Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Amino/guanidino- functionalized *N*-(pyrrolidinyl-2-ethyl)glycine-based pet-PNA: Design, synthesis and binding with DNA/RNA

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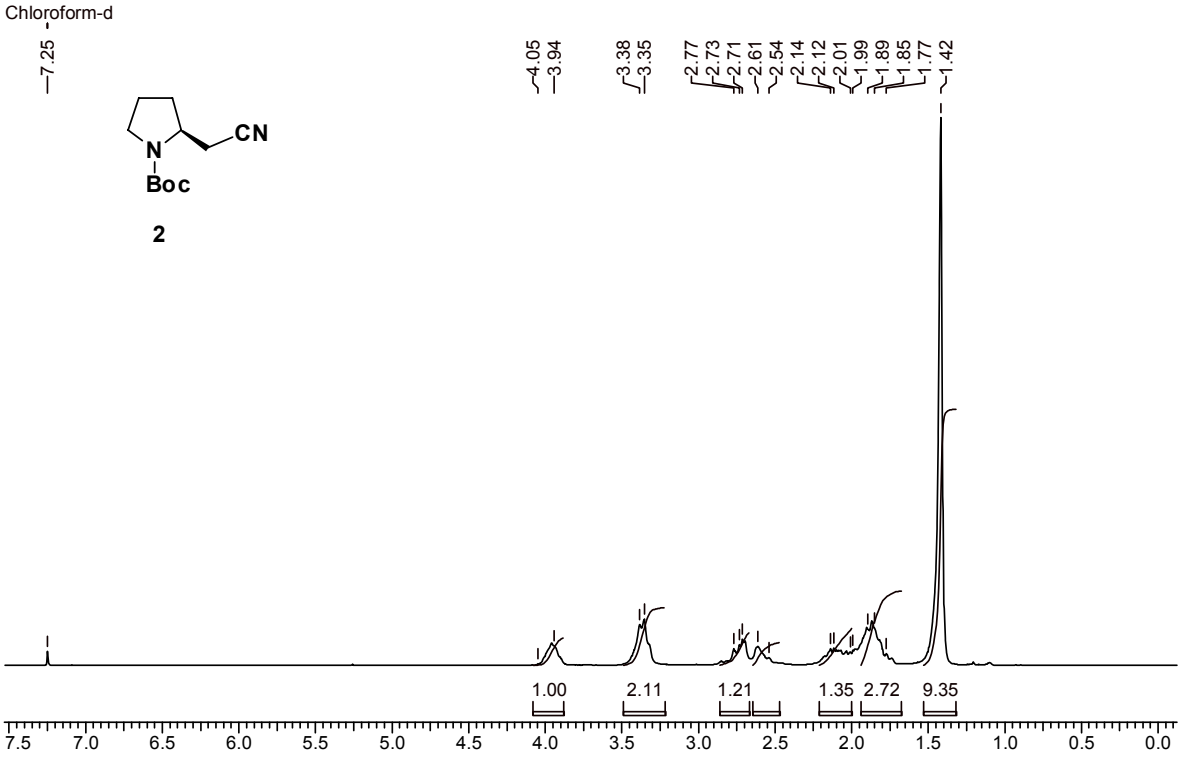


