Oligonucleotides Containing 7-Deazaadenines: The Influence of the 7-Substituent Chain Length and Charge on the Duplex Stability

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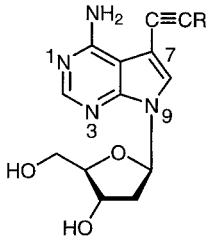
Oligonucleotides carrying alkynyl and aminoalkynyl chains at the position 7 of 7-deazaadenine are synthesized, and the chain lengths as well as the bulkiness of the substituents are varied. The corresponding nucleosides **1a**–**f** are prepared from 7-deaza-2′-deoxy-7-iodoadenosine and the particular alkynes by the Pd^0-catalyzed cross-coupling reaction. The nucleosides are converted to the phosphoramidites **2a**–**f**, which are used in solid-phase oligonucleotide synthesis. The stability of the duplexes is determined by the T_m values and the thermodynamic data. Compared to adenine or the unsubstituted 7-deazaadenine, the incorporation of a 7-ethynyl chain in a 7-deazaadenine moiety increases the duplex stability significantly, while a dodecynyl residue or a bulky steroid moiety leads to a duplex destabilization. A 3-aminoprop-1-ynyl residue (see **1g**) or a 5-aminopent-1-ynyl residue (see **1h**), which are charged under neutral conditions, lead to zwitterionic DNA. A high density of charged residues as found in homomers impairs duplex formation, most probably by counter-ion condensation.

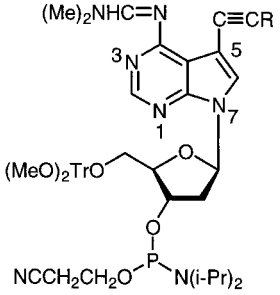
Introduction. – Bulky iodo substituents or hydrophobic hexynyl chains at the 7-position of a 7-deazapurine moiety (purine numbering is used throughout the general section) are well accommodated in the major groove of oligonucleotide duplexes [1–3]. Generally, an increased duplex stability is observed when such residues are attached to 7-deazaadenine or 7-deazaguanine moieties [1–5]. Comparable effects were reported when the 7-deazaguanine moiety carries a 5-aminopent-1-ynyl side chain which is positively charged under physiological conditions [3]. Nature has implemented this type of structure in bacteriophage DNA where half of the thymidine residues are replaced by α-putrescinylthymidine (=α-[(4-aminobutyl)amino]thymidine) [6][7]. This zwitterionic DNA has been shown to resist a variety of endonucleases and exonucleases [6][8][9], and in the case of 5-aminoalkylated pyrimidines, it was found that these oligomers hybridize with natural single-stranded DNA as well or even better than natural DNA itself does [10]. Furthermore, these partially neutralized DNA fragments are membrane-permeable [11–13] and can find application in the transfection of DNA molecules [14][15].

In the case of the alkynylated pyrimidines, it was observed that the length and the structure of the side chain is of decisive importance for the duplex stability [16]. This paper describes the influence of the various 7-substituents of 7-deazaadenine on the stability of DNA duplexes. With regard to this, a series of phosphoramidites (see **2a**–**f**) were synthesized containing 7-deazaadenine as base which carries an ethynyl (see **1a**) [17], dodecynyl (see **1b**), phenylethynyl (see **1c**) [17], or an estradiol-modified ethynyl residue (see **1d**) at position 7. Also, 3-aminoprop-1-ynyl- or 5-aminopent-1-ynyl side chains (see **1g**,**h**) were introduced, yielding zwitterionic DNA. In this case, the positive

charge is located in the major groove in the vicinity of the sugar-phosphate backbone. These aminoalkynylated compounds are precursor molecules for the introduction of fluorescence labels or other reporter groups [18–27]. This matter will be described elsewhere [23].



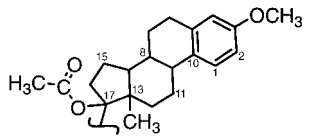
purine numbering



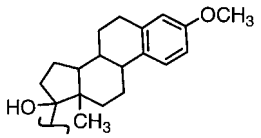
systematic numbering

1a R = H
b R = $Me(CH_2)_9$
c R = Ph
d R = 17β-acetoxy-3-methoxy-estra-1,3,5(10)-trien-17α-yl
e R = $CF_3CONHCH_2$
f R = $CF_3CONH(CH_2)_3$
g R = H_2NCH_2
h R = $H_2N(CH_2)_3$
i R = 17β-hydroxy-3-methoxy-estra-1,3,5(10)-trien-17α-yl

2a R = H
b R = $Me(CH_2)_9$
c R = Ph
d R = 17β-acetoxy-3-methoxy-estra-1,3,5(10)-trien-17α-yl
e R = $CF_3CONHCH_2$
f R = $CF_3CONH(CH_2)_3$



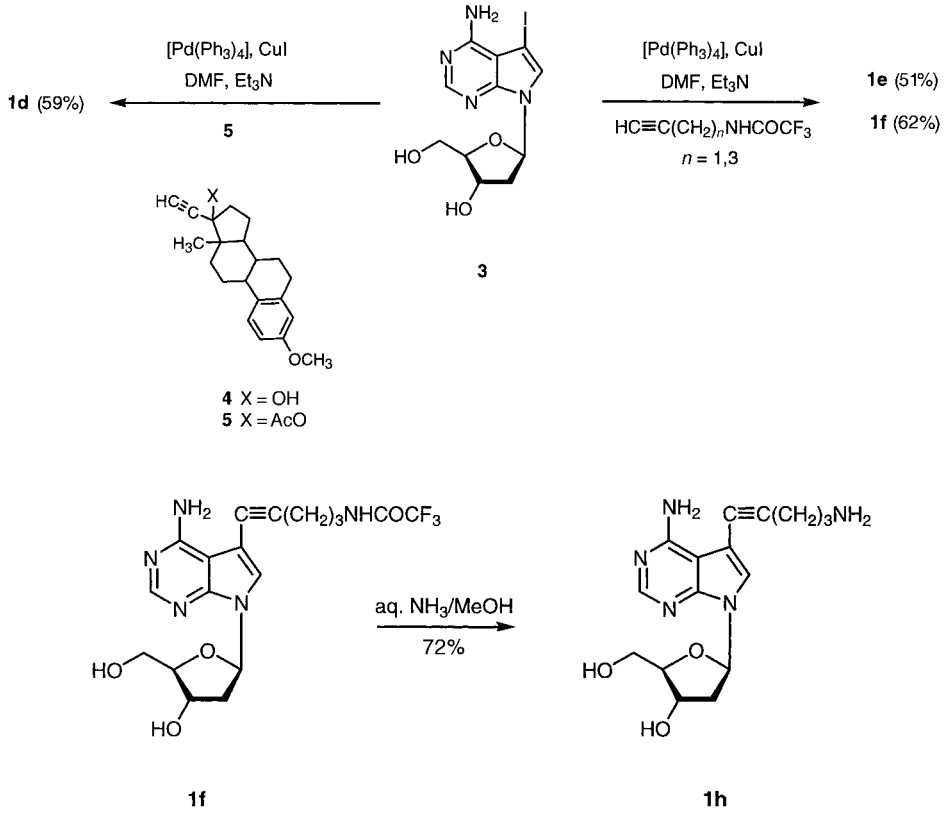
d 17β-acetoxy-3-methoxyestra-1,3,5(10)-trien-17α-yl



i 17β-hydroxy-3-methoxyestra-1,3,5(10)-trien-17α-yl

Results and Discussion. – 1. *Monomers.* The 7-deaza-2′-deoxy-7-ethynyl- (**1a**) and 7-deaza-2′-deoxy-7-(phenylethynyl)adenosine (**1c**) have already been described [17]. The nucleosides **1b**,**e**–**f** were synthesized using 7-deaza-2′-deoxy-7-iodoadenosine (**3**) [17] as starting material by the palladium(0)-catalyzed cross-coupling reaction [26] with alkynes or protected aminoalkynes (*Scheme 1*). A 7-(dodec-1-ynyl) (see **1b**) as well as a 7-[ω-(trifluoroacetamido)alkynyl] (see **1e**,**f**) side chain was introduced. The trifluoroacetyl group of compound **1f** was sufficiently labile and could be removed – if necessary – in aqueous ammonia/MeOH yielding **1h**. Compound **1h** can serve as a precursor for nucleoside triphosphates labeled with reporter groups [23]. When the [(17β-hydroxy-3-methoxyestra-1,3,5(10)-trien-17α-yl)ethynyl]-containing nucleoside **1i** was used in the solid-phase synthesis, a temporary protection of the 17β-OH group was required. Thus, the 17α-ethynyl-3-methoxyestra-1,3,5(10)-trien-17β-ol (**4**) was

Scheme 1



protected with an acetyl residue to yield **5**, which was then used in the Pd^0-catalyzed cross-coupling [26] with nucleoside **3** to yield compound **1d**.

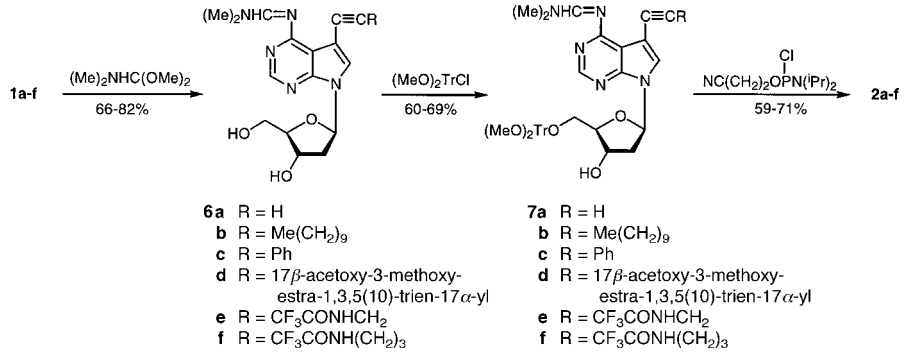
Next, the protection of the nucleosides was performed. The (dimethylamino)methylidene residue [28] was used for this purpose as this residue had already been successfully employed in the case of 7-deaza-2′-deoxy-7-(hex-1-ynyl)adenosine ($hxy^7c^7A_d$) [1]. Compounds **1a**–**f** were treated with dimethylformamide dimethyl acetal yielding the (dimethylamino)methylidene compounds **6a**–**f** (*Scheme 2*). To ensure the applicability of this group, the half-life of the deprotection was measured in conc. ammonia by UV spectrophotometry. According to *Table 1*, the formamidine residue of the nucleosides **6c**,**e**,**f** was found to be more labile than that of the parent 2′-deoxy-N^6-[(dimethylamino)methylidene]-7-iodotubercidin ($fma^6I^7c^7A_d$) [1] and also than that of 2′-deoxy-N^6-[(dimethylamino)methylidene]-7-(hex-1-ynyl)tubercidin ($fma^6hxy^7c^7A_d$) [1], while for **6a** and **6d**, a higher stability of the protecting group was determined. Next, the derivatives **6a**–**f** were converted to the 4,4′-dimethoxytrityl ($(MeO)_2Tr$) derivatives **7a**–**f**. Phosphitylation with chloro(2-cyanoethyl)(diisopropylamino)phosphine (= 2-cyanoethyl diisopropylphosphoramidochloridite) in THF in the presence of $^iPr_2EtN$ furnished the phosphoramidites **2a**–**f**.

Table 1. *Half-Life Values ($t_{1/2}$) of Deprotection of 7-Deazaadenine 2′-Deoxyribonucleosides in 25% Aq. NH_3 Solution at 40°* [a])

	fma^{6}I^{7}c^{7}A$_d$ [1]	fma^{6}hxy^{7}c^{7}A$_d$ [1]	**6a**	**6c**	**6d**	**6e**	**6f**
λ [nm]	323	321	318	316	323	320	320
$t_{1/2}$ [min]	82	110	150	50	170	47	58

[a]) The data were determined UV-spectrophotometrically at the wavelength indicated.

Scheme 2



All compounds were characterized by ^{1}H-, ^{13}C-, or ^{31}P-NMR spectra (*Table 2* and *Exper. Part*) as well as by elemental analyses or mass spectra. The assignment of the ^{13}C-NMR chemical shifts of the 7-substituted 7-deaza-2′-deoxyadenosine derivatives resulted from the coupling pattern taken from the gated-decoupled ^{1}H/^{13}C-NMR spectra. Also heteronuclear correlation spectra confirmed the assignments. *Table 2* summarizes the ^{13}C-NMR data of the monomers.

