Nucleosides and Oligonucleotides with Diynyl Side Chains: Base Pairing and Functionalization of 2′-Deoxyuridine Derivatives by the Copper(I)-Catalyzed Alkyne–Azide 'Click' Cycloaddition

by **Frank Seela*** and **Venkata Ramana Sirivolu**

Laboratorium für Organische und Bioorganische Chemie, Universität Osnabrück, Barbarastrasse 7, D-49069 Osnabrück, and
Laboratory for Bioorganic Chemistry and Chemical Biology, Center for Nanotechnology, Heisenbergstrasse 11, D-48149 Münster
(phone: +49(0)541 969 2791; mobil: +49(0)173 725 0297; fax: +49(0)251 3540 6501; e-mail: Frank.Seela@uni-osnabrueck.de, Seela@uni-muenster.de; www.seela.net)

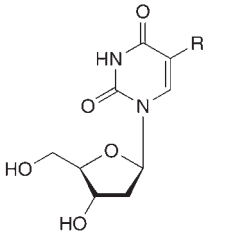
Dedicated to Professor *Wolfgang Pfleiderer*

Oligonucleotides containing the 5-substituted 2′-deoxyuridines **1b** or **1d** bearing side chains with terminal C≡C bonds are described, and their duplex stability is compared with oligonucleotides containing the 5-alkynyl compounds **1a** or **1c** with only one nonterminal C≡C bond in the side chain. For this, 5-iodo-2′-deoxyuridine (**3**) and diynes or alkynes were employed as starting materials in the *Sonogashira* cross-coupling reaction (*Scheme 1*). Phosphoramidites **2b**–**d** were prepared (*Scheme 3*) and used as building blocks in solid-phase synthesis. T_m Measurements demonstrated that DNA duplexes containing the octa-1,7-diynyl side chain or a diprop-2-ynyl ether residue, *i.e.*, containing **1b** or **1d**, are more stable than those containing only one triple bond, *i.e.*, **1a** or **1c** (*Table 3*). The diyne-modified nucleosides were employed in further functionalization reactions by using the protocol of the Cu^I-catalyzed *Huisgen–Meldal–Sharpless* [2+3] cycloaddition ('click chemistry') (*Scheme 2*). An aliphatic azide, *i. e.*, 3′-azido-3′-deoxythymidine (AZT; **4**), as well as the aromatic azido compound **5** were linked to the terminal alkyne group resulting in 1*H*-1,2,3-triazole-modified derivatives **6** and **7**, respectively (*Scheme 2*), of which **6** forms a stable duplex DNA (*Table 3*). The *Husigen–Meldal–Sharpless* cycloaddition was also performed with oligonucleotides (*Schemes 4* and *5*).

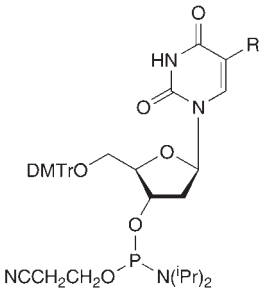
Introduction. – Modified oligonucleotides have the potential to widen the diagnostic application of synthetic DNA such as in primer probe interactions, DNA chip technology, or to enlarge the repertoire of therapeutic applications (antisense therapy) [1–4]. This led to the development of nucleic acid mimics containing a wide variety of modified bases [5–8], sugar residues [9], or altered phosphodiester backbones [10]. These structural modifications can influence hybridization strength, binding affinity, mismatch discrimination, nuclease resistance, or cellular uptake. Oligonucleotide–alkynyl conjugates are of particular importance because of their antiviral and anticancer activity [11–13]. They can also be used to link any kind of reporter groups [14] or duplex-stabilizing residues to DNA.

Among the various base modifications, the 5-position of pyrimidine nucleosides is an ideal place for introducing structural diversity in oligonucleotides [15–17]. This site has steric freedom as it lies in the major groove of the B-DNA duplex. Thus, substituents of moderate size can be introduced without perturbing the DNA structure. Recently, we have shown that halogen substituents present at the 5-position of

pyrimidines show very little influence or even no effect on the DNA duplex stability [18]. A significant stabilization is observed when alkynyl residues are introduced. A series of such nucleosides containing propynyl groups (*e.g.*, **1a**) have been synthesized and incorporated into duplex DNA [19–24]. It was found that an increase in the chain length from propynyl to hexynyl groups has an unfavorable influence on the base-pair stability most likely due to the increasing hydrophobic character of the alkynyl chain [17]. As the hydrophobic character of a side chain can dry out the grooves from H_2O molecules, an increasing hydrophilic character might have a favorable influence on the duplex structure. As triple bonds or O-functionalities can fulfill these requirements, we decided to use this principle for the functionalization of oligonucleotides carrying long flexible linker arms, such as those present in the nucleosides, **1b–d**.



1a R = MeC≡C
1b R = HC≡C(CH$_2$)$_4$C≡C
1c R = Me(CH$_2$)$_5$C≡C
1d R = HC≡CCH$_2$OCH$_2$C≡C



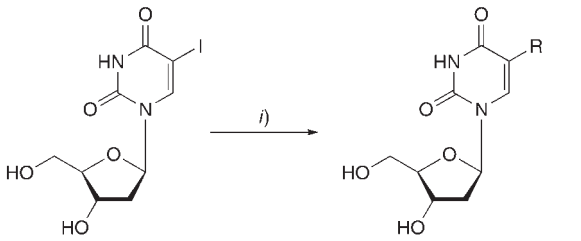
2a R = MeC≡C
2b R = HC≡C(CH$_2$)$_4$C≡C
2c R = Me(CH$_2$)$_5$C≡C
2d R = HC≡CCH$_2$OCH$_2$C≡C

We have already reported on the construction of polymeric networks by the functionalization of the 2′-deoxyuridine side chain by a terminal alkyne [25]. For this purpose the 5-octadiynyl derivative **1b** was prepared containing two C≡C bonds. This compound was employed in the *Sonogashira* cross-coupling reaction resulting in the formation of cross-linked 5,5′-bis(nucleosides). The side chains with such a terminal unsaturation could also be used for further functionalization by means of the Cu^I-catalyzed alkyne–azide coupling described independently by the *Meldal* [26] and *Sharpless* [27] groups ('click' reaction). Cu^I strongly catalyzes this coupling and improves the regioselectivity by giving the 1,4-disubstituted 1*H*-1,2,3-triazole regioisomer exclusively. So, the 'click' functionalization of oligonucleotides can be used to introduce almost any reporter group in nucleic acids.

Now, we performed the *Huisgen–Meldal–Sharpless* cycloaddition with nucleosides and oligonucleotides. In the case of oligonucleotides, the reaction was carried out in solution as well as on solid support. Details of the synthesis of the phosphoramidite building blocks **2b–d** are given, and the stability of duplexes containing such modifications was studied. After the appearance of a short communication [28] in which we described the functionalization and incorporation of four octa-1,7-diynyl nucleosides in oligonucleotides mimicking the canonical DNA constitutents, the functionalization of alkyne-modified DNA by means of one base modification was reported [29].

Results and Discussion. – 1. *Synthesis and Properties of Nucleosides.* The synthesis of nucleoside **1a** was already reported [23]. The nucleosides **1b**–**d** were synthesized from 5-iodo-2′-deoxyuridine (**3**) [30] by the *Sonogashira* cross-coupling reaction [31] (*Scheme 1*). The reaction was performed in dry DMF in the presence of Et_3N, of $[Pd^0(PPh_3)_4]$ and CuI as catalyst in a 1:2 ratio, and of an excess of the diyne or alkyne. As a result, only one terminal C≡C bond of the diyne was functionalized leading to the almost exclusive formation of the mono-substituted diynyl nucleosides **1b** and **1d** in 72 and 84% yield, respectively. The presence of traces of a fluorescent by-product was observed [32]. Its formation depended on the type of reactant used as well as on the reaction time. Reactions at the second C≡C bond occurred when compounds **1b** or **1d** were isolated and employed in a second cross-coupling reaction. The structure of the nucleosides was confirmed by the ^{1}H-, and ^{13}C-NMR spectra (*Table 1*) and by elemental analysis.

Scheme 1



3 **1b** R = HC≡C(CH$_2$)$_4$C≡C (72%)
c R = Me(CH$_2$)$_5$C≡C (78%)
d R = HC≡CCH$_2$OCH$_2$C≡C (84%)

i) $[Pd^0(PPh_3)_4]$, CuI, Et_3N, DMF, octa-1,7-diyne (→**1b**), oct-1-yne (→**1c**), or diprop-2-ynyl ether (→**1d**).

The ^{13}C-NMR chemical shifts were assigned by means of gated-decoupled spectra. The terminal alkynyl C-atom of the side chain of **1b** showed a C,H coupling (^1J(C,H) = 250 Hz) and the chain CH_2 groups were observed in the range of δ 17–27 (J(C,H) = 125–129 Hz). Because of the presence of an O-atom in the dipropynyl ether moiety of **1d**, the CH_2 signals were shifted downfield to δ(C) 56.0 and 56.7; the δ(C) of the C≡C moieties were in agreement with literature data [31]. In the ^{1}H-NMR spectra of **1b**, the presence of an alkyne proton at δ(H) 2.74 reveals the formation of a 5-diynyl nucleoside, while for the dipropynyl ether derivative **1d** the O-atom of the side chain significantly shifts the adjacent proton signals downfield to δ(H) 4.22–4.37.

