Electronic Supplementary Material (ESI) for Chemical Science.

LNA-Enhanced DNA FIT-Probes for Multicolour RNA Imaging

Supporting Information

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1.) General aspects **S1**
2.) Synthesis **S2**
3.) DNA-synthesis and labelling **S7**
4.) Analytical data of *H1N1-neuraminidase* probes **S8**
5.) Analytical and fluorescence data of *vasa*-probes **S22**
6.) Multi-colour RNA Detection **S24**
7.) Fluorescence *in-situ* hybridization **S25**
8.) NMR-spectra **S28**

1.) General aspects

General information: Reagents were purchased from Sigma-Aldrich (St. Louis, USA), Acros Organics (Geel, Belgium or Link Technologies (Bellshill, Scotland). Synthetic oligoribonucleotides were obtained from Noxxon-Pharma AG (Berlin, Germany). CH_2Cl_2 was dried by using the solvent purification system *SPS 800* of *MBraun*. Aqueous solutions were made of water purified using a *Milli-Pore* purification device. TLC plates *Silica Gel 60 F254* were obtained from Merck (Darmstadt, Germany).

DNA-Synthesis, work-up and purification for Ser(BO), Ser(TO) and LNA-enhanced TO- and QB-FIT probes: The DNA FIT probes were assembled by using a *Bioautomation MerMade-4.*. CPG support was purchased from *Link Technologies* (1 µmol, pore size 500 Å) and DNA synthesis reagents were purchased from *Roth* (Karlsruhe, Germany) and *EMP-Biotech (Berlin, Germany).* DNA phosphoramidites (dT, dG(DMF), dC(Bz) and dA(Bz)) were obtained from Thermo Fisher Scientific (Waltham, USA), 2'-*O*-Me-building blocks were purchased from Link Technologies and LNA phosphoramdites were obtained from Exiqon (Vedbak, Denmark). All phosphoramidites were used according to manufactures instructions. The quality of each coupling step was monitored by measuring the absorbance of DMTr cleavage solutions. The synthesizer was programmed to yield oligomers carrying the terminal DMTr protective group „trityl-on". Synthesis of LNA/2'-O-Me-Oligos was carried out on 3'-C3-modified CPG (*Link Technologies*). After synthesis the resulting CPGs were dried under reduced pressure and then transferred to 2 mL tubes. 1 mL of aqueous ammonia (32%) was added and the tubes were agitated for 2 h at 55 °C. After centrifugation the supernatant was collected. The volatiles were removed at reduced pressure and the residues were dissolved in water. The crude product was purified by preparative RP-HPLC (DMTr-on, 15-40% B in 10 min, A = 0.1 M triethylammonium acetate, aq. pH 7.5; B = MeCN). Afterwards, the DMTr group was removed upon treatment with 300 µL of 80% aqueous AcOH for 30 min at room temperature. The detritylation mixture was treated with *i*PrOH and the resulting precipitate, if necessary, again purified by RP-HPLC (DMTr-off, 5-20% B in 10 min, A = 0.1 M triethylammonium acetate, aq. pH 7.5; B = MeCN). Finally, the oligonucleotides were desalted by precipitation with ammonium acetate and *i*PrOH. The pellets were dissolved in water (Milli-Pore) and the purity was determined by using analytical RP-HPLC-UV (260 nm) and MALDI-TOF mass spectrometry.

preparative HPLC was carried out on an *1105 HPLC System* from *Gilson*, using a Waters *X-Bridge BEH130 C18 (10x150mm, 5 µm)* at a flow rate of 8 mL/min at 55 °C.

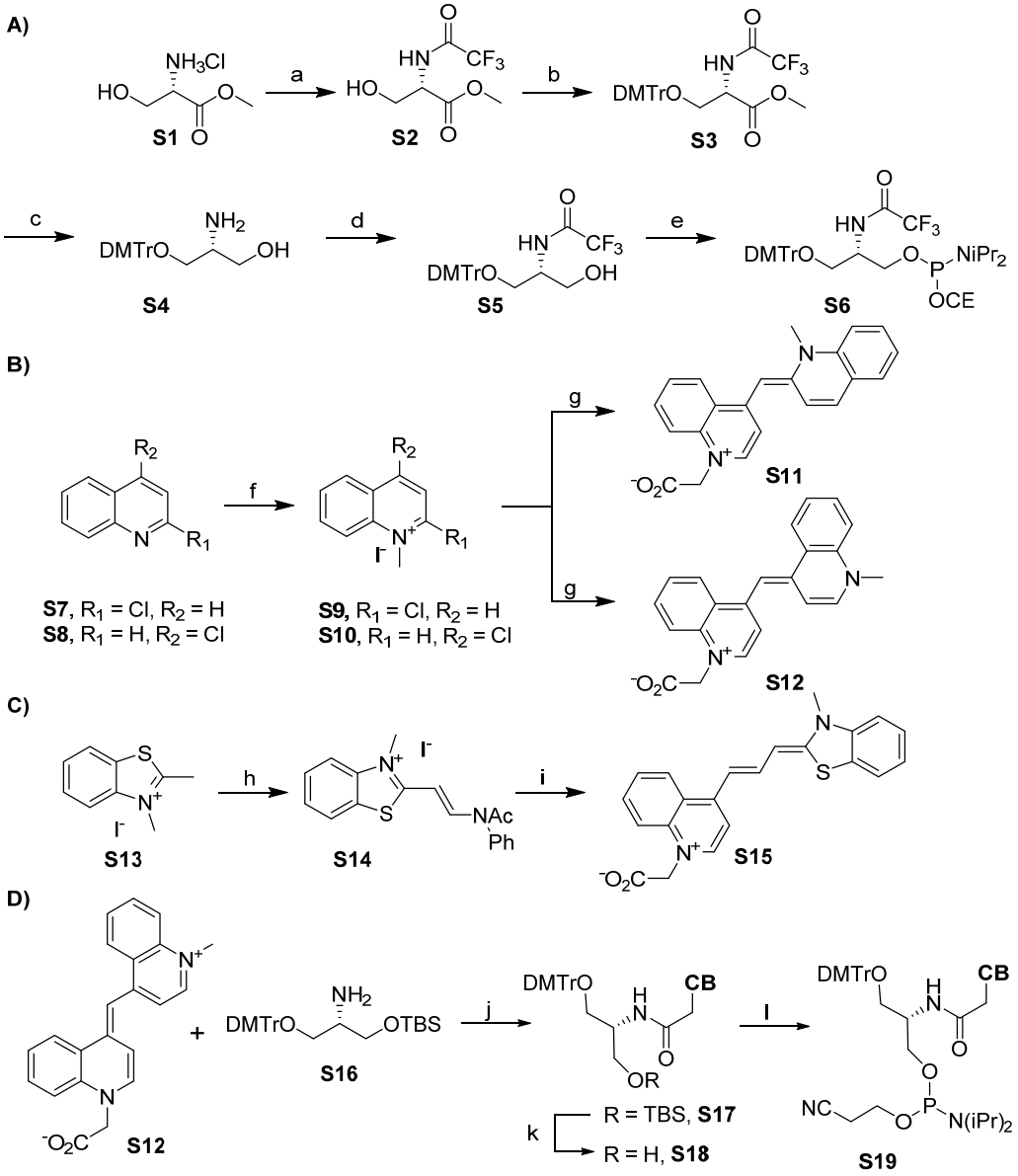
Analytical HPLC was carried out on an *1105 HPLC System* from *Gilson* by using a Waters *X-Bridge BEH130 C18 (4.6x250mm, 5 µm)* column or on an *Acquity-UPLC*®-System from Waters using a binary mixture of A (0.1 M triethylammonium acetate, aq. pH 7.5) and B (MeCN).
For analysis via UPLC®-RP-UV a *BEH 130 C18-column 1.7 mm* (*2.1x50mm,* 130 Å) from *Waters* was used at 50 °C. **Gradient I:** 3% B to 30% B in 4 min, 0.6 mL/min.
For analysis via Gilson HPLC-RP-UV a *X-Bridge BEH130 C18-column* (*4.6x250mm, 5 µm*) from *Waters* was used at 55 °C. **Gradient II:** 3% B to 30% B in 20 min, 1.0 mL/min; g**radient III:** 5% B to 35% B in 12 min, 1.5 mL/min.

MALDI-TOF mass spectra were measured on a Shimadzu Axima Confidence in positive or negative mode. For measurements in positive mode HPA Matrix was used (1:1 mixture of 50 mg/mL 3-hydroxy picolinic acid in MeCN/H_2O, 1:1 and 50 mg/mL diammonium citrate in MeCN/H_2O, 1:1). For measurements in negative mode THAP-Matrix was used (1:1 mixture of 50 mg/mL 2,4,6-trihydroxy acetophenone in MeCN/H_2O, 1:1 and 50 mg/mL diammonium citrate in MeCN/H_2O, 1:1).

Fluorescence spectroscopy: Fluorescence emission spectra were measured by using a *Varian Cary Eclipse* fluorescence spectrometer and 10 mm quartz cuvettes in phosphate buffer (10 mM Na_2HPO_4, 100 mM NaCl, pH 7) and are corrected for buffer fluorescence. FIT-probes and target RNA were added as specified. Prior to measurement, samples were allowed to equilibrate for 2 min. Settings: λ_{ex} = 485 nm, λ_{em} = 500-700 nm, $slit_{ex}$ = 5 nm, $slit_{em}$ = 5 nm. The spectra are the average of 3 measurement cycles. Quantum yields were assessed by using ATTO 520 for TO and ATTO 590 for QB (purchased from ATTO-Tec GmbH, Siegen, Germany) as a standard, using excitation at 485 nm and 560 nm, respectively. All fluorescence measurements were corrected according to their concentration in the cuvette (determined by UV-Vis absorption measurements). The fluorescence measurements were reproducible within 5-10% error.

UV-Vis spectroscopy: Melting temperatures and probe concentrations were measured on a *Varian Cary Bio 100 UV-Vis* spectrometer. For melting analysis the absorbance at 260 nm was monitored during a thermal cycle (3 times 25-85 °C in 0.5 °C/min). To determine melting temperatures T_M, the inflexion points of the resulting melt curves were calculated by the Varian Cary software. Melting temperatures were average values of three cycles. Complete absorption spectra (620-220 nm, 1 nm steps) were measured directly before or after the fluorescence experiments in the same cuvettes.

2.) Synthesis

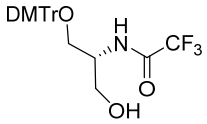


Scheme S1: Synthesis of chemical building blocks. A) Ser(Tfa)-phosphoramidite: a) ethyl trifluoroacetate, NEt_3, CH_2Cl_2, 87%; b) DMTrCl, pyridine, 98%; c) $LiBH_4$, MeOH, THF, 72%; d) ethyl trifluoroacetate, NEt_3, CH_2Cl_2, 76%; e) 2-cyanoethyl-*N,N*-diisopropylchloro phosphoramidite, Et*N*iPr_2, CH_2Cl_2, 73%; B) QV and QB: f) MeI, reflux, 60-83%; g) carboxymethylated 4-methylquinoline, NEt_3, CH_2Cl_2, 26% (QV), 77% (QB); C) TR: h) *N,N*-diphenylformamidine, Ac_2O, 150 °C, 78%; i) carboxymethylated 4-methylquinoline, NEt_3, CH_2Cl_2, 84%; D) Ser(QB)-phosphoramidite: j) PyBOP, NMM, PPTS, DMF, 72%; k) TBAF·$3H_2O$, THF, 54%; l) 2-cyanoethyl-*N,N*-diisopropylchloro phosphoramidite, Et*N*iPr_2, CH_2Cl_2, 75%.

DMTr-Ser-OH (S4)

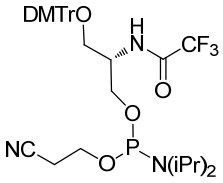
(*R*)-2-Amino-3-dimethoxytritylpropane-1-ol (**DMTr-Ser-OH**) prepared according to K. S. Ramasamy and W. Seifert, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 1799-1804 within four steps from L-serine in overall 61% yield. Analytical data were identical with those previously published.

DMTr-Ser(Tfa)-OH (S5)



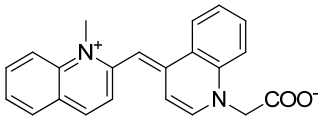
To a solution of (*R*)-2-Amino-3-dimethoxytritylpropane-1-ol (**S4**) (8.50 g, 21.60 mmol, 1.0 eq.)in CH_2Cl_2 (50 mL), NEt_3 (3.05 mL, 21.60 mmol, 1.0 eq.) was added ethyl trifluoroacetate (3.11 mL, 25.92 mmol, 1.2 eq.). After 3 h stirring at room temperature the mixture was diluted with CH_2Cl_2 (100 mL) and extracted with sat. $NaHCO_3$-solution (100 mL, 3x). The organic layer was dried over Mg_2SO_4, the solvent was evaporated and the crude product was purified by flash chromatography using a linear gradient of cHex/EtOAc/NEt_3 (1/1/0.02, v/v/v).
Yield: 8.00 g (16.35 mmol, 76%), yellow oil.
R_f (cHex/EtOAc/NEt_3, 1:1:0.01, v/v/v) = 0.63.
m/z (ESI(+)-ICT-HRMS): 512.1654 (100%), (calcd. for $C_{26}H_{26}F_3NNaO_5^+$ $[M+Na]^+$ 512.1655).
^{1}*H-NMR:* (500 MHz, $CDCl_3$) δ [ppm] = 7.32 – 7.28 (m, 2H, CH_{aryl}), 7.24 – 7.13 (m, 7H, CH_{aryl}), 6.82 (d, *J* = 7.9 Hz, 1H, NH), 6.76 (dd, *J* = 8.9, 1.1 Hz, 4H, CH_{aryl}), 4.08 – 3.97 (m, 1H, CH), 3.80 (dd, *J* = 11.5, 3.8 Hz, 1H, ½ CH_2ODMTr), 3.74 – 3.68 (m, 6H, 2xDMTr-OMe), 3.65 (dd, *J* = 11.5, 4.6 Hz, 1H, ½ CH_2-ODMTr), 3.36 – 3.27 (m, 2H, CH_2OH), 2.11 (s, 1H, OH).
^{13}*C-NMR:* (126 MHz, $CDCl_3$) δ [ppm] = 158.9 (2xC_q-OMe), 158.7 (C_q, C=O), 144.3 (1xC_q), 135.4 (2xC_q), 130.1 (4xC_{aryl}), 128.2 (2xC_{aryl}), 128.0 (2xC_{aryl}), 127.3 (1xC_{aryl}), 113.5 (4xC_{aryl}), 113.4 (CO-CF_3) 87.0 (DMTr-C_q), 62.8 (CH_2ODMTr), 62.6 (CH_2OH), 55.4 (2xDMTr-OMe), 51.4 (CH).

DMTr-Ser(Tfa)-phosphoramidite (S6)



To **S4** (489.5 mg, 1.0 mmol, 1.0 eq.) in CH_2Cl_2 (10 mL) were added $EtN(iPr)_2$ (680 µL, 4.0 mmol, 4.0 eq.) and 2-cyanoethyl *N,N*-diisopropylchloro phosphoramidite (446 µL, 2.0 mmol, 2.0 eq.). After 30 min stirring at ambient temperature the volatiles were removed in vacuo and the residue was purified by flash chromatography using a linear gradient of cyclohexane/ethyl acetate/pyridine (2/1/0.02, v/v/v).
Yield: 503 mg (0.73 mmol, 73%), colourless oil.
R_f (cHex/EtOAc/Pyridine, 2:1:0.01, v/v/v) = 0.62.
^{1}*H-NMR:* (300 MHz, $CDCl_3$) δ [ppm] = 7.49 – 7.41 (m, 2H, DMTr), 7.39 – 7.20 (m, 8H, DMTr), 6.91 – 6.85 (m, 4H, DMTr), 6.78 (d, *J* = 8.5 Hz, 1H, NH), 4.42 – 4.25 (m, 1H, CH), 4.01 – 3.84 (m, 2H, CH_2-ODMTr), 3.81 (s, 6H, 2xDMTr-OMe), 3.81 – 3.66 (m, 3H, CH_2, 1/2xCH_2), 3.58 (ddd, *J* = 13.3, 10.4, 6.7 Hz, 2H, CH_2), 3.49 – 3.36 (m, 1H, ½CH_2), 3.34 – 3.20 (m, 1H, ½CH_2), 2.63 – 2.51 (m, 2H, CH_2-CN), 1.26 – 1.11 (m, 12H, 4xCH_3(N*i*Pr_2)).
^{19}*F-NMR:* (282 MHz, $CDCl_3$) δ [ppm] = -76.10, -76.15 (CF_3, two diastereomers).
^{31}*P-NMR:* (121 MHz, $CDCl_3$) δ [ppm] = 149.47, 149.45 (P(III), two diastereomers).

Quinoline Violet (QV, S11)



N-Methyl-2-chlorquinolinium iodide (synthesized according to R. Lartia, U. Asseline, *Chem. Eur. J.* **2006**, *12*, 2270-2281) (250 mg, 820 µmol, 1.0 eq.) and *N*-carboxymethyl-4-methquinolinium bromide (280 mg, 984 µmol,

1.2 eq.) were suspended CH_2Cl_2 (2 mL). After addition of NEt_3 (400 µL, 2886 µmol, 3.5 eq.) the mixture was stirred at room temperature overnight. The volatiles were removed under vacuum and the residue was purified by flash chromatography (CH_2Cl_2/MeOH: 8/2, v,v).

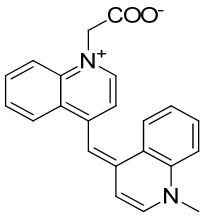
Yield: 26%, 73 mg, 213 µmol, violet solid

m/z (ESI(+)-ICT-HRMS): 343.1441 $[M+H]^+$ (100%) (calcd. for $C_{22}H_{19}N_2O_2^+$: 343.447), 365.1260 $[M+Na]^+$ (50%) (calcd. for $C_{22}H_{18}N_2O_2Na^+$: 365.1260).

^{1}H-NMR: (500 MHz, DMSO-d_6) δ [ppm] = 8.53 (1H, d, *J* = 7.8 Hz), 8.23 (1H, d, *J* = 9.4 Hz), 8.12 – 8.04 (3H, m), 7.98 (1H, d, *J* = 6.6 Hz), 7.89 – 7.80 (2H, m), 7.69 (1H, d, *J* = 8.3 Hz), 7.63 – 7.55 (2H, m), 7.34 (1H, d, *J* = 7.4 Hz), 6.59 (1H, s, methine), 5.33 (2H, s, CH_2), 4.10 (2H, s, CH_3).

^{13}C-NMR: (126 MHz, DMSO-d_6) δ[ppm] = 169.1 (C_q), 155.1 (C_q), 148.7 (C_q), 143.0 (CH_{aryl}), 140.5 (C_q), 140.0 (C_q), 138.3 (CH_{aryl}), 132.8 (CH_{aryl}), 129.1 (CH_{aryl}), 125.9 (CH_{aryl}), 125.6 (CH_{aryl}), 125.0 (C_q), 123.9 (C_q), 121.8 (CH_{aryl}), 117.2 (CH_{aryl}), 117.2 (CH_{aryl}), 107.5 (CH_{aryl}), 95.7 (CH), 53.9 (CH_2), 39.5 (CH_3).

Quinoline Blue (QB, S12)



N-Methyl-4-chlorquinolinium iodide (synthesized according to R. Lartia, U. Asseline, *Chem. Eur. J.* **2006**, *12*, 2270-2281) (250 mg, 820 µmol, 1.0 eq.) and *N*-carboxymethyl-4-methquinolinium bromide (280 mg, 984 µmol, 1.2 eq.) were suspended CH_2Cl_2 (2 mL). After addition of NEt_3 (400 µL, 2886 µmol, 3.5 eq.) the mixture was stirred at room temperature overnight. The volatiles were removed under vacuum and the residue was purified by flash chromatography (CH_2Cl_2/MeOH: 8/2, v,v). Product containing fractions were combined, the solvent was evaporated and the residue was recrystallized from water.

