7-Deazaadenine-DNA: Bulky 7-Iodo Substituents or Hydrophobic 7-Hexynyl Chains Are Well Accommodated in the Major Groove of Oligonucleotide Duplexes

Frank Seela* and Matthias Zulauf

Abstract: Oligonucleotides containing 7-deaza-7-(hex-1-ynyl)-2′-deoxyadenosine (**1**) and 7-deaza-7-iodo-2′-deoxyadenosine (**2**) are described. The corresponding phosphoramidites (**3a,b**) were synthesized and employed in solid-phase oligonucleotide synthesis. The modified 7-deazaadenine residues show selective base-pairing with dT. According to the T_m values and the thermodynamic data of duplex formation, the 7-iodo and the 7-(hex-1-ynyl) residues of a 7-deazaadenine moiety increase the duplex stability with retention of the particular DNA structure. This is different from 8-substituted adenine, which destabilizes the nucleic acid duplex significantly. The nucleoside **1** fluoresces strongly.

Keywords: DNA structures · duplex stability · nucleosides · oligonucleotides

Introduction

Modified oligonucleotides are useful tools with which to probe the influence of specific changes to bases or to sugar moieties on the structural, spectroscopic, and thermodynamic properties of nucleic acids. They have also attracted interest as gene expression inhibitors (antisense oligonucleotides) and are used as hybridization probes.[1] In particular, the 7-deazapurine (pyrrolo[2,3-d]pyrimidine) nucleosides, nucleotides, and oligonucleotides (purine numbering is used throughout the discussion section) have already made a significant contribution to various areas of this research.[2–4]

Previously, we observed that less bulky substituents introduced at the 7-position of 7-deazaadenine-containing oligodeoxynucleotides stabilize the oligonucleotide duplex structure compared with the parent adenine-containing DNA fragments.[5] These findings are in line with the effects of 5-substituted pyrimidine nucleosides with less bulky groups.[6, 7] Nevertheless, very bulky substituents or lipophilic side chains in 7-deazapurine nucleosides can affect the major groove in various ways and can thereby change the DNA structure. Up to now, such studies have only been performed on 7-deazaguanine and 2,6-diamino-7-deazapurine containing oligonucleotides.[8, 9] Thermal denaturation profiles of oligophosphorothioates containing C-7 propyne derivatives of 7-deaza-2′-deoxyguanosine and 7-deaza-2′-deoxyadenosine hybridized to RNA have been reported.[10]

This work focuses on the properties of oligonucleotides with rather bulky iodo or lipophilic (hex-1-ynyl, hxy) substituents in the 7-position of the 7-deazaadenine moiety (**1** and **2**, shown in Scheme 1). Evidence for the influence of modified residues on the stability of duplexes will be presented and thermodynamic data will be determined. The results of these studies can have practical applications: a) a 7-iodo substituent, when used in the form of the isotope ^{135}I, can be valuable for the isotopic labeling of DNA; b) the lipophilic 7-alkynyl side-chains can improve the delivery of antisense constructs through the cell membrane, and c) the rodlike structures of the 7-(hex-1-ynyl) side-chain have the potential to allow the identification of particular nucleobases by atomic force microscopy (AFM).

Here, we report the synthesis of the phosphoramidites **3a,b**. A series of oligonucleotides with different sequence patterns are prepared and their physicochemical properties, as well as their duplex structure and stability, are studied.

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Results and Discussion

Monomers: Single-crystal X-ray analysis as well as ^1H NMR and NOE measurements have already been used for the conformational analysis of 7-deaza-7-iodo-2′-deoxyadenosine ($I^7c^7A_d$, **2**).[11] It has been demonstrated that the bulky iodo

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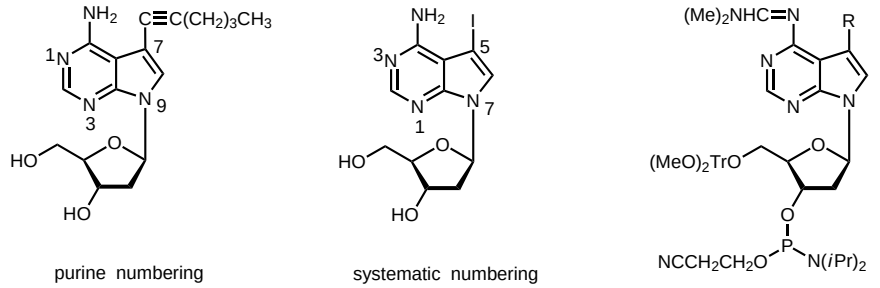


1 **2** **3a,b**

Scheme 1. Nucleosides and phosphoramidite building blocks used for the synthesis of oligonucleotides [**a**: R = I, **b**: R = C≡C(CH$_2$)$_3$CH$_3$].

substituent does not affect the favorable *anti* conformation of the nucleoside (**2**)[11] as has been reported for 8-substituted purines.[12] The 7-deaza-7-(hex-1-ynyl)-2′-deoxyadenosine (hxy^{7}c^{7}A$_d$, **1**) was prepared by the palladium-catalyzed cross-coupling reaction of 7-deaza-7-iodo-2′-deoxyadenosine (**2**) with hex-1-yne.[13] It was observed that compound **1** exhibits strong fluorescence.[14] More than 30 7-alkynyl-7-deazaadenine nucleosides have been synthesized since and studied with regard to their fluorescence, quantum yields, and lifetimes.[15] Figure 1 shows the excitation and emission spectra of compound **1**. The quantum yield was found to be 0.27 and the lifetime 5.8 ns; both were measured in aqueous solution. A typical feature of the fluorescence spectrum is the large Stokes shift, which can be related to a charged transition state (**1**: excitation: 280 nm; emission: 397 nm).[16]

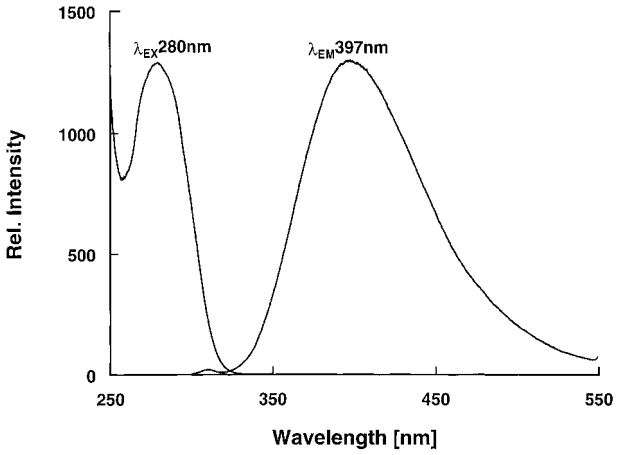
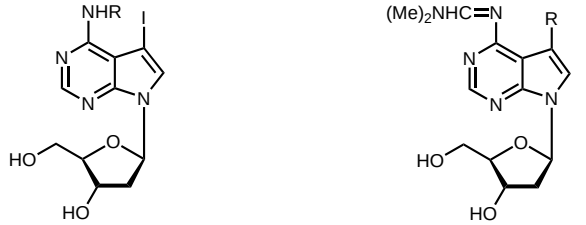


Figure 1. Fluorescence spectra (excitation and emission) of 7-deaza-7-(hex-1-ynyl)-2′-deoxyadenosine (**1**) measured in double-distilled H$_2$O with 10^{-5} M nucleoside concentration.

Abstract in German: *Oligonucleotide, die 7-Desaza-7-(hex-1-inyl)-2′-desoxyadenosin (**1**) oder 7-Desaza-7-iodo-2′-desoxyadenosin (**2**) enthalten, werden beschrieben. Entsprechende Phosphoramidite (**3a,b**) wurden synthetisiert und in der Festphasensynthese eingesetzt. Die modifizierten 7-Desazaadenine gehen eine selektive Basenpaarung mit dT ein. Laut den T_m-Werten und den thermodynamischen Daten der Duplexbildung stabilisieren die 7-Iod- und 7-(Hex-1-inyl)-Reste Oligonucleotid-Duplexe unter weitgehender Erhaltung der DNA-Struktur. Dies unterscheidet sie von 8-substituierten Adeninen, die die Duplexstruktur signifikant destabilisieren. Das Nucleosid **1** weist eine starke Fluoreszenz auf.*

Regarding oligonucleotide synthesis, it has been reported that the NH$_2$-protecting groups of 7-deaza-2′-deoxyadenosine are more stable than those of 2′-deoxyadenosine.[17] The same was expected for the 7-substituted derivatives **1** and **2**. Several acyl derivatives (**4a–d**) were prepared as well as the (dimethylamino)methylidene derivatives **5a,b** (Scheme 2).[18] The increased basicity of the 6-amino group of **2** prompted us to try the acylation reaction in EtOH solution with acid anhydrides,



4a-e **5a,b**

Scheme 2. N^6-amino-protected nucleosides [**4a**: R = Bz, **b**: R = Ac, **c**: R = *i*Bu, **d**: R = Piv, **e**: R = Pac; **5a**: R = I, **b**: R = C≡C(CH$_2$)$_3$CH$_3$].