The introduction of the alkynyl or diynyl substituents at the 5-position of 2′-deoxyuridines influences their physical properties, such as the pK_a values, the hydrophobic character, and the polarizability. As this may lead to changes in the physical and biological properties of oligonucleotides, containing these nucleosides, these properties were studied. *Fig. 1*, *a*, shows that the side chain has a significant effect on nucleoside mobility in reversed-phase HPLC, compound **1c** being the slowest migrating nucleoside when compared with **1b** and **1d**. Retention times are correlated to the hydrophobic character of the nucleosides; the presence of an additional C≡C bond

Table 1. *^{13}C-NMR Chemical Shifts of 5-Substituted Pyrimidine 2′-Deoxyribonucleosides at 298 K*[a])

	1a	**1b**[b])	**1c**	**1d**	**6**	**4**	**7**[b])	**8**	**9**	**10**
C(2)	149.4	149.4 (6.7)	149.4	149.4	149.5	150.5	149.5 (8.2)	149.4	149.7	149.3
C(4)	161.8	161.7 (9.4)	161.7	161.5	161.8	163.8	161.8 (9.7)	161.7	162	161.5
C(5)	99.1	98.9 (< 1.9)	99.1	97.5	99	109.7	99 (< 1.9)	99.3	99.7	97.8
C(6)	142.6	142.6 (184)	142.5	144.2	142.8	136.3	142.7 (184)	142	142.2	143.6
C≡C	89.1	92.9, 84.2	93.2	87.8, 79.5	93.1	–	93	93.0, 84.2	93.7	87.9, 79.4
C≡C	72	72.9, 71.3 (250)	72.8	79.1, 77.7	73	–	73	72.4, 71.2	72.5	78.6, 77.7
CH_2	–	27.2, 27.1, 18.3, 17.2	31.6, 28.8	56.7, 56	28.0, 27.6, 24.5, 18.6	–	27.8, 27.6, 24.5, 18.6	27.1, 27.0, 18.3, 17.1	31, 28.3	56.7, 56
Triazole	–	–	–	–	147.5, 121.5	–	148.6, 121.1 (198)	–	–	–
C(1′)	84.5	84.6 (168.5)	85.4	84.8	84.6	83.9	84.6 (171.1)	84.8	85.1	85
C(2′)	[c])	[c])	[c])	[c])	[c])	37.1	[c])	[c])	[c])	[c])
C(3′)	70.2	70.2 (149.2)	71	70	70.2	59.1	70.1 (148.0)	70.4	70.7	70.4
C(4′)	87.5	87.5 (147.6)	88.4	87.6	87.5	84.5	87.5 (148.1)	85.8	86.2	85.8
C(5′)	61	60.9 (141.1)	61.8	60.8	61	60.8	61 (141.1)	63.6	63.9	63.6
MeO	–	–	–	–	–	–	–	55	55.3	55
C=O	–	–	–	–	–	–	195.7 (6.3)	–	–	–
Me	–	–	14.7	–	–	12.3	27.2	–	14.2	–

[a]) Measured in (D_6)DMSO. [b]) ^{13}C,^{1}H-Coupling constants given in parentheses. [c]) Superimposed by the DMSO signal.

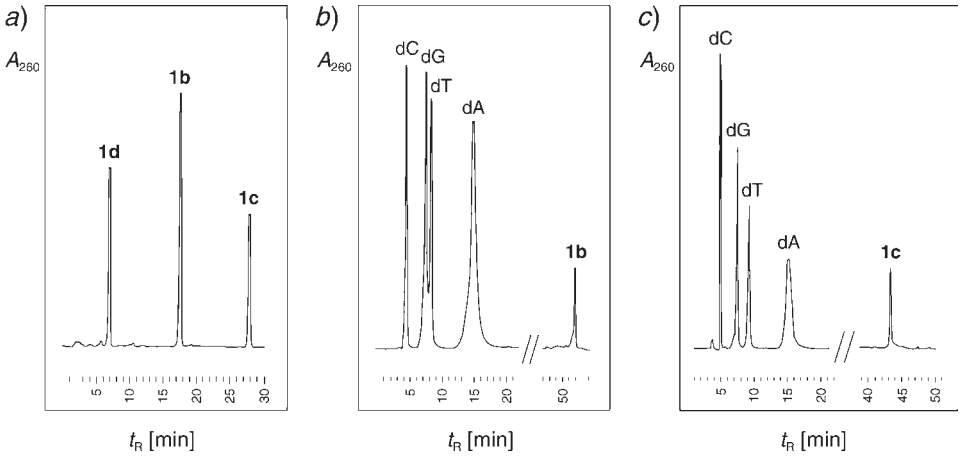


Fig. 1. *HPLC Profile* a) *of the nucleosides* **1b**–**d**, b) *of the enzymatic-hydrolysis products of the oligonucleotide 5′-d(TAG G***1b***C AA***1b*** ACT)-3′, and* c) *of the oligonucleotide 5′-d(AGT AT***1c*** GAC CTA)-3′ obtained by digestion with snake-venom phosphodiesterase and alkaline phosphatase in* 1M Tris·*HCl buffer* (pH 8.3) *at 37°*. Gradient for *a*): within 0–30 min 10–60% *B* in *A*; for *b*): 25 min 100% *A*, within 25–60 min 0–50% *B* in *A*; for *c*): 25 min 100% *A*, within 25–55 min 0–60% *B* in *A*; flow rate 0.7 ml/min; *A* = 0.1M (Et_3NH)OAc (pH 7.0)/MeCN 95:5, *B* = MeCN.

as in the octa-1,7-diynyl side chain of **1b** decreases the lipophilicity when compared to the oct-1-ynyl-substituted nucleoside **1c**. In the case of nucleoside **1d**, the O-atom in the side chain makes the molecule even more hydrophilic.

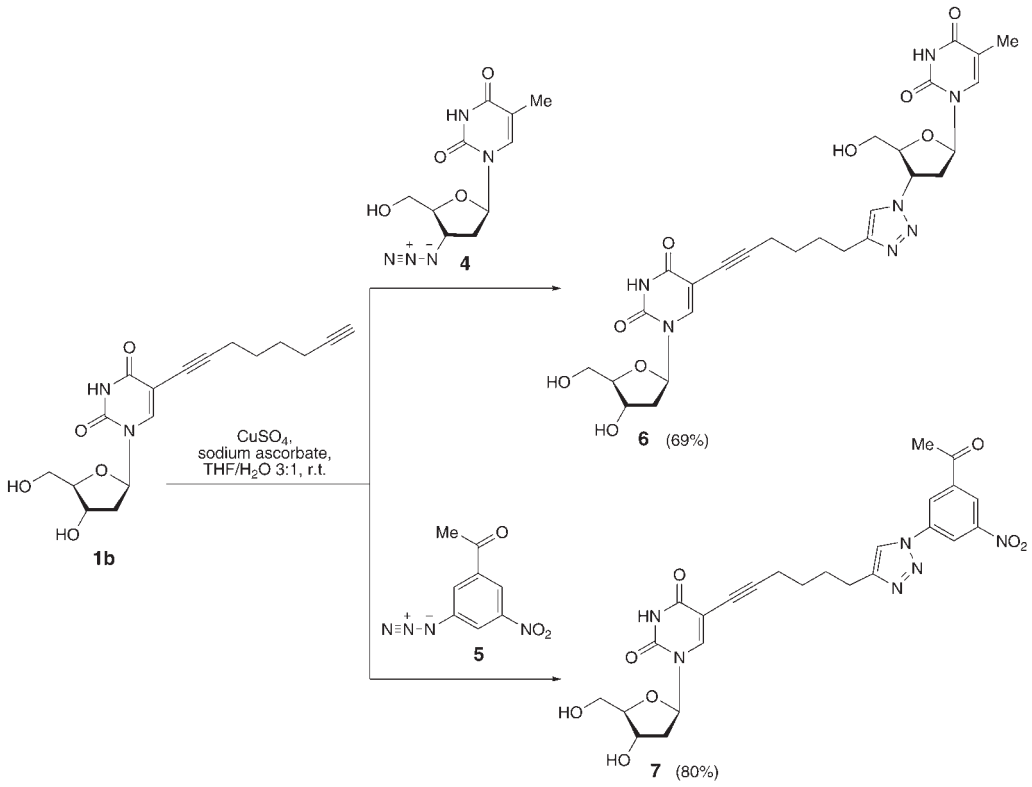
To evaluate the impact of these side chains, the log*P* values were calculated for the bases with the help of the ACD/log*P* 1.0 program. For the natural bases, the log*P* values were negative which reflects the hydrophilicity of these compounds. The highest log*P* value was calculated for 5-(oct-1-ynyl)uracil (base of **1c**; 3.42 ± 0.75); an additional C≡C bond as in the 5-(octa-1,7-diynyl)uracil (base of **1b**) decreased the value (2.23 ± 0.75), while the corresponding dipropynyl ether derivative led to the lowest value (1.45 ± 0.75). Moreover, the presence of a long hydrophobic substituent enhanced the nucleobase's polarizability $= \alpha_m/10^{-24}$ cm^3; the calculated data (Hyperchem 7.0) were 23.23 for **1b**, 24.10 for **1c**, and 20.24 for **1d**. These values are higher than that of the 5-propynylated 2′-deoxyuridine **1a** (14.92). The pK_a-value changes were also noticeable; they were determined UV-spectrophotometrically [33] (from pH 1.5 to 11.50) at 220–350 nm. The 5-alkynyl-2′-deoxyuridines showed the following pK_a values: 8.7 for **1b** and 8.8 for **1c**. These values are significantly lower than that of thymidine (dT) (9.5) or of the nonfunctionalized 2′-deoxyuridine (9.34).

