Modification of DNA with Octadiynyl Side Chains: Synthesis, Base Pairing, and Formation of Fluorescent Coumarin Dye Conjugates of Four Nucleobases by the Alkyne−Azide "Click" Reaction

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Oligonucleotides incorporating 5-(octa-1,7-diynyl)-2′-deoxycytidine **1a**, 5-(octa-1,7-diynyl)-2′-deoxyuridine **2a** and 7-deaza-7-(octa-1,7-diynyl)-2′-deoxyguanosine **3a**, 7-deaza-7-(octa-1,7-diynyl)-2′-deoxyadenosine **4a** were prepared. For this, the phosphoramidites **7**, **10**, and **13** were synthesized and employed in solid-phase oligonucleotide synthesis. The octa-1,7-diynyl nucleosides **1a**−**4a** were obtained from their corresponding iodo derivatives using the palladium-assisted Sonogashira cross-coupling reaction. The T_m values demonstrated that DNA duplexes containing octa-1,7-diynyl nucleosides show a positive influence on the DNA duplex stability when they are introduced at the 5-position of pyrimidines or at the 7-position of 7-deazapurines. The terminal alkyne residue of oligonucleotides were selectively conjugated to the azide residue of the nonfluorescent 3-azido-7-hydroxycoumarin (**38**) using the protocol of copper(I)-catalyzed [3 + 2] Huisgen−Sharpless−Meldal cycloaddition "click chemistry" resulting in the formation of strongly fluorescent 1,2,3-triazole conjugates. The fluorescence properties of oligonucleotides with covalently linked coumarin−nucleobase assemblies were investigated. Among the four modified bases, the 7-deazapurines show stronger fluorescence quenching than that of pyrimidines.

INTRODUCTION

The chemical modification of DNA of defined sequence is an invaluable tool to study DNA structure and recognition. Modified DNAs find applications as therapeutic and diagnostic agents. Areas of antisense and antigene therapy (*1–3*) or DNA detection and sequencing (*4, 5*) are studied intensively; new fields such as DNA-based nanotechnology are emerging (*6–9*). The most common DNA modification sites are the sugar moieties, the phosphodiester backbone, and the nucleobases. Minimal changes of the DNA-duplex structure by nucleoside-shaped mimics (*10–13*) are the prerequisites for a modified DNA to behave as the parent molecule. However, in a number of cases, larger residues, e.g., dye reporters, have to be introduced that can alter the structure of DNA. The 5-position of the pyrimidine bases (*14–17*) and 7-position of 7-deazapurines (*18, 19*) are "ideal" sites for DNA modifications (purine numbering is used throughout the discussion). Such groups are well-accommodated in the DNA major groove of the B-DNA duplex not disturbing the DNA helical structure. The introduction of substituents of moderate size–7-substituents into 7-deazapurines or 5-substituents into pyrimidines–can even cause duplex stabilization when compared to the canonical DNA constituents. These residues strengthen the base pairs as was shown for halogeno or alkynyl groups (*20–25*). A series of such modified nucleosides containing propynyl groups (**1b**−**4b**) have been synthesized and incorporated into DNA (*26–32*).

Recently, the copper(I)-catalyzed Huisgen−Sharpless−Meldal alkyne−azide 1,3-dipolar cycloaddition reaction has gained significant importance because of its applications in organic synthesis, molecular biology, and materials science (*33, 34*). The high potential energy of the azide and alkyne components make this a thermodynamically favored reaction ($\Delta H° = -45$ to −55 kcal/mol). Although both azides and alkynes are highly reactive, they are orthogonal to a broad range of reagents, solvents, and other functional groups. Copper(I) dramatically enhances the reaction rate of azides with terminal alkynes leading to the formation of stable regiospecific 1,4-disubstituted-[1,2,3]-triazoles. To apply this chemistry to the four building blocks of DNA, nucleosides bearing the octa-(1,7-diynyl) group at the 5-position of pyrimidine bases (**1a**, **2a**) or at the 7-position of 7-deazapurines (**3a**, **4a**) were synthesized (Scheme 1).

In a recent Communication, we reported on the synthesis and properties of 5-(octa-1,7-diynyl)-2′-deoxyuridines and performed the "click" reaction with AZT or aromatic azides (*35*); we outlined the principle for dG, dC, and dT modification (*36*). Now, we present the syntheses of the three building blocks related to dC, dG, and dA in order to complete a set of canonical DNA building blocks bearing octadiynyl side chains and report on their duplex stability. They all contain the same Watson–Crick recognition sites as those of canonical DNA. For dye labeling, we introduce a nonfluorescent coumarin reporter molecule for the above-mentioned octadiynyl derivatives, which becomes fluorescent after the "click" reaction (Scheme 2). These reporter groups occupy well-defined positions within the DNA helix, without disturbing the native DNA structure (*35–38*). On the basis of this principle of the 1,3-dipolar cycloaddition, we performed the "click" reaction on oligonucleotides containing nucleoside residues bearing side chains with terminal triple bonds (**1a**−**4a**) with the nonfluorescent coumarin azide **38** to generate strongly fluorescent 1,2,3-triazole coumarin conjugates. The dye conjugates containing the 7-deazapurines show a strong photoinduced intramolecular electron transfer between the coumarin dye and the 7-deazapurine system, which is almost negligible in the case of pyrimidines. A general outcome of the work is the design of the four DNA building blocks **1a**−**4a** showing the same recognition pattern as the canonical DNA constituents; they can be labeled with almost any reporter group

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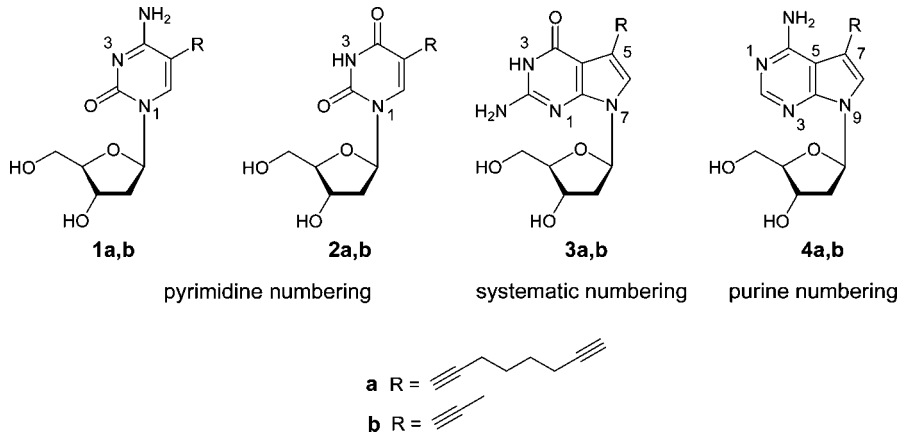
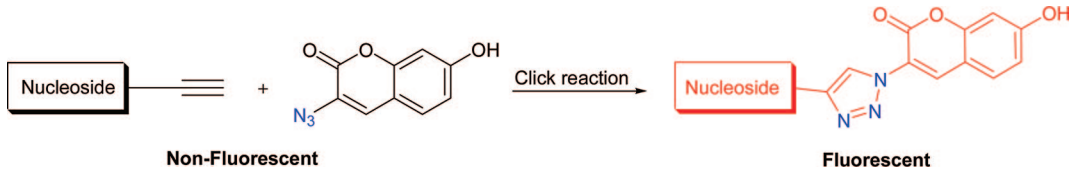
Scheme 1



Scheme 2. Huisgen−Sharpless−Meldal Cycloaddition of Terminal Alkyne with Azide Generating a Fluorescent 1,4-Disubstituted 1,2,3-Triazole Product



or an otherwise active moiety leading to the structurally "undisturbed" DNA duplexes.

EXPERIMENTAL SECTION

General. All chemicals were purchased from Acros, Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany). Solvents were of technical grade and freshly distilled before use. Thin layer chromatography (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 fraction collector (LKB Instruments, Sweden). UV spectra: U-3200 spectrometer (Hitachi, Tokyo, Japan); λ_{max} (ϵ) in nm. NMR spectra: Avance-DPX-250 or AMX-500 spectrometers (Bruker, Karlsruhe, Germany) at 250.13 MHz for ^{1}H and ^{13}C; chemical shifts (δ) are in ppm relative to Me_4Si as internal standard or external 85% H_3PO_4 for ^{31}P; J values in Hz. Elemental analyses were performed by Mikroanalytisches Laboratorium Beller (Göttingen, Germany). Electron spray ionization (ESI) MS for the nucleosides: Bruker-Daltonics-MicroTOF spectrometer with loop injection (Bremen, Germany). The molecular masses of the oligonucleotides were determined by MALDI-TOF-MS with a Biflex-III mass spectrometer (Bruker Saxonia, Leipzig, Germany) and Applied Biosystems Voyager DE PRO with 3-hydroxypicolinic acid (3-HPA) as a matrix. The detected masses were identical to the calculated values. The fluorescence measurements were performed in bidistilled water at 20 °C. Fluorescence spectra were recorded in the wavelength range between 320 and 600 nm using the fluorescence spectrophotometer F-2500 (Hitachi, Tokyo, Japan).

Synthesis, Purification, and Characterization of the Oligonucleotides. The oligonucleotide synthesis was performed on a DNA synthesizer, model 392–08 (Applied Biosystems, Weiterstadt, Germany) at 1 μmol scale using the phosphoramidites **7**, **10**, and **13** following the synthesis protocol for 3′-(2-cyanoethyl phosphoramidites) (user's manual for the 392 DNA synthesizer, Applied Biosystems, Weiterstadt, Germany). The coupling efficiency was always higher than 95%. After cleavage from the solid support, the oligonucleotides were deprotected in 25% aqueous NH_3 solution for 14–16 h at 60 °C; during this process, the amine protecting groups of the nucleobases—adenine, cytosine with tBPA (4-tert-butylphenoxy)acetyl) protecting group, and guanine, **4a** with iB (isobutyryl)—were removed. The purification of 5′-dimethoxytrityl oligomers was carried out on reversed-phase HPLC (Merck-Hitachi-HPLC; RP-18 column; 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; flow rate 1.0 mL/min. The purified "trityl-on" oligonucleotides were 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 again by reversed-phase HPLC (gradient: 0–20 min 0–20% B in A; flow rate 1 mL/min). The oligomers were desalted on a short column (RP-18, silica gel) and lyophilized on a Speed-Vac evaporator to yield colorless solids which were frozen at −24 °C.

Melting curves were measured with a Cary-1/3 UV/vis spectrophotometer (Varian, Australia) equipped with a Cary thermoelectrical controller. The temperature 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. The Cary 100 Bio UV–vis spectrophotometer was used for the UV-melting curves of oligonucleotides containing **4a** with a heating rate of 1 °C.

