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Supporting Information

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Target-Catalyzed Transfer Reactions for the Amplified Detection of RNA

Tom N. Grossmann, L. Röglin and O. Seitz*

Institut für Chemie, Humboldt-Universität zu Berlin, Brook-Taylor-Str. 2, D-12489 Berlin, Germany

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1 Materials and general procedures

Materials: PNA monomers were purchased from Applied Biosystems. PyBOP, HCTU, resins and protected amino acids were purchased from Novabiochem. DNA was purchased from BioTeZ Berlin-Buch GmbH, Germany in HPLC quality. RNA was purchased from Invitrogen in HPLC quality. Biotin-AHX-OH and dry DMF (H_2O < 0.01%) were purchased from Fluka. Water was purified with a Milli-Q Ultra Pure Water Purification System, Membrapure, Germany. Boc-*i*Cys(Trt)-OH was synthesized following reported procedures.[1]

Synthesis: Preparative HPLC was performed on an Agilent 1100 system (column: Nucleodur Gravity C18 A 5µ (VP 250/21)) using eluents A (98.9% H_2O, 1% acetonitrile, 0.1% TFA) and B (98.9% acetonitrile, 1% H_2O, 0.1 % TFA) in a linear gradient (0% B – 40% B in 30 min) with a flow rate of 13 mL/min. Detection of the signals was achieved with a UV-detector at wavelength λ = 280 nm. TLC was performed with E. Merck Silica Gel 60 F254 plates. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker DPX 300 spectrometer. The signal of the residual protonated solvent (DMSO-d_6, 2.50 ppm) was used as reference signal. ^{13}C-spectra were calibrated to 39.43 ppm (DMSO-d_6). High resolution mass spectra (HRMS) were measured with a Hewlett-Packard GCMS 5995-A (ESI+) spectrometer.

Solid-phase synthesis: Manual solid phase synthesis was performed using 5 mL polyethylene syringe reactors equipped with a fritted disc. Automated linear solid-phase Fmoc-synthesis was performed using an Intavis ResPep parallel synthesizer equipped with micro scale columns for PNA synthesis.

Preloading of resins: The resins used in solid phase synthesis were loaded with the protected amino acids (ca. 0.1 mmol/g) according to standard protocols (see: *NovaBiochem Catalog* 2004/2005).

Automated solid-phase synthesis according to the Fmoc-strategy: *Fmoc cleavage*: DMF/piperidine (4:1, 200 µL) was added to the resin. After 2 min, the procedure was repeated once. The resin was washed (7× 200 µL DMF). *Coupling*: A preactivation vessel was charged with a 0.6 M HCTU solution in NMP (12 µL), a 4 M NMM solution in DMF (4 µL), and a 0.2 M PNA monomer solution in NMP (40 µL). After 8 min, 50 µL of preactivation solution were transferred to the resin. After 30 min, the resin was washed (2× 200 µL DMF). *Capping*: Ac_2O/2,6-lutidine/DMF (5:6:89, 200 µL) was added. After 3 min the resin was washed (2× 200 µL DMF).

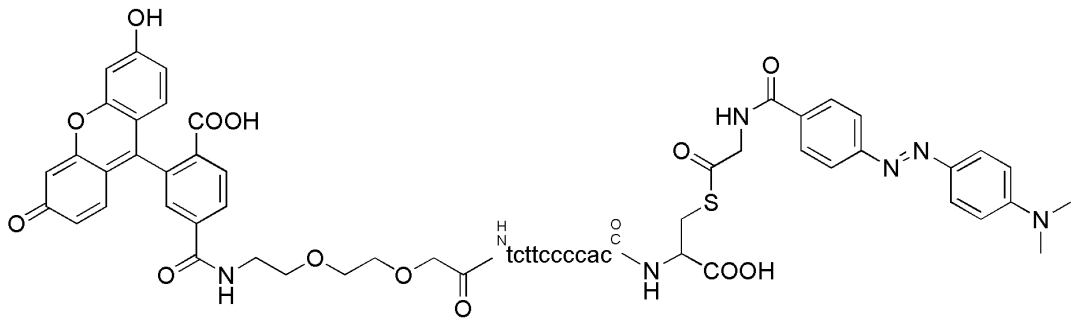
Purification of probes: For purification the combined TFA solutions were concentrated *in vacuo* before addition of diethylether. The precipitated crude product was dissolved in water and purified by semi preparative HPLC, performed on an Agilent 1100 series instrument (column: Varian Polaris C18 A 5µ 250x100, pore size 220 Å) using eluents A (98.9% H_2O, 1% acetonitrile, 0.1% TFA) and B (98.9% acetonitrile, 1% H_2O, 0.1% TFA) in a linear gradient with a flow rate of 6 mL/min. After the eluent was removed *in vacuo*, the product was dissolved in degassed H_2O.