Fig. 1:[1]H NMR spectra for compound **2**

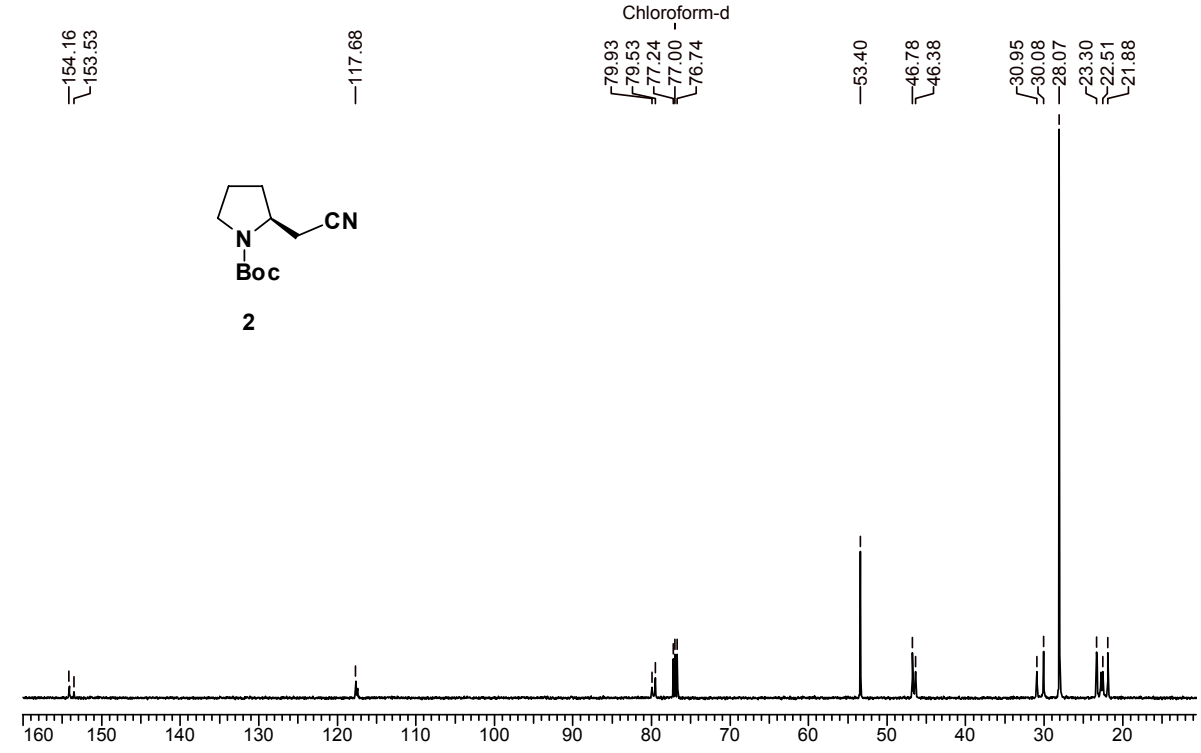


Fig. 2:[13]C NMR spectra for compound **2**

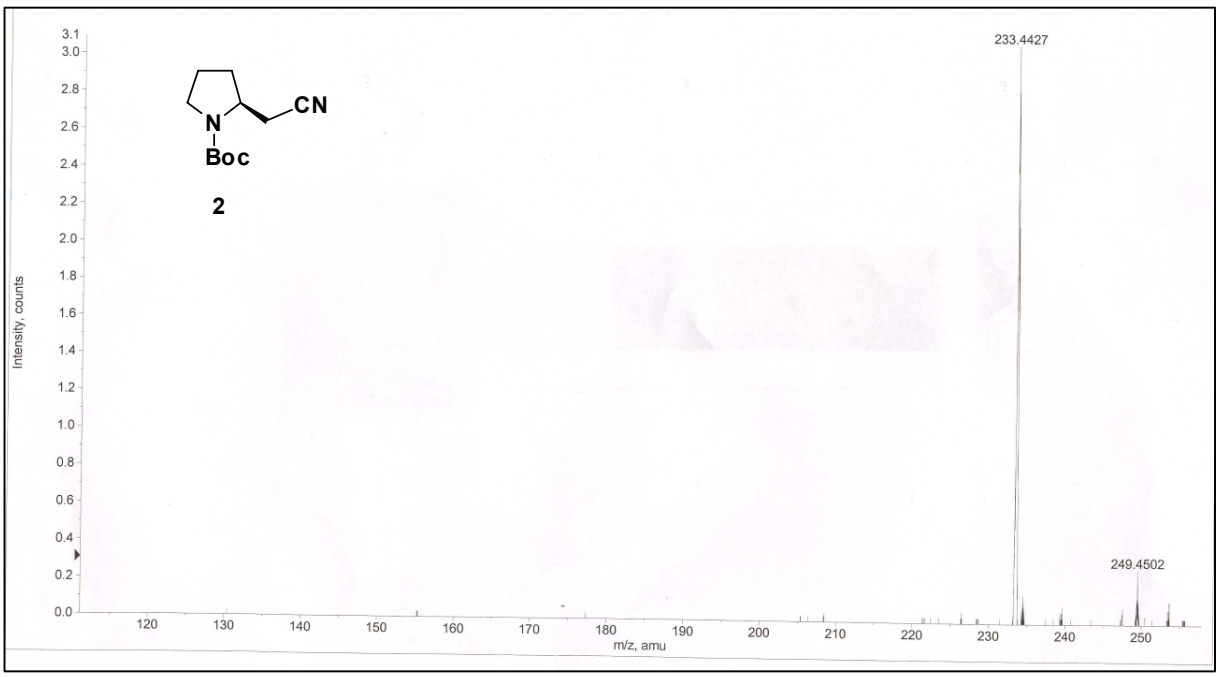


Fig. 3: Mass spectra for compound **2**

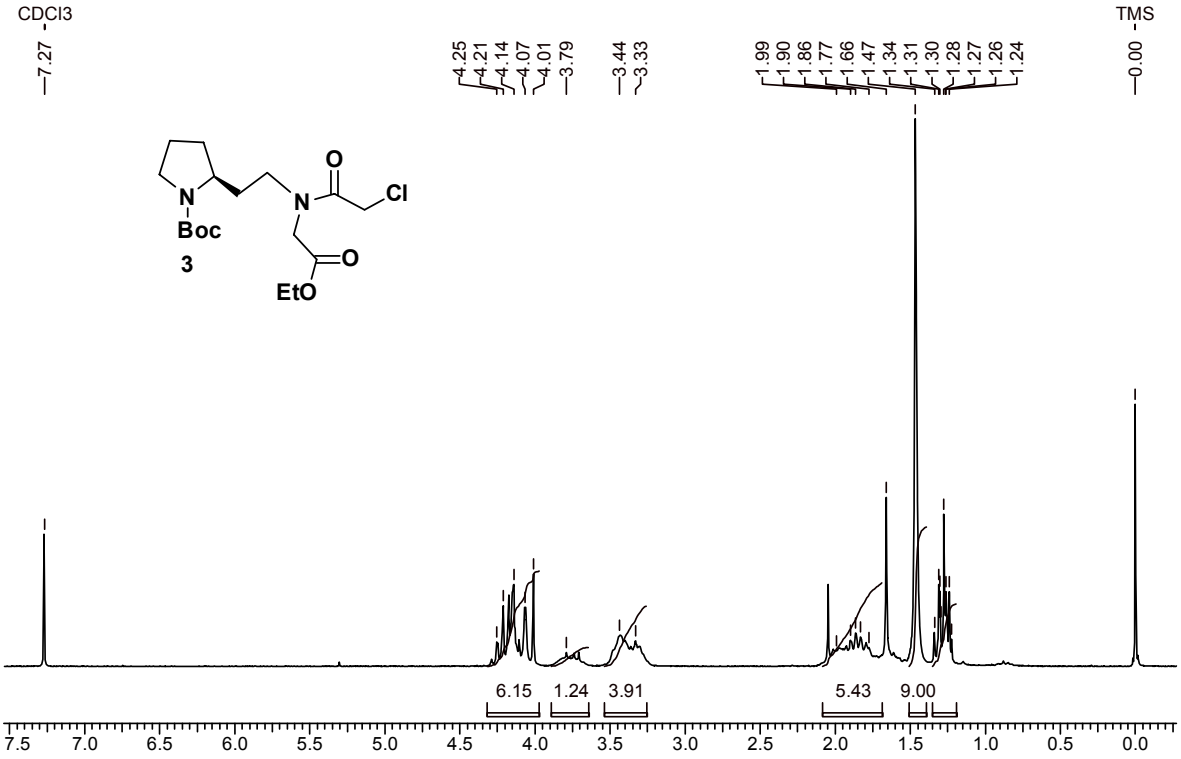


Fig. 4:[1]H NMR spectra for compound **3**

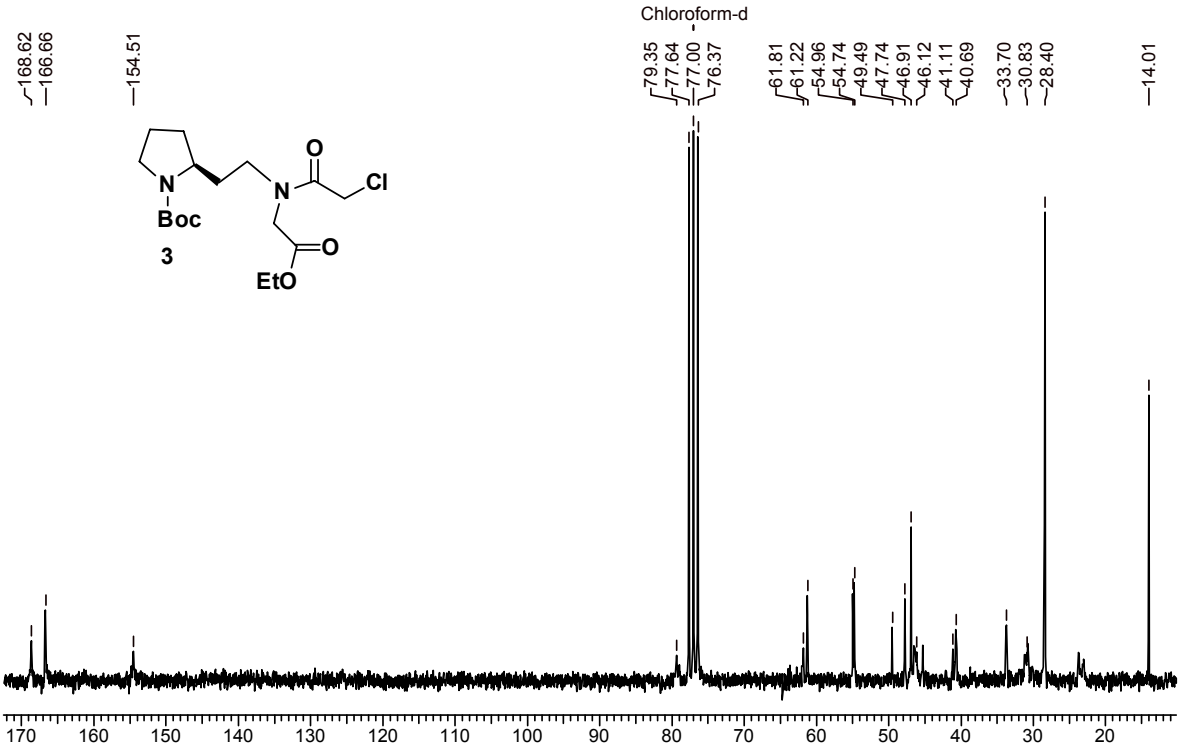


Fig. 5: ^{13}C NMR spectra for compound **3**

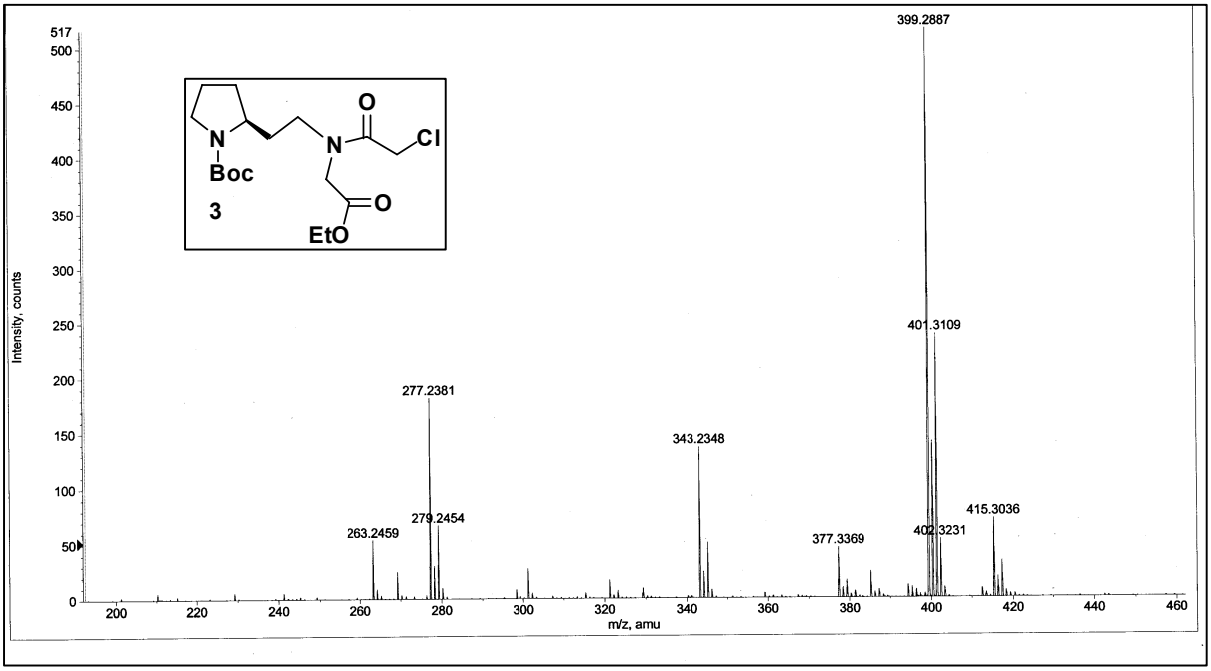


Fig. 6: Mass spectra for compound **3**

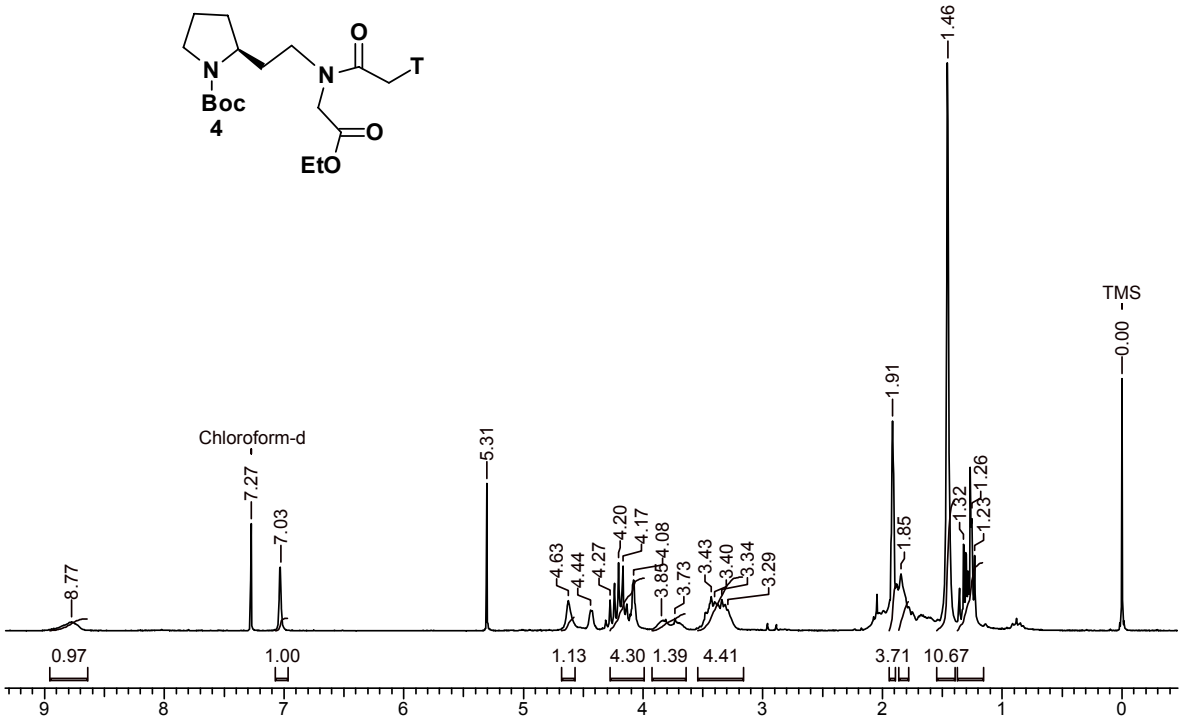


Fig. 7:[1]H NMR spectra for compound **4**

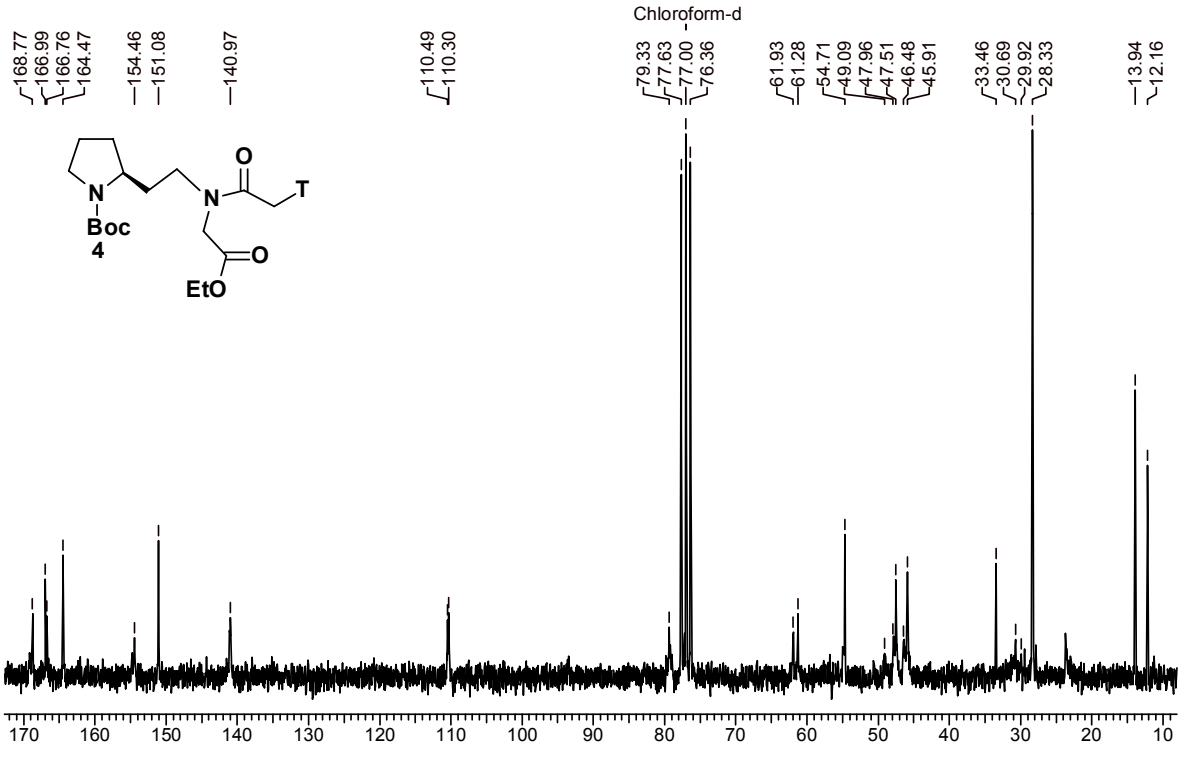


Fig. 8:[13]C NMR spectra for compound **4**

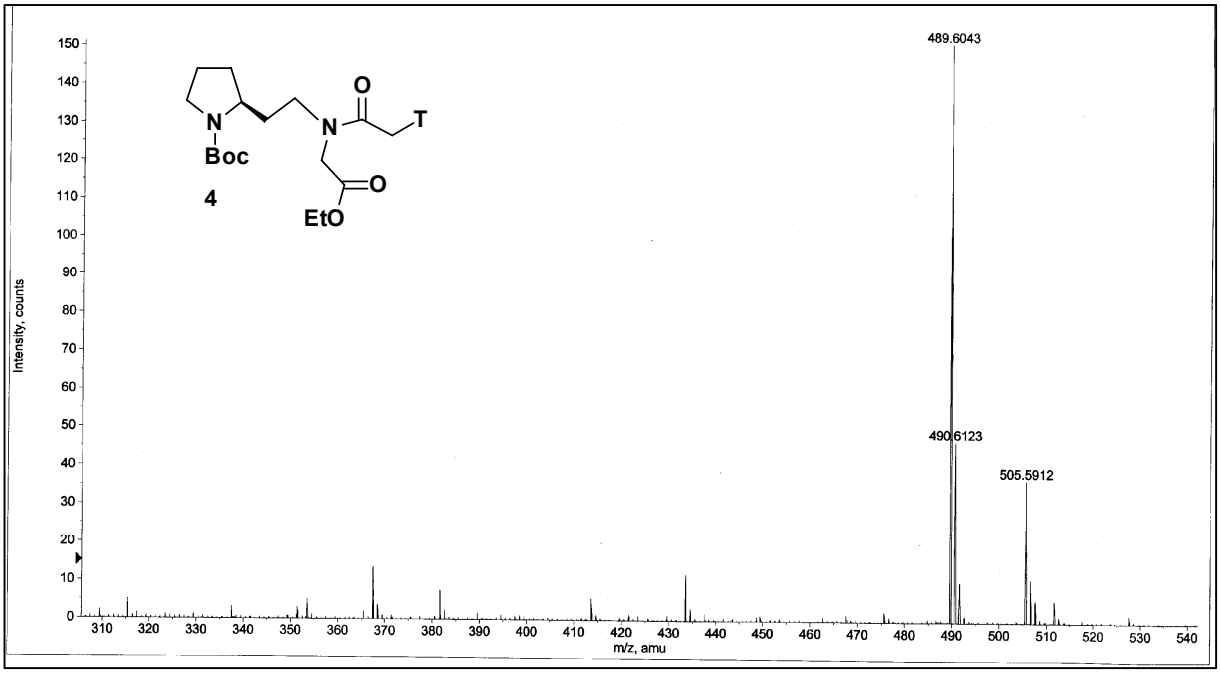


Fig. 9: Mass spectra for compound **4**

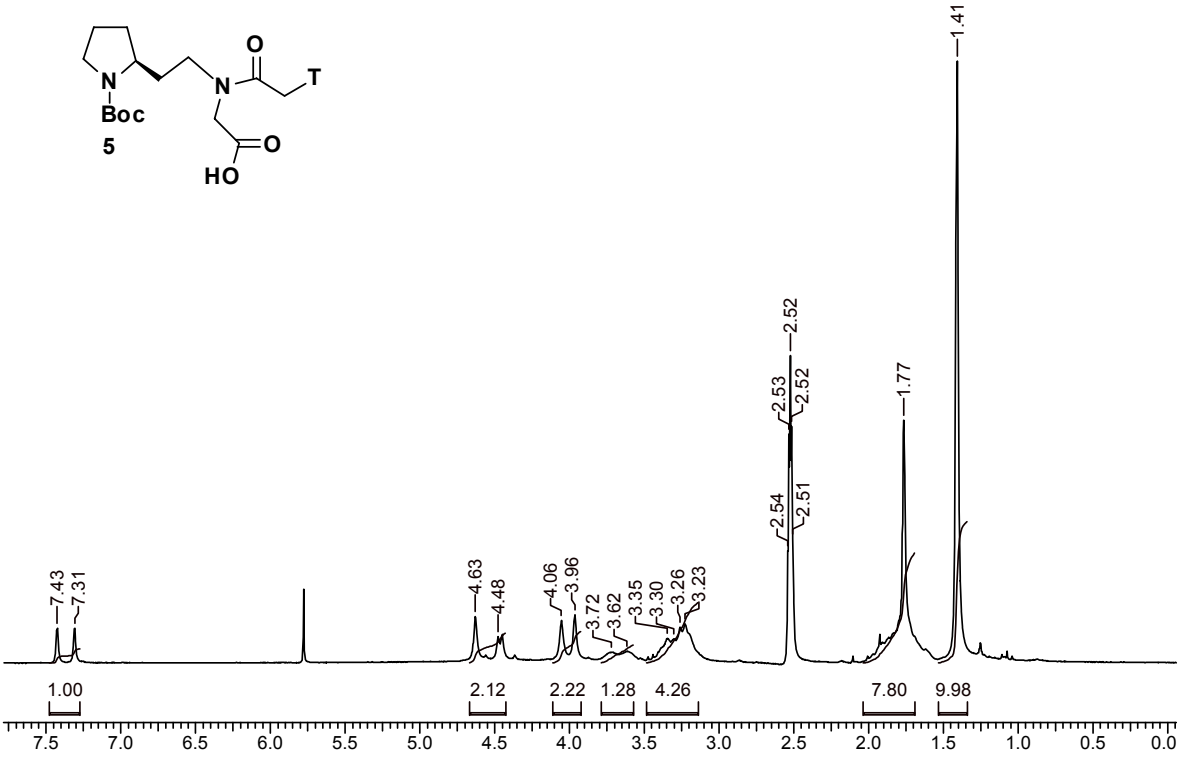


Fig. 10: ^{1}H NMR spectra for compound **5**

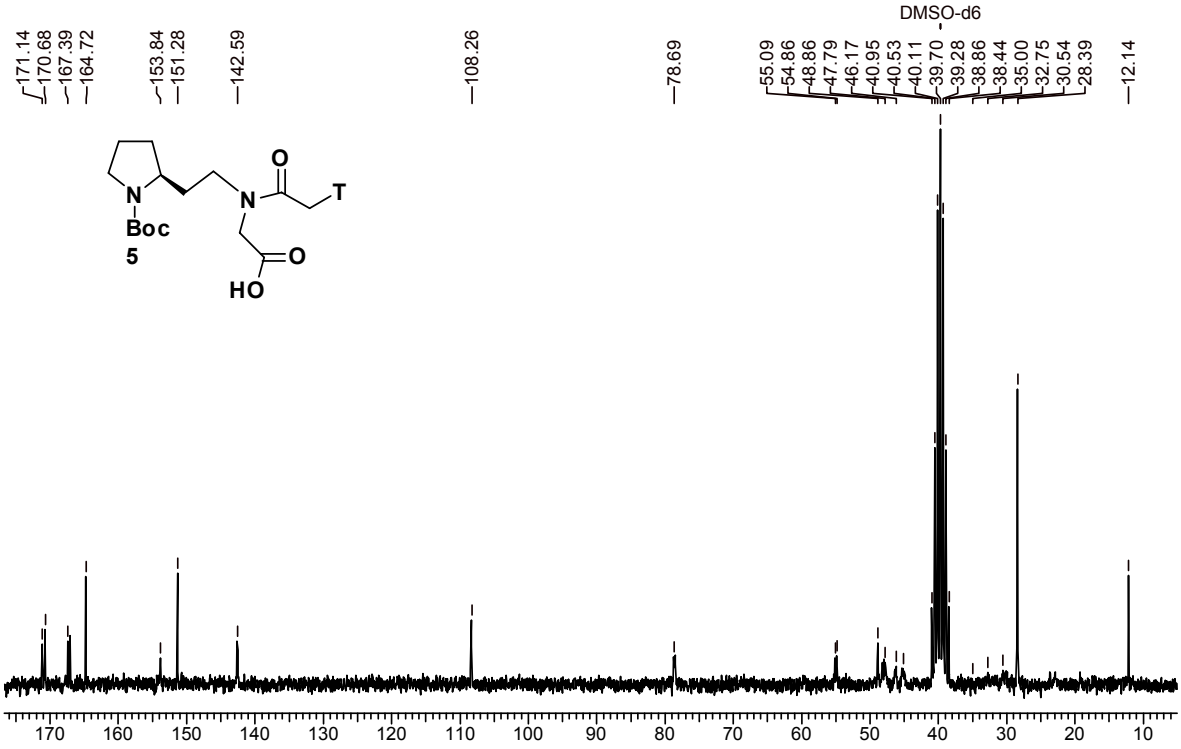


Fig. 11:[13]C NMR spectra for compound **5**

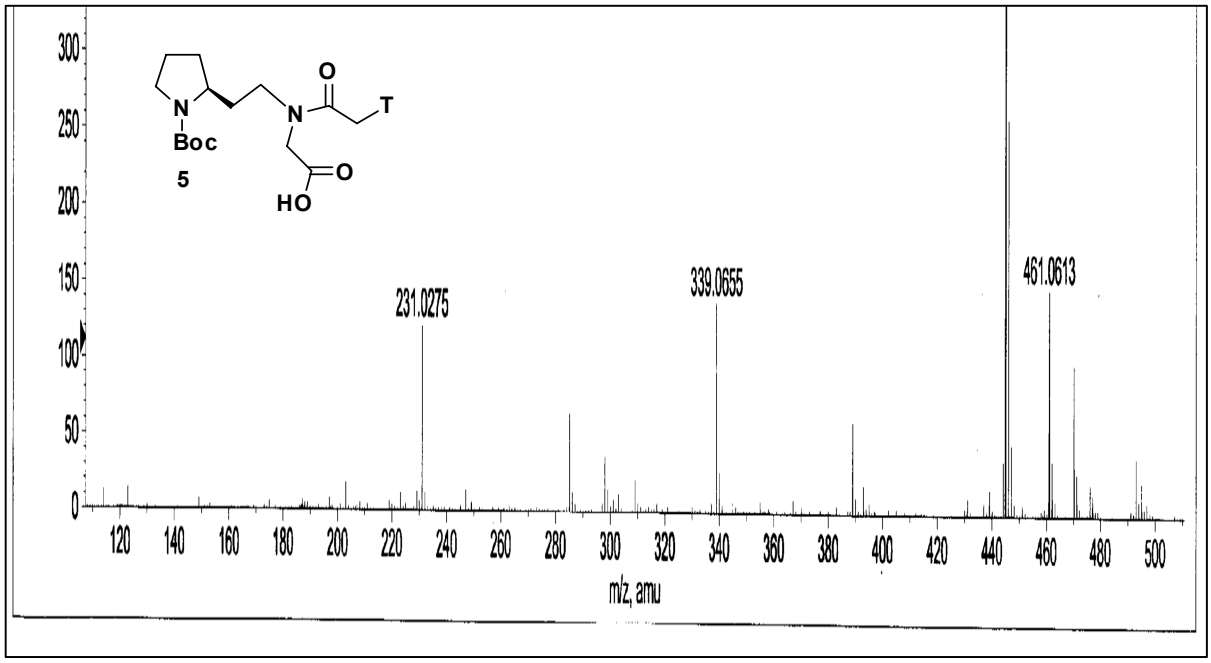


Fig. 12: Mass spectra for compound **5**

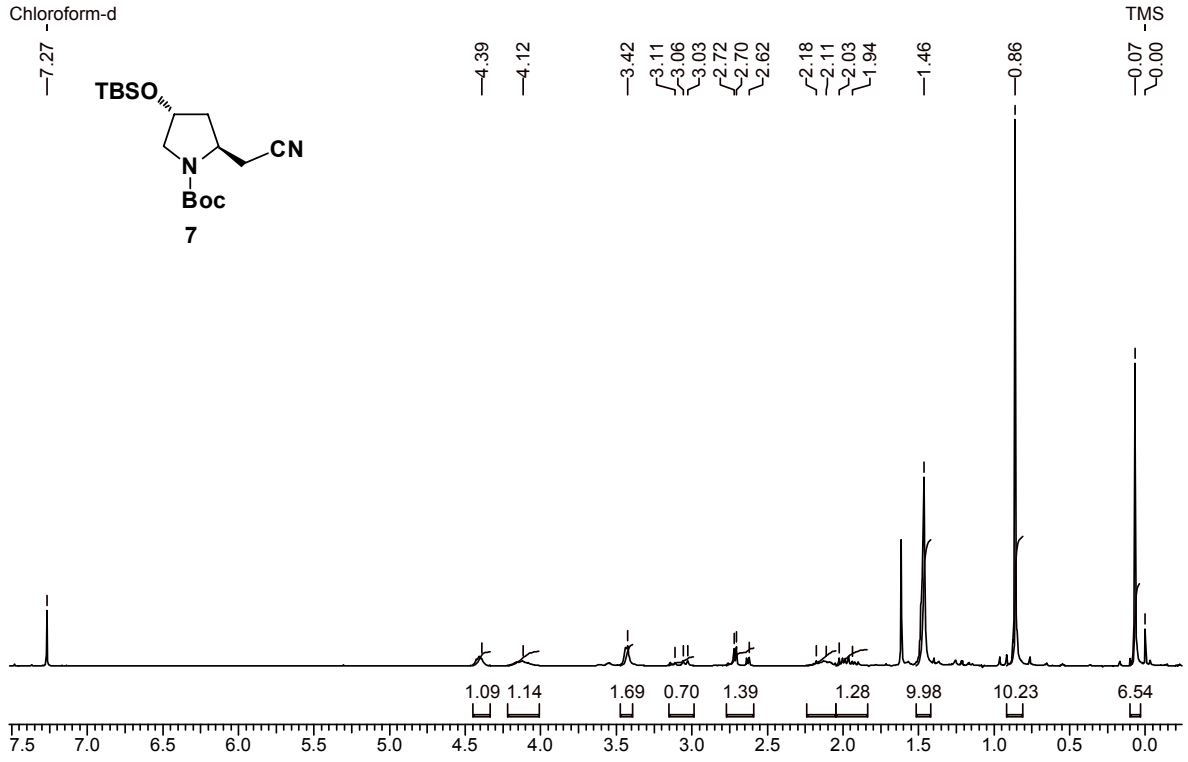


Fig. 13:[1]H NMR spectra for compound **7**

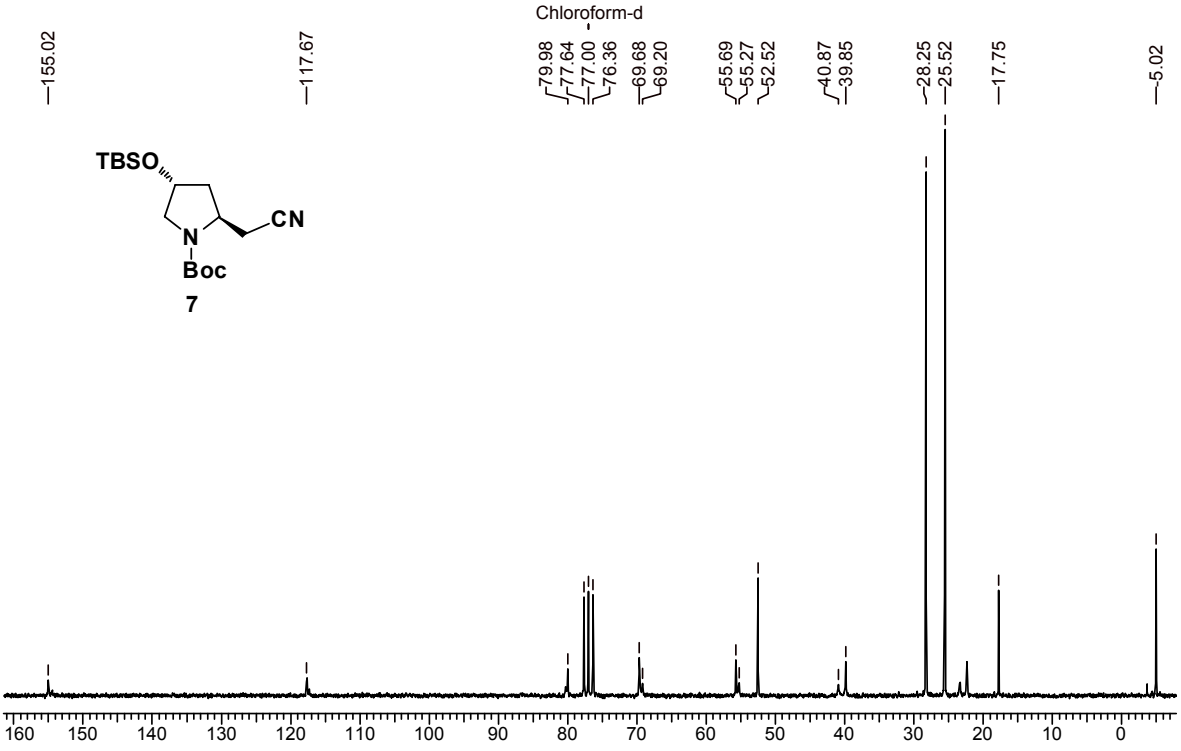


Fig. 14:[13]C NMR spectra for compound **7**

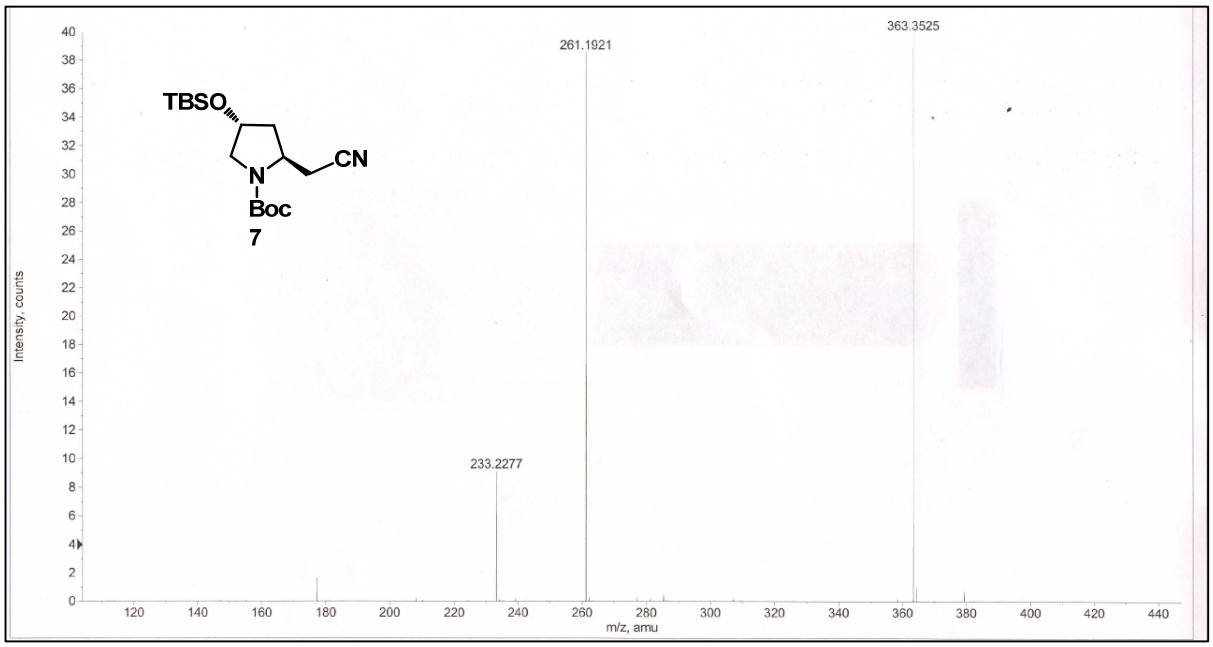


Fig. 15: Mass spectra for compound **7**

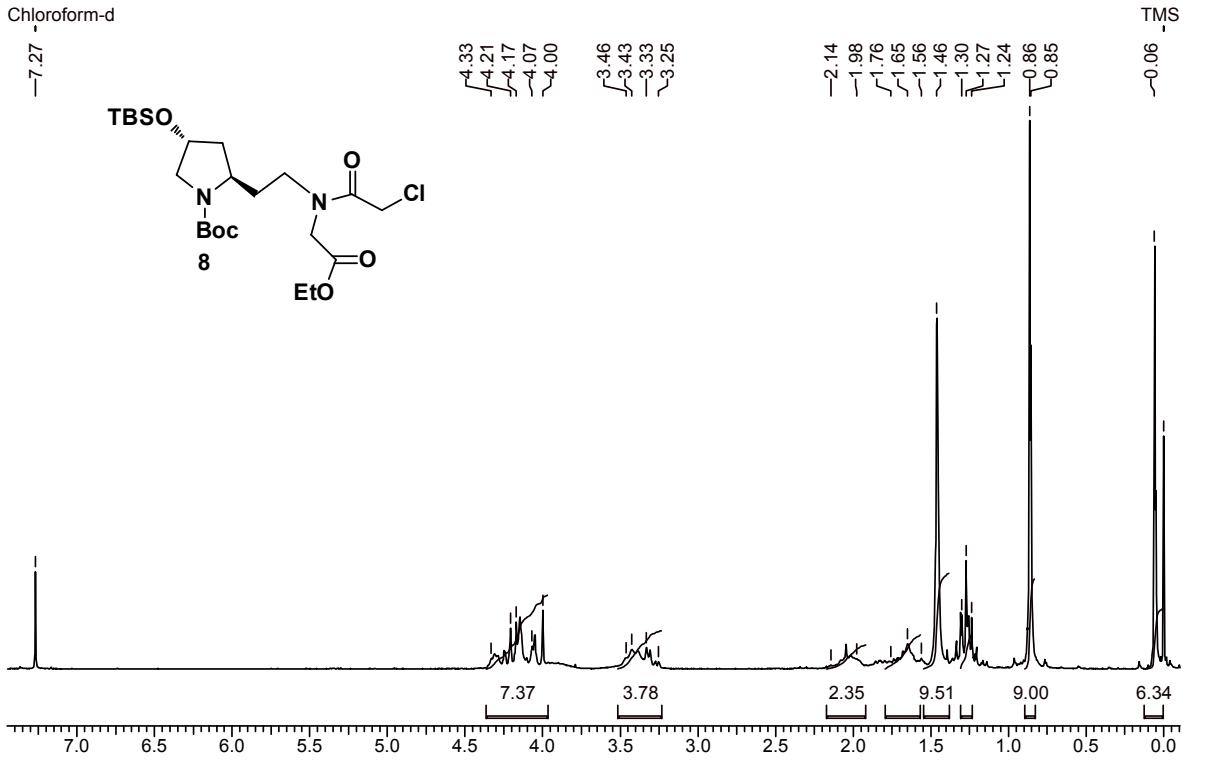


Fig. 16: ^{1}H NMR spectra for compound **8**

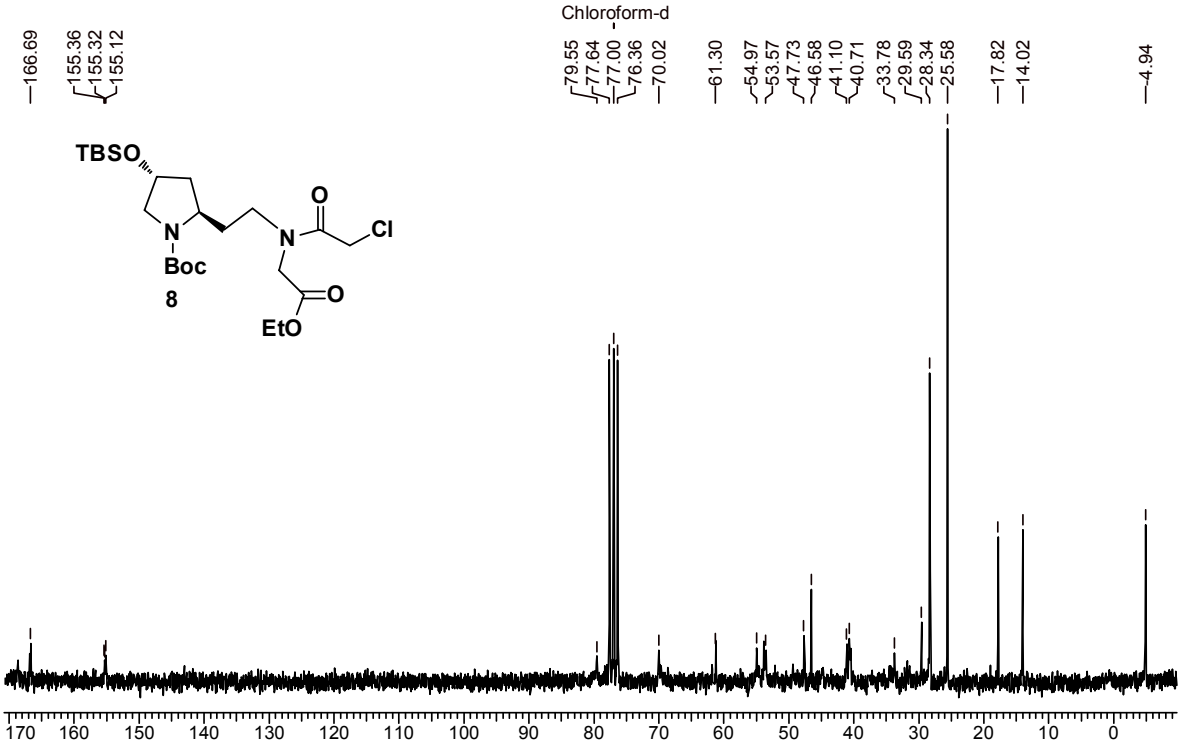


Fig. 17:[13]C NMR spectra for compound **8**

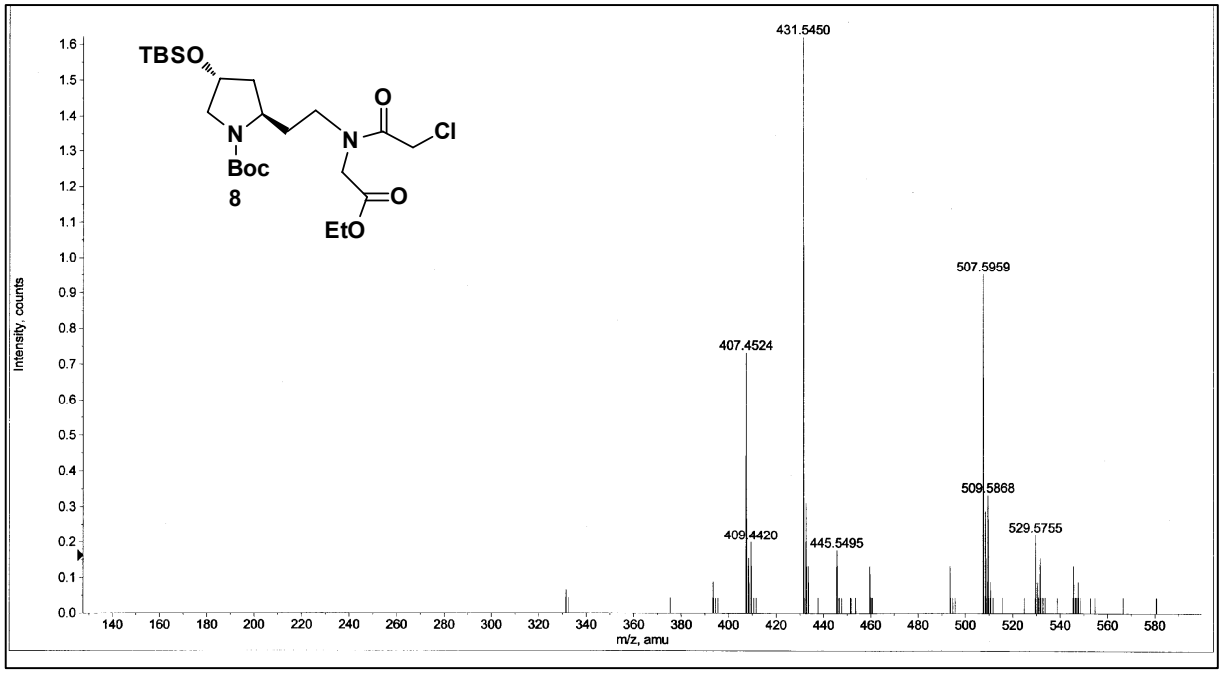


Fig. 18: Mass spectra for compound **8**

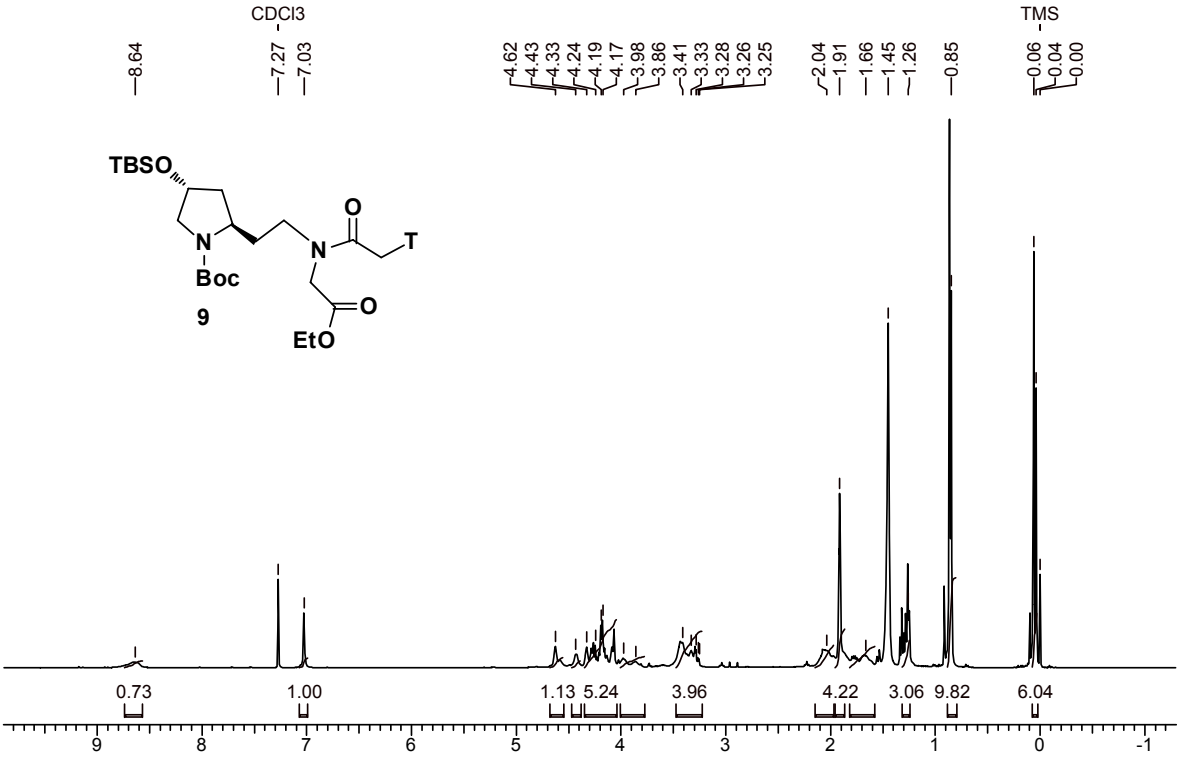