using conditions developed earlier for the relatively nucleophilic amino group of 2′-deoxycytidine.[19] However, these conditions resulted in low reaction yields (≈20%). Higher yields (≈50%) were obtained when compounds **4a–d** were prepared by the protocol of transient protection.[20] Only the phenoxyacetyl derivative **4e** was prepared by peracylation followed by selective deprotection of the sugar moiety.[21]

The stability of the various protecting groups (**4a–e** and **5a,b**) was studied by UV spectrophotometry in 25% aqueous NH$_3$ (Table 1). From these data it is apparent that the benzoylated derivative **4a** is too stable for oligonucleotide synthesis, whereas the phenoxyacetyl nucleoside **4e** is too labile. Because the (dimethylamino)methylidene compounds **5a,b** were readily available, further investigations were performed with these derivatives. The intermediates **5a,b**

Table 1. Half-life values ($\tau_{1/2}$) for the deprotection of 7-deazaadenine 2′-deoxyribonucleosides in 25% aq. ammonia.[a]

	λ [nm]	$\tau_{1/2}$ [min]
bz^{6}I^{7}c^{7}A$_d$ (**4a**)	312	290
ac^{6}I^{7}c^{7}A$_d$ (**4b**)	299	7
ibu^{6}I^{7}c^{7}A$_d$ (**4c**)	299	12
piv^{6}I^{7}c^{7}A$_d$ (**4d**)	303	103
pac^{6}I^{7}c^{7}A$_d$ (**4e**)	301	4
fma^{6}I^{7}c^{7}A$_d$ (**5a**)	323	82
fma^{6}hxy^{7}c^{7}A$_d$ (**5b**)	321	110

[a] Measured at 40 °C.

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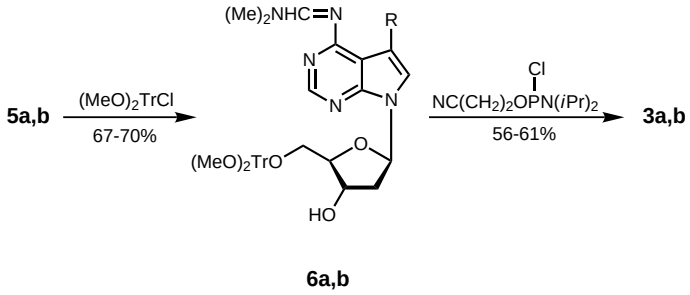
were treated with 4,4′-dimethoxytriphenylmethyl chloride to give the $(MeO)_2$Tr derivatives **6a,b** (Scheme 3).[22] The phosphoramidites **3a,b** were prepared from the DMT derivatives under standard conditions from chloro((2-cyanoethyl)-diisopropylamino)phosphine.



Scheme 3. Synthesis of the 7-deazaadenine nucleoside building blocks for DNA synthesis [**a**: R = I, **b**: R = C≡C$(CH_2)_3CH_3$].

The structures of the new compounds were determined by ^{1}H, ^{13}C, and ^{31}P NMR spectra and by elemental analyses (Table 2 and Experimental Section). The ^{13}C chemical shifts of the derivatives **4a–e**, **5a,b**, and **6a,b** were assigned from the coupling pattern of the corresponding gated-decoupled ^{13}C NMR spectra. According to Table 2, carbon-7 of the 7-iodo derivatives shows a strong upfield shift upon iodination, which is due to the strong mesomeric effect of the iodo substituent.

The nucleosides **1** and **2** were analyzed by ^{1}H NOE spectroscopy to elucidate the base conformation around the *N*-glycosylic bond. Vicinal ^{1}H,^{1}H coupling constants of the sugar protons revealed the most favored puckering (*N* vs *S*-conformer populations) of these nucleosides. It was found that the 7-(hex-1-ynyl) chain influences the conformation of the parent 7-deazaadenine in a similar way to the bulky iodo substituent. The conformation of the 7-(hex-1-ynyl) nucleoside **1** around the *N*-glycosylic bond is *anti* and the sugar confomation is 69 % *S*-type compared to 71 % determined for the iodo compound **2**.[23] Thus, it can be anticipated that both nucleosides form B-like DNA structures if incorporated in a DNA-duplex.

Oligonucleotides: Oligonucleotides were synthesized on a solid phase by means of an automated synthesizer with the phosphoramidites **3a,b,** as well as those of the regular DNA constituents.[24] The oligonucleotides were prepared and purified by oligonucleotide purification cartridges,[25] and their homogeneity was determined by reverse-phase HPLC. Figure 2 shows that the mobility of the parent oligomer **7** was decreased by a 7-iodo substituent (→**11**) and even more by the lipophilic hexynyl side chain of **12**. The nucleoside composition of the oligomers was determined by hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase (see Figure 3, for example), as well as by MALDI-TOF mass spectroscopy (Table 3).

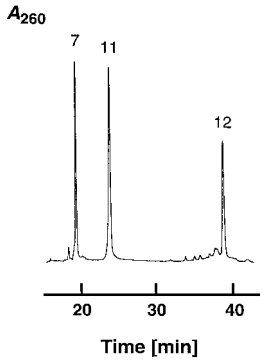


Figure 2. HPLC profiles of the oligonucleotides 5′-d(A–T)$_6$ (**7**), 5′-d(I^7c^7A–T)$_6$ (**11**), and 5′-d(hxy$^7c^7$A–T)$_6$ (**12**) after purification by reverse-phase (RP-18) chromatography, gradient *I*; for details see Experimental Section.

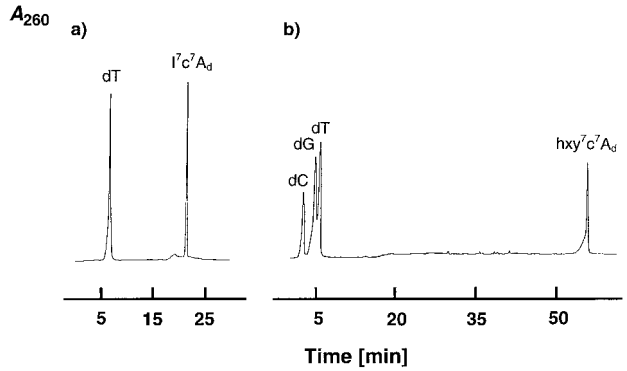


Figure 3. HPLC profiles of the oligonucleotides a) **11** and b) **24** after enzymatic hydrolysis with snake-venom phosphodiesterase, followed by alkaline phosphatase in 1M Tris–HCl buffer (pH 8.3), gradient *II*; for details see Experimental Section.

Table 2. ^{13}C NMR chemical shifts of 7-deazaadenine 2′-deoxyribofuranosides.[a,b]

	C(2)[c] C(2)[d]	C(6)[c] C(4)[d]	C(5)[c] C(4a)[d]	C(7)[c] C(5)[d]	C(8)[c] C(6)[d]	C(4) C(7a)[d]	C(1′)	C(3′)	C(4′)	C(5′)
c^7A_d	151.6	157.5	102.9	99.6	121.6	149.6	83.3	71.1	87.3	62.1
1	152.5	157.5	102.3	95.5	125.4	149.0	83.1	70.9	87.4	61.9
2	152.0	157.3	103.2	51.9	126.9	149.8	83.0	71.0	87.5	62.0
4a	151.9	167.3	114.5	54.6	131.9	151.1	83.1	71.0	87.8	61.8
4b	150.9	151.1	112.7	53.8	131.3	151.5	83.0	70.8	87.5	61.7
4c	150.8	151.3	113.3	54.0	131.4	151.6	82.9	70.8	87.5	61.7
4d	150.7	151.5	114.0	54.0	131.4	152.0	82.9	70.8	87.5	61.7
4e	151.1	157.6	112.0	53.2	131.6	151.7	83.1	70.9	87.7	61.8
5a	151.5	160.2	110.3	53.6	128.6	150.9	83.0	71.1	87.5	61.9
5b	151.6	160.9	110.1	97.5	127.1	150.7	82.9	71.0	87.4	61.9
6a	151.2	160.2	110.3	53.9	128.3	151.0	82.8	70.9	85.6	64.3
6b	151.9	161.0	110.2	98.0	127.1	151.0	82.7	70.8	85.5	64.3

[a] Measured in [D_6]DMSO at 25 °C. [b] Superimposed by DMSO. [c] Purine numbering. [d] Systematic numbering.

Table 3. Molecular weights determined from MALDI-TOF mass spectra of oligonucleotides.

	M^+ (calcd)	M^+ (found)
5′-d($I^7c^7A-T)_6$ (**11**)	4391.9	4392.0
5′-d[$(I^7c^7A)_6$-$(T)_6$] (**14**)	4391.9	4394.8
5′-d[$(I^7c^7A_{11})$-A] (**29**)	5070.5	5071.2
5′-d($hxy^7c^7A-T)_6$ (**12**)	4117.3	4117.1
5′-d[$(hxy^7c^7A)_6$-$(T)_6$] (**15**)	4117.3	4116.5
5′-d($hxy^7c^7A_{11}$)-A] (**30**)	4567.1	[a]
5′-d(hxy^7c^7A-$A)_6$ (**31**)	4171.4	4173.0
5′-d(CGCG($hxy^7c^7A)_2$TTCGCG) (**20**)	3804.7	3801.2
5′-d(GTI$^7c^7$AG($I^7c^7A)_2$TTCTI$^7c^7$AC) (**23**)	4144.0	4144.3

[a] Not detected.