2. *Oligonucleotides.* 2.1. *Synthesis.* The phosphoramidites **2a**–**f** and those of the regular DNA constituents were employed in solid-phase synthesis. The lipophilic nucleosides **1a**–**d**,**i**, the 3-aminopropynyl derivative **1g** as well as the 5-aminopentynyl derivative **1h** were incorporated into oligonucleotides representing various sequence motifs. The average coupling yield of the modified phosphoramidites **2a**–**c**,**e**,**f** was >90%, while that of the bulky **2d** was below 80%. The oligonucleotides were deprotected and then purified using oligonucleotide-purification cartridges [29]. Their purity was established by reversed-phase HPLC. In only a few cases, additional purification of the oligonucleotide was necessary (by HPLC (*RP-18*)). The mobility of the parent adenine-containing oligonucleotide was decreased by an ethynyl substituent and even more by lipophilic phenylethynyl or dodecynyl side chains (see, *e.g.*, *Fig. 1*,*a*). The nucleoside composition of oligomers containing the nucleosides **1a**–**d** was determined by hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase (see, *e.g.*, *Fig. 1*,*b*–*d*), while enzymatic cleavage of oligonucleotides containing **1g**,**h** failed. The molecular mass of a series of oligonucleotides was determined by MALDI-TOF mass spectra (see *Exper. Part*, *Table 11*).

2.2. *Self-complementary Oligonucleotides with Alternating 7-Alkynyl Residues Different in Chain Length and Size.* Environmental factors such as dehydration and counter-ion binding influence the secondary structure of alternating duplexes 5′-d(A-

Table 2. *^{13}C-NMR Chemical Shifts ((D$_6$)DMSO[a])) of 7-Deazaadenine 2′-Deoxyribofuranosides*

	C(2)[b]) C(2)[c])	C(6)[b]) C(4)[c])	C(5)[b]) C(4a)[c])	C(7)[b]) C(5)[c])	C(8)[b]) C(6)[c])	C(4)[b]) C(7a)[c])	C(1′)	C(3′)	C(4′)	C(5′)	C≡C	MeO	Me_2N	HC=N
1a	152.7	157.5	102.3	93.9	127.0	149.1	83.2	70.9	87.5	61.8	77.3, 82.9			
b	152.5	157.5	102.3	95.5	125.3	149.0	83.1	70.9	87.4	61.9	73.6, 92.5			
c	152.9	153.7	102.4	94.8	126.9	149.5	83.3	71.0	87.7	62.0	83.2, 91.2			
d	152.8	157.5	102.5	94.1	126.2	149.3	83.1	70.8	87.5	61.8	90.9, [e])	54.8		
e	152.7	157.4	102.2	94.0	126.5	149.2	83.2	70.9	87.5	61.8	76.2, 86.7			
f	152.7	157.6	102.3	95.5	125.7	149.2	83.2	71.1	87.6	62.0	74.0, 91.6			
h	152.5	157.5	102.2	95.2	125.7	149.0	83.1	70.9	87.5	61.8	74.2, 91.1			
6a	151.9	160.9	110.2	96.0	128.8	150.6	83.0	70.9	87.5	61.8	78.2, 80.6		34.4, 40.2	156.4
b	151.6	160.9	110.1	97.6	127.1	150.7	83.1	71.0	87.4	61.9	74.6, 90.1		34.3, 40.2	156.4
c	151.8	160.9	110.1	96.7	127.8	150.8	83.2	71.1	87.6	62.0	84.7, 89.5		34.5, 40.4	156.7
d	151.7	160.9	109.8	[d])	128.8	150.8	83.1	70.9	87.4	61.8	96.4, [e])		34.4, 40.4	156.7
e	151.9	160.8	110.0	96.0	128.4	150.7	83.1	70.9	87.5	61.8	77.3, 84.6		34.4, 40.3	156.4
f	151.7	160.9	110.1	97.3	127.3	150.7	82.9	71.0	87.4	61.9	75.1, 89.1		34.3, 40.3	156.4
7a	152.0	160.9	110.0	96.3	128.5	150.8	82.7	70.6	85.4	64.1	78.0, 80.7	55.0	34.4, 40.2	156.4
b	151.9	161.0	110.3	98.0	127.0	151.0	82.7	70.8	85.6	64.3	74.6, 90.3	55.0	34.4, 40.6	156.5
c	152.1	161.0	110.0	96.8	128.0	151.1	82.8	70.6	85.5	64.1	84.6, 89.4	54.9	34.4, 40.3	156.6
d	151.9	160.9	109.8	[d])	128.2	151.0	82.9	70.9	85.5	64.1	96.6, [e])	54.8, 54.9	34.5, 40.3	156.7
e	152.0	160.8	110.1	96.2	128.2	150.9	82.8	70.7	85.4	64.2	77.1, 84.7	54.9	34.4	156.4
f	152.0	161.0	110.3	97.6	127.2	151.0	82.7	70.8	85.5	64.3	75.1, 89.3	55.1	34.3, 40.2	156.6

[a]) The signal of C(2′) is superimposed by the solvent. [b]) Purine numbering. [c]) Systematic numbering, [d]) Not detectable. [e]) Not assigned.

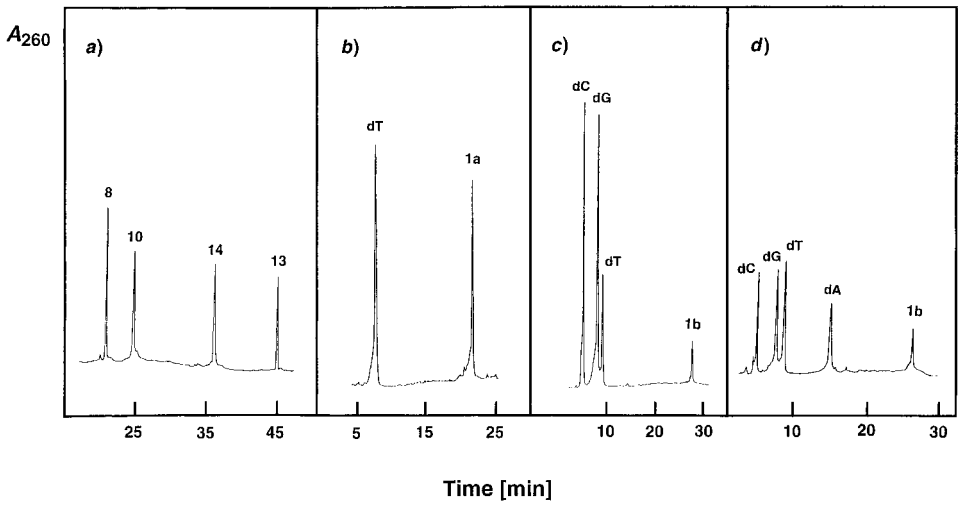


Fig. 1. a) *HPLC Profiles* (*RP-18*) *of the oligonucleotides 5′-d(A-T)*$_6$ (**8**), *5′-d(***1a***-T)*$_6$ (**10**), *5′-d(***1b***-T)*$_6$ (**13**), *and 5′-d(***1c***-T)*$_6$ (**14**) *after purification by reversed-phase* (*RP-18*) *chromatography* (gradient *I*). b) c) d) *HPLC profiles* (*RP-18*) *of the oligonucleotides* **10** (*b*), **28** (*c*), *and* **35** (*d*) *after enzymatic hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase in* 1M Tris · *HCl buffer* (pH 8.3; gradient *II* for *c*) and *d*) and gradient *III* for *b*)). For details, see *Exper. Part*; for sequences see *Tables 3*, *5*, and *6*.

T)$_n$ [30]. Also structural modifications have a strong impact on the stability of such duplexes. Therefore, in a first series of experiments, 7-deazaadenine residues carrying alkynyl groups different in length or size of the alkynyl chain were used to replace the adenine base of 5′-d(A-T)$_6$ (**8**). The T_m values and thermodynamic data of the various oligonucleotide duplexes were determined for buffer solutions containing Mg^{2+} ions and 1.0M or 0.1M NaCl (see *Table 3*). The thermodynamic data were generally obtained by curve-shape analysis and the T_m values from temperature-dependent UV-absorption profiles (see *Exper. Part*); in the case of **14** · **14** data were also extracted from concentration-dependent T_m values. In all cases, the thermodynamic data were calculated by the program 'MeltWin 3.0' [31].

Comparison of the thermal stability of the duplex 5′-d[(**1a**-T)$_6$]$_2$ (**10** · **10**) (T_m 52°) with the parent unmodified compound 5′-d[(A-T)$_6$]$_2$ (**8** · **8**) or the 7-deazaadenine-containing duplex 5′-d[c^{7}A-T)$_6$]$_2$ (**9** · **9**) revealed a significant stabilization by the introduction of an ethynyl residue (see **10** · **10**) (*Table 3*). In this case, and also in the case of the sequence motifs shown later, the stabilization caused by 7-ethynyl residues is driven entropically. Residues with longer alkynyl chains (dodecynyl, see **1b**) led to duplex destabilization (see **12** · **12**), while a complete substitution (see **13** · **13**) produced an oligomer which did not show sigmoidal duplex melting. Phenyl residues attached directly to the ethynyl moiety led to unusual biphasic melting profiles (see **14** · **14**). The bulky estradiol-modified ethynyl residue (see **1i**) resulted in significant duplex destabilization when only one modified base was incorporated (*Table 3*). For self-complementary oligonucleotide duplexes containing a modified dA$_6$ tract, enhanced duplex stability was observed when a 7-deaza-7-ethynyladenine was introduced (see 5′-

Table 3. T_m *Values and Thermodynamic Data of Self-complementary Oligonucleotides Containing Adenine, 7-Deazaadenine, or 7-Substituted 7-Deazaadenines*[a])[b])

	T_m [°]	ΔH^0 [kcal/mol]	ΔS^0 [cal/mol·K]	ΔG^0_{298} [kcal/mol]
5′-d[(A-T)$_6$]$_2$ (**8**·**8**)	33(26)	−45(−44)	−125(−127)	−6.3(−5.5)
5′-d[(c^{7}A-T)$_6$]$_2$ (**9**·**9**) [32]	33(29)	−41(−34)	−113(−89)	−6.1(−6.3)
5′-d[(**1a**-T)$_6$]$_2$ (**10**·**10**)	52(46)	−43(−41)	−110(−103)	−9.2(−8.7)
5′-d[(hxy^{7}c^{7}A-T)$_6$]$_2$ (**11**·**11**) [1]	52(49)	−47(−62)	−123(−170)	−9.3(−9.4)
5′-d[(A-T)$_3$-(**1b**-T)-(A-T)$_2$]$_2$ (**12**·**12**)	22	−33	−91	−5.1
5′-d[(**1b**-T)$_6$]$_2$ (**13**·**13**)	[c])			
5′-d[(**1c**-T)$_6$]$_2$ (**14**·**14**)	26+51(36)	(−58) (−67)[d])	(−165) (−195)[d])	(−7.0) (−6.9)[d])
5′-d[(**1i**-T)-(A-T)$_5$]$_2$ (**15**·**15**)	26	−32	−84	−5.6
5′-d[(A-T)$_3$-(**1i**-T)-(A-T)$_2$]$_2$ (**16**·**16**)	(21)	(−30)	(−79)	−5.4
5′-d[(A)$_6$-(T)$_6$]$_2$ (**17**·**17**)	46(40)	−81(−75)	−232(−219)	−9.1(−7.4)
5′-d[(**1a**)$_6$-(T)$_6$]$_2$ (**18**·**18**)	51	−64	−175	−9.7
5′-d[(**1b**)$_6$-(T)$_6$]$_2$ (**19**·**19**)	[c])			

[a]) 7-Ethynyl- (**1a**), 7-(dodec-1-ynyl)- (**1b**), 7-(phenylethynyl)- (**1c**), and 7-[(17β-hydroxy-3-methoxyestra-1,3,5(10)-trien-17α-yl)ethynyl]-substituted (**1i**) 7-deaza-2′-deoxyadenosine; hxy^{7}c^{7}A$_d$ = 7-deaza-2′-deoxy-7-(hex-1-ynyl)adenosine. [b]) Determined at 270 nm. Data without parentheses are measured in 1M NaCl containing 100 mM $MgCl_2$ and 60 mM Na-cacodylate (pH 7.0) with 5 + 5 μM oligonucleotide concentration. Data in parentheses are measured in 0.1M NaCl containing 10 mM $MgCl_2$ and 10 mM Na-cacodylate (pH 7.0) with 5 + 5 μM oligonucleotide concentration. [c]) No cooperative melting. [d]) Determined from the concentration dependence of the T_m value.

d[(**1a**)$_6$-(T)$_6$] (**18**)), while the oligonucleotide 5′-d[(**1b**)$_6$-(T)$_6$] (**19**) did not form a duplex at all. From the data shown in *Table 3*, it can be concluded that alkynyl side chains up to a length of 6–7 Å, which is about the depth of a B-DNA major groove (8.8 Å; [33]), lead to duplex stabilization, while longer residues destabilize the duplex structure.