Fig. 19:[1]H NMR spectra for compound **9**

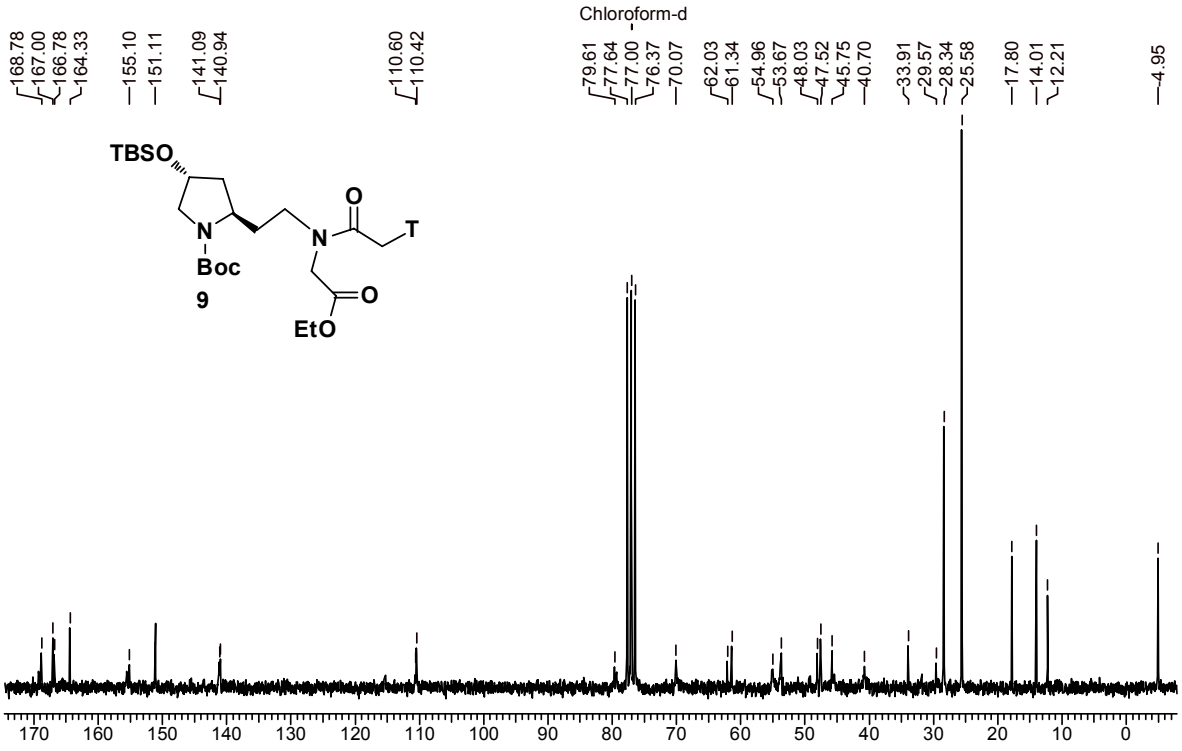


Fig. 20:[13]C NMR spectra for compound **9**

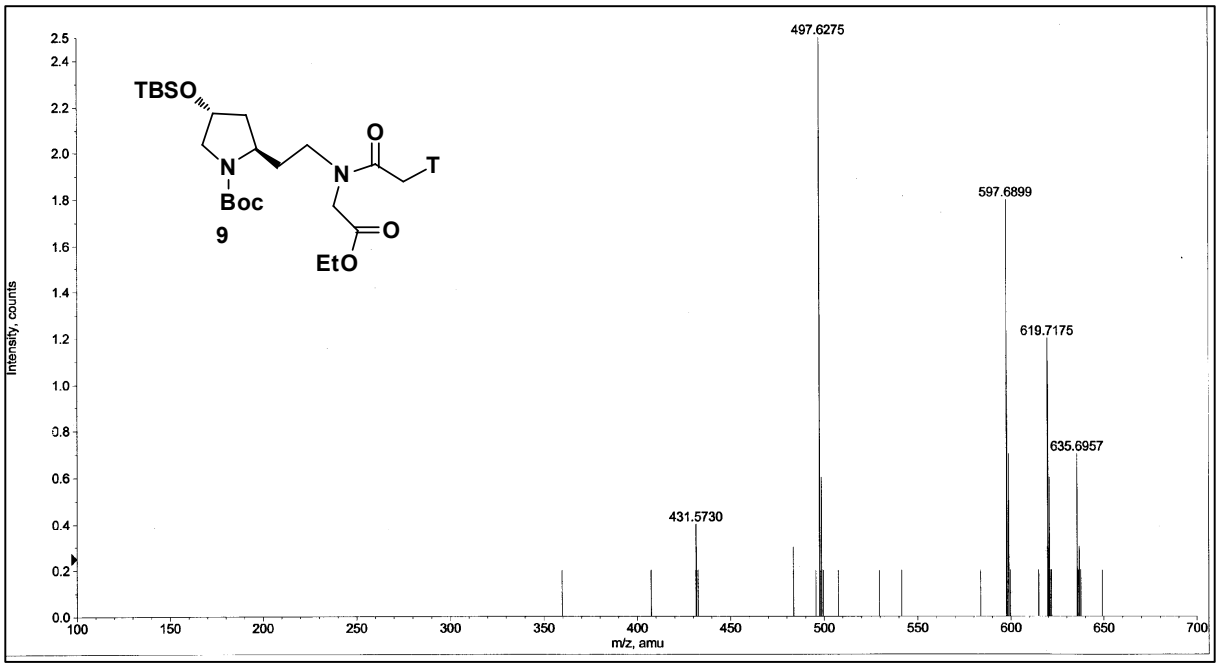


Fig. 21: Mass spectra for compound **9**

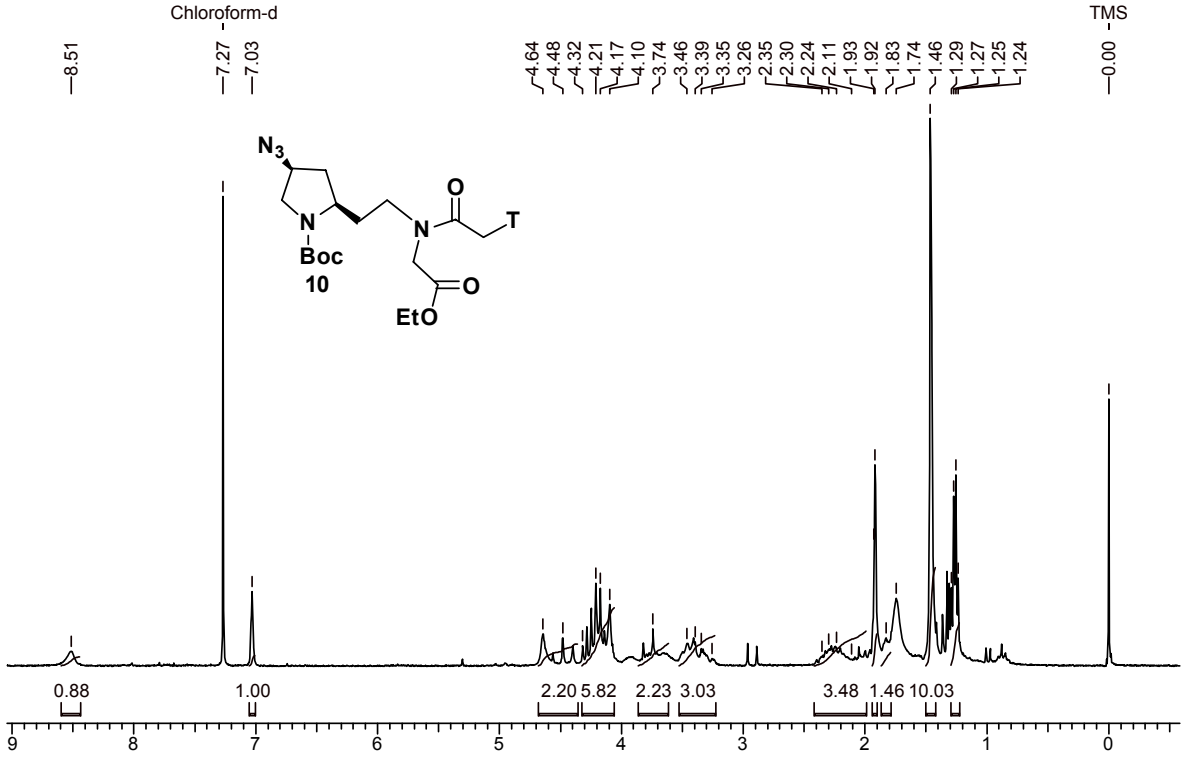


Fig. 22: ^{1}H NMR spectra for compound **10**

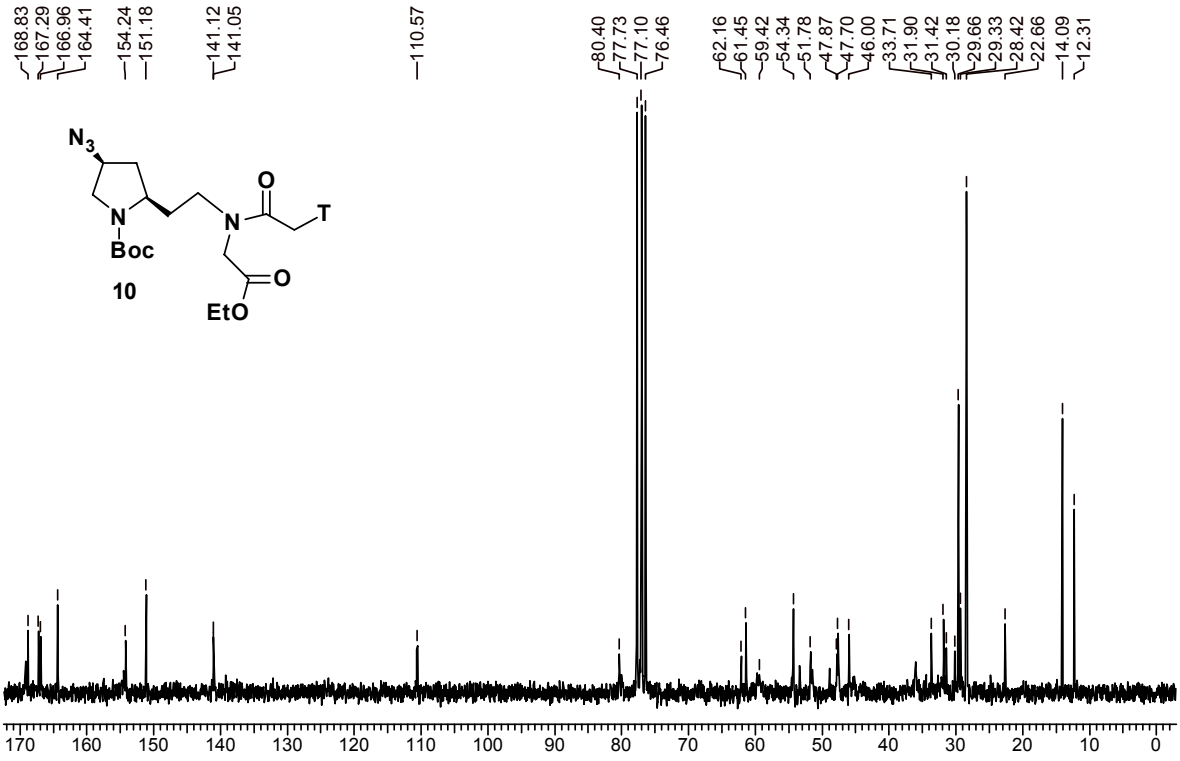


Fig. 23:[13]C NMR spectra for compound **10**

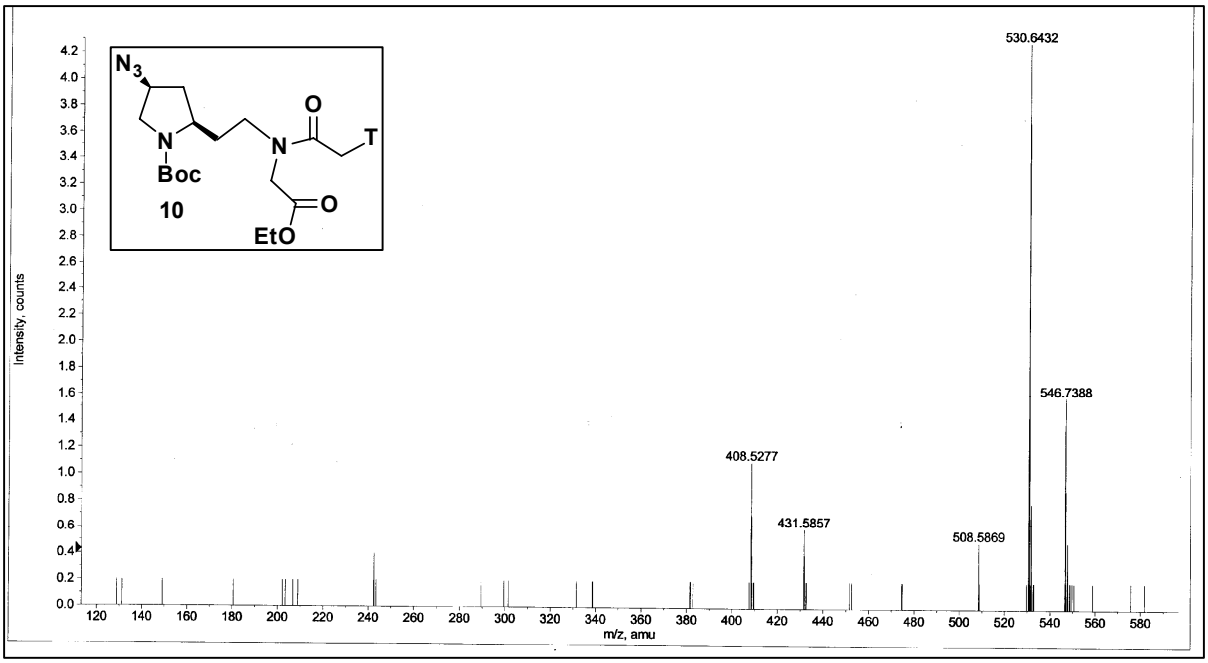


Fig. 24: Mass spectra for compound **10**

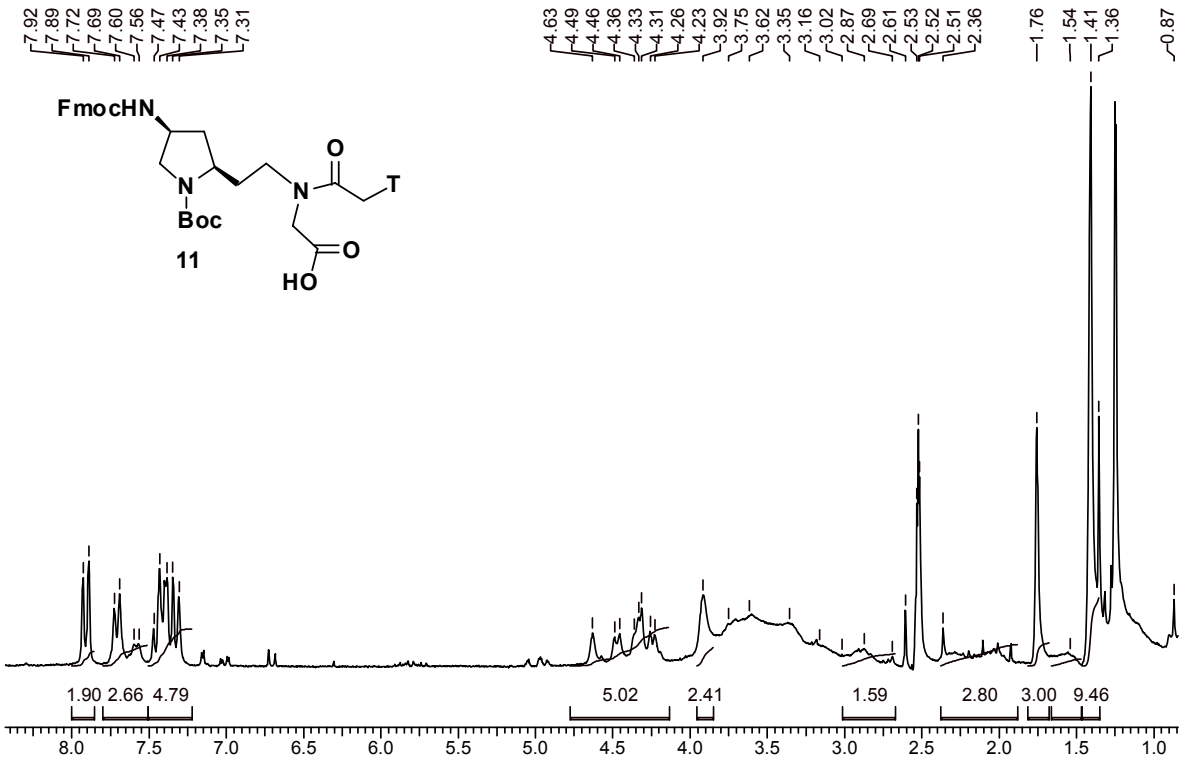


Fig. 25:[1]H NMR spectra for compound **11**

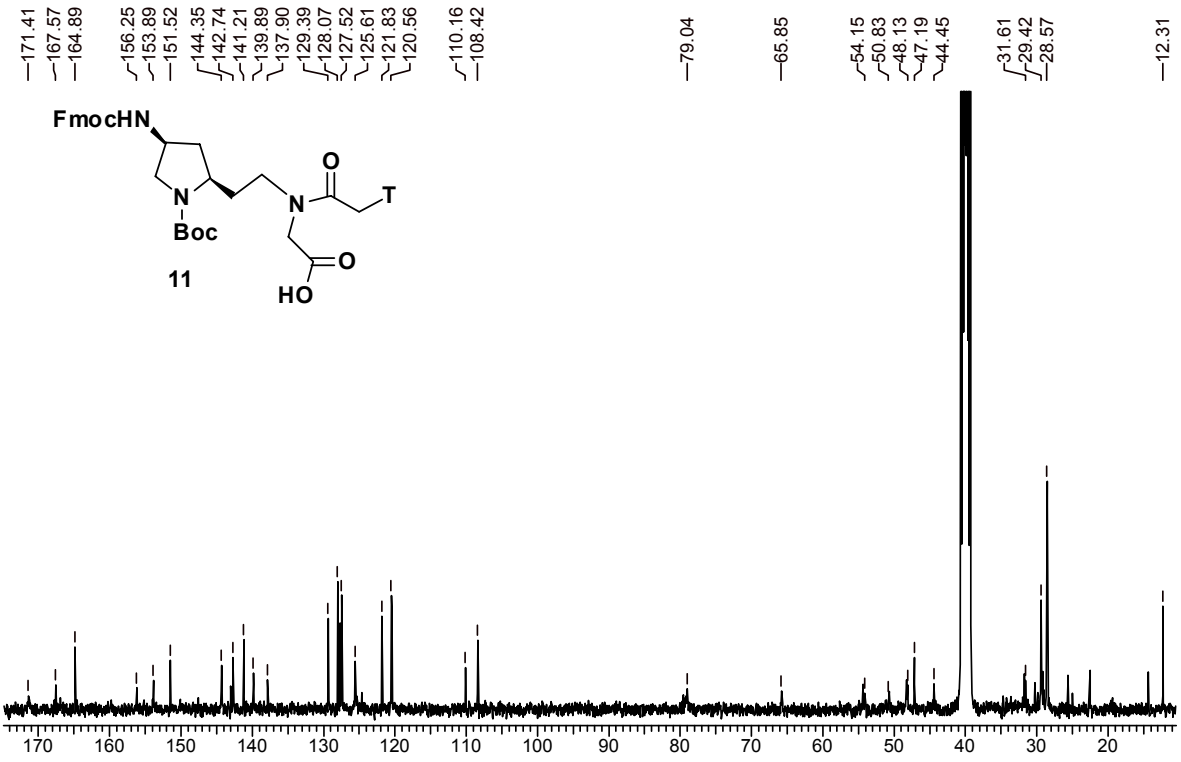


Fig. 26:[13]C NMR spectra for compound **11**

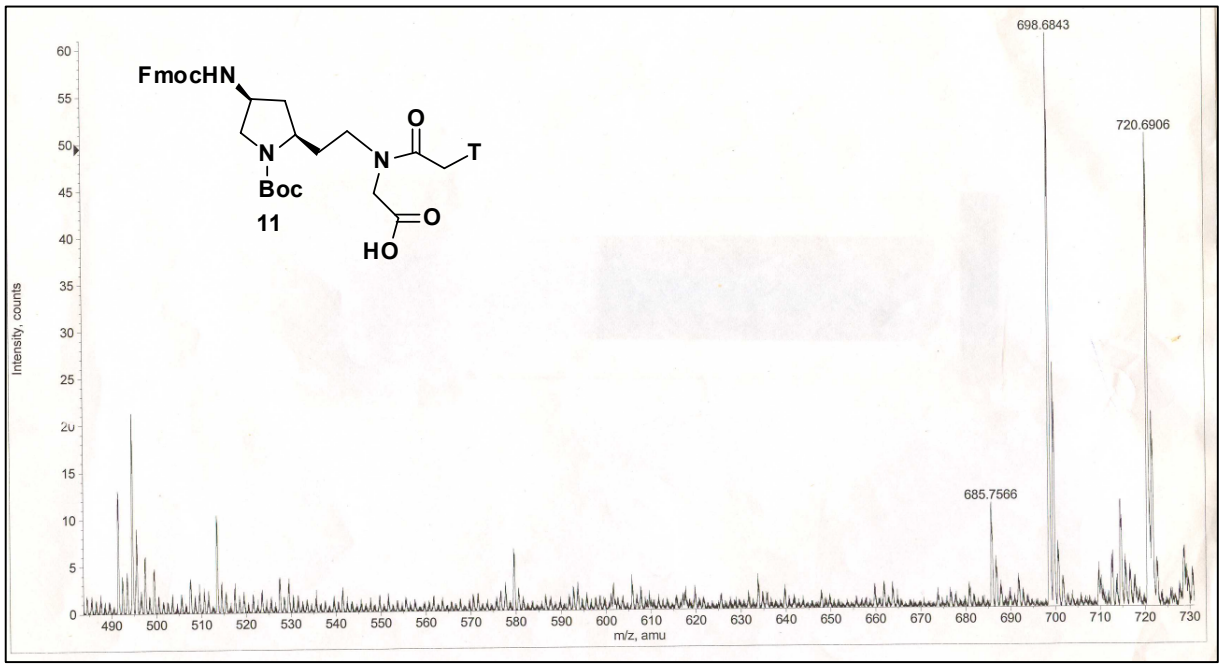


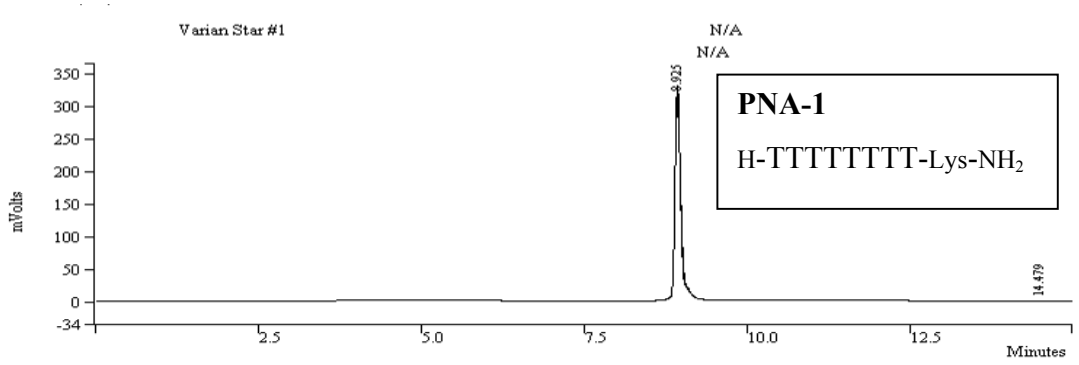
Fig. 27: Mass spectra for compound **11**

Oligomer Synthesis: The PNA oligomers were synthesized by standard Boc-solid phase peptide strategy. Purification of all the PNA oligomers was carried out by Varian dual pump PROSTAR model No.210 HPLC on RP-C18 column with Water: acetonitrile-0.1% TFA system. The gradient used for HPLC analysis is (A: 95% Water: 5% acetonitrile+0.1% TFA, B: 50% Water: 5% acetonitrile-0.1% TFA) 0-20 min 100% B. PNA oligomers were characterized by MALDI-TOF mass spectrometry by using Voyager-DE-STR (Applied Biosystems) MALDI-TOF. A nitrogen laser (337 nm) was used for desorption and ionization. Spectra were acquired in linear mode. The matrix used for analysis was CHCA (α-cyano hydroxyl cinnamic acid)

Table.1 PNA Oligomers synthesized their HPLC t_R and MALDI-TOF mass characterization.

