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Oligonucleotides containing 7-propynyl-7-deazaguanine: synthesis and base pair stability

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Abstract—Oligonucleotides incorporating the propynyl derivative of 7-deaza-2′-deoxyguanosine (**1**) were synthesized by solid-phase oligonucleotide synthesis. As building blocks the phosphoramidites **7a**,**b** were prepared. The incorporation of **1** into oligonucleotides exerts a positive effect on the DNA duplex stability. The duplex stabilization by **1** was higher than that of 7-iodo-7-deaza-2′-deoxyguanosine (**2b**). The stabilizing effect of the 7-propynyl group introduced in the 7-deazapurines is similar to that reported for 8-aza-7-deazapurines. From CD spectra it was deduced that the B-DNA structure is not significantly altered by compound **1**.

1. Introduction

Structural modifications on nucleic acids constituents have been examined to increase base pair stability or nucleobase pairing selectivity or to change base pair recognition as well as to increase antiviral or anticancer activity. Such modifications were also applied to oligonucleotides hybridization probes used for diagnostic purposes.[1] The structural perturbation include modifications on the oligonucleotide backbone,[2] the sugar moiety,[3–5] or the nucleobase.[6–11] Among the various groups used for duplex stabilization, the propynyl group gained particular attention.[12–14] Their introduction has been shown to increase duplex stability which can be useful for antisense oligonucleotide application or primer probe interactions.[15–17] Furthermore, it was demonstrated that this modification enhances the mismatch penalties[13,14,18,19] as well as the stability of DNA triplexes.[20,21]

Earlier, the propynyl group was introduced in the 5-position of pyrimidine nucleosides, e.g. in 2′-deoxycytidine[11–13,22,23] and 2′-deoxyuridine.[24–26] Also 8-propynylated 2′-deoxyadenosine and 2′-deoxyguanosine derivatives were studied.[26,27] However, the 8-propynyl residues destabilize duplex DNA and drive the molecule into the *syn* conformation.[28,29] Recently, it was shown that 7-propynyl residues of 8-aza-7-deazapurines are well accommodated in duplex DNA.[30,31] However, longer hex-1-ynyl chains do not show such favourable properties.[32]

Earlier, oligophosphorothioates with propynyl groups substituting the 7-position of 7-deaza-2′-deoxyguanosine (**2a**) have been investigated.[33,34] Nevertheless, oligodeoxyribonucleotides with a natural phosphodiester backbone in which 2′-deoxyguanosine was replaced by compound **1** have not been studied. This manuscript reports on the synthesis and hybridization of such oligodeoxyribonucleotides. For this purpose, an improved synthetic route for compound **1** was developed starting from the 7-iodo nucleoside **2b** which was subsequently converted into phosphoramidite building blocks in an excellent overall yield (67%). Also an amidine protected phosphoramidite was synthesized from which oligonucleotides can be obtained using mild deprotection conditions. Finally, the effect of the propynyl group at the 7-position of 7-deaza-2′-deoxyguanosine (**2a**) and 8-aza-7-deaza-2′-deoxyguanosine (**3a**) on the DNA duplex stability will be compared (Scheme 1).

2. Results and discussion

The synthesis of the 7-deaza-7-propynyl-2′-deoxyguanosine (**1**) was reported earlier using 4-chloro-2-(methylthio)-pyrrolo[2,3-*d*]pyrimidine as a starting material, followed by glycosylation and the exchange of the 2-methylthio group by an amino residue.[33] As the yields of the particular

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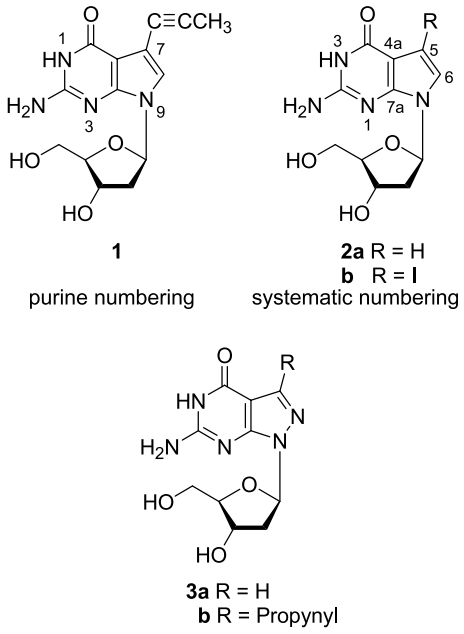
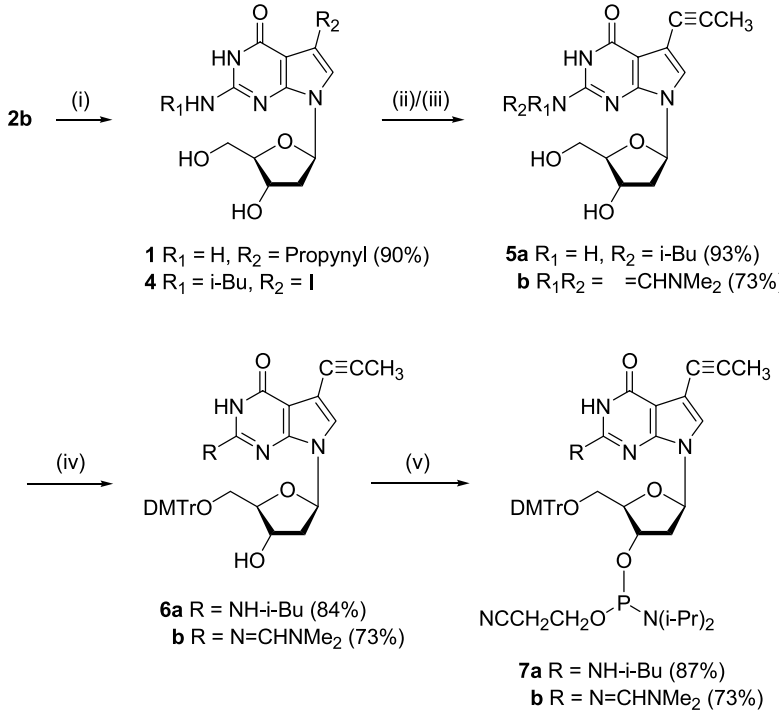


