# Supporting information

# for

# Labelling of nucleosides and oligonucleotides by solvatochromic 4-aminophthalimide fluorophore for studying DNA-protein interactions

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# 1. Additional figures

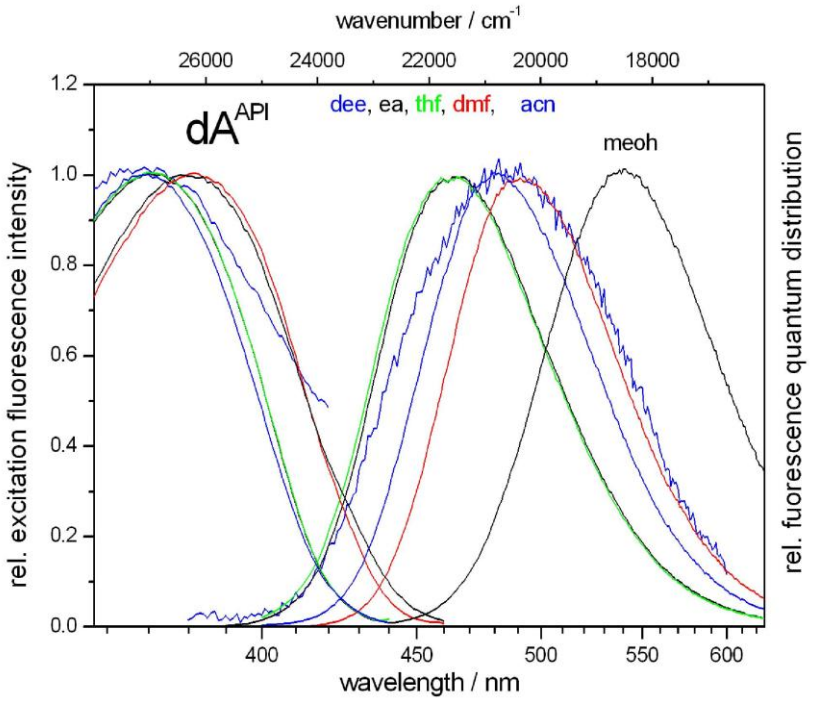



**Figure S1.** Excitation (left) and fluorescence spectra (right, quantum distribution over wavenumbers) of **dA$^{API}$** (**4**) in several solvents (*dee* – diethyl ether, *ea* – ethyl acetate, *thf* – tetrahydrofurane, *acn* – acetonitrile, *dmf* – dimethyl formamide, *meoh* – methanol, *w* – water) upon excitation at 340 nm in all cases.

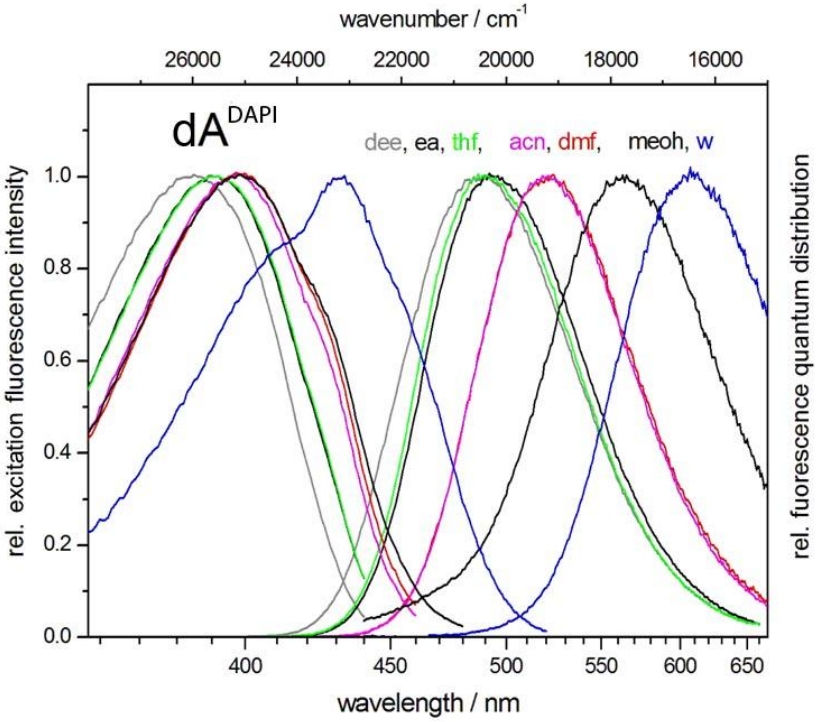



**Figure S2.** Excitation (left) and fluorescence spectra (right, quantum distribution over wavenumbers) of **dA$^{DAPI}$** (**6**) in several solvents (*dee* – diethyl ether, *ea* – ethyl acetate, *thf* – tetrahydrofurane, *acn* – acetonitrile, *dmf* – dimethyl formamide, *meoh* – methanol, *w* – water) upon excitation at 340 nm in all cases. Excitation at 380 nm was chosen for water solution.

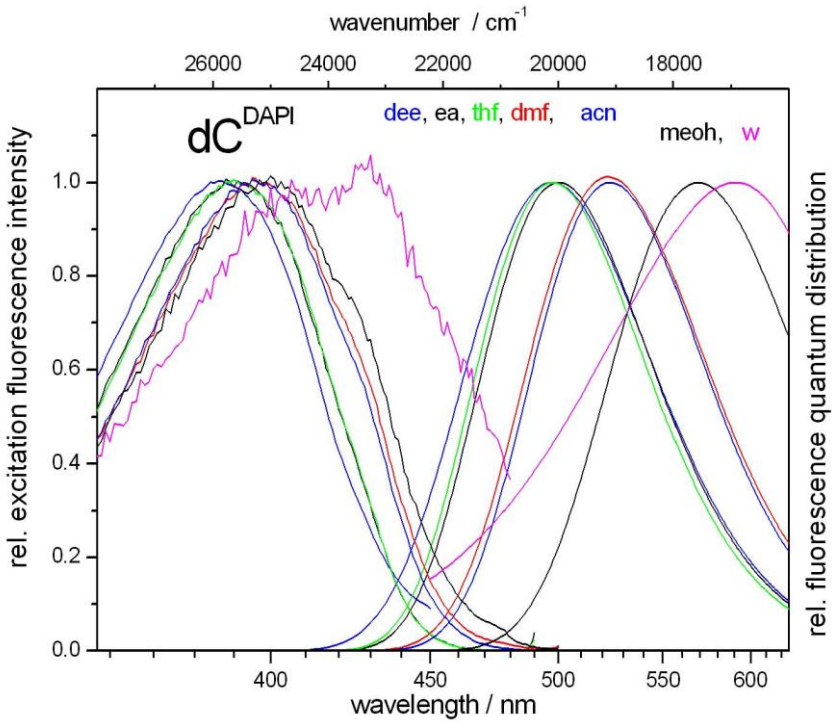



**Figure S3.** Excitation (left) and fluorescence spectra (right, quantum distribution over wavenumbers) of **dC$^{DAPI}$** (**7**) in several solvents (*dee* – diethyl ether, *ea* – ethyl acetate, *thf* – tetrahydrofurane, *acn* – acetonitrile, *dmf* – dimethyl formamide, *meoh* – methanol, *w* – water) upon excitation at 340 nm in all cases. Excitation at 380 nm was chosen for water solution.

