123. 7-Deazaguanine DNA: Oligonucleotides with Hydrophobic or Cationic Side Chains

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Dedicated to Prof. Dr. *H. Seliger* on the occasion of his 60th birthday

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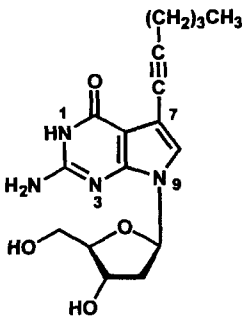
The phosphoramidites **6b** and **9** as well as the phosphonate **6a** derived from 7-(hex-1-ynyl)- and 7-[5-(trifluoroacetamido)pent-1-ynyl]-substituted 7-deaza-2'-deoxyguanosines **1** and **10**, respectively, were prepared (*Scheme 1*). They were employed in solid-phase oligodeoxynucleotide synthesis of the alternating octamers d(hxy^{7}c^{7}G-C)$_4$ (**12**), d(C-hxy^{7}c^{7}G)$_4$ (**13**), and d(npey^{7}c^{7}G-C)$_4$ (**15**) as well as of other oligonucleotides (see **22**–**25**; *Table 2*; hxy = hex-1-ynyl, npey = 5-aminopent-1-ynyl). The T_m values and the thermodynamic data of duplex formation were determined and correlated with the major-groove modification of the DNA fragments. A hexynyl side chain introduced into the 7-position of a 7-deazaguanine residue (see **1**) was found to fit into the major groove without any protrusion. The incorporation of the (5-aminopent-1-ynyl)-modified 7-deaza-2'-deoxyguanosine **2** into single-stranded oligomers of the type **24** and **25** did not lead to change in duplex stability compared to the parent oligonucleotides. The self-complementary oligomer **15** with alternating npey^{7}c^{7}G$_d$ (**2**) and dC units did not lead to a cooperative melting, either due to orientational disorder or interaction of the 5-aminopent-1-ynyl moiety with a base or with phosphate residues nearby or on the opposite strand.

Introduction. – Much efforts have been made in the design of antisense oligonucleotides by replacing the negatively charged phosphodiester group of the DNA backbone while leaving the remainder of the structure chemically unchanged [1] [2]. A different strategy of design was described neutralizing the phosphate negative charge by attaching a cationic group on a lipophilic spacer to each nucleotide base [3]. It was reported that oligonucleotides bearing ω-aminohexyl groups at the 5-position of the natural pyrimidine residues bind to natural DNA as well or even better than does natural DNA with itself. This appeared even when all of the nucleotide residues in a given single strand were rendered zwitterionic. Interestingly, nature has implemented a design of this general type in bacteriophage DNA where half of the thymidine residues are replaced by positively charged α-putrescinylthymidine (= 5-{[(4-aminobutyl)amino]methyl}thymidine), resulting in one positively charged base every eight nucleotides on average [4] [5]. Contrarily, the comparison of DNA duplexes bearing just unbranched hexyl groups showed that the hexyl tether itself has a severe and cumulative problematic effect on the duplex stability of short oligonucleotides [3].

Recently, *Froehler* and coworkers reported that replacement of dG or dA residues within diastereoisomer mixtures of model oligodeoxyphosphorothioates by 7-deaza-2'-deoxy-7-(prop-1-ynyl)guanosines or -adenosines results generally in an increased stability of these oligomers hybridized to RNA relative to 7-unsubstituted 7-deazapurines and non-modified oligonucleotide controls [6]. Despite the fact that these results are not directly comparable, they are in line with our findings on oligodeoxynucleotide duplexes

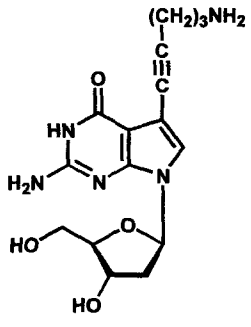
with a regular phosphodiester backbone which are stabilized by 7-substituted 7-deaza-purine residues [7–9].

One of the outstanding features of the 7-position of guanine residues is its deepest electrostatic potential minimum as well as its highest steric accessibility within the major groove of B-DNA. In this regard it was of interest to study the stability of oligonucleotides containing 7-(hex-1-ynyl)- and 7-(5-aminopent-1-ynyl)-substituted 7-deazaguanines, synthesized *e.g.* by means of the deoxynucleoside building blocks **1** (hxy^{7}c^{7}G) and **2** (npey^{7}c^{7}G), and to compare them with those of the non-modified oligomers. Moreover, the latter are central intermediates for the labeling of a preformed DNA molecule carrying side chains which can be functionalized.



1

purine numbering



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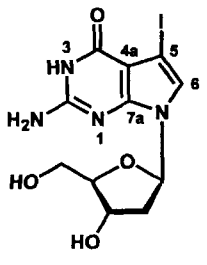


