CHEMISTRY
A European Journal

Supporting Information

Excess Electron Transfer through DNA Duplexes Comprising a Metal-Mediated Base Pair

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chem_201702241_sm_miscellaneous_information.pdf

Author Contributions

S.H. Investigation: Equal; Writing – original draft: Supporting
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P.S. Investigation: Supporting
N.M. Investigation: Supporting
U.K. Conceptualization: Supporting; Resources: Equal; Supervision: Equal
J.M. Conceptualization: Lead; Funding acquisition: Lead; Project administration: Lead; Supervision: Equal; Writing – original draft: Lead; Writing – review & editing: Lead.

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Experimental section

2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-β-D-*erythro*-pentafuranosyl azide was synthesized according to a literature procedure.[1] Phosphoramidites of the canonical nucleosides were purchased from Glen Research. Oligonucleotide synthesis and purification were performed as previously reported.[2] The desalted oligonucleotides were characterized by MALDI-TOF (matrix-assisted laser desorption-ionization time-of-flight) mass spectrometry. MALDI-TOF mass spectra were recorded on a Bruker Reflex IV instrument using a 3-hydroxypicolinic acid/ammonium citrate matrix. Electrospray ionization experiments (ESI-TOF) were performed using the oa-TOF mass spectrometer MicrOTOF (Bruker Daltonics GmbH, Bremen, Germany). The MicrOTOF was equipped with a standard ESI source. All mass spectra are quasi-internally mass calibrated by the measurement of an infused calibrant (ammonium formate) prior to the compound of interest. During the quantification of the oligonucleotides, a molar extinction coefficient ε_{260} of 10 cm^2 μmol^{-1} was used for d**N**. NMR spectra were recorded using Bruker Avance(I) 400 and Avance(III) 400 spectrometers at 300 K. Chemical shifts were recorded with reference to TMS (tetramethylsilane) ($CDCl_3$, δ = 0 ppm) or to the residual solvent peak ($CDCl_3$, δ = 7.26 ppm). CD spectra were recorded at 10 °C on a Jasco J-815 spectrometer. The spectra were smoothed. The UV melting experiments were carried out on a CARY 100 Bio instrument. In the melting curves, the absorbance was normalized ($A_{norm} = (A–A_{min})/(A_{max}–A_{min})$ at 260 nm). Oligonucleotide solutions for the spectroscopic characterization contained 1.5 μM oligonucleotide duplex, 150 mM $NaClO_4$ and 5 mM MOPS buffer (pH 6.8). Irradiation measurements were performed using a 500 W Hg/Xe arc lamp (Newport) equipped with a 1.5 inch water filter and a 335 nm longpass filter (Schott) with water-cooled (12 °C) solutions (100 μL) containing 1.5 μM oligonucleotide duplex, 150 mM $NaClO_4$, and 5 mM MOPS buffer (pH 6.8). Subsequent enzymatic digestion to the respective nucleosides was performed following standard protocols.[3] In particular, an enzyme mix composed of Benzonase® (250 U), phosphodiesterase I (300 mU) and alkaline phosphatase (200 U) in a buffered solution (TRIS-HCl (20 mM, pH 7.9) with NaCl (100 mM) and $MgCl_2$ (20 mM)) was added to 100 μL of the respective oligonucleotide solution. The mixture was incubated for 6 h at 37 °C, followed by deactivation of the enzymes at 60 °C. After ultrafiltration using Nanosep® 10K Omega centrifugal filters, the digested nucleosides were ready for the LC/ICP-MS analysis.

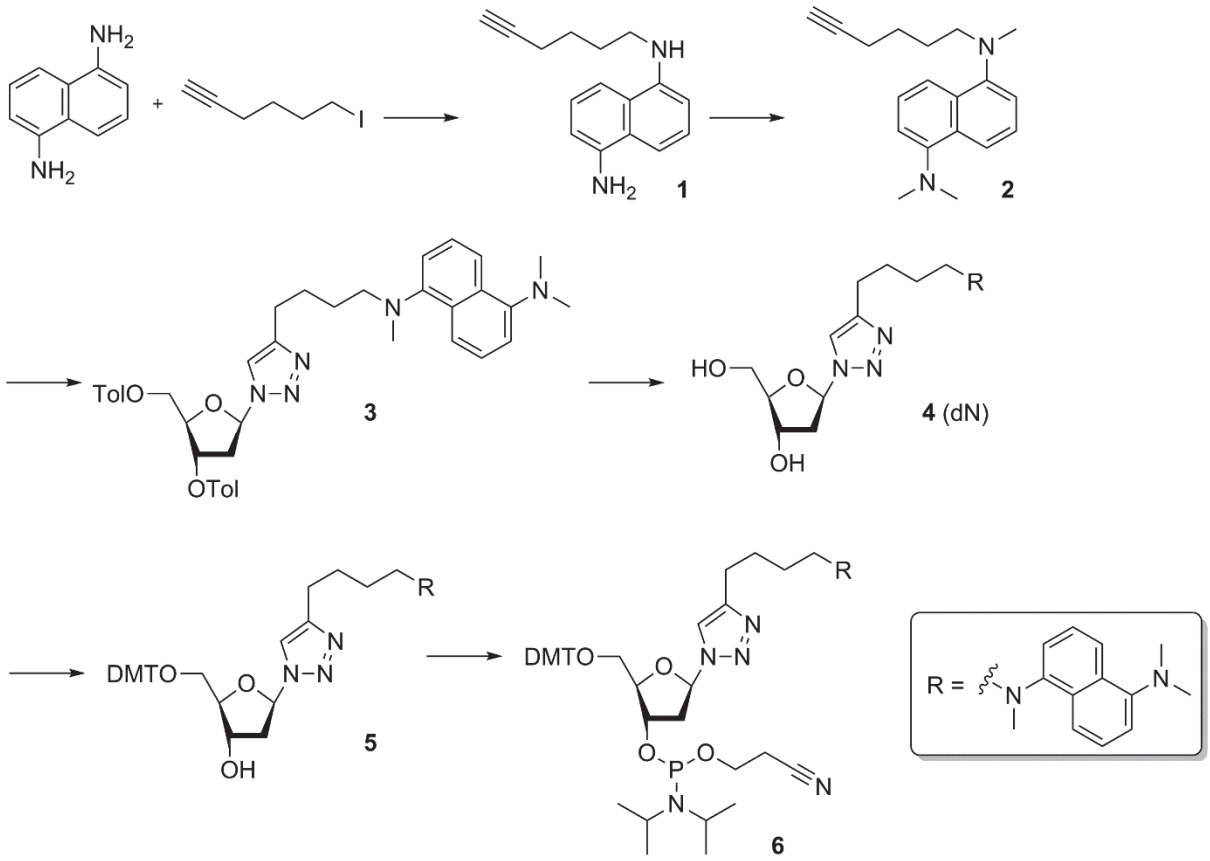
For the detection and quantification of d**B**, a high performance liquid chromatographic separation coupled to an inductively coupled plasma mass spectrometer (HPLC/ICP-MS) was used. For the chromatographic separation, a Shimadzu LC system (Duisburg, Germany) consisting of an SCL-10AVP controller, two LC-10ADVP pumps, a SIL-10A autosampler, a DGU-14A degasser, and a CTO-10AS column oven were utilized. 20 µL of sample solution were injected onto a reversed-phase C18 column (Hypersil Gold, 150 × 2.1 mm, 3 μm particle size, 175 Å, Thermo Scientific, Bremen, Germany). The isocratic separation was carried out at 40 °C using 10% methanol in bidistilled water as eluent. The total flow rate was set to 300 μL min^{-1}. Detection of the two mass traces m/z = 79 and m/z = 81 for bromine were carried out using a PlasmaQuant MS Elite ICP-MS (Analytik Jena, Jena, Germany) with a PFA micromist Nebulizer and a Scott-geometry spray chamber. Detailed mass spectrometric parameters are given in Table S1. The samples were quantified using an external calibration consisting of diluted

bromide ICP-MS standard solutions in bidistilled water with 10% methanol to receive bromine concentrations of 1, 5, 10, 50, 100, 200, 500 and 1000 μg L^{-1}. The bromide ICP-MS standard (≥99.9%) was obtained from High Purity Standards (Charleston, SC, USA), methanol (HiPerSolv Chromanorm) from VWR (Leuven, Belgium). Prior to use, water was purified using an Aquatron A4000D system (Barloworld Scientific, Nemours, France).

In a typical measurement, two to three samples were injected into the LC/ICP-MS. As a result, four to six concentration values were determined for each data point based on the ^{79}Br and ^{81}Br traces. The arithmetic mean was calculated for each data point. To allow an easy comparison of the different duplexes, the dataset for each duplex was normalized.

Table S1. Mass spectrometric parameters for the determination of exact masses of d**B** analyzed by ICP-MS.

RF Power	1.20 kW
Plasma Flow	8 mL min^{-1}
Auxiliary Flow	1.50 mL min^{-1}
Sheath Gas Flow	0.10 mL min^{-1}
Nebulizer Gas Flow	1.00 mL min^{-1}
Spray chamber temperature	2 °C
Sampling Depth	5.0 mm
Extraction Lens 1 voltage	–55 V
Extraction Lens 2 voltage	–420 V
Extraction Lens 3 voltage	–460 V
Corner Lens voltage	–440 V
Dwell time per *m*/*z*	300 ms
CRI Gas	He
CRI Gas Flow	120 mL min^{-1}
Nitrox 200 Gas Flow	20 mL min^{-1}



Scheme S1. Synthesis of d**N** and its phosphoramidite.

