Covalent Modification of 2′-Deoxyuridine with Two Different Molecular Switches

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Abstract: Two different molecular switches, a spiropyran and a diarylethene, were attached synthetically to the 5-position of 2′-deoxyuridine. The diarylethene-modified nucleoside can be incorporated synthetically into DNA while preserving its characteristic photochromism.

Key words: oligonucleotide, palladium, DNA, photochromism, phosphoramidite

Switching optical properties by light represents an important task for the development of new photoreactive nanostructured materials including those that are based on nucleic acids. Especially diazobenzenes, spirobenzopyrans and diarylethenes[1–3] were prepared and applied as molecular switches for all kinds of biomolecules.[4–13] With respect to nucleic acids mainly diazobenzene derivatives were developed as artificial and photoswitchable nucleosides that are suitable to control a variety of different functions in DNA.[6–8] Both alternatives, spiropyrans and diarylethenes, remain rather unexplored as covalent photochromic labels for nucleic acids, despite their great potential as molecular switches.

In contrast to simple *cis-trans* switching of diazobenzenes the ring opening of spiropyrans to merocyanines represents not only a major structural change but includes also a large change in polarity.[3] In fact, studies with spiropyrans as non-covalently acting DNA and RNA binders have revealed that strong ground state interactions occur only between the merocyanine form and the DNA base stack.[14,15] The description of covalent attachment of spiropyran to DNA can be found only very rarely in the literature[12,13] and includes our approach to incorporate synthetically the spiropyran chromophore as an artificial DNA base into oligonucleotides.[16]

The second alternative, diarylethenes, are applied in the context of switching binding properties of proteins and peptides[17] but are still nearly completely unexplored with respect to nucleic acids. There is only one recent report on how to combine 7-deazaadenosine with thiophene to obtain a nucleosidic diarylethene switch.[18] Other examples include simpler stilbene-like switches that do not exhibit the good photochromic performance of diarylethenes. Herein, we present the synthesis of the spiropyrane-modified 2′-deoxyuridine **1** and the corresponding diarylethene-modified nucleoside **2** and their photoswitching behavior. With respect to several disadvantages of spiropyrans as covalent labels in DNA we incorporated only the diarylethene **2** into one representative oligonucleotide using automated phosphoramidite chemistry, and characterized its photochromism in **DNA1** preliminarily.

The synthesis of the spiropyrane **1** (Scheme 1) applied our previously improved protocol for the preparation of spirobenzopyrans by ultrasonic irradiation.[19] With this protocol, typically good yields were obtained, and the reaction time is significantly reduced compared to standard reaction conditions. Accordingly, the reaction of freshly distilled Fischer base **3**[19] with **4**[19] under ultrasonic irradiation conditions in EtOH for one hour gave the corresponding *N*-methyl spirobenzopyran **5**[20] in 76% yield. The 6-bromo compound **8**[21] was prepared from the indolium iodide **6**[19] and **7**[19] with addition of excess triethylamine under ultrasonic conditions in EtOH and obtained as a pale pink solid in 77% yield. With the spirobenzopyrans **5** and **8** in hand, the Sonogashira cross-coupling was performed subsequently in two different ways. First, commercially available 5-iodo-2′-deoxyuridine (**9**) and spirobenzopyran **5** were heated at 55 °C for three hours in Et_3N in the presence of $Pd(PPh_3)_2Cl_2$ (3 mol%) and CuI (5 mol%) as catalysts to provide nucleoside **1** in a yield of 27%. The similarly performed reaction of nucleoside **10**[22] with the spirobenzopyran **8** gave product **1** in comparable low yield (24%). Interestingly, a better cross-coupling efficiency was observed with increased amounts of catalysts: **5** and **9** were dissolved in a mixture of DMF and Et_3N with a relatively high amount of $Pd(dppf)Cl_2$ (17 mol%) and CuI (22 mol%), and the reaction was carried out at room temperature for 26 hours. Under these optimized reaction conditions, the desired nucleoside **1**[23] was isolated in 67% yield.

The photoswitching properties of the synthesized spirobenzopyran-modified nucleoside **1** were studied by UV/Vis spectroscopy (Figure 1). Upon irradiation with UV light (λ = 312 nm) for one minute an increase in the visible range was observed. The formation of the merocyanine form with the characteristic absorption at λ = 589 nm was also observable by eye, since the former colorless solution turned magenta-red. Further irradiation of the sample for one more minute did not give any further increase; obviously the photostationary equilibrium between the spiropyran and the merocyanine isomers was

