Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Figures

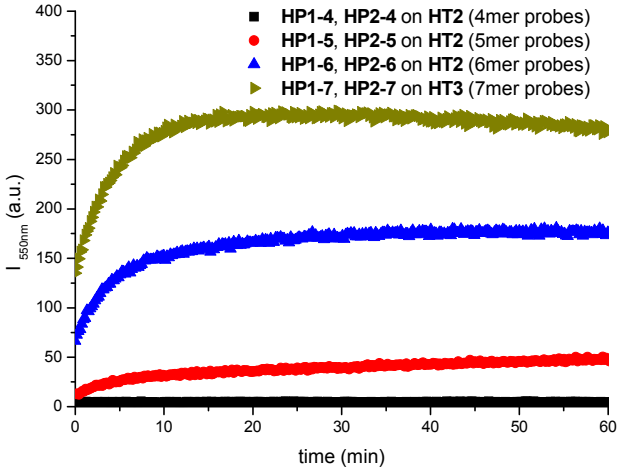


Figure S1. Time course of the fluorescence signal development with probes of different lengths. Experiments were performed at 35°C in buffer 1 (50 mM KCl, 10 mM Tris, 3.5 mM $MgCl_2$, pH 8.0) with the indicated probes (400 nM) and templates (200 nM).

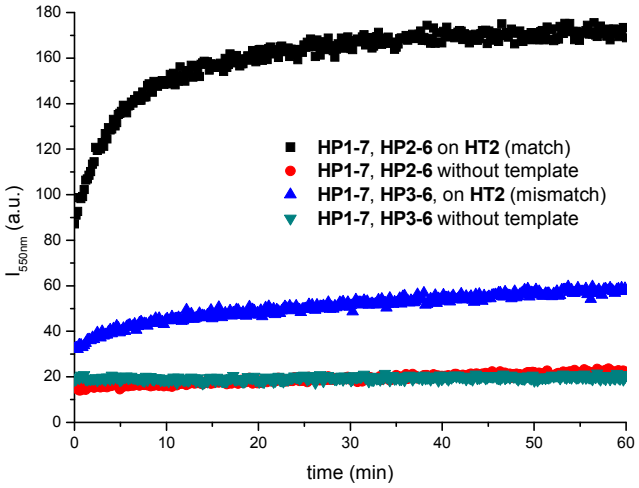


Figure S2. Time course of the fluorescence signal development of either **HP2-6** or **HP3-6** by **HP1-7** on **HT2** (mismatch discrimination). Experiments were performed at 35°C in buffer 1 (50 mM KCl, 10 mM Tris, 3.5 mM $MgCl_2$, pH 8.0) with the indicated probes (400 nM) and templates (200 nM).

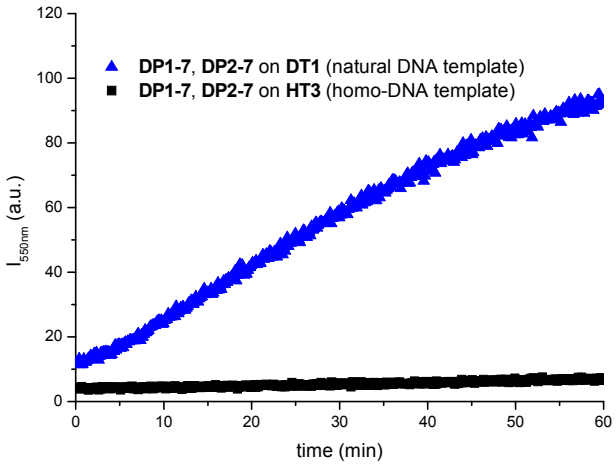


Figure S3. Time course of the fluorescence signal development of **DP2-7** by **DP1-7** (natural DNA probes) in presence of the natural DNA or homo-DNA template. Experiments were performed at 15°C in buffer 1 (50 mM KCl, 10 mM Tris, 3.5 mM $MgCl_2$, pH 8.0) with **DP2-7** (400 nM), **DP1-7** (800 nM) and the indicated templates (200 nM).

