Designed thiazole orange nucleotides for the synthesis of single labelled oligonucleotides that fluorescence upon matched hybridization

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Supplementary Material

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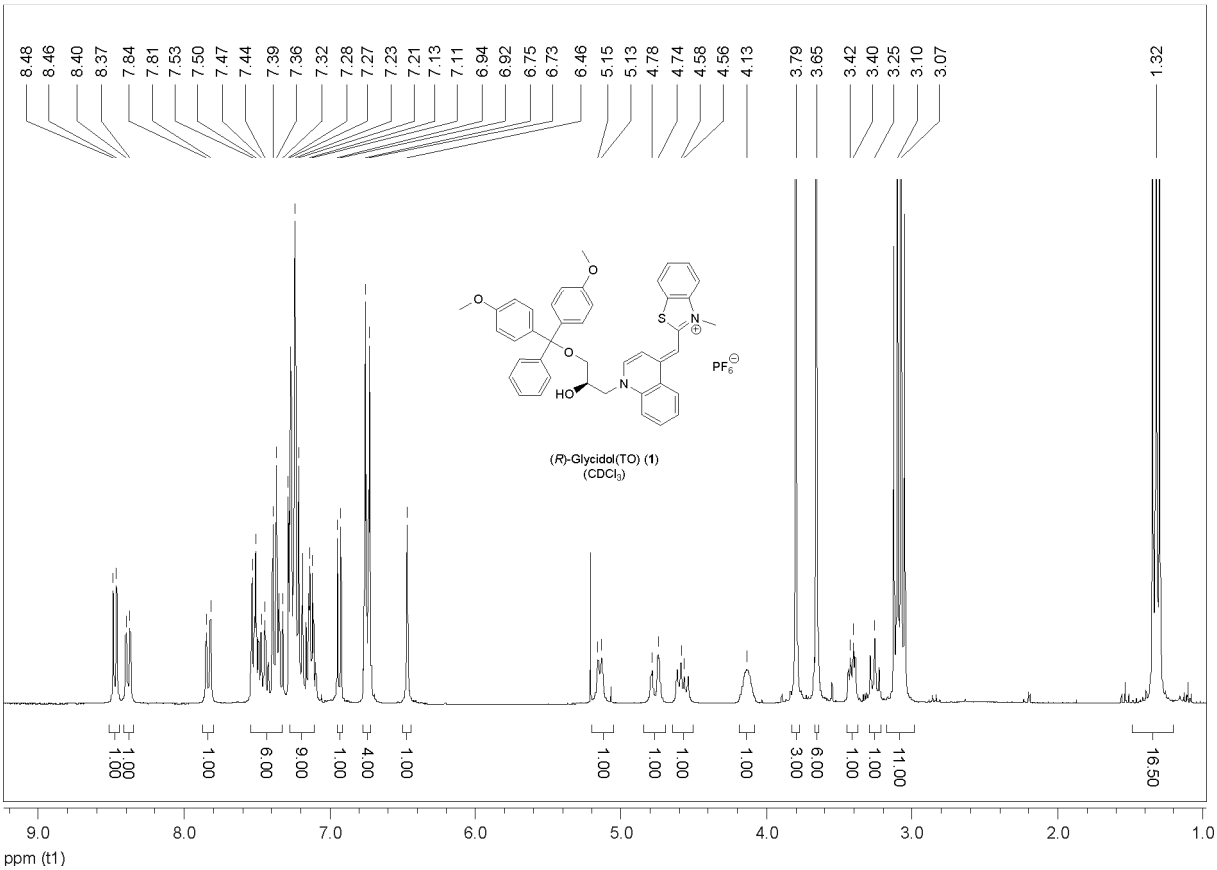
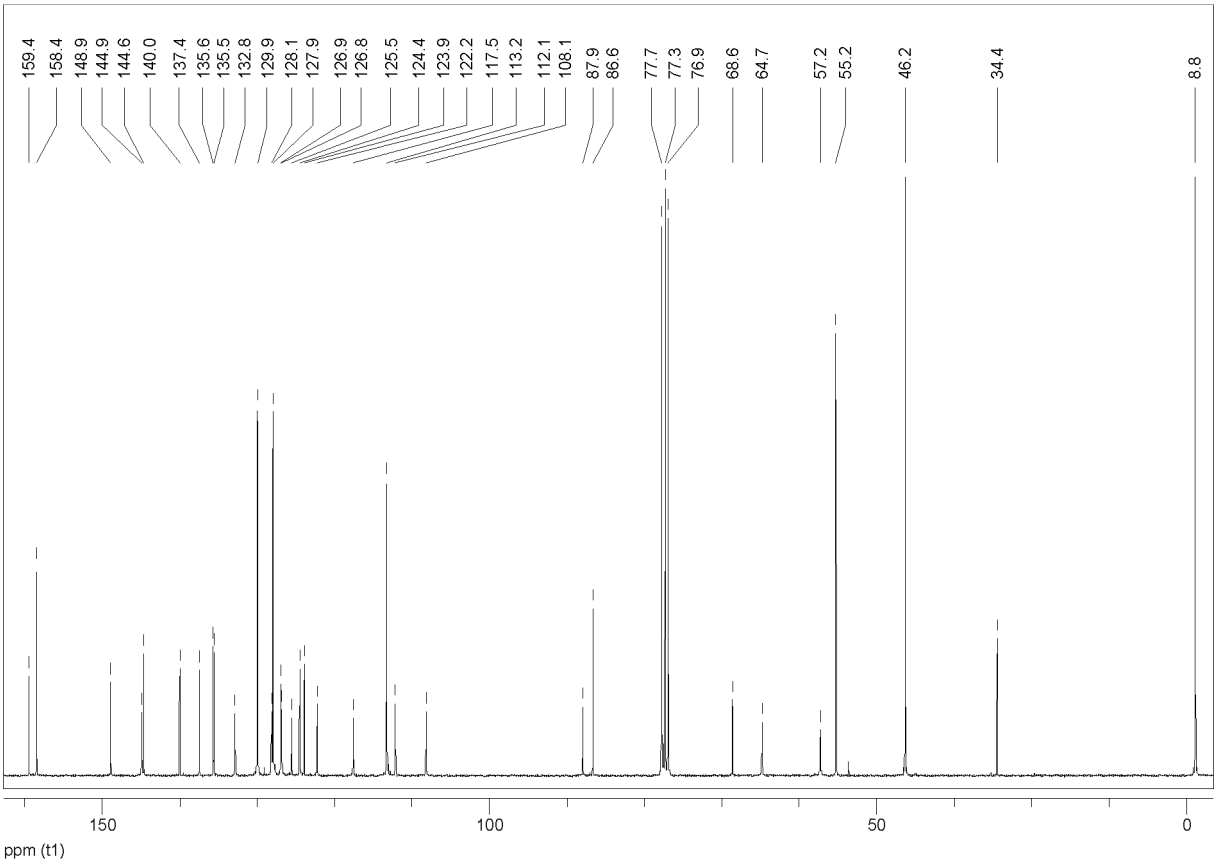
1 NMR Spectra of TO-DNA-Monomers



