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

Supporting Information

Synthesis and Properties of GuNA Purine/Pyrimidine Nucleosides and Oligonucleotides

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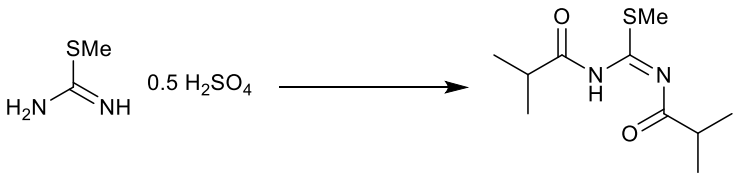
Yuuki Arai, Shuhei Yamakoshi, Katsuya Yamada, Tetsuya Ohta, Eiji Kawanishi,
Naohiro Horie, Takao Yamaguchi, and Satoshi Obika*

Contents

1. Synthesis of *N*,*N*'-diisobutyryl-*S*-methylisothiourea

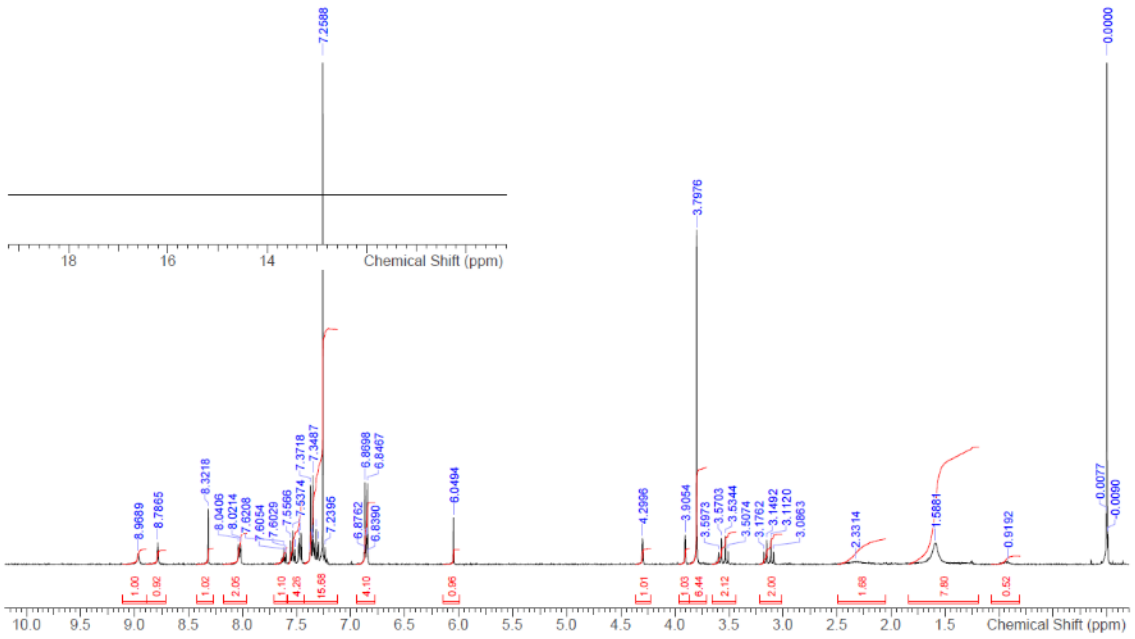


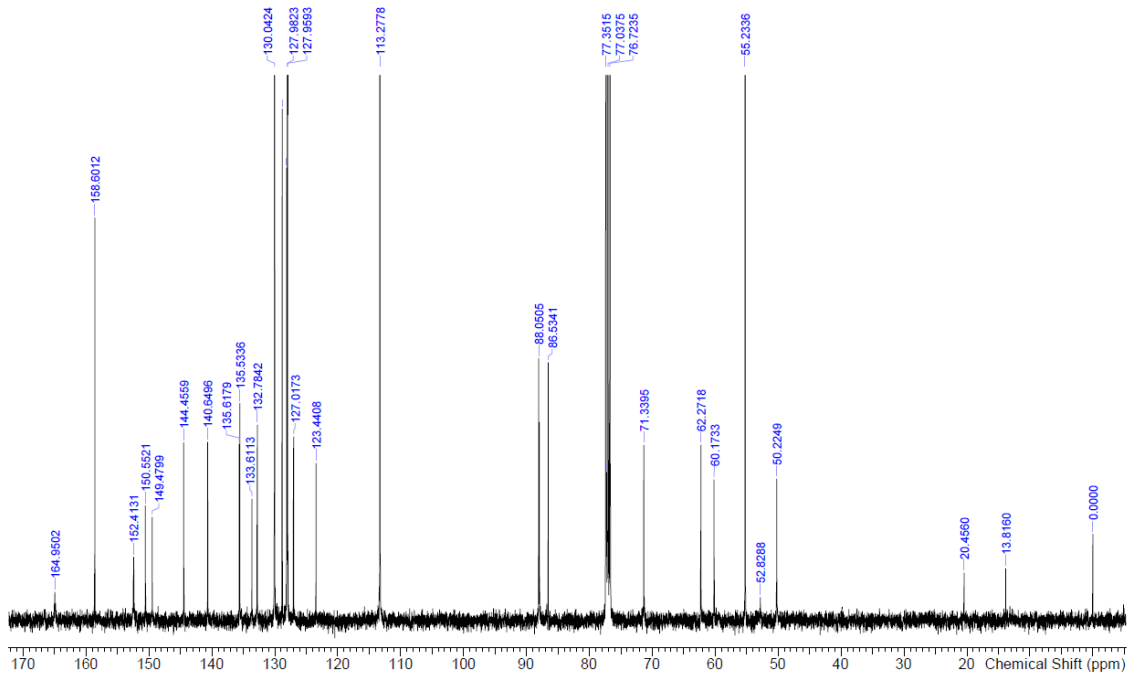
Pyridine (8.7 mL, 108.00 mmol) was added to a suspension of *S*-methylisothiouronium hemisulfate (3.00 g, 21.55 mmol) in dichloromethane (43 mL), and the mixture was cooled in an ice bath. Isobutyryl chloride (6.77 mL, 64.66 mmol) was then added, and the mixture was stirred for two hours. The reaction was quenched with water (20 mL), and the organic layer was separated and concentrated. The resulting residue was purified by column chromatography (hexane/ ethyl acetate, 95/5 to 85/15) to obtain the title compound (4.65 g, yield 93%) as a colorless oil.

^{1}H NMR ($CDCl_3$) δ: 13.19 (1H, brs), 2.65–2.62 (2H, m), 2.41 (3H, s), 1.23 (12H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ: 190.09, 176.39, 171.14, 39.10, 37.57, 19.06, 14.68; HRMS (ESI) Calcd. for $C_{10}H_{19}N_2O_2S$ $[M+H]^+$: 231.1162, Found 231.1159.

2. ^{1}H, ^{13}C, and ^{31}P-NMR spectra of synthesized compounds

5'-*O*-DMTr-2'-amino-LNA-A(bz) (1A)





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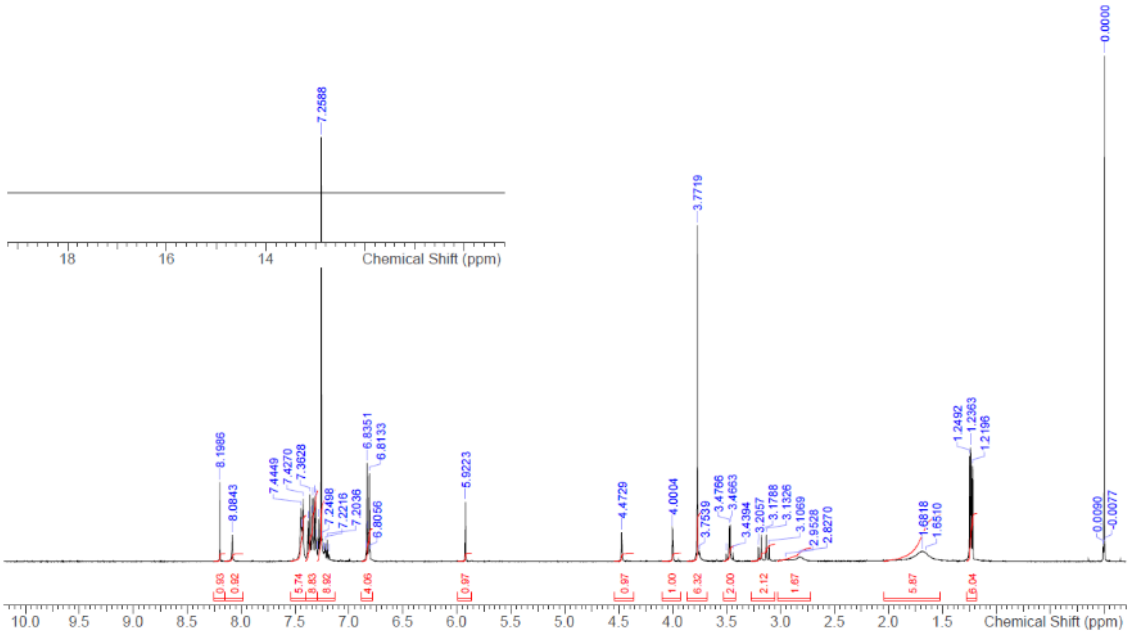

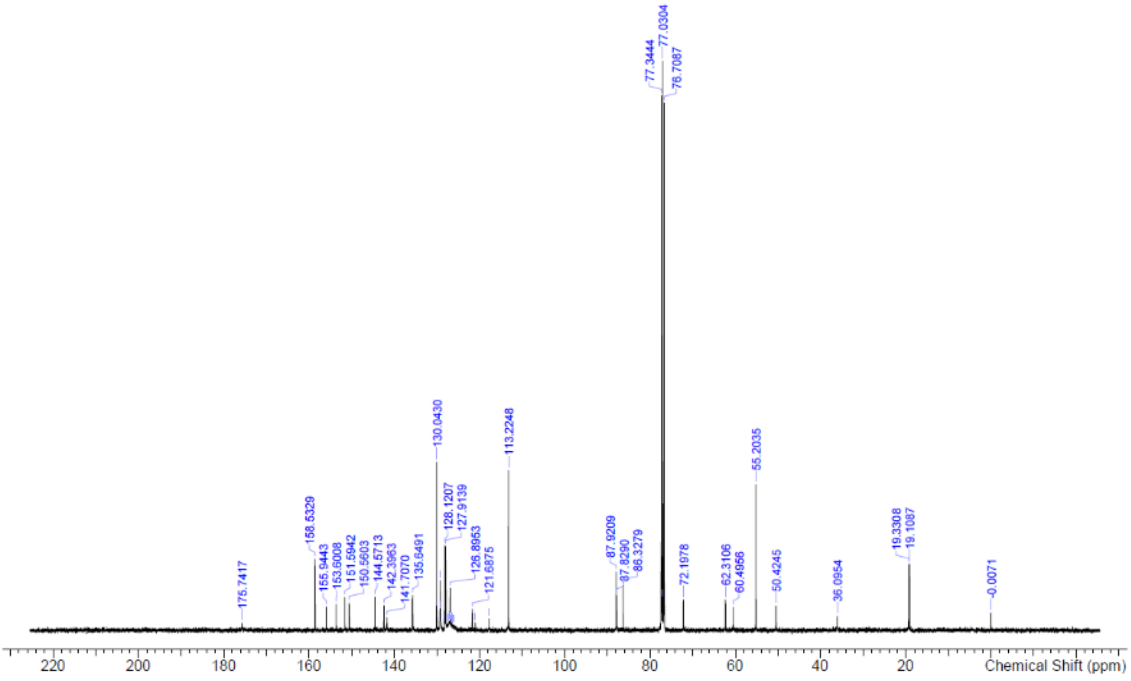

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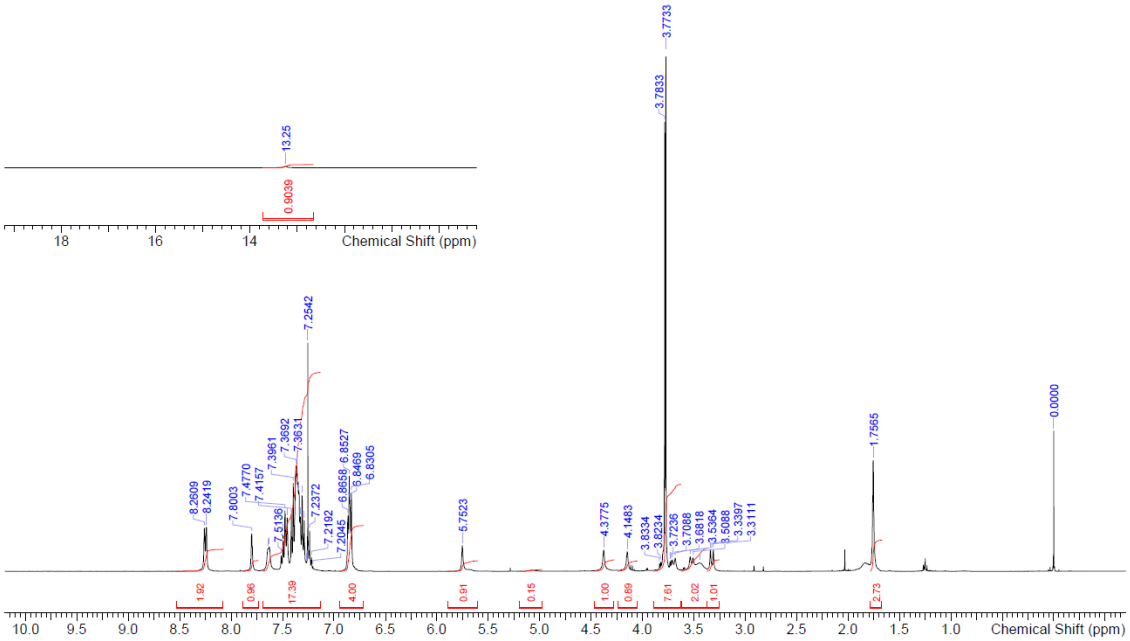

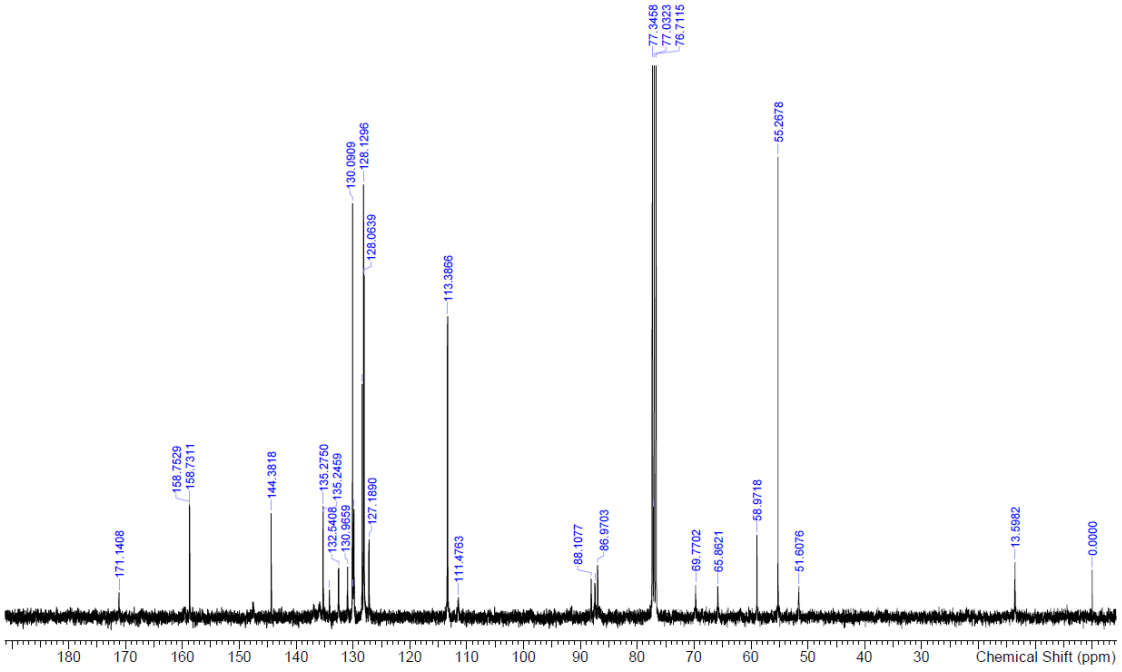

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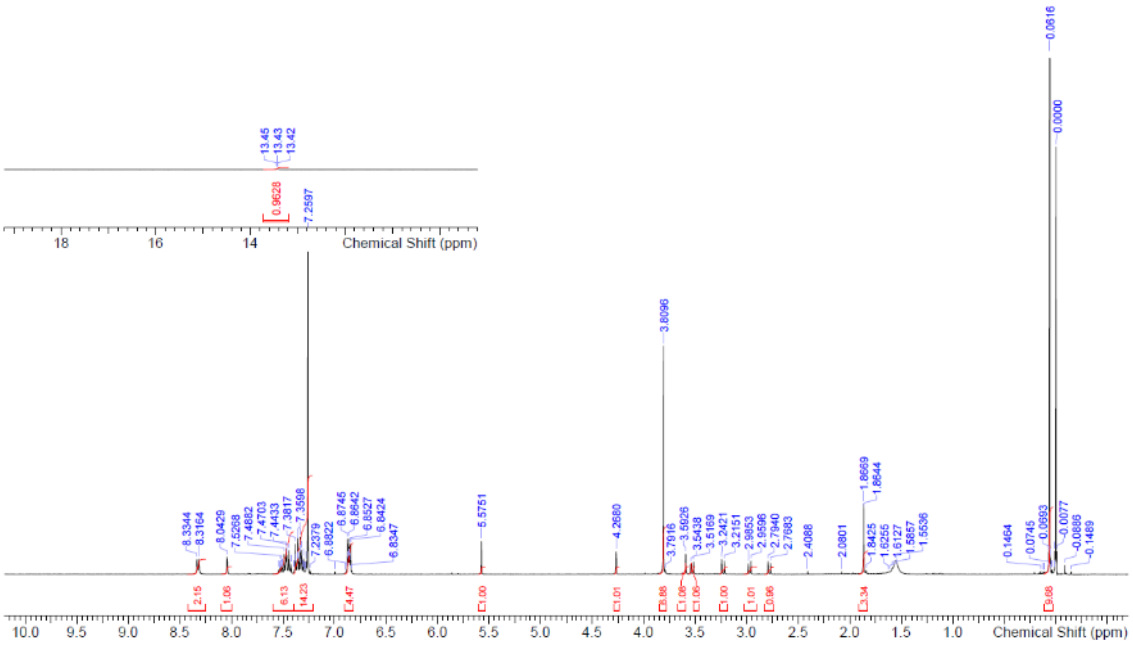

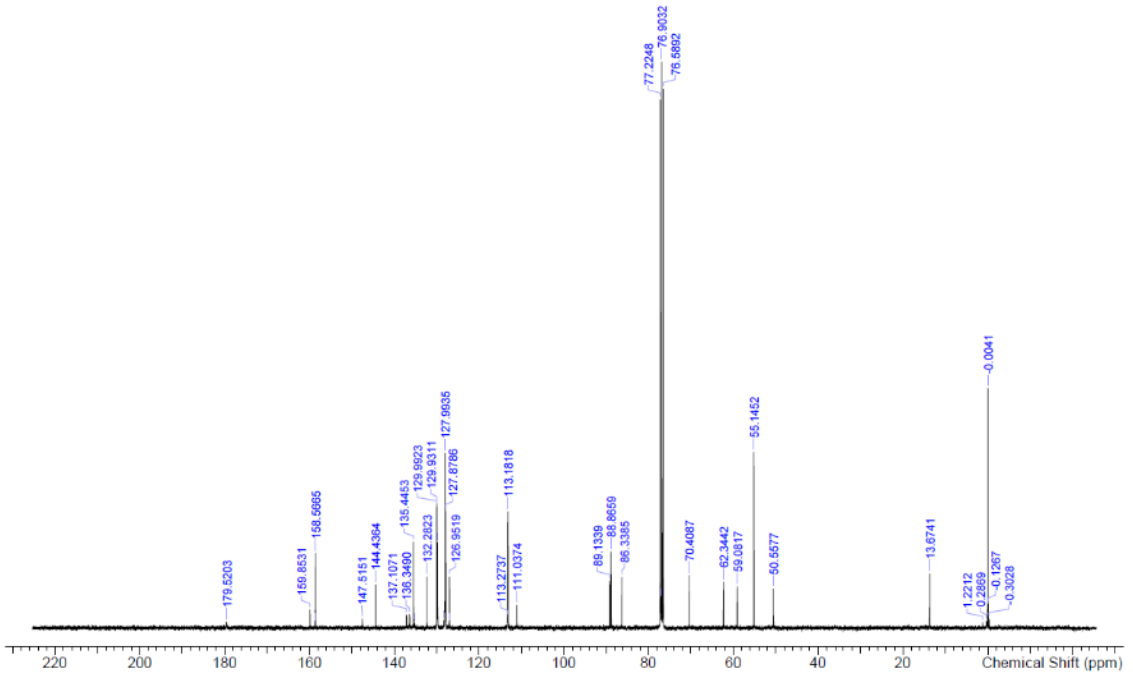

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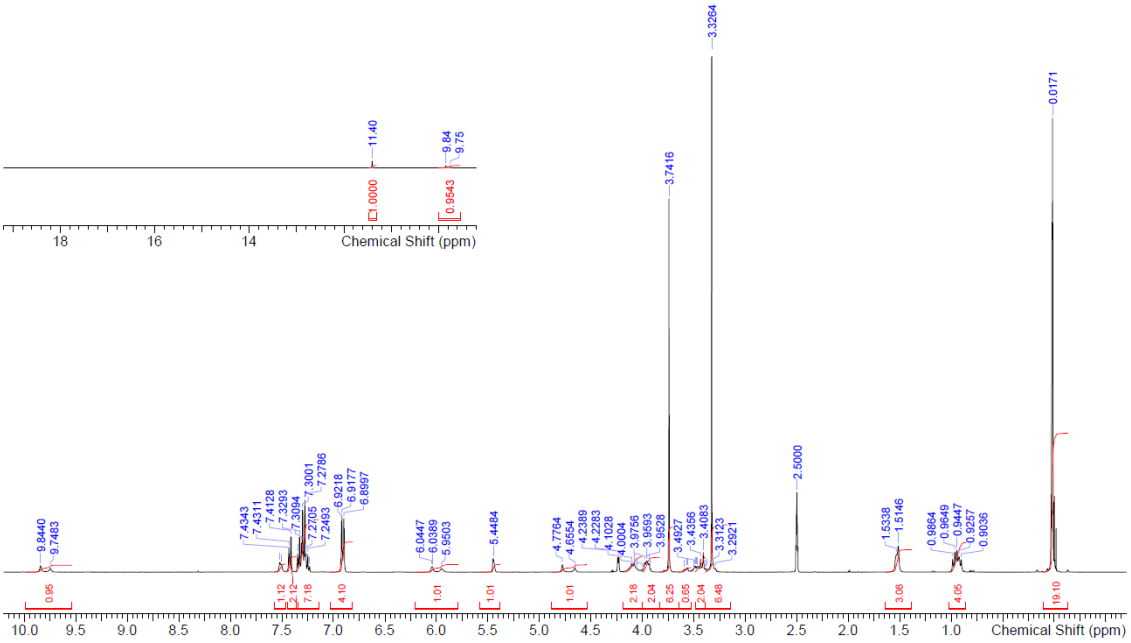

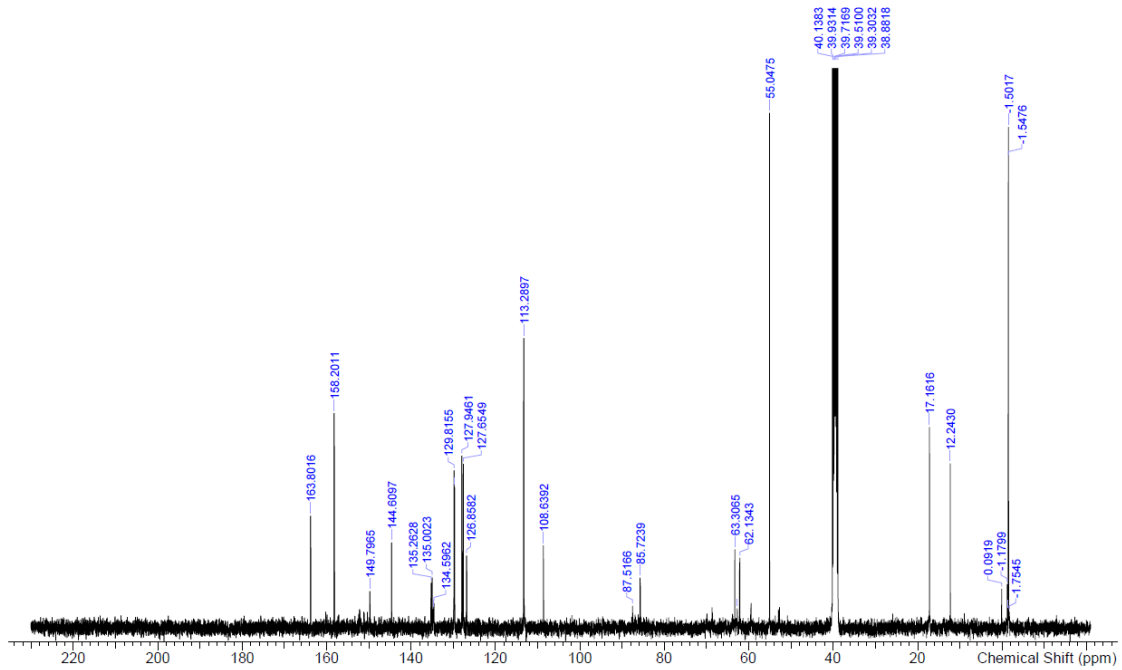

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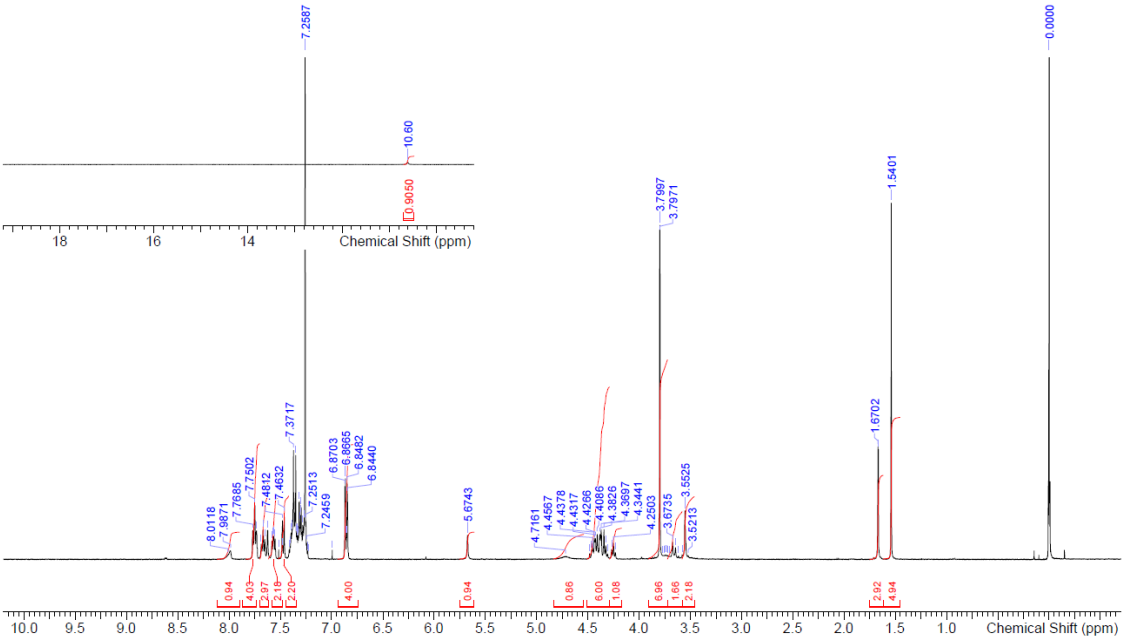

