Oligonucleotides Containing 7-Deaza-2′-deoxyinosine as Universal Nucleoside: Synthesis of 7-Halogenated and 7-Alkynylated Derivatives, Ambiguous Base Pairing, and Dye Functionalization by the Alkyne – Azide 'Click' Reaction

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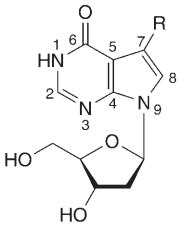
Oligonucleotides containing 7-deaza-2′-deoxyinosine derivatives bearing 7-halogen substituents or 7-alkynyl groups were prepared. For this, the phosphoramidites **2b**–**2g** containing 7-substituted 7-deaza-2′-deoxyinosine analogues **1b**–**1g** were synthesized (*Scheme 2*). Hybridization experiments with modified oligonucleotides demonstrate that all 2′-deoxyinosine derivatives show ambiguous base pairing, as 2′-deoxyinosine does. The duplex stability decreases in the order $C_d > A_d > T_d > G_d$ when **2b**–**2g** pair with these canonical nucleosides (*Table 6*). The self-complementary duplexes 5′-d(F^7c^7I-C)$_6$, d(Br^7c^7I-C)$_6$, and d(I^7c^7I-C)$_6$ are more stable than the parent duplex d(c^7I-C)$_6$ (*Table 7*). An oligonucleotide containing the octa-1,7-diyn-1-yl derivative **1g**, *i.e.*, **27**, was functionalized with the nonfluorescent 3-azido-7-hydroxycoumarin (**28**) by the *Huisgen*–*Sharpless*–*Meldal* cycloaddition 'click' reaction to afford the highly fluorescent oligonucleotide conjugate **29** (*Scheme 3*). Consequently, oligonucleotides incorporating the derivative **1g** bearing a terminal C≡C bond show a number of favorable properties: *i*) it is possible to activate them by labeling with reporter molecules employing the 'click' chemistry. *ii*) Space demanding residues introduced in the 7-position of the 7-deazapurine base does not interfere with duplex structure and stability (*Table 8*). *iii*) The ambiguous pairing character of the nucleobase makes them universal probes for numerous applications in oligonucleotide chemistry, molecular biology, and nanobiotechnology.

Introduction. – The naturally occurring ribonucleoside inosine is known to form wobble base pairs at ambiguous positions of the anticodon of tRNAs [1][2]. The corresponding 2′-deoxyinosine (I_d) is the classical universal nucleoside which shows ambiguous base pairing with the four natural components of DNA [1]. The unnatural nucleosides of 5-nitroindole [3a] and 3-nitropyrrole [3b] lacking the H-bonding capability stabilize the DNA equally well as an unusually linked 8-aza-7-deazaadenine nucleoside does [3c], thereby showing base-pairing ambiguity. Recently, the base-pairing properties of 7-deaza-2′-deoxyinosine (c^7I_d; **1a**) were investigated and found to be similar to those of 2′-deoxyinosine [3d]. Due to the absence of the 2-amino group, d(I·C) and d(c^7I·C) base pairs are less stable than the d(G·C) pair [3d][4][5]. Consequently, oligonucleotides containing I_d or the 7-deazapurine analogue **1a** exhibit lower T_m values when used in hybridization probes. Thus, it was considered to introduce substituents into the 7-deazahypoxanthine moiety which enhance base-pair stability and/or have the capability to be used as linkers for the functionalization of DNA.

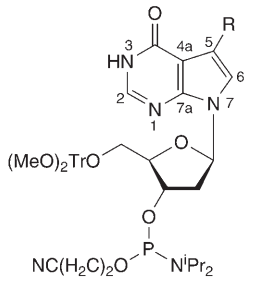
Earlier, our laboratory has shown that halogenated 7-deaza-2′-deoxyadenosine and 7-deaza-2′-deoxyguanosine derivatives bearing 7-substituents enhance the duplex stability compared to those incorporating canonical purine bases [6]. While 7-alkynyl groups show the same favorable properties, the side chains have the potential to be functionalized with reporter groups. A 3-aminoprop-2-yn-1-yl group can be derivatized by amide-bond formation, and side chains with terminal triple bonds can be linked to other molecular units by the copper(I)-catalyzed *Huisgen – Meldal – Sharpless* cycloaddition 'click' reaction [7 – 10]. This reaction is chemoselective, thereby forming 1,4-substituted triazole derivatives exclusively, and is bioorthogonal regarding other functional groups present in biomolecules. Thus, almost any molecule can be applied for this reaction. Furthermore, the 'click' chemistry performed in the absence of copper(I) catalyst is tolerated by living systems. A copper-free 'click' protocol was described by *Bertozzi* by using a fluorinated cyclooctynyl moiety instead of an octadiynyl chain [11].

Recently, we reported on the 'click' functionalization of four DNA constituents by using monomeric phosphoramidite building blocks with octadiynyl side chains introduced in the 5-position of pyrimidine bases or the 7-position of 7-deazapurines [12a – c]. Now we investigate the effect of 7-substituted 7-deaza-2′-deoxyinosines with halogen substituents (see **1b** – **1e**) or 7-alkynyl side chains (see **1f** and **1g**) on duplex stabilization and/or the functionalization with reporter groups. The 7-halogenated 7-deaza-2′-deoxyinosine derivatives **1b** – **1f** have already been described [13][14]. The nucleosides **1f** and **1g** with an alkynyl chain are now synthesized. The nucleosides are transformed to the phosphoramidites **2b** – **2g** which are employed in solid-phase oligonucleotide synthesis. The nucleoside **1g** as well as the oligonucleotide **27**, both bearing an octadiynyl side chain, are functionalized by the nonfluorescent dye 3-azido-7-hydroxycoumarin (= 3-azido-7-hydroxy-2*H*-1-benzopyran-2-one; **28**) yielding the fluorescent conjugates **30** and **29** by the alkyne – azide 'click' reaction (*Scheme 1*). The



purine numbering

1a – **1g**

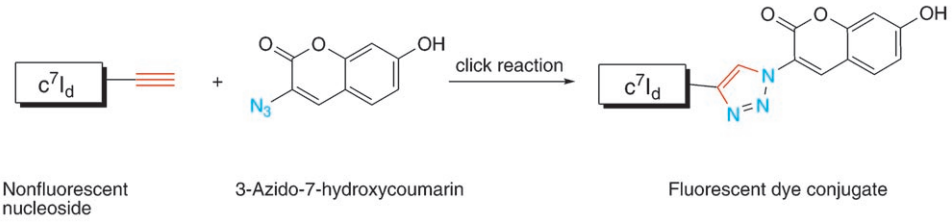


systematic numbering

2a – **2g**

a R = H
b R = F
c R = Cl
d R = Br
e R = I
f R = $F_3CCONHCH_2C{\equiv}C$
g R = $HC{\equiv}C(CH_2)_4C{\equiv}C$

Scheme 1



base pairing of **1b**–**1g** with the four canonical nucleosides is studied, and the photophysical properties of the dye-labeled oligonucleotides are investigated.

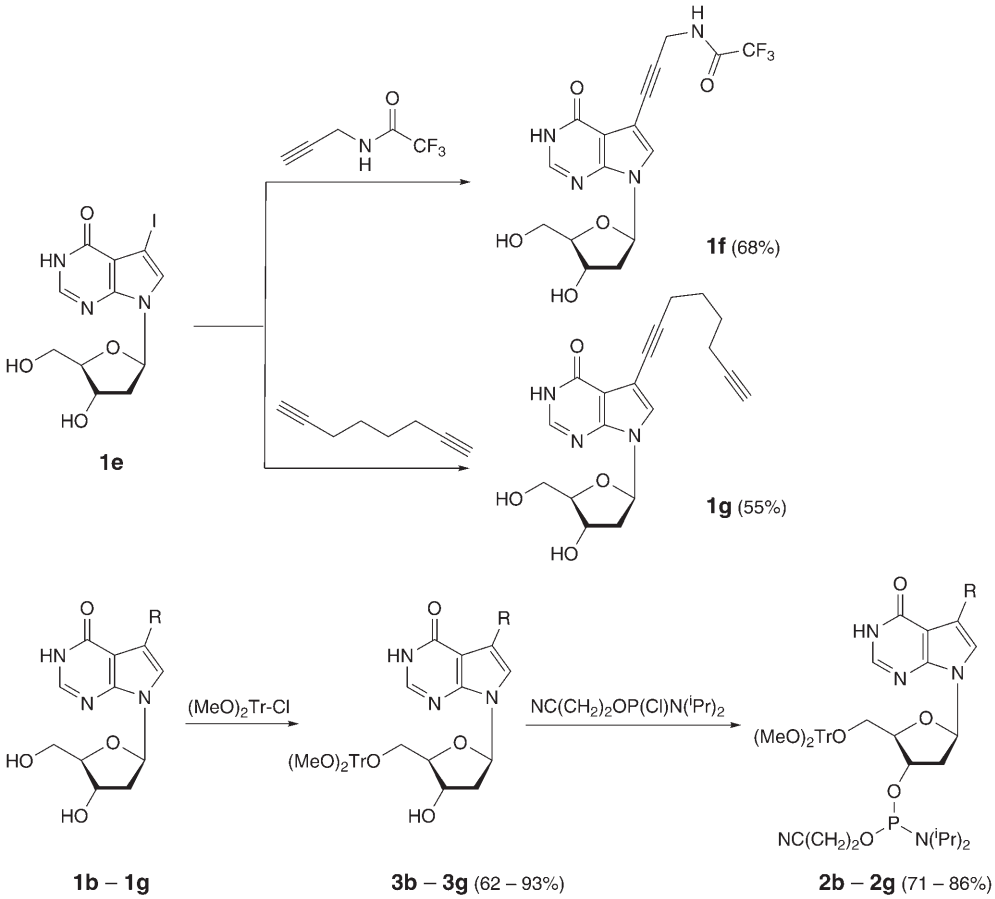
Results and Discussion. – *Monomers.* The nucleosides **1a**–**1g** were prepared according to literature protocols [13a,b]. The 7-[3-(2,2,2-trifluoroacetamido)prop-1-yn-1-yl] and 7-(octa-1,7-diyn-1-yl) derivatives **1f** and **1g** of 7-deaza-2′-deoxyinosine were synthesized from the 7-iodonucleoside **1e** with 2,2,2-trifluoro-*N*-(prop-2-yn-1-yl)acetamide or octa-1,7-diyne by the *Sonogashira* cross-coupling reaction. The reaction was performed in DMF and catalyzed by the [$(PPh_3)_4$Pd]/CuI catalyst (*Scheme 2*). Then, the nucleosides **1b**–**1g** were converted to the phosphoramidites **2b**–**2g** *via* the 5′-*O*-dimethoxytritylated derivatives **3b**–**3g** (70–93%) [15a]. All compounds were characterized by elemental analysis and spectroscopic means. *Table 1* summarizes the UV/VIS data of **1a**–**1g** measured in MeOH. Accordingly, alkynyl substituents induce a shift from *ca.* 263 nm to *ca.* 272 nm compared to the halogenated nucleosides.

