Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

C5-Pyrimidine-Functionalized Morpholino Oligonucleotides Exhibit Differential Binding Affinity, Target Specificity and Lipophilicity

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‡A.D. and A.G. contributed equally to this work. AD synthesized the modified-U PMOs and AG synthesized modified-C PMOs.

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Contents

1. Materials and Methods

1.1. General chemical methods

All reagents were purchased from commercially available sources and used without further purification, unless specified. Reactions were carried out in oven dried glassware under argon atmosphere. Solvents were purified and dried according to recommended procedures. Thin layer chromatography (TLC) was carried out on sheets of silica gel 60 F254 on aluminium (layer thickness 0.25 mm, Merck). Visualization of the TLC was achieved under UV light and staining of TLC was carried out in standard staining solutions (CAM, Ninhydrin, $KMnO_4$, 2,4-DNP). Chromatographic purification of products was accomplished by column chromatography on silica gel (mesh 60-120 and 100-200). ^{1}H, ^{13}C and ^{31}P-NMR spectra were recorded on Bruker NMR spectrometers (300 MHz, 400 MHz and 500 MHz). Chemical shifts (δ) are given in ppm relative to the solvent residual peak or TMS as internal standard. The following abbreviations are used for multiplicity of NMR signals: s = singlet, d = doublet, t = triplet, m = multiplet. High Resolution Mass Spectra (HRMS) were recorded out in a QTOF I (Quadrupole hexapole TOF) mass spectrometer with an orthogonal Z spray electrospray interface on Micro (YA 263) mass spectrometer (Manchester, UK). Matrix-Assisted Laser Desorption Ionization (MALDI) mass spectra were recorded on Bruker UltrafleXtreme MALDITOF/ TOF system. Sinapinic acid (SA) or 3-hydroxypocolinic acid (HPA) was used as a matrix. The LCMS separation was performed on the Agilent 1290 Infinity LC system which was coupled to Agilent 6545 Accurate-Mass Quadrupole Time-of-Flight (QTOF) with an Agilent jet stream Thermal Gradient Technology with electrospray ionization (ESI) source. The suitable MS parameters were optimized and high resolution mass spectra were obtained by performing the analysis in negative ionization mode. HPLC purification of the functionalized 12-mer morpholino oligonucleotides was done on Shimadzu SP-20AD system with C18 (Ascentis) column using 0.1M Ammonium acetate buffer (in H_2O)-CH_3CN gradient system (10-50%).

Synthesis of Morpholino-Oligomers

All PMOs were synthesized in Automated Oligosynthesizer, K&A H-8 DNA/RNA/LNA Synthesizer, K&A Laborgeräte GbR, Germany. Coupling with functionalized chlorophosphoramidate monomers were carried out manually with a coupling time of 1 h (20 min x 3 times).

DNA and RNA melting experiments and thermodynamic properties

All PMO-DNA and PMO-RNA UV-melting experiments were performed using 2 μM concentration of each strand and 0.04M phosphate buffer (pH 7). UV-Vis spectra were recorded on Cary 3500 UV-Visible spectrometer equipped with Peltier temperature controlling device and thermal software. All the samples were allowed to anneal at 65^{o}C for 5 minutes and then cooled slowly to 15^{o}C at a rate of 1^{o}C/min at 260 nm absorption.

CD-spectral experiments

Circular Dichroism experiment of all PMO-DNA and PMO-RNA was performed using 2 μM concentration of each strand and 0.04M phosphate buffer in a JASCO J-1500 Spectropolarimeter. All the samples were allowed to anneal at 65^{o}C for 5 minutes and then cooled slowly to 15^{o}C at a rate of 1^{o}C/min. Then all the duplexes were stored at 4^{o}C. All data collection were carried out at 10^{o}C

1.2. General synthesis methods

In case of compound **1**, Pd catalyzed Sonogashira cross coupling reactions were carried out using earlier reported procedure[1].
In case of compound **2**, Pd catalyzed Sonogashira, Suzuki, and Heck coupling reactions were carried out using earlier reported procedure[2].

General method for the Suzuki reaction of morpholino uridine:

Compound **1** (0.1 mmol) was dissolved in 3ml of dioxane followed by the addition of boronic acid (0.2 mmol). After that NaOH (0.3 mmol, 1.5M in water) was added drop wise in the reaction mixture and 1 ml of water was added to maintain the ratio. Finally $Pd(PPh_3)_4$ (4-5 mol%) was added in the reaction mixture and heated at 65-70^{o}C (oil bath) for 4-5 hrs. The reaction was monitored by TLC. After completion of the reaction the solvent was evaporated to dryness and extracted with EtOAc. Then solvent was washed repeatedly with water and with brine. Collected organic layer was dried over anhydrous Na_2SO_4 and solvent was evaporated in vacuo. The compound was purified by silica gel (100-200 mesh) column chromatography using MeOH-DCM as eluent. Trace amount of catalyst impurity was further removed by second time column chromatography eluting with EtOAc-petroleum ether (if necessary).

Entry	**Solvent**	**Base(Eq)**	**Catalyst(Eq)**	**Temperature**	**Result**
1	Dry DMF	Et_3N (4)	$Pd(PPh_3)_4$ (0.1)	70^0C	In all cases starting materials were present along with other spots
2		Cs_2CO_3 (4)	$Pd(PPh_3)_4$(0.1)	70^0C	
3		Cs_2CO_3 (4)	$Pd(OAc)_2/(O\text{-tolyl})_3P$ (0.05/.1)	80^0C	
4	Dry Dioxane	KO^tBu (4)	$Pd(PPh_3)_4$ (0.1)	90^0C	
5	Distilled Dioxane	DABCO (4)	$Pd(dba)_2$ (0.1)	90^0C	
6		NaOH (4)			
7		K_3PO_4 (4)			
8		DABCO (4)	$Pd(PPh_3)_4$ (0.1)		
9		NaOH (4)			
10		K_3PO_4 (4)			
11	Dioxane water in 3:1 ratio	DABCO (4)	$Pd(PPh_3)_4$ (0.1)	80^0C	Desired product was obtained in 58% yield
12		DABCO (4)	$Pd(PPh_3)_4$ (0.1)	80^0C/3hrs	58%
13		K_2CO_3 (3)		65^0C/10hrs	82%
14		Et_3N (12)		65^0C	Starting materials was mainly present
15		**NaOH (3)**	$Pd(PPh_3)_4$ (0.1)	65^0C/30mins	61%
16			$Pd(PPh_3)_4$ (0.07)	65^0C/30mins	70%
17			**$Pd(PPh_3)_4$ (0.05)**	**65^0C/1.5hrs**	**89%**
18			$Pd(PPh_3)_4$ (0.01)	65^0C/7 hrs	82%
19			$Pd(PPh_3)_2Cl_2$(0.05)	65^0C/1hr	74%
20			$Pd(dppf)Cl_2.CH_2Cl_2$ (0.05)	65^0C/30mins	73%
21			$Pd(dba)_2$(0.05)	65^0C/1hr	67%

Table S1: various conditions for Suzuki cross-coupling reaction

Synthetic procedure for m5C morpholino cytidine (Compound 2c)

Compound **2a** (2g, 2.7 mmol) was dissolved in dry DCM/CH_3CN (1:1) under Ar atmosphere followed by the addition of dry Et_3N (15 equiv, 40.5 mmol). Then the reaction mixture was cooled to 0^oC in an ice bath. Freshly distilled $POCl_3$ (3 equiv, 8.1 equiv) was added to the reaction mixture in a drop wise manner. After that the reaction mixture was stirred for 5 min at 0^oC. Then 1,2,4 triazole (10 equiv, 27 mmol) was added to the reaction mixture portion wise. After that it was stirred at 0^oC for 30 min more and left for overnight at room temperature. After complete consumption of the starting material (TLC analysis), the solvent was evaporated to dryness and diluted with EtOAc. The organic layer was then washed with saturated $NaHCO_3$ (4 times), then with water (2 times) and finally with saturated NH_4Cl (2 times). Collected organic layer was dried on anhydrous Na_2SO_4 and solvent was evaporated *in vacuo*. The crude was then used for the next step without further purification.

The obtained crude product was then transferred to a sealed tube dissolved in THF (15 ml) followed by the addition of 33% aqueous NH_3 (15 ml). After that the reaction mixture was stirred for 3 hrs. On complete consumption of the starting material the reaction mixture was evaporated to dryness. The reaction mixture was then diluted with water and extracted by DCM (5 times). Collected organic layer was dried on anhydrous Na_2SO_4 and solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (60-120 mesh) to obtain the compound **2c** in 83% yield. ($R_f = 0.5$ in 5% MeOH-DCM)

General method for the benzoylation of functionalized morpholino cytidines 2, 2c and 3a-j.

Cytidine was dissolved in dry CH_3CN followed by addition of phenyl(1H-tetrazol-1-yl)methanone (2 equiv) and then DMAP (1 equiv) under Ar-atmosphere. The reaction mixture was then allowed to stir at 65-70^oC for 1.5 hrs under Ar. After completion of the reaction (TLC analysis) the solvent was evaporated to dryness and re-dissolved in EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution and then water and brine. Collected organic layer was dried on anhydrous Na_2SO_4 and solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (100-200 mesh) to obtain the compounds **4a-l**. EtOAc-Hexane was used as eluent.

General method for the Silyl Deprotection of functionalized morpholino cytidine

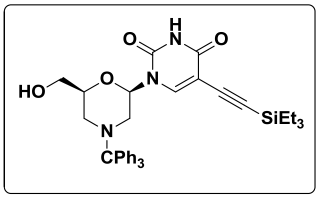
Compounds **4a-l** was dissolved in freshly dried THF followed by the addition of tetrabutylammonium fluoride solution (1.5 equiv, 1M in THF) under Ar atmosphere in ice cold condition. Then the reaction mixture was allowed to stir at room temperature for 4-5 hrs. After completion of the reaction (TLC analysis), the solvent was evaporated *in vacuo* and re-dissolved in EtOAc. The organic layer was washed with water for 5-6 times and finally with saturated NH_4Cl. The collected organic layer was dried on anhydrous Na_2SO_4 and solvent was evaporated *in vacuo*. The crude product was purified by silica gel (60-120 mesh) column chromatography eluting with Acetone-DCM to obtain the compounds **5a-l**,.

General method for dimer formation reaction

Compound **8** (1 equiv) was dissolved in dry DMF, cooled to 0^oC followed by the addition of *N*-ethyl morpholine (3 equiv) and ETT (2 equiv). Then the active monomer (1.5 equiv) was added in the reaction medium. The reaction was then left for 45 mins to 1 hr at room temperature. After completion (as analyzed by TLC), the reaction mixture was diluted with ethyl acetate and washed with water and brine. Collected organic layer was dried over anhydrous Na_2SO_4 and dried *in vacuo*. The crude mixture was purified by column chromatography and characterized by spectroscopic techniques.

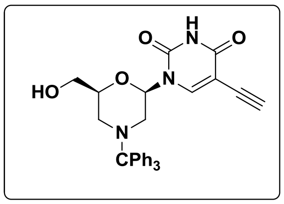
2. Spectral Data

Compound **1a**



^{1}H –NMR (300 MHz, CDCl$_3$): δ 8.48 (s, 1H), 7.52 – 7.16 (m, 16H), 6.13 (dd, J = 9.5, 2.3 Hz, 1H), 4.33 – 4.20 (m, 1H), 3.62 (dd, J = 5.1, 1.9 Hz, 2H), 3.38 (dd, J = 11.3, 2.5 Hz, 1H), 3.15 – 3.01 (m, 1H), 1.45 (ddd, J = 17.5, 11.6, 10.1 Hz, 2H), 1.04 – 0.93 (m, 9H), 0.64 (qd, J = 7.8, 0.9 Hz, 6H).
^{13}C –NMR (75 MHz, CDCl$_3$): δ 160.8, 148.7, 142.5, 129.1, 127.9, 126.5, 100.3, 97.6, 96.1, 81.1, 77.9, 77.4, 77.2, 77.0, 76.7, 76.5, 63.5, 52.1, 48.7, 7.4, 4.2.
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{36}H_{41}N_3NaO_4Si$ 630.2764, found 630.2764.

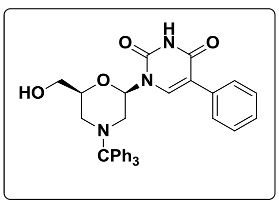
Compound **1b**



^{1}H-NMR (300 MHz, CDCl$_3$): δ 8.53 (s,1H), 7.53 (s,1H), 7.47-7.17 (m, 15H), 6.13 (dd, J = 9.6, 2.2 Hz), 3.61-3.59 (m, 2H), 3.41 (d, J = 12 Hz), 3.14 (s,1H), 3.13-3.09 (d, J = 12 Hz, 1H), 1.92 (s, 1H), 1.50-1.36 (m, 2H)
^{13}C –NMR (75 MHz, CDCl$_3$): δ 160.9, 148.5, 143.6, 129.3, 128.9, 128.1, 127.9, 127.0, 126.8, 99.1, 82.3, 81.2, 78.1, 77.4, 77.3, 77.1, 77.0, 76.8, 74.5, 63.7, 52.4, 48.9
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{30}H_{27}N_3NaO_4$ 516.1899, found 516.1897

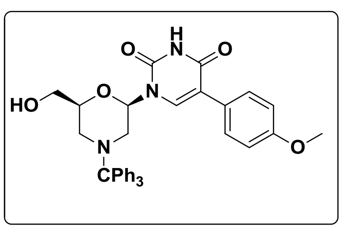
Compound **1c Reported previously**[1]

Compound **1d**



^{1}H-NMR (300 MHz, CDCl$_3$): δ 8.25 (s, 1H), 7.65 – 7.20 (m, 21H), 6.24 (dd, J = 9.7, 2.4 Hz, 1H), 4.38 – 4.24 (m, 1H), 3.60 (dt, J = 8.2, 4.5 Hz, 2H), 3.48 – 3.39 (m, 1H), 3.14 – 3.01 (m, 1H), 1.83 (d, J = 6.7 Hz, 1H), 1.52-1.41 (m, 2H).
^{13}C –NMR (100 MHz, CDCl$_3$): δ 161.5, 149.2, 136.9, 132.3, 132.2, 132.0, 132.0, 129.4, 129.4, 129.3, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 126.7, 115.4, 81.0, 78.0, 77.6, 77.4, 77.3, 77.1, 76.9, 76.8, 76.5, 76.3, 63.8, 52.3, 48.9
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{34}H_{31}N_3NaO_4$ 568.2212, found 568.2211.

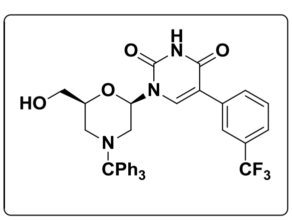
Compound **1e**



^{1}H –NMR (300 MHz, CDCl$_3$): δ 8.63 (s, 1H), 7.62 – 7.12 (m, 20H), 6.84 (d, J = 8.8 Hz, 2H), 6.24 (dd, J = 9.6, 2.4 Hz, 1H), 4.31 (ddd, J = 6.4, 4.4, 2.2 Hz, 1H), 3.78 (s, 3H), 3.59 (d, J = 6.2 Hz, 2H), 3.42 (d, J = 11.3 Hz, 1H), 3.10 (d, J = 11.8 Hz, 1H), 2.25 (s, 1H), 1.52-1.40 (m, 2H).
^{13}C –NMR (100 MHz, CDCl$_3$): δ 161.9, 159.6, 149.3, 136.0, 132.3, 132.2, 129.5, 129.3, 128.7, 128.5, 128.0, 126.7, 124.5, 115.1, 114.0, 80.9, 77.9, 77.4, 77.1, 76.8, 63.7, 55.4, 52.3, 48.9

HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{35}H_{33}N_3NaO_5$ 598.2318, found 598.2316 .

Compound **1f**

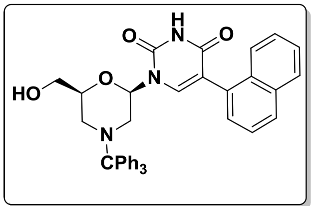


^{1}H –NMR (300 MHz, CDCl$_3$): δ 8.86 (s, 1H), 7.59 – 7.22 (m, 20H), 6.26 (dd, J = 9.7, 2.4 Hz, 1H), 4.40 – 4.25 (m, 1H), 3.62 (d, J = 5.2 Hz, 2H), 3.45 (d, J = 11.3 Hz, 1H), 3.12 (d, J = 11.9 Hz, 1H), 2.18 (s, 1H), 1.48 (dt, J = 11.9, 9.9 Hz, 2H).

^{13}C –NMR (100 MHz, $CDCl_3$): δ 161.4, 149.1, 137.6, 132.9, 132.3, 132.2, 132.1, 132.0, 131.7, 131.4, 131.1, 130.8, 130.4, 129.3, 129.0, 128.7, 128.6, 128.1, 126.7, 125.1, 125.0, 124.8, 124.8, 122.6, 114.1, 81.0, 78.1, 77.4, 77.3, 77.1, 77.0, 76.8, 63.7, 52.3, 48.8

HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{35}H_{30}F_3N_3NaO_4$ 636.2086, found 636.2087

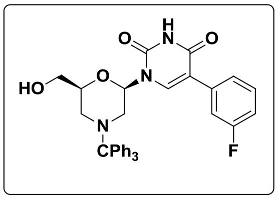
Compound **1g**



^{1}H –NMR (300MHz, $CDCl_3$): δ 8.73 (s, 1H), 7.85 (d, *J* = 7.3 Hz, 2H), 7.58 – 7.09 (m, 22H), 6.29 (dd, *J* = 9.6, 2.3 Hz, 1H), 4.36 – 4.23 (m, 1H), 3.63 – 3.41 (m, 3H), 3.07 (d, *J* = 11.9 Hz, 1H), 1.92 (s, 1H), 1.54 – 1.33 (m, 2H).
^{13}C –NMR (100 MHz, $CDCl_3$): δ 161.9, 149.6, 139.1, 133.8, 132.2, 129.3, 128.6, 128.4, 128.0, 126.7, 126.5, 126.1, 125.3, 125.2, 114.5, 80.9, 77.9, 77.4, 77.3, 77.1, 76.9, 76.8, 63.7, 52.3, 48.9.

HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{38}H_{33}N_3NaO_4$ 618.2369, found 618.2366

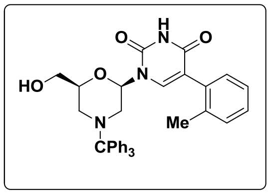
Compound **1h**



^{1}H –NMR (300 MHz, $CDCl_3$): δ 9.13 (d, *J* = 16.7 Hz, 1H), 7.59 – 7.09 (m, 20H), 6.98-6.92 (m,1H), 6.25 (dd, *J* = 9.6, 2.4 Hz, 1H), 4.35-4.31 (m,1H), 3.61 (d, *J* = 5.4 Hz, 2H), 3.49 – 3.38 (m, 1H), 3.11 (d, *J* = 11.9 Hz, 1H), 2.44 (s, 1H), 1.51-1.43 (m, 2H).
^{13}C –NMR (100 MHz, $CDCl_3$): δ 164.3, 161.6, 161.0, 149.2, 137.4, 134.2, 134.0, 132.2, 132.1, 130.0, 129.9, 129.4, 129.2, 128.7, 128.6, 128.0, 126.7, 123.8, 123.7, 115.5, 115.2, 115.1, 114.8, 114.1, 81.0, 78.0, 77.5, 77.3, 77.1, 76.9, 76.7, 63.6, 52.3, 48.8

HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{34}H_{30}FN_3NaO_4$ 586.2118, found 586.2119

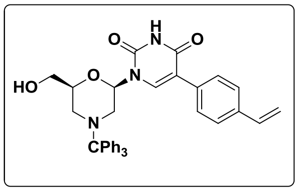
Compound **1i**



^{1}H –NMR (300 MHz, $CDCl_3$):) δ 9.07 (s, 1H), 7.56 – 7.07 (m, 20H), 6.26 (dd, *J* = 9.7, 2.4 Hz, 1H), 4.28 (dq, *J* = 6.4, 2.2 Hz, 1H), 3.54 (t, *J* = 5.1 Hz, 2H), 3.43 (dt, *J* = 11.3, 2.4 Hz, 1H), 3.09 (dt, *J* = 12.0, 2.3 Hz, 1H), 2.16 (s, 3H), 1.85 (s, 1H), 1.49-1.36 (m,2H).
^{13}C –NMR (100 MHz, $CDCl_3$): δ 161.7, 149.7, 138.2, 137.7, 132.2, 132.1, 131.7, 130.6, 130.4, 129.2, 128.7, 128.6, 128.5, 128.0, 126.7, 125.9, 115.7, 80.6, 77.7, 77.4, 77.3, 77.1, 76.9, 76.8, 63.6, 52.3, 48.9, 20.1

HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{35}H_{33}N_3NaO_4$ 582.2369, found 582.2365.

Compound **1j**

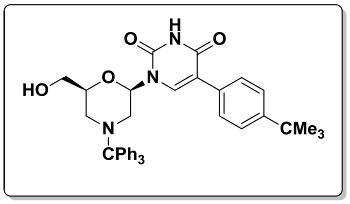


^{1}H –NMR (300 MHz, $CDCl_3$): δ 9.01 (s, 1H), 7.52 – 7.18 (m, 20H), 6.67 (dd, *J* = 17.6, 10.9 Hz, 1H), 6.25 (dd, *J* = 9.6, 2.3 Hz, 1H), 5.72 (dd, *J* = 17.6, 0.9 Hz, 1H), 5.24 (dd, *J* = 10.8, 0.9 Hz, 1H), 4.32 (ddd, *J* = 8.5, 4.4, 2.1 Hz, 1H), 3.59 (d, *J* = 5.7 Hz, 2H), 3.43 (d, *J* = 11.3 Hz, 1H), 3.10 (d, *J* = 11.9 Hz, 1H), 2.47 (s, 1H), 1.51-1.40 (m, 2H).

^{13}C –NMR (100 MHz, $CDCl_3$): δ 161.8, 149.3, 137.4, 136.8, 136.3, 132.2, 132.1, 132.1, 131.5, 129.3, 128.7, 128.5, 128.3, 128.0, 126.7, 126.3, 115.0, 114.4, 80.9, 78.0, 77.4, 77.3, 77.1, 76.9, 76.8, 63.7, 52.3, 48.9

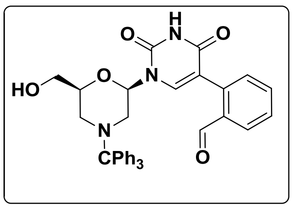
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{36}H_{33}N_3NaO_4$ 594.2369, found 594.2367

Compound **1k**



^{1}H –NMR (300 MHz, CDCl$_3$): δ 8.96 (s, 1H), 7.59 – 7.12 (m, 21H), 6.27 (dd, J = 9.6, 2.3 Hz, 1H), 4.40 – 4.27 (m, 1H), 3.58 (dt, J = 7.4, 3.9 Hz, 2H), 3.44 (dt, J = 11.4, 2.4 Hz, 1H), 3.10 (dt, J = 12.0, 2.3 Hz, 1H), 2.31 (t, J = 5.9 Hz, 1H), 1.55 – 1.36 (m, 2H), 1.30 (s, 9H).
^{13}C –NMR (100 MHz, CDCl$_3$): δ 162.0, 151.2, 149.4, 136.7, 129.2, 128.0, 126.7, 125.5, 115.3, 80.9, 77.9, 77.4, 77.1, 77.0, 76.9, 63.7, 52.3, 48.9, 34.6, 31.3.
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{38}H_{39}N_3NaO_4$ 624.2838, found 624.2839

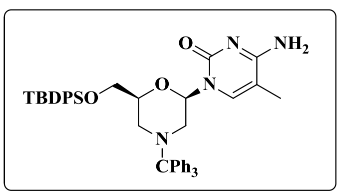
Compound **1l**



^{1}H –NMR (300 MHz,CDCl$_3$): δ 9.88 (s, 1H), 8.78 (s, 1H), 7.89 (d, J = 7.4 Hz, 1H), 7.58 – 7.18 (m, 19H), 6.25 (dd, J = 9.6, 2.4 Hz, 1H), 4.31 (dd, J = 6.9, 3.7 Hz, 1H), 3.68 – 3.51 (m, 2H), 3.47 (d, J = 11.5 Hz, 1H), 3.08 (d, J = 11.9 Hz, 1H), 2.15 – 2.00 (m, 1H), 1.53 – 1.35 (m, 2H).
^{13}C–NMR (100 MHz,CDCl$_3$): δ 191.2, 161.8, 161.7, 149.4, 138.5, 135.1, 133.8, 131.5, 130.3, 129.3, 129.1, 128.1, 126.7, 113.3, 81.0, 77.9, 77.4, 77.3, 77.1, 77.0, 76.8, 63.7, 52.3, 48.9

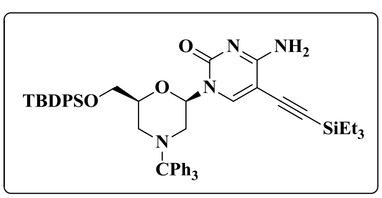
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{35}H_{31}N_3NaO_5$ 596.2161, found 596.2163

Compound **2c**



^{1}H –NMR (300 MHz,CDCl$_3$): δ 7.66 – 7.12 (m, 26H), 7.01 (d, J = 1.1 Hz, 1H), 6.19 (dd, J = 9.4, 2.3 Hz, 1H), 4.32 – 4.19 (m, 1H), 3.79 – 3.69 (m, 1H), 3.60 (dd, J = 10.5, 5.9 Hz, 1H), 3.47 – 3.39 (m, 1H), 3.32 – 3.22 (m, 1H), 2.06 (d, J = 5.8 Hz, 1H), 1.75 (s, 3H), 1.51 (d, J = 1.4 Hz, 1H), 1.22 (d, J = 1.6 Hz, 1H), 0.97 (s, 9H).
^{13}C–NMR (100 MHz,CDCl$_3$): δ 165.2, 154.6, 138.5, 135.6, 135.5, 133.4, 133.2, 129.8, 129.7, 129.3, 127.8, 127.7, 127.7, 126.4, 81.3, 77.5, 77.3, 77.1, 76.9, 76.9, 76.7, 64.7, 52.8, 50.1, 26.8, 19.3, 13.0.
HRMS (ESI) [M + H]$^+$: Calculated mass for $C_{45}H_{49}N_4O_3Si$ 721.3576, found 721.3575

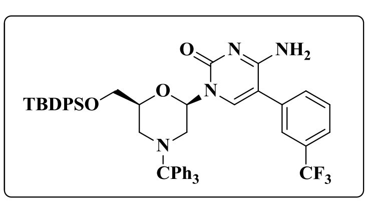
Compound **3a**



^{1}H –NMR (300 MHz,CDCl$_3$): δ 7.60 – 7.18 (m, 27H), 6.16 (dd, J = 9.2, 2.2 Hz, 1H), 5.70 (s, 1H), 4.32 – 4.16 (m, 1H), 3.73 (dd, J = 10.7, 4.6 Hz, 1H), 3.59 (dd, J = 10.6, 5.5 Hz, 1H), 3.48 (d, J = 11.0 Hz, 1H), 3.30 – 3.17 (m, 1H), 1.56 – 1.40 (m, 1H), 1.19 (dd, J = 11.1, 9.3 Hz, 1H), 1.02 – 0.90 (m, 18H), 0.72 – 0.59 (m, 6H).
^{13}C-NMR (75 MHz, CDCl$_3$): δ 158.9, 143.8, 141.6, 135.6, 135.6, 133.3, 133.1, 132.8, 129.9, 129.3, 128.3, 128.0, 127.9, 127.8, 126.6, 96.5, 82.0, 77.8, 77.5, 77.3, 77.1, 76.9, 76.8, 76.7, 64.5, 52.7, 49.7, 26.8, 19.3, 7.6, 4.4.
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{52}H_{61}N_4O_3Si_2$ 845.4282, found 845.4280

Compound **3b-d, 3f-j reported previously**[2]

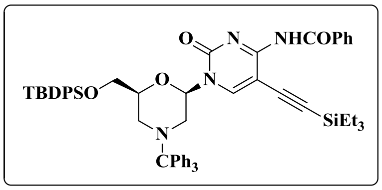
Compound **3e**



^{1}H –NMR (300 MHz,CDCl$_3$): δ 8.49 (s, 1H), 7.64 – 7.17 (m, 31H), 6.20 (d, J = 7.1 Hz, 1H), 5.17 (s, 1H), 4.33 – 4.18 (m, 1H), 3.70 (dd, J = 10.6, 4.4 Hz, 1H), 3.60 – 3.54 (m, 1H), 3.50 (dt, J = 11.2, 2.6 Hz, 1H),

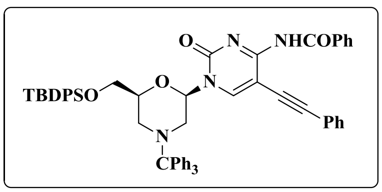
3.25 (d, *J* = 11.4 Hz, 1H), 1.47 (t, *J* = 11.1 Hz, 1H), 1.27 – 1.18 (m, 1H), 0.91 (s, 9H).
^{13}C–NMR (100 MHz,CDCl$_3$): δ 163.8, 154.5, 140.0, 135.5, 135.5, 134.1, 133.3, 132.8, 130.0, 129.8, 129.7, 129.4, 127.9, 127.7, 126.4, 126.1, 125.4, 122.4, 107.2, 81.7, 77.4, 77.3, 77.2, 77.1, 77.0, 76.9, 76.8, 64.6, 53.0, 50.0, 26.7, 19.2.
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{51}H_{49}F_3N_4O_3SiNa$ 873.3424, found 873.3422

Compound **4a**



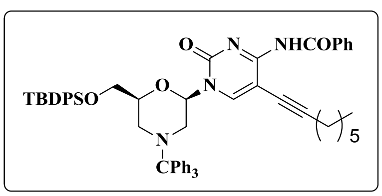
^{1}H-NMR (300 MHz, CDCl$_3$): δ 13.28 (s, 1H), 8.27 (s, 2H), 7.73 – 7.12 (m, 31H), 6.12 (d, *J* = 7.1 Hz, 1H), 4.28 (dt, *J* = 7.2, 2.1 Hz, 1H), 3.75 (dd, *J* = 10.8, 4.6 Hz, 1H), 3.63 (dd, *J* = 10.8, 5.3 Hz, 1H), 3.47 (d, *J* = 11.1 Hz, 1H), 3.31 – 3.19 (m, 1H), 1.64 – 1.49 (m, 2H), 1.35 (dd, *J* = 13.2, 5.8 Hz, 1H), 1.08 – 0.89 (m, 17H), 0.77 – 0.58 (m, 6H).
^{13}C-NMR (75 MHz, CDCl$_3$): δ 158.9, 143.8, 141.6, 135.6, 135.6, 133.3, 133.1, 132.8, 129.9, 129.3, 128.3, 127.9, 127.8, 126.6, 96.5, 82.0, 77.8, 77.5, 77.3, 77.1, 76.9, 76.8, 76.7, 64.5, 52.7, 49.7, 26.8, 19.3, 7.6, 4.4.
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{59}H_{64}N_4NaO_4Si_2$ 971.4364, found 971.4367

Compound **4b**



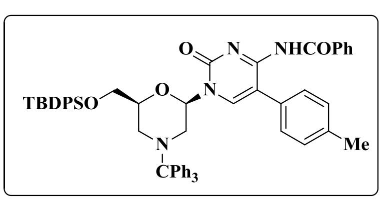
^{1}H-NMR (300 MHz, CDCl$_3$): δ 8.32 (d, *J* = 7.5 Hz, 2H), 7.73 – 7.15 (m, 36H), 6.15 (dd, *J* = 9.3, 2.3 Hz, 1H), 4.32 (dt, *J* = 7.4, 2.2 Hz, 1H), 3.77 (dd, *J* = 10.7, 4.8 Hz, 1H), 3.65 (dd, *J* = 10.8, 5.3 Hz, 1H), 3.51 (dt, *J* = 11.2, 2.4 Hz, 1H), 3.28 (dt, *J* = 12.0, 2.4 Hz, 1H), 1.55 (dd, *J* = 12.0, 10.4 Hz, 1H), 1.33 (dd, *J* = 11.2, 9.3 Hz, 1H), 0.99 (s, 9H).
^{13}C –NMR (75 MHz, CDCl$_3$): δ 158.6, 143.1, 135.6, 133.2,132.8, 131.6, 129.9, 129.3, 128.8, 128.5, 128.4, 128.0, 127.9, 127.8, 126.6, 81.9, 80.6, 77.8, 77.5, 77.3, 77.1, 76.9, 76.7, 64.6, 52.8, 49.7, 26.8, 19.3.
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{59}H_{54}N_4NaO_4Si$ 933.3812, found 933.3815

Compound **4c**



^{1}H-NMR (300MHz, CDCl$_3$): δ 8.26 (d, *J* = 7.6 Hz, 2H), 7.69 – 7.16 (m, 31H), 6.13 (dd, *J* = 9.4, 2.3 Hz, 1H), 4.30 (dt, *J* = 7.4, 2.1 Hz, 1H), 3.83 – 3.69 (m, 1H), 3.61 (dd, *J* = 10.7, 5.5 Hz, 1H), 3.54 – 3.43 (m, 1H), 3.28 (dd, *J* = 11.9, 2.3 Hz, 1H), 2.43 (t, *J* = 7.0 Hz, 2H), 1.62 (dd, *J* = 8.4, 7.0 Hz, 2H), 1.47 (dd, *J* = 5.9, 3.0 Hz, 2H), 1.36 – 1.24 (m, 5H), 0.99 (s, 8H), 0.91 (d, *J* = 6.3 Hz, 2H).
^{13}C-NMR (100 MHz, CDCl$_3$): δ 169.0, 160.5, 159.2, 153.4, 144.6, 142.8, 135.6, 135.6, 133.2, 133.2, 132.7, 130.8, 129.9, 129.8, 129.3, 128.6, 128.3, 128.0, 127.8, 127.8, 127.8, 126.6, 126.4, 108.4, 101.2, 96.2, 83.1, 81.7, 77.6, 77.4, 77.1, 76.9, 76.8, 71.4, 67.1, 64.8, 64.6, 53.3, 52.7, 50.0, 49.8, 31.5, 31.4, 28.7, 26.8, 22.6, 22.5, 19.7, 19.3, 14.1.
HRMS (ESI) [M + H]$^+$: Calculated mass for $C_{59}H_{63}N_4O_4Si$ 919.4619, found 919.4616

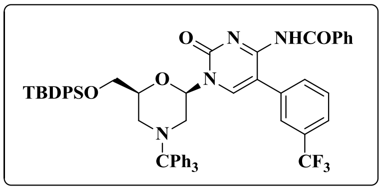
Compound **4d**



^{1}H-NMR (300 MHz, CDCl$_3$) : δ 13.62 (s, 1H), 8.14 (d, *J* = 7.6 Hz, 2H), 7.58 – 7.15 (m, 35H), 6.19 (dd, *J* = 9.4, 2.3 Hz, 1H), 4.36 – 4.19 (m, 1H), 3.71 (dd, *J* = 10.6, 4.6 Hz, 1H), 3.58 (dd, *J* = 10.6, 5.8 Hz, 1H), 3.47 (d, *J* = 11.2 Hz, 1H), 3.26 (d, *J* = 12.0 Hz, 1H), 2.38 (s, 3H), 1.51 – 1.31 (m, 2H), 0.94 (s, 9H).
^{13}C-NMR (100 MHz, CDCl$_3$): δ 158.5, 138.3, 137.9, 135.6, 135.5, 133.2, 132.5, 130.1, 130.0, 129.9, 129.8, 129.5, 129.3, 128.9, 128.2, 128.0, 127.8, 126.6, 81.4, 77.4, 77.3, 77.1, 76.8, 64.6, 52.6, 50.0, 26.8, 21.3, 19.3

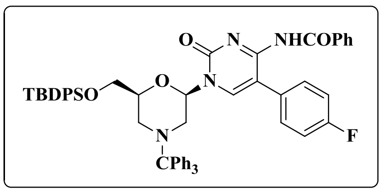
HRMS (ESI) [M + H]⁺: Calculated mass for $C_{58}H_{57}N_4O_4Si$ 901.4149, found 901.4146

Compound **4e**



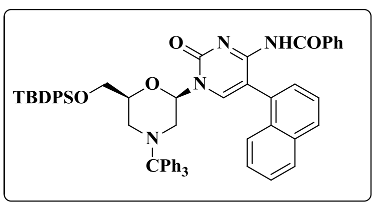
^{1}H –NMR (300 MHz, $CDCl_3$): δ 13.65 (s, 1H), 8.17 (d, *J* = 7.6 Hz, 2H), 7.98 (s, 1H), 7.43 (m, ,7.60-7.18,33H), 6.33 – 6.19 (m, 1H), 4.41 – 4.27 (m, 1H), 3.77 (q, *J* = 5.0, 3.0 Hz, 1H), 3.68 (dd, *J* = 9.1, 3.7 Hz, 1H), 3.56 (d, *J* = 11.2 Hz, 1H), 3.31 (d, *J* = 11.9 Hz, 1H), 1.60 (t, *J* = 11.2 Hz, 1H), 1.50 – 1.39 (m, 1H), 0.97 (s, 9H).
^{13}C–NMR (100 MHz, $CDCl_3$): δ 179.8, 157.9, 146.9, 138.8, 136.9, 135.5, 135.4, 133.7, 133.2, 132.7, 132.4, 130.1, 130.0, 129.9, 129.8, 129.3, 128.7, 128.7, 128.2, 128.2, 128.0, 127.7, 127.2, 127.2, 126.7, 124.6, 114.8, 81.5, 77.7, 77.4, 77.3, 77.1, , 77.0, 76.8, 64.5, 52.7, 49.8, 26.8, 26.7, 19.3.
HRMS (ESI) [M + H]⁺: Calculated mass for $C_{58}H_{54}F_3N_4O_4Si$ 955.3866, found 955.3863

Compound **4f**



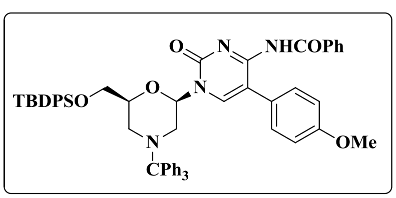
^{1}H-NMR (300 MHz, $CDCl_3$): δ 13.60 (s, 1H), 8.12 (d, *J* = 7.0 Hz, 2H), 7.62 – 7.16 (m, 31H), 7.04 (t, *J* = 8.7 Hz, 2H), 6.20 (dd, *J* = 9.4, 2.3 Hz, 1H), 4.36 – 4.22 (m, 1H), 3.73 (dd, *J* = 10.7, 4.5 Hz, 1H), 3.62 (dd, *J* = 10.7, 5.6 Hz, 1H), 3.50 (dt, *J* = 11.2, 2.4 Hz, 1H), 3.27 (dd, *J* = 11.9, 2.4 Hz, 1H), 1.53 (d, *J* = 1.5 Hz, 1H), 1.38 (dd, *J* = 11.2, 9.4 Hz, 1H), 0.94 (s, 9H).
^{13}C-NMR (100 MHz, $CDCl_3$): δ 163.9, 161.4, 158.3, 147.0, 138.4, 135.6, 135.5, 133.2, 132.6, 131.5, 131.4, 130.1, 129.9, 129.8, 129.3, 128.2, 128.0, 127.8, 126.7, 115.2, 115.0, 81.5, 77.7, 77.4, 77.3, 77.1, 77.0, 76.8, 64.6, 52.7, 49.9, 26.8, 19.3.
HRMS (ESI) [M + H]⁺: Calculated mass for $C_{57}H_{54}FN_4O_4Si$ 905.3898, found 905.3892

Compound **4g**



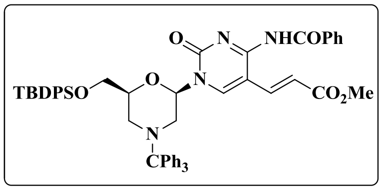
^{1}H-NMR (300 MHz, $CDCl_3$): δ 13.47 (s, 1H), 7.89 (s, 2H), 7.72 – 7.09 (m, 37H), 6.27 (td, *J* = 9.7, 4.7 Hz, 1H), 4.38 – 4.22 (m, 1H), 3.69 (td, *J* = 10.0, 9.2, 5.1 Hz, 1H), 3.52 (ddd, *J* = 19.7, 10.5, 5.8 Hz, 2H), 3.27 (d, *J* = 11.9 Hz, 1H), 1.51 – 1.36 (m, 2H), 0.89 (d, *J* = 3.5 Hz, 9H).
^{13}C-NMR (100 MHz, $CDCl_3$): δ 158.8, 140.1, 135.6, 135.5, 135.4, 133.6, 132.3, 129.9, 129.8, 129.7, 129.3, 129.1, 129.1, 128.7, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.7, 127.6, 126.6, 126.1, 125.9, 125.3, 125.2, 81.6, 81.3, 77.4, 77.3, 77.1, 77.0, 76.8, 64.5, 52.7, 49.9, 26.8, 19.2.
HRMS (ESI) [M + H]⁺: Calculated mass for $C_{61}H_{57}N_4O_4Si$ 937.4149, found 937.4146

Compound **4h**



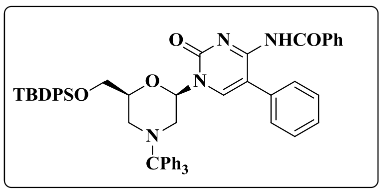
^{1}H-NMR (300 MHz, $CDCl_3$): δ 13.59 (s, 1H), 8.20 – 8.06 (m, 2H), 7.59 – 7.17 (m, 33H), 6.88 (d, *J* = 8.7 Hz, 2H), 6.20 (dd, *J* = 9.5, 2.3 Hz, 1H), 4.29 (d, *J* = 9.8 Hz, 1H), 3.83 (s, 3H), 3.73 (dd, *J* = 10.7, 4.5 Hz, 1H), 3.59 (dd, *J* = 10.6, 5.8 Hz, 1H), 3.48 (d, *J* = 11.2 Hz, 1H), 3.27 (d, *J* = 12.0 Hz, 1H), 1.47 – 1.30 (m, 2H), 0.94 (s, 8H).
^{13}C-NMR (100 MHz, $CDCl_3$): δ 159.6, 158.6, 137.9, 137.3, 135.6, 135.6, 133.33, 132.5, 130.9, 130.1, 129.9, 129.8, 129.3, 128.2, 128.0, 127.9, 127.8, 126.6, 125.4, 113.7, 81.5, 77.6, 77.4, 77.3, 77.1, 77.0, 76.8, 64.6, 55.5, 52.6, 50.0, 26.9, 19.3.
HRMS (ESI) [M + Na]⁺: Calculated mass for $C_{58}H_{56}N_4NaO_5SiNa$ 939.3918, found 939.3915

Compound **4i**



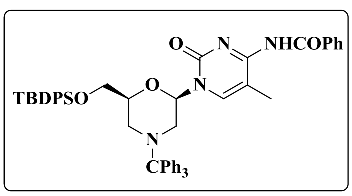
^{1}H-NMR (300 MHz, CDCl$_3$): δ 13.51 (s, 1H), 8.30 – 8.21 (m, 2H), 7.62 – 7.23 (m, 32H), 6.88 (d, *J* = 15.9 Hz, 1H), 6.14 (dd, *J* = 9.4, 2.3 Hz, 1H), 4.32 (d, *J* = 9.5 Hz, 1H), 3.78 (s, 4H), 3.65 (dd, *J* = 10.7, 5.8 Hz, 1H), 3.48 (d, *J* = 11.6 Hz, 1H), 3.31 (d, *J* = 12.0 Hz, 1H), 1.51 (t, *J* = 11.2 Hz, 1H), 1.31 (dd, *J* = 11.0, 9.1 Hz, 1H), 1.00 (s, 9H).
^{13}C-NMR (100 MHz, CDCl$_3$): δ 180.1, 167.5, 157.2, 146.3, 141.3, 136.7, 136.3, 135.6, 135.5, 133.2, 132.8, 130.1, 129.9, 129.2, 128.4, 128.0, 127.9, 127.8, 126.7, 119.7, 110.0, 81.6, 77.8, 77.4, 77.3, 77.1, 76.9, 76.8, 64.6, 52.8, 51.7, 49.9, 26.8, 19.3.
HRMS (ESI) [M + H]$^+$: Calculated mass for $C_{55}H_{55}N_4O_6Si$ 895.3891, found 895.3887

Compound **4j**



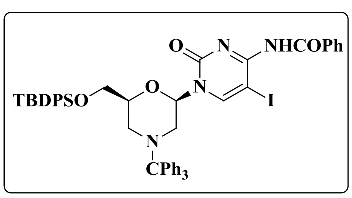
^{1}H-NMR (300 MHz, CDCl$_3$): δ 13.25 (s, 1H), 8.42 – 8.30 (m, 2H), 7.69 (s, 1H), 7.64 – 7.20 (m, 31H), 6.08 (dd, *J* = 9.5, 2.3 Hz, 1H), 4.35 – 4.21 (m, 1H), 3.75 (dd, *J* = 10.7, 4.6 Hz, 1H), 3.63 (dd, *J* = 10.7, 5.6 Hz, 1H), 3.45 (d, *J* = 11.3 Hz, 1H), 3.27 (d, *J* = 11.9 Hz, 1H), 1.55 – 1.46 (m, 1H), 1.30 (dd, *J* = 11.6, 2.1 Hz, 1H), 0.99 (s, 9H).
^{13}C-NMR (75 MHz, CDCl$_3$): δ 156.7, 146.9, 145.4, 136.7, 135.6, 133.2, 133.1, 132.9, 130.3, 129.9, 129.2, 128.3, 128.0, 127.9, 127.8, 127.8, 126.6, 81.7, 77.8, 77.5, 77.3, 77.1, 76.9, 76.7, 64.5, 52.6, 49.8, 26.8, 19.3.
HRMS (ESI) [M + H]$^+$: Calculated mass for $C_{57}H_{55}N_4O_4Si$ 887.3993, found 887.3993

Compound **4k**

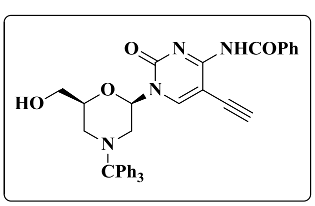


^{1}H-NMR (300 MHz, CDCl$_3$): δ 13.28 (s, 1H), 8.30 (dd, *J* = 7.0, 1.3 Hz, 2H), 7.68 – 7.08 (m, 30H), 6.24 – 6.08 (m, 1H), 4.35 – 4.19 (m, 1H), 3.75 (ddd, *J* = 10.6, 4.4, 1.3 Hz, 1H), 3.63 (ddd, *J* = 10.6, 5.6, 1.3 Hz, 1H), 3.40 (d, *J* = 11.1 Hz, 1H), 3.28 (d, *J* = 11.8 Hz, 1H), 1.94 (s, 3H), 1.64 – 1.49 (m, 2H), 1.41 – 1.33 (m, 1H), 0.97 (d, *J* = 1.2 Hz, 9H).
^{13}C-NMR (75 MHz, CDCl$_3$): δ 179.6, 159.7, 147.5, 137.3, 136.8, 135.6, 135.6, 133.4, 133.2, 132.5, 130.0, 129.9, 129.8, 129.3, 128.2, 128.0, 127.8, 126.6, 111.4, 81.0, 77.6, 77.3, 77.1, 76.9, 76.7, 64.6, 52.4, 49.9, 26.8, 19.3, 13.5.
HRMS (ESI) [M + H]$^+$: Calculated mass for $C_{52}H_{53}N_4O_4Si$ 825.3836, found 825.3834

Compound **4l**



^{1}H-NMR (300 MHz, CDCl$_3$): δ 13.26 (s, 1H), 8.46 – 8.22 (m, 2H), 7.73 – 7.15 (m, 31H), 6.08 (dd, *J* = 9.3, 2.3 Hz, 1H), 4.28 (dt, *J* = 7.6, 2.2 Hz, 1H), 3.75 (dd, *J* = 10.7, 4.6 Hz, 1H), 3.71 – 3.60 (m, 1H), 3.45 (d, *J* = 11.2 Hz, 1H), 3.36 – 3.24 (m, 1H), 1.53 (d, *J* = 1.5 Hz, 1H), 1.37 – 1.25 (m, 1H), 0.99 (s, 9H).
^{13}C-NMR (75 MHz, CDCl$_3$): δ 156.7, 147.0, 145.4, 136.7, 135.6, 133.2, 133.1, 132.9, 130.4, 129.9, 129.3, 128.3, 128.0, 127.9, 127.8, 126.7, 81.7, 77.8, 77.5, 77.3, 77.1, 76.9, 76.7, 64.5, 52.7, 49.8, 26.9, 19.3.
HRMS (ESI) [M + H]$^+$: Calculated mass for $C_{51}H_{50}IN_4O_4Si$ 937.2646, found 937.2649

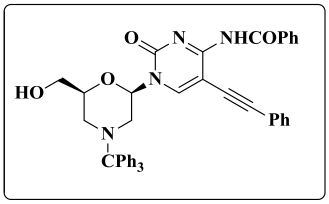


Compound **5a**

^{1}H-NMR (300 MHz, CDCl$_3$): δ 8.25 (d, *J* = 7.6 Hz, 2H), 7.69 (s, 1H), 7.57 – 7.10 (m, 19H), 6.18 (dd, *J* = 9.4, 2.3 Hz, 1H), 4.30 (ddd, *J* = 6.3, 4.3, 2.2 Hz, 1H), 3.63 (dd, *J* = 5.1, 3.2 Hz, 2H), 3.49 (dt, *J* = 11.3, 2.4 Hz, 1H), 3.27 (s, 1H), 3.13 (d, *J* = 11.9 Hz, 1H), 1.91 (s, 1H), 1.50 (t, *J* = 11.3 Hz, 1H),

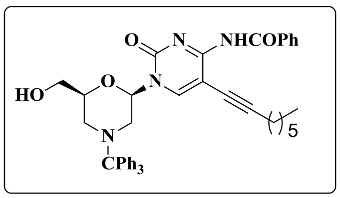
1.37 (dd, J = 11.4, 9.4 Hz, 1H).
^{13}C NMR (100 MHz, CDCl$_3$): δ 158.6, 147.0, 144.6, 132.9, 129.9, 129.2, 128.4, 128.0, 128.0, 126.75, 82.9, 81.8, 78.2, 77.4, 77.3, 77.1, 76.9, 76.8, 74.7, 63.6, 52.6, 48.9.
HRMS (ESI) [M + H]$^+$: Calculated mass for $C_{37}H_{33}N_4O_4$ 597.2502, found 597.2501

Compound **5b**



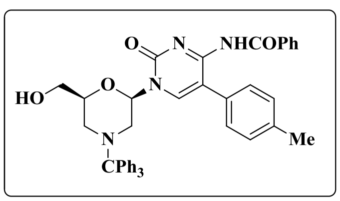
^{1}H-NMR (300MHz, CDCl$_3$): δ 8.25 (d, J = 7.6 Hz, 2H), 7.74 (s, 1H), 7.60 – 7.04 (m, 24H), 6.23 (dd, J = 9.4, 2.3 Hz, 1H), 4.45 – 4.27 (m, 1H), 3.67 (dd, J = 5.0, 3.0 Hz, 2H), 3.53 (d, J = 11.2 Hz, 1H), 3.20 – 3.09 (m, 1H), 2.76 – 2.24 (m, 1H), 1.59 – 1.48 (m, 1H), 1.40 (dd, J = 11.3, 9.4 Hz, 1H).
^{13}C-NMR (75 MHz, CDCl$_3$): δ 158.5, 143.1, 132.8, 131.5, 129.7, 129.2, 128.8, 128.5, 128.4, 128.0, 126.6, 122.5, 81.9, 80.3, 78.3, 77.5, 77.1, 76.9, 76.7, 63.7, 52.6, 48.9.
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{43}H_{36}N_4NaO_4$ 695.2634, found 695.2635

Compound **5c**



^{1}H-NMR (300 MHz, CDCl$_3$): δ 8.23 (d, J = 7.6 Hz, 2H), 7.65 – 7.14 (m, 20H), 6.20 (dd, J = 9.4, 2.3 Hz, 1H), 4.31 (ddt, J = 8.1, 3.9, 2.3 Hz, 1H), 3.63 (t, J = 4.3 Hz, 2H), 3.48 (d, J = 11.3 Hz, 1H), 3.12 (d, J = 11.9 Hz, 1H), 2.40 (t, J = 7.1 Hz, 2H), 2.12 – 1.94 (m, 1H), 1.67 – 1.55 (m, 2H), 1.45 (q, J = 3.3, 2.4 Hz, 2H), 1.35 – 1.22 (m, 5H), 0.97 – 0.82 (m, 3H).
^{13}C-NMR (100 MHz, CDCl$_3$): δ 159.1, 142.7, 132.8, 129.3, 128.3, 128.0, 126.7, 81.7, 78.1, 77.4, 77.3, 77.1, 77.0, 76.9, 76.8, 71.3, 63.7, 52.6, 48.9, 31.4, 28.7, 28.6, 22.6, 19.7, 14.1.
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{43}H_{44}N_4NaO_4$ 703.3260, found 703.3262

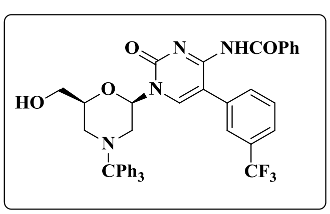
Compound **5d**



^{1}H-NMR (300 MHz, CDCl$_3$): δ 13.55 (s, 1H), 8.13 (d, J = 7.0 Hz, 2H), 6.29 (dd, J = 9.6, 2.3 Hz, 1H), 4.34 (dd, J = 7.2, 3.9 Hz, 1H), 3.61 (t, J = 4.8 Hz, 2H), 3.51 (d, J = 11.3 Hz, 1H), 3.13 (d, J = 12.1 Hz, 1H), 2.40 (s, 3H), 1.95 (s, 1H), 1.57 – 1.38 (m, 2H).

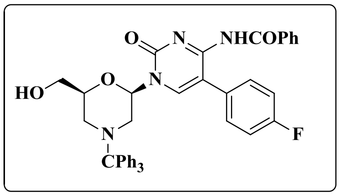
^{13}C-NMR (100 MHz, CDCl$_3$): δ 179.6, 158.4, 147.2, 138.2, 138.1, 137.0, 132.5, 130.1, 129.9, 129.5, 129.2, 128.9, 128.2, 128.0, 126.7, 116.4, 81.3, 78.0, 77.4, 77.3, 77.1, 76.9, 76.8, 63.7, 52.4, 48.9, 21.3.
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{42}H_{38}N_4NaO_4$ 685.2791, found 685.2793

Compound **5e**



^{1}H-NMR (300 MHz, CDCl$_3$): δ 13.57 (s, 1H), 8.17 – 8.03 (m, 2H), 7.91 (s, 1H), 7.63 – 7.16 (m, 23H), 6.31 (dd, J = 9.5, 2.4 Hz, 1H), 4.36 (ddt, J = 6.5, 4.4, 2.2 Hz, 1H), 3.65 (t, J = 4.5 Hz, 2H), 3.54 (d, J = 11.2 Hz, 1H), 3.16 (d, J = 11.9 Hz, 1H), 2.21 – 1.89 (m, 1H), 1.62 – 1.45 (m, 2H).
^{13}C-NMR (100 MHz, CDCl$_3$): δ 179.7, 157.7, 146.8, 138.7, 136.6, 133.5, 132.7, 132.4, 130.5, 130.2, 130.0, 129.2, 128.6, 128.1, 127.9, 127.1, 127.0, 126.6, 125.4, 124.7, 124.6, 122.7, 114.8, 81.3, 78.1, 77.3, 77.2, 77.0, 76.9, 76.7, 63.6, 52.4, 48.8.
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{42}H_{35}F_3N_4NaO_4$ 739.2508, found 739.2506

Compound **5f**

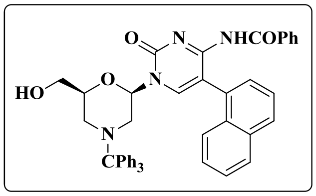


^{1}H-NMR (300 MHz, $CDCl_3$): δ 13.52 (s, 1H), 8.10 (d, J = 7.0 Hz, 2H), 7.59 – 7.16 (m, 21H), 7.08 (t, J = 8.6 Hz, 2H), 6.28 (dd, J = 9.5, 2.3 Hz, 1H), 4.39 – 4.27 (m, 1H), 3.63 (t, J = 4.7 Hz, 2H), 3.52 (d, J = 11.3 Hz, 1H), 3.14 (d, J = 12.0 Hz, 1H), 1.88 (s, 1H), 1.56 – 1.37 (m, 2H).

^{13}C-NMR (100 MHz, $CDCl_3$): δ 179.8, 163.9, 161.5, 158.29, 147.0, 138.2, 137.0, 132.7, 131.5, 131.4, 130.1, 129.3, 128.9, 128.8, 128.2, 128.1, 126.7, 115.6, 115.3, 115.0, 81.4, 78.1, 77.4, 77.3, 77.1, 77.0, 76.8, 63.8, 52.5, 49.0.

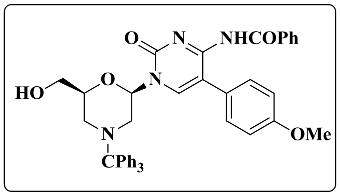
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{41}H_{35}FN_4NaO_4$ 689.2540, found 689.2542

Compound **5g**



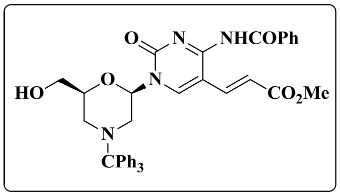
^{1}H NMR (300 MHz, $CDCl_3$) : δ 13.45 (s, 1H), 7.91 (d, J = 8.2 Hz, 2H), 7.72 – 7.15 (m, 25H), 6.34 (td, J = 9.5, 2.4 Hz, 1H), 4.32 (t, J = 7.5 Hz, 1H), 3.65 – 3.45 (m, 3H), 3.16 – 3.03 (m, 1H), 1.78 (s, 1H), 1.55 – 1.34 (m, 2H).
^{13}C-NMR (100 MHz, $CDCl_3$): δ 158.7, 147.7, 139.9, 136.7, 133.7, 132.5, 132.4, 129.9, 129.3, 128.8, 128.5, 128.4, 128.0, 128.0, 126.7, 126.3, 126.2, 126.1, 126.0, 125.3, 125.2, 115.1, 81.5, 81.3, 78.0, 77.4, 77.3, 77.1, 77.0, 76.8, 63.7, 52.5, 48.9.
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{45}H_{38}N_4NaO_4$ 721.2791, found 721.2794

Compound **5h**



^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.18 – 8.08 (m, 2H), 7.60 – 7.17 (m, 23H), 6.92 (dd, J = 8.7, 1.7 Hz, 2H), 6.29 (dd, J = 9.5, 2.3 Hz, 1H), 4.39 – 4.25 (m, 1H), 3.83 (s, 3H), 3.62 (t, J = 5.0 Hz, 2H), 3.50 (d, J = 11.3 Hz, 1H), 3.13 (d, J = 12.0 Hz, 1H), 2.05 (s, 1H), 1.58 – 1.42 (m, 2H).
^{13}C-NMR (100 MHz, $CDCl_3$): δ 179.6, 159.6, 158.5, 147.2, 137.8, 137.1, 132.5, 130.8, 130.1, 129.3, 128.2, 128.0, 126.7, 125.2, 116.1, 113.6, 81.3, 78.0, 77.4, 77.3, 77.1, 77.0, 76.8, 63.8, 55.4, 52.4, 49.0.
HRMS (ESI) [M + H]$^+$: Calculated mass for $C_{42}H_{39}N_4O_5$ 679.2920, found 679.2913

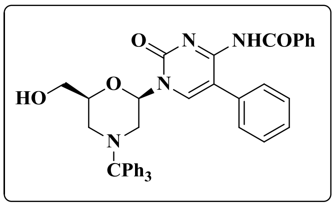
Compound **5i**



^{1}H-NMR (300 MHz, $CDCl_3$): δ 13.44 (s, 1H), 8.24 (d, J = 7.0 Hz, 2H), 7.63 (s, 1H), 7.59 – 7.18 (m, 20H), 6.91 (d, J = 15.9 Hz, 1H), 6.19 (dd, J = 9.5, 2.3 Hz, 1H), 4.31 (td, J = 7.5, 6.5, 3.4 Hz, 1H), 3.76 (s, 3H), 3.71 – 3.62 (m, 2H), 3.48 (dt, J = 11.3, 2.4 Hz, 1H), 3.17 – 3.05 (m, 1H), 2.11 (s, 1H), 1.52 (dd, J = 12.0, 10.6 Hz, 1H), 1.39 (dd, J = 11.3, 9.5 Hz, 1H).

^{13}C-NMR (100 MHz, $CDCl_3$): δ 180.1, 167.6, 157.1, 146.3, 141.4, 136.6, 136.2, 132.9, 130.1, 129.2, 129.2, 128.4, 128.1, 128.0, 126.83, 119.9, 110.2, 81.6, 78.3, 77.4, 77.3, 77.1, 77.0, 76.8, 63.7, 52.7, 51.8, 48.9.
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{39}H_{36}N_4NaO_6$ 679.2533, found 679.2535

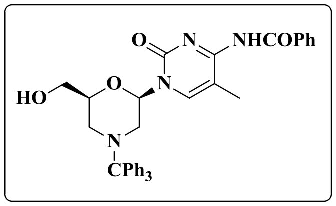
Compound **5j**



^{1}H-NMR (300 MHz, $CDCl_3$): δ 13.56 (s, 1H), 8.13 (d, J = 7.3 Hz, 2H), 7.61 – 7.17 (m, 26H), 6.31 (dd, J = 9.5, 2.3 Hz, 1H), 4.43 – 4.31 (m, 1H), 3.63 (t, J = 4.4 Hz, 2H), 3.58 – 3.49 (m, 1H), 3.16 (d, J = 11.8 Hz, 1H), 2.30 (s, 1H), 1.61 – 1.45 (m, 2H).
^{13}C NMR (75 MHz, $CDCl_3$): δ 158.2, 147.1, 138.5, 136.9, 132.8, 132.5, 130.0, 129.6, 129.2, 128.1, 128.1, 128.0, 128.0, 126.6, 116.3, 81.3, 78.0,

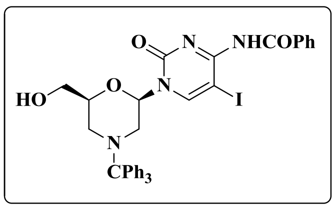
77.5, 77.3, 77.1, 76.9, 76.7, 63.6, 52.4, 48.9.
HRMS (ESI) [M + H]$^+$: Calculated mass for $C_{41}H_{37}N_4O_4$ 649.2815, found 649.2816

Compound **5k**



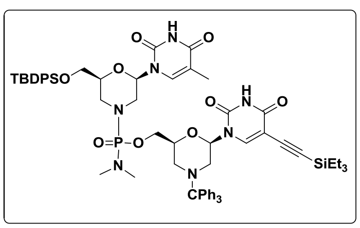
^{1}H-NMR (300 MHz, CDCl$_3$): δ 8.28 (d, *J* = 7.0 Hz, 2H), 7.58 – 7.16 (m, 19H), 6.22 (dd, *J* = 9.6, 2.4 Hz, 1H), 4.38 – 4.22 (m, 1H), 3.64 (qd, *J* = 11.9, 5.0 Hz, 2H), 3.41 (d, *J* = 11.2 Hz, 1H), 3.12 (d, *J* = 11.9 Hz, 1H), 2.00 (d, *J* = 1.1 Hz, 3H), 1.47 (ddd, *J* = 18.1, 11.6, 10.1 Hz, 2H).
^{13}C NMR (75 MHz, CDCl$_3$): δ 159.6, 147.5, 137.1, 136.7, 132.6, 130.0, 129.3, 128.2, 128.0, 126.7, 111.7, 81.0, 77.9, 77.5, 77.3, 77.1, 76.9, 76.7, 63.8, 52.2, 48.9, 13.6.
HRMS (ESI) [M + H]$^+$: Calculated mass for $C_{36}H_{35}N_4O_4$ 587.2658, found 587.2659

Compound **5l**



^{1}H-NMR (300 MHz, CDCl$_3$): *δ* 13.22 (s, 1H), 8.41 – 8.30 (m, 2H), 7.73 (s, 1H), 7.58 – 7.17 (m, 20H), 6.16 (d, *J* = 7.1 Hz, 1H), 4.37 – 4.22 (m, 1H), 3.64 (dd, *J* = 5.0, 2.6 Hz, 2H), 3.46 (d, *J* = 11.2 Hz, 1H), 3.13 (d, *J* = 11.9 Hz, 1H), 1.45 (ddd, *J* = 29.5, 11.7, 10.1 Hz, 2H).
^{13}C NMR (75 MHz, CDCl$_3$): *δ* 156.6, 147.0, 145.3, 136.6, 133.0, 130.4, 129.2, 128.3, 128.1, 128.0, 126.7, 81.6, 78.2, 77.5, 77.3, 77.1, 76.9, 76.7, 63.7, 52.5, 48.9.
HRMS (ESI) [M + H]$^+$: Calculated mass for $C_{35}H_{32}IN_4O_4$ 699.1468,found 699.1466

Compound **9a**

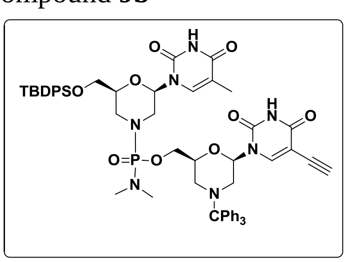


^{1}H–NMR (300 MHz,CDCl$_3$): *δ* 9.39 (dd, *J* = 40.8, 27.8 Hz, 2H), 7.69 – 7.10 (m, 28H), 6.13 (ddd, *J* = 22.5, 9.5, 2.3 Hz, 1H), 5.61 (ddd, *J* = 23.9, 9.9, 2.7 Hz, 1H), 4.43 (s, 1H), 4.04 – 3.66 (m, 6H), 3.49 – 3.26 (m, 3H), 3.16 (dd, *J* = 12.8, 3.6 Hz, 1H), 2.63 (dd, *J* = 12.0, 9.7 Hz, 6H), 2.12 – 1.96 (m, 1H), 1.89 (d, *J* = 1.2 Hz, 3H), 1.55 – 1.32 (m, 2H), 1.06 (d, *J* = 3.5 Hz, 9H), 0.97 (td, *J* = 7.8, 3.2 Hz, 9H), 0.66 – 0.57 (m, 6H).
^{13}C-NMR (125 MHz, CDCl$_3$): *δ* 163.8, 163.7, 161.0, 149.8, 149.8, 149.0, 148.9, 147.0, 142.8, 142.7, 135.6, 135.5, 135.1, 133.0, 133.0, 132.9, 130.0, 130.0, 129.2, 129.2, 128.8, 128.0, 128.0, 127.9, 127.3, 126.7, 126.6, 111.0, 110.9, 100.3, 100.2, 97.3, 96.5, 82.1, 81.2, 81.0, 79.8, 79.8, 77.8, 77.4, 77.1, 76.9, 75.8, 75.7, 75.5, 75.5, 65.6, 65.4, 65.4, 64.2, 64.2, 52.2, 52.1, 49.2, 48.9, 47.5, 47.3, 45.3, 45.2, 36.8, 36.7, 26.8, 19.4, 19.3, 12.6, 7.5, 4.3

^{31}P–NMR (121 MHz, CDCl$_3$): *δ* 16.29, 16.51.

HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{64}H_{78}N_7NaO_9PSi_2$1198.5035, found 1198.5037
Yield: 61%

Compound **9b**



^{1}H–NMR (300 MHz,CDCl$_3$): *δ* 9.18 – 8.91 (m, 2H), 7.76 – 7.04 (m, 28H), 6.20 – 6.05 (m, 1H), 5.66 – 5.51 (m, 1H), 4.49 – 4.32 (m, 1H), 4.01 – 3.63 (m, 5H), 3.49 – 3.25 (m, 3H), 3.12 (dd, *J* = 12.1, 9.6 Hz, 1H), 3.03 (s, 1H), 2.63 (dd, *J* = 18.8, 9.8 Hz, 7H), 1.87 (dd, *J* = 5.4, 1.1 Hz, 3H), 1.43 (dt, *J* = 27.5, 10.6 Hz, 2H), 1.06 (d, *J* = 3.1 Hz, 9H).

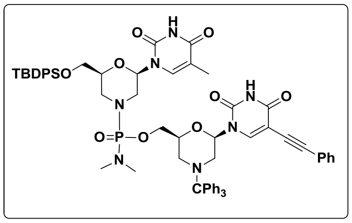
^{13}C–NMR (125MHz, $CDCl_3$): δ 163.6, 161.1, 155.8, 151.5, 149.8, 149.7, 148.8, 148.7, 147.0, 143.7, 135.6, 135.1, 133.0, 130.1, 129.2, 128.0, 127.9, 127.3, 126.7, 111.1, 98.8, 82.0, 81.2, 79.8, 77.4, 77.1, 76.9, 64.2, 52.2, 49.1, 47.4, 45.3, 36.8, 36.7, 26.9, 19.4, 12.6

^{31}P–NMR (121 MHz, $CDCl_3$): δ 16.32, 16.52.

HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{58}H_{64}N_7NaO_9PSi$ 1084.4170, found 1084.4171

Yield: 53%

Compound **9c**



^{1}H–NMR (300 MHz,$CDCl_3$): δ 9.15 (d, J = 42.7 Hz, 1H), 7.75 – 7.08 (m, 32H), 6.27 – 6.06 (m, 1H), 5.69 – 5.49 (m, 1H), 4.47 (d, J = 11.4 Hz, 1H), 4.06 – 3.63 (m, 5H), 3.45 (d, J = 10.7 Hz, 2H), 3.16 (d, J = 11.6 Hz, 1H), 2.93 – 2.74 (m, 1H), 2.69 – 2.49 (m, 6H), 1.84 (d, J = 1.2 Hz, 3H), 1.76 (s, 2H), 1.46 (dt, J = 25.4, 10.4 Hz, 2H), 1.06 (s, 9H).

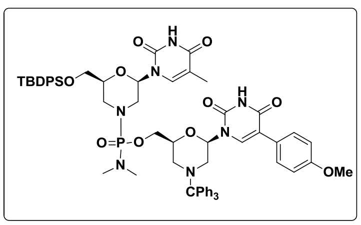
^{13}C–NMR (75 MHz,$CDCl_3$): δ 163.7, 161.0, 149.8, 148.9, 142.2, 135.6, 135.0, 133.1, 131.7, 130.0, 129.9, 129.6, 129.2, 128.9, 128.7, 128.4, 128.1, 127.9, 126.7, 122.5, 111.1, 111.0, 100.2, 93.7, 81.1, 80.3, 79.9, 79.8, 77.5, 77.3, 77.1, 77.0, 76.7, 65.6, 64.3, 52.4, 49.0, 47.5, 45.3, 36.8, 36.8, 26.9, 19.4, 12.6

^{31}P–NMR (121 MHz, $CDCl_3$): δ 16.53, 16.29.

HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{64}H_{68}N_7NaO_9PSi$ 1160.4483, found 1160.4487

Yield: 57%

Compound **9e**



^{1}H–NMR (300 MHz,$CDCl_3$): δ 8.72 (d, J = 8.3 Hz, 1H), 7.67 – 7.09 (m, 31H), 6.83 (dd, J = 8.9, 2.8 Hz, 2H), 6.28 – 6.15 (m, 1H), 5.63 – 5.49 (m, 1H), 4.50 – 4.32 (m, 1H), 3.92 (dd, J = 10.5, 6.1 Hz, 2H), 3.75 (s, 3H), 3.69 (s, 1H), 3.47 – 3.25 (m, 3H), 3.15 (d, J = 11.4 Hz, 1H), 2.65 – 2.52 (m, 6H), 1.86 (d, J = 1.2 Hz, 3H), 1.55 – 1.41 (m, 2H), 1.05 (d, J = 4.4 Hz, 8H).

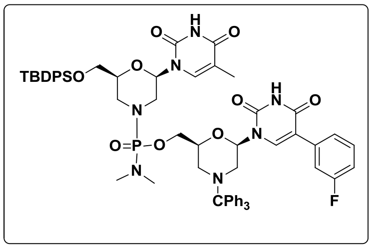
^{13}C–NMR (100 MHz, $CDCl_3$): δ 163.4, 161.9, 159.5, 149.6, 149.4, 136.1, 136.1, 135.6, 135.0, 133.0, 130.0, 129.5, 129.4, 129.3, 128.0, 127.9, 126.7, 126.6, 124.6, 124.5, 115.0, 114.0, 111.0, 80.9, 79.8, 79.7, 77.4, 77.3, 77.1, 77.0, 76.8, 75.5, 75.5, 65.5, 65.4, 64.2, 55.4, 52.1, 49.3, 47.4, 45.3, 36.8, 36.8, 26.9, 19.4, 12.5

^{31}P–NMR (121 MHz, $CDCl_3$): δ 16.42,16.51

HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{63}H_{70}N_7NaO_{10}PSi$ 1166.4589, found 1166.4587

Yield: 63%

Compound **9h**



^{1}H–NMR (300 MHz,$CDCl_3$): δ 9.00 (s, 1H), 8.89 (s, 1H), 7.68 – 7.10 (m, 31H), 6.98 – 6.86 (m, 1H), 6.26 – 6.12 (m, 1H), 5.51 (dd, J = 9.9, 2.7 Hz, 1H), 4.42 (s, 1H), 4.01 – 3.87 (m, 2H), 3.84 – 3.59 (m, 4H), 3.52 – 3.21 (m, 4H), 3.16 (d, J = 11.6 Hz, 1H), 2.72 – 2.59 (m, 6H), 1.86 (s, 3H), 1.75 (s, 3H), 1.50 (q, J = 11.6, 11.1 Hz, 2H), 1.05 (s, 9H).

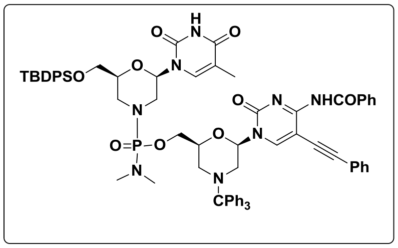
^{13}C–NMR (100 MHz,$CDCl_3$): δ 163.5, 161.5, 149.6, 149.3, 147.0, 137.7, 135.6, 135.0, 133.0, 133.0, 130.1, 129.9, 129.9, 129.3, 129.2, 128.0, 127.9, 127.3, 126.6, 123.8, 123.8, 115.3, 115.1, 114.9, 111.0, 81.1, 79.8, 77.4, 77.1, 76.8, 75.6, 75.5, 65.4, 65.3, 64.2, 52.1, 49.1, 47.4, 45.3, 36.8, 36.8, 26.9, 19.4, 12.5

^{31}P–NMR (162 MHz, $CDCl_3$): δ 16.42, 16.26.

HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{62}H_{67}FN_7NaO_9PSi$ 1154.4389, found 1154.4385

Yield: 64%

Compound **10b**

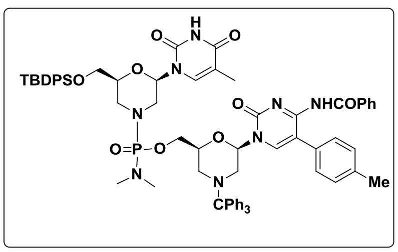


^{1}H NMR (300 MHz, $CDCl_3$): δ 8.36 (s, 1H), 8.29 (d, J = 7.2 Hz, 2H), 7.76 (s, 1H), 7.63 (dd, J = 7.7, 1.8 Hz, 4H), 7.56 – 7.24 (m, 27H), 7.20 – 7.13 (m, 3H), 6.27 – 6.14 (m, 1H), 5.58 (td, J = 11.8, 10.9, 2.8 Hz, 1H), 4.44 (s, 1H), 4.06 – 3.89 (m, 2H), 3.88 – 3.64 (m, 4H), 3.58 – 3.33 (m, 3H), 3.17 (d, J = 11.7 Hz, 1H), 2.89 – 2.75 (m, 1H), 2.64 (t, J = 10.1 Hz, 6H), 1.82 (dd, J = 6.3, 1.2 Hz, 3H), 1.42 (q, J = 11.4, 10.4 Hz, 2H), 1.04 (d, J = 6.0 Hz, 9H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 163.2, 149.6, 135.6, 135.6, 135.6, 135.0, 133.1, 132.9, 131.6, 130.0, 129.9, 129.2, 128.9, 128.6, 128.5, 128.4, 128.1, 128.0, 127.9, 127.4, 126.8, 122.6, 110.9, 81.7, 79.8, 77.8, 77.4, 77.3, 77.1, 77.0, 77.0, 76.8, 75.8, 64.3, 52.5, 49.1, 47.5, 45.3, 36.9, 36.8, 36.8, 26.9, 19.4, 12.5.
^{31}P NMR (162 MHz, $CDCl_3$): δ 16.41, 16.19.
HRMS (ESI) [M + H]$^+$: Calculated mass for $C_{71}H_{74}N_8O_9PSi$ 1241.5086 found 1241.5083

Yield: 63%

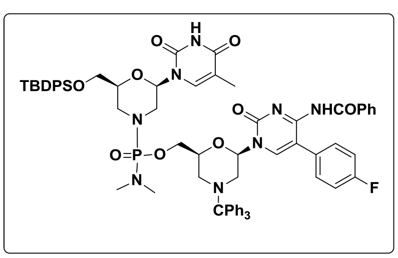
Compound **10d**



^{1}H NMR (300 MHz, $CDCl_3$): δ 13.58 (s, 1H), 8.66 (d, J = 35.1 Hz, 1H), 8.21 – 8.06 (m, 2H), 7.63 (ddd, J = 7.9, 4.9, 1.7 Hz, 4H), 7.55 – 7.24 (m, 25H), 7.24 – 7.09 (m, 6H), 6.26 (ddd, J = 9.6, 7.3, 2.3 Hz, 1H), 5.55 (ddd, J = 19.4, 9.9, 2.7 Hz, 1H), 4.41 (d, J = 11.6 Hz, 1H), 3.93 (t, J = 6.5 Hz, 2H), 3.85 – 3.62 (m, 3H), 3.48 (d, J = 10.0 Hz, 1H), 3.37 (dd, J = 10.5, 6.0 Hz, 2H), 3.17 (d, J = 11.8 Hz, 1H), 2.86 – 2.70 (m, 1H), 2.62 (dd, J = 9.7, 8.4 Hz, 6H), 2.37 (d, J = 6.1 Hz, 3H), 1.86 (dd, J = 2.9, 1.1 Hz, 3H), 1.57 – 1.41 (m, 2H), 1.06 (d, J = 5.7 Hz, 9H).
^{13}C NMR (101 MHz, $CDCl_3$): δ 163.4, 163.2, 149.6, 149.5, 138.0, 138.0, 135.6, 135.6, 135.5, 135.0, 133.1, 133.0, 132.5, 130.1, 130.0, 129.4, 129.2, 128.9, 128.9, 128.2, 128.0, 128.0, 127.9, 127.9, 127.9, 127.3, 126.7, 126.7, 110.9, 110.9, 81.3, 79.9, 79.7, 79.7, 77.8, 77.7, 77.4, 77.3, 77.1, 77.0, 77.0, 76.8, 75.8, 75.7, 75.6, 75.5, 64.2, 52.3, 52.1, 49.3, 49.2, 47.3, 45.3, 36.8, 36.8, 36.7, 26.9, 26.8, 21.3, 19.4, 19.3, 12.5.
^{31}P NMR (162 MHz, $CDCl_3$): δ 16.39, 16.26.
HRMS (ESI) [M + Na]$^+$: Calculated mass for $C_{70}H_{76}N_8O_9Psi$ 1231.5242, found=1231.5243

Yield: 70%

Compound **10f**

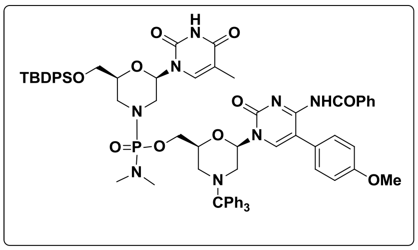


^{1}H NMR (300 MHz, $CDCl_3$): δ 13.57 (s, 1H), 8.52 (d, J = 29.0 Hz, 1H), 8.11 (ddd, J = 8.6, 2.7, 1.4 Hz, 2H), 7.70 – 7.57 (m, 4H), 7.54 – 7.23 (m, 24H), 7.19 – 6.99 (m, 6H), 6.25 (ddd, J = 9.1, 6.3, 2.3 Hz, 1H), 5.54 (ddd, J = 15.0, 9.9, 2.8 Hz, 1H), 4.41 (d, J = 9.3 Hz, 1H), 4.03 – 3.86 (m, 2H), 3.85 – 3.62 (m, 3H), 3.53 (d, J = 9.6 Hz, 1H), 3.44 – 3.26 (m, 2H), 3.18 (d, J = 11.9 Hz, 1H), 2.86 – 2.69 (m, 1H),

2.61 (dd, J = 9.7, 4.5 Hz, 6H), 2.55 – 2.46 (m, 1H), 1.86 (d, J = 1.1 Hz, 3H), 1.57 – 1.42 (m, 2H), 1.06 (d, J = 6.0 Hz, 9H).
^{13}C NMR (100 MHz, $CDCl_3$): δ 163.9, 163.4, 163.2, 161.4, 158.3, 149.66, 149.5, 147.1, 138.5, 138.4, 135.6, 135.0, 133.1, 133.0, 132.7, 131.4, 131.4, 130.1,, 130.0, 129.2, 128.8, 128.2, 128.0, 127.9, 127.9, 127.8, 126.7, 115.4, 115.2, 115.0, 111.0, 110.9, 81.4, 79.9, 79.8, 79.7, 77.8, 77.4, 77.3, 77.1, 77.0, 76.8, 75.8, 75.7, 75.6, 75.5, 65.3, 64.2, 64.2, 52.3, 52.2, 50.9, 49.3, 49.2, 47.4, 47.4, 45.3, 36.8, 36.7, 26.9, 19.4, 19.3, 12.5.
^{31}P NMR (162 MHz, $CDCl_3$): δ 16.45, 16.28.
HRMS (ESI) [M + H]$^+$: Calculated mass for $C_{69}H_{73}FN_8O_9PSi$ 1235.4991, found 1235.4984

Yield: 75%

Compound **10h**

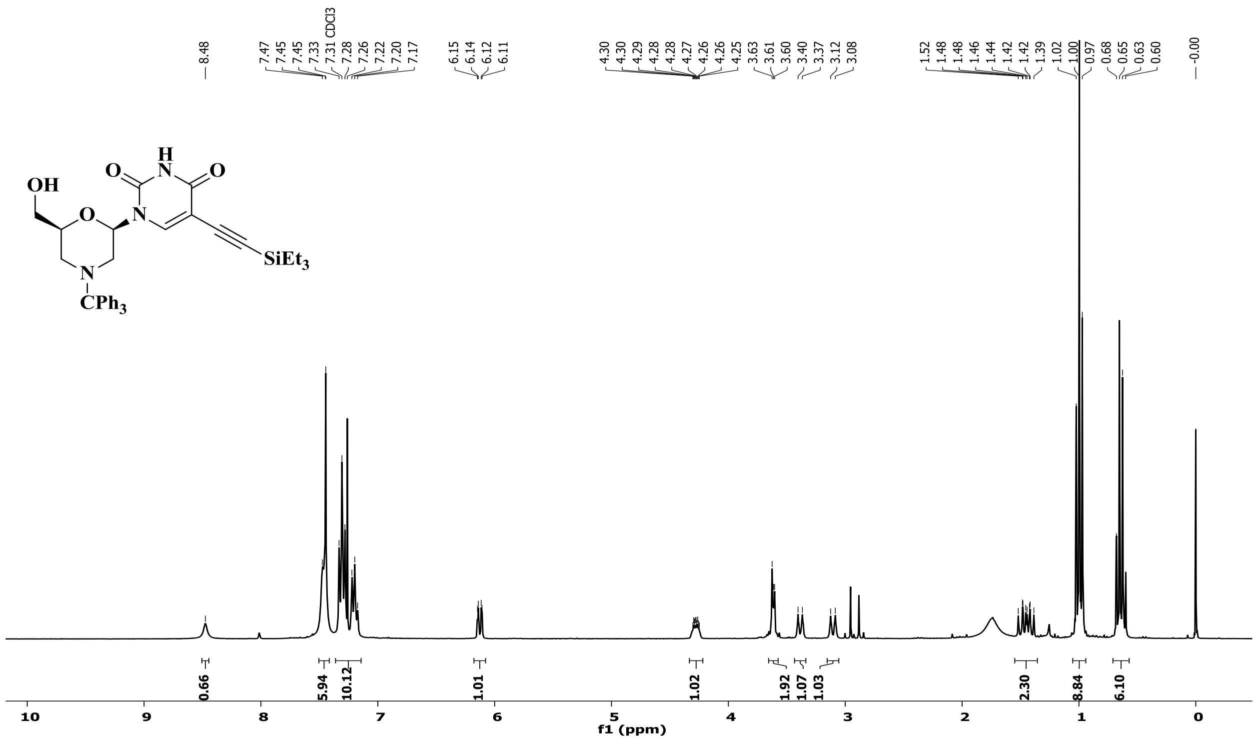


^{1}H NMR (300 MHz, $CDCl_3$): δ 13.54 (s, 1H), 8.26 (d, J = 21.8 Hz, 1H), 8.19 – 8.09 (m, 2H), 7.67 (s, 5H), 7.53 – 7.23 (m, 26H), 7.22 – 7.09 (m, 4H), 6.92 (d, J = 8.4 Hz, 2H), 6.25 (t, J = 8.1 Hz, 1H), 5.61 – 5.45 (m, 1H), 4.41 (s, 1H), 3.99 – 3.87 (m, 2H), 3.82 (d, J = 6.5 Hz, 3H), 3.80 – 3.66 (m, 3H), 3.49 (t, J = 10.0 Hz, 1H), 3.36 (t, J = 9.7 Hz, 2H), 3.15 (s, 1H), 2.76 (dd, J = 17.5, 5.8 Hz, 1H), 2.61 (dd, J = 9.7, 7.7 Hz, 6H), 1.86 (dd, J = 3.3, 1.1 Hz, 3H), 1.48 (td, J = 11.5, 9.0 Hz, 2H), 1.05 (d, J = 5.9 Hz, 9H).
^{13}C NMR (101 MHz, $CDCl_3$): δ 163.1, 159.6, 159.6, 158.5, 149.5, 147.0, 137.9, 135.6, 135.1, 133.1, 133.0, 132.6, 130.8, 130.1, 130.0, 129.3, 128.2, 128.0, 128.0, 127.9, 127.3, 126.7, 125.2, 113.7, 113.6, 110.9, 110.9, 81.3, 77.8, 77.4, 77.3, 77.1, 77.0, 76.8, 65.5, 65.3, 64.2, 55.4, 55.4, 52.3, 52.1, 49.3, 47.4, 45.3, 36.8, 36.8, 26.9, 19.4, 19.4, 12.5.
^{31}P NMR (121 MHz, $CDCl_3$): δ 16.52, 16.37.
HRMS (ESI) [M + H]$^+$: Calculated mass for $C_{70}H_{76}N_8O_{10}PSi$ 1247.5191 found 1247.5187

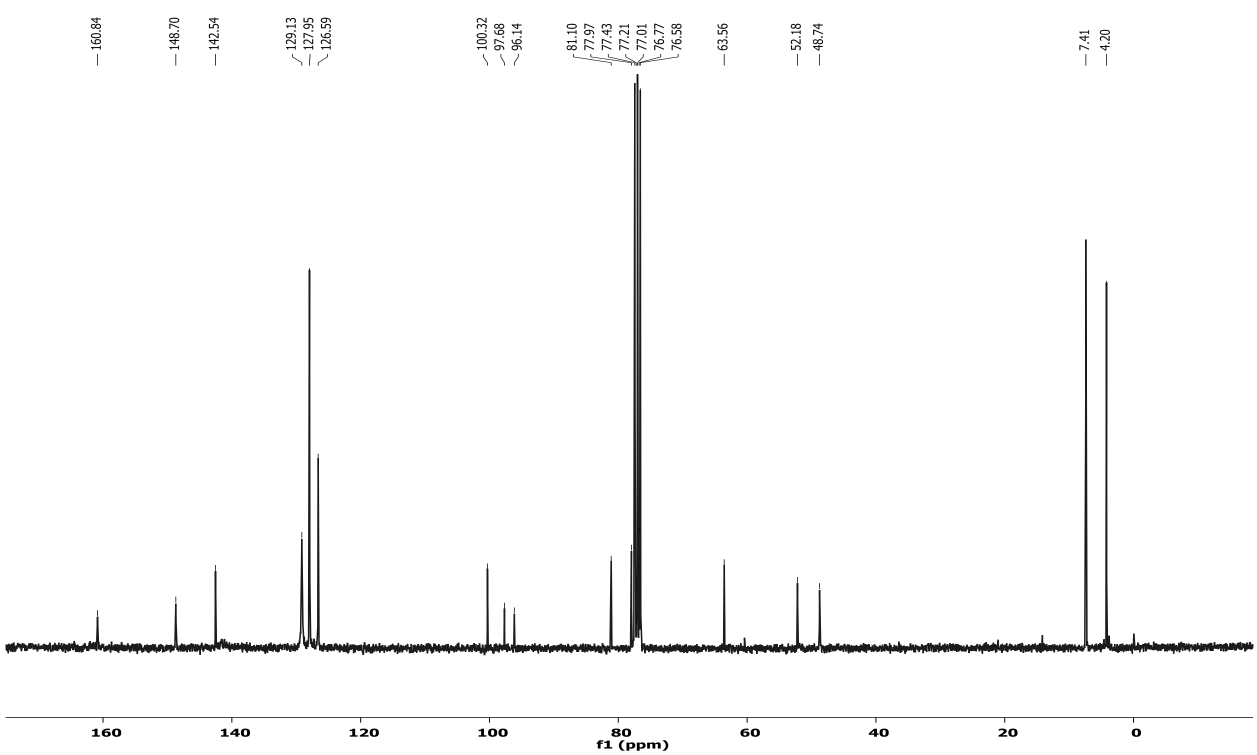
Yield: 72%

3. NMR spectra

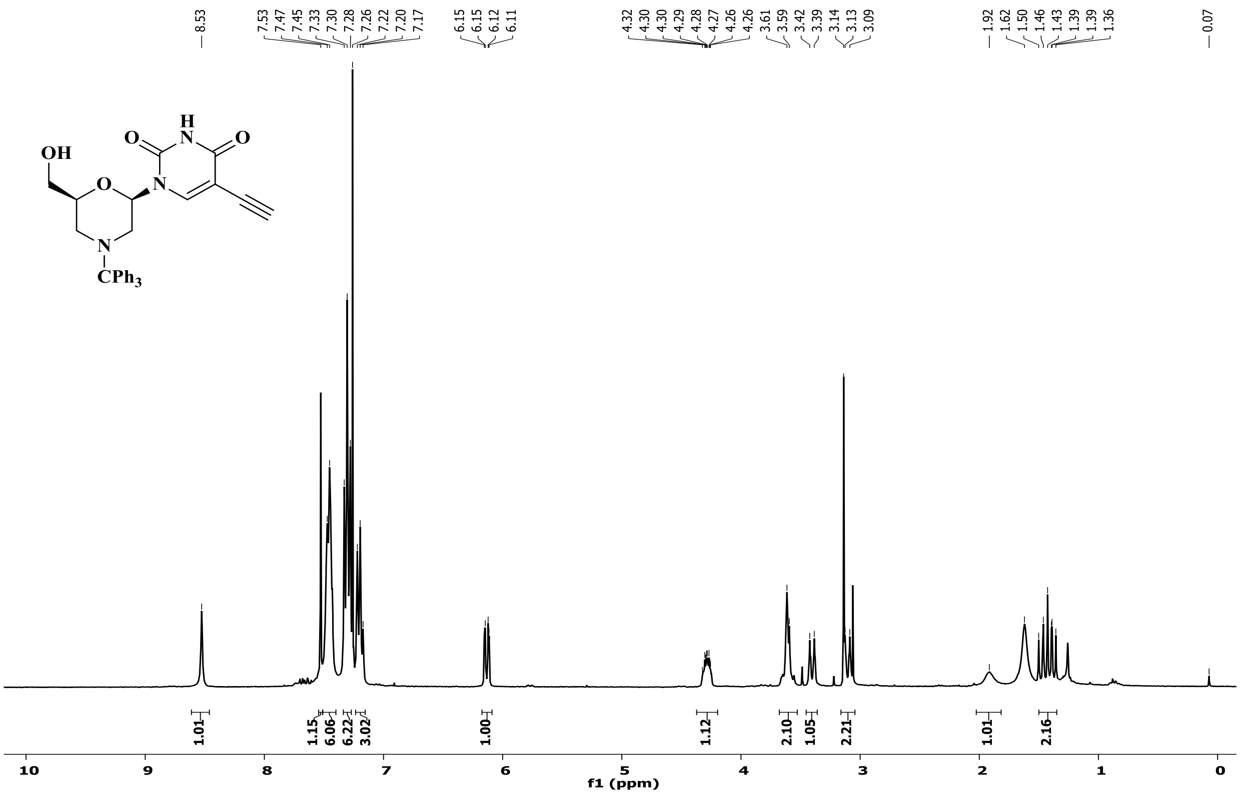
1H-NMR of compound **1a**, $CDCl_3$, 300 MHz



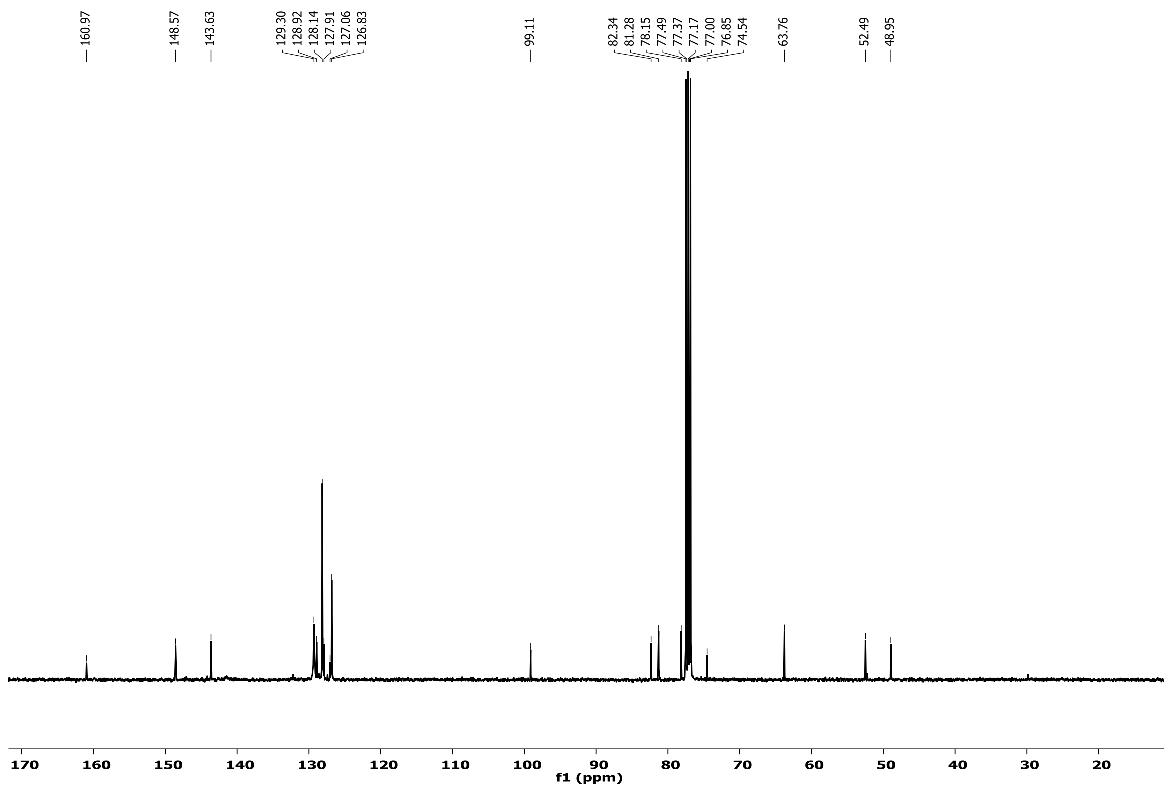
13C-NMR of compound **1a**, $CDCl_3$, 75 MHz



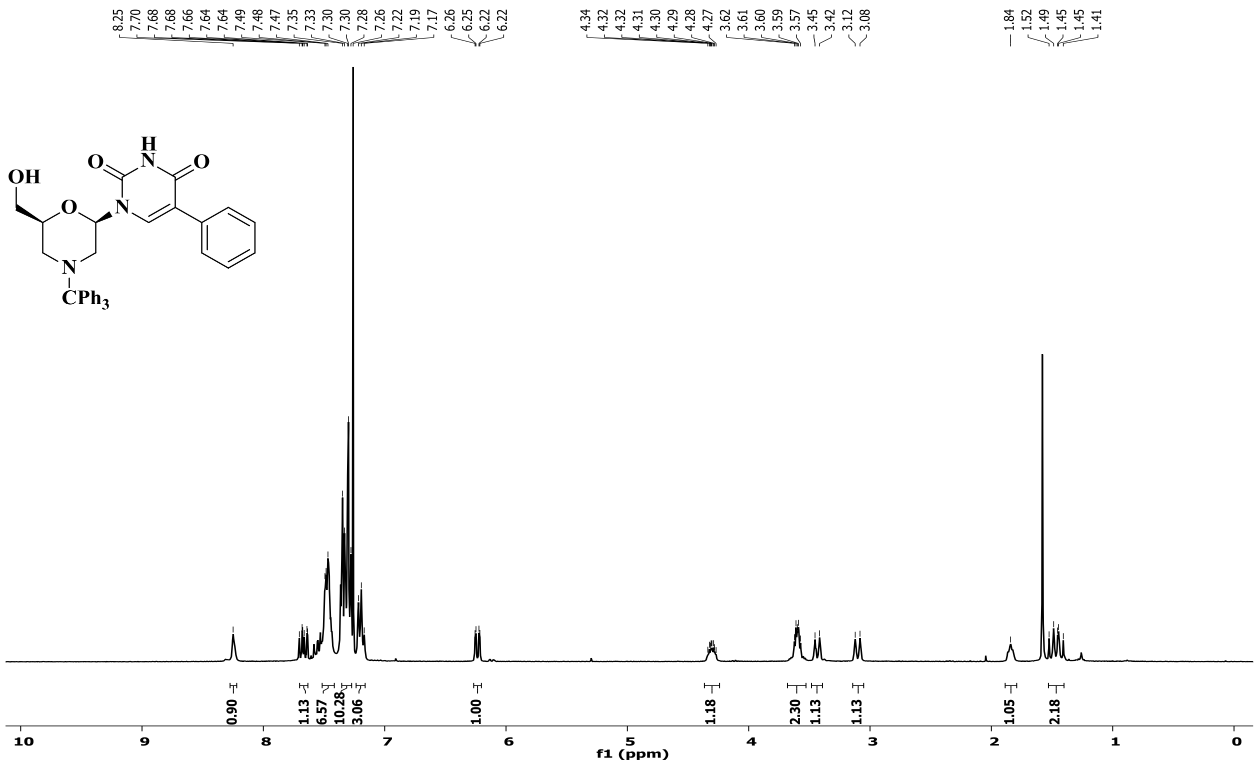
1H-NMR of compound **1b**, $CDCl_3$, 300 MHz



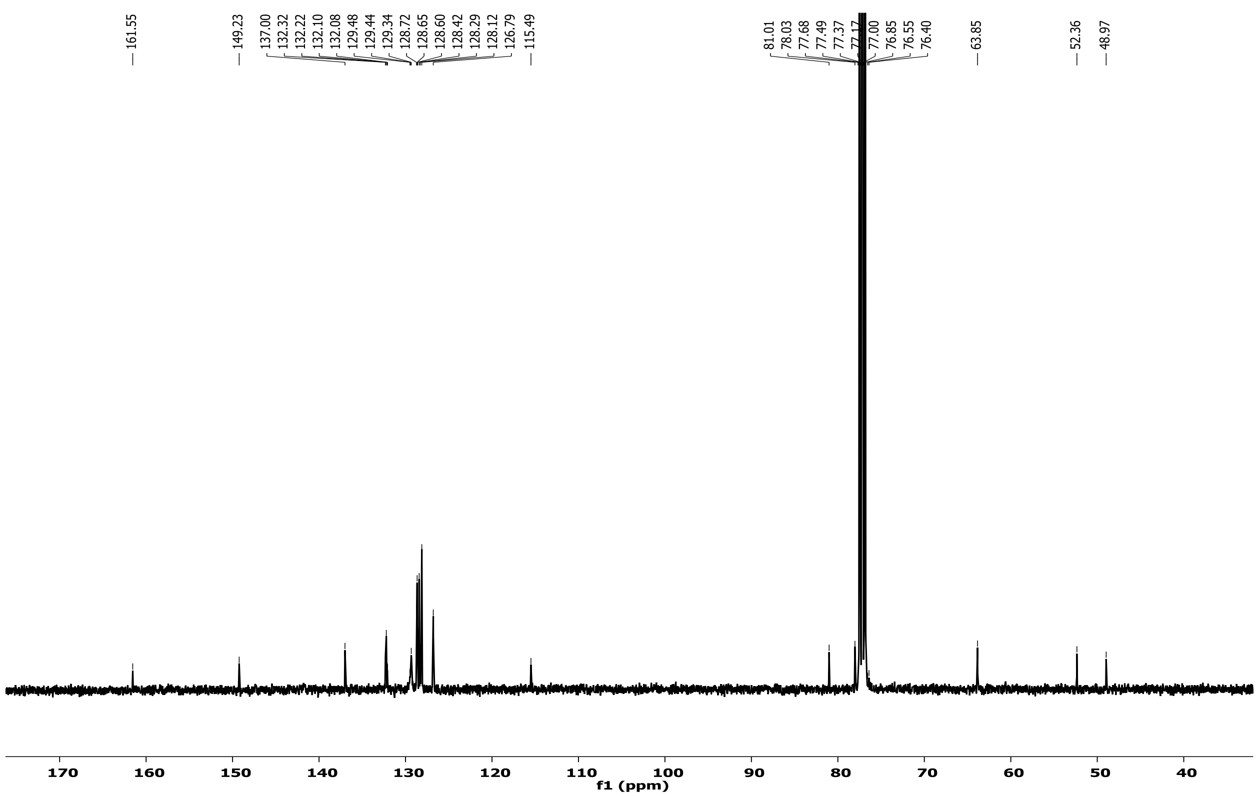
13C-NMR of compound **1b** $CDCl_3$, 100 MHz



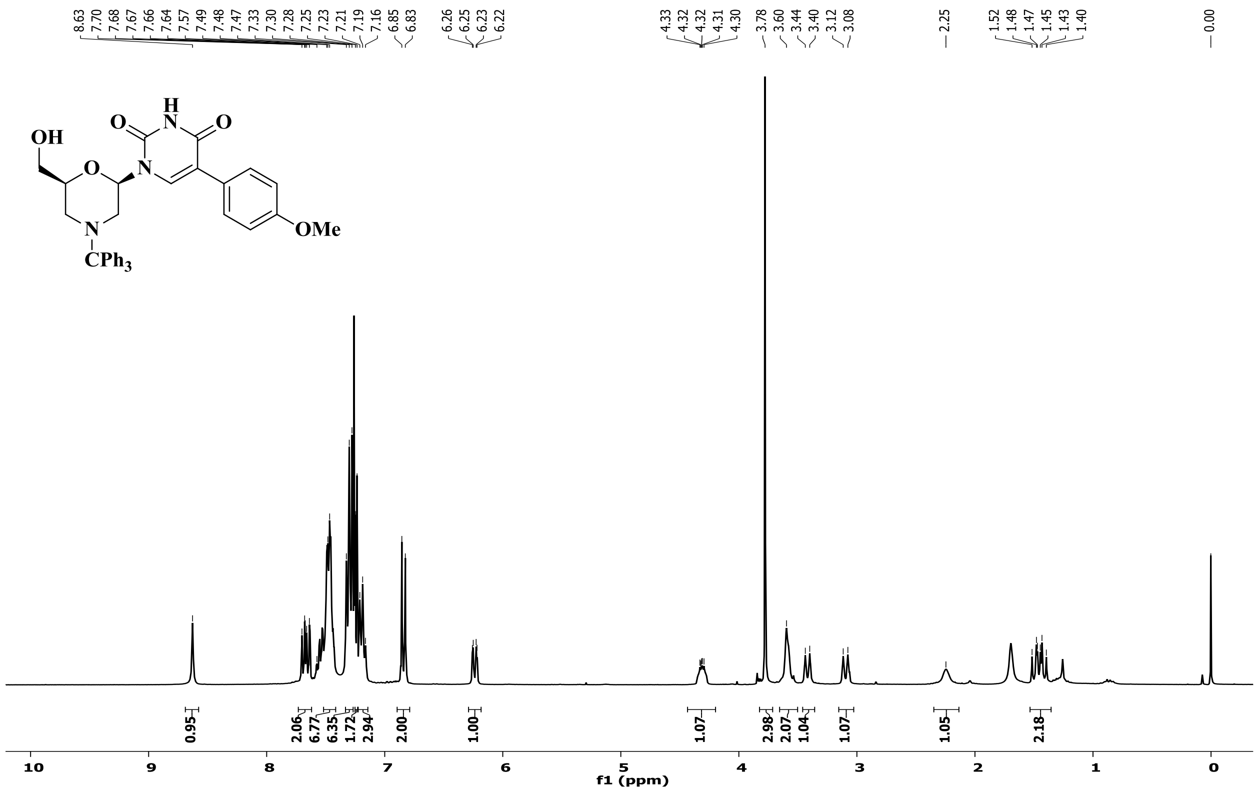
1H-NMR of compound **1d**, $CDCl_3$, 300 MHz



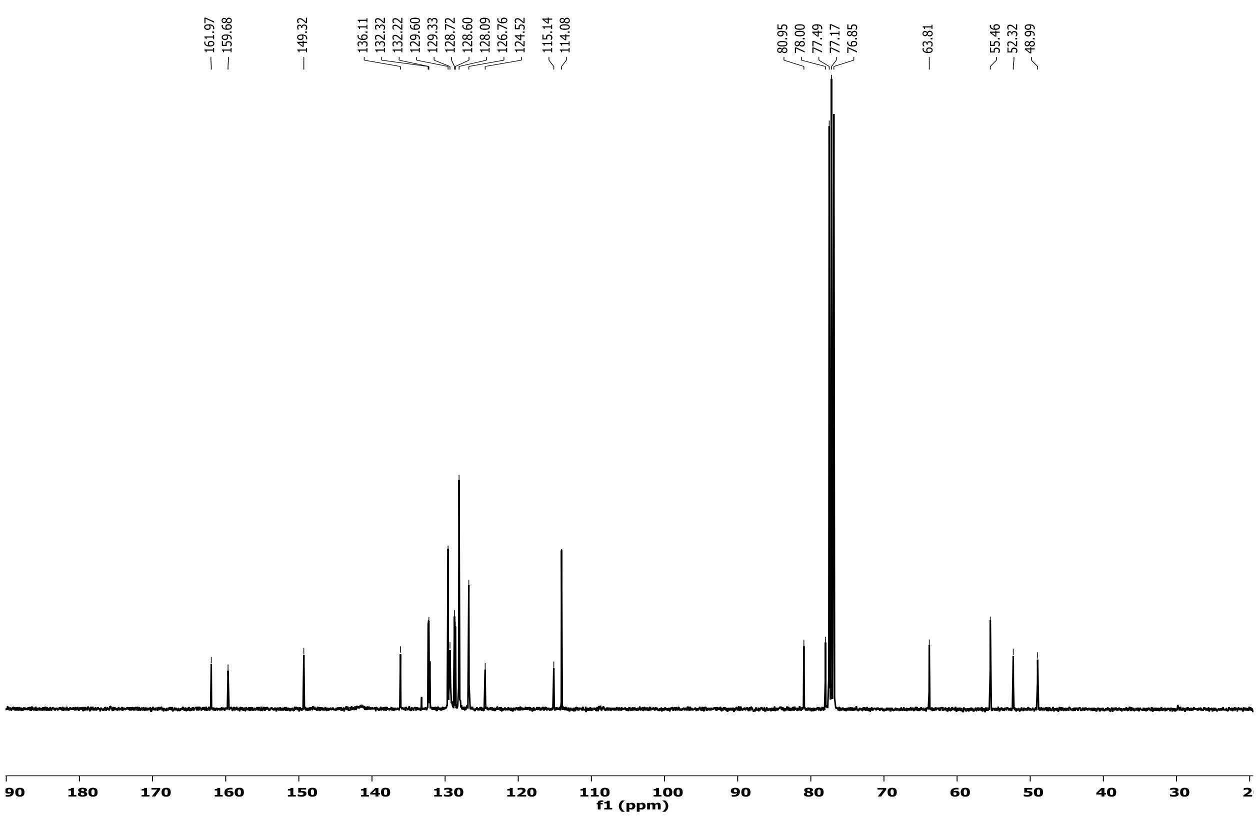
13C-NMR of compound **1d**, $CDCl_3$, 100 MHz



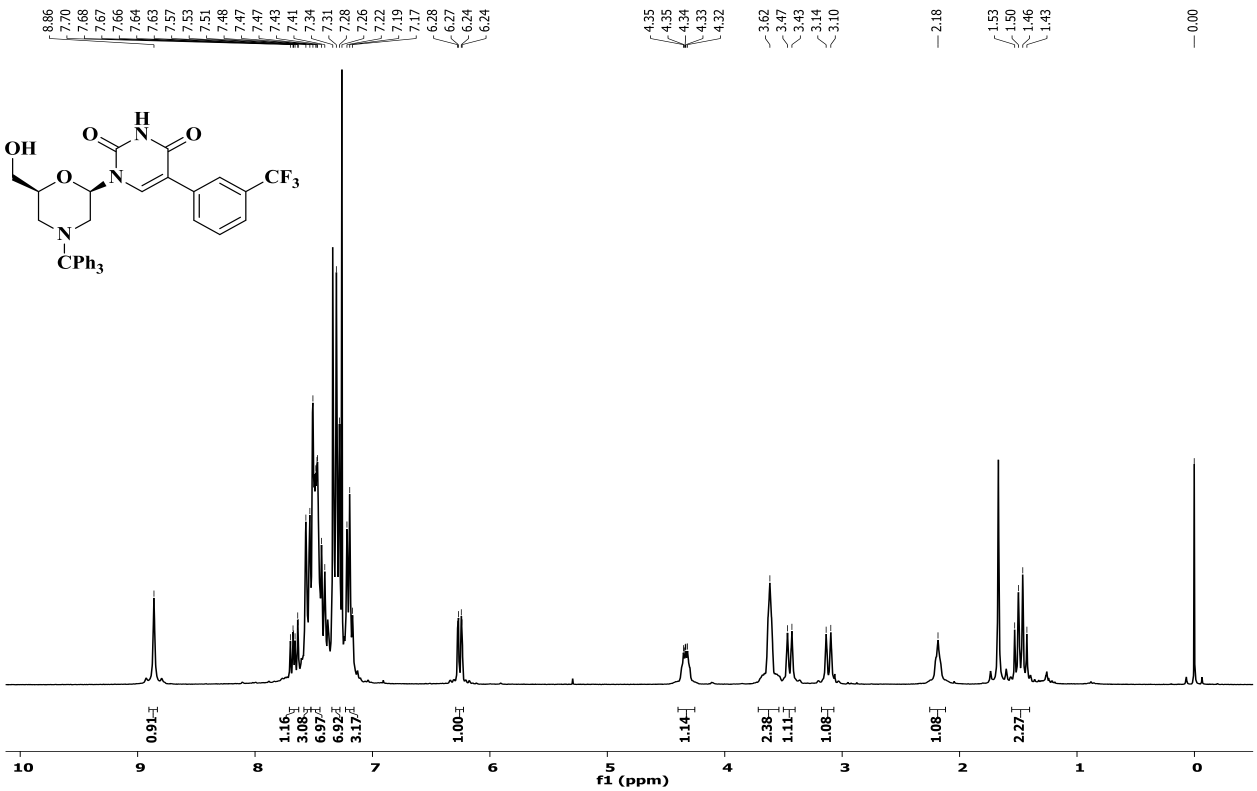
1H-NMR of compound **1e**, $CDCl_3$, 300 MHz