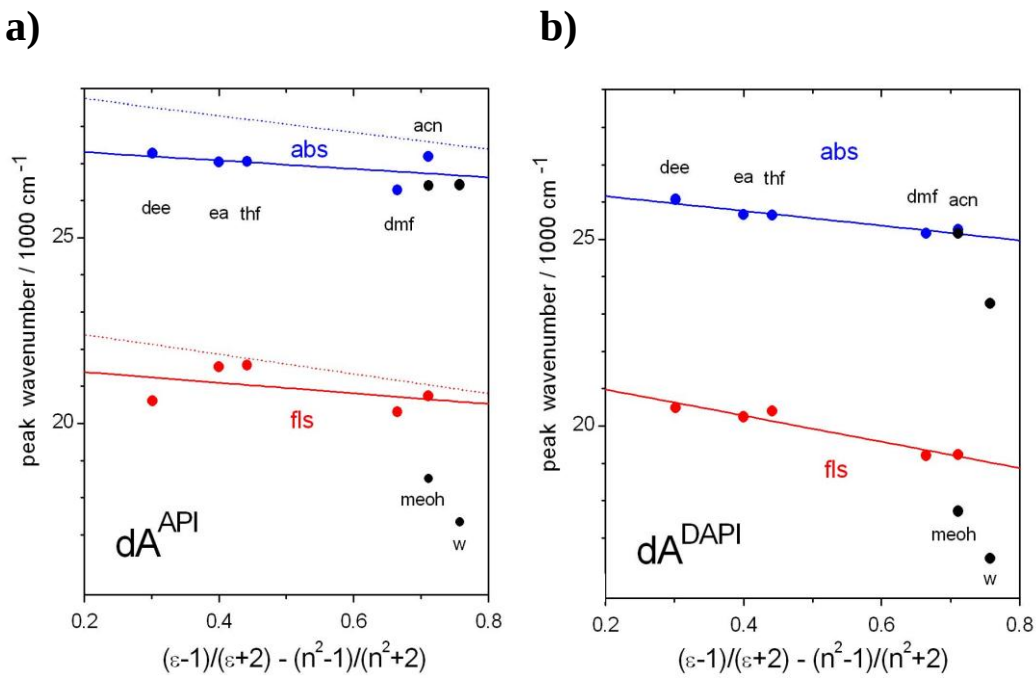



**Figure S4.** a) Sovatochromism of **dA$^{API}$** (**4**). Shown are the peak positions of the excitation (labelled abs) and emission (fls) bands of Figure S1 against a measure of solvent polarity. b) Sovatochromism of **dA$^{DAPI}$** (**6**). Shown are the peak positions of the excitation (labelled abs) and emission (fls) bands of Figure S2 against a measure of solvent polarity. Regression lines were calculated from the solvents *dee, ea, thf, dmf* and *acn*. Dashed lines represent the behavior of unsubstituted 4-amino-phthalimide.

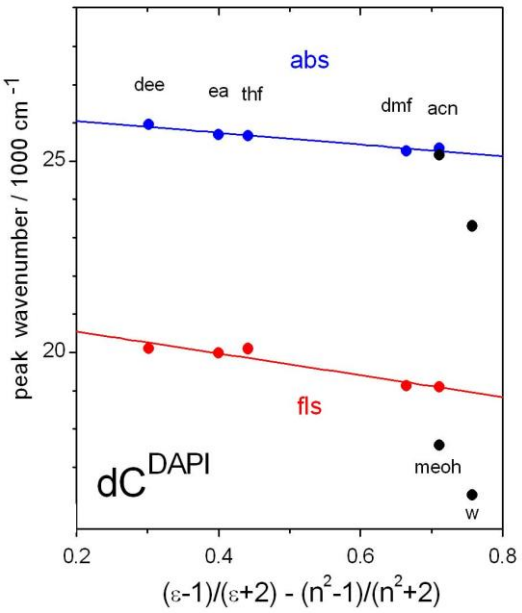



**Figure S5.** Sovatochromism of **dC$^{DAPI}$** (**7**). Shown are the peak positions of the excitation (labelled abs) and emission (fls) bands of Figure S4 against a measure of solvent polarity. Regression lines were calculated from the solvents *dee*, *ea*, *thf*, *dmf* and *acn*. Dashed lines represent the behavior of unsubstituted 4-amino-phthalimide.

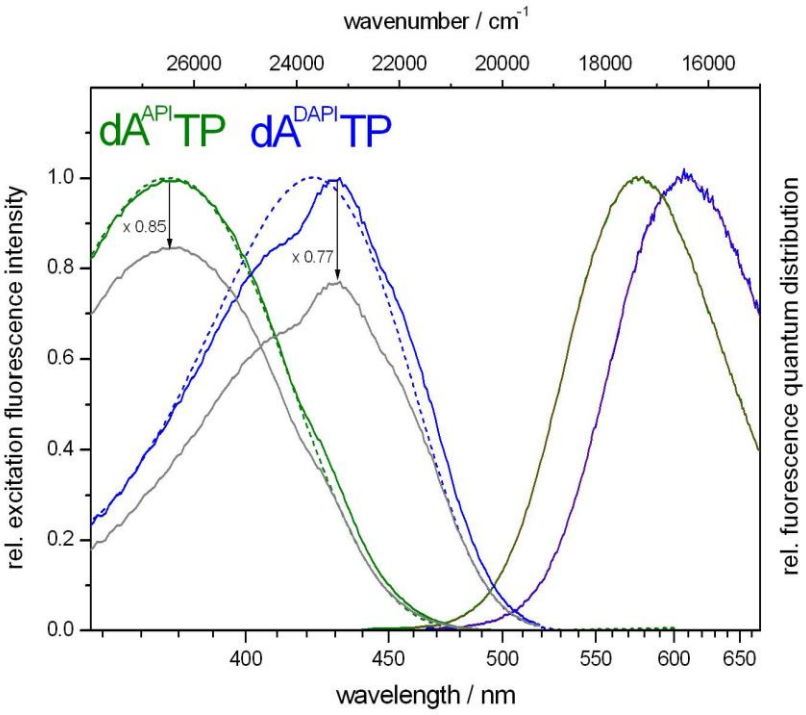



**Figure S6**. Comparison of fluorescence excitation (solid lines at left) and absorption bands (dashed) for the **dA$^{API}$TP** (**8**) and **dA$^{DAPI}$TP** (**10**) in water. Florescence excitation bands are structured and appear slightly red-shifted, compared to the unstructured absorption bands (see text).

**Study of p53 binding at 1 μM concentration**

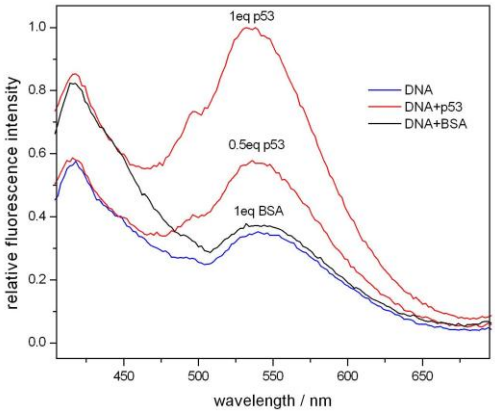



**Figure S7.** Increase of fluorescence intensity of 1 μM **dC**$^{\mathbf{API}}$-labeled DNA (pex$^{p53}$ **dC**$^{\mathbf{API}}$) upon p53 binding. The **API**-labeled DNA was titrated by 0.5 and 1 equivalent of p53 protein.