Determination of yields: An aliquot was diluted with buffer (10 mM NaH_2PO_4, 10 mM NaCl, pH 7.0) to 1 mL and the optical density measured at λ = 260 nm using a quartz cuvette with a 1 cm path length. The sample concentration was calculated using oligo calculation at http://proligo2.proligo.com/Calculation/calculation.html.

Characterization: Analytical HPLC was performed on a Merck–Hitachi Elite LaChrom instrument (column: Varian Polaris C18 A 5µ 250x46, pore size 220 Å) using eluents A (98.9% H_2O, 1% acetonitrile, 0.1% TFA) and B (98.9% acetonitrile, 1% H_2O, 0.1 % TFA) in a linear gradient (I: 3% B – 30% B in 30 min, II: 3% B – 60% B in 30 min, III: 0% B – 40% B in 30 min) at 55 °C with a flow rate of 1 mL/min. Detection of the signals was achieved with a photodiode array detector at wavelength λ = 280 nm. MALDI-TOF mass spectra were recorded with a Voyager-DE Pro Biospectrometry Workstation of PerSeptive Biosystems (matrix: sinapinic acid).

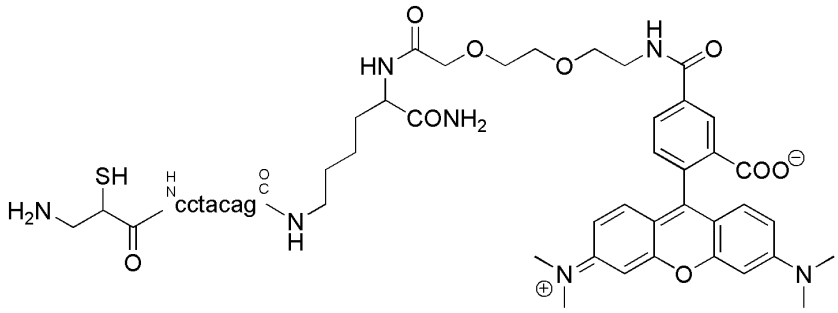
2 Quencher transfer reaction

2.1 Synthesis of probe 6 (FAM-AEEA-tcttccccac-Cys(Dabcyl-Gly)-OH)



The synthesis of probe **6** was performed as described before.[2] OD_{260} = 8.16 (81.0 nmol, 8.1%). HPLC: t_R = 19.2 (gradient II). MALDI/TOF-MS: *m/z* = 3515.8 ($[M+H]^+$, calculated 3515.4). $C_{151}H_{178}N_{56}O_{44}S$ (MW = 3513.44).

2.2 Synthesis of probe 7 (TMR-AEEA-Lys(*i*Cys-cctacag)-NH_2)



The synthesis of probe **7** was performed as described before.[2] OD_{260} = 30.4 (407 nmol, 20%). HPLC: t_R = 16.4 (gradient II). MALDI/TOF-MS: *m/z* = 2669.3 ($[M+H]^+$, calculated 2668.7). $C_{114}H_{143}N_{47}O_{29}S$ (MW = 2667.72).

2.3 Distance dependence of the transfer reaction

Probes **6** and **7** were allowed to react in the presence of target DNA or RNA with varying numbers of unpaired nucleotides (nt) between the recognition segments (Figure S1). Time courses of fluorescence intensities were recorded using a BioRad IQ5-PCR-Cycler. The following filters were used: FAM (EX: 485/30 nm, EM: 530/30 nm), TMR (EX: 545/30 nm, EM: 585/20 nm). Kinetic measurements were carried out in 96-well plates in a buffered solution (50 µL: 10 mM K_2HPO_4, 200 mM NaCl, 1 mM TCEP and 0.2 mg/mL roche blocking reagent). The buffer was adjusted to pH 7.0 using a 2 M HCl solution. Experiments including RNA and the corresponding controls in absence of RNA were carried out under RNase-free conditions. In addition, the RNase inhibitor RiboLock from Fermentas was added to the buffer (final concentration: 0.1 u/µL RiboLock).

