Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information To:

A Turn-On Dual Emissive Nucleobase Sensitive to Mismatches and Duplex Conformational Changes

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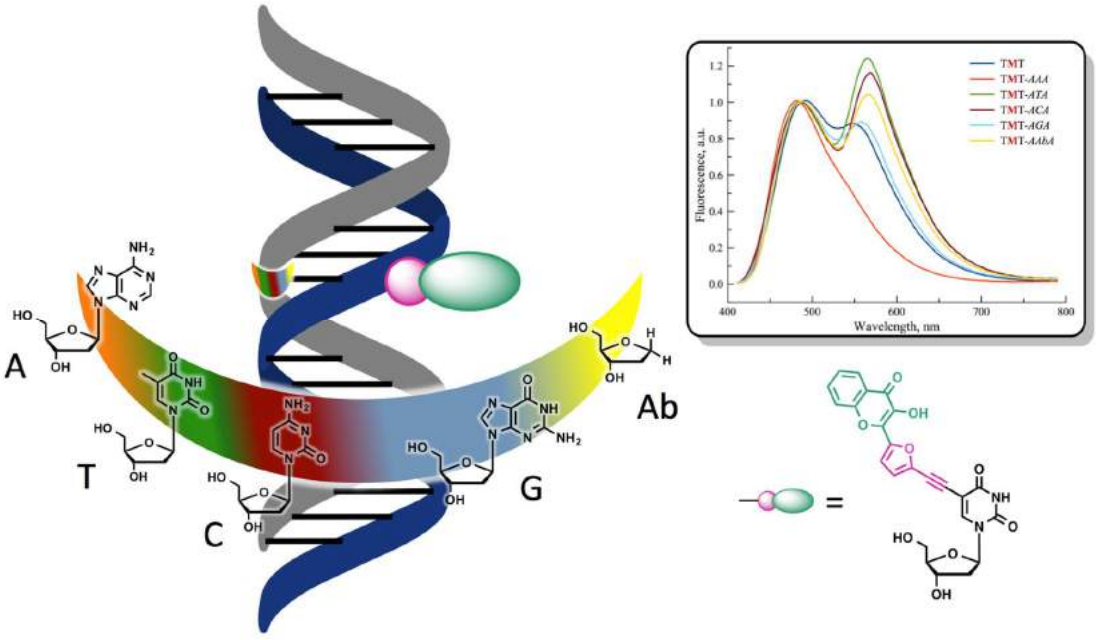

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Experimental section

1. Synthetic Procedures

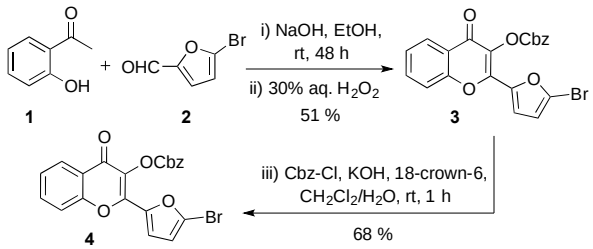
General methods:

All reactions involving water-sensitive reagents were performed in oven-dried glassware under argon using dry solvents. The synthetic intermediates were co-evaporated twice with toluene beforehand and dried in *vacuo* before use. All chemical reagents were obtained from commercial sources and were used as supplied. Anhydrous solvents were obtained according to standard procedures.[1] The reactions were monitored by thin-layer chromatography (TLC, Merck silica gel 60 F254 plates) and visualized both by UV radiation (254 & 365 nm) and by spraying with vanillin in ethanol and then sulfuric acid (5 %) in ethanol followed by a subsequent warming with a heat gun. Column chromatography [2] was performed with flash silica gel (40ï 63 mm). All NMR spectra (^{1}H, ^{13}C, ^{2}D) were recorded on 200 or 500 Bruker Advance Spectrometers (200 or 500 MHz). ^{1}H NMR (200 and 500 MHz), ^{13}C NMR (50 and 125 MHz, recorded with complete proton decoupling) spectra were obtained with samples dissolved in $CDCl_3$, CD_3OD, or DMSO-d^6, with the solvent signals used as internal references: 7.26 ppm for C*H*Cl_3, 3.31 ppm for CD_2*H*OD, 2.50 ppm for (CD_3)(CD_2*H*)S(O) for ^{1}H NMR experiments, and 77.0 ppm for $CDCl_3$, 49.0 ppm for CD_3OD, 39.4 ppm for $(CD_3)_2S(O)$ for ^{13}C NMR experiments.[3] Chemical shifts (δ) are given in ppm to the nearest 0.01 (1H) or 0.1 ppm (^{13}C). The coupling constants (*J*) are given in Hertz (Hz). The signals are reported as follows: (s=singlet, d=doublet, t=triplet, m=multiplet, br=broad). Assignments of ^{1}H and ^{13}C NMR signals were achieved with the help of D/H exchange, COSY, DEPT, APT, HMQC, HSQC, HMBC experiments. Regular mass spectra (MS) were recorded on an Esquire 3000 Plus apparatus with ESI in both positive and negative mode. High-resolution mass spectrometry was conducted with a FINIGAN MAT 95 spectrometer with EI or ESI ionization techniques. Supplementary data associated with this article: the experimental protocols for the synthesis of intermediates **2-7** and **10-11**, the ^{1}H NMR, ^{13}C NMR and (in part) ^{1}Hï ^{13}C COSY, ^{1}Hï ^{13}C HMQC and ^{1}Hï ^{13}C HMBC spectra of all compounds. Systematic nomenclatures are used below for the assignments of the flavone and nucleoside.

1.1 Preparation of the amidite 10

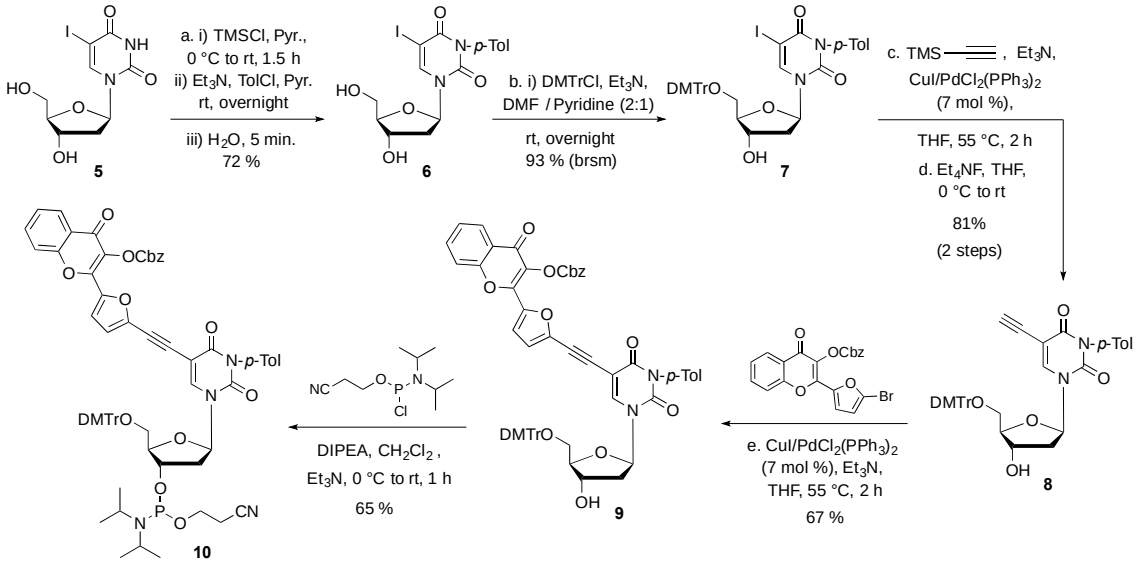
Retrosynthesis: The emissive amidite **6** was engineered through a convergent strategy involving on one hand for the 3HC preparation, a Claisen-Schmidt condensation followed by an Algar-Flynn-Oyamada reaction and, on the other hand for the final assembly, two Sonogashira cross-couplings.

Synthesis: The 3HC-coupling partner, 3-Benzyloxycarbonyloxy-2-(5-bromofur-2-yl)-chromen-4-one **4** was synthesized according to our robust procedure.[4]



Scheme S1. Preparation of the 3HC-coupling partner **10**.

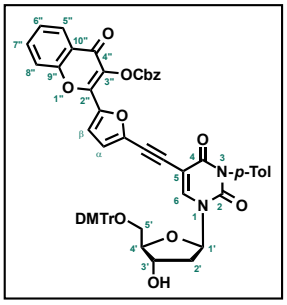
The 6-step convergent synthesis of the target amidite **10** required for the preparation of labelled ODNs was adapted and optimized from our reported protocol.[5] 5-iododeoxyuridine **5** was engaged in a transient protection to provide the desired 3-imido-protected derivative **6**. Masking the *N*-3 position is a prerequisite to avoid 5-*endo-dig* cyclization as a side reaction during the final assembly of the fluorophore and the alkynyl nucleoside. After tritylation of the 5ô-OH group leading to **7**, trimethylsilylacetylene was efficiently coupled to the 5-iodouracil moiety. Tetraethylammonium fluoride was employed for the chemoselective removal of the silyl group delivering **8** in appreciable yields. The furylchromone **4**, prepared from a gram-scalable procedure, was then connected on the 5-ethynyluridine **8** through a convenient Sonogashira coupling. Phosphitylation of **9** under classical conditions yielded the amidite **10** which allowed the preparation of 15-mer labelled oligonucleotides via solid-phase synthesis. HPLC, UV and MS analysis ascertained the purity and integrity of the synthesized ODNs.



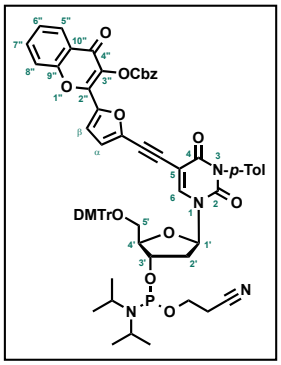
Scheme S2. Synthetic access to the fluorescent amidite **10**.

1.2 Protocols

NMR characterizations and synthetic procedures leading to derivatives **5**, **6**, **7** and **8** were previously described in a similar approach.[5]



