Electronic Supplementary Material (ESI) for ChemComm.

Control of helical chirality in supramolecular chromophore-DNA architectures

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Supporting Information

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1. Materials

General

All solvents used for synthesis and analysis were p.A (*pro analysi*) grade. All chemicals, which were used for synthesis, had the grade "for synthesis".

Water used for assembly experiments was deionized and ultra filtrated using a *Millipore Direct 8/16* from MERCK MILLIPORE.

Commercially available DNA strands were bought HPLC-purified and lyophilized from METABION. They were dissolved in *MilliQ*-water and used without further purification. Concentration of the resulting solution was measured on a NANODROP *ND-1000* spectrophotometer, using the *Nucleic Acids* mode.

Mass spectrometry

FAB-Mass spectra were recorded on a FINNIGAN *MAT 95* spectrometer. MALDI-TOF spectra were rerecorded either on a BRUKER DALTRONICS *Biflex-IV* or a SHIMADZU *AXIMA Confidence* spectrometer. As matrix 3-HPA (3-Hydroxypicolinic acid (sat. solution in acetonitrile:water = 1:1) / diammonium hydrogen citrate (0.44 M in water) = 9:1). The peaks were reported as mass/charge-ratio (m/z).

NMR

NMR spectra were recorded on a BRUKER *Advance 400* (400 MHz ^{1}H-NMR; 101 MHz. ^{13}C-NMR) or BRUKER *Advance 500* (500 MHz ^{1}H-NMR; 126 MHz. ^{13}C-NMR). For this purpose a NMR-tube with 5 mm diameter was filled with 5-10 mg of the sample and roughly 0.6 mL of the deuterated solvent. The chemical shifts were reported in parts per million (ppm) relative to the tetramethylsilane standard (TMS; δ = 0.00 ppm). The spectrum was calibrated against the residual H-signal of the incompletely deuterated solvents.

[*d6*]-DMSO: 1H-NMR: δ = 2.50 ppm 13C-NMR: δ = 39.52 ppm

The coupling constant *J* was reported in Hz. The following abbreviations were used for the signal pattern: s (singlet), d (soublet), dd (doublet of doublet), t (Triplett), m (multiplet).

Absorption Spectra

All absorption spectra were recorded with semi-micro quartz glass cuvettes (width 1 cm, volume 1.4 mL) from STARNA at 25 °C on a *Cary 100 Scan* spectrometer from VARIAN, which is equipped with a *Cary 100* temperature controller. All spectra were corrected against the absorption of the pure solvent. Following settings were used: SBW: 2.0 nm, Average time 0.1 s, Data interval 1.0, Light source changeover 350 nm.

Fluorescence Spectra

Emission spectra and excitation spectra were recorded with a HORIBA JOBIN-YVON *Fluoromax-3* spectrofluorometer (equipped with *LFI-3751* Peltier-Element from WAVELENGTH ELECTRONICS) in semi-micro quartz glass cuvettes (width 1 cm, volume 1.4 mL) from STARNA. The spectrometer was calibrated against the raman peak of water (λ = 397 nm). All spectra were corrected against the raman scattering of the pure solvent. Following parameters were used: slits 3 nm, increment 1.0 nm, integration time 0.1 s, acquisitions 3 (average scans).

CD Spectra

CD spectra were recorded with a JASCO J-1500 CD spectrometer (0.2 nm resolution, 3 accumulations). All photophysical measurements were conducted in a Suprasil fluorescence quartz cuvette by HELLMA ANALYTICS with a light path of 1 cm or in a semi-micro quartz glass cuvettes (width 1 cm, volume 1.4 mL) from STARNA. All spectra were corrected against the pure solvent. Following parameters were used: CD scale 200 mdeg/1.0 dOD, data integration time 4 s, bandwidth 8.50 nm, data pitch 0.2 nm, scanning speed 100 nm/min, accumulation 3 times.

DNA-strands

In the following, the DNA strands used for the assembly experiments are listed. The *D*-configured strands were bought from METABION (HPLC purified, lyophilized). They were dissolved in MILLI-Q water and the concentration of the solutions were determined via the characteristic absorption at λ = 260 nm. For the β-*L*-Desoxyadenosin building block the abbreviation a, and for the *L*-configured poly-dA strand the abbreviation da_{20} were used. They were synthesized on a *Expedite 8909 Nucleic Acid Synthesizer* from APPLIED BIOSYSTEMS using the standard protocol. The commercially available β-*L*-desoxyadenosine phosphoramidite and CPG-columns (1 µmol, 500 Å) loaded with β-*L*-desoxyadenosine were bought from CHEMGENES. For easy purification the 5'-terminal DMT protecting group was not removed during synthesis. Cleavage and deprotection the DNA strands were achieved by heating the oligonucleotide strands in 0.7 mL conc. aque. Ammonia-Solution (>25 %, *trace select,* FLUKA) to 55 °C for 16 h. The solvents were removed under reduced pressure and the DNA was purified using *Glen-Pak™ DNA Purification Cartridges* from GLEN RESEARCH with the manufacturer given standard procedure. As a final step the DNA was desalted with *illustra NAP-10* columns from GE HEALTHCARE using the manufacturer given procedure.

A_{20} 5'—A-A-A-A-A-A-A-A-A-A-A-A-A-A-A-A-A-A-A-A—3'

a_{20} 5'—a-a-a-a-a-a-a-a-a-a-a-a-a-a-a-a-a-a-a-a—3'

C_{20} 5'—C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C—3'

G_{20} 5'—G-G-G-G-G-G-G-G-G-G-G-G-G-G-G-G-G-G-G-G—3'

T_{20} 5'—T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T—3'

Table S1: Masses and extinction coefficients of the DNA strands used.

DNA	Mass calc. [g/mol]	Mass found. [g/mol]	ε_{260} [$mM^{-1}cm^{-1}$]
A_{20}	6199.2	bought	277.2
a_{20}	6199.2	6204.4	277.2
C_{20}	5719.0	bought	131.4
G_{20}	6519.1	bought	210.6
T_{20}	6019.0	bought	158.4

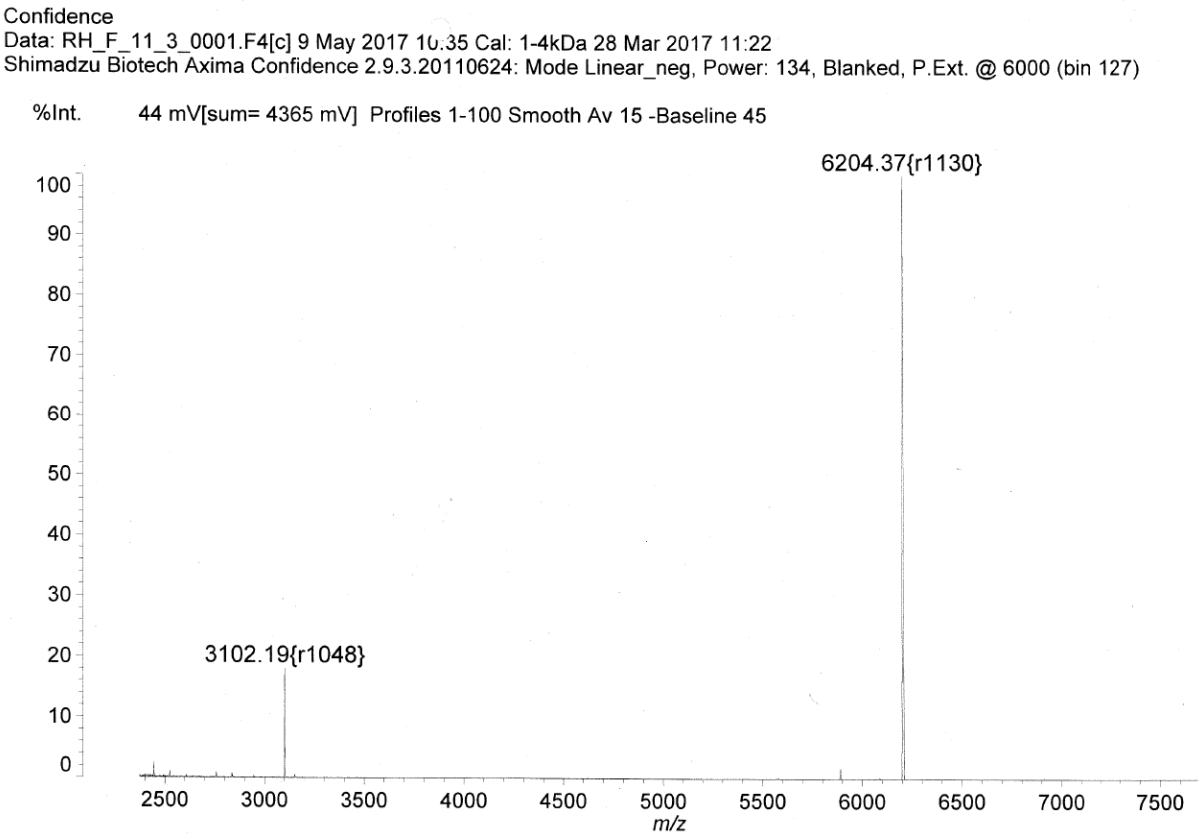


Figure S1: MALDI-TOF MS of a_{20}.