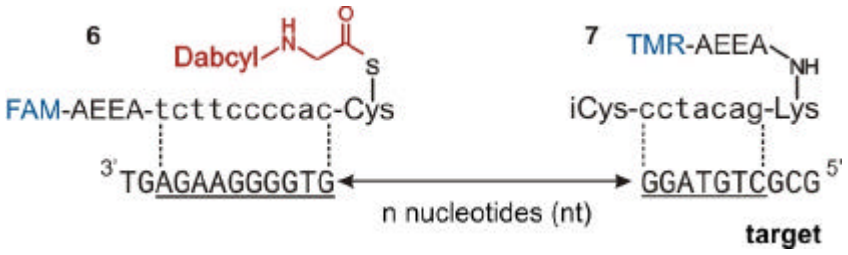


Figure S1. Probe **6** and **7** hybridized with **target** (for RNA: T = U) including varying numbers of unpaired nucleotides (nt, for sequences see Table S1) between recognized segments.

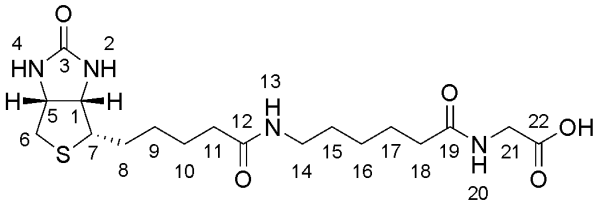
A solution of 100 nM **6** and 100 nM **7** was prepared using the buffer. 50 µL of this solution was placed in the wells and the appropriate amount of **target** was added. The reactions were followed in the IQ5-PCR-cycler at 32 °C for DNA and 37 °C for RNA. Based on the linear phase of FAM-fluorescence time courses the initial rates (v) were determined in absence and presence of **target**. In table S1 the background corrected initial rates are shown.

Table S1. Background corrected initial rates (v = v(with target) – v(without target) / pM·s^{-1}) of the **target**-catalyzed transfer with varying spacing nucleotides (for RNA: T = U). For empty entries (-) no measurements were performed. Values are average of a triplicate of runs. Errors represent the standard deviation. Conditions: 100 nM **6**, 100 nM **7**, 10 mM K_2HPO_4, 200 mM NaCl, 1 mM TCEP, 0.2 mg/mL roche blocking reagent, pH 7.0 (for RNA: 0.1 u/µL RiboLock).

		DNA (32 °C)		**RNA** (37 °C)	
target sequence (5' → 3')	spacing	1 eq.	0.05 eq.	1 eq.	0.05 eq.
GCGCTGTAGG GTGGGGAAGAGT	0 nt	23.9±0.9	0.57±0.04	4.40±0.12	0.45±0.07
GCGCTGTAGGTGTGGGGAAGAGT	1 nt	96.2±3.2	6.91±0.19	-	-
GCGCTGTAGGTTGTGGGGAAGAGT	2 nt	153±10	18.4±1.1	-	-
GCGCTGTAGGTTAGTGGGGAAGAGT	3 nt	196±5	24.7±0.6	186±15	1.00±0.07
GCGCTGTAGGTTATCGTGGGGAAGAGT	5 nt	111±4	15.7±1.2	-	-
GCGCTGTAGGTTATCGAGTGGGGAAGAGT	7 nt	100±8	11.7±0.7	-	-
GCGCTGTAGGTTATCGATTAGTGGGGAAGAGT	10 nt	77.8±3.8	5.66±0.27	69.9±11	0.86±0.10

3 Biotin transfer reaction

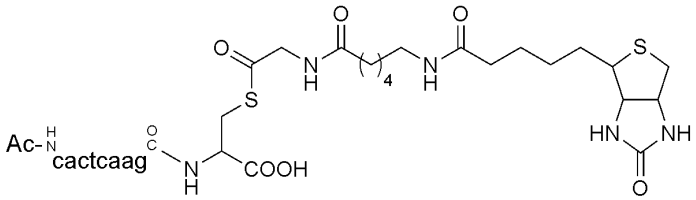
3.1 Synthesis of *N*-(+)-biotinyl-6-aminohexanoylglycine (Biotin-AHX-Gly-OH)