5′-*O*-(4,4′-Dimethoxytrityl)-3-*N*-(4-methylbenzoyl)-5-(5-(3-Benzylcarbonate-4-oxo-chromen-2-yl)fur-2-yl)ethynyl-2'-deoxyuridine (9): To a stirred solution of **8** (300 mg, 0,45 mmol, previously azeotropically coevaporated with dry toluene) and **4** (269 mg, 0.58 mmol, 1.3 eq) in THF (10 mL) under argon, were sequentially added triethylamine (311 µL, 2.23 mmol, 5 eq), and CuI (7 mol %, 0.03 mmol, 6 mg,)/$PdCl_2(PPh_3)_2$ (7 mol %, 0.03 mmol, 22 mg) all together. The reaction mixture was warmed up to 60 °C for 2 h. The volatiles were removed *in vacuo* and the residue was purified by flash chromatography on silica gel eluted with toluene/EtOAc (93:7 Ÿ 3:2, v/v) to provide the desired compound **9** as a yellow foam (307 mg, 67 %). $C_{61}H_{48}N_2O_{14}$ (1033.05). R_f=0.41 (toluene/EtOAc=7:3). ^{1}H-NMR ($CDCl_3$, 200 MHz): δ=8.41 (1H, s, H6), 8.23 (1H, dd, ^3J=8.3 Hz and ^4J=1.6 Hz, H5[illegible]) 7.83 (2H, d, ^3J=8.2 Hz, *o-H*-Tol), 7.67 (1H, ddd, ^3J=8.3 Hz, 7.2 Hz and ^4J=1.6 Hz, H7[illegible]) 7.50ï 7.13 (16H, m, H6[illegible]H8[illegible]C*H*-Cbz, *H*-Ph, *o-H*-PhOMe, *m-H*-Tol), 7.04 (1H, d, ^3J=3.8 Hz, Hβ), 6.83 (2H, d, ^3J=8.9 Hz, *m-H*-PhOMe), 6.81 (2H, d, ^3J=8.9 Hz, *m-H*-PhOMe), 6.36ï 6.28 (1H, m, H1[illegible]) 6.30 (2H, d, ^3J=3.8 Hz, Hα), 5.32 (2H, s, CH_2-Cbz), 4.42ï 4.61 (1H, m, H3[illegible]) 4.19ï 4.11 (1H, m, H4[illegible]) 3.70 (3H, s, *Me*O), 3.69 (3H, s, *Me*O), 3.45ï 3.37 (2H, m, H5[illegible]) 2.65ï 2.46 (2H, m, H2[illegible]) 2.42 (3H, s, *p-Me*-Tol); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ=171.7 (C4[illegible]) 167.8 (*C*(O)-Tol), 160.2 (C4), 158.9 (*p-C*-PhOMe), 158.9 (*p-C*-PhOMe), 155.3 (C9[illegible]) 152.2 (*C*(O)-Cbz), 148.3 (C2), 147.3 (*p-C*-Tol), 147.2 (C2[illegible]) 144.5 (*i-C*-Ph), 143.6 (C6), 143.3 (*Fur*-C[illegible]), 139.8 (C2[illegible]*Fur*), 135.7 (*i-C*-PhOMe), 135.5 (*i-C*-PhOMe), 134.7 (*i-C*-Cbz), 134.4 (C7[illegible]) 132.1, 131.0 (*o-C*-Tol), 130.2 (*o-C*-PhOMe), 130.1, 129.3, 129.0, 128.9, 128.7, 128.6, 128.5, 128.3, 128.1, 127.3, 126.2 (C5[illegible]) 125.7 (C6[illegible]) 124.0, 118.5 (Cα), 118.4 (C8[illegible]) 118.3 (Cβ), 113.6 (*m-C*-PhOMe), 99.1 (C5), 87.5 ($C_{1'}$-O-5[illegible]) 87.2 (Fur-C[illegible] *C*), 87.2 (C4[illegible]) 86.8 (C1[illegible]) 83.2 (Fur-*C*[illegible]), 72.4 (C3[illegible]) 71.5 (*C*H_2-Cbz), 63.5 (C5[illegible]) 55.4 (*Me*O), 42.3 (C2[illegible]) 22.2 (*p-Me*-Tol); MS (ESI^+, MeOH) *m/z*: 1072.0 $[M+K]^+$. HRMS (ESI^+): *m/z* calcd for $C_{61}H_{48}N_2NaO_{14}$: 1055.2998 $[M+Na]^+$; found 1055.3000 $[M+Na]^+$.



5′-*O*-(4,4′-Dimethoxytrityl)-3-*N*-(4-methylbenzoyl)-5-(5-(3-Benzylcarbonate-4-oxo-chromen-2-yl)fur-2-yl)ethynyl-2′-deoxyuridine, 3'-[(2-cyanoethyl)-*N*,*N*-diisopropyl]-phosphoramidite (10): To a stirred solution of **9** (0.30 mmol, 309 mg, previously azeotropically coevaporated with dry toluene) in CH_2Cl_2 (2.1 mL), previously cooled down to 0 °C, were sequentially added DIPEA (1.20 mmol, 211 µL, 4 eq) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.60 mmol, 135 µL, 2 eq). The reaction mixture was stirred at rt for 1 h. The volatiles were removed *in vacuo* and the residue was purified by flash chromatography on silica gel eluted with toluene/EtOAc (95:5 Ÿ 65:35, v/v) to provide the desired compound **10** as a yellow foam (241 mg, 65 %). $C_{70}H_{65}N_4O_{15}P$ (1233.28). R_f=0.55

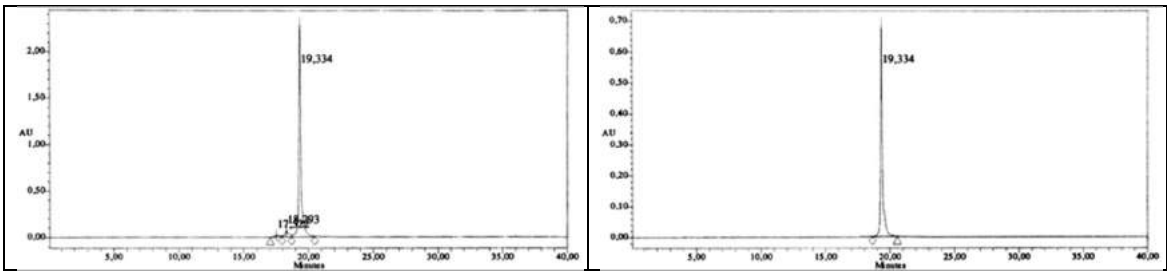
(toluene/EtOAc=8:2). ^{1}H-NMR (CD_3CN, 200 MHz): δ=8.44 (1H, s, H6), 8.13 (1H, dd, ^3J=8.2 Hz and ^4J=1.6 Hz, H5Ñj 7.93 (2H, d, ^3J =8.1 Hz, *o-H*-Tol), 7.79 (1H, ddd, ^3J=8.2 Hz, 7.4 Hz and ^4J=1.6 Hz, H7Ñj 7.56ï 7.18 (18H, m, H6Ñj H8Ñj *H*-Ph, *o-H*-PhOMe, *H*-Ph-Cbz, *m-H*-Tol), 7.16 (1H, d, ^3J=3.8 Hz, Hβ), 6.85 (4H, m, *m-H*-PhOMe), 6.43 (1H, d, ^3J=3.8 Hz, Hα), 6.13 (1H, m, H1Ñj 5.29 (2H, s, CH_2-Cbz), 4.90ï 4.70 (1H, m, H3Ñj 4.26ï 4.13 (1H, m, H4Ñj 3.83ï 3.70 (2H, m, CH_2-O), 3.68 (3H, s, *Me*O), 3.67 (3H, s, *Me*O), 3.60ï 3.45 (2H, m, N-C<u>H</u>(CH_3)$_2$), 3.38 (2H, ddd, ^2J=12.0 Hz, ^3J=9.0 and ^4J=3.2 Hz, Hz H5Ñj 2.71ï 2.51 (4H, m, H2Ñj *C*H_2-CN), 2.45 (3H, s, *p-Me*-Tol), 1.22ï 1.05 (12H, m, N-CH(<u>CH_3</u>)$_2$); ^{31}P-NMR (CD_3CN, 81 MHz): δ=148.16 (1P), 148.03 (1P); HRMS (ESI$^+$): *m/z* calcd for $C_{70}H_{65}N_4NaO_{15}P$: 1255.4076 $[M+Na]^+$; found 1255.4036 $[M+Na]^+$.

2. ODN synthesis, purification & physical characterization

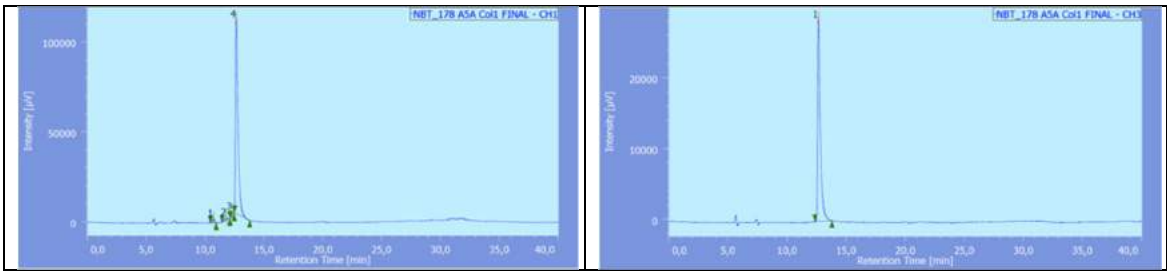
2.1 ODN synthesis and purification:

The ODN synthesis was performed on an Expedite 8900 DNA synthesizer (Applied Biosystem) using the ñtrityl offò mode and mild phosphoramidite chemistry on a 0.2 ε mol scale. Reagents and solvents, as well as dT, Ac-dC, Pac-dA, and dmf-dG phosphoramidites were purchased from Link Technologies. The standard DNA assembly protocol ñDMT-offò was used except for the following modifications: 5-Ethylthio-1H-tetrazole (ETT) was used as activating agent; Pac-anhydride was used for capping; a longer coupling time (1200 s) was applied to the 3HC phosphoramidite as well as for the following nucleotide (600 s). Non-labelled ODNs were purchased from Microsynth AG. The ODNs were cleaved from the solid support and deprotected with concentrated aqueous ammonia at room temperature for 12 h. The ODNs were analysed (0.5 mL/min) and purified (2.5 mL/min) by RP HPLC (HPLC apparatus: Waters™ 600 Controller with Waters™ 996 Photodiode Array Detector and Jasco LC-Net II / ADC apparatus. Columns: analytical, 300 × 4.60 mm, 5 ε m particle size, Clarity® 100Å, Phenomenex®; semi-preparative, Clarity® 5u Oligo-RP column 250 x 10 mm Phenomenex®). The following gradient system was used: 100 % A ï (30 min)Ÿ 60 % A / 40 % B ï (5 min)Ÿ 100 % B ï (5 min)Ÿ 100 % A with A=Buffer pH 7.0 (1.9 L of MilliQ® water, 160 mL acetonitrile, 28 mL triethylamine, 12 mL of acetic acid) and B=0.2 CH_3CN:0.8 Buffer.

<u>Fig S1. HPLC profile of TMT single strand ODN (254 nm left – 390 nm right):</u>



<u>Fig S2. HPLC profile of AMA single strand ODN (254 nm left – 390 nm right):</u>



<u>Fig S3. HPLC profile of CMC single strand ODN (254 nm left – 390 nm right):</u>

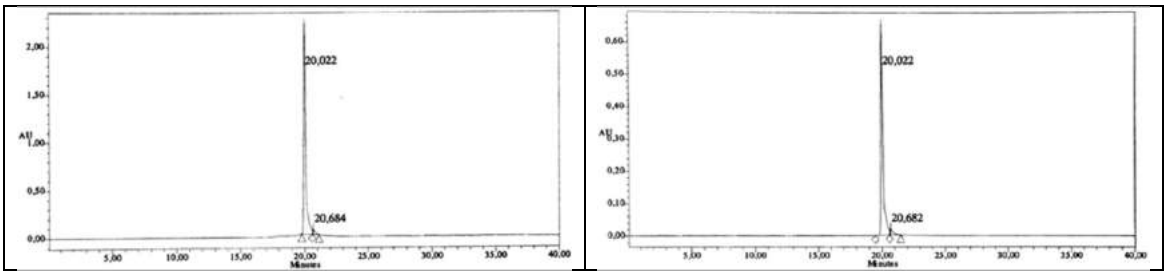
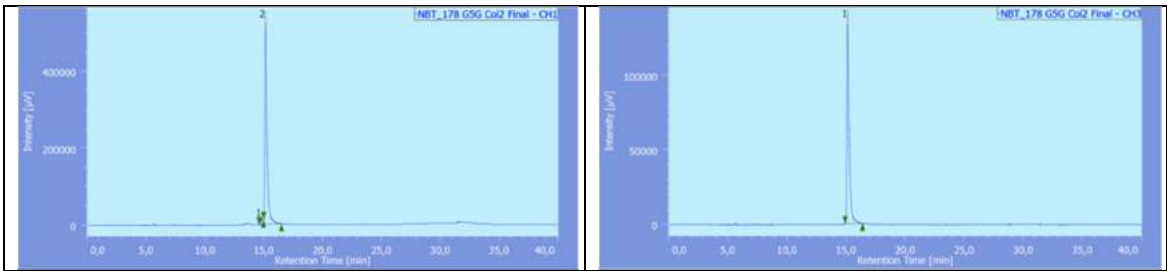


Fig S4. HPLC profile of GMG single strand ODN (254 nm left – 390 nm right):



2.2 MALDI TOF/TOF analysis of ODNs:

Ammonium Citrate dibasic (DAC) (98% capillary GC) was obtained from Sigma. CH_3CN of HPLC grade was purchased from VWR chemical. Ultrapure 3-Hydroxypicolinic Acid (3-HPA) MALDI matrix was purchased from protea Biosciences. C4 pipette tips (Zip-Tip) were from Millipore.
The samples (500 pmol) was diluted to 10 µL of water and was desalted with a C4 pipette Tips (Zip-tip). The Zip-tip was activated before use with 2 x 5 µL of water: CH_3CN (50:50) and 2 x 5µL of DAC (50 mg/mL diluted in water). The 10 µL of the ODN solution was loaded on Zip-tip by drawing and expelling ten times. Next the zip-tip was washed with 3 x 5 µL of DAC (50 mg/mL) and 3 x 5 µL of water. Elution was performed with 1.5 µL of 3-HPA matrix (80 mg/mL, 50:50 CH_3CN:DAC) directly on MALDI plate. The ODN profile was obtained on a ABSciex MALDI-TOF/TOF mass spectrometer in reflector mode with external calibration mixture (cal Mix 1+2 distributed by ABSciex). MALDI-TOF/TOF-MS analysis: MS spectra were recorded manually in a mass range of 500-6000 Da resulting from 400 laser shots of constant intensity fixed at 6200. Data were collected using 4000 series Explorer (AB SCIEX) experiments.