2. Syntheses and Characterizations

The synthesis and properties of **1D**, **2D** and 2-Ethynylnilered were previously reported.[1, 2]

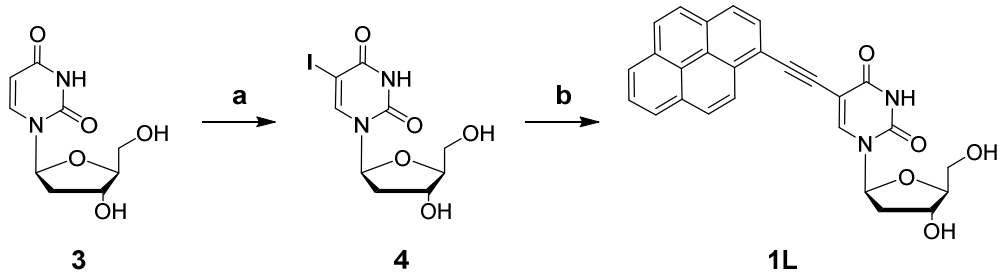


Figure S2: Synthesis of **1L**. a) Lithium iodide, ceric ammonium nitrate, acetic acid, 80 °C, 30 min (37 %); b) 1-Ethynylpyren, $Pd(PPh_3)_4$, CuI, NEt_3, abs. DMF, 60 °C, 19 h (89 °%).

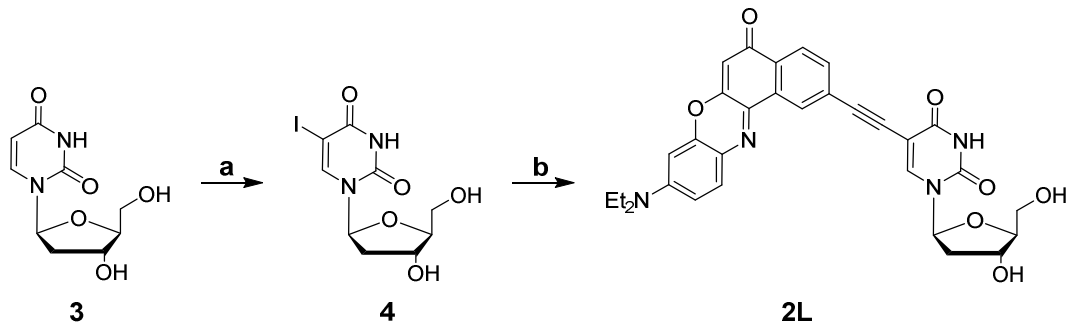
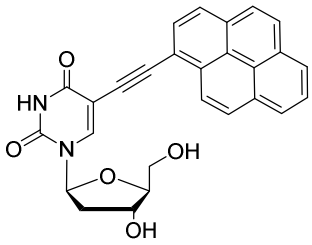


Figure S3: Synthesis of **2L**. a) Lithium iodide, ceric ammonium nitrate, acetic acid, 80 °C, 30 min (37 %); b) 2-Etynylnilered, $Pd(PPh_3)_4$, CuI, NEt_3, abs. DMF, 60 °C, 22 h (34 °%).

5-(Pyren-1-ylethynyl)-β-*L*-2'-desoxyuridine (1L)



1L
$C_{27}H_{20}N_2O_5$
M = 452.47 g/mol

A mixture of 40.5 mg 1-ethynylpyren (1.00 eq., 0.179 mmol), 69.1 mg **4** (1.10 eq., 0.195 mmol), 41.3 mg tetrakis(triphenylphosphin)palladium(0) (0.20 eq., 0.035 mmol) and 7.30 mg copper(I)iodide (0.20 eq., 0.038 mmol) were dissolved in 6.7 mL DMF and 100 µL triethylamine under argon. The resulting mixture was degassed by injection of argon under stirring for 5 min and was heated to 60 °C for 19 h. The solvents were removed under reduced pressure and the crude product was purified by flash-column chromatography (*silica*, DCM:MeOH = 1:0 – 10:1). The remove last traces of triethylammonium impurities, the product was washed with small amounts of DCM (p.a.) and dried *in vacuo*. The desired product **1L** could be obtained as yellow solid in a yield of 89 % (79.0 mg, 0.174 mmol).

DC (*silica*, DCM:MeOH = 10:1): R_f = 0.44

^{1}H-NMR (600 MHz, [*d6*]-DMSO): δ (ppm) = 11.80 (s, 1H), 8.64 (d, *J* = 9.1 Hz, 1H), 8.60 (s, 1H), 8.41 – 8.33 (m, 3H), 8.31 (d, *J* = 8.0 Hz, 1H), 8.26 (d, *J* = 8.9 Hz, 1H), 8.22 (d, *J* = 8.9 Hz, 1H), 8.17 (d, *J* = 7.9 Hz, 1H), 8.13 (t, *J* = 7.6 Hz, 1H), 6.20 (t, *J* = 6.5 Hz, 1H), 5.32 – 5.18 (m, 2H), 4.38 – 4.26 (m, 1H), 3.86 (q, *J* = 3.4 Hz, 1H), 3.77 – 3.55 (m, 2H), 2.31 – 2.18 (m, 2H).

^{13}C NMR (151 MHz, [*d6*]-DMSO): δ (ppm) = 161.6, 149.5, 143.8, 130.9, 130.8, 130.8, 130.5, 129.0, 128.7, 128.3, 127.2, 126.8, 125.9, 125.9, 125.0, 124.9, 123.6, 123.4, 117.0, 98.4, 91.1, 88.5, 87.7, 85.0, 69.8, 60.8, 40.1.

FAB-MS m/z: 452.2 [M^+].

HR-MS (FAB): m/z calc. for $C_{27}H_{20}O_5N_2^+$ [M^+] = 452.1367; found = 452.1369.

$\varepsilon_{375,\ DMSO}$ = 35.8 $mM^{-1}cm^{-1}$

$\varepsilon_{398,\ DMSO}$ = 40.3 $mM^{-1}cm^{-1}$

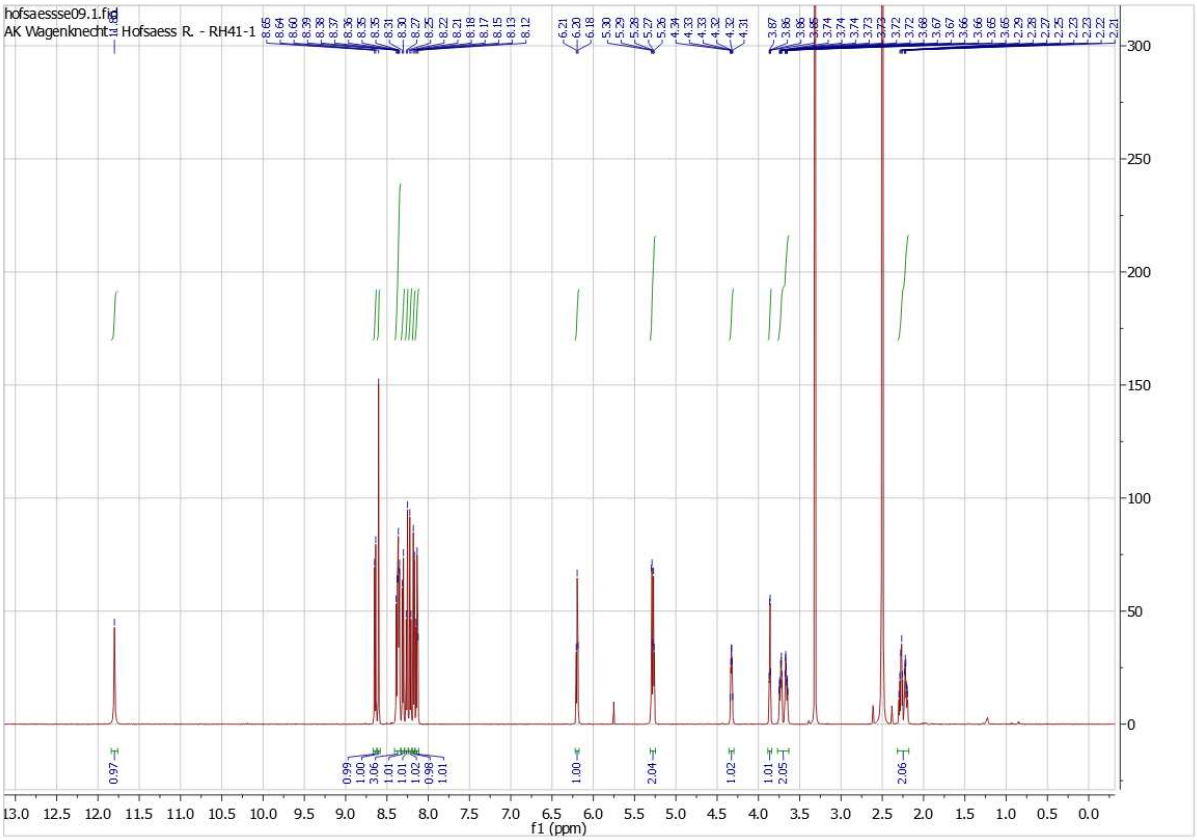


Figure S4: ^{1}H-NMR spectrum of **1L**.