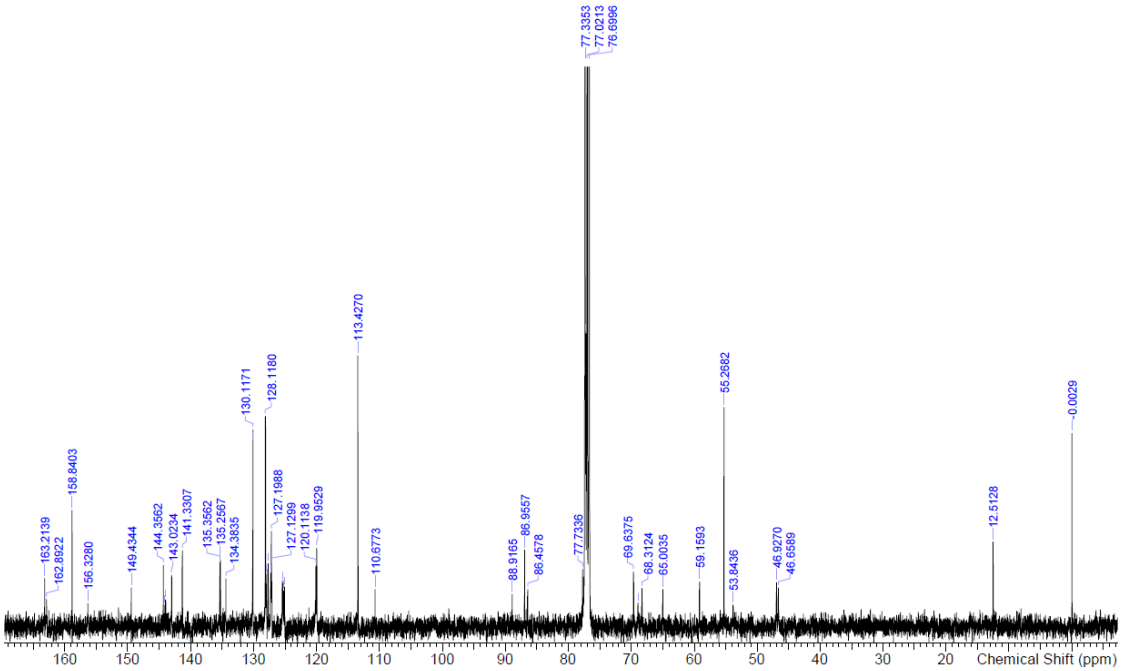

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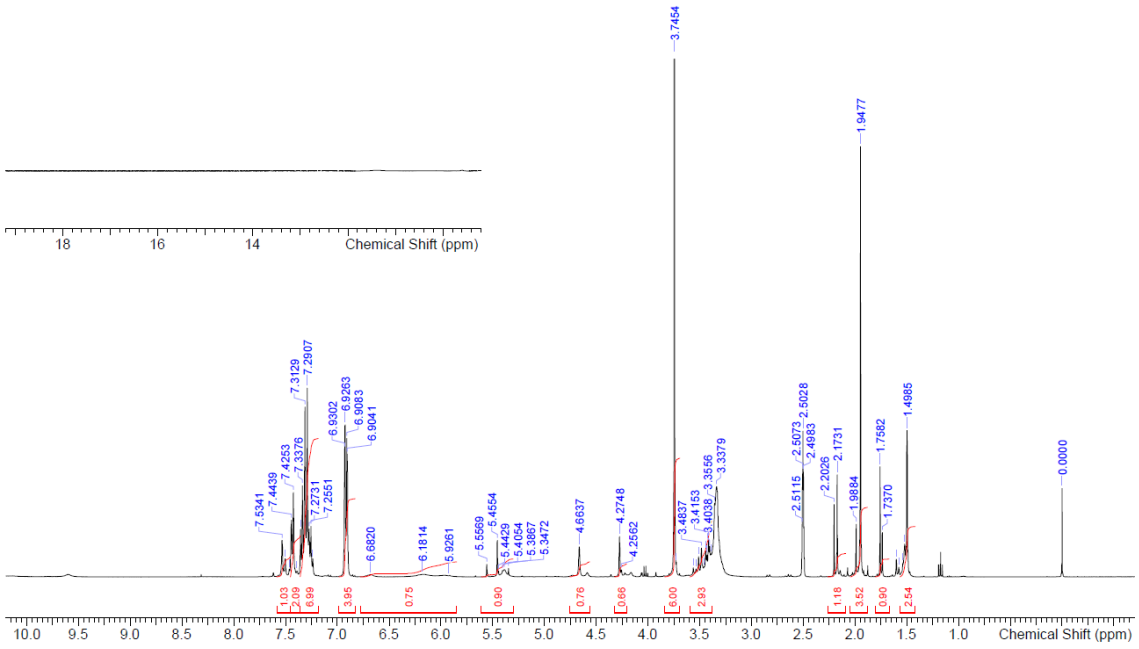

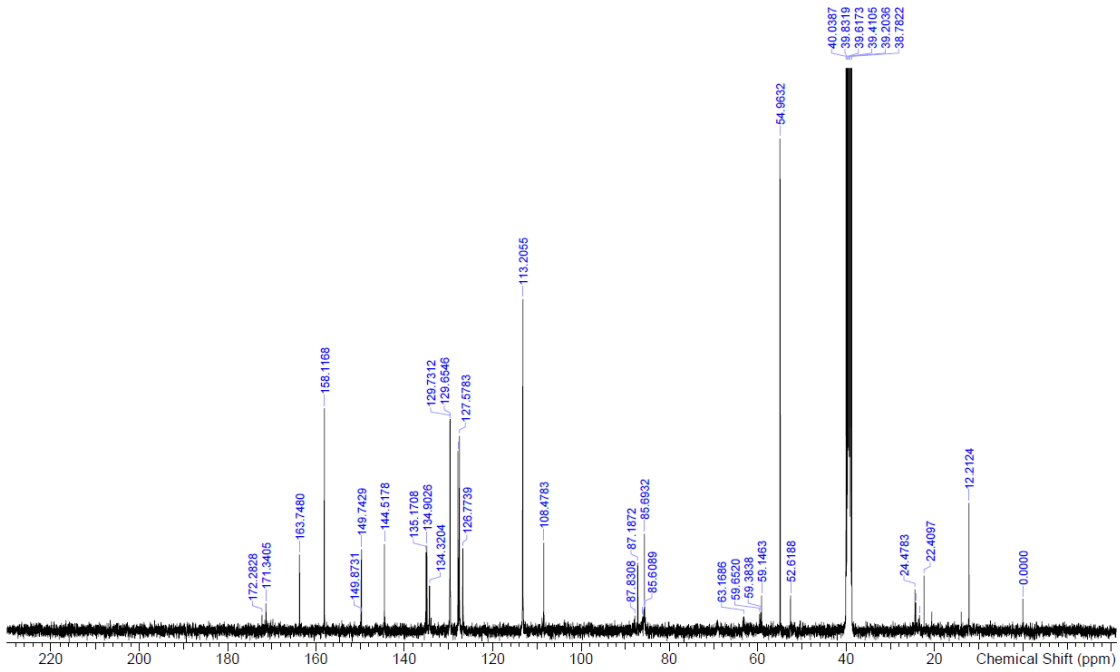

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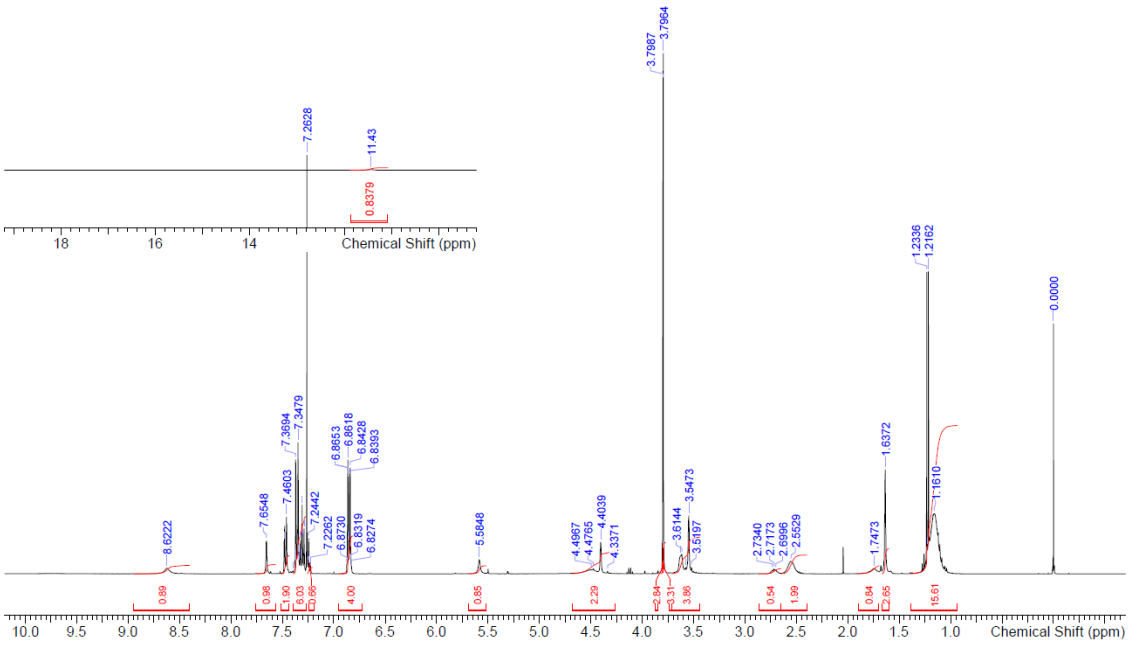

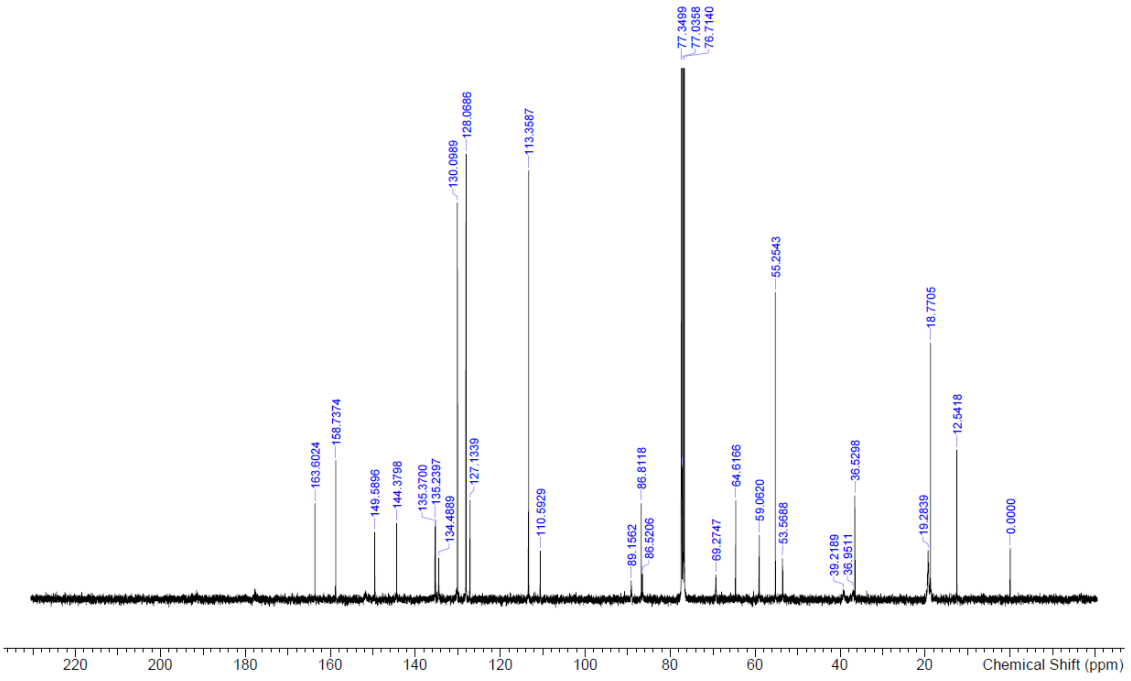

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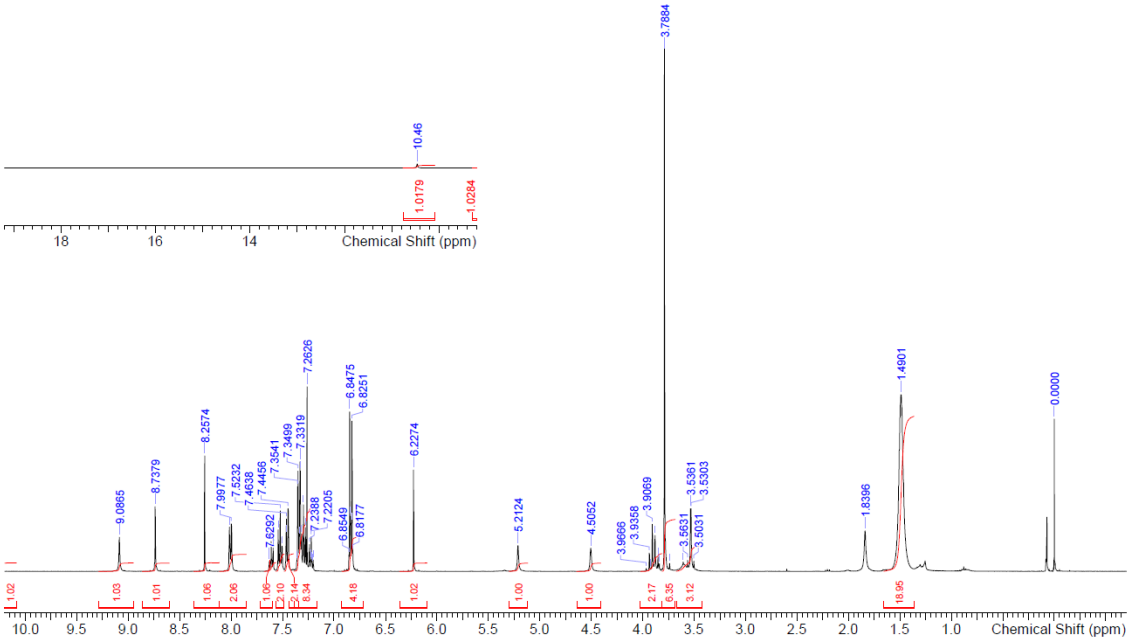

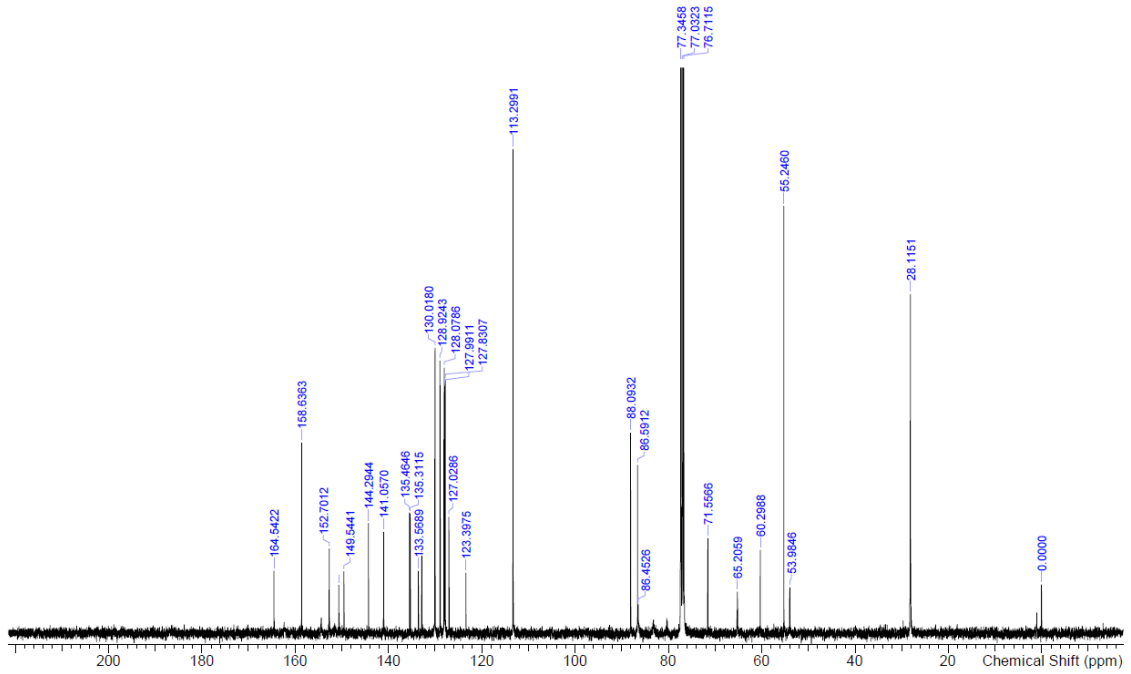

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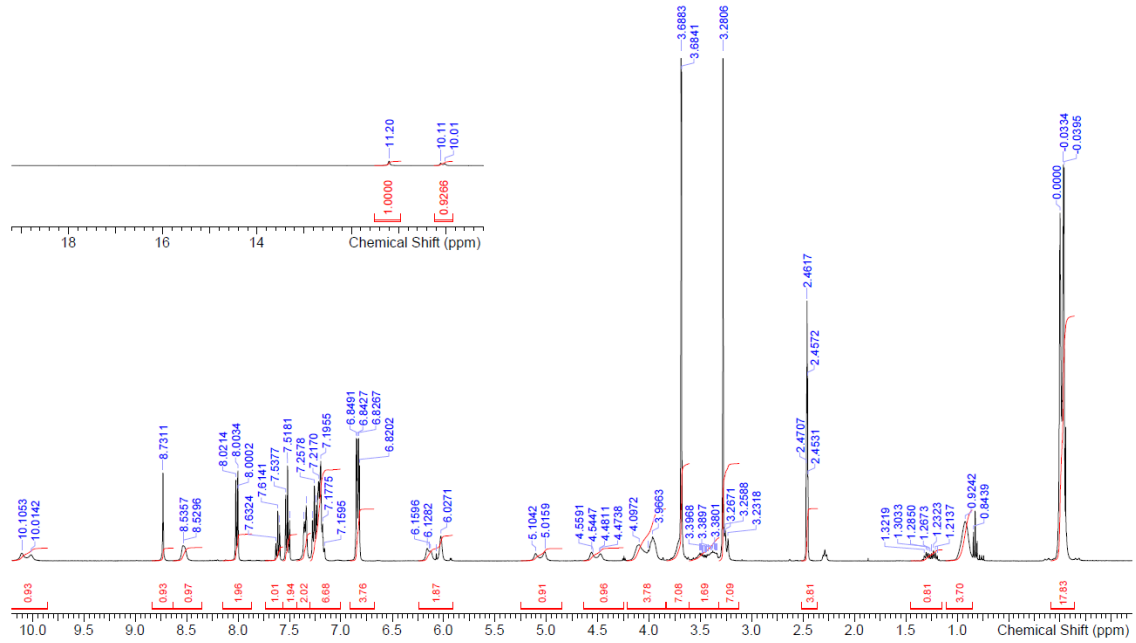

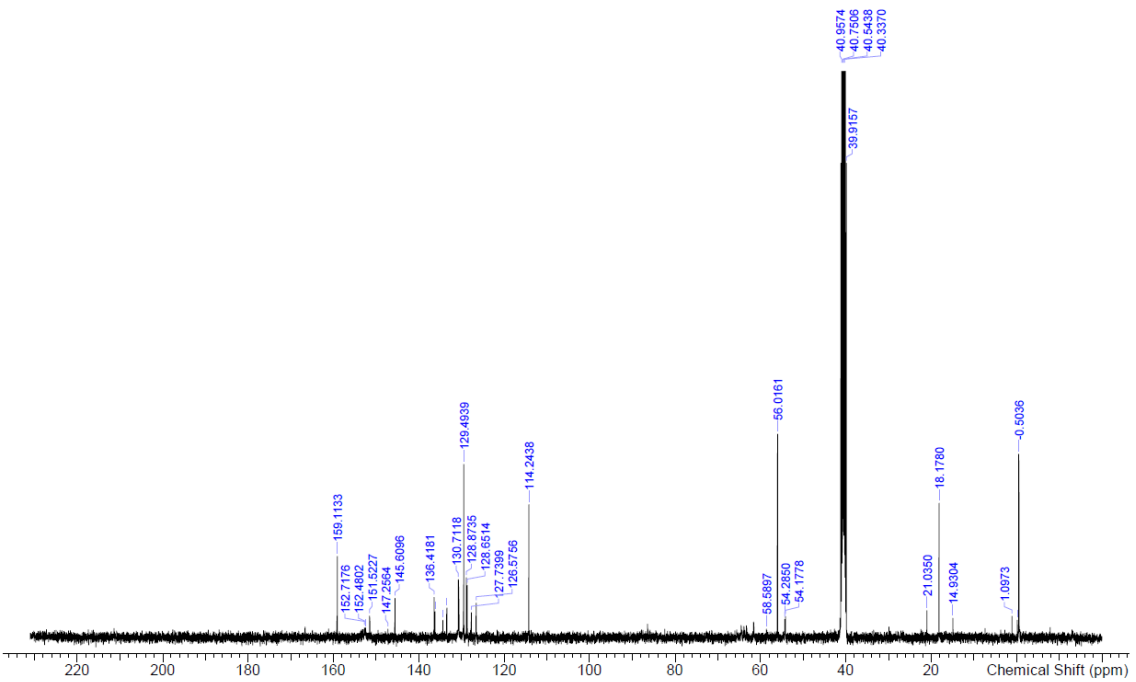

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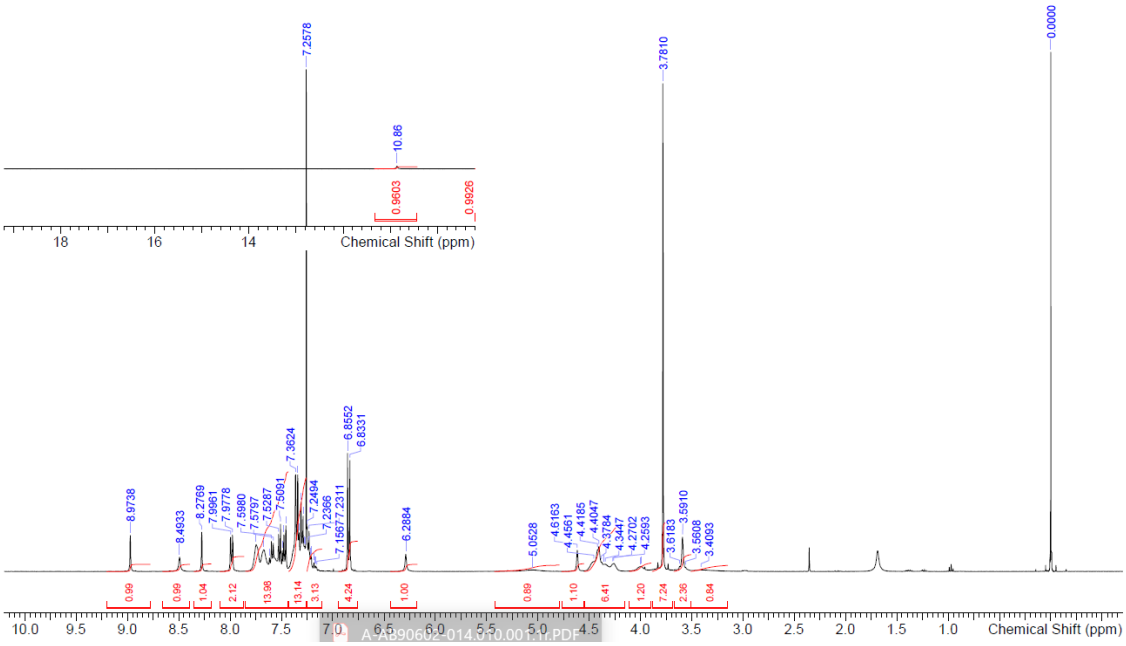

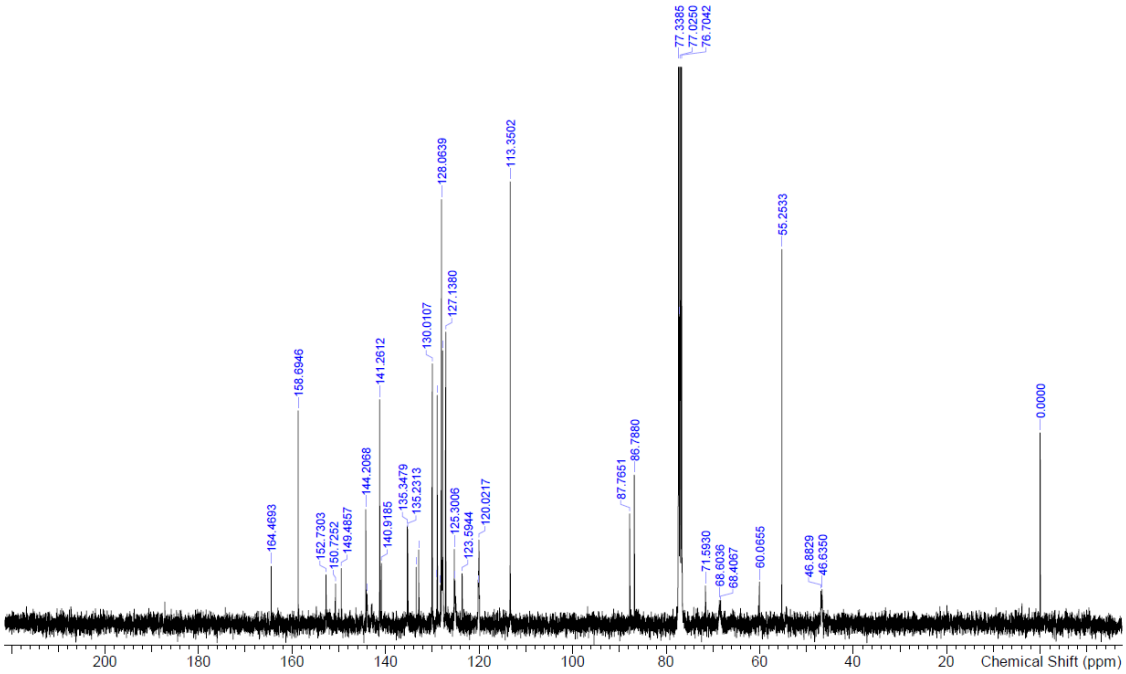

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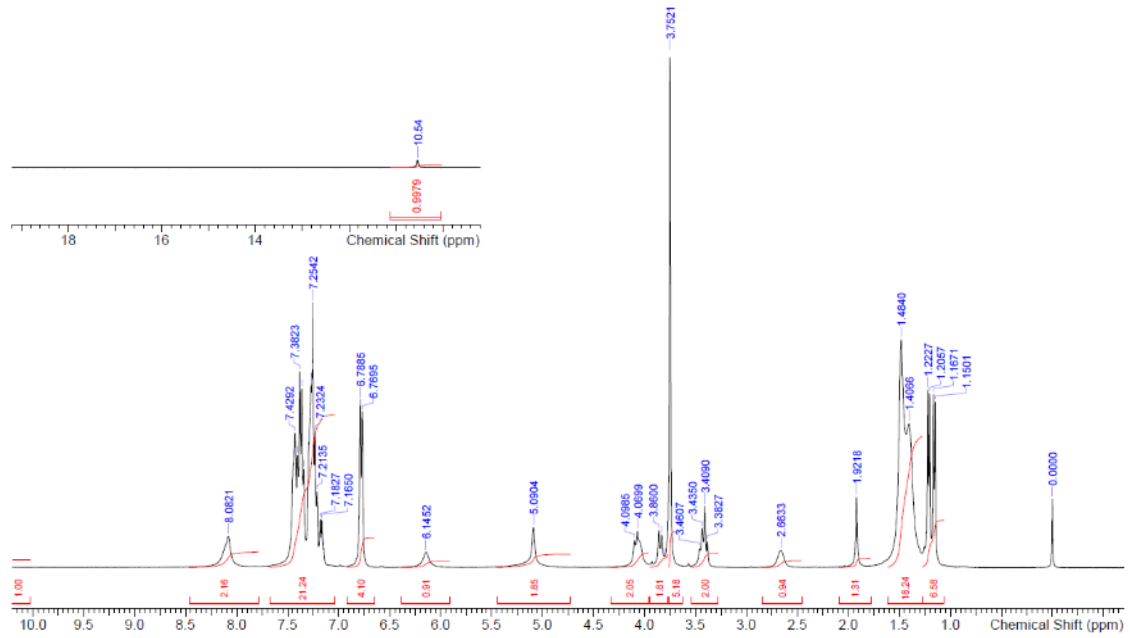

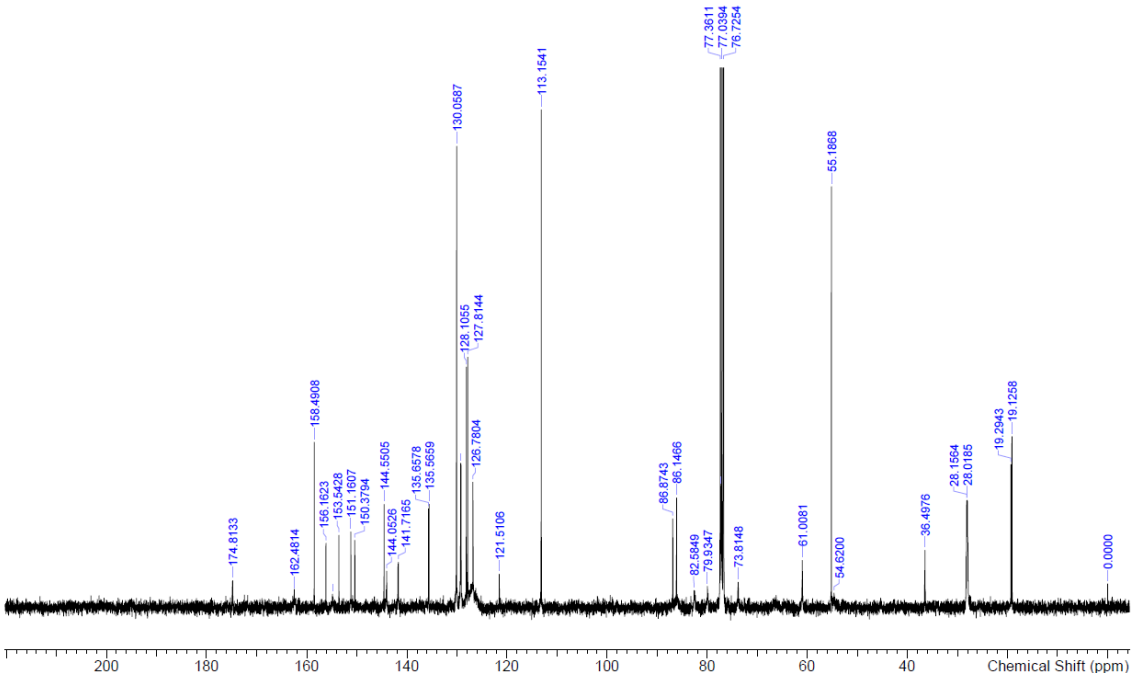

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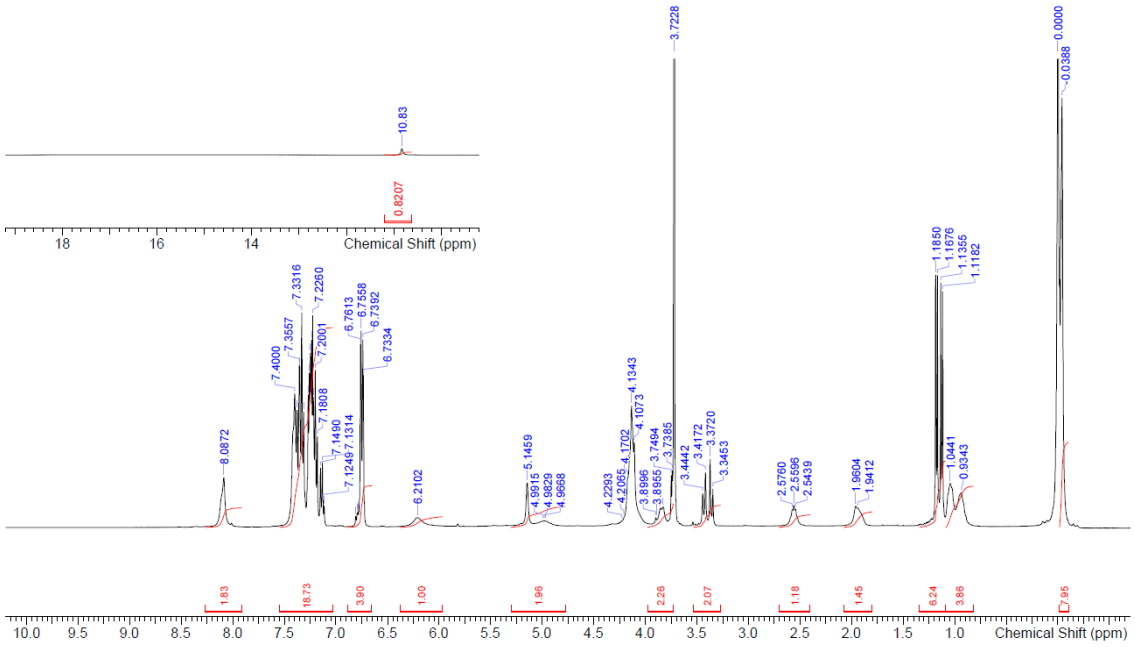