N[1]-(Hex-5-yn-1-yl)naphthalene-1,5-diamine (**1**)

6-Iodo-hexyne (80 µL, 0.32 mmol) was dissolved in EtOH (10 mL) and added dropwise to a solution of 1,5-diaminonaphthalene (200 mg, 1.26 mmol, 4 equiv.) and $NaHCO_3$ (246 mg, 3.14 mmol, 10 equiv.) in EtOH/water (10 mL/ 5 mL) at 75 °C. The reaction mixture was stirred overnight at 75 °C. The solvent was removed under reduced pressure, the residue was suspended in DCM (30 mL) and the organic layer was washed with water (3 × 30 mL). The solvent was removed under reduced pressure and the crude material purified via column chromatography (cyclohexane:EtOAc, 1:1). Yield 85% (76 mg, 0.27 mmol). ^{1}H NMR ($CDCl_3$): δ = 7.29 (m, 4H, H2/H3/H8/H7), 6.79 (d, 1H, H1), 6.71 (d, 1H, H6), 3.32 (t, 2H, H11/H11'), 2.29 (m, 2H, H14/H14'), 1.99 (t, 1H, H16), 1.91 (m, 2H, H12/H12'), 1.73 (m, 2H, H13/H13') ppm. ^{13}C NMR ($CDCl_3$): δ = 143.9 (C5), 142.7 (C10), 125.6 (C9), 125.1 (C4), 124.2 (C7), 124.2 (C2), 110.5 (C8), 109.8 (C3), 109.7 (C6), 104.4 (C1), 84.1 (C15), 68.8 (C16), 43.7 (C11), 28.3 (C12), 26.2 (C13), 18.3 (C14) ppm. MS (ESI-TOF) calc. [*M*+H]$^+$: 239.1543, found 239.1542.

N[1]-(Hex-5-yn-1-yl)-*N*[1],*N*[5],*N*[5]-trimethylnaphthalene-1,5-diamine (**2**)

Compound **1** (64 mg, 0.27 mmol) was dissolved in MeOH/water/THF (4 mL/2 mL/ 2 mL). Next, Na_2CO_3 (114 mg, 1.08 mmol, 4 equiv.) and DMS (136 mg, 1.08 mmol, 4 equiv.) were added and the solution was stirred over night at rt. The solution was neutralized with aqueous NaOH solution (1 M) and extracted with EtOAc (3 × 30 mL). The solvent was removed under reduced pressure. Yield 92% (72 mg, 0.25 mmol). ^{1}H NMR ($CDCl_3$): δ = 7.98 (d, 2H, H1/H6), 7.40 (m, 2H, H2/H7), 7.10 (dd, 2H, H3/H8),

3.10 (t, 2H, H11/H11‘), 2.91 (s, 6H, 2 × CH_3), 2.85 (s, 3H, CH_3), 2.21 (m, 2H, H14/H14’), 1.95 (m, 1H, H16), 1.76 (m, 2H, H12/H12’), 1.61 (dd, 2H, H13/H13’) ppm. ^{13}C NMR ($CDCl_3$): δ = 131.2 (C5), 130.3 (C10), 124.9 (C4), 124.9 (C9), 119.3 (C3), 115.6 (C2/C7), 113.9 (C1/C6), 84.4 (C15), 68.3 (C16), 56.3 (C11), 45.3 (2 × CH_3), 42.8 (CH_3), 26.6 (C13), 26.1 (C12), 18.3 (C14) ppm. MS (ESI-TOF) calc. [*M*+H]$^+$: 281.2021, found 281.2023.

5-(4-(4-((5-(Dimethylamino)naphthalen-1-yl)(methyl)amino)butyl)-1*H*-1,2,3-triazol-1-yl)-β-2’-deoxy-3’,5’-di-*O*-(*p*-toluoyl)-ribonucleoside (**3**)

2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-β-D-*erythro*-pentafuranosyl azide[1] (709 mg, 1.79 mmol, 1.3 equiv.) and compound **2** (501 mg, 1.79 mmol) were dissolved in THF/isopropanol (50 mL, 4:1), then sodium ascorbate (140 mg, 0.706 mmol, 0.4 equiv.) and $CuSO_4 \cdot 5\ H_2O$ (92.0 mg, 0.368 mmol, 0.2 equiv.) were added. The solution was stirred over night at rt, then EtOAc (40 mL) was added and the organic layer was washed with aqueous EDTA solution (0.5%, 2 × 50 mL). The organic layer was dried ($MgSO_4$), the solvent was removed under reduced pressure and the crude material was purified via column chromatography (pentane:EtOAc, 10:7). Yield 40% (480 mg, 0.710 mmol). ^{1}H NMR ($CDCl_3$): δ = 7.94 (m, 2H, Tol), 7.94 (m, 2H, H3/H8), 7.84 (m, 2H, Tol), 7.39 (m, 2H, H2/H7), 7.36 (s, 1H, H16), 7.17 (m, 2H, H1/H6), 7.07 (m, 2H, Tol), 6.41 (pt, 1H, H1’), 5,74 (m, 1H, H4’), 4.61 (m, 2H, H5’/H5’’),4.52 (m, 1H, H3’), 3.10 (m, 2H, H11/H11’), 2.89 (s, 6H, 2 × CH_3), 2.82 (s, 3H, CH_3), 2.64 (t, 2H, H2’/H2’’), 2.43 (s, 3H, Tol-CH_3), 2.33 (s, 3H, Tol-CH_3), 1.64 (m, 6H, H12/H12’/H13/H13’/H14/H14’) ppm. ^{13}C NMR ($CDCl_3$): δ = 166.1 (CO), 165.9 (CO), 148.7 (C15), 144.5 (Tol), 144.1 (Tol), 129.8 (Tol), 129.7 (Tol), 129.3 (Tol), 129.2 (Tol), 126.7 (Tol), 126.5 (Tol), 125.1 (C4/C9), 124.9 C16), 119.2 (C2/C7), 115.6 (C3/C8), 114.0 (C1/C6), 88.7 (C1’), 83.4 (C4’), 74.9 (C3’), 64.0 (C5’), 56.6 (C11), 45.3 (CH_3), 42.9 (CH_3), 38.3 (C2’), 27.1 (C14), 26.8 (C12), 25.4 (C13), 21.7 (CH_3), 21.6 (CH_3) ppm. MS (ESI-TOF) calc. [*M*+H]$^+$: 676.3493, found 676.3501.

5-(4-(4-((5-(Dimethylamino)naphthalen-1-yl)(methyl)amino)butyl)-1*H*-1,2,3-triazol-1-yl)-β-2’-deoxyribonucleoside (**4**)

Compound **3** (480 mg, 0.710 mmol) was dissolved in MeOH (40mL) and aqueos ammonia (25%, 20 mL) was added. The reaction mixture was stirred over night, the solvent was removed under reduced pressure and the crude material was purified via column chromatography (DCM:EtOAc:MeOH, 7:3:2). Yield 83% (259 mg, 0.59 mmol). ^{1}H NMR ($CDCl_3$): δ = 7.99 (m, 2H, H3/H8), 7.40 (m, 2H, H2/H7), 7.20 (s, 1H, H16), 7.09 m, 2H, H1/H6), 6.13 (m, 1H, H1’), 4.69 (m, 1H, H4’), 4.07 (m, 1H, H3’), 3.74 (m, 2H, H5’/H5’’), 3.15 (m, 2H, H11/H11’), 2.90 (s, 6H, 2 × CH_3) , 2.85 (s, 3H, CH_3), 2.75 (m, 1H, H14) , 2.69 (m, 2H, H2’/H2’’), 2.47 (m, 1H, H14’), 1.68 (m, 4H, H12/H12’/H13/H13’) ppm. ^{13}C NMR ($CDCl_3$): δ = 151.0 (C15), 150.4 (C10), 148.0 (C5), 131.3 (C9), 130.2 (C4), 119.2 (C2/C7), 119.0 (C3/C8), 115.8 (C3/C8), 113.9 (C1/C6), 88.3 (C1’), 88.0 (C4’), 71.2 (C3’), 62.5 (C5’), 56.1 (C11), 45.4 (CH_3), 43.4 (CH_3), 41.3 (C2’), 26.8 (C14), 26.7 (C12), 25.2 (C13) ppm. MS (ESI-TOF) calc [*M*+H]$^+$: 440.2656, found 440.2654.

5-(4-(4-((5-(Dimethylamino)naphthalen-1-yl)(methyl)amino)butyl)-1*H*-1,2,3-triazol-1-yl)-β-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-ribonucleoside (**5**)

Compound **4** (728 mg, 1.65 mmol) was co-evaporated with pyridine (3 × 15 mL) and dissolved in dry pyridine (20 mL). DMT-Cl (1.22 g, 3.30 mmol, 2 equiv.) and DMAP (cat.) were added and the mixture was stirred for 3 h at rt under argon atmosphere. DCM (50 mL) was added and the organic layer was washed with aqueous $NaHCO_3$ solution (3 × 30 mL). The product was obtained as a yellowish foam after column chromatography (DCM:MeOH, 100:1→95:5). Yield 44% (530 mg, 0.715 mmol). ^{1}H NMR ($CDCl_3$): δ = 7.92 (m, 2H, H3, H8), 7.32 (m, 12H, H2/H7/H16/DMT), 7.03 (m, 2H, H1/H6), 6.75 (m, 4H, DMT), 6.28 (dd, 1H, H1'), 4.55 (m, 1H, H4'), 4.09 (m, 1H, H3'), 3.72 (s, 6H, 2 × DMT-CH_3), 3.28 (m, 2H, H5'/H5''), 3.02 (m, 2H, H11/H11'), 2.86 (s, 6H, 2 × CH_3), 2.77 (s, 3H, CH_3), 2.71 (m, 2H, H14/H14'), 2.56 (m, 2H, H2'/H2''), 1.57 (m, 4H, H12/H12'/H13/H13') ppm. MS (ESI-TOF) calc. [*M*+Na]$^+$: 764.3788, found 764.3783.