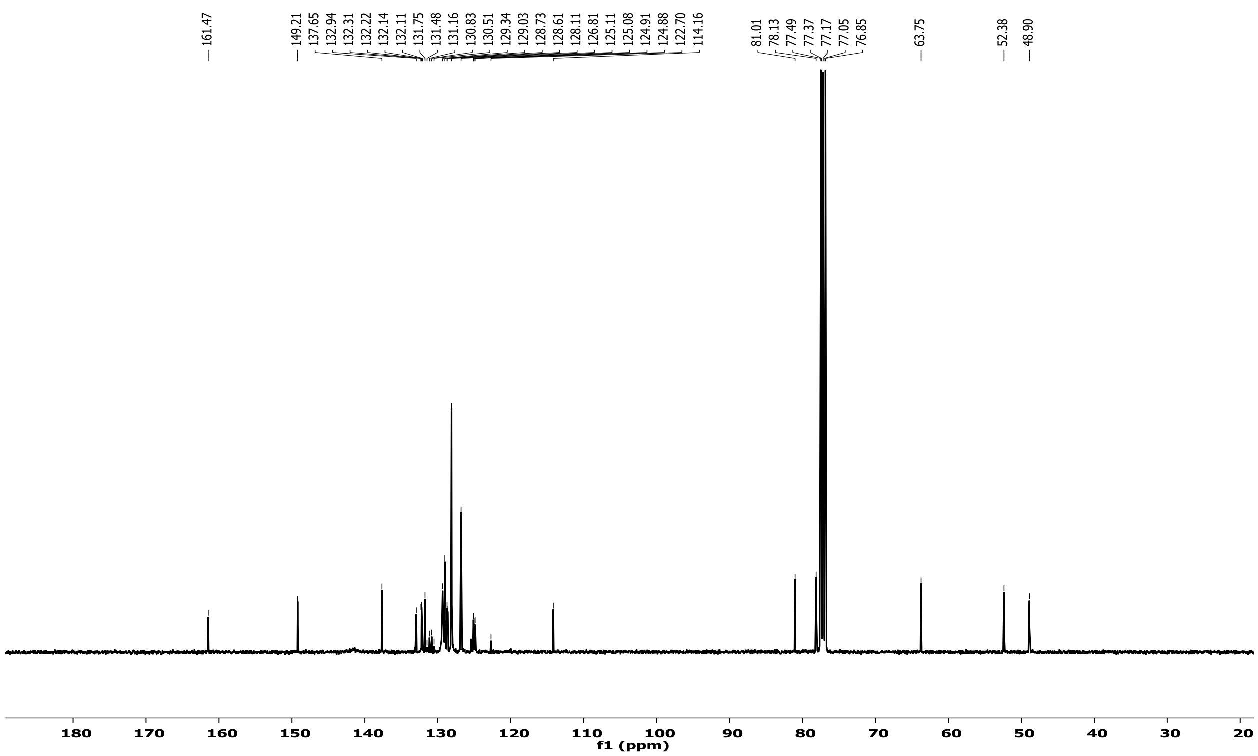
13C-NMR of compound **1e**, $CDCl_3$, 100 MHz



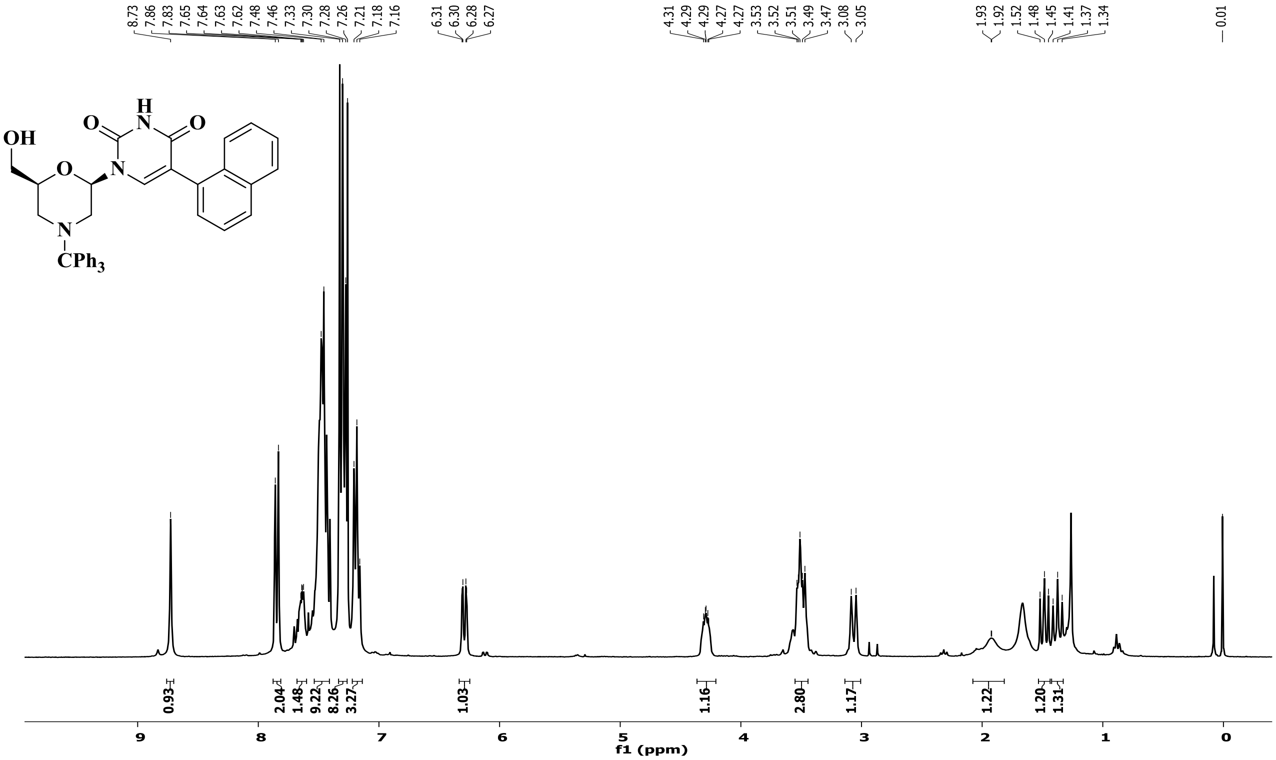
1H-NMR of compound **1f**, $CDCl_3$, 300 MHz



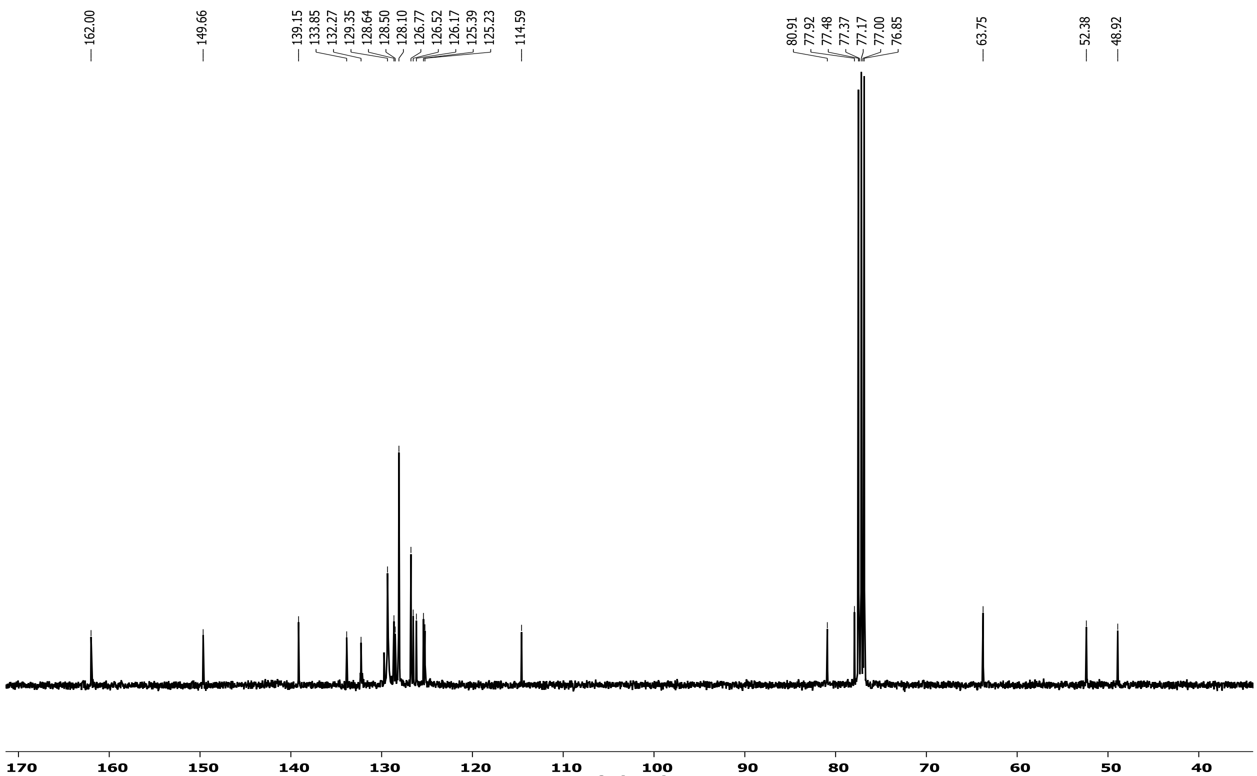
13C-NMR compound **1f**, $CDCl_3$, 100 MHz



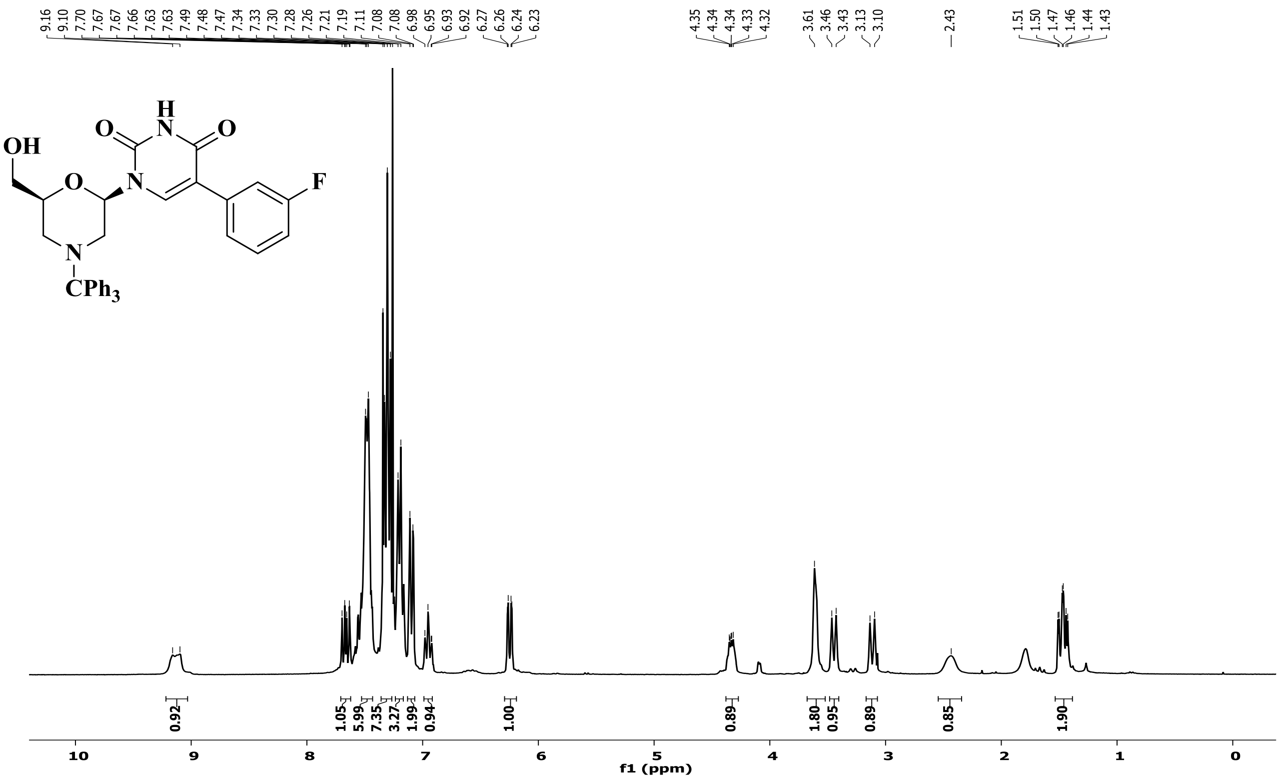
1H-NMR of compound **1g**, $CDCl_3$, 300 MHz



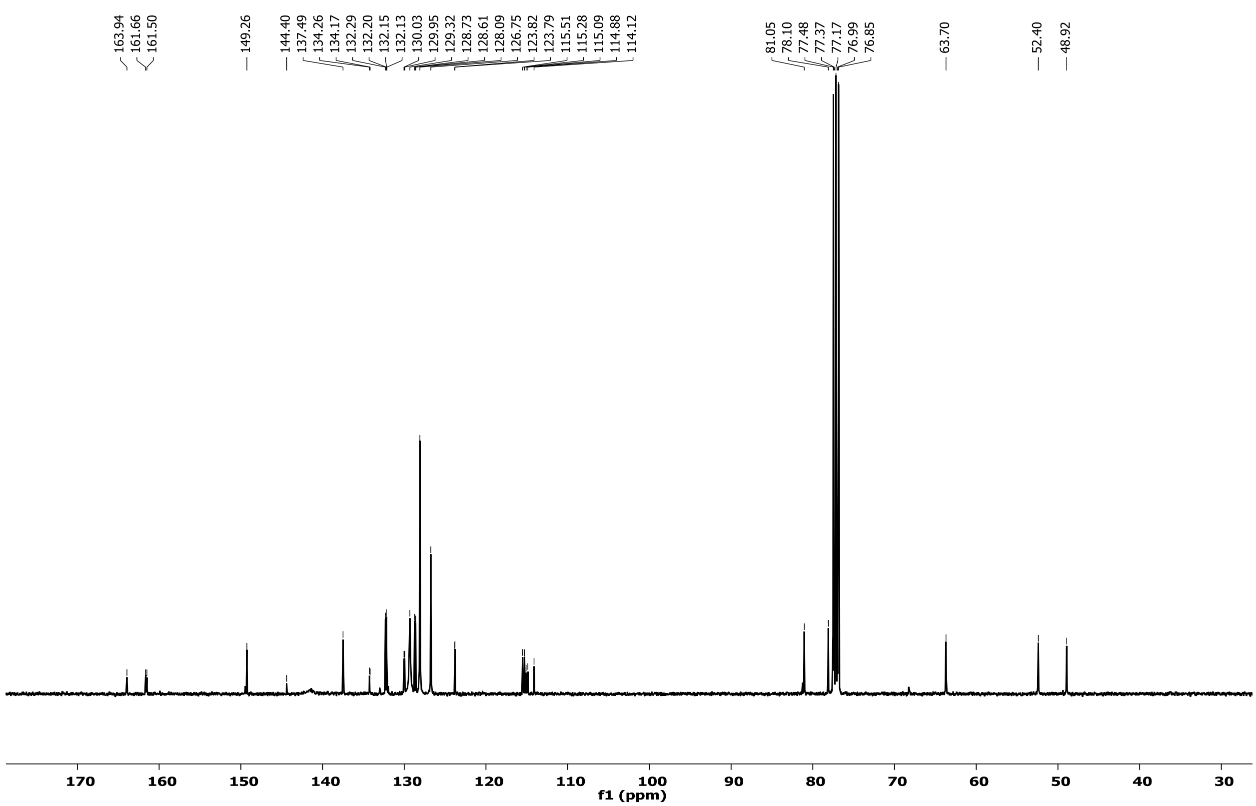
13C-NMR of compound **1g**, $CDCl_3$, 100 MHz



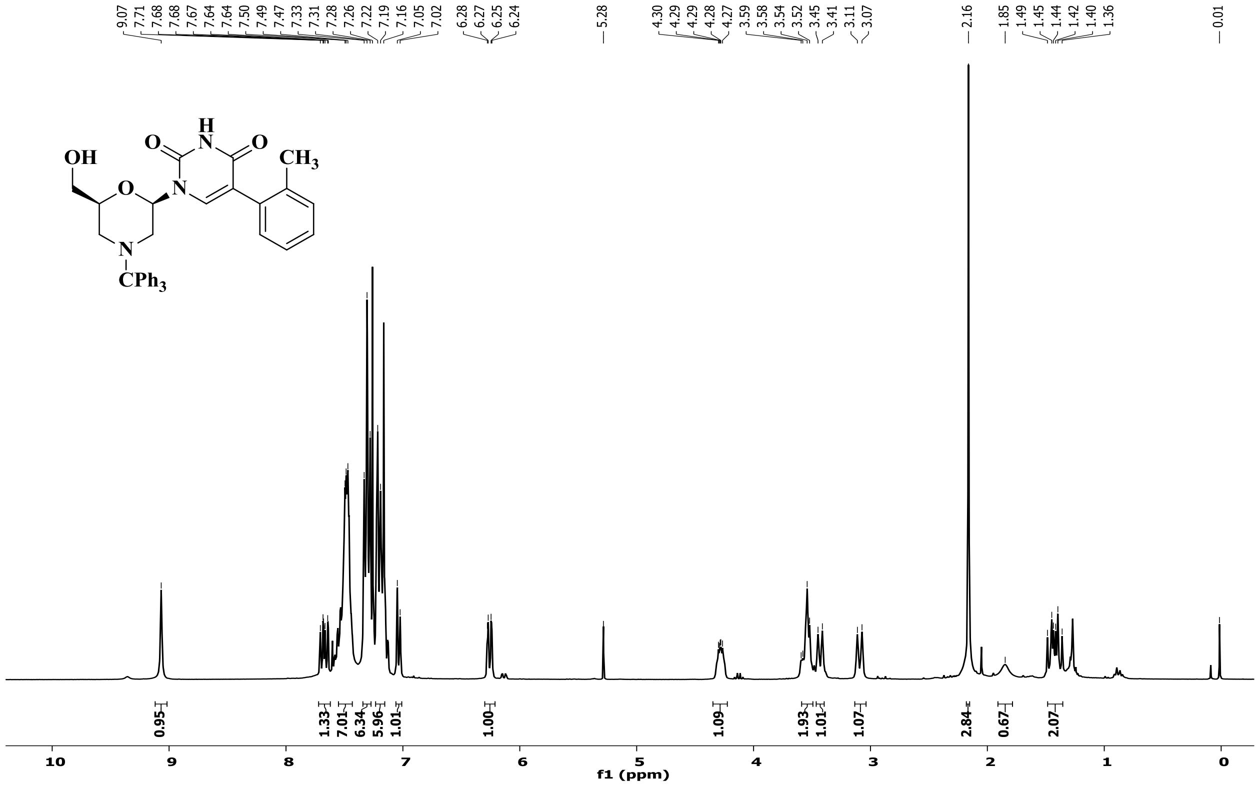
1H-NMR of compound **1h**, $CDCl_3$, 300 MHz



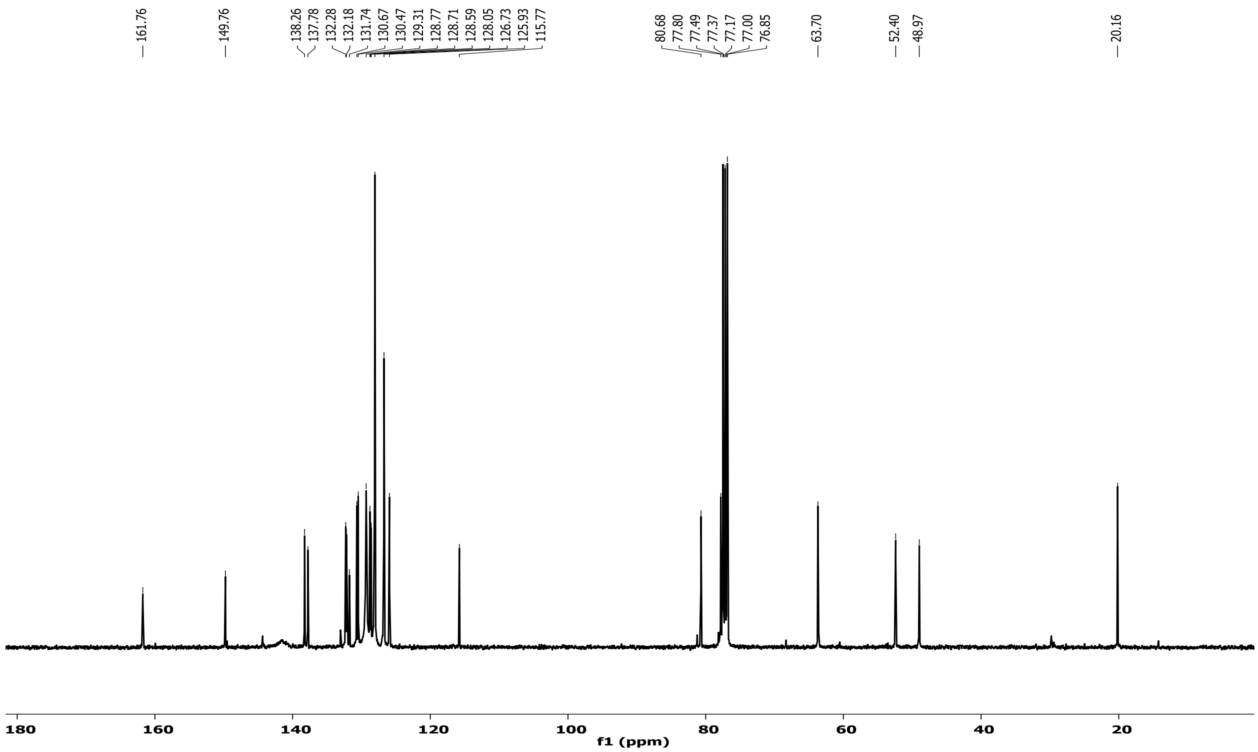
13C-NMR of compound **1h**, $CDCl_3$, 100 MHz



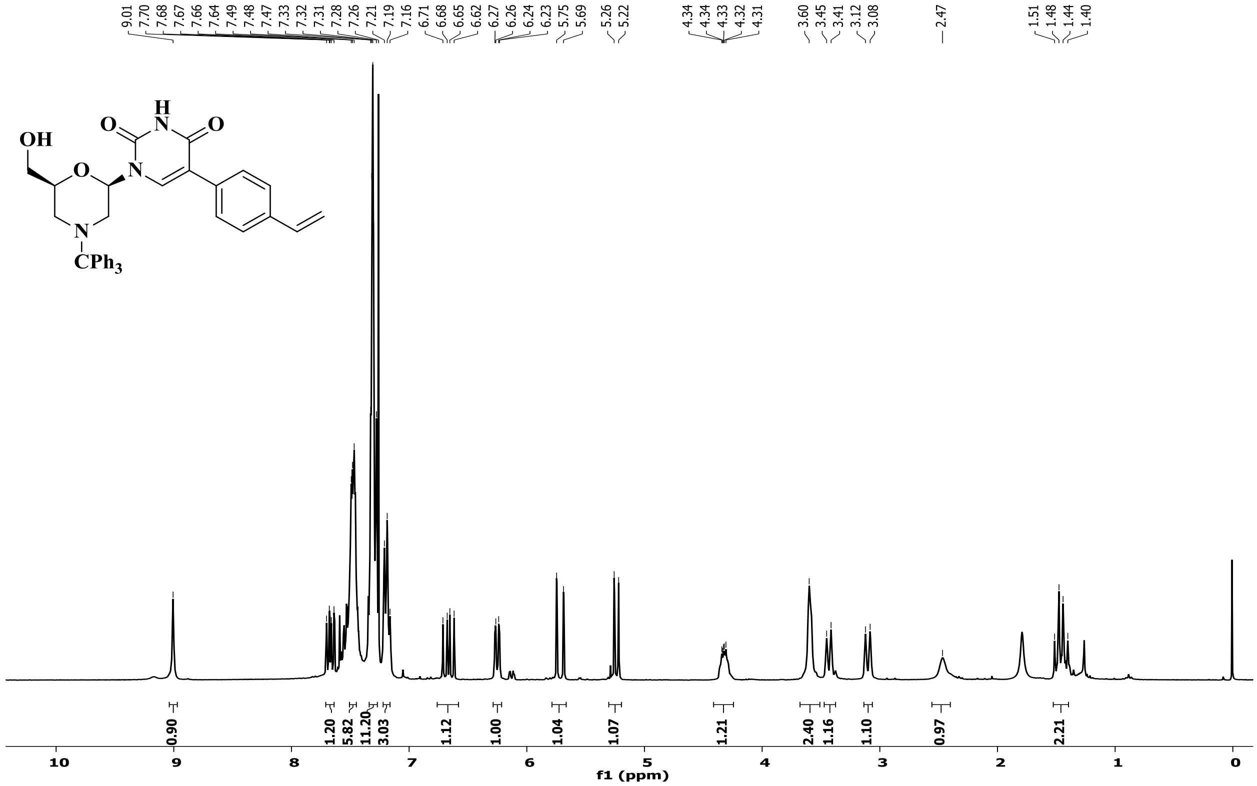
1H-NMR of compound **1i**, $CDCl_3$, 300 MHz



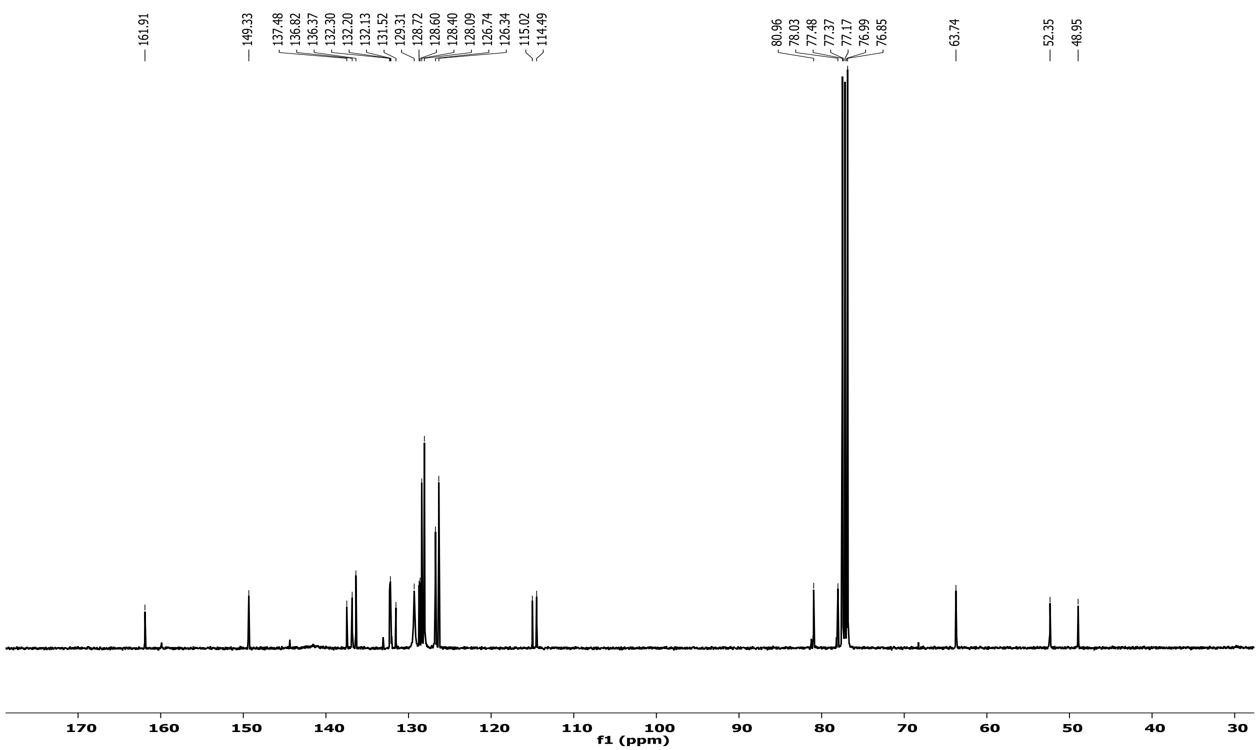
13C-NMR of compound **1i**, $CDCl_3$, 100 MHz



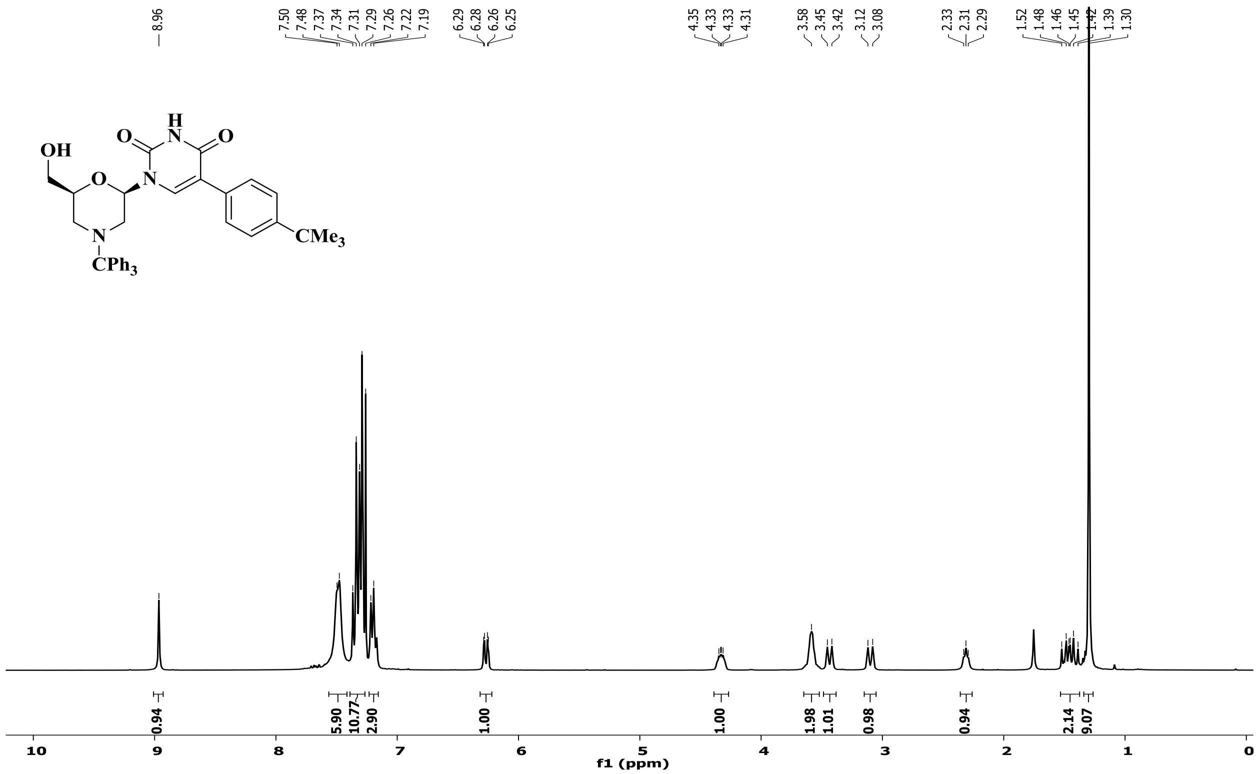
1H-NMR of compound **1j**, $CDCl_3$, 300 MHz



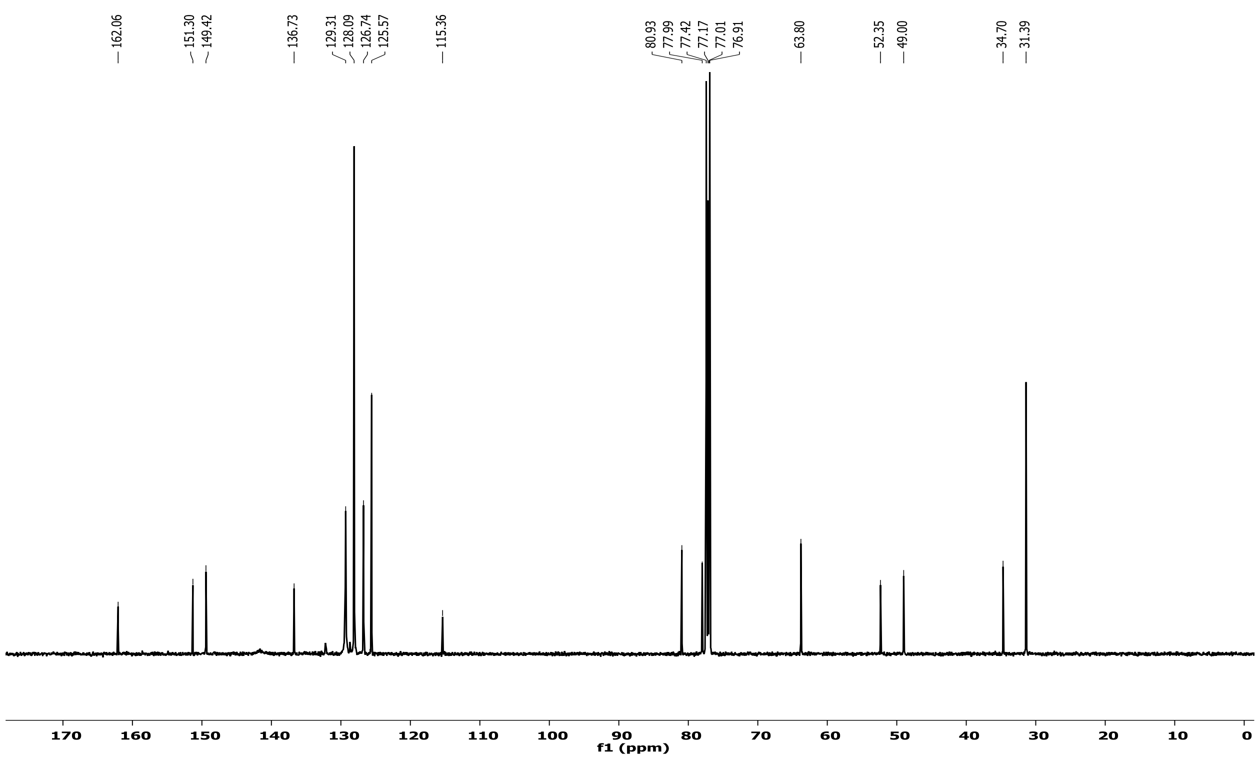
13C-NMR of compound **1j**, $CDCl_3$, 100 MHz



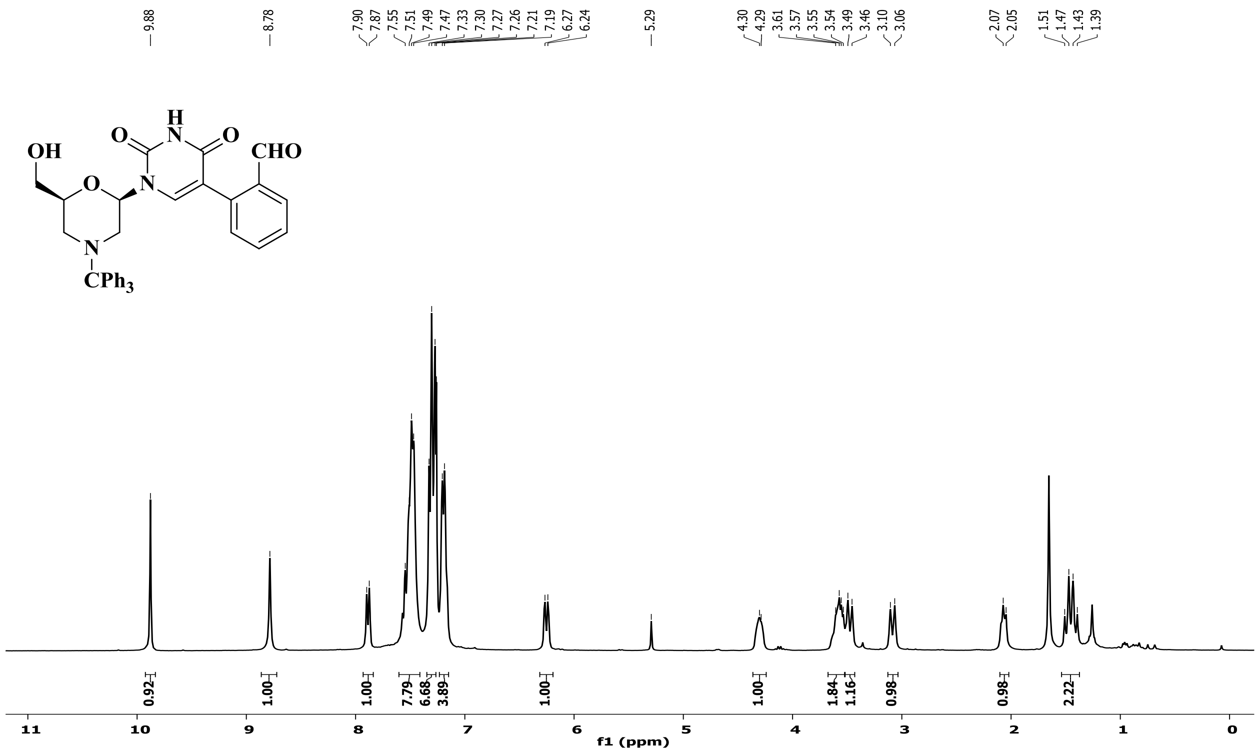
1H-NMR of compound **1k**, $CDCl_3$, 300 MHz



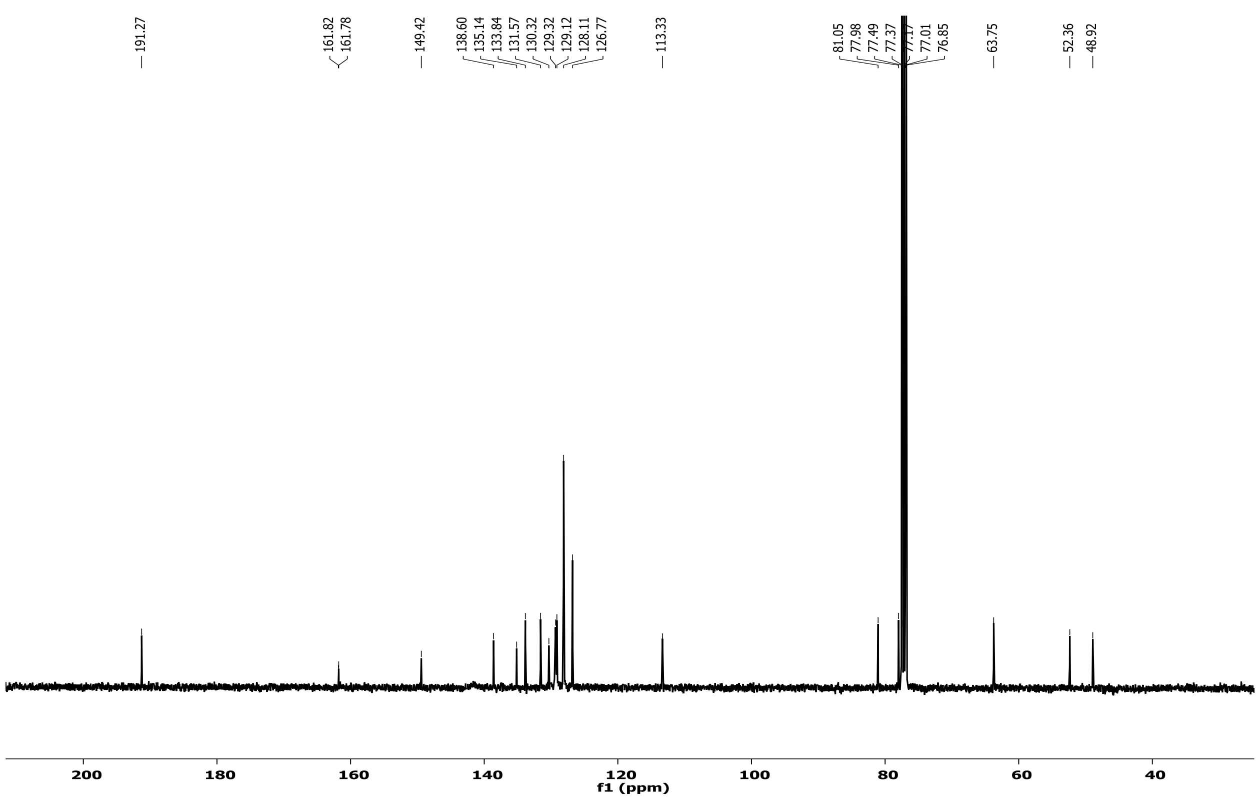
13C-NMR of compound **1k**, $CDCl_3$, 100 MHz



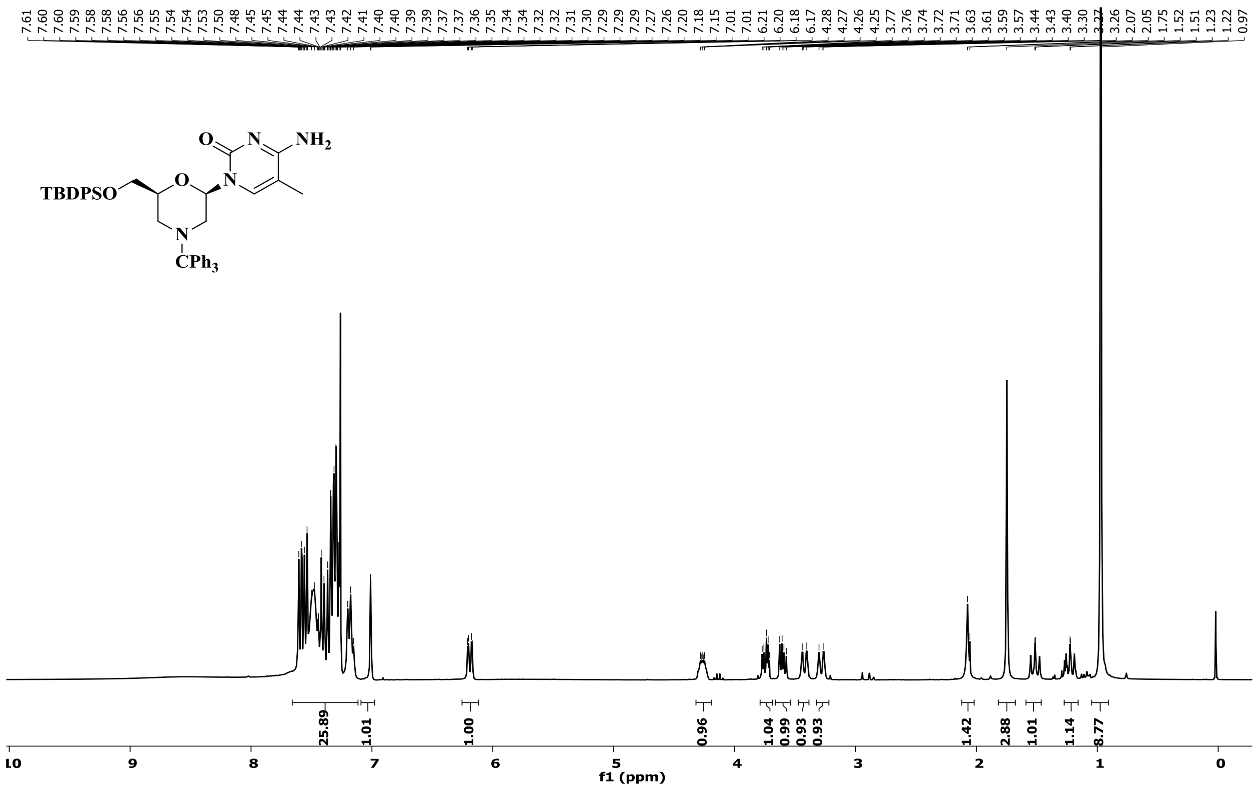
1H-NMR of compound **1l**, $CDCl_3$, 300 MHz



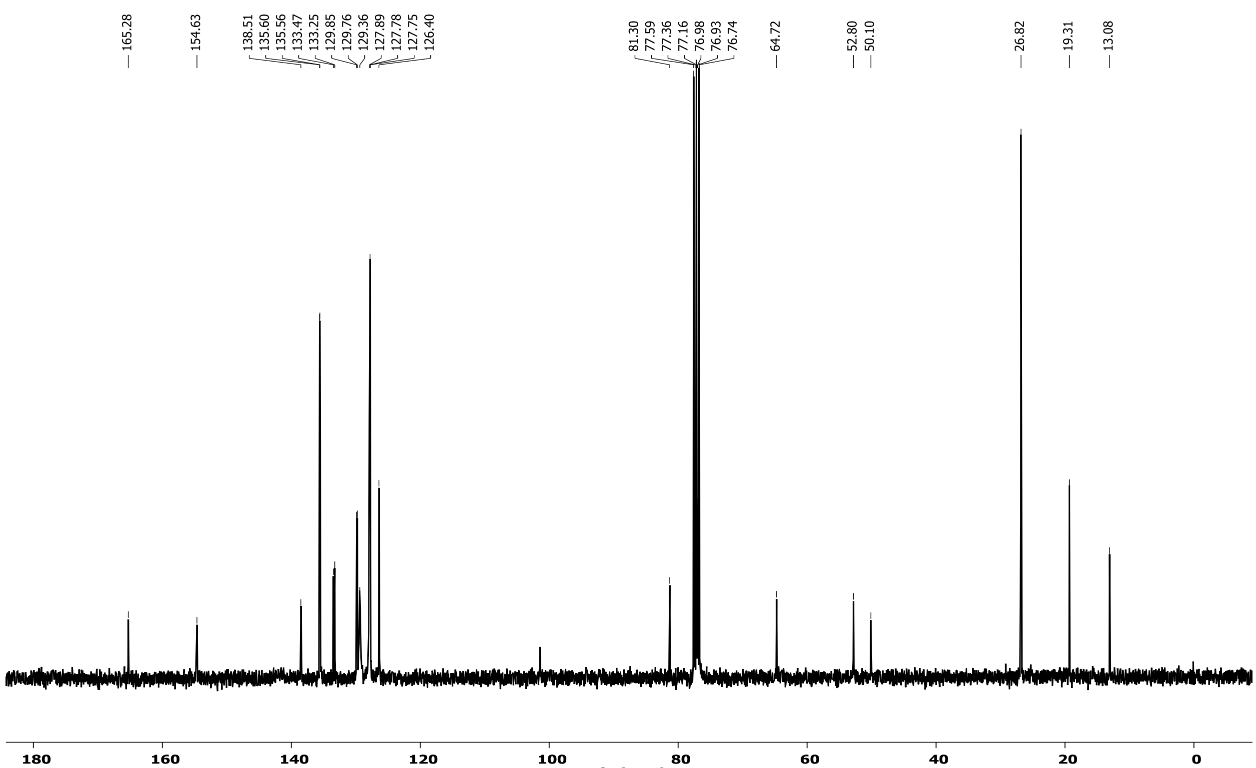
13C-NMR of compound **1l**, $CDCl_3$, 100 MHz



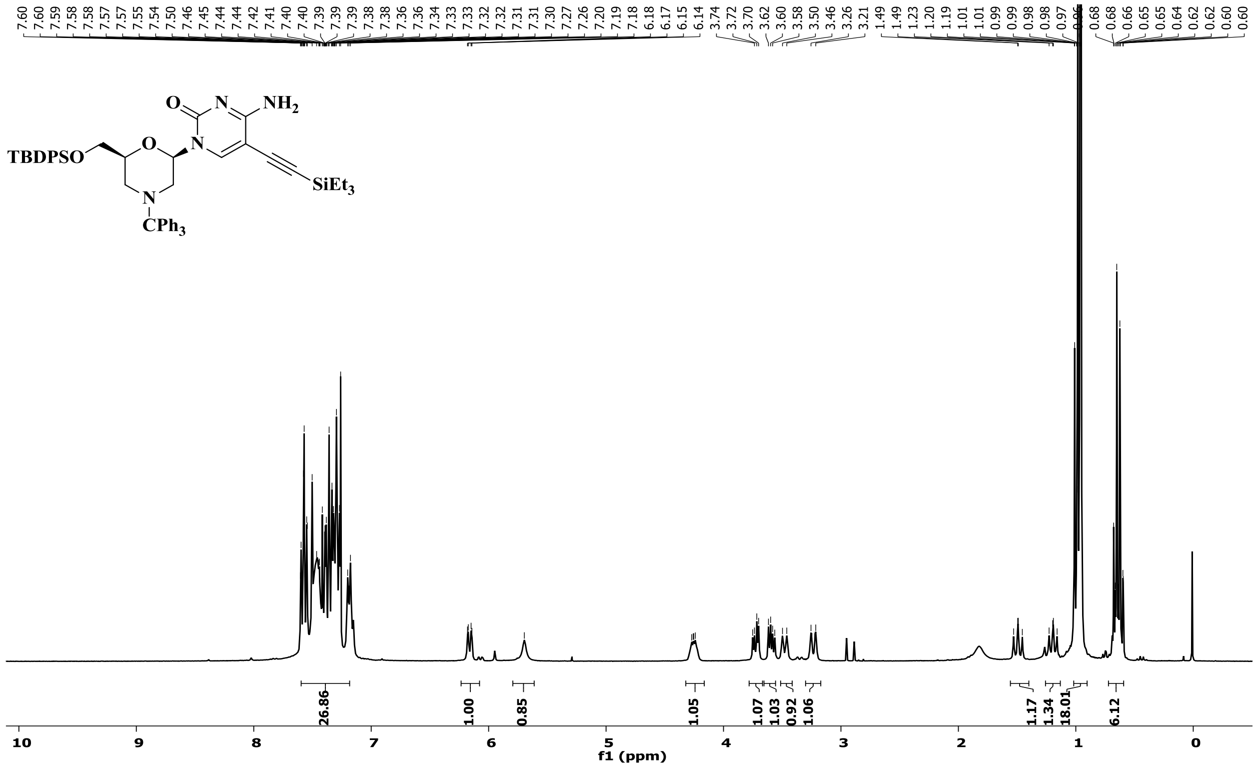
1H-NMR of compound **2c**, $CDCl_3$, 300 MHz



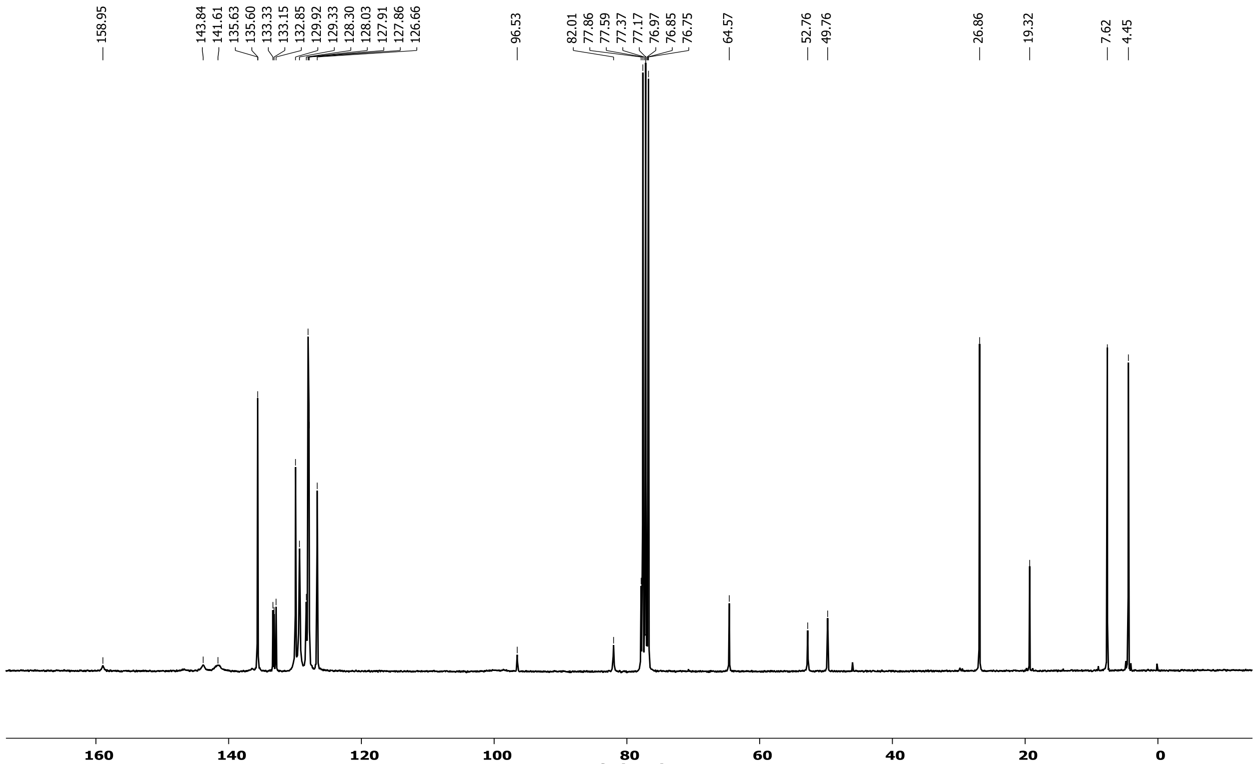
13C-NMR of compound **2c**, $CDCl_3$, 100 MHz



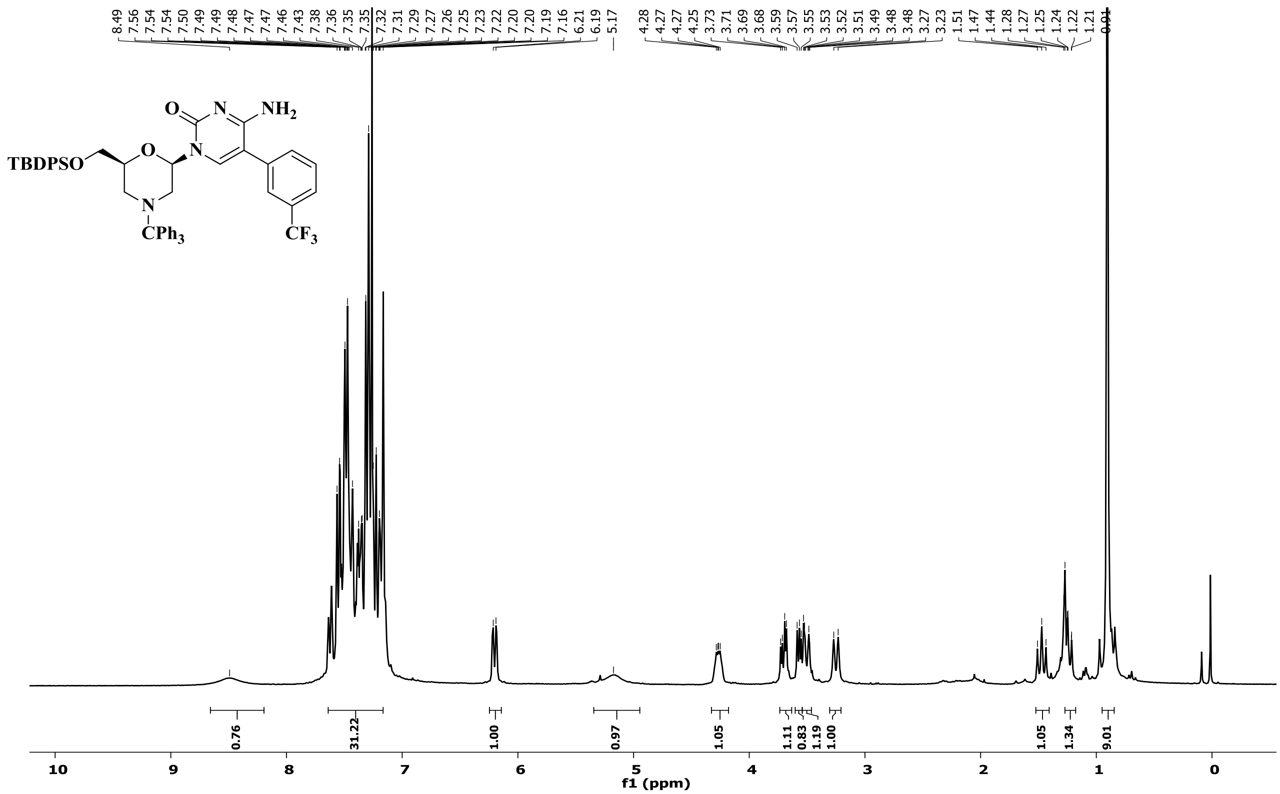
1H-NMR of compound **3a**, CDCl$_3$, 300 MHz



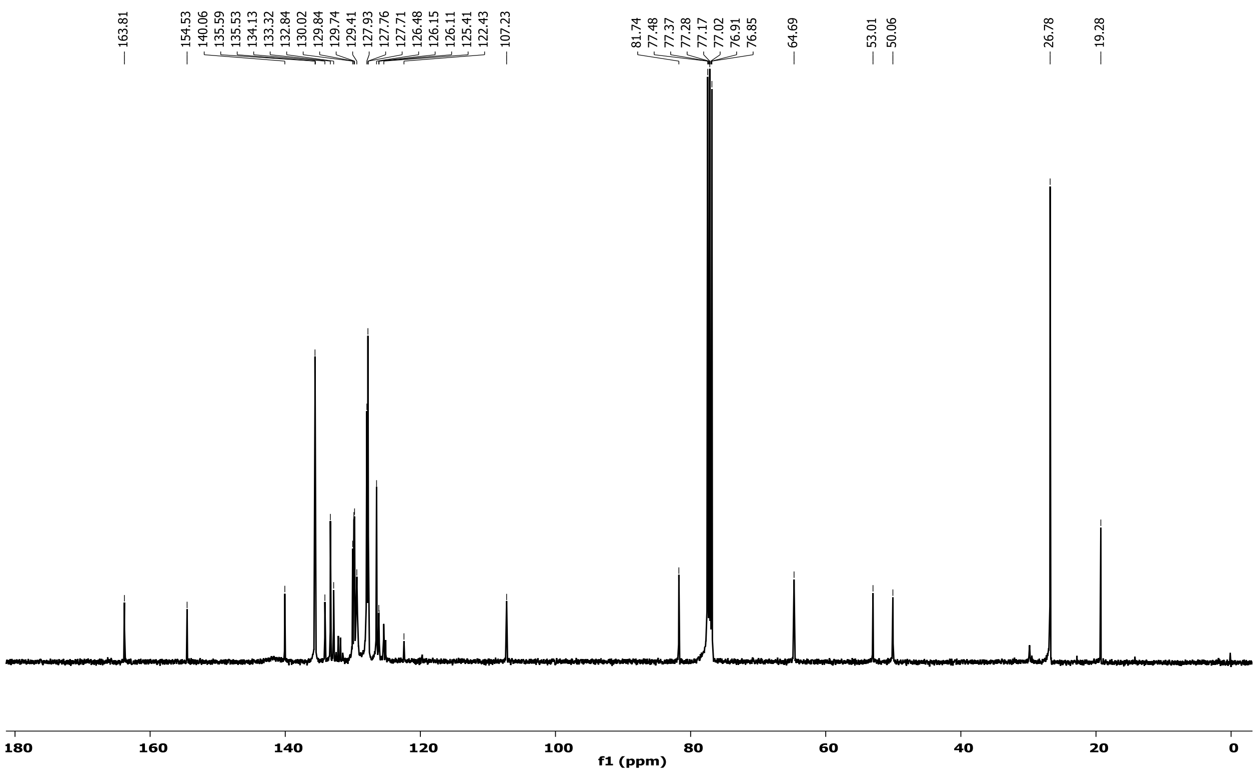
1H-NMR of compound **3a**, CDCl$_3$, 75 MHz



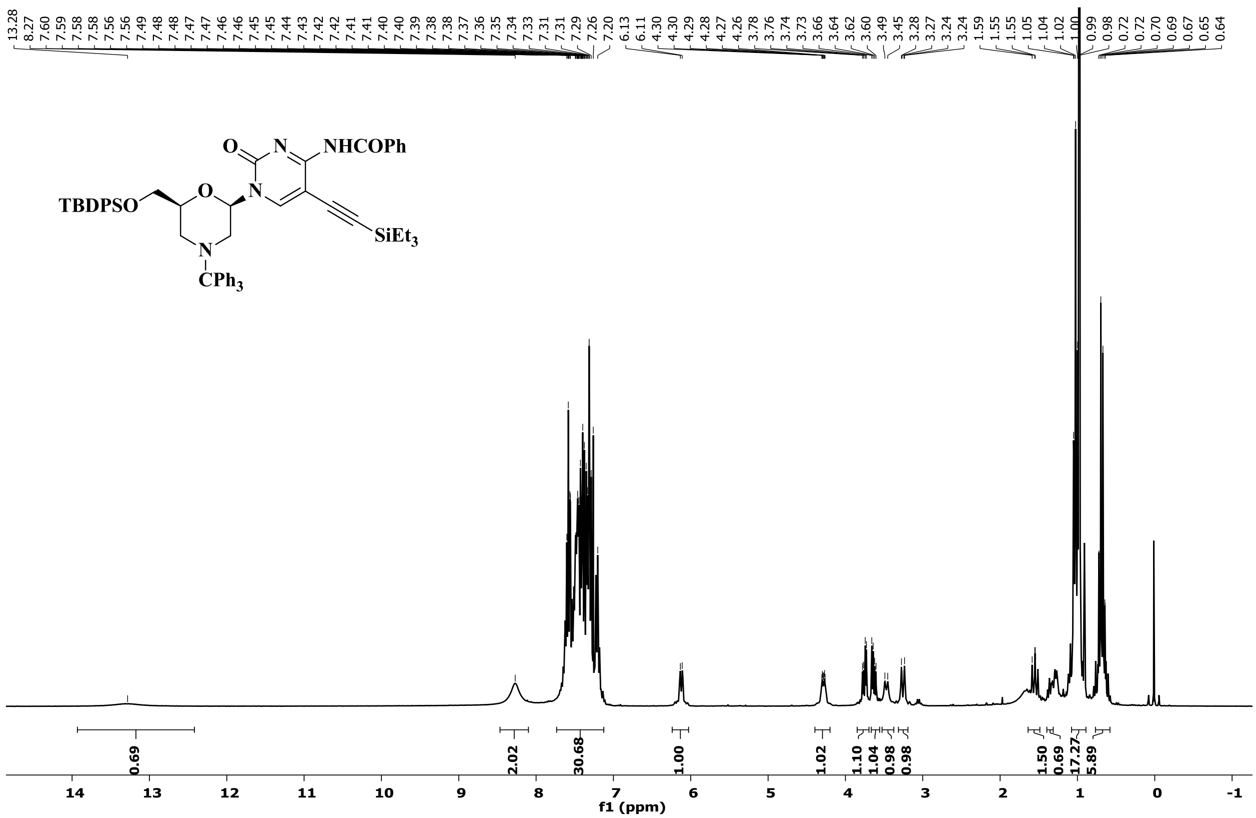
1H-NMR of compound **3e**, $CDCl_3$, 300 MHz



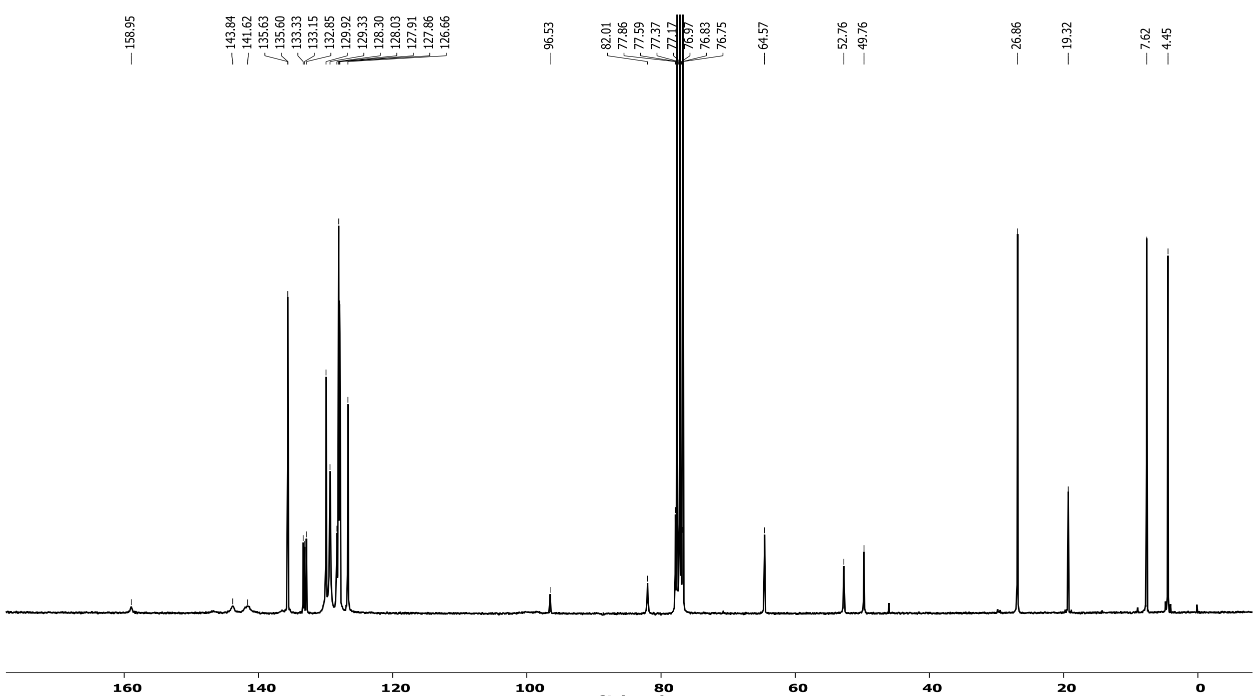
13C-NMR of compound **3e**, $CDCl_3$, 100 MHz



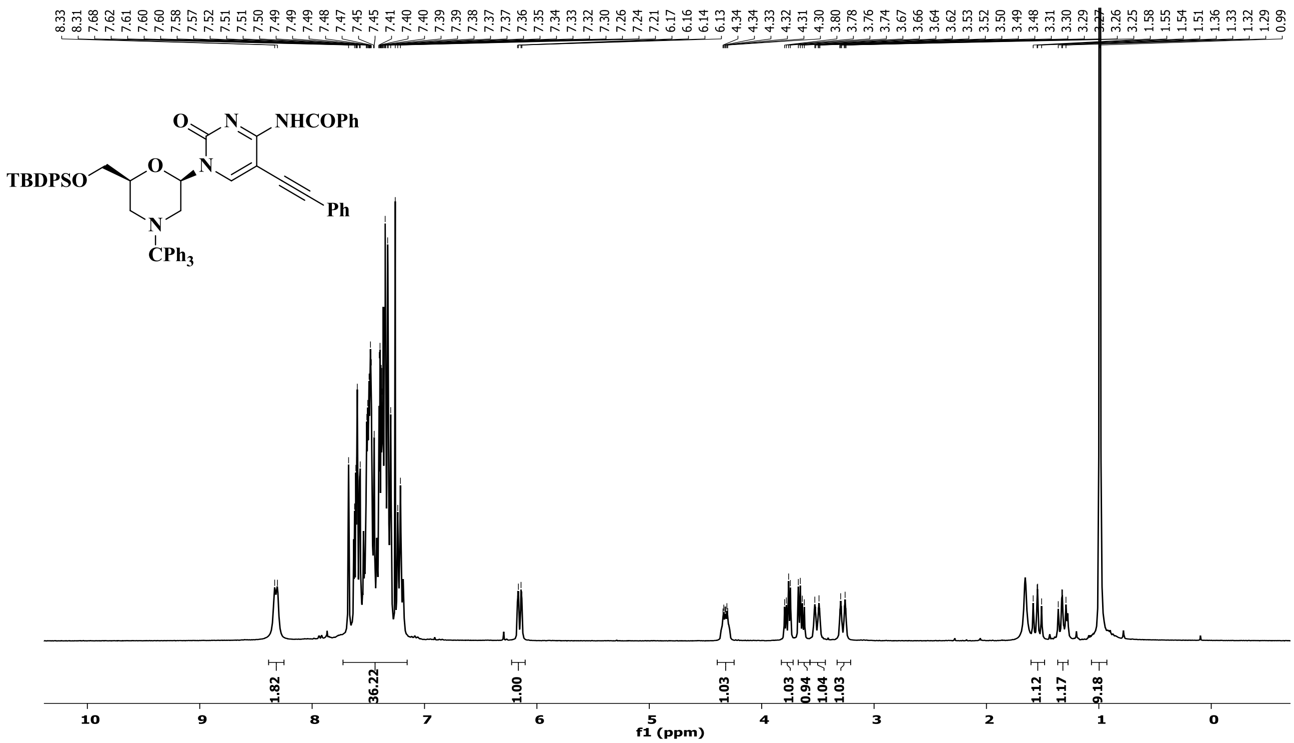
1H-NMR of compound **4a**, $CDCl_3$, 300 MHz



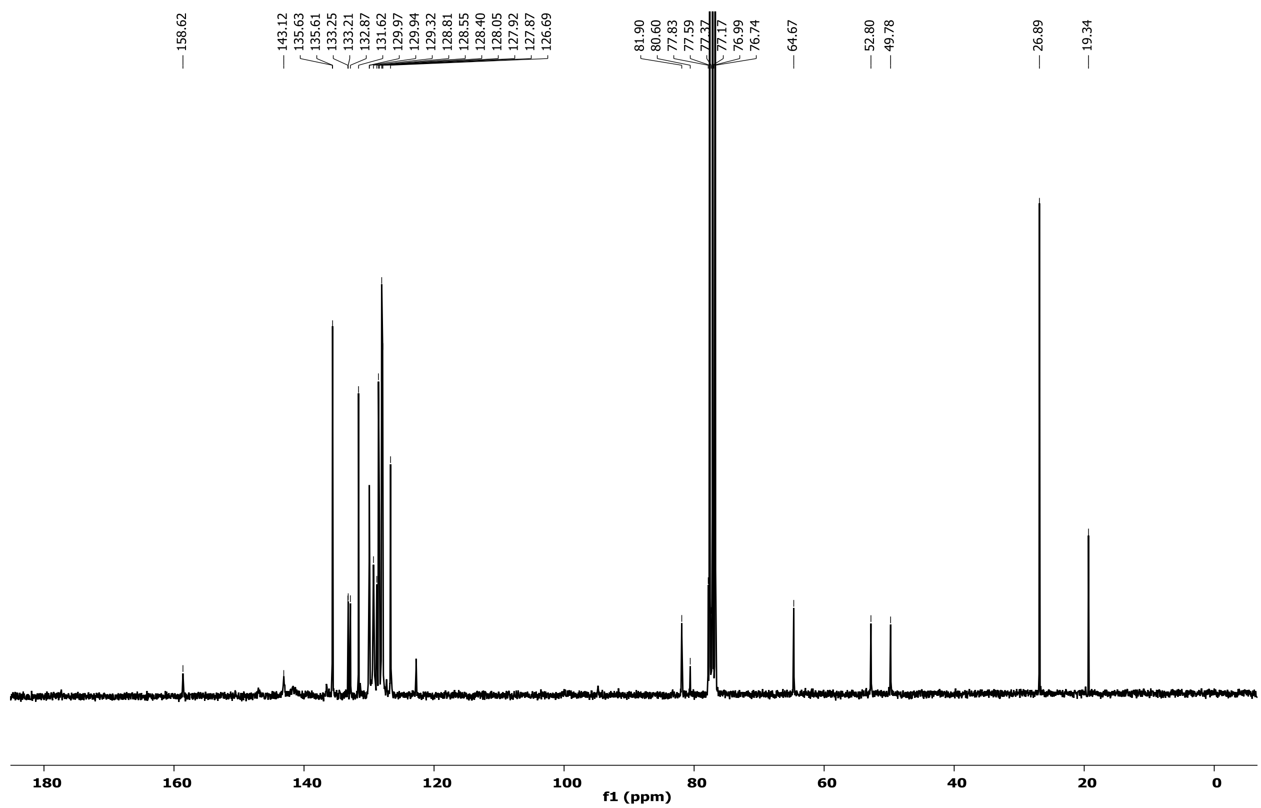
13C-NMR of compound **4a**, $CDCl_3$, 75 MHz



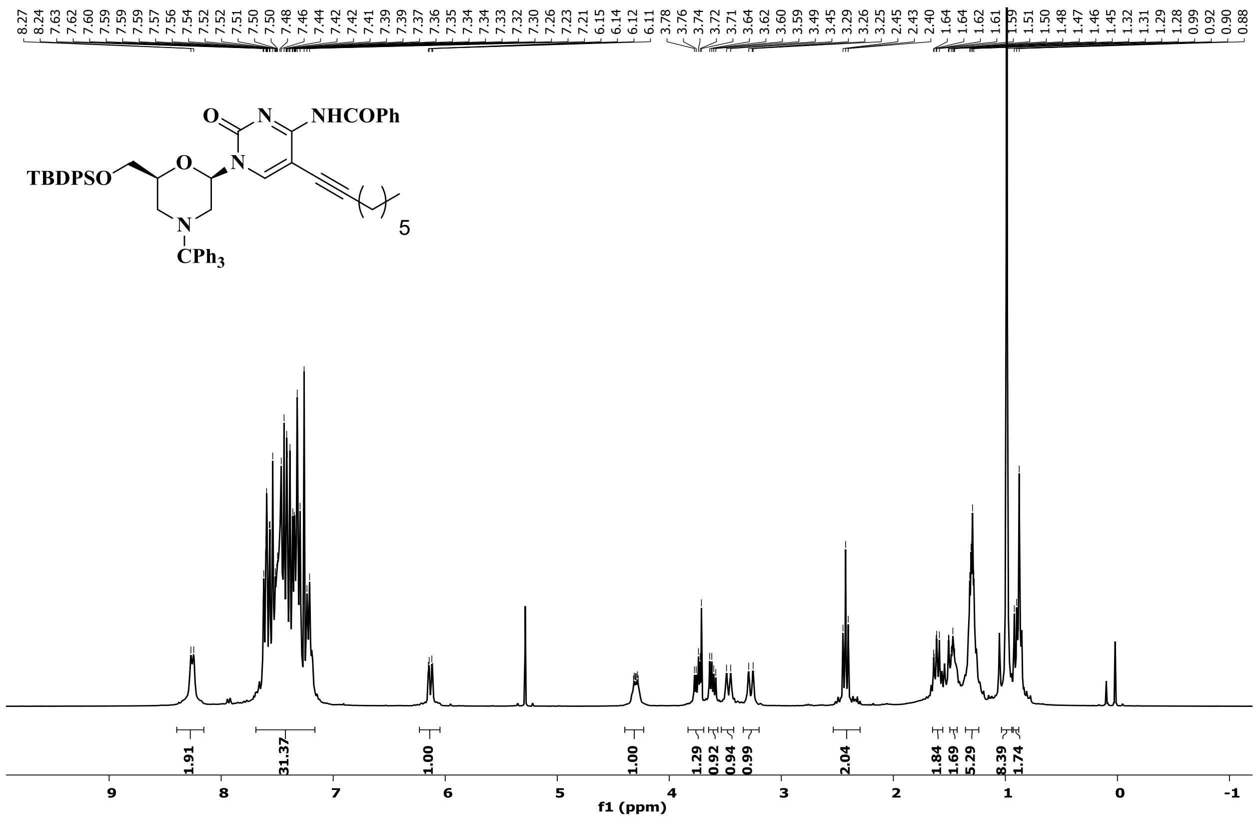
1H-NMR of compound **4b**, $CDCl_3$, 300 MHz



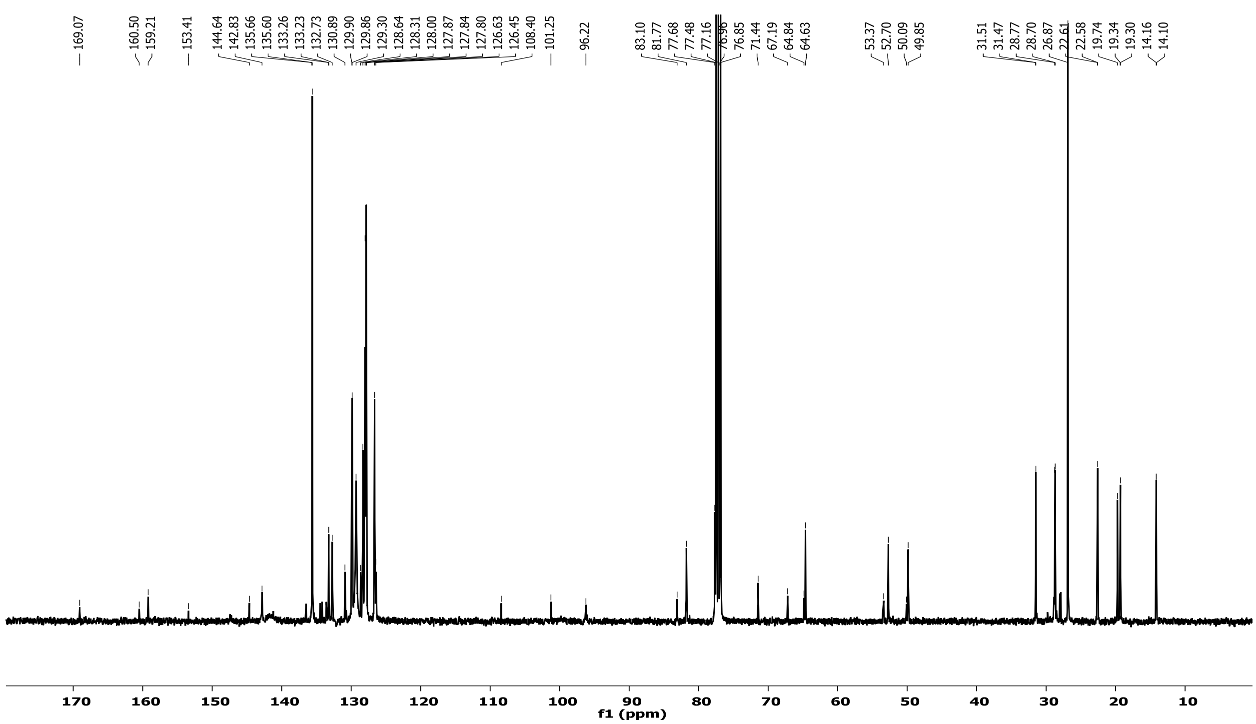
13C-NMR of compound **4b**, $CDCl_3$, 75 MHz



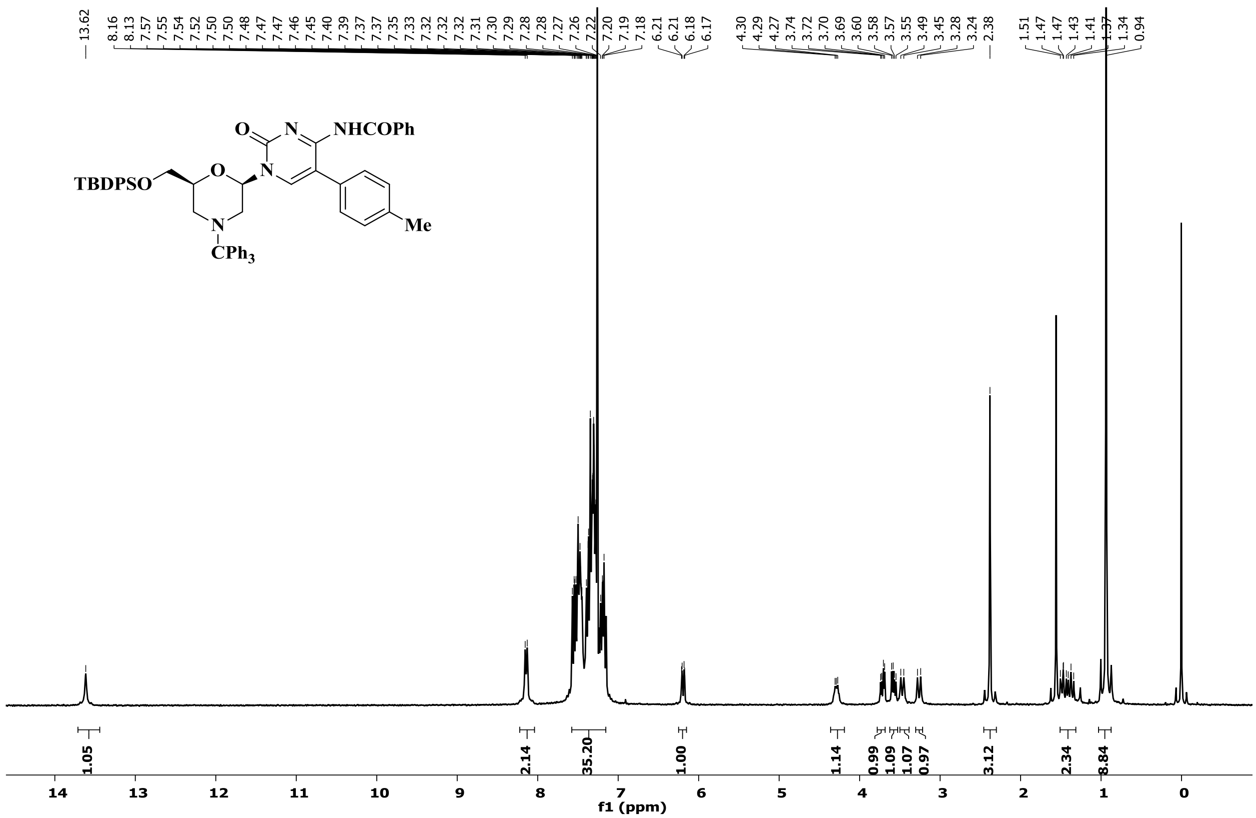
1H-NMR of compound **4c**, $CDCl_3$, 300 MHz



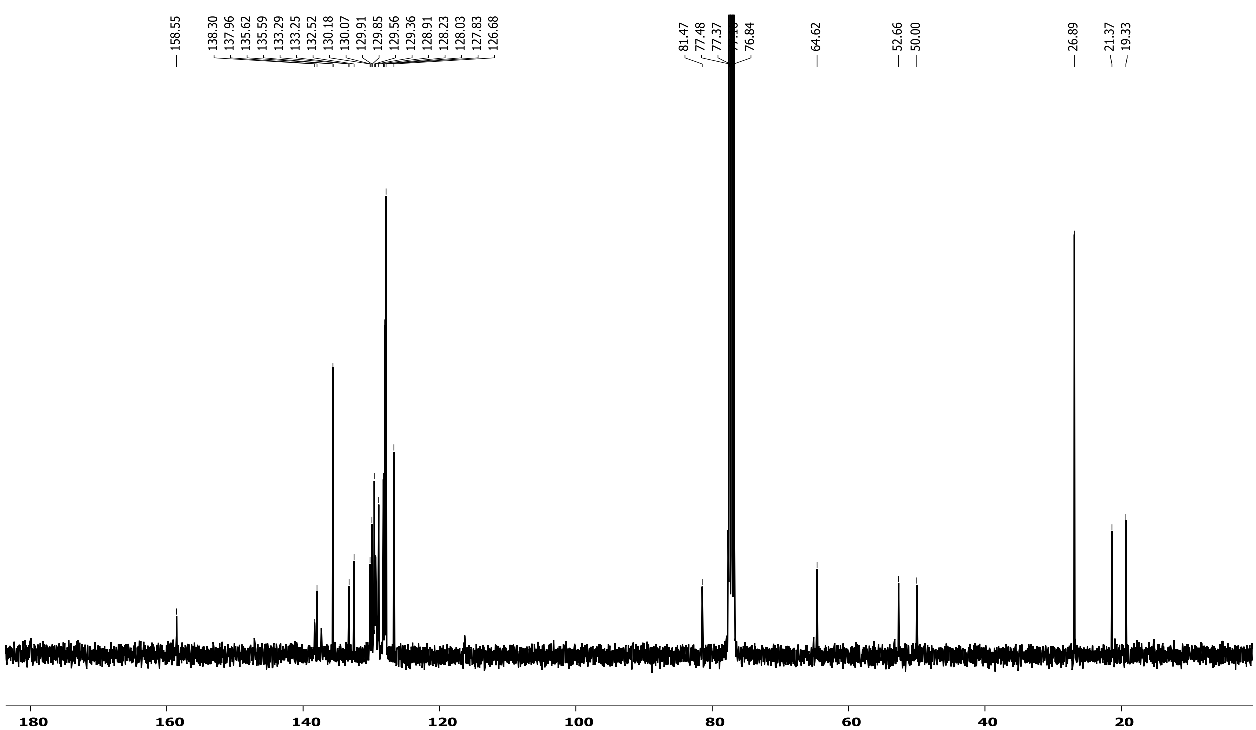
13C-NMR of compound **4c**, $CDCl_3$, 100 MHz



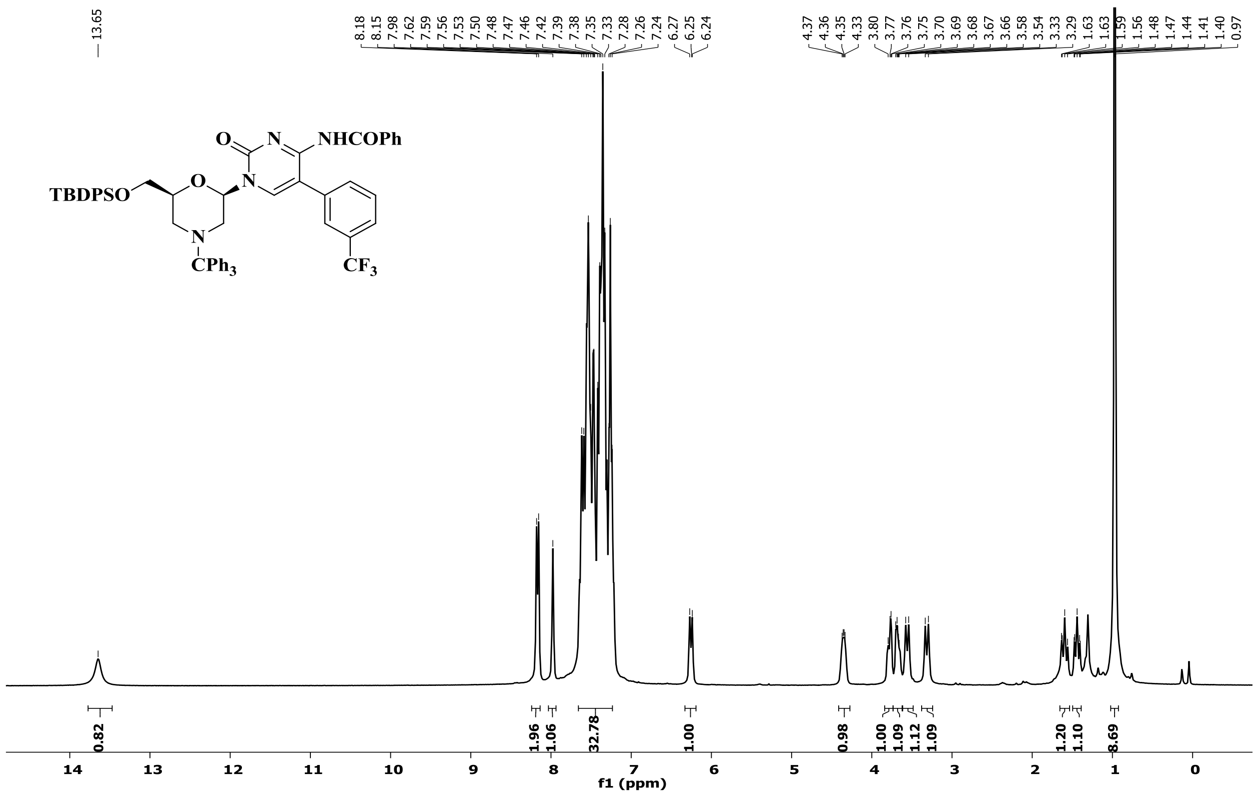
1H-NMR of compound **4d**, $CDCl_3$, 300 MHz



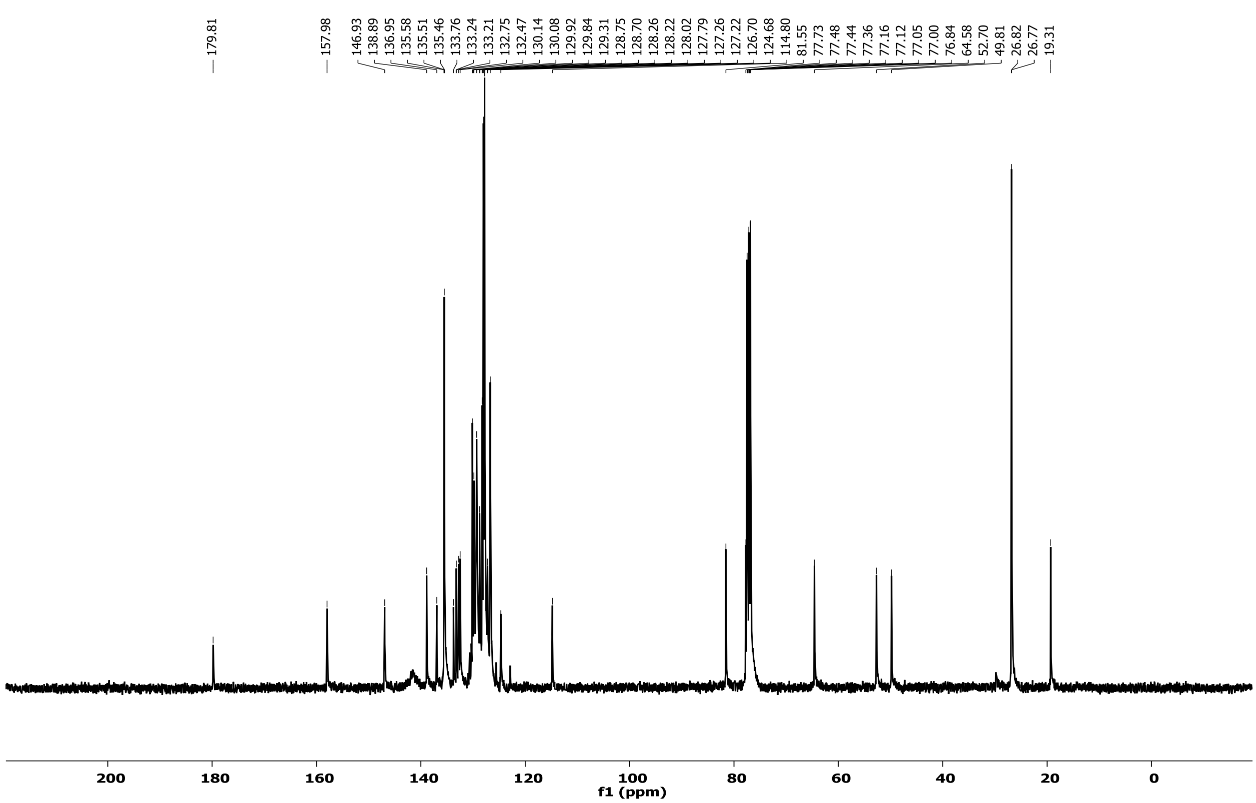
13C-NMR of compound **4d**, $CDCl_3$, 100 MHz



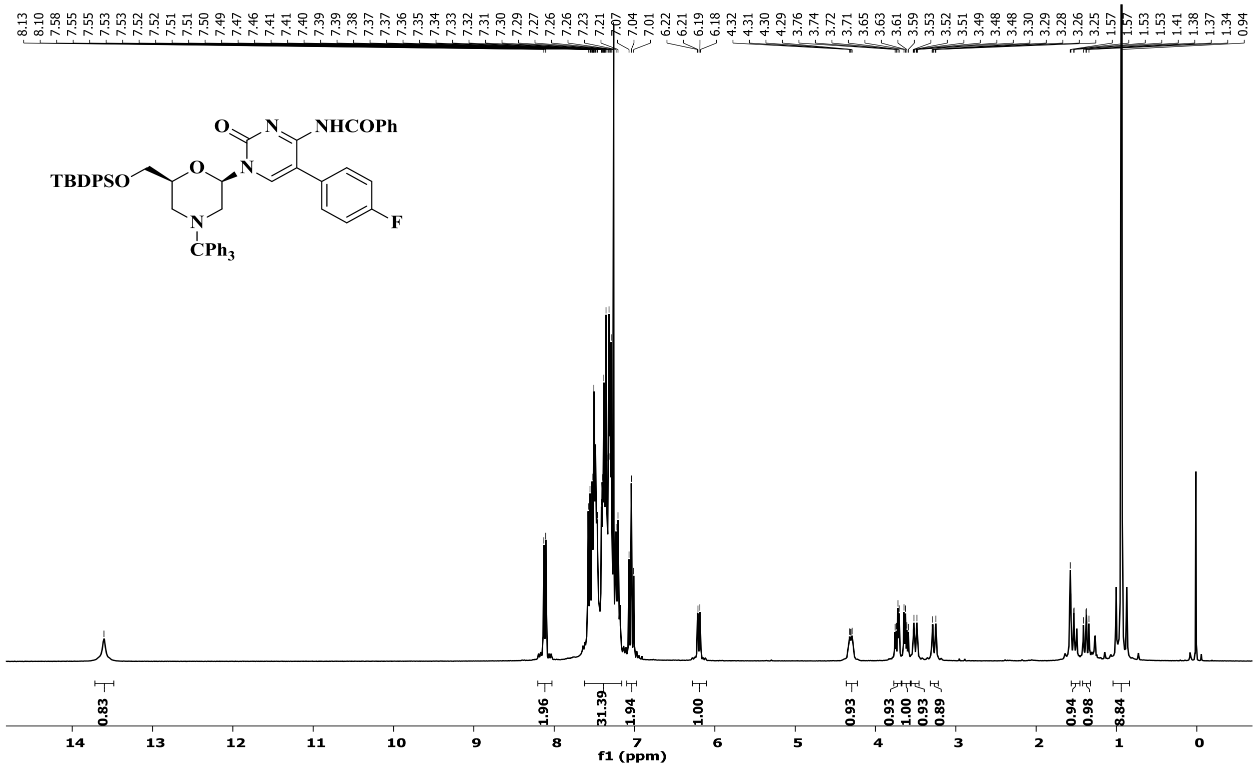
1H-NMR of compound **4e**, $CDCl_3$, 300 MHz



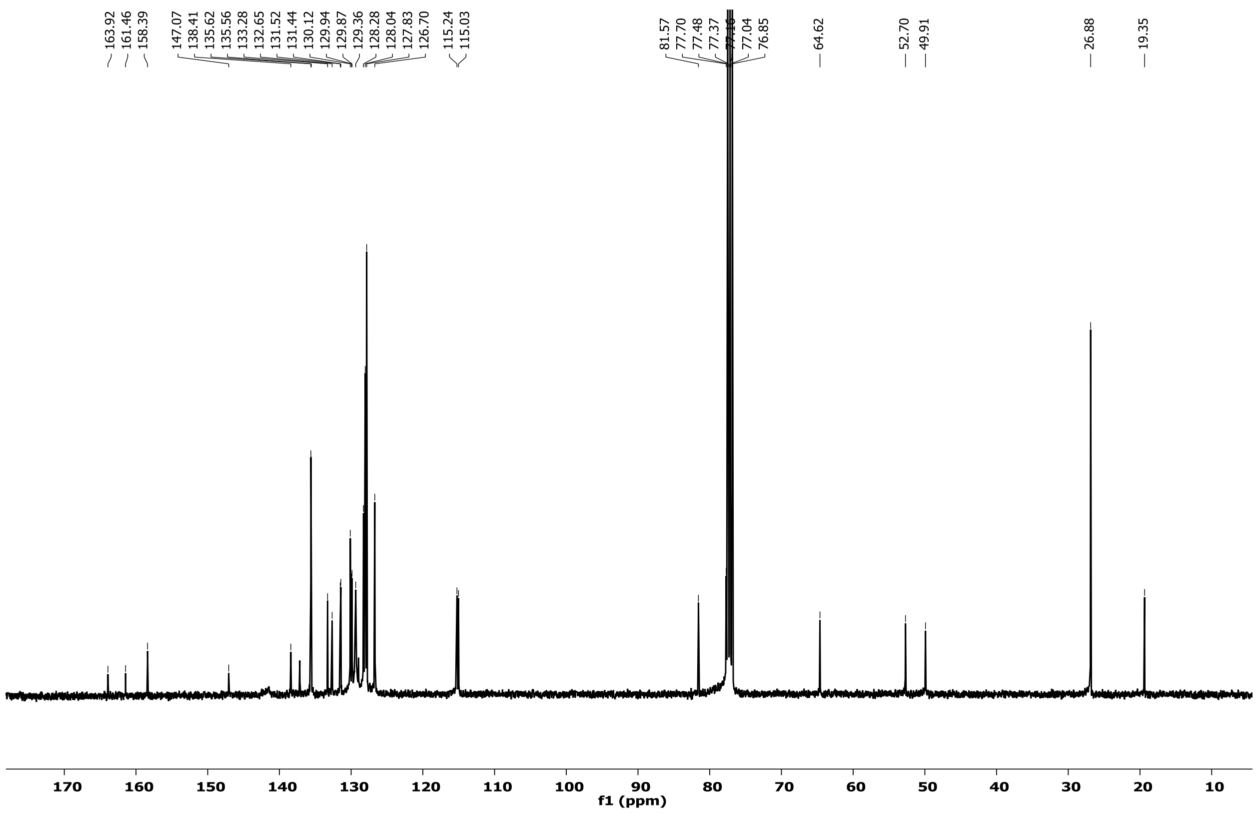
13C-NMR of compound **4e**, $CDCl_3$, 100 MHz



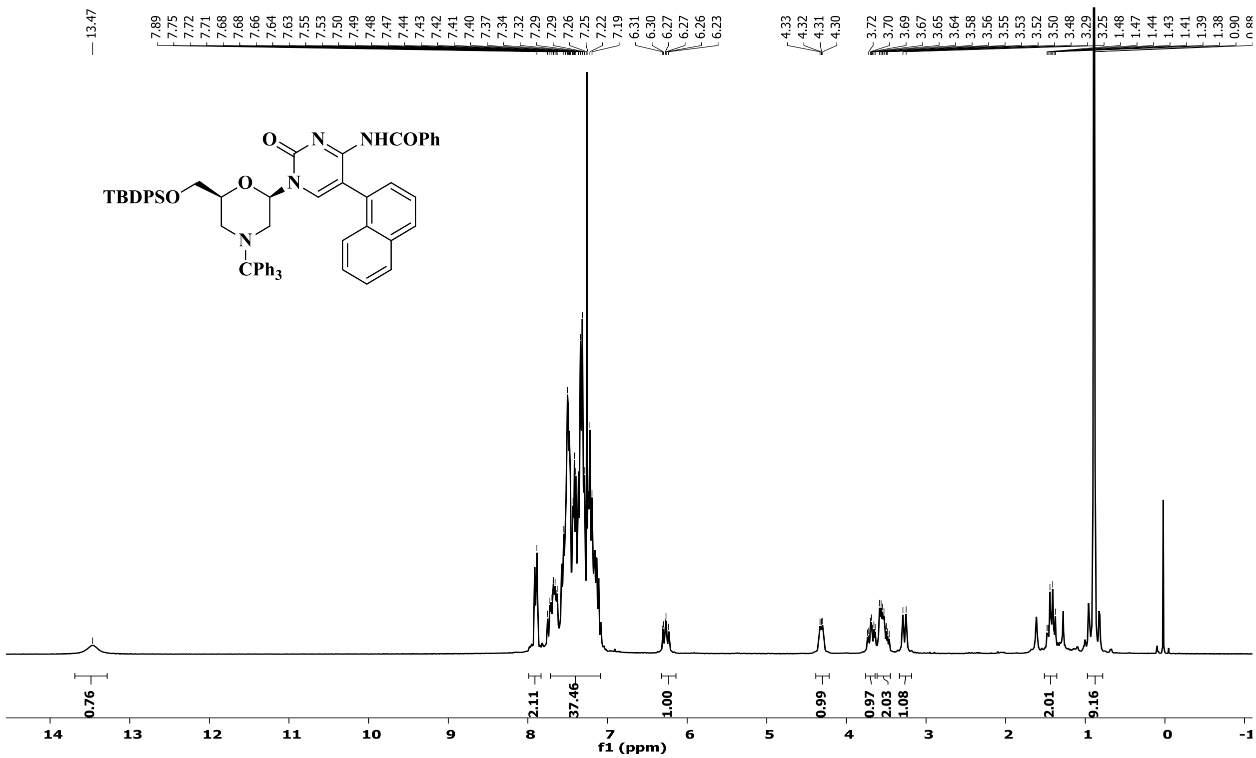
1H-NMR of compound **4f**, $CDCl_3$, 300 MHz



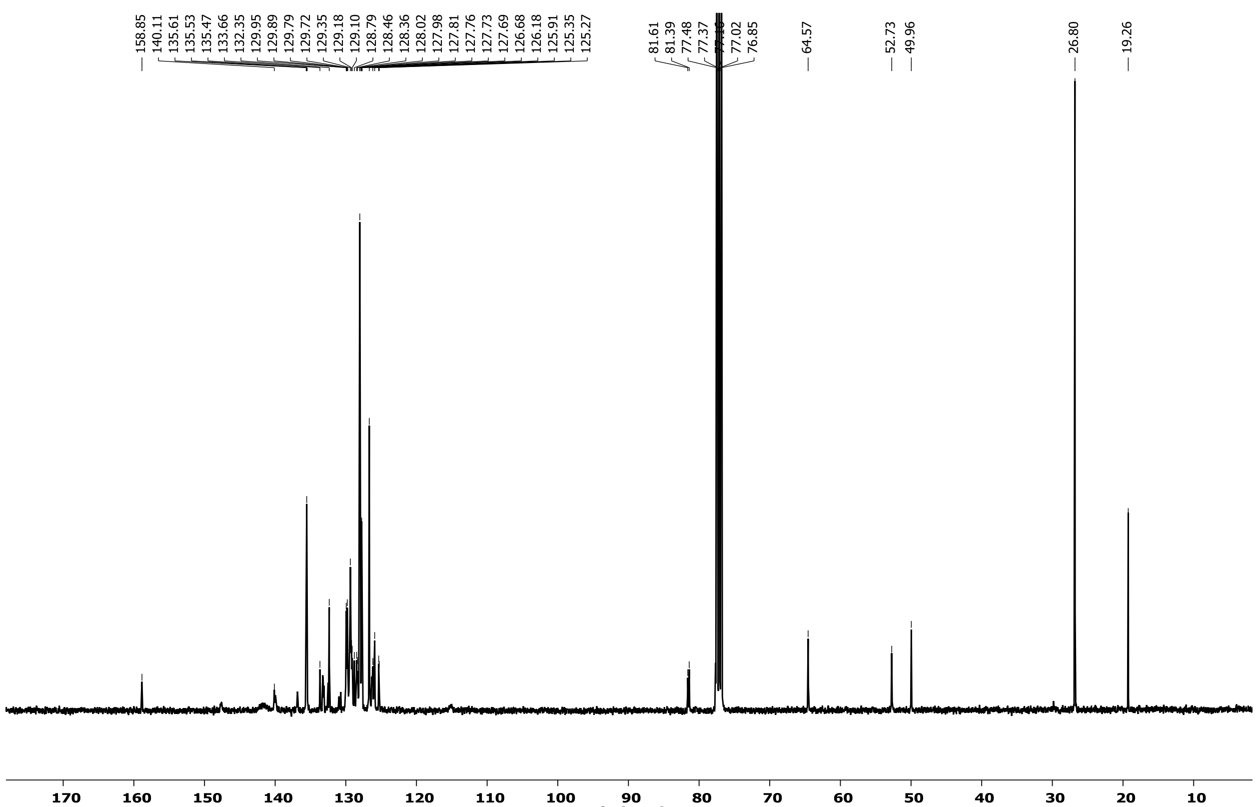
13C-NMR of compound **4f**, $CDCl_3$, 100 MHz



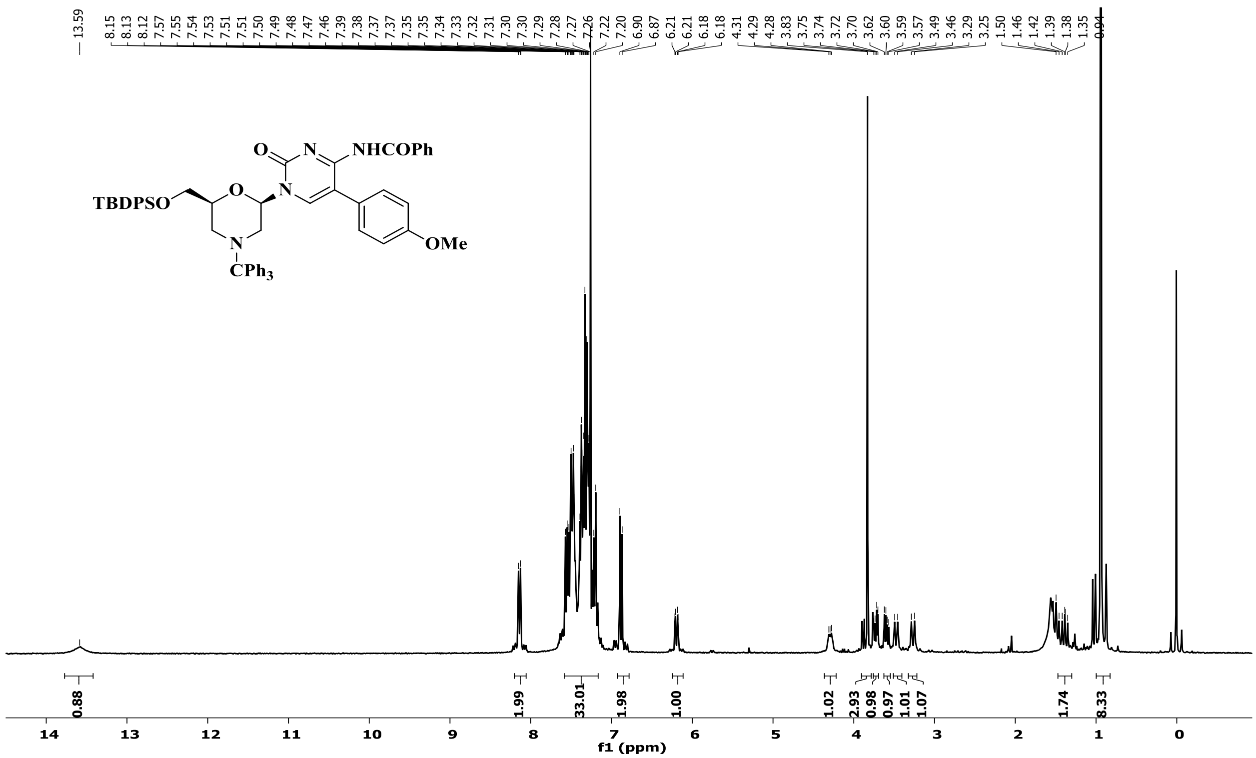
1H-NMR of compound **4g**, $CDCl_3$, 300 MHz



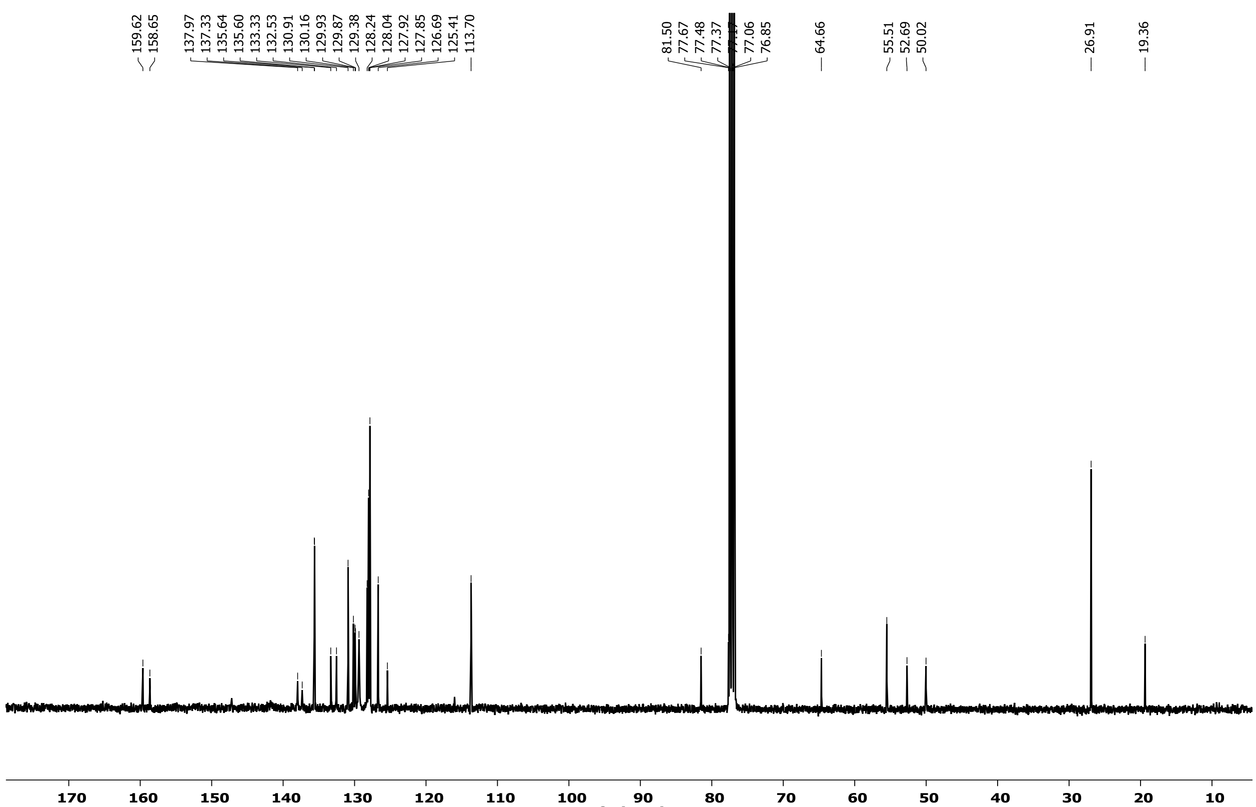
13C-NMR of compound **4g**, $CDCl_3$, 100 MHz



