ChemPhotoChem

Supporting Information

Photoswitchable 2-Phenyldiazenyl-Purines and their Influence on DNA Hybridization

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1. Chemical synthesis

1.1 Devices and Materials

Conditions

If necessary, reactions were carried out under Schlenk-conditions, which means only dry solvents stored over molecular sieve were used in oven-dried Schlenk-glassware. Reactions were carried out under argon atmosphere to exclude moisture.

Solvents

Dry solvents were purchased from the company Acros and used without any further purification in the synthesis. If not otherwise stated, solvents of technical grade were used.

Reagents

All chemicals used were purchased from Sigma-Aldrich, Fluka, Alfa Aesar, TCI, ChemPur, Carbosynth or Fluorochem and used without any further purification in the synthesis.

NMR-Spectroscopy

The NMR spectra were measured on the devices AM 250, AV 300, AV400 and AV 500 from Bruker. As suitable solvents for the NMR sample DMSO-*d*6 (^{1}H, δ = 2.49 ppm, ^{13}C, δ = 39.52 ppm) and $CDCl_3$ (^{1}H, δ = 7.26 ppm, ^{13}C, δ = 77.16 ppm) were used. The chemical shift is given in ppm. The abbreviations of the multiplicity of the signals are as follows: singlet = s, doublet = d, triplet = t, quartet = q, quintet = qn, multiplet = m. Corresponding combinations are called deductively, for example, dt for doublet of triplets.

TLC

To check the reaction progress of all reactions, thin layer chromatography plates silica gel 60 F254 on aluminum foil from Macherey-Nagel were used. The evaluation was performed visually using a UV lamp having a wavelength of λ = 254 nm or 365 nm.

Column-Chromatography

Purification by using column chromatography was performed using silica gel 60 (particle size: 40-63 µm) from Macherey-Nagel and technical solvents.

Mass-Spectrometry

ESI were measured on a VG Platform II Fisons and high resolution mass spectra were measured on a MALDI LTQ Orbitrap XL from Thermo Fisher Scientific. Mass spectra were measured by MS service department of University Frankfurt (Matthias Brandl, Andreas Münch, Uwe Hener and Simon Zenglein), which we would like thank for their work.

1.2 Synthesis of 2-Phenyldiazenylpurine-deoxyriboside-phosphoramidites

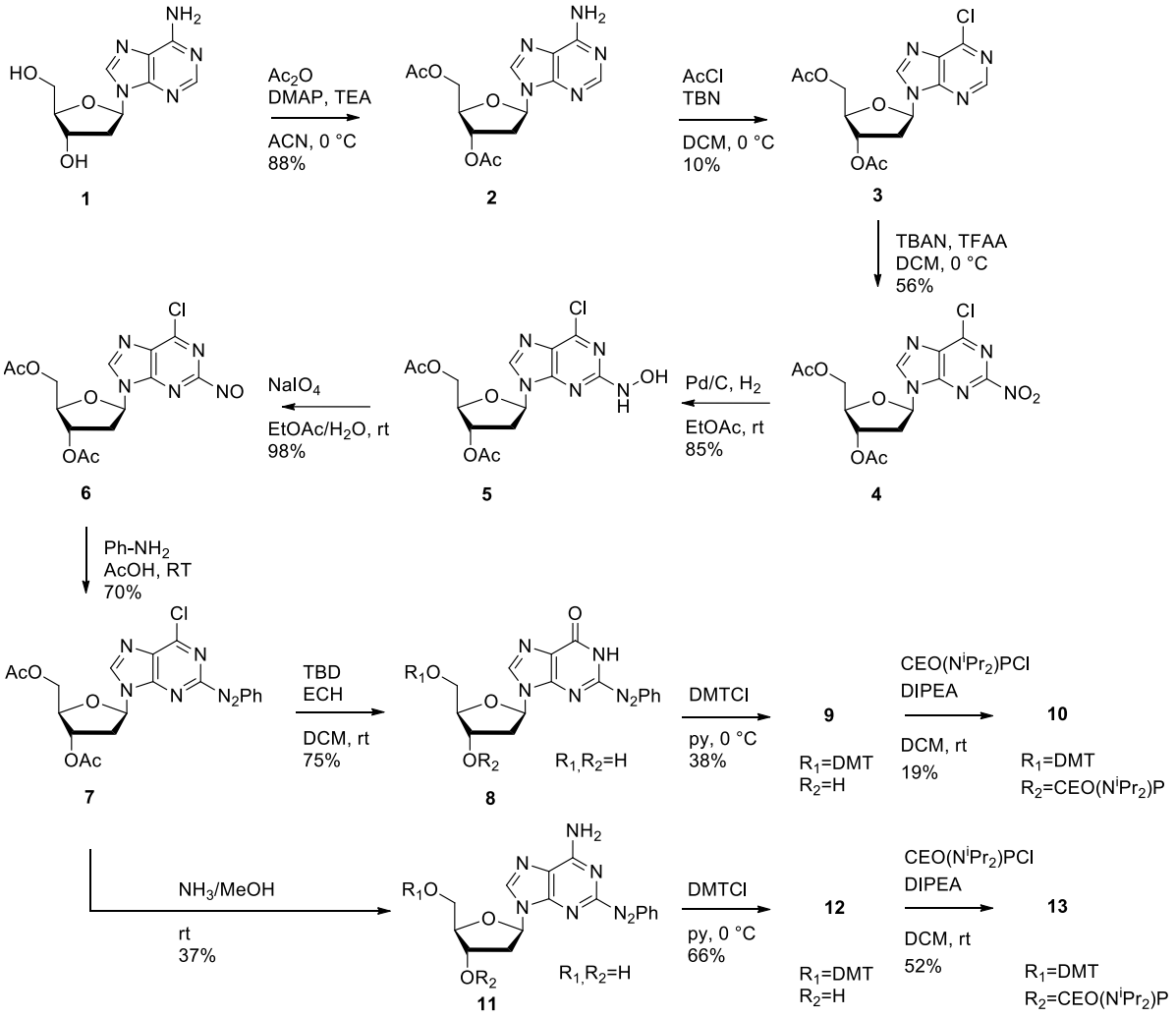

Fig. S1 Synthetic approach to afford the 2-phenyldiazenylpurine-deoxyriboside-phosphoramidites used in this study.

3',5'-Di-*O*-acetyl-2'-deoxy-adenosine (**2**)

To a solution of 62.190 g (247.5 mmol, 1.0 eq.) of 2'-deoxyadenosine, 1.512 g (12.377 mmol, 0.05 eq.) 4-*N*,*N*-dimethylamino-pyridine and 137 mL (0.99 mol, 4.0 eq.) triethylamine in 270 mL acetonitrile, 49 mL (53.068 mmol, 2.1 eq.) acetic acid anhydride was added dropwise at 0 °C, then stirred for 3 h at room temperature. Upon completion, excess anhydride was quenched with methanol and solvents where evaporated at a rotary evaporator. The residue was dissolved in DCM, washed with 10 mM hydrochloric acid, dried over Na_2SO_4, and evaporated to dryness. Flash column chromatography (DCM/MeOH 19:1 → 9:1) afforded the product as colorless powder.

Yield: 72.65 g (216.657 mmol, 88%)
^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.34 (s, 1H, 8), 8.15 (s, 1H, 2), 7.32 (s, 2H, NH_2), 6.36 (dd, *J* = 8.0, 6.3 Hz, 1H, 1'), 5.40 (dt, *J* = 5.2, 2.3 Hz, 1H, 3'), 4.31 (dt, *J* = 11.2, 5.6 Hz, 1H, 5'), 4.25 – 4.22 (m, 1H, 4'), 4.20 (dd, *J* = 10.8, 5.9 Hz, 1H, 5'), 3.16 (ddd, *J* = 14.4, 8.0, 6.6 Hz, 1H, 2'), 2.09 (s, 3H, CH_3), 2.01 (s, 3H, CH_3).
^{13}C NMR (126 MHz, DMSO) δ 170.17, 170.07, 156.13, 152.68, 149.21, 139.58, 119.23, 83.52, 81.60, 74.41, 63.61, 21.32, 20.84, 11.04.
TLC (DCM/MeOH) R_f = 0.48.
HRMS (MALDI) $[M+H]^+$ m/z = calculated: 336.13025, found: 336.13050.

6-Chloropurine-3',5'-di-*O*-acetyl-2'deoxyriboside (**3**)

To a solution of 72.65 g (216.66 mmol, 1.0 eq.) of compound **2** in 300 mL DCM, 87 mL (1.22 mol, 5.5 eq.) acetyl chloride and 100 mL (1.12 mol, 4.5 eq.) tert-butyl nitrite where added dropwise subsequently at 0 °C and then stirred at 0 °C. Upon completion, the reaction was quenched with 1 M NaOH, extracted with DCM, dried over Na_2SO_4 and evaporated to dryness. Flash column chromatography (Cy/EA 1:2 → 100% EA) afforded the product as pale yellow oil.

Yield: 7.73 g (21.790 mmol, 10%)
^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.90 (s, 1H, 8), 8.82 (s, 1H, 2), 6.51 (t, *J* = 6.9 Hz, 1H, 1'), 5.49 – 5.41 (m, 1H, 3'), 4.31 (ddd, *J* = 12.5, 8.4, 3.7 Hz, 2H, 4'+5'), 4.27 – 4.19 (m, 1H, 5'), 3.18 (dt, *J* = 14.2, 7.0 Hz, 1H, 2'), 2.68 – 2.59 (m, 1H, 2'), 2.10 (s, 3H, CH_3), 1.99 (s, 3H, CH_3).
^{13}C-NMR (126 MHz, DMSO-d_6) δ 170.13, 170.07, 151.78, 151.40, 149.44, 146.08, 131.55, 84.28, 81.99, 74.04, 63.44, 35.48, 20.81, 20.51.
TLC (DCM/MeOH) R_f = 0.66.
HRMS (MALDI) $[M+H]^+$ m/z = calculated: 355.08037, found: 355.08047.

2-Nitro-6-chloropurine-3',5'-di-*O*-acetyl-2'deoxyriboside (**4**)

The nitration mixture was prepared by adding 0.21 mL (1.450 mmol, 1.5 eq.) of trifluoro acetic anhydride to a solution of 0.442 g (1.450 mmol, 1.5eq.) of tetrabutyl ammonium nitrate in 10 mL of DCM at 0 °C dropwise and stirred for 20 minutes. The nitration mixture was then added to a solution of compound **3** in 20 mL DCM at 0 °C and was stirred for 2 hours at 0 °C. Upon completion, quenching was carried out by pouring the reaction mixture into an ice-cooled aqueous sodiumcarbonate solution, extracted with DCM, dried over Na_2SO_4 and evaporated to dryness. Flash column chromatography (DCM/MeOH 99:1→9:1) afforded the product as pale yellow foam.

Yield: 262 mg (0,546 mmol, 56%)
^{1}H-NMR (500 MHz, DMSO-d_6) δ 9.22 (s, 1H, 8), 6.56 (q, *J* = 6.7 Hz, 1H, 1'), 5.51 – 5.46 (m, 1H, 3'), 4.37 – 4.31 (m, 2H, 4'+5'), 4.28 (td, *J* = 7.5, 1.9 Hz, 1H, 5'), 3.12 (dt, *J* = 14.0, 6.8 Hz, 1H, 2'), 2.70 (ddd, *J* = 14.4, 6.6, 3.5 Hz, 1H, 2'), 2.11 (s, 3H, CH_3), 1.99 – 1.97 (m, 3H, CH_3).
^{13}C-NMR (126 MHz, DMSO-d_6) δ 170.11, 170.06, 152.30, 151.62, 149.95, 149.73, 134.77, 84.70, 82.34, 73.81, 63.44, 54.93, 20.81, 20.51.
TLC (DCM/MeOH 19:1) R_f = 0.5.
HRMS (MALDI) $[M+Na]^+$ m/z = calculated: 422.04740, found: 422.04744.

2-Hydroxyamino-6-chloropurine-3‘,5‘-di-*O*-acetyl-2’deoxyriboside (**5**)

To a solution of 1.840 g (4.603 mmol, 1.0 eq.) of compound **4** in a mixture of 10 mL EA and 5 mL EtOH, 0.211 g palladium on charcoal where added and was left to stir under hydrogen atmosphere for 2 hours. Upon completion, the catalyst was filtered off and the solution was evaporated to dryness. The crude product was directly used for subsequent reaction without further purification and yielded an off-white foam.

Yield: 1.510 g (3.913 mmol, 85%)
^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.85 (s, 1H, OH), 8.91 (s, 1H, NH) 8.44 (s, 1H, 8), 6.33 (t, *J* = 6.9 Hz, 1H, 1’), 5.37 (d, *J* = 4.3 Hz, 1H, 3’), 4.28 – 4.20 (m, 3H, 4’+5’), 3.19 – 3.03 (m, 1H, 2’), 2.69 (dt, *J* = 16.6, 6.7 Hz, 1H, 2’), 2.02 (s, 3H, CH_3), 1.98 (s, 3H, CH_3).
^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.10, 161.78, 153.25, 151.58, 149.88, 141.87, 81.66, 73.77, 63.54, 55.99, 35.18, 20.78, 20.51, 18.52.
TLC (DCM/MeOH 19:1) R_f = 0.29.
HRMS (MALDI) $[M+H]^+$ m/z = calculated: 386.08619, found: 386.08493.

2-Nitroso-6-chloropurine-3‘,5‘-di-*O*-acetyl-2’deoxyriboside (**6**)

To a solution of 1.510 g (3.913 mmol, 1.0 eq.) of compound **5** in 10 mL EA a solution of 1.181 g (5.523 mmol, 1.2 eq.) of sodium periodate in 5 mL water was added and stirred for 30 minutes at room temperature. Upon completion, the mixture was extracted with EA, dried over Na_2SO_4 and evaporated to dryness. Flash column chromatography (Cy/EA 1:1 → 100% EA) afforded the product as pale yellow foam.

Yield: 1.465 g (3.818 mmol, 98%)
^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 1H, 8), 6.63 (t, *J* = 6.7 Hz, 1H, 1’), 5.55 – 5.47 (m, 1H, 3’), 4.31 (m, 3H, 4’+5’), 3.20 (dt, *J* = 14.2, 6.9 Hz, 1H, 2’), 2.72 (ddd, *J* = 14.1, 7.2, 3.5 Hz, 1H, 2’), 2.12 (s, 3H, CH_3), 1.97 (s, 3H, CH_3).
^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.61 (s, 1H, 8), 6.58 (t, *J* = 6.8 Hz, 1H, 1’), 5.45 (dd, *J* = 11.4, 8.5 Hz, 1H, 3’), 4.48 – 4.36 (m, 3H, 4’+5’), 2.91 – 2.84 (m, 1H, 2’), 2.79 (ddd, *J* = 14.2, 6.0, 2.7 Hz, 1H, 2’), 2.18 – 2.14 (m, 3H, CH_3), 2.09 (s, 3H, CH_3).
^{13}C-NMR (126 MHz, $CDCl_3$) δ 170.37, 170.32, 153.37, 152.85, 151.30, 147.18, 135.09, 85.71, 83.51, 74.07, 63.58, 38.47, 21.03, 20.90.
TLC (DCM/MeOH 19:1) R_f = 0.43.
HRMS (MALDI) $[M+H]^+$ m/z = calculated: 384.07058, found: 384.07058.

2-Phenyldiazenyl-6-chloropurine-3‘,5‘-di-*O*-acetyl-2’deoxyriboside (**7**)

To a solution of 0.342 g (0,891 mmol, 1.0 eq.) of compound **6** in 5 mL ACN, 80 µL (1.18 mmol, 1.2 eq.) aniline where added and stirred for 10 minutes, before 100 µL acetic acid where added and stirred for 30 minutes. Upon completion, the solution was neutralized by addition of aqueous sodium carbonate solution, extracted with DCM, dried over Na_2SO_4 and evaporated to dryness. Flash column chromatography (Cy/EA 1:1 → 100% EA) afforded the product as orange foam.

Yield: 0.286 mg (0.624 mmol, 70 %)
^{1}H-NMR (500 MHz, DMSO-d_6) δ 9.03 (s, 1H, 8), 8.05 (dd, *J* = 7.9, 1.7 Hz, 2H, Ar), 7.72 – 7.66 (m, 3H, Ar), 6.57 (t, *J* = 6.8 Hz, 1H, 1’), 5.51 (dd, *J* = 6.3, 3.1 Hz, 1H, 3’), 4.38 – 4.27 (m, 3H, 4’+5’), 3.17 (dt, *J* = 14.1, 6.9 Hz, 1H, 2’), 2.68 (ddd, *J* = 14.3, 6.5, 3.3 Hz, 1H, 2’), 2.10 (s, 3H, CH_3), 1.98 (s, 3H, CH_3).
^{13}C-NMR (126 MHz, DMSO-d_6) δ 165.54, 165.51, 156.52, 147.85, 147.78, 147.42, 139.85, 128.66, 127.12, 124.52, 123.84, 122.26, 119.50, 80.27, 78.31, 69.54, 58.96, 33.69, 16.19, 16.09.
TLC (DCM/MeOH 19:1) R_f = 0.28.
HRMS (MALDI) $[M+Na]^+$ m/z = calculated: 481.09909, found: 481.09977.

2-Phenyldiazenyl-2’-deoxyguanosine (**8**)

To a solution of 100 mg (0.218 mmol, 1.0 eq.) of compound **7** and 58 µL (0.872 mmol, 4.0 eq.) 3-hydroxypropionitrile in 5 mL DCM, 61 mg (0.436 mmol, 2.0 eq.) 1,5,7-triazabicyclo(4.4.0)dec-5-ene was added and left to stir over night. Upon completion, solvents where evaporated and submitted to flash column chromatography (DCM/MeOH 19:1) to afford the product as orange solid.

Yield: 58 mg (0.163 mmol, 75 %)
^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.54 (s, 1H, NH), 8.50 (d, *J* = 5.5 Hz, 1H, 8), 8.07 – 8.03 (m, 2H, Ar), 7.76 – 7.67 (m, 3H, Ar), 6.43 (dd, *J* = 13.6, 6.9 Hz, 1H, 1‘), 5.40 (t, *J* = 4.6 Hz, 1H, 3‘-OH), 5.01 (dd, *J* = 11.7, 6.2 Hz, 1H, 5‘-OH), 4.44 (td, *J* = 6.9, 3.5 Hz, 1H, 3‘), 3.94 – 3.88 (m, 1H, 4‘), 3.67 – 3.61 (m, 1H, 5‘), 3.59 – 3.53 (m, 1H, 5‘), 2.72 – 2.65 (m, 1H, 2‘), 2.42 – 2.35 (m, 1H, 2‘).
^{13}C-NMR (126 MHz, DMSO-d_6) δ 156.12, 154.51, 151.01, 147.53, 140.42, 134.36, 129.89, 124.96, 123.80, 123.28, 88.12, 83.70, 70.60, 61.53, 20.93, 20.61.
TLC (DCM/MeOH 9:1) R_f = 0.22.
HRMS (MALDI) $[M+H]^+$ m/z = calculated: 357.13058, found: 357.13059.

2-Phenyldiazenyl-5’(4,4‘-dimethoxytrityloxymethyl)-2’-deoxyguanosine (**9**)

After coevaporating 1.139 g (3.196 mmol, 1.0 eq.) of compound **8** two times with pyridine and dissolving it in 40 mL pyridine, a solution of 1.191 g (3.516 mmol, 1.1 eq.) of 4,4‘-dimethoxytrityloxymethylchloride in 20 mL pyridine where added dropwise at 0 °C and stirred for 3 hours at room temperature. Upon completion, reaction was quenched with methanol and solvents where coevaporated with toluene. The residue was dissolved in DCM, washed with aqueous sodium carbonate solution, dried over Na_2SO_4 and evaporated to dryness. Flash column chromatography (DCM/MeOH 49:1→19:1 + 1% TEA) afforded the product as orange foam.

Yield: 0.806 g (1.223 mmol, 38%)
^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.60 – 8.55 (m, 1H, Ar), 8.37 (s, 1H, 8), 8.03 – 7.97 (m, 1H, Ar), 7.82 – 7.76 (m, 1H, Ar), 7.76 – 7.67 (m, 2H, Ar), 7.38 (ddd, *J* = 7.6, 4.3, 1.4 Hz, 1H, Ar), 7.28 (dd, *J* = 7.8, 1.7 Hz, 2H, Ar), 7.20 – 7.10 (m, 6H, Ar), 6.71 (dd, *J* = 22.0, 8.9 Hz, 4H, Ar), 6.46 (t, *J* = 6.3 Hz, 1H, 1’), 5.39 (d, *J* = 4.7 Hz, 1H, 3’-OH), 4.52 – 4.46 (m, 1H, 3’), 4.05 – 3.99 (m, 1H, 4’), 3.37 – 3.34 (m, 1H, 5’), 3.12 (dd, *J* = 10.2, 3.1 Hz, 1H, 5’), 2.88 – 2.80 (m, 1H, 2’), 2.45 – 2.38 (m, 1H, 2’).
^{13}C-NMR (126 MHz, DMSO-d_6) δ 157.95, 157.91, 156.28, 154.39, 151.02, 149.63, 147.44, 144.91, 140.86, 136.15, 135.55, 135.45, 134.25, 129.85, 129.66, 129.58, 127.63, 127.59, 126.53, 125.43, 123.92, 123.71, 112.95, 112.88, 86.38, 85.31, 84.03, 70.56, 54.95, 54.92, 45.69, 10.60.
TLC (DCM/MeOH 9:1) R_f = 0.30.
HRMS (MALDI) $[M+Na]^+$ m/z = calculated: 681.24320, found: 681.24500.