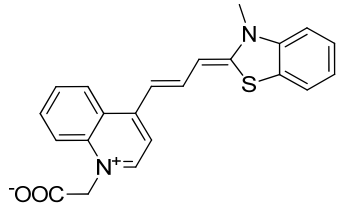
Yield: 77%, 216 mg, 631 µmol, blue solid.

m*/z (ESI(+)-ICT-HRMS):* 343.1441 $[M+H]^+$ (100%) (calcd. for $C_{22}H_{19}N_2O_2^+$: 343.447), 365.1260 $[M+Na]^+$ (55%) (calcd. for $C_{22}H_{18}N_2O_2Na^+$: 365.1260).

^{1}H-NMR: (500 MHz, DMSO-d_6) δ [ppm] = 8.71 (1H, d, *J* = 8.6 Hz), 8.64 (1H, dd, *J* = 8.7, 1.0 Hz), 8.31 (1H, d, *J* = 7.2 Hz), 8.02 (1H, d, *J* = 7.4 Hz), 7.98 – 7.92 (2H, m), 7.81 (1H, ddd, *J* = 8.5, 7.0, 1.3 Hz), 7.74 (1H, d, *J* = 7.2 Hz), 7.70 (1H, ddd, *J* = 8.3, 5.9, 2.2 Hz), 7.66 – 7.62 (1H, m), 7.57 (2H, dd, *J* = 11.4, 4.1 Hz), 7.27(s, 1H, methine), 5.23 (s, 2H, CH_2), 4.11 (s, 3H, Me).

^{13}C-NMR: (126 MHz, DMSO-d_6) δ 169.0 (C_q), 149.9 (C_q), 148.2 (C_q), 144.1 (CH_{aryl}), 143.0 (CH_{aryl}), 138.6 (C_q), 138.4(C_q), 133.0 (CH_{aryl}), 132.5 (CH_{aryl}), 126.6 (CH_{aryl}), 125.9 (CH_{aryl}), 125.8 (CH_{aryl}), 125.7 (CH_{aryl}), 125.4 (C_q), 124.7 (C_q), 117.9 (CH_{aryl}), 117.1 (CH_{aryl}), 109.8 (CH_{aryl}), 107.7 (CH_{aryl}), 96.9 (CH), 54.6 (CH_2), 42.1 (CH_3).

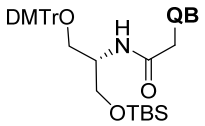
Thiazole Red (TR, S15)



To a suspension of *N*-Carboxymethyl-4-methquinolinium bromide (1.52 mg, 5.36 mmol, 1.0 eq.) and hemicyanine S**14** (synthesized according to S. Ikeda, H. Yanagisawa, A. Nakamura, D. O. Wang, M. Yuki, A. Okamoto, *Org. Biomol. Chem.* **2011**, *9*, 4199-4204) (2.34 g, 5.36 mmol, 1.0 eq.) in CH_2Cl_2 (100 mL) was added NEt_3 (7.6 mL, 54.3 mmol, 10 eq.). The resulting blue suspension was stirred at room temperature overnight. The blue solid was filtered off, washed twice with CH_2Cl_2 (20 mL) and dried under vacuum.

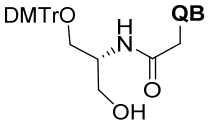
Yield: 84%, 1.68 g, 4.50 mmol), blue solid.
m/z (ESI(+)-ICT-HRMS): 375.1161 (100%) $[M+H]^+$ (calcd. for $C_{22}H_{19}O_2N_2S^+$: 375.1162).
^{1}H-NMR: (500 MHz, DMSO-d_6) δ [ppm] = 8.38 (1H, d, *J* = 8.7 Hz), 8.24 – 8.15 (2H, m, methine), 7.90 (1H, d, *J* = 7.7 Hz), 7.88 – 7.80 (1H, m), 7.76 (1H, d, *J* = 7.5 Hz), 7.72 (1H, d, *J* = 8.7 Hz), 7.62 (2H, t*, J_{app} = 8.8 Hz, quinoline-CH), 7.53 – 7.44 (1H, m), 7.36 – 7.29 (1H, m), 7.07 (1H, d, *J* = 13.1 Hz,), 6.59 (1H, d, *J* = 12.6 Hz,), 5.37 (2H, s, CH_2), 3.77 (3H, s, CH_3).
^{13}C-NMR: (126 MHz, DMSO-d_6) δ [ppm] = 168.8 (CO_2H), 144.6 (CH), 142.5 (CH), 141.9 (C_q), 138.7 (C_q), 133.2 (CH), 128.6 (CH), 127.8 (CH), 126.4 (CH), 125.0 (C_q), 124.9 (CH), 124.6 (CH), 122.7 (CH), 118.8 (C_q), 117.4 (CH), 116.5 (C_q), 114 (C_q), 113.0 (CH), 109.3 (CH), 108.9(CH), 100.4 (CH), 54.3 (CH_2), 33.2 (CH_3).

DMTr-Ser(QB)-OTBS (S17)



To a suspension of **QB** (**S12**) (1.21 g, 3.55 mmol, 1.0 eq.) in DMF (50 mL) were added pyridinium *p*-toluene sulfonate (0.90 g, 3.55 mmol, 1.0 eq.), PyBOP (2.23 g, 4.29 mmol, 1.2 eq.) and *N*-methylmorpholine (1.54 mL, 1.42 g, 14.01 mmol, 4.0 eq.). The mixture was stirred for 2 min, which provided a clear solution. Subsequently, a solution of **S16** (prepared according to L. Bethge, I. Singh and O. Seitz, *Org. Biomol. Chem.*, 2010, **8**, 2439-2448.) (1.80 g, 3.55 mmol, 1.0 eq.) in DMF (10 mL) was added. The mixture was stirred over night before the solvent was removed under reduced pressure. The remaining blue oil was dissolved in ethyl acetate (100 mL) and washed two times with saturated $NaHCO_3$ (aq.) solution, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was purified by flash chromatography.
Yield: 72%, 2.35 g, 2.57 mmol, blue amorphous solid.
R_f (CH_2Cl_2/MeOH/NEt_3, 89.9/10/0.1, v/v/v) = 0.58.
m/z (ESI(+)-ICT-HRMS): 832.4131 (100%) $[M]^+$ (calcd. for $C_{52}H_{58}N_3O_5Si^+$: 832.4140).
^{1}H-NMR: (500 MHz, CD_3CN,) δ [ppm] = 8.44 (1H, d, *J* = 8.0 Hz, H_{Aryl}), 8.38 (1H, d, *J* = 7.8 Hz, H_{Aryl}), 7.94 (1H, d, *J* = 7.1 Hz, H_{Aryl}), 7.89 (1H, ddd, *J* = 8.4, 6.9, 1.3 Hz, H_{Aryl}), 7.84 – 7.79 (1H, m, H_{Aryl}), 7.66 – 7.60 (2H, m, H_{Aryl}), 7.60 – 7.55 (2H, m, H_{Aryl}), 7.49 – 7.44 (1H, m, H_{Aryl}), 7.44 – 7.37 (4H, m, H_{Aryl}), 7.29 – 7.18 (m, 7H, CH-DMTr), 7.09 (1H, s, CH-methine), 6.88 – 6.78 (5H, m, 4xCH-DMTr, NH), 4.87 (2H, d, *J* = 0.9 Hz, CH_2-CO), 4.13 – 4.05 (1H, m, CH-NH), 4.00 (3H, s, CH3), 3.80 – 3.71 (m, 7H, 2xOMe, ½ CH_2), 3.65 (1H, dd, *J* = 10.2, 6.0 Hz, ½ CH_2), 3.16 (1H, dd, *J* = 9.1, 5.8 Hz, ½ CH_2), 3.09 – 3.02 (1H, m, ½ CH_2), 0.80 (9H, s, Si-*t*Bu), -0.01 (6H, d, *J* = 4.4 Hz, 2xSi-Me).
^{13}C-NMR: (126 MHz, CD_3CN) δ [ppm] = 166.6 (C_q), 159.6 (C_q), 152.1 (C_q), 149.3 (C_q), 146.2 (C_q), 144.9 (CH_{Aryl}), 143.5 (CH_{Aryl}), 139.9 (C_q), 139.5 (C_q), 137.1 (C_q), 136.9 (C_q), 134.1 (CH_{Aryl}), 133.5 (CH_{Aryl}), 131.0 (CH_{Aryl}), 131.0 (CH_{Aryl}), 128.9 (CH_{Aryl}), 128.8 (CH_{Aryl}), 127.8 (CH_{Aryl}), 127.7 (CH_{Aryl}), 126.8 (C_q), 126.7 (CH_{Aryl}), 126.7 (CH_{Aryl}), 126.6 (CH_{Aryl}), 125.9(C_q) , 118.6 (CH_{Aryl}), 117.5 (CH_{Aryl}), 114.0 (CH_{Aryl}), 114.0 (CH_{Aryl}), 111.4 (CH_{Aryl}), 108.6 (CH_{Aryl}), 98.8 (CH-methine), 86.8 (CH_q), 62.7(2xCH_2), 56.8 (CH_2CO), 55.9 (2xOMe), 52.4 (CH), 43.3 (N-CH_3), 26.8 (C_q Si-*t*Bu), 26.2 (Si-*t*Bu), -5.2, -5.3 (2xSi-Me).

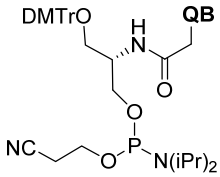
DMTr-Ser(QB)-OH (S18)



To a solution of **S17** (2.35 g, 2.57 mmol, 1.0 eq.) in THF (50 mL) tetrabutylammonium fluoride trihydrate (1.62 g, 5.14 mmol, 2.0 eq.) was added. After stirring for 1 h, 200 ml of half-saturated aq. $NaHCO_3$ was added.) After stirring for further 15 min the blue precipitate formed was collected by filtration, washed with H_2O (2x), EtOAc (4x) and Et_2O (2x) and finally dried under reduced pressure to yield a fine blue powder.
Yield: 54%, 1.18 g, 1.39 mmol, blue solid.
m/z (ESI(+)-ICT-HRMS): 718.3272 (100%) $[M]^+$ (calcd. for $C_{46}H_{44}N_3O_5^+$: 718.3275).

[1]H-NMR: (500 MHz, CD_3CN) δ [ppm] = 8.47 (1H, d, *J* = 8.6 Hz, H_{Aryl}), 8.42 (1H, d, *J* = 8.5 Hz, H_{Aryl}), 7.96 (1H; d, *J* = 7.1 Hz, H_{Aryl}), 7.90 (1H, ddd, *J* = 8.3, 6.9, 1.3 Hz, H_{Aryl}), 7.84 (1H, d, *J* = 8.7 Hz, H_{Aryl}), 7.68 – 7.55 (4H, m, H_{Aryl}), 7.51 – 7.41 (3H, m, H_{Aryl}), 7.38 (2H, dt, *J* = 8.4, 1.8 Hz, H_{Aryl}), 7.31 – 7.19 (7H, m, DMTr), 7.13 (1H, s, CH-Methin), 6.93 (1H, d, *J* = 8.6 Hz, NH), 6.85 – 6.79 (4H, m, DMTr), 4.88 (2H, s, CH_2CO), 4.10 – 4.03 (1H, m, CH), 4.03 (3H, s, N-Me), 3.74 (6H, d, *J* = 0.7 Hz, 2xOMe), 3.62 (2H, ddd, *J* = 21.6, 11.0, 5.5 Hz, CH_2), 3.13 (1H, dd, *J* = 9.3, 5.6 Hz, ½ CH_2), 3.07 (1H, dd, *J* = 9.3, 5.1 Hz, ½ CH_2), 2.96 (1H, d, *J* = 5.2 Hz, CH_2OH).
[13]C-NMR: (126 MHz, CD_3CN) δ [ppm] = 166.8 (C_q), 159.6 (C_q), 152.1 (C_q), 149.5 (C_q), 146.1 (C_q), 144.9 (CH_{Aryl}), 143.6 (CH_{Aryl}), 140.0 (C_q), 139.6 (C_q), 137.0 (C_q), 137.0 (C_q), 134.1 (CH_{Aryl}), 133.5 (CH_{Aryl}), 131.0 (CH_{Aryl}), 129.0 (CH_{Aryl}), 128.8 (CH_{Aryl}), 127.8 (CH_{Aryl}), 127.7 (CH_{Aryl}), 126.7 (CH_{Aryl}), 126.6 (CH_{Aryl}), 126.0 (C_q), 118.6 (CH_{Aryl}), 117.6 (CH_{Aryl}), 114.0 (CH_{Aryl}), 111.4 (CH_{Aryl}), 108.6 (CH_{Aryl}), 98.8 (CH-Methin), 86.9 (C_q), 63.2 (CH_2), 62.2 (CH_2), 56.9 (CH_2-CO), 55.9 (2xOMe), 52.8 (CH), 43.3 (N-Me).

DMTr-Ser(QB)-phosphoramidite (S19)



Compound **S18** (253 mg, 0.3 mmol, 1.0 eq.) was twice coevaporated with pyridine (10 mL). Dry CH_2Cl_2 (6 mL), Et*N*iPr_2 (300 µL, 1.80 mmol, 6 eq.) and 2-cyanoethyl chloro *N*,*N*-diisopropyl phosphoramidite (200 µL, 0.90 mmol, 3 eq.) were added. The reaction was monitored by 31P-NMR. After 30 min of stirring at room temperature the reaction was quenched by addition of saturated $NaHCO_3$ (aq.) solution. The organic layer was separated and washed two times with saturated $NaHCO_3$ (aq.) solution, dried over $MgSO_4$ and filtered. The filtrate was collected and the solvent was evaporated. The crude product was coevaporated twice with dry benzene, dissolved in 20 mL of dry benzene and lyphoyllized to give a fine powder which was dissolved in dry CH_3CN to a final concentration of 0.15 M and transferred into a reagent bottle for subsequent use in DNA-synthesis.

3.) DNA-synthesis and labelling

Synthesis of Ser(NH_2)-oligonucleotides

Ser(NH_2)-containing oligonucleotides were assembled on 1 µmol scale as described above using the Ser(Tfa)-phosphoramidite (**S6**) at a concentration of 0.1 M in MeCN. Coupling times and cleavage conditions were carried out according to standard DNA-synthesis procedures. During cleavage with aq. ammonia (32%, aq.) the Tfa group on serinol was removed. The Ser(NH_2)-oligonucleotides were purified by RP-HPLC (see general aspects) with the 5'-DMTr group on. Afterwards, the DMTr group was removed upon treatment with 300 µL of 80% aqueous AcOH for 30 min at room temperature. To the detritylation mixture were added 10 vol% of 1M NaCl (aq.) and 1 mL of *i*PrOH (1 mL). The precipitation was repeated once in order to completely remove AcOH, which might interfere in the following coupling reaction. The final pellet was dissolved in water.

Synthesis and coupling of dye-NHS-esters

To the respective dye-carboxylate (20 µmol), pyridinium para-toluene sulfonate (20 µmol) and DMF (1 mL) were added. Ultrasound was applied until the mixture cleared up (5-10 min). *N*-Hydroxysuccinimide (40 µmol) and diisopropylcarbodiimide (40 µmol) were added and the mixture was incubated for 2 h at 30 °C.

In 2 mL-Sarsted®-tubes, oligonucleotides (20 nmol) were dissolved in aqueous $NaHCO_3$ (0.1 M, 100 µL), the crude solution of activated dye was added (100 µL, 2 µmol, 100 eq.) and the mixture was incubated for 1 h. Isopropanol (1.6 mL) was added, followed by centrifugation (14.000 rpm for 10 min) in order to precipitate the oligonucleotides. The supernatant was discarded and the pellet (containing oligonucleotide and excess reagent) was re-suspended in aqueous ammonium acetate (0.3 M, 150 µL). Isopropanol was added (1 mL), followed by centrifugation (14.000 rpm for 10 min) to obtain a pellet. The supernatant was discarded and the pellet was re-suspended in water, insoluble material was removed by a syringe filter and the mixture was subjected to semi-preparative RP-HPLC purification (5-30% B in 12 min) on an *1105 HPLC System* from *Gilson* by using a Waters *X-Bridge BEH130 C18 (4.6x250mm, 5 µm)* column with binary mixture of A (0.1 M triethylammonium acetate, aq. pH 7.5) and B (MeCN).

Product containing fraction were combined, dried and desalted by precipitation (ammonium acetate/ isopropanol) and analysed by RP-HPLC-UV/Vis and MALDI-TOF-MS as mentioned before.

4.) Analytical data *H1N1-neuraminidase* probes

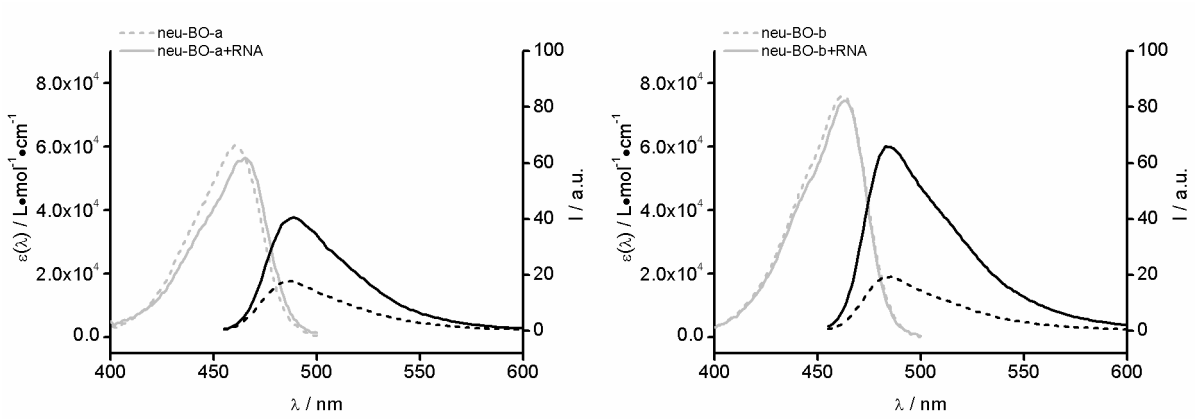
Table S1: Analytical data of BO-labelled *neuraminidase* probes.

