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

Supporting Information

Methyl group configuration on acyclic threoninol nucleic acids (*a*TNAs) impacts supramolecular properties

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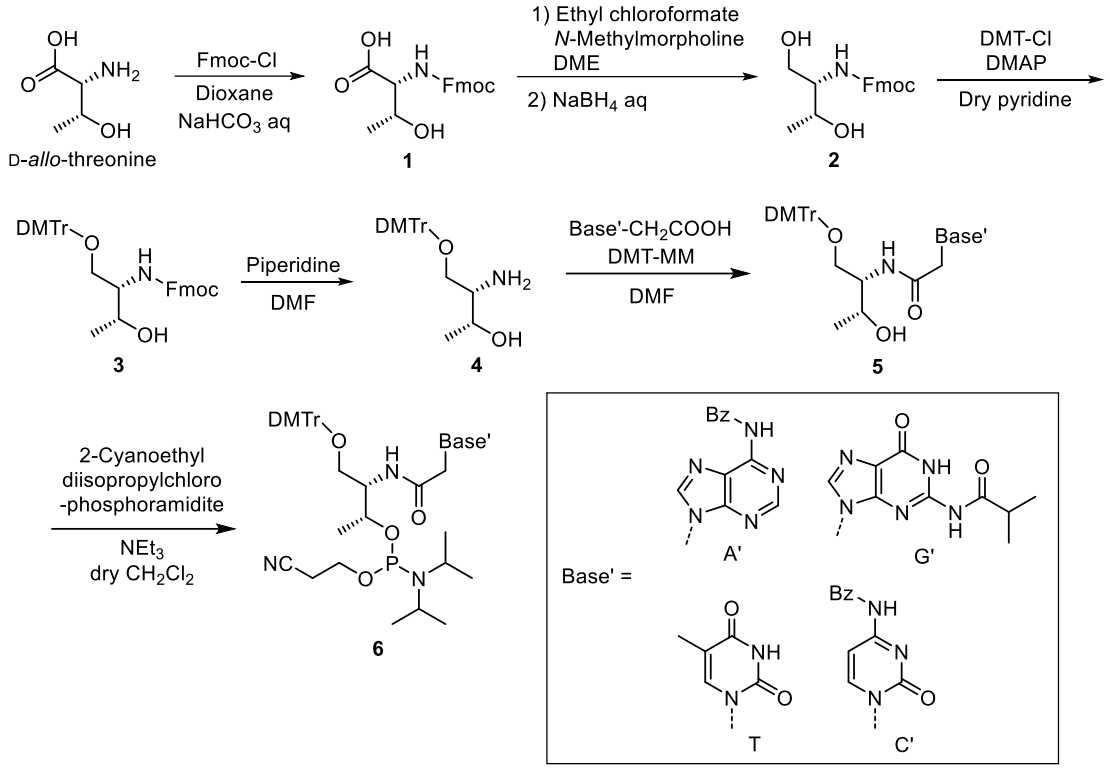


Scheme S1. Synthesis of the phosphoramidite monomer of D-*allo*-*a*TNA.

Synthesis of 1: D-*allo*-Threonine (7.30 g, 61.3 mmol) was dissolved in 200 mL saturated $NaHCO_3$ solution. After addition of 9-fluorenylmethyl chloroformate (16.6 g, 64.2 mmol) dissolved in 150 mL 1,4-dioxane, the mixture was vigorously stirred 1 h at room temperature. The solvent was reduced by evaporation until a clear solution was obtained, and the aqueous layer was extracted twice with diethyl ether. The pH was adjusted to around 2 using concentrated H_2SO_4, and ethyl acetate was added to dissolve precipitated product. The organic layer was washed twice with a saturated aqueous solution of NaCl. After drying over $MgSO_4$, the solid was removed by filtration, and the solvent was removed by evaporation. The residue was co-evaporated with acetonitrile to afford **1** (21.5 g, quant.).

^{1}H-NMR [DMSO-d_6, 500 MHz] δ = 7.92 (d, *J* = 7.5 Hz, 2H), 7.79 (d, *J* = 7.4 Hz, 2H), 7.54 (d, *J* = 8.6 Hz, 1H), 7.45 (t, *J* = 7.5 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 4.33 (d, *J* = 6.9 Hz, 2H), 4.28 (t, *J* = 7.0 Hz, 1H), 4.06-3.98 (m, 2H), 1.18 (d, *J* = 6.2 Hz, 3H). ^{13}C-NMR [DMSO-d_6,125 MHz] δ = 172.8, 156.7, 144.3, 114.2, 128.1, 127.6, 125.8, 120.6, 67.0, 66.8, 60.9, 47.1, 20.2.

HRMS (FAB) Calcd for $C_{19}H_{20}NO_5$ ($M+H^+$) 342.1336. Found 342.1335.

Synthesis of 3: 1 (61.3 mmol) was dissolved in 110 mL dry 1,2-dimethoxyethane under argon. The mixture was cooled to -20 °C, and 4-methylmolphorine (6.88 mL, 63.7 mmol) and ethyl chloroformate (5.95 mL, 62.5 mmol) were added dropwise. After stirring for 10 min at -20 °C, mixture was further stirred for 20 min at room temperature. The solid was removed by filtration, and the collected filtrate was slowly added to $NaBH_4$ (3.72 g, 98.4 mmol) dissolved in 120 mL deionized H_2O at 0 °C. After addition of 300 mL deionized

H_2O and 300 mL ethyl acetate, the organic layer was washed with twice with a saturated aqueous solution of NaCl. The solid was removed by filtration, and the solvent was removed by evaporation. The residue was co-evaporated with acetonitrile to afford **2** (17.8 g, 54.4 mmol, yield: 89%), which was used in the next step without further purification. **2** (17.8 g, 54.4 mmol) and *N,N*-diisopropylethylamine (10.0 mL, 59.1 mmol) were dissolved in 100 mL dry pyridine under argon. 4,4'-Dimethoxytrityl chloride (14.8 g, 53.3 mmol) and *N*,*N*-dimethyl-4-aminopyridine (0.61 g, 5.0 mmol) dissolved in 80 mL of dry CH_2Cl_2 were added to the reaction mixture at 0 °C. The mixture was stirred for 30 min at 0 °C and then for 2.0 h at room temperature. The reaction was quenched by addition of MeOH (20 ml). The solvent was removed under vacuum. The residue was dissolved with ethyl acetate and washed with an aqueous 4% citric acid and saturated aqueous solution of $NaHCO_3$ and then with a saturated aqueous solution of NaCl. The solvent was reduced under vacuum, and purification by silica gel column chromatography (hexane/EtOAc (2:1, v/v), 3% Et_3N) afforded **3** (24.9 g, 39.6 mmol, yield: 73%).

^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 7.76 (d, *J* = 7.5 Hz, 2H), 7.62 (m, 2H), 4.70 (m, 5H), 7.33-7.20 (m, 10H), 6.80 (m, 4H), 5.58 (d, *J* = 8.4 Hz, 1H), 4.45-4.36 (m, 2H), 4.24 (t, *J* = 7.0 Hz, 1H), 3.93 (br, 1H), 3.73-3.70 (m, 7H), 3.48-3.30 (m, 2H), 1.10 (d, *J* = 6.3 Hz, 3H). ^{13}C-NMR [$CDCl_3$, 125 MHz] δ = 158.7, 156.4, 144.4, 143.98, 143.94, 141.3, 135.6, 135.3, 129.97, 129.95, 128.1, 128.0, 127.8, 127.2, 127.0, 125.2, 125.1, 120.0, 113.3, 86.8, 69.4, 67.0, 62.8, 55.6, 55.22, 55.21, 47.3, 20.1. HRMS (FAB) Calcd for $C_{40}H_{39}NO_6$ (M) 629.2777. Found 629.2775.

Synthesis of 4: 3 (24.9 g, 39.6 mmol) was dissolved in 80 mL *N,N*-dimethyl formamide (DMF) and 20 mL piperidine was added. The mixture was stirred for 2.0 h at room temperature, and then solvent was removed under vacuum. After purification by silica gel column chromatography (ethyl acetate, 3% Et_3N to $CHCl_3$/MeOH (25:1, v/v), 3% Et_3N), the solvent was removed and co-evaporated twice with diethyl ether to afford **4** (12.1 g, 29.7 mmol, yield: 75%).

^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 7.42 (m, 2H), 7.34-7.19 (m, 7H), 6.73 (m, 4H), 3.79 (s, 6H), 3.75 (m, 1H), 3.18 (m, 2H), 2.92 (m, 1H), 1.01 (d, *J* = 6.5 Hz, 3H). ^{13}C-NMR [$CDCl_3$, 125 MHz] δ = 158.5, 144.8, 135.99, 135.95, 130.0, 128.1, 127.9, 126.8, 113.2, 86.4, 69.5, 65.5, 55.9, 55.2, 18.5. HRMS (FAB) Calcd for $C_{25}H_{29}NO_4$ (M) 407.2097. Found 407.2066.

General procedures for the synthesis of 5: To a stirred solution of **Base'-CH_2COOH**[1-3] (1.2 eq.), triethylamine (2.0 eq.) and **4** (1.0 eq.) in 10 mL DMF were added 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) (1.5 eq.), and the mixture was stirred for 2 h. Then 20 mL $CHCl_3$ was added, and the mixture was washed with a saturated aqueous solution of $NaHCO_3$ and a saturated aqueous solution of NaCl. The solvent was removed in vacuo. The residue was purified by silica gel column chromatography using chloroform and methanol as eluent (3% triethylamine was added). After column chromatography, products were co-evaporated with acetonitrile to afford **5**.

5-A': 1.58 g (2.30 mmol, yield: 82%), ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 9.25 (br, 1H), 8.70 (s, 1H), 8.11 (s, 1H), 8.00 (m, 2H), 7.59 (m, 1H), 7.49 (m, 2H), 7.35-7.16 (m, 9H), 6.80 (m, 4H), 4.92 (dd, 2H), 3.98 (m, 1H),

3.90 (m, 1), 3.76 (s, 6H), 3.41-3.35 (m, 2H), 3.22 (m, 1H), 1.04 (d, *J* = 6.5 Hz, 3H). ^{13}C-NMR [$CDCl_3$, 125 MHz] δ = 165.5, 164.7, 158.6, 152.8, 152.0, 149.6, 144.2, 143.6, 135.4, 135.1, 133.5, 132.8, 129.85, 129.82, 128.8, 128.0, 127.9, 127.8, 127.0, 122.5, 113.3, 86.8, 68.9, 62.0, 55.2, 54.4, 46.6, 19.9. HRMS (FAB) Calcd for $C_{39}H_{39}N_6O_6$ ($M+H^+$) 687.2926. Found 687.2907.

5-T: 2.00 g (3.49 mmol, quant.), ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 9.70 (br, 1H), 7.40 (m, 2H), 7.31-7.18 (m, 9H), 7.04 (s, 1H), 6.82 (m, 4H), 7.49 (m, 2H), 7.35-7.16 (m, 9H), 6.80 (m, 4H), 4.33 (dd, 2H), 3.99 (m, 1H), 3.90 (m, 1), 3.78 (s, 6H), 3.42-3.29 (m, 2H), 1.85 (s, 3H), 1.05 (d, *J* = 6.5 Hz, 3H). ^{13}C-NMR [$CDCl_3$, 125 MHz] δ = 166.6, 164.3, 162.6, 158.6, 151.3, 144.4, 140.9, 135.5, 135.3, 129.9, 128.0, 127.9, 127.0, 113.28, 113.26, 111.0, 86.8, 69.1, 62.1, 55.2, 54.2, 50.3, 36.5, 31.5, 30.9, 19.8, 12.3. HRMS (FAB) Calcd for $C_{32}H_{35}N_3O_7$ (M) 573.2475. Found 573.2478.

5-C': 1.76 g (2.66 mmol, yield: 87%), ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 8.96 (br, 1H), 7.91 (d, *J* = 7.6 Hz, 2H), 7.70-7.17 (m, 15H), 6.82 (m, 4H), 4.53 (dd, 2H), 4.00 (m, 1H), 3.94 (br, 1H), 3.75 (s, 6H), 3.40-3.30 (m, 2H), 1.07 (d, *J* = 6.5 Hz, 3H). ^{13}C-NMR [$CDCl_3$, 125 MHz] δ = 166.5, 162.8, 162.6, 158.5, 149.7, 144.5, 135.6, 135.4, 133.1, 132.9, 130.01, 129.95, 128.9, 127.96, 127.94, 127.87, 127.78, 126.9, 113.2, 97.1, 86.9, 86.6, 68.9, 61.9, 55.2, 54.8, 52.8, 36.5, 31.4, 19.6. HRMS (FAB) Calcd for $C_{38}H_{39}N_4O_7$ ($M+H^+$) 663.2814. Found 663.2832.

5-G': 1.90 g (2.84 mmol, yield: 95%), ^{1}H-NMR [DMSO-d_6, 500 MHz] δ = 12.2-11.5 (br, 2H), 8.12 (d, *J* = 9.0 Hz, 1H), 7.91 (s, 1H), 7.44-7.24 (m, 9H), 6.92 (m, 4H), 4.86 (dd, 2H), 4.69 (d, *J* = 5.5 Hz, 1H), 3.98 (m, 1H), 3.79 (m, 1H + s, 6H),3.11 (m, 2H), 2.78 (m, 1H), 1.14 (m, 6H), 1.06 (d, *J* = 6.4 Hz, 3H). ^{13}C-NMR [DMSO-d_6, 125 MHz] δ = 178.4, 164.2, 156.3, 153.3, 147.4, 146.3, 143.4, 139.0, 134.2, 134.1, 128.1, 126.10, 126.05, 124.9, 117.9, 116.4, 111.4, 83.6, 77.5, 64.4, 64.1, 60.8, 53.3, 44.4, 43.7, 33.0, 18.3, 17.21, 17.16. HRMS (FAB) Calcd for $C_{36}H_{41}N_6O_7$ ($M+H^+$) 669.3032. Found 669.3076.

General procedures for the synthesis of phosphoramidite monomers 6: 5 (1.0 eq.) and triethylamine (5.0 eq.) were dissolved in 10 mL dry dichloromethane under argon. 2-Cyanoethyl diisopropylchlorophosphoramidite (2.0 eq.) was added to the mixture at 0 °C. After 10 min at 0 °C, the reaction mixture was stirred for 20 min at room temperature. The reaction mixture was diluted with 10 mL solvent, and the product was isolated by silica gel column chromatography. Fractions containing product were dried and co-evaporated twice with dry acetonitrile to afford **6**. Chloroform and acetone were used as eluent (3% triethylamine was added) for **6-G'**. Hexane, ethyl acetate, and triethylamine were used for the others. Before the synthesis of *allo-a*TNA on a DNA synthesizer, phosphoramidite monomers **6** were dried by co-evaporation with dry acetonitrile.

6-A': 0.67 g (0.76 mmol, yield: 52%), ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 9.21 (br, 1H), 8.70 (m, 1H), 8.18,8.12 (s, 1H), 8.04 (m, 2H), 7.62-7.20 (m, 12H), 6.82 (m, 4H), 6.65,6.48 (d, *J* = 8.9 Hz, 1H), 5.00-4.84 (m, 2H), 4.29-4.11 (m, 2H), 3.78-3.45 (m, 12H), 2.65-2.53 (m, 2H), 1.18-1.03 (m, 15H). ^{13}C-NMR [$CDCl_3$, 125 MHz] δ = 165.3, 165.2, 164.60, 164.56, 158.48, 158.45, 152.8, 152.7, 152.0, 151.9, 149.6, 149.5, 144.7, 144.6, 143.8, 143.5, 135.93, 135.88, 135.84, 135.78, 133.73, 133.68, 132.8, 132.7, 130.03, 129.99, 129.97, 129.90, 128.9, 128.1, 128.0, 127.9, 127.8, 126.82, 126.79, 122.38, 122.36, 118.2, 117.9, 113.2, 113.1,

86.3, 71.3, 71.1, 70.6, 70.5, 61.3, 61.2, 58.2, 58.1, 57.9, 57.8, 54.83, 54.78, 54.5, 54.4, 46.8, 46.6, 45.4, 45.3, 43.24, 43.12, 43.04, 43.02, 24.64, 24.58, 24.54, 24.4, 24.3, 22.98, 22.97, 22.90, 22.88, 20.49, 20.47, 20.44, 20.42, 20.12, 20.06, 19.27, 19.25, 18.8, 18.83, 18.81. ^{31}P-NMR [$CDCl_3$, 121 MHz] δ = 147.7, 147.0. HRMS (FAB) Calcd for $C_{48}H_{56}N_8O_7P$ ($M+H^+$) 887.4005. Found 887.4047.

6-T: 0.77 g (0.99 mmol, yield: 57%), ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 9.13 (br, 1H), 7.43-7.19 (m, 9H), 7.08,7.02 (s, 1H), 6.82 (m, 4H), 6.52,6.29 (d, 1H), 4.43-4.09 (m, 4H), 3.80-3.63 (m, 8H), 3.50 (m, 2H), 3.25 (m, 2H), 2.68-2.51 (m, 2H), 1.88 (d, *J* = 8.1 Hz, 3H), 1.20-1.05 (m, 15H). ^{13}C-NMR [$CDCl_3$, 125 MHz] δ = 166.3, 166.2, 164.1, 164.0, 158.5, 151.1, 151.0, 144.83, 144.77, 140.9, 140.6, 136.0, 135.94, 135.89, 130.1, 130.0, 128.2, 128.1, 127.9, 127.8, 126.81, 126.77, 118.4, 117.9, 113.2, 113.1, 111.0, 110.8, 86.3, 71.3, 71.1, 70.6, 70.5, 61.4, 58.0, 57.9, 57.8, 55.2, 54.7, 54.2, 50.5, 50.3, 43.2, 43.1, 24.63, 24.58, 24.53, 24.4, 24.3, 20.44, 20.40, 20.35, 19.3, 18.8, 12.3. ^{31}P-NMR [$CDCl_3$, 121 MHz] δ = 147.5, 147.0. HRMS (FAB) Calcd for $C_{41}H_{52}N_5O_8P$ (M) 773.3554. Found 773.3549.