Scheme 1.

steps were moderate or were not mentioned[34] we developed an alternative synthetic route. 7-Iodo-7-deaza-2′-deoxyguanosine (**2b**)[35] was selected as precursor which was subjected to the palladium-catalyzed Sonogashira cross-coupling reaction yielding compound **1**.[36–38] The reaction was carried out in anhydrous DMF in the presence of [$(PPh_3)_4Pd(0)$]/CuI/triethylamine. The solution was saturated with propyne (0 °C) to give the nucleoside **1** in 90% yield (Scheme 2).

Next, the amino group of **1** was blocked with either an isobutyryl residue[39] or a formamidine protecting group. For this purpose, the isobutyryl protected compound **4** was used for the cross-coupling reaction furnishing compound **5a** in 93% yield, and compound **1** was treated with *N*,*N*-dimethylaminoformamide dimethylacetal in MeOH at 40 °C yielding **5b** in 73% yield. The half-life of the isobutyryl protecting group of **5a** was found to be 95 min (at 40 °C, 25% aq. NH_3) while that of the dimethylaminoformamidine residue of **5b** was 7 min. As the formamidine protection results in a lower half-life when compared to the isobutyryl group, mild deprotection conditions can be used when compound **5b** is incorporated. Subsequently, the intermediates **5a**,**b** were converted into the 4,4′-dimethoxytrityl derivatives **6a**,**b** under standard conditions. Phosphitylation of the DMT derivatives **6a**,**b** with 2-cyanoethyl diisopropylphosphoramidochloridite in CH_2Cl_2 in the presence of $(i\text{-}Pr)_2EtN$ furnished the phosphoramidites **7a**,**b** in 86 and 73% yields, respectively (Scheme 2 and Section 3). Structural proof of all compounds was performed by ^1H-, ^{13}C- and ^{31}P NMR spectra (Table 1) as well as by elemental analyses. According to Table 1, the 7-propynyl substituent changes the chemical shift of C(7) of compound **1** and its derivatives. This is due to a positive mesomeric effect of the 7-propynyl-substituent on the pyrrolo[2,3-*d*]pyrimidine system, which is similar in the case of the 7-iodo substituted nucleoside **2b**. As the base pair stability is influenced by the pK_a value the effect of the 7-propynyl residue on the pyrrolo[2,3-*d*]pyrimidine nucleoside was studied; pK_a values of the compound **1** were determined UV-spectrophotometrically. The 7-propynyl group does not change the pK_a values significantly, compound **1** has pK_a values of 1.6 and 10.2 while the non-functionalized nucleoside **2a** shows pK_a values of 1.7 and 10.2; the pK_a values for the 7-iodo nucleoside **2b** are 0.6 and 10.3. The UV-spectra of compound **1** was significantly



Scheme 2. (i) and (ii) [$Pd(0)(PPh_3)_4$], CuI, Et_3N, DMF, propyne, rt, 18 h. (iii) *N*,*N*-dimethylformamide dimethylacetal, MeOH, 40 °C, 30 min. (iv) $(MeO)_2TrCl$, pyridine, rt, 15 h. (v) 2-cyanoethyl-diisopropylphosphoramido chloridite, $(i\text{-}Pr)_2NEt$, CH_2Cl_2, rt, 30 min.