The enzymatic hydrolysis of the oligonucleotides containing **1a** and **3a** was performed as described (*9*) 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) at 37 °C, which was analyzed by reversed-phase HPLC (RP-18, at 260 nm) showing the peaks of the modified and unmodified nucleosides (Figure 1a,b). Quantification of the constituents was made on the basis of the peak areas, which were divided by the extinction coefficients ϵ_{260} of the nucleosides: dA 15 400 (H_2O), dC 7300 (H_2O), dG 11 700 (H_2O), dT 8800 (H_2O), **1a** 4900 (MeOH), **3a** 7300 (MeOH). For the fluorescence quenching experiments, the enzymatic hydrolysis was performed using 1.7 μM single-stranded oligonucleotide dissolved in 1 mL of 0.1 M Tris−HCl buffer (pH 8.3). To this solution, 3–10 μL of snake venom phosphodiesterase and 3–10

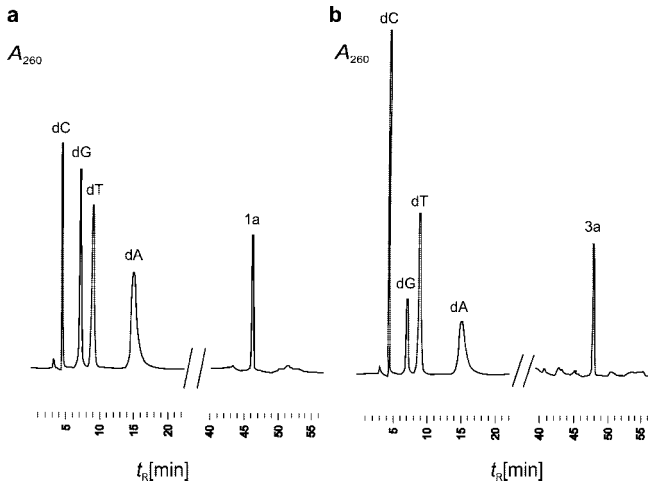


Figure 1. HPLC profiles of the enzymatic hydrolysis products (a) of the oligonucleotide 5′-d(TAG GT**1a** AAT ACT); (b) of oligonucleotide 5′-d(A**3a**T ATT GAC CTA) obtained by digestion with snake venom phosphodiesterase and alkaline phosphatase in 0.1 M Tris−HCl buffer (pH 8.3) at 37 °C. Gradient for (a) 25 min 100% A, 25–60 min 0–50% B in A; (b) 25 min 100% A, 25–60 min 0–60% B in A; flow rate 0.7 mL/min (A = 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN 95:5, B = MeCN).

μL alkaline phosphatase were added and the fluorescence spectra measured at different time intervals with an emission 479 nm.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-(octa-1,7-diynyl)-cytosine (1a). To a suspension of 5-iodo-2′-deoxycytidine (0.6 g, 1.7 mmol) and CuI (65.5 mg, 0.34 mmol) in anhydrous DMF (9 mL) was added successively [$Pd(PPh_3)_4$] (196 mg, 0.17 mmol), anhydrous Et_3N (344 mg, 3.4 mmol), and octa-1,7-diyne (1.8 g, 17.0 mmol). The mixture was stirred at room temperature under argon atmosphere and allowed to proceed until the starting material was consumed (TLC monitoring). The reaction mixture was diluted with MeOH/CH_2Cl_2 1:1 (20 mL), and Dowex 1 × 8 (100–200 mesh; 0.6 g, hydrogen carbonate form) was introduced. After additional stirring for 1 h, the mixture was filtered and the resin washed with MeOH/CH_2Cl_2 1:1 (30 mL). The combined filtrate was concentrated and the residue purified by FC (silica gel, column 15 × 3 cm, CH_2Cl_2/MeOH 96:4): **1a** (0.42 g, 75%). Colorless amorphous solid. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.40. UV λ_{max} (MeOH)/nm (ϵ/dm^3 mol^{-1} cm^{-1}) 236 (15900), 299 (7200). ^{1}H NMR ((D$_6$) DMSO): 1.54–1.62 (m, 4 H, 2CH$_2$), 2.0–2.15 (m, 3 H, H$_\alpha$-C(2′), CH$_2$), 2.22–2.44 (m, 3 H, H$_\beta$-C(2′), CH$_2$), 2.76 (s, 1 H, C≡CH), 3.54–3.62 (m, 2 H-C(5′)), 3.79 (m, H-C(4′)), 4.20 (m, H-C(3′)), 5.04 (t, J = 4.96, OH-C(5′)), 5.20 (d, J = 4.1, OH-C(3′)), 6.12 (t, J = 6.5, H-C(1′)), 6.71 (s, NH$_a$), 7.67 (s, NH$_b$), 8.07 (s, H-C(6)). Anal. Calcd for $C_{17}H_{21}N_3O_4$ (331.15): C 61.62, H 6.39, N 12.68. Found: C 61.44, H 6.77, N 12.45.

2-Amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-(octa-1,7-diynyl)-3,7-dihydro-4-*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (3a). As described for **1a**, with 7-deaza-7-iodo-2′-deoxyguanosine (0.7 g, 1.78 mmol) and CuI (68 mg, 0.36 mmol) in anhydrous DMF (10 mL) were added successively [$Pd(PPh_3)_4$] (208 mg, 0.18 mmol), anhydrous Et_3N (360 mg, 3.56 mmol), and octa-1,7-diyne (1.89 g, 17.8 mmol). FC (silica gel, column 15 × 3 cm, CH_2Cl_2/MeOH 94:6), afforded **3a** (0.47 g, 71%) as a colorless amorphous solid. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.19. UV λ_{max} (MeOH)/nm (ϵ/dm^3 mol^{-1} cm^{-1}) 238 (22600), 273 (10600), 290 (8800). ^{1}H NMR ((D$_6$) DMSO): 1.46 (m, 4 H, 2 CH$_2$), 1.95–2.18 (m, 3 H, H$_\alpha$-C(2′), CH$_2$), 2.25–2.38 (m, 3 H, H$_\beta$-C(2′), CH$_2$), 2.64 (s, 1 H, C≡CH), 3.28 (m, 2 H-C(5′)), 3.62 (m, H-C(4′)), 4.14 (m, H-C(3′)), 4.81 (t, OH-C(5′)), 5.11 (d, OH-C(3′)), 6.14 (t, J = 6.3 Hz, H-C(1′)), 6.20 (s, NH$_2$), 7.01 (s, H-C(8)), 10.31 (s, NH). ESI-HR-MS: 393.15 ([M + Na])$^+$, $C_{19}H_{22}N_4O_4^+$; calcd 370.16.

1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-5-(octa-1,7-diynyl)cytosine (5). Compound **1a** (0.5 g 1.5 mmol) was dried by repeated coevaporation with anhydrous pyridine (2 × 5 mL) before dissolving in anhydrous pyridine (10 mL). Then, 4,4′-dimethoxytrityl chloride (0.61 g, 1.80 mmol) was added in three portions to the remaining solution at room temperature under stirring for 6 h. Thereupon, MeOH (2 mL) was added, and the mixture was stirred for another 30 min. The reaction mixture was dissolved in CH_2Cl_2 (2 × 50 mL) and extracted with 5% aqueous $NaHCO_3$ solution (100 mL) followed by H_2O (80 mL), dried over Na_2SO_4, and then concentrated. Purification by FC (silica gel, column 15 × 3 cm, CH_2Cl_2/acetone 70:30) gave a colorless foam of **5** (0.82 g, 86%). TLC (CH_2Cl_2/MeOH 95:5): R_f 0.25. UV λ_{max} (MeOH)/nm (ϵ/dm^3 mol^{-1} cm^{-1}) 235 (32000), 83 (6500), 299 (6600). ^{1}H NMR ((D$_6$) DMSO): 1.39 (m, 2 CH$_2$), 2.06–2.26 (m, 6 H, H-C(2′)), 2 CH$_2$), 2.74 (s, 1 H, C≡CH), 3.10–3.23 (m, 2 H-C(5′)), 3.73 (s, 6 H, 2 MeO), 3.92 (m, H-C(4′)), 4.27 (m, H-C(3′)), 5.33 (d, J = 3.9, OH-C(3′)), 6.12 (t, J = 6.5, H-C(1′)), 6.76 (s, NH$_a$), 6.86–7.42 (m, H-arom), 7.71 (s, NH$_b$), 7.89 (s, H-C(6)). Anal. Calcd for $C_{38}H_{39}N_3O_6$ (633.28): C 72.02, H 6.20, N 6.63. Found: C 71.86, H 6.20, N 6.58.

N^4-Acetyl-1-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-5-(octa-1,7-diynyl)cytosine (6). To a suspension of compound **5** (200 mg, 0.32 mmol) in *N,N*-dimethylformamide (2 mL) was added acetic anhydride (36 μL, 0.38 mmol) and the reaction mixture stirred at room temperature for 20 h. After evaporation of DMF under reduced pressure, the residue was applied to FC (silica gel, column 15 × 3 cm, CH_2Cl_2/acetone 80:20). Compound **6** was isolated as colorless foam (0.18 g, 84%). TLC (CH_2Cl_2/MeOH 95:5): R_f 0.73. UV λ_{max} (MeOH)/nm (ϵ/dm^3 mol^{-1} cm^{-1}) 236 (40000), 282 (4200), 317 (5300). ^{1}H NMR ((D$_6$) DMSO): 1.42 (m, 2 CH$_2$), 2.10–2.17 (m, 2 H-C(2′)), 2.22 (s, 3 H, COMe), 2.36 (m, 2 CH$_2$), 2.77 (s, 1 H, C≡CH), 3.20 (m, 2 H-C(5′)), 3.75 (s, 6 H, 2-MeO), 4.0 (m, H-C(4′)), 4.3 (m, H-C(3′)), 5.39 (d, J = 4.0, OH-C(3′)), 6.10 (t, J = 6.2, H-C(1′)), 6.89–7.44 (m, H-arom), 8.24 (s, H-C(6)), 9.24 (s, NH). Anal. Calcd for $C_{40}H_{41}N_3O_7$ (675.29): C 71.09, H 6.12, N 6.22. Found: C 71.15, H 6.20, N 6.35.

N^4-Acetyl-1-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-5-(octa-1,7-diynyl)cytosine 3′-(2-Cyanoethyl)-*N,N*-diisopropylphosphoramidite (7). A stirred solution of **6** (250 mg, 0.37 mmol) in anhydrous CH_2Cl_2 (5 mL) was preflushed with argon and treated with (i-Pr)$_2$EtN (100 μL, 0.58 mmol) followed by 2-cyanoethyl-*N,N*-diisopropylphosphoramidochloridite (130 μL, 0.60 mmol). After stirring for 45 min at room temperature, the solution was diluted with CH_2Cl_2 (30 mL) and extracted with 5% aqueous $NaHCO_3$ solution (20 mL). The organic layer was dried over Na_2SO_4 and concentrated. FC (silica gel, column 10 × 2 cm, CH_2Cl_2/acetone 95:5) gave **7** (260 mg, 80%). Colorless foam. TLC (CH_2Cl_2/acetone 95:5): R_f 0.74. ^{31}P NMR ($CDCl_3$): 150.25, 149.6.