In *Table 2*, the ^{13}C-NMR chemical shifts of 7-deaza-2′-deoxyinosine derivatives are compiled. The characteristic signal for the terminal C≡C H-atom of **1g** at δ(H) 2.76 reveals the formation of the 7-(octa-1,7-diyn-1-yl)nucleoside. The ^{13}C-NMR data of **1g** show the presence of eight C-atoms for the side chain, and the δ(C) of the terminal C≡C moiety can be clearly identified by the coupling pattern (^1J(C(8″),H–C(8″)) = 254.5 Hz) taken from the gated-decoupled ^{1}H,^{13}C-NMR spectra (*Table 3*). The successful incorporation of the side chain in the corresponding nucleoside is confirmed by the characteristic signals for the CH_2 group at δ(C) 27.4, 27.1, 18.4, and 17.3 (*Table 2*) showing inverted signals in the DEPT-135 spectra. For the heterocyclic part of the nucleosides, the introduction of an I-atom at C(7) of 7-deaza-2′-deoxyinosine results in an upfield shift of C(7) by *ca.* 47 ppm for compound **1e**, whereas the 7-alkynylated side chains induce a minor shift of only *ca.* 4 ppm for **1f** and *ca.* 3 ppm for **1g**. The upfield shift of C(7) increases in the order I > Br, and the introduction of an F or Cl substituent at C(7) leads to a downfield shift compared to the nonfunctionalized **1a**.

The graph of *Fig. 1* correlates the C(7) and C(8) chemical shifts of the halogenated 7-deaza-2′-deoxyinosines to the substituent electronegativity (values according to *Shoolery* and *Dailey* [16]). The δ(C) of C(7) is increasing with increasing electronegativity of the substituent, while that of C(8) is decreasing. In both cases, a linear relationship of δ(C) and the electronegativity is observed.

Next, the conformation of the sugar moiety of the 7-alkynyl-7-deaza-2′-deoxyinosines was studied in aqueous solution applying the program PSEUROT (version 6.3) [17]. A minimization of the differences between the experimental and calculated

Scheme 2



b R = F **e** R = I
c R = Cl **f** R = $F_3CCONHCH_2C{\equiv}C$
d R = Br **g** R = $HC{\equiv}C(CH_2)_4C{\equiv}C$

Table 1. *UV Data* (MeOH) *of 7-Substituted 7-Deaza-2′-deoxyinosine Derivatives*

	λ_{max} [nm]	ε		λ_{max} [nm]	ε
1a [15b]	258	9200	**1e** [13a]	265	13100
	280	6400		282	12000
1b [13b]	263	7400	**1f**	229	20000
	275	7200		269	13000
1c [13a]	263	9000	**1g**	233	21000
	282	7600 (sh)		272	11000
1d [13a]	264	10900			
	287	8700			

Table 2. *^{13}C-NMR Chemical Shifts of 7-Deaza-2′-deoxyinosine Nucleoside Derivatives*[a])

	C(2)[b]) C(2)[c])	C(4)[b]) C(7a)[c])	C(5)[b]) C(4a)[c])	C(6)[b]) C(4)[c])	C(7)[b]) C(5)[c])	C(8)[b]) C(6)[c])	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C≡C	CH_2
1a [13a]	143.7	147.4	108.4	158.2	102.5	120.7	83.2	40.2	71.0	87.4	62.0	–	
1b [13b]	144.8	143.1	97.3	156.3	144.9	103.4	82.6	[d])	70.8	87.4	61.8	–	
1c [13b]	144.9	146.2	104.8	156.9	106.3	117.8	82.9	[d])	70.7	87.5	61.7	–	
1d [13b]	145.3	146.8	105.9	157.7	90.2	120.1	83.0	[d])	70.7	87.5	61.7	–	
1e [13b]	144.6	147.2	108.0	157.7	55.4	125.6	83.2	[d])	70.9	87.6	61.8	–	
1f	145.2	146.9	107.6	157.4	98.4	125.8	83.2	[d])	70.9	87.6	61.7	76.3, 85.2	
1g	144.8	146.7	107.6	157.4	99.7	124.3	83.0	[d])	70.8	87.5	61.7		
3b	144.9	145.9	97.5	156.4	143.5	103.5	82.6	[d])	70.5	85.5	64.2	–	
3c	145.0	146.3	105.0	157.0	106.5	117.8	82.9	[d])	70.5	85.6	64.1	–	
3d	144.9	146.7	106.1	157.8	90.4	120.3	83.0	[d])	70.8	85.5	64.1	–	
3e	144.6	147.2	108.1	157.6	55.4	125.3	83.0	[d])	70.7	85.6	64.1	–	
3f	145.0	147.1	107.9	157.5	98.4	125.8	83.1	[d])	70.6	85.5	64.2	76.1, 85.2	29.7
3g	144.9	146.8	107.8	157.4	99.8	124.2	82.9	[d])	70.6	85.5	64.1		
30	144.8	146.9	107.6	157.4	99.7	124.3	83.0		70.8	87.5	61.7		

Side chain	C(1″)	C(2″)	C(3″)[e])	C(4″)[e])	C(5″)[e])	C(6″)[e])	C(7″)	C(8″)
1g	84.3	90.3	27.1	18.4	17.3	27.4	73.9	71.2
3g	84.3	90.3	27.1	18.4	17.2	27.4	73.7	71.2
30[f])	84.3	90.5	27.8	18.7	17.2	24.4	146.7	125.6

[a]) Measured in (D_6)DMSO. [b]) Purine numbering. [c]) Systematic numbering. [d]) Superimposed by (D_6)DMSO. [e]) Tentative. [f]) Data of the coumarin moiety of **30**: 162.3 (C(2)); 156.3 (C(7)); 154.6 (C(8a)); 136.0 (C(4)); 130.8 (C(5)); 114.2 (C(6)); 110.4 (C(4a)); 102.1 (C(8)).

Table 3. *Selected ^{13}C,^{1}H-Coupling Constants* [Hz] *of 7-Deaza-2′-deoxyinosine Derivatives*[a])[b])

^{13}C,^{1}H-Coupling constant	*J* [Hz]			^{13}C,^{1}H-Coupling constant	*J* [Hz]		
	1e	**1f**	**1g**		**1e**	**1f**	**1g**
^{1}J(C(2),H−C(2))	204.2	225.0	203.8	^{1}J(C(5′),H−C(5′))	139.5	138.9	139.2
^{1}J(C(8),H−C(8))	202.5	191.7	191.4	^{2}J(C(2″),H−C(3″))	–	9.45	–
^{3}J(C(5),H−C(8))	6.9	6.7	6.7	^{1}J(C(3″),H−C(3″)[c])	–	–	128.3
^{3}J(C(6),H−C(2))	5.4	6.6	5.6	^{1}J(C(4″),H−C(4″))	–	–	129.8
^{2}J(C(7),H−C(8))	4.9	5.7	5.8	^{1}J(C(5″),H−C(5″))	–	–	129.8
^{3}J(C(8),H−C(1′))	4.6	–	–	^{1}J(C(6″),H−C(6″)[c])	–	–	128.0
^{1}J(C(1′),H−C(1′))	164.1	163.9	166.4	^{2}J(C(7″),H−C(8″))	–	–	2.8
^{1}J(C(3′),H−C(3′))	149.3	148.5	148.7	^{1}J(C(8″),H−C(8″))	–	–	254.5
^{1}J(C(4′),H−C(4′))	145.6	145.5	146.8				

[a]) Measured in (D_6)DMSO. [b]) Purine numbering. [c]) Tentative.

coupling constants is accomplished by a nonlinear *Newton–Raphson* minimization, and the quality of the fit is expressed by the root-mean-square (r.m.s.) difference. This procedure presupposes the existence of a two-state *N*/*S* equilibrium. The input contained the following ^{1}H,^{1}H-coupling constants: ^{3}J(1′,2′), ^{3}J(1′,2″), ^{3}J(2′,3′), ^{3}J(2″,3′), and ^{3}J(3′,4′) (2′ = short form of H−C(2′), 2″ = short form of H′−C(2′)). During the interactions either the puckering parameters (*P* and *Ψ*) of the minor conformer (*N*) or the puckering amplitudes of both conformers were constrained. The coupling constants

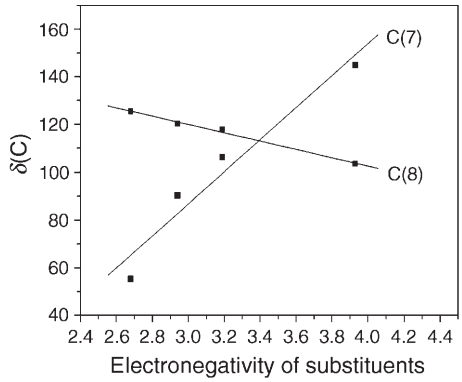


Fig. 1. *^{13}C-NMR Chemical shift of C(7) and C(8)* vs. *substituent electronegativity for the 7-halogenated 7-deaza-2′-deoxyinosines* **1b**–**1e**

and the result of the pseudorotation (*N*/*S*) are shown in *Table 4*. Thus, the $N \rightleftharpoons S$ equilibrium of the 7-unsubstituted 7-deaza-2′-deoxyinosine (**1a**) is biased towards the *S*-conformation, and the 7-halogen and 7-alkynyl substituents of **1b** and **1e**–**g** have only a minor influence on this equilibrium.