The temperature-dependent CD spectra of the duplex **14**·**14** containing 7-deaza-7-(phenylethynyl)adenine show significant differences in buffers of high or low ionic strength. While the CD spectra recorded in low-salt-concentration buffer exhibit a nearly uniform temperature profile, a discontinuous change of the spectra is observed at high salt concentration (*Fig. 2*,*a* and *b*). This points to the presence or formation of more than two species during the melting. This is supported by a biphasic UV/melting profile of **14**·**14** measured at high-salt-concentration buffer, whereas a monophasic melting is observed under low-salt-concentration conditions (*Table 3*). In the case of the duplex **10**·**10**, containing 7-deaza-7-ethynyladenine, no changes are observed under these conditions (*Fig. 2*,*c* and *d*).

2.3. *Oligonucleotides with Mismatches.* It was of interest to study the base-pair selectivity of the modified oligonucleotides. For this purpose, compound **1c** was incorporated into the center of d(T)$_{12}$. This oligomer was hybridized with a 'd(A)$_{12}$-homomer' containing dT, dA, dC, or dG opposite the 7-deazaadenine moiety (*Table 4*). Compared to the parent duplex **20**·**21**, the modified duplex **21**·**22** showed no significant change in thermal stability. However, with dC, dG, and dA opposite **1c** (see **22**·**23**, **22**·**24**, and **22**·**25**, resp.), the duplex stability was significantly decreased. This confirms a stable **1c**·dT base pair and its specificity.

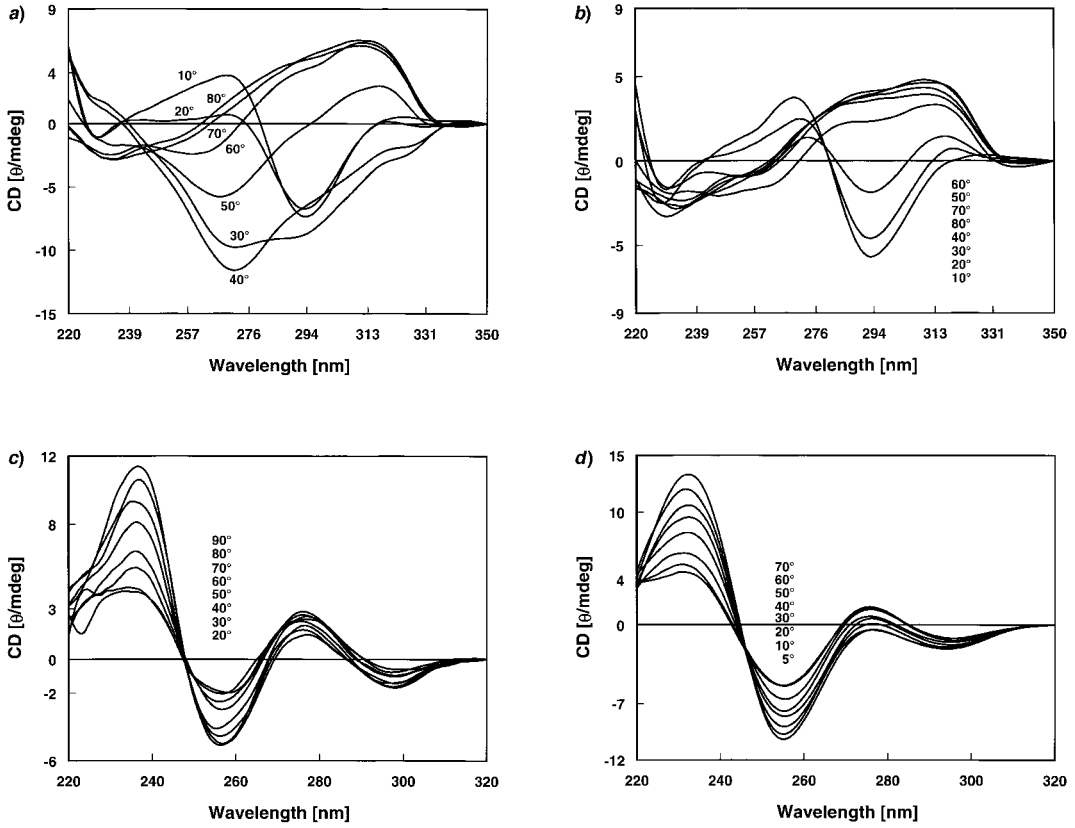


Fig. 2. *Temperature-dependent CD spectra of the alternating duplexes* a) b) *5′-d[(***1c***-T)$_6$]$_2$* (**14**·**14**) *and* c) d) *5′-d[(***1a***-T)$_6$]$_2$* (**10**·**10**). *a*) *c*) Measured in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) with 5 + 5 μM oligomer concentration. *b*) *d*) Measured in 0.1M NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) with 5 + 5 μM oligomer concentration.

Table 4. T_m *Values and Thermodynamic Data of Non-self-complementary Oligonucleotides with Mismatches Opposite to 7-Deaza-2′-deoxy-7-(phenylethynyl)adenosine* (**1c**)[a])

	T_m [°]	ΔH^0 [kcal/mol]	ΔS^0 [cal/mol·K]	ΔG^0_{298} [kcal/mol]
5′-d[(T)$_5$-A-(T)$_6$] (**20**) 3′-d[(A)$_5$-T-(A)$_6$] (**21**)	39(33)	−97(−110)	−284(−301)	−8.4(−6.6)
5′-d[(T)$_5$-**1c**-(T)$_6$] (**22**) 3′-d[(A)$_5$-T-(A)$_6$] (**21**)	39(34)	−83(−88)	−239(−261)	−8.5(−7.1)
5′-d[(T)$_5$-**1c**-(T)$_6$] (**22**) 3′-d[(A)$_5$-C-(A)$_6$] (**23**)	29	−78	−232	−5.3
5′-d[(T)$_5$-**1c**-(T)$_6$] (**22**) 3′-d[(A)$_5$-G-(A)$_6$] (**24**)	27	−71	−214	−5.0
5′-d[(T)$_5$-**1c**-(T)$_6$] (**22**) d(A$_{12}$) (**25**)	30	−59	−171	−6.0

[a]) Determined at 270 nm. Data without parentheses are measured in 1M NaCl containing 100 mM $MgCl_2$ and 60 mM Na-cacodylate (pH 7.0) with 5 μM single-strand concentration. Data in parentheses are measured in 0.1M NaCl containing 10 mM $MgCl_2$ and 10 mMNa-cacodylate (pH 7.0) with 5 μM single-strand concentration.

2.4. *Self-Complementary Palindromic Oligonucleotides.* Next, the influence of the 7-alkynyl side chains on the duplex stability was studied on the *Dickerson-Drew* dodecamer 5′-d(CGCGAATTCGCG) (**26**) [34]. The replacement of the central adenosine residues by compounds **1a**–**c** resulted in duplex stabilization (*Table 5*). However, the incorporation of only one estradiol-modified ethynyl-substituted base (see **1i**) reduced the T_m value significantly (see **30**·**30**). It is worth mentioning that, in the case of the duplex **28**·**28** with two dodecynyl side chains, duplex stabilization occurred that was not found for all other duplexes containing **1b** (see, *e.g.*, examples in *Tables 3* and *6*). As the *Dickerson-Drew* dodecamer forms a hairpin at low salt and oligomer concentration [34][35], concentration-dependent T_m measurements were performed. Duplex melting was confirmed from the concentration dependence of the T_m values leading to similar thermodynamic data as those obtained by curve-shape analysis (see *Fig. 3*). The CD-spectra of the duplexes **28**·**28**, **29**·**29**, and **30**·**30** show a similar shape but differences in the CD amplitudes (*Fig. 4*).

Table 5. T_m *Values and Thermodynamic Data of the* 'Dickerson-Drew' *Dodecamer Containing 7-Ethynyl-* (**1a**), *7-(Dodec-1-ynyl)-* (**1b**), *7-(Phenylethynyl)-* (**1c**), *or 7-[(17β-Hydroxy-3-methoxyestra-1,3,5(10)-trien-17α-yl)ethynyl]-Substituted* (**1i**) *7-Deaza-2′-deoxyadenosine*[a])

	T_m [°]	ΔH^0 [kcal/mol]	ΔS^0 [cal/mol·K]	ΔG^0_{298} [kcal/mol]
5′-d(CGCGAATTCGCG)$_2$ (**26**·**26**)	64(63)	−83(−73)	−224(−195)	−13.7(−12.6)
5′-d[CGCG(**1a**)$_2$TTCGCG]$_2$ (**27**·**27**)	67(65)	−75(−70)	−200(−185)	−13.4(−12.7)
		(−77)[b])	(−206)[b])	(−13.2)[b])
5′-d[CGCG(**1b**)$_2$TTCGCG]$_2$ (**28**·**28**)	71(67)	−62(−74)	−159(−194)	−12.7(−13.4)
		(−76)[b])	(−200)[b])	(−13.6)[b])
5′-d[CGCG(**1c**)$_2$TTCGCG]$_2$ (**29**·**29**)	68(66)	−98(−93)	−265(n-253)	−16.0(−15.0)
		(−93)[b])	(−252)[b])	(−15.0)[b])
5′-d(CGCG**1i**ATTCGCG)$_2$ (**30**·**30**)	59(58)	−62(−51)	−164(−131)	−11.2(−10.2)

[a]) Determined at 270 nm. Data without parentheses are measured in 1M NaCl containing 100 mM $MgCl_2$ and 60 mM Na-cacodylate (pH 7.0) with 5 + 5 μM oligonucleotide concentration. Data in parentheses are measured in 0.1M NaCl containing 10 mM $MgCl_2$ and 10 mM Na-cacodylate (pH 7.0) with 5 + 5 μM oligonucleotide concentration. [b]) Determined from the concentration dependence of the T_m value.

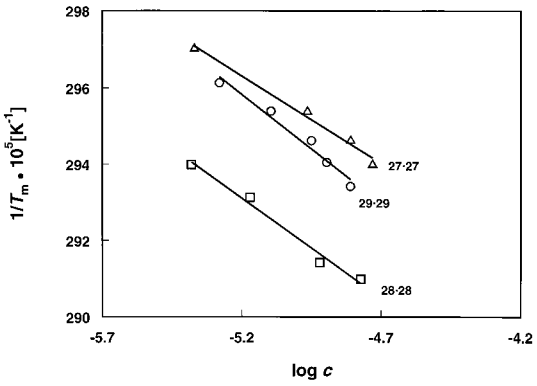


Fig. 3. $1/T_m$ vs. *log* c *plot of the self-complementary duplexes* **27**·**27**, **28**·**28**, *and* **29**·**29**. For sequences, see *Table 5*.

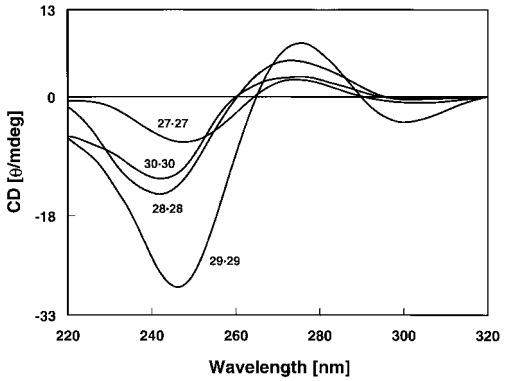


Fig. 4. *CD Spectra of the self-complementary duplexes* **27**·**27**, **28**·**28**, **29**·**29**, *and* **30**·**30**. Measured at 10° in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) with 5 + 5 μM oligomer concentration; for sequences, see *Table 5*.