7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-(octa-1,7-diynyl)-3,7-dihydro-2-[(*N,N*-dimethylamino)methylidene]amino-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (8). To a solution of **3a** (400 mg, 1.08 mmol) in MeOH (12 mL) was added *N,N*-dimethylformamide dimethylacetal (425 μL, 3.2 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 95:5). Compound **8** was isolated as a colorless solid (335 mg, 73%). TLC (CH_2Cl_2/MeOH, 9:1): R_f 0.31. UV λ_{max} (MeOH)/nm (ϵ/dm^3 mol^{-1} cm^{-1}) 232 (17000), 253 (24000), 316 (18000). ^{1}H NMR ((D$_6$) DMSO): 1.60 (m, 2 CH$_2$), 2.20 (m, 3 H, H$_\alpha$-C(2′), CH$_2$), 2.39 (m, 3 H, H$_\beta$-C(2′), CH$_2$), 2.77 (s, 1 H, C≡CH), 3.01 (s, 3 H, NCH$_3$),

3.14 (s, 3 H, NCH_3), 3.50 (m, 2 H-C(5′)), 3.76 (m, H-C(4′)), 4.30 (m, H-C(3′)), 4.91 (t, OH-C(5′)), 5.25 (d, OH-C(3′)), 6.41 (t, J = 6.3, H-C(1′)), 7.27 (s, H-C(8)), 8.54 (s, H, N=CH), 11.05 (s, NH). Anal. Calcd for $C_{22}H_{27}N_5O_4$ (425.21): C 62.10, H 6.40, N 16.46. Found: C 62.14, H 6.50, N 16.34.

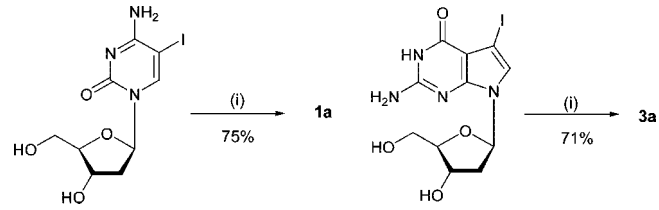
7-(2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)-5-(octa-1,7-diynyl)-3,7-dihydro-2-[(*N,N*-dimethylamino)methylidene]amino-4 *H*-pyrrolo-[2,3-*d*]pyrimidin-4-one (9). As described for **5**, compound **8** (0.35 g 0.82 mmol) in anhydrous pyridine (6 mL) was treated with 4,4′-dimethoxytrityl chloride (370 mg, 1.09 mmol). Purification by FC (silica gel, column 15 × 3 cm, CH_2Cl_2/acetone, 1:1) gave a colorless foam of **9** (0.45 g, 75%). TLC (CH_2Cl_2/MeOH 97:3): (B): R_f 0.25: UV λ_{max} (MeOH)/nm (ϵ/dm^3 mol^{-1} cm^{-1}) 232 (45000), 279 (16000), 296 (15000). ^{1}H NMR ((D$_6$) DMSO): 1.61 (m, 2 CH_2), 2.09–2.19 (m, 3 H, H$_\alpha$-C(2′), CH_2), 2.40 (m, 3 H, H$_\beta$-C(2′), CH_2), 2.77 (s, 1 H, C≡CH), 3.02 (s, 3 H, NCH_3), 3.15 (s, 3 H, NCH_3), 3.74 (s, 6 H, 2-MeO), 3.90 (m, H-C(4′)), 4.31 (m, H-C(3′)), 5.33 (d, OH-C(3′)), 6.46 (t, J = 6.52, H-C(1′)), 6.85–7.32 (m, H-arom), 7.4 (s, H-C(8)), 8.57 (s, H, N=CH), 11.09 (s, NH). Anal. Calcd for $C_{43}H_{45}N_5O_6$ (727.34): C 70.96, H 6.23, N 9.62. Found: C 71.08, H 6.26, N 9.70.

7-(2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)-5-(octa-1,7-diynyl)-3,7-dihydro-2-[(*N,N*-dimethylamino)methylidene]amino-4-*H*-pyrrolo-[2,3-*d*]pyrimidin-4-one 3′-(2-Cyanoethyl)-*N,N*-diisopropylphosphoramidite (10). As described for **7**, with **9** (200 mg, 0.27 mmol), (i-Pr)$_2$EtN (72 μL, 0.42 mmol), and 2-cyanoethyl-*N,N*-diisopropylphosphoramidochloridite (103 μL, 0.47 mmol). FC (silica gel, 10 × 2 cm, solvent system, CH_2Cl_2/acetone 93:7) gave a colorless foam of **10** (160 mg, 62%). TLC (CH_2Cl_2/acetone 90:10): R_f 0.68. ^{31}P NMR ($CDCl_3$): 149.95, 149.65.

7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-4-(iso-butyrylamino)-5-(octa-1,7-diynyl)-7-*H*-pyrrolo[2,3-*d*]pyrimidine (11). To a solution of compound **4a** (*19*) (248 mg, 0.7 mmol) in anhydrous pyridine (4 mL) was added Me_3SiCl (910 μL, 7.14 mmol) and stirred at room temperature. After 45 min, the isobutyric anhydride (770 mg, 4.9 mmol) was introduced, and the solution stirred for another 2 h. The mixture was cooled to 0 °C, diluted with H_2O (3 mL), and stirred for 10 min. After the addition of 12% aq NH_3 (3 mL), stirring was continued for 1 h at room temperature. The solution was evaporated, and the residue was applied to FC (silica gel, column 10 × 3 cm, CH_2Cl_2/MeOH 95:5). Compound **11** was isolated as a colorless solid (210 mg, 71%). TLC (CH_2Cl_2/MeOH, 90:10): R_f 0.64. UV λ_{max} (MeOH)/nm (ϵ/dm^3 mol^{-1} cm^{-1}) 213 (19000), 241 (26000), 305 (7000). ^{1}H NMR ((D$_6$) DMSO): 1.17 (s, 2 CH_3), 1.60 (m, 2 CH_2), 2.20 (m, 3 H, H$_\alpha$-C(2′), CH_2), 2.38–2.58 (m, 3 H, H$_\beta$-C(2′), CH_2), 2.77 (s, 1 H, C≡CH), 2.88 (m, 1 H, CH), 3.50 (m, 2 H-C(5′)), 3.83 (m, H-C(4′)), 4.35 (m, H-C(3′)), 4.99 (t, J = 4.87, OH-C(5′)), 5.30 (d, OH-C(3′)), 6.61 (t, J = 6.7, H-C(1′)), 7.98 (s, H-C(6)), 8.59 (s, 1 H, H-C(2)), 9.96 (s, 1H, NH). Anal. Calcd for $C_{23}H_{28}N_4O_4$ (424.21): C 65.08, H 6.65, N 13.20. Found: C 65.22, H 6.70, N 13.11.

7-(2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)-4-(isobutyrylamino)-5-(octa-1,7-diynyl)-7-*H*-pyrrolo-[2,3-*d*]pyrimidine (12). As described for **5**, compound **11** (250 mg 0.59 mmol) in anhydrous pyridine (5 mL) was treated with 4,4′-dimethoxytrityl chloride (275 mg, 0.81 mmol). Purification by FC (silica gel, column 12 × 3 cm, CH_2Cl_2/acetone, 7:3) gave a colorless foam of **12** (365 mg, 85%). TLC (CH_2Cl_2/MeOH 95:5) R_f 0.33. UV λ_{max} (MeOH)/nm (ϵ/dm^3 mol^{-1} cm^{-1}) 214 (36400), 237 (42000), 275 (9400). ^{1}H NMR ((D$_6$) DMSO): 1.17 (s, 2 CH_3), 1.58 (m, 2 CH_2), 2.14–2.41 (m, 6 H, H-C(2′), 2 CH_2), 2.77 (s, 1 H, C≡CH), 2.88 (m, 1 H, CH), 3.2 (m, 2 H-C(5′)), 3.72 (s, 6 H, 2-MeO), 3.96 (m, 1 H-C(4′)), 4.39 (m, H-C(3′)), 5.37 (d, J = 4.20, OH-C(3′)), 6.62 (t, J = 6.7, H-C(1′)), 6.82 (m, 4 H, H-arom), 7.20–7.38 (m, 9 H, H-arom), 7.86 (s, 1 H, H-C(6)), 8.57 (s, 1 H, H-C(2)), 9.96 (s, 1 H, NH). Anal. Calcd for $C_{44}H_{46}N_4O_6$ (726.34): C 72.71, H 6.38, N 7.71. Found: C 72.82, H 6.48, N 7.70.

Scheme 3[a]



[a] (i) [Pd^0(PPh$_3$)$_4$], CuI, Et$_3$N, DMF, octa-1,7-diyne, rt.

7-(2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)-4-(isobutyrylamino)-5-(octa-1,7-diynyl)-7-*H*-pyrrolo-[2,3-*d*]pyrimidine-3′-(2-cyanoethyl)-*N,N*-diisopropylphosphoramidite (13). As described for **7**, with **12** (300 mg, 0.41 mmol), (i-Pr)$_2$EtN (150 μL, 0.87 mmol), and 2-cyanoethyl-*N,N*-diisopropylphosphoramidochloridite (210 μL, 0.96 mmol). FC (silica gel, 10 × 2 cm, solvent system, CH_2Cl_2/acetone 94:6) gave colorless foam of **13** (230 mg, 60%). TLC (CH_2Cl_2/acetone 90:10): R_f 0.70. ^{31}P NMR ($CDCl_3$): 149.97, 149.81.

Huisgen−Sharpless−Meldal [3 + 2] Cycloaddition Performed in Aqueous Solution for Oligonucleotides 23, 30, 34, and 42 with the Nonfluorescent Coumarin Azide 38. To the single-stranded oligonucleotide (5 A_{260} units), $CuSO_4$−TBTA ligand complex (35 μL of a 20 mM stock solution in *t*-BuOH/H_2O 1: 9), tris(carboxyethyl)phosphine (TCEP) (35 μL of a 20 mM stock solution in water), 3-azido-7-hydroxycoumarin (**38**; 50 μL of a 20 mM stock solution in dioxane/H_2O 1: 1), and 35 μL of DMSO were added, and the reaction was run at room temperature for 15–20 h. The "click" products **39**, **40**, **41**, and **43** were further purified by reversed-phase HPLC in the trityl-off modus (see above). The molecular masses of the oligonucleotides were determined by MALDI-TOF spectroscopy.

RESULTS AND DISCUSSION

Synthesis and Physical Properties of Monomers. The nucleosides **1a** and **3a** were synthesized from the corresponding 5-iodo 2′-deoxycytidine or 7-deaza-7-iodo-2′-deoxyguanosine (*23*) employing the Sonogashira cross-coupling reaction. The reaction was carried out in dry DMF in the presence of triethylamine using the palladium catalyst Pd^0(PPh$_3$)$_4$ and CuI. An excess amount of octa-1,7-diyne was used for the efficient cross-coupling reaction (*20*), leading to the exclusive formation of the monofunctionalized octa-1,7-diynyl nucleosides **1a** and **3a** in 75% and 71% yield, respectively (Scheme 3). The synthesis of the corresponding c^{7}dA$_d$ (**4a**) and 2′-deoxyuridine (**2a**) nucleosides were already reported (*20, 35*). Reaction at the second terminal C≡C bond can be accomplished when compounds **1a**−**4a** are isolated and employed as intermediates in a second cross-coupling reaction to form bis-nucleosides (*7*). The structure of the isolated nucleosides was confirmed on the basis of ^{1}H and ^{13}C NMR spectroscopy as well as by elemental analysis or mass spectra. The characteristic signal for the terminal C≡C proton at δ(H) 2.76 (for **1a**) and 2.64 (for **3a**) reveals the formation of 5- or 7-octa-1,7-diynylated nucleosides. The chemical shifts and the terminal triple bonds of the nucleosides can be clearly identified on the basis of coupling pattern taken from the gated-decoupled ^{1}H/^{13}C NMR spectra. The side chain carbon atom of terminal C≡C of **1a** showed a C, H coupling (^1J(C,H) = 250 Hz).