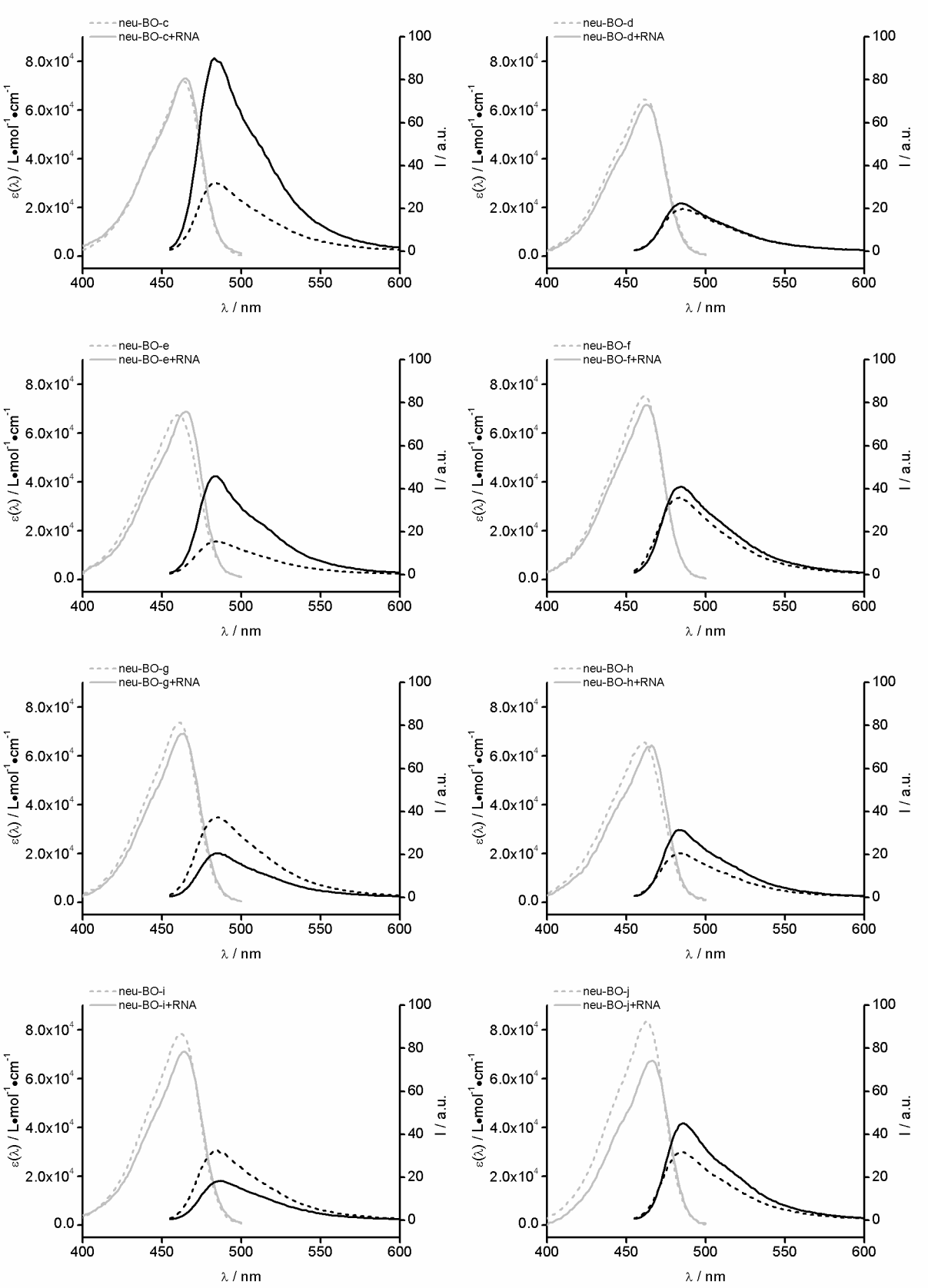
	sequence, **X = Ser(BO)**	MALDI-TOF-MS		R_T / min
		calcd. $[M-2H]^-$	found	
neu-BO-a	GGTTTC**X**GTTATTATGCCGTTGTATTT	8412	8415	18.2[a]
neu-BO-b	GGTTTCA**X**TTATTATGCCGTTGTATTT	8398	8398	9.7[b]
neu-BO-c	GGTTTCAG**X**TATTATGCCGTTGTATTT	8421	8425	18.2[c]
neu-BO-d	GGTTTCAGT**X**ATTATGCCGTTGTATTT	8423	8422	9.74[c]
neu-BO-e	GGTTTCAGTT**X**TTATGCCGTTGTATTT	8412	8416	18.17[a]
neu-BO-f	GGTTTCAGTTA**X**TATGCCGTTGTATTT	8423	8423	9.72[b]
neu-BO-g	GGTTTCAGTTAT**X**ATGCCGTTGTATTT	8423	8423	9.78[b]
neu-BO-h	GGTTTCAGTTATT**X**TGCCGTTGTATTT	8412	8416	18.29[a]
neu-BO-i	GGTTTCAGTTATTA**X**GCCGTTGTATTT	8421	8427	18.54[a]
neu-BO-j	GGTTTCAGTTATTAT**X**CCGTTGTATTT	8398	8399	9.63[b]
neu-BO-k	GGTTTCAGTTATTATG**X**CGTTGTATTT	8436	8439	9.62[b]
neu-BO-l	GGTTTCAGTTATTATGC**X**GTTGTATTT	8436	8434	1.90[c]
neu-BO-m	GGTTTCAGTTATTATGCC**X**TTGTATTT	8396	8395	1.90[c]
neu-BO-n	GGTTTCAGTTATTATGCCG**X**TGTATTT	8423	8422	9.6[b]
neu-BO-o	GGTTTCAGTTATTATGCCGT**X**GTATTT	8423	8423	9.7[b]

[a] Analytical HPLC-RP-UV, gradient II, [b] analytical HPLC-RP-UV, gradient III, [c] analytical UPLC-RP-UV, gradient I

Please note that different analytical systems were used for the analysis of different probes. In general, the probes show similar retention on RP material.

Analytical HPLC was carried out on an *1105 HPLC System* from *Gilson* by using a Waters *X-Bridge BEH130 C18 (4.6x250mm, 5 µm)* column or on an *Acquity-UPLC*®-System from Waters using a binary mixture of A (0.1M triethylammonium acetate, aq. pH 7.5) and B (MeCN).
For analysis via UPLC®-RP-UV a *BEH 130 C18-column 1.7 mm* (*2.1x50mm,* 130 Å) from *Waters* was used at 50 °C. **Gradient I:** 3% B to 30% B in 4 min, 0.6 mL/min.
For analysis via Gilson HPLC-RP-UV a *X-Bridge BEH130 C18-column* (*4.6x250mm, 5 µm*) from *Waters* was used at 55 °C. **Gradient II:** 3% B to 30% B in 20 min, 1.0 mL/min; g**radient III:** 5% B to 35% B in 12 min, 1.5 mL/min.

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neu-BO-g
neu-BO-g+RNA
neu-BO-h
neu-BO-h+RNA
neu-BO-i
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ε(λ) / L•mol-1•cm-1
I / a.u.
λ / nm

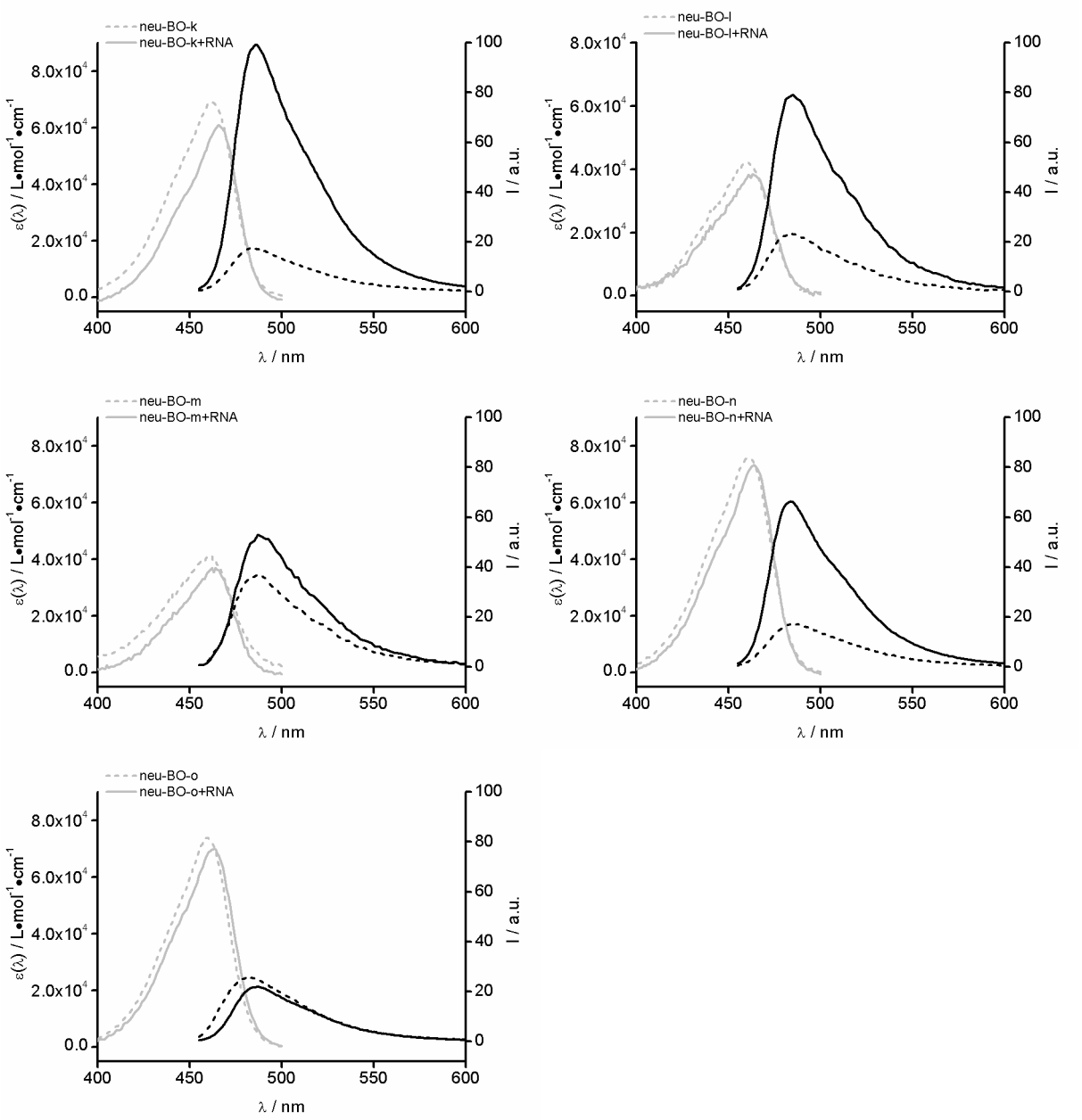
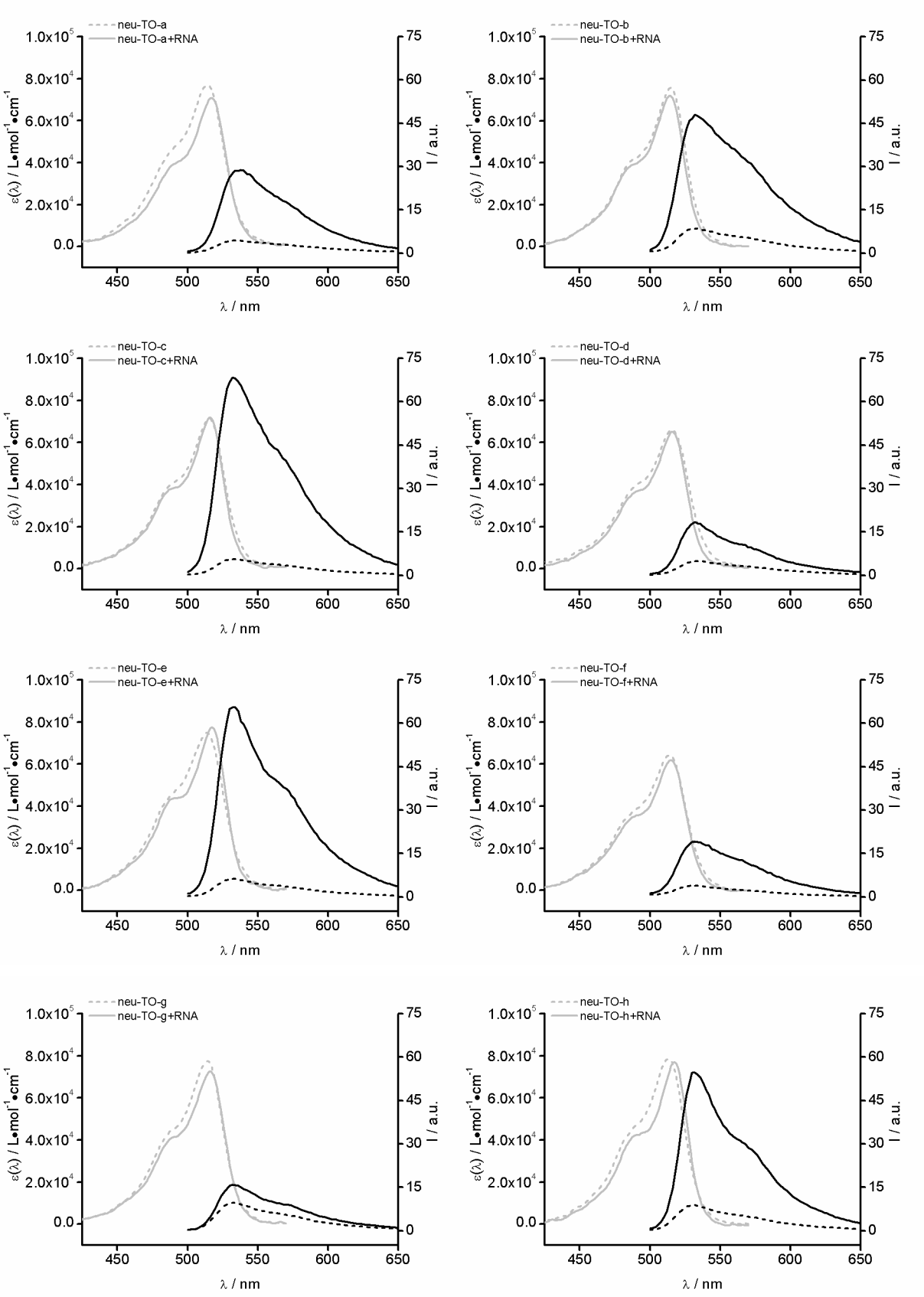


Figure S1: Absorption (grey) and emission spectra (black) of BO-labelled neuraminidase-probes in absence (dotted) and presence of complementary RNA target (solid). Conditions: 0.5 μM probe and 5 eq. RNA target when added, in PBS (100 mM NaCl, 10 mM Na_2HPO_4, pH 7), in 1 mL-cuvette (path length = 1 cm); λ(ex) = 440 nm, λ(em) = 455-600 nm, slit(ex) = 5 nm, slit(em) = 5 nm, 37 °C.

Table S2: Analytical data of TO-labelled *neuraminidase* probes.

	sequence, **X** = **Ser(TO)**	MALDI-TOF-MS		R_T / min[a]
		calcd. $[M-2H]^-$	found	
neu-TO-a	GGTTTC**X**GTTATTATGCCGTTGTATTT	8464	8463	2.08
neu-TO-b	GGTTTCA**X**TTATTATGCCGTTGTATTT	8448	8446	2.11
neu-TO-c	GGTTTCAG**X**TATTATGCCGTTGTATTT	8473	8471	2.04
neu-TO-d	GGTTTCAGT**X**ATTATGCCGTTGTATTT	8473	8471	2.08
neu-TO-e	GGTTTCAGTT**X**TTATGCCGTTGTATTT	8464	8463	2.14
neu-TO-f	GGTTTCAGTTA**X**TATGCCGTTGTATTT	8473	8470	2.11
neu-TO-g	GGTTTCAGTTAT**X**ATGCCGTTGTATTT	8473	8478	2.09
neu-TO-h	GGTTTCAGTTATT**X**TGCCGTTGTATTT	8464	8460	2.12
neu-TO-i	GGTTTCAGTTATTA**X**GCCGTTGTATTT	8473	8469	2.11
neu-TO-j	GGTTTCAGTTATTAT**X**CCGTTGTATTT	8448	8444	2.09
neu-TO-k	GGTTTCAGTTATTATG**X**CGTTGTATTT	8488	8490	2.08
neu-TO-l	GGTTTCAGTTATTATGC**X**GTTGTATTT	8488	8488	2.10
neu-TO-m	GGTTTCAGTTATTATGCC**X**TTGTATTT	8448	8447	2.13
neu-TO-n	GGTTTCAGTTATTATGCCG**X**TGTATTT	8473	8476	2.08
neu-TO-o	GGTTTCAGTTATTATGCCGT**X**GTATTT	8473	8470	2.05

[a] analytical UPLC-RP-UV, gradient I.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neu-TO-b
neu-TO-b+RNA
neu-TO-c
neu-TO-c+RNA
neu-TO-d
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neu-TO-e
neu-TO-e+RNA
neu-TO-f
neu-TO-f+RNA
neu-TO-g
neu-TO-g+RNA
neu-TO-h
neu-TO-h+RNA
ε(λ) / L•mol-1•cm-1
I / a.u.
λ / nm

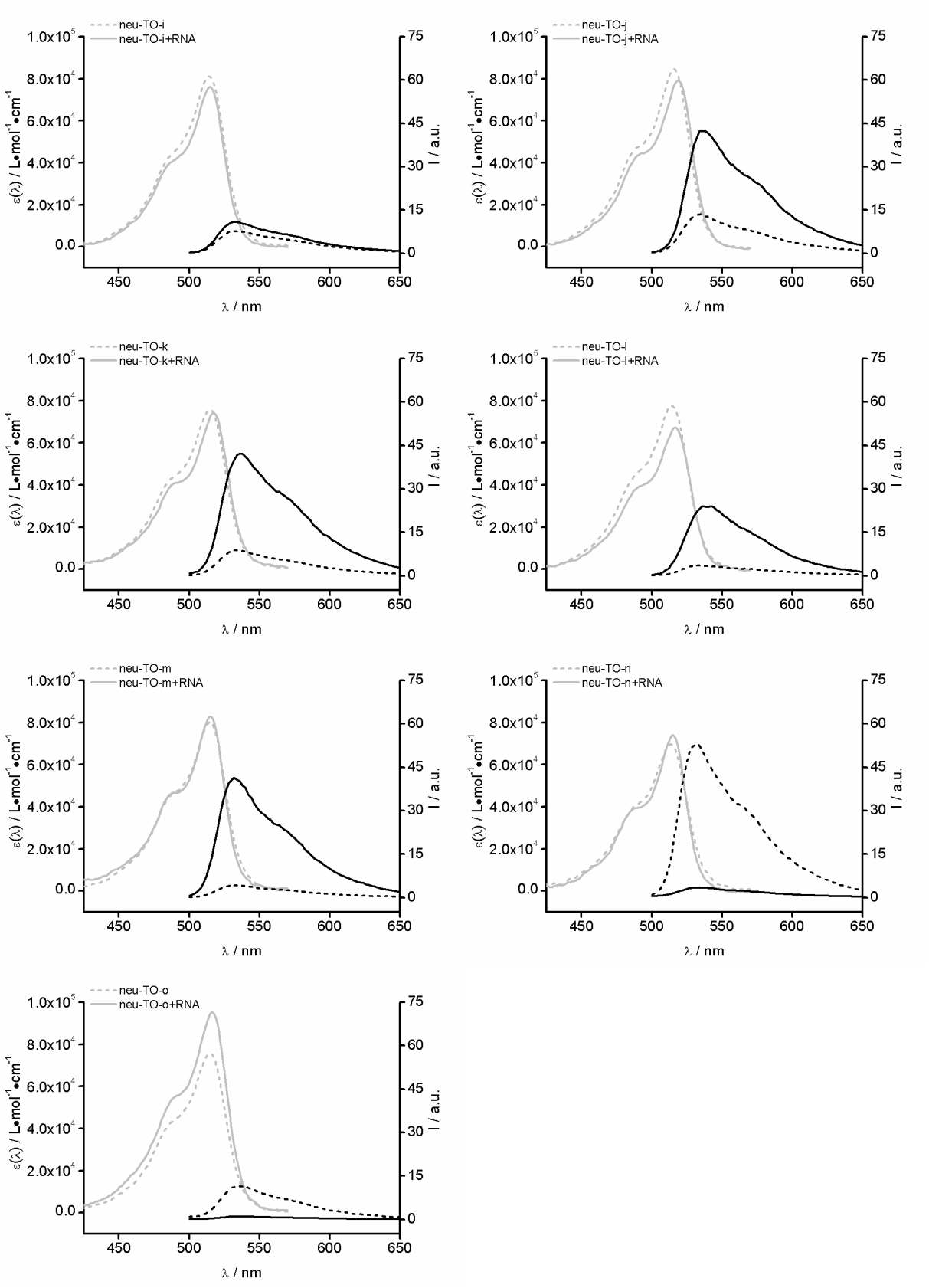
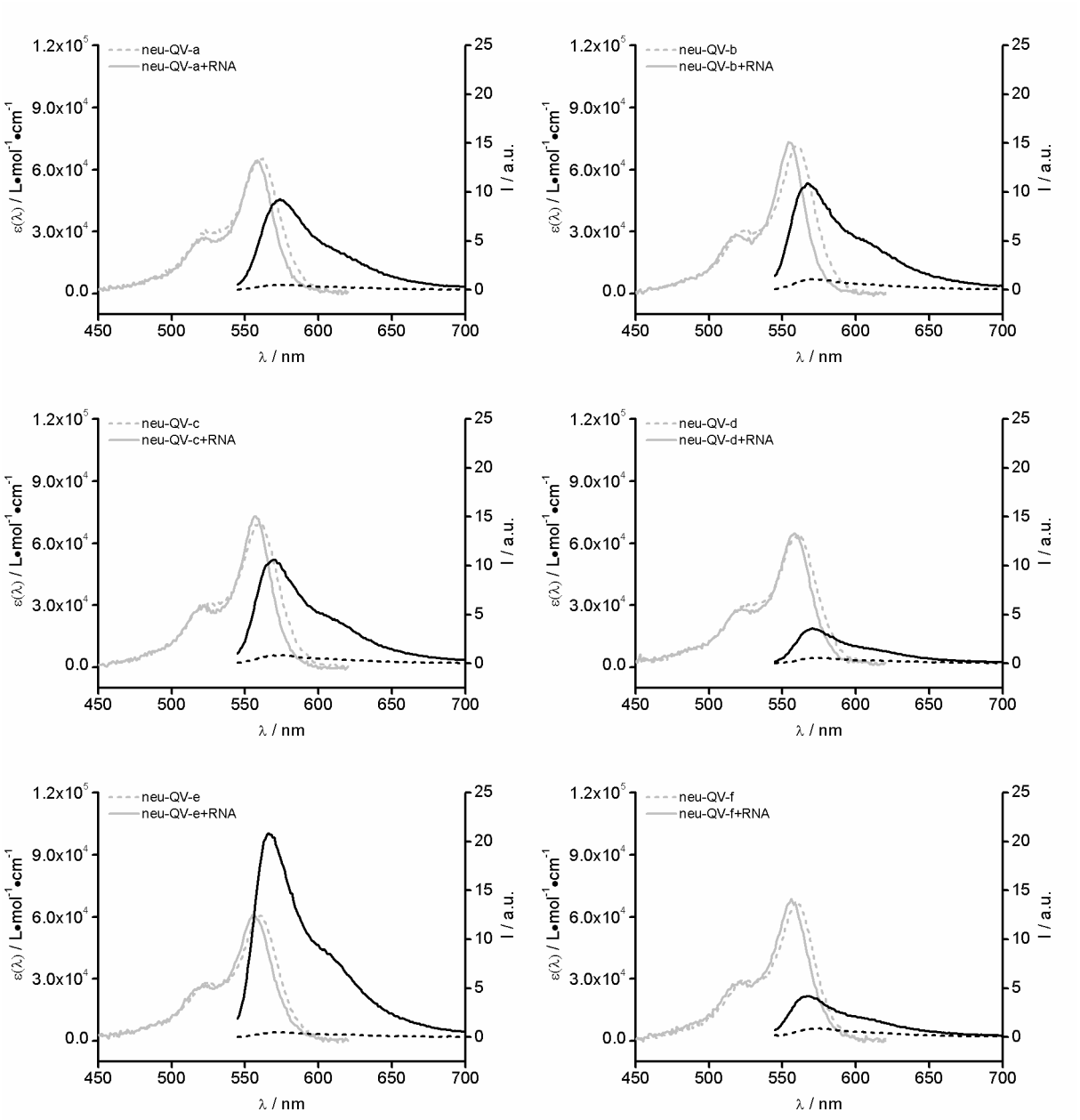


