Propynyl groups in duplex DNA: stability of base pairs incorporating 7-substituted 8-aza-7-deazapurines or 5-substituted pyrimidines

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ABSTRACT

Oligonucleotides incorporating the 7-propynyl derivatives of 8-aza-7-deaza-2′-deoxyguanosine (3b) and 8-aza-7-deaza-2′-deoxyadenosine (4b) were synthesized and their duplex stability was compared with those containing the 5-propynyl derivatives of 2′-deoxycytidine (1) and 2′-deoxyuridine (2). For this purpose phosphoramidites of the 8-aza-7-deazapurine (pyrazolo[3,4-*d*]pyrimidine) nucleosides were prepared and employed in solid-phase synthesis. All propynyl nucleosides exert a positive effect on the DNA duplex stability because of the increased polarizability of the nucleobase and the hydrophobic character of the propynyl group. The propynyl residues introduced into the 7-position of the 8-aza-7-deazapurines are generally more stabilizing than those at the 5-position of the pyrimidine bases. The duplex stabilization of the propynyl derivative 4b was higher than for the bromo nucleoside 4c. The extraordinary stability of duplexes containing the 7-propynyl derivative of 8-aza-7-deazapurin-2,6-diamine (5b) is attributed to the formation of a third hydrogen bond, which is apparently not present in the base pair of the purin-2,6-diamine 2′-deoxyribonucleoside with dT.

INTRODUCTION

The thermal stability of oligonucleotide duplexes depends on their length and their base pair composition (1). Efforts were undertaken to increase the duplex stability by chemical modification of the DNA constituents. The sugar moiety (2) or the nucleobase (1,3–7) of the DNA constituents as well as the oligonucleotide backbone (8) were structurally modified. These changes have broadened the application of oligonucleotides in the field of antisense technology (9–11) or in hybridization techniques used for diagnostic purposes (12). All approaches focused either on strengthening the hydrogen bonds between the bases and/or the increase of the stacking interactions among them. Our search for more stable base pairs led us to the pyrazolo[3,4-*d*]pyrimidine (8-aza-7-deazapurine)

2′-deoxyribonucleosides (13–16), which can be considered as ideal shape mimics of the parent purine nucleosides. Their 7-halogenated and alkynylated derivatives induce a positive effect on the base pair stability of duplex DNA (1,17,18).
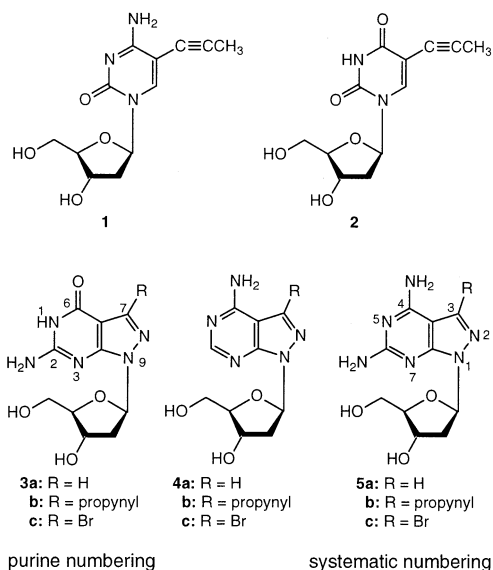
Among the various groups introduced into oligonucleotides for duplex stabilization, the propynyl group found particular attention (19–21). This group was introduced in the 5-position of the pyrimidine nucleosides, e.g. in 2′-deoxycytidine (**1**) (19–23) and 2′-deoxyuridine (**2**) (24–26) or in the 7-position of pyrrolo[2,3-*d*]pyrimidine nucleosides (27). Also, 8-propynylated 2′-deoxyadenosine and 2′-deoxyguanosine derivatives were studied (26,28). However, 8-substituted purine derivatives destabilize DNA due to steric hindrance. As the 7-substituents of 8-aza-7-deazapurines have steric freedom within a DNA-duplex, propynyl residues were now introduced into the 7-position of oligonucleotides incorporating pyrazolo[3,4-*d*]pyrimidines (8-aza-7-deazapurines) (1,13,18). This study reports on the synthesis of 7-propynyl derivatives of the 8-aza-7-deaza-2′-deoxyguanosine (**3b**) and the corresponding 2′-deoxyadenosine derivative (**4b**). The work also pays attention to the extraordinary stability of duplexes incorporating the propynyl derivative of 8-aza-7-deazapurin-2,6-diamine 2′-deoxyribonucleoside (**5b**) (18) and compares the stabilities of 'dA–dT' versus 'dG–dC' base pairs replacing either one or two of the propynylated base analogs shown in Scheme 1.

MATERIALS AND METHODS

General

For general remarks see Seela and Becher (1). Thin-layer chromatography (TLC) was performed on TLC aluminium sheets coated with silica gel 60 F_{254} (0.2 mm; Merck, Darmstadt, Germany). Flash chromatography (FC) was performed on silica gel 60 H at 0.4 bar (Merck). Reverse-phase HPLC was carried out on a 4×250 mm RP-18 (10 μm) LiChrosorb column (Merck) with a Merck-Hitachi HPLC pump (model 655 A-12) connected with a variable wavelength monitor (model 655-A), a controller (model L-5000), and an integrator (model D-2000). UV spectra were recorded on a U-3200 spectrophotometer (Hitachi, Japan), λ_{max} in nm, ε in $dm^3\ mol^{-1}\ cm^{-1}$. NMR spectra were measured on an Avance DPX 250 and an AMX 500 spectrometer (Bruker, Germany);

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Scheme 1.

Table 1. Molecular masses [M+H]$^+$ of selected oligonucleotides determined by MALDI-TOF mass spectrometry

Oligonucleotide	MH$^+$ (calc.)	MH$^+$ (found)
3′-d(ATC CA**3b** TTA TGA) (**18**)	3682	3682
3′-d(ATC CA**3b** TTA T**3b**A) (**19**)	3720	3721
5′-d(TA**3b 3b**TC AAT ACT) (**20**)	3720	3722
5′-d(TAG GT**1** AAT ACT) (**21**)	3682	3683
3′-d(AT**1 1**AG TTA TGA) (**22**)	3720	3720
5′-d(TAG GT**1** AAT A**1**T) (**23**)	3720	3720
3′-d(ATC CA**10c** TTA TGA) (**24**)	3697	3698
5′-d(TA**10c 10c**TC AAT ACT) (**25**)	3750	3752
5′-d(**1**TAG GTC AAT ACT) (**26**)	3979	3971
5′-d(TAG GTC **4b**AT ACT) (**27**)	3682	3682
3′-d(ATC C**4b**G TT**4b** TGA) (**28**)	3720	3720
5′-d(TAG GTC **4b4b**T ACT) (**29**)	3720	3720
5′-d(T**4b**G GTC **4b4b**T **4b**CT) (**30**)	3796	3795
5′-d(TAG G**2**C AA**2** ACT) (**33**)	3692	3694
5′-d(AGT A**22** GAC CTA) (**34**)	3692	3696
5′-d(TAG G**2**C AAT ACT) (**35**)	3668	3668
5′-d(**2**TA GGT CAA TAC T) (**36**)	3979	3979
5′-d(TAG GTC **5b**AT ACT) (**37**)	3697	3697
3′-d(ATC C**5b**G TT**5b** TGA) (**38**)	3750	3750
5′-d(TAG GTC **5b5b**T ACT) (**39**)	3750	3749
5′-d(T**5b**G GTC **5b5b**T **5b**CT) (**40**)	3858	3857

chemical shifts (δ) are in p.p.m. downfield from internal TMS (^{1}H, ^{13}C) or external 85% H_3PO_4 (^{31}P). The J values are given in Hz. CD spectra of duplexes were measured on a Jasco J-600 CD spectropolarimeter (Jasco, Japan) in a thermostatted cell with 1 cm path length at 20°C. Microanalyses were performed by Mikroanalytisches Labor Beller (Göttingen, Germany). The solvents were purified and dried according to standard procedures.

The UV melting of oligonucleotides was performed on a Cary-1/1E UV/VIS spectrophotometer (Varian, Australia) equipped with a Cary thermoelectrical controller. The T_m values were obtained from the melting curves. Each melting curve was fit to a non-self-complementary two-state model, and the thermodynamic parameters were obtained with the Meltwin 3.0 software package (29); UV spectra: 150-20 spectrometer (Hitachi, Japan); MALDI-TOF mass spectra: Biflex III spectrometer (Bruker Saxonia, Leipzig, Germany), HPA (3-hydroxypicolinic acid) as matrix.

Oligonucleotides

The oligonucleotide syntheses were carried out on an ABI 392-08 DNA synthesizer (Applied Biosystems, Weiterstadt, Germany) in a 1 μmol scale (Applied Biosystems) following the synthesis protocol for 3′-cyanoethylphosphoramidites. After cleavage from the solid support, the oligonucleotides were deprotected in 25% aqueous ammonia solution for 12–16 h at 60°C; room temperature (r.t.) deprotection (3 h) was used in the case of the oligonucleotides incorporating compound **2**.

Purification of the 5′-dimethoxytrityl oligomers was performed by reversed-phase HPLC (RP-18) with the following solvent gradient system [A: 0.1 M (Et$_3$NH)OAc (pH 7.0)/MeCN 95:5; B: MeCN]: 0–3 min, 15% B in A with a flow rate of 1.0 ml/min, 3–15 min, 15–60% B in A with a flow rate of 1 ml/min. The solution was dried and treated with 2.5% CHCl$_2$COOH/CH$_2$Cl$_2$ for 5 min at r.t. to remove the 4,4′-dimethoxytrityl residues. The detritylated oligomers were purified by reversed-phase HPLC with the gradient: 0–20 min, 0–20% B in A, with a flow rate of 1 ml/min. The oligomers were desalted and lyophilized on a Speed-Vac evaporator to yield colorless solids.