2.5. *Non-self-complementary Oligonucleotides with Random Base Composition*. The duplex of 5′-d(TAGGTCAATACT) (**31**) with 3′-d(ATCCAGTTATGA) (**32**) is used in our laboratory as a standard to study the influence of modified bases on the duplex structure and stability. Because of the random base distribution, the hybrid is representative for a common DNA. The oligonucleotides shown in *Table 6* were

Table 6. T_m *Values and Thermodynamic Data of Non-self-complementary Oligonucleotides Containing 7-Ethynyl-* (**1a**), *7-(Dodec-1-ynyl)-* (**1b**), *7-(Phenylethynyl)-* (**1c**), *and [(17β-Hydroxy-3-methoxyestra-1,3,5(10)-trien-17α-yl)ethynyl]-Substituted* (**1i**) *7-Deaza-2′-deoxyadenosine*[a])

	T_m [°]	ΔH^0 [kcal/mol]	ΔS^0 [cal/mol·K]	ΔG^0_{298} [kcal/mol]
5′-d(TAGGTCAATACT) (**31**) 3′-d(ATCCAGTTATGA) (**32**)	50(47)	−90(−82)	−252(−230)	−11.8(−10.4)
5′-d(T**1a**GGTCAAT**1a**CT) (**33**) 3′-d(ATCCAGTTATGA) (**32**)	52(49)	−86(−59)	−238(−158)	−12.2(−10.3)
5′-d(T**1a**GGTC(**1a**)$_2$T**1a**CT) (**34**) 3′-d(ATCCAGTTATGA) (**32**)	53(50)	−74(71)	−200(−198)	−12.0(−10.0)
5′-d(T**1a**GGTC(**1a**)$_2$T**1a**CT) (**34**) 3′-d(ATCC**1a**GTT**1a**TGA) (**33**)	53	−67	−182	−10.8
5′-d(TAGGTCAATACT) (**31**) 3′-d(ATCC**1b**GTT**1b**TGA) (**35**)	39	−80	−231	−8.3
5′-d(T**1b**GGTCAAT**1b**CT) (**36**) 3′-d(ATCCAGTTATGA) (**32**)	41	−71	−198	−9.7
5′-d(T**1b**GGTC(**1b**)$_2$T**1b**CT) (**37**) 3′-d(ATCC**1b**GTT**1b**TGA) (**35**)	< 15			
5′-d(T**1i**GGTCAAT**1i**CT) (**38**) 3′-d(ATCCAGTTATGA) (**32**)	40(38)	−56(−66)	−160(−188)	−8.4(−8.2)
3′-r(AUCCAGUUAUGA) (**39**) 5′-d(TAGGTCAATACT) (**31**)	48	−65	−176	−10.2
3′-r(AUCCAGUUAUGA) (**39**) 5′-d(T**1i**GGTCAAT**1i**CT) (**38**)	35	−59	−167	−7.5

[a]) Determined at 270 nm. Data without parentheses are measured in 1M NaCl containing 100 mM $MgCl_2$ and 60 mM Na-cacodylate (pH 7.0) with 5 μM single-strand concentration. Data in parentheses are measured in 0.1M NaCl containing 10 mM $MgCl_2$ and 10 mM Na-cacodylate (pH 7.0) with 5 μM single-strand concentration.

hybridized with the complementary strand, and the T_m values were measured. A duplex stabilization caused by the 7-ethynyl residue of **1a** was observed in all cases when the modified strand was hybridized with its unmodified counterpart (see **32**·**33**, **32**·**34**, and **33**·**34**). For the single-stranded oligomers **35**–**37** containing 7-deaza-7-(dodec-1-ynyl)adenine, similar experiments were performed (see **31**·**35**, **32**·**36**, and **35**·**37**). According to *Table 6*, less stable duplexes were formed, even when **1b** residues were incorporated into only one strand of the heteroduplex. Also when the duplex was tethered to an estradiol-modified ethynyl-substituted residue, the stability decreased (see **32**·**38**). When the oligodeoxynucleotide **38** was hybridized with the oligoribonucleotide **39**, a further destabilization took place (see **38**·**39**) which agrees with observations on regular DNA·RNA hybrids [36]. The heteroduplexes shown in *Table 6* form B-like DNA structures as shown by CD spectra, except that of the DNA·RNA duplex **38**·**39**, which forms an A-like DNA as observed for other DNA·RNA hybrids (*Figures* not shown) [1].

2.6. *Zwitterionic Oligonucleotides Containing Aminoalkynyl Residues.* Next, oligonucleotide duplexes with a zwitterionic structure were investigated. Earlier, it was shown in the case of (6-aminohexyl)uridylate and (6-aminohexyl)cytidylate that an ammonium-ion tether has a significant influence on the stability of oligonucleotide duplexes [10]. The zwitterionic character of these oligonucleotides leads to a reduced net charge of the duplex. As a consequence, fewer counter ions, *e.g.*, sodium or potassium ions, are bound to the single-stranded as well as to double-stranded DNA. A lower ionic strength of the environment is necessary to overcome the repulsion of the negatively charged phosphodiester anions. The DNA secondary structure becomes stabilized at lower salt concentrations as found for DNA not carrying tethered ammonium ions.

The comparison of the thermal stability of the self-complementary dodecameric duplex 5′-d[(**1g**-T)$_6$]$_2$ (**40**·**40**) containing 3-aminopropynyl side chains with that of 5′-d[(A-T]$_6$]$_2$ (**8**·**8**) or 5′-d[(c^{7}A-T)$_6$]$_2$ (**9**·**9**) [32] revealed a significant stabilization effect of the aminoalkynyl tether (*Table 7*). The stabilization of the duplex was also slightly higher than that observed for the duplex **10**·**10** substituted with an ethynyl side chain. In particular, this stabilization was noticeable when the measurements were performed

Table 7. T_m *Values and Thermodynamic Data of Self-complementary Oligonucleotide Duplexes Containing 7-(3-Aminoprop-1-ynyl)-7-deaza-2′-deoxyadenosine* (**1g**; npry^{7}c^{7}A$_d$)[a])

	T_m [°]	ΔH^0 [kcal/mol]	ΔS^0 [cal/mol·K]	ΔG^0_{298} [kcal/mol]
5′-d[(A-T)$_6$]$_2$ (**8**·**8**)	33(26)	−45(−44)	−125(−127)	−6.3(−5.5)
5′-d[(c^{7}A-T)$_6$]$_2$ (**9**·**9**) [32]	33(29)	−41(−34)	−113(−89)	−6.1(−6.3)
5′-d[(**1a**-T)$_6$]$_2$ (**10**·**10**)	52(46)	−43(−41)	−110(−103)	−9.2(−8.7)
5′-d[(**1g**-T)$_6$]$_2$ (**40**·**40**)	54(50)	−62(−70) −60[b])	−168(−193) −160[b])	−10.2(−9.7) −10.0[b])
5′-d(CGCGAATTCGCG)$_2$ (**26**·**26**)	64(63)	−83(−73)	−224(−195)	−13.7(−12.6)
5′-d[CGCG(**1g**)$_2$TTCGCG]$_2$ (**41**·**41**)	66(65)	−65(−61)	−170(−157)	−12.7(−12.3)

[a]) Determined at 270 nm. Data without parentheses are measured in 1M NaCl containing 100 mM $MgCl_2$ and 60 mM Na-cacodylate (pH 7.0) with 5 μM single-strand concentration. Data in parentheses are measured in 0.1M NaCl containing 10 mM $MgCl_2$ and 10 mM Na-cacodylate (pH 7.0) with 5 μM single-strand concentration. [b]) Determined from the concentration dependence of the T_m value.

Table 8. T_m *Values and Thermodynamic Data of Non-self-complementary Oligonucleotide Duplexes Containing 7-(3-Aminoprop-1-ynyl)-7-deaza-2′-deoxyadenosine* (**1g**; npry^{7}c^{7}A$_d$)[a])

	T_m [°]	ΔH^0 [kcal/mol]	ΔS^0 [cal/mol·K]	ΔG^0_{298} [kcal/mol]
5′-d(TAGGTCAATACT) (**31**) 3′d(ATCCAGTTATGA) (**32**)	50(47)	−90(−82)	−252(−230)	−11.8(−10.4)
5′-d(Tc^{7}AGGTCc^{7}AATACT) (**42**) 3′-d(ATCCc^{7}AGTTc^{7}ATGA) (**43**)	(41)	(−60)	(−165)	(−8.9)
5′-d(T**1g**GGTCAAT**1g**CT) (**44**) 3′-d(ATCC**1g**GTT**1g**TGA) (**45**)	53(51)	−77(−74)	−210(−201)	−11.8(−11.3)
5′-d(A)$_{12}$·d(T)$_{12}$ (**25**·**46**)	44(37)	−84(−91)	−238(−267)	−9.8(−7.9)
5′-d[(c^{7}A)$_{11}$-A]·d(T)$_{12}$ (**47**·**46**) [32]	30[b])			
5′-d(**1a**-A)$_6$·d(T)$_{12}$ (**48**·**46**)	48(40)	−71(−69)	−197(−195)	−10.4(−8.2)
5′-d[(**1g**)$_{11}$-A]·d(T)$_{12}$ (**49**·**46**)	[c])			

[a]) Determined at 270 nm. Data without parentheses are measured in 1M NaCl containing 100 mM $MgCl_2$ and 60 mM Na-cacodylate (pH 7.0) with 5 μM oligonucleotide single-strand concentration. Data in parentheses are measured in 0.1M NaCl containing 10 mM $MgCl_2$ and 10 mM Na-cacodylate (pH 7.0) with 5 μM oligonucleotide single-strand concentration. [b]) See [32]. [c]) No sigmoidal melting was observed.

at low salt concentration (values in parentheses). The T_m increase was lower in the case of the *Dickerson-Drew* dodecamer **41**, as there are only two 3-aminopropynyl residues present, as compared to six in the case of **40**. Similar effects were observed in the case of the non-self-complementary duplexes shown in *Table 8*. It should be noted that the duplex stability of **31**·**32** was reduced by the replacement of adenine by 7-deazaadenine (c^{7}A) residues (see **42**·**43**). Already four 3-aminopropynyl residues compensated this destabilization and led to a duplex **44**·**45**, which melted at higher temperature than the parent duplex **31**·**32**. The situation was different in the homomeric duplex **46**·**49**. In this case, it was not possible to obtain a sigmoidal melting profile, probably due to counter-ion condensation of the ammonium-ion-tethered oligomer. On the other hand, the duplex **46**·**48** containing ethynyl residues exhibited an increased stability that was superior to that of the parent **46**·**47** [32].

Experiments similar to those performed with the 3-aminopropynyl nucleoside **1g** (npry^{7}c^{7}A$_d$) were carried out with the 5-aminopentynyl derivative **1h** (npey^{7}c^{7}A$_d$) (*Table 9*). One or three dA replacements by **1h** in the sequence **8**·**8** led to a duplex stabilization (see **50**·**50** and **51**·**51**), whereas, in contrast to the duplex 5′-d[(**1g**-T)$_6$]$_2$ (**40**·**40**), no cooperative melting was observed for 5′-d[(**1h**-T)$_6$]$_2$ (**52**·**52**). Such an observation has already been made for the corresponding 7-(5-aminohex-1-ynyl) derivative of 7-deaza-2′-deoxyguanosine [3]. Also in this case, counter-ion condensation of the single strands affected duplex formation. As the incorporation of such residues in high-molecular DNA does not result in such problems [37], the kinetics of duplex nucleation might be the reason for this phenomenon. In palindromic or non-self-complementary oligonucleotides containing **1h**, the duplex stability was similar to that observed for oligonucleotides containing **1g**. As the thermodynamic data obtained by curve-shape analysis or from concentration-dependent T_m measurements (see **53**·**53**) were similar, duplex melting can be anticipated in this case (*Table 9*).