Table S1. MALDI-TOF and photophysical characterization of the synthesized FCU-labelled oligonucleotides.

ODN	Sequence[b,c]	MALDI-TOF found (calcd) [M^+]	λ_{abs} (nm)[c]	λ_{N^*} (nm)[d]	λ_{T^*} (nm)[e]	I_{N^*}/I_{T^*}	Φ (%)[f]
1	5´d-CGTTTTTMTTTTTGC-3´	4757.7 (4757.2)	397	490	549	1.15	14
2	5´d-CGTTTTCMCTTTTGC-3´	4727.8 (4727.2)	398	490	554	1.21	10
3	5´d-CGTTTTAMATTTTGC-3´	4775.7 (4775.2)	400	489	557	1.01	8.7
4	5´d-CGTTTTGMGTTTTGC-3´	4807.7 (4807.2)	401	486	555	1.22	1.8

3. Spectroscopic characterization

3.1 Preparation of the samples:

For spectroscopic measurements, the ODNs prepared in phosphate buffer pH 7.0 (20 mM phosphate, 150 mM NaCl, 1 mM EDTA).

Preparation of the single strand solution: the solution of the sample was prepared by mixing 400 µL of a stock solution of 40 mM phosphate buffer solution pH 7.0, 80 µL of 1.5 M NaCl solution, 10 µL of 80 mM EDTA solution, 25 µL of 64 µM ssODN and 285 µL of MilliQ® water.

Preparation of the double strand solution: 400 µL of a stock solution of 40 mM phosphate buffer solution pH 7.0 was mixed with 80 µL of 1.5 M NaCl solution, 10 µL of 80 mM EDTA solution, 25 µL of 64 µM ODN 1, 25 µL of 64 µM ODN 2 and 260 µL of MilliQ® water.

3.2 Denaturation studies and melting temperatures:

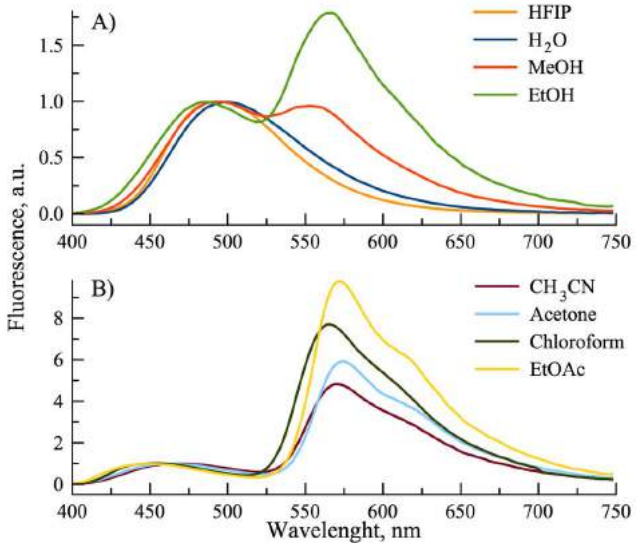
Melting curves were recorded by following the temperature-dependence of the absorbance changes at 260 nm of the sample (2 ε M concentration of each strand). Absorption spectra were recorded in a Peltier thermostated cell holder on a Cary 4000 spectrophotometer (Varian). The pathlength of cell was 1 cm. The temperature range for denaturation measurement was 5 ï 80 °C. Speed of heating was 0.3 °C/min. Cacodylate buffer pH 7 was used (10 mM cacodylate buffer, 150 mM NaCl, 1 mM EDTA). The melting curves were converted into a plot of Ŭ versus temperature, where Ŭ represents the fraction of single-strands in the duplex state. The melting temperatures were extracted from these curves after differentiation as described elsewhere.[6]

Table S2. Absorption and fluorescence properties of **FCU** in different solvents

Solvent	λ_{abs} (nm) [a]	λ_{N^*} (nm) [b]	λ_{T^*} (nm) [c]	I_{N^*}/I_{T^*}	Φ (%) [d]
HFIP[e]	387	493	ī	ī	33
H_2O	391	499	ī	ī	13
MeOH	387	497	551	1.03	24
EtOH	388	488	566	0.56	22
CH_3CN	382	466	569	0.20	17
Acetone	383	462	574	0.17	16
$CHCl_3$	390	454	564	0.15	23
EtOAc	384	444	573	0.10	22

[a] Position of the absorption maximum
[b] Position of the emission maximum of the N* band
[c] Position of the emission maximum of the T* band
[d] Mean standard deviation of quantum yields, ±10%
[e] Hexafluoroisopropanol

Fig S5. Normalized emission spectra of FCU in protic (A) and aprotic (B) solvents:



The emission spectra were normalized at the N* band. Excitation wavelength was 390 nm.

Table S3. Absorption, fluorescence and thermal melting properties of the FCU-labelled DNA duplexes

Duplexes	T_m (°C)[c]			λ_{abs} (nm)[d]	λ_{N^*} (nm)[e]	λ_{T^*} (nm)[f]	I_{N^*}/I_{T^*}	Φ (%)[g]
	M[a]	*Wild Type*	*ΔTm* (°C)[b]					
TMT·*AAA*	45.9	49.5	-3.6	392	482	-	-	14.9
TMT·*ATA*	37.8	40.5	-2.7	397	487	566	0.80	17.6
TMT·*ACA*	39.7	39	0.7	397	488	567	0.86	19.9
TMT·*AGA*	39.1	40.7	-1.6	399	488	560	1.15	7.1
TMT·*AAbA*	39.2	34.5	4.7	401	489	567	0.96	19.6
CMC·*GAG*	46.7	51.3	-4.6	392	483	-	-	10
CMC·*GTG*	39.4	41.1	-1.7	400	484	562	1.37	3.4
CMC·*GCG*	43.2	42.1	1.1	401	484	561	1.29	2.4
CMC·*GGG*	42.7	46.5	-3.8	403	488	549	1.28	5.5
CMC·*GAbG*	43.1	37.2	5.9	403	488	558	1.25	2.1
AMA·*TAT*	41.9	47.3	-5.4	395	486	-	-	16.8
AMA·*TTT*	38.1	39.8	-1.7	398	487	567	0.79	18.9
AMA·*TCT*	40.4	38.5	1.9	398	486	567	0.76	20.8
AMA·*TGT*	38.7	42.7	-4.0	400	487	562	0.83	8.3
AMA·*TAbT*	37.5	30.5	7.0	402	487	561	1.23	22.9
GMG·*CAC*	52.1	54.8	-2.7	395	484	-	-	1.2
GMG·*CTC*	46.6	46.6	0	400	485	569	0.91	1.6
GMG·*CCC*	48.6	43.5	5.1	401	485	570	0.96	1.7
GMG·*CGC*	47.9	49.7	-1.8	402	484	566	0.74	1.7
GMG·*CAbC*	45.8	37.5	8.3	403	487	567	1.08	1.6

Duplexes[b]	T_m (°C)[c]			λ_{abs} (nm)[d]	λ_{N^*} (nm)[e]	λ_{T^*} (nm)[f]	I_{N^*}/I_{T^*}	Φ (%)[g]
	M	*Wild Type*	*ΔTm* (°C)					
TMT·*rAAA*	35.9	41.4	-5.5	402	485	552	1.38	23.2
AMA·*rUAU*	34.5	40.9	-6.4	403	485	548	1.35	16.5

[a] M denotes the FCU.
[b] ΔT_m refers to the difference of Tm between the labelled and non labelled ODNs
[c] T_m Melting temperature; ±0.5 °C
[d] Position of the absorption maximum
[e] Position of the emission maximum of the N* band
[f] Position of the emission maximum of the T* band
[g] Mean standard deviation of quantum yields, ±10%

Fig S6. Melting curves of the non labelled duplexes:

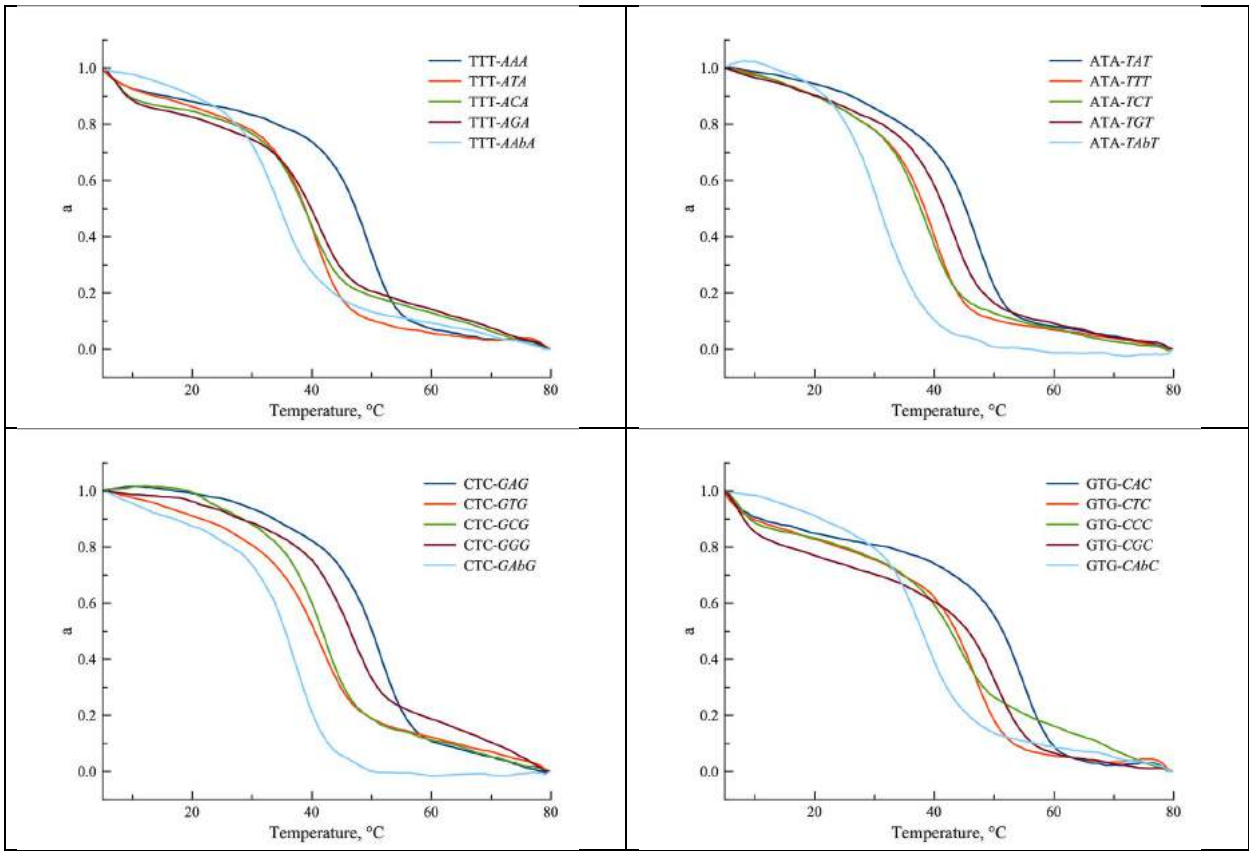
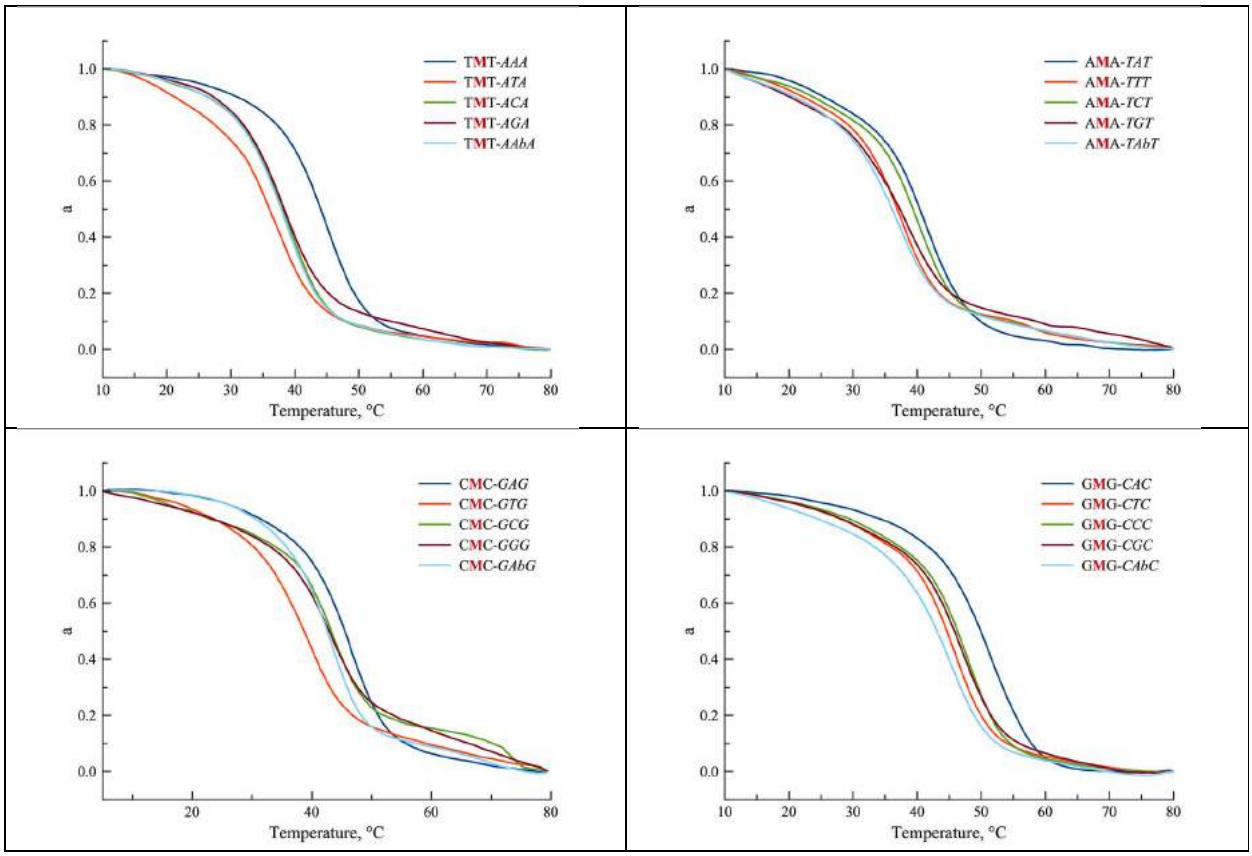


Fig S7. Melting curves of the FCU-labelled duplexes:



3.3. Circular dichroism

Circular dichroism spectra were recorded with 2 εM solution of the non labeled dsDNA and labeled dsDNA (FCU opposite **A**, **T** or **Ab**) in phosphate buffer pH 7 (20 mM phosphate buffer, 150 mM NaCl, 1 mM EDTA) at 25 °C on a Jasco J-810 spectropolarimeter. Two maxima were observed in CD spectra: a negative one at 249 nm and a positive one at 282 nm.

Fig S8. Representative CD spectra of non labelled and FCU-labelled duplexes:

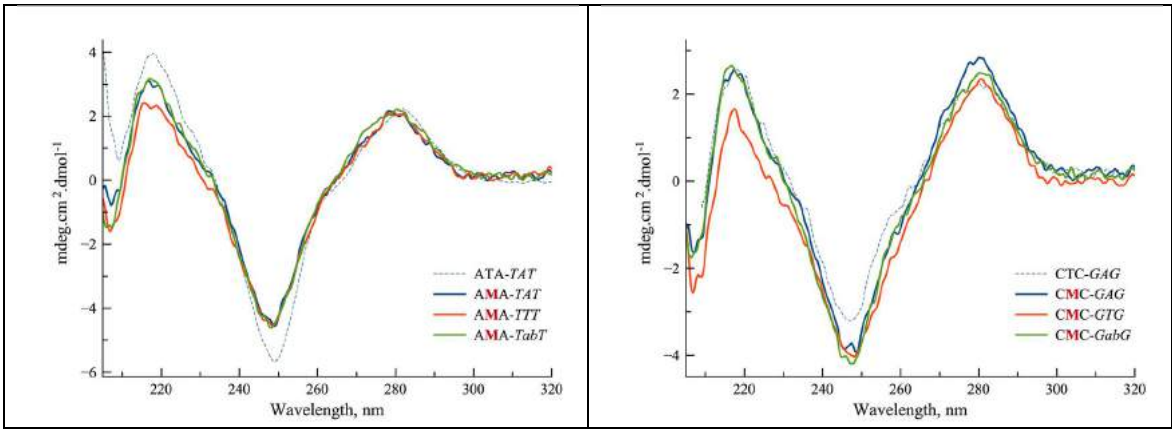
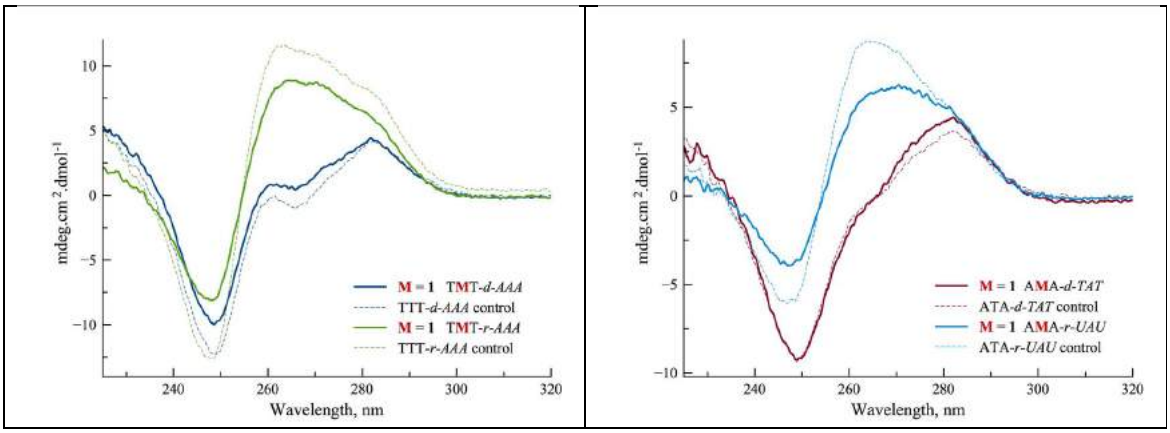


Fig S9. CD spectra of B DNA/DNA and A DNA/RNA:



3.4. Absorption and Fluorescence spectra

The absorption and fluorescence spectra were recorded in phosphate buffer of pH 7 (20 mM phosphate buffer, 150 mM NaCl, 1 mM EDTA). The absorption spectra were recorded on a Cary 300 Scan spectrophotometer (Varian) using 1 cm quartz cells at 20 °C. The fluorescence spectra were recorded on a FluoroMax 4.0 spectrofluorometer (Jobin Yvon, Horiba) using excitation and emission slits of 2 nm and were corrected at both excitation and emission. The solutions had an absorbance of about 0.05 at 20 °C at the 390 nm excitation wavelength. The quantum yields were corrected according to the variation of the refractive index of the solvents.

Fig S10. UV spectra of FCU-labelled single and double strands:

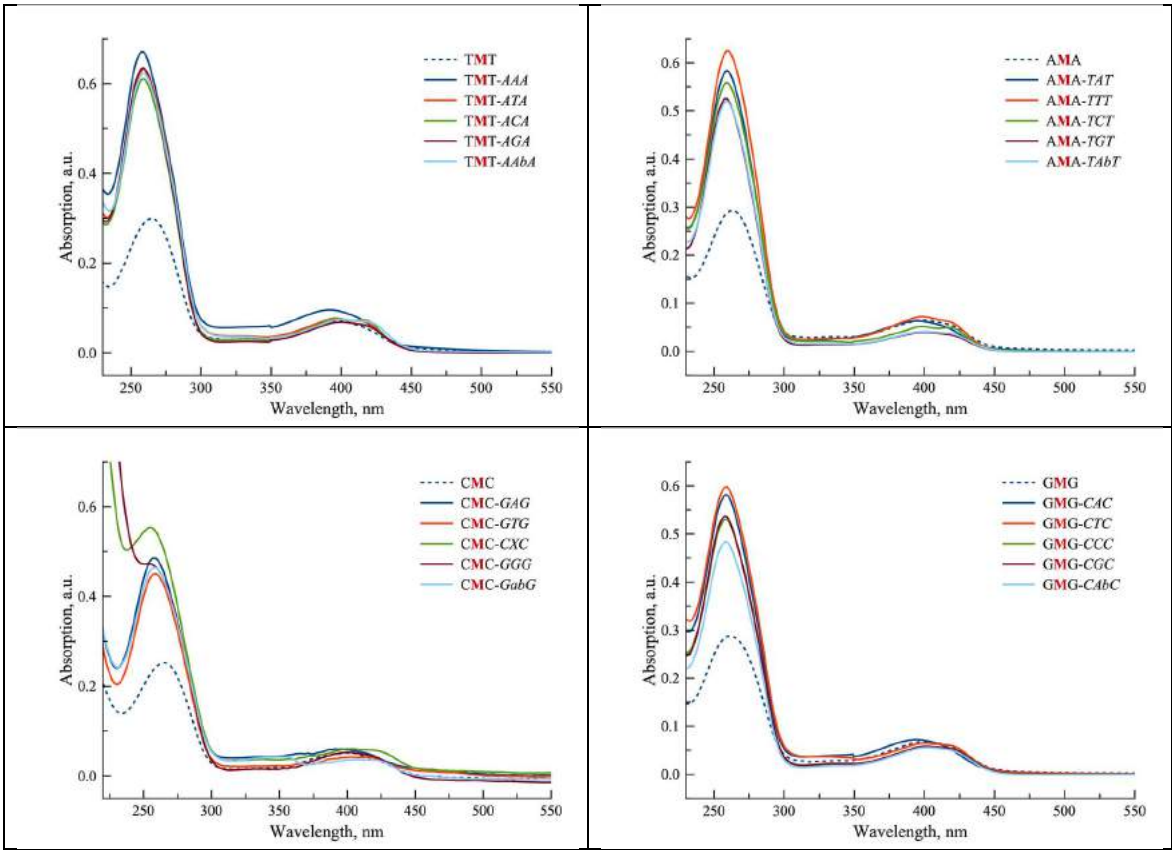


Fig S11. Fluorescence spectra of single and double strands with TMT:

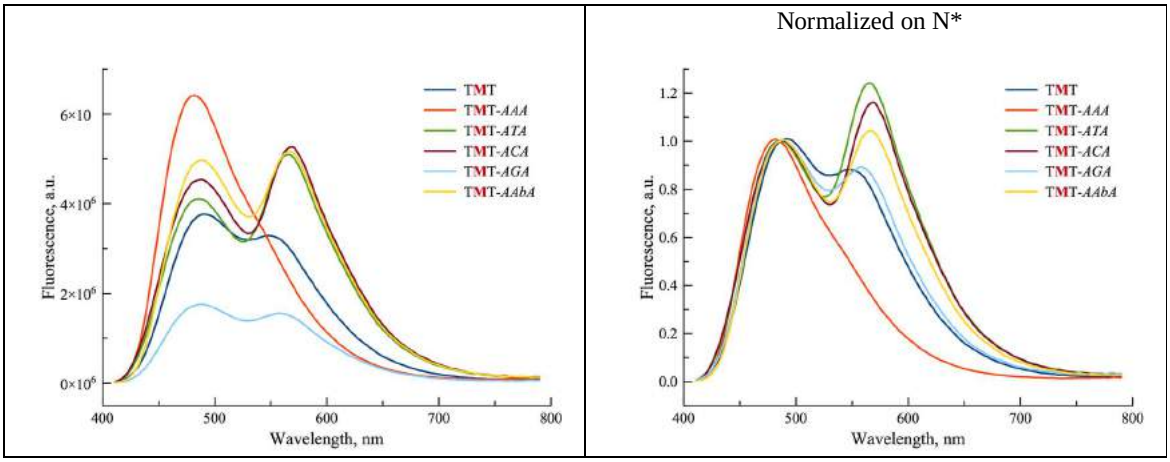


Fig S12. Fluorescence spectra of single and double strands with AMA:

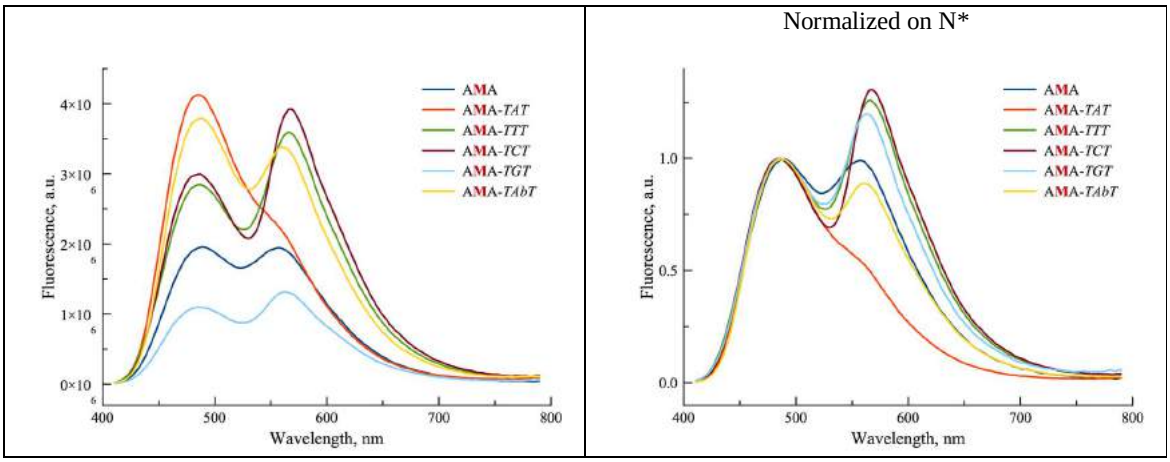


Fig S13. Fluorescence spectra of single and double strands with CMC:

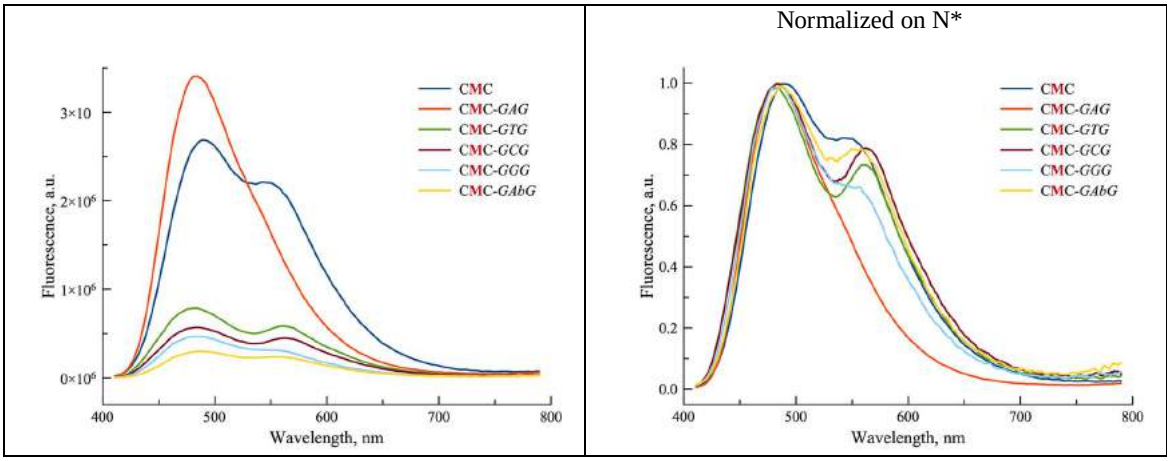


Fig S14. Fluorescence spectra of single and double strands with GMG:

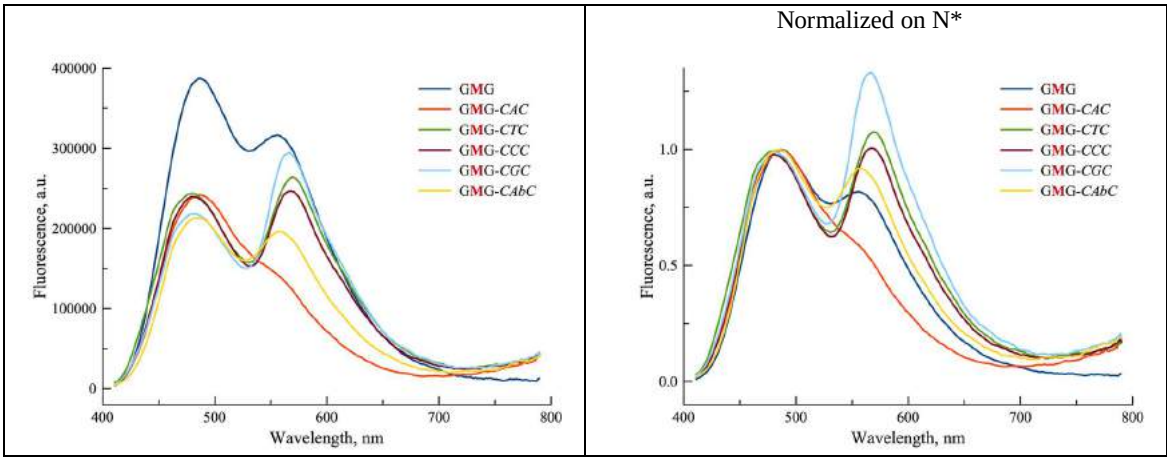
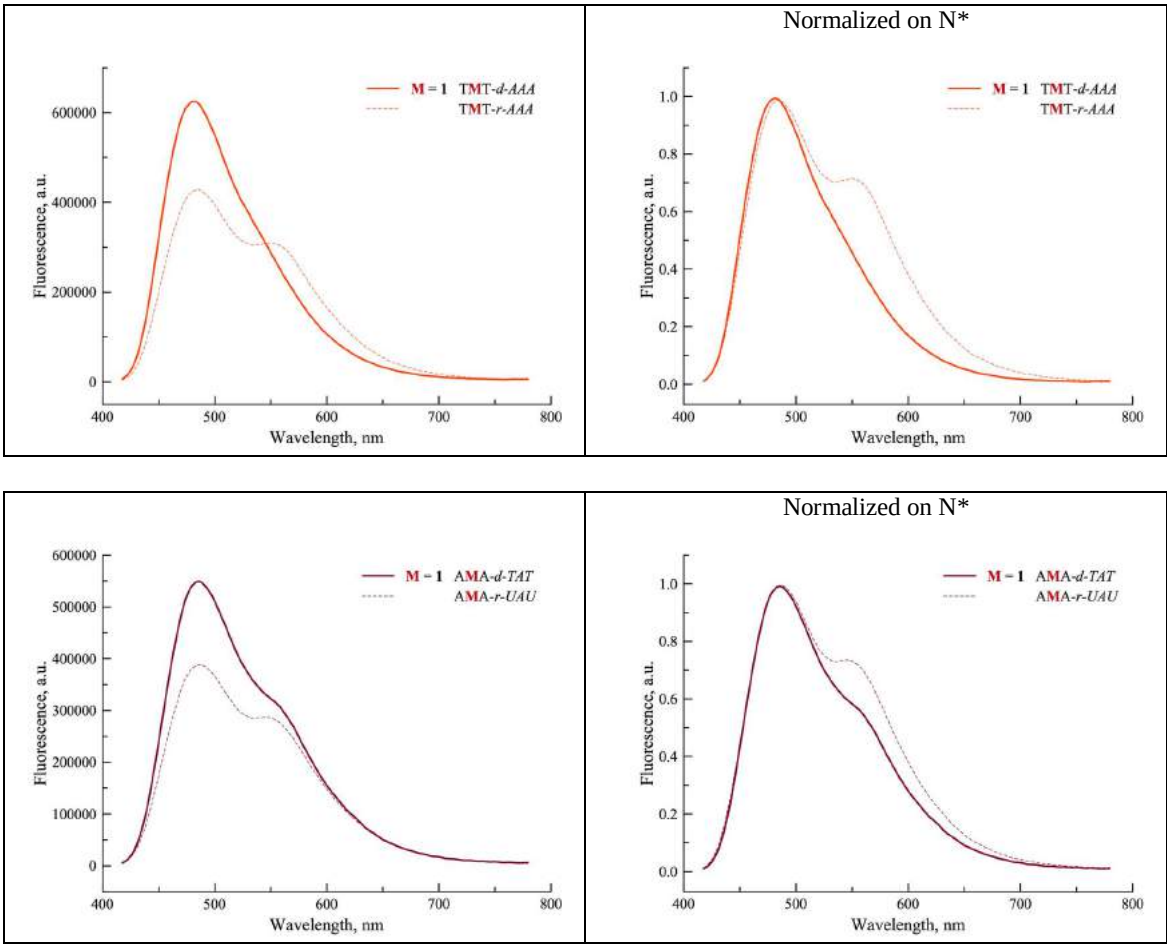


Fig S15. Fluorescence spectra of B DNA/DNA and A DNA/RNA:



3.5. Time resolved fluorescence measurements

Time-resolved fluorescence measurements were performed with the time-correlated, single-photon counting technique (TCSPC).[7] Excitation at 420 nm was provided by a pulse-picked frequency doubled Ti-sapphire laser (Tsunami, Spectra Physics), pumped by a Millenia X laser(Spectra Physics). Emission was collected through a polarizer set at magic angle and an 8 nm band-pass monochromator (Jobin-Yvon H10) set at emission maximum. The single photon events were detected with a microchannel plate Hamamatsu R3809Uphotomultiplier coupled to a Philips 6954 pulse preamplifier and recorded on a multichannel analyzer (Ortec 7100), calibrated at 25.5 ps/channel. The instrumental response function was recorded with a polished aluminum reflector,and its full width at half-maximum was 40 ps. Fluorescence intensity decays [$I(t)$] were analyzed as a sum of exponentials :$I(t) = \sum \alpha_i \exp(-t/\tau_i)$, where τ_i values are the fluorescence lifetimes and α_i values are the associated amplitudes such that $\sum \alpha_i = 1$. The mean lifetime was calculated according to the relationship $\langle\tau\rangle = \sum \alpha_i \tau_i$.