2-Phenyldiazenyl-2’(cyanoethoxy(*N*,*N*-diisopropylamino)phosphinoxyl)-5’(4,4‘-dimethoxytrityloxymethyl)-2’-deoxyguanosine (**10**)

To a solution of 806 mg (1.223 mmol, 1.0 eq.) of compound **9** and 243 µL (6.118 mmol, 5.0 eq.) *N*,*N*-diisopropylethylamine in 8 mL DCM, 348 µL (1.835 mmol, 1.5 eq.) CEO(N^iPr_2)PCl was added and let to stir at room temperature for 2 hours. Upon quenching with methanol the reaction was evaporated to dryness, extracted with DCM, dried over Na_2SO_4 and evaporated to dryness again. The crude product was subjected to flash column chromatography (DCM/MeOH 99:1 → 49:1) to afford the product as orange foam.

Yield: 292 mg (0.340 mmol, 19%)
^{1}H-NMR (500 MHz, $CDCl_3$) δ 10.28 (s, 1H, NH), 8.11 – 8.05 (m, 1H, 8), 8.03 – 8.00 (m, 1H, Ar), 7.64 – 7.60 (m, 1H, Ar), 7.58 – 7.54 (m, 2H, Ar), 7.39 – 7.35 (m, 2H, Ar), 7.29 – 7.17 (m, 8H, Ar), 6.77 – 6.72 (m, 4H, Ar), 6.56 (t, *J* = 6.7 Hz, 1H, 1’), 4.75 – 4.66 (m, 1H, 3’), 4.34 – 4.26 (m, 1H, 4’), 3.73 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 3.62 – 3.53 (m, 2H, CH), 3.45 – 3.30 (m, 2H, 5’), 2.80 – 2.63 (m, 2H, 2’), 2.60 (t, *J* = 6.3 Hz, 2H, CH_2), 2.45 (t, *J* = 6.4 Hz, 2H, CH_2), 1.19 – 1.11 (m, 12H, CH_3).
^{13}C NMR (126 MHz, $CDCl_3$) δ 158.62, 155.49, 152.88, 150.69, 148.03, 144.51, 140.17, 135.66, 135.55, 134.87, 130.18, 130.16, 129.79, 128.25, 128.18, 127.94, 127.01, 126.76, 124.76, 117.64, 117.53, 116.51, 113.23, 86.65, 86.40, 86.20, 84.86, 74.13, 73.42, 63.72, 58.54, 58.32, 55.29, 43.41, 43.37, 43.27, 40.54, 40.51, 40.49, 40.45, 24.76, 24.74, 24.68, 24.66, 24.59, 23.07, 23.05, 22.98, 22.96, 20.52, 20.46, 20.35, 20.29, 1.98.
^{31}P NMR (202 MHz, $CDCl_3$) δ 148.85, 148.83.
TLC (DCM/MeOH 19:1) R_f = 0.38.
HRMS (MALDI) $[M+Na]^+$ m/z = calculated: 881.35105, found: 881.35462.

2-Phenyldiazenyl-2'-deoxyadenosine (**11**)

205 mg (0.447 mmol) of compound **7** was dissolved in ammonia saturated methanol (7 M) and stirred over night at room temperature. After evaporation of the solvent the residue was submitted to flash column chromatography (DCM/MeOH 19:1→ 99:1) to afford the product as orange solid.
Yield: 59 mg (0.166 mmol, 37%)
^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.49 (d, *J* = 4.1 Hz, 1H, 8), 7.97 – 7.91 (m, 2H, Ar), 7.75 (s, 2H, NH_2), 7.67 – 7.61 (m, 3H, Ar), 6.42 (dd, *J* = 7.6, 6.2 Hz, 1H, 1'), 5.35 (d, *J* = 4.0 Hz, 1H, 3'-OH), 5.09 (t, *J* = 5.7 Hz, 1H, 5'-OH), 4.44 (d, *J* = 2.5 Hz, 1H, 3'), 3.93 – 3.88 (m, 1H, 4'), 3.65 (dt, *J* = 11.6, 4.5 Hz, 1H, 5'), 3.59 – 3.52 (m, 1H, 5'), 2.75 (ddd, *J* = 13.3, 7.7, 5.8 Hz, 1H, 2'), 2.35 – 2.29 (m, 1H, 2').
^{13}C-NMR (126 MHz, DMSO-d_6) δ 162.34, 156.60, 151.99, 149.93, 141.06, 132.49, 129.63, 122.93, 119.00, 88.15, 83.88, 70.94, 61.88, 48.64, 39.61.
TLC (DCM/MeOH 9:1) R_f = 0.31.
HRMS (MALDI) $[M+H]^+$ m/z = calculated: 356.14656, found: 356.14677.

2-Phenyldiazenyl-5'(4,4'-dimethoxytrityloxymethyl)-2'-deoxyadenosine (**12**)

After coevaporating 100 mg (0.281 mmol, 1.0 eq.) of compound **11** two times with pyridine and dissolving it in 5 mL pyridine, a solution of 105 mg (0.310 mmol, 1.1 eq.) of 4,4'-dimethoxytrityloxymethylchloride in 5 mL pyridine where added dropwise at 0 °C and stirred for 3 hours at room temperature. Upon completion, reaction was quenched with methanol and solvents where coevaporated with toluene. The residue was dissolved in DCM, washed with aqueous sodium carbonate solution, dried over Na_2SO_4 and evaporated to dryness. Flash column chromatography (DCM/MeOH 49:1→19:1 + 1% TEA) afforded the product as orange foam.
Yield: 122 mg (0.185 mmol, 66%)
^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.59 – 8.55 (m, 1H, Ar), 8.39 (s, 1H, 8), 7.91 – 7.87 (m, 1H, Ar), 7.70 (s, 2H, NH_2), 7.67 – 7.62 (m, 2H, Ar), 7.38 (ddd, *J* = 7.6, 4.3, 1.5 Hz, 1H, Ar), 7.28 (dd, *J* = 8.0, 1.5 Hz, 1H, Ar), 7.21 – 7.13 (m, 7H, Ar), 6.71 (dd, *J* = 18.9, 8.9 Hz, 4H, Ar), 6.44 (t, *J* = 6.3 Hz, 1H, 1'), 5.36 (d, *J* = 4.7 Hz, 1H, 3'-OH), 4.51 (dt, *J* = 10.1, 4.9 Hz, 1H, 3'), 4.00 – 3.94 (m, 1H, 4'), 3.68 (s, 6H, CH_3) 3.28 (dd, *J* = 10.3, 6.5 Hz, 1H, 5'), 3.12 (dd, *J* = 10.3, 3.4 Hz, 1H, 5'), 2.87 (dt, *J* = 12.6, 6.1 Hz, 1H, 2'), 2.43 – 2.36 (m, 1H, 2').
^{13}C NMR (126 MHz, DMSO-d_6) δ 162.31, 157.95, 157.91, 156.52, 152.01, 149.94, 149.63, 148.64, 144.92, 136.15, 135.60, 135.46, 129.66, 129.59, 128.67, 127.74, 127.64, 126.52, 123.92, 122.85, 119.58, 119.06, 113.09, 112.98, 112.93, 86.09, 85.34, 83.79, 83.41, 70.79, 70.61, 64.29, 54.95, 45.70, 20.05, 13.99.
TLC (DCM/MeOH 9:1) R_f = 0.58.
HRMS (MALDI) $[M+Na]^+$ m/z = calculated: 680.25919, found: 680.26127.

2-Phenyldiazenyl-2'(cyanoethoxy(*N*,*N*-diisopropylamino)phosphinoxyl)-5'(4,4'-dimethoxytrityloxymethyl)-2'-deoxyadenosine (**13**)

To a solution of 122 mg (0.185 mmol, 1.0 eq.) of compound **12** and 37 µL (0.927 mmol, 5.0 eq.) *N*,*N*-diisopropylethylamine in 3 mL DCM, 44 µL (1.835 mmol, 1.5 eq.) CEO(N^iPr_2)PCl was added and let to stir at room temperature for 1 hour. Upon quenching with methanol the reaction was evaporated to dryness, extracted with DCM, dried over Na_2SO_4 and evaporated to dryness again. The crude product was subjected to flash column chromatography (DCM/MeOH 49:1 → 19:1) to afford the product as orange foam.
Yield: 125 mg (0.146 mmol, 52%)
^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.16 – 8.10 (s, 1H, 8), 8.03 (m, 2H, Ar), 7.52 – 7.48 (m, 2H, Ar), 7.40 – 7.34 (m, 2H, Ar), 7.29 – 7.14 (m, 6H, Ar), 6.81 – 6.71 (m, 6H, Ar), 6.64 – 6.59 (m, 1H, 1'), 6.38 (s, 2H, NH_2), 4.76 (dq, *J* = 6.1, 3.5 Hz, 1H, 3'), 4.25 (ddd, *J* = 10.9, 7.2, 3.6 Hz, 1H, 4'), 3.73 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 3.59 – 3.52 (m, 2H, CH), 3.43 – 3.38 (m, 1H, 5'), 3.27 (m, 1H, 5'), 2.79 – 2.73 (m, 1H, 2'), 2.73 – 2.69 (m, 1H, 2'), 2.57 (t, *J* = 6.4 Hz, 2H, CH_2), 2.43 – 2.39 (m, 2H, CH_2), 1.15 (m, 12H, CH_3).
^{13}C-NMR (126 MHz, $CDCl_3$) δ 162.29, 158.59, 156.20, 152.61, 150.64, 144.56, 140.57, 135.72, 132.52, 130.13, 129.17, 128.28, 128.21, 127.93, 126.98, 123.92, 120.17, 119.98, 117.67, 117.54, 113.23, 86.59, 86.59, 85.83, 84.97, 84.31, 74.20, 73.43, 63.67, 63.45, 58.49, 58.44, 58.34, 58.28, 58.25, 58.20, 55.28, 45.35, 43.39, 43.24, 24.66, 23.07, 20.50, 20.44, 20.31, 20.25, 20.15, 1.97.
^{31}P-NMR (202 MHz, $CDCl_3$) δ 148.79, 148.76.
TLC (DCM/MeOH 19:1) R_f = 0.38.
HRMS (MALDI) $[M+H]^+$ m/z = calculated: 858.38509, found: 858.38488.

2. Oligonucleotide synthesis

DNA synthesis

For the synthesis of DNA containing azobenzene modified nucleosides, phosphoramidites were purchased from Sigma–Aldrich (DMTr-CE-dA-PAC, DMTr-CE-dG-iPrPAC, DMTr-CE-dC-Ac). The strands were synthesized in the 4,4′-dimethoxytrityl (DMT)-On mode on an ABI392 synthesizer by using standard protocols and columns (1.0 µmol; Applied Biosystems). For the cleavage of nucleobase protection groups, a wash with diethylamine (25% in acetonitrile, 20 minutes) followed by an acetonitrile wash and evaporation under reduced pressure were applied. Ammonia (25% in water) was used for resin-cleavage and simultaneous deprotection of nucleobase protection groups (over night, room temperature). After evaporation and resuspension in ultrapure water, the strands were purified by means of RP-HPLC with 0.4M hexafluoroisopropanol and 15 mM trimethylamine in ultrapure water and methanol (gradient: 5 → 40 % methanol over 40 min). Incubation with 80 % acetic acid for 20 min at room temperature cleaved the DMT-group. After evaporation, the strands were again purified by means of RP-HPLC as mentioned before. Mass-spectrometric analyses were recorded on a Bruker micrOTOF-Q device. The strands containing no azobenzene modifications were purchased from biomers.

Oligonucleotide sequences and masses

Oligo type	Name	Sequence 5'→3'	X =	$M_{calculated}$ [g·mol^{-1}]	M_{found} [g·mol^{-1}]
DNA	Strand 1	FAM-GTG-GCA-AGG-T		3646	3645
DNA	Strand 2	ACC-TTG-CCA-C-DAB		3408	3409
DNA	Strand 3	ACC-TTX-CCA-C-DAB	DNAzo	3439	3439
DNA	Strand 4	ACC-TTX-CCA-C-DAB	dG_{Azo}	3497	3496
DNA	Strand 5	FAM-GTG-GCX-AGG-T	dA_{Azo}	3746	3746

3. Spectroscopic studies

3.1 Photostationary states of 2-Phenyldiazenylpurine-deoxyribosides

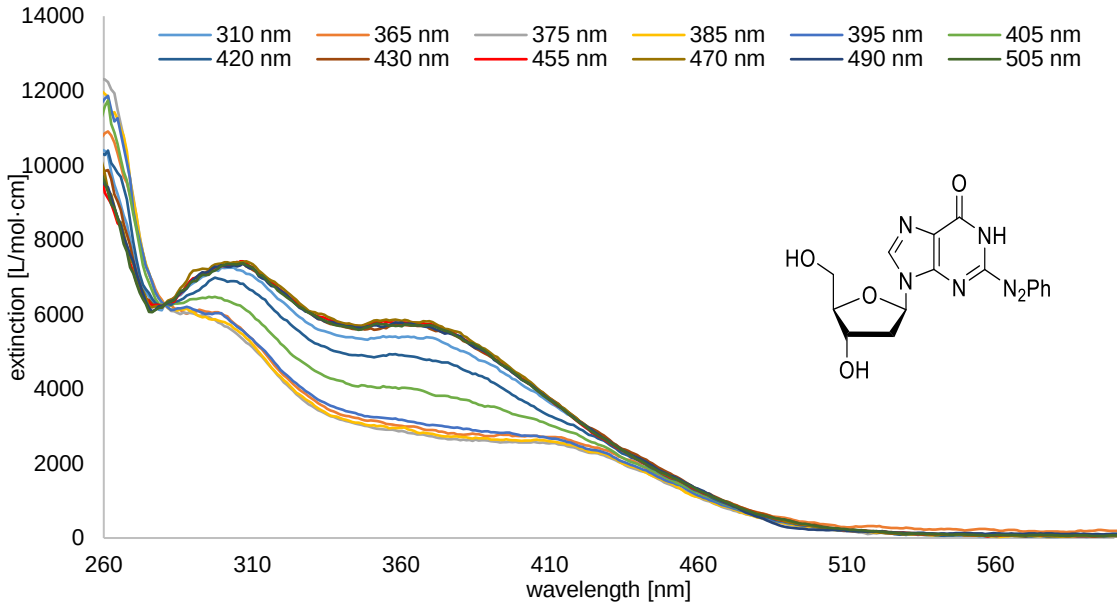

Fig. 12 Photostationary states of free dG_{Azo} nucleoside **8** (100 µM in 1x PBS). 1 mL sample was irradiated until no absorbance changes could be seen anymore, then the spectrum was recorded. Each spectrum corresponds to the wavelength stated above in nm.

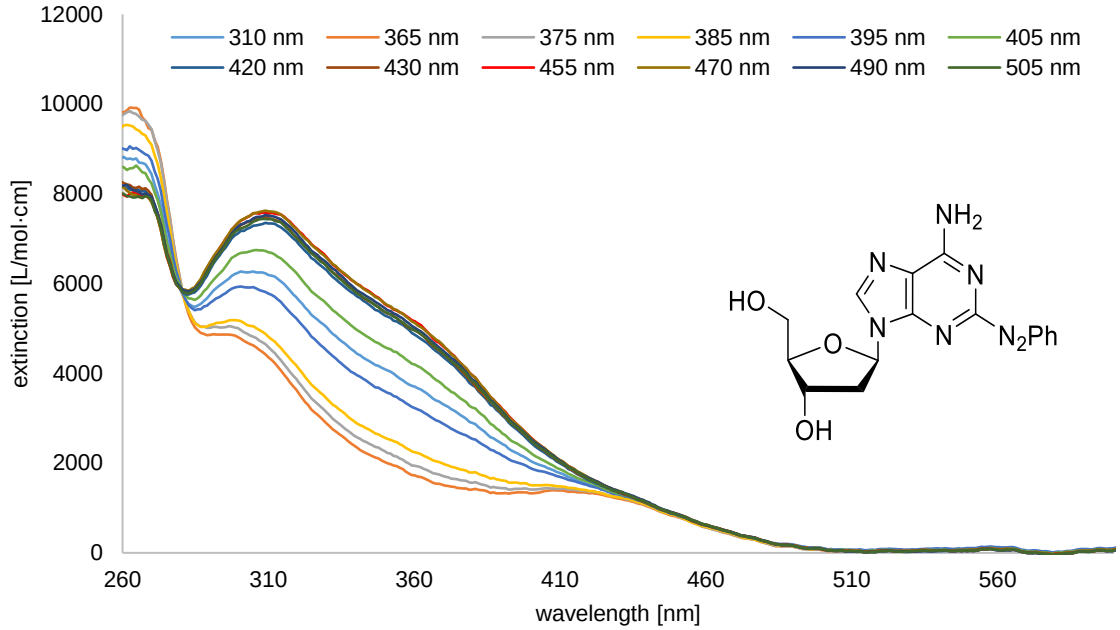

Fig. 2 Photostationary states of free dA_{Azo} nucleoside **11** (100 µM in 1x PBS). 1 mL sample was irradiated until no absorbance changes could be seen anymore, then the spectrum was recorded. Each spectrum corresponds to the wavelength stated above in nm.

3.2 Spectra of the pure photoisomers of 2-phenyldiazenyl purines

Three independent samples of compounds **dG$_{Azo}$** and **dA$_{Azo}$** (100 nmol) were irradiated either at 365 nm or 455 nm until the photostationary state was reached (365 nm: 220 mW, 60 s; 455 nm: 300 mW, 60 s). Photoisomers were then baseline-separated on a short precolumn by means of RP-HPLC under isocratic conditions (**dG$_{Azo}$**: 100% 0.4 M trimethylammonium acetate buffer pH 7; **dA$_{Azo}$**: 95% 0.4 M trimethylammonium acetate buffer pH 7, 5% ACN) and spectra were recorded with an inline diode array detector. The spectra of separate peaks from both photoisomers were superimposed and normalized to the extinction coefficient at the isosbestic point (278 nm; Fig. S3, S4).

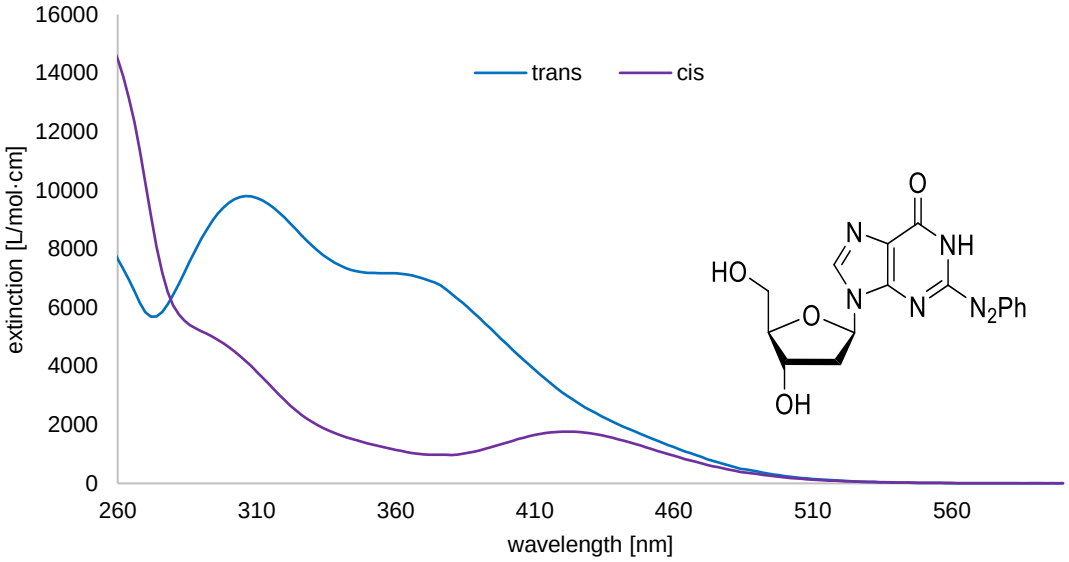

Fig. S3 Spectra of the pure photoisomers of **dG$_{Azo}$**.

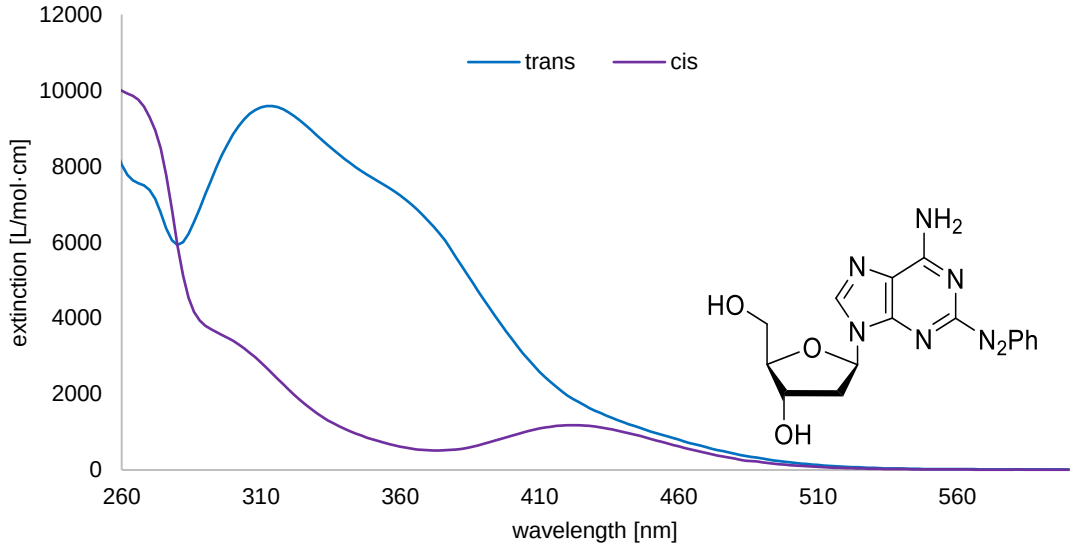

Fig. S4 Spectra of the pure photoisomers of **dA$_{Azo}$**.