6-C': 0.68 g (0.79 mmol, yield: 52%), ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 8.79 (br, 1H), 7.90 (m, 2H), 7.77-7.15 (m, 14H), 6.85-6.68 (m, 5H), 4.66-4.41 (m, 2H), 4.27-4.09 (m, 2H), 3.78-3.67 (m, 8H), 3.48 (m, 2H), 3.25 (m, 2H), 2.70-2.57 (m, 2H), 1.18-1.03 (m, 15H). ^{13}C-NMR [$CDCl_3$, 125 MHz] δ = 166.2, 166.0, 162.7, 158.5, 144.9, 144.8, 136.1, 135.98, 135.95, 133.2, 130.12, 130.06, 129.0, 128.2, 128.1, 127.9, 127.8, 127.7, 126.8, 118.5, 118.0, 113.2, 113.1, 86.30, 86.28, 70.9, 70.8, 70.5, 70.3, 61.6, 61.5, 58.3, 58.1, 58.0, 57.9, 55.2, 54.81, 54.77, 54.38, 54.35, 53.1, 43.19, 43.15, 43.09, 43.05, 24.7, 24.62, 24.56, 24.4, 24.3, 20.5, 20.42, 20.37, 19.22, 19.20, 18.63, 18.61. ^{31}P-NMR [$CDCl_3$, 121 MHz] δ = 147.6, 146.9. HRMS (FAB) Calcd for $C_{47}H_{54}N_6O_8P$ ($M-H^+$) 861.3746. Found 861.3755.

6-G': 0.85 g (0.98 mmol, yield: 65%), ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 11.8-12.1 (br, 1H), 8.94-8.40 (br, 1H), 7.78, 7.69 (s, 1H), 7.42-7.00 (m, 10H), 6.80-6.75 (m, 4H), 4.90-4.77 (m, 2H), 4.29 (m, 1H), 4.06 (m, 1H), 3.77-3.62 (m, 8H), 3.52-3.41 (m, 2H), 3.33-3.18 (m, 2H), 2.63-2.55 (m, 2H), 2.26-1.95 (m, 1H), 1.19-0.96 (m, 21H). ^{13}C-NMR [$CDCl_3$, 125 MHz] δ = 179.0, 178.7, 166.1, 165.9, 158.5, 158.52, 158.51, 158.48, 155.6, 148.81, 148.78, 147.8, 147.6, 144.83, 144.80, 140.1, 139.9, 136.1, 136.0, 135.8, 130.2, 130.11, 130.07, 130.03, 128.3, 128.2, 127.78, 127.72, 126.9, 120.5, 118.5, 118.1, 116.4, 113.1, 113.04, 112.98, 112.9, 86.0, 71.3, 71.1, 70.3, 61.8, 61.5, 57.9, 57.8, 57.7, 55.2, 54.8, 54.3, 46.6, 46.5, 43.2, 43.09, 43.06, 43.0, 35.9, 35.8, 24.6, 24.5, 24.3, 24.2, 24.5, 20.6, 20.51, 20.48, 20.43, 19.3, 19.1, 19.0, 18.9, 18.6, 18.4. ^{31}P-NMR [$CDCl_3$, 121 MHz] δ = 147.6, 146.7. HRMS (FAB) Calcd for $C_{45}H_{58}N_8O_8P$ ($M+H^+$) 869.4110. Found 869.4147.

Synthesis of the phosphoramidite monomers of L-*allo*-*a*TNA was performed by same procedures with D-*allo*-*a*TNA, described above. All NMR data of L-*allo*-*a*TNA phosphoramidites and synthetic intermediates corresponded to those of D-*allo*-*a*TNA, because they are enantiomers. Yields of synthesis relating L-*allo*-*a*TNA monomers were shown below; **1**: 21.7 g, (61.3 mmol, quant), **2**: 16.1 g, (49.2 mmol, yield: 80%), **3**: 20.4 g, (32.4 mmol, yield: 66%), **4**: 9.90 g, (24.3 mmol, 75%), **5-A':** 1.75 g (2.55 mmol, yield: 92%), **5-T:** 1.88 g (3.28 mmol, quant.), **5-C':** 1.71 g (2.58 mmol, yield: 91%), **5-G':** 1.56 g (2.33 mmol, yield: 77%), **6-**

A': 0.67 g (0.76 mmol, yield: 52%), **6-T:** 0.76 g (0.98 mmol, yield: 56%), **6-C':** 0.70 g (0.81 mmol, yield: 54%), **6-G':** 0.79 g (0.91 mmol, yield: 61%).

References

[1] L. Christensen, H. F. Hansen, T. Koch, P. E. Nielsen, Inhibition of PNA triplex formation by N4-benzoylated cytosine. *Nucleic Acids Res.* 1998, **26**, 2735.

[2] Z. Timár, L. Kovács, G. Kovács, Z. Schmél, Fmoc/acyl protecting groups in the synthesis of polyamide (peptide) nucleic acid monomers. *J. Chem. Soc. Perkin Trans.* 2000, **1**, 19.

[3] T. Kofoed, H. F. Hansen, H. Ørum, T. Koch, PNA synthesis using a novel Boc/acyl protecting group strategy. *J. Peptide Sci.* 2001, **7**, 402.

2. Supporting Figures and Tables

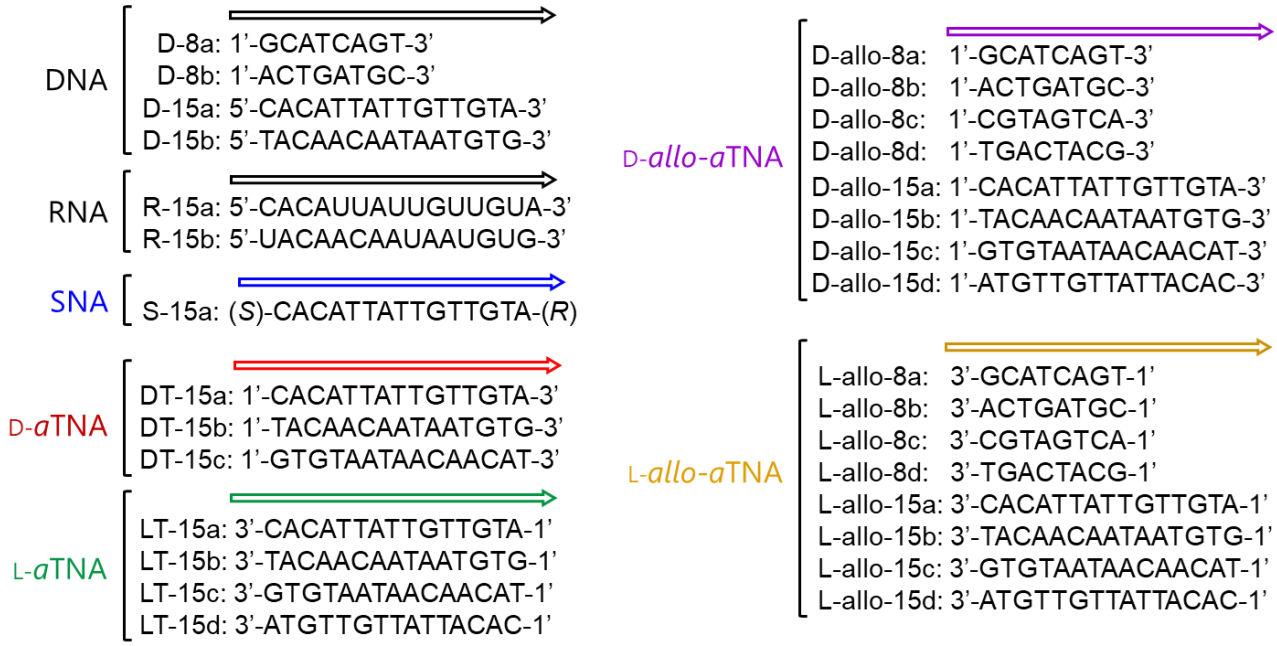


Figure S1. XNA sequences used in this study.

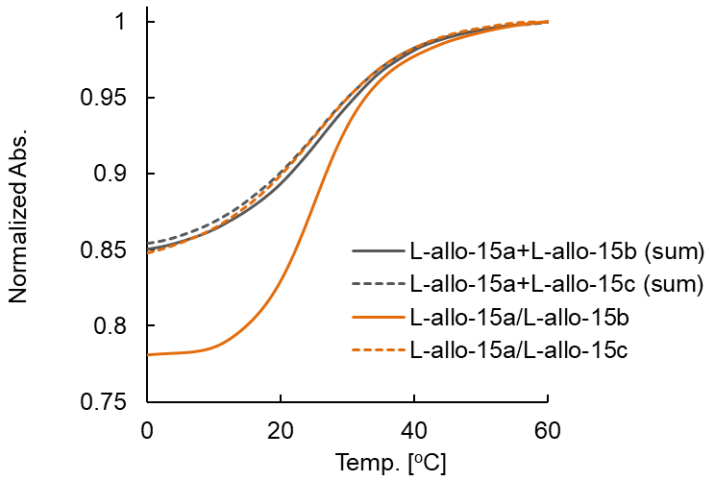


Figure S2. Comparison of the sums of the melting curves of single strands and of the duplex melting curves.

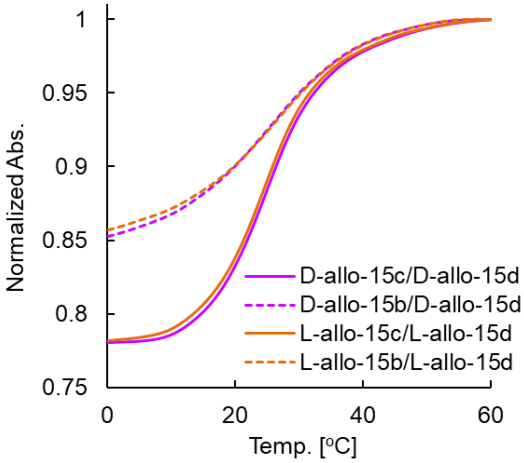


Figure S3. Melting profiles of *allo-a*TNA homo-duplexes with 15b/15d and 15c/15d.

Table S1. Melting temperatures of 15-mer homo-duplexes.

Sequences	Direction of strands	T_m (°C)[a]
D-allo-15c / D-allo-15d	Antiparallel	24.1
D-allo-15b / D-allo-15d	Parallel	- [b]
L-allo-15c / L-allo-15d	Antiparallel	23.9
L-allo-15b / L-allo-15d	Parallel	- [b]
DT-15a / DT-15b	Antiparallel	78.1
LT-15a / LT-15b	Antiparallel	81.1
S-15a / S-15b	Antiparallel	68.7

[a] 100 mM NaCl, 10 mM phosphate buffer (pH 7.0), 2.0 μM oligonucleotide. [b] Melting transition was not observed.

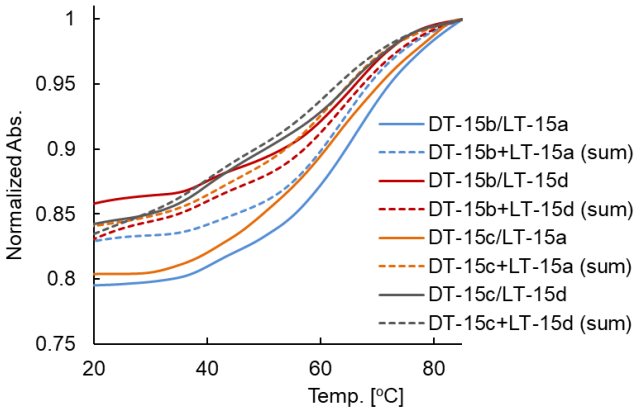


Figure S4. Melting profiles of hetero-duplexes between D-aTNA and L-aTNA and the sums of spectra of each single strand. Solid lines indicate two strands are mixed in solution; broken line indicate summed spectra.

Table S2. Melting temperatures of hetero-duplexes.

Sequences	Direction of strands	T_m (°C)[a]	Figure number
DT-15b / LT-15a	Parallel	- [b]	Figure S4
DT-15b / LT-15d	Antiparallel	- [b]	Figure S4
DT-15c / LT-15a	Parallel	- [b]	Figure S4
DT-15c / LT-15d	Antiparallel	- [b]	Figure S4
S-15a / DT-15b	Antiparallel	74.9	Figure S5
S-15a / DT-15c	Parallel	- [b]	Figure S5
S-15a / LT-15b	Antiparallel	73.6	Figure S5
S-15a / LT-15c	Parallel	- [b]	Figure S5
DT-15c / D-allo-15a	Parallel	- [b]	Figure S6a
DT-15c / D-allo-15d	Antiparallel	51.7	Figure S6a
LT-15c / D-allo-15a	Parallel	- [b]	Figure S6a
LT-15c / D-allo-15d	Antiparallel	42.5	Figure S6a
S-15a / D-allo-15b	Antiparallel	44.8	Figure S6a
S-15a / D-allo-15d	Parallel	- [b]	Figure S6a
DT-15c / L-allo-15a	Parallel	- [b]	Figure S6b
DT-15c / L-allo-15d	Antiparallel	45.6	Figure S6b
LT-15c / L-allo-15a	Parallel	- [b]	Figure S6b
LT-15c / L-allo-15d	Antiparallel	50.7	Figure S6b
S-15a / L-allo-15b	Antiparallel	46.2	Figure S6b
S-15a / L-allo-15d	Parallel	- [b]	Figure S6b
D-allo-15a / L-allo-15b	Antiparallel	18.2	Figure S7
D-allo-15a / L-allo-15c	Parallel	- [b]	Figure S7
D-allo-15c/ L-allo-15d	Antiparallel	18.2	Figure S7
D-allo-15b / L-allo-15d	Parallel	- [b]	Figure S7
D-15a / L-allo-15b	Antiparallel	20.8	Figure S8
D-15a / L-allo-15c	Parallel	- [b]	Figure S8
D-15a / D-allo-15b	Antiparallel	- [b]	Figure S8
D-15a / D-allo-15c	Parallel	- [b]	Figure S8
R-15a / L-allo-15b	Antiparallel	21.5	Figure S8
R-15a / L-allo-15c	Parallel	- [b]	Figure S8
R-15a / D-allo-15b	Antiparallel	- [b]	Figure S8
R-15a / D-allo-15c	Parallel	- [b]	Figure S8

[a] 100 mM NaCl, 10 mM phosphate buffer (pH 7.0), 2.0 μM oligonucleotide. [b] Melting transition was not observed. [c] Melting curves were similar to single-stranded oligomers, indicating no duplex formation.

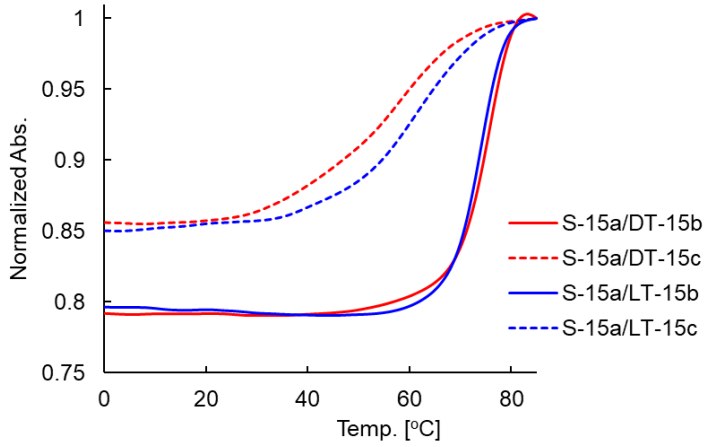


Figure S5. Melting profiles of hetero-duplexes between SNA and L-aTNA (blue lines) and D-aTNA (red lines). Solid lines and broken lines indicate antiparallel and parallel pairings, respectively.

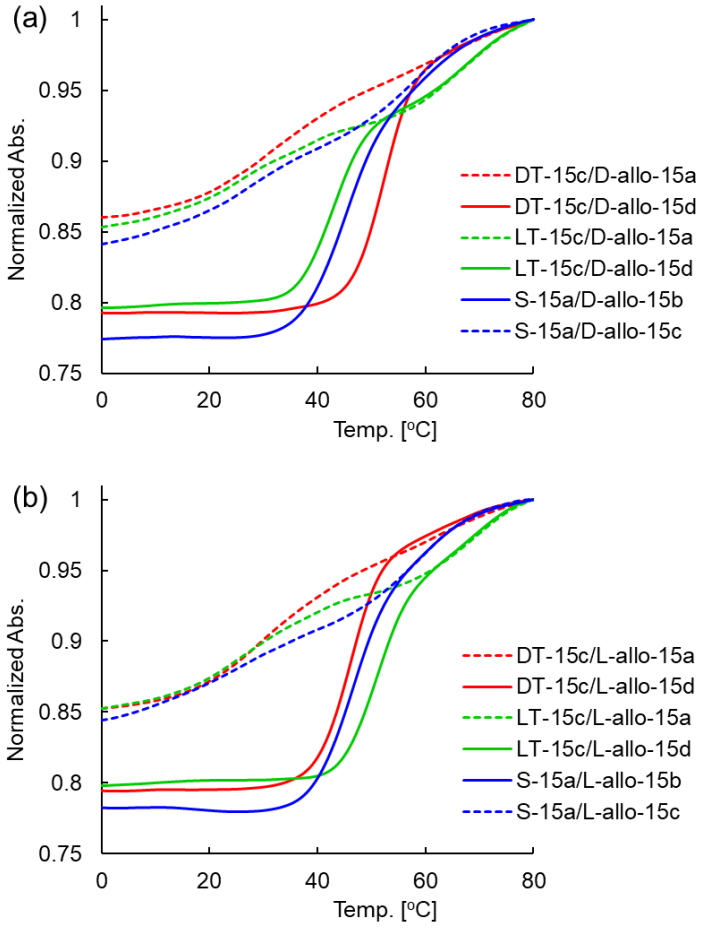


Figure S6. Melting profiles of hetero-duplexes between allo-aTNAs and aTNA/SNA. (a) D-allo-aTNA hetero duplexes; (b) L-allo-aTNA hetero-duplexes with D-aTNA (red lines), L-aTNA (green lines), and SNA (blue lines). Solid lines and broken lines indicate antiparallel and parallel pairings, respectively.

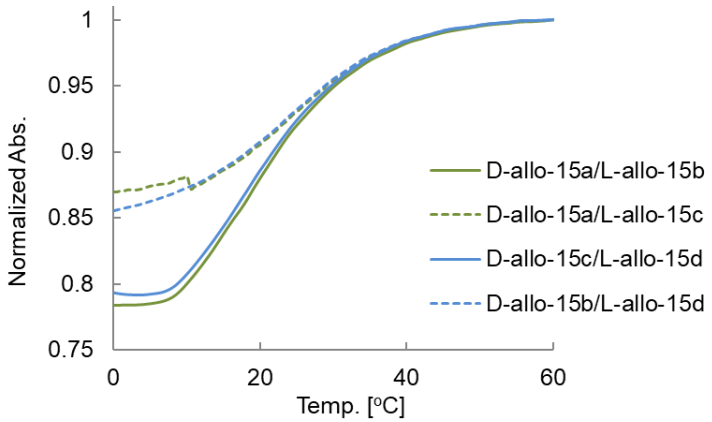


Figure S7. Melting profiles of hetero-duplexes between D-allo-aTNA and L-allo-aTNA. Solid lines and broken lines indicate antiparallel and parallel pairings, respectively.

