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Syntheses and characterization of building blocks

Phosphoramidite building blocks

The syntheses of **1**[1], **16a**[1, 2] and **17a**[3] were reported previously. The synthesis of **16b** was reported previously,[4] but without any analytical data given.

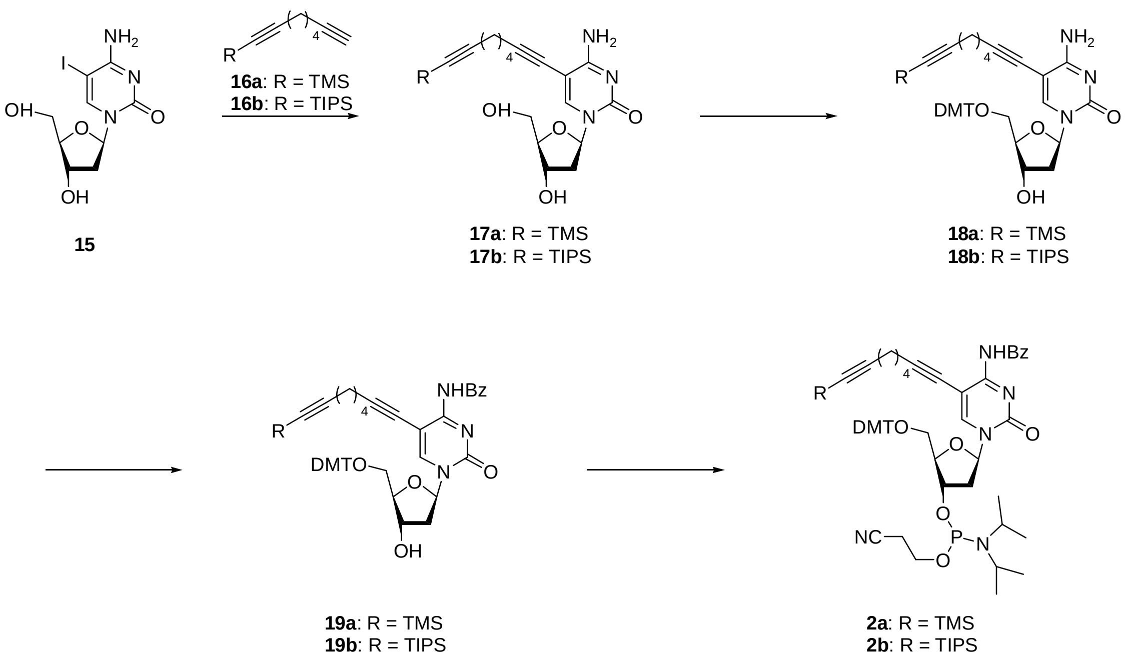

Fig. S1: Syntheses of building blocks **2a** and **2b**.

16b: To a solution of 1,7-octadiyne (3.10 mL, 23.3 mmol) in dry THF (50 mL) at -78 °C was added LiHMDS (1.0 M in THF, 23.3 mL). The solution was stirred for 30 min, and TIPS-Cl (4.95 mL, 23.3 mmol) was added over 1 h. The solution was warmed to room temperature and stirred for 2.5 h, quenched with water (100 mL), extracted with diethyl ether and washed with 1 M HCl. The organic layer was dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was distilled (bath temp = 115-135 °C, HV pump) to give 2.82 g (10.8 mmol, 46%) of **16b** as a colorless liquid. ^{1}H-NMR (CDCl$_3$, 600 MHz): δ 1,04-1,08 (m, 21H, *i*Pr), 1.63-1.70 (m, 4H, 2 × $\mathrm{CH_2}$), 1.94 (t, 1H, *J* = 2.6 Hz, HCC), 2.23 (dt, 2H, *J* = 2.6 Hz, *J* = 6.6 Hz, H_2CCCH), 2.29 (t, 1H, *J* = 6.6 Hz, $\mathrm{H_2CCCSi}$). ^{13}C-NMR ($\mathrm{CDCl_3}$, 75 MHz) δ 11.3 (CH), 17.9 ($\mathrm{CH_2}$), 18.6 ($\mathrm{CH_3}$), 19.3 ($\mathrm{CH_2}$), 27.4 ($\mathrm{CH_2}$), 27.7 ($\mathrm{CH_2}$), 68.4, 80.5, 84.1, 108.5. HRMS (EI): calcd. for $\mathrm{C_{10}H_{17}Si^+}$ $[\mathrm{M}]^+$: 262.2117, found: 262.2131.