Self-complementary oligonucleotides with alternating bases or alternating base tracts: Oligonucleotide duplexes with alternating 5′-d$(A-T)_n$, for example poly[5′-d(A–T)]·poly[5′-d(A–T)], have a more flexible secondary structure than other oligonucleotide duplexes. They have a small helical twist on the 5′-d(A–T) steps and a larger one on the 5′-d(T–A) steps.[26, 27] For this kind of duplexes [5′-d$(A-T)_n$], environmental factors such as dehydration and counterion binding will influence their secondary as well as their tertiary structure. Therefore, structural modification of the base residues has a major impact on the stability of such duplexes.

In order to study this behavior, alternating self-complementary oligonucleotides 5′-d$(A-T)_6$ (**7**), 5′-d[$(A)_6-(T)_6$] (**13**), and 5′-d[$(A)_3-(T)_3-(A)_3-(T)_3$] (**16**) were prepared and the influence of 7-substituents on the T_m values as well as on the thermodynamic data was determined (Table 4). From Table 4, it is apparent that the alternating oligonucleotides with 7-halogenated 7-deazaadenine residues have T_m values generally about 20 °C higher than those with the nonsubstituted 7-deazaadenine residue. This amounts to a T_m increase of about 1.7 °C per modified base residue, which is almost independent of whether the halogen substituent is Cl, Br, or I. The stabilization of duplexes containing 7-halogenated 7-deazaadenines reflects a favorable enthalpic term compared with the duplex of 5′-d$(A-T)_6$ (**7**). However, when the halogenated compounds **9**, **10**, and **11** are compared with 5′-d$(c^7A-T)_6$ (**8**), the change of entropy seems to play the major role. The duplex of 5′-d[$(hxy^7c^7A)_6-(T)_6$] (**15**), which also shows a significant T_m increase, is stabilized by a more favorable entropy term. Some of these observations on the duplexes derived from the analogues of 5′-d$(A-T)_6$ (**7**) can also be made for oligomers in which larger segments alternate (Table 4, Figure 4).

The retention of the helix geometry of the duplexes, shown in Table 4, can be seen from their CD spectra. The oligonu-

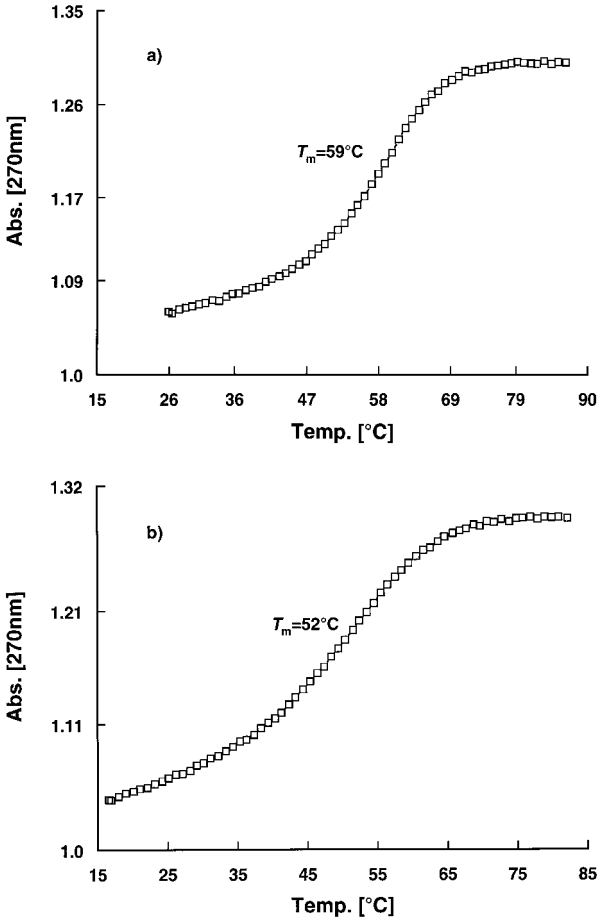


Figure 4. Melting profiles of the alternating duplexes a) 5′-d[$(I^7c^7A-T)_6]_2$ (**11**·**11**), and b) 5′-d[$(hxy^7c^7A-T)_6]_2$ (**12**·**12**), measured at 270 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na cacodylate (pH = 7.0) with 10 μM oligomer concentration.

Table 4. T_m values and thermodynamic data for self-complementary oligonucleotides with alternating bases or base tracts.

	T_m [°C][a,b]	ΔH [kcal mol^{-1}]	ΔS [cal mol^{-1} K^{-1}]	$\Delta G°$ [kcal mol^{-1}]
5′-d[$(A-T)_6]_2$ (**7**·**7**)	33(26)	−45(−44)	−125(−127)	−6.3(−5.5)
5′-d[$c^7A-T)_6]_2$[5] (**8**·**8**)	36(27)	−65(−53)	−186(−153)	−6.8(−5.5)
5′-d[$(Cl^7c^7A-T)_6]_2$[5] (**9**·**9**)	57(51)	−60(−63)	−159(−173)	−10.6 (−9.8)
5′-d[$(Br^7c^7A-T)_6]_2$[5] (**10**·**10**)	57(51)	−62(−55)	−165(−148)	−10.9(−9.4)
5′-d[$(I^7c^7A-T)_6]_2$ (**11**·**11**)	59(53)	−65(−70) (−71)[c]	−174(−192) (−193)[c]	−11.4(−10.6) (−10.8)[c]
5′-d[$(hxy^7c^7A-T)_6]_2$ (**12**·**12**)	52(49)	−47(−62)	−123(−170)	−9.3(−9.4)
5′-d[$(A)_6$-$(T)_6]_2$ (**13**·**13**)	46(40)	−81(−75)	−232(−219)	−9.1(−7.4)
5′-d[$(I^7c^7A)_6$-$(T)_6]_2$ (**14**·**14**)	58(52)	−76(−76)	−206(−212)	−11.9(−10.4)
5′-d[$(hxy^7c^7A)_6$-$(T)_6]_2$ (**15**·**15**)	58(52)	−64(−54)	−169(−144)	−11.3(−9.0)
5′-d[$(A)_3$-$(T)_3$-$(A)_3$-$(T)_3]_2$ (**16**·**16**)	40(35)	−71(−69)	−203(−201)	−7.8(−6.4)
5′-d[$(hxy^7c^7A)_3$-$(T)_3$-$(hxy^7c^7A)_3$-$(T)_3]_2$ (**17**·**17**)	61(57)	−66(−57)	−175(−149)	−12.1(−10.8)

[a] Measured at 270 nm in 1M NaCl containing 100 mM $MgCl_2$ and 60 mM Na cacodylate (pH = 7.0) with 10 μM oligonucleotide concentration. [b] Values in parentheses measured at 270 nm in 0.1M NaCl, 100 mM $MgCl_2$, and 10 mM Na cacodylate (pH = 7.0) with 10 μM oligonucleotide concentration. [c] Determined from the concentration dependence of the T_m values.

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cleotide duplexes **9**, **10**, and **12** exhibit the characteristics of a B-like DNA structure, with a positive band at 275 nm and a negative lobe around 250 nm (Figure 5). Only the CD spectrum of the iodine-containing duplex **11** differs significantly from the others (Figure 5).

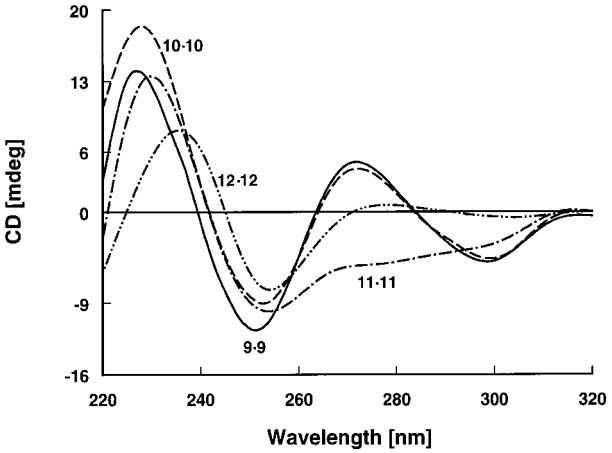

Figure 5. CD spectra of the alternating duplexes 5′-d[$(Cl^7c^7A-T)_6$]$_2$ (**9**·**9**), 5′-d[$(Br^7c^7A-T)_6$]$_2$ (**10**·**10**), 5′-d[$(I^7c^7A-T)_6$]$_2$ (**11**·**11**), and 5′-d[$(hxy^7c^7A-T)_6$]$_2$ (**12**·**12**), measured at 10 °C in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na cacodylate (pH = 7.0) with 10 μM oligomer concentration.

Self-complementary oligonucleotides with palindromic sequences: The next oligonucleotides to be investigated were also self-complementary but contained a palindromic sequence, which represents the recognition site of the endodeoxyribonuclease Eco RI 5′-d(GAATTC).[28] Hairpin formation has been previously reported, in particular for the Dickerson–Drew oligomer 5′-d(CGCGAATTCGCG)[29] measured at low salt concentration in the absence of Mg^{2+}.[30] Since our measurements were performed at 1 M or 0.1 M NaCl in the presence of 0.1 M $MgCl_2$, only duplex formation can be anticipated for all cases. The thermodynamic data of the oligonucleotides were determined by curve shape analysis of the melting profiles by means of the program Meltwin 3.0[31] and in a few cases also from the concentration dependence of the T_m values (Table 5, Figure 6). The thermodynamic data obtained by these two methods are in sufficient agreement. This underlines the fact that duplex formation follows a two-state process.