Table S4. Fluorescence decays fitting parameters of FCU in solvents

Collecting Band	τ_1 (ns)	a_1	τ_2 (ns)	a_2	$\langle\tau\rangle$
Water					
N*	1.11	-	-	-	1.11
Methanol					
N*	0.65	0.90	1.35	0.10	0.72
T*	0.55	-0.35	1.29	0.65	1.03
Ethanol					
N*	0.44	0.96	1.60	0.04	0.49
T*	0.31	-0.35	1.50	0.65	1.08
EtOAc					
N*	0.07	0.98	2.15	0.02	0.11
T*	0.07	-0.48	2.10	0.52	1.13

Table S5. Fluorescence decays fitting parameters of matched and mismatched duplexes

Collecting Band	τ_1 (ns)	a_1	τ_2 (ns)	a_2	τ_3 (ns)	a_3	$\langle\tau\rangle$
T**M**T (ss DNA)							
N*	0.20	0.42	0.86	0.45	1.83	0.13	0.71
T*	0.25	-0.23	-	-	2.26	0.77	1.80
T**M**T-*AAA*							
N*	0.62	0.56	0.87	0.44	-	-	0.73
T*	0.61	-0.47	0.73	0.52	2.65	0.01	0.68
T**M**T-*AAbA*							
N*	0.38	0.56	0.97	0.35	2.34	0.09	0.77
T*	0.36	-0.38	-	-	3.35	0.62	2.207
T**M**T-*ACA*							
N*	0.18	0.61	0.75	0.27	2.36	0.12	0.59
T*	0.21	-0.36	-	-	3.41	0.64	2.26
T**M**T-*ATA*							
N*	0.20	0.61	0.73	0.30	2.01	0.09	0.52
T*	0.21	-0.37	-	-	3.30	0.63	2.16

Table S6. Fluorescence decays fitting parameters of DNA/DNA and DNA/RNA duplexes

Collecting Band	τ_1 (ns)	a_1	τ_2 (ns)	a_2	τ_3 (ns)	a_3	$\langle\tau\rangle$
T**M**T-*AAA*							
N*	0.62	0.56	0.87	0.44	-	-	0.73
T*	0.61	-0.47	0.73	0.52	2.65	0.01	0.68
T**M**T-r*AAA*							
N*	0.24	0.22	0.61	0.71	1.59	0.07	0.60
T*	0.22	-0.32	1.25	0.39	3.42	0.28	1.53

3.6. pH dependence of the absorption and fluorescence spectra

pH of the solution was adjusted by adding diluted acid or base to the phosphate buffer solution (20 mM phosphate buffer, 150 mM NaCl). The absorption spectra were recorded on a Cary 4000 spectrophotometer (Varian) using 1 cm quartz cells at 20 °C. The fluorescence spectra were recorded on a FluoroMax 4.0 spectrofluorometer (Jobin Yvon, Horiba) by using excitation and emission slits of 2 nm and were corrected at excitation and emission. Excitation wavelength was 390 nm.

Figure S16. UV (left) and Fluorescence (right) spectra of FCU at different pH.

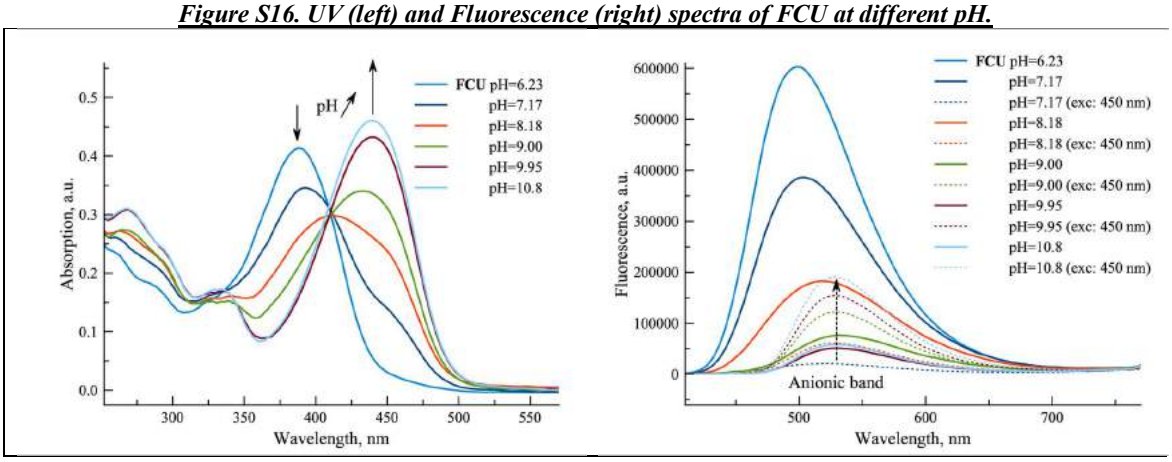


Figure S17. UV (left) and Fluorescence (right) spectra of CMC at different pH

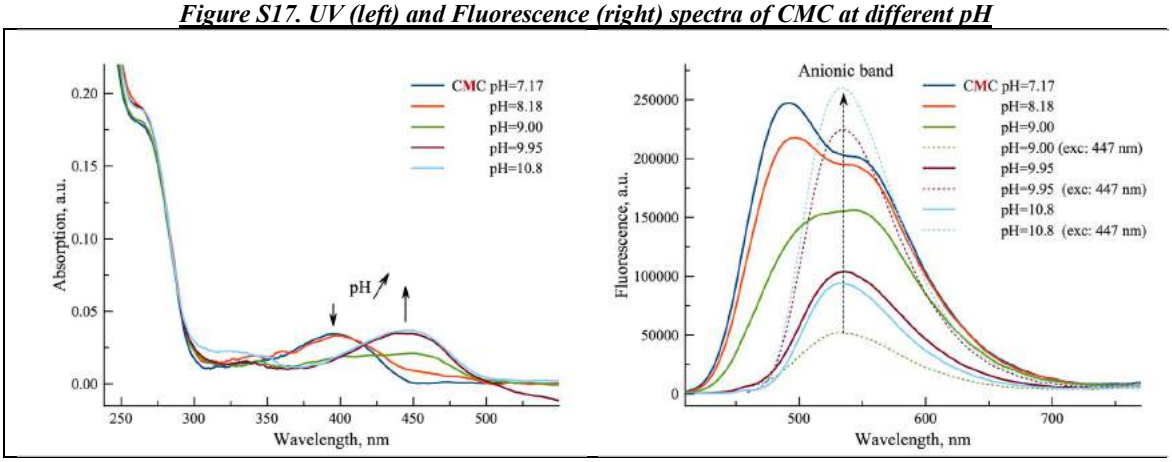


Figure S18. UV (left) and Fluorescence (right) spectra of CMC-GAG (match) at different pH.

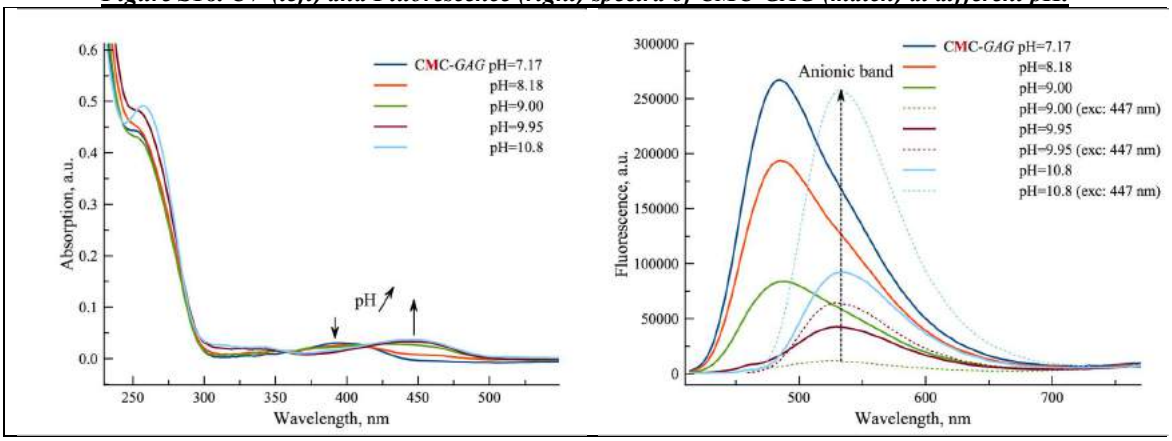


Figure S19. UV (left) and Fluorescence (right) spectra of CMC-GCG (mismatch) at different pH.

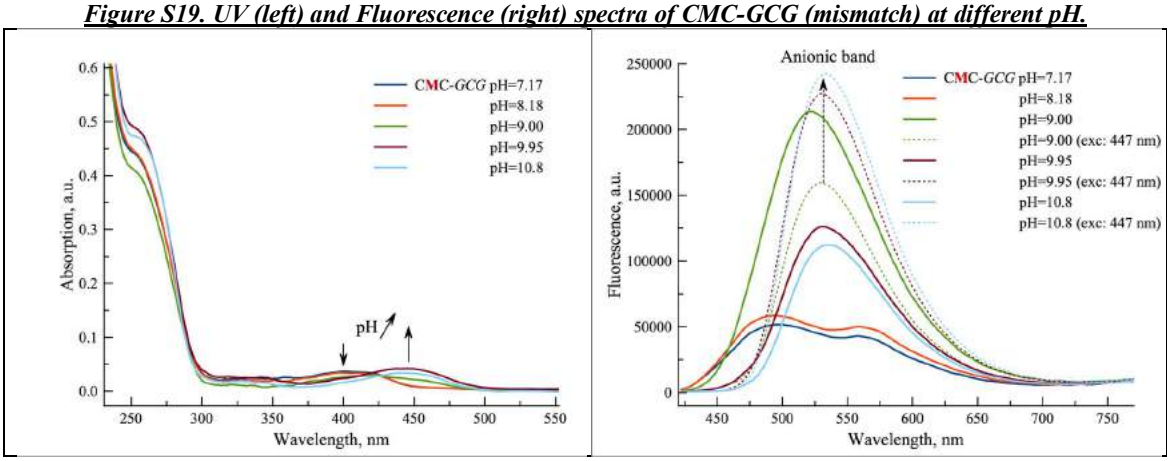
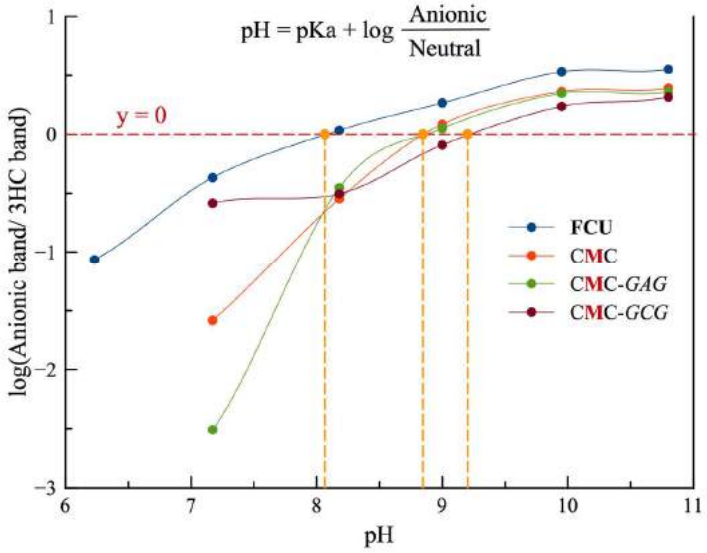


Figure S20. pH titration curves of FCU (blue), CMC (orange), CMC-GAG (green) and CMC-GCG (brown).



Sample	pK_a 3-O*H*
FCU	å 8.1
C**M**C	å 8.8
C**M**C-*GAG*	å 8.8
C**M**C-*GCG*	å 9.2

References:

[1] W. L. F. Armarego and C. L. L. Chai, *Purification of Laboratory Chemicals*, 7th ed.; Butterworth-Heinemann: Oxford, 2013.

