Electronic Supplementary Information

Inhibiting guanine oxidation and enhancing excess-electron-transfer efficiency of a pyrene-modified oligonucleotide by introducing an electron-donating group on pyrene

Takumi Okuda,[a] Yusuke Kawashima,[a] Yuuya Kasahara,[ab] Tatsuya Takagi,[a] Junpei Yamamoto,[c] Shigenori Iwai[c] and Satoshi Obika*[ab]

* Corresponding author; E-mail: obika@phs.osaka-u.ac.jp

Present/permanent address

[a] Graduate School of Pharmaceutical Sciences, Osaka University, 1–6, Yamadaoka, Suita, Osaka 565–0871, Japan

[b] National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), 7-6-8 Saito-Asagi, Ibaraki, Osaka 567-0085, Japan

[c] *Graduate School of Engineering Science, Osaka University, 1-3 Machikaneyama, Toyonaka, Osaka, 560-8531, Japan.*

Contents

General

Reagents and solvents were purchased from commercial suppliers and were used without purification unless otherwise specified. All experiments were carried out under an Ar atmosphere. All reactions were monitored with analytical TLC (Merck Kieselgel 60 F254; Merck, Darmstadt, Germany). Flash column chromatography was carried out using EPCLC–W–Prep 2XY (YAMAZEN, Osaka, Japan). Physical data were measured as follows: NMR spectra were recorded on an AVHD 400 NB (Bruker Daltonics, Billerica, MA, USA; ^{1}H, ^{13}C ^{31}P) using $CDCl_3$ or DMSO–d_6 as the solvents with tetramethylsilane (TMS) or 10% phosphoric acid aqueous solution as an internal standard. FAB mass spectra were measured using a JEOL JMS–700 mass spectrometer. Solid-phase oligonucleotide (ON) synthesis was performed using an nS–8 Oligonucleotide Synthesizer (GeneDesign, Osaka, Japan). ESI masses were recorded on an ultrafleXtreme mass spectrometer (Bruker Daltonics, MA, USA). Moreover, ON UV/Vis absorption measurements and UV melting experiments were performed using a UV–1650PC UV–Vis spectrophotometer equipped with a TMSPC–8 T_m analysis accessory (SHIMADZU, Kyoto, Japan). HPLC was performed by SLC-20A3R, LC-20AD, CTO-20AC, SPD-20A, FRC-10A.

Oligonucleotide (ON) synthesis

Phosphroamidites of four nucleosides (dA^{Bz}, dG^{ibu}, dC^{Bz}, dU) were purchased from Sigma-Aldrich. Phosphroamidites of d^{Py}U or CPD were chemically synthesized by previously reported methods.[S1, S2] ONs were synthesized under trityl-OFF conditions on a 1000 Å CPG solid support column (1.0 µmol scale). 5-Ethylthio-1*H*-tetrazole (0.25 M in MeCN) was used as the activator. Cleavage from CPG and deprotection of nucleobases were accomplished with ammonia solution (28%) at 55 °C for 6 h. After removal of ammonia, the deprotected residues were isolated by gel filtration (Nap™-5, GE-healthcare). These ONs were purified by RP-HPLC on XBridge™ OST C18 column, 2.5 µm, 10 × 50 mm (Waters) using MeCN in 0.1 M triethylammonium acetate buffer (pH = 7.2). The purified ONs were quantified by UV absorption at 260 nm and their purity and composition were analyzed by LC/MS.

UV melting experiments

Melting temperatures (T_m) were determined by absorbance fluctuation at 260 nm. The ONs were denatured at 95 °C and slowly cooled to room temperature for 1 h. The melting curve was recorded from 5 °C to 90 °C with a scan rate of 0.5 °C/min. Sample conditions: 4 μM ONs, 100 mM NaCl, 10 mM phosphate buffer (pH = 7.2).

Fluorescent spectra measurements

Fluorescent spectra were measured using FP-8500 spectrometers (JASCO, Tokyo, Japan) with a quartz cuvette. Sample conditions: 4 μM ONs, 100 mM NaCl, 10 mM phosphate buffer (pH = 7.2).

CD spectra measurements

CD spectra were acquired on the J-720W spectrophotometer (JASCO, Tokyo, Japan). The spectra were recorded at room temperature in a quartz cuvette. Sample conditions: 4 μM ONs, 100 mM NaCl, 10 mM phosphate buffer (pH = 7.2).

Photo-irradiation experiments

50 μL duplex solutions (10 μM ONs, 100 mM NaCl, 10 mM phosphate buffer (pH = 7.2)) were irradiated with a 300 W Xe lamp (MAX-303, Asahi Spectra, Tokyo, Japan) through a UV cut-off filter (400 nm). Irradiated samples were digested by 0.1 U/μL phosphodiesterase l (WOR) and 0.1 U/μL alkaline phosphatase (TaKaRa) at 37 °C for 1 h. Reaction samples were analyzed by RP-HPLC, and the decomposition were calculated using dC as the internal standard. In the case of ^{Br}U, the amount of ^{Br}U from no photoirradiated **ON3 (^{Br}U)** :3'-d(CGCUGCAAAU**^{Br}U**UUCAGCAGGCA)-5' was defined as 0 % decomposition, and the ^{Br}U decomposition (%) was calculated from the area ratio of ^{Br}U generated by enzymatic degradation against no photoirradiated samples. T formation (%) was expressed as a percentage based on the amount of T produced when **ON3 (TT)** (3'-d(CGCUGCAAATTUUCAGCAGGCA)-5') is decomposed with an enzyme. dG decomposition (%) was calculated based on the amount of dG produced by enzymatic degradation against no photoirradiated **ON4 (G)**. Each experiment had to be repeated at least three times, to obtain the result as an average value.

Computational method

Entries were generated from the SMILES in Table x. Initial structures of these entries were generated and optimized using molecular mechanics method at MMFF level (conditions: num_confs=100 prune_rms_thresh=2).[S4] Conformers were re-optimized using DFT (Density Functional Theory) calculations at B3LYP/6-31g(d) level and the most stable conformers of each entries[S3] were obtained.

Time dependent DFT method (TD-DFT) was applied to our optimized compounds to evaluate transition moments of excitation at TD-B3LYP/6-311+g(2d,p) level.[S3]

Table S1 Molecular orbital energies and SMILES of each entries.

Entry	LUMO+1 (eV)	LUMO (eV)	HOMO (eV)	SMILES
^{Py}U	-1.0961	-1.7478	-5.1849	Cn1cc(C#Cc2ccc3ccc4cccc5ccc2c3c45)c(=O)[nH]c1=O
^{OMe}PyU	-0.9867	-1.6172	-4.9046	COc1ccc2ccc3c(C#Cc4cn(C)c(=O)[nH]c4=O)ccc4ccc1c2c43
^{PipPy}U	-1.0389	-1.7064	-4.9378	Cn1cc(C#Cc2ccc3ccc4c(N5CCCCC5)ccc5ccc2c3c54)c(=O)[nH]c1=O
G	0.3456	0.2414	-5.4972	Cn1cnc2c(=O)[nH]c(N)nc21
A	0.2773	-0.4201	-5.9653	Cn1cnc2ncnc(N)c21
U	0.5614	-0.8561	-6.4124	Cc1cn(C)c(=O)[nH]c1=O

Table S2 Transition moments of excitation of entries.

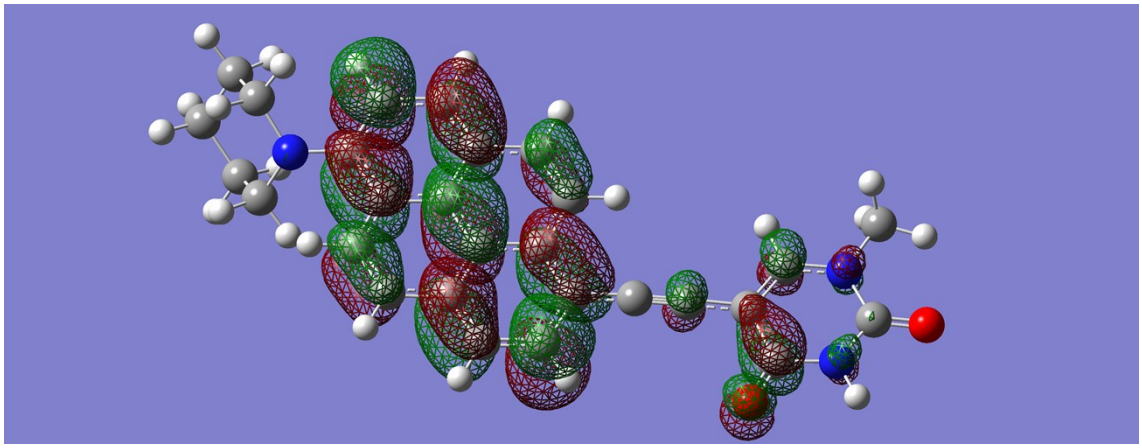
^{OMePy}U	
Excited State 1: 2.7545 eV (450.12 nm) f=0.6922 <S2>=0.000**	
HOMO ->LUMO	0.69248
HOMO ->LUMO+1	□□□□0.11543
Excited State 2: 3.2658 eV (379.64 nm) f=0.1655 <S2>=0.000**	
HOMO ->LUMO	□□□-0.10685
HOMO ->LUMO+1	0.62629
HOMO ->LUMO+2	0.14827
HOMO-1 ->LUMO	0.21773
Excited State 3: 3.4430 eV (360.10 nm) f=0.0189 <S2>=0.000**	

HOMO ->LUMO+1	-0.23915
HOMO ->LUMO+2	0.56444
HOMO-1 ->LUMO	0.18885
HOMO-2 ->LUMO	0.24972
HOMO-2 ->LUMO+1	-0.10958
^{PipPy}U	
Excited State 1: 2.6127 eV (474.55 nm) f=0.6818 <S2>=0.000**	
HOMO -> LUMO	0.69525
Excited State 2: 3.1422 eV (394.57 nm) f=0.1463 <S2>=0.000**	
HOMO -> LUMO+1	0.65651
HOMO -> LUMO+2	0.13792
HOMO-2 -> LUMO	-0.18158
Excited State 3: 3.3524 eV (369.84 nm) f=0.0289 <S2>=0.000**	
HOMO -> LUMO+1	-0.20101
HOMO -> LUMO+2	0.59169
HOMO-2 -> LUMO	-0.28244
HOMO-2 -> LUMO+1	0.10971
HOMO-3 -> LUMO	0.10080
^{Py}U	
Excited State 1: 2.9128 eV (425.66 nm) f=0.7580 <S2>=0.000**	
HOMO -> LUMO	0.69308
HOMO -> LUMO+1	0.11203
Excited State 2: 3.3685 eV (368.07 nm) f=0.0963 <S2>=0.000**	
HOMO -> LUMO+1	0.56493
HOMO -> LUMO+2	0.19028
HOMO-1 -> LUMO	-0.34660
Excited State 3: 3.5138 eV (352.84 nm) f=0.0112 <S2>=0.000**	
HOMO -> LUMO+1	-0.34881
HOMO -> LUMO+2	0.45264
HOMO-1 -> LUMO	-0.31067
HOMO-2 -> LUMO	-0.23031

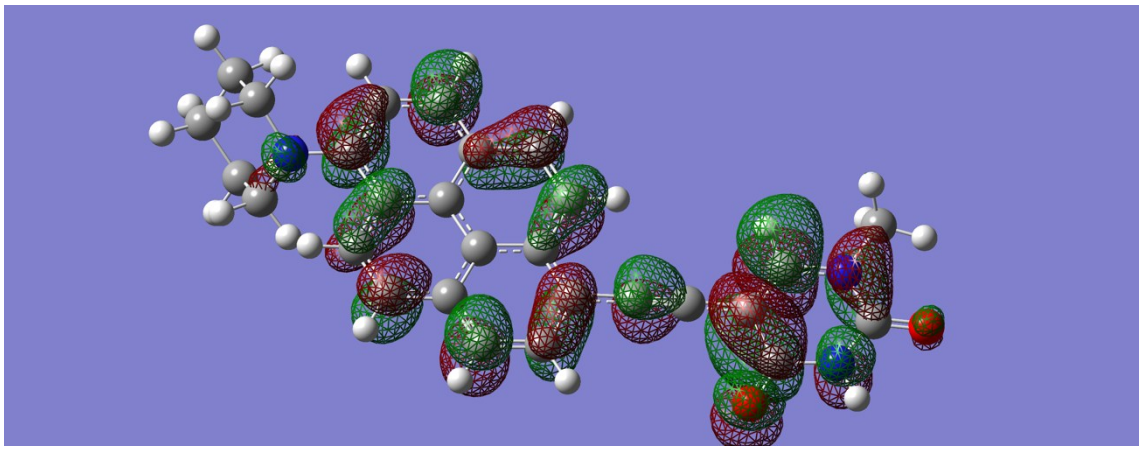
Fig. S1 MO images of each entries of Table S2.