17b: To a solution of **15** (2.00 g, 5.66 mmol), $\mathrm{Pd(PPh_3)_4}$ (870 mg, 0.750 mmol) and CuI (280 mg, 1.47 mmol) in DMF (40 mL) was added triethylamine (2.10 mL, 15.0 mmol). **16b** (3.00 g, 11.45 mmol) was added to the reaction mixture over 5 min. After complete addition, the reaction mixture was stirred at room temperature overnight. The solvent was removed *in vacuo* and the residue was directly applied to a flash column chromatography ($\mathrm{CH_2Cl_2}$/MeOH 12:1). The product was obtained as a pale yellow powder (2.09 g, 4.28 mmol, 76%). ^{1}H-NMR ($\mathrm{CD_3OD}$, 400 MHz): δ 1.05-1.10 (m, 21H, *i*Pr), 1.63-1.72 (m, 2H, $\mathrm{CH_2}$), 1.73-1.81 (m, 2H, $\mathrm{CH_2}$), 2.13 (td, 1H, *J* = 6.5 Hz, *J* = 13.3 Hz, $\mathrm{C_{2'}H}$), 2.30-2.42 (m, 3H, $\mathrm{CH_2}$, $\mathrm{C_{2'}H}$,), 2.49 (t, 2H, *J* = 6.9 Hz, $\mathrm{CH_2}$), 3.73 (dd, 1H, *J* = 3.7 Hz, *J* = 12.0 Hz, $\mathrm{C_{5'}H}$), 3.82 (dd, 1H, *J* = 3.2 Hz, *J* = 12.0 Hz, $\mathrm{C_{5'}H}$), 3.94 (q, 1H, *J* = 3.5 Hz, $\mathrm{C_{4'}H}$), 4.36 (td, 1H, *J* = 3.8 Hz, *J* = 6.4 Hz, $\mathrm{C_{3'}H}$), 6.21 (t, 1H, *J* = 6.4 Hz, $\mathrm{C_{1'}H}$), 8.23 (s, 1H, NH). ^{13}C-NMR ($\mathrm{CD_3OD}$, 100 MHz): δ 12.5 (CH), 19.1 ($\mathrm{CH_3}$), 19.7 ($\mathrm{CH_2}$), 20.0 ($\mathrm{CH_2}$), 28.7 ($\mathrm{CH_2}$), 29.1 ($\mathrm{CH_2}$), 42.4 ($\mathrm{CH_2}$), 62.6 ($\mathrm{CH_2}$), 71.8 (CH), 72.3, 81.3, 87.9 (CH), 89.1 (CH), 93.6, 97.1, 110.2, 145.0 (CH), 156.8, 166.6. HRMS (ESI): calcd. for $\mathrm{C_{26}H_{42}N_3O_4Si^+}$ $[\mathrm{M+H}]^+$: 488.2939, found: 488.2936.

18a: **17a** (1.00 g, 2.48 mmol) was co-evaporated twice with pyridine and dissolved in dry pyridine (14 mL). The solution was cooled to 0 °C. DMAP (60 mg, 0.49 mmol), 4,4´-dimethoxytrityl chloride (1.09 g, 3.22 mmol) and triethylamine (0.69 mL, 5.0 mmol) were added and the solution stirred for 15 min at 0 °C. The solution was allowed to warm to room temperature and stirred for 5 h. The reaction mixture was quenched with MeOH (30 mL) and the solvent evaporated. The residue was extracted with dichloromethane, washed with aqueous $\mathrm{NaHCO_3}$ solution and dried over $\mathrm{NaSO_4}$. The residue was purified by flash column chromatography ($\mathrm{CH_2Cl_2}$/MeOH/$\mathrm{NEt_3}$ 200:7:1) to give **18a** (1.12 g, 1.69 mmol, 68%) as a white powder. ^{1}H-NMR (acetone, 200 MHz): δ 0.10 (s, 9H, $\mathrm{H_3CSi}$), 1.45-1.52 (m, 4H, 2 × $\mathrm{CH_2}$), 2.11-2.16 (m, 2H, $\mathrm{CH_2}$), 2.18-2.29 (m, 3H, $\mathrm{CH_2}$, $\mathrm{C_{2'}H}$), 2.47 (ddd, 1H, *J* = 2.7 Hz, *J* = 5.9 Hz, *J* = 13.7 Hz, $\mathrm{C_{2'}H}$), 3.32 (dd, 1H, *J* = 3.0 Hz, *J* = 10.5 Hz, $\mathrm{C_{5'}H}$), 3.36 (dd, 1H, *J* = 4.3 Hz, *J* = 10.5 Hz, $\mathrm{C_{5'}H}$), 3.80 (s, 6H, OMe), 4.09-4.13 (m, 1H, $\mathrm{C_{4'}H}$), 4.52-4.57 (m, 1H, $\mathrm{C_{3'}H}$), 6.27 (dd, 1H, *J* = 6.0 Hz, *J* = 7.2 Hz, $\mathrm{C_{1'}H}$), 6.35 (s, 1H, NH/OH), 6.90 (dd, 4H, *J* = 1.6 Hz, *J* = 8.9 Hz, ArH), 7.08 (s, 1H, OH/NH), 7.22-7.27 (m, 1H, ArH), 7.31-7.36 (m, 2H, ArH), 7.39-7.44 (m, 4H, ArH), 7.51-7.55 (m, 2H, ArH), 8.09 (s, 1H, $\mathrm{C_6H}$). ^{13}C-NMR ($\mathrm{CD_3OD}$, 100 MHz): δ 0.3 ($\mathrm{CH_3}$), 19.8 ($\mathrm{CH_2}$), 19.9 ($\mathrm{CH_2}$), 28.5 ($\mathrm{CH_2}$), 28.9 ($\mathrm{CH_2}$), 43.1 ($\mathrm{CH_2}$), 55.8 ($\mathrm{CH_3}$), 64.6 ($\mathrm{CH_2}$), 71.9, 72.5 (CH), 85.2, 88.2, 88.3 (CH), 93.7, 97.5, 108.3, 114.3 (CH), 128.0 (CH), 129.0 (CH), 129.3 (CH), 131.3 (CH), 131.3 (CH), 137.1, 137.2, 144.3 (CH), 146.2, 156.7, 160.2, 160.2, 166.6. HRMS (ESI): calcd. for $\mathrm{C_{41}H_{48}N_3O_6Si^+}$ $[\mathrm{M+H}]^+$: 706.3307, found: 706.3297.