Table 1. ^{13}C NMR chemical shifts of 7-substituted 7-deaza-2′-deoxyguanosines at 298 K[a]

Compound	C(2)[b] C(2)[c]	C(4)[b] C(6)[c]	C(4a)[b] C(5)[c]	C(5)[b] C(7)[c]	C(6)[b] C(8)[c]	C(7a)[b] C(4)[c]	C=O/C=N
1	152.9[d]	157.8	99.3	85.4	120.9	150.0[d]	—
2a	152.5	158.5	100.0	102.0	116.6	150.5	—
2b	152.7	158.0	99.8	54.9	121.6	150.5	—
5a	147.4[d]	155.9	103.4	86.6	123.4	147.0[d]	180.0
6a	147.4[d]	155.9	103.6	85.4	123.3	147.0[d]	180.0
5b	157.4	158.7	102.4	85.6	122.5	156.6	148.8
6b	158.0[d]	158.8	102.5	85.3	122.2	157.4[d]	148.9
	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C≡C	C≡C
1	82.1	[e]	70.8	87.0	61.8	99.3	73.7
2a	82.2	39.5	70.8	86.8	61.9	—	—
2b	82.2	[e]	70.9	87.1	61.8	—	—
5a	82.6	[e]	70.8	87.3	61.7	100.1	72.9
6a	82.5	[e]	70.4	86.6	64.1	100.2	72.7
5b	82.3	[e]	70.9	87.2	61.9	99.5	73.6
6b	81.9	[e]	70.6	85.6	64.3	99.7	73.5

[a] Measured in (D_6) DMSO.
[b] Systematic numbering.
[c] Purine numbering.
[d] Tentative.
[e] Overlaped in DMSO signal.

different from that of the non-functionalized nucleoside **2a** (**1**: λ_{max}=236 and 271 nm; **2a**: λ_{max}=257 and 281 nm).

Next, the role of the 7-propynyl group on the duplex stability was studied on the duplex 5′-d(TAG GTC AAT ACT) (**8**) 3′-d(ATC CAG TTA TGA) (**9**). Earlier, it was shown that the non-functionalized nucleoside **2a** reduces the stability of the Watson–Crick base pair thereby reducing the duplex stability of **8·9** by about 1 °C per modification (**9·10** and **10·13**). The incorporation of the 7-iodo derivative **2b** increases the duplex stability (**9·11** and **11·14**) to the level of standard duplex **8·9** (Table 2)[32] when compared with that of **2a**. Now, it is shown that the 7-propynyl group is more effective than a halogen substituent for duplex stabilization. The average T_m-increase per modification is around 1.5 °C when the data of the duplex **9·12** and **12·15** containing nucleoside **1** are related to the standard duplex **8·9**. If one relates it to the duplexes **9·10** or **10·13** containing 7-deaza-2′-deoxyguanosine (**2a**) in place of dG, the T_m-increase is around 2.5 °C per modification (Table 2). Also the T_m-values of the duplexes **8·17**, **9·16** and **9·18** containing **1** at various positions clearly indicate a nearest neighbour effect. According to Table 3, the ΔT_m for the 8-aza-7-deaza-7-propynyl-2′-deoxyguanosine (**3b**) is also around 2.5 °C per

Table 2. T_m-values and thermodynamic data of duplexes containing 7-propynyl-7-deaza-2′-deoxyguanosine (**1**)

Duplexes	T_m [°C]	ΔH° [kcal/mol]	ΔS° [cal/mol K]	ΔG°_{310} [kcal/mol]
5′-d(TAG GTC AAT ACT)(**8**)[a] 3′-d(ATC CAG TTA TGA) (**9**)	47	−89	−253	−10.9
5′-d(TA**2a** **2a**TC AAT ACT) (**10**)[a,b] 3′-d(ATC CAG TTA TGA) (**9**)	45	−101	−317	−2.7
5′-d(TA**2b** **2b**TC AAT ACT) (**11**)[a,b] 3′-d(ATC CAG TTA TGA) (**9**)	46	−99	−310	−2.9
5′-d(TA**1** **1**TC AAT ACT) (**12**)[a] 3′-d(ATC CAG TTA TGA) (**9**)	51	−99	−280	−11.7
5′-d(TA**2a** **2a**TC AAT ACT) (**10**)[a,b] 3′-d(ATC CA**2a** TTA T**2a**A) (**13**)	44	−91	−284	−2.9
5′-d(TA**2b** **2b**TC AAT ACT) (**11**)[a,b] 3′-d(ATC CA**2b** TTA T**2b**A) (**14**)	48	−112	−348	−4.12
5′-d(TA**1** **1**TC AAT ACT) (**12**)[a] 3′-d(ATC CA**1** TTA T**1**A) (**15**)	53	110	−314	−12.8
5′-d(TAG **1**TC AAT ACT) (**16**)[c] 3′-d(ATC CAG TTA TGA) (**9**)	52	−91	−284	−12.1
5′-d(TAG GTC AAT ACT)(**8**)[c] 3′-d(ATC CAG TTA T**1**A) (**17**)	51	−88	−247	−11.6
5′-d(TA**1** GTC AAT ACT) (**18**)[c] 3′-d(ATC CAG TTA TGA) (**9**)	50	−86	−241	−11.3
5′-d(TAG GTC AAT ACT) (**8**)[c] 3′-d(ATC CA**1** TTA T**1**A) (**15**)	53	−91	−225	−12.2
5′-d(TA**1** GTC AAT ACT) (**18**)[c] 3′-d(ATC CA**1** TTA T**1**A) (**15**)	52	−85	−237	−11.8
5′-d(TA**1** **1**TC AAT ACT) (**12**)[c] 3′-d(ATC CA**1** TTA TGA) (**19**)	54.5	−95	−264	−12.8