2 Eq. of NEt_3 added for stabilization of DMT ether in $CDCl_3$



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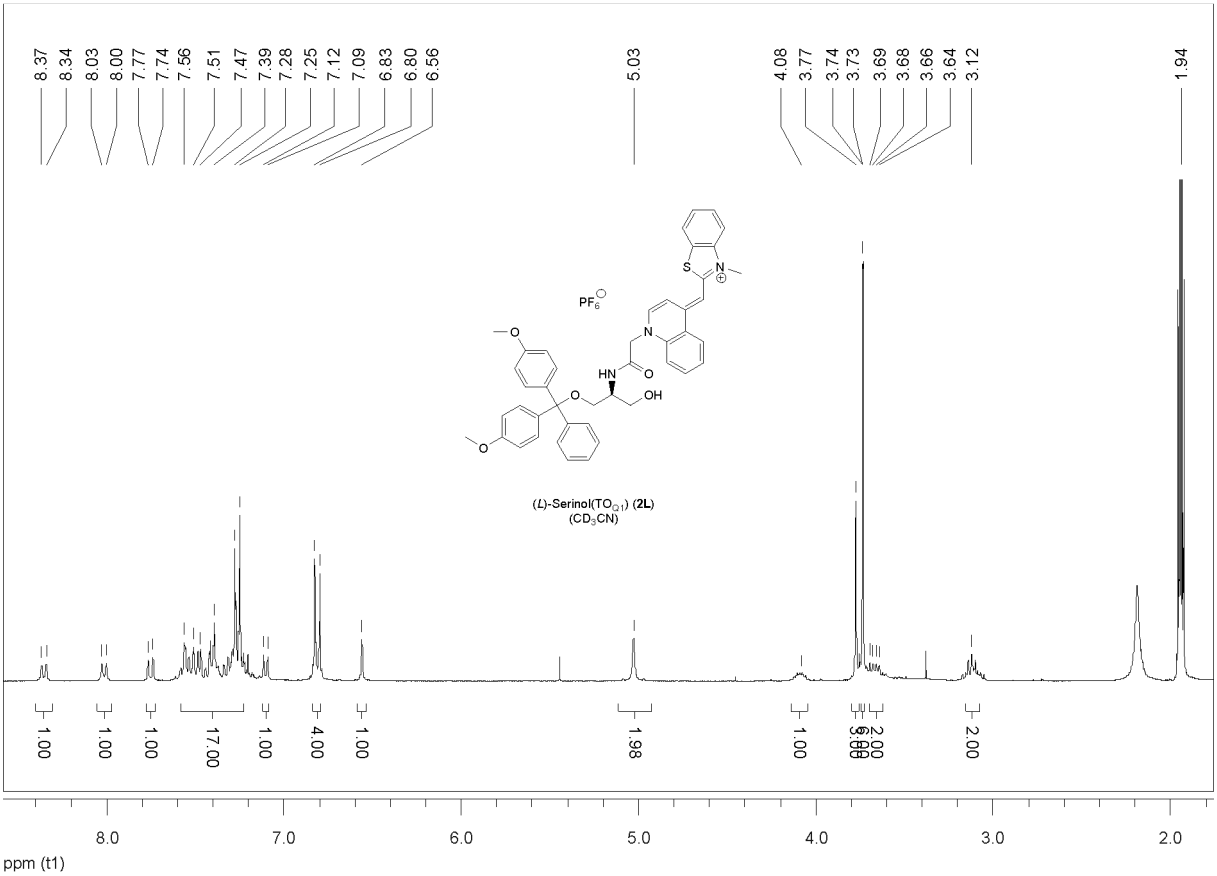
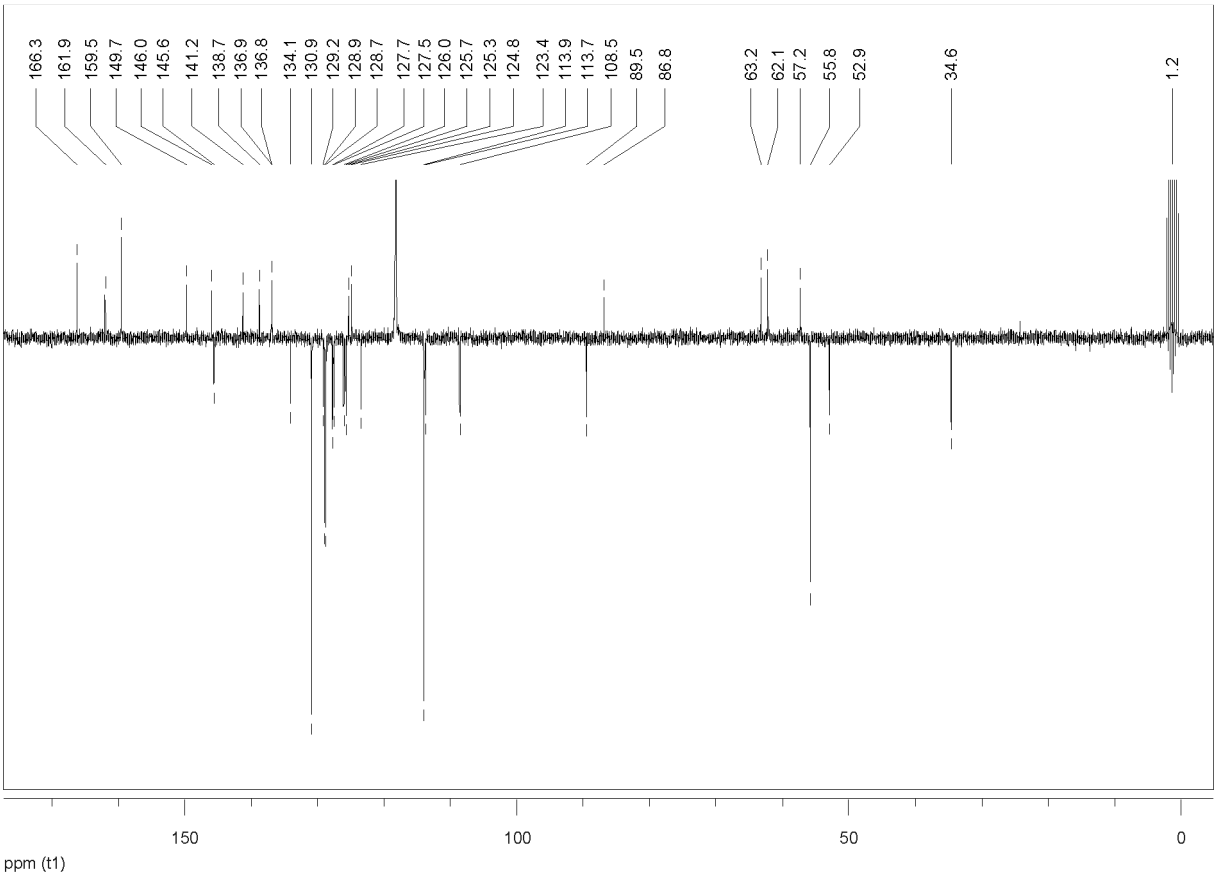


(*L*)-Serinol(TO$_{Q1}$) (**2L**)
(CD$_3$CN)