The physical and biological properties of oligonucleotides can be influenced by introducing various alkyne or diyne

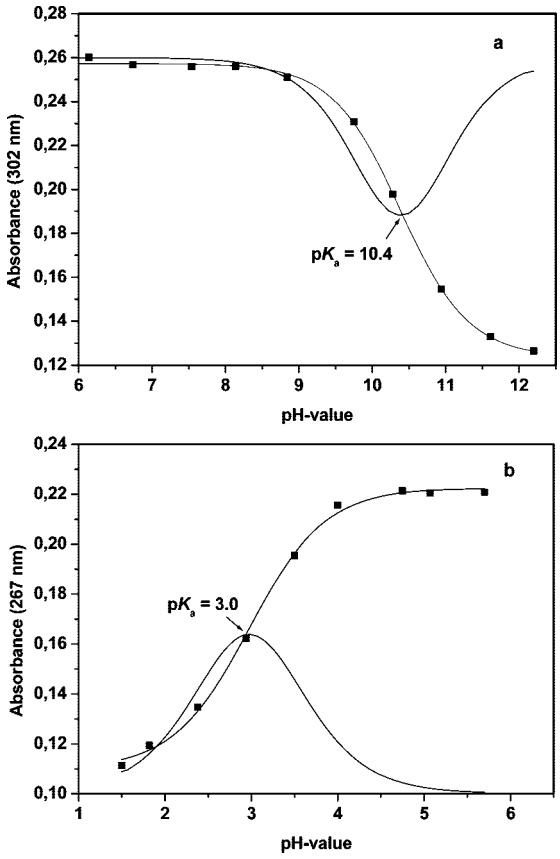


Figure 2. UV spectra of compounds **3a** and **1a** measured in phosphate buffer (7.8 g $NaH_2PO_4 \cdot H_2O$ in 500 mL H_2O). Absorbance (a) of compound **3a** as a function of pH values measured at 302 nm from pH 1.5 to 12.5 and (b) of compound **1a** at 267 nm.

substituents at the 5-position of pyrimidine or at the 7-position of 7-deazapurine nucleosides. To evaluate the impact of these side chain units on the hydrophobicity, the log *P* values are calculated and compared for the heterocyclic bases with the help of *ACD/log P 1.0* program. For the natural bases, the log *P* values are negative, which reflects the hydrophilicity of these compounds. The maximum log *P* value is observed for the octa-1,7-diynyl nucleosides (1.23 ± 0.75 for **1a**, 1.51 ± 1.30 for **3a**, 2.69 ± 1.43 for **4a**) when compared with the propynylated nucleosides (−0.24 ± 0.75 for **1b**, 0.03 ± 1.29 for **3b**, 1.22 ± 1.42 for **4b**). Moreover, the presence of long-chain hydrophobic substituents enhances the polarizability, $\alpha_m/10^{-24}$ cm^3; the calculated data (*ACD/ChemSketch 2.51*) are of the nucleobases (25.36 for **1a**, 29.22 for **3a**, and 27.87 for **4a**). These values are higher when compared with the propynylated nucleobases (16.23 for **1b**, 20.08 for **3b**, and 19.56 for **4b**).

In order to understand the relative base-pairing strength of DNA duplexes, it is necessary to know the pK_a values of the monomeric donor and acceptor residues involved in hydrogen bonding (*39*). The effect of octa-1,7-diynyl residue on the pyrrolo[2,3-*d*]pyrimidine as well as on the pyrimidine system was studied. The pK_a values were determined by spectrophotometric titration (*40*) (pH 1.5 to 11.50) at 220–350 nm (see Figure 2). The octa-1,7-diynyl group of **1a** (pK_a = 3.0), **2a** (pK_a = 8.7), **3a** (pK_a = 10.4), and **4a** (pK_a = 4.4) does not change the pK_a value significantly when compared with the propynylated nucleosides (3.3 for **1b**, 8.7 for **2b**, 10.2 for **3b**, and 4.3 for **4b**). The pK_a values of the natural pyrimidine 2′-deoxyribonucleosides dC (pK_a = 4.2) and dT (pK_a = 9.3) and purine 2′-deoxyribonucleosides dG (pK_a = 9.2) and dA (pK_a = 3.8) (*41*) are significantly different when compared to the octadiynylated nucleosides. The octa-1,7-diynyl side chains of pyrimidine 2′-deoxyribonucleosides make them more acidic than the parent compounds and have an impact on the base pair stability and base discrimination. Moreover, the octadiynyl side-chain containing nucleosides show rather different UV spectra and extinction coefficients than those of natural nucleosides (Figure 3a−d and Table 1).

Phosphoramidite Synthesis. In order to investigate the properties of oligonucleotides containing the octa-1,7-diynyl nucleosides, a series of phosphoramidites **7**, **10**, and **13** were prepared. The synthesis of 5-octadiynyl 2′-deoxyuridine phosphoramidite has already been reported (*35*). Various protecting groups were studied for the protection of the amino group of different monomers. First, we used the transient protection protocol to protect the amino group of **1a** with a benzoyl residue at the nucleoside level but obtained a rather low yield. Next, isobutyric anhydride was employed for protection and was also discarded for poor reactivity and hygroscopic nature. Thus, it became clear that the presence of a bulky alkynyl side chain influences protection. To avoid this, nucleoside **1a** was first converted into the 4,4′-dimethoxytrityl derivative **5**, which increased the solubility, and then the amino group was protected with the less bulky acetyl group using acetic anhydride in DMF. This results in the formation of the derivative **6** in 84% yield, which was subsequently converted into the phosphoramidite buiding block **7** (Scheme 4).

In the case of 7-deaza-2′-deoxyguanosine **3a**, the amino group was protected with the formamidine residue furnishing compound **8**, which is subsequently converted into the DMT derivative **9**. Phosphitylation of the DMT derivative results in the formation of phosphoramidite building block **10** (Scheme 5). We have already reported on the use of different protecting groups for the amino group of 7-alkynylated 7-deaza 2′-deoxyadenosines (*21*). In the case of **4a**, formamidine and acetamidine protection was not successful due to the unstable products which turned brown when exposed to the light. On the basis of these observations, the isobutyric residue was chosen. The nucleoside **4a** was treated with trimethylsilyl chloride in pyridine followed by isobutyric anhydride (transient protection) (*42*), yielding the derivative **11**. The protected nucleoside was obtained in 71% yield, which was further converted into phosphoramidite building block **13** via the DMT derivative **12** (Scheme 6).

All the compounds were characterized by ^1H, ^{13}C, and ^{31}P NMR spectra and by elemental analysis (Table 2 and Experimental Section). The δ(C) of the C≡C bonds were in good agreement with the literature data of the related compounds (*20, 35*). Moreover, the nucleobase as well as the protecting group carbon atom signals of 7-deazapurines and pyrimidines were assigned on the basis of literature data (*31*). The above synthesized phosphoramidites were further employed in solid-phase oligonucleotide synthesis.

Duplex Stability of Tridentate "dG−dC" Base Pair Modified by 5-Octadiynyl-2′-deoxycytdine (1a) and 7-Deaza-7-octadiynyl-2′-deoxyguanosine (3a). To evaluate the influence of base-modified nucleosides on the duplex stability, hybridization experiments were performed by using the oligonucleotide duplex 5′-d(TAGGTCAATACT)-3′ (**14**) 3′-d(ATCCAGTTATGA)-5′ (**15**) as a reference. Earlier, it was reported from our laboratory that the pyrimidine nucleoside **1b** increase the thermal stability of duplexes by 2–3 °C per modification (*32*) when incorporated in the place of dC (motif I, Figure 4). Herein, we studied the role of octa-1,7-diyne in the duplexes **14** and **15** by incorporating compound **1a** instead of 2′-deoxycytidine. The 5-(octa-1,7-diynyl) nucleoside **1a** enhances the stability of a dG−dC base pair by 2–3 °C per modification (Table 3).

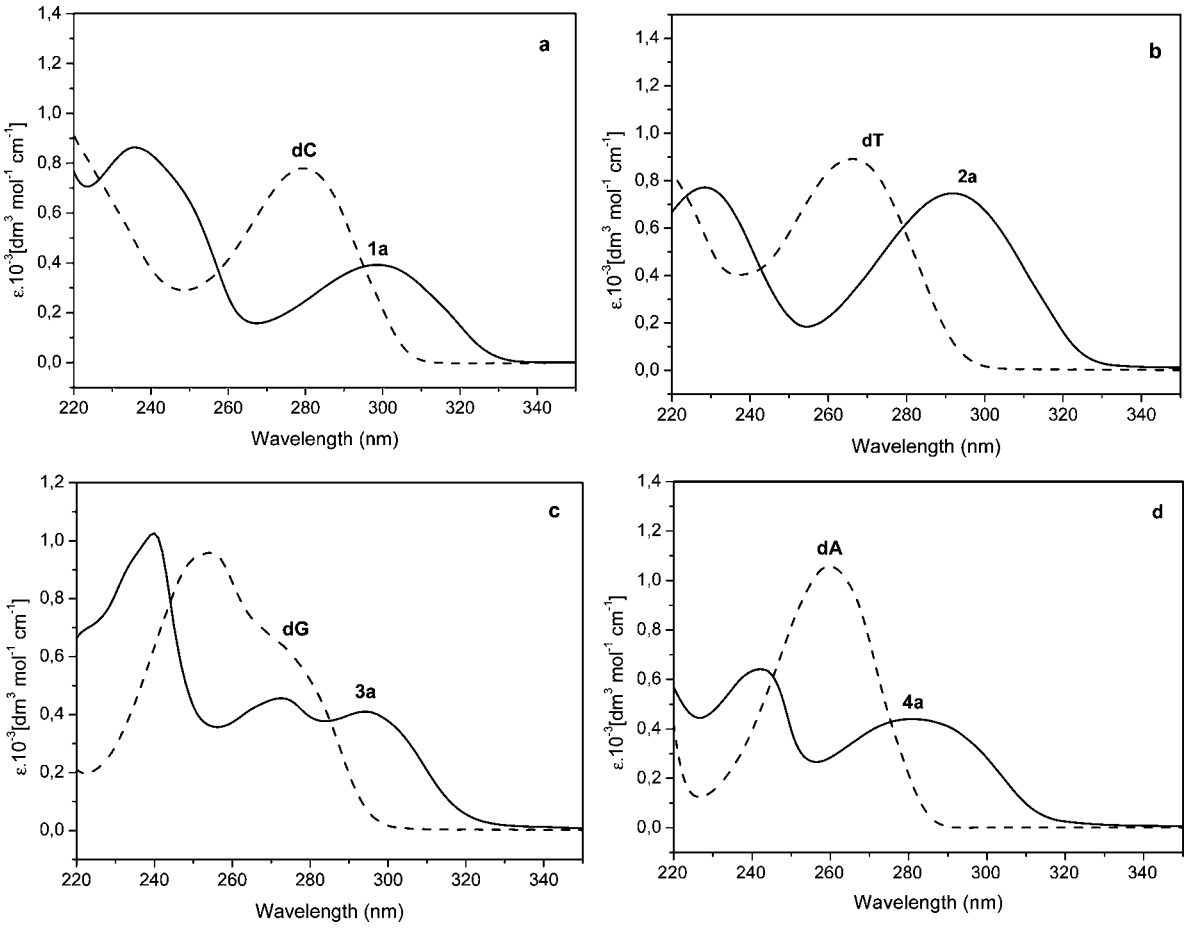


Figure 3. UV/vis spectra of (a) compounds dC and **1a**; (b) dT, **2a**; (c) dG, **3a**; (d) dA, **4a** measured in MeOH.