^{OMePy}**U**

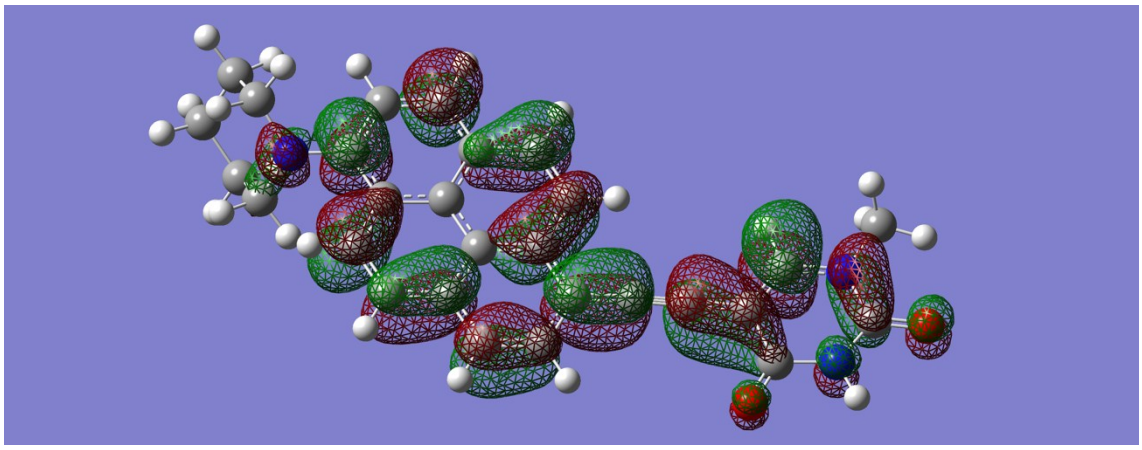
LUMO+2



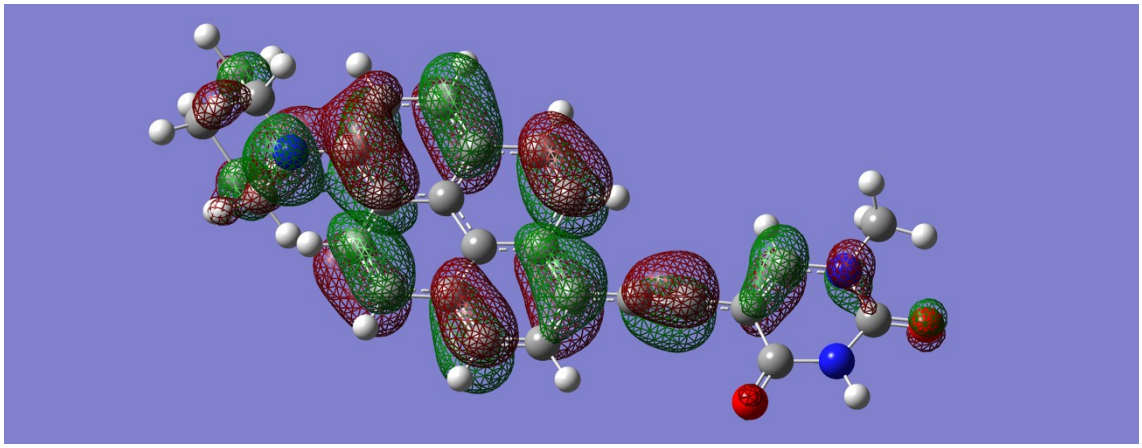
LUMO+1



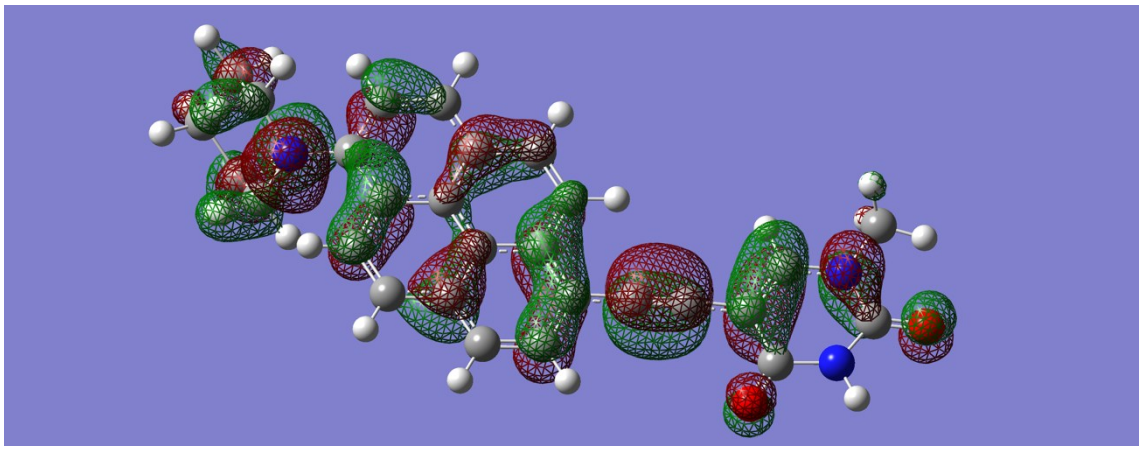
LUMO



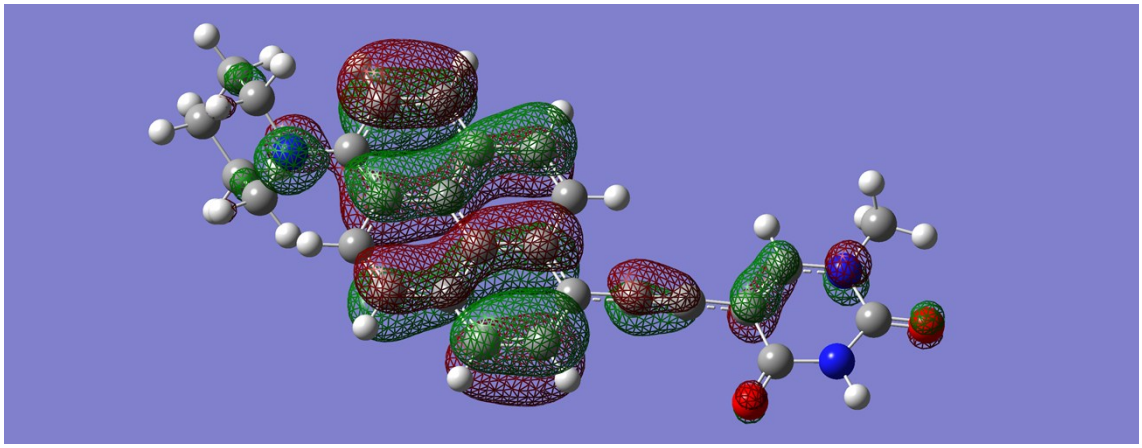
HOMO



HOMO-1

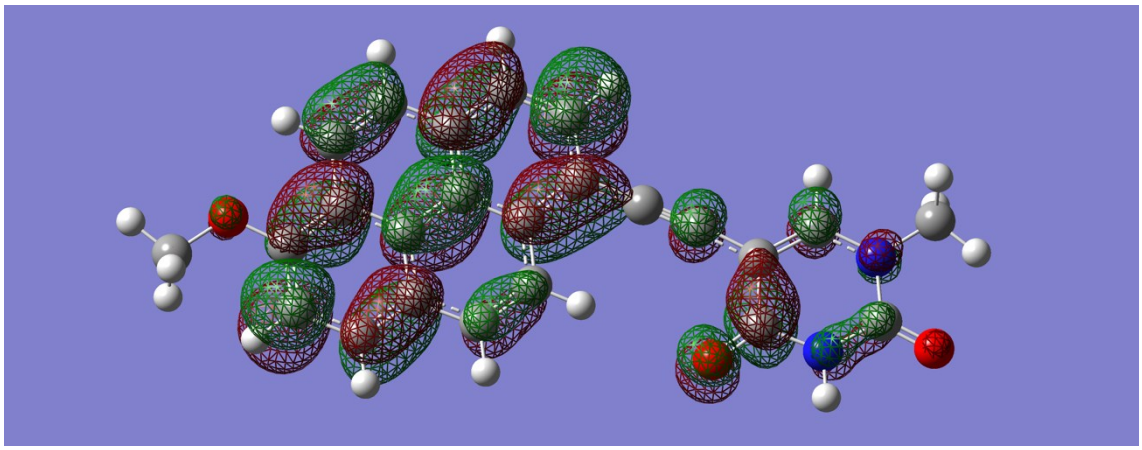


HOMO-2

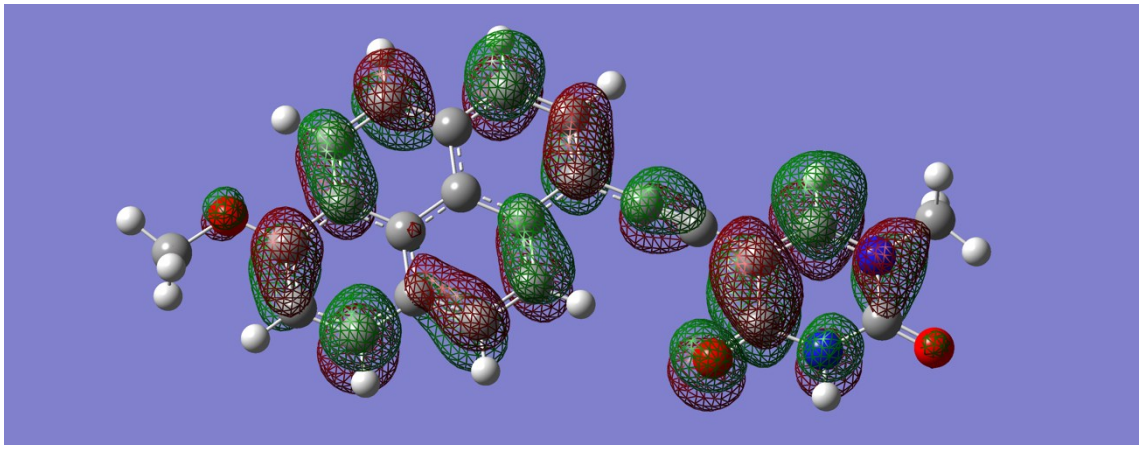


^{PipPy}U

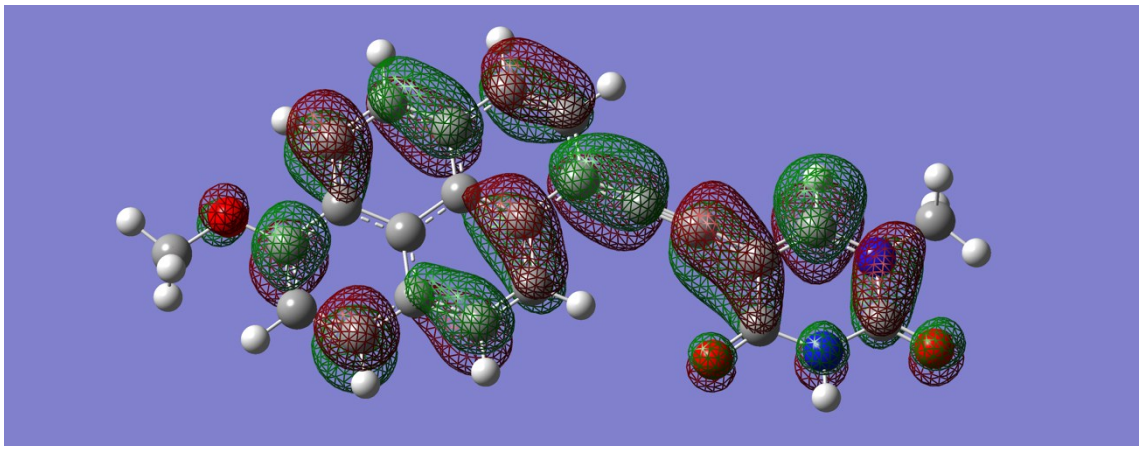
LUMO+2



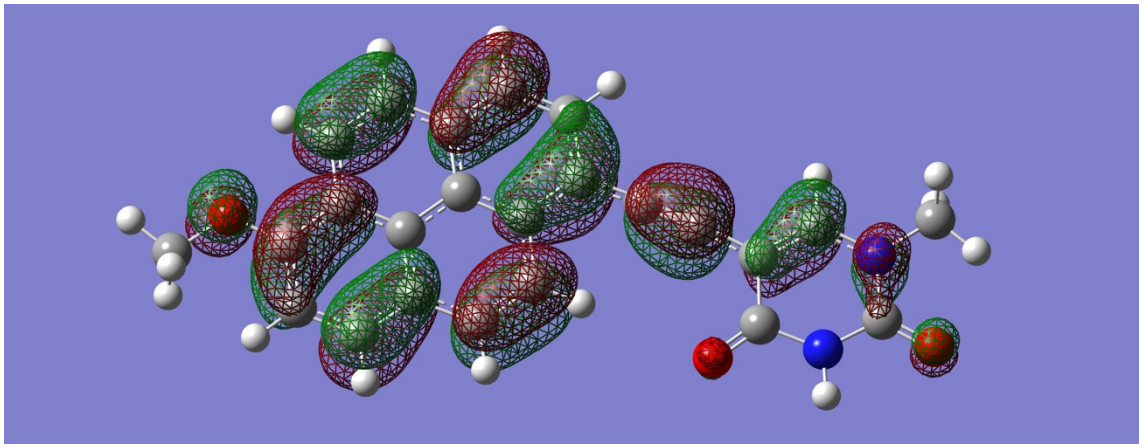
LUMO+1



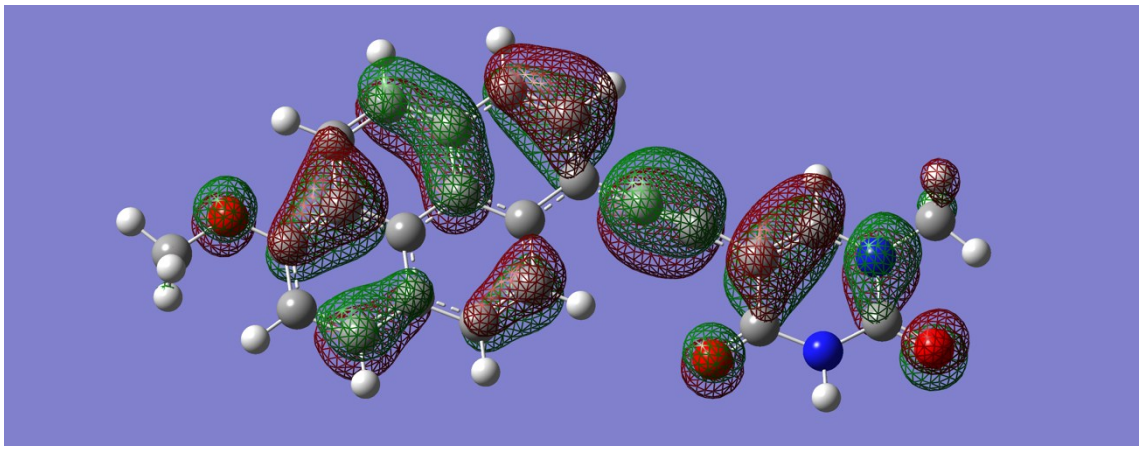
LUMO



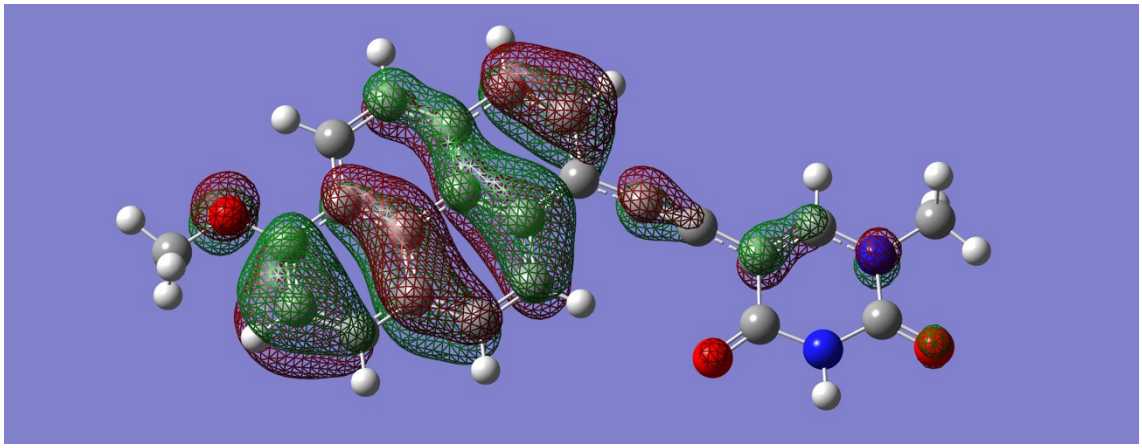
HOMO



HOMO-1

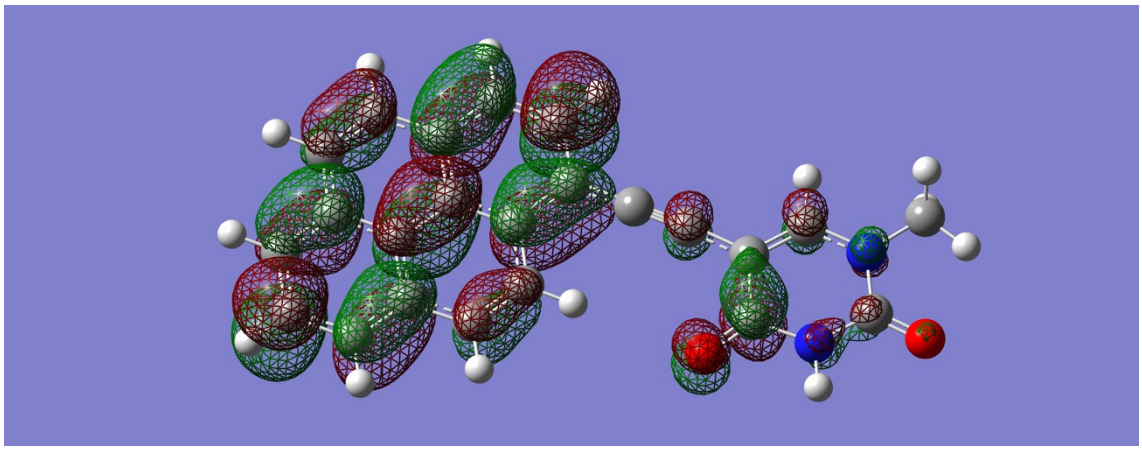


HOMO-2

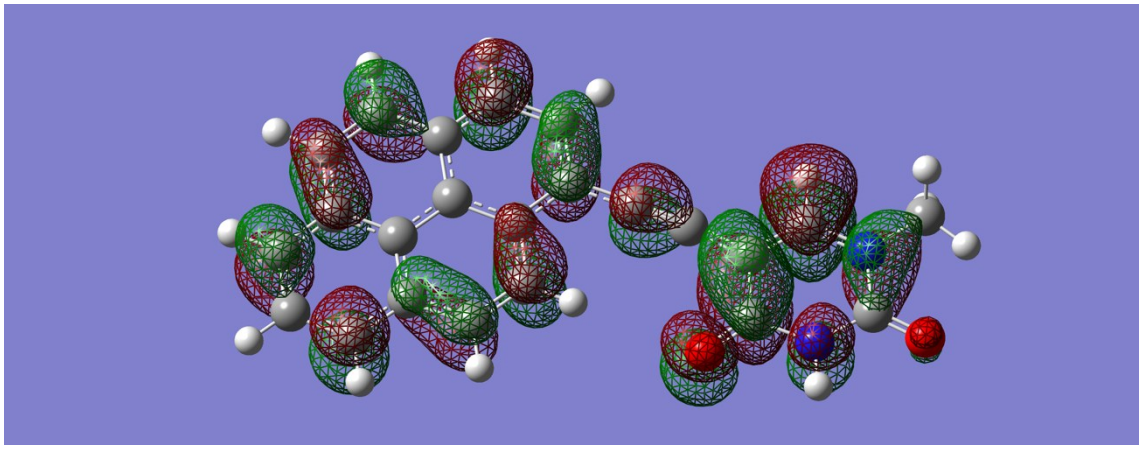


^{Py}U

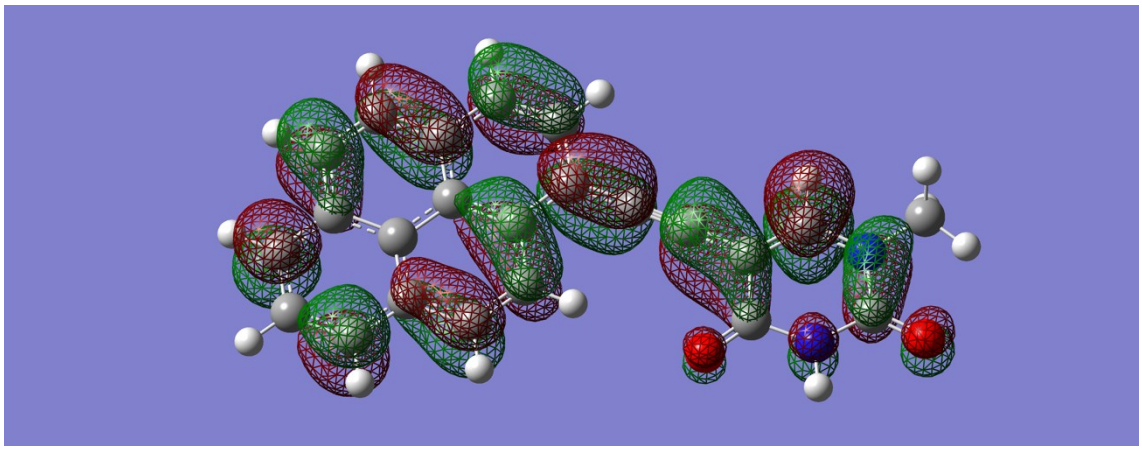
LUMO+2



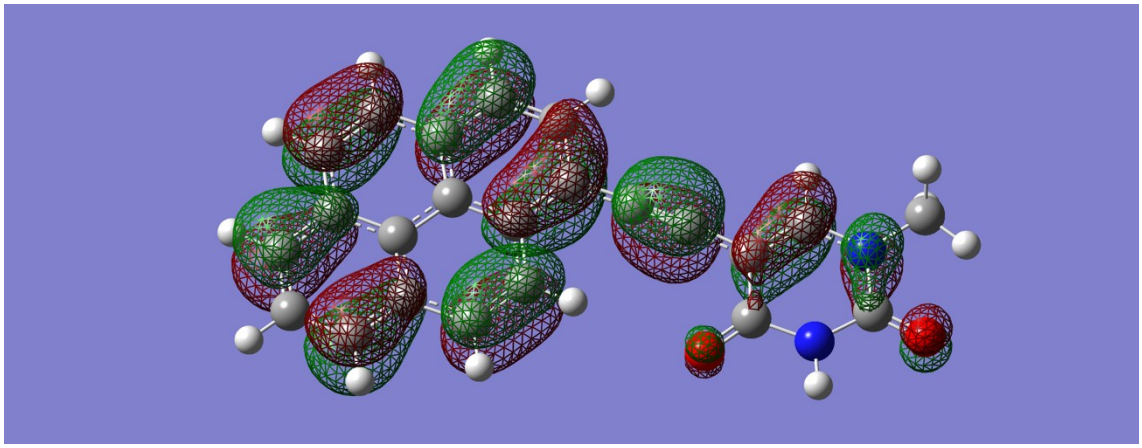
LUMO+1



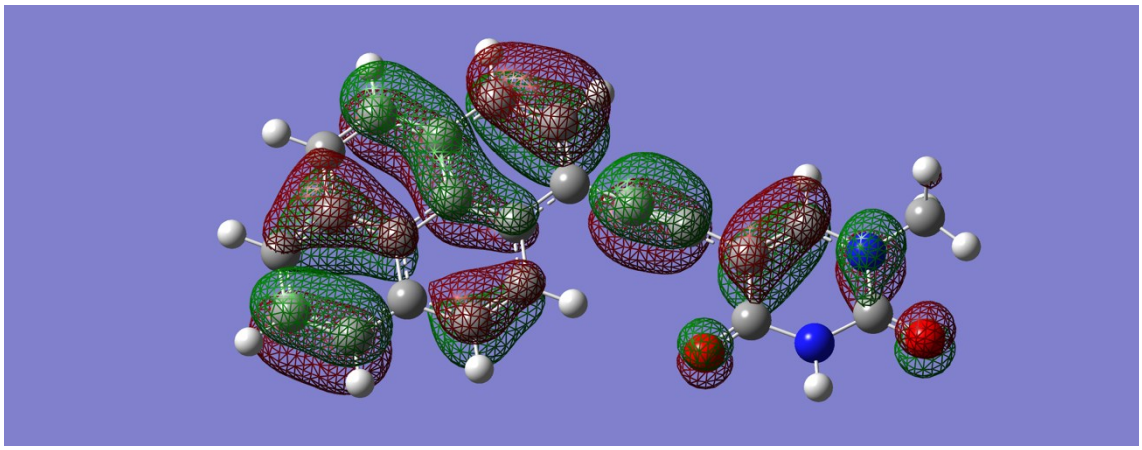
LUMO



HOMO



HOMO-1



HOMO-2

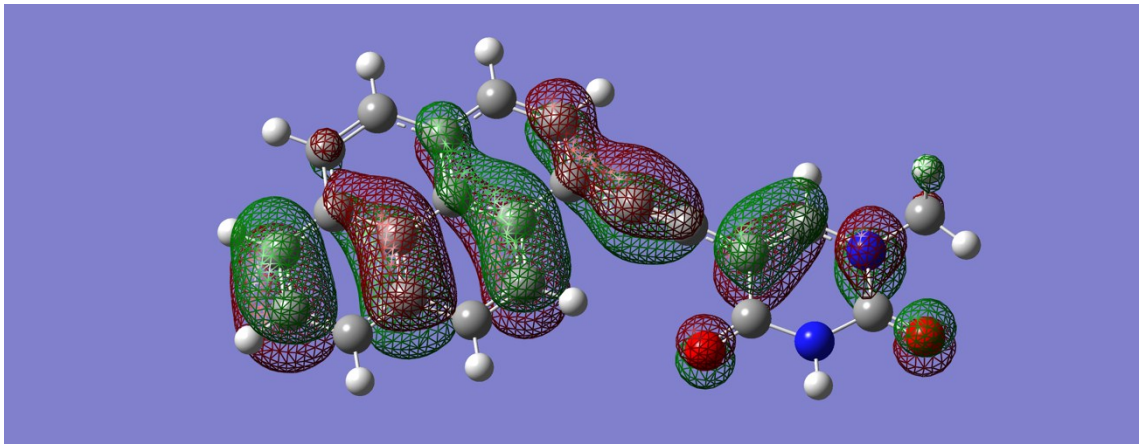
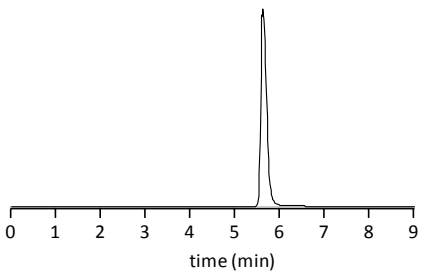


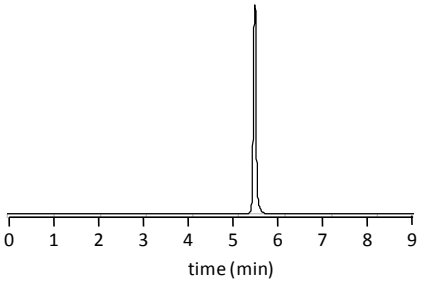
Table S3 Sequences of oligonucleotides used in this study.

Name	Sequence	calcd. [M-H]$^-$	found [M-H]$^-$
ON 1 (T)	5′-d(GCGACGUU**T**AAAAGUCGUCCGU)-3′		
ON 2 (T)	5′-d(GCGACGUU**T**ACCAGUCGUCCGU)-3′		
ON 1 (^{Py}U)	5′-d(GCGACGUU^{Py}**U**AAAAGUCGUCCGU)-3′	6886.1	6886.6
ON 2 (^{Py}U)	5′-d(GCGACGUU^{Py}**U**ACCAGUCGUCCGU)-3′	6838.1	6838.5
ON 1 (^{PipPy}U)	5′-d(GCGACGUU^{PipPy}**U**AAAAGUCGUCCGU)-3′	6979.2	6979.2
ON 2 (^{PipPy}U)	5′-d(GCGACGUU^{PipPy}**U**ACCAGUCGUCCGU)-3′	6921.2	6921.5
ON 1 (^{OMePy}U)	5′-d(GCGACGUU^{OMePy}**U**AAAAGUCGUCCGU)-3′	6916.5	6916.5
ON 2 (^{OMePy}U)	5′-d(GCGACGUU^{OMePy}**U**ACCAGUCGUCCGU)-3′	6968.1	6968.2
ON 3 (^{Br}U)	3′-d(CGCUGCAAA^{Br}**UU**UUCAGCAGGCA)-5′	6722.9	6723.0
ON 3 (CPD)	3′-d(CGCUGCAAA**T[]T**UUCAGCAGGCA)-5′	6673.1	6673.0
ON 4 (U)	3′-d(CGCUGCAAAU**UU**UCAGCAGGCA)-5′		
ON 4 (G)	3′-d(CGCUGCAAAU**GG**UCAGCAGGCA)-5′		
