Angewandte Chemie

Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

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Photoinduced Reductive Electron Transfer in LNA:DNA Hybrids: A Compromise between Conformation and Base Stacking**

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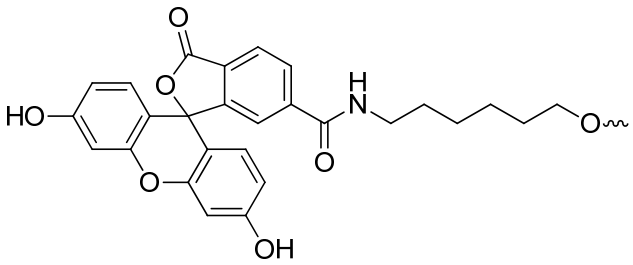
Oligonucleotide Synthesis

Unmodified and fluorescein-modified oligonucleotides were purchased. Pyrene-modified deoxyribonucleic acids were prepared on a DNA synthesizer on controlled pore glass (CPG) (1 µmol). Fluorescein and BrdU phosphoramidites were commercially available. The pyrene phosphoramidite was synthesized according to our previously published protocol.[1] After deprotecting with aq. NH_3 (25%) at r.t. over 24 h, the oligonucleotides were purified by HPLC on a semi-preparative RP-C18 column (300 Å), using the following conditions: A) NH_4OAc buffer (50 mM, pH 6.5); B) MeCN, gradient 0 – 20 % B over 45 min, flow rate 2.5 mL/min. UV/VIS detection at 260 nm and 510 nm. The oligonucleotides were lyophilized, identified by MALDI mass spectrometry, and quantified by UV/Vis absorption using extinction coefficients of 18600 L/mol·cm for 2PydU, 5100 L/mol·cm for BrdU and 20900 L/mol·cm for fluorescein, each at 260 nm. Duplexes were formed by heating in the presence of 1.0 equiv. counterstrand for UV/Vis absorption spectroscopy and 1.2 equiv. counterstrand for strand cleavage experiments, each at 90 °C for 5 min followed by slow cooling to r.t.

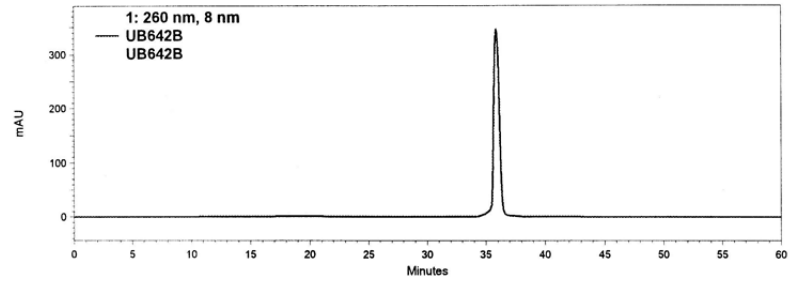
5'-Fluorescein modification (Fluo):



LNA-modified oligonucleotides were synthesized in 1.0 µmol scale using a DNA synthesizer- Standard cycle procedures (2 min coupling time; ~99% stepwise coupling yield) were applied for unmodified phosphoramidites using 0.45 M solution of 1*H*-tetrazole as activator whereas 6 min coupling time (~99% stepwise coupling yield) was applied for LNA phosphoramidites. The oligonucleotides were purified by DMT-ON RP-HPLC using a C18-column (10 µm, 300 mm × 7.8 mm) and the following eluent system: eluent-A, 95% 0.1 M Et_3N, 5% CH_3CN; eluent-B, 25% 0.1 M $Et_3NH{\cdot}HCO_3$, 75% CH_3CN; gradient, 0-5 min isocratic hold of 100% eluent-A, followed by a linear gradient to 55% eluent-B over 75 min at a flow rate of 1.0 mL/min. Fractions containing pure oligonucleotides were collected and evaporated on speed-vac followed by detritylation (80% aq. AcOH, 20 min), precipitation (anhydrous acetone, 1000 µL, 18 °C, 12 h) and washing with anhydrous acetone (3×1000 µL). Oligonucleotides were finally desalted using commercially available NAP-10 columns. Their composition and purity were verified by MALDI-MS analysis and ion-exchange HPLC (100 mm × 4.6 mm column size), respectively. The following eluent system was used: eluent-A, 25 mM Tris-Cl, 1 mM EDTA (pH 8.0); eluent-B, 1 M NaCl, gradient, 0-5 min isocratic hold of 95% eluent-A followed by a linear gradient to 70% eluent-B over 41 min at a flow rate of 0.75 mL/min.