5-(4-(4-((5-(Dimethylamino)naphthalen-1-yl)(methyl)amino)butyl)-1*H*-1,2,3-triazol-1-yl)-β-2'-deoxy-3'-(*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite)-5'-*O*-(4,4'-dimethoxytrityl)-ribonucleoside (**6**)

Compound **5** (250 mg, 0.337 mmol) was dissolved in dry DCM (20 mL). Under argon atmosphere, DIPEA (230 µL, 1.35 mmol, 4 equiv.) and CEDIP-Cl (95 µL, 0.40 mmol, 1.2 equiv.) were added and the reaction mixture was stirred for 30 min at rt. EtOAc (50 mL) was added and the organic layer was washed with aqueous $NaHCO_3$ solution (3 × 30 mL). The product was obtained as a colourless foam after column chromatography (DCM:EtOAc:Net3, 75:25:2). Yield 75% (238 mg, 0.25 mmol). ^{1}H NMR ($CDCl_3$): δ = 7.92 (t, 2H, H3/H8), 7.35 (m, 5H, H2/H7/DMT) , 7.21 (m, 7H, DMT/H16), 7.04 (d, 2H, H1/H6), 6.78 (m, 4H, DMT), 6.32 (dd, 1H, H1'), 4.72 (m, 1H, H4'), 4.23 (m, 1H, H3'), 3.74 (s, 6H, 2 × DMT-CH_3), 3.59 (m, 4H, H5'/CEDIP), 3.01 (m, 2H, H11/H11'), 2.88 (s, 6H, 2 × CH_3), 2.83 (m, 2H, H14/H14'), 2.77 (s, 3H, CH_3), 2.59 (m, 4H, H2'/H2''/CEDIP), 2.43 (m, 1H, CEDIP), 1.56 (s, 12H, CEDIP-^{i}Pr), 1.27 (m, 4H, H12/H12'/H13/H13') ppm. ^{31}P NMR ($CDCl_3$): δ =149.3, 148.9. MS (ESI-TOF) calc. [*M*+Na]$^+$: 964.4866, found 964.4869.

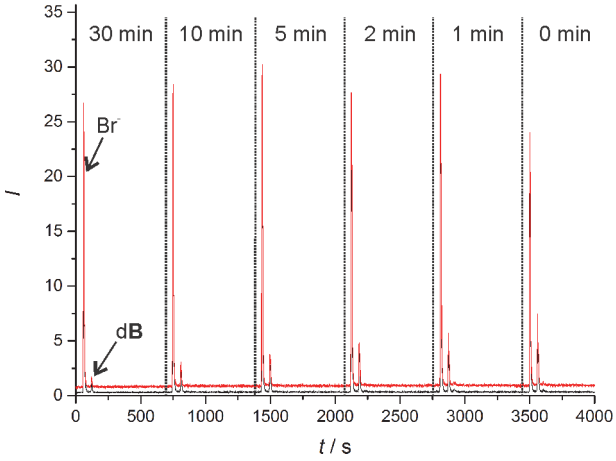


Figure S1. Sequential plot of six chromatograms of enzymatically digested duplex **I** at different irradiation times (^{79}Br trace in black, ^{81}Br trace in red). Peaks for free bromide ions (Br^-) and 5-bromo-2'-deoxyuridine (d**B**) are clearly discernible. Samples were injected via an auto-sampler with continuous detection, hence a sequential plot of chromatograms was obtained.

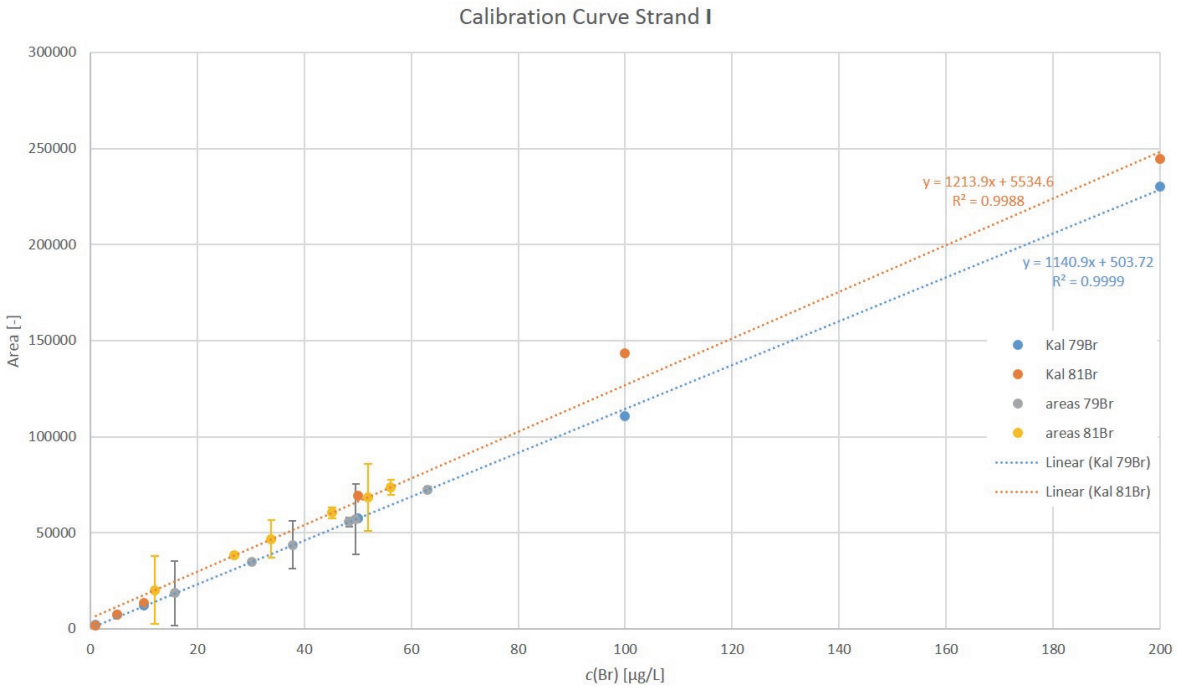


Figure S2. Exemplary calibration curve used for the calibration of the ^{79}Br and ^{81}Br traces.

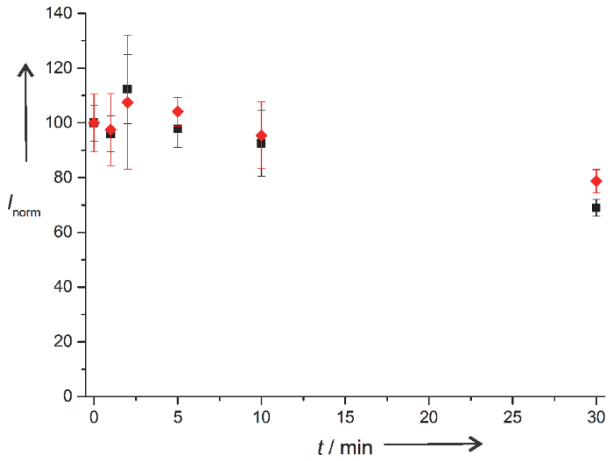


Figure S3. Change of the amount of d**B** of enzymatically digested duplex **IV** at different irradiation times (black ■: without Hg^{II}, red ◆: with Hg^{II}). These data cannot be fitted reliably to an exponential decay.

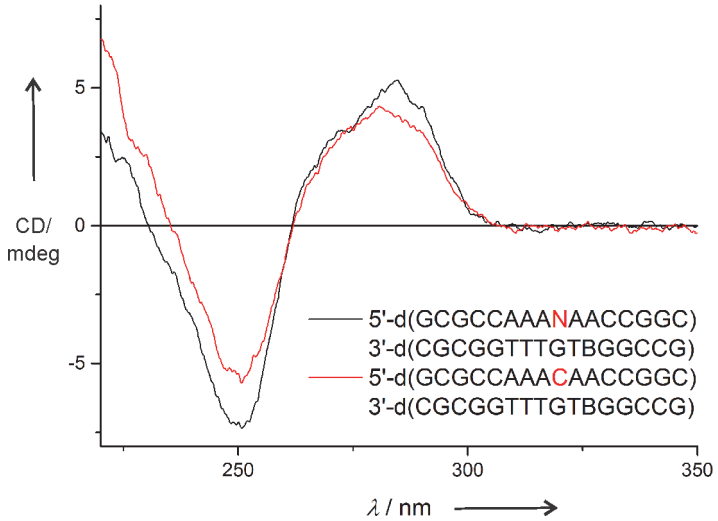


Figure S4. CD spectra of duplex **I** (black) and of a reference duplex (red) comprising a canonical G:C instead of the G:N base pair (experimental conditions: 1.5 μM oligonucleotide duplex, 150 mM $NaClO_4$ and 5 mM MOPS buffer (pH 6.8)). The more negative circular dichroism at ~250 nm and the more positive Cotton effect at ~285 nm have previously been associated with the presence of an intercalator,[4] suggesting that in duplex **I**, the diaminonaphthalene derivative intercalates into the base pair stack, too.

