Supporting Information

for

A postsynthetically 2’-“clickable” uridine with arabino configuration and its application for fluorescent labeling and imaging of DNA

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Additional data and spectra

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1. Images of NMR and MS analyses

Compound 5

Figure S1: $^1$H NMR spectrum of 5.
Figure S2: $^{13}$C NMR spectrum of 5.

Figure S3: MS (FAB) analysis of 5.
**Figure S4:** HR-MS (FAB) analysis of 5.

**Compound 2**

![Compound 2](image)

**Figure S5:** $^1$H NMR spectrum of 2.
Figure S6: $^{13}$C NMR spectrum of 2.

Figure S7: MS (FAB) analysis of 2.
Compound 6

Figure S8: $^1$H NMR spectrum of 6.
Figure S9: $^{13}$C NMR spectrum of 6.

Figure S10: MS (FAB) analysis of 6.
Figure S11: HR-MS (FAB) analysis of 6.

### Compound 7

![Chemical structure of Compound 7](image)

Figure S12: MS (APCI) analysis of 7.

<table>
<thead>
<tr>
<th>m/z</th>
<th>Intensity</th>
<th>Relative Intensity</th>
<th>Theo. Mass (amu)</th>
<th>Delta Mass (amu)</th>
<th>Composition</th>
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<td>585.2231</td>
<td>109150.0</td>
<td>100.00</td>
<td>585.2231</td>
<td>-0.04</td>
<td>C_{13}H_{11}O_{3}N_{2}</td>
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</table>
2. HPLC purification of modified DNA and images of HPLC analytics

The labelled DNA strands were purified via HPLC Reversed Phase Supelcosil™ LC-C18 column (250 × 10 mm, 5 µm) on a Shimadzu HPLC system (autosampler, SIL-10AD, pump LC-10AT, controller SCL-10A, diode array detector SPD-M10A) using the following conditions:

- eluent A: NH₄OAc buffer (0.05 M in water, pH 6.5)
- eluent B: acetonitrile
- flow rate: 2.5 mL/mL

For gradients see Table S1: UV–vis detection at 260 nm and the characteristic absorption wavelengths for each dye which are listed below:

- DNA2aD1/DNA2rD1: 385 nm
- DNA2aD2/DNA2rD2: 459 nm
- DNA2aD3/DNA2rD3: 444 nm
- DNA2aD4/DNA2rD4: 461 nm
- DNA3aD5/DNA3rD5: 497 nm
- DNA3aD6/DNA3rD6: 509 nm
- DNA3aD7/DNA3rD7: 542 nm
- DNA3aD8/DNA3rD8: 585 nm
- DNA3aD9/DNA3rD9: 530 nm

Table S1: HPLC gradients for semi-preparative purification of oligonucleotides DNA2aD1–DNA2aD9 and DNA2rD1 – DNA2rD9. [a] DNA1a/DNA1r; [b] DNA2aD1/DNA2rD1; [c] DNA3aD6/DNA3rD6; [d] DNA2aD2/DNA2rD2/DNA2aD3/DNA2rD3/DNA2aD4/DNA2rD4/DNA3aD5/DNA3rD5/DNA3aD8/DNA3rD8; [e] DNA3aD7/DNA3rD7/DNA3aD9/DNA3rD9.

<table>
<thead>
<tr>
<th>time [min]</th>
<th>eluent B [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>45</td>
<td>8[a]/10[b]/12[c]/15[d]/17[e]</td>
</tr>
<tr>
<td>66</td>
<td>80</td>
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<td>75</td>
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<td>76</td>
<td>0</td>
</tr>
<tr>
<td>85</td>
<td>0</td>
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</table>

Analytical HPLC of the purified DNA samples were performed with reversed phase Supelcosil™ LC-C18 column (250 × 4.5 mm, 5 µm) on a Shimadzu HPLC system (autosampler, SIL-10AD, pump LC-10AT, controller SCL-10A, diode array detector SPD-M10A) using the following conditions:
eluent A: NH₄OAc buffer (0.05 M in water, pH 6.5)
eluent B: acetonitrile
flow rate: 1.0 mL/min

For gradients see Table S2. UV–vis detection at 260 nm and the characteristic absorption wavelengths for each dye (see chapter 0)

Table S2: HPLC-gradients for analytical determination of purified oligonucleotides DNA1a, DNA1r, DNA2aD1–DNA2aD4, DNA2rD1–DNA2rD4, DNA3aD5–DNA2aD9 and DNA3rD5–DNA3rD9.