Table 1. Extinction Coefficients of Octadiynylated 2′-Deoxyribonucleosides[a]

compd	wavelength (λ_{max}) [nm]	extinction coefficient (ϵ)
1a	236	15900
	260	4900
	299	7200
2a	228	12800
	260	3800
	292	12400
3a	240	20200
	260	7300
	273	9000
	294	8100
4a	239	15700
	260	5300
	281	10600

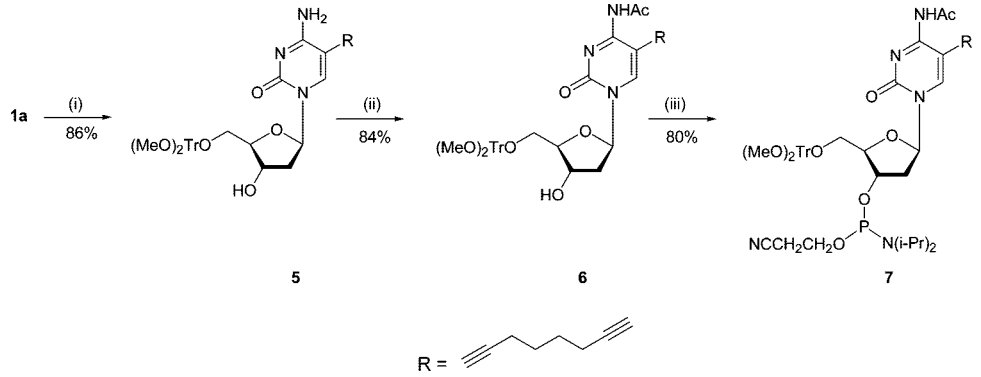
[a] Measured in MeOH.

The oligonucleotides with longer alkynyl chains linked to the 7-position of 7-deaza-2′-deoxyguanosine show an unfavorable influence on the DNA duplex stability (*24*). Moreover, it was found that introduction of the propynyl group at this position enhances the DNA duplex stability significantly (*31*). Recently, we reported the single-crystal X-ray analysis of the 7-propynylated nucleoside **3b** (*43*) which shows an anti [$\chi = -117.2$ (5)°] orientation of the nucleobase related to the sugar moiety with pseudorotational parameters $P = 152.5°$ and $\tau_m = 41.9°$. The linear 7-propynyl residue is placed almost in-plane with the base moiety and protruding into the major groove with steric freedom. From these findings, it was concluded that the duplex stability can be tuned by an appropriate selection of a 7-substituent. To evaluate this, nucleoside **3a** was incorporated in the standard duplex 5′-d(TAGGTCAATACT)-3′ (**14**) 3′-d(ATCCAGTTATGA)-5′ (**15**) in place of dG. The incorporation of the **3a** residue results in a T_m increase of 2–3 °C per modification (Table 3). These values are more or almost similar when compared with the oligonucleotides containing the 7-propynyl derivative **3b** (Table 1 in Supporting Information). Thus, it is shown that the octa-1,7-diynyl group is a more effective substituent for duplex stabilization. These results led us to study the influence of the octa-1,7-diynyl group at the 7-position of 7-deazaadenine.

Duplex Stability of the Bidentate dA−dT Base Pair Modified by 7-Deaza-7-octadiynyl-2′-deoxyadenosine (4a) and 5-Octadiynyl-2′-deoxyuridine (2a). The 7-deaza-7-(octa-1,7-diynyl)-2′-deoxyadenosine **4a** was introduced into the duplex 5′-d(TAGGTCAATACT)-3′ (**14**) 3′-d(ATCCAGTTATGA)-5′ (**15**) replacing dA. From Table 4, it is concluded that single or double incorporation of **4a** (motif VI, Figure 4) does not show much influence on the stability, whereas three incorporations enhances the T_m value by 3 °C (**30•31**) when compared to the standard sequence in high-salt buffer (Table 4). In the low-salt buffer, there is no significant influence of this side chain on the duplex stability when compared to **4b** (data not shown). In the case of 5-octadiynyl 2′-deoxyuridine (**2a**), a T_m increase of 1–2 °C per modification was observed, similar to that of **2b** (motif VIII, Figure 4).

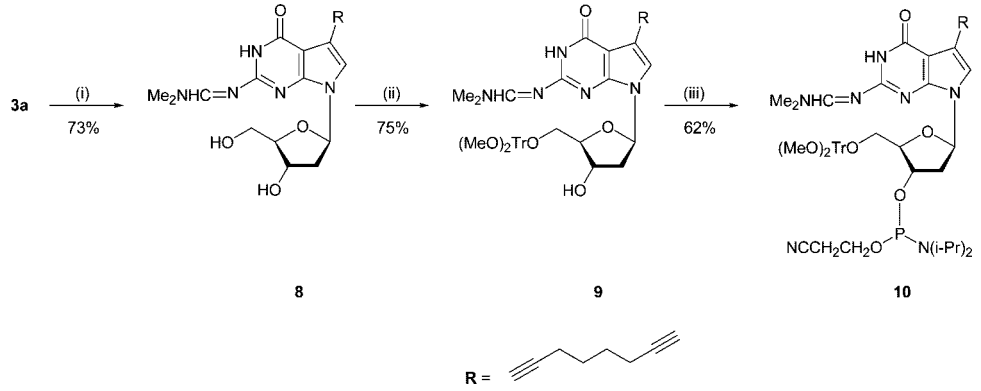
The above results clearly demonstrate that the octa-1,7-diynyl nucleosides with side chains located at the 7-position of 7-deazapurine nucleosides or at the 5-position of pyrimidine nucleosides show a very similar behavior to that of four canonical DNA constituents with a tendency of duplex stabilization. From the above data, it can be concluded that bidentate base pair reacts more sensitively to this change than the

Scheme 4[a]



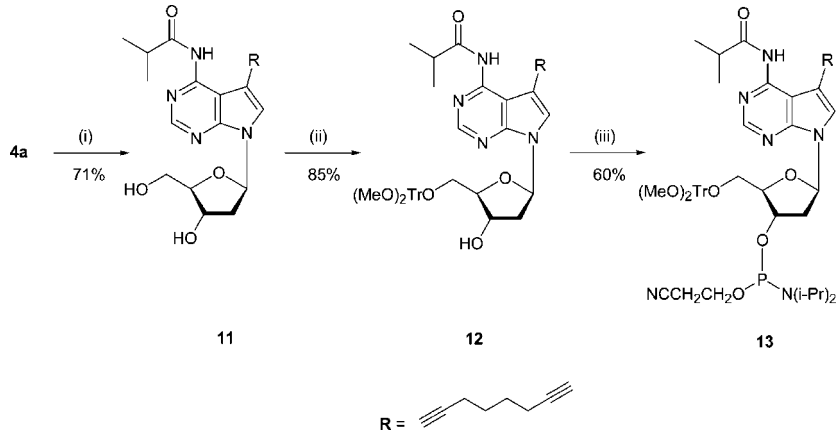
[a] (i) 4,4′-Dimethoxytriphenylmethyl chloride, anhydrous pyridine, rt, 6 h; (ii) acetic anhydride, DMF, rt, 20 h; (iii) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite, *N,N*-diisopropylethylamine, CH_2Cl_2, rt, 45 min.

Scheme 5[a]



[a] (i) *N,N*-Dimethylformamide dimethylacetal, MeOH, 40 °C, 1 h; (ii) 4,4′-dimethoxytriphenylmethyl chloride, anhydrous pyridine, rt, 6 h; (iii) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite, *N,N*-diisopropylethylamine, CH_2Cl_2, rt, 45 min.

Scheme 6[a]



[a] (i) Me_3SiCl, isobutyric anhydride, anhydrous pyridine, rt; (ii) 4,4′-dimethoxytriphenylmethyl chloride, anhydrous pyridine, rt, 6 h; (iii) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite, *N,N*-diisopropylethylamine, CH_2Cl_2, rt, 45 min.

tridentate pair. The octa-1,7-diynyl groups are protruding into the major groove, which does not distort the base pairs significantly. The higher stability for such a long chain is due to the presence of terminal triple bonds.

Functionalization of Terminal C≡C Bonded Oligonucleotides in Solution with the Coumarin Azide 38 Employing the Huisgen−Sharpless−Meldal [3 + 2] Cycloaddition ("Click" Chemistry). The Huisgen−Sharpless−Meldal "click" chemistry is defined as a selective chemical reaction between alkynes and organic azides (*44*). The reaction is tolerant of a variety of solvents and functional groups to form stable 1,2,3-triazoles. Independently, the Sharpless (*45*) and Meldal (*46*) groups reported the copper(I)-catalyzed coupling protocol which enhances the reaction rate significantly and leads to regioselective ring closure. Previous work includes the demonstration of this chemistry in nucleic acids (*36, 47–49*) for the construction of fluorescent oligonucleotides for DNA sequencing (*50*), for the intramolecular circularization and catenation of DNA (*51*), and also to attach oligonucleotide probes on monolayers (*52*). Recently, we reported on the functionalization of alkynyl-modified 2′-deoxyuridines using "click" chemistry at the nucleoside as well as at the oligonucleotide levels (*35*).

