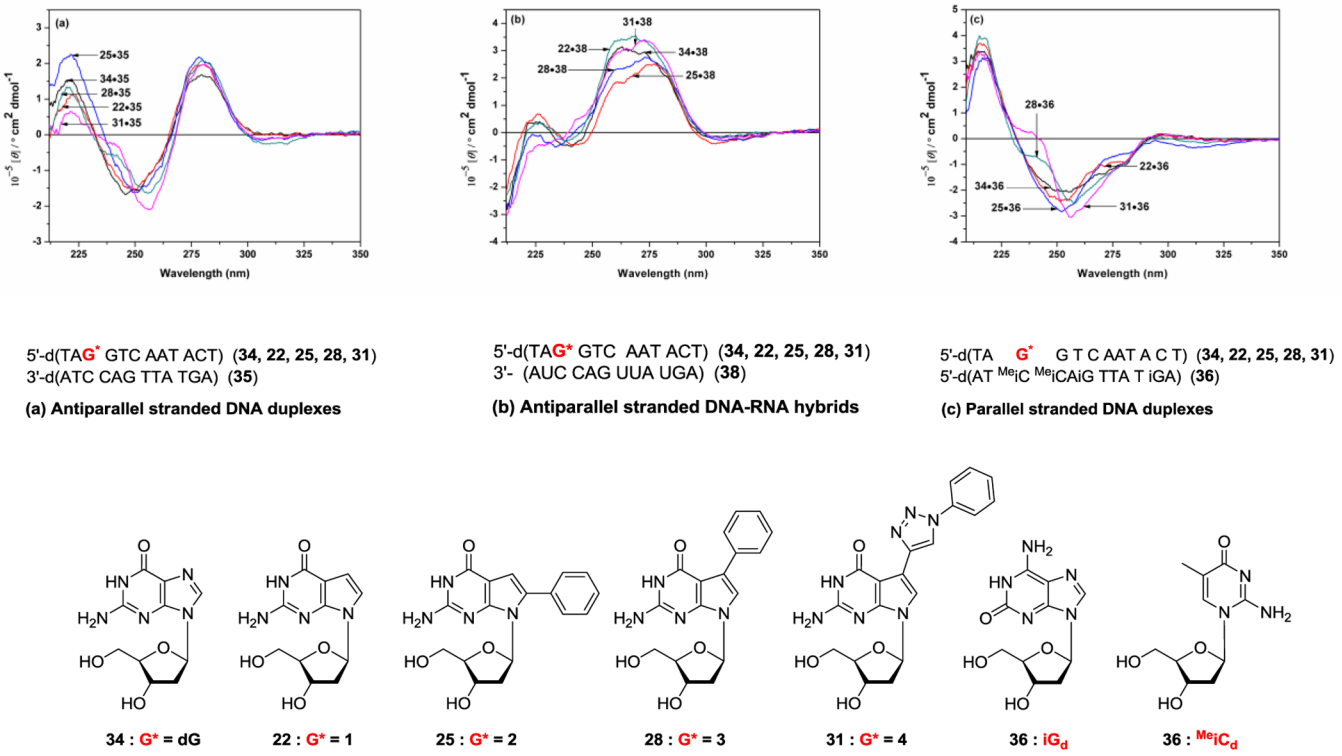

Figure 3. CD spectra of duplexes containing nucleosides **1**–**4** of (a) antiparallel-stranded DNA duplexes, (b) antiparallel-stranded DNA-RNA hybrids, and (c) parallel-stranded DNA duplexes. Measurements were performed in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) with 5 μM duplex concentration at 5 °C.

hybrids was slightly lower for nucleosides **2**–**4** when compared to DNA-DNA duplexes; (v) when the phenyl residue was replaced by the 7-phenyltriazolyl residue (two consecutive incorporations of **4**) in a DNA-RNA hybrid duplex, stability was increased.

Duplexes with Parallel Strand Orientation. Ps DNA is characterized by its sugar–phosphate backbone chains pointing in the same direction as originally suggested by Pattabiraman[10] and later realized by Jovin,[11] Seela,[12] and others.[13,14] In ps DNA, the two grooves have almost identical widths of 8–9 Å, whereas significantly different widths are found in aps DNA for the minor (6 Å) and major (12 Å) grooves.[15] As the groove size of ps DNA differs from that of aps DNA, it was decided that the impact of space-occupying residues in the 7- or 8-positions of 7-deazapurines in the ps double helix should be studied and compared to the stability in aps DNA.

Recently, it was observed for ps DNA that modification of the sugar moiety has a negative impact on the helix stability.[35] As the influence of space-occupying nucleobase substituents at the 7- or 8-position of 7-deazapurines is unknown for ps DNA, corresponding duplexes were constructed, and their thermal stabilities were studied (Table 3). Duplexes **34**·**36** and **37**·**35** were used as references.