The enzymatic hydrolysis of the oligonucleotides was performed as described (1) using snake-venom phosphodiesterase (EC 3.1.15.1, *Crotallus adamanteus*) and alkaline phosphatase (EC 3.1.3.1, *Escherichia coli*) provided by Roche Diagnostics GmbH, Germany. The molecular masses of the oligonucleotides were determined by MALDI-TOF mass spectrometry (Table 1).

6-Amino-1-(2-deoxy-β-D-erythro-pentofuranosyl)-1,5-dihydro-3-propynyl-4*H*-pyrazolo[3,4-*d*]-pyrimidin-4-one (3b)

A mixture of 8-aza-7-deaza-7-iodo-2′-deoxyguanosine (**6a**) (3) (0.27 g, 0.69 mmol), tetrakis(triphenylphosphine)palladium(0) [(PPh$_3$)$_4$Pd(0)] (94 mg, 0.08 mmol), CuI (31.1 mg, 0.16 mmol), and triethylamine (0.24 ml, 1.72 mmol) in DMF (2.5 ml) was saturated with propyne at 0°C, sealed, and stirred at r.t. for 24 h. Afterwards, a second portion of the same amounts of reagents was added, and the solution was saturated with propyne again. After stirring for an additional 24 h, the reaction mixture was absorbed on silica gel (20 g) and subjected to FC (CH$_2$Cl$_2$/MeOH, 20:1) to obtain compound **3b** as a colorless solid (0.15 g, 72%). TLC (CH$_2$Cl$_2$/MeOH, 9:1): R_f 0.49. UV (MeOH): λ$_{max}$ 216 (18 700), 242 (27 300). ^{1}H-NMR [(D$_6$)DMSO]: 2.06 (s, CH$_3$); 2.15, 2.64 [m, H$_2$-C(2′)]; 3.44 [m, H$_2$-C(5′)]; 3.76 [m, H-C(4′)]; 4.35 [m, H-C(3′)]; 4.72 [t, J = 5.7, HO-C(5′)]; 5.23 [d, J = 4.3, HO-C(3′)]; 6.27 ['t', J = 6.5, H-C(1′)]; 6.72 (br, NH$_2$); 10.72 (m, arom. H). Anal. calc. for C$_{13}$H$_{15}$N$_5$O$_4$ (305.3): C, 51.14; H, 4.95; N, 22.94; found: C, 51.18; H, 5.03; N, 22.10.

1-(2-Deoxy-β-D-erythro-pentofuranosyl)-1,5-dihydro-6-[(2-methylpropanoyl)amino]-3-propynyl-4*H*-pyrazolo-[3,4-*d*]-pyrimidin-4-one (7)

Compound **6b** (15) (0.75 g, 1.62 mmol) was reacted with tetrakis(triphenylphosphine)-palladium(0) [(PPh$_3$)$_4$Pd(0)] (94 mg, 0.08 mmol), CuI (65.5 mg, 0.34 mmol), triethylamine (0.24 ml, 1.72 mmol) and propyne in anhydrous DMF under Ar as described for **3b**. The reaction mixture was subjected to FC (CH$_2$Cl$_2$/MeOH, 20:1). Compound **7** was obtained as a colorless solid (0.73 g, 90%). R_f (CH$_2$Cl$_2$/MeOH, 20:1): 0.66. UV (MeOH): λ$_{max}$ 202 (17 000), 250 (23 000), 270 (16 000). ^{1}H-NMR [(D$_6$)DMSO]: 1.12 [m, CH(C*H$_3$*)$_2$]; 2.10 (s, CH$_3$); 2.19 [m, H$_\alpha$-C(2′)]; 2.68 [m, H$_\beta$-C(2′)]; 2.76 [m, C*H*(CH$_3$)$_2$]; 3.47 [m, H$_2$-C(5′)]; 3.78 [m, H-C(4′)]; 4.39 [*m*, H-C(3′)]; 4.73 [t, *J* = 5.6, HO-C(5′)]; 5.27 [d, *J* = 4.1, HO-C(3′)]; 6.36 ['t', *J* = 6.3, H-C(1′)]; 11.79, 11.91 (2s, 2NH). Anal. calc. for C$_{17}$H$_{21}$N$_5$O$_5$ (375.4): C, 54.39; H, 5.64; N, 18.66; found: C, 54.10; H, 5.80; N, 18.47.

1-[2-Deoxy-5-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-1,5-dihydro-6-[(2-methylpropanoyl)amino]-3-propynyl-4*H*-pyrazolo[3,4-*d*]-pyrimidin-4-one (8)

Compound **7** (0.3 g, 0.8 mmol) was co-evaporated three times with anhydrous pyridine before dissolving in anhydrous pyridine (1 ml). DMT-Cl (0.3 g, 0.89 mmol) was added in portions. After stirring for 3 h at r.t., the reaction was quenched with methanol (1 ml) and the solvents removed in vacuum. The residue was dissolved in CH$_2$Cl$_2$, washed with 5% NaHCO$_3$, dried with Na$_2$SO$_4$, and the solvent was removed. The residue was applied to FC (CH$_2$Cl$_2$/MeOH, 20:1) to give a colorless foam of **8** (0.47 g, 87%). TLC (CH$_2$Cl$_2$/MeOH, 95:5): R_f 0.38. UV (MeOH): λ$_{max}$ 202 (83 000), 234 (36 000), 273 (18 000). ^{1}H-NMR [(D$_6$)DMSO]: 1.14 [m, 2(C*H$_3$*)$_2$CH]; 2.10 (s, CH$_3$); 2.28 [m, H$_\alpha$-C(2′)]; 2.77 [m, H$_\beta$-C(2′), C*H*(CH$_3$)$_2$]; 3.02 [m, H$_2$-C(5′)]; 3.71 (s, OCH$_3$); 3.91 [m, H-C(4′)]; 4.51 [m, H-C(3′)]; 5.34 [d, *J* = 4.6, HO-C(3′)]; 6.38 [m, H-C(1′)]; 6.76–7.32 (m, arom. H); 11.88, 11.93 (*s*, N*H*CO). Anal. calc. for C$_{38}$H$_{39}$N$_5$O$_7$ (677.8): C, 67.34; H, 5.80; N, 10.33; found: C, 67.27; H, 5.70; N, 10.36.

1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-1,5-dihydro-6-[(2-methylpropanoyl)amino]-3-propynyl-4*H*-pyrazolo[3,4-*d*]-pyrimidin-4-one 3′-[(2-cyanoethyl)-*N,N*-diisopropylphosphoramidite (9)

To the solution of compound **8** (0.4 g, 0.59 mmol) in anhydrous dichloromethane (25 ml) *N,N*-diisopropylethyl-amine (DIPEA) (0.2 ml, 1.15 mmol) and 2-cyanoethyl diisopropylphosphoramido chloridite (0.2 ml, 0.9 mmol) were added under an Ar atmosphere. The reaction mixture was stirred at r.t. for 1 h, diluted with dichloromethane (20 ml), was washed with 5% aq. NaHCO$_3$, and dried over Na$_2$SO$_4$. Upon evaporation, the residue was submitted to FC (CH$_2$Cl$_2$/acetone, 9:1) yielding a colorless foam of **9** (0.45 g, 87%). TLC (CH$_2$Cl$_2$/acetone, 9:1): R_f 0.56, 0.65. ^{31}P-NMR (CDCl$_3$): 149.12. ^{1}H-NMR [(D$_6$)DMSO], 1.19 [m, CH(C*H$_3$*)$_2$]; 2.16 (s, CH$_3$); 2.35–3.35 [m, H$_2$-C(2′), C*H*(CH$_3$)$_2$, H$_2$-C(5′)]; 3.45–3.94 (m, CH$_2$CH$_2$); 3.80 (s, CH$_3$O); 4.23 [m, H-C(4′)];

4.82 [m, H-C(3′)]; 6.45 [m, H-C(1′)]; 6.77–7.47 (m, arom. H); 9.65, 11.73 (br, NH).

6-Amino-1-(2-deoxy-β-D-erythro-pentofuranosyl)-1,5-dihydro-3-(3-phthaloylaminopropynyl)-4*H*-pyrazolo-[3,4-*d*]-pyrimidin-4-one (10a)

Compound **6a** (0.27g, 0.69 mmol) (3) was reacted with (PPh$_3$)$_4$Pd(0) (94 mg, 0.08 mmol), CuI (31.1 mg, 0.16 mmol), and phthaloylamidopropyne (1.5 g, 8.1 mmol) (30) in anhydrous DMF under Ar at r.t. as described for **3b**. TLC indicated that the reaction was finished after 24 h, the second addition of the reagents was not necessary. The reaction mixture was subjected to FC (CH$_2$Cl$_2$/MeOH, 20:1); compound **3c** was obtained as colorless solid (0.18 g, 58%). TLC (CH$_2$Cl$_2$/MeOH, 9:1): R_f 0.19. UV (MeOH): λ$_{max}$ 240 (36 900), 217 (51 080). ^{1}H-NMR [(D$_6$)DMSO], 2.13, 2.63 [m, 2H-C(2′)]; 3.32 [m, 2H-C(5′)]; 3.74 [m, H-C(4′)]; 4.32 [m, H-C(3′)]; 4.66 (s, CH$_2$), 4.72 [t, *J* = 5.7, HO-C(5′)]; 5.23 [d, *J* = 4.3, HO-C(3′)]; 6.26 ['t', *J* = 6.5, H-C(1′)]; 6.77 (br, NH$_2$); 7.93 (m, arom. H). Anal. calc. for C$_{21}$H$_{18}$N$_6$O$_6$ (450.4): C, 56.00; H, 4.03; N, 18.66; found: C, 55.84; H, 4.13; N, 18.27.