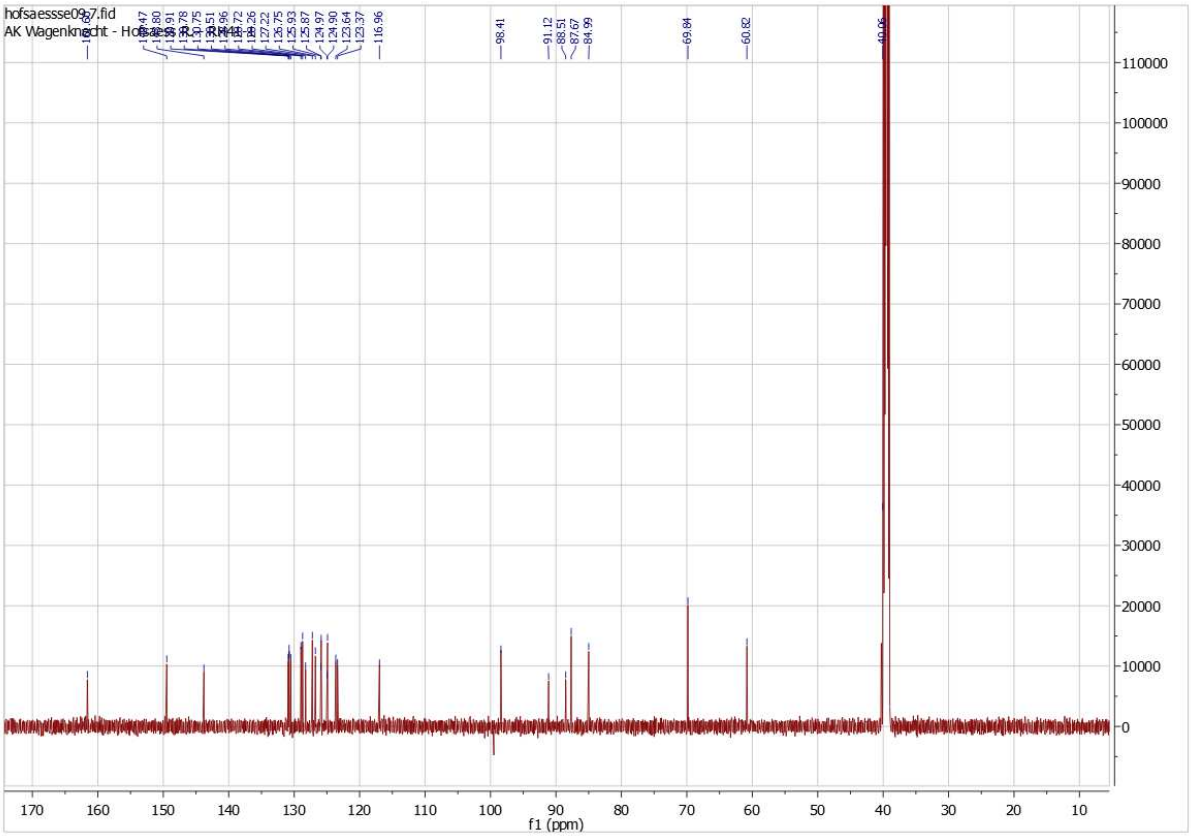


Figure S5: ^{13}C-NMR spectrum of **1L**.

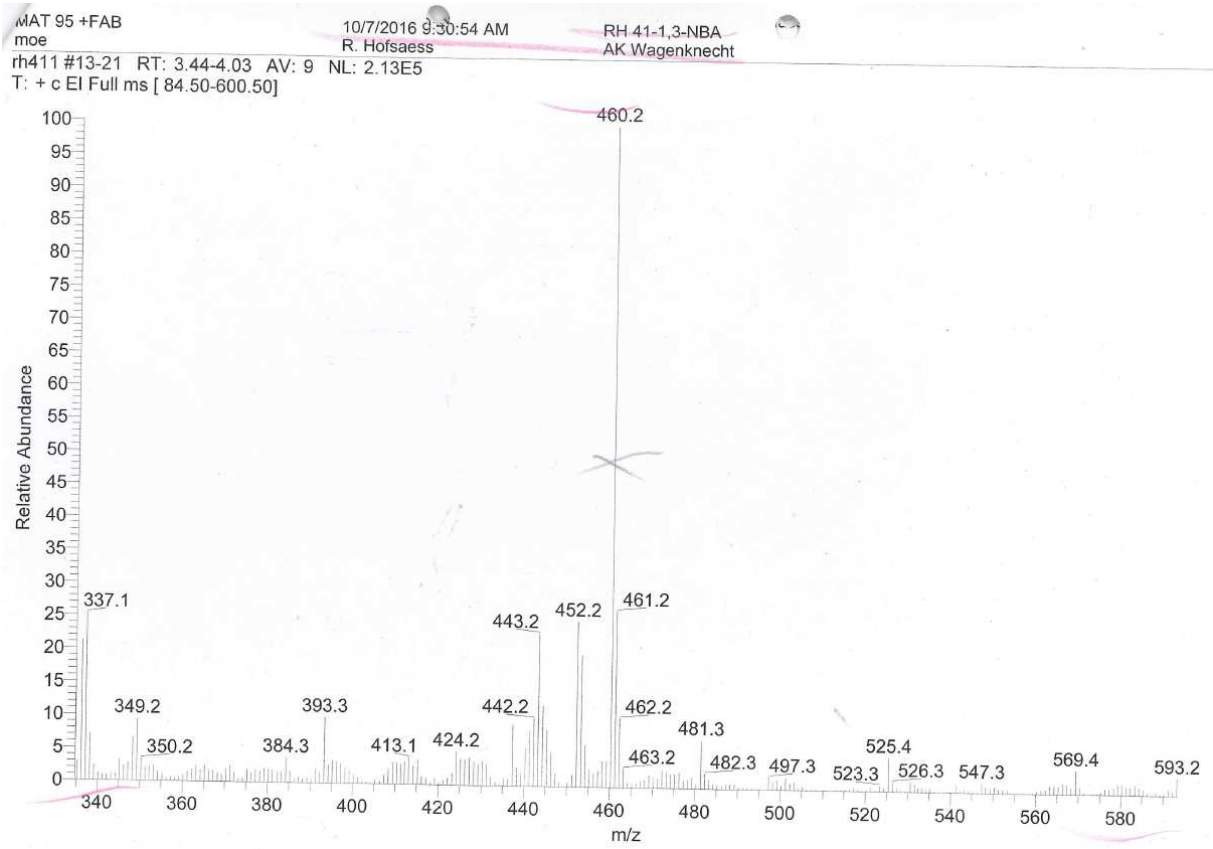


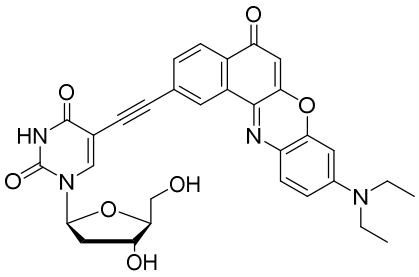
Figure S6: FAB-MS of **1L**.

rh411-c6#15 RT: 3.59
T: + c EI Full ms [84.44-600.44]
m/z= 452.1204-452.1540

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
452.1369	53956.0	100.00	452.1367	0.22	$C_{27}H_{20}O_5N_2$

Figure S7: HR-FAB-MS of **1L**.

5-((9-Diethylamino-5-benzo[a]phenoxazinon-2-yl)ethinyl)-β-*L*-2'-desoxyuridine (2L)



2L
$C_{31}H_{28}N_4O_7$
M = 568.59 g/mol

A mixture of 60.3 mg 2-ethynylnilered (1.00 eq., 0.176 mmol), 68.1 mg **4** (1.10 eq., 0.192 mmol), 40.6 mg tetrakis(triphenylphosphine)palladium(0) (0.20 eq., 0.035 mmol) and 7.60 mg copper(I)iodide (0.20 eq., 0.040 mmol) were dissolved in 6.7 mL DMF and 100 µL triethylamine under argon. The resulting mixture was degassed by injection of argon under stirring for 5 min and was heated to 60 °C for 21 h. The solvents were removed under reduced pressure and the crude product was purified by flash-column chromatography (*silica*, DCM:MeOH = 1:0 – 18:1). The remove last traces of triethylammonium impurities, the product was washed with small amounts of DCM (p.a.) and dried *in vacuo*. The desired product **2L** was isolated as shiny green-black solid in a yield of 34 % (37.4mg, 0.065 mmol).

DC (*silica*, DCM:MeOH = 10:1): R_f = 0.36

^{1}H NMR (400 MHz, [*d6*]-DMSO): δ (ppm) = 11.79 (s, 1H), 8.58 (d, *J* = 1.6 Hz, 1H), 8.50 (s, 1H), 8.13 (d, *J* = 8.2 Hz, 1H), 7.73 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.69 (d, *J* = 9.2 Hz, 1H), 6.87 (dd, *J* = 9.2, 2.7 Hz, 1H), 6.69 (d, *J* =2.6 Hz, 1H), 6.31 (s, 1H), 6.15 (t, *J* = 6.5 Hz, 1H), 5.29 (d, *J* = 4.3 Hz, 1H), 5.23 (t, *J* = 4.9 Hz, 1H), 4.27 (q, *J* = 4.4 Hz, 1H), 3.83 (q, *J* = 3.4 Hz, 1H), 3.74 - 3.56 (m, 2H), 3.52 (q, *J* = 7.0 Hz, 4H), 2.28 – 2.10 (m, 2H), 1.17 (t, *J* = 7.0 Hz, 6H).

^{13}C-NMR (101 MHz, [*d6*]-DMSO): δ (ppm) = 181.1, 161.4, 152.1, 151.2, 149.4, 146.7, 144.6, 137.2, 131.8, 131.7, 131.2, 130.2, 125.7, 125.4, 124.5, 110.6, 104.7, 97.7, 96.0, 91.4, 87.7, 85.4, 85.0, 69.9, 60.8, 44.6, 40.2, 12.5.

FAB-MS m/z: 569.2 [$(M+H)^+$].

HR-MS (FAB): m/z calc. for $C_{31}H_{29}O_7N_4^+$ [$(M+H)^+$] = 569.2031; found = 569.2032.