In ps DNA, the influence of space-occupying phenyl groups show similarities and also differences to aps DNA (Table 3): (i) 8-phenyl groups introduced in 7-deaza-2′-deoxyguanosine destabilize the duplex more strongly than in aps DNA; (ii) contrary to the 8-phenyl residue, the 7-phenyl group maintained duplex stability; (iii) when the 7-phenyl group was linked to the nucleobase via a triazole linkage, thereby giving the phenyl moiety more flexibility, the stability of the ps duplexes was increased compared to that with the phenyl residue attached directly to the nucleobase; (iv) two consecutive incorporations of phenyltriazolyl groups (nucleoside **4**) increase the duplex stability further compared to nonfunctionalized ps DNA and the ps duplex containing 7-phenyl nucleoside **3**. This result was rather unexpected and shows that the space-occupying phenyltriazolyl group in the 7-deazapurine "click" conjugate is well-accommodated at the 7-position of ps DNA. This finding might be useful for ps DNA functionalization with fluorescent dyes or other space-occupying reporter groups by "click" chemistry or for stabilization of ps DNA to overcome stability problems caused by sugar functionalization at the 2′-position by fluoro, *O*-Me, and *O*-propargyl residues.[35]

4. CD Spectra of Oligonucleotide Duplexes with Antiparallel and Parallel Strand Orientation Containing Nucleosides 1–4. Next, CD spectra were measured (Figure 3, Figure S22). All spectra of the antiparallel-stranded duplex DNA containing nucleosides **1**–**4** show the typical shape of a B-DNA with a positive lobe at 220 and 280 nm and a negative lobe at 250 nm (Figure 3a). The CD spectra of the DNA-RNA hybrids show an intermediate type of spectra. The curves approach A-type structure with a positive band at 265 nm and a central negative band at around 245 nm (Figure 3b). As all the CD spectra were measured at the same temperature, a static picture of the DNA helix is provided, and functionalization leads only to local changes of the spectra. This is different to thermal melting experiments where a cooperative process of strand separation or association takes place.

The CD spectra of parallel DNA containing dA-dT oligonucleotides were already reported by Jovin et al., whereas the CD spectra of parallel oligonucleotide duplexes containing all nucleosides necessary for the construction of parallel DNA, namely dA, dG, dC, dT, isodG, and isodC, were reported by our group and others.[13g,33,36] For the influence of substituents

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of nucleosides **1**−**4** in ps duplex DNA to be studied, the CD spectra of parallel-stranded DNA duplexes were measured under identical conditions as used for aps DNA (Figure 3c). According to the altered structure of ps DNA, the CD spectra of the ps duplexes look entirely different than those with aps chain orientation. They show negative lobes around 250 nm and exhibit only weak positive lobes around 285 nm (Figure 3c). This positive lobe is hypsochromically shifted compared to that in aps DNA.[33] As in aps DNA, all modifications in ps DNA also led only to minor changes of the overall shape of the CD spectra and indicate that even modification at the 8-position causes only local changes in the DNA structure.

CONCLUSIONS AND OUTLOOK

7-Deaza-2′-deoxyguanosine (**1**) was used as starting material for the synthesis of 8-iodo-7-deaza-2′-deoxyguanosine (**1a**) and 7-iodo-7-deaza-2′-deoxyguanosine (**1b**). Both iodination reactions were performed in a selective way. Treatment of **1** with iodine/KOH furnished 8-iodo nucleoside **1a** whereas treatment of isobutyrylated 7-deaza-2′-deoxyguanosine (**7**) with *N*-iodo succinimide gave 7-deaza-2′-deoxy-7-iodoguanosine **8**. For the utility of the reaction to be verified, iodination was also performed on ribonucleoside **5**.

Phenyl residues were introduced in both positions by Suzuki−Miyaura cross-coupling and a phenyltriazolyl residue by click reaction in position 7. The phenyl group attached to position 8 generates fluorescence whereas all other 7-substituted nucleosides are nonfluorescent. Significantly higher fluorescence quantum yields are observed in polar aprotic solvents (DMSO and DMF) compared to those in polar protic solvents. The space-occupying 8-phenyl substituent shifted the conformation of the sugar moiety toward *S* compared to that of the 7-isomer. Nucleosides **2**−**4** were converted to phosphoramidites (**12**, **15**, and **20**), and oligonucleotides were synthesized. 8-Phenyl-7-deaza-2′-deoxyguanosine (**2**) strongly destabilizes aps as well as ps DNA, whereas incorporation of 7-phenyl-7-deaza-2′-deoxyguanosine (**3**) maintained duplex stability. On the contrary, 7-phenyltriazolyl "click" residues introduced in ps DNA are well-accommodated in the groove of the parallel double helix and have a positive impact on the helix stability. CD spectra of aps and ps DNA duplexes indicate that the functionalizations lead only to local changes of the double helices but maintain the overall structure of aps and ps DNA. Consequently, click functionalization with fluorescent dyes or other space-occupying reporter groups could be used to functionalize ps DNA without significantly perturbing the double helix structure.

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 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 were recorded on a UV spectrophotometer with λ_{max} (ε) in nm and ε in dm^3 mol^{-1} cm^{-1}. Fluorescence spectra were recorded on a fluorescence spectrophotometer. NMR spectra were measured at 300.15 MHz for ^{1}H, 75.48 MHz for ^{13}C, and 121.52 MHz for ^{31}P. The ^{13}C NMR signals (Table S1) were assigned on the basis of DEPT-135 and ^{1}H−^{13}C gated-decoupled NMR spectra (for coupling constants, see Table S2). The *J* values are given in Hz, and δ values are 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 ^{1}H NMR and 39.50 ppm for ^{13}C NMR. Reversed-phase HPLC was carried out on a 4 × 250 mm RP-18 (10 μm) LiChrospher 100 column with an HPLC pump connected with a variable wavelength monitor, a controller, and an integrator. CD spectra were recorded on a CD spectrometer. ESI-TOF mass spectra of nucleosides were recorded on a Micro-TOF spectrometer. Molecular masses of oligonucleotides were determined by MALDI-TOF mass spectrometry in the linear positive mode with 3-hydroxypicolinic acid (3-HPA) as a matrix (Table 2). The thermal melting curves were measured with a UV/vis spectrophotometer equipped with a thermos electrical controller. The temperature was measured continuously in the reference cell with a Pt-100 resistor with a heating rate of 1 °C min^{-1}. T_m values were determined from the melting curves using the software MELTWIN 3.0.[37]