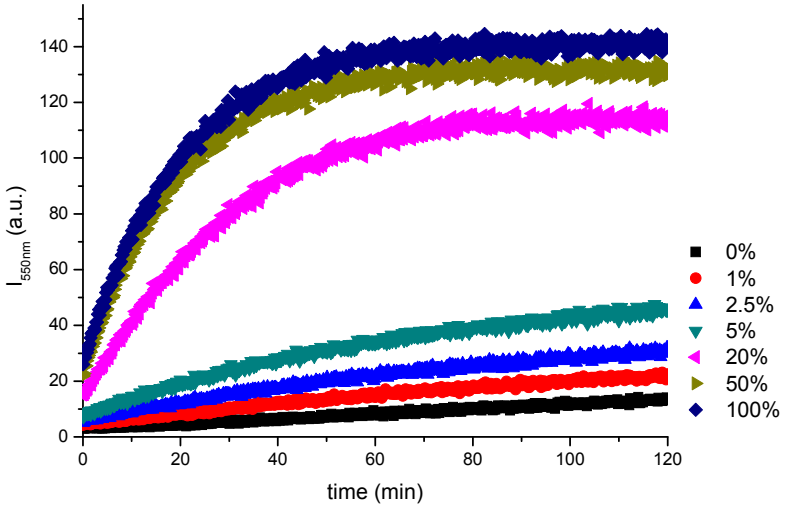


Figure S4. Time course of the fluorescence signal development of **HP2-7** by **HP1-7** in presence of various concentrations of **HT3**. Experiments were performed at 16°C in buffer 2 (50 mM KCl, 10 mM Tris, 3.5 mM $MgCl_2$, pH 7.1) with **HP2-7** (400 nM), **HP1-7** (800 nM) and template **HT3** (indicated as percentage of the concentration of **HP2-7**).

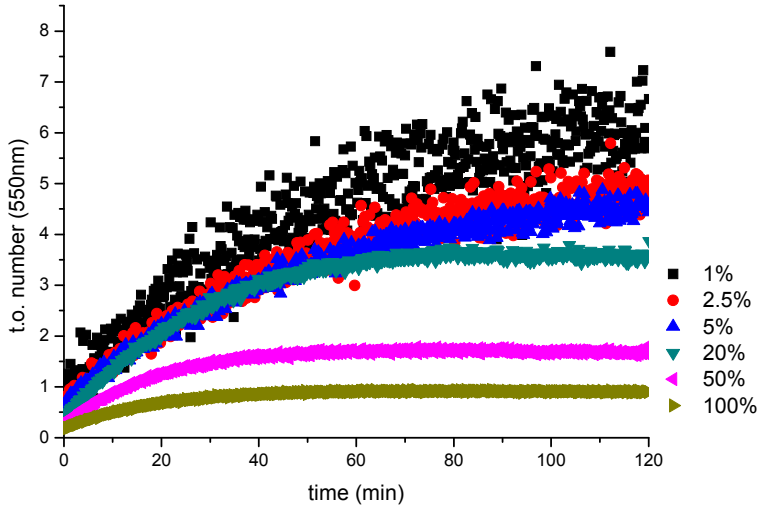


Figure S5. Calculated turnover (t.o.) numbers for the templated reaction between **HP1-7** and **HP2-7** at various concentrations of **HT3**. Experiments were performed at 16°C in buffer 2 (50 mM KCl, 10 mM Tris, 3.5 mM $MgCl_2$, pH 7.1) with **HP2-7** (400 nM), HP1-7 (800 nM) and template **HT3** (indicated as percentage of the concentration of HP2-7). The turnover was calculated from the percentage of the full conversion after background subtraction of the untemplated reaction.