ON 4 (^{Br}U)	3′-d(CGCUGCAAAU^{Br}**UU**UCAGCAGGCA)-5′	6722.9	6723.2
ON 4 (CPD)	3′-d(CGCUGCAAAU**T[]T**UCAGCAGGCA)-5′	6673.1	6673.0
ON 5 (^{Br}U)	3′-d(CGCUGCAAAUU^{Br}**UU**CAGCAGGCA)-5′	6722.9	6723.2
ON 5 (CPD)	3′-d(CGCUGCAAAUU**T[]T**CAGCAGGCA)-5′	6673.1	6673.0
ON 6 (^{Br}U)	3′-d(CGCUGCAAAUUU^{Br}**U**CAGCAGGCA)-5′	6722.9	6723.6

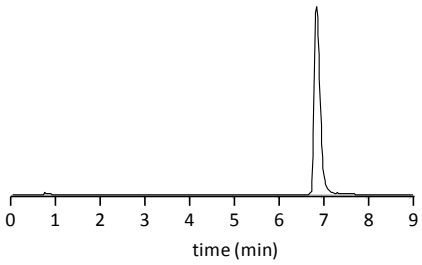
ON 1 (^{Py}U) : 5′-d(GCGACGUU^{Py}UAAAAGUCGUCCGT)-3′



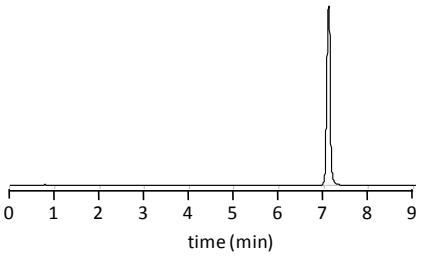
ON 2 (^{Py}U) : 5′-d(GCGACGUU^{Py}UACCAGUCGUCCGT)-3′



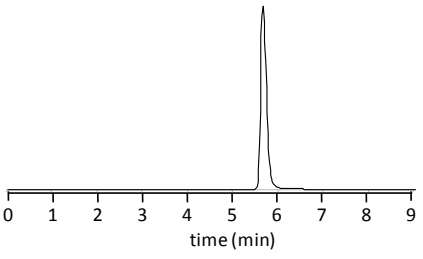
ON 1 (^{PipPy}U) : 5′-d(GCGACGUU^{PipPy}UAAAAGUCGUCCGT)-3′



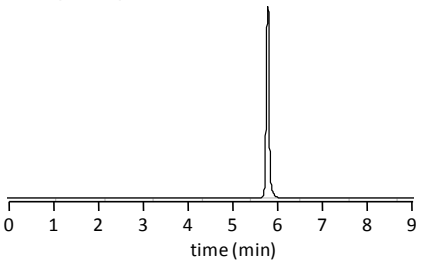
ON 2 (^{PipPy}U) : 5′-d(GCGACGUU^{PipPy}UACCAGUCGUCCGT)-3′



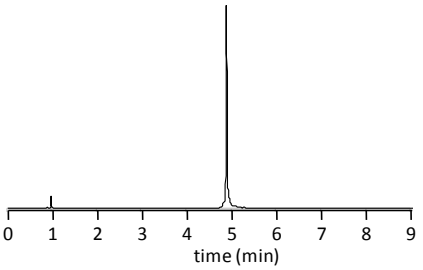
ON 1 (^{OMePy}U) : 5′-d(GCGACGUU^{OMePy}UAAAAGUCGUCCGT)-3′



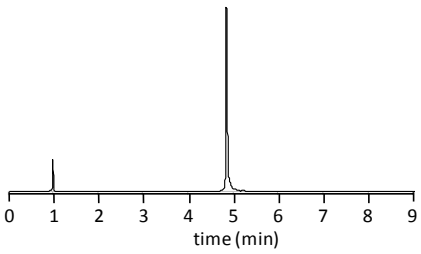
ON 2 (^{OMePy}U) : 5′-d(GCGACGUU^{OMePy}UACCAGUCGUCCGT)-3′



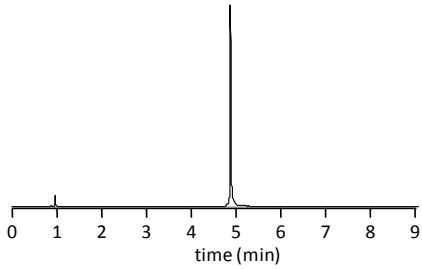
ON 3 (^{Br}U) : 3′-d(CGCUGCAAA^{Br}UUUUCAGCAGGCA)-5′



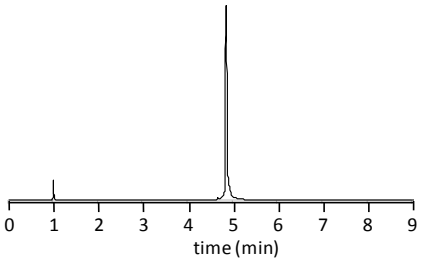
ON 3 (CPD) : 3′-d(CGCUGCAAAT[]TUUCAGCAGGCA)-5′



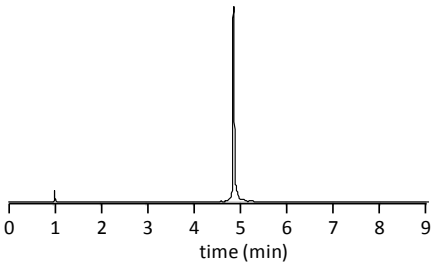
ON 4 (^{Br}U) : 3′-d(CGCUGCAAAU^{Br}UUUCAGCAGGCA)-5′



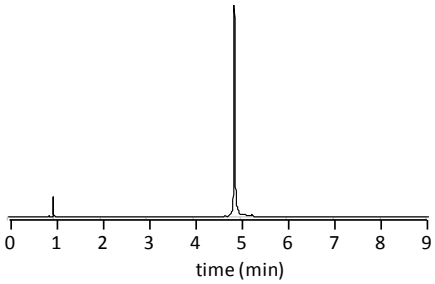
ON 4 (CPD) : 3′-d(CGCUGCAAAUT[]TUCAGCAGGCA)-5′



ON 5 (CPD) : 3′-d(CGCUGCAAAUU**T[]T**CAGCAGGCA)-5′



ON 5 (^{Br}U) : 3′-d(CGCUGCAAAUU^{Br}**U**U**U**CAGCAGGCA)-5′



ON 6 (^{Br}U) : 3′-d(CGCUGCAAAUUU^{Br}**U**CAGCAGGCA)-5′

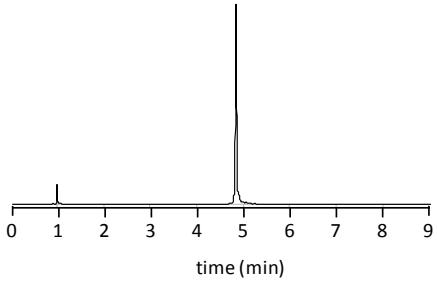


Fig. S2 LC profiles of synthesized oligonucleotides. RP-UPLC conditions (linear gradient: A conc.: B conc. = 99:1 to 80:20, A = 400 mM HFIP, 15 mM TEAA, B = MeOH over 10 min, pH = 7.0, room temperature, detection: 260 nm).

Table S4 Duplex stability of oligonucleotides.