18b: **17b** (1.95 g, 4.00 mmol) was co-evaporated twice with pyridine and dissolved in dry pyridine (20 mL). The solution was cooled to 0 °C. DMAP (100 mg, 0.82 mmol), 4,4´-dimethoxytrityl chloride (2.03 g, 5.23 mmol) und triethylamine (1.11 mL, 7.98 mmol) were added and the solution stirred for 45 min at 0 °C. The solution was allowed to warm to room temperature and stirred for 4 h. The reaction mixture was quenched with MeOH (40 mL) and the solvent evaporated. The residue was extracted with dichloromethane, washed with aqueous $\mathrm{NaHCO_3}$ solution and dried over $\mathrm{NaSO_4}$. The residue was purified by flash column chromatography ($\mathrm{CHCl_3}$/MeOH/$\mathrm{NEt_3}$ 200:5:1) to give **18b** (2.59 g, 3.28 mmol, 82%) as a white powder. ^{1}H-NMR (acetone, 400 MHz): δ 1.03-1.09 (m, 21H, *i*Pr), 1.47-1.60 (m, 4H, 2 × $\mathrm{CH_2}$), 2.16-2.32 (m, 5H, 2 × $\mathrm{CH_2}$, $\mathrm{C_{2'}H}$), 2.46 (ddd, 1H, *J* = 2.8 Hz, *J* = 5.9 Hz, *J* = 13.4 Hz, $\mathrm{C_{2'}H}$), 3.32 (dd, 1H, *J* = 3.0 Hz, *J* = 10.5 Hz, $\mathrm{C_{5'}H}$), 3.36 (dd, 1H, *J* = 4.3 Hz, *J* = 10.5 Hz, $\mathrm{C_{5'}H}$), 3.79 (s, 6H, OMe), 4.08-4.13 (m, 1H, $\mathrm{C_{4'}H}$), 4.50-4.56 (m, 1H, $\mathrm{C_{3'}H}$), 6.26 (dd, 1H, *J* = 6.1 Hz, *J* = 7.1 Hz, $\mathrm{C_{1'}H}$), 6.90 (dd, 4H, *J* = 1.6 Hz, *J* = 8.9 Hz, ArH), 7.21-7.54 (m, 9H, ArH), 8.08 (s, 1H, $\mathrm{C_6H}$). ^{13}C-NMR (acetone, 100 MHz): δ 13.0 (CH), 20.0 ($\mathrm{CH_3}$), 20.4 ($\mathrm{CH_2}$), 20.6 ($\mathrm{CH_2}$), 29.2 ($\mathrm{CH_2}$), 29.7 ($\mathrm{CH_2}$), 43.6

(CH_2), 56.5 (CH_3), 65.6 (CH_2), 73.2 (CH), 73.3 (CH), 73.6, 81.6, 88.1 (CH), 88.4 (CH), 88.4, 97.1, 110.0, 115.0 (CH), 115.0 (CH), 128.6 (CH), 129.7 (CH), 130.0 (CH), 131.9 (CH), 132.0 (CH), 137.8, 137.9, 145.0 (CH), 147.0, 155.5, 160.6. HRMS (ESI): calcd. for $C_{47}H_{60}N_3O_6Si^+$ $[M+H]^+$: 790.4246, found: 790.4283.