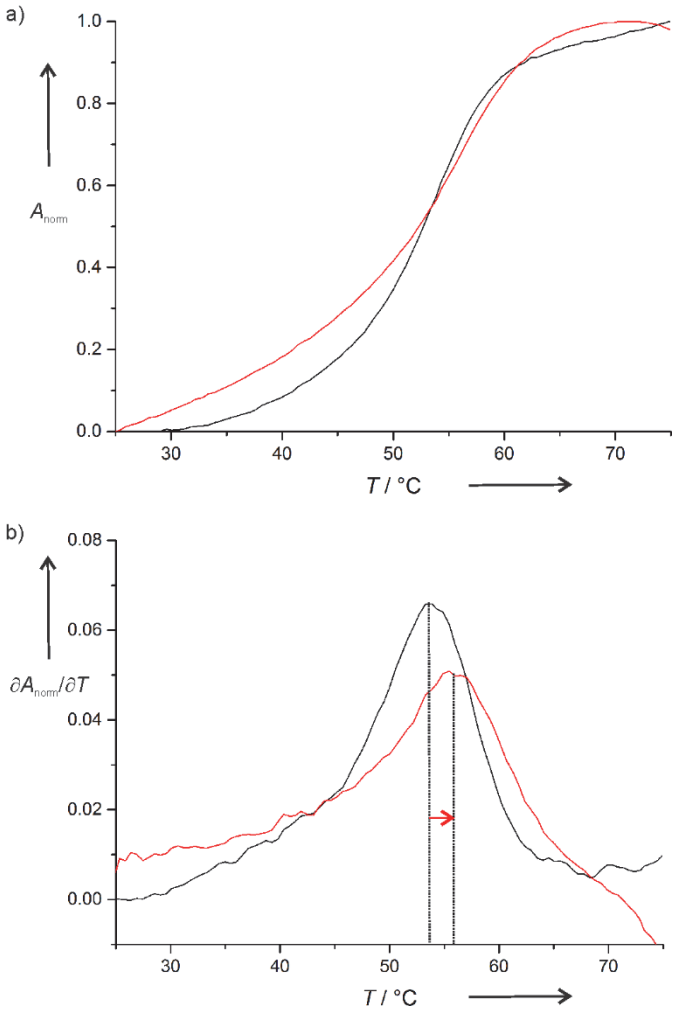
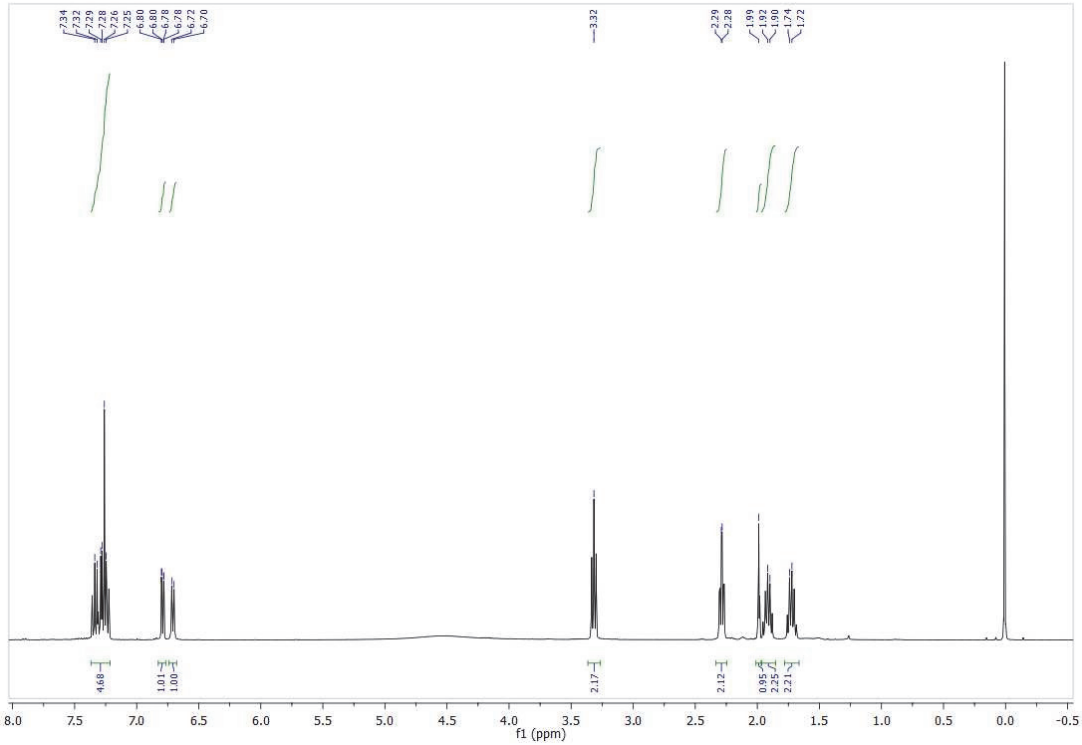
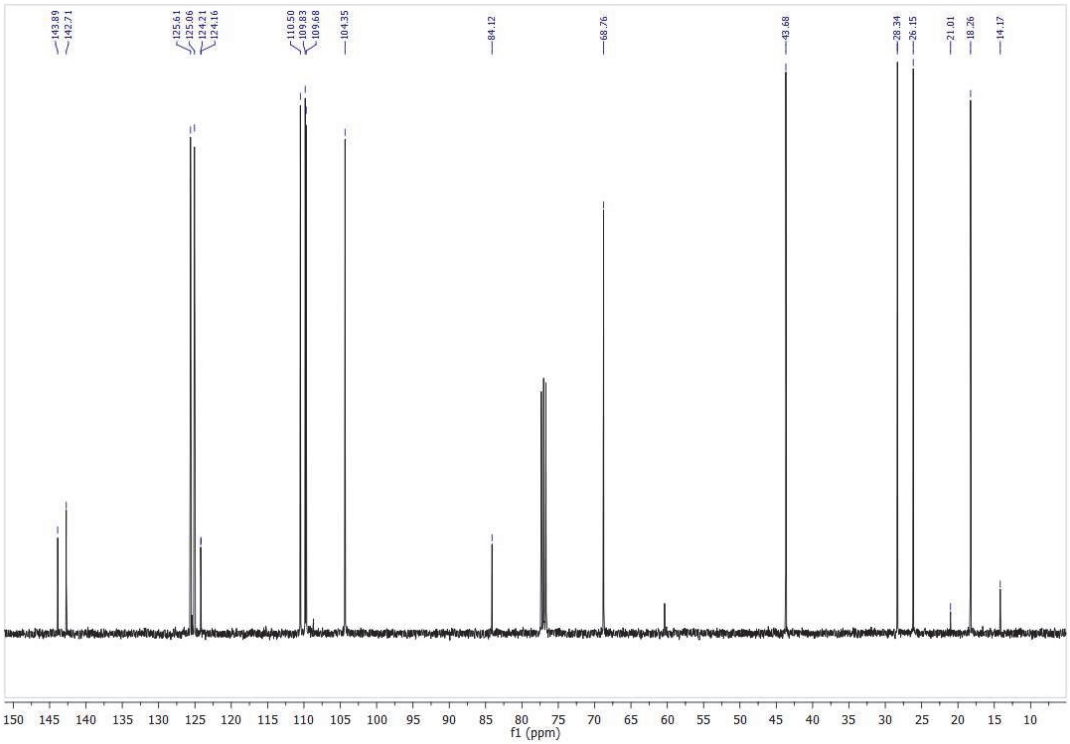


Figure S5. a) Melting curves of duplex **III** in the absence (black) and presence (red) of one equivalent of Hg^{II}. In the presence of Hg^{II}, the DNA duplex melting occurs less cooperatively, but at a higher melting temperature T_m. Importantly, the melting temperatures are significantly higher than the temperature of the irradiation experiments (12 °C). b) Derivatives of the melting curves, indicating an increase T_m upon the addition of Hg^{II}, indicative of the formation of a T–Hg^{II}–T base pair.