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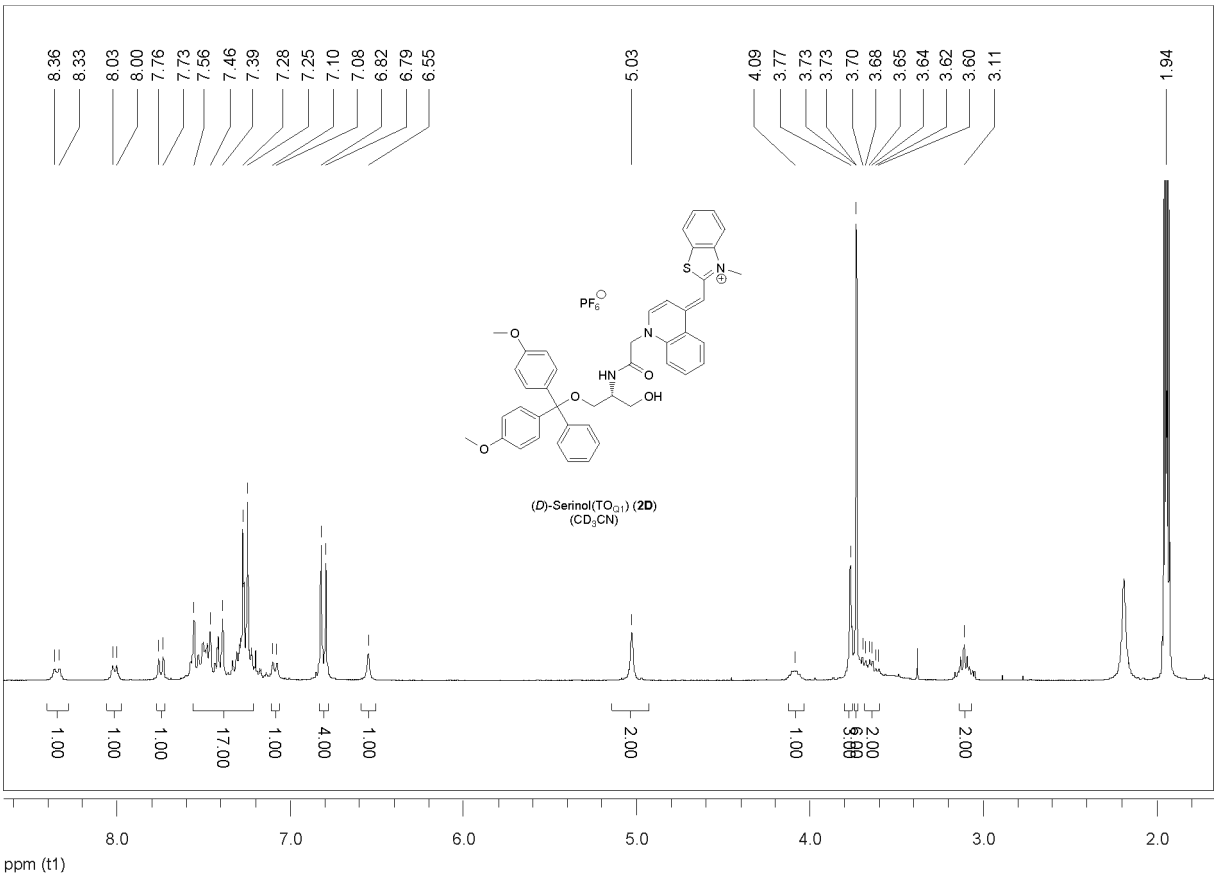
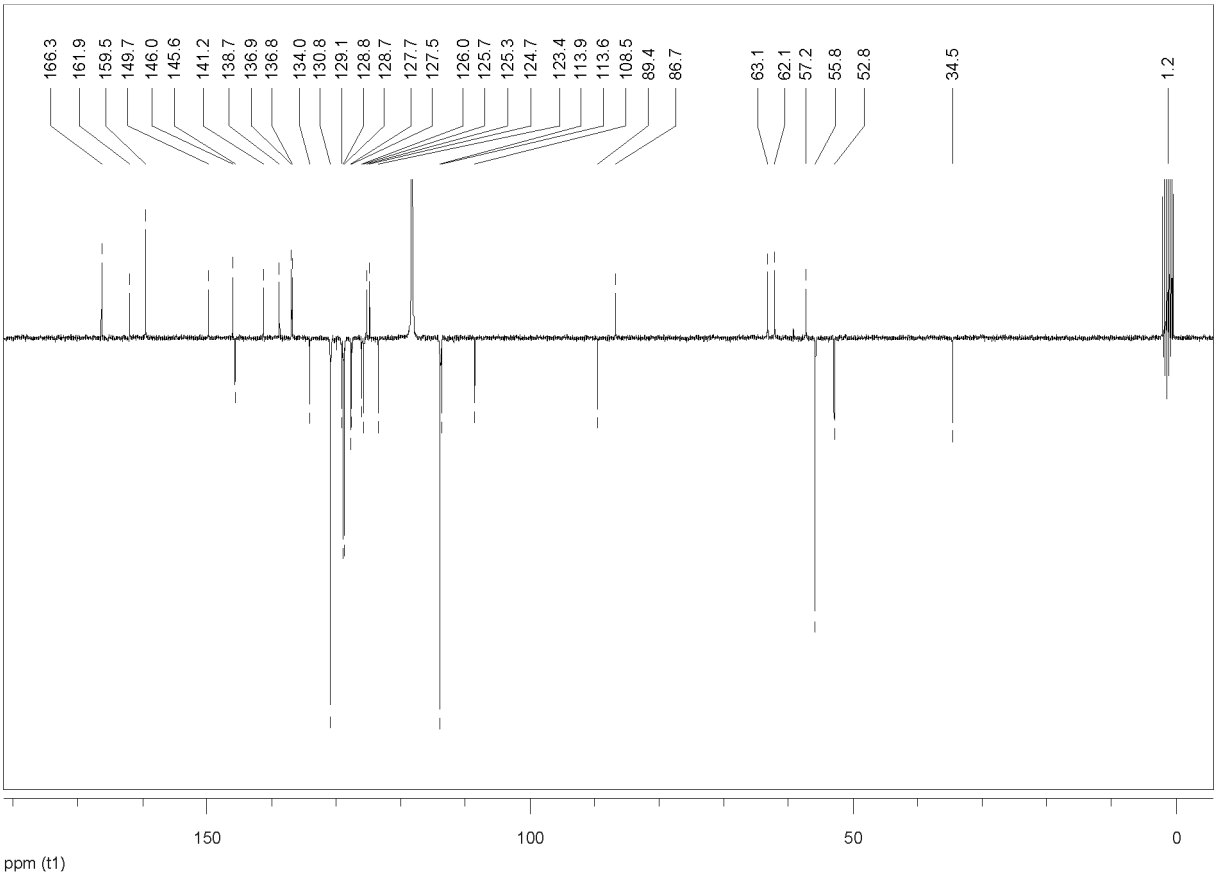


(*D*)-Serinol(TO$_{Q1}$) (**2D**)
(CD$_3$CN)



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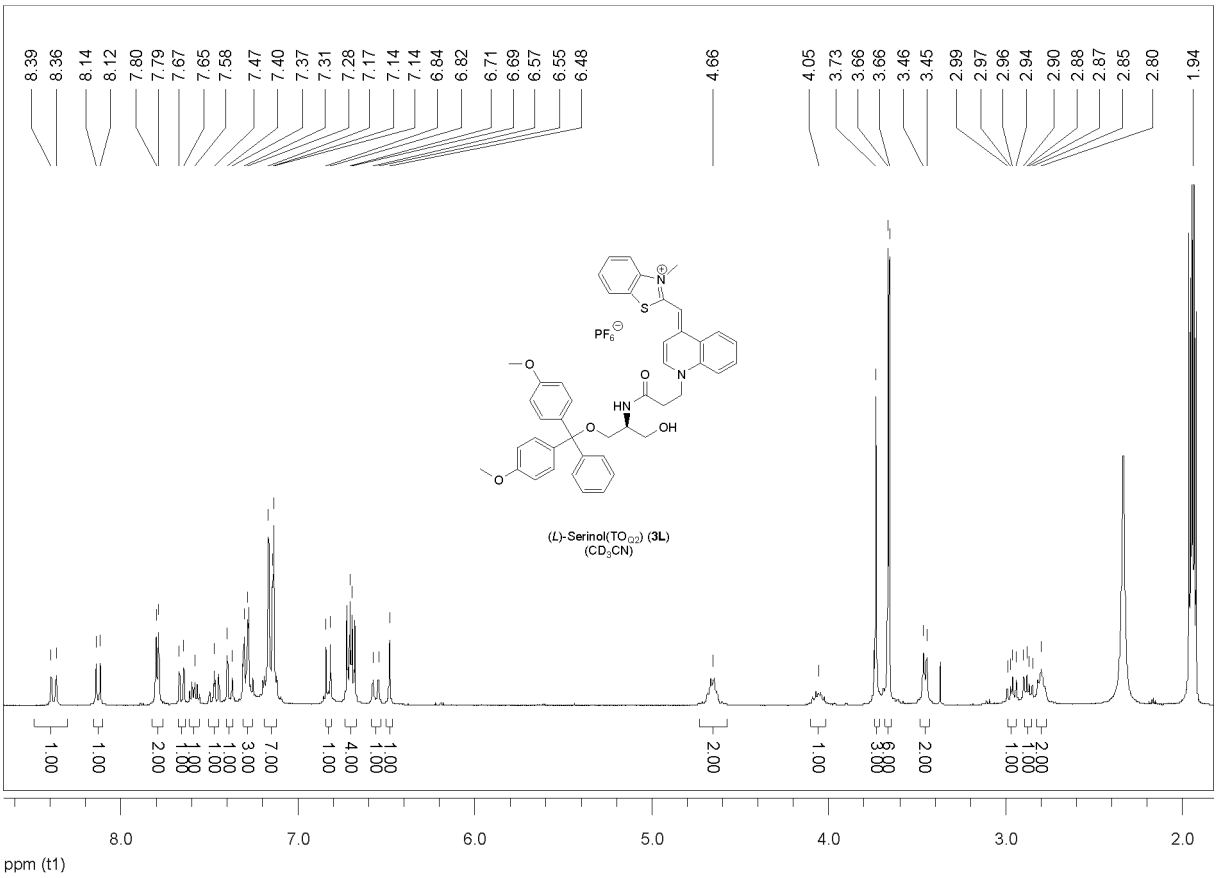