19a: **18a** (555 mg, 0.786 mmol) was co-evaporated twice with pyridine and dissolved in dry pyridine (3 mL). Trimethylsilyl chloride (357 µL, 2.80 mmol) was added and the solution stirred for 2.5 h at room temperature. The reaction mixture was cooled on an ice-water bath while benzoyl chloride (143 µL, 1.23 mmol) was added dropwise. The solution was stirred for 2.5 h at room temperature. The reaction was quenched by addition of methanol (1 mL) and all the solvent removed *in vacuo*. Purification by silica gel chromatography ($CHCl_3$/MeOH/pyridine 200:4:1) gave **19a** (639 mg, 0.788 mmol, 100%) as pale yellow solid. ^{1}H-NMR (acetone, 400 MHz): δ 0.08 (s, 9H, H_3CSi), 1.50-1.66 (m, 4H, 2 × CH_2), 2.17-2.30 (m, 5H, 2 × CH_2, $C_{2'}H$), 2.41-2.58 (m, 1H, $C_{2'}H$), 3.36 (dd, 1H, J = 2.7 Hz, J = 10.7 Hz, $C_{5'}H$), 3.41 (dd, 1H, J = 4.2 Hz, J = 10.7 Hz, $C_{5'}H$), 3.79 (s, 6H, OMe), 4.17 (q, 1H, J = 3.3 Hz, $C_{4'}H$), 4.60-4.65 (m, 1H, $C_{3'}H$), 6.28 (t, 1H, J = 6.6 Hz, $C_{1'}H$), 6.91 (dd, 1H, J = 1.6 Hz, J = 8.7 Hz, ArH), 7.22-7.64 (m, 12H, ArH), 8.26 (s, 1H, C_6H), 8.55-8.63 (m, 2H, ArH). ^{13}C-NMR (acetone, 100 MHz): δ 1.3 (CH_3), 20.5 (CH_2), 20.8 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 43.1 (CH_2), 55.5 (CH_3), 65.4 (CH_2), 70.2 (CH), 70.3, 73.0 (CH), 73.8, 85.8, 88.3, 88.5 (CH), 88.9, 109.0, 115.0 (CH), 115.0 (CH), 128.6 (CH), 129.7 (CH), 129.9 (CH), 130.1 (CH), 130.8 (CH), 131.1 (CH), 131.9 (CH), 131.9 (CH), 134.3 (CH), 137.8, 146.9, 160.6, 160.6, 210.9. HRMS (ESI): calcd. for $C_{48}H_{52}N_3O_7Si^+$ $[M+H]^+$: 810.3569, found: 810.3547.

19b: **18b** (3.00 g, 3.79 mmol) was co-evaporated twice with pyridine and dissolved in dry pyridine (12 mL). Trimethylsilyl chloride (1.46 mL, 11.5 mmol) was added and the solution stirred for 2.5 h at room temperature. The reaction mixture was cooled on an ice-water bath while benzoyl chloride (580 µL, 5.00 mmol) was added dropwise. The solution was stirred for 2.5 h at ambient temperature. The reaction was quenched by addition of methanol (10 mL) and all the solvent removed *in vacuo*. Purification by column chromatography (CH_2Cl_2/pyridine 200:1 → CH_2Cl_2/MeOH/pyridine 200:8:1) gave **19b** (1.68 g, 1.88 mmol, 49%) as pale yellow solid. ^{1}H-NMR (acetone, 400 MHz): δ 1.01-1.07 (m, 21H, *i*Pr), 1.57-1.71 (m, 4H, 2 × CH_2), 2.23-2.29 (m, 4H, 2 × CH_2), 2.41-2.50 (m, 1H, $C_{2'}H$), 2.51 (ddd, 1H, J = 3.5 Hz, J = 6.1 Hz, J = 13.5 Hz, $C_{2'}H$), 3.36 (dd, 1H, J = 2.9 Hz, J = 10.7 Hz, $C_{5'}H$), 3.36 (dd, 1H, J = 4.2 Hz, J = 10.7 Hz, $C_{5'}H$), 3.79 (s, 3H, OMe), 3.79 (s, 3H, OMe), 4.15-4.18 (m, 1H, $C_{4'}H$), 4.60-4.66 (m, 1H, $C_{3'}H$), 6.28 (t, 1H, J = 6.6 Hz, $C_{1'}H$), 6.91 (dd, 4H, J = 1.4 Hz, J = 8.9 Hz, ArH), 7.22-7.61 (m, 12H, ArH), 8.26 (s, 1H, C_6H), 8.32 (d, 2H, J = 7.2 Hz, ArH). ^{13}C-NMR (acetone, 100 MHz): δ 13.0 (CH), 20.0 (CH_3), 20.4 (CH_2), 20.7 (CH_2), 29.2 (CH_2), 29.7 (CH_2), 43.2 (CH_2), 56.5 (CH_3), 65.5 (CH_2), 73.2 (CH), 73.9, 81.7, 88.4 (CH), 88.6, 89.0, 110.9, 115.0 (CH), 115.0 (CH), 128.6 (CH), 129.8 (CH), 129.9 (CH), 130.2 (CH), 131.6 (CH), 131.9 (CH), 132.0 (CH), 134.4 (CH), 137.7, 137.8, 147.0, 160.7. HRMS (ESI): calcd. for $C_{54}H_{64}N_3O_7Si^+$ $[M+H]^+$: 894.4508, found: 894.4503.