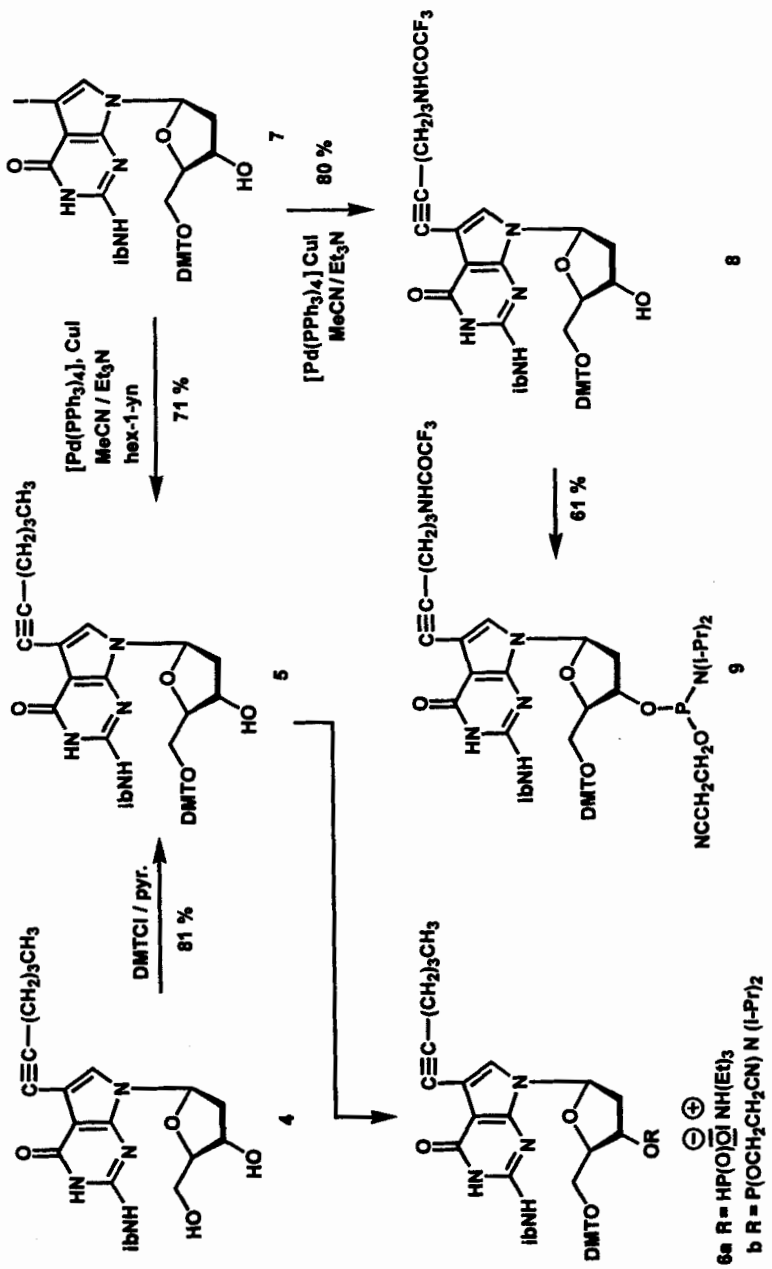
3

systematic numbering

Results and Discussion. – *Monomers.* The 7-deaza-2′-deoxy-7-iodoguanosine (**3**) [10] was used as starting material for the synthesis of the 7-deaza-2′-deoxy-7-(hex-1-ynyl)-guanosine (**1**) employing the palladium-catalyzed cross-coupling reaction [10] [11]. The corresponding protected phosphoramidite **6b** as well as the phosphonate **6a** were then prepared from **1** by a three-step route (*Scheme 1*). First, isobutyryl (i-Bu) protecting group was introduced using the protocol of transient protection [12] (→ **4**). The half-life for the deprotection of the amino group of **4** was determined to be 80 min (25% aq. NH_3, 40°), which is slightly shorter than that of the parent 2-isobutyrylated 2′-deoxyguanosine ($\tau_{1/2}$ 112 min). Subsequently, the intermediate **4** was treated with 4,4′-dimethoxytriphenylmethyl chloride ($(MeO)_2TrCl$) in pyridine to give compound **5**. The latter was also prepared directly by cross-coupling reaction of the 5′-protected 7-deaza-2′-deoxy-7-iodonucleoside **7** [8] with hex-1-yne in MeCN solution. This procedure was particularly useful for the synthesis of a number of different, already protected 7-alkynyl-7-deazapurines from the same precursor which was earlier synthesized by us. From the dimethoxytrityl derivative **5**, the phosphonate **6a** was prepared using PCl_3/*N*-methylmorpholine/1*H*-1,2,4-triazole. Phosphitylation of **5** with 2-cyanoethyl diisopropylphosphoramidochloridite in CH_2Cl_2 in the presence of $(i\text{-}Pr)_2EtN$ furnished the phosphoramidite **6b**.

The 7-deaza-2′-deoxy-7-[5-(trifluoroacetamido)pent-1-ynyl]guanosine derivative **8** was prepared from **7** in a similar fashion as compound **5** (*Scheme 1*). The half-life for the NH_2-protecting group was taken to be that of derivative **4**. The phosphoramidite **9** was subsequently synthesized from **8** analogously to the hexynyl compound **6b**.

Scheme 1

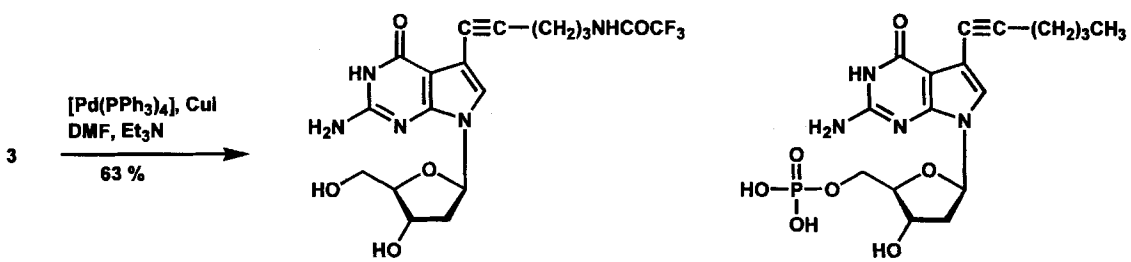


DMT = $(MeO)_2Tr$; ib = $Me_2CHC(O)$, i-Pr = Me_2CH

The 7-deaza-2′-deoxy-7-[5-(trifluoroacetamido)pent-1-ynyl]guanosine **10** was also prepared from 7-deaza-7-iodo-2′-deoxyguanosine **3** (*Scheme 2*) for the conversion into the corresponding 5′-triphosphate which will be described elsewhere. The monophosphate **11** which was needed for identification purposes was obtained from **1** (see *Exper. Part*).

Structural proof of all new compounds was accomplished by ^{1}H-, ^{13}C-, and ^{31}P-NMR spectra (*Table 1* and *Exper. Part*) as well as by elemental analyses. *Table 1* summarizes the ^{13}C-NMR data of the various monomers.

Scheme 2



10 **11**

Table 1. *^{13}C-NMR Shifts of 7-Substituted 7-Deaza-2′-deoxyguanosines in (D_6)DMSO* (303 K)[a])

	C(2)[b]) C(2)[c])	C(6) C(4)	C(5) C(4a)	C(7) C(5)	C(8) C(6)	C(4) C(7a)
1	153.0	157.8	99.4	89.7	120.6	150.0
4	147.4	155.8	100.1	90.7	123.2	146.9
5	147.4	155.8	100.2	90.7	123.1	147.0
6a	147.5	155.9	100.4	90.8	123.0	147.2
8	147.4	155.8	99.9	89.8	123.2	147.1
10	153.0	157.9	99.2	88.9	120.9	150.1
	C(1′)	C(3′)	C(4′)	C(5′)	C(1″)[d])	C(2″)[d])
1	81.9	70.6	86.8	61.6	103.5	73.5
4	82.5	70.8	87.3	61.7	103.4	73.6
5	82.6	70.5	85.5	64.1	103.6	73.5
6a	82.7	72.8	85.5	63.9	103.5	73.4
8	82.6	70.5	85.5	64.1	103.7	73.9
10	82.2	70.9	87.1	61.9	99.5	75.0

[a]) In all cases, the C(2′) signals are superimposed by the solvent. [b]) Purine numbering. [c]) Systematic numbering. [d]) C≡C Atoms.

As can be seen, isobutyrylation of compound **1** (→ **4**) causes typical upfield shifts of C(2), C(6), and C(4) (5.6, 2.0, and 3.1 ppm, resp.) and a slight downfield shift (2.6 ppm) of C(8) (purine numbering). Subsequent dimethoxytritylation of compound **4** (→ **5**) results in a typical downfield shift of C(5′) (2.4 ppm) while all other ^{13}C resonances remain almost unchanged.

Oligonucleotides. The oligonucleotides were prepared by solid-phase synthesis on an automated synthesizer using either H-phosphonate or phosphoramidite chemistry [13]

[14], and were purified on oligonculeotide-purification cartridges. The 3'-terminus-modified oligonucleotides **13** and **14** were synthesized using *'Rainbow Universal CPG'* [15]. The composition of the newly synthesized oligonucleotides **12–26** was proved by MALDI-TOF mass spectrometry which gave the expected molecular weights in all cases (*Table 2*).

Table 2. *Synthesized Oligonucleotides* **12–26**, *Molecular Weights Determined by MALDI-TOF Mass Spectra, and Chromatographic Retention Times* t_R (see *Exper. Part*)

	Oligomer	M^+ (calc.)	M^+ (found)	t_R [min]
12	5'-d(hxy^{7}c^{7}G-C)$_4$-3'	2728.2	2728.2	15.00
13	5'-d(C-hxy^{7}c^{7}G)$_4$-3'	2728.2	2725.7	15.04
14	5'-d(C-I^{7}c^{7}G)$_4$-3'	2911.5	2911.0	12.18
15	5'-d(npey^{7}c^{7}G-C)$_4$-3'	2732.1	[a])	[a])
16	5'-d(TAGGTCAATACT)-3'	3644.4	3644.0	9.10
17	5'-d(ATCCAGTTATGA)-3'	3644.4	3644.8	10.20
18	5'-d(TAc^{7}Gc^{7}GTCAATACT)-3'	3642.5	3642.5	9.54
19	5'-d(ATCCAc^{7}GTTATc^{7}GA)-3'	3642.5	3643.3	9.15
20	5'-d(TAI^{7}c^{7}GI^{7}c^{7}GTCAATACT)-3'	3894.3	3895.7	9.43
21	5'-d(ATCCAI^{7}c^{7}GTTATI^{7}c^{7}GA)-3'	3894.3	3894.2	9.48
22	5'-d(TAhxy^{7}c^{7}Ghxy^{7}c^{7}GTCAATACT)-3'	3802.7	3799.9	9.27
23	5'-d(ATCCAhxy^{7}c^{7}GTTAThxy^{7}c^{7}GA)-3'	3802.7	3800.1	9.89
24	5'-d(TAnpey^{7}c^{7}Gnpey^{7}c^{7}GTCAATACT)-3'	3804.7	3803.7	9.38
25	5'-d(ATCCAnpey^{7}c^{7}GTTATnpey^{7}c^{7}GA)-3'	3804.7	3806.1	10.24
26	5'-d[(hxy^{7}c^{7}G)$_5$-G]-3'	2308.6	2310.1	26.45

[a]) Not detectable.