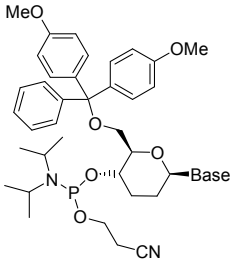
Experimental part

General

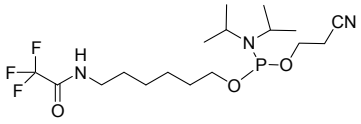
Flash chromatography (FC) was performed with silica gel 60 (230-400 mesh) from Fluka. ^{1}H NMR spectra were recorded at 300 MHz on a *Bruker AC-300* or on a *Bruker Avance* spectrometer. Chemical shifts are reported in ppm using the residual undeuterated solvent as an internal reference. Multiplicities are abbreviated as follows: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet. ^{31}P NMR spectra were recorded at 121 MHz on a *Bruker Avance* spectrometer using 85% H_3PO_4 as an external standard.

Phosphoramidite synthesis

For the synthesis of the four phosphoramidites **1**-**4** we followed the published procedures of Eschenmoser and coworkers.[1] The phosphoramidite of the C_6-aminolinker **5** was purchased from Glen Research.

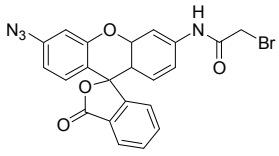


1-4 (with A, T, C, G as base)



5

Building block synthesis for templated chemistry



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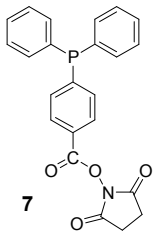

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Building block **6** was synthesized according to literature procedures.[2] Triphenylphosphine NHS-ester **7** was obtained from the commercial available 4-(diphenylphosphino)-benzoic acid (Aldrich) as follows:

4-(Diphenylphosphino)-benzoic acid (400 mg, 1.3 mmol) was dissolved in dry DMF (3.5 ml) together with DCC (295 mg, 1.43 mmol, 1.1 eq.) and NHS (150 mg, 1.3 mmol, 1 eq.). The mixture was stirred at

r.t. during 2 h. After filtration, the solvent was evaporated at high-vacuum. The residue was dissolved in 2 ml *iso*-propanol and precipitated with 40 ml hexane. The solid was filtered off and washed with 50 ml of *iso*-propanol/hexane (1:20) solution to yield pure product **7** (250 mg, 48%). ^{1}H NMR ($CDCl_3$) δ(ppm): 8.05 (2H, dd, J_1=8.5 Hz, J_2=1.3 Hz), 7.40-7.32 (12 H, m), 2.90 (4H, s); ^{31}P NMR ($CDCl_3$) δ(ppm): -4.28.

Oligonucleotide synthesis

All oligonucleotides (Table S1) were prepared from phosphoramidites **1**-**5** and the natural building blocks for dA, dC, dG, dT (Vivotide). Solid supports (Universal Support 500 was used for homo-DNA Sequences) were purchased from Glen Research. The oligonucleotides were all synthesized on the 1.3 µmol scale on a *Pharmacia LKB Gene Assembler Special* DNA synthesizer. The solutions used for coupling (0.1 M solutions of phosphoramidites in CH_3CN) as well as for activation, detritylation, capping and oxidation were prepared according to the manufacturer's protocols. As activator 5-(ethylthio)-1H-tetrazole was used (0.25M in CH_3CN). The coupling time was extended from 30 s to 6 min for all phosphoramidites except for the natural dA-, dC-, dG-, dT-building blocks. Otherwise, standard protocols were used. To obtain 3'-thiophosphates the oligonucleotides were synthesized on a 3'-phosphate-CPG solid support, and phenylacetyl disulfide (PADS, 0.2 M solution in pyridine/ACN (1:1)) instead of iodine was used for oxidation. The sulfur oxidation was performed before the capping step with 2 ml of the PADS solution during a period of 90 seconds. This protocol is used only for the first phosphoramidite incorporation. After chain elongation and final detritylation, the synthesized oligonucleotides were cleaved from the solid support following protocols a-e:

a) 55°C, 16 h, 33% NH_3
b) 55°C, 60 h, 33% NH_3
c) r.t., 3 h then 55°C, 16 h, 33% NH_3
d) r.t., 4 h, DBU (1.5 ml) then 55°C, 60 h, 33% NH_3 (before ammonia treatment the DBU solution was removed by centrifuging and washing 3x with 1 ml ACN)
e) r.t., 4 h, DBU then 55°C, 16 h, 33% NH_3 (before ammonia treatment the DBU solution was removed by centrifuging and washing 3x with 1 ml ACN)