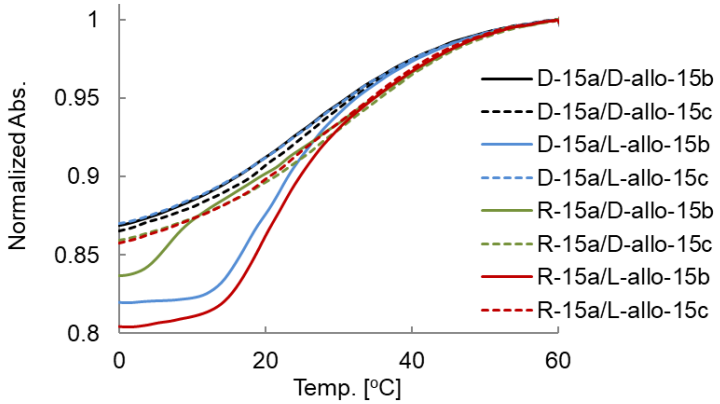


Figure S8. Melting profiles of hetero-duplexes between allo-aTNAs and DNA/RNA. Solid lines and broken lines indicate antiparallel and parallel pairings, respectively.

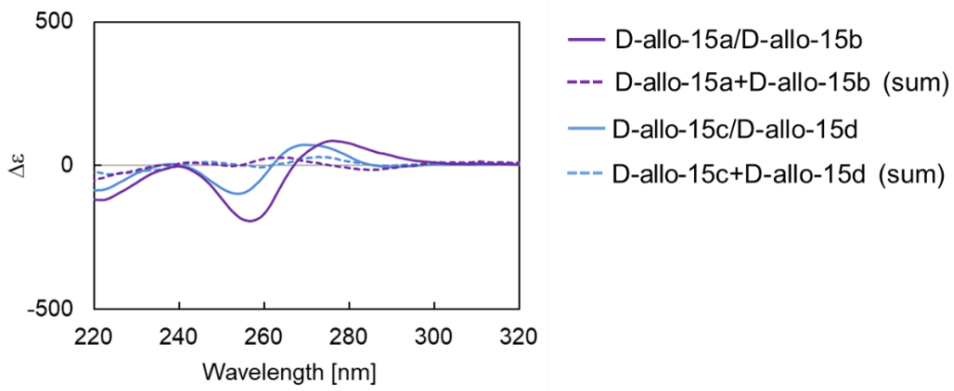
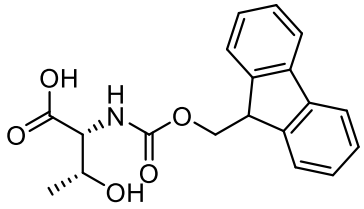


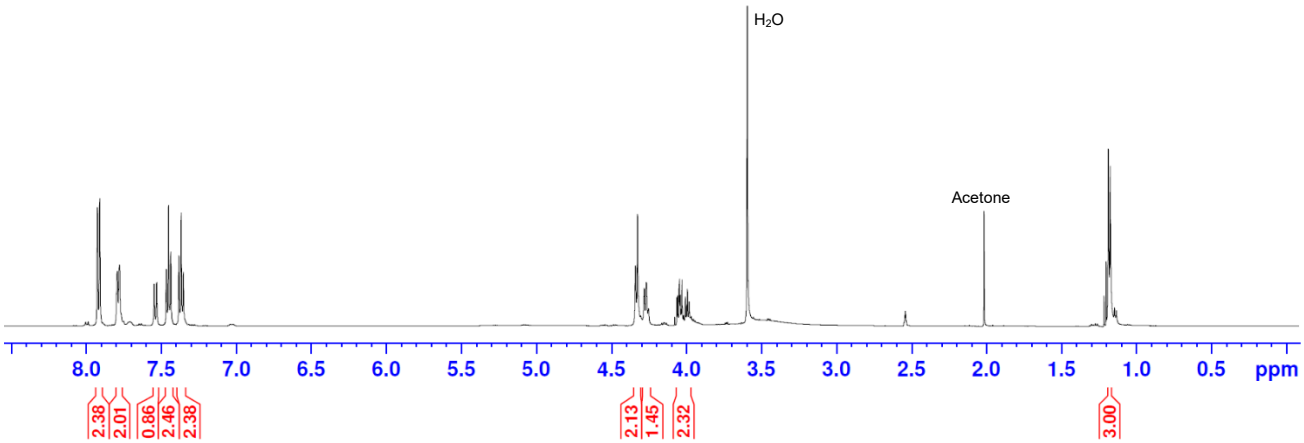
Figure S9. CD spectra of D-*allo*-*a*TNA homo-duplexes (solid lines) and sum of spectra of single strands (broken lines).

3. Appendix

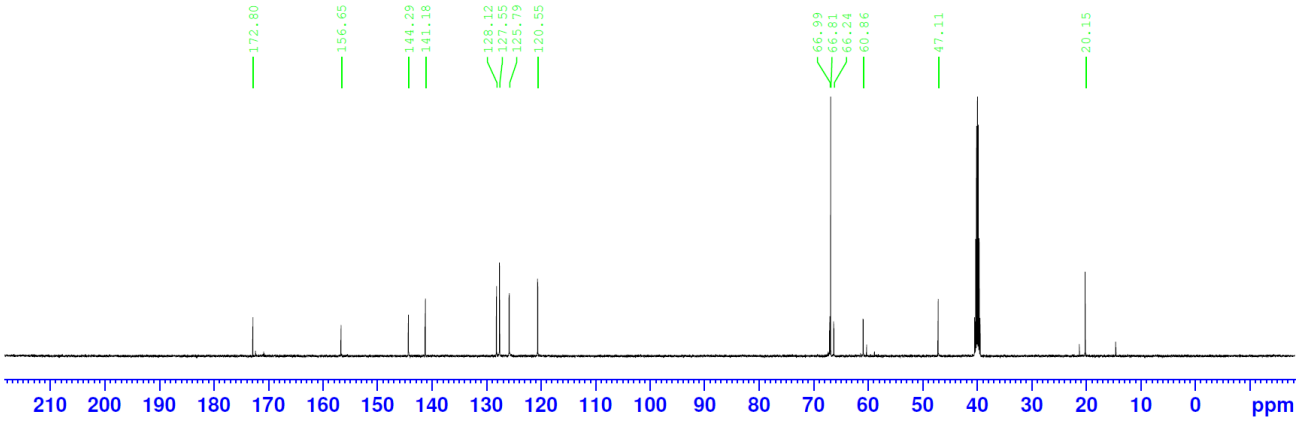
3-1. NMR spectra



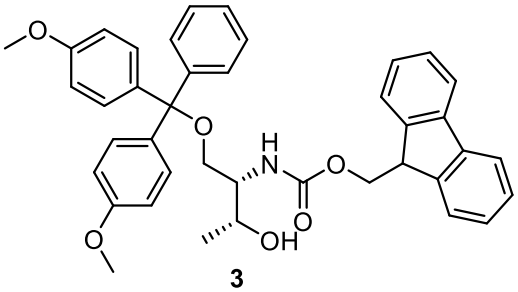
1



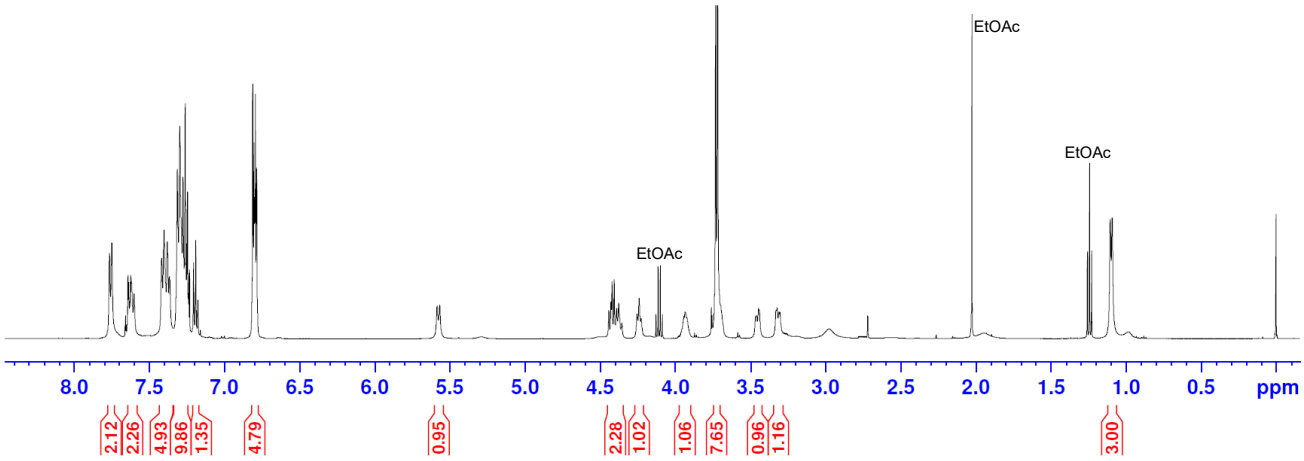
^{1}H-NMR spectrum of **1**



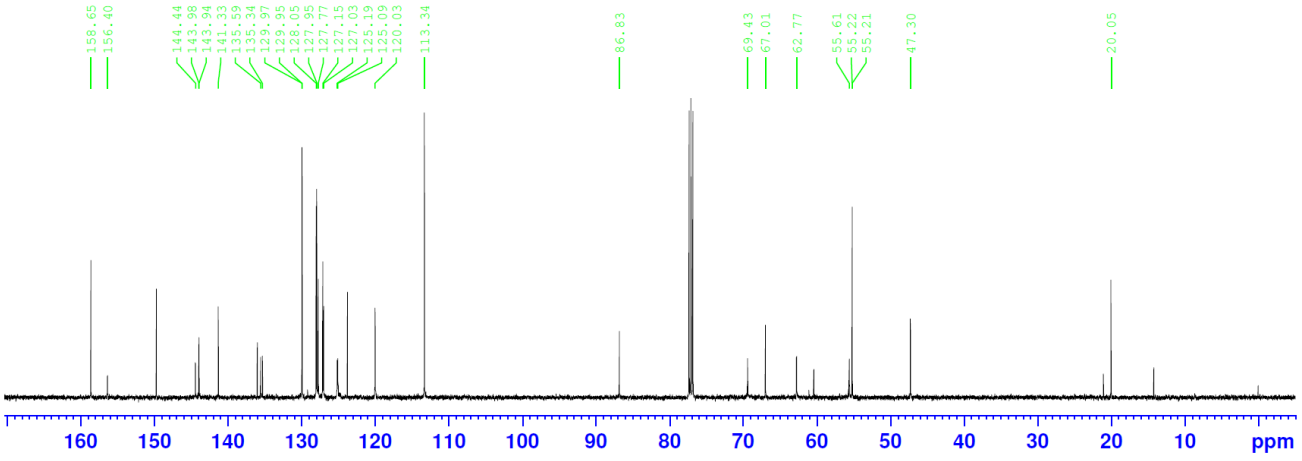
^{13}C-NMR spectrum of **1**



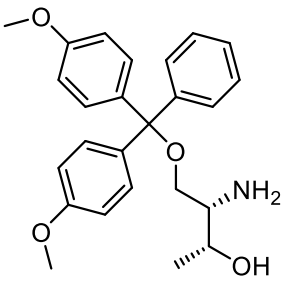
3



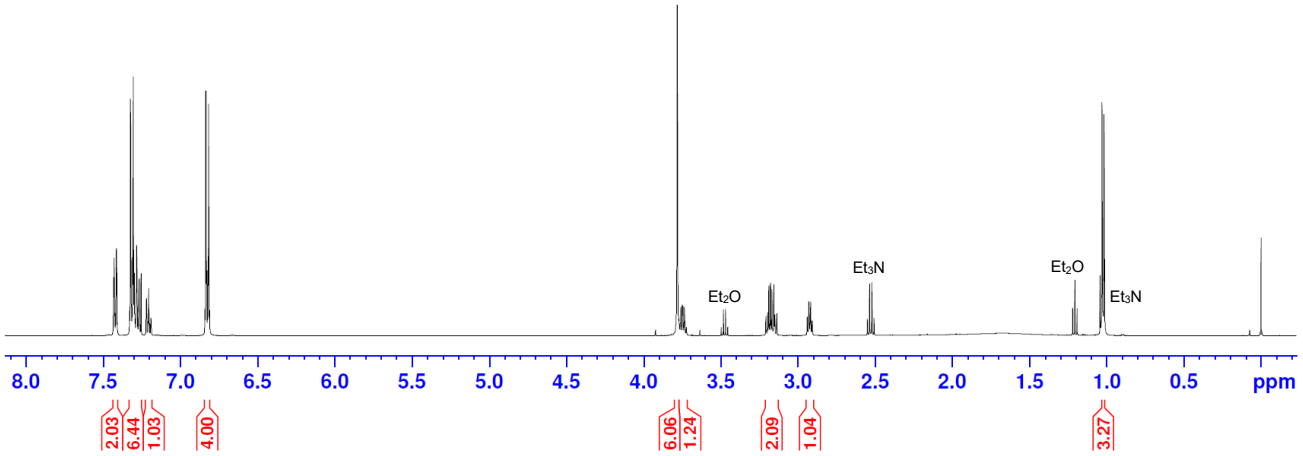
^{1}H-NMR spectrum of **3**



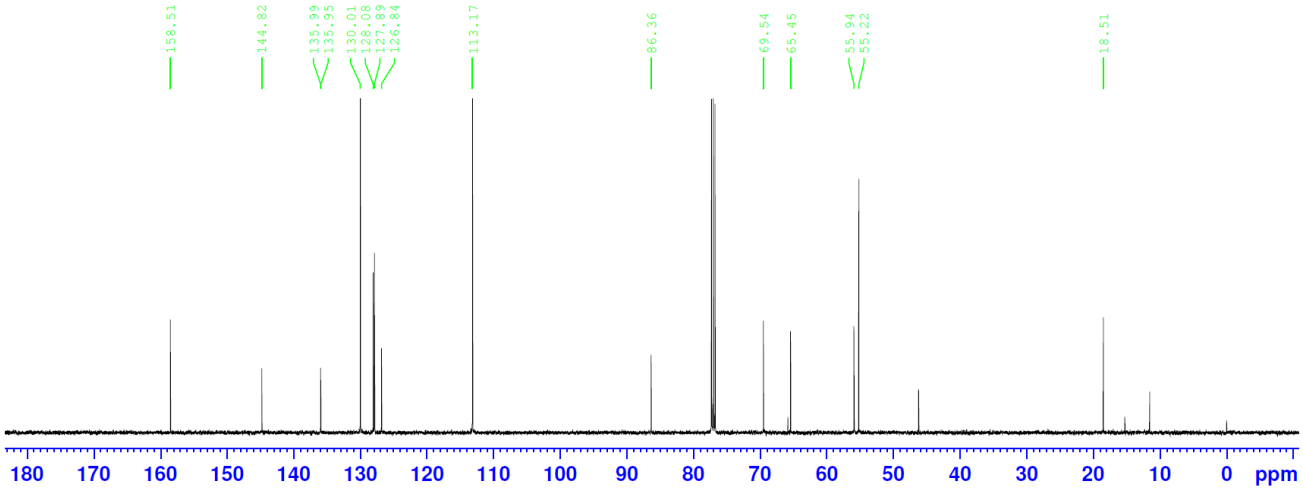
^{13}C-NMR spectrum of **3**



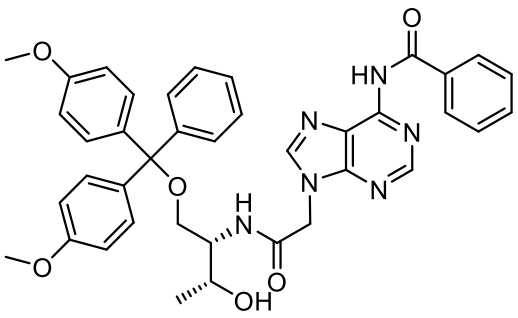
4



^{1}H-NMR spectrum of **4**

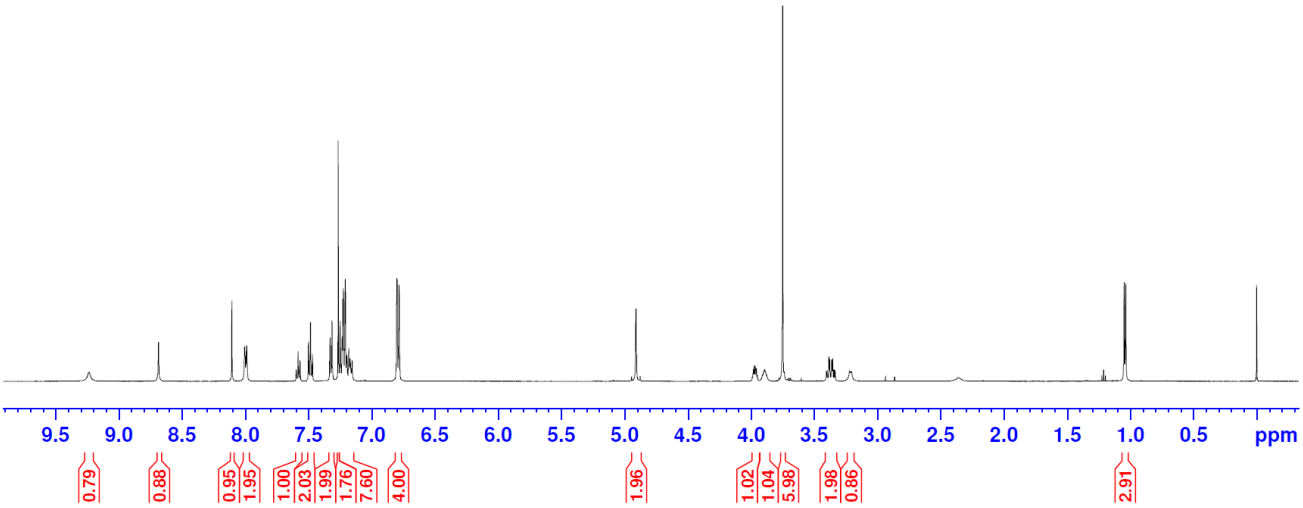


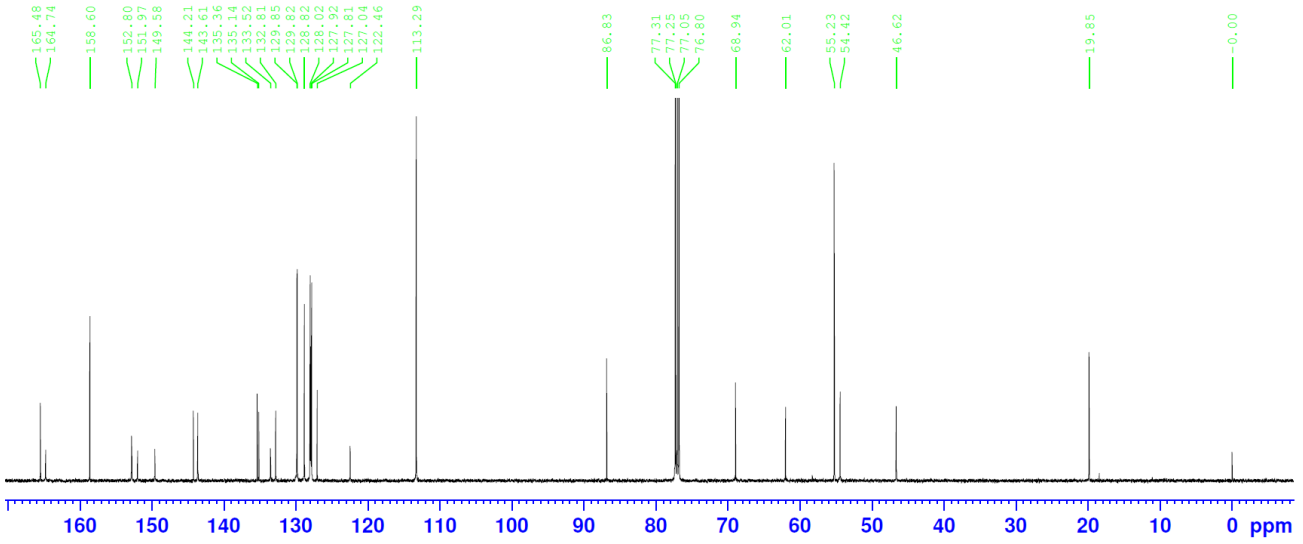
^{13}C-NMR spectrum of **4**



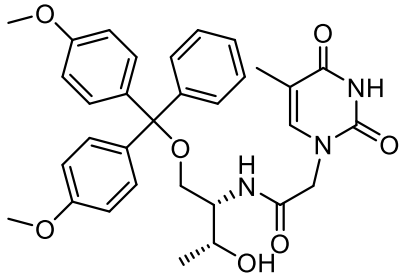
5-A'