To investigate the stability of alternating as well as of random duplexes containing 7-deaza-2'-deoxy-7-(hex-1-ynyl)guanosine (**1**, hxy^{7}c^{7}G$_d$) or 7-(5-aminopent-1-ynyl)-7-deaza-2'-deoxyguanosine (**2**, npey^{7}c^{7}G$_d$) opposite to the conventional bases, the hybrids shown in *Tables 3* and *4* were formed, and their T_m values were compared with those of the corresponding regular counterparts as well as with those containing 7-deaza-2'-deoxyguanosine (c^{7}G$_d$) or 7-deaza-2'-deoxy-7-iodoguanosine (**3**, I^{7}c^{7}G$_d$). From the melting profiles, thermodynamic data of duplex formation of all duplexes were calculated applying a stacked-unstacked two-state model as described in [16].

Table 3. T_m *Values and Thermodynamic Data of Self-complementary Oligodeoxynucleotides*[a])

		T_m[b]) [°C]	$\Delta H°$ [kcal/mol]	$\Delta S°$ [cal/mol · K]	H [%]
27 [8]	d(I^{7}c^{7}G-C)$_4$	70	−91.0	−260	26
14	d(C-I^{7}c^{7}G)$_4$	67	−71.6	−210	23
12	d(hxy^{7}c^{7}G-C)$_4$	59	−62.6	−189	23
13	d(C-hxy^{7}c^{7}G)$_4$	59	−75.4	−227	23
28 [8]	d(G-C)$_4$	60	−81.0	−244	26
29 [20]	d(C-G)$_4$	63	−88.8	−264	27
30 [8]	d(c^{7}G-C)$_4$	53	−53.0	−162	26

[a]) Oligomer concentration, 7.5 μM of single strands.
[b]) Measured in 0.1M NaCl containing 10 mM Na-cacodylate and 10 mM $MgCl_2$, pH 7.0.

It has been observed earlier that 7-deaza-2′-deoxyguanosine (c^7G_d) destabilizes a duplex structure of short oligodeoxynucleotides such as $d(c^7G\text{-}C)_4$ (**30**) significantly compared to the parent oligonucleotide $d(G\text{-}C)_4$ (**28**) containing 2′-deoxyguanosine. Much more stable duplexes are, however, formed when a 7-bromo or 7-iodo substituent was introduced (see **27**) [8]. From this finding, it was concluded that the duplex stability can be tuned by an appropriate selection of a 7-substituent within the 7-deazaguanine-cytosine base pair. The structure of a B-DNA is such that a 7-substituent or a linker arm from the 7-position of a base-paired c^7G_d to a reporter group should extent into the major groove with minimal distortion of the helix. To evaluate a critical limit of the size of the 7-substituent within the duplexes described above, the alternating octanucleotides $d(hxy^7c^7G\text{-}C)_4$ (**12**), $d(C\text{-}hxy^7c^7G)_4$ (**13**) as well as $d(C\text{-}I^7c^7G)_4$ (**14**) were examined. A similar stabilization as described above was now also observed for the oligomers **12**–**14** containing 7-iodo- or 7-(hex-1-ynyl)-substituted 7-deazaguanine residues in comparison to oligomers containing 7-unsubstituted 7-deazaguanine (*Table 3*). In comparison with the parent oligonucleotides **28** and **29**, the $hex^7c^7G_d$-containing oligomers exhibit similar T_m values. This is in line with the topology of the major groove of regular B-DNA which reveals a depth of 8.5 Å. The length of a hexynyl chain amounts to 8.4 Å ('Alchemy III' force field, *Tripos Co.*, St. Louis) so that it fits exactly into the major groove without any protrusion. From the CD spectra of alternating $d(C\text{-}I^7c^7G)_4$ (**14**) and $d(I^7c^7G\text{-}C)_4$ (**27**) as well as of $d(C\text{-}hxy^7c^7G)_4$ (**13**) and $d(hxy^7c^7G\text{-}C)_4$ (**12**) (*Fig. 1, b*), it is concluded that all duplexes form B-DNA-type structures. They exhibit similar shapes with a positive *Cotton* effect around either 270 (**13**) or 290 nm (**12**) and a negative lobe at 250 nm (**12** and **13**).

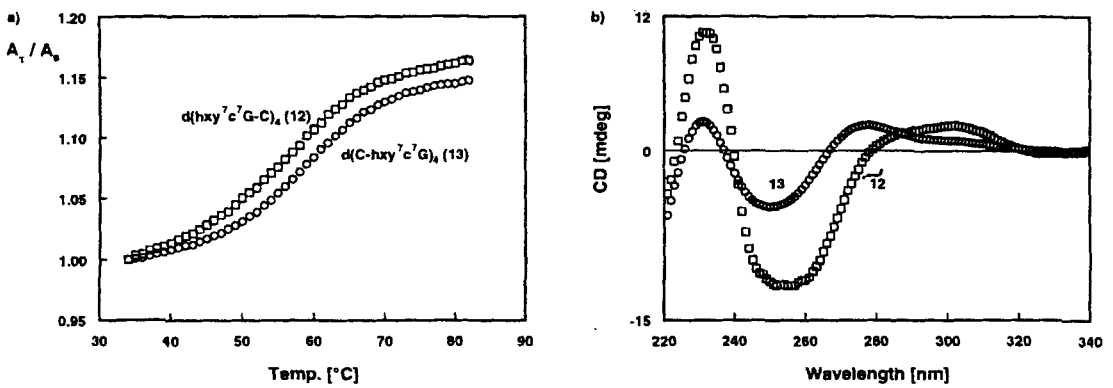


Fig. 1. a) *Normalized melting profiles of self-complementary duplexes* $d(hxy^7c^7G\text{-}C)_4$ (**12**) *and* $d(C\text{-}hxy^7c^7G)_4$ (**13**) *measured at 285 nm in 10 mM Na-cacodylate, 0.1 M NaCl, and 10 mM* $MgCl_2$ *at pH 7.0* (oligomer concentration, 7.5 μM of single strands) *and* b) *CD spectra of self-complementary oligomers* **12** *and* **13** *measured at 20° at 15 μM oligomer concentration* (conditions, see *Fig. 1, a*)

Next, the thermodynamic data of duplex formation (*Table 3*) were examined. The 7-iodo substituents of oligomer **27** stabilize the duplex enthalpically compared to $d(c^7G\text{-}C)_4$ (**30**) as well as to the parent oligomer **28** [8]. Comparison of the thermodynamic data of the oligomers **27** and **14** shows striking differences: the latter, having $I^7c^7G_d$ on the 3′-terminal position, exhibits a significantly lower reaction enthalpy which

is compensated by a more favorable entropy term. On the other hand, a comparison of the oligonucleotides d(hxy^{7}c^{7}G-C)$_4$ (**12**) and d(C-hxy^{7}c^{7}G)$_4$ (**13**) showed that in both cases, the T_m values are similar to those of the parent oligomers **28** and **29** and that the thermodynamic data of duplex formation are mainly driven by a favorable reaction entropy. Thus, analysis of sequences containing either I^{7}c^{7}G$_d$ or hxy^{7}c^{7}G$_d$ suggests that thermal stability of the oligomers **12**–**14** is induced by a hydrophobization of the major groove thus expelling water molecules rather then by increased stacking interactions of the modified bases which takes place in the case of d(I^{7}c^{7}G-C)$_4$.

