Supporting Information for

Fluorescent oligonucleotides with bis (prop-2-yn-1-yloxy) butane-1,3-diol scaffold rapidly detect disease associated nucleic acids

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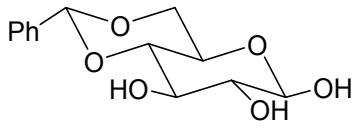
Experimental Procedures

1. Synthesis

General synthetic methods

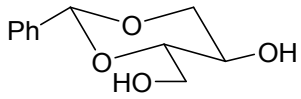
All commercial reagents and solvents were used as received. The reactions were monitored by TLC using Merck silica 60 F_{254} aluminum sheets. The TLC plates were visualized by UV-light at 254 nm and/or by staining by an appropriate staining agent, either vanillin in ethanol and/or 5% sulfuric acid in ethanol, followed by heating. Column chromatography was performed using silica gel (0.040-0.063 mm), Merck. ^{1}H, ^{13}C and ^{31}P NMR were recorded on Bruker Avance III 400 spectrometer at 400.12 MHz, 100.62 MHz and 161.97 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane ($\delta_{H,C}$ 0 ppm) or the solvents (DMSO-d_6 δ_C 39.5 ppm, $CDCl_3$ δ_C 77.16 ppm) The coupling constants (*J*) are reported in Hz. As external standard for ^{31}P NMR spectra, 85% H_3PO_4 was used. 2DNMR spectra (HSQC, COSY) have been used in assigning the ^{1}H and ^{13}C NMR signals. High resolution MS-ESI was recorded on a Bruker microTOF-Q II in a positive ion mode with an accuracy of ±5 ppm. Oligonucleotides were synthesized on an automated DNA synthesizer - PerSpective Bio-systems Expedite 8909. MALDI-TOF mass spectrometry was performed on a Ultraflex II TOF/TOF instrument, Bruker, using 3-hydroxypicolinic acid matrix (10 mg/mL 3-hydroxypicolinic acid, 50mM ammonium citrate in 70% aqueous acetonitrile). IE HPLC was performed using a Merck Hitachi LaChrom instrument equipped with a Dionex DNAPac Pa-100 column (250mm x 4 mm).

Preparation of 4,6-O-benzylidene-D-glucose (2)



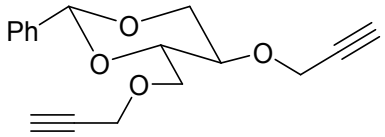
Anhydrous glucose (67.5 g, 374 mmol) and 270 g of dry zinc chloride were stirred in 675 mL of benzaldehyde at room temperature (rt) for 5 h. Then the reaction mixture was cooled to 0 °C and 1350 mL of water were added. The solution was stored at 4 °C for 24 hours. The resulting white precipitate was filtrated, washed with cold water and dried under high vacuum overnight to obtain **2** as a white solid (9 g, 33.5 mmol).
Yield: 9%. ***Rf*** = 0.30 (ethyl acetate).
HRMS-ESI (M+Na$^+$): found m/z: 291.0845, calcd: 291.0845.

Preparation of 1,3-O-benzylidene-L-erythriol (3)



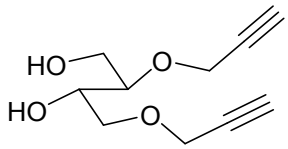
Compound **2** (9 g, 33.5 mmol) was dissolved in 180 mL of water and 67 mL of tetrahydrofuran. Sodium metaperiodate (14.3 g) and sodium hydrogen carbonate (7.4 g) were added and the reaction mixture was stirred at rt for 1 h. Then the solution, that presented a white precipitate, was cooled to 0°C and sodium borohydride (2.7 g) was added. The resulting mixture was stirred for 1 h. The precipitate was filtered and the product was extracted with ethyl acetate. The extract was washed with a solution of sodium thiosulfate and a solution of sodium hydrogen carbonate. The organic fraction was concentrated under reduced pressure and the crude material was purified by column chromatography (40→100% Ethyl acetate in Petroleum ether) to give a white compound **3** (4.9 g, 23.3 mmol).
Yield: 69%. ***Rf*** = 0.40 (ethyl acetate).
HRMS-ESI (M+Na$^+$): found m/z: 233.0795, calcd: 233.0892.
^{1}H NMR (400 MHz, DMSO): δ 7.46-7.43 (m, 2H), 7.38-7.33 (m, 3H), 5.50 (s, 1H), 5.18 (d, *J* = 5.0 Hz, 1H), 4.65 (t, *J* = 5.7 Hz, 1H), 4.10 (dd, *J* = 9.3 Hz, 6.4 Hz, 1H), 4.08 (dd, *J* = 9.3 Hz, 6.4 Hz, 1H), 3.75 (td, *J* = 9.8 Hz, 4.2 Hz, 1H), 3.57-3.46 (m, 3H); **^{13}C NMR** (101 MHz, DMSO): δ 138.3, 128.6, 127.8, 126.3, 100.1, 83.1, 70.7, 60.8.

Preparation of (2R,4S,5R)-2-phenyl-5-(prop-2-yn-1-yloxy)-4-((prop-2-yn-1-yloxy)methyl)-1,3-dioxane (4)



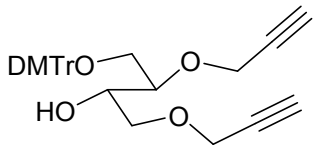
Compound 3 (4.5 g, 21.4 mmol) was dissolved in 112 mL dimethylformamide and 6 g of sodium hydride (60%) was added. The solution was stirred for 1 h under reflux and then was cooled down to rt before adding 13.7 mL propargyl bromide (80%). The solution was left under stirring overnight. The resulted brown precipitate was disolved after addition of 100 mL of ethyl acetate and 100 ml water. The yellowish organic phase collected with two more times of extraction with ethyl acetate was concentrated under reduced pressure and the crude material was purified by silica gel column chromatography (40→100% Ethyl acetate in Petroleum ether) affording compound **4** (4 g, 13.9 mmol) as a light orange oil.
Yield: 65%. ***Rf*** = 0.44 (ethyl acetate).
HRMS-ESI (M+Na$^+$): found m/z: 309.1099, calcd: 309.1103.
^{1}H NMR (400 MHz, DMSO): δ 7.43-7.40 (m, 2H), 7.40-7.36 (m, 3H), 5.56 (s, 1H), 4.46-4.38 (m, 1H), 4.27 (d, *J* = 2.4 Hz, 2H), 4.20 (d, *J* = 2.3 Hz, 2H), 3.85 (m, 1H), 3.76 (dd, *J* = 11.0 Hz, 1.7 Hz, 1H), 3.64 (dd, *J* = 11.0 Hz, 5.7 Hz, 1H), 3.60-3.56 (m, 1H), 3.51 (t, *J* = 2.3 Hz, 1H), 3.44 (t, *J* = 2.3 Hz, 1H), 3.41-3.38 (m, 1H); **^{13}C NMR** (101 MHz, DMSO): δ 137.8, 128.8, 128.0, 126.2, 100.1, 80.3, 80.1, 79.0, 77.4, 77.3, 68.5, 68.2, 68.0, 57.8, 57.1.

Preparation of 2,4-bis(prop-2-yn-1-yloxy)butane-1,3-diol (5)



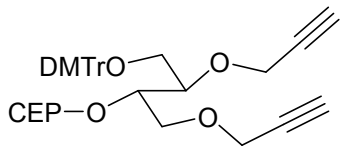
Compound **4** (0.4 g, 1.39 mmol) was dissolved in 12 mL anhydrous dichloromethane and cooled to 0 °C. 3 mL trifluoroacetic acid diluted in 12 mL anhydrous dichloromethane was added drop-wise over 1 h. The reaction mixture was left under stirring for 16 h, The reaction was quenched with 8 mL (2.5 eq) dry pyridine. It was concentrated under reduced pressure and the crude material was purified by flash chromatography (0→10% methanol in dichloromethane) to give a light yellow oil (0.12 g, 0.60 mmol)
Yield: 43%. ***Rf*** = 0.41 (ethyl acetate).
HRMS-ESI (M+Na$^+$): found m/z: 221.0774, calcd: 221.0790.
^{1}H NMR (400 MHz, DMSO): δ 4.91 (dd, *J* = 5.6, 2.9 Hz, 1H), 4.56 (t, *J* = 5.5 Hz, 1H), 4.22 (dd, *J* = 15.5, 2,4 Hz, 2H), 4.15 (d, *J* = 2.4 Hz, 2H), 3.70-3.55-3.65 (m, 4H, H4), 3.50-3.35 (m, 4H); **^{13}C NMR** (101 MHz, DMSO): δ 80.9, 80.4, 80.1, 76.9, 76.5, 71.3, 68.9, 60.3, 57.8, 57.7.

Preparation of 2,4-bis(prop-2-yn-1-yloxy)butane-1-O-dimetoxytrityl-3-ol (6)



Compound **5** (0.12 g, 0.060 mmol) was co-evaporated with anhydrous pyridine (2 x 10 mL) and dissolved in 4 mL anhydrous pyridine. Dimethoxytrityl chloride (0.45 mg) was added and the reaction mixture was stirred at rt for 24 h. Ethanol (99.6%, 2 mL) was added and it was stirred for 15 min and evaporated to dryness under reduced pressure. The crude mixture was dissolved in dichloromethane 20 mL and washed with water (2 x 20 mL) and a solution of saturated sodium hydrogen carbonate (2 x 20 ml). The resulting organic phase was dried over sodium sulfate and concentrated under reduced pressure The crude material was purified by column chromatography (0→30% Ethyl acetate in Petroleum ether) affording compound **6** (0.17 g, 0.034 mmol) as a light yellow oil.
Yield: 57%. ***Rf*** = 0.24 (40% ethyl acetate in petroleum ether).
HRMS-ESI (M+Na$^+$): found: m/z 523.2089 , calcd: 523.2100.
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46-7.43 (m, 2H), 7.34 (dt, J = 8.9 Hz, 3,1 Hz 4H), 7.30 – 7.20 (m, 3H), 6.83 (dt, *J* = 8.9 Hz, 2.6 Hz, 4H), 4.33 (dd, *J* = 15.7 Hz, 2.4 Hz, 1H), 4.25 (dd, *J* = 15.7 Hz, 2.4 Hz, 1H), 4.16 (d, *J* = 2.4 Hz, 2H), 3.95 (td, *J* = 6.1 Hz, 3.7 Hz, 1H), 3.78 (s, 6H), 3.75 – 3.71 (m, 1H), 3.64 (dd, *J* = 9.8 Hz, 3.8 Hz, 1H), 3.59 (dd, *J* = 9.8 Hz, 6.0 Hz, 1H), 3.40 (dd, *J* = 10.3 Hz, 3.9 Hz, 1H), 3.29 (d, *J* = 10.3 Hz, 5.3 Hz, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 158.5, 144.8, 135.9, 135.9, 130.1, 128.2, 127.9, 126.8, 113.2, 86.5, 80.0, 79.5, 78.0, 77.3, 77.0, 76.7, 74.7, 74.4, 70.8, 70.7, 63.2, 58.6, 57.9, 55.2.

Preparation of 4-(bis(4-methoxyphenyl)(phenyl)methoxy)-1,3-bis(prop-2-yn-1-yloxy)butan-2-yl(2-cyanoethyl) diisopropylphosphoramidite (7)

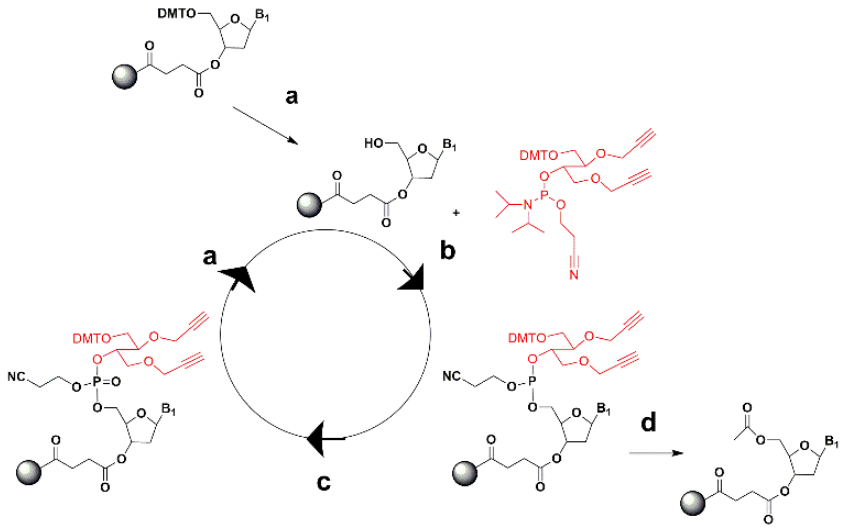


Compound **6** (0.17 g, 0.034 mmol) was coevaporated with dry dichloroethane (2x10 mL) and dissolved in anhydrous dichloromethane 16 mL. Diisopropylammonium tetrazolide (60 mg) and 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphoroamidite (110 µL) were added and the reaction mixture was stirred under argon for 16 h. The mixture was concentrated under reduced pressure and the crude material was purified by flash column chromatography (20→50% ethyl acetate in petroleum ether) to give compound **7** (0.2 g, 0.028 mmol) as a light yellow oil.
Yield: 82%. ***Rf*** = 0.71 (50% ethyl acetate in petroleum ether).
HRMS-ESI (M+Na$^+$): found: m/z: 723.3170 , calcd: 723.3200.
^{31}P NMR (162 MHz, $CDCl_3$): δ 150.15, 150.03.