Table 4. *^{3}J(H,H) Coupling Constants of the Sugar Moiety and Conformer Population of Nucleosides* **1a**, **1b**, *and* **1e**–**1g**

Compound	^3J(H,H) [Hz][a])					Conformation	
	1′,2′	1′,2″	2′,3′	2″,3′	3′,4′	*N* [%]	*S* [%]
1a [13a]	7.15	6.60	6.40	3.40	3.50	31	69
1b [13b]	6.82	6.49	6.65	3.39	3.61	30	70
1e [13a]	6.90	6.60	6.40	3.70	3.60	34	66
1f	6.83	6.56	6.59	3.48	3.63	31	69
1g	6.91	6.90	6.59	3.34	3.59	29	71

[a]) 2′ = short form of H–C(2′), 2″ = short form of H′–C(2′).

Oligonucleotides. – Oligonucleotide Synthesis and Characterization. The standard oligonucleotides **4**–**7** and **22**–**25**, and the oligonucleotides **8**–**21**, **26**, and **27** containing compounds **1a**–**1g** were synthesized by solid-phase synthesis by means of the standard protocol of automatized DNA synthesis (*Applied Biosystems*, Weiterstadt, Germany) [18] (*cf. Table 5*). The coupling efficiency of the phosphoramidites **2a**–**2g** was always higher than 95%. Deprotection of the oligonucleotides was performed in 25% aqueous NH_3 solution for 16 h at 60°. The oligonucleotides were purified before and after detritylation by reversed-phase HPLC (for purification conditions, see *Exper. Part*). MALDI-TOF Mass spectra of the oligonucleotides were measured and found to be in agreement with the calculated data (*Table 5*). The composition of the oligonucleotides was determined by reversed-phase HPLC (*RP-18*) after tandem enzymatic hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase in 0.1M *Tris*·HCl buffer (pH 8.3) at 37°. *Fig. 2* shows the composition analyses of the oligonucleo-

Table 5. *Molecular Masses* ($[M+H]^+$) *of Oligonucleotides Containing 7-Deazapurine Nucleosides* c^7I_d (**1a**), $F^7c^7I_d$ (**1b**), $Cl^7c^7I_d$ (**1c**), $Br^7c^7I_d$ (**1d**), $I^7c^7I_d$ (**1e**), $pro^7c^7I_d$ (**1f**), *and* $oct^7c^7I_d$ (**1g**), *Measured by MALDI-TOF Mass Spectrometry*

	$[M+H]^+$ (calc.)	$[M+H]^+$ (found)
5′-d(CCTTTTCTTTTCC)-3′ (**4**)	3818	3818
5′-d(CCTTTTATTTTCC)-3′ (**5**)	3840	3840
5′-d(CCTTTTTTTTTCC)-3′ (**6**)	3833	3833
5′-d(CCTTTTGTTTTCC)-3′ (**7**)	3858	3858
5′-d(GGAAAA F^7c^7IAAAAGG)-3′ (**8**)	4092	4092
5′-d(GGAAAA Cl^7c^7IAAAAGG)-3′ (**9**)	4109	4110
5′-d(GGAAAA Br^7c^7IAAAAGG)-3′ (**10**)	4153	4154
5′-d(GGAAAA I^7c^7IAAAAGG)-3′ (**11**)	4200	4200
5′-d(GGAAAA pro^7c^7IAAAAGG)-3′ (**12**)	4127	4127
5′-d(GGAAAA oct^7c^7IAAAAGG)-3′ (**13**)	4177	4177
5′-d(c^7I-C)$_6$-3′ (**14**)	3553	3552
5′-d(F^7c^7I-C)$_6$-3′ (**15**)	3661	3660
5′-d(Br^7c^7I-C)$_6$-3′ (**16**)	4027	4025
5′-d(I^7c^7I-C)$_6$-3′ (**17**)	4309	4309
5′-d(TAG GTC A c^7IT ACT)-3′ (**18**)	3646	3646
5′-d(AGT ATT G c^7IC CTA)-3′ (**19**)	3646	3646
5′-d(TAG GTC A I^7c^7IT ACT)-3′ (**20**)	3772	3771
5′-d(AGT ATT G I^7c^7IC CTA)-3′ (**21**)	3772	3771
5′-d(AGT ATT GAC CTA)-3′ (**22**)	3646	3645
5′-d(AGT AAT GAC CTA)-3′ (**23**)	3655	3654
5′-d(AGT AGT GAC CTA)-3′ (**24**)	3671	3670
5′-d(AGT ACT GAC CTA)-3′ (**25**)	3631	3632
5′-d(AGT ATT G oct^7c^7IC CTA)-3′ (**26**)	3748	3748
5′-d(TAG GTC A oct^7c^7IT ACT)-3′ (**27**)	3748	3747
Oligonucleotide–dye conjugate **29**	3952	3951

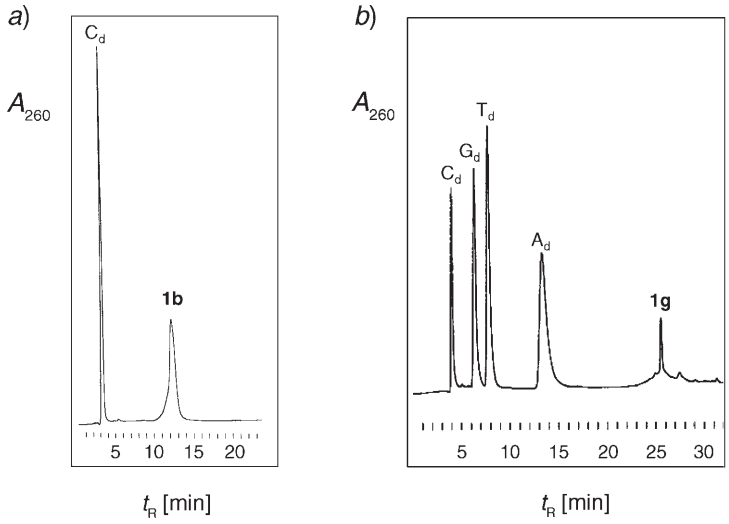


Fig. 2. *HPLC-Elution profile of enzymatic digests of the oligonucleotides* a) *5′-d(*F^7c^7I-C)$_6$*-3′* (**15**) *and* b) *5′-d(AGT ATT Goct*$^7c^7$*IC CTA)-3′* (**26**) *containing* $F^7c^7I_d$ (**1b**) *and* $oct^7c^7I_d$ (**1g**), *resp.* Column *RP-18* (250 × 10 mm); gradient: 0–15 min 100 % *B*, 15–40 min 0–50% *A* in *B*; *A* = MeCN, *B* = 0.1M AcO· Et_3NH buffer, pH 7.0.

tides **15** and **26** indicating the lipophilic character conferred by the 7-halogeno substituent (see **1b**) or the 7-alkynyl group (see **1g**).

Base Pairing and Duplex Stability of Oligonucleotides Incorporating 7-Deaza-2′-deoxyinosine Derivatives. Earlier, the pairing properties of 2′-deoxyinosine (I_d) and its 7-deazapurine derivative c^7I_d (**1a**) were investigated in our laboratory and by other authors [3d][6g][15a][19]. It was demonstrated that the 7-deazapurine nucleoside is an efficient substitute of I_d because of its similar ambiguous pairing properties accompanied with an extraordinary stability of its *N*-glycosylic bond [3d][4][6g]. Now, studies were undertaken in which the modified 7-deaza-2′-deoxyinosine derivatives **1b**–**1g** are embedded in the same environment to exclude nearest-neighbor effects [3d]. For this, we used a duplex described by *Ohtsuka* [4]. In the duplex 5′-d(GGAAAA**X**AAAAGG)-3′·3′-d(CCTTTT**Y**TTTTCC)-5′, $\mathbf{X_d}$ stands for the modified residues **1b**–**1g** as well as for 2′-deoxyinosine (I_d), and $\mathbf{Y_d}$ represents the four canonical bases C_d, A_d, T_d, and G_d. The T_m values of the corresponding duplex melting (*Table 6*) show a similar base-pairing ambiguity of all 7-substituted 7-deaza-2′-deoxyinosines **1b**–**1g** with C_d, A_d, G_d, and T_d compared to that of I_d or **1a**. The duplex stability decreases in the order $d(X^7c^7I\cdot C) > d(X^7c^7I\cdot A) > d(X^7c^7I\cdot T) > d(X^7c^7I\cdot G)$ (X^7c^7I = **1b**–**1g**). These findings are in line with earlier observations made with c^7I_d (**1a**) [3d]. A significant stabilization by the 7-modification is not observed, most likely due to a single replacement within the duplex. Nevertheless, even the 7-octadiynylated nucleoside **1g** with a space-demanding side chain shows an almost identical behavior as the unmodified 7-deaza-2′-deoxyinosine **1a**. Thus, all new 7-substituted 7-deaza-2′-deoxyinosine analogues are excellent candidates for universal base pairing. To demonstrate the influence of the 7-modification more clearly, we used the self-complementary 12-mer $d(I\text{-}C)_6$ and replaced all I_d residues by the modified nucleosides **1a**, **1b**, **1d**, or **1e**. From *Table 7*, it is apparent that the self-complementary dodecanucleotide $d(c^7I\text{-}C)_6$ (T_m 13°) forms a less stable duplex than $d(I\text{-}C)_6$ (T_m 19°). The self-complementary duplexes containing the 7-substituted 7-deaza-2′-deoxyinosine residues **1b**, **1d**, or **1e** show an increase of the duplex stability depending on the various substituents, compared to the incorporation of c^7I_d (**1a**) or I_d. Among them, the duplex

Table 6. T_m *Values* [°] *of Duplex Formation of 5′-d(GGAAAA**X**AAAAGG)-3′·3′-d(CCTTTT-**Y**TTTTCC)-5′*[a])

$\mathbf{X_d}$	$\mathbf{Y_d} = C_d$	$\mathbf{Y_d} = A_d$	$\mathbf{Y_d} = T_d$	$\mathbf{Y_d} = G_d$
I_d [3d]	47	44	38	37
c^7I_d (**1a**) [3d]	45	43	40	37
$F^7c^7I_d$ (**1b**)	46	43	39	35
$Cl^7c^7I_d$ (**1c**)	46	43	39	34
$Br^7c^7I_d$ (**1d**)	47	45	41	35
$I^7c^7I_d$ (**1e**)	46	43	38	36
$pro^7c^7I_d$ (**1f**)	48	45	38	35
$oct^7c^7I_d$ (**1g**)	46	44	38	36

[a]) Measured at 260 nm in 0.1M NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) with 5 μM + 5 μM single-strand concentrations.