To examine not only the steric but also charge effects of 7-substituted 7-deazaguanines on the duplex stability of self-complementary oligonucleotides, the alternating octamer **15**, containing npey^{7}c^{7}G$_d$ (**2**), was studied. In contrast to d(hxy^{7}c^{7}G-C)$_4$ (**12**) which forms a stable duplex (T_m 59°), no cooperative melting was observed for d(npey^{7}c^{7}G-C)$_4$ (**15**). Some analogy may be drawn between the interactions of biogenic polyamines with DNA and the interaction of tethered amine in the present case. For example, in a crystallographic study, spermine (= *N*,*N'*-bis(3-aminopropyl)butane-1,4-diamine) was found to bind across the major groove of a B-DNA dodecamer interacting with the phosphate groups of the opposite strands and with a guanine base [17]. It is conceivable that the effect observed with npey^{7}c^{7}G$_d$ in the alternating octamer **15** may be traced back to the orientational disorder where the 5-aminopent-1-ynyl moiety interacts with the base moieties nearby or with the phosphates of the opposite strand. However, there has to be some sequence specificity involved in the thermal stability of oligonucleotides containing npey^{7}c^{7}G$_d$ residues. Thus, incorporation of the (5-aminopent-1-ynyl)-modified 7-deaza-2'-deoxyguanosine **2** into single-strand oligomers of the type **24** and **25** (*Table 4*) led to the formation of the duplex **24** · **25** almost without change in stability compared to the native DNA duplex **16** · **17** as well as those containing I^{7}c^{7}G$_d$ (see **20** · **21**) or hxy^{7}c^{7}G$_d$ (see **22** · **23**) in place of dG (*Fig. 2,a*, *Table 4*).

According to the thermodynamic data of duplex formation (*Table 4*), enthalpic and entropic contributions to duplex stabilities where one or both strands bear modified nucleosides such as I^{7}c^{7}G$_d$ or hxy^{7}c^{7}G$_d$ are not strongly different from those of the

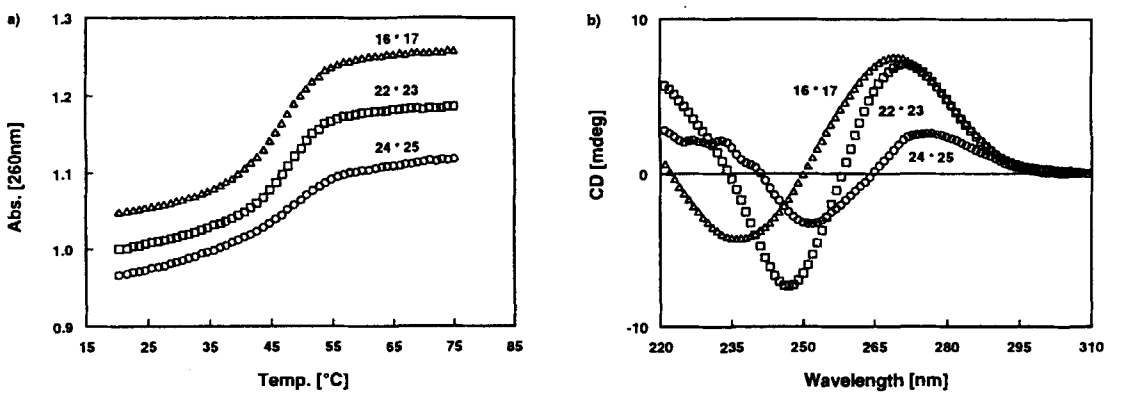


Fig. 2. a) *Melting profiles of hybrids* **16** · **17**, **22** · **23**, *and* **24** · **25** (see *Table 4*) *measured at 260 nm* (oligomer concentration, 10 μM of single strands; conditions, see *Fig. 1,a*) *and* b) *CD spectra of hybrids* **16** · **17**, **22** · **23**, *and* **24** · **25** *measured at 20° at 10 μM single-strand concentration* (conditions, see *Fig. 1,a*)

Table 4. T_m *Values and Thermodynamic Data of Oligonucleotide Duplexes*[a])

		T_m[b]) [°C]	ΔH [kcal/mol]	ΔS [cal/mol · K]	H [%]
16 · 17	5′-d(TAGGTCAATACT)-3′ 3′-d(ATCCAGTTATGA)-5′	47	−103.3	−323	17
18 · 17	5′-d(TAc^{7}Gc^{7}GTCAATACT)-3′ 3′-d(ATCCAGTTATGA)-5′	45	−101.0	−317	13
16 · 19	5′-d(TAGGTCAATACT)-3′ 3′-d(ATCCAc^{7}GTTATc^{7}GA)-5′	46	−106.2	−333	14
18 · 19	5′-d(TAc^{7}Gc^{7}GTCAATACT)-3′ 3′-d(ATCCAc^{7}GTTATc^{7}GA)-5′	44	−103.9	−328	14
20 · 17	5′-d(TAI^{7}c^{7}GI^{7}c^{7}GTCAATACT)-3′ 3′-d(ATCCAGTTATGA)-5′	46	−99.4	−310	14
16 · 21	5′-d(TAGGTCAATACT)-3′ 3′-d(ATCCAI^{7}c^{7}GTTATI^{7}c^{7}GA)-5′	49	−117.9	−366	12
20 · 21	5′-d(TAI^{7}c^{7}GI^{7}c^{7}GTCAATACT)-3′ 3′-d(ATCCAI^{7}c^{7}GTTATI^{7}c^{7}GA)-5′	48	−111.7	−348	15
22 · 17	5′-d(TAhxy^{7}c^{7}Ghxy^{7}c^{7}GTCAATACT)-3′ 3′-d(ATCCAGTTATGA)-5′	47	−90.6	−284	13
16 · 23	5′-d(TAGGTCAATACT)-3′ 3′-d(ATCCAhxy^{7}c^{7}GTTAThxy^{7}c^{7}GA)-5′	46	−102.8	−322	14
22 · 23	5′d(TAhxy^{7}c^{7}Ghxy^{7}c^{7}GTCAATACT)-3′ 3′-d(ATCCAhxy^{7}c^{7}GFTTAThxy^{7}c^{7}GA)-5′	49	−120.4	−374	15
24 · 17	5′-d(TAnpey^{7}c^{7}Gnpey^{7}c^{7}GTCAATACT)-3′ 3′-d(ATCCAGTTATGA)5′	48	−51.8	−162	15
16 · 25	5′-d(TAGGTCAATACT)-3′ 3′-d(ATCCAnpey^{7}c^{7}GTTATnpey^{7}c^{7}GA)-5′	50	−83.0	−257	13
24 · 25	5′-d(TAnpey^{7}c^{7}Gnpey^{7}c^{7}GTCAATACT)-3′ 3′-d(ATCCAnpey^{7}c^{7}GTTATnpey^{7}c^{7}GA)-5′	48	−70.3	−220	13

[a]) Oligomer concentration, 10 μM of single strands.
[b]) Conditions, see *Table 3*.

natural duplex. Apparently, both 7-iodo- and 7-(hex-1-ynyl) substituents stabilize the duplex structure (see **20 · 21** and **22 · 23**) enthalpically but lead to an entropic destabilization. This is, however, opposite to the data observed for the oligonucleotides with 7-(5-aminopent-1-ynyl)-7-deazaguanine residues (see **24 · 17**, **16 · 25**, and **24 · 25**). The incorporation of two consecutive npey^{7}c^{7}G$_d$ residues (see **24**) into the duplex results in a significantly lower enthalpy and a more favorable entropy value of duplex formation (see **24 · 17**). In the case of the duplex **16 · 25** having also two npey^{7}c^{7}G$_d$ residues but in non-consecutive positions, the thermodynamic data are more similar to those of the non-modified duplex **16 · 17**. When both strands contain two npey^{7}c^{7}G$_d$ residues each (see **24 · 25**), the thermodynamic data of duplex formation are in-between the above described data. In the latter case, the accumulation of four tethered ammonium ions is obviously responsible for the favorable entropy term. Generally, the solvation of oligomers having tethered ammonium ions is better in the single- than in the double-

stranded form. Base-tethered cations should displace metal ions normally neutralizing negative phosphate charges, resulting in attendant changes in charge and size of monomer units [18]. The four tethered ammonium ions of the duplex **24** · **25** clearly have a substantial electrostatic influence on the stability of the oligonucleotide. This becomes obvious when the T_m values of **24** · **25** as well as of the parent duplex **16** · **17** were measured as a function of NaCl concentration over a range of 0.1 to 4M.