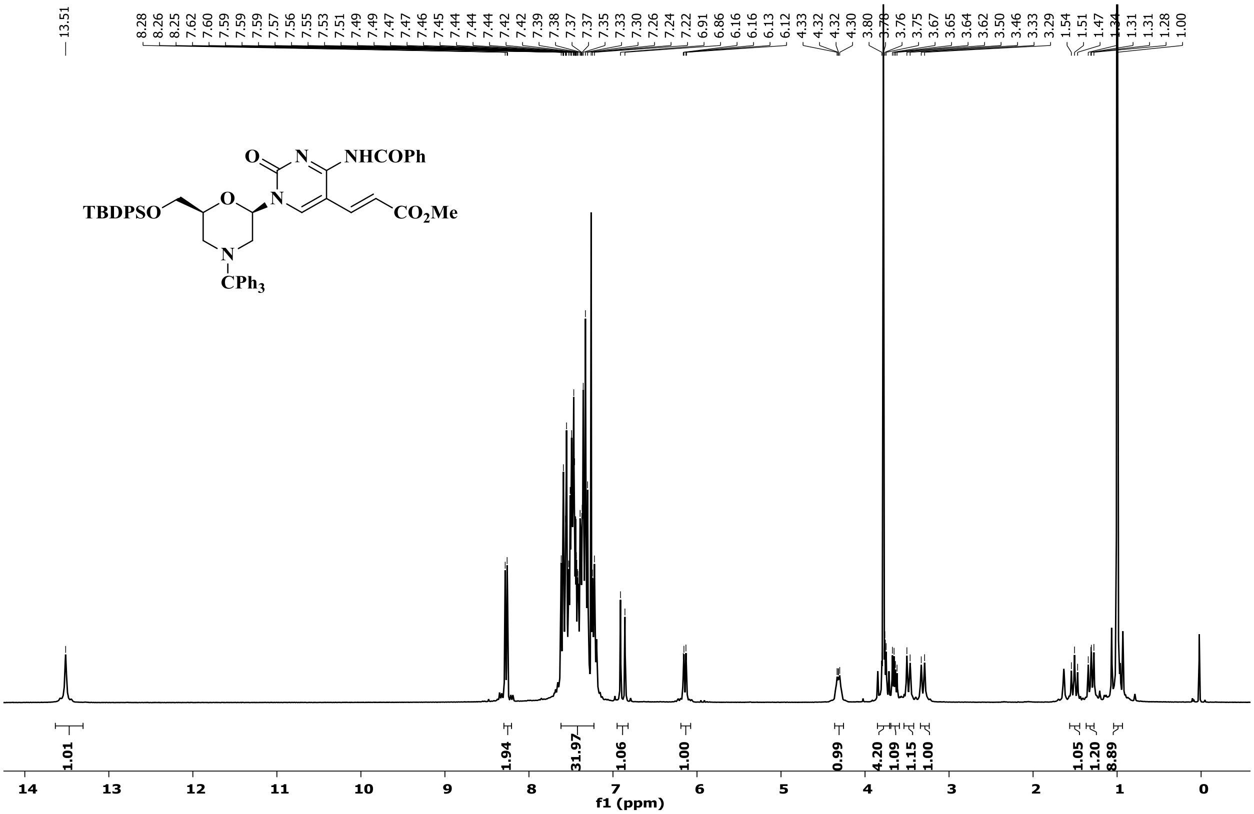
1H-NMR of compound **4h**, $CDCl_3$, 300 MHz



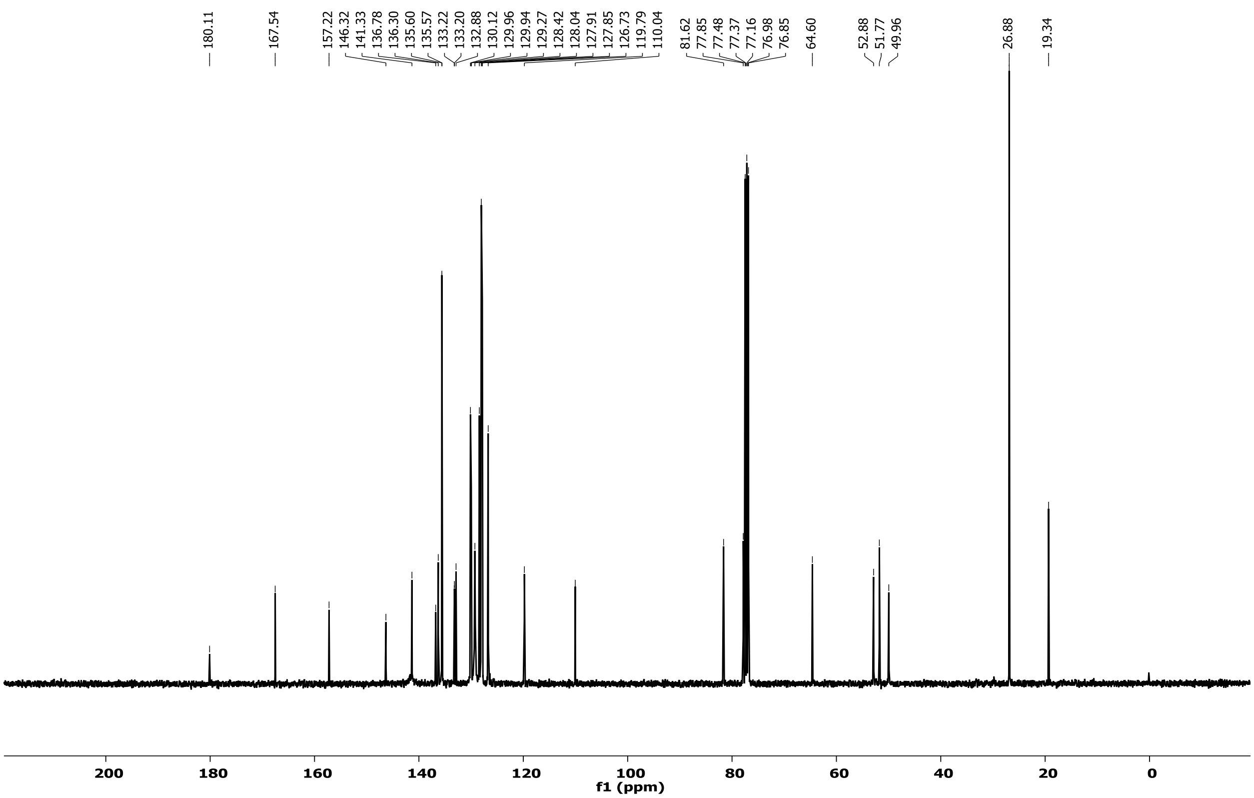
1H-NMR of compound **4h**, $CDCl_3$, 100 MHz



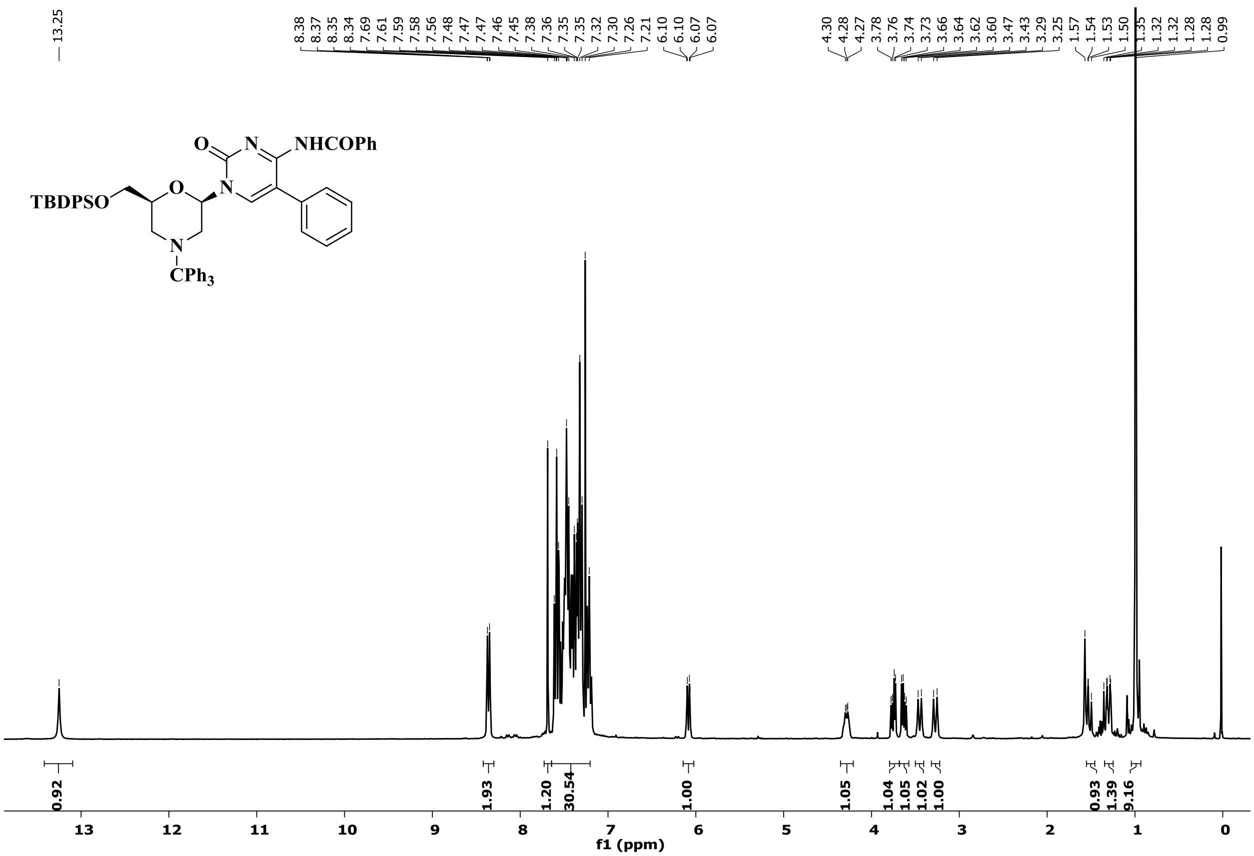
1H-NMR of compound **4i**, $CDCl_3$, 300 MHz



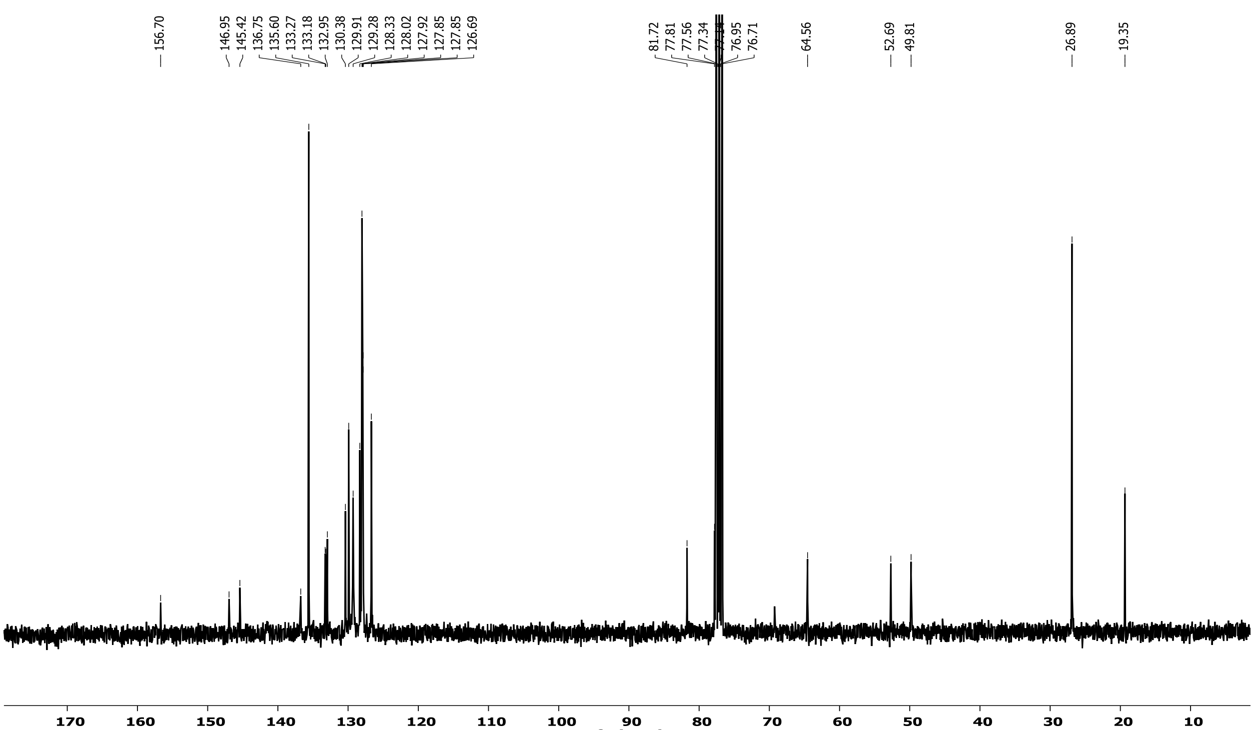
13C-NMR of compound **4i**, $CDCl_3$, 100 MHz



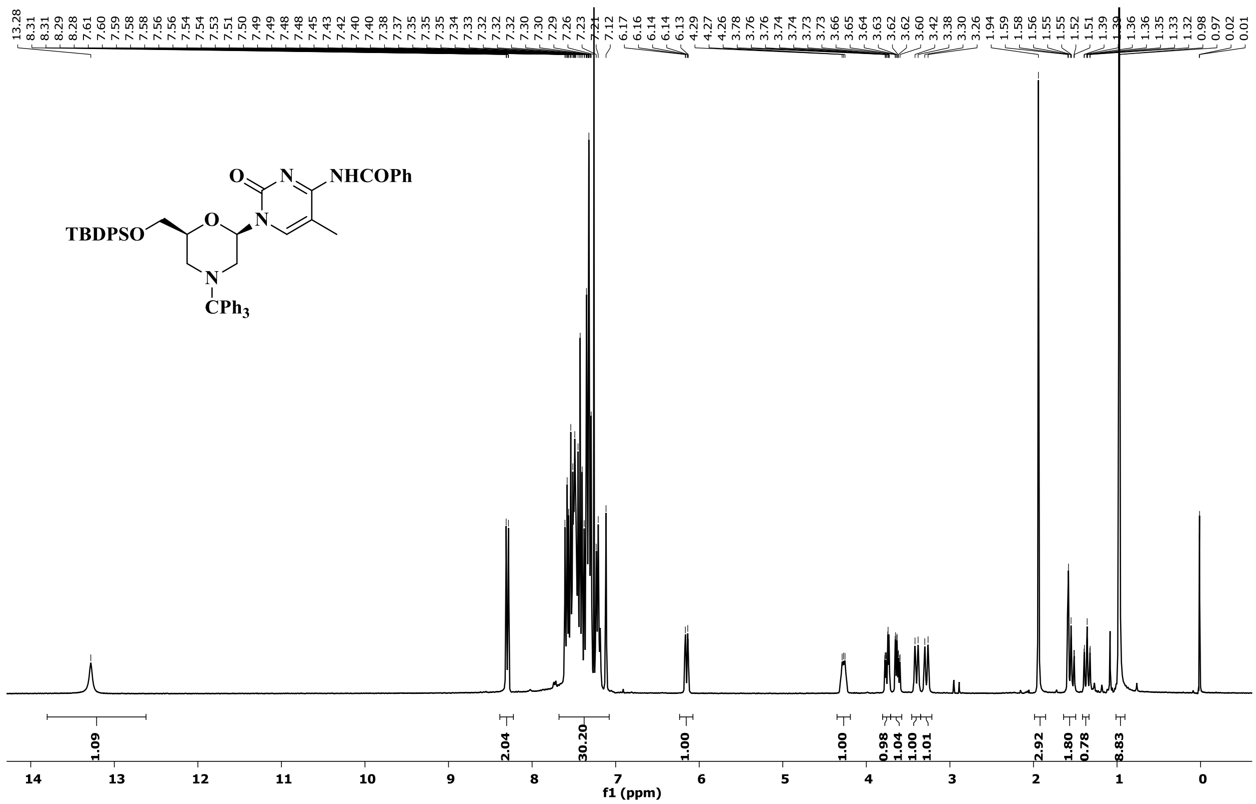
1H-NMR of compound **4j**, $CDCl_3$, 300 MHz



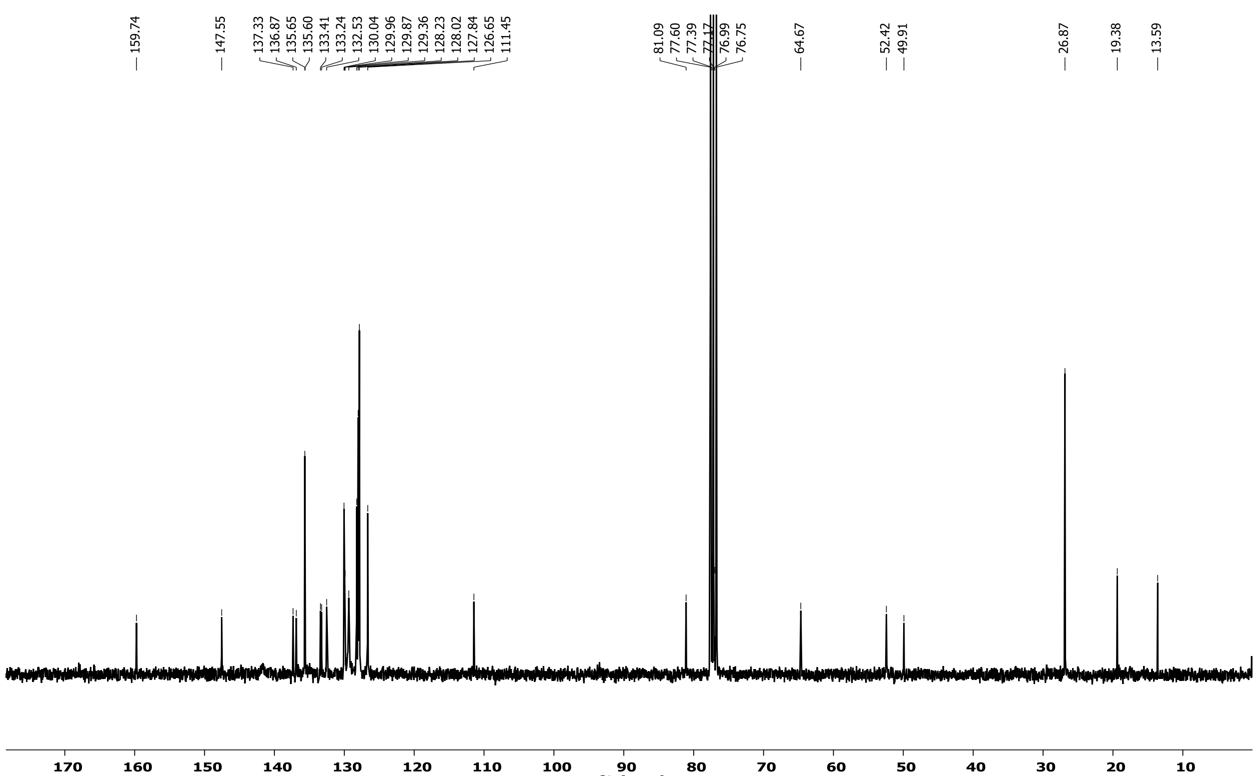
13C-NMR of compound **4j**, $CDCl_3$, 100 MHz



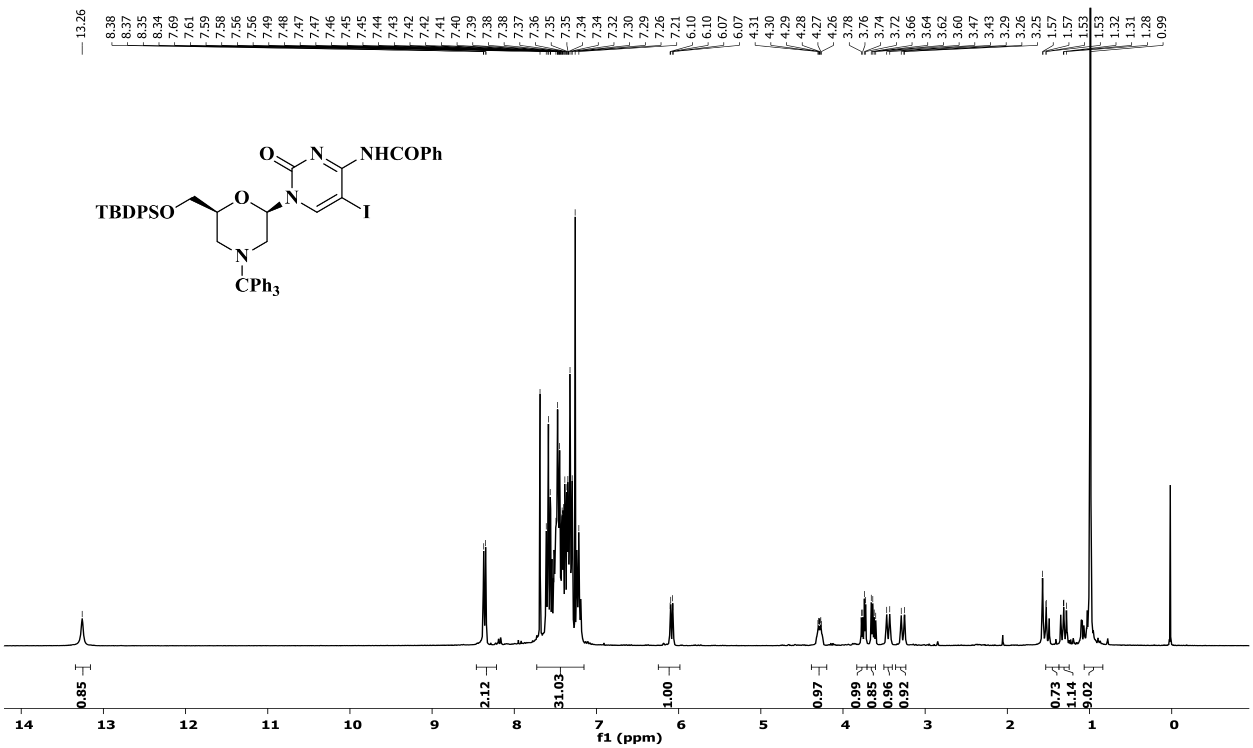
1H-NMR of compound **4k**, $CDCl_3$, 300 MHz



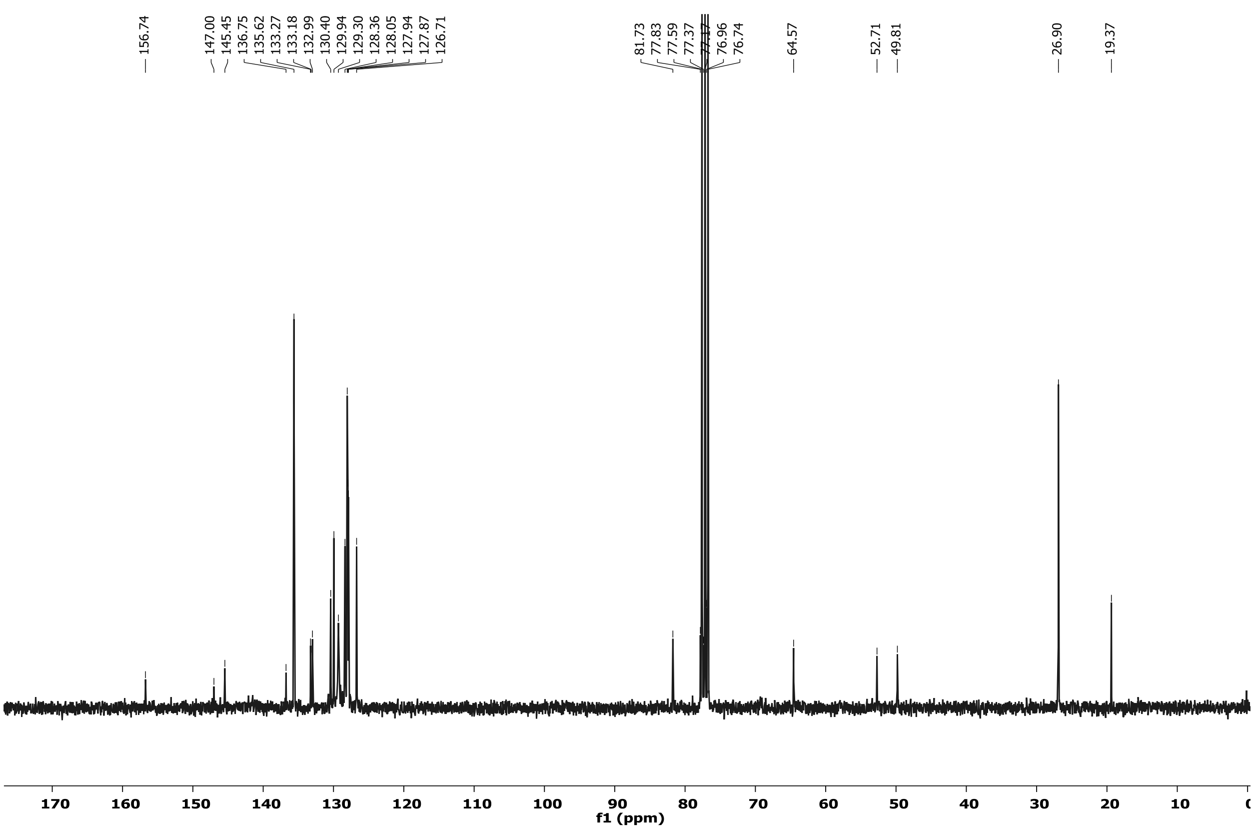
13C-NMR of compound **4k**, $CDCl_3$, 100 MHz



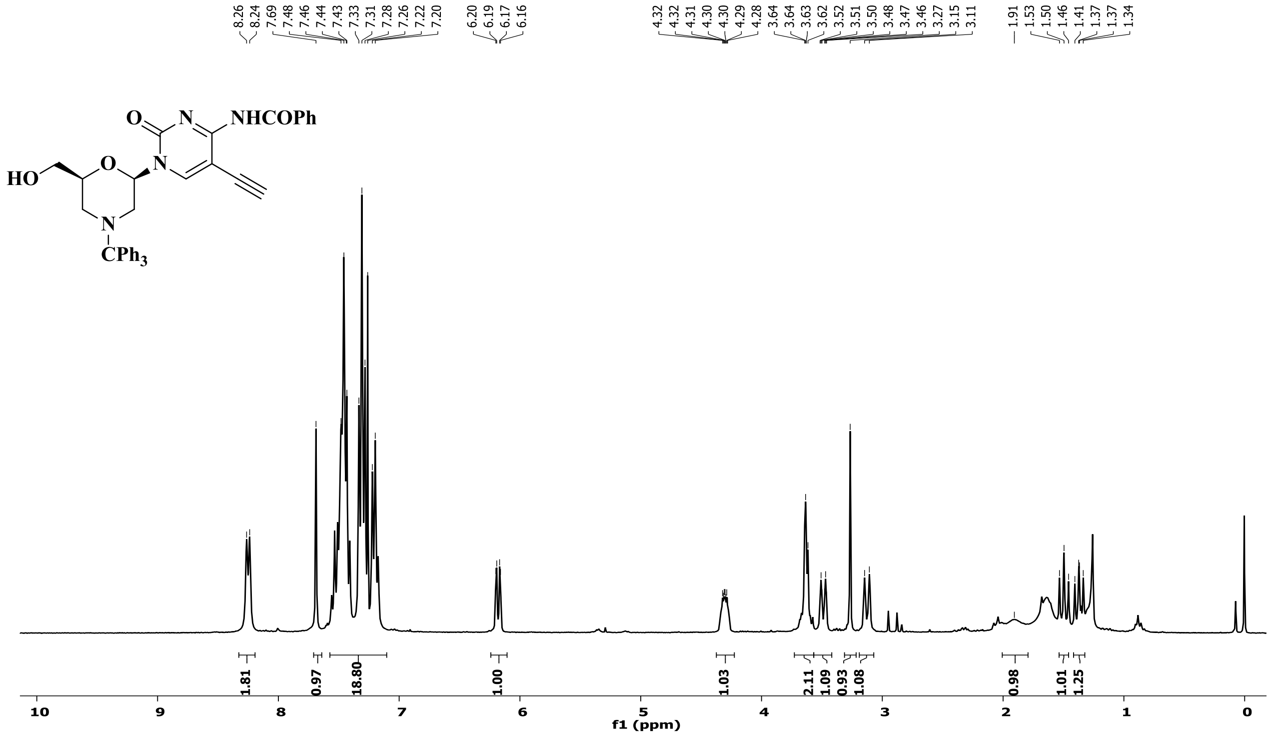
1H-NMR of compound **4l**, $CDCl_3$, 300 MHz



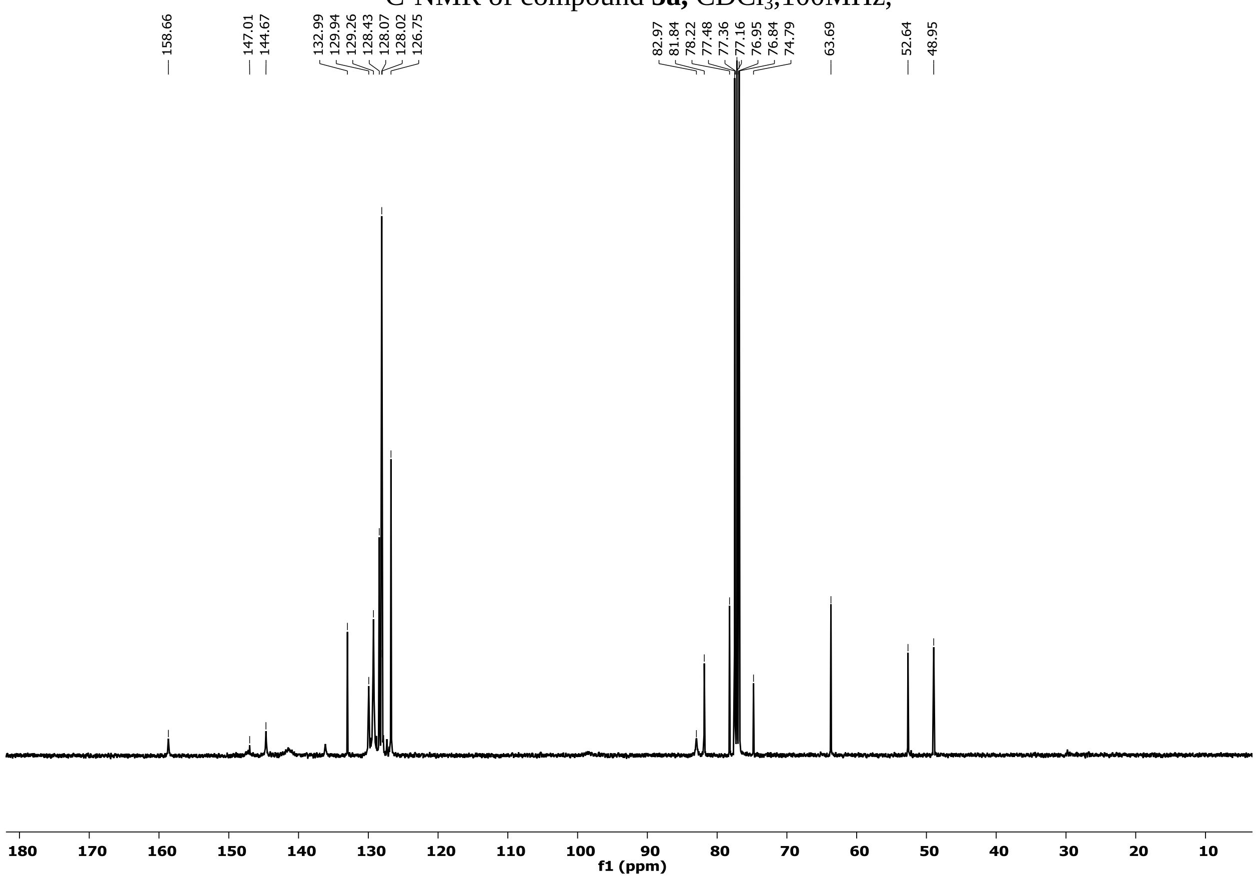
13C-NMR of compound **4l**, $CDCl_3$, 100 MHz



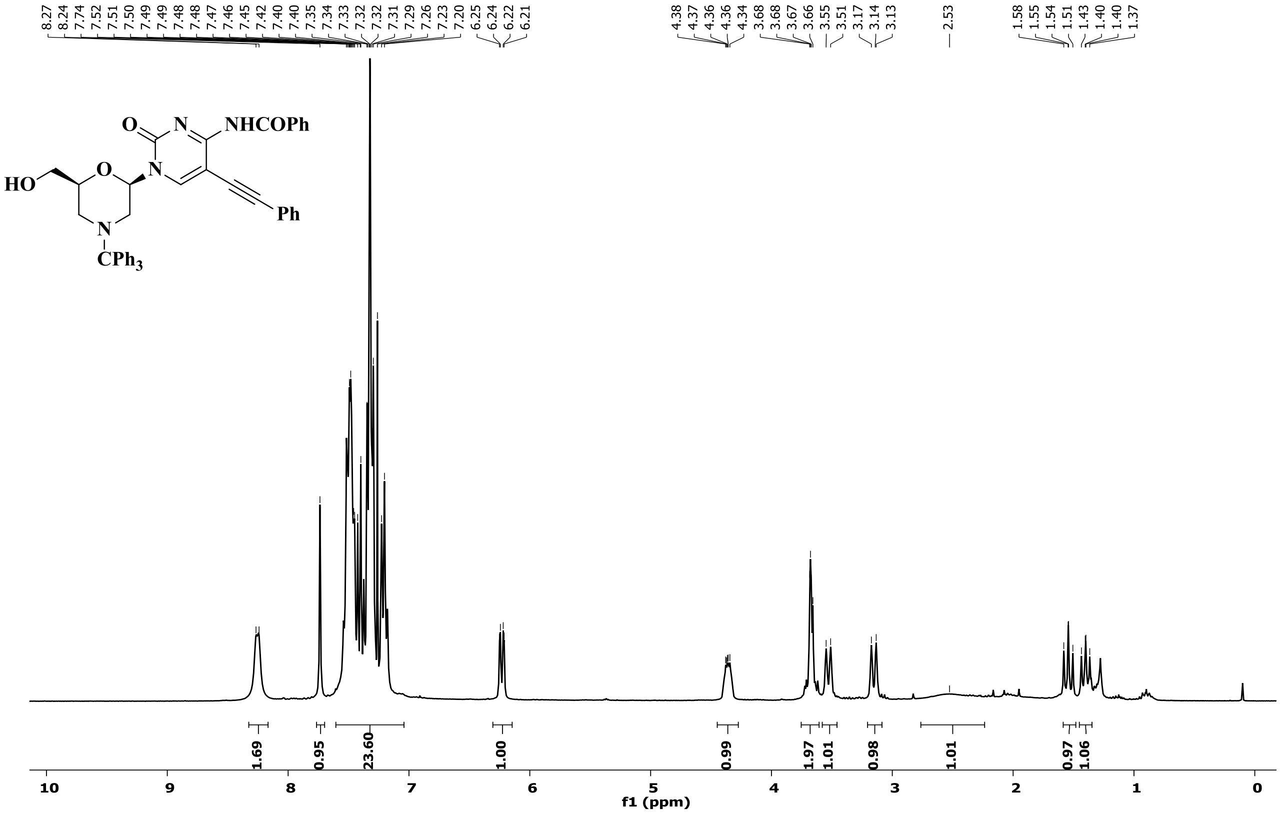
1H-NMR of compound **5a**, $CDCl_3$, 300 MHz



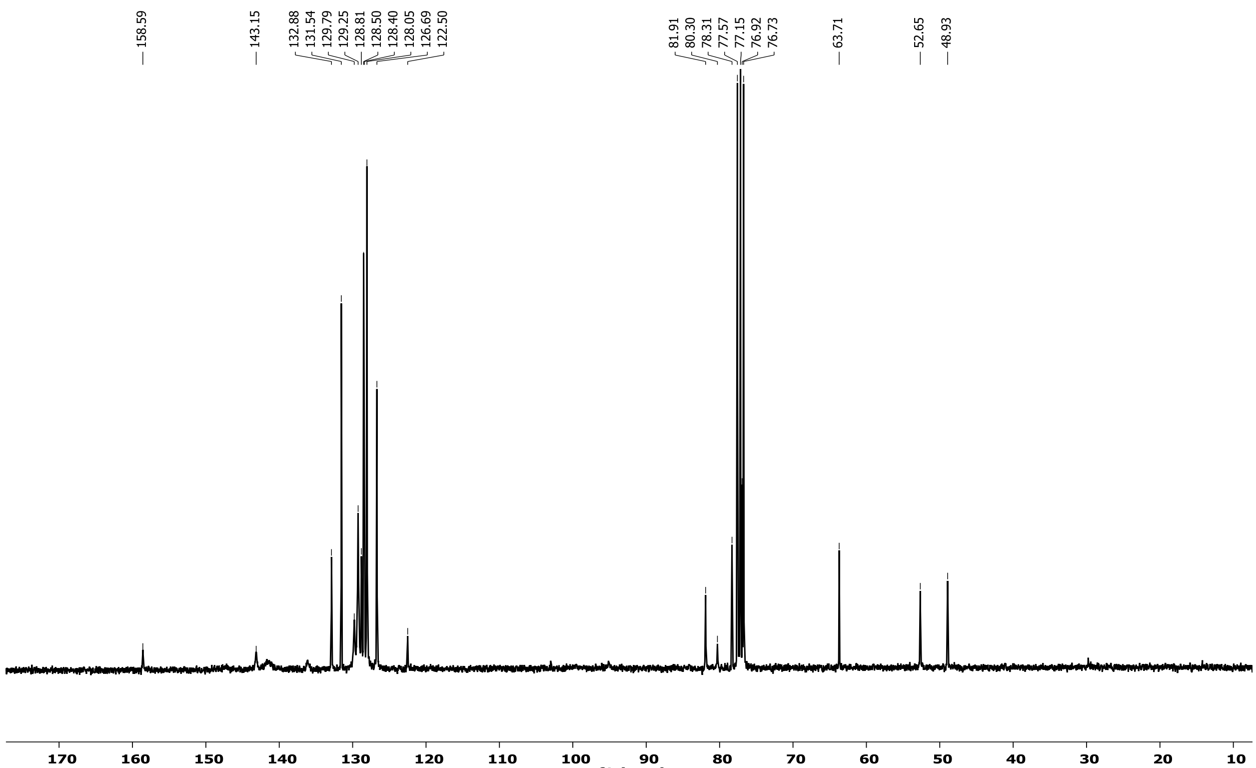
^{13}C-NMR of compound **5a,** $CDCl_3$,100MHz,



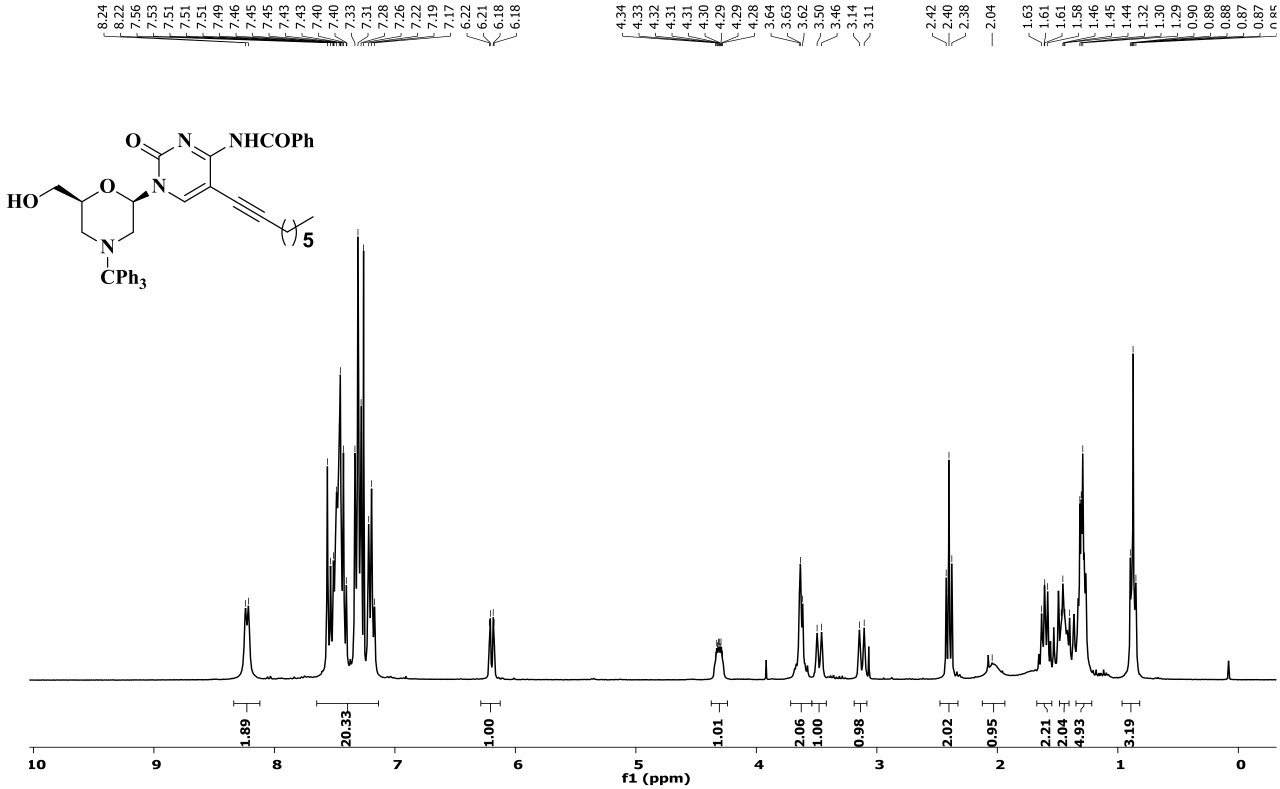
1H-NMR of compound **5b**, $CDCl_3$, 300 MHz



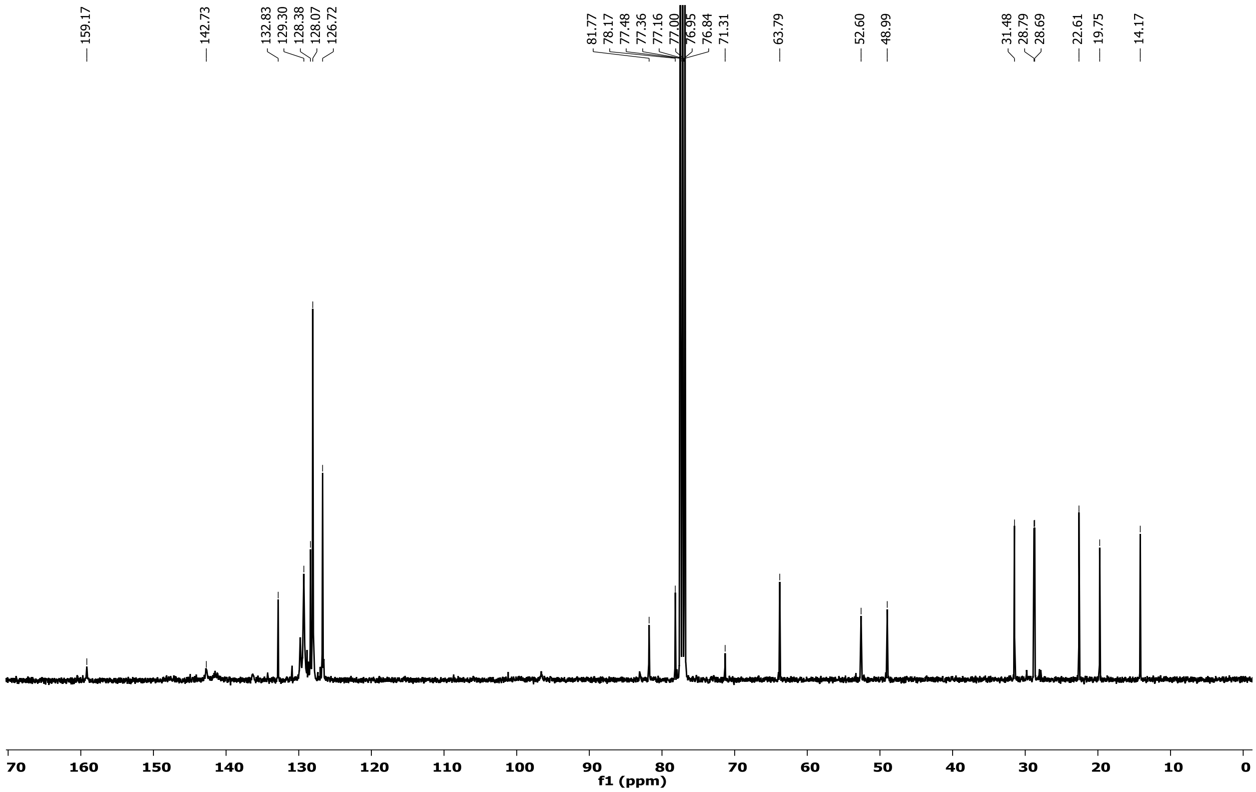
13C-NMR of compound **5b**, $CDCl_3$, 75 MHz



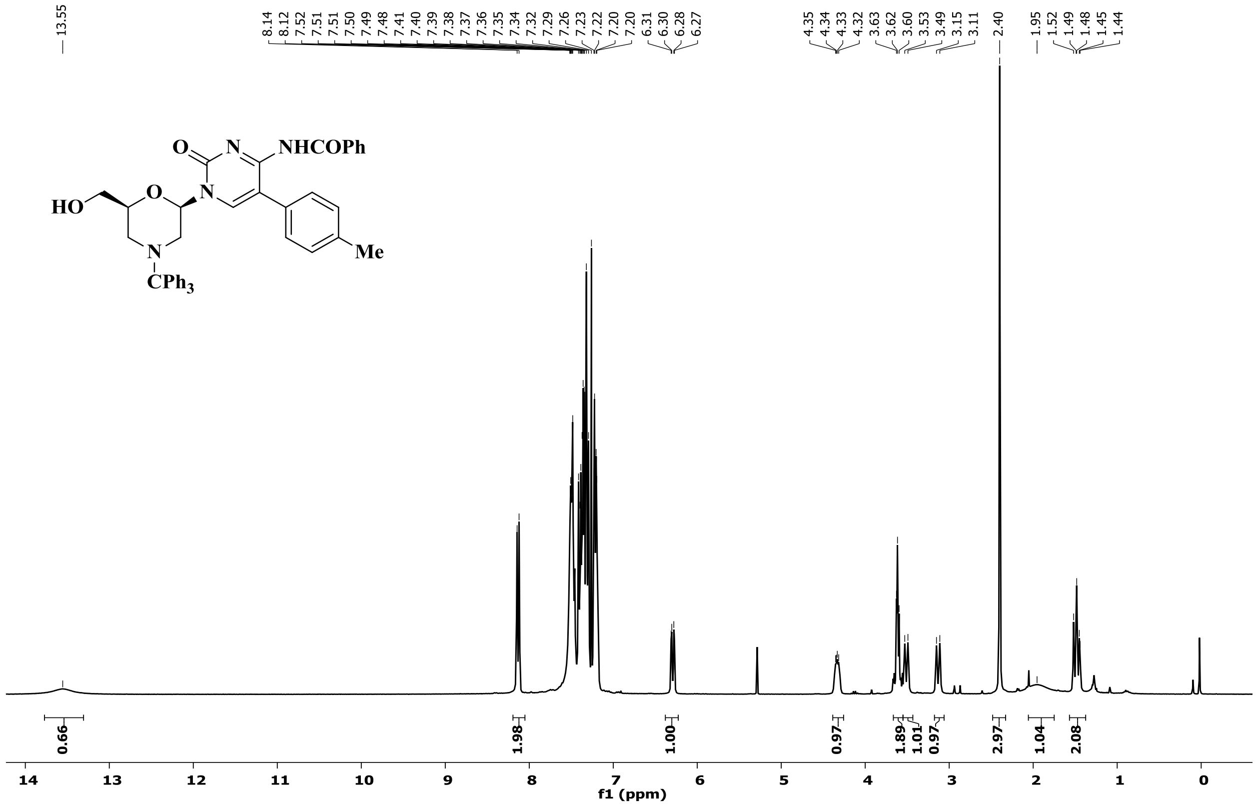
1H-NMR of compound **5c**, $CDCl_3$, 300 MHz



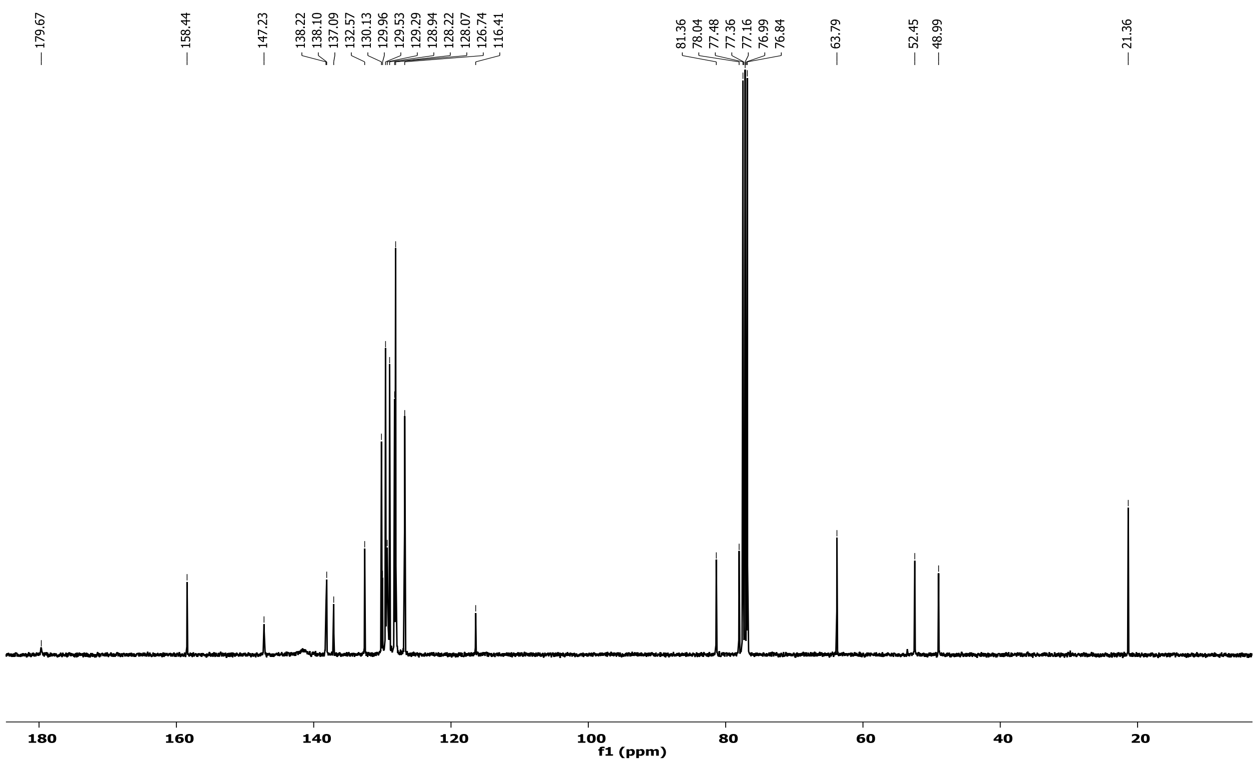
1H-NMR of compound **5c**, $CDCl_3$, 100 MHz



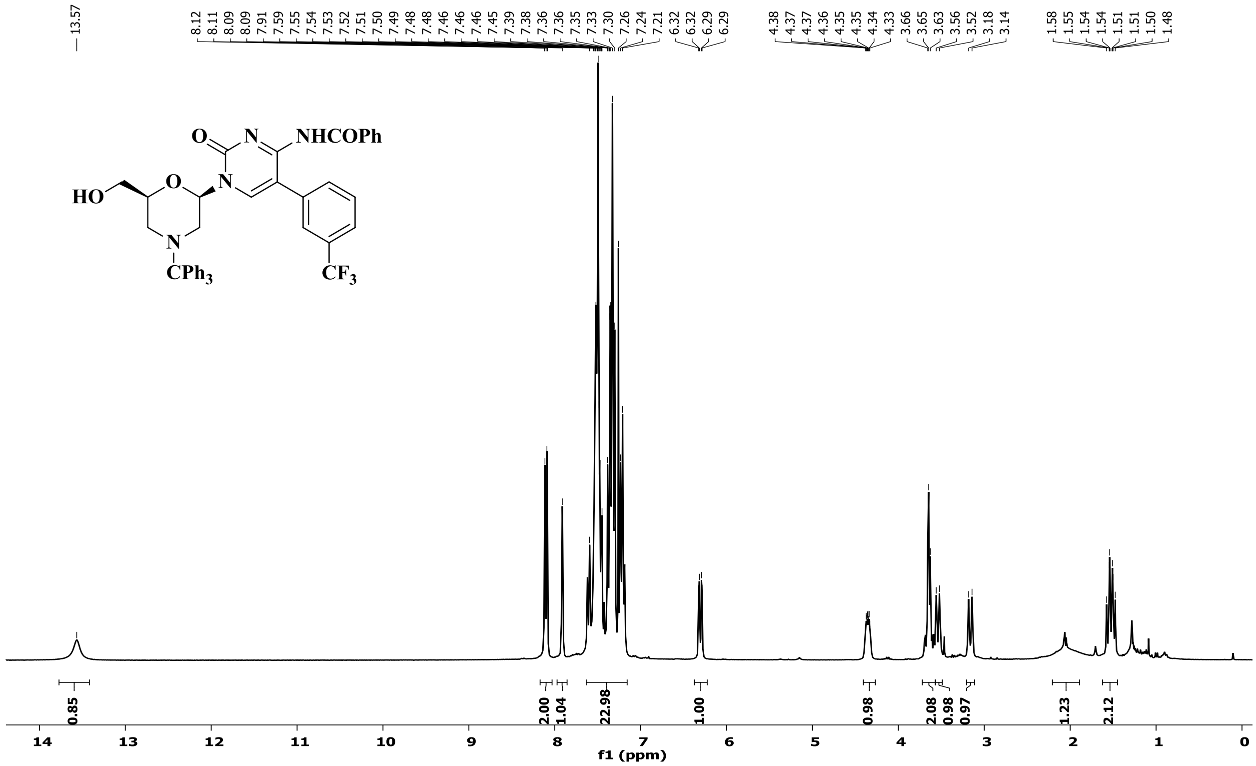
1H-NMR of compound **5d**, $CDCl_3$, 300 MHz



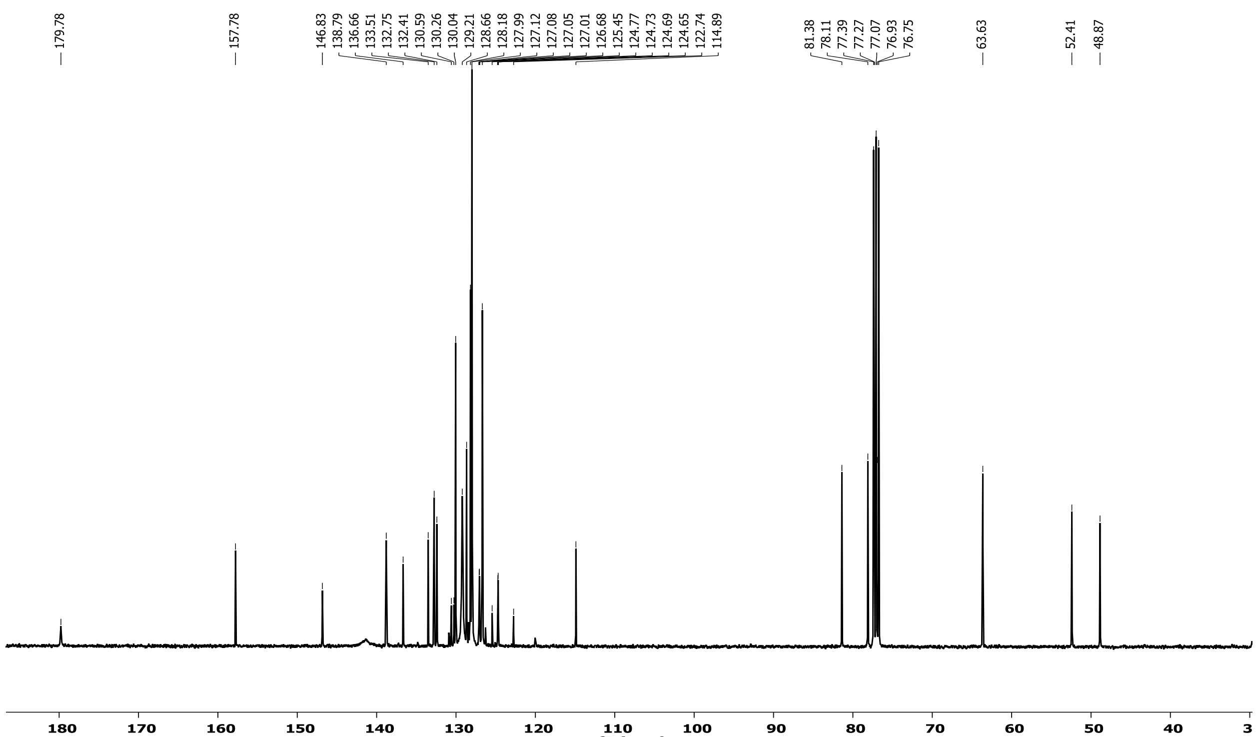
13C-NMR of compound **5d**, $CDCl_3$, 100 MHz



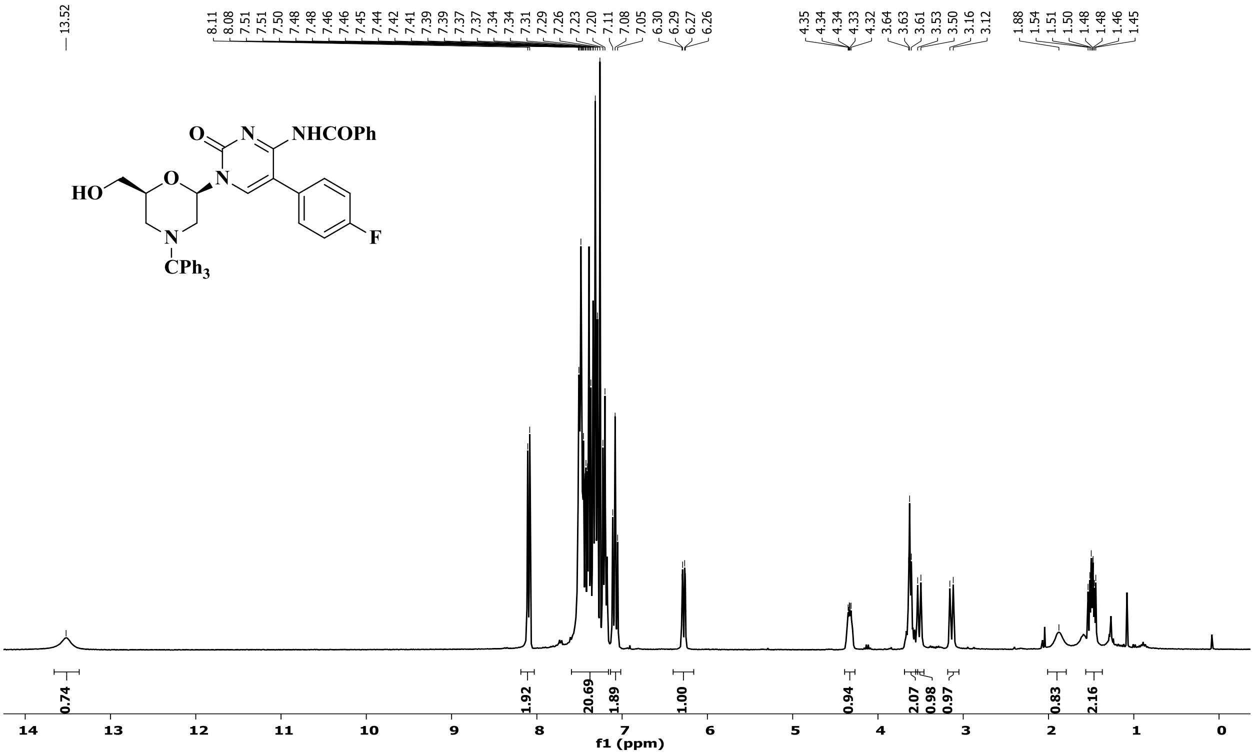
1H-NMR of compound **5e**, $CDCl_3$, 300 MHz



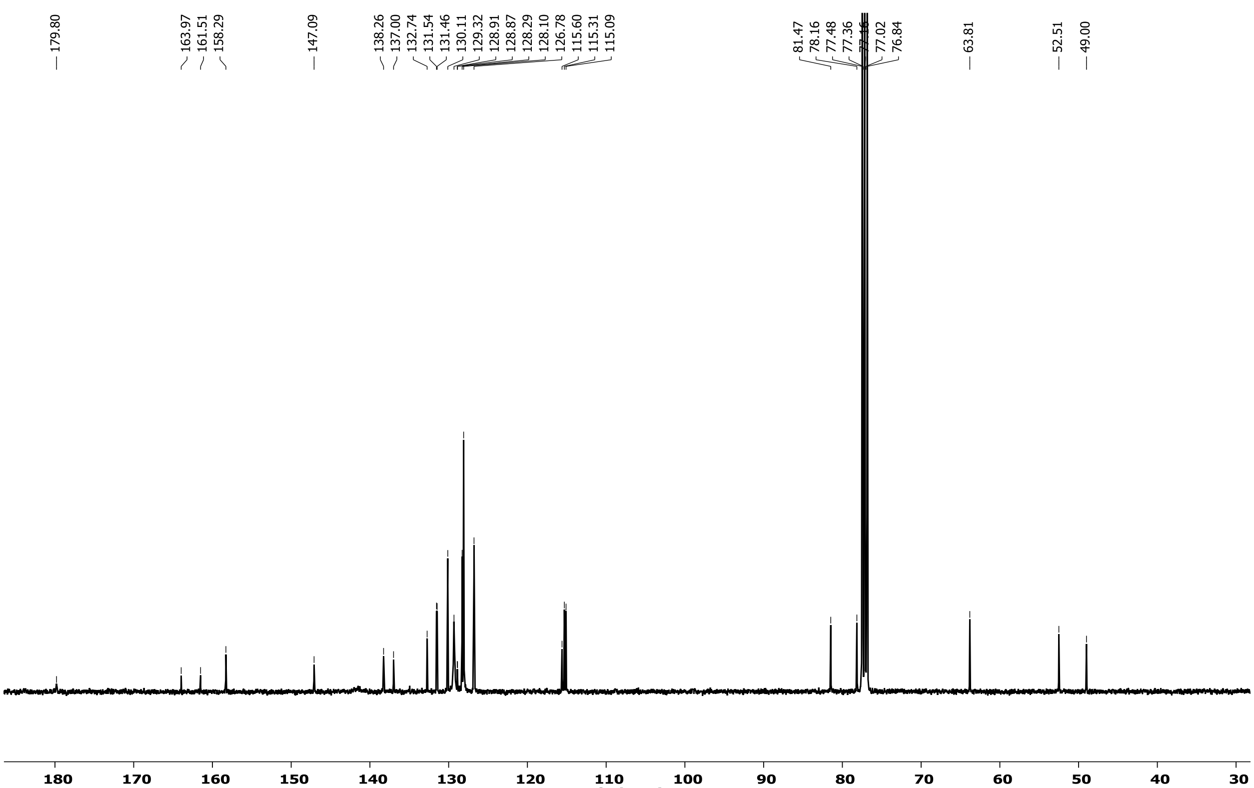
13C-NMR of compound **5e**, $CDCl_3$, 100 MHz



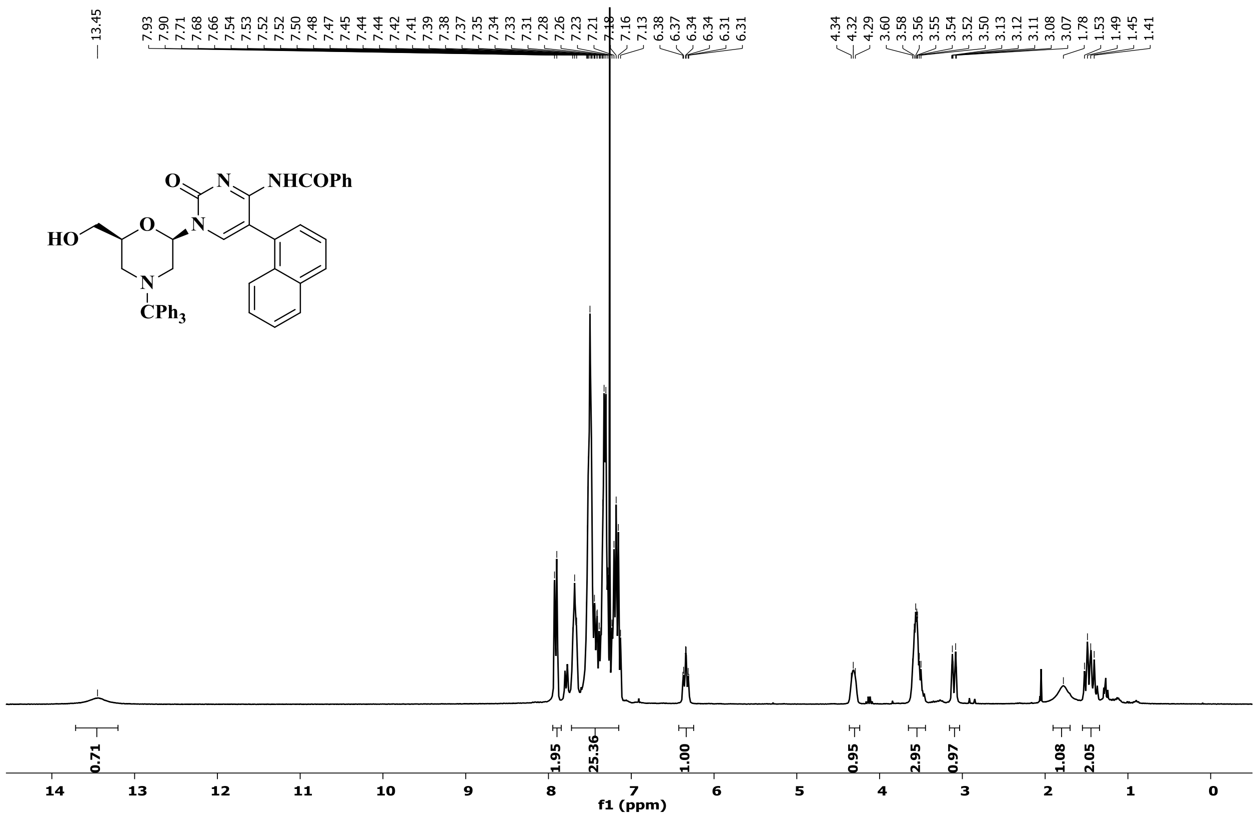
1H-NMR of compound **5f**, $CDCl_3$, 300 MHz



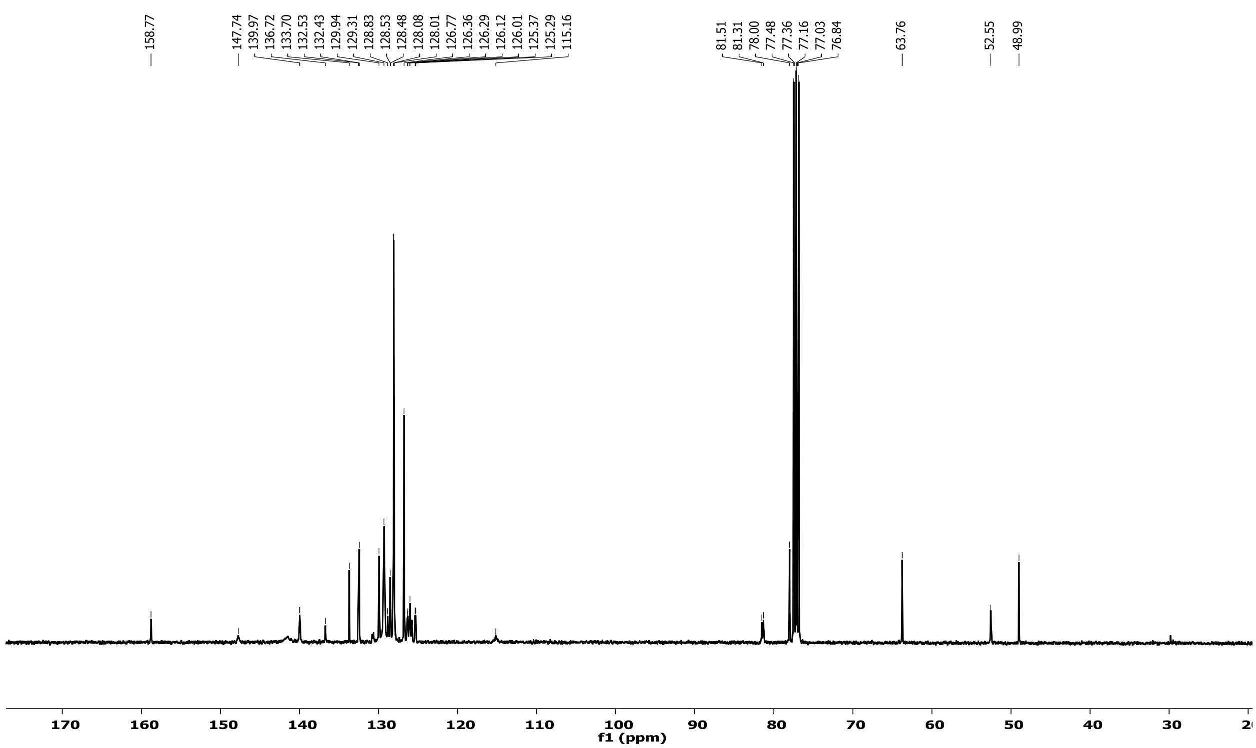
13C-NMR of compound **5f**, $CDCl_3$, 100 MHz



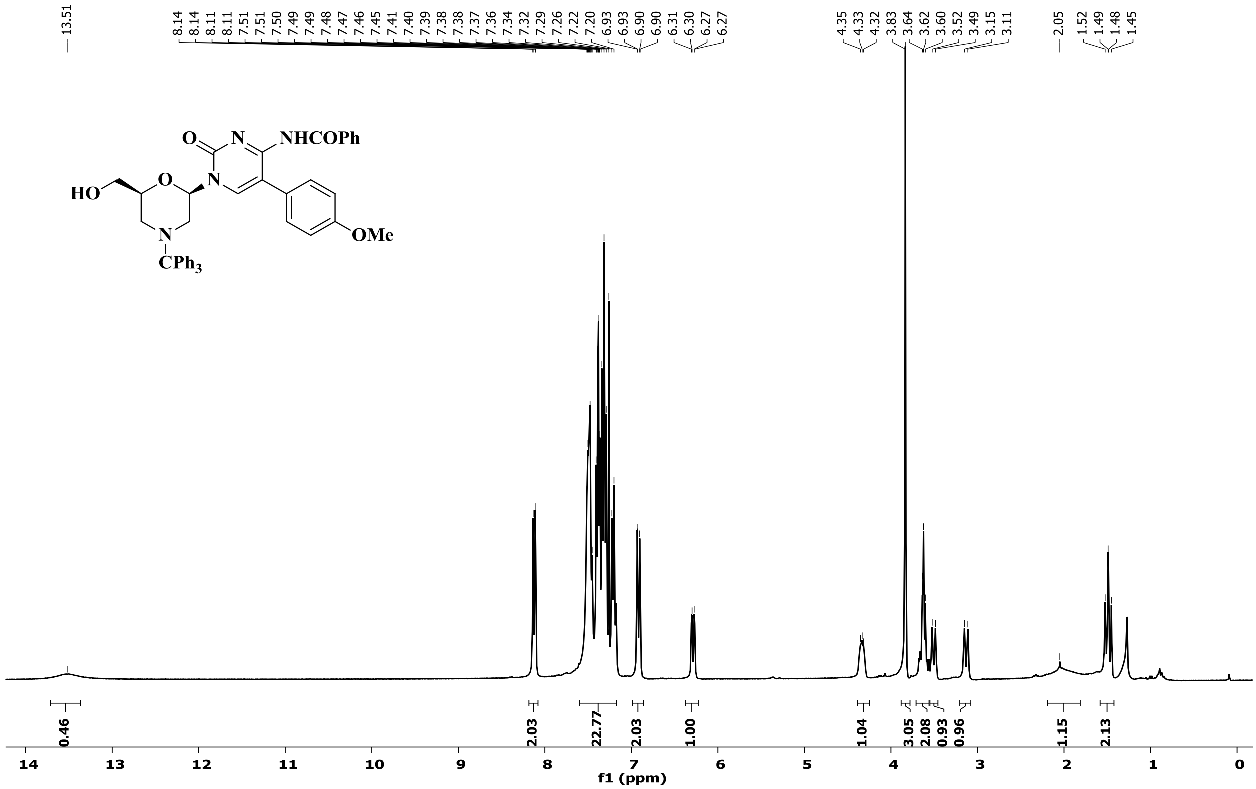
1H-NMR of compound **5g**, $CDCl_3$, 300 MHz



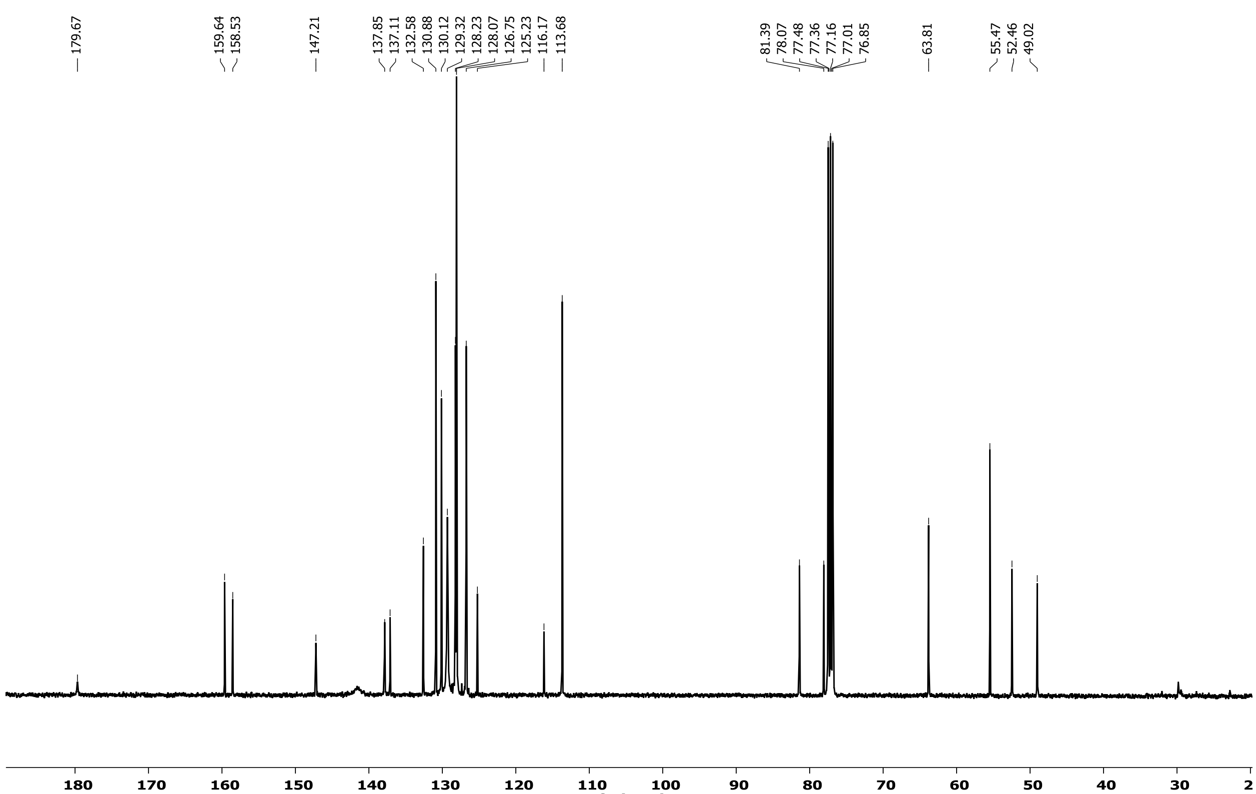
1H-NMR of compound **5g**, $CDCl_3$, 100 MHz



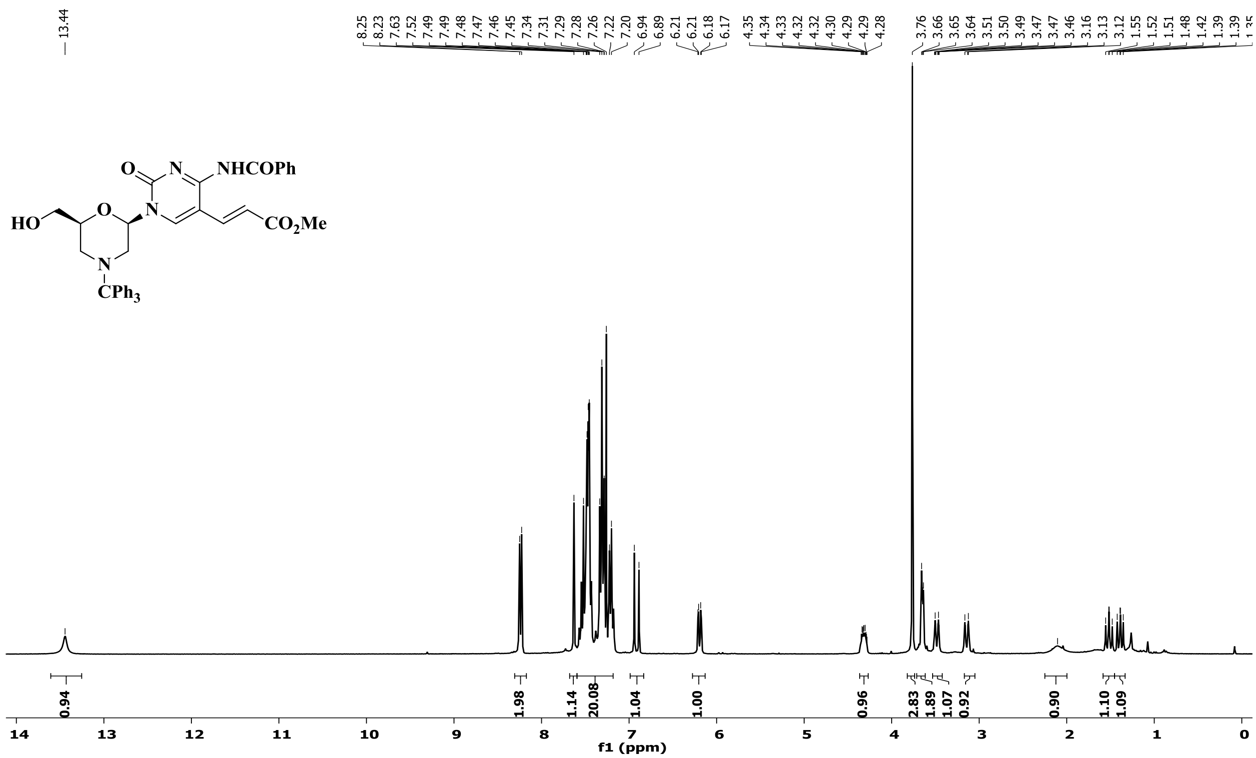
1H-NMR of compound **5h**, $CDCl_3$, 300 MHz



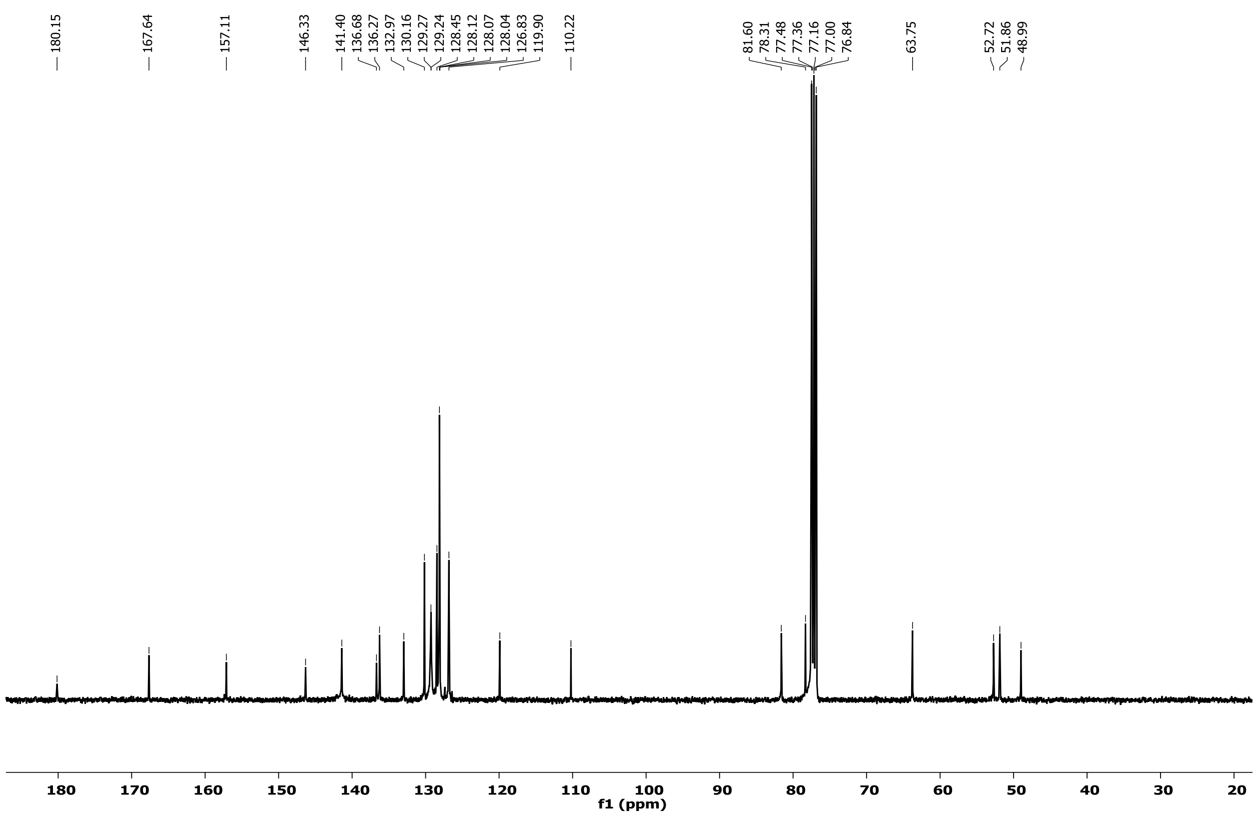
13C-NMR of compound **5h**, $CDCl_3$, 100 MHz



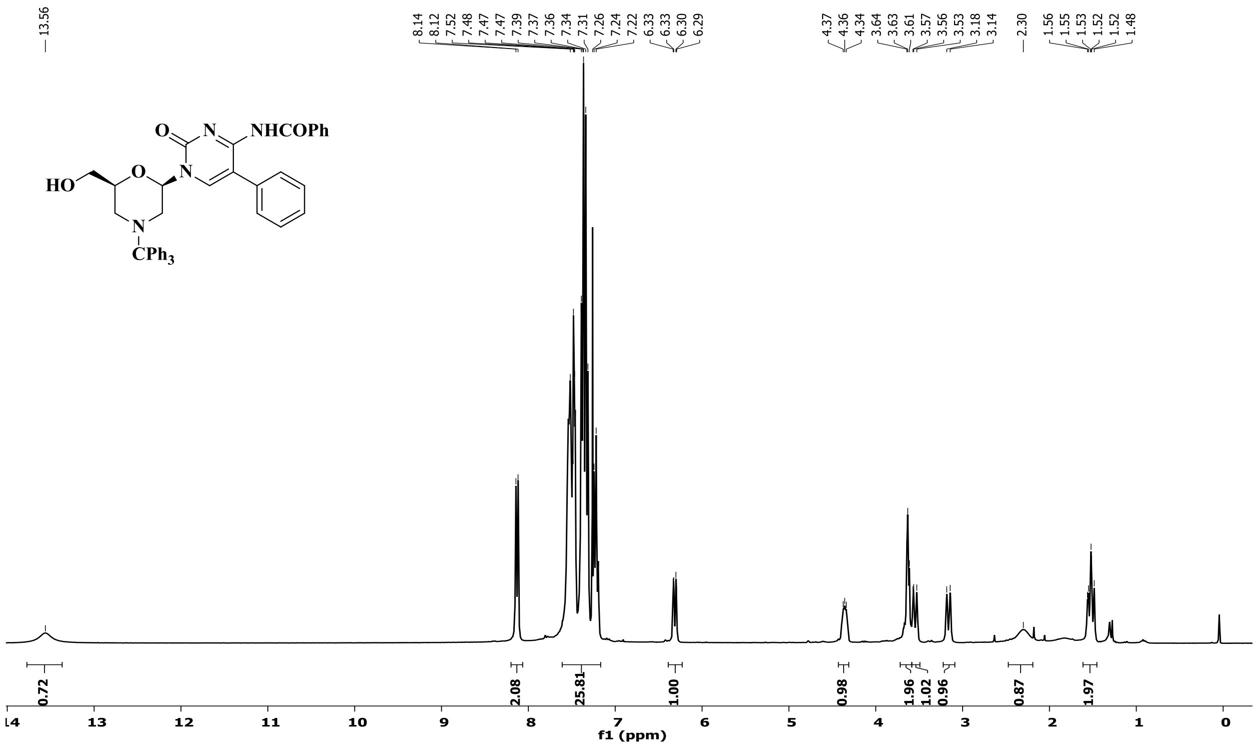
1H-NMR of compound **5i**, $CDCl_3$, 300 MHz



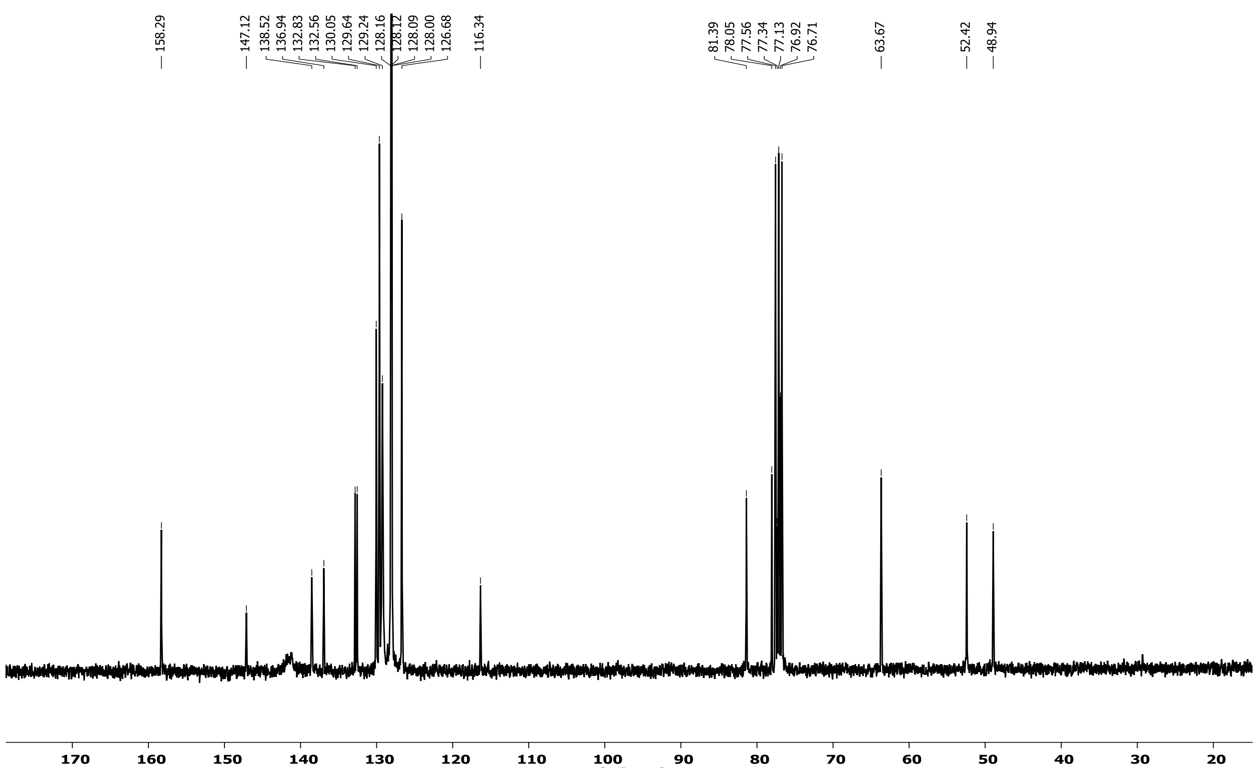
13C-NMR of compound **5i**, $CDCl_3$, 100 MHz



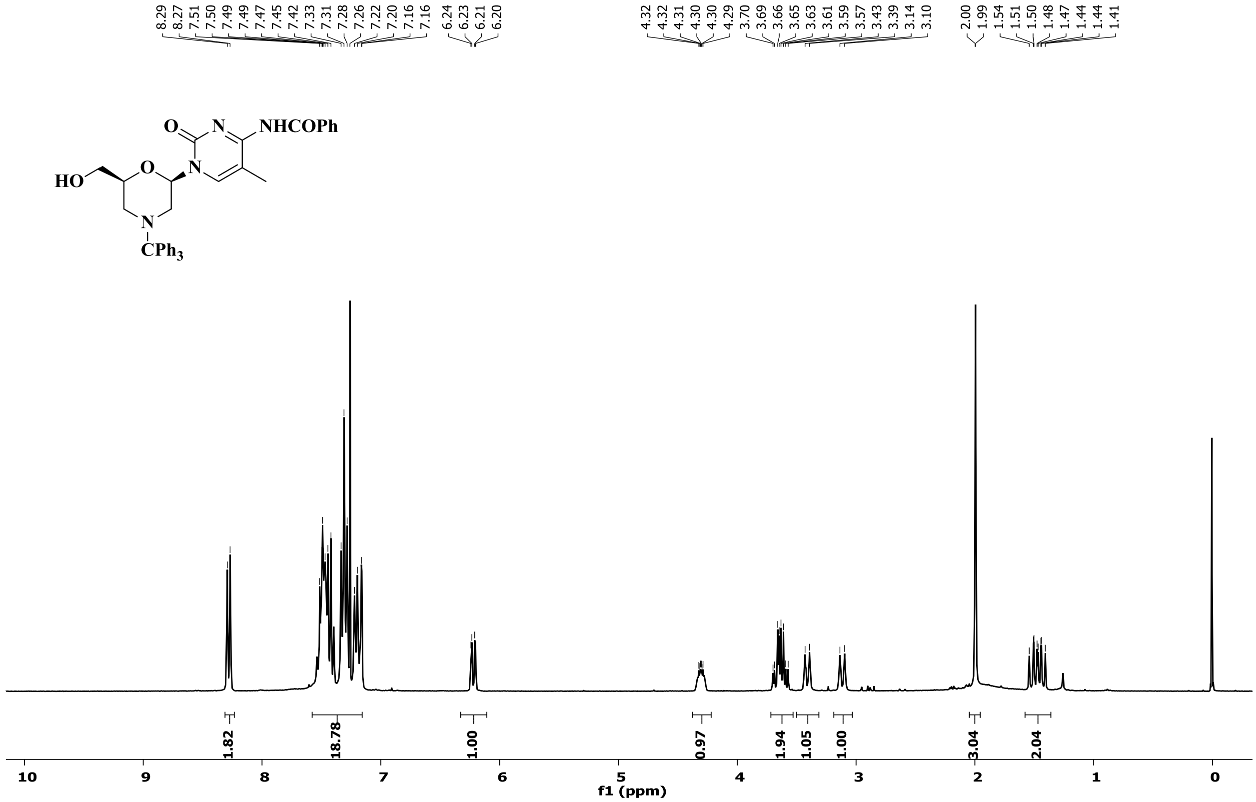
1H-NMR of compound **5j**, $CDCl_3$, 300 MHz



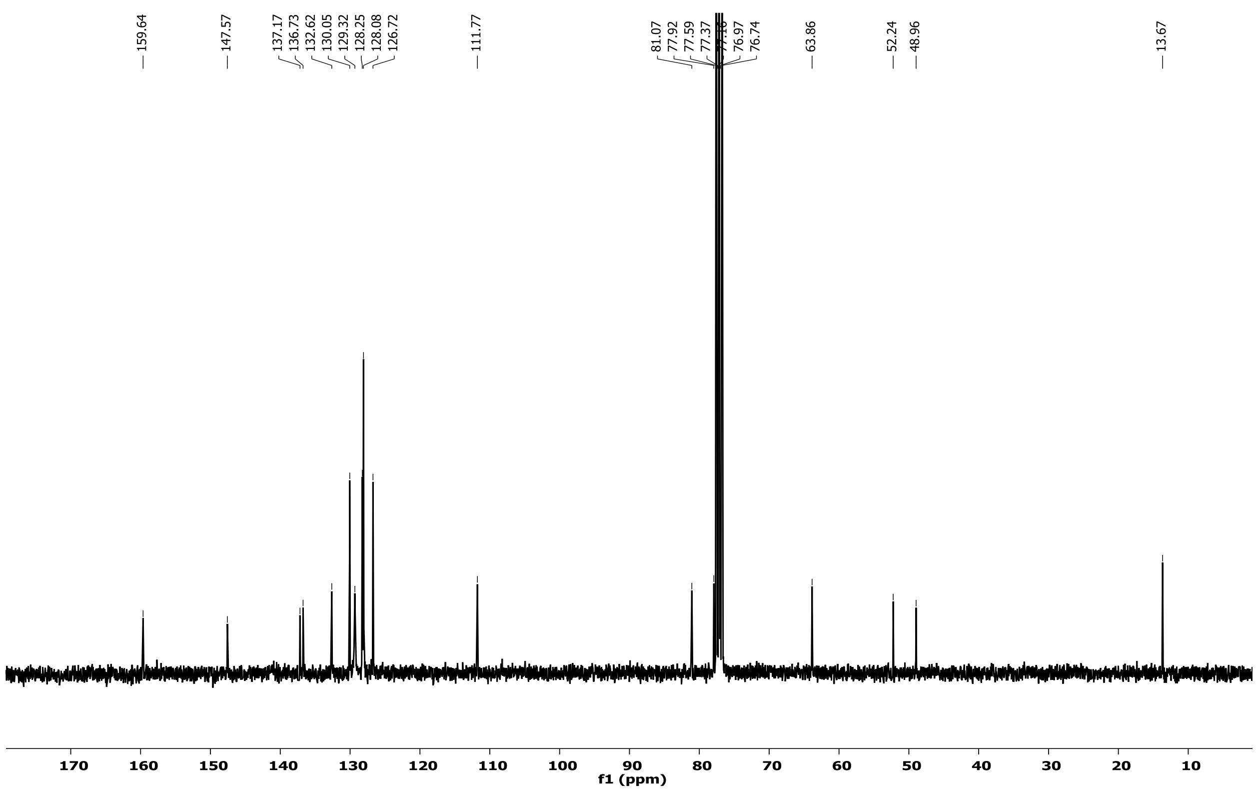
13C-NMR of compound **5j**, $CDCl_3$,75 MHz



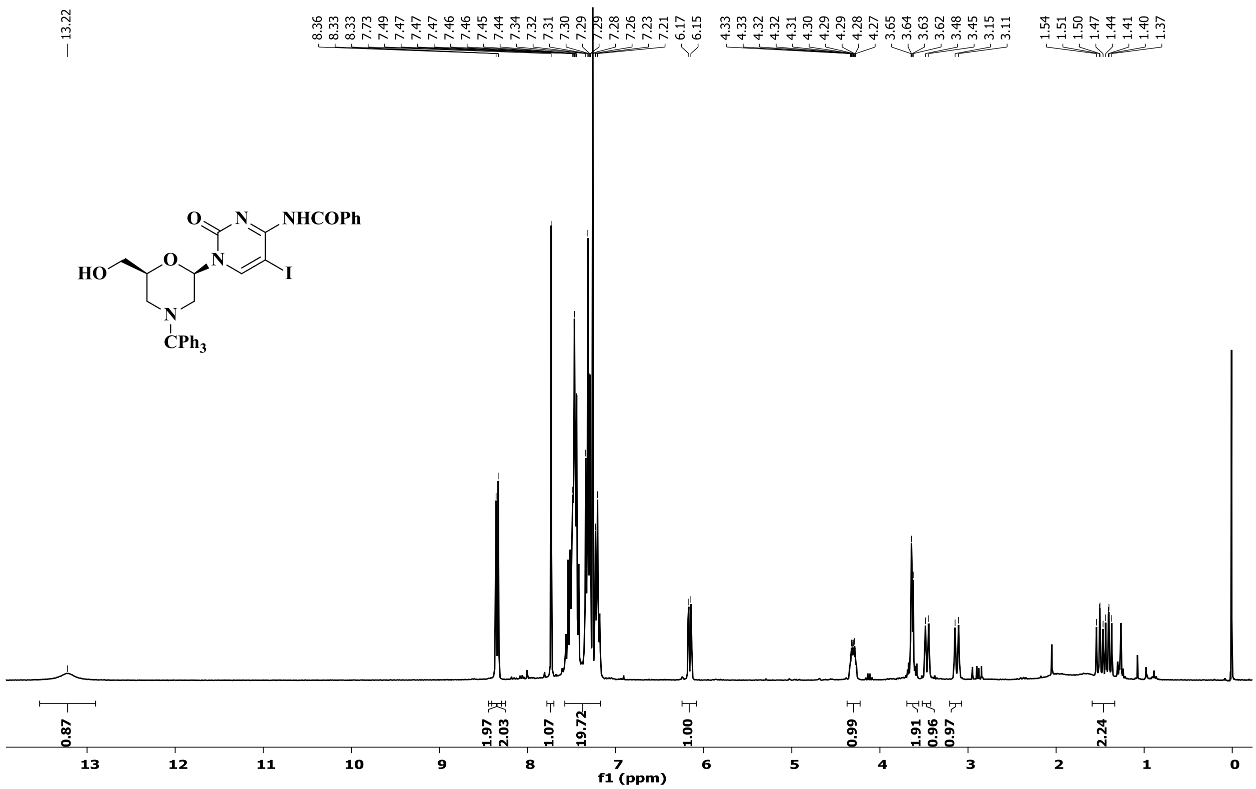
1H-NMR of compound **5k**, $CDCl_3$, 300 MHz



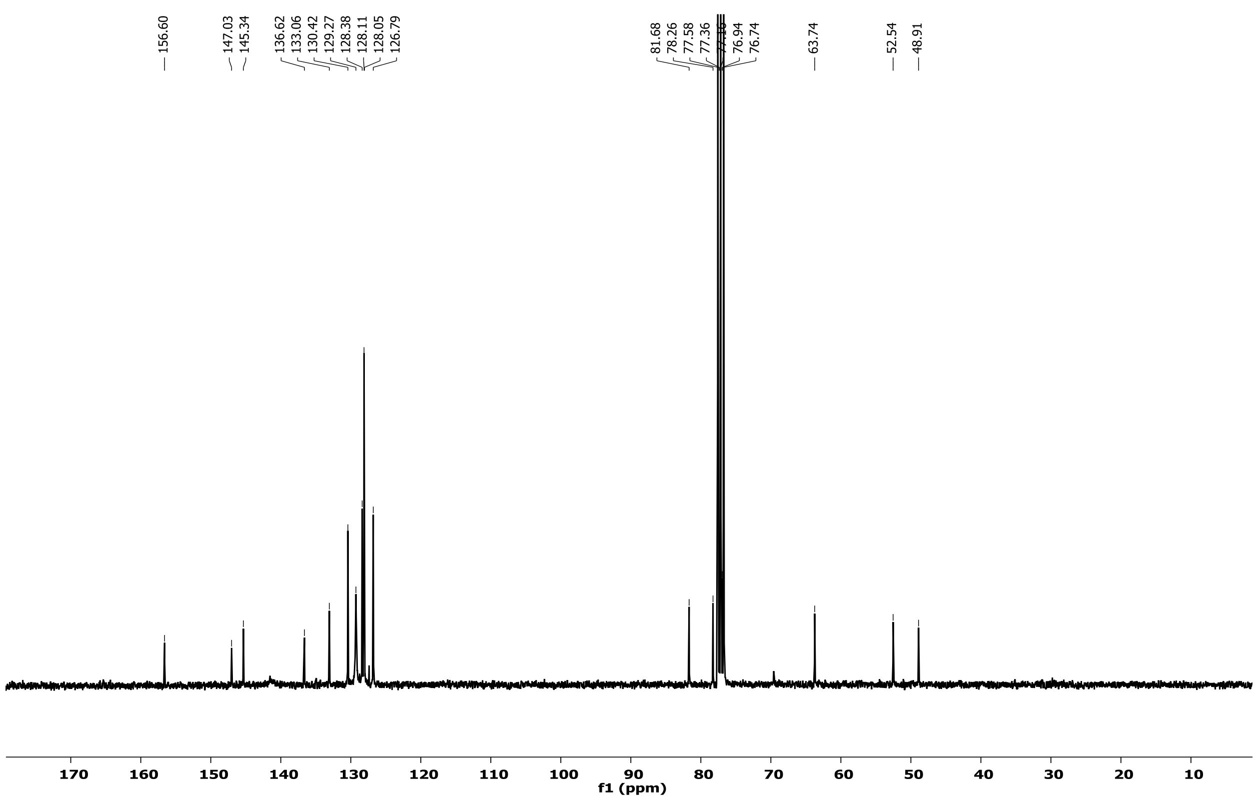
13C-NMR of compound **5k**, $CDCl_3$, 75 MHz



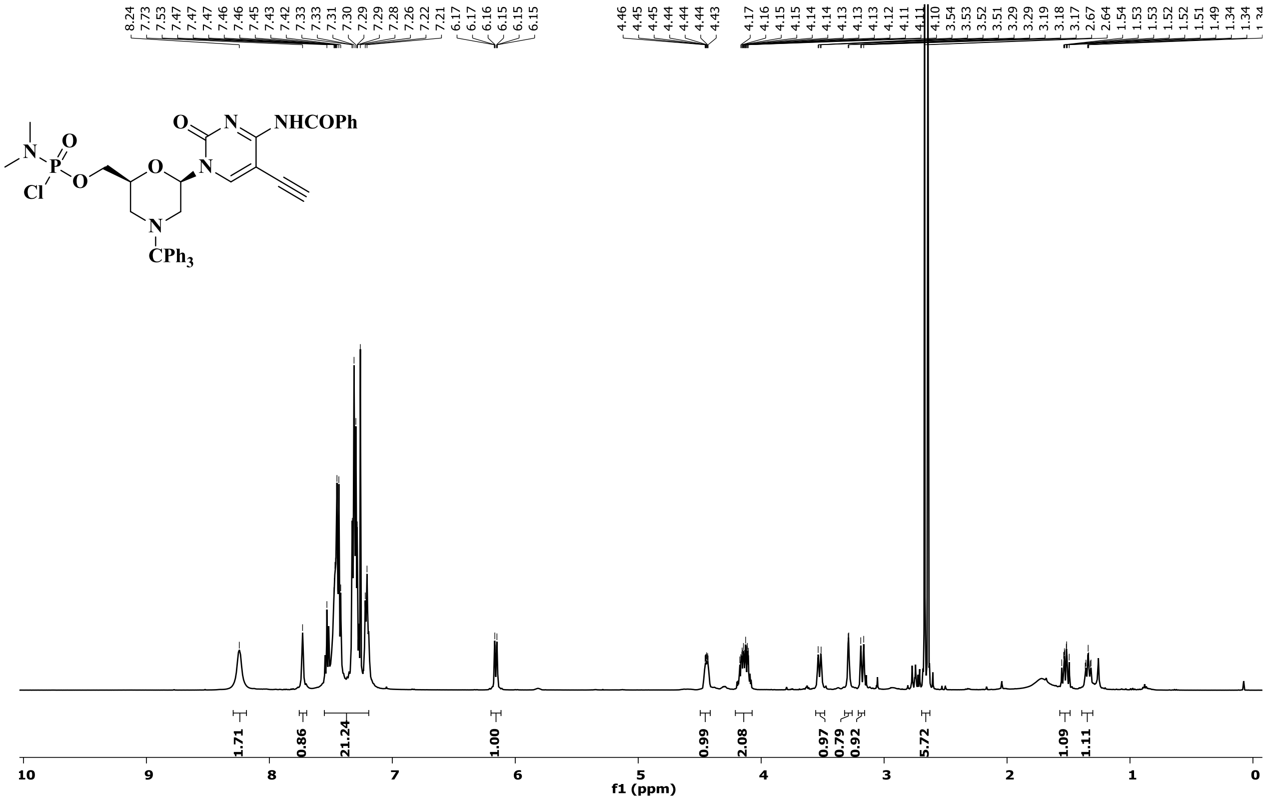
1H-NMR of compound **5l**, $CDCl_3$, 300 MHz



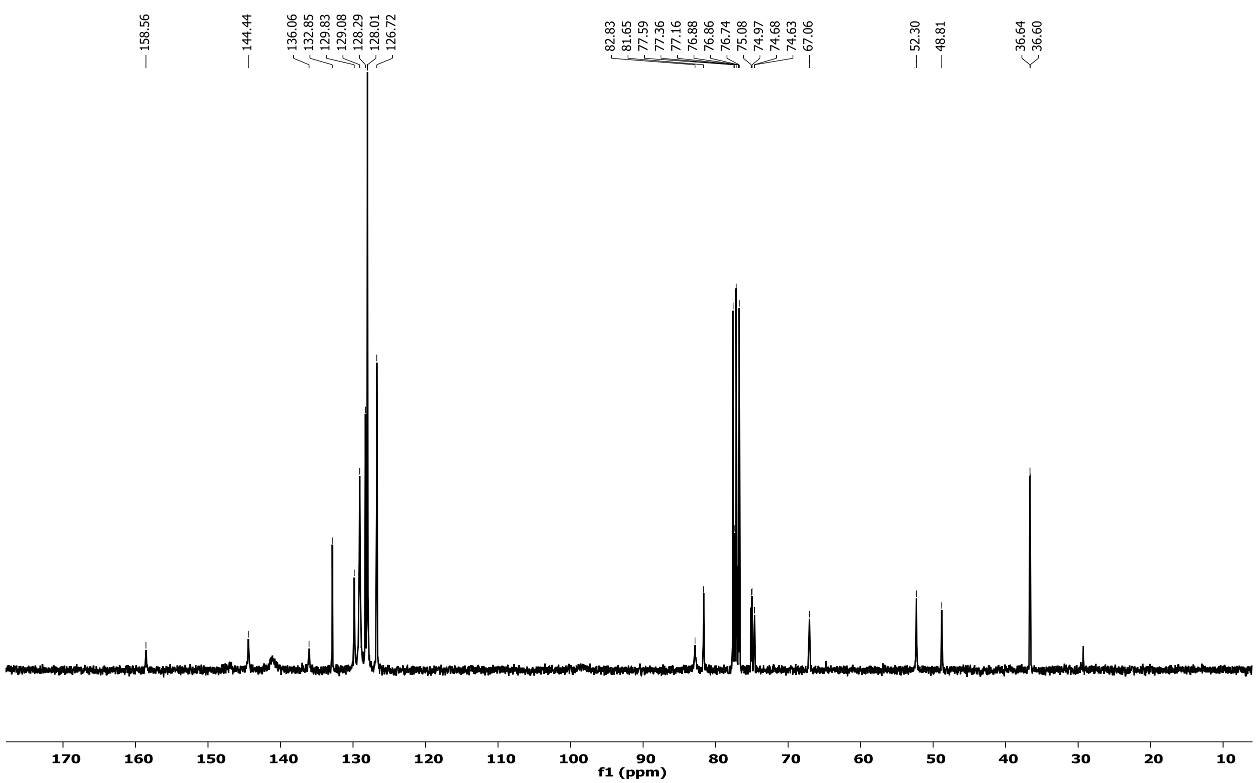
13C-NMR of compound **5l**, $CDCl_3$, 75 MHz



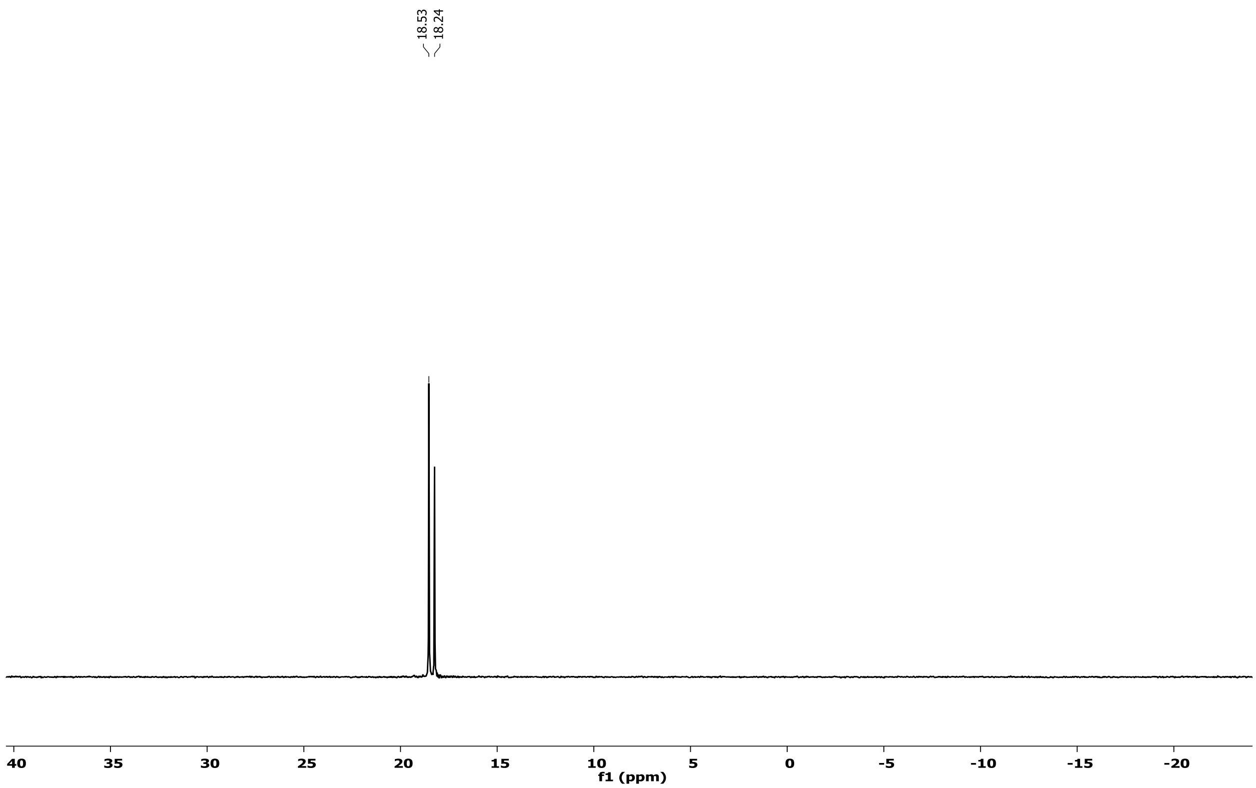
1H-NMR of compound **6a**, $CDCl_3$, 500 MHz