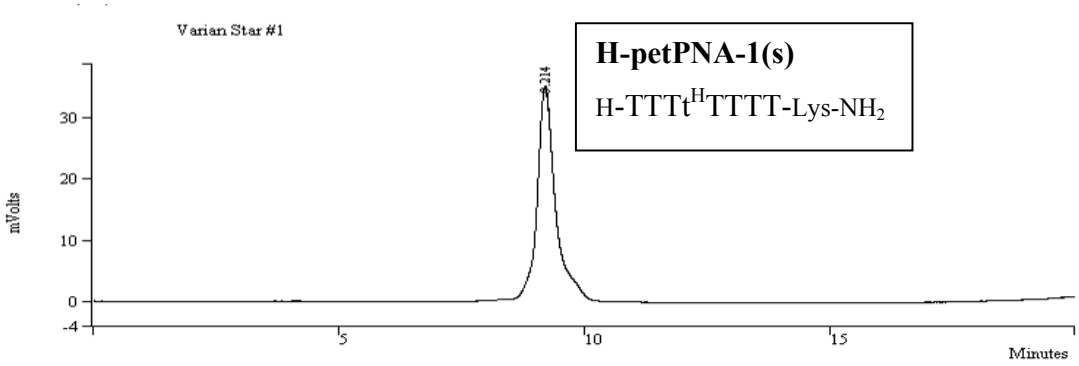
Entry	Sequence	HPLC t_R (min)	Mass $_{calcd/obsd}$
PNA-1	H-TTTTTTTT-Lys-NH_2($C_{94}H_{127}N_{35}O_{33}$)	8.90	2273.93/2274.91
	H-TTTTt^RTTT-Lys-NH_2		
H-petPNA-1(s)	R = H($C_{98}H_{134}N_{35}O_{33}$)	9.21	2328.98/2329.1
Am-petPNA-1(s)	R= NH_2($C_{98}H_{135}N_{36}O_{33}$)	14.16*	2343.99/2343.34
Gu-petPNA-1(s)	R= NH-C(= NH)NH_2 ($C_{99}H_{137}N_{38}O_{33}$)	14.58*	2386.02/2382.33
	H-TTTTTTTt^R-Lys-NH_2		
H-petPNA-1(s*)	R = H($C_{98}H_{134}N_{35}O_{33}$)	9.48	2328.98/2329.20
Am-petPNA-1(s*)	R= NH_2($C_{98}H_{135}N_{36}O_{33}$)	8.48	2343.99/2366.13($M+Na^+$)
Gu-petPNA-1(s*)	R= NH-C(= NH)NH_2 ($C_{99}H_{137}N_{38}O_{33}$)	8.66	2386.02/2386.57
PNA-2	H-TTACCTCAGT-Lys-NH_2 ($C_{113}H_{149}N_{55}O_{33}$)	9.04	2804.16/2827.0($M+Na^+$)
H-petPNA-2(s)	H-TTACCt^RCAGT-Lys-NH_2 R=H($C_{117}H_{156}N_{55}O_{33}$)	9.30	2859.22/2859.38
	H-TTACCt^RCAGt^R-Lys-NH_2		
H-petPNA-2(d)	R=H ($C_{121}H_{163}N_{55}O_{33}$)	9.60	2914.27/2910.43
Am-petPNA-2(d)	R= NH_2 ($C_{121}H_{165}N_{57}O_{33}$)	12.98*	2944.29/2940.6
Gu-petPNA-2(d)	R= NH-C(=NH)NH_2 ($C_{123}H_{169}N_{61}O_{33}$)	14.12*	3028.34/3024.5
PNA-3	H-CTTCTTCCTT-Lys-NH_2 ($C_{112}H_{151}N_{47}O_{37}$)	11.19	2746.13/2741.15
	H-CTt^RCTt^RCCt^Rt^R-Lys-NH_2		
Am-petPNA-3(t)	R= NH_2 ($C_{128}H_{183}N_{51}O_{37}$)	13.47*	3026.40/3034.50
Gu-petPNA-3(t)	R= NH-C(= NH) NH_2 ($C_{132}H_{191}N_{59}O_{37}$)	14.64*	3194.35/3201.40

* The gradient use for HPLC analysis is (A: 95% Water: 5% acetonitrile-0.1% TFA, B: 50% Water: 50% acetonitrile-0.1% TFA) 0-20 min- 0%-50%B, 20-30min-50%-100% B