2. *Functionalization of Nucleoside* **1b** *and of Oligonucleotides Containing* **1b** *by the* Huisgen–Meldal–Sharpless *[2 + 3] Cycloaddition* (*'Click' Chemistry*). Recent studies of the Cu^I-catalyzed [2 + 3] dipolar cycloaddition between an organic azide and an alkyne has attracted our attention [34][35]. Azides and alkynes are highly energetic functional groups with a particularly narrow distribution of reactivity. The irreversible *Huisgen–Meldal–Sharpless* cycloaddition of azides and alkynes is thermodynamically favorable by *ca.* 30 kcal/mol. The reaction is characterized by mild and simple reaction conditions and O_2 and H_2O tolerance, even in the presence of a large variety of other functional groups. It is chemoselective affording only the desired 1,4-disubstituted 1*H*-1,2,3-triazole. We report here on the use of nucleosides containing terminal C≡C bonds for the functionalization of nucleosides as well as of oligonucleotides employing the protocol of such a [2 + 3] cycloaddition. This kind of coupling chemistry was already used to construct fluorescent oligonucleotides [36] and also to attach oligonucleotides at a monolayer [37]. The functionalization of oligonucleotides can be employed for primers and probes used in DNA detection, hybridization, sequencing, and nanotechnology applications.

We functionalized the octadiynylated nucleoside **1b** with the antivirally active 3′-azido-3′-dideoxythymidine (AZT; **4**) having the azido group attached to the sugar moiety and with the aromatic system **5** [38] (*Scheme 2*). Standard reaction conditions were employed, and the reaction was performed in THF/H_2O 3 : 1 in the presence of copper(II) sulfate and sodium ascorbate. The 'click' products **6** and **7** were obtained in excellent yields, *i.e.*, regiospecifically, only the mono-functionalized 1,4-disubstituted 1*H*-1,2,3-triazole derivatives were formed. Bis-functionalization under participation of the second C≡C bond was not found, most likely due to steric reasons. The structures of **6** and **7** formed by the cycloaddition were confirmed by comparison of their NMR (*Table 1*) and UV spectra (*Fig. 2*, *Table 2*) with those of **1b** and **4**.

Due to triazole-ring formation, the acetylene proton of **1b** (δ(H) 2.74) disappeared, and new olefinic protons arising from the formed triazole moiety were identified at δ(H) 8.0–9.0. In the ^{13}C-NMR spectra

Scheme 2



of **7**, the signals of the terminal C≡C bond of **1b** disappeared, and the signals of the olefinic C-atoms of the 1,2,3-triazole moiety were identified at δ(C) 121.1 and 148.6 (quaternary C-atom) (*Table 1*). The unsaturated side chain of compound **1b** has a strong impact on the UV spectrum as can be seen from *Fig. 2*,*a*, the UV maximum being bathochromically shifted by 26 nm with respect to that of dT, with the minimum at *ca.* 260 nm. The UV data of **1b** are similar to those of 2′-deoxy-5-propynyluridine (**1a**) (*Table 2*). The 'click' product **6** shows a rather different UV spectrum as this is formed by the combination of the UV absorbances of AZT (**4**), **1b**, and the 1,2,3-triazole moiety (*Fig. 2*,*b*). A complete set of data is shown in *Table 2*.

The mobility of the 'click' nucleosides **4** and **6** in reversed-phase HPLC was determined by injecting an artificial mixture of starting materials and products onto the column; the starting material AZT (**4**) and 'click' product **6** move faster when compared to the 5-(octa-1,7-diynyl)-2′-deoxyuridine **1b** (*Fig. 3*,*a*). The presence of a triazole ring increases the hydrophilic character of the functionalized nucleoside **6** when compared to **1b**.

To study the properties of oligonucleotides containing the modified nucleosides **1b** or the derivatives **1c**,**d**, a series of phosphoramidites were prepared. For this purpose,

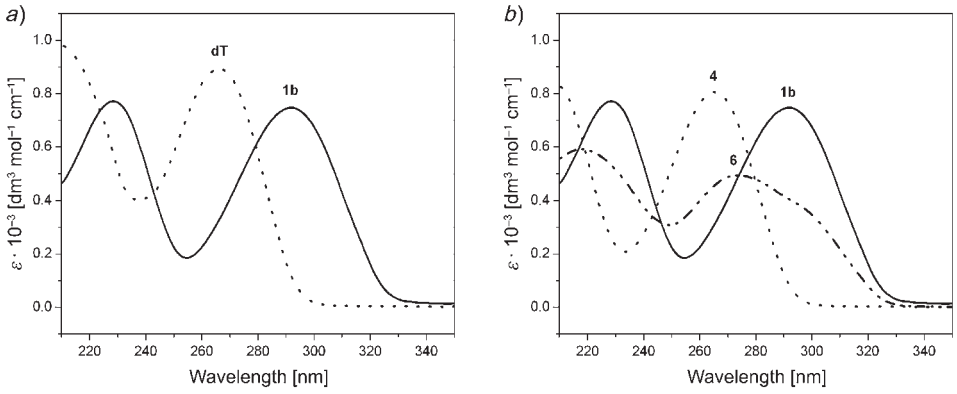


Fig. 2. *UV/VIS Spectra of* a) *dT and* **1b**, *and* b) **1b**, **4**, *and* **6** *in MeOH*

Table 2. *Extinction Coefficients of 5-Substituted Pyrimidine 2′-Deoxyribonucleosides*[a])

	Wavelength (λ_{max}) [nm]	Extinction coefficient (ε)		Wavelength (λ_{max}) [nm]	Extinction coefficient (ε)
1a	228	13000	**1b**	228	12800
	260	4000		260	3800
	291	13700		292	12400
1c	229	13000	**6**	218	17000
	260	3800		260	12000
	292	12700		274	14000

[a]) Measured in MeOH.

nucleosides **1b**–**d** were converted to the 5′-*O*-(dimethoxytrityl) derivatives **8**–**10** under standard reaction conditions. They were phosphitylated to yield the phosphoramidite building blocks **2b**–**d** (*Scheme 3*). All compounds were characterized by elemental analyses or mass spectra, as well as by ^{1}H- and ^{13}C-NMR. The ^{13}C-NMR chemical shifts are summarized in *Table 1*, for other data, see *Exper. Part*.

3. *Synthesis and Characterization of Oligonucleotides.* The oligonucleotides **11**–**25** (see *Schemes 4* and *5* and *Table 3* for *Formulae*) were prepared by solid-phase synthesis employing the phosphoramidites **2b**–**d** as well as standard building blocks. The coupling yields were higher than 95%. The oligonucleotides were purified by reversed-phase HPLC (see *Exper. Part*). The base composition of the oligonucleotides was determined by tandem enzymatic hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase in 0.1M Tris · HCl buffer (pH 8.3) at 37°. The HPLC profiles of the reaction products obtained after enzymatic digestion clearly demonstrate that the newly incorporated nucleosides **1b** or **1c** migrate much slower than the canonical DNA constituents (*Fig. 1*, *b*, and *c*). The oligonucleotides were also characterized by MALDI-TOF mass spectra. The detected masses were identical with the calculated values (see *Exper. Part*, *Table 4*).

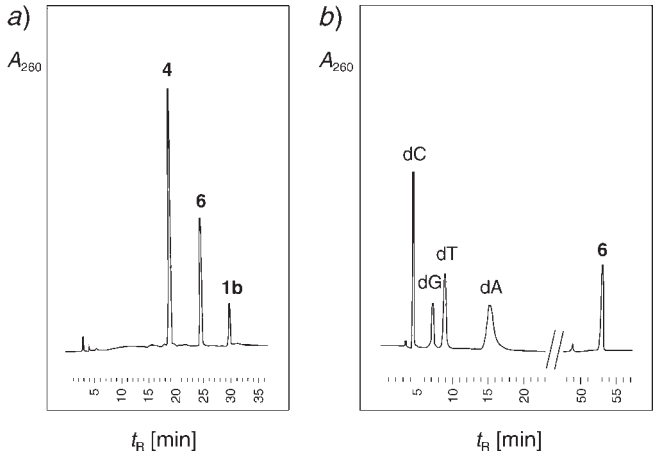
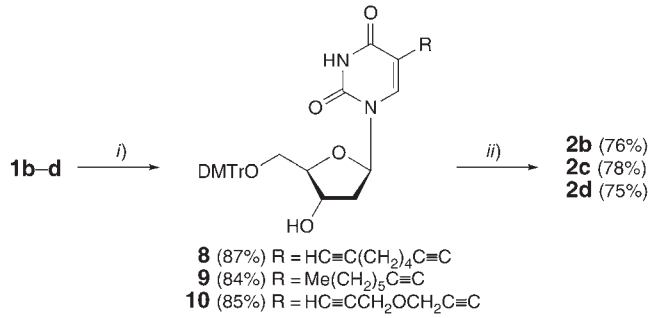


Fig. 3. *HPLC Profile* a) *of the artificial mixture of nucleosides* **1b**, **4**, *and* **6** *and* b) *of the enzymatic-hydrolysis products of the oligonucleotide* **18** *obtained by digestion with snake-venom phosphodiesterase and alkaline phosphatase in* 1M Tris · *HCl buffer* (pH 8.3) *at 37°*. Gradient for *a*): within 0 – 40 min 0 – 40% *B* in *A*; for *b*): 20 min 100% *A*, within 20 – 60 min 0 – 30% *B* in *A*; flow rate 0.7 ml/min; *A* = 0.1M (Et_3NH)OAc (pH 7.0)/MeCN 95 : 5, *B* = MeCN.