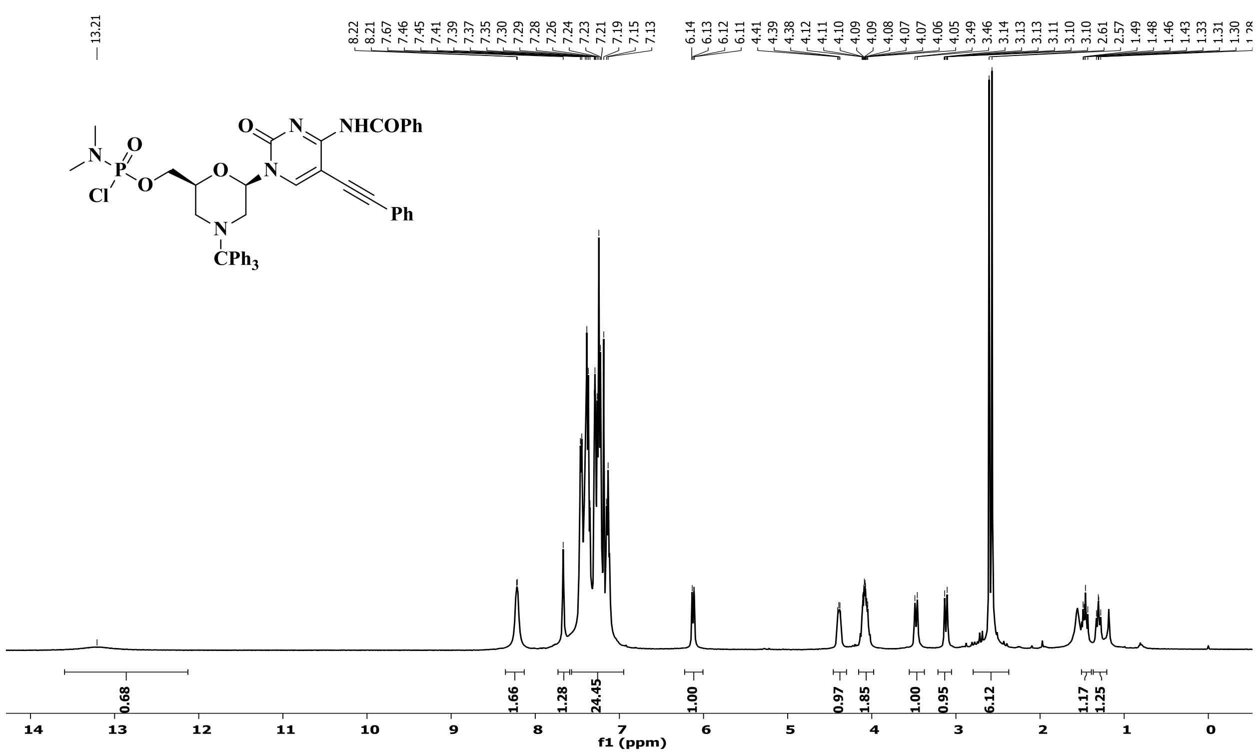
13C-NMR of compound **6a**, $CDCl_3$, 75 MHz



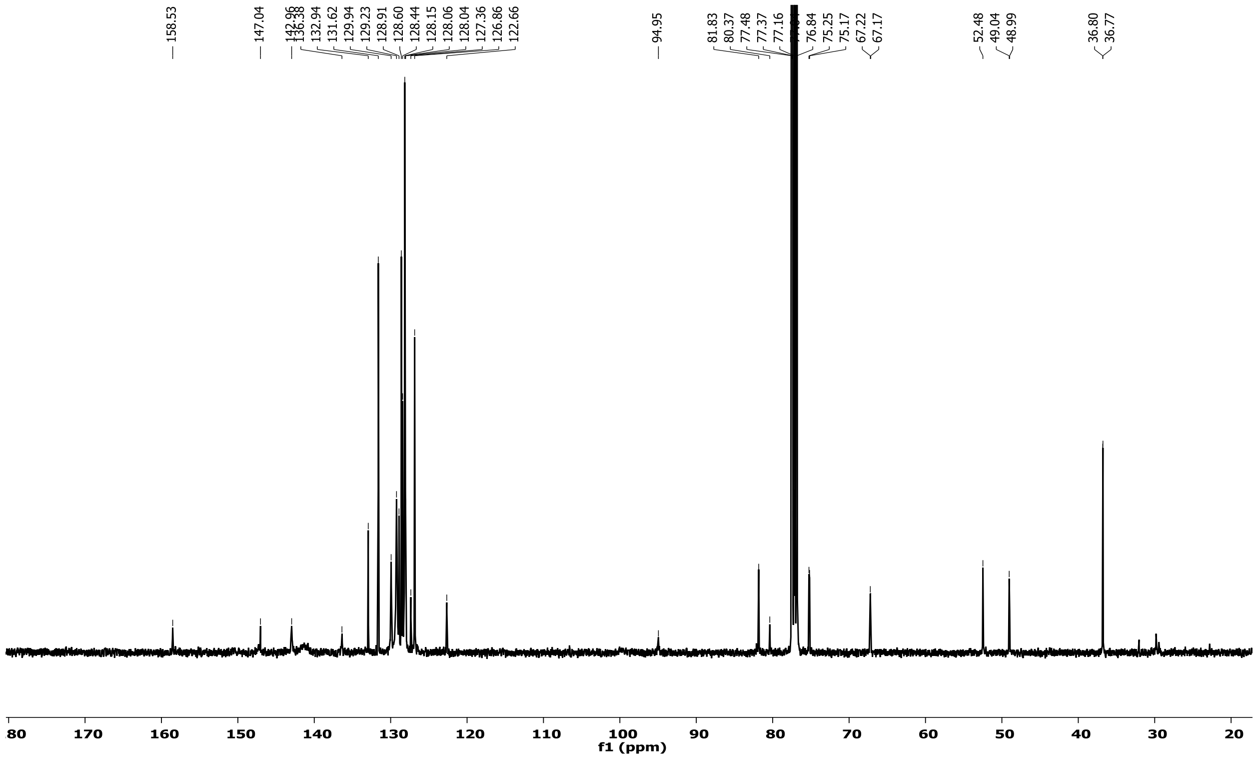
31P-NMR of compound **6a,** $CDCl_3$, 121 MHz



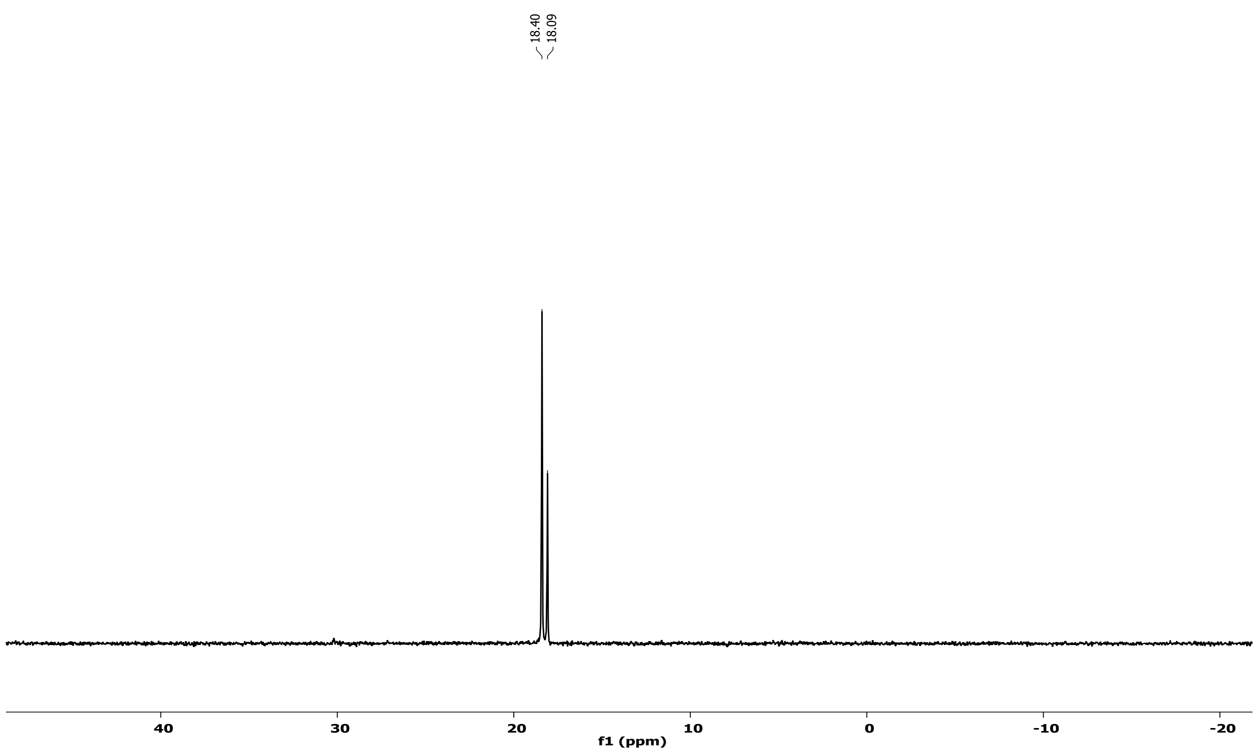
1H-NMR of compound **6b**, $CDCl_3$, 400 MHz



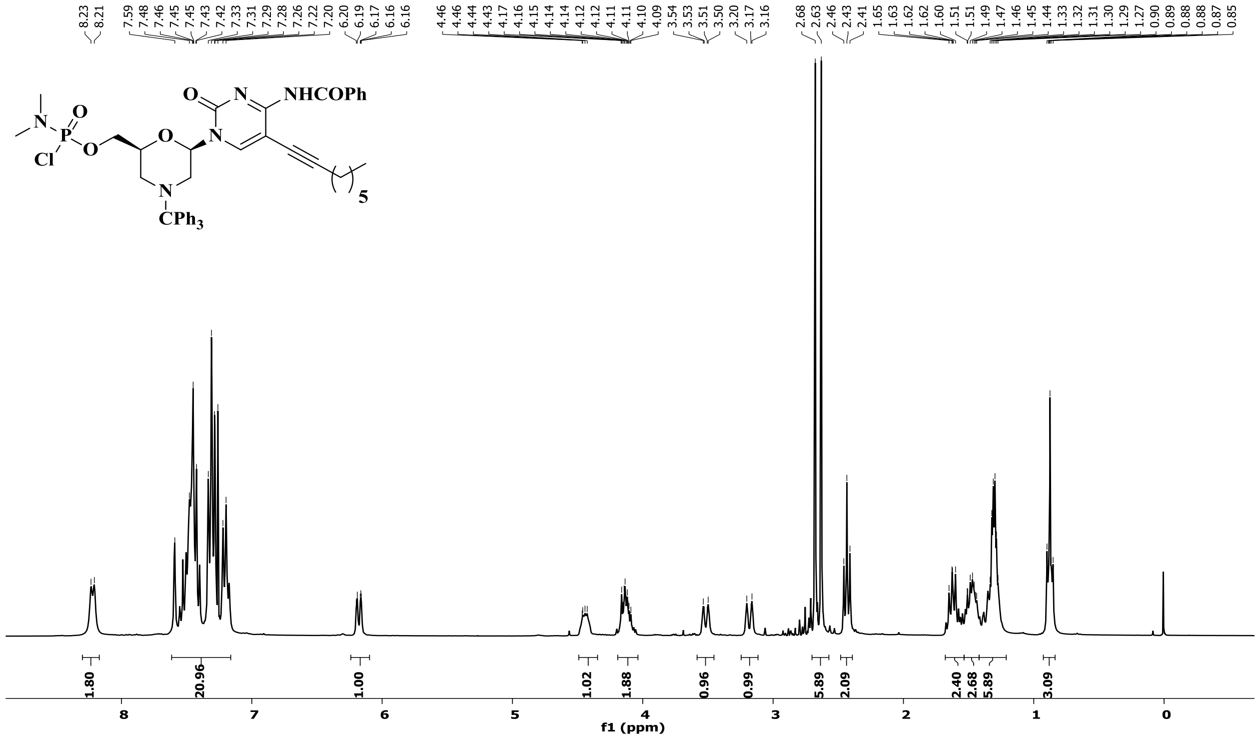
13C-NMR of compound **6b**, $CDCl_3$, 100 MHz



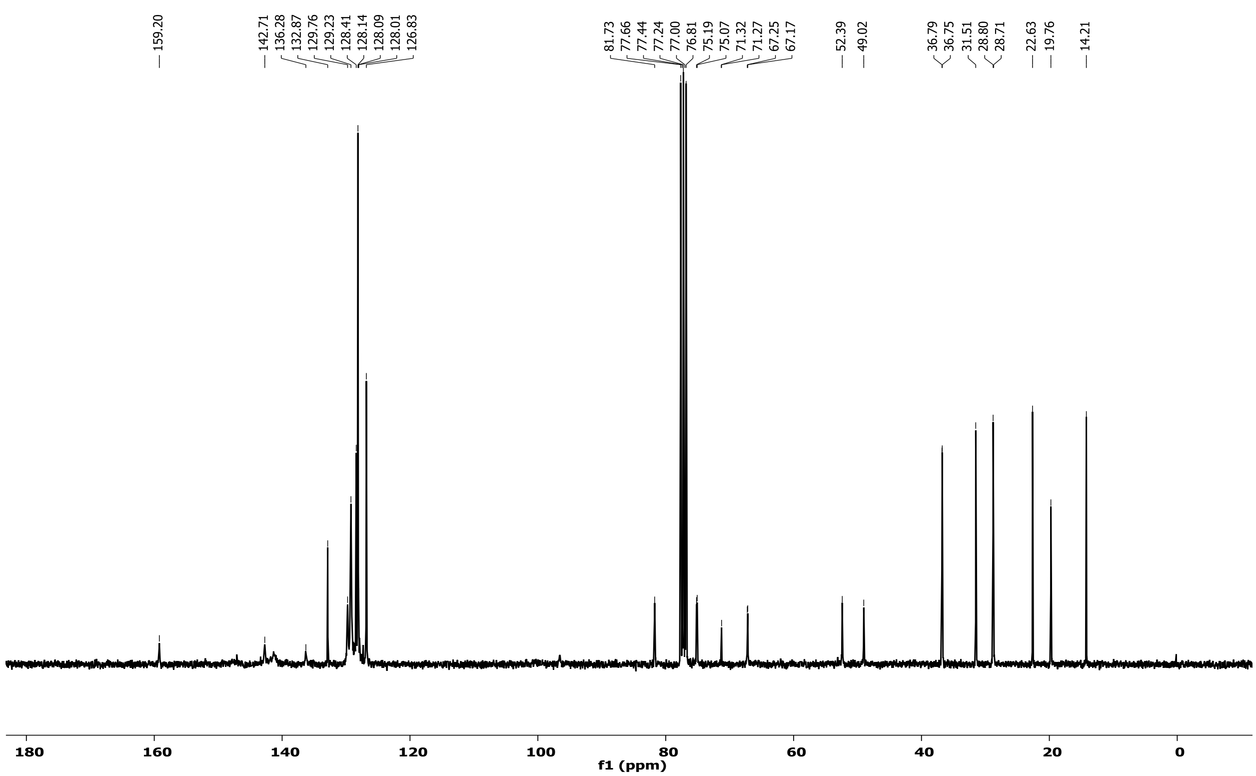
31P-NMR of compound **6b**, $CDCl_3$, 162 MHz



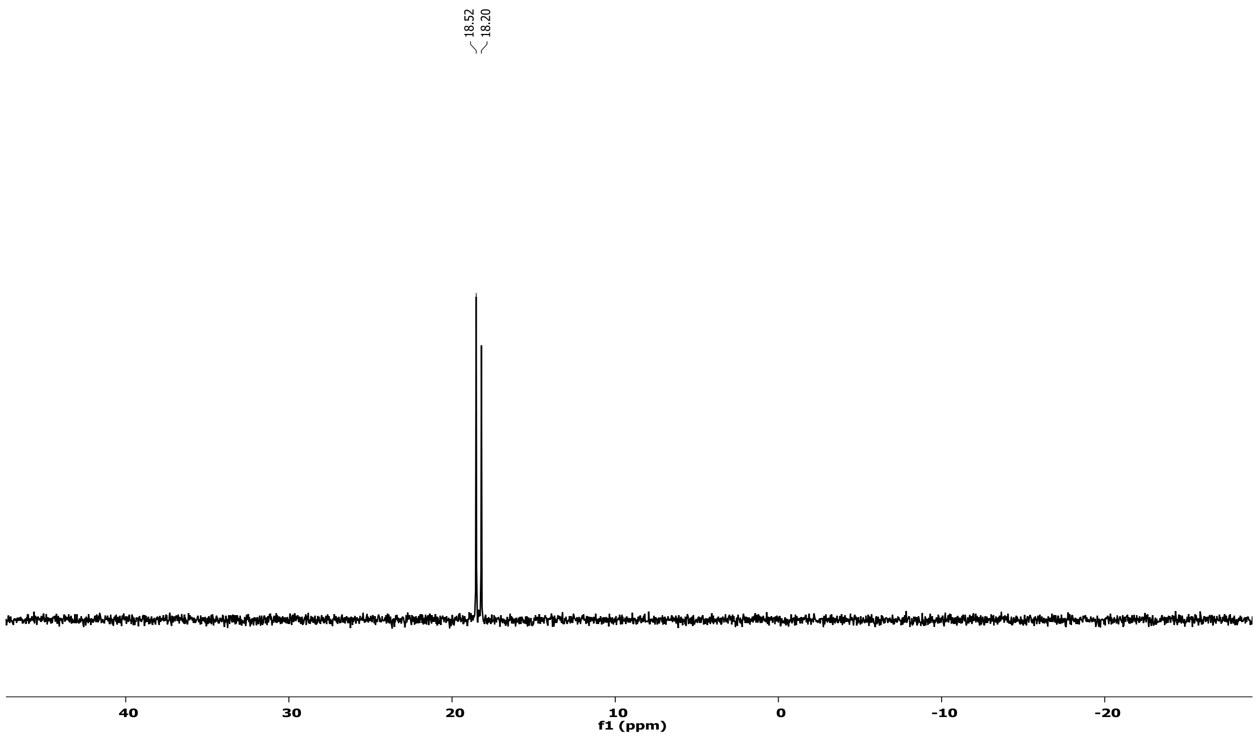
1H-NMR of compound **6c**, $CDCl_3$, 300 MHz



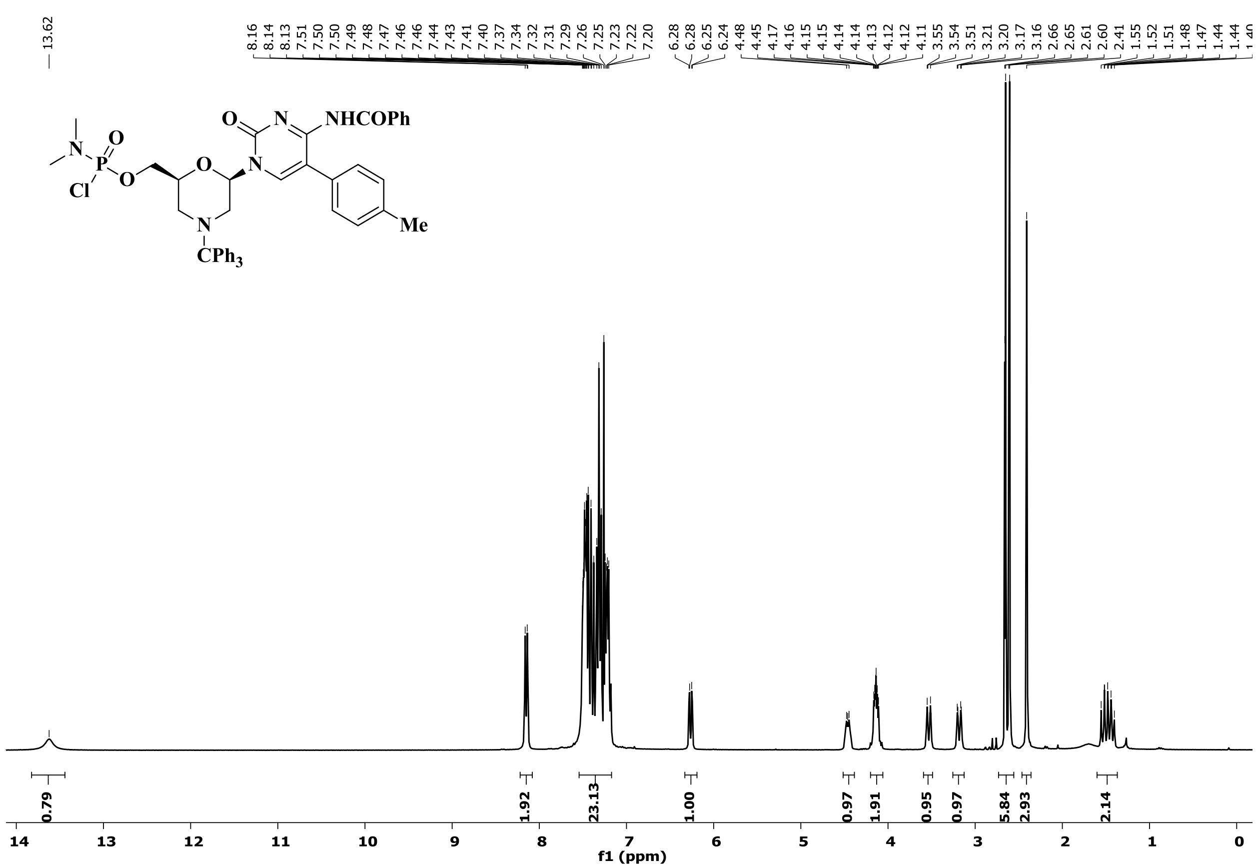
13C-NMR of compound **6c,** $CDCl_3$, 75 MHz



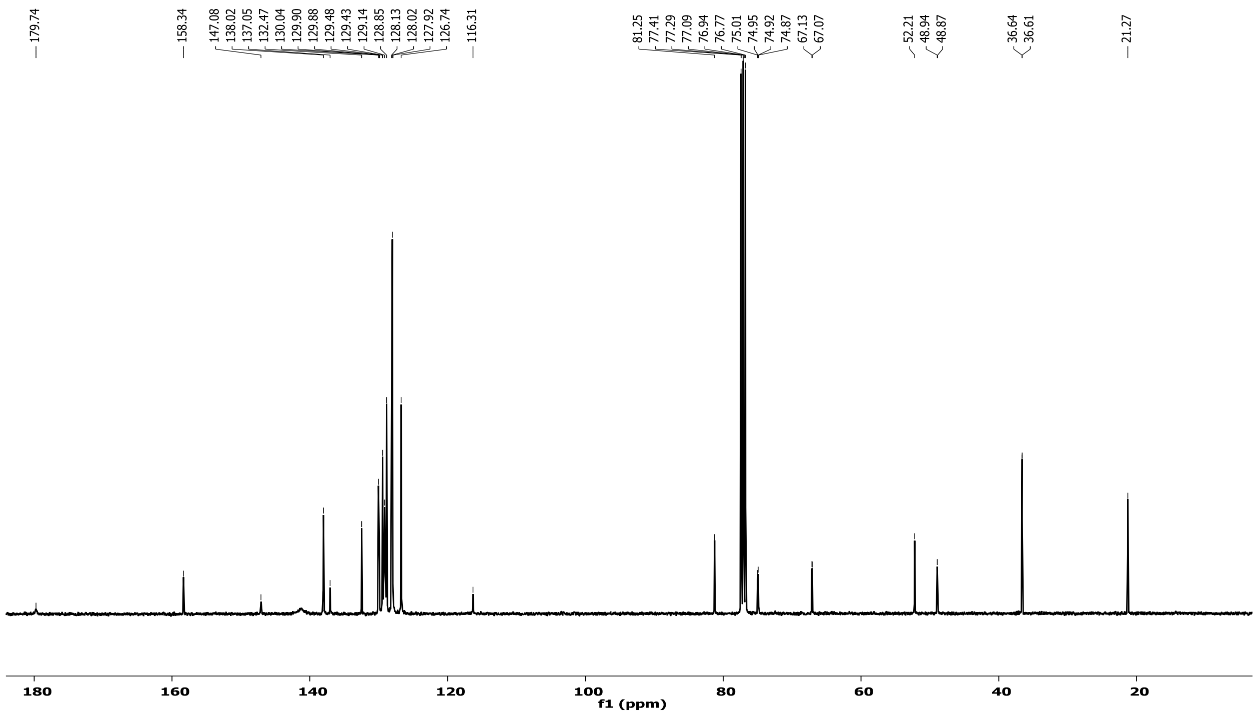
31P-NMR of compound **6c**, $CDCl_3$,121 MHz



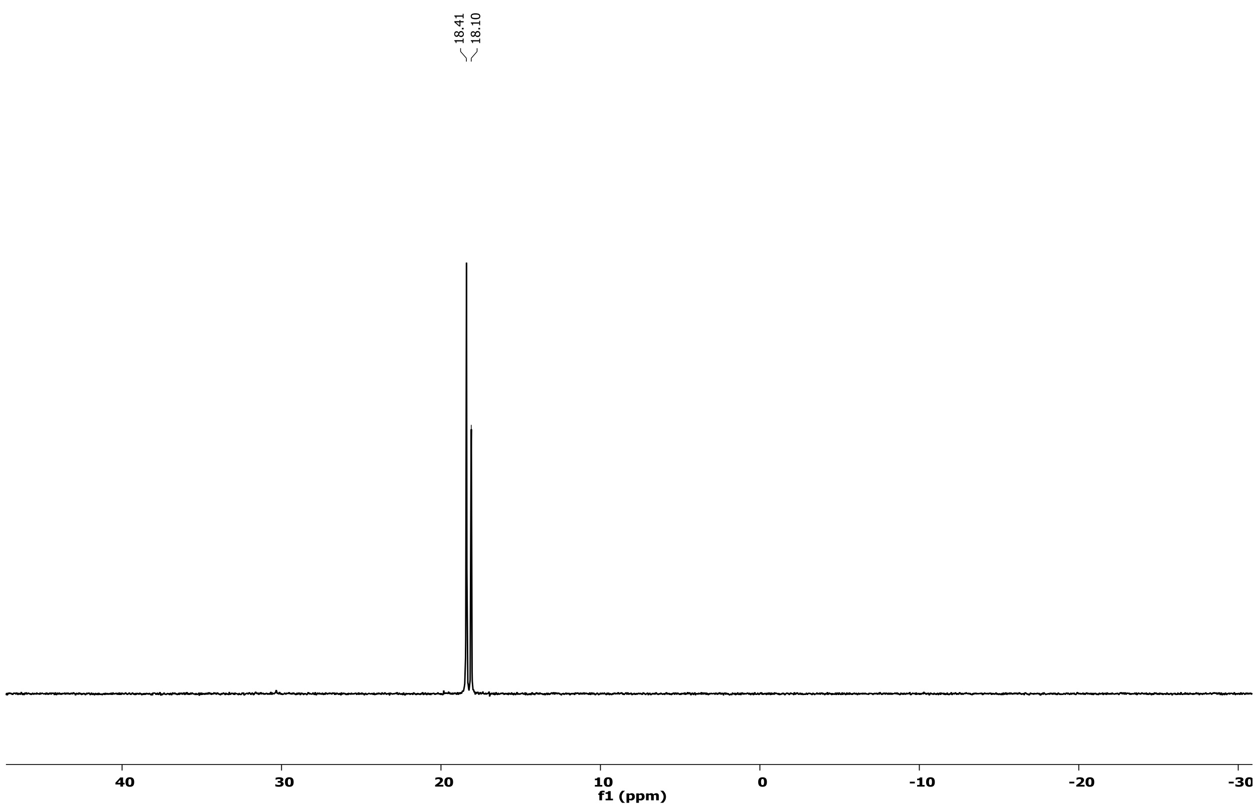
1H-NMR of compound **6d**, $CDCl_3$, 300 MHz



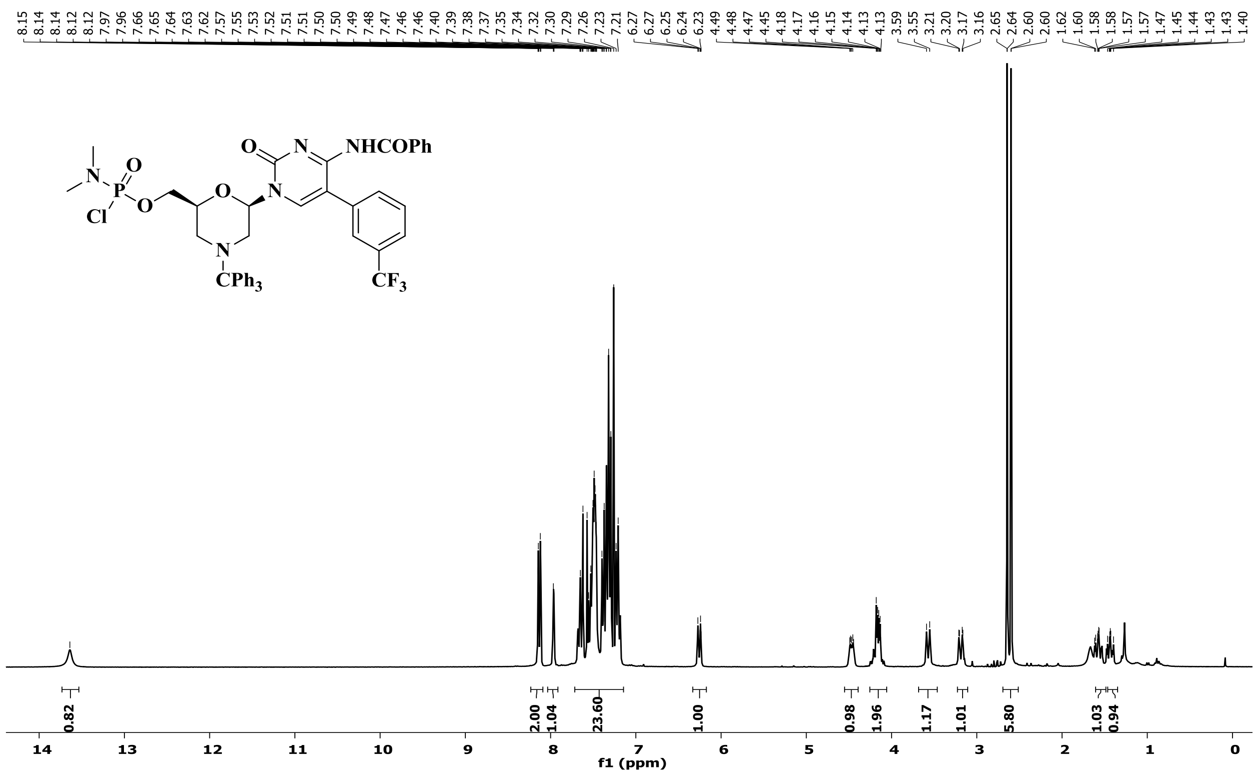
13C-NMR of compound **6d**, $CDCl_3$, 100 MHz



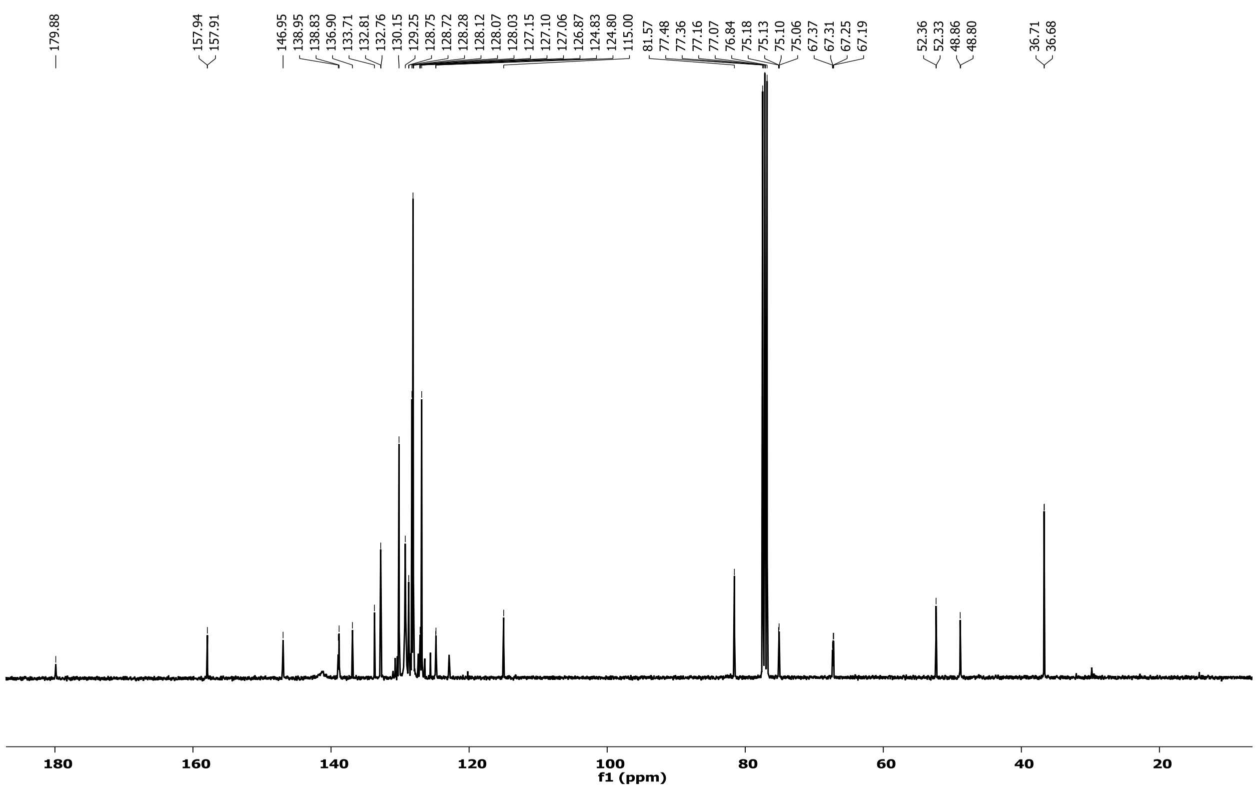
31P-NMR of compound **6d**, $CDCl_3$, 162 MHz



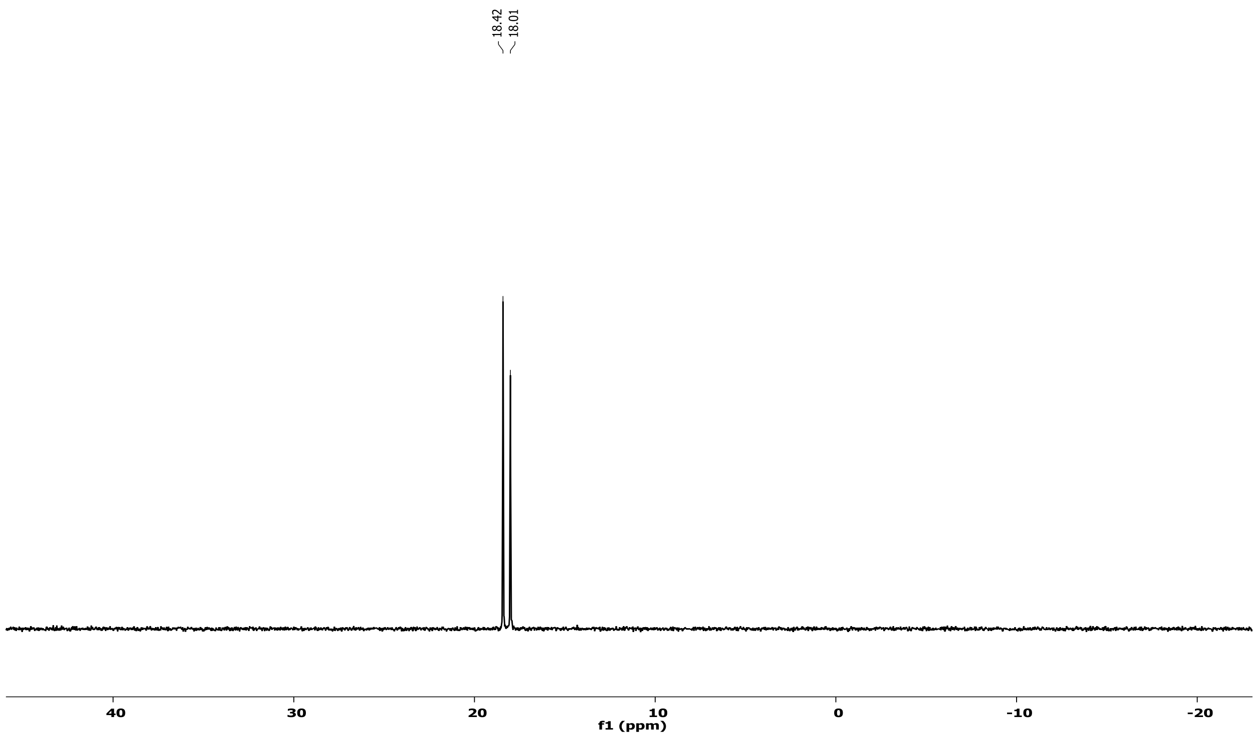
1H-NMR of compound **6e**, $CDCl_3$, 300 MHz



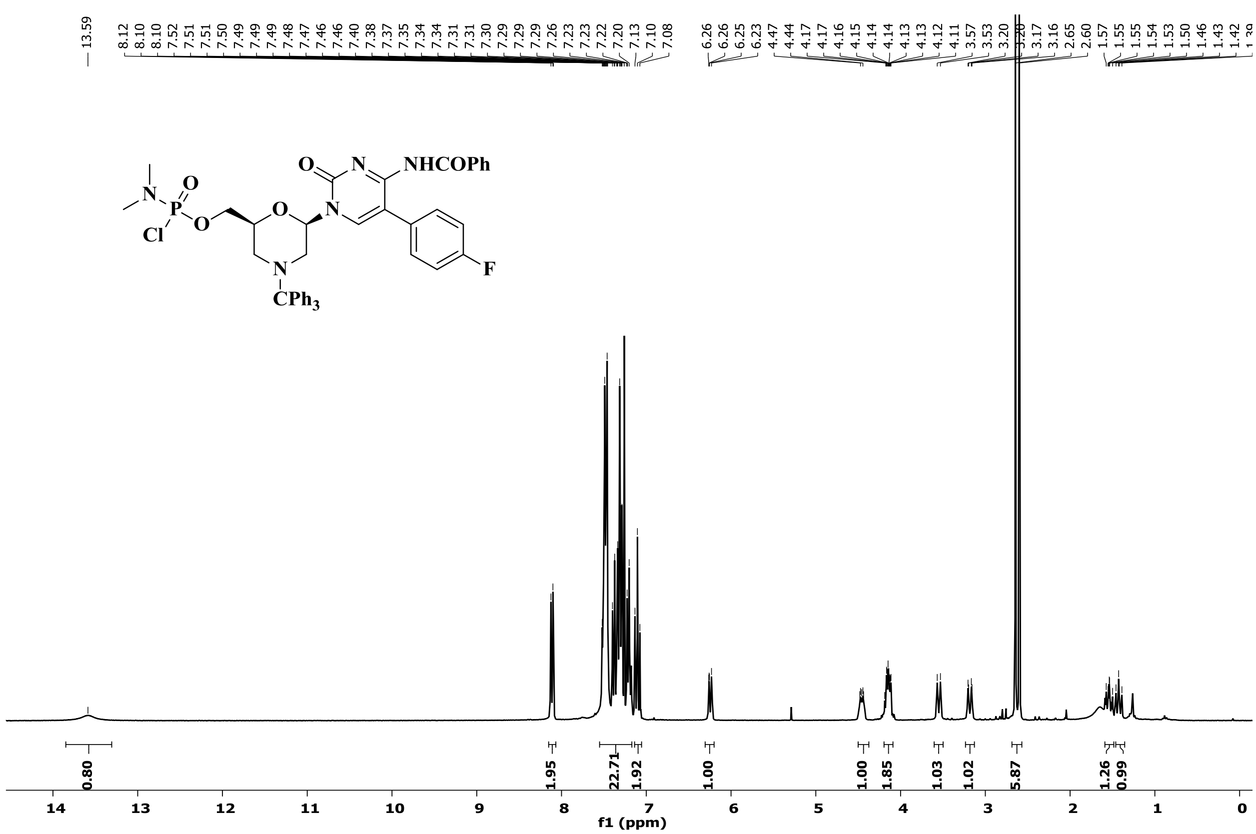
13C-NMR of compound **6e**, $CDCl_3$, 100 MHz



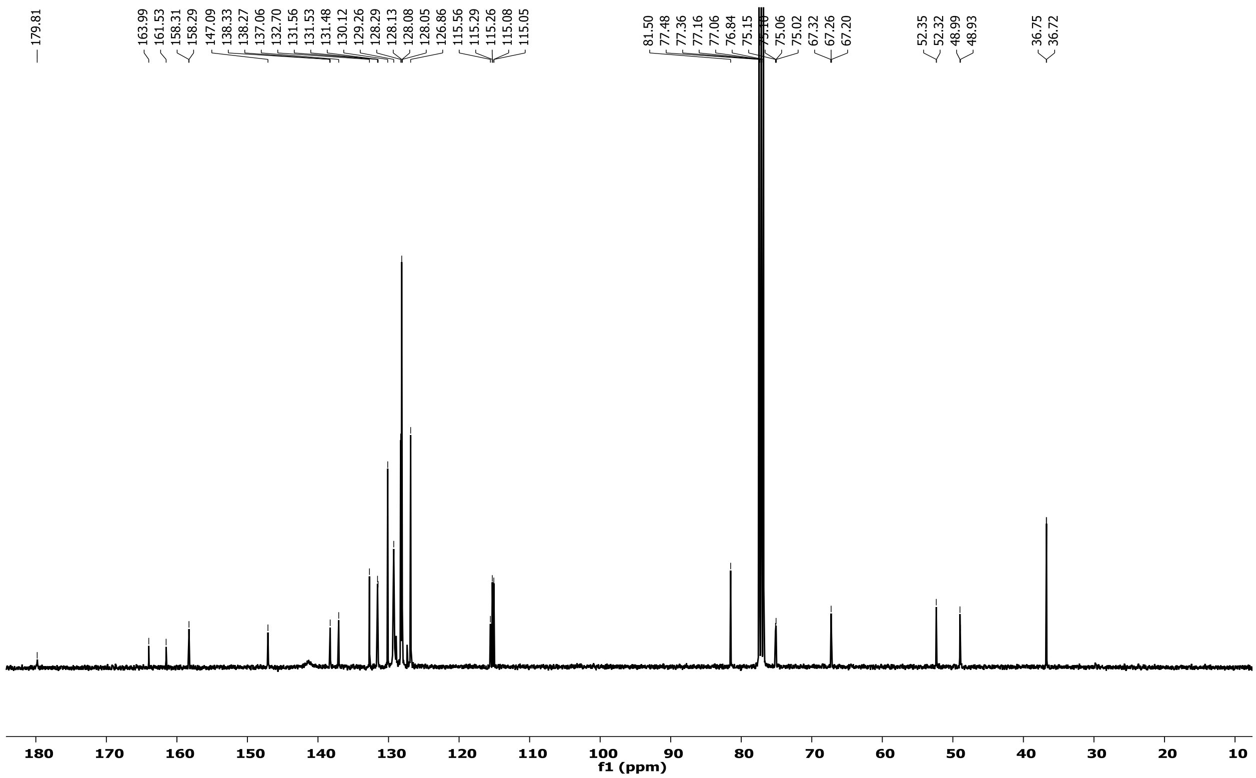
31P-NMR of compound **6e**, $CDCl_3$, 162 MHz



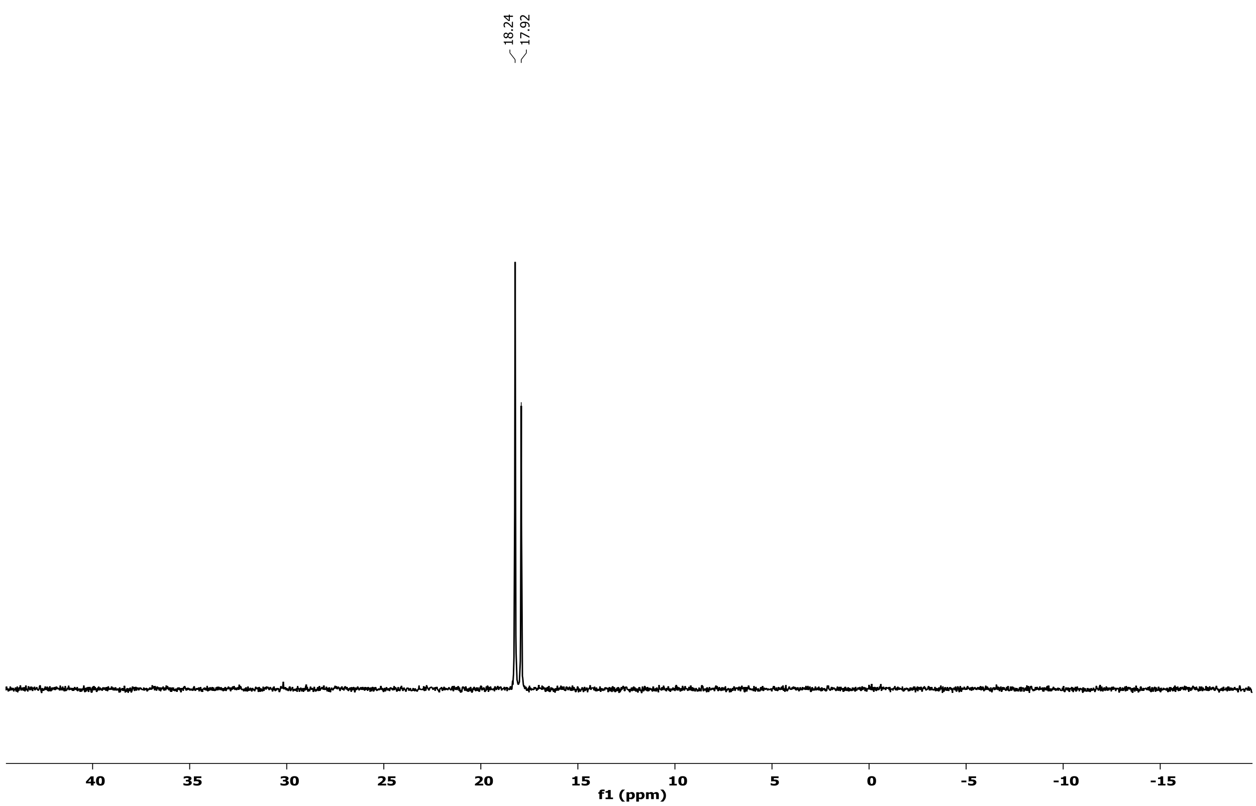
1H-NMR of compound **6f**, $CDCl_3$, 300 MHz



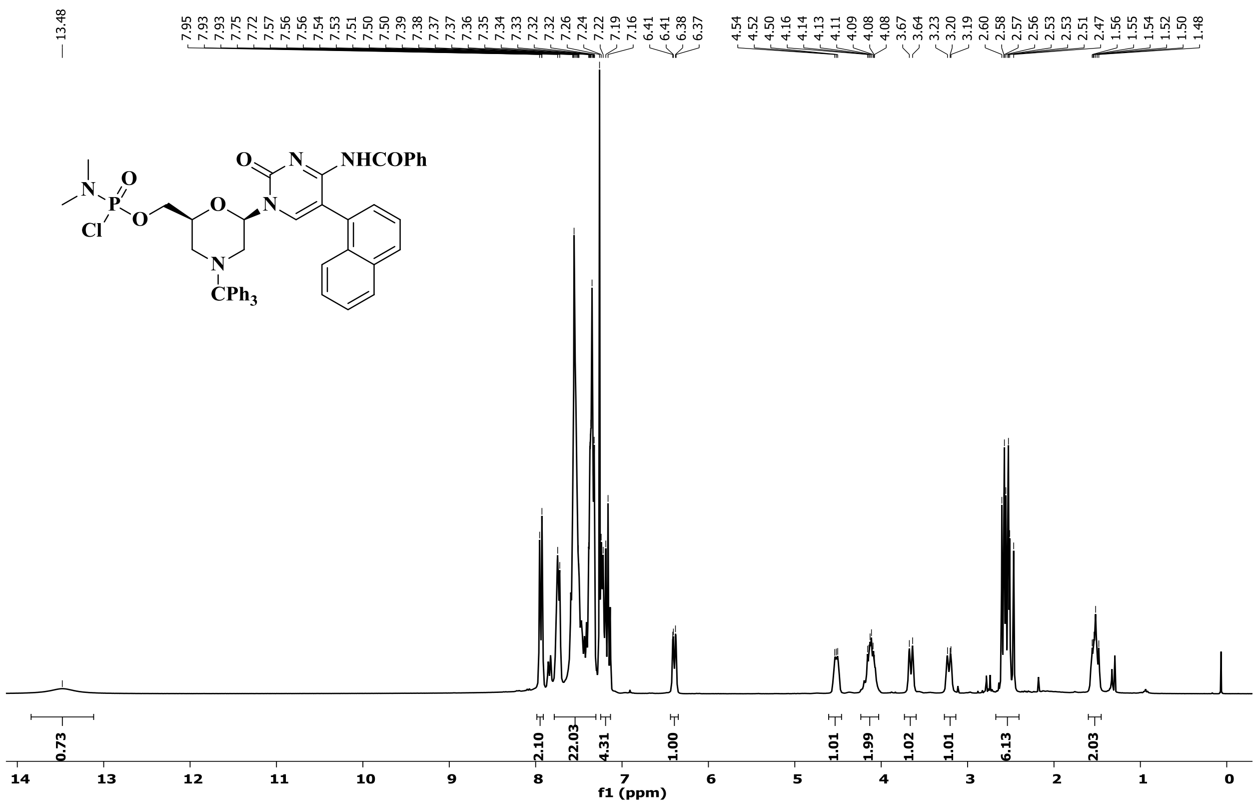
13C-NMR of compound **6f**, $CDCl_3$, 100 MHz



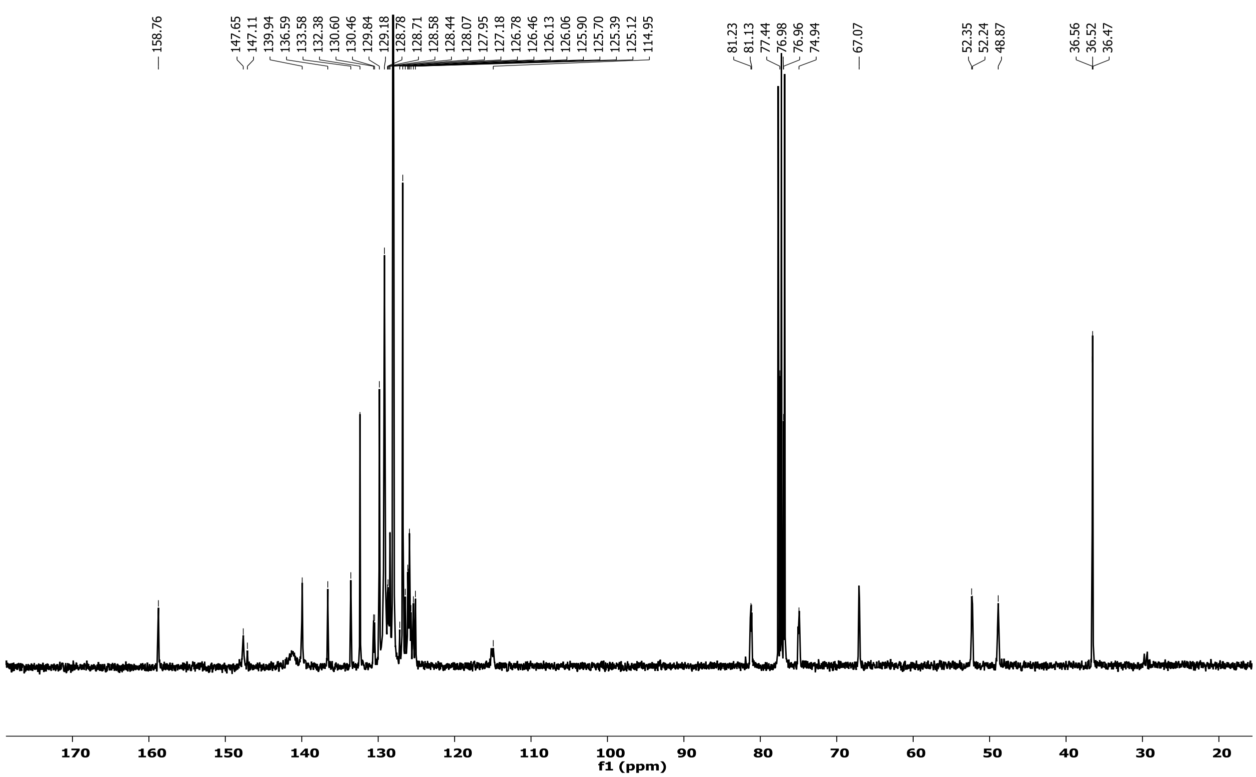
31P-NMR of compound **6f**, $CDCl_3$, 162 MHz



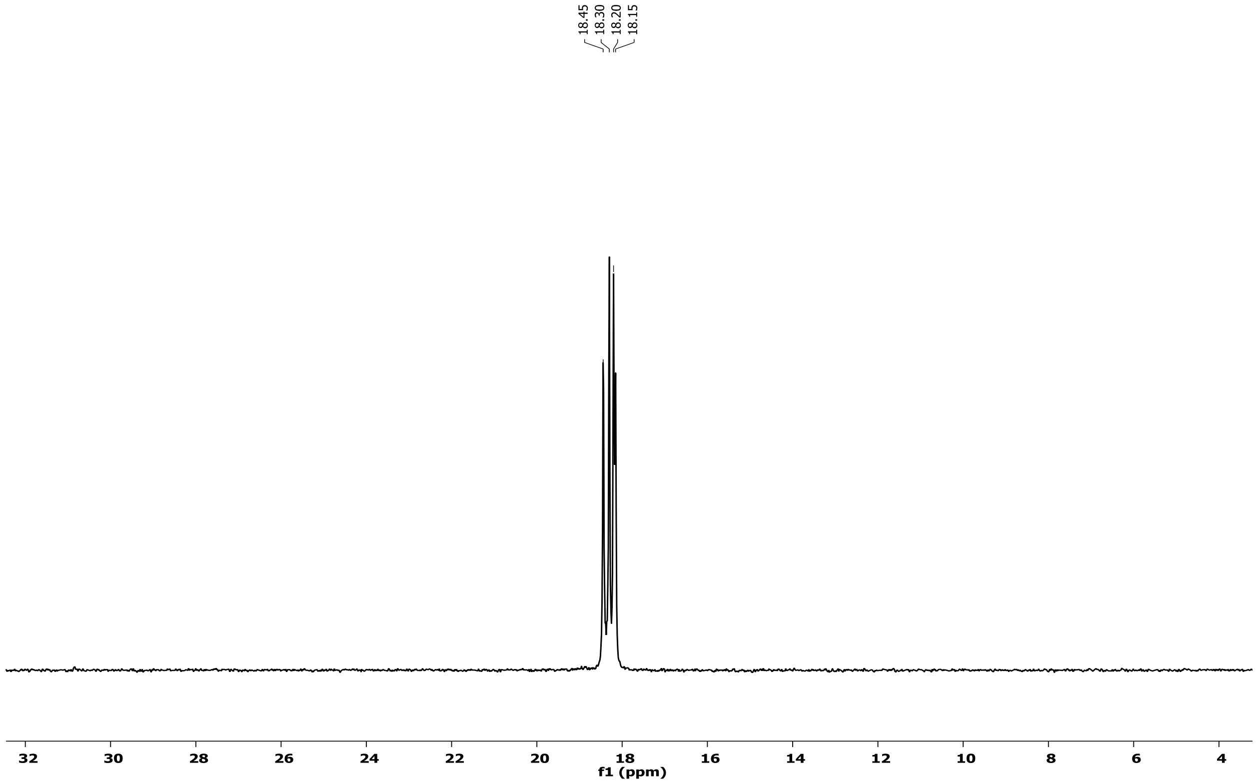
1H-NMR of compound **6g**, $CDCl_3$, 300 MHz



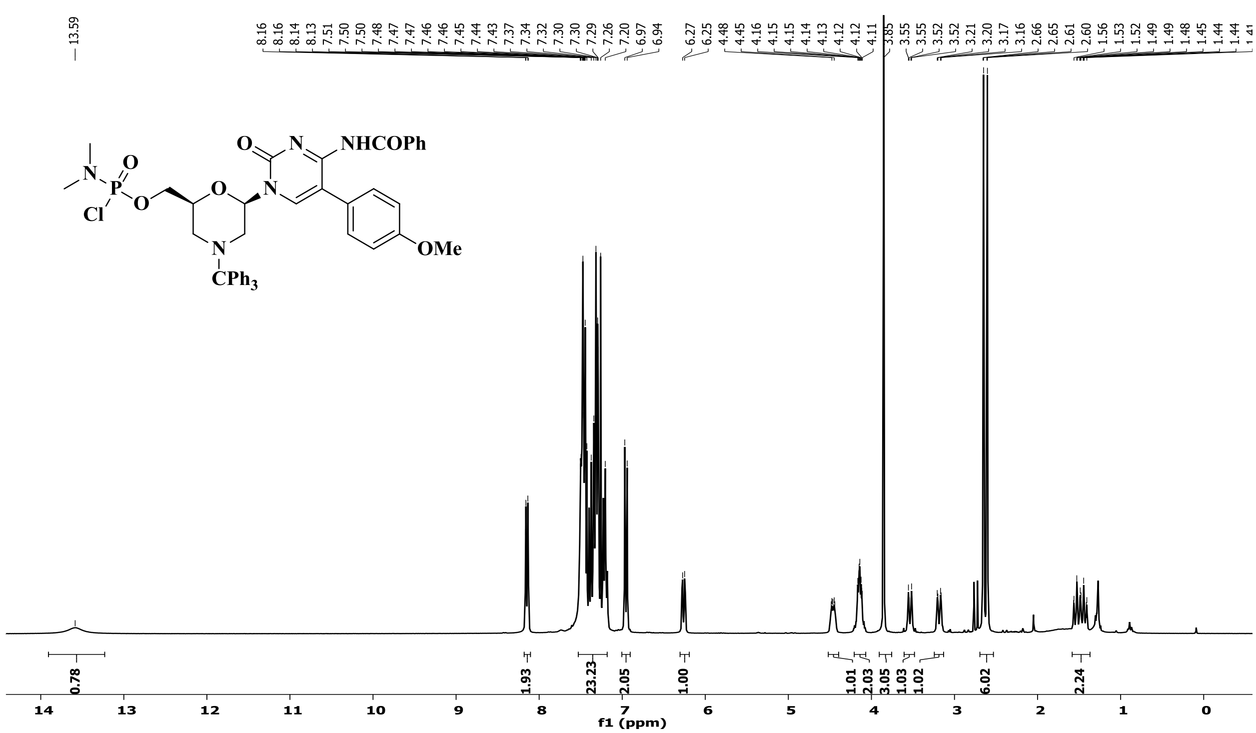
13C-NMR of compound **6g**, $CDCl_3$, 75 MHz



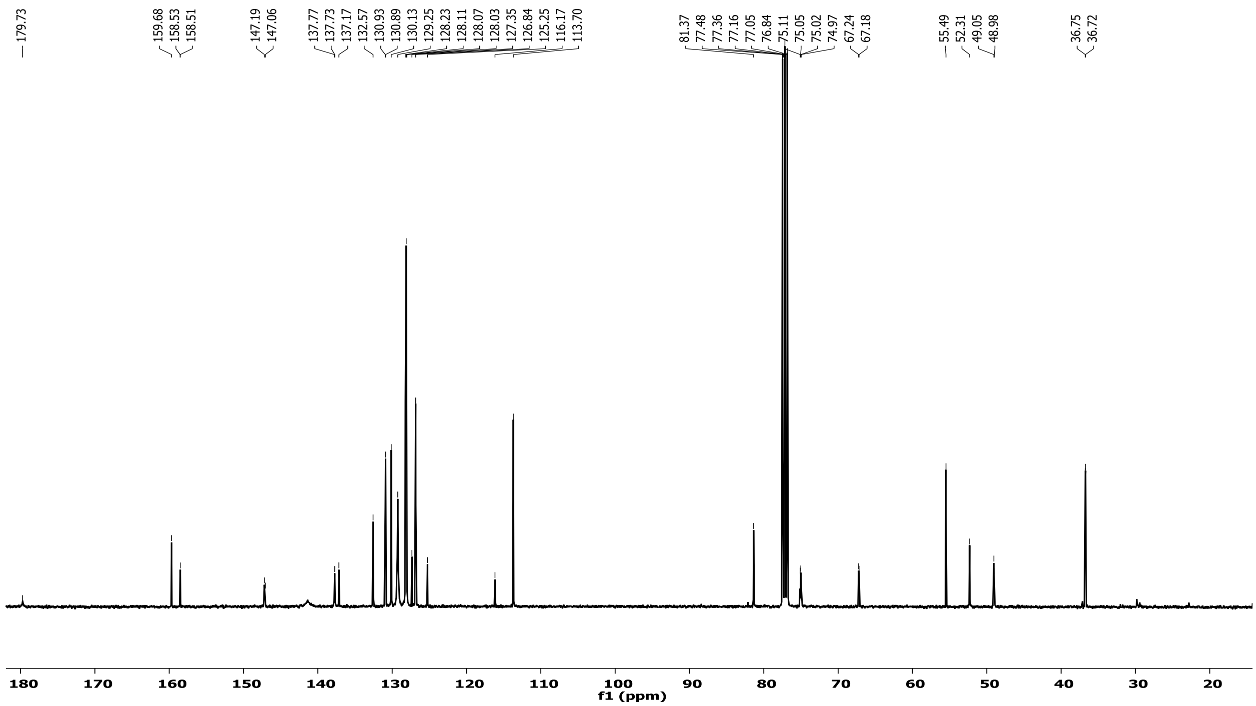
31P-NMR of compound **6g**, $CDCl_3$, 121 MHz



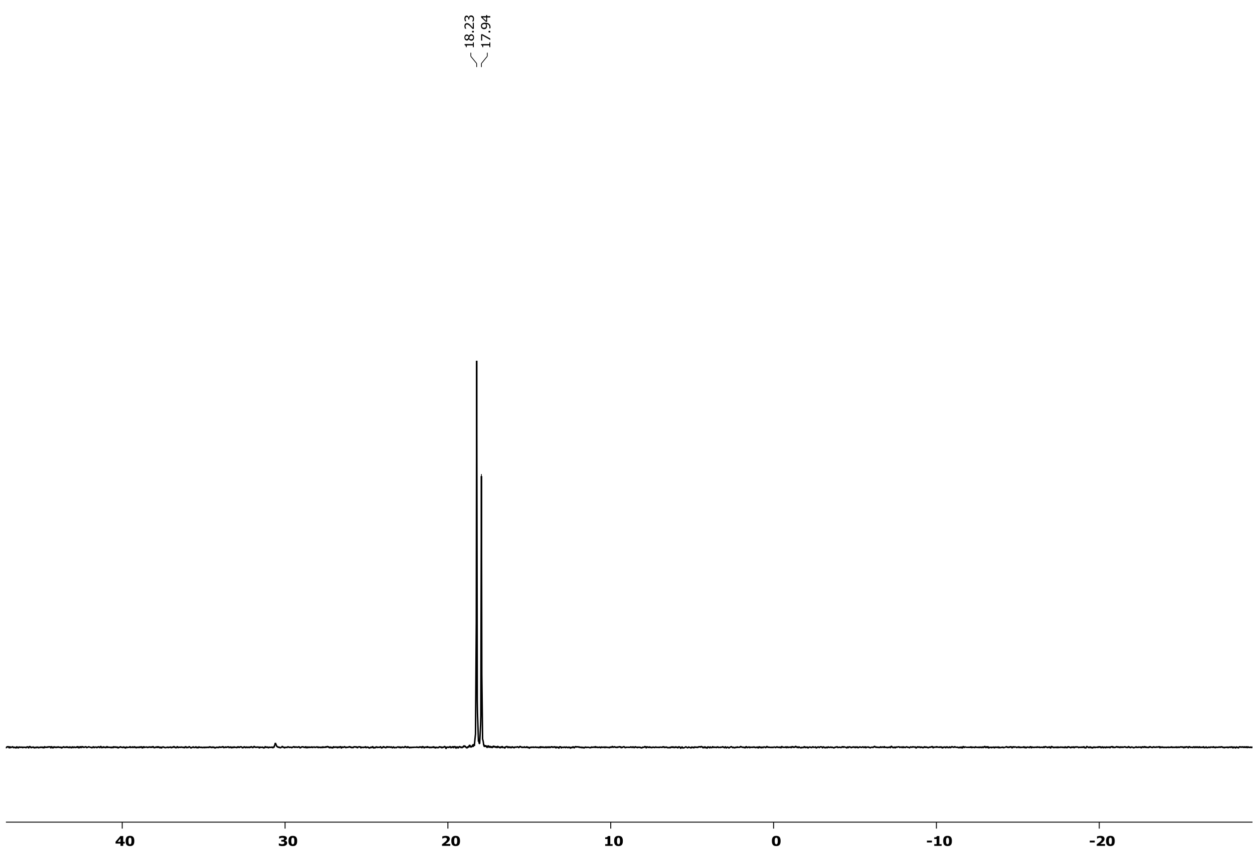
1H-NMR of compound **6h**, $CDCl_3$, 300 MHz



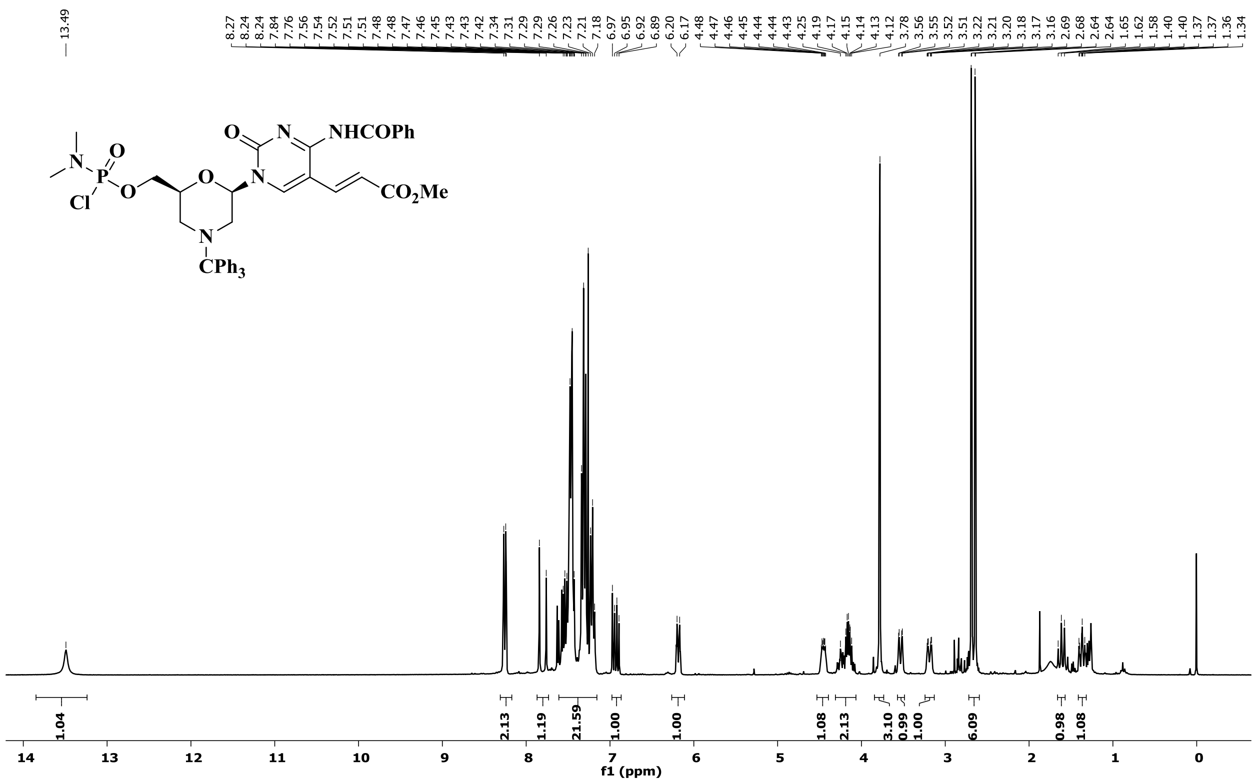
13C-NMR of compound **6h**, $CDCl_3$, 100 MHz



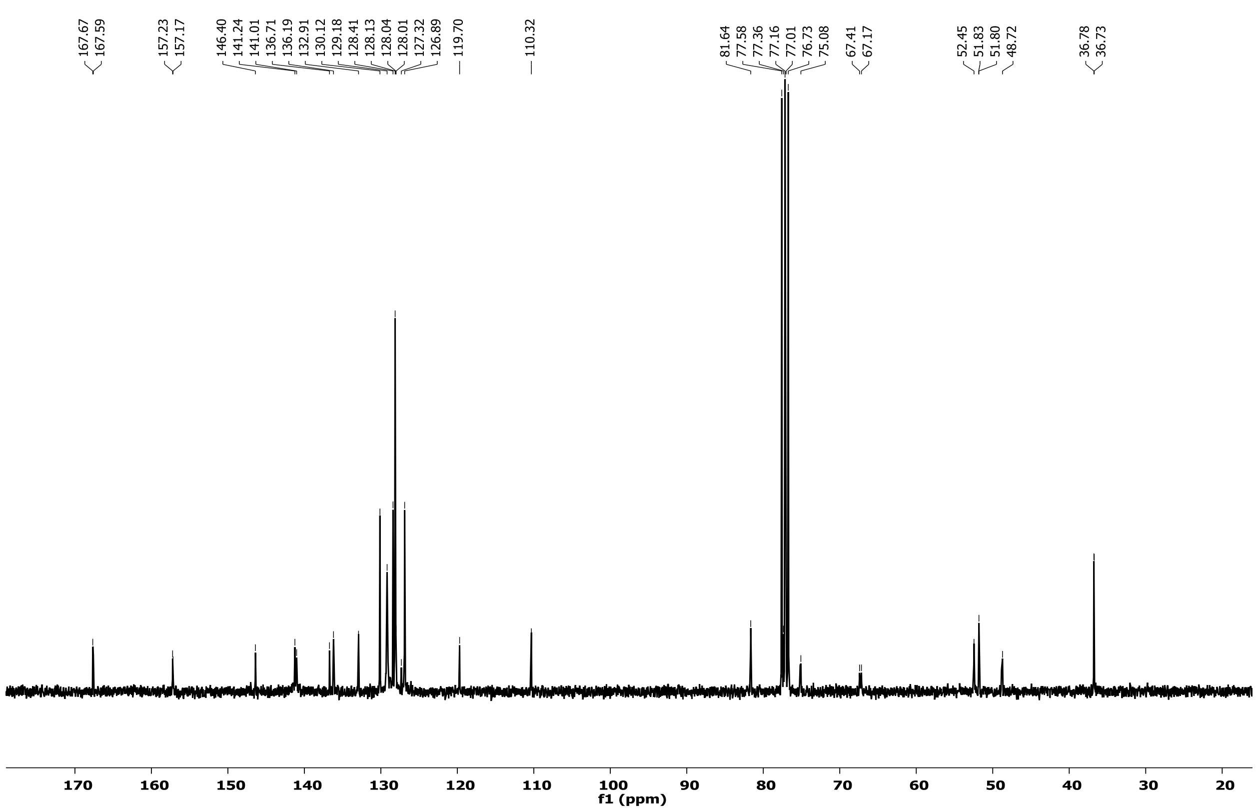
31P-NMR of compound **6h**, $CDCl_3$, 162 MHz



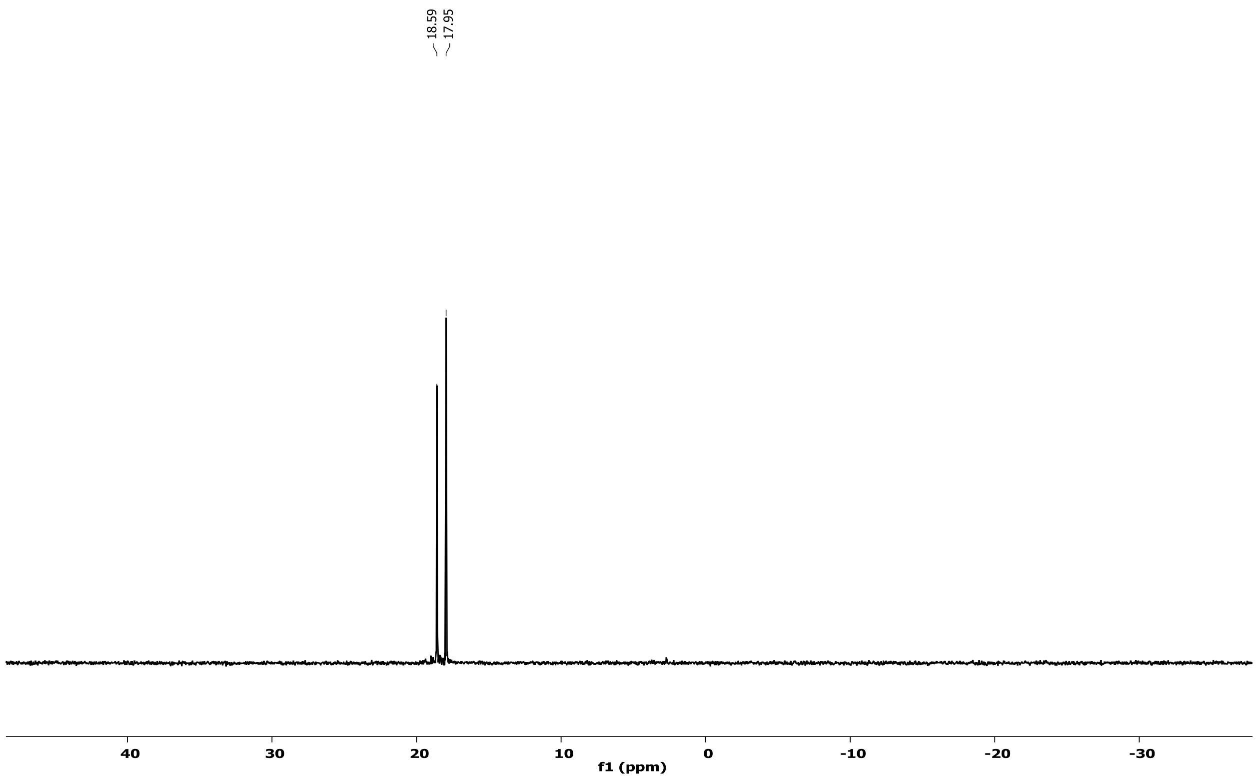
1H-NMR of compound **6i**, $CDCl_3$, 300 MHz



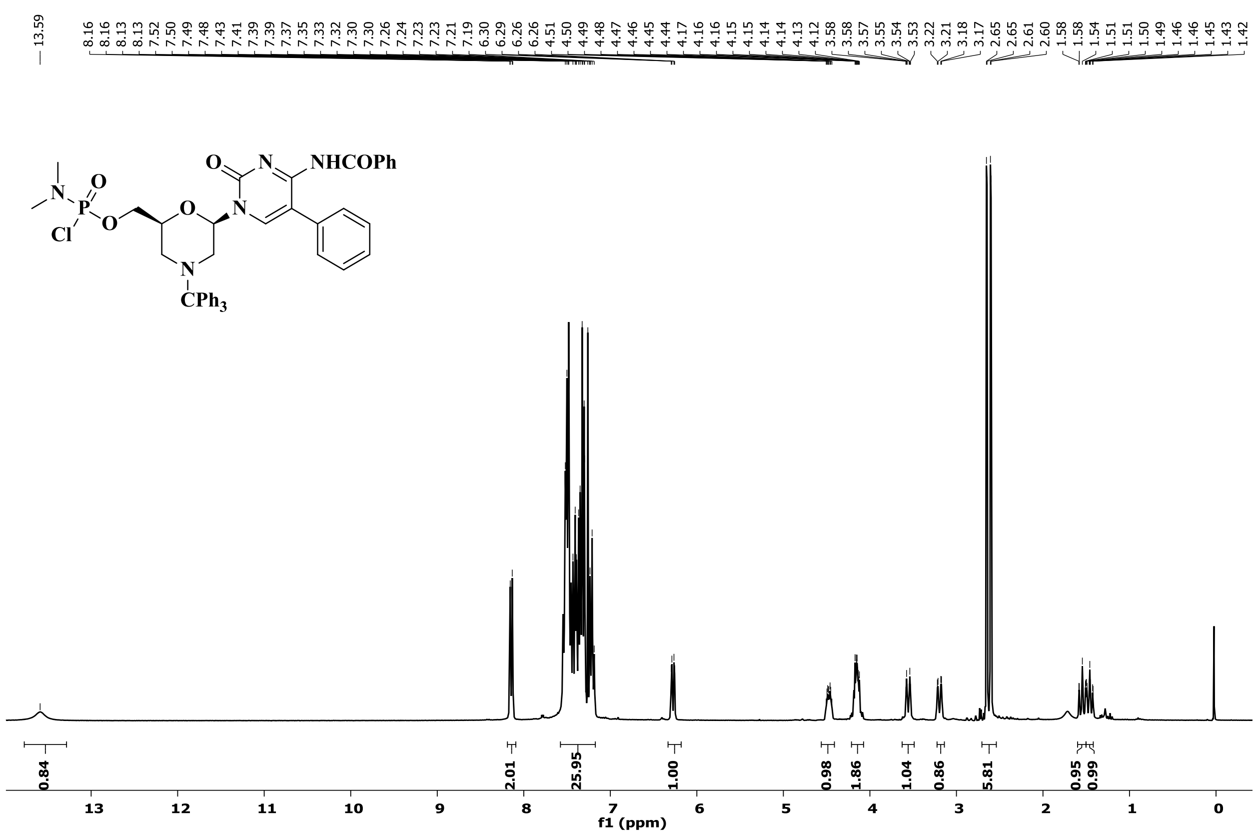
13C-NMR of compound **6i,** $CDCl_3$, 75 MHz



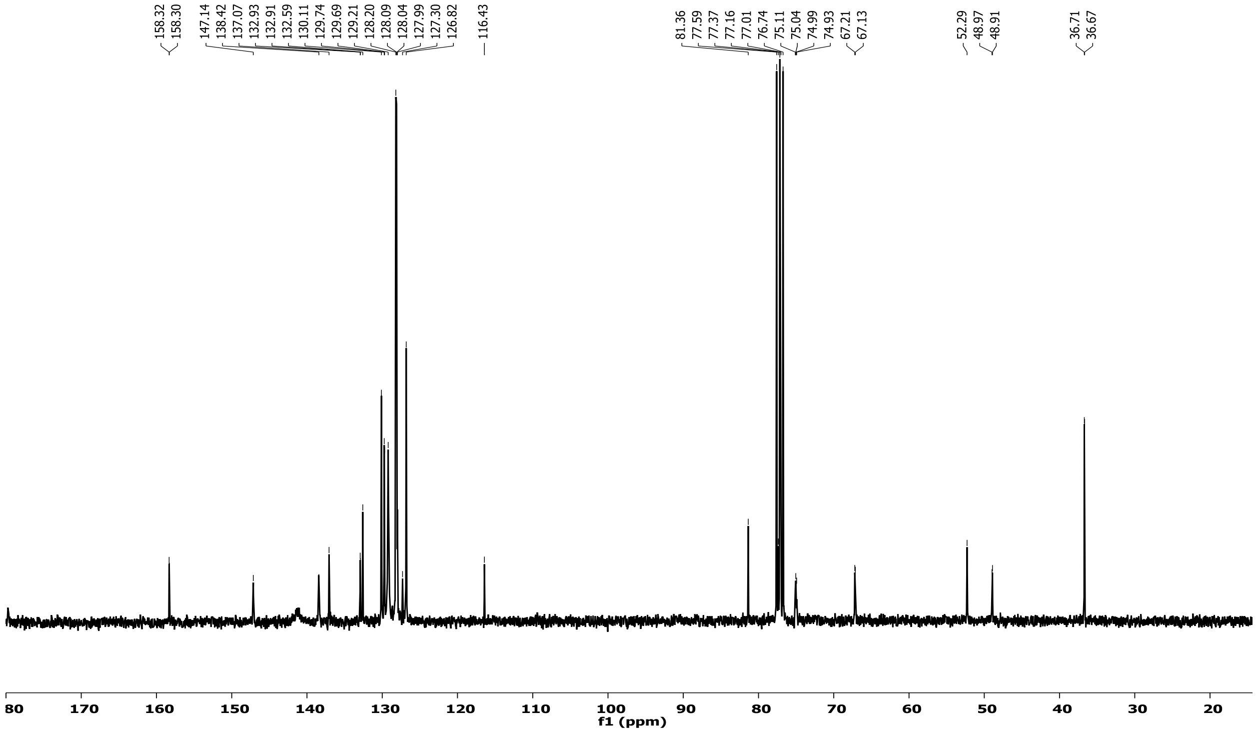
31P-NMR of compound **6i**, $CDCl_3$, 162 MHz



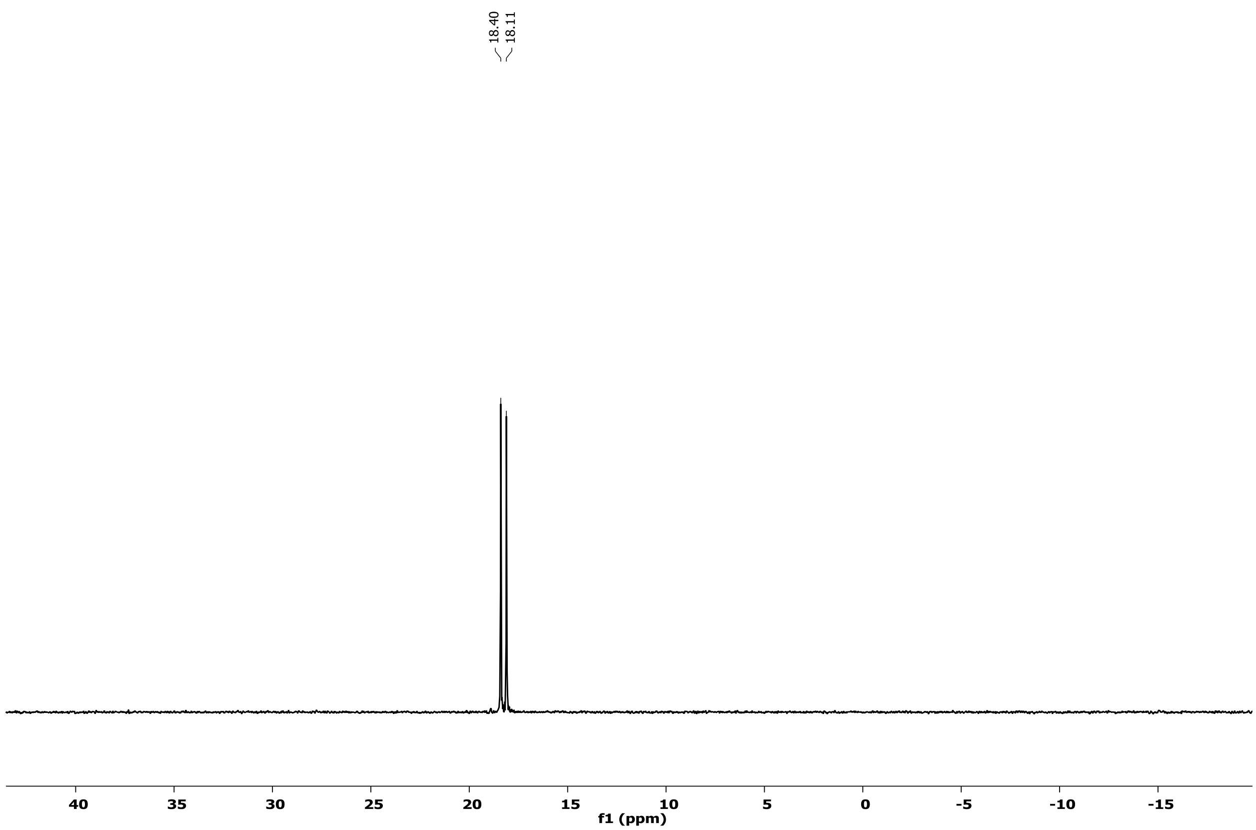
1H-NMR of compound **6j**, $CDCl_3$, 300 MHz



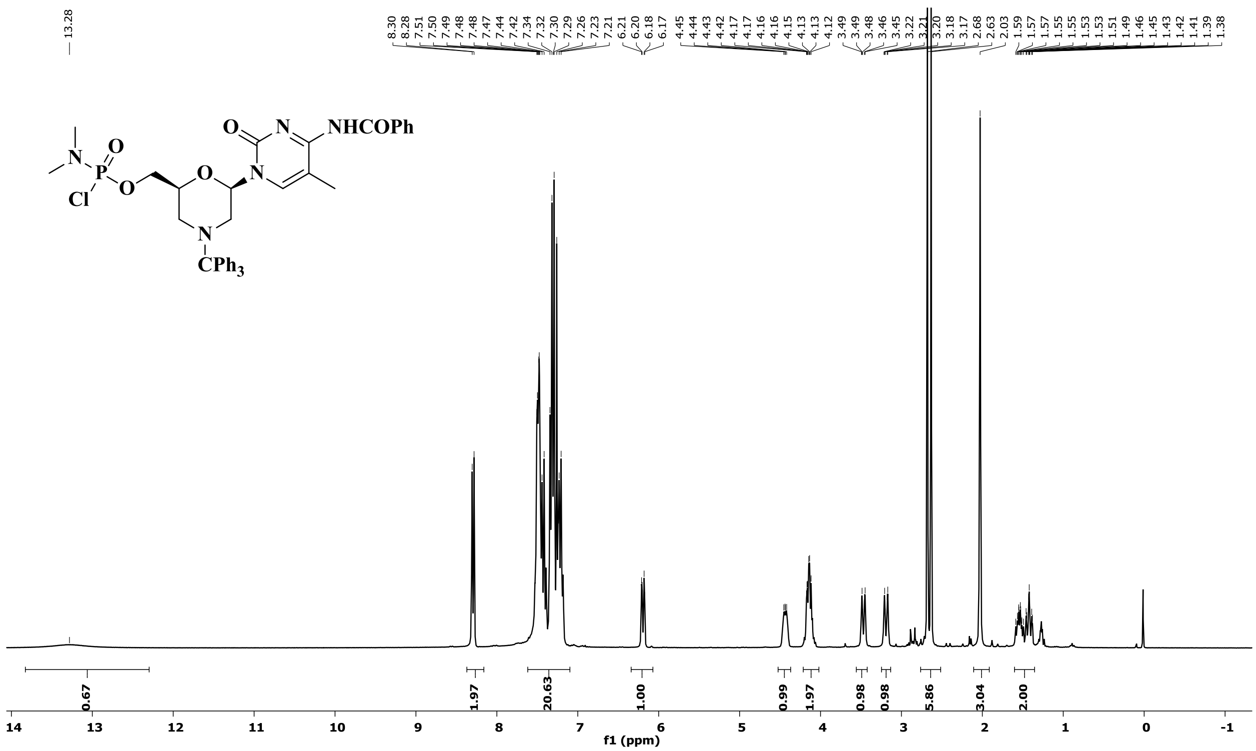
13C-NMR of compound **6j**, $CDCl_3$, 75 MHz



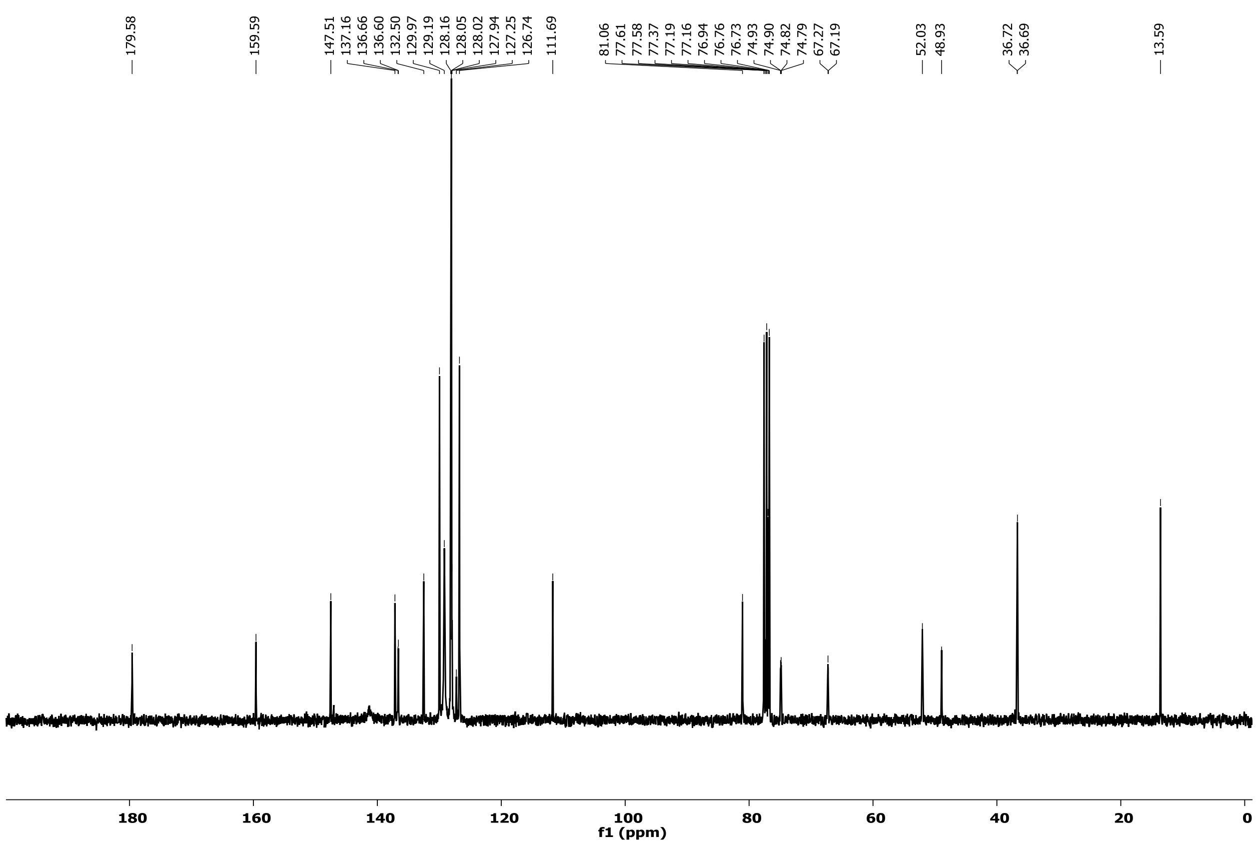
31P-NMR of compound **6j**, $CDCl_3$, 121 MHz



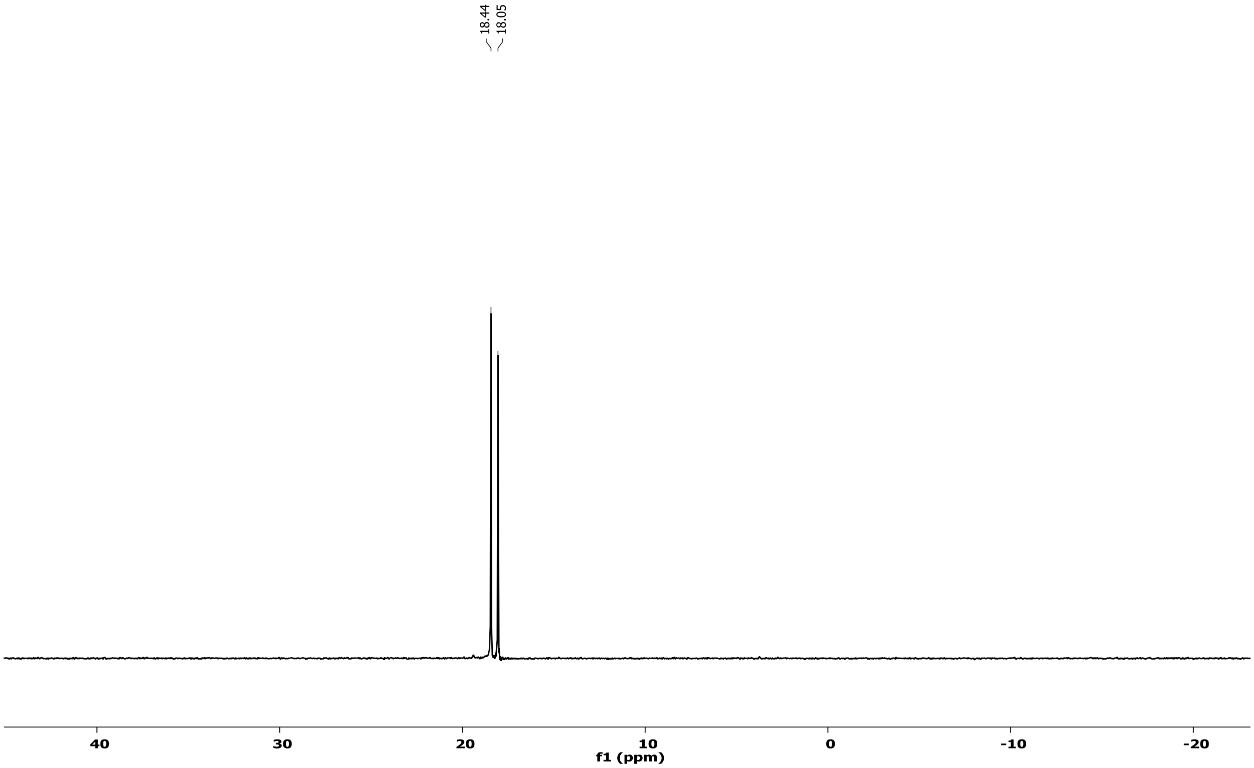
1H-NMR of compound **6k**, $CDCl_3$, 300 MHz



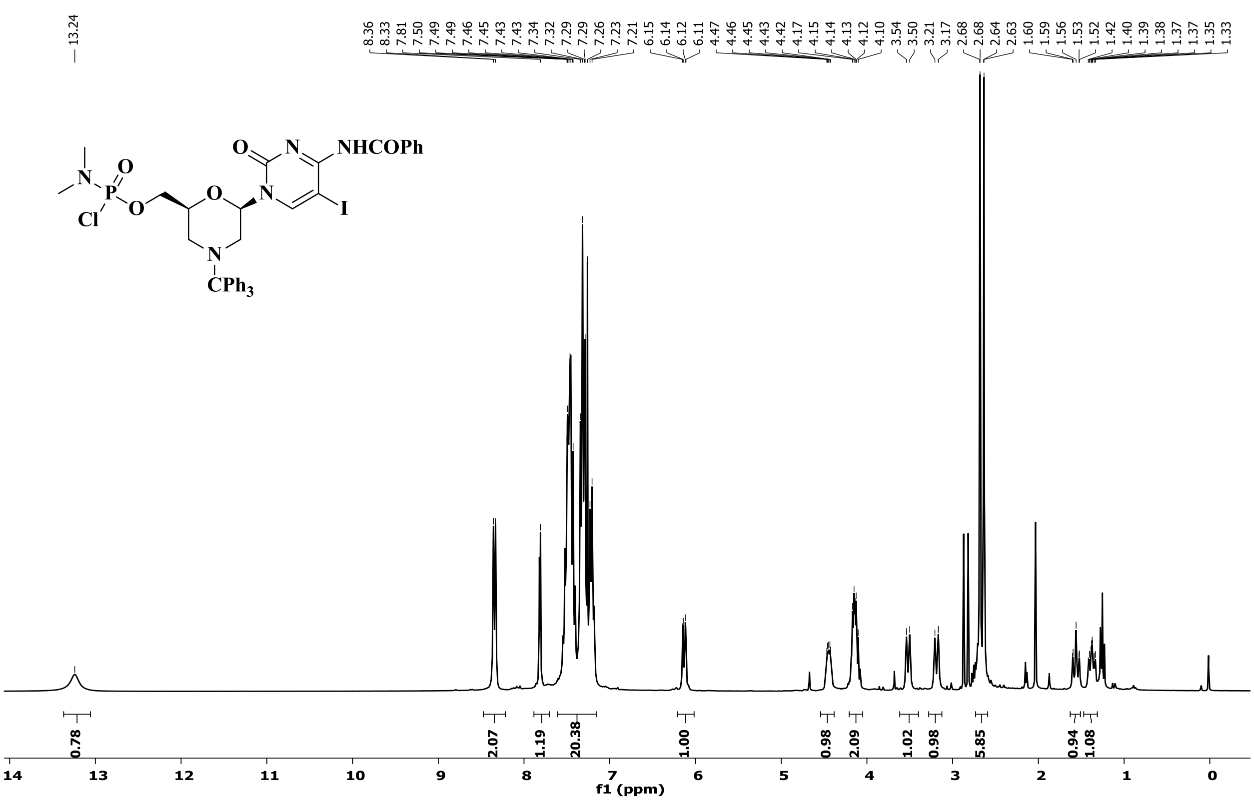
13C-NMR of compound **6k**, $CDCl_3$, 75 MHz



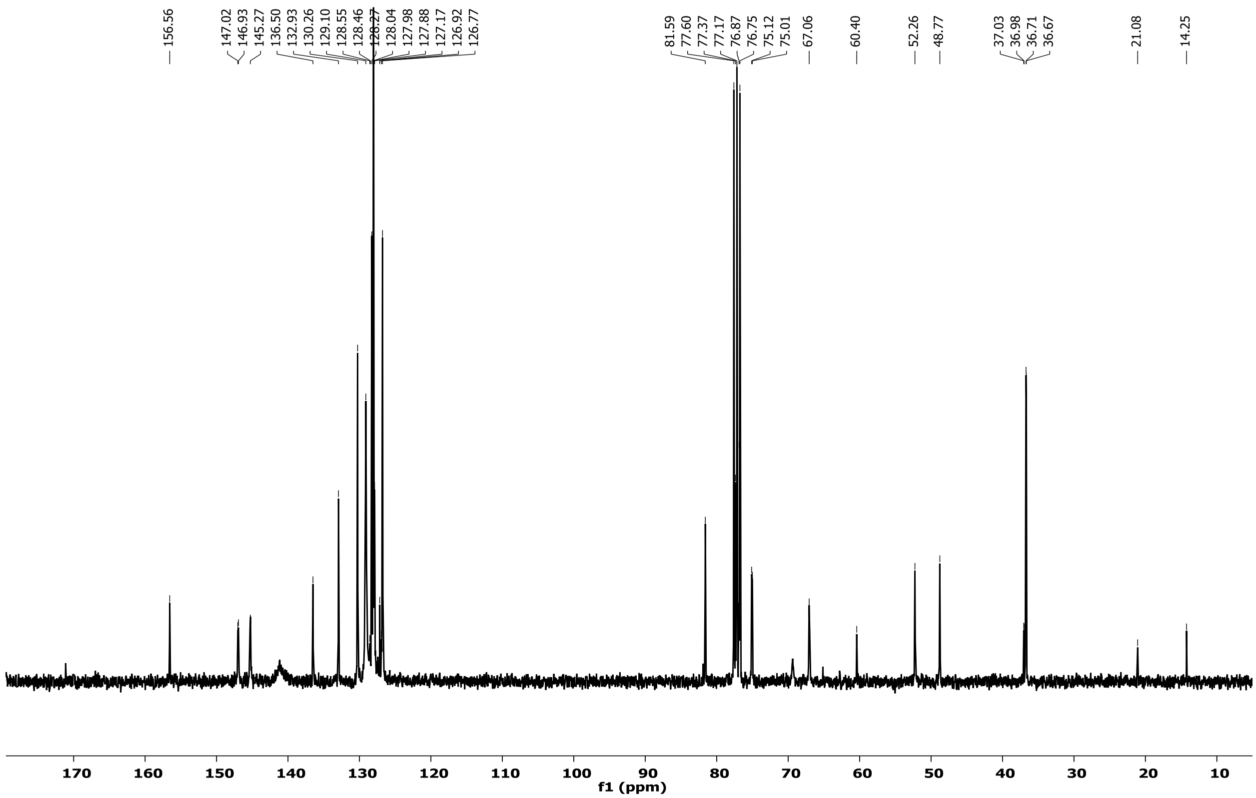
31P-NMR of compound **6k**, $CDCl_3$, 121 MHz



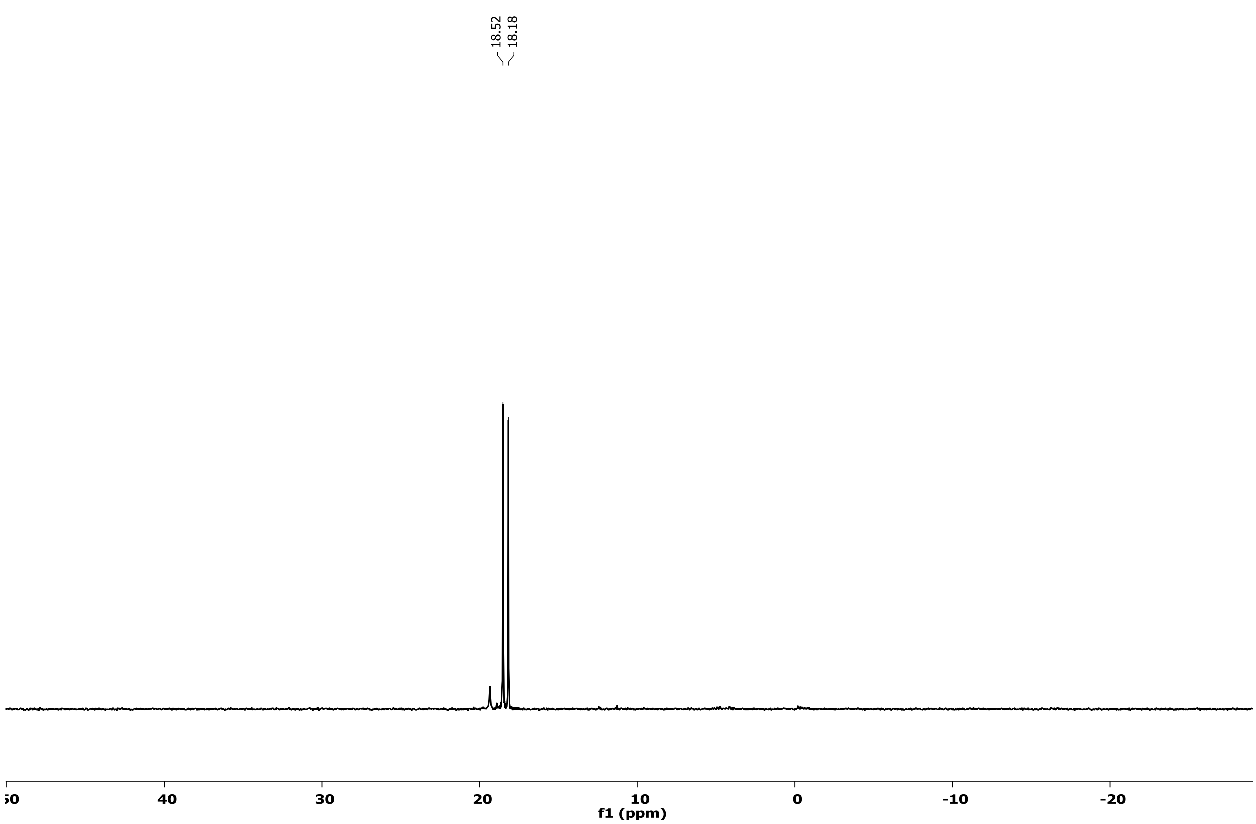
1H-NMR of compound **6l**, $CDCl_3$, 300MHz



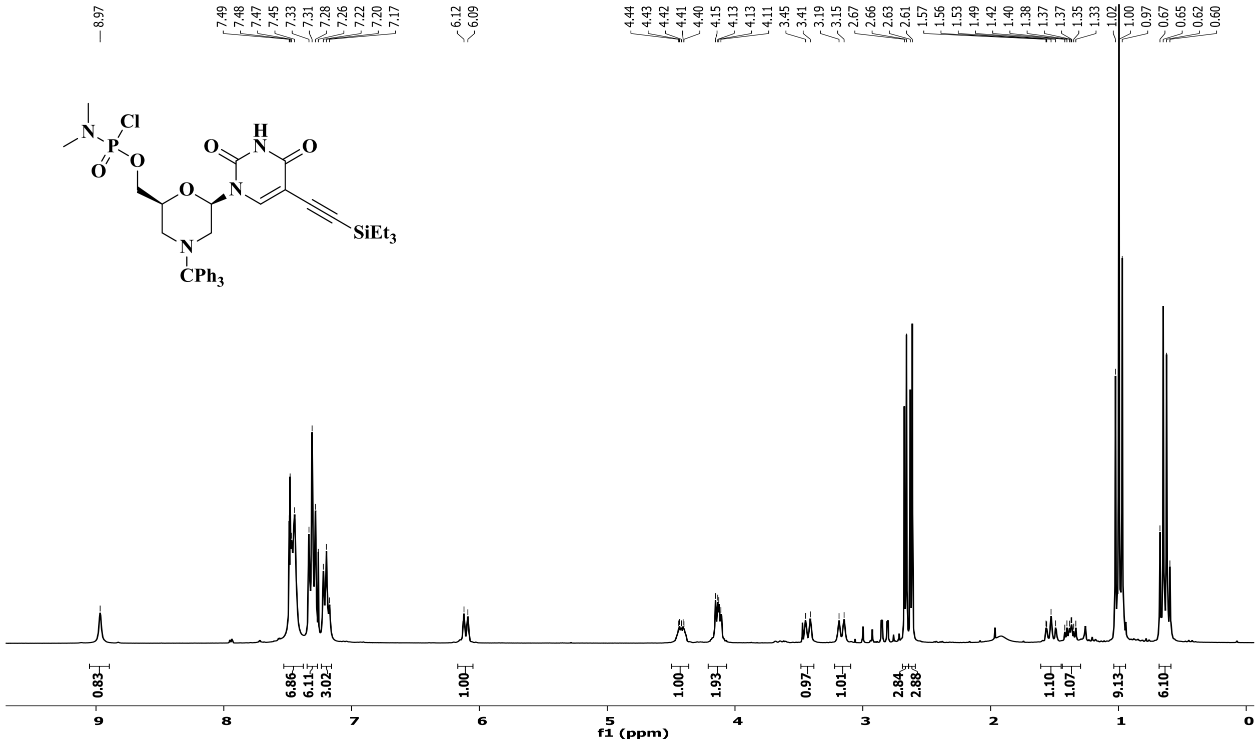
13C-NMR of compound **6l**, $CDCl_3$, 75 MHz



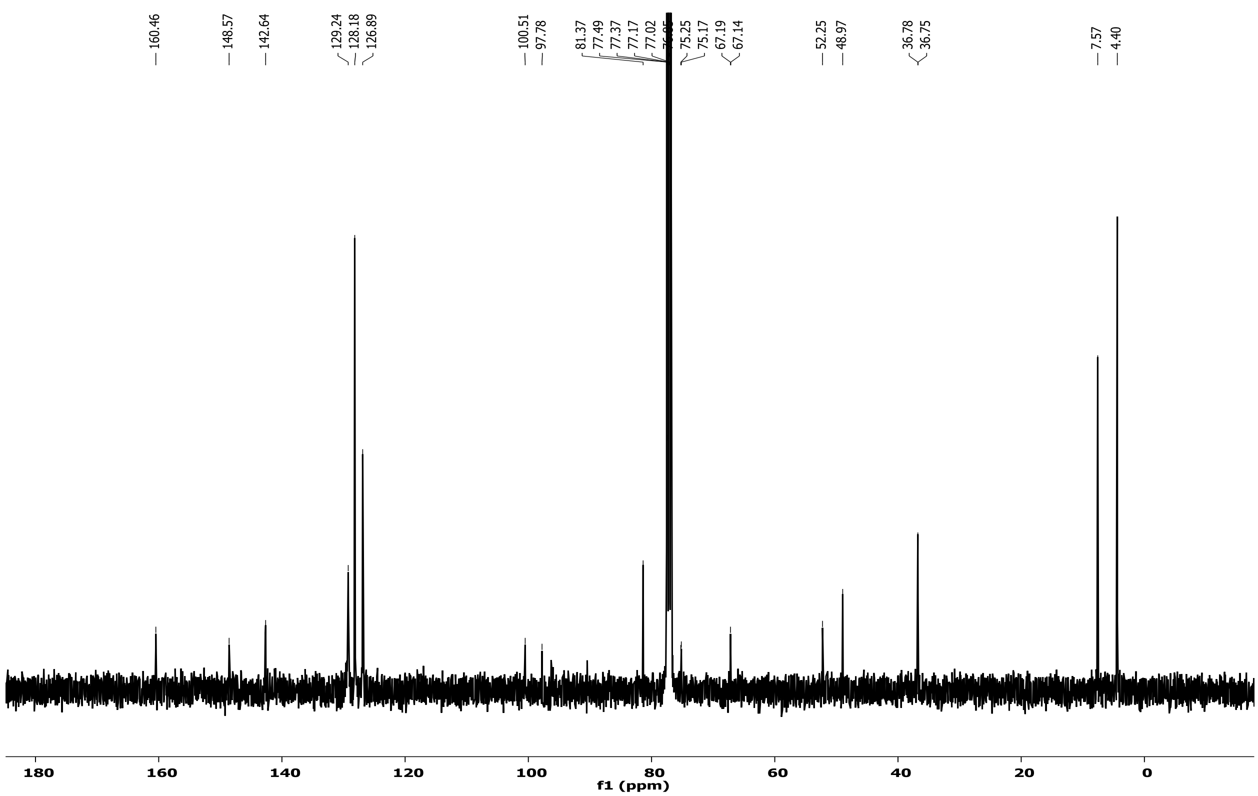
31P-NMR of compound **6l**, $CDCl_3$, 121 MHz