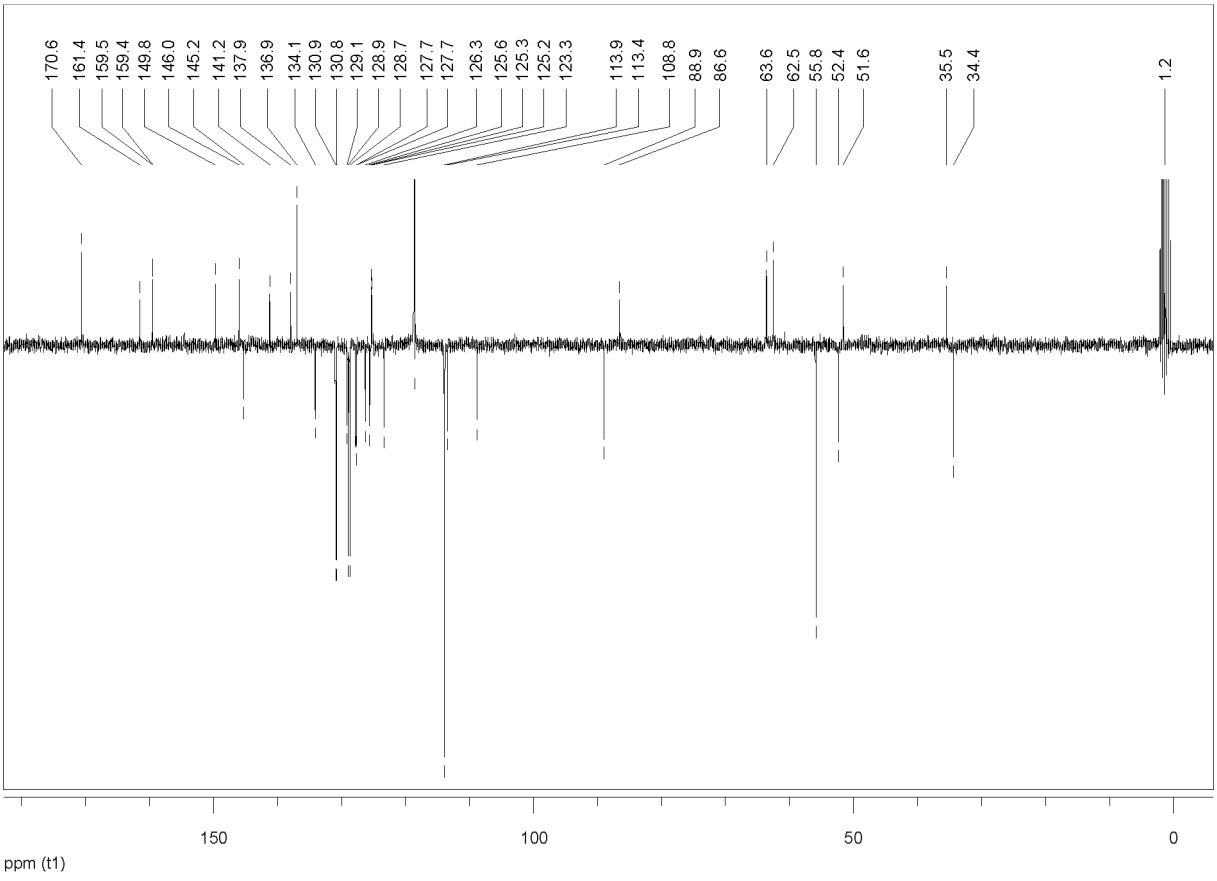

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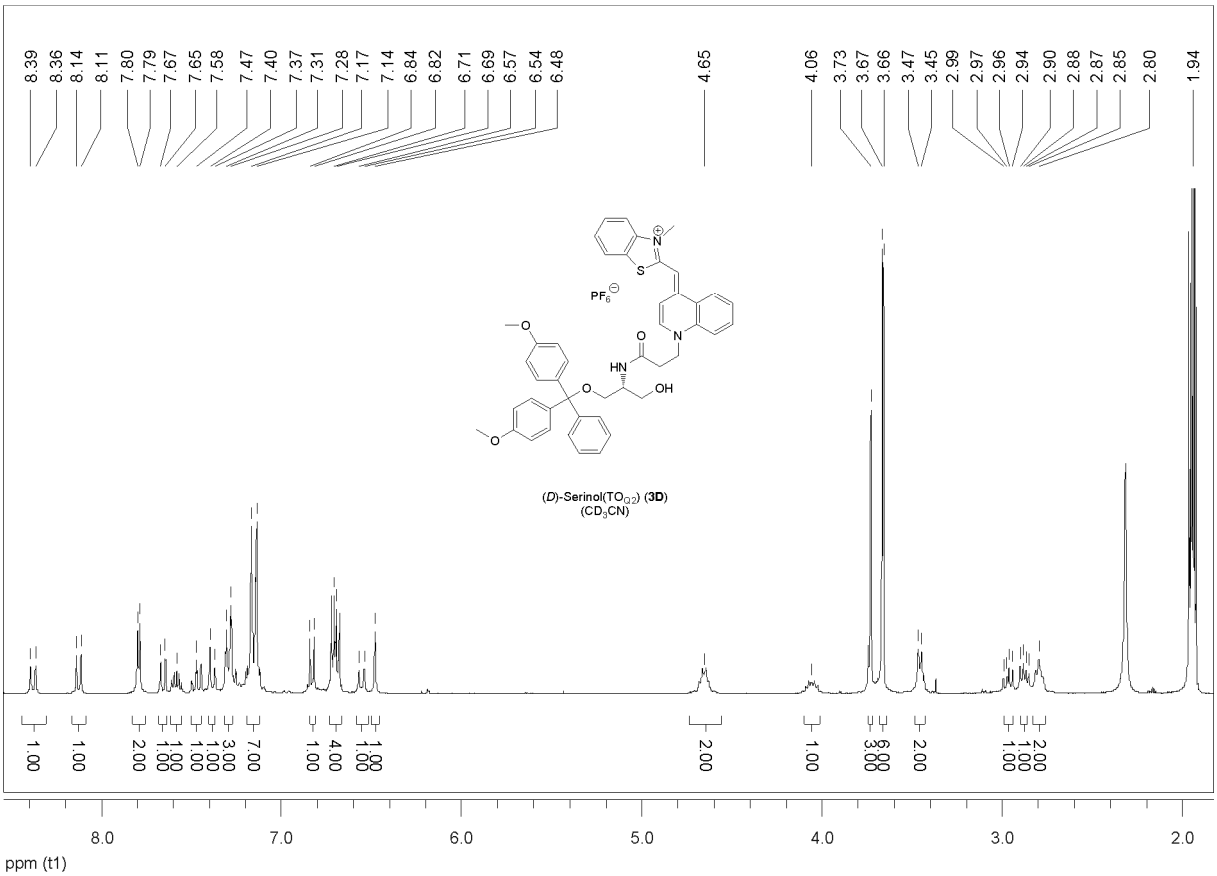