Duplex	T_m (ΔT_m) (°C)[a]
ON 1 (T) · ON 4 (U)	69
ON 2 (T) · ON 4 (G)	75
ON 1 (T) · ON 4 (^{Br}U)	67 (-2)
ON 1 (T) · ON 4 (CPD)	57 (-12)
ON 1 (^{PyU}) · ON 4 (U)	65 (-4)
ON 2 (^{PyU}) · ON 4 (G)	70 (-5)
ON 1 (^{PyU}) · ON 4 (^{Br}U)	66 (-3)
ON 1 (^{PyU}) · ON 4 (CPD)	58 (-11)
ON 1 (^{PipPyU}) · ON 4 (U)	62 (-7)
ON 2 (^{PipPyU}) · ON 4 (G)	72 (-3)
ON 1 (^{PipPyU}) · ON 4 (^{Br}U)	63 (-6)
ON 1 (^{PipPyU}) · ON 4 (CPD)	60 (-9)
ON 1 (^{OMePyU}) · ON 4 (U)	66 (-3)
ON 2 (^{OMePyU}) · ON 4 (G)	70 (-5)
ON 1 (^{OMePyU}) · ON 4 (^{Br}U)	65 (-4)
ON 1 (^{OMePyU}) · ON 4 (CPD)	58 (-11)

UV melting temperature was measured in buffer (4 µM oligonucleotide, 10 mM sodium phosphate, 100 mM NaCl, pH 7.4) at a scan rate of 0.5 °C/min at 260. (a) ΔT_m values were calculated relative to the T_m values of unmodified complimentary duplex (**ON 1 (T) · ON 4 (U)** = 69 °C, **ON 2 (T) · ON 4 (G)** = 75 °C)

ON 1 (X) : 5′–d(GCGACGUU**X**AAAAGUCGUCCGU)–3′
ON 4 (Y) : 3′–d(CGCUGCAAAU**YY**UCAGCAGGCA)–5′

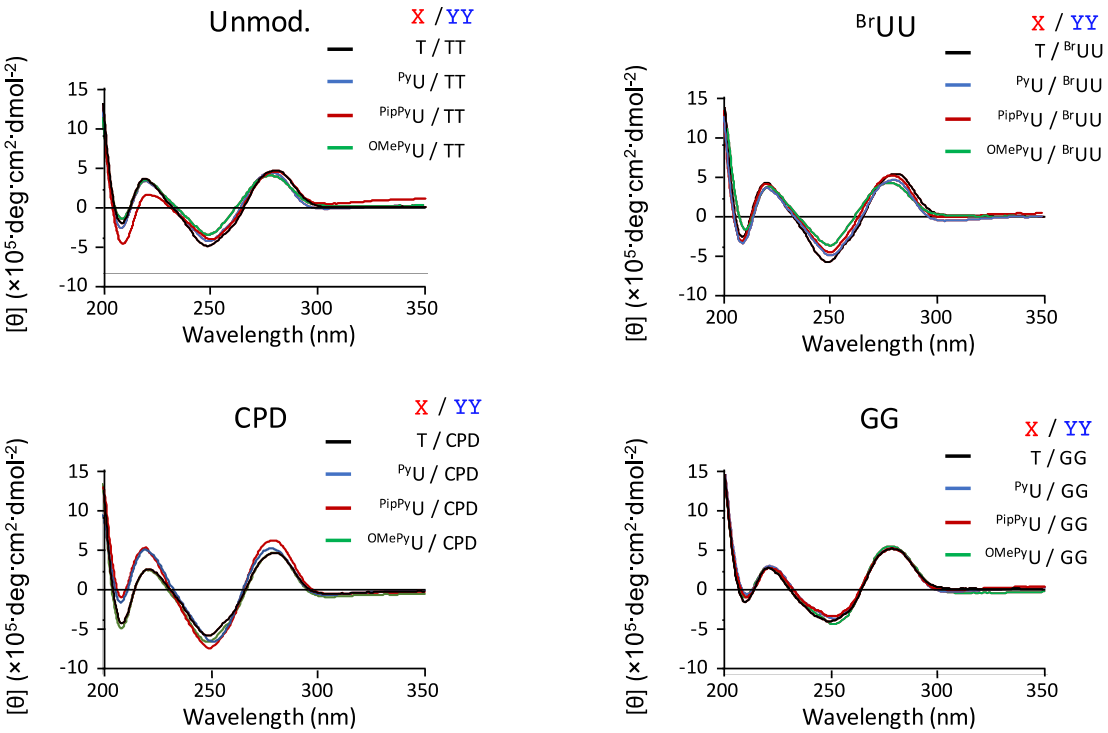


Fig. S3 CD spectra of duplexes. CD spectra were measured in buffer (10 mM sodium phosphate, 100 mM NaCl, pH 7.4) at 24 °C. The concentration of oligonucleotide was 4 µM for each strand.

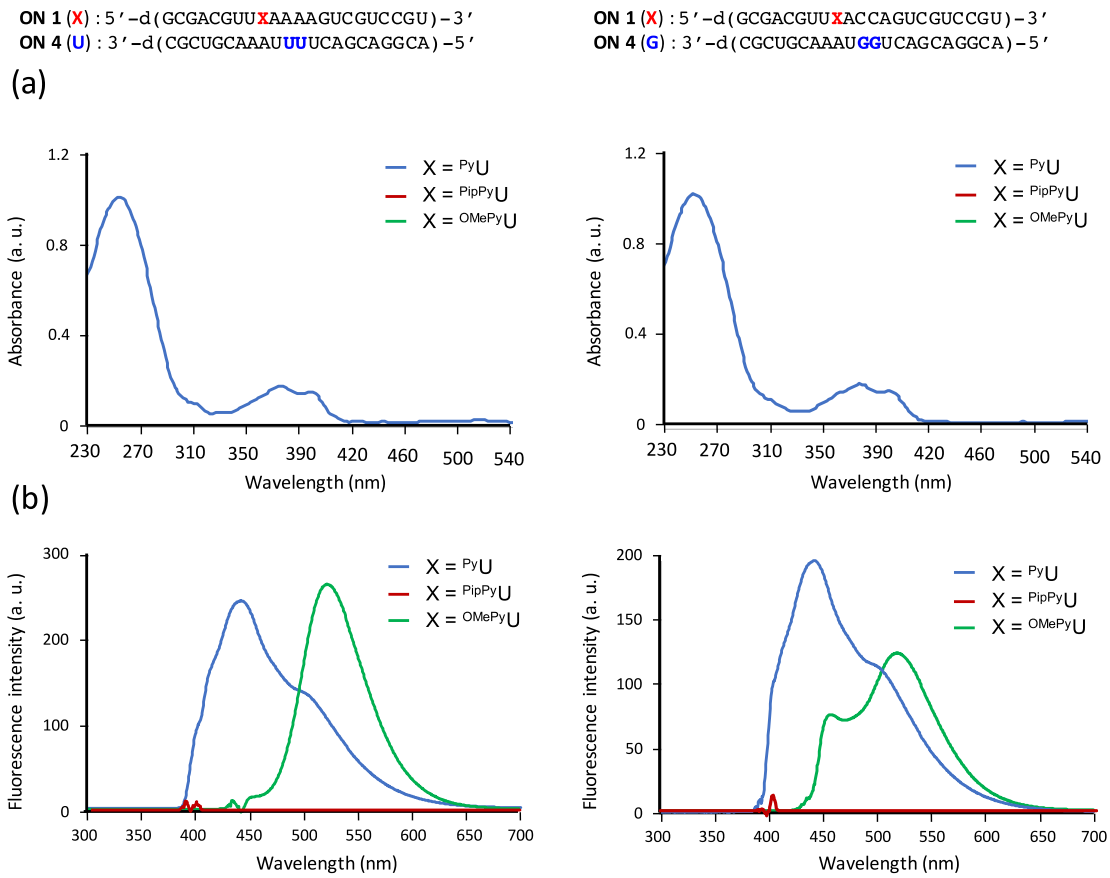
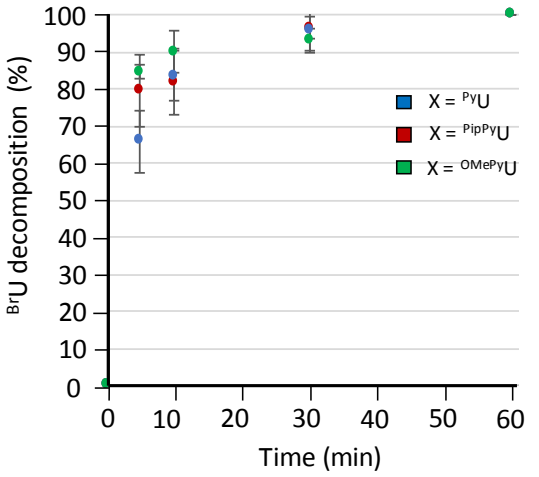


Fig. S4 Absorption (a) and fluorescent (b) spectra of duplexes. Spectra were measured in buffer (10 mM sodium phosphate, 100 mM NaCl, pH 7.4) at 24 °C. The concentration of each strand of oligonucleotide was 4 μM. The excitation wavelength was 400 nm.

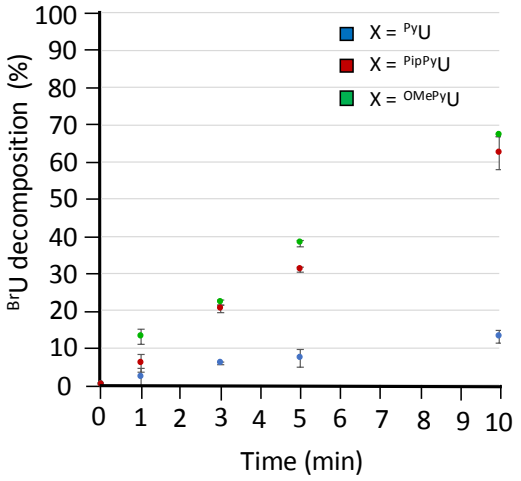
(a) Y = ^{Br}U (300 W)

ON 1 (X) : 5′-d(GCGACGUU**X**A AAAGUCGUCCGU)-3′
ON 4 (^{Br}U) : 3′-d(CGCUGCAAAU^{Br}**UU**UCAGCAGGCA)-5′



(b) Y = ^{Br}U (150 W)

ON 1 (X) : 5′-d(GCGACGUU**X**A AAAGUCGUCCGU)-3′
ON 4 (^{Br}U) : 3′-d(CGCUGCAAAU^{Br}**UU**UCAGCAGGCA)-5′



(c) Y = **CPD**

ON 1 (X) : 5′-d(GCGACGUU**X**AA AAGUCGUCCGU)-3′
ON 4 (**CPD**) : 3′-d(CGCUGCAAAU**T[]T**UCAGCAGGCA)-5′

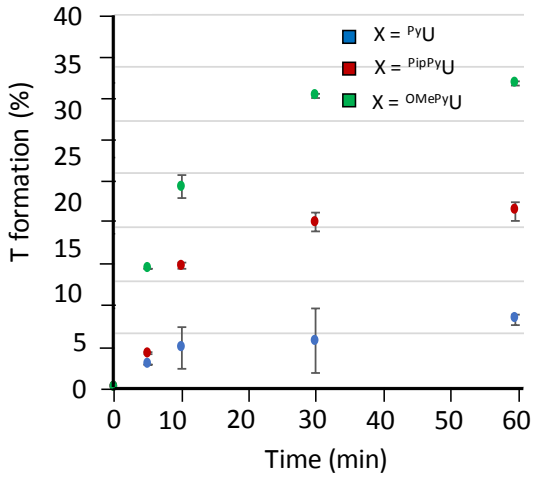


Fig. S5 Time-dependent decomposition of ^{Br}U (300 W) (a), ^{Br}U (150 W) (b) and formation of T (c) as a function of each pyrene-modified uridine in buffer (10 µM oligonucleotide, 100 mM NaCl, 10 mM phosphate, pH 7.4). A 300 W Xe lamp with a cut-off filter (400 nm) was used. The data are given as the average of three independent experiments.

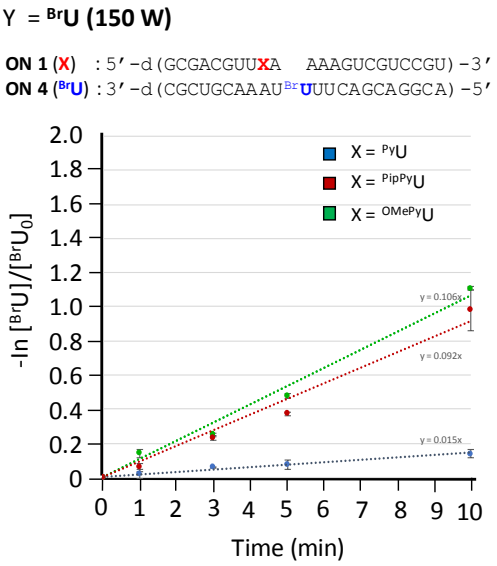
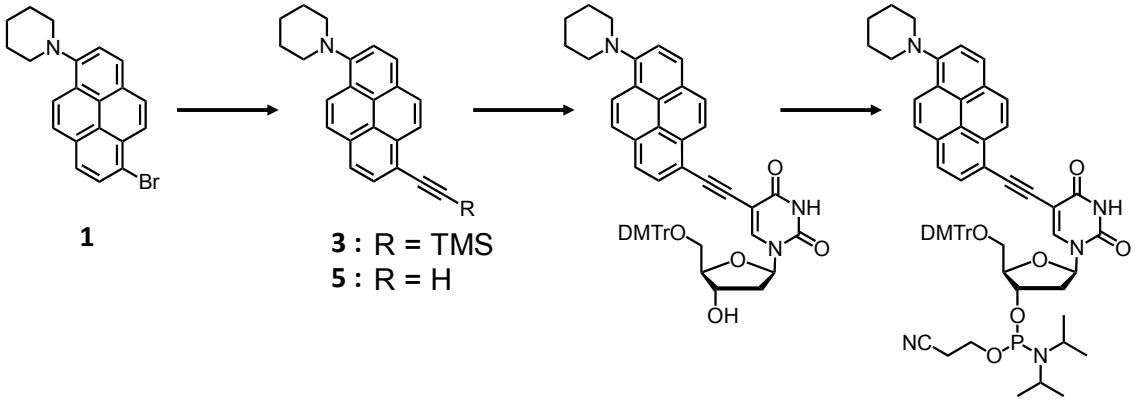


Fig. S6 Initial decomposition rates of ^{Br}U as a function of each pyrene-modified uridine in buffer (10 μM oligonucleotide, 100 mM NaCl, 10 mM phosphate, pH 7.4). A 150 W Xe lamp with a cut-off filter (400 nm) was used. $-\ln[^{Br}U]/[^{Br}U_0]$ are given as the average of three independent experiments.

Synthesis of piperidine-modified ^{Py}U analog (^{PipPy}U)



1 **3** : R = TMS **5** : R = H **7** **9**

Synthesis of 6-piperidyl-1-(trimethylsilyl)ethynylpyrene (3)

CH_2Cl_2 (83 mL) was placed in a round-bottom flask (RBF) and deoxygenated by continuous bubbling of Ar. Pyrene **1** (3 g, 8.3 mmol) was then added and dissolved under Ar. TMSA (6 mL, 42.5 mmol), $Pd(PPh_3)_4$ (982 mg, 0.8 mmol), CuI (162 mg, 0.8 mmol), and TEA (5.9 mL, 42.5 mmol) were added to the solution and the reaction mixture was stirred at reflux for 15 h. The resultant black solution was filtered through a pad of Celite and evaporated to dryness. Thereafter the residue was partitioned between EtOAc and water and extracted several times. The organic solution was dried over sodium sulfate, filtered, and evaporated to dryness. The crude product was dissolved in a small amount of $CHCl_3$ and subjected to column chromatography with a gradient of 0 to 50 % toluene in hexanes (R_f: 0.3 in 5% toluene in hexanes) to give **3** as a yellow solid (2.6 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.75 (d, *J* = 9.1 Hz, 1H), 8.70 (d, *J* = 9.1 Hz, 1H), 8.41 (d, *J* = Hz, 1H), 8.37 (d, *J* = 8.0 Hz, 1H), 8.35-8.24, (m, 4H), 7.93 (d, *J* = 8.0 Hz, 1H), 3.45 (br, 4H), 2.20 (q, *J* = 5.6 Hz, 4H), 1.98 (br, 2H), 0.34 (s, *J* = Hz, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.86, 132.96, 131.79, 130.03, 128.53, 126.94, 126.27, 126.19, 125.60, 125.07, 124.94, 124.28, 123.78, 123.69, 117.40, 116.83, 104.70, 99.81, 55.13, 26.82, 24.65, 0.41. HRMS (MALDI) calcd. for $C_{26}H_{27}N_1Si_1$ 381.1918, found 381.1907.

Synthesis of 6-piperidyl-1-ethynylpyrene (5)

The TMS-protected pyrene analog **3** (2.5 g, 6.6 mmol) was dissolved in THF (66 mL) in an RBF (200 mL) under Ar. Tetrabutylammonium fluoride (1 M in THF, 6 mL) was slowly added and the reaction mixture was stirred in an ice bath for a minute before being brought to room temperature. After 12 h, the reaction was quenched with water (10

mL) and the solution was extracted with EtOAc and water. The pooled organic extracts were dried over sodium sulfate, filtered, and evaporated to dryness. The residue was dissolved in a small amount of $CHCl_3$ and subjected to column chromatography using a gradient of 0 to 20 % $CHCl_3$ in hexanes (R_f: 0.3 in 20% $CHCl_3$ in hexanes) to afford **5** as a yellow solid (2.0 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (d, *J* = 3.1 Hz, 1H), 8.41 (d, *J* = 3.1 Hz, 1H), 8.13 (d, *J* = 8.2 Hz, 1H), 8.10-7.99 (m, 4H), 7.73 (d, *J* = 8.2 Hz, 1H), 3.59 (s, 1H), 3.20 (br, 4H), 1.92 (q, *J* = 5.7 Hz, 4H), 1.71 (br, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.04, 133.22, 132.03, 130.30, 128.71, 126.95, 126.39, 126.21, 125.63, 125.10, 124.99, 124.52, 123.82, 123.51, 117.57, 115.77, 83.24, 82.36, 55.23, 26.87, 24.70. HRMS (MALDI) calcd. for $C_{23}H_{19}N_1$ 309.1509, found 309.1512.