Scheme 3



i) $(MeO)_2TrCl$, pyridine, r.t., 5 h. *ii*) $^iPr_2NP(Cl)OCH_2CH_2CN$, $^iPr_2EtN$, CH_2Cl_2, r.t, 45 min.

Next, the *Huisgen – Meldal – Sharpless* cycloaddition was performed with oligonucleotides containing **1b**. For this, the self-complementary 6-mer oligonucleotide 5′-d(GCA**1b**GC)-3′ (**11**) was synthesized in which dT was replaced by **1b**. The reaction was carried out with 86 nmol of the purified oligomer **11** and AZT (**4**) in ^{t}BuOH/H_2O 1 : 9 in the presence of a 1 : 1 complex $CuSO_4$ · TBTA (tris(benzyltriazoylmethyl)amine) [39][40] and TCEP (tris(carboxyethyl)phosphine), a H_2O-soluble reducing agent, to give the functionalized oligonucleotide **12** (*Scheme 4*). The oligonucleotide conjugate was purified by reversed-phase HPLC (*RP-18* column) in the trityl-off modus (see *Exper. Part*). The oligonucleotide conjugate was identified by MALDI-TOF-MS (see

Table 3. T_m *Values and Thermodynamic Data of Oligonucleotide Duplexes Containing the Nucleosides* **1a–d** *and* **6** *Located Opposite dA*[a])

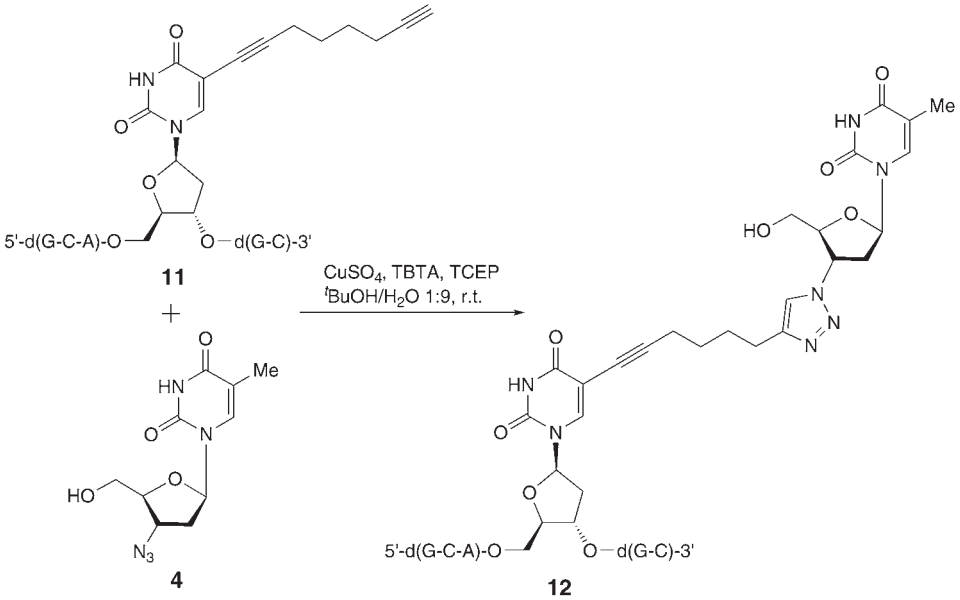
Duplex				T_m [°]	ΔT_m[b]) [kcal/mol]	ΔG°_{310} [kcal/mol]
5′-d(TAG	GTC	AAT	ACT)-3′ (**15**)	50		−11.8
3′-d(ATC	CAG	TTA	TGA)-5′ (**16**)			
5′-d(TAG	G**1b**C	AAT	ACT)-3′ (**17**)	52	+2	−11.9
3′-d(ATC	CAG	TTA	TGA)-5′ (**16**)			
5′-d(TAG	G**6**C	AAT	ACT)-3′ (**18**)	51	+1	−11.8
3′-d(ATC	CAG	TTA	TGA)-5′ (**16**)			
5′-d(TAG	GTC	AAT	ACT)-3′ (**15**)	53	+1.5	−12.4
3′-d(ATC	CAG	**1b1b**A	TGA)-5′ (**19**)			
5′-d(TAG	GTC	AAT	ACT)-3′ (**15**)	53	+1.5	−12.0
3′-d(A**1b**C	CAG	TTA	**1b**GA)-5′ (**20**)			
5′-d(TAG	G**1b**C	AAT	ACT)-3′ (**17**)	53	+1.0	−12.0
3′-d(A**1b**C	CAG	TTA	**1b**GA)-5′ (**20**)			
5′-d(TAG	G**1b**C	AAT	ACT)-3′ (**17**)	55	+1.6	−12.3
3′-d(ATC	CAG	**1b1b**A	TGA)-5′ (**19**)			
5′-d(TAG	G**1d**C	AAT	ACT)-3′ (**21**)	52	+2	−11.8
3′-d(ATC	CAG	TTA	TGA)-5′ (**16**)			
5′-d(TAG	GTC	AAT	ACT)-3′ (**15**)	53	+1.5	−11.5
3′-d(A**1d**C	CAG	TTA	**1d**GA)-5′ (**22**)			
5′-d(TAG	G**1d**C	AAT	ACT)-3′ (**21**)	53	+1.0	−11.8
3′-d(A**1d**C	CAG	TTA	**1d**GA)-5′ (**22**)			
5′-d(TAG	GTC	AAT	ACT)-3′ (**15**)	50.5	+0.5	−11.6
3′-d(ATC	CAG	**1c**TA	TGA)-5′ (**23**)			
5′-d(TAG	GTC	AAT	ACT)-3′ (**15**)	50.5	+0.25	−11.7
3′-d(A**1c**C	CAG	TTA	**1c**GA)-5′ (**24**)			
5′-d(TAG	G**1c**C	AAT	ACT)-3′ (**25**)	51	+0.3	−11.4
3′-d(A**1c**C	CAG	TTA	**1c**GA)-5′ (**24**)			
5′-d(TAG	G**1a**C	AAT	ACT)-3′ (**26**)	52	+2	−11.9
3′-d(ATC	CAG	TTA	TGA)-5′ (**16**)			
5′-d(TAG	GTC	AAT	ACT)-3′ (**15**)	52	+1	−12.2
3′-d(ATC	CAG	**1a1a**A	TGA)-5′ (**27**)			
5′-d(TAG	G**1a**C	AAT	ACT)-3′ (**26**)	53	+1	−12.5
3′-d(ATC	CAG	**1a1a**A	TGA)-5′ (**27**)			

[a]) Measured at 260 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) with 5 μM single-strand concentration. [b]) T_m increase per modification.

Exper. Part) and by enzymatic hydrolysis with snake-venom phosphodiesterase and alkaline phosphatase (see *Exper. Part* and *Fig. 4*). *Fig. 4*, *a*, shows the HPLC profile of the starting oligomer **11** after enzymatic hydrolysis, while *Fig. 4*, *b*, represents the HPLC profile after enzymatic digestion of the crude reaction mixture of the 'click' product **12**, which clearly demonstrates the formation of the 'click' nucleoside **6**. However, AZT (**4**) exhibits the same mobility as the oligonucleotide **12** causing difficulties in the separation during chromatographic purification.

In the second experiment, the reaction was performed with the CPG-bound oligonucleotide **13** and AZT (**4**). For this purpose the CPG-bound 12-mer oligonu-