$\varepsilon_{569,\ DMSO}$ = 41.5 $mM^{-1}cm^{-1}$

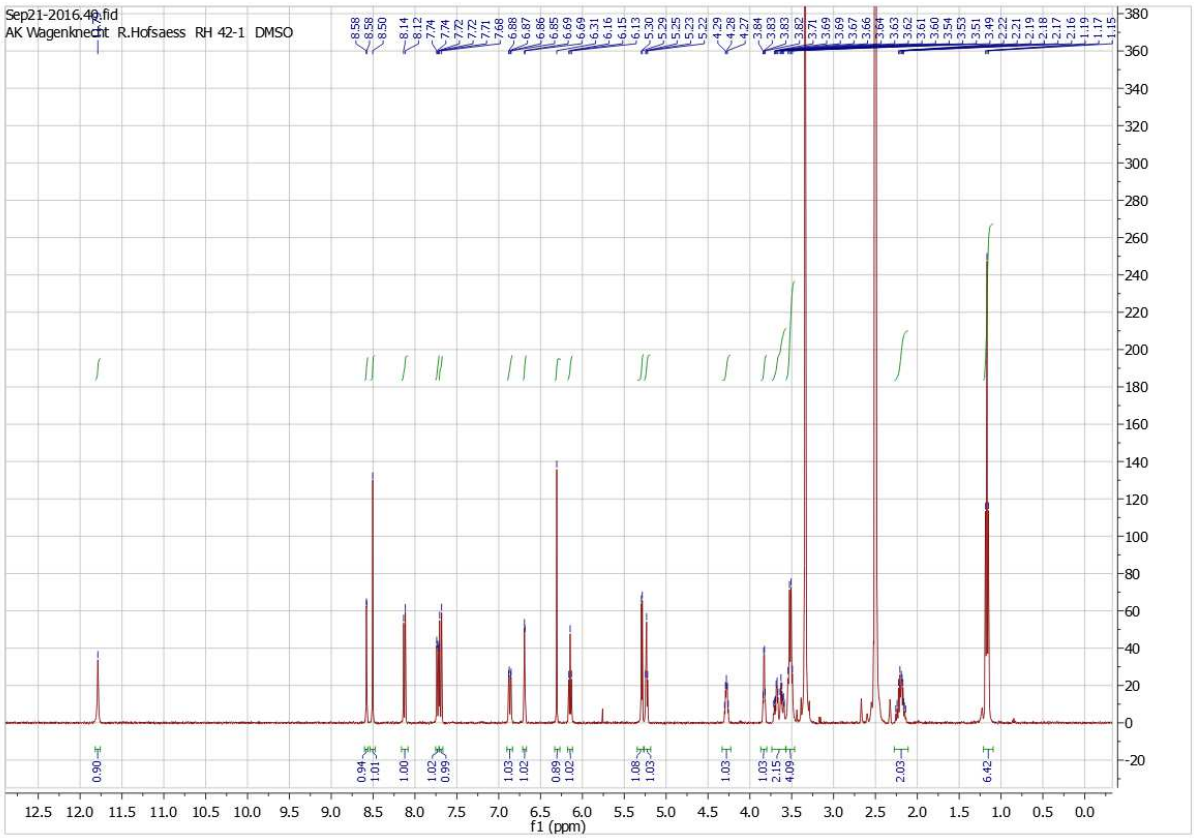


Figure S8: ^{1}H-NMR spectrum of **2L**.

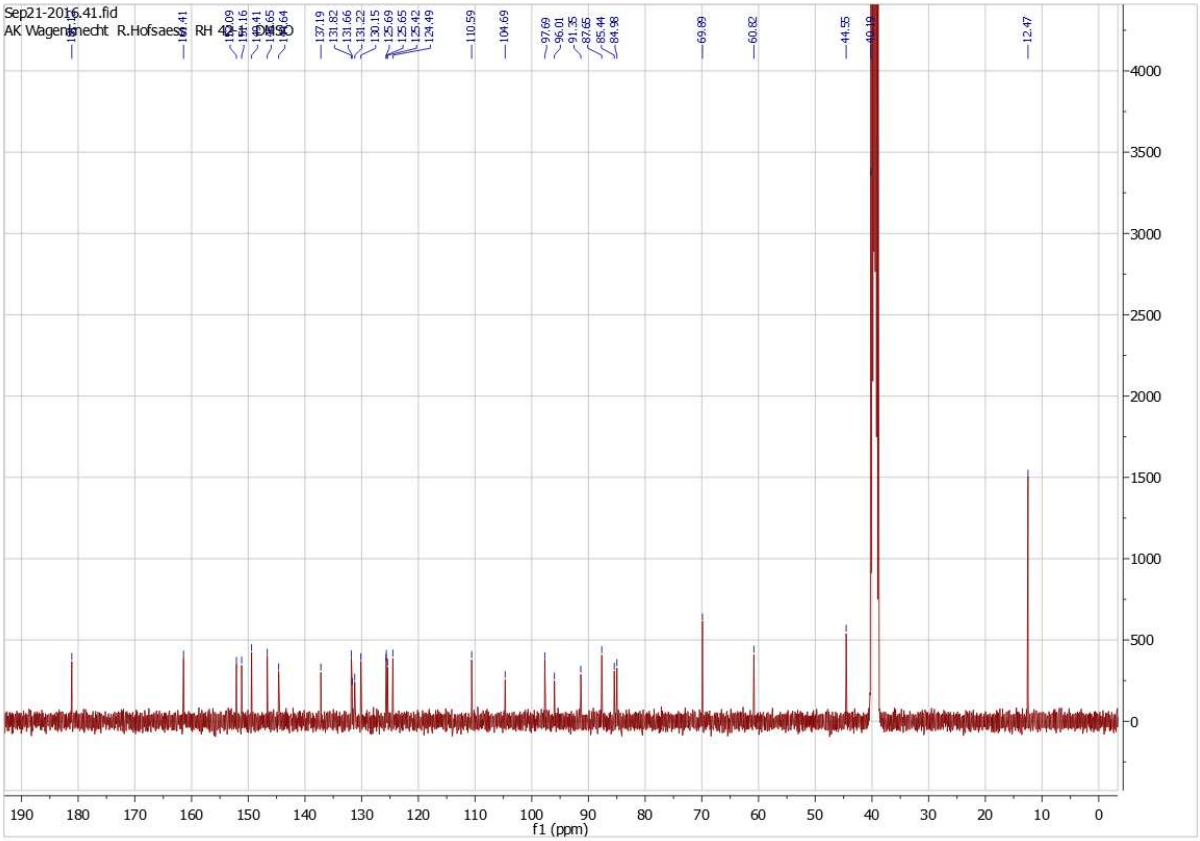


Figure S9: ^{13}C-NMR spectrum of **2L**.

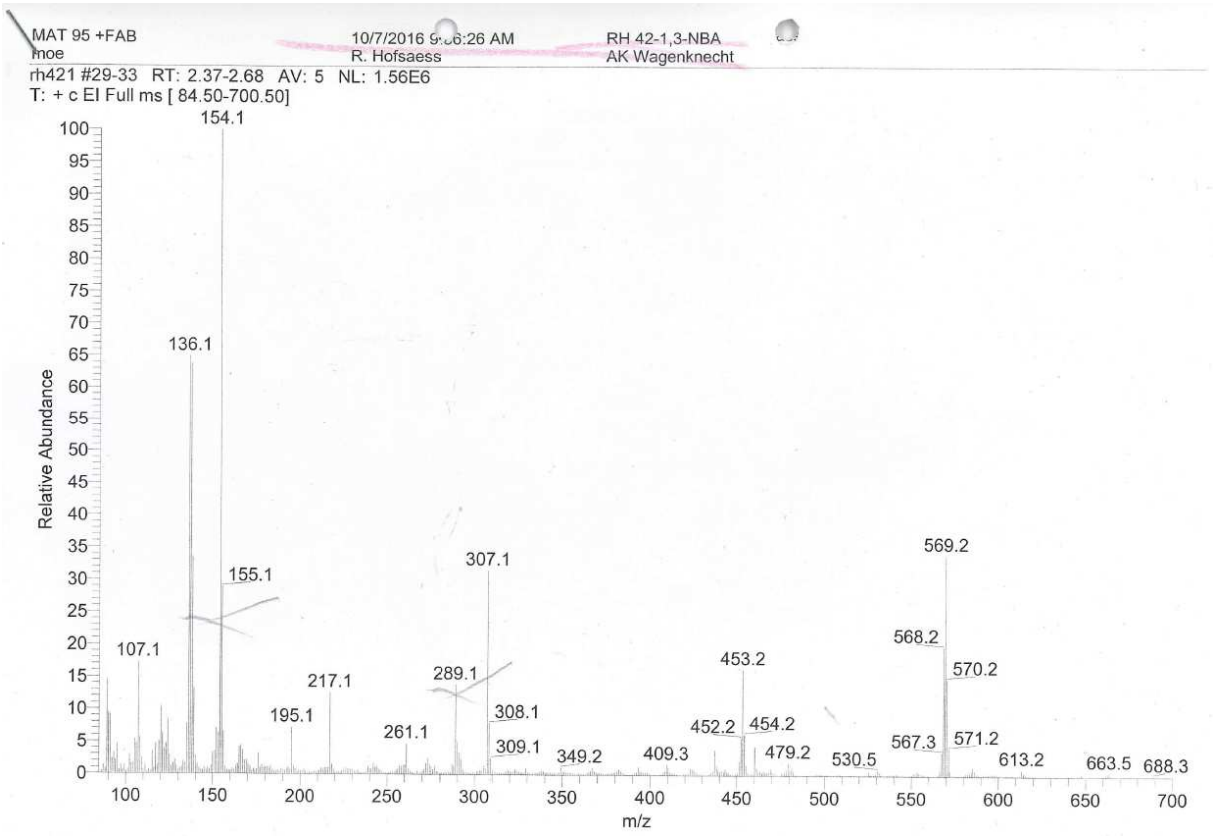


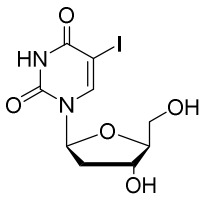
Figure S10: FAB-MS of **2L**.

rh421-c3#26 RT: 2.13
T: + c EI Full ms [84.45-700.45]
m/z= 569.1966-569.2109

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
569.2032	416837.0	100.00	569.2031	0.13	$C_{31}H_{29}O_7N_4$

Figure S11: HR-FAB-MS of **2L**.