Table 7. T_m *Values of Duplex Formation of Alternating Sequences*[a])

	T_m [°]		T_m [°]
d(I-C)$_6$·d(I-C)$_6$	19	d(Br^{7}c^{7}I-C)$_6$·d(Br^{7}c^{7}I-C)$_6$ (**16**·**16**)	33
d(c^{7}I-C)$_6$·d(c^{7}I-C)$_6$ (**14**·**14**)	13	d(I^{7}c^{7}I-C)$_6$·d(I^{7}c^{7}I-C)$_6$ (**17**·**17**)	27
d(F^{7}c^{7}I-C)$_6$·d(F^{7}c^{7}I-C)$_6$ (**15**·**15**)	28	d(A-T)$_6$·d(A-T)$_6^7$ [6]	33

[a]) Measured at 260 nm in 1.0M NaCl, 100 mM $MgCl_2$, and 100 mM Na-cacodylate (pH 7.0) with 5 μM single-strand concentration.

containing the Br derivative **1d** is the most stable one showing comparable stability (T_m 33°) as the dodecanucleotide duplex of d(A–T)$_6$.

Next, the effect of the groups neighboring the modification site on the base pairing was investigated. The duplex 5′-d(TAGGTCAATACT)-3′·3′-d(ATCCAGTTATGA)-5′ which is used in our laboratory to study the influence of the modified bases on the duplex stability was chosen for these experiments. One of the A_d residues of both oligomers was replaced by residue **1a**, **1e**, or **1g**. Thus, two new series of duplexes, 5′-d(TAGG**Y**CAATACT)-3′·3′-d(ATCC**X**GTTATGA)-5′ and 5′-d(TAGGTCA**X**-TACT)-3′·3′-d(ATCCAGT**Y**ATGA)-5′ were formed wherein $\mathbf{X_d}$ stands for **1a**, **1e**, and **1g**, and $\mathbf{Y_d}$ represents the four canonical nucleosides C_d, A_d, T_d, and G_d. In one series, the modified base pairs are positioned between d(G·C) pairs, while in the second the d(A·T) pairs are the nearest neighbours. The most striking observation in the experiments conducted with these two series is that the nearest neighbors (two d(A·T) *vs.* two d(G·C) base pairs) of the base pairs containing the modified 7-deaza-2′-deoxyinosine derivatives $\mathbf{X_d}$ have only very little influence on the duplex stability when they are paired with the pyrimidine nucleosides $\mathbf{Y_d} = C_d$ and T_d, while significant differences are observed when pairing occurs with $\mathbf{Y_d} = G_d$ or A_d (*Table 8*). Furthermore, the base pairs of the 7-functionalized 7-deaza-2′-deoxyinosines with C_d or A_d are almost as strong as a d(A·T) pair. These observations are in line with data

Table 8. T_m *Values* [°] *of Duplex Formation*

5′-d(TAGG**Y**CAATACT)-3′·3′-d(ATCC**X**GTTATGA)-5′[a])				
$\mathbf{X_d}$	$\mathbf{Y_d} = C_d$	$\mathbf{Y_d} = A_d$	$\mathbf{Y_d} = T_d$	$\mathbf{Y_d} = G_d$
A_d	n.m.	n.m.	46	n.m.
c^{7}I$_d$ (**1a**)	46	47	42	38
I^{7}c^{7}I$_d$ (**1e**)	45	48	41	37
oct^{7}c^{7}I$_d$ (**1g**)	44	46	39	35
5′-d(TAGGTCA**X**TACT)-3′·3′-d(ATCCAGT**Y**ATGA)-5′[a])				
$\mathbf{X_d}$	$\mathbf{Y_d} = C_d$	$\mathbf{Y_d} = A_d$	$\mathbf{Y_d} = T_d$	$\mathbf{Y_d} = G_d$
c^{7}I$_d$ (**1a**)	46	43	40	34
I^{7}c^{7}I$_d$ (**1e**)	47	45	42	34
oct^{7}c^{7}I$_d$ (**1g**)	45	43	40	34
dye^{7}c^{7}I$_d$ (**30**)	45	43	40	34

[a]) Measured at 260 nm in 1.0M NaCl, 100 mM $MgCl_2$, and 100 mM Na-cacodylate (pH 7.0) with 5 μM + 5 μM single-strand concentrations. N.m.: not measured.

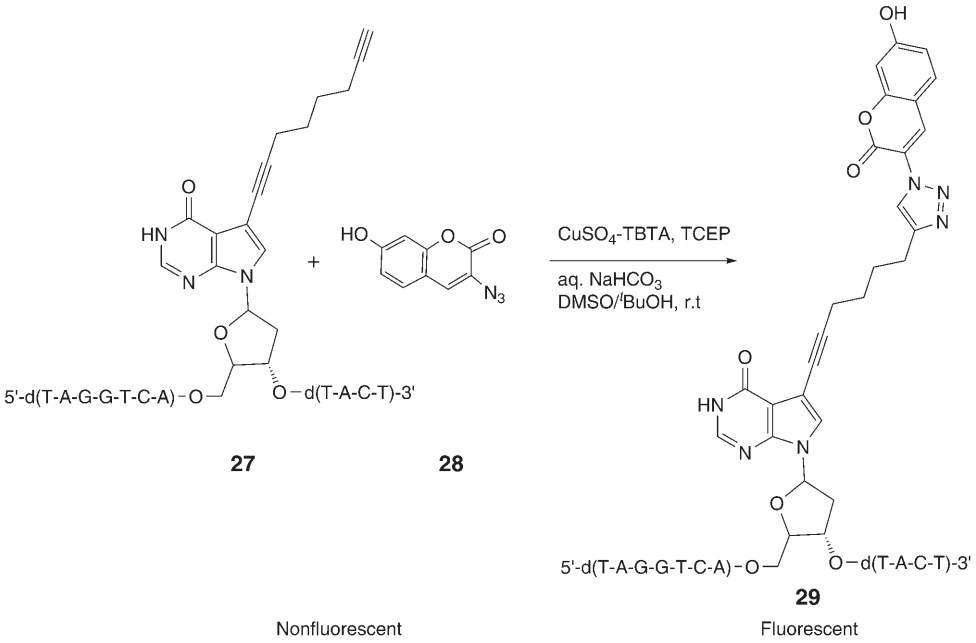
reported by *SantaLucia* and co-workers [20]. The trends indicate an interplay between H-bonding, nearest-neighbor stacking, and mismatch geometry on the observed stability of the 7-functionalized 7-deaza-2′-deoxyinosines.

Functionalization of the Terminal C≡C Bond of Oligonucleotides bearing an Octadiynyl Chain with 3-Azidocoumarin (*Huisgen–Sharpless–Meldal* [3+2] Cycloaddition 'Click' Reaction). The *Huisgen–Sharpless–Meldal* 'click' chemistry is known as a chemoselective bioorthogonal reaction between alkynes and organic azides [21]. Previous work has demonstrated the application in nucleic acids [7][9][22] for the construction of fluorescent oligonucleotides for DNA sequencing [8], for the intramolecular circularization and catenation of DNA [23], as well as for attaching oligonucleotide probes on monolayers [9]. Recently, our group reported on the functionalization of alkynyl-modified 2′-deoxycytidine, 2′-deoxyuridine, and 7-deaza-2′-deoxyguanosine, 7-deaza-2′-deoxyadenosine by means of a 'click' reaction with the nonfluorescent 3-azido-7-hydroxycoumarin (**28**) to produce highly fluorescent oligonucleotides [12a][12b]. Here, we report on the use of oligonucleotides containing the octa-1,7-diynyl nucleoside **1g** with a terminal C≡C bond for further functionalization with the nonfluorescent azidocoumarin **28**. The protocol consists of a 1,3-dipolar [3+2] cycloaddition of an alkyne moiety and the azido group of a 3-azidocoumarin [24] to generate a fluorescent (1,2,3-triazolyl)-substituted oligonucleotide. This type of reaction has already been used for the preparation of fluorogenic probes by *Zhou* and *Fahrni* [25] and also for the fluorescence visualization of synthesized mammalian proteins [26].

The 'click' reaction was performed on the 7-(octa-1,7-diyn-1-yl)-substituted nucleoside residue **1g** within oligonucleotide **27** with nonfluorescent 3-azidocoumarin **28** in aqueous solution (H_2O/$NaHCO_3$/^{t}BuOH/DMSO) in the presence of a 1:1 complex of $CuSO_4$ and TBTA (tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine) [27] and of TCEP (tris(2-carboxyethyl)phosphine = 3,3′,3″-phosphinidynetris[propanoic acid]), as H_2O-soluble reducing agent (*Scheme 3*). The conversion was complete after 14 h at room temperature when aqueous $NaHCO_3$ solution and an excess of **28** was used. The obtained strongly fluoresent oligonucleotide–dye conjugate **29** was purified by reversed-phase HPLC.