The respective deprotection protocol used is indicated in Table S1. In general, protocol a) was used for natural oligonucleotides without modifications. Protocol b) was applied when universal solid support was used for 3'-homo-DNA modified oligonucleotides. Protocol c) was used together with 3'-phosphate solid support for the 3'-thiophosphates. Protocols d) and e) have to be applied for 5'-amino modified oligonucleotides to induce β-elimination by DBU before the amino-group is deprotected. After separation of the solid support from the dissolved oligonucleotides by filtration (Titan HPLC-filters, Teflon, 0.45 µm, Infochroma AG) the solvent was evaporated.

Oligonucleotide purification

The oligonucleotides were dissolved in H_2O (1ml) and filtered (Titan HPLC-filters, Teflon, 0.45 µm, Infochroma AG) before purification by RP-HPLC. For RP-HPLC we were using the indicated gradients of buffer B in buffer A:

RP20: 0%→20% B in 30’ (Flow: 1 ml/min)
RP30: 0%→30% B in 30’ (Flow: 1 ml/min)
RP40: 0%→40% B in 30’ (Flow: 1 ml/min)
RP50: 0%→50% B in 30’ (Flow: 1 ml/min)

Buffer A: 0.1 M Et_3N/CH_3COOH in H_2O, pH 7.0.
Buffer B: 0.1 M Et_3N/CH_3COOH in H_2O/ACN (1:4), pH 7.0.

Either column A (colA) or column B (colB) were used as indicated in Table S1
ColA: Source 15 RPC ST 4.6/100 from Pharmacia Biotech
ColB: VA 150/4.6 Nucleogel RP 300-5 from Macherey Nagel

The purification was performed at r.t. Deviations in temperature are otherwise indicated in Table S1. After RP-purification the oligonucleotides were desalted over Sep-Pack C18 cartridges (Waters) according to the manufacturer's protocol.

Post-synthetic derivatization of oligonucleotides

3’- or 4'-Rhodamine azide conjugated oligonucleotides (**HP2-4**, **HP2-5**, **HP2-6**, **HP2-7**, **DP2-7**, **HP3-6, MBP1**)

50 nmol of the respective 4'-phosphothioate modified oligonucleotide were dissolved in 33 µl of 200 mM NaH_2PO_4 buffer (pH 7.2). Then 2.1 µmol of rhodamine-azide **6** dissolved in 123 µl DMF were added and the reaction mixture was incubated at 20°C over night in a thermomixer. The mixture was centrifuged (1 min, 14000 rpm) and the supernatant was purified by RP-HPLC.

5'- or 6’-Triphenylphosphine conjugated oligonucleotides (**HP1-4**, **HP1-5**, **HP1-6**, **HP1-7**, **DP1-7**, **MB1**)

50 nmol of the respective oligonucleotide containing the 6'-NH_2-C-6 linker were dissolved in 135 µl of 100 mM $Na_2B_4O_7$ buffer (pH 8.5). Then 2 µmol of TPP-ester **7** dissolved in 95 µl DMF were added. The mixture was degassed in an ultrasonic bath (30 s) to prevent TPP from oxidation and then incubated at 20°C during 1 h in a thermomixer. The mixture was centrifuged (1 min, 14000 rpm) and the supernatant was purified by RP-HPLC.