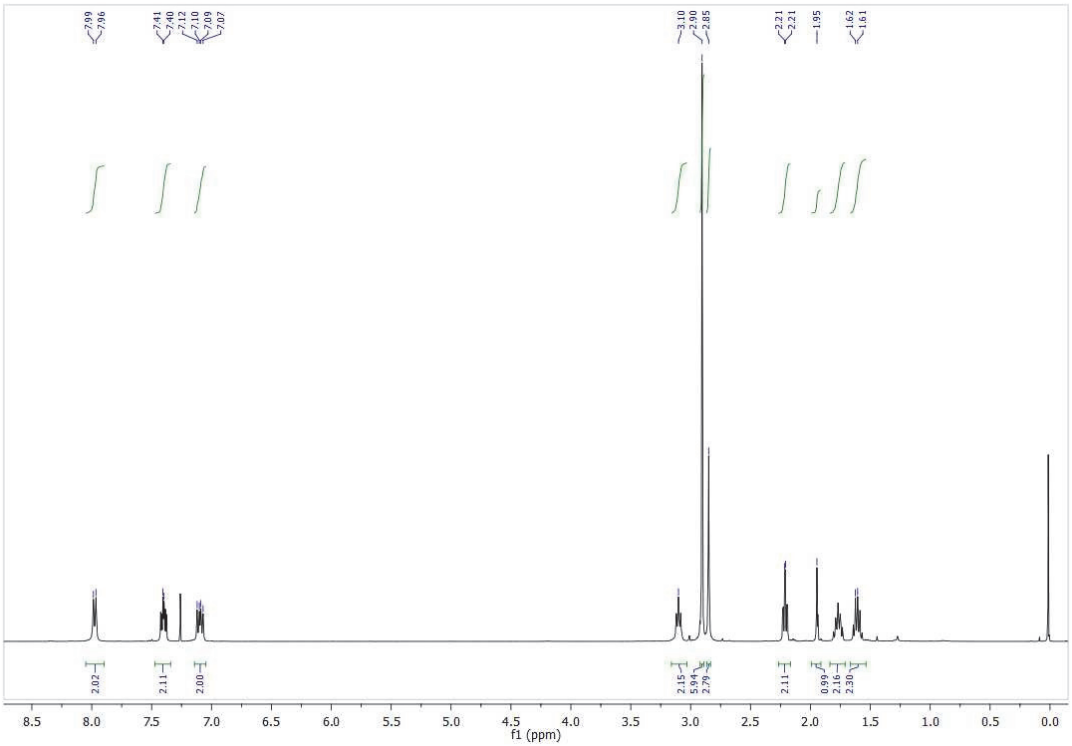
NMR spectra



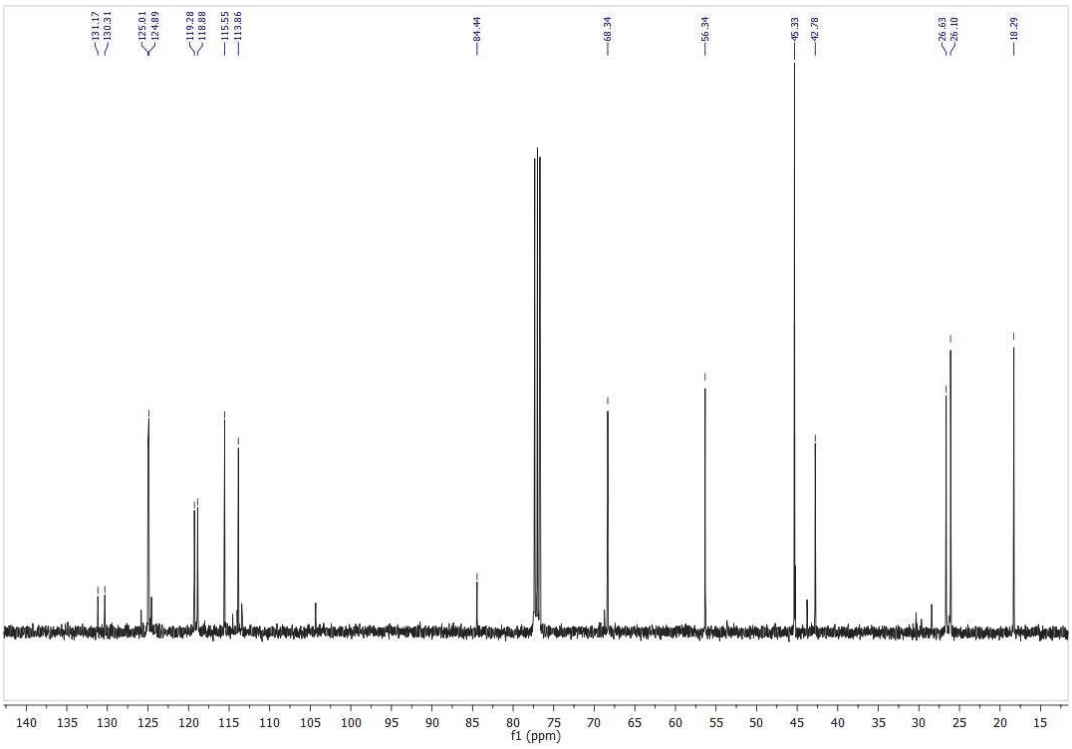
^{1}H NMR spectrum of compound **1**.



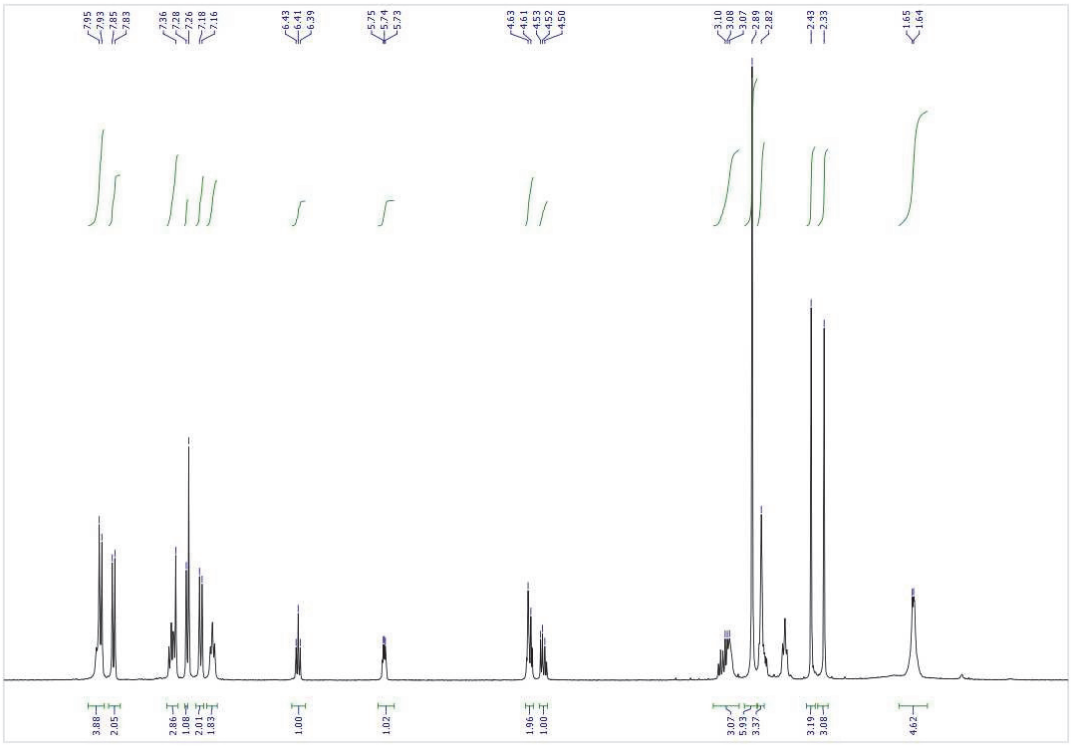
^{13}C NMR spectrum of compound **1**.



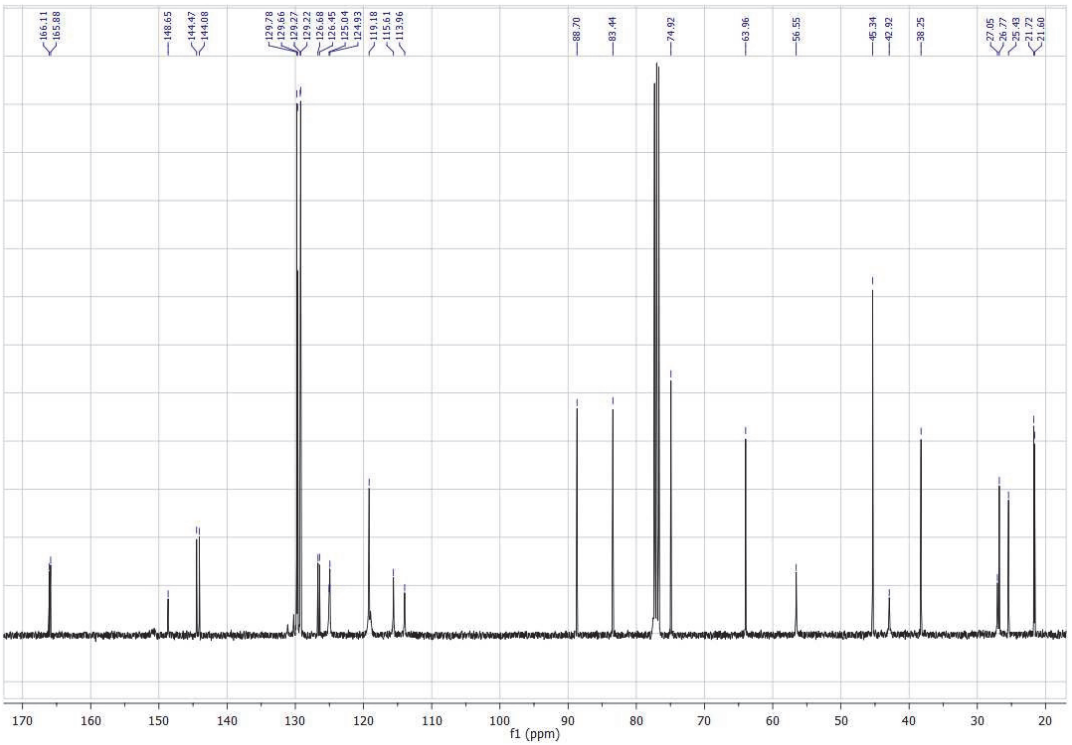
^{1}H NMR spectrum of compound **2**.



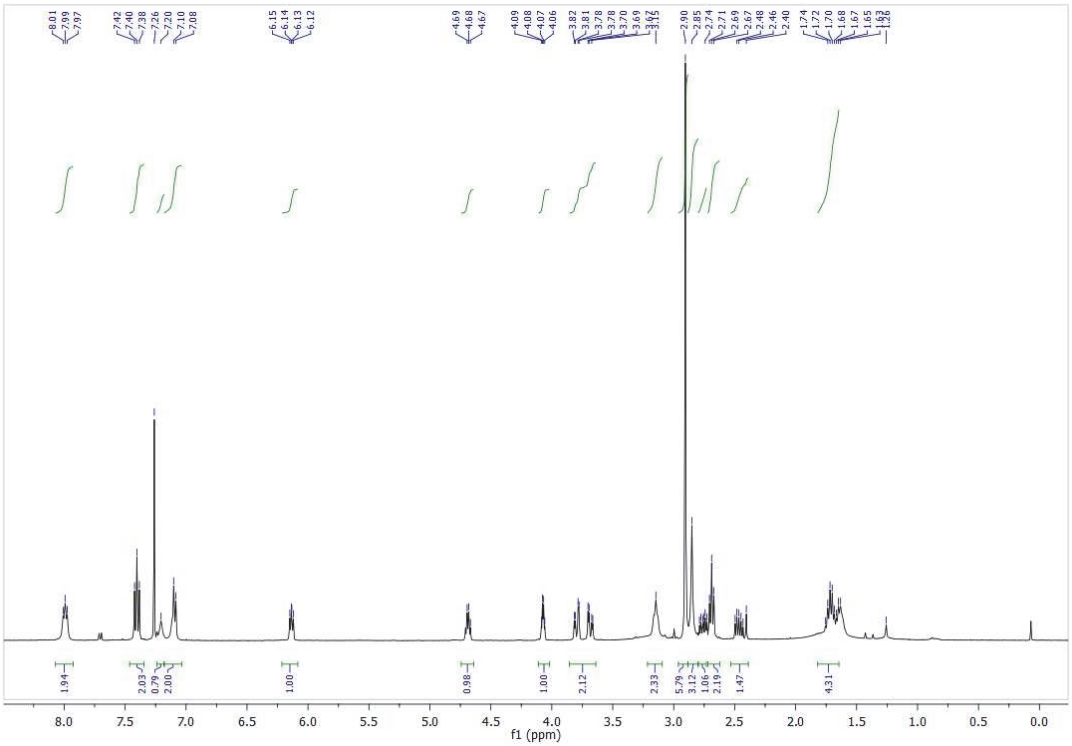
^{13}C NMR spectrum of compound **2**.