A plot of T_m *vs.* $^{10}\log c_{NaCl}$ (*Fig. 3*) shows that the non-modified duplex shows a linear relationship up to 0.5M of c_{NaCl} with a slope of 4 K/decade of salt concentration. Above this value, T_m passes through a maximum and then decreases with further increase of NaCl concentration which is in accordance with counter ion condensation theory [19]. In case of the modified duplex **24** · **25** this linearity of T_m *vs.* $^{10}\log c_{NaCl}$ holds up to 2M of NaCl with about the same slope (3.6 K/decade of c_{NaCl}). This finding on the modified duplex implies that the tethered ammonium ions exert a kind of 'buffering' on the duplex stability with respect to ionic strength. The CD spectra of the modified base containing duplexes **22** · **23** and **24** · **25** do not differ significantly from that of the parent **16** · **17** which shows that **22** · **23** and **24** · **25** form a B-like duplex as well (*Fig. 2, b*).

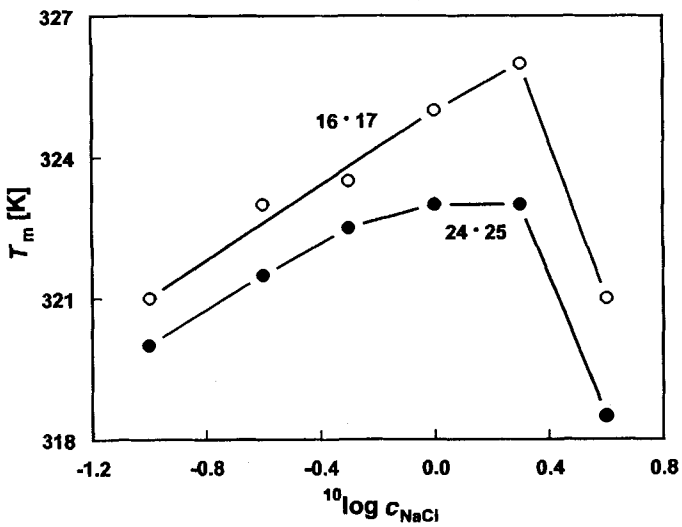


Fig. 3. *Plot of* T_m vs. $^{10}\log c_{NaCl}$ *of duplexes* **16** · **17** *and* **24** · **25** *measured at 260 nm in 10 mM Na-cacodylate and 10 mM* $MgCl_2$ *at pH 7.0.* Oligomer concentration, 10 μM of single strands.

To confirm the composition of the oligomers **12**, **13**, **22**, and **23**, enzymatic hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase was performed. According to the HPLC pattern (*Fig. 4*) and according to earlier findings [7] [20], oligonucleotides containing 7-substituted 7-deaza-2′-deoxyguanosines are hydrolyzed significantly slower by exonucleases compared to the unmodified oligonucleotide controls. Moreover, as can be seen from *Fig. 4, d*, the oligonucleotide **23** shows – after subsequent addition of alkaline phosphatase – a cleavage pattern with five peaks instead of four which was expected from the structure. The additional peak was assigned to 7-deaza-2′-deoxy-7-(hex-1-ynyl)guanosine 5′-monophosphate (**11**) as established by in-

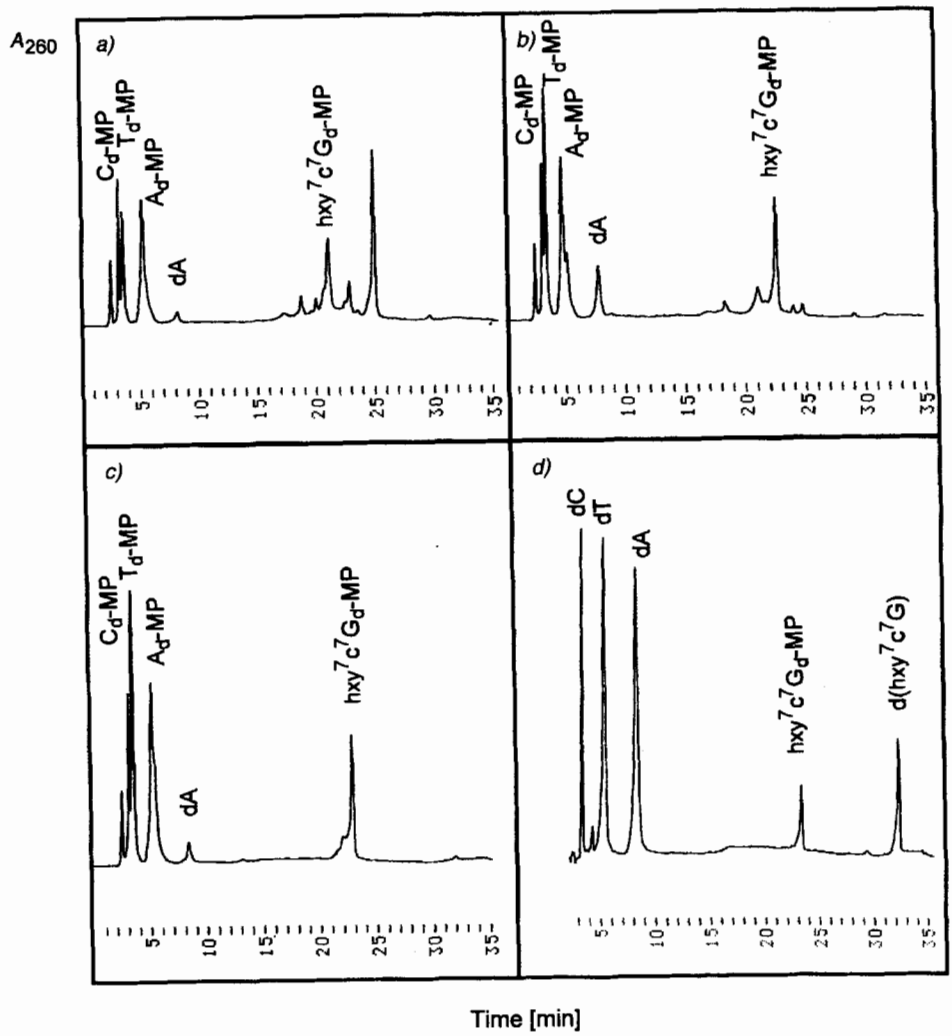


Fig. 4. *Reversed-phase HPLC pattern (RP-18) of the reaction products obtained during enzymatic hydrolysis of oligonucleotide* **23** *by snake-venom phosphodiesterase at 37° after* a) *10 min*, b) *1 h*, c) *30 h, and* d) *30 h after subsequent addition of alkaline phosphatase.* For details, see *Exper. Part.*

dependent synthesis (see *Exper. Part*). Similar results were obtained also from the enzymatic cleavage of the oligomers **12**, **13**, and **22** (data not shown). This suggests that 7-deaza-2′-deoxy-7-(hex-1-ynyl)guanosine monophosphate is either difficult to be dephosphorylated or even can act as alkaline phosphatase inhibitor preventing the enzymatic cleavage to go to completion.