Scheme 4



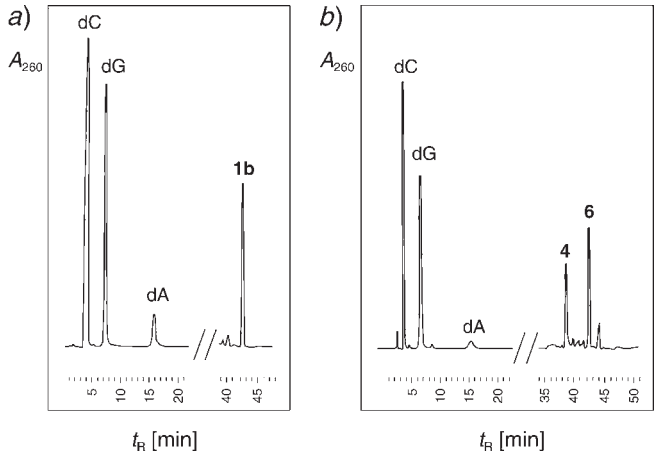
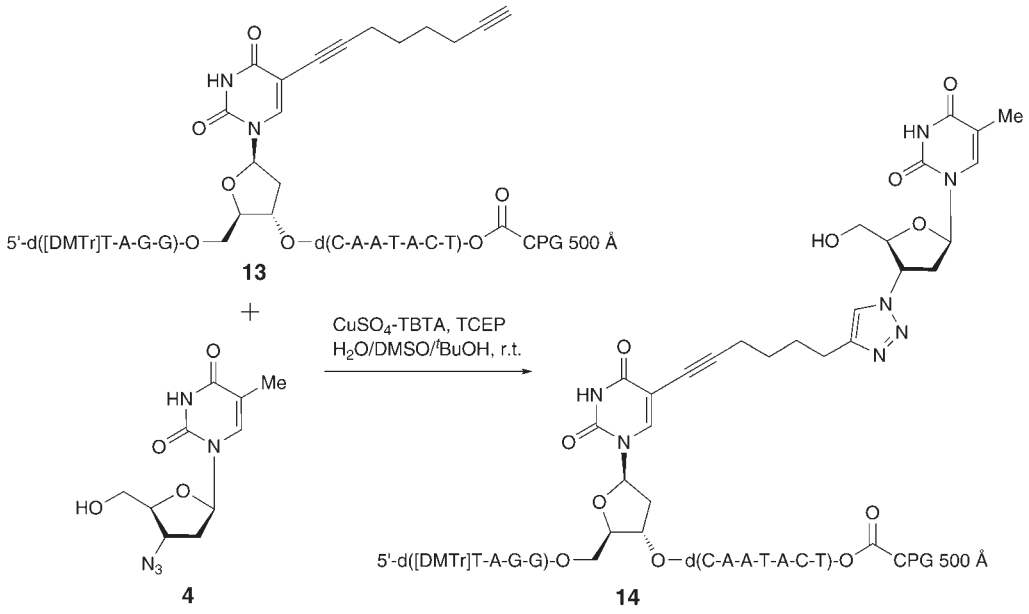


Fig. 4. *HPLC Profile* a) *of the enzymatic-hydrolysis products of the oligonucleotide* **11** *and* b) *of oligonucleotide* **12** *obtained by digestion with snake-venom phosphodiesterase and alkaline phosphatase in* 1M Tris · *HCl buffer* (pH 8.3) *at 37°*. Gradient for *a*): 20 min 100% *A*, within 55 min 0–35% *B* in *A*; for *b*): 20 min 100% *B*, within 50 min 0–35% *B* in *A*; flow rate 0.7 ml/min; *A* = 0.1M (Et_3NH)OAc (pH 7.0)/MeCN 95 : 5, *B* = MeCN.

cleotide 5′-d((5′-*O*-$(MeO)_2$Tr)TAG G**1b**C AAT ACT)-3′ **13** was synthesized by solid-phase synthesis by means of the protocol of phosphoramidite chemistry and the regular phosphoramidites and modified building block **1b** (*Scheme 5*). The $(MeO)_2$Tr

protecting group was preserved on the CPG-bound oligonucleotide, and the 'click' reaction was performed with AZT (**4**) in H_2O/DMSO/^{t}BuOH 3:1:1 for 12 h and the above mentioned reagents (see *Exper. Part*) to give the crude modified oligomer **14**. To remove excess **4** and other starting materials present in the reaction mixture, the crude matrix-bound **14** was washed with MeOH/H_2O 1:1. Thereafter, the solid support was removed by using standard deprotection conditions (25% aqueous NH_3 solution, 60°, 14 h). During this procedure, the protecting groups were also removed (*Scheme 5*). Purification of the obtained 5′-*O*-(dimethoxytrityl)oligonucleotide conjugate was performed by reversed-phase HPLC (*RP-18* column). The $(MeO)_2$Tr protecting group was then removed and the formed oligonucleotide **18** purified further (see *Exper. Part*). The structure of the ligation product **18** was confirmed by MALDI-TOF-MS (*Table 4*) and by enzymatic hydrolysis (*Fig. 3,b*). From the HPLC profile it is apparent that the compound obtained after the digest was the only modified residue and was identical to the nucleoside **6** prepared earlier. The complete conversion of oligonucleotide **18** to the respective triazole product **6** was observed within 12 h.

Scheme 5. *Cycloaddition Performed with the Oligonucleotide* **13**



4. *Duplex Stability of Oligonucleotides Containing 5-Substituted 2′-Deoxyuridines* **1a–d** *and the 'Click'-Functionalized Nucleoside* **6**. Earlier work performed with oligonucleotides containing the propynylnucleoside **1a** showed an increase of the duplex stability resulting in a ΔT_m of 1–2° per modification when **1a** was incorporated in the place of dT [24] (*Table 3*). To evaluate the influence of the base modification on the duplex stability, hybridization experiments were performed with the oligonucleo-

tide duplex 5′-d(TAGGTCAATACT)-3′ (**15**) and 3′-d(ATCCAGTTATGA)-5′ (**16**); the dT residues were replaced by the modified nucleosides **1b**–**d**. The oct-5-ynylated nucleoside **1c** shows very little influence on the duplex stability with an increase of the T_m value of 0.25–0.50° per modification. According to the previous reports, the destabilizing effect dominates when alkynyl groups higher than hexynyl were introduced at the 5-position of 2′-deoxyuridines [17]. To study the influence of terminal unsaturated diynes on the DNA duplex stability, the octa-1,7-diynyl residue was chosen instead of oct-1-ynyl. The 5-(octa-1,7-diynyl)nucleoside **1b** enhances the stability of the dA·dT base pair resulting in a T_m increase of 1–2° per modification depending on the position of the substitution (*Table 3*). This increase is similar to that found for the propynylnucleoside **1a**. In a similar way, the dipropynyl ether derivative **1d** increases the stability like **1b**, with a T_m increase of 1–2° per modification. This study was extended to multiple incorporations of compound **1b** into a series of oligonucleotide duplexes at various positions. Replacement of two dT residues by **1b** enhances the T_m value by 3° (**15**·**19**, **15**·**20**, *Table 3*). With three incorporations of **1b** in duplex **17**·**19**, the T_m increases from 53 to 55° when compared with **1a** in the corresponding duplex **26**·**27**. The T_m values of a series of oligonucleotides are summarized in *Table 3*.

To study the influence of the modified nucleoside **6** containing the 1,2,3-triazole moiety as well as an AZT residue on the duplex stability, the oligonucleotide **18** was hybridized with **16**. This duplex **16**·**18** (*Fig. 5*) shows a T_m value (51°) similar to the one of duplex **16**·**17** containing the 5-octadiynylated nucleoside **1b** (T_m 52°, *Table 3*). From the T_m values shown above, it can be concluded that the octadiynyl and dipropynyl ether moieties at C(5) are better stabilizers than the octynyl residue (*Table 3*), they almost behave like propynyl residues, and in a few sequences, they are superior to the propynyl group. We were surprised to see that such a bulky side chain does not interfere with base-pair stability implying that the solvation of the side chain is of utmost importance for the duplex stability.

Conclusions and Outlook. – Nucleosides incorporating the octa-1,7-diynyl or dipropynyl ether moiety at the 5-position of 2′-deoxyuridine were synthesized by the *Sonogashira* cross-coupling reaction resulting in a selective mono-derivatization of the diyne starting material. The (1,7-diynyl)uracil derivative **1b** bearing a long flexible linker arm was further functionalized by the *Huisgen*–*Meldal*–*Sharpless* [2+3] cycloaddition ('click' chemistry) at the stages of the nucleoside and oligonucleotides. The reactions were performed in solution as well as on solid support. Oligonucleotides incorporating the octa-1,7-diynyl and dipropynyl ether linkers at the 5-position of 2′-deoxyuridine (base-pair motifs **Ic** and **Id**, *Fig. 6*) stabilize the DNA duplexes stronger than 5-alkynyl nucleosides such as 5-propynyl-2′-deoxyuridine or 5-octynyl-2′-deoxyuridine (motifs **Ia** and **Ib**). Even a long linker arm formed by the cycloaddition of AZT (**4**) with the (1,7-diynyl)oligonucleotide **17** (→**18**) yields an oligonucleotide–nucleoside conjugate forming a stable base pair (motif **II**). Therefore, side chains of this complex structure are not disturbing the duplex structure if the environment around the DNA helix allows the solvation by H_2O molecules. In the case of motif **II**, the side chain fulfills these requirements because of the polar character of the nucleoside residues as well as of the highly polar 1,2,3-triazole moiety. Thus, for an unperturbated

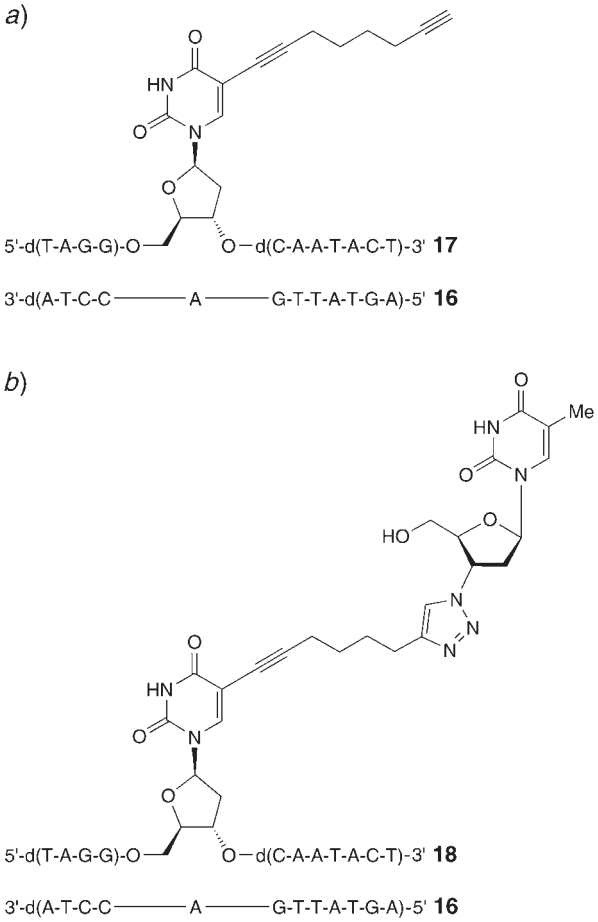


Fig. 5. a) *DNA Duplex* **16**·**17** *containing 2′-deoxy-5-(octa-1,7-diynyl)uridine* (**1b**). b) *DNA Duplex* **18**·**16** *containing post-modified nucleoside* **6**.