3.3 Photostationary distributions of 2-phenyldiazenyl purines

Three independent samples of compounds **dG_{Azo}** and **dA_{Azo}** (100 nmol) were irradiated either at 365 nm or 455 nm until the photostationary state was reached (365 nm: 220 mW, 60 s; 455 nm: 300 mW, 60 s). Photoisomers were then separated on a short precolumn by means of RP-HPLC under isocratic conditions (**dG_{Azo}**: 100% 0.4 M triethylammonium acetate buffer pH 7; **dA_{Azo}**: 95% 0.4 M triethylammonium acetate buffer pH 7, 5% ACN) and spectra were recorded with an inline diode array detector. A chromatogram trace at the isosbestic point (278 nm) was extracted (Figure S5). From the integrals of the peaks, the relative amount of the photoisomers was determined. Results including standard deviation are shown below.

	dG_{Azo}		dA_{Azo}	
	cis [%]	*trans* [%]	*cis* [%]	*trans* [%]
PSS 455 nm	38.2 ± 0.3	61.8 ± 0.3	34.8 ± 0.5	65.2 ± 0.5
PSS 365 nm	77 ± 1	23 ± 1	81.2 ± 0.8	18.8 ± 0.8

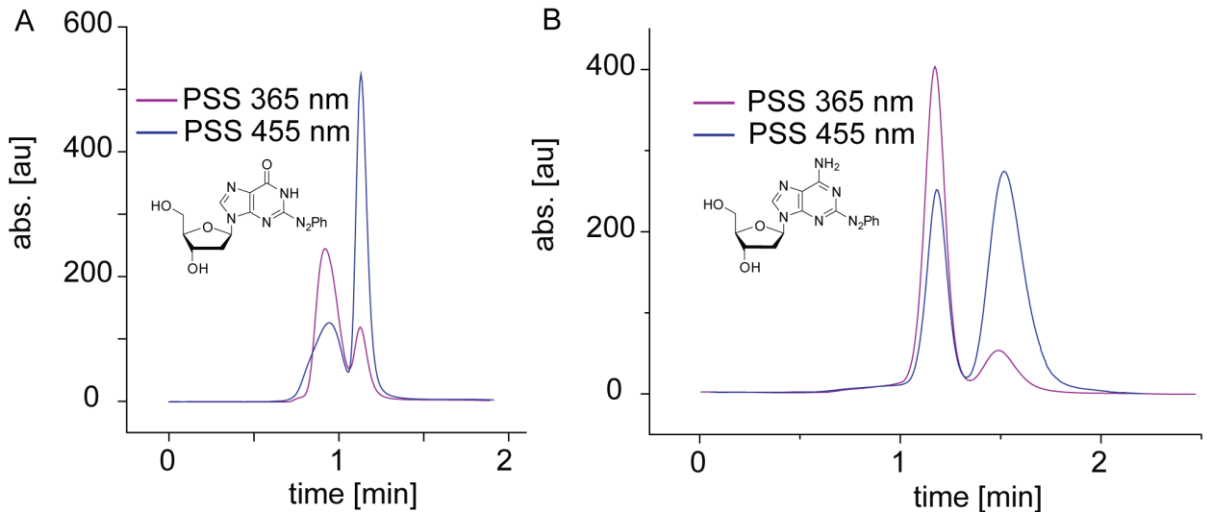

Fig. S5 RP-HPLC chromatograms of the photostationary states at 365 nm and 455 nm of dG_{Azo} (A) and dA_{Azo} (B).

3.4 Photofatigue studies of 2-phenyldiazenylpurine-deoxyribosides

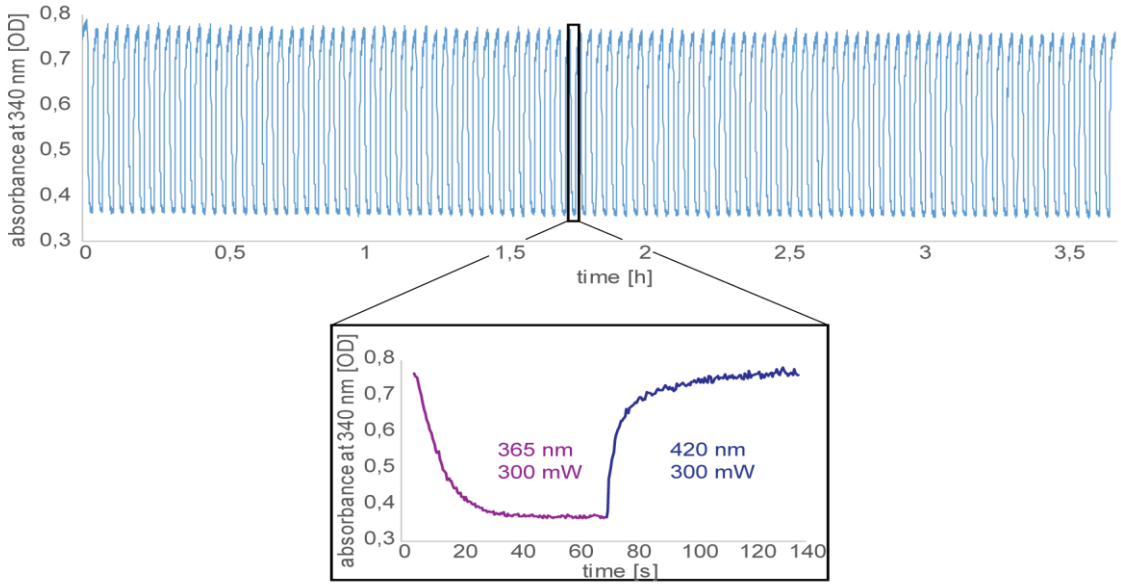

Fig. S6 Photofatigue-study of **dG$_{Azo}$**. A 100 µM sample in 1x PBS-buffer was alternately irradiated at 365 nm (300 mW, 1 min) and at 420 nm (300 mW, 1 min) for 100 cycles, while absorbance change was monitored at 340 nm. No photofatigue could be observed over 100 switching cycles.

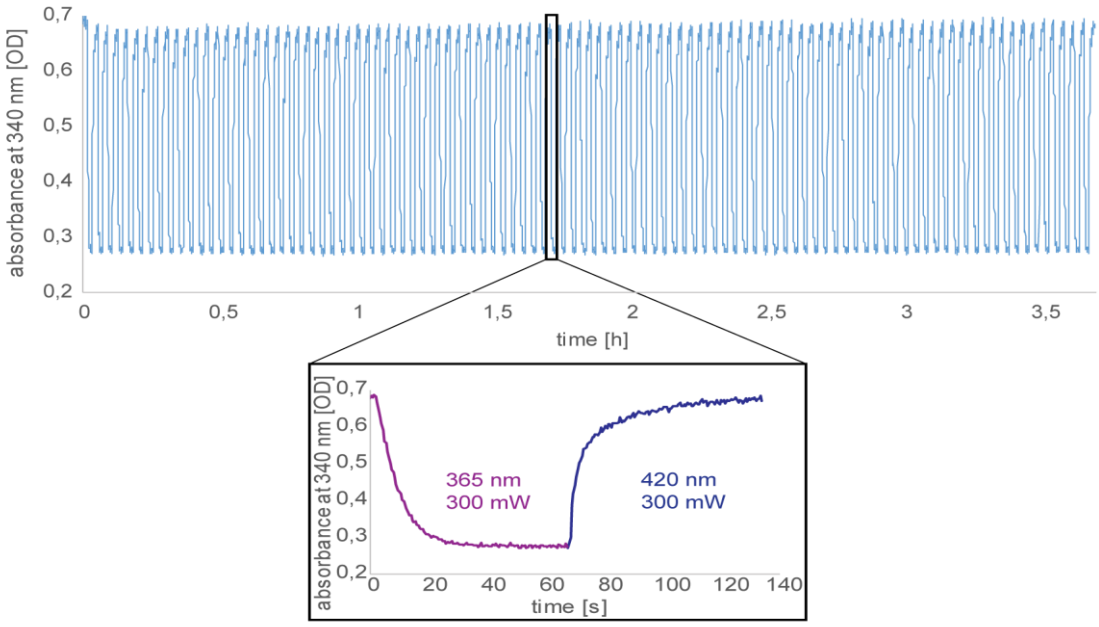

Fig. S7 Photofatigue-study of **dA$_{Azo}$**. A 100 µM sample in 1x PBS-buffer was alternately irradiated at 365 nm (300 mW, 1 min) and at 420 nm (300 mW, 1 min) for 100 cycles, while absorbance change was monitored at 340 nm. No photofatigue could be observed over 100 switching cycles.

3.5 Thermal relaxation of the *cis*-states

The thermal relaxation of the *cis*-state to the thermodynamically favored *trans*-state without irradiation follows a first order reaction kinetic. Formulating the reaction as $Azo_{cis} \rightarrow Azo_{trans}$ with the reaction rate k in [s^{-1}], the concentration of Azo_{cis} is proportional to the time dependent concentration change of Azo_{cis}.

$$-\frac{dAzo_{cis}}{dt} = k[Azo_{cis}] \quad \text{(equation 1)}$$

To solve this first order differential equation, separation of variables and integration is necessary.

$$\frac{d[Azo_{cis}]}{[Azo_{cis}]} = -kdt \quad \text{(equation 2)}$$

$$\ln[Azo_{cis}] - l\,n[Azo_{cis}]_0 = -ln\frac{[Azo_{cis}]_0}{[Azo_{cis}]} = -kt \quad \text{(equation 3)}$$

The concentration of the *cis*-species can be determined with the absorption at 380 nm according to the following rearrangements.[1]

$$\frac{[Azo_{cis}]_0}{[Azo_{cis}]} = \frac{[Azo_{cis}]_0(\varepsilon_{trans,380}-\varepsilon_{cis,380})}{[Azo_{cis}](\varepsilon_{trans,380}-\varepsilon_{cis,380})} \quad \text{(equation 4)}$$

$$= \frac{([Azo_{trans}]_0+[Azo_{cis}]_0)\varepsilon_{trans,380}-([Azo_{trans}]_0+[Azo_{cis}]_0)\varepsilon_{cis,380})}{([Azo_{trans}]_t+[Azo_{cis}]_t)\varepsilon_{trans,380}-([Azo_{trans}]_t+[Azo_{cis}]_t)\varepsilon_{cis,380})} \quad \text{(equation 5)}$$

$$= \frac{\varepsilon_{trans,380}-([Azo_{trans}]_0\varepsilon_{trans,380}+[Azo_{cis}]_0\varepsilon_{cis,380})}{\varepsilon_{trans,380}-([Azo_{trans}]_t\varepsilon_{trans,380}+[Azo_{cis}]_t\varepsilon_{cis,380})} \quad \text{(equation 6)}$$

$$= \frac{A_{\infty,380}-A_{0,380}}{A_{\infty,380}-A_{t,380}} \quad \text{(equation 7)}$$

The absorption at the thermal ground state at 380 nm is defined as $A_{\infty,380}$, the absorption at 380 nm at the beginning of the measurement as $A_{0,380}$ and $A_{t,380}$ as the absorption at 380 nm during the time t. To calculate the thermal relaxation rate k, equation 8 and 3 are merged.

$$ln\frac{A_{\infty,380}-A_{0,380}}{A_{\infty,380}-A_{t,380}} = kt \quad \text{(equation 8)}$$

Plotting $ln\frac{A_{\infty,350}-A_{0,350}}{A_{\infty,350}-A_{t,350}}$ against the time t gives the thermal relaxation rate k as the slope. Thermal relaxation has been measured at 25, 37 and 60 °C. As thermal relaxation at 25 and 37 °C has been very slow, time dependent absorption change has been extrapolated in order to calculate the relaxation rate and thermal half-life times.

The half-time of a first order reaction is defined by equation 9.

$$\boldsymbol{t_{\frac{1}{2}}} = \frac{\boldsymbol{ln(2)}}{\boldsymbol{k}} \quad \text{(equation 9)}$$

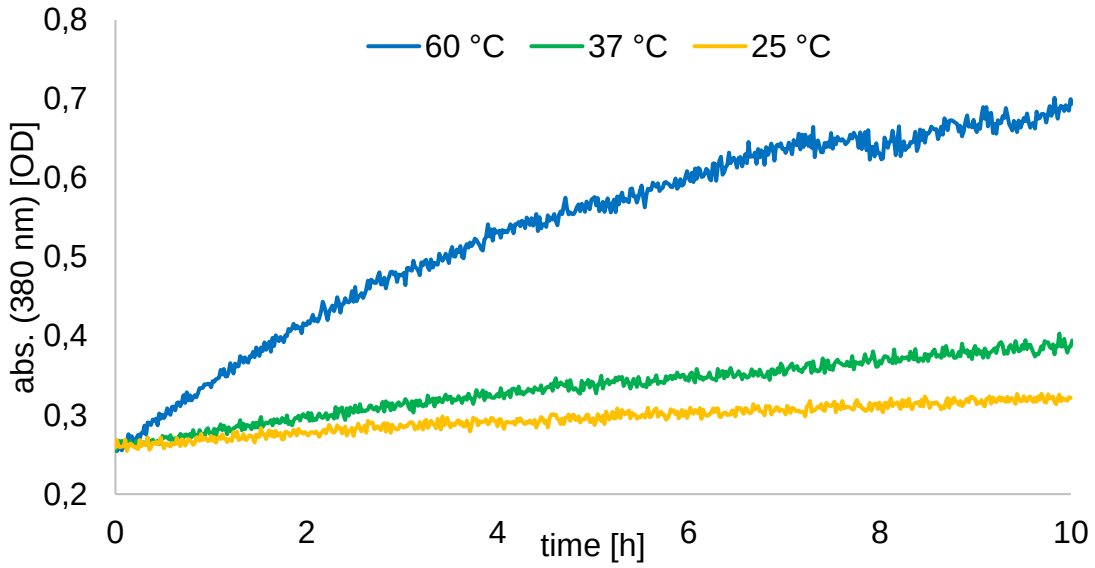

Fig. S8 Thermal relaxation of dG_{Azo} observed as absorption change at 380 nm.

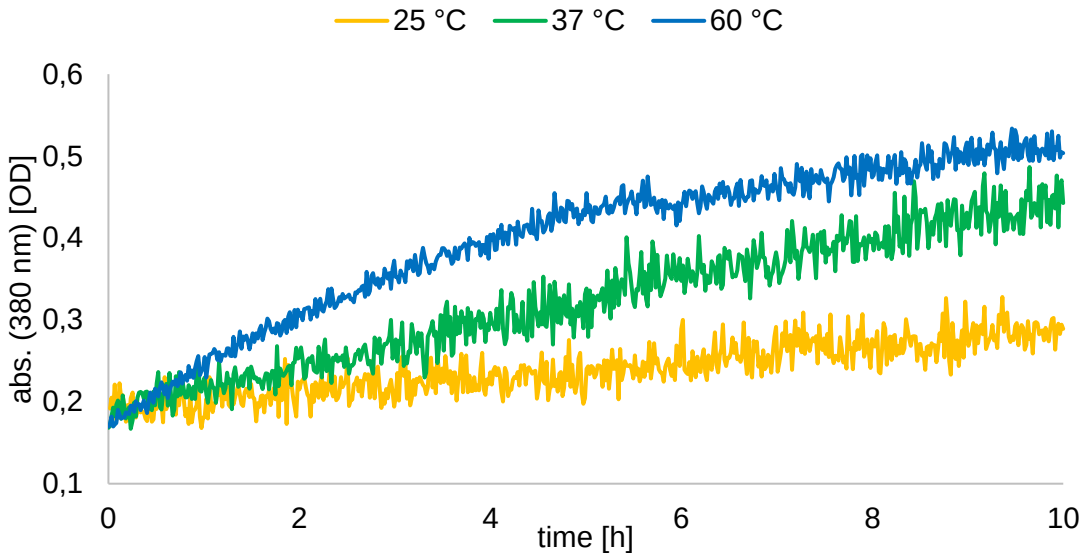

Fig. S9 Thermal relaxation of dA_{Azo} observed as absorption change at 380 nm.

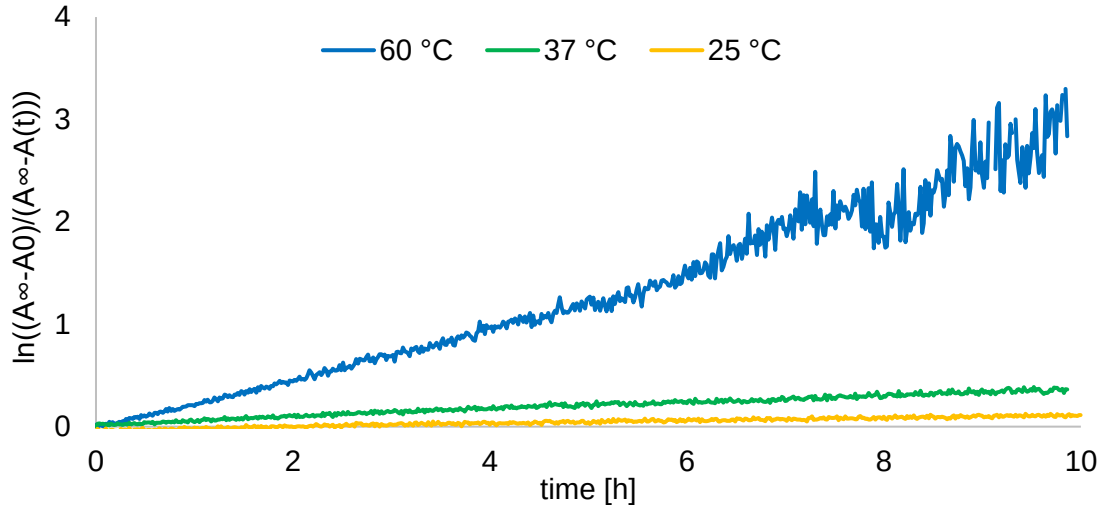

Fig. S10 Plot to calculate thermal relaxation rate k and half-life $t_{1/2}$ of dG_{Azo} in accordance to equation 8.

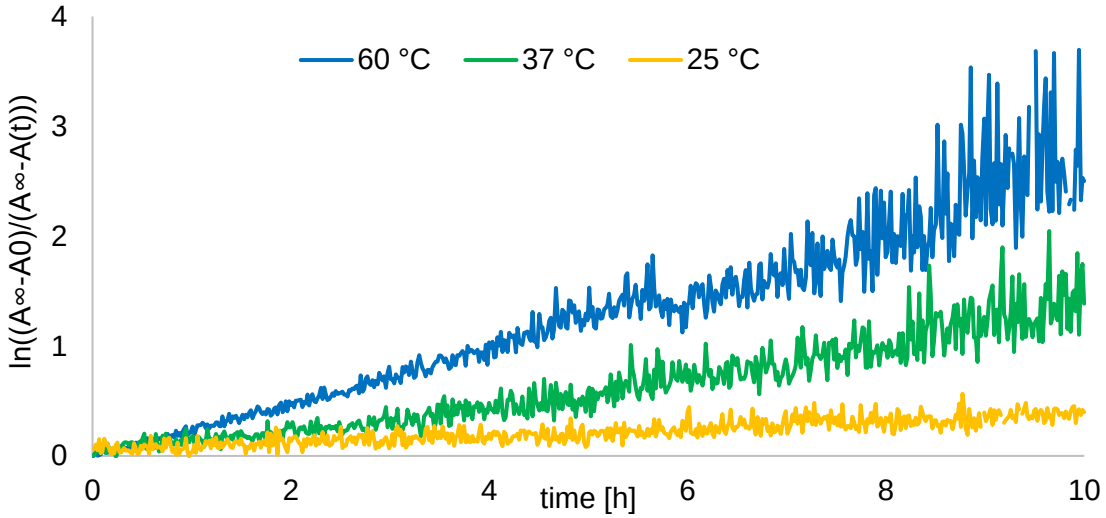

Fig. S11 Plot to calculate thermal relaxation rate k and half-life $t_{1/2}$ of dA_{Azo} in accordance to equation 8.

dG_{Azo}	k=	0.29±0.01 s^{-1}	(60 °C)	dA_{Azo}	k=	0.27±0.02 s^{-1}	(60 °C)
		0.034±0.001 s^{-1}	(37 °C)			0.14±0.02 s^{-1}	(37 °C)
		0.015±0.001 s^{-1}	(25 °C)			0.03±0.01 s^{-1}	(25 °C)
$t_{1/2}$=		2.39±0.08 h	(60 °C)		$t_{1/2}$=	2.6±0.2 h	(60 °C)
		20.4±0.5 h	(37 °C)			5.0±0.6 h	(37 °C)
		46±3 h	(25 °C)			23±6 h	(25 °C)

3.6 Quantum yields of the photoisomerization processes

For calculation of the quantum yields the method of Reinfelds *et al.* was used.[2] The described fulgide was dissolved in toluene to give a solution with an OD of approximately 0.5, then irradiated between the photostationary states of 595 nm, 455 nm and 365 nm. With the known quantum yields and extinction coefficients for these processes, photon flux of used LED's could be calculated with a custom software (Photoswitch Irradiation Test Suite – PHITS). The photon flux was calculated from five independent irradiation processes each for best results.

wavelength λ [nm]	LED type	LED power [mW]	photon flux [nmol/s]
365	Thorlabs M365L2	44.3 (100 mA)	39.6 ± 0.5
455	Thorlabs M455L2	112.5 (100 mA)	21.1 ± 0.6

With the known photon flux the quantum yield of the 2-phenyldiazenyl purines could be calculated. Solutions of the 2-phenyldiazenyl purines (100 µM in 1x PBS, pH7.4, 2 mL) where irradiated, until photostationary states at 365 nm or 455 nm where reached. The quantum yield was calculated with the above mentioned software and the spectra of pure photoisomers from five independent irradiation processes each for best results.

	process	wavelength λ [nm]	quantum yield Φ [%]
dA_{Azo}	*cis* → *trans*	455	43.5 ± 0.5
	trans → *cis*	365	16.9 ± 0.2
dG_{Azo}	*cis* → *trans*	455	58.9 ± 0.2
	trans → *cis*	365	18.7 ± 0.1

4. Melting temperature measurements

For melting temperature measurements 1 mL samples were prepared with 1µM of strand and counterstrand in 1x PBS-buffer for each modification (5 in total). The absorbance changes at 260 nm were measured by a V-650 UV/vis-spectrometer from JASCO. Samples were irradiated as single strands at 70 °C with either 365 nm or 455 nm until photostationary state (PSS) was reached to prevent mismatches. Temperature gradient was 1 °C per minute. To avoid effects of hysteresis, melting temperatures were calculated by sigmoidal fit from cooling and heating measurements. At least five independent heating and cooling measurements were performed for precise results. For experimental values with error bars see chapter 4.2.3. The most important melting curves of duplexes are shown below (Fig. S13-S16), each containing five cooling (1-5) and heating (1r-5r) curves.