[a] Measured in 0.1 M NaCl, 10 mM $mgCl_2$ and 10 mM Na-cacodylate (pH 7.0) with 7.5 μM single-strand concentration.
[b] Ref. 32.
[c] Measured in 1 M NaCl, 100 mM $mgCl_2$ and 60 mM Na-cacodylate (pH 7.0) with 5 μM single-strand concentration.

Table 3. T_m-values and thermodynamic data of the oligonucleotides containing 7-deaza-7-propynyl-2′-deoxyguanosine (**1**) and 8-aza-7-deaza-7-propynyl -2′-deoxyguanosine (**3**)[a]

Duplexes	T_m [°C]	ΔT_m [°C] per modification	$\Delta G°_{310}$ [kcal/mol]
5′-d(TAG GTC AAT ACT) (**8**) 3′-d(ATC CAG TTA TGA) (**9**)	50	0	−10.9
5′-d(TAG GTC AAT ACT) (**8**) 3′-d(ATC CA**1** TTA TGA) (**20**)	52	2	−11.6
5′-d(TAG GTC AAT ACT) (**8**) 3′-d(ATC CA**3** TTA TGA) (**21**)	53	3	−12.5
5′-d(TA**1** **1**TC AAT ACT) (**12**) 3′-d(ATC CAG TTA TGA) (**9**)	53	1.5	−12.5
5′-d(TA**3** **3**TC AAT ACT) (**22**) 3′-d(ATC CAG TTA TGA) (**9**)	56	3	−14.5
5′-d(TA**1** **1**TC AAT ACT) (**12**) 3′-d(ATC CAG TTA T**1**A) (**17**)	54	1.3	−13.0
5′-d(TA**3** **3**TC AAT ACT) (**22**) 3′-d(ATC CAG TTA T**3**A) (**23**)	58	2.6	−14.0
5′-d(TA**1** **1**TC AAT ACT) (**12**) 3′-d(ATC CA**1** TTA T**1**A) (**15**)	55	1.25	−12.8
5′-d(TA**3** **3**TC AAT ACT) (**22**) 3′-d(ATC CA**3** TTA T**3**A) (**24**)	60	2.5	−14.6

[a] Measured in 1 M NaCl, 100 mM $mgCl_2$ and 60 mM Na-cacodylate (pH 7.0) with 5 μM single-strand concentration.

modification. As the incorporation of 8-aza-7-deaza-2′-deoxyguanosine (**3a**) causes already a T_m-increase of about 1 °C per modification.[40] The final effect of compound **3a** is stronger than that observed for **2a**. Nevertheless, when the T_m data of the propynylated oligonucleotide duplexes are correlated to those of non-propynylated duplexes, the average increase of the T_m-value is very similar (2.5 °C) in both series of heterocycles (pyrrolo[2,3-*d*]pyrimidines and pyrazolo[3,4-*d*]pyrimidines).

The enhancement of duplex stability may be caused by increased molecular polarizability of the nucleobase, the hydrophobic character and coplanarity of the propynyl group to the heterocyclic base which can increase stacking interactions.

Recently, we have performed a single crystal X-ray analysis of the nucleoside **1** (Fig. 1). In the crystalline state, the orientation of the nucleobase related to the sugar moiety of **1** is *anti* [$\chi = -117.2(5)°$], and the sugar moiety shows *S*-type sugar puckering with pseudorotational parameters $P = 152.5°$ and $\tau_m = 41.9°$. The linear propynyl group is almost in plane with the base moiety.[41] As this group is protruding into the major groove it has steric freedom. The conformation in solution was also determined from the vicinal [^{1}H, ^{1}H] NMR coupling constants using the PSEUROT 6.3 program. Here, the favored conformation is *S* (71%, $^2T_3$) which is the actual conformation of the nucleoside residue in B-DNA.

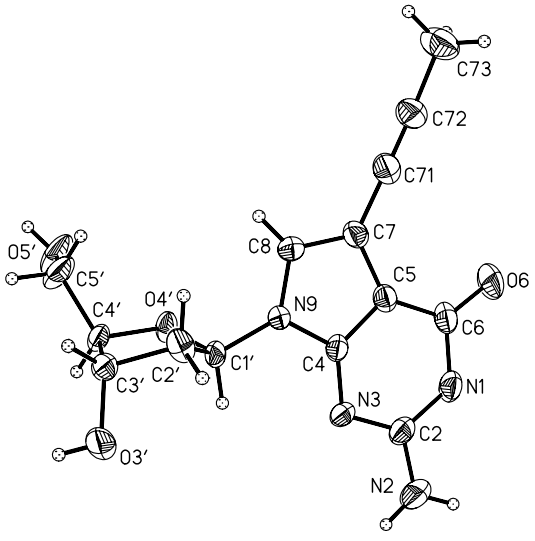


Figure 1. The perspective view of the single crystal X-ray analysis of the 7-deaza-7-propynyl-2′-deoxyguanosine (**1**).