^{1}H-NMR spectrum of **5-A'**

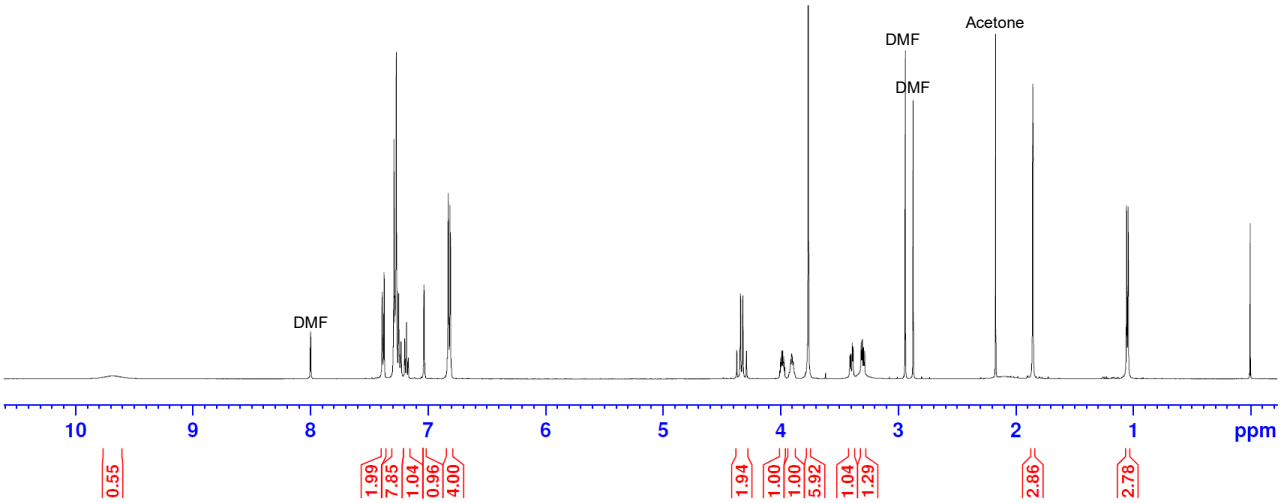




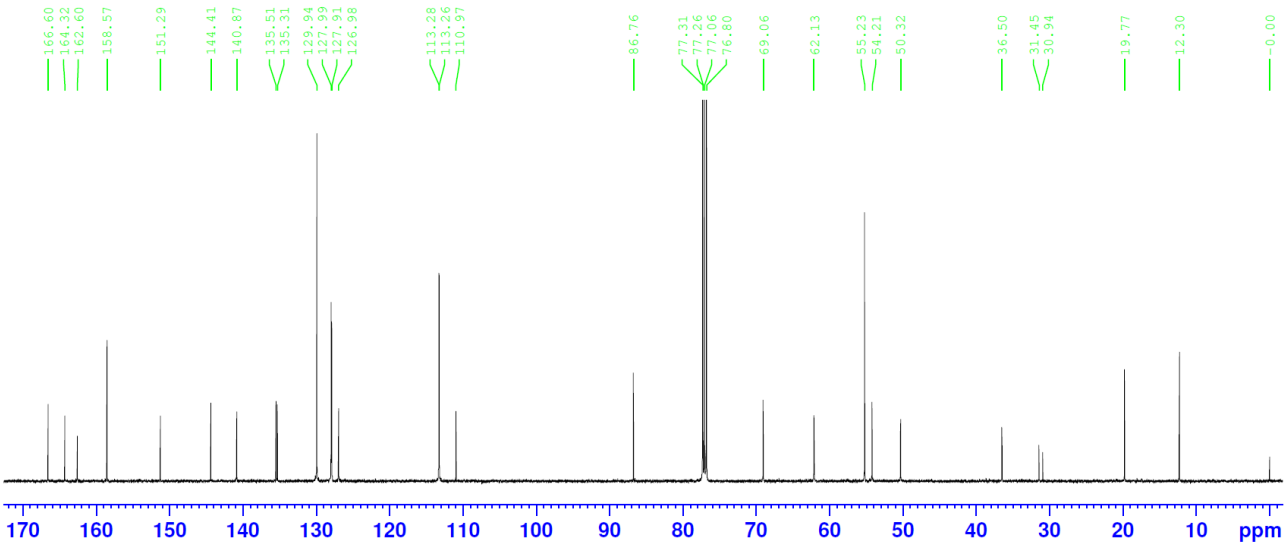
^{13}C-NMR spectrum of **5-A'**



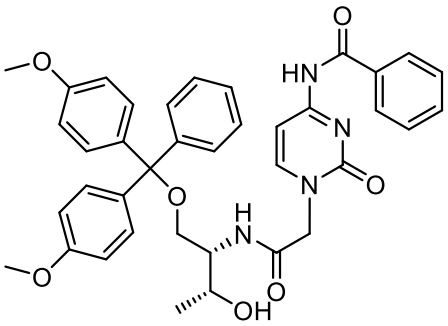
5-T



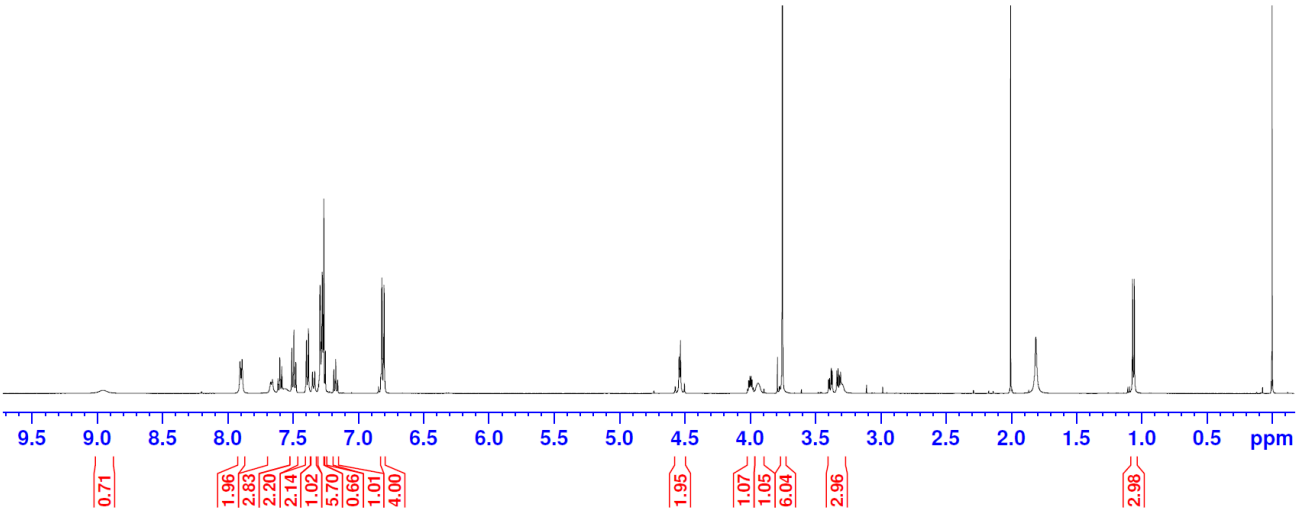
^{1}H-NMR spectrum of **5-T**



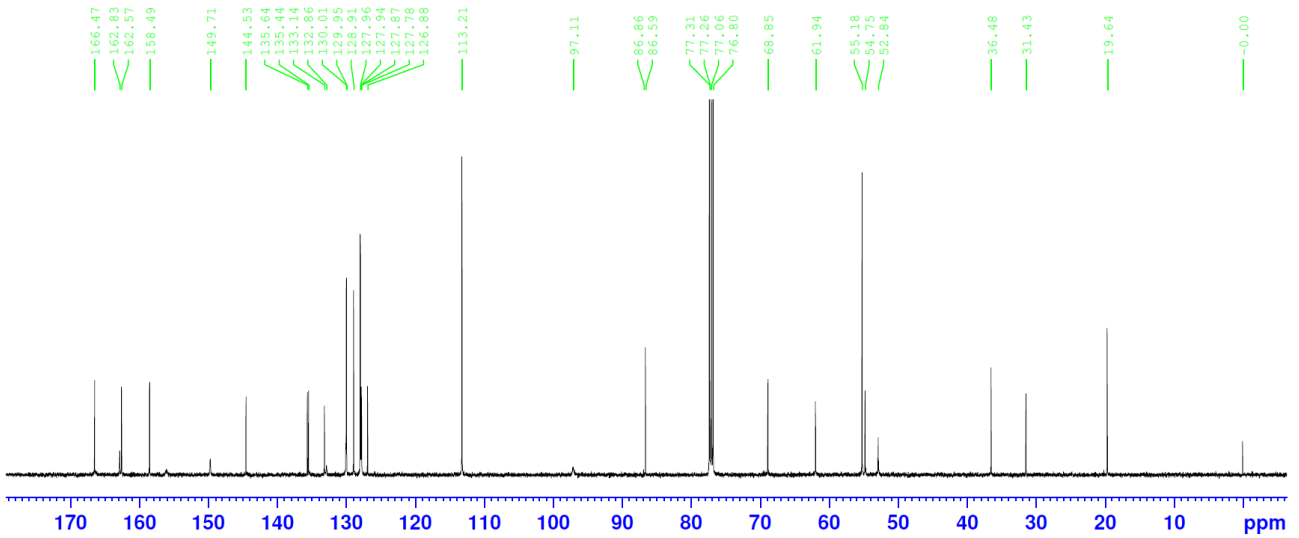
^{13}C-NMR spectrum of **5-T**



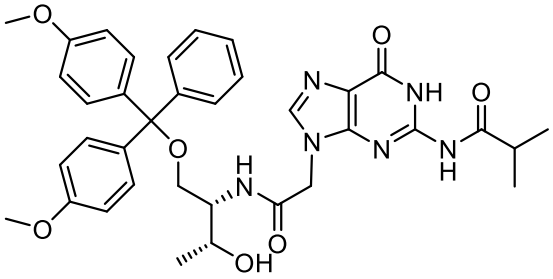
5-C'



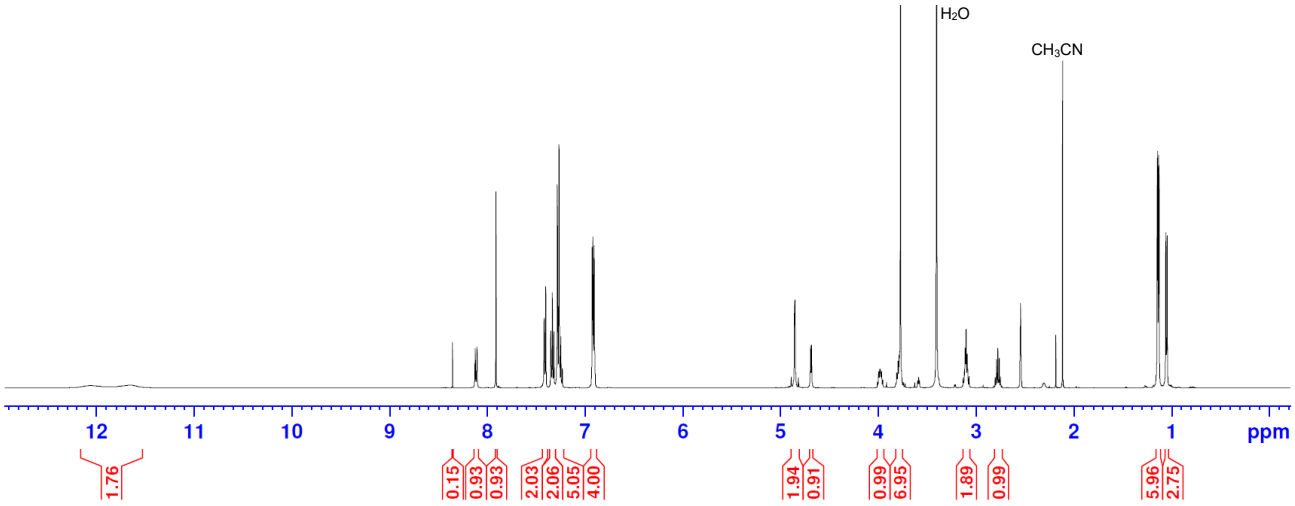
^{1}H-NMR spectrum of **5-C'**



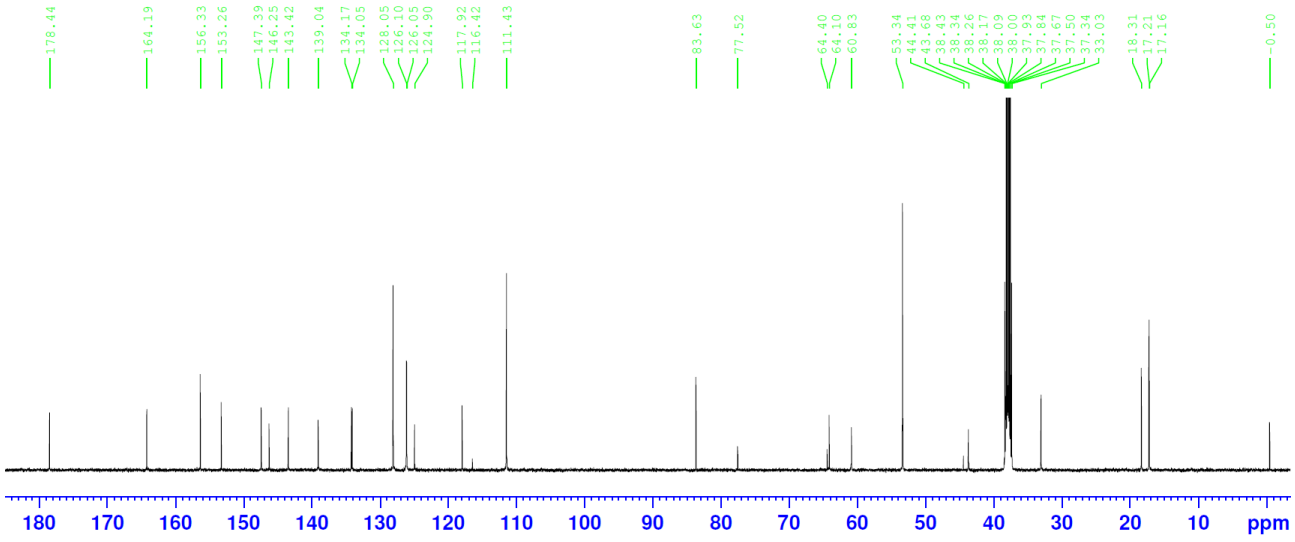
^{13}C-NMR spectrum of **5-C'**



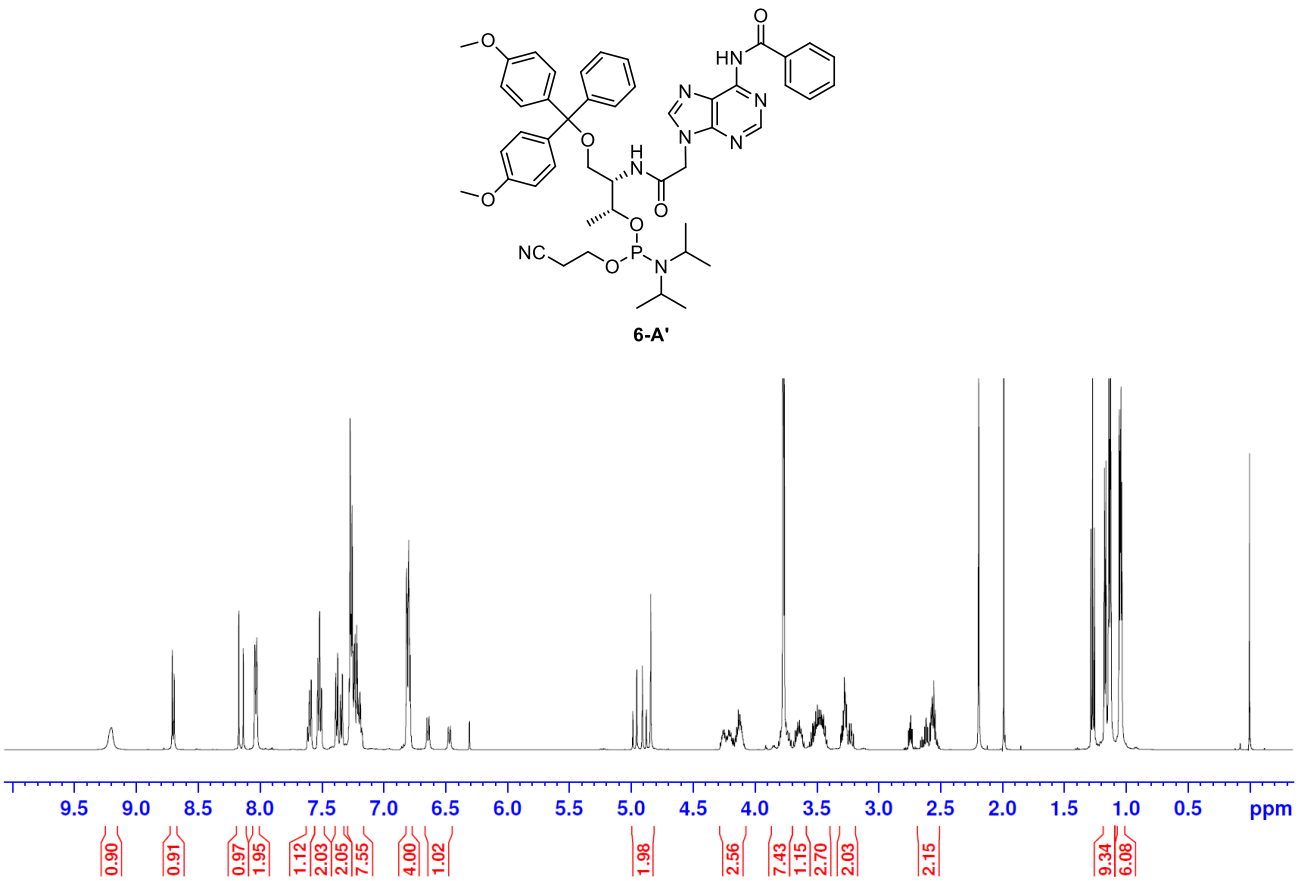
5-G'