1-(2-Deoxy-β-D-erythro-pentofuranosyl)-1,5-dihydro-6-[(2-methylpropanoyl)amino]-3-(3-phthaloylaminopropynyl)-4*H*-pyrazolo[3,4-*d*]-pyrimidin-4-one (10b)

The cross-coupling reaction of compound **6b** (15) (0.8 g, 1.73 mmol) with phthaloylamidopropyne (3.0 g, 16.2 mmol) was performed in anhydrous DMF (20 ml) under Ar in the presence of (PPh$_3$)$_4$Pd(0) (207 mg, 0.18 mmol), CuI (67.2 mg, 0.35 mmol) and triethylamine (0.5 ml, 3.59 mmol) at r.t. as described for **3b**. After chromatographic purification (CH$_2$Cl$_2$/MeOH, 20:1) a colorless solid (0.89 g, quantitative yield) was obtained. TLC (CH$_2$Cl$_2$/CH$_3$OH, 9:1): R_f 0.41. UV (MeOH): λ$_{max}$ 217 (51 400), 239 (30 000), 249 (26 000), 271 (16 300). ^{1}H-NMR [(D$_6$)DMSO], 1.11 (m, 2CH$_3$); 2.25 [m, H-C(2′)]; 2.73 [m, H-C(2′), C*H*(CH$_3$)$_2$)]; 3.45 [m, 2H-C(5′)]; 3.77 [m, H-C(4′)]; 4.37 [m, H-C(3′)]; 4.70 (s, CH$_2$); 4.73 [HO-C(5′)]. Anal. calc. for C$_{25}$H$_{24}$N$_6$O$_7$ (520.5): C, 57.69; H, 4.65; N, 16.15; found: C, 57.45; H, 4.56; N, 15.95.

1-[2-Deoxy-5-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-1,5-dihydro-6-[(2-methylpropanoyl)-amino]-3-(3-phthaloylaminopropynyl)-4*H*-pyrazolo-[3,4-*d*]-pyrimidin-4-one (11)

1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-erythro-pentofur-anosyl]-1,5-dihydro-3-iodo-6-[(2-methylpropanoyl)amino]-4*H*-pyrazolo[3,4-*d*]-pyrimidin-4-one (15) (130 mg, 0.17 mmol) was subjected to the cross-coupling reaction with phthaloyl-amidopropyne (351.5 mg, 1.9 mmol) under the same conditions as for **3b**. CuI (7.3 mg, 0.04 mmol), (PPh$_3$)$_4$Pd(0) (22.5 mg, 0.02 mmol), and triethylamine (57 μl, 0.41 mmol) were used. The reaction mixture was subjected to FC (CH$_2$Cl$_2$/MeOH, 20:1) to furnish **11** as a colorless solid (0.1 g, 72%). TLC (CH$_2$Cl$_2$/MeOH 9:1): R_f 0.76. UV (MeOH): λ$_{max}$ 202 (110 000), 274 (19 000). ^{1}H-NMR [(D$_6$)DMSO], 1.13 (m, 2CH$_3$); 2.26 [m, H-C(2′)]; 2.73 [m, H-C(2′)]; 3.01 [m, C*H*(CH$_3$)$_2$, 2H-C(5′)]; 3.68 (s, OCH$_3$); 3.88 [m, H-C(4′)]; 4.42 [m, H-C(3′)]; 4.69 (s, CH$_2$); 5.33 [d, *J* = 4.3, HO-C(3′)]; 6.39 ['t', *J* = 6.5, H-C(1′)]; 6.70–7.27 (m, arom. H); 11.90,

11.96 (s, 1-N*H*, N*H*CO). Anal. calc. for $C_{46}H_{42}N_6O_9$ (822.9): C, 67.14; H, 5.14; N, 10.21; found: C, 67.18; H, 5.09; N, 9.99.

1-[2-Deoxy-5-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-1,5-dihydro-6-[(2-methylpropanoyl)amino]-3-(3-phthaloylaminopropynyl)-4*H*-pyrazolo-[3,4-*d*]-pyrimidin-4-one 3′-(2-cyanoethyl diisopropylphosphoramidite) (12)

The conversion from **11** (0.51 g, 0.62 mmol) was conducted as for **9**, with DIPEA (0.25 ml, 1.44 mmol) and 2-cyanoethyl diisopropylphosphoramido chloridite (250 µl, 1.12 mmol) in anhydrous CH_2Cl_2 (20 ml) under Ar. The product was purified by FC (CH_2Cl_2/acetone, 85:15) to yield a colorless foam (0.51 g, 80%). TLC (CH_2Cl_2/acetone, 85:15): R_f 0.61, 0.67. ^{1}H-NMR (CDCl₃): 1.15 (m, 2CH₃); 2.46 [m, H-C(2′), C*H*(CH₃)₂]; 3.12 [m, H-C(2′)], 3.69 (m, CH₂CH₂); 3.79 [m, 2H-C(5′)]; 3.79 (s, OCH₃); 4.25 [m, H-C(4′)]; 4.76 [m, H-C(3′)]; 4.79 (s, CH₂); 6.40 ['t', *J* = 6.5, H-C(1′)]; 6.74–8.26 (m, arom. H); 11.73 (*s*, N*H*CO). ³¹P-NMR: 149.04, 149.11.

1-(2-Deoxy-β-D-erythro-pentofuranosyl)-4-{[(dimethylamino)methylidene]amino}-3-propynyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (13)

A solution of compound **4b** (7) (250 mg, 0.87 mmol) in MeOH (30 ml) was stirred with *N,N*-dimethylformamide dimethyl acetal (3 ml, 21.4 mmol) for 30 min at r.t. After evaporation, the residue was applied to FC (CH_2Cl_2/MeOH, 95:5). Compound **13** was isolated as a colorless foam (270 mg, 91%). TLC (CH_2Cl_2/MeOH, 9:1): R_f 0.43. UV (MeOH) λ_max: 321 nm (26 500), 223 (26 100). ^{1}H-NMR [(D₆)DMSO]: δ 2.27 [m, 1H, H_α-C(2′)]; 2.77 [m, 1H, H_β-C(2′)]; 3.18, 3.21 (2s, 6H, Me₂N); 3.72–3.81 [m, 2H, H-C(4′), H-C(5′)]; 4.42 [m, 1H, H-C(3′)]; 4.87 [m, *J* = 5.6, 1H, HO-C(5′)]; 5.40 [d, *J* = 4.5, 1H, HO-C(3′)]; 6.56 [t, *J* = 6.4, 1H, H-C(1′)]; 8.42 [s, 1H, H-C(6)]; 8.87 (s, 1H, N=CH). Anal. calc. for $C_{16}H_{20}N_6O_3$ (344.4): C, 55.80; H, 5.85; N, 24.40; found: C, 55.75; H, 5.95; N, 24.25.

1-(2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl)-4-{[(di-methylamino)-methylidene]amino}-3-propynyl-1*H*-pyrazolo-[3,4-*d*]pyrimidine (14)

Compound **14** was obtained by the tritylation of compound **13** (0.2 g, 0.58 mmol) with DMT-Cl (0.24 g, 0.7 mmol) as described for **8** as a colorless foam purified by FC (CH_2Cl_2/MeOH, 20:1) (350 mg, 93%). R_f (CH_2Cl_2/MeOH, 9:1): 0.29. UV (MeOH) λ_max: 224 nm (41 500), 320 (25 900). ^{1}H-NMR [(D₆)DMSO]: δ 2.33 [m, 1H, H_α-C(2′)]; 2.79 [m, 1H, H_β-C(2′)]; 2.94, 3.04 [m, 2H, H-C(5′)]; 3.17, 3.21 (2s, 6H, Me₂N); 3.68, 3.69 (2s, 6H, 2 MeO); 3.90 [m, 1H, H-C(4′)], 4.56 [m, 1H, H-C(3′)]; 5.44 [d, *J* = 5.0, 1H, HO-C(3′)]; 6.58 [m, 1H, H-C(1′)]; 6.69–7.29 (m, arom. H); 8.45 [s, 1H, H-C(6)]; 8.86 (s, 1H, N=CH). Anal. calc. for $C_{37}H_{38}N_6O_5$ (646.7): C, 68.71; H, 5.92; N, 12.99; found: C, 68.59; H, 6.00; N, 13.02.

1-(2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl)-4-{[(di-methylamino)-methylidene]amino}-3-propynyl-1*H*-pyrazolo-[3,4-*d*]pyrimidine 3′-[(2-cyanoethyl)-*N,N*-diisopropylphosphoramidite] (15)

Compound **15** was synthesized from **14** (300 mg, 0.47 mmol) in anhydrous CH_2Cl_2 (5 ml) with anhydrous DIPEA (0.16 ml,

0.92 mmol) and 2-cyanoethyl diisopropylphosphoramido chloridite (0.21 ml, 0.94 mmol) under Ar. The residue was applied to FC (CH_2Cl_2/Me₂CO, 10:1) to yield the title compound **15** as a colorless foam (290 mg, 74%). TLC (CH_2Cl_2/Me₂CO, 8:1): R_f 0.18, 0.24. ^{1}H-NMR [(D₆)DMSO]: δ 1.16 [m, 6H, 2CH*Me*₂]; 2.18 (s, 3H, Me); 2.48, 2.66 [m, 2H, H₂-C(2′)]; 3.23, 3.30 [2s, 6H, 2CH₃]; 3.25 [m, H₂-C(5′)]; 3.73 (m, CH₂CH₂); 3.79 (2s, OCH₃); 4.22 [m, 1H, H-C(4′)]; 4.81 [m, 1H, H-C(3′)]; 6.74–7.42 [m, H-C(1′), arom. H]; 8.54 [s, 1H, H-C(6)]; 8.84 (s, 1H, N=CH). ³¹P-NMR (CDCl₃): 149.61, 149.71.