As a result of their zwitterionic structure, aminoalkynyl-containing oligomers show a smaller dependence of the T_m values from the ionic strength than molecules not

Table 9. T_m *Values and Thermodynamic Data of Oligonucleotides Containing 7-(5-Aminopent-1-ynyl)-7-deaza-2′-deoxyadenosine* (**1h**; npey^{7}c^{7}A$_d$)[a])

	T_m [°]	ΔH^0 [kcal/mol]	ΔS^0 [cal/mol·K]	ΔG^0_{298} [kcal/mol]
5′-d[(A-T)$_6$]$_2$ (**8**·**8**)	33(26)	−45(−44)	−125(−127)	−6.3(−5.5)
5′-d[(A-T)$_3$-(**1h**-T)-(A-T)$_2$]$_2$ (**50**·**50**)	37(33)	−52(−50)	−145(−141)	−7.1(−6.8)
5′-d[(A-T-**1h**-T)$_3$]$_2$ (**51**·**51**)	41	−43	−113	−7.8
5′-d[(**1h**-T)$_6$]$_2$ (**52**·**52**)	[b])			
5′-d[(hxy^{7}c^{7}A-T)$_6$]$_2$ (**11**·**11**) [1]	52(49)	−47(−62)	−123(−170)	−9.3(−9.4)
5′-d(CGCGAATTCGCG)$_2$ (**26**·**26**)	64(63)	−83(−73)	−224(−195)	−13.7(−12.6)
5′-d[CGCG(**1h**)$_2$TTCGCG]$_2$ (**53**·**53**)	68(67)	−86(−94)	−230(−253)	−14.9(−15.6)
	–	−87[c])	−233[c])	−14.3[c])
5′-d[CGCG(hxy^{7}c^{7}A)$_2$TTCGCG]$_2$ (**54**·**54**)	70(69)	−89(−84)	−238(−225)	−15.5(−14.8)
5′-d(TAGGTCAATACT) (**31**)	50(47)	−90(−82)	−252(−230)	−11.8(−10.4)
3′-d(ATCCAGTTATGA) (**32**)				
5′-d(T**1h**GGTCAAT**1h**CT) (**55**)	52	−55	−143	−10.4
3′-d(ATCCAGTTATGA) (**32**)				
5′-d(T**1h**GGTCAAT**1h**CT) (**55**)	53	−80	−217	−12.4
3′-d(ATCC**1h**GTT**1h**TGA) (**56**)				
5′-d(TAGGTCAATACT) (**32**)	52	−93	−260	−12.2
3′-d(ATCChxy^{7}c^{7}AGTThxy^{7}c^{7}ATGA) (**57**) [1]				

[a]) Determined at 270 nm. Data without parentheses are measured in 1M NaCl containing 100 mM $MgCl_2$ and 60 mM Na-cacodylate (pH 7.0) with 5 μM oligonucleotide single-strand concentration. Data in parentheses are measured in 0.1M NaCl containing 10 mM $MgCl_2$ and 10 mM Na-cacodylate (pH 7.0) with 5 μM oligonucleotide single-strand concentration. [b]) No sigmoidal melting was observed. [c]) Determined from the concentration dependence of the T_m value.

Table 10. T_m *Values of Oligonucleotide Duplexes Containing 7-(Aminoalkynylated) 7-Deaza-2′-deoxyadenosines* **1g** *or* **1h**[a])

	T_m [°]	
	low salt concentration[b])	high salt concentration[c])
5′-d[(A-T)$_6$]$_2$ (**8**·**8**)	[d])	33
5′-d[(c^{7}A-T)$_6$]$_2$ (**9**·**9**) [32]	[d])	32
5′-d[(**1a**-T)$_6$]$_2$ (**10**·**10**)	38	52
5′-d[(hxy^{7}c^{7}A-T)$_6$]$_2$ (**11**·**11**) [1]	35	52
5′-d[(**1g**-T)$_6$]$_2$ (**40**·**40**)	54	53
5′-d[(A-T-**1h**-T)$_3$]$_2$ (**51**·**51**)	[d])	41
5′-d(TAGGTCAATACT) (**31**)	40	53
3′-d(ATCCAGTTATGA) (**32**)		
5′-d(Thxy^{7}c^{7}AGGTCAAThxy^{7}c^{7}CT) (**58**)	37	52
3′-d(ATCChxy^{7}c^{7}AGTThxy^{7}c^{7}ATGA) (**57**) [1]		
5′-d(T**1h**GGTCAAT**1h**CT) (**55**)	[e])	53
3′-d(ATCC**1h**GTT**1h**TGA) (**56**)		
5′-d(T**1h**GGTCAAT**1h**CT) (**55**)	40	52
3′-d(ATCCAGTTATGA) (**32**)		
5′-d(Thxy^{7}c^{7}AGGTCAAThxy^{7}c^{7}CT) (**58**)	40	52
3′-d(ATCCAGTTATGA) (**32**)		

[a]) Determined at 270 nm. [b]) Measured in 50 mM NaCl, 10 mM Na-phosphate buffer (pH 7.0) containing 0.1 mM EDTA with 5 μM single-strand concentration. [c]) Measured in 1M NaCl, 10 mM Na-phosphate buffer (pH 7.0) containing 0.1 mM EDTA with 5 μM single-strand concentration. [d]) No sigmoidal melting. [e]) Not determined.

carrying a positively charged residue [10]. Therefore, the T_m values of selected oligonucleotide duplexes were measured in a Mg^{2+}-free Na-phosphate buffer and were compared with the data found in Mg^{2+}-containing NaCl solution. It was verified that the replacement of dA by **1g** (see 5′-d(**1g**-T)$_6$ (**40**)) led to a duplex structure the T_m value of which was independent from the ionic strength of the buffer (*Table 10*). For duplexes containing only a few aminoalkynyl residues, the stability was already influenced by the ionic strength of the buffer. An explanation for this behavior can be given by differences in the release of counter ions during duplex formation. This amount is considered to be significantly decreased in the case of a single strand tethered with ammonium ions compared to the unmodified DNA, an effect which will lead to a favorable entropy change during the formation of DNA duplexes.

The retention of the helix geometry of the duplexes shown in *Tables 7–9* can be seen from their CD spectra. The self-complementary oligonucleotide duplexes **40**·**40**, **41**·**41**, **51**·**51**, and **53**·**53** exhibit the characteristics of a B-like DNA with a positive band at 275 nm and a negative loop around 250 nm (*Figs. 5* and *6*).

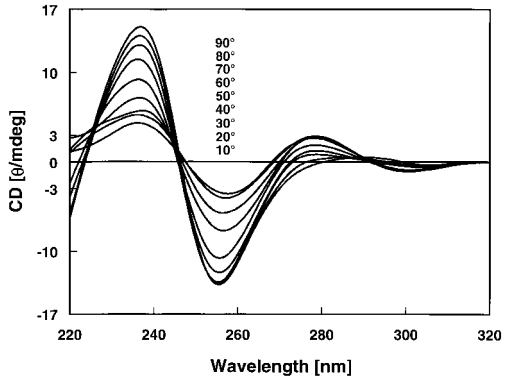


Fig. 5. *Temperature-dependent CD spectra of the alternating duplex 5′-d[(***1g***-T)$_6$]$_2$* (**40**·**40**). Measured in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) with 5 + 5 μM oligomer concentration.

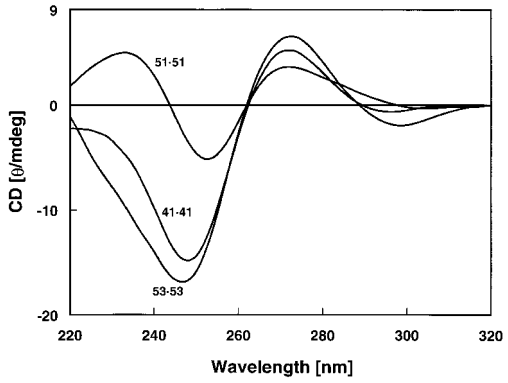
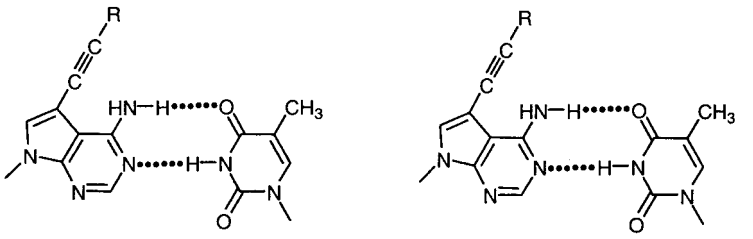


Fig. 6. *CD Spectra of the self-complementary duplexes* **41**·**41**, **51**·**51**, *and* **53**·**53**. Measured at 10°C in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) with 10 μM oligomer concentration; for sequences, see *Tables 7* and *9*.



Antiparallel A*·T base pair with duplex stabilizing residues

Antiparallel A*·T base pair with duplex destabilizing residues

a R = H

b R = $Me(CH_2)_9$

c R = Ph

i R = 17β-hydroxy-3-methoxy-estra-1,3,5(10)-trien-17α-yl

g R = $^{+}H_3NCH_2$ [a])

h R = $^{+}H_3N(CH_2)_3$ [a])

[a]) Multiple replacements leading to duplex destabilization

Fig. 7. *Antiparallel A*·T base pairs with* a) *duplex-stabilizing and* b) *duplex-destabilizing residues.* A* = 7-substituted 7-deaza-2′-deoxyadenosine residue.

3. Conclusion and Perspective. – The incorporation of short alkynyl (*e.g.*, ethynyl) residues at position 7 of 7-deaza-2′-deoxyadenosine-containing oligonucleotide duplexes enhances their stability if the side chain is not much longer than the depth of the major groove of B-DNA (see base pairs **a** and **c** in *Fig. 7*). Long dodecynyl chains or bulky steroid residues lead to duplex destabilization (see base pairs **b** and **i** in *Fig. 7*). The 7-(3-aminoprop-1-ynyl) or 7-(5-aminopent-1-ynyl) residue (see base pairs **g** and **h** in *Fig. 7*) which introduce a positive charge into the side chain was found to be even more effective – particularly at low counter-ion concentration. However, 7-(3-aminoprop-1-ynyl) residues introduced in homomers was problematic as it resulted in counter-ion condensation. The experiments described above make the aminoalkynyl modifications performed on 7-deazaadenine residues attractive for the introduction of reporter groups into the chain of duplex DNA [23]. Furthermore, the duplex stabilization of alkynyl or aminoalkynyl residues can be used to improve the binding of short primers to high-molecular-weight DNA. Also, the T_m values of dA · dT *vs.* dG · dC pairs can be equalized by this method, which will be valuable for the hybridization sequencing performed on a chip surface. The incorporation of related dideoxynucleoside triphosphates has been shown to occur with various DNA-polymerases [24–26] and represents the common technique in the commercially available sequencing machines [38]. Therefore, the use of the triphosphates of 7-substituted 7-deazapurine 2′-deoxyribonucleosides has the potential to generate fully labeled transcripts of DNA fragments [37].

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Experimental Part

1. *General*. All chemicals were supplied by *Aldrich*, *Sigma*, or *Fluka* (*Sigma-Aldrich Chemie GmbH*; Deisenhofen, Germany). Solvents were of laboratory grade. Thin-layer chromatography (TLC): aluminum sheets, silica gel *60* F_{254}, 0.2 mm layer (*Merck*, Germany). Column flash chromatography (FC): silica gel *60* (*Merck*, Germany) at 0.4 bar ($4 \cdot 10^4$ Pa) using the following solvent systems: CH_2Cl_2/MeOH 9 : 1 (*A*), CH_2Cl_2/MeOH 95 : 5 (*B*), and petroleum ether (40–60°)/acetone 1 : 1 (*C*); sample collection with an *UltroRac-II* fractions collector (*LKB Instruments*, Sweden). M.p.'s: *Büchi-SMP-20* apparatus (*Büchi*, Switzerland); uncorrected. UV Spectra: *U-3200* spectrometer (*Hitachi*, Japan). NMR Spectra: *AC-250* or *AMX-500* spectrometers (*Bruker*, Karlsruhe, Germany); ^{1}H at 250.13 and 500.14 MHz, ^{13}C at 125.13 MHz, chemical shifts δ in ppm rel. to $SiMe_4$ or H_3PO_4 as internal standard, *J* values in Hz; st. refers to steroidal protons. Elemental analyses were performed by *Mikroanalytisches Labor Beller* (Göttingen, Germany). Positive-ion fast-atom-bombardment (FAB) MS were provided by Dr. *M. Sauer*, Universität Heidelberg, Germany, using 3-nitrobenzyl alcohol (3-NOBA) as matrix.

2. *Syntheses of Monomers. 7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-5-(dodec-1-ynyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidin-4-amine* (**1b**). A suspension of 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]-pyrimidin-4-amine [17] (**3**) (200 mg, 0.53 mmol) and CuI (30.3 mg, 0.16 mmol) in anh. DMF (2 ml) was treated with dodec-1-yne (10 equiv.), anh. Et_3N (162 mg, 1.6 mmol), and $[Pd(PPh_3)_4]$ (94 mg, 0.08 mmol) at r.t. under stirring for 4 h under Ar. The soln. was diluted with MeOH/CH_2Cl_2 1 : 1 (20 ml), and *Dowex 1X8* (800 mg, 100–200 mesh; hydrogen carbonate form) was added. After additional stirring for 45 min, the mixture was filtered, the resin washed with MeOH/CH_2Cl_2 1 : 1 (50 ml), the combined filtrate evaporated, and the residue purified by FC (silica gel, column 15 × 4 cm, 2–10% MeOH/CH_2Cl_2): **1b** (112 mg, 51%). Colorless amorphous solid. TLC (*A*): R_f 0.5. UV (MeOH): 280 (10500), 238 (14700). ^{1}H-NMR ((D$_6$)DMSO): 0.81 (*t*, *J* = 7.0, Me); 1.22 (br. *m*, several CH_2); 1.41 (*quint.*, *J* = 7.0, CH_2); 1.53 (*quint.*, *J* = 7.0, CH_2); 2.16 (*m*, H_α–C(2′)); 2.43–2.47 (*m*, H_β–C(2′), CH_2); 3.55 (*m*, 2 H–C(5′)); 3.81 (*m*, H–C(4′)); 4.33 (*m*, H–C(3′)); 5.05 (*t*, *J* = 5.4, OH–C(5′)); 5.24 (*d*, *J* = 3.9, OH–C(3′)); 6.46 (‘*t*’, *J* = 6.9, H–C(1′)); 6.70 (br. *s*, NH_2); 7.62 (*s*, H–C(6)); 8.09 (*s*, H–C(2)). Anal. calc. for $C_{23}H_{34}N_4O_3$ (414.55): C 66.64, H 8.27, N 13.52; found: C 66.32, H 8.13, N 13.43.