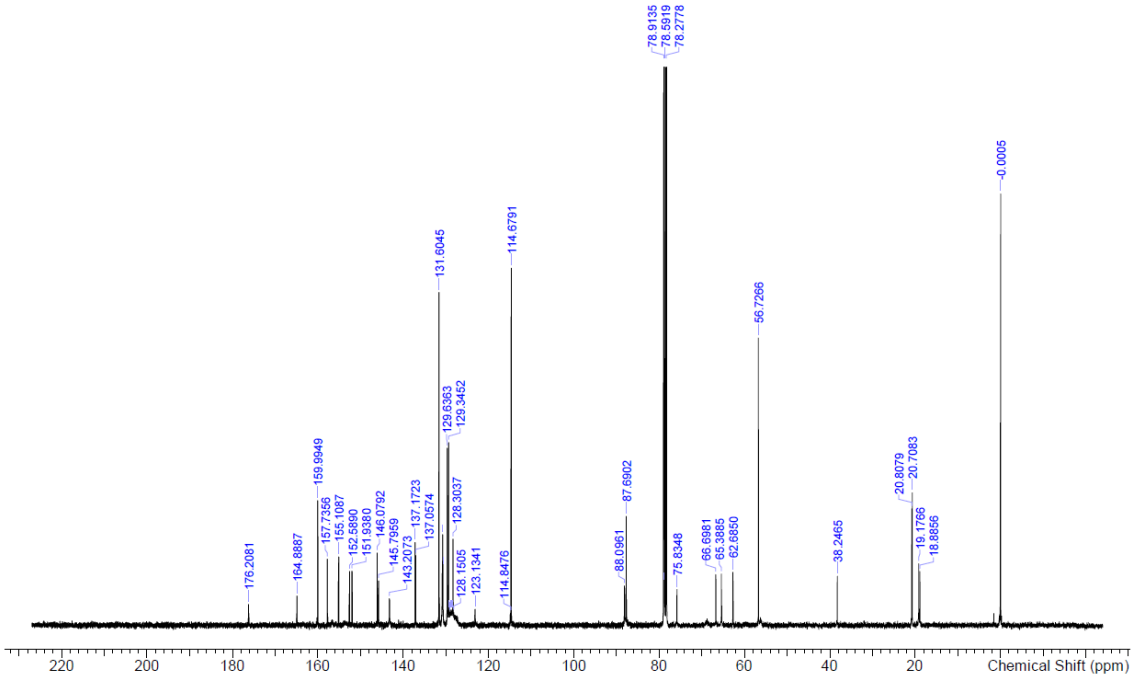

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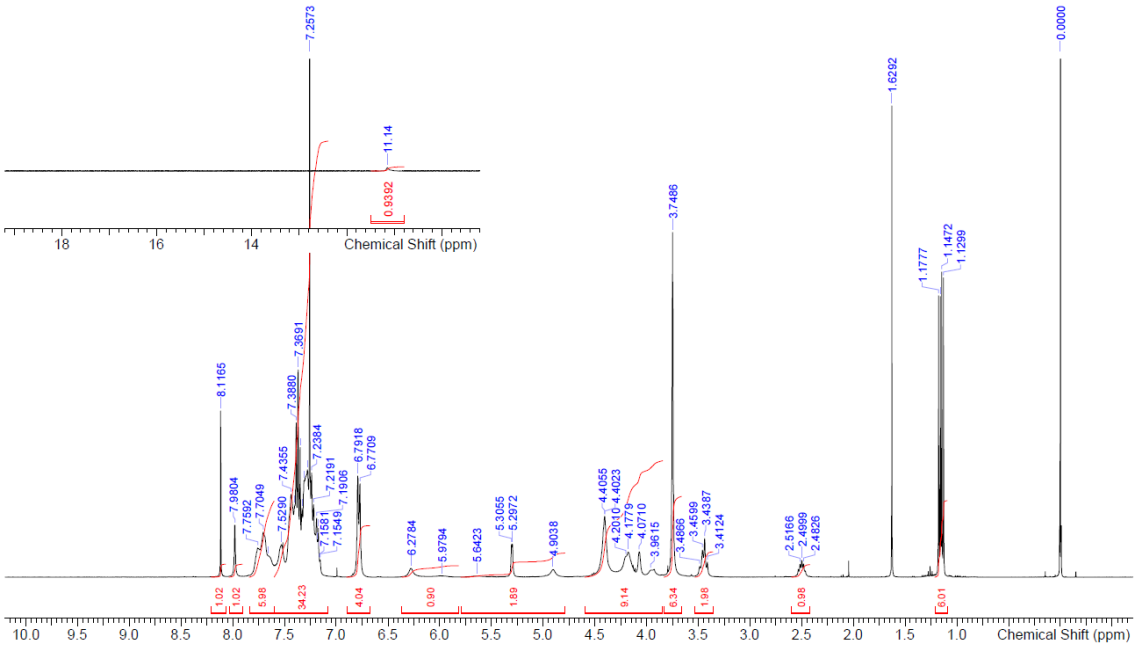

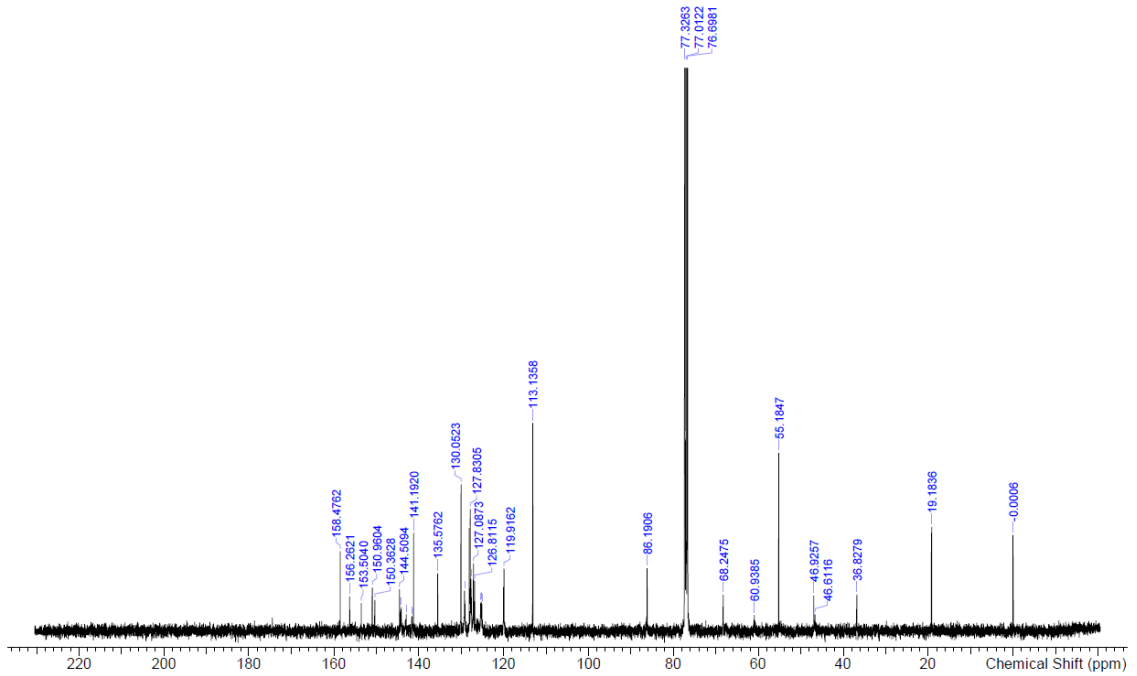

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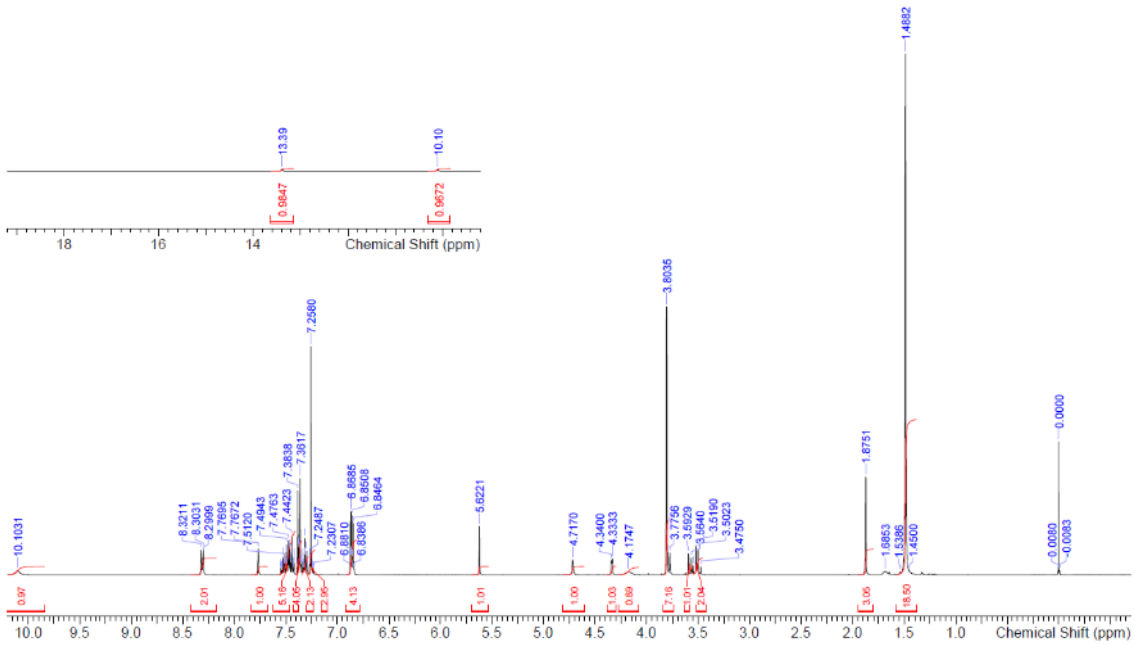

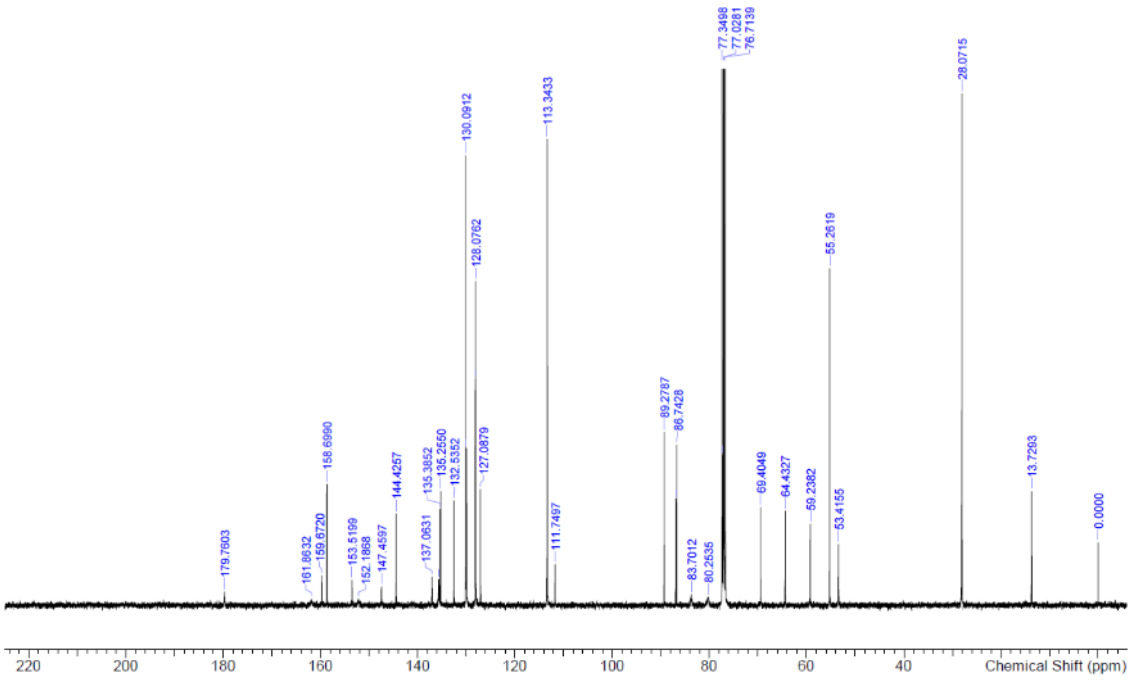

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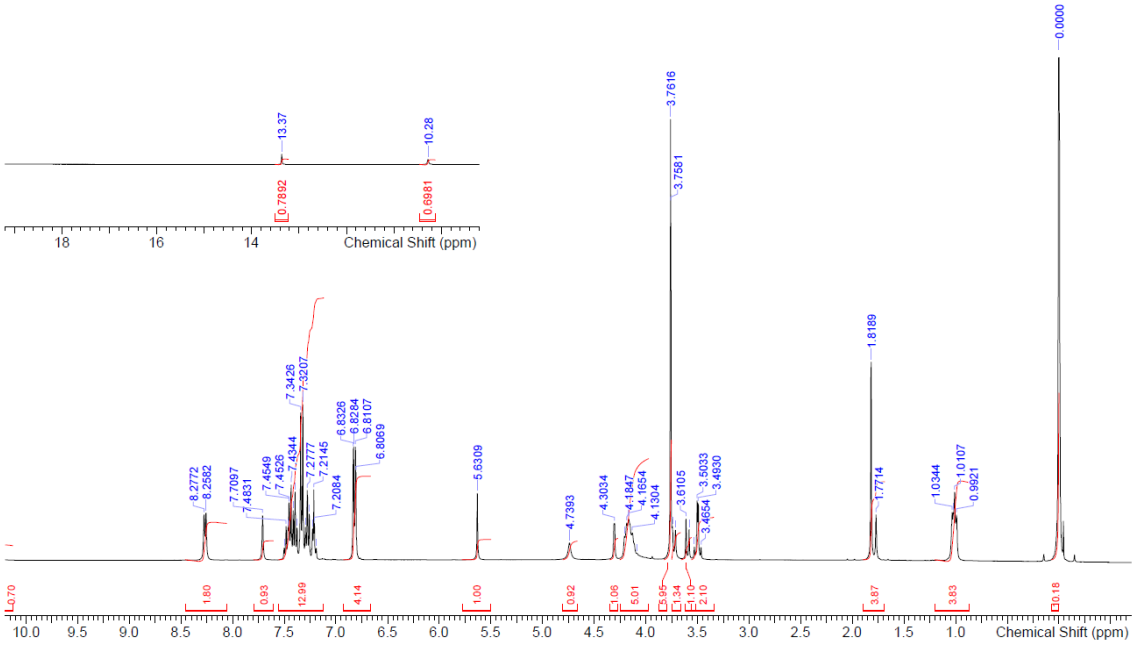

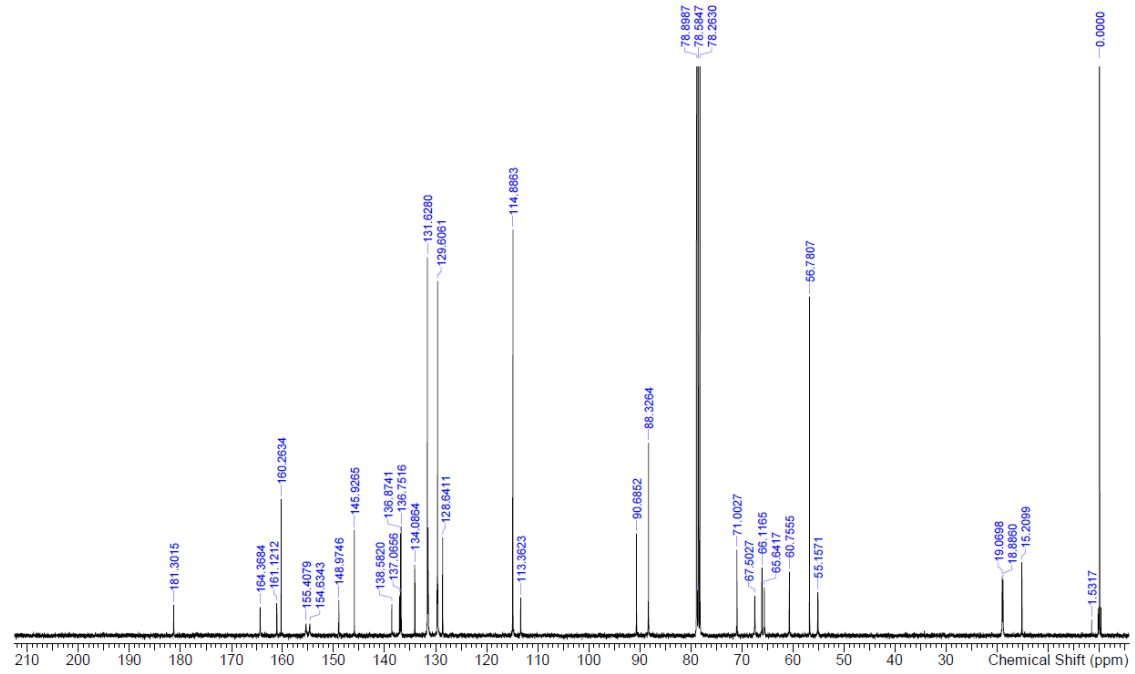

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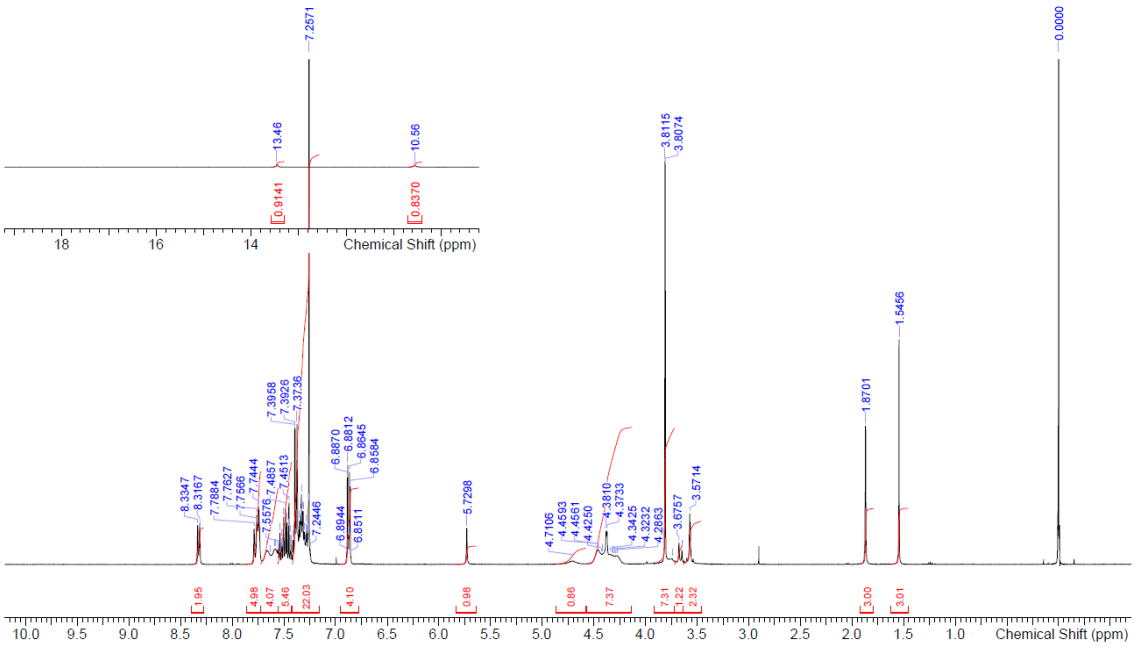

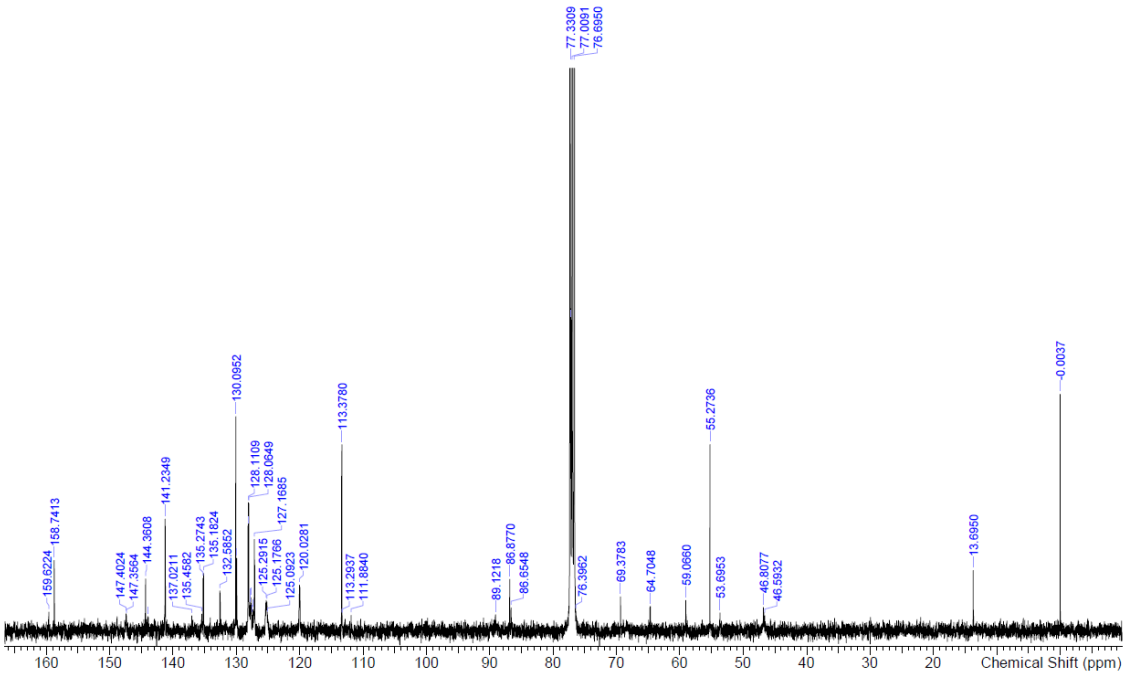

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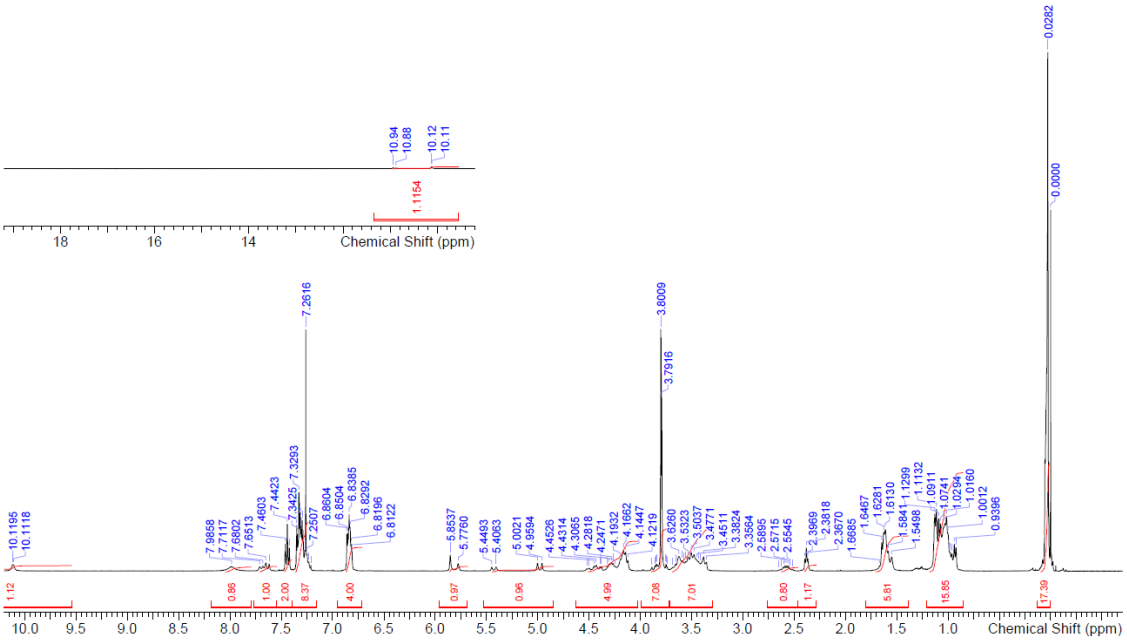

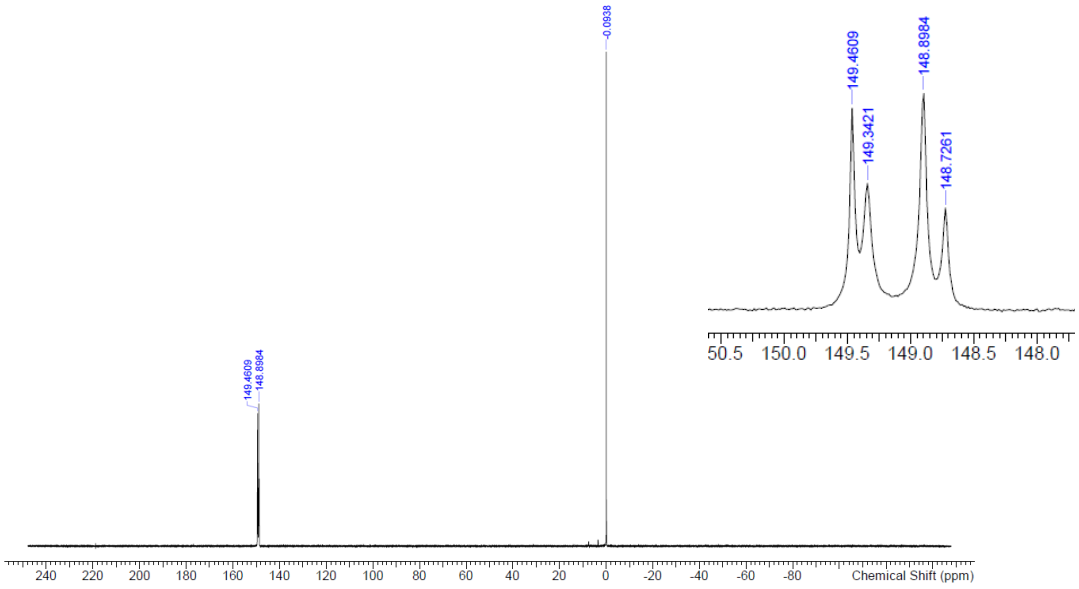

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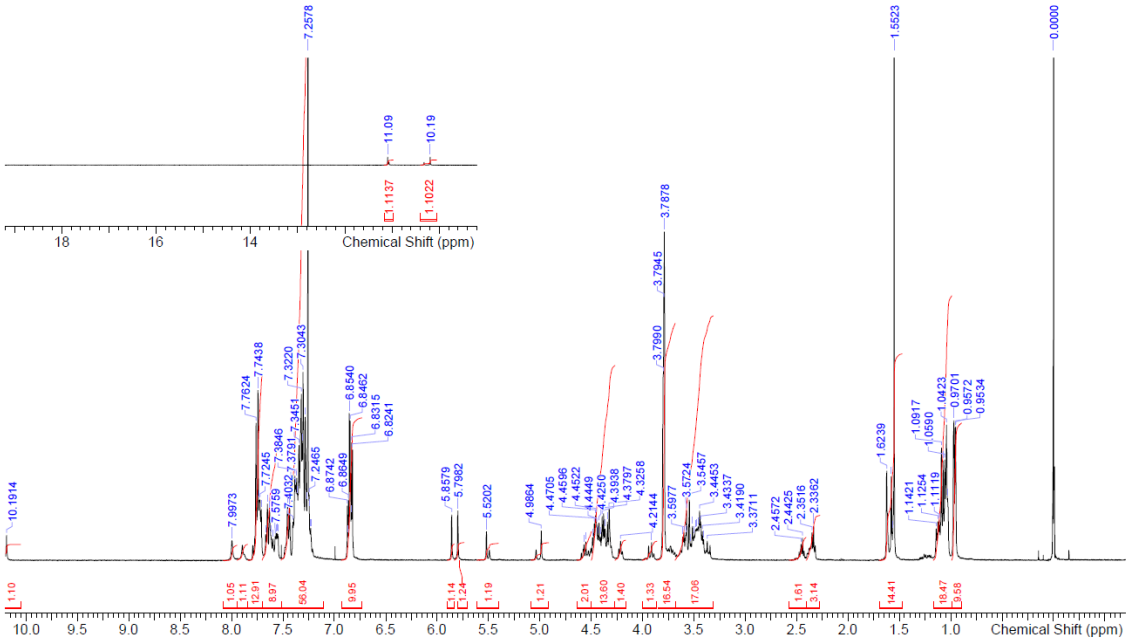