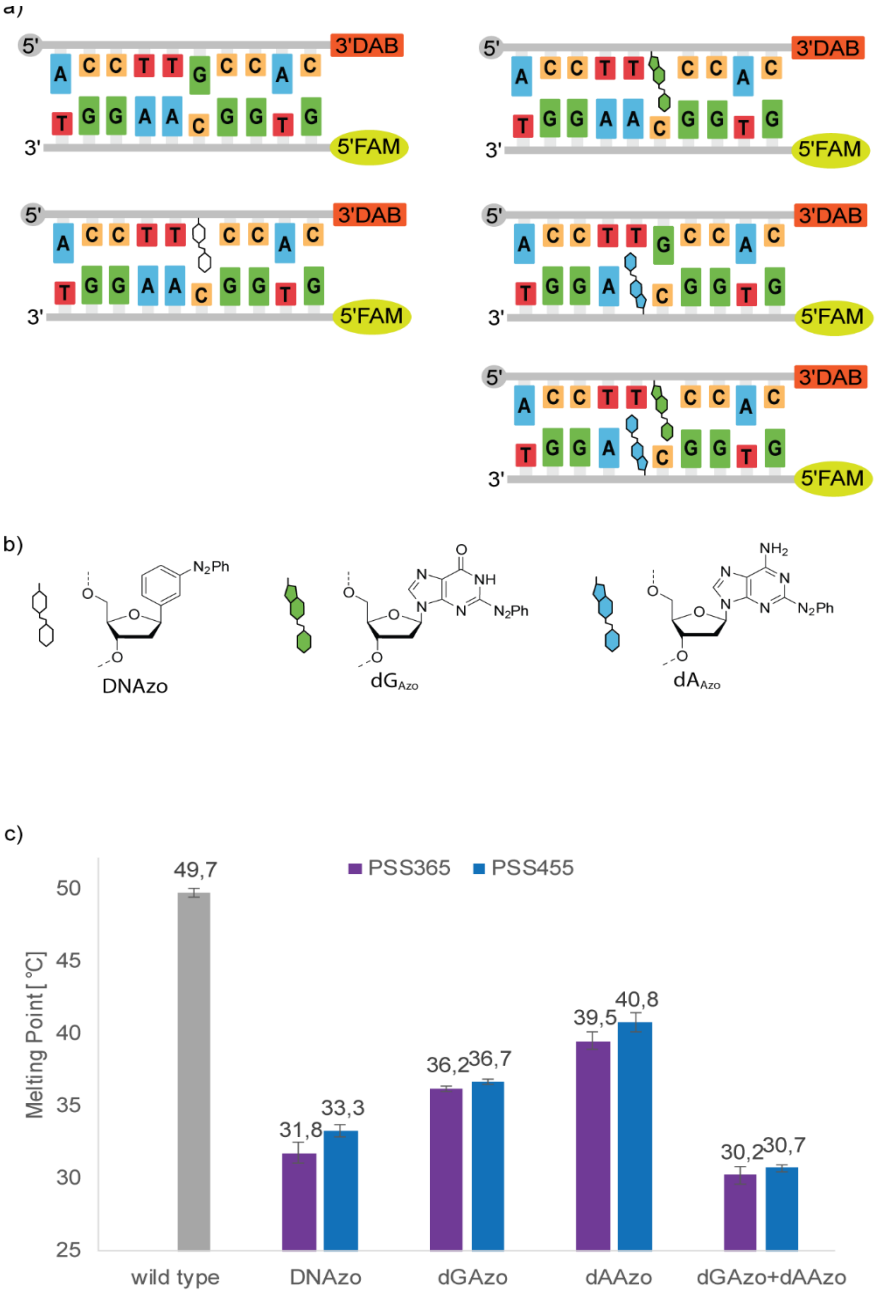

Fig. S12 Oligonucleotides used in this study (a), modifications in the oligonucleotides shown above (b) and melting points measured by measuring temperature dependent absorption changes at 260 nm summarized in a column graph (c).

	wild type	TM						
	1	48,1						
	1r	51,6						
	2	47,5						
	2r	51,5						
	3	47,7						
	3r	51,8						
	4	47,9						
	4r	51,7						
	5	47,6						
	5r	51,8						
	80->10 °C	47,8						
	10->80 °C	51,7						
	average	49,7						
	σ	0,3						
	DNAzo	TM	dGAzo	TM	dAAzo	TM	dGAzo+dAAzo	TM
	1	25,9	1	34,3	1	36,3	1	26,7
	1r	30,2	1r	38,4	1r	40,9	1r	33,0
	2	25,8	2	34,2	2	36,5	2	27,9
	2r	30,2	2r	38,5	2r	42,7	2r	32,6
	3	26,4	3	34,0	3	36,7	3	28,0
	3r	30,7	3r	38,3	3r	42,6	3r	32,5
PSS 365 nm	4	26,9	4	34,1	4	36,7	4	28,0
	4r	31,1	4r	38,3	4r	42,9	4r	32,7
	5	27,1	5	34,0	5	36,7	5	27,9
	5r	30,9	5r	38,3	5r	43,1	5r	32,9
	80->10 °C	31,8	80->10 °C	34,1	80->10 °C	36,6	80->10 °C	27,7
	10->80 °C	31,8	10->80 °C	38,4	10->80 °C	42,4	10->80 °C	32,8
	average	31,8	average	36,2	average	39,5	average	30,2
	σ	0,8	σ	0,2	σ	0,6	σ	0,7
	DNAzo	TM	dGAzo	TM	dAAzo	TM	dGAzo+dAAzo	TM
	1	31,0	1	34,5	1	37,3	1	27,9
	1r	34,9	1r	38,6	1r	42,8	1r	33,0
	2	31,3	2	34,8	2	38,0	2	28,2
	2r	35,0	2r	38,7	2r	43,4	2r	33,1
	3	31,4	3	34,8	3	38,2	3	28,3
	3r	35,2	3r	38,6	3r	43,6	3r	33,2
PSS 420 nm	4	31,5	4	35,0	4	38,4	4	28,4
	4r	35,3	4r	38,6	4r	43,7	4r	33,2
	5	31,8	5	34,7	5	38,4	5	28,4
	5r	35,4	5r	38,6	5r	44,0	5r	33,3
	80->10 °C	31,4	80->10 °C	34,8	80->10 °C	38,1	80->10 °C	28,2
	10->80 °C	35,2	10->80 °C	38,6	10->80 °C	43,5	10->80 °C	33,2
	average	33,3	average	36,7	average	40,8	average	30,7
	σ	0,4	σ	0,2	σ	0,7	σ	0,3

Table 1: Measured melting temperatures of heating (1r-5r) and cooling (1-5) curves in °C, averaged heating and cooling melting temperatures and averaged total melting temperatures with error calculated from mean standard deviation.

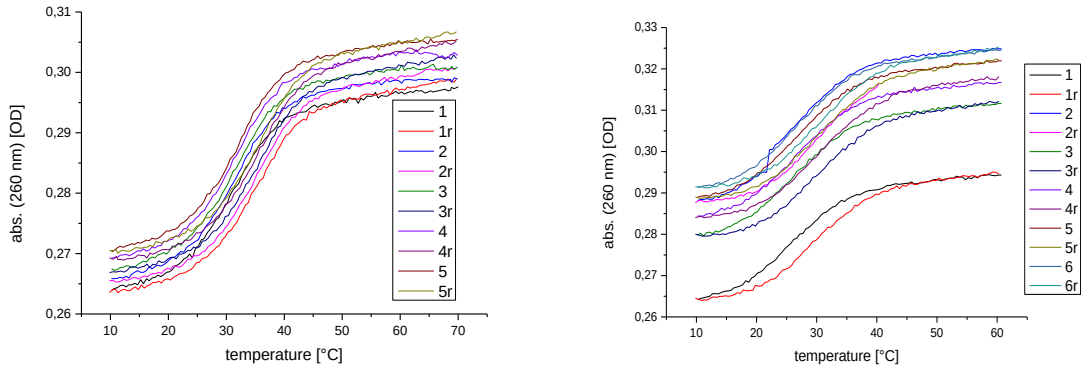

Fig. S13 Melting curves of DNAzo-modified strands (strand 1+3) in PSS 455 nm (left) and PSS 365 nm (right).

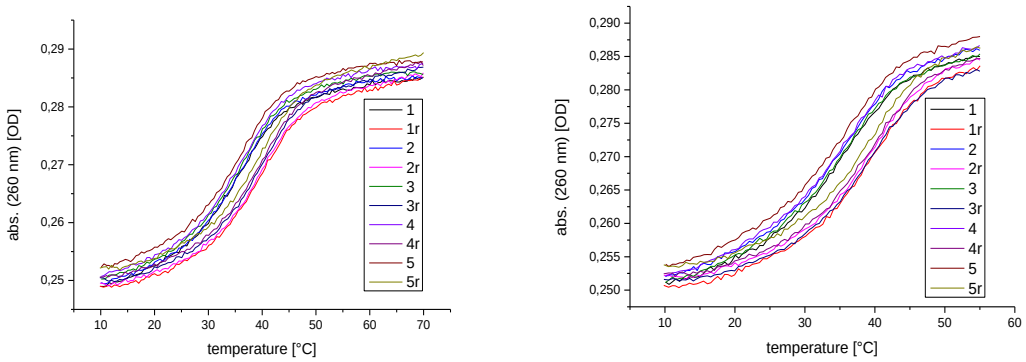

Fig. S14 Melting curves of dG_{Azo}-modified strands (strand 1+4) in PSS 455 nm (left) and PSS 365 nm (right).

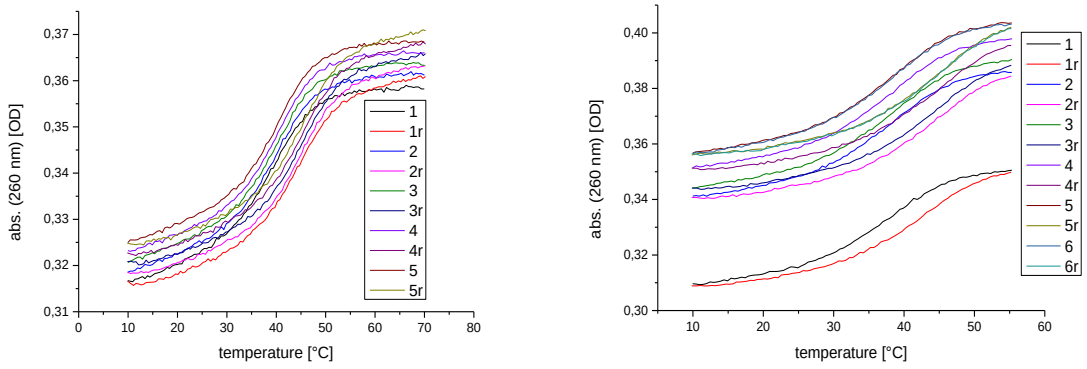

Fig. S15 Melting curves of dA_{Azo}-modified strands (strand 2+5) in PSS 455 nm (left) and PSS 365 nm (right).

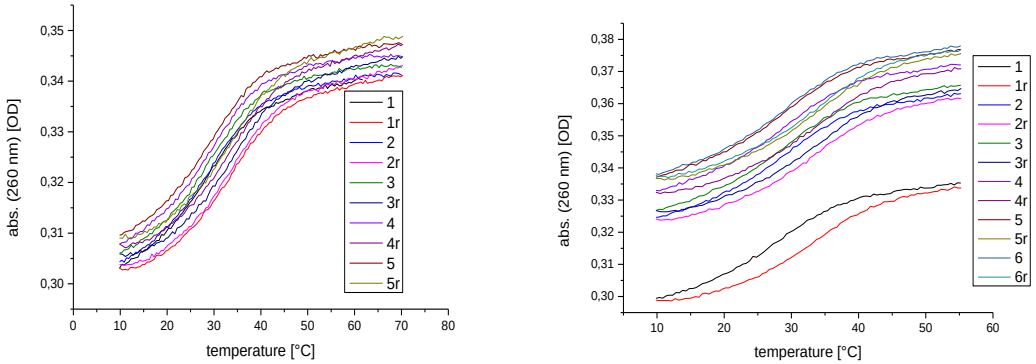

Fig. S16 Melting curves of dG_{Azo}+dA_{Azo}-modified strands (strand 4+5) in PSS 455 nm (left) and PSS 365 nm (right).

5. CD-spectroscopic studies

Samples for CD-measurements were 10 µM in 1x PBS-buffer, CD-spectra were recorded on a J-710 CD-spectrometer from JASCO. Samples were irradiated at 70 °C, then annealed and measured at 20 °C. Ten individual measurements were accumulated for each spectrum for best results.

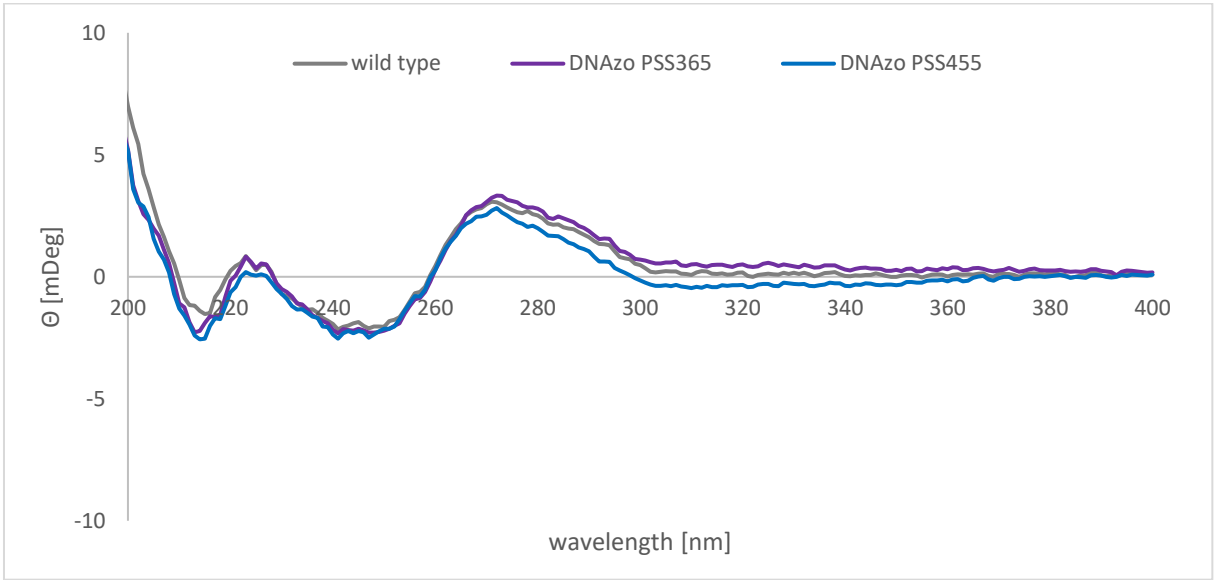

Fig. S17 CD-spectrum of strand 1+3 compared to strand 1+2.

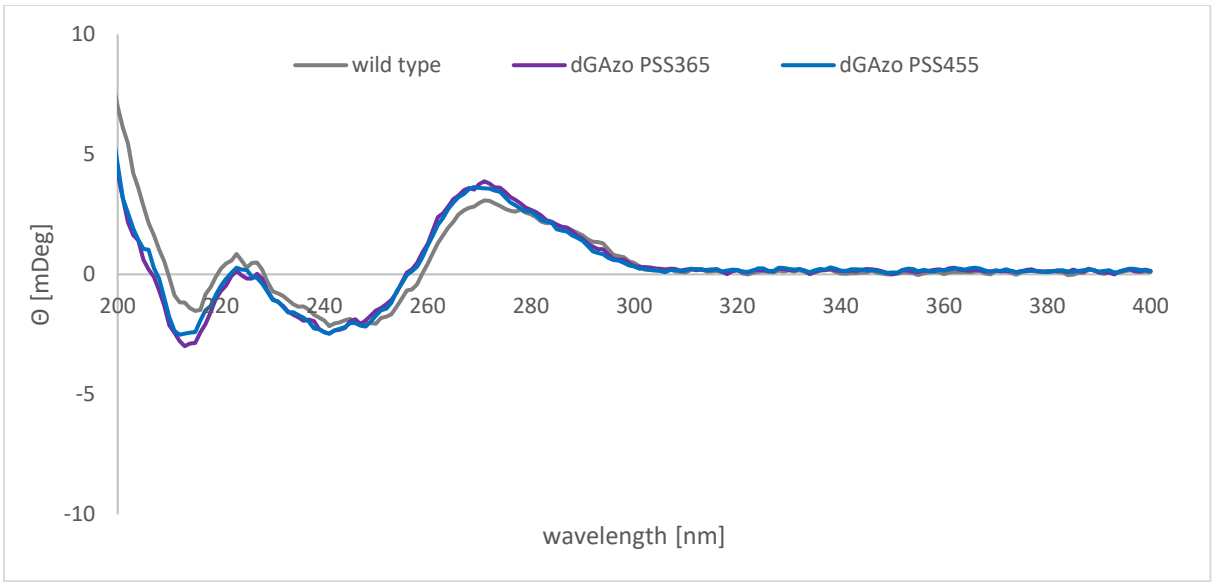

Fig. S18 CD-spectrum of strand 1+4 compared to strand 1+2.

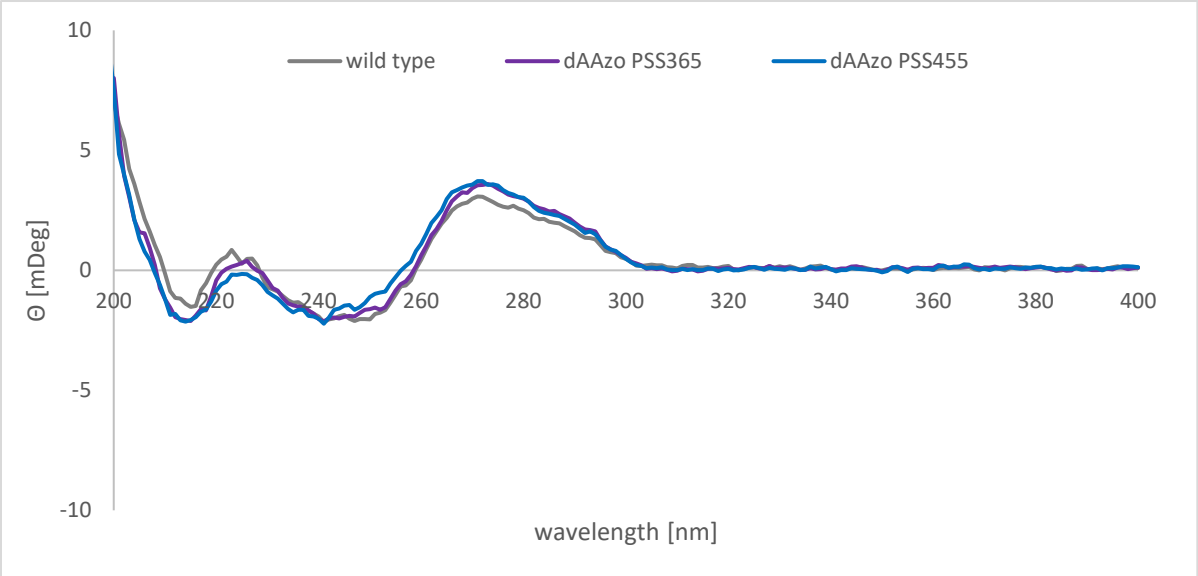

Fig. S19 CD-spectrum of strand 2+5 compared to strand 1+2.