5-Iodo-β-*L*-2'-desoxyuridine (4)



4
$C_9H_{11}IN_2O_5$
M = 354.10 g/mol

The synthesis was carried out analogous to the reported iodination of *R*-2'-desoxyuridine by Robins *et al.*.[3] Therefore, 402 mg β-*L*-2'-desoxyuridine (1.00 eq., 1.77 mmol) and 321 mg lithiumiodide (1.20 eq., 2.11 mmol) were dissolved in 1 mL glacial acetic acid. Subsequently, 890 mg cer(IV)ammoniumnitrat (2.00 eq., 3.51 mmol) were added and the mixture was stirred at 80 °C for 30 min. The solvent was removed under reduced pressure, the crude product washed with ethanol/toluene = 1:2 (3 × 2.5 mL) and co-evaporated with water:ethanol = 1:2 (3 × 6 mL). Finally the residue was recrystallized two times from 6 mL water:methanol = 1:1. The desired product **4** could be obtained as colorless solid in a yield of 37 % (229 mg, 0.649 mmol).

DC (*silica*, DCM:MeOH = 10:1): R_f = 0.27

^{1}H-NMR (500 MHz, [*d6*]-DMSO): δ (ppm) = 11.66 (s, 1H), 8.39 (s, 1H), 6.09 (t, *J* = 6.5 Hz, 1H), 5.24 (d, *J* = 4.3 Hz, 1H), 5.15 (t, *J* = 4.8 Hz, 1H), 4.23 (quin, *J* = 4.2 Hz, 1H), 3.79 (q, *J* = 3.2 Hz, 1H), 3.63 – 3.56 (m, 2H), 2.18 – 2.05 (m, 2H).

^{13}C-NMR (126 MHz, [*d6*]-DMSO): δ (ppm) = 160.5, 150.1, 145.1, 87.5, 84.6, 70.0, 69.3, 60.8, 40.2.

FAB-MS m/z: 355.1 [$(M+H)^+$].

HR-MS (FAB): m/z calc. for $C_9H_{12}O_5N_2{}^{127}I^+$ [$(M+H)^+$] = 354.9784; found = 354.9786.

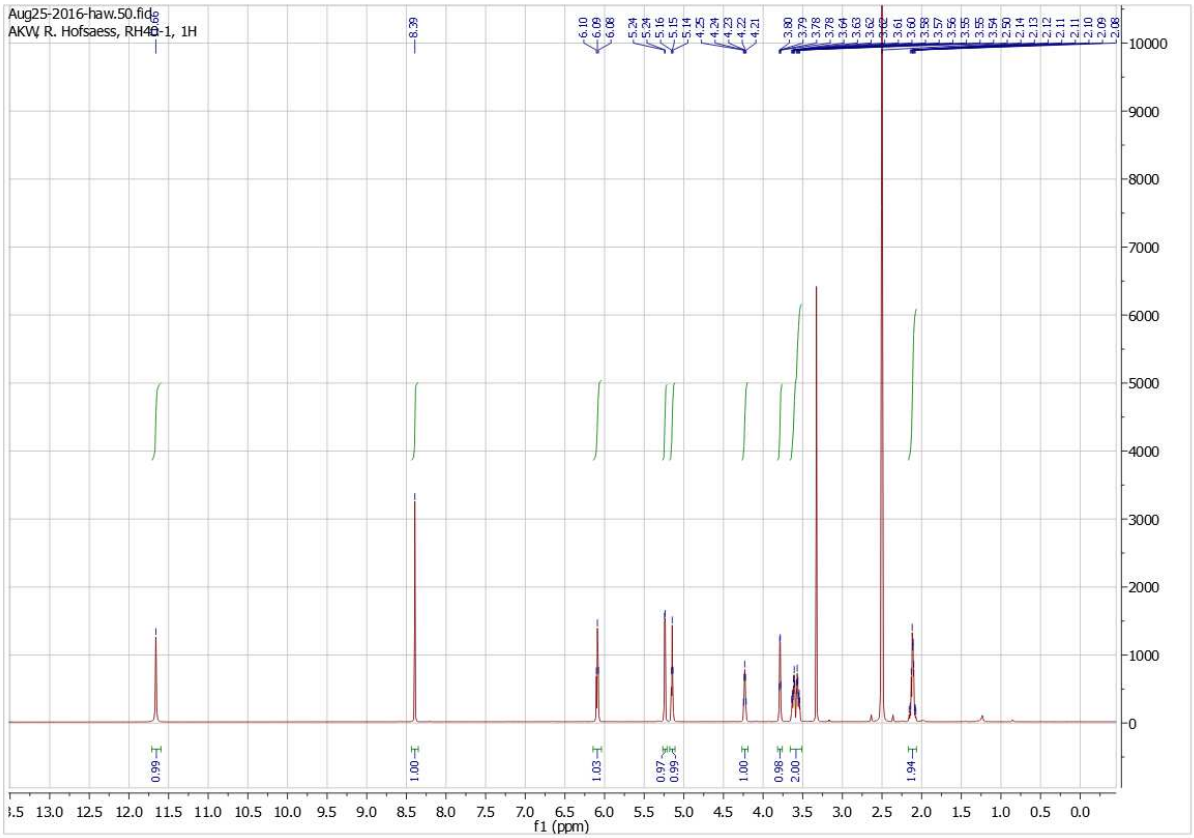


Figure S12: ^{1}H-NMR spectrum of **4**.

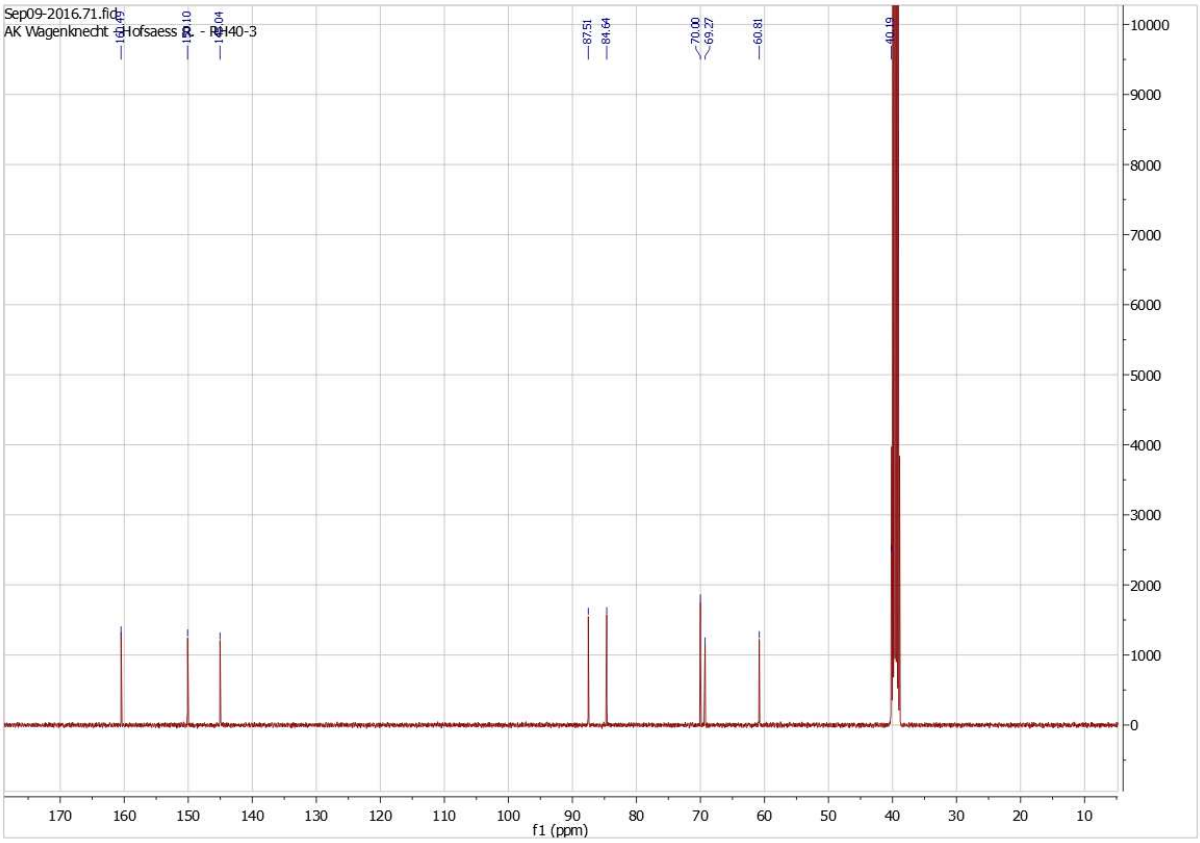


Figure S13: ^{13}C-NMR spectrum of **4**.