Oligonucleotide synthesis
Oligonucleotides K1 and K2 were synthesized on automated DNA synthesizer PerSpective Bio-systems Expedite 8909. The synthesis was done using standard protocol in a DMT-ON mode, scale of 1.0 µmol on 500 Å CPG support (Scheme S1). LNA monomers and new bisalkyne nucleic acid scaffold were incorporated using hand-coupling procedure. These reagents were dissolved in acetonitrile and coupled for 18 min using 1*H*-tetrazole as an activator. Coupling efficiencies were measured using absorbance of the dimethoxytrityl cation realized after each coupling. Cleavage of the oligonucleotides from the CPG solid support was performed with 30% aqueous ammonia solution at 55 °C, overnight. Detritylation of the oligonucleotides was done with 80% acetic acid, 30 min, followed by addition of water, sodium acetate 3M, sodium perchlorate 5M and precipitation with cold acetone (-20 °C, 10 min). Work up was finished by washing two times with cold acetone. The identity and purity of ONs were verified by MALDI-TOF mass spectrometry and IE HPLC, respectively.

Bioconjugation
The oligonucleotide-dye conjugates (ODCs) were synthetized by CuAAC "click" reaction between the K1/K2 oligonucleotide modified with the bisalkyne nucleic acid scaffold and azide reagents: perylene, 5JOE and phenylethynylpyrene (Scheme 2). 10 nmol Oligonucleotide was mixed with 1 M triethylammonium acetate buffer, 200/100 nmol azide dye for K1 and K2, respectively, 10 µL 10 mM Cu (II):TBTA and 4 µL 50 mM ascorbic acid solution in a final volume of 200 µL, DMSO:MQ water, 0.8:1.2. The reaction mixture was deaerated, heated on 75 °C for 10 min, placed in a microwave reactor for 45 min, 60 °C and afterwards kept at rt overnight. The products were purified on a Illustra NAP-5 column (GE Healthcare), using the protocol from the manufacturer. The obtained solution was dried under N_2 flow and dissolved in Mili-Q water. Concentration of ODCs was calculated by measuring the absorbance at 260 nm. The products were analyzed by MALDI-TOF mass spectrometry and IE HPLC.

Scheme S1. Oligonucleotide synthesis: main steps and coupling of the bisalkyne scaffold



*Reagents and conditions for solid-phase oligonucleotide synthesis: (a) CCl_3COOH, DCM (3/100, v/v); (b) 1H-tetrazole, CH_3CN, DCM 18 min; (c) THF, H_2O, pyridine, I_2, (90.54/9.05/0.41/0.43, v/v/v/v); (d) $(CH_3CO)_2O$, THF (Cap A, 9.1/90.9, v/v), THF, $C_4H_6N_2$, pyridine, (Cap B, 8/1/1, v/v/v).

Table S1. Commercial DNA and RNA targets used in this study*

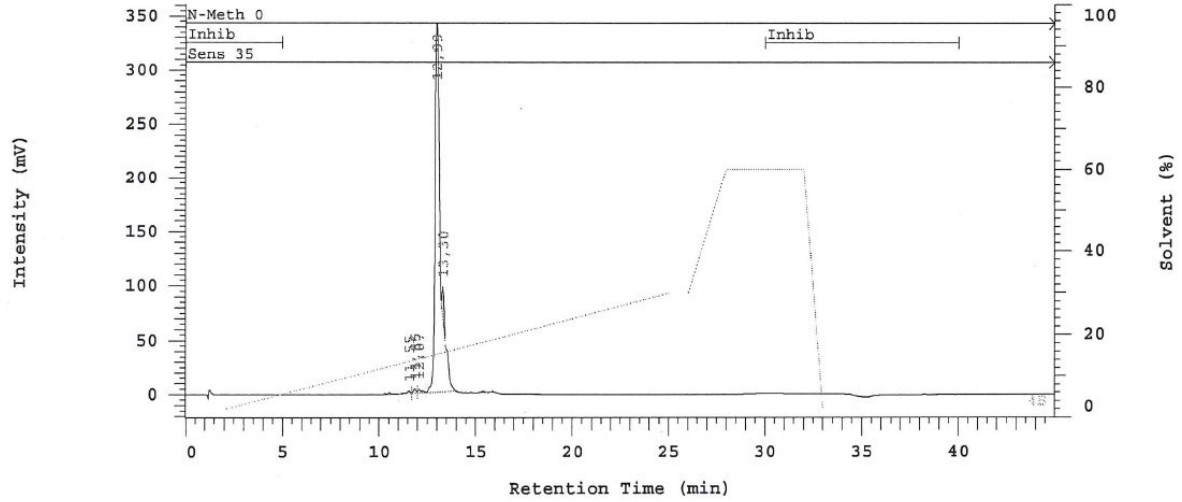
#	Sequence
DT1sh	5'- AGC TAC AGA GAA ATC-3'
DT2sh	5'- AGC TAC AGT GAA ATC-3'
DT3A	5'- AGC TAC AAA GAA ATC-3
DT3U	5'- AGC TAC AUA GAA ATC-3'
DT3C	5'- AGC TAC ACA GAA ATC-3'
RT1sh	5'- r(AGC UAC AGA GAA AUC)-3'
RT2sh	5'- r(AGC UAC AGU GAA AUC)-3'
RT3A	5'- r(AGC UAC AAA GAA AUC)-3
RT3U	5'- r(AGC UAC AUA GAA AUC)-3'
RT3C	5'-r(AGC UAC ACA GAA AUC)-3'

* Mismatched nucleotides are shown in red.

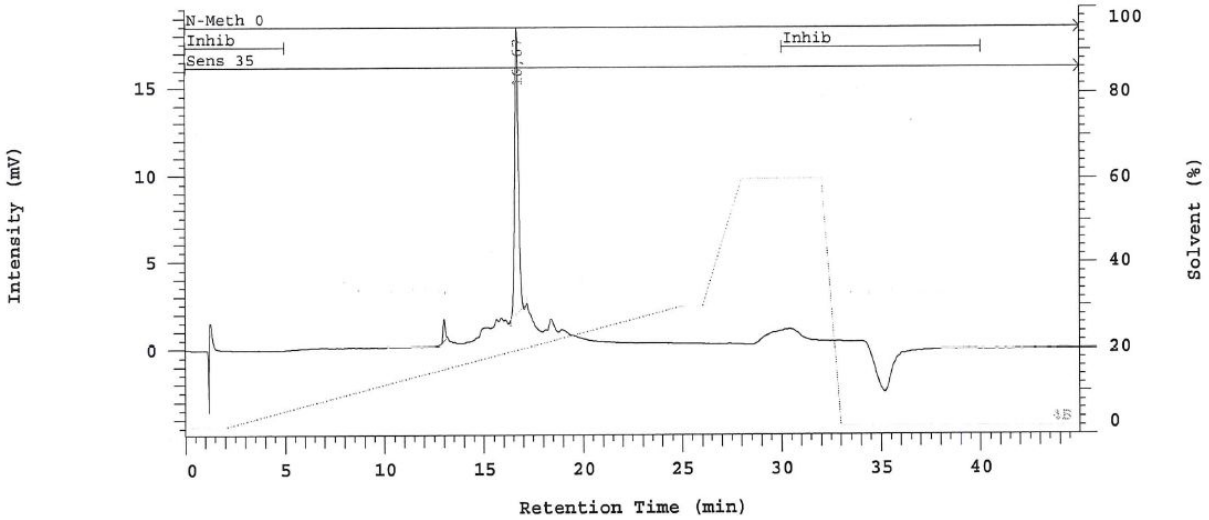
2. Characterization of oligonucleotide precursors and oligonucleotide-dye conjugates

Figure S1. Representative IC HPLC of the bisalkyne-modified oligonucleotides and ODCs

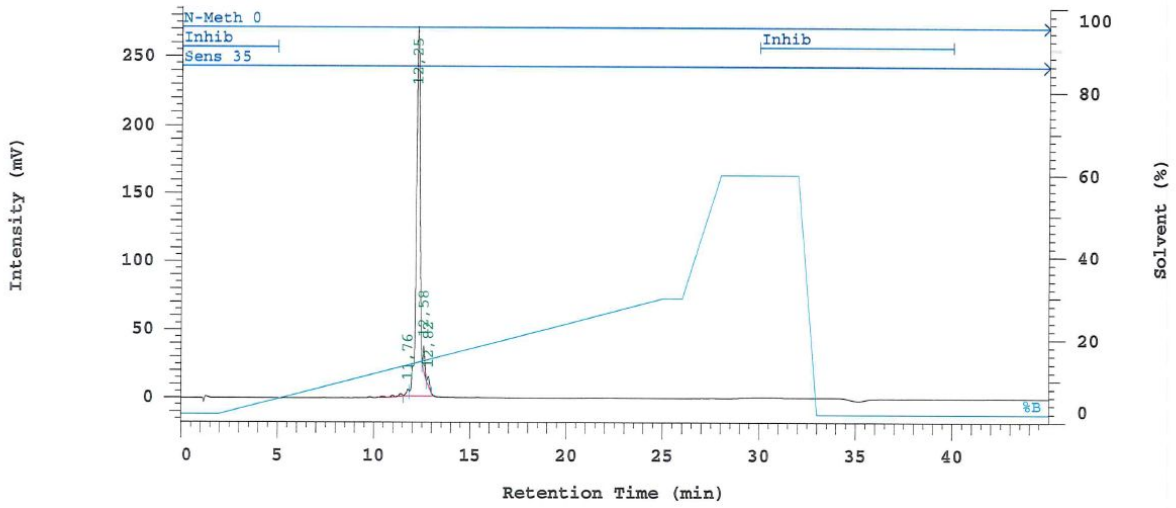
K1



K1-Per



K2


N-Meth 0
Inhib
Sens 35
Inhib
12,25
11,76
12,82
12,58
%B
Intensity (mV)
Solvent (%)
Retention Time (min)

K2-Per

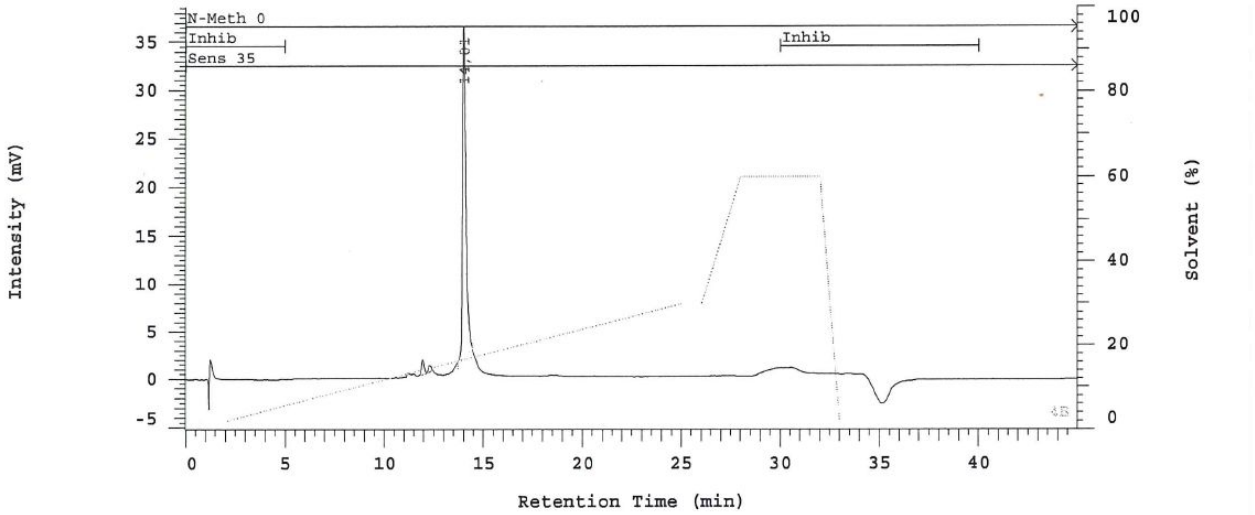
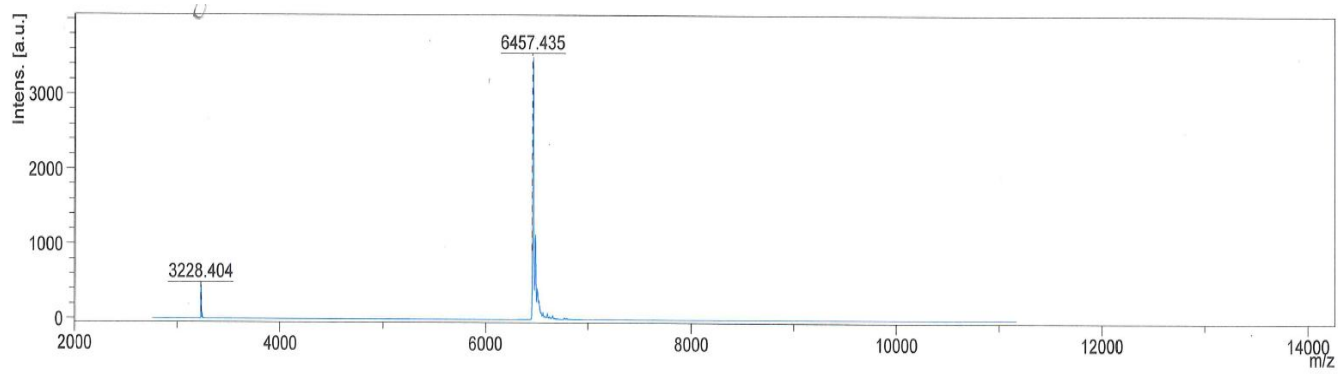
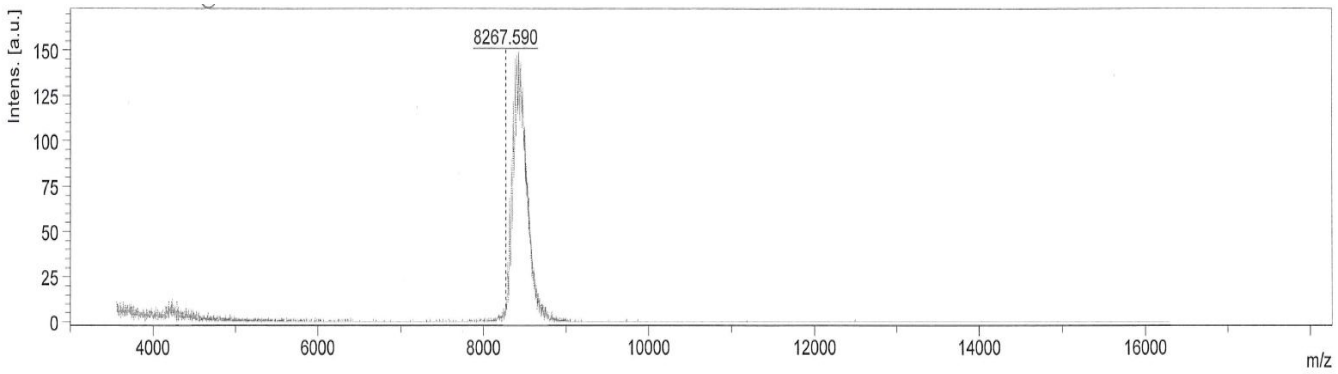