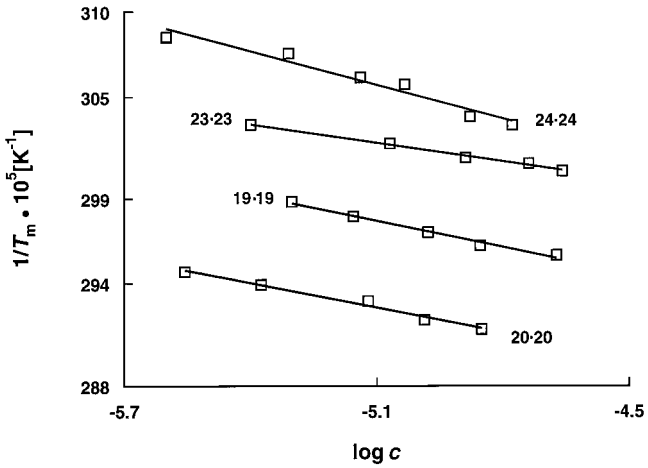

Figure 6. $1/T_m$ vs log c plot of self-complementary duplexes **19**·**19**, **20**·**20**, **23**·**23**, and **24**·**24**; for sequences see Table 5.

In the case of the oligomers related to the Dickerson–Drew dodecamer 5′-d(CGCGAATCGCG) (**18**) (Table 5), the replacement of dA residues by either **1** (→**20**·**20**) or **2** (→**19**·**19**) increases the T_m values above the value of the parent duplex **18**·**18**. The same is observed for 5′-d(GTAGAATTCTAC) (**21**). The T_m increase is between 1–1.5 °C per modified base residue, depending on the sequence and the type of substituent. A 7-(hex-1-ynyl) residue has less of a stabilizing effect than the 7-iodo substituent in the case of the sequence type **21**. Again, the stabilization by the alkynyl residue is caused by more favorable entropy, even when the enthalpy change is unfavorable.

Non-self-complementary Oligonucleotides: homooligonucleotides: For the studies of homooligonucleotide duplexes, the oligomers 5′-d[$(I^7c^7A)_{11}$–A] (**29**) and 5′-d[$hxy^7c^7A)_{11}$-A] (**30**) were hybridized with $d(T)_{12}$ (**26**), and their T_m values were compared with those of $d(A)_{12}\cdot d(T)_{12}$ (**25**·**26**) (Table 6). Stabilization for the hybrid **29**·**26** (T_m 54 °C) containing 7-iodo-7-deazaadenine residues was similar to alternating 5′-d$(I^7c^7A-T)_6$ (**11**), compared with the parent duplex $d(A)_{12}\cdot d(T)_{12}$ (**25**·**26**) (T_m 44 °C). As can be seen from Table 6, the

Table 5. T_m values and thermodynamic data of self-complementary oligonucleotides containing 7-iodo- and 7-(hex-1-ynyl)-7-deazaadenine.

	T_m [°C][a,b]	ΔH [kcal mol^{-1}]	ΔS [cal mol^{-1} K^{-1}]	$\Delta G°$ [kcal mol^{-1}]
5′-d[(CGCGAATTCGCG)]$_2$ (**18**·**18**)	64(63)	−83(−73)	−224(−195)	−13.7(−12.6)
5′-d[(CGCG(I^7c^7A)$_2$TTCGCG)]$_2$ (**19**·**19**)	65(63)	−94(−90)	−255(−247)	−14.5(−13.9)
		−95[c]	−258[c]	−14.5[c]
5′-d[(CGCG(hxy^7c^7A)$_2$TTCGCG)]$_2$ (**20**·**20**)	70(69)	−89(−84)	−238(−225)	−15.5(−14.8)
		−94[c]	−253[c]	−16.0[c]
5′-d[(GTAGAATTCTAC)]$_2$ (**21**·**21**)	46(43)	−79(−84)	−226(−225)	−9.2(−8.6)
		(−75)[c]	(−215)[c]	(−8.5)[c]
5′-d[(GTAGI^7c^7AATTCTAC)]$_2$ (**22**·**22**)	47	−82	−231	−9.9
5′-d[(GTI^7c^7AG(I^7c^7A)$_2$TTCTI^7c^7AC)]$_2$ (**23**·**23**)	55(50)	−80(−81)	−221(−229)	−11.1(−10.0)
		−85(−85)[c]	−236(−242)[c]	−12.0(−10.3)[c]
5′-d[(GThxy^7c^7AG(hxy^7c^7A)$_2$TTCThxy^7c^7AC)]$_2$ (**24**·**24**)	52(48)	−56(−63)	−149(−173)	−9.4(−9.1)
		−62[c]	−168[c]	−10.3[c]

[a] Measured at 270 nm in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na cacodylate (pH = 7.0) with 10 μM single-strand concentration. [b] Measured at 270 nm in 0.1 M NaCl, 100 mM $MgCl_2$, and 10 mM Na cacodylate (pH = 7.0) with 10 μM single-strand concentration. [c] Determined from the concentration dependence of the T_m values.

Table 6. T_m values and thermodynamic data for non-self-complementary oligonucleotides containing consecutive base residues.

	T_m [°C][a,b]	ΔH [kcal mol^{-1}]	ΔS [cal mol^{-1} K^{-1}]	$\Delta G°$ [kcal mol^{-1}]
d(A)$_{12}$ · d(T)$_{12}$ (**25** · **26**)	44(37)	−84(−91)	−238(−267)	−9.8(−7.9)
5′-d[(c^{7}A)$_{11}$-A] · d(T)$_{12}$[5] (**27** · **26**)	30(24)	−73(−67)	−215(−199)	−6.3(−5.0)
5′-d[(Br^{7}c^{7}A)$_{11}$-A] · d(T)$_{12}$[5] (**28** · **26**)	54(48)	−85(−79)	−234(−219)	−12.7(−10.7)
5′-d[(I^{7}c^{7}A)$_{11}$-A] · d(T)$_{12}$ (**29** · **26**)	54(49)	−98(−80)	−275(−222)	−13.7(−10.9)
5′-d[(hxy^{7}c^{7}A)$_{11}$-A] · d(T)$_{12}$ (**30** · **26**)	[c]			
5′-d[(hxy^{7}c^{7}A-A)$_6$] · d(T)$_{12}$ (**31** · **26**)	47	−59	−160	−9.5
5′-d[(hxy^{7}c^{7}A)$_3$-(A)$_3$-(hxy^{7}c^{7}A)$_3$-(A$_3$)] · d(T)$_{12}$ (**32** · **26**)	50	−59	−157	−10.3

[a] Measured at 270 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na cacodylate (pH = 7.0) with 5 μM single-strand concentration. [b] Values in parentheses measured at 270 nm in 0.1M NaCl, 100 mM $MgCl_2$, and 10 mM Na cacodylate (pH = 7.0) with 5 μM single-strand concentration. [c] No melting was observed.

stabilization by iodinated bases reflects a favorable enthalpy term. In contrast to **29**, no cooperative melting profile was observed for 5′-d[(hxy^{7}c^{7}A)$_{11}$−A] · d(T)$_{12}$ (**30** · **26**). This phenomenon has already been observed in the case of 5-substituted pyrimidines, for example by the introduction of a hexyl tether into synthetic DNA, which led to an unfavorable effect on the duplex stability.[32] These results also agree with our finding that a consecutive arrangement of bulky and hydrophobic 7-substituents, such as hex-1-ynyl of hxy^{7}c^{7}G$_d$ along the major groove of a double helix hybridized with oligo(dC), leads to duplex destabilization. The destabilization is not found for c^{7}G$_d$.[33] This is probably due to desiccation of the molecule, causing a shrinking of the major groove. However, when alternating hxy^{7}c^{7}A$_d$ residues and dA (oligomers **31** and **32**) are present, stable duplexes with d(T)$_{12}$ (**31** · **26** and **32** · **26**) are formed. These are stabilized by a more favorable entropy term (Table 6).

It has been reported that base-stacking interactions within single-stranded oligomers can be quite strong and can lead to preorganised single-strand helices.[34] This preorganization of a single strand influences the duplex stability in such a way that the more stable secondary structure of one of the single strands determines the secondary structure of the duplex. As the CD spectra give information on the secondary structure of such single strands, the spectra of compounds d(A)$_{12}$ (**25**), 5′-d[(I^{7}c^{7}A)$_{11}$−A] (**29**), 5′-d[(hxy^{7}c^{7}A)$_{11}$−A] (**30**), and 5′-d[(hxy^{7}c^{7}A−A)$_6$] (**31**) were measured (Figure 7). The CD spectra of the alternating oligonucleotide **31** is somewhat similar to that of d(A)$_{12}$ (**25**), which shows the characteristics of a right-handed B-DNA helix. These spectra are significantly different from that of the homooligomer 5′-d(I^{7}c^{7}A$_{11}$−A) (**29**). However, the latter is also typical for the CD spectrum of normal (A/T)-paired duplexes of a homo-DNA.[35] On the other hand, the oligomer 5′-d[(hxy^{7}c^{7}A)$_{11}$−A] (**30**) exhibits a CD spectrum that looks different from the spectra mentioned above.