DNA structure, it is of utmost importance to select reporter groups – dyes or other functionalizing residues which do not change the water shells of a DNA molecule.

We thank Dr. *H. Rosemeyer* and Dr. *Y. He* for measuring the NMR spectra. We also thank Mr. *K. I. Shaikh* for the MALDI-TOF mass spectra, Mrs. *E. Michalek* for the oligonucleotide syntheses, Mrs. *P. Chittepu* for measurement of the pK_a values, and Dr. *P. Leonard* and Mrs. *S. Budow* for reading the manuscript. Financial support from *ChemBiotech*, Münster, is gratefully acknowledged.

Experimental Part

General. All chemicals were purchased from *Aldrich*, *Sigma*, or *Fluka* (*Sigma-Aldrich Chemie GmbH*, Deisenhofen, Germany). Solvents were of technical grade and freshly distilled before use. Thin layer chromatography (TLC): aluminium sheets, silica gel *60* F_{254}, 0.2 mm layer (*VWR*, Germany). Column flash chromatography (FC): silica gel *60* (*VWR*, Germany), at 0.4 bar; sample collection with an

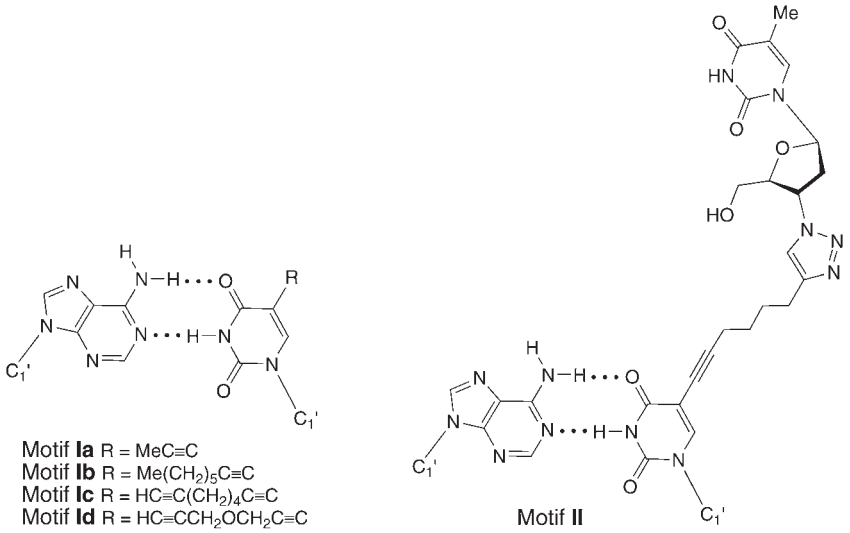


Fig. 6. *Base-pair motifs formed by* **1a**–**d** *and* **6**

UltroRac-II fraction collector (*LKB Instruments*, Sweden). UV Spectra: *U-3200* spectrometer (*Hitachi*, Tokyo, Japan); λ_{max} (ε) in nm. NMR Spectra: *Avance-DPX-250* or *AMX-500* spectrometers (*Bruker*, Karlsruhe, Germany); at 250.13 MHz for ^{1}H and ^{13}C; δ in ppm rel. to Me_4Si as internal standard or external 85% H_3PO_4 for ^{31}P; *J* values in Hz. Elemental analyses were performed by the *Mikroanalytisches Laboratorium Beller* (Göttingen, Germany). Electron-spray-ionization (ESI) MS for the nucleosides: *Bruker-Daltonics-MicroTOF* spectrometer with loop injection (Bremen, Germany). MALDI-TOF-MS for the oligonucleotides: *Biflex-III* mass spectrometer (*Bruker Saxonia*, Leipzig, Germany), with 3-hydroxypicolinic acid (3-HPA) as a matrix; detected masses were identical with the calculated values (*Table 4*).

Synthesis, Purification, and Characterization of the Oligonucleotides **11**–**25**. The oligonucleotide synthesis was performed on a DNA synthesizer, model *392-08* (*Applied Biosystems*, Weiterstadt,

Table 4. *Molecular Masses [M + H]$^+$ of the Oligonucleotides Measured by MALDI-TOF Mass Spectrometry*

	$[M + H]^+$ (calc.)	$[M + H]^+$ (found)
5′-d(GCA **1b**GC)-3′ (**11**)	1882.4	1882.9
5′-d(GCA **6**GC)-3′ (**12**)	2148.1	2147.2
5′-d(TAG GTC AAT ACT)-3′ (**15**)	3645.4	3645.0
5′-d(AGT ATT GAC CTA)-3′ (**16**)	3645.4	3645.1
5′-d(TAG G**1b**C AAT ACT)-3′ (**17**)	3735.5	3735.4
5′-d(TAG G**6**C AAT ACT)-3′ (**18**)	4002.4	4002.4
5′-d(AGT A**1b1b** GAC CTA)-3′ (**19**)	3825.7	3825.4
5′-d(AG**1b** ATT GAC C**1b**A)-3′ (**20**)	3825.7	3826.1
5′-d(TAG G**1d**C AAT ACT)-3′ (**21**)	3721.6	3721.8
5′-d(AG**1d** ATT GAC C**1d**A)-3′ (**22**)	3801.6	3801.6
5′-d(AGT AT**1c** GAC CTA)-3′ (**23**)	3739.6	3739.4
5′-d(AG**1c** ATT GAC C**1c**A)-3′ (**24**)	3831.8	3832.5
5′-d(TAG G**1c**C AAT ACT)-3′ (**25**)	3739.6	3739.0

Germany) at a 1-μmol scale, with the phosphoramidites **2b**–**d** and following the synthesis protocol for 3′-(2-cyanoethyl phosphoramidites) (user's manual for the *392* DNA synthesizer, *Applied Biosystems*, Weiterstadt, Germany). The coupling efficiency was always higher than 95%. After cleavage from the solid support with 25% aq. NH_3 soln. for 14–16 h at 60°, the oligonucleotides were deprotected in the same soln. for 14–16 h at 60°. The purification of the 5′-*O*-(dimethoxytrityl)oligomers was carried out on reversed-phase HPLC (*Merck-Hitachi-HPLC*; *RP-18* column; gradient system (*A* = 0.1M $(Et_3NH)OAc$ (pH 7.0)/MeCN 95:5, *B* = MeCN): 3 min 20% *B* in *A*, 12 min 20–50% *B* in *A*, and 25 min 20% *B* in *A*; flow rate 1.0 ml/min. The purified 'trityl-on' oligonucleotides were treated with 2.5% $CHCl_2COOH$/CH_2Cl_2 for 5 min at 0° to remove the 4,4′-dimethoxytrityl residues. The detritylated oligomers were purified again by reversed-phase HPLC (gradient: 0–20 min 0–20% *B* in *A*; flow rate 1 ml/min). The oligomers were desalted on a short column (*RP-18*, silica gel) and lyophilized on a *Speed-Vac* evaporator to yield colorless solids which were frozen at −24°.

Melting curves were measured with a *Cary-1/3* UV/VIS spectrophotometer (*Varian*, Australia) equipped with a *Cary* thermoelectrical controller. The temp. was measured continuously in the reference cell with a *Pt-100* resistor, and the thermodynamic data of duplex formation were calculated by the Meltwin 3.0 program [41].

The enzymatic hydrolysis of the oligonucleotides containing **1b**, **1c**, and **6** was performed as described [5] with snake-venom phosphodiesterase (EC 3.1.15.1, *Crotallus adamanteus*) and alkaline phosphatase (EC 3.1.3.1, *Escherichia coli* from *Roche Diagnostics GmbH*, Germany) in 0.1M *Tris*·HCl buffer (pH 8.3) at 37°, which was analyzed by reversed-phase HPLC (*RP-18*, at 260 nm) showing the peaks of the modified and unmodified nucleosides (*Fig. 1*,*b* and *c*; *Fig. 3*,*b*; *Fig. 4*,*b*). Quantification of the constituents were made on the basis of the peak areas, which were divided by the extinction coefficients ε_{260} of the nucleosides: dA 15400 (H_2O), dC 7300 (H_2O), dG 11700 (H_2O), dT 8800 (H_2O), **1b** 3800 (MeOH), **1c** 3800 (MeOH), and **6** 12000 (MeOH).