Purification of post-synthetically modified oligonucleotides

All modified oligonucleotides were purified by RP-HPLC after the post-synthetic reaction steps (see section oligonucleotide purification and Table S1). For the TPP modified oligonucleotides always two peaks were observed by HPLC. Analysis by mass spectrometry showed that the faster eluting fraction is the oxidized product whereas the desired product has a longer retention time. Also in that slower eluting fraction we found a significant amount of the oxidized product after isolation, showing that post HPLC manipulations also lead to partial oxidation. Therefore, for the assays we used the TPP modified oligonucleotide fractions after HPLC purification without further treatment. The oligonucleotides were freshly prepared for each set of experiments, shock frozen and stored in the

freezer (-20°C) between single measurements. As an example the HPLC chromatogram of the purification of **HP1-7** is shown in *Figure S6*. Peak **a** at 11 min is assigned to the unmodified oligonucleotide. The oxidized TPP product is eluting at 18 min (peak **b**) and the desired TPP product at 24 min (peak **c**). The rhodamine modified oligonucleotides were desalted with Sep-Pack C18 cartridges (Waters) before further use according to the manufacturer's protocol.

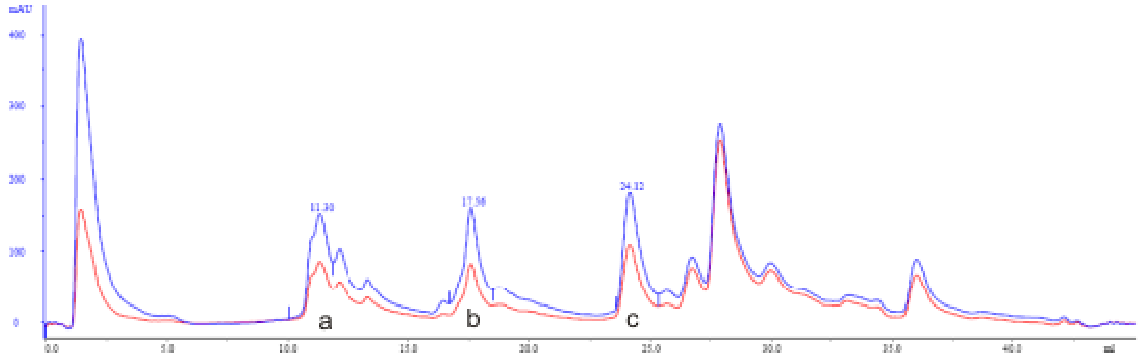

Figure S6.

Oligonucleotide analysis by ESI-MS

The integrity of the synthesized and purified oligonucleotides was confirmed with ESI-MS (negative ion mode) on a *Sciex QSTAR Pulsar* mass spectrometer from Applied Biosystems. The corresponding values are indicated in Table S1. TPP-modified oligonucleotides contained significant amounts of the oxidized product (calculated mass +16).

Fluorescence measurements were performed on a *Cary Eclipse Fluorescence Spectrophotometer* with a *Cary Temperature Controller* (both from Varian) in fluorescence quartz cuvettes from Hellma with a path length of 10 mm. The photomultiplier voltage was set to 800 V and the emission and excitation slits were set to 5 nm. The fluorescence properties of the rhodamine derivate was previously described [2] and we were measuring at λ_{ex} = 490 nm and λ_{em} = 550 nm.

The experimental parameters for all templated reactions are indicated for each experiment. The samples were prepared as 1 ml solutions in Eppendorf tubes and mixed before transferring the mixture to the fluorescence cuvettes. The TPP modified oligonucleotids were always added at last immediately before mixing the sample.