To gain more information on the effect of the 7-propynyl group of **1** on the DNA duplex structure circular dichroism (CD) spectra of the duplexes **16**·**9**, **8**·**15**, **12**·**19**, **12**·**15** containing **1** were measured. The B-DNA structure is perturbated only very little when compound **1** is replacing dG, as it is demonstrated by the CD spectra shown in Figure 2(a). This clearly demonstrates that the B-DNA structure is maintained even with four modified residues (**1**) present in the duplex. Next, the composition of oligonucleotides containing **1** were determined by the enzymatic hydrolysis of the oligonucleotides using snake venom phosphodiesterase followed by alkaline phosphatase. According to the HPLC profile shown in Figure 2(b) compound **1** is much more hydrophobic than 2′-deoxyguanosine as indicated in the composition analysis of the oligonucleotide **12** (see also Section 3).

From the points discussed above it can be concluded that the introduction of the propynyl group in the position-7 of 7-deazapurine 2′-deoxyribonucleoside enhances the DNA duplex stability significantly. The stability increase is attributed to the linear and coplanar nature of the 7-propynyl group towards the heterocyclic base, which increase stacking interactions and makes the major groove hydrophobic by expelling water molecules. The B-DNA structure is not perturbated significantly when compound **1** is replacing dG. These favourable properties can broaden the applications of such modifications into oligonucleotides hybridization probes used for diagnostic purposes or in antisense technology.

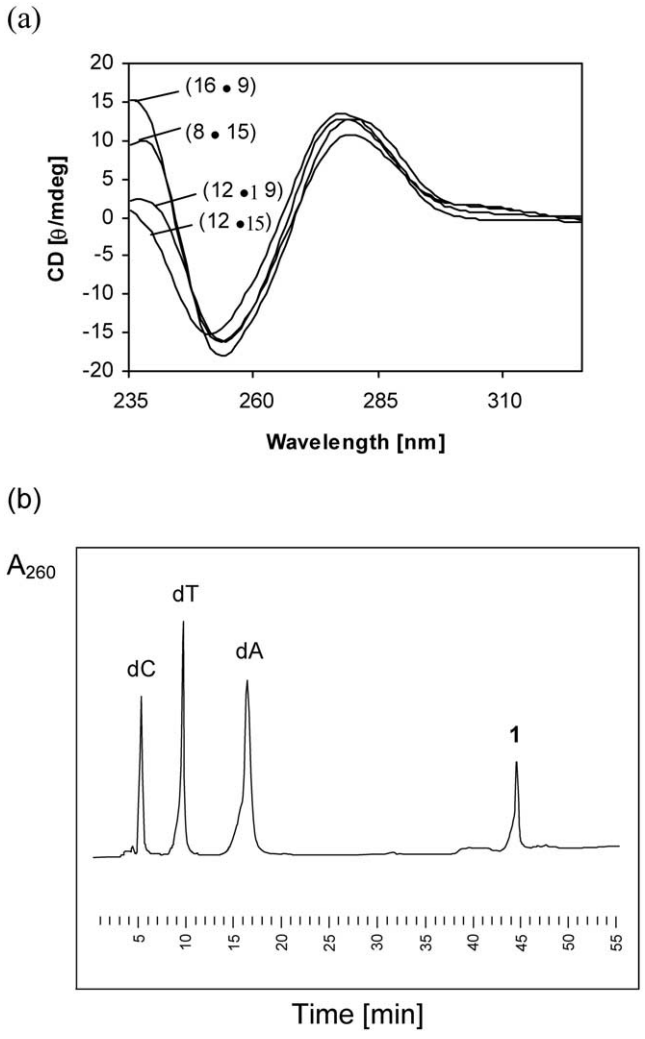


Figure 2. (a) The CD spectra of oligonucleotides containing **1**, measured at 20 °C in buffer as indicated in Table 3. (b) HPLC profile of enzymatic analysis of oligonucleotide **12** containing **1** by phosphodiesterase followed by alkaline phosphatase in 0.1 M Tris–HCl buffer (pH 8.3) at 37 °C.