To a suspension of *N*-(+)-biotinyl-6-aminohexanoic acid (75 mg, 0.210 mmol) and *N*,*N*-diisopropylethylamine (0.117 mL, 0.735 mmol) in DMF (17 mL) was added HOBt (31.2 mg, 0.231 mmol) and EDC (63 mg, 0.330 mmol). After stirring at r.t. for 20 min glycine methylester hydrochloride (53 mg, 0.420 mmol) was added and stirred for another 21 h at r.t.. The solvent was evaporated, the residue coevaporated with CH_2Cl_2 (2× 10 mL) and dissolved in H_2O/CH_3CN (3:5, 16 mL). The solution was treated with 2 M NaOH (3.5 mL, 7.0 mmol) and stirred for 2 h at r.t. followed by treatment with 2 M HCl (7.5 mL, 14 mmol). The mixture was concentrated to 2 mL, filtered and the filtrate purified by preparative HPLC to give the product in 40 % yield (30 mg, 84 µmol). HPLC: t_R = 20.4 min (gradient III). HRMS: *m/z* = 415.2016 ($C_{18}H_{31}N_4O_5S$ [M+H$^+$], calculated 415.2010). ^{1}H-NMR (400 MHz, DMSO-d_6): d [ppm] = 1.21 – 8.41 (m, 12 H; 8 – 10, 15 – 17-C**H**$_2$), 2.03 and 2.10 (m, 4 H; 11-, 18-C**H**$_2$), 2.55 – 2.84 (m, 2 H; 6- C**H**$_2$), 3.00 (m, 2 H; 14-C**H**$_2$), 3.09 (m, 1 H; 7 C**H**), 3.71 (d, 2 H; 21-C**H**$_2$, $^3J_{H,H}$ = 5.9 Hz), 4.12 (m, 1 H; 1-C**H**), 4.30 (m, 1 H; 5-C**H**$_2$), 6.37 (m, 1 H; 4-N**H**), 6.43

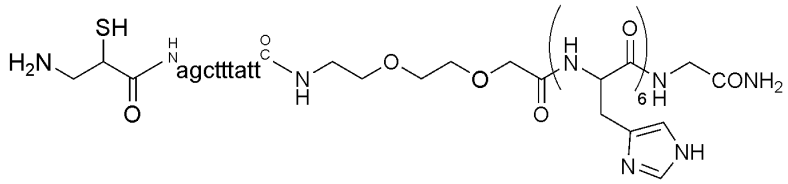
(m, 1 H; 2-N**H**), 7.74 (m, 1 H; 13-N**H**), 8.11 (t, 1 H; 20-N**H**, $^{3}J_{H,H}$ = 5.9 Hz). ^{13}C-NMR (75 MHz, DMSO-d_6): d [ppm] = 24.8, 25.3, 26.0, 28.0, 28.2 and 28.9 (8 – 10, 15 – 17-CH_2), 34.9 und 35.1 (11-, 18-CH_2), 38.2, 39.8 and 40.4 (6-, 14-, 22-CH_2), 55.4, 59.1 and 61.0 (1-, 5-, 7-CH), 162.7 (3-C_q), 171.4, 171.7 and 172.4 (12-, 19-, 22-C_q)

3.2 Synthesis of probe 8 (Ac-cactcaag-Cys(Biotin-AHX-Gly)-OH)



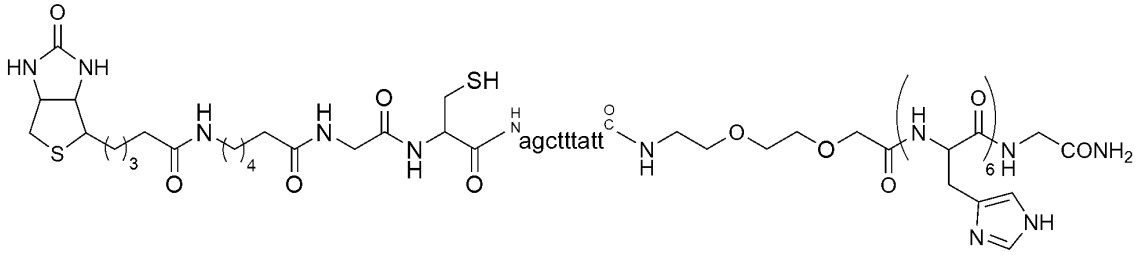
The PNA sequence Fmoc-c^{Bhoc}a^{Bhoc}c^{Bhoc}tc^{Bhoc}a^{Bhoc}a^{Bhoc}g^{Bhoc} was assembled via automated solid-phase Fmoc-synthesis in 2 µmol scale on an Fmoc-Cys(Mmt)-TGA resin (21.5 mg, 0.093 mmol/g). The subsequent synthesis was continued in a syringe reactor. After treatment with DMF/piperidine (4:1, 2× 5 min) the resin was washed (5× DMF, 5× CH_2Cl_2, 5× DMF), treated with pyridine/Ac_2O (10:1) and washed (5× DMF, 10× CH_2Cl_2). For Mmt-deprotection the resin was treated with CH_2Cl_2/TIS/TFA (93:5:2, 4× 10 min), washed (10× CH_2Cl_2, 5× DMF), and reacted with 5 eq. Biotin-AHX-Gly-OH (final concentration ca. 0.05 M in DMF), 5 eq. PyBOP and 12.5 eq. NMM (2× 1.5 h). After washing (5× DMF, 10× CH_2Cl_2) the product was cleaved from the resin by adding TFA/H_2O/*m*-cresol (18:1:1, 0.6 mL + 3 mg Cys-OMe·HCl, 1.5 h) and further extracted with TFA (4× 0.2 mL). OD_{260} = 9.30 (120 nmol, 6.0 %). HPLC: t_R = 18.4 min (gradient I). MALDI/TOF-MS: *m/z* = 2697.0 ($[M+H]^+$, calculated 2697.8). $C_{108}H_{142}N_{52}O_{29}S_2$ (MW = 2696.74).