## RESULTS AND DISCUSSION

Monomers

The propynyl nucleosides **1** (25), **2** (25), **4b** (7) and **5b** (18) were prepared according to the literature. The 7-propynyl and 7-(3-phthaloylamidopropynyl) substituted 8-aza-7-deaza-2′-deoxyguanosine derivatives **3b**, **10a** were synthesized from the 7-iodo nucleosides **6a** (3) or **6b** (15) by the Sonogashira cross-coupling reaction (16,31–33) (Schemes 2 and 3). The reaction was catalyzed by Pd(0)[(PPh)₃]₄/CuI and was performed in DMF in the presence of triethylamine. For **3b**, DMF was saturated with propyne at 0°C. Its amino group was protected with an isobutyryl group by transient protection (34) (**7**). The protected **7** was converted into the DMT derivative **8** and further transformed to the phosphoramidites **9** under standard conditions. The phthaloylamino derivatives **10a,b** and **11** were prepared from **6a,b**, and the DMT derivative (**15**) employing phthaloylamidopropyne in the cross-coupling reaction. Compound **11** was converted into the phosphoramidite **12**.
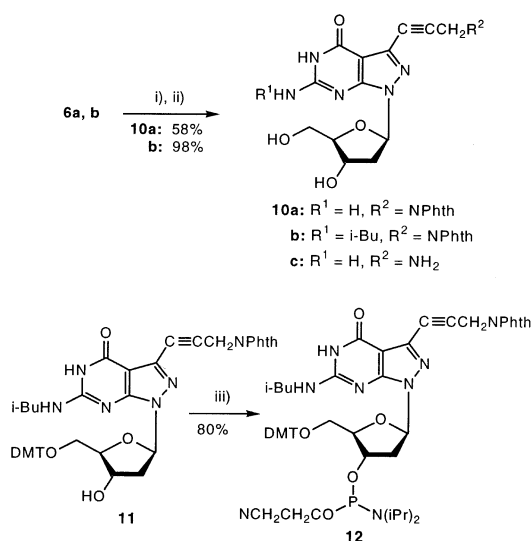
Finally, the phosphoramidite **15** was prepared (Scheme 4). For this purpose, compound **4b** was protected at the amino group with the dimethylaminomethylidene residue resulting in **13**. Then, the DMT residue was introduced to the 5′-hydroxyl group yielding compound **14**, which was transformed into the phosphoramidite **15**. All compounds were characterized by elemental analysis as well as by ^{1}H- and ^{13}C-NMR spectra (Table 2 and Materials and Methods).

Oligonucleotides

Synthesis and characterization. The oligonucleotides **16–47** were prepared in a 1 µmol scale on an ABI 392-08 synthesizer (trityl-on mode) employing phosphoramidite chemistry (35). Apart from the phosphoramidites **9**, **12** and **15**, standard phosphoramidites were used. In the case of the oligonucleotides containing 5-propynyl-2′-deoxyuridine (**2**), the tac [(4-(*tert*-butyl)phenoxyacetyl]-protected building blocks were used. The coupling efficiency was always higher than 95%. The deprotection of the oligonucleotides was performed in 25% aq. NH₃ for 12–18 h at 60°C or at r.t. for 2–3 h for oligonucleotides containing compound **2**. The oligonucleotides were purified before and after detritylation by reversed-phase HPLC (for conditions of purification see Materials and Methods). The composition analysis of oligomers was performed by enzymatic hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase (Fig. 1). Also, MALDI-TOF mass spectrometry was performed confirming the constitution of the oligonucleotides.
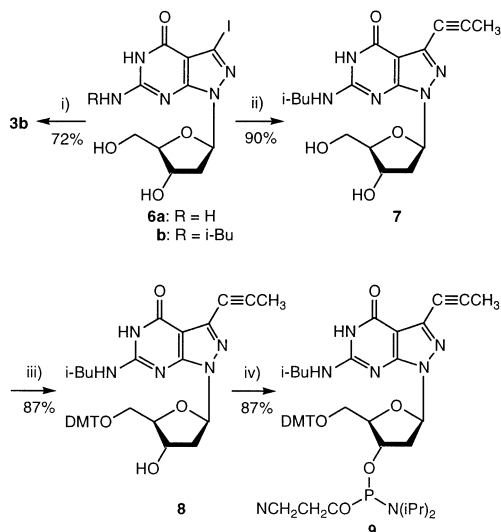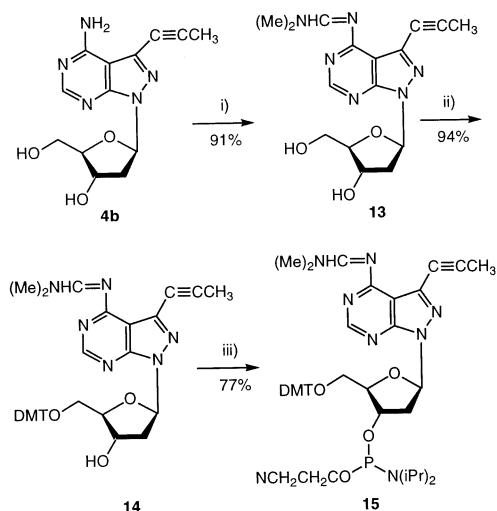
Scheme 2. (i) and (ii) Propyne, Pd(0)(PPh₃)₄, CuI, Et₃N, Ar, r.t., 18 h. (iii) (MeO)₂TrCl, pyridine, r.t.; 3 h. (iv) 2-Cyanoethyl diisopropylphosphoramido chloridite, CH₂Cl₂, r.t., 30 min.

Scheme 4. (i) *N,N*-Dimethylformamide dimethyl acetal in methanol, r.t. (ii) DMT-Cl, dry pyridine, 3 h at r.t. (iii) 2-Cyanoethyl diisopropylphosphoramido chloridite, diisopropylethylamine, CH₂Cl₂, 1 h, at r.t.

Scheme 3. (i) and (ii) Phthaloylamidopropyne Pd(0)(PPh₃)₄, CuI, Et₃N, Ar, r.t., 21 h. (iii) 2-Cyanoethyl diisopropylphosphoramido chloridite, CH₂Cl₂, r.t., 30 min.

Duplex stability of oligonucleotides containing propynyl residues and related side chains. Earlier, it was reported that 8-aza-7-deazapurine nucleosides with bromo or iodo substituents and also longer alkynyl chains linked to the 7-position are well accommodated in the major groove of DNA, thereby increasing the thermal stability of duplexes (1,18,36). Moreover, it was found that alkynyl chains with three to six carbon atoms led to a significant stabilization of duplex DNA while longer chains are too hydrophobic to fulfill this objective, in particular when multiple incorporations were made. This study makes use of propynyl residues being part of the 8-aza-7-deazapurine nucleosides **3b**, **4b** and **5b**. The already known pyrimidine nucleosides **1** and **2** were employed to study the role of the propynyl group on both sides of the

'dG–dC' or the 'dA–dT' base pair (motifs **I–XVI**, Schemes 5 and 6). As all four propynyl nucleosides related to the canonical DNA constituents were now accessible, a rather complete picture of the stabilizing effects of propynyl groups on Watson–Crick base pairs of oligonucleotide duplexes was developed.

The stability of the tridentate 'dG–dC' base pair modified by 8-aza-7-deaza-7-propynyl-2′-deoxyguanosine (3b) and/or 5-propynyl-2′-deoxycytidine (1). In a first series of experiments, the role of the propynyl group was studied with the duplex 5′-d(TAG GTC AAT ACT) (**16**) 3′-d(ATC CAG TTA TGA) (**17**) incorporating compound **3b** in the place of dG. The T_m values of a series of oligonucleotides are shown in Table 3. According to Table 3, the 7-propynyl nucleoside **3b** increases the stability of a 'dG–dC' base pair (Scheme 5, motif **I**) significantly. The incorporation of **3b** (motif **II**) residue results in a T_m increase of 2.5–3°C per modification (data taken in the high salt buffer) depending on the position of substitution. The contribution of the 7-aminopropyl residue (**10c**) is smaller (1–2°C), which is ~1°C less than for **3b**. In a similar way oligonucleotide duplexes were studied in which the dC residues of dG–dC base pairs were replaced by 5-propynyl-2′-deoxycytidine (**1**). The stability increase of such a modified base pair (motif **III**) was found to be between 2.0 and 3.0°C, which is slightly lower than that found for the **3b**–dC pair. Also, both bases of the 'dG–dC' pair were substituted by the propynyl nucleosides **1** and **3b** (motif **IV**) in which the T_m increase was 5.0–5.5°C for two propynyl modifications per base pair, which corresponds to ~2.5–2.75°C for each single nucleoside substitution (Tables 3 and 4).

From the T_m values shown above, it can be concluded that a 7-propynyl residue linked to the 7-position of an 8-aza-7-deaza-2′-deoxyguanosine residue (**3b**) causes a slightly better stabilization of a DNA duplex than that of the 5-propynyl residue of a 2′-deoxycytidine moiety (**1**). The modification at both sites of the 'dG–dC' base pair (motif **IV**) shows an addition of propynyl effects (Table 3). The 3-amino propynyl

Table 2. ^{13}C-NMR chemical shifts of pyrazolo[3,4-*d*]-pyrimidine 2′-deoxyribonucleosides[a]

Compound	C(2)[b,d] C(6)[c,d]	C(4)[b,d] C(7a)[c,d]	C(5)[b] C(3a)[c]	C(6)[b,d] C(4)[c,d]	C(7)[b] C(3)[c]	C≡C	CH$_3$/CH$_2$	N(CH$_3$)$_2$/C=N	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
3a (4)	154.6	155.2	99.7	157.4	134.9				83.1	37.9	71.0	87.3	62.4
3b	155.1	155.4	99.8	157.0	130.4	72.2, 89.4	3.9		83.0	37.6	70.9	87.5	62.4
4a (7)	156.9	154.5	101.3	158.9	133.9				84.9	38.8	72.0	88.4	63.3
4b	156.5	153.5	100.8	157.6	127.4	71.1, 92.9	4.0		83.3	38.0	71.3	87.5	62.7
5a	156.9	158.3	95.5	162.7	133.3				83.0	37.6	71.0	87.3	62.5
5b	156.7	157.8	95.6	162.7	127.3	72.0, 91.5	3.8		83.1	37.6	70.8	87.4	62.3
6a (3)	154.9	155.8	93.9	157.3	101.9				83.5	37.7	70.7	87.7	62.2
7	152.7	150.3	102.8	155.0	130.7	71.5, 90.6	4.18		83.7	37.8	70.2	87.7	63.9
8	152.6	150.3	103.0	155.1	130.5	71.6, 90.4	4.0		87.3	37.6	70.9	83.1	62.4
10a	155.2	155.5	100.2	156.8	123.4	74.5, 86.5	27.4		83.8	37.8	70.9	87.6	62.3
10b	152.8	150.5	103.2	154.9	129.3	73.8, 87.5	27.4		84.5	37.6	70.6	87.5	62.1
11	152.6	150.4	103.4	154.9	123.4	73.9, 85.0	27.4		87.3	37.8	70.2	83.9	63.9
13	155.8	154.3	107.7	162.2	129.1	72.5, 90.7	3.8	34.6, 157.5	83.8	37.8	70.9	87.5	62.3
14	155.8	154.3	107.8	162.2	129.0	72.7, 90.4	3.9	34.5, 157.8	83.5	[e]	70.3	85.0	63.9