<table>
<thead>
<tr>
<th>time [min]</th>
<th>eluent B [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>80</td>
</tr>
<tr>
<td>71</td>
<td>0</td>
</tr>
<tr>
<td>75</td>
<td>0</td>
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Figure S13: HPLC analysis of purified DNA1r.

Figure S14: HPLC analysis of purified DNA1a.

Figure S15: HPLC analysis of purified DNA2aD1.
Figure S16: HPLC analysis of purified DNA2rD1.

Figure S17: HPLC analysis of purified DNA2aD2.

Figure S18: HPLC analysis of purified DNA2rD2.
Figure S19: HPLC analysis of purified DNA2aD3.

Figure S20: HPLC analysis of purified DNA2rD3.

Figure S21: HPLC analysis of purified DNA2aD4.
Figure S22: HPLC analysis of purified DNA2rD4.

Figure S23: HPLC analysis of purified DNA3aD5.

Figure S24: HPLC analysis of purified DNA3rD5.
Figure S25: HPLC analysis of purified DNA3aD6.

Figure S26: HPLC analysis of purified DNA3rD6.

Figure S27: HPLC analysis of purified DNA3aD7.
Figure S28: HPLC analysis of purified DNA3rD7.

Figure S29: HPLC analysis of purified DNA3aD8.

Figure S30: HPLC analysis of purified DNA3rD8.
**Figure S31**: HPLC analysis of purified DNA3aD9.

**Figure S32**: HPLC analysis of purified DNA3rD9.
3. Images of MALDI-TOF MS analyses of modified DNA

**Figure S33**: MALDI-TOF MS analysis of DNA1a; calculated: 6153.2 Da, found: 6158.2 Da.

**Figure S34**: MALDI-TOF MS analysis of DNA2aD1; calculated: 6445.2 Da, found: 6451.1 Da.
Figure S35: MALDI-TOF MS analysis of DNA2rD1; calculated: 6445.2 Da, found: 6446.8 Da.

Figure S36: MALDI-TOF MS analysis of DNA2aD2; calculated: 6471.2 Da, found: 6475.5 Da.
Figure S37: MALDI-TOF MS analysis of DNA2rD2; calculated: 6471.2 Da, found: 6472.0 Da.

Figure S38: MALDI-TOF MS analysis of DNA2aD3; calculated: 6470.2 Da, found: 6476.2 Da.
Figure S39: MALDI-TOF MS analysis of DNA2rD3; calculated: 6470.2 Da, found: 6474.2 Da.

Figure S40: MALDI-TOF MS analysis of DNA2aD4; calculated: 6546.3 Da, found: 6553.7 Da.
Figure S41: MALDI-TOF MS analysis of DNA2rD4; calculated: 6546.3 Da, found: 6548.8 Da.

Figure S42: MALDI-TOF MS analysis of DNA2aD5; calculated: 6550.2 Da, found: 6556.7 Da.
Figure S43: MALDI-TOF MS analysis of DNA2rD5; calculated: 6550.2 Da, found: 6551.6 Da.

Figure S44: MALDI-TOF MS analysis of DNA2aD6; calculated: 6584.3 Da, found: 6590.0 Da.
Figure S45: MALDI-TOF MS analysis of DNA2rD6; calculated: 6584.3 Da, found: 6586.0 Da.

Figure S46: MALDI-TOF MS analysis of DNA2aD7; calculated: 6597.3 Da, found: 6603.8 Da.
Figure S47: MALDI-TOF MS analysis of DNA2rD7; calculated: 6597.3 Da, found: 6597.9 Da.

Figure S48: MALDI-TOF MS analysis of DNA2aD8; calculated: 6577.2 Da, found: 6584.4 Da.
Figure S49: MALDI-TOF MS analysis of DNA2rD8; calculated: 6577.2 Da, found: 6580.2 Da.

Figure S50: MALDI-TOF MS analysis of DNA2aD9; calculated: 6520.2 Da, found: 6525.9 Da.
Figure S51: MALDI-TOF MS analysis of DNA2rD9; calculated: 6520.2 Da, found: 6521.8 Da.
4. Optical spectra for single- and double stranded modified DNA

Figure S52: Fluorescence (left) and absorption (right) of single- (solid line) and double stranded (dotted line) DNA modified with D1–D4.
Figure S53: Fluorescence (left) and absorption (right) of single- (solid line) and double-stranded (dotted line) DNA modified with D5–D9.
Figure S54: Fluorescence (left) and absorption (right) of single- (dotted line) and double-modified (solid line) DNA modified with D1 as donor dye and D5–D9 as acceptor dyes.
Figure S55: Fluorescence (left) and absorption (right) of single- (dotted line) and double-modified (solid line) DNA modified with D2 as donor dye and D7–D9 as acceptor dyes.
Figure S56: Fluorescence (left) and absorption (right) of single- (dotted line) and double-modified (solid line) DNA modified with D3 as donor dye and D7–D9 as acceptor dyes.
Figure S57: Fluorescence (left) and absorption (right) of single- (dotted line) and double-modified (solid line) DNA modified with D4 as donor dye and D7–D9 as acceptor dyes.
5. Melting temperatures of modified DNA double strands

Table S3: Melting temperatures $T_m$.