Synthesis, Purification, and Characterization of Oligonucleotides. The oligonucleotides were synthesized on an automated DNA synthesizer on a 1 μmol scale employing standard phosphoramidites as well as the phosphoramidites **12**, **15**, and **20**. After cleavage from the solid support, the oligonucleotides were deprotected in concentrated aqueous ammonia solution for 16 h at 55 °C. The DMT-containing oligonucleotides were purified by reversed-phase HPLC (RP-18) with the gradient system at 260 nm: (A) MeCN, (B) 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN, 95:5; gradient I: 0−3 min 10−15% A in B, 3−15 min 15−50% A in B; flow rate 0.8 mL/min. The purified "trityl-on" oligonucleotides were treated with 2.5% $CHCl_2COOH$/CH_2Cl_2 for 2 min at 0 °C to remove the 4,4′-dimethoxytrityl residues. The detritylated oligomers were purified again by reversed-phase HPLC with gradient II: 0−20 min 0−20% A in B; 20−25 min, 20% A in B; flow rate 0.8 mL/min. The oligonucleotides were desalted on a short column (RP-18) using water for elution of salt, while the oligonucleotides were eluted with H_2O/MeOH (2:3). The oligonucleotides were lyophilized on a Speed-Vac evaporator to yield colorless solids, which were frozen at −24 °C. Extinction coefficients ε_{260} (H_2O) of nucleosides are dA, 15 400; dG, 11 700; dT, 8800; dC, 7300; iG_d 4300;[34] $^{Me}iC_d$ 6100;[33] **2** (10000 in MeOH); **3** (13400 in MeOH); **4** (12900 in MeOH).

*2-Amino-7-(2-deoxy-β-D-erythro-pentofuranosyl)-3,7-dihydro-6-iodo-4H-pyrrolo[2,3-d]pyrimidin-4-one (**1a**).*[19d] Compound **1**[5a] (1.000 g, 3.76 mmol) was dissolved (by using sonication) in 20 mL of DMF (flask was covered by aluminum foil), and potassium hydroxide (0.527 g, 9.39 mmol) was added to this solution followed by dropwise addition of iodine (1.096 g, 4.32 mmol, dissolved in 20 mL of DMF) over 30 min. The resulting reaction mixture was subsequently stirred for 1.5 h at room temperature. The organic solvent was evaporated to dryness, and the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 92:8) to give compound **1a** (0.533 g, 36%) as a colorless solid. TLC (silica gel; CH_2Cl_2/MeOH, 85:15) R_f = 0.47. λ_{max} (MeOH)/nm 265 (ε/dm^3 mol^{-1} cm^{-1} 18300), 260 (17500). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.00−2.02 (m, 1H, H_α-2′), 3.09−3.18 (m, 1H, H_β-2′), 3.50−3.71 (m, 2H, H-5′), 3.76−3.78 (m, 1H, H-4′), 4.37−4.38 (m, 1H, H-3′), 5.04 (t, *J* = 6.3 Hz, 1H, OH-5′), 5.22 (d, *J* = 4.2 Hz, 1H, OH-3′), 6.18−6.22 (m, 3H, H-1′, NH_2), 6.56 (s, 1H, H-5), 10.55 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.5, 62.3, 71.3, 75.5, 86.9, 87.4, 103.7, 112.9, 151.3, 151.9, 157.2. ESI-TOF *m*/*z* calcd for $C_{11}H_{13}IN_4O_4$ $[M + Na]^+$ 414.9874, found 414.9879.

*2-Amino-3,7-dihydro-6-iodo-7-(β-D-ribofuranosyl)-4H-pyrrolo-[2,3-d]pyrimidin-4-one (**6**).* Compound **5**[5c] (0.040 g, 0.14 mmol) was dissolved in 1.5 mL of DMF (flask was covered by aluminum foil), and potassium hydroxide (0.020 g, 0.36 mmol) was added to this solution followed by dropwise addition of iodine (0.041 g, 0.16 mmol, dissolved in 0.5 mL of DMF) over 2 min. The resulting reaction mixture was subsequently stirred for 8 h at room temperature. The organic solvent was evaporated to dryness, and the residue was purified by column chromatography (silica gel; CH_2Cl_2/MeOH, 91:9) to give compound **6** (0.030 g, 52%) as a colorless solid. TLC (silica gel; CH_2Cl_2/MeOH, 85:15) R_f = 0.36. λ_{max} (MeOH)/nm 265 (ε/dm^3 mol^{-1} cm^{-1} 18600), 260 (17700). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.51−3.65 (m, 2H, H-5′), 3.79 (brs, 1H, H-4′), 4.16 (brs, 1H, H-3′), 4.94−5.02 (m, 3H, H-2′, OH-3′ and OH-5′), 5.29 (d, *J* = 5.1 Hz, 1H,

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OH-2′), 5.67 (d, J = 4.8 Hz, 1H, H-1′), 6.20 (s, 2H, NH_2), 6.56 (s, 1H, H-5), 10.55 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 62.3, 70.4, 70.8, 73.1, 85.1, 92.0, 103.7, 112.8, 151.3, 152.0, 157.2. ESI-TOF m/z calcd for $C_{11}H_{13}IN_4O_5$ $[M + Na]^+$ 430.9823, found 430.9831.