## 2. p53-DNA binding assay

Binding of p53 protein to **API**-modified 50-nt ds oligonucleotide substrate (pex$^{p53}$ prepared from temp$^{p53}$ template using **dC$^{API}$TP (9)**, see sequence in figure below: top strand is template temp$^{p53}$, primer stretch is denoted by *italics*, p53 recognition site is **bold**, cytosines bearing the **API** labels are red and extension of the primer by arrow) was checked by electrophoretic mobility shift assay (EMSA). The p53 protein was incubated with pex$^{p53}$ in 50 mM KCl, 5 mM Tris (pH 7.6), 2 mM DTT, 0.01% Triton-X100 for 30-min at 10 °C. The reaction mixture contained 20 ng of the $^{32}$P-labeled pex$^{p53}$ and 120 ng of p53 protein. The protein- pex$^{p53}$ complexes were detected by EMSA in 5% native polyacrylamide gel followed by autoradiography. Unmodified pex$^{p53}$ was prepared using mixture of all four natural dNTPs. As evident from the autoradiogram below, the p53 protein formed stable complexes with both unmodified and **API**-modified DNA substrates.

5'-GACGGTATCGATAAG**AGGCATGTCTAGGCATGTCT**CTTGATATCGAATTC-3'
3'-CTGCCATAGCTATTC**TCCGTACAGATCCGTACAGA***GAACTATAGCTTAAG*-5'

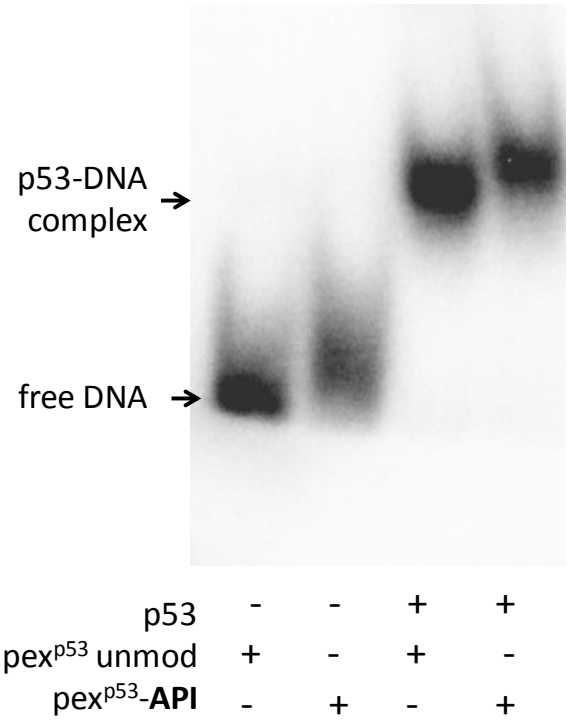



**Figure S8**. Electrophoretic mobility shift assay of the binding of p53 to unmodified and modified DNA.

## 3. Full emission spectrum

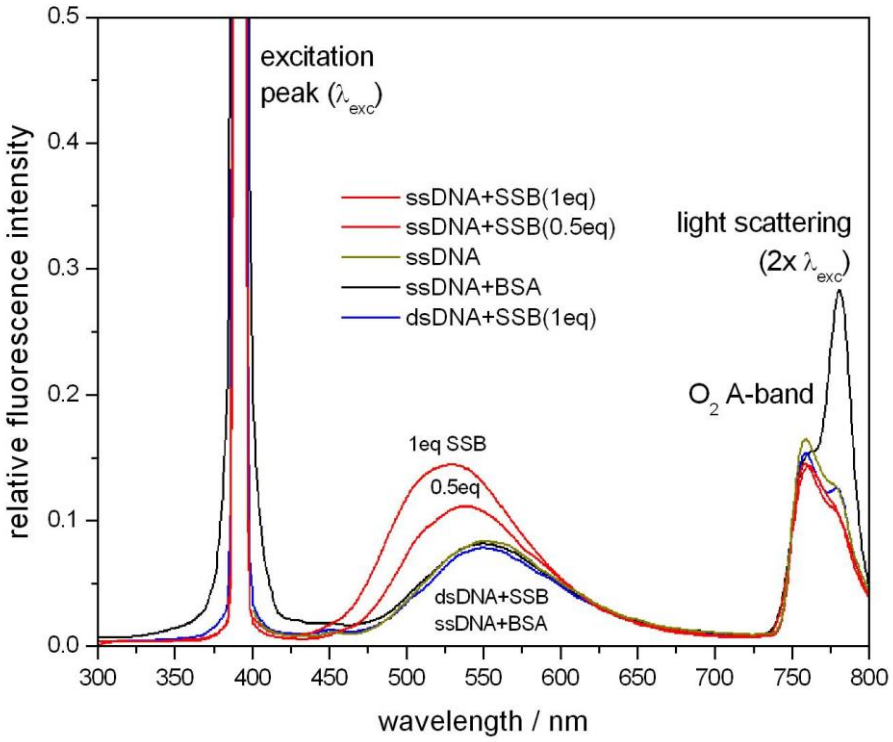
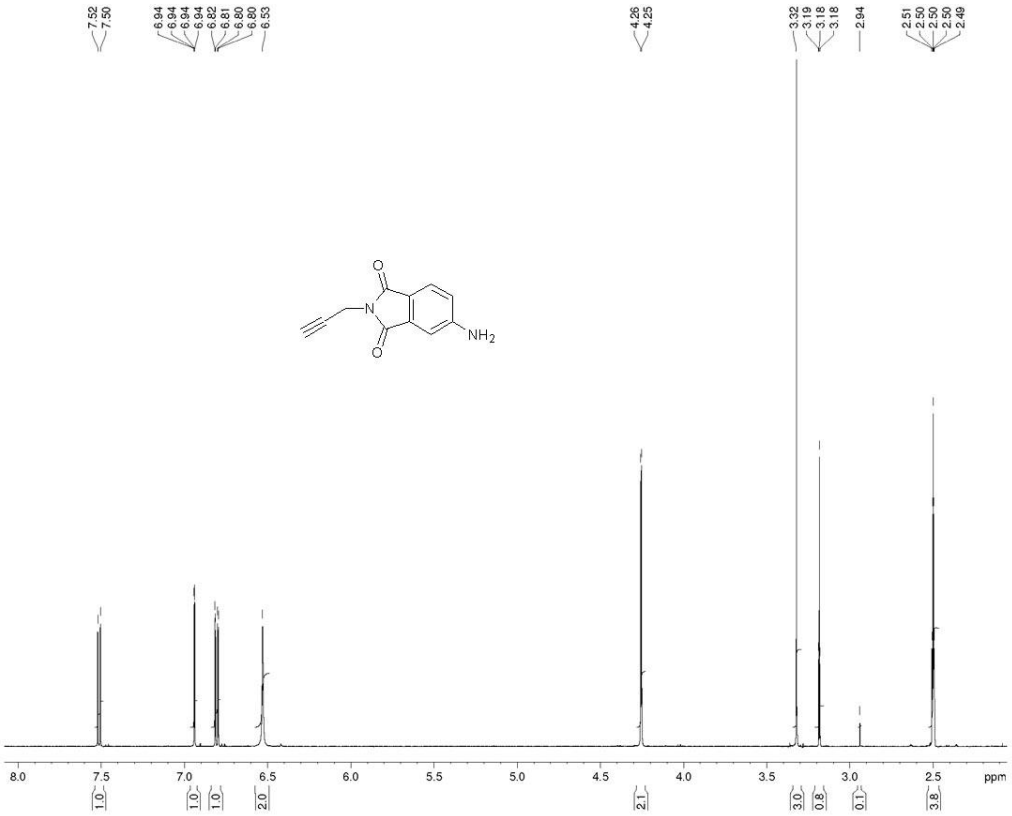
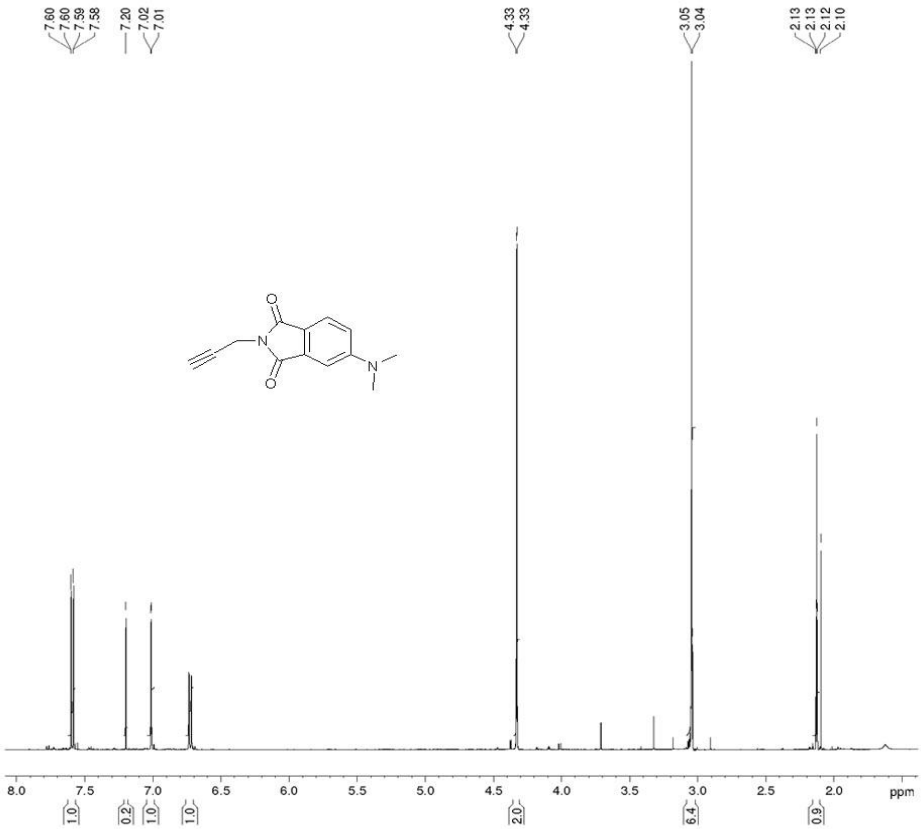