The UV/VIS absorption spectrum of the oligonucleotide–dye conjugate **29** (*Fig. 3*,*b*) shows two maxima, one (at 261 nm) resulting from the oligonucleotide bases, while the other (at 346 nm) arises from the dye conjugate. The corresponding UV/VIS spectrum of the oligonucleotide **27** bearing the octadiynyl side chain but lacking the dye shows only the 261 nm absorption (*Fig. 3*,*a*). Next, the fluorescence spectra of the single-stranded oligonucleotide **29** were measured (*Fig. 4*). A strong fluorescence is observed with an emission at 479 nm when **29** is irradiated at 346 or 399 nm. The fluorescence excitation maxima depend strongly on the pH value of the solution: At pH 7.0, the excitation maximum is at 346 nm with an emission at 479 nm (*Stoke* shift of 133 nm) (*Fig. 4*,*a*), and at pH 8.0 and 9.0, the excitation maximum is shifted to 399 nm, while the emission maximum remains unchanged at 479 nm (*Stoke* shift of 80 nm) (*Figs. 4*,*b* and *c*). The fluorescence intensity increases significantly with increasing pH values (*Fig. 4*,*d*). The two different excitation wavelengths (346 and 399 nm) of the coumarin residue result from the presence of the neutral dye or of its monoanion [28]. During DNA-duplex formation of **29** (→ **22**·**29**, **23**·**29**, **24**·**29**, and **25**·**29**), the fluorescence decreases significantly (*ca.* 40%), which is similar to the

Scheme 3



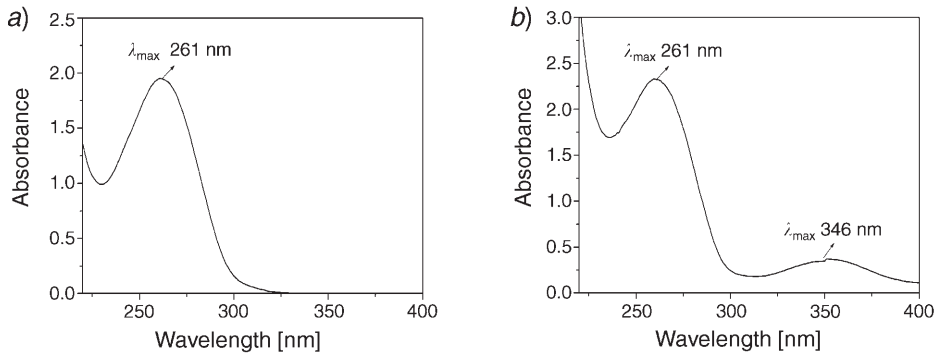


Fig. 3. a) *UV/VIS Spectra of oligonucleotide* **27** *in* H_2O *at pH 7.0 at room temperature,* b) *oligonucleotide* **29** *in* H_2O *at pH 7.0 at room temperature*

situation of oligonucleotides containing 7-deazaguanosine [12a] (*Fig. 5*). Regarding the duplex stability, the space-demanding coumarin conjugate (see, *e.g.*, **25** · **29** in *Fig. 6*) does not affect it as indicated by the unchanged T_m value (*Table 8*). This establishes that the spacious dye is not interfering with the duplex, *i.e.*, no interaction by intercalation or by ionic effects with the phosphodiester backbone occurs. This demonstrates the applicability of the dye-modified nucleoside dye^{7}c^{7}I$_d$ (**30**) for labeling experiments.

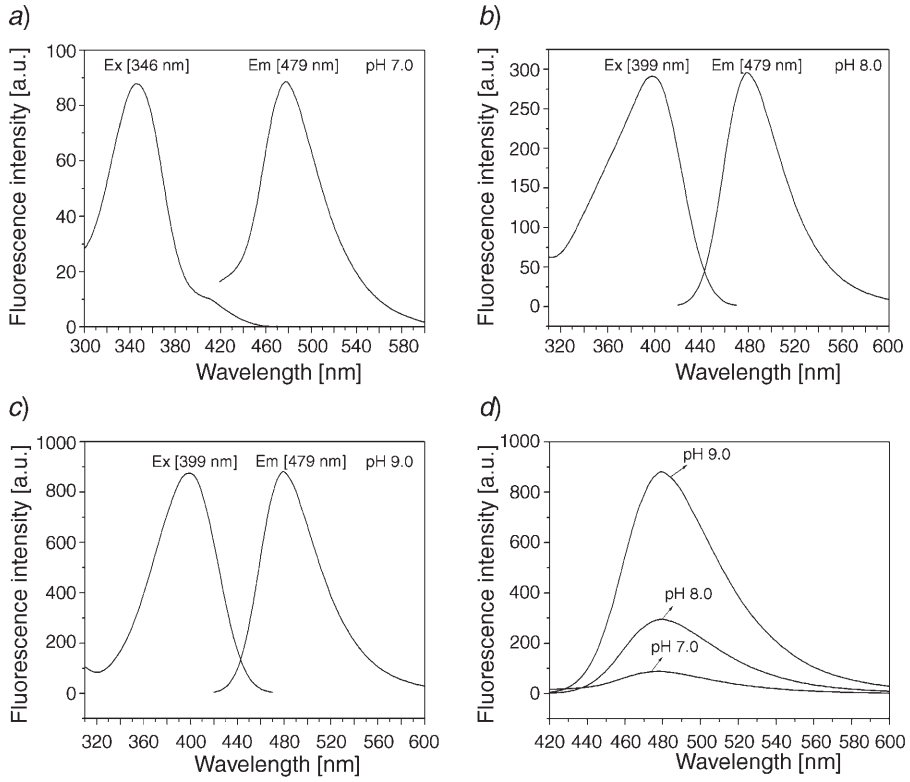


Fig. 4. a–c) *The emission and excitation spectra of oligonucleotide* **29** *at room temperature in* Tris · *HCl buffer of different pH.* d) *The influence of pH on the fluorescence intensity of* **29** *at room temperature in* Tris · *HCl buffer of different pH*

It has been reported that the fluorescence of some dyes is quenched by the interaction between the dye and the nucleobases. The quenching of 3-azido-7-hydroxycoumarin dyes by nucleobase derivatives in aqueous solution is caused by the photoinduced electron transfer from the nucleobase to the dye, which depends on the oxidation- and reduction-potential values of the nucleobases and the dye molecule [29]. Our laboratory reported that the fluorescence of 7-deazaguanosine and 7-deazaadenosine is quenched by this phenomenon, while that of the pyrimidine nucleosides show only minor effects [13a]. To verify the quenching behavior of the coumarin dye attached to a 7-deaza-2′-deoxyinosine moiety in single-stranded DNA, the fluorescence-quenching properties of the click-functionalized oligonucleotide **29** were studied by enzymatic hydrolysis. Oligomer **29** containing the 7-deaza-2′-deoxyinosine conjugate $dye^7c^7I_d$ (**30**) was digested with snake venom phosphodiesterase and alkaline phosphatase, and the fluorescence spectra ($\lambda_{excitation}$ 399 nm; $\lambda_{emission}$ 479 nm) were recorded within 8 h of cleavage. We observed a decrease in fluorescence intensity after addition of enzyme up to 80 min; afterwards, the fluorescence remained constant (*Fig. 7*). The HPLC profile of enzymatically digested oligonucleotide **29**

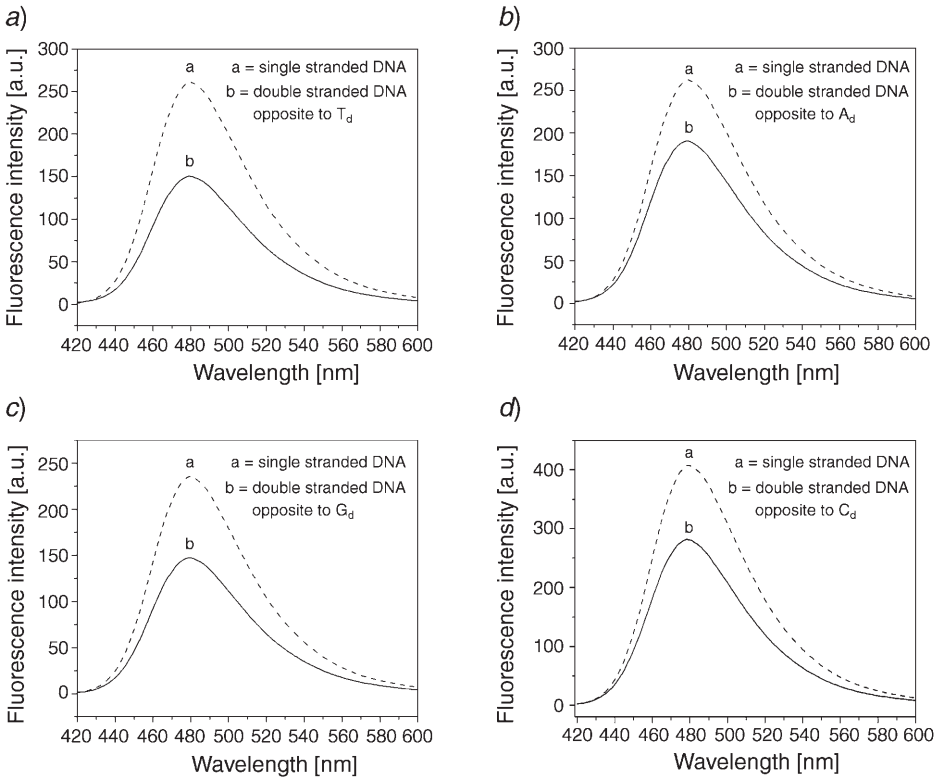


Fig. 5. *Fluorescence intensity changes on DNA-duplex formation from single strands and* **29***:* a) **22** · **29**, b) **23** · **29**, c) **24** · **29**, *and* d) **25** · **29**

clearly indicated the formation of the modified and unmodified nucleosides; a complete digestion pattern was observed after 80 min. To identify the enzymatic digestion product **30**, the 7-(octa-1,7-diyn-1-yl)nucleoside **1g** was conjugated with coumarin **28** in the presence of $CuSO_4$ and sodium ascorbate to form the strongly fluorescent 1,4-disubstituted 1,2,3-1*H*-triazole product dye^{7}c^{7}I$_d$ (**30**) in 85% yield (*Scheme 4*). Conjugate **30** was used to identify the unchanged monomeric dye conjugate in the enzymatic digest by HPLC co-injection. Normally, a fluorescence increase is observed when an oligonucleotide is cleaved to the monomeric components. The reason for the fluorescence decrease after phosphodiester hydrolysis of **29** is most likely due to an intramolecular photoinduced electron transfer from the nucleobase to the coumarin-dye moiety attached to it, which quenches the fluorescence when these moieties are in close proximity [12a]. The transfer occurs within the monomeric conjugate **30**, while it is not occurring when the nucleobase is part of the π-stack of the oligonucleotide chain of **29**.