*7-(2-Deoxy-β-D-erythro-pentofuranosyl)-3,7-dihydro-5-iodo-2-(isobutyrylamino)-4H-pyrrolo[2,3-d]pyrimidin-4-one (**8**).* A solution of compound 7[21] (1.100 g, 3.27 mmol) and *N*-iodosuccinimide (0.957 g. 4.25 mmol) in DMF (4 mL) was stirred at room temperature for 24 h under nitrogen atmosphere. After completion of the reaction (monitored by TLC), the solvent was evaporated, and the residue was applied to FC (silica gel; CH_2Cl_2/MeOH, 95:5). The main zone (slower migrating spot) afforded **8** (0.667 g, 44%) as a colorless solid. TLC (CH_2Cl_2/MeOH, 90:10) R_f = 0.49. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.10, 1.12 (2s, 6H, 2 x CH_3), 2.08−2.14 (m, 1H, H_α-2′), 2.33−2.42 (m, 1H, H_β −2′), 2.69−2.76 (m, 1H, CH), 3.46−3.53 (m, 2H, H-5′), 3.77−3.78 (m, 1H, H-4′), 4.30−4.31 (m, 1H, H-3′), 4.93 (t, J = 5.4 Hz, 1H, OH-5′), 5.24 (d, J = 3.0 Hz, 1H, OH-3′), 6.36 (dd, J = 5.7 Hz, 8.4 Hz, 1H, H-1′), 7.45 (s, 1H, H-6), 11.54 (s, 1H, NH), 11.78 (s, 1H, NH). The obtained NMR data correspond to earlier reported literature values.[6a] ESI-TOF m/z calcd for $C_{15}H_{19}IN_4O_5$ $[M + Na]^+$ 485.0292, found 485.0290.

*7-(2-Deoxy-β-D-erythro-pentofuranosyl)-3,7-dihydro-5,6-diiodo-2-(isobutyrylamino)-4H-pyrrolo[2,3-d]pyrimidin-4-one (**9**).* Evaporation of the minor zone (faster migrating spot) followed by crystallization in methanol gave **9** as a colorless solid (0.287 mg, 15%). TLC (CH_2Cl_2/MeOH, 90:10) R_f = 0.62. λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 6800), 304 (13100). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.11, 1.13 (2s, 6H, 2 x CH_3), 2.01−2.06 (m, 1H, H_α-2′), 2.72−2.79 (m, 1H, CH), 3.15−3.24 (m, 1H, H_β-2′), 3.49−3.71 (m, 2H, H-5′), 3.71−3.75 (m, 1H, H-4′), 4.40−4.41 (m, 1H, H-3′), 4.75 (t, J = 5.4 Hz, 1H, OH-5′), 5.25 (d, J = 3.9 Hz, 1H, OH-3′), 6.42 (t, J = 7.2 Hz, 1H, H-1′), 11.34 (s, 1H, NH), 11.89 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 18.9, 34.7, 37.3, 62.0, 70.9, 74.2, 87.2, 89.6, 107.6, 146.3, 148.9, 154.8, 180.1. ESI-TOF m/z calcd for $C_{15}H_{18}I_2N_4O_5$ $[M + Na]^+$ 610.9259, found 610.9268.

*2-Amino-7-(2-deoxy-β-D-erythro-pentofuranosyl)-3,7-dihydro-5-iodo-4H-pyrrolo[2,3-d]pyrimidin-4-one (**1b**).* A suspension of compound **8** (0.500 g, 1.08 mmol) in aqueous 2 N NaOH (40 mL) was refluxed for 3 h. The solution was neutralized with acetic acid, and the product was collected by filtration, washed with water, and dried to give **1b** as a colorless solid (0.395 g, 93%). The NMR data are identical to earlier reported literature values.[19a]

*2-Amino-7-(2-deoxy-β-D-erythro-pentofuranosyl)-3,7-dihydro-6-phenyl-4H-pyrrolo[2,3-d]pyrimidin-4-one (**2**).* A solution of compound **1a** (0.270 g, 0.69 mmol), phenylboronic acid (0.839 g, 6.88 mmol), sodium carbonate (0.365 g, 3.44 mmol), and $Pd(PPh_3)_4$ (0.080 g, 0.07 mmol) in a mixture of water and acetonitrile (2:1, 18 mL) was heated to 105 °C for 2 h. After cooling, the mixture was evaporated to dryness and purified by column chromatography (silica gel; CH_2Cl_2/MeOH, 94:6) to give compound **2** (0.157 g, 67%) as a colorless solid. TLC (silica gel; CH_2Cl_2/MeOH, 85:15) R_f = 0.54. λ_{max} (MeOH)/nm 289 (ε/dm^3 mol^{-1} cm^{-1} 21500), 260 (10000). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.86−1.93 (m, 1H, H_α-2′), 2.97−3.06 (m, 1H, H_β-2′), 3.47−3.64 (m, 2H, H-5′), 3.69−3.72 (m, 1H, H-4′), 4.20−4.21 (m, 1H, H-3′), 5.05 (d, J = 3.9 Hz, 1H, OH-3′), 5.12 (t, J = 6.0 Hz, 1H, OH-5′), 6.05−6.14 (m, 3H, H-1′, NH_2), 6.27 (s, 1H, H-5), 7.34−7.47 (m, 5H, Ar-H), 10.54 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.0, 61.9, 70.9, 83.9, 87.0, 100.8, 102.2, 127.3, 128.2, 128.6, 132.0, 133.0, 151.2, 151.8, 158.1. ESI-TOF m/z calcd for $C_{17}H_{18}N_4O_4$ $[M + Na]^+$ 365.1220, found 365.1215.