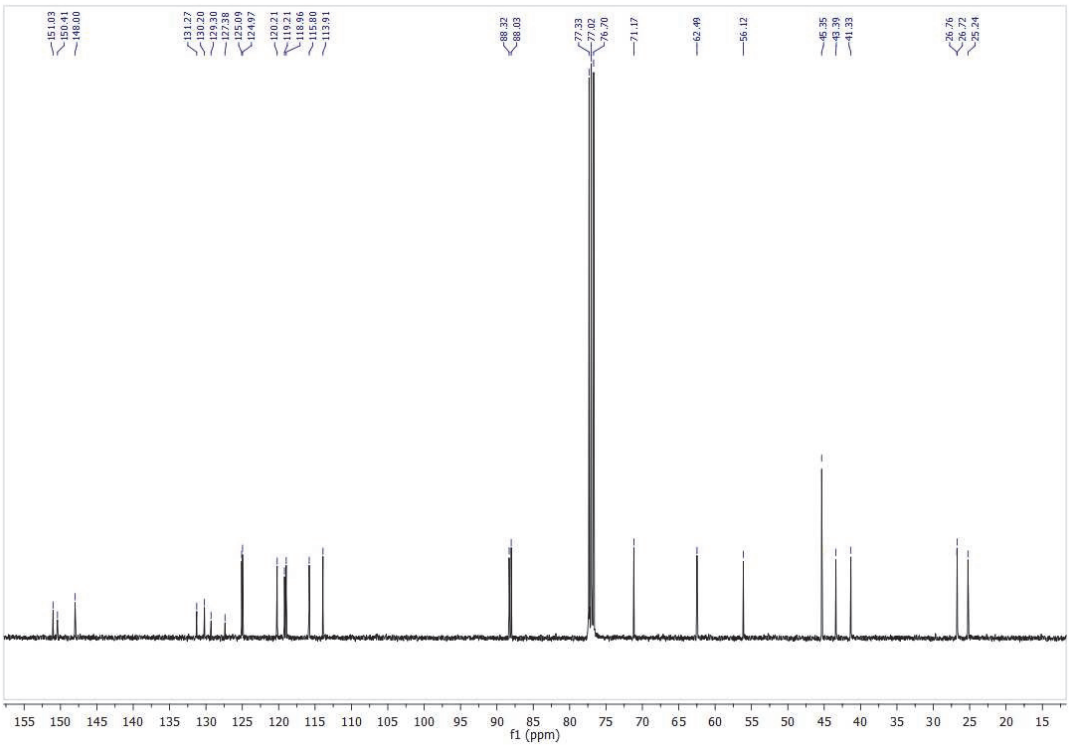
^{1}H NMR spectrum of compound **3**.



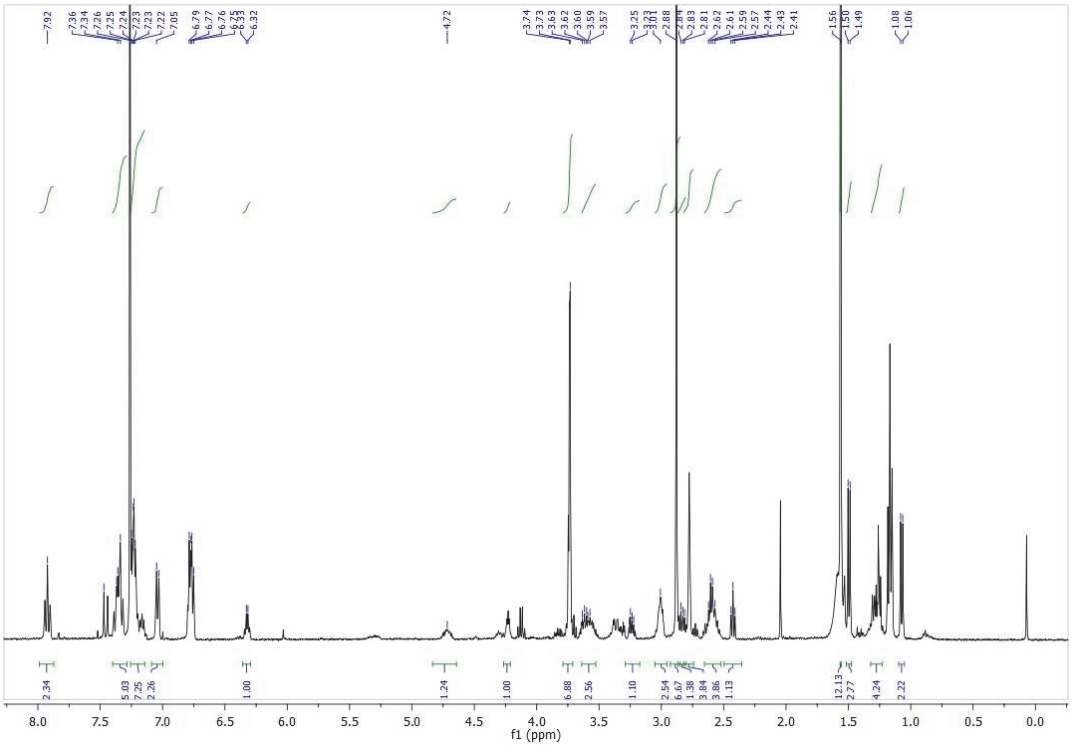
^{13}C NMR spectrum of compound **3**.



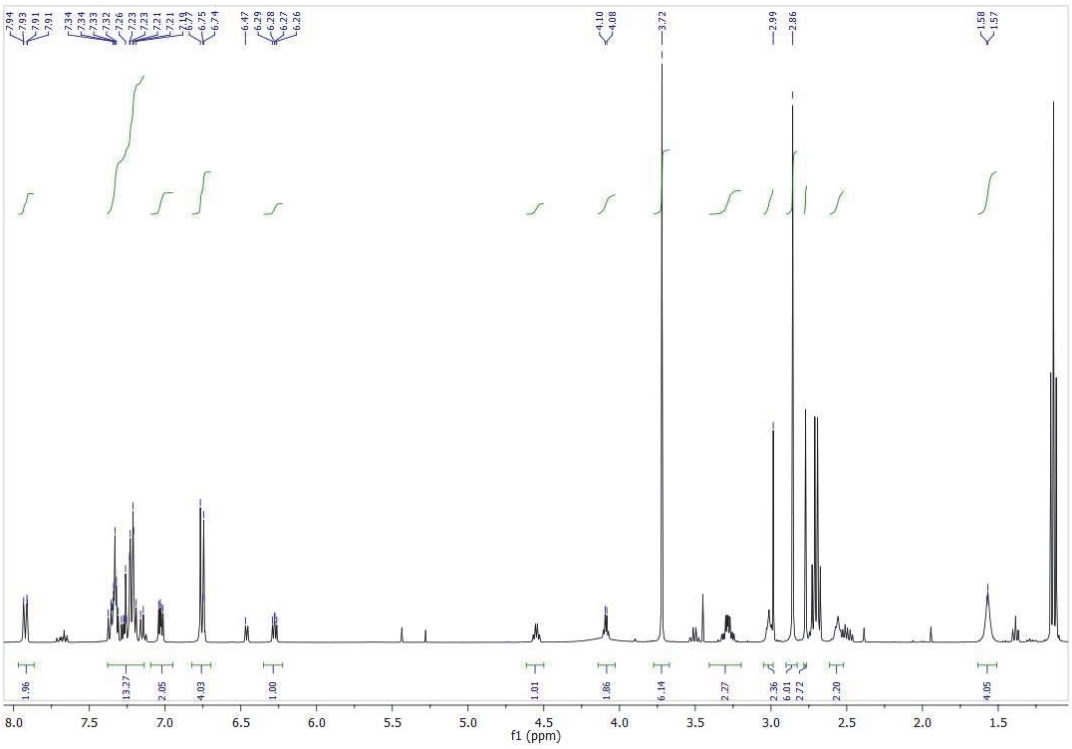
^{1}H NMR spectrum of compound **4**.