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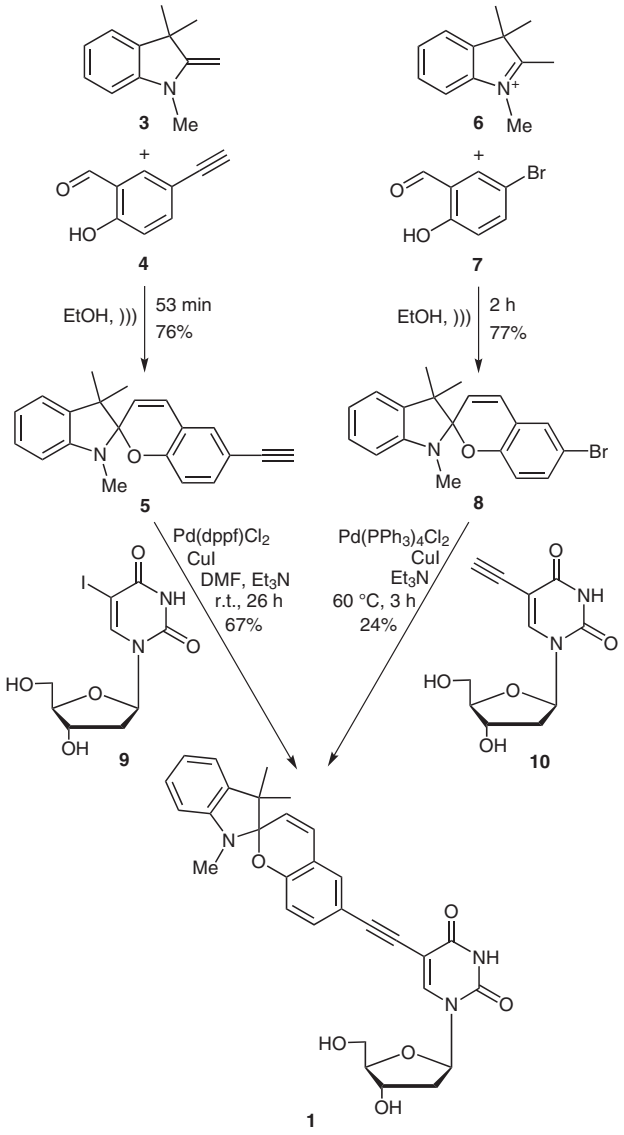

Scheme 1 Synthesis of the spiropyran-modified nucleoside **1**

reached. When the irradiation was abandoned, thermal reformation of the spiropyran form was observed within 10 minutes at room temperature.

Although the spiropyran-modified nucleoside **1** exhibits promising optical properties, we did not incorporate it into oligonucleotides for two different reasons: (i) It is reported that spiropyrans decompose in aqueous buffer solutions,[24] and (according to our experience) the DNA environment does not efficiently prevent this decomposition. (ii) In another study that was performed at the same time as the part of work presented herein we found out that the spiropyran chromophore in the DNA environment loses its photoswitching abilities.[16] Hence, we focused our efforts on the second alternative which is the diarylethene-modified nucleoside **2**.

The preparation of nucleoside **2** was also based on a Sonogashira-type cross-coupling as the key step to attach the photoactive chromophore to the nucleoside (Scheme 2). The synthesis started with commercially

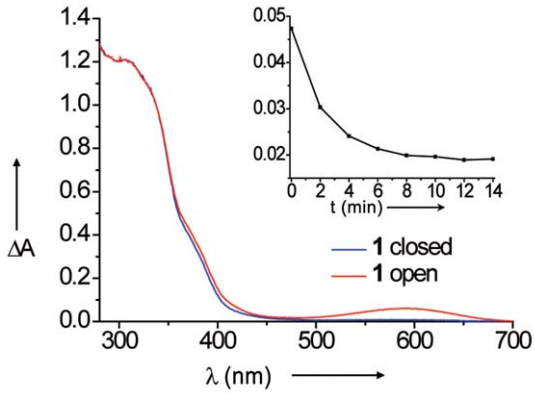

Figure 1 UV/Vis absorption of spiropyran (closed) form of **1** and interconversion into merocyanine (open) form, in MeOH (100 μM **1**) at r.t.: after 2 min irradiation with λ = 312 nm (red), after thermal reclosing (blue). Inset shows the time dependence of absorption change at λ = 589 nm due to thermal interconversion of **1** from the merocyanine to the spiropyran form at r.t.

available 5-bromo benzothiazole (**11**) that was methylated by methyl iodide after deprotonation with LDA to obtain **12**[25] in quantitative yield. A double Friedel–Crafts-type acylation using glutaryl chloride linked together two benzothiazoles **12** to compound **13**[26] in 74% yield. Subsequently the central cyclopentene group of **14**[27] was built by a double McMurry-type reaction, that meant treatment of **13** with Zn and $TiCl_4$. The ethynyl linker was attached by TMS-protected acetylene in a Pd-catalyzed cross-coupling reaction. Although the yield of **15**[28] was only moderate (43%), attempts to improve it failed. The competitively formed double substituted by-product could not be avoided. Thus the potentially usable amount of TMS-protected acetylene decreased. The synthesis of the diarylethene building block **16**[29] was concluded by removal of the TMS protecting group of **15** under standard conditions using K_2CO_3 in MeOH. Finally, diarylethene **16** was coupled to 5-iodo-2′-deoxyuridine by the already mentioned Sonogashira-type coupling to the diarylethene-modified nucleoside **2**.[30]