*7-(2-Deoxy-β-D-erythro-pentofuranosyl)-3,7-dihydro-2-(isobutyrylamino)-6-phenyl-4H-pyrrolo[2,3-d]pyrimidin-4-one (**10**).* Compound **2** (0.500 g, 1.46 mmol) was dried by repeated coevaporation with pyridine (2 × 8 mL) and dissolved in pyridine (15 mL). Then, trimethylsilyl chloride (0.793 g, 0.93 mL, 7.30 mmol) was added to the solution. The reaction mixture was stirred for 15 min at room temperature; then, isobutyric anhydride (1.155 g, 1.21 mL, 7.30 mmol) was added, and the solution was stirred for an additional 4 h at room temperature. Then, the reaction mixture was cooled in an ice bath; H_2O (1.5 mL) and subsequently (5 min later) 28−30% aqueous NH_3 solution (1 mL) were added, and stirring was continued for 30 min at room temperature. The solvent was evaporated to near dryness and coevaporated with toluene (2 × 10 mL), and the residue was purified by FC (silica gel; CH_2Cl_2/MeOH, 93:7) to give **10** (0.442 g, 73%) as a colorless solid. TLC (CH_2Cl_2/MeOH, 90:10) R_f = 0.66. λ_{max} (MeOH)/nm 303 (ε/dm^3 mol^{-1} cm^{-1} 25000). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.12, 1.14 (2s, 6H, 2 x CH_3), 1.89−1.95 (m, 1H, H_α-2′), 2.78−2.82 (m, 1H, CH), 2.94−2.98 (m, 1H, H_β-2′), 3.46−3.56 (m, 2H, H-5′), 3.64−3.67 (m, 1H, H-4′), 4.13−4.15 (m, 1H, H-3′), 4.74 (t, J = 5.4 Hz, 1H, OH-5′), 5.07 (d, J = 4.2 Hz, 1H, OH-3′), 6.21 (dd, J = 6.9 Hz, 8.4 Hz, 1H, H-1′), 6.47 (s, 1H, H-5), 7.39−7.56 (m, 5H, Ar-H), 11.30 (s, 1H, NH), 11.95 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 18.6, 34.8, 36.4, 61.4, 70.3, 83.2, 86.6, 102.9, 104.6, 127.9, 128.2, 129.0, 131.6, 135.7, 145.8, 148.5, 156.2, 179.6. ESI-TOF m/z calcd for $C_{21}H_{24}N_4O_5$ $[M + Na]^+$ 435.1639, found 435.1636.

*7-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-6-phenyl-4H-pyrrolo[2,3-d]pyrimidin-4-one (**11**).* Compound **10** (0.330 g, 0.80 mmol) was dried by repeated coevaporation with pyridine (2 × 8 mL). The residue was dissolved in pyridine (2 mL) and stirred with 4,4′-dimethoxytrityl chloride (0.407 g, 1.20 mmol) at room temperature for 20 h. The solution was poured into 5% aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (2 × 50 mL). The combined extracts were dried (Na_2SO_4), and the solvent was evaporated. The residue was purified by FC (silica gel; CH_2Cl_2/acetone, 92:8) to give product **11** (0.208 g, 36%) as a colorless foam. TLC (CH_2Cl_2/acetone, 90:10) R_f = 0.18. λ_{max} (MeOH)/nm 303 (ε/dm^3 mol^{-1} cm^{-1} 21400). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.10, 1.12 (2s, 6H, 2 x CH_3), 2.00−2.05 (m, 1H, H_α-2′), 2.69−2.74 (m, 1H, CH), 2.93−3.02 (m, 2H, H-5′, H_β-2′), 3.41−3.47 (m, 1H, H-5′), 3.68, 3.70 (2s, 6H, 2 x OCH_3), 3.86−3.90 (m, 1H, H-4′), 4.12−4.13 (m, 1H, H-3′), 5.06 (d, J = 4.8 Hz, 1H, OH-3′), 6.26 (t, J = 6.9 Hz, 1H, H-1′), 6.51 (s, 1H, H-5), 6.70−6.78 (m, 4H, Ar-H), 7.11−7.20 (m, 7H, Ar-H), 7.30−7.37 (m, 5H, Ar-H), 7.49−7.51 (m, 2H, Ar-H), 11.07 (s, 1H, NH), 11.89 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 18.8, 19.0, 34.6, 37.5, 54.8, 54.9, 64.8, 71.0, 83.9, 85.2, 85.8, 103.0, 105.0, 112.7, 112.8, 126.4, 127.5, 127.8, 128.1, 128.5, 129.3, 129.6, 129.8, 131.8, 135.6, 135.7, 135.9, 144.9, 145.9, 148.6, 156.5, 157.8, 157.9, 179.8. ESI-TOF m/z calcd for $C_{42}H_{42}N_4O_7$ $[M + Na]^+$ 737.2946, found 737.2922.