Table 2. ^{13}C NMR Chemical Shifts (δ) of 5-Substituted Pyrimidine 2′-Deoxyribonucleosides and 7-Substituted 7-Deazapurines[a]

compd	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(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C=O/C=N	C≡C	CH_2
1	153.9	164.3		95.4	143.5		84.6	[e]	70.2	87.5	60.9		90.3, 84.2	17.2, 18.5
													72.1, 71.2	27.1, 27.2
3a	153.1	157.9	99.5[d]	89.6	121.0	150.2	82.2	[e]	71.0	87.1	61.9		99.4[d], 84.4	17.3, 18.5
													74.7, 71.3[d]	27.2, 27.5
3b (*31*)	152.9[d]	157.8	99.3	85.4	120.9	150.0[d]	82.1	[e]	70.8	87.0	61.8		99.3, 73.7	
4a (*20*)	152.5	157.4	102.3	95.4	125.4	149.0	83.0	[e]	70.9	87.4	61.8		95.4, 71.3	
4b	152.5[d]	157.5	102.3	95.6	125.4	148.9[d]	83.1	[e]	70.9	87.4	61.9		88.4, 72.6	
5	153.5	164.4		95.3	143.0		85.7[d]	[e]	70.3	85.4[d]	63.6		90.7, 84.2	17.2, 18.5
													71.6, 71.3	26.9, 27.2
6	152.4	161.0		96.8	145.6		86.3[d]	[e]	70.1	85.9[d]	63.3		93.6, 84.1	17.1, 18.3
													71.3, 70.8	26.8, 27.1
8	157.4	158.7	102.5	89.6	122.4	156.5	82.2	[e]	71.0	87.2	61.9	148.9	99.4, 84.3	17.8, 18.5
													74.6, 71.3	27.1, 27.5
9	158.0	158.8	102.5	89.6	122.1	157.5[d]	82.0	[e]	70.8	85.4	64.3	148.9	99.7, 84.3	17.3, 18.5
													74.5, 71.3	27.0, 27.5
11	150.9	151.3	110.4	97.0	129.8	151.0[d]	83.0	[e]	70.9	87.5	61.8	175.7	91.2, 84.2	17.3, 18.7
													73.8, 71.4	27.3, 27.4
12	150.9	151.3	110.4	97.1	129.8	151.0[d]	83.0	[e]	70.8	85.5	64.2	175.7	38.4, 37.8	17.3, 18.7
														27.3, 27.4

[a] Measured in DMSO-d_6. [b] Systematic numbering. [c] Purine numbering. [d] Tentative. [e] Superimposed by DMSO.

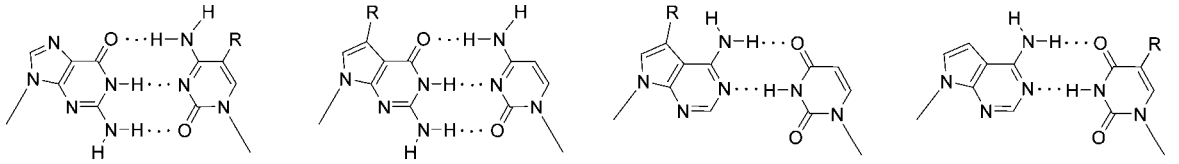


Figure 4. Base Pair Motifs in Duplexes Containing Octadiynyl and Propynyl Nucleosides.

Table 3. T_m Values and Thermodynamic Data of Oligonucleotide Duplexes Containing 5-Octadiynyl-2′-deoxycytidine 1a and 7-Deaza-7-octadiynyl-2′-deoxyguanosine 3a[a]

duplex	T_m [°C]	$\Delta G\,^\circ_{310}$ [kcal/mol]
5′-d(TAG GTC AAT ACT) (**14**)	50	−11.8
3′-d(ATC CAG TTA TGA) (**15**)		
5′-d(TAG GT **1a** AAT ACT) (**16**)	53	−12.1
3′-d(ATC CAG TTA TGA) (**15**)		
5′-d(TAG GT **1a** AAT A **1a**T) (**17**)	54	−12.0
3′-d(ATC CAG TTA TGA) (**15**)		
5′-d(TAG GTC AAT ACT) (**14**)	55	−12.9
3′-d(AT **1a** **1a**AG TTA TGA) (**18**)		
5′-d(TAG GT **1a** AAT ACT) (**16**)	57	−12.6
3′-d(AT **1a** **1a**AG TTA TGA) (**18**)		
5′-d(TA **3a** GTC AAT ACT) (**22**)	52	−11.8
3′-d(ATC CAG TTA TGA) (**15**)		
5′-d(TAG GTC AAT ACT) (**14**)	52	−11.8
3′-d(ATC CAG TTA T **3a**A) (**23**)		
5′-d(TA **3a** **3a**TC AAT ACT) (**24**)	53	−12.3
3′-d(ATC CAG TTA TGA) (**15**)		
5′-d(TA **3a** **3a**TC AAT ACT) (**24**)	55	−12.7
3′-d(ATC CAG TTA T **3a**A) (**23**)		
5′-d(TA **3a** **3a**TC AAT ACT) (**24**)	58	−14.0
3′-d(ATC CA **3a** TTA T **3a**A) (**25**)		

[a] Measured at 260 nm in 1 M NaCl, 100 mM $MgCl_2$ and 60 mM Na cacodylate (pH 7.0) with 5 μM + 5 μM single-strand concentration.

Table 4. T_m Values and Thermodynamic Data of Oligonucleotide Duplexes Containing 7-Deaza-7-octadiynyl-2′-deoxyadenosine 4a, 5-Octadiynyl-2′-deoxyuridine 2a[a]

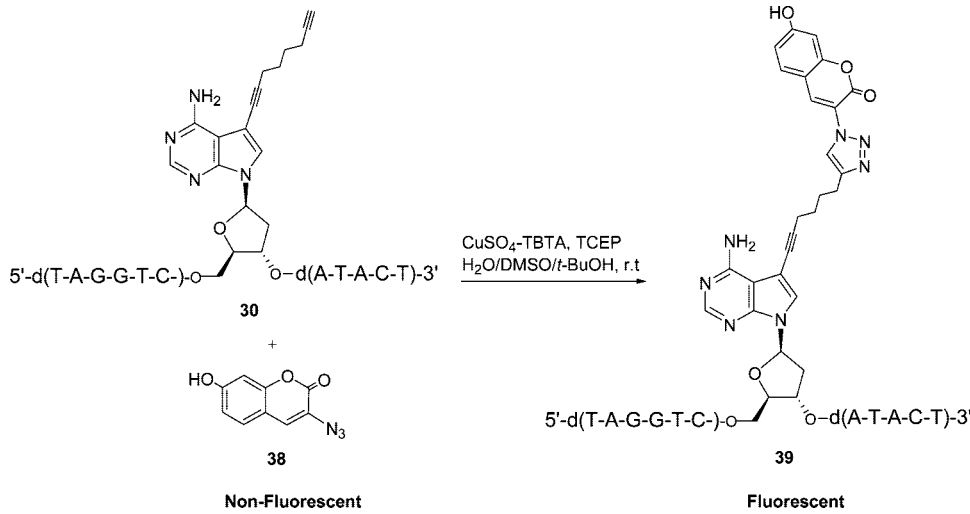
duplex	T_m [°C]	$\Delta G\,^\circ_{310}$ [kcal/mol]
5′-d(TAG GTC AAT ACT) (**14**)	51	−12.2
3′-d(ATC CAG TTA TGA) (**15**)		
5′-d(TAG GTC **4a**AT ACT) (**30**)	51	−12.2
3′-d(ATC CAG TTA TGA) (**15**)		
5′-d(TAG GTC AAT ACT) (**14**)	51	−12.1
3′-d(ATC C **4a**G TT **4a** TGA) (**31**)		
5′-d(TAG GTC **4a**AT ACT) (**30**)	54	−12.8
3′-d(ATC C **4a**G TT **4a** TGA) (**31**)		
5′-d(TAG G **2a**C AAT ACT) (**34**)	52	−11.9
3′-d(ATC CAG TTA TGA) (**15**)		
5′-d(TAG GTC AAT ACT) (**14**)	53	−12.4
3′-d(ATC CAG **2a2a**A TGA) (**35**)		
5′-d(TAG G **2a**C AAT ACT) (**34**)	55	−12.3
3′-d(ATC CAG **2a2a**A TGA) (**35**)		

[a] Measured at 260 nm in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na cacodylate (pH 7.0) with 5 μM + 5 μM single-strand concentration.

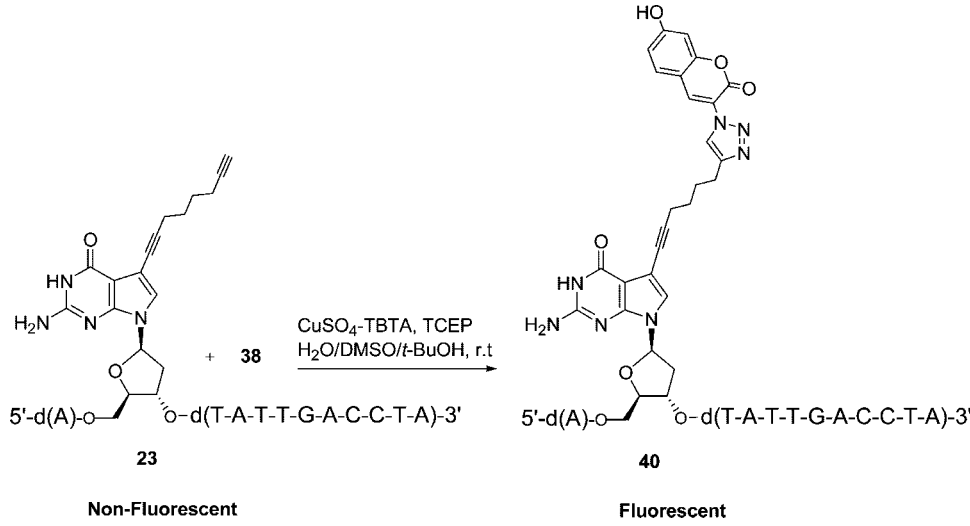
Here, we report on the use of oligonucleotides containing octa-1,7-diynyl nucleosides **1a**−**4a** with terminal C≡C bonds for further functionalization using the protocol of [3 + 2] cycloaddition with a nonfluorescent coumarin azide to produce highly fluorescent 1,2,3-triazolyl oligonucleotides. Coumarin is a compound found in many plants, notably as the sweet scent of newly mown hay. It has clinical value as the precursor for several anticoagulants and is used as a gain medium in some dye lasers. Among many fluorophores, coumarin derivatives have been extensively studied because of their special photophysical properties with no low-lying electronic states that interact with their first excited states (*53*). Moreover, the fluorescence properties of coumarin derivatives depend on the nature of the substituents present at the 6- or 7-position of the coumarin ring (*54–56*) and can be explained in terms of the intramolecular charge transfer (ICT) mechanism (*57*). Coumarins were used as potential fluorescent probes to study the structural dynamics of DNA (*58*), for the specific labeling of nucleic acids (*59*) and mainly as nucleobase-specific quenchers (*60, 61*).