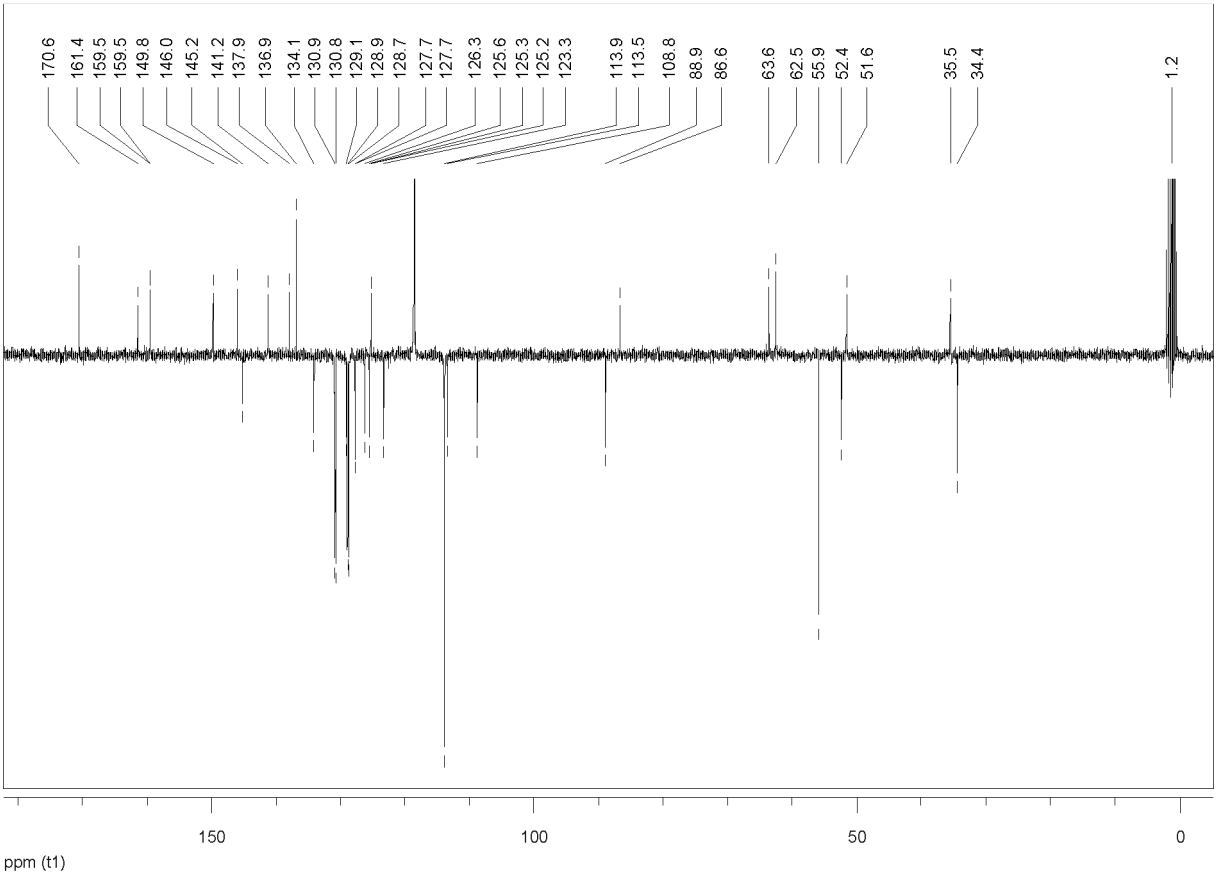

2 DNA-Synthesis, Workup, Purification and Characterization

The oligodesoxynucleotides were assembled by using an *AB Applied Biosystems Synthesyzer Model 3400* and phosphoramidite methodolody. CPGs were purchased from *Applied Biosystems* and *Link Technologies* (1 µmol, pore size 500 Å) and DNA syntheses reagents from *Applied Biosystems* and *Roth* (dry acetonitrile, 2 % dichloroacetic acid in CH_2Cl_2, 4 % tetrazole in acetonitrile, acetic anhydrid in 2,6-Lutidin/THF (1/1/8), 16 % 1-methylimidazole in THF, iodine in water/pyridine/THF (3/2/20/75)). The phosphoramidites dT-, dA^{Pac}-, dC^{Ac}-, $dG^{iPr\text{-}Pac}$, dA^{Bz}-, dC^{Bz} and dG^{DMF} were used following the manufacturers instructions (0.1 mol/L dry acetonitrile). The synthesized phosphoramidites **15**, **16D**, 1**6L**, **17D** and **17L** were used in 0.2 M solution. The quality of each coupling step was monitored by measuring the conductivity of DMT cleavage solutions. The synthesizer was programmed to yield oligomers carrying the terminal DMT protective group („trityl-on“).

After synthesis the resulting CPGs were dried under reduced pressure for 1h and then transferred to 2 mL eppendorf tubes. 1 mL of saturated aqueous NH_4OH was added and the tubes were shaken for 4h at RT. Subsequently, the tubes were centrifuged and the supernatant was collected. The volatiles were evaporated by using a *Uniequip Speed-vac Unijet II*. The samples were then dissolved in 0.1 TEAA buffer (pH = 7) and the crude product was further purified by RP-HPLC (gradient I). Afterwards, DMT removal was induced through the addition of 50 % AcOH aqueous solution over 30 min. The reaction mixtures were neutralized with NEt_3 and the crude product was again purified by RP-HPLC (gradient II). The resulting oligomers were concentrated to an overall volume of 0.5 mL and desalted using *NAP-5 Sephadex* columns of *GE Healthcare* or *Amersham Biosciences*. Finally, the oligomers were freeze dried with a *Christ LDC 1m* lyophilizer. The residues were dissolved in water (Milli-Q-Pore) to reach a final concentration of 0.1 mM. Identity and purity was determined by using analytical RP-HPLC (gradient II) or UPLC (gradient III) and MALDI-TOF mass spectroscopy.

Semi preparative was carried out on a *1105 HPLC System* from *Gilson*, for analytical RP-HPLC a *1105 HPLC System* of *Gilson* and a *Acquity UPLC* System of *Waters* were used. A UV-detector at a wavelength λ = 260 nm and λ = 520 nm was used for the detection. Semi preparative separations were carried out by using a *Polaris C18 A 5µ (PN A 2000-250x100)*-Column of *Varian* (Pore size 220 angstrom) at a flow rate of 4 mL/min at 55°C (“trityl-on”: Gradient I, “trityl-off”: Gradient II). Analytical HPLC was carried out by using a *XBridge*

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C18 5µ (250x046)-column of *Waters* (Pore size 130 angstrom) at a flow rate of 1 mL/min at 55°C ("trityl-off": Gradient II) or a *BEH 130 C18 1.7µm (2.1x50)*-column of *Waters* (pore size 130 angstrom) at a flow rate of 1mL/min at 55°C ("trityl-off": Gradient III).

As mobile phase a binary mixture of A (0.1 M TEAA buffer, pH = 7, aq.) and B (acetonitrile) was used. All aqueous solutions were made of water of Milli-Q-Pore purity.

Gradient I: 0-1 min 3% B, 1-21 min 3% B → 40% B
Gradient II: 0-1 min 3% B, 1-21 min 3% B → 20 % B
Gradient III: 0-4 min 3 % B → 20 % B

MALDI-TOF mass spectra were measured on a *Voyager-DE^{TM} Pro Biospectrometry Workstation* of *PerSeptive Biosystems*. For ionisation a nitrogen UV-laser with a wavelength of λ = 337 nm was used. Acceleration voltage: 20.000 V, grid: 95 %, guide wire: 0.025 %, delay time: 100 ns. As matrix a solution of 2 parts of a solution of 50 mg 2',4',6'-trihydroxyacetophenone in 1 mL EtOH and 1 part of a solution of 50 mg diammonium citrate in 1 mL water (Milli-Q-Pore) was used.