To complete the study of the sequence dependence of $hxy^7c^7G_d$-dC base pair stability, also the homomeric oligonucleotide d[$(hxy^7c^7G)_5$-G] (**26**) was hybridized with $d(C_6)$. The stoichiometry of this complex was determined from a UV-mixing curve at 5° and was found to be 1:1 (*Fig. 5a*). UV-Spectrophotometric melting experiments of **26** · $d(C)_6$ at

different oligomer concentrations demonstrated that the thermal stability of the duplex is low ($T_m < 15°$). These results are in line with our finding that a consecutive arrangement of bulky and hydrophobic 7-substituents such as Br or I of $Br^7c^7G_d$ and $I^7c^7G_d$ along the major groove of a double helix formed by hybridization with oligo(dC) leads to duplex destabilization compared to c^7G_d [8]. This is probably due to a desiccation and therewith to a shrinking of a major groove. As a consequence, the DNA evades from a B-type structure into other DNA structures with less steric stress. In the case of **26** · $d(C_6)$ – like in the cases of $d[(Br^7c^7G)_5\text{-}G] \cdot d(C_6)$ and $d[(I^7c^7G)_5\text{-}G] \cdot d(C_6)$ [8] – an A-type secondary structure is formed which is confirmed by CD spectroscopy. Already the single-stranded $d[(hxy^7c^7G)_5\text{-}G]$ exhibits the typical pattern of A-DNA, and this pattern is conserved in the case of **26** · $d(C_6)$ (*Fig. 5, b*).

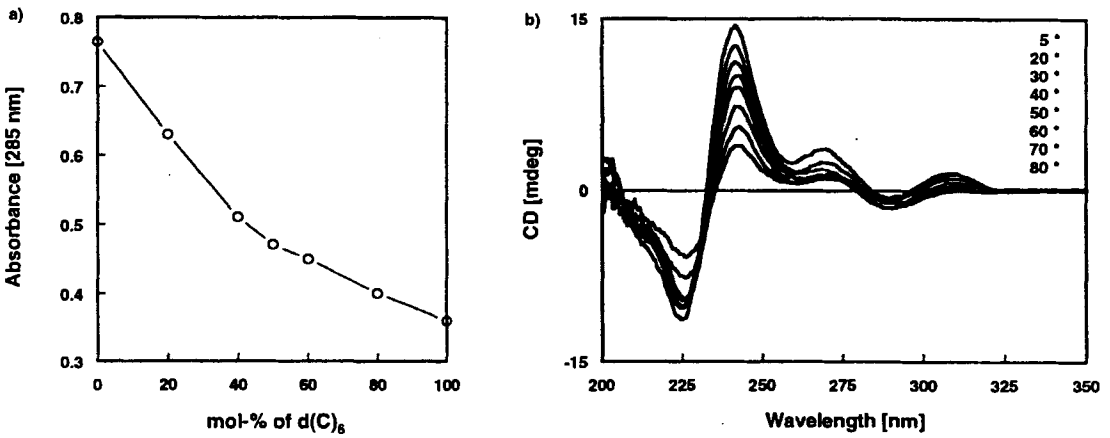
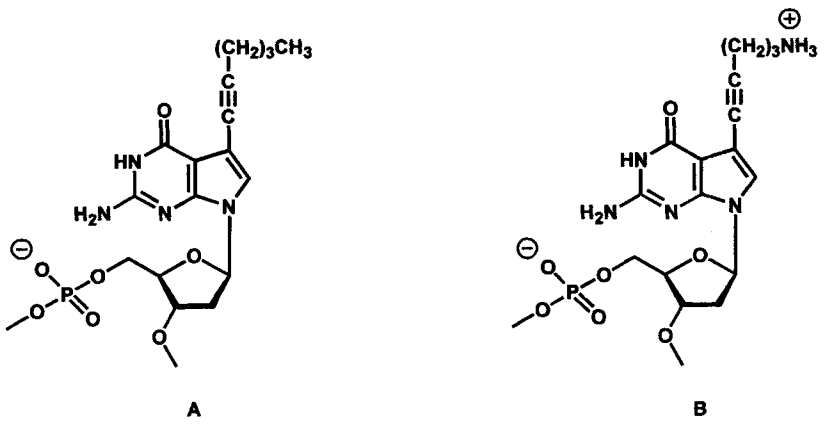


Fig. 5. a) *UV Mixing curve of* $d[(hxy^7c^7G)_5\text{-}G]$ (**26**) *with* $d(C_6)$ *in 10 mM Na-cacodylate, 0.1 M NaCl, and 10 mM* $MgCl_2$ *at pH 7 measured at 5°* (UV monitoring at 285 nm for the mol fraction of **26** rel. to $d(C_6)$ (100 = pure $d(C_6)$, 0 = pure **26**)) *and* b) *temperature-dependent CD spectrum of the complex* **26** · $d(C_6)$ *at 10 μM single-strands concentrations* (conditions, see *Fig. 5, a*)

Conclusion. – From the results described above it is concluded that neither the incorporation of 7-deaza-2′-deoxy-7-(hex-1-ynyl)guanosine (see **A**) nor that of 7-(5-aminopent-1-ynyl)-7-deaza-2′-deoxyguanosine (see **B**) or 7-deaza-2′-deoxy-7-iodoguanosine (**3**) instead of dG into a duplex with a random sequence (see **16** · **17**) alters its duplex stability or the secondary structure (B-DNA) significantly. Only in the case of alternating or homomeric sequences, a stabilization or destabilization of the corresponding modified oligodeoxynucleotides compared to their unmodified counterparts can be observed. These findings are important in the context with recent results from our laboratory that 7-deaza-2′-deoxy-7-(hex-1-ynyl)guanosine triphosphate as well as 7-(5-aminopent-1-ynyl)-7-deaza-2′-deoxyguanosine triphosphate are accepted by various DNA-polymerases upon the polymerization on a single-stranded template. By this way, DNA fragments of more than 500 base pairs in length containing several consecutive $hxy^7c^7G_d$ have been synthesized [21]. This indicates that the restriction to the hybridization of oligonucleotides with consecutive 7-substituted 7-deaza-2′-deoxyguanosine residues is limited to short duplexes and is not problematic in the case of high-molecular DNA.



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Experimental Part

General. See [8]. Flash chromatography (FC): at 0.5 bar on silica gel *60 H* (*Merck*, Germany); solvent systems for FC and TLC: CH_2Cl_2/MeOH 80:20 (*A*); CH_2Cl_2/MeOH 90:10 (*B*); CH_2Cl_2/acetone 85:15 (*C*); CH_2Cl_2/MeOH/Et_3N 88:10:2 (*D*); CH_2Cl_2/AcOEt/Et_3N 69:30:1 (*E*). CD Spectra: *Jasco-600* spectropolarimeter, thermostatically controlled 1-cm cuvettes with a *Lauda-RCS-6* bath. Melting curves: *Cary-1 E-UV/VIS* spectrophotometer (*Varian*, Australia) equipped with a *Cary* thermoelectrical controller; the actual temp. was measured in the reference cell with a Pt-*100* resistor. NMR Spectra: *AMX-500* and *AC-250* spectrometers (*Bruker*, Germany); δ in ppm rel. to internal $SiMe_4$ (^1H, ^{13}C) and to external 85% H_3PO_4 soln. (^{31}P). MALDI-TOF Mass spectra of the oligonucleotides **12**–**26** were measured by Mrs. *Stephanie Hahner* and Mrs. *Julia Gross* (Institute of Medical Physics and Biophysics, Westfälische Wilhelms-Universität Münster) on a home-built apparatus.