*7-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-6-phenyl-4H-pyrrolo[2,3-d]pyrimidin-4-one 3′-(2-Cyanoethyl)-N,N-diisopropylphosphoramidite (**12**).* To a solution of **11** (0.150 g, 0.21 mmol) in dry CH_2Cl_2 (3 mL) were added (*i*-Pr)$_2$NEt (0.068 g, 90 μL, 0.53 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.069 g, 65 μL, 0.29 mmol). After stirring for 25 min at room temperature, the solution was diluted with CH_2Cl_2 (30 mL) and extracted with 5% aqueous $NaHCO_3$ solution (20 mL), and the combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by FC (silica gel; CH_2Cl_2/acetone, 95:5), affording product **12** (0.123 g, 64%) as a colorless foam. TLC (CH_2Cl_2/acetone, 95:5) R_f = 0.17. ^{31}P NMR ($CDCl_3$, 121 MHz) δ 148.0, 147.3. ESI-TOF: m/z calcd for $C_{51}H_{59}N_6O_8P$ $[M + Na]^+$ 937.4024, found 937.4004.

*2-Amino-7-(2-deoxy-β-D-erythro-pentofuranosyl)-3,7-dihydro-5-phenyl-4H-pyrrolo[2,3-d]pyrimidin-4-one (**3**).*[22] A solution of compound **8** (0.580 g, 1.25 mmol), phenylboronic acid (1.531 g, 12.56 mmol), sodium carbonate (0.665 g, 6.27 mmol), and $Pd(PPh_3)_4$ (0.145 g, 0.12 mmol) in a mixture of water and acetonitrile (2:1, 30 mL) was heated to 105 °C for 6 h. After cooling, the mixture was evaporated to dryness and purified by column chromatography (silica gel; petroleum ether/ethyl acetate/MeOH, 40:50:10) to give compound **3** (0.210 g, 49%) as a colorless solid. TLC (silica gel; CH_2Cl_2/MeOH, 85:15) R_f = 0.47. λ_{max} (MeOH)/nm 299 (ε/dm^3 mol^{-1} cm^{-1} 12400), 260 (13400), 242 (20500). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.07−2.13 (m, 1H, H_α-2′), 2.37−2.46 (m, 1H, H_β-2′), 3.41−3.57 (m, 2H, H-5′), 3.76−3.77 (m, 1H, H-4′), 4.31 (brs, 1H, H-3′), 4.91 (t, J = 5.4 Hz, 1H, OH-5′), 5.21 (d, J = 3.6 Hz, 1H, OH-3′), 6.29 (s, 2H, NH_2), 6.38 (dd, J = 5.7 Hz, 8.1 Hz, 1H, H-1′), 7.16 (t, J = 7.2 Hz, 1H, Ar-H), 7.27−7.32 (m, 3H, 2 x Ar-H, H-6), 7.90 (d, J = 7.5 Hz, 2H, Ar-H), 10.44 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ

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62.0, 71.0, 82.1, 87.0, 97.3, 115.0, 120.1, 125.6, 127.6, 127.8, 134.3, 151.9, 152.6, 158.8. ESI-TOF m/z calcd for $C_{17}H_{18}N_4O_4$ $[M + Na]^+$ 365.1220, found 365.1227.

*7-(2-Deoxy-β-D-erythro-pentofuranosyl)-3,7-dihydro-2-(isobutyrylamino)-5-phenyl-4H-pyrrolo[2,3-d]pyrimidin-4-one (**13**).* Compound **3** (0.140 g, 0.41 mmol) was dried by repeated coevaporation with pyridine (2 × 4 mL) and dissolved in pyridine (5 mL). Then, trimethylsilyl chloride (0.222 g, 0.26 mL, 2.04 mmol) was added to the solution. The reaction mixture was stirred for 15 min at room temperature; then, isobutyric anhydride (0.323 g, 0.34 mL, 2.04 mmol) was added, and the solution was stirred for an additional 4 h at room temperature. Then, the reaction mixture was cooled in an ice bath; H_2O (0.7 mL) and subsequently (5 min later) 28−30% aqueous NH_3 solution (0.5 mL) were added, and stirring was continued for 30 min at room temperature. The solvent was evaporated to near dryness and coevaporated with toluene (2 × 5 mL), and the residue was purified by FC (silica gel; CH_2Cl_2/MeOH, 93:7) to give **13** (0.093 g, 55%) as a colorless solid. TLC (CH_2Cl_2/MeOH, 90:10) R_f = 0.55. λ_{max} (MeOH)/nm 302 (ε/dm^3 mol^{-1} cm^{-1} 16300). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.12, 1.14 (2s, 6H, 2 x CH_3), 2.14−2.21 (m, 1H, H_α-2′), 2.45−2.54 (m, 1H, H_β-2′), 2.72−2.81 (m, 1H, CH), 3.48−3.59 (m, 2H, H-5′), 3.81−3.82 (m, 1H, H-4′), 4.36−4.37 (m, 1H, H-3′), 4.95 (t, J = 5.4 Hz, 1H, OH-5′), 5.27 (d, J = 3.3 Hz, 1H, OH-3′), 6.49 (dd, J = 5.7 Hz, 8.4 Hz, 1H, H-1′), 7.22 (t, J = 7.2 Hz, 1H, Ar-H), 7.35 (t, 2H, J = 7.8 Hz, Ar-H), 7.60 (s, 1H, H-6), 7.91 (d, J = 7.2 Hz, 2H, Ar-H), 11.57 (s, 1H, NH), 11.81 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 18.8, 18.9, 34.7, 61.8, 70.8, 82.4, 87.3, 101.1, 127.4, 120.6, 126.1, 127.8, 128.0, 133.6, 146.9, 148.7, 156.8, 180.0. ESI-TOF m/z calcd for $C_{21}H_{24}N_4O_5$ $[M + Na]^+$ 435.1639, found 435.1647.