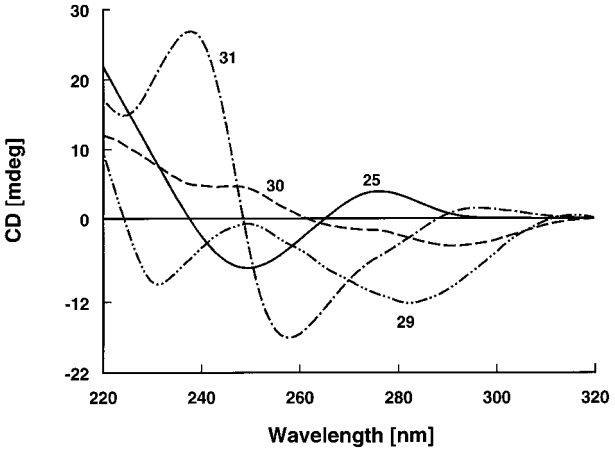

Figure 7. CD spectra of the single-stranded homooligonucleotides d(A)$_{12}$ (**25**), 5′-d[(I^{7}c^{7}A)$_{11}$-A] (**29**), 5′-d[(hxy^{7}c^{7}A)$_{11}$-A] (**30**), and 5′-d[(hxy^{7}c^{7}A-A)$_6$] (**31**). Measured at 10 °C in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na cacodylate (pH = 7.0) with 7.5 μM single-strand concentration.

As it was of interest whether the temperature-induced changes of the CD spectra resulted from a cooperative destacking, the CD values of 5′-d(I^{7}c^{7}A$_{11}$−A) (**29**) at 283 nm were plotted against the temperature. The linear dependence indicates that no cooperative destacking occurred (Figure 8).

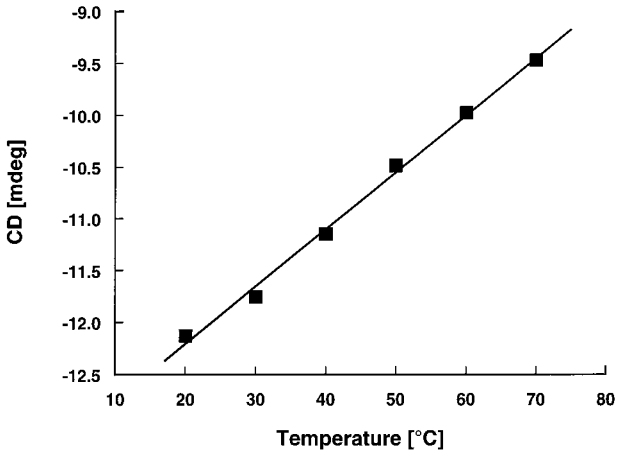

Figure 8. Temperature-dependent CD data (ellipticities) of single-stranded 5′-d[(I^{7}c^{7}A)$_{11}$-A] (**29**), measured at 283 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na cacodylate (pH = 7.0) with 7.5 μM single-strand concentration.

Non-self-complementary oligonucleotides with mismatches: It was also of interest to study the base-pair selectivity of the modified oligonucleotides. For this purpose, a hxy^{7}c^{7}A$_d$ residue was incorporated into the center of dT$_{12}$. This oligomer was hybridized with dA$_{12}$ homomers containing the dT, dA, dC, and dG bases opposite to the 7-deazaadenine moiety (Table 7). The modified duplex (**34** · **35**) showed a slightly higher stability than the parent duplex (**33** · **34**), caused by more favorable entropy and a slightly lower enthalpy change. With dC (**35** · **36**), dG (**35** · **37**), and dA (**25** · **35**), the duplex stability is significantly decreased. These results show that only the hxy^{7}c^{7}A$_d$-dT base pair is stable and

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Table 7. T_m values and thermodynamic data of non-self-complementary oligonucleotides containing mismatches opposite to 7-(hex-1-ynyl)-7-deazaadenine.

	T_m [°C][a,b]	ΔH [kcal mol^{-1}]	ΔS [cal mol^{-1} K^{-1}]	ΔG° [kcal mol^{-1}]
5′-d[(T)$_5$-A-(T)$_6$] (**33**) 3′-d[(A)$_5$-T-(A)$_6$] (**34**)	39(33)	−97(−110)	−284(−301)	−8.4(−6.6)
5′-d[(T)$_5$-hxy^{7}c^{7}A-(T)$_6$] (**35**) 3′-d[(A)$_5$-T-(A)$_6$] (**34**)	41(35)	−81(−90)	−232(−268)	−8.7(−7.1)
5′-d[(T)$_5$-hxy^{7}c^{7}A-(T)$_6$] (**35**) 3′-d[(A)$_5$-C-(A)$_6$] (**36**)	28	−44	−146	−6.0
5′-d[(T)$_5$-hxy^{7}c^{7}A-(T)$_6$] (**35**) 3′-d[(A)$_5$-G-(A)$_6$] (**37**)	28	−46	−127	−5.7
5′-d[(T)$_5$-hxy^{7}c^{7}A-(T)$_6$] (**35**) 3′-d(A)$_{12}$ (**25**)	27	−64	−188	−5.9
5′-d[(T)$_5$-Br^{8}A-(T)$_6$] (**38**) 3′-d[(A)$_5$-T-(A)$_6$] (**34**)	34(28)	−77(−81)	−226(−245)	−7.1(−5.4)

[a] Measured at 270 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na cacodylate (pH = 7.0) with 5 μM single-strand concentration. [b] Values in parentheses measured at 270 nm in 0.1M NaCl, 100 mM $MgCl_2$, and 10 mM Na cacodylate (pH = 7.0) with 5 μM single-strand concentration.

that mismatches can be observed. Duplexes with 8-bromo-2′-deoxyadenosine are strongly destabilized (**34**·**38**). In this case, the 8-substituent forces the nucleoside into the *syn* conformation and can therefore interfere with the sugar–phosphate backbone.[12, 36]

Non-self-complementary oligonucleotides with random base composition: The oligonucleotides discussed above have special sequences and are not representative of the common regions of natural DNA. Therefore, two oligonucleotides, 5′-d(TAGGTCAATACT) (**39**) and 5′-d(AGTATTGACCTA) (**40**), were constructed to form a stable hybrid with a T_m value of 50 °C. This duplex is used in our laboratory as a standard to study the influence of modified bases on duplex structure and stability. The oligonucleotides **39**–**45** were synthesized. They were then hybridized with the complementary strand, and the T_m values were measured. Duplex formation was observed in all cases (Table 8). The overall increase in stability of the deoxyoligonucleotide duplexes was 1.5 °C per I^{7}c^{7}A$_d$ residue, although the duplex stabilization caused by a hxy^{7}c^{7}A$_d$ residue is somewhat lower. The highest T_m values were found for the hybrids **42**·**43**, **44**·**45**, and **42**·**45**. When single-stranded oligomers containing 7-iodo-7-deazaadenine residues are hybridized with those containing a 7-(hex-1-ynyl) chain, for example in **42**·**45**, stable duplexes are also formed. No final decision can be made as to whether duplex stabilization results from more favorable enthalpic or entropic terms. The deoxyoligonucleotide single strands **39**, **42**, and **44** were also hybridized with the ribooligonucleotide **41**. In these cases, a less stable helix was formed than for the oligodeoxynucleotide duplexes **39**·**40**, **40**·**42**, and **40**·**44**. This agrees with observations on regular DNA–RNA hybrids.[37] The oligodeoxyheteroduplexes shown in Table 8 form B-DNA structures, as observed by CD spectroscopy, whereas the hybrids with the ribooligonucleotide strand **41** form an A-DNA structure (Figure 9).

Table 8. T_m values and thermodynamic data for non-self-complementary oligonucleotides containing 7-iodo- and 7-(hex-1-ynyl)-7-deazaadenine.