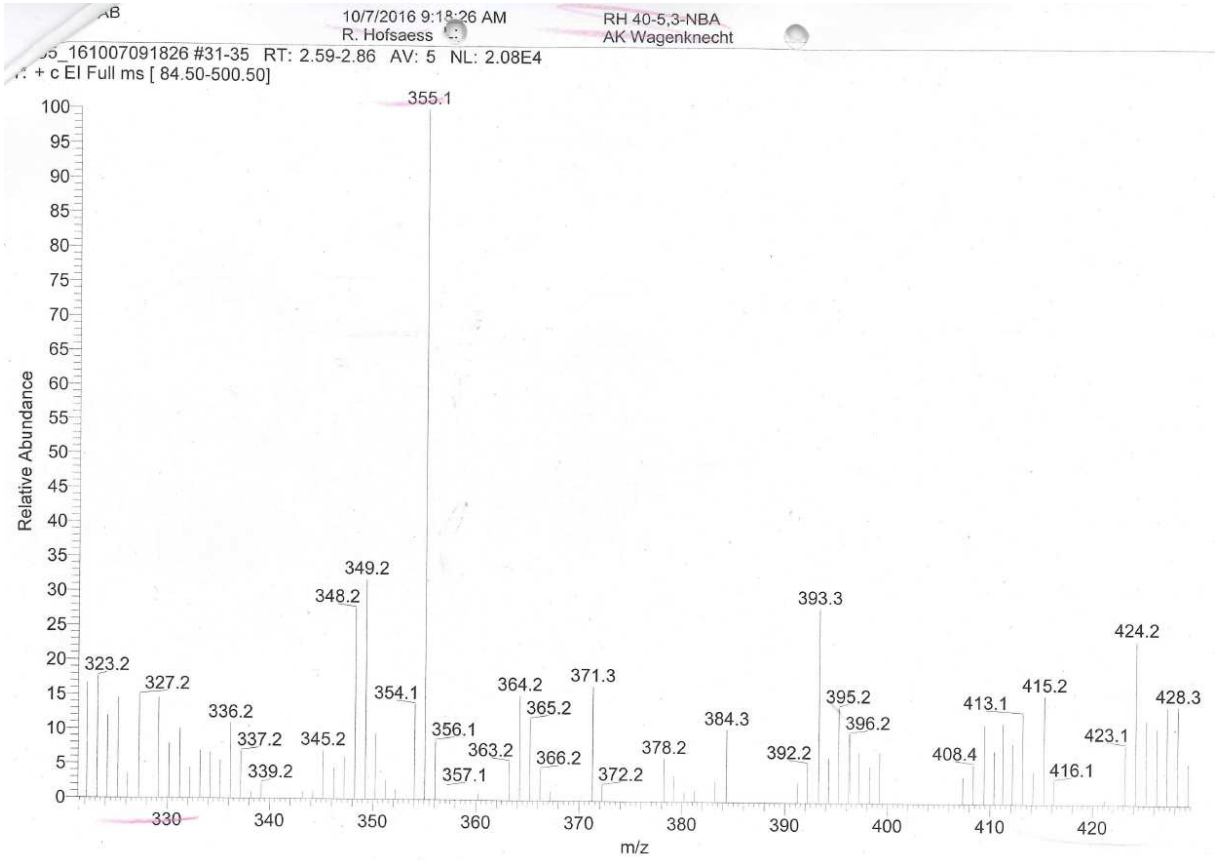


Figure S14: FAB-MS of **4**.

rh405_161007091826-c1#19 RT: 1.78
T: + c EI Full ms [84.43-500.43]
m/z= 354.9637-354.9964

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
354.9784	14551.0	100.00	354.9786	-0.11	$C_9 H_{12} O_5 N_2 {}^{127}I_1$

Figure S15: HR-FAB-MS of **4**.

3. Sample preparation

First, stock solutions of the chromophore building blocks in DMSO (c(**1D**/**1L**) = 2.40 mM and c(**2D**/**2L**) = 3.19 mM), and a 1.25 µM solution of the respective template strands in 1000 µL *MilliQ*-water were prepared. Subsequently, the respective stock solution was added to the template solution until a chromophore concentration of 37.5 µM was reached. This equals 30 binding sites in the template and should there maximize the occupation of the dN_{20}-templates. After 60 min of assembly time, the samples were centrifuged for 1 min at 16000 g. In this step, the unbound chromophore-nucleosides are removed, since they precipitate upon centrifugation. Only the bound chromophores are kept in solution by the DNA template. The supernatant was collected and used for optical spectroscopy without any further purification. All preparations were done at r.t.. For the negative sample (only dyes, no DNA) the same volume of deionized water (instead of the DNA solution) was used.

4. Additional optical spectra

1L

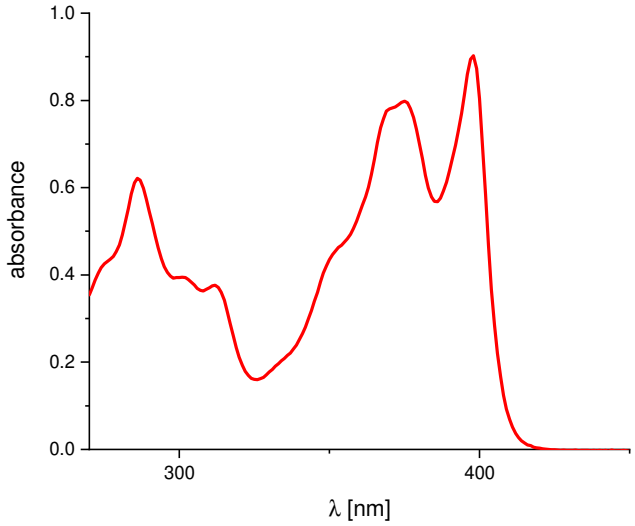


Figure S16: UV/Vis absorption of **1L** in DMSO (c(**1L**) = 2.2 × 10^{-2} mmol/L).

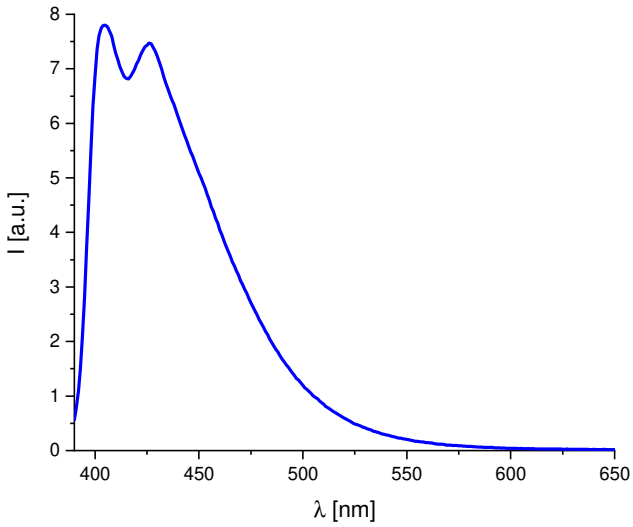


Figure S17: Fluorescence of **1L** in DMSO (c(**1L**) = 5.5 × 10^{-3} mmol/L, λ_{exc} = 375 nm, slits: 2 nm)

2L

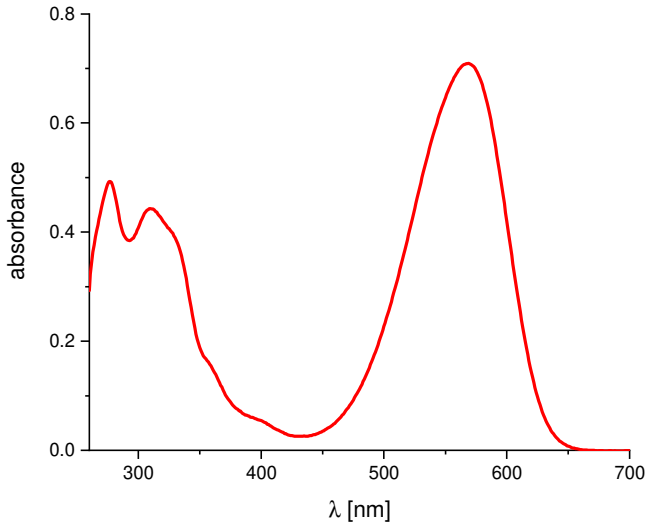


Figure S18: UV/Vis bsorption of **2L** in DMSO (c(**2L**) = 1.71 × 10^{-2} mmol/L).

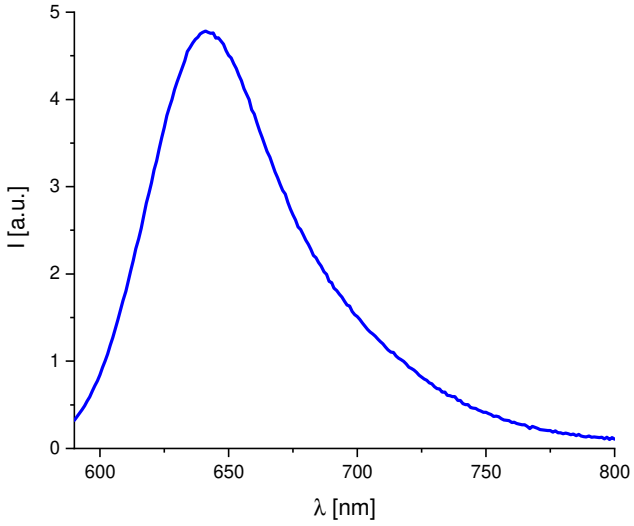


Figure S19: Fluorescence of **2L** in DMSO (c(**2L**) = 5.7 × 10^{-3} mmol/L, λ_{exc} = 596 nm, slits: 2 nm)

Assemblies

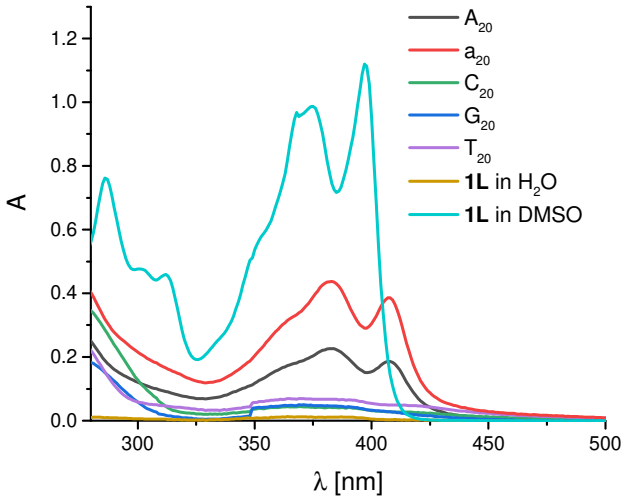


Figure S20: UV/Vis absorption of **1L** in DMSO (turquoise, c(**1L**) = 25 µM), without template strand in water (brown, c(**1L**) = 25 µM), in water + 2% DMSO with the complementary A_{20} (black) and a_{20} (red) stands and with the non-complementary G_{20} (blue), C_{20} (green) and T_{20} (purple) templates (c(template) = 1.25 µM und c(**1L**) = 25 µM) after centrifugation for 1 min at 16000 g.