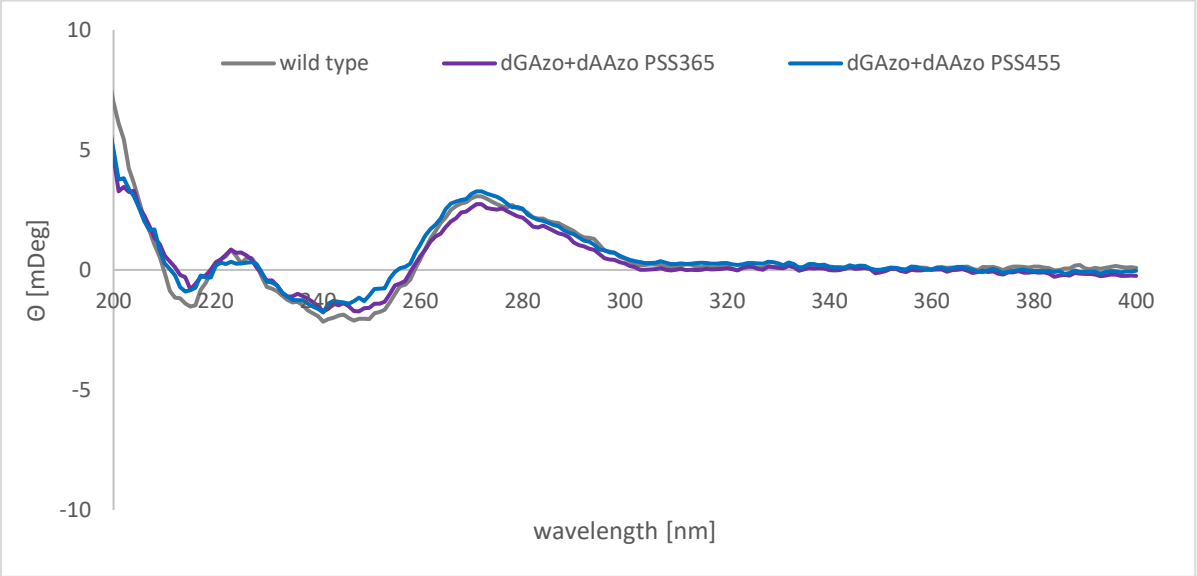

Fig. S20 CD-spectrum of strand 4+5 compared to strand 1+2.

6. Fluorescence-based studies

Temperature-dependent fluorescence measurements have been recorded in a PikoReal real-time PCR system from *Thermo Scientific.* Triplikates were irradiated at 80 °C, then temperature dependent fluorescence was measured from 80 °C to 5 °C within one hour. Values given are averaged over these three individual samples. Measured spectra were normalized with the spectra of free fluorescein in PBS buffer of the corresponding concentration to level out temperature-dependence of fluorescein fluorescence followed by sigmoidal fit with Origin. The inflection points of resulting fits are given as melting points. Melting points will be given as column graphs in Figure S20-34, corresponding melting points in [°C] above the graphs, concentrations in [mol/L] below the graphs starting on the following page. The corresponding wild type was included as a negative control, as irradiation at different wavelength should not alter melting points. In some cases sigmoidal fit to curves with abnormal course could not be executed, indicated by missing columns. In the case of dG_{Azo} + dA_{Azo} combined, no reasonable values could be gained out of fluorescence spectra.

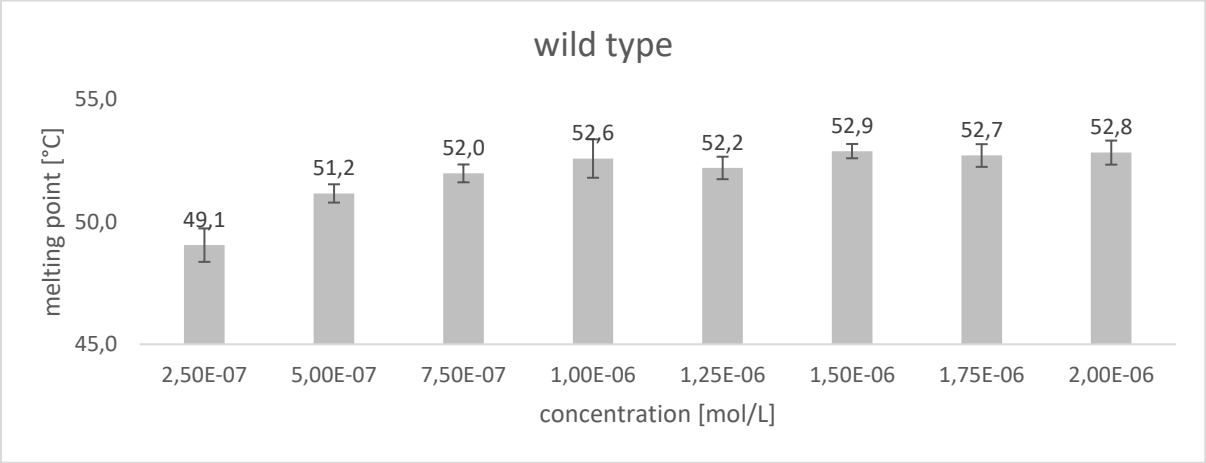

Fig. S21 Bar-graph of fluorescence-based melting temperatures of the wild type duplex.

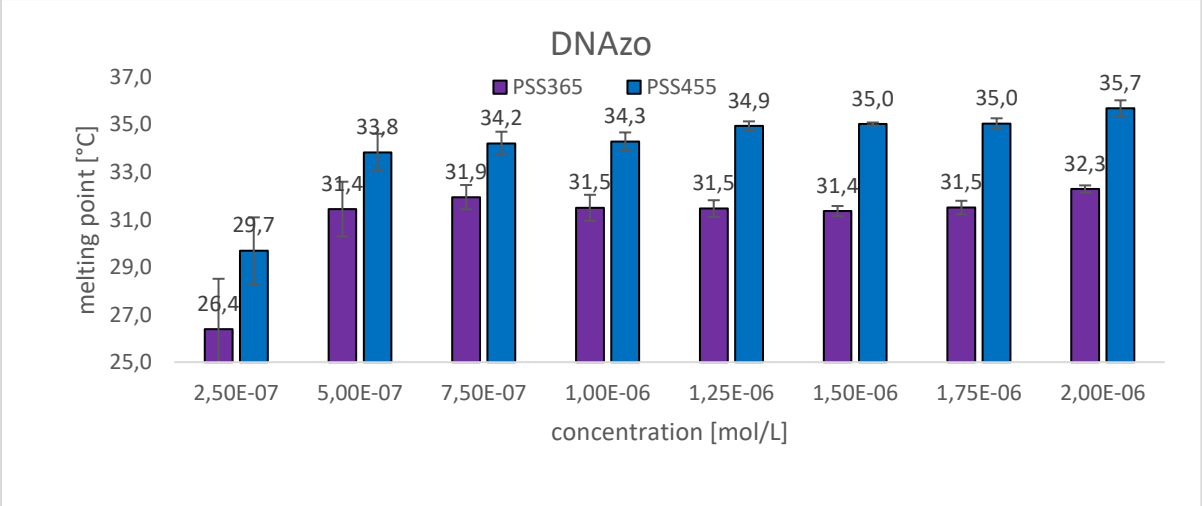

Fig. S22 Bar-graph of fluorescence-based melting temperatures of the DNAzo-containing duplex.

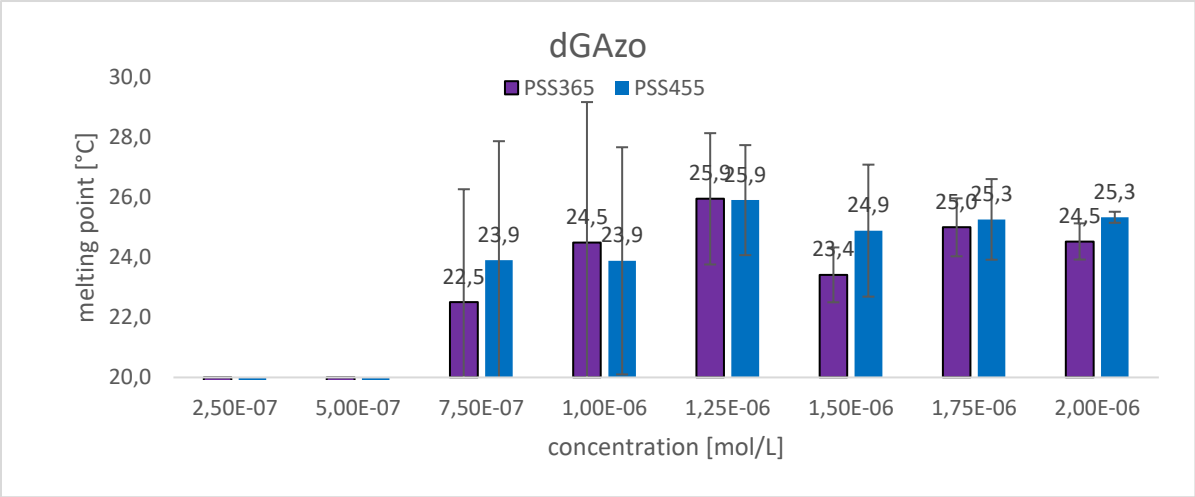

Fig. S23 Bar-graph of fluorescence-based melting temperatures of the dGAzo-containing duplex.

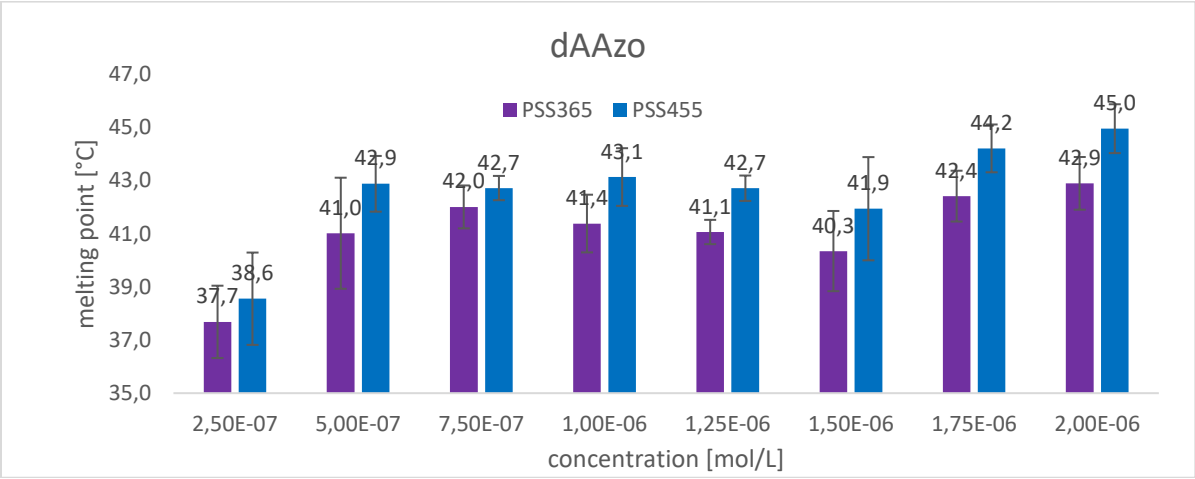

Fig. S24 Bar-graph of fluorescence-based melting temperatures of the dAAzo-containing duplex.

7. NMR-spectra of synthesized compounds

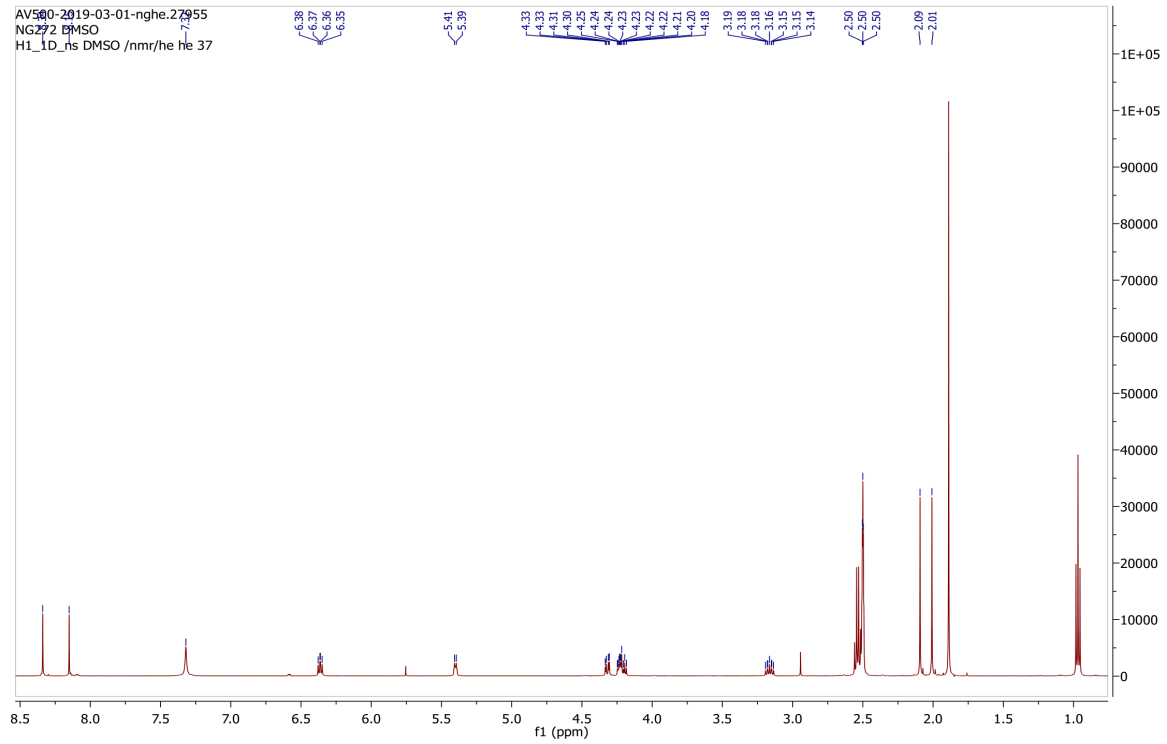

Fig. S25 ^{1}H spectrum of compound 2.

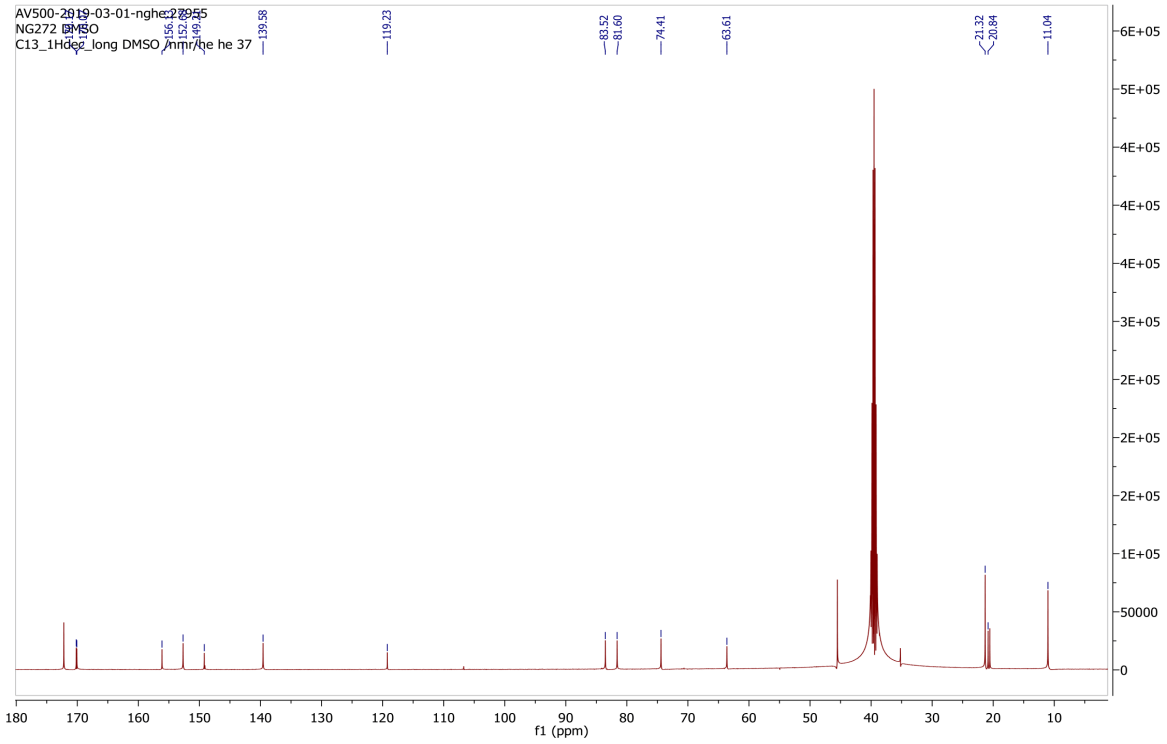

Fig. S26 ^{13}C spectrum of compound 2.

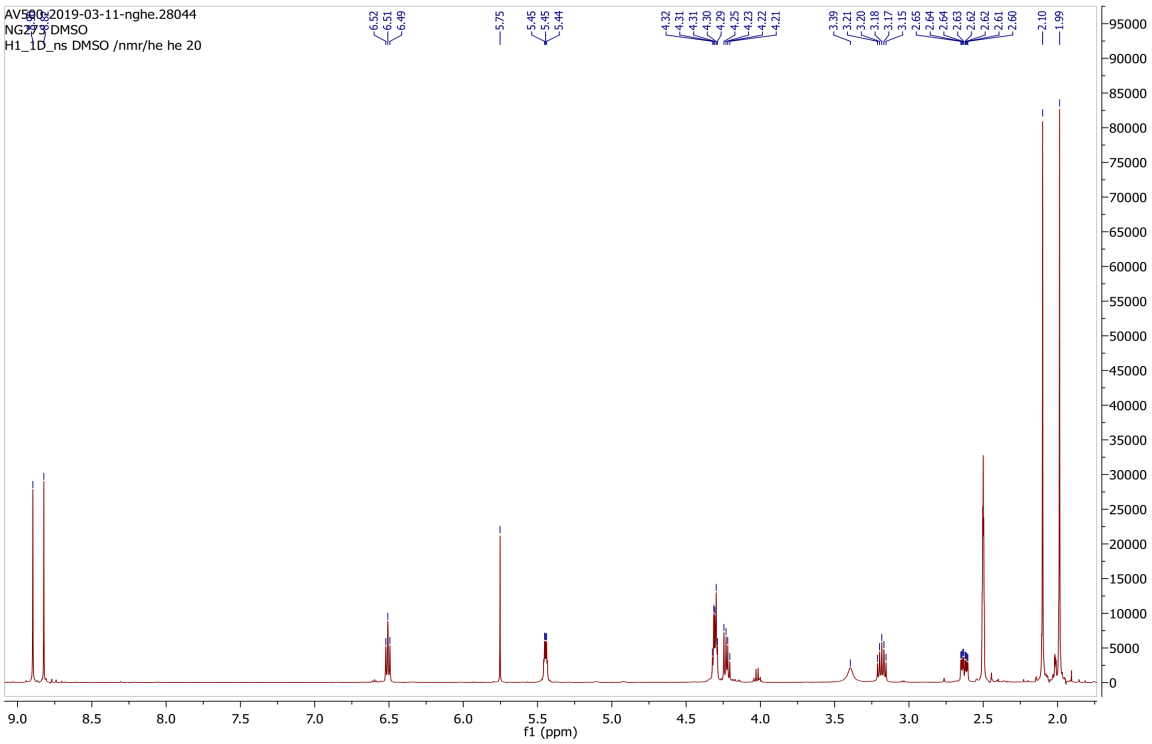

Fig. S27 ^{1}H spectrum of compound 3.

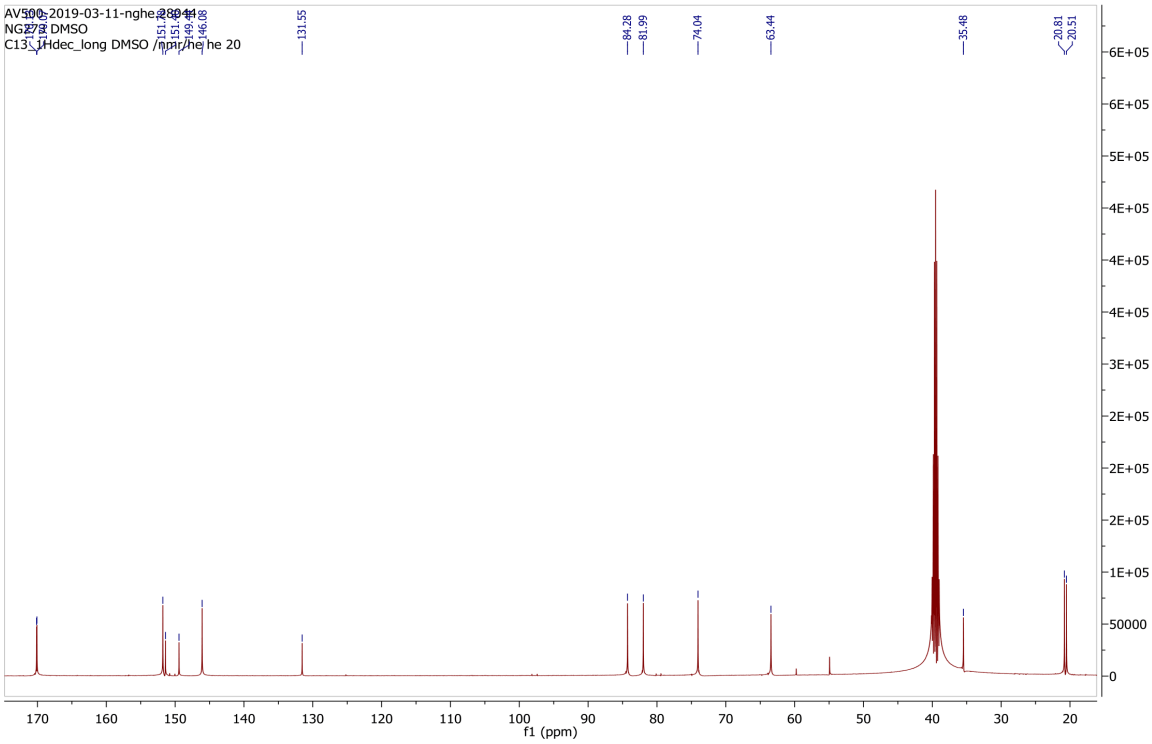

Fig. S28 ^{13}C spectrum of compound 3.