At first, the "click" reaction was performed using the purified oligonucleotide **30** containing one 7-deaza-7-(octa-1,7-diynyl)-2′-deoxyadenynyl residue and the nonfluorescent coumarin azide **38**. The reaction was carried out in aqueous solution (H_2O/*t*-BuOH/DMSO) in the presence of a 1:1 complex of $CuSO_4$−TBTA (tris(benzyltriazoylmethyl)amine) (*62*) and TCEP (tris(carboxyethyl)phosphine), a water-soluble reducing agent, to give the strongly fluorescent functionalized

Scheme 7



Scheme 8



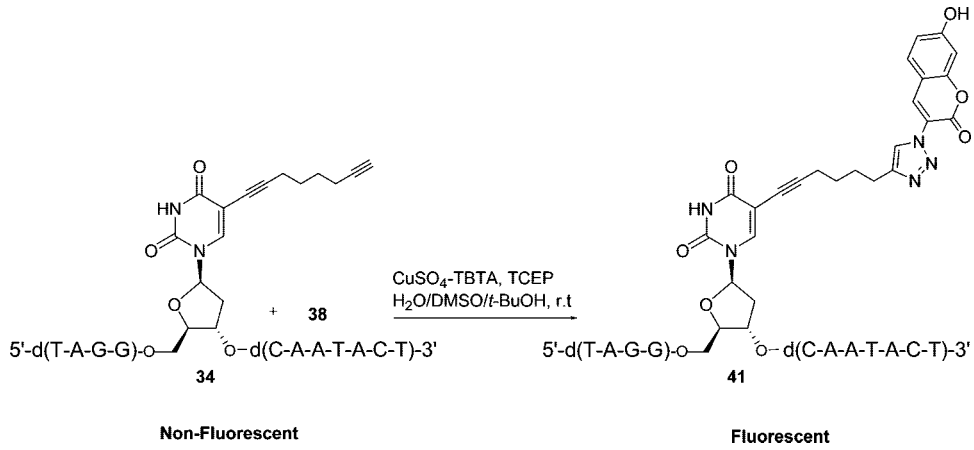
oligonucleotide **39**. The reaction was complete in 15 h at room temperature when an excess molar ratio of coumarin azide was used. The reaction mixture was purified by reversed-phase HPLC, and the strongly fluorescent oligonucleotide **39** was isolated (Scheme 7). The 1,2,3-triazole ring formed by the 1,3-dipolar cycloaddition of the alkyne and azido derivatives of coumarin was found to be strongly fluorescent (*63*). Such reactions are already used for the preparation of fluorogenic probes (*64*) and also for the fluorescence visualization of synthesized mammalian proteins (*65*).

Next, a similar reaction was performed on the oligonucleotide 5′-d(A**3a**T ATT GAC CTA)-3′ (**23**) containing one 7-deazaguanine residue **3a** resulting in the formation of the highly flourescent 7-deazaguanine coumarin conjugate **40** (Scheme 8). The same reaction conditions were used for the click reaction as mentioned above. After the reaction was performed on the 7-deazapurine derivatives, the corresponding pyrimidines were investigated. For this, the oligonucleotide **34** containing **2a** was used, which was conjugated with the coumarin azide **38** to give the click product **41** (Scheme 9). In a similar fashion, 5′-d(AGT ATT GA**1a** CTA)-3′ (**42**) containing 5-(octa-1,7-diynyl)-2′-deoxycytidine was functionalized with the coumarin azide **38** to give **43** (Scheme 10). The ligated 1,2,3-triazolyl "click" oligonucleotides were identified by MALDI-TOF mass spectrometry (Table 3, Supporting Information).

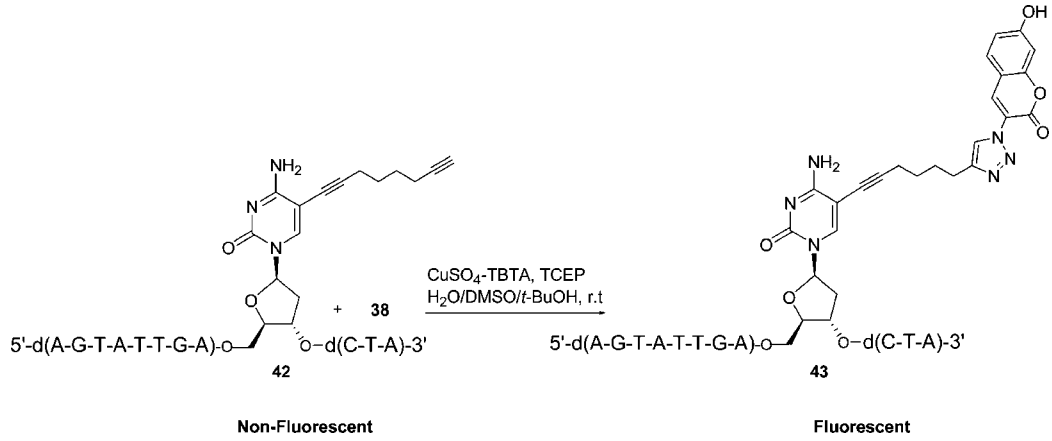
Fluorescence Properties of Oligonucleotides Containing 1,2,3-Triazole Coumarin Derivatives. The fluorescence spectra of the single-stranded oligonucleotide **39** show a strong fluorescence with an emission at 479 nm and an excitation at 400 nm. In the case of the DNA duplex **15•39**, the fluorescence was strongly decreased, a quenching of 85% is observed (Figure 5). This arises from the stacking of dye with the neighboring bases, which is significantly higher in duplex DNA when compared to the single-stranded DNA. In the case of single strands, the dye has a higher degree of steric freedom with negligible stacking with the nucleobases, leading to a higher fluorescence.

It has been reported that the fluorescence of some fluorescent dyes is quenched by the interaction between the dyes and the nucleobases. This quenching is useful to monitor the conformational dynamics of oligonucleotides in solution. However, the dependency of fluorescence quenching is mainly based on the distance between a dye and a nucleobase; in this perspective, several fluorescent dyes like acridine, stilbene, and pyrene were studied (*66, 67*). Moreover, the quenching of coumarin dyes by nucleobase derivatives in aqueous solution is caused by the photoinduced electron transfer, which is dependent on the oxidation and reduction potential values of the nucleobases (*60*) and the dye. In order to understand the quenching behavior of coumarin dye attached to the modified canonical nucleosides **1a**−**4a** on single-stranded DNA, a series of the above-prepared

Scheme 9



Scheme 10



click-functionalized oligonucleotides were used, and the fluorescence quenching properties of such covalently linked coumarin-nucleobase assemblies were investigated by performing enzymatic hydrolysis. The oligomer **39** containing 7-deazaadenine conjugate was digested with snake venom phosphodiesterase and alkaline phosphatase, and fluorescence spectra were measured before and after 3–10 h of cleavage (Figure 6) with an emission observed at 479 nm. Surprisingly, we observed a decrease in fluorescence intensity after addition of enzyme from 3 to 10 h; even after the addition of excess enzyme, the fluorescence was further decreased by 10% and remained constant afterward.

Then, the oligonucleotide **40** containing the 7-deazaguanine conjugate was subjected to enzymatic hydrolysis, and fluorescence emission was recorded at different time intervals (Figure 7) with an emission observed at 479 nm, when excited at 400 nm. From Figure 7, it is clear that the 7-deazaguanine moiety strongly quenches the fluorescence of the coumarin dye attached to it, similar to that of the 7-deazaadenine moiety. Apparently, an intramolecular photoinduced electron transfer plays a role in fluorescence quenching. The 7-deazapurines can act as electron donors, and the 7-deazapurine nucleobase quenches the triazolyl coumarin dye attached to it. The purine bases quench

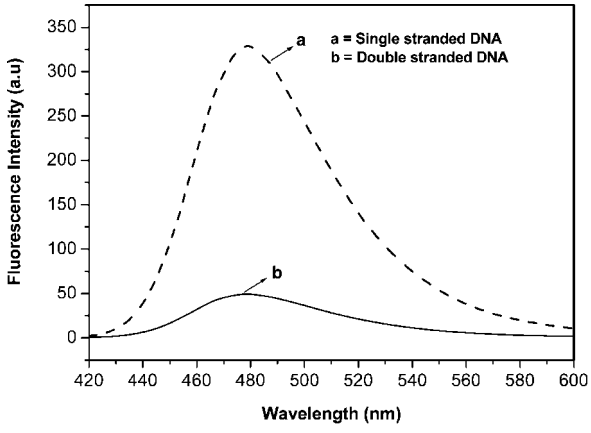


Figure 5. Fluoresence emission spectra of **39** (2 μM single strand) and duplex DNA **15•39** (2 μM of each strand) in bidistilled water at 25 °C when excited at 400 nm.

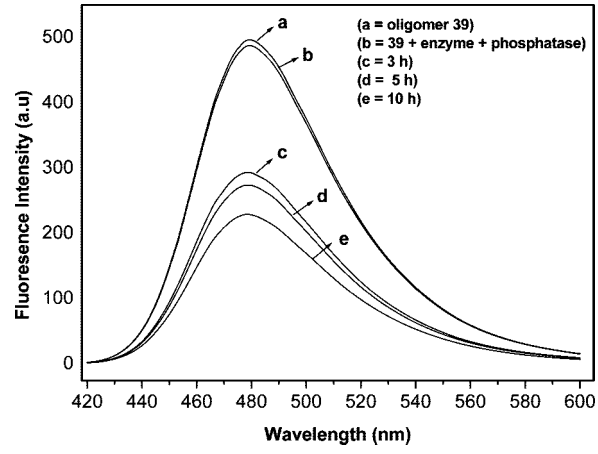


Figure 6. Enzymatic digestion of 1.7 μM single-stranded oligonucleotide **39** with snake venom phosphodiesterase followed by alkaline phosphatase in 0.1 M Tris−HCl buffer (pH 8.3) at 37 °C.

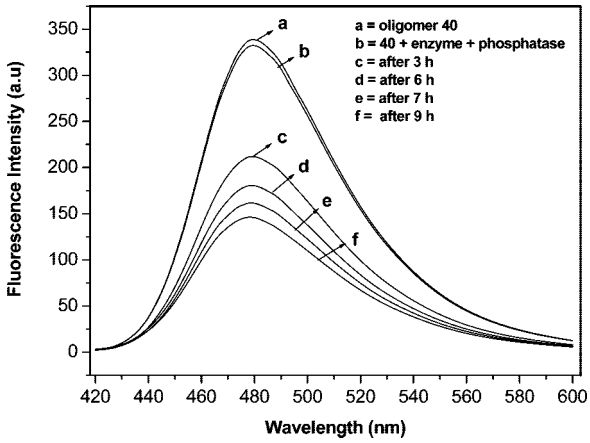


Figure 7. Enzymatic digestion of 1.7 μM single-stranded oligonucleotide **40** with snake venom phosphodiesterase followed by alkaline phosphatase in 0.1 M Tris−HCl buffer (pH 8.3) at 37 °C.