N-Meth 0
Inhib
Sens 35
Inhib
14,61
45
Intensity (mV)
Solvent (%)
Retention Time (min)

Figure S2. Representative Maldi-TOF MS of the bisalkyne modified oligonucleotides and ODCs

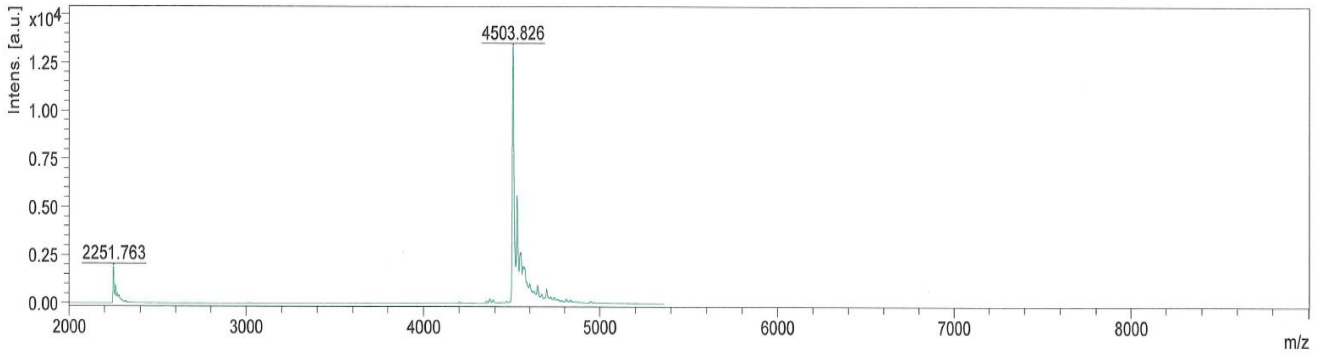
K1



K1-Per



K2



K2-Per

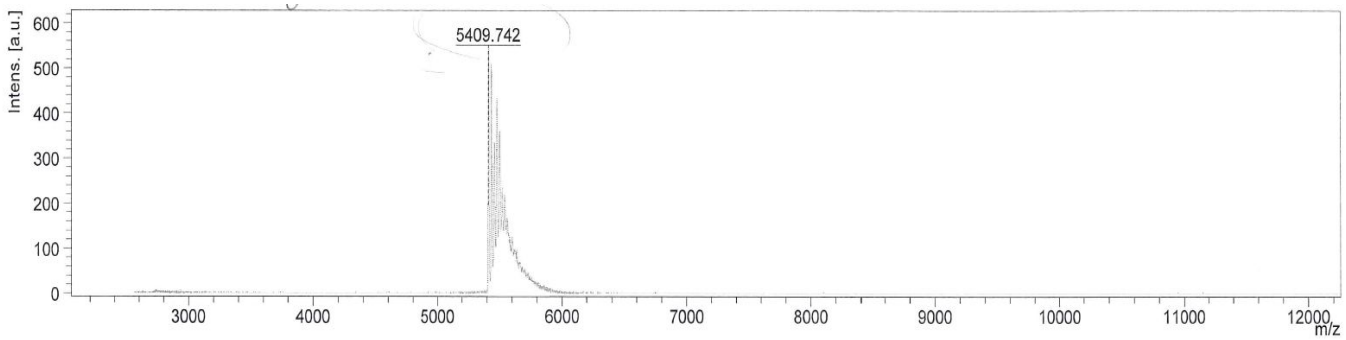


Table S2. Oligonucleotide dyes conjugates MALDI results

#	Mw calc.	Mw exp.
K1-Per	8267.0	8267.6
K1-5JOE	8806.2	8805.9
K1-PEP	8635.4	8637.3
K2-Per	5408.0	5409.7
K2-5JOE	5677.6	5677.8
K2-PEP	5592.2	5595.1

3. Thermal denaturation results (T_m) and representative curves for modified duplexes

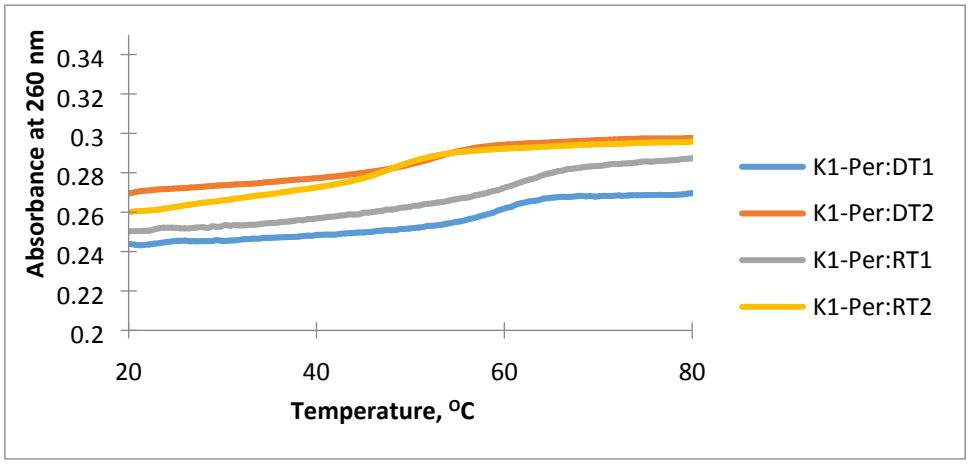
Table S3. T_m values*

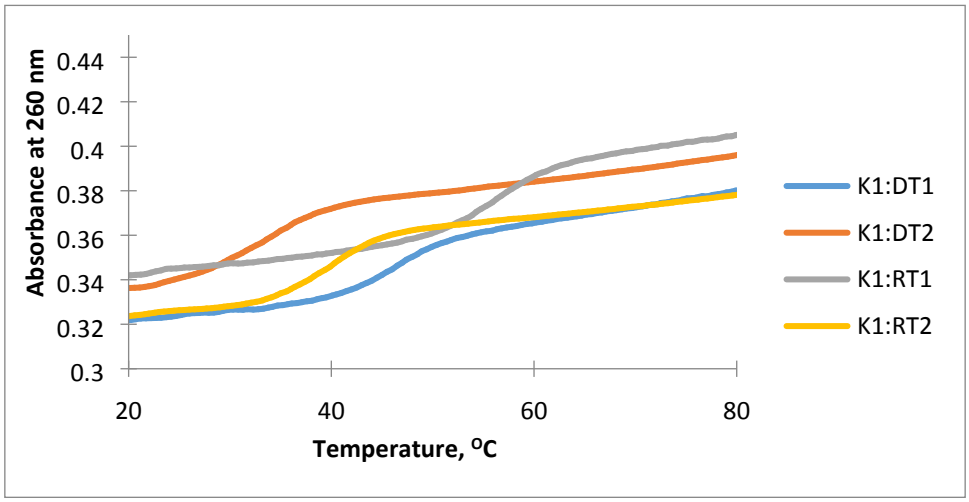
Probe/Target	DT1	DT2	RT1	RT2
K1	46.6	33.8	56.2	40.1
K1-Per	59.0	49.9	61.5	45.9
K1-5JOE	nct	nct	nct	nct
K1-PEP	nct	nct	nct	nct
RK1	62.7	53.4	67.3	55.2
K2	38.1	37.1	39.7	35.7
K2-Per	49.3	49.1	46.2	45.2
K2-5JOE	nct	nct	nct	nct
K2-PEP	nct	nct	nct	nct
RK2	49.5	42.8	52.2	41.9

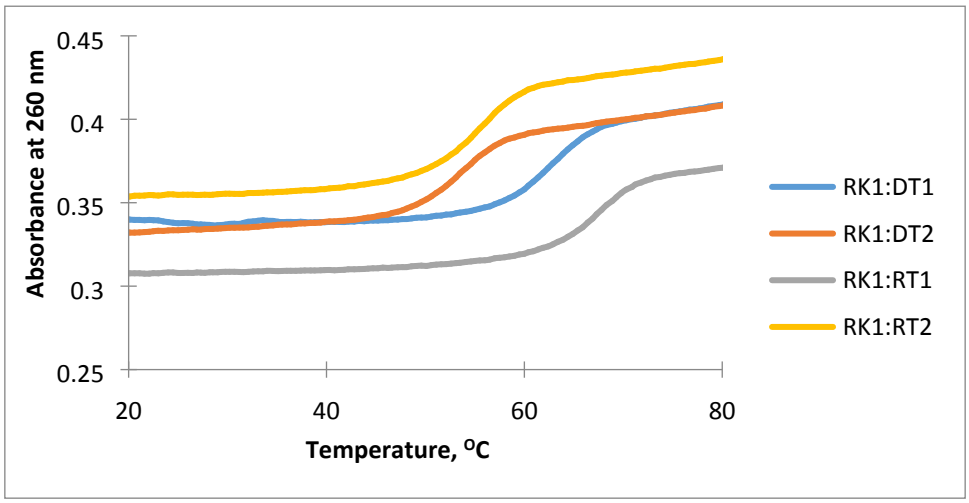
Probe/Target	DT1sh	DT2sh	DT3A	DT3U	DT3C	RT1sh	RT2sh	RT3A	RT3U	RT3C
K2	34.6	32.5	25.6	26.6	25.2	36.9	31.9	24.2	23.6	22.9
K2-Per	49.8	49.4	nct	nct	nct	42.5	43.1	nct	nct	nct
K2-5JOE	nct	nct	nct	nct	nct	nct	nct	nct	nct	nct
K2-PEP	nct	nct	nct	nct	nct	nct	nct	nct	nct	nct
RK2	48.6	39.7	33.6	35.6	32.6	50.6	39.6	35.4	34.0	32.4