	T_m [°C][a,b]	ΔH [kcal mol^{-1}]	ΔS [cal mol^{-1} K^{-1}]	ΔG° [kcal mol^{-1}]
5′-d(TAGGTCAATACT) (**39**) 3′-d(ATCCAGTTATGA) (**40**)	50(47)	−90(−82)	−252(−230)	−11.8(−10.4)
5′-d(TAGGTCAATACT) (**39**) 3′-r(AUCCAGUUAUGA) (**41**)	48(46)	−65(−82)	−176(−230)	−10.2(−10.1)
5′-d(TI^{7}c^{7}AGGTC(I^{7}c^{7}A)$_2$TI^{7}c^{7}ACT) (**42**) 3′-d(ATCCAGTTATGA) (**40**)	55	−81	−221	−12.2
5′-d(TAGGTCAATACT) (**39**) 3′-d(ATCCI^{7}c^{7}AGTTI^{7}c^{7}ATGA) (**43**)	54	−85	−236	−12.3
5′-d(TI^{7}c^{7}AGGTC(I^{7}c^{7}A)$_2$TI^{7}c^{7}ACT) (**42**) 3′-d(ATCCI^{7}c^{7}AGTTI^{7}c^{7}ATGA) (**43**)	58(54)	−85(−81)	−231(−224)	−13.1(−12.0)
5′-d(TI^{7}c^{7}AGGTC(I^{7}c^{7}A)$_2$TI^{7}c^{7}ACT) (**42**) 3′-r(AUCCAGUUAUGA) (**41**)	48	−80	−225	−10.1
5′-d(Thxy^{7}c^{7}AGGTC(hxy^{7}c^{7}A)$_2$Thxy^{7}c^{7}ACT) (**44**) 3′-d(ATCCAGTTATGA) (**40**)	54	−79	−217	−11.9
5′-d(TAGGTCAATACT) (**39**) 3′-d(ATCChxy^{7}c^{7}AGTThxy^{7}c^{7}ATGA) (**45**)	52	−93	−260	−12.2
5′-d(Thxy^{7}c^{7}AGGTC(hxy^{7}c^{7}A)$_2$Thxy^{7}c^{7}ACT) (**44**) 3′-d(ATCChxy^{7}c^{7}AGTThxy^{7}c^{7}ATGA) (**45**)	58(55)	−103(−91)	−285(−253)	−14.2(−12.8)
5′-d(Thxy^{7}c^{7}AGGTC(hxy^{7}c^{7}A)$_2$Thxy^{7}c^{7}ACT) (**44**) 3′-r(AUCCAGUUAUGA) (**41**)	48(43)	−96(−74)	−273(−209)	−11.5(−9.5)
5′-d(TI^{7}c^{7}AGGTC(I^{7}c^{7}A)$_2$TI^{7}c^{7}ACT) (**42**) 3′-d(ATCChxy^{7}c^{7}AGTThxy^{7}c^{7}ATGA) (**45**)	59(54)	−82(−81)	−223(−222)	−13.0(−12.0)

[a] Measured at 270 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na cacodylate (pH = 7.0) with 5 μM single-strand concentration. [b] Values in parentheses measured at 270 nm in 0.1M NaCl, 100 mM $MgCl_2$, and 10 mM Na cacodylate (pH = 7.0) with 5 μM single-strand concentration.

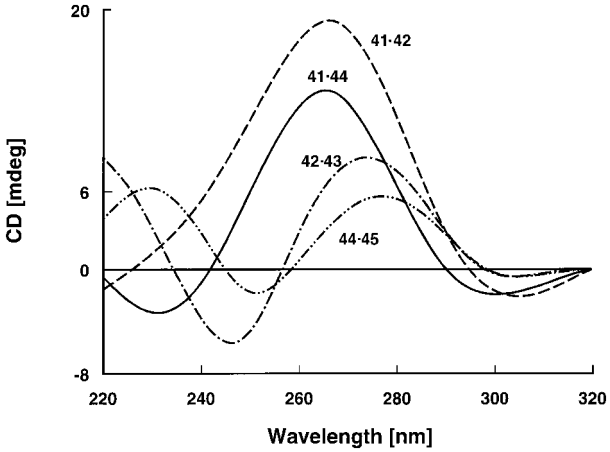
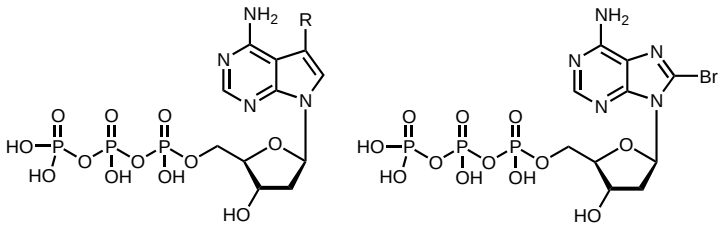


Figure 9. CD spectra of the heteroduplexes (**41** · **42**), (**41** · **44**), (**42** · **43**), and (**44** · **45**) (for sequences see Table 8),



46a,b **47**

Scheme 4. Triphosphates of 7-deazaadenine and adenine [**a**: R = I, **b**: R = C≡C(CH$_2$)$_3$CH$_3$].

Conclusion and Outlook

From these experiments, it can be concluded that bulky iodo substituents or lipophilic hexynyl residues attached to the 7-position of a 7-deazaadenine moiety stabilize the oligonucleotide duplex structure. The stabilization occurs both in the series of self-complementary and non-self-complementary oligonucleotide duplexes. The increase of T_m is sequence-dependent and can be due to more favorable enthalpy or entropy changes.

In all cases, the bulky 7-substituents have steric freedom within the grooves of the DNA. This is different from the 8-substituents of purine residues, which unlike the 7-substituents interfere with the sugar–phosphate backbone. Furthermore, the halogen and the alkynyl 7-substituents reduce the basicity of nitrogen 1 of the 7-deazaadenine moiety (pK_a values: $c^7A_d = 5.3$,[38] $I^7c^7A_d = 4.5$, $hxy^7c^7A_d = 4.3$). At the same time, the 6-amino group can become a better proton donor. With regard to these properties, compounds **1** and **2** are closer analogues of dA ($pK_a = 3.8$[38]) than the nonsubstituted 7-deaza-2′-deoxyadenosine (c^7A_d).

The duplex stability of oligonucleotides with 7-substituted 7-deazaadenine residues is significant for hybridization studies and for the stability of antisense oligonucleotides. It is also important for the complete replacement of a purine base by base-modified nucleosides with a large substituent or a reporter group on the purine base. However, oligonucleotides with bulky substituents at position 8 of an adenine base cannot be used for such purposes, as they make the duplex rather unstable (Table 7). Recently, it was shown that compounds **1** and **2** can be used in the triphosphate form (**46a,b**, Scheme 4) as 100% substitutes for dATP during the DNA-polymerase-catalyzed elongation of a growing oligonucleotide chain on a single-stranded DNA matrix.[39] The 8-bromo dATP **47** cannot be incorporated in such a way and will cause chain termination when used instead of ATP.[39]

In addition to the observations made for the oligonucleotides, the 7-alkynyl derivatives of 7-deaza-2′-deoxyadenosine represent a new class of highly fluorescent nucleosides. They have bulky hydrophobic bases in the favorable *anti* conformation required for hybridization. The bright fluorescence of 7-deaza-7-(hex-1-ynyl)-2′-deoxyadenosine (**1**) makes this molecule useful for fluorescence studies on DNA, even on the single molecule level.

Experimental Section

Monomers: Flash chromatography (FC): at 0.4 bar with silica gel 60 (Merck, Darmstadt, Germany). Solvent systems for FC and TLC: CH_2Cl_2/MeOH 9:1 (*A*), petroleum ether (boiling range 40–60 °C)/acetone 1:1 (*B*). Samples were collected with an UltroRac II fractions collector (LKB Instruments, Sweden). Melting points: Büchi SMP-20 apparatus (Büchi, Switzerland). UV spectra: 150-20 spectrometer (Hitachi, Japan). NMR spectra: AC 250 and AMX-500 spectrometers (Bruker, Germany); δ values are relative to internal Me_4Si or external H_3PO_4. Microanalyses were performed by Mikroanalytisches Laboratorium Beller (Göttingen, Germany).

Oligonucleotides: Oligonucleotides were synthesized with a 380B DNA synthesizer (Applied Biosystems, Germany) according to the standard protocol. The oligonucleotides were purified by oligonucleotide purification cartridges.[25] The enzymatic hydrolysis of the oligomers was performed as described in ref. [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, $I^7c^7A_d$ 5000, $hxy^7c^7A_d$ 6600). Snake-venom phosphodiesterase (EC 3.1.15.1, *Crotallus durissus*) and alkaline phosphatase (EC 3.1.3.1., *E. coli*) were generous gifts of Boehringer Mannheim, Germany). MALDI-TOF spectra were provided by Dr. S. Hahner and J. Gross (Prof. Hilgenkamp, Institute of Medicinal Physics and Biophysics, University of Münster, Germany). RP-18 HPLC: 250 × 4 mm RP-18 column; Merck-Hitachi HPLC; 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^{-1}; gradient *II*: 20 min 0–20% *B* in *A*; 40 min 20–40% *B* in *A*, flow rate 1 mL min^{-1}.

Determination of melting curves and thermodynamics: Absorbance vs. temperature profiles were measured on a Cary 1/1E UV/VIS spectrophotometer (Varian, Australia) with a Cary thermoelectrical controller. The T_m values were measured in the reference cell with a Pt-100 resistor, and the thermodynamic data (ΔH, ΔS, $\Delta G°$) were calculated with the program MeltWin 3.0.[31] Circular dichroism (CD) spectra were recorded on a Jasco-600 (Jasco, Japan) spectropolarimeter, a thermostatically controlled bath (Lauda-RCS-6) and 1 cm cuvettes.

General procedure for the acylation of 4-amino-7-(2-deoxy-β-D-erythro-pentofuranosyl)-5-iodo-7*H*-pyrrolo[2,3-d]pyrimidine (**2**): Me_3SiCl (650 μL, 5.1 mmol) was added to a solution of compound **2**[13] (188 mg, 0.5 mmol) in anhydrous pyridine (3 mL), and was stirred at r.t. After 30 min, the acid chloride (2.5 mmol) was introduced and the solution was kept at r.t. for another 2 h. The mixture was cooled to 0 °C, diluted with H_2O (2 mL), and stirred for 10 min. After the addition of 25% aq. NH_3 (2 mL), stirring was continued for 2 h at r.t. The solution was evaporated and the residue was purified by using flash chromatography (column 10 × 3 cm, solvent *A*).