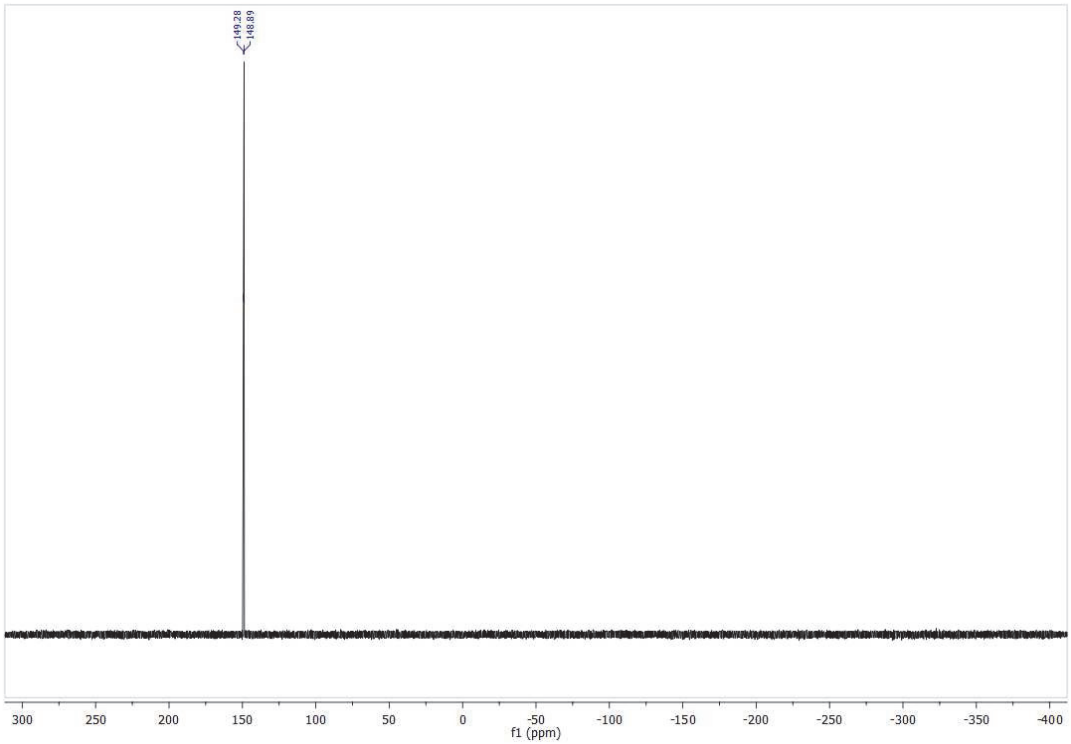
^{13}C NMR spectrum of compound **4**.



^{1}H NMR spectrum of compound **5**.

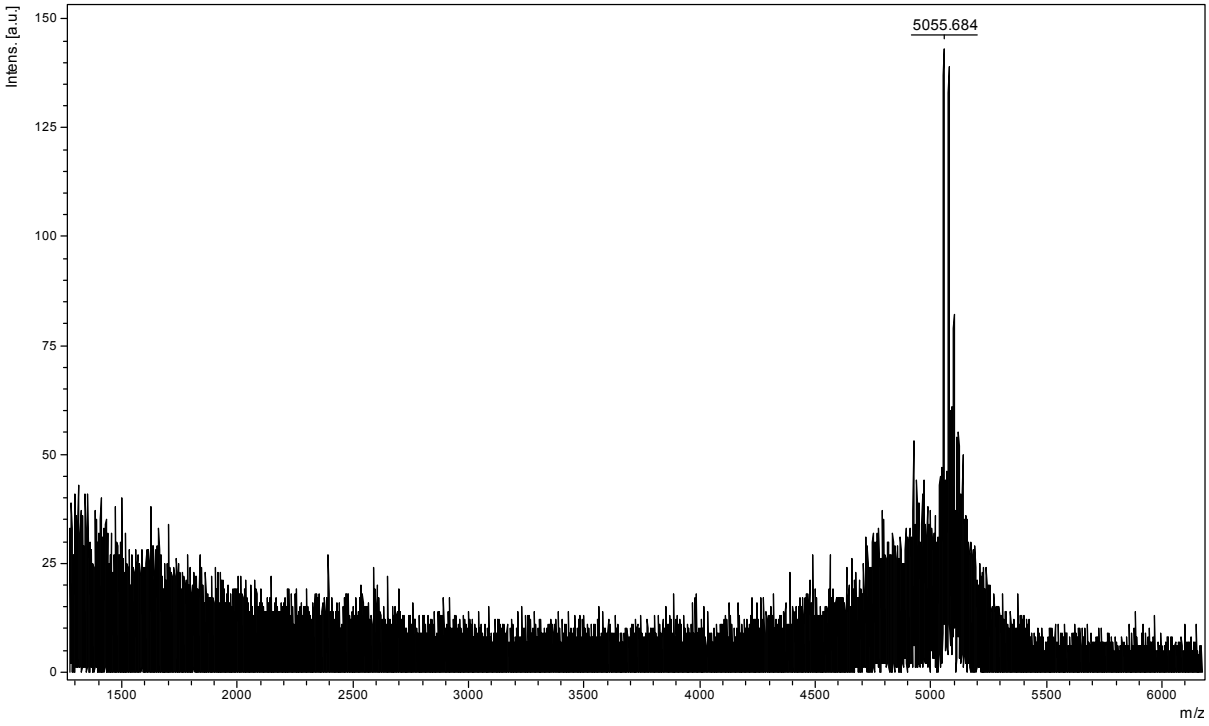


^{1}H NMR spectrum of compound **6**.

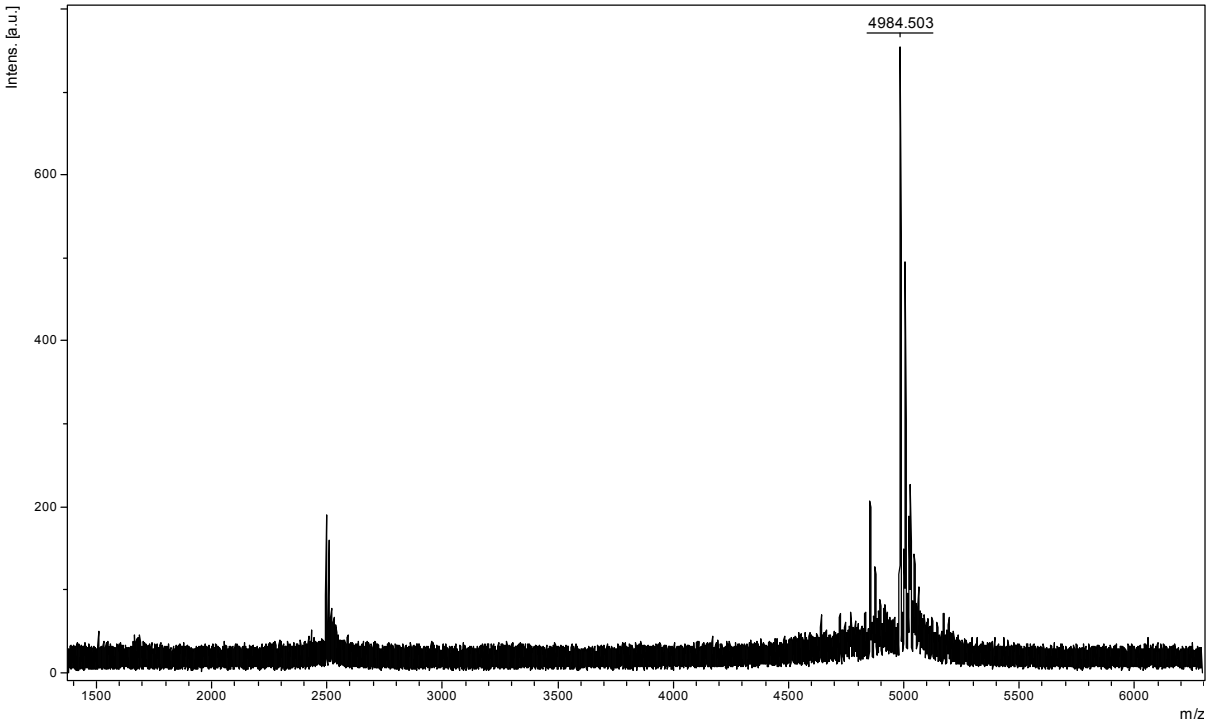


^{31}P NMR spectrum of compound **6**.