*7-(2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl)-3,7-dihydro-2-(isobutyrylamino)-5-phenyl-4H-pyrrolo[2,3-d]-pyrimidin-4-one (**14**).* Compound **13** (0.210 g, 0.51 mmol) was dried by repeated coevaporation with pyridine (2 × 5 mL). The residue was dissolved in pyridine (8 mL) and stirred with 4,4′-dimethoxytrityl chloride (0.293 g, 0.87 mmol) at room temperature for 20 h. The solution was poured into 5% aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (2 × 40 mL). The combined extracts were dried (Na_2SO_4), and the solvent was evaporated. The residue was purified by FC (silica gel; CH_2Cl_2/acetone, 95:5) to give product **14** (0.228 g, 63%) as a colorless foam. TLC (CH_2Cl_2/acetone, 90:10) R_f = 0.29. λ_{max} (MeOH)/nm 302 (ε/dm^3 mol^{-1} cm^{-1} 17100). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.12, 1.14 (2s, 6H, 2 x CH_3), 2.27−2.32 (m, 1H, H_α-2′), 2.61−2.67 (m, 1H, H_β-2′), 2.73−2.80 (m, 1H, CH), 3.09−3.27 (m, 2H, H-5′), 3.65, 3.67 (2s, 6H, 2 x OCH_3), 3.95−3.96 (m, 1H, H-4′), 4.45−4.46 (m, 1H, H-3′), 5.34 (d, J = 3.6 Hz, 1H, OH-3′), 6.51 (t, J = 6.9 Hz, 1H, H-1′), 6.77−6.81 (m, 4H, Ar-H), 7.14−7.28 (m, 10H, Ar-H), 7.37 (d, 2H, J = 6.9 Hz, Ar-H), 7.43 (s, 1H, H-6), 7.71−7.74 (m, 2H, Ar-H), 11.59 (s, 1H, NH), 11.83 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 18.8, 18.9, 34.7, 54.9, 55.0, 64.1, 70.9, 82.5, 85.6, 101.2, 113.1, 117.1, 120.8, 126.1, 126.7, 127.6, 127.7, 127.8, 127.9, 129.6, 129.7, 133.3, 135.4, 135.5, 144.7, 147.0, 148.8, 156.8, 157.9, 158.0, 180.0. ESI-TOF m/z calcd for $C_{42}H_{42}N_4O_7$ $[M + Na]^+$ 737.2946, found 737.2918.

*7-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-5-phenyl-4H-pyrrolo[2,3-d]-pyrimidin-4-one 3′-(2-Cyanoethyl)-N,N-diisopropylphosphoramidite (**15**).* To a solution of **14** (0.175 g, 0.25 mmol) in dry CH_2Cl_2 (8 mL) were added (i-Pr)$_2$NEt (0.079 g, 104 μL, 0.61 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.081 g, 76 μL, 0.34 mmol). After stirring for 40 min at room temperature, the solution was diluted with CH_2Cl_2 (30 mL) and extracted with 5% aqueous $NaHCO_3$ solution (20 mL), and the combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by FC (silica gel; CH_2Cl_2/acetone, 95:5), affording product **15** (0.173 g, 77%) as a colorless foam. TLC (CH_2Cl_2/acetone, 95:5) R_f = 0.46. ^{31}P NMR ($CDCl_3$, 121 MHz) δ 148.0, 147.4. ESI-TOF: m/z calcd for $C_{51}H_{59}N_6O_8P$ $[M + Na]^+$ 937.4024, found 937.4049.