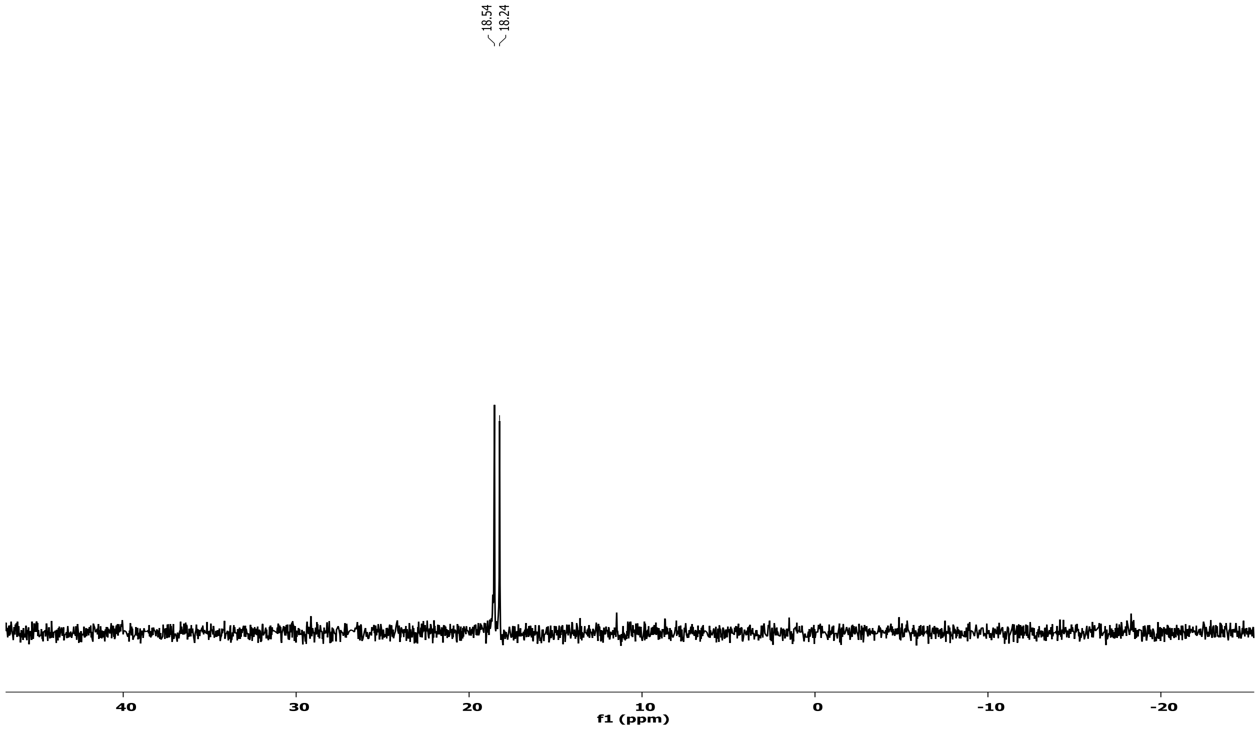
1H-NMR of compound **7a**, $CDCl_3$, 300 MHz



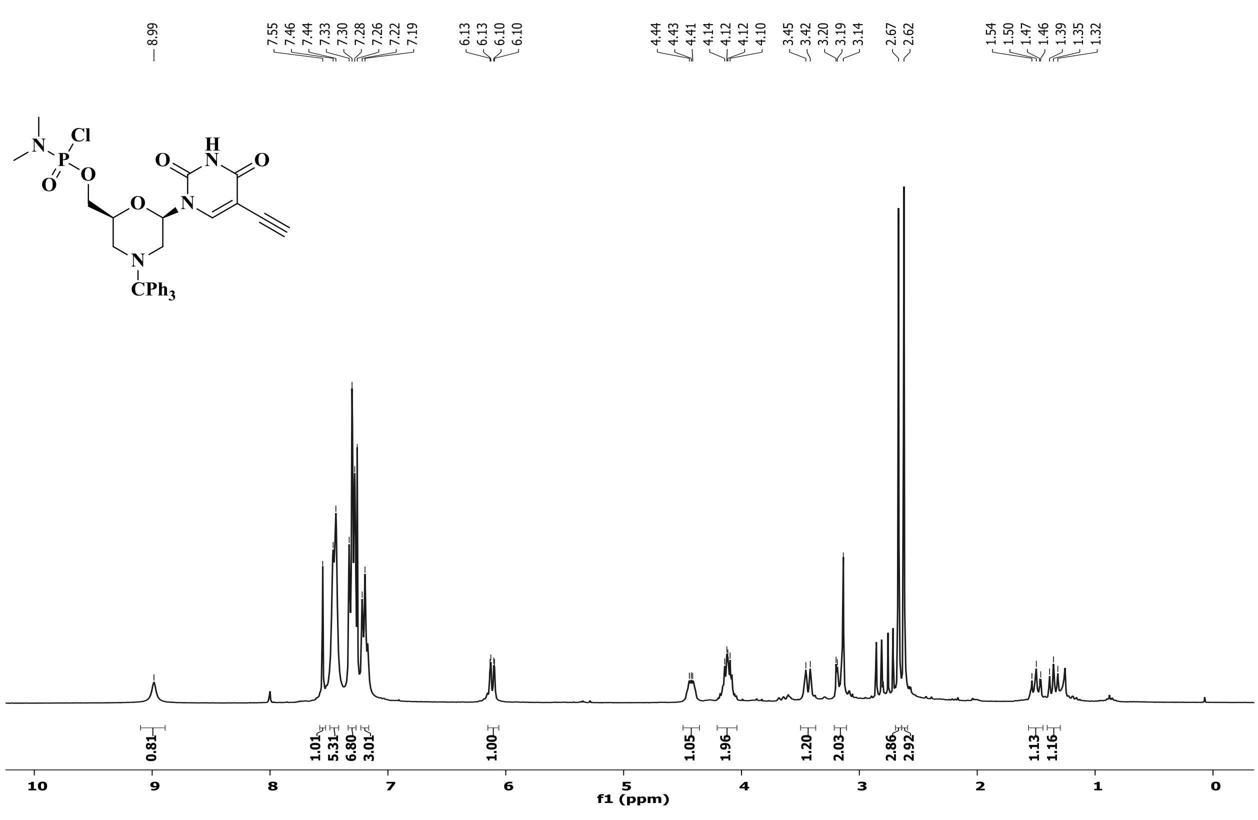
13C-NMR of compound **7a**, $CDCl_3$, 100 MHz



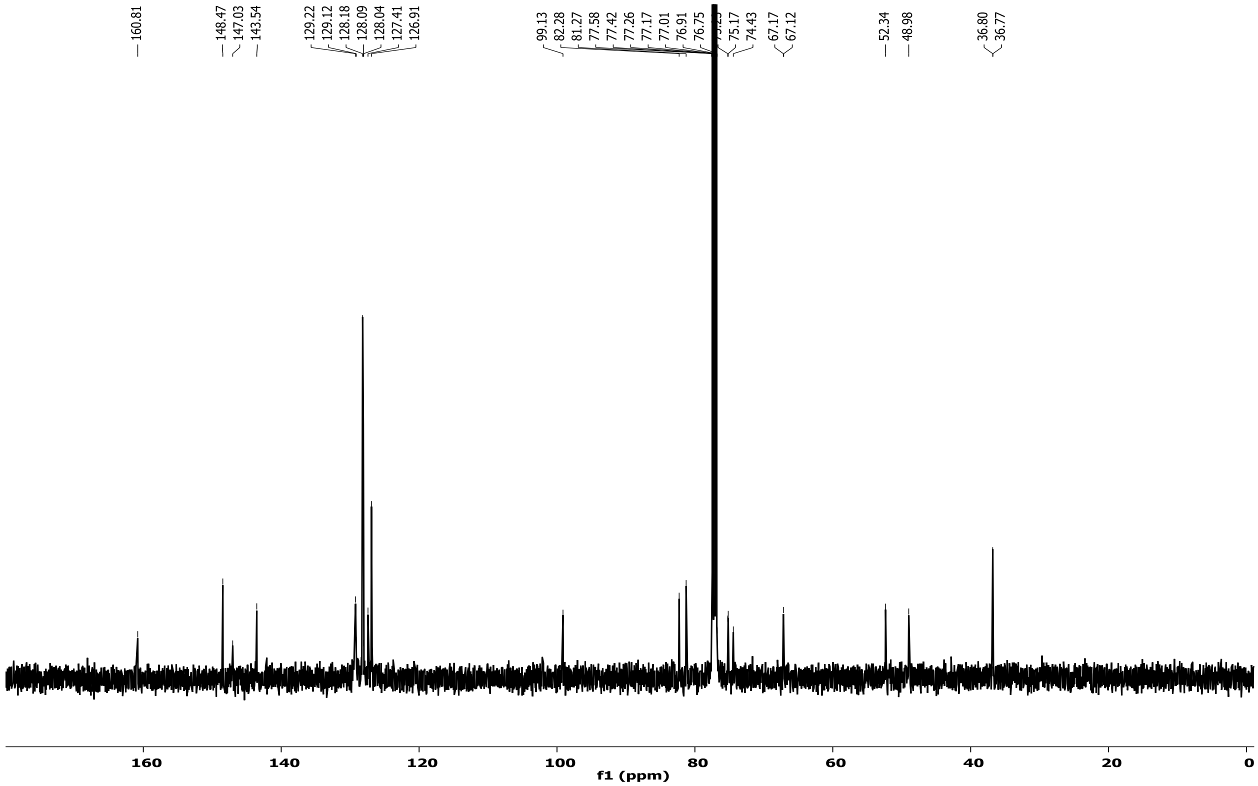
31P-NMR of compound **7a**, $CDCl_3$, 121 MHz



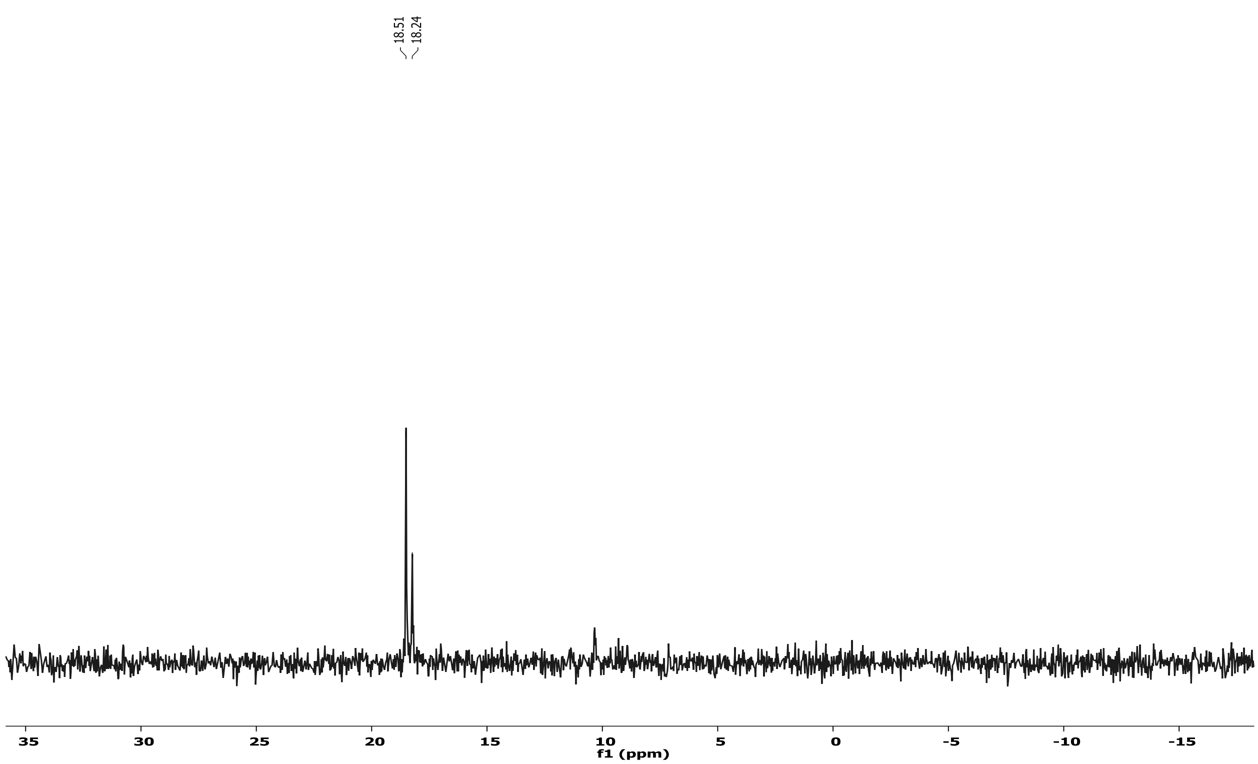
1H-NMR of compound **7b**, $CDCl_3$, 300 MHz



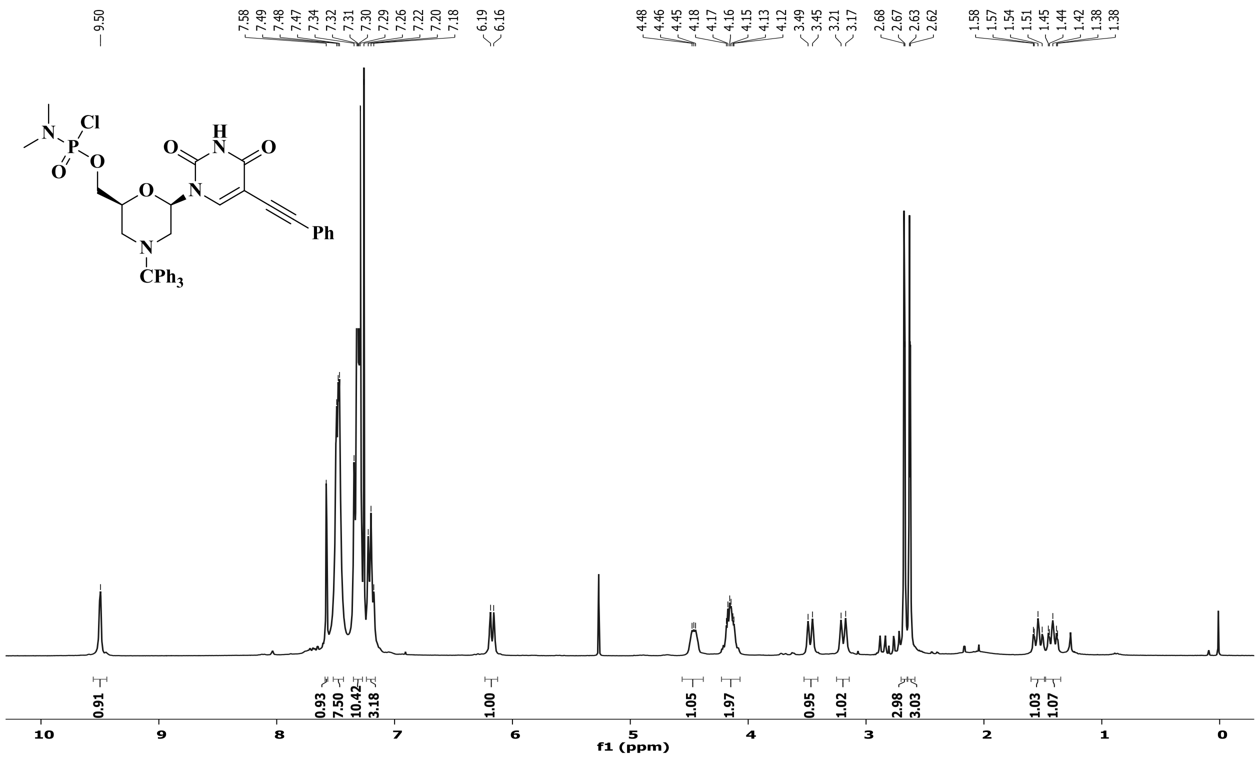
13C-NMR of compound **7b**, $CDCl_3$, 125 MHz



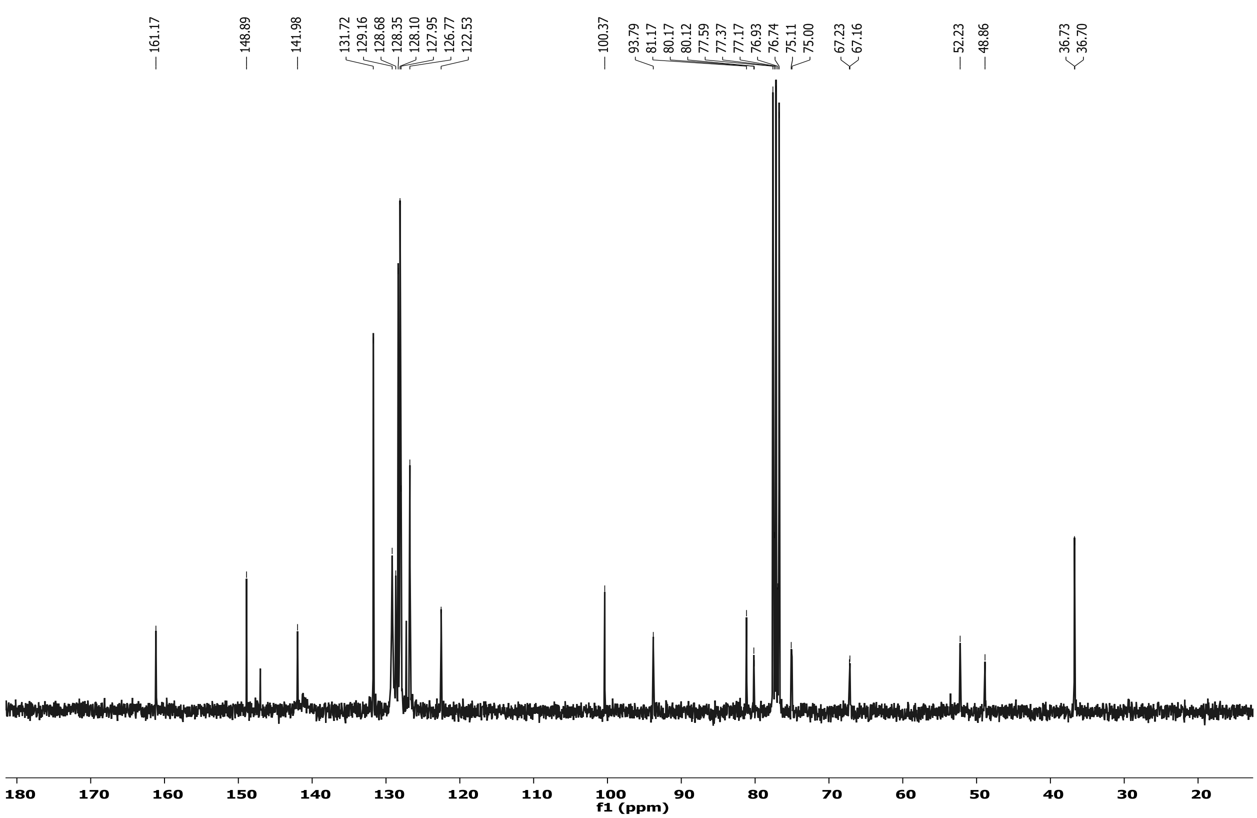
31P-NMR of compound **7b**, $CDCl_3$, 121 MHz



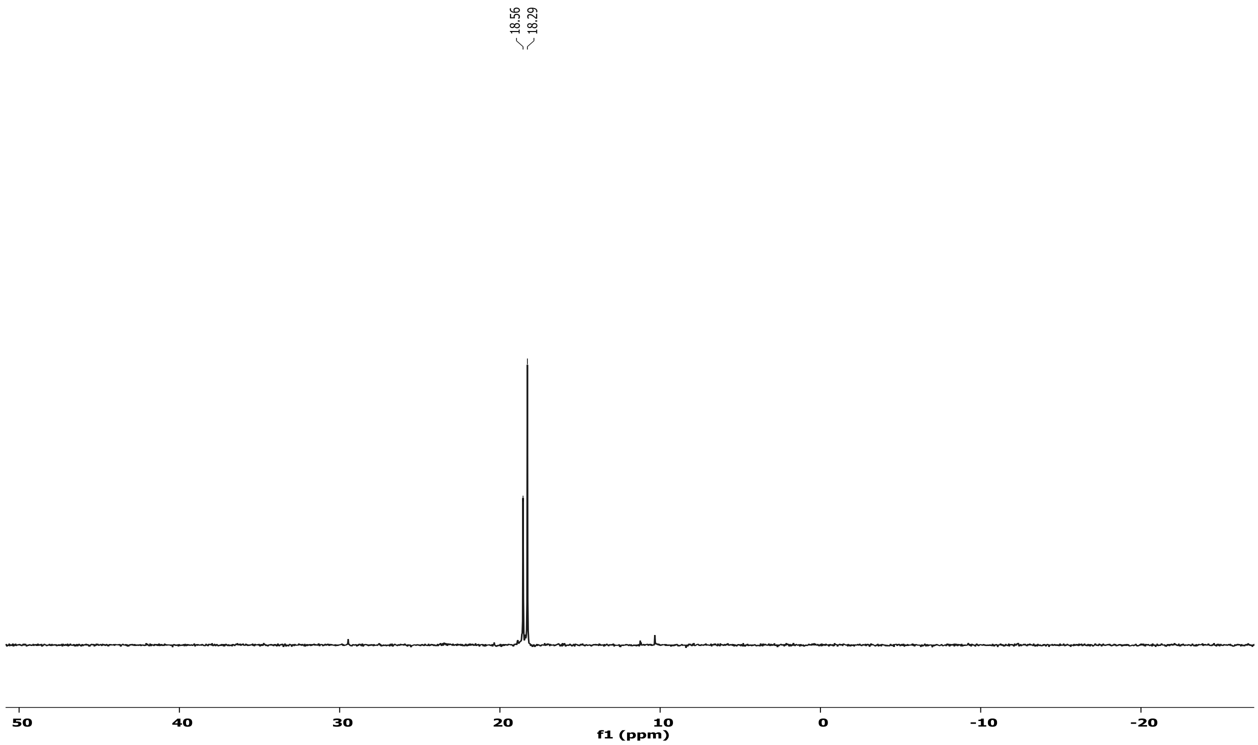
1H-NMR of compound **7c**, $CDCl_3$, 300 MHz



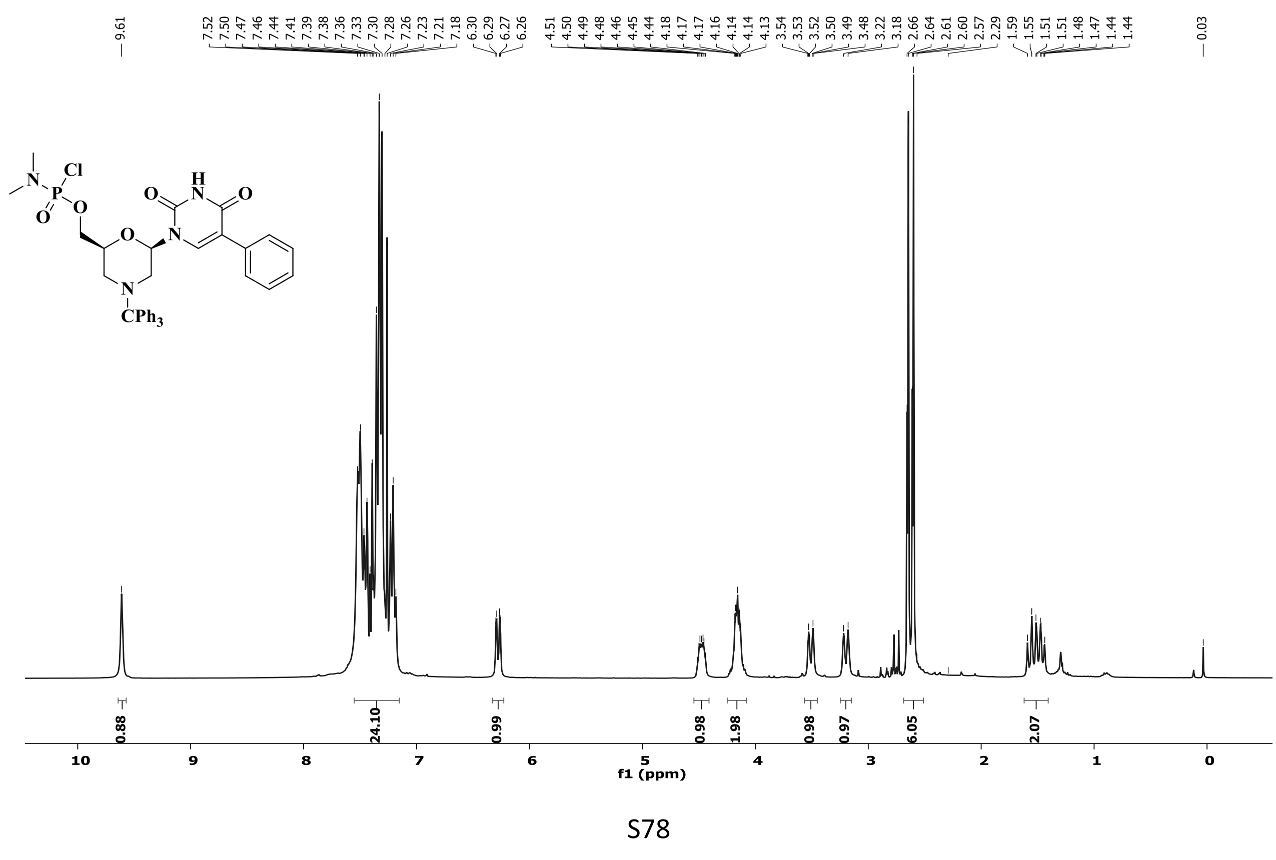
13C-NMR of compound **7c**, $CDCl_3$, 75 MHz



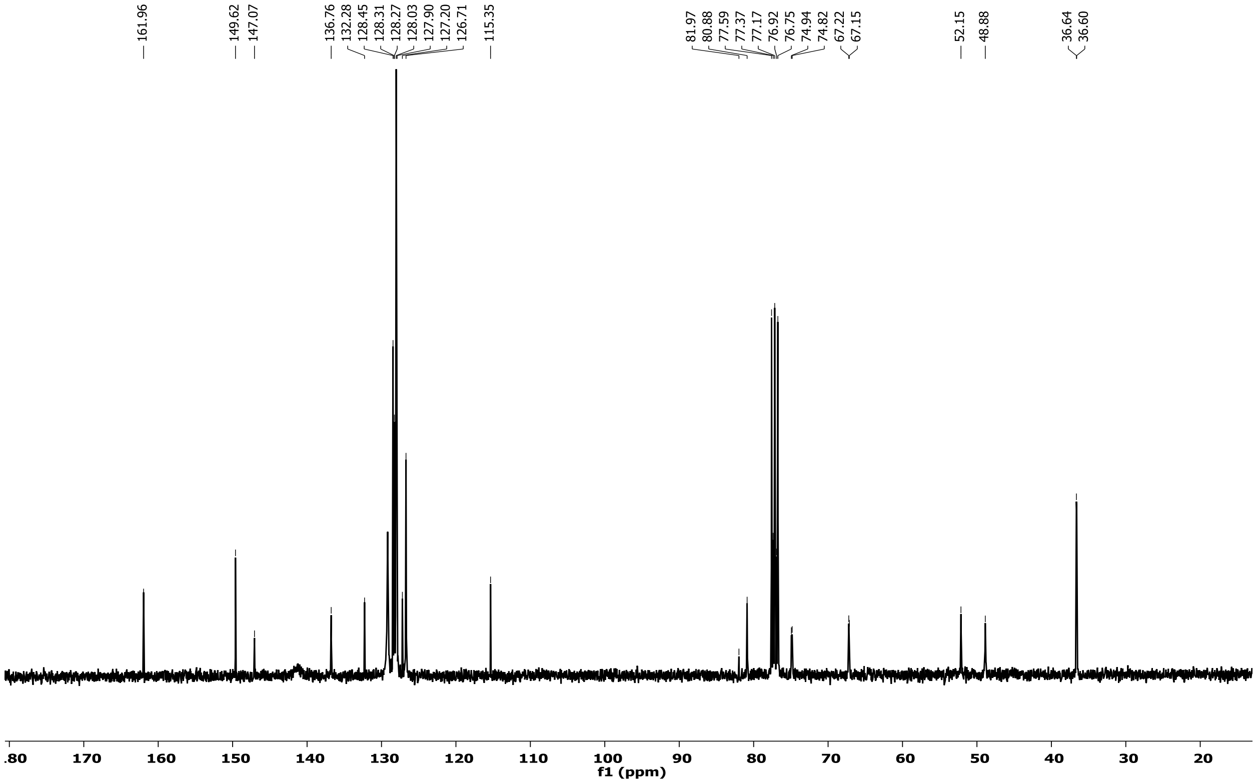
31P-NMR of compound **7c**, $CDCl_3$, 121 MHz



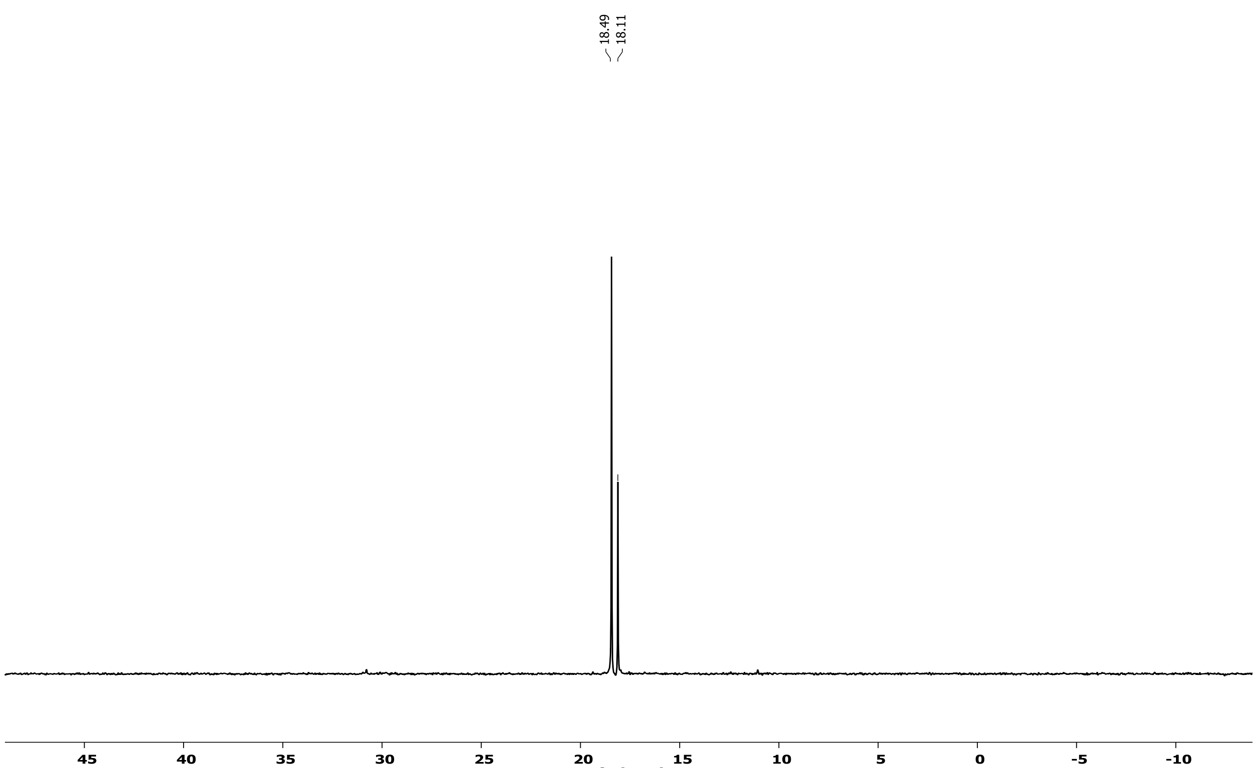
1H-NMR of compound **7d**, $CDCl_3$, 300 MHz



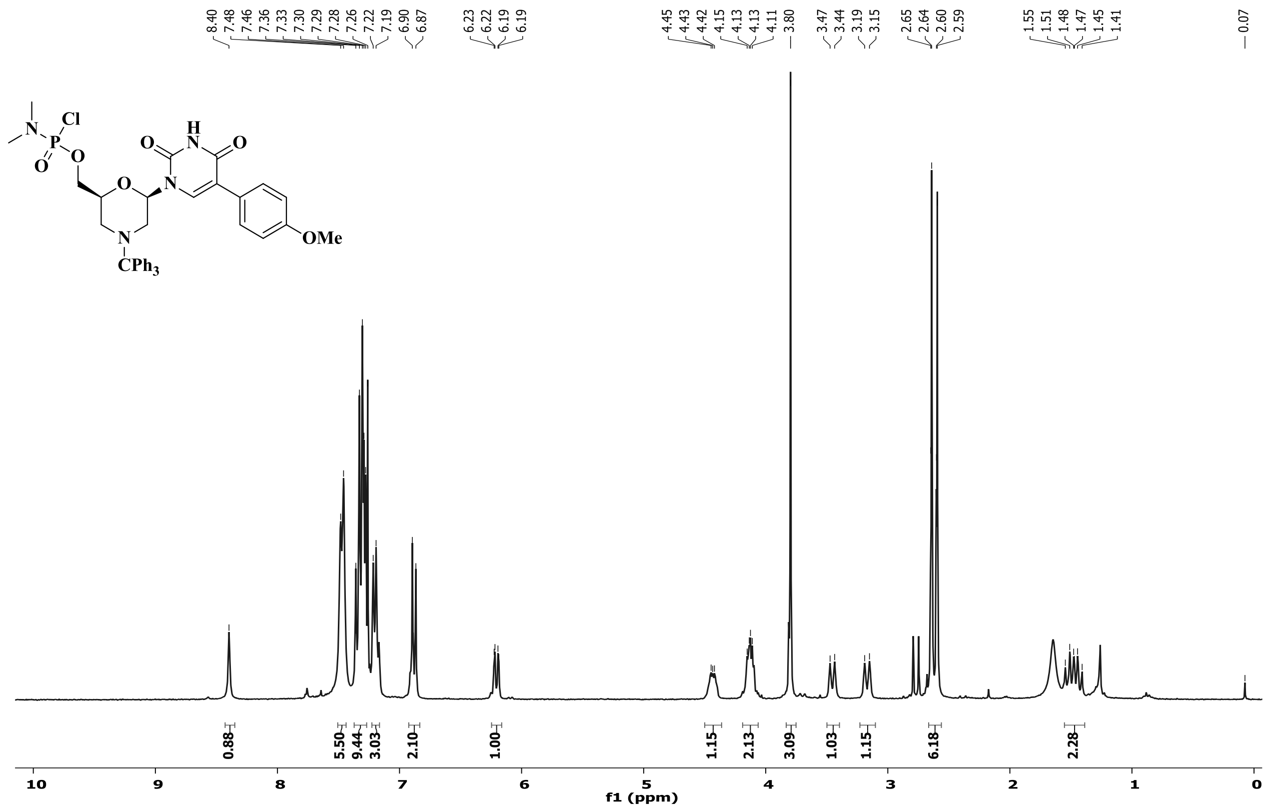
13C-NMR of compound **7d**, $CDCl_3$, 75 MHz



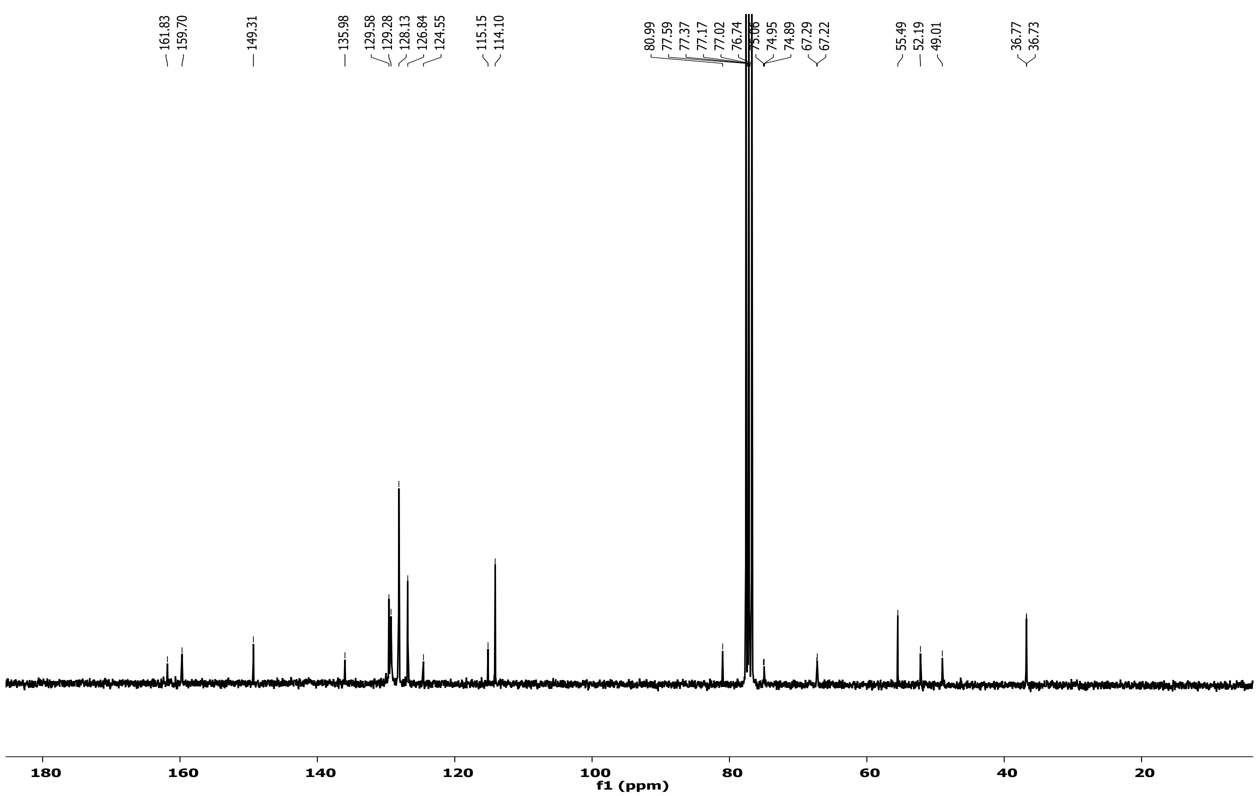
31P-NMR of compound **7d**, $CDCl_3$, 121 MHz



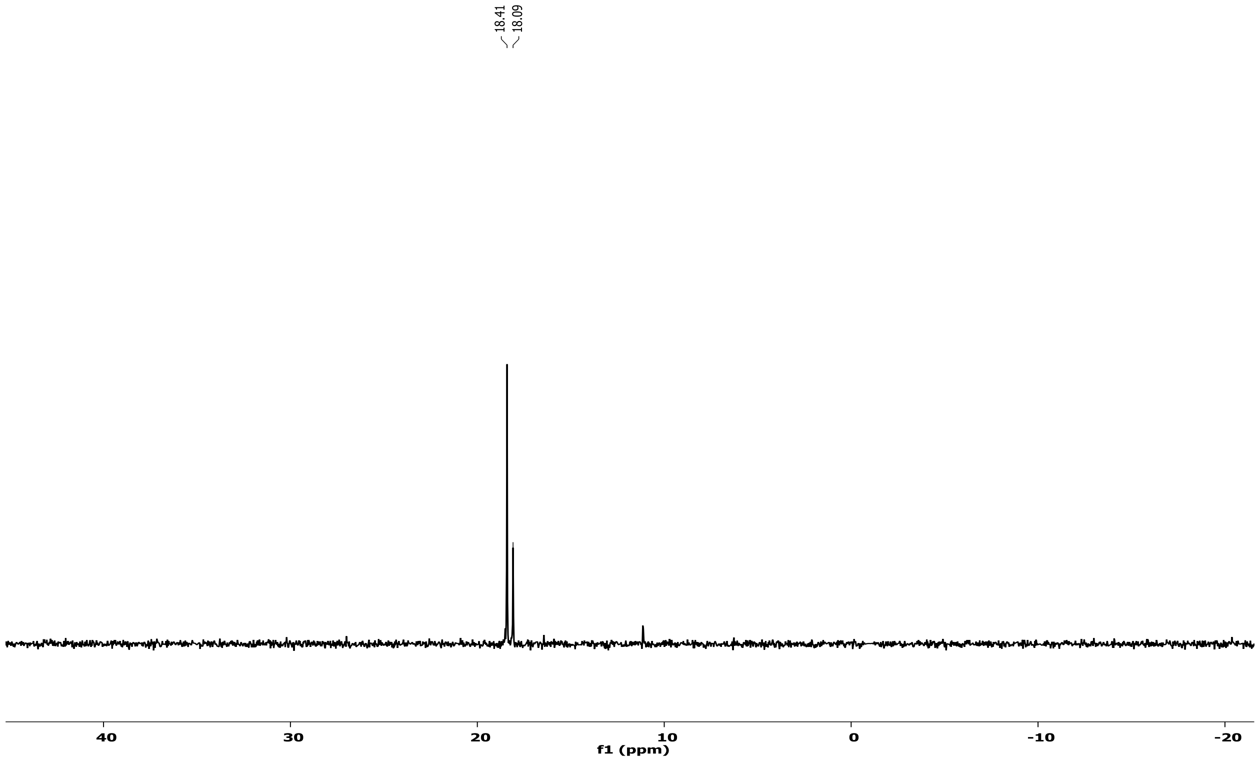
1H-NMR of compound **7e**, $CDCl_3$, 300 MHz



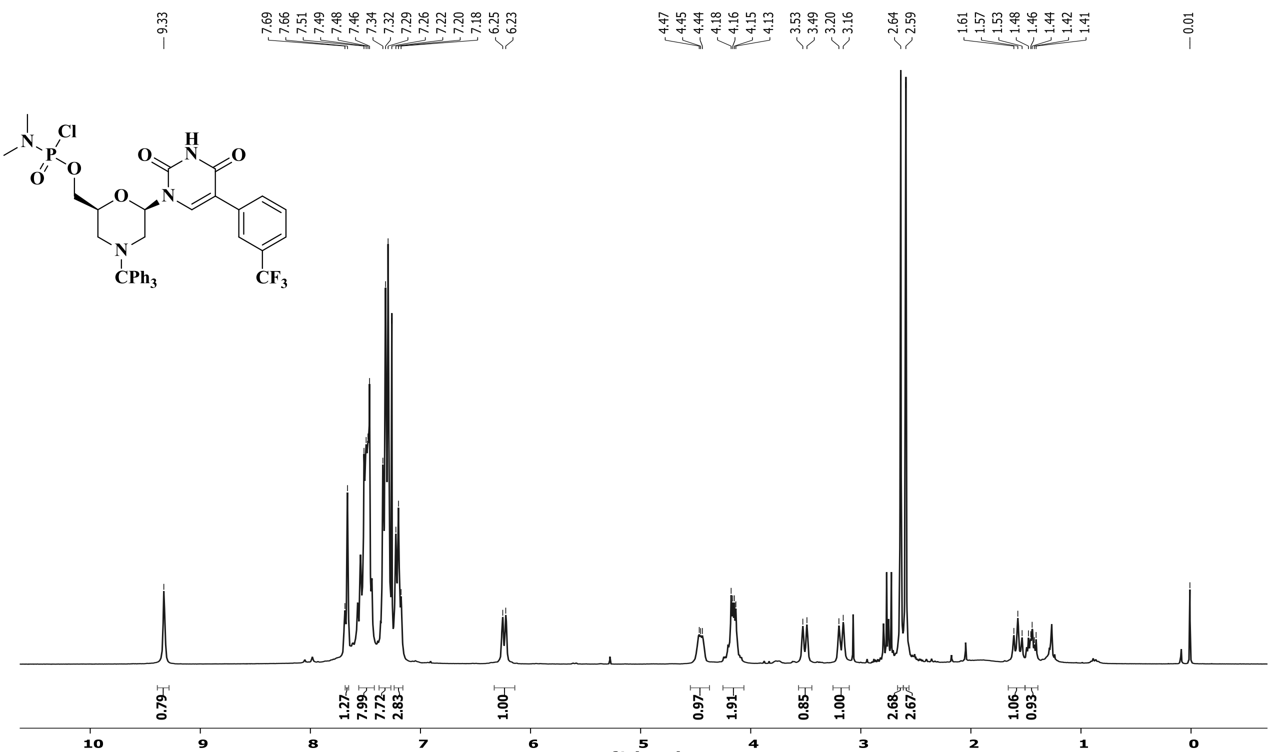
13C-NMR of compound **7e**, $CDCl_3$, 75 MHz



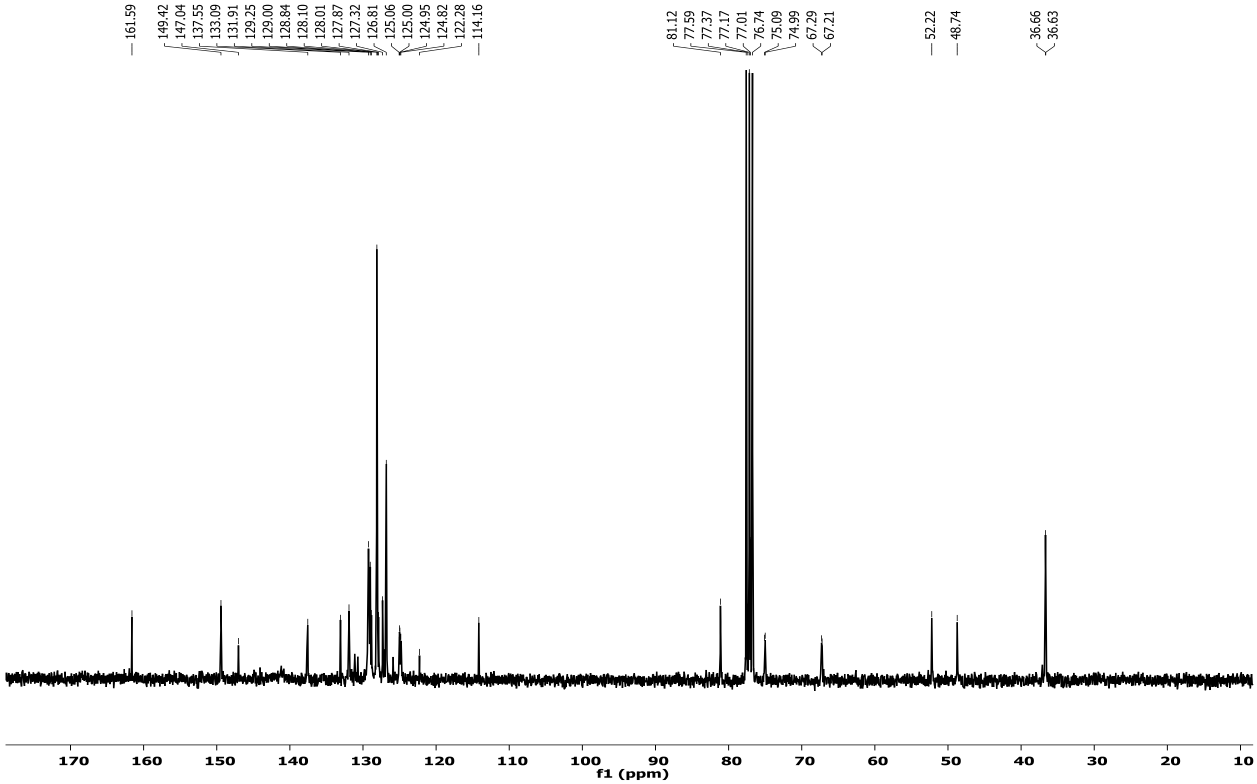
31P-NMR of compound **7e**, $CDCl_3$, 121 MHz



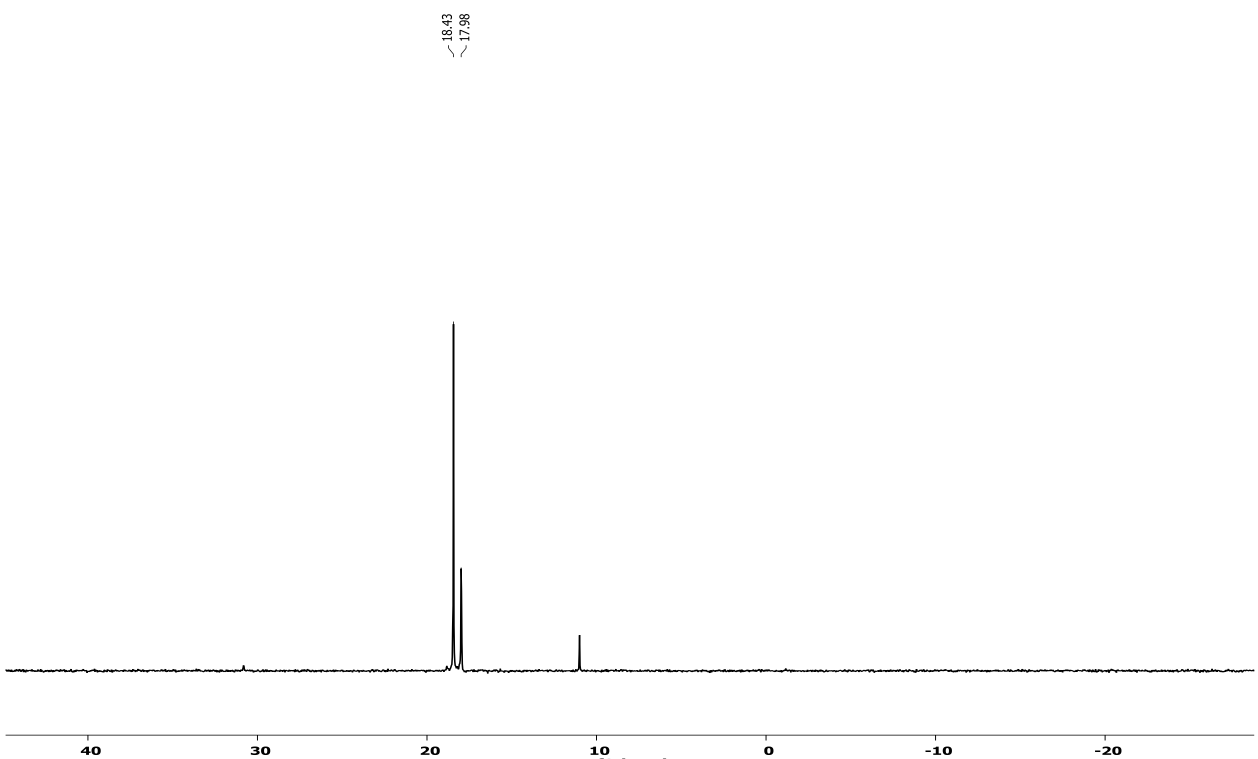
1H-NMR of compound **7f**, $CDCl_3$, 300 MHz



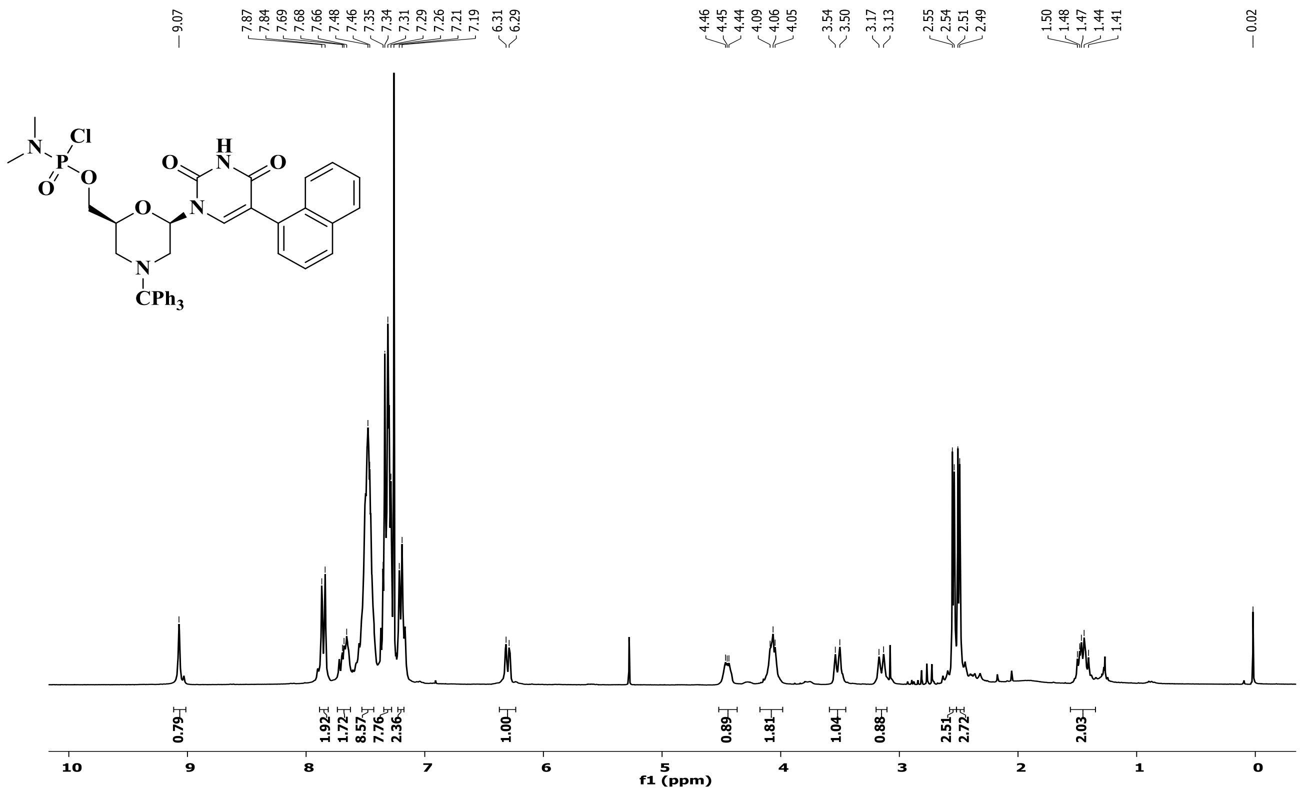
13C-NMR of compound **7f**, $CDCl_3$, 75 MHz



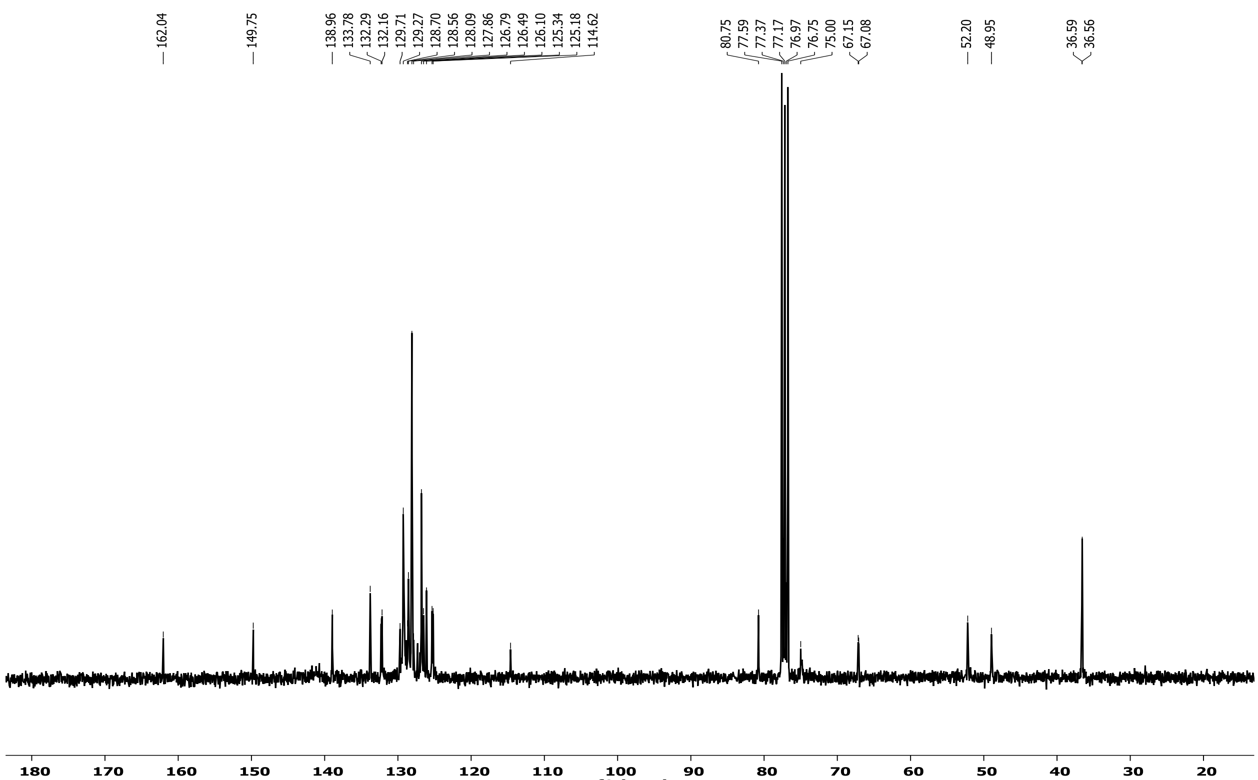
31P-NMR of compound **7f**, $CDCl_3$, 121 MHz



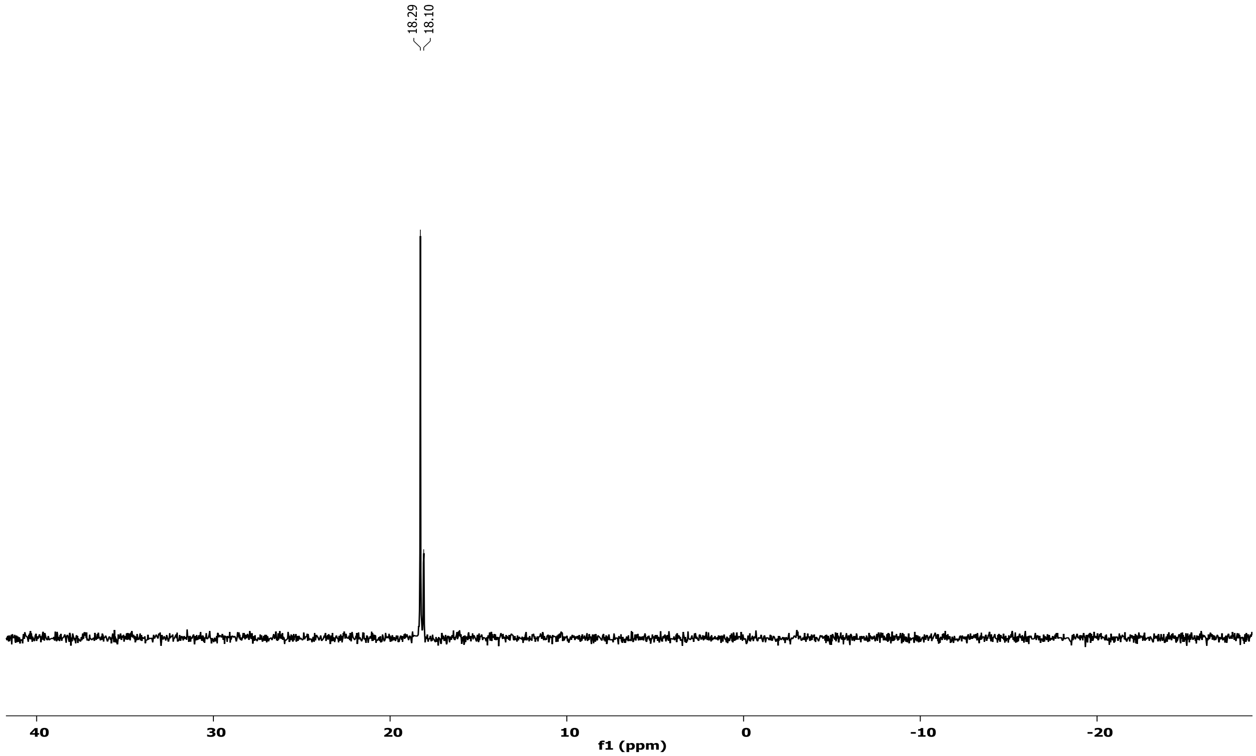
1H-NMR of compound **7g**, $CDCl_3$, 300 MHz



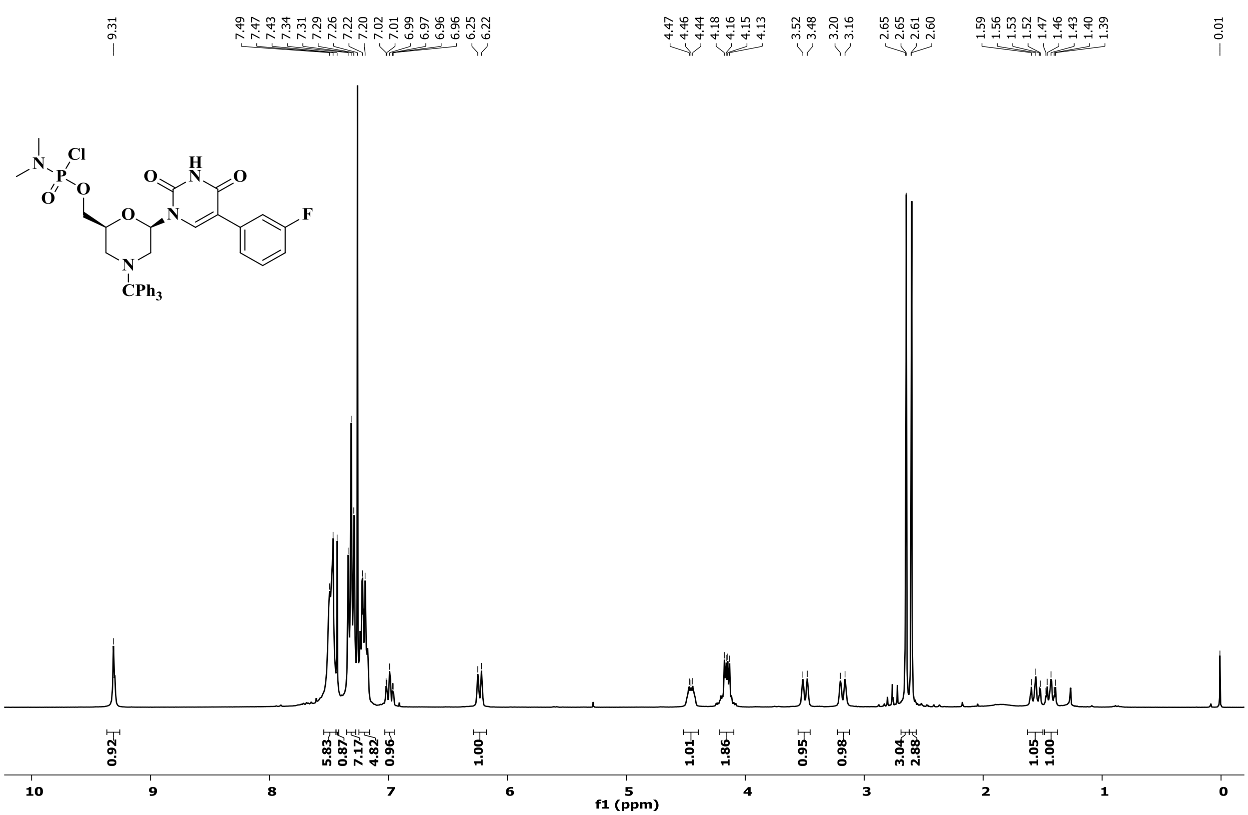
13C-NMR of compound **7g**, $CDCl_3$, 75 MHz



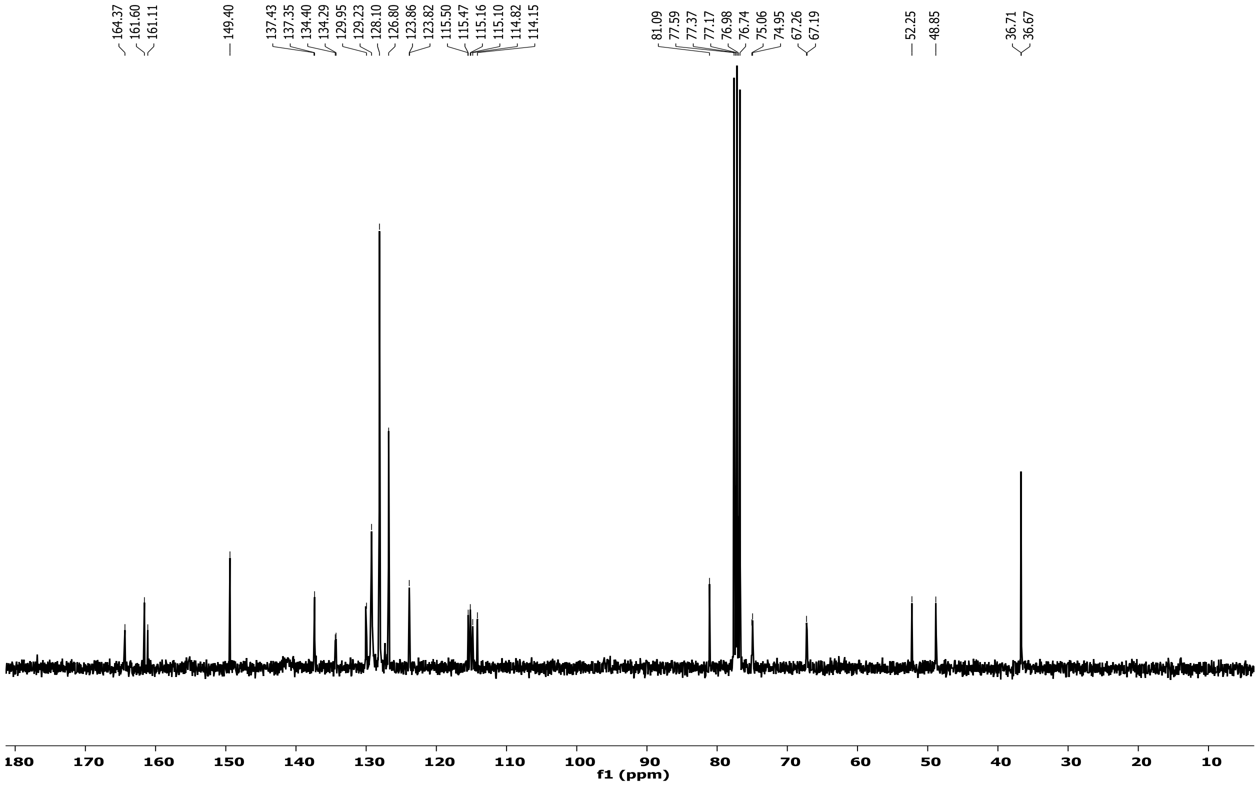
31P-NMR of compound **7g**, $CDCl_3$, 121 MHz



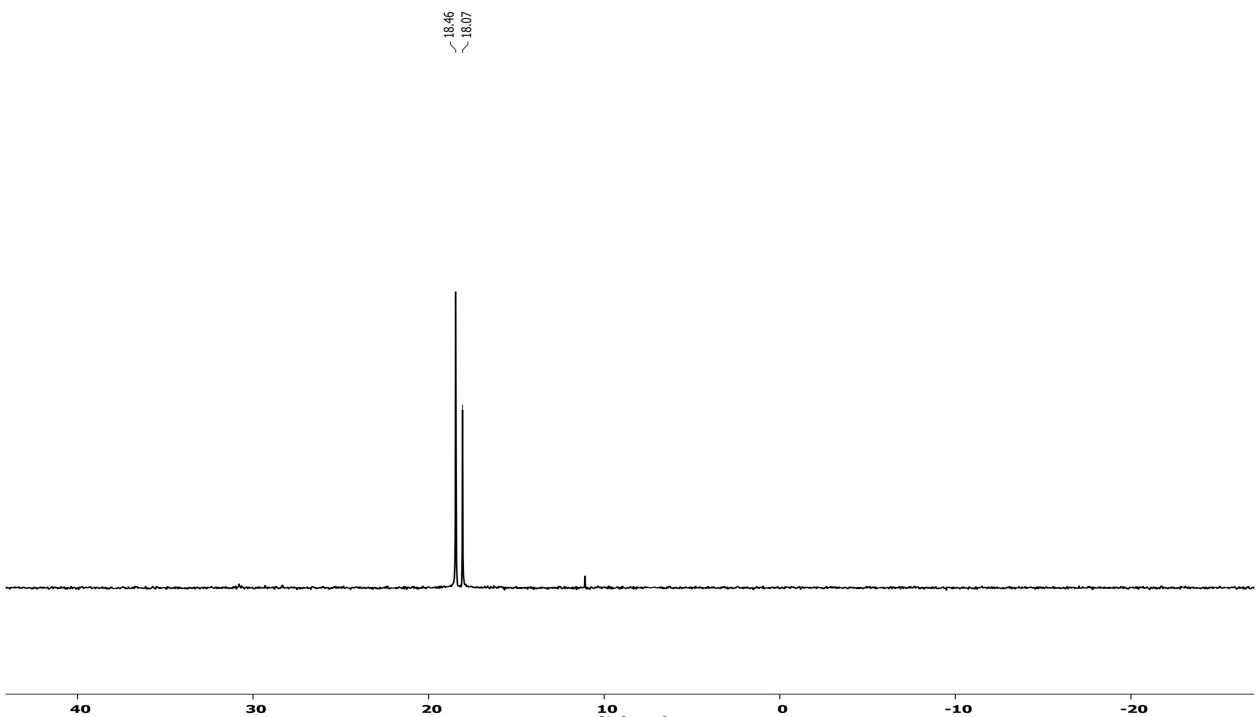
1H-NMR of compound **7h**, $CDCl_3$, 300 MHz



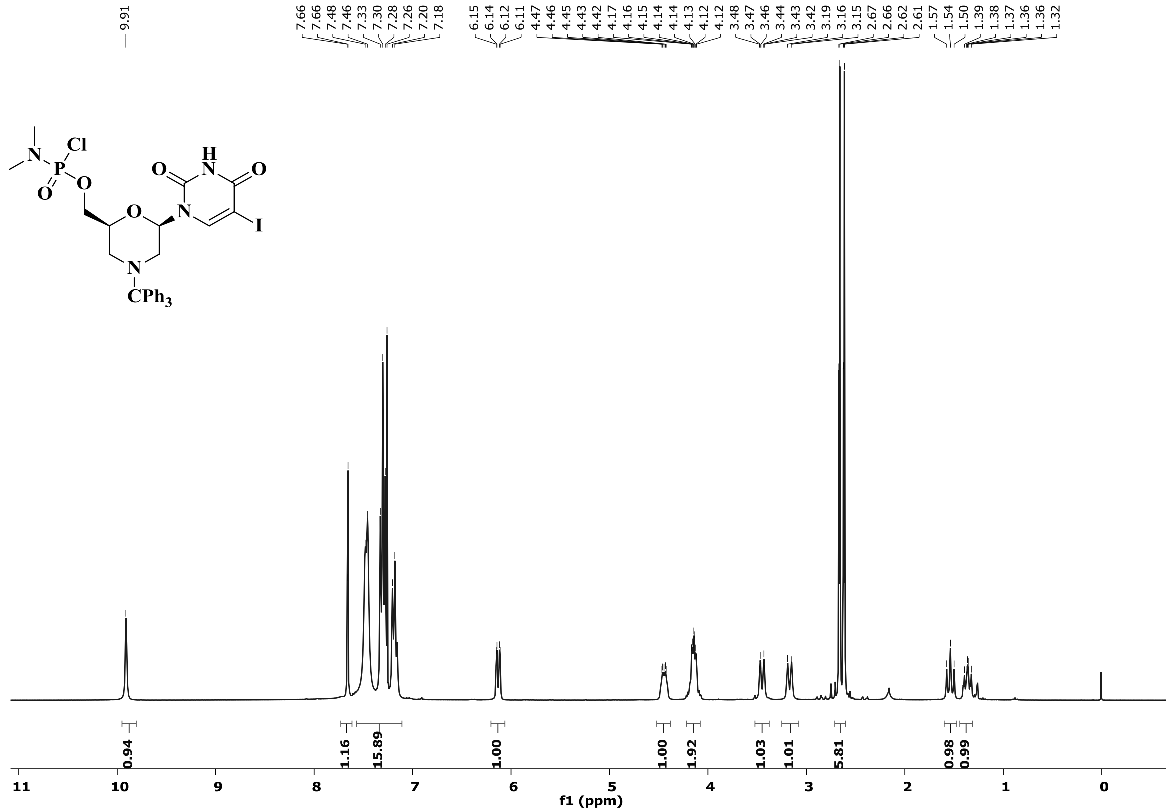
13C-NMR of compound **7h**, $CDCl_3$, 75 MHz



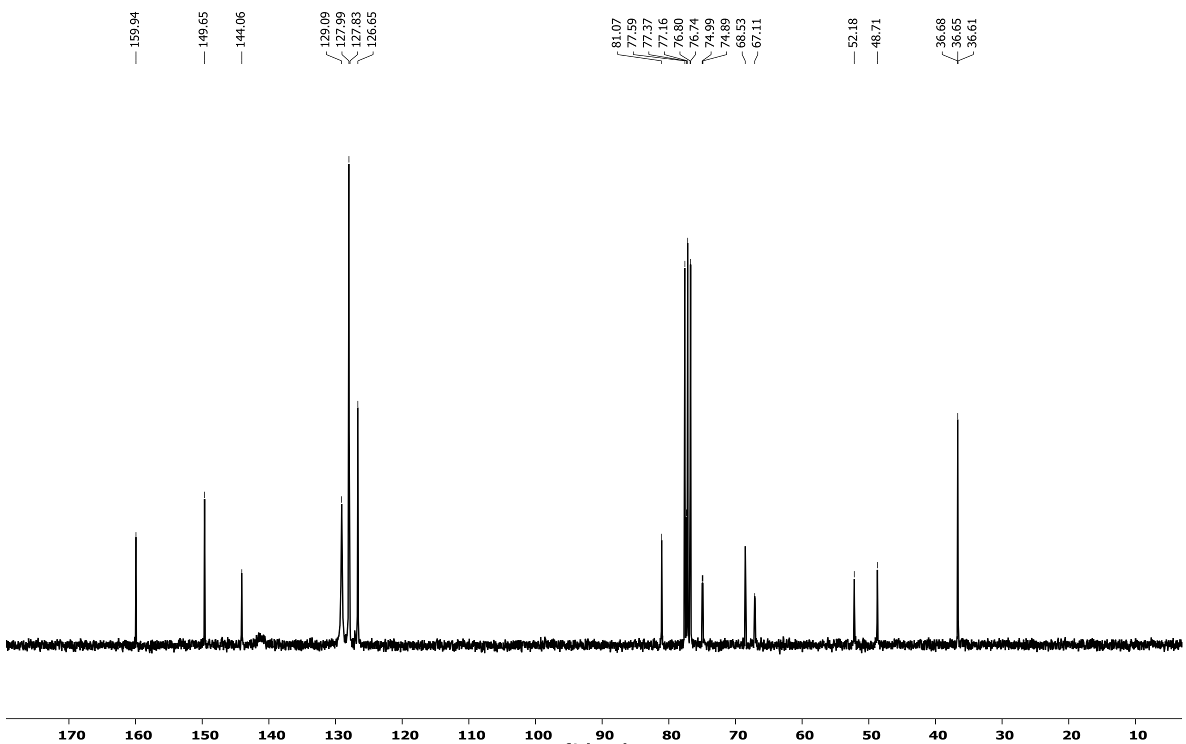
31P-NMR of compound **7h**, $CDCl_3$, 121 MHz



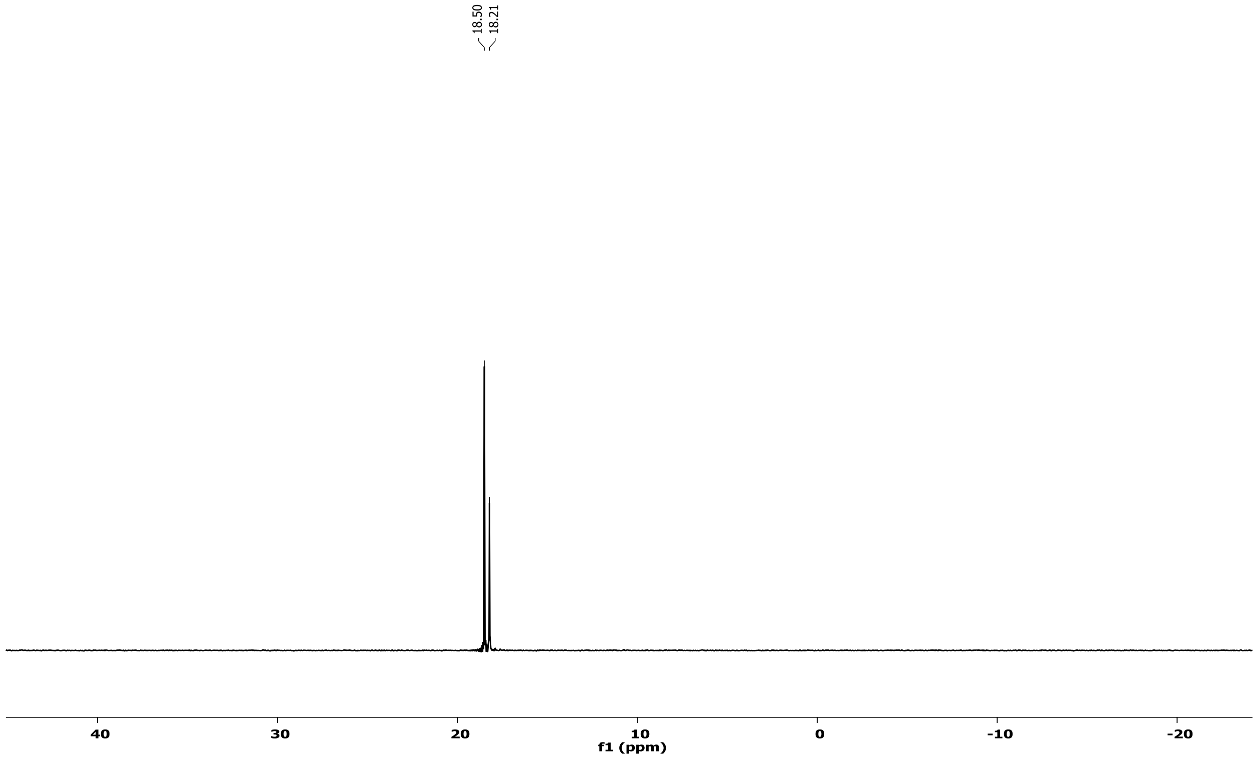
1H-NMR of compound **7i**, $CDCl_3$, 300 MHz



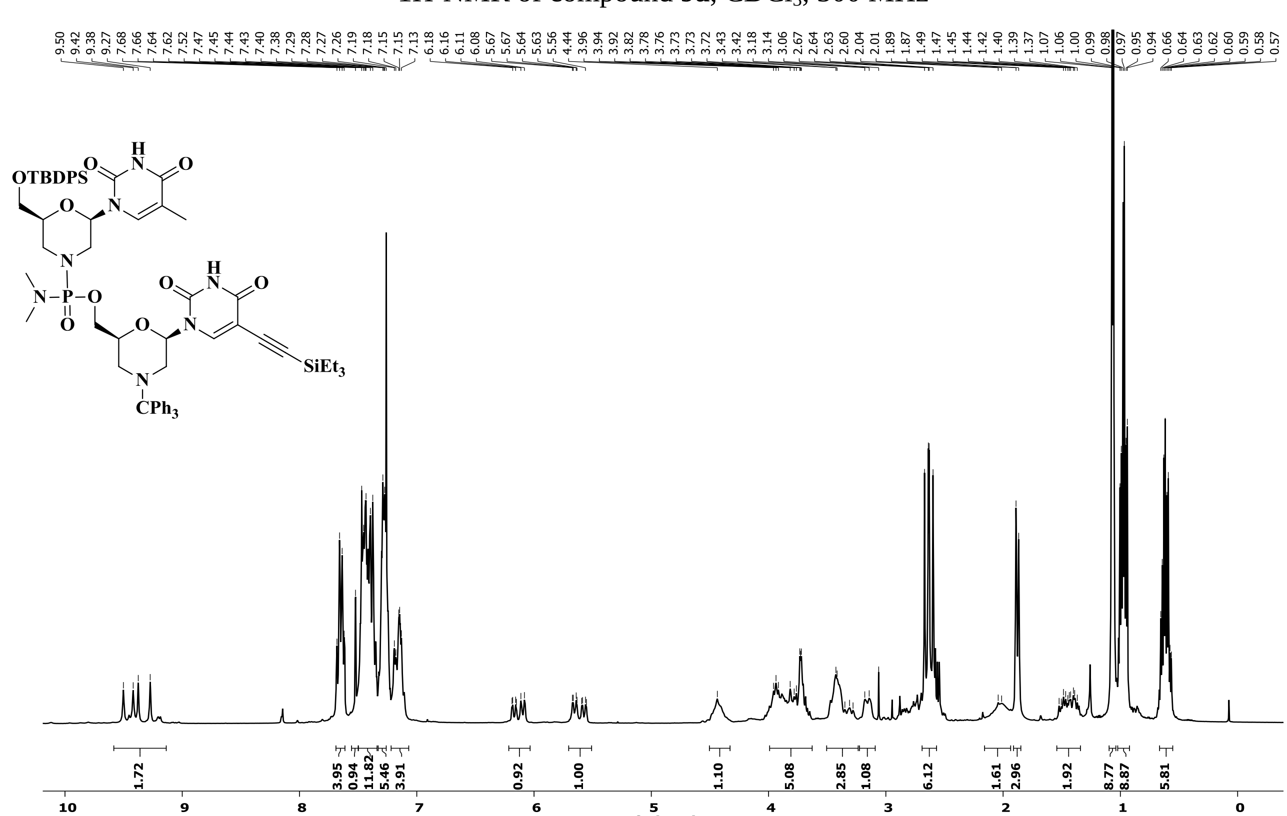
13C-NMR of compound **7i**, $CDCl_3$, 75 MHz



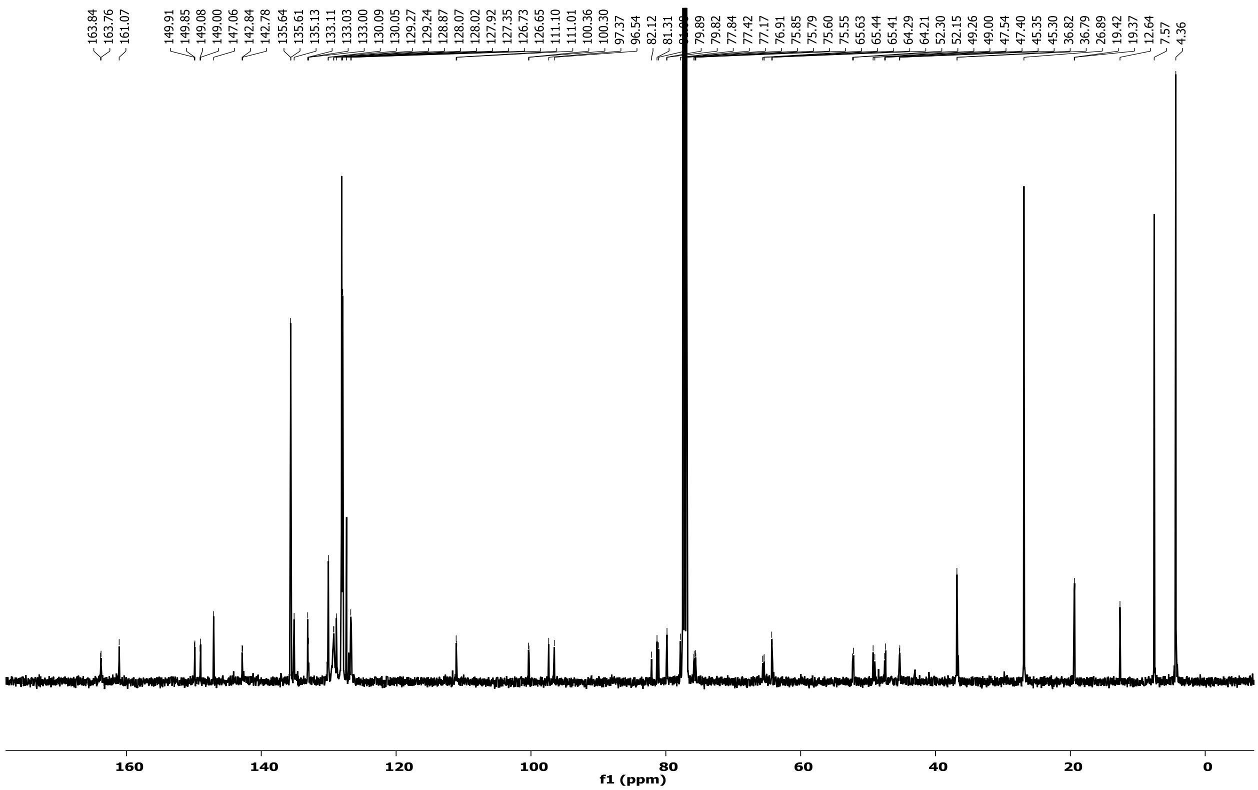
31P-NMR of compound **7i**, $CDCl_3$, 121 MHz



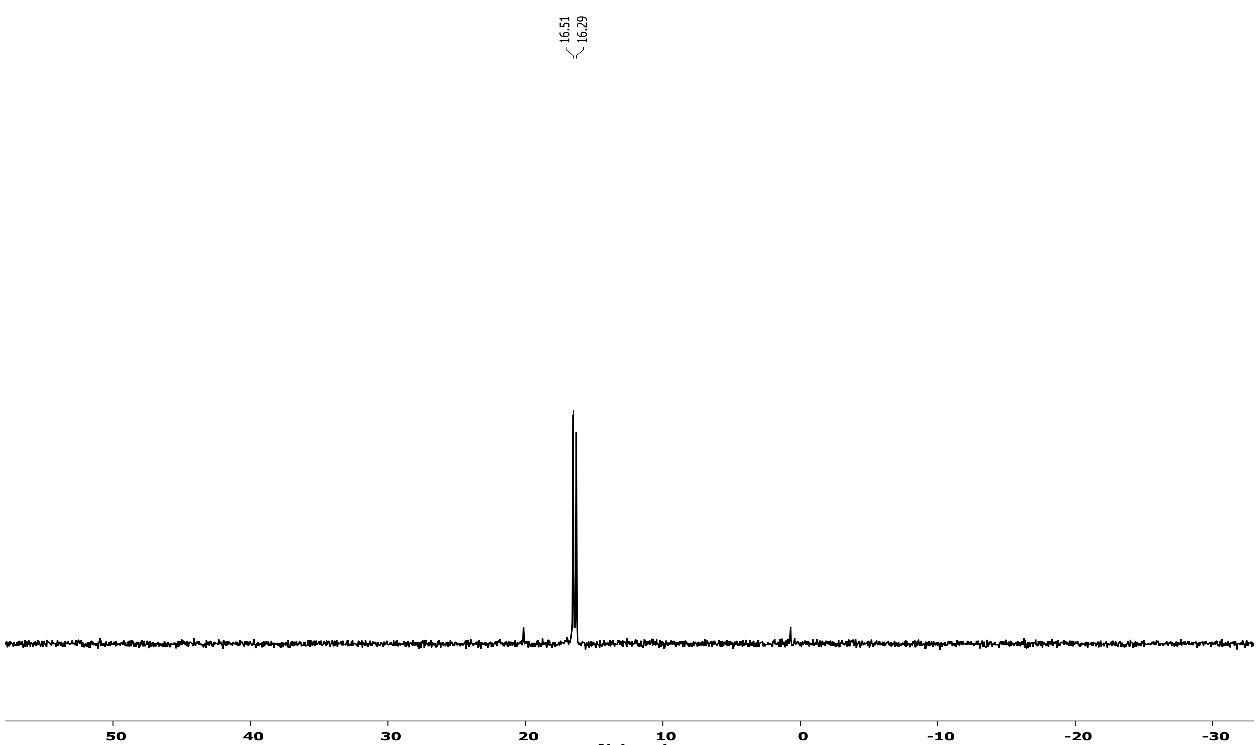
1H-NMR of compound **9a**, $CDCl_3$, 300 MHz



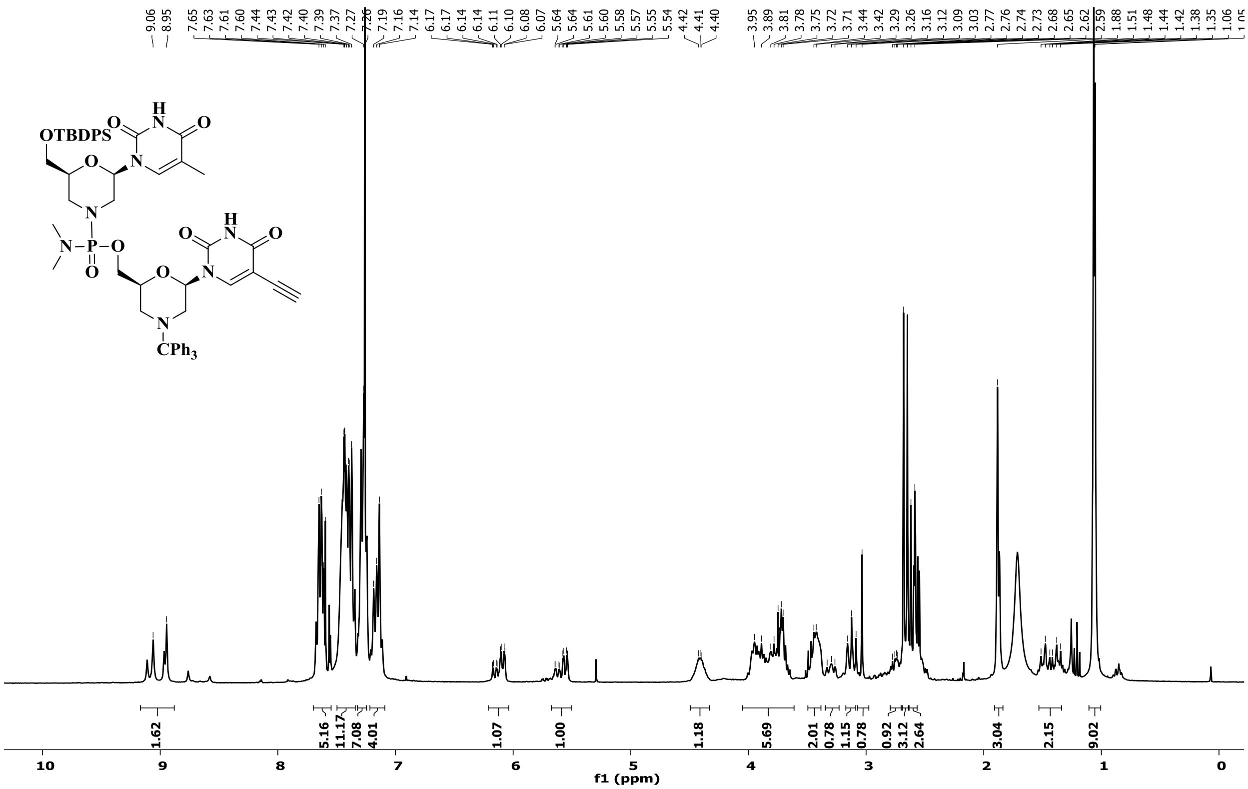
13C-NMR of compound **9a**, $CDCl_3$, 125 MHz



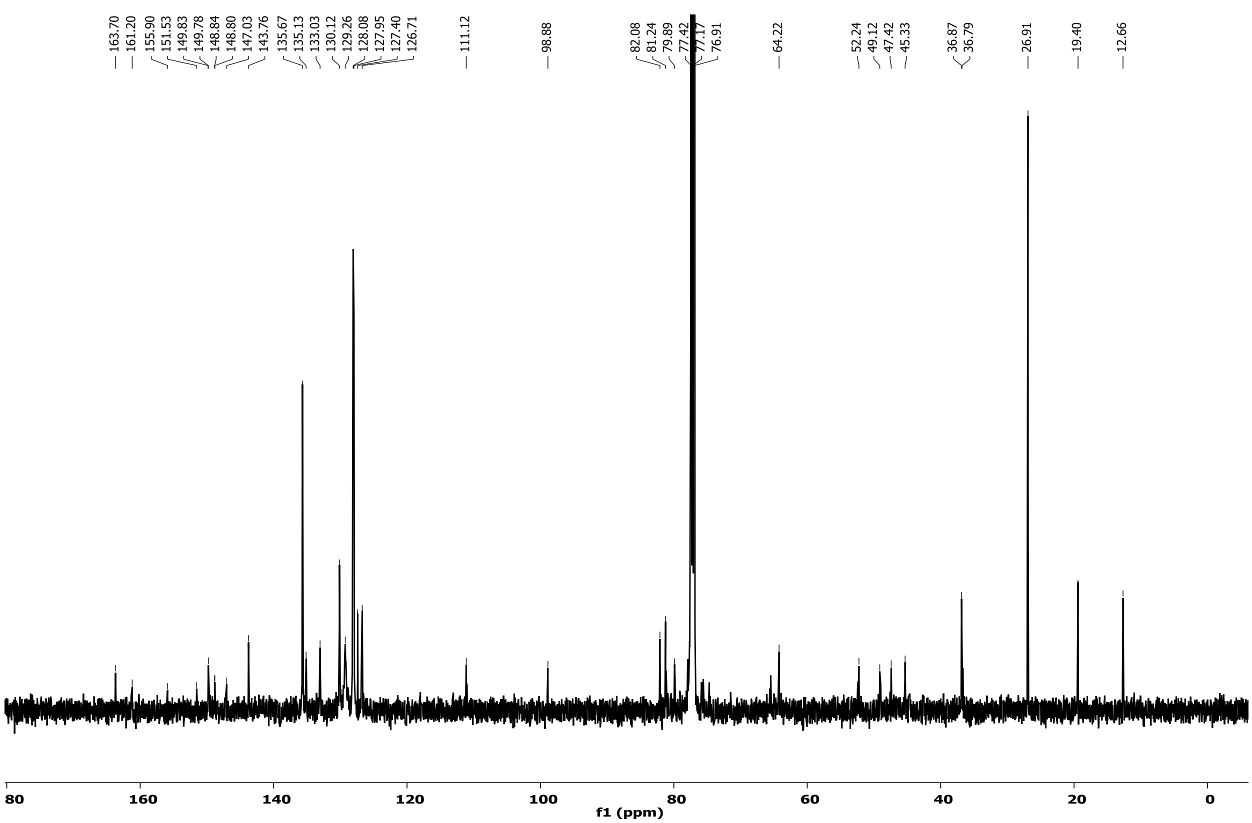
31P-NMR of compound **9a**, $CDCl_3$, 121 MHz



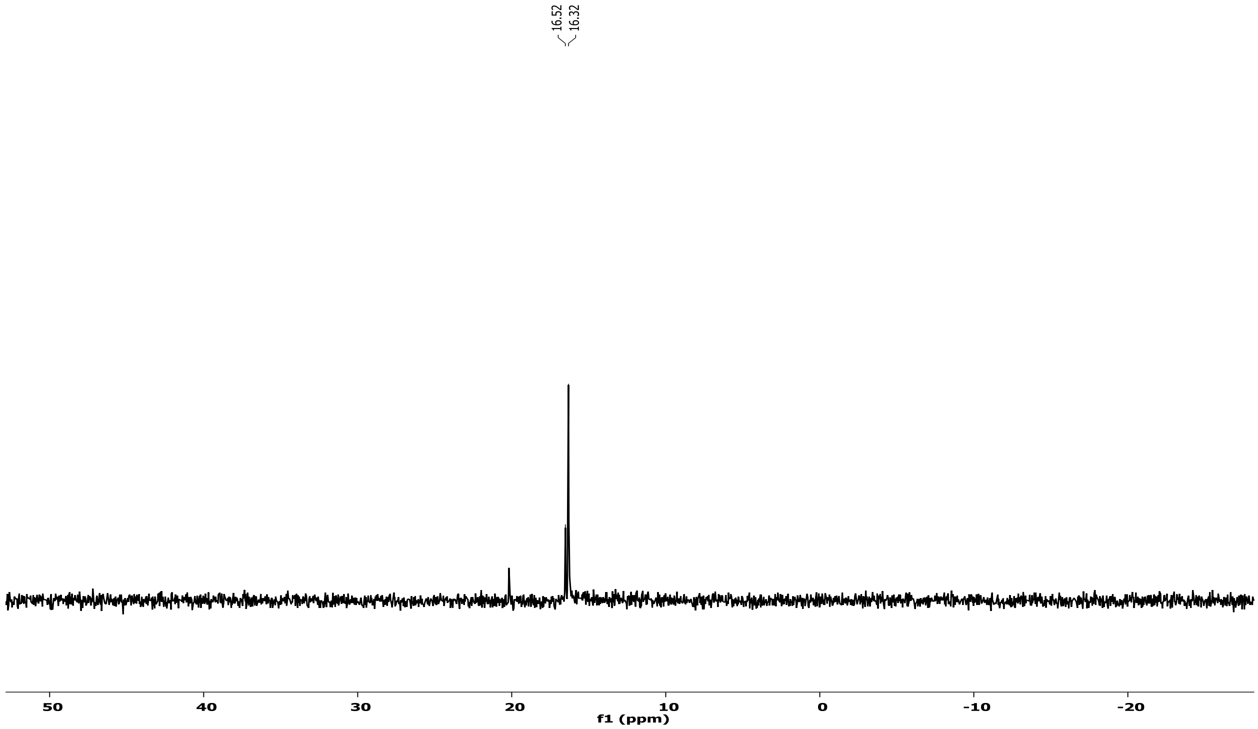
1H-NMR of compound **9b**, $CDCl_3$, 300 MHz



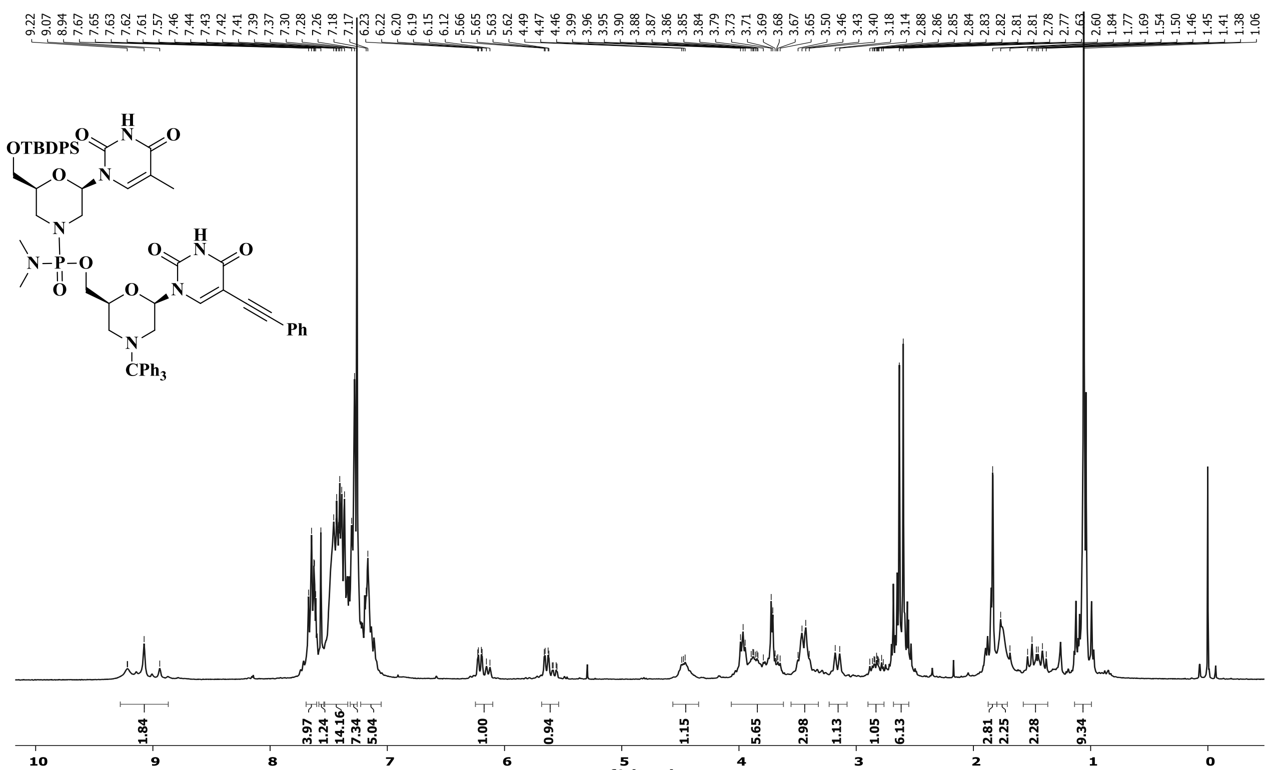
13C-NMR of compound **9b**, $CDCl_3$, 125 MHz



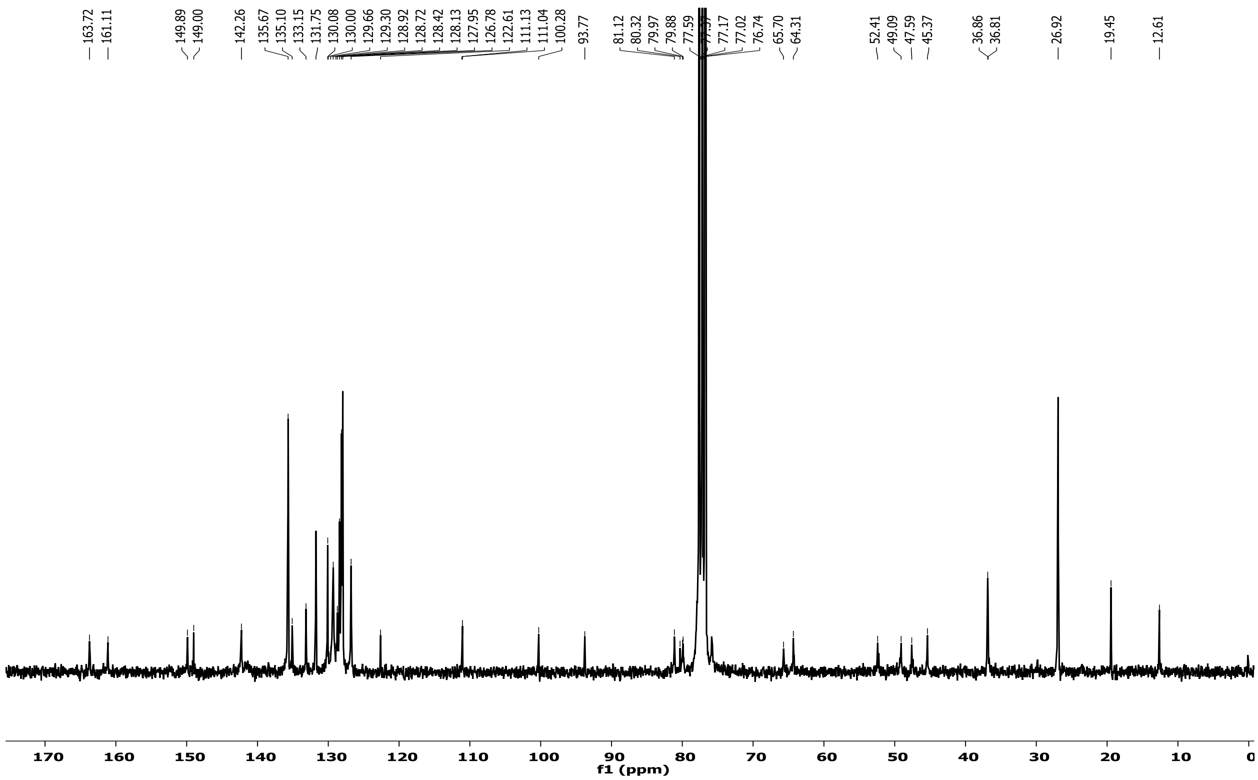
31P-NMR of compound **9b**, $CDCl_3$, 121 MHz



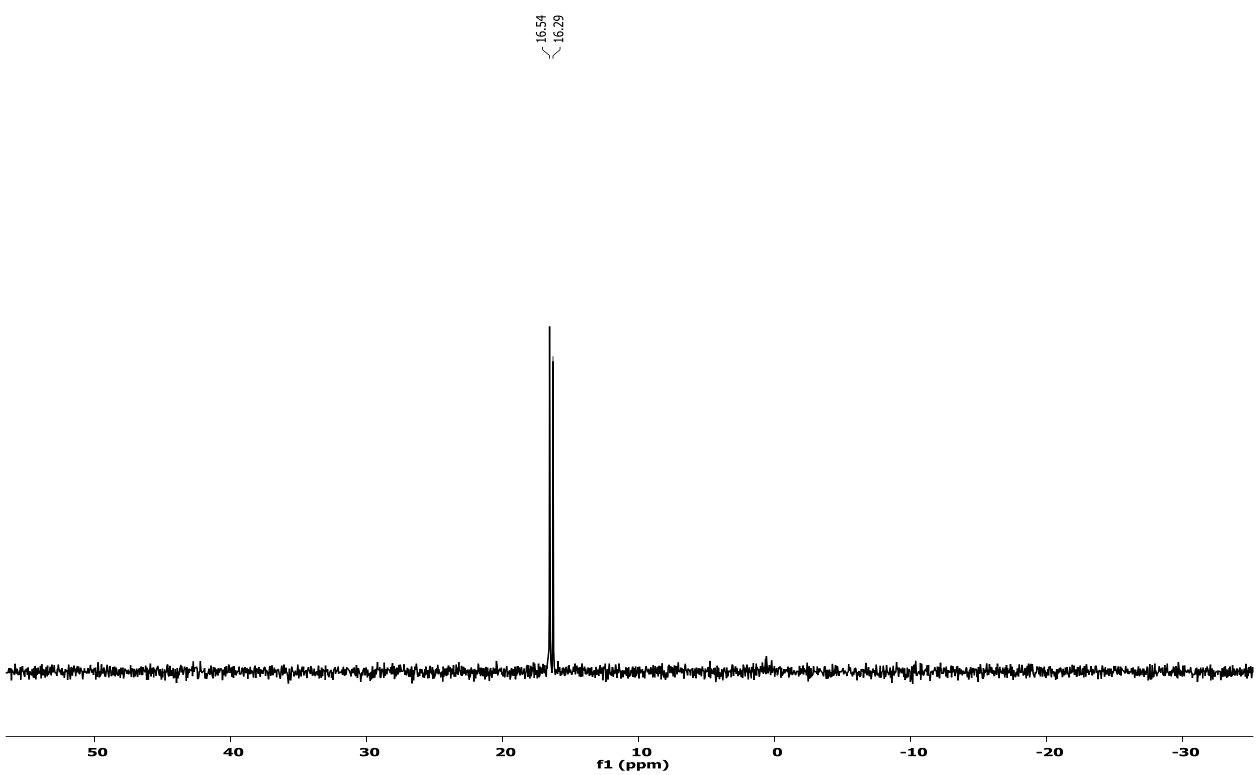
1H-NMR of compound **9c**, $CDCl_3$, 300 MHz



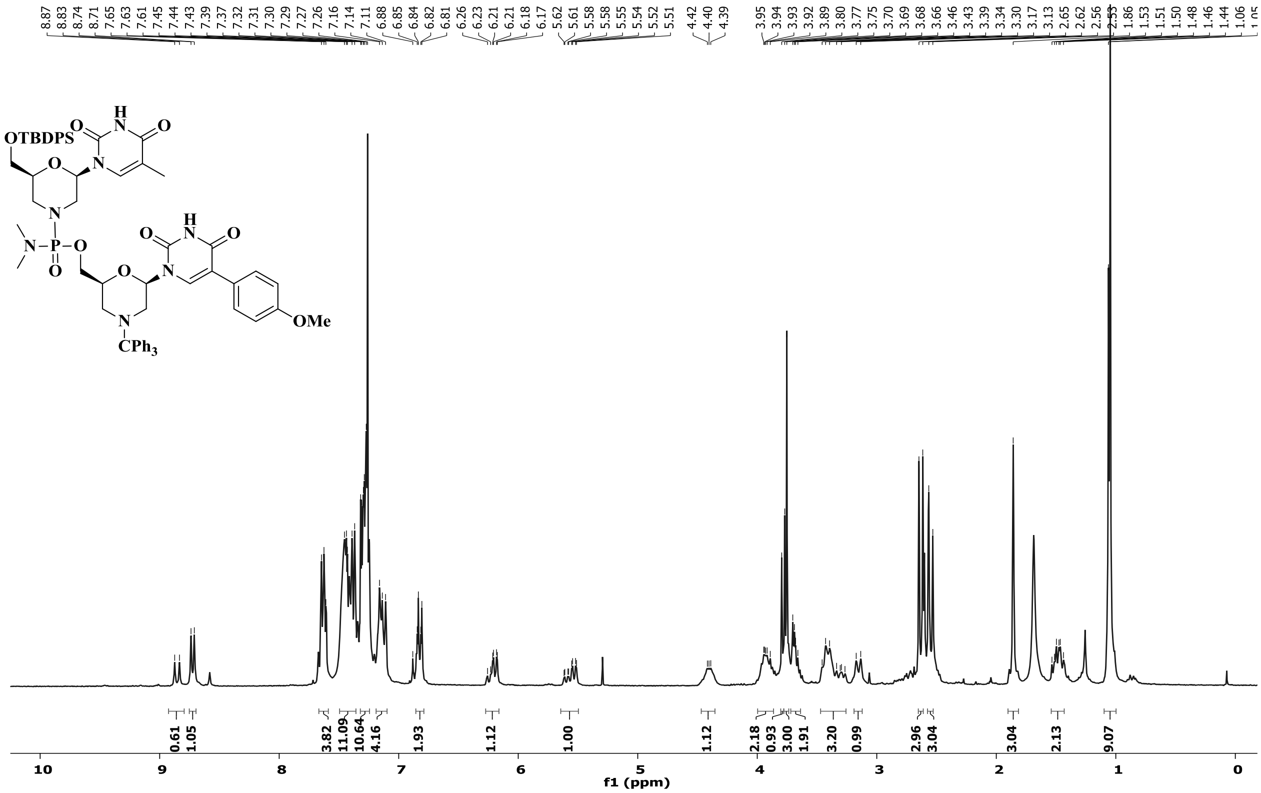
13C-NMR of compound **9c**, $CDCl_3$, 75 MHz