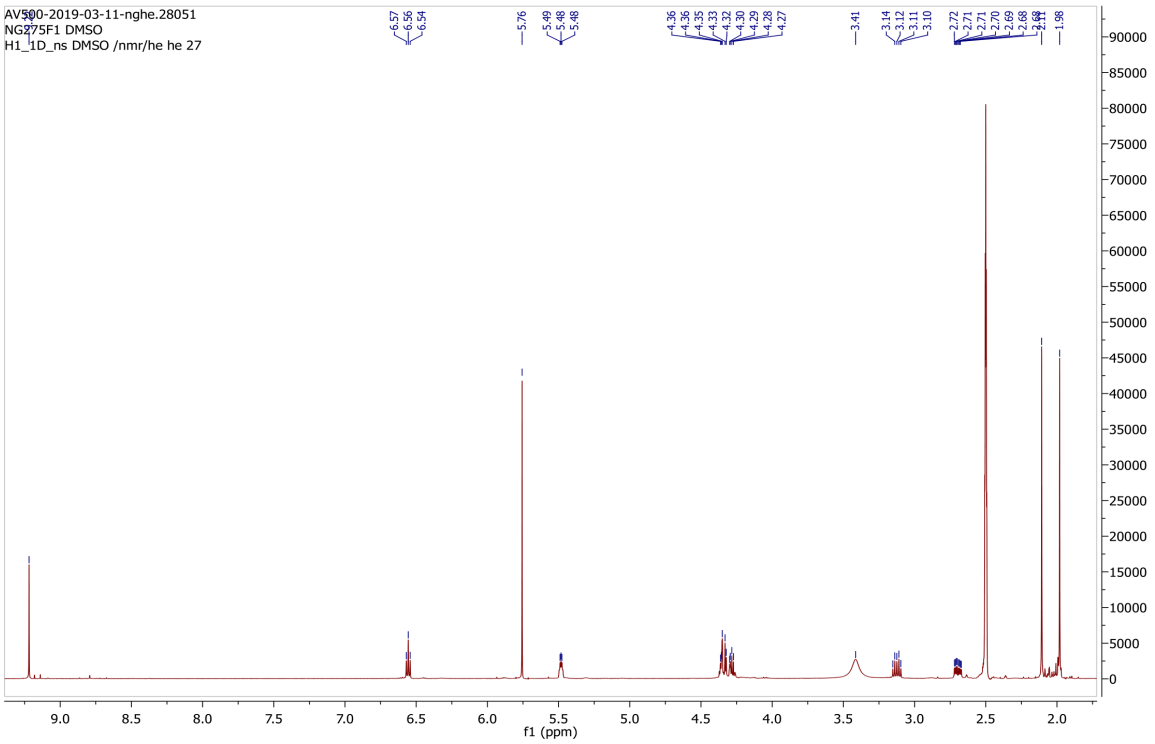

Fig. S29 ^{1}H spectrum of compound 4.

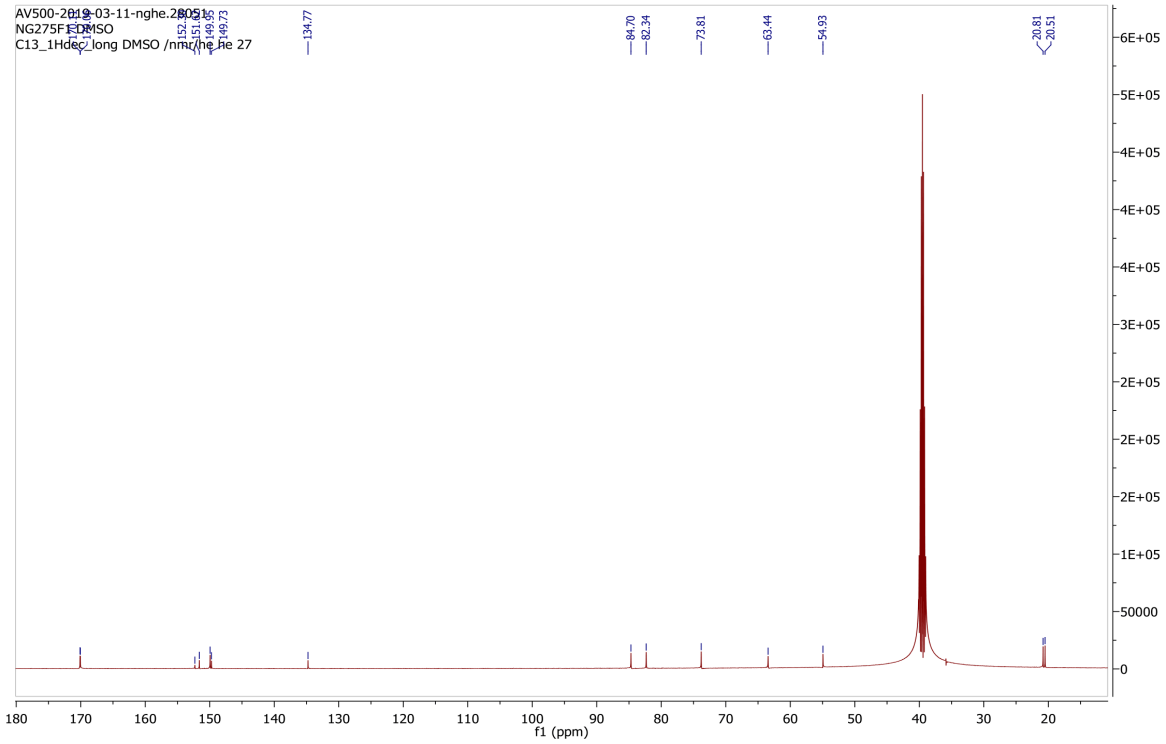

Fig. S30 ^{13}C spectrum of compound 4.

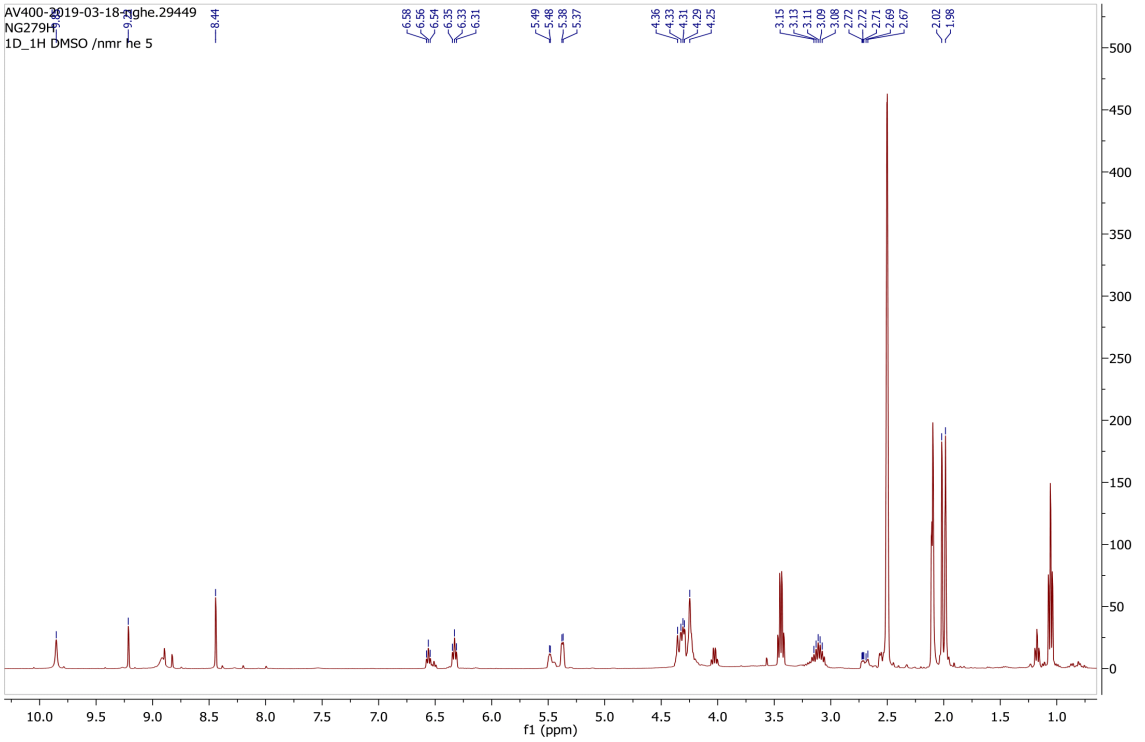

Fig. S31 ^{1}H spectrum of compound 5.

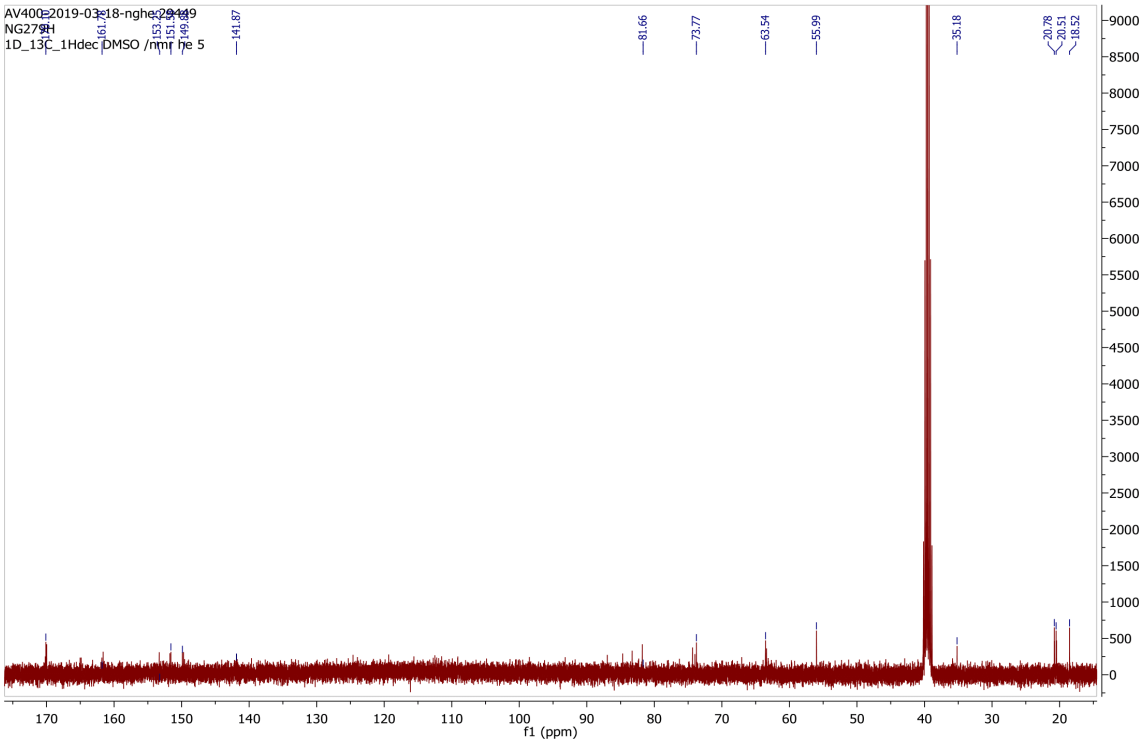

Fig. S32 ^{13}C spectrum of compound 5.

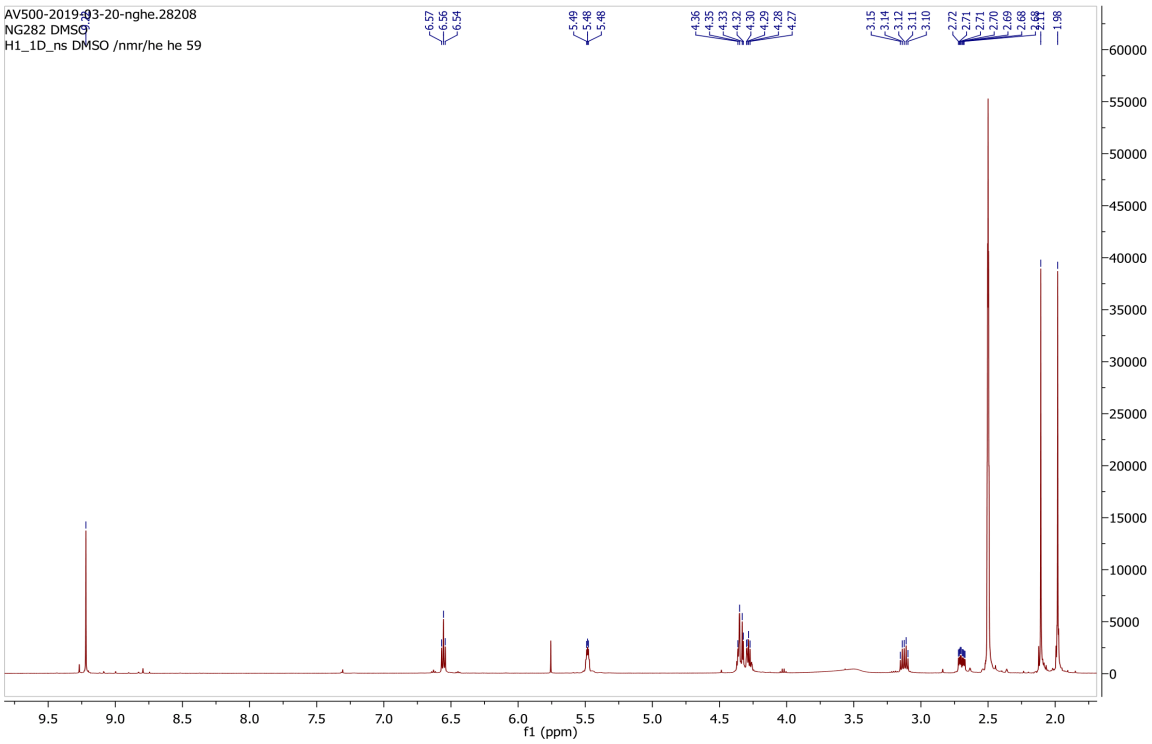

Fig. S33 ^{1}H spectrum of compound 6.

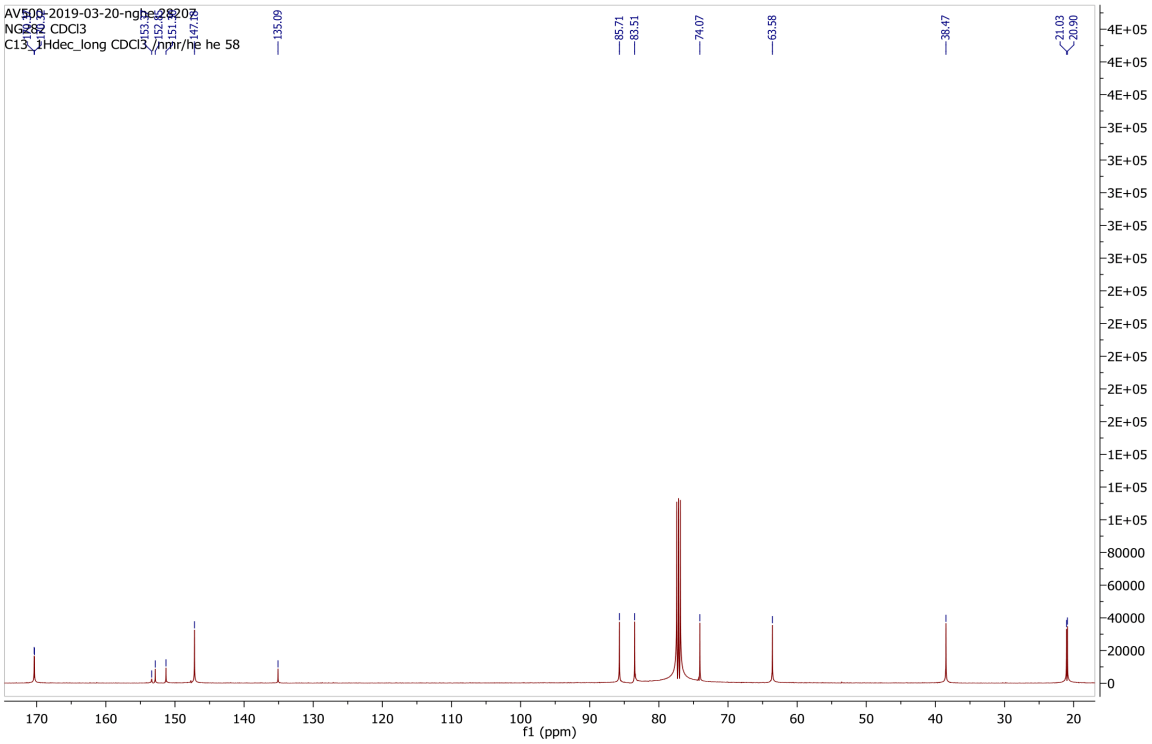

Fig. S34 ^{13}H spectrum of compound 6.

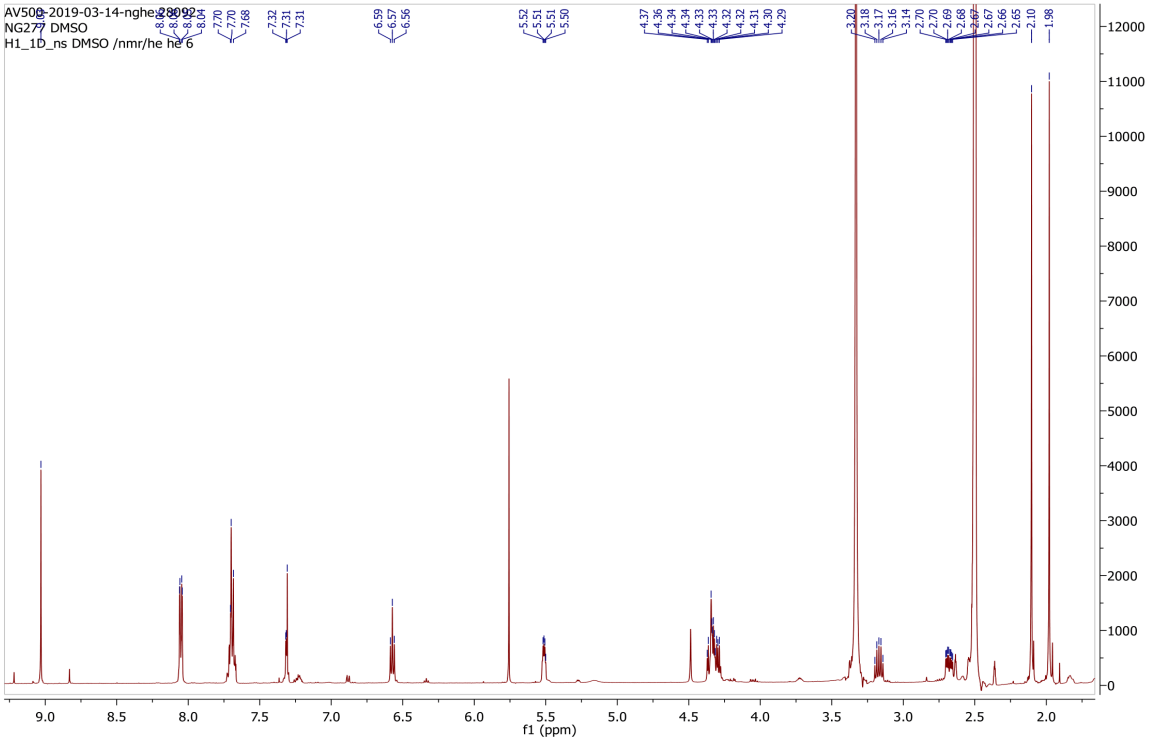

Fig. S35 ^{1}H spectrum of compound 7.