[a]Measured in (D$_6$)DMSO.
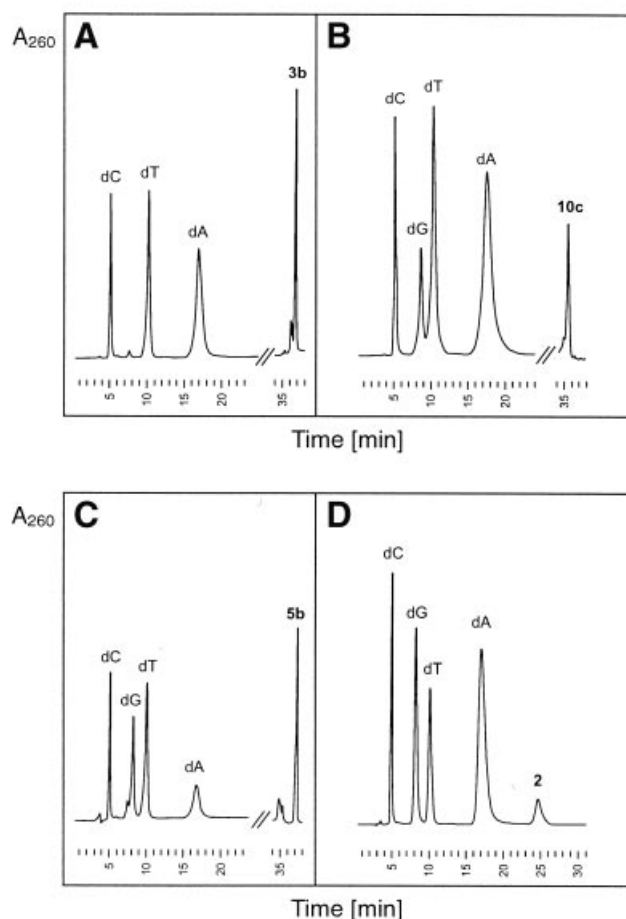[b]Purine numbering.
[c]Systematic numbering.
[d]Tentative.
[e]Superimposed by DMSO.

Figure 1. HPLC profile of enzymatic analysis of oligonucleotides **20** containing **3b** (**A**), **24** containing **10c** (**B**), **39** containing **5b** (**C**), **34** containing **2** (**D**) by phosphodiesterase and alkaline phosphatase in 0.1 M Tris–HCl buffer (pH 8.3) at 37°C. Condition: reversed-phase HPLC at 260 nm on an RP-18 column (200 × 10 mm) with 100% B as the eluent, 0.7 ml/min (for composition of B, see Materials and Methods).
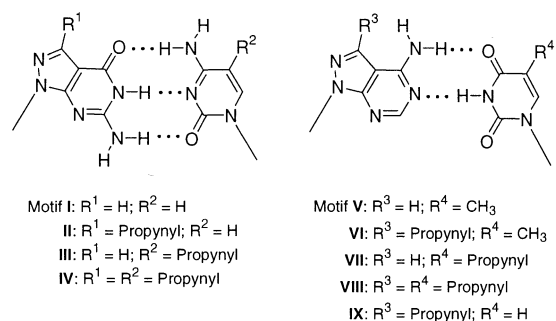
Scheme 5.
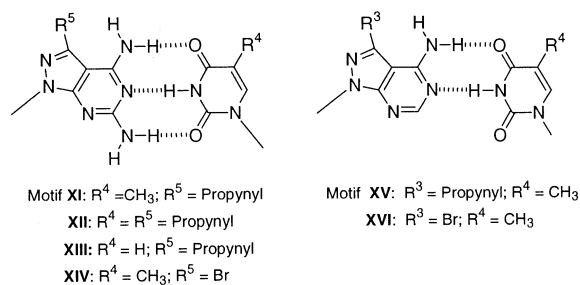
Scheme 6.

residues destabilize the duplex compared to the propynyl groups (37). The thermodynamic data taken from the shape analyses of the melting profiles have to be interpreted with care. An error of ~15% has to be taken into account for the thermodynamic data while the accuracy of the T_m measurements is within ±0.5°C.

The bidentate 'dA–dT' base pair modified by 7-propynylated 8-aza-7-deazapurin-6-amine 2′-deoxyribonucleoside (4b) and/or 5-propynyl-2′-deoxyuridine (2). Similar experiments

Table 3. T_m values and thermodynamic data of oligonucleotides containing 8-aza-7-deaza-7-propynyl-2′-deoxyguanosine **3b**, **1**, or the aminopropynyl derivative **10c**[a]

Duplex	T_m (°C)	ΔT_m[b] (°C)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol K)	$\Delta G°_{310}$ (kcal/mol)
5′-d(TAG GTC AAT ACT) (**16**)	50		−90	−252	−11.8
3′-d(ATC CAG TTA TGA) (**17**)	(47)		−89	−253	−10.9
5′-d(TAG GTC AAT ACT) (**16**)	53	3	−96	−270	−12.5
3′-d(ATC CA**3b** TTA TGA) (**18**)	(49)	2			
5′-d(TAG GTC AAT ACT) (**16**)	56	3	−105	−293	−13.8
3′-d(ATC CA**3b**TTA T**3b**A) (**19**)	(53)	3			
5′-d(TA**3b 3b**TC AAT ACT) (**20**)	56	3	−105	−294	−14.5
3′-d(ATC CAG TTA TGA) (**17**)	(52)	2.5			
5′-d(TA**3b 3b**TC AAT ACT) (**20**)	58	2.7	−98	−272	−14.0
3′-d(ATC CA**3b** TTA TGA) (**18**)	(56)	3			
5′-d(TA**3b 3b**TC AAT ACT) (**20**)	60	2.5	−99	−272	−14.6
3′-d(ATC CA**3b** TTA T**3b**A) (**19**)	(58)	2.8	−93	−256	−13.7
5′-d(TAG GT**1** AAT ACT) (**21**)	55	2.5	−89	−245	−12.8
3′-d(ATC CA**3b** TTA TGA) (**18**)					
5′-d(TA**3b 3b**TC AAT ACT) (**20**)	61	2.75	−90	−243	−14.3
3′-d(AT**1 1**AG TTA TGA) (**22**)	(58)	2.75			
5′-d(TAG GT**1** AAT A**1**T) (**23**)	60	2.5	−97	−265	−14.6
3′-d(ATC CA**3b** TTA T**3b**A) (**19**)	(57)	2.5			
5′-d(TAG GTC AAT ACT) (**16**)	52	2.0	−87	−242	−11.8
3′-d(ATC CA**10c** TTA TGA) (**24**)	(48)	1.0			
5′-d(TA**10c 10c**TC AAT ACT) (**25**)	54	2.0	−90	−250	−12.6
3′-d(ATC CAG TTA TGA) (**17**)					
5′-d(TA**10c 10c**TC AAT ACT) (**25**)	55	1.75	−97	−269	−13.3
3′-d(ATC CA**10c** TTA TGA) (**24**)					

[a]Measured in 1 M NaCl, 100 mM MgCl$_2$ and 60 mM Na-cacodylate (pH 7.0) with 5 µM single-strand concentration. Data in parentheses were measured in 100 mM NaCl, 10 mM MgCl$_2$ and 10 mM Na-cacodylate (pH 7.0) with 5 µM oligonucleotide.
[b]Refers to the contribution of the modified residues divided by the number of replacements.

as performed for the 'dG–dC' base pair were undertaken on the 'dA–dT' pair (Scheme 5, motifs **V–IX**). Single and multiple substitutions of dA residues by the propynyl derivative **4b** are shown in Table 5. A ΔT_m of 2–3°C is observed for each modification of a dA residue by compound **4b** when measured in low salt buffer (motif **VI** versus **V**) (18). These values are lower than for the propynylated dG derivative **3b**. Even lower are the ΔT_m values (0.75–1.5°C) for the modification of dT by the propynyl nucleoside **2** (motif **VII**). However, in these cases it has to be taken into account that the values refer to a dA–dT base pair containing already a methyl group at the dT residue. The propynyl group at both sides of the base pair **4b–2** (motif **VIII**) shows an additive effect on the T_m increase. The 5-propynyl group is more effective than the 5-methyl group (**4b–2** versus **4b**–dT, motif **VIII** versus **VI**). The base pair **4b**–dU (motif **IX**) shows the lowest T_m value (Tables 5 and 6).