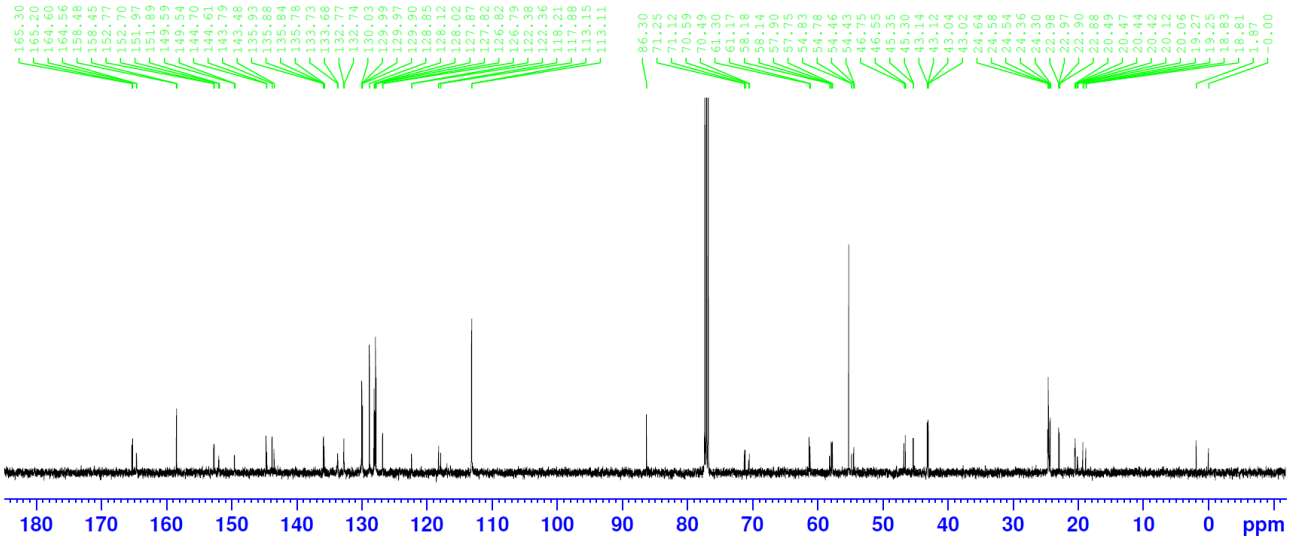
^{1}H-NMR spectrum of **5-G'**



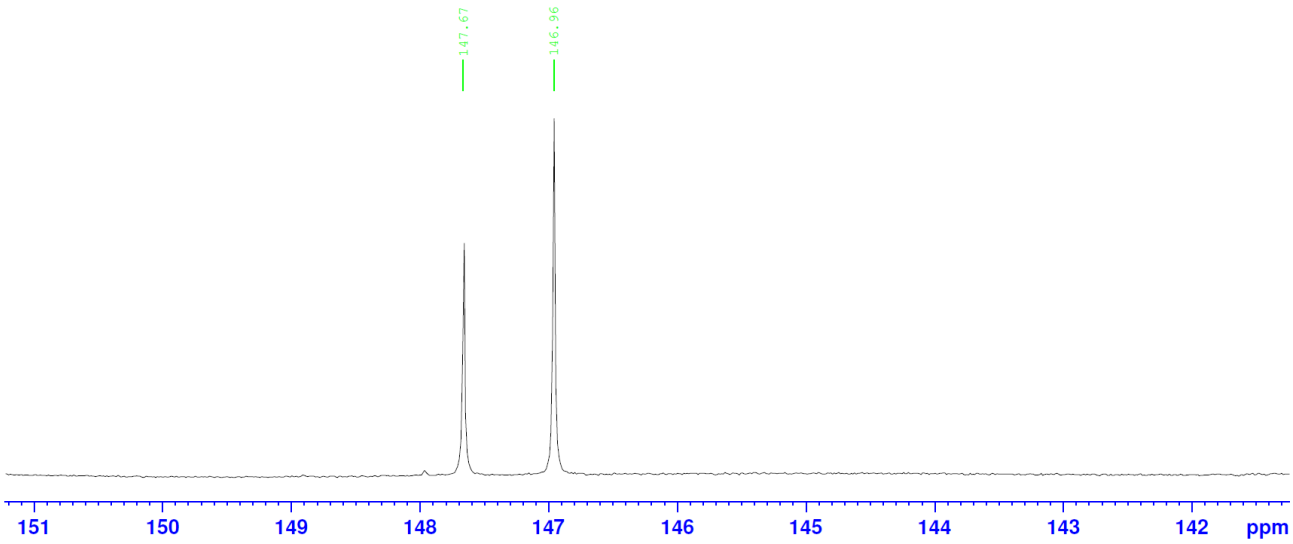
^{13}C-NMR spectrum of **5-G'**



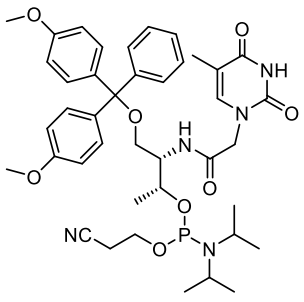
^{1}H-NMR spectrum of **6-A'**



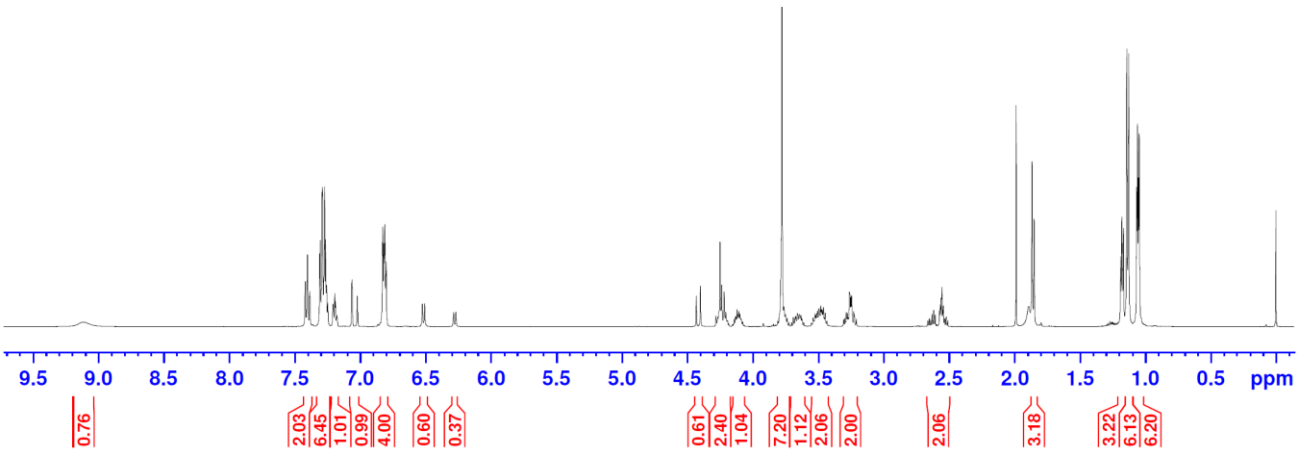
^{13}C-NMR spectrum of **6-A'**



^{31}P-NMR spectrum of **6-A'**



6-T



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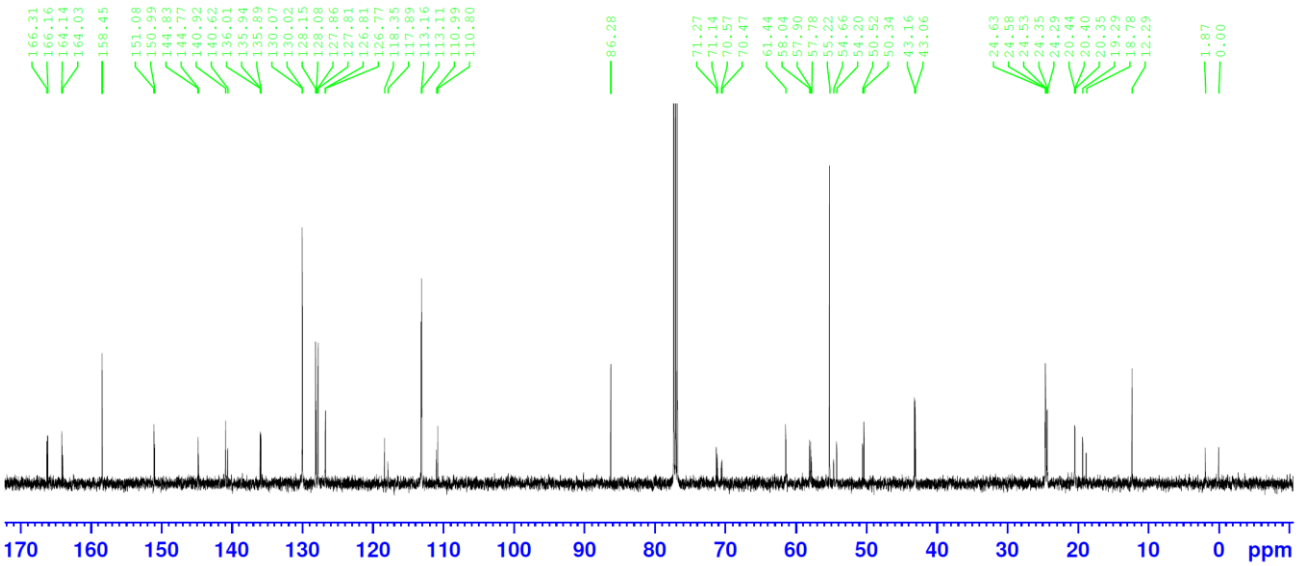
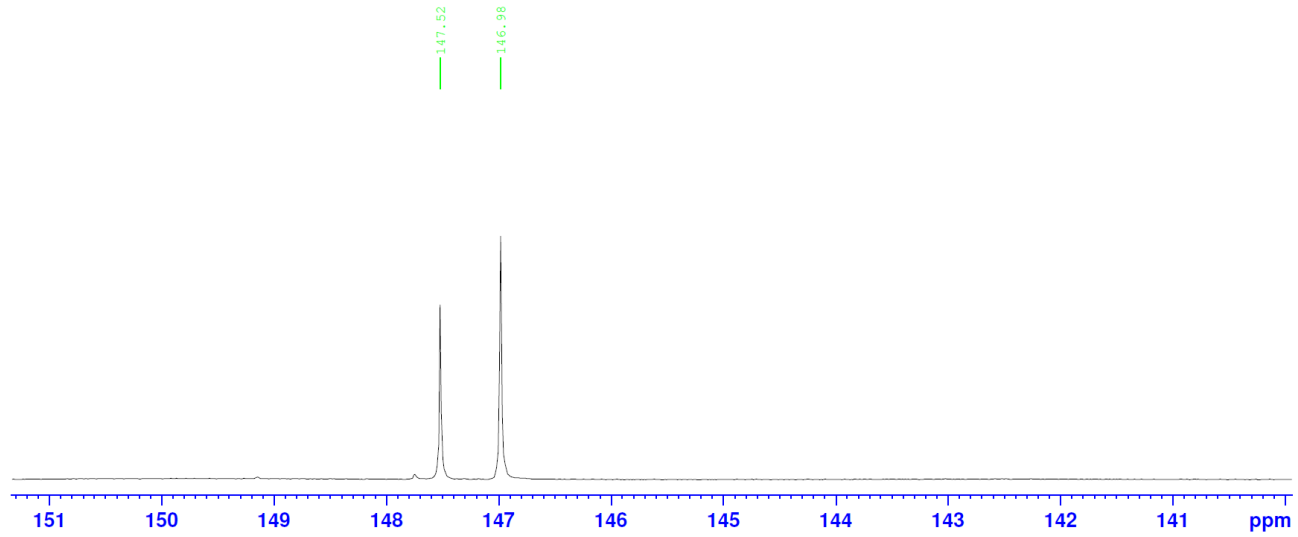
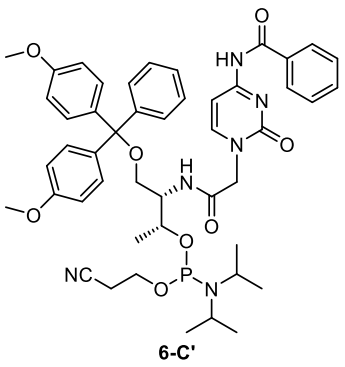


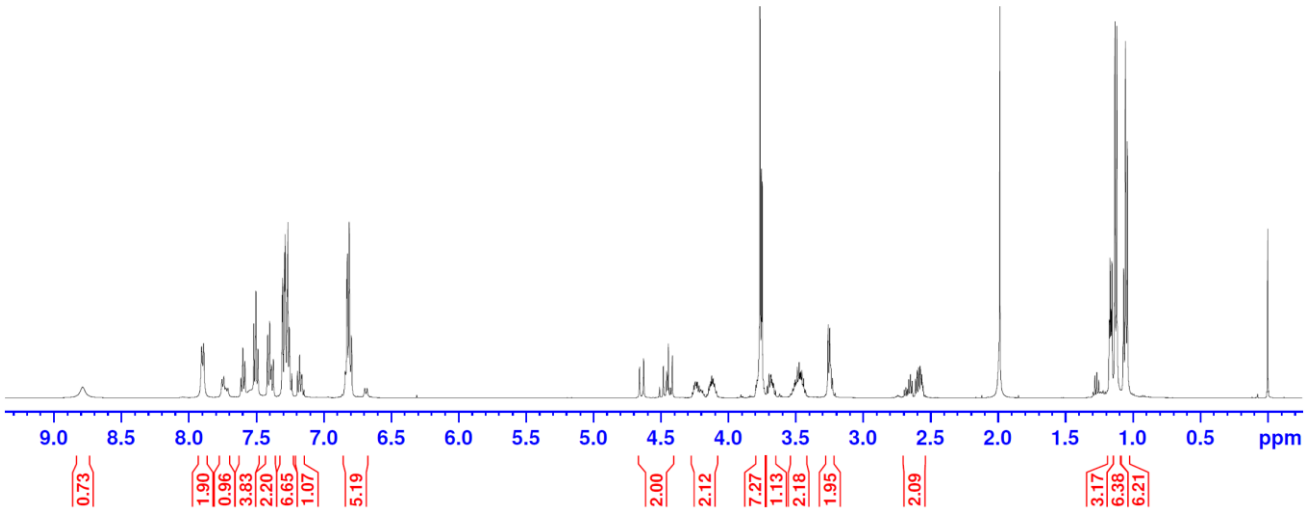
^{13}C-NMR spectrum of **6-T**



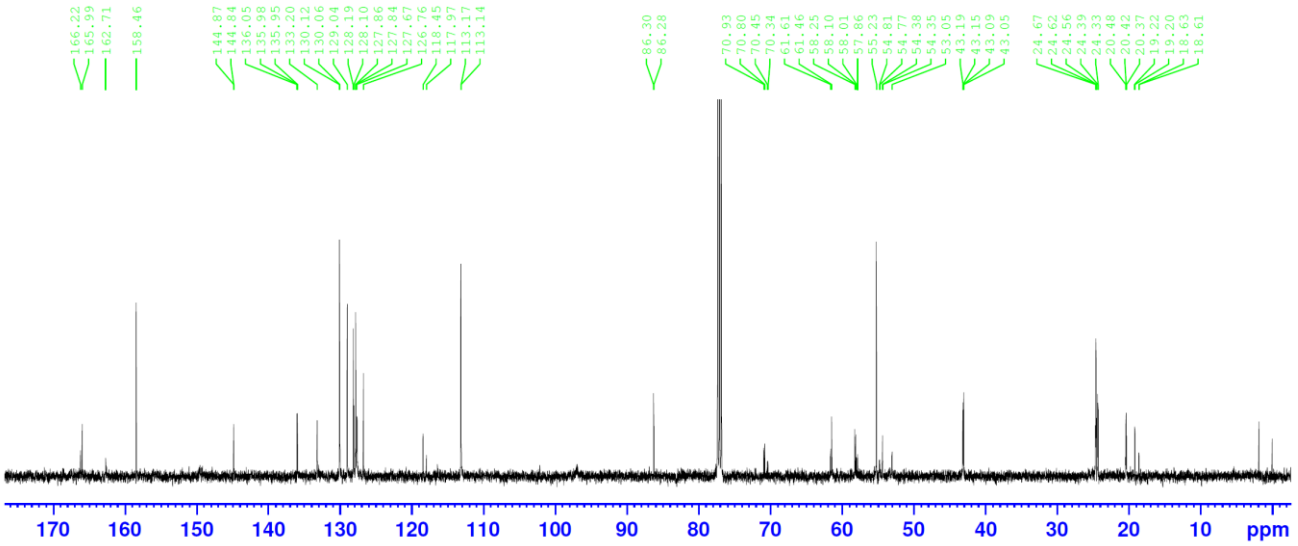
^{31}P-NMR spectrum of **6-T**



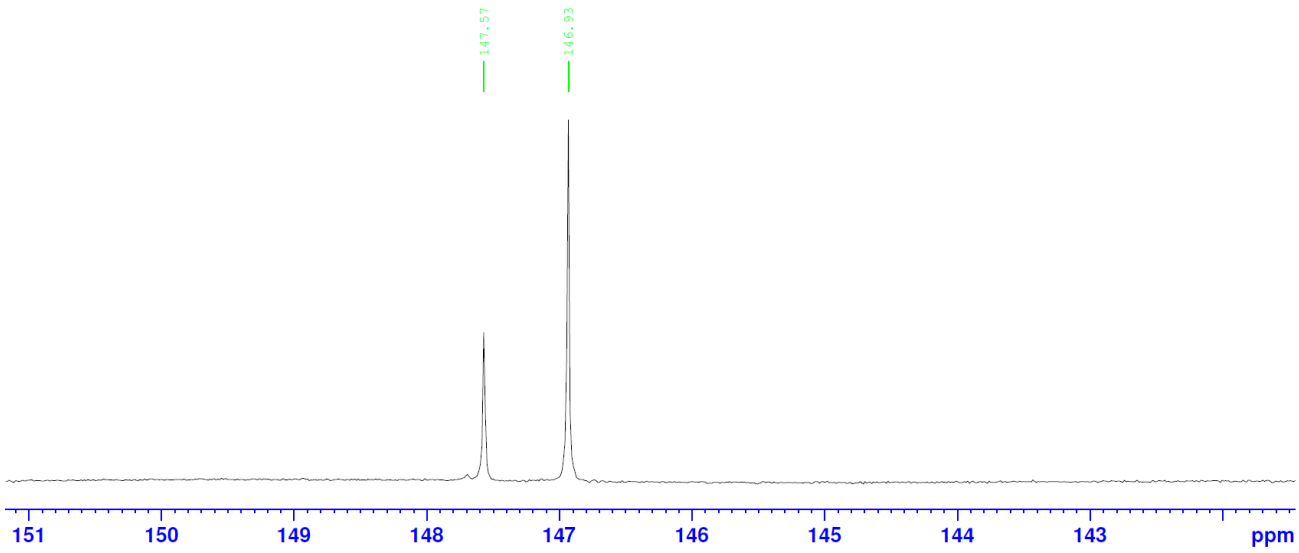
6-C'