17α-Ethynyl-3-methoxyestra-1,3,5(10)-trien-17β-ol Acetate (**5**). To a soln. of *17α-ethynyl-3-methoxyestra-1,3,5(10)-trien-17β-ol* (**4**; 4.0 g, 12.9 mmol) in pyridine (60 ml), Ac_2O (8 ml) was added. The mixture was heated under reflux for 12 h and then evaporated and the residue chromatographed (silica gel, column 15 × 5 cm, 0 → 5% MeOH/CH_2Cl_2: **5** (3.8 g, 84%)). Colorless foam. ^{1}H-NMR ((D$_6$)DMSO): 0.86 (*s*, Me); 1.37–2.49 (several *m*, CH_2(6), CH_2(7), H–C(8), H–C(9), CH_2(11), CH_2(12), H–C(14), CH_2(15), CH_2(16)); 2.00 (*s*, COMe); 3.55 (*s*, C≡CH); 3.70 (*s*, MeO); 6.62 (*d*, *J* = 2.5, H–C(2)); 6.68 (*m*, H–C(4)); 7.17 (*d*, *J* = 8.6, H–C(1)).

*5-[2-(17β-Acetoxy-3-methoxyestra-1,3,5(10)-trien-17α-yl)ethynyl]-7-(2-deoxy-β-*D*-*erythro*-pentofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine* (**1d**). As described for **1b**, with **3** [17] (400 mg, 1.06 mmol), CuI (60.6 mg, 0.32 mmol), anh. DMF (20 ml), **5** (2.5 g, 7 mmol), anh. Et_3N (324 mg, 3.2 mmol), and $[Pd(PPh_3)_4]$ (188 mg, 0.16 mmol) for 3 h. After workup as described for **1b**, crystallization from MeOH/H_2O furnished **1d** (376 mg, 59%). Colorless crystals. M.p. 179–180°. TLC (*A*): R_f 0.5. UV (MeOH): 280 (10800), 231 (12700). ^{1}H-NMR ((D$_6$)DMSO): 0.93 (*s*, Me); 1.37–2.49 (several *m*, H_α–C(2′), H_β–C(2′), CH_2(6)(st.), CH_2(7)(st.), H–C(8)(st.), H–C(9)(st.), CH_2(11)(st.), CH_2(12)(st.), H–C(14)(st.), CH_2(15)(st.), CH_2(16)(st.)); 2.07 (*s*, COMe); 3.55 (*m*, 2 H–C(5′)); 3.70 (*s*, MeO); 3.83 (*m*, H–C(4′)); 4.34 (*m*, H–C(3′)); 5.03 (*t*, *J* = 5.5, OH–C(5′)); 5.24 (*d*, *J* = 4.1, OH–C(3′)); 6.48 (‘*t*’, *J* = 7.0, H–C(1′)); 6.62 (*d*, *J* = 2.3, H–C(4)(st.)); 6.69 (*m*, H–C(2)(st.)); 6.75 (br. *s*, NH_2); 7.19 (*d*, *J* = 8.6, H–C(1)(st.)); 7.75 (*s*, H–C(6)); 8.12 (*s*, H–C(2)). Anal. calc. for $C_{34}H_{40}N_4O_6 \cdot 0.5\ H_2O$ (609.72): C 66.98, H 6.78, N 9.19; found: C 67.01, H 6.98, N 8.98.

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-5-[3-(trifluoroacetamido)prop-1-ynyl]-7*H*-pyrrolo-[2,3-*d*]pyrimidin-4-amine* (**1e**). As described for **1b**, with **3** [17] (800 mg, 2.13 mmol), CuI (121 mg, 0.64 mmol), anh. DMF (20 ml), 2,2,2-trifluoro-*N*-(prop-2-ynyl)acetamide [25] (1.5 g, 10 mmol), anh. Et_3N (650 mg, 6.4 mmol), and $[Pd(PPh_3)_4]$ (376 mg, 0.32 mmol) for 3 h. Workup as described for **1b** gave **1e** (430 mg, 51%). Amorphous solid. TLC (*A*): R_f 0.3. UV (MeOH): 237 (14400), 279 (14200). ^{1}H-NMR ((D$_6$)DMSO): 2.19 (*m*, H_α–C(2′)); 2.47 (*m*, H_β–C(2′)); 3.56 (*m*, 2 H–C(5′)); 3.84 (*m*, H–C(4′)); 4.32 (*d*, *J* = 5.3, CH_2NH); 4.35 (*m*, H–C(3′)); 5.05 (*t*, *J* = 4.5, OH–C(5′)); 5.25 (*d*, *J* = 3.0, OH–C(3′)); 6.49 (‘*t*’, *J* = 6.6, H–C(1′)); 6.79 (br. *s*, NH_2); 7.76

(*s*, H–C(6)); 8.12 (*s*, H–C(2)); 10.07 (*s*, NH). Anal. calc. for $C_{16}H_{16}F_3N_5O_4$ (399.33): C 48.13, H 4.04, N 17.54; found: C 48.26, H 4.13, N 17.58.

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-5-[5-(trifluoroacetamido)pent-1-ynyl]-7*H*-pyrrolo-[2,3-*d*]pyrimidin-4-amine* (**1f**). As described for **1b**, with **3** [17] (800 mg, 2.13 mmol), CuI (121 mg, 0.64 mmol), anh. DMF (20 ml), 2,2,2-trifluoro-*N*-(pent-4-ynyl)acetamide [25] (2 g, 11 mmol), anh. Et_3N (650 mg, 6.4 mmol), and $[Pd(PPh_3)_4]$ (376 mg, 0.32 mmol): **1f** (564 mg, 62%). Colorless foam. TLC (*A*): R_f 0.35. UV (MeOH): 239 (14300), 280 (10900). ^{1}H-NMR ((D$_6$)DMSO): 1.76 (*quint.*, *J* = 7.0, $CH_2CH_2CH_2$); 2.17 (*m*, H_α–C(2′)); 2.47 (*m*, $CH_2C{\equiv}C$, H_β–C(2′), superimposed by DMSO); 3.31 (*m*, CH_2NH, superimposed by H_2O); 3.50 (*m*, 2 H–C(5′)); 3.80 (*m*, H–C(4′)); 4.32 (*m*, H–C(3′)); 5.05 (br. *s*, OH–C(5′)); 5.20 (br. *s*, OH–C(3′)); 6.46 (‘*t*’, *J* = 6.9, H–C(1′)); 6.60 (br. *s*, NH_2); 7.61 (*s*, H–C(6)); 8.07 (*s*, H–C(2)); 9.46 (*s*, NH). Anal. calc. for $C_{18}H_{20}F_3N_5O_4$ (427.38): C 50.59, H 4.72, N 16.39; found: C 50.65, H 4.82, N 16.32.

*5-(5-Aminopent-1-ynyl)-7-(2-deoxy-β-*D-erythro-*pentofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine* (**1h**). Compound **1f** (75 mg, 0.18 mmol) was stirred in MeOH/aq. NH_3 soln. 1:4 (30 ml) for 5 h at r.t. After evaporation, the residue was purified by FC (silica gel, column 10 × 2 cm, CH_2Cl_2/MeOH/NH_3 soln. 75 : 20 : 5): **1h** (42 mg, 72%). Colorless foam. TLC (CH_2Cl_2/MeOH/NH_3 soln. 65 : 30 : 5): R_f 0.5. UV (MeOH): 238 (11000), 280 (8300). ^{1}H-NMR ((D$_6$)DMSO): 1.84 (*quint.*, *J* = 7.0, $CH_2CH_2CH_2$); 2.20 (*m*, H_α–C(2′)); 2.47 (*m*, H_β–C(2′), superimposed by DMSO); 2.60 (*m*, $CH_2C{\equiv}C$); 2.91 (*m*, CH_2NH); 3.55 (*m*, 2 H–C(5′)); 3.83 (*m*, H–C(4′)); 4.35 (*m*, H–C(3′)); 5.05–5.70 (2 br, *s*, OH–C(5′), OH–C(3′)); 6.48 (‘*t*’, *J* = 7.0, H–C(1′)); 6.65 (br. *s*, NH_2); 7.67 (*s*, H–C(6)); 8.11 (*s*, H–C(2)). FAB-MS (3-NOBA): 332.2 ($[M+H]^+$, $[C_{16}H_{21}N_5O_3+H]^+$; calc. 332.37).

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-*N^4*-[(dimethylamino)methylidene]-5-ethynyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine* (**6a**). A soln. of 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-ethynyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine [17] (**1a**; 400 mg, 1.46 mmol) in MeOH (20 ml) was stirred with dimethylformamide dimethyl acetal (2.0 g, 16.8 mmol) for 2 h at 40°. After evaporation, the residue was applied to FC (silica gel, column 12 × 4 cm, *A*): **6a** (384 mg, 80%). Colorless foam. TLC (*A*): R_f 0.4. UV (MeOH): 278 (11500), 319 (13200). ^{1}H-NMR ((D$_6$)DMSO): 2.20 (*m*, H_α–C(2′)); 2.47 (*m*, H_β–C(2′)); 3.15, 3.18 (2*s*, Me_2N); 3.56 (*m*, 2 H–C(5′)); 3.84 (*m*, H–C(4′)); 3.94 (*s*, C≡CH); 4.36 (*m*, H–C(3′)); 5.04 (*t*, *J* = 5.6, OH–C(5′)); 5.26 (*d*, *J* = 4.1, OH–C(3′)); 6.54 (‘*t*’, *J* = 6.5, H–C(1′)); 7.87 (*s*, H–C(6)); 8.34 (*s*, H–C(2)); 8.78 (*s*, N=CH). Anal. calc. for $C_{16}H_{19}N_5O_3$ (329.36): C 58.35, H 5.81, N 21.26; found: C 58.48, H 5.71, N 21.36.

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-*N^4*-[(dimethylamino)methylidene]-5-(dodec-1-ynyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine* (**6b**). As described for **6a**, from **1b** (500 mg, 1.21 mmol) and dimethylformamide dimethyl acetal (2.0 g, 16.8 mmol). FC (silica gel, column 12 × 3 cm, *A*) gave **6b** (371 mg, 66%). Colorless foam. TLC (*A*): R_f 0.4. UV (MeOH): 236 (14300), 279 (11400), 322 (13800). ^{1}H-NMR ((D$_6$)DMSO): 0.85 (*t*, *J* = 7.1, Me); 1.39 (br. *m*, 5 CH_2); 1.41 (*quint.*, *J* = 7.0, CH_2); 1.53 (*quint.*, *J* = 7.2, CH_2); 2.18 (*m*, H_α–C(2′)); 2.40 (*t*, *J* = 7.0, CH_2); 2.47 (*m*, H_β–C(2′), CH_2); 3.16, 3.18 (2*s*, Me_2N); 3.58 (*m*, 2 H–C(5′)); 3.83 (*m*, H–C(4′)); 4.35 (*m*, H–C(3′)); 5.03 (*t*, *J* = 5.5, OH–C(5′)); 5.24 (*d*, *J* = 4.1, OH–C(3′)); 6.54 (‘*t*’, *J* = 6.4, H–C(1′)); 7.70 (*s*, H–C(6)); 8.32 (*s*, H–C(2)); 8.76 (*s*, N=CH). FAB-MS (3-NOBA): 470.4 ($[M+H]^+$, $[C_{26}H_{39}N_5O_3+H]^+$; calc. 470.63).