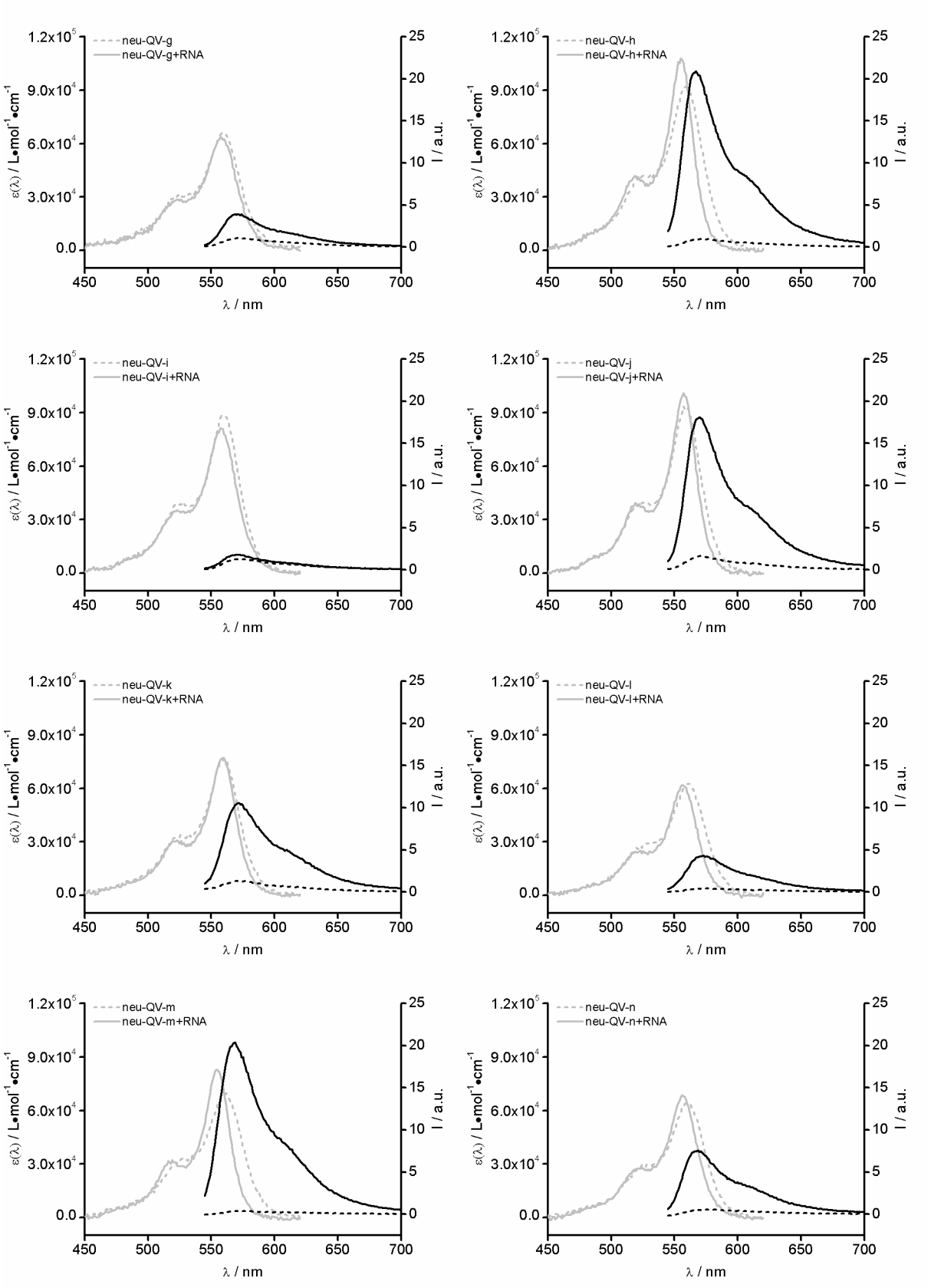
Figure S2: Absorption (grey) and emission spectra (black) of TO-labelled neuraminidase-probes in absence (dotted) and presence of complementary RNA target (solid). Conditions: 0.5 μM probe and 5 eq. RNA target when added, in PBS (100 mM NaCl, 10 mM Na_2HPO_4, pH 7), in 1 mL-cuvette (path length = 1 cm); λ(ex) = 485 nm, λ(em) = 500-660 nm, slit(ex) = 5 nm, slit(em) = 5 nm, 37 °C.

Table S3: Analytical data of QV-labelled *neuraminidase* probes.

	sequence, **X = Ser(QV)**	MALDI-TOF-MS		R_T / min[a]
		calcd. $[M]^+$	found	
neu-QV-a	GGTTTC**X**GTTATTATGCCGTTGTATTT	8458	8454	9.1
neu-QV-b	GGTTTCA**X**TTATTATGCCGTTGTATTT	8442	8439	9.5
neu-QV-c	GGTTTCAG**X**TATTATGCCGTTGTATTT	8467	8464	9.5
neu-QV-d	GGTTTCAGT**X**ATTATGCCGTTGTATTT	8467	8466	9.7
neu-QV-e	GGTTTCAGTT**X**TTATGCCGTTGTATTT	8458	8461	10.0
neu-QV-f	GGTTTCAGTTA**X**TATGCCGTTGTATTT	8467	8465	9.8
neu-QV-g	GGTTTCAGTTAT**X**ATGCCGTTGTATTT	8467	8466	9.6
neu-QV-h	GGTTTCAGTTATT**X**TGCCGTTGTATTT	8458	8456	9.8
neu-QV-i	GGTTTCAGTTATTA**X**GCCGTTGTATTT	8467	8466	9.0
neu-QV-j	GGTTTCAGTTATTAT**X**CCGTTGTATTT	8442	8439	9.2
neu-QV-k	GGTTTCAGTTATTATG**X**CGTTGTATTT	8482	8480	9.1
neu-QV-l	GGTTTCAGTTATTATGC**X**GTTGTATTT	8482	8480	9.6
neu-QV-m	GGTTTCAGTTATTATGCC**X**TTGTATTT	8442	8439	9.2
neu-QV-n	GGTTTCAGTTATTATGCCG**X**TGTATTT	8467	8465	9.3
neu-QV-o	GGTTTCAGTTATTATGCCGT**X**GTATTT	8467	8466	9.9

[a] analytical HPLC-RP-UV, gradient III.

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I / a.u.
λ / nm

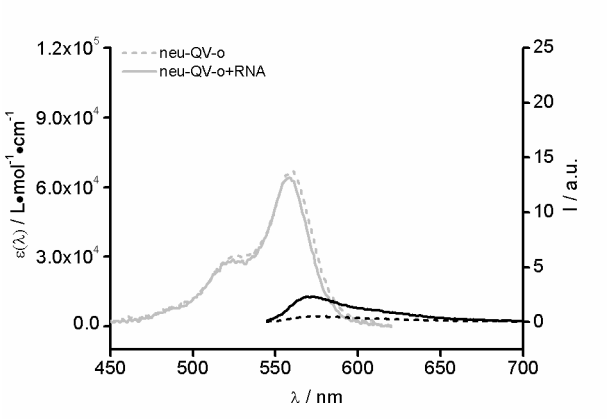


Figure S3: Absorption (grey) and emission spectra (black) of QV-labelled neuraminidase-probes in absence (dotted) and presence of complementary RNA target (solid). Conditions: 0.5 μM probe and 5 eq. RNA target when added, in PBS (100 mM NaCl, 10 mM Na_2HPO_4, pH 7), in 1 mL-cuvette (path length = 1 cm); λ(ex) = 530 nm, λ(em) = 545-700 nm, slit(ex) = 5 nm, slit(em) = 5 nm, 37 °C.

Table S4: Analytical data of QB-labelled *neuraminidase* probes.

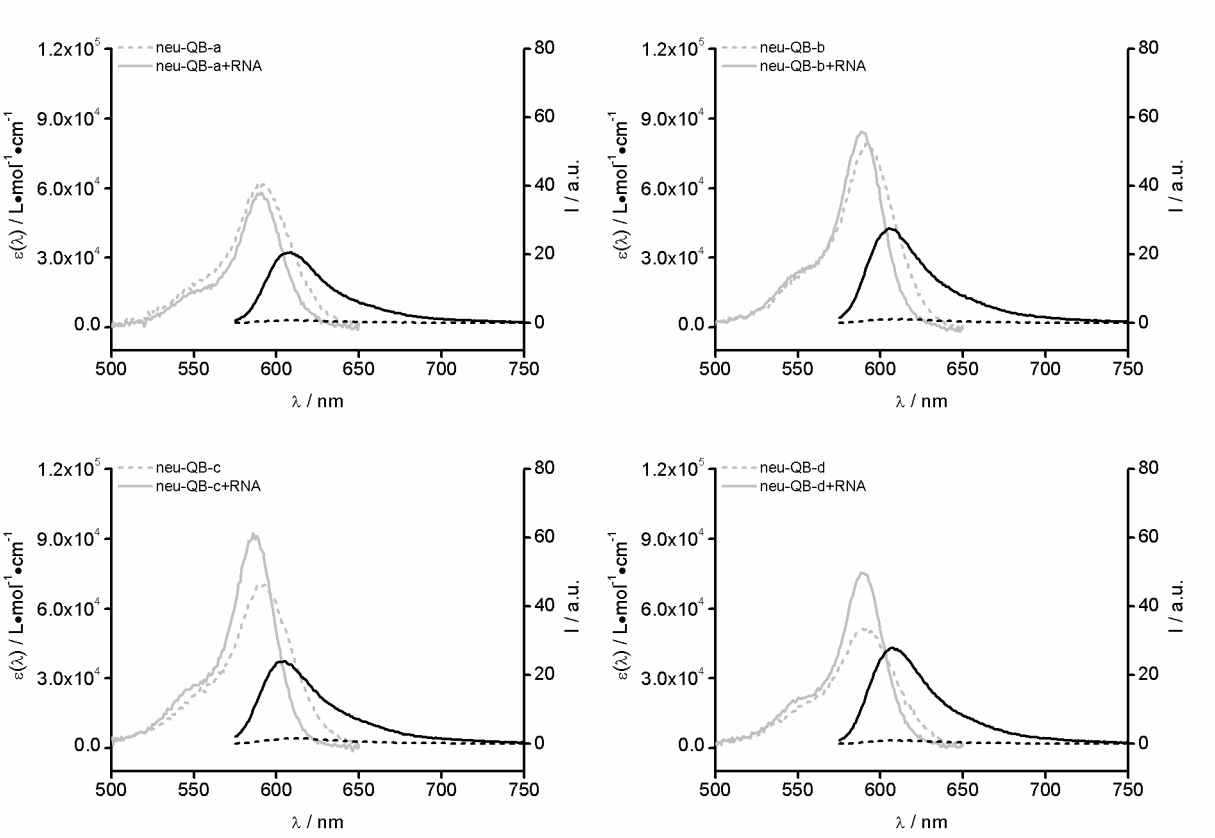
	sequence, **X = Ser(QB)**	MALDI-TOF-MS		R_T / min[a]
		calcd. $[M]^+$	found	
neu-QB-a	GGTTTC**X**GTTATTATGCCGTTGTATTT	8458	8456	9.4
neu-QB-b	GGTTTCA**X**TTATTATGCCGTTGTATTT	8442	8439	9.7
neu-QB-c	GGTTTCAG**X**TATTATGCCGTTGTATTT	8467	8465	9.5
neu-QB-d	GGTTTCAGT**X**ATTATGCCGTTGTATTT	8467	8465	9.8
neu-QB-e	GGTTTCAGTT**X**TTATGCCGTTGTATTT	8458	8455	9.9
neu-QB-f	GGTTTCAGTTA**X**TATGCCGTTGTATTT	8467	8465	9.5
neu-QB-g	GGTTTCAGTTAT**X**ATGCCGTTGTATTT	8467	8468	9.8
neu-QB-h	GGTTTCAGTTATT**X**TGCCGTTGTATTT	8458	8460	9.5
neu-QB-i	GGTTTCAGTTATTA**X**GCCGTTGTATTT	8467	8466	9.4
neu-QB-j	GGTTTCAGTTATTAT**X**CCGTTGTATTT	8442	8440	9.4
neu-QB-k	GGTTTCAGTTATTATG**X**CGTTGTATTT	8482	8480	9.8
neu-QB-l	GGTTTCAGTTATTATGC**X**GTTGTATTT	8482	8481	9.9
neu-QB-m	GGTTTCAGTTATTATGCC**X**TTGTATTT	8442	8441	9.5
neu-QB-n	GGTTTCAGTTATTATGCCG**X**TGTATTT	8467	8465	9.4
neu-QB-o	GGTTTCAGTTATTATGCCGT**X**GTATTT	8467	8466	10.0

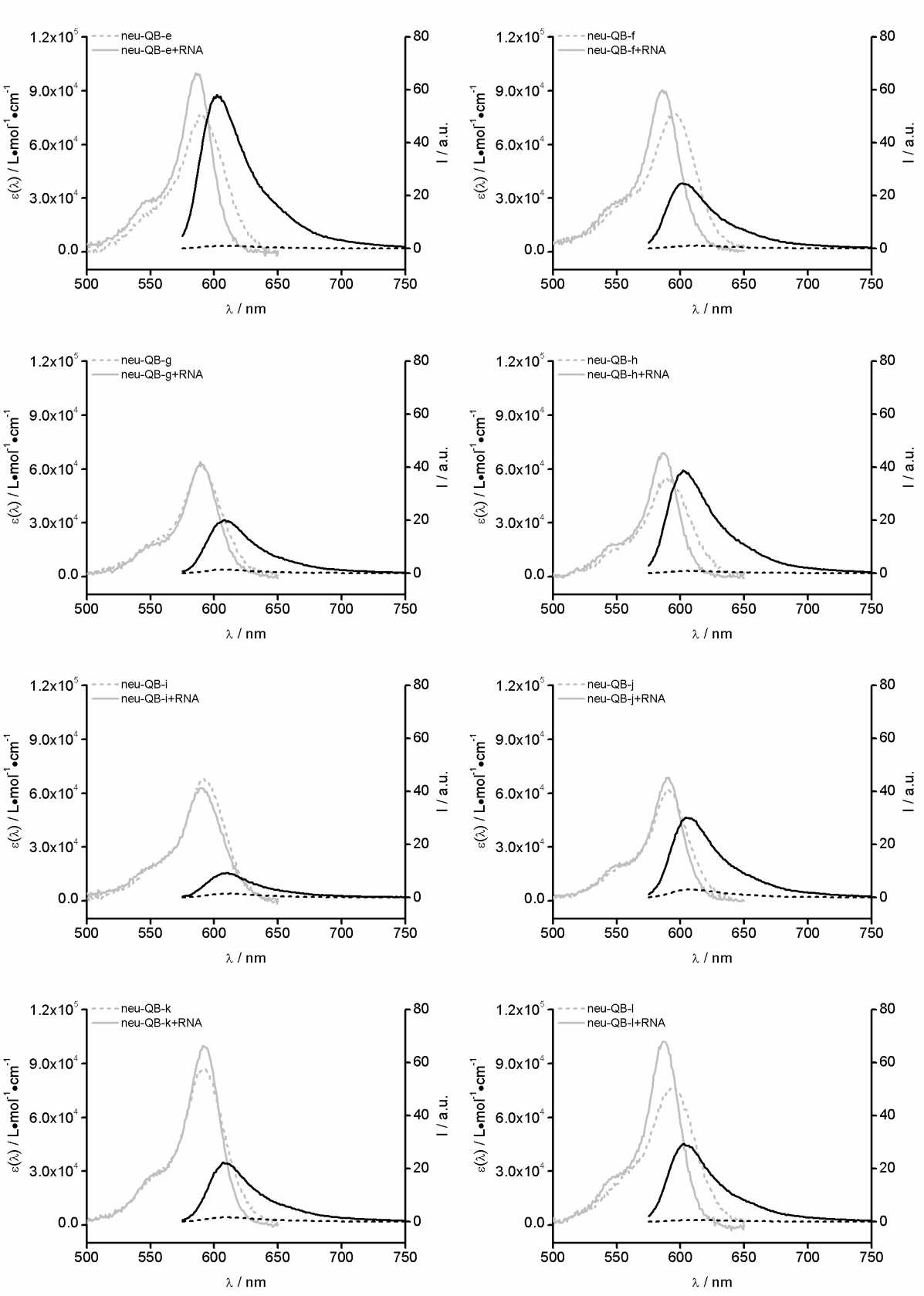
[a] analytical HPLC-RP-UV, gradient III.