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[3] (a) H. E. Gottlieb, V. Kotlyar and A. Nudelman, *J. Org. Chem.*, 1997, **62**, 7512ï 7515; (b) G. R. Fulmer, A. Miller, N. H. Sherden and H. E. Gottlieb, *Organometallics*, 2010, **29**, 2176ï 2179.

[4] D. Dziuba, I. A. Karpenko, N. P. F. Barthes, B. Y. Michel, A. S. Klymchenko, R. Benhida, R., A. P. Demchenko, Y. Mely and A. Burger, *Chem. Eur. J.*, **2014**, *20*, 1998ï 2009.

[5] N. P. F. Barthes, K. Gavvala, D. Dziuba, D. Bonhomme, I. A. Karpenko, A. S. Dabert-Gay, D. Debayle, A. P. Demchenko, R. Benhida, B. Y. Michel, Y. Mely and A. Burger, *J. Mater. Chem. C*, **2016**, *4*, 3010ï 3017.

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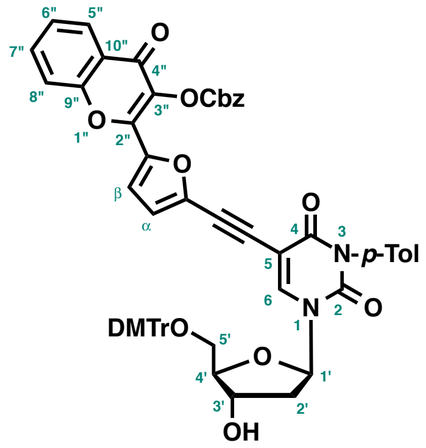
[7] J.R. Lakowicz. *Principles of Fluorescence Spectroscopy*; Kluwer Academic, NewYork, 2006.

4. NMR spectra of compounds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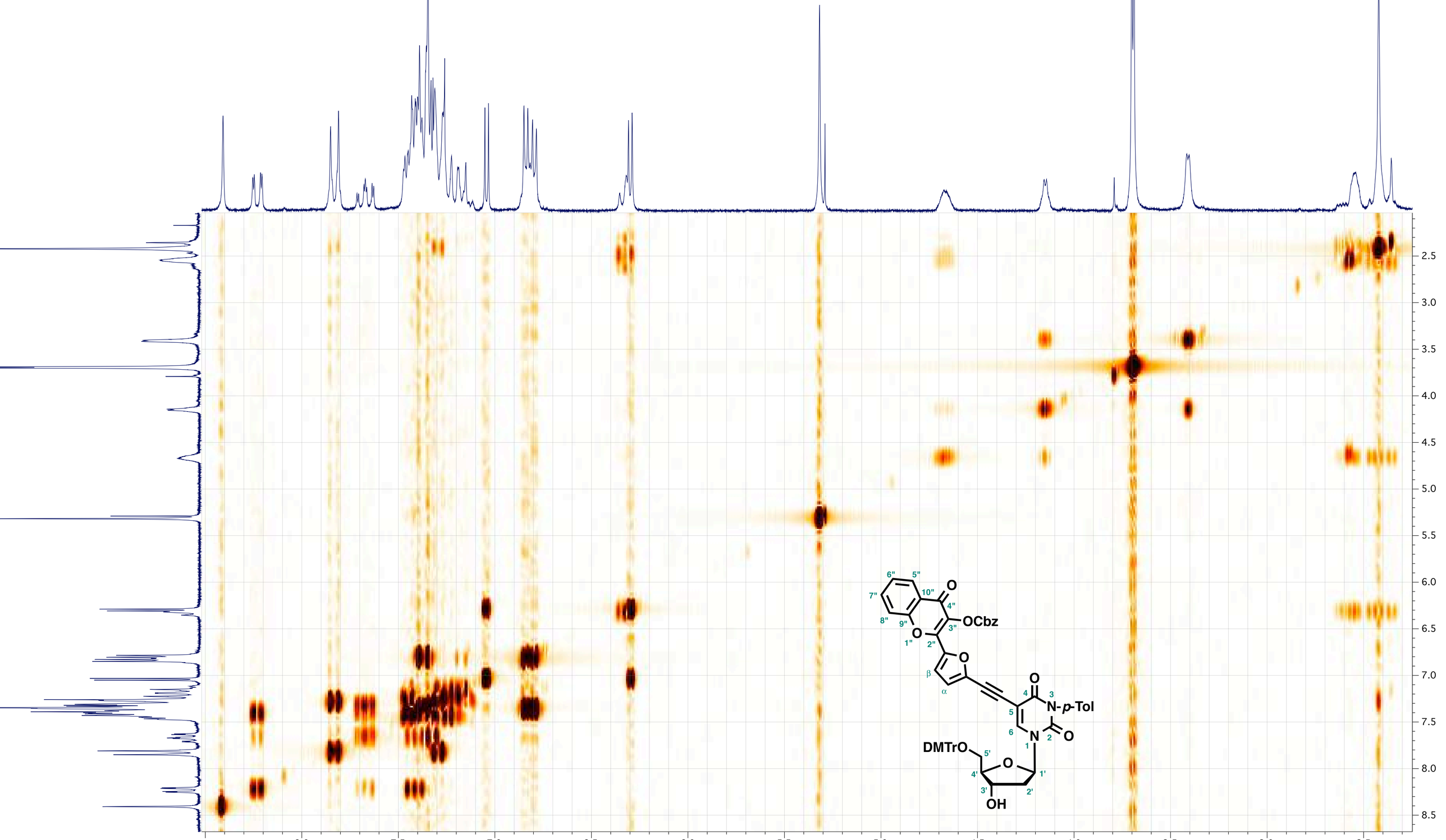
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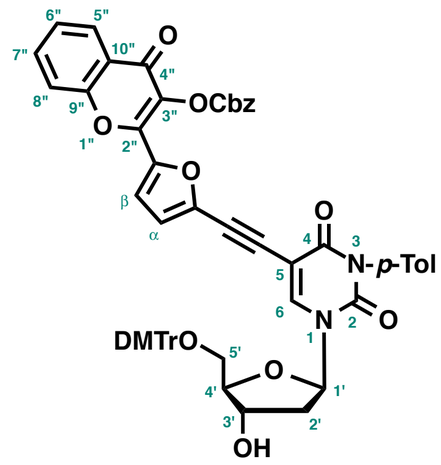