Comparison of the duplex stabilities of base pairs incorporating the 7-propynyl as well as the bromo derivatives of 8-aza-7-deaza-2′-deoxyguanosine (3b,c), 8-aza-7-deaza-2′-deoxyadenosines (4b,c) and the 8-aza-7-deazapurin-2,6-amine 2′-deoxyribonucleosides (5b,c). Earlier, it was already reported on the extraordinary stability of oligonucleotide duplexes

incorporating the diamino nucleoside **5b** (18). It forms a stable base pair with dT (Scheme 6, motif **XI**) as the tridentate dG–dC pair. This study was now extended to a series of oligonucleotide duplexes incorporating compound **5b** at various positions of the oligonucleotide chain and containing the modified nucleoside in one, the other, or in both strands (Table 7). From the data shown in Table 7 (low salt buffer), it is apparent that the T_m increase amounts to 4.3–6°C for a single modification of compound **5b**. As the corresponding non-propynylated nucleoside **5a** and the parent purin-2,6-diamine 2′-deoxyribonucleoside have only a minor influence on the duplex stability (see Table 7), this stabilization is caused by the combination of two favorable effects: one caused by the propynyl group and the other by the 2-amino group, which forms the tridentate base pair with the 2-oxo group of the uracil base (motif **XI**). The propynyl effect of the nucleoside **5b** is diluted by the rather low stabilizing propynylated pyrimidine nucleoside **2**, as is seen by the comparison of four incorporations of compound **5b** (duplex **38·39**) with four incorporations (2 × compound **5b** and 2 × compound **2**) in the duplex **33·38** (motif **XII**). The former amounts to a ΔT_m of 21°C compared to the unmodified duplex **16·17** and only to 13°C for the duplex **33·38**. The propynyl effect of compound **2** is seen when comparing the duplexes

Table 4. T_m values and thermodynamic data of oligonucleotides incorporating 5-propynyl-deoxycytidine **1** and **3b**[a]

Duplex	T_m (°C)	ΔT_m[b] (°C)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol K)	$\Delta G°_{310}$ (kcal/mol)
5′-d(TAG GT1 AAT ACT) (**21**) 3′-d(ATC CAG TTA TGA) (**17**)	53	3	–90	–250	–12.4
5′-d(TAG GT1 AAT A1T) (**23**) 3′-d(ATC CAG TTA TGA) (**17**)	54 (52)	2 2.5	–93	–259	–12.8
5′-d(TAG GTC AAT ACT) (**16**) 3′-d(AT1 1AG TTA TGA) (**22**)	55.5	2.75	–87	–239	–12.8
5′-d(1 TAG GTC AAT ACT) (**26**) 3′-d(ATC CAG TTA TGA) (**17**)	52 (49)	2.0 2.0	–96	–271	–12.2
5′-d(TAG GT1 AAT ACT) (**21**) 3′-d(AT1 1AG TTA TGA) (**22**)	58.5	2.7	–87	–237	–13.4
5′-d(TAG GT1 AAT A1T) (**23**) 3′-d(AT1 1AG TTA TGA) (**22**)	60	2.5	–91	–248	–14.0
5′-d(1 TAG GTC AAT ACT) (**26**) 3′-d(AT1 1AG TTA TGA) (**22**)	57	2.3	–90	–247	–13.1
5′-d(1 TAGGTC AAT ACT) (**26**) 3′-d(ATC CA**3b**TTA TGA) (**18**)	54 (51)	2.0 2.0	–89	–247	–12.6
5′-d(1 TAG GTC AATACT) (**26**) 3′-d(ATC CA**3b**TTA T**3b**A) (**19**)	57	2.3	–92	–255	–13.4

[a]Measured in 1 M NaCl, 100 mM MgCl$_2$ and 60 mM Na-cacodylate (pH 7.0) with 5 µM single-strand concentration. Data in parentheses were measured in 100 mM NaCl, 10 mM MgCl$_2$ and 10 mM Na-cacodylate (pH 7.0) with 5 µM oligonucleotides.
[b]See Table 3.

Table 5. T_m values and thermodynamic data of oligonucleotides containing 8-aza-7-deaza-7-propynyl-2′-deoxyadenosine **4b**[a]

Duplex	T_m (°C)	ΔT_m[b] (°C)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol K)	$\Delta G°_{310}$ (kcal/mol)
5′-d(TAG GTC **4b**AT ACT) (**27**) 3′-d(ATC CAG TTA TGA) (**17**)	50	3	–83	–233	–11.0
5′-d(TAG GTC AAT ACT) (**16**) 3′-d(ATC C**4b**G TT**4b** TGA) (**28**)	53	3	–94	–263	–12.6
5′-d(TAG GTC **4b4b**T ACT) (**29**) 3′-d(ATC CAG TTA TGA) (**17**)	52	2.5	–85	–234	–11.8
5′-d(TAG GTC **4b**AT ACT) (**27**) 3′-d(ATC C**4b**G TT**4b** TGA) (**28**)	56	3	–94	–262	–13.2
5′-d(TAG GTC **4b4b**T ACT) (**29**) 3′-d(ATC C**4b**G TT**4b** TGA) (**28**)	59	3	–88	–240	–13.6
5′-d(T**4b**G GTC **4b4b**T **4b**CT) (**30**) 3′-d(ATC CAG TTA TGA) (**17**)	56	2.5	–94	–261	–13.2
5′-d(T**4b**G GTC **4b4b**T **4b**CT) (**30**) 3′-d(ATC C**4b**G TT**4b** TGA) (**28**)	63	2.7	–99	–269	–15.7
5′-d(TAG GTC **4b4b**T ACT) (**29**) 3′-d(ATC CAG UUA TGA) (**31**)	51	2	–93	–263	–11.8
5′-d(TAG GUC AAU ACT) (**32**) 3′-d(ATC C**4b**G TT**4b** TGA) (**28**)	51	2	–83	–231	–11.8

[a]Measured in 100 mM NaCl, 10 mM MgCl$_2$ and 10 mM Na-cacodylate (pH 7.0) with 5 µM + 5 µM single-strand concentration.
[b]See Table 3.

34·39 with **31·39**. The propynyl group amounts only to 1°C per modification in comparison to the unsubstituted dU (motif **XIII**).

Next, the base pair stabilization of the 7-propynyl group versus the 7-bromo substituent was studied. Table 8 contains a series of the duplexes incorporating the propynylated

Table 6. T_m values and thermodynamic data of oligonucleotides containing 5-propynyl-2′-deoxyuridine (**2**) and **4b**[a]

Duplex	T_m (°C)	ΔT_m[b] (°C)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol K)	$\Delta G°_{310}$ (kcal/mol)
5′-d(TAG G2C AA2 ACT) (**33**) 3′-d(ATC CAG TTA TGA) (**17**)	53 (50)	1.5 1.5	–98	–274	–12.6
5′-d(TAG GTC AAT ACT) (**16**) 3′-d(ATC CAG **22**A TGA) (**34**)	52 (50)	1.0 1.5	–93	–262	–12.2
5′-d(TAG G2C AA2 ACT) (**33**) 3′-d(ATC CAG **22**A TGA) (**34**)	53 (53)	0.75 1.5	–101	–285	–12.8
5′-d(TAG G2C AAT ACT) (**35**) 3′-d(ATC CAG TTA TGA) (**17**)	52 (50)	2.0 3.0	–91	–256	–11.9
5′-d(**2** TAG GTC AAT ACT) (**36**) 3′-d(ATC CAG TTA TGA) (**17**)	52 (48)	2.0 1.0	–96	–271	–12.2
5′-d(TAG G2C AAT ACT) (**35**) 3′-d(ATC CAG **22**A TGA) (**34**)	53 (51)	1.0 1.3	–91	–254	–12.5
5′-d(**2** TAG GTC AAT ACT) (**36**) 3′-d(ATC CAG **22**A TGA) (**34**)	53 (51)	1.0 1.3	–100	–280	–12.8
5′-d(TAG GTC **4b4b**T ACT) (**29**) 3′-d(ATC CAG **22**A TGA) (**34**)	56	1.5	–100	–278	–13.6

[a]Measured in 1 M NaCl, 100 mM MgCl$_2$ and 60 mM Na-cacodylate (pH 7.0) with 5 μM single-strand concentration. Data in parentheses were measured in 100 mM NaCl, 10 mM MgCl$_2$ and 10 mM Na-cacodylate (pH 7.0) with 5 μM oligonucleotides.
[b]See Table 3.

Table 7. T_m values and thermodynamic data of oligonucleotides incorporating the base pairs of the 8-aza-7-deazapurin-2,6-diamine 2′-deoxyribonucleosides **5b**–dT and **2**–**5b**[a]

Duplex	T_m (°C)	ΔT_m[b] (°C)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol K)	$\Delta G°_{310}$ (kcal/mol)
5′-d(TAG GTC **5a5a**T ACT)[c] 3′-d(ATC CAG TTA TGA) (**17**)	50	1.5	–93	–263	–11.7
5′-d(TAG GT C **5a5a**T ACT)[c] 3′-d(ATC C**5a**G TT**5a** TGA)[c]	51	2.0	–99	–279	–12.3
5′-d(TAG GTC **5b**AT ACT) (**37**) 3′-d(ATC CAG TTA TGA) (**17**)	53	6	–88	–244	–11.9
5′-d(TAG GTC AAT ACT) (**16**) 3′-d(ATC C**5b**G TT**5b**TGA) (**38**)	58	5.5	–101	–279	–14.4
5′-d(TAG GTC **5b5b**T ACT) (**39**) 3′-d(ATC CAG TTA TGA) (**17**)	58	5.5	–100	–277	–13.8
5′-d(TAG GTC **5b**AT ACT) (**37**) 3′-d(ATC C**5b**G TT**5b** TGA) (**38**)	64	6	–104	–284	–16.0
5′-d(TAG GT C **5b5b**T ACT) (**39**) 3′-d(ATC C**5b**G TT**5b** TGA) (**38**)	68	5	–111	–300	–18.0
5′-d(T**5b**G GTC **5b5b**T **5b**CT) (**40**) 3′-d(ATC CAG TTA TGA) (**17**)	65	4.3	–109	–297	–17.1
5′-d(T**5b**G GTC **5b5b**T **5b**CT) (**40**) 3′-d(ATC C**5b**G TT **5b** TGA) (**38**)	75	4.7	–115	–303	–20.6
5′-d(TAG G2C AA2 ACT) (**33**) 3′-d(ATC C**5b**GTT**5b** TGA) (**38**)	60	3.25	–96	–264	–14.3
5′-d(TAG GTC **5b5b**T ACT) (**39**) 3′-d(ATC CAG **22**A TGA) (**34**)	59	3.00	–91	–249	–14.0
5′-d(TAG GTC **5b5b**T ACT) (**39**) 3′-d(ATC CAG **UU**A TGA) (**31**)	56	2.25	–104	–289	–13.8

[a]Measured in 100 mM NaCl, 10 mM MgCl$_2$ and 10 mM Na-cacodylate (pH 7.0) with 5 μM + 5 μM oligonucleotide.
[b]See Table 3.
[c]See Becher *et al.* (17).