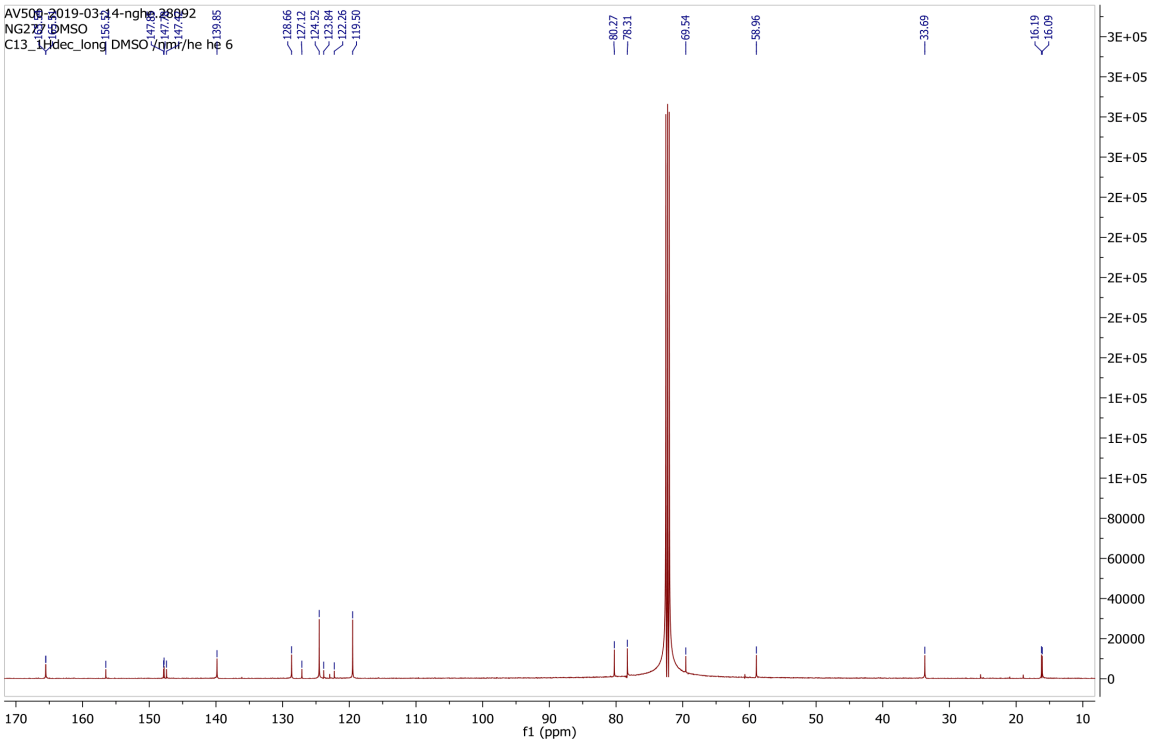

Fig. S36 ^{13}C spectrum of compound 7.

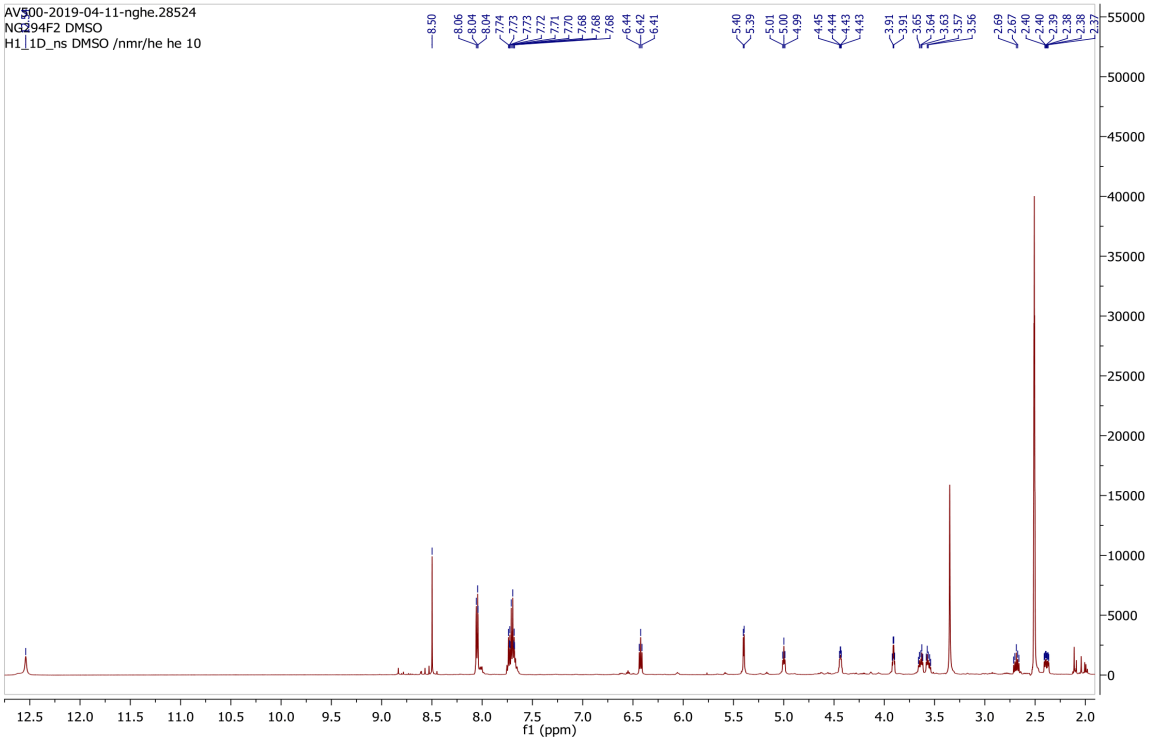

Fig. S37 ^{1}H spectrum of compound 8.

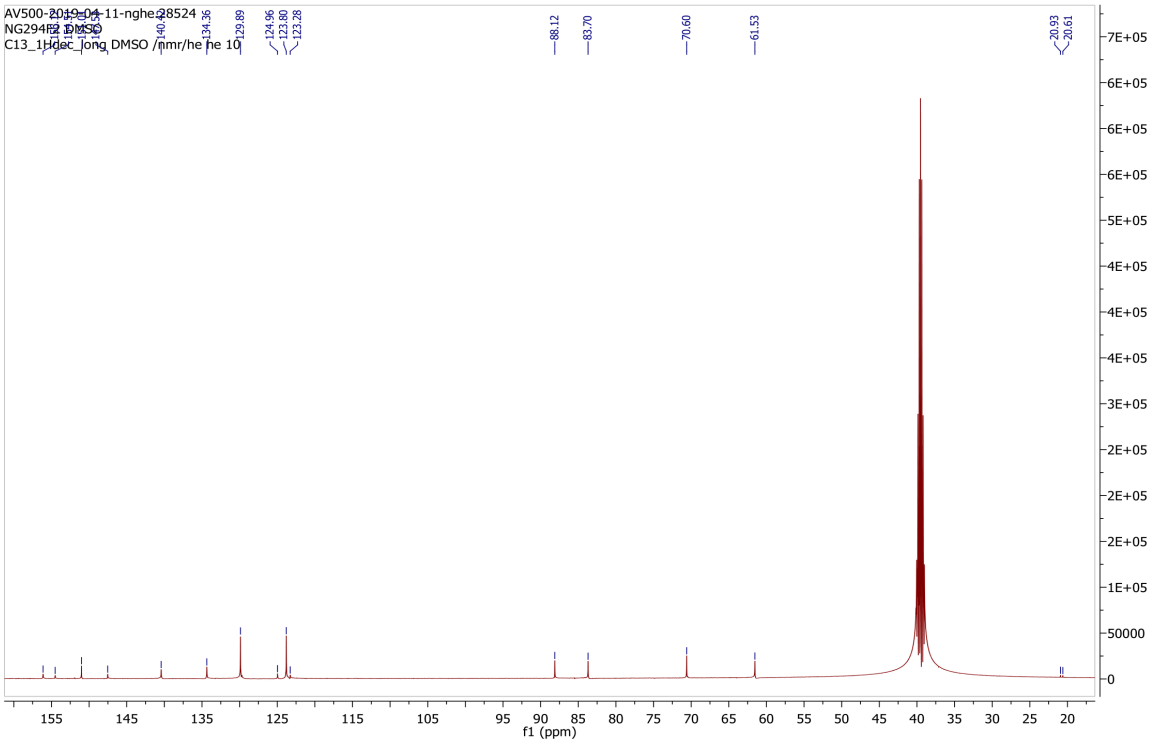

Fig. S38 ^{13}C spectrum of compound 8.

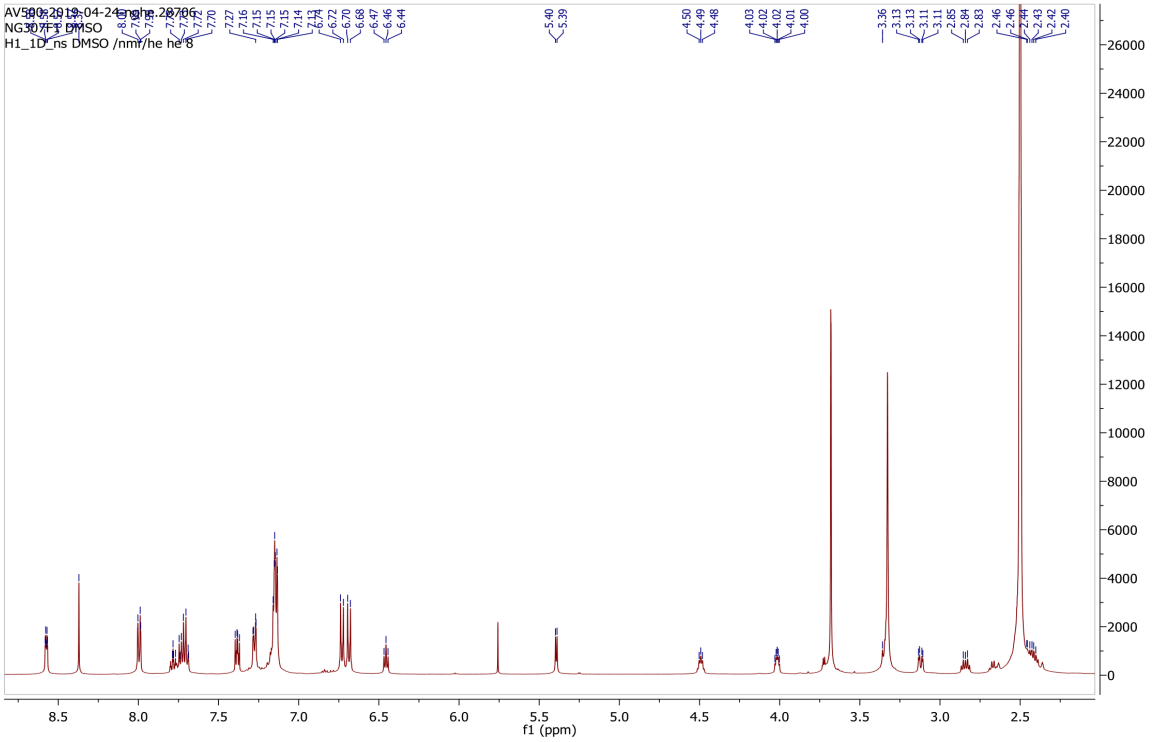

Fig. S39 ^{1}H spectrum of compound 9.

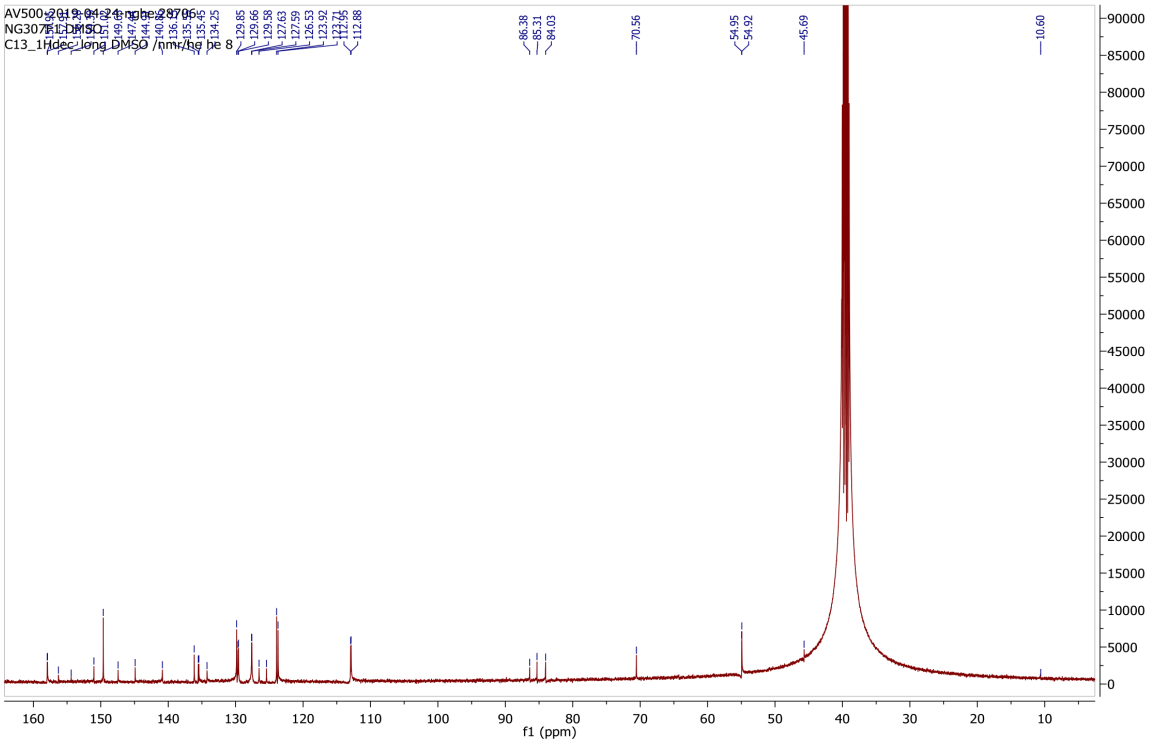

Fig. S40 ^{13}C spectrum of compound 9.

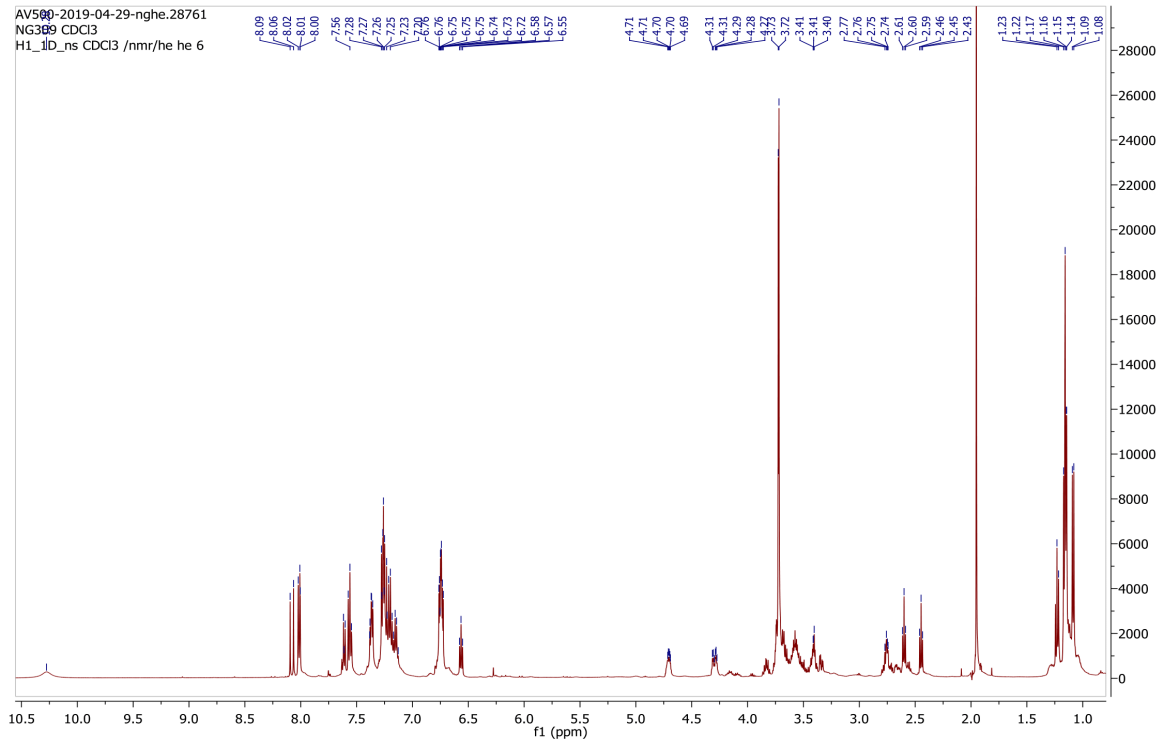

Fig. S41 ^{1}H spectrum of compound 10.

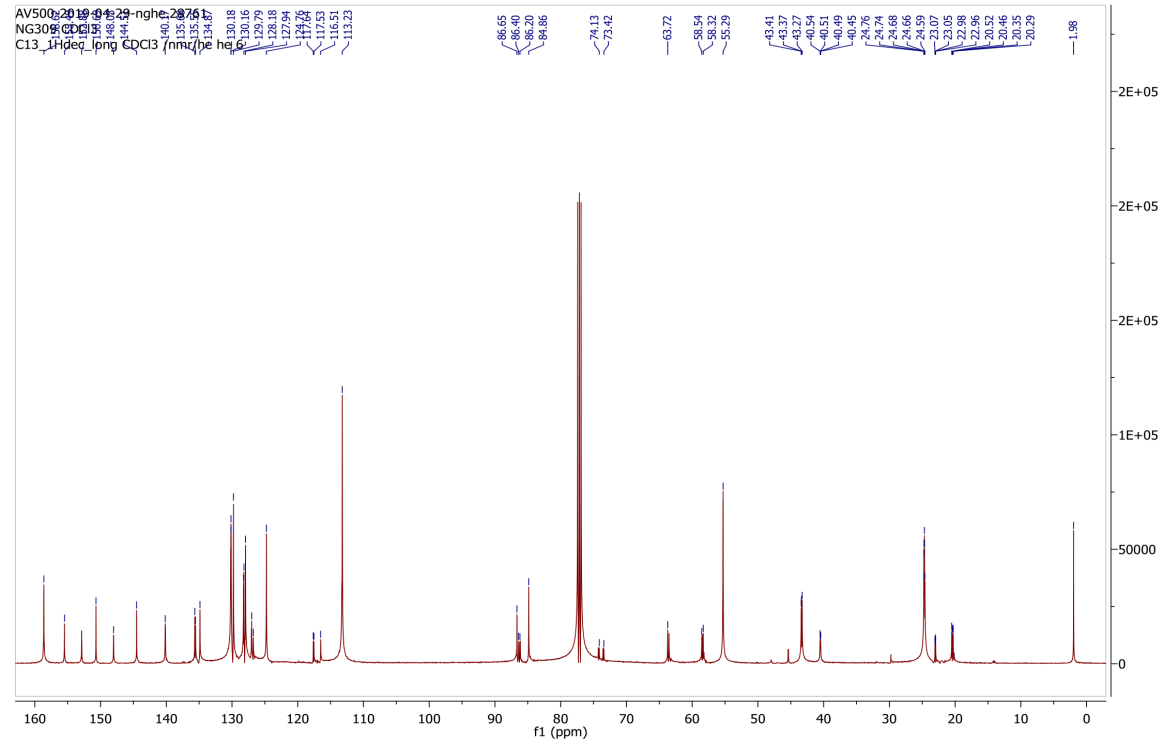

Fig. S42 ^{13}C spectrum of compound 10.

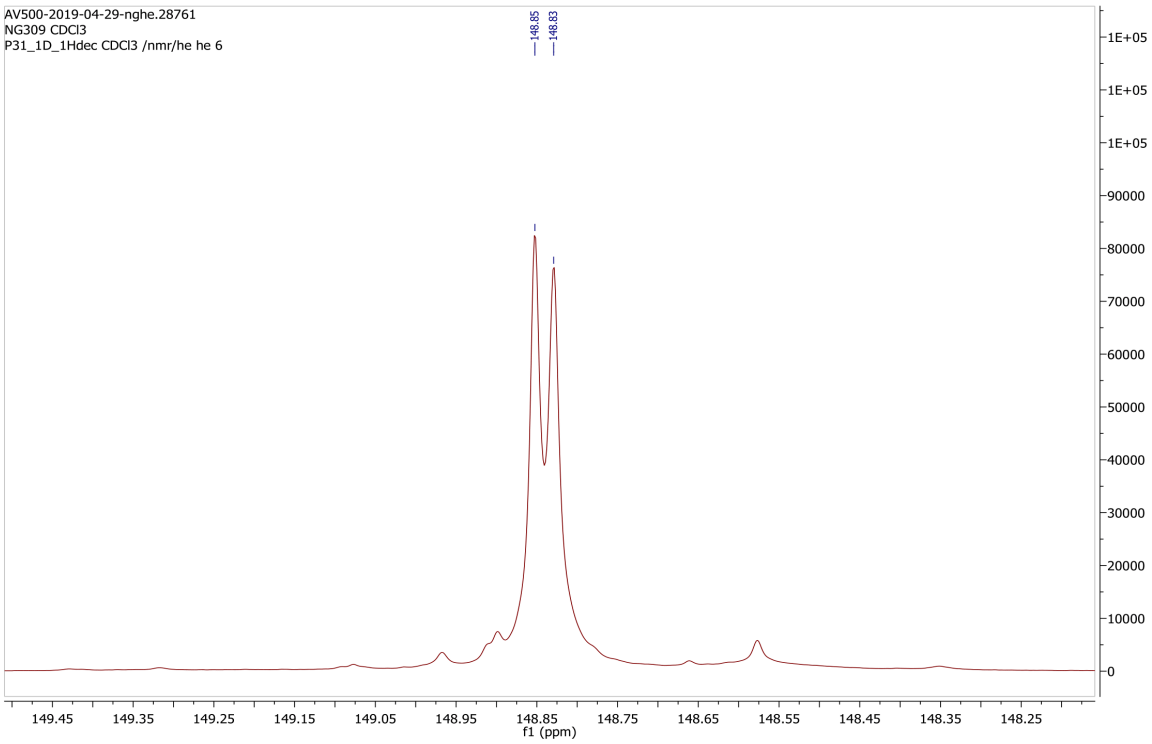

Fig. S43 ^{31}P spectrum of compound 10.