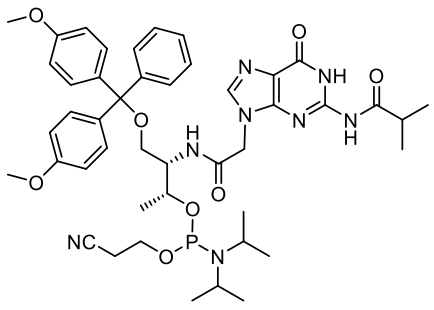
^{1}H-NMR spectrum of **6-C'**



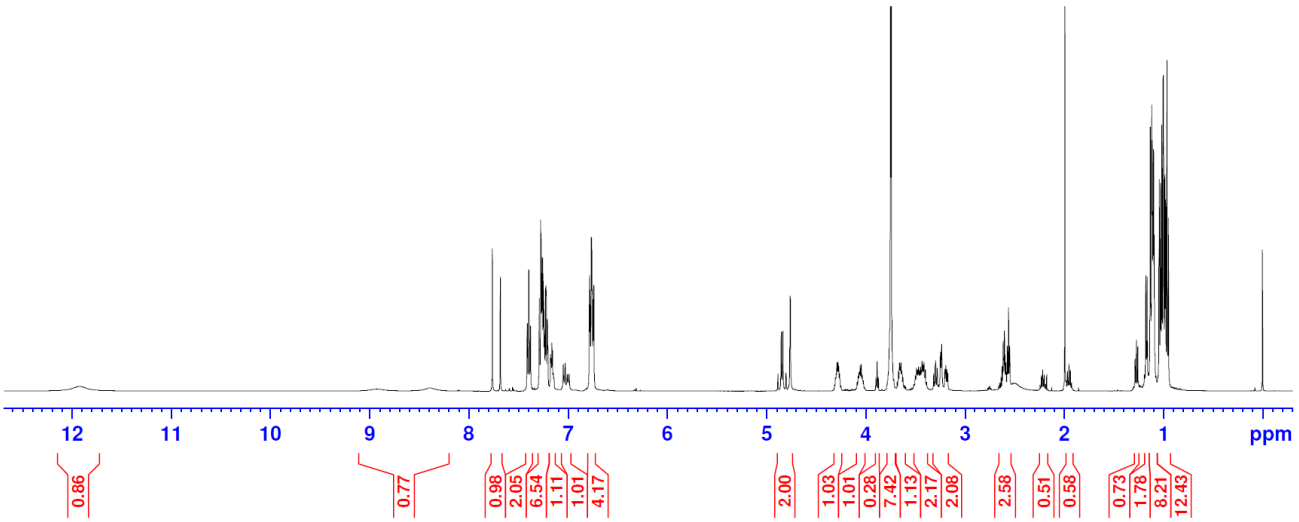
^{13}C-NMR spectrum of **6-C'**



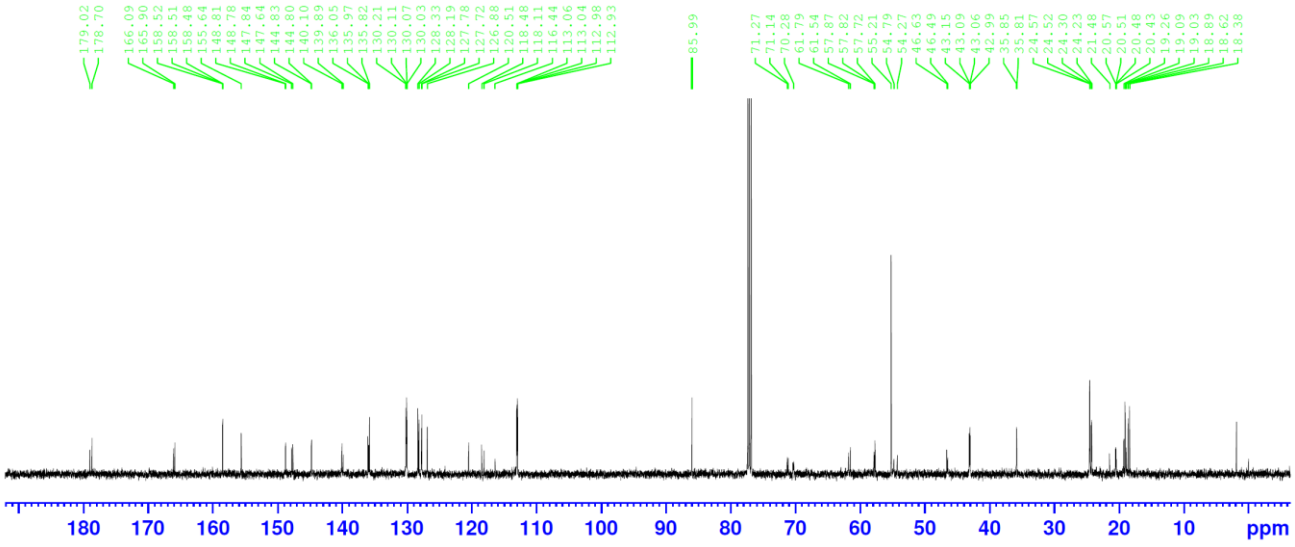
^{31}P-NMR spectrum of **6-C'**



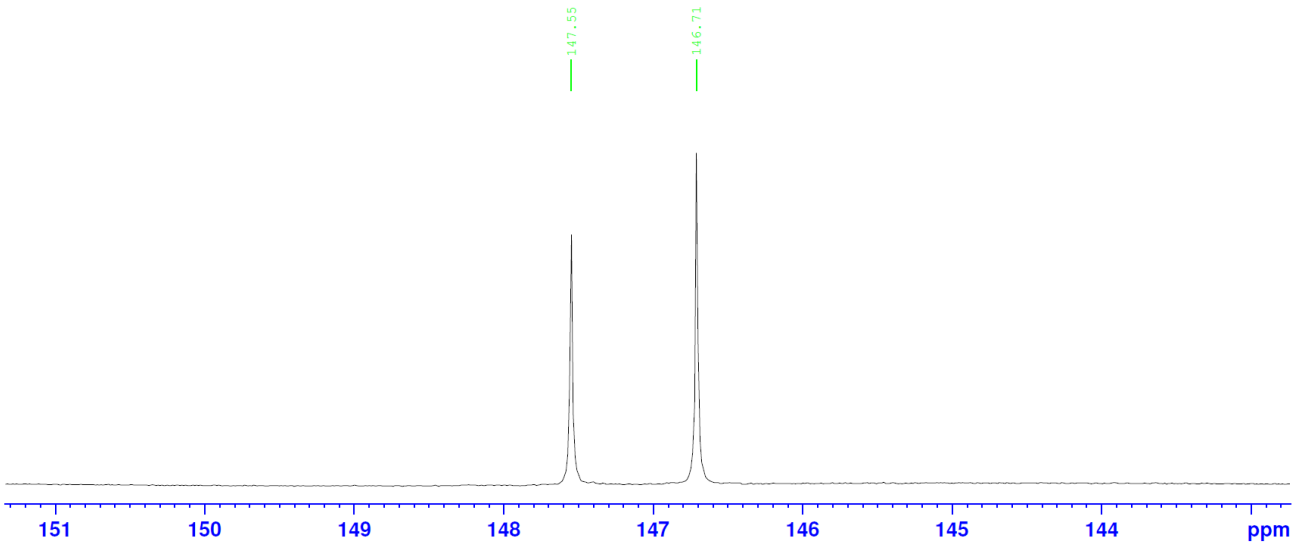
6-G'



^{1}H-NMR spectrum of **6-G'**



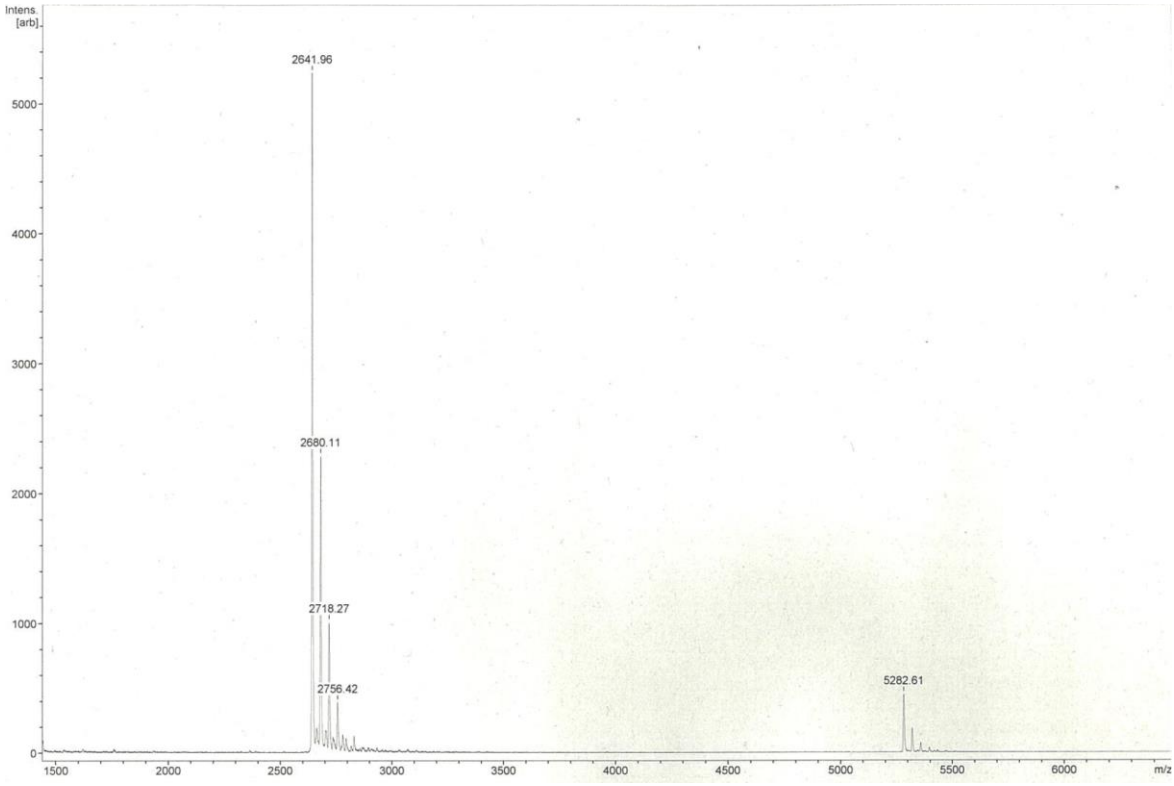
^{13}C-NMR spectrum of **6-G'**



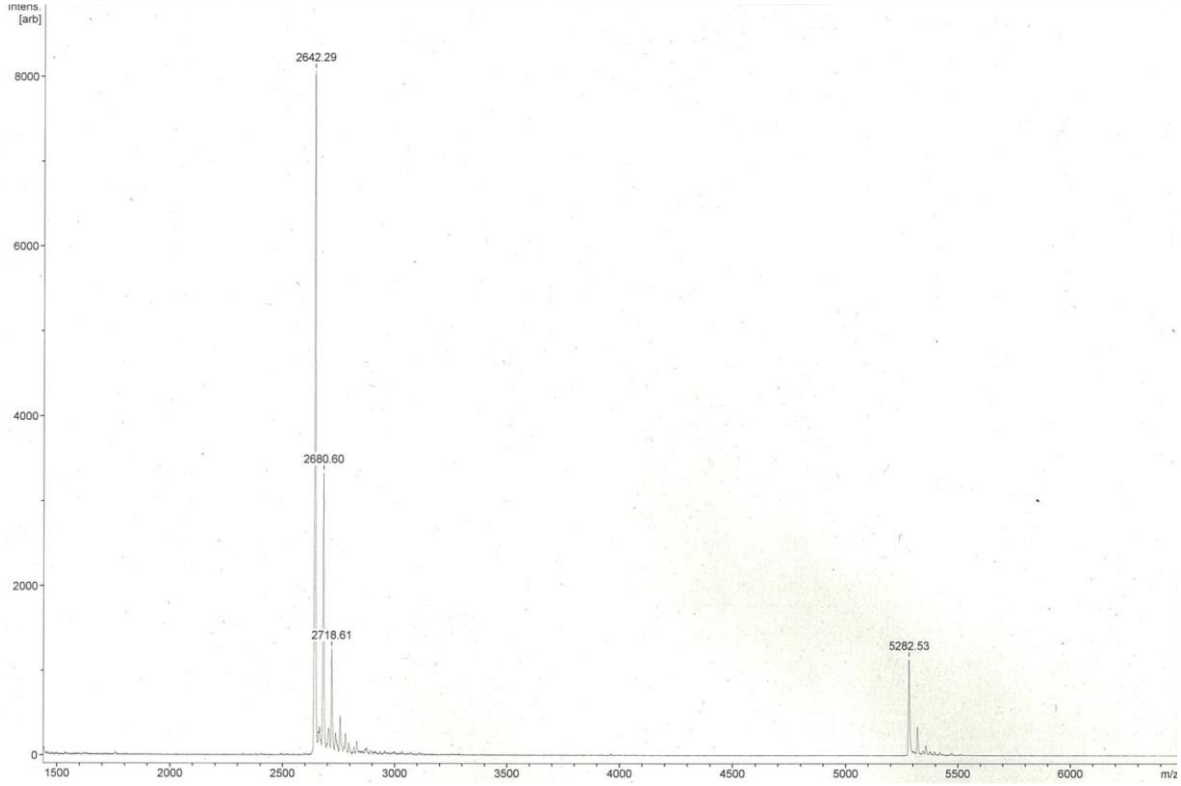
^{31}P-NMR spectrum of **6-G'**

3-2. Results of MALDI-TOF MS:

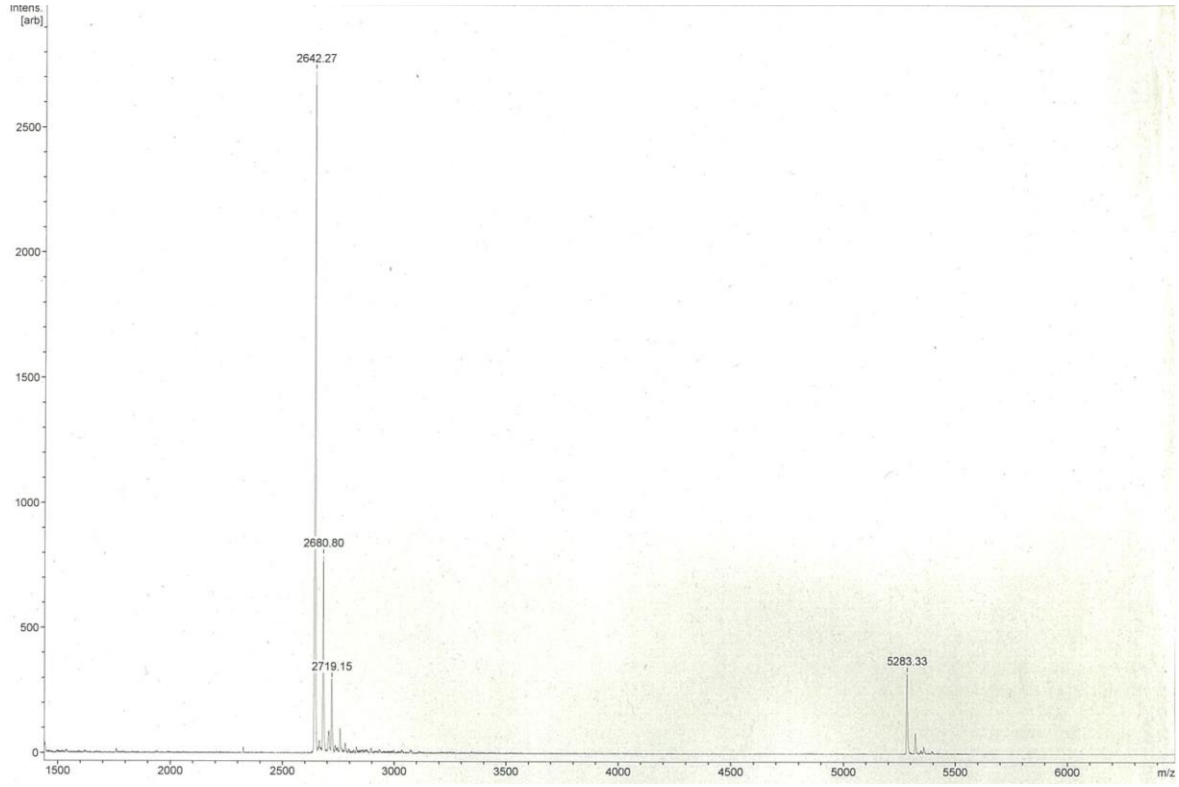
m/z calcd for D-allo-8a [M+H$^+$]: 2641.7, found: 2642.0