3. Experimental

3.1. General

All chemicals were purchased from Aldrich, Sigma, or Fluka (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany). Solvents were of laboratory grade. TLC: aluminum sheets, silica gel 60 F_{254}, 0.2 mm layer (VWR, Germany). Column flash chromatography (FC): silica gel 60 (VWR, Germany) at 0.4 bar; Sample collection with an UltroRac II fractions collector (LKB Instruments, Sweden). UV spectra: U-3200 spectrometer (Hitachi, Tokyo, Japan); λ_{max} (ε) in nm. CD Spectra: Jasco 600 (Jasco, Japan) spectropolarimeter with thermostatically (Lauda RCS-6 bath) controlled 1-cm quartz cuvettes. NMR Spectra: Avance-250 or AMX-500 spectrometers (Bruker, Karlsruhe, Germany), at 250.13 MHz for ^{1}H and ^{13}C; δ in ppm rel. to Me_4Si as internal standard; ^{31}P rel. to ext. 85% H_3PO_4, *J* values in Hz. Elemental analyses were performed by Mikroanalytisches Laboratorium Beller (Göttingen, Germany). The melting temperatures were measured with a Cary-1/3 UV/Vis spectrophotometer (Varian, Australia) equipped with a Cary thermoelectrical controller. The temp. was measured continuously in the reference cell with a Pt-100 resistor, and the thermodynamic data of duplex formation were calculated by the Meltwin 3.0 program.[42]

3.2. Synthesis, purification and characterization of the oligonucleotides

The oligonucleotide syntheses were carried out in an ABI 392-08 DNA synthesizer (Applied Biosystems, Weiterstadt, Germany) at 1-μmol scale using the phosphoramidites **7a**,**b** following the synthesis protocol for 3′-cyanoethyl-phosphoramidites (user manual for the 392 DNA synthesizer Applied Biosystems, Weiterstadt, Germany). The coupling efficiency was always higher than 97%. After cleavage from the solid support, the oligonucleotides were deprotected in 25% aq. ammonia solution for 14–16 h at 60 °C.[43]

Purification of 5′-dimethoxytrityl oligomers was performed by reversed-phase HPLC (RP-18) with the following solvent gradient system [A: 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN 95:5; B: MeCN]: 3 min, 20% B in A, 12 min, 20–50% B in A and 25 min, 20% B in A with a flow rate of 1.0 ml/min. The solution was dried and treated with 2.5% $CHCl_2COOH/CH_2Cl_2$ for 5 min at 0 °C to remove the 4,4′-dimethoxytrityl residues. The detritylated oligomers were purified by reverse phase HPLC with the gradient: 0–20 min, 0–20% B in A with a flow rate of 1.0 ml/min. The oligomers were desalted (RP-18, silica gel) and lypophilized on a Speed Vac evaporator to yield colorless solids which were frozen at −24 °C.

The enzymatic hydrolysis of the oligonucleotides was performed as described by Seela and Becher[10] with snake venom phosphodiesterase (EC 3.1.15.1, *Crotallus adamanteus*) and alkaline phosphatase (EC 3.1.3.1, *Escherichia coli* from *Roche Diagnostics GmbH*, Germany) in 0.1 M Tris–HCl buffer (pH 8.3), which was carried out on reverse phase HPLC by gradient: 20 min A, 20–60 min 50% B in A. Quantification of the constituents was made on the basis of the peak areas, which were divided by the extinction coefficients of the nucleosides [(ε_{260}): dT 8800, dC 7300, dA 15400, **1** 11400]. The molecular masses of the oligonucleotides were determined by MALDI-TOF Biflex-III mass spectrometry (Bruker Saxonia, Leipzig, Germany) with 3-hydroxypicolinic acid (3-HPA) as a matix (Table 4).

3.2.1. 2-Amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-(prop-1-ynyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (1). To a soln. of compound **2b** (500 mg, 1.28 mmol)[35] in anhydrous DMF (4 ml), tetrakis(triphenylphosphine)palladium(0) [$(PPh_3)_4Pd(0)$] (116 mg, 0.1 mmol), CuI (68 mg, 0.36 mmol) and triethylamine (240 μl, 1.71 mmol) were added while stirring. The sealed suspension was saturated with propyne at 0 °C and stirred at rt for 24 h. The solvent was evaporated in vacuo, the reaction mixture dissolved in MeOH (2 ml) and adsorbed on silica gel (2 g). The resulting powder was subjected to FC. (silica gel, column 15×3 cm, CH_2Cl_2/MeOH, 95:5). From the main zone compound **1** was isolated as colorless solid (350 mg, 90%) which gave colorless crystals from MeOH.

Table 4. Molecular weight of selected oligonucleotides determined by MALDI-TOF mass spectrometry

Oligonucleotide	MH^+ (calcd)	MH^+ (found)
5′-d(TA**1** **1**TC AAT ACT) (**12**)	3718.4	3719.1
3′-d(ATC CA**1** TTA T**1**A) (**15**)	3718.4	3718.2
5′-d(TAG **1**TC AAT ACT) (**16**)	3682.4	3681.7
3′-d(ATC CAG TTA T**1**A) (**17**)	3682.4	3681
5′-d(TA**1** GTC AAT ACT) (**18**)	3682.4	3681
3′-d(ATC CA**1** TTA TGA) (**19**)	3682.4	3682.4
3′-d(ATC CA**1** TTA T**1**A) (**20**)	3718.4	3718.2