Images of HPLC analyses

II



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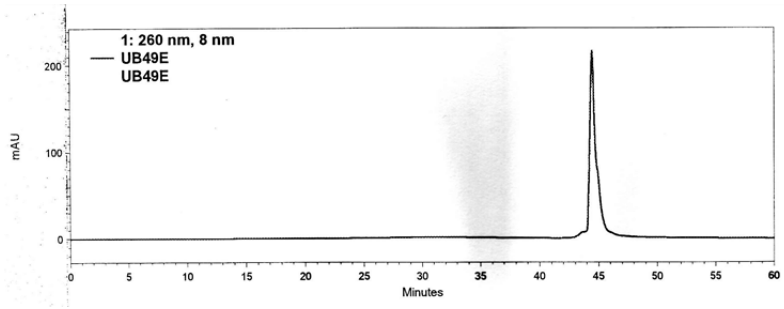

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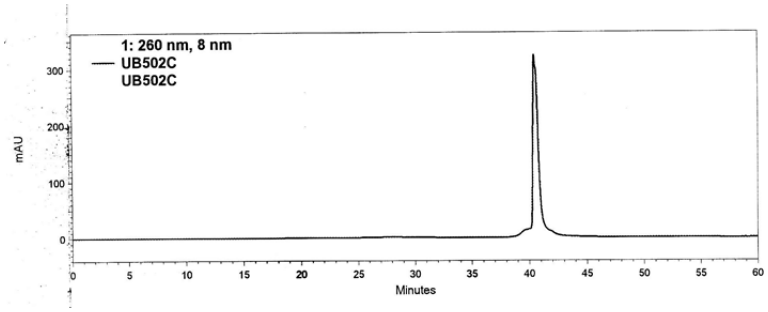

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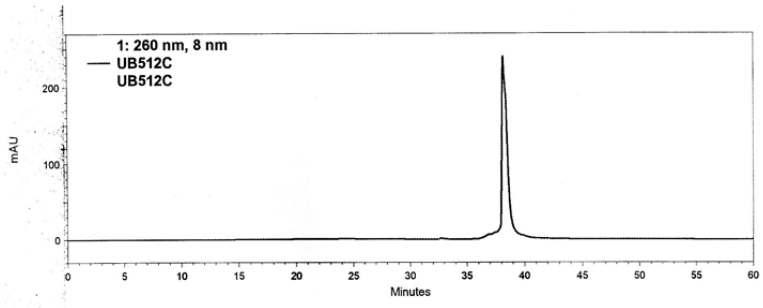

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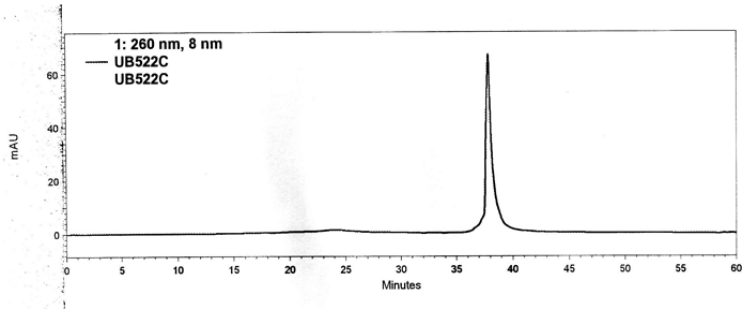