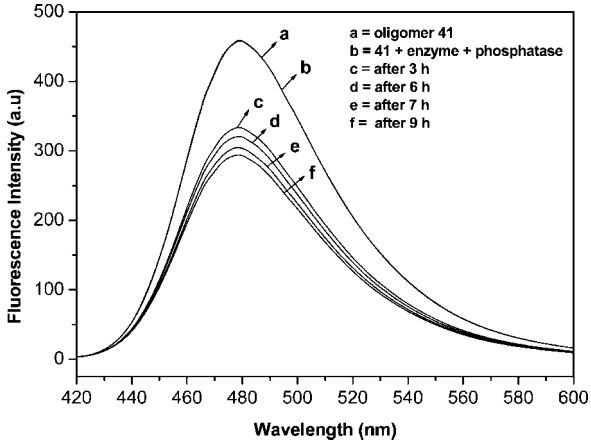


Figure 8. Enzymatic digestion of 1.7 μM single-stranded oligonucleotide **41** with snake venom phosphodiesterase followed by alkaline phosphatase in 0.1 M Tris−HCl buffer (pH 8.3) at 37 °C.

the excited singlet state of coumarins more effectively than pyrimidine bases (*68*). The above results prompted us to study the quenching properties of pyrimidine nucleoside dye conjugates. For that purpose, the enzymatic hydrolysis of the single-stranded oligonucleotide **41** containing the uracil moiety was performed under conditions as described earlier. When oligonucleotide **41** was cleaved, an unexpected fluorescence quenching was observed (Figure 8). However, this quenching was less effective than that observed for the oligonucleotides containing the 7-deazapurine nucleosides **3a** and **4a**. From the HPLC profile of the enzymatic digest of oligomer **41** which contains additional unidentified peaks, it can be concluded that side reactions occurred during irradiation. Such side reactions could be caused by the photoinduced cycloaddition involving the dT−coumarin conjugate within the oligonucleotide chain (*69*). The monomeric click product of octadiynyl 2′-deoxyuridine with azidocoumarin did not form side products under those conditions (data not shown).

In contrary to the above results, a rather different result was observed for oligonucleotide **43** (Figure 9), after the enzymatic digestion for 12 h. There is very little quenching for the 5-(octa-1,7-diynyl)-2′-deoxycytidine dye conjugate when compared with 7-(octa-1,7-diynyl) 7-deazapurines. This shows that the 7-deazapurine bases are strong quenchers of fluorescence when compared to pyrimidines. By changing the oligonucleotide concentration of **40** and **43** from 1.7 μM to 4 μM without changing the enzyme concentration, similar results were obtained in both series of experiments (Figures 7, 9, 10, and 11).

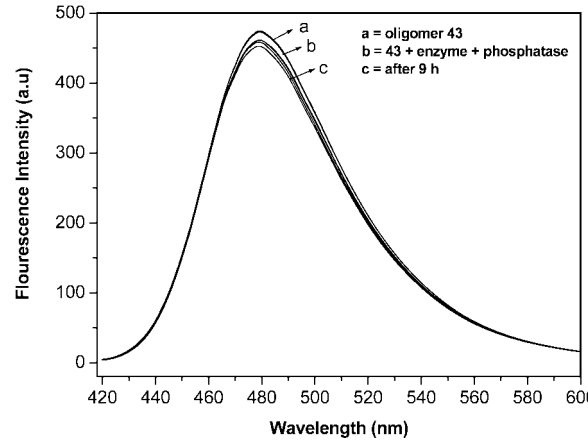


Figure 9. Enzymatic digestion of 1.7 μM single-stranded oligonucleotide **43** with snake venom phosphodiesterase followed by alkaline phosphatase in 0.1 M Tris−HCl buffer (pH 8.3) at 37 °C.

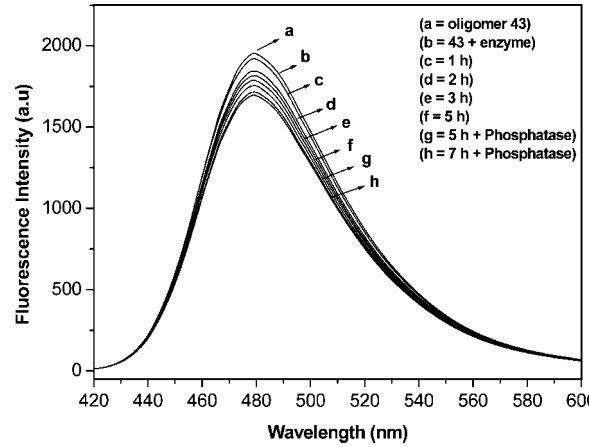


Figure 10. Enzymatic digestion of 4 μM single-stranded oligonucleotide **43** with snake venom phosphodiesterase followed by alkaline phosphatase in 0.1 M Tris−HCl buffer (pH 8.3) at 37 °C.

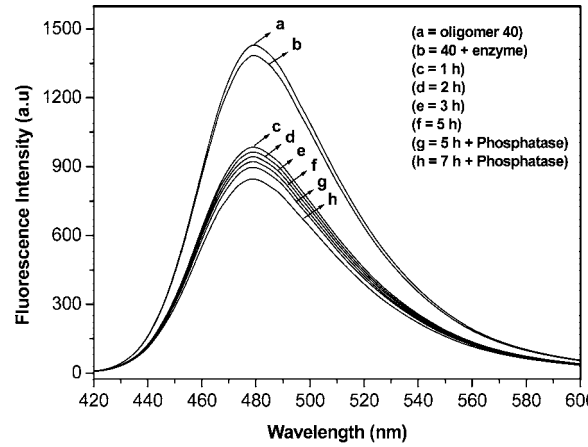


Figure 11. Enzymatic digestion of 4 μM single-stranded oligonucleotide **40** with snake venom phosphodiesterase followed by alkaline phosphatase in 0.1 M Tris−HCl buffer (pH 8.3) at 37 °C.

The HPLC profile of the enzymatically digested oligonucleotide **43** clearly indicates the formation of free nucleosides, and the complete digestion pattern was observed after 7 h.

The most probable reason for this behavior can be explained in terms of intramolecular photoinduced electron transfer from the nucleobase to the coumarin dye attached to it, which strongly quenches the fluorescence when they are in close proximity (Figure

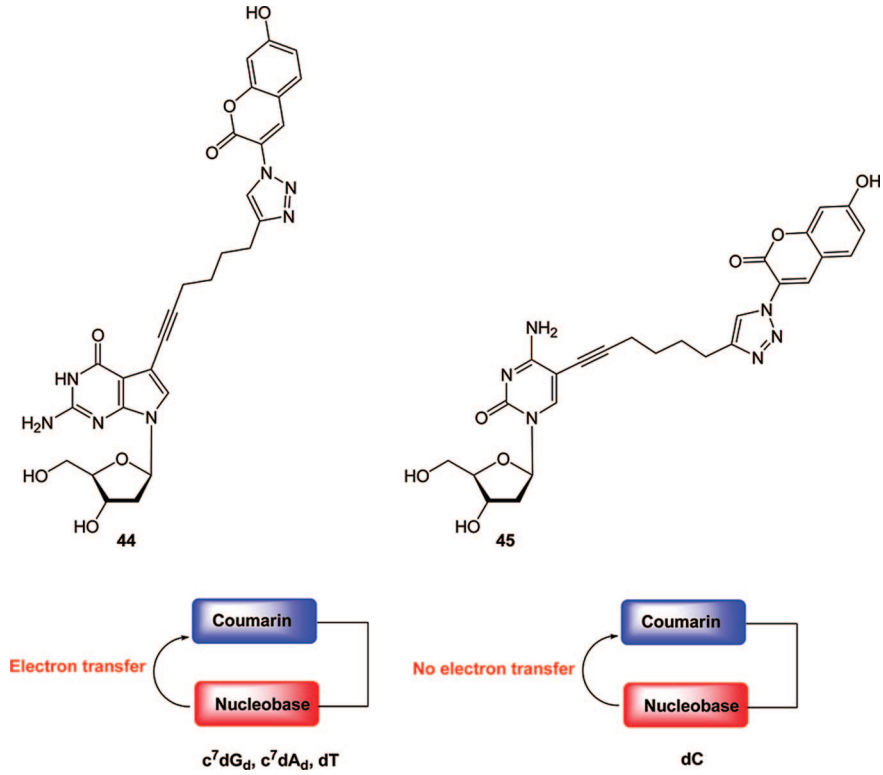


Figure 12. Electron transfer between the nucleobases and coumarin dye attached to it when they are in close proximity.

12). This is evidenced from the fluorescence quantum yield measurements of the monomeric nucleoside−coumarin conjugates in bidistilled water. The 7-deaza-2′-deoxyguanosine−coumarin conjugate **44** shows a quantum yield (Φ) of only 0.11 when compared to that of 2′-deoxycytidine−coumarin conjugate **45** (Φ = 0.22). The quantum yield of fluorescence for nucleoside−dye conjugates was determined relative to quinine sulfate in 0.1 N H_2SO_4 as the standard with Φ = 0.53. The excited coumarins serve as an electron acceptor and the nucleobase as a ground-state donor. A stacked arrangement of the nucleobase and the coumarin dye appears to be a prerequisite for fluorescence quenching. This quenching occurs much more efficiently in the monomeric nucleosides dye conjugates than within a single-stranded oligonucleotide. The latter already shows a preformed helical structure with weakly stacked nucleobases. In this form, the bases are positioned in a way that they are apparently not able to interact with the coumarin dye, while in the duplex structure, the dye molecule interacts with the stacked base pair. Consequently, quenching occurs either in the form of a duplex or within the coumarin−nucleobase assembly, which is generated after the enzymatic digestion of the oligonucleotide. The intramolecular quenching is highly efficient only at short distances; collisional quenching is expected to be comparably inefficient. One should note that the octadiynyl groups might have an influence on the redox potentials of the nucleobases.

CONCLUSION

Nucleosides containing the octa-1,7-diynyl residue at the 5-position of 2′-deoxycytidine or at the 7-position of 7-deazapurines were synthesized by the palladium-assisted Sonogashira cross-coupling reaction resulting in a selective monoderivatization of the diyne starting material. The oligonucleotides incorporating the octa-1,7-diynyl derivatives of 2′-deoxycytidine and 7-deazapurines stabilize the DNA duplexes significantly when compared with the alkynyl counterparts. This group enhances the stability more or equal to that of propynyl residue. Such oligonucleotides containing octa-1,7-diynyl derivatives **1a**−**4a** were further annelated with the nonfluorescent coumarin azide **38** by Huisgen−Sharpless−Meldal "click" chemistry to generate a set of highly fluorescent oligomers **39**, **40**, **41**, and **43**. The enzymatic hydrolysis of such 1,2,3-triazolyl oligonucleotide conjugates revealed that strong fluorescence quenching was observed for 7-deazapurines compared to pyrimidines. The quenching efficiency is in the order $c^7dA_d \approx c^7dG_d > dU > dC$. So, the nucleobase specific quenching of base-modified nucleosides is useful to monitor conformational dynamics of oligonucleotides in solution. The click methodology allows the introduction of various reporter groups like dyes or other functionalized residues, which do not change the water shells of a DNA molecule by retaining stable duplexes. The generation of fluorescence by the "click" reaction can be used for the footprinting of DNA accessible in solution or buried in DNA–protein complexes. The reaction kinetics of this bioorthogonal chemical reporter strategy (nonfluorescent dye becomes fluorescent) can be used for the labeling and visualization of biomolecules in vivo.

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Supporting Information Available: T_m values and thermodynamic data of oligonucleotide duplexes containing propynyl nucleosides **1b**−**4b** (Tables 1 and 2); molecular masses ($[M + H]^+$) of oligonucleotides measured by MALDI-TOF mass spectrometry (Table 3). This material is available free of charge via the Internet at http://pubs.acs.org.

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