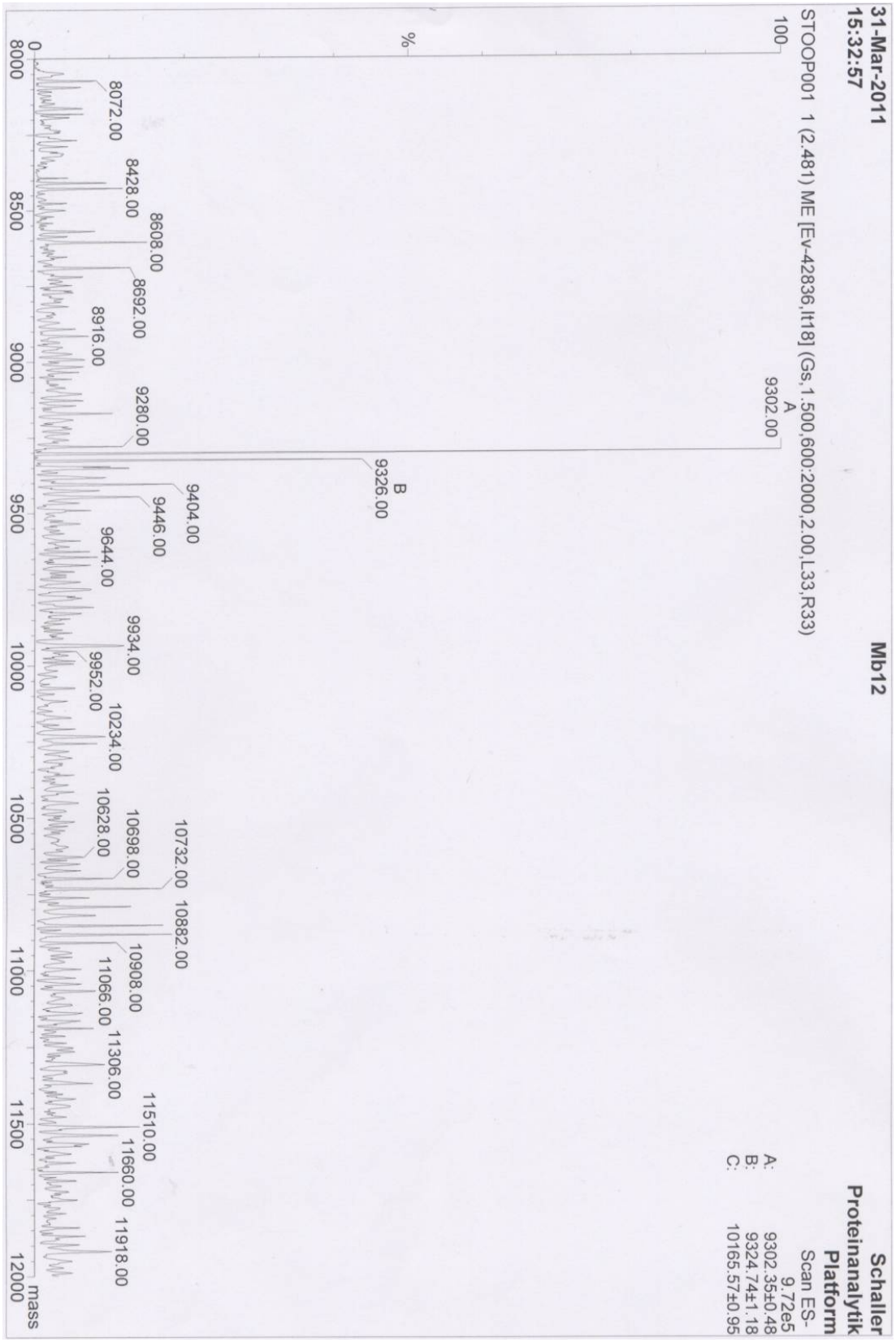

Figure S7a: ESI$^-$-MS of **MB1** containing the 6'-amino linker without the triphenylphosphine unit. m/z found: 9302; m/z calcd: 9302