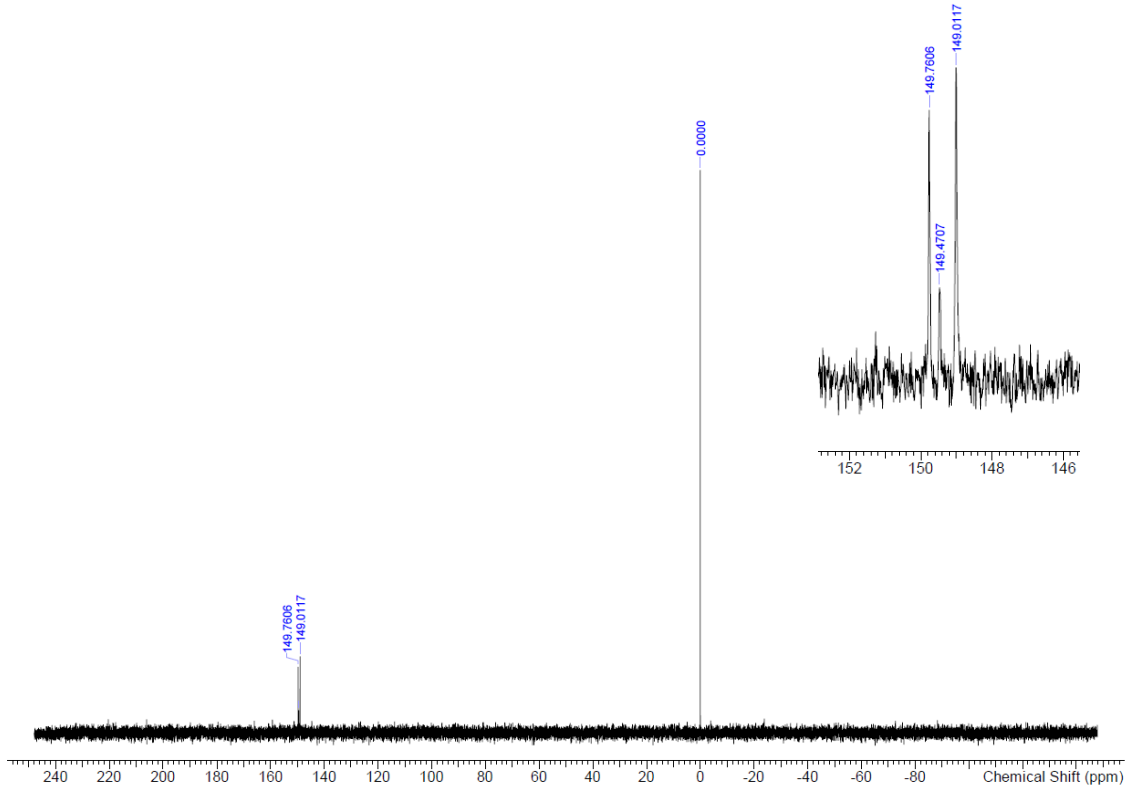

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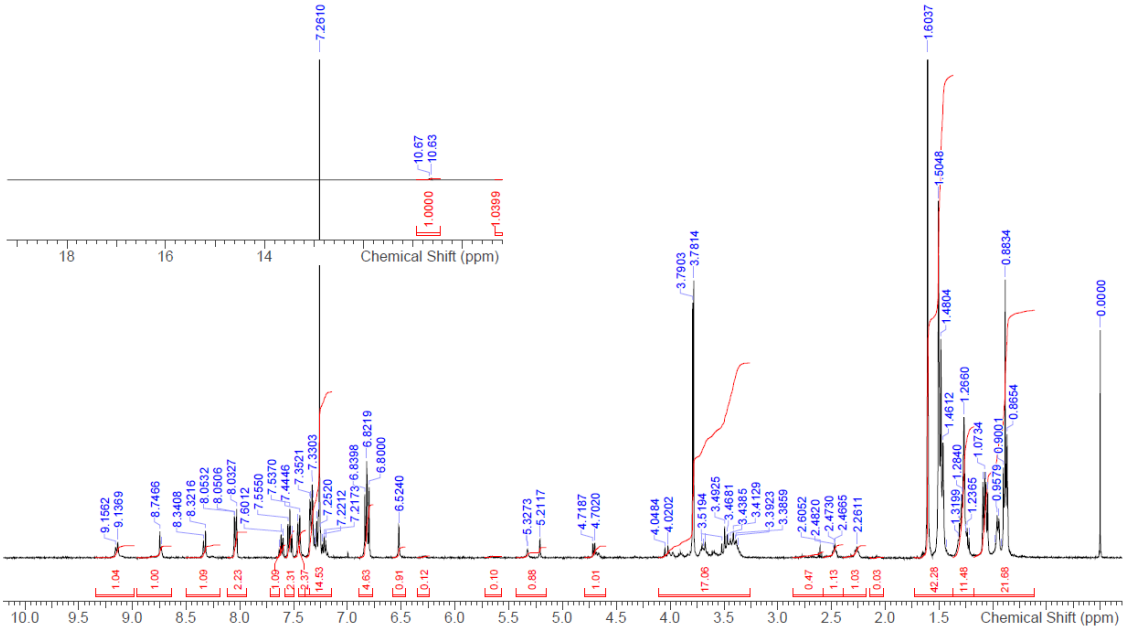

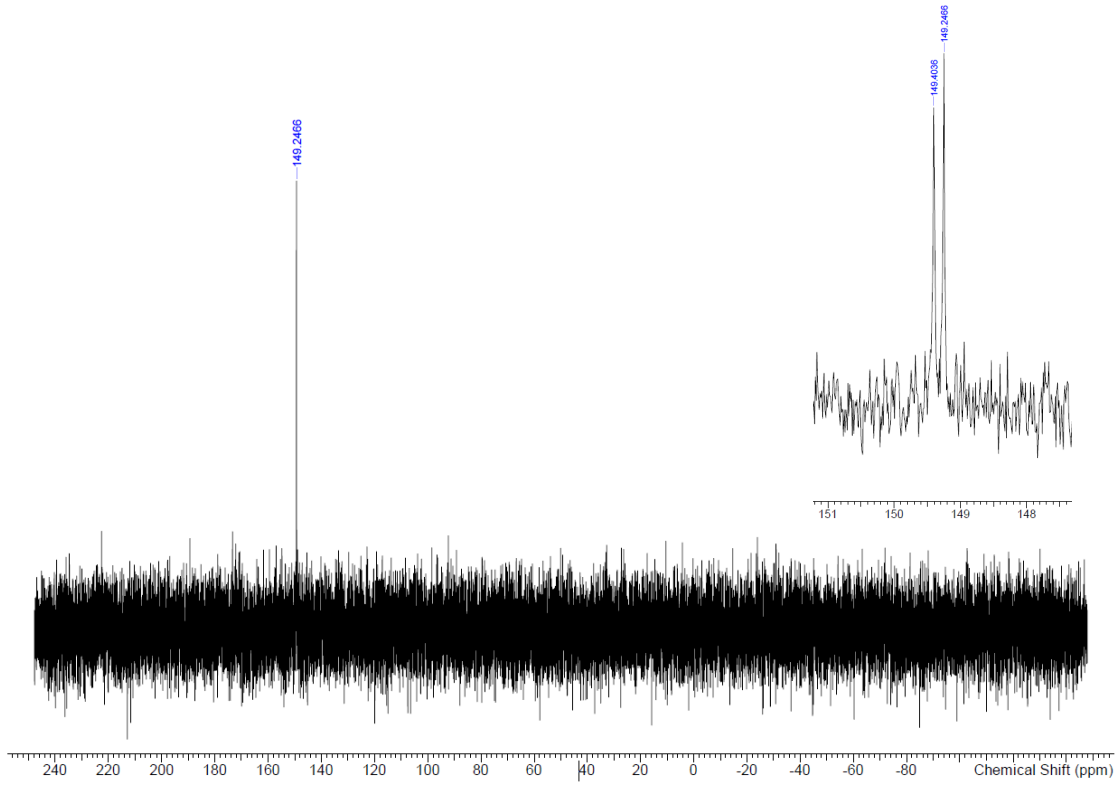

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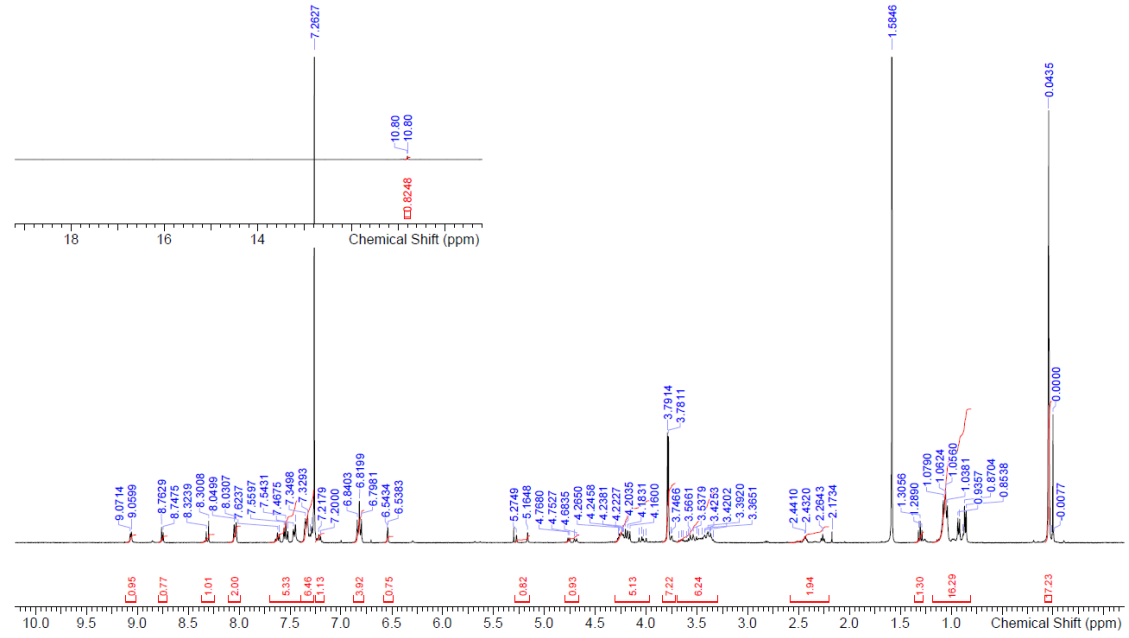

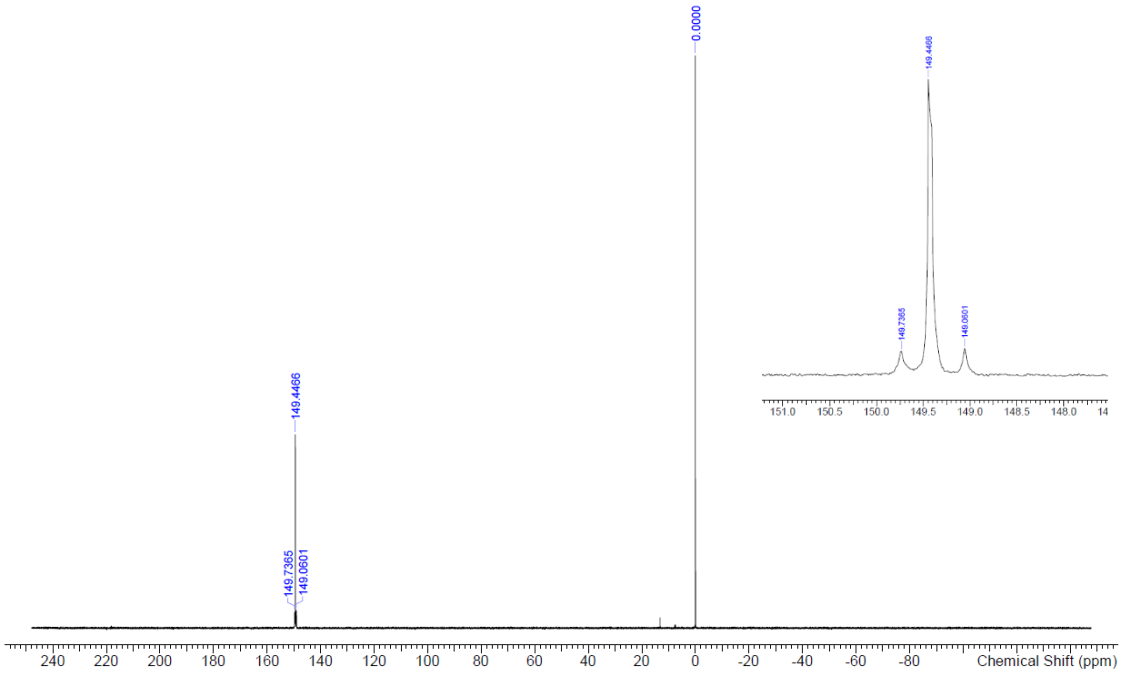

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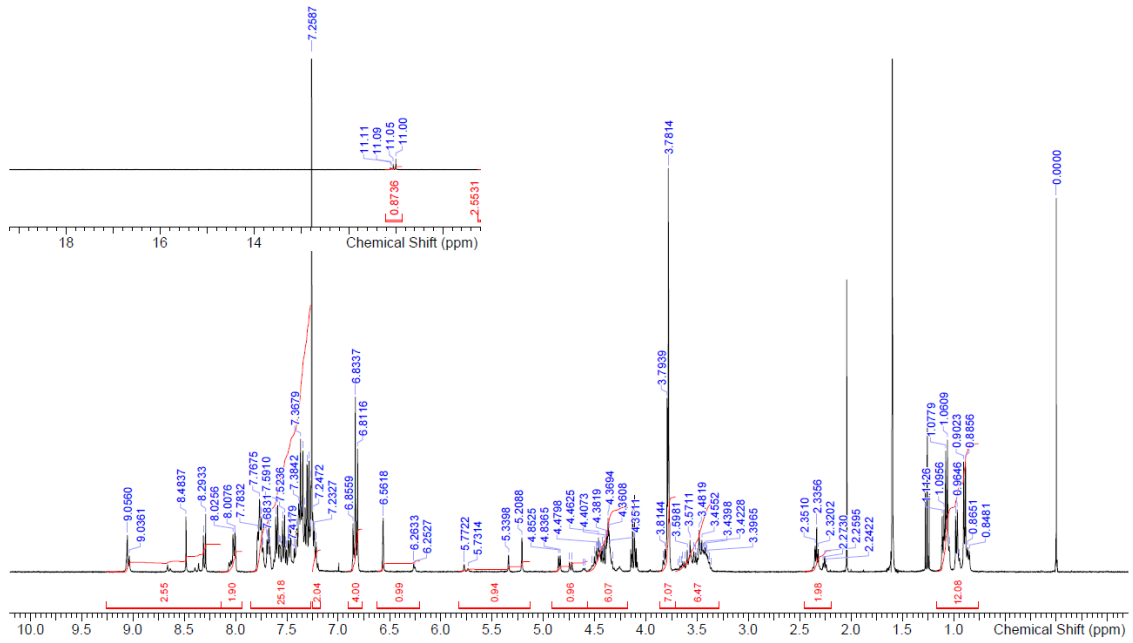

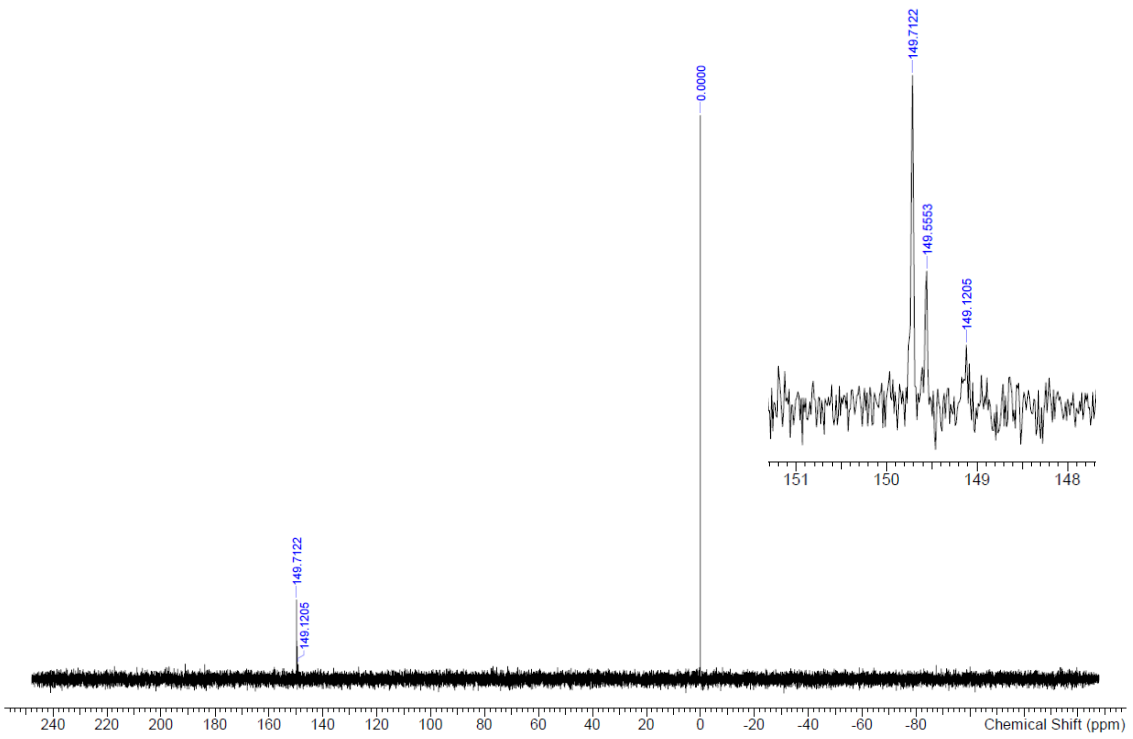

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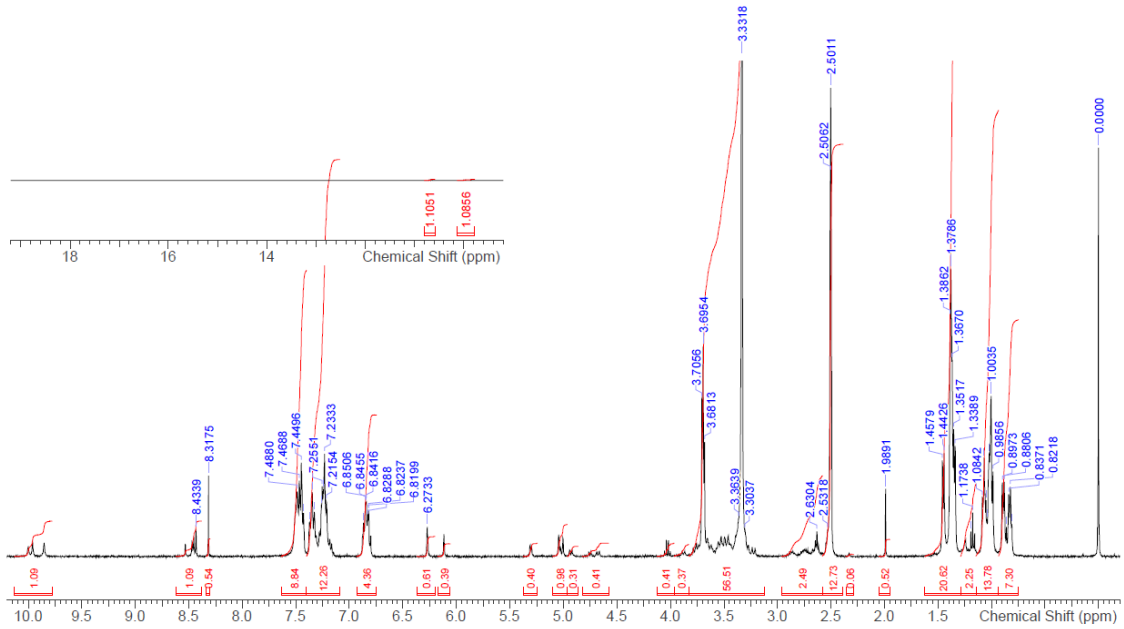

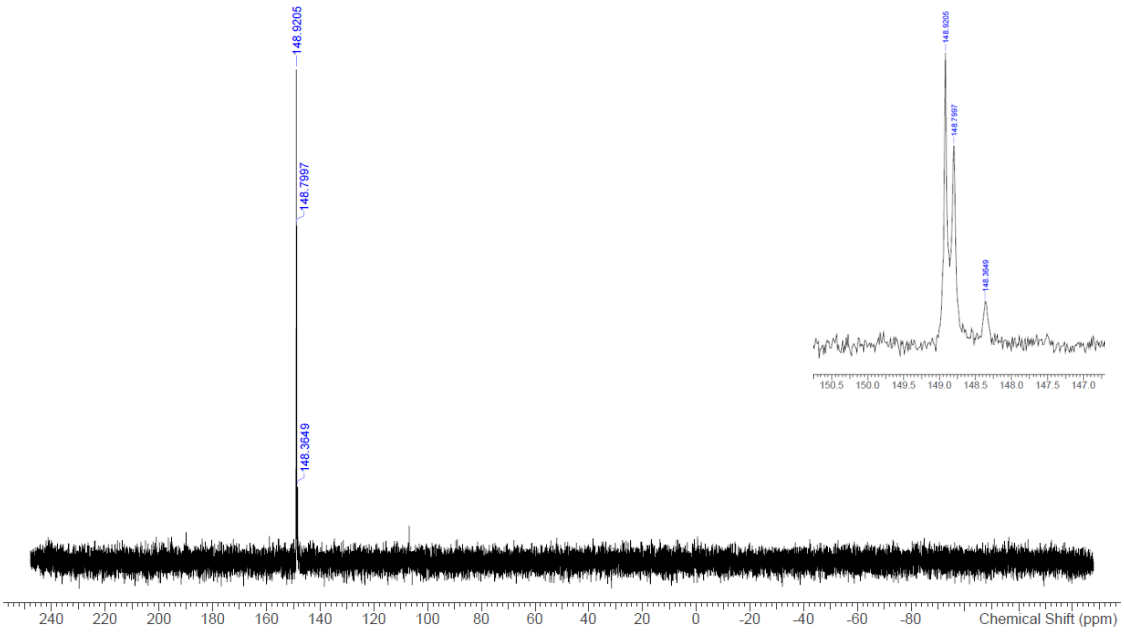

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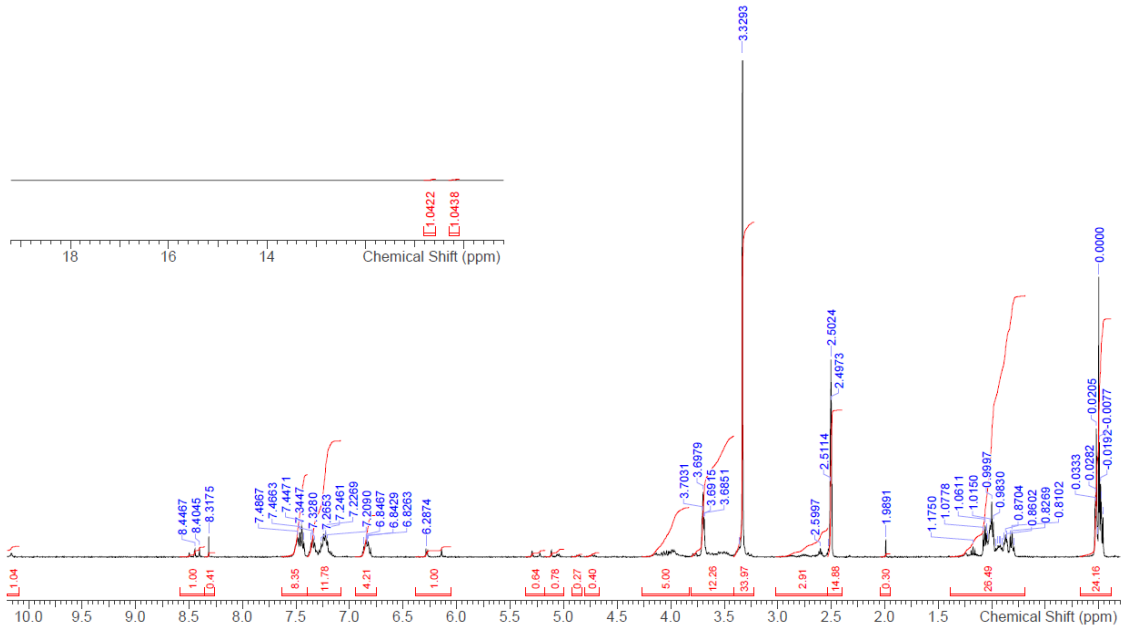