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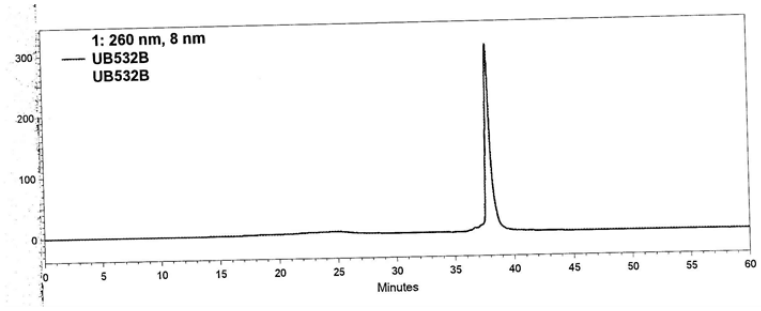
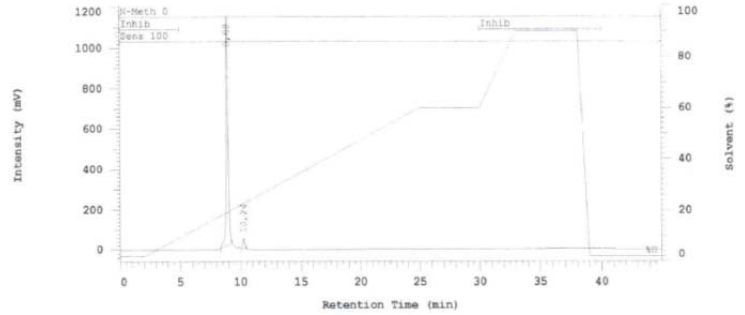
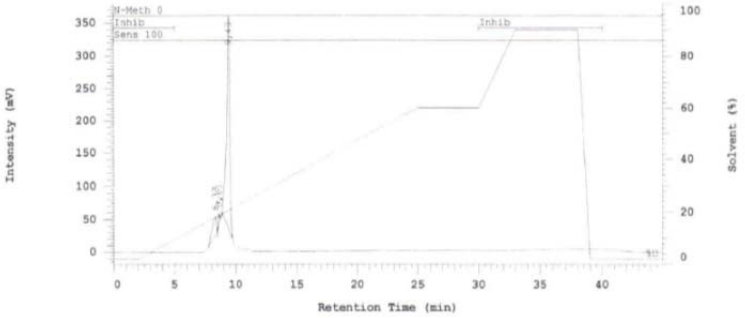


lna

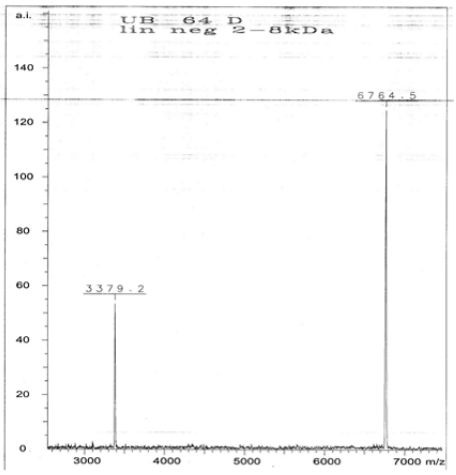


LNA



Images of MALDI mass spectrometry

II



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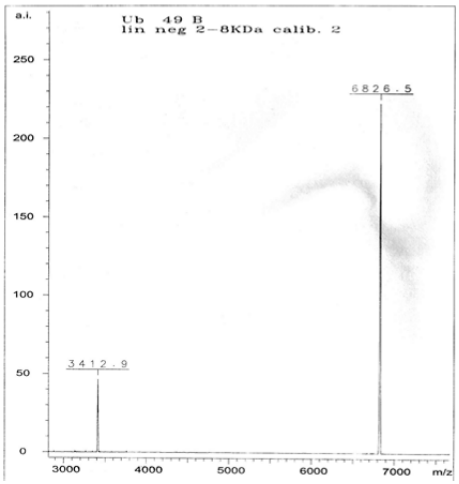