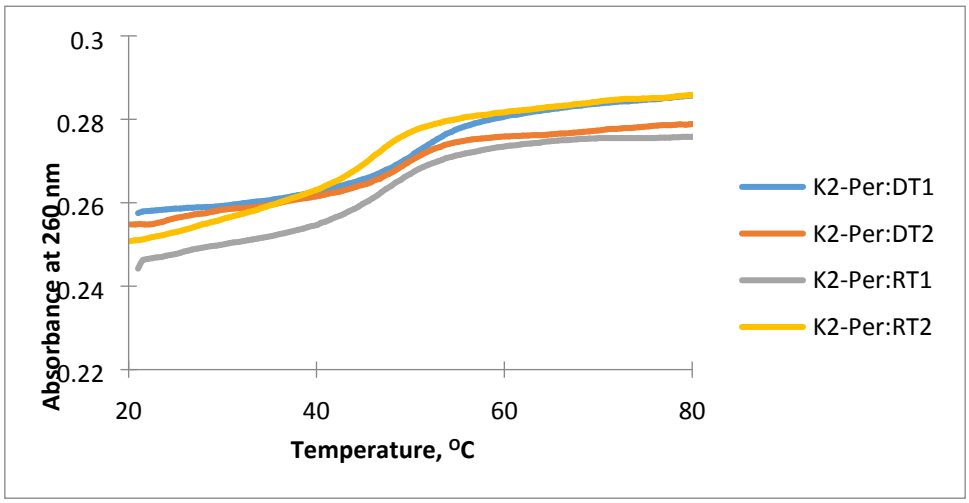
* nct = no clear transition

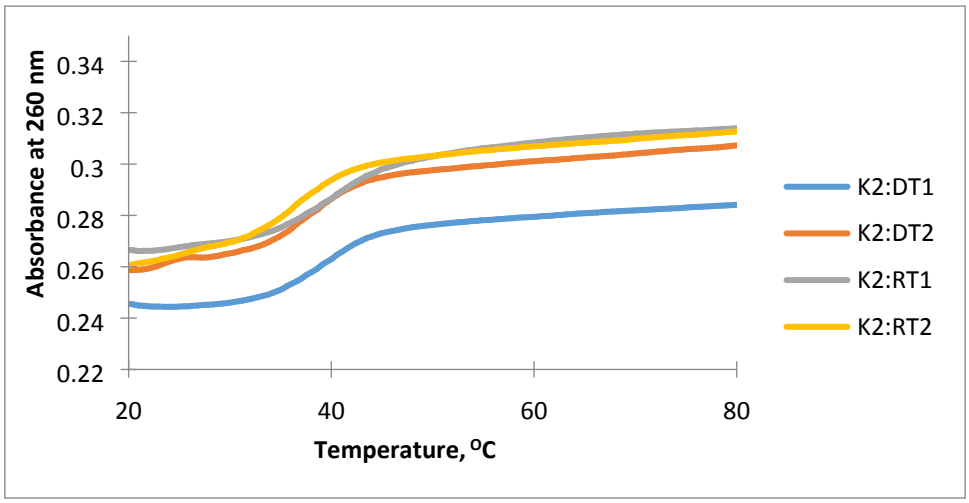
Figure S3. Representative T_m graphs

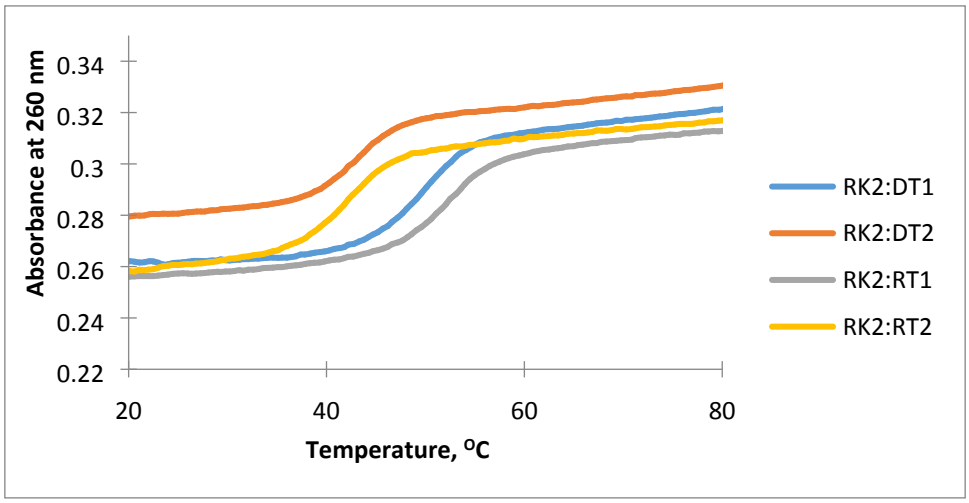






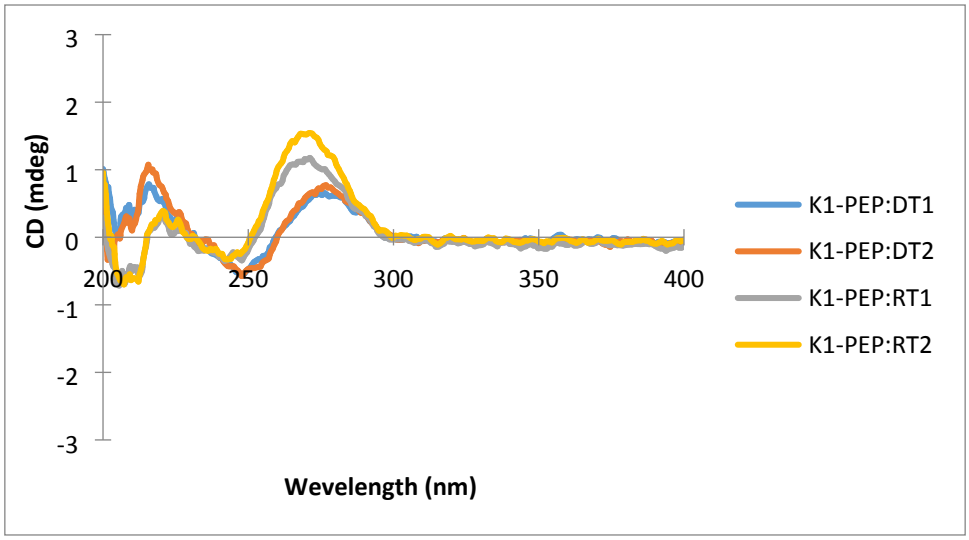

0.3
0.28
0.26
0.24
0.22
Absorbance at 260 nm
20
40
60
80
Temperature, °C
K2-Per:DT1
K2-Per:DT2
K2-Per:RT1
K2-Per:RT2

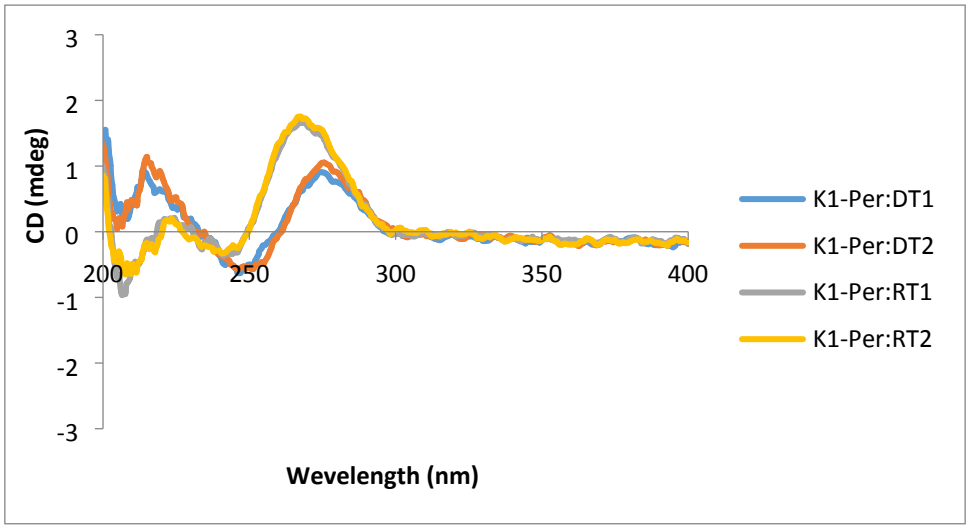

0.34
0.32
0.3
0.28
0.26
0.24
0.22
Absorbance at 260 nm
20
40
60
80
Temperature, °C
K2:DT1
K2:DT2
K2:RT1
K2:RT2

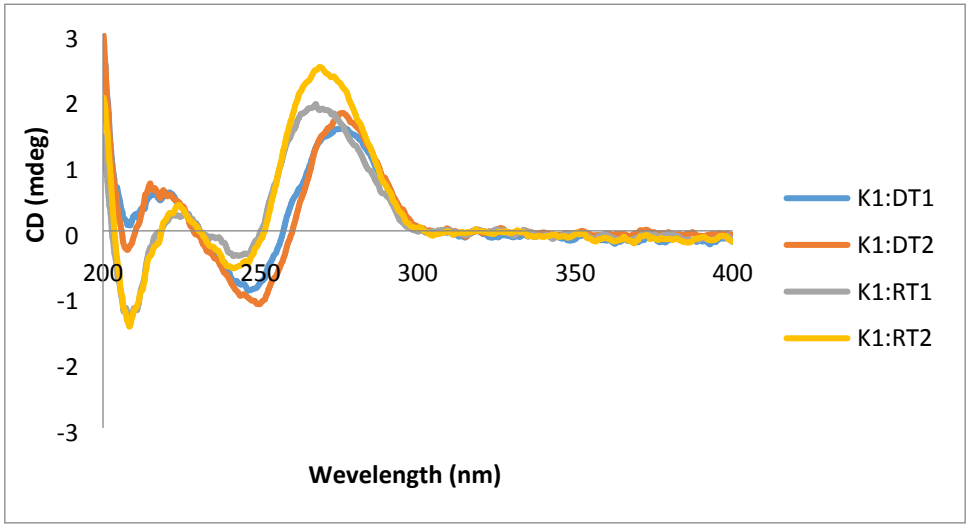

0.34
0.32
0.3
0.28
0.26
0.24
0.22
Absorbance at 260 nm
20
40
60
80
Temperature, °C
RK2:DT1
RK2:DT2
RK2:RT1
RK2:RT2

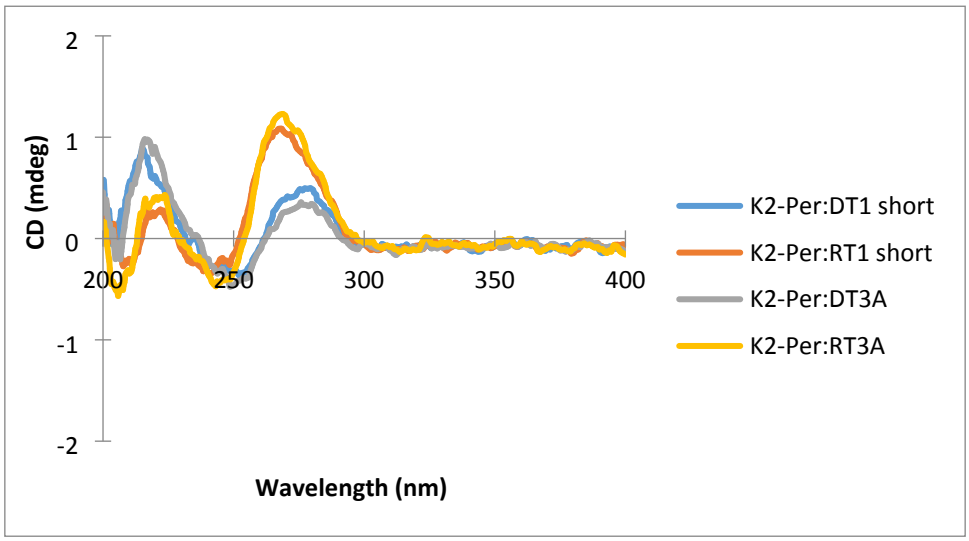
4. Circular Dichroism (CD) results and representative graphs

Figure S4. Representative CD graphs









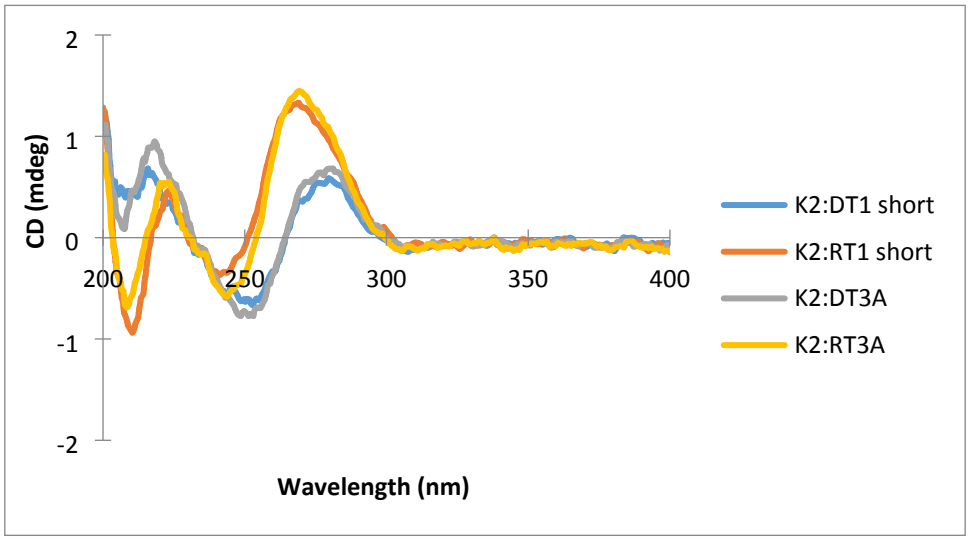


Table S4. CD results: numerical intensities at certain wavelengths.