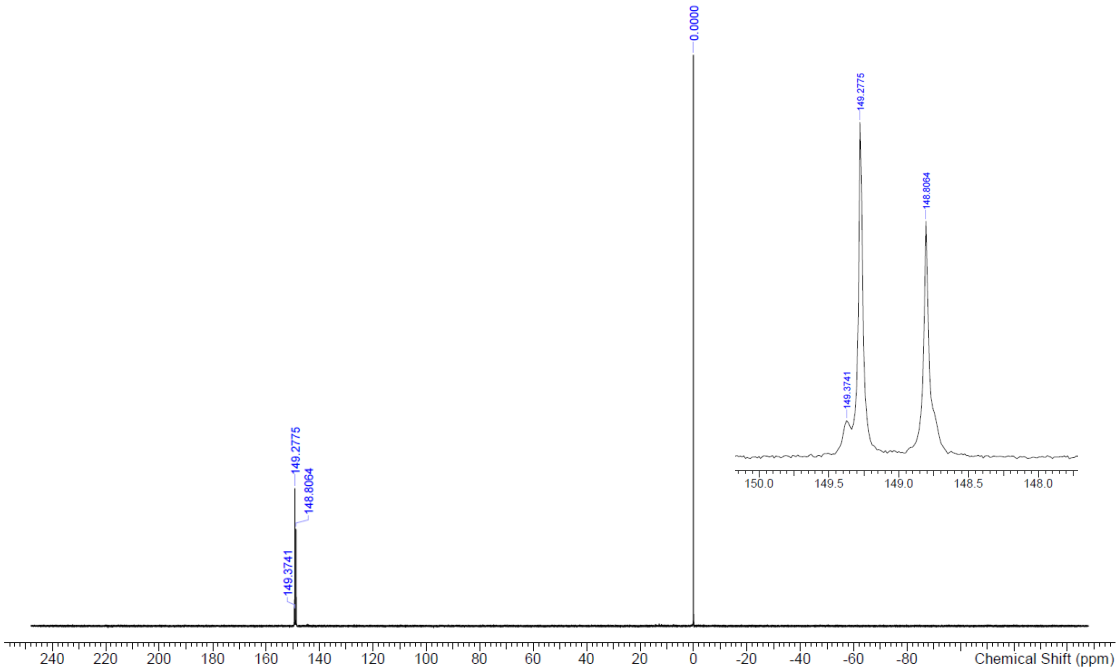

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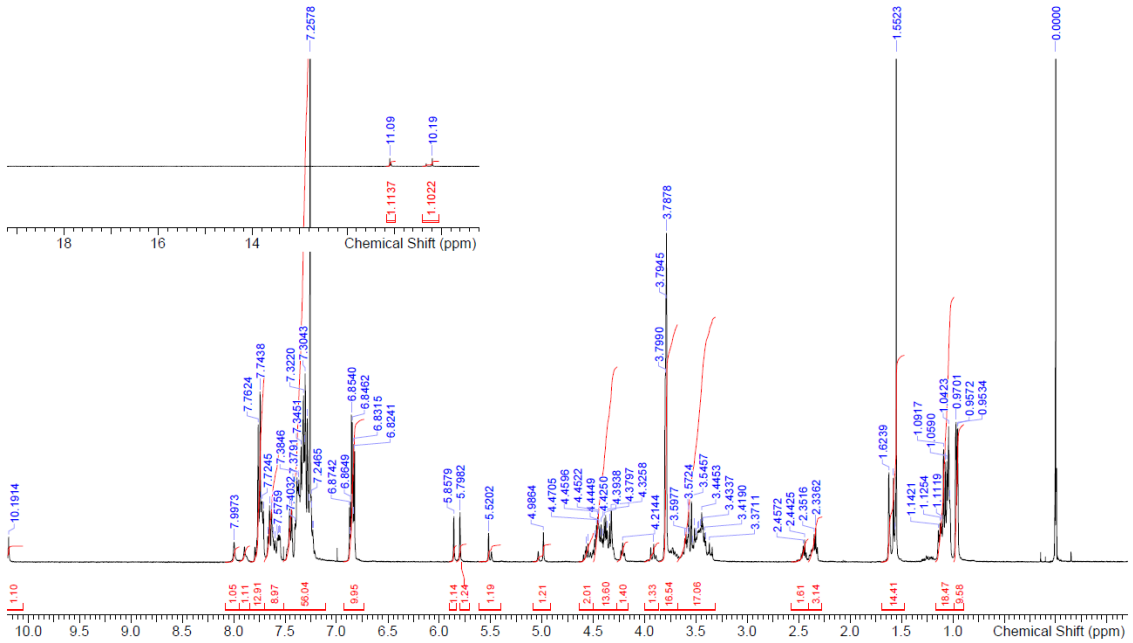
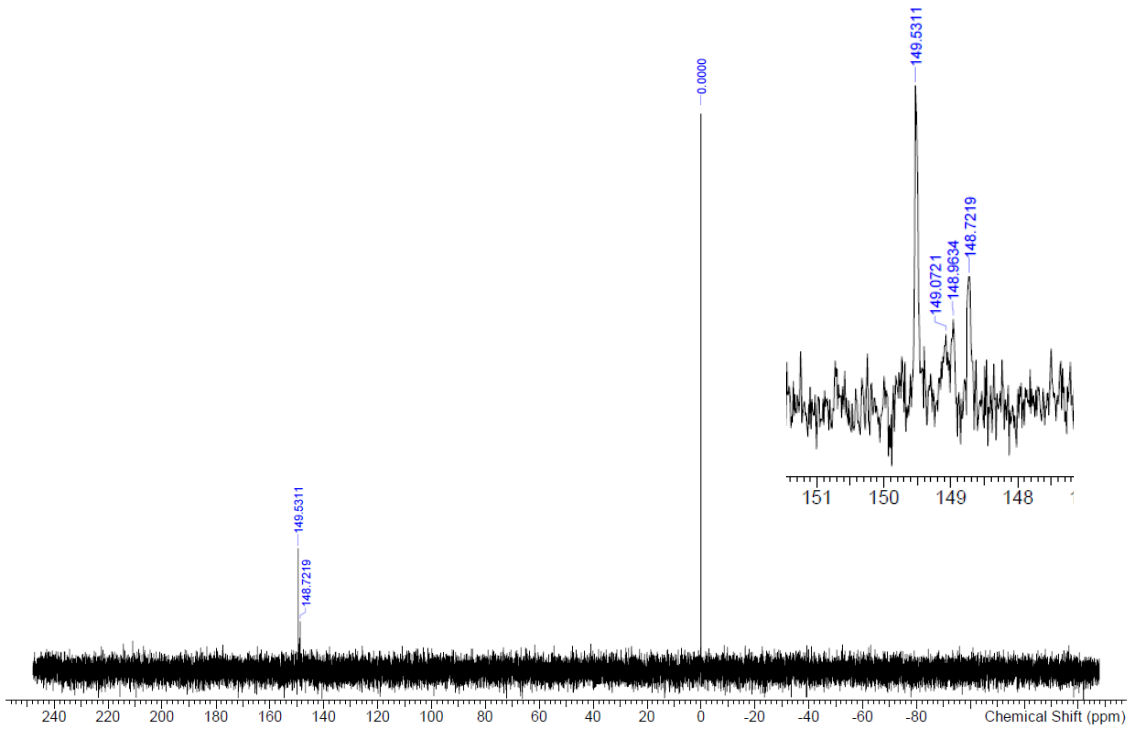
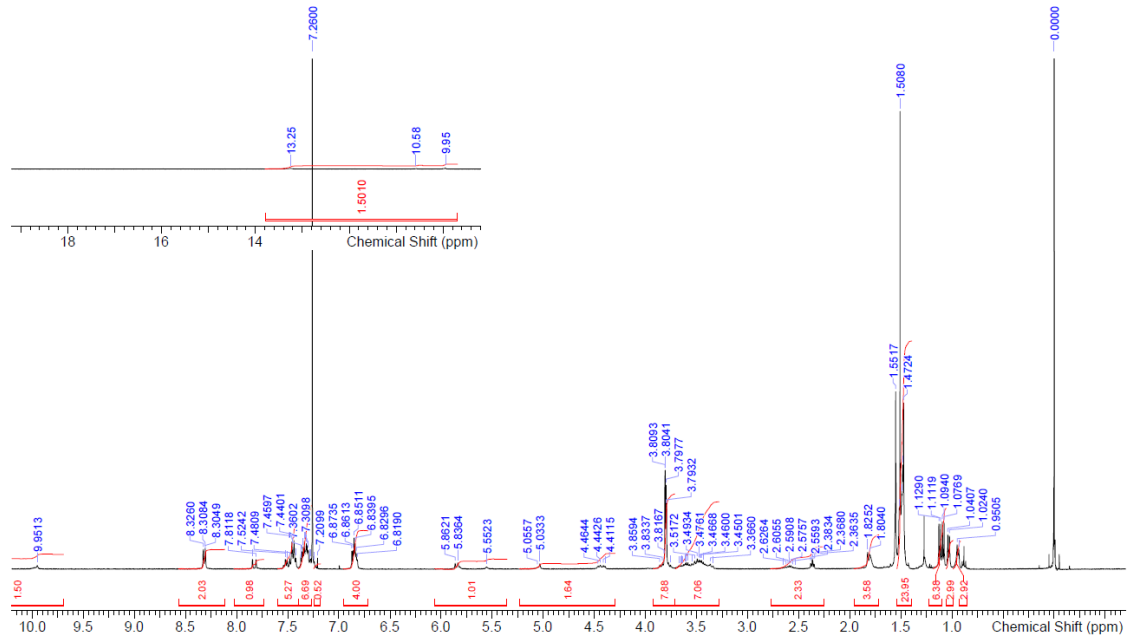
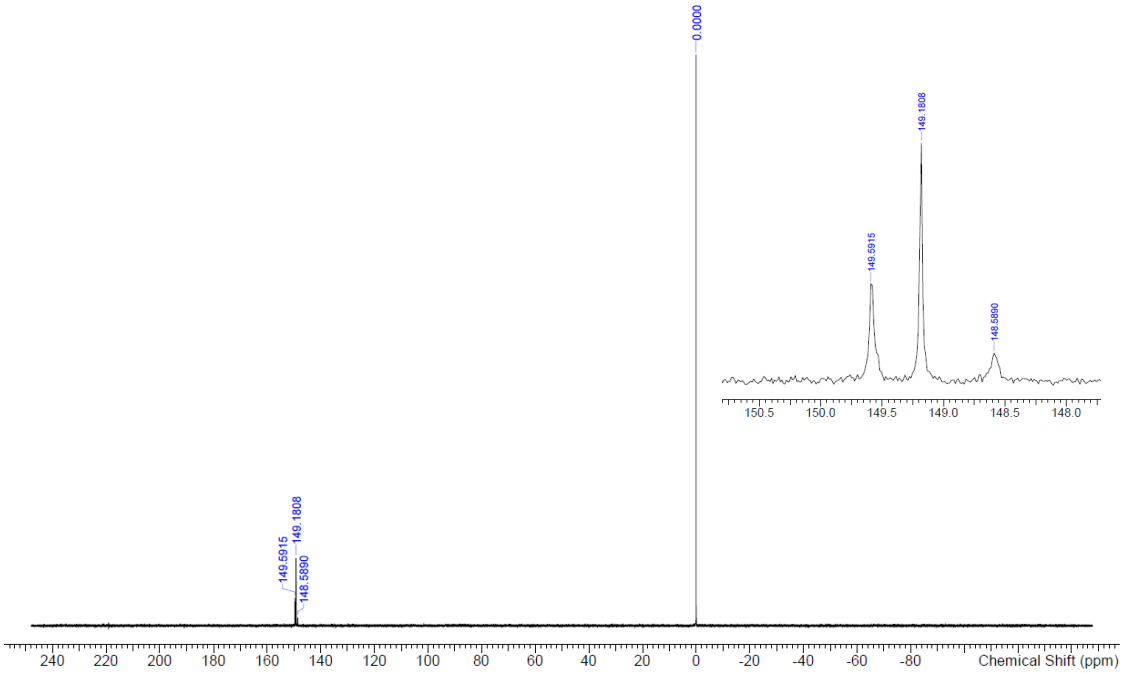


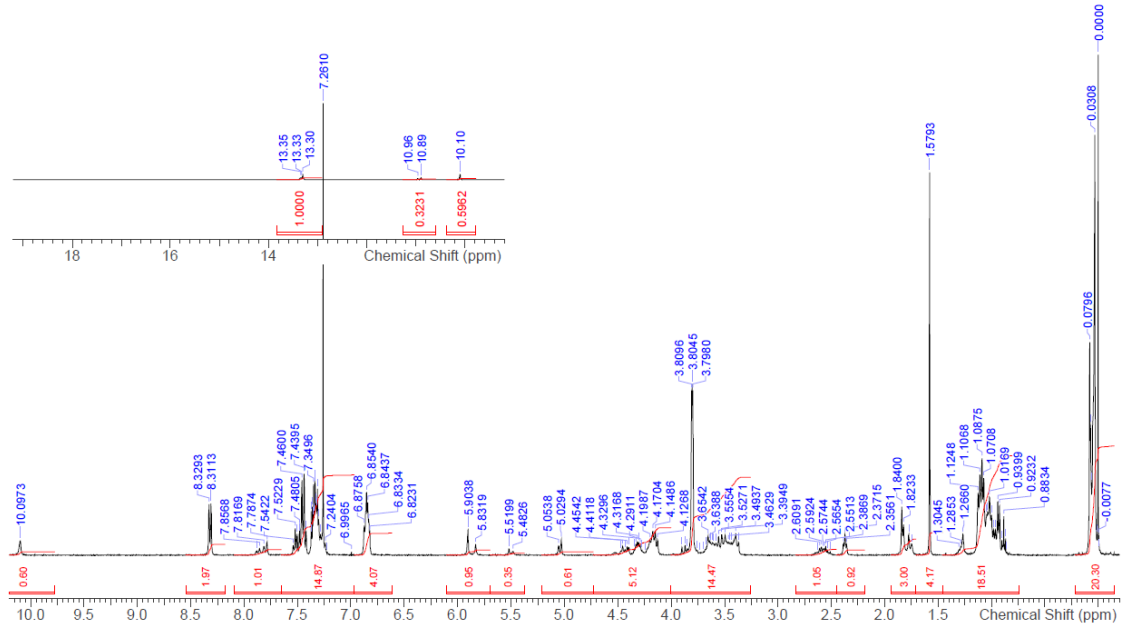


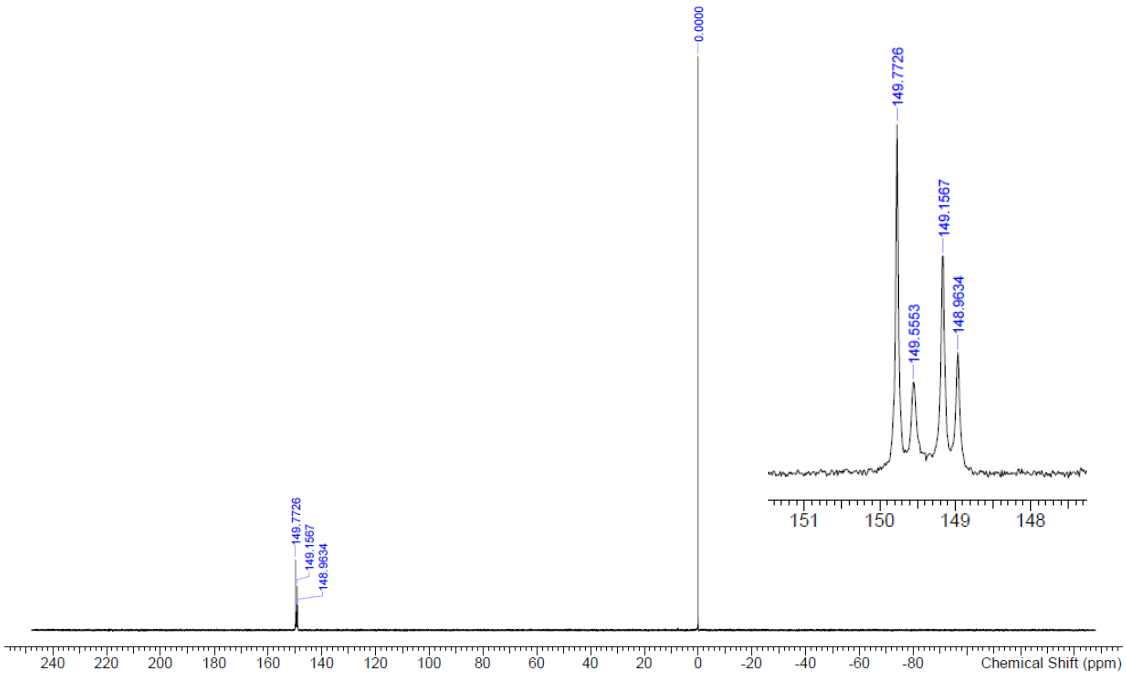
5'-*O*-DMTr-GuNA(boc)-^{m}C(bz) phosphoramidite (4^{m}C-Boc)



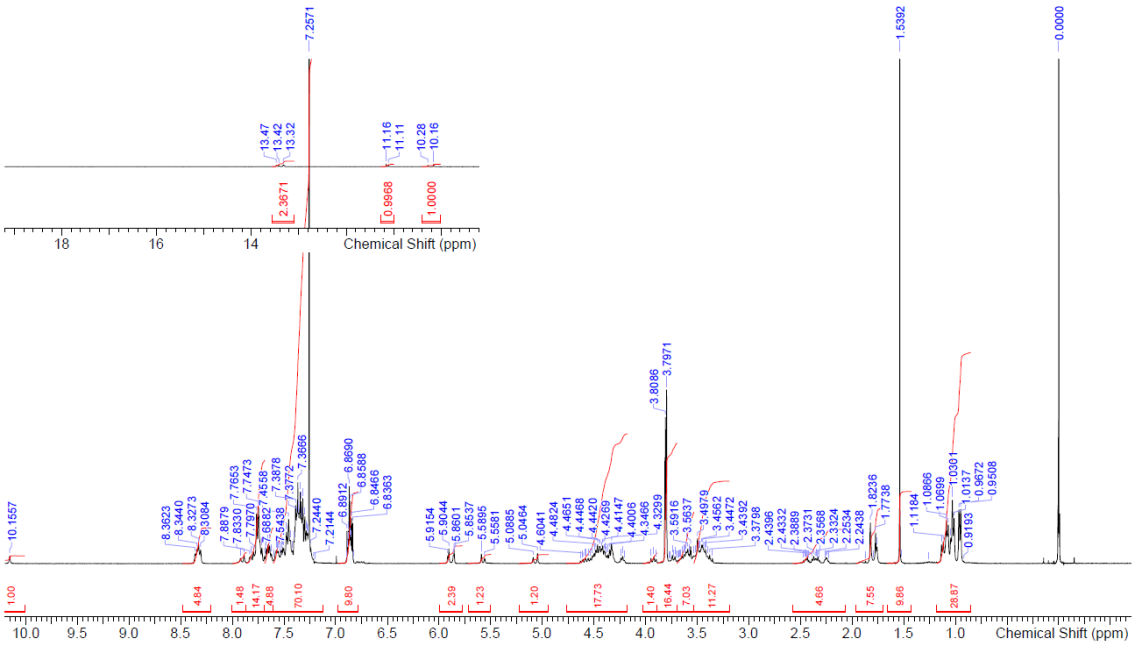


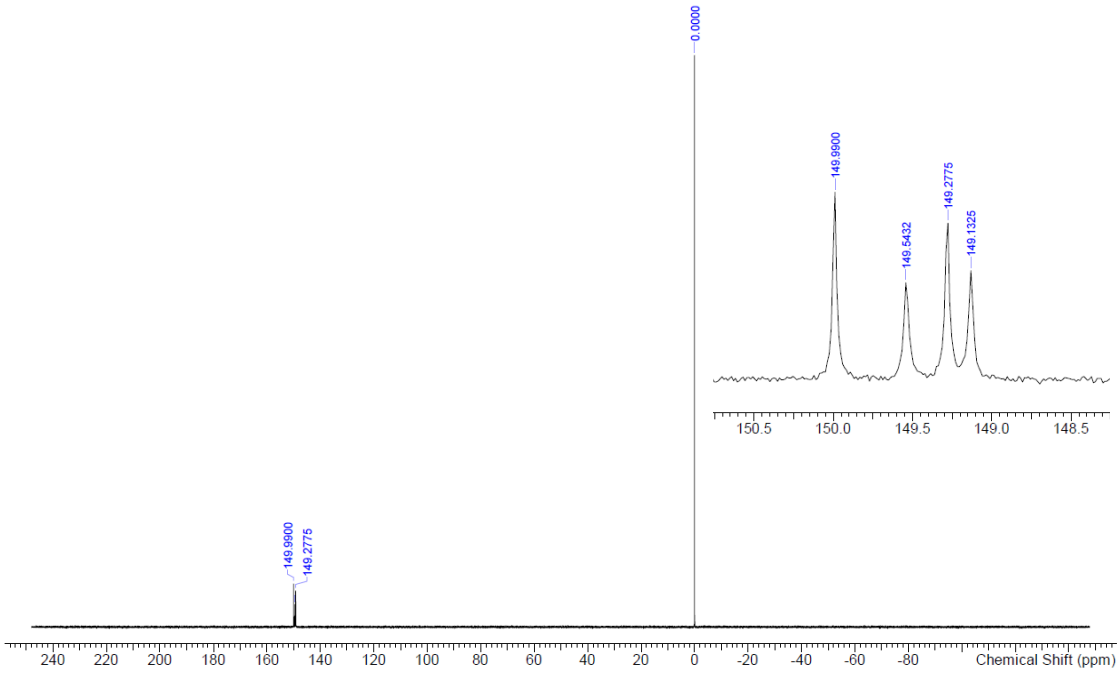
5'-*O*-DMTr-GuNA(teoc)-^{m}C(bz) phosphoramidite (4^{m}C-Teoc)



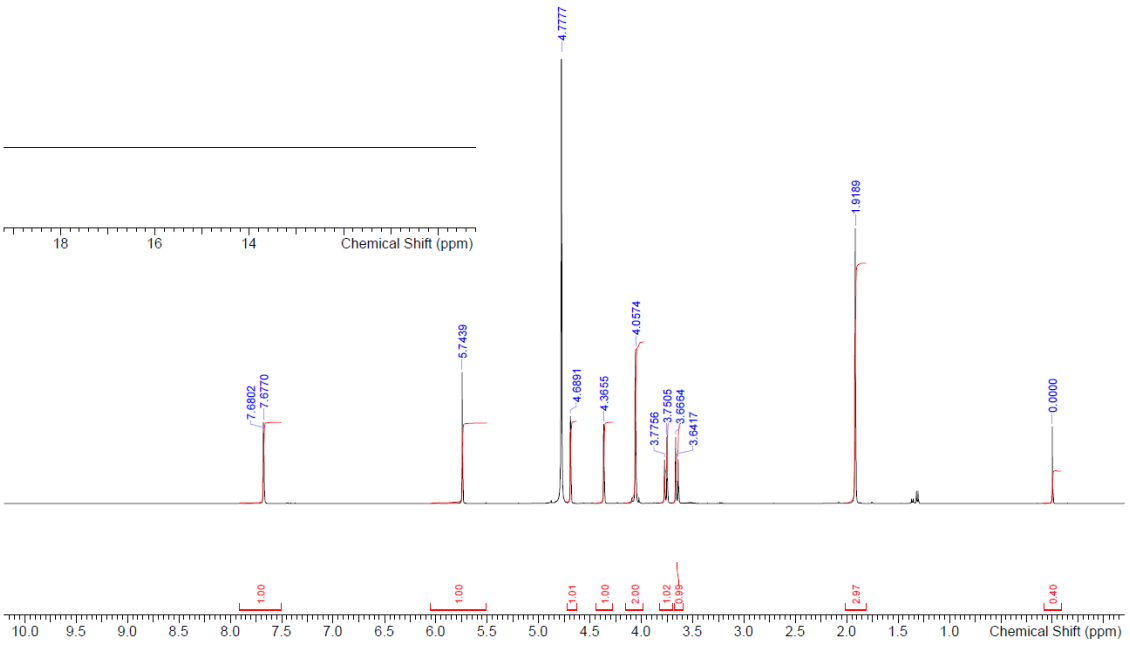


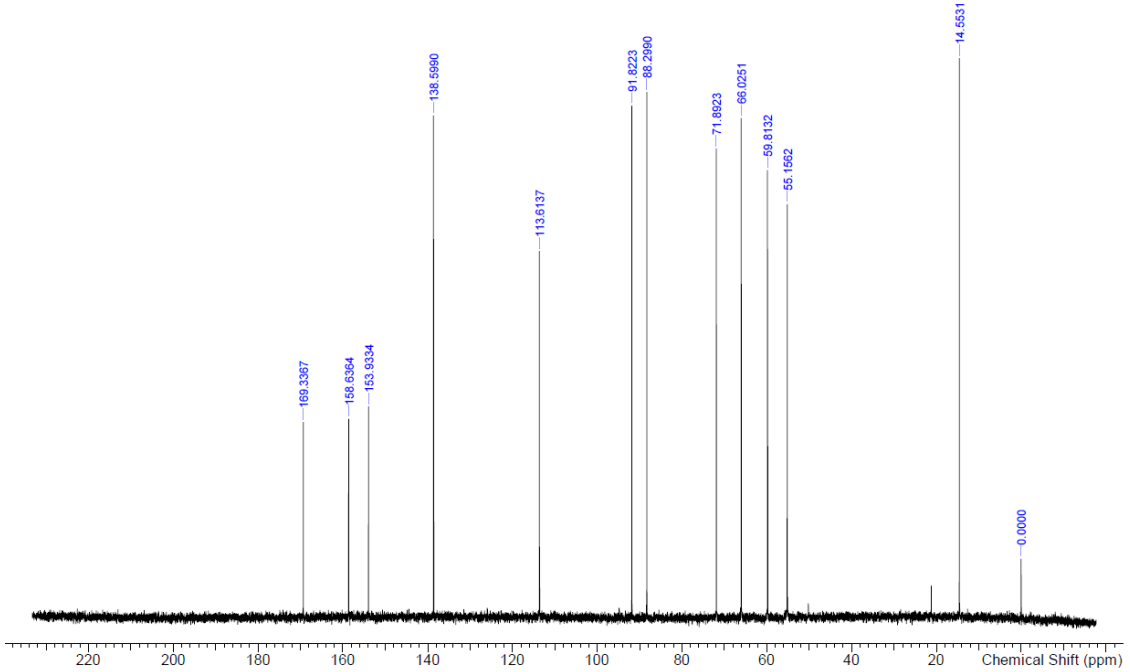
5'-*O*-DMTr-GuNA(fmoc)-^{m}C(bz) phosphoramidite (4^{m}C-Fmoc)



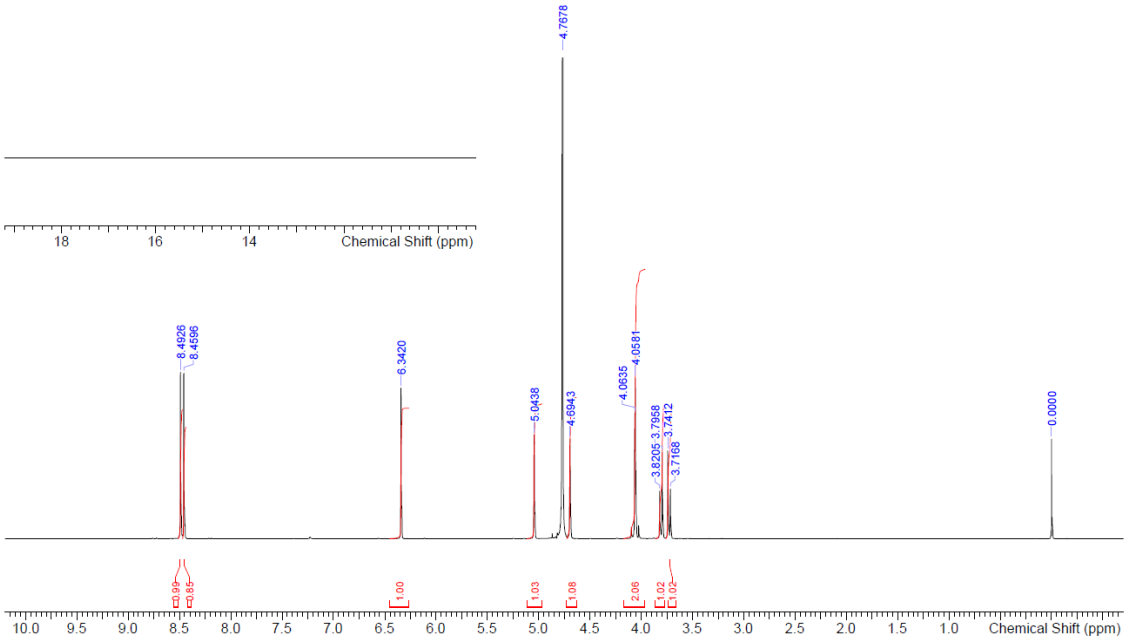


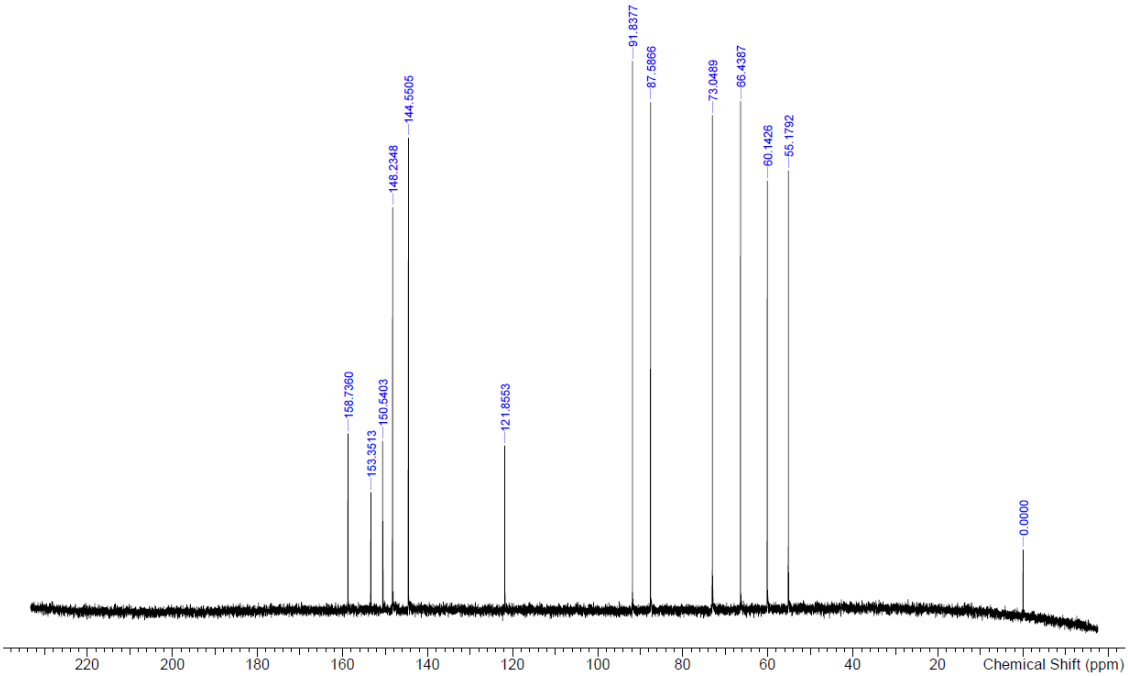
GuNA-T nucleoside (5T)



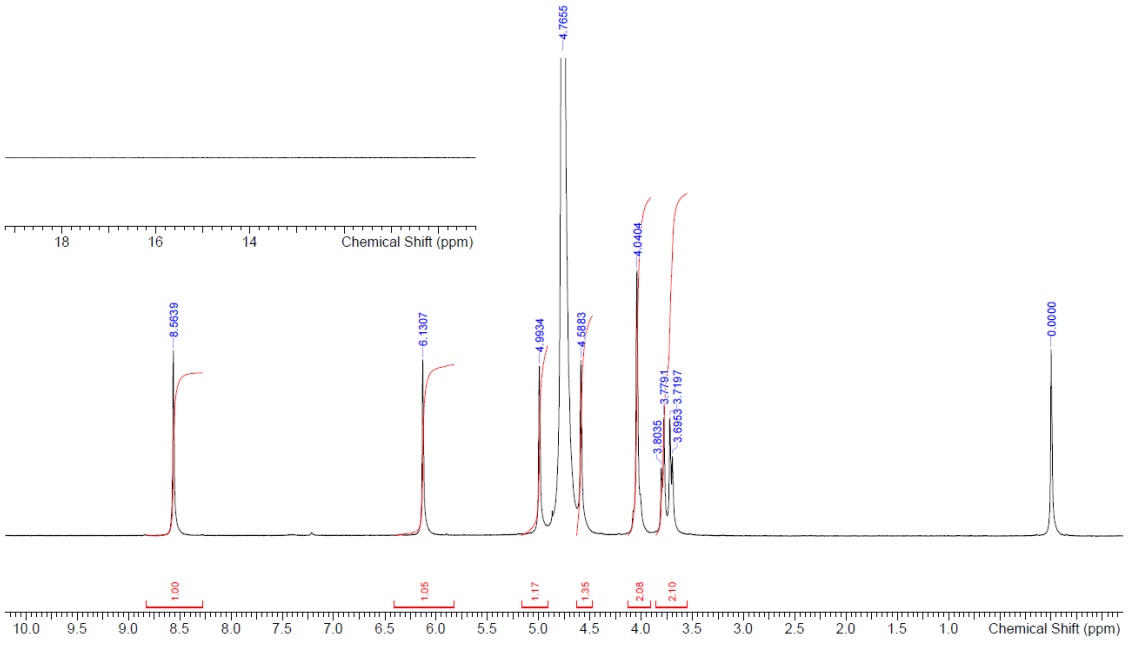


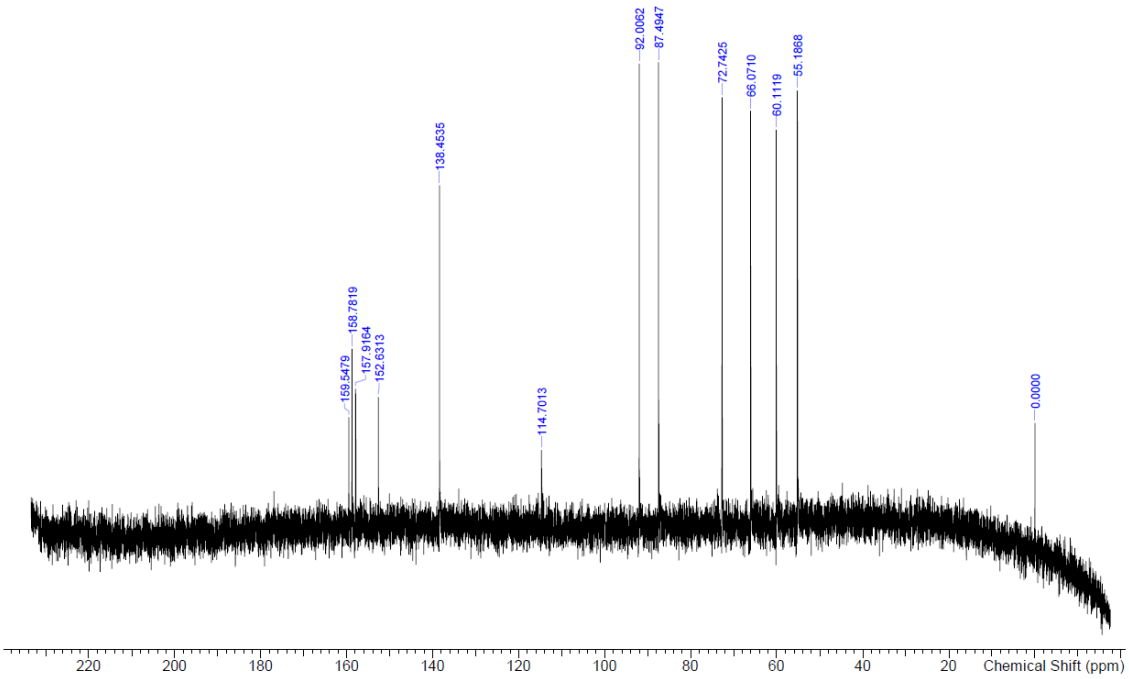
GuNA-A nucleoside (5A)