The characteristic absorption bands of the diarylethene chromophore at λ = 242 nm and λ = 305 nm are clearly detectable in the UV/Vis spectrum of the modified nucleoside **2** if the spectrum is compared with that of **14** (Figure 2). Irradiation of **2** in the UV range, either at λ = 242 nm or at λ = 310 nm, closed the diarylethene moiety and the visible absorption band rose at λ = 450 nm. The color of the solution turned yellow. After 30 minutes, the photostationary state was reached. Irradiation of the sample at λ = 450 nm opened again the chromophore in a few minutes. In contrast to spiropyrane **1**, thermally induced opening to the diarylethene-modified isomer of **2** was not observed. The irradiation cycle of closing and subsequent opening could be repeated several times. The modified nucleoside **2** stayed similarly stable as the molecular switch **14**. Moreover, the nucleoside **2** shows overall very similar optical properties as **14**. This is a remarkable observation since the diarylethene chromophore in **2** is con-

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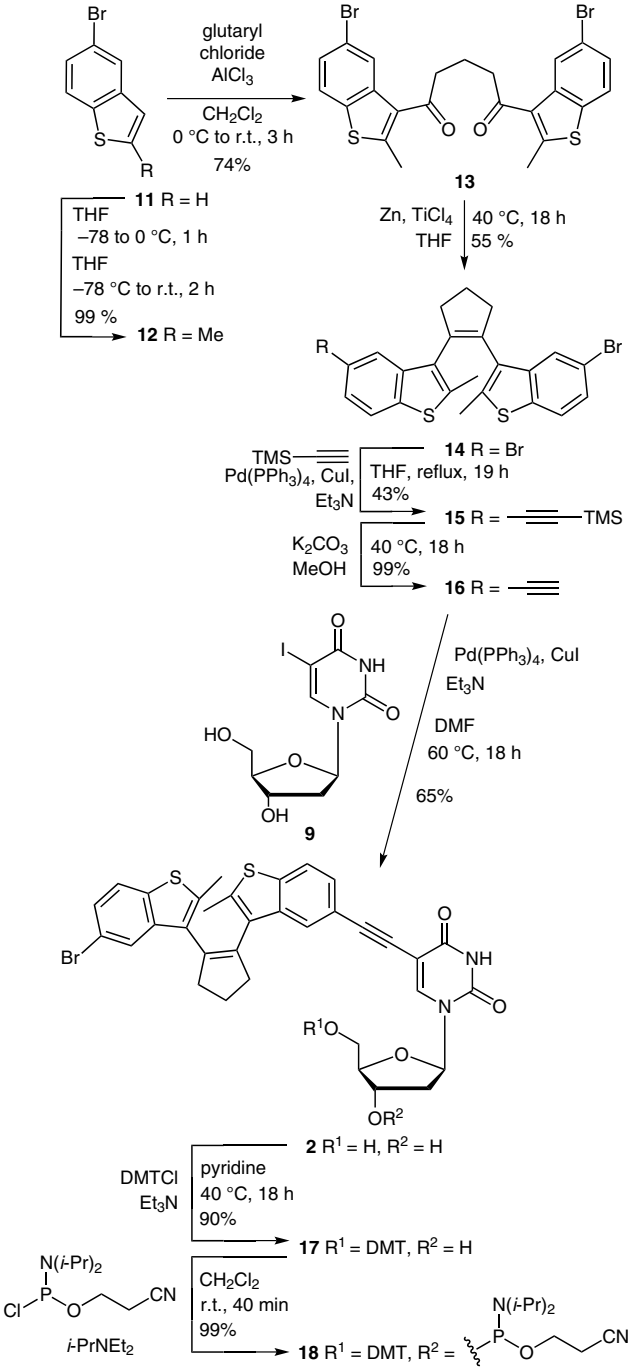

Scheme 2 Synthesis of diarylethene nucleoside **2** and the corresponding DNA building block **18**

jugated with the uracil aromatic system via the acetylene bridge.

The preparation of the DNA building block **18** follows standard procedures, including the protection of the 5′-OH group of **2** by DMT and subsequent phosphitylation of the intermediate **17**[31] to phosphoramidite **18**.[32] We prepared one representative oligonucleotide[33] in order to report preliminarily the optical properties of **2** in double-stranded **DNA1**. It is remarkable to observe that the photoswitching behavior of **2** is maintained in the DNA environment (Figure 3). Irradiation of **DNA1** with λ = 310 nm