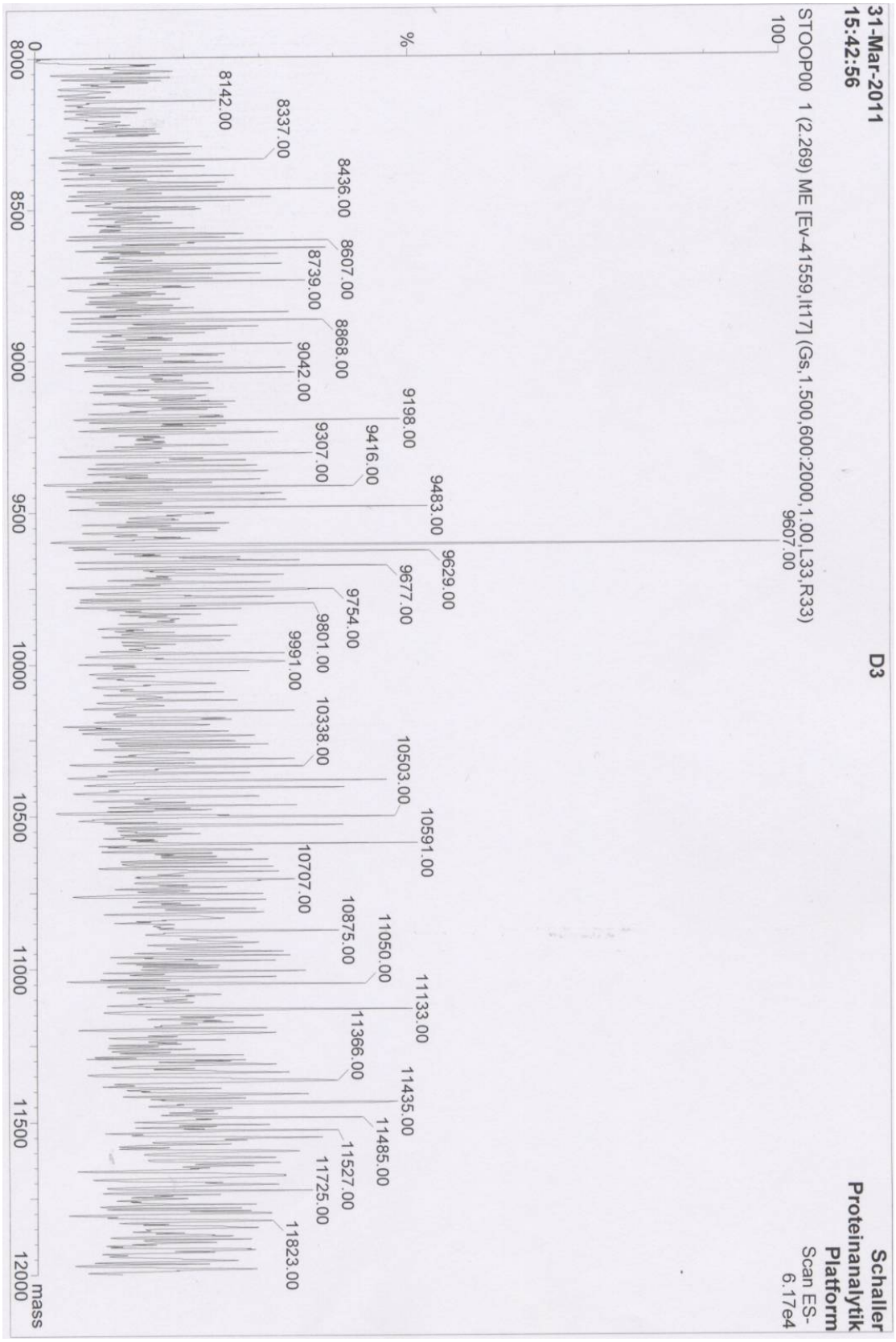

Figure S7b: ESI$^-$-MS of the oxidized (triphenylphosphinoxide) form of **MB1**: m/z found: 9607; m/z calcd: 9606.