*2-Amino-7-(2-deoxy-β-*D-erythro-*pentofuranosyl)-5-(1-hex-1-ynyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**1**). Compound **1** was synthesized according to [10] starting from **3** (1.5 g, 3.8 mmol) and was further characterized: 1.04 g (74%) of colorless crystals. M.p. 203–205°. TLC (silica gel, *A*): R_f 0.75. UV (H_2O): 272 (10100), 291 (sh, 8800). Anal. calc. for $C_{17}H_{22}N_4O_4$ (346.4): C 58.95, H 6.40, N 16.17; found: C 58.88, H 6.54, N 16.16.

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-5-(hex-1-ynyl)-3,7-dihydro-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-*d*]-pyrimidin-4-one* (**4**). Compound **1** (520 mg, 1.5 mmol) was dried by co-evaporation with pyridine (3 × 5 ml). The residue was suspended in pyridine (8 ml), and Me_3SiCl (0.96 ml, 7.5 mmol) was added at r.t. After 15 min stirring, the soln. was treated with isobutyric anhydride (1.23 ml, 7.6 mmol) and maintained at r.t. for 3 h. The mixture was cooled in an ice bath, H_2O (1.5 ml) and subsequently (5 min later) 25% aq. NH_3 soln. (1.5 ml) were added, and stirring was continued for 15 min. The soln. was evaporated to near dryness and the residue crystallized from H_2O. The solid was filtered, washed with Et_2O and dried by vacuum desiccation: 580 mg (93%) of **4** colorless crystals. TLC (silica gel, *B*): R_f 0.46. UV (H_2O): 300 (9400), 286 (9700), 240 (14900). ^{1}H-NMR ((D$_6$)DMSO): 11.74 (*s*, NH); 11.49 (*s*, NH); 7.43 (*s*, H–C(6)); 6.36 (*t*, H–C(1')); 5.20 (*d*, OH–C(3')); 4.88 (*t*, OH–C(5')); 4.31 (*m*, H–C(3')); 3.78 (*m*, H–C(4')); 3.51 (*m*, 2H–C(5')); 2.74 (*m*, Me_2CH); 2.37 (*m*, H_b–C(2'), CH_2); 2.12 (*m*, H_a–C(2')); 1.47 (*m*, CH_2CH_2); 1.10 (*m*, Me_2CH); 0.89 (*m*, Me). Anal. calc. for $C_{21}H_{28}N_4O_5$ (416.5): C 60.56, H 6.78, N 13.45; found: C 60.38, H 6.90, N 13.29.

*7-[2-Deoxy-5-*O*-(4,4'-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-5-(hex-1-ynyl)-3,7-dihydro-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**5**). *Method A.* Compound **4** (570 mg, 1.37 mmol) was dried by repeated co-evaporation from anh. pyridine and then dissolved in pyridine (15 ml). After addition of 4,4'-dimethoxytrityl chloride (567 mg, 1.67 mmol), the soln. was stirred overnight at r.t. MeOH (5 ml) and 5% aq.

$NaHCO_3$ soln. (60 ml) were added. The aq. layer was extracted 3× with CH_2Cl_2 and the org. layer dried (Na_2SO_4) and evaporated. FC (silica gel, 0–20% acetone/CH_2Cl_2) afforded 800 mg (81%) of **5** as colorless foam.

Method B. A soln. of **7** (765 mg, 1 mmol) in dry MeCN (5 ml) was flushed with Ar. CuI (38.1 mg, 0.2 mmol), Et_3N (0.28 ml, 2 mmol), tetrakis(triphenylphosphine)palladium(0) (40.5 mg, 0.1 mmol), and hex-1-yne (492 mg, 6 mmol) were added. After stirring overnight at r.t., 5% aq. $NaHCO_3$ soln. (5 ml) was added and the mixture extracted with CH_2Cl_2 (2 × 15 ml). The org. layer was dried (Na_2SO_4), filtered, and evaporated. FC (silica gel, 0–5% MeOH/CH_2Cl_2) afforded 511 mg (71%) of **5**. Colorless foam. TLC (silica gel, *C*): R_f 0.5. ^{1}H-NMR ((D$_6$)DMSO): 11.78 (*s*, NH); 11.53 (*s*, NH); 7.36–7.25 (*m*, 9 arom. H, H–C(6)); 6.85 (*m*, 4 arom. H); 6.39 (*t*, H–C(1′)); 5.28 (*d*, OH–C(3′)); 4.33 (*m*, H–C(3′)); 3.92 (*s*, 2 MeO); 3.17 (*m*, H_a–C(5′)); 3.11 (*m*, H_b–C(5′)); 2.76 (*m*, Me_2CH); 2.37 (*m*, H_b–C(2′), CH_2); 2.22 (*m*, H_a–(2′)); 1.48–1.42 (*m*, CH_2CH_2); 1.12 (*m*, Me_2CH); 0.88 (*m*, Me). Anal. calc. for $C_{42}H_{46}N_4O_7$ (718.9): C 70.18, H 6.45, N 7.79; found: C 70.18, H 6.59, N 7.71.

*7-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-5-(hex-1-ynyl)-3,7-dihydro-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-d]pyrimidin-4-one 3′-(Triethylammonium Phosphonate)* (**6a**). To a soln. of PCl_3 (225 µl, 2.5 mmol) and *N*-methylmorpholine (2.7 ml) in CH_2Cl_2 (15 ml) was added 1*H*-1,2,4-triazole (600 mg, 8.7 mmol). The soln. was cooled to 0°, **5** (360 mg, 0.5 mmol) in CH_2Cl_2 (10 ml) added slowly, stirring continued for 30 min at 0°, and then the mixture poured into 1M (Et_3NH)HCO_3 (TBK, pH 8.0, 25 ml). The aq. layer was extracted with CH_2Cl_2 (3 × 30 ml) and the combined org. extract dried (Na_2SO_4) and evaporated. FC (silica gel, CH_2Cl_2/Et_3N 98:2, then *D*) furnished a main zone which was evaporated. The residue was dissolved in CH_2Cl_2, extracted with aq. 0.1M TBK (8 × 20 ml), dried (Na_2SO_4), and evaporated: **6a** (220 mg, 50%). Colorless foam. TLC (silica gel, *D*): R_f 0.7. ^{1}H-NMR ((D$_6$)DMSO): 11.77 (*s*, NH); 11.73 (*s*, NH); 7.36–7.22 (*m*, 9 arom. H, H–C(6), PH); 6.85 (*m*, 4 arom. H); 6.43 (*t*, H–C(1′)); 6.05 (*s*, PH); 4.73 (*m*, H–C(3′)); 4.09 (*m*, H–C(4′)); 3.73 (*s*, 2 MeO). ^{31}P-NMR ((D$_6$)DMSO): 2.89 (*dd*, ^1J (P, H) = 584.6, ^3J (P, H) = 8.1). Anal. calc. for $C_{48}H_{62}N_5O_9P$ (884.0): C 65.22, H 7.07, N 7.92; found: C 65.28, H 7.22, N 8.10.

*7-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-5-(hex-1-ynyl)-3,7-dihydro-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-d]pyrimidin-4-one 3′-[2-Cyanoethyl* N,N-*Diisopropylphosphoramidite]* (**6b**). A soln. of **5** (300 mg, 0.42 mmol) in anh. CH_2Cl_2 (5 ml) was pre-flushed with Ar. Then (i-Pr)$_2$EtN (140 µl, 0.84 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (125 µl, 0.55 mmol) were added under Ar. After stirring for 0.5 h, 5% aq. $NaHCO_3$ soln. (5 ml) was added and the mixture extracted with CH_2Cl_2 (2 × 10 ml). The org. layer was dried (Na_2SO_4), filtered, and evaporated. FC (silica gel, *E*) gave a mixture **6b** of diastereoisomers as colorless foam (220 mg, 57%). TLC (silica gel, *E*): R_f 0.8. ^{31}P-NMR ($CDCl_3$): 148.71, 148.24.