3.3 Synthesis of probe 9 (*i*Cys-agctttatt-AEEA-His_6-Gly-NH_2)



Using standard Fmoc-based solid phase synthesis Fmoc-AEEA[3]-$(His(Trt))_6$-Gly-rink amid tentagel resin (loading: 0.087 mmol/g) was assembled. The PNA-peptide sequence was built up in 2 µmol scale using automated solid-phase synthesis. For cleavage of the product the resin was washed (5× DMF, 10× CH_2Cl_2), treated with TFA/H_2O/*m*-cresol (90:5:5, 1 mL + 5 mg Cys-OMe·HCl, 1 h) and subsequently extracted with TFA (2× 0.15 mL). OD_{260} = 5.64 (62.1 nmol, 3.1 %). HPLC: t_R = 14.3 min (gradient I). MALDI/TOF-MS: *m/z* = 3569.7 ($[M+H]^+$, calculated 3570.5). $C_{145}H_{186}N_{68}O_{41}S$ (MW = 3569.52).

3.4 Synthesis of probe 15 (Biotin-AHX-Gly-Cys-agctttatt-AEEA-His_6-Gly-NH_2)



Using standard Fmoc-based solid phase synthesis Fmoc-AEEA[3]-$(His(Trt))_6$-Gly-rink amid tentagel resin (loading: 0.087 mmol/g) was assembled. The PNA-peptide sequence Fmoc-Gly-Cys(Trt)-$a^{Bhoc}g^{Bhoc}c^{Bhoc}ttta^{Bhoc}tt$ was built up in 2 µmol scale using automated solid-phase synthesis. The subsequent synthesis was continued in a syringe reactor. After treatment with DMF/piperidine (4:1, 2× 5 min) the resin was washed (5× DMF, 5× CH_2Cl_2, 5× DMF) and reacted with 10 eq. Biotin-AHX-OH, 10 eq. PyBOP and 25 eq. NMM (2× 1.5 h). After washing (5× DMF, 10× CH_2Cl_2) the product was cleaved from the resin by adding TFA/H_2O/*m*-cresol (18:1:1, 0.72 mL + 3 mg Cys-OMe·HCl, 1.5 h) and further extracted with TFA (4× 0.2 mL). OD_{260} = 13.6 (150 nmol, 7.5 %). HPLC: t_R = 18.8 min (gradient I). MALDI/TOF-MS: *m/z* = 3967.5 ($[M+H]^+$, calculated 3967.0). $C_{163}H_{214}N_{72}O_{45}S_2$ (MW = 3966.03).

3.5 HPLC- and plate reader-based readout

The following RNA oligonucleotides were purchased in HPLC quality from Invitrogen:

HIV: 5'-UCAAUAAAGCUUGCCUUGAGUGCUU-3'

RND: 5'-UGCUAUUGGAGUCAGUAUACGCGA-3' (random sequence)

For RNA stock solutions 1 mM ammonium citrate buffer (pH 6.4) was used. Aliquots were stored at -20 °C. All experiments including RNA were carried out under RNase-free conditions. In addition, the RNase inhibitor RiboLock from Fermentas was added to the transfer buffer (**TB**). The following buffer solutions were used:

transfer buffer (TB): 10 mM K_2HPO_4, 200 mM NaCl, 1 mM TCEP, 0.2 mg/mL roche blocking reagent, 0.2 u/µL RiboLock, pH 7.0

1st wash buffer (WB1): 10 mM NaH_2PO_4, 137 mM NaCl, 2.7 mM KCl, 2 % Tween 20, pH 7.0

blocking buffer (BB): 50 mM NaH_2PO_4, 25 mM lysine, 1 mM EDTA, 0.05 % Tween 20, 3 % BSA, pH 7.0

2nd wash buffer (WB2): 10 mM NaH_2PO_4, 137 mM NaCl, 2.7 mM KCl, 0.05 % Tween 20, pH 7.0