**Figure S9.** Full emission spectra of API during SSB-DNA binding study.

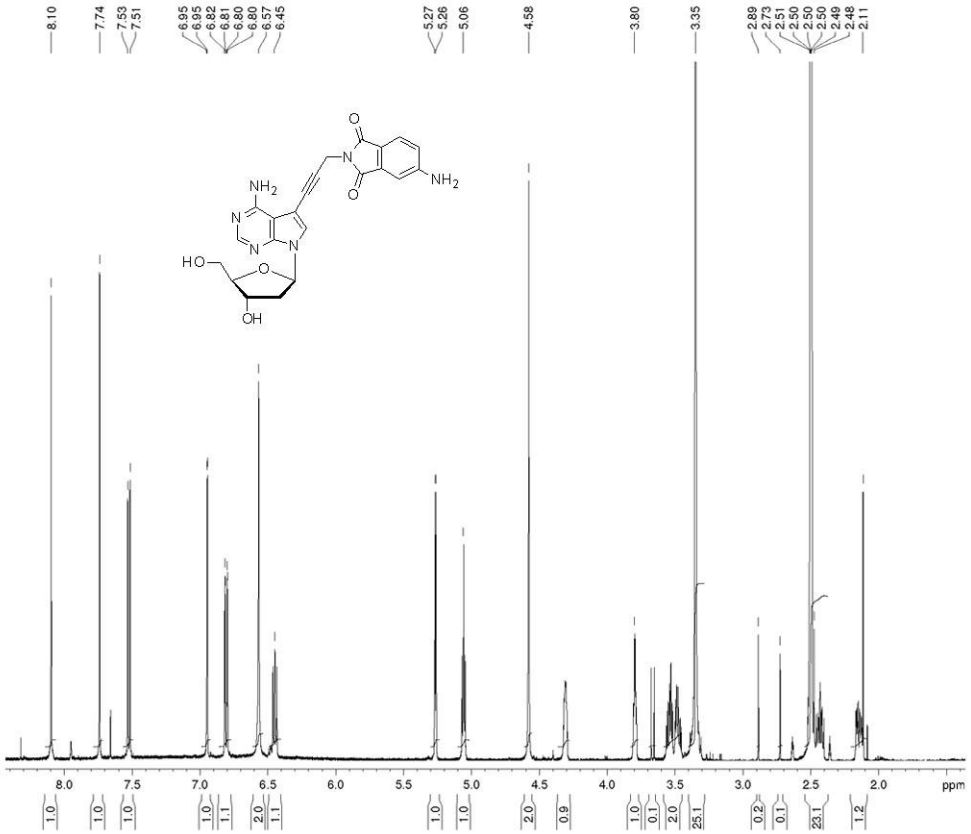
# 4. Copies of $^{1}H$, $^{31}P$-NMR spectra