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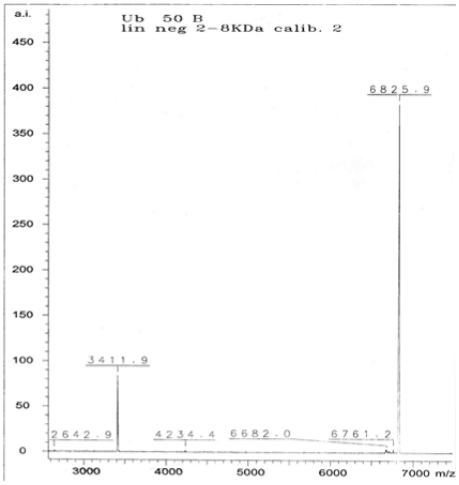

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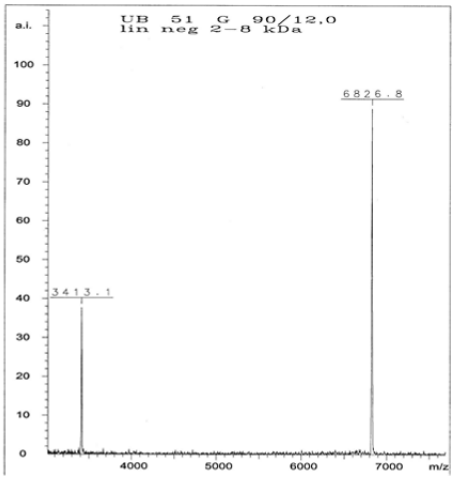

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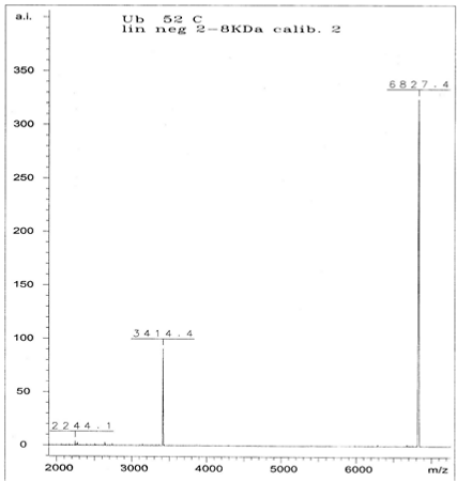

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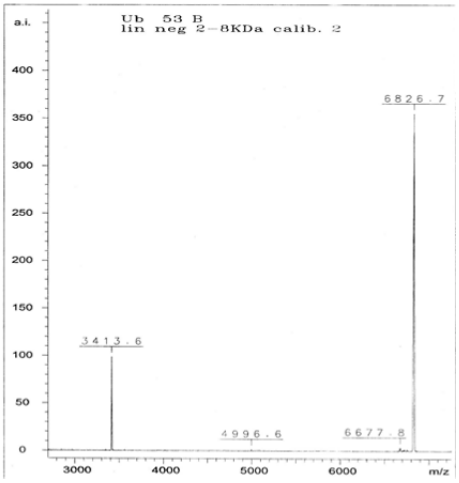
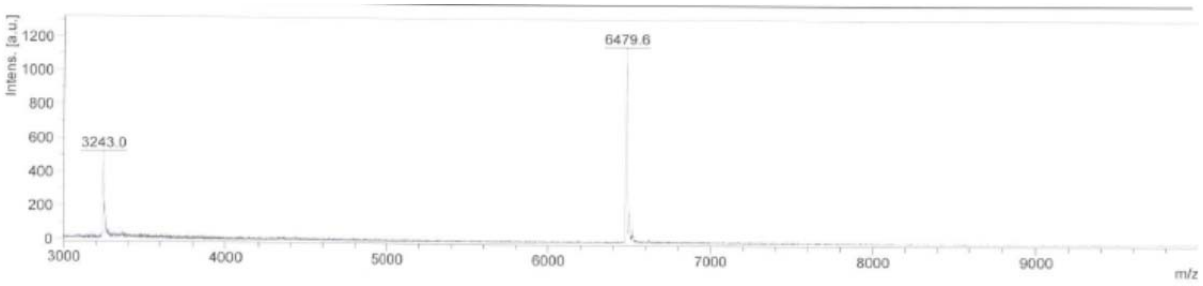