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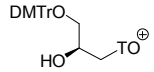
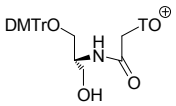
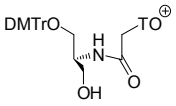
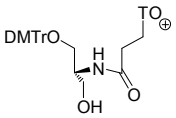
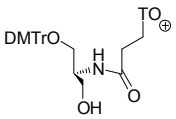
Monomer Z	Nr	Sequence	Yield	m/z calc.	m/z found	R_t [a] (min)
 *(R)*-Glycerol(TO) **1**	**21A**	5'-GCCGTA **Z** ATAGCCG-3'	13.7%	4387.0	4382.6	16.65[a]
	21T	5'-GCCGTT **Z** TTAGCCG-3'	13.6%	4369.0	4365.7	17.17[a]
	21C	5'-GCCGTC **Z** CTAGCCG-3'	15.0%	4339.0	4337.7	16.40[a]
	21G	5'-GCCGTG **Z** GTAGCCG-3'	11.2%	4419.0	4418.3	15.67[a]
 *(L)*-Serinol(TO_{Q1}) **2L**	**22A**	5'-GCCGTA **Z** ATAGCCG-3'	5.3%	4444.1	4443.6	17.09[a]
	22T	5'-GCCGTT **Z** TTAGCCG-3'	1.0%	4426.0	4425.9	17.54[a]
	22C	5'-GCCGTC **Z** CTAGCCG-3'	2.1%	4396.0	4398.4	15.70[a]
	22G	5'-GCCGTG **Z** GTAGCCG-3'	2.1%	4476.1	4478.9	15.89[a]
	30	5'-ACACC **Z** ACGGCGC-3'	4.0%	4084.8	4084.7	17.60[a]
 *(D)*-Serinol(TO_{Q1}) **2D**	**23A**	5'-GCCGTA **Z** ATAGCCG-3'	27.4%	4444.1	4445.6	17.05[a]
	23T	5'-GCCGTT **Z** TTAGCCG-3'	18.1%	4426.0	4428.6	17.23[a]
	23C	5'-GCCGTC **Z** CTAGCCG-3'	20.0%	4396.0	4397.1	16.07[a]
	23G	5'-GCCGTG **Z** GTAGCCG-3'	20.1%	4476.1	4477.7	15.60[a]
 *(L)*-Serinol(TO) **3L**	**24A**	5'-GCCGTA **Z** ATAGCCG-3'	5.1%	4458.1	4460.2	1.95[b]
	24T	5'-GCCGTT **Z** TTAGCCG-3'	6.3%	4440.1	4442.8	1.95[b]
	24C	5'-GCCGTC **Z** CTAGCCG-3'	6.5%	4410.1	4412.6	1.81[b]
	24G	5'-GCCGTG **Z** GTAGCCG-3'	5.7%	4490.1	4492.4	1.76[b]
 *(D)*-Serinol(TO) **3D**	**25A**	5'-GCCGTA **Z** ATAGCCG-3'	7.1%	4458.1	4460.7	1.97[b]
	25T	5'-GCCGTT **Z** TTAGCCG-3'	6.9%	4440.1	4442.3	1.95[b]
	25C	5'-GCCGTC **Z** CTAGCCG-3'	6.7%	4410.1	4410.7	1.75[b]
	25G	5'-GCCGTG **Z** GTAGCCG-3'	5.8%	4490.1	4490.1	1.73[b]

[a] Gradient II, [b] Gradient III,

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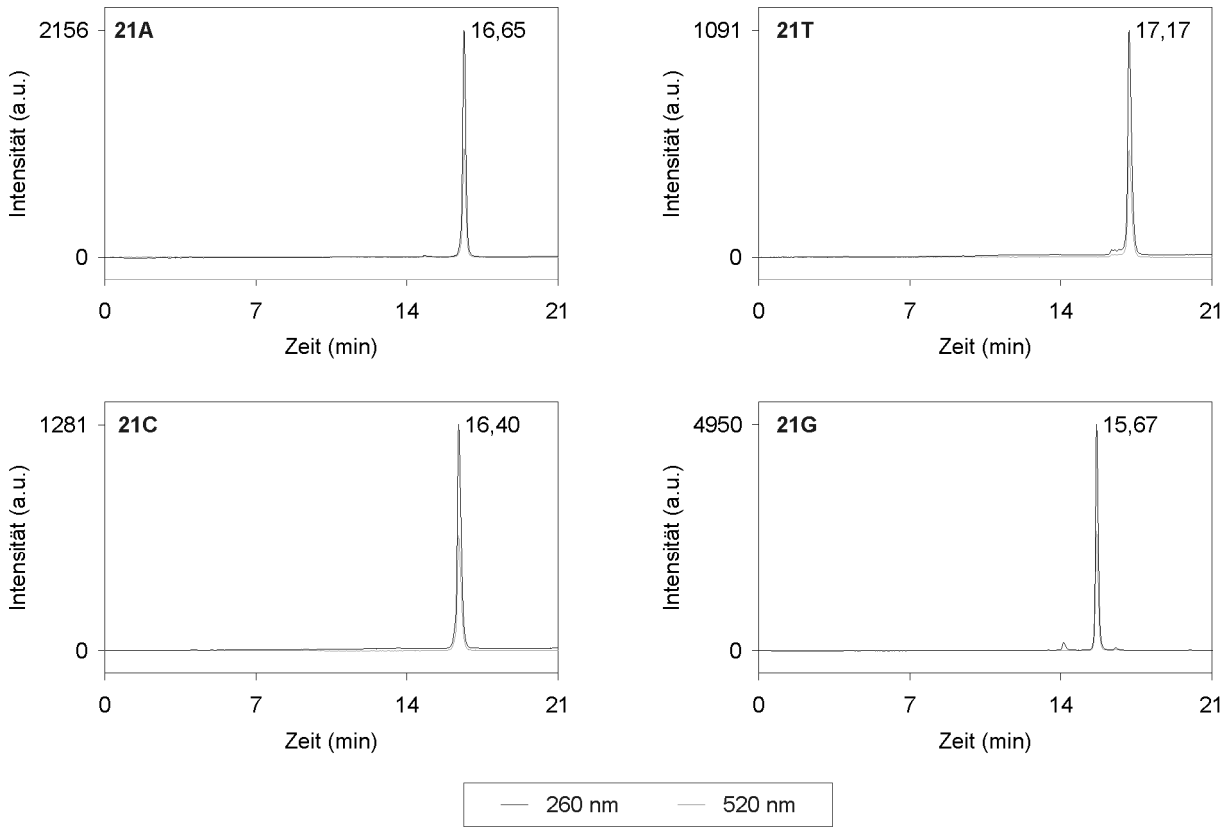