Huisgen–Meldal–Sharpless *[2+3] Cycloaddition of* **11** *with* **4** *Performed in Aqueous Solution Yielding* **12**. To the single-stranded oligonucleotide **11** (5 A_{260} units, 87 nmol), $CuSO_4$·TBTA ligand complex (20 μl of a 10 mM stock soln. in ^{t}BuOH/H_2O 1:9), tris(carboxyethyl)phosphine (TCEP; 20 μl of a 10 mM stock soln. in H_2O), AZT (**4**; 25 μl of a 10 mM stock soln. in dioxane/H_2O 1:1) and 35 μl of 10% ^{t}BuOH were added, and the reaction was run at r.t. for 12 h. The product 5′-d(GCA**6**GC)-3′ (**12**) was purified by reversed-phase HPLC (see above). Composition by enzymatic hydrolysis: *Fig. 4*,*b*. MALDI-TOF-MS: *Table 4*.

Huisgen–Meldal–Sharpless *[2+3] Cycloaddition of* **13** *with* **4** *Performed on Solid Support Yielding* **18**. To the single-stranded oligonucleotide **13** attached to a solid support (40 mg, 36 μmol/g loading 500 Å) bearing the $(MeO)_2$Tr protected residue as well as protected nucleobases adenine and cytosine with tBPA (=(4-*tert*-butylphenoxy)acetyl) protecting group and guanine with iB (=isobutyryl) protecting group, an aq. soln. of 70 μl of H_2O/DMSO/^{t}BuOH 3:1:1, $CuSO_4$·TBTA ligand complex (40 μl of a 10 mM stock soln. in ^{t}BuOH/H_2O 1:9), tris(carboxyethyl)phosphine (TCEP; 40 μl of a 10 mM stock soln. in H_2O), AZT (**4**; 50 μl of a 10 mM stock soln. in dioxane/H_2O 1:1) were added, and the mixture was stirred at r.t. for 12 h and then concentrated. The crude modified CPG-bound **14** was shaken with 4 ml of H_2O/MeOH 1:1. After cleavage from the CPG by 25% aq. NH_3 soln. for 14 h at 60° the 'trityl-on' oligonucleotide was further purified by reversed-phase HPLC (see above). The detritylated oligonucleotide **18** was also purified by reversed-phase HPLC (see above). MALDI-TOF: *Table 4*. Enzymatic hydrolysis: *Fig. 3*,*b*.

*1-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-5-(octa-1,7-diynyl)uracil* (=*α-(Hept-6-ynylidyne)thymidine*; **1b**). To a suspension of 5-iodo-2′-deoxyuridine (**3**; 1.0 g, 2.82 mmol) and CuI (108 mg, 0.57 mmol) in anh. DMF (15 ml) was added successively $[Pd(PPh_3)_4]$ (323 mg, 0.28 mmol), anh. Et_3N (570 mg, 5.64 mmol), and octa-1,7-diyne (3.0 g, 28.3 mmol). The mixture was stirred at r.t. under Ar until the starting material was consumed (TLC monitoring). The mixture was diluted with MeOH/CH_2Cl_2 1:1 (30 ml), and *Dowex 1X8* (100–200 mesh; 1.0 g, hydrogen carbonate form) was introduced. After additional stirring for 1 h, the mixture was filtered and the resin washed with MeOH/CH_2Cl_2 1:1 (50 ml). The combined filtrate was concentrated and the residue purified by FC (silica gel, column 15 × 3 cm, CH_2Cl_2/MeOH 95:5): **1b** (0.68 g, 72%). Colorless amorphous solid. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.42. UV (MeOH): 228 (12800), 292 (12400). ^{1}H-NMR ((D_6)DMSO): 1.55–1.57 (*m*, 2 CH_2); 2.11 (*m*,

2 H–C(2′)); 2.19 (*m*, CH_2); 2.38 (*m*, CH_2); 2.74 (*s*, C≡CH); 3.55–3.60 (*m*, 2 H–C(5′)); 3.77 (*m*, H–C(4′)); 4.22 (*m*, H–C(3′)); 5.06 (*t*, *J* = 4.8, OH–C(5′)); 5.21 (*d*, *J* = 4.2, OH–C(3′)); 6.10 (*t*, *J* = 6.6, H–C(1′)); 8.10 (*s*, H–C(6)); 11.53 (*s*, NH). Anal. calc. for $C_{17}H_{20}N_2O_5$ (332.14): C 61.44, H 6.07, N 8.43; found: C 61.42, H 6.03, N 8.38.

*1-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-5-(oct-1-ynyl)uracil* ((=*α-Heptylidynethymidine*; **1c**) [42]. As described for **1b**, with **3** (1.0 g, 2.82 mmol) and oct-1-yne (1.86 g, 16.9 mmol). FC (silica gel, column 15 × 3 cm, CH_2Cl_2/MeOH 94 : 6) gave **1c** (0.74 g, 78%). Colorless amorphous solid. TLC (CH_2Cl_2/MeOH 95 : 5): R_f 0.23. UV (MeOH): 229 (13000), 292 (12700). ^{1}H-NMR ((D$_6$)DMSO): 0.84 (*t*, Me); 1.34–1.48 (*m*, 4 CH_2); 2.10 (*m*, 2 H–C(2′)); 2.35 (*t*, CH_2); 3.57 (*m*, 2 H–C(5′)); 3.78 (*m*, H–C(4′)); 4.22 (*m*, H–C(3′)); 5.08 (*t*, OH–C(5′)); 5.24 (*d*, *J* = 3.7, OH–C(3′)); 6.12 (*t*, *J* = 6.6, H–C(1′)); 8.10 (*s*, H–C(6)); 11.60 (*s*, NH).

*1-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-5-[3-(prop-2-ynyloxy)prop-1-ynyl]uracil* ((=*α-[2-(Prop-2-ynyloxy)ethylidyne]thymidine*; **1d**). As described for **1b**, with **3** (700 mg, 1.98 mmol), CuI (76 mg, 0.40 mmol), anh. DMF (10 ml), [Pd(PPh$_3$)$_4$] (230 mg, 0.20 mmol), anh. Et_3N (398 mg, 3.94 mmol), and diprop-2-ynyl ether (1.5 g, 16.0 mmol). Purification by FC (silica gel, column 15 × 3 cm, CH_2Cl_2/MeOH 96 : 4) yielded **1d** (0.53 g, 84%). Yellowish amorphous solid. TLC (CH_2Cl_2/MeOH 9 : 1): R_f 0.46. UV (MeOH): 230 (12000), 291 (12500). ^{1}H-NMR ((D$_6$)DMSO): 2.08–2.15 (*m*, 2 H–C(2′)); 3.47 (*s*, C≡CH); 3.53–3.64 (*m*, 2 H–C(5′)); 3.79 (*m*, H–C(4′)); 4.22 (*m*, 3 H, CH_2, H–C(3′)); 4.37 (*m*, CH_2); 5.10 (*t*, OH–C(5′)); 5.23 (*d*, *J* = 3.3, OH–C(3′)); 6.10 (*t*, *J* = 6.3, H–C(1′)); 8.26 (*s*, H–C(6)); 11.63 (*s*, NH). Anal. calc. for $C_{15}H_{16}N_2O_6$ (320.10): C 56.25, H 5.04, N 8.75; found: C 56.02, H 5.10, N 8.56.

Copper(I)-Catalyzed Cycloaddition: General Procedure. To a mixture of **1b** (100 mg, 0.30 mmol) and 1-(5-azido-3-nitrophenyl)ethan-1-one (**5**; 86.5 mg, 0.42 mmol) in THF/H_2O 3 : 1 (8 ml), was added 1M aq. sodium ascorbate (90 µl, 0.09 mmol; freshly prepared, followed by 7.5% aq. copper(II) sulfate pentahydrate soln. (70 µl, 0.021 mmol). The emulsion was stirred for 20 h at r.t., concentrated and applied to FC (silica gel, column 10 × 3 cm, CH_2Cl_2/MeOH 94 : 6).

*α-{5-[1-(3-Acetyl-5-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]pentylidyne}thymidine* (**7**). As described in the *General Procedure*. From the main zone of the FC **7** (130 mg, 80%) was isolated. Yellowish solid. TLC (CH_2Cl_2/MeOH 9 : 1): R_f 0.49. UV (MeOH): 227 (30000), 288 (12000). ^{1}H-NMR ((D$_6$)DMSO): 1.57–1.85 (*m*, 2 CH_2); 2.08–2.12 (*m*, 2 H–C(2′)); 2.44 (*m*, CH_2); 2.76 (*s*, COMe); 2.81 (*m*, CH_2); 3.56 (*m*, 2 H–C(5′)); 3.77 (*m*, H–C(4′)); 4.23 (*m*, H–C(3′)); 5.09 (*t*, *J* = 4.8, OH–C(5′)); 5.25 (*d*, *J* = 4.2, OH–C(3′)); 6.10 (*t*, *J* = 6.6, H–C(1′)); 8.12 (*s*, H–C(6)); 8.65–8.91 (*m*, arom. H); 8.99 (*s*, C=CH); 11.54 (*s*, NH). ESI-HR-MS: 561.17 ([*M* + Na]$^+$, $C_{25}H_{26}N_6O_8^+$; calc. 538.18).