Mp>210 °C. TLC (CH_2Cl_2/MeOH, 9:1)): R_f 0.35. UV (MeOH): λ_{max} 236 (27800), 271 (13200). ^{1}H NMR.[33]

3.2.2. 7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-(prop-1-ynyl)-3,7-dihydro-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (5a). As described for **1** with compound **4** (400 mg, 0.87 mmol),[44] tetrakis(triphenylphosphine)palladium(0) [$(PPh_3)_4Pd(0)$] (58 mg, 0.05 mmol), CuI (34 mg, 0.18 mmol), triethylamine (0.14 ml, 1.0 mmol) and DMF (3 ml). FC (silica gel, column 15×3 cm, CH_2Cl_2/MeOH, 95:5) afforded compound **5a** as a colorless solid (300 mg, 93%). TLC (CH_2Cl_2/MeOH, 9:1): R_f 0.68. UV (MeOH): λ_{max} 237 (22600), 281 (1600), 296 (16300). ^{1}H NMR ((D_6) DMSO): 1.09 (d, J= 6.7 Hz, 2 CH_3), 2.00 (s, CH_3); 2.14 (m, H_α-C(2′)); 2.36 (m, H_β-C(2′)); 2.73 (m, CH); 3.49 (t, J=2.3, 5.9 Hz, H_2-C(5′)); 3.78 (m, H-C(4′)); 4.30 (m, H-C(3′)); 4.96 (t, J=5.3, 5.3 Hz, OH-C(5′)); 5.26 (d, J=3.4 Hz, OH-C(3′)); 6.35 (dd, J=5.9, 5.9 Hz, H-C(1′)); 7.46 (s, H-C(8)); 11.54 (s, NH); 11.78 (s, NH). Anal. calcd for $C_{18}H_{22}N_4O_5$ (374.39): C 57.75, H 5.92, N 14.96. Found: C 57.48, H 5.92, N 14.62.

3.2.3. 7-(2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)-5-(prop-1-ynyl)-3,7-dihydro-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (6a). Compound **5a** (250 mg, 0.67 mmol) was dried by repeated co-evaporation with anhydrous pyridine and then dissolved in pyridine (5 ml). After addition of 4,4′-dimethoxytrityl chloride (338 mg, 1.0 mmol) the soln. was stirred overnight at rt. The reaction was quenched by adding 5% aq. $NaHCO_3$ soln. (25 ml), and the soln. was extracted with CH_2Cl_2 (30 ml). The aq. layer was washed with CH_2Cl_2 (25 ml×3). The combined org. phase was dried over Na_2SO_4 and evaporated. The residue was subjected to FC (silica gel, column 15×3 cm, CH_2Cl_2/acetone, 95:5). The main zone afforded compound **6a** as a colorless solid (379 mg, 84%). TLC (CH_2Cl_2/MeOH, 95:5): R_f 0.41. UV (MeOH): λ_{max} 234 (39400), 281 (1700), 340 (1100). ^{1}H NMR ((D_6) DMSO): 1.10 (d, J=6.4 Hz, 2 CH_3); 2.00 (s, CH_3); 2.21 (m, H_α-C(2′)); 2.42 (m, H_β-C(2′)); 2.75 (m, CH); 3.13 (m, H_2-C(5′)); 3.71, 3.73 (2s, 2 OCH_3); 3.89 (m, H-C(4′)); 4.31 (m, H-C(3′)); 5.30 (s, OH-C(3′)); 6.38 (t, J= 6.5, 6.1 Hz, H-C(1′)); 6.8 (m, Ar 4H); 7.21–7.58 (m, H-C(8) + Ar 9H); 11.57 (s, NH); 11.81 (s, NH). Anal. calcd for $C_{39}H_{40}N_4O_7$ (676.76): C 69.21, H 5.96, N 8.28. Found: C 69.11, H 6.00, N 8.32.

3.2.4. 7-(2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)-5-(prop-1-ynyl)-3,7-dihydro-2-(isobutyrylamino)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 3′-[2-Cyanoethyl *N*,*N*-diisopropylphosphoramidite] (7a). To a soln. of compound **6a** (200 mg, 0.30 mmol) in anhydrous CH_2Cl_2 (10 ml), *N*,*N*-diisopropylethylamine (DIPEA) (0.1 ml, 0.57 mmol) and 2-cyanoethyl-diisopropylphosphoramido chloridite (0.1 ml, 0.45 mmol) were added under Ar atmosphere. After stirring for 0.5 h, 5% aq. $NaHCO_3$ soln. was added, and it was extracted with CH_2Cl_2 (10 ml×2). The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was subjected to FC (silica gel, column 7×1.5 cm, CH_2Cl_2/acetone, 95:5). The main zone afforded compound **7a** as a colorless foam (225 mg, 87%).TLC (CH_2Cl_2/$(CH_3)_2CO$, 9:1): R_f 0.7. ^{31}P NMR ($CDCl_3$): 148.69, 149.19.