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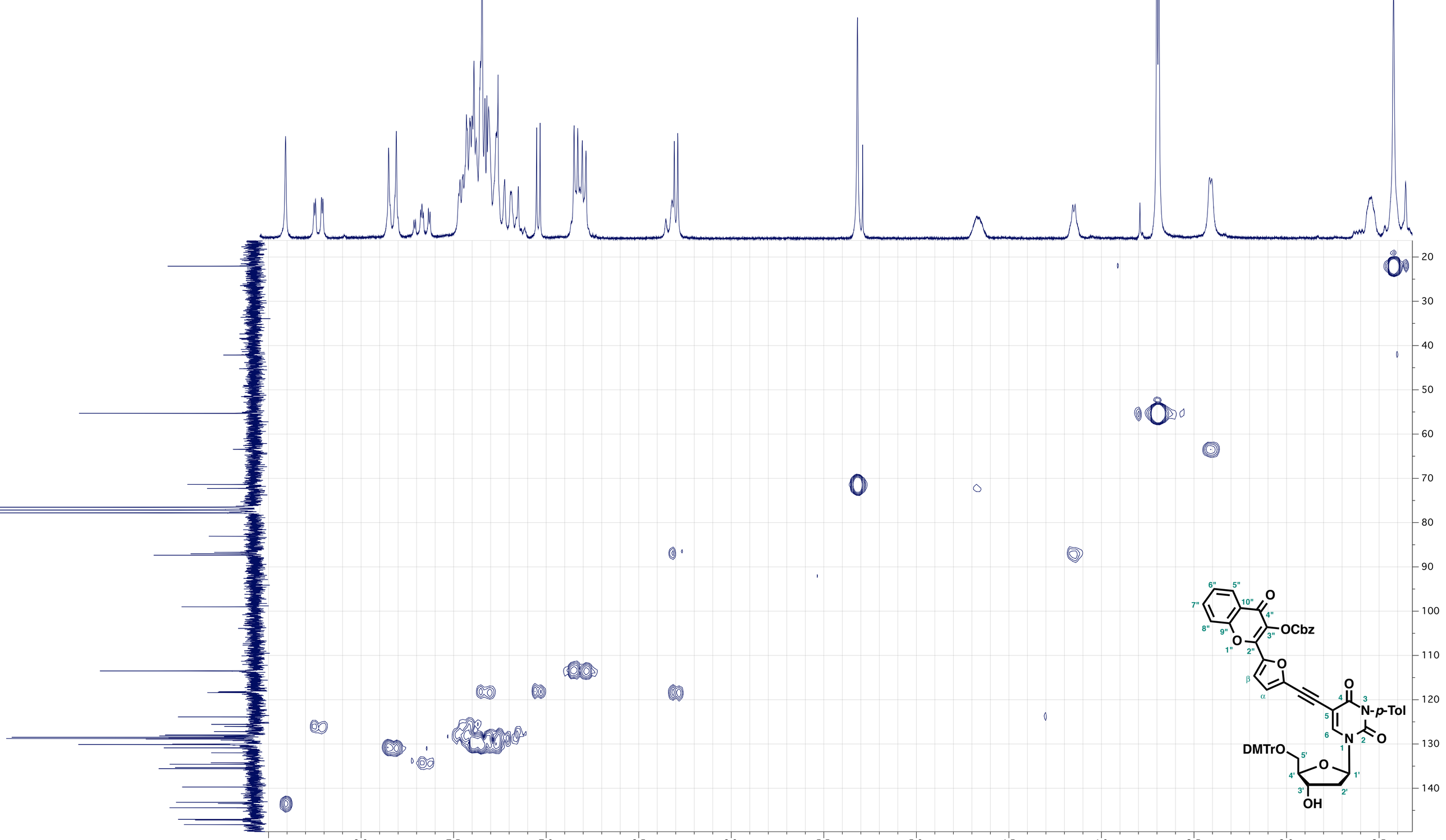
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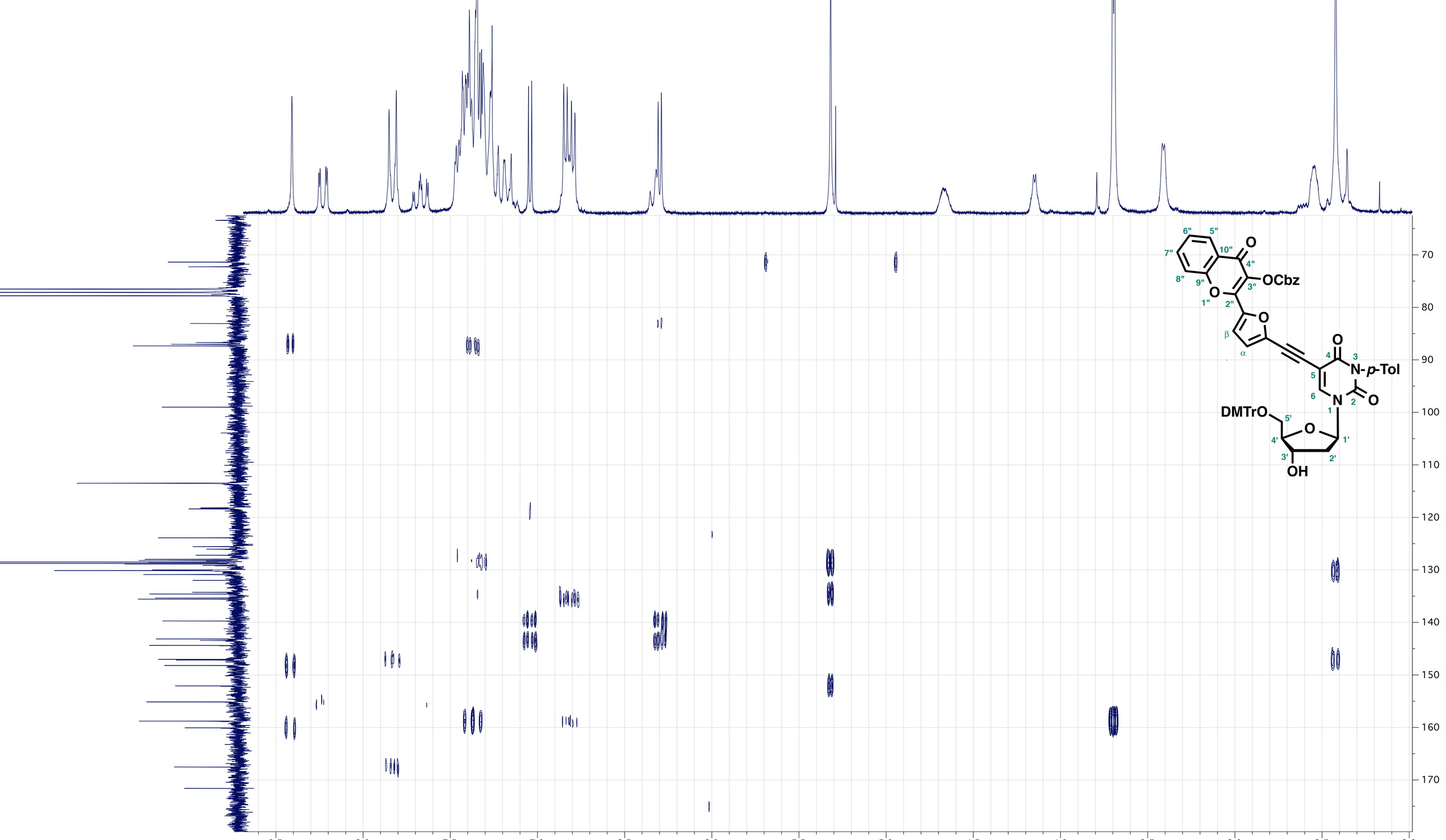
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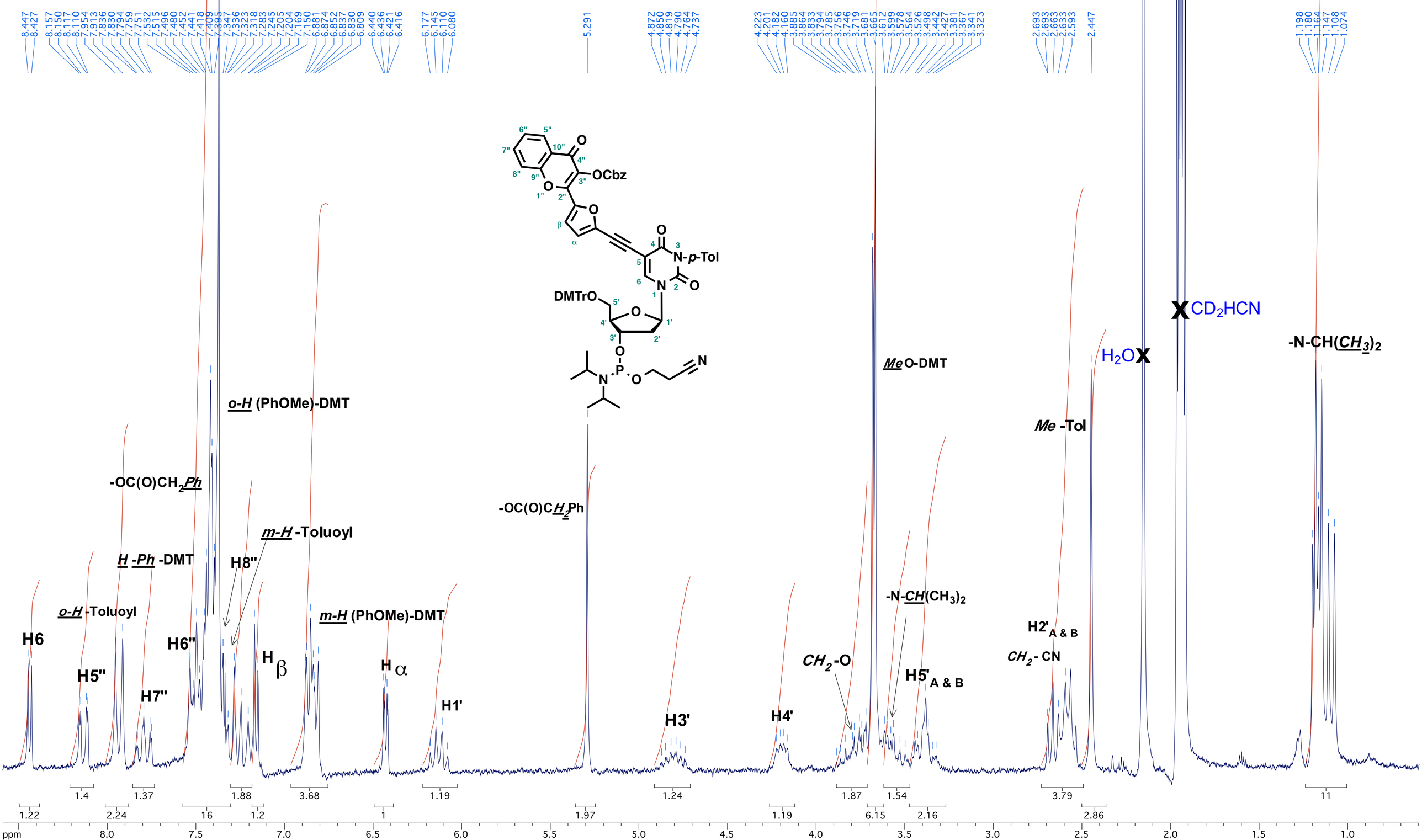
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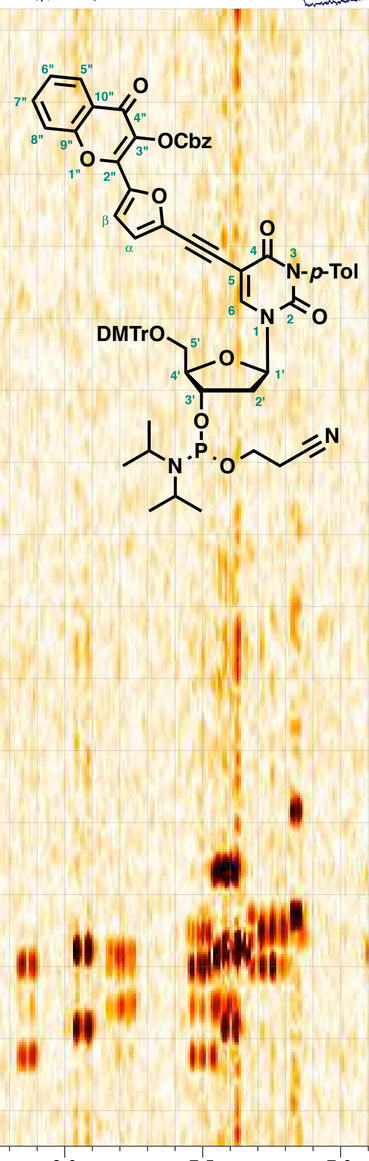
H2'A & B
CH2- CN
Me -Tol
H2O
CD2HCN
-N-CH(CH3)2
1.4
1.37
1.88
3.68
1.19
1.24
1.87
1.54
3.79
11
1.22
2.24
16
1.2
1
1.97
1.19
6.15
2.16
2.86
ppm
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0


6"
5"
10"
O
7"
4"
OCbz
8"
9"
3"
O
1"
2"
O
β
α
O
4
3
N-p-Tol
5
6
N
1
2
O
DMTrO
5'
O
4'
1'
3'
2'
O
P
N
O
N

ppm
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
1.0
1.5
2.0
2.5
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3.5
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4.5
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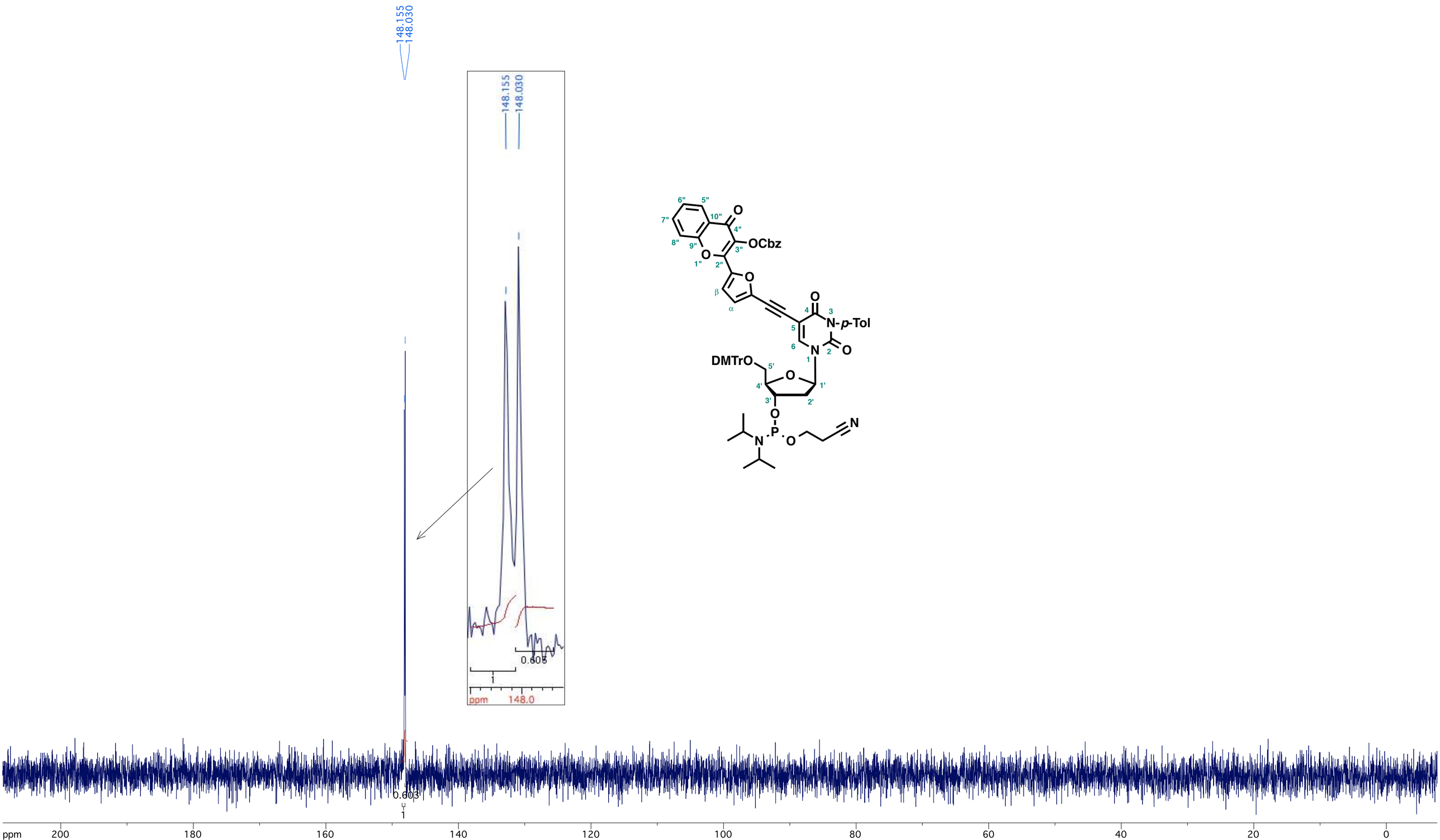
6.5
7.0
7.5
8.0
8.5


148.155
148.030
148.155
148.030
0.605
1
ppm 148.0
0.603
1
ppm
200
180
160
140
120
100
80
60
40
20
0
6"
5"
7"
10"
O
4"
8"
9"
O
3"
OCbz
1"
2"
O
β
α
4
3
N-p-Tol
5
6
N
1
2
O
DMTrO
5'
O
4'
1'
3'
2'
O
P
N
O
N