HPLC/MS-based readout: The transfer reaction was monitored by HPLC/MS. Probes **8** and **9** were used in a concentrations between 500 and 750 nM to obtain detectable signals at λ = 260 nm. Using **TB** a 1.2/1.5 µM solution of probe **8** and a 1 µM solution of probe **9** were prepared. 50 µL of each solution were combined in an Eppendorf tube and the appropriate amount of RNA was added. The probes were allowed to react at 25 °C. For HPLC/MS analysis the reaction was quenched by adding formic acid (2 µL). Aliquots of 100 µL were analyzed by analytical HPLC/MS on an Agilent 1100 system (column: Varian Polaris C18 A 5µ 250×46, pore size 220 Å) with a VL-quadrupol mass spectrometer using eluents A (98.9% H_2O, 1% acetonitrile, 0.1% formic acid) and B (98.9% acetonitrile, 1% H_2O, 0.1 % formic acid) in a linear gradient (3% B – 18% B in 20 min at 55 °C) with a flow rate of 1 mL/min. Detection of the signals was achieved with a UV-detector at wavelength λ = 260 nm.

Figure S2A shows the chromatograms of the reaction of 600 nM **8** with 500 nM **9**. At the beginning (bottom) the two reactant peaks (**8** and **9**) were detected. After 30 min in absence of RNA (middle) no product formation was observed. After the same time in presence of 1 eq. **HIV** (500 nM, top) a decrease of the reactant peaks (**8** and **9**) and the appearance of the product peaks (**10** and **11**) was observed. The found product masses were in agreement with the calculated values for **10** and **11** (Figure S2B).

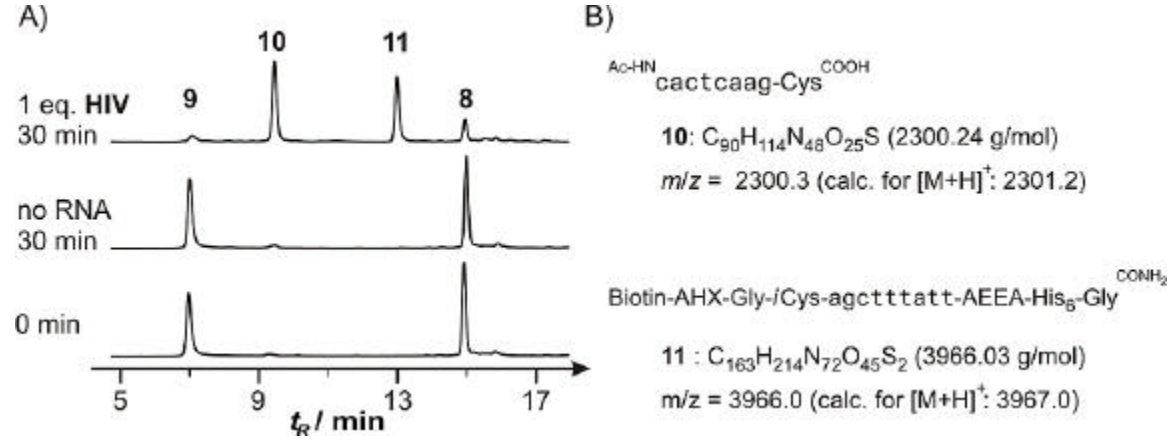
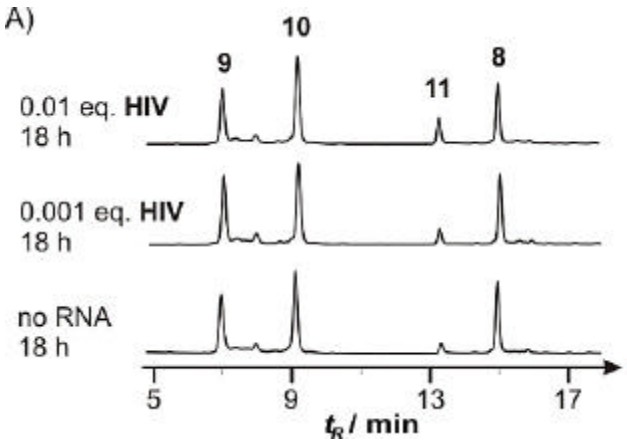


Figure S2. A) HPLC traces of the reaction of 600 nM **8** with 500 nM **9** after 30 min in the presence and absence of **HIV**. Conditions: 10 mM K_2HPO_4, 200 mM NaCl, 1 mM TCEP, 0.2 mg/mL Roche blocking reagent, 0.2 u/µL RiboLock, pH 7.0, 25 °C; B) Found and calculated masses for product probes **10** and **11**.