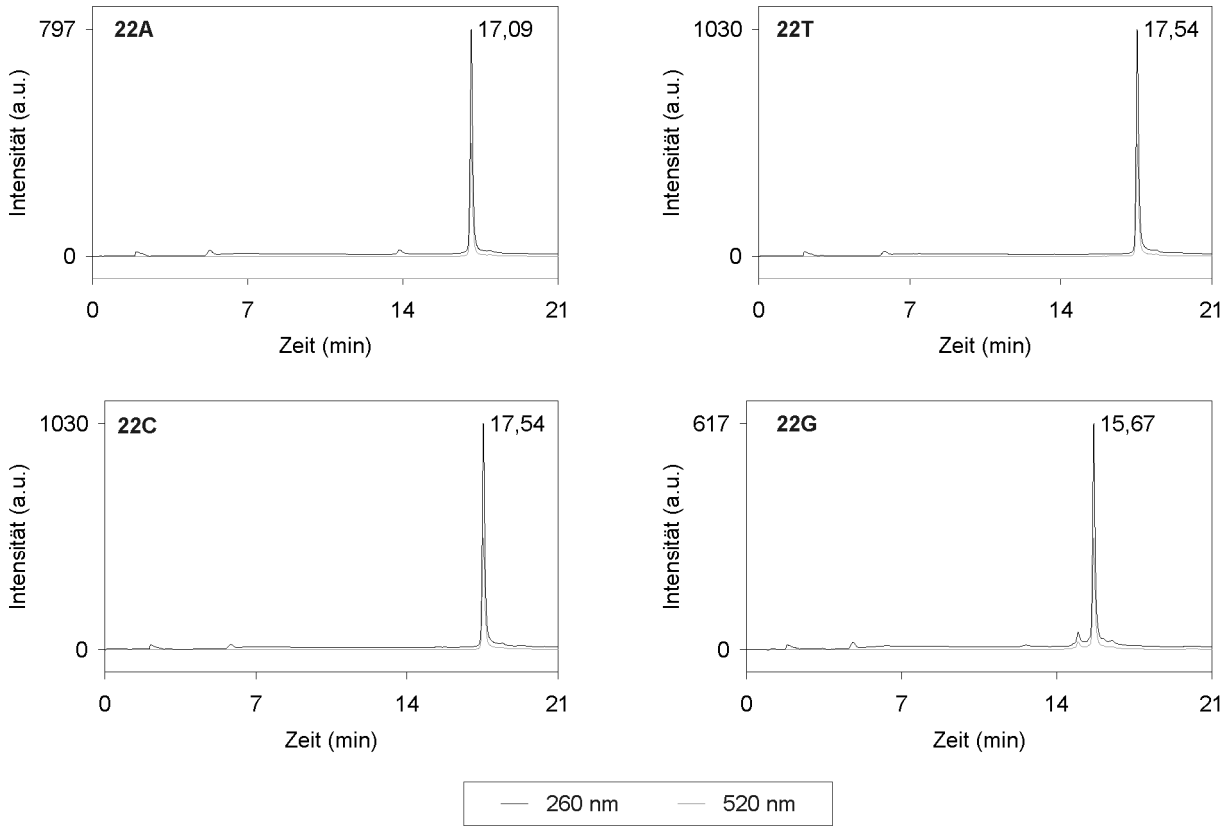

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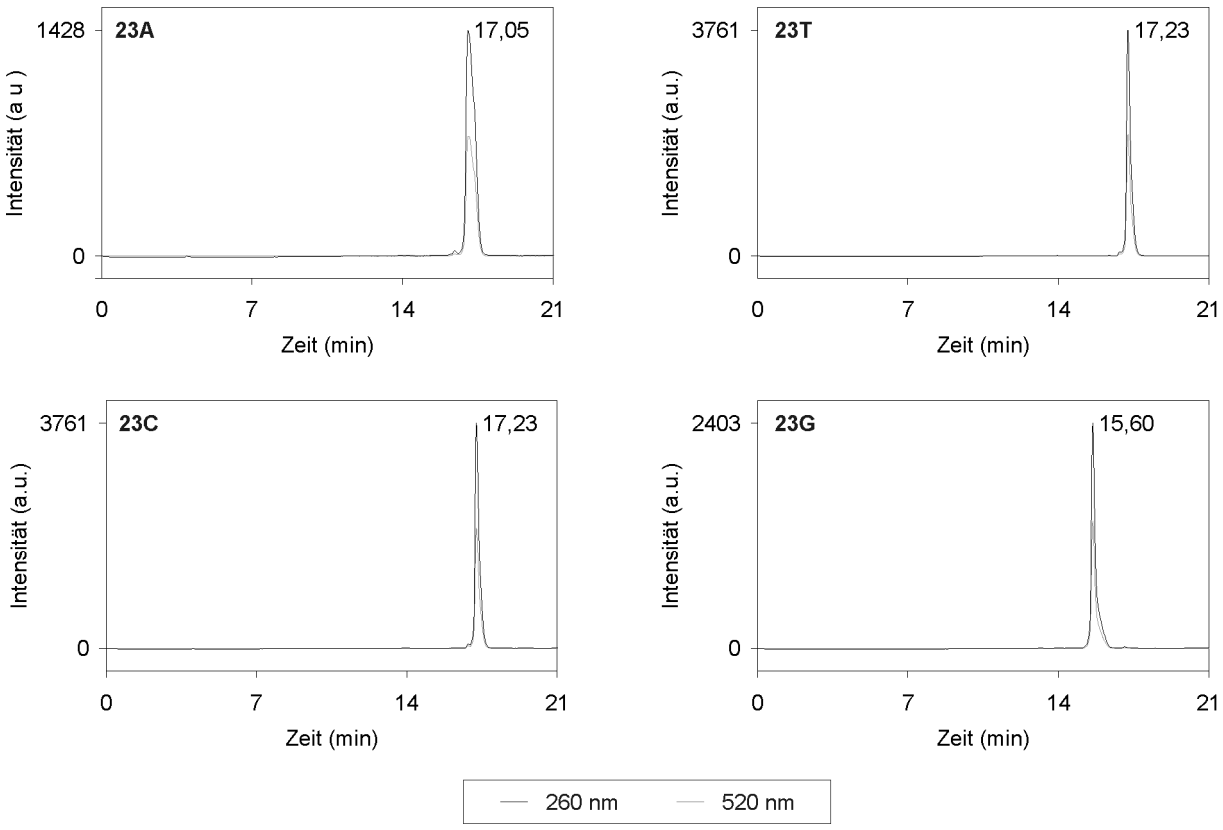