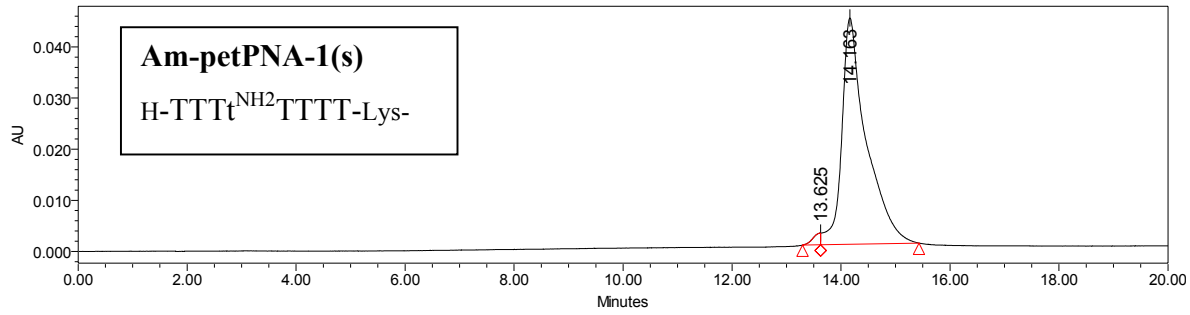
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	8.925	5.5	2083421	99.7167
2	14.479	7.9	5919	0.2833
			2089340	**100.0000**

Fig. 28: HPLC chromatogram for **PNA-1**



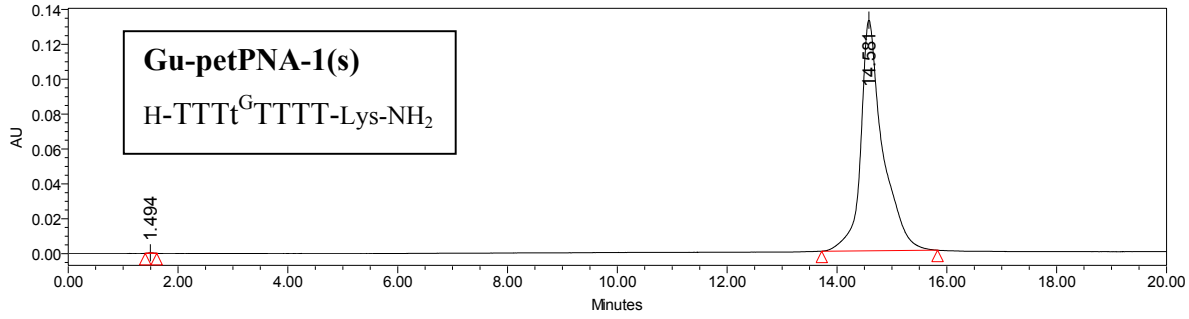
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	9.214	22.1	960647	100.0000
			960647	**100.0000**

Fig. 29: HPLC chromatogram for **H-petPNA-1(s)**



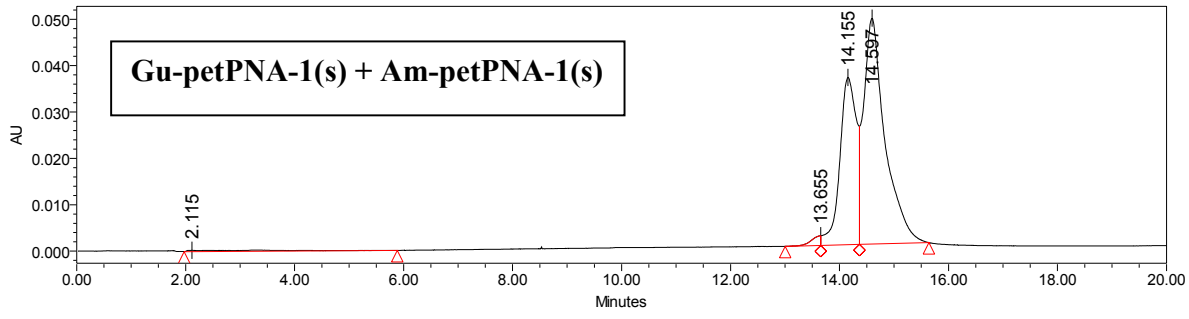
	Name	Retention Time	Area	% Area	Height	Int Type
1		13.625	21779	1.55	2293	BV
2		14.163	1379306	98.45	44243	VB

Fig. 30: HPLC chromatogram for **Am-petPNA-1(s)**



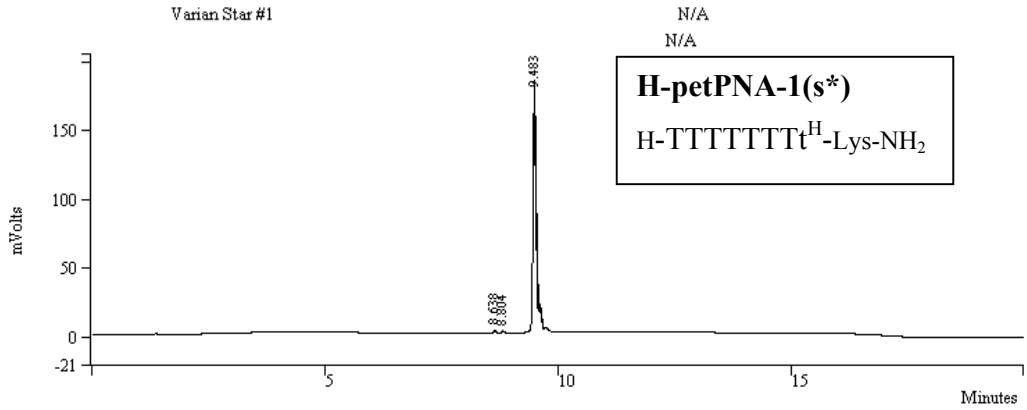
	Name	Retention Time	Area	% Area	Height	Int Type
1		1.494	1969	0.05	288	BB
2		14.581	3658315	99.95	132240	BB

Fig. 31: HPLC chromatogram for **Gu-petPNA-1(s)**



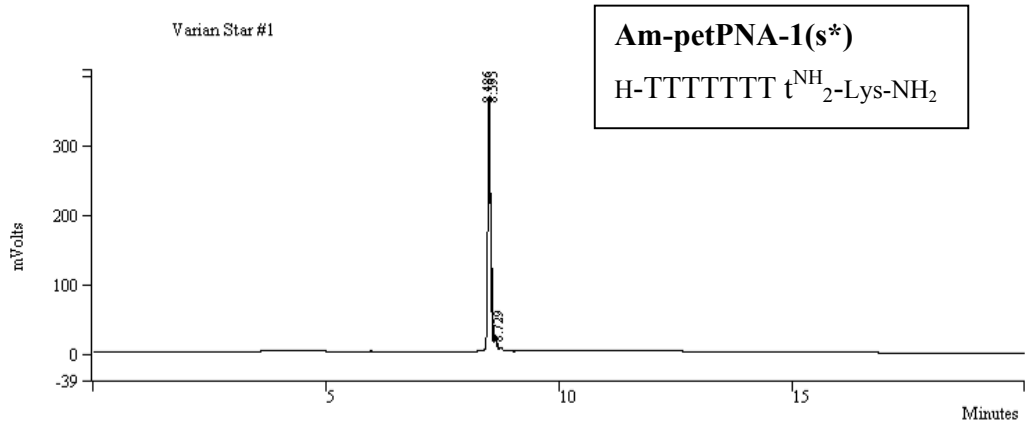
	Name	Retention Time	Area	% Area	Height	Int Type
1		2.115	26271	1.23	275	BB
2		13.655	24430	1.14	2094	BV
3		14.155	765374	35.71	36101	VV
4		14.597	1327210	61.92	48743	VB

Fig. 32: HPLC chromatogram for **Am-petPNA-1(s) +Gu-petPNA-1(s)**



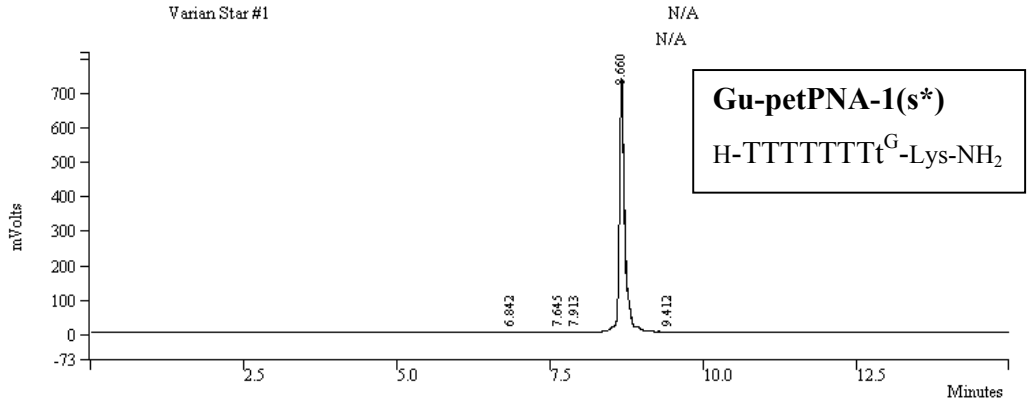
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	8.638	4.5	8315	0.8698
2	8.804	4.8	6818	0.7132
3	9.483	4.4	940848	98.4170
			955981	**100.0000**

Fig. 33: HPLC chromatogram for **H-petPNA-1(s*)**



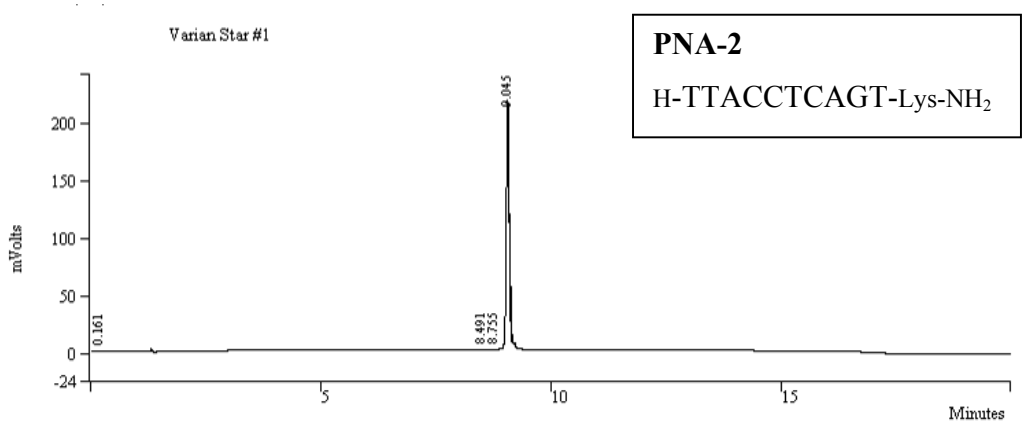
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	8.486	3.9	1635273	98.1050
2	8.595	0.0	23869	1.4320
3	8.729	0.0	7718	0.4630
			1666860	**100.0000**