Synthesis of 5-(6-piperizyl-pyrenylethynyl)-5'-*O*-dimethoxytrityl-2'-deoxyuridine (7)

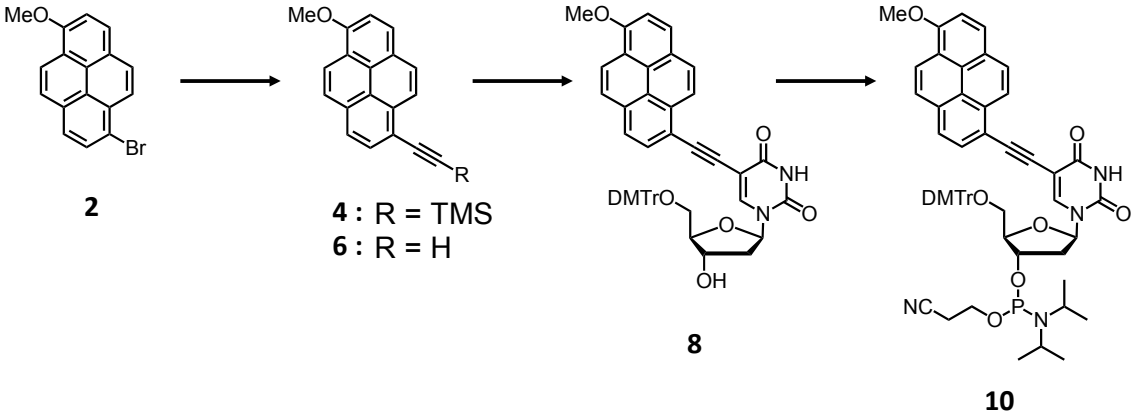
To a solution of **5** (1.0 g, 3.2 mmol) in THF (32 mL) were added 5-iodo-5'-*O*-dimethoxytrityl-2'-deoxyuridine (1.1 g, 1.6 mmol), $Pd(PPh_3)_4$ (184 mg, 0.2 mmol), CuI (38 mg, 0.2 mmol) and TEA (1.1 mL, 8.0 mmol) and the reaction mixture was stirred at room temperature for 24 h. The resulting mixture was filtered through a pad of Celite and the filtrate concentrated *in vacuo*. The residue was partitioned between EtOAc and water and the separated organic layer washed with brine. It was then dried over sodium sulfate, filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography with a gradient of 0 to 10 % MeOH in $CHCl_3$ (NH_2 silica gel, R_f: 0.3 in 5% MeOH in $CHCl_3$) to give **7** (1.2 g, 92 %) as a yellow foam.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (d, *J* = 9.1 Hz, 1H), 8.33 (d, *J* = 9.1 Hz, 1H), 8.26 (s, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.93 (d, *J* = 9.1 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.81 (d, *J* = 9.1 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.45 (d, *J* = 7.4 Hz 2H), 7.34(d, *J* = 8.8 Hz, 4H), 7.20 (t, *J* = 8.0 Hz, 2H), 7.03 (t, *J* = 7.4 Hz, 1H), 6.70 (dd, *J* = 5.9 Hz, 8.8 Hz, 4H) 6.43 (m, 1H), 4.56 (br, 1H), 4.20 (br, 1H), 3.46 (s, 3H), 3,44 (s, 3H), 3.34-3,30 (m, 1H), 3.28 (br, 1H), 3.15 (br, 4H), 2.67-2.62 (m, 1H), 2.39-2.31 (m, 1H), 1.89 (q, *J* = 5.7 Hz, 4H), 1.68 (br, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.97, 158.63, 158.61, 149.80, 149.71, 144.60, 141.65, 135.68, 135.53, 132.62, 131.65, 130.13, 130.04, 129.68, 128.64, 128.14, 128.05, 127.08, 127.00, 126.33, 126.15, 125.51, 124.91, 124.87, 124.22, 123.95, 123.53, 117.37, 116.37, 113.42, 101.27, 93.91, 87.14, 86.96, 86.26, 85.51, 72.64, 63.73, 55.15, 55.12, 55.09, 41.76, 26.81, 24.64. HRMS (MALDI) calcd. for $C_{53}H_{47}N_3O_7$ 837.3397, found 837.3401.

Synthesis of 5-(6-piperizyl-pyrenylethynyl)-5'-*O*-dimethoxytrityl-3'-*O*-{2-cyanoethyl(diisopropylamino)phosphino}-2'-deoxyuridine (9)

To a solution of **7** (770 mg, 0.8 mmol) in CH_2Cl_2 (8 mL), were added 2-cyanoethyl-*N,N'*-diisopropylchlorophosphoramidite (220 µL, 0.9 mmol) and DIPEA (156 µL, 0.9 mmol). The reaction mixture was stirred at room temperature for 1.5 h and then partitioned between EtOAc and water. The separated organic layer was washed with brine, followed by drying over sodium sulfate, filtration, and concentration *in vacuo.* The residue was purified by flash column chromatography with a gradient of 0 to 10 % MeOH in $CHCl_3$ (NH_2 silica gel, R_f: 0.3 in 5% MeOH in $CHCl_3$) to give diastereo-mixture **9** (730 mg, 90 %) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (d, *J* = 9.1 Hz, 1H), 8.33 (s, 1H), 8.27 (d, *J* = 9.1 Hz, 1H), 8.07 (d, *J* = 8.3 Hz, 1H), 7.95 (d, *J* = 9.1 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.79 (d, *J* = 9.1 Hz, 1H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.49-7.45 (m, 1H), 7.34 (d, *J* = 8.0 Hz, 4H), 7.24 (t, *J* = 8.0 Hz, 2H), 7.08 (t, *J* = 7.3 Hz, 1H), 6.72 (dd, *J* = 5.9 Hz, 8.8 Hz, 4H), 6.37 (m, 1H), 4.66-4.63 (m, 1H), 4.27 (br, 1H), 3.69-3.52 (m, 5H), 3.51 (s, 3H), 3.48 (s, 3H), 3.37-3.33 (m, 1H), 3.20 (br, 4H), 2.65-2.60 (br, 1H), 2.46-2.37 (br, 3H), 1.93 (q, *J* = 5.7 Hz, 4H), 1.72 (br, 2H), 1.20 (d, *J* = 1.5 Hz, 6H), 1.18 (d, *J* = 1.5 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.18, 158.75, 158.73, 149.83, 149.16, 148.65, 144.57, 141.57, 135.67, 135.52, 132.67, 131.73, 130.26, 130.14, 129.79, 128.58, 128.21, 128.18, 127.21, 127.06, 126.30, 126.22, 125.58, 124.99, 124.29, 123.98, 123.50, 117.49, 117.43, 116.34, 113.46, 101.29, 93.96, 87.27, 86.51, 86.45, 86.17, 86.12, 85.21, 77.37, 73.85, 70.73, 63.31, 58.45, 58.26, 55.24, 55.18, 55.16, 43.56, 43.41, 40.09, 26.89, 24.81, 24.77, 24.75, 24.72, 24.68, 20.41, 20.35. ^{31}P NMR (120 MHz, CDCl3): δ 149.27, 148.69. HRMS (MALDI) calcd. for $C_{62}H_{64}N_5O_8P_1$ 1037.4488, found 1037.4487.

Synthesis of methoxy-modified ^{Py}U analog ($^{OMePy}U$)



Synthesis of 6-methoxy-1-(trimethylsilyl)ethynylpyrene (4)

Pyrene **2** (1.3 g, 4.3 mmol) was dissolved in CH_2Cl_2 (43 mL, deoxygenated by bubbling Ar) in an RBF (100 mL) under Ar. TMSA (1.5 mL, 21.5 mmol), $Pd(PPh_3)_4$ (450 mg, 0.4 mmol), CuI (75 mg, 0.4 mmol), and TEA (3.0 mL, 42.5 mmol) were added to the reaction mixture and the solution was stirred at reflux for 15 h. The brown solution was filtered through a pad of Celite and evaporated to dryness. The material was partitioned between EtOAc and water and extracted several times. The organic solution was dried over sodium sulfate, filtered, and evaporated to dryness. The residue was dissolved in a small amount of $CHCl_3$ and subjected to column chromatography with a gradient of 0 to 50 % toluene in hexanes (R_f: 0.3 in 5% toluene in hexanes) to give **4** as a yellow solid (875 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.49 (d, *J* = 9.1 Hz, 1H), 8.41 (d, *J* = 9.1 Hz, 1H), 8.16 (d, *J* =8.5 Hz, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 8.07 (d, *J* = 9.1 Hz, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 8.01 (d, *J* = 9.1 Hz, 1H), 7.56 (d, *J* = 8.5 Hz, 1H), 4.19 (s, 3H), 0.43 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.15, 133.03, 131.97, 130.16, 128.33, 126.36, 126.20, 125.37, 125.13, 124.63, 123.57, 123.16, 122.05, 116.70, 108.40, 104.43, 99.79, 56.18, 0.24. HRMS (MALDI) calcd. for $C_{22}H_{20}O_1Si_1$ 328.1281, found 328.1277.

Synthesis of 6-methoxy-1-ethynylpyrene (6)

The TMS-protected pyrene analog **4** (800 mg, 2.4 mmol) was dissolved in THF (24 ml) in an RBF (200 mL) under Ar. Tetrabutylammonium fluoride (1 M in THF, 2 mL) was slowly added and the reaction mixture was stirred in an ice bath for a minute before being brought to room temperature. After 12 h, the reaction was quenched with water and the solution was extracted with EtOAc and water. The organic solution was dried over

sodium sulfate, filtered and evaporated to dryness. The residue was dissolved in a small amount of toluene and subjected to column chromatography in a gradient of 0 to 20 % toluene in hexanes (R_f: 0.4 in 20% toluene in hexanes) to yield **6** as a yellow solid (522 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (d, *J* = 9.1 Hz, 1H), 8.35 (d, *J* = 9.1 Hz, 1H), 8.08 (d, *J* = 8.4 Hz, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 7.99 (d, *J* = 9.1 Hz, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.94 (d, *J* = 9.1 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 4.11 (s, 3H), 3.58 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.26, 133.35, 132.25, 130.45, 128.55, 126.53, 126.20, 125.37, 125.12, 124.68, 123.64, 122.95, 122.30, 120.39, 115.66, 108.50, 83.18, 82.36, 56.24. HRMS (MALDI) calcd. for $C_{19}H_{12}O_1$ 256.0873, found 256.0882.

Synthesis of 5-(6-methoxy-pyrenylethynyl)-5'-*O*-dimethoxytrityl-2'-deoxyuridine (8)

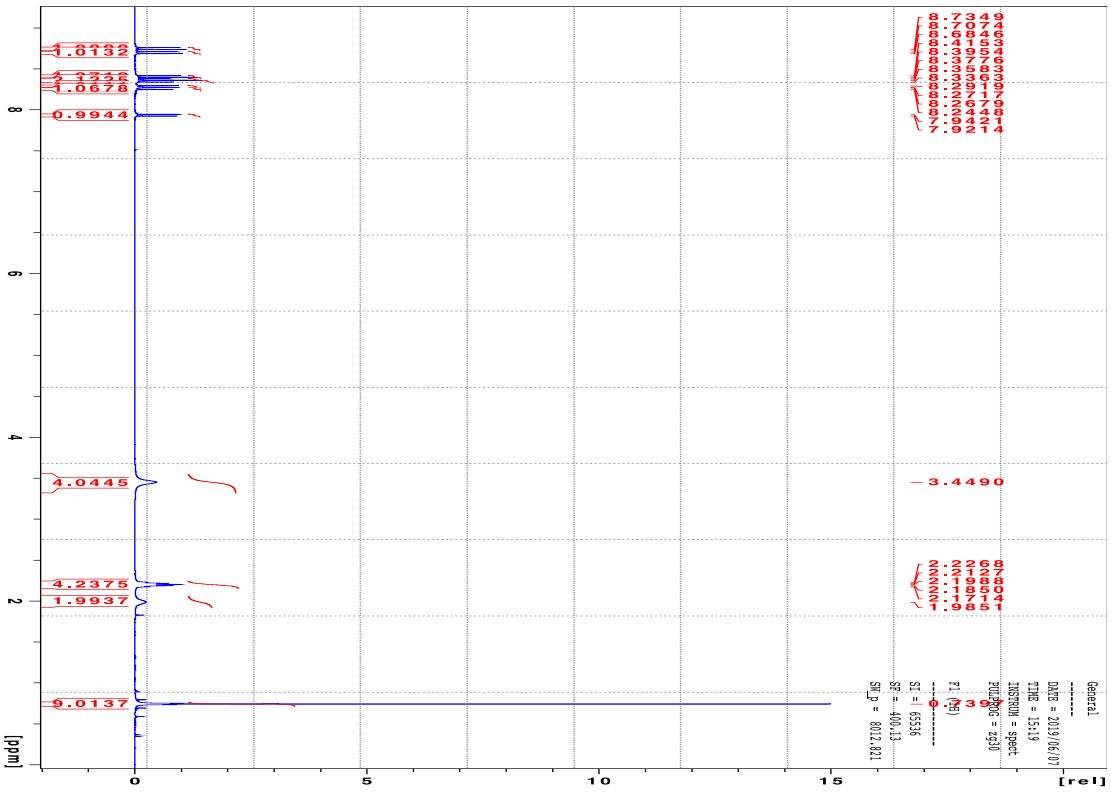
To a solution of **6** (500 mg, 2.0 mmol) in THF (32 mL) were added 5-iodo-5'-*O*-dimethoxytrityl-2'-deoxyuridine (1.1 g, 1.0 mmol), $Pd(PPh_3)_4$ (92 mg, 0.1 mmol), CuI (19 mg, 0.1 mmol) and TEA (378 mL, 4.0 mmol), and the reaction mixture was stirred at room temperature for 24 h. The resulting mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo*. The reaction mixture was partitioned between EtOAc and water and the separated organic layer washed with brine. The organic layer was dried over sodium sulfate and concentrated *in vacuo.* The residue was purified by flash column chromatography using a gradient of 0 to 10 % MeOH in $CHCl_3$ (NH_2 silica gel, R_f: 0.3 in 5% MeOH in $CHCl_3$) to give **8** (502 mg, 66%) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.05 (br, 1H) 8.38 (d, *J* = 9.1 Hz, 1H), 8.29 (d, *J* = 9.1 Hz, 1H), 8.25 (s, 1H), 7.97 (d, *J* = 8.6 Hz, 1H), 7.99 (d, *J* = 9.1 Hz, 1H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.77 (d, *J* = 9.1 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.44 (d, *J* = 7.5 Hz, 2H), 7.39 (d, *J* = 8.8 Hz, 1H), 7.33 (d, *J* = 8.8 Hz, 4H), 7.19 (t, *J* = 7.8 Hz, 1H), 7.03 (t, *J* = Hz, 1H), 6.71-6.67 (m, 2H), 6.45-6.41 (m, 1H), 4.56 (br, 1H), 4.21 (br, 1H), 4.05 (s, 3H), 3.44 (s, 3H), 3.42 (s, 3H), 3.33-3.29 (m, 1H), 2.68-2.63 (m, 1H), 2.38-2.31 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.58, 154.03, 149.83, 144.62, 141.65, 135.69, 135.55, 132.76, 131.88, 130.11, 130.02, 129.82, 129.12, 128.51, 128.31, 128.12, 128.06, 127.06, 126.49, 126.15, 125.38, 123.28, 125.19, 124.52, 123.40, 123.36, 122.04, 120.28, 116.28, 113.40, 108.39, 101.21, 93.81, 87.10, 86.97, 86.28, 85.59, 72.60, 63.74, 56.16, 55.07, 55.05, 41.72. HRMS (MALDI) calcd. for $C_{49}H_{40}N_2O_8$ 784.2758, found 784.2779.