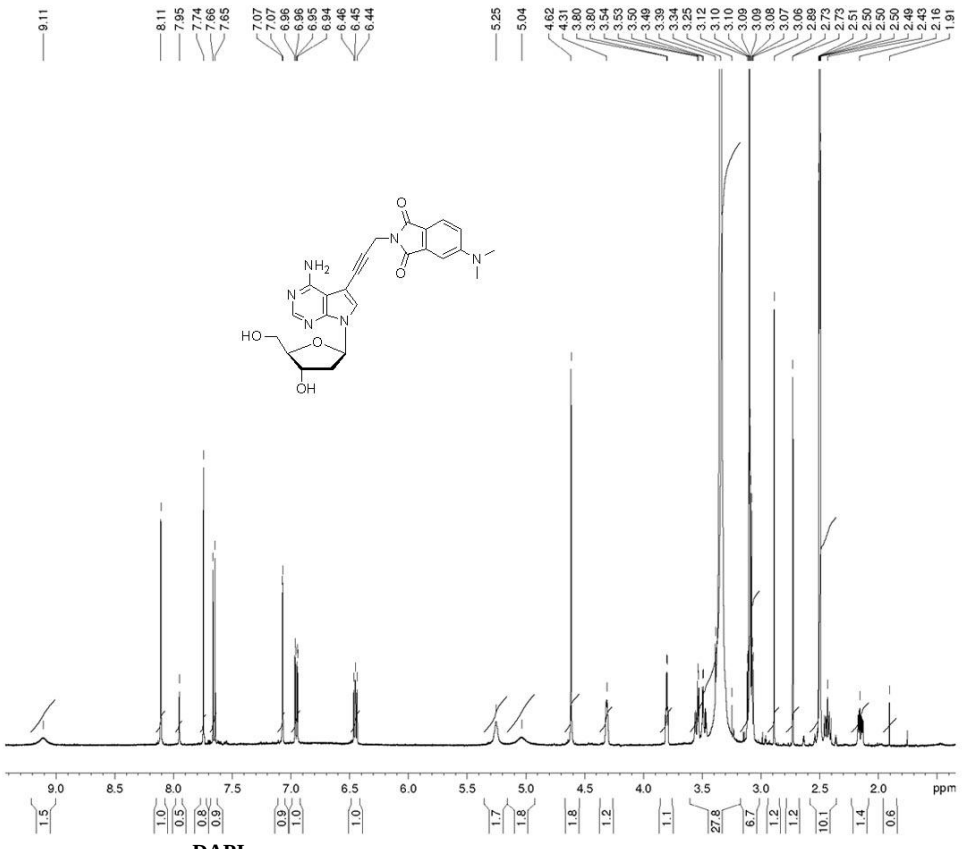

$^{1}H$ NMR spectrum of **P-API (2)**



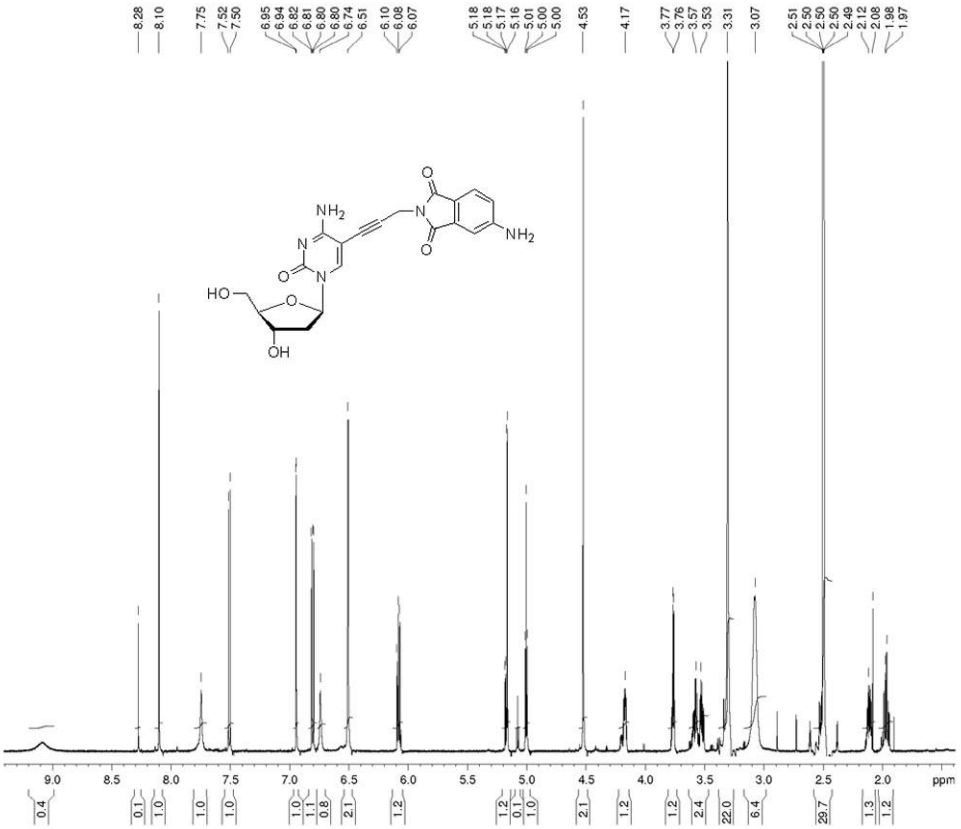

$^{1}H$ NMR spectrum of **P-DAPI (3)**



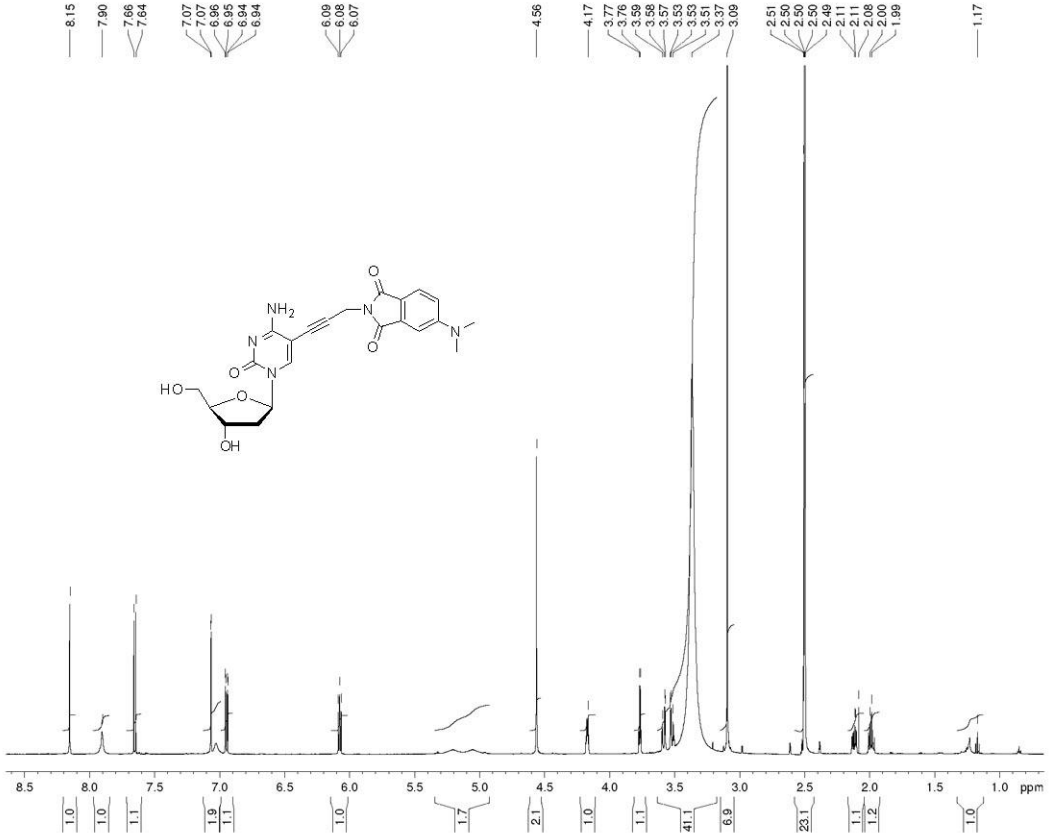
$^1$H NMR spectrum of **dA$^{API}$ (4)**



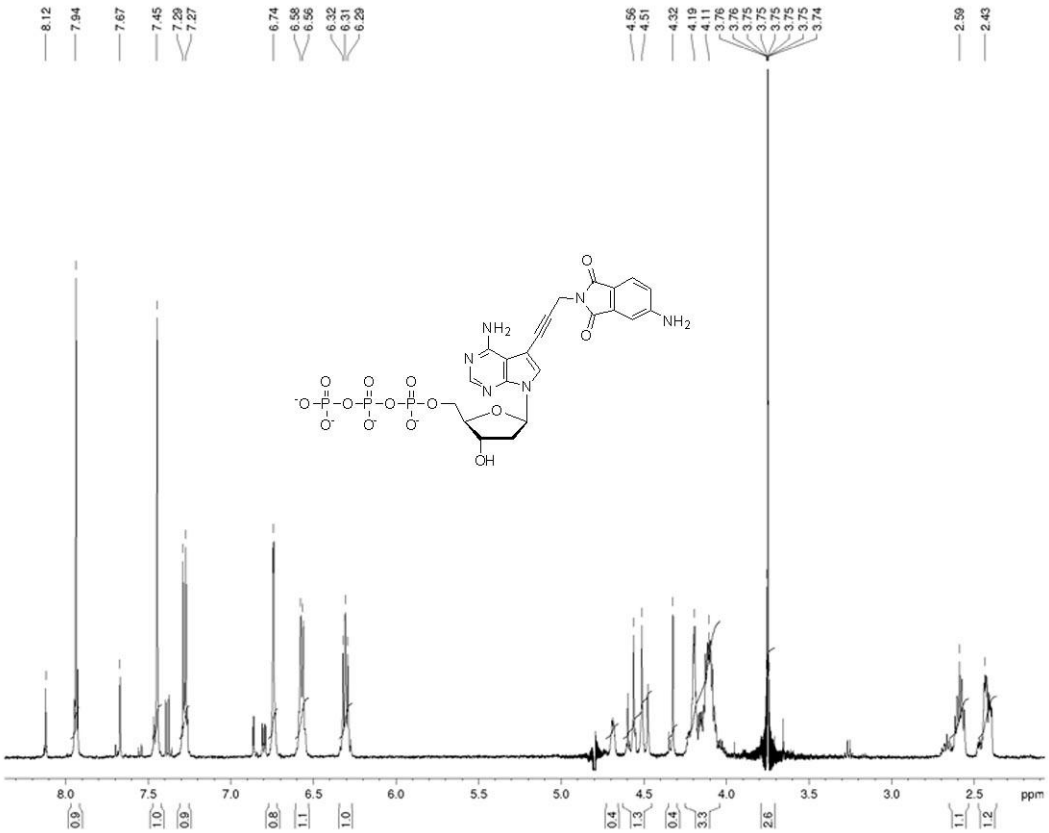
$^1$H NMR spectrum of **dA$^{DAPI}$ (6)**