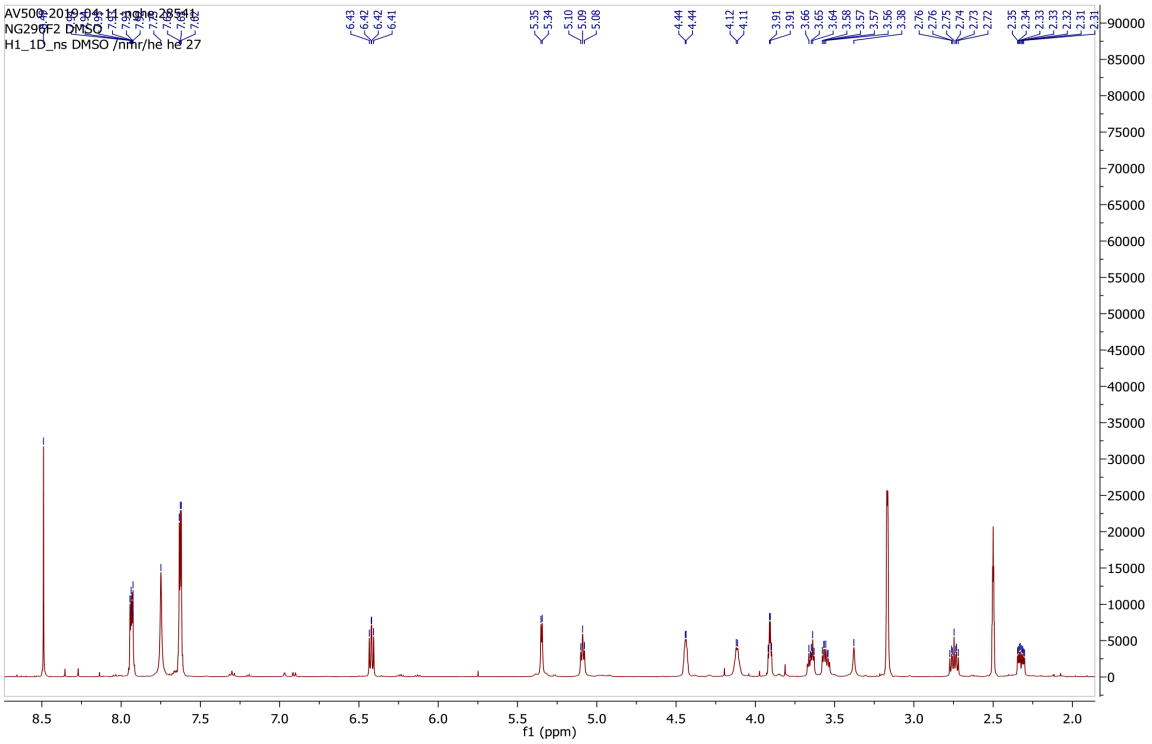

Fig. S44 ^{1}H spectrum of compound 11.

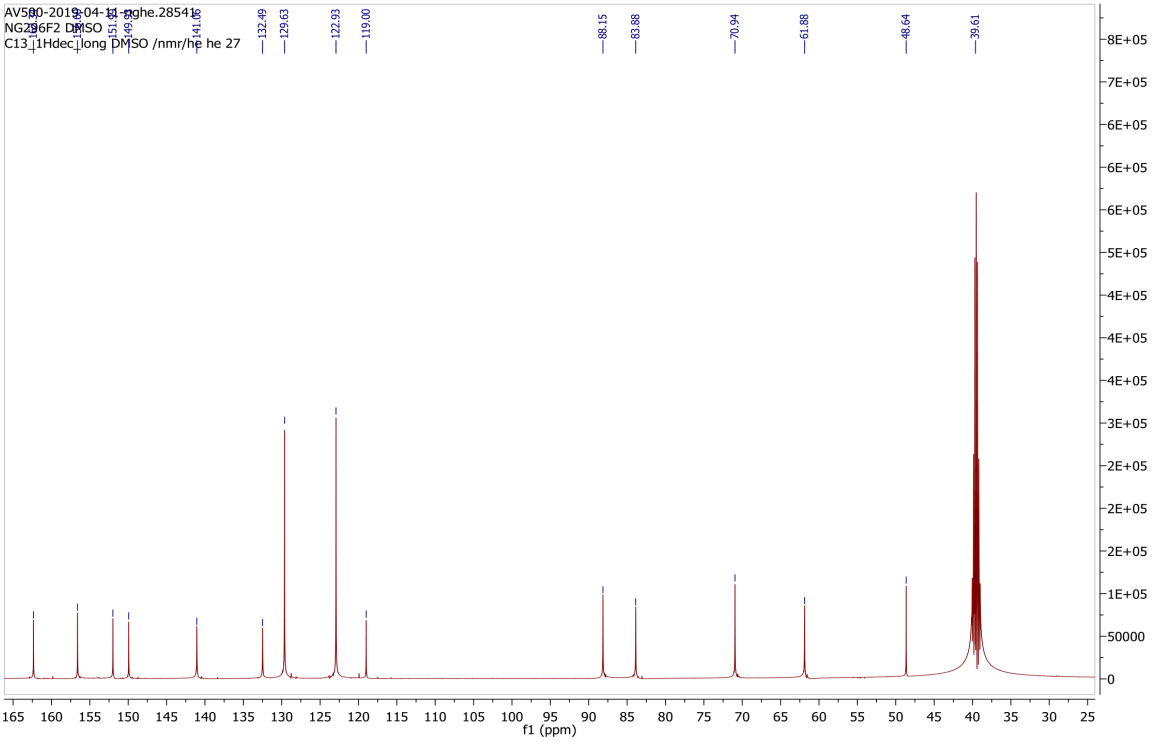

Fig. S45 ^{13}C spectrum of compound 11.

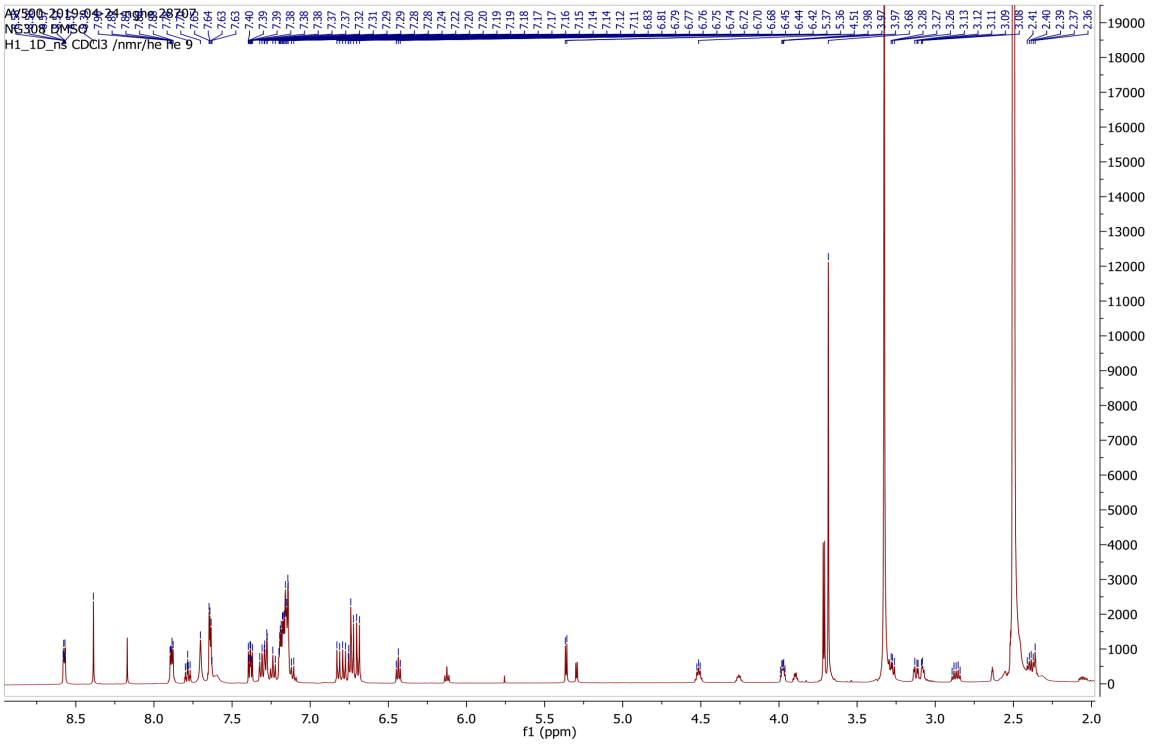

Fig. S46 ^{1}H spectrum of compound 12.

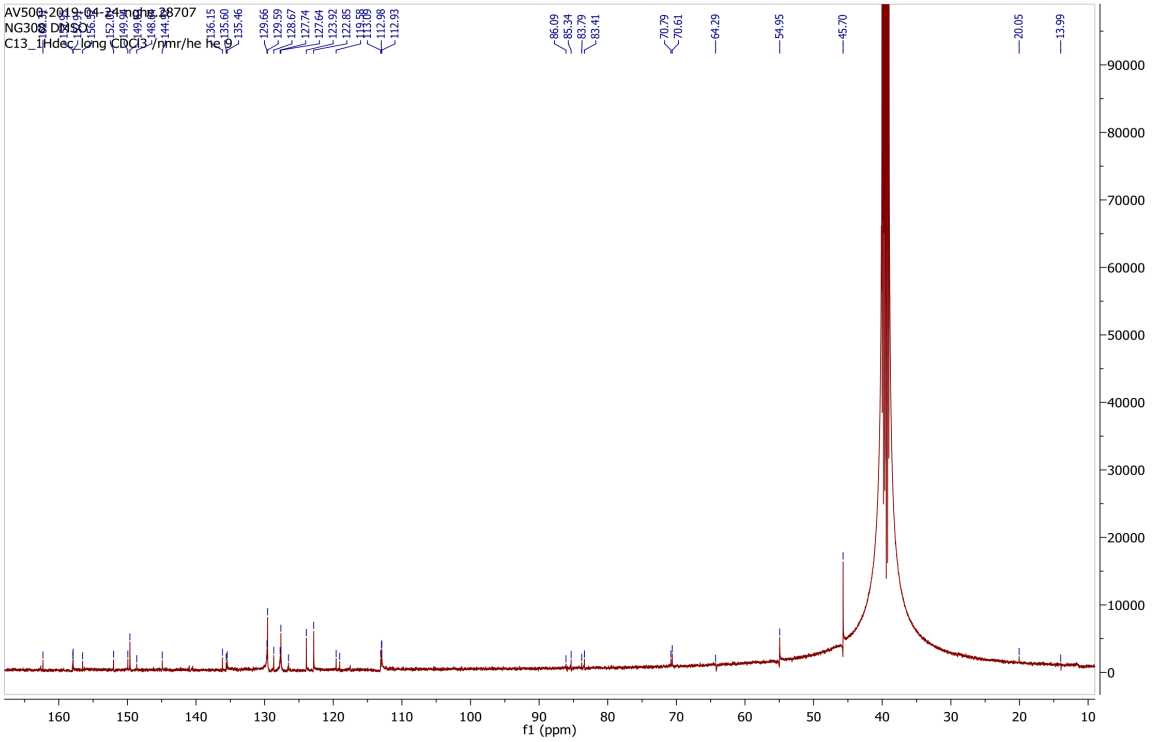

Fig. S47 ^{13}C spectrum of compound 12.

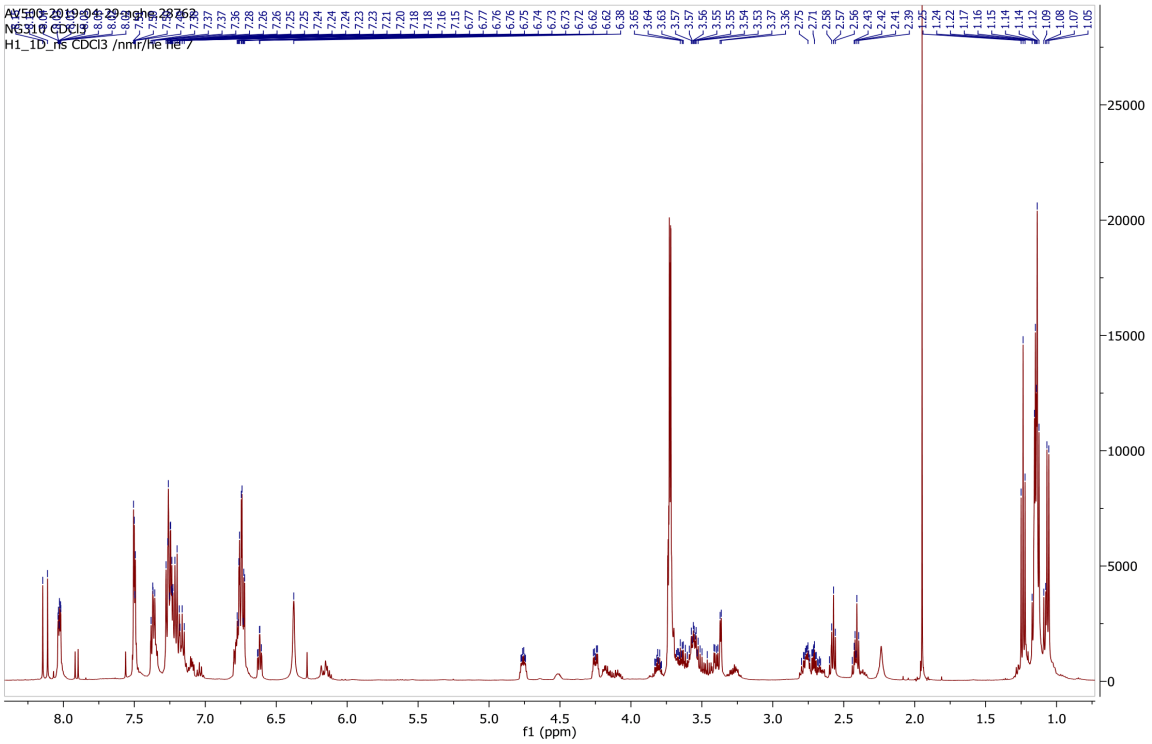

Fig. S48 ^{1}H spectrum of compound 13.

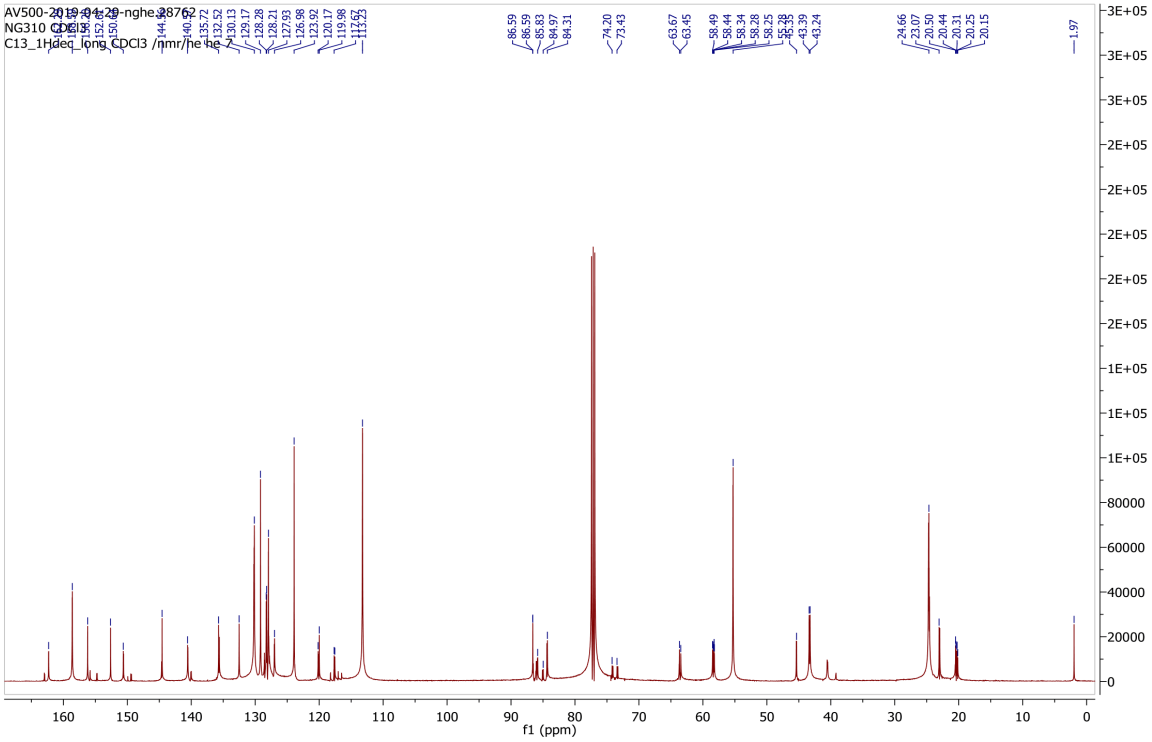

Fig. S49 ^{13}C spectrum of compound 13.

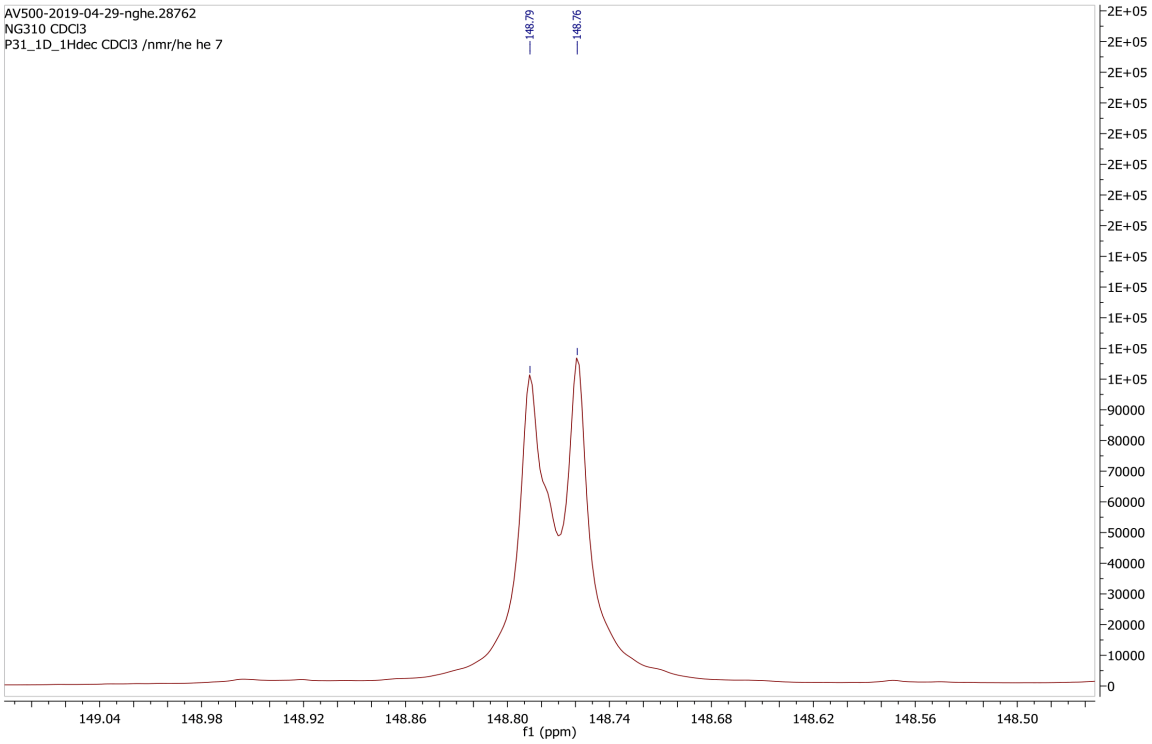

Fig. S50 ^{31}P spectrum of compound 13.

8. List of abbreviations

Ac	acyl
Azo	azobenzene
CE	cyanoethyl
Cy	cyclohexane
dA_{Azo}	2-Phenyldiazenyl-2'-deoxyadenosine
DAB	3'-dabcyl modification
DCM	dichloromethane
dG_{Azo}	2-Phenyldiazenyl-2'-deoxyguanosine
DIPEA	di-*iso*propyl ethyl amine
DMAP	4-*N*,*N*-dimethylamino-pyridine
DNAzo	desoxyribonucleic acid analogue azobenzene *C*-nucleoside
DMT	4,4'-Dimethoxytrityl
EA	ethyl acetate
ECH	3-hydroxypropionitrile
Et	ethyl
FAM	5' 6-fluorescein modification
Me	methyl
MeOH	methanol
*mi*RNA	*micro*-RNA
^{i}Pr	*iso*propyl
^{n}Bu	*n*-Butyl
PBS	phosphate buffered saline
Ph	phenyl
py	pyridine
PSS	photostationary state (equilibrium state, at which no change of isomeric distribution of a photoswitch occurs anymore during irradiation with a specified wavelength)
PSS365	photostationary state of the azobenzenes at 365 nm wavelength
PSS455	photostationary state of the azobenzenes at 455 nm wavelength
RP-HPLC	reversed phase high performance liquid chromatography
rt	room temperature (25 °C)
*t*Azo	*D*-threoninol-azobenzene
TBAN	tetrabutyl ammonium nitrate
TBD	1,5,7-triazabicyclo(4.4.0)dec-5-ene
TEA	triethylamine
T_M	melting point (temperature, were the same amount of single stranded and duplex oligonucleotide is present)

9. References

[1] J. Parsch, J. W. Engels, *J. Am. Chem. Soc.* 2002, *124*, 5664–5672.

[2] M. Reinfelds, V. Hermanns, T. Halbritter, J. Wachtveitl, M. Braun, T. Slanina, A. Heckel, *ChemPhotoChem* 2019, DOI 10.1002/cptc.201900010.