2a: **19a** (920 mg, 1.14 mmol) and diisopropylammonium tetrazolide (93 mg, 0.55 mmol) were co-evaporated twice with pyridine and dissolved in dry dichloromethane (57 mL). 2-cyanoethyl N,N´diisopropylchlorophosphoramidite (400 µL, 1.26 mmol) was added and the solution stirred for 3 h at room temperature. The reaction mixture was concentrated *in vacuo* to 1/4 of the original volume. The residue was purified by flash chromatography on passivated silica gel ($CHCl_3$/MeOH/NEt_3 200:4:1). **2a** was obtained as a yellow solid (1.03 g, 1.02 mmol, 90%). ^{1}H-NMR (acetone, 200 MHz): δ 0.08-0.10 (2 × s, 9H, H_3CSi), 1.50-1.65 (m, 4H, 2 × CH_2), 2.15-2.27 (m, 5H, 2 × CH_2, $C_{2'}H$), 2.54-2.70 (m, 3H, H_2CCN, $C_{2'}H$), 2.73-2.82 (m, 2H, H_2COP), 3.41-3.48 (m, 2H, $C_{5'}H$), 3.80 (2 × s, 6H, OMe), 4.22-4.34 (m, 1H, $C_{4'}H$), 4.70-4.84 (m, 1H, $C_{3'}H$), 6.22-6.32 (m, 1H, $C_{1'}H$), 6.88-6.97 (m, 4H, ArH), 7.24-7.60 (m, 12H, ArH), 8.24-8.35 (m, 3H, C_6H, ArH). ^{31}P-NMR (acetone, 81 MHz): δ 149.2, 149.5.

2b: **19b** (134 mg, 0.149 mmol) and diisopropylammonium tetrazolide (15 mg, 88 µmol) were co-evaporated twice with pyridine and dissolved in dry dichloromethane (10 mL). 2-cyanoethyl

N,N´diisopropylchlorophosphoramidite (60 µL, 0.19 mmol) was added and the solution stirred for 3 h at room temperature. The reaction mixture was concentrated *in vacuo* to 1/4 of the original volume. The residue was purified by flash chromatography on passivated silica gel ($CHCl_3$/MeOH/NEt_3 200:4:1). **2b** was obtained as a yellow solid (104 mg, 95 µmol, 63%). ^{1}H-NMR (acetone, 200 MHz): δ 1.02-1.07 (m, 21H, *i*Pr), 1.57-1.68 (m, 4H, 2 × CH_2), 2.20-2.30 (m, 5H, 2 × CH_2, $C_{2'}H$), 2.57-2.69 (m, 3H, H_2CCN, $C_{2'}H$), 2.73-2.82 (m, 2H, H_2COP), 3.41-3.48 (m, 2H, $C_{5'}H$), 3.80 (s, 6H, OMe), 4.23-4.33 (m, 1H, $C_{4'}H$), 4.69-4.84 (m, 1H, $C_{3'}H$), 6.22-6.33 (m, 1H, $C_{1'}H$), 6.87-6.98 (m, 4H, ArH), 7.21-7.62 (m, 12H, ArH), 8.25 (s, 1H, C_6H), 8.27-8.36 (m, 2H, ArH). ^{31}P-NMR (acetone, 81 MHz): δ 149.2, 149.5.

Syntheses of azide building blocks

3[5]**, 4**[6]**, 5**[7]**, 6**[8], **7**[9], **10**[10], **11**[11], and were prepared according to literature procedures.

Dabcyl azide 8:

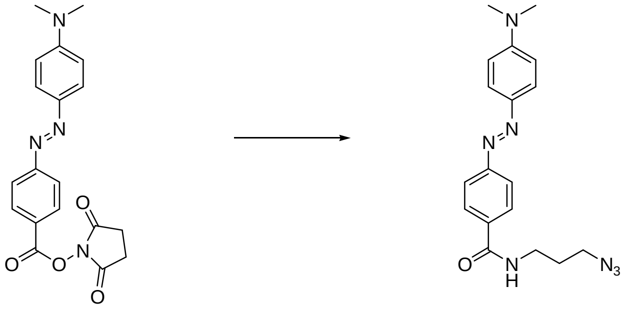

Fig. S3: Synthesis of dabcyl azide **8**.

Dabcyl-*N*-hydroxysuccinimide ester (29 mg, 79 µmol) and DMAP (9.5 mg, 78 µmol) were dissolved in dry pyridine (2 mL). A solution of 3-azido-propylamine (1.3 M in toluene, 95 µL, 124 µmol) was added dropwise and the solution stirred for one day at room temperature. The solvent was removed and the raw product purified by column chromatography (*iso*-hexane/ethyl acetate 3:1) to give 28 mg **8** (79 µmol, 100%) as a red powder.

^{1}H-NMR (600 MHz, $CDCl_3$): *d* 1.93 (quin, 2H, *J* = 6.6 Hz, CH_2), 3.10 (s, 6H, CH_3), 3.46 (t, 2H, *J* = 6.5 Hz, H_2C-N_3), 3.57 (q, 2H, *J* = 6.5 Hz, H_2C-NH), 6.48 (t, 1H, *J* = 5.0 Hz, NH), 6.75 (d, 2H, *J* = 9.1 Hz, ArH), 7.86-7.91 (m, 6H, ArH). ^{13}C-NMR (150 MHz, $CDCl_3$): δ 28.8 (CH_2), 37.9 (CH_2), 40.3 (CH_3), 49.6 (CH_2), 111.4 (CH), 122.2 (CH), 125.4 (CH), 127.7 (CH), 134.3, 143.6, 152.8, 155.1, 167.2. HRMS (EI) calcd. for $C_{18}H_{21}N_7O^+$ $[M]^+$: 351.1802, found: 351.1815.