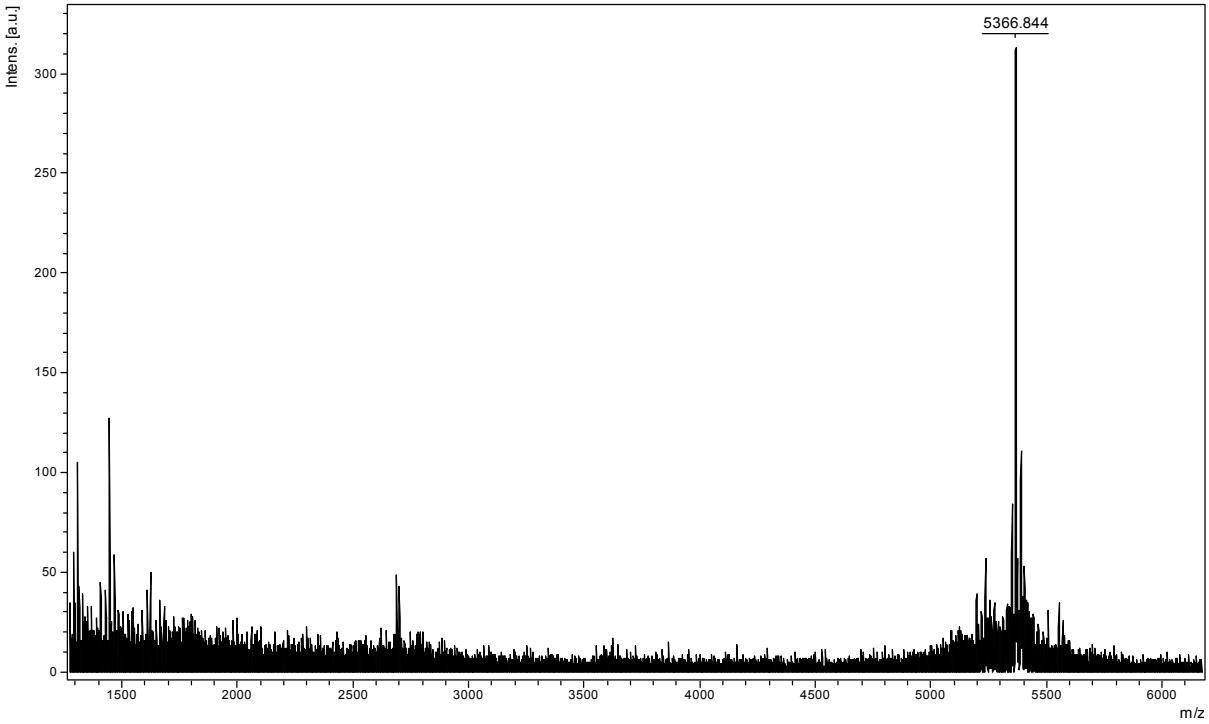
Mass spectra of the oligonucleotides



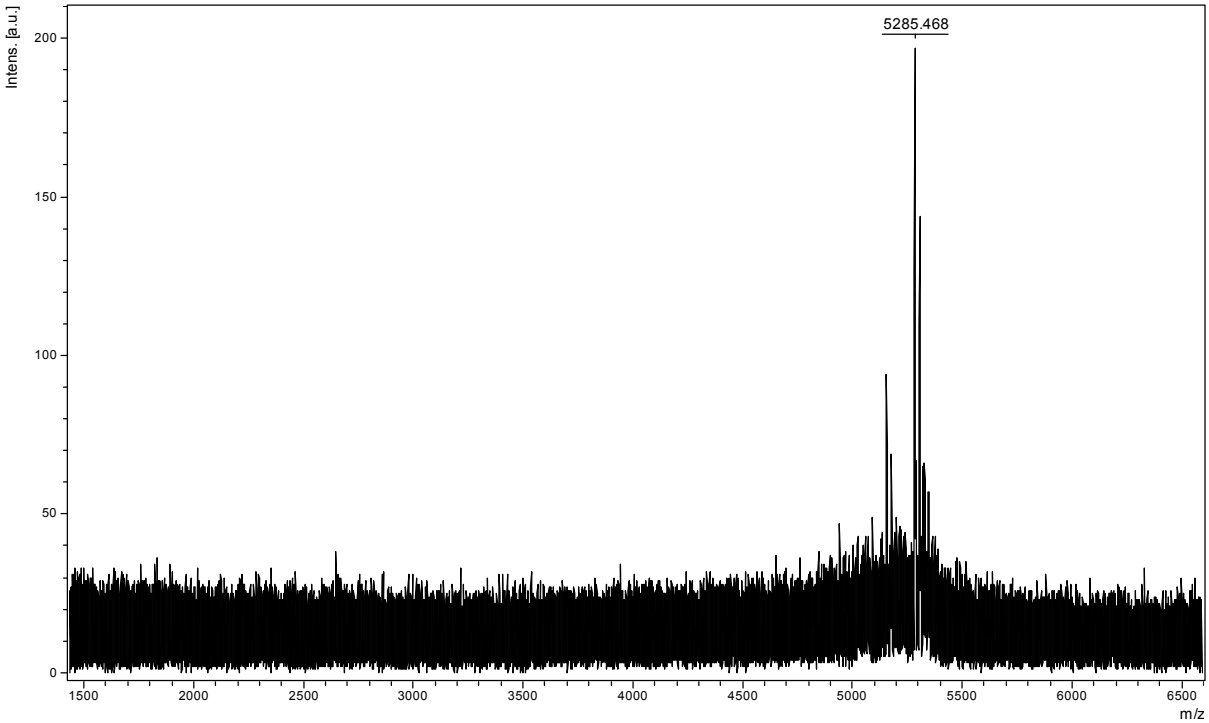
Mass spectrum of ODN1. Calcd for ($C_{168}H_{213}N_{68}O_{88}P_{15}$) [*M*+H]$^+$: 5056 Da.



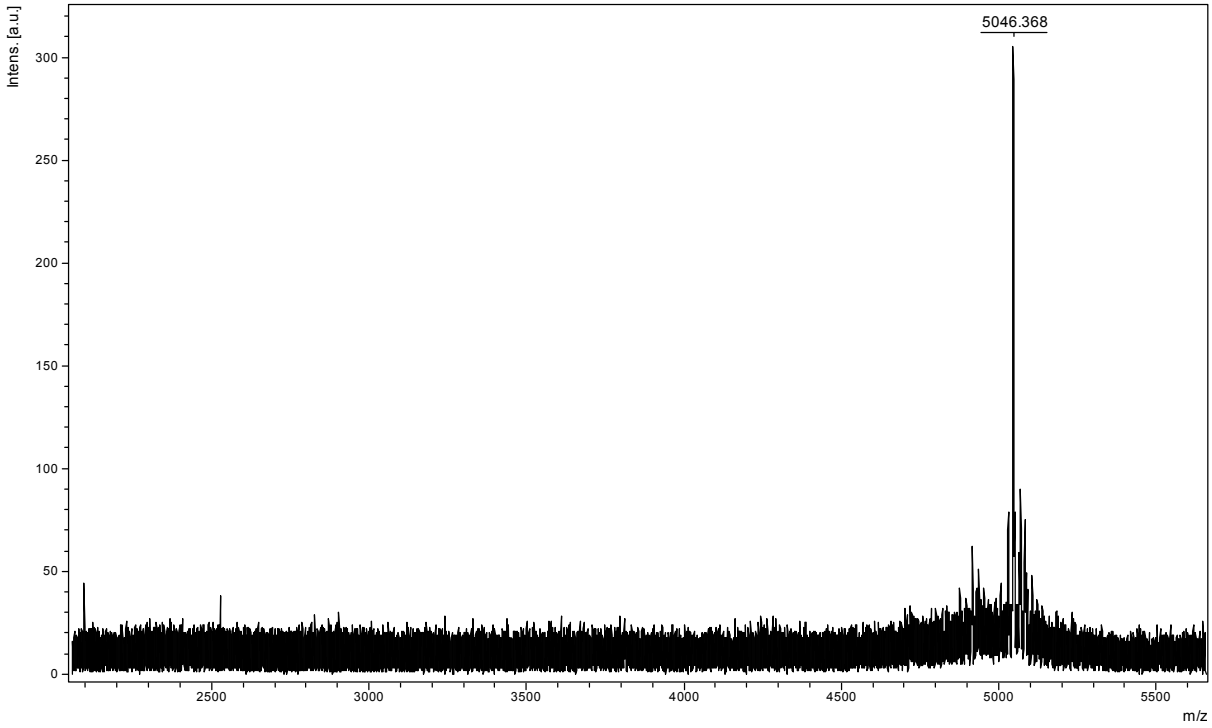
Mass spectrum of ODN2. Calcd for ($C_{155}H_{195}N_{57}O_{99}P_{15}Br$) [*M*+H]$^+$: 4983 Da.



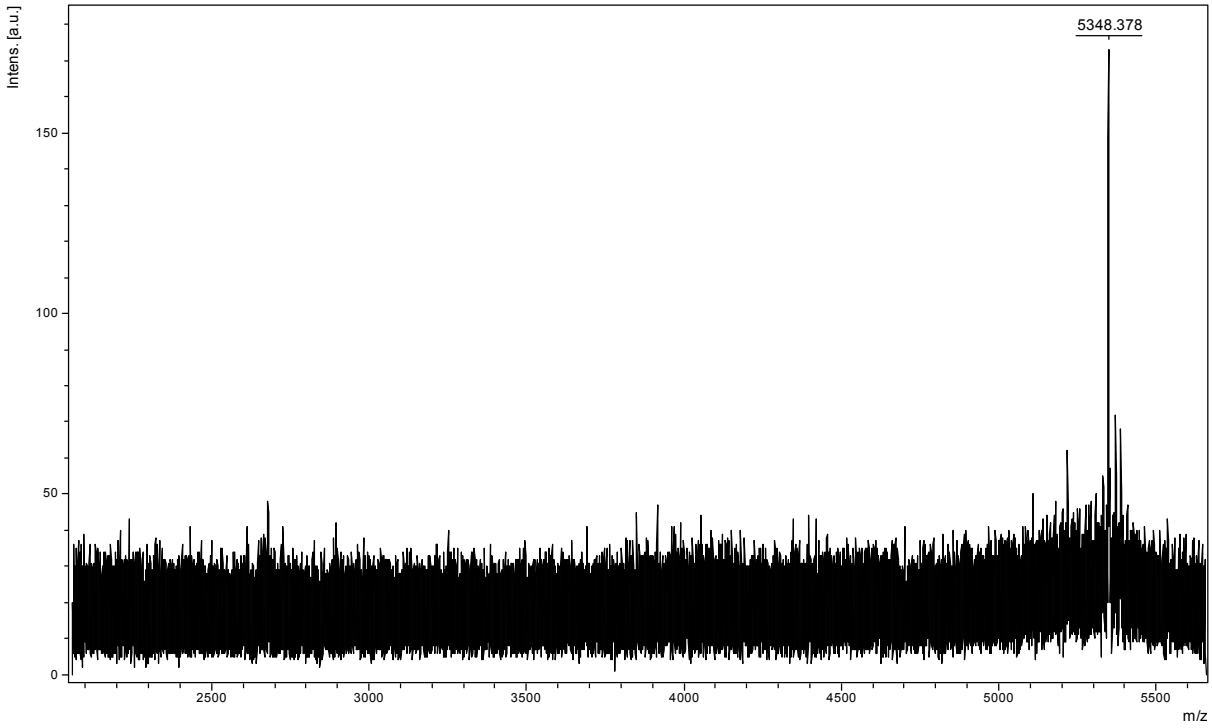
Mass spectrum of ODN3. Calcd for ($C_{178}H_{225}N_{73}O_{93}P_{16}$) [*M*+H]$^+$: 5369 Da.



Mass spectrum of ODN4. Calcd for ($C_{165}H_{208}N_{59}O_{106}P_{16}Br$) [*M*+H]$^+$: 5287 Da.



Mass spectrum of ODN5. Calcd for ($C_{168}H_{214}N_{65}O_{90}P_{15}$) [*M*+H]$^+$: 5047 Da.



Mass spectrum of ODN6. Calcd for ($C_{178}H_{227}N_{67}O_{97}P_{16}$) [*M*+H]$^+$: 5351 Da.

References

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