Fig. 34: HPLC chromatogram for **Am-petPNA-1(s*)**



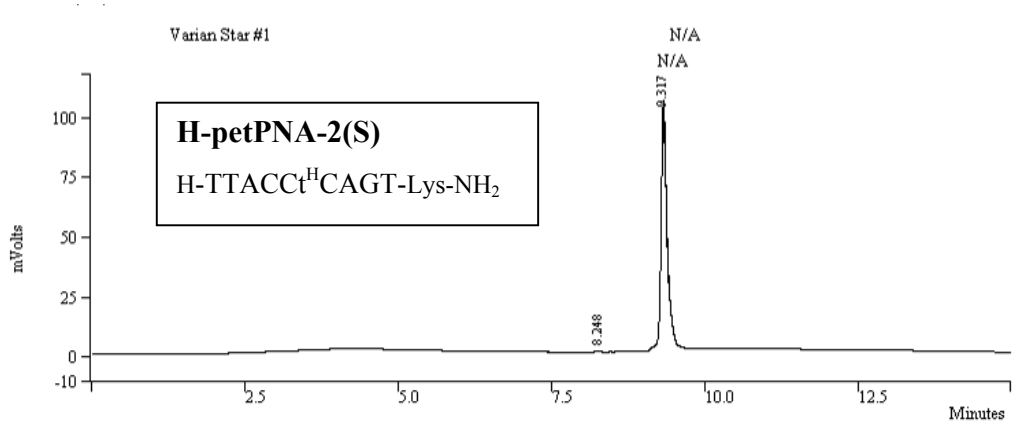
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	6.842	15.2	5699	0.1217
2	7.645	25.4	2483	0.0530
3	7.913	31.6	1858	0.0397
4	8.660	4.8	4668231	99.6864
5	9.412	0.0	4645	0.0992
			4682916	**100.0000**

Fig. 35: HPLC chromatogram for **Gu-petPNA-1(s*)**



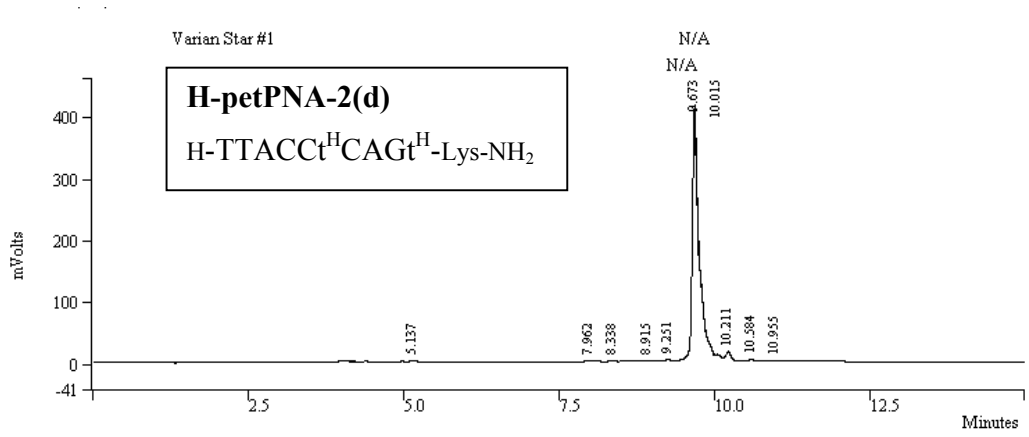
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	0.161	8.2	4322	0.4342
2	8.491	7.6	3737	0.3753
3	8.755	5.5	1909	0.1917
4	9.045	4.1	985526	98.9988
			995494	**100.0000**

Fig. 36: HPLC chromatogram for **PNA-2**



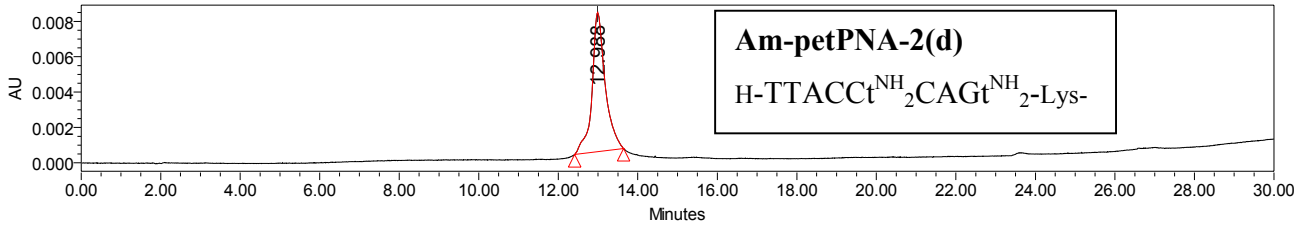
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	8.248	4.5	1280	0.1790
2	9.317	5.4	713738	99.8210
			715018	**100.0000**

Fig. 37: HPLC chromatogram for **H-petPNA-2(s)**



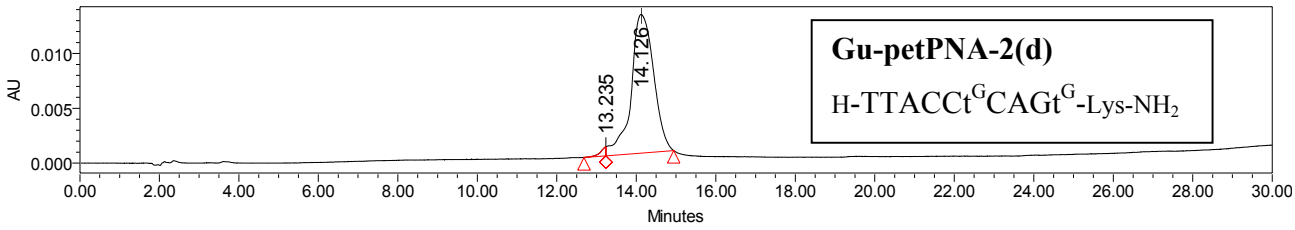
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	5.137	4.8	8074	0.2335
2	7.962	5.9	12045	0.3484
3	8.338	4.9	6379	0.1845
4	8.915	7.3	17218	0.4980
5	9.251	12.2	28734	0.8311
6	9.673	5.4	3305292	95.6013
7	10.015	0.0	7901	0.2285
8	10.211	0.0	58628	1.6957
9	10.584	0.0	11256	0.3256
10	10.955	0.0	1844	0.0533
			3457371	**99.9999**

Fig. 38: HPLC chromatogram for **H-pet PNA-2(d)**



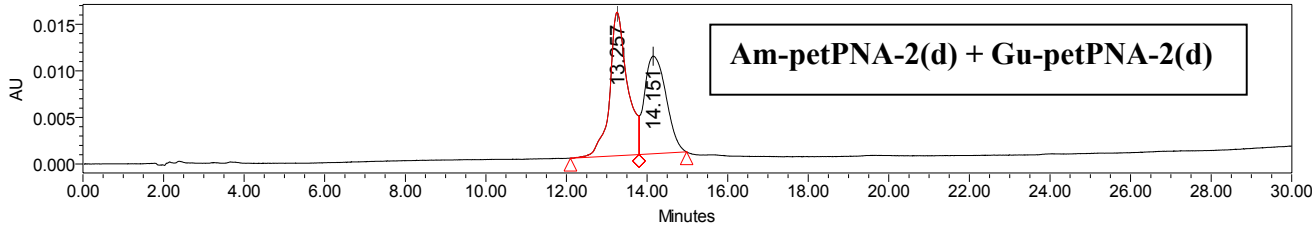
	Name	Retention Time	Area	% Area	Height	Int Type
1		12.988	180969	100.00	7851	BB

Fig. 39: HPLC chromatogram for **Am-petPNA-2(d)**



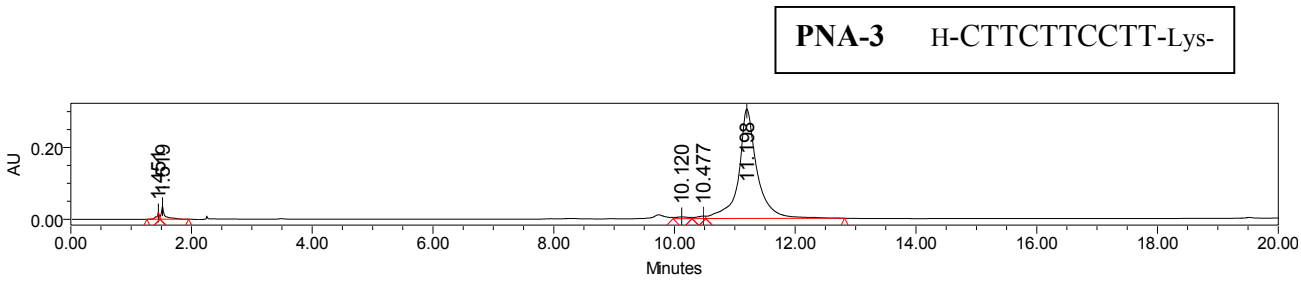
	Name	Retention Time	Area	% Area	Height	Int Type
1		13.235	7454	1.47	826	BV
2		14.126	500307	98.53	12658	VB

Fig. 40: HPLC chromatogram for **Gu-petPNA-2(d)**



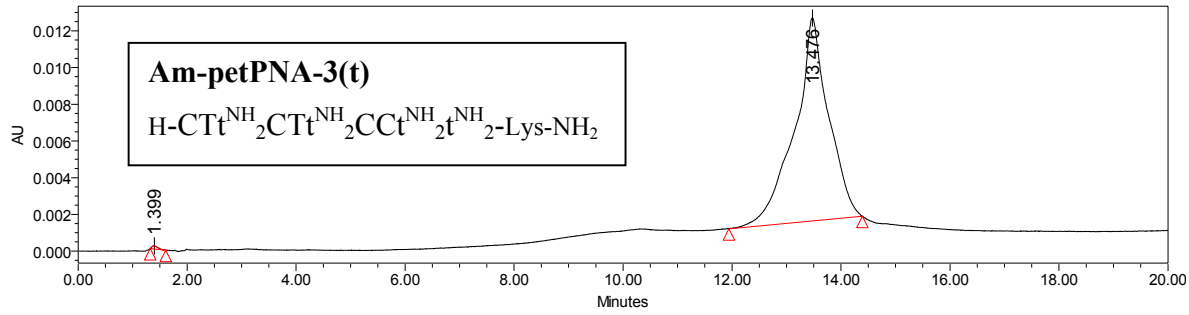
	Name	Retention Time	Area	% Area	Height	Int Type
1		13.257	473221	54.61	15409	BV
2		14.151	393267	45.39	10479	VB

Fig. 41: HPLC chromatogram for **Am-petPNA-2(d) +Gu-petPNA-2(d)**



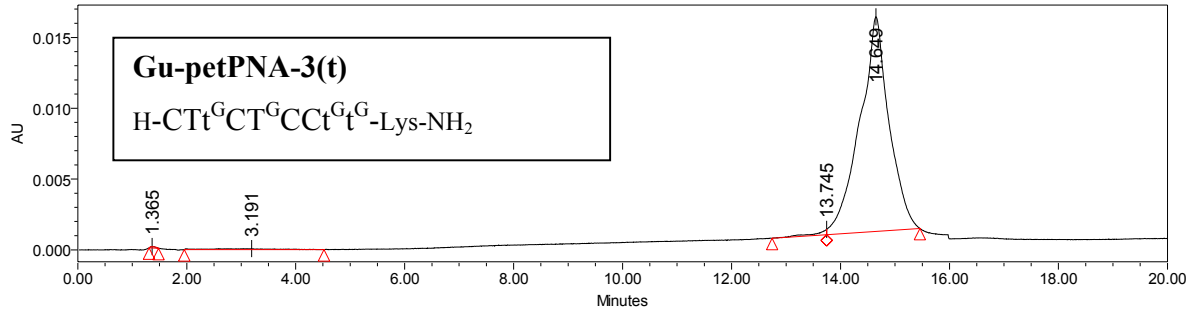
	Name	Retention Time	Area	% Area	Height	Int Type
1		1.451	56443	0.83	18197	BV
2		1.519	153164	2.24	35130	VB
3		10.120	78711	1.15	4946	VV
4		10.477	71552	1.05	6835	VV
5		11.198	6471200	94.73	305003	VB

Fig. 42: HPLC chromatogram for **PNA-3**



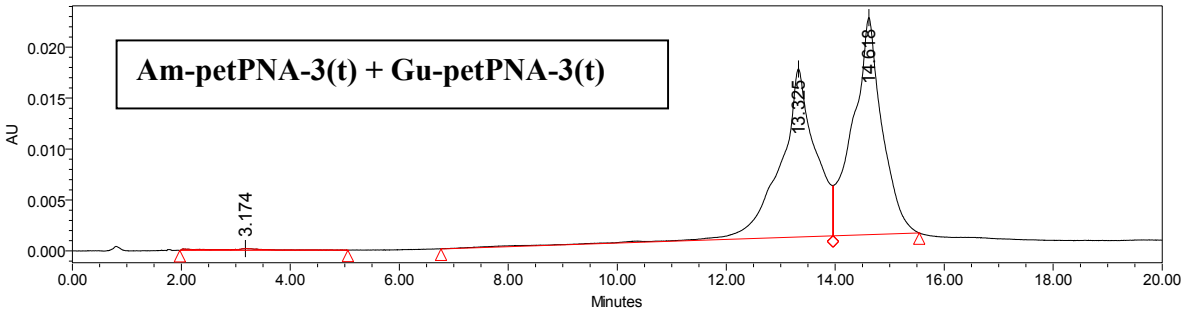
	Name	Retention Time	Area	% Area	Height	Int Type
1		1.399	1277	0.27	165	BB
2		13.476	478492	99.73	11055	BB

Fig. 43: HPLC chromatogram for **Am-petPNA-3(t)**



	Name	Retention Time	Area	% Area	Height	Int Type
1		1.365	700	0.12	105	BB
2		3.191	5183	0.90	70	BB
3		13.745	5278	0.91	375	BV
4		14.649	565951	98.07	15153	VB

Fig. 44: HPLC chromatogram for **Gu-petPNA-3(t)**



	Name	Retention Time	Area	% Area	Height	Int Type
1		3.174	12032	0.78	176	BB
2		13.325	739822	47.81	16480	BV
3		14.618	795616	51.41	21296	VB