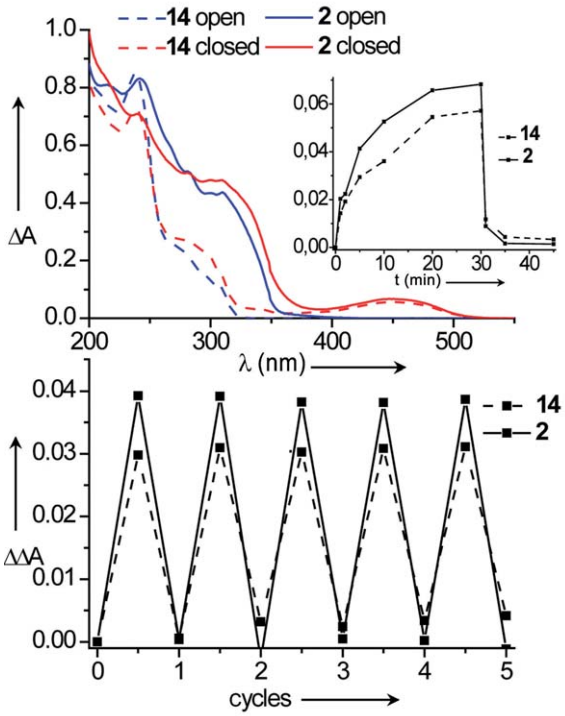

Figure 2 Top: UV/Vis absorption of open form of **2** and **14** and interconversion into closed form, in acetonitrile (20 μM) at r.t.; after 30 min irradiation with λ = 310 nm (red), after 30 min irradiation at λ = 450 nm (blue). Inset shows time dependence of absorption change at λ = 450 nm due to the interconversion of **2** and **14** from the open to the closed form and back to the opened form. Bottom: Absorption changes at λ = 450 nm due to irradiation cycles between the open and closed form of **2** and **14**.

yields the characteristic absorption of the closed form at λ = 450 nm, and irradiation at the latter wavelength drives the reaction back to the open form of the switch in **DNA1**. It is important to note here that the kinetics for opening and closing of **2** cannot directly be compared to **DNA1** since the solvent in both measurements is different. **DNA1** bearing the open form of the diarylethene switch exhibited a melting temperature of 60.6 °C. After irradiation at λ = 310 nm, the melting temperature decreased significantly to 56.8 °C. At the moment this observation lacks any plausible explanation and needs to be further explored in the future.

In conclusion it becomes clear that spiropyrane and diarylethene as well-known molecular switches can be attached covalently to the 5-position of 2′-deoxyuridine. Sonogashira-type cross-couplings represent the key steps in the syntheses of the modified nucleosides **1** and **2** yielding short ethynyl linkers between chromophore and uridine. The photoactive properties of the molecular switches are maintained in the corresponding nucleosides **1** and **2**. The preliminary investigation of the representatively prepared **DNA1** shows clearly that diarylethene-modified 2′-deoxyuridine can be switched from the open to closed form and backwards by irradiation in the DNA environment. The latter result represents one important

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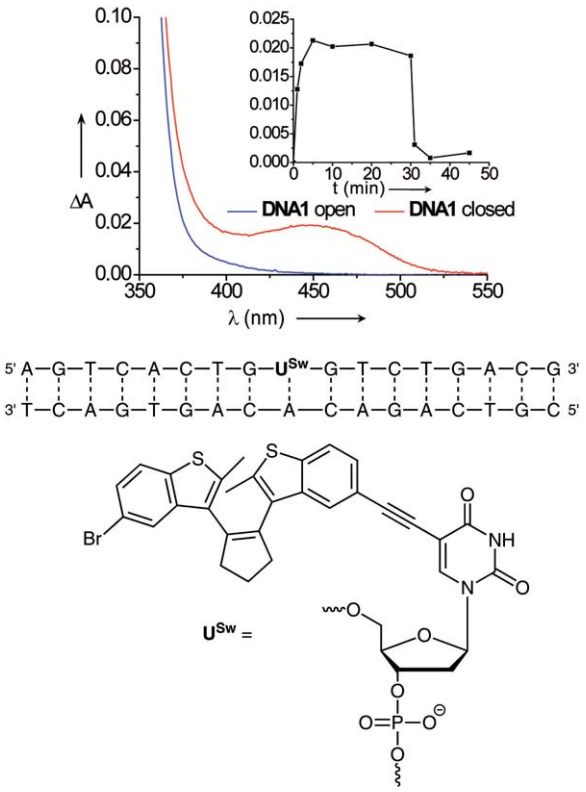

Figure 3 Top: UV/Vis absorption spectra of open form of **DNA1** and interconversion into closed form (20 μM in 50 mM Na-P_i buffer, pH 7, 250 mM NaCl, pH 7) at r.t.; after 30 min irradiation with λ = 310 nm (red), after 30 min irradiation at λ = 450 nm (blue). Inset shows time dependence of absorption change at λ = 450 nm due to the interconversion of **DNA1** from the open to the closed form and back to the opened form. Bottom: Sequence of **DNA1**.

step further in the preparation of photoreactive nanostructures that are based on nucleic acids as structural scaffold.