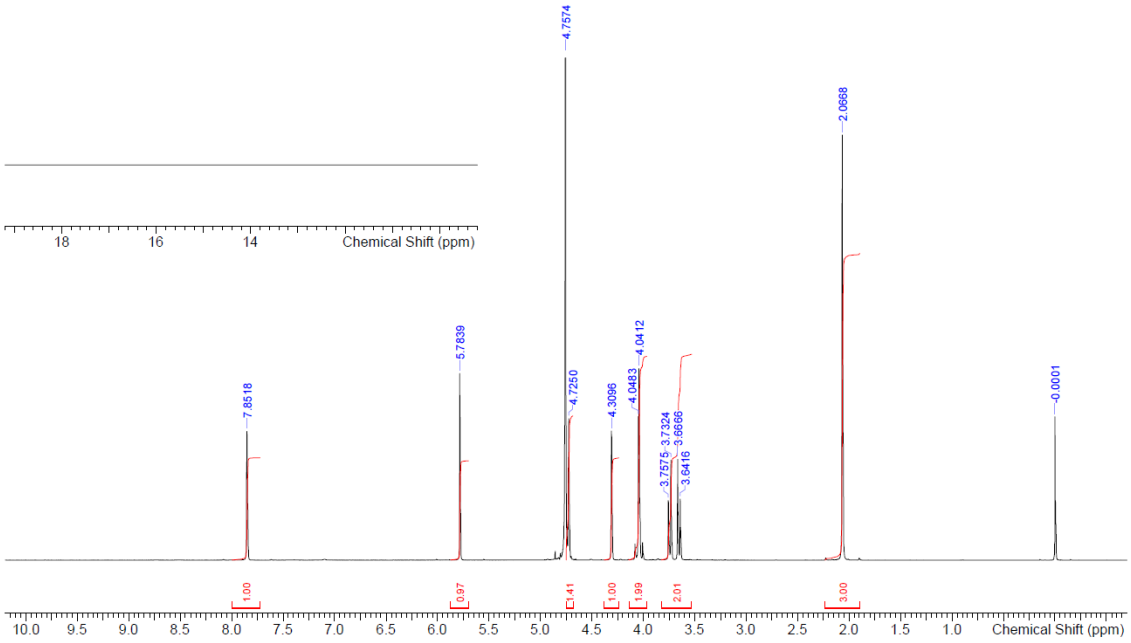


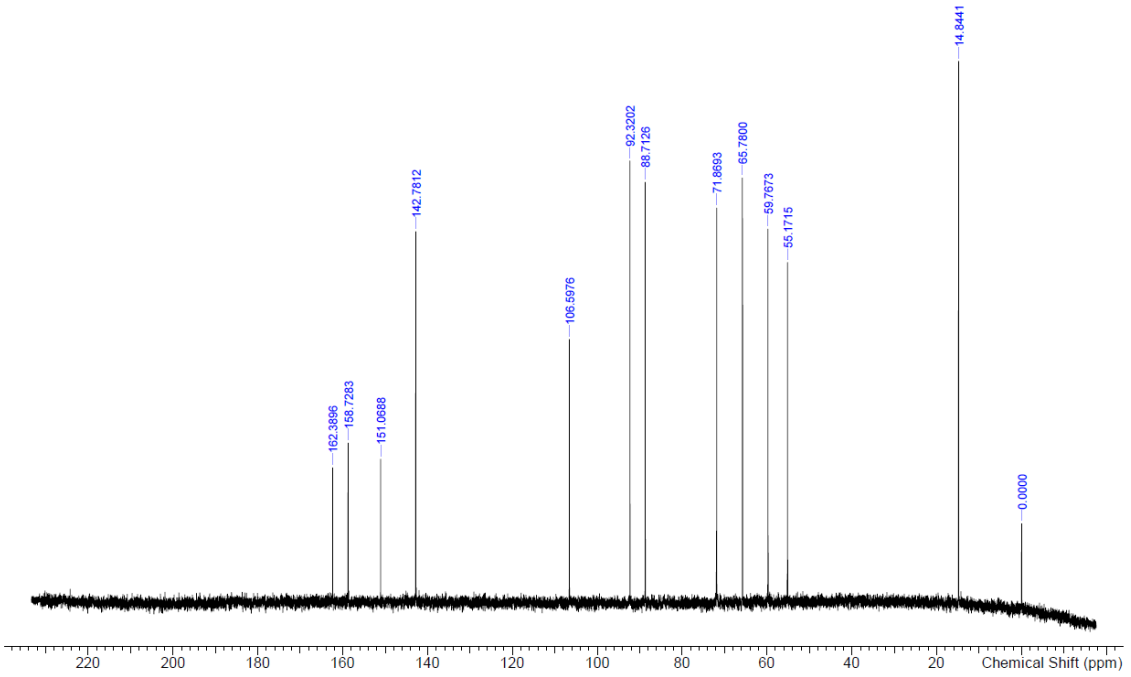
GuNA-G nucleoside (5G)



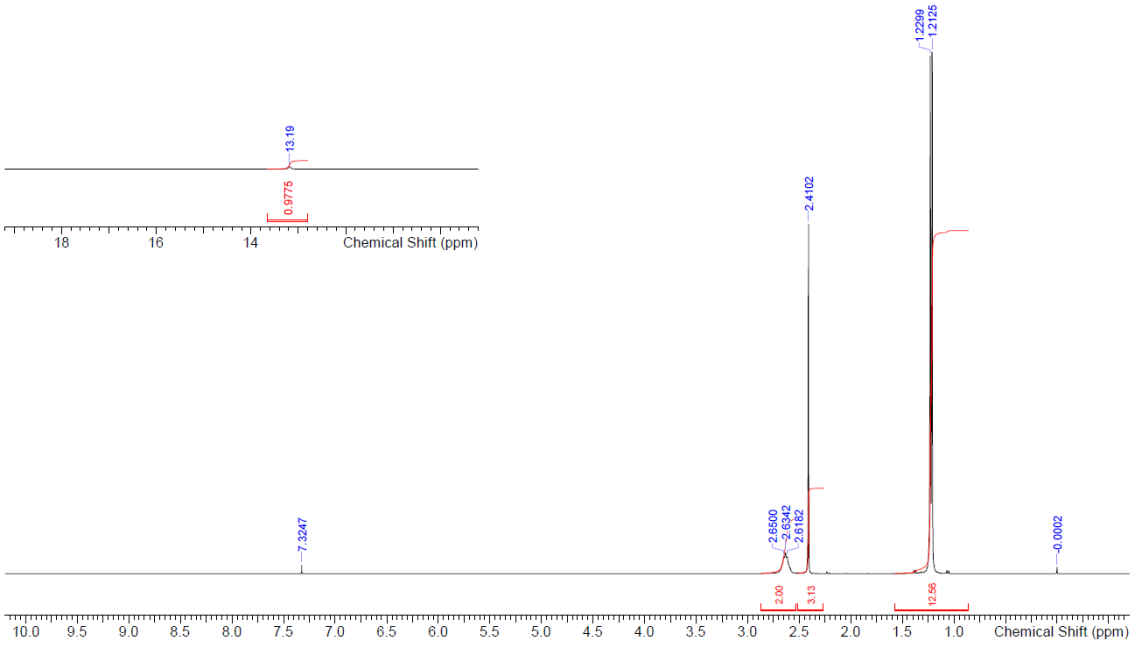


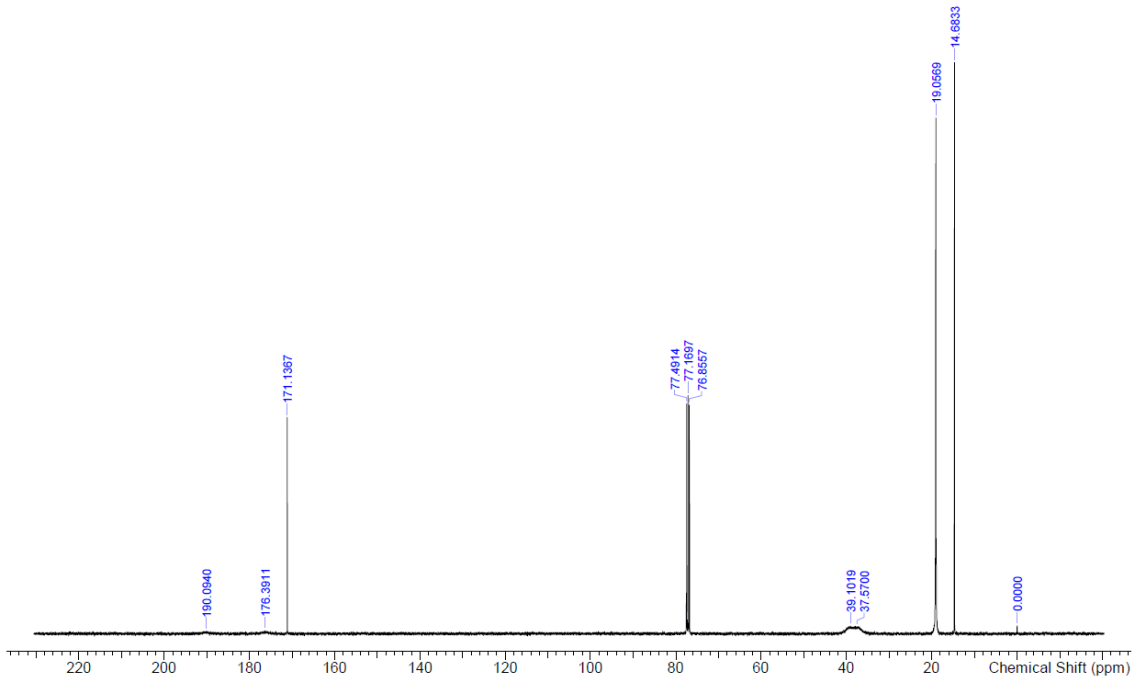
GuNA-^{m}C nucleoside (5^{m}C)





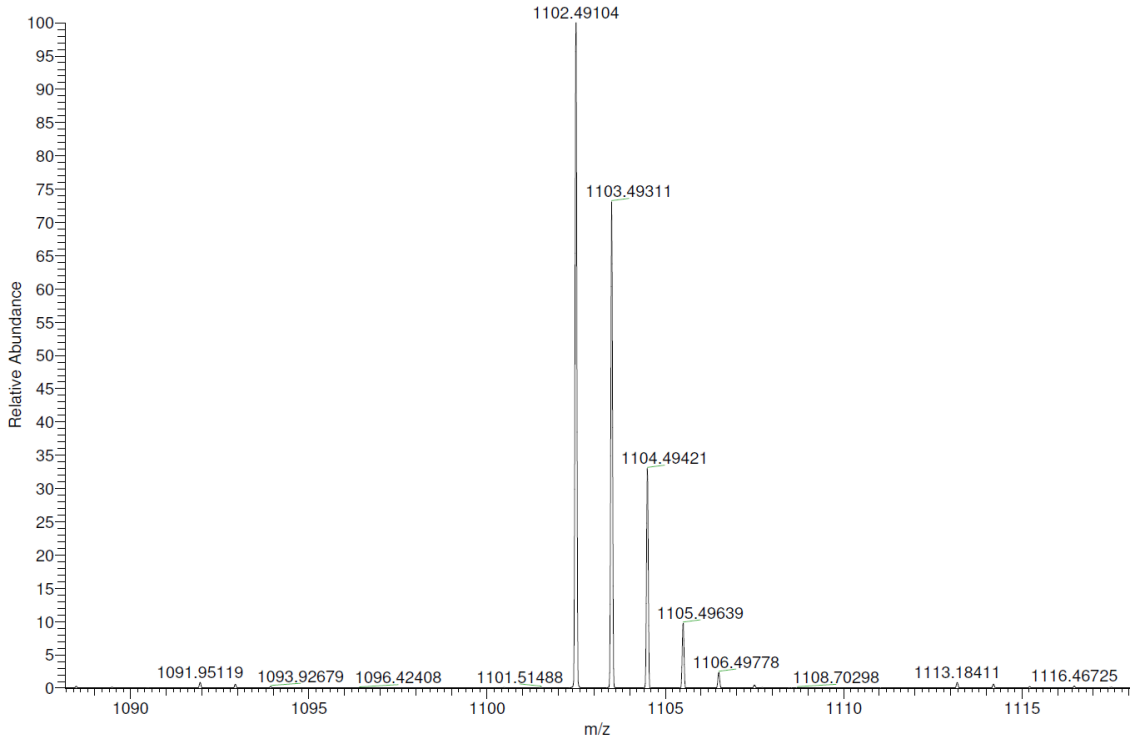
***N,N'*-Diisobutyryl-*S*-methylisothiourea**





3. High resolution mass spectra of phosphoramidites

5'-*O*-DMTr-GuNA(teoc)-T phosphoramidite (4T-Teoc)

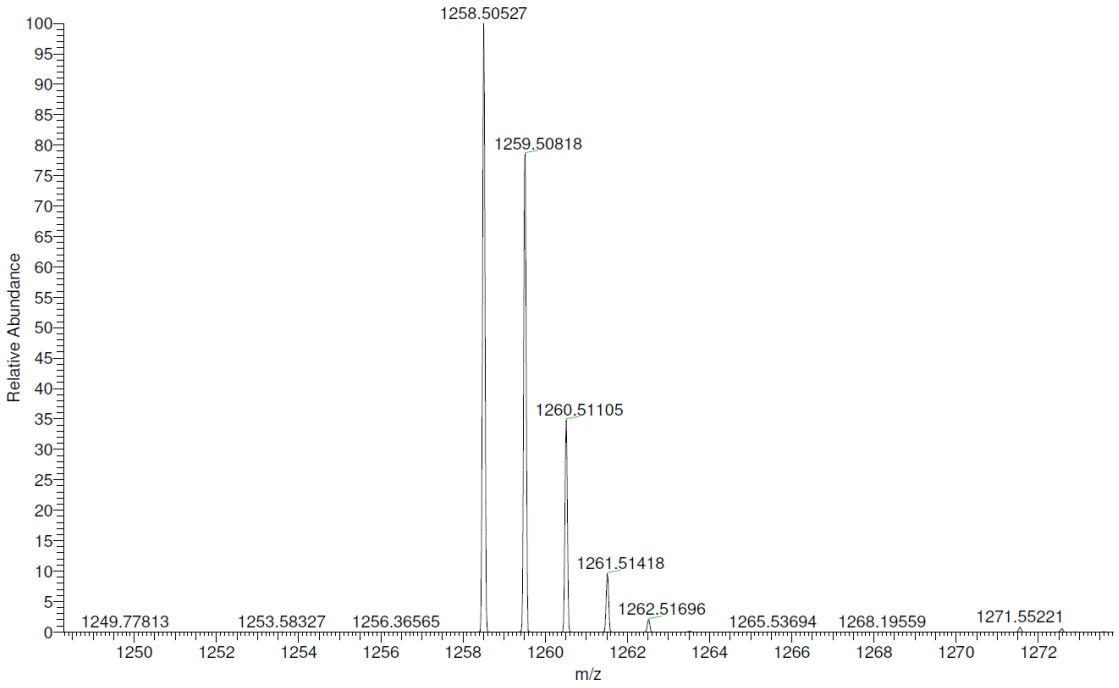


Elemental composition search on mass 1102.49104

m/z= 1097.49104-1107.49104

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1102.49104	1102.49009	0.86	22.5	$C_{54}H_{77}O_{12}N_7PSi_2$

5'-*O*-DMTr-GuNA(fmoc)-T phosphoramidite (4T-Fmoc)

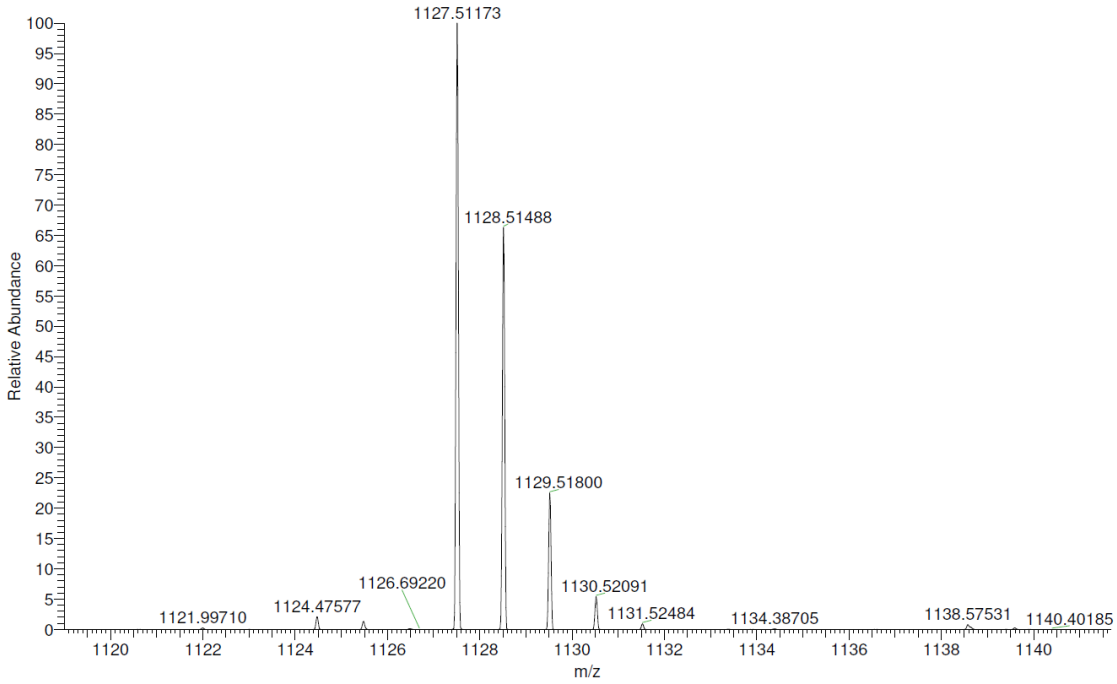


Elemental composition search on mass 1258.50527

m/z= 1253.50527-1263.50527

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1258.50527	1258.50493	0.27	40.5	$C_{72}H_{73}O_{12}N_7P$

5'-*O*-DMTr-GuNA(boc)-A(bz) phosphoramidite (4A-Boc)

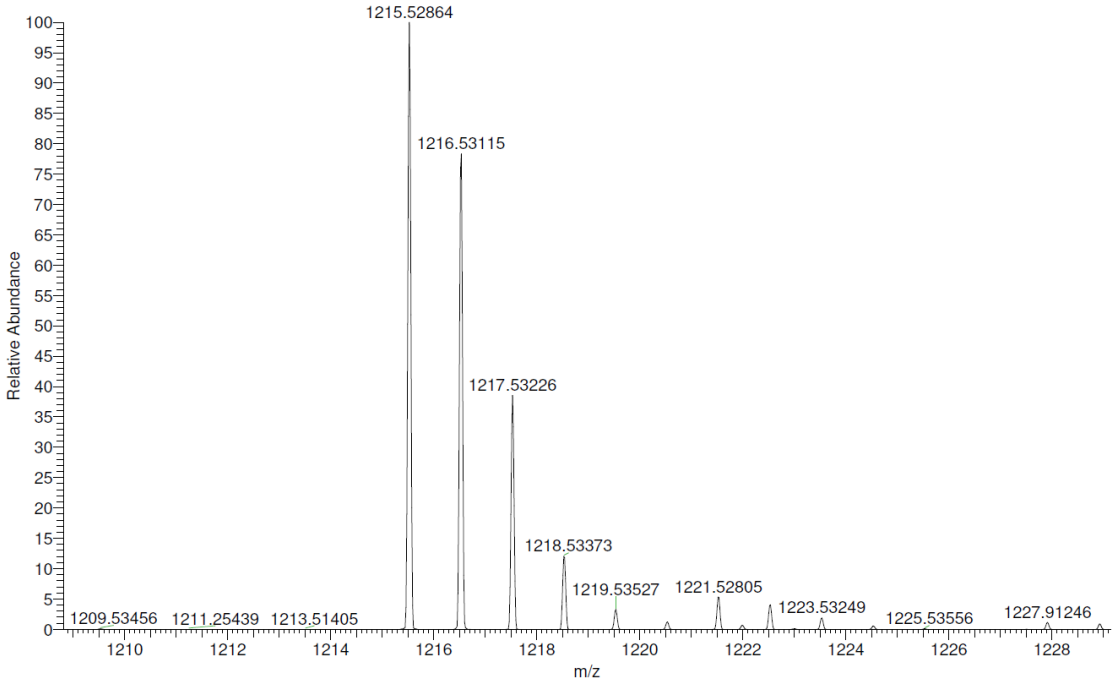


Elemental composition search on mass 1127.51173

m/z= 1122.51173-1132.51173

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1127.51173	1127.51142	0.28	29.5	$C_{59}H_{72}O_{11}N_{10}P$

5'-*O*-DMTr-GuNA(teoc)-A(bz) phosphoramidite (4A-Teoc)

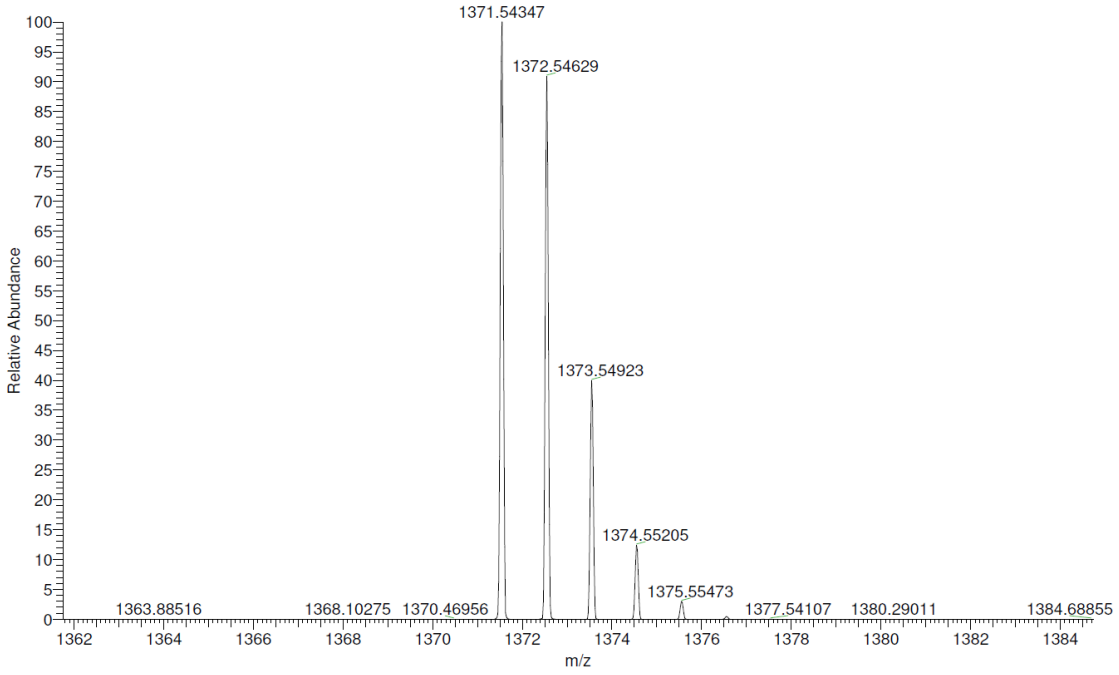


Elemental composition search on mass 1215.52864

m/z= 1210.52864-1220.52864

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1215.52864	1215.52787	0.63	29.5	$C_{61}H_{80}O_{11}N_{10}PSi_2$

5'-*O*-DMTr-GuNA(fmoc)-A(bz) phosphoramidite (4A-Fmoc)

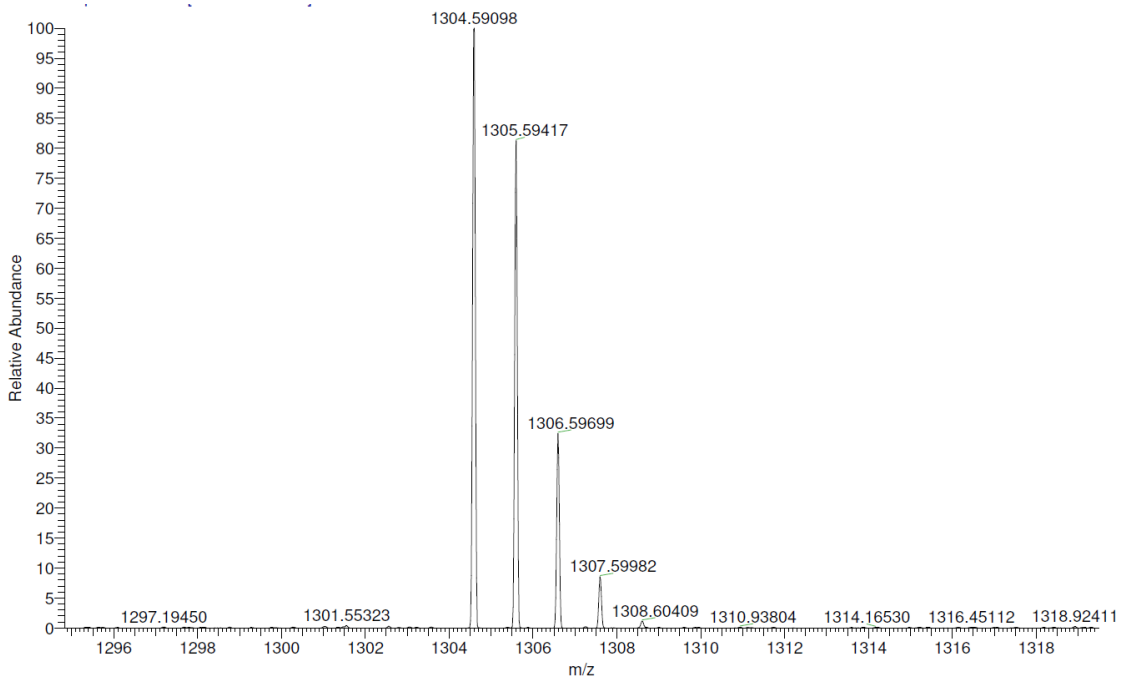


Elemental composition search on mass 1371.54347

m/z= 1366.54347-1376.54347

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1371.54347	1371.54272	0.55	47.5	$C_{79}H_{76}O_{11}N_{10}P$

5'-*O*-DMTr-GuNA(boc)-G(ib,dpc) phosphoramidite (4G-Boc)

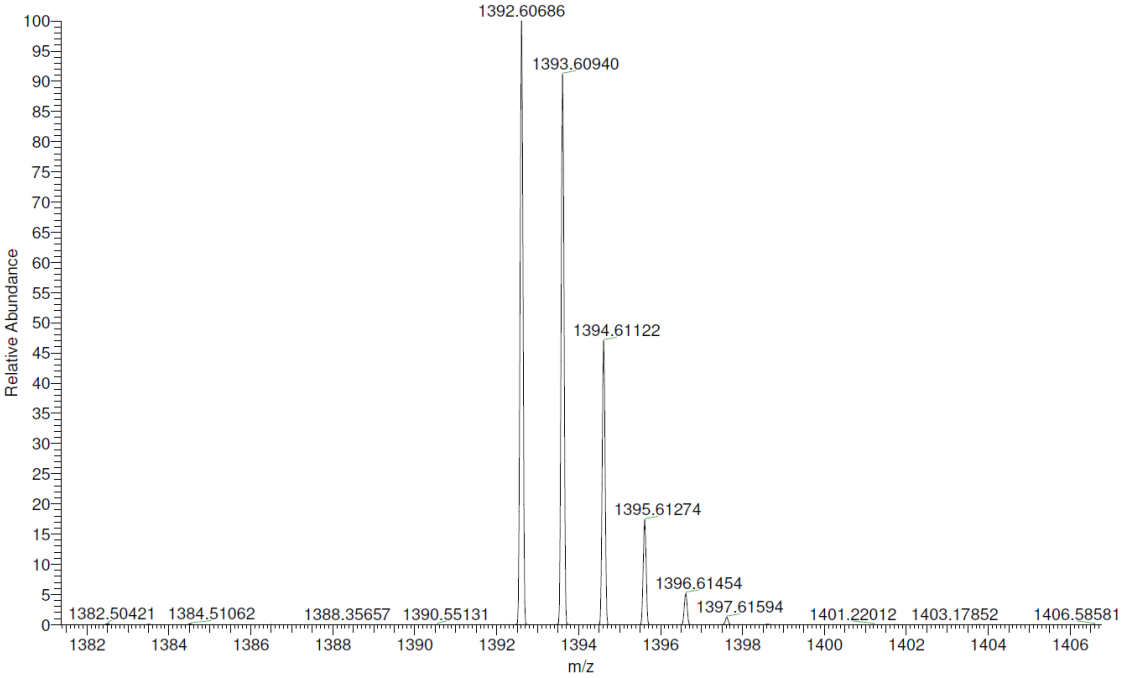


Elemental composition search on mass 1304.59098

m/z= 1299.59098-1309.59098

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1304.59098	1304.59039	0.45	34.5	$C_{69}H_{83}O_{13}N_{11}P$

5'-*O*-DMTr-GuNA(teoc)-G(ib,dpc) phosphoramidite (4G-Teoc)