<table>
<thead>
<tr>
<th>DNA3a and DNA3r</th>
<th>D1 $T_m$ [°C]</th>
<th>D2 $T_m$ [°C]</th>
<th>D3 $T_m$ [°C]</th>
<th>D4 $T_m$ [°C]</th>
<th>- $T_m$ [°C]</th>
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<tbody>
<tr>
<td>D5 a-a</td>
<td>63.0</td>
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<td>D6 a-a</td>
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<td>r-r</td>
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</tr>
</tbody>
</table>
6. Additional cell images

Figure S58: Confocal microscopy of HeLa cells after transfection with DNA2aD1–DNA3rD5 (row 1), DNA2rD1–DNA3aD8 (row 2), DNA2aD2–DNA3aD8 (row 3) and DNA2rD4–DNA2aD8 (row 4). The visualization was performed using a Leica TCS-SPE (DMi8) inverted microscope with an ACS APO 63×/1.30 oil objective. For DNA2aD1–DNA3rD5 λ<sub>ex</sub> = 405 nm (UV laser), λ<sub>em</sub> = 435–470 nm (blue) and 575–750 nm (yellow), for DNA2rD1–DNA3aD8 λ<sub>ex</sub> = 405 nm (UV laser), λ<sub>em</sub> = 415–550 nm (blue) and 575–750 nm (red), for DNA2aD2–DNA3aD8 λ<sub>ex</sub> = 488 nm (argon ion laser), λ<sub>em</sub> = 490–550 nm (green) and 550–675 nm (red), for DNA2rD4–DNA2aD8 λ<sub>ex</sub> = 488 nm (argon ion laser), λ<sub>em</sub> = 490–550 nm (green) and 675–800 nm (red).
6. Cytotoxicity in HeLa cells

To determine the toxicity of the respective fluorophores (used in the DNA FRET pair constructs) in HeLa cells, the viability was tested using the CellTiter 96® Non-Radioactive Cell Proliferation Assay (Promega) according to the manufacturer’s instructions. This assay is based on the intracellular reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazoliumbromide), MTT) into a violet formazan product by mitochondrial dehydrogenases, which only takes place in metabolic active cells. Therefore the amount of the generated formazan which can be determined by absorbance measurements is directly linked to cell viability.

$1 \times 10^4$ HeLa cells were seeded in each well of a 96 well plate (Costar 3596, 96 Well Cell Culture Cluster, sterile) and cultured in 100 µL Dulbecco’s modified Eagle’s medium (DMEM, Gibco) supplemented with 10% fetal calf serum (FCS, Sigma-Aldrich) and 1% penicillin/streptomycin at 37 °C, 5% CO₂. After 24 hours the cells were treated with different concentrations of the dyes D1, D2, D4, D5, D7 and D8 (0.0375–0.30 µM). For each amount 6 wells were prepared and as a positive control (living cells) 6 wells were treated with respected DMSO concentrations to 0.30 µM dye samples. After an incubation time of 72 h another 6 wells were treated with 5 µL of 20% Triton X-100 (Serva) as a negative control for 100% dead cells for 5 min before all cells were treated with 15 µL of MTT reagent (Dye Solution according to manufacturer’s instructions) per well and incubated for 2.5 h. Subsequently, 100 µL Solubilization Solution/Stop Mix was added to each well to lyse cells and dissolve the formed formazan crystals. After 24 h incubation at 37 °C the absorbance was measured at 595 nm using a 96-well plate reader (Ultra Microplate Reader ELx808, BioTEK Instruments, INC). Mean values and standard deviation were calculated from $n = 6$ experiments.

![Cytotoxicity test with dyes D1, D2, D4, D5 and D8 that were applied for imaging experiments with live cells at two different concentrations (0.0150 µM and 0.075 µM).](image)

**Figure S59:** Cytotoxicity test with dyes D1, D2, D4, D5 and D8 that were applied for imaging experiments with live cells at two different concentrations (0.0150 µM and 0.075 µM).