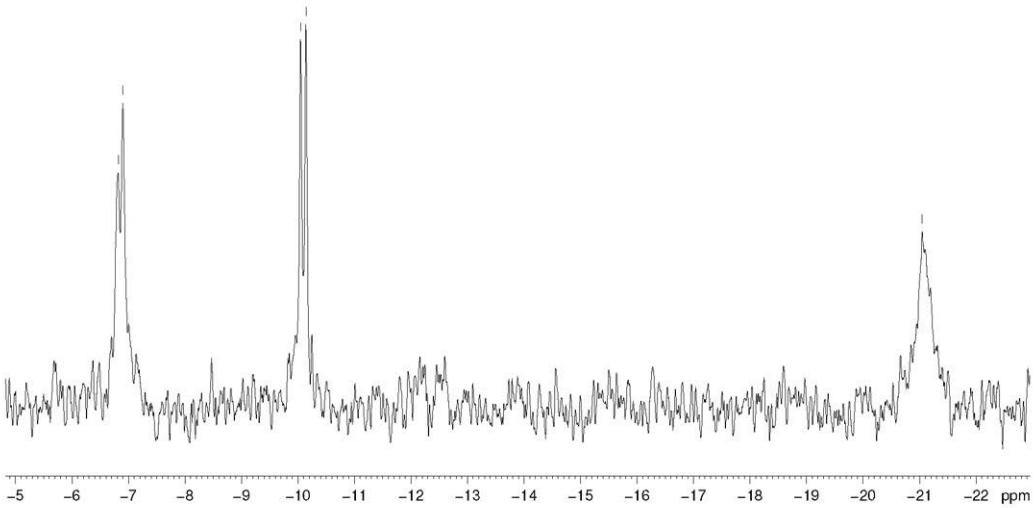

$^1$H NMR spectrum of **dC$^{API}$ (5)**



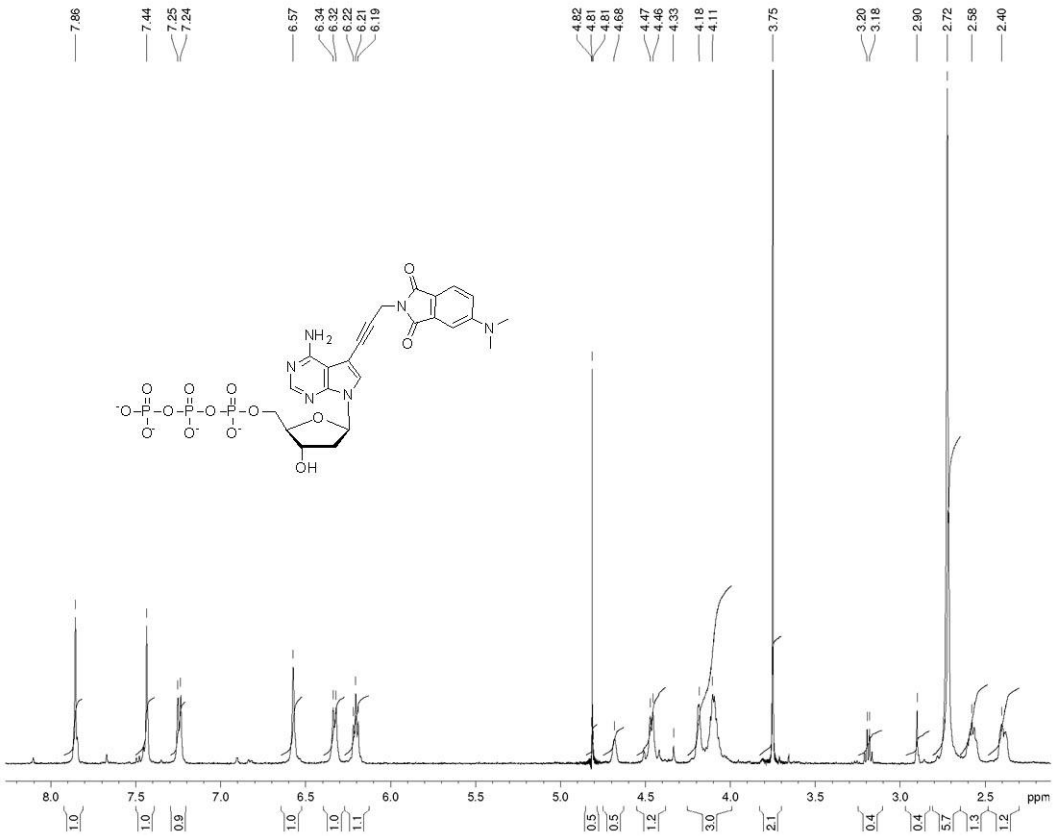

$^1$H NMR spectrum of **dC$^{DAPI}$ (7)**



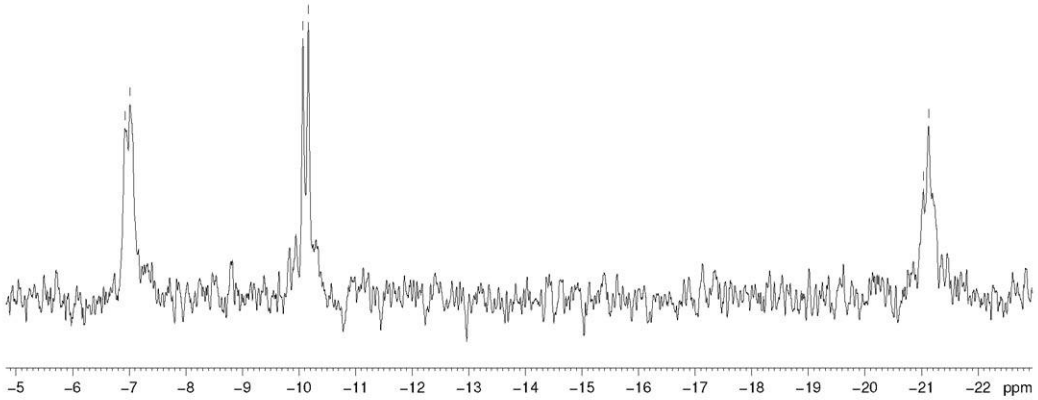

$^{1}$H NMR spectrum of **dA$^{\mathbf{API}}$TP (8)**



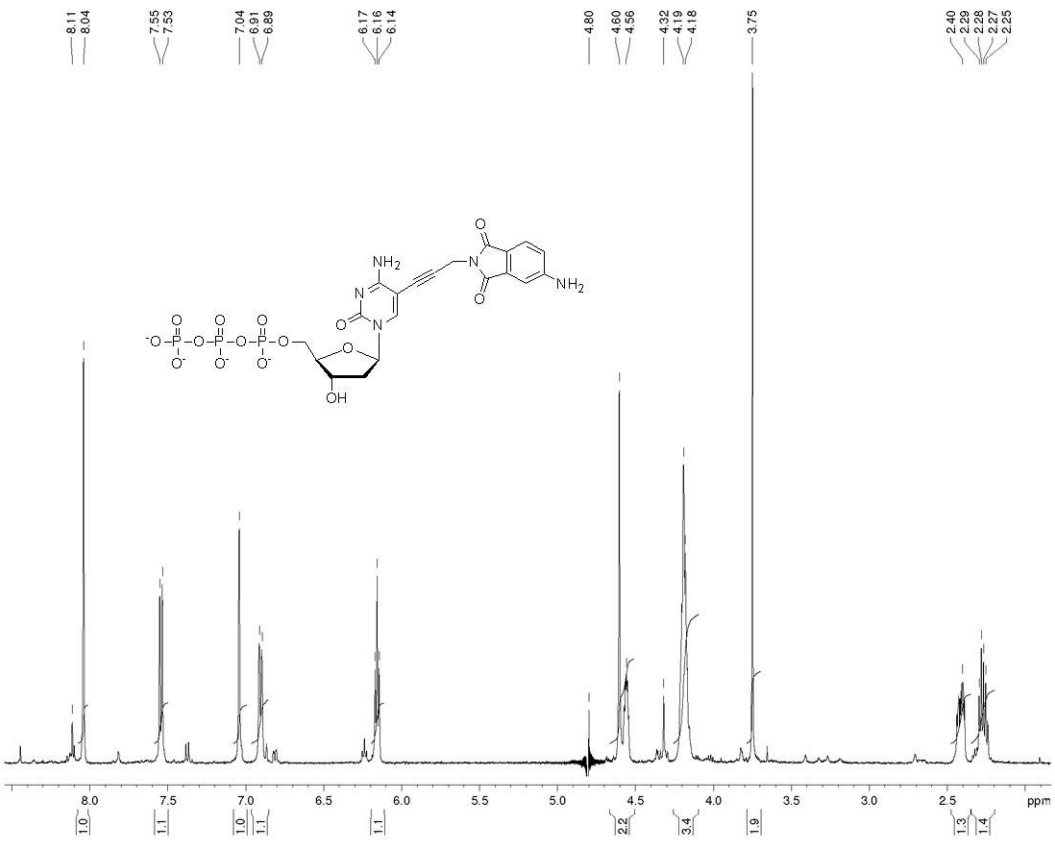

$^{31}$P NMR spectrum of **dA$^{\mathbf{API}}$TP (8)**



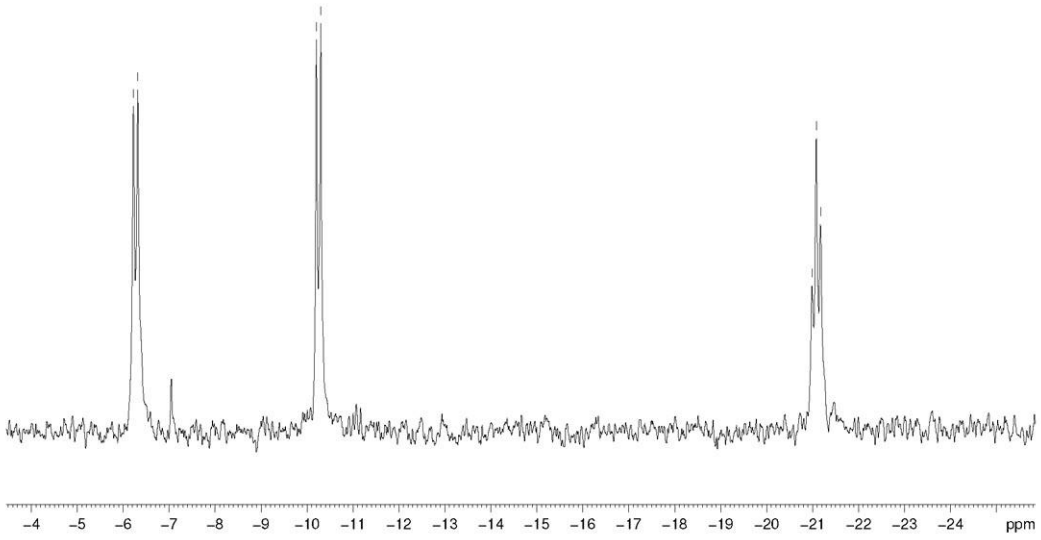