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-*N^4*-[(dimethylamino)methylidene]-5-(phenylethynyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine* (**6c**). As described for **6a**, from 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-(phenylethynyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**1c**) [17] (500 mg, 1.43 mmol) and dimethylformamide dimethyl acetal (2.0 g, 16.8 mmol). FC (silica gel, column 12 × 3 cm, *A*) gave **6c** (474 mg, 82%). Colorless foam. TLC (*A*): R_f 0.4. UV (MeOH): 247 (23000), 316 (23600). ^{1}H-NMR ((D$_6$)DMSO): 2.22 (*m*, H_α–C(2′)); 2.52 (*m*, H_β–C(2′)); 3.15, 3.16 (2*s*, Me_2N); 3.58 (*m*, 2 H–C(5′)); 3.86 (*m*, H–C(4′)); 4.38 (*m*, H–C(3′)); 5.04 (*t*, *J* = 5.5, OH–C(5′)); 5.27 (*d*, *J* = 4.1, OH–C(3′)); 6.58 (‘*t*’, *J* = 6.5, H–C(1′)); 7.35–7.49 (*m*, arom. H); 7.94 (*s*, H–C(6)); 8.37 (*s*, H–C(2)); 8.81 (*s*, N=CH). Anal. calc. for $C_{22}H_{23}N_5O_3$ (405.46): C 65.17, H 5.72, N 17.27; found: C 64.67, H 5.41, N 16.83.

*5-[(17β-Acetoxy-3-methoxyestra-1,3,5(10)-trien-17α-yl)ethynyl]-7-(2-deoxy-β-*D-erythro-*pentofuranosyl)-*N^4*-[(dimethylamino)methylidene]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine* (**6d**). A mixture of **1d** (300 mg, 0.50 mmol) and dimethylformamide dimethyl acetal (4 g, 33.6 mmol) in DMF (10 ml) was stirred at 80° for 24 h. The solvent was evaporated and the residue applied to FC (silica gel, column 12 × 4 cm, *A*): **6d** (230 mg, 70%). Colorless foam. TLC (*A*): R_f 0.5. UV (MeOH): 278 (17900). ^{1}H-NMR ((D$_6$)DMSO): 0.93 (*s*, Me); 1.37–2.49 (several *m*, H_α–C(2′), H_β–C(2′), CH_2(6)(st.), CH_2(7)(st.), H–C(8)(st.), H–C(9)(st.), CH_2(11)(st.), CH_2(12)(st.), H–C(14)(st.), CH_2(15)(st.), CH_2(16)(st.)); 2.00 (*s*, COMe); 3.20, 3.22 (2*s*, Me_2N); 3.58 (*m*, 2 H–C(5′)); 3.69 (*s*, MeO); 3.84 (*m*, H–C(4′)); 4.35 (*m*, H–C(3′)); 5.04 (*t*, *J* = 5.5, OH–C(5′)); 5.24 (*d*, *J* = 3.8, OH–C(3′)); 6.54 (‘*t*’, *J* = 6.9, H–C(1′)); 6.61 (br. *s*, H–C(4)(st.)); 6.68 (*d*, *J* = 8.6, H–C(2)(st.));

7.18 (*d*, *J* = 8.6, H–C(1)(st.)); 7.80 (*s*, H–C(6)); 8.33 (*s*, H–C(2)); 8.82 (*s*, N=CH). FAB-MS (3-NOBA): 656.4 ($[M+H]^+$, $[C_{37}H_{45}N_5O_6+H]^+$; calc. 656.79).

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-N⁴-[(dimethylamino)methylidene]-5-[3-(trifluoroacetamido)prop-1-ynyl]-7H-pyrrolo[2,3-d]pyrimidin-4-amine* (**6e**). As described for **6a**, from **1e** (400 mg, 1.0 mmol) and dimethylformamide dimethyl acetal (2.0 g, 16.8 mmol). FC (silica gel, column 15 × 3 cm, *A*) gave **6e** (364 mg, 80%). Colorless foam. TLC (*A*): R_f 0.4. UV (MeOH): 230 (24900), 275 (11100), 320 (17700). ^{1}H-NMR ((D$_6$)DMSO): 2.22 (*m*, H$_\alpha$–C(2′)); 2.48 (*m*, H$_\beta$–C(2′)); 3.14, 3.17 (2*s*, Me$_2$N); 3.55 (*m*, 2 H–C(5′)); 3.85 (*m*, H–C(4′)); 4.30 (*d*, *J* = 5.3, C*H*$_2$NH); 4.36 (*m*, H–C(3′)); 5.03 (*t*, *J* = 5.5, OH–C(5′)); 5.26 (*d*, *J* = 4.0, OH–C(3′)); 6.55 (‘*t*’, *J* = 7.0, H–C(1′)); 7.85 (*s*, H–C(6)); 8.34 (*s*, H–C(2)); 8.80 (*s*, N=CH); 10.04 (*s*, NH). Anal. calc. for $C_{19}H_{21}F_3N_6O_4$ (454.41): C 50.22, H 4.66, N 18.49; found: C 50.42, H 4.91, N 18.49.

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-N⁴-[(dimethylamino)methylidene]-5-[5-(trifluoroacetamido)pent-1-ynyl]-7H-pyrrolo[2,3-d]pyrimidin-4-amine* (**6f**). As described for **6a** from **1f** (400 mg, 0.94 mmol) and dimethylformamide dimethyl acetal (2.0 g, 16.8 mmol). FC (silica gel, column 15 × 3 cm, *A*) gave **6f** (354 mg, 78%). Colorless foam. TLC (*A*): R_f 0.4. UV (MeOH): 232 (24900), 278 (11500), 320 (14700). ^{1}H-NMR ((D$_6$)DMSO): 1.78 (*quint.*, *J* = 7.1, CH$_2$C*H*$_2$CH$_2$); 2.20 (*m*, H$_\alpha$–C(2′)); 2.47 (*m*, CH$_2$C≡C, H$_\beta$–C(2′), superimposed by DMSO); 3.14, 3.18 (2*s*, Me$_2$N); 3.33 (*m*, C*H*$_2$NH, superimposed by H$_2$O); 3.55 (*m*, 2 H–C(5′)); 3.84 (*m*, H–C(4′)); 4.35 (*m*, H–C(3′)); 5.02 (*t*, *J* = 5.5, OH–C(5′)); 5.25 (*d*, *J* = 4.0, OH–C(3′)); 6.54 (‘*t*’, *J* = 7.0, H–C(1′)); 7.72 (*s*, H–C(6)); 8.33 (*s*, H–C(2)); 8.76 (*s*, N=CH); 9.47 (*s*, NH). Anal. calc. for $C_{21}H_{25}F_3N_6O_4$ (482.46): C 52.28, H 5.22, N 17.42; found: C 52.50, H 5.19, N 17.40.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-N⁴-[(dimethylamino)methylidene]-5-ethynyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine* (**7a**). To a soln. of **6a** (300 mg, 0.91 mmol) in anh. pyridine (2 ml), 4,4′-dimethoxytriphenylmethyl chloride (360 mg, 1.1 mmol) was added. After stirring at 50° for 1 h, the mixture was poured into 5% aq. NaHCO$_3$ soln. (10 ml) and extracted with CH$_2$Cl$_2$ (twice, 50 ml each). The combined org. layers were dried (Na$_2$SO$_4$) and evaporated. The residue was applied to FC (silica gel, column 15 × 4 cm, *A*): **7a** (389 mg, 67%). Colorless foam. TLC (*A*): R_f 0.6. UV (MeOH): 231 (45700), 275 (14800), 318 (17900). ^{1}H-NMR ((D$_6$)DMSO): 2.29 (*m*, H$_\alpha$–C(2′)); 2.64 (*m*, H$_\beta$–C(2′)); 3.15, 3.18 (2*s*, Me$_2$N); 3.19 (*m*, 2 H–C(5′)); 3.74 (*s*, 2 MeO); 3.95 (*m*, H–C(4′), C≡CH); 4.40 (*m*, H–C(3′)); 5.33 (*d*, *J* = 4.1, OH–C(3′)); 6.57 (‘*t*’, *J* = 6.5, H–C(1′)); 6.82 (*m*, 4 arom. H); 7.20–7.36 (*m*, 9 arom. H); 7.73 (*s*, H–C(6)); 8.35 (*s*, H–C(2)); 8.78 (*s*, N=CH). Anal. calc. for $C_{37}H_{37}N_5O_5$ (631.73): C 70.35, H 5.90, N 11.09; found: C 70.31, H 5.80, N 10.72.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-N⁴-[(dimethylamino)methylidene]-5-(dodec-1-ynyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine* (**7b**). As described for **7a**, with **6b** (320 mg, 0.68 mmol) and 4,4′-dimethoxytriphenylmethyl chloride (270 mg, 0.80 mmol). FC (silica gel, column 15 × 4 cm, *A*) furnished **7b** (341 mg, 65%). Colorless foam. TLC (*A*): R_f 0.6. UV (MeOH): 244 (31500), 279 (14800), 323 (14300). ^{1}H-NMR ((D$_6$)DMSO): 0.83 (*t*, *J* = 7.1, Me); 1.22 (br. *m*, 5 CH$_2$); 1.37 (*quint.*, *J* = 7.1, CH$_2$); 1.51 (*quint.*, *J* = 7.2, CH$_2$); 2.25 (*m*, H$_\alpha$–C(2′)); 2.38 (*t*, *J* = 7.1, CH$_2$); 2.47 (*m*, CH$_2$); 2.72 (*m*, H$_\beta$–C(2′)); 3.14 (*m*, Me$_2$N, 2 H–C(5′)); 3.72 (*s*, 2 MeO); 3.93 (*m*, H–C(4′)); 4.36 (*m*, H–C(3′)); 5.32 (*d*, *J* = 3.9, OH–C(3′)); 6.56 (‘*t*’, *J* = 6.5, H–C(1′)); 6.81 (*m*, 4 arom. H); 7.22–7.35 (*m*, 9 arom. H); 7.57 (*s*, H–C(6)); 8.31 (*s*, H–C(2)); 8.87 (*s*, N=CH). FAB-MS (3-NOBA): 772.5 (M^+, $C_{47}H_{57}N_5O_5^+$; calc. 772.0).

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-N⁴-[(dimethylamino)methylidene]-5-(phenylethynyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine* (**7c**). As described for **7a**, with **6c** (350 mg, 0.86 mmol) and 4,4′-dimethoxytriphenylmethyl chloride (340 mg, 1.0 mmol). FC (silica gel, column 15 × 4 cm, *A*) furnished **7c** (422 mg, 69%). Colorless foam. TLC (*A*): R_f 0.6. UV (MeOH): 238 (34200), 282 (20200), 308 (20900). ^{1}H-NMR ((D$_6$)DMSO): 2.33 (*m*, H$_\alpha$–C(2′)); 2.67 (*m*, H$_\beta$–C(2′)); 3.16, 3.17 (2*s*, Me$_2$N); 3.19 (*m*, 2 H–C(5′)); 3.69, 3.70 (2*s*, 2 MeO); 3.98 (*m*, H–C(4′)); 4.43 (*m*, H–C(3′)); 5.35 (*d*, *J* = 4.4, OH–C(3′)); 6.62 (‘*t*’, *J* = 6.5, H–C(1′)); 6.82 (*m*, 4 arom. H); 7.19–7.46 (*m*, 14 arom. H); 7.84 (*s*, H–C(6)); 8.38 (*s*, H–C(2)); 8.82 (*s*, N=CH). Anal. calc. for $C_{43}H_{41}N_5O_5$ (707.83): C 72.97, H 5.84, N 9.89; found: C 73.04, H 6.02, N 9.78.

*5-[(17β-Acetoxy-3-methoxyestra-1,3,5(10)-trien-17α-yl)ethynyl]-7-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-N⁴-[(dimethylamino)methylidene]-7H-pyrrolo[2,3-d]pyrimidin-4-amine* (**7d**). As described for **7a**, with **6d** (300 mg, 0.46 mmol) and 4,4′-dimethoxytriphenylmethyl chloride (180 mg, 0.53 mmol). FC (silica gel, 15 × 4 cm, *A*) furnished **7d** (299 mg, 68%). Colorless foam. TLC (*A*): R_f 0.6. UV (MeOH): 277 (20800). ^{1}H-NMR ((D$_6$)DMSO): 0.90 (*s*, Me); 1.34–2.49 (several *m*, H$_\alpha$–C(2′), H$_\beta$–C(2′), CH$_2$(6)(st.), CH$_2$(7)(st.), H–C(8)(st.), H–C(9)(st.), CH$_2$(11)(st.), CH$_2$(12)(st.), H–C(14)(st.), CH$_2$(15)(st.), CH$_2$(16)(st.)); 1.96 (*s*, COMe); 3.19 (*m*, 2 H–C(5′)); 3.20, 3.22 (2*s*, Me$_2$N); 3.70 (*m*, 3 MeO); 3.95 (*m*, H–C(4′)); 4.38 (*m*, H–C(3′)); 5.32 (*d*, *J* = 4.2, OH–C(3′)); 6.56 (‘*t*’, *J* = 6.8, H–C(1′)); 6.60 (br. *s*,

H–C(4)(st.)); 6.68 (*d*, *J* = 8.6, H–C(2)(st.)); 6.82 (*m*, 4 arom. H); 7.13 (*d*, *J* = 8.6, H–C(1)(st.)); 7.19–7.39 (*m*, 14 arom. H); 7.64 (*s*, H–C(6)); 8.32 (*s*, H–C(2)); 8.83 (*s*, N=CH). FAB-MS (3-NOBA): 958.5 (M^+, $C_{58}H_{63}N_5O_8^+$; calc. 958.16).