Table 8. Comparison of T_m values and thermodynamic data of oligonucleotide duplexes incorporating 7-propynyl versus 7-bromo nucleosides in tridentate and bidentate base pairs[a]

Duplex	T_m (°C)	ΔT_m[b] (°C)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol K)	$\Delta G°_{310}$ (kcal/mol)
5′-d(TA**3b 3b**TC AAT ACT) (**20**) 3′-d(ATC CA**3b** TTA T**3b**A) (**19**)	58	2.75	–95	–262	–13.8
5′-d(TA**3c 3c**TC AAT ACT) (**41**) 3′-d(ATC CA**3c** TTA T**3c**A) (**42**)	55	2.00	–78	–219	–14.3
5′-d(TA**3b 3b**TC AAT ACT) (**20**) 3′-d(ATC CAG TTA TGA) (**17**)	52	2.5	–94	–264	–12.1
5′-d(TA**3c 3c**TC AAT ACT) (**41**) 3′-d(ATC CAG TTA TGA) (**17**)	52	2.5	–105	–301	–12.4
5′-d(T**4b**G GTC **4b4b**T **4b**CT) (**30**) 3′-d(ATC C**4b**G TT**4b** TGA) (**28**)	63	2.7	–99	–269	–15.7
5′-d(T**4c**G GTC **4c4c**T **4c**CT) (**43**) 3′-d(ATC C**4c**G TT**4c** TGA) (**44**)	57	1.7	–111	–308	–15.2
5′-d(T**4b**G GTC **4b4b**T **4b**CT) (**30**) 3′-d(ATC CAG TTA TGA) (**17**)	56	2.25	–94	–261	–13.2
5′-d(T**4c**G GTC **4c4c**T **4c**CT) (**43**) 3′-d(ATC CAG TTA TGA) (**17**)	52	1.25	–87	–242	–12.3
5′-d(TAGGTC AAT ACT) (**16**) 3′-d(ATC C**4b**G TT**4b** TGA) (**28**)	55	4.0	–89	–246	–12.4
5′-d(TAGGTC AAT ACT) (**16**) 3′-d(ATC C**4c**G TT**4c** TGA) (**44**)	51	2.0	–	–	–
5′-d(T**5b**G GTC **5b5b**T **5b**CT) (**40**) 3′-d(ATC C**5b**G TT**5b** TGA) (**38**)	75	4.7	–115	–303	–20.6
5′-d(T**5c**G GTC **5c5c**T **5c**CT) (**45**) 3′-d(ATC C**5c**G TT**5c** TGA) (**46**)	75	4.7	–107	–283	–19.5
5′-d(TAGGTC **5b5b**T ACT) (**39**) 3′-d(ATC C**5b**G TT**5b** TGA) (**38**)	68	5.25	–111	–300	–18.0
5′-d(TAGGTC **5c5c**T ACT) (**47**) 3′-d(ATC C**5c**G TT**5c** TGA) (**46**)	67	5.0	–105	–285	–17.0
5′-d(TAGGTC **5b5b**T ACT) (**39**) 3′-d(ATC CAG TTA TGA) (**17**)	58	5.5	–100	–277	–13.8
5′-d(TAGGTC **5c5c**T ACT) (**47**) 3′-d(ATC CAG TTA TGA) (**17**)	56	4.5	–91	–251	–13.4

[a]Measured in 100 mM NaCl, 10 mM MgCl$_2$ and 10 mM Na-cacodylate (pH 7.0) with 5 µM + 5 µM oligonucleotide.
[b]See Table 3.

8-aza-7-deazapurine nucleosides **3b**, **4b** and **5b** and the brominated compounds **3c**, **4c** and **5c** for comparison, which are substituted at exactly the same positions. In oligonucleotide duplexes incorporating either **3b** or **3c**, four duplexes were available for comparison. In one pair the propynyl effect is greater than that of a bromo substituent, in the other case the T_m values are identical so it is not possible to make a clear cut conclusion in this series, incorporating the tridentate base pair related to the dG–dC pair (in high salt buffer). However, when we go to oligonucleotide duplexes incorporating either the propynylated **4b** (motif **XV**) or the brominated **4c** (motif **XVI**) the stabilization of the propynyl group is definitely superior to that of the halogen substituent. It seems to us that a bidentate base pair reacts more sensitively to this change than a tridentate pair. Only due to a hydrogen bond is this pair more

flexible, and the various substituents directed into the major groove can distort the base pair to a much greater extent than that within a tridentate pair. This idea is supported by the data of duplexes incorporating the propynylated compound **5b** (motif **XI**) in comparison to the brominated **5c** (motif **XIV**). Here, an almost identical contribution for the two kinds of residues are observed, which amount to a ΔT_m of +4.5 –6°C in both cases. Thus, the use of either **5b** or **5c** is equally favorable for the stabilization of oligonucleotide duplexes being used in antisense technology or biochips for diagnostic purposes employing hybridization either in solution or on a solid surface.

Oligonucleotide duplexes incorporating base pairs with propynyl residues show rather different CD spectra than their unmodified counterparts (Fig. 2A–C). In particular, in the
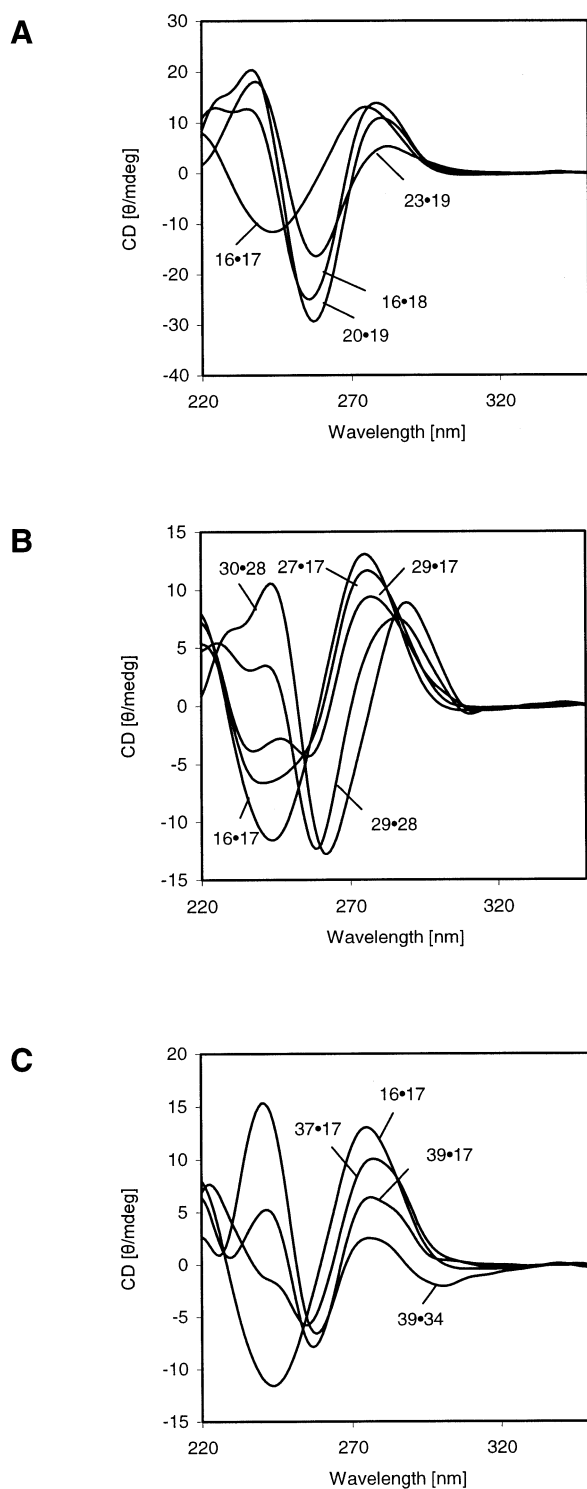
Figure 2. The CD spectra of oligonucleotides containing **1** and **3b** (**A**), **4b** (**B**) and **5b** (**C**). Measured at 10°C in buffers as indicated in Tables 3–8.

of the nucleobases. Thus, water molecules being present in the major groove are expelled, which can effect base pairing and base stacking.

CONCLUSIONS

The synthesis of the phosphoramidites **12** and **15**, together with the already known analogs of dC and dU, made oligonucleotides accessible, incorporating propynyl groups at the pyrimidine and/or the purine bases of the dA–dT and dG–dC base pairs. The propynyl group increases the stability of DNA no matter if this group is linked to the 5-position of a pyrimidine or to the 7-position of a 'purine' (8-aza-7-deazapurine) base. Because of its linear structure and coplanarity towards the heterocyclic base, it increases stacking interactions. It also tends to make the major groove hydrophobic and expels water molecules. The contribution of the propynyl group to the 'dG–dC' versus the 'dA–dT' base pairs shows differences. While the introduction of this group in the dG and dC analogs (**3b** and **1**) causes a similar duplex stabilization (2–3°C for a 12mer duplex), it is less stabilizing in the case of dA and dT residues **4b** and **2**. The propynyl modification causes an extraordinary stabilization when the group is part of the pyrazolo[3,4-*d*]pyrimidine nucleoside **5b**. Although the propynyl group, as well as the bromo substituent, increase the polarizibility of the various nucleobases, significant differences are observed regarding their stabilizing effects. The propynyl group is more stabilizing than a bromo substituent within the bidentate '**4b,c**–dT' base pairs, while they show an almost identical behavior in the tridentate **5b,c**–dT pairs.