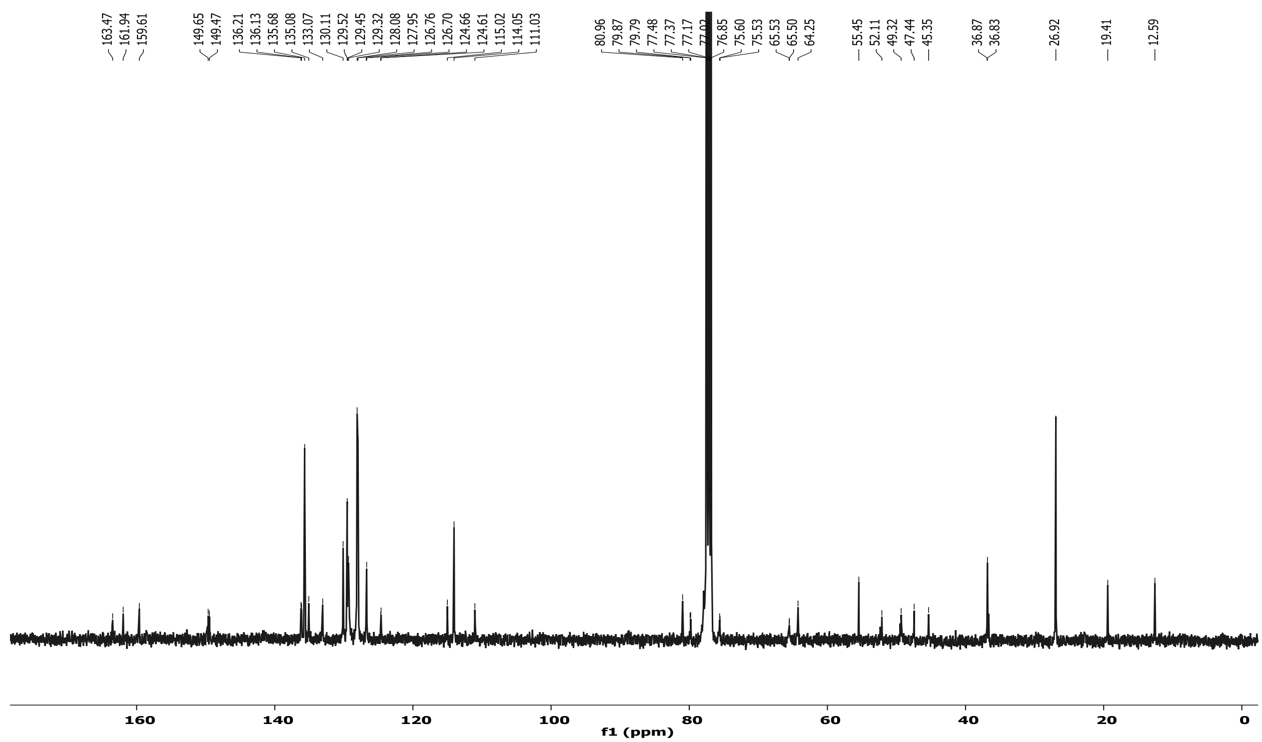
31P-NMR of compound **9c**, $CDCl_3$, 121 MHz



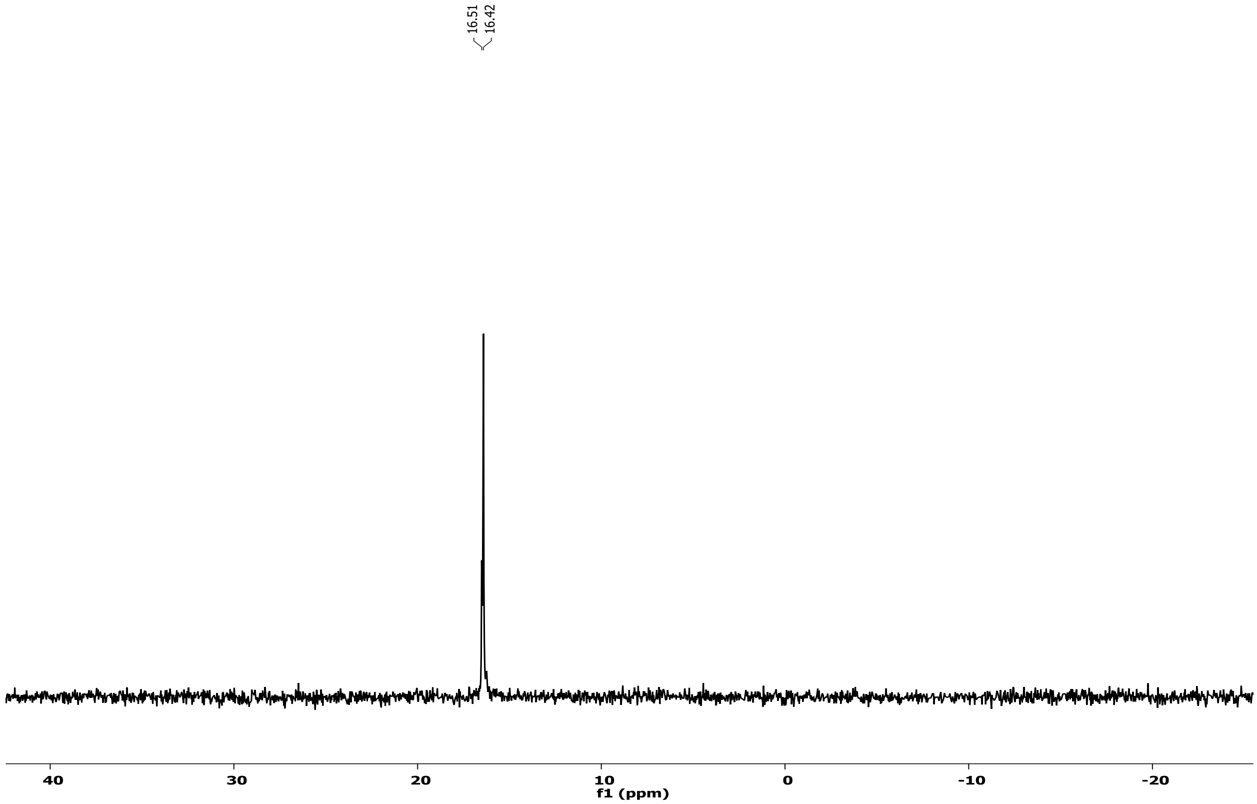
1H-NMR of compound **9e**, $CDCl_3$, 300 MHz



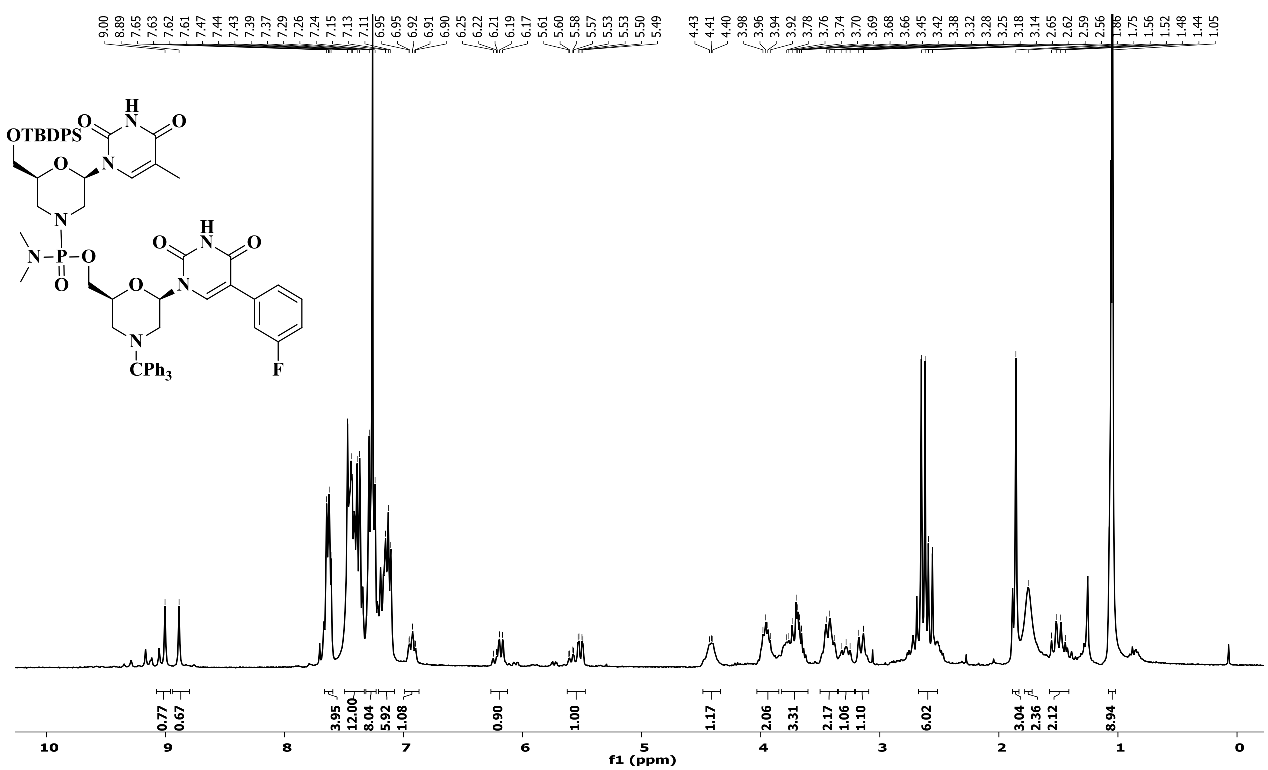
13C-NMR of compound **9e**, $CDCl_3$, 100 MHz



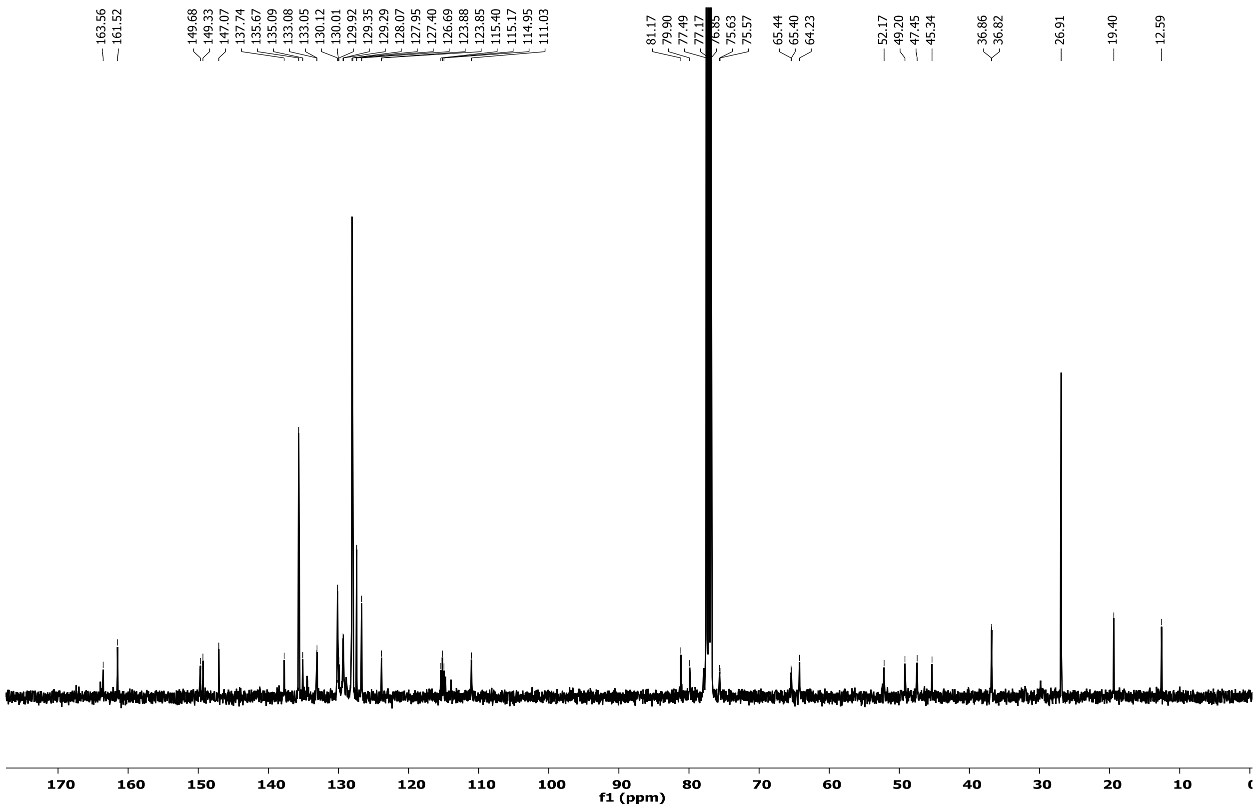
31P-NMR of compound **9e**, $CDCl_3$, 121 MHz



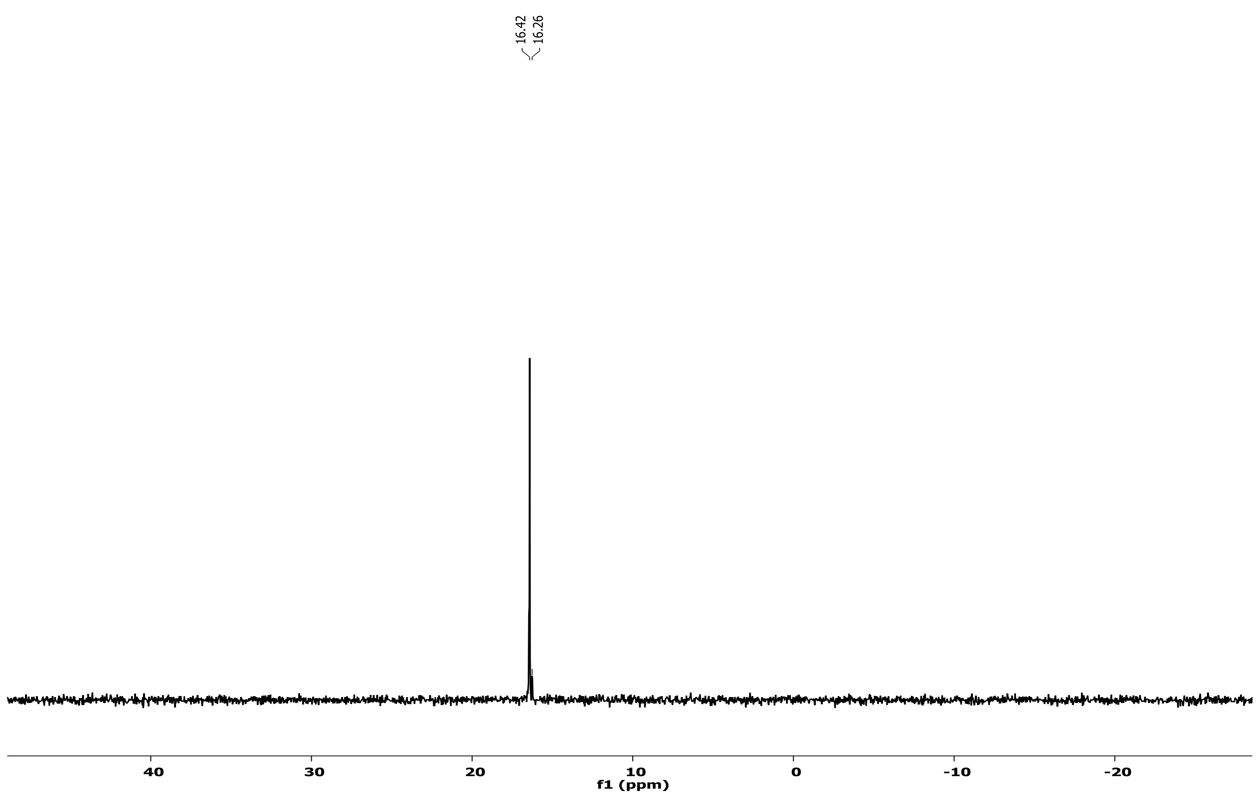
1H-NMR of compound **9h**, $CDCl_3$, 300 MHz



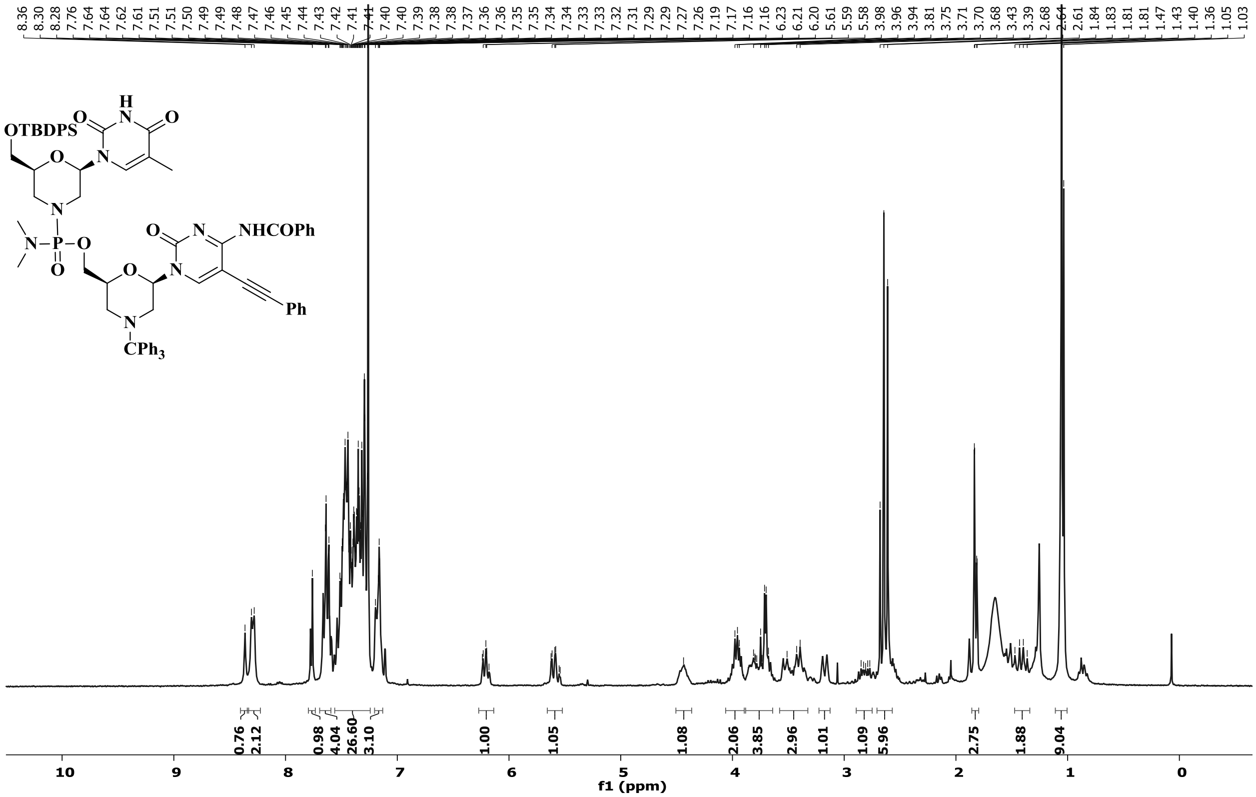
13C-NMR of compound **9h**, $CDCl_3$, 100 MHz



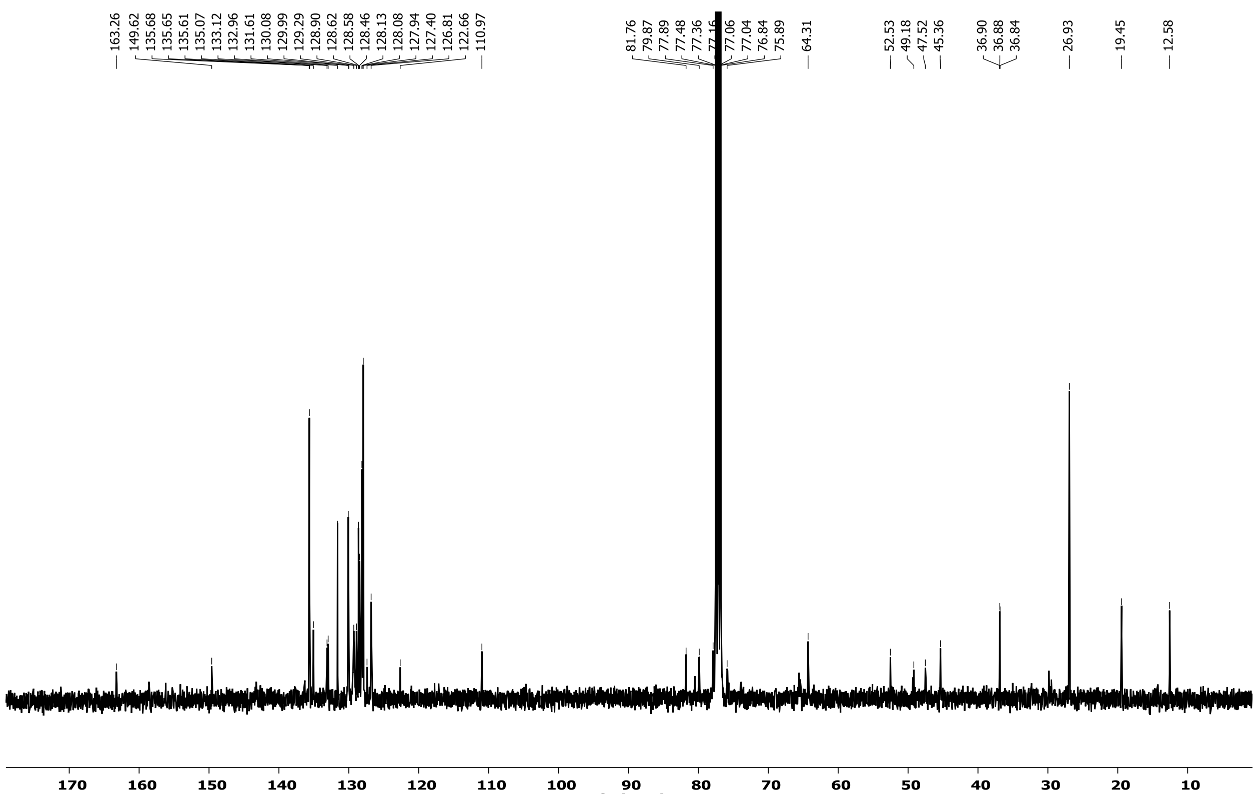
31P-NMR of compound **9h**, $CDCl_3$, 162 MHz



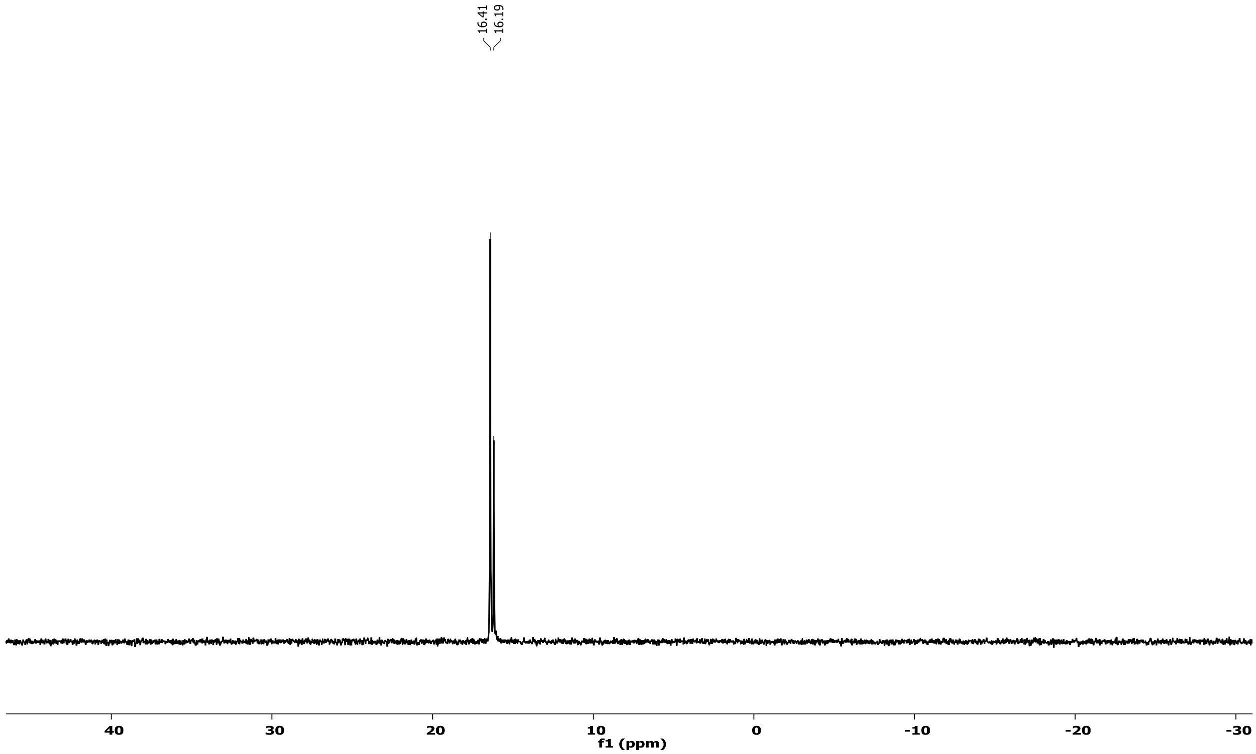
1H-NMR of compound **10b**, $CDCl_3$, 300 MHz



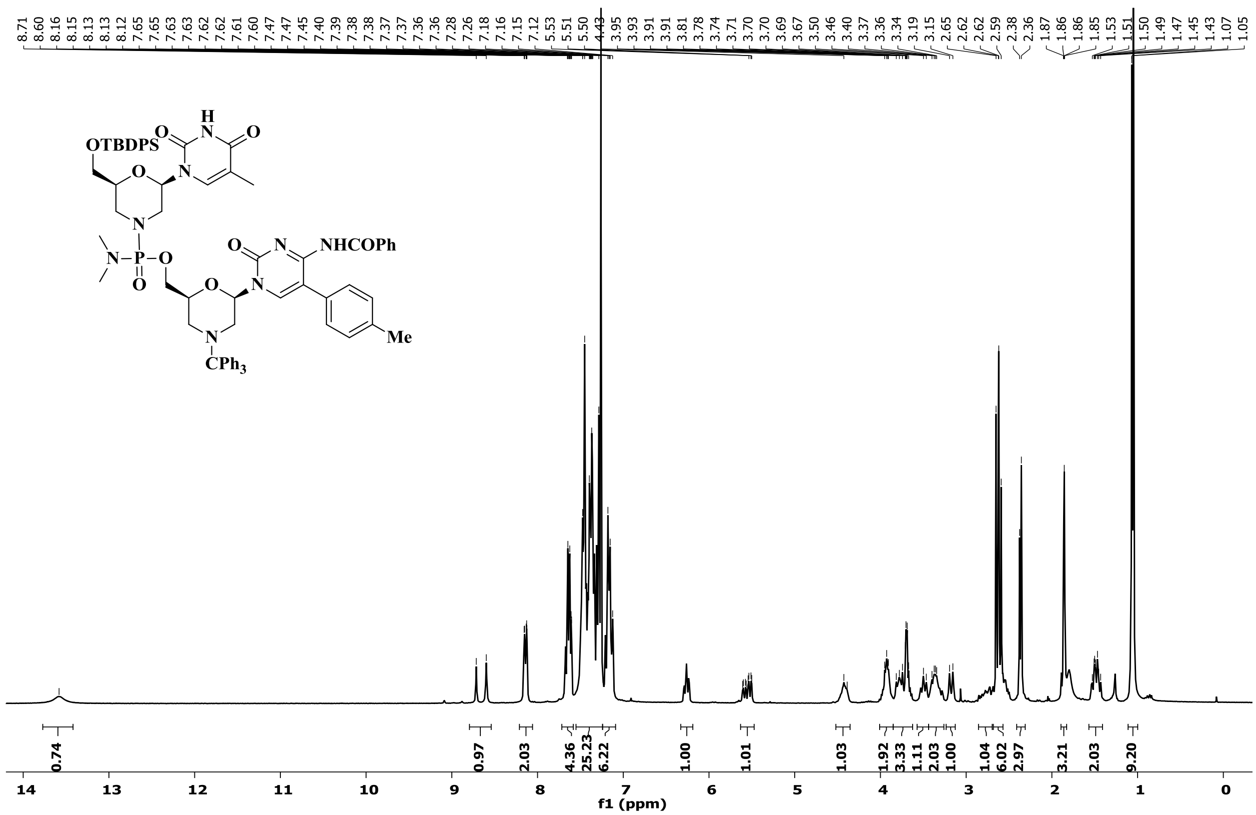
13C-NMR of compound **10b**, $CDCl_3$, 100 MHz



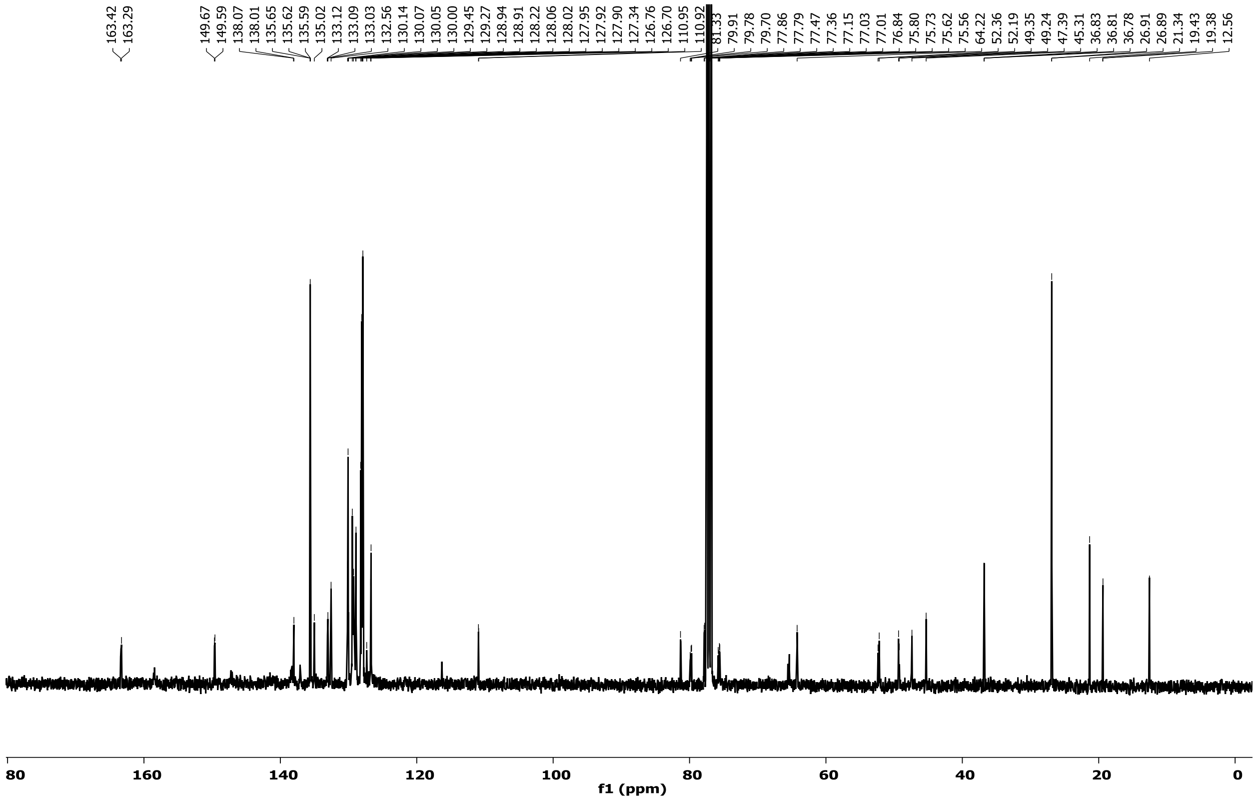
31P-NMR of compound **10b**, $CDCl_3$, 100 MHz



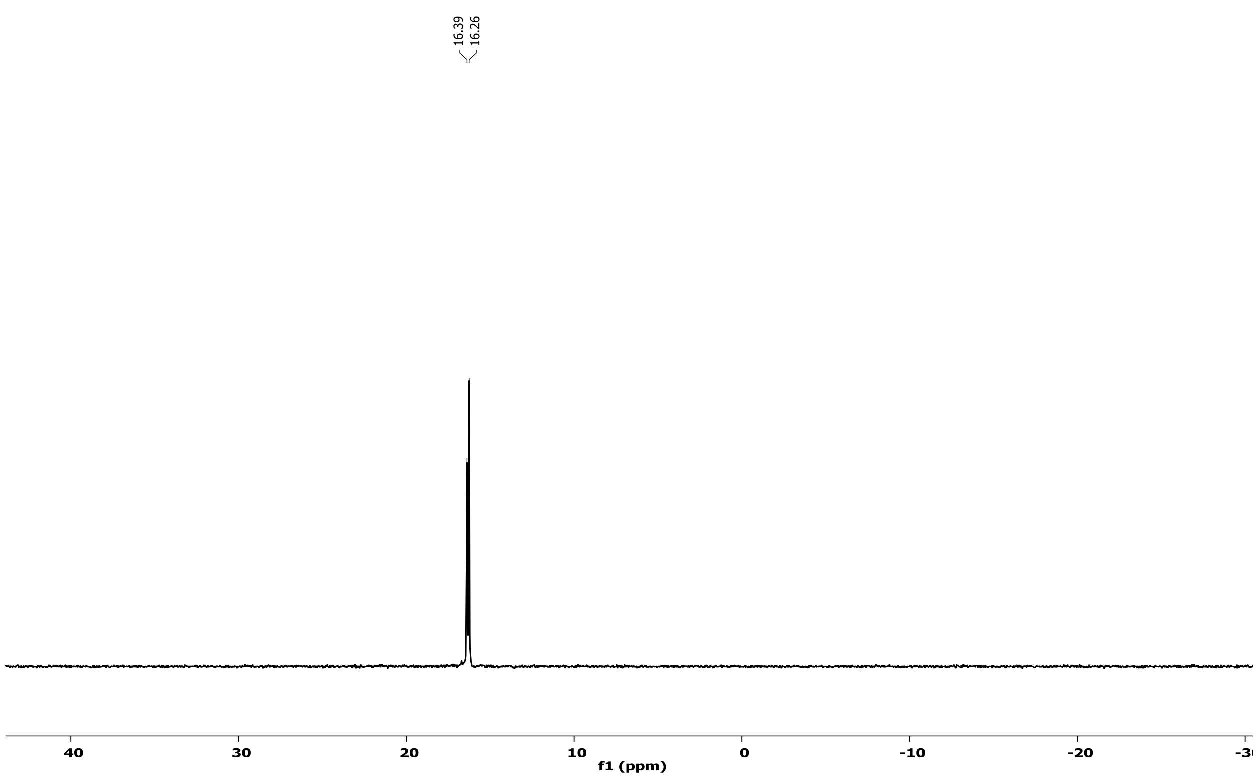
1H-NMR of compound **10d**, $CDCl_3$, 300 MHz



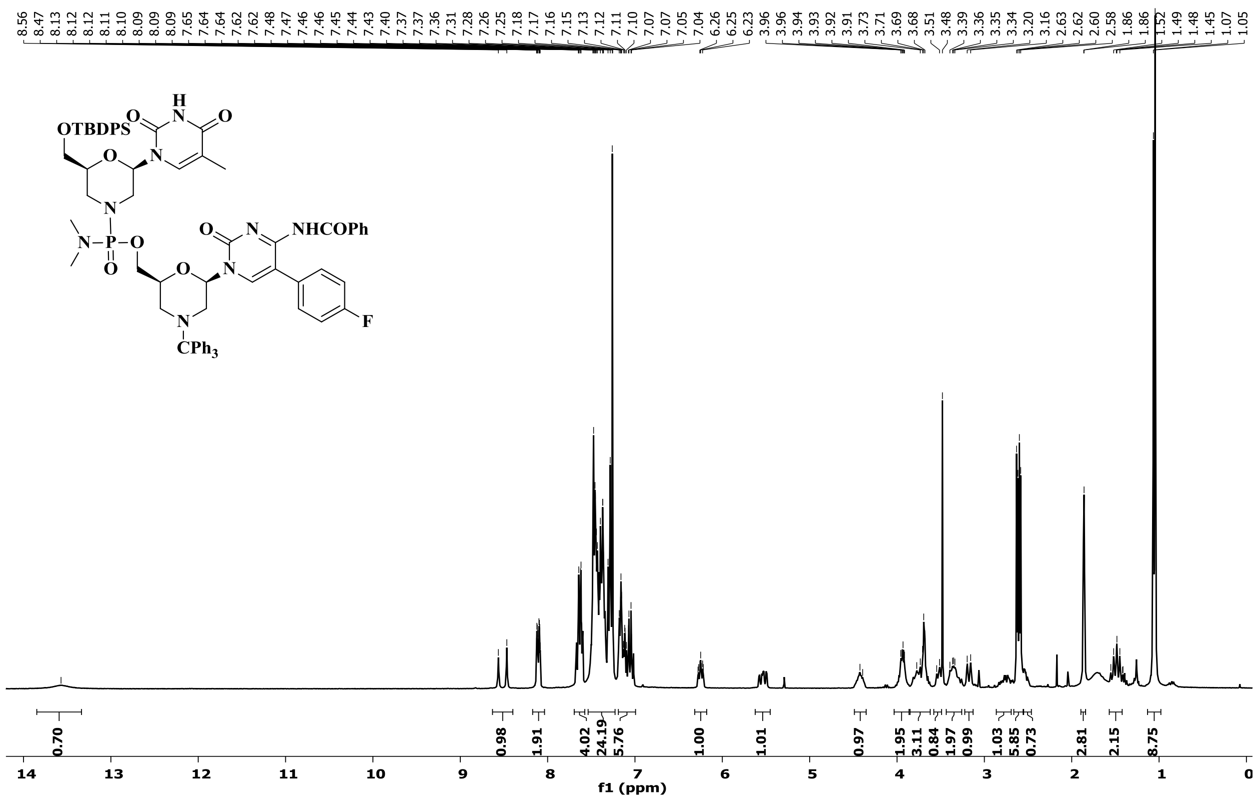
13C-NMR of compound **10d**, $CDCl_3$, 100 MHz



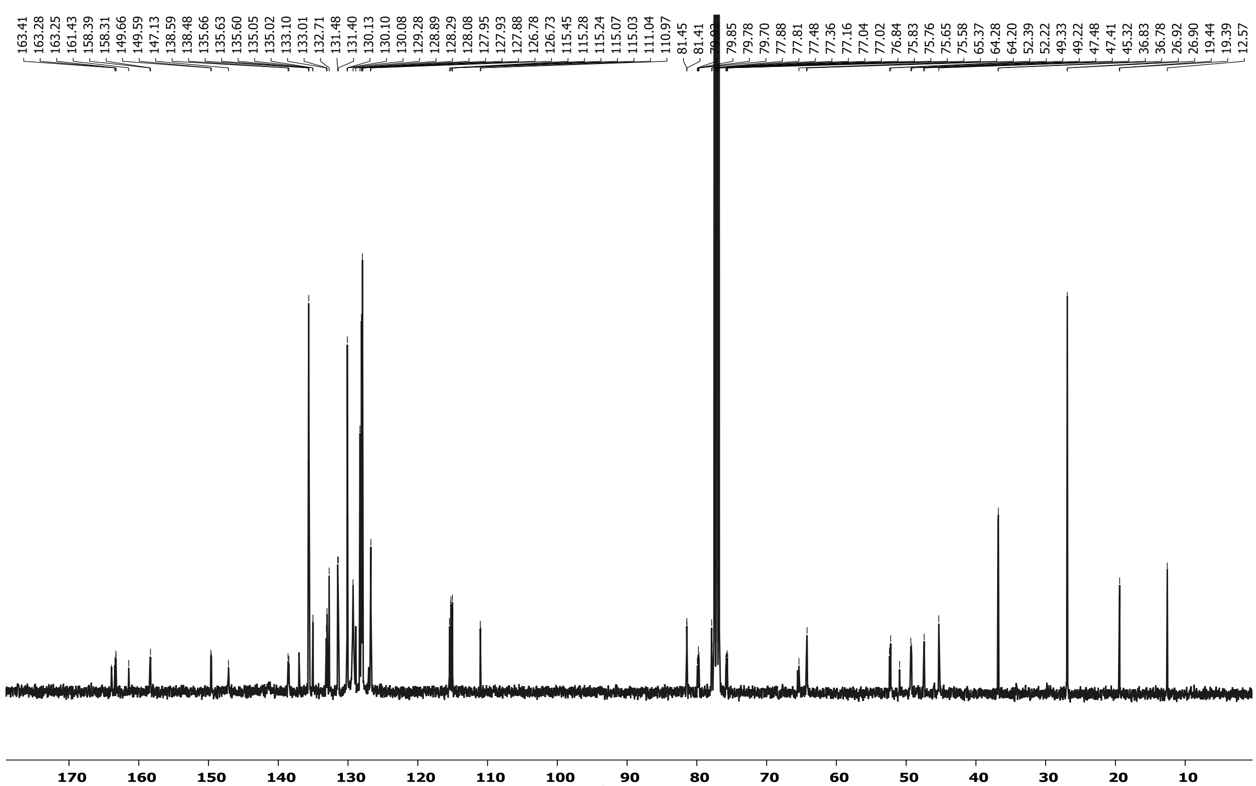
31P-NMR of compound **10d**, $CDCl_3$, 162 MHz



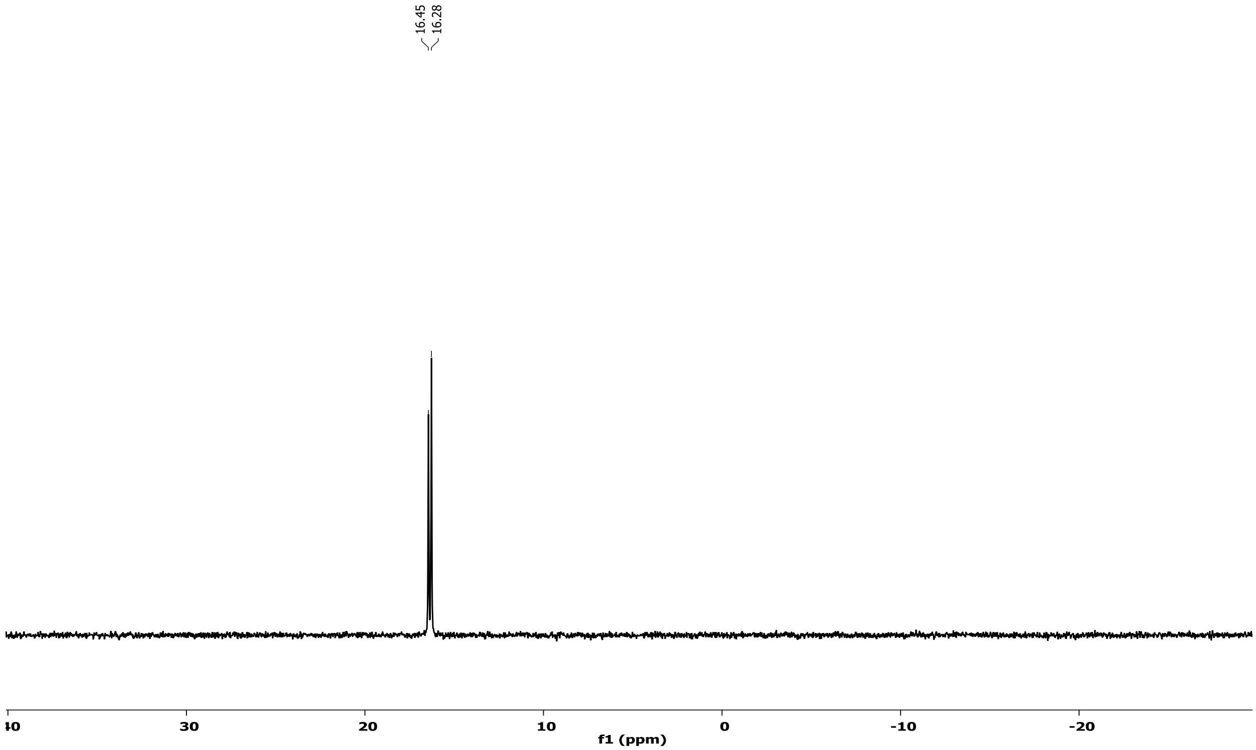
1H-NMR of compound **10f**, $CDCl_3$, 300 MHz



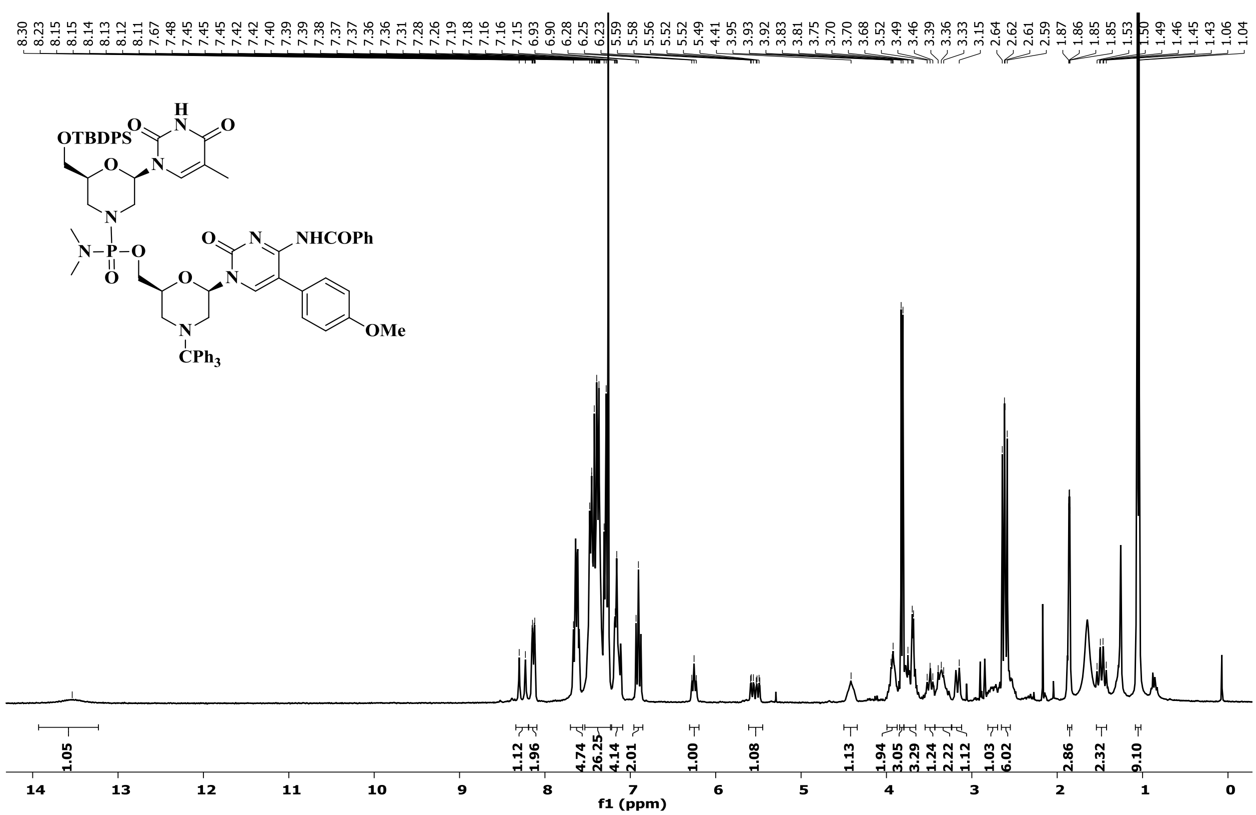
13C-NMR of compound **10f**, $CDCl_3$, 100 MHz



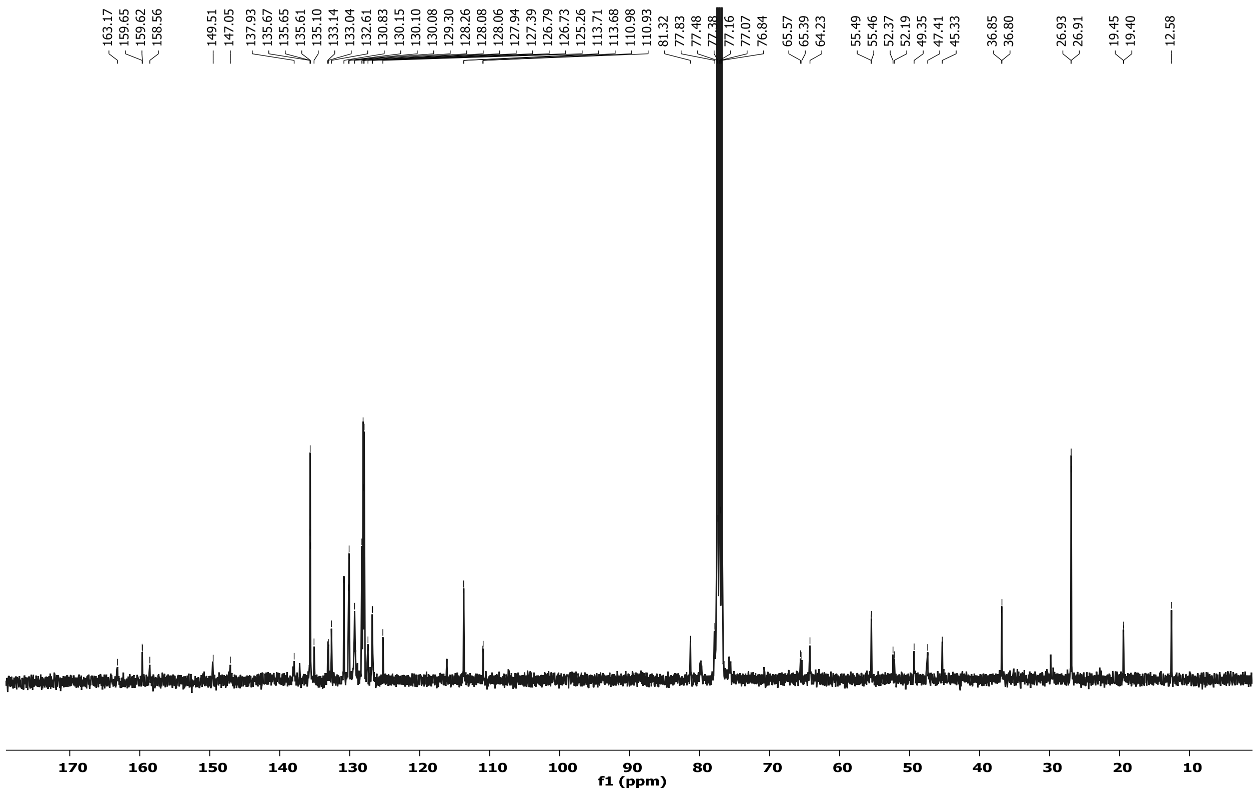
31P-NMR of compound **10f**, $CDCl_3$, 162 MHz



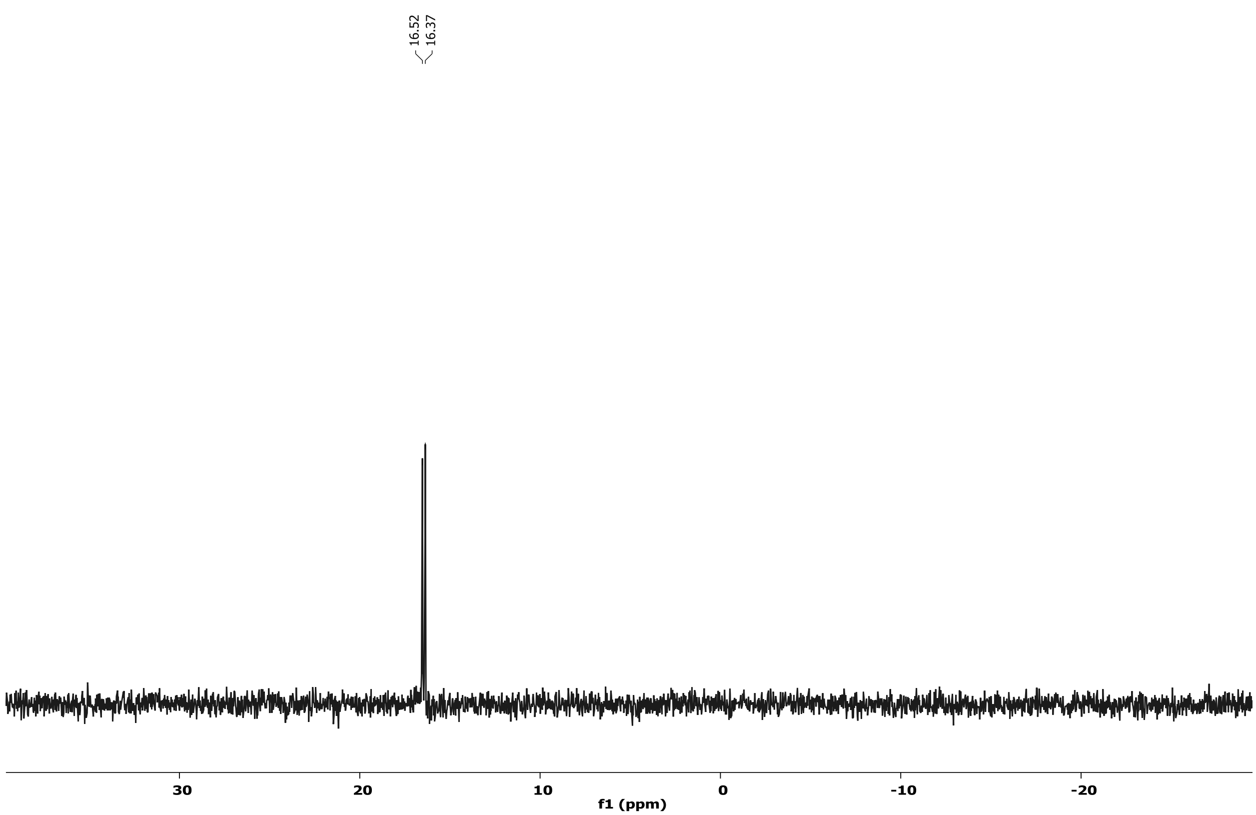
1H-NMR of compound **10h**, $CDCl_3$, 300 MHz



13C-NMR of compound **10h**, $CDCl_3$, 100 MHz



31P-NMR of compound **10h**, $CDCl_3$, 162 MHz

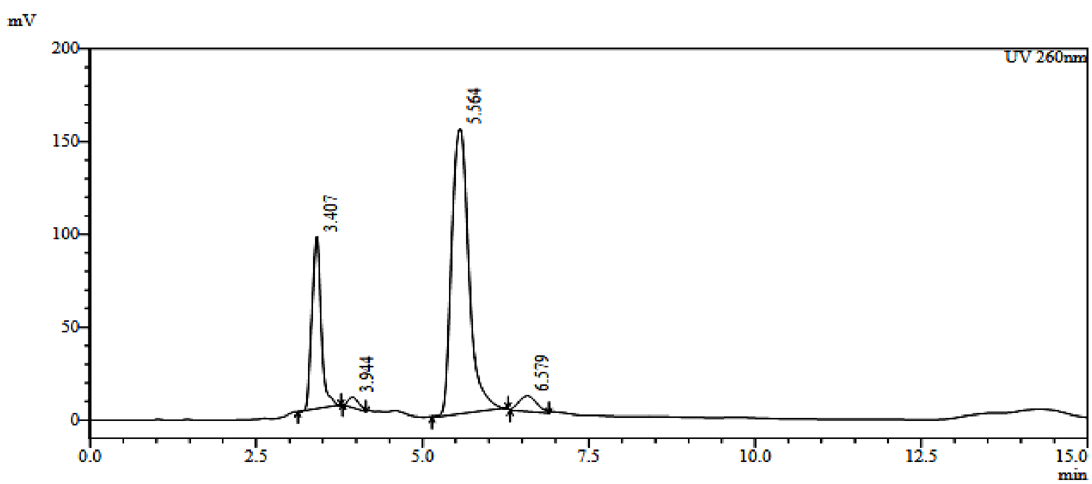


4. Dimer Formation reaction and HPLC analysis

Sl. No	Reaction condition	Additive	HPLC analysis (Retention time, % purity of dimer peak)
1	Compound **8** + **Additive (2 eq)** + Compound **7c** (1.5 eq) + NEM (3 eq)	LiBr	5.56 min, 71.2%
2		ETT	5.55 min, 79.7%
3	Compound **8** + **Additive (2 eq)** + Compound **7h** (1.5 eq)+ NEM (3 eq)	LiBr	5.50 min, 81.7%
4		ETT	5.42 min, 82.9%

Table S2: HPLC analysis of the reaction mixture of dimer formation reaction. HPLC was carried out by C-18 (Ascentis) column using Acetonitrile as eluent (Flow rate = 1ml / min)

Chromatogram



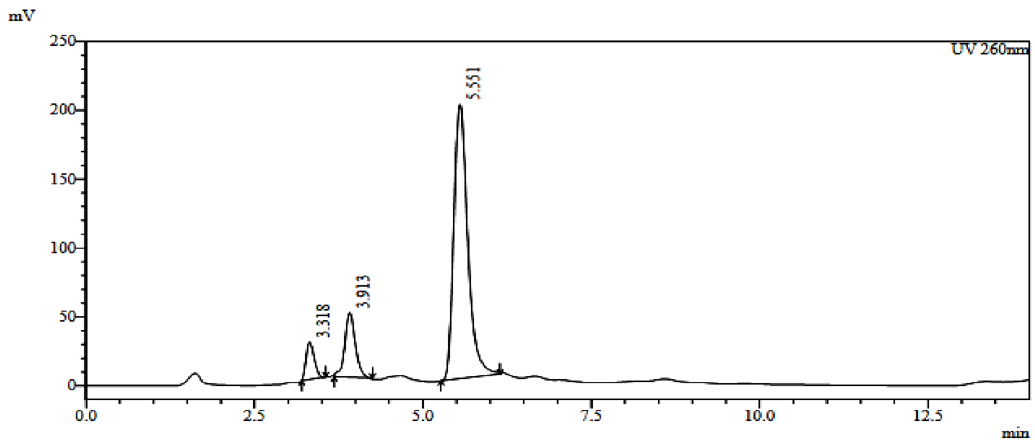
Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	3.407	887984	92765	22.941
2	3.944	63767	5717	1.647
3	5.564	2757521	153583	71.240
4	6.579	161462	8588	4.171
Total		3870735	260653	100.000

Figure S1: HPLC chromatogram of the crude reaction mixture of entry 1 (LiBr) of Table S2.

Chromatogram



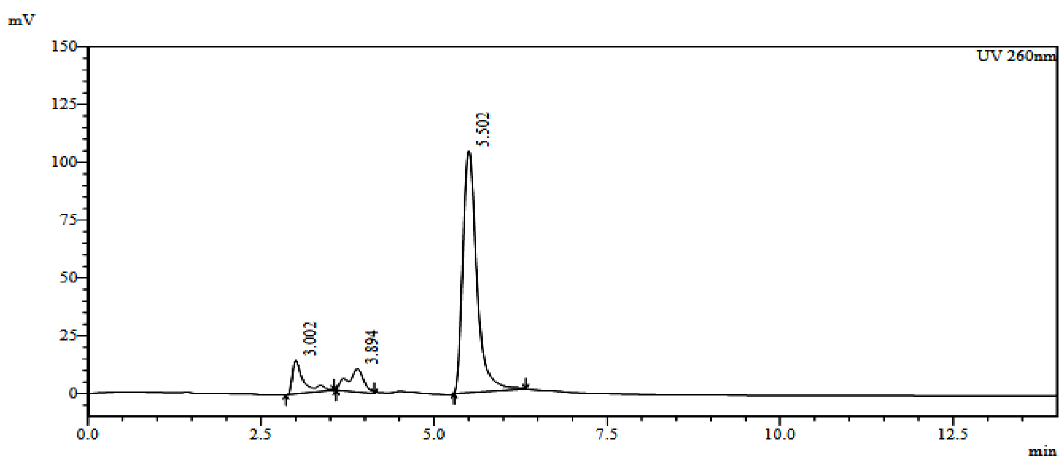
Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	3.318	225619	27035	6.252
2	3.913	505857	46667	14.017
3	5.551	2877480	199016	79.732
Total		3608956	272718	100.000

Figure S2: HPLC chromatogram of the crude reaction mixture of entry 2 (ETT) of Table S2.

Chromatogram



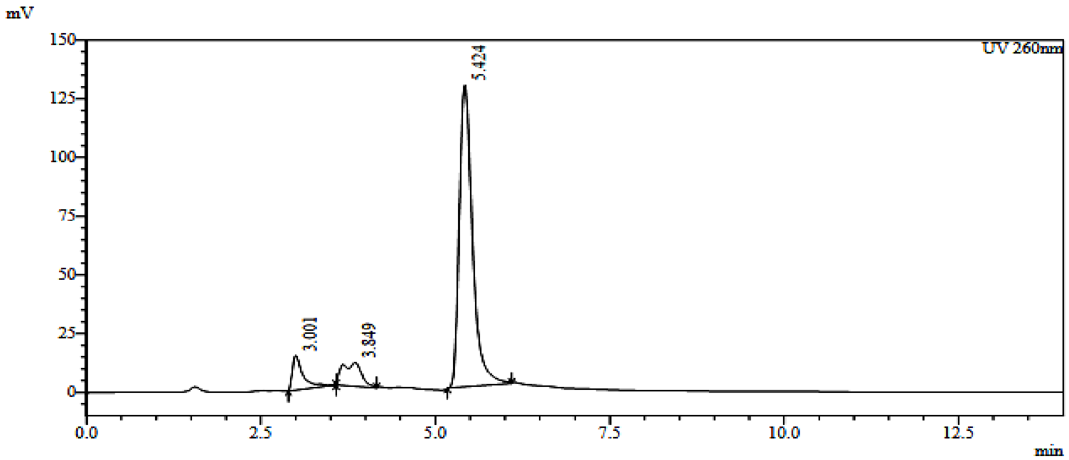
Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	3.002	167044	14395	9.262
2	3.894	161618	10030	8.961
3	5.502	1474866	104584	81.777
Total		1803527	129010	100.000

Figure S3: HPLC chromatogram of the crude reaction mixture of entry 3 (LiBr) of Table S2.

Chromatogram



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	3.001	157237	14619	7.673
2	3.849	193387	10141	9.437
3	5.424	1698606	128572	82.890
Total		2049230	153332	100.000

Figure S4: HPLC chromatogram of the crude reaction mixture of entry 4 (ETT) of Table S2

5. General PMO Synthesis Cycle in Solid phase[a,3] :

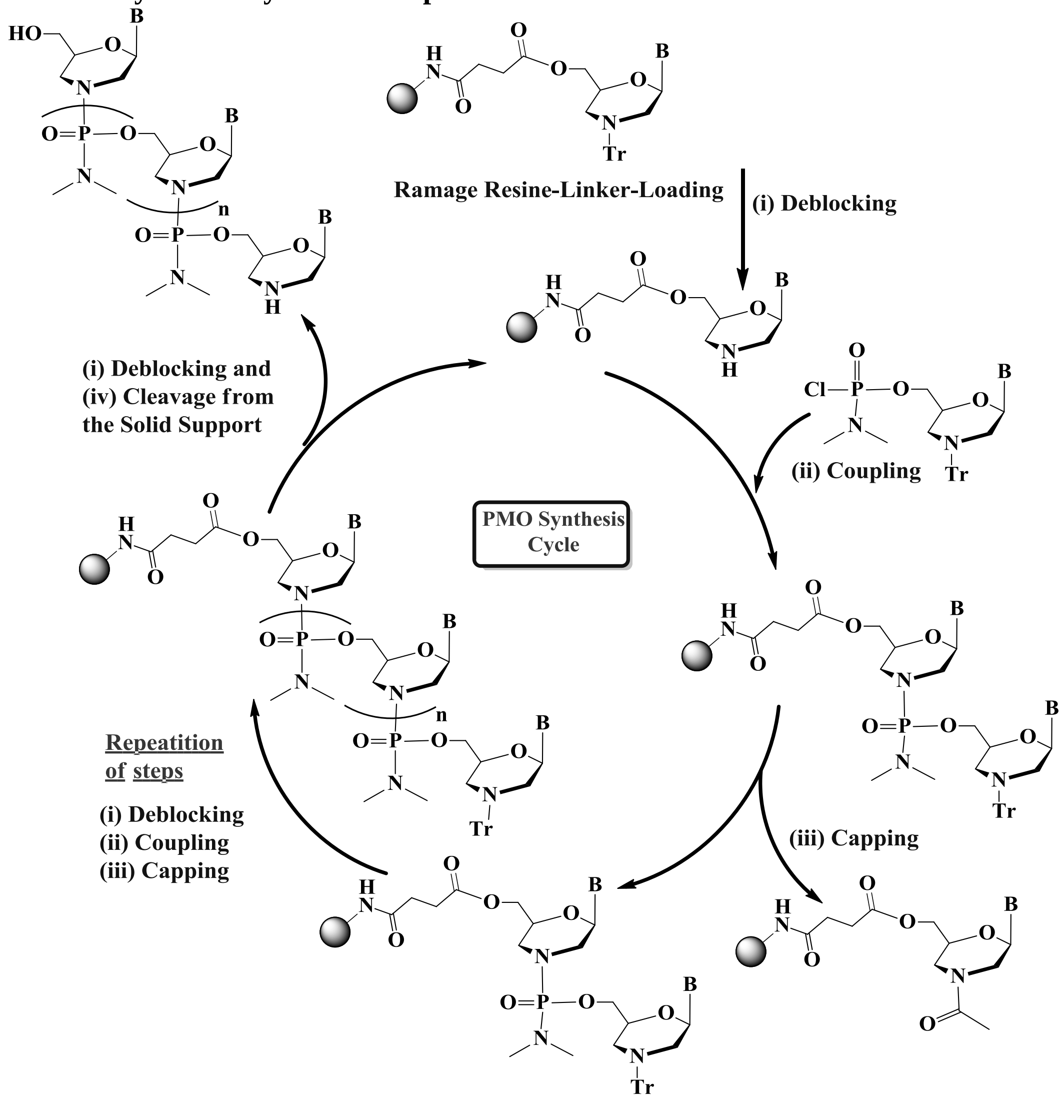
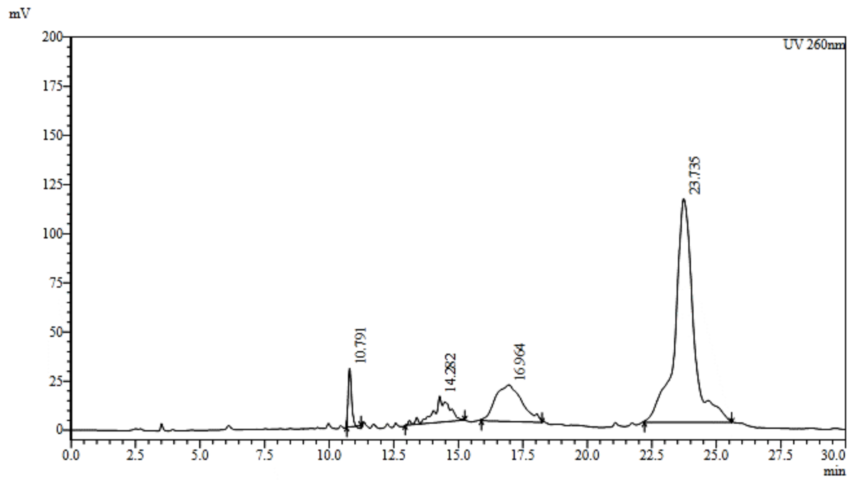


Figure S5: PMO synthesis cycle in solid phase

[a]Reagents and conditions **(i) Deblocking:** CYPTFA (3-cyano pyridine, TFA, Trifluro ethanol, DCM) 3 x 1 min = 3 min; **(ii) Coupling:** Chlorophosphoramidate morpholino monomer, ETT (5-ethylthio tetrazole and NEM (4-ethyl morpholine), NMP, 30 min, for functionalized chlorophosphoramidate morpholino monomer (3 x 30 min = 1.5 hr) **(iii) Capping:** (1:1)- 10% Ac_2O-NMP and 10% DIPEA-NMP (3 x 1 = 3 min); **(iv) Cleavage from the solid support:** 30% aq NH_3, 55°C, 16 h .

6. HPLC chromatogram of the crude PMO-8 in Trityl on mode:

5′-TTTTACT***C***ACAT-Tr-3′(C-Ph-Acetylene, 6b) (PMO-8-Tr)



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	10.791	260701	29884	3.327
2	14.282	552895	13348	7.056
3	16.964	1339907	18596	17.100
4	23.735	5682070	113857	72.516
Total		7835573	175685	100.000

Figure S6: HPLC analysis of crude mixture of **PMO-8-Tr** in trityl on mode. HPLC was carried out by C-18 (Agilent) column using 0.1M Ammonium acetate buffer (in H_2O)-CH_3CN gradient system (10-50%) as eluent (Flow rate = 1ml / min)

7. HPLC and Mass analysis of the oligonucleotides:

Sequence	HPLC Pure Yield (%)	Molecular formula	Calculated mass	Observed mass	HPLC Retention Time R_t (min)
5′-TTTTACTCACAT-3′ [a] (PMO-1)					11.7
5′-TTTTACT***C***ACAT-3′ (C-Acetylene,6a)(PMO-2)	25	$C_{141}H_{218}N_{59}Na_3O_{50}P_{11}$	3947.314	3946.176	12.2
5′-TTTTAC***U***CACAT-3′ (U-Acetylene,7b)(PMO-3)	22	$C_{140}H_{218}N_{59}Na_2O_{50}P_{11}$	3945.494	3945.204	12.3
5′-T***U***TTACTCACAT-3′ (U-Acetylene,7b)(PMO-4)	30	$C_{140}H_{217}K_2N_{59}O_{50}P_{11}$	3912.324	3912.043	11.7
5′-T***U***TTACT***C***ACAT-3′ (Acetylene,7b,6a)(PMO-5)	21	$C_{142}H_{217}N_{59}Na_2O_{50}P_{11}$	3937.299	3937.143	12.2
5′-TTTTAC***UC***ACAT-3′ (Acetylene,7b,6a)(PMO-6)	18	$C_{142}H_{216}KN_{59}Na_3O_{50}P_{11}$	3998.379	3997.367	12.4
5′-TTTTA***C***T***C***ACAT-3′ (C-Acetylene,6a)(PMO-7)	20	$C_{143}H_{218}N_{59}Na_2O_{50}P_{11}$	3948.324	3948.236	14.5
5′-TTTTACT***C***ACAT-3′ (C-Ph-Acetylene ,6b)(PMO-8)	27	$C_{147}H_{224}N_{59}O_{50}P_{11}$	3958.428	3958.656	14.4
5′-TTTTAC***U***CACAT-3′ (U-Ph-Acetylene,7c)(PMO-9)	24	$C_{146}H_{220}K_4N_{59}NaO_{50}P_{11}$	4119.205	4120.251	13.9
5′-TTTTACT***C***ACAT-3′ (C-Phenyl,6j)(PMO-10)	28	$C_{145}H_{222}K_2N_{59}NaO_{50}P_{11}$	4031.293	4032.393	12.5
5′-TTTTAC***U***CACAT-3′ (U-Phenyl,7d)(PMO-11)	26	$C_{144}H_{220}K_3N_{59}NaO_{50}P_{11}$	4056.241	4057.995	13.3
5′-TTTTACT***C***ACAT-3′ (C-Phenyl-CF_3,6e)(PMO-12)	30	: $C_{146}H_{221}F_3K_2N_{59}NaO_{50}P_{11}$	4101.575	4101.682	14.6
5′-TTTTAC***U***CACAT-3′ (U-Phenyl-CF_3,7f)(PMO-13)	29	$C_{145}H_{219}F_3N_{59}Na_3O_{50}P_{11}$	4053.317	4050.514	15.2
5′-TTTTACT***C***ACAT-3′ (C-Phenyl-OMe,6h)(PMO-14)	29	$C_{146}H_{225}N_{59}Na_2O_{51}P_{11}$	4007.374	4007.161	13.3
5′-TTTTAC***U***CACAT-3′ (U-Phenyl-OMe,6e)(PMO-15)	27	$C_{145}H_{224}K_2N_{59}O_{51}P_{11}$	4026.314	4026.208	13.5
5′-TTTTACT***C***ACAT-3′ (C- Napthyl,6g)(PMO-16)	28	$C_{149}H_{224}N_{59}NaO_{50}P_{11}$	4003.381	4002.551	13.8
5′-TTTTAC***U***CACAT-3′ (U-Naphthyl,7g)(PMO-17)	24	$C_{148}H_{223}N_{59}NaO_{50}P_{11}$	3990.373	3990.059	13.1
5′-TTTTAC***C***TACAT-3′ (C-Acetylene,6a)(PMO-23)	24	$C_{141}H_{218}K_3N_{59}O_{50}P_{11}$	3997.611	3997.079	13.6
5′-TTTTACC***U***ACAT-3′ (U-Acetylene,7b)(PMO-24)	21	$C_{140}H_{216}K_4N_{59}Na2O_{50}P_{11}$	4068.662	4068.270	11.6

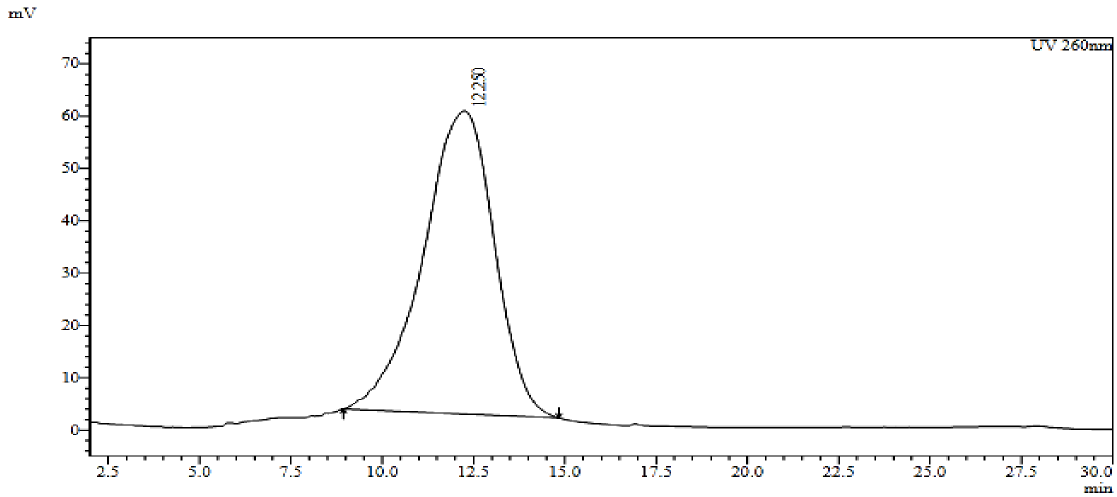
Table S3: HPLC retention time and MALDI-TOF mass of the oligonucleotides (PMO1-PMO17, PMO23 and 24).

Sequence	HPLC Pure Yield (%)	Molecular formula	Calculated mass	Observed mass	HPLC Retention Time R_t (min)
5′-TTTTAC***U***CACAT-3′ U-iodo,7i (PMO-18)	22	$C_{138}H_{213}IN_{59}O_{50}P_{11}$	1982.1	1982.4	12.7
5′-TTTTACU***C***ACAT-3′ m5C,6k (PMO-19)	26	$C_{140}H_{221}N_{59}NaO_{50}P_{11}$	1947.1	1948.1	13.0
5′-TTTTACU***C***ACAT-3′ C-iodo,6l (PMO-20)	24	$C_{139}H_{219}IN_{59}O_{50}P_{11}$	1989.1	1989.3	12.6
5′-TTTTACCCACAT-3' (Single mismatch Regular PMO)(PMO-21)	29	$C_{138}H_{219}N_{60}O_{49}P_{11}$	1918.7	1919.0	10.9
5′-TTTTAC***C***CACAT-3' (Single mismatch modified PMO)(C-Acetylene,6a)((PMO-22)	23	$C_{140}H_{219}N_{60}O_{49}P_{11}$	1930.7	1931.0	12.9

Table S4: HPLC retention time and LCMS mass of the oligonucleotides (PMO18-PMO22).
HPLC purification of the functionalized 12-mer morpholino oligonucleotides was done on Shimadzu SP-20AD system with C18 (Ascentis) column using 0.1M Ammonium acetate buffer (in H_2O)-CH_3CN gradient system (10-50%) (2 ml/min).
[a] Reference-3

(A)

Chromatogram



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	12.250	7887212	57975	100.000
Total		7887212	57975	100.000

(B)

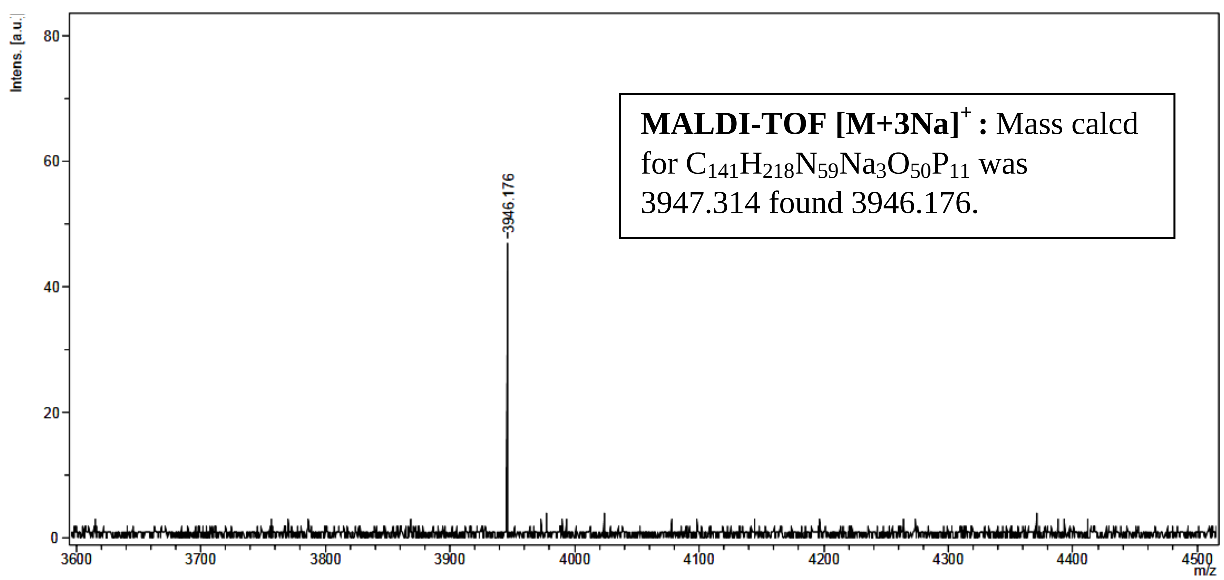
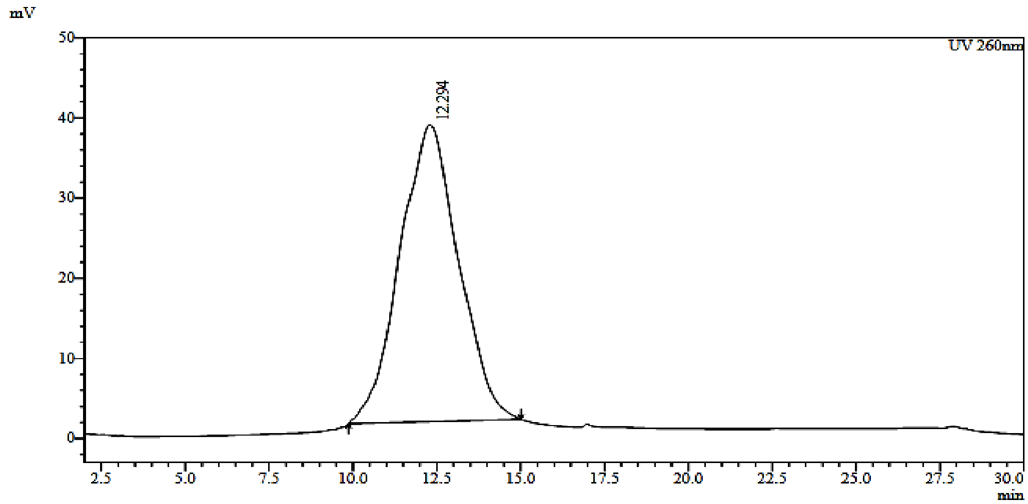


Figure S7: **(A)** HPLC Chromatogram of PMO 2 [5′-TTTTACT*C*ACAT-3′ **(C-Acetylene, 6a)], (B)** MALDI-TOF spectrum of PMO 2.

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	12.294	4507048	37032	100.000
Total		4507048	37032	100.000

(B)

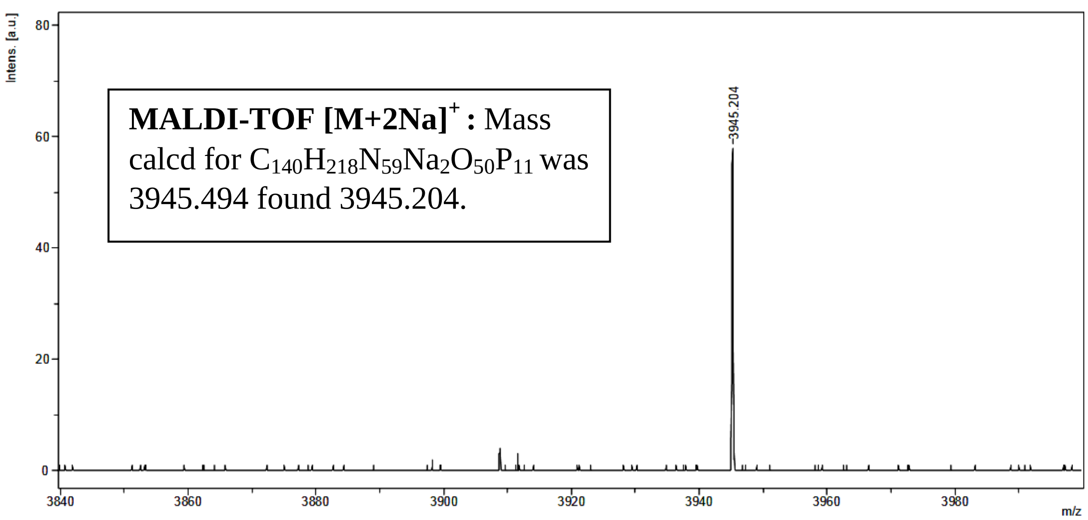
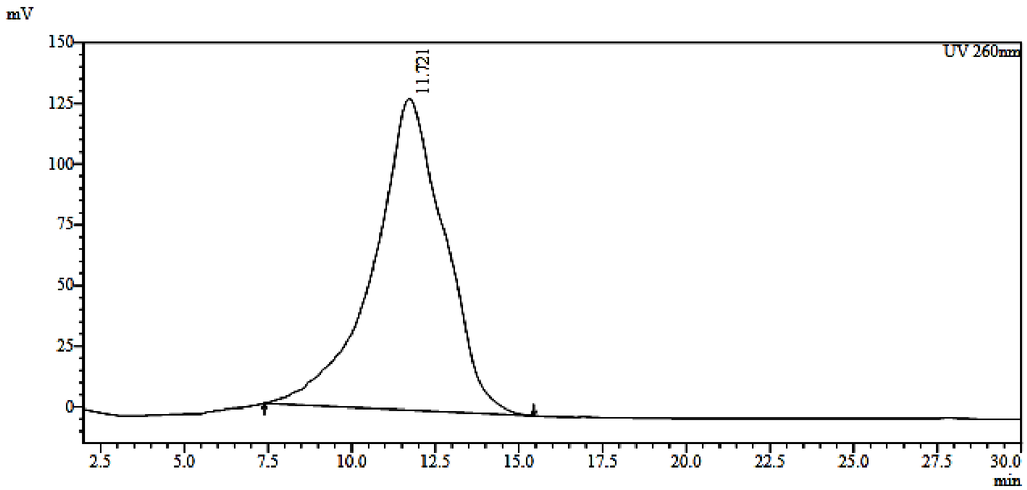


Figure S8: **(A)** HPLC Chromatogram of PMO 3 **[**5′-TTTTAC***U***CACAT-3′ **(U-Acetylene, 7b)], (B)** MALDI-TOF spectrum of PMO 3.

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	11.721	19030365	128176	100.000
Total		19030365	128176	100.000

(B)

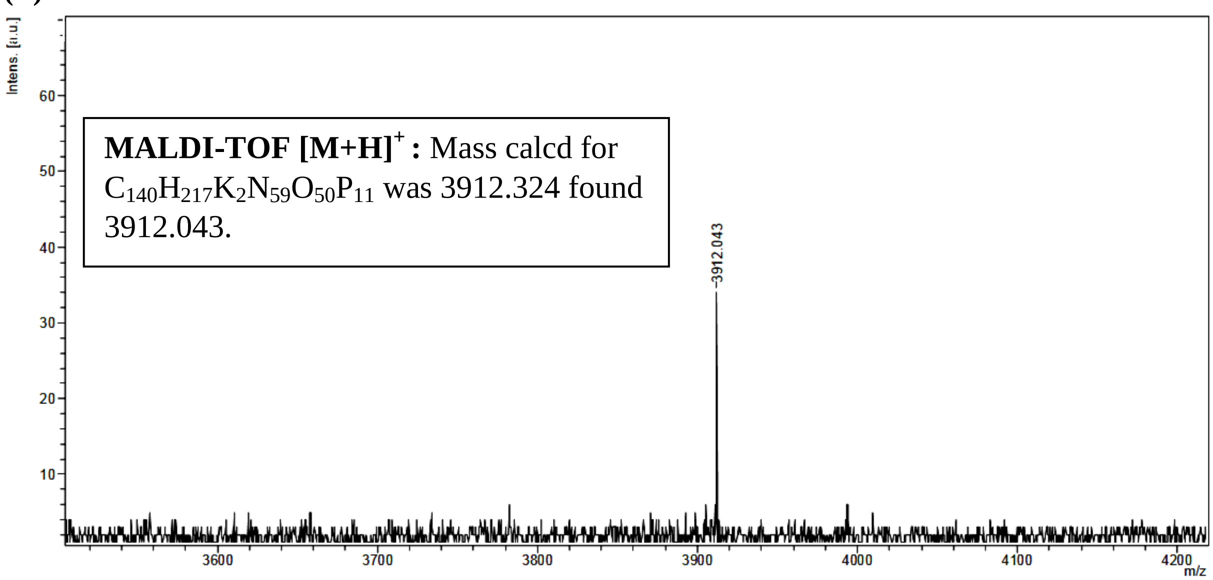
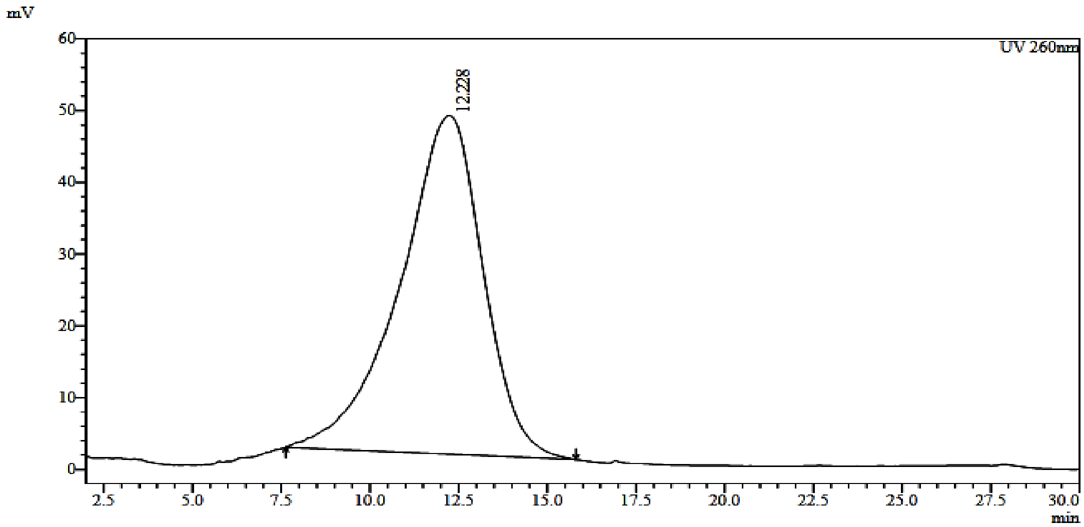


Figure S9: (A) HPLC Chromatogram of PMO 4 [5′-T*U*TTACTCACAT-3′ **(U-Acetylene, 7b)], (B)** MALDI-TOF spectrum of PMO 4.

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	12.228	7697301	47152	100.000
Total		7697301	47152	100.000

(B)

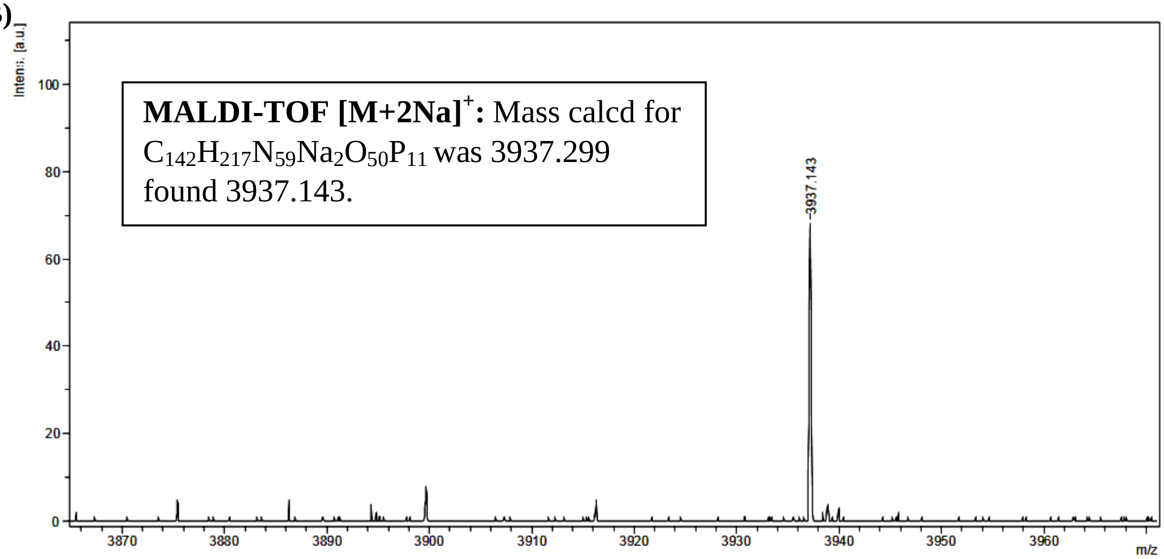
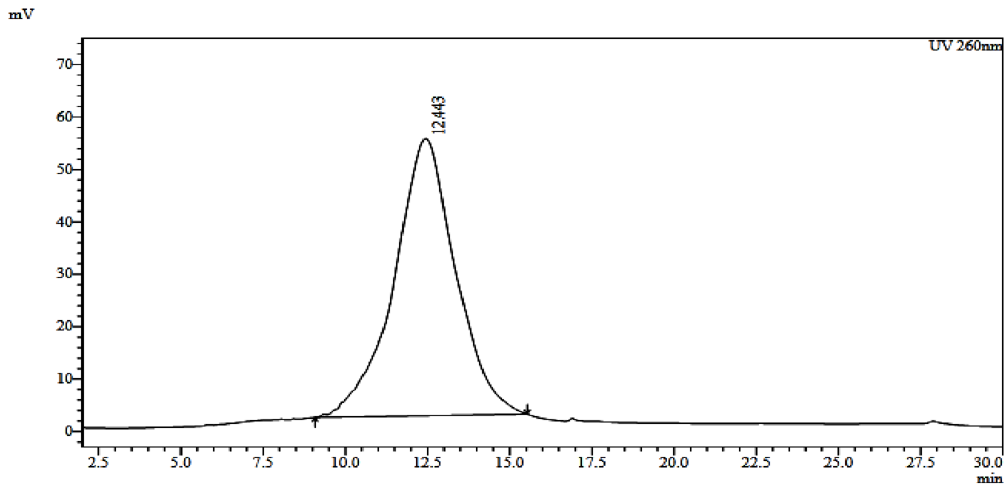


Figure S10: (A) HPLC Chromatogram of PMO 5 **[**5′-T***U***TTACT***C***ACAT-3′ **(Acetylene, 7b, 6a)], (B)** MALDI-TOF spectra of PMO 5.

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	12.443	6873592	52831	100.000
Total		6873592	52831	100.000

(B)

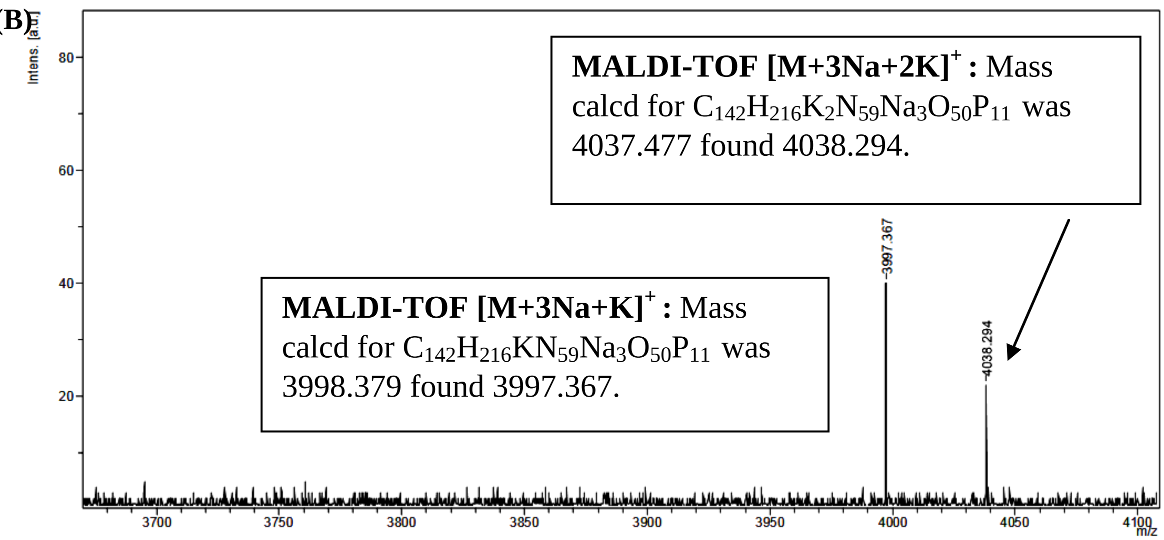
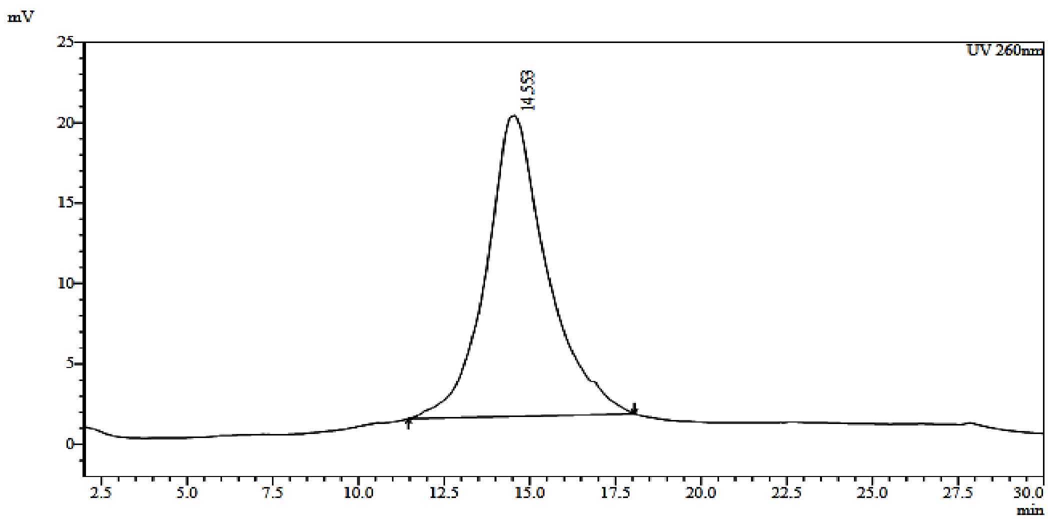


Figure S11: (A) HPLC Chromatogram of PMO 6 [5′-TTTTAC*UC*ACAT-3′ **(Acetylene, 7b, 6a)], (B)** MALDI-TOF spectrum of PMO 6.

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	14.553	2316527	18699	100.000
Total		2316527	18699	100.000

(B)

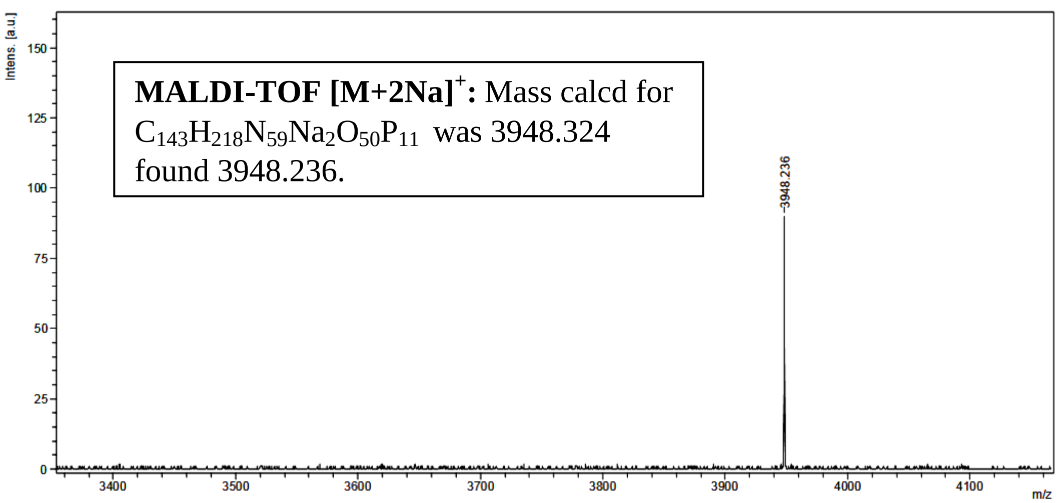
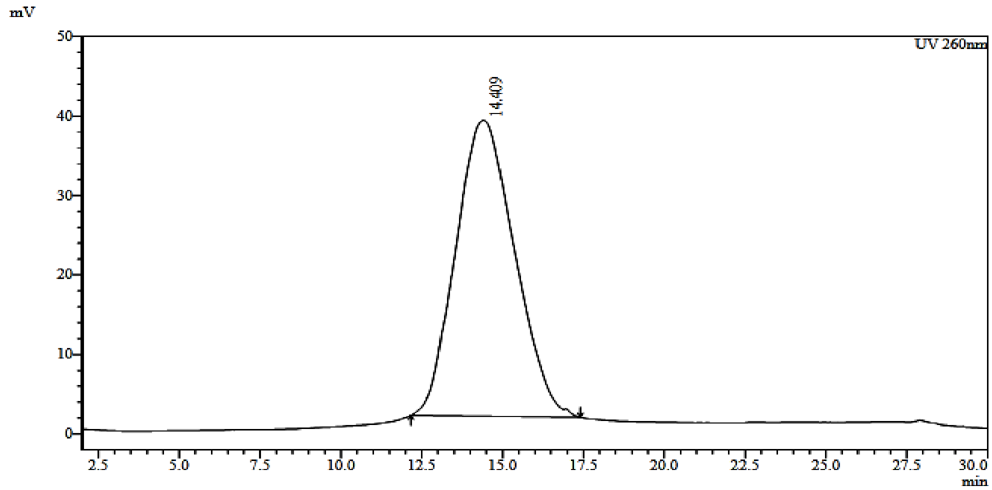


Figure S12: (A) HPLC Chromatogram of PMO 7 [5′-TTTTA***C***T***C***ACAT-3′ **(C-Acetylene, 6a)], (B)** MALDI-TOF spectrum of PMO 7.

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	14.409	4729493	37244	100.000
Total		4729493	37244	100.000

(B)

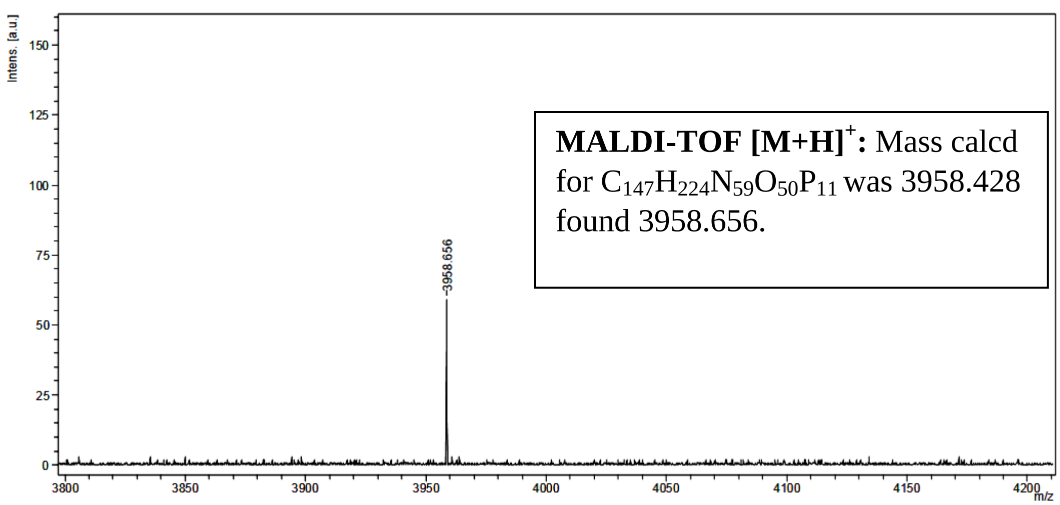
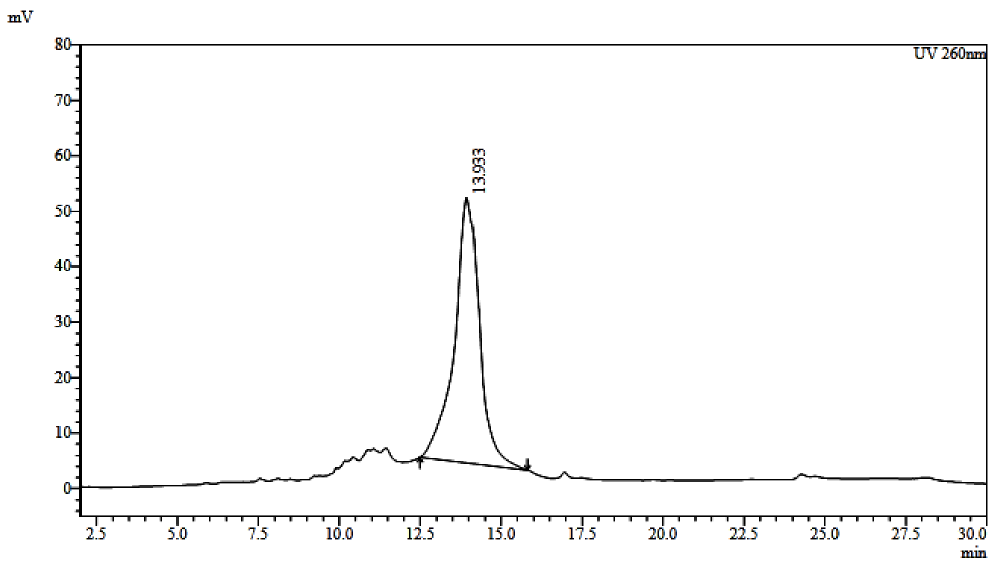


Figure S13: (A) HPLC Chromatogram of PMO 8 [5′-TTTTACT***C***ACAT-3′ **(C-Ph-Acetylene, 6b)],** (**B)** MALDI-TOF spectrum of PMO 8.

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	13.933	2578103	47793	100.000
Total		2578103	47793	100.000

(B)

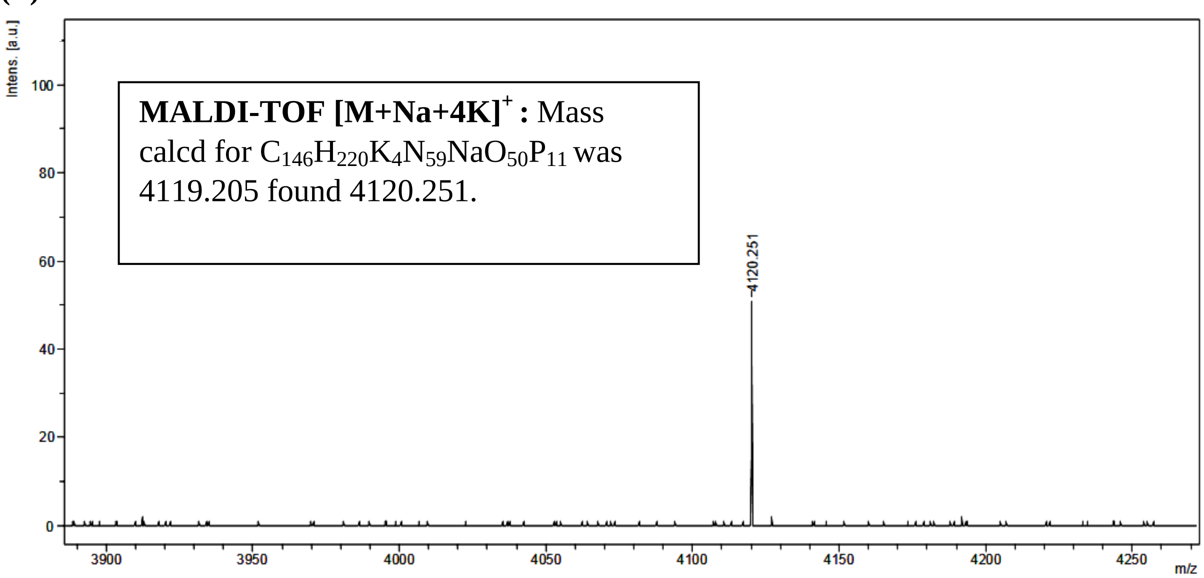
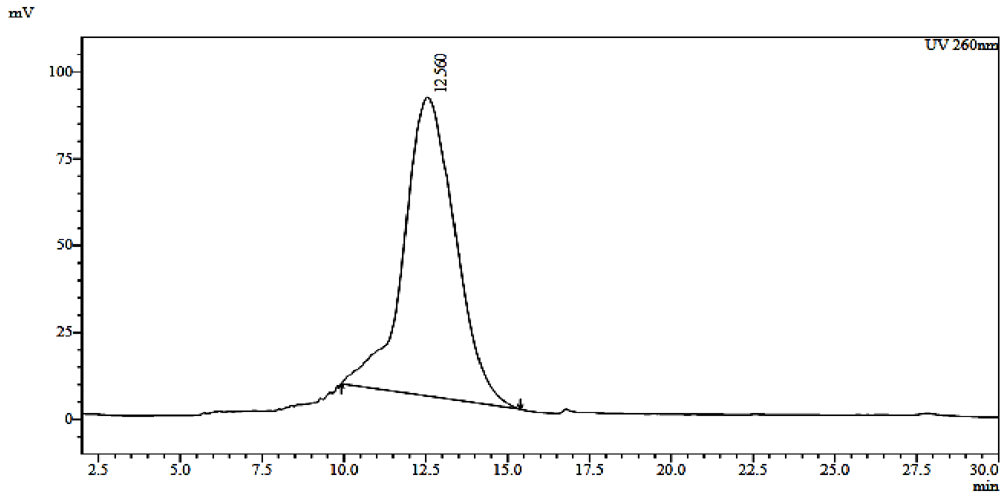


Figure S14: (A) HPLC Chromatogram of PMO 9 [5′-TTTTAC*U*CACAT-3′ **(U-Ph-Acetylene, 7c]**, (**B)** MALDI-TOF spectrum of PMO 9.

(A) Chromatogram



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	12.560	9456213	86038	100.000
Total		9456213	86038	100.000

(B)

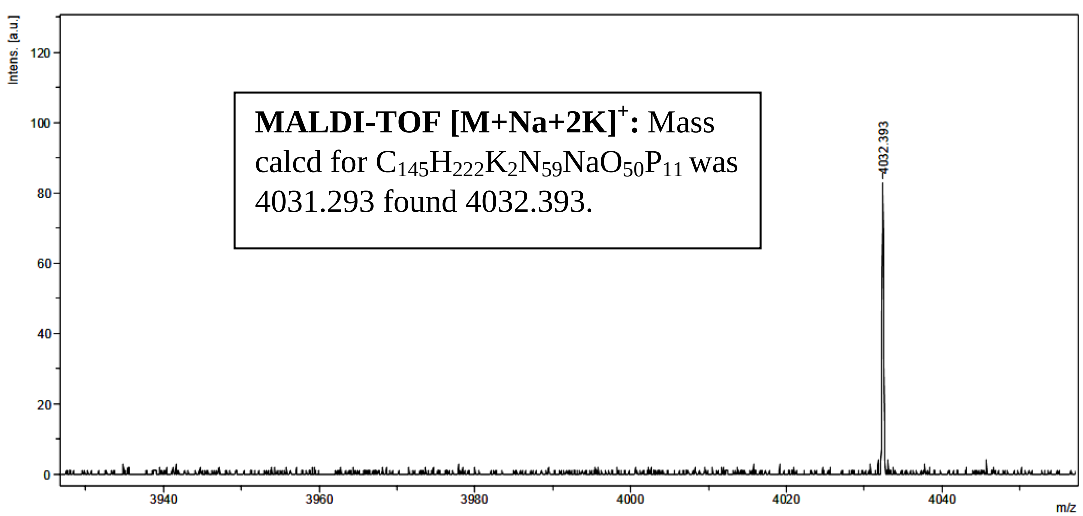
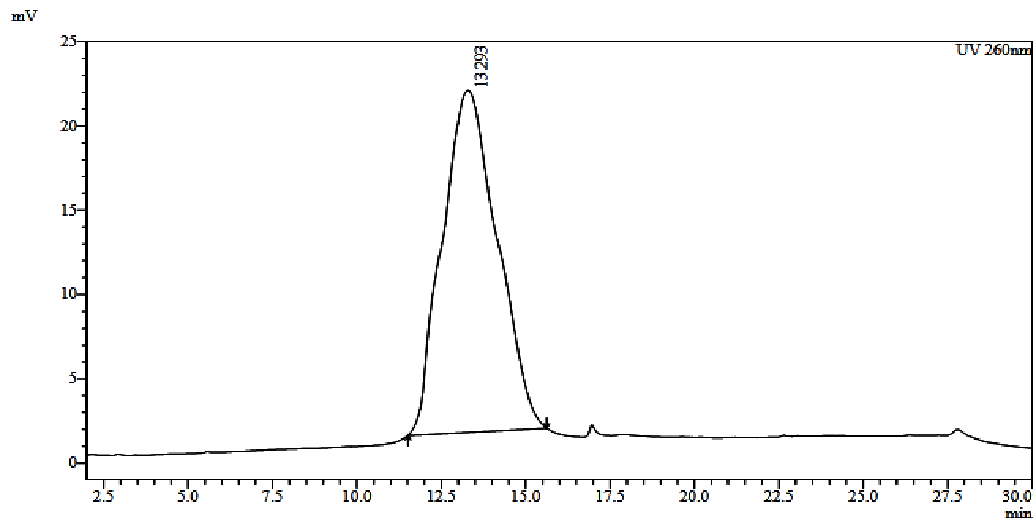


Figure S15: (A) HPLC Chromatogram of PMO 10 [5′-TTTTACT***C***ACAT-3′ **(C-Phenyl, 6j)]**, (**B**) MALDI-TOF spectrum of PMO 10.