lna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1000
800
600
400
200
0
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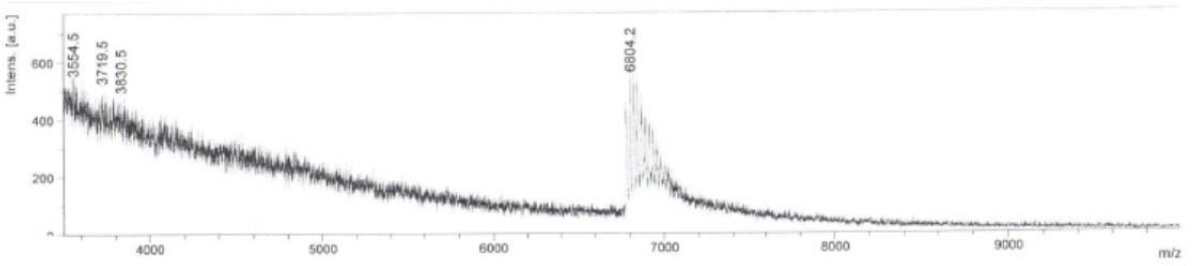
6479.6
3000
4000
5000
6000
7000
8000
9000
m/z

LNA


Intens. [a.u.]
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400
200
3554.5
3719.5
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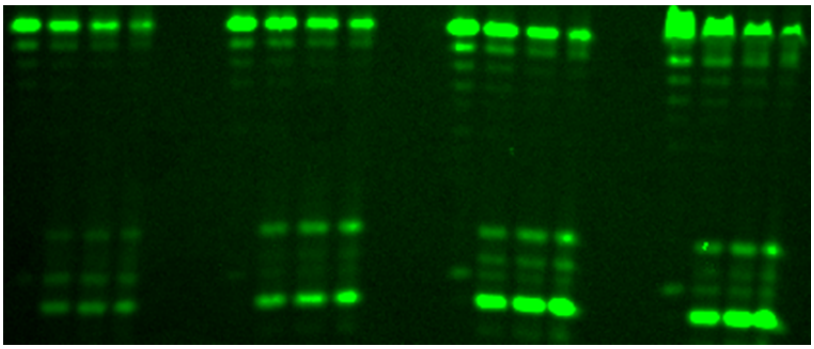
6804.2
4000
5000
6000
7000
8000
9000
m/z

Strand cleavage Experiments

500 µL of oligonucleotide solution (1.0 µM, 10 mM NaP_i, 250 mM NaCl) were irradiated using a 200 W Xe/Hg Lamp equipped with a 320 nm cutoff filter (WG 320, 3.0 mm, air cooled). Over 30 min. every 5 min. aliquots (20 µL) were taken, 20 µL piperidine solution (20 %) were added, and the samples heated to 90 °C for 30 min. The solvent was removed using a speedvac concentrator. The samples were dissolved in 12µL loading buffer (80 % formamide, 20 mM EDTA, 0,02 % xylene cyanol FF) and a denaturating polyacrylamide gel electrophoresis (12%) was performed. The gels were analyzed by an imaging system equipped with adequate excitation and emission filter for fluorescein. The data analysis was performed using AIDA evaluation software package.