Pyrene azide 9:

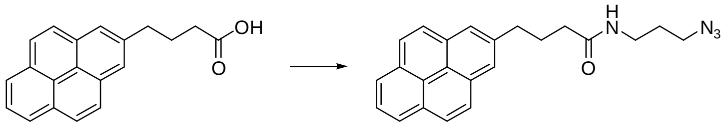

Fig. S4: Synthesis of pyrene azide **9**.

4-Pyren-2-yl-butyric acid (288 mg, 1.00 mmol), HOBt (162 mg, 1.20 mmol) and DCI (176 µL, 1.14 mmol) were dissolved in DMF (5 mL) and stirred for 24 h at room temperature. A solution of 3-azido-propylamine (1.3 M in toluene, 1.15 mL, 1.50 mmol) was added dropwise and the reaction mixture stirred for an additional 48 h. Water (100 mL) was added and the reaction mixture extracted with EtOAc

(2×100 mL). The combined organic phases were washed with water and dried over Na_2SO_4. **9** was obtained as light yellow solid (110 mg, 0.296 mmol, 30%) after column chromatography ($CHCl_3$/MeOH 20:1). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.62 (quin, 2H, $J = 6.7$ Hz, CH_2), 2.10-2.16 (m, 4H, $2 \times CH_2$), 3.15-3.24 (m, 4H, $2 \times CH_2$), 3.28 (t, 2H, $J = 7.3$ Hz, CH_2), 5.72 (t, 1H, $J = 5.6$ Hz, NH), 7.77 (d, 1H, $J = 7.8$ Hz, ArH), 7.94-8.00 (m, 3H, ArH), 8.03-8.08 (m, 2H, ArH), 8.13 (d, 2H, $J = 7.7$ Hz, ArH), 8.22 (d, 1H, $J = 9.3$ Hz, ArH). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 27.2 (CH_2), 28.7 (CH_2), 32.6 (CH_2), 35.8 (CH_2), 36.9 (CH_2), 49.2 (CH_2), 123.2 (CH), 124.7 (CH), 124.8 (CH), 125.8 (CH), 126.6 (CH), 127.2 (CH), 127.3 (CH), 127.4 (CH), 128.6, 129.8, 130.8, 131.3, 135.7, 172.7. HRMS (EI) calcd. for $C_{23}H_{22}N_4O^+$ $[M]^+$: 370.1788, found: 370.1775.

Cy3-azide 12:

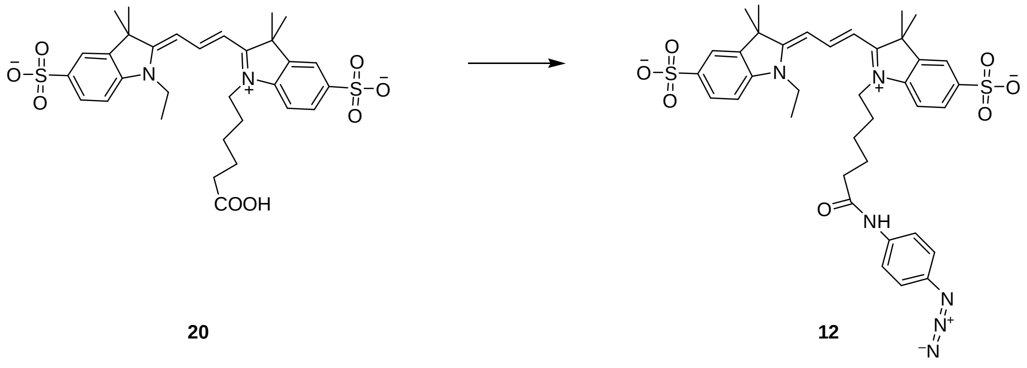

Fig. S5: Synthesis of Cy3 azide **12**.

20 was prepared according to literature procedures.[12] **20** (325 mg, 0.52 mmol), 4-azidoaniline hydrochloride (352 mg, 2.1 mmol) and HATU (785 mg, 2.1 mmol) were dissolved in dry DMF (10 mL) and DIEA (0.5 mL). The solution was stirred under Argon at room temperature overnight. The raw product was precipitated with dry ethyl acetate (50 mL) and separated by RP-HPLC. The separation was performed using 0.1 M $HNEt_3$/OAc buffers (buffer A: H_2O, buffer B: MeCN/H_2O 4:1). The flow rate was 5 mL with a linear gradient from 100% A to 60% A in 45 min. **12** (0.31 mmol, 60%) was eluted at 45.0 min as its bistriethylammonium salt.