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	13.293	2292479	20285	100.000
Total		2292479	20285	100.000

(B)

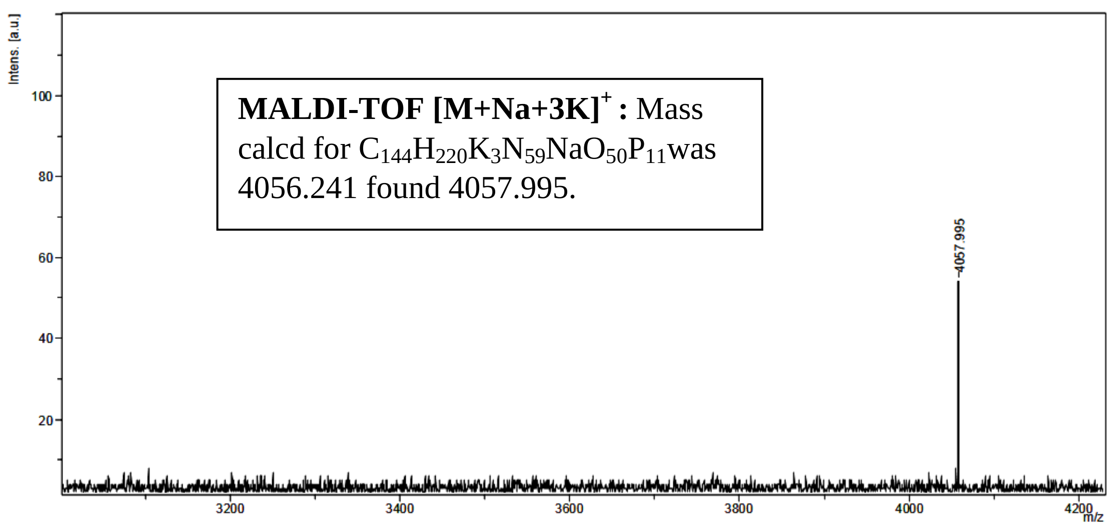
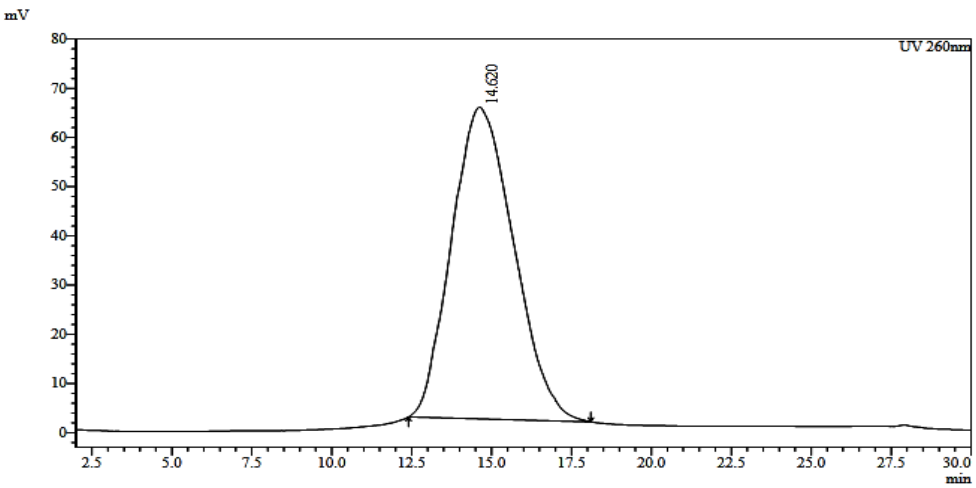


Figure S16: (A) HPLC Chromatogram of PMO 11 [5′-TTTTAC*U*CACAT-3′ **(U-Phenyl, 7d**], (**B)** MALDI-TOF spectrum of PMO 11.

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	14.620	8594840	63377	100.000
Total		8594840	63377	100.000

(B)

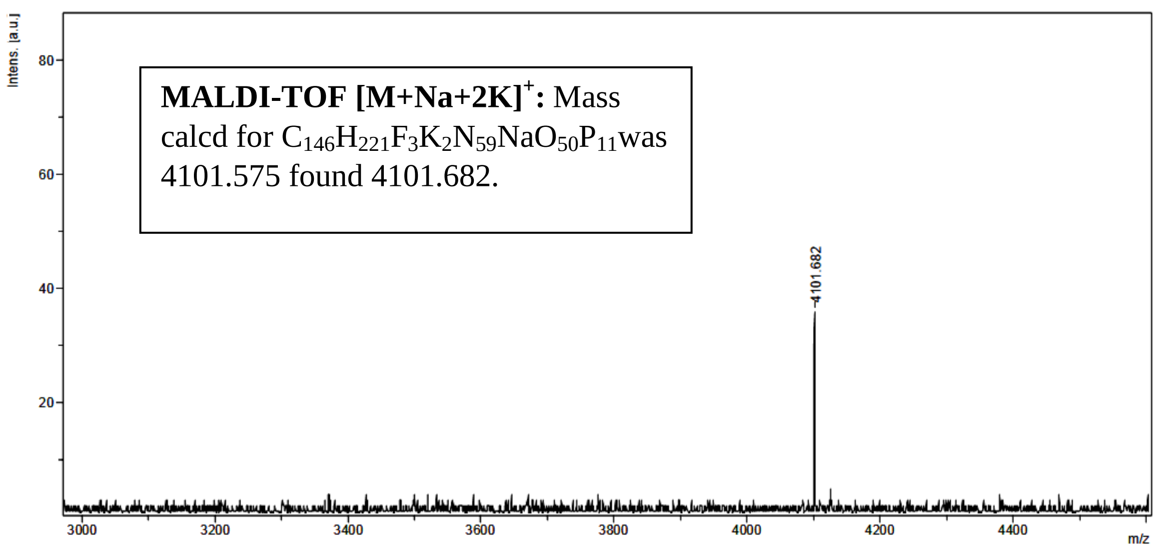
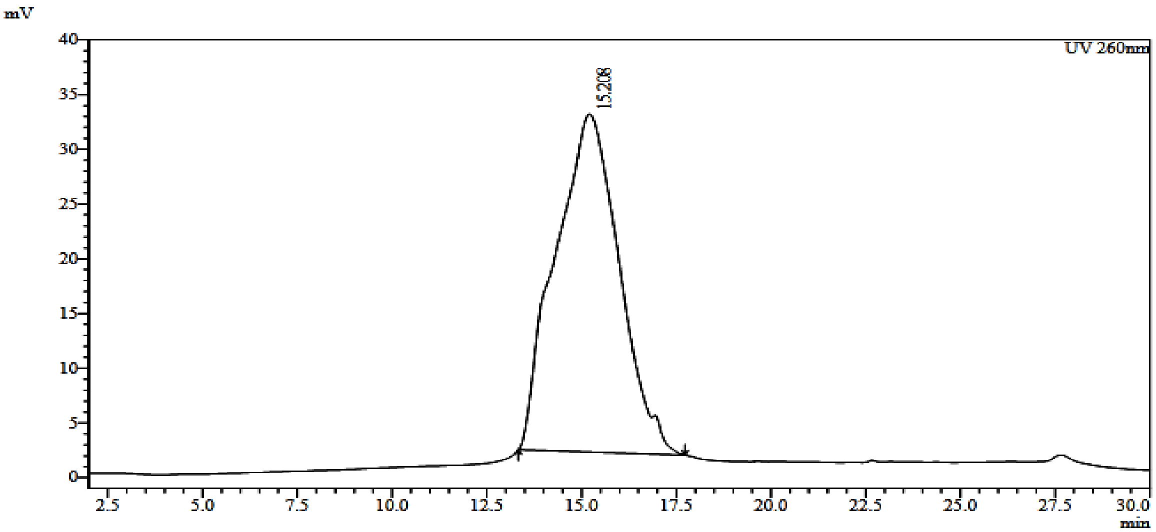


Figure S17: (A) HPLC Chromatogram of PMO 12 [5′-TTTTACT***C***ACAT-3′ **(C-Phenyl-CF$_3$, 6e)], (B)** MALDI-TOF spectrum of PMO 12.

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	15.208	3610020	30860	100.000
Total		3610020	30860	100.000

(B)

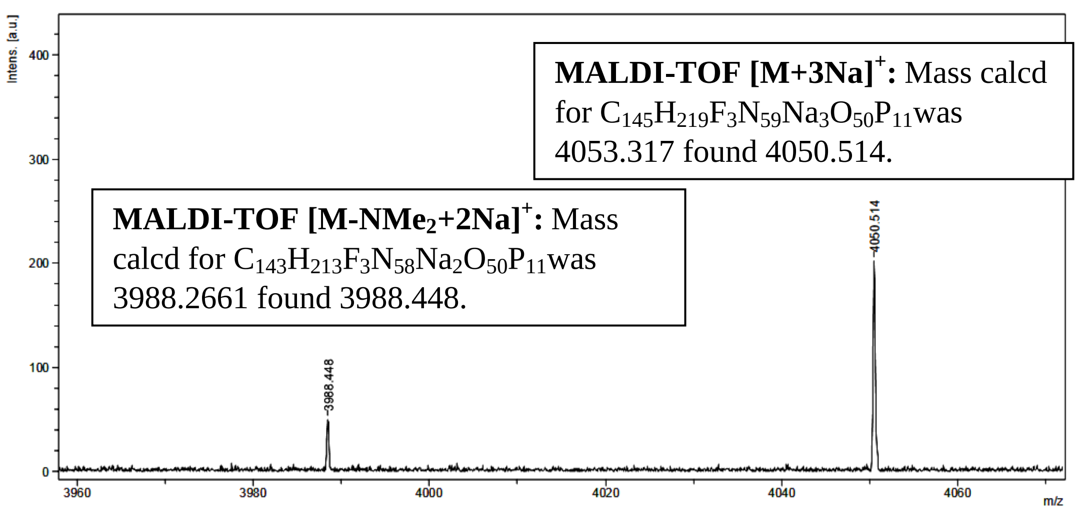
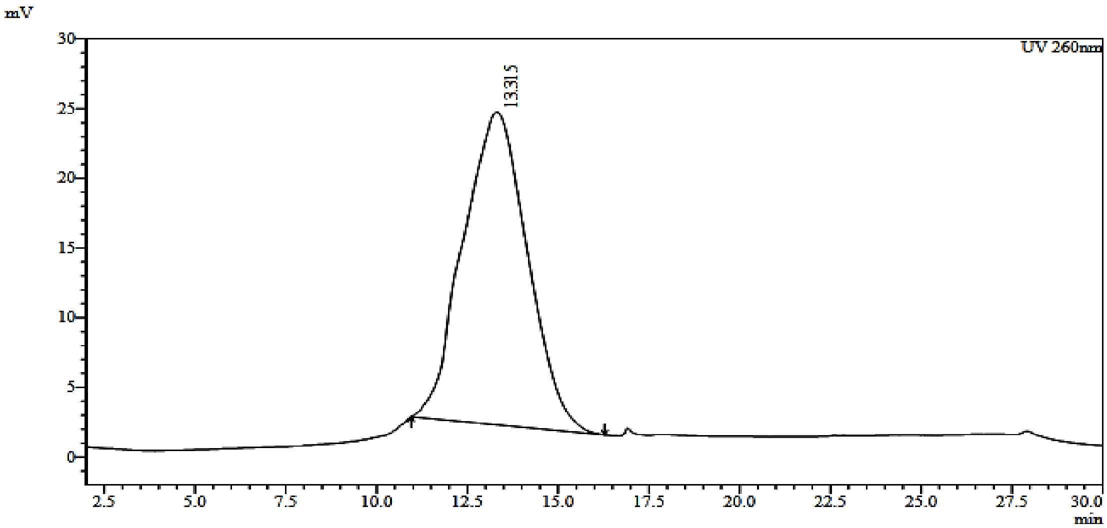


Figure S18: (A) HPLC Chromatogram of PMO 13 [5′-TTTTAC*U*CACAT-3′ **(U-Phenyl-CF$_3$, 7f)],** **(B)** MALDI-TOF spectrum of PMO 13.

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	13.315	2763215	22435	100.000
Total		2763215	22435	100.000

(B)

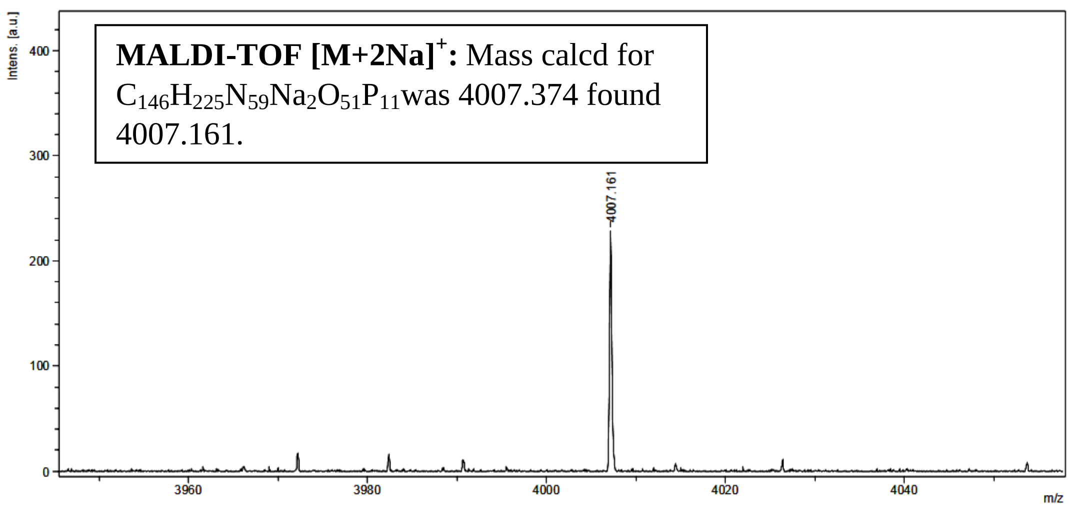
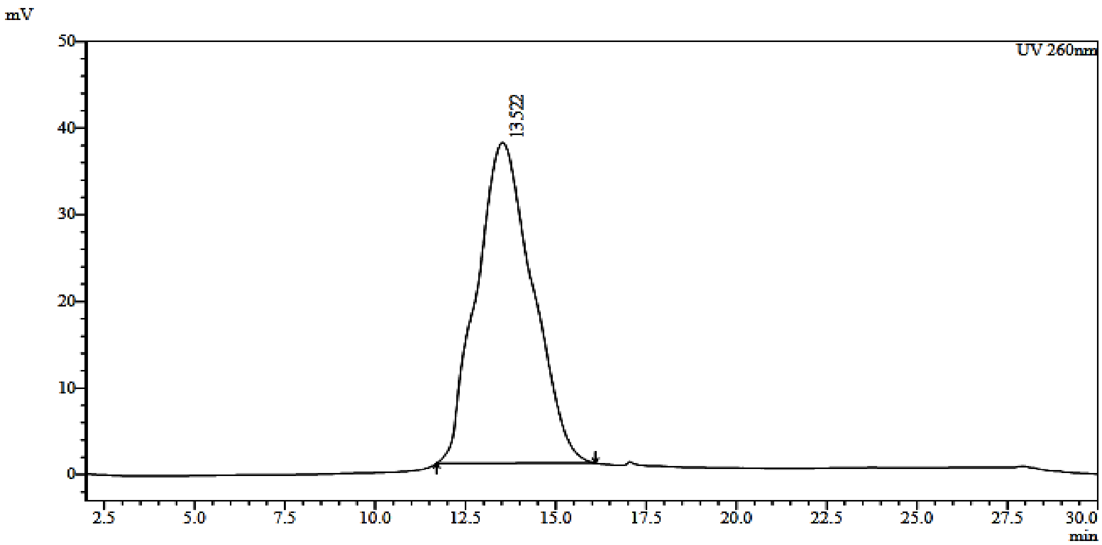


Figure S19: (A) HPLC Chromatogram of PMO 14 [5′-TTTTACT***C***ACAT-3′(**C-Phenyl-OMe, 6h)],** **(B)** MALDI-TOF spectrum of PMO 14.

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	13.522	3980289	36993	100.000
Total		3980289	36993	100.000

(B)

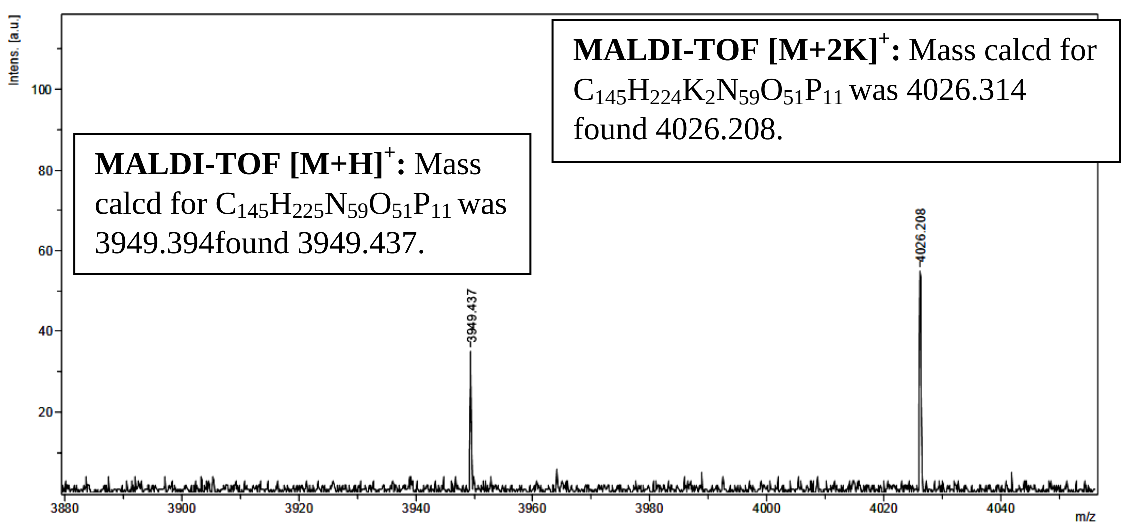
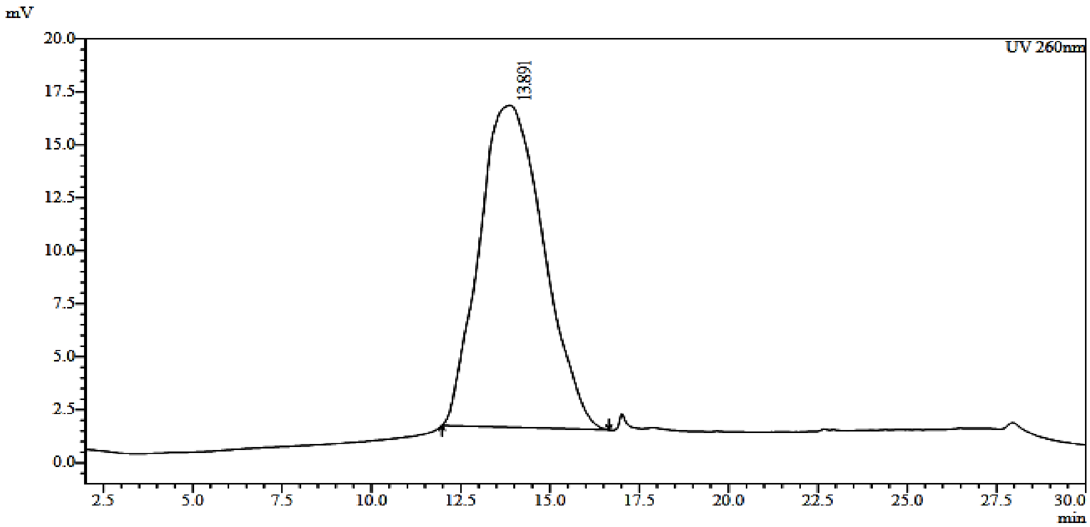


Figure S20: (A) HPLC Chromatogram of PMO 15 [5′-TTTTAC*U*CACAT-3′ **(U-Phenyl-OMe, 6e)], (B)** MALDI-TOF spectrum of PMO 15.

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	13.891	1917308	15188	100.000
Total		1917308	15188	100.000

(B)

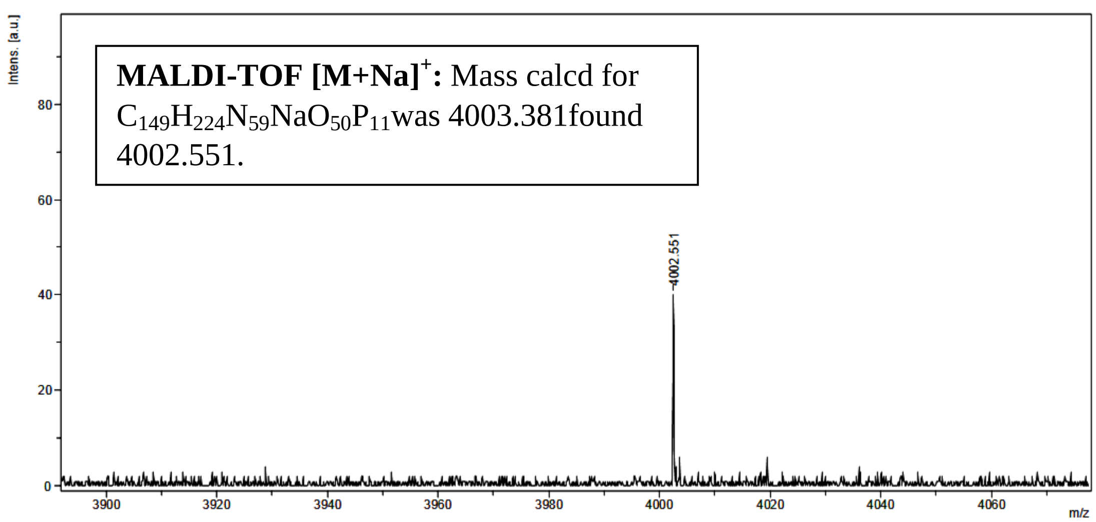
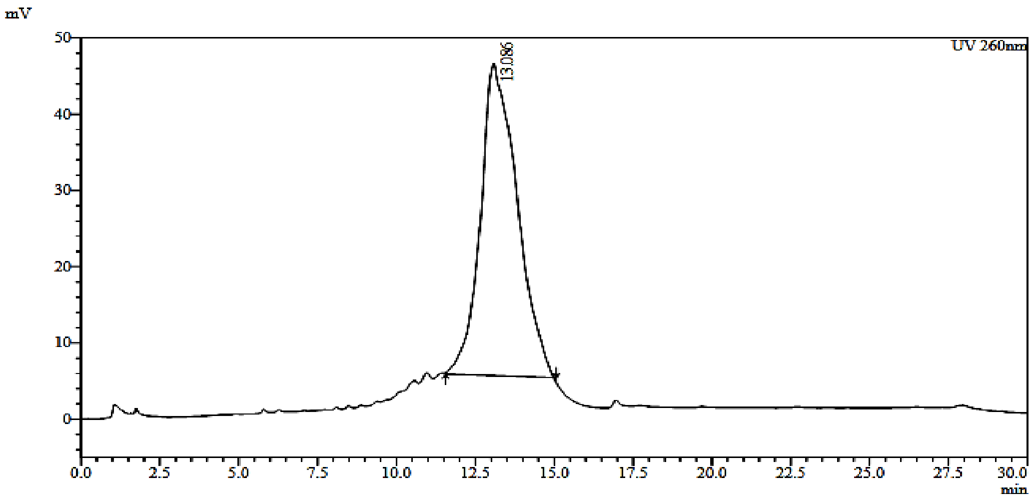


Figure S21: (A) HPLC Chromatogram of PMO 16 [5′-TTTTACT***C***ACAT-3′ **(C-Napthyl, 6g)], (B)** MALDI-TOF spectrum of PMO 16.

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	13.086	3323186	40984	100.000
Total		3323186	40984	100.000

(B)

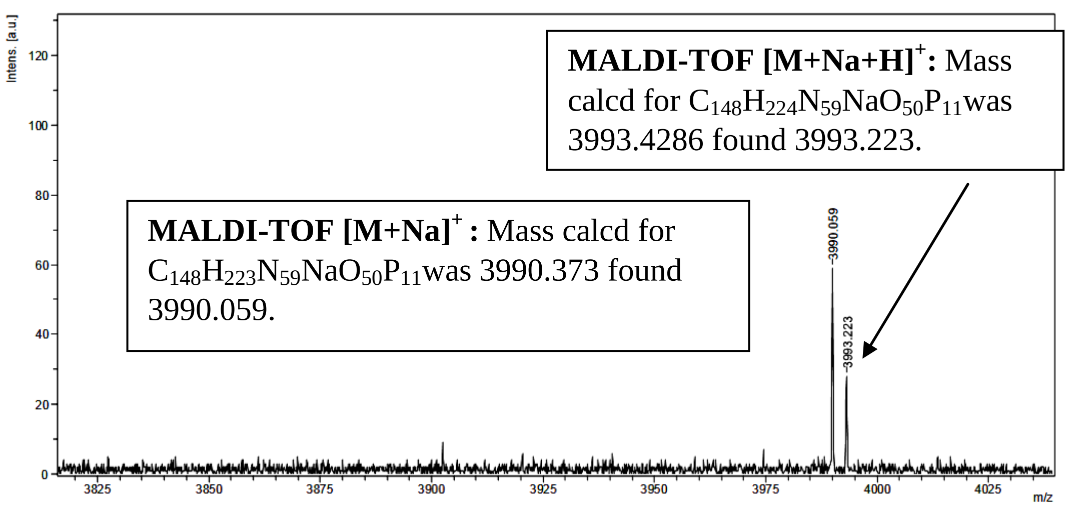
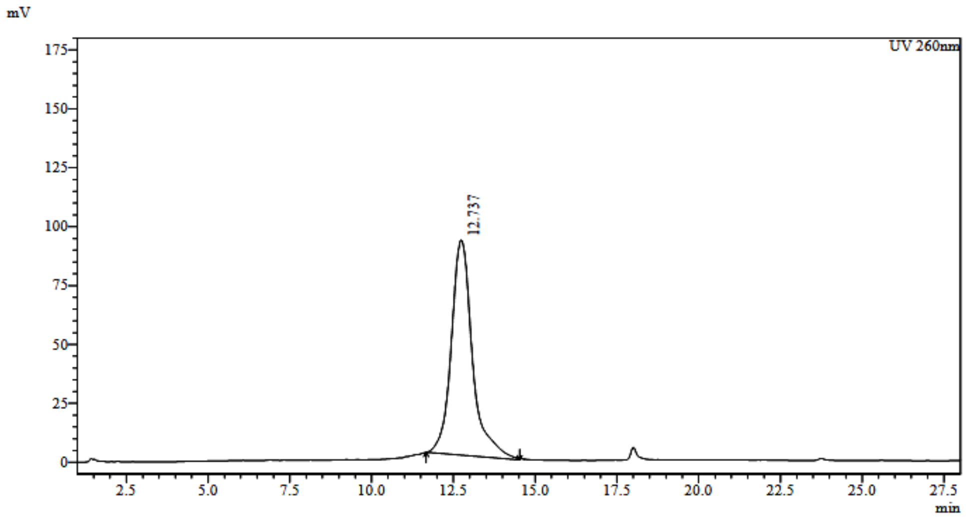


Figure S22: **(A)** HPLC Chromatogram of PMO 17 [5′-TTTTAC***U***CACAT-3′ **(U-Naphthyl, 7g)]**, **(B)** MALDI-TOF spectrum of PMO 17

Chromatogram

(A)



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	12.737	4113798	91256	100.000
Total		4113798	91256	100.000

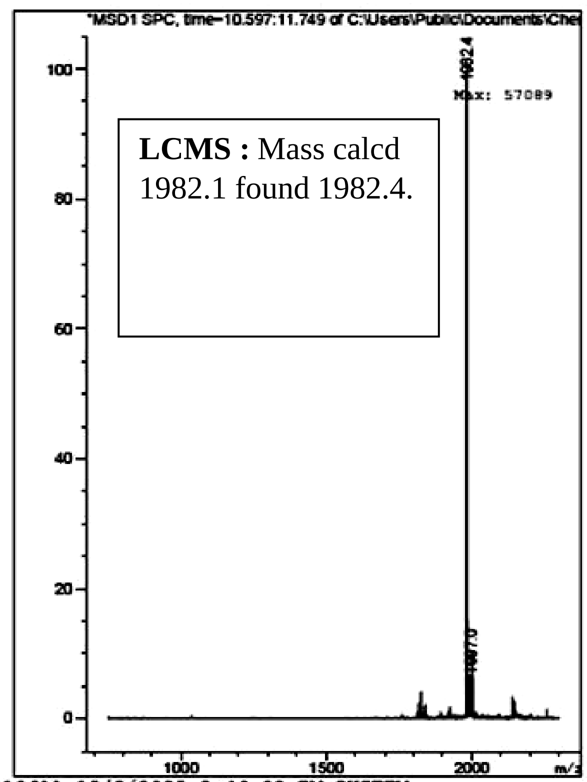
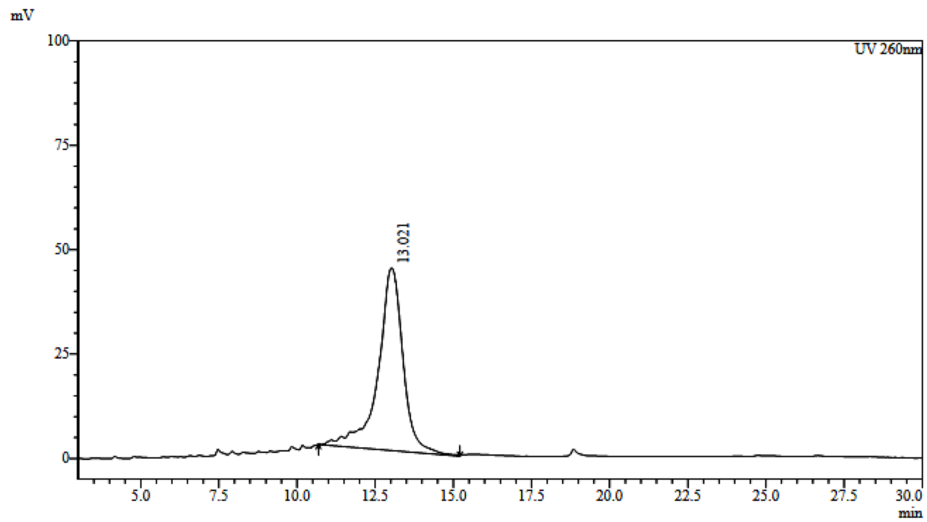


Figure S23: **(A)** HPLC Chromatogram of PMO 18 [5′-TTTTAC*U*CACAT-3′ **(U-iodo, 7i)]**, **(B)** LCMS-spectrum of PMO 18

Chromatogram

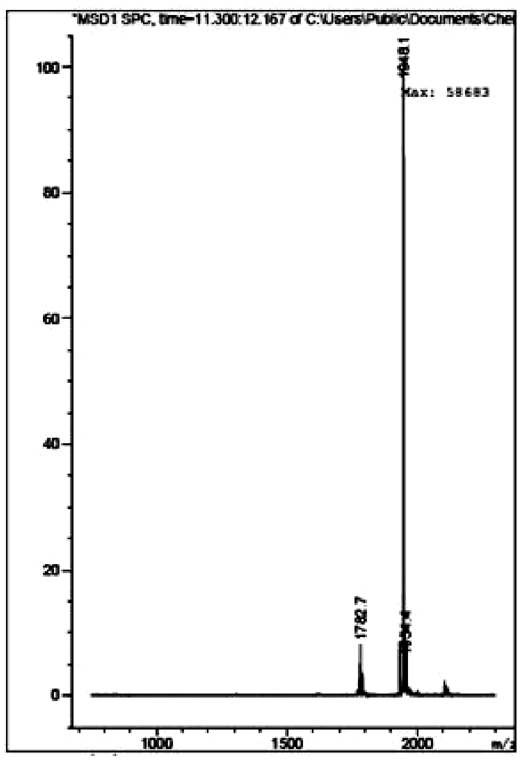
(A)



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	13.021	2432376	43722	100.000
Total		2432376	43722	100.000

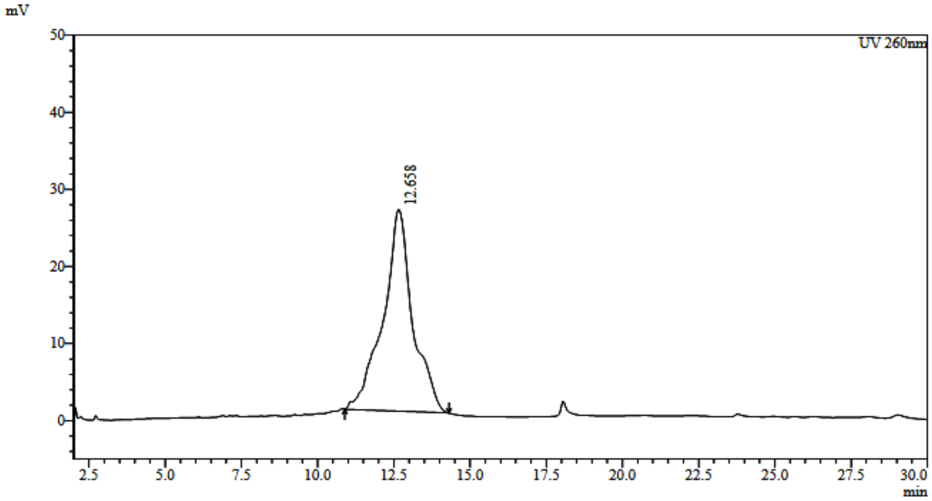


LCMS : Mass calcd was 1947.1 found 1948.1

Figure S24: **(A)** HPLC Chromatogram of PMO 19 [5′-TTTTACU***C***ACAT-3′ **(m5C, 6k)],**
(B) LCMS spectrum of PMO 19

Chromatogram

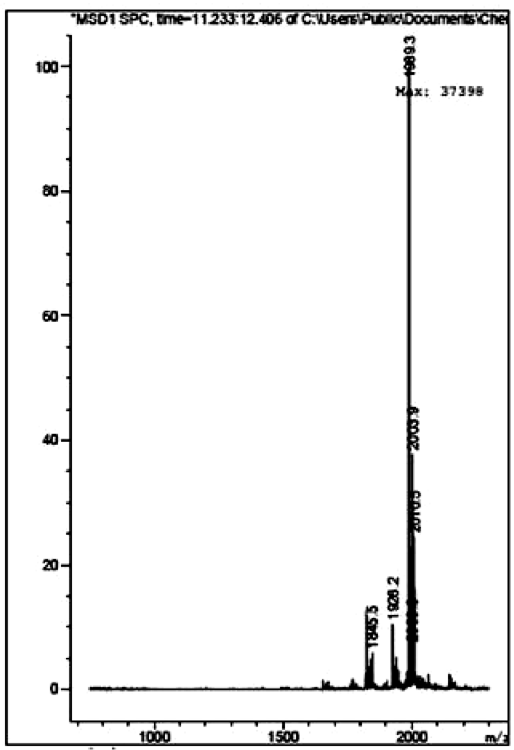
(A)



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	12.658	1764394	26170	100.000
Total		1764394	26170	100.000

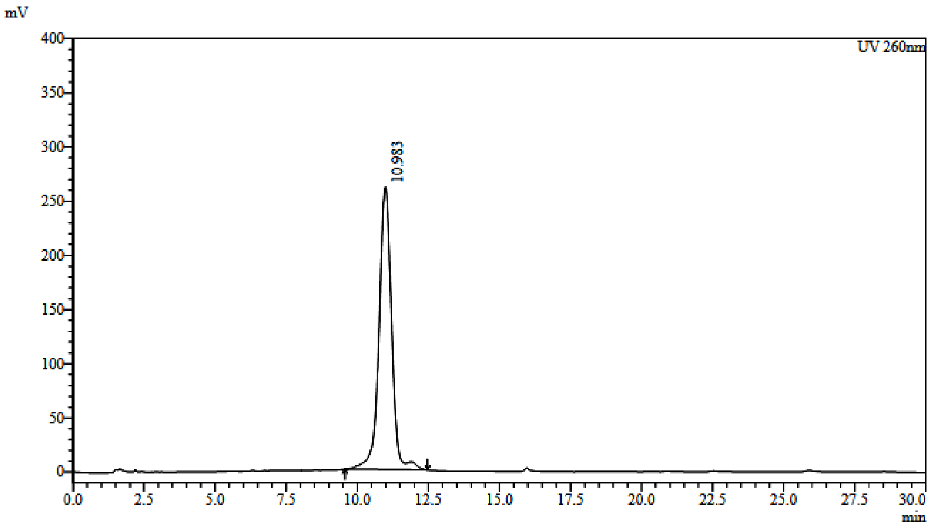


LCMS : Mass calcd was 1989.1 found 1989.3

Figure S25: **(A)** HPLC Chromatogram of PMO 20 [5′-TTTTACU***C***ACAT-3′ **(C-iodo, 6k)],**
(B) LCMS spectrum of PMO 20

(A)

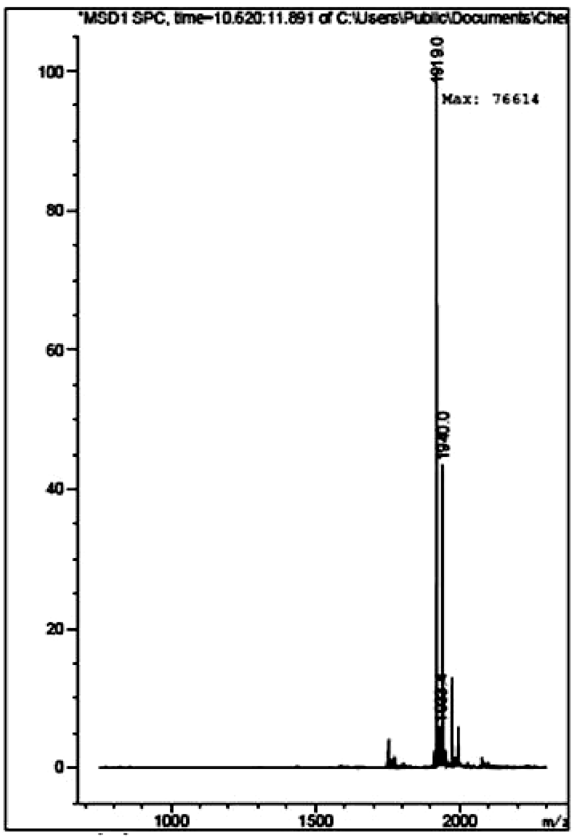
Chromatogram



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	10.983	8019065	260530	100.000
Total		8019065	260530	100.000

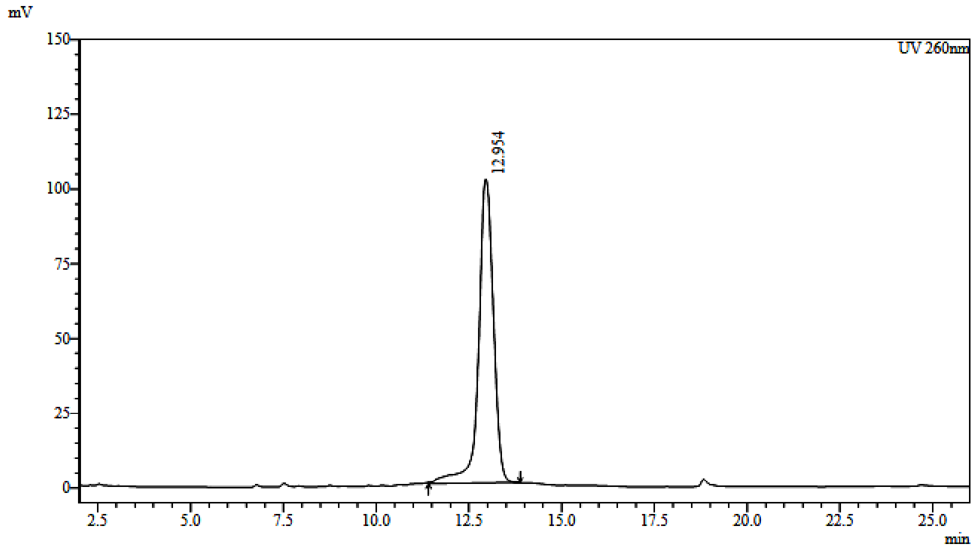


LCMS: Mass calcd was 1918.7 found 1919.0

Figure S26: **(A)** HPLC Chromatogram of PMO 21 [5′-TTTTACCCACAT-3′ **(Single mismatch regular PMO)]**, **(B)** LCMS spectrum of PMO 21

(A)

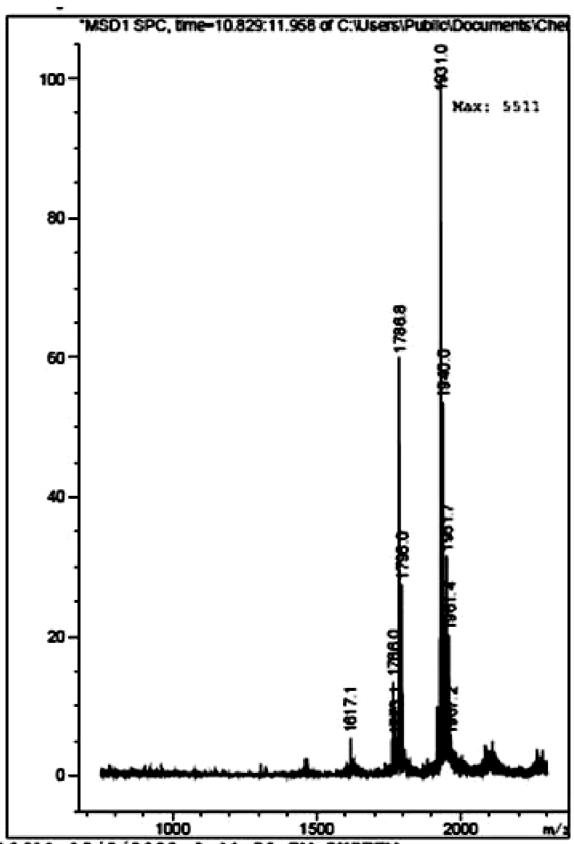
Chromatogram



Peak Table

UV 260nm

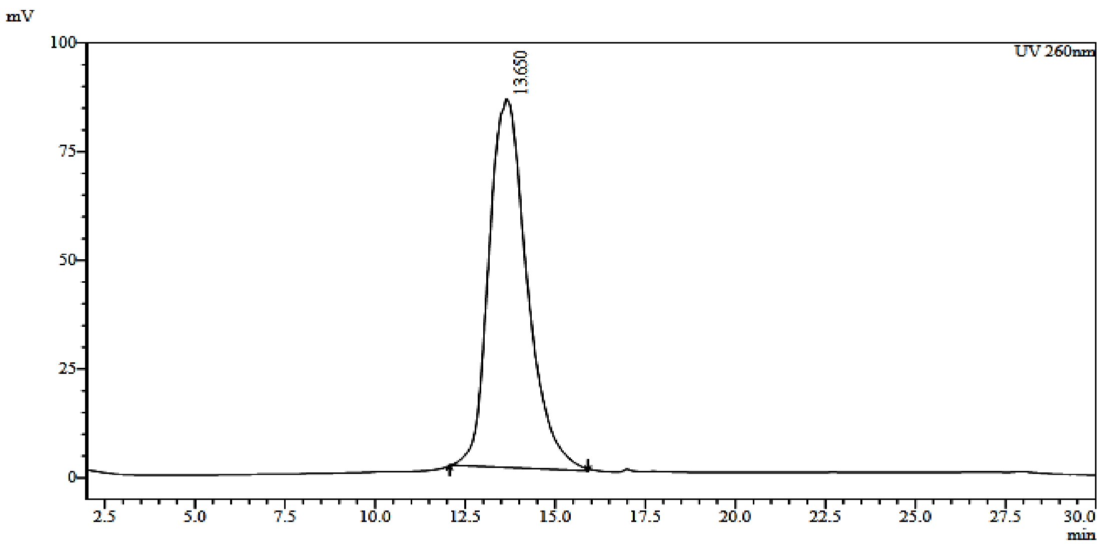
Peak#	Ret. Time	Area	Height	Area%
1	12.954	2814128	101620	100.000
Total		2814128	101620	100.000



LCMS: Mass calcd was 1930.7 found 1931.0

Figure S27: **(A)** HPLC Chromatogram of PMO 22 [5′-TTTTAC<u>*C*</u>CACAT-3′ **(Single mismatch C-acetylene, 6a)]**, **(B)** LCMS spectrum of PMO 22

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	13.650	6242974	84763	100.000
Total		6242974	84763	100.000

(B)

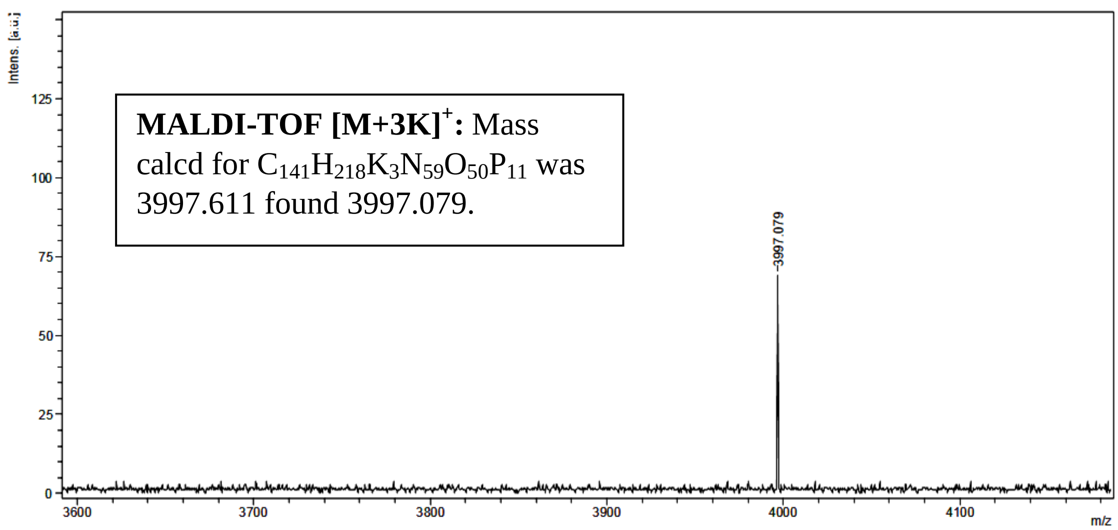
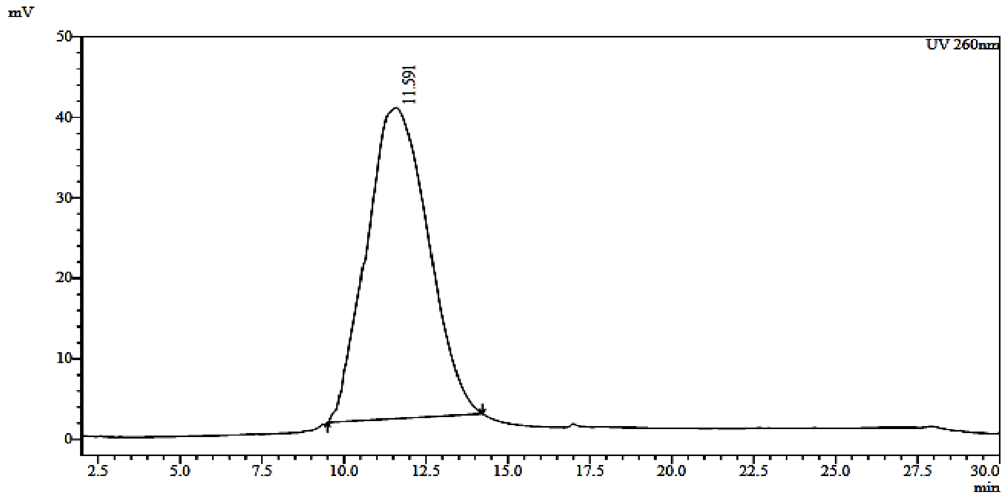


Figure S28: **(A)** HPLC Chromatogram of PMO 23 [5′-TTTTAC***C***TACAT-3′ **(C-Acetylene, 6a)],** **(B)** MALDI-TOF spectrum of PMO 23

(A) **Chromatogram**



Peak Table

UV 260nm

Peak#	Ret. Time	Area	Height	Area%
1	11.591	5062785	38591	100.000
Total		5062785	38591	100.000

(B)

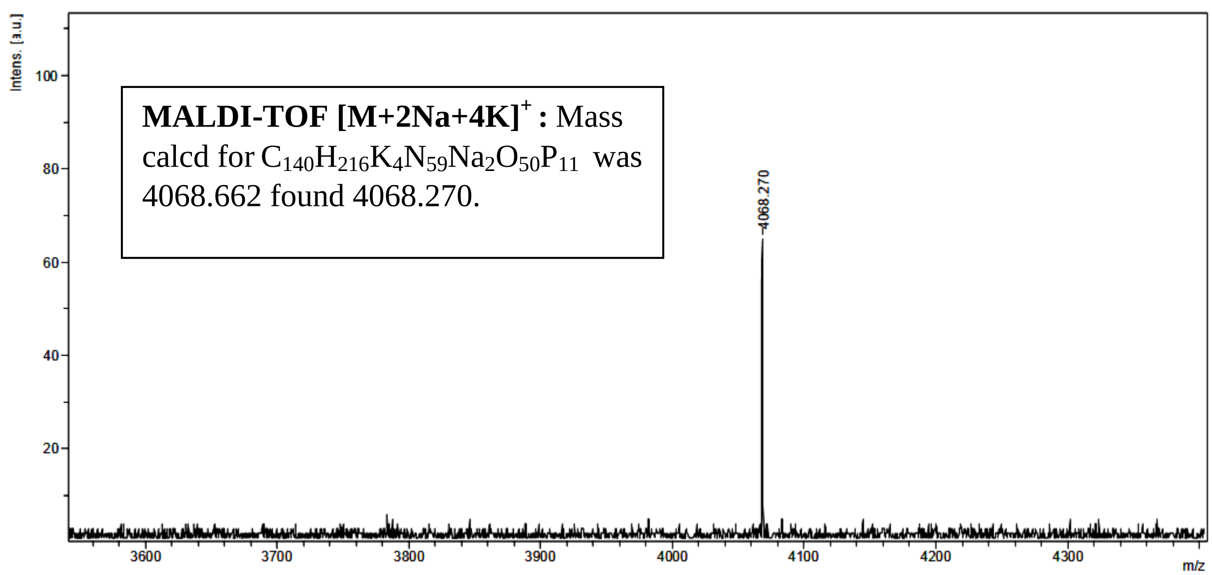
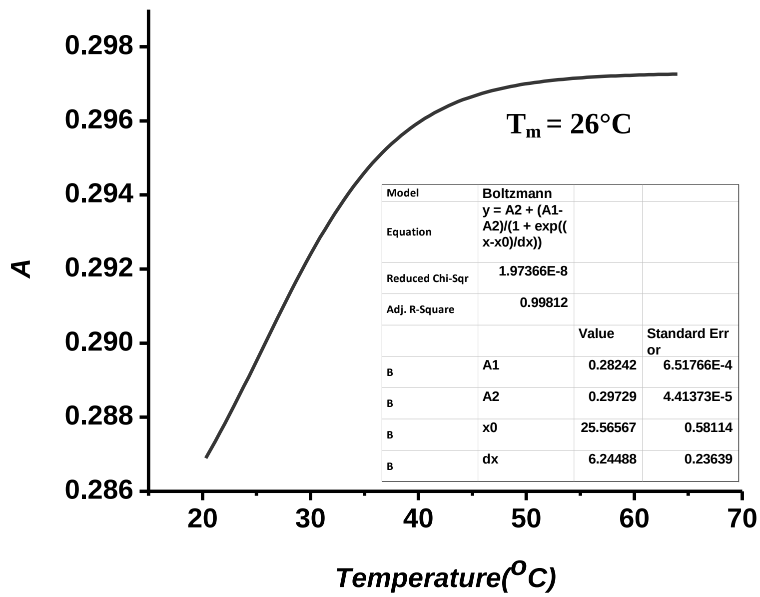


Figure S29: **(A)** HPLC Chromatogram of PMO 24 [5′-TTTTACC***U***ACAT-3′ **(U-Acetylene, 7b)],** **(B)** MALDI-TOF spectrum of PMO 24.

8. Melting temperature Curves

(A) **5′-T-T-T-T-A-C-T-C-A-C-A-T-3′**

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



(B)

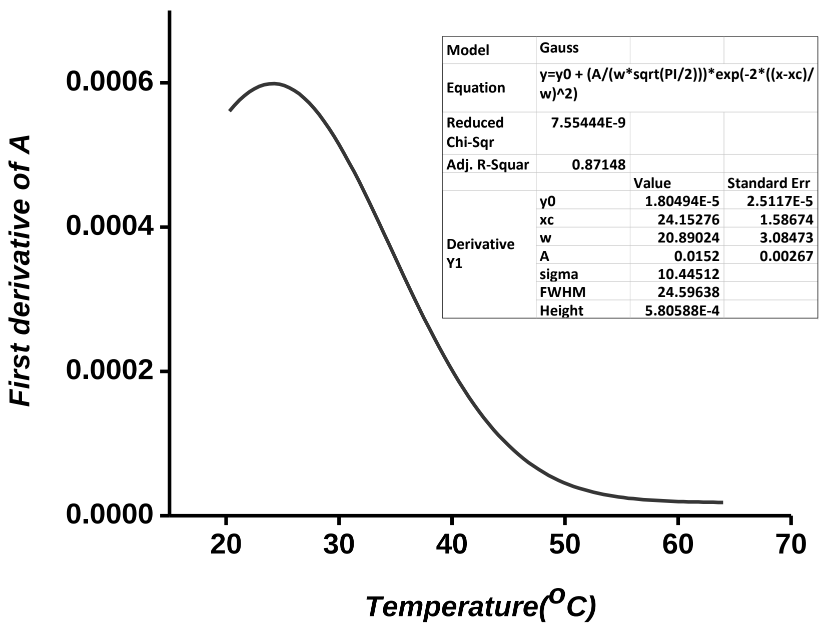
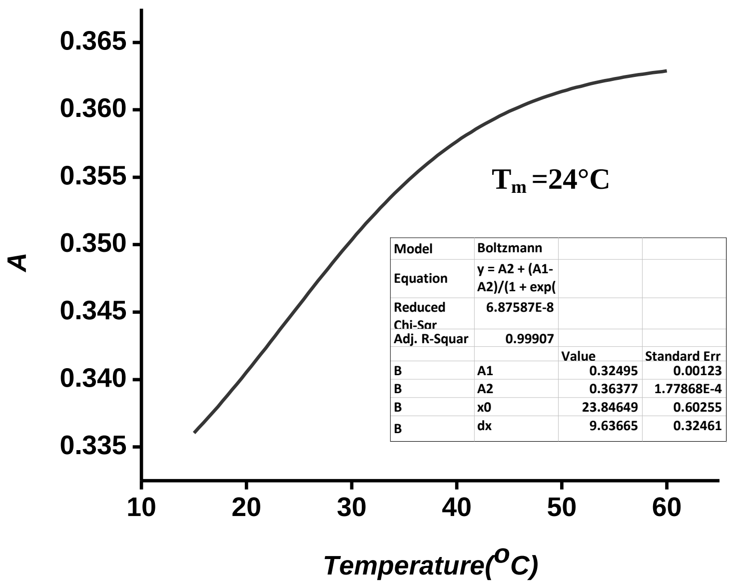


Figure S30: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO1-DNA **(B)** First derivative plot PMO1-DNA

(A)

5′-T-T-T-T-A-C-T-C-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

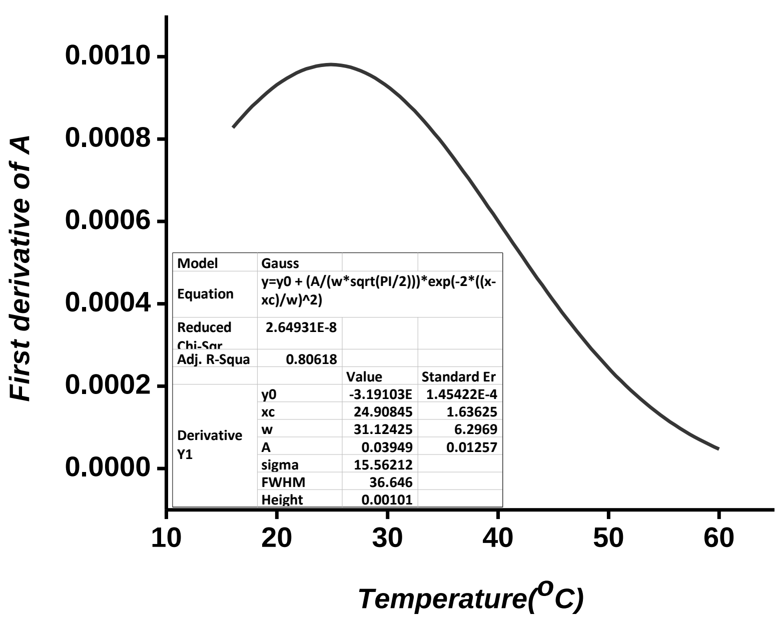
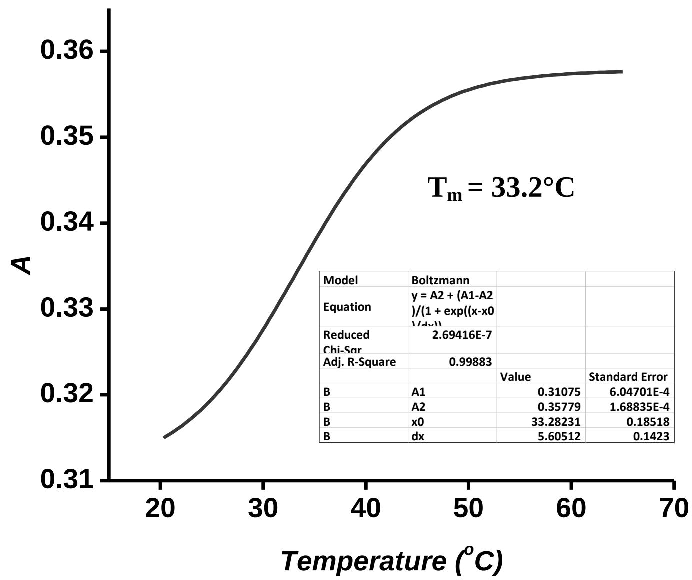


Figure S31: Thermal melting curve of duplexes in 40 mM phosphate buffer (pH = 7) **(A)** PMO1-RNA **(B)** First derivative plot PMO1-RNA

(A) 5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

$3'\text{-}A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d\text{-}5'$



(B)

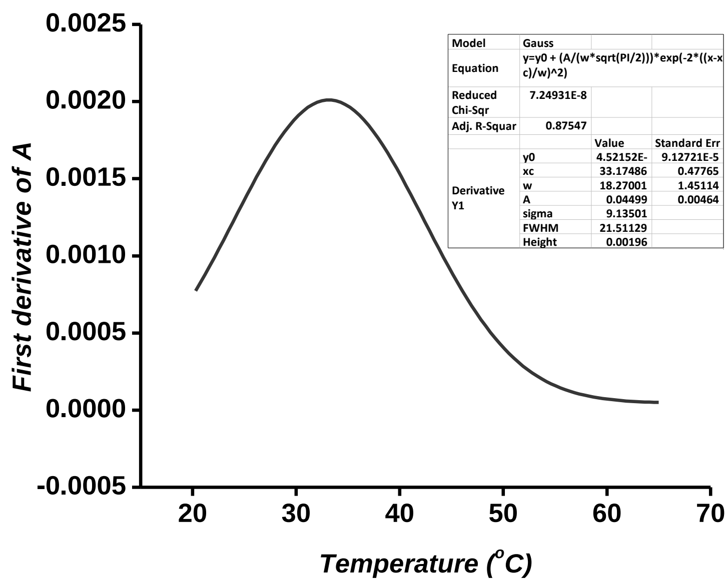
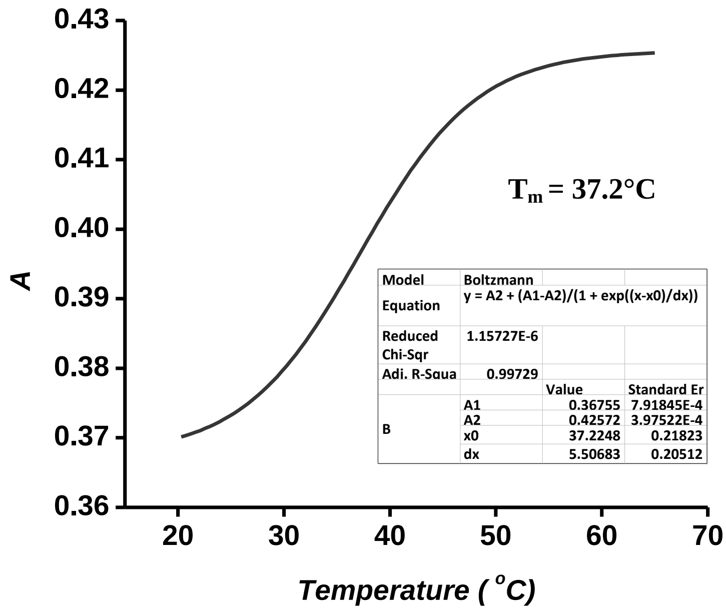


Figure S32: Thermal melting curve of duplexes in 40 mM phosphate buffer (pH = 7) **(A)** PMO2-DNA **(B)** First derivative plot PMO2-DNA

(A)

5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

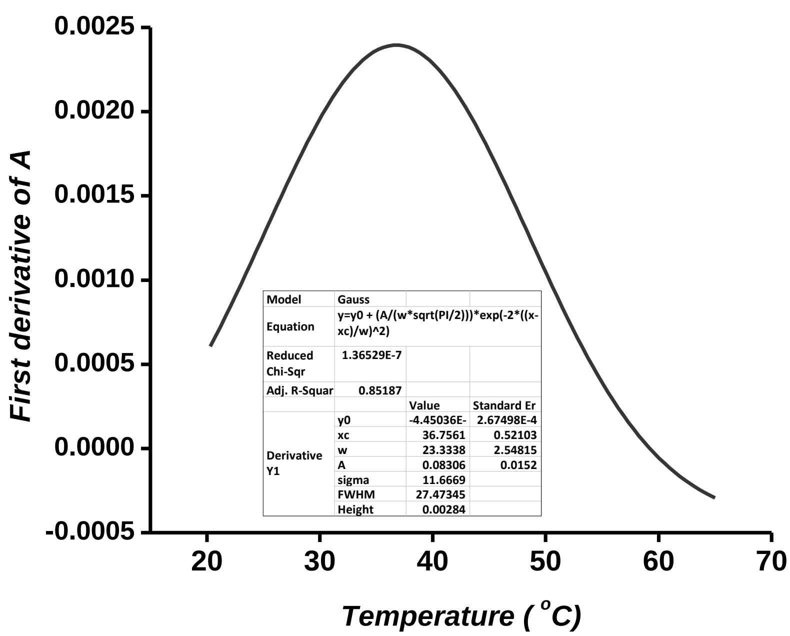
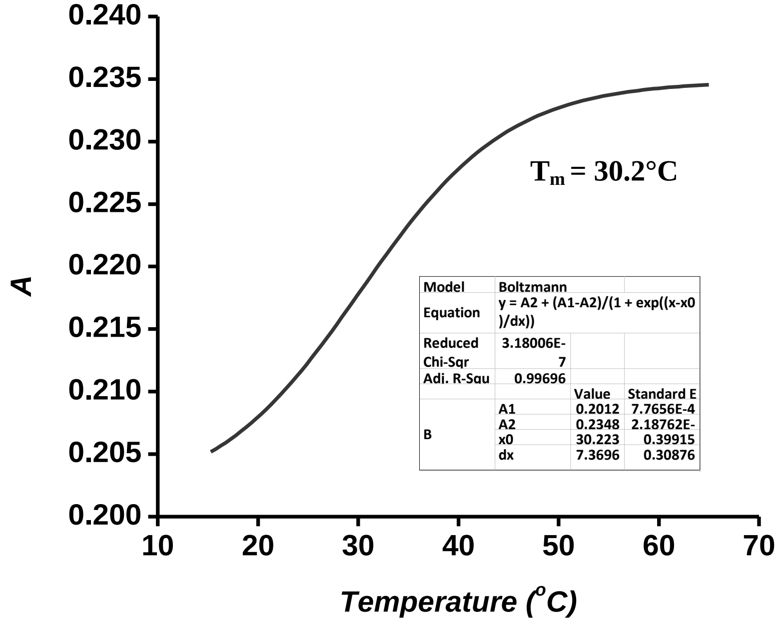


Figure S33: Thermal melting curve of duplexes in 40 mM phosphate buffer (pH = 7) **(A)** PMO2-RNA **(B)** First derivative plot PMO2-RNA

(A) 5′-T-T-T-T-A-C-***U***-C-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



(B)

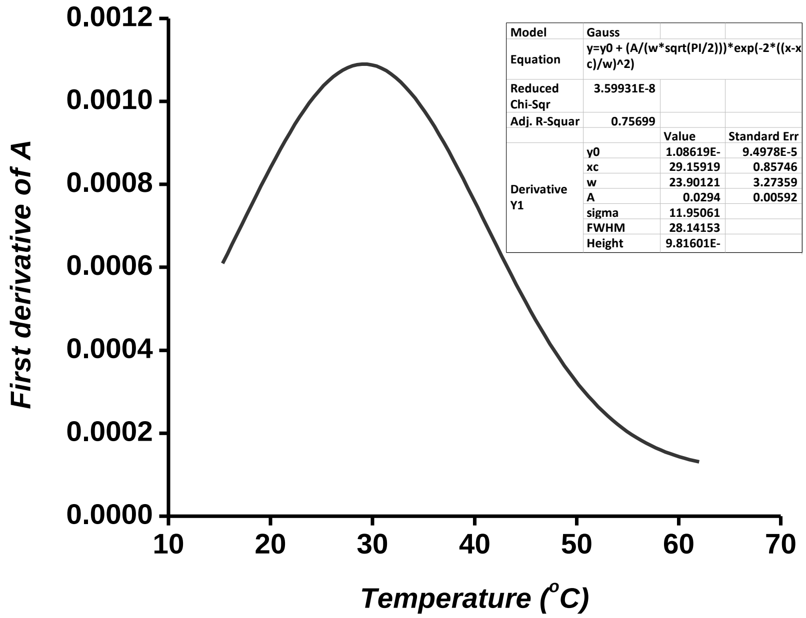
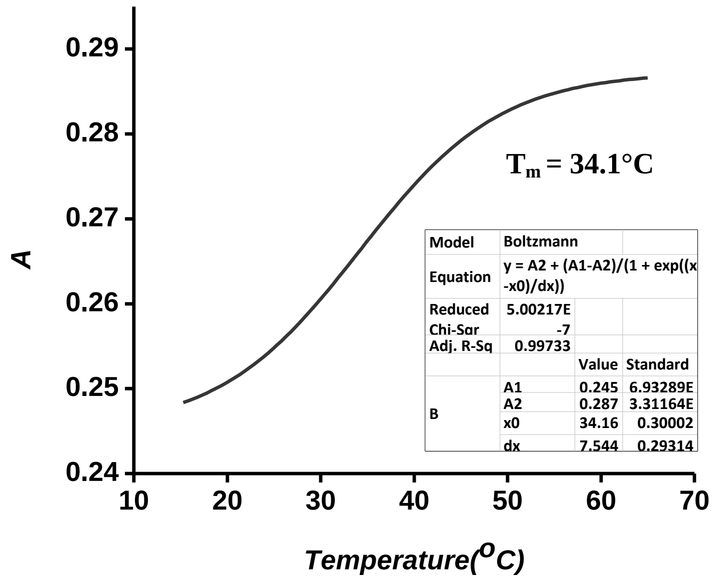


Figure S34: Thermal melting curve of duplexes in 40 mM phosphate buffer (pH = 7) **(A)** PMO3-DNA **(B)** First derivative plot PMO3-DNA

5′-T-T-T-T-A-C-***U***-C-A-C-A-T-3′

(A) 3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

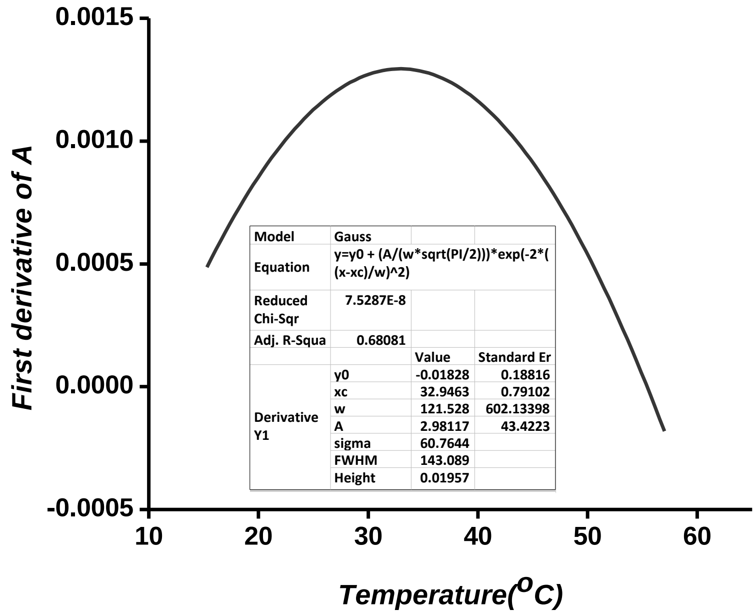
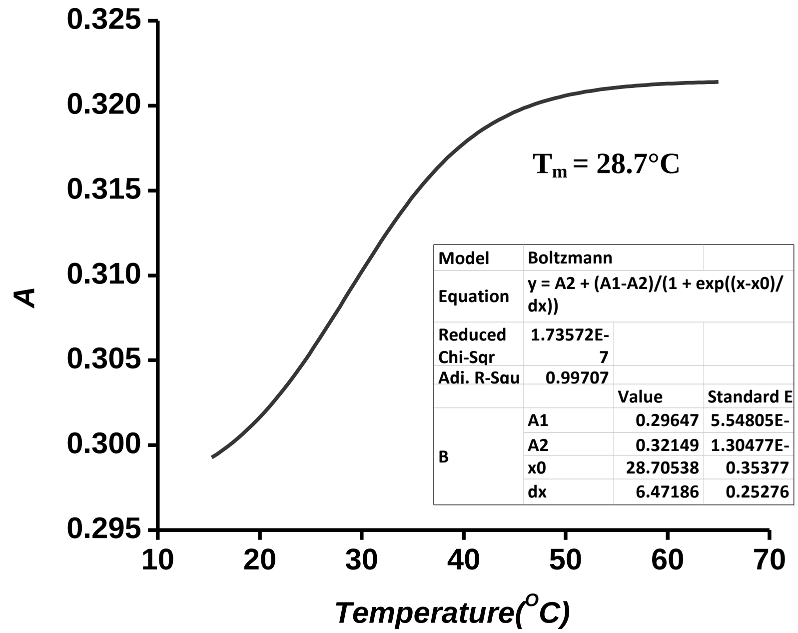


Figure S35: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO3-RNA **(B)** First derivative plot PMO3-RNA

(A) 5′-T-***U***-T-T-A-C-T-C-A-C-A-T-3′

$3'\text{-}A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d\text{-}5'$



(B)

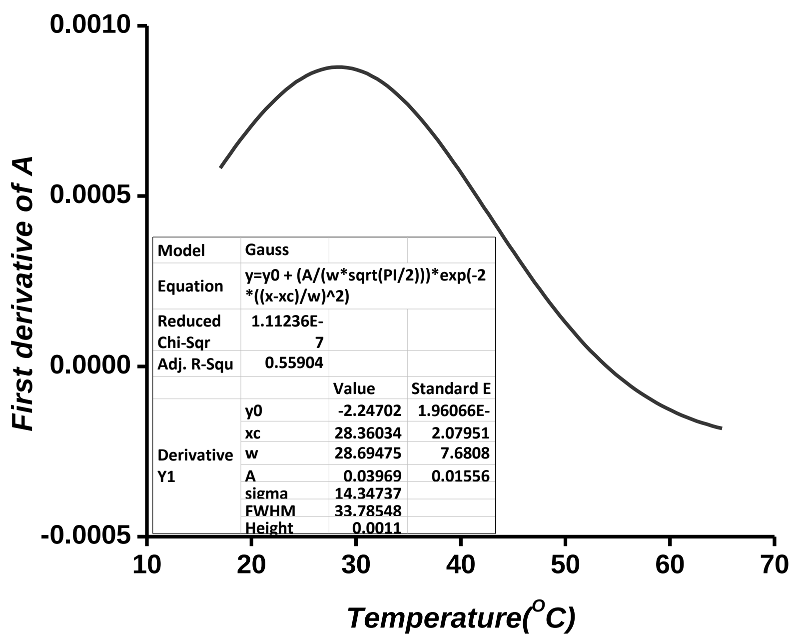
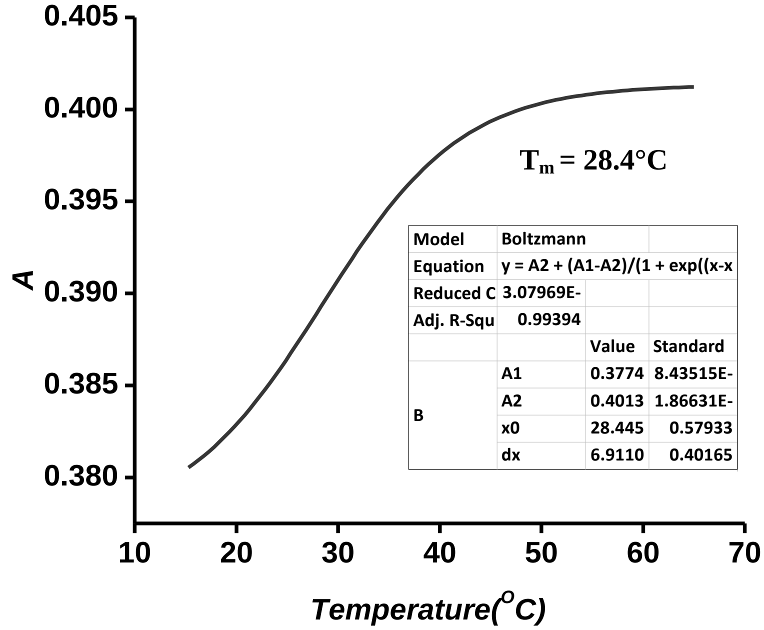


Figure S36: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO4-DNA **(B)** First derivative plot PMO4-DNA

(A)

5′-T-***U***-T-T-A-C-T-C-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

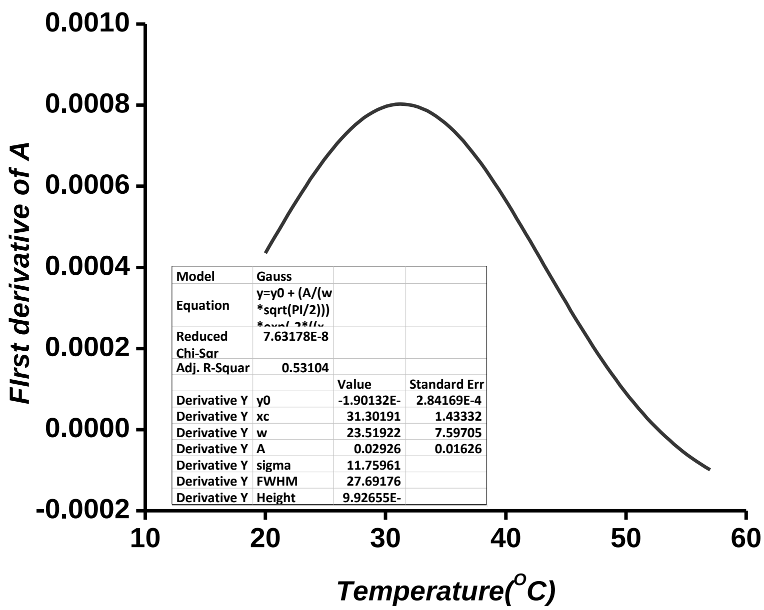
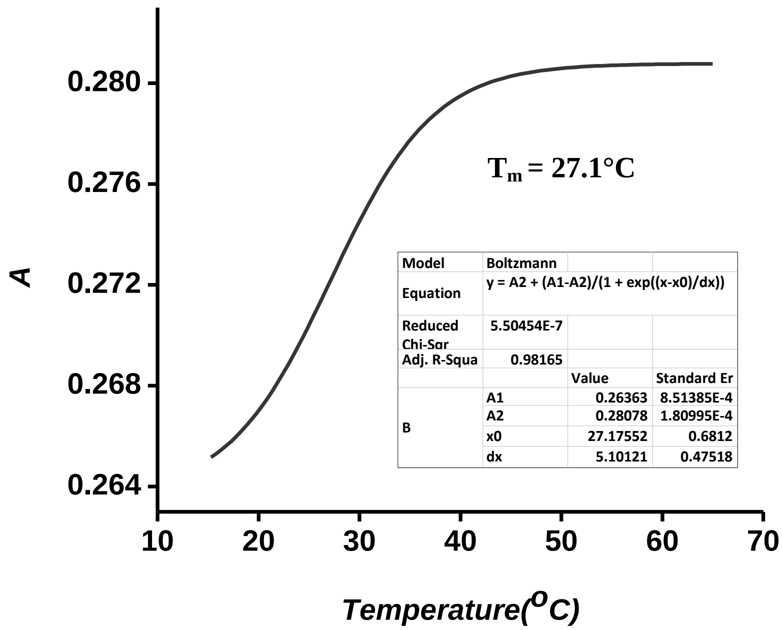


Figure S37: Thermal melting curve of duplexes in 40 mM phosphate buffer (pH = 7) **(A)** PMO4-RNA **(B)** First derivative plot PMO4-RNA

(A) 5′-T-***U***-T-T-A-C-T-***C***-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



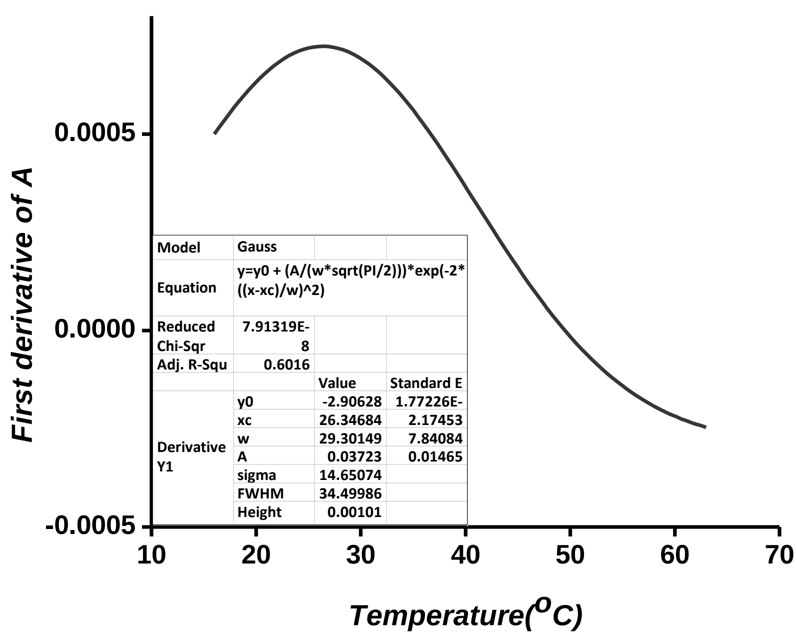
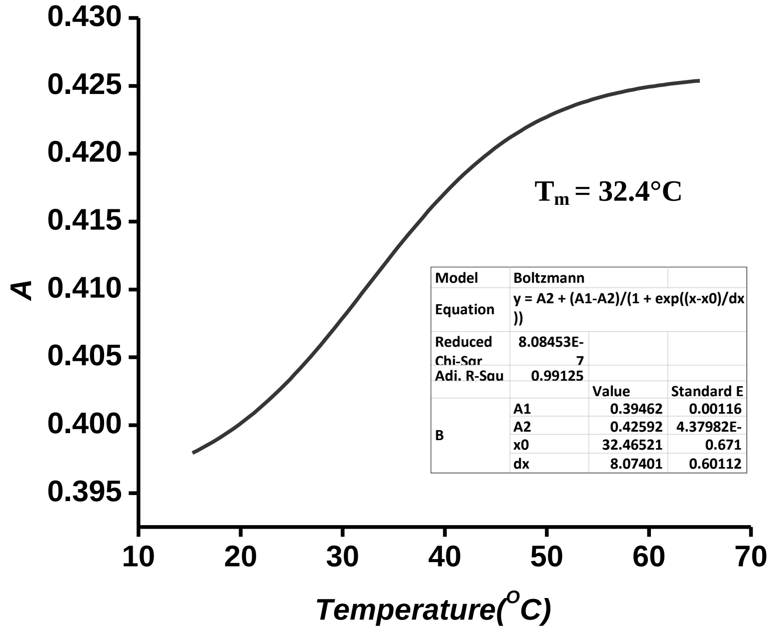


Figure S38: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO5-DNA **(B)** First derivative plot PMO5-DNA

(B)

5′-T-***U***-T-T-A-C-T-***C***-A-C-A-T-3′

$3'\text{-}A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r\text{-}5'$



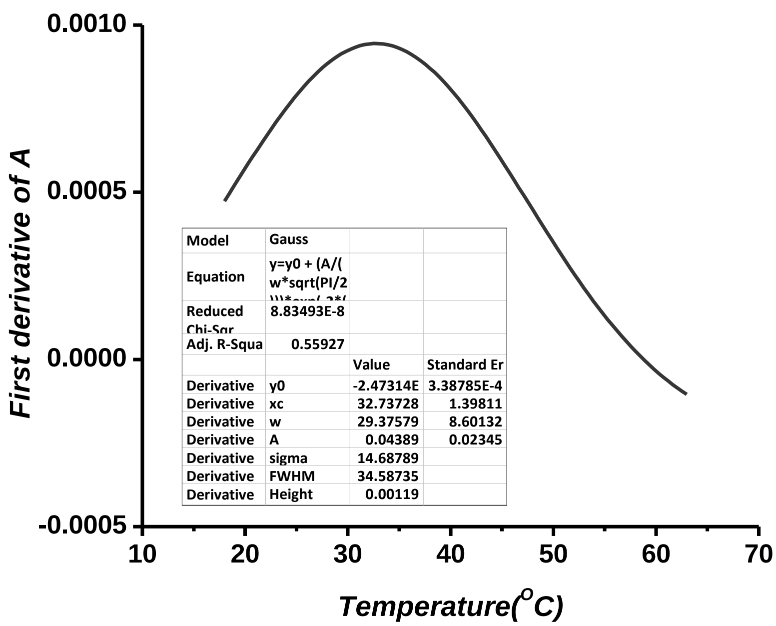
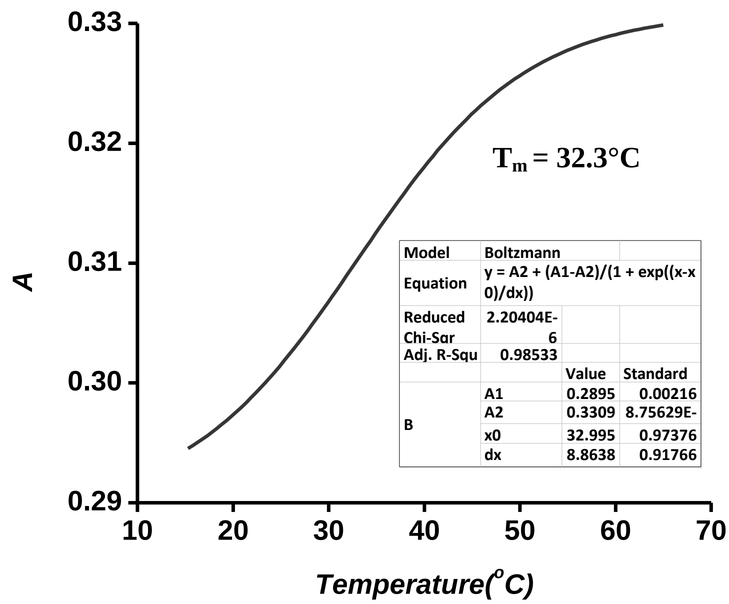


Figure S39: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO5-RNA **(B)** First derivative plot PMO5-RNA

(A)

5′-T-T-T-T-A-C-***U***-***C***-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



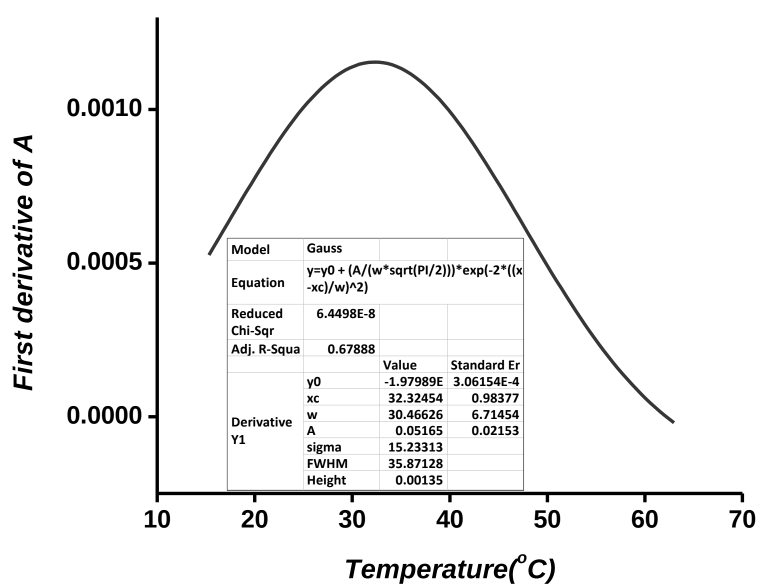
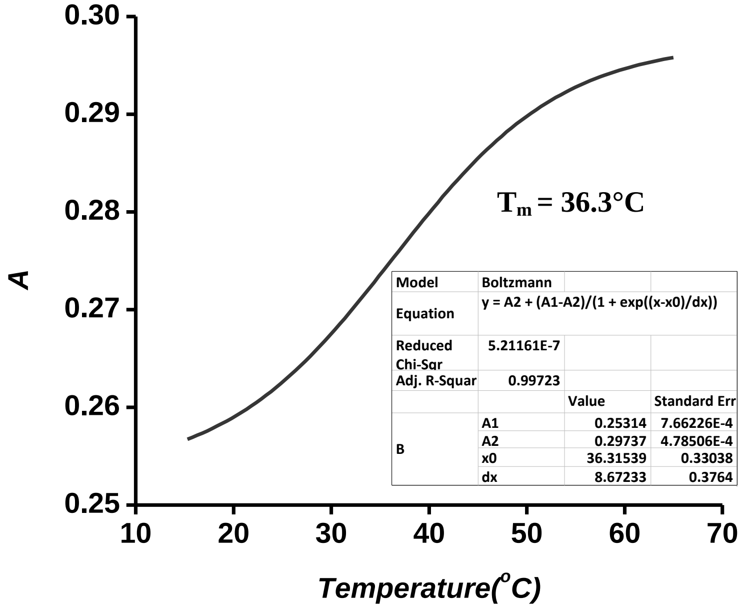


Figure S40: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO6-DNA **(B)** First derivative plot PMO6-DNA

(B) 5′-T-T-T-T-A-C-***U***-***C***-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



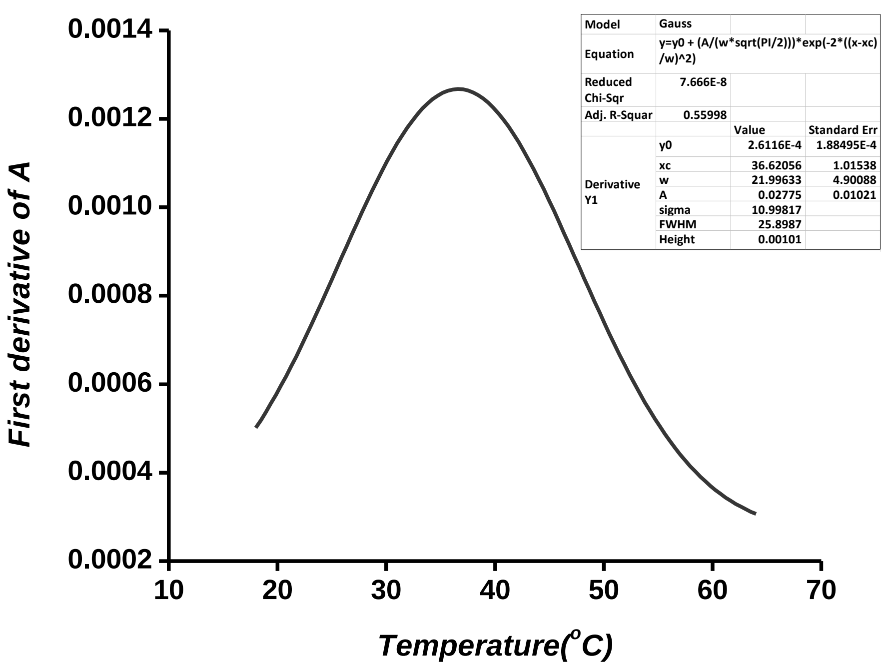
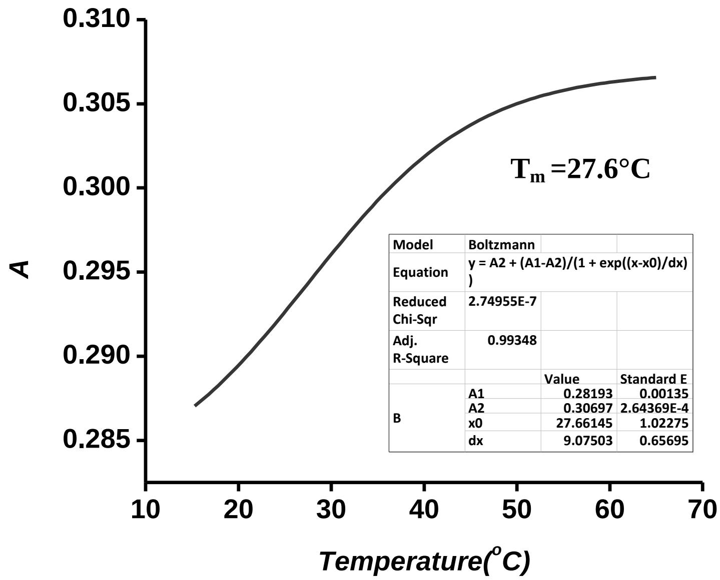


Figure S41: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO6-RNA **(B)** First derivative plot PMO6-RNA

(A) 5′-T-T-T-T-A-***C***-T-***C***-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



(B)

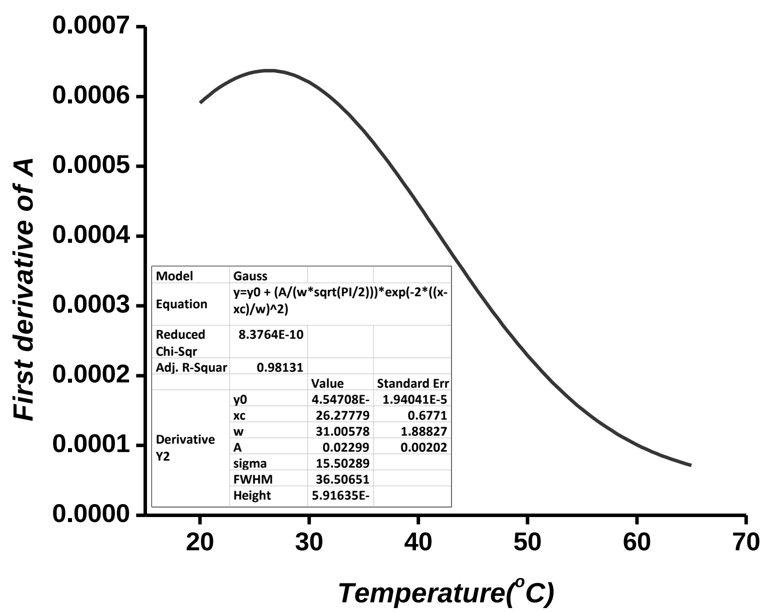
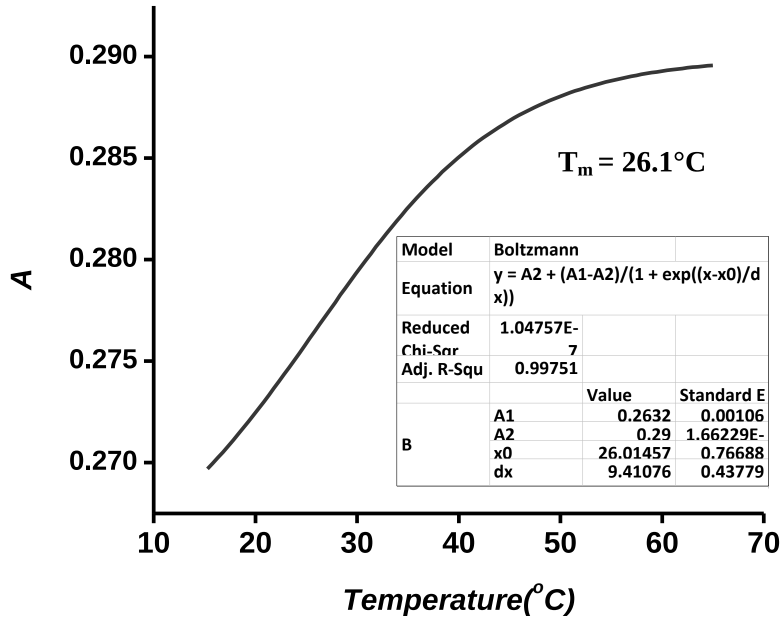


Figure S42: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO7-DNA **(B)** First derivative plot PMO7-DNA

(A) 5′-T-T-T-T-A-***C***-T-***C***-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

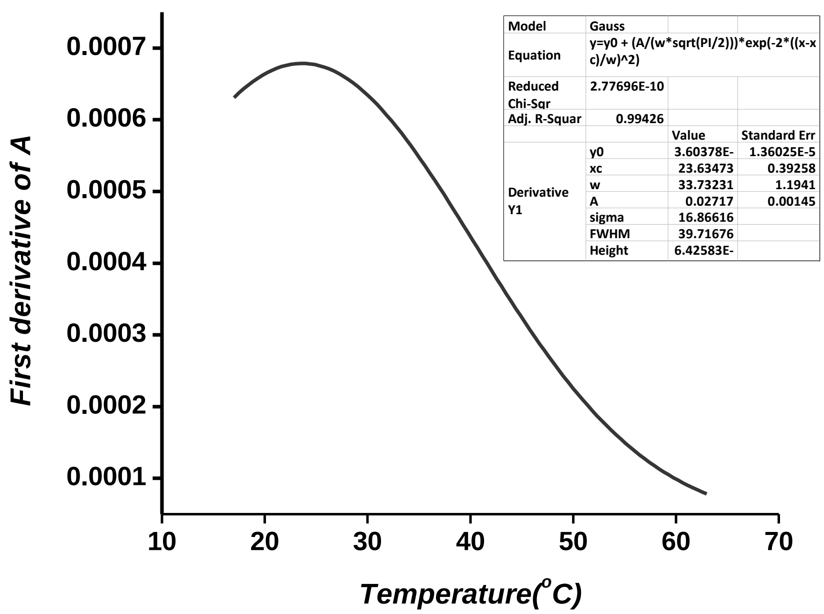
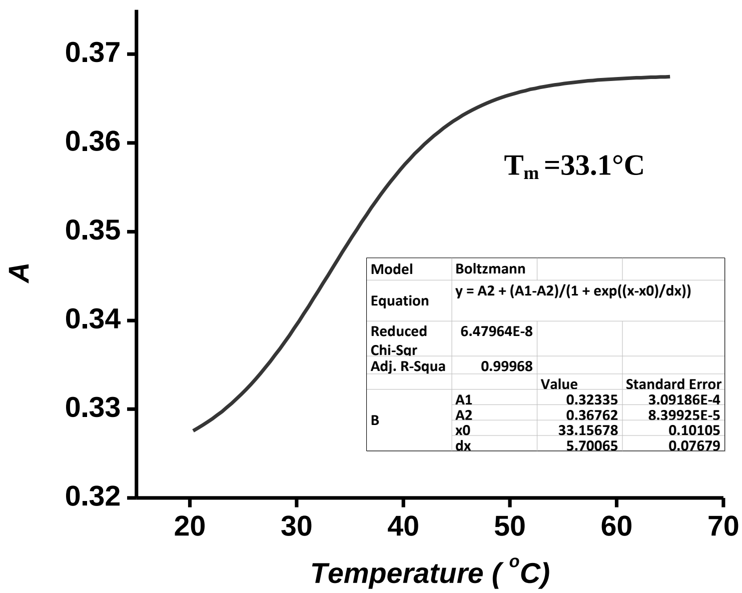


Figure S43: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO7-RNA **(B)** First derivative plot PMO7-RNA

(A)

5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



(B)

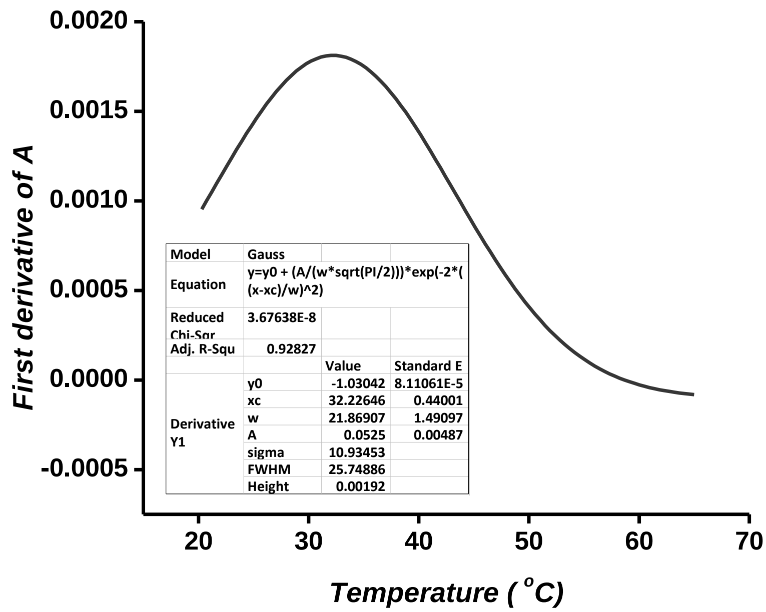
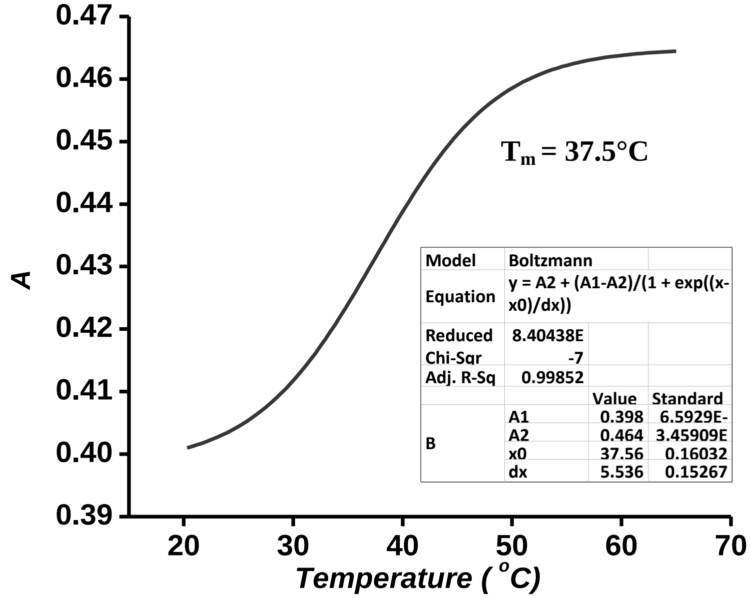


Figure S44: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO8-DNA **(B)** First derivative plot PMO8-DNA

(A)

5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

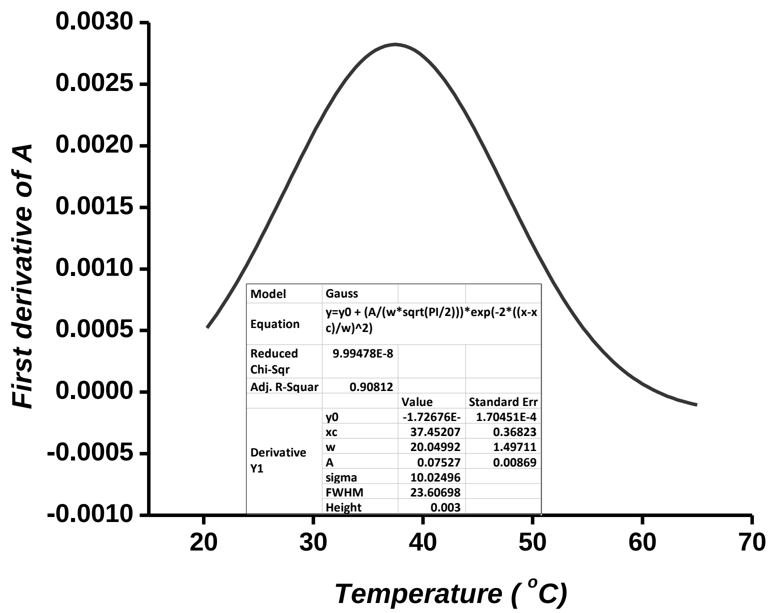
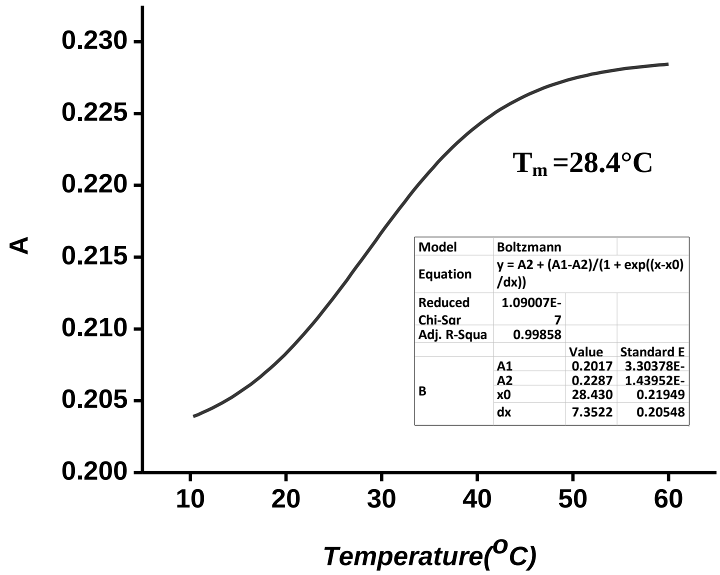


Figure S45: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO8-RNA **(B)** First derivative plot PMO8-RNA

(A) 5′-T-T-T-T-A-C-***U***-C-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



(B)

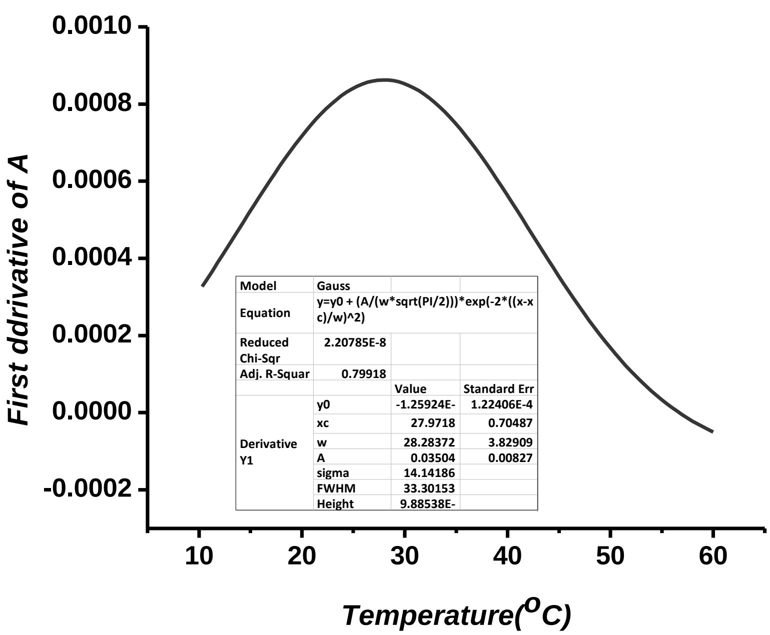
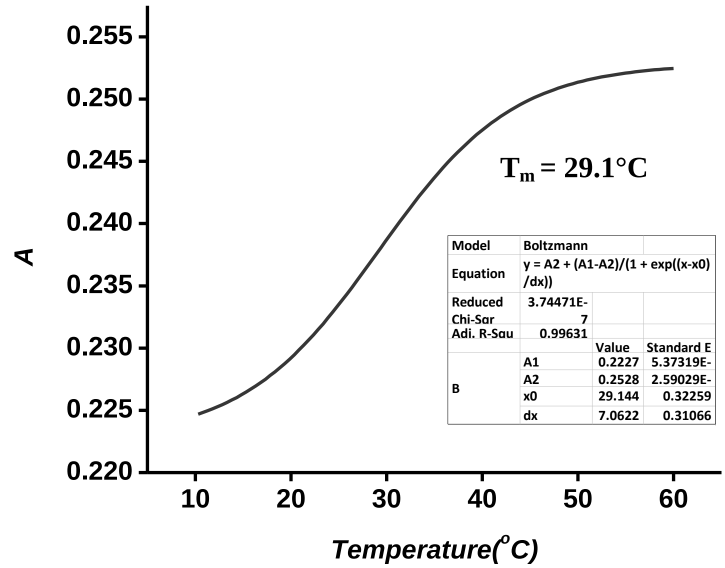


Figure S46: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO9-DNA **(B)** First derivative plot PMO9-DNA

(A) 5′-T-T-T-T-A-C-***U***-C-A-C-A-T-3′

$3'$-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-$5'$



(B)

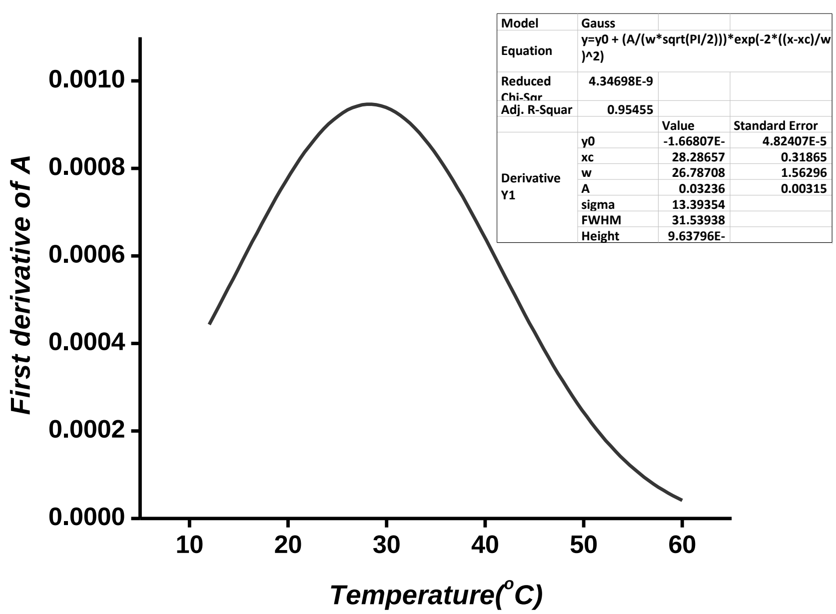
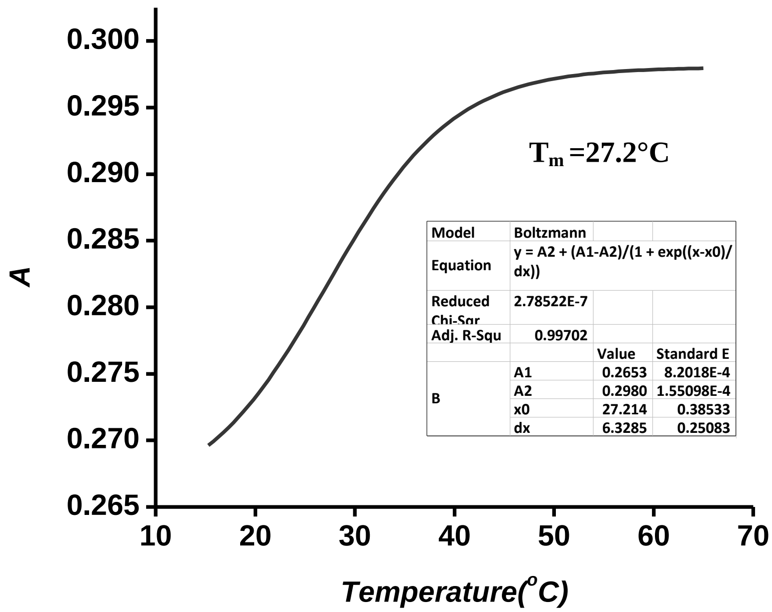


Figure S47: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO9-RNA **(B)** First derivative plot PMO9-RNA

5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

(A) 3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



(B)

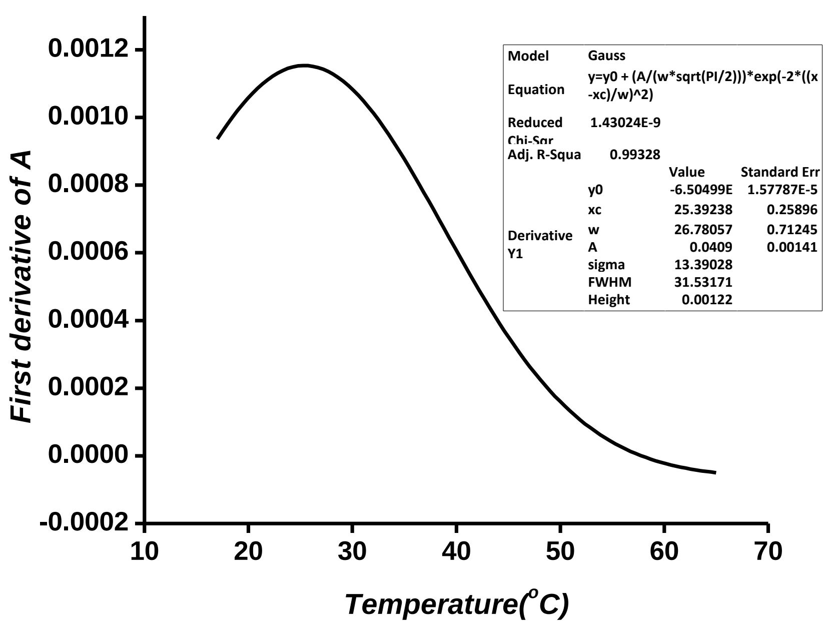
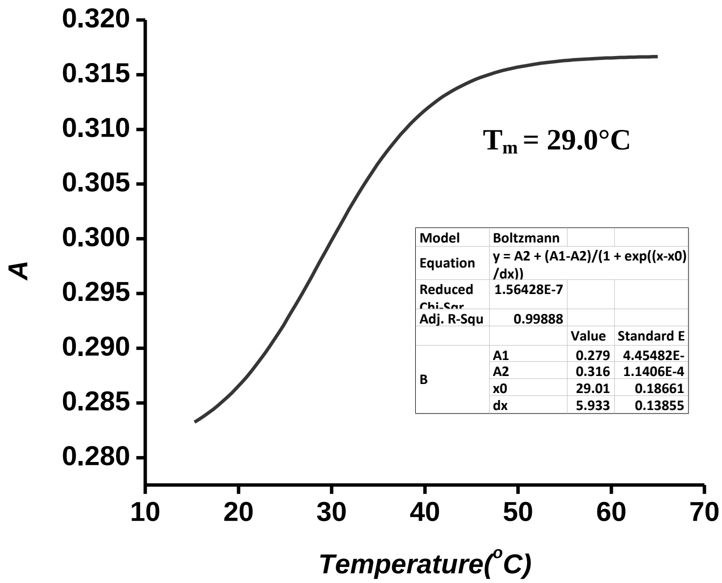