Figure S3A shows the chromatograms of the reaction of 750 nM **8** with 500 nM **9** after 18 h in absence of RNA (bottom) and in presence of 0.001 eq. (middle) and 0.01 eq. (top) **HIV**. A triplicate of runs provided the yields of transfer product **11** in Figure S3B. After 18 h in presence of 0.001 eq. **HIV** a transfer yield of 21.1 % was observed. Subtraction of the yield in absence of RNA (10.8 %) provides the yield based on RNA-catalysis (10.3 %). This yield corresponds to a turnover number of 103. The unselective hydrolysis of the thioester bond in

probe **8** contributes to the formation of **10**. Therefore, **10** is formed faster than **11**, providing 57 % – 63 % yield of **10** after 18 h.



B)

amount of **HIV**	yield of **11** after 18 h	turnover number
0.01 eq. (5 nM)	31.1±0.9 %	20±1
0.001 eq. (0.5 nM)	21.1±1.0 %	103±12
-	10.8±0.7 %	-

Figure S3. A) HPLC traces of the reaction of 750 nM **8** with 500 nM **9** after 18 h in the presence and absence of **HIV**. Conditions: 10 mM K_2HPO_4, 200 mM NaCl, 1 mM TCEP, 0.2 mg/mL Roche blocking reagent, 0.2 u/µL RiboLock, pH 7.0, 25 °C; B) Yields and turnover numbers after 18 h. Values are average of a triplicate of runs. Errors represent the standard deviation.

Plate reader-based readout: The RNA-catalyzed transfer of the biotin group (reaction **I**) was detected by using an enzyme-based readout (Figure S4A). Transfer product **11** was immobilized on Ni-coated wells and incubated with a horseradish peroxidase-streptavidin conjugate (HRP-SA). The following catalytic oxidation of TMB provided the amplified signal (reaction **II**). In control experiments biotin(**BT**)- and His_6-labeled probe **15** (a mimic of transfer product **11**) was detected without performing the catalyzed transfer reaction (Figure S4B).

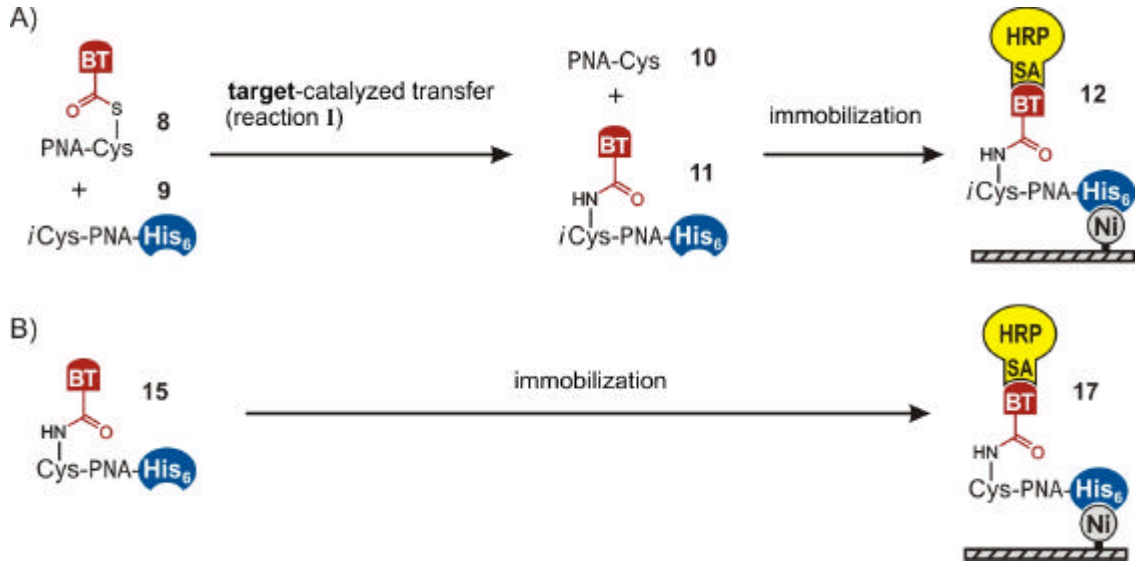


Figure S4. A) **target**-catalyzed transfer of the biotin (**BT**) group yields probe **11** that is subsequently immobilized and binds a horseradish peroxidase-streptavidin conjugate (HRP-SA); B) **BT**- and His_6-labelled probe **15** is immobilized.