Acknowledgment

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(20) Compound **5**: Compound **4**[19] (490 mg, 3.35 mmol) was dissolved in anhyd EtOH (50 mL), freshly distilled **3**[19] (0.59 mL, 3.33 mmol) was added in one portion, and the reaction mixture was sonicated at 35 kHz under Ar. After 53 min the solvent was removed under reduced pressure. The crude product was purified by gradient flash chromatography on silica gel (hexane–THF, 70:1 → 50:1) yielding **5** (762 mg, 76%) as a pale blue foam; R_f 0.36 (hexane–THF, 50:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.15–7.27 (m, 2 H, ArH), 7.20 (dt, 1 H, *J* = 1.2, 7.7 Hz, ArH), 7.09 (d, 1 H, *J* = 7.2 Hz, ArH), 6.87 (t, 1 H, *J* = 7.4 Hz, ArH), 6.83 (d, 1 H, *J* = 10.3 Hz, ArH), 6.67 (d, 1 H, *J* = 8.3 Hz, ArH), 6.55 (d, 1 H, *J* = 7.7 Hz, ArH), 5.74 (d, 1 H, *J* = 10.2 Hz, ArH), 2.98 (s, 1 H, C≡CH), 2.74 (s, 3 H, NMe), 1.31 (s, 3 H, Me), 1.18 (s, 3 H, Me). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 155.0, 148.1, 136.6, 133.7, 130.5, 128.8, 127.6, 121.5, 120.3, 119.3, 118.8, 115.2, 113.5, 106.9, 104.8, 83.6, 75.5, 51.9, 28.9, 25.9, 20.1. MS (CI, NH_3): *m/z* (%) = 302.1 (100) [MH^+]. HRMS (EI–MS): *m/z* [M^+] calcd for $C_{21}H_{19}NO$: 301.1467; found: 301.1465.
(21) Compound **8**: Compound **6**[19] (335 mg, 1.11 mmol) was dissolved in freshly distilled EtOH (10 mL) and anhyd Et_3N (200 μL, 1.43 mmol). Compound **7**[19] (220 mg, 1.09 mmol) was added and the reaction mixture was sonicated at 35 kHz under Ar. After 2 h the solvent was removed under reduced pressure, the residue was dissolved in CH_2Cl_2 and dried over anhyd Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was dried in vacuo and purified by gradient flash chromatography on silica gel (hexane–EtOAc, 30:1 → 15:1) to yield **8** (300 mg, 77%) as a pale pink solid; R_f 0.49 (hexane–EtOAc, 19:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.15–7.27 (m, 3 H, ArH), 7.09 (d, 1 H, *J* = 7.2 Hz, ArH), 6.87 (t, 1 H, *J* = 7.4 Hz, ArH), 6.80 (d, 1 H, *J* = 10.3 Hz, ArH), 6.61 (d, 1 H, *J* = 9.2 Hz, ArH), 6.55 (d, 1 H, *J* = 7.7 Hz, ArH), 5.74 (d, 1 H, *J* = 10.2 Hz, ArH), 2.74 (s, 3 H, NMe), 1.32 (s, 3 H, 3-Me), 1.18 (s, 3 H, 3-Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 153.6 (C_{quat}), 148.0 (C_{quat}), 136.5 (C_{quat}), 132.2 (+, CH), 129.1 (+, CH), 128.4 (+, CH), 127.7 (+, CH), 121.5 (+, CH), 120.7 (+, CH), 120.6 (C_{quat}), 119.3 (+, CH), 116.9 (+, CH), 111.8 (C_{quat}), 106.9 (+, CH), 104.5 (C_{quat}), 51.9 (C_{quat}), 28.9 (+, Me), 25.9 (+, Me), 20.1 (+, Me). MS (EI, 70 eV): *m/z* (%) = 357.0(100) [$M^{+\cdot}$], 339.9 (47) [M^+ – $Me^{\cdot}$]. HRMS (EI–MS): *m/z* [M^+] calcd for $C_{19}H_{18}BrNO$: 355.0572; found: 355.0570.
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(23) Compound **1**: Compound **5** (53 mg, 0.176 mmol), **9** (50 mg, 0.141 mmol), CuI (6 mg, 0.0315 mmol) and Pd(dppf)Cl_2 (20 mg, 0.0245 mmol) were dissolved in anhyd DMF (2.0 mL) and anhyd Et_3N (100 μL, 0.717 mmol). The mixture was degassed and stirred at r.t. for 26 h. Then all was poured into an EtOAc–H_2O mixture (20 mL, 1:1). After separation, the aqueous phase was extracted with CH_2Cl_2, and the combined organic layers were dried over anhyd Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was dried in vacuo and purified by gradient flash chromatography on silica gel (CH_2Cl_2–MeOH, 10:1 → 5.1) to yield **1** (50 mg, 67%) as glistening green crystals; R_f 0.29 (CH_2Cl_2–MeOH, 10:1). ^{1}H NMR (600 MHz, MeOD): δ = 8.36 (s, 1 H, H-6), 7.27 (d, 1 H, *J* = 2.0 Hz), 7.23 (dd, 1 H, *J* = 8.4 Hz), 7.10 (dt, 1 H, *J* = 7.6 Hz), 7.03 (d, 1 H, *J* = 7.2 Hz), 6.92 (d, 1 H, *J* = 10.3 Hz), 6.78 (t, 1 H, *J* = 7.1 Hz), 6.61 (d, 1 H, *J* = 8.4 Hz), 6.52 (d, 1 H, *J* = 7.8 Hz), 6.26 (t, 1 H, *J* = 6.6 Hz, H-1′), 5.80 (d, 1 H, *J* = 10.3 Hz), 4.40–4.43 (m, 1 H, H-3′), 3.94 (q, 1 H, *J* = 3.3 Hz, H-4′), 3.83 (dd, 1 H, *J* = 3.0, 12.0 Hz, H-5′), 3.75 (dd, 1 H, *J* = 3.4, 12.0 Hz, H-5′), 2.70 (s, 3 H, NMe), 2.29–2.34 (m, 1 H, H-2′), 2.22–2.28 (m, 1 H, H-2′), 1.26 (s, 3 H, MeC), 1.14 (s, 3 H, Me). ^{13}C NMR (150 MHz, MeOD): δ = 164.4, 156.2, 151.2, 149.5, 144.5 (C6), 137.8, 134.2, 131.1, 130.0, 128.6, 122.4, 121.4, 120.5, 120.4, 116.0, 107.9, 106.3, 100.9, 93.9, 89.1 (C-4′), 87.0 (C-1′), 80.1, 72.0 (C-3′), 62.6 (C-5′), 52.9, 41.8 (C-2′), 29.2 (NMe), 26.3 (Me), 20.4 (Me), 9.3. MS (ESI): *m*/*z* (%) = 528.2 (100) [MH^+].