4-(Benzoylamino)-7-(2-deoxy-β-D-erythro-pentofuranosyl)-5-iodo-7*H*-pyrrolo[2,3-d]pyrimidine (**4a**): The reaction was performed according to the general procedure. When benzoyl chloride (351 mg, 290 μL) was used,

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colorless needles were obtained, which were crystallized from MeOH (159 mg, 66%). M.p. 195 °C; TLC (*A*): R_f 0.5; UV (MeOH): λ_{max} (ε) = 230 (28400), 273 (6500), 312 nm (6100); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 30 °C, TMS): δ = 2.24 (m, 1H; H$_\alpha$-C(2′)), 2.47 (m, 1H; H$_\beta$-C(2′)), 3.55 (m, 2H; 2 H-C(5′)), 3.84 (m, 1H; H-C(4′)), 4.36 (m, 1H; H-C(3′)), 5.03 (t, ^3J(H,H) = 5.3 Hz, 1H; OH-C(5′)), 5.33 (d, ^3J(H,H) = 3.9 Hz, 1H; OH-C(3′)), 6.66 (t, ^3J(H,H) = 6.9 Hz, 1H; H-C(1′)), 7.57 (m, 3H; 3 arom. H), 8.05 (d, ^3J(H,H) = 8 Hz, 2H; 2 arom. H), 8.05 (s, 1H; H-C(6)), 8.71 (s, 1H; H-C(2)), 10.90 (s, 1H; NH); $C_{18}H_{17}IN_4O_4$ (480.3): calcd. C 45.02, H 3.57, N 11.67; found C 45.10, H 3.62, N 11.85.

4-(Acetylamino)-7-(2-deoxy-β-D-erythro-pentofuranosyl)-5-iodo-7*H*-pyrrolo[2,3-d]pyrimidine (4b): As described for **4a** with acetyl chloride (196 mg, 178 μL). A 6% aq. NH_3 solution (2 mL, 20 min, r.t.) was used. Crystallization (MeOH/EtOAc, 8:2) produced colorless needles (101 mg, 48%). M.p. 201 °C; TLC (*A*): R_f 0.5; UV (MeOH): λ_{max} (ε) = 273 (6500), 298 nm (6000); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 30 °C, TMS): δ = 2.18 (s, 3H; CH$_3$), 2.24 (m, 1H; H$_\alpha$-C(2′)), 2.44 (m, 1H; H$_\beta$-C(2′)), 3.54 (m, 2H; 2 H-C(5′)), 3.83 (m, 1H; H-C(4′)), 4.35 (m, 1H; H-C(3′)), 4.95 (t, ^3J(H,H) = 5.3 Hz, 1H; OH-C(5′)), 5.29 (d, ^3J(H,H) = 3.9 Hz, 1H; OH-C(3′)), 6.62 (t, ^3J(H,H) = 6.9 Hz, 1H; H-C(1′)), 8.00 (s, 1H; H-C(6)), 8.62 (s, 1H; H-C(2)), 10.21 (s, 1H; NH); $C_{13}H_{15}IN_4O_4$ (418.2): calcd C 37.34, H 3.62, N 13.40; found C 37.37, H 3.74, N 13.48.

7-(2-Deoxy-β-D-erythro-pentofuranosyl)-5-iodo-4-(*iso*-butyrylamino)-7*H*-pyrrolo[2,3-d]pyrimidine (4c): The reaction was performed as described for **4a** but with *iso*-butyryl chloride (266 mg, 260 μL). In this case, 12% aq. NH_3 (2 mL, r.t., 30 min) was used. Colorless needles from MeOH (114 mg, 51%). M.p. 192 °C; TLC (*A*): R_f 0.5; UV (MeOH): λ_{max} (ε) = 272 (6200), 299 nm (5700); ^{1}H NMR (250 MHz, [D$_6$]DMSO, 30 °C, TMS): δ = 1.21 (s, 6H; 2 CH$_3$), 2.24 (m, 1H; H$_\alpha$-C(2′)), 2.44 (m, 1H; H$_\beta$-C(2′)), 2.80 (m, 1H; CH), 3.55 (m, 2H; 2 H-C(5′)), 3.85 (m, 1H; H-C(4′)), 4.37 (m, 1H; H-C(3′)), 4.96 (t, ^3J(H,H) = 5.3 Hz, 1H; OH-C(5′)), 5.29 (d, ^3J(H,H) = 3.9 Hz, 1H; OH-C(3′)), 6.64 (t, ^3J(H,H) = 6.9 Hz, 1H; H-C(1′)), 8.01 (s, 1H; H-C(6)), 8.63 (s, 1H; H-C(2)), 10.16 (s, 1H; NH); $C_{15}H_{19}IN_4O_4$ (446.2): calcd C 40.37, H 4.29, N 12.56; found C 40.25, H 4.30, N 12.46.

7-(2-Deoxy-β-D-erythro-pentofuranosyl)-5-iodo-4-(pivaloylamino)-7*H*-pyrrolo[2,3-d]pyrimidine (4d): As described for **4a** with **2**[13] (94 mg, 0.25 mmol), pivaloyl chloride (150 mg, 150 μL), and treatment with 12% aq. NH_3 (2 mL, r.t. 30 min). An amorphous solid was obtained (58 mg, 51%). TLC (*A*): R_f 0.5; UV (MeOH): λ_{max} (ε) = 274 (5800), 300 nm (5300); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 30 °C, TMS): δ = 1.10 (s, 3H; 3 CH$_3$); 2.23 (m, 1H; H$_\alpha$-C(2′)), 2.47 (m, 1H; H$_\beta$-C(2′)), 3.56 (m, 2H; 2 H-C(5′)), 3.84 (m, 1H; H-C(4′)), 4.36 (m, 1H; H-C(3′)), 4.98 (br, 1H; OH-C(5′)), 5.30 (br, 1H; OH-C(3′)), 6.63 (t, ^3J(H,H) = 6.9 Hz, 1H; H-C(1′)), 8.00 (s, 1H; H-C(6)), 8.63 (s, 1H; H-C(2)), 9.90 (s, 1H; NH). FAB-MS (NBA): *m*/*z* = 461.1 [*M*+H]$^+$.

7-(2-Deoxy-β-D-erythro-pentofuranosyl)-5-iodo-4-(phenoxyacetylamino)-7*H*-pyrrolo[2,3-d]pyrimidine (4e): Phenoxyacetic anhydride (1.7 g, 6.0 mmol) was added to a solution of **2**[13] (376 mg, 1.0 mmol) in dry pyridine (10 mL) under stirring at r.t. After 2 h, H_2O (2 mL) was added and the stirring was continued for another 15 min. The mixture was evaporated and the residue dissolved in CH_2Cl_2 (100 mL). The solution was washed with 5% aq. $NaHCO_3$ (2 × 25 mL) and H_2O (2 × 25 mL). The organic layers were combined, dried over Na_2SO_4, filtered and evaporated to dryness. The resulting oil was treated with Et_3N/pyridine/H_2O (1:1:3) (30 mL) and was stirred (30 min). The solution was evaporated to dryness and was analyzed using FC (column 10 × 3 cm, *A*). Crystallization (MeOH/EtOAc, 3:1) produced colorless crystals (265 mg, 52%). M.p. 199 °C; TLC (*A*): R_f 0.5; UV (MeOH): λ_{max} (ε) = 236 (21600), 275 (6100), 301 nm (5800); ^{1}H NMR (250 MHz, [D$_6$]DMSO, 30 °C, TMS): δ = 2.23 (m, 1H; H$_\alpha$-C(2′)), 2.47 (m, 1H; H$_\beta$-C(2′)), 3.53 (m, 2H; 2 H-C(5′)), 3.84 (m, 1H; H-C(4′)), 4.36 (m, 1H; H-C(3′)), 4.88 (s, 2H; CH$_2$OPh), 4.99 (t, ^3J(H,H) = 5.3 Hz, 1H; OH-C(5′)), 5.32 (d, ^3J(H,H) = 3.9 Hz, 1H; OH-C(3′)), 6.63 (t, ^3J(H,H) = 6.9 Hz, 1H; H-C(1′)), 7.01 (m, 3H; 3 arom. H), 7.32 (m, 2H; 2 arom. H), 8.05 (s, 1H; H-C(6)), 8.66 (s, 1H; H-C(2)), 10.37 (s, 1H; NH); $C_{19}H_{19}IN_4O_5$ (510.3): calcd C 44.72, H 3.75, N 10.98; found C 44.75, H 3.61, N 10.79.