The transfer probes were applied in concentrations between 10 and 15 nM as low concentrations minimize the rate of the off-target transfer reaction. In each Eppendorf tube 750 fmol probe **8** (15 nM), 500 fmol probe **9** (10 nM) and target RNA were premixed in 50 µL **TB**. The aqueous solutions were allowed to react at 23 °C for 2.5 h and 5 h, respectively. Then each 50 µL were loaded into a well of transparent nickel coated HisGrab 8-well strips (96-well format, Pierce). The wells were incubated at 23 °C for 30 min to immobilize His_6-labeld probes and washed (subsequently with water, **WB1** and water). For the immobilization of HRP-SA (Pierce) on biotin-labeled probes the wells were incubated with 0.5 µg/mL or 2 µg/mL HRP-SA in **BB** (70 µL) at 37 °C for 30 min. After washing (subsequently with water, **WB2** and water) the wells were incubated with 1-step ultra TMB-ELISA (80 µL, Pierce) at 30 °C for 25 min. To stop the oxidation reaction 1 M H_2SO_4 (40 µL) was added. The wells were scanned at λ = 450 nm using a BMG Labtech FLUOstar OPTIMA. The background absorbance (wells without RNA) was subtracted. The control experiments including probe **15** (Figure S4B) followed this protocol, but lacked the initial RNA-catalyzed reaction. Therefore, the transparent nickel coated HisGrab wells were directly incubated with **probe 15** at 23 °C for 30 min.

Table S3 gives the background subtracted absorbance at λ = 450 nm including the standard deviation (triplicate of runs). The transfer reaction (reaction **I**) was performed for 2.5 h or 5 h followed by the addition of 0.5 µg/mL HRP-SA. As a control 500 fmol RNA sequence **RND** were added but did not provide a detectable signal (0.02±0.02).

Table S3. Background subtracted absorbance at λ = 450 nm in presence of varying amounts of RNA following the detection protocol described above (0.5 µg/mL HRP-SA). For empty entries (-) no measurements were performed. Values are average of a triplicate of runs. Errors represent the standard deviation.

conditions (in 50 µL TB)	**500 fmol**	**50 fmol**	**5 fmol**
Figure S4A			
HIV (750 fmol **8**, 500 fmol **9**, 2.5 h reaction **I**)	3.18±0.05	2.13±0.09	0.23±0.03
HIV (750 fmol **8**, 500 fmol **9**, 5 h reaction **I**)	-	3.04±0.08	0.31±0.06
RND (750 fmol **8**, 500 fmol **9**, 2.5 h reaction **I**)	0.02±0.02	-	-
Figure S4B			
immobilization of biotin product **15**	3.49±0.11	0.49±0.04	0.07±0.02

To increase the sensitivity for target RNA reaction **I** was performed for 5 h followed by the addition of 2 µg/mL HRP-SA. Table S4 gives the background subtracted absorbance at λ = 450 nm including the standard deviation (triplicate of runs). These conditions allowed the

detection of **HIV** amounts as low as 500 attomol. As a control 5 fmol RNA sequence **RND** were added but did not provide a detectable signal (0.00±0.02).

Table S4. Background subtracted absorbance at λ = 450 nm in presence of varying amounts of RNA following the detection protocol described above (2 µg/mL HRP-SA). For empty entries (-) no measurements were performed. Values are average of a triplicate of runs. Errors represent the standard deviation.

conditions (in 50 µL TP)	5 fmol	2 fmol	0.5 fmol	0.1 fmol
Figure S4A				
HIV (750 fmol **8**, 500 fmol **9**, 5 h reaction **I**)	0.59±0.05	0.34±0.03	0.13±0.02	0.04±0.03
RND (750 fmol **8**, 500 fmol **9**, 5 h reaction **I**)	0.00±0.02	-	-	-

4 Abbreviations

AEEA	[2-(2-aminoethoxy)ethoxy]acetyl
AHX	6-aminohexanoyl
Dabcyl	4-[4-dimethylamino)phenylazo]benzoyl
DMF	*N*,*N*-dimethylformamide
EDC	*N*-(dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride
FAM	6-carboxyfluorescein
Fmoc	9-fluorenylmethoxycarbonyl
HCTU	2-(6-Chloro-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate
HOBt	*N*-hydroxybenzotriazole
HRP-SA	horseradish peroxidase-streptavidin conjugate
Mmt	4-monomethoxytrityl
NMM	*N*-methylmorpholine
NMP	*N*-methyl-2-pyrrolidon
PNA	peptide nucleic acid
PyBOP	benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
r.t.	room temperature
TCEP	tris(2-carboxyethyl)phosphine
TFA	trifluoroacetic acid
TIS	triisopropylsilane
TMB	3,3',5,5'-tetramethylbenzidine
TMR	5-carboxytetramethylrhodamine

5 References

[1] C. Dose, O. Seitz, *Org. Biomol. Chem.* **2004**, *2*, 59-65.

[2] T. N. Grossmann, O. Seitz, *J. Am. Chem. Soc.* **2006**, *128*, 15596-15597.

[3] J. V. Aldrich, V. Kumar, US 2003/0134989 A1.