Conclusions. – From T_m measurements of oligonucleotide duplexes containing the 7-substituted 7-deazainosine analogues **1b**–**1g** bearing halogen substituents or alkynyl

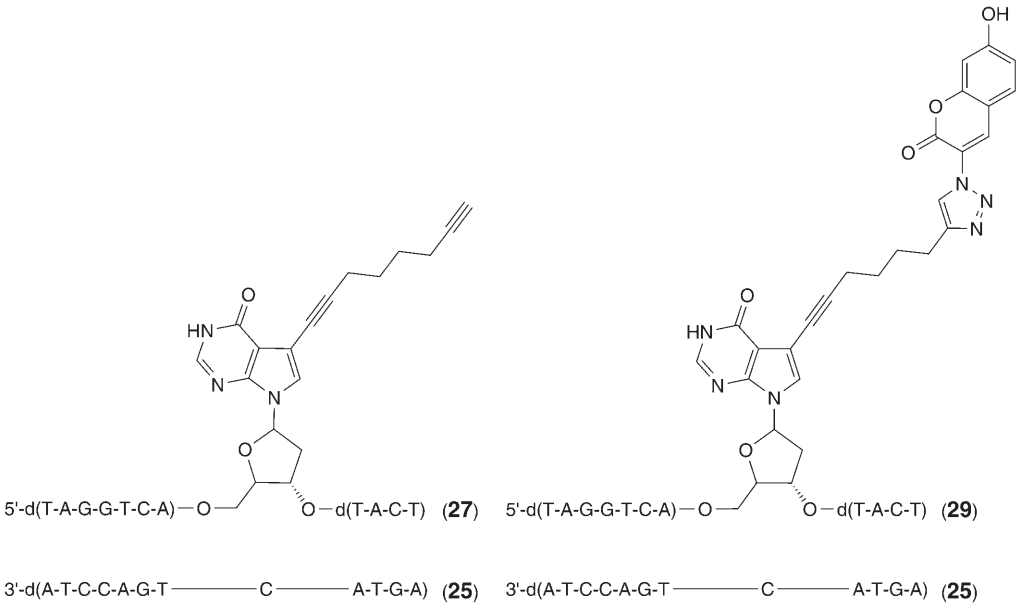


Fig. 6. a) *DNA Duplex* **25**·**27** *containing the 7-deaza-2′-deoxy-7-(octa-1,7-diyn-1-yl-)inosine* (**1g**); b) *DNA duplex* **25**·**29** *containing the 7-deaza-7-(octa-1,7-diyn-1-yl)-2′-deoxyinosine-coumarin conjugate* **30**

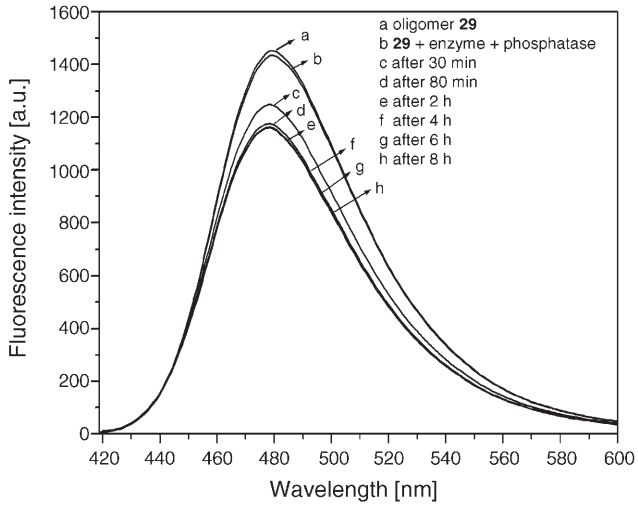
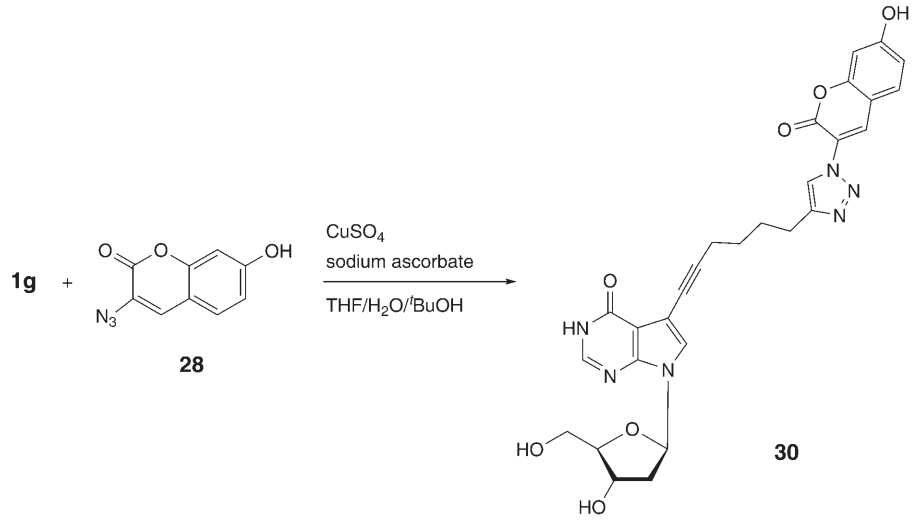


Fig. 7. *Fluorescence change of 0.3* μM *oligonucleotide* **29** *on enzymatic digestion with snake-venom phosphodiesterase followed by alkaline phosphatase in 0.1*M Tris · *HCl buffer* (pH 8.3) *at 37*°

groups at the 7-position of the pyrrolo[2,3-*d*]pyrimidine base, it is obvious that base pairing with the four canonical 2′-deoxynucleosides shows the same paring ambiguity as 7-deaza-2′-deoxyinosine (**1a**) or 2′-deoxyinosine. Self-complementary duplexes such as

Scheme 4



5′-d(F^7c^7I-C)$_6$-3′, 5′-d(Br^7c^7I-C)$_6$-3′, and 5′-d(I^7c^7I-C)$_6$-3′ are more stable than the parent duplex 5′-d(c^7I-C)$_6$-3′. Oligonucleotides containing the octa-1,7-diyn-1-yl derivative **1g** can be functionalized in a chemoselective way at the terminal C≡C bond of the side chain by employing the orthogonal 'click' chemistry. The conjugation of DNA by the nonfluorescent 3-azidocoumarin **28** yields a highly fluorescent conjugate **29**. Due to the ambiguous pairing character of the nucleobase and the 'clickable' side chain, almost any reporter molecule can be linked to single-stranded or duplex DNA (*Fig. 8*). According to the observation that purine 2′-deoxyribonucleosides are degraded in acidic medium while 7-deazapurine compounds are stable, the latter can be used in footprinting experiments. The reported phosphoramidites **2b**–**2g** are applicable in solid-phase synthesis of oligonucleotides, while the corresponding triphosphates can be incorporated in DNA by enzymatic polymerization. Thus, the new building blocks are expected to find broad application in nucleic acid chemistry, molecular biology, and nanotechnology.

We thank Dr. *Simone Budow* for the measurement of the NMR spectra and Dipl.-Chem. *Nhât Quang Tran* for the oligonucleotide synthesis. We also thank Dr. *Peter Leonard* and Dr. *Budow* for useful discussions. Financial support from the *Roche Diagnostics GmbH* and *ChemBiotech*, Münster, Germany, is gratefully acknowledged.

Experimental Part

General. Melting curves: *Cary 100-Bio*-UV/VIS spectrophotometer (*Varian*, Australia) equipped with a *Cary* thermoelectrical controller. Calculation of thermodynamic data: program MeltWin (version 3.0) by using the curve fitting of the melting profiles according to a two-state model. The following extinction coefficients (ε_{260}) were used: A_d 15400, G_d 11700, T_d 9200, and C_d 7500. TLC: aluminium sheets covered with silica gel (SiO_2) *60 F_{254}* (0.2 mm; *VWR International*, Darmstadt, Germany). Flash column chromatography (FC): SiO_2 *60* (*VWR International*, Darmstadt, Germany) at 0.4 bar. UV Spectra: *U-*

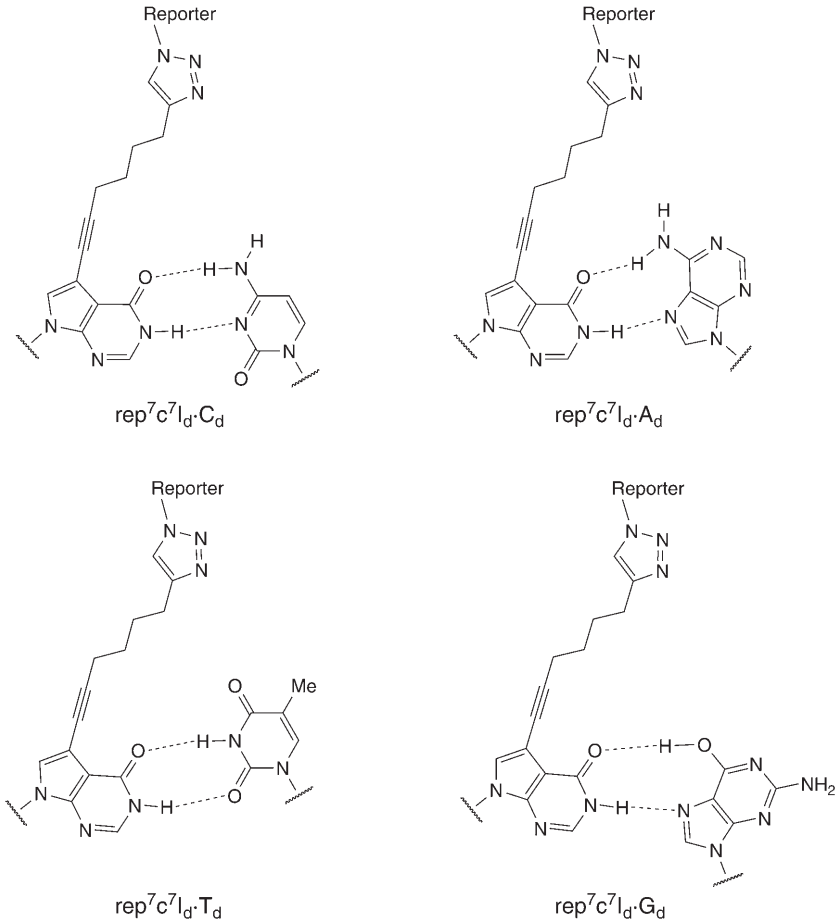


Fig. 8. *7-Deaza-2′-deoxyinosine reporter conjugates showing ambiguous pairing properties with the canonical DNA constituents*

3200 spectrophotometer (*Hitachi*, Japan). NMR Spectra: *Avance-250* or *AMX-500* spectrometers (*Bruker*, Rheinstetten, Germany); chemical shifts δ in ppm rel. to internal Me_4Si or external H_3PO_4 (^{31}P) and *J* values in Hz. Elemental analyses were performed by the *Mikroanalytisches Laboratorium Beller*, Göttingen, Germany. MALDI-TOF-MS: *Biflex-III* spectrometer (*Bruker Saxonia-Analytik GmbH*, Leipzig, Germany).