Fig. 45: HPLC chromatogram for **Am-petPNA-3(t)** + **Gu-petPNA-3(t)**

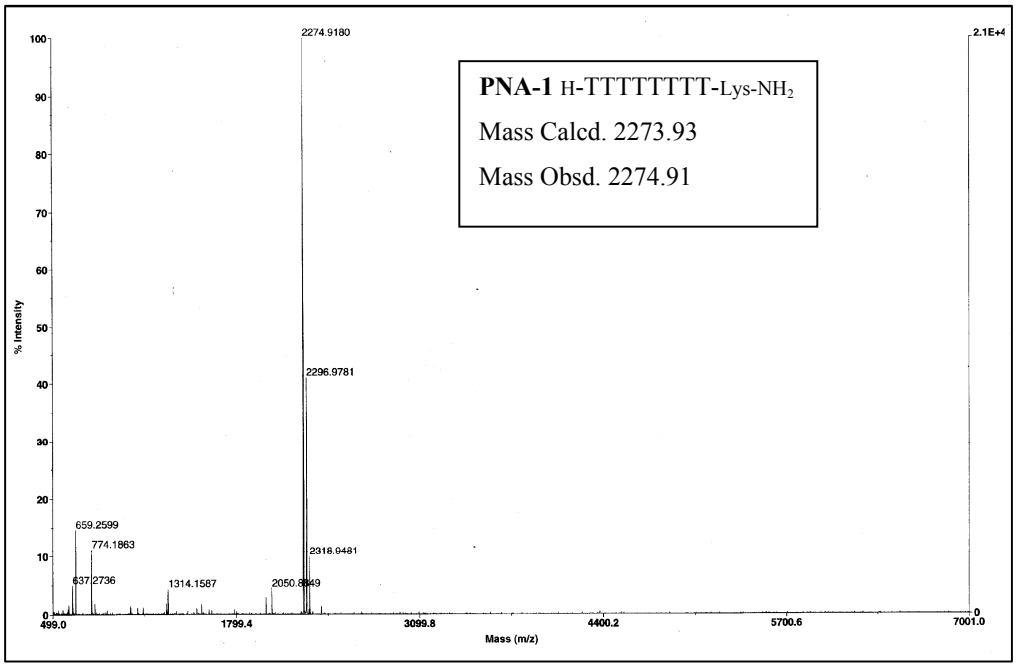


Fig. 46: MALDI-TOF spectra for **PNA-1**

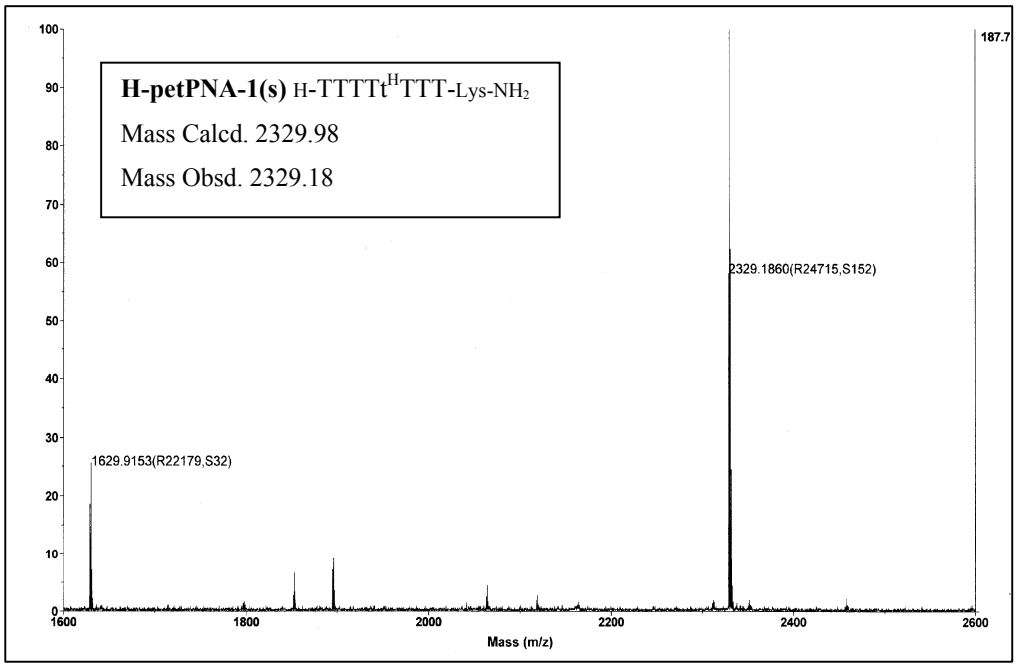


Fig. 47: MALDI-TOF spectra for **H-petPNA-1(s)**

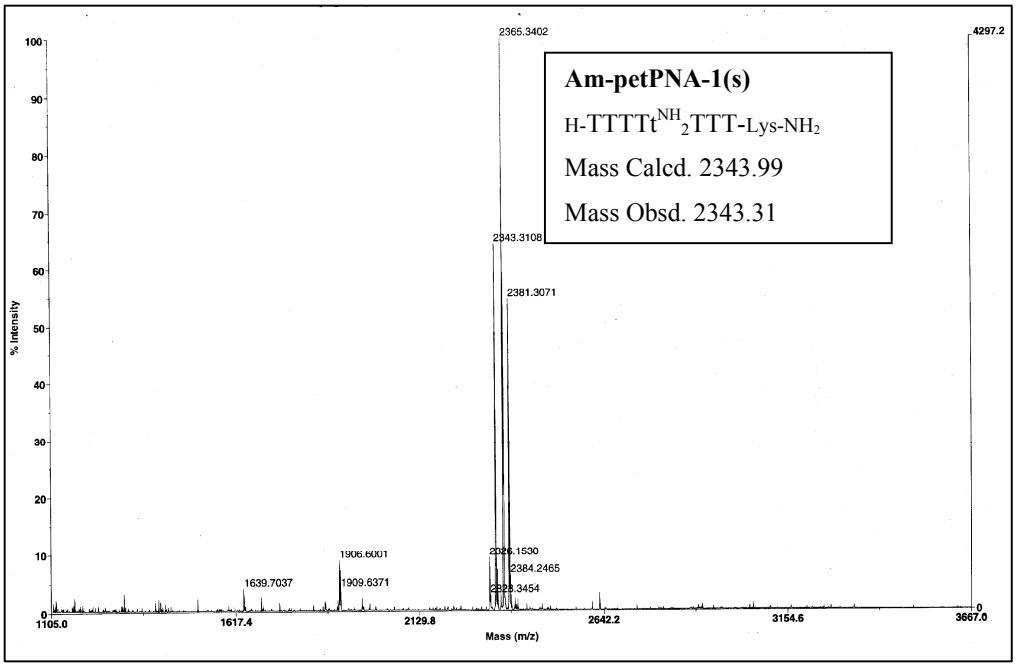


Fig. 48: MALDI-TOF spectra for **Am-petPNA-1(s)**

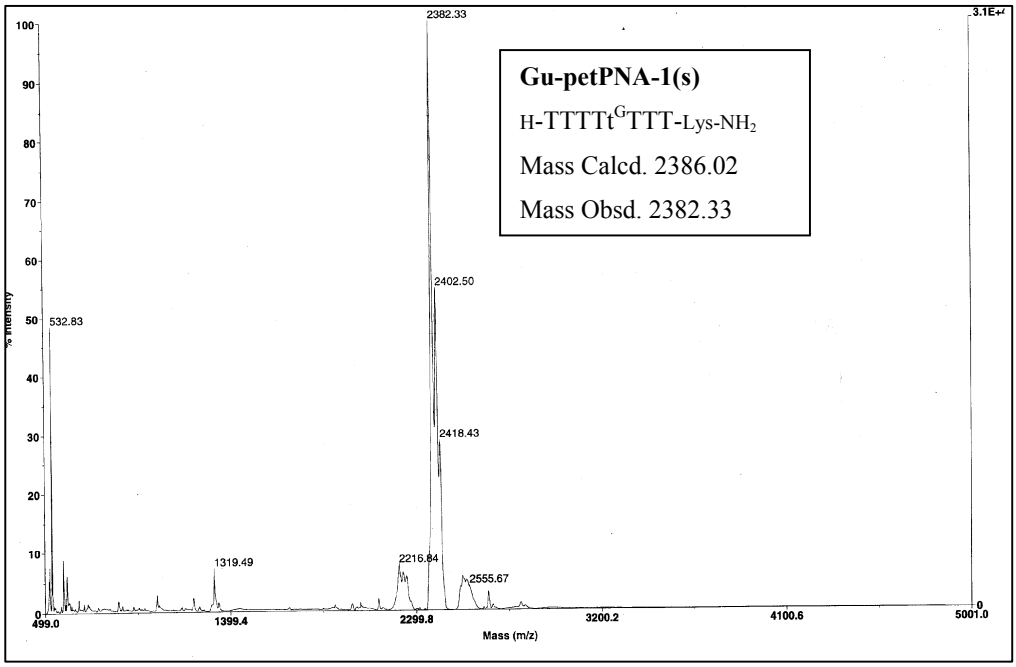


Fig. 49: MALDI-TOF spectra for **Gu-petPNA-1(s)**

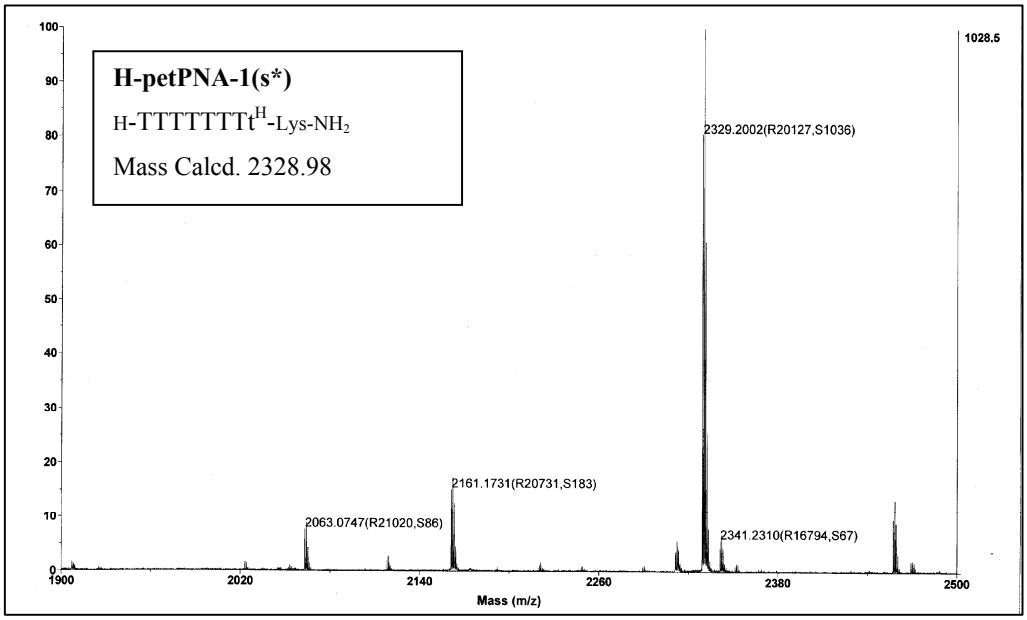


Fig. 50: MALDI-TOF spectra for **H-petPNA-1(s*)**

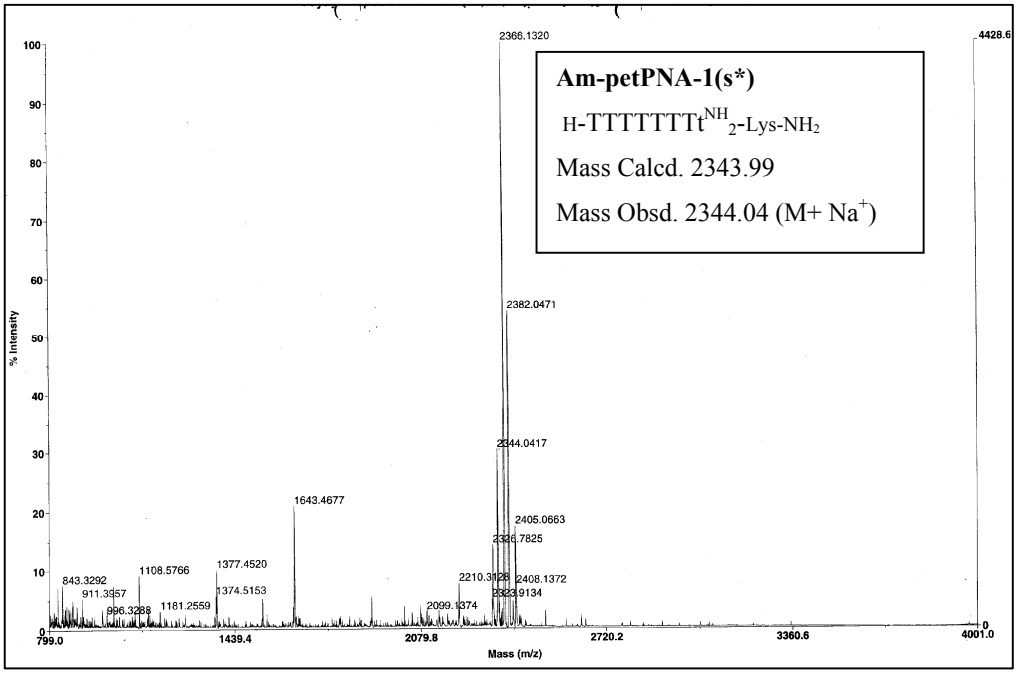


Fig. 51: MALDI-TOF spectra for **Am-petPNA-1(s*)**

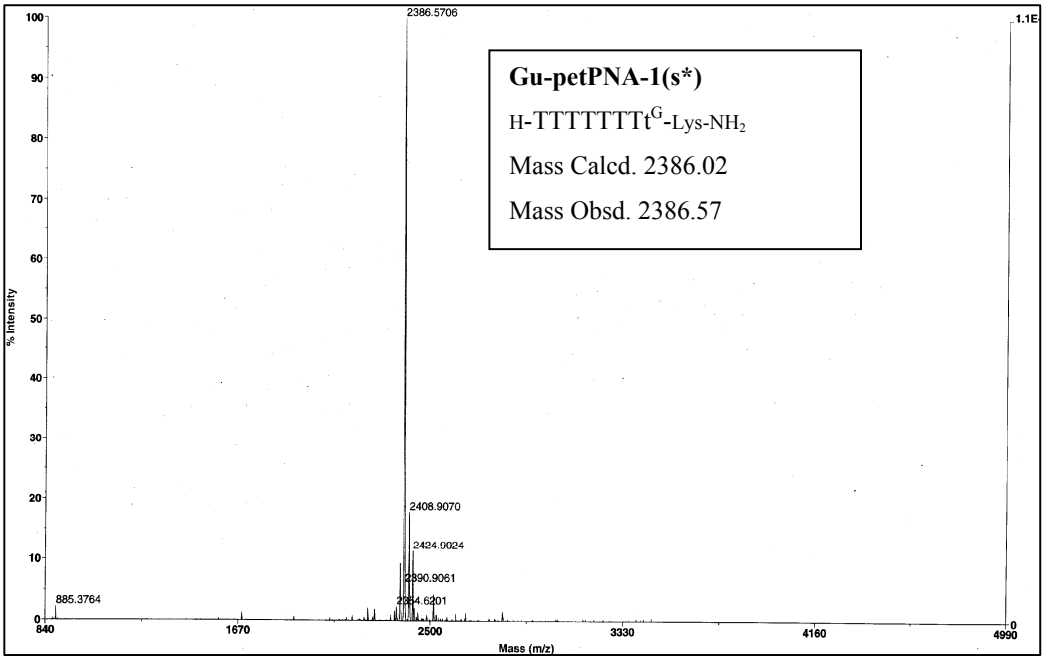


Fig. 52: MALDI-TOF spectra for **Gu-petPNA-1(s*)**

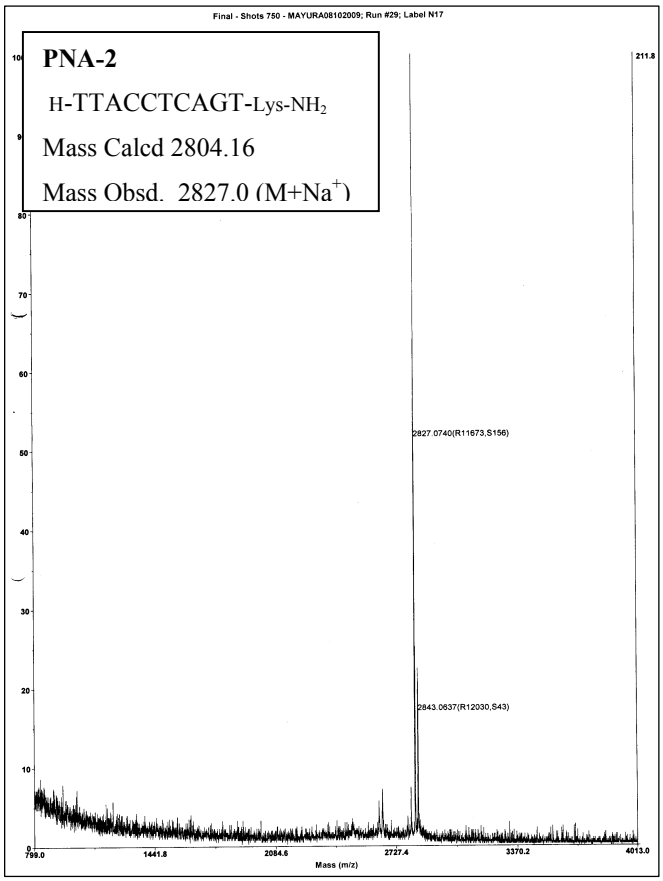


Fig. 53: MALDI-TOF spectra for **PNA-2**

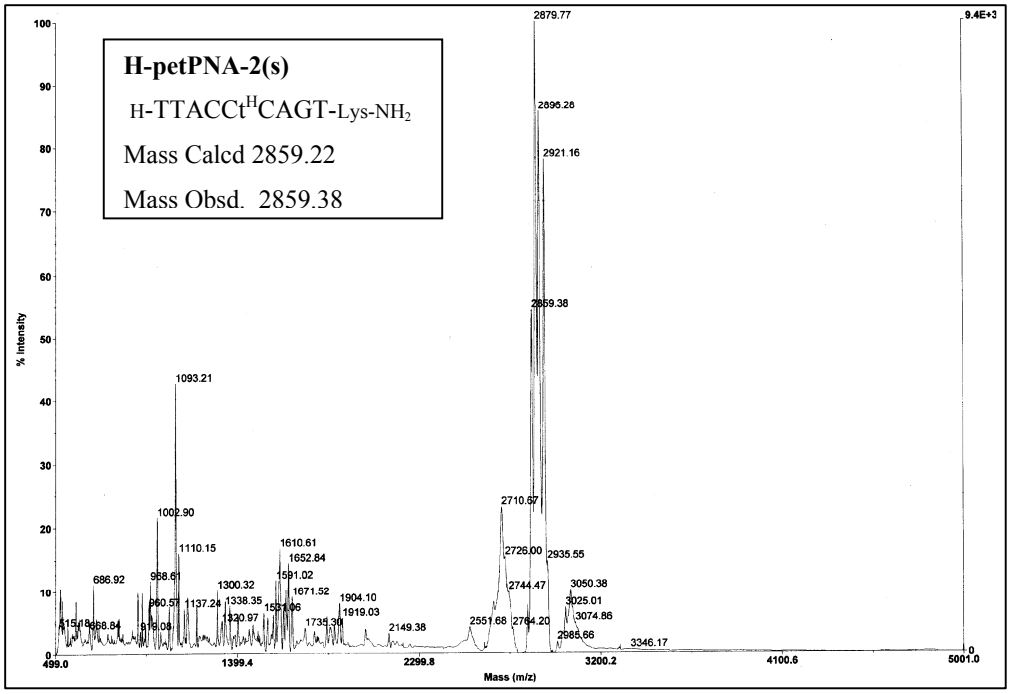


Fig. 54: MALDI-TOF spectra for **H-petPNA-2(s)**

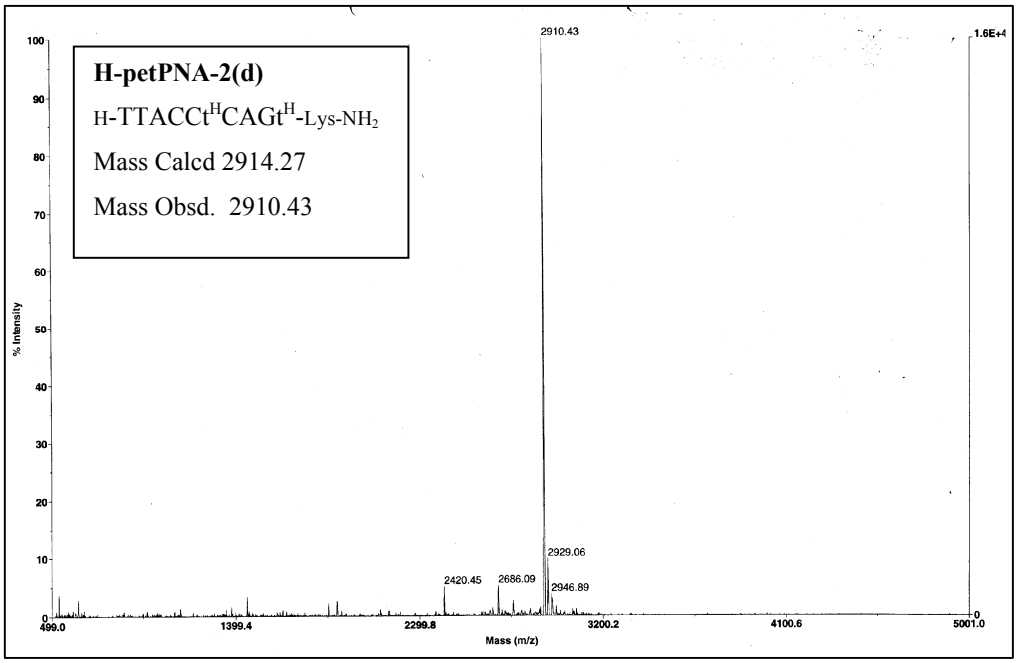


Fig. 55: MALDI-TOF spectra for **H-petPNA-2(d)**

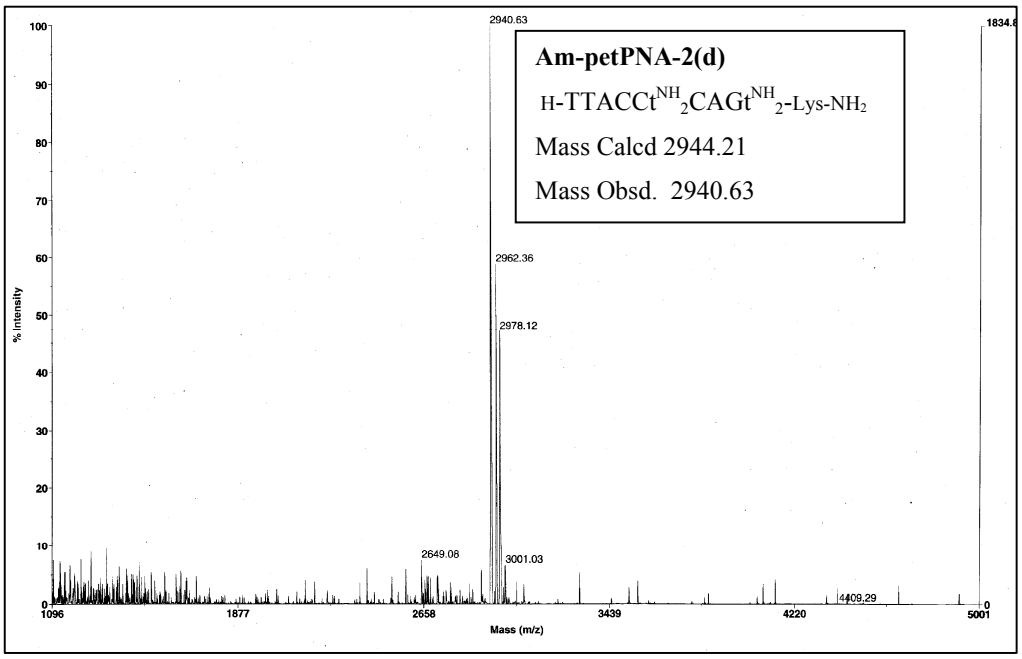


Fig. 56: MALDI-TOF spectra for **Am-petPNA-2(d)**

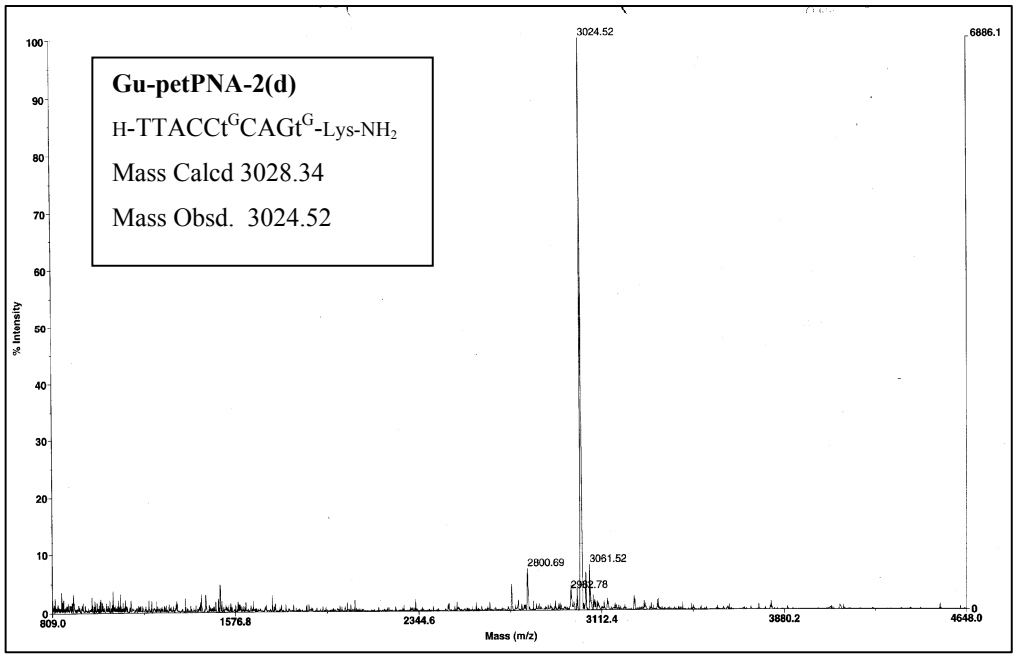


Fig. 57: MALDI-TOF spectra for **Gu-petPNA-2(d)**

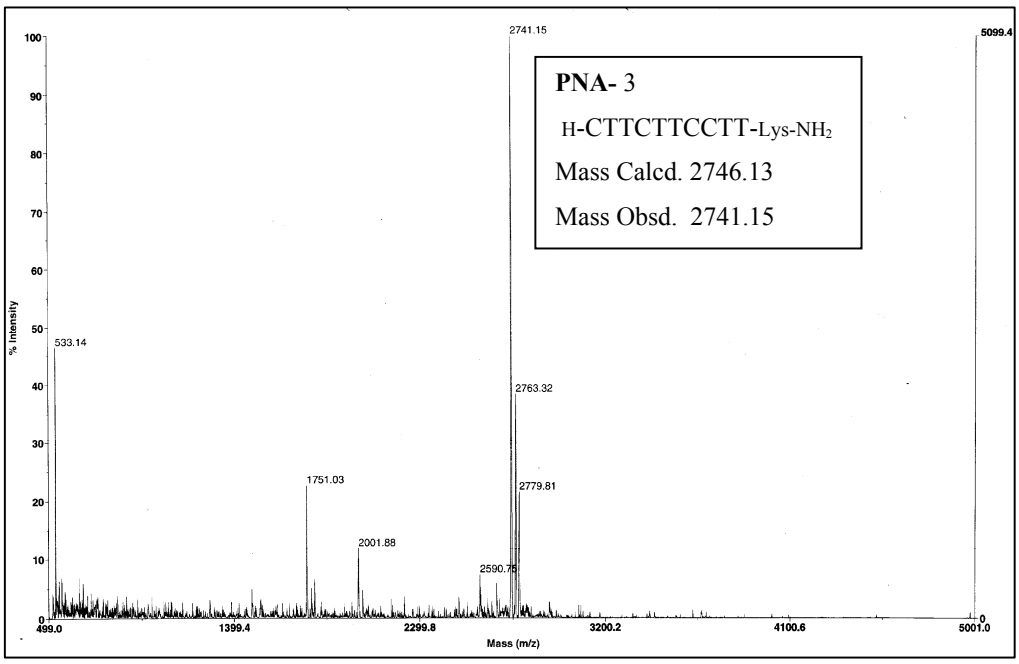


Fig. 58: MALDI-TOF spectra for **PNA-3**

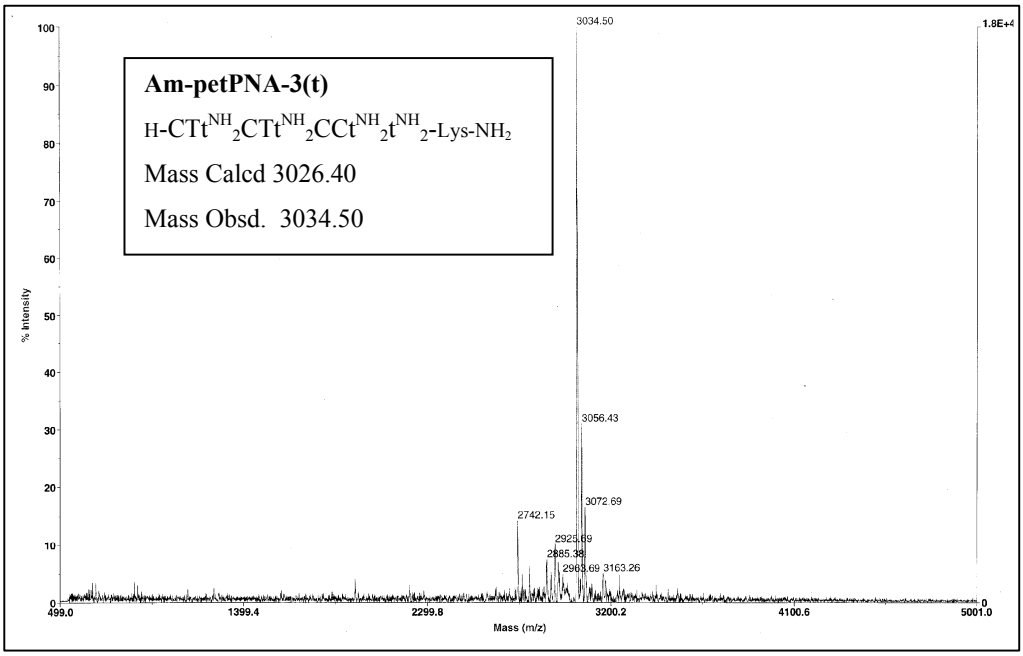


Fig. 59: MALDI-TOF spectra for **Am-petPNA-3(t)**