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(25) Compound **12**: Compound **11** (2.50 g, 11.7 mmol) was dissolved under argon atmosphere in anhyd THF (30 mL) and cooled to –78 °C. A 2 M lithium diisopropylamide solution in THF–heptane–ethylbenzene (8.79 mL, 17.6 mmol) was added dropwise and the mixture was stirred for 1 h at 0 °C. After cooling down to –78 °C, MeI (1.10 mL, 17.6 mmol) was added dropwise. The mixture was stirred for 2 additional hours. Then H_2O (30 mL) was added, the phases were separated and the aqueous phase was extracted with EtOAc. The combined organic layers were dried over anhyd Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was dried in vacuo and purified by flash chromatography on silica gel(hexanes) to yield **12** (2.66 g, 99%) as a white solid; R_f 0.57 (hexanes). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.78 (d, 1 H, *J* = 1.9 Hz, ArH), 7.59 (d, 1 H, *J* = 8.5 Hz, ArH), 7.34 (dd, 1 H, *J* = 8.5, 1.9 Hz, ArH), 6.90 (s, 1 H, ArH), 2.59 (d, 3 H, *J* = 1.2 Hz, Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 143.1 (C_{quat}), 142.2 (C_{quat}), 138.4 (C_{quat}), 126.5 (C_{arom}), 125.3 (C_{arom}), 123.4 (C_{arom}), 121.0 (C_{arom}), 118.2 (C_{arom}), 16.3 (Me). MS (EI, 70 eV): *m*/*z* (%) = 228.0(100) [M^+], 147.1 (24) [M – Me].

(26) Compound **13**: Compound **12** (969 mg, 4.3 mmol) and glutaryl chloride (0.27 mL, 2.1 mmol) were dissolved under argon atmosphere in anhyd CH_2Cl_2 (20 mL) and the solution was cooled to 0 °C. Then $AlCl_3$ (626 mg 4.7 mmol) was added and the reaction mixture was stirred for 3 h at r.t. After completion of the reaction, a mixture of ice and 1 M aq HCl was added. The solution was let to be warmed to r.t., the phases were separated and the aqueous phase was extracted with EtOAc. The combined organic layers were dried over anhyd Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was dried in vacuo and purified by flash chromatography on silica gel (hexanes–EtOAc, 10:1) to yield **13** (896 mg, 74%) as a white solid; R_f 0.31 (hexanes–EtOAc, 10:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.36 (d, 2 H, *J* = 1.8 Hz, ArH), 7.57 (d, 2 H, *J* = 8.5 Hz, ArH), 7.42 (dd, 2 H, *J* = 8.5, 1.8 Hz, ArH), 3.06 (t, 4 H, *J* = 6.9 Hz, CH_2), 2.77 (s, 6 H, Me), 2.27 (quin, 2 H, *J* = 7.0 Hz, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.6 (CO), 146.1 (C_{quat}), 142.9 (C_{quat}), 140.5 (C_{quat}), 136.5 (C_{quat}), 127.2 (C_{arom}), 125.0 (C_{arom}), 123.4 (C_{arom}), 118.8 (C_{arom}), 28.6 (CH_2), 19.6 (CH_2), 15.3 (Me). MS (EI, 70 eV): *m*/*z* (%) = 549.9 (14) [M^+].

(27) Compound **14**: To a suspension of zinc dust (301 mg, 4.6 mmol, stabilized, grading <63 μm) in anhyd THF (50 mL) $TiCl_4$ (0.26 mL, 2.3 mmol) was added under argon atmosphere dropwise at 0 °C. The suspension was heated to 40 °C and stirred for 1 h. Compound **13** (631 mg, 1.1 mmol) was added and the mixture was stirred for 18 h. After cooling to r.t. the reaction mixture was poured through a silica gel pallet and rinsed with hexanes. The solvent was removed under reduced pressure and the residue was dried in vacuo and purified by flash chromatography on silica gel(hexanes) to yield **14** (328 mg, 55%) as a white solid; R_f 0.25 (hexanes). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.64 (s, 2 H, ArH), 7.46 (d, 2 H, *J* = 8.4 Hz, ArH), 7.24 (s, 2 H, ArH), 2.88–3.02 (m, 4 H, CH_2), 2.28 (quin, 2 H, *J* = 7.22 Hz, CH_2), 2.12 (s, 6 H, Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 140.9 (C_{quat}), 137.0 (C_{quat}), 136.8 (C_{quat}), 129.72 (C_{arom}), 126.5 (C_{arom}), 125.1 (C_{arom}), 123.3 (C_{arom}), 118.1 (C_{arom}), 37.8 (CH_2), 24.1 (CH_2), 15.2 (Me). MS (EI, 70 eV): *m*/*z* (%) = 517.9 (100) [M^+].