*α-{5-{1-[(2*S*,3*S*,5*R*)-Tetrahydro-2-(hydroxymethyl)-5-(1,2,3,4-tetrahydro-5-methyl-2,4-dioxopyrimidin-1-yl)furan-3-yl]-1*H*-1,2,3-triazol-4-yl}pentylidyne}thymidine* (**6**). As described in the *General Procedure*, with **1b** (50 mg, 0.15 mmol), AZT (**4**; 40 mg, 0.15 mmol), 1M of sodium ascrobate (120 µl, 0.12 mmol), and copper(II) sulfate pentahydrate soln. (100 µl, 0.03 mmol of a 7.5% stock soln. in H_2O) dissolved in THF/H_2O 3 : 1 (4 ml) for 30 h. FC (silica gel, column 10 × 3 cm, CH_2Cl_2/MeOH 9 : 1) gave **6** (62 mg, 69%) from the main zone. Colorless foam. TLC (CH_2Cl_2/MeOH 9 : 1): R_f 0.24. UV (MeOH): 218 (17000), 274 (14000). ^{1}H-NMR ((D$_6$)DMSO; nucleoside atom numbering for the AZT-derived moiety, *i.e.*, doubly (″) and triply (‴) primed locants): 1.54–1.72 (*m*, 2 CH_2); 1.80 (*s*, Me–C(5″)); 2.08–2.68 (*m*, 8 H, H–C(2′), H–C(2‴), CH_2); 3.55–3.62 (*m*, 4 H, H–C(5′), H–C(5‴)); 3.77 (*m*, 2 H, H–C(4′), H–C(4‴)); 4.17–4.22 (*m*, 2 H, H–C(3′), OH–C(5‴)); 5.14 (*t*, *J* = 4.9, OH–C(5′)); 5.25 (*d*, *J* = 4.17, OH–C(3′)); 5.30 (*m*, H–C(3‴)); 6.10 (*t*, *J* = 6.55, H–C(1′)); 6.40 (*t*, *J* = 6.55, H–C(1‴)); 7.81 (*s*, H–C(6″)); 8.06 (*s*, C=CH); 8.13 (*s*, H–C(6)); 11.36 (*s*, HN(3″)); 11.56 (*s*, HN(3)). ESI-HR-MS: 622.22 ([*M* + Na]$^+$, $C_{27}H_{33}N_7O_9^+$; calc. 599.23).

*1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl*]*-5-(octa-1,7-diynyl)uracil* (=*5′-O-*[Bis(4-methoxyphenyl)phenylmethyl]-α-(hept-6-ynylidyne)thymidine*; **8**). Compound **1b** (0.5 g, 1.5 mmol) was dried by repeated co-evaporation with anh. pyridine (2 × 5 ml) before dissolving in anh. pyridine (10 ml). Then 4,4′-dimethoxytrityl chloride (0.61 g, 1.80 mmol) was added in three portions to the remaining soln. at r.t. under stirring for 5 h. Thereupon, MeOH (2 ml) was added, and the mixture was stirred for another 30 min. The mixture was dissolved in CH_2Cl_2 (2 × 50 ml) and extracted with 5% aq. $NaHCO_3$ soln. (100 ml) followed by H_2O (80 ml), dried (Na_2SO_4), and then concentrated. Purification by FC (silica gel, column 15 × 3 cm, CH_2Cl_2/acetone 80 : 20) gave **8** (0.83 g, 87%). Colorless

foam. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.31. UV (MeOH): 233 (28000), 284 (10300). ^{1}H-NMR ((D$_6$)DMSO)): 1.39 (*m*, 2 CH_2); 2.06–2.26 (*m*, 6 H, H–C(2′), CH_2); 2.72 (*s*, C≡CH); 3.10–3.23 (*m*, 2 H–C(5′)); 3.73 (*s*, 2 MeO); 3.91 (*m*, H–C(4′)); 4.28 (*m*, H–C(3′)); 5.33 (*d*, *J* = 3.63, OH–C(3′)); 6.12 (*t*, *J* = 6.6, H–C(1′)); 6.86–7.42 (*m*, arom. H); 7.87 (*s*, H–C(6)); 11.60 (*s*, NH). Anal. calc. for $C_{38}H_{38}N_2O_7$ (634.27): C 71.91, H 6.03, N 4.41; found: C 71.75, H 6.05, N 4.37.

*1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-5-(octa-1-ynyl)uracil* (= *5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-α-heptylidynethymidine*; **9**). As described for **8**, with **1c** (0.4 g, 1.19 mmol) and 4,4′-dimethoxytrityl chloride (0.48 g, 1.42 mmol). Purification by FC (silica gel, column 15 × 3 cm, CH_2Cl_2/MeOH 95:5) furnished **9** (0.64 g, 84%). Colorless foam. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.31. UV (MeOH): 233 (26000), 284 (9600). ^{1}H-NMR ((D$_6$)DMSO): 0.83 (*t*, Me); 1.18–1.23 (*m*, 4 CH_2); 2.11 (*m*, 2 H–C(2′)); 2.20 (*t*, CH_2); 3.10–3.20 (*m*, 2 H–C(5′)); 3.73 (*s*, 2 MeO); 3.91 (*m*, H–C(4′)); 4.28 (*m*, H–C(3′)); 5.33 (*d*, *J* = 3.63, OH–C(3′)); 6.12 (*t*, *J* = 6.62, H–C(1′)); 6.86–7.42 (*m*, arom. H); 7.87 (*s*, H–C(6)); 11.60 (*s*, NH). Anal. calc. for $C_{38}H_{42}N_2O_7$ (638.30): C 71.45, H 6.63, N 4.39; found: C 71.29, H 6.70, N 4.40.

*1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-5-[3-(prop-2-ynyloxy)prop-1-ynyl]uracil* (= *5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-α-[2-(prop-2-ynyloxy)ethylidyne]thymidine*; **10**). As described for **8**, with **1d** (0.35 g, 1.09 mmol) anh. pyridine (8 ml), and 4,4′-dimethoxytrityl chloride (0.44 g, 1.3 mmol). Purification by FC (silica gel, column 15 × 3 cm, CH_2Cl_2/acetone 80:20) gave **10** (0.58 g, 85%). Colorless foam. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.34. UV (MeOH): 232 (29000), 283 (11000). ^{1}H-NMR ((D$_6$)DMSO): 2.08–2.30 (*m*, 2 H–C(2′)); 3.10–3.28 (*m*, 2 H–C(5′)); 3.47 (*s*, C≡CH); 3.73 (*s*, 2 MeO); 3.92 (*m*, H–C(4′)); 4.07 (*m*, CH_2); 4.17 (*m*, CH_2); 4.28 (*m*, H–C(3′)); 5.33 (*d*, *J* = 4.4, OH–C(3′)); 6.12 (*t*, *J* = 6.52, H–C(1′)); 6.86–7.42 (*m*, arom. H); 7.97 (*s*, H–C(6)); 11.69 (*s*, NH). Anal. calc. for $C_{36}H_{34}N_2O_8$ (622.23): C 69.44, H 5.50, N 4.50; found: C 69.34, H 5.70, N 4.66.

*1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-5-(octa-1,7-diynyl)uracil 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (= *5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-α-(hept-6-ynylidyne)thymidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)*; **2b**). A stirred soln. of **8** (110 mg, 0.17 mmol) in anh. CH_2Cl_2 (5 ml) was pre-flushed with Ar and treated with ^{i}Pr$_2$EtN (55 µl, 0.32 mmol) at r.t. followed by 2-cyanoethyl diisopropylphosphoramidochloridite (59 µl, 0.27 mmol). After stirring for 45 min at r.t., the soln. was diluted with CH_2Cl_2 (30 ml) and extracted with 5% aq. $NaHCO_3$ soln. (20 ml). The org. layer was dried (Na_2SO_4) and concentrated. FC (silica gel, column 10 × 2 cm, CH_2Cl_2/acetone 95:5) gave **2b** (110 mg, 76%). Colorless foam. TLC (CH_2Cl_2/acetone 95:5): R_f 0.70. ^{31}P-NMR ($CDCl_3$): 150.25; 149.76.

*1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-5-(oct-1-ynyl)uracil 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (= *5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-α-heptylidynethymidine 3′-(2-Cynoethyl Diisopropylphosphoramidite)*; **2c**). As described for **2b**, with **9** (110 mg, 0.17 mmol), ^{i}Pr$_2$EtN (55 µl, 0.32 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (59 µl, 0.27 mmol). FC (silica gel, column 10 × 2 cm, CH_2Cl_2/acetone 95:5) gave **2c** (113 mg, 78%). Colorless foam. TLC (CH_2Cl_2/acetone 95:5): R_f 0.66. ^{31}P-NMR ($CDCl_3$): 150.21; 149.74.

*1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-5-[3-(prop-2-ynyloxy)prop-1-ynyl)uracil 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (= *5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-α-[2-(prop-2-ynyloxy)ethylidyne]thymidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)*; **2d**). As described for **2b**, with **10** (250 mg, 0.40 mmol), ^{i}Pr$_2$EtN (130 µl, 0.75 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (122 µl, 0.56 mmol). FC (silica gel, column 10 × 2 cm, CH_2Cl_2/acetone 95:5) gave **2d** (248 mg, 75%). Colorless foam. TLC (CH_2Cl_2/acetone 95:5): R_f 0.71. ^{31}P-NMR ($CDCl_3$): 150.19; 149.65.

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