Example for PAGE (III-1_DNA, III-1_RNA, III-1_lna, III-1_LNA)



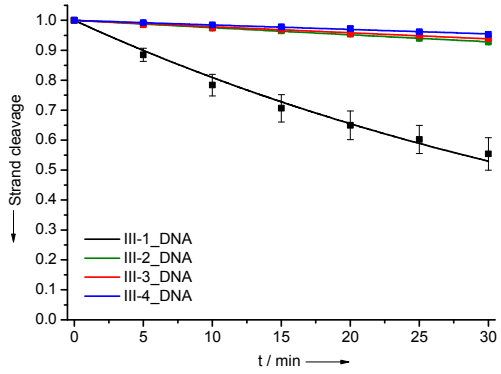
The cleavage yield was calculated from the intensity of the cleavage relative to the total intensities. The values determined at 0 min. irradiation were used as background for subsequent determinations. All experiments were repeated three times to get mean and SD values.

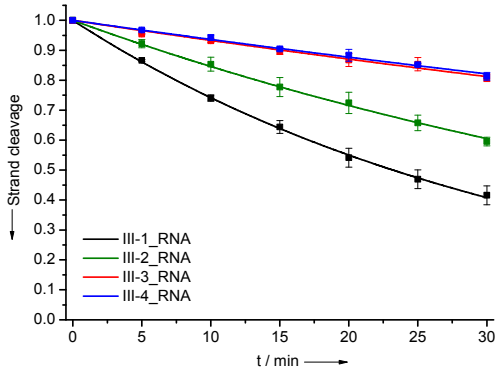
	III-1_DNA		III-2_DNA		III-3_DNA		III-4_DNA	
t / min	mean	SD	mean	SD	mean	SD	mean	SD
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
5	0.885	0.022	0.989	0.004	0.985	0.005	0.992	0.004
10	0.784	0.036	0.974	0.001	0.975	0.004	0.985	0.004
15	0.706	0.046	0.964	0.004	0.969	0.006	0.978	0.007
20	0.649	0.048	0.954	0.005	0.957	0.011	0.973	0.008
25	0.602	0.047	0.939	0.003	0.950	0.007	0.962	0.004
30	0.554	0.054	0.927	0.005	0.939	0.002	0.953	0.003

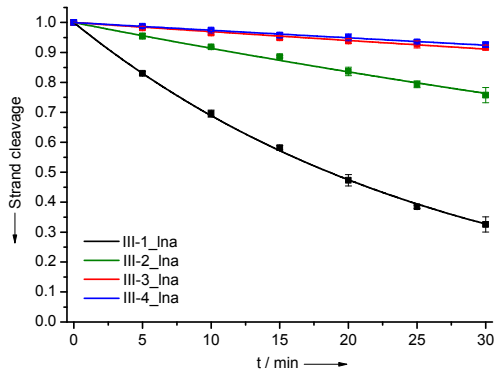
	III-1_RNA		III-2_RNA		III-3_RNA		III-4_RNA	
t / min	mean	SD	mean	SD	mean	SD	mean	SD
0	1.000	0.000	1.000	0.000	1.000	0.001	1.000	0.000
5	0.866	0.003	0.922	0.014	0.954	0.002	0.968	0.006
10	0.740	0.011	0.854	0.023	0.932	0.006	0.942	0.009
15	0.644	0.022	0.777	0.032	0.897	0.011	0.904	0.006
20	0.541	0.031	0.724	0.036	0.868	0.022	0.883	0.020
25	0.469	0.031	0.657	0.026	0.854	0.023	0.852	0.004
30	0.416	0.031	0.595	0.014	0.808	0.013	0.813	0.013
	III-1_lna		III-2_lna		III-3_lna		III-4_lna	
t / min	mean	SD	mean	SD	mean	SD	mean	SD
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
5	0.830	0.006	0.955	0.009	0.982	0.006	0.987	0.004
10	0.696	0.012	0.919	0.003	0.964	0.006	0.974	0.005
15	0.582	0.007	0.886	0.004	0.949	0.006	0.958	0.009
20	0.473	0.019	0.837	0.014	0.938	0.008	0.951	0.012
25	0.384	0.006	0.794	0.011	0.927	0.012	0.935	0.007
30	0.326	0.025	0.758	0.025	0.917	0.008	0.926	0.009
	III-1_LNA		III-2_LNA		III-3_LNA		III-4_LNA	
t / min	mean	SD	mean	SD	mean	SD	mean	SD
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
5	0.760	0.036	0.976	0.006	0.997	0.002	0.998	0.002
10	0.577	0.030	0.959	0.002	0.994	0.002	0.994	0.001
15	0.452	0.037	0.942	0.006	0.991	0.002	0.992	0.002
20	0.359	0.018	0.930	0.004	0.986	0.001	0.990	0.001
25	0.301	0.025	0.915	0.006	0.984	0.001	0.987	0.002
30	0.235	0.030	0.903	0.005	0.981	0.002	0.983	0.002