(28) Compound **15**: Compound **14** (131 mg, 0.25 mmol), trimethylsilylacetylene (72 μL, 0.51 mmol), anhyd Et_3N (41 μL, 0.51 mmol), Pd(PPh_3)$_4$ (30 mg, 0.03 mmol) and CuI (5 mg, 0.03 mmol) were dissolved in anhyd THF (3 mL) under argon. The mixture was degassed and refluxed for 19 h. After cooling to r.t. the solvent was removed under reduced pressure, the residue was dried in vacuo and purified by flash chromatography on silica gel(hexanes) to yield **15** (58 mg, 43%) as a white solid; R_f 0.26 (hexanes). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.67 (s, 2 H, ArH), 7.54 (d, 1 H, *J* = 8.2 Hz, ArH), 7.47 (d, 1 H, *J* = 8.0 Hz, ArH), 7.29 (s, 2 H, ArH), 2.83–3.12 (m, 4 H, CH_2), 2.28 (quin, 2 H, *J* = 7.8 Hz, CH_2), 2.04 (s, 6 H, Me), 0.29 (s, 9 H, $SiMe_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 139.1 (C_{quat}), 138.6 (C_{quat}), 137.3 (C_{quat}), 136.7 (C_{quat}), 127.1 (C_{arom}), 126.4 (C_{arom}), 125.9 (C_{arom}), 125.1 (C_{arom}), 123.3 (C_{arom}), 121.9 (C_{arom}), 118.0 (C_{arom}), 105.9 (C≡C), 37.9 (CH_2), 29.9 (C≡C), 24.2 (CH_2), 15.3 (Me), 0.3 ($SiMe_3$). MS (EI, 70 eV): *m*/*z* (%) = 536.0(100) [M^+].

(29) Compound **16**: Compound **15** (35 mg, 0.07 mmol) was dissolved in anhyd MeOH (3 mL) and K_2CO_3 (27 mg, 0.19 mmol) was added. The reaction mixture was stirred for 18 h at 40 °C. After cooling to r.t. MeOH was removed under reduced pressure. The residue was dried in vacuo and purified by flash chromatography on silica gel (hexanes) to yield **16** (30 mg, 99%) as a white solid; R_f 0.65 (hexanes–EtOAc, 20:1) ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.71 (s, 2 H, ArH), 7.57 (d, 2 H, *J* = 8.0 Hz, ArH), 7.46 (d, 1 H, *J* = 7.5 Hz, ArH), 7.28 (s, 1 H, ArH), 3.07 (s, 1 H, C≡CH), 2.84–3.01 (m, 4 H, CH_2), 2.28 (quin, 2 H, *J* = 8.0 Hz, CH_2), 2.02 (s, 6 H, Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 146.8 (C_{quat}), 139.2 (C_{quat}), 137.2 (C_{quat}), 136.9 (C_{quat}), 126.9 (C_{arom}), 126.4 (C_{arom}), 125.1 (C_{arom}), 123.3 (C_{arom}), 122.0 (C_{arom}), 118.1 (C_{arom}), 117.6 (C_{arom}), 76.5 (C≡C), 37.8 (CH_2), 29.9 (C≡C), 24.1 (CH_2), 15.2 (Me). MS (EI, 70 eV): *m*/*z* (%) = 461.9 (100) [M^+].

(30) Compound **2**: Compound **16** (126 mg, 0.27 mmol), **9** (97 mg, 0.27 mmol), anhyd Et_3N (45 μL, 0.54 mmol), Pd(PPh_3)$_4$ (63 mg, 0.05 mmol) und CuI (11 mg, 0.05 mmol) were dissolved in anhyd DMF (10 mL). The mixture was degassed and stirred for 18 h at 60 °C. After cooling to r.t. the solvent was removed under reduced pressure, the residue was dried in vacuo and purified by flash chromatography on silica gel (CH_2Cl_2–MeOH, 25:1) to yield **2** (121 mg, 65%) as a light yellow foam; R_f 0.40 (CH_2Cl_2–MeOH, 10:1). ^{1}H NMR (300