^{1}H-NMR (600 MHz, D_2O): δ 1.00-1.03 (m, 2H, CH_2), 1.14 (t, 21H, $J = 7.2$ Hz, CH_3, 2×3 CH_3 (TEA)), 1.33 (s, 12H, $4 \times CH_3$), 1.50-1.55 (m, 2H, CH_2), 2.00-2.08 (m, 2H, CH_2), 3.06 (q, 14H, $J = 7.2$ Hz, H_2CCONHAr, 2×3 CH_2 (TEA)), 3.75-3.90 (m, 4H, α, α'-CH_2), 6.08 (d, 1H, $J = 13.8$ Hz, α proton of the bridge), 6.14 (d, 1H, $J = 13.8$ Hz, α' proton of the bridge), 6.42-6.47 (m, 2H, ArH), 7.04-7.16 (m, 4H, ArH), 7.66-7.73 (m, 4H, ArH), 8.08 (t, 1H, $J = 13.8$ Hz, β proton of the bridge). ^{13}C-NMR (150 MHz, $CDCl_3$): δ 11.7, 24.9, 25.5, 26.7, 27.1, 27.2, 36.3, 39.6, 42.4, 44.2, 49.2, 59.1, 103.2, 103.6, 105.0, 111.2, 111.6, 118.7, 119.1 , 120.0, 121.6, 126.9, 134.9, 140.3, 141.3, 141.4, 143.4, 143.9, 151.5, 175.0, 175.7, 181.6. HRMS (ESI) calc. for $C_{37}H_{41}N_6O_7S_2^+$ $[M]^-$: 745.2489, found: 745.2496.

Solid phase synthesis of alkyne-modified ODNs[1]

ODNs were prepared by the DMT- and β-(cyanoethyl) phosphoramidite method on CPG supports (500 Å) with an *Expedite* DNA synthesizer (*Applied Biosystems*) or on an *Äkta Oligopilot* from *Amersham Biosciences*. A double coupling protocol (10 equivalents each) was applied for the coupling of modified bases and the coupling time was elongated to 10 min. As activator, benzylthiotetrazole (BTT) gave the best coupling yields. After automated synthesis, ODNs were cleaved from the solid support by soaking in concentrated aqueous ammonia/ethanol solution (3:1) for 24 hours at 25 °C. The aqueous ammonia was removed in a SpeedVac, and the crude ODN was purified by RP-HPLC. UV/Vis spectroscopy and MALDI-TOF mass spectrometry were used to characterize the ODNs.

Click-deprotect-click procedure

Click reaction on the resin[a]

Approx. 0.02 µmol DNA on CPG resin was dried under high vacuum after DNA synthesis and placed in a 1.5 mL vial together with 20 µL benzyl azide **3**. In a separate vial, 40 µL CuBr solution (10 mM in DMSO/*t*BuOH 3:1), 10 µL sodium ascorbate (100 mM in water) and 80 µL ligand[13] solution (10 mM in DMSO/*t*BuOH 3:1) were vortexed and added to the DNA. The reaction vial was gently rotated over night,[b] centrifuged and the solution carefully removed and discarded. The resin was washed repeatedly (2 × DMSO, 2 × H_2O, 2 × ethanol) by adding the solvent, vortexing, centrifuging and discarding the supernatant. The DNA was subsequently deprotected as described above.

Click reaction in solution

DNA (0.38 mM, 200 µL) and azide[c] (10 mM, 114 µL) were placed in a 1.5 mL vial. In a separate vial, 17 µL CuBr solution (100 mM in DMSO/*t*BuOH 3:1) and 34 µL ligand[13] solution (100 mM in DMSO/*t*BuOH 3:1) were vortexed and added to the DNA.[d] The solution was shaken at 25 °C for 4 h[e] and evaporated to near-dryness in a SpeedVac. Sodium acetate solution (0.3 M, 100 µL) was added and the suspension left standing for 1 h with occasional vortexing[f]. 1 mL ethanol was added, the vial vortexed and placed in a freezer (-20 °C) over night. After centrifugation (15 min at 13 000 rpm) the supernatant was carefully removed from the DNA pellet. 70% ethanol (-20 °C) was added, the vial vortexed, centrifuged and the supernatant removed. This washing step was repeated twice. After the last washing step the pellet was left drying on air and taken up in water or buffer, as preferred.

Deprotection of the TIPS-alkyne

Lyophilized DNA was dissolved in dry acetonitrile (400 µL) and dry DMF (100 µL). Two drops of TBAF (1.0 M in THF) were added and the solution shaken at 45 °C for 2 h. Excess fluoride ions are quenched with MeOTMS (10 µL). If an additional Click reaction is to be performed on the DNA strand, the organic solvents should be exchanged to water as follows: the reaction solution is evaporated to near-dryness in a SpeedVac. Water (1 mL) is added, the solution frozen, lyophilized to dryness and taken up in an appropriate amount of water.

MALDI-TOF analyses of entries 1-17 of table 2 (main text)

Table S1. Exact and average masses of the functionalized strands **1-17**.

Entry	exact parent mass	average mass	mass found
1	5540.1808	5542.9746	5539.9
2	5035.0095	5037.5220	5034.2
3	5585.1217	5587.9905	5582.5
4	5522.0386	5524.8236	5523.9
5	5464.0390	5466.7413	5465.5

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[a] The following procedures describe Click reactions on ODNs bearing one alkyne. For DNA with more than one alkyne the amounts of reagents have to be adjusted accordingly.