$^{1}$H NMR spectrum of **dA$^{DAPI}$TP (10)**



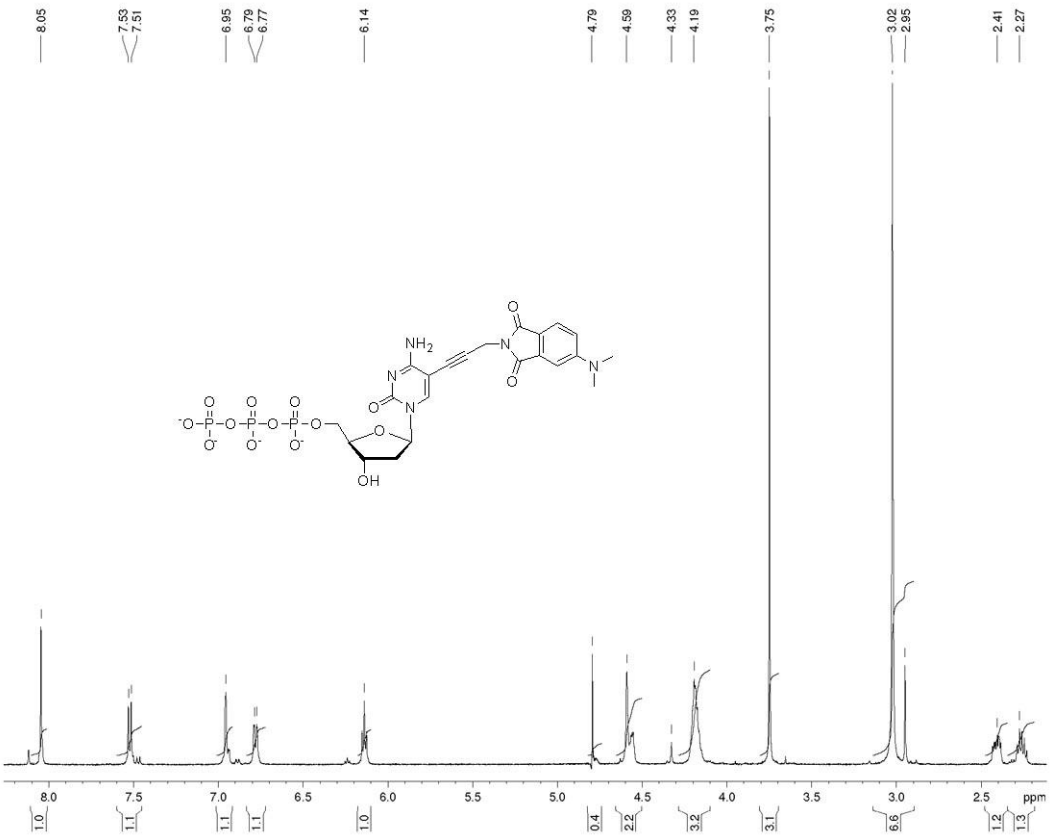

$^{31}$P NMR spectrum of **dA$^{DAPI}$TP (10)**



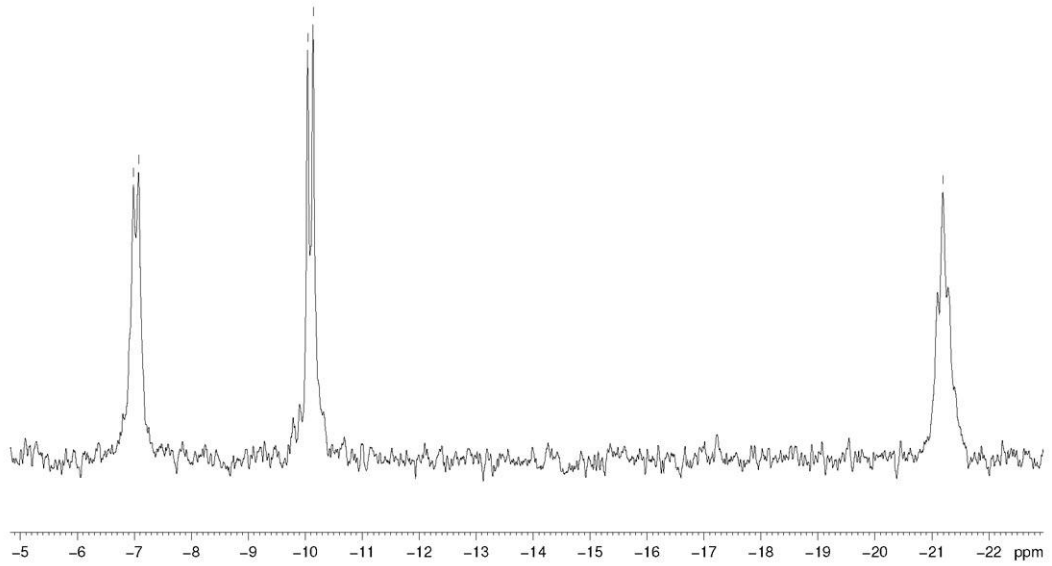

$^{1}$H NMR spectrum of **dC$^{API}$TP (9)**




$^{31}$P NMR spectrum of **dC$^{API}$TP (9)**

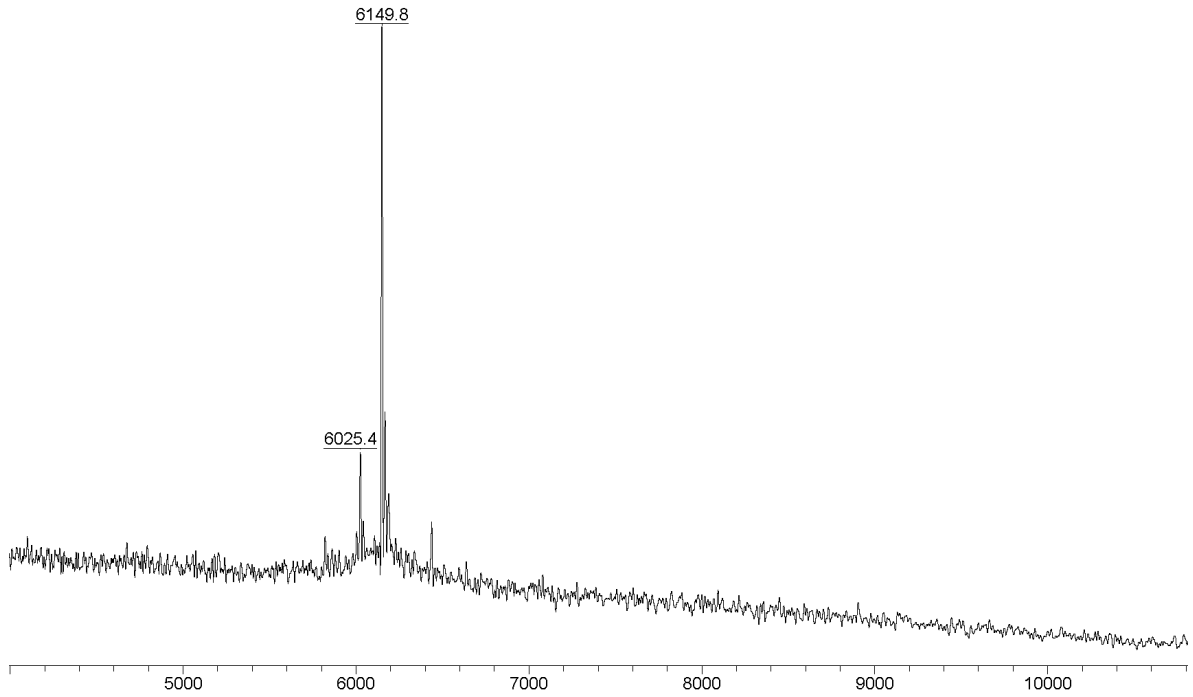



$^{1}$H NMR spectrum of **dC$^{DAPI}$TP (11)**

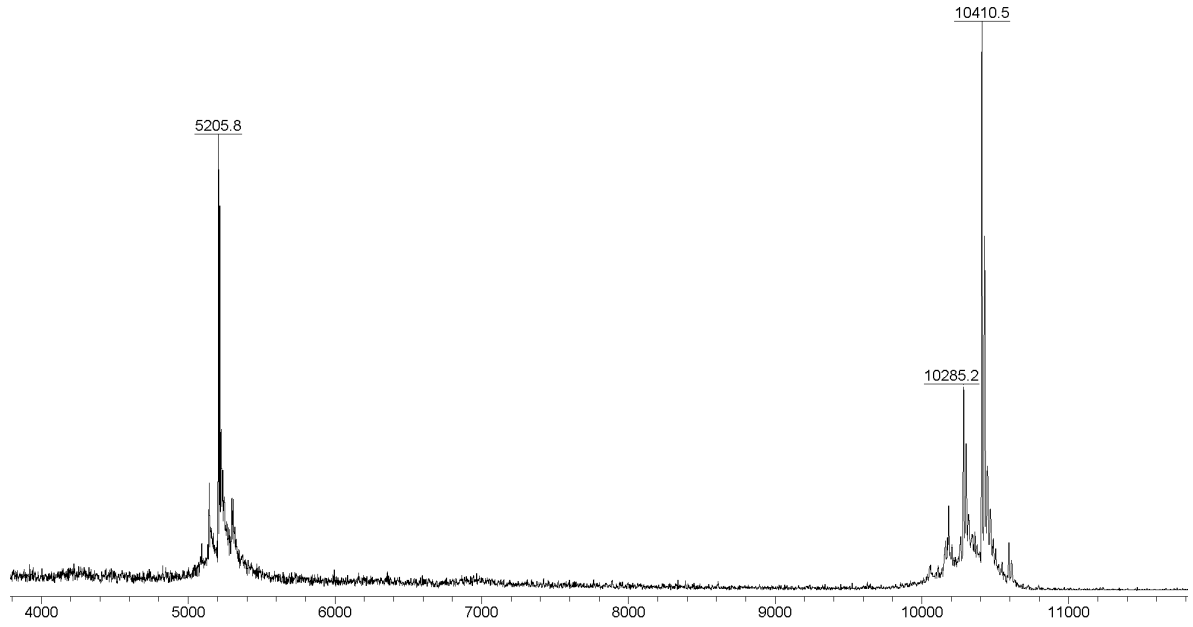



$^{31}$P NMR spectrum of **dC$^{DAPI}$TP (11)**

## 5. Copies of MALDI-TOF spectra




MALDI-TOF spectrum of pex$^{C}$(**dC$^{API}$**) oligonucleotide




MALDI-TOF spectrum of pex$^{rnd16}$(**dC$^{API}$**) oligonucleotide, peak at 5205.8 represents double charged ion-radical.

Both spectra contain fragmentation products which have lower molecular weight by 125 Da as result of single loss of uracil.