Oligonucleotide Synthesis. The oligonucleotides were prepared on a 1 μmol scale (trityl-on mode) on an *ABI-392-08* synthesizer employing phosphoramidite chemistry. The phosphoramidites **2a**–**2g** as well as the standard phosphoramidites were employed in solid-phase synthesis. The average coupling yield of the modified building blocks was always higher than 95%. After cleavage from the solid support, the oligonucleotides were deprotected in 25% aq. NH_3 soln. for 16 h at 60°. The $(MeO)_2$Tr-containing oligonucleotides were purified by reversed-phase HPLC (*RP-18*, *Lichrospher*, 5 μM, *VWR*) with the following solvent gradient system (A = 0.1M AcO · $NHEt_3$ (pH 7.0)/MeCN 95 : 5, B = MeCN): 0–3 min 10–15% B in A, 3–15 min 15–50% B in A, and 15–20 min 50–10% B in A, flow rate 1.0 ml/min. Then, the mixture was concentrated, and the oily residue was treated with 2.5% $Cl_2CHCOOH/CH_2Cl_2$ for

4 min at r.t. to remove the $(MeO)_2Tr$ residues. The detritylated oligonucleotides were purified by reversed-phase HPLC with the following gradient: 0–25 min 0–20% *B* in *A*, 25–30 min 20% *B* in *A*, and 30–35 min 20–0% *B* in *A*, flow rate 1.0 ml/min. Then, they were desalted on a short column (*RP-18*, *Lichrospher*, 5 μm, 125 × 5 mm, *VWR*; H_2O for salt elution, MeOH/H_2O 3:2 for elution of the pure oligonucleotides). Lyophilization on a *Speed-Vac* evaporator yielded colorless solids, which were stored frozen at −18°.

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-3,7-dihydro-5-{3-[(2,2,2-(trifluoroacetyl)amino]prop-1-yn-1-yl}-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**1f**). A soln. of **1e** [24] (450 mg, 1.2 mmol) in DMF was treated with $[(PPh_3)_4Pd]$ (152 mg, 0.13 mmol) and CuI (60 mg, 0.32 mmol). Et_3N (0.33 ml, 2.4 mmol) was added, followed by 2,2,2-trifluoro-*N*-(prop-2-yn-1-yl)acetamide (2.05 g, 13.5 mmol). The soln. was stirred at r.t. under N_2 for 18 h. SiO_2 (20 g) was added, and the solvent was evaporated; then the material was subjected to FC (SiO_2, column 10 × 2.5 cm, CH_2Cl_2/MeOH 9:1): **1f** (326 mg, 68%). White solid. R_f (CH_2Cl_2/MeOH 6:1) 0.41. UV (MeOH): 269 (13000), 229 (20000). ^{1}H-NMR ((D$_6$)DMSO): 12.15 (*s*, NH); 10.12 (*s*, NH); 7.95 (*s*, H–C(2)); 7.70 (*s*, H–C(6)); 6.44 (*q*, *J* = 5.12, 4.12, H–C(1′)); 5.32 (*d*, *J* = 3.2, OH–C(3′)); 5.01 (*dd*, *J* = 4.40, 4.24, OH–C(5′)); 4.33 (*m*, H–C(3′)); 4.26 (*m*, NCH_2); 3.82 (*m*, H–C(4′)); 3.53 (*m*, 2 H–C(5′)); 2.43 (*m*, H_β–C(2′)); 2.20 (*m*, H_α–C(2′)). Anal. calc. for $C_{16}H_{15}F_3N_4O_5$ (400.31): C 48.01, H 3.78, N 14.00; found: C 47.93, H 3.69, N 13.89.

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-3,7-dihydro-5-(octa-1,7-diyn-1-yl)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**1g**). As described for **1f**, with **1e** [24] (663 mg, 1.76 mmol), DMF (10 ml), $[(PPh_3)_4Pd]$ (206 mg, 0,178 mmol), CuI (72 mg, 0.38 mmol), Et_3N (0.45 ml, 6.13 mmol), and octa-1,7-diyne (2.5 ml, 18.84 mmol) for 24 h: **1g** (344 mg, 55%). White solid. R_f (CH_2Cl_2/MeOH 6:1) 0.41. UV (MeOH): 348 (18200). ^{1}H-NMR ((D$_6$)DMSO): 12.01 (*s*, NH); 7.91 (*s*, H–C(2)); 7.62 (*s*, H–C(6)); 6.44 (*dd*, *J* = 7.1, 6.7, H–C(1′)); 5.26 (*d*, *J* = 4.0, OH–C(3′)); 4.94 (*dd*, *J* = 5.2, 5.4, OH–C(5′)); 4.32 (*m*, H–C(3′)); 3.81 (*m*, H–C(4′)); 3.53 (*m*, 2 H–C(5′)); 2.76 (*m*, ≡CH); 2.41 (*m*, CH_2, H_β–C(2′)); 2.20 (*m*, CH_2, H_α–C(2′)); 1.61 (*s*, 2 CH_2). Anal. calc. for $C_{19}H_{21}N_3O_4$ (355.39): C 64.21, H 5.96, N 11.82; found: C 64.33, H 5.96, N 11.72.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-5-fluoro-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**3b**). To a soln. of 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-fluoro-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (**1b**) [25] (340 mg, 1.27 mmol; dried by repeated co-evaporation with dry pyridine (3 × 10 ml)) in dry pyridine (20 ml), 4,4′-dimethoxytriphenylmethyl chloride ($(MeO)_2Tr$–Cl; 942 mg, 2.78 mmol) was added at r.t., and the soln. was stirred for 3 h. Then, MeOH (2 ml) was added, and the mixture was stirred for additional 5 min. The soln. was poured into 5% aq. $NaHCO_3$ soln. (15 ml) and extracted with CH_2Cl_2 (3 × 10 ml). The org. layer was dried (Na_2SO_4) and concentrated, and the residue was submitted to FC (SiO_2, column 10 × 2.5 cm, CH_2Cl_2/acetone 1:1): **3b** (566 mg, 78%). Colorless foam. R_f (CH_2Cl_2/acetone 1:1) 0.34. UV (MeOH): 276 (15000), 282 (14900). ^{1}H-NMR ((D$_6$)DMSO): 12.16 (*s*, NH); 7.94 (*s*, H–C(2)); 7.37–6.83 (*m*, 14 arom. H, H–C(6)); 6.53 (*dd*, *J* = 5.16, 4.92, H–C(1′)); 5.38 (*d*, *J* = 3.64, OH–C(3′)); 4.33 (*m*, H–C(3′)); 3.91 (*m*, H–C(4′)); 3.73 (*s*, 2 MeO); 3.14 (*m*, 2 H–C(5′)); 2.47 (*m*, H_α–C(2′)); 2.27 (*m*, H_β–C(2′)). Anal. calc. for $C_{32}H_{30}FN_3O_6$ (571.60): C 67.24, H 5.29, N 7.35; found: C 67.35, H 5.40, N 7.53.

*5-Chloro-7-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**3c**). As described for **3b**, with **1c** (220 mg, 0.77 mmol), pyridine (10 ml), and $(MeO)_2Tr$–Cl (450 mg, 1.33 mmol): **3c** (419 mg, 93%). Colorless foam. R_f (CH_2Cl_2/acetone 1:1) 0.43. UV (MeOH): 263 (12000), 274 (11700). ^{1}H-NMR ((D$_6$)DMSO): 12.15 (*s*, NH); 7.95 (*s*, H–C(2)); 7.38–6.47 (*m*, 14 arom. H, H–C(6)); 6.48 (*d*, *J* = 5.28, H–C(1′)); 5.35 (*d*, *J* = 3.64, OH–C(3′)); 4.35 (*m*, H–C(3′)); 3.93 (*m*, H–C(4′)); 3.74 (*s*, 2 MeO); 3.12 (*m*, 2 H–C(5′)); 2.52 (*m*, H_α–C(2′)); 2.27 (*m*, H_β–C(2′)). Anal. calc. for $C_{32}H_{30}ClN_3O_6$ (588.05): C 65.36, H 5.14, N 7.15; found: C 65.30, H 5.30, N 7.29.