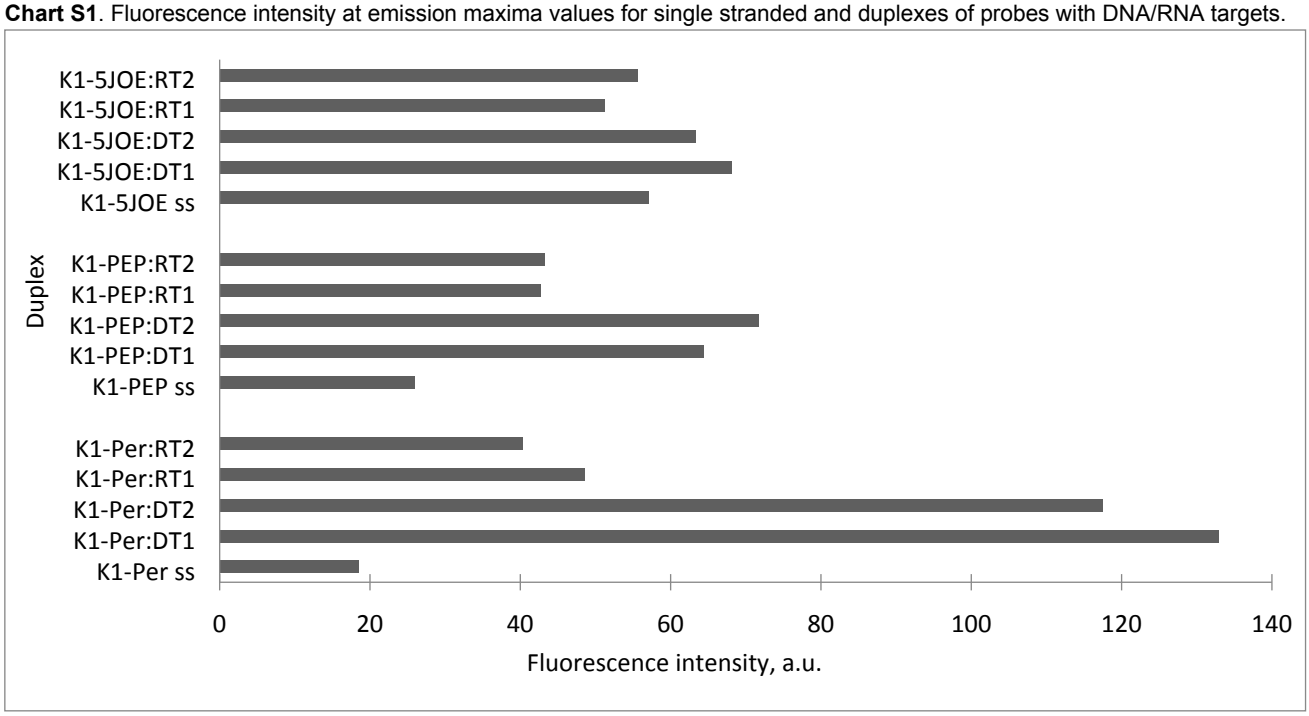
Probe	Target: DNA					RNA				
	Wavelength, nm:									
	205	220	245	275	**SUM**	210nm	225nm	240nm	265nm	**SUM**
RK1	0.00	0.25	-1.25	1,25	**0.25**	-2.25	0.00	-0.75	2.25	**-0.75**
K1	-0.25	0.50	-1.25	1.75	**0.30**	-1.50	0.25	-0.50	2.00	**0.25**
K1-Per	0.00	1.00	-0.5	1.00	**1.50**	-0.75	0.25	-0.25	1.75	**1.00**
K1-PEP	0.00	1.00	-0.5	0.75	**1.25**	-0.5	0.5	-0.50	1.25	**0.75**
K1-5JOE	0.00	0.75	-0.75	1.00	**1.00**	-1	0.25	-0.25	1.50	**0.50**

Table S5. CD results, numerical intensities at certain wavelength

Probe	Target: DNA					RNA				
	Wavelength, nm:									
	210	220	245	275	**SUM**	210	225	240	270	**SUM**
RK2	0.00	0.50	-0.75	0.75	**0.50**	-1.50	0.00	-0.25	1.50	**-0.25**
K2	0.25	0.75	-0.75	0.75	**1.00**	-0.75	0.50	-0.50	1.50	**0.75**
K2-Per	-0.25	1.00	-0.50	+0.5	**0.75**	-0.50	0.50	-0.50	1.25	**0.75**
K2-5JOE	0.00	1.00	-0.50	0.25	**0.75**	-0.50	0.50	-0.50	1.00	**0.50**

5. Fluorescence results

Chart S1. Fluorescence intensity at emission maxima values for single stranded and duplexes of probes with DNA/RNA targets.



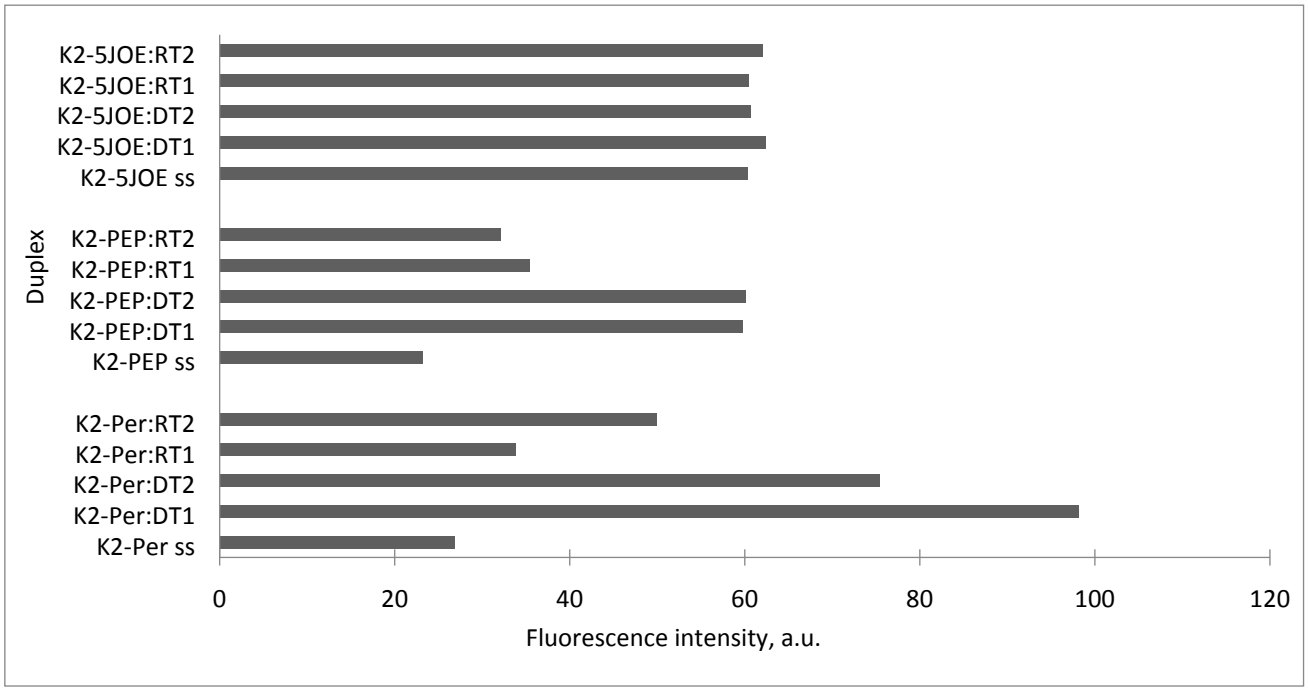
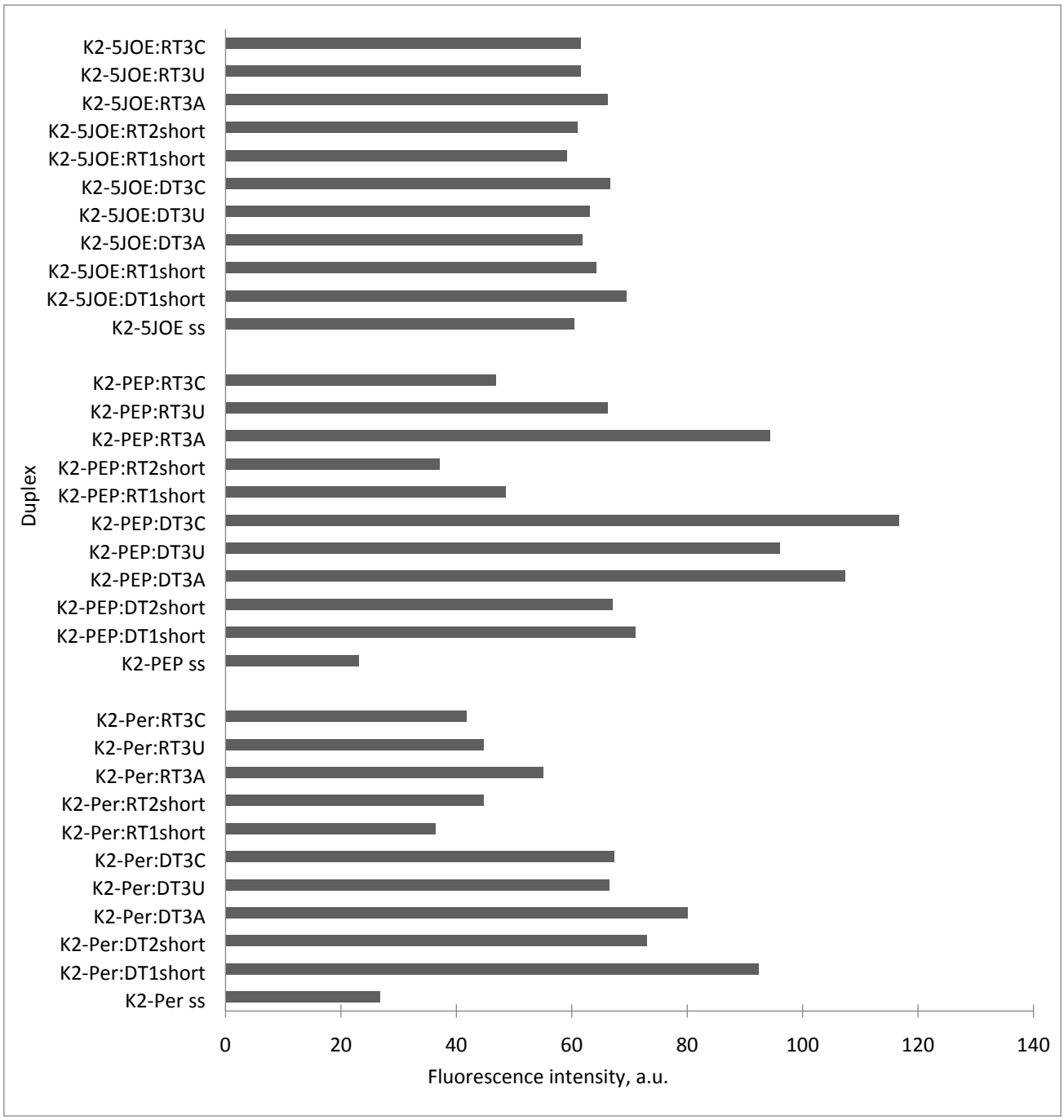


Chart S2. Fluorescence intensity at emission maxima values for single stranded and duplexes of probes with DNA/RNA targets.



6. Capturing (bead) assay

We designed target specific capturing and derection probes to *BRAF* V600E and *EGFR* L858R regions of human genome, following previously described strategy.[1]
The resulting probes were as follows:

BRAF capturing probe:
5'-/Biosg/ GAA AAT ACT ATA GTT GAG ACC TTC AAT GAC TTT CTA GTA ACT CAG CAG CAT CTC AGG GCC AAA AAT TTA ATC AGT GGA AAA ATA GCC TCA ATT CTT ACC ATC CAC AAA ATG GAT CCA GAC

EGFR capturing probe:
5'-/Biosg/ CTG GAG AGC ATC CTC CCC TGC ATG TGT TAA ACA ATA CAG CTA GTG GGA AGG CAG CCT GGT CCC TGG TGT CAG GAA AAT GCT GGC TGA CCT AAA GCC ACC TCC TTA CTT TGC CTC CTT CTG

E1-Per:
Mw calc: 7668,5 g/mol; Mw exp:7665.0 g/mol, sequence given in the main article.

The sequences of *BRAF* V600E specific probes K1 and K2 are given in Table 1 (main article).

For the assay, we used purified genomic DNA from A101D – human skin malignant melanoma cell line (mutated *BRAF*, ATCC) and NCI-H1975 – human long non-small cell line carcinoma (mutant *EGFR*, ATCC). As a negative control we used DNA extracted from healthy cell line HMC-1 (ATCC). The capturing probes were purchesed by Qiagen and the E1-Per probe was synthetised on a Expedite as described above (handcoupled with the bisalkyne scaffold) and bioconjugated with the perylene-azide using CuAAC click chemistry (described above). For fluorescence quantification we used LightCycler® 480 Real-Time PCR System (Roche), LightCycler® Cyan 500 chanel (440 nm-488 nm).