3.2.5. 7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-(prop-1-ynyl)-3,7-dihydro-2-[(*N*,*N*-dimethylamino)methylidene]amino-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (5b). To a soln. of **1** (500 mg, 1.87 mmol) in MeOH (20 ml) was added *N*,*N*-dimethylformamide dimethylacetal (750 µl, 5.6 mmol). The reaction mixture was stirred at 40 °C for 1 h, and the solvent was evaporated to dryness. The resulting residue was applied to FC (silica gel, column 15×3 cm, CH_2Cl_2/MeOH, stepwise gradient, 95:5, 9:1). Compound **5b** was isolated as a colorless solid (430 mg, 73%). TLC (CH_2Cl_2/MeOH, 9:1)): R_f 0.65. UV (MeOH): λ_{max} 231 (17000), 253 (22000), 314 (16800). ^{1}H NMR ((D_6) DMSO): 1.99 (s, CH_3); 2.14 (m, H_α-C(2′)); 2.34 (m, H_β-C(2′)); 3.00, 3.14 (2s, 2 NCH_3); 3.50 (d, J=4.0 Hz, H_2-C(5′)); 3.77 (m, H-C(4′)); 4.31 (m, H-C(3′)); 4.89 (t, J=4.8, 5.0 Hz, OH-C(5′)); 5.22 (d, J=3.3 Hz, OH-C(3′)); 6.40 (t, J=6.5, 7.0 Hz, H-C(1′)); 7.27 (s, H-C(8)); 8.53 (s, N=CH); 11.02(s, NH). Anal. calcd for $C_{17}H_{21}N_5O_4$ (359.38): C 56.82, H 5.89, N 19.49. Found: C 56.58, H 5.75, N 19.55.

3.2.6. 7-(2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)-5-(prop-1-ynyl)-3,7-dihydro-2-[(*N*,*N*-dimethylamino)methylidene]amino-4*H*-pyrrolo-[2,3-*d*]pyrimidin-4-one (6b). Compound **5b** (350 mg, 0.97 mmol) was dried by repeated co-evaporation with anhydrous pyridine (4 ml×2) and dissolved in pyridine (4 ml). After addition of 4,4′-dimethoxytrityl chloride (440 mg, 1.3 mmol) the soln. was stirred for 30 min at rt. The reaction was quenched by adding 5% aq. $NaHCO_3$ soln. (25 ml) and it was extracted with CH_2Cl_2 (30 ml). The aq. layer was extracted with CH_2Cl_2 (25 ml×3), the combined org. phase dried over Na_2SO_4 and evaporated. The residue was subjected to FC (silica gel, column 15×3 cm, CH_2Cl_2/acetone, stepwise gradient, 9:1, 8:2, 1:1). The main zone afforded compound **6b** as a colorless solid (470 mg, 73%). TLC (CH_2Cl_2/MeOH, 97:3): R_f 0.38. UV (MeOH): λ_{max} 233 (37400), 313 (1700). ^{1}H NMR ((D_6) DMSO): 1.98 (s, CH_3); 2.16 (m, H_α-C(2′)); 2.42 (m, H_β-C(2′)); 3.01 (s, NCH_3); 3.13 (m, NCH_3 + H_2-C(5′)), 3.73 (s, 2 OCH_3); 3.88 (m, H-C(4′)); 4.30 (m, H-C(3′)); 5.30 (d, J=4.0 Hz, OH-C(3′)); 6.45 (t, J=6.4, 6.5 Hz, H-C(1′)); 6.84 (m, Ar 4H); 7.13–7.38 (m, H-C(8)+Ar 9H); 8.55 (s, N=CH); 11.07 (s, NH). Anal. calcd for $C_{38}H_{39}N_5O_6$ (661.75): C 68.97, H 5.94, N 10.58. Found: C 68.85, H 6.03, N 10.48.

3.2.7. 7-(2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)-5-(prop-1-ynyl)-3,7-dihydro-2-[(*N*,*N*-dimethylamino)methylidene]amino-4*H*-pyrrolo-[2,3-*d*]pyrimidin-4-one 3′-[2-Cyanoethyl *N*,*N*-diisopropylphosphoramidite] (7b). To a soln. of compound

6b (250 mg, 0.38 mmol) in anhydrous CH_2Cl_2 (5 ml), *N*,*N*-diisopropylethylamine (DIPEA) (116 µl, 0.67 mmol) and 2-cyanoethyl-diisopropylphosphoramido chloridite (112 µl, 0.55 mmol) were added under Ar atmosphere. After stirring for 0.5 h, 5% aq. $NaHCO_3$ soln. was added, and it was extracted with CH_2Cl_2 (10 ml×2). The org. layer was dried over Na_2SO_4, filtered and evaporated. The residue was subjected to FC (CH_2Cl_2/acetone, 9:1). The main zone afforded compound **7b** as a colorless foam (240 mg, 73%). TLC (CH_2Cl_2/$(CH_3)_2CO$, 8:2): R_f 0.7. ^{31}P NMR ($CDCl_3$): 149.79, 150.12.

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