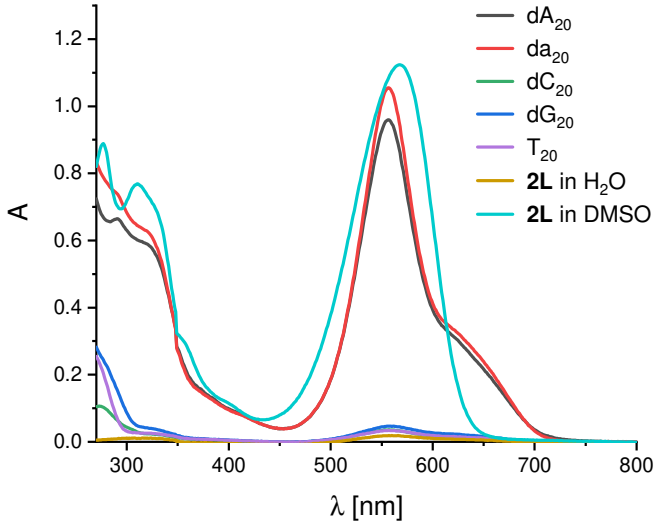


Figure S21: UV/Vis absorption of **2L** in DMSO (turquoise, c(**2L**) = 25 µM), without template strand in water (brown, c(**2L**) = 25 µM), in water + 2% DMSO with the complementary A_{20} (black) and a_{20} (red) stands and with the non-complementary G_{20} (blue), C_{20} (green) and T_{20} (purple) templates (c(template) = 1.25 µM und c(**2L**) = 25 µM) after centrifugation for 1 min at 16000 g.

Titration experiments

Titration experiments were carried out as described in literature.[2] The building blocks were added from a DMSO stock solution, so that each addition equaled one binding site in the template strand. The sample was incubated for 1 min after each addition. After the final titration step, the sample was incubated for 1 h, and excess or unbound nucleosides were removed by centrifugation (1 min at 16000 g).

1D / 1L

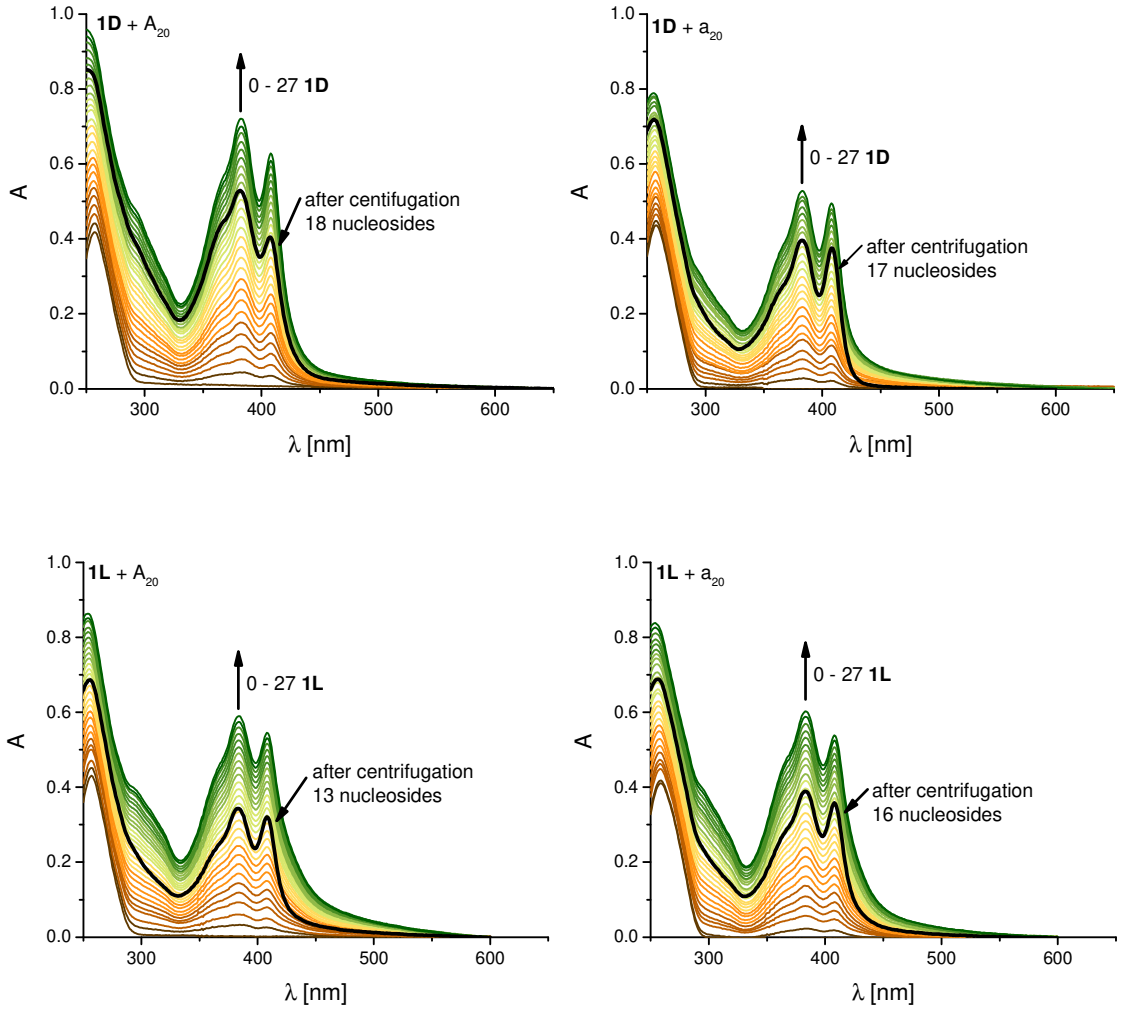


Figure S22: UV/Vis absorption of the titration experiments of **1D** to A_{20} (top left), **1D** with a_{20} (top right), **1L** with A_{20} (bottom left) and **1L** with a_{20} (bottom right) (all in water + 2% DMSO, c(template) = 1.25 µM). Spectra of the supernatant after centrifugation are plotted in bold and black into the respective experiments.

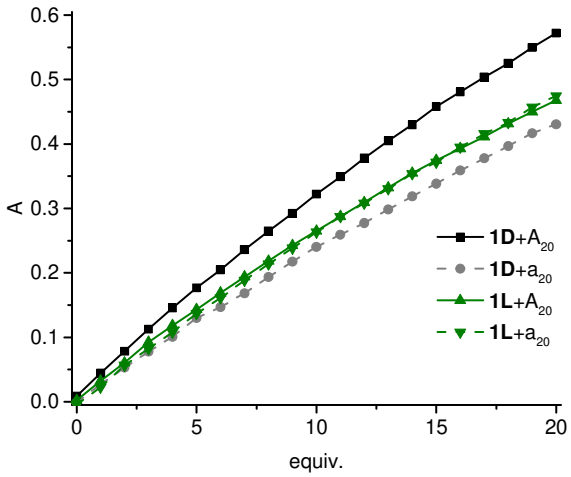


Figure S23: UV/Vis absorption at 382 nm of the titration experiments with **1D**/**1L** and A_{20}/a_{20} (all in water + 2% DMSO, 1 equiv = c(template) = 1.25 µM).