[b] This can conveniently be achieved by attaching the vial onto a rotary evaporator with sticky tape.

[c] Azide solutions should be as concentrated as possible. The azides reported here were all soluble in water, DMSO or DMSO/*t*BuOH (3:1).

[d] No additional solvent is added to achieve complete dissolution of all components.

[e] In order to check the progress of the Click reaction, 1.5 µL of the reaction solution can be removed during the reaction, spotted on a desalting membrane and analyzed by MALDI-TOF MS (HPA matrix).

[f] At this point it is not necessary to obtain a clear solution, the precipitation is performed from the suspension.

6	5515.1526	5517.9868	5515.1
7	5392.0332	5394.7233	5396.7
8	5394.0699	5396.7376	5394.0
9	5559.1794	5562.0160	5560.3
10	5610.1499	5612.9783	5607.8
11	5559.1794	5562.0160	5562.5
12	5752.2679	5755.2832	5756.9
13	5629.1485	5632.0197	5631.0
14	5840.2112	5843.2591	5844.9
15	5921.3497	5924.3996	5921.7
16	5849.3133	5852.2924	5849.5
17	5824.2850	5827.2850	5826.0

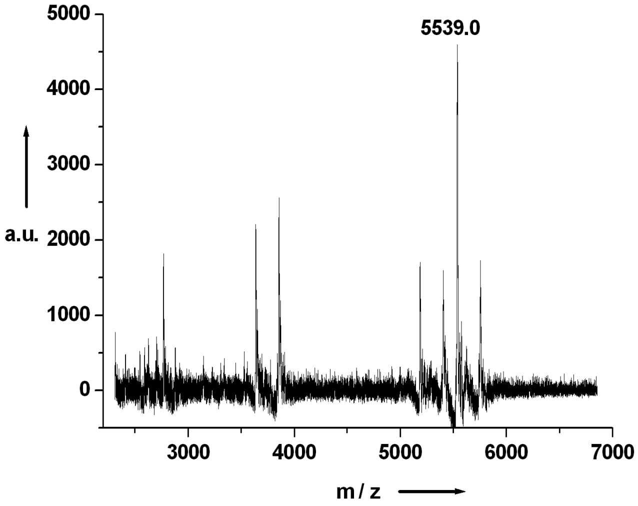

Fig. S6: MALDI-TOF analysis of entry 1.

References

[1] J. Gierlich, G. A. Burley, P. M. E. Gramlich, D. M. Hammond, T. Carell, *Org. Lett.* **2006**, *8*, 3639.

[2] B. M. Trost, M. T. Rudd, *Org. Lett.* **2003**, *5*, 4599

[3] M. Fischler, U. Simon, H. Nir, Y. Eichen, G. A. Burley, J. Gierlich, P. M. E. Gramlich, T. Carell, *Small* **2007**, *3*, 1049.

[4] J. Xiang, W. Jiang, P. L. Fuchs, *Tetrahedron Lett.* **1997**, *38*, 6635; A. Orsini, A. Viterisi, A. Bodlenner, J.-M. Weibel, P. Pale, *Tetrahedron Lett.* **2005**, *46*, 2259.

[5] S. G. Alvarez, M. T. Alvarez, *Synthesis* **1997**, 413.

[6] K. Sivakumar, F. Xie, B. M. Cash, S. Long, H. N. Barnhill, Q. Wang, *Org. Lett.* **2004**, *6*, 4603.

[7] P.-C. Lin, S.-H. Ueng, M.-C. Tseng, J.-L. Ko, K.-T. Huang, S.-C. Yu, A. K. Adak, Y.-J. Chen, C.-C. Lin, *Angew. Chem.* **2006**, *118*, 4392.

[8] L. V. Lee, M. L. Mitchell, S.-J. Huang, V. V. Fokin, K. B. Sharpless, C.-H. Wong, *J. Am. Chem. Soc.* **2003**, *125*, 9588; T. Furuta, H. Torigai, M. Sugimoto, M. Iwamura, *J. Org. Chem.* **1995**, *60*, 3953.

[9] F. Moris-Varas, X.-H. Qian, C.-H. Wong, *J. Am. Chem. Soc.* **1996**, *118*, 7647.

[10] Q. Wang, T. R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless, M. G. Finn, *J. Am. Chem. Soc.* **2003**, *125*, 3192; M. Adamczyk, J. R. Fishpaugh, K. J. Heuser, *Bioconjugate Chem.* **1997**, *8*, 253.

[11] A. E. Speers, B. F. Cravett, *Chem. Biol.* **2004**, *11*, 535.

[12] R. B. Mujumdar, L. A. Ernst, S. R. Mujumdar, C. J. Lewis, A. S. Waggoner, *Bioconjugate Chem.* **1993**, *4*, 105.

[13] T. R. Chan, R. Hilgraf, K. B. Sharpless, V. V. Fokin, *Org. Lett.* **2004**, *6*, 2853.