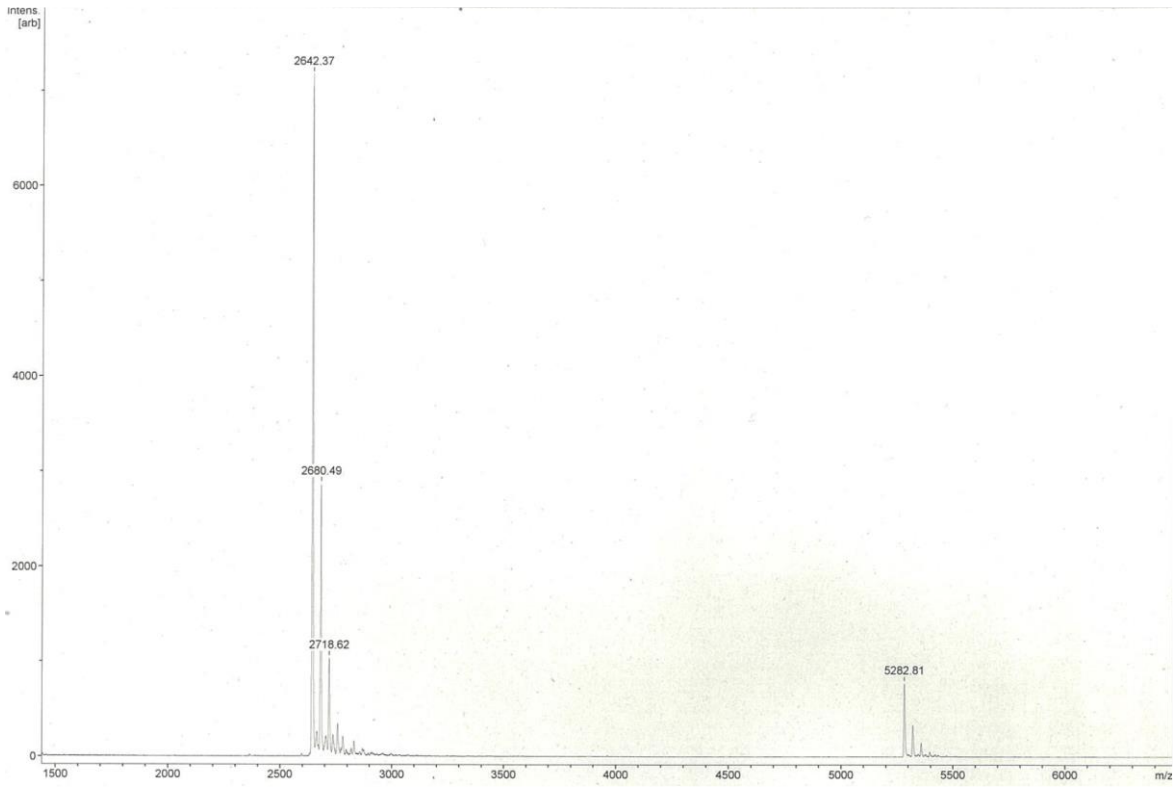
m/z calcd for D-allo-8b [M+H$^+$]: 2641.7, found: 2642.3



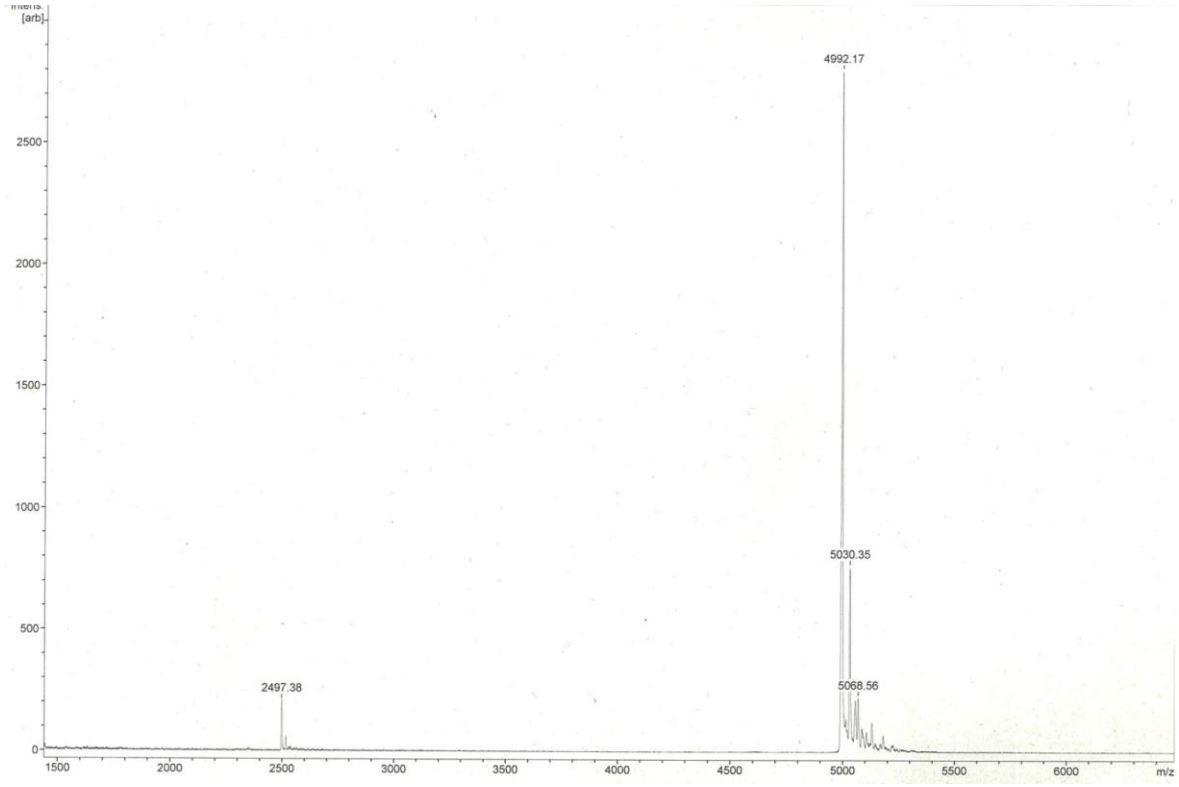
m/z calcd for D-allo-8c [M+H$^+$]: 2641.7, found: 2642.3



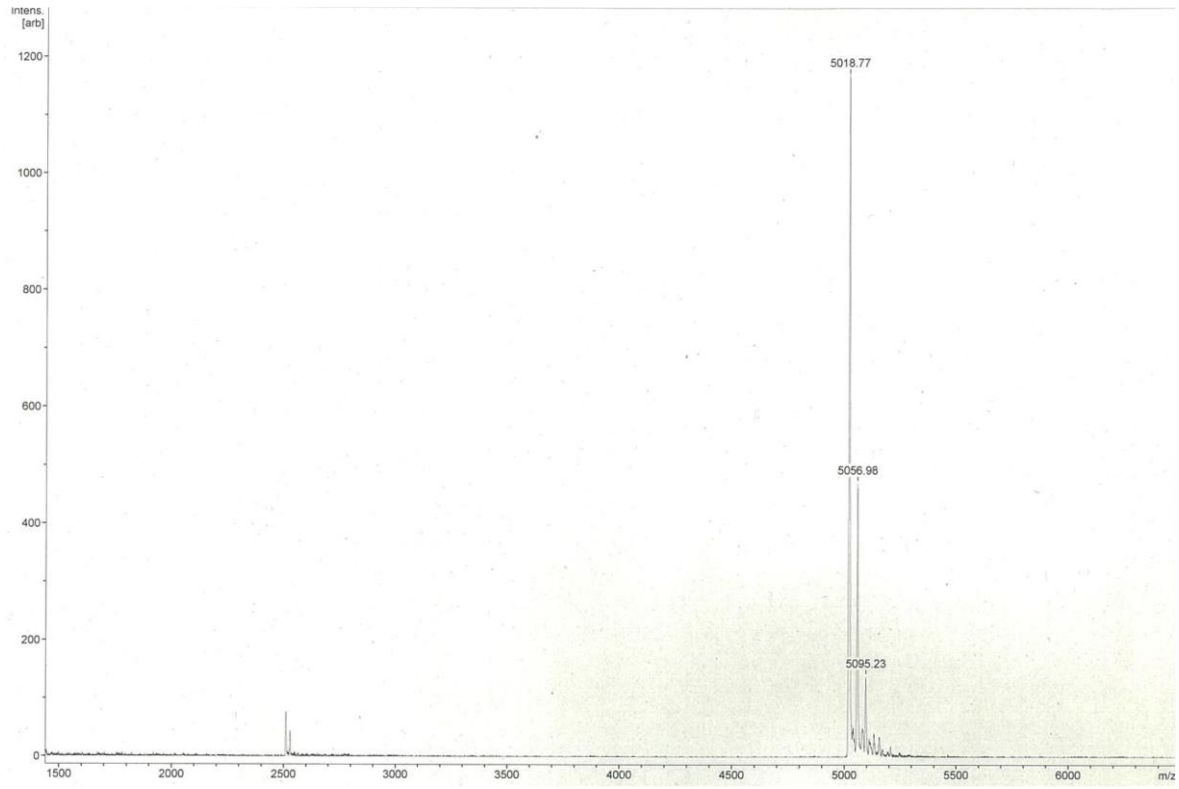
m/z calcd for D-allo-8d [M+H$^+$]: 2641.7, found: 2642.4



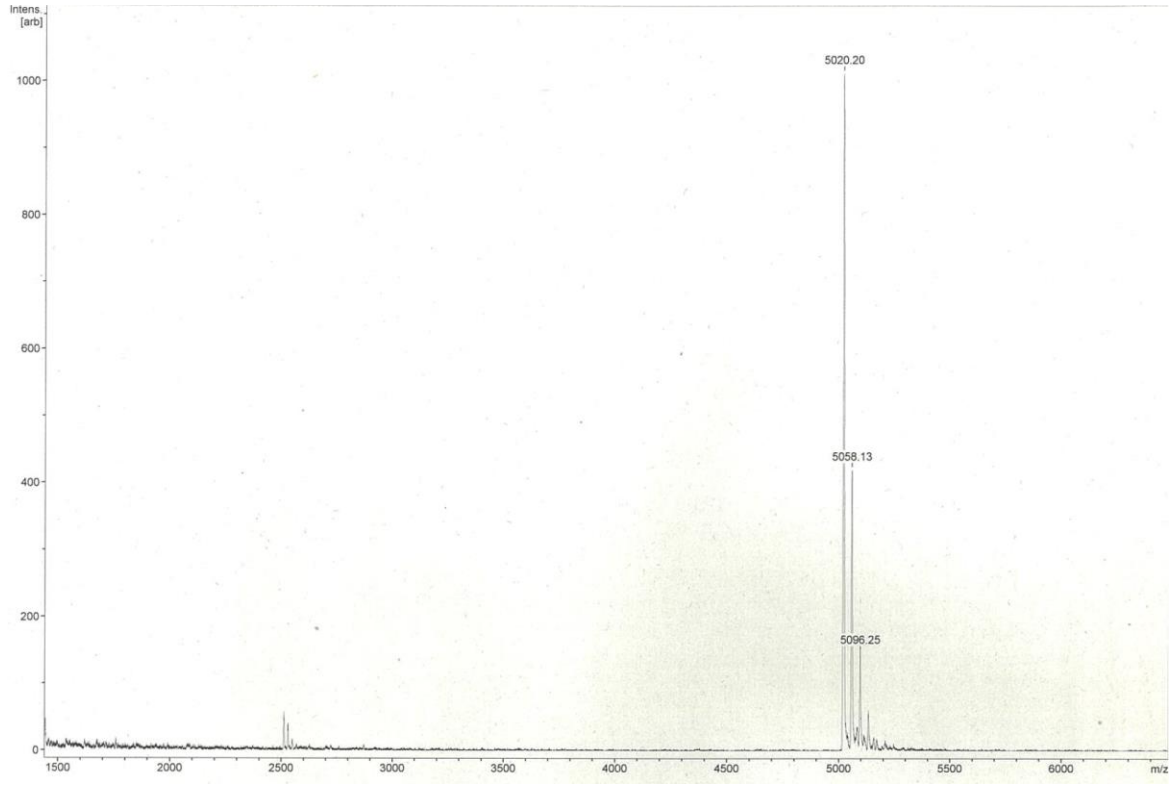
m/z calcd for D-allo-15a [M+H$^+$]: 4991.3, found: 4992.2



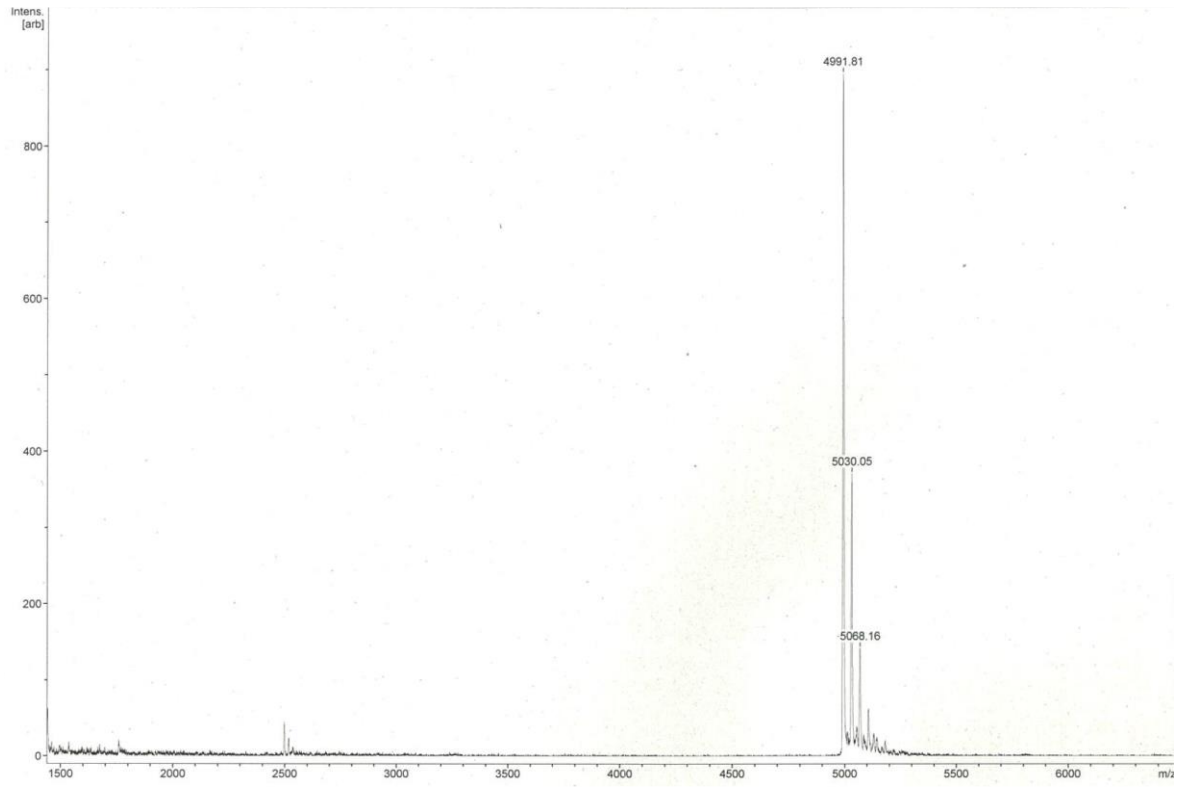
m/z calcd for D-allo-15b [M+H$^+$]: 5018.4, found: 5018.8



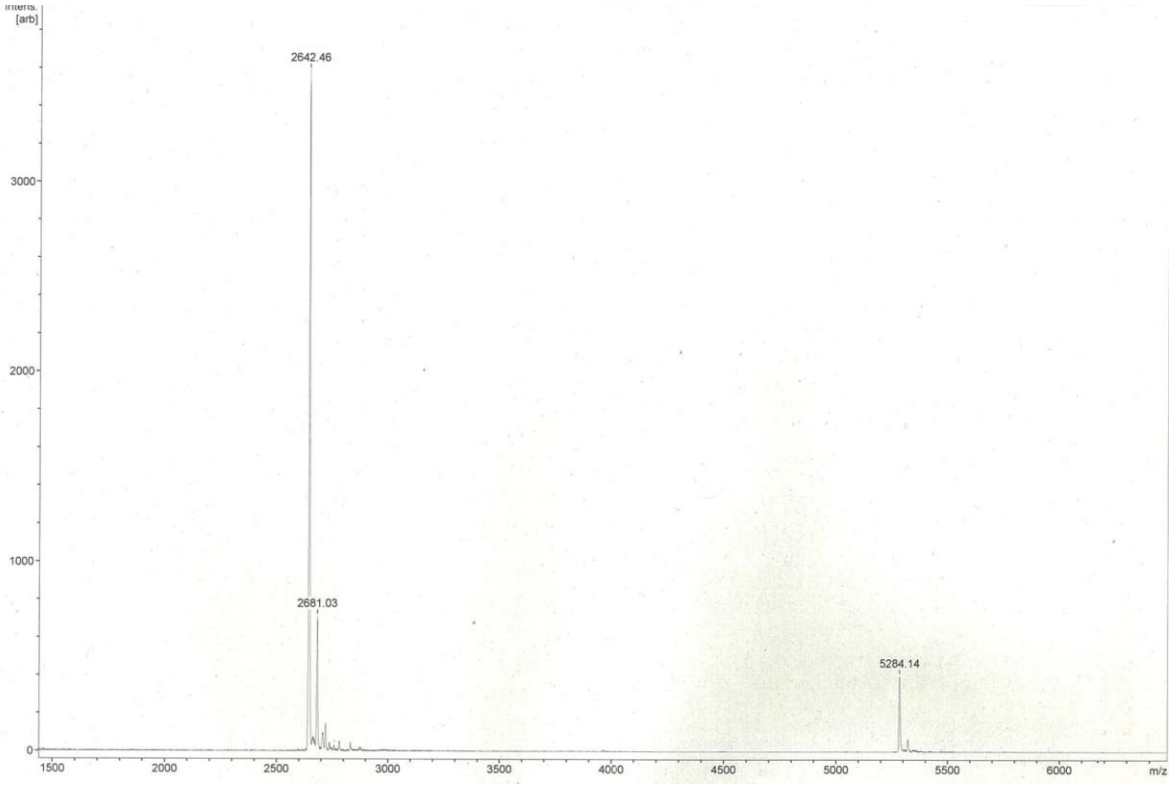
m/z calcd for D-allo-15c [M+H$^+$]: 5018.4, found: 5020.2