Synthesis of 5-(6-methoxy-pyrenylethynyl)-5'-*O*-dimethoxytrityl-3'-*O*-{2-cyanoethyl(diisopropylamino)phosphino}-2'-deoxyuridine (10)

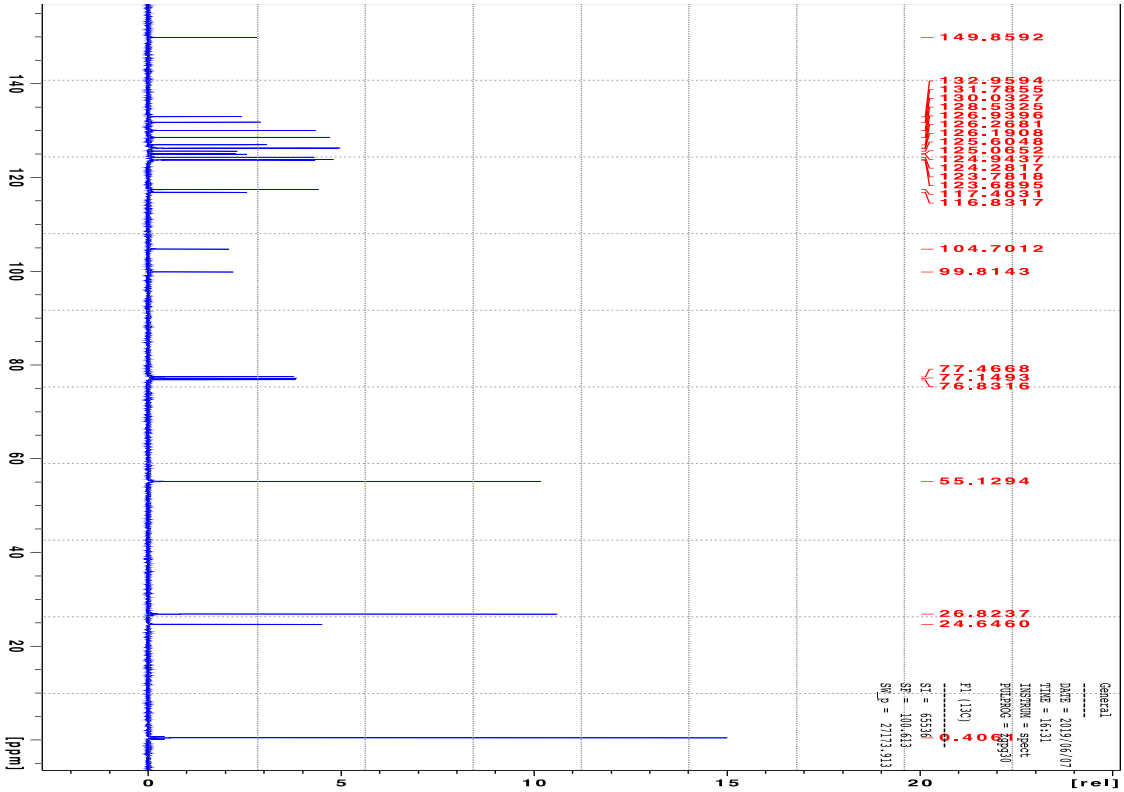
To a solution of **8** (500 mg, 0.7 mmol) in CH_2Cl_2 (8 mL) were added 2-cyanoethyl-*N,N'*-diisopropylchlorophosphoramidite (220 µL, 0.9 mmol) and DIPEA (156 µL, 0.9 mmol). The reaction mixture was stirred at room temperature for 1.5 h and then partitioned between EtOAc and water. The separated organic layer was washed with brine, dried over sodium sulfate, filtered, and concentrated *in vacuo.* The residue was purified by flash column chromatography using a gradient of 0 to 10 % MeOH in $CHCl_3$ (R_f: 0.4 in 5% MeOH in $CHCl_3$) to give diastereo-mixture **10** (484 mg, 72%) as a yellow foam.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40-8.37 (m, 1H), 8.34-8.30 (m, 2H), 8.01-7.99 (m, 1H), 7.91-7.88 (m, 1H), 7.83-7.78 (m, 2H), 7.54-7.48 (m, 3H), 7.43-7.37 (m, 5H), 7.27-7.22 (m, 2H), 7.15-7.06 (m, 1H), 6.76-6.73 (m, 4H), 6.42-6.38 (m, 1H), 4.67-4.63 (m, 1H), 4.27-4.22 (m, 1H), 4.08 (s, 3H), 3.69-3.53 (m, 5H), 3.49-3.49(m, 3H), 3.47-3.46 (m, 3H), 3.36-3.31 (m, 1H), 2.72-2.64 (m, 1H), 2.63-2.60 (m, 1H), 2.43-2.32 (m, 2H), 1.18-1.06 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.83, 158.57, 153.93, 149.63, 144.46, 141.40, 135.55, 135.40, 132.70, 131.77, 130.11, 130.06, 130.00, 129.72, 129.04, 128.40, 128.22, 128.07, 128.01, 127.03, 126.34, 126.04, 125.29, 125.11, 125.11, 124.43, 123.36, 123.21, 121.93, 120.19, 117,71, 117.48, 116.25, 113.32, 108.29, 101.20, 93.69, 87.07, 86.28, 85.96, 85.50, 77.35, 73.68, 63.21 58.34, 56.08, 54.60, 43.25, 40.81, 24.56, 21.45, 20.40, 20.21. ^{31}P NMR (120 MHz, CDCl3): δ 148.75, 148.35. HRMS (MALDI) calcd. for $C_{58}H_{57}N_4O_9P_1$ 984.3855, found 984.3857.