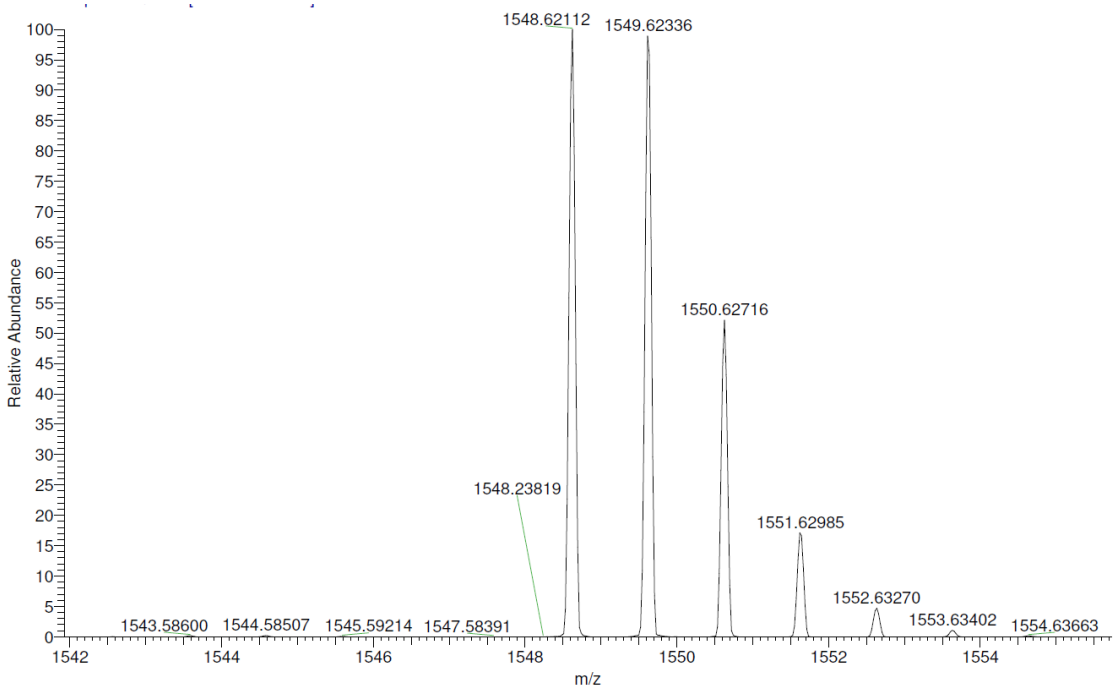


Elemental composition search on mass 1392.60686

m/z= 1387.60686-1397.60686

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1392.60686	1392.60685	0.01	34.5	$C_{71}H_{91}O_{13}N_{11}PSi_2$

5'-*O*-DMTr-GuNA(fmoc)-G(ib,dpc) phosphoramidite (4G-Fmoc)

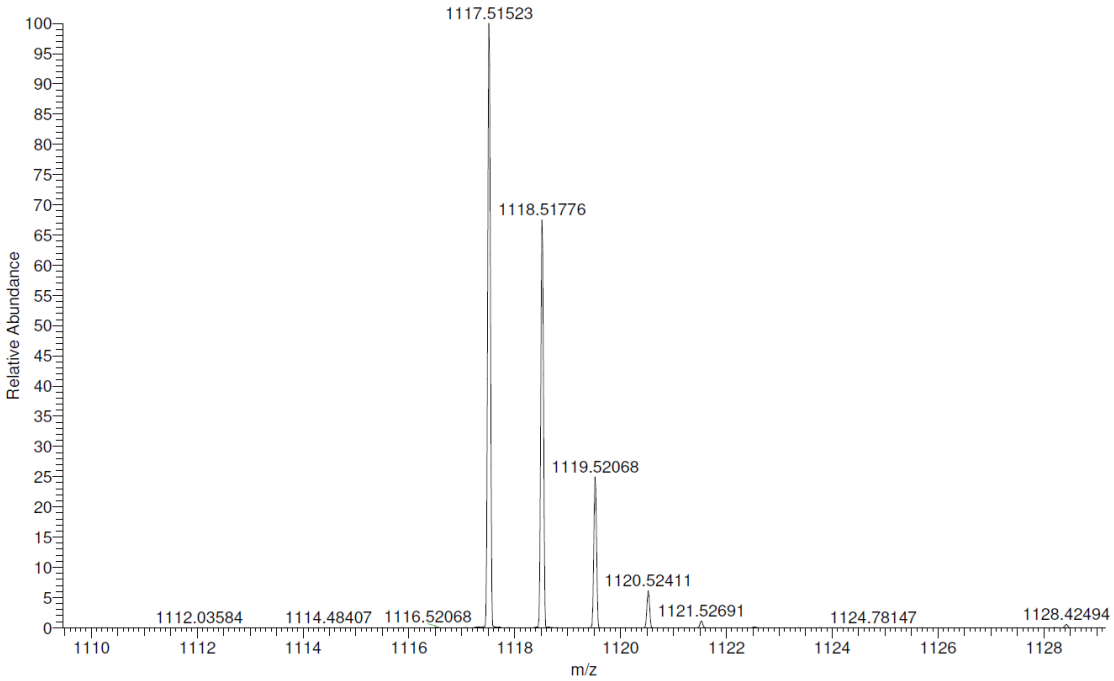


Elemental composition search on mass 1548.62112

m/z= 1543.62112-1553.62112

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1548.62112	1548.62169	-0.37	52.5	$C_{89}H_{87}O_{13}N_{11}P$

5'-*O*-DMTr-GuNA(boc)-^{m}C(bz) phosphoramidite (4^{m}C-Boc)

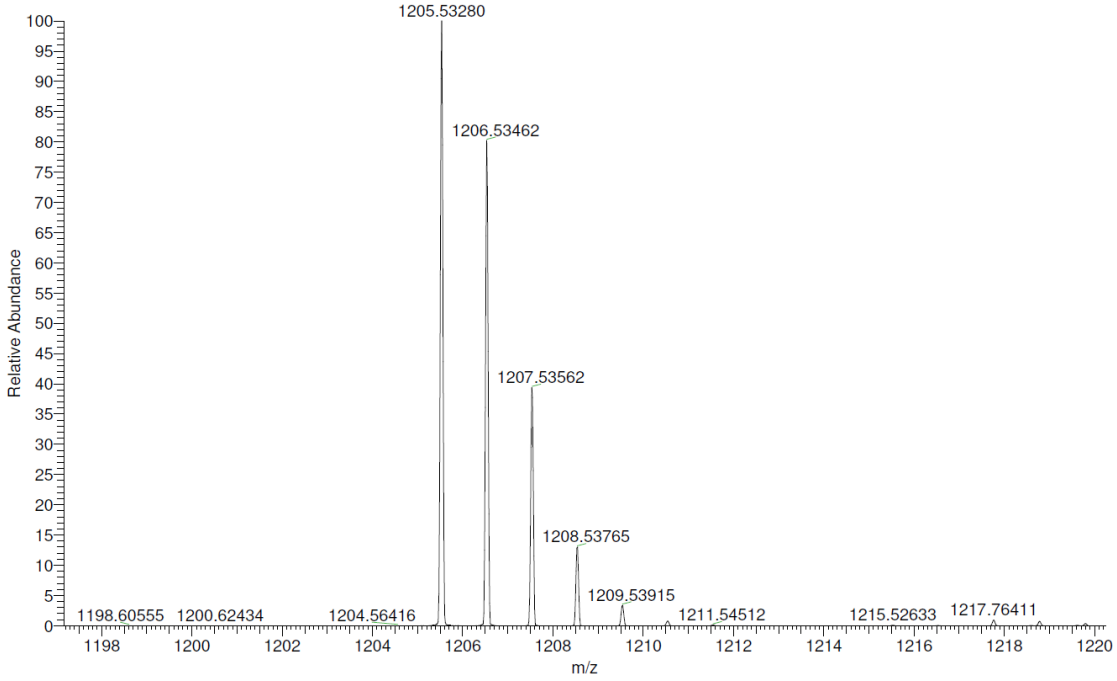


Elemental composition search on mass 1117.51523

m/z= 1112.51523-1122.51523

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1117.51523	1117.51583	-0.54	27.5	$C_{59}H_{74}O_{12}N_8P$

5'-*O*-DMTr-GuNA(teoc)-^{m}C(bz) phosphoramidite (4^{m}C-Teoc)

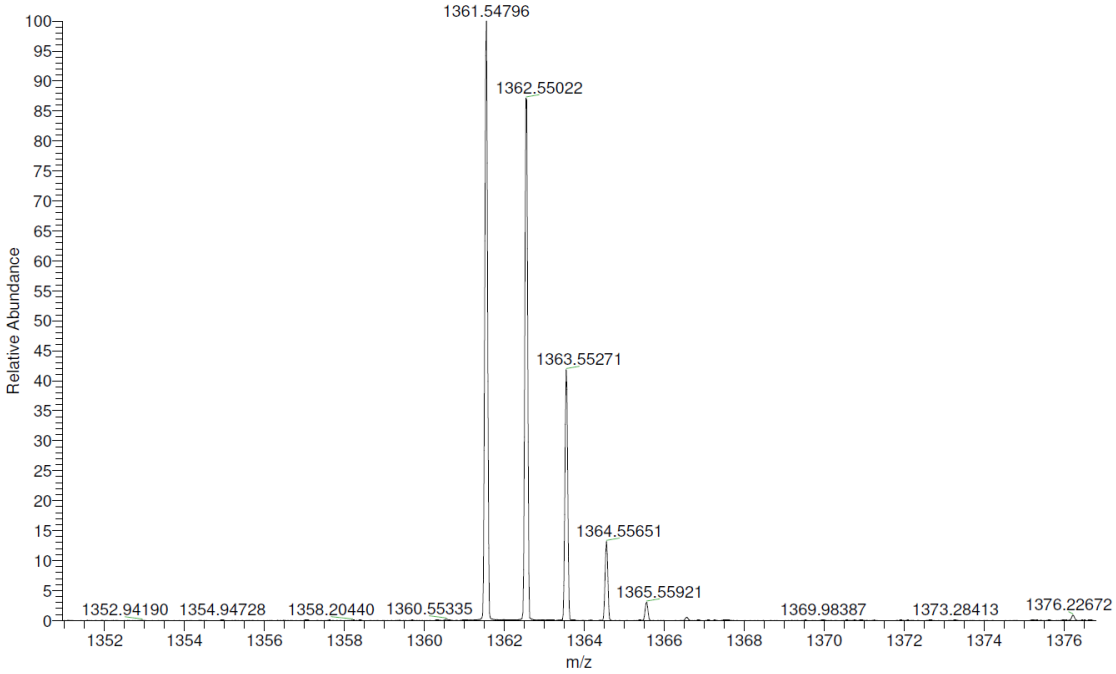


Elemental composition search on mass 1205.53280

m/z= 1200.53280-1210.53280

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1205.53280	1205.53229	0.43	27.5	$C_{61}H_{82}O_{12}N_8PSi_2$

5'-*O*-DMTr-GuNA(fmoc)-^{m}C(bz) phosphoramidite (4^{m}C-Fmoc)



Elemental composition search on mass 1361.54796

m/z= 1356.54796-1366.54796

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1361.54796	1361.54713	0.61	45.5	$C_{79}H_{78}O_{12}N_8P$

4. HPLC charts and MALDI-TOF MS spectra of oligonucleotides

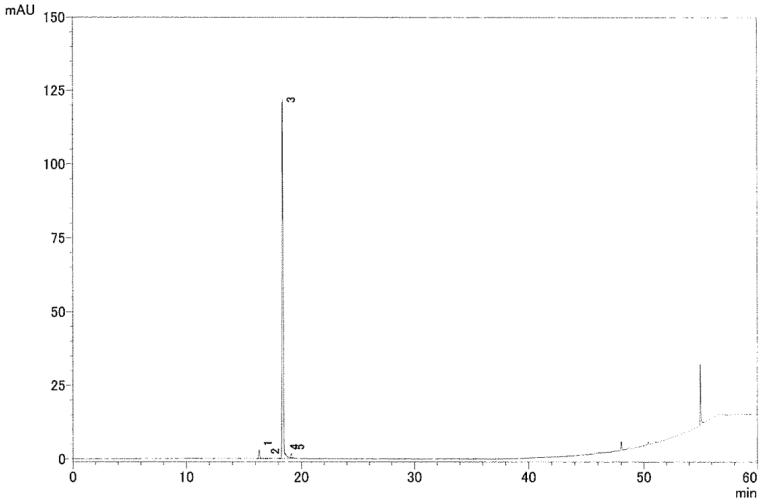
Each mass spectrum corresponds to the major peak of the HPLC chart.

HPLC conditions

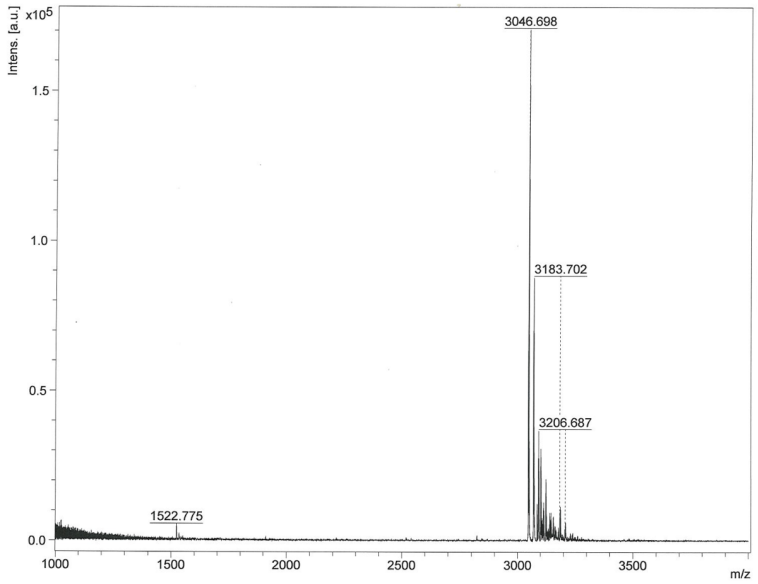
Mobile phase A: 100 mM HFIP / 8mM TEA in water
Mobile phase B: 100 mM HFIP / 8mM TEA in methanol
Flow rate: 1 mL/min
Column: Waters XBridge™ BEH C18 2.5 μm (4.6 × 75 mm)
Column oven temperture: 60 °C
Detector: UV 260 nm

ON1

HPLC (gradient condition: B% 5–20% in 30 min, then 20–98% in 20 min)

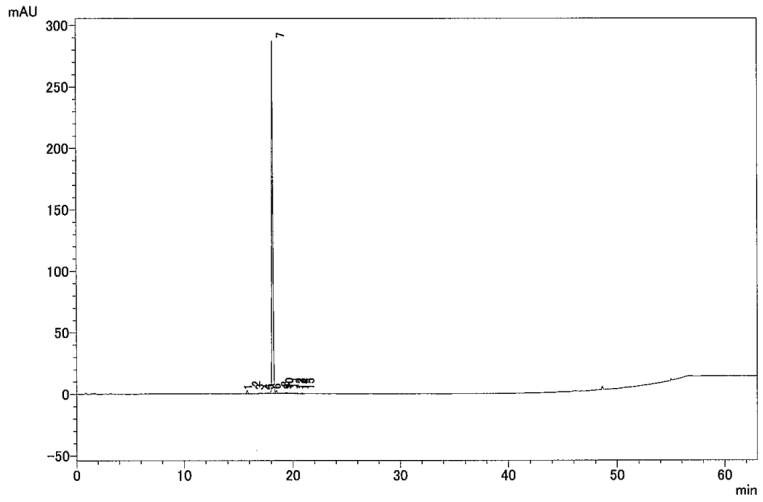


MALDI-TOF-MS

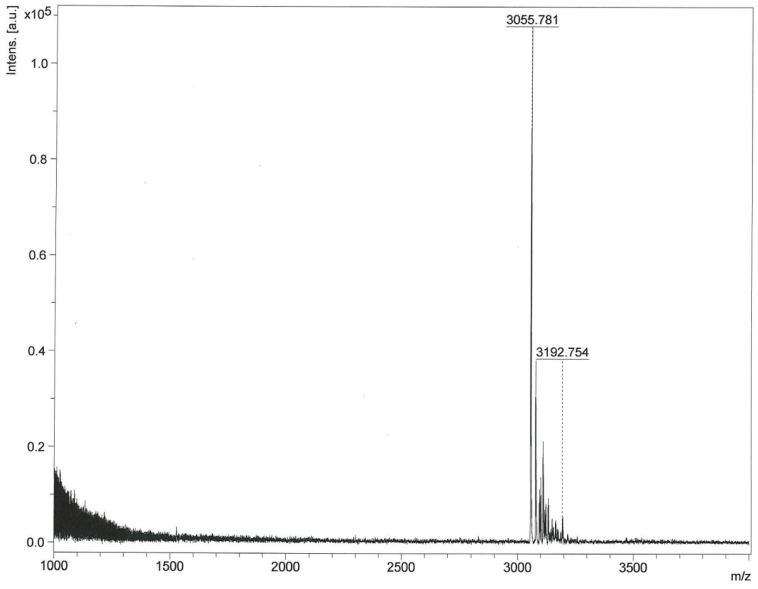


ON2

HPLC (gradient condition: B% 5–20% in 30 min, then 20–98% in 20 min)

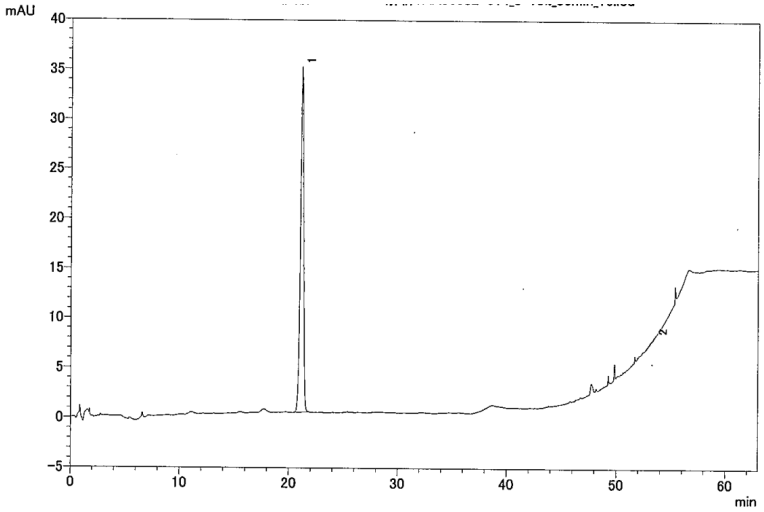


MALDI-TOF-MS

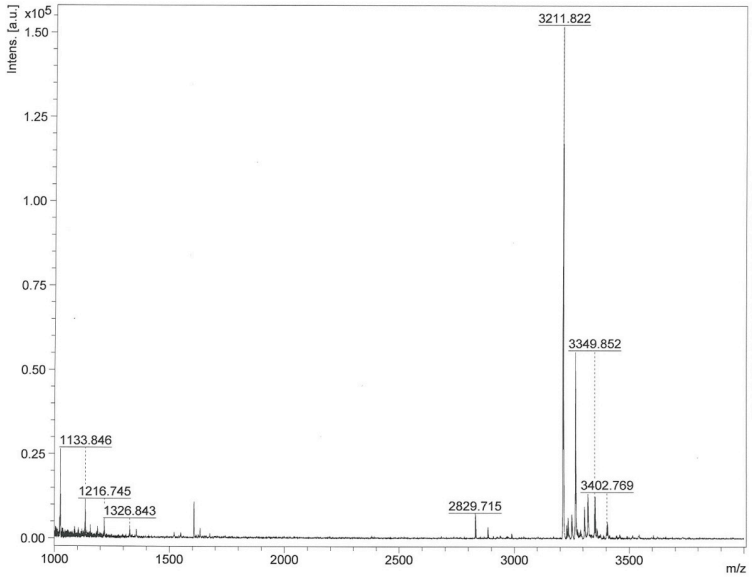


ON3

HPLC (gradient condition: B% 3–10% in 30 min, then 10–98% in 20 min)

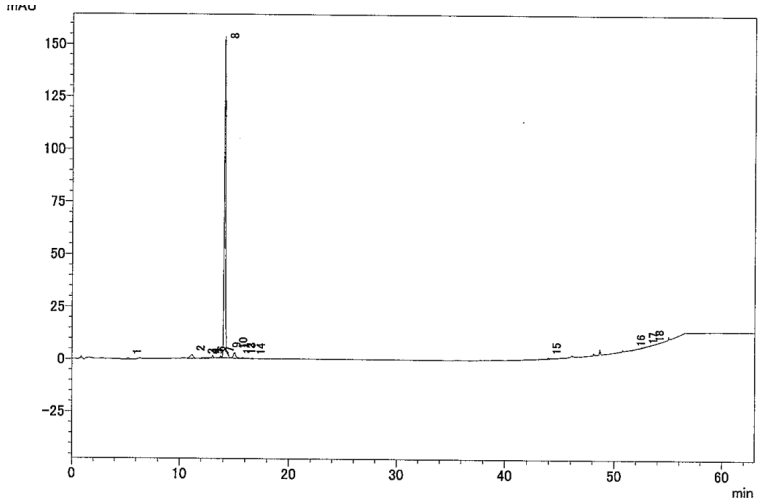


MALDI-TOF-MS

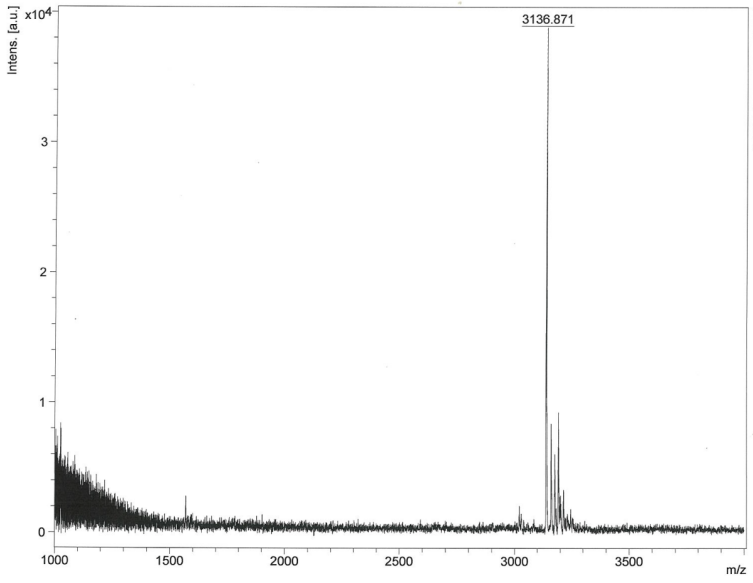


ON4

HPLC (gradient condition: B% 5–20% in 30 min, then 20–98% in 20 min)

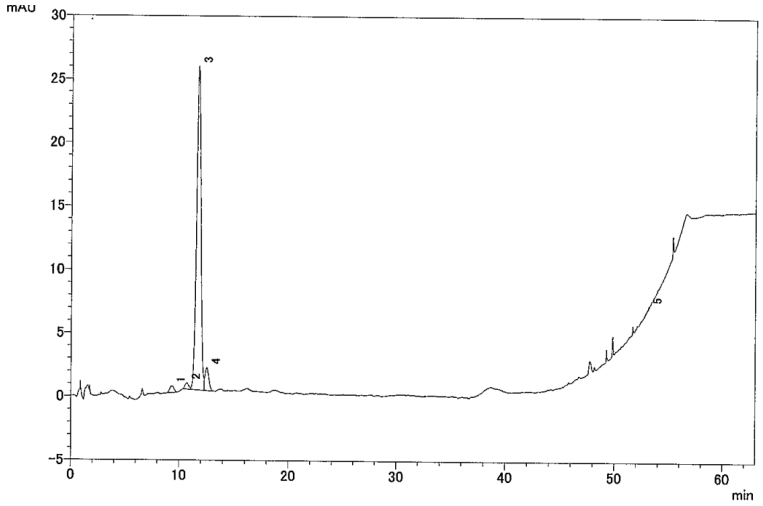


MALDI-TOF-MS

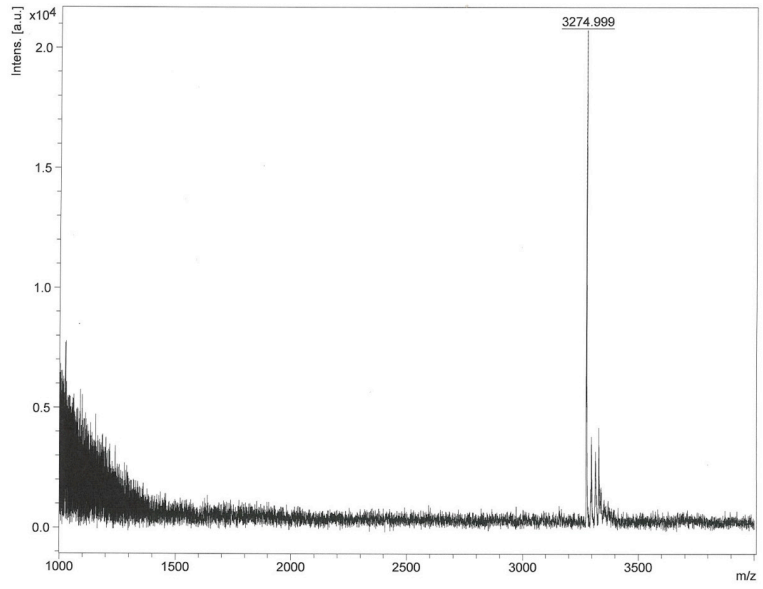


ON5

HPLC (gradient condition: B% 3–10% in 30 min, then 10–98% in 20 min)

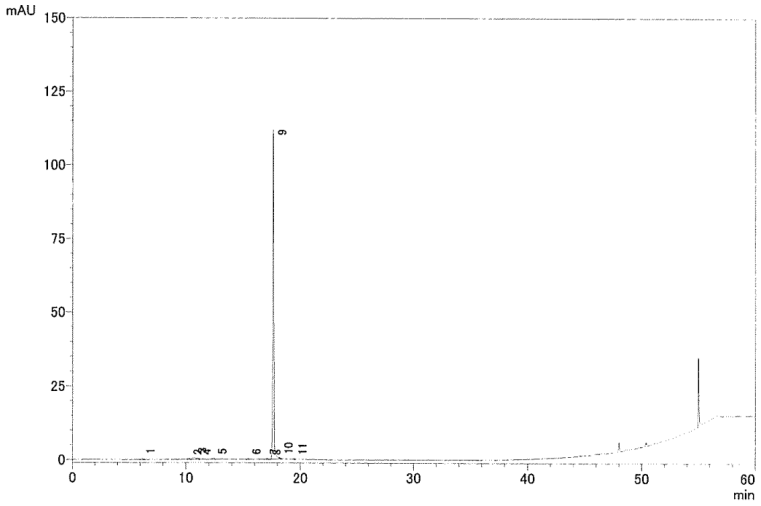


MALDI-TOF-MS

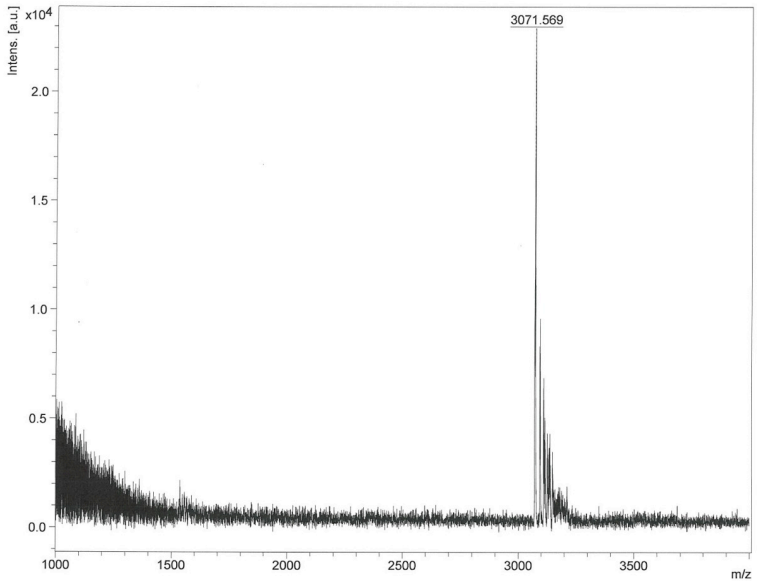


ON6

HPLC (gradient condition: B% 5–20% in 30 min, then 20–98% in 20 min)

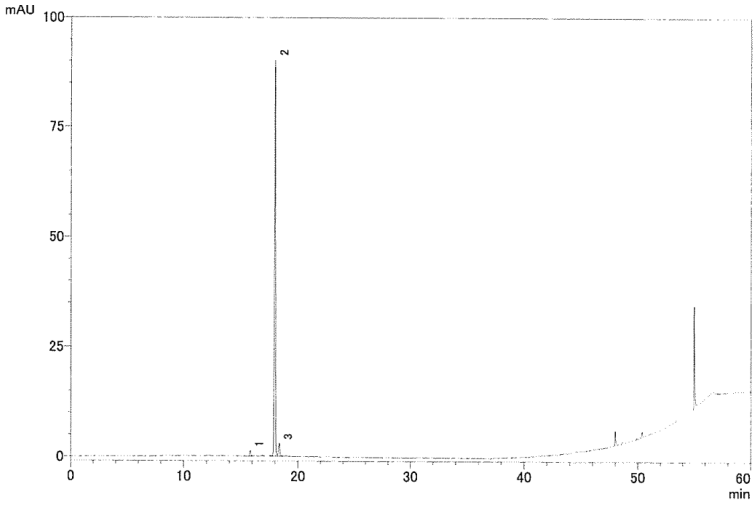


MALDI-TOF-MS

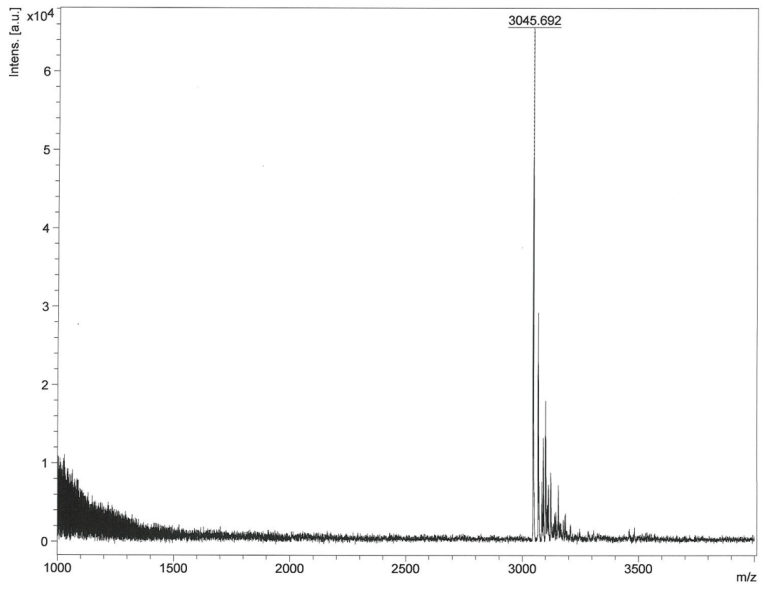


ON7

HPLC (gradient condition: B% 5–20% in 30 min, then 20–98% in 20 min)