2D / 2L

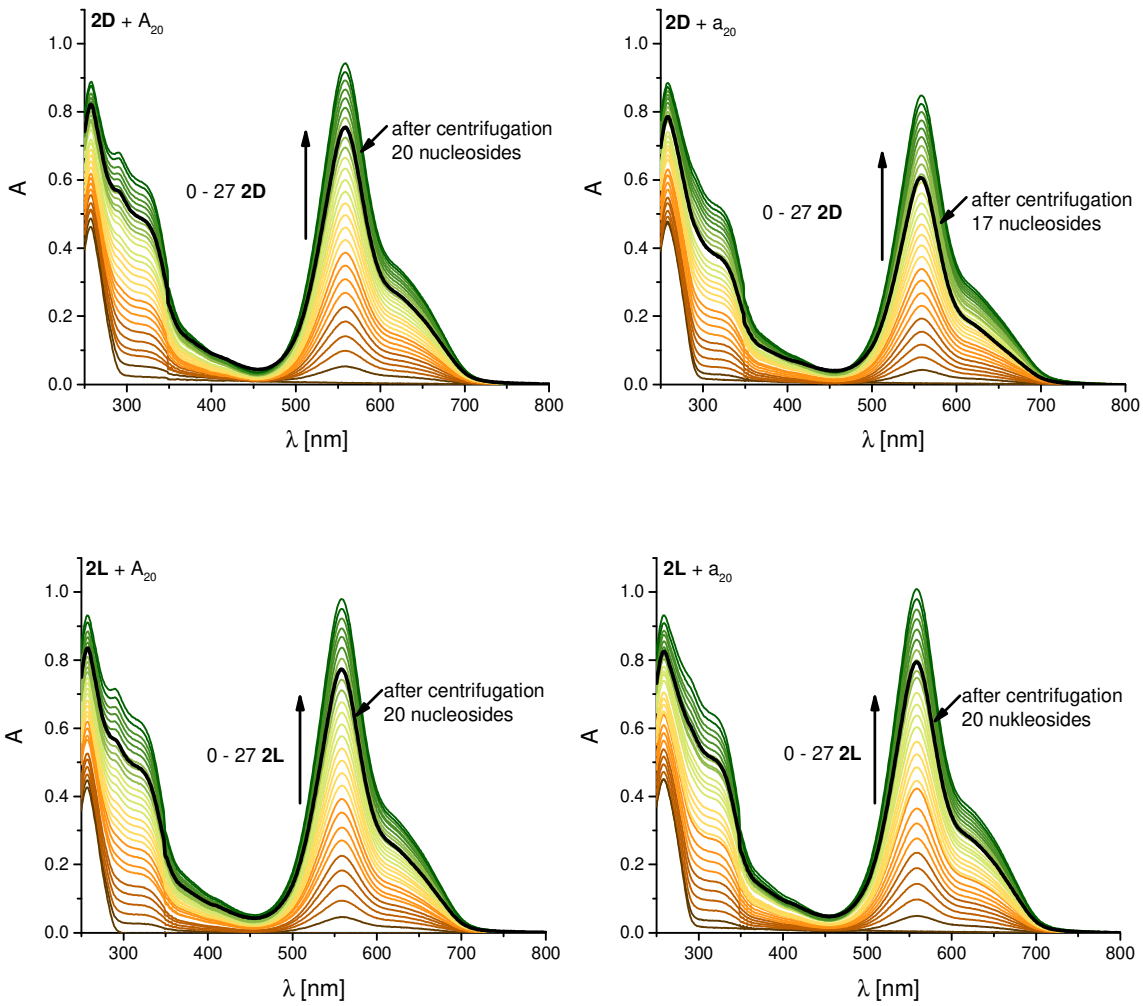


Figure S24: UV/Vis absorption of the titration experiments of **2D** to A_{20} (top left), **2D** with a_{20} (top right), **2L** with A_{20} (bottom left) and **2L** with a_{20} (bottom right) (all in water + 2% DMSO, c(template) = 1.25 µM). Spectra of the supernatant after centrifugation are plotted in bold and black into the respective experiments.

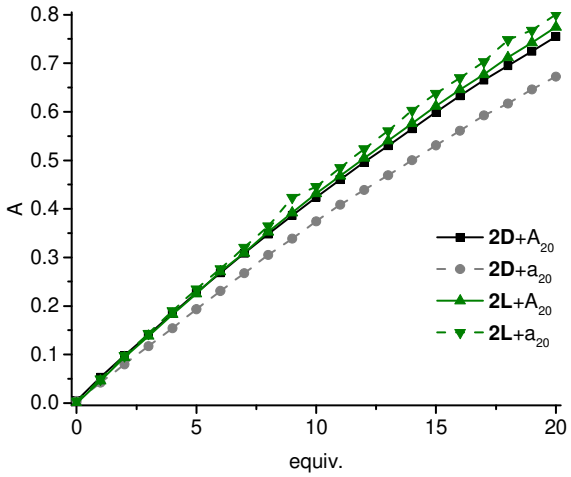


Figure S25: UV/Vis absorption at 588 nm of the titration experiments with **2D**/**2L** and A_{20}/a_{20} (all in water + 2% DMSO, 1 equiv = c(template) = 1.25 µM).

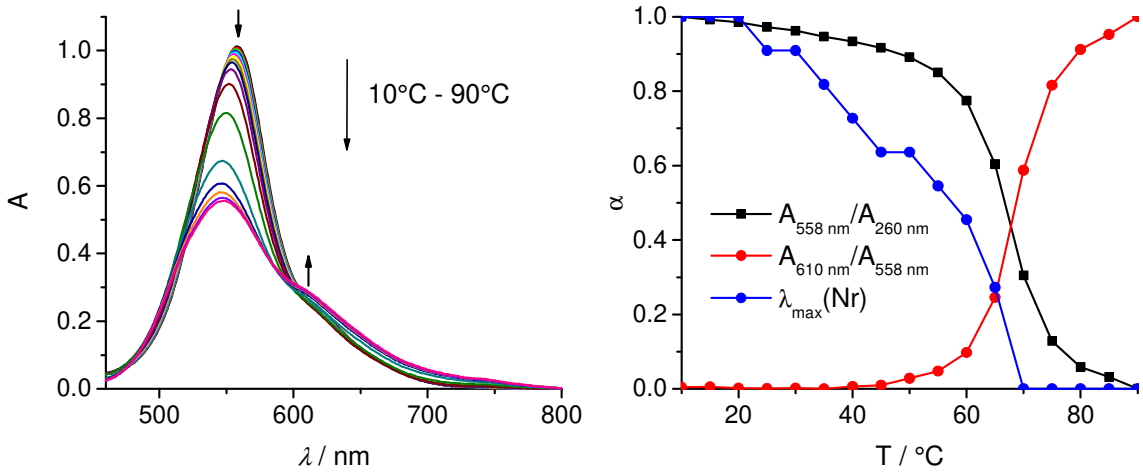


Figure S26: Left: Temperature-dependent UV/Vis absorption of the **2D** assembly along A_{20} (all in water + 2% DMSO, c(template) = 1.25 µM). Right: Analysis a with respect to the Nr/DNA absorption ratios at 558 nm/260 nm, 610 nm/260 nm and λ_{max} of the Nr moiety of **2D**.

CD

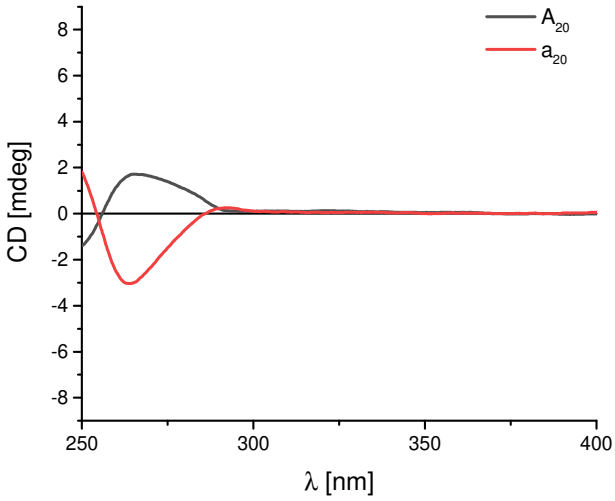


Figure S27: CD of the pure templates A_{20} and a_{20} without assembled chromophore building blocks (c(template) = 1.25 µM in water). The slight differences in the CD signals of A_{20} and a_{20} are probably due to small conformational differences that result from different preparations. We bought A_{20}, but prepared and purified a_{20} by ourselves as described above.

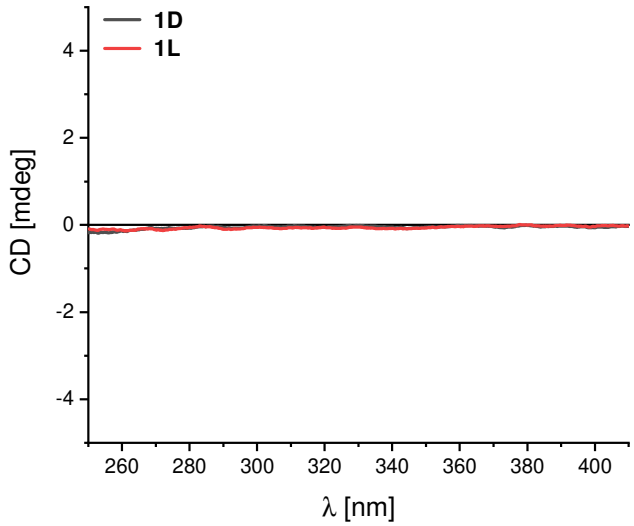


Figure S28: CD of **1D** and **1L** in DMSO without template strands (c(**1D**/**1L**) = 25 µM, respectively).

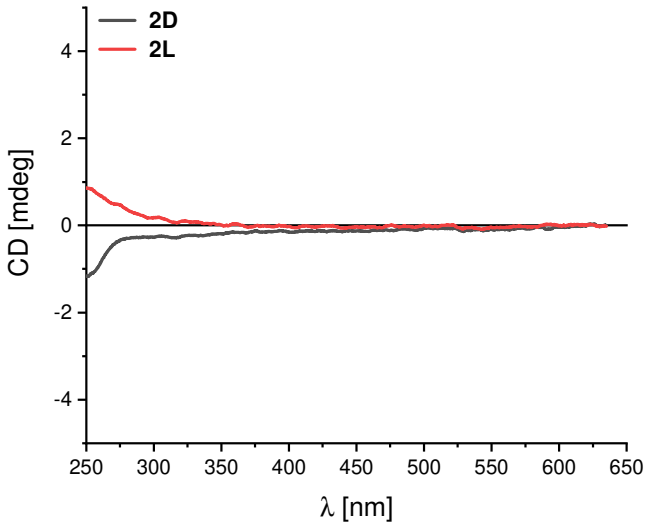


Figure S29: CD **2D** and **2L** in DMSO without template strands (c(**2D**/**2L**) = 25 µM, respectively). The CD spectrum of **2D** was previously reported.[4]

5. References

[1] R. Varghese, H.-A. Wagenknecht, *Chem. Eur. J.* **2010**, *16*, 9040-9046.
[2] P. Ensslen, Y. Fritz, H.-A. Wagenknecht, *Org. Biomol. Chem.* **2015**, *13*, 487-492.
[3] J. Asakura, M. J. Robins, *J. Org. Chem.* **1990**, *55*, 4928-4933.
[4] R. Hofsäß, S. Sinn, F. Biedermann, H.-A. Wagenknecht, *Chem. Eur. J.* **2018**, *24*, 16257.