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-*N*^{4}-[(dimethylamino)methylidene]-5-[3-(trifluoroacetamido)prop-1-ynyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine* (**7e**). As described for **7a**, with **6e** (300 mg, 0.66 mmol) and 4,4′-dimethoxytriphenylmethyl chloride (250 mg, 0.74 mmol). FC (silica gel, column 20 × 3 cm, *A*) furnished **7e**. Colorless foam (310 mg, 62%). TLC (*A*): R_f 0.5. UV (MeOH): 235 (32800), 281 (13600), 319 (14400). ^{1}H-NMR ((D$_6$)DMSO): 2.26 (*m*, H$_\alpha$–C(2′)); 2.47 (*m*, H$_\beta$–C(2′)); 3.13, 3.19 (2*s*, Me$_2$N); 3.17 (*m*, 2 H–C(5′)); 3.69 (*s*, 2 MeO); 3.94 (*m*, H–C(4′)); 4.28 (*d*, *J* = 5.3, C*H*$_2$NH); 4.37 (*m*, H–C(3′)); 5.33 (*d*, *J* = 3.9, OH–C(3′)); 6.55 (‘*t*’, *J* = 6.7, H–C(1′)); 6.84 (*m*, 4 arom. H); 7.20–7.38 (*m*, 9 arom. H); 7.69 (*s*, H–C(6)); 8.33 (*s*, H–C(2)); 8.80 (*s*, N=CH); 10.04 (*s*, NH). Anal. calc. for $C_{40}H_{39}F_3N_6O_6$ (756.78): C 63.48, H 5.19, N 11.11; found: C 63.36, H 5.04, N 11.01.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-*N*^{4}-[(dimethylamino)methylidene]-5-[5-(trifluoroacetamido)pent-1-ynyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine* (**7f**). As described for **7a**, with **6f** (300 mg, 0.62 mmol) and 4,4′-dimethoxytriphenylmethyl chloride (250 mg, 0.74 mmol). FC (silica gel, column 20 × 3 cm, *B*) yielded **7f** (293 mg, 60%). Colorless foam. TLC (*A*): R_f 0.6. UV (MeOH): 238 (45800), 276 (14800), 320 (14700). ^{1}H-NMR ((D$_6$)DMSO): 1.74 (*quint.*, *J* = 7.1, CH$_2$C*H*$_2$CH$_2$); 2.23 (*m*, H$_\alpha$–C(2′)); 2.47 (*m*, CH$_2$C≡C, H$_\beta$–C(2′), superimposed by DMSO); 3.11, 3.15 (2*s*, Me$_2$N); 3.13 (*m*, 2 H–C(5′)); superimposed by Me$_2$N); 3.31 (*m*, C*H*$_2$NH, superimposed by H$_2$O); 3.71 (*s*, 2 MeO); 3.91 (*m*, H–C(4′)); 4.34 (*m*, H–C(3′)); 5.29 (*d*, *J* = 4.4, OH–C(3′)); 6.54 (‘*t*’, *J* = 6.7, H–C(1′)); 6.82 (*m*, 4 arom. H); 7.16–7.36 (*m*, 9 arom. H); 7.56 (*s*, H–C(6)); 7.56 (*s*, H–C(6)); 8.29 (*s*, H–C(2)); 8.73 (*s*, N=CH); 9.43 (*s*, NH). Anal. calc. for $C_{42}H_{43}F_3N_6O_6$ (784.83): C 64.28, H 5.52, N 10.71; found: C 64.42, H 5.73, N 10.68.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-*N*^{4}-[(dimethylamino)methylidene]-5-ethynyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**2a**). To a soln. of **7a** (300 mg, 0.47 mmol) and anh. ^{i}Pr$_2$EtN (200 mg, 1.55 mmol) in dry THF (2 ml), 2-cyanoethyl diisopropylphosphoramidochloridite (140 mg, 0.59 mmol) was added under stirring at r.t. under Ar. The mixture was allowed to stir for another 30 min and was then filtered. The filtrate was diluted with AcOEt (30 ml) and extracted with ice-cold 10% aq. Na$_2$CO$_3$ soln. (2 × 10 ml) and H$_2$O (10 ml). The combined org. phase was dried (Na$_2$SO$_4$) and evaporated. The solid material was applied to FC (silica gel, column 10 × 3 cm, *C*): **2a** (262 mg, 67%). Colorless foam. TLC (*C*): R_f 0.4, 0.5. ^{31}P-NMR (CDCl$_3$): 149.2, 149.4.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-*N*^{4}-[(dimethylamino)methylidene]-5-(dodec-1-ynyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**2b**). As described for **2a**, with **7b** (300 mg, 0.39 mmol), ^{i}Pr$_2$EtN (150 mg, 1.16 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (110 mg, 0.46 mmol): **2b** (269 mg, 71%). Colorless oil. TLC (*C*): R_f 0.4, 0.5. ^{31}P-NMR (CDCl$_3$): 149.1, 149.4.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-*N*^{4}-[(dimethylamino)methylidene]-5-(phenylethynyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**2c**). As described for **2a**, with **7c** (350 mg, 0.49 mmol), ^{i}Pr$_2$EtN (190 mg, 1.47 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (140 mg, 0.59 mmol): **2c** (276 mg, 61%). Colorless foam. TLC (*C*): R_f 0.4, 0.5. ^{31}P-NMR (CDCl$_3$): 149.1, 149.3.

*5-[(17β-Acetoxy-3-methoxyestra-1,3,5(10)-trien-17α-yl)ethynyl]-7-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-*N*^{4}-[(dimethylamino)methylidene]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**2d**). As described for **2a**, with **7d** (250 mg, 0.26 mmol), ^{i}Pr$_2$EtN (100 mg, 0.77 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (76 mg, 0.32 mmol): **2d** (211 mg, 70%). Colorless foam. TLC (*C*): R_f 0.4, 0.5. ^{31}P-NMR (CDCl$_3$): 149.0, 149.2.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-*N*^{4}-[(dimethylamino)methylidene]-5-[3-(trifluoroacetamido)prop-1-ynyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**2e**). As described for **2a**, with **7e** (250 mg, 0.33 mmol), ^{i}Pr$_2$EtN (120 mg, 0.93 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (95 mg, 0.40 mmol): **2e** (187 mg, 59%). Colorless foam. TLC (*C*): R_f 0.3, 0.4. ^{31}P-NMR (CDCl$_3$): 149.2, 149.4.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-*N*^{4}-[(dimethylamino)methylidene]-5-[5-(trifluoroacetamido)pent-1-ynyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**2f**). As described for **2a**, with **7f** (300 mg, 0.38 mmol), ^{i}Pr$_2$EtN (149 mg, 1.15 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (118 mg, 0.50 mmol): **2f** (241 mg, 64%). Colorless foam. TLC (*C*): R_f 0.3, 0.4. ^{31}P-NMR (CDCl$_3$): 149.1, 149.3.

3. *Synthesis and Purification of the Oligonucleotides* **8**, **10**, **12**–**32**, **44**–**46**, *and* ***48***–**56**. Oligonucleotide synthesis was performed on an *ABI-392-08* DNA synthesizer (*Applied Biosystems*, Weiterstadt, Germany) on a 1-μmol scale using the phosphoramidites **2a**–**f** and those of the regular 2′-deoxynucleosides (*Applied Biosystems*, Weiterstadt, Germany) following the synthesis protocol for 3′-phosphoramidites [39]. The crude oligonucleotides were purified and detritylated on an oligonucleotide-purification cartridge following the standard protocol for purification [29]. The oligonucleotides were lyophilized on a *Speed-vac* evaporator to yield colorless solids, which were dissolved in 100 μl of double-dest. H_2O and stored frozen at $-18°$. The enzymatic hydrolysis of the oligomers was performed as described in [40]. Quantification of the constituents was made on the basis of the peak areas, which were divided by the extinction coefficients of the nucleoside (ε_{260} values: dA 15400, dC 7300, dG 11400, dT 8800, **1a** 6100, **1b** 6700, and **1c** 12600). Snake-venom phosphodiesterase (EC 3.1.15.1., *Crotallus durissus*) and alkaline phosphatase (EC 3.1.3.1., *E. coli*) were generous gifts of *Roche Diagnostics GmbH*, Germany. MALDI-TOF mass spectra (see *Table 11*) were provided by Prof. *Dr. Hillenkamp*, Dr. *S. Hahner*, and Mrs. *J. Gross* (Institut für Medizinische Physik and Biophysik, Universität Münster, Germany) and by Dr. *J. Gross* (Universität Heidelberg, Germany). Oligonucleotide analysis was carried out by reversed-phase HPLC with a *Merck-Hitachi*-HPLC: 250 × 4 mm *RP-18* column; gradients of 0.1M (Et_3NH)OAc (pH 7.0)/MeCN 95 : 5 (*A*) and MeCN (*B*); gradient *I:* 50 min 0–50% *B* in *A*, flow rate 1 ml/min; gradient *II:* 20 min 0–25% *B* in *A*, flow rate 0.7 ml/min; 30 min 25–40% *B* in *A*, flow rate 1 ml/min; gradient *III:* 20 min 0–25% *B* in *A*, flow rate 1 ml/min.

Table 11. M^+ *Data of Oligonucleotides*[a]) *Determined by MALDI-TOF Mass Spectrometry*[a])

	M^+ [Da]	
	calc.	found
5′-d(**1c**-T)$_6$ (**14**)	4237	4237
5′-d(**1b**-T)$_6$ (**13**)	4622	4625
5′-d[(A-T)$_3$-**1i**-T-(A-T)$_2$] (**16**)	3950	3949
5′-d[CGCG(**1a**)$_2$TTCGCG] (**27**)	3692	3689
5′-d[CGCG(**1b**)$_2$TTCGCG] (**28**)	3973	3969
5′-d[CGCG(**1c**)$_2$TTCGCG] (**29**)	3845	3844
5′-d(CGCG**1i**ATTCGCG) (**30**)	3954	3951
3′-d(ATCC**1b**GTT**1b**TGA) (**35**)	3971	3970
5′-d(T**1b**GGTCAAT**1b**CT) (**36**)	3971	3969
5′-d(T**1i**GGTCAAT**1i**CT) (**38**)	4259	4254
5′-d(**1g**-T)$_6$ (**40**)	3955	3952
5′-d(T**1g**GGTCAAT**1g**CT) (**44**)	3748	3751
3′-d(ATCC**1g**GTT**1g**TGA) (**45**)	3748	3746
5′-d(A-T-**1h**-T)$_3$ (**51**)	3883	3873
5′-d(**1h**-T)$_6$ (**52**)	4123	[b])
5′-d(CGCG(**1h**)$_2$CGCG)$_2$ (**53**)	3805	3800
5′-d(T**1h**GGTCAAT**1h**CT) (**55**)	3805	3801
3′-d(ATCC**1h**GTT**1h**TGA) (**56**)	3805	3804

[a]) Oligonucleotides containing 7-ethynyl-(**1a**), 7-(dodec-1-ynyl)-(**1b**), 7-(phenylethynyl)-(**1c**), 7-(3-amino-prop-1-ynyl)-(**1g**), 7-(5-aminopent-1-ynyl)-(**1h**), or 7-[(17β-hydroxy-3-methoxyestra-1,3,5(10)-trien-17α-yl)-ethynyl]-substituted (**1i**) 7-deaza-2′-deoxyadenosine units. [b]) Could not be detected.

4. *Determination of* T_m *Values and Thermodynamic Data*. Absorbance *vs.* temperature profiles were measured on a *Cary-1/1E* UV/VIS spectrophotometer (*Varian*, Australia) equipped with a *Cary* thermo-electrical controller. The T_m values were measured in the reference cell with a Pt-*100* resistor, and the thermodynamic data (ΔH^0, ΔS^0, ΔG°_{298}) were calculated using the MeltWin 3.0 program [31]. CD Spectra: *Jasco-600* (*Jasco*, Japan) spectropolarimeter with thermostatically (*Lauda-RCS-6* bath) controlled 1-cm cuvettes.

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