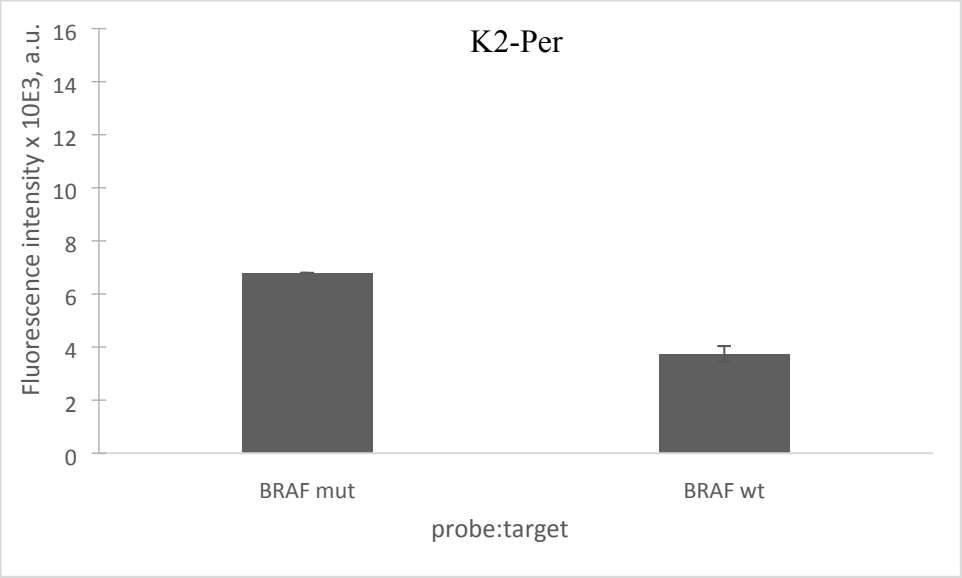
In addition, we examined the performance of comercialy available fluorescent oligonucleotides conjugated with Fam dye (purchased from Qiagen). Sequences of these control oligonucleotides are given in the main article:

Protocol

The capturing assay was performed using 50 µL (10 mg/mL) magnetic beads per analysis. The beads were stabilized at room temperature for 1 h prior to the assay, washed three times with 150 µL 1x PBS and redisolved in 25 µL 1x PBS. Next, the beads were incubated for 30 min with biotin-labeled capturing probe (10 µL, 500 nM concentration) with rotation. This was followed with three times wash with 150 µL 1x PBS and redisolving in 32.5 µL 2x PBS buffer. Afterwards, 2.5 µL (19 ng/µL) purified genomic DNA (from A101D/NCI-H1975/HMC) was added and the mixture was placed for 10 min at 85 °C followed by 3 h at 65 °C and 20 min cooling down to room temperature. This step was finished with subsequent three times washing with 150 µL 1x PBS and redisolving in 25 µL 2x PBS. Then, 10 µL (50 nM concentration) of detection probe (K1-Per/ K2-Per /E1-Per/K1-Fam or E1-Fam) was added, followed with subsequent treatment program, 10 min at 85 °C, 40 min at 60 °C and 10 min at 40 °C. Immediately, warm wash step was performed washing four times with 150 µL 1x PBS buffer (buffer kept at 45 °C, minimum 2 h before the wash and during the wash). Finally, the beads were redisolved in 30 µL 2x PBS, placed for 10 min at 92 °C with immediate separation of the supernatant from the beads. The fluorescence intensity was measured using excitation/emission of 500 nm/530 nm, respectively.
LOD was measured following the same protocol as desciebed above. The mutated *BRAF*/*EGFR* DNA was used in conentration range 290 pM-4 fM.

[1] Miotke, Astakhova et al. PLoS ONE 2015.

Chart S3. Capturing assay results for probe K2-Per and the negative control HMC.*



* Each data point is an average of two independent measurements with CV < 5%.

Chart S4. Capturing assay results for the probes K1-Fam, E1-Fam and the negative control HMC.

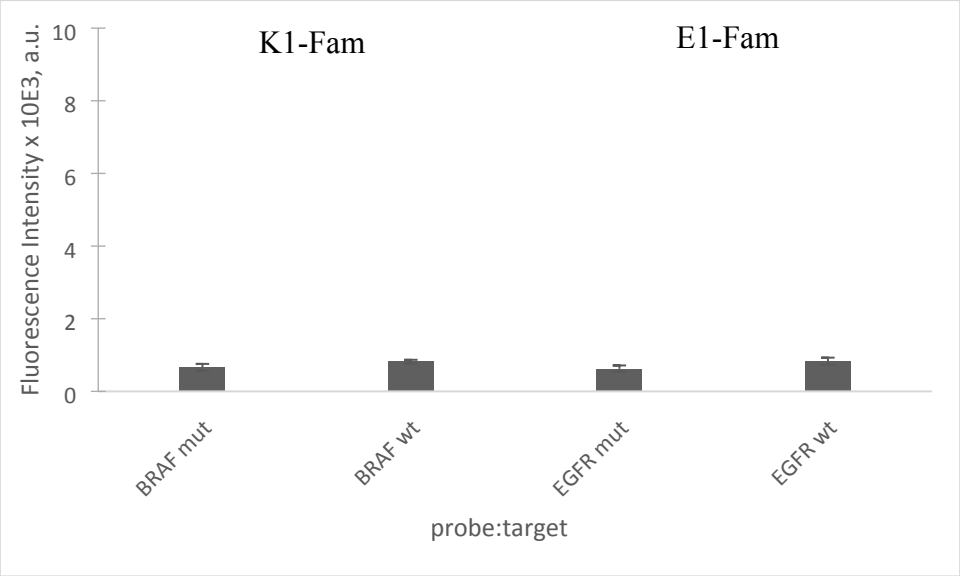
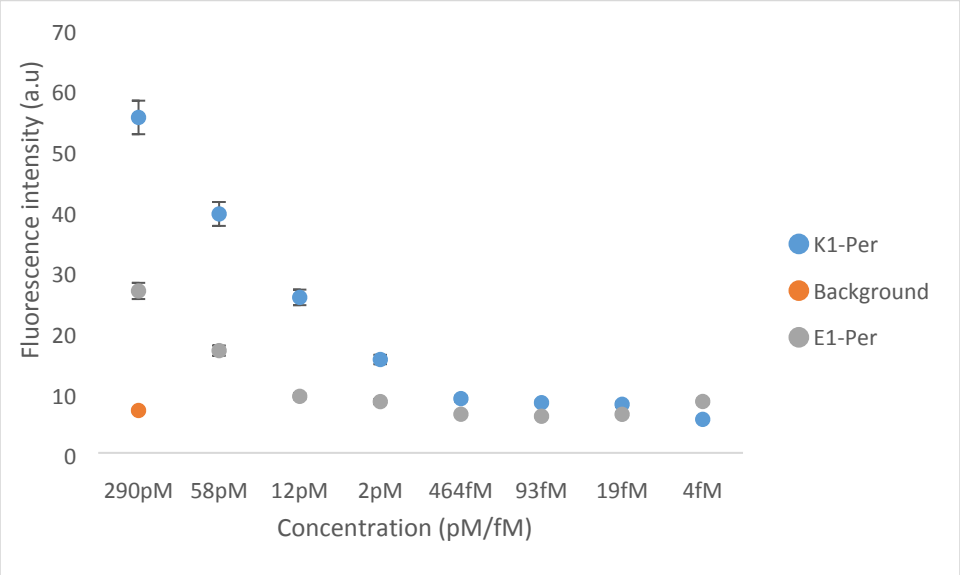


Chart S5. Limit of detection (LOD) determination for K1-Per and E1-Per.

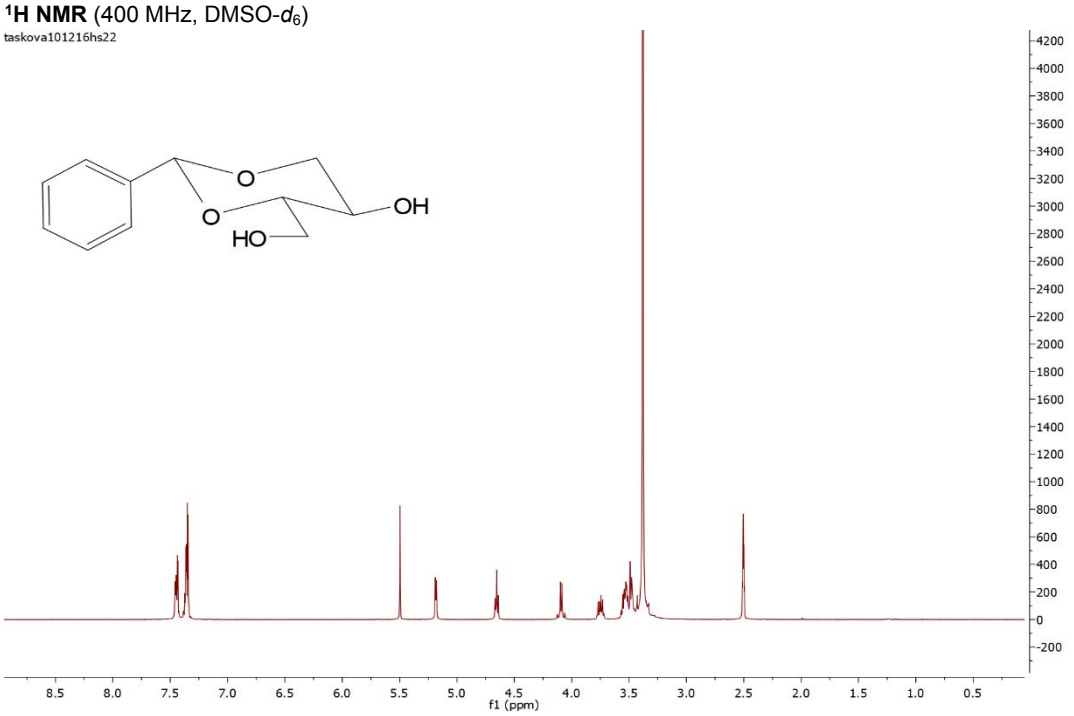


*LOD evaluated performing the capturing assay (described above) using mutated *BRAF*/*EGFR* DNA at 290 pM to 4 fM concentrations.The estimated LOD was calculated to give minimum three times higher fluorescent response compared to the background. Background has been measured using 1x PBS in the plate well instead of DNA sample.

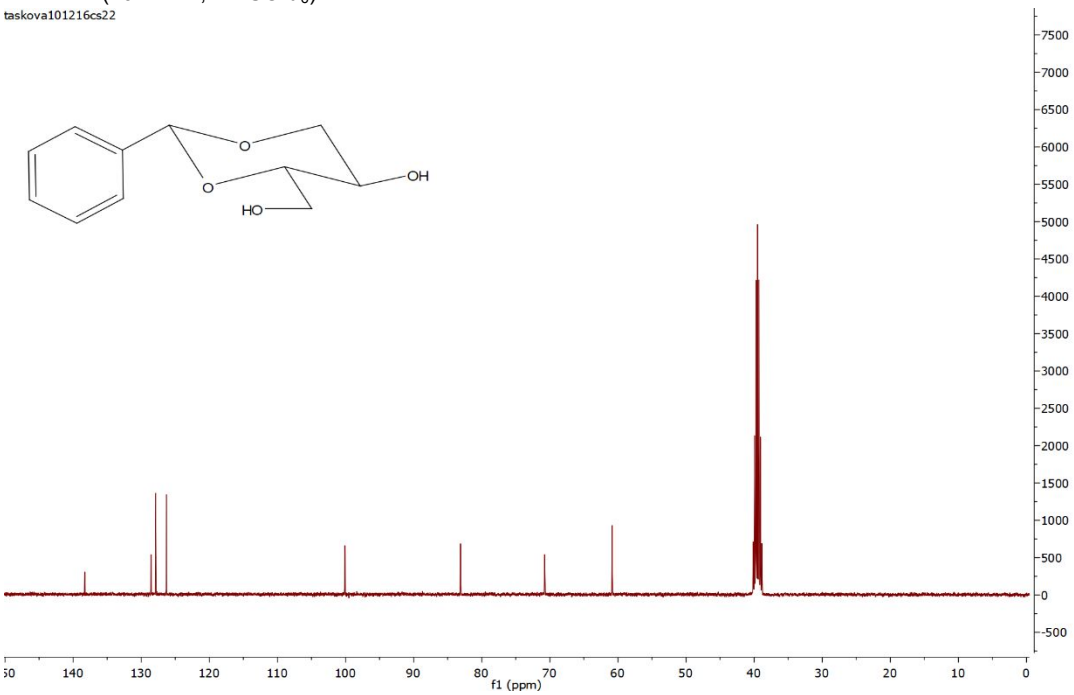
7. ^{1}H and ^{13}C NMR spectra

Preparation of 1,3-O-benzylidene-L-erythriol (3)

^{1}H NMR (400 MHz, DMSO-d_6)

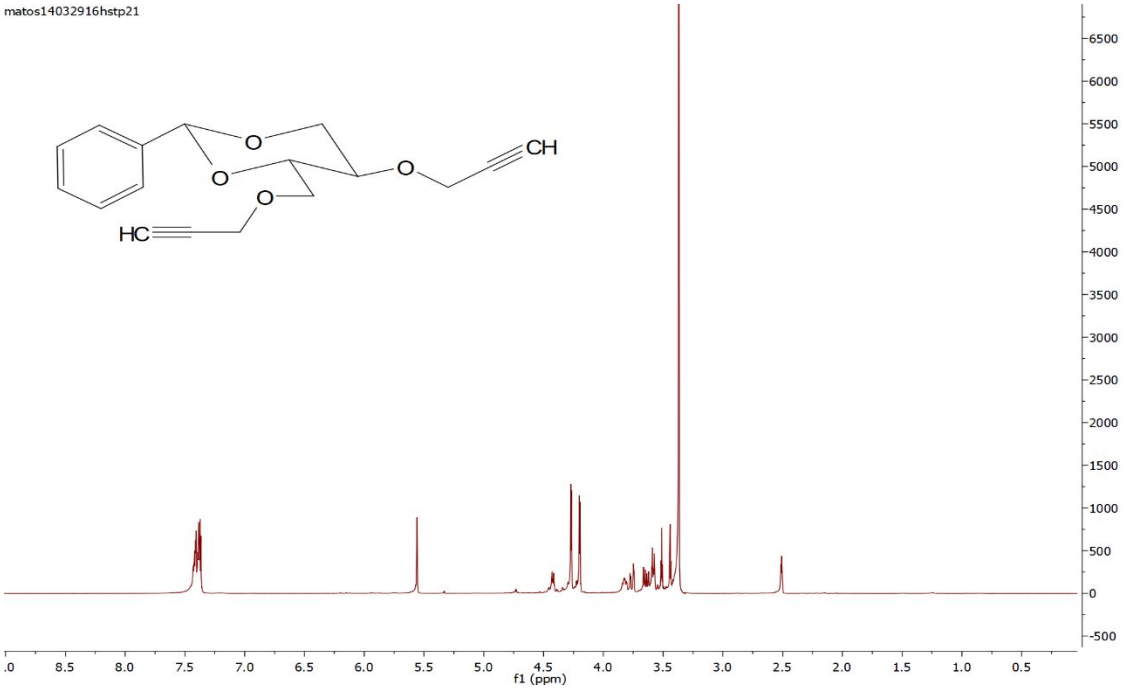


^{13}C NMR (101 MHz, DMSO-d_6)