Table S1

Entry	Sequence [1)]	RP-HPLC method, retention time, column [2)]	ESI$^-$-MS found mass (Calculated mass) [3)]	Deprotection conditions [4)]
HT1	6' - cat agg cac gtg cc - 4'	RP40, 13 min, colB	4445.0 (4445.2)	b
HT2	6' - ata ggc tac gtg c - 4'	RP40, 15 min, colA	4154.0 (4157.0)	b
HT3	6' - cat agg cta cgt gcc - 4'	RP20, 25 min, colA	4763.0 (4763.0)	b
HT4	6' - cat agg ctt acg tgc c - 4'	RP40, 14 min, colA	5082.0 (5081.6)	b
HP1-6	6' - TPP - gcc tat - 4'	RP50, 26 min, colA	2334.5 (100%, ox.) (2318.8)	d
HP2-6	6' - gca cgt - Rhd-N_3 - 4'	RP50, 24 min, colA	2368.5 (2368.8)	c
HP1-7	6' - TPP - gcc tat g - 4'	RP50, 24 min, colA	2661.0 and 2677.0 (70% ox.) (2662.0)	d
HP2-7	6' - ggc acg t - Rhd-N_3 - 4'	RP50, 23 min, colA	2712.0 (2713.0)	c
HP3-6	6' - gc<u>t</u> cgt - Rhd-N_3 - 4'	RP50, 21 min, colB	2360.0 (2359.8)	c
HP1-4	6' - TPP - gcc t - 4'	RP40, 22 min, colB	1689.0 (100%, ox.) (1673.3)	d
HP2-4	6' - acg t - Rhd-N_3 - 4'	RP50, 26 min, colB	1722.0 (1722.3)	c
HP1-5	6' - TPP - gcc ta - 4'	RP50, 26 min, colA	2000.0 and 2016.0 (40%, ox.) (2000.5)	d
HP2-5	6' - cac gt - Rhd-N_3 – 4'	RP50, 26 min, colB	2025.0 (2025.5)	c
DT1	5'- CAT AGG CTA CGT GCC - 3'	RP20, 20 min, colA	4552.0 (4553.0)	a
DP1-7	5' - TPP - GCC TAT G - 3'	RP50, 19 min, colB	2564.0 and 2580.0 (40% ox.) (2564.0)	e
DP2-7	5' - GGC ACG T - Rhd-N_3 - 3'	RP50, 21 min, colA	2614.0 (2615.0)	c
MB1	6' - TPP - agg cac g AAG TTA AGA CCT ATG cgt gcc t - 4'	RP40, 28 min, colA, 55°C	See Figure S7a,b	d
MBP1	6' - acg tgc ct - Rhd-N_3 - 4'	RP50, 25 min, colA	2990.0 (2990.2)	c
MBI1	6' - NH_2-C6 - agg cac gt - 4'	RP30, 17 min, colA	2725.0 (2726.0)	d
MBT1	5' - TTT TTC ATA GGT CTT AAC TTT TTT T - 3'	RP30, 19 min, colA	7584.0 (7584.0)	a
MBT2	5' - TTT TTC ATA GGT <u>A</u>TT AAC TTT TTT T - 3'	RP30, 19 min, colA	7606.0 (7608.0)	a
MBT3	5' - TTT TTC ATA GGT <u>T</u>TT AAC TTT TTT T - 3'	RP40, 16 min, colA	7599.0 (7599.0)	a
MBT4	5' - TTT TTC ATA GGT <u>G</u>TT AAC TTT TTT T - 3'	RP40, 15 min, colA	7623.0 (7624.0)	a

1) Homo-DNA: lower case letters; natural DNA: capital letters; mismatches: underlined.

2) For details about buffers and columns see the section about oligonucleotide purification.

3) TPP modified oligonucleotides were detected partially in the oxidized form (see also the comments in the section about the purification of post synthetic modified oligonucleotides).

4) For details about the deprotection conditions see the section about the oligonucleotides synthesis.

1. M. Böhringer, H. J. Roth, J. Hunziker, M. Göbel, R. Krishnan, A. Giger, B. Schweizer, J. Schreiber, C. Leumann and A. Eschenmoser, *Helv. Chim. Acta*, 1992, **75**, 1416-1477.
2. H. Abe, J. Wang, K. Furukawa, K. Oki, M. Uda, S. Tsuneda and Y. Ito, *Bioconjug Chem*, 2008, **19**, 1219-1226.