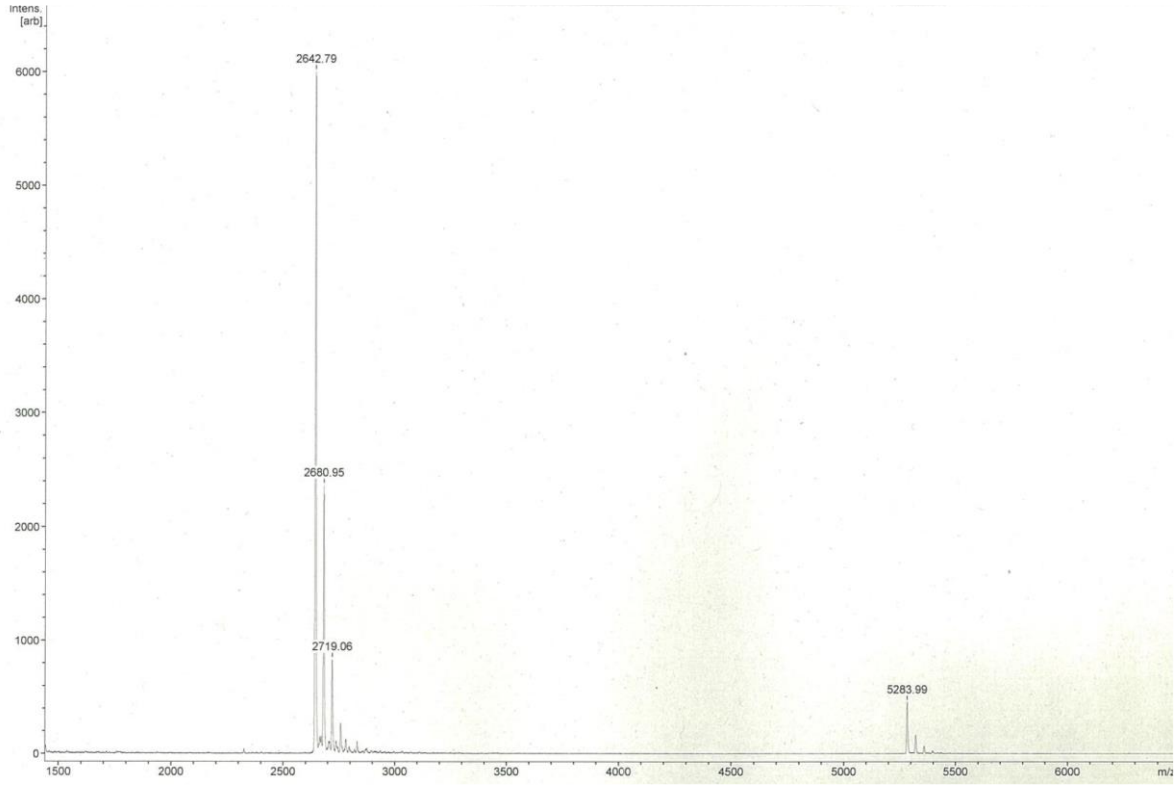
m/z calcd for D-allo-15d [M+H$^+$]: 4991.3, found: 4991.8



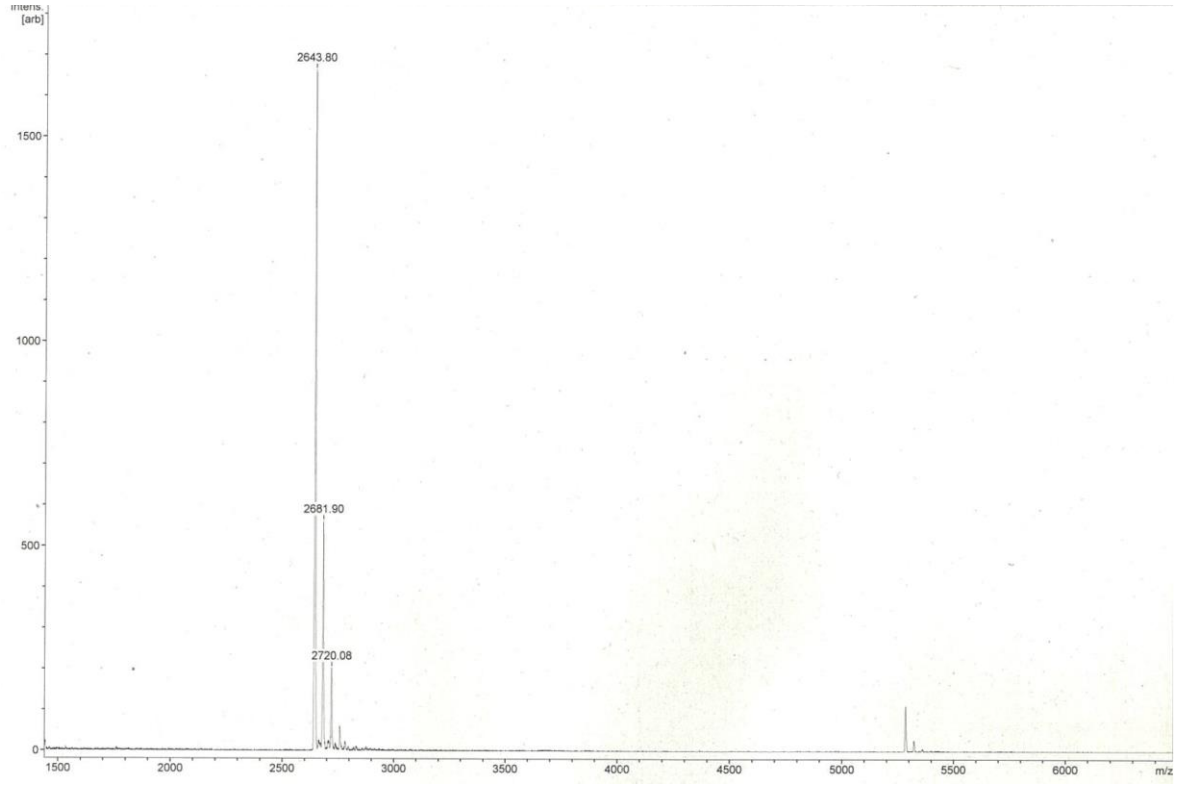
m/z calcd for L-allo-8a [M+H$^+$]: 2641.7, found: 2642.5



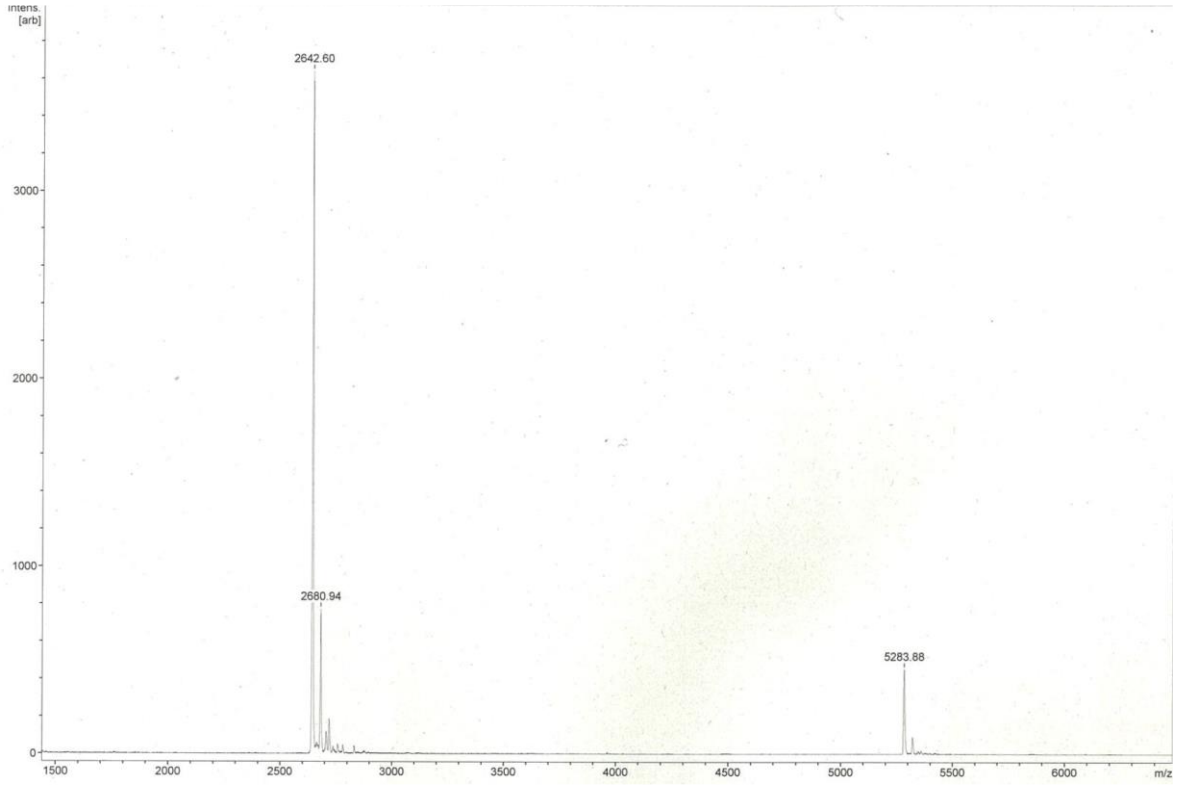
m/z calcd for L-allo-8b [M+H$^+$]: 2641.7, found: 2642.8



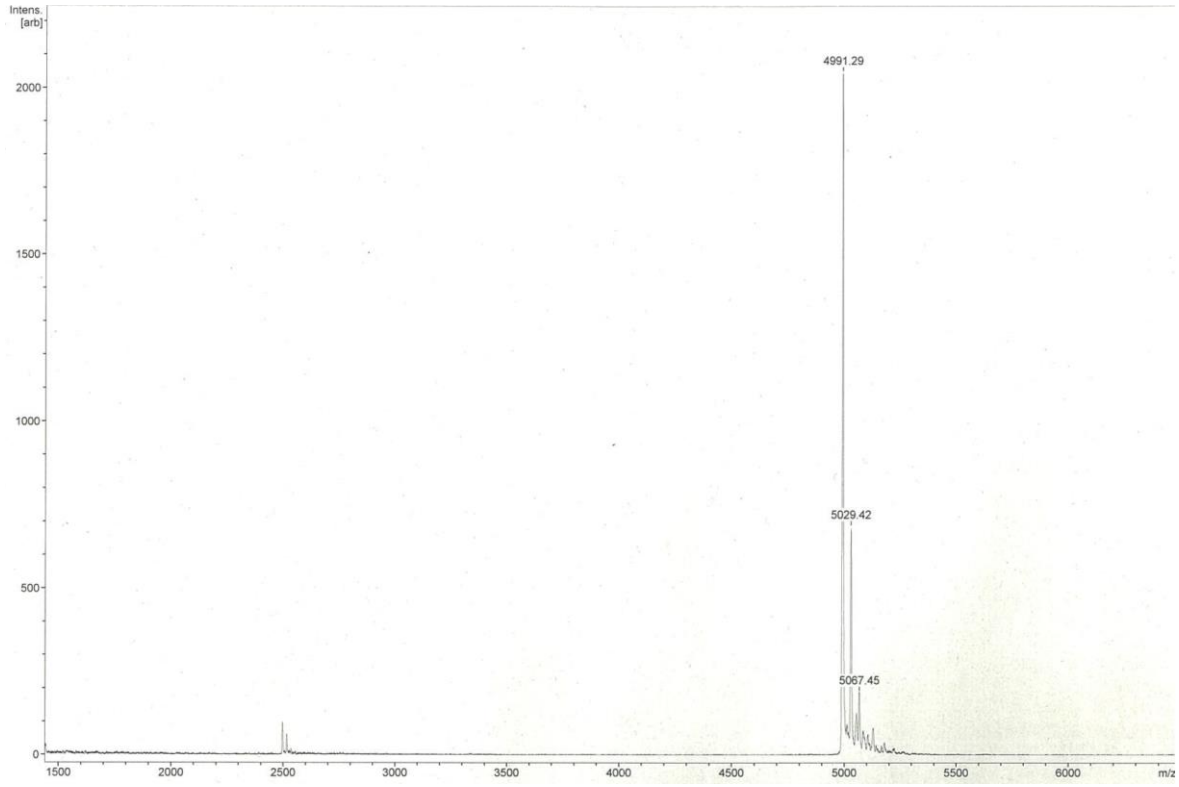
m/z calcd for L-allo-8c [M+H$^+$]: 2641.7, found: 2643.8



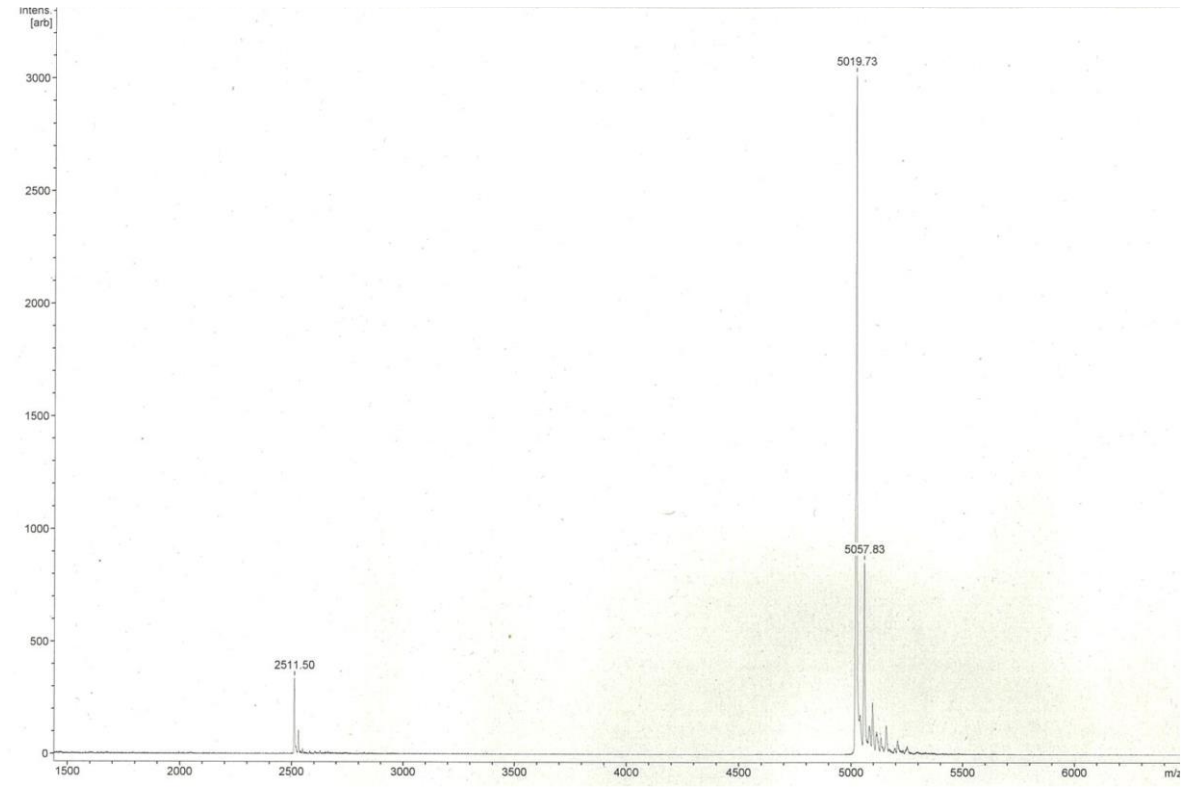
m/z calcd for L-allo-8d [M+H$^+$]: 2641.7, found: 2642.6



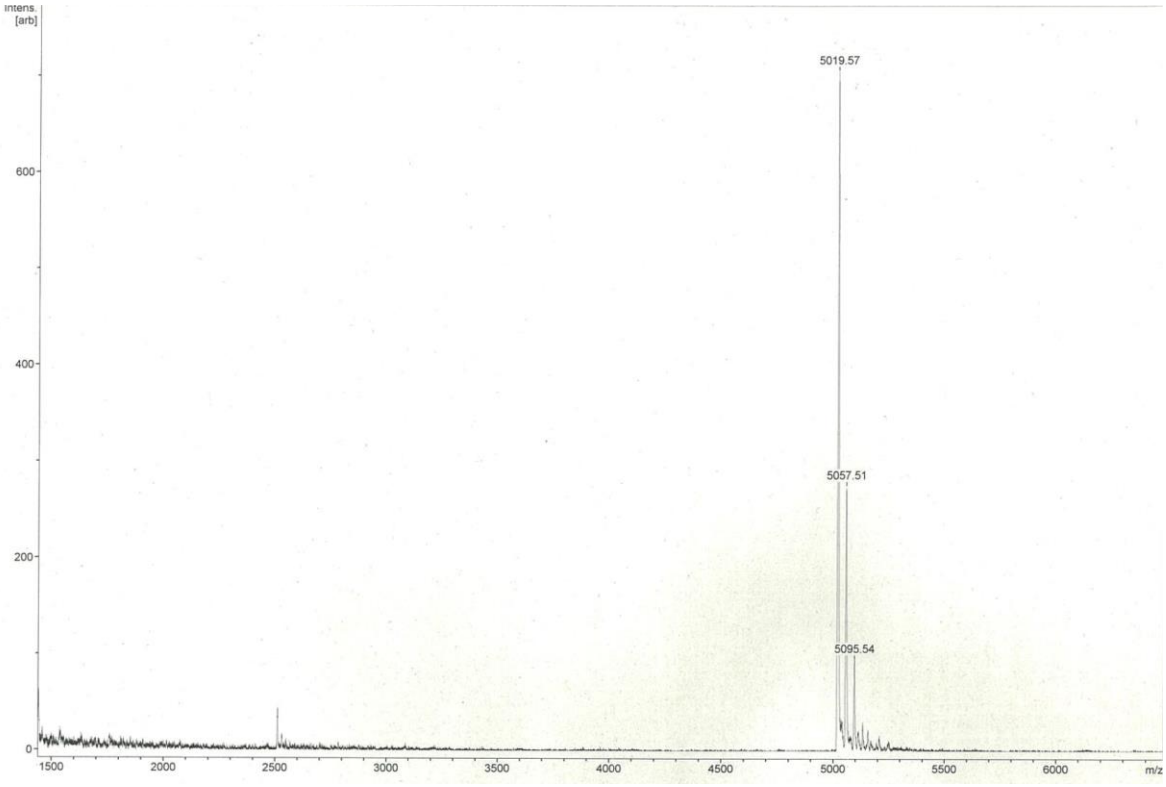
m/z calcd for L-allo-15a [M+H$^+$]: 4991.3, found: 4991.3



m/z calcd for L-allo-15b [M+H$^+$]: 5018.4, found: 5019.7



m/z calcd for L-allo-15c [M+H$^+$]: 5018.4, found: 5019.6



m/z calcd for L-allo-15d [M+H$^+$]: 4991.3, found: 4992.2