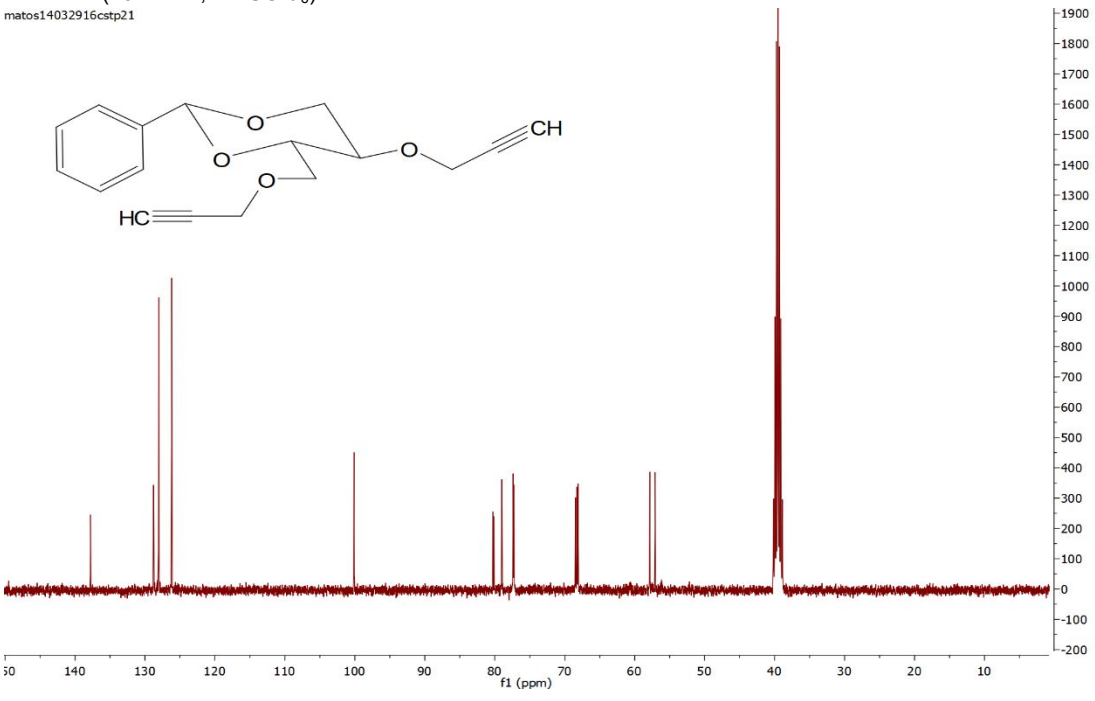


Preparation of (2R,4S,5R)-2-phenyl-5-(prop-2-yn-1-yloxy)-4-((prop-2-yn-1-yloxy)methyl)-1,3-dioxane (4)

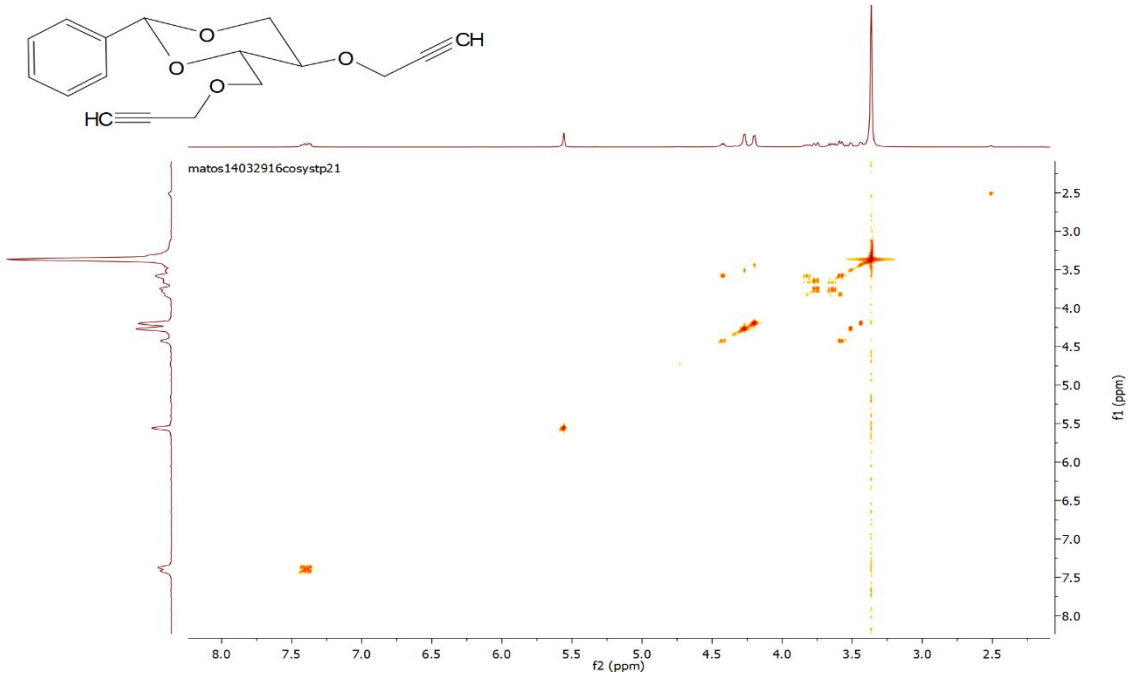
^{1}H NMR (400 MHz, DMSO-d_6):



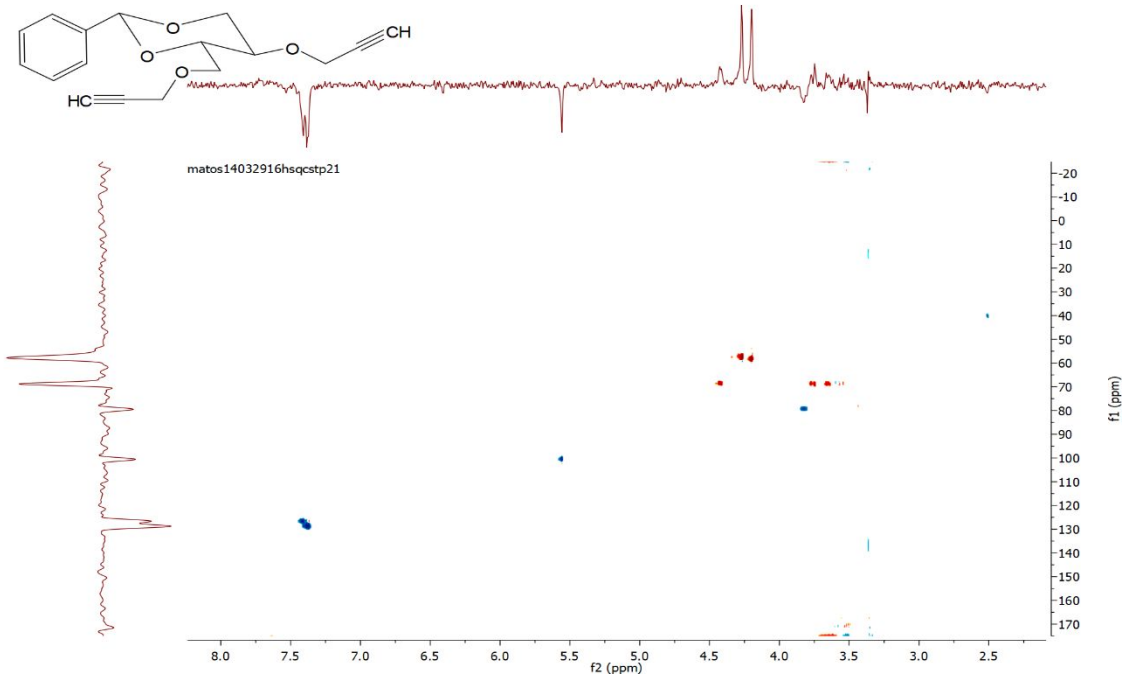
^{13}C NMR (101 MHz, DMSO-d_6):



COSY (400 MHz, DMSO-d_6):

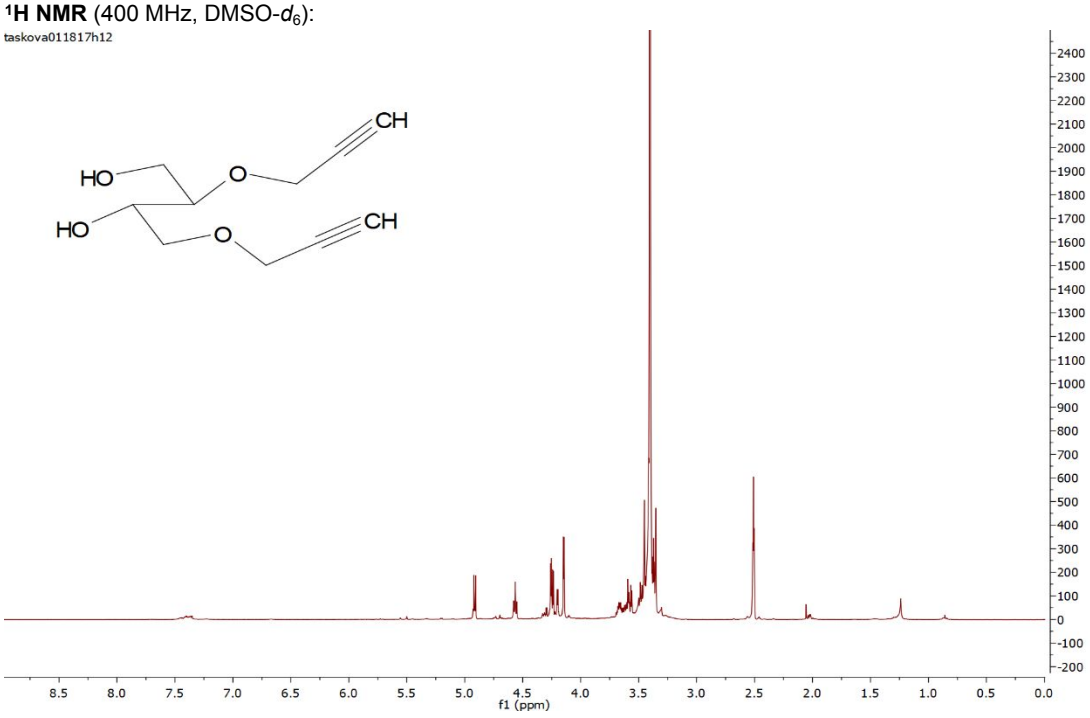


HSQC (400 MHz, DMSO-d_6):

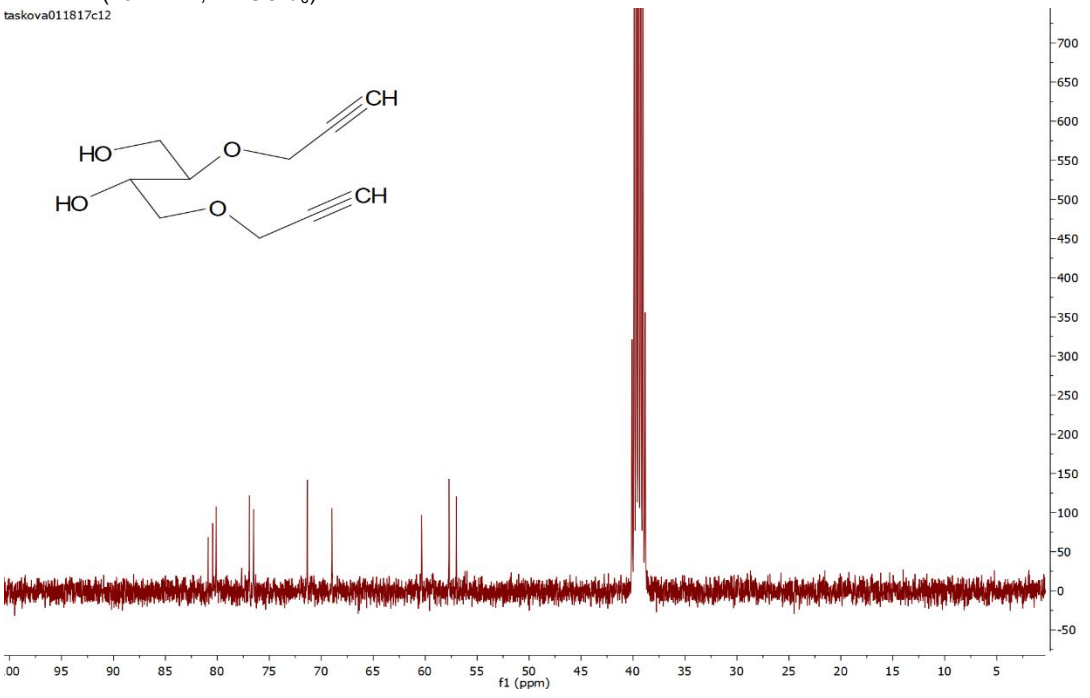


Preparation of 2,4-bis(prop-2-yn-1-yloxy)butane-1,3-diol (5)