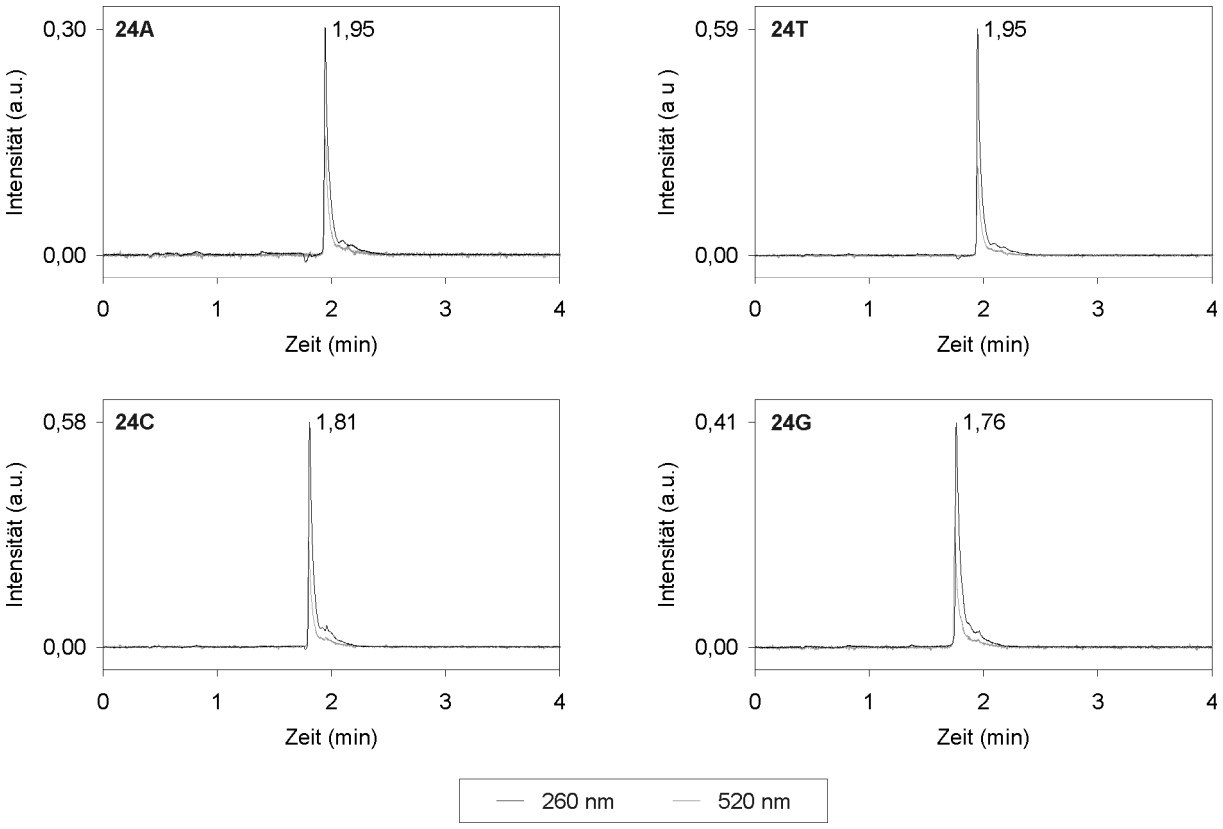

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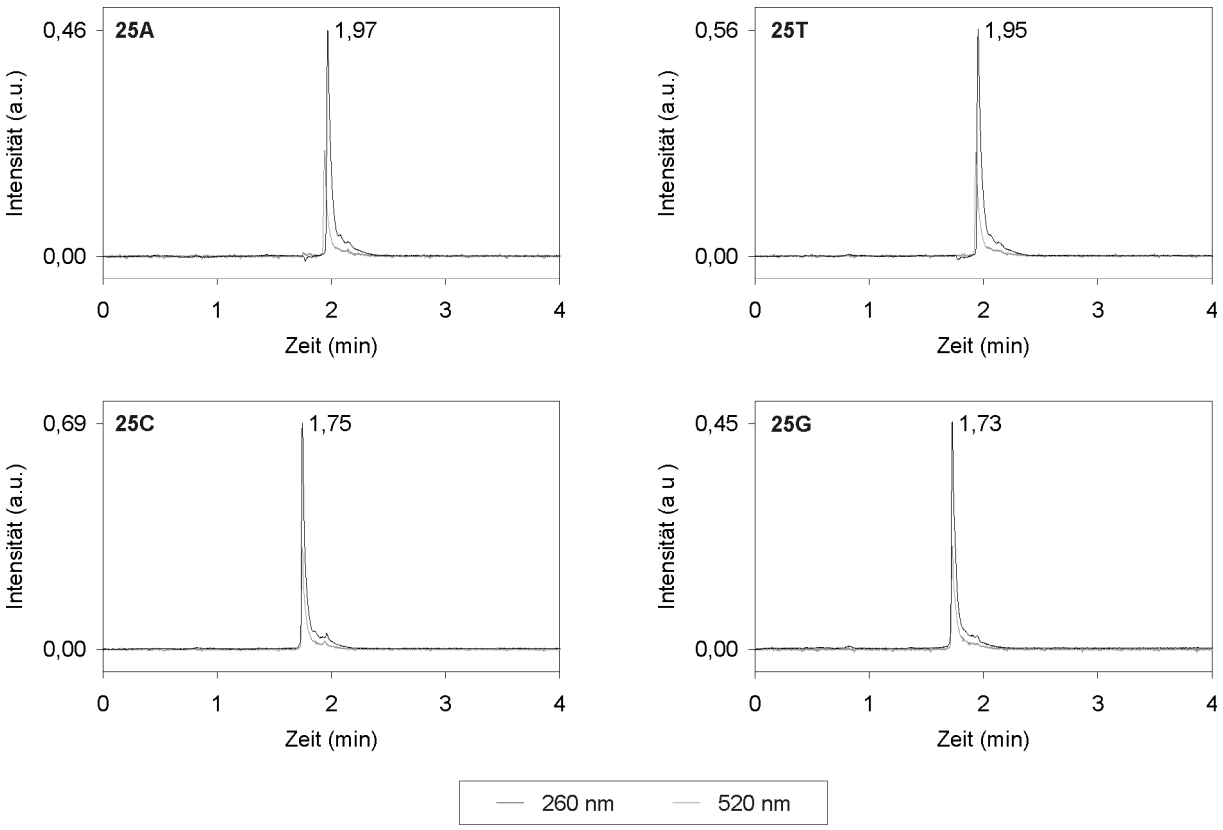

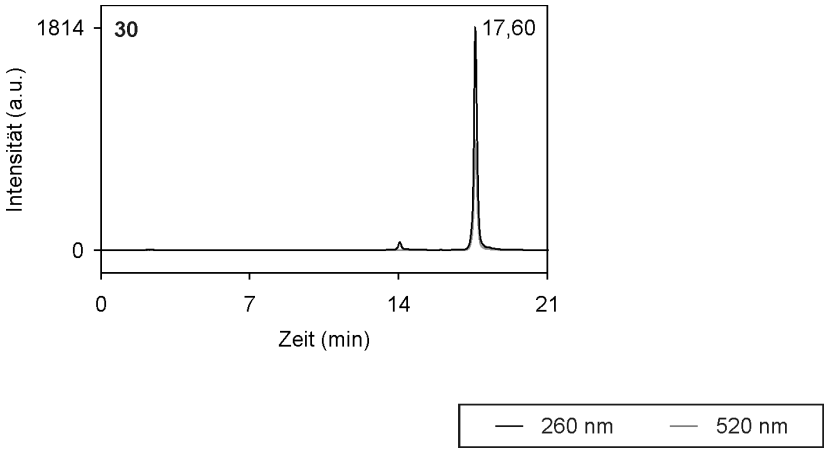

3 Fluorescence Measurements

Table S1: Fluorescence enhancement of TO-DNA conjugates **21X-25X** (X = A, T, C, G) upon addition of fully complementary DNA **26-29** at λ = 525 nm. Conditions: 25 °C, λ_{ex} = 495 nm, $Slit_{Ex}$ = 5, $Slit_{Em}$ = 2.5, **21X-25X**, **26-29** 1 µM in 10 mM NaH_2PO_4 buffer, 100 mM NaCl, pH = 7.

Fluorescence Enhancement F_{ds}/F_0 (F_0, fluorescence intensity of single strand; F_{ds} = fluorescence intensity matched duplex)			
21A·26	**21T·27**	**21C·28**	**21G·29**
1.1	0.4	0.3	0.5
22A·26	**22T·27**	**22C·28**	**22G·29**
4.0	3.7	1.2[a]	1.4[a]
23A·26	**23T·27**	**23C·28**	**23G·29**
1.4	0.8	0.9	1.0
24A·26	**24T·27**	**24C·28**	**24G·29**
1.3	0.4	0.2	0.7
25A·26	**25T·27**	**25C·28**	**25G·29**
0.6	0.3	0.3	0.3

[a] This sequence has been studied with peptide nucleic acid-based probes that contained thiazole orange as base surrogate (D.V. Jarikote, N. Krebs, S. Tannert, B. Röder, O. Seitz, *Chem. Eur. J.* **2007**, *13*, 300-310). It was shown that within the chosen sequence context highest fluorescence enhancements are obtained when the thiazole orange is flanked by at least one AT base pair.

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Table S2: Fluorescence enhancement of TO-DNA conjugates **21X-25X** (X = A, T, C, G) upon addition of fully complementary DNA **26-29** at λ = 525 nm. Conditions: 50 °C, λ_{ex} = 495 nm, $Slit_{Ex}$ = 5, $Slit_{Em}$ = 2.5, **21X-25X**, **26-29** 1 μM in 10 mM NaH_2PO_4 buffer, 100 mM NaCl, pH = 7.

Fluorescence Enhancement F_{ds}/F_0 (F_0, fluorescence intensity of single strand; F_{ds} = fluorescence intensity matched duplex)			
21A·26	**21T·27**	**21C·28**	**21G·29**
1.4	0.9	0.5	1.0
22A·26	**22T·27**	**22C·28**	**22G·29**
3.4	5.3	1.6[a]	1.8[a]
23A·26	**23T·27**	**23C·28**	**23G·29**
1.9	1.0	1.1	1.0
24A·26	**24T·27**	**24C·28**	**24G·29**
1.7	0.8	0.5	0.7
25A·26	**25T·27**	**25C·28**	**25G·29**
1.0	0.6	0.5	0.5

[a] This sequence has been studied with peptide nucleic acid-based probes that contained thiazole orange as base surrogate (D.V. Jarikote, N. Krebs, S. Tannert, B. Röder, O. Seitz, *Chem. Eur. J.* **2007**, *13*, 300-310). It was shown that within the chosen sequence context highest fluorescence enhancements are obtained when the thiazole orange is flanked by at least one AT base pair.