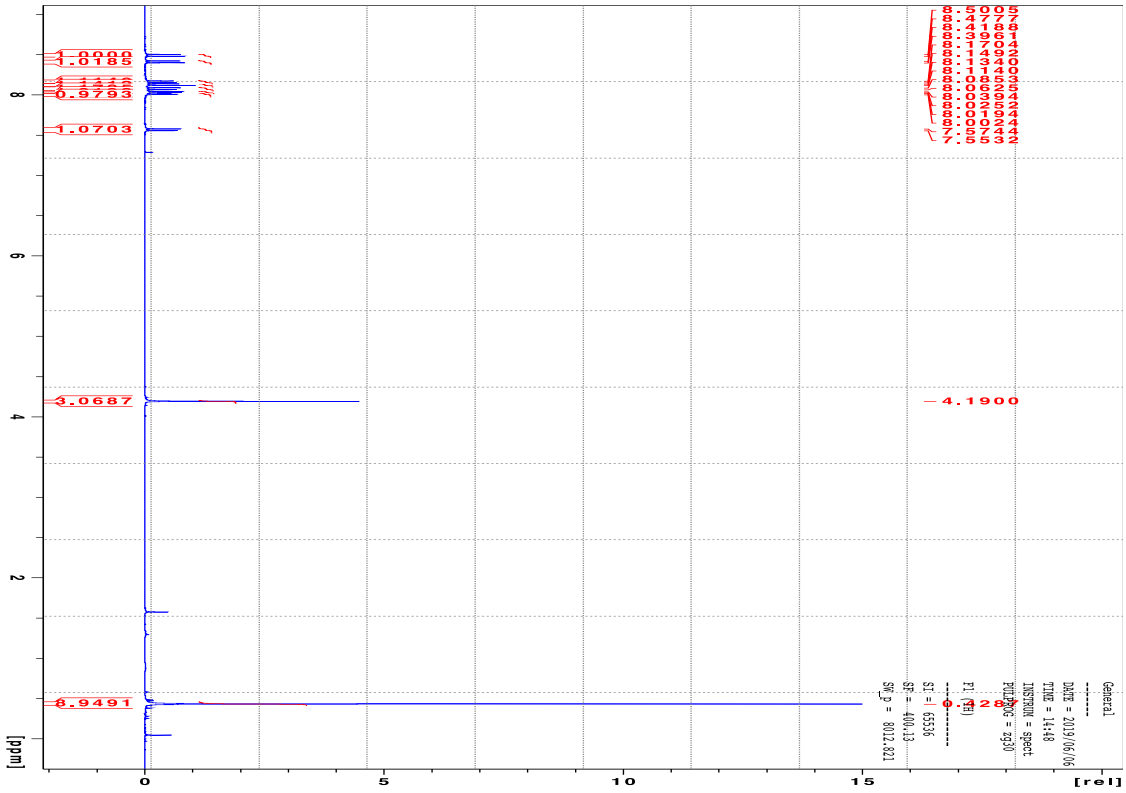
^{1}H spectrum of 3



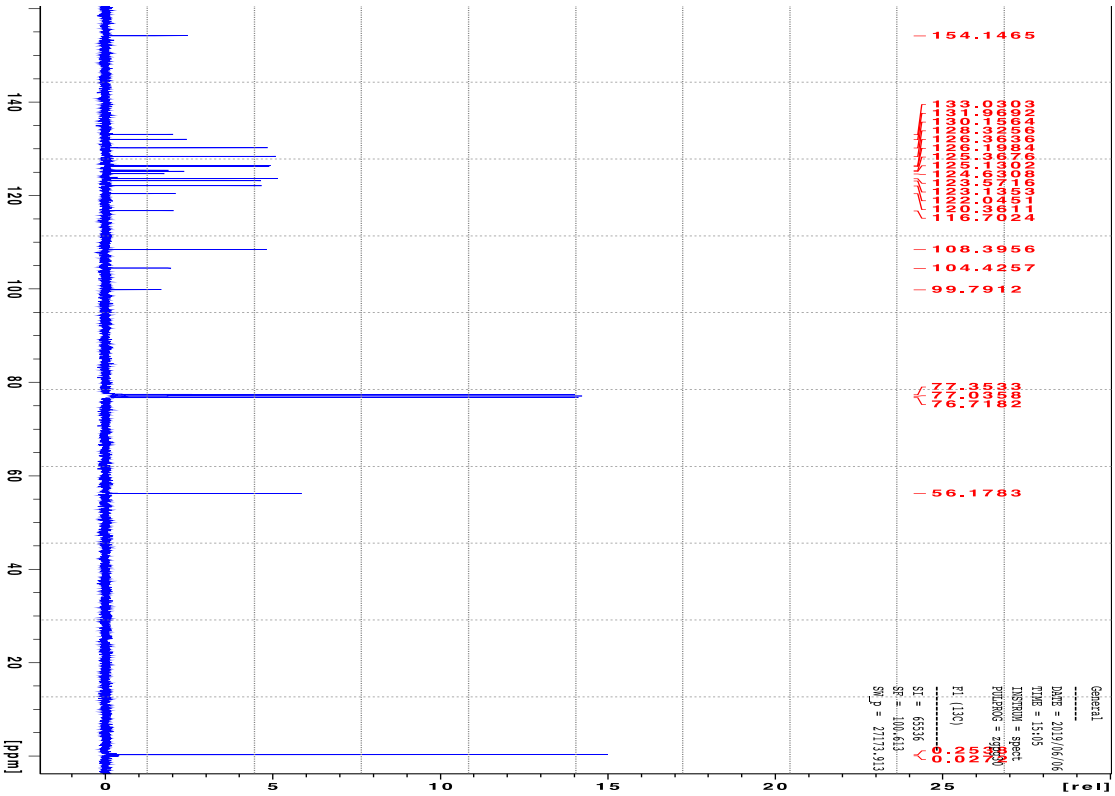
^{13}C spectrum of compound 3



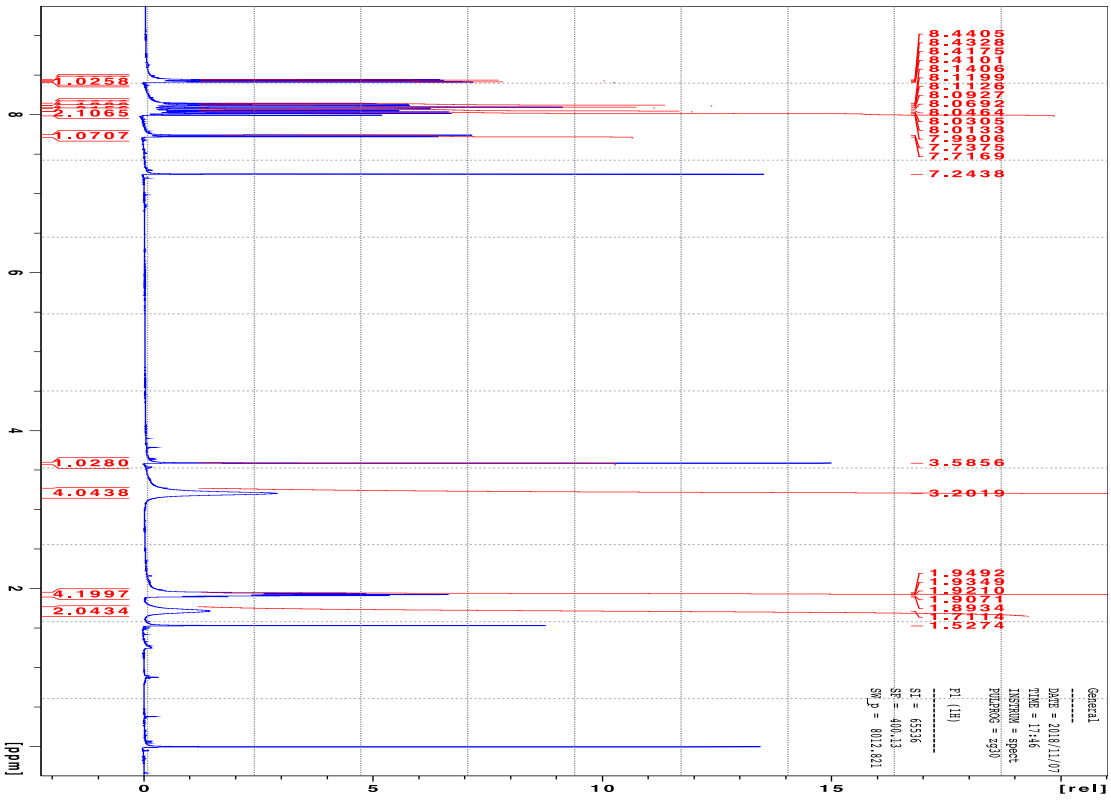
^{1}H spectrum of 4



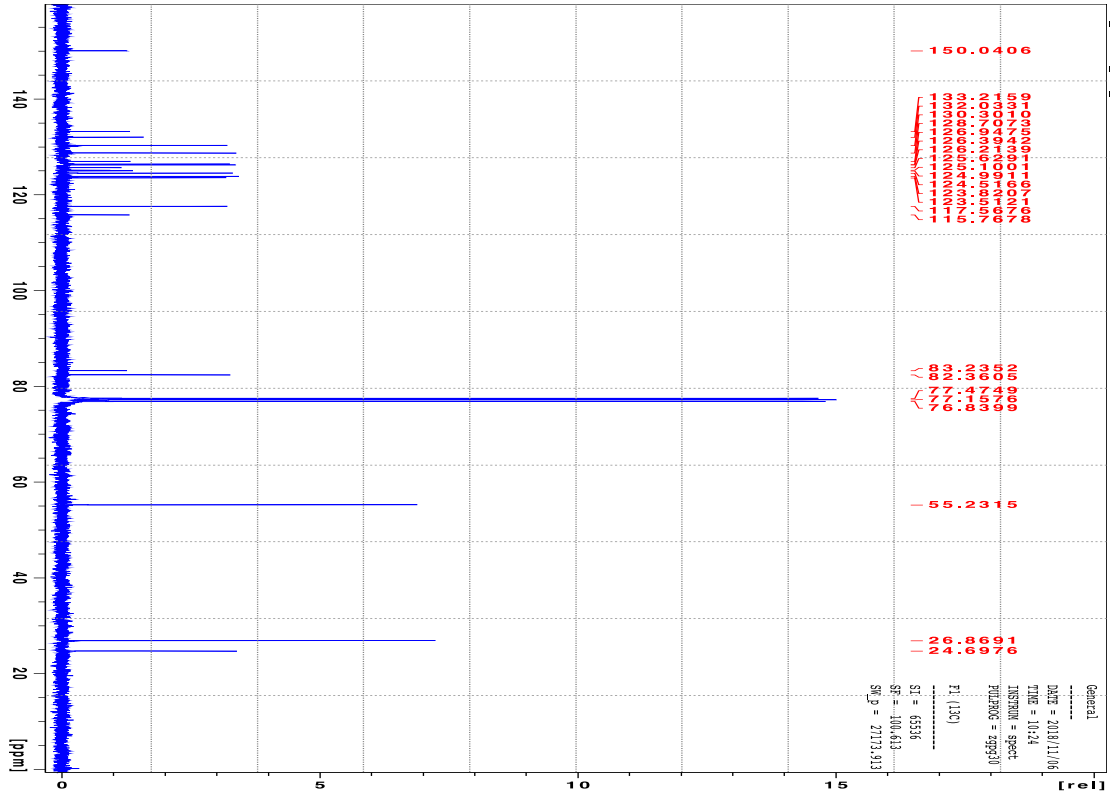
^{13}C spectrum of 4



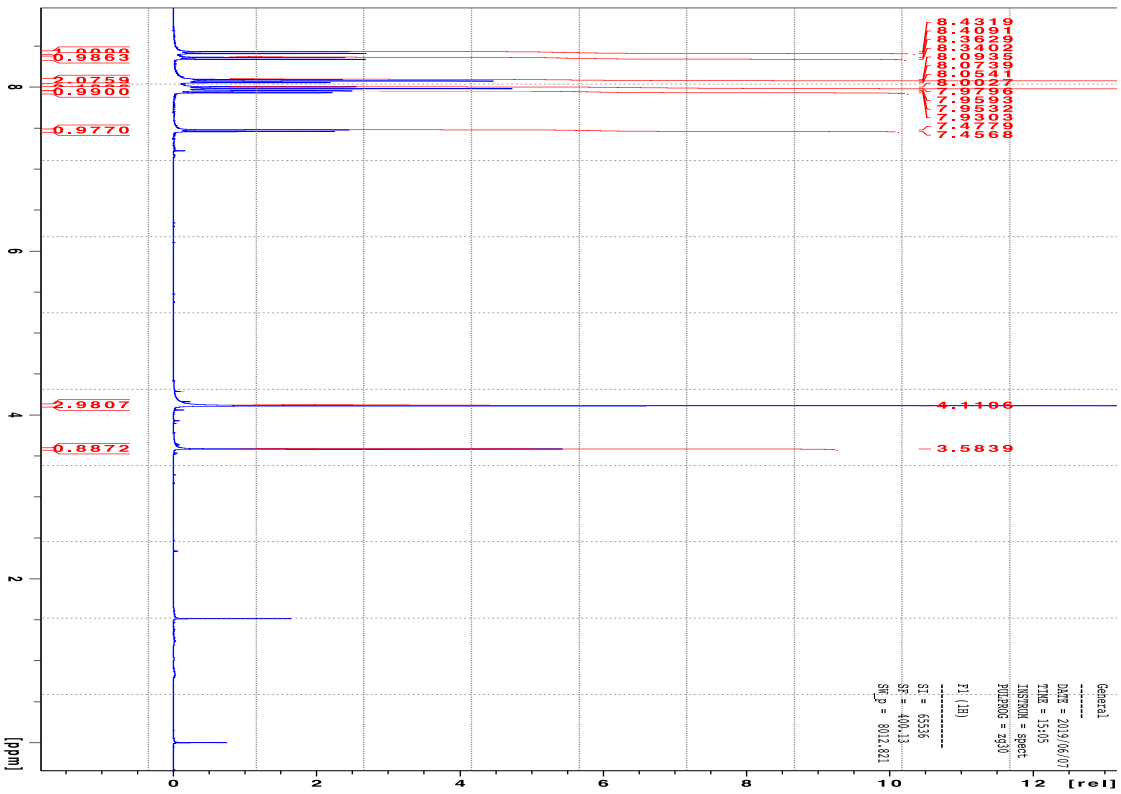
^{1}H spectrum of 5



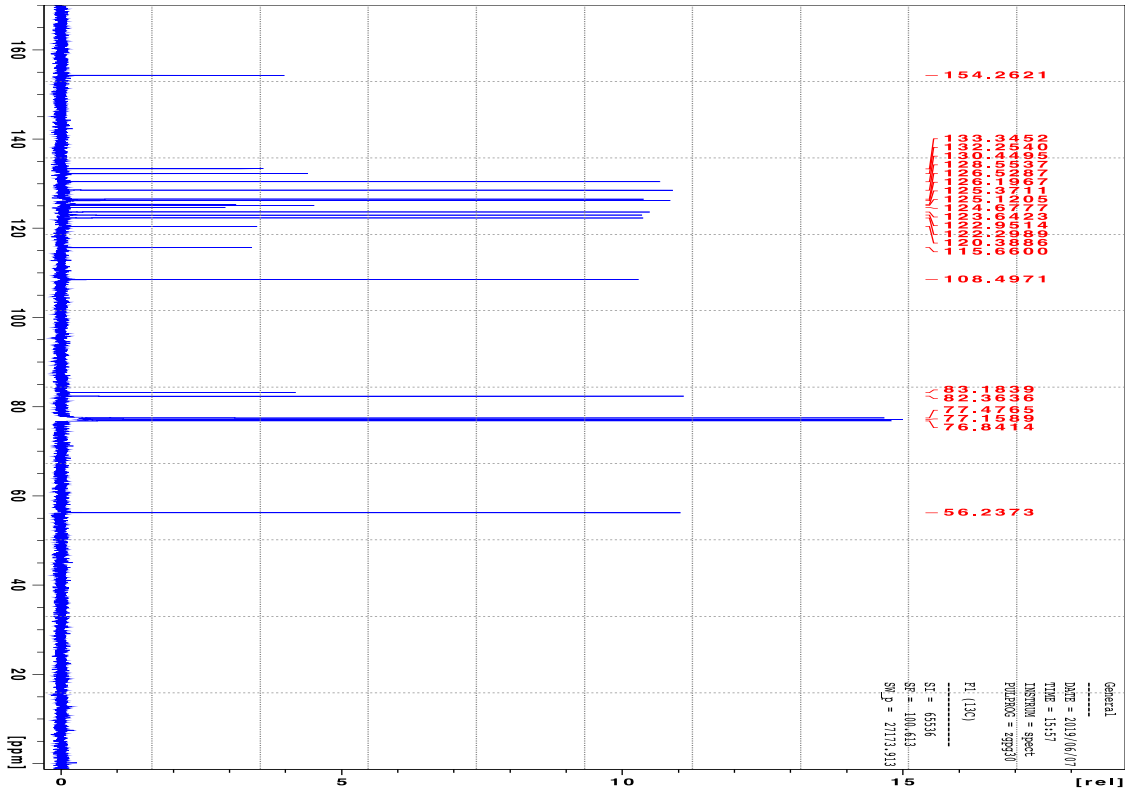
^{13}C spectrum of 5



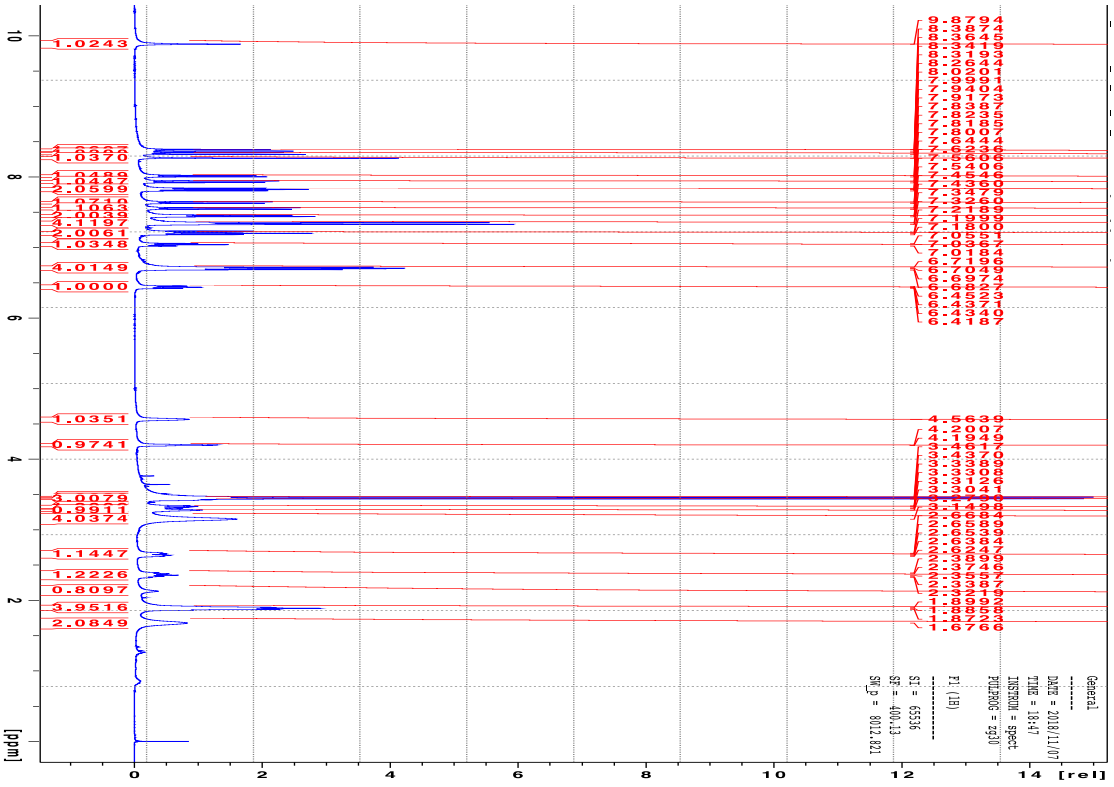
^{1}H spectrum of 6



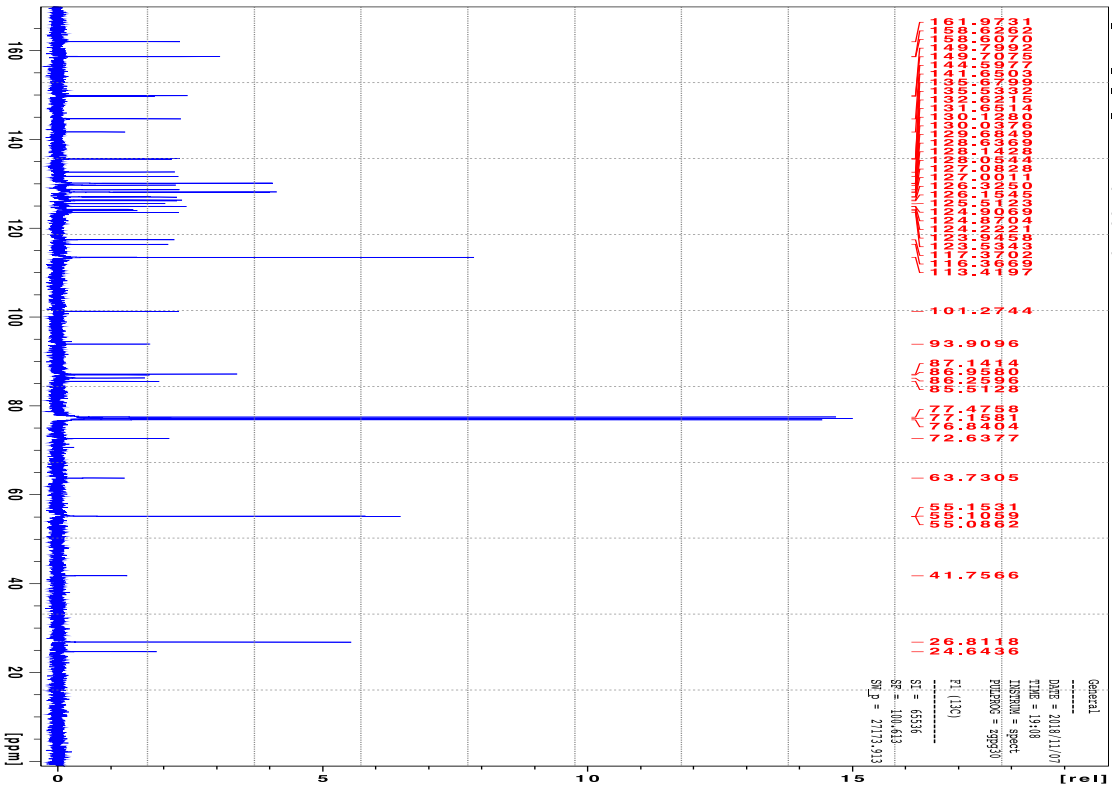
^{13}C spectrum of 6



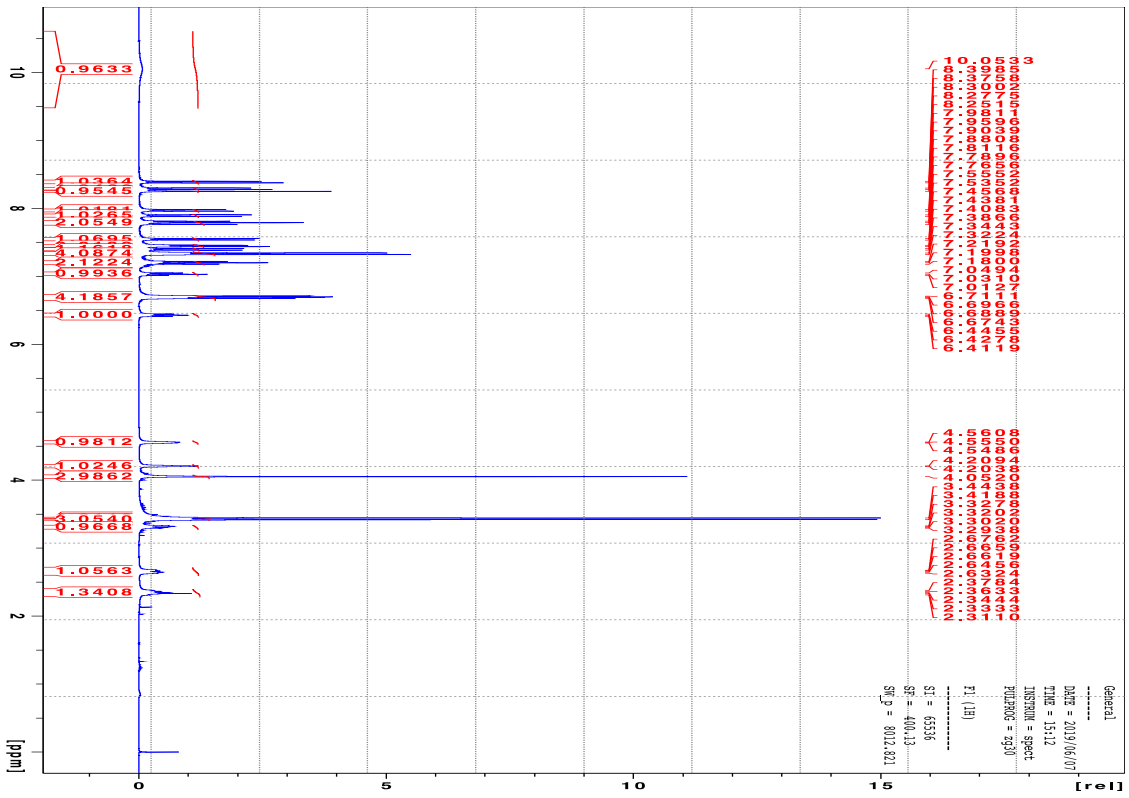
^{1}H spectrum of 7



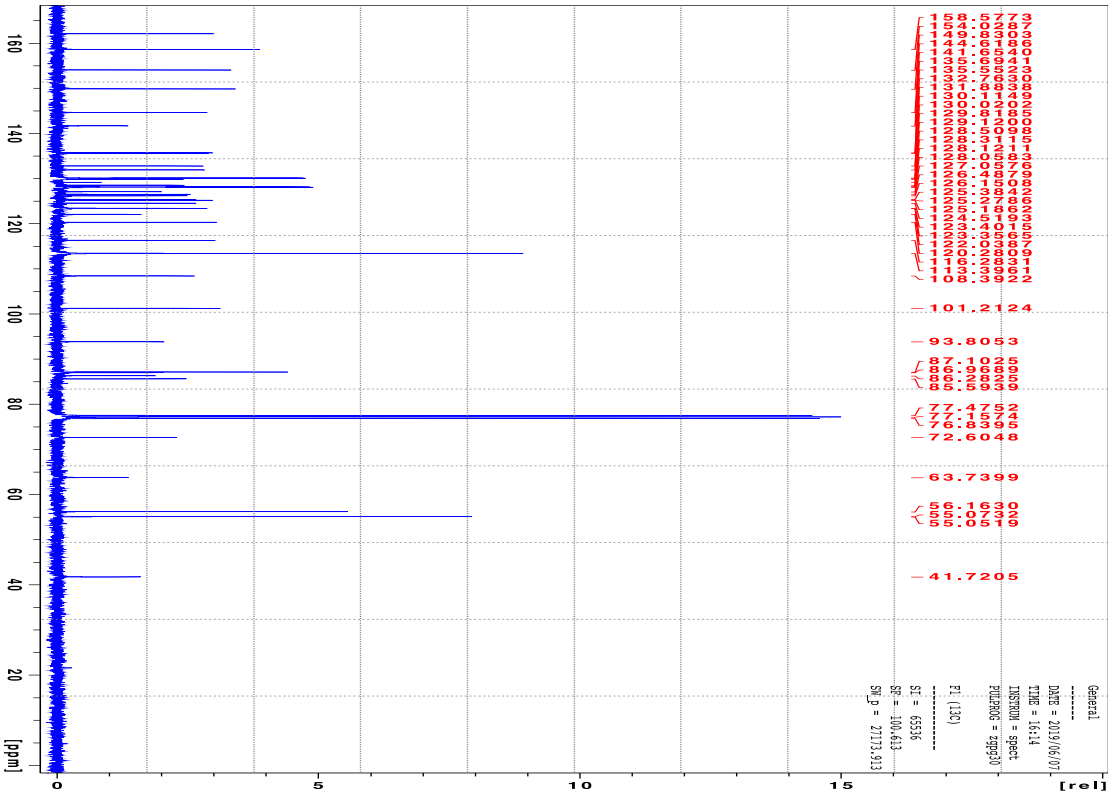
^{13}C spectrum of 7



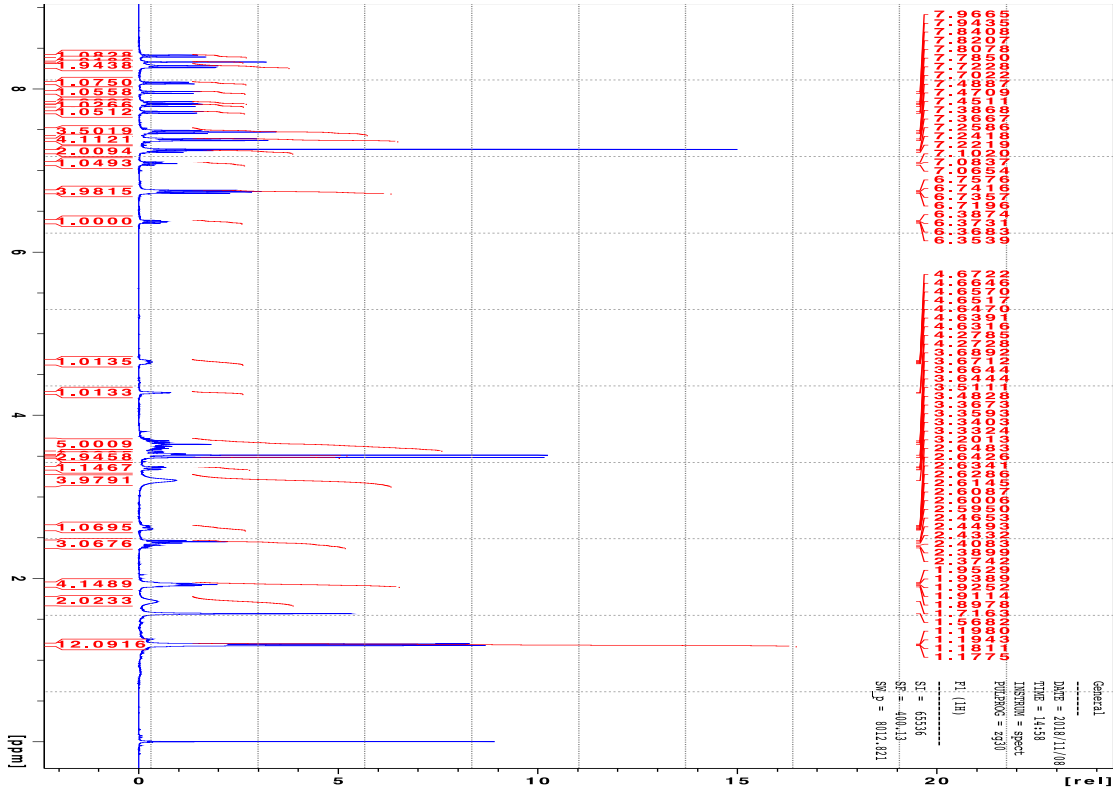
^{1}H spectrum of 8



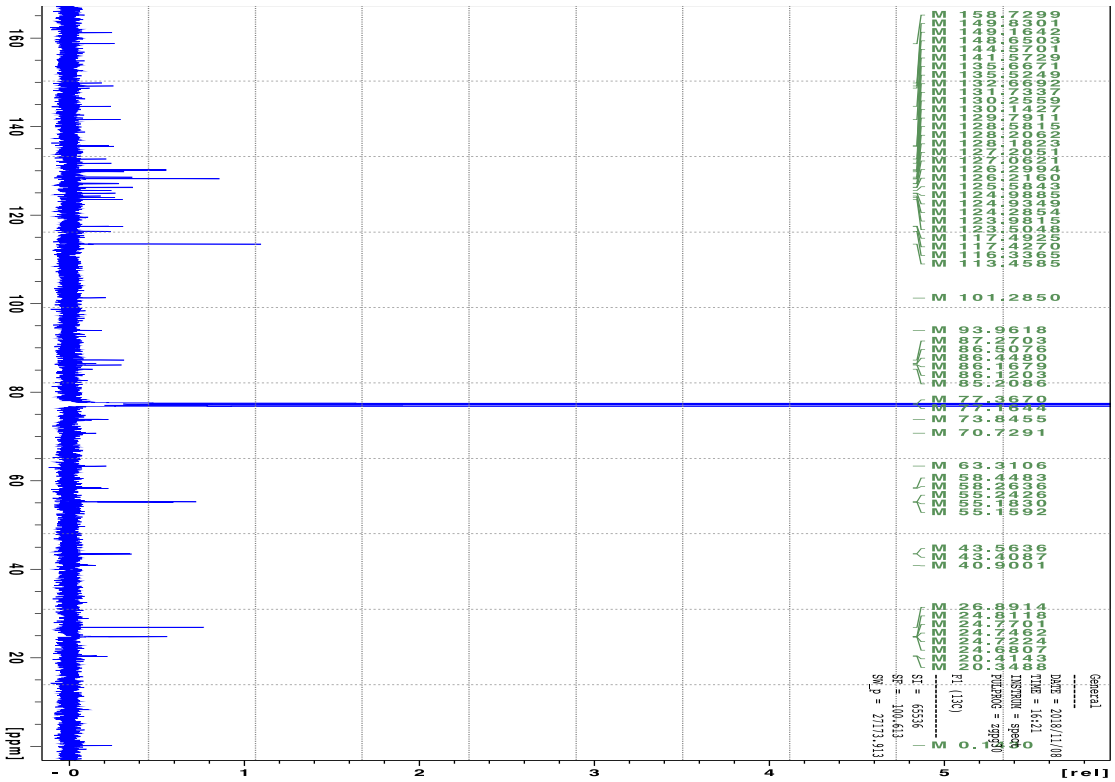