Table S5: Fluorescence and absorption data of QB-labelled *neuraminidase* probes.

neu-	single strand				double strand				I/I_0	Φ/Φ_0	$\Delta\lambda$ / nm
	I_0	ε_0[a]	λ_0[b]	Φ_0[c]	I	ε[a]	λ[b]	Φ[c]			
QB-a	0.7	62	592	**0.004**	20	58	591	**0.13**	**28**	**29**	1
QB-b	1.1	79	591	**0.006**	28	84	588	**0.11**	**25**	**20**	3
QB-c	1.6	70	593	**0.008**	24	92	586	**0.09**	**15**	**11**	7
QB-d	0.9	51	592	**0.006**	27	76	588	**0.13**	**30**	**22**	4
QB-e	0.9	75	590	**0.005**	58	100	586	**0.21**	**64**	**43**	4
QB-f	1.1	77	596	**0.005**	24	90	586	**0.08**	**23**	**18**	10
QB-g	1.4	62	589	**0.007**	19	64	589	**0.11**	**14**	**15**	0
QB-h	0.9	55	592	**0.007**	38	69	587	**0.20**	**42**	**31**	5
QB-i	1.4	63	590	**0.008**	9	68	592	**0.04**	**6**	**6**	2
QB-j	3.0	62	591	**0.017**	30	69	591	**0.14**	**10**	**9**	0
QB-k	1.6	87	591	**0.007**	21	100	591	**0.08**	**14**	**12**	0
QB-i	0.7	76	595	**0.004**	29	102	588	**0.12**	**44**	**32**	7
QB-m	0.5	66	594	**0.002**	74	107	585	**0.24**	**152**	**102**	9
QB-n	0.8	70	593	**0.004**	25	90	589	**0.09**	**32**	**23**	4
QB-o	1.2	77	590	**0.006**	43	100	590	**0.16**	**35**	**26**	0

[a] extinction coefficient at absorbance maximum, (in $L \cdot mmol^{-1} \cdot cm^{-1}$), [b] wavelength of maximum absorbance in the VIS rang. [c] quantum yield, determined by using ATTO 590 as a reference dye.

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λ / nm

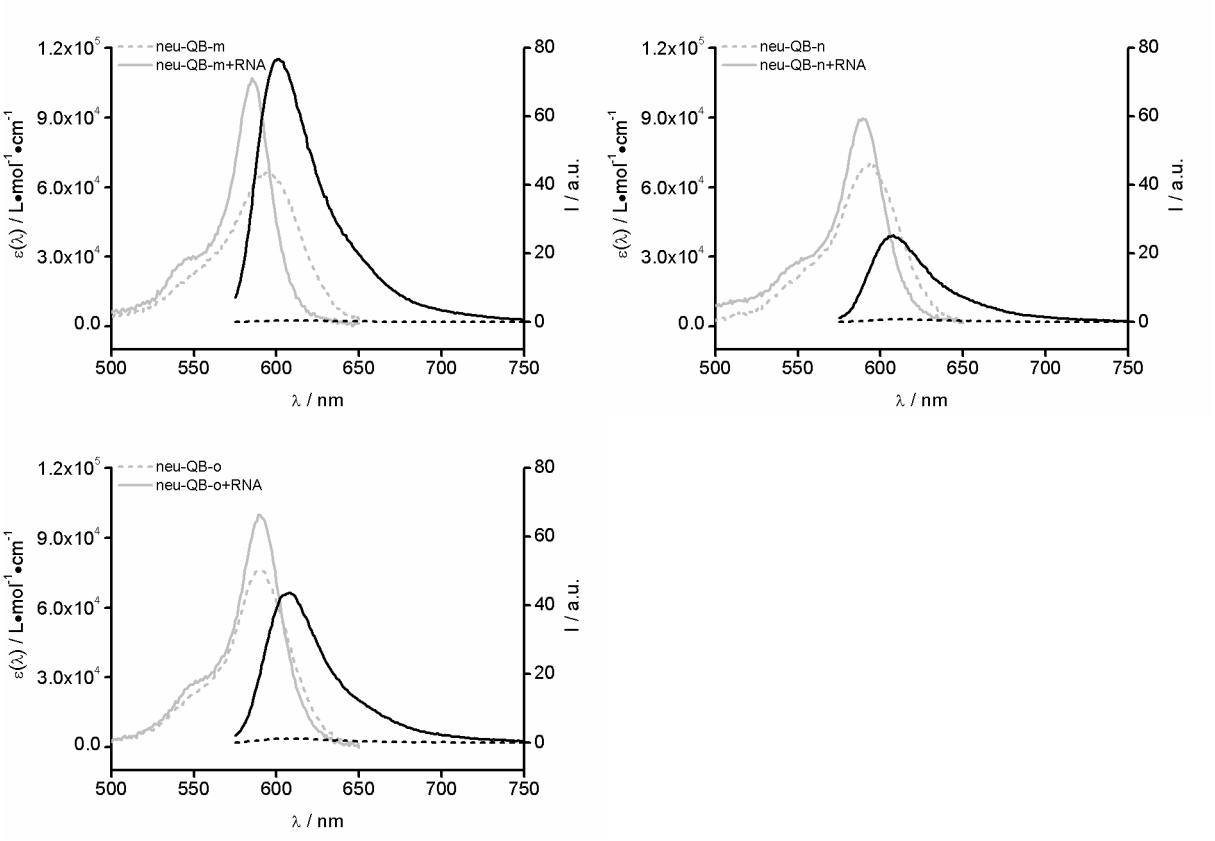
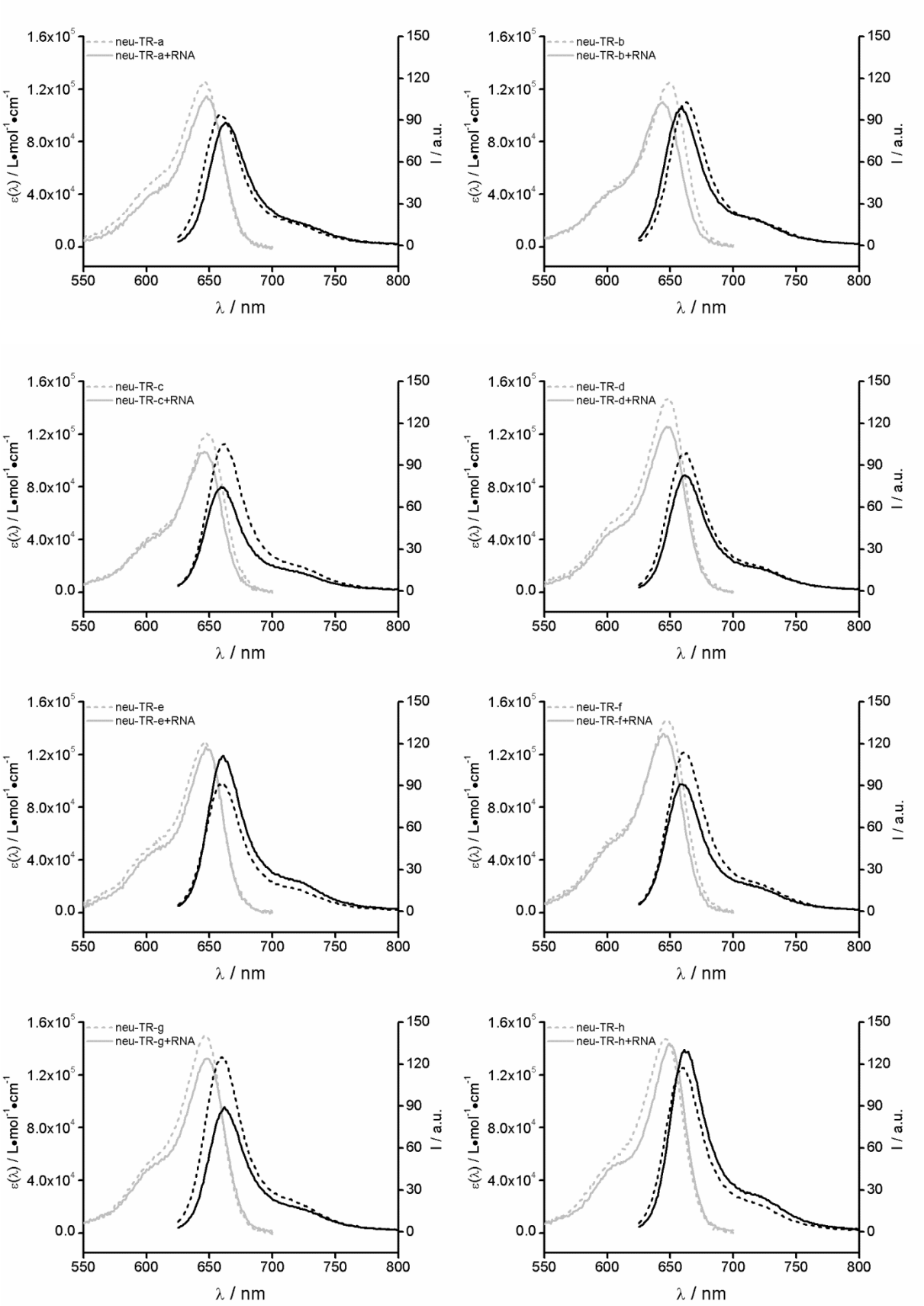


Figure S4: Absorption (grey) and emission spectra (black) of QB-labelled neuraminidase-probes in absence (dotted) and presence of complementary RNA target (solid). Conditions: 0.5 μM probe and 5 eq. RNA target when added, in PBS (100 mM NaCl, 10 mM Na_2HPO_4, pH 7), in 1 mL-cuvette (path length = 1 cm); λ(ex) = 560 nm, λ(em) = 575-750 nm, slit(ex) = 5 nm, slit(em) = 5 nm, 37 °C.

Table S6: Analytical data of TR-labelled *neuraminidase* probes

	sequence, **X = Ser(TR)**	MALDI-TOF-MS		R_T / min[a]
		calcd. $[M]^+$	found	
neu-TR-a	GGTTTC**X**GTTATTATGCCGTTGTATTT	8490	8489	10.1
neu-TR-b	GGTTTCA**X**TTATTATGCCGTTGTATTT	8474	8470	10.2
neu-TR-c	GGTTTCAG**X**TATTATGCCGTTGTATTT	8499	8497	9.9
neu-TR-d	GGTTTCAGT**X**ATTATGCCGTTGTATTT	8499	8498	10.1
neu-TR-e	GGTTTCAGTT**X**TTATGCCGTTGTATTT	8490	8489	10.2
neu-TR-f	GGTTTCAGTTA**X**TATGCCGTTGTATTT	8499	8498	10.1
neu-TR-g	GGTTTCAGTTAT**X**ATGCCGTTGTATTT	8499	8499	10.1
neu-TR-h	GGTTTCAGTTATT**X**TGCCGTTGTATTT	8490	8487	10.2
neu-TR-i	GGTTTCAGTTATTA**X**GCCGTTGTATTT	8499	8496	10.2
neu-TR-j	GGTTTCAGTTATTAT**X**CCGTTGTATTT	8474	8475	10.1
neu-TR-k	GGTTTCAGTTATTATG**X**CGTTGTATTT	8514	8514	10.0
neu-TR-l	GGTTTCAGTTATTATGC**X**GTTGTATTT	8514	8516	10.0
neu-TR-m	GGTTTCAGTTATTATGCC**X**TTGTATTT	8474	8475	10.2
neu-TR-n	GGTTTCAGTTATTATGCCG**X**TGTATTT	8499	8497	10.1
neu-TR-o	GGTTTCAGTTATTATGCCGT**X**GTATTT	8499	8498	10.1

[a] analytical HPLC-RP-UV, gradient III.


neu-TR-a
neu-TR-a+RNA
neu-TR-b
neu-TR-b+RNA
neu-TR-c
neu-TR-c+RNA
neu-TR-d
neu-TR-d+RNA
neu-TR-e
neu-TR-e+RNA
neu-TR-f
neu-TR-f+RNA
neu-TR-g
neu-TR-g+RNA
neu-TR-h
neu-TR-h+RNA
ε(λ) / L•mol-1•cm-1
I / a.u.
λ / nm

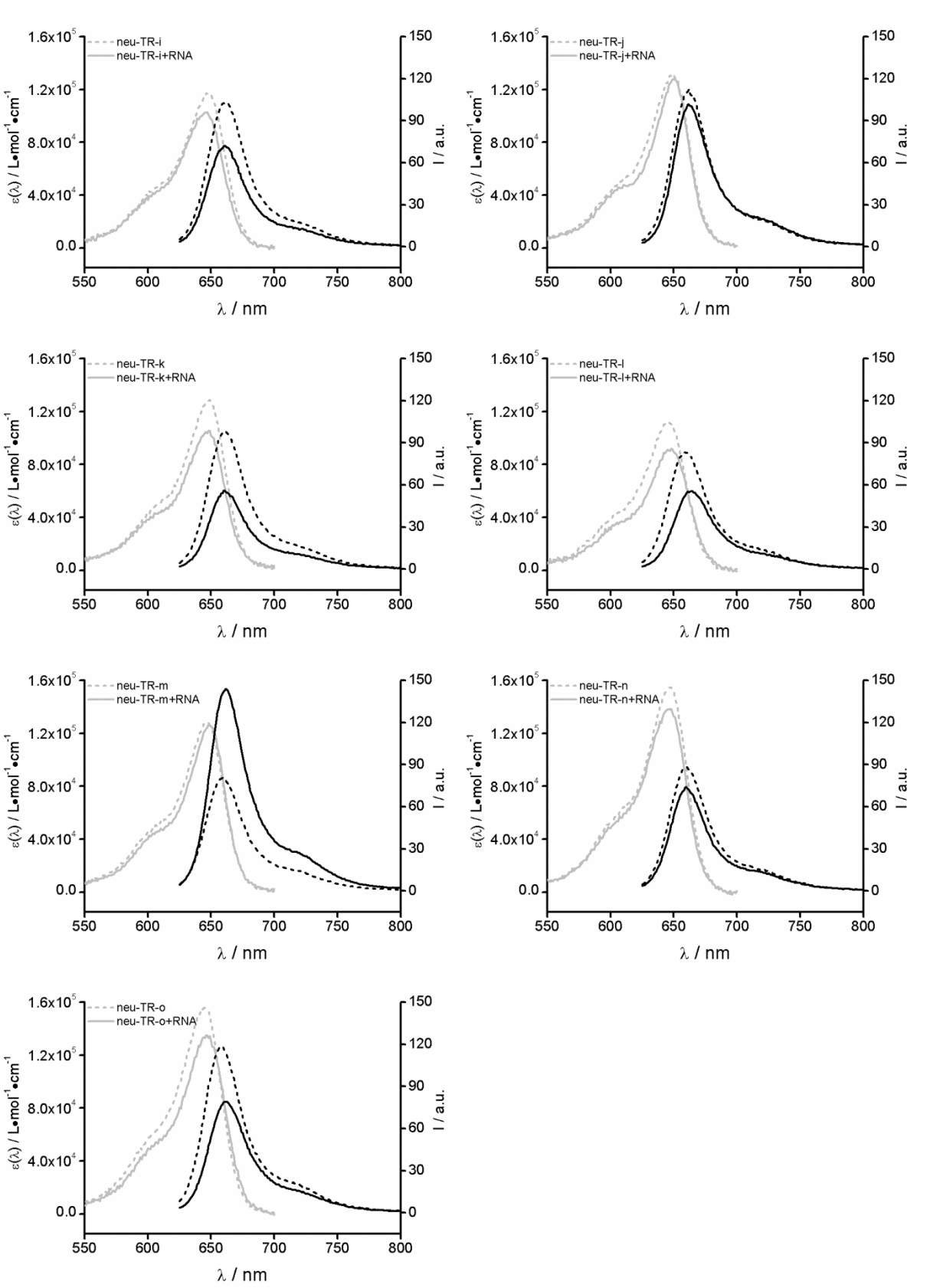


Figure S5: Absorption (grey) and emission spectra (black) of TR-labelled neuraminidase-probes in absence (dotted) and presence of complementary RNA target (solid). Conditions: 0.5 μM probe and 5 eq. RNA target when added, in PBS (100 mM NaCl, 10 mM Na_2HPO_4, pH 7), in 1 mL-cuvette (path length = 1 cm); λ(ex) = 615 nm, λ(em) = 625-800 nm, slit(ex) = 5 nm, slit(em) = 5 nm, 37 °C.

5.) Analytical and fluorescence data of *vasa*-probes

Table S7: analytical data of QB-labelled FIT probes directed against *vasa* mRNA.

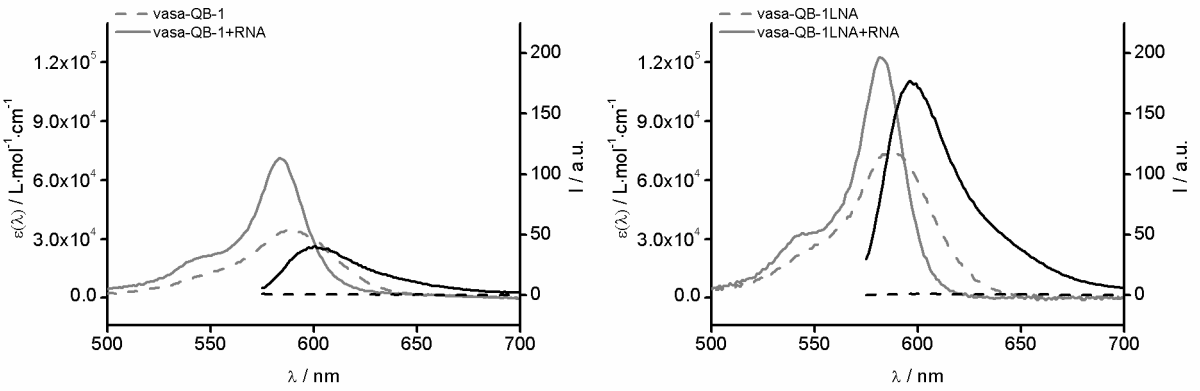
name	sequence[a], **X = Ser(QB)**	MALDI-TOF-MS		R_T / min
		calcd. $[M]^+$	found	
vasa-QB-1	UCUAUUUUC**X**TCAUUUUCA	6308	6308	8.75[b]
vasa-QB-2	UCCAUUUUC**X**TTAUUUUCU	6299	6300	8.91[b]
vasa-QB-3	GGCCGCCGUT**X**TTCCUG	5820	5820	8.86[b]
vasa-QB-4	GGAACUAC**X**TGUUGGGC	5903	5903	8.53[b]
vasa-QB-1LNA	UCUAUUUUC**XT$_L$**CAUUUUCA	6335	6335	10.1[c]
vasa-QB-2LNA	UCCAUUUUC**XT$_L$**TAUUUUCU	6326	6326	10.1[c]
vasa-QB-3LNA	GGCCGCCGUT**XT$_L$**TCCUG	5847	5846	9.9[c]
vasa-QB-4LNA	GGAACUAC**XT$_L$**GUUGGGC	5930	5929	9.8[c]

[a] LNA nucleotides are printed in boldface and indicated by subscript "L", 2'-O-Me nucleotides are underlined; [b] analytical HPLC-RP-UV, gradient IV (10-50% B); [c] analytical HPLC-RP-UV, gradient III.

Table S8: Fluorescence data of QB - 2'-O-Me gapmer probes with and without LNA. Conditions: 0.5 µM probe and 5 eq. RNA target when added, in PBS, in 1 mL cuvette (path length = 1 cm); TO: λ(ex) = 485 nm, λ(em) = 500-675 nm, QB: λ(ex) = 560 nm, λ(em) = 575-700 nm, slit(ex) = 5 nm, slit(em) = 5 nm, 37 °C.

name	I_0	I	I/I_0	**ε(max)[a]**	Φ_0	Φ	Φ/Φ_0	**Br(560nm)**	**Br(max)[b]**
vasa-QB-1	0.55	35.5	65	71	0.007	0.18	25.7	5.0	12.8
vasa-QB-2	0.47	32.8	70	55	0.004	0.31	77.5	4.6	17.1
vasa-QB-3	0.85	36.5	43	48	0.006	0.27	45.0	4.9	13.0
vasa-QB-4	2.42	33.8	14	67	0.020	0.24	12.0	4.7	16.1
vasa-QB-1LNA	0.79	154	195	122	0.004	0.45	112.5	19.1	54.9
vasa-QB-2LNA	2.10	151	72	119	0.009	0.47	53.5	19.1	55.9
vasa-QB-3LNA	1.26	158	125	119	0.007	0.47	71.4	19.5	55.9
vasa-QB-4LNA	2.38	164	69	129	0.012	0.45	38.8	20.2	58.1

[a] extinction coefficient at absorbance maximum, (in $L \cdot mmol^{-1} \cdot cm^{-1}$); [b] Br(max) = maximum achievable brightness as the product of ε(max)*Φ/1000.