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MHz, MeOD): δ = 8.37 (s, 1 H, NH), 7.72 (s, 2 H, ArH), 7.58–7.68 (m, 1 H, C=CH), 7.55 (d, 1 H, *J* = 8.2 Hz, ArH), 7.46 (d, 1 H, *J* = 6.8 Hz, ArH), 6.95–7.29 (m, 2 H, ArH), 6.26 (t, 1 H, *J* = 6.2 Hz, 1′-H), 4.28–4.50 (m, 1 H, 3′-H), 3.88–4.03 (m, 1 H, 4′-H), 3.78–3.87 (m, 1 H, 5′-H), 3.66–3.78 (m, 1 H, 5′-H), 2.19–2.61 (m, 4 H, CH_2), 2.00–2.48 (m, 9 H, 2′-H, CH_2, Me), 1.95 (s, 1 H, 2′-H). ^{13}C NMR (75 MHz, MeOD): δ = 151.2 (CO), 144.7 (C=C), 140.4 (CO), 139.8 (C_{quat}), 138.6 (C_{quat}), 138.5 (C_{quat}), 138.1 (C_{quat}), 131.3 (C_{quat}), 127.3 (C_{arom}), 126.0 (C_{arom}), 124.3 (C_{arom}), 122.9 (C_{arom}), 118.9 (C_{arom}), 94.5 (C≡C), 89.2 (4′-C), 87.1 (1′-C), 72.1 (3′-C), 62.6 (5′-C), 41.8 (2′-C), 38.6 (CH_2), 25.0 (CH_2), 15.1 (Me). MS (FAB): *m*/*z* (%) = 689.1 (6) [M^+]. HRMS (ESI): *m*/*z* [M + H^+] calcd for $C_{34}H_{30}BrN_2O_5S_2$: 691.0759; found: 691.0766.

(31) Compound **17**: Compound **2** (50 mg, 0.07 mmol), dimethoxytrityl chloride (37 mg, 0.11 mmol) and anhyd Et_3N (10 μL, 0.12 mmol) were dissolved in anhyd pyridine (2 mL) under argon atmosphere. The solution was stirred for 18 h at 40 °C. The solvent was removed, the residue was dried in vacuo and purified by flash chromatography on silica gel (CH_2Cl_2–MeOH, 100:3 + 0.1% Et_3N) to yield **17** (62 mg, 90%) as a light yellow foam; R_f 0.61 (CH_2Cl_2–MeOH, 10:1). ^{1}H NMR (300 MHz, DMSO): δ = 8.05 (s, 1 H, NH), 7.70 (d, 2 H, *J* = 8.2 Hz, ArH_{bt}), 7.63 (s, 1 H, C=CH), 7.43 (d, 2 H, *J* =7.4 Hz, ArH_{bt}), 7.18–7.37 (m, 8 H, ArH_{DMT}), 7.14 (d, 2 H, *J* = 7.3 Hz, ArH_{bt}), 6.75–6.96 (m, 5 H, ArH_{DMT}), 6.16 (t, 1 H, *J* = 6.7 Hz, 1′-H), 4.26–4.34 (m, 1 H, 3′-H), 3.93–398 (m, 1 H, 4′-H), 3.66–3.80 (m, 2 H, 5′-H), 3.61 (d, 6 H, *J* = 2.5 Hz, $CH_{3,DMT}$), 2.79–2.93 (m, 4 H, CH_2), 2.06–2.37 (m, 9 H, 2′-H, CH_2, Me), 1.95–2.03 (m, 1 H, 2′-H). ^{13}C NMR (75 MHz, acetone): δ = 163.9 (C_{quat}), 161.9 (C_{quat}), 159.6 (C_{quat}), 157.7 (C_{quat}), 150.4 (CO), 146.0 (C_{quat}), 143.1 (C=C), 140.3 (CO), 139.0 (C_{quat}), 138.1 (C_{quat}), 137.9 (C_{quat}), 136.6 (C_{quat}), 131.0 (C_{arom}), 128.9 (C_{arom}), 127.6 (C_{arom}), 127.1 (C_{arom}), 126.0 (C_{arom}), 122.7 (C_{arom}), 114.1 (C≡C), 87.7 (4′-C), 86.6 (1′-C), 64.8 (5′-C), 58.4 (3′-C), 55.4 (2′-C), 46.4 (5′-C), 41.9 (s), 38.3 (CH_2), 24.4 (CH_2), 15.4 (Me), 8.9 (Me). MS (ESI): *m*/*z* (%) = 1015.2 (4)[(M + $Na)^+$].

(32) Compound **18**: Compound **17** (62 mg, 0.06 mmol), diisopropylethylamine (36 μL, 0.16 mmol) and 2-cyanoethyldiisopropylphosphoramidite (27 μL, 0.16 mmol) were dissolved in anhyd CH_2Cl_2 (3 mL). The solution was stirred at r.t. for 40 min. The product was purified by flash chromatography on silica gel (CH_2Cl_2–MeOH, 100:4 + 0.1% Et_3N) and additionally lyophilized out of benzene to yield **18** (74 mg, 99%) as a light yellow foam; R_f 0.85, 0.91 (CH_2Cl_2–MeOH, 25:1). ^{31}P NMR (101 MHz, DMSO): δ = 150.6 [P(III), 37%], 16.3 [P(V), 63%]. MS (MALDI): *m*/*z* (%) = 1191.3 (28) [M^+].

(33) **DNA 1**: MS (MALDI): *m*/*z* (%) = 5950.0(100) [(M + $DMT)^+$], 5647.9 (38) [M^+], 2974.6 (17) [(M + $DMT)^{2+}$/2], 2823.6 (7) [M^{2+}/2].

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