7-(2-Deoxy-β-D-erythro-pentofuranosyl)-4-{[(dimethylamino)methylidene]amino}-5-iodo-7*H*-pyrrolo[2,3-d]pyrimidine (5a): *N*,*N*-dimethylformamide dimethylacetal (2.0 g, 16.8 mmol) was added to a solution of 7-(2-deoxy-β-D-erythro-pentofuranosyl)-5-iodo-7H-pyrrolo[2,3-d]pyrimidine (**2**)[13] (400 mg, 1.06 mmol) in methanol (20 mL) and the solution was stirred for 2 h at 40 °C. After evaporation, the residue was purified by FC (column 10 × 5 cm, *A*), yielding a colorless foam (389 mg, 85%), which was isolated from the main zone. TLC (*A*): R_f 0.5; UV (MeOH): λ_{max} (ε) = 229 (17400), 277 (10400), 323 nm (19000); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 30 °C, TMS): δ = 2.18 (m, 1H; H$_\alpha$-C(2′)), 2.47 (m, 1H; H$_\beta$-C(2′)), 3.18, 3.22 (2s, 6H; Me$_2$N), 3.54 (m, 2H; 2 H-C(5′)), 3.81 (m, 1H; H-C(4′)), 4.32 (m, 1H; H-C(3′)); 5.00 (t, ^3J(H,H) = 5.4 Hz, 1H; OH-C(5′)), 5.23 (d, ^3J(H,H) = 3.9 Hz, 1H; OH-C(3′)), 6.52 (t, ^3J(H,H) = 7.0 Hz, 1H; H-C(1′)), 7.70 (s, 1H; H-C(6)), 8.30 (s, 1H; H-C(2)), 8.82 (s, 1H; N=CH); $C_{14}H_{18}IN_5O_3$ (431.2): calcd C 38.99, H 4.21, N 16.24; found C 39.09, H 4.27, N 16.10.

7-(2-Deoxy-β-D-erythro-pentofuranosyl)-4-{[(dimethylamino)methylidene]amino}-5-(hex-1-ynyl)-7*H*-pyrrolo[2,3-d]pyrimidine (5b): Compound **5b** was prepared from 7-(2-deoxy-β-D-erythro-pentofuranosyl)-5-(hex-1-ynyl)-7*H*-pyrrolo[2,3-d]pyrimidine (**1**)[13] (400 mg, 1.21 mmol) and *N*,*N*-dimethylformamide dimethylacetal (2.0 g, 16.8 mmol) as described for **5a**. On FC, a colorless foam (373 mg, 80%) was obtained from the main zone. TLC (*A*): R_f 0.5; UV (MeOH): λ_{max} (ε) = 278 (12100), 321 nm (14300); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 30 °C, TMS): δ = 0.91 (t, ^3J(H,H) = 7.3 Hz, 3H; CH$_3$), 1.45 (sextet, ^3J(H,H) = 7.2 Hz, 2H; CH$_2$-CH$_3$), 1.53 (quintet, ^3J(H,H) = 7.3 Hz, 2H; CH$_2$-CH$_2$-CH$_3$), 2.18 (m, 1H; H$_\alpha$-C(2′)), 2.42 (t, ^3J(H,H) = 6.8 Hz, 2H; CH$_2$), 2.47 (m, 1H; H$_\beta$-C(2′)), 3.16, 3.18 (2s, 6H; Me$_2$N), 3.56 (m, 2H; 2 H-C(5′)), 3.84 (m, 1H; H-C(4′)), 4.35 (m, 1H; H-C(3′)), 5.02 (t, ^3J(H,H) = 5.3 Hz, 1H; OH-C(5′)), 5.24 (d, ^{3}J(H,H) = 3.7 Hz, 1H, OH-C(3′)); 6.53 (t, ^3J(H,H) = 6.8 Hz, 1H, H-C(1′)), 7.71 (s, 1H; H-C(6)), 8.32 (s, 1H; H-C(2)), 8.76 (s, 1H; N=CH); $C_{20}H_{27}N_5O_3$ (385.5): calcd C 62.32, H 7.06, N 18.17; found C 62.48, H 7.03, N 18.53.

7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl]-4-[{(dimethylamino)methylidene}amino]-5-iodo-7*H*-pyrrolo-[2,3-d]pyrimidine (6a): 4,4′-dimethoxytriphenylmethyl chloride (256 mg, 0.76 mmol) was added to a solution of **5a** (300 mg, 0.70 mmol) in dry pyridine (3 mL). After stirring at 50 °C for 1 h, the mixture was poured into 5% aq. $NaHCO_3$ soln. (10 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and evaporated. The residue was analyzed by FC (column 12 × 4 cm, *A*), yielding a colorless foam (360 mg, 70%). TLC (*A*): R_f 0.6; UV (MeOH): λ_{max} (ε) = 236 (29200), 275 (12200), 322 nm (19600); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 30 °C, TMS): δ = 2.24 (m, 1H; H$_\alpha$-C(2′)), 2.57 (m, 1H; H$_\beta$-C(2′)), 3.18 (m, 2H; 2 H-C(5′)), 3.18, 3.22 (2s, 6H; Me$_2$N), 3.72 (s, 6H; 2 MeO), 3.92 (m, 1H; H-C(4′)), 4.37 (m, 1H; H-C(3′)), 5.30 (d, ^3J(H,H) = 4.0 Hz, 1H; OH-C(3′)), 6.54 (t, ^3J(H,H) = 6.6 Hz, 1H; H-C(1′)), 6.84 (m, 4H; 4 arom. H), 7.22-7.38 (m, 9H; 9 arom. H), 7.56 (s, 1H; H-C(6)), 8.31 (s, 1H; H-C(2)), 8.82 (s, 1H; N=CH); $C_{35}H_{36}IN_5O_5$ (733.6): calcd C 57.30, H 4.95, N 9.55; found C 57.48, H 5.12, N 9.44.

7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl]-4-[{(dimethylamino)methylidene}amino]-5-(hex-1-ynyl)-7*H*-pyrrolo[2,3-d]pyrimidine (6b): Compound **6b** was prepared from **5a** (300 mg, 0.78 mmol) as described for **6a** but with 4,4′-dimethoxytriphenylmethyl chloride (290 mg, 0.86 mmol). FC (column 12 × 4 cm, *A*) produced a colorless foam (360 mg, 67%). TLC (*A*): R_f 0.6; UV (MeOH): λ_{max} (ε) = 276 (17500), 320 nm (12900); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 30 °C, TMS): δ = 0.91 (t, ^3J(H,H) = 7.3 Hz, 3H; CH$_3$), 1.45 (sextet, ^3J(H,H) = 7.2 Hz, 2H; CH$_2$-CH$_3$), 1.53 (quintet, ^3J(H,H) = 7.3 Hz, 2H; CH$_2$-CH$_2$-CH$_3$), 2.18 (m, 1H; H$_\alpha$-C(2′)), 2.41 (t, ^3J(H,H) = 6.8 Hz, 2H; CH$_2$), 2.53 (m, 1H; H$_\beta$-C(2′)), 3.16, 3.18 (2s, 6H; Me$_2$N), 3.18 (m, 2H, H-C(5′)), 3.71 (s, 6H; 2 MeO), 3.91 (m, 1H; H-C(4′)), 4.34 (m, 1H; H-C(3′)), 5.28 (d, ^3J(H,H) = 3.9 Hz, 1H; OH-C(3′)), 6.53 (t, ^3J(H,H) = 7.0 Hz, 1H; H-1′), 6.82 (m, 4H; 4 arom. H), 7.20–7.36 (m, 9H; 9 arom. H), 7.56 (s, 1H; H-C(6)), 8.30 (s, 1H; H-C(2)), 8.73 (s, 1H; N=CH); $C_{41}H_{45}N_5O_5$ (687.8): calcd C 71.59, H 6.59, N 10.18; found C 71.64, H 6.63, N 10.28.

7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl]-4-[{(dimethylamino)methylidene}amino]-5-iodo-7*H*-pyrrolo[2,3-d-]pyrimidine-3′-[(2-cyanoethyl)-*N*,*N*-diisopropyl phosphoramidite] (3a): Chloro-(2-cyanoethoxy)-*N*,*N*-diisopropylaminophosphine (126 mg, 0.53 mmol) was added to a stirred solution of **6a** (300 mg, 0.41 mmol) and anhydrous *N*,*N*-diisopropylethylamine (212 mg, 1.64 mmol) in dry THF (2 mL) under an Ar atmosphere at room temperature. The reaction mixture was stirred for another 30 min and was filtered. The filtrate was diluted with ethyl acetate (30 mL) and extracted twice with ice-cold aq. 10% Na_2CO_3 solution (2 × 10 mL) and H_2O (10 mL). The organic phases were dried over Na_2SO_4 and evaporated to dryness. The solid material was purified by FC (column 8 × 3 cm, *B*), yielding a colorless foam (222 mg,

61%) from the main zone. TLC (*B*): R_f 0.4, 0.5; ^{31}P NMR (101 MHz, $CDCl_3$, 30 °C, 85% H_3PO_4): $\delta = 149.0, 149.2$.

7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl]-4-[{(dimethylamino)methylidene}amino]-5-(hex-1-ynyl)-7*H*-pyrrolo[2,3-d]pyrimidine-3′-[(2-cyanoethyl)-*N*,*N*-diisopropyl phosphoramidite] (**3b**): Compound **6b** (300 mg, 0.44 mmol) was treated with anhydrous *N*,*N*-diisopropylethylamine (220 mg, 1.70 mmol) and chloro-(2-cyanoethoxy)-*N*,*N*-diisopropylamino phosphine (135 mg, 0.57 mmol) as described for **3a**. A colorless foam (220 mg, 56%) was obtained. TLC (*B*): R_f 0.4, 0.5; ^{31}P NMR (101 MHz, $CDCl_3$, 30 °C, 85% H_3PO_4): $\delta = 149.1, 149.3$.

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