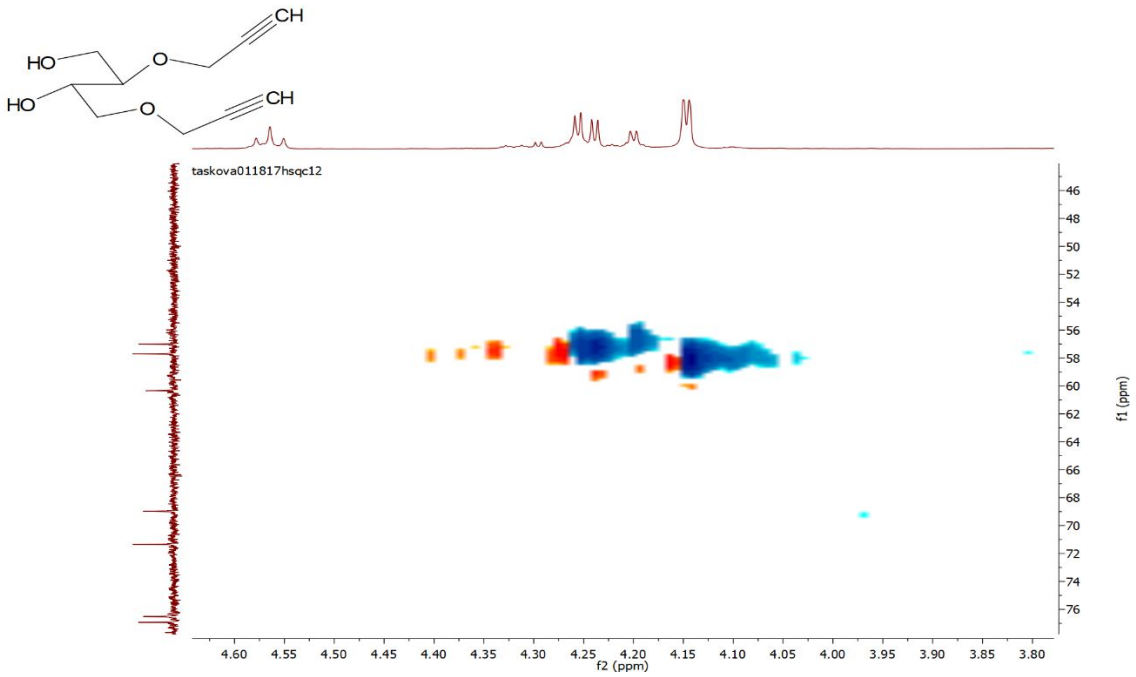
^{1}H NMR (400 MHz, DMSO-d_6):



^{13}C NMR (101 MHz, DMSO-d_6):

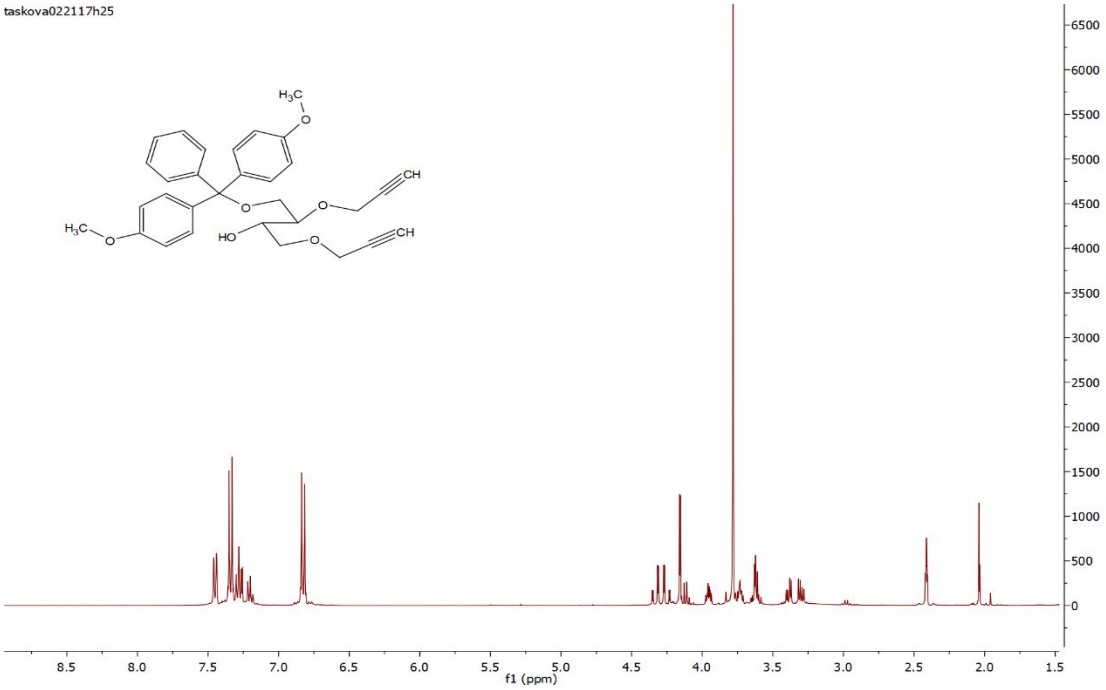


HSQC (400 MHz, DMSO-d_6):

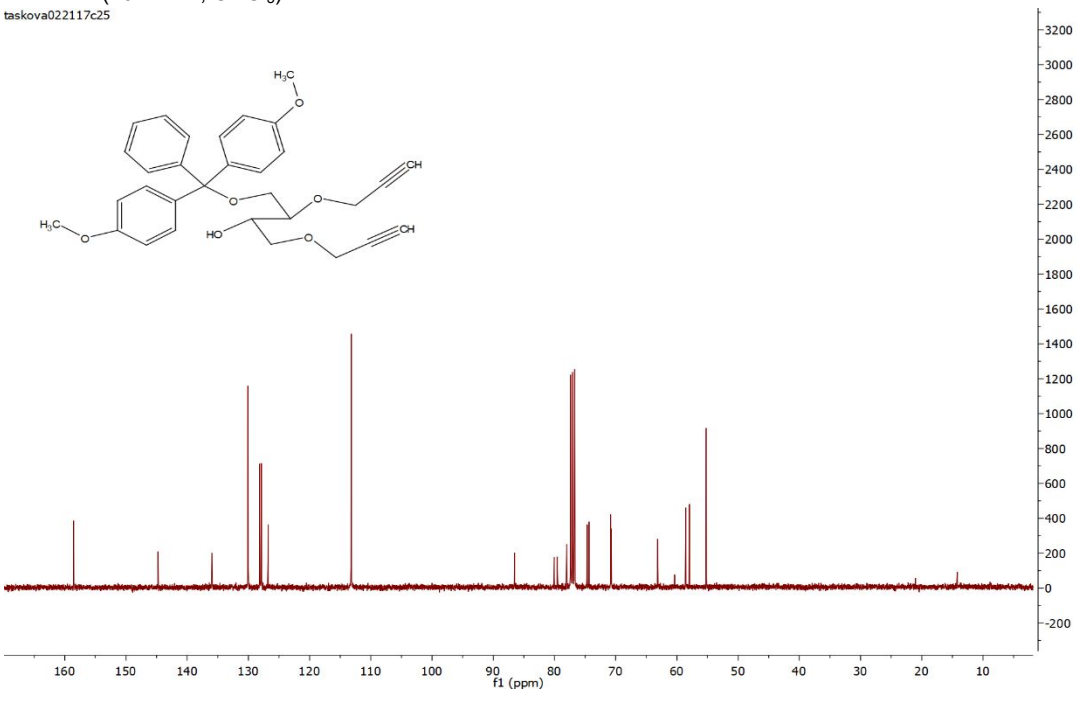


Preparation of 2,4-bis(prop-2-yn-1-yloxy)butane-1-O-dimetoxytrityl-3-ol (6)

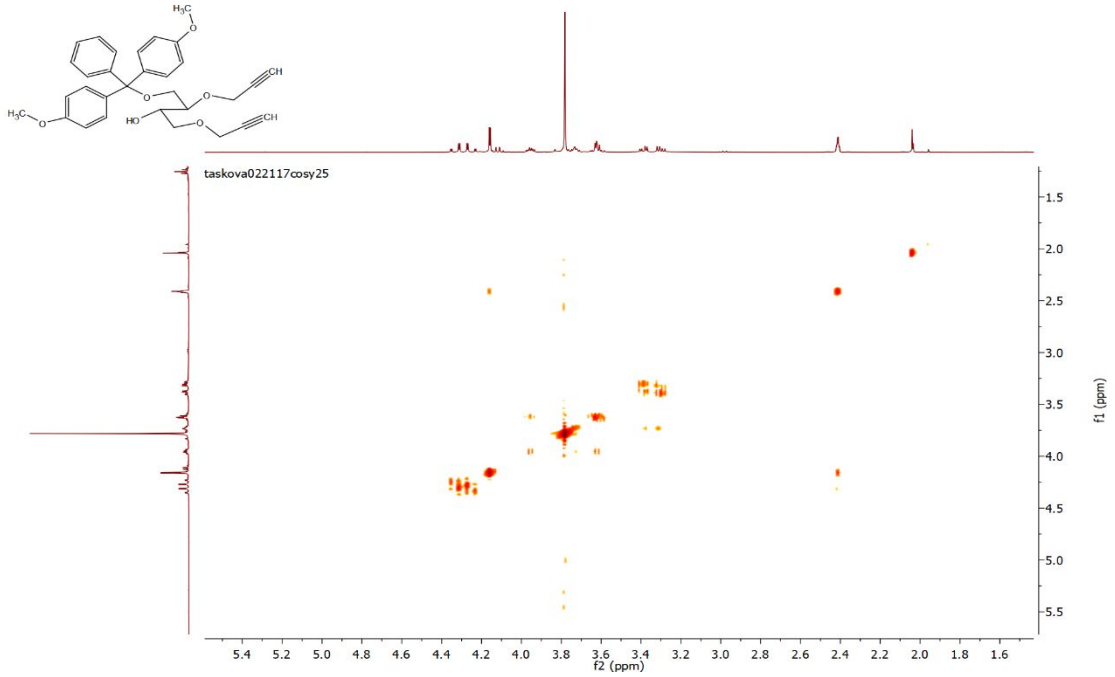
^{1}H NMR (400 MHz, $CDCl_3$):



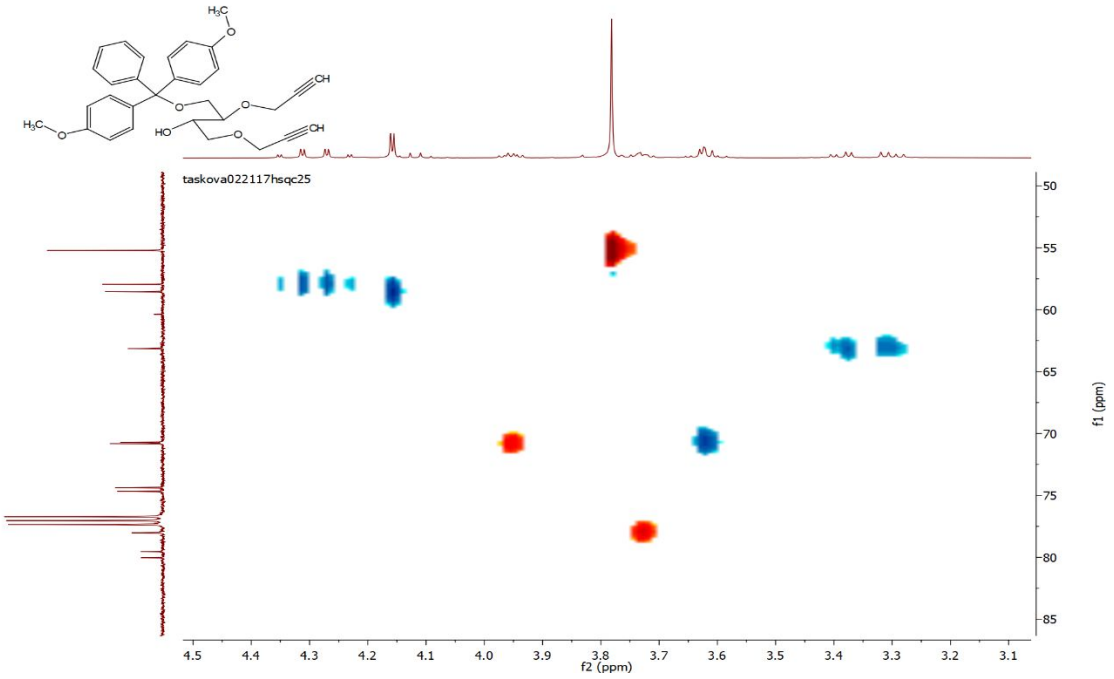
^{13}C NMR (101 MHz, $CDCl_3$):



COSY (400 MHz, $CDCl_3$):



HSQC (400 MHz, $CDCl_3$):



Preparation of 4-(bis(4-methoxyphenyl)(phenyl)methoxy)-1,3-bis(prop-2-yn-1-yloxy)butan-2-yl(2-cyanoethyl) diisopropylphosphoramidite (7)

^{31}P NMR (162 MHz, $CDCl_3$):