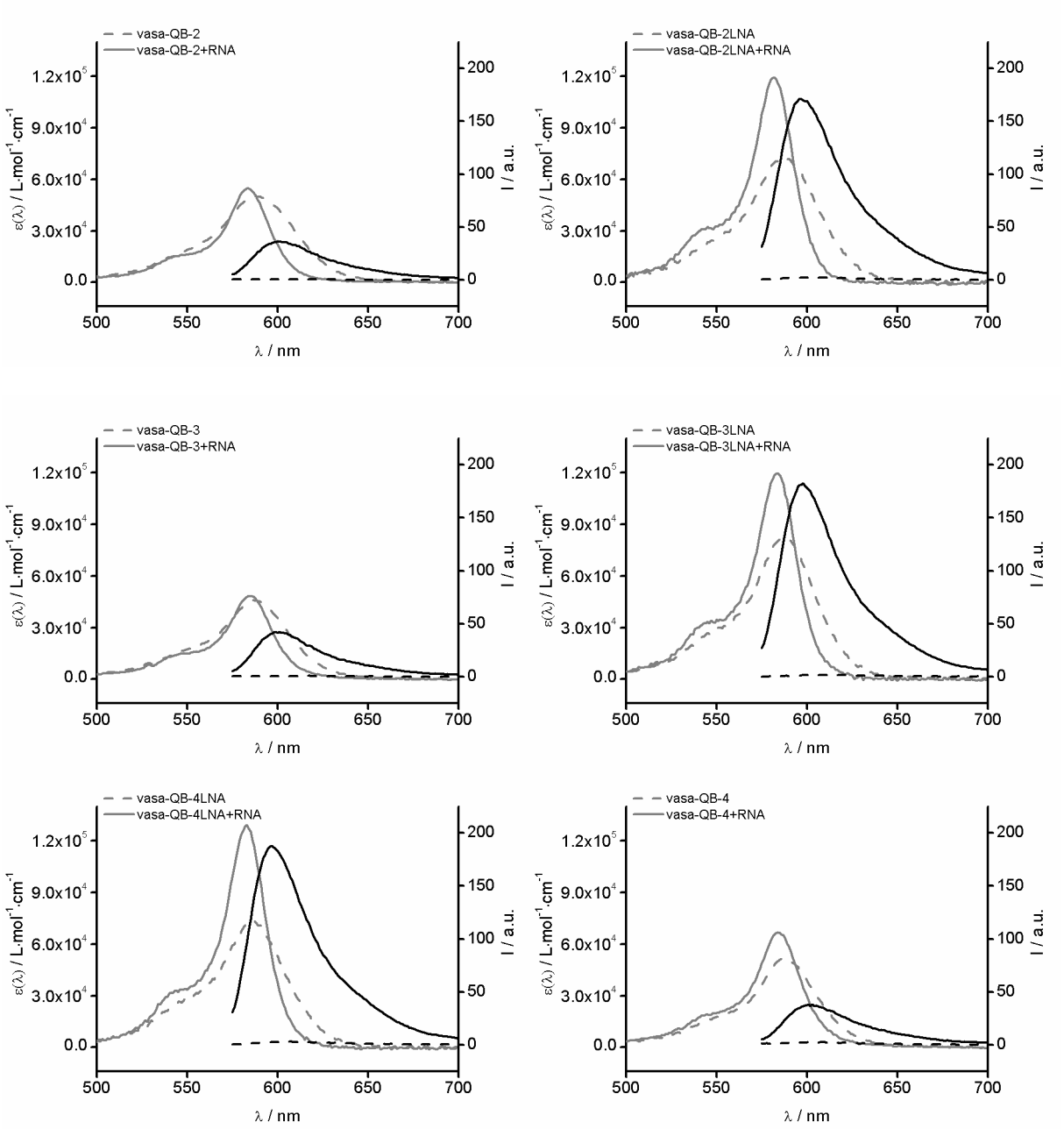
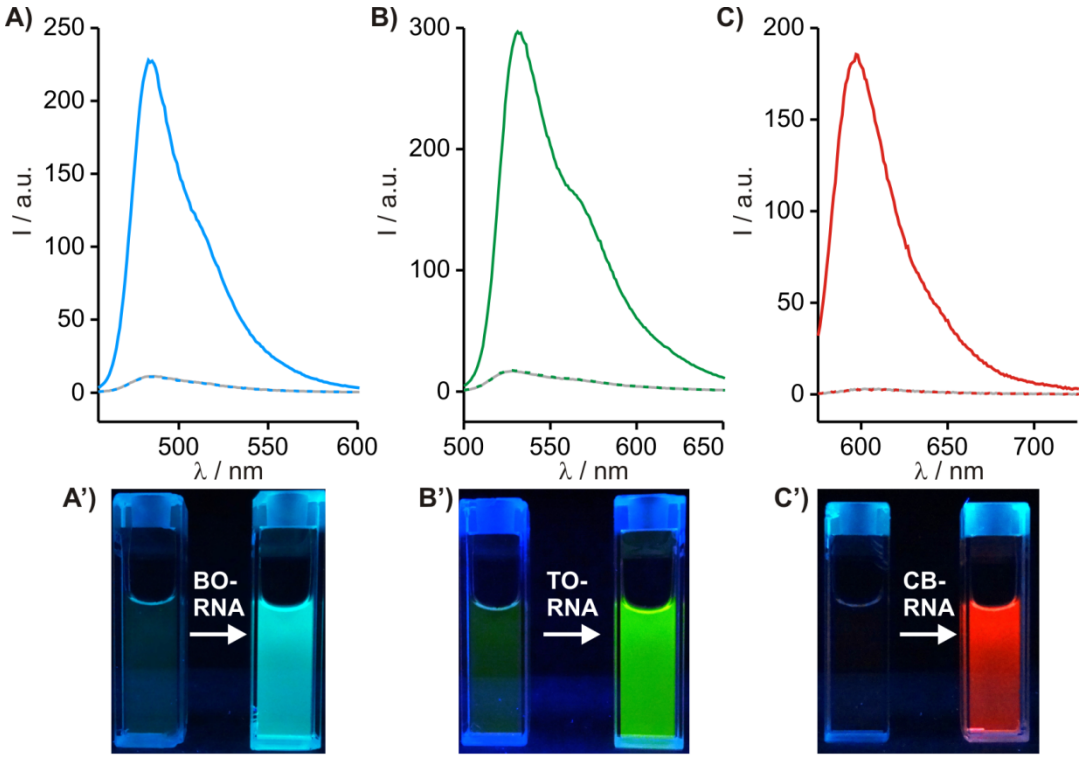


Figure S6: Absorption (grey) and emission spectra (black) of 2′-O-Me-modified and LNA/2′-O-Me-modified *vasa*-QB-probes in absence (dotted) and presence of complementary RNA target (solid). Conditions: see Table S8.

6.) Multi-colour RNA detection



BO-probe: ATG**C**$_L$ACG**XC**$_L$CG
BO-RNA: GACGGACGUGCAUAUUUGCG

TO-probe: TCTATTTTC**XT**$_L$CATTTTCA
TO-RNA: UGAAAAUGAUGAAAAUAGA

QB-probe: GGAACTAC**XT**$_L$GTTGGGC
QB-RNA: GCCCAACAAGUAGUUCC

Figure S7: A-C) Investigation of sequence specificity. Fluorescence emissions of probe in the absence of target (grey), in the presence of 3 eq. of both of the non-complementary targets (colored dashed) and upon addition of 3 eq. of complementary RNA (colored solid). A'-C') Photos of cuvettes corresponding to A-C before and after addition of complementary RNA; illumination with a handheld UV Lamp (5W). Conditions: 0.5 μM probe in PBS and 3 eq. of RNA, when added. BO: λ(ex) = 440 nm, λ(em) = 455-600 nm, TO: λ(ex) = 485 nm, λ(em) = 500-650 nm, CB: λ(ex) = 560 nm, λ(em) = 575-725 nm, slit(ex) = 5 nm, slit(em) = 5 nm, 37 °C.

7.) Fluorescence *in-situ* hybridization

dT(18)-QB probe

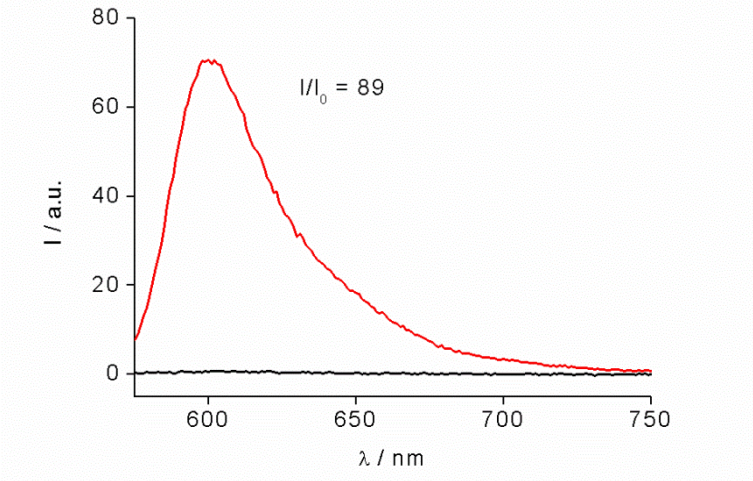


Figure S8: Fluorescence emission of LNA-modified poly-dT-QB-probes in absence (black) and presence (red) of 5 eq. complementary RNA. Conditions: 0.5 µM probe in PBS and 5 eq. of RNA, when added. λ(ex) = 560 nm, λ(em) = 575-725 nm, slit(ex) = 5 nm, slit(em) = 5 nm, 37 °C, I/I_0 at 605 nm.

Oskar-TO-LNA probes

The following probes have been used for the detection of *oskar* mRNA in Oozytes. Detailed information for the synthesis, characterization and application of these probes was previously published: F. Hövelmann, I. Gaspar, S. Loibl, E. A. Ermilov, B. Röder, J. Wengel, A. Ephrussi and O. Seitz, *Angew. Chem. Int. Ed.*, 2014, **53**, 11370-11375.

Table S9: TO and LNA labelled probes for the detection of *oskar*-mRNA.

	sequence, X = Ser(TO)
osk-TO-LNA-1	**T**$_L$AA**G**$_L$ATA**X****T**$_L$AGG**T**$_L$T
osk-TO-LNA-2	**G**$_L$CG**G**$_L$AAAAG**X****T**$_L$TGAA**G**$_L$
osk-TO-LNA-3	**C**$_L$TCG**X****T**$_L$TCAA**T**$_L$A
osk-TO-LNA-4	ACCGAT**X****T**$_L$TGUUCCAGAAC
osk-TO-LNA-5	CGGUUUUCUGG**X****T**$_L$TUGGGU

LNA nucleotides are labelled with subscript "L", 2'-O-Me-building blocks are underlined.

Wash free FISH and imaging

Wash free FISH was performed as described by F. Hövelmann, I. Gaspar, A. Ephrussi and O. Seitz, *J. Am. Chem. Soc.*, 2013, **135**, 19025-19032. Briefly, w^{1118} ovaries were dissected into 2% paraformaldehyde (PFA), 0.05% Triton-X100 in PBS and were fixed for 20 minutes. Fixative was removed and ovaries were rinsed twice with IBEX (10 mM HEPES, pH=7.7, 100 mM KCl, 1mM EDTA, 0.3% Triton-X). All probes were diluted in IBEX to 0.5 µM final concentration (for osk1-5, this means 0.1 µM concentration per individual probe). This mixture was applied to ovaries (50 µL/ovary) at 35 °C from 30 minutes. Following the incubation, ovaries were transferred onto slides and were

mounted in the staining solution by covering with high precision (170±5µm) coverslips and were sealed from air. Imaging was carried out on a Leica SP8 TCS microscope using 63x 1.4 NA oil immersion objective. Fluorescence of TO and QB were excited with 514 nm and 592 nm laser lines of a white-light laser source, emission of the dyes were detected between 525-575 nm and 605-650 nm, respectively. To minimize bleed-through, the two dyes were imaged sequentially. For single colour imaging, emission and excitation settings of QB were used and all probes were excited with constant 0.7% of maximal output of the 592nm laser line. For super resolution STED imaging, we used a Leica SP8 gated-STED 3x microscope with a 100x 1.4 NA oil immersion objective. To prevent premature photobleaching, we first acquired the entire stack of images of the QB labelled probe (λ_{ex}=575 nm, λ_{ex}=605-650 nm, $\lambda_{depletion}$=775 nm) then followed with the imaging of TO (λ_{ex}=514 nm, λ_{ex}=525-570 nm, $\lambda_{depletion}$=592 nm). All dual color FISH images were deconvoluted with Huygens Professional to maximize image quality.

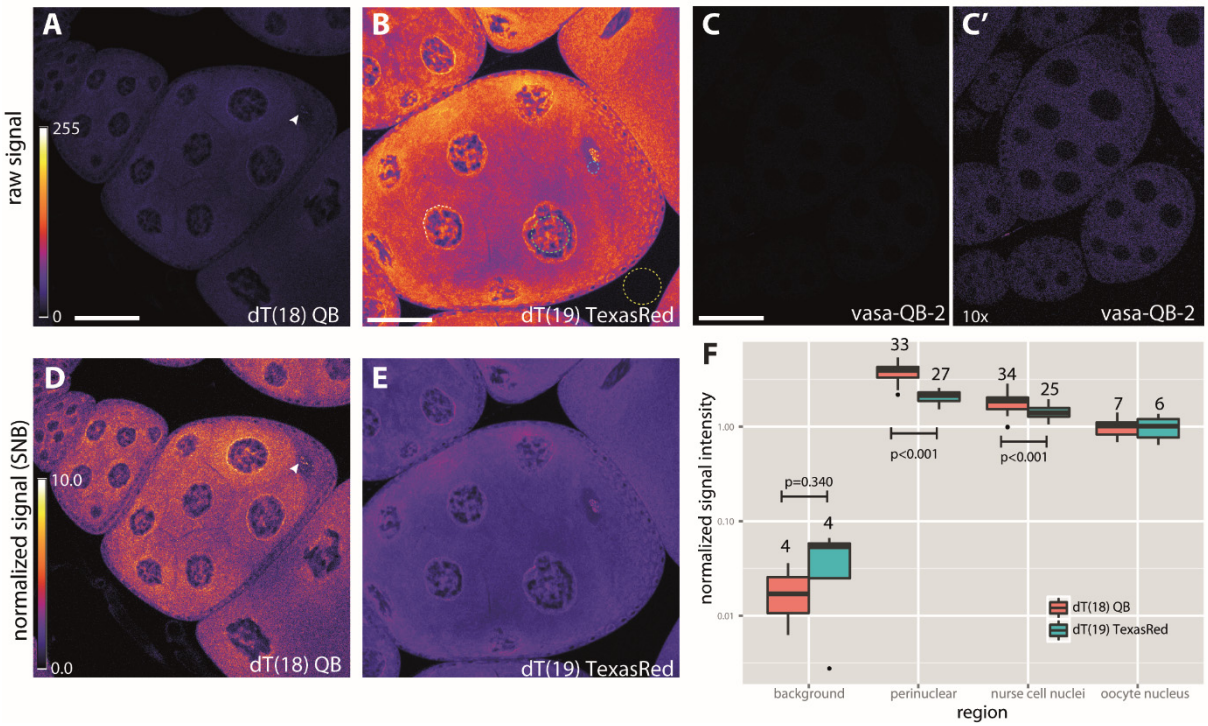


Figure S9: Wash-free FISH of fixed *Drosophila* egg chambers. (A-C′) Raw images of egg chambers labelled with (A) dT(18) QB, (B) dT(19) TexasRed or (C and C′) *Danio rerio* Vasa 2-4 QB. Images were acquired using the same microscope settings. (C′) 10-fold enhanced brightness of (C) Vasa 2-4 QB labelling. Scale bars represent 50 microns (A-C). (D and E) Normalized images of A and B. Normalization was done against the background signal measured within the oocyte nuclei (B, cyan dashed circle). (F) Comparison of normalized signal intensities of dT_{18}-QB and TexasRed-labelled dT_{19} measured in the cell free background (yellow dashed circle, B), within nurse cell nuclei (green dashed circle, B) and in the nurse cell perinuclear region (white dashed polyline, B). Numbers above the boxplots indicate sample size. P values indicate the p values of pairwise Mann Whitney U tests.

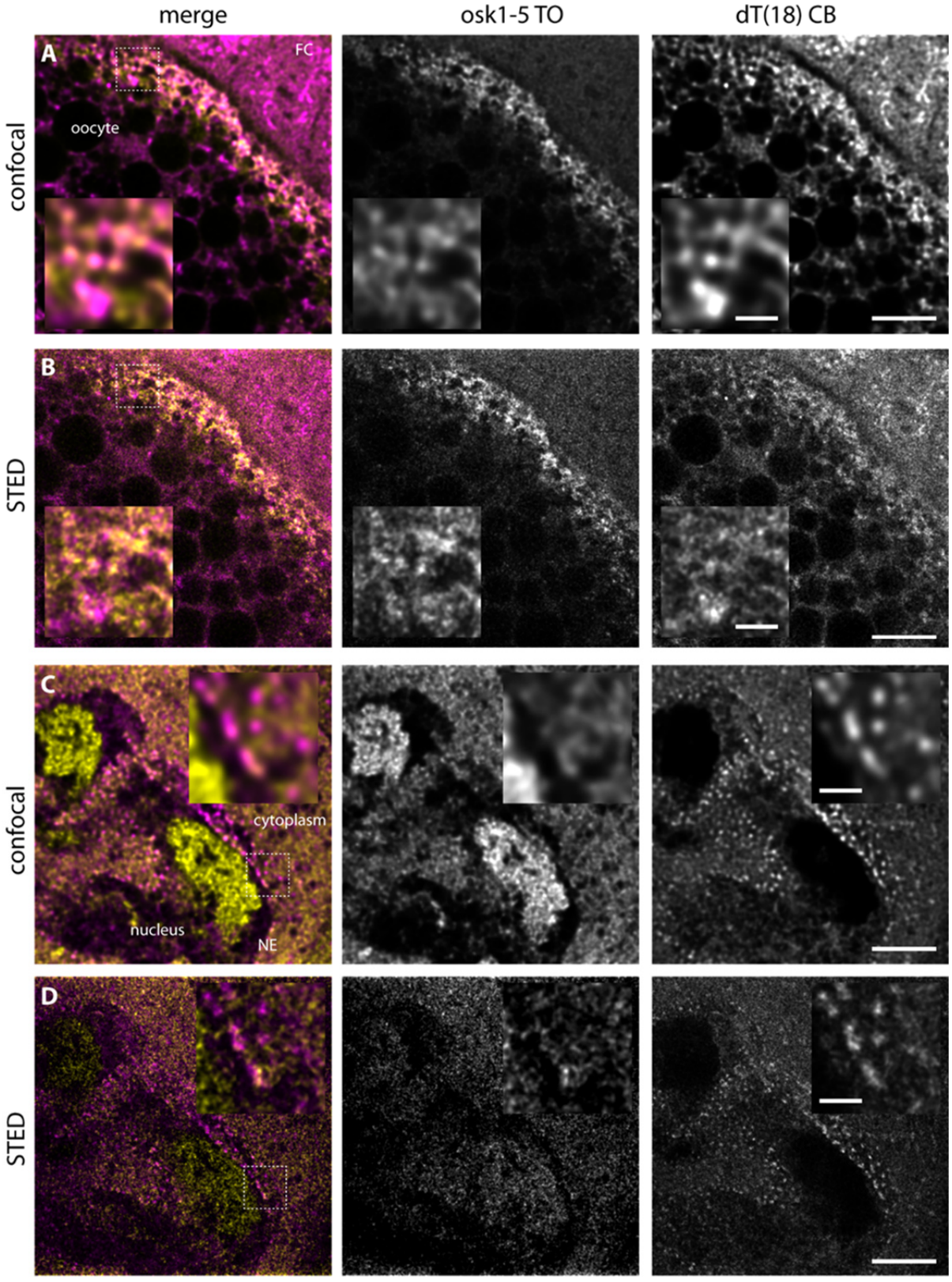
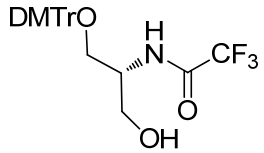