Figure S48: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO10-DNA **(B)** First derivative plot PMO10-DNA

(A)

5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

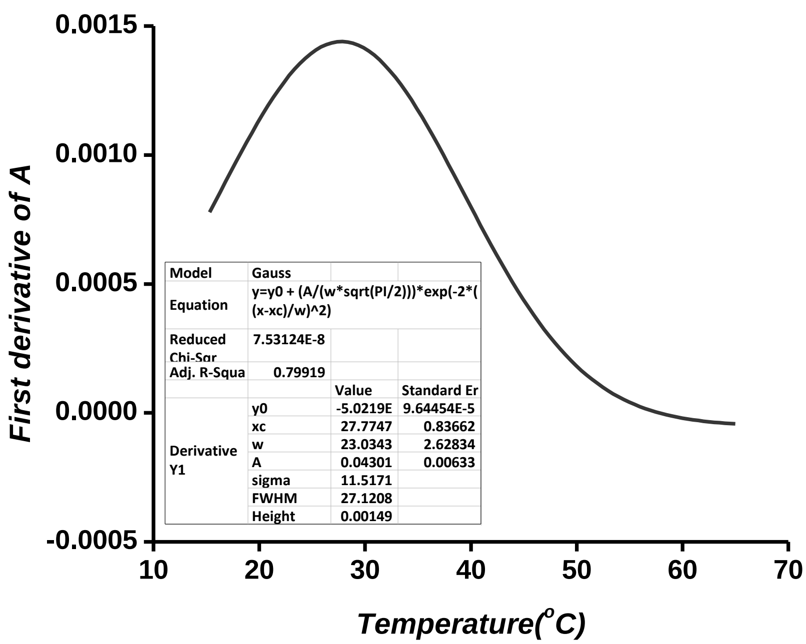
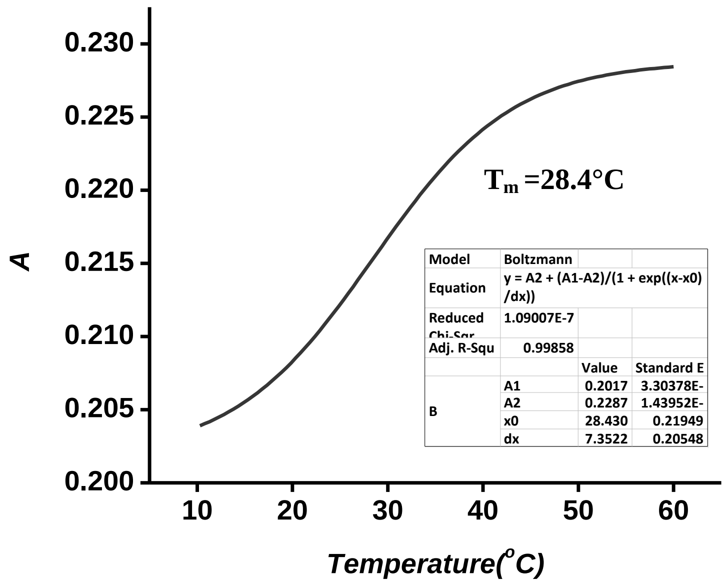


Figure S49: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO10-RNA **(B)** First derivative plot PMO10-RNA

(A) 5′-T-T-T-T-A-C-*U*-C-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dA_dG_dT_dG_dT_dA_d$-5′



(B)

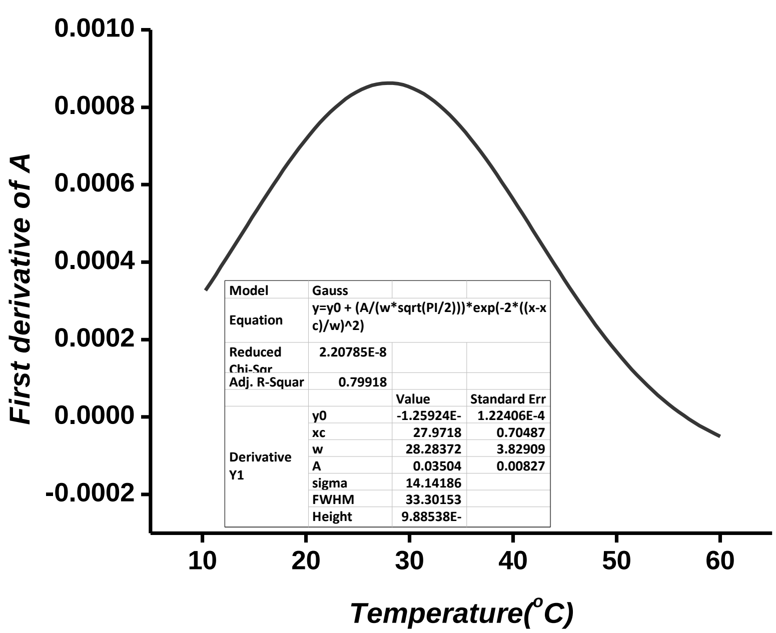
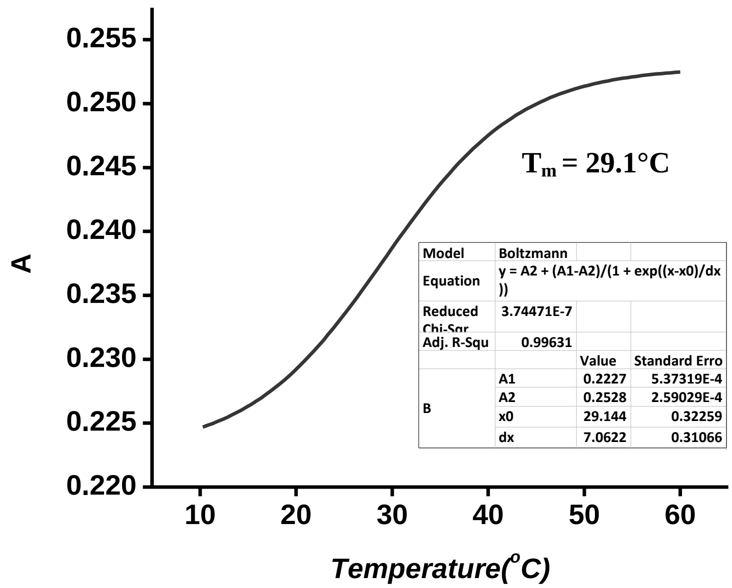


Figure S50: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO11-DNA **(B)** First derivative plot PMO11-DNA

5′-T-T-T-T-A-C-*U*-C-A-C-A-T-3′

(A) 3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



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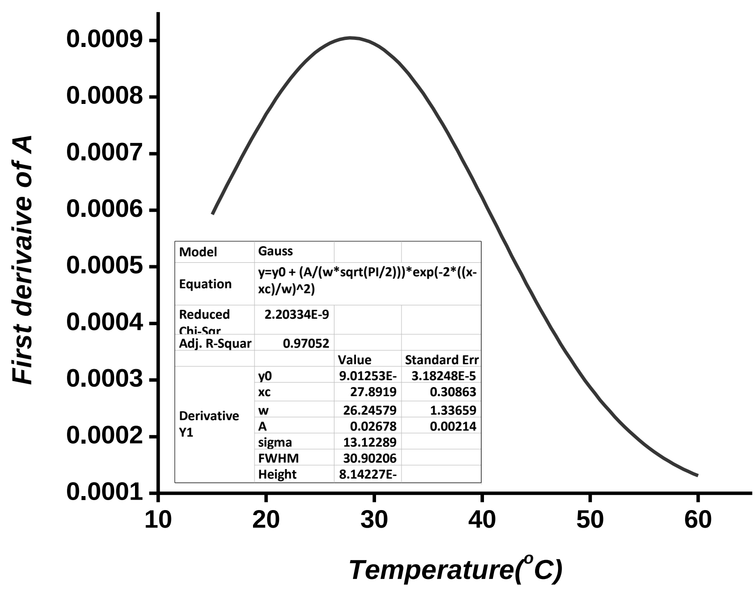
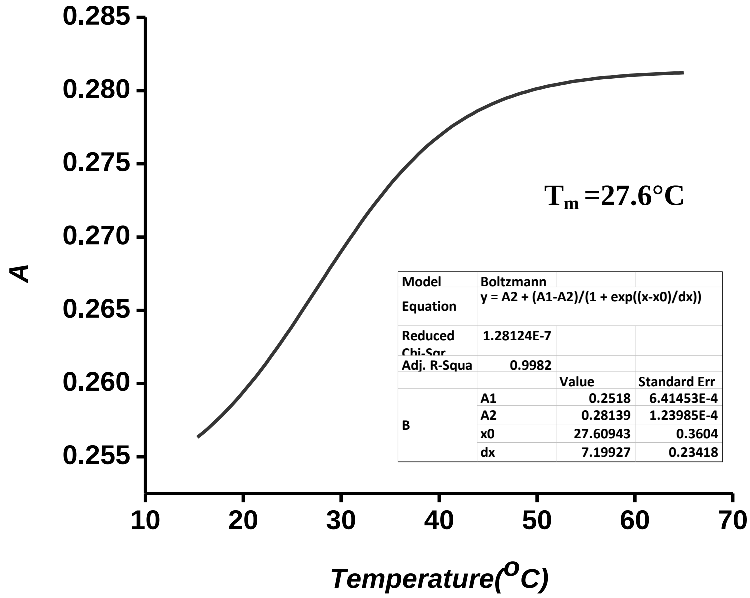


Figure S51: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO11-RNA **(B)** First derivative plot PMO11-RNA

(A) 5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



(B)

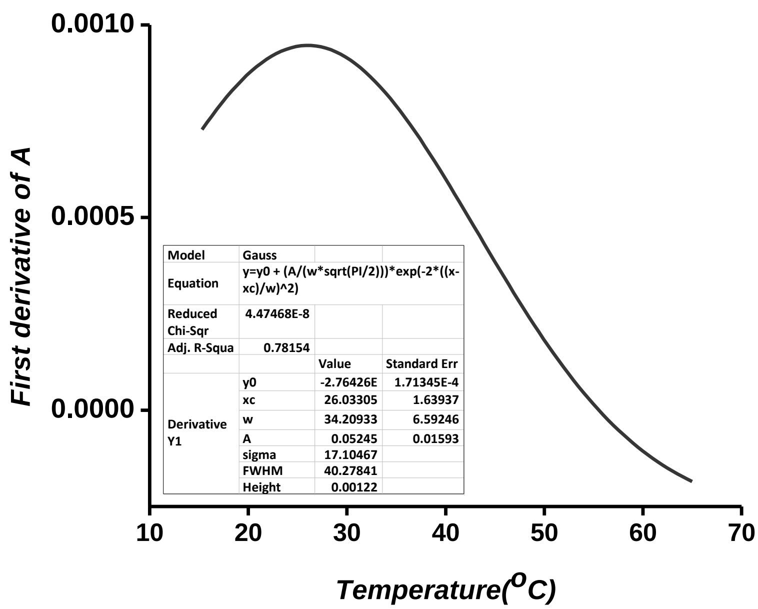
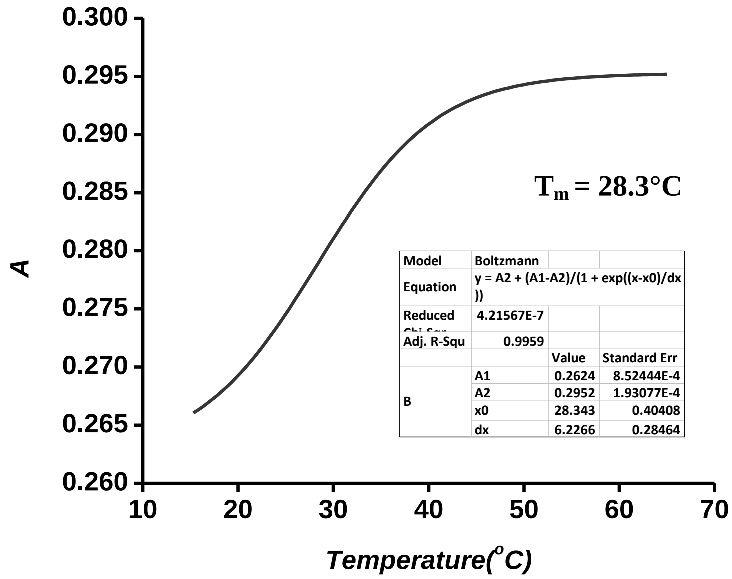


Figure S52: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO12-DNA **(B)** First derivative plot PMO12-DNA

(A) 5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

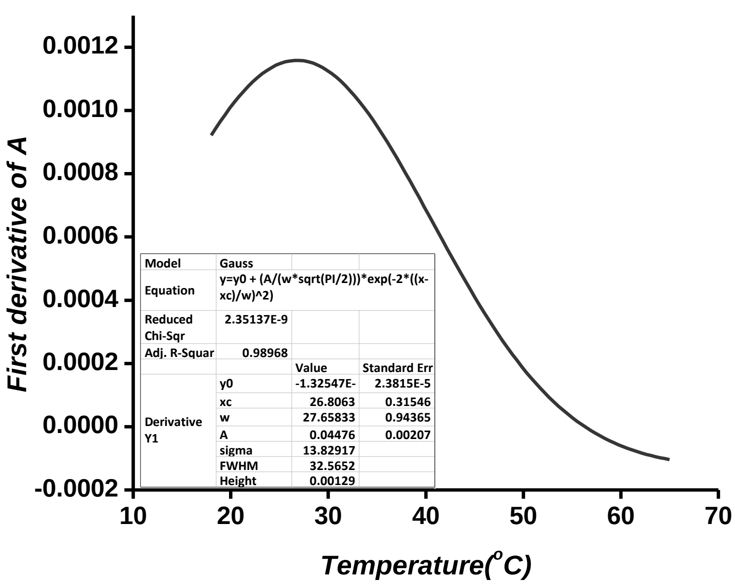
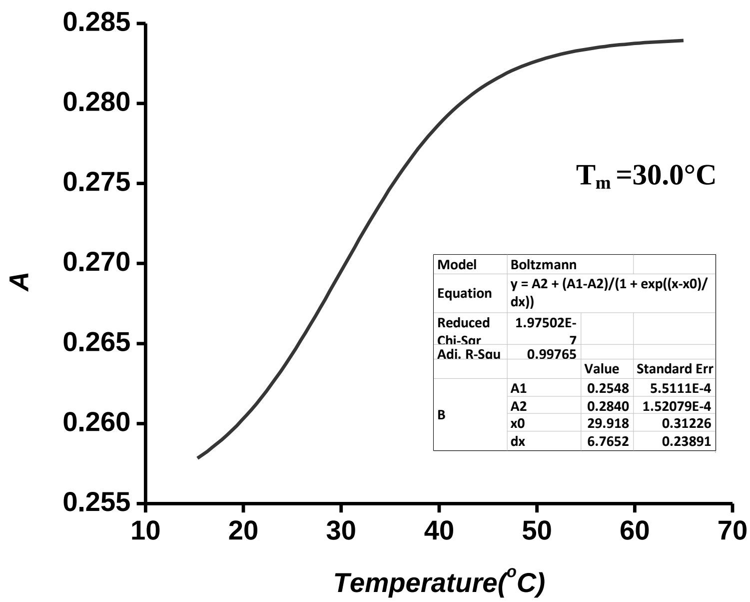


Figure S53: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO12-RNA **(B)** First derivative plot PMO12-RNA

(A) 5′-T-T-T-T-A-C-***U***-C-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



(B)

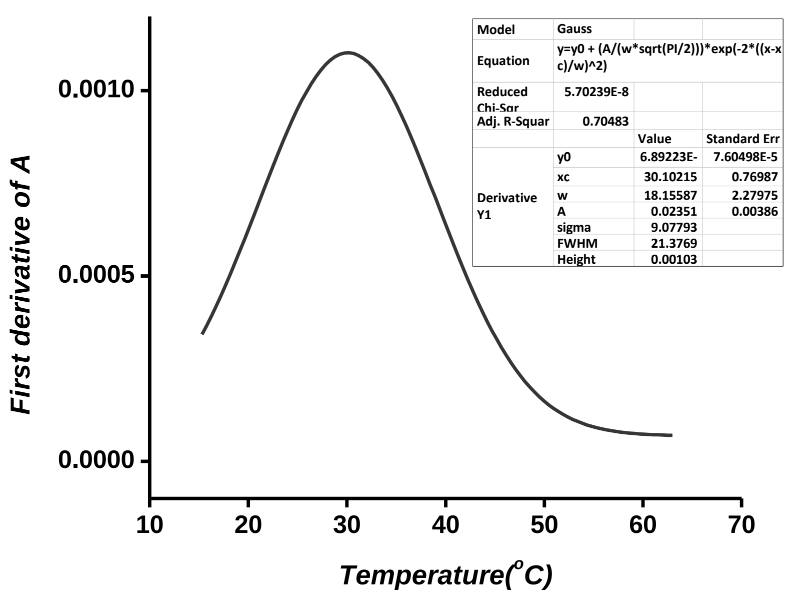
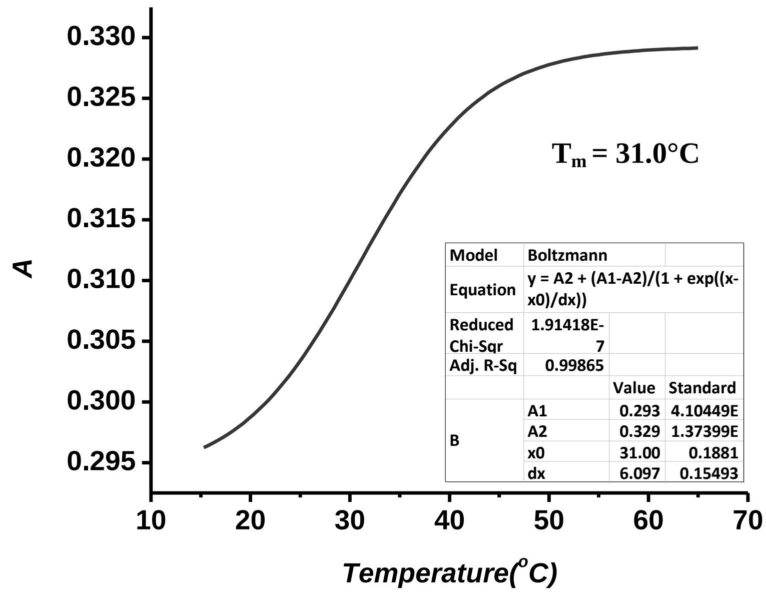


Figure S54: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO13-DNA **(B)** First derivative plot PMO13-DNA

(A)

5′-T-T-T-T-A-C-*U*-C-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

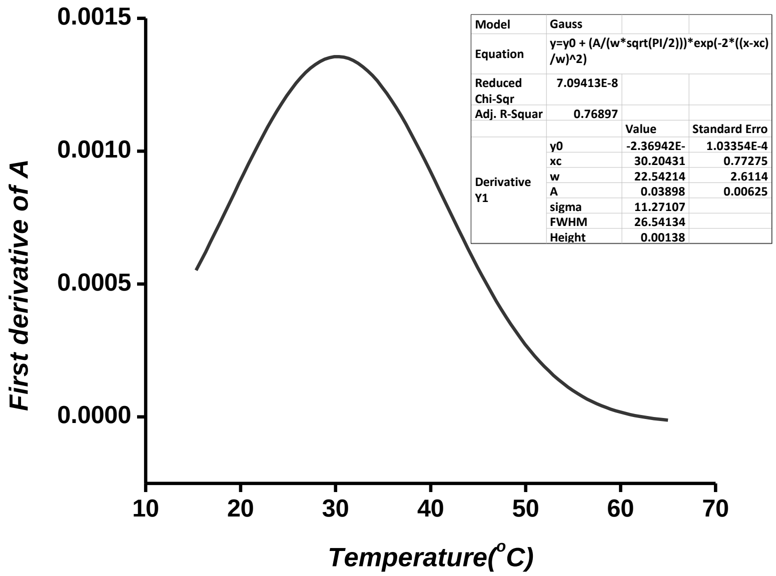
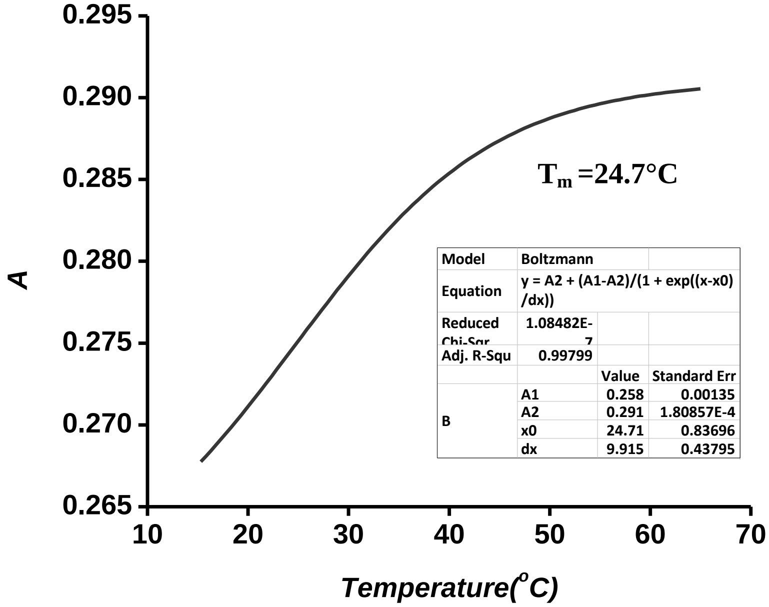


Figure S55: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO13-RNA **(B)** First derivative plot PMO13-RNA

(A) 5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

$3'\text{-}A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d\text{-}5'$



(B)

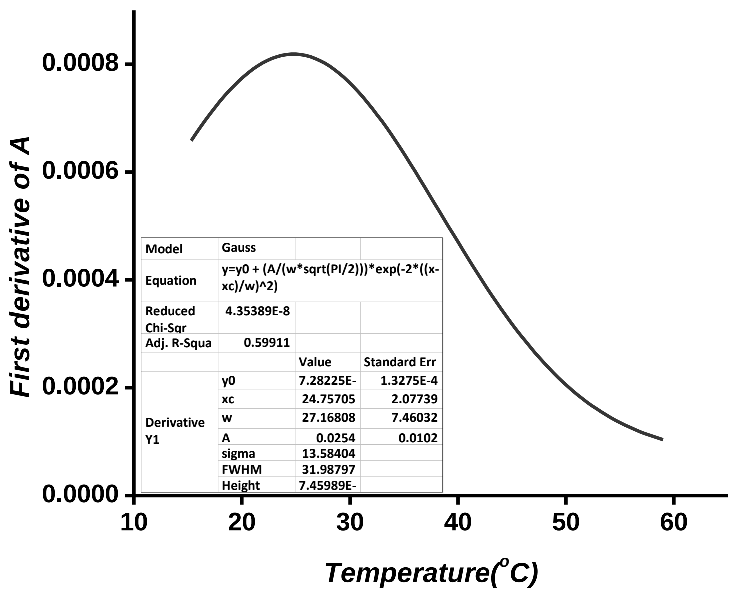
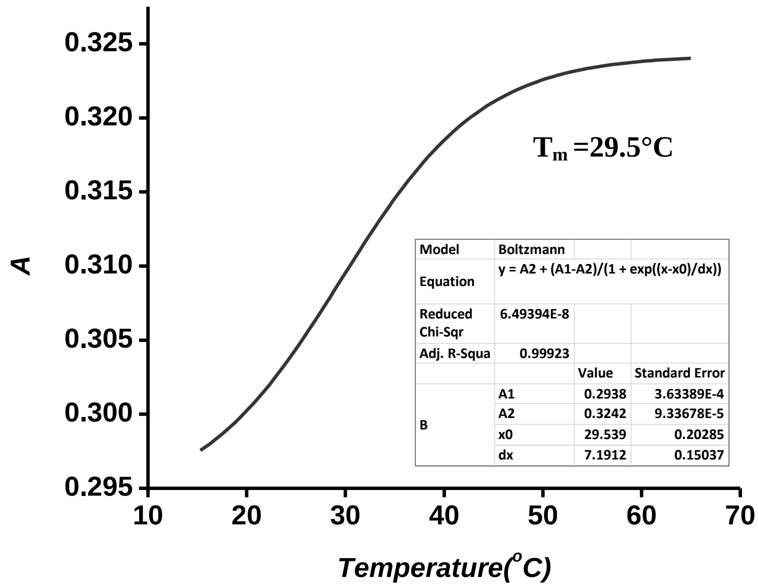


Figure S56: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO14-DNA **(B)** First derivative plot PMO14-DNA

(A) 5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

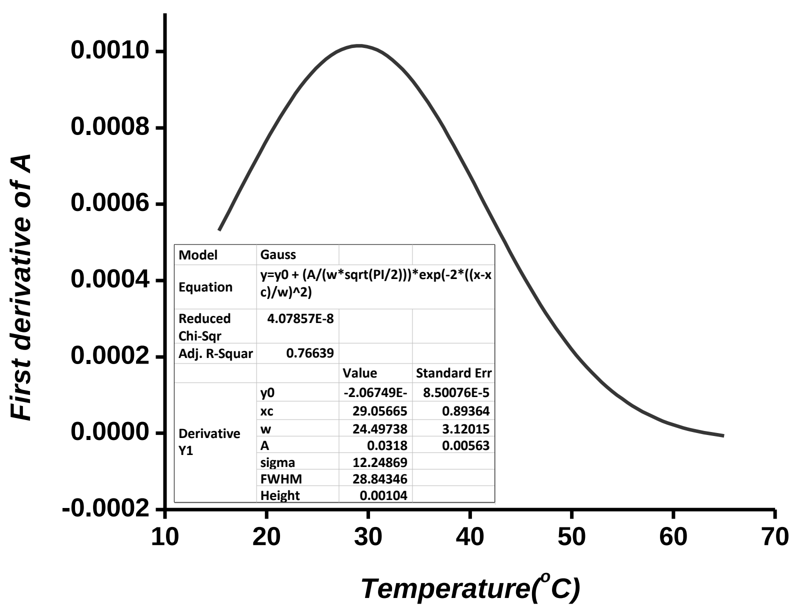
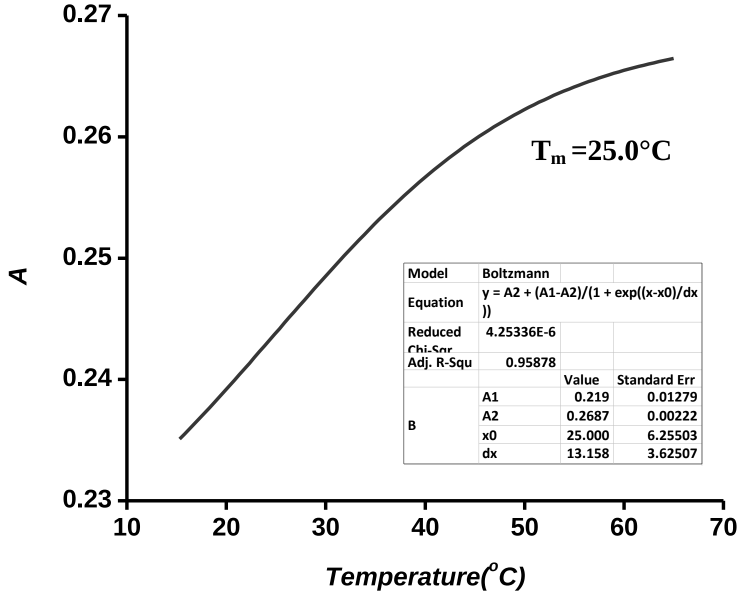


Figure S57: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO14-RNA **(B)** First derivative plot PMO14-RNA

(A)

5′-T-T-T-T-A-C-***U***-C-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



(B)

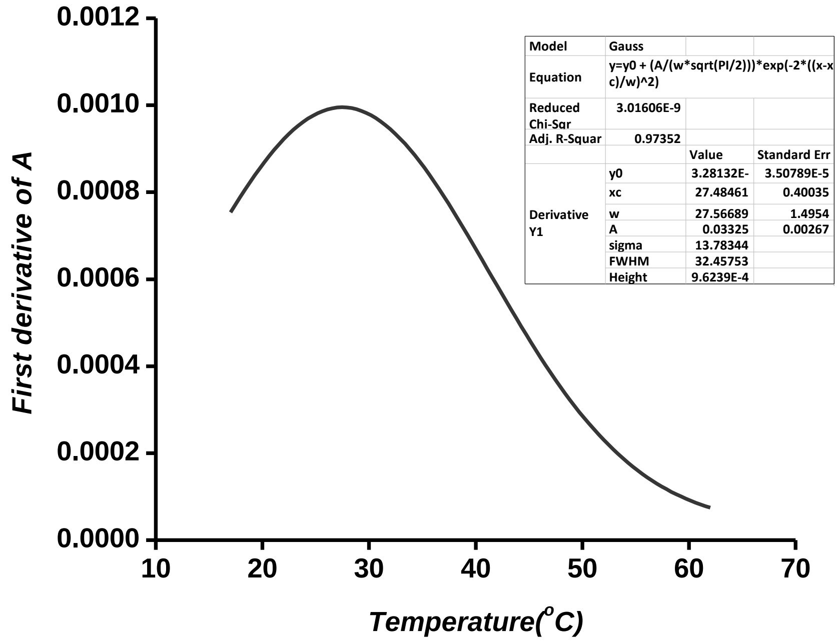
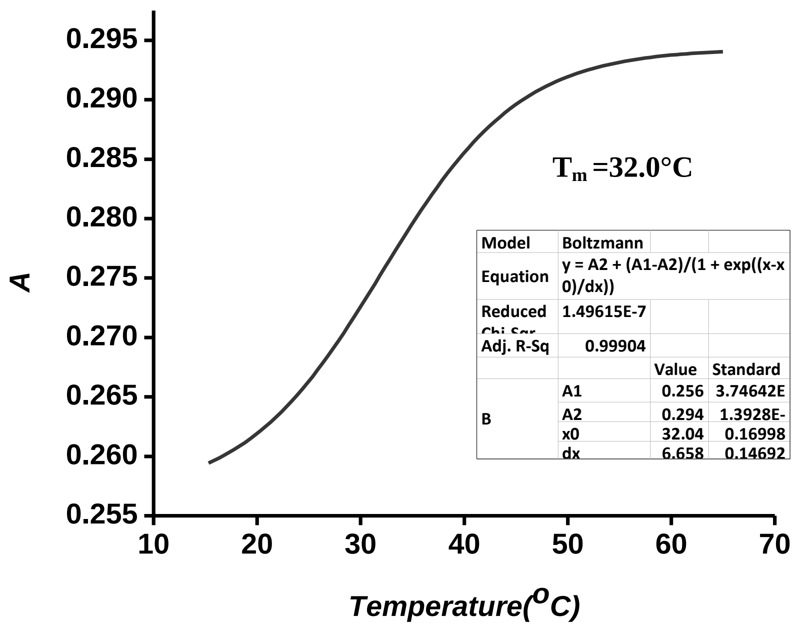


Figure S58: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO15-DNA **(B)** First derivative plot PMO15-DNA

(A)

5′-T-T-T-T-A-C-***U***-C-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

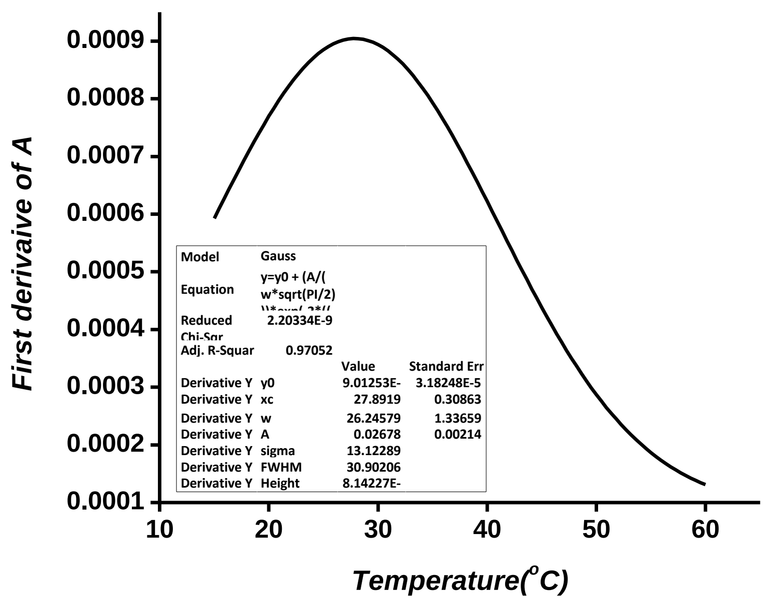
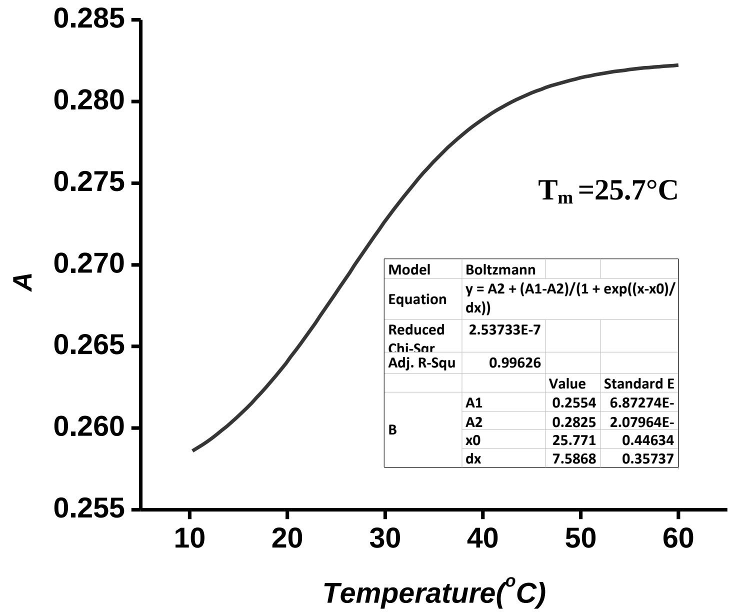


Figure S59: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO15-RNA **(B)** First derivative plot PMO15-RNA

(A) 5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



(B)

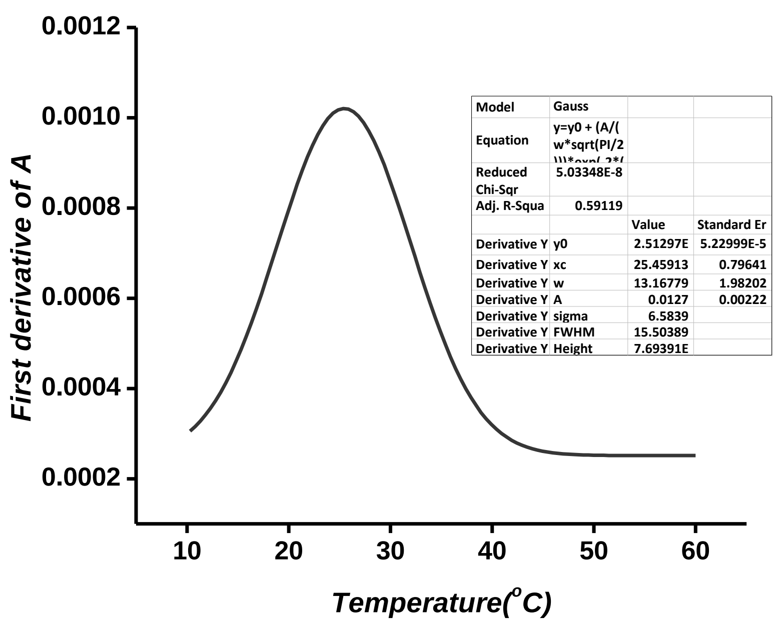
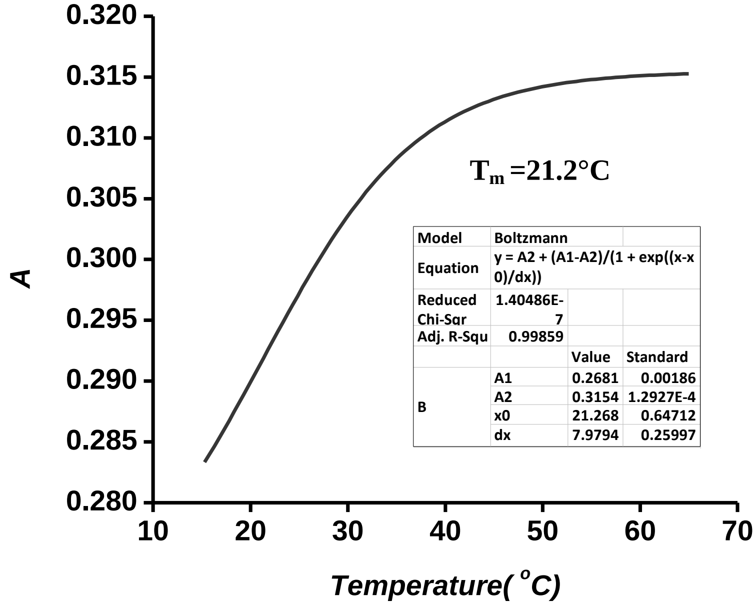


Figure S60: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO16-DNA **(B)** First derivative plot PMO16-DNA

(A) 5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

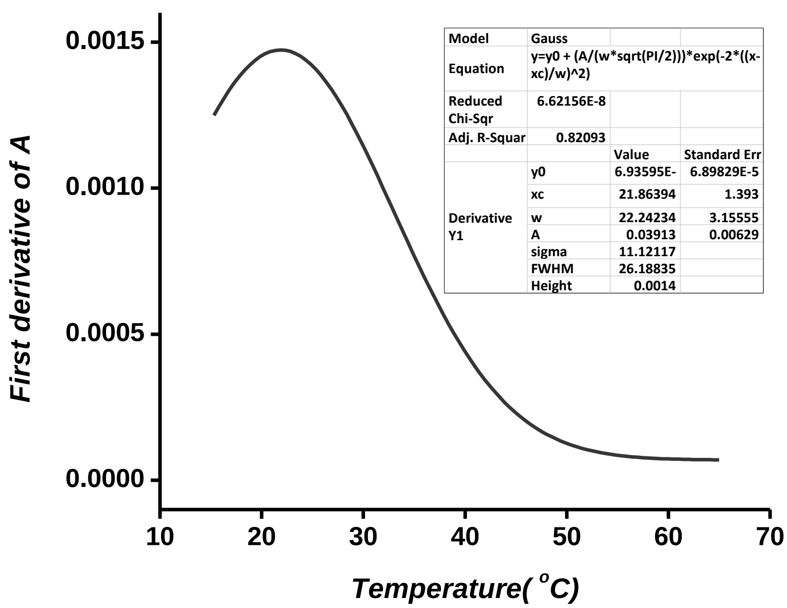
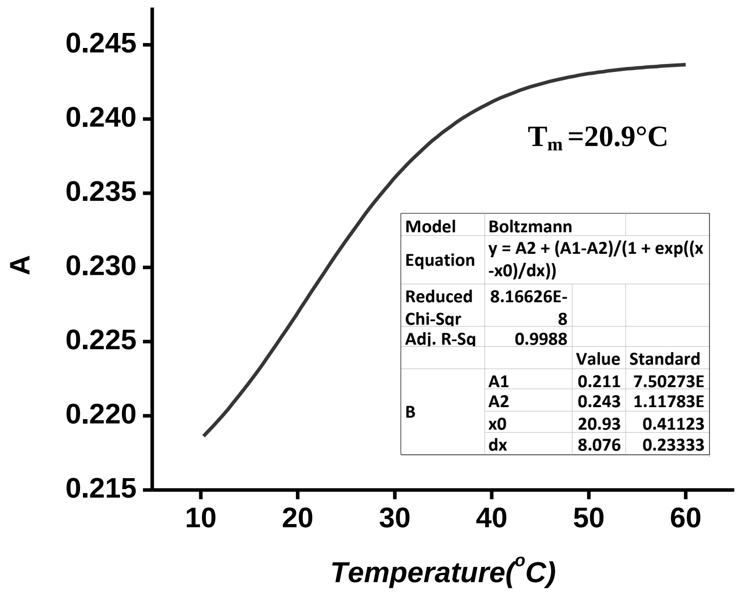


Figure S61: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO16-RNA **(B)** First derivative plot PMO16-RNA

(A) 5′-T-T-T-T-A-C-***U***-C-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



(B)

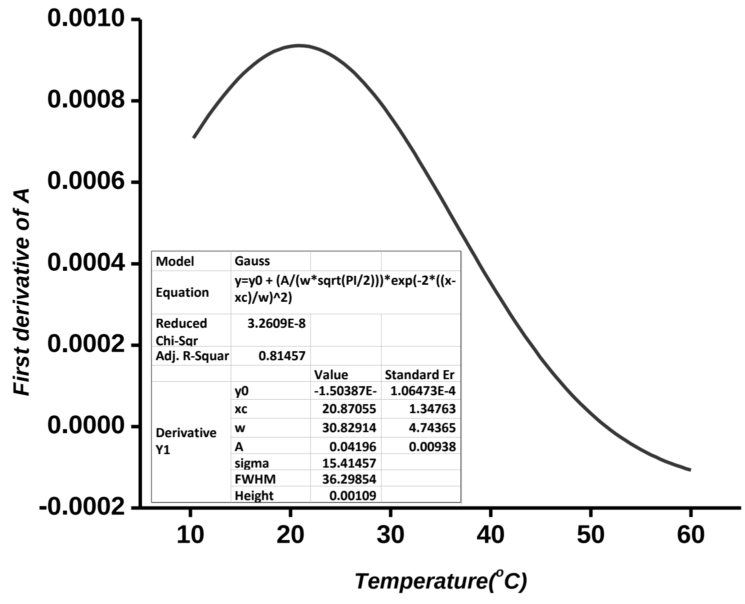
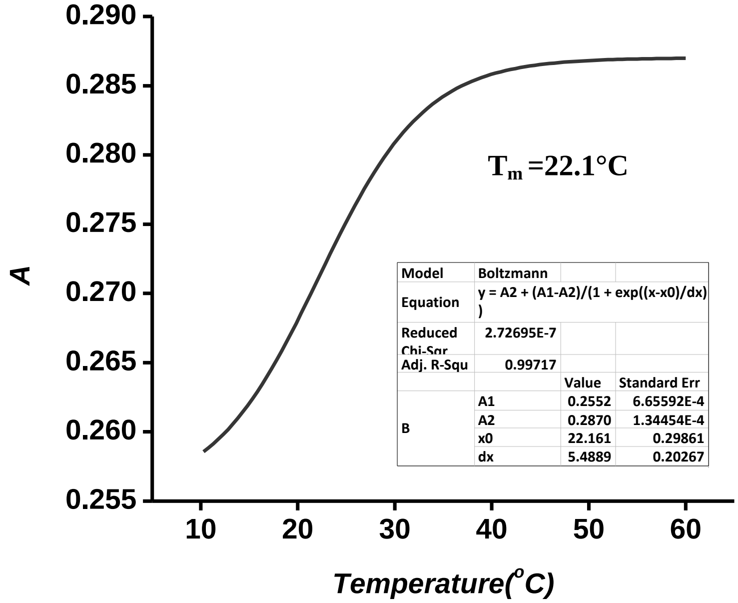


Figure S62: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO17-DNA **(B)** First derivative plot PMO17-DNA

(A) 5′-T-T-T-T-A-C-***U***-C-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



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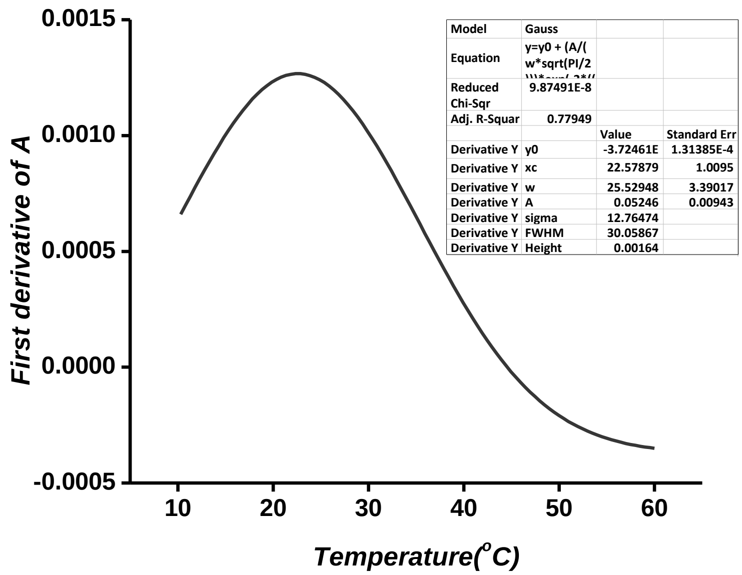
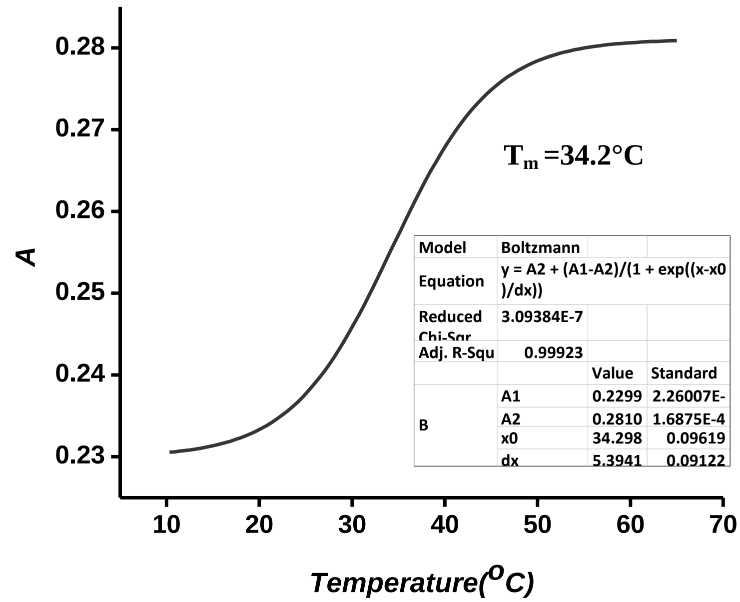


Figure S63: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO17-RNA **(B)** First derivative plot PMO17-RNA

5′-T-T-T-T-A-C-***U***-C-A-C-A-T-3′

(A) 3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



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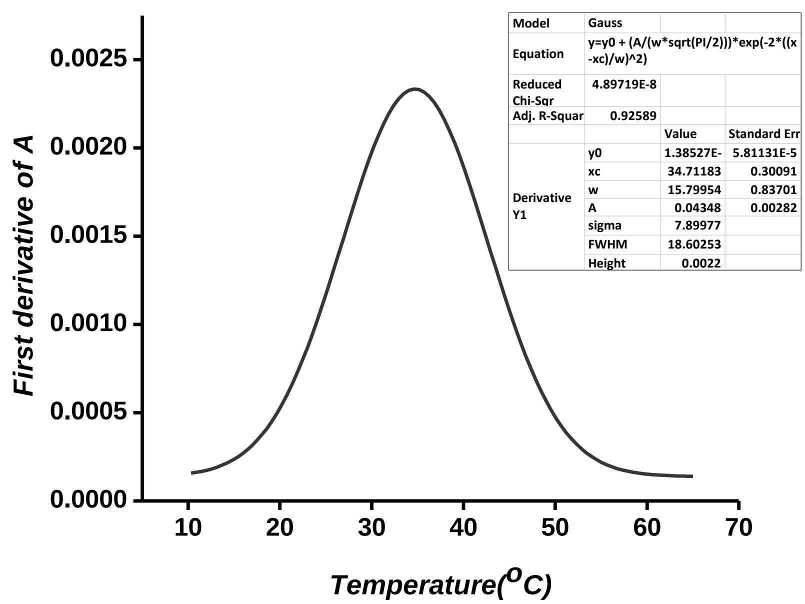
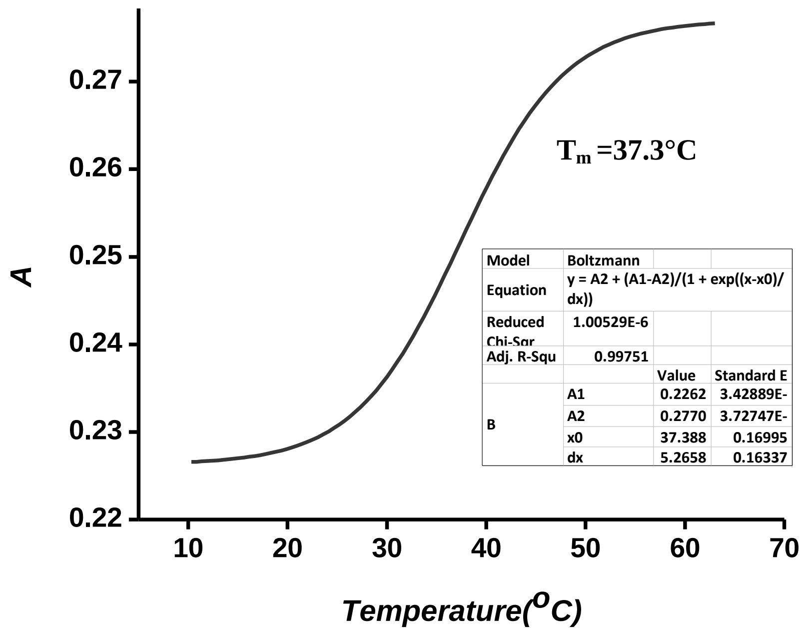


Figure S64: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO18-DNA **(B)** First derivative plot PMO18-DNA

(A)

5′-T-T-T-T-A-C-***U***-C-A-C-A-T-3′

$3'$-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-$5'$



(B)

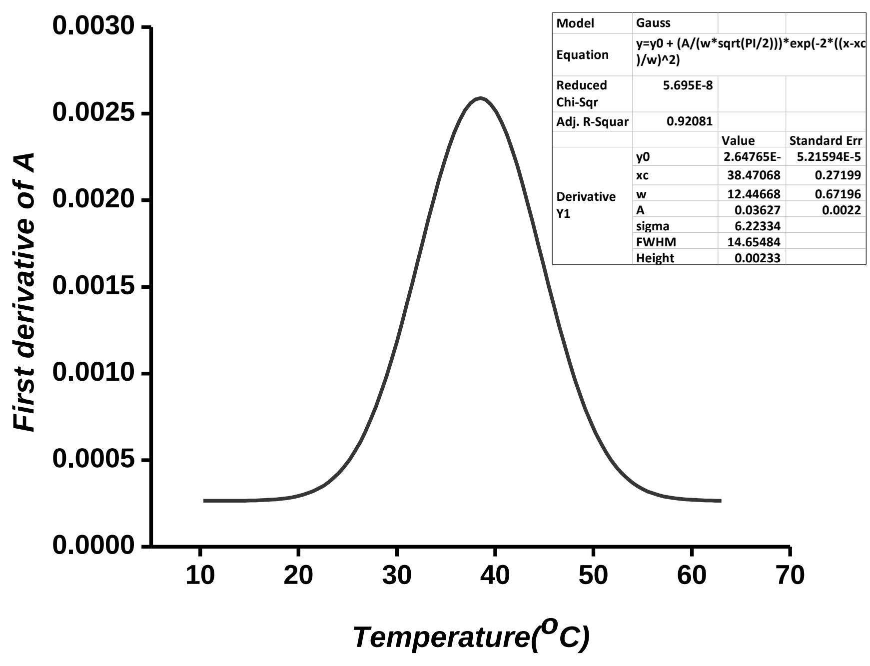
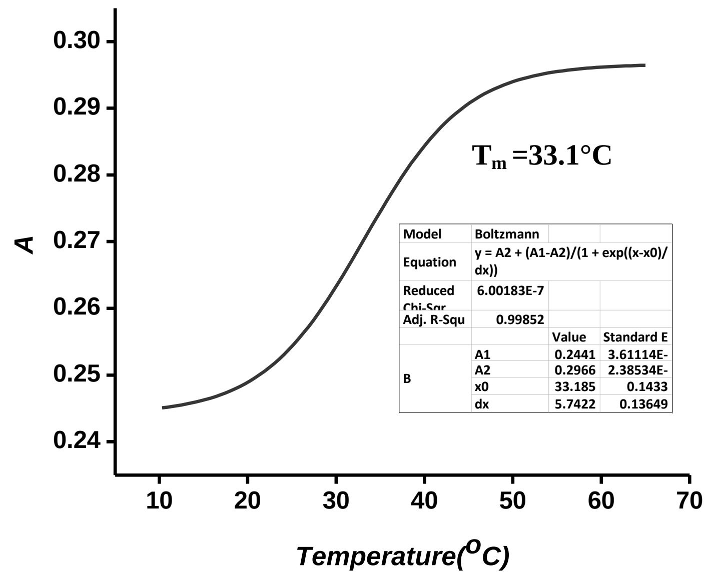


Figure S65: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO18-RNA **(B)** First derivative plot PMO18-RNA

(A)

5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



(B)

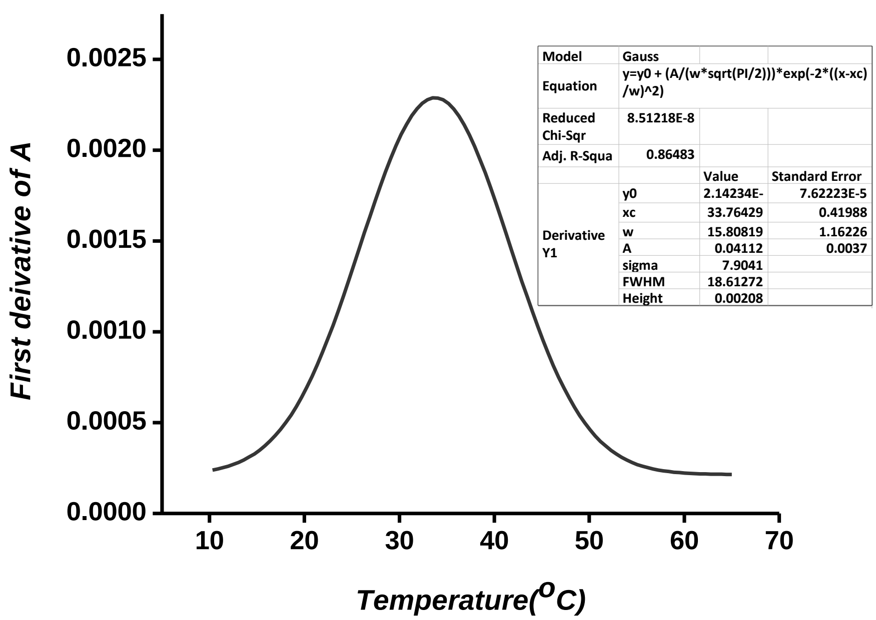
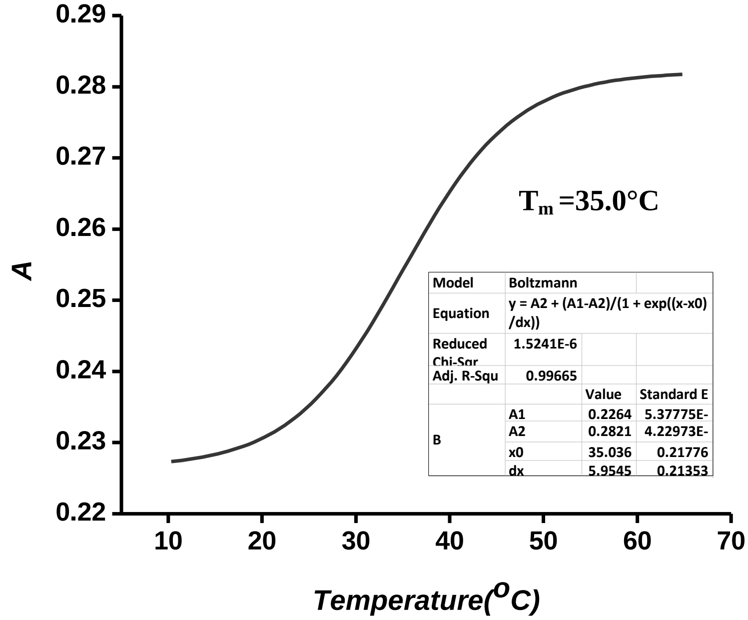


Figure S66: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO19-DNA **(B)** First derivative plot PMO19-DNA

(A) 5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

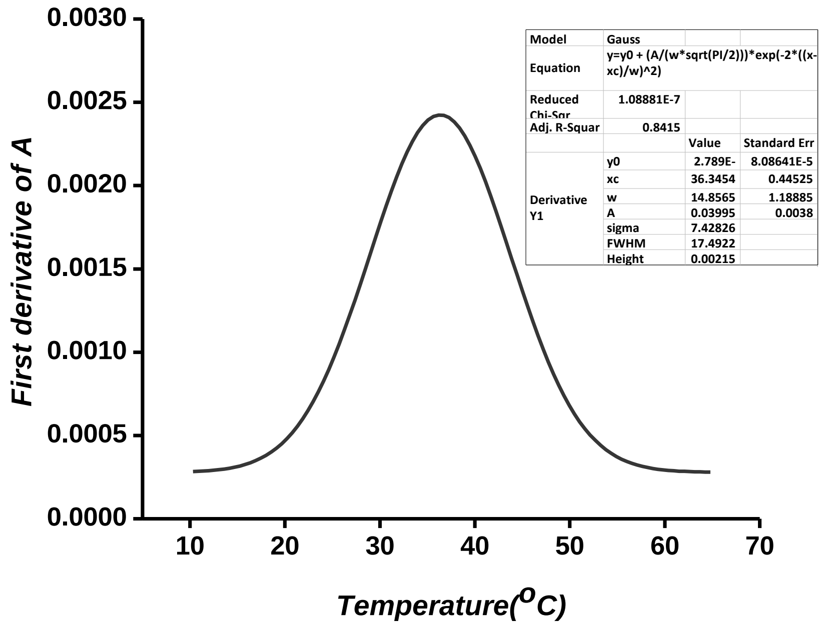
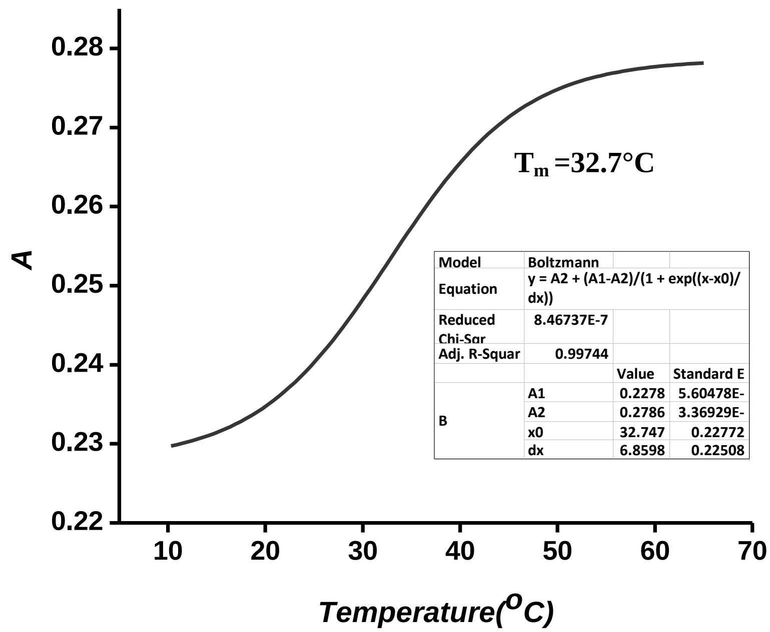


Figure S67: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO19-RNA **(B)** First derivative plot PMO19-RNA

(A)

5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



(B)

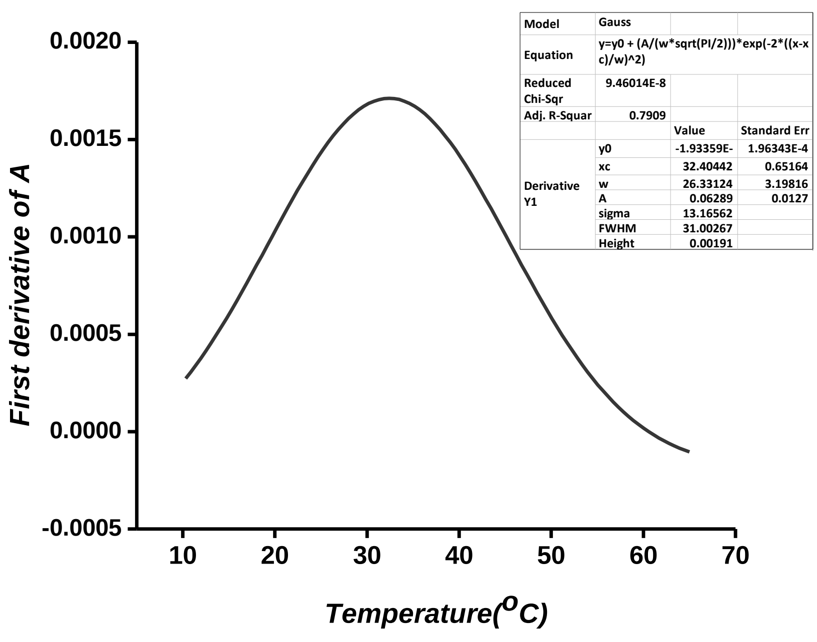
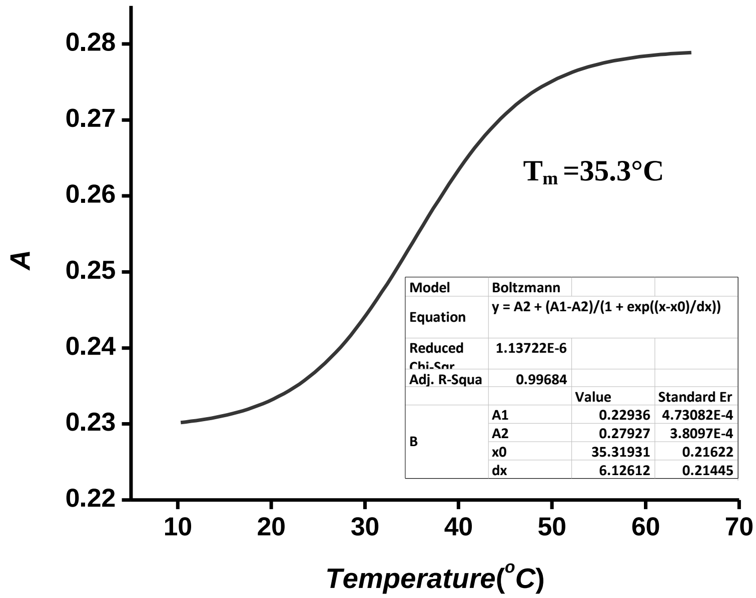


Figure S68: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO20-DNA **(B)** First derivative plot PMO20-DNA

(A)

5′-T-T-T-T-A-C-T-***C***-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

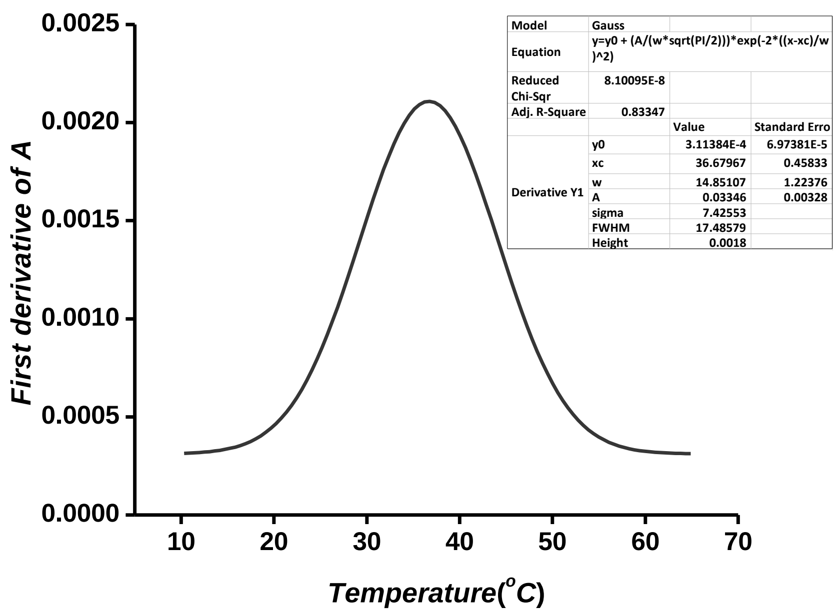
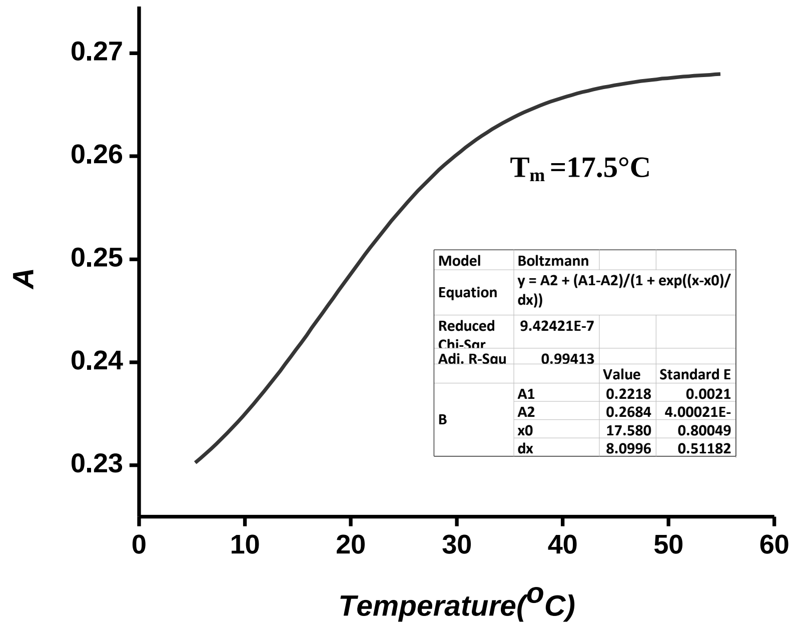


Figure S69: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO20-RNA **(B)** First derivative plot PMO20-RNA

(A)

5′-T-T-T-T-A-C-C-C-A-C-A-T-3′

$3'\text{-}A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d\text{-}5'$



(B)

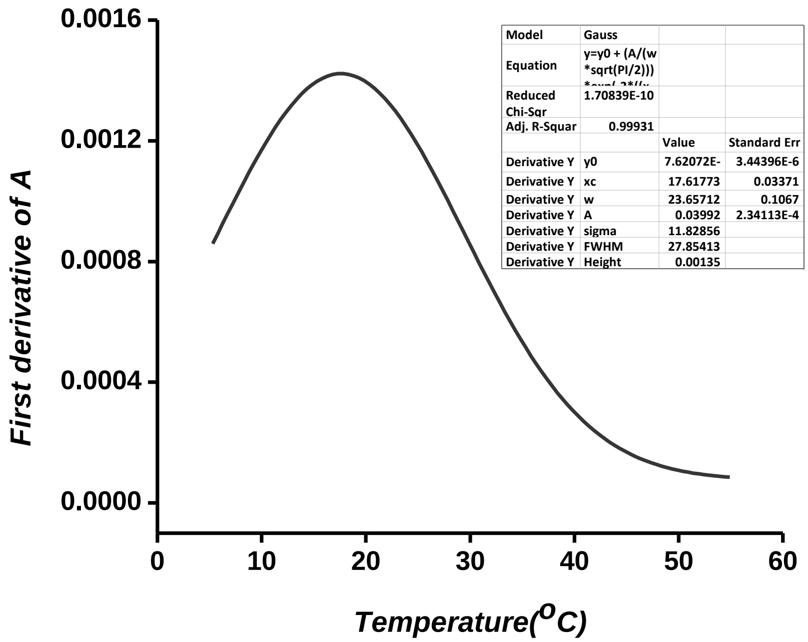
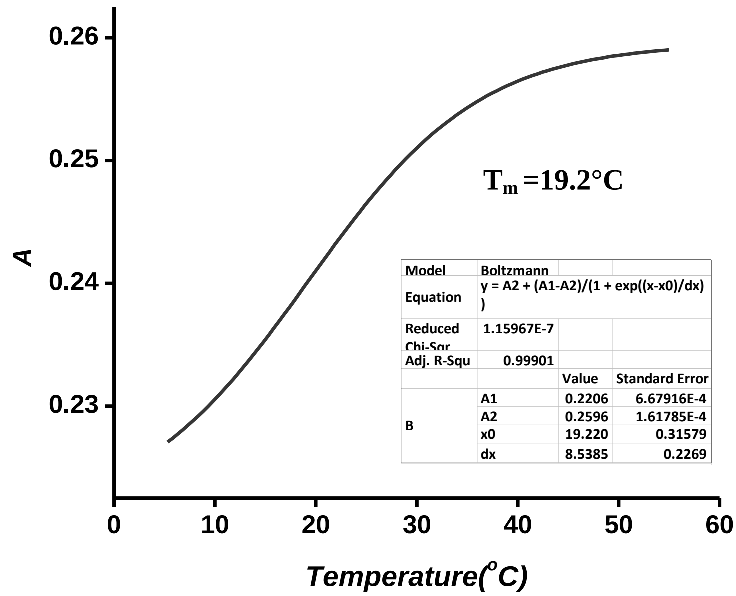


Figure S70: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO21-DNA **(B)** First derivative plot PMO21-DNA

5′-T-T-T-T-A-C-***C***-C-A-C-A-T-3′

(A)

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

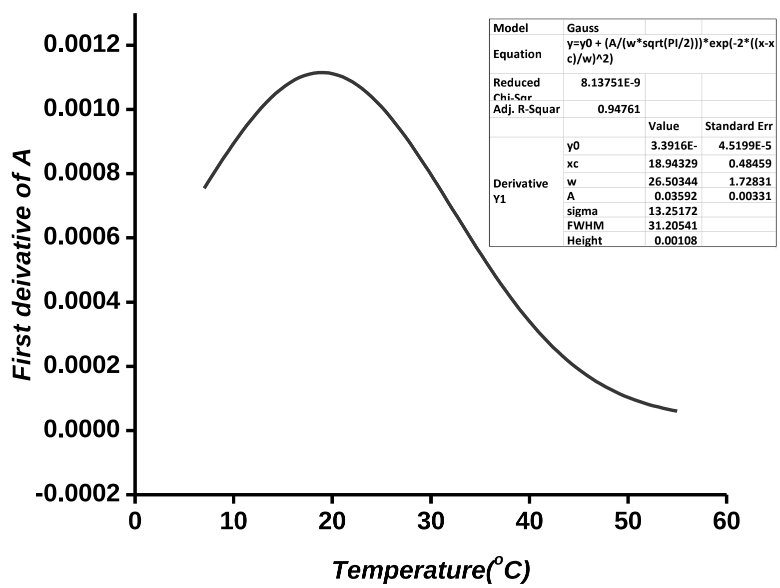
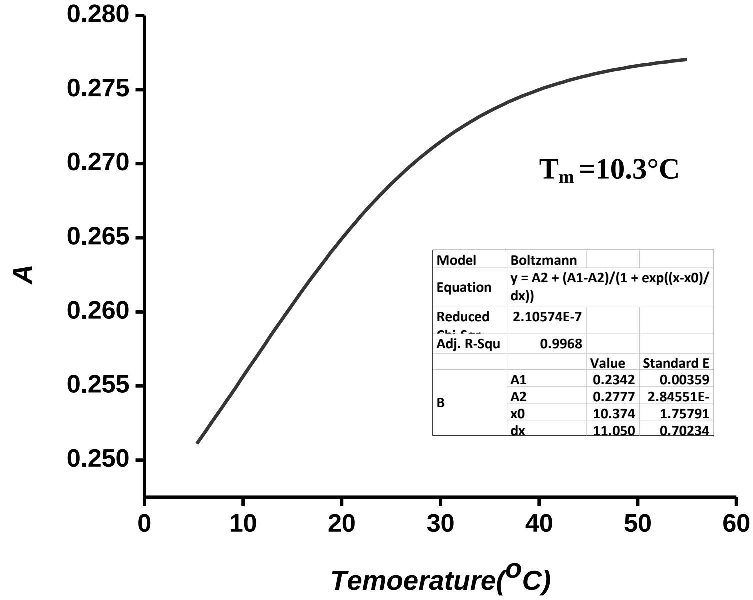


Figure S71: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO21-RNA **(B)** First derivative plot PMO21-RNA

5′-T-T-T-T-A-C-***C***-C-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′

(A)



(B)

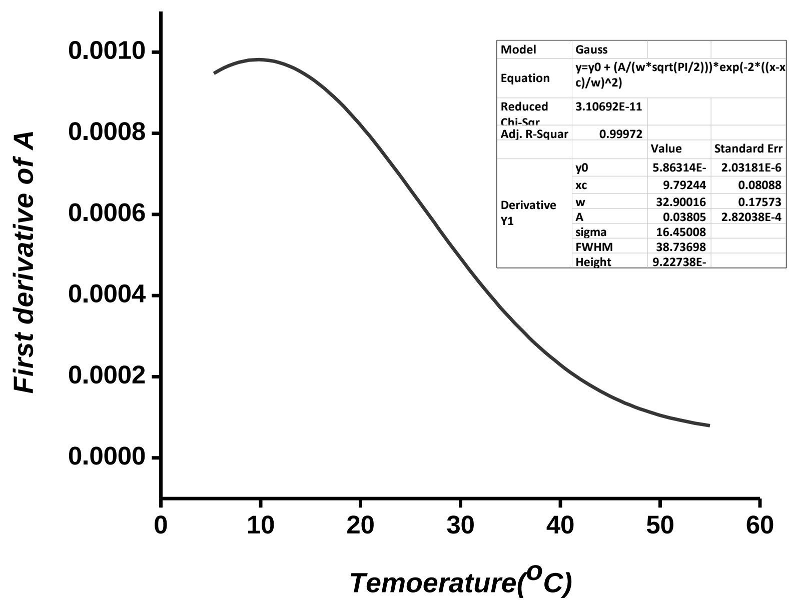
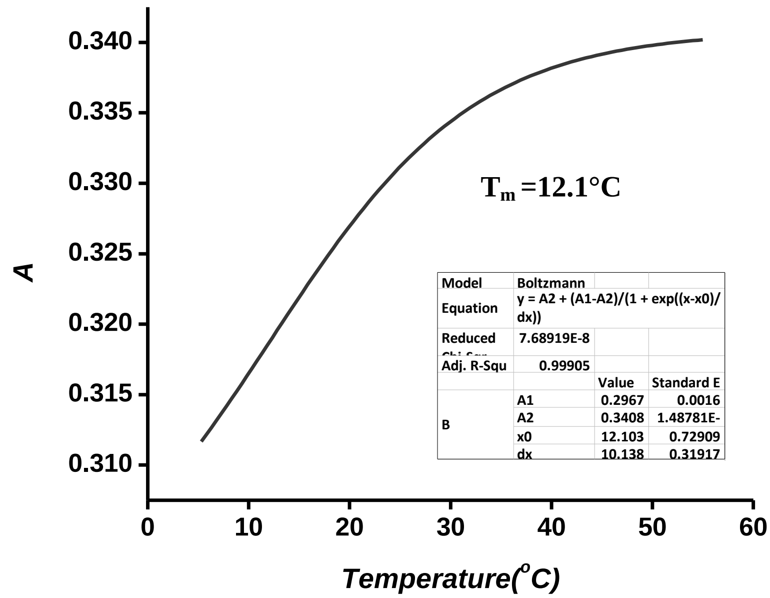


Figure S72: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO22-DNA **(B)** First derivative plot PMO22-DNA

5′-T-T-T-T-A-C-***C***-C-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′

(A)



(B)

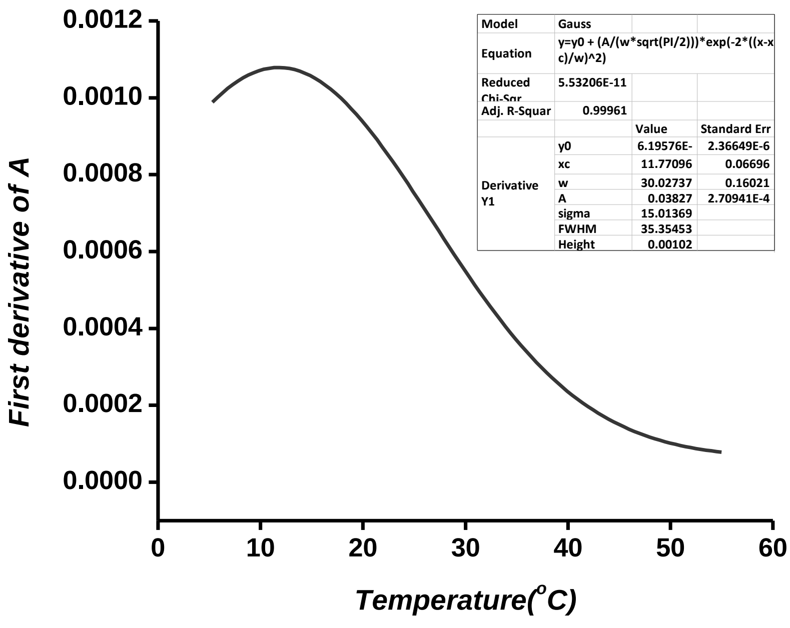
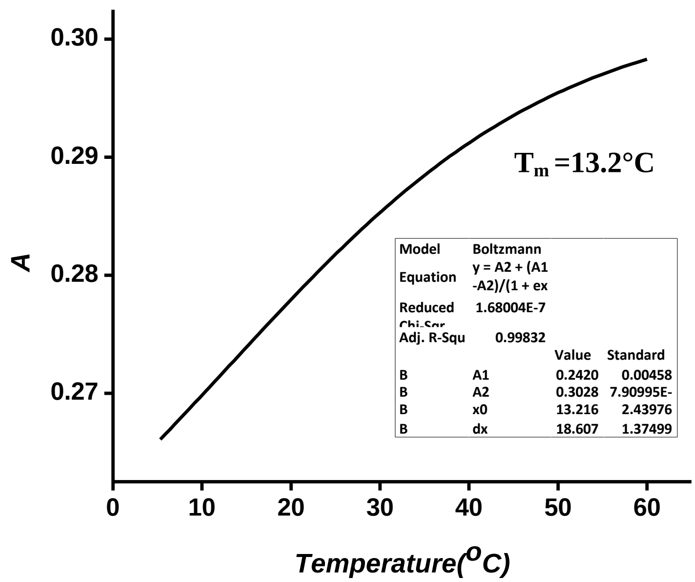


Figure S73: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO22-RNA **(B)** First derivative plot PMO22-RNA

(A)

5′-T-T-T-T-A-C-***C***-T-A-C-A-T-3′

3′-A$_d$A$_d$A$_d$A$_d$T$_d$G$_d$A$_d$G$_d$T$_d$G$_d$T$_d$A$_d$-5′



(B)

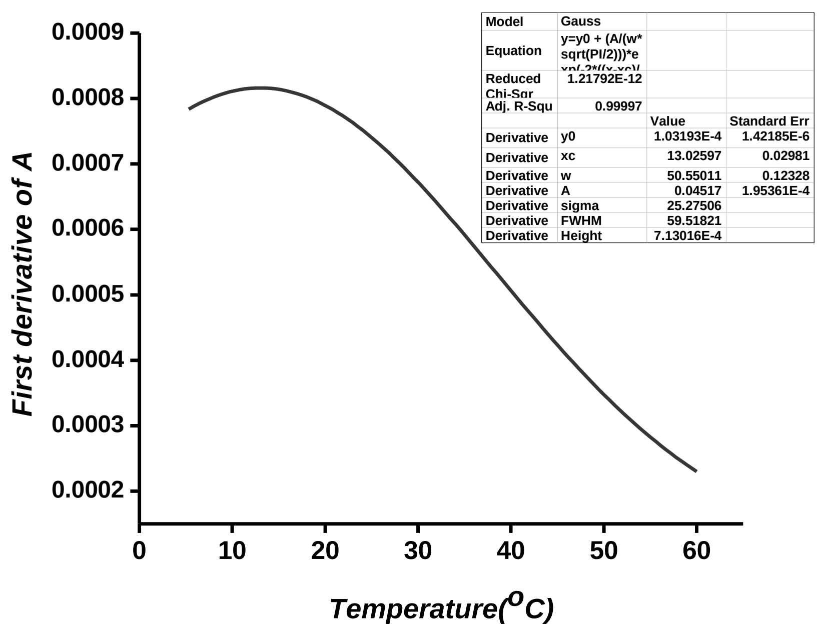


Figure S74: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO23-DNA **(B)** First derivative plot PMO23-DNA

5′-T-T-T-T-A-C-***C***-T-A-C-A-T-3′

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′

(A)

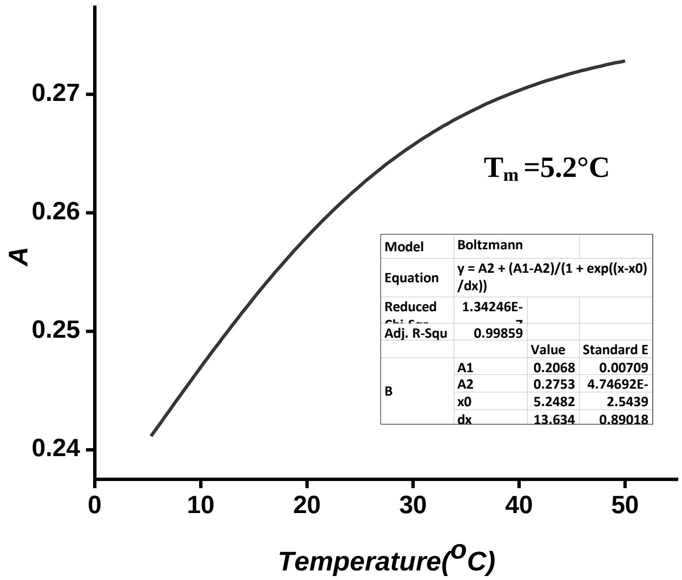
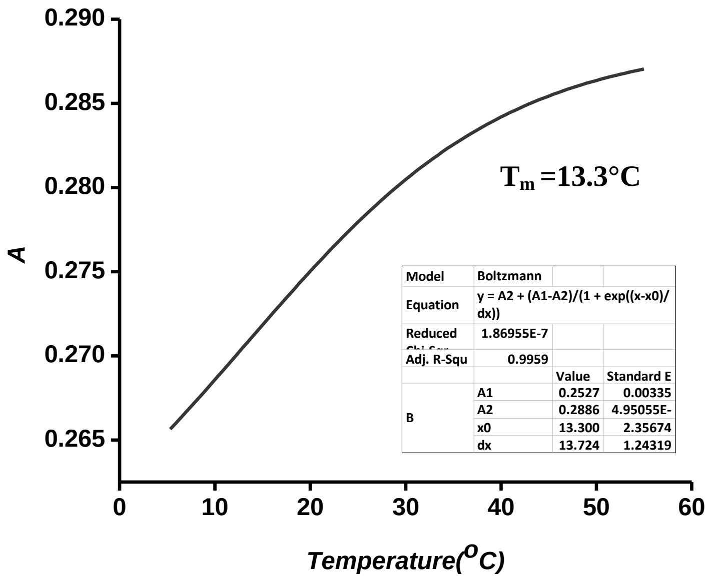


Figure S75: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO23-RNA

(A)

5′-T-T-T-T-A-C-C-***U***-A-C-A-T-3′

3′-$A_dA_dA_dA_dT_dG_dA_dG_dT_dG_dT_dA_d$-5′



(B)

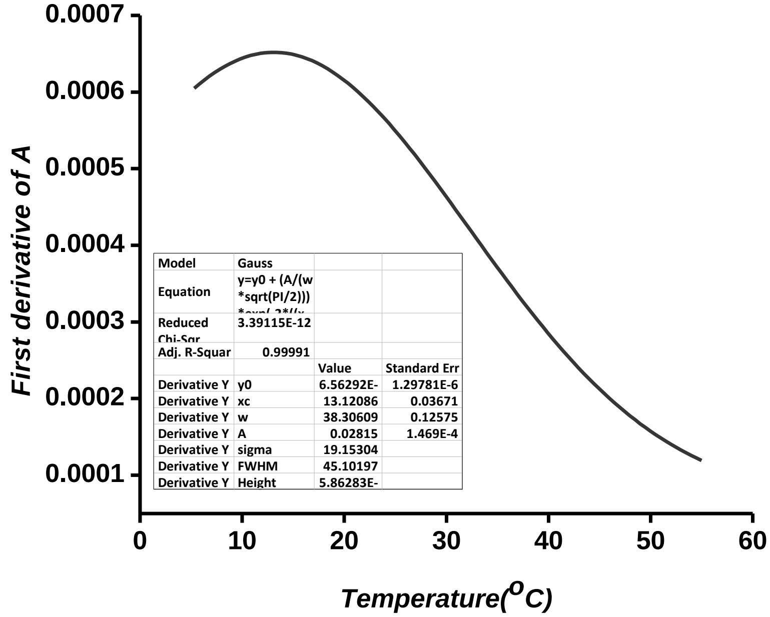
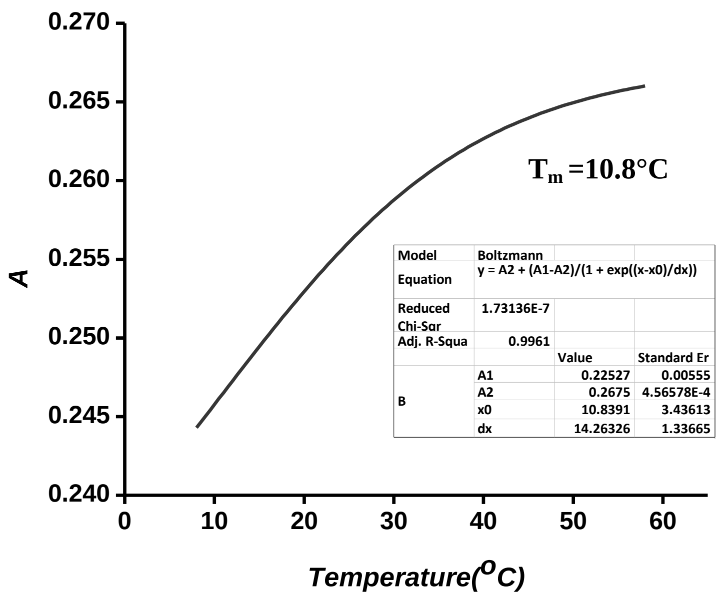


Figure S76: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO24-DNA **(B)** First derivative plot PMO24-DNA

5′-T-T-T-T-A-C-C-***U***-A-C-A-T-3′

(A)

3′-$A_rA_rA_rA_rU_rG_rA_rG_rU_rG_rU_rA_r$-5′



(B)

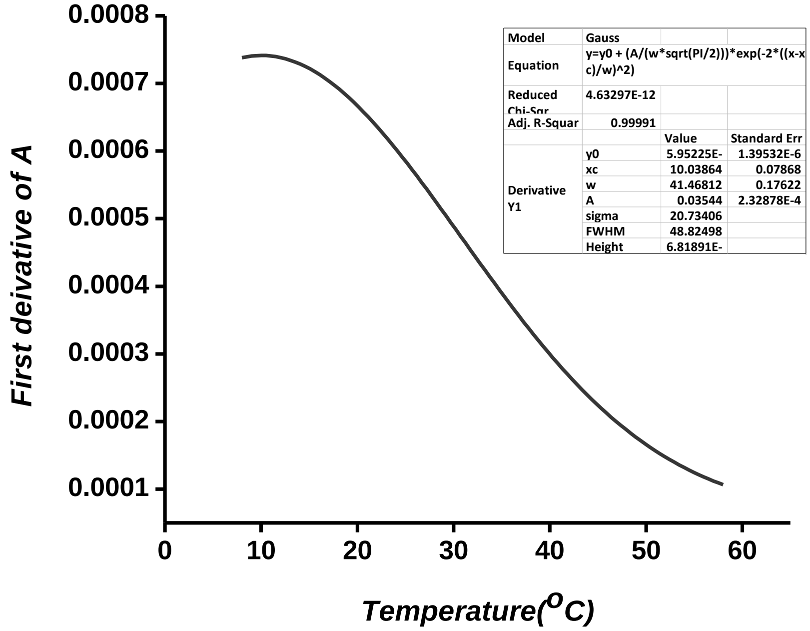


Figure S77: Thermal melting curve of duplexes in 40mM phosphate buffer (pH = 7) **(A)** PMO24-RNA **(B)** First derivative plot PMO24-RNA

9. CD Spectra of duplexes

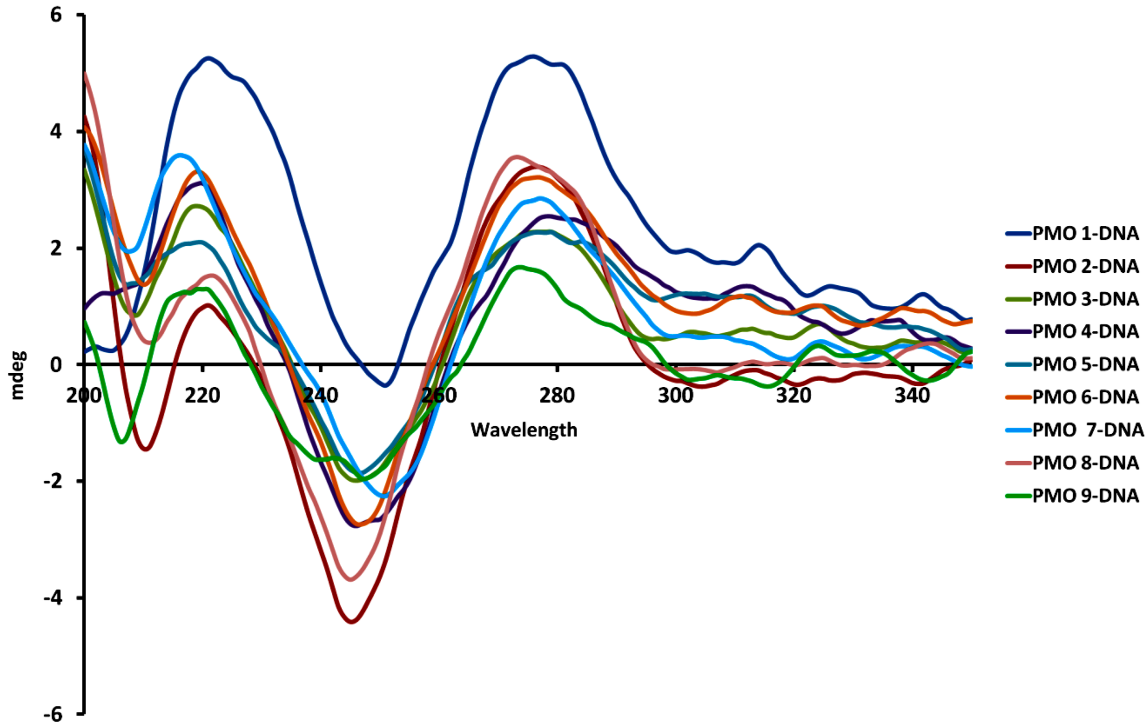


Figure S78: CD-spectra of PMOs **(PMO 2-PMO 9, C-alkynes and U-alkynes)** with DNA

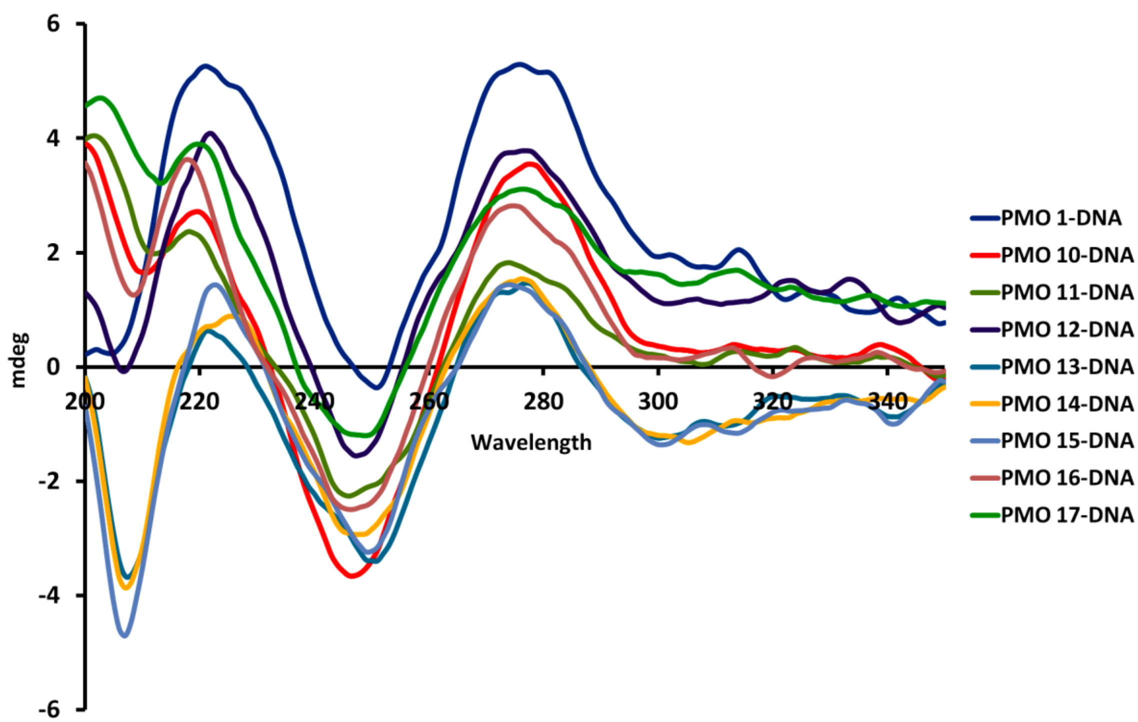


Figure S79: CD-spectra of PMOs **(PMO 10-PMO 17, C and U-substituted aryls)** with DNA

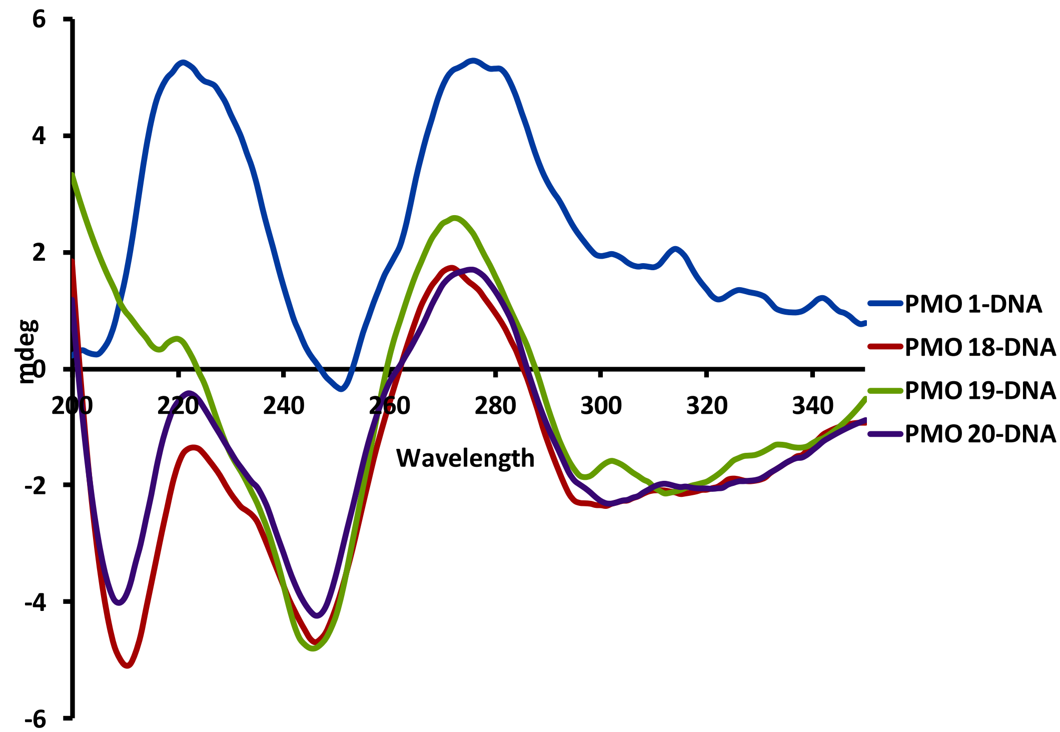


Figure S80: CD-spectra of PMOs **(PMO 18, PMO 19, PMO 20, U-iodo, m5C and C-iodo)** with DNA

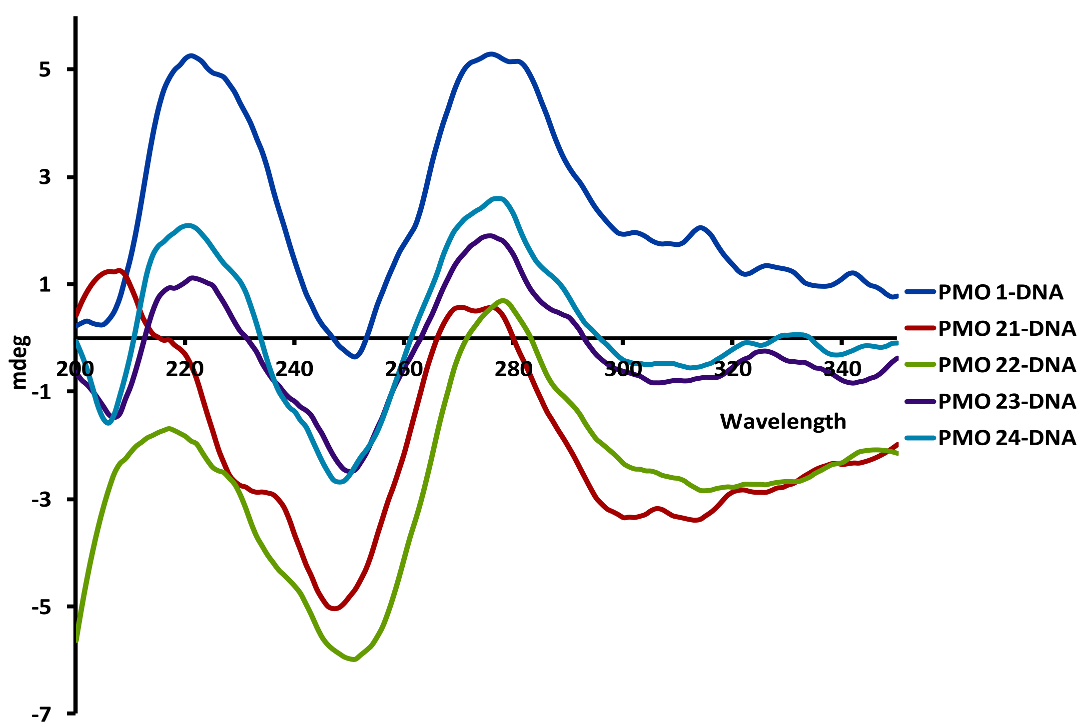


Figure S81: CD-spectra of PMOs **(PMO 21-24, mismatch sequences)** with DNA

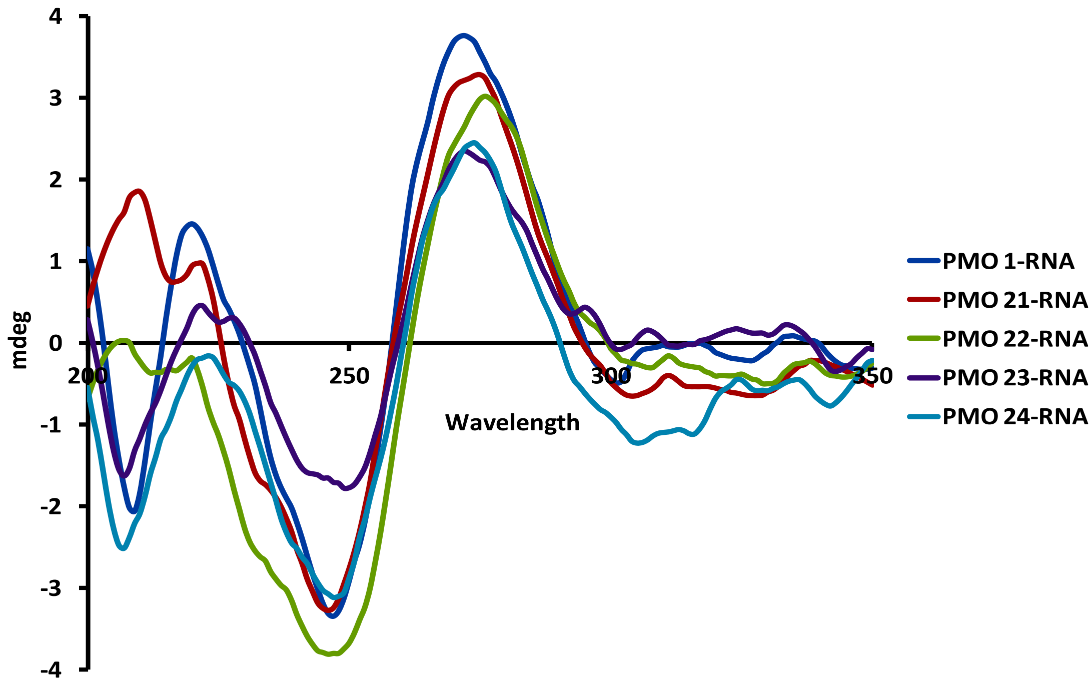


Figure S82: CD-spectra of PMOs **(PMO 21-24, mismatch sequences)** with RNA

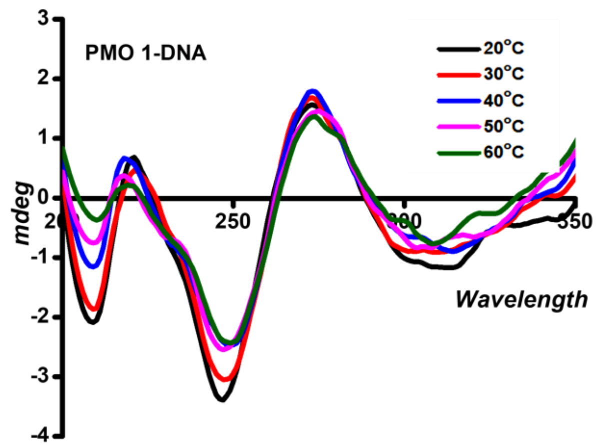


Figure S83: Variable temperature CD-spectra of PMO 1with DNA showing the melting profile. Conditions: 40 mM phosphate buffer (pH 7). The concentration of duplex is 2 µM.

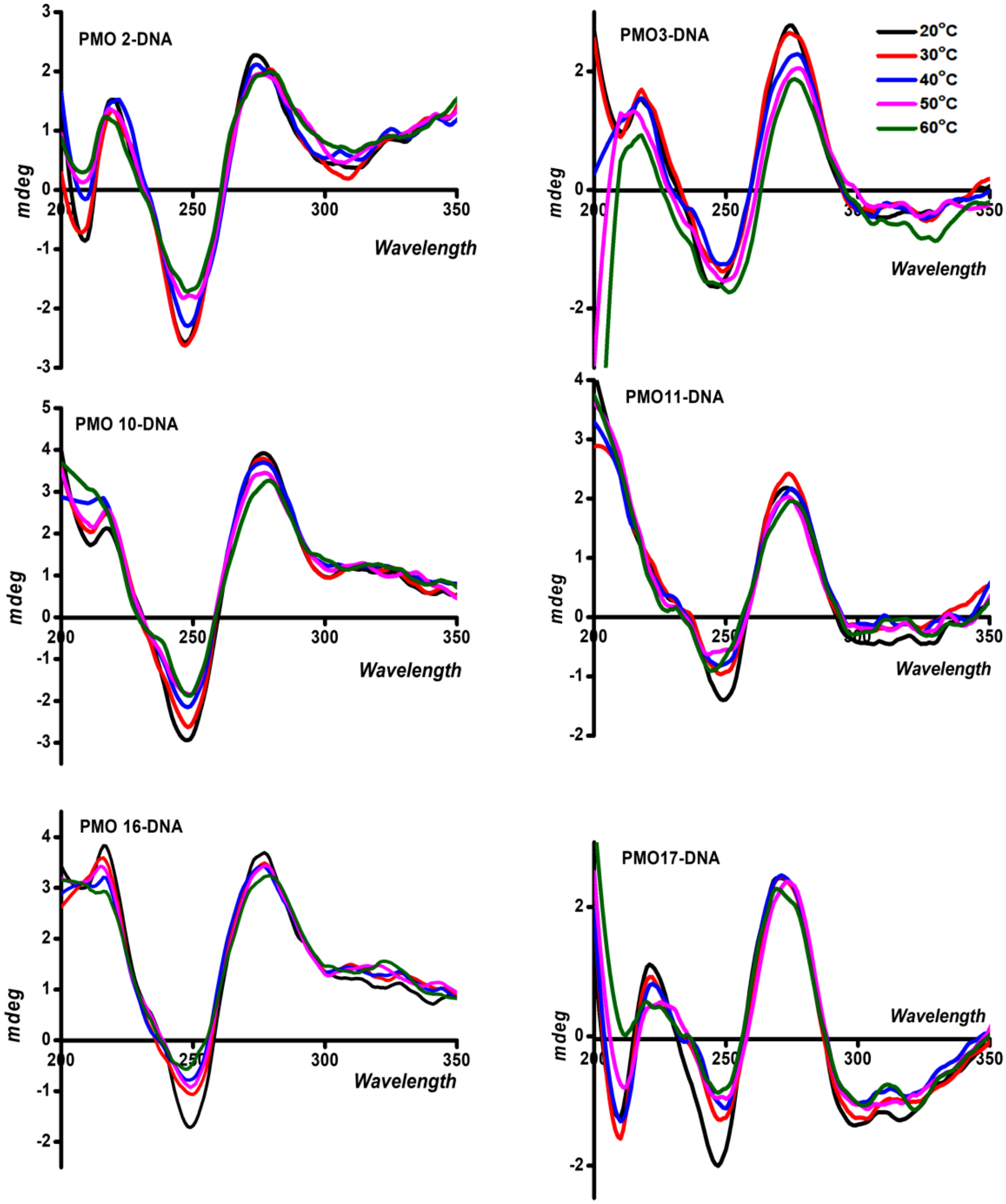


Figure S84: Variable temperature CD-spectra of PMOs (PMO 2,3,10,11,16 and 17) with DNA showing the melting profile. Conditions: 40 mM phosphate buffer (pH 7). The concentration of duplex is 2 µM.

10. X-ray crystallographic data of the compound 1g

Single crystal of compound **1g** was obtained via slow evaporation from EtOAc.

Crystal data for the compound 1**g** was collected at 145 K on a Bruker D8VENTURE Micro-focus diffractometer equipped with PHOTON II Detector, with Mo K_{α} radiation (λ = 0.71073 Å), controlled by the APEX3 (v2017.3-0) software package. The raw data were integrated and corrected for Lorentz and polarization effects with the aid of the Bruker APEX III program suite. Absorption corrections were performed by using SADABS. Space groups were assigned by systematic absences (determined by XPREP) and analysis of metric symmetry and were further checked by PLATON for additional symmetry. Structures were solved by direct methods and refined against all data in the reported 2θ ranges by full-matrix least squares on F2 using the SHELXL program suite in the OLEX 2 interface. Hydrogen atoms at idealized positions were included in final refinements. The OLEX 2 interface was used for structure visualization as well as for drawing ORTEP plots.

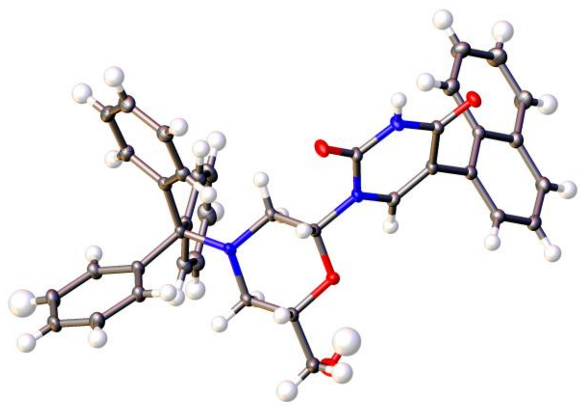


Figure S85: Single crystal structure of compound **1g**

Table S5: Crystal data and structure refinement for mo_SS290422_0ma_a.	
Identification code	mo_SS290422_0ma_a
Empirical formula	$C_{168}H_{164}N_{12}O_{24}$
Formula weight	2735.10
Temperature/K	144.98
Crystal system	orthorhombic
Space group	$P2_12_12$
a/Å	15.094(3)
b/Å	18.129(3)
c/Å	14.040(2)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	3841.9(12)
Z	1
ρ_{calc}g/cm^3	1.182

μ/mm^{-1}	0.079
F(000)	1448.0
Crystal size/mm^3	0.035 × 0.032 × 0.028
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	4.494 to 50.124
Index ranges	-17 ≤ h ≤ 17, -21 ≤ k ≤ 21, -14 ≤ l ≤ 16
Reflections collected	25718
Independent reflections	6777 [R_{int} = 0.0913, R_{sigma} = 0.0857]
Data/restraints/parameters	6777/0/472
Goodness-of-fit on F^2	1.026
Final R indexes [I>=2σ (I)]	R_1 = 0.0493, wR_2 = 0.1117
Final R indexes [all data]	R_1 = 0.0805, wR_2 = 0.1314
Largest diff. peak/hole / e Å^{-3}	0.33/-0.19
Flack parameter	-0.5(10)
Deposition Number	2207095

11. References

1. S. Paul, B. Nandi, S. Pattanayak, S. Sinha, Synthesis of 5-alkynylated uracil–morpholino monomers using Sonogashira coupling. *Tetrahedron Lett.* 2012, **53**, 4179-4183.
2. B. Nandi, S. Pattanayak, S. Paul, S. Sinha, Synthesis of Nucleobase-Functionalized Morpholino-Modified Nucleoside Monomers Through Palladium-Catalyzed Cross-Coupling Reactions. *Eur. J. Org. Chem.* 2013, **7**, 1271–1286.
3. J. Kundu, A. Ghosh, U. Ghosh, A. Das, D. Nagar, S. Pattanayak, A. Ghose, S. Sinha, Synthesis of Phosphorodiamidate Morpholino Oligonucleotides Using Trityl and Fmoc Chemistry in an Automated Oligo Synthesizer. *J. Org. Chem.* 2022, **87,** 9466–9478.