ACKNOWLEDGEMENTS

REFERENCES

1. Seela,F. and Becher,G. (2001) Pyrazolo[3,4-*d*]pyrimidine nucleic acids: adjustment of dA–dT to dG–dC base pair stability. *Nucleic Acids Res.*, **29**, 2069–2078.
2. Boudou,V., Kerremans,L., De Bouvere,B., Lescrinier,E., Schepers,G., Busson,R., Van Aerschot,A. and Herdewijn,P. (1999) Base pairing of anhydrohexitol nucleosides with 2,6-diaminopurine, 5-methylcytosine and uracil as base moiety. *Nucleic Acids Res.*, **27**, 1450–1456.
3. Seela,F. and Becher,G. (1998) Synthesis of 7-halogenated 8-aza-7-deaza-2′-deoxy-guanosines and related pyrazolo[3,4-*d*]pyrimidine 2′-deoxyribonucleosides. *Synthesis*, 207–214.
4. Seela,F. and Steker,H. (1986) Synthesis of 2′-deoxyribofuranosides of 8-aza-7-deazaguanine and related pyrazolo[3,4-*d*]pyrimidines. *Helv. Chim. Acta*, **69**, 1602–1613.
5. Seela,F., Winkeler,H.-D., Driller,H. and Menkhoff,S. (1984) Synthesis of 7-deaza-2′-deoxyguanosine by phase-transfer glycosylation and preparation of suitable derivatives for oligonucleotide synthesis. *Nucleic Acids Res.*, **14**, 245–246.
6. Seela,F. and Steker,H. (1985) Facile synthesis of 2′-deoxyribofuranosides of allopurinol and 4-amino-1*H*-pyrazolo-[3,4-*d*]pyrimidine via phase-transfer glycosylation. *Helv. Chim. Acta*, **68**, 563–570.

region around 250 nm rather strong changes are observed. It is not clear whether these changes are due to the hydrophobic character of the propynyl residues or the altered polarizability

7. Seela,F. and Zulauf,M. (1998) Synthesis of 7-alkynylated 8-aza-7-deaza-2′-deoxy-adenosines via the Pd-catalysed cross-coupling reaction. *J. Chem. Soc. [Perkin 1]*, 3233–3239.

8. Haaima,G., Hansen,H.F., Christensen,L., Dahl,O. and Nielsen,P.E. (1997) Increased DNA binding and sequence discrimination of PNA oligomers containing 2,6-diaminopurine. *Nucleic Acids Res.*, **25**, 4639–4643.

9. Sanghvi,Y.S., Hoke,G.D., Freier,S.M., Zounes,M.C., Gonzalez,C., Cummins,L., Sasmor,H. and Cook,P.D. (1993) Antisense oligodeoxynucleotides: synthesis, biophysical and biological evaluation of oligodeoxynucleotides containing modified pyrimidines. *Nucleic Acids Res.*, **21**, 3197–3203.

10. Uhlmann,E., Peyman,A., Ryte,A., Schmidt,A. and Buddecke,E. (2000) Use of minimally modified antisense oligonucleotides for specific inhibition of gene expression. *Methods Enzymol.*, **313**, 268–284.

11. Lamm,G.M., Blencowe,B.J., Sproat,B.S., Iribarren,A.M., Ryder,U. and Lamond,A.I. (1991) Antisense probes containing 2-aminoadenosine allow efficient depletion of U5 snRNP from HeLa splicing extracts. *Nucleic Acids Res.*, **19**, 3193–3197.

12. Bailly,C. and Waring,M.J. (1998) The use of diaminopurine to investigate structural properties of nucleic acids and molecular recognition between ligands and DNA. *Nucleic Acids Res.*, **26**, 4309–4314.

13. Seela,F. and Zulauf,M. (1998) 7-Deazaadenine-DNA: bulky 7-iodo substituents or hydrophobic 7-hexynyl chains are well accommodated in the major groove of oligonucleotide duplexes. *Chem. Eur. J.*, **4**, 1781–1790.

14. Seela,F. and Zulauf,M. (1999) Synthesis of oligonucleotides containing pyrazolo[3,4-*d*]pyrimidines: the influence of 7-substituted 8-aza-7-deazaadenines on the duplex structure and stability. *J. Chem. Soc. [Perkin 1]*, 479–488.

15. Seela,F. and Becher,G. (1999) Oligonucleotides containing pyrazolo-[3,4-*d*]pyrimidines: the influence of 7-substituted 8-aza-7-deaza-2′-deoxyguanosines on the duplex structure and stability. *Helv. Chim. Acta*, **82**, 1640–1655.

16. Seela,F., Becher,G. and Zulauf,M. (1999) 8-Aza-7-deazapurine DNA: synthesis and duplex stability of oligonucleotides containing 7-substituted bases. *Nucl. Nucl.*, **18**, 1399–1400.

17. Becher,G., He,J. and Seela,F. (2001) Major-groove-halogenated DNA: the effects of bromo and iodo substituents replacing H-C(7) of 8-aza-7-deazapurine-2,6-diamine or H-C(5) of uracil residues. *Helv. Chim. Acta*, **84**, 1048–1065.

18. He,J. and Seela,F. (2002) 8-Aza-7-deazapurine-pyrimidine base pairs: the contribution of 2- and 7-substituents to the stability of duplex DNA. *Tetrahedron*, **58**, 4535–4542.

19. Sági,J., Szemzö,A., Ébinger,K., Szabolcs,A., Sági,G., Ruff,É. and Ötvös,L. (1993) Base-modified oligodeoxynucleotides. I. Effect of 5-alkyl, 5-(1-alkenyl) and 5-(1-alkynyl) substitution of the pyrimidines on duplex stability and hydrophobicity. *Tetrahedron Lett.*, **34**, 2191–2194.

20. Barnes,T.W.,III and Turner,D.H. (2001) Long-range cooperativity in molecular recognition of RNA by oligodeoxynucleotides with multiple C5-(1-propynyl) pyrimidines. *J. Am. Chem. Soc.*, **123**, 4107–4118.

21. Barnes,T.W.,III and Turner,D.H. (2001) C5-(1-propynyl)-2′-deoxy-pyrimidines enhance mismatch penalties of DNA:RNA duplex formation. *Biochemistry*, **40**, 12738–12745.

22. Wagner,R.W., Matteucci,M.D., Lewis,J.G., Gutierrez,A.J., Moulds,C. and Froehler,B.C. (1993) Antisense gene inhibition by oligonucleotides containing C-5 propyne pyrimidines. *Science*, **260**, 1510–1513.

23. Froehler,B.C., Wadwani,S., Terhorst,T.J. and Gerrard,S.R. (1992) Oligodeoxynucleotides containing C-5 propyne analogs of 2′-deoxyuridine and 2′-deoxycytidine. *Tetrahedron Lett.*, **33**, 5307–5310.

24. Gutierrez,A.J., Matteucci,M.D., Grant,D., Matsumura,S., Wagner,R.W. and Froehler,B.C. (1997) Antisense gene inhibition by C-5-substituted deoxyuridine-containing oligodeoxynucleotides. *Biochemistry*, **36**, 743–748.

25. Ahmadian,M., Zhang,P. and Bergstrom,D.E. (1998) A comparative study of the thermal stability of oligodeoxyribonucleotides containing 5-substituted 2′-deoxyuridines. *Nucleic Acids Res.*, **26**, 3127–3135.

26. Graham,D., Parkinson,J.A. and Brown,T. (1998) DNA duplexes stabilized by modified monomer residues: synthesis and stability. *J. Chem. Soc. [Perkin 1]*, 1131–1138.

27. Buhr,C.A., Wagner,R.W., Grant,D. and Froehler,B.C. (1996) Oligodeoxynucleotides containing C-7 propyne analogs of 7-deaza-2′-deoxyguanosine and 7-deaza-2′-deoxyadenosine. *Nucleic Acids Res.*, **24**, 2974–2980.

28. Catalanotti,B., Galeone,A., Gomez-Paloma,L., Mayol,L. and Pepe,A. (2000) 2′-deoxy-8-(propyn-1-yl)adenosine-containing oligonucleotides: effects on stability of duplex and quadruplex structures. *Bioorg. Med. Chem. Lett.*, **10**, 2005–2009.

29. McDowell,J.A. and Turner,D.H. (1996) Investigation of the structural basis for thermodynamic stabilities of tandem GU mismatches: solution structure of (rGAGGUCUC)$_2$ by two-dimensional NMR and simulated annealing. *Biochemistry*, **35**, 14077–14089.

30. Seela,F., Ramzaeva,N., Leonard,P., Chen,Y., Debelak,H., Feiling,E., Kröschel,R., Zulauf,M., Wenzel,T., Fröhlich,T. and Kostrzewa,M. (2001) Phosphoramidites and oligonucleotides containing 7-deazapurines and pyrimidines carrying aminopropargyl side chains. *Nucleosides Nucleotides Nucleic Acids*, **20**, 1421–1424.

31. Hobbs,F.W. (1989) Palladium-catalyzed synthesis of alkynylamino nucleosides. A universal linker for nucleic acids. *J. Org. Chem.*, **54**, 3420–3422.

32. Robins,M.J., Vinayak,R.S. and Wood,S.G. (1990) Solvent, not palladium oxidation state, is the primary determinant for successful coupling of terminal alkynes with iodo-nucleosides. *Tetrahedron Lett.*, **31**, 3731–3734.

33. Korshun,V.A., Manasova,E.V. and Berlin,Yu.A. (1997) Alkynylated nucleosides and their analogues. I. Methods of synthesis. *Russian J. Bioorg. Chem.*, **23**, 300–362.

34. Ti,G.S., Gaffney,B.L. and Jones,R.A. (1982) Transient protection: efficient one-flask syntheses of protected deoxynucleosides. *J. Am. Chem. Soc.*, **104**, 1316–1319.

35. *User Manual of the DNA Synthesizer.* Applied Biosystems, Weiterstadt, Germany.

36. Seela,F. and Zulauf,M. (1999) Oligonucleotides containing 7-deazaadenines: the influence of the 7-substituent chain length and charge on the duplex stability. *Helv. Chim. Acta*, **82**, 1878–1898.

37. Ramzaeva,N., Mittelbach,C. and Seela,F. (1997) 7-Deazaguanine DNA: oligonucleotides with hydrophobic or cationic side chains. *Helv. Chim. Acta*, **80**, 1809–1822.