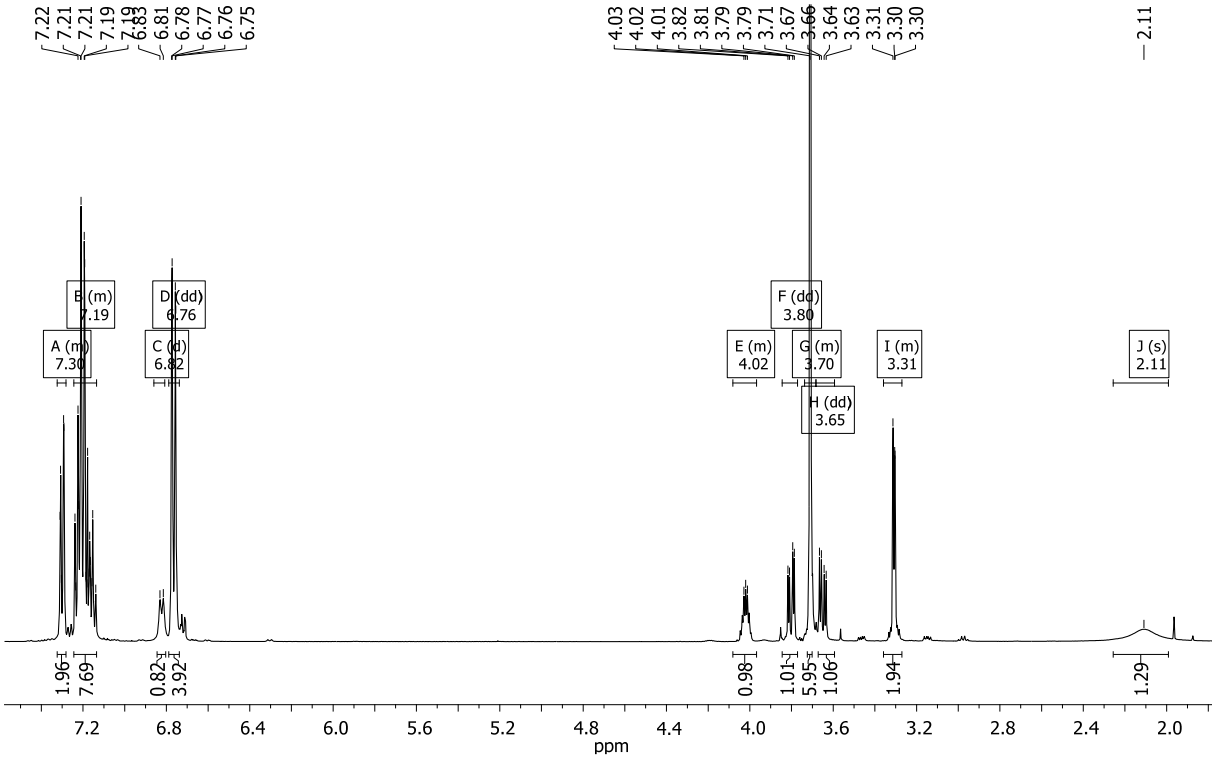
Figure S10: High and super-resolution images of dual colour wash free FISH. Oocyte posterior region (A and B) and nurse cell (C and D) of a stage 10 egg chamber imaged with point scanning confocal (A and C) and STED (B and D) microscopy. FC represent follicle cells (A), NE represent the nuclear envelope (C). Insets are enlarged versions of the image areas within the dashed boxes (A-D). Scale bars in main panels and in insets represent five and one microns, respectively. Intensive signal within the nurse cell nuclei in the TO channel (yellow, C) represent non-specific accumulation of the probe in the nucleolus, which is observed occasionally.

8.) NMR-spectra

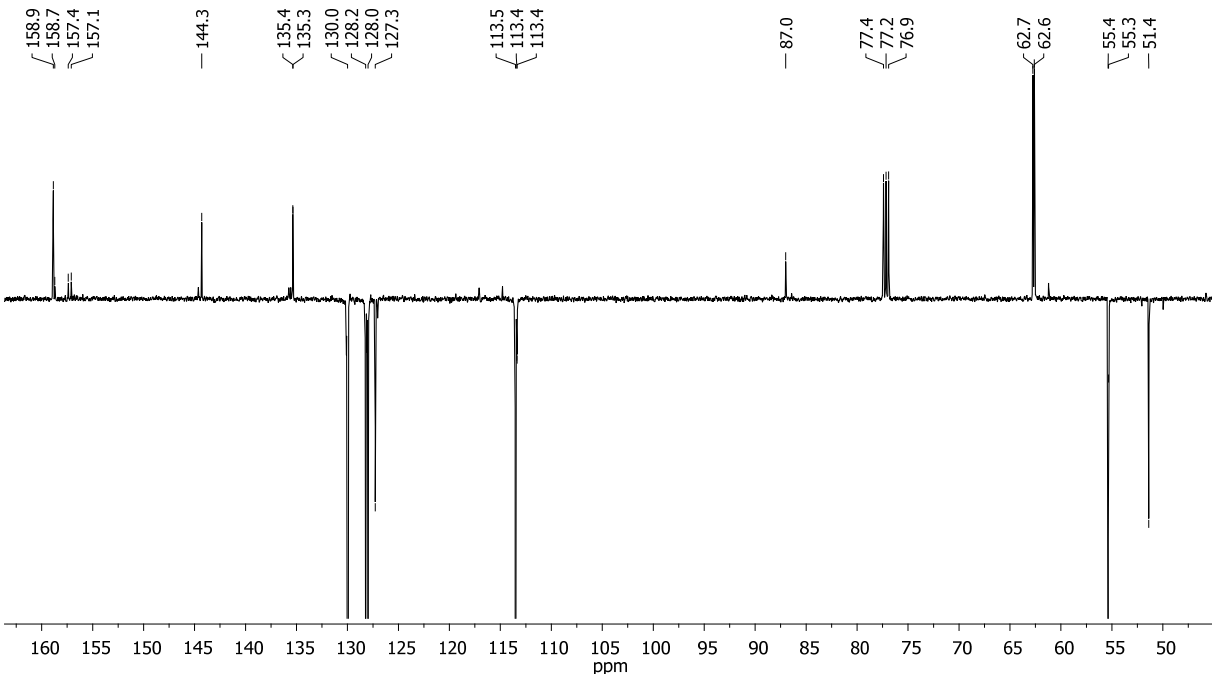
DMTr-Ser(Tfa)-OH (S5)



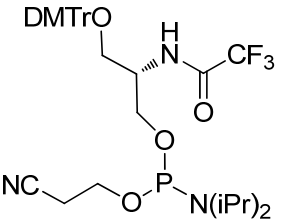
^{1}H-NMR



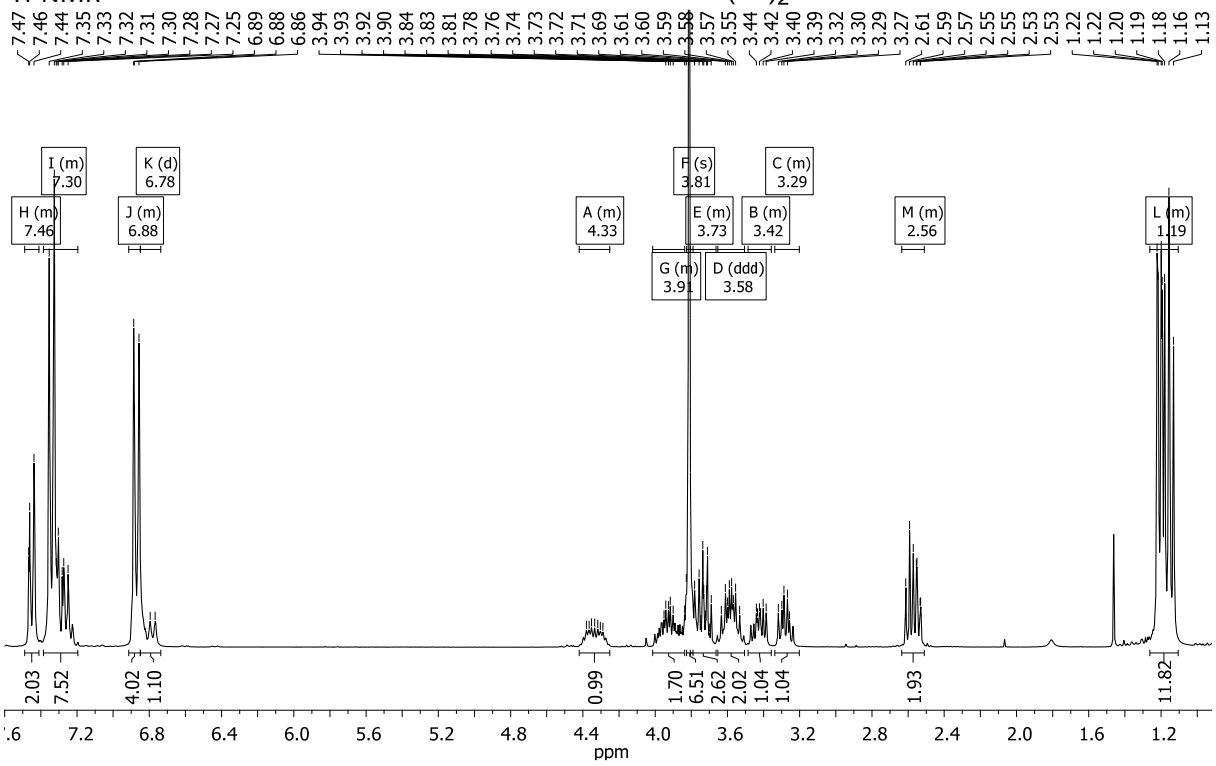
^{13}C-NMR



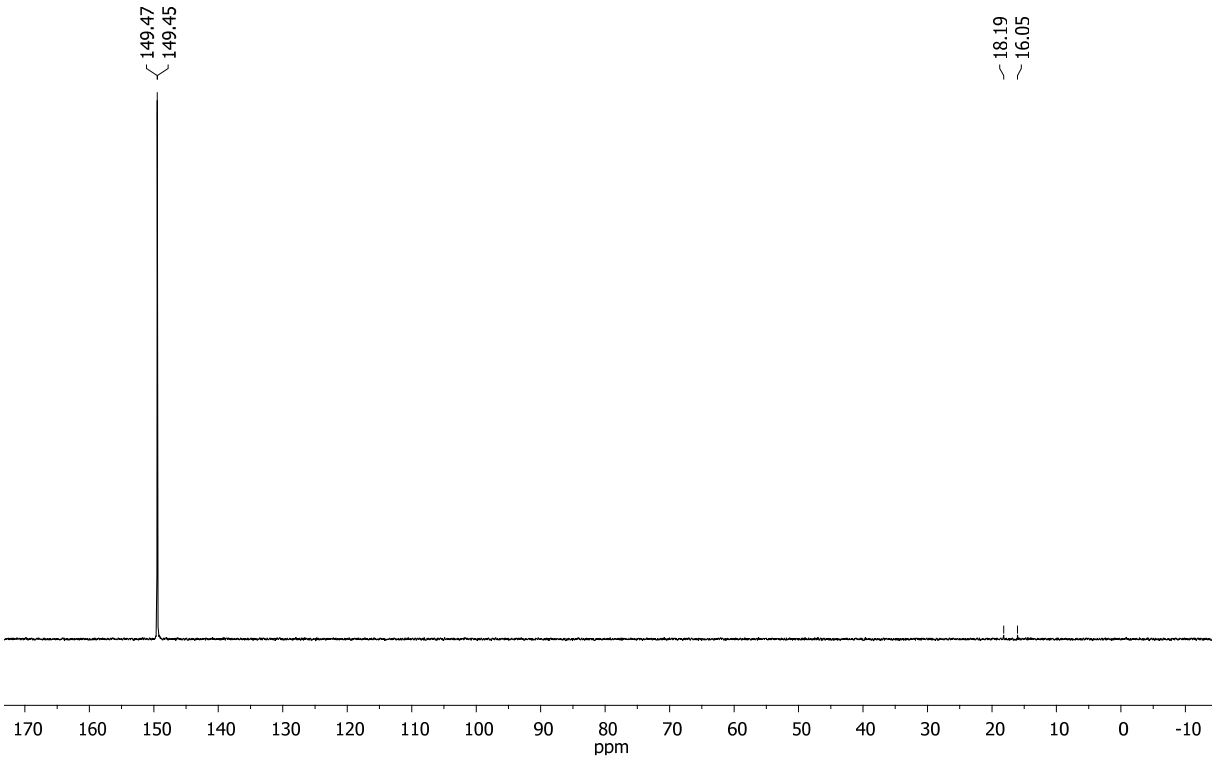
DMTr-Ser(Tfa)-phosphoramidite (S6)



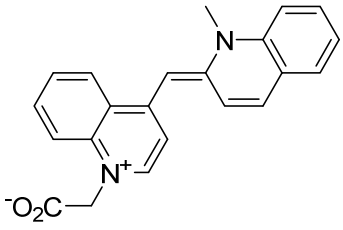
^{1}H-NMR



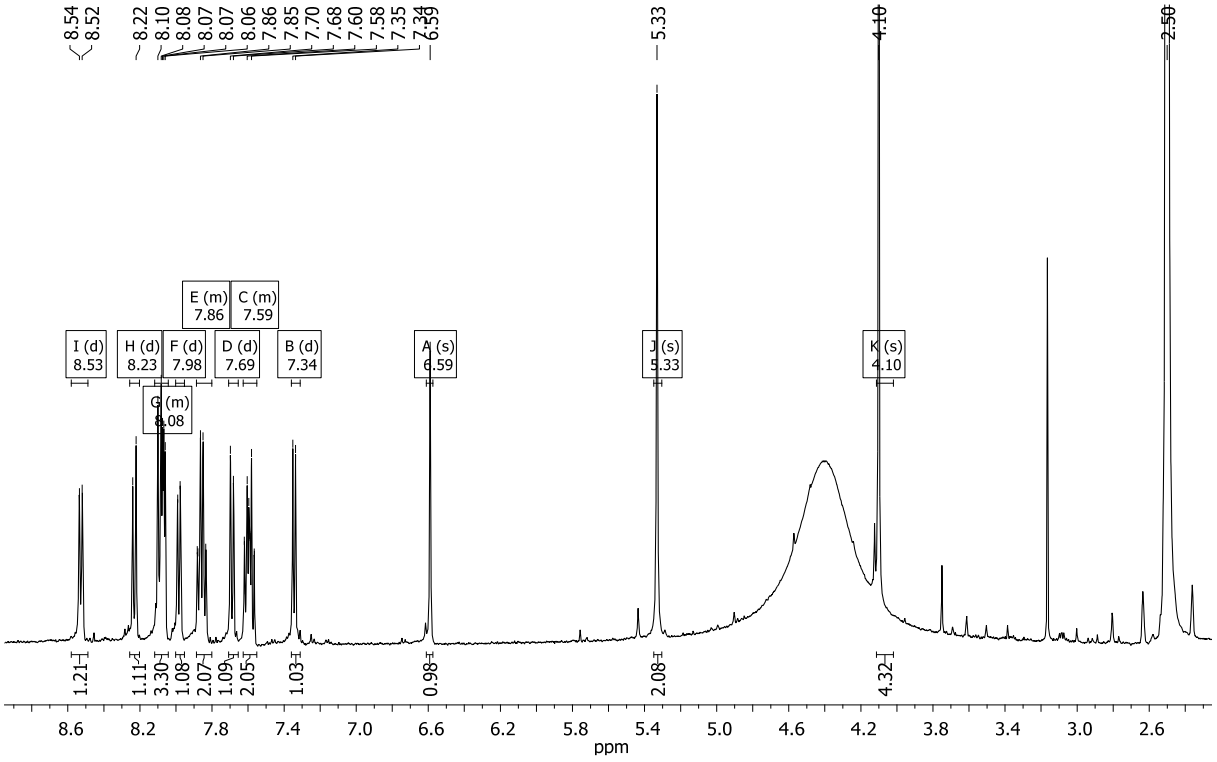
^{31}P-NMR



Quinoline violet (QV, S11)



^{1}H-NMR



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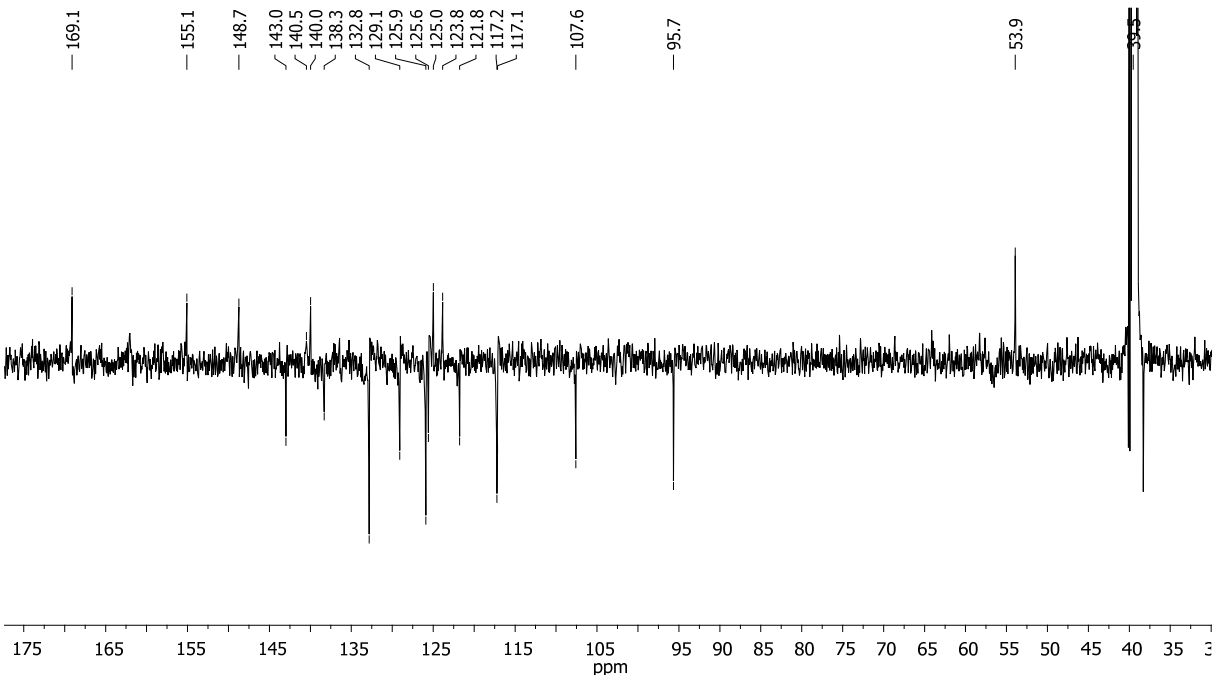
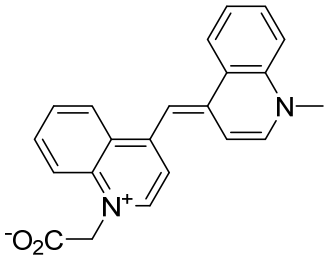
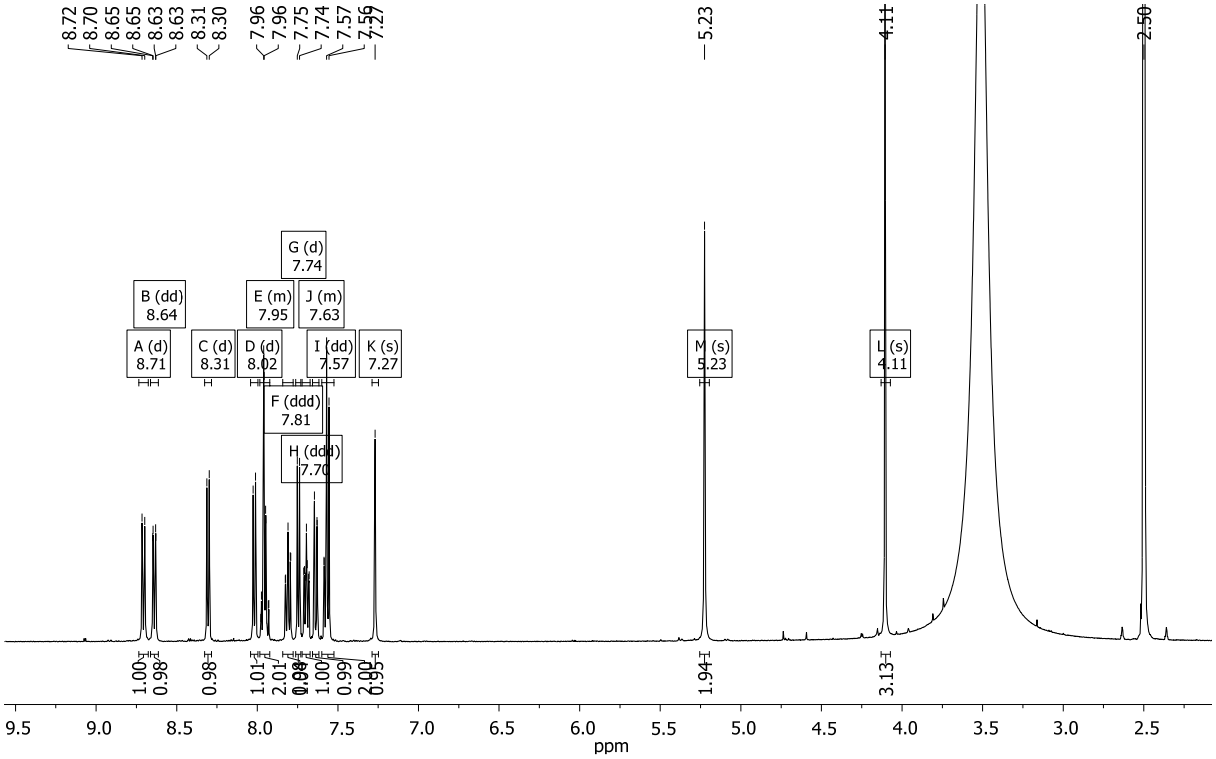