*5-Bromo-7-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**3d**). As described for **3b**, with **1d** (230 mg, 0.70 mmol), pyridine (10 ml), and $(MeO)_2Tr$–Cl (355 mg, 1.05 mmol): **3d** (310 mg, 70%). Colorless foam. R_f (CH_2Cl_2/acetone 1:1) 0.50. UV (MeOH): 264 (12000), 274 (12000). ^{1}H-NMR ((D$_6$)DMSO): 12.15 (*s*, NH); 7.95 (*s*, H–C(2)); 7.39–6.83 (*m*, 14 arom. H, H–C(6)); 6.47 (*dd*, *J* = 6.55, 6.43, H–C(1′)); 5.35 (*d*, *J* = 4.40, OH–C(3′)); 4.35 (*m*, H–C(3′)); 3.93 (*m*, H–C(4′)); 3.73 (*s*, 2 MeO); 3.15 (*m*, 2 H–C(5′)); 2.52 (*m*, H_α–C(2′)); 2.27 (*m*, H_β–C(2′)). Anal. calc. for $C_{32}H_{30}BrN_3O_6$ (632.50): C 60.77, H 4.78, N 6.64; found: C 60.79, H 4.87, N 6.75.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-5-iodo-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**3e**). As described for **3b**, with **1e** (360 mg, 0.96 mmol), pyridine (10 ml), and $(MeO)_2Tr-Cl$ (650 mg, 1.92 mmol): **3e** (590 mg, 91%). Colorless foam. R_f (CH_2Cl_2/acetone 1:1) 0.57. UV (MeOH): 228 (32500), 274 (11000). ^{1}H-NMR ((D$_6$)DMSO): 12.09 (*s*, NH); 7.94 (*s*, H–C(2)); 7.41–6.85 (*m*, 14 arom. H, H–C(6)); 6.45 (*m*, H–C(1′)); 5.35 (*m*, OH–C(3′)); 4.36 (*m*, H–C(3′)); 3.93 (*m*, H–C(4′)); 3.74 (*s*, 2 MeO); 3.18 (*m*, 2 H–C(5′)); 2.52 (*m*, H_α–C(2′)); 2.27 (*m*, H_β–C(2′)). Anal. calc. for $C_{32}H_{30}IN_3O_6$ (579.50): C 56.56, H 4.45, N 6.18; found: C 56.74, H 4.49, N 6.35.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-5-{3-[(2,2,2-trifluoroacetyl)amino]prop-1-yn-1-yl}-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**3f**). As described for **3b**, with **1f** (266 mg, 0.66 mmol), pyridine (10 ml), and $(MeO)_2Tr-Cl$ (270 mg, 0.80 mmol): **3f** (400 mg, 86%). Colorless foam. R_f (CH_2Cl_2/acetone 1:1) 0.57. UV (MeOH): 232 (36400), 272 (14400). ^{1}H-NMR ((D$_6$)DMSO): 12.36 (*s*, NH); 10.11 (*s*, NH); 7.94 (*s*, H–C(2)); 7.53 (*s*, H–C(6)); 7.36–6.81 (*m*, 13 arom. H); 6.44 (*dd*, *J* = 6.43, 6.35, H–C(1′)); 5.39 (*d*, *J* = 4.35, OH–C(3′)); 4.32 (*m*, H–C(3′)); 4.25 (*m*, NCH_2); 3.91 (*m*, H–C(4′)); 3.72 (*s*, 2 MeO); 3.10 (*m*, 2 H–C(5′)); 2.56 (*m*, H_α–C(2′)); 2.25 (*m*, H_β–C(2′)). Anal. calc. for $C_{37}H_{33}F_3N_4O_7$ (702.68): C 63.24, H 4.73, N 7.97; found: C 63.27, H 4.85, N 7.80.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-5-(octa-1,7-diyn-1-yl)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**3g**). As described for **3b**, with **1g** (383 mg, 1.08 mmol), pyridine (10 ml), and $(MeO)_2Tr-Cl$ (720 mg, 1.85 mmol): **3g** (526 mg, 74%). Colorless foam. R_f (CH_2Cl_2/acetone 1:1) 0.57. UV (MeOH): 233 (40200), 273 (13300). ^{1}H-NMR ((D$_6$)DMSO): 12.05 (*s*, NH); 7.91 (*s*, H–C(2)); 7.43 (*s*, H–C(6)); 7.36–6.83 (*m*, 13 arom. H); 6.46 (*dd*, *J* = 6.6, 6.5, H–C(1′)); 5.34 (*d*, *J* = 4.3, OH–C(3′)); 4.34 (*m*, H–C(3′)); 3.94 (*m*, H–C(4′); 3.73 (*s*, 2 MeO); 3.10 (*m*, 2 H–C(5′)); 2.76 (*t*, *J* = 2.6, ≡CH); 2.56 (*m*, H_β–C(2′)); 2.40 (*m*, CH_2); 2.29 (*m*, CH_2, H_α–C(2′)); 1.60 (*s*, 2 CH_2). Anal. calc. for $C_{40}H_{39}N_3O_6$ (657.75): C 73.04, H 5.98, N 6.39; found: C 72.92, H 5.85, N 6.42.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-5-fluoro-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 3′-(2-Cyanoethyl* N,N*-Diisopropylphosphoramidite)* (**2b**). To a soln. of **3b** (384 mg, 0.67 mmol) in anh. CH_2Cl_2 (10 ml), $Et(^iPr)_2N$ (291 µl, 1.70 mmol) and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (350 µl, 1.57 mmol) were added, and the mixture was stirred at r.t. for 30 min. Then 5% aq. $NaHCO_3$ soln. (15 ml) was added, and the mixture was extracted with CH_2Cl_2 (2 × 15 ml). The org. phase was dried (Na_2SO_4) and concentrated. The product was separated by FC (SiO_2, 2.5 × 10 cm, CH_2Cl_2/acetone 3:1): **2b** (380 mg, 73%). Colorless foam. R_f (CH_2Cl_2/acetone 3:1) 0.37. ^{31}P-NMR ($CDCl_3$): 150.0; 149.8.

*5-Chloro-7-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 3′-(2-Cyanoethyl* N,N*-Diisopropylphosphoramidite)* (**2c**). As described for **2b**, with **3c** (288 mg, 0.49 mmol), anh. CH_2Cl_2 (10 ml), $Et(^iPr)_2N$ (226 µl, 1.30 mmol), and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (290 µl, 1.30 mmol): **2c** (322 mg, 83%). Colorless foam. R_f (CH_2Cl_2/acetone 3:1) 0.44. ^{31}P-NMR ($CDCl_3$): 150.0; 149.9.

*5-Bromo-7-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 3′-(2-Cyanoethyl* N,N*-Diisopropylphosphoramidite)* (**2d**). As described for **2b**, with **3d** (200 mg, 0.32 mmol), anh. CH_2Cl_2 (10 ml), $Et(^iPr)_2N$ (108 µl, 0.63 mmol), and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (150 µl, 0.67 mmol): **2d** (221 mg, 83%). Colorless foam. R_f (CH_2Cl_2/acetone 3:1) 0.57. ^{31}P-NMR ($CDCl_3$): 150.0; 149.9.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-5-iodo-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 3′-(2-Cyanoethyl* N,N*-Diisopropylphosphoramidite)* (**2e**). As described for **2b**, with **3e** (360 mg, 0.53 mmol), anh. CH_2Cl_2 (20 ml), $Et(^iPr)_2N$ (253 µl, 1.48 mmol), and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (320 µl, 1.43 mmol): **2e** (380 mg, 82%). Colorless foam. R_f (CH_2Cl_2/acetone 3:1) 0.57. ^{31}P-NMR ($CDCl_3$): 150.0; 149.9.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-5-{3-[(2,2,2-trifluoroacetyl)amino]prop-1-yn-1-yl}-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 3′-(2-Cyanoethyl* N,N*-Diisopropylphosphoramidite)* (**2f**). As described for **2b**, with **3f** (306 mg, 0.44 mmol), anh. CH_2Cl_2 (10 ml), $Et(^iPr)_2N$ (150 µl, 0.86 mmol), and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (200 µl, 0.89 mmol): **2f** (342 mg, 87%). Colorless foam. R_f (CH_2Cl_2/acetone 3:1) 0.57. ^{31}P-NMR ($CDCl_3$): 150.0; 149.9.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-erythro-pentofuranosyl]-3,7-dihydro-5-(octa-1,7-diyn-1-yl)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 3′-(2-Cyanoethyl* N,N*-Diisopropylphosphoramidite*) (**2g**). As described for **2b**, with **3g** (334 mg, 0.51 mmol), anh. CH_2Cl_2 (10 ml), $Et(^iPr)_2N$ (210 µl, 1.25 mmol), and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite) (250 µl, 1.12 mmol): **2g** (310 mg, 71%). Colorless foam. R_f (CH_2Cl_2/acetone 3:1) 0.57. ^{31}P-NMR ($CDCl_3$): 150.0; 149.8.

*Dye Conjugate 7-(2-Deoxy-β-*D*-erythro-pentofuranosyl)-3,7-dihydro-5-[6-[1-(7-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)-1*H*-1,2,3-triazol-4-yl]hex-1-yn-1-yl]-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**30**). To a soln. of **1g** (80 mg, 0.23 mmol) and 3-azido-7-hydroxycoumarin (= 3-azido-7-hydroxy-2*H*-1-benzopyran-7-one; **28**, 56.9 mg, 0.28 mmol) in THF/H_2O/^{t}BuOH 3:1:1 (4 ml), a freshly prepared 1M soln. of sodium ascorbate (210 µl, 0.21 mmol) in H_2O was added, followed by $CuSO_4 \cdot 5\,H_2O$ (173 µl, 0.052 mmol of a 7.5% stock soln. in H_2O). The emulsion was stirred for 24 h at r.t., concentrated, and applied to FC (SiO_2, column 10 × 3 cm, CH_2Cl_2/MeOH 5:1): **30** (109.1 mg, 85%). Yellowish solid. R_f (SiO_2, CH_2Cl_2/MeOH 5:1) 0.36. UV (MeOH): 348 (18200). ^{1}H-NMR ((D$_6$)DMSO): 12.0 (*s*, NH); 8.55 (*s*, 1 arom. H); 8.34 (*s*, 1 arom. H); 7.89 (*s*, H−C(2)); 7.72 (*d*, *J* = 8.6, 1 arom. H); 7.55 (*s*, H−C(6)); 6.89 (*dd*, *J* = 8.5, 2.2, 1 arom. H); 6.84 (*d*, *J* = 2.1, 1 arom. H); 6.42 (*dd*, *J* = 7.0, 6.9, H−C(1′)); 5.25 (br., OH−C(3′)); 4.93 (br., OH−C(5′)); 4.31 (*m*, H−C(3′)); 3.81 (*m*, H−C(4′)); 3.53 (*m*, 2 H−C(5′)); 2.79 (*dd*, *J* = 7.4, 7.3, CH_2); 2.51–2.36 (*m*, 2 CH_2, H_β−C(2′)); 2.17 (*m*, H_α−C(2′)); 1.85 (*m*, CH_2,); 1.60 (*m*, CH_2). ESI-HR-MS: 581.16 ($[M + Na]^+$, $C_{28}H_{26}N_6NaO_7^+$; calc. 581.18).

Huisgen–Sharpless–Meldal [3 + 2] Cycloaddition of Oligonucleotide **27** *and the Nonfluorescence 3-Azidocoumarin* **28**. To the soln. of single-stranded oligonucleotide **27** (5 A_{260} units) in 20 mM aq. $NaHCO_3$ soln./^{t}BuOH/DMSO 1:1:3 (120 µl), $CuSO_4 \cdot$TBTA ligand complex (35 µl of a 20 mM stock soln. in ^{t}BuOH/H_2O 1:9), TCEP (35 µl of a 20 mM stock soln. in H_2O), and **28** (50 µl of a 20 mM stock soln. in dioxane/H_2O 1:1) were added, and the reaction was run at r.t. overnight. The 'click' product **29** was further purified by reversed-phase HPLC.

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