^{13}C spectrum of 8



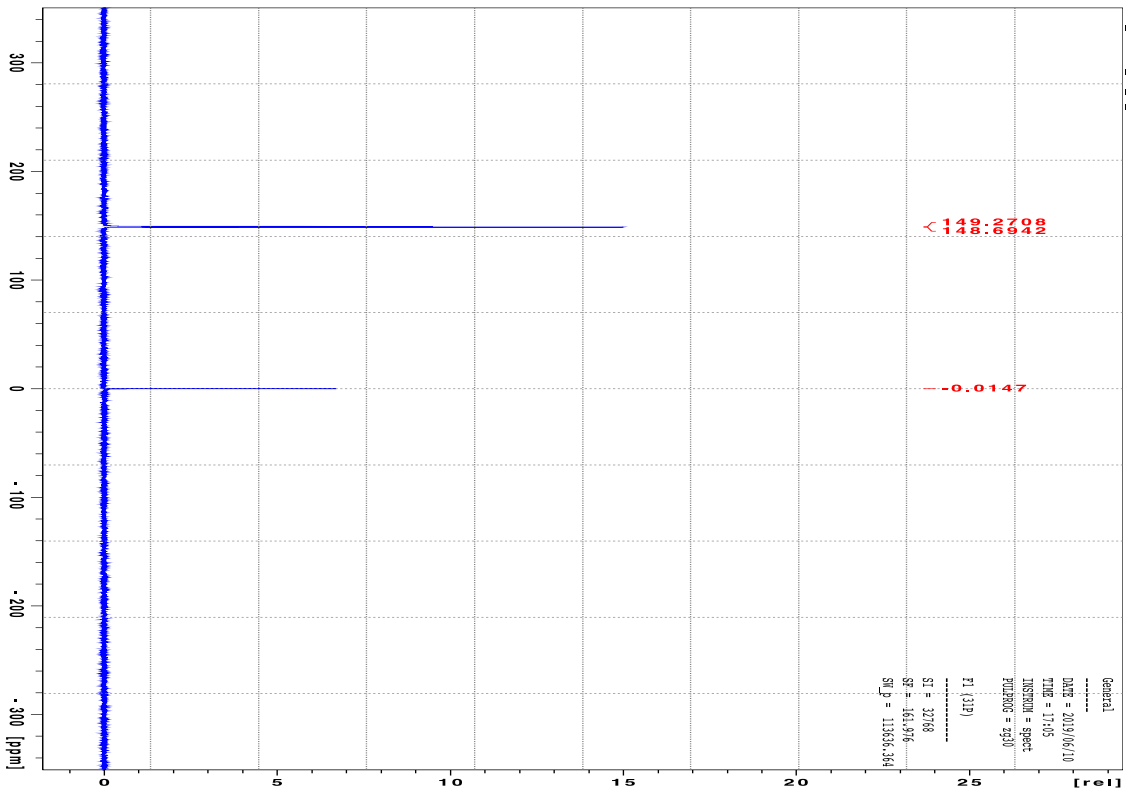
^{1}H spectrum of 9



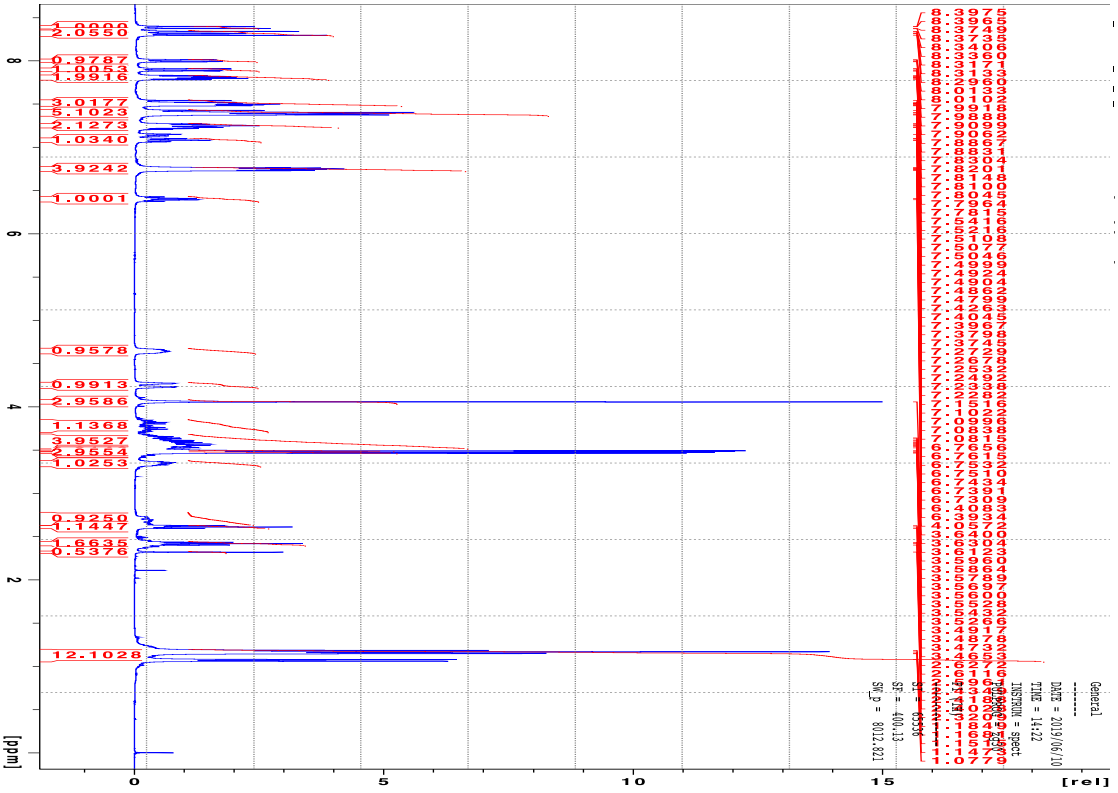
^{13}C spectrum of 9



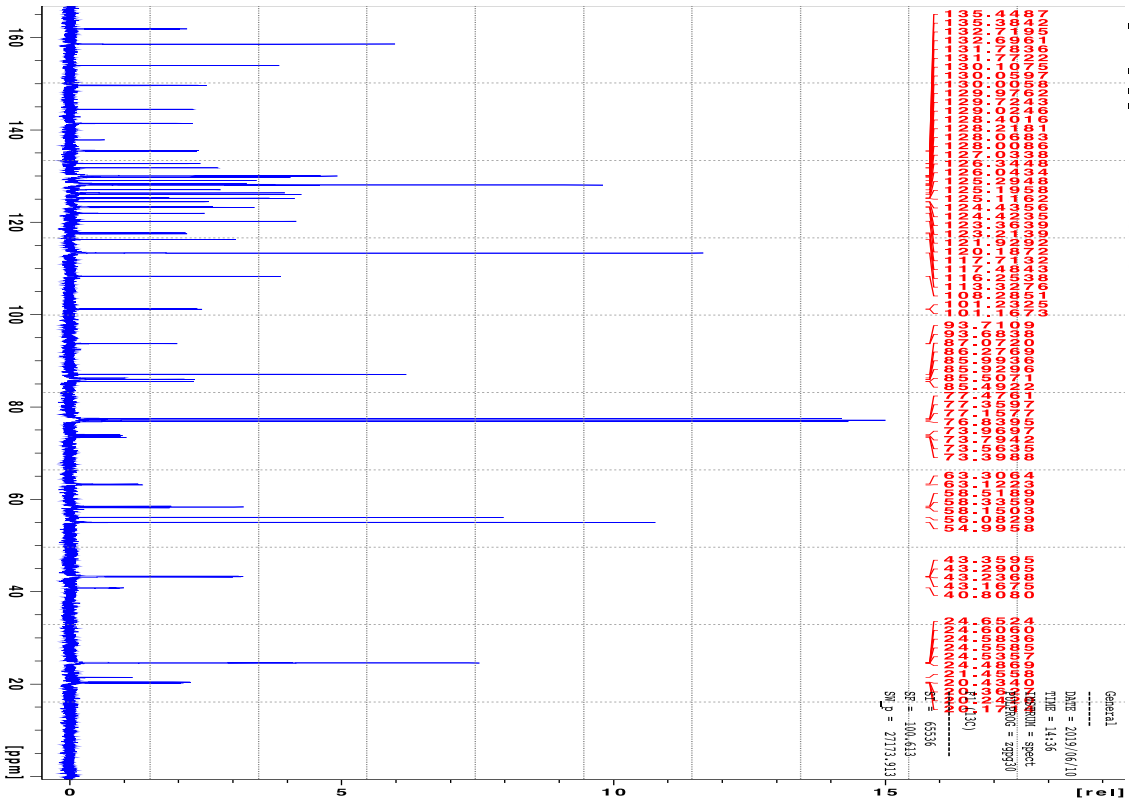
^{31}P spectrum of 9



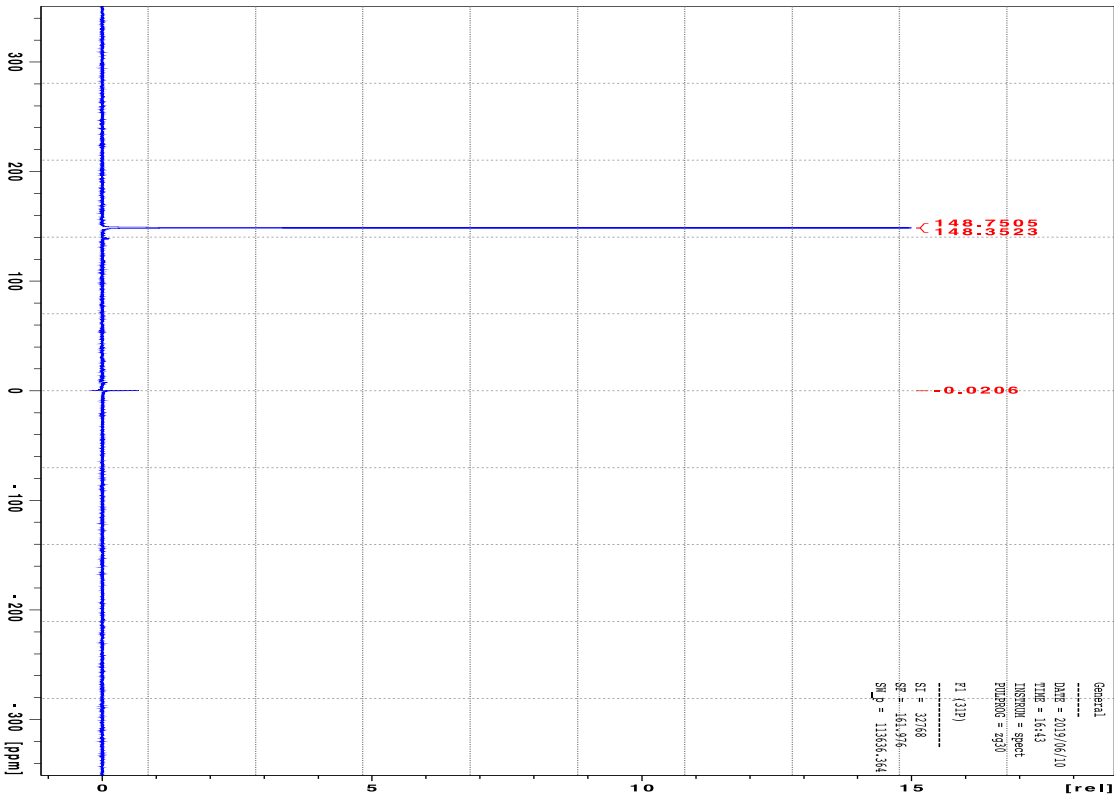
^{1}H spectrum of 10



^{13}C spectrum of 10



^{31}P spectrum of 10



References

S1. A. D. Malakhov, E. V. Malakhova, S. V. Kuznitsova, I. V. Grechishnikova, I. A. Prokhorenko, M. V. Skorobogatyi, V. A. Korshun, Yu. A. Berlin, *Russian J. bioorg. Chem.*, 2000, **26**, 34.

S2. T. Murata, S. Iwai, E. Ohtsuka, Nucleic Acids Res., 1990, **18**, 7279.

S3. Gaussian 09, Revision C.01 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2010.

S4. RDKit 2019.03.1: Open-source cheminformatics; http://www.rdkit.org