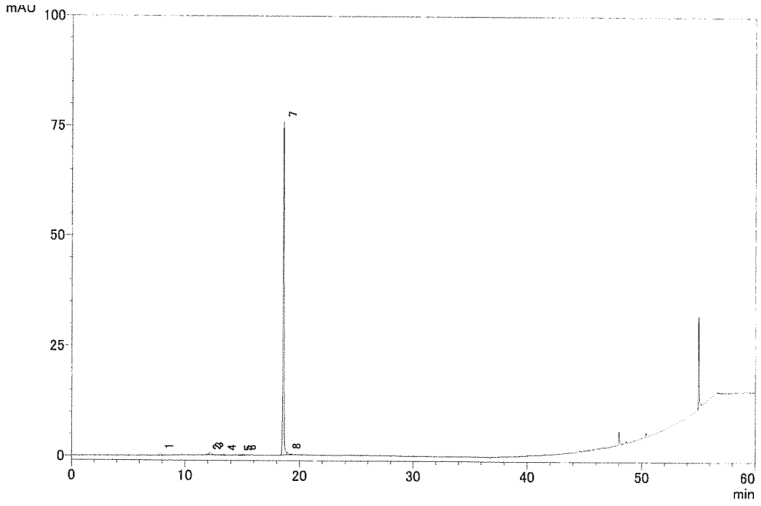


MALDI-TOF-MS

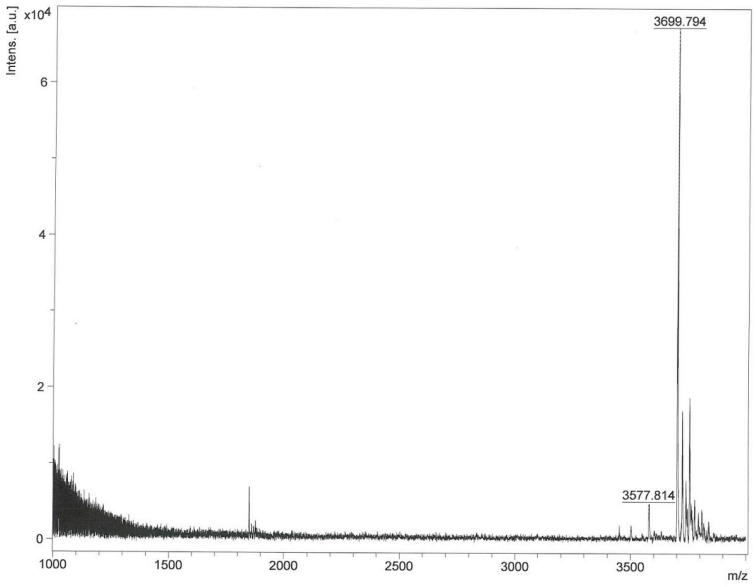


ON8

HPLC (gradient condition: B% 5–20% in 30 min, then 20–98% in 20 min)

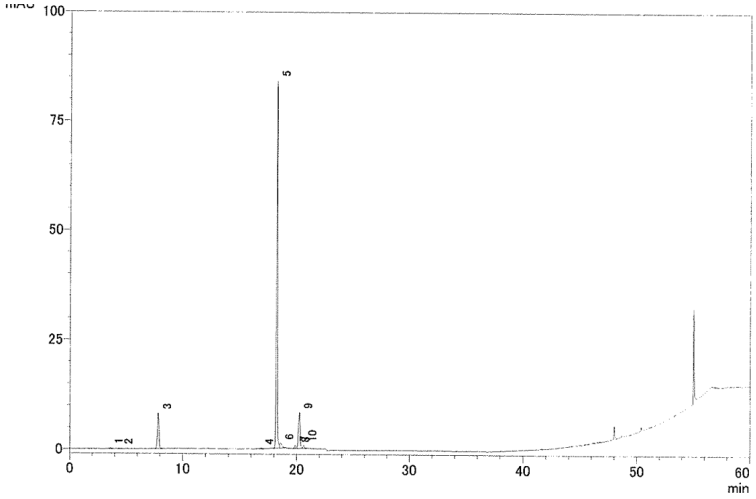


MALDI-TOF-MS

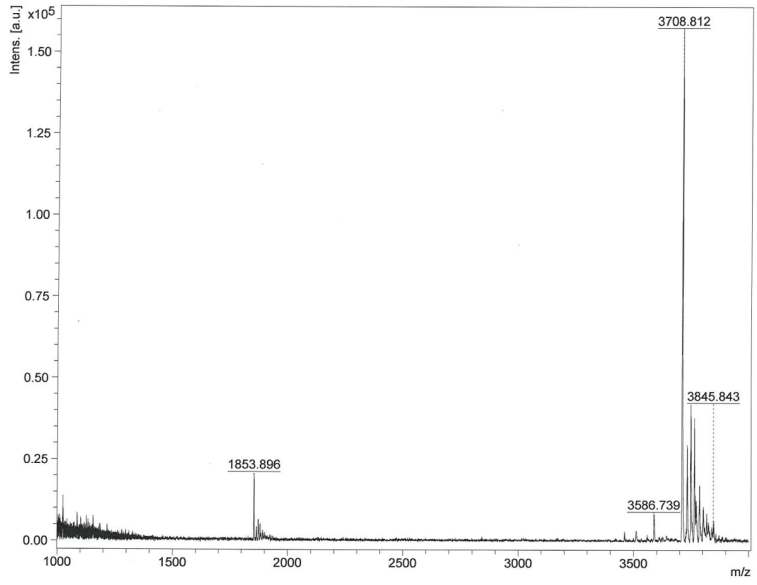


ON9

HPLC (gradient condition: B% 5–20% in 30 min, then 20–98% in 20 min)

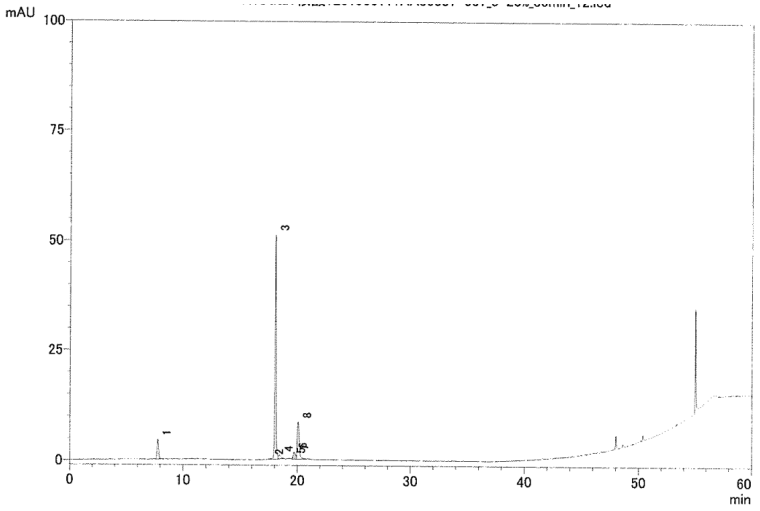


MALDI-TOF-MS

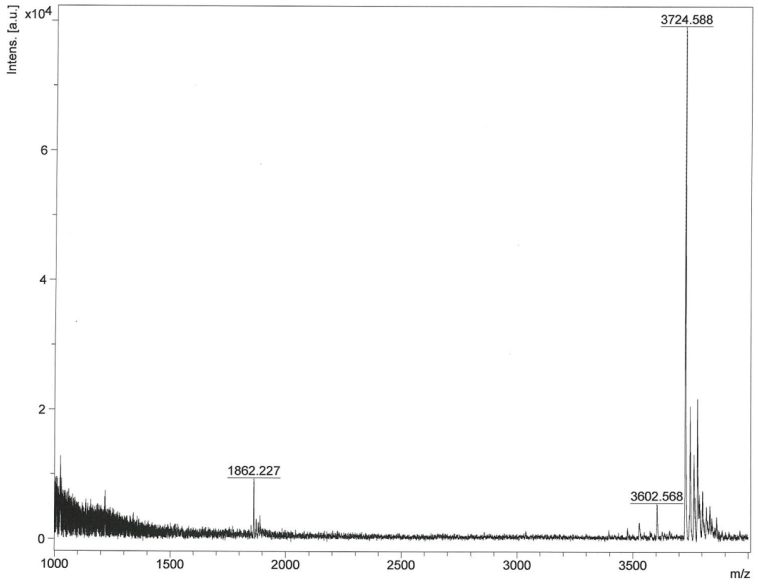


ON10

HPLC (gradient condition: B% 5–20% in 30 min, then 20–98% in 20 min)

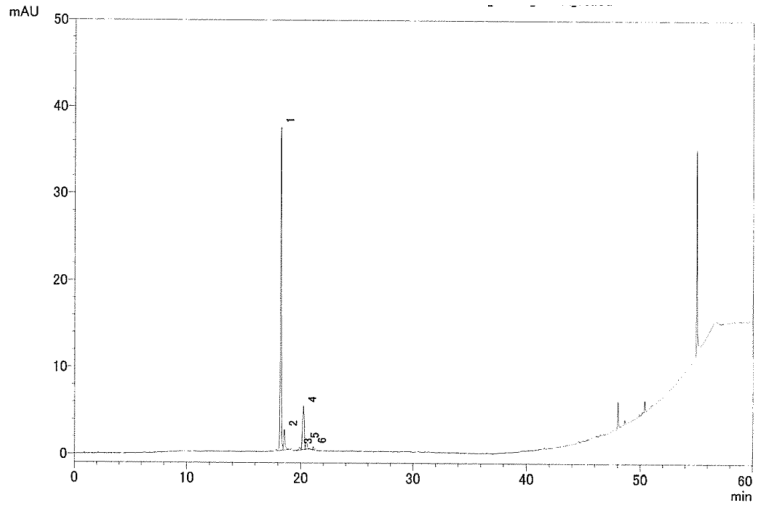


MALDI-TOF-MS

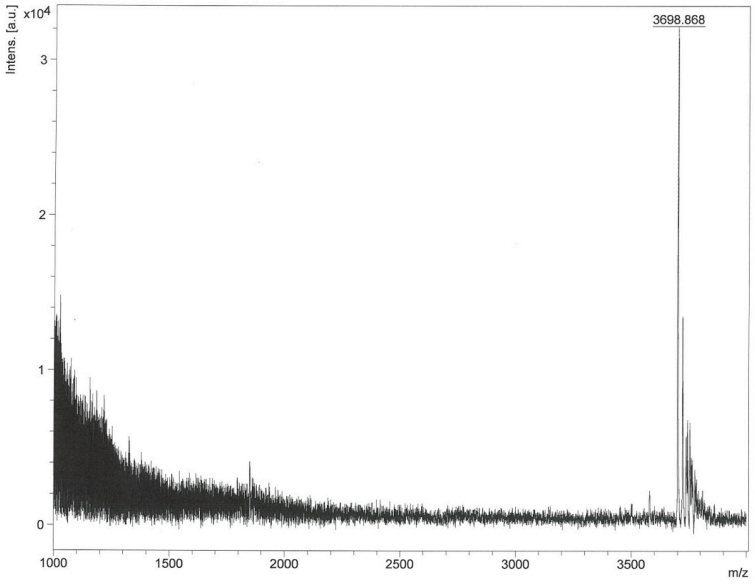


ON11

HPLC (gradient condition: B% 5–20% in 30 min, then 20–98% in 20 min)

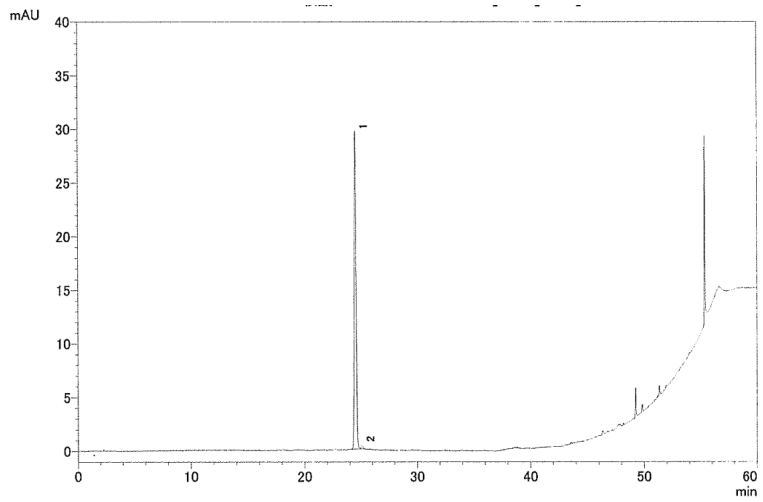


MALDI-TOF-MS

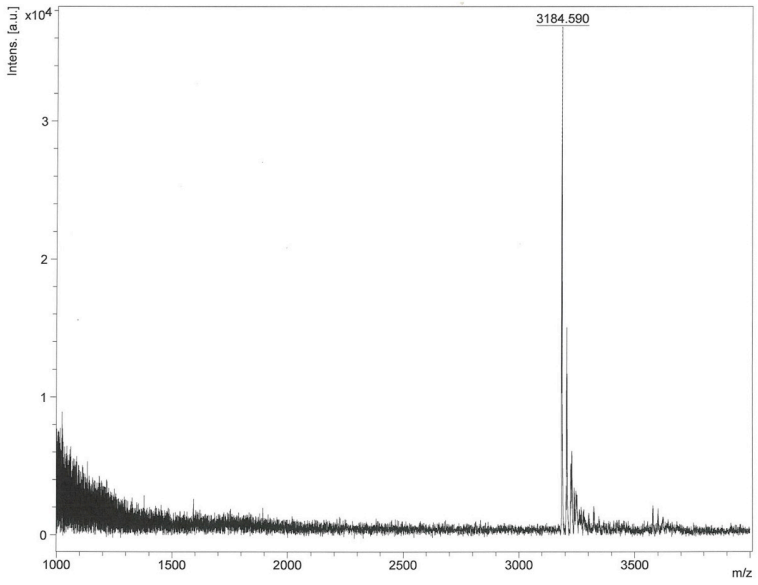


ON12

HPLC (gradient condition: B% 3–10% in 30 min, then 10–98% in 20 min)

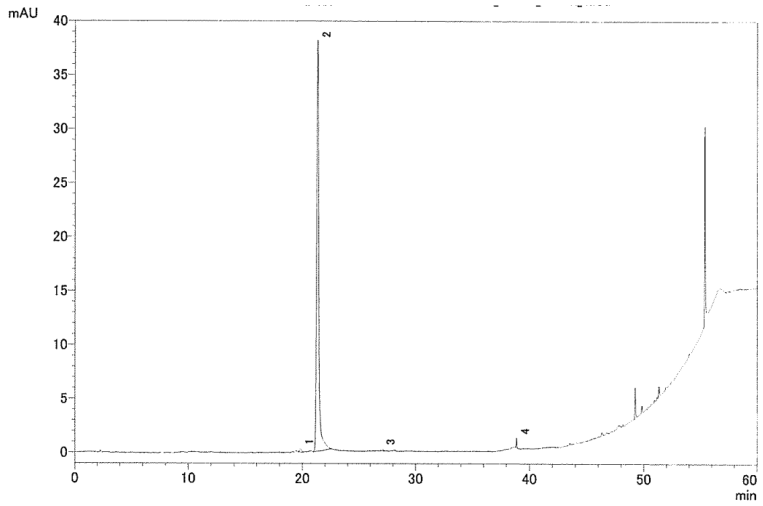


MALDI-TOF-MS

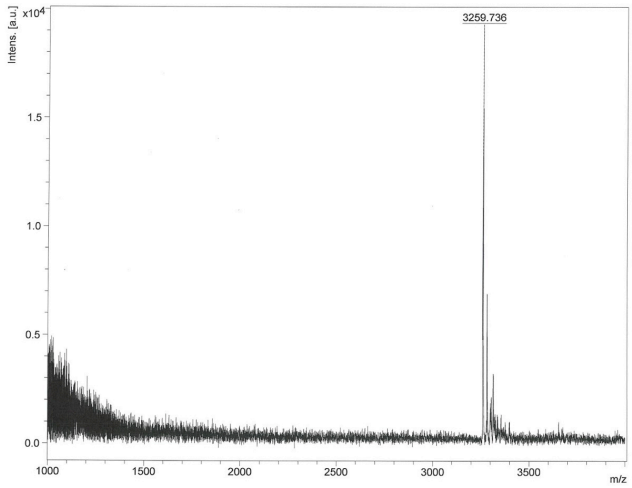


ON13

HPLC (gradient condition: B% 3–10% in 30 min, then 10–98% in 20 min)

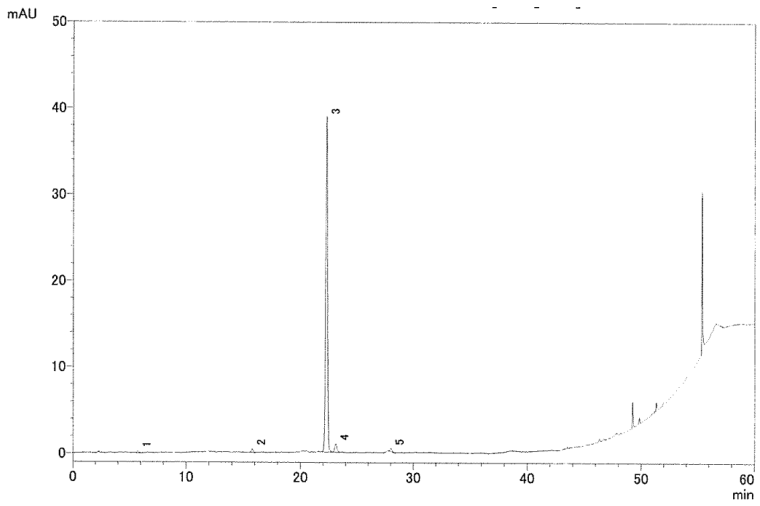


MALDI-TOF-MS

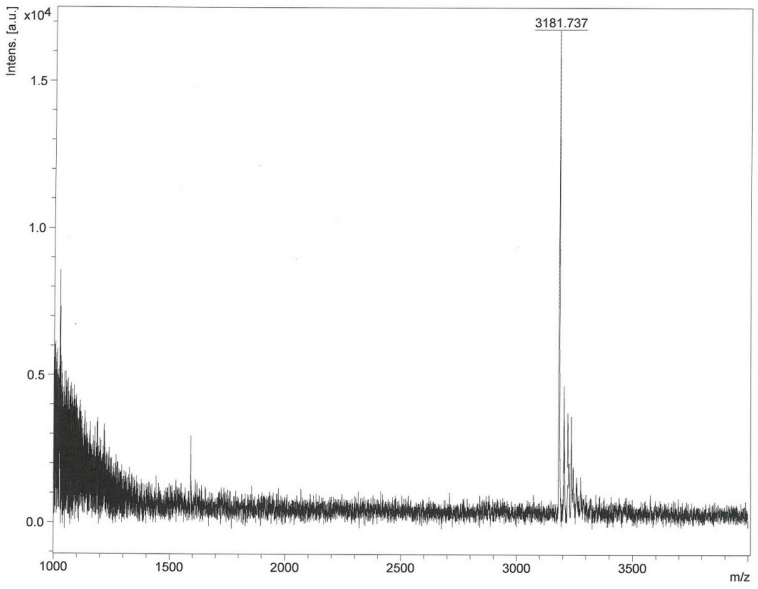


ON14

HPLC (gradient condition: B% 5–20% in 30 min, then 20–98% in 20 min)



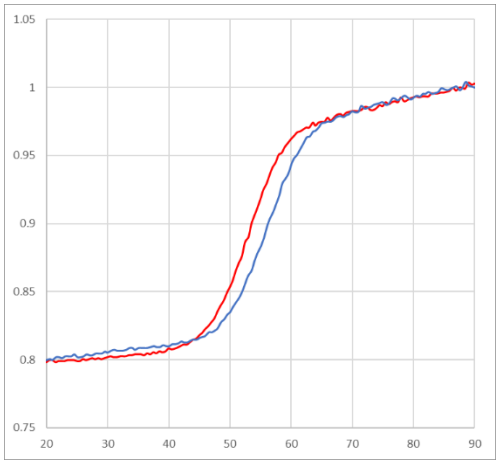
MALDI-TOF-MS



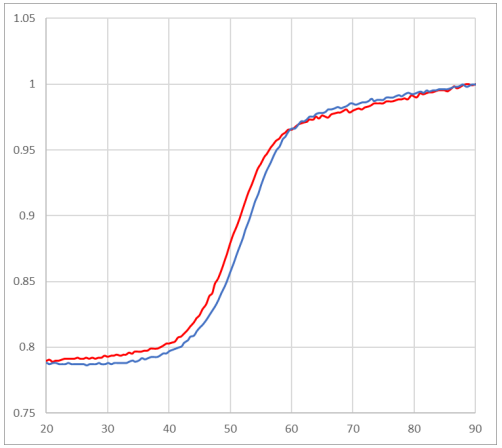
5. Representative UV melting curves

Thermal stability of the duplexes formed between GuNA-modified oligonucleotides and fully-matched ssDNAs

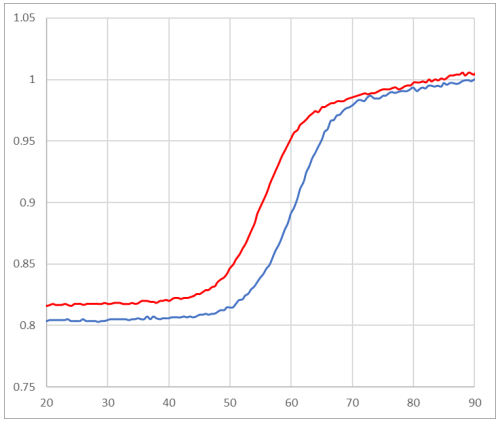
DNA-T (red) and GuNA-T (ON8, blue)



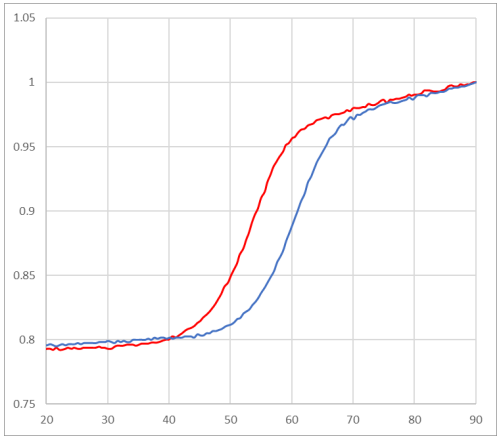
DNA-A (red) and GuNA-A (ON9, blue)



DNA-G (red) and GuNA-G (ON10, blue)

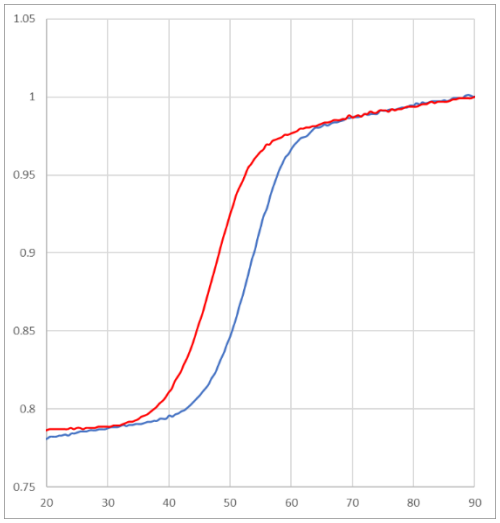


DNA-C (red) and GuNA-^{m}C (ON11, blue)

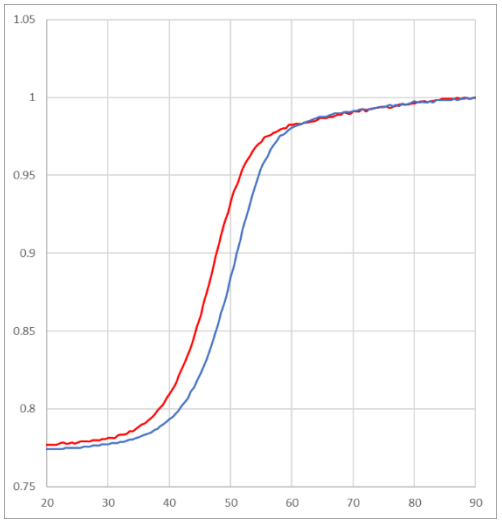


Thermal stability of the duplexes formed between GuNA-modified oligonucleotides and fully-matched ssRNAs

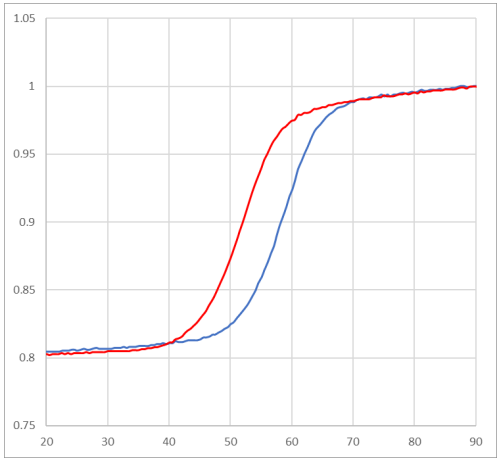
DNA-T (red) and GuNA-T (ON8, blue)



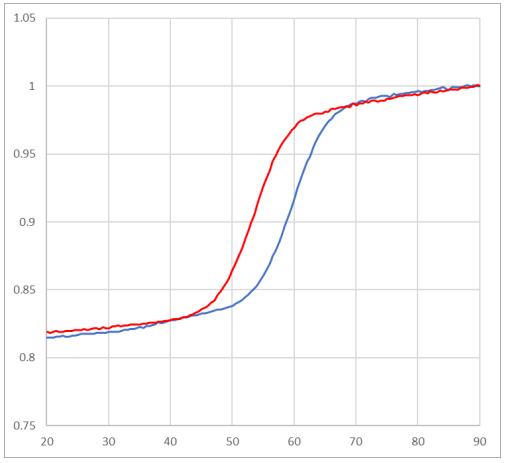
DNA-A (red) and GuNA-A (ON9, blue)



DNA-G (red) and GuNA-G (ON10, blue)

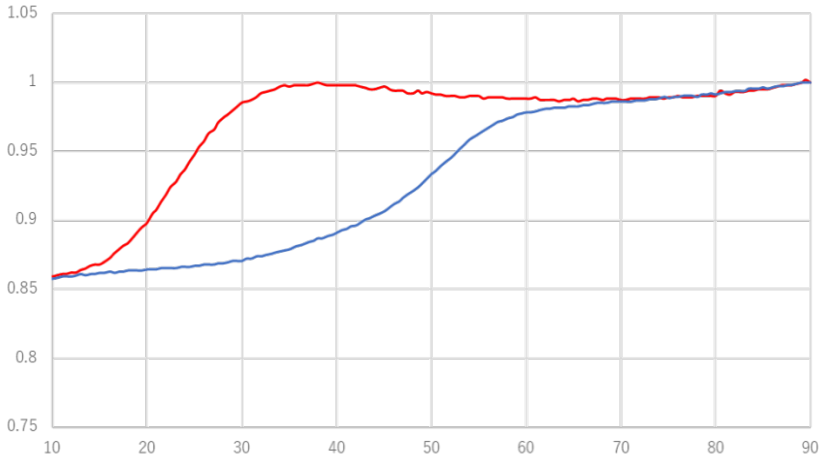


DNA-C (red) and GuNA-^{m}C (ON11, blue)

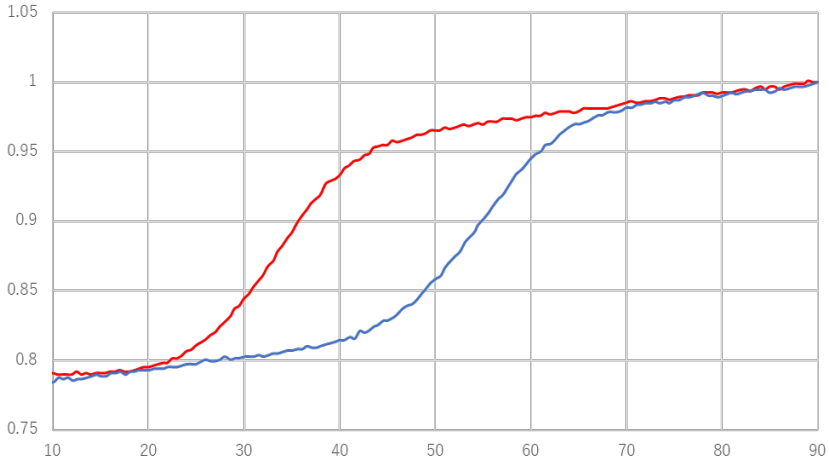


Thermal stability of the duplexes formed between multiply GuNA-modified oligonucleotides and their complimentary ssDNAs

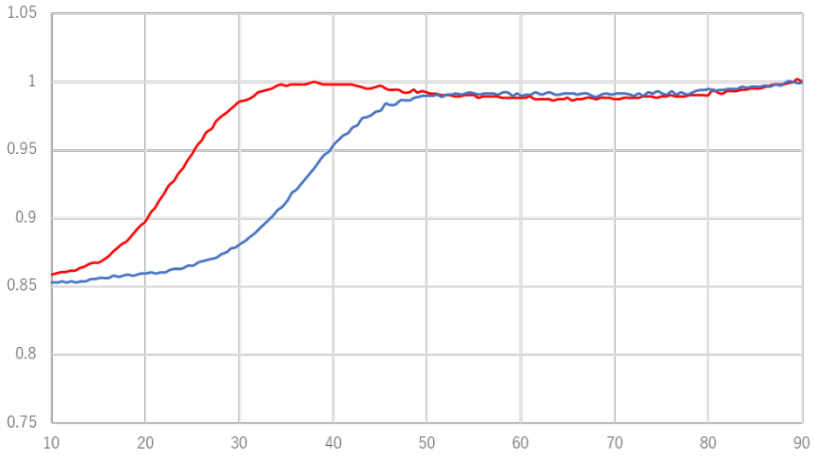
5′-d(TTTTTTTTTT)-3′(red) and 5′-d(TTTTTTTTTT)-3′ (ON12, blue)



5′-d(TTTGGGTTTT)-3′(red) and 5′-d(TTTGGGTTTT)-3′ (ON13, blue)



5′-d(AAAAAAAAAA)-3′(red) and 5′-d(AAAAAAAAAA)-3′ (ON5, blue)



5′-d(TTTCCCTTTT)-3′(red) and 5′-d(TTT^{m}C^{m}C^{m}CTTTT)-3′ (ON14, blue)