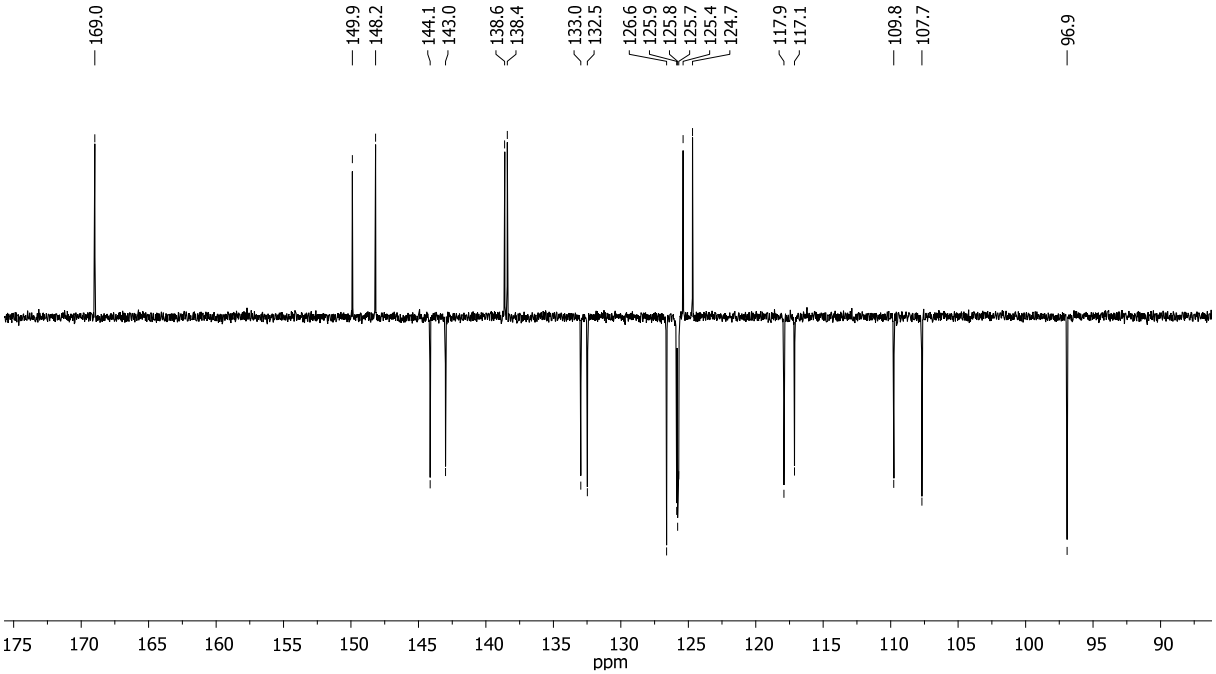
Quinoline Blue (QB, S12)



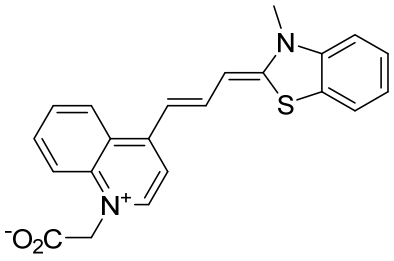
^{1}H-NMR



^{13}C-NMR



Thiazole Red (TR, S15)



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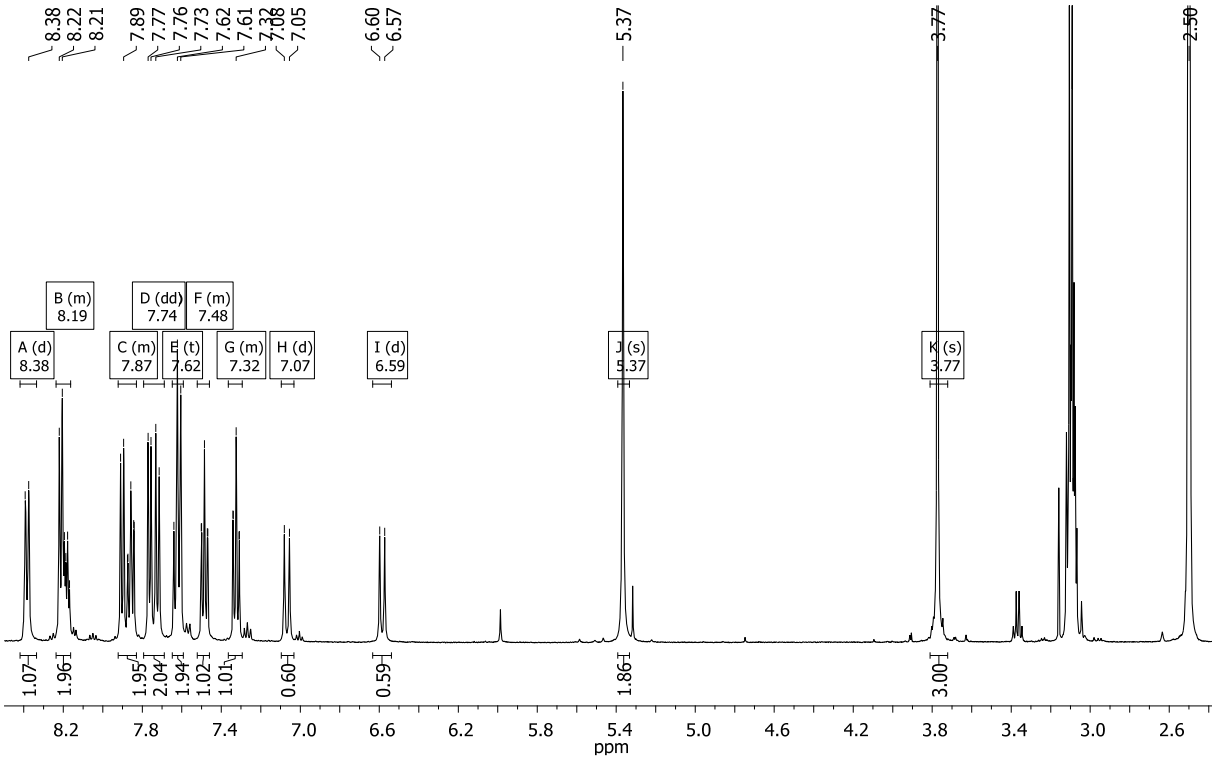

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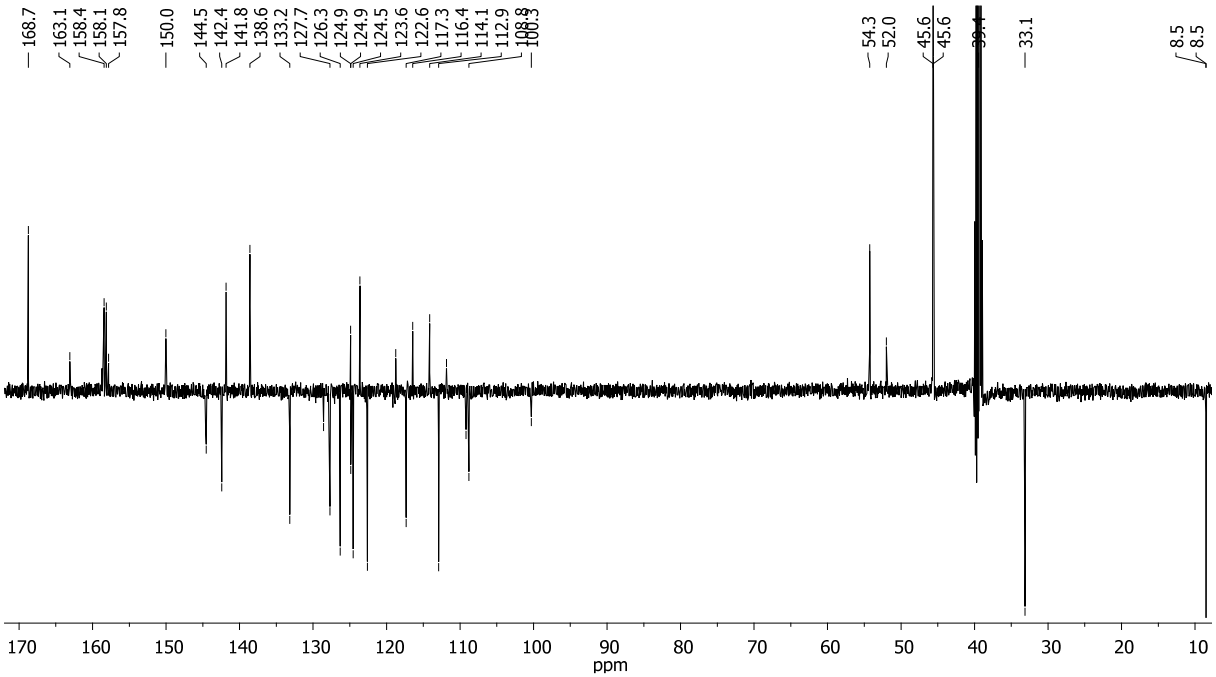
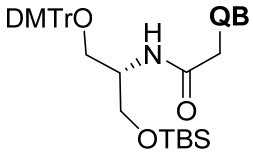


DMTr-Ser(QB)-OTBS (S17)



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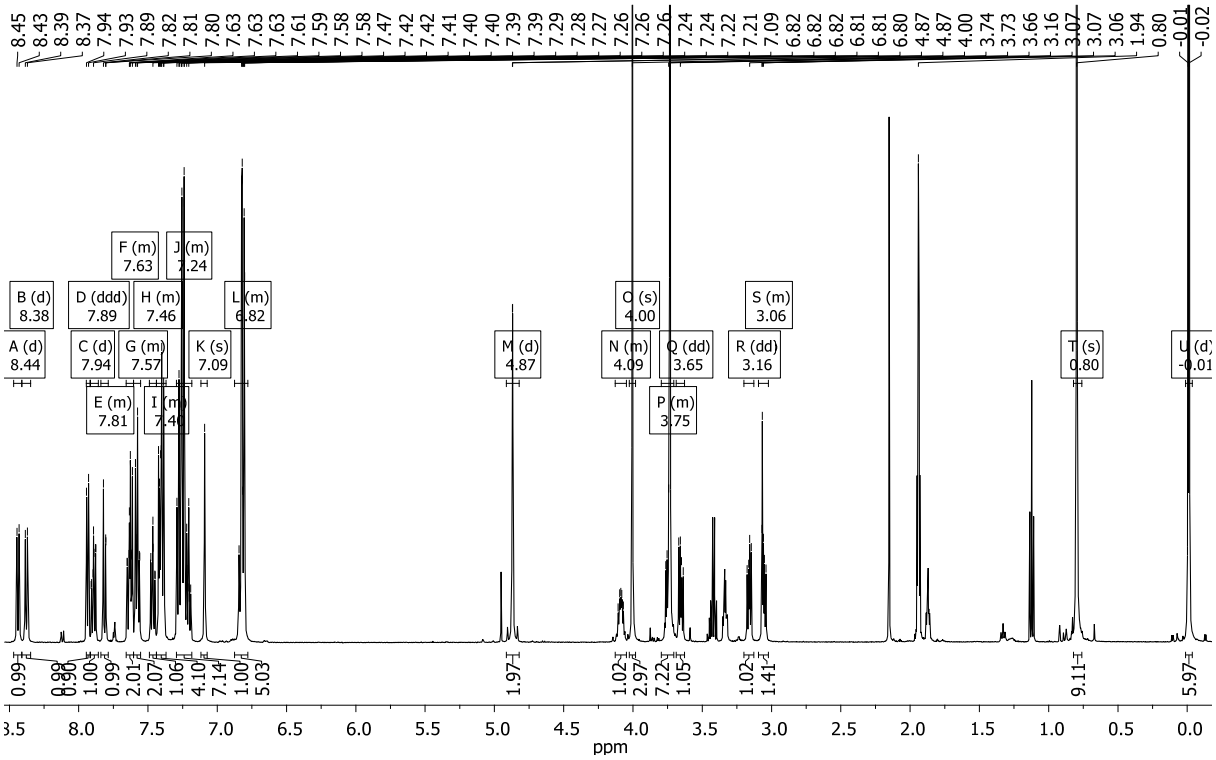

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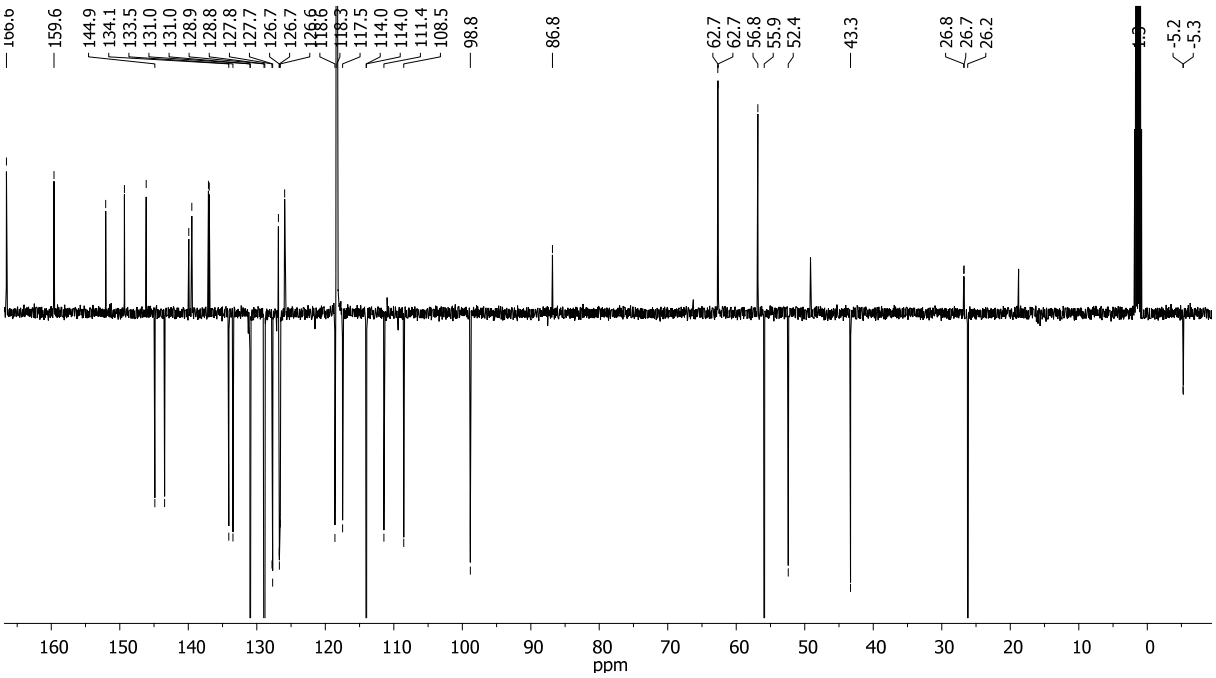
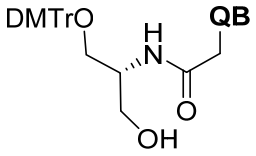
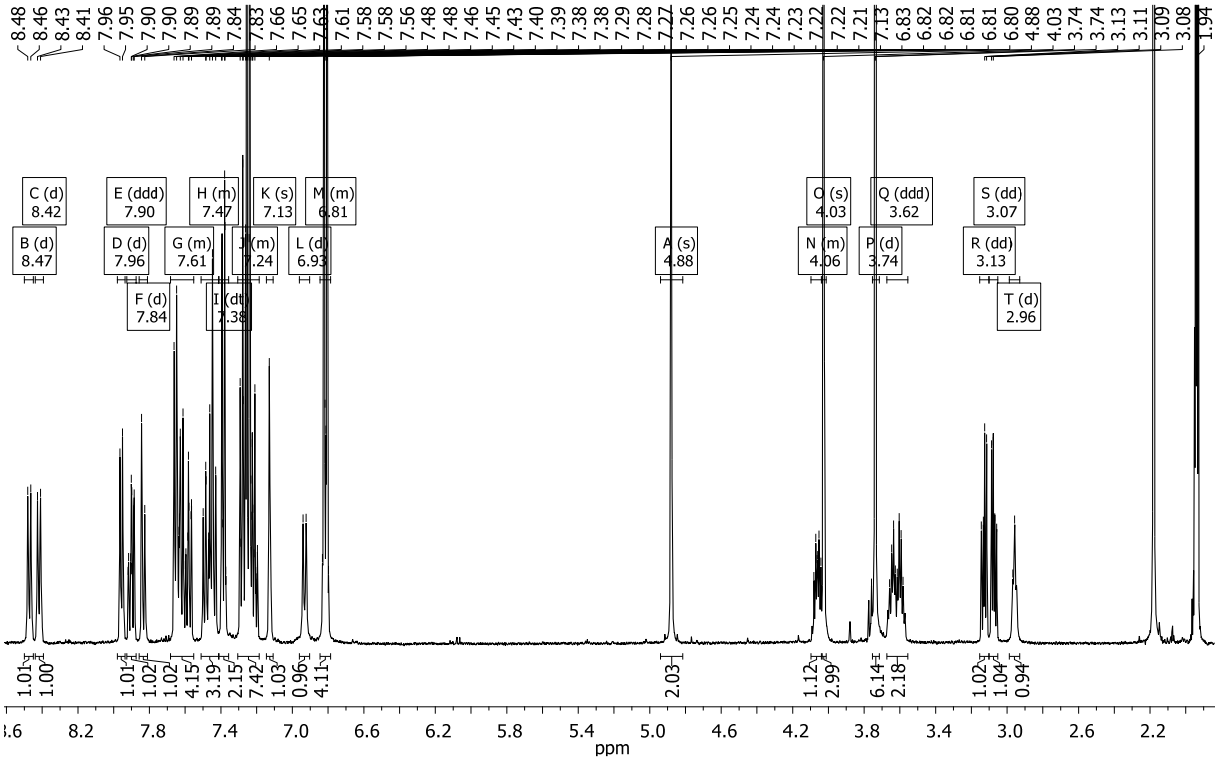


DMTr-Ser(QB)-OH (S18)



^{1}H-NMR



^{13}C-NMR