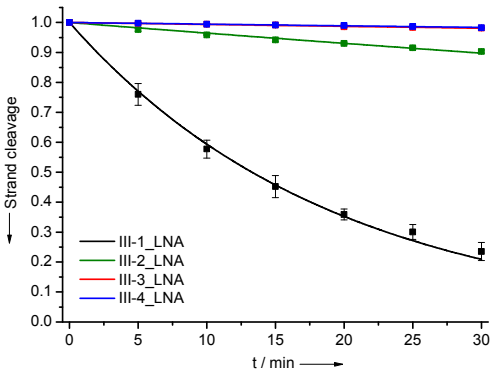
The data were fitted monoexponential. $y = e^{-k/t}$

Duplex	k	error	COD R^2
III-1_DNA	2.117E-02	6.46E-04	0.986
III-2_DNA	2.470E-03	3.42E-05	0.997
III-3_DNA	2.130E-03	6.10E-05	0.983
III-4_DNA	1.540E-03	3.44E-05	0.991
III-1_RNA	2.988E-02	2.43E-04	0.999
III-2_RNA	1.673E-02	1.98E-04	0.998
III-3_RNA	6.930E-03	1.89E-04	0.986
III-4_RNA	6.560E-03	1.25E-04	0.994
III-1_lna	3.724E-02	3.07E-04	0.999
III-2_lna	9.000E-03	1.62E-04	0.995
III-3_lna	3.100E-03	9.44E-05	0.981
III-4_lna	2.630E-03	4.14E-05	0.995
III-1_LNA	5.214E-02	1.16E-03	0.996
III-2_LNA	3.600E-03	1.08E-04	0.981
III-3_LNA	6.460E-04	1.75E-05	0.987
III-4_LNA	5.509E-04	1.21E-05	0.991









To obtain strand cleavage rates, the plots of the initial phase of the reaction were fitted linearily.

Duplex	strand cleavage rate / min^{-1}	error	COD R^2	ln (strand cleavage rate / min^{-1})
III-1_DNA	1.85E-02	8.58E-05	1.00	-3.99
III-2_DNA	2.41E-03	1.74E-06	1.00	-6.03
III-3_DNA	2.08E-03	1.30E-06	1.00	-6.18
III-4_DNA	1.52E-03	6.84E-07	1.00	-6.49
III-1_RNA	2.58E-02	1.47E-04	1.00	-3.66
III-2_RNA	1.46E-02	6.03E-05	1.00	-4.23
III-3_RNA	6.42E-03	1.29E-05	1.00	-5.05
III-4_RNA	6.10E-03	1.16E-05	1.00	-5.10
III-1_lna	3.25E-02	2.01E-04	1.00	-3.43
III-2_lna	8.15E-03	2.11E-05	1.00	-4.81
III-3_lna	2.99E-03	2.70E-06	1.00	-5.81
III-4_lna	2.55E-03	1.95E-06	1.00	-5.97
III-1_LNA	4.71E-02	2.99E-04	1.00	-3.06
III-2_LNA	3.46E-03	3.63E-06	1.00	-5.67
III-3_LNA	6.41E-04	1.21E-07	1.00	-7.35
III-4_LNA	5.47E-04	8.84E-08	1.00	-7.51

[1]Wanninger-Weiß, C.; Wagenknecht, H.-A. *Eur. J. Org. Chem*. **2008**, 64-71.