*2-Amino-7-(2-deoxy-β-D-erythro-pentofuranosyl)-3,7-dihydro-5-(1-phenyl-1H-1,2,3-triazol-4-yl)-4H-pyrrolo[2,3-d]pyrimidin-4-one (**4**).* A solution of **16**[25] (0.100 g, 0.34 mmol) and 1-azidobenzene[26] (0.050 g, 0.42 mmol) in THF:H_2O (1:1, 2 mL) was cooled to 0 °C. To that solution were added a freshly prepared 1 M solution of sodium ascorbate (0.27 mL, 0.27 mmol) in water and copper(II) sulfate pentahydrate 7.5% in water (0.23 mL, 0.07 mmol). After addition, the reaction mixture was stirred vigorously in the dark at room temperature (16 h) and monitored by TLC. After completion of the reaction, the solvent was evaporated, and the residue was purified by FC (silica gel; column 10 cm × 3 cm, CH_2Cl_2/MeOH, 92:8) to give **4** as a colorless solid (0.072 g, 51%). TLC (CH_2Cl_2/MeOH, 85:15) R_f = 0.41. λ_{max} (MeOH)/nm 235 (ε/dm^3 mol^{-1} cm^{-1} 27200), 260 (12900), 297 (11400). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.10−2.16 (m, 1H, H_α-2′), 2.38−2.46 (m, 1H, H_β-2′), 3.54 (brs, 2H, H-5′), 3.80 (brs, 1H, H-4′), 4.33 (brs, 1H, H-3′), 4.97 (t, J = 5.1 Hz, 1H, OH-3′), 5.26 (d, J = 3.3 Hz, 1H, OH-3′), 6.37−6.41 (m, 3H, NH_2, H-1′), 7.48−7.64 (m, 4H, Ar-H,, H-6), 7.86 (d, J = 8.1 Hz, 2H, Ar-H), 9.15 (s, 1H, triazole-CH), 10.56 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 62.0, 71.1, 82.3, 87.1, 96.8, 109.0, 114.2, 120.1, 120.3, 128.5, 130.0, 136.7, 142.4, 151.5, 152.9, 159.0. ESI-TOF m/z calcd for $C_{19}H_{19}N_7O_4$ $[M + Na]^+$ 432.1391, found 432.1396.

*7-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-5-(1-phenyl-1H-1,2,3-triazol-4-yl)-4H-pyrrolo[2,3-d]pyrimidin-4-one (**19**).* To a solution of **18**[25] (0.200 g, 0.30 mmol) and 1-azidobenzene[26] (0.043 g, 0.36 mmol) in THF:H_2O:*t*-BuOH (3:1:1, 5 mL) were added a freshly prepared 1 M solution of sodium ascorbate (0.24 mL, 0.24 mmol) in water and copper(II) sulfate pentahydrate 7.5% in water (0.20 mL, 0.06 mmol). The reaction mixture was stirred vigorously in the dark at room temperature for 3 h and monitored by TLC. After completion of the reaction, the solvent was evaporated, and the residue was purified by FC (silica gel; column 10 cm × 3 cm, CH_2Cl_2:acetone, 85:15) to give **19** as a colorless solid (0.166 g, 70%). TLC (CH_2Cl_2/acetone, 85:15) R_f = 0.41. λ_{max} (MeOH)/nm 282 (ε/dm^3 mol^{-1} cm^{-1} 20500). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.13, 1.15 (2s, 6H, 2 x CH_3), 2.24−2.31 (m, 1H, H_α-2′), 2.55−2.64 (m, 1H, H_β-2′), 2.72−2.83 (m, 1H, CH), 3.16−3.18 (m, 2H, H-5′), 3.67, 3.68 (2s, 6H, 2 x OCH_3), 3.93−3.94 (m, 1H, H-4′), 4.37 (brs, 1H, H-3′), 5.37 (d, J = 3.6 Hz, 1H, OH-3′), 6.49 (t, J = 6.6 Hz, 1H, H-1′), 6.81−6.85 (m, 4H, Ar-H), 7.14−7.28 (m, 7H, Ar-H), 7.37 (d, J = 7.5 Hz, 2H, Ar-H), 7.51 (t, J = 7.2 Hz, 1H, Ar-H), 7.62 (t, J = 7.8 Hz, 2H, Ar-H), 7.77 (s, 1H, H-6), 7.88 (d, J = 7.8 Hz, 2H, Ar-H), 9.14 (s, 1H, triazole-CH), 11.68 (s, 1H, NH), 11.98 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 18.9, 34.8, 54.9, 64.0, 70.5, 82.7, 85.5, 101.0, 109.8, 113.1, 116.4, 120.1, 120.6, 126.6, 127.7, 127.8, 128.7, 129.6, 129.8, 130.0, 135.5, 135.7, 136.6, 141.7, 144.9, 147.3, 148.4, 157.1, 157.9, 158.0, 180.1. ESI-TOF m/z calcd for $C_{44}H_{43}N_7O_7$ $[M + Na]^+$ 804.3116, found 804.3136.

*7-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-5-(1-phenyl-1H-1,2,3-triazol-4-yl)-4H-pyrrolo[2,3-d]pyrimidin-4-one 3′-(2-Cyanoethyl)-N,N-diisopropylphosphoramidite (**20**).* To a solution of **19** (0.175 g, 0.22 mmol) in dry CH_2Cl_2 (5 mL) were added (i-Pr)$_2$NEt (0.072 g, 95 μL, 0.56 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.074 g, 70 μL, 0.31 mmol). After stirring for 30 min at room temperature, the solution was diluted with CH_2Cl_2 (30 mL) and extracted with 5% aqueous $NaHCO_3$ solution (20 mL), and the combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by FC (silica gel; CH_2Cl_2/acetone, 92:8), affording product **20** (0.159 g, 72%) as a colorless foam. TLC (CH_2Cl_2/acetone, 95:5) R_f = 0.35. ^{31}P NMR ($CDCl_3$, 121 MHz) δ 147.9, 147.5. ESI-TOF: m/z calcd for $C_{53}H_{60}N_9O_8P$ $[M + Na]^+$ 1004.4195, found 1004.4192.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01498.

^{13}C NMR chemical shifts, ^{1}H−^{13}C coupling constants, HPLC profiles, melting profiles, and copies of ^{1}H, ^{13}C, ^{31}P, DEPT-135, and ^{1}H−^{13}C gated-decoupled NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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