*7-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-5-[5-(trifluoroacetamido)pent-1-ynyl]-4*H*-pyrrolo[2,3-d]pyrimidin-4-one* (**8**). As described for **5** (*Method B*), with **7** (1.53 g, 2 mmol), [Pd(0)(PPh$_3$)$_4$] (116 mg, 0.1 mmol), CuI (40.4 mg, 0.2 mmol), Et_3N (0.56 ml, 4 mmol), and 2,2,2-trifluoro-*N*-(pent-4-ynyl)acetamide (1.1 g, 6 mmol). FC (silica gel, 0–20% acetone/CH_2Cl_2) gave 1.3 g (80%) of **8**. Colorless foam. TLC (silica gel, *C*): R_f 0.5. ^{1}H-NMR ((D$_6$)DMSO): 11.81 (*s*, NH); 11.53 (*s*, NH); 9.47 (*t*, NHCOCF$_3$); 7.37–7.20 (*m*, 9 arom. H, H–C(6)); 6.85 (*m*, 4 arom. H); 6.39 (*t*, H–C(1′)); 5.27 (*d*, OH–C(3′)); 4.32 (*m*, H–C(3′)); 3.90 (*m*, H–C(4′)); 3.72 (*s*, 2 MeO); 3.30 (*m*, CH_2); 3.17 (*m*, H_a–C(5′)); 3.10 (*m*, H_b–C(5′)); 2.76 (*m*, Me_2CH); 2.41 (*m*, CH_2); 2.23 (*m*, H–C(2′)); 1.74 (*m*, CH_2); 1.12 (*m*, Me_2CH).

*7-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-5-[5-(trifluoroacetamido)pent-1-ynyl]-4*H*-pyrrolo[2,3-d]pyrimidin-4-one 3′-[2-Cyanoethyl* N,N-*Diisopropylphosphoramidite]* (**9**). As described for **6b**, **8** (342 mg, 0.42 mmol) was reacted with (i-Pr)$_2$EtN (140 µl, 0.84 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (130 µl, 0.57 mmol): 230 mg (61%), of **9**. Yellowish foam. TLC (silica gel, *E*): R_f 0.6. ^{31}P-NMR ($CDCl_3$): 148.77, 148.15.

*2-Amino-7-(2-deoxy-β-*D*-*erythro*-pentofuranosyl)-3,7-dihydro-5-[5-(trifluoroacetamido)pent-1-ynyl]-4*H*-pyrrolo[2,3-d]pyrimidin-4-one* (**10**). A suspension of **3** (1.6 g, 4 mmol) and CuI (152.4 mg, 0.8 mmol) in anh. DMF (20 ml) was treated with 2,2,2-trifluoro-*N*-(pent-4-ynyl)acetamid (2.15 g, 12 mmol), anh. Et_3N (1.12 ml, 8 mmol), and [Pd(0)(PPh$_3$)$_4$] (162 mg, 0.4 mmol) under Ar at r.t. overnight. The mixture was diluted with MeOH/CH_2Cl_2 1:1 (40 ml) and *Dowex 1 × 8* (100–200 mesh; 4 g, hydrogen carbonate form) was introduced. After 15 min, stirring, the evaluation of gas ceased. Stirring was continued for another 30 min, the mixture filtered, and the resin washed with MeOH/CH_2Cl_2 1:1 (120 ml). The combined filtrates were evaporated and the residue submitted to column chromatography (silica gel, 10–20% MeOH/CH_2Cl_2): 1.1 g (63%) of **10**. Yellowish solid. TLC (silica gel, *A*): R_f 0.8. UV (H_2O): 289 (9200), 272 (10800). ^{1}H-NMR ((D$_6$)DMSO): 10.41 (*s*, NH); 9.46 (*t*, NHCOCF$_3$); 7.12 (*s*, H–C(6)); 6.28–6.24 (*m*, H–C(1′), NH_2); 5.17 (*d*, OH–C(3′)); 4.86 (*m*, OH–C(5′)); 4.25 (*m*, H–C(3′)); 3.74 (*m*, H–C(4′)); 3.48 (*m*, 2 H–C(5′)); 3.32 (*m*, CH_2); 2.39 (*m*, CH_2); 2.27 (*m*, H_b–C(2′)); 2.06 (*m*, H_a–C(2′)); 1.73 (*m*, CH_2).

*2-Amino-7-(2-deoxy-β-*D*-*erythro*-pentofuranosyl)-5-(hex-1-ynyl)-3,7-dihydro-4*H*-pyrrolo[2,3-d]pyrimidin-4-one 5'-[Bis(triethylammonium) Monophosphate]* (**11** · $2Et_3N$). A soln. of **1** (100 mg, 0.3 mmol) in trimethyl phosphate (2.5 ml, 10.6 mmol) was cooled to 0°. Freshly distilled $POCl_3$ (116 µl, 1.24 mmol) was added and the mixture stored at 4°. After 7 h, the mixture was neutralized with 1M aq. $(Et_3NH)HCO_3$ (TBK). The residue obtained upon evaporation was chromatographed on *DEAE Sephadex* (column 30 × 2.5 cm, HCO_3^- form) with H_2O (1 l) and then with a linear gradient of $(Et_3NH)HCO_3$ (0–0.5M, 2 l). From the main zone **11** (78.2 mg, 42%) was isolated. ^{13}P-NMR (D_2O/0.1M *Tris* · HCl buffer 1:1): 3.65.

Oligonucleotide Synthesis and Characterization. Oligonucleotide synthesis was carried out on DNA synthesizers, model *380 B* for phosphonates and model *392* (*Applied Biosystems*, Weiterstadt, Germany) for phosphoramidites on a 1-µmol scale. Purification of the oligonucleotides was performed using oligonucleotide purification cartridges (*OPC*, *Applied Biosystems*). After desalting, 19–29 A_{260} units of **12**–**26** were obtained (see *Table 2*).

The oligomers were characterized by MALDI-TOF mass spectrometry as well as by chromatographic mobility on ion-exchange (*NucleoPac PA-100*, *Dionex*) HPLC (*Table 2*); for details see [22]. The following buffers were used for ion-exchange HPLC: buffer *A*: 25 mM *Tris* · HCl, 1 mM EDTA (pH 8)/MeCN 9:1; buffer *B*: 25 mM *Tris*-HCl, 1M NaCl, 1 mM EDTA (pH 8)/MeCN 9:1. For chromatography, a linear gradient was applied: 0–30 min, 20–80% *B* in *A*; 30–35 min, 80% *B* in *A*; flow rate, 0.75 ml/min.

The following extinction coefficients (ε_{260}) were used to calculate the ε_{260} values of the oligodeoxynucleotides: dA (15200), dT (8800), dC (7300), dG (11700), c^7G_d (13000), $I^7c^7G_d$ (11000), $hxyc^7G_d$ (7600). For the ε_{260} of $npey^7c^7G_d$, the same value as for $hxy^7c^7G_d$ was taken. Thermal hypochromicity values (10–80°) of the single-stranded oligonucleotides **16**–**25** amount to 15–20%. Hypochromicity values of double-stranded oligonucleotides are given in *Tables 3* and *4*.

Enzymatic Oligonucleotide Hydrolysis. The oligonucleotides (0.8 A_{260} units) in 1 ml of 0.1M *Tris* · HCl buffer (pH 8.3) were hydrolyzed by snake-venom phosphodiesterase (0.009 units). The reaction was followed by reversed-phase (*RP-18*) chromatography. The following buffer was used for a linear gradient: 0.1M $(Et_3NH)OAc$ (pH 7)/MeCn 95:5 (*A*); MeCN (*B*). Gradient: 0–25 min, 5–20% *B* in *A*; 25–35 min. 20% *B* in *A*; 35–40 min, 20–5% *B* in *A*.

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