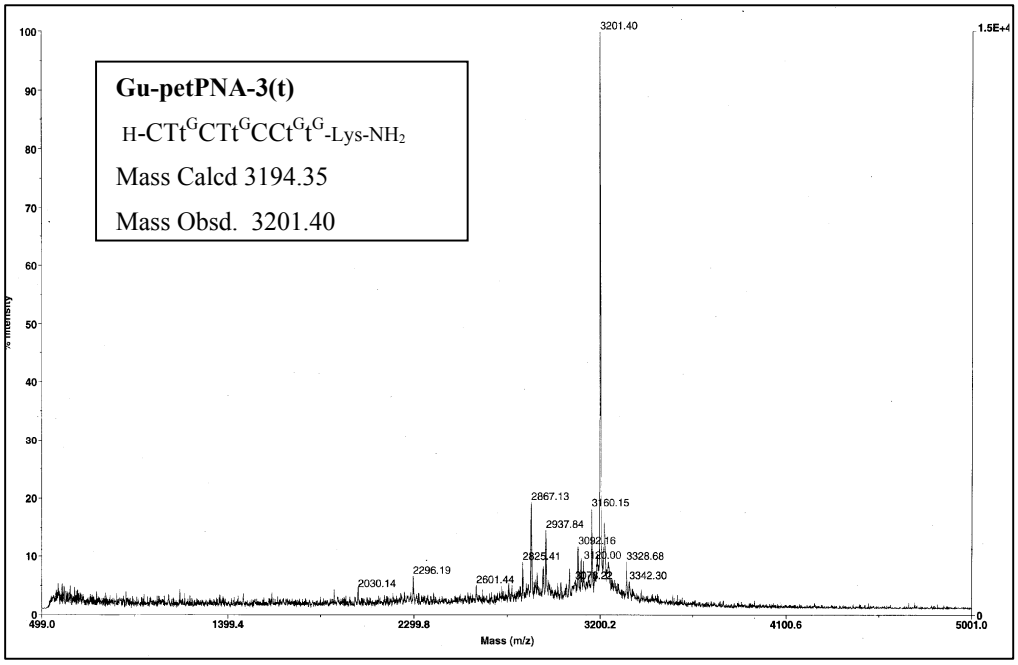


Fig. 60: MALDI-TOF spectra for **Gu-petPNA-3(t)**

UV-*Tm* measurements:

Entry	Sequence	DNA-1 (DNA-3)	RNA-1 (RNA-3)
PNA-1.	H-TTTTTTTT-Lys-NH_2	41.6 (30.0)	41.2 (29.8)
	H-TTTTt^{R}TTT-Lys-NH_2		
H-petPNA-1(s)	R= H	27.2 (nd)	37.4(16.5)
Am-petPNA-1(s)	R= NH_2	40.7 (19.4)	36.8(18.2)
Gu-petPNA-1(s)	R= NH-C(= NH) NH_2	36.01 (21.4)	36.4(18.9)

DNA-1: 5'GCAAAAAAACG 3' **RNA-1**: 5'GCAAAAAAACG 3'
DNA-3: 5'GCAAATAAACG 3' **RNA-3**: 5'GCAAAUAAACG

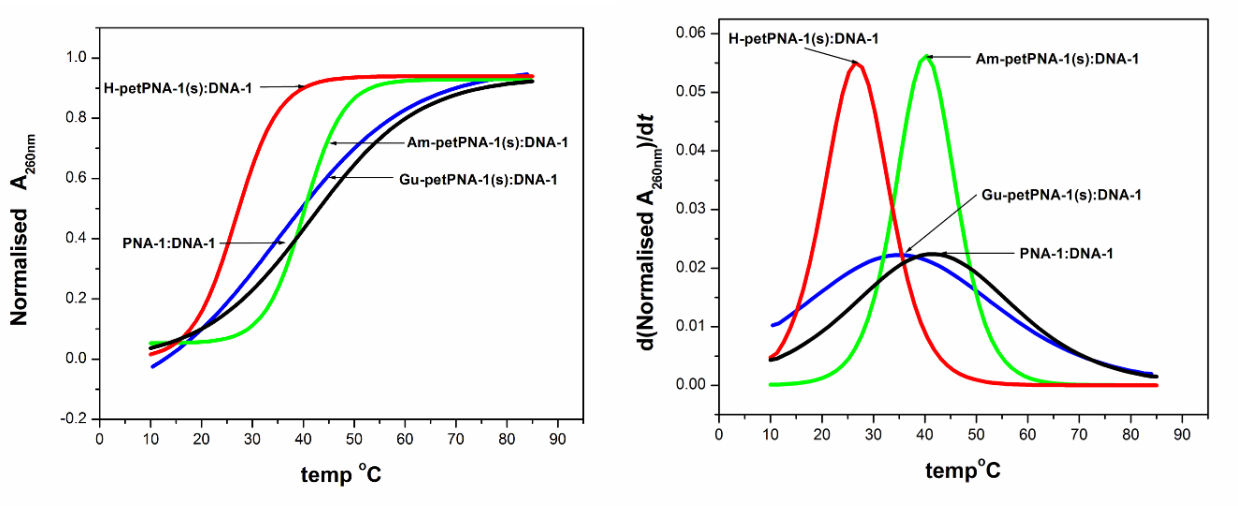


Fig. 61: A UV melting Curves of **PNA-1-Gu-petPNA-1(s)** with DNA-1 and **B** corresponding derivative graphs

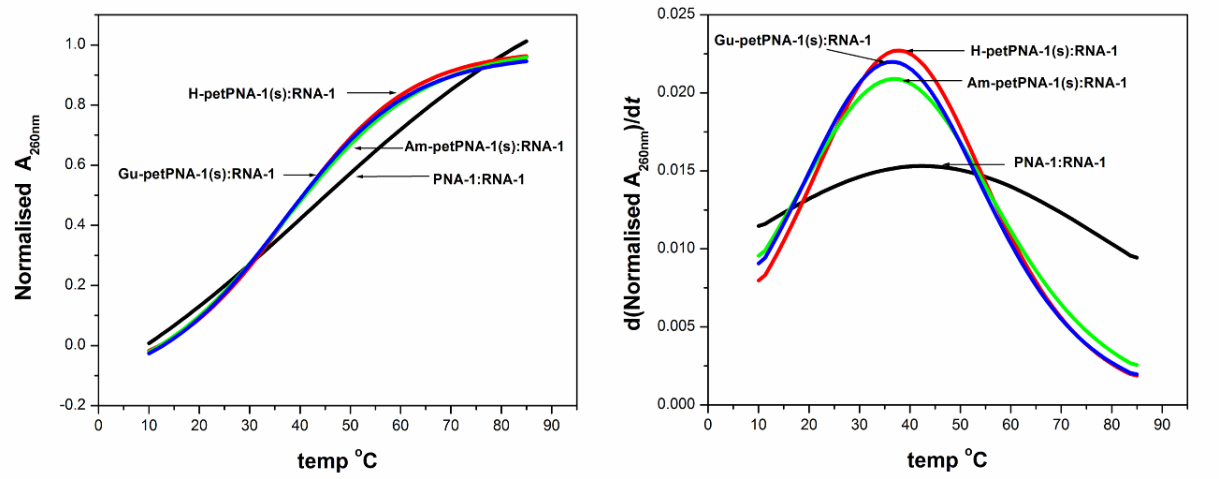


Fig. 62: A UV melting Curves of **PNA-1-Gu-petPNA-1(s)** with **RNA-1** and **B** corresponding derivative graphs

Entry	Sequence	DNA-1 (DNA-3)	RNA-1 (RNA-3)
PNA-1.	H-TTTTTTTT-Lys-NH_2	41.6 (30.0)	41.2 (29.8)
	H-TTTTTTTt^R-Lys-NH_2		
H-petPNA-1(s*)	R= H	26.7 (nd)	48.7(20.9)
Am-petPNA-1(s*)	R= NH_2	45.7 (26.8)	49.9(28.5)
Gu-petPNA-1(s*)	R= NH-C(= NH) NH_2	51.6 (32.6)	58.7(31.8)

DNA-1: 5'GCAAAAAAACG 3' **RNA-1**: 5'GCAAAAAAACG 3'
DNA-3: 5'GCAAATAAACG 3' **RNA-3**: 5'GCAAAUAAACG

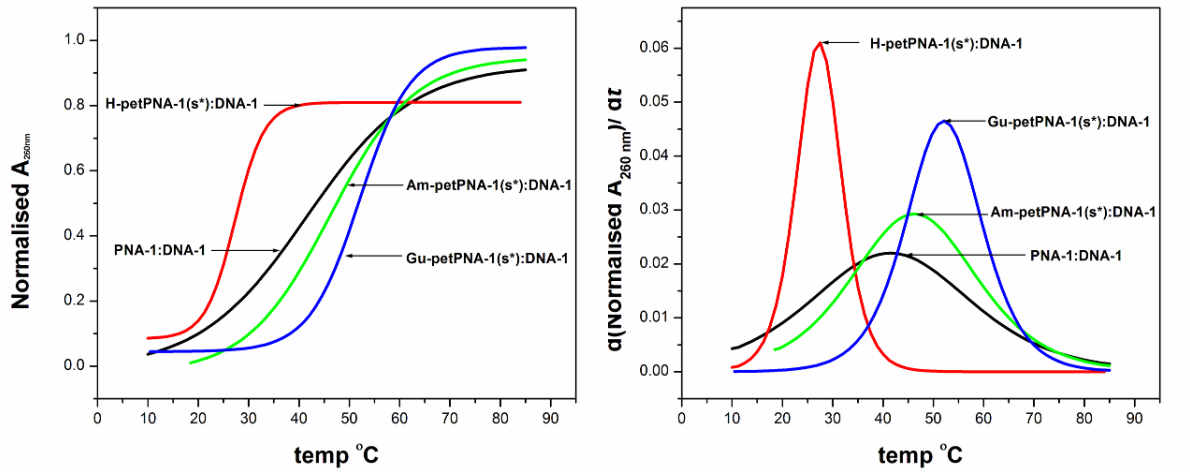


Fig. 63: A UV melting Curves of **PNA-1-Gu-petPNA-1(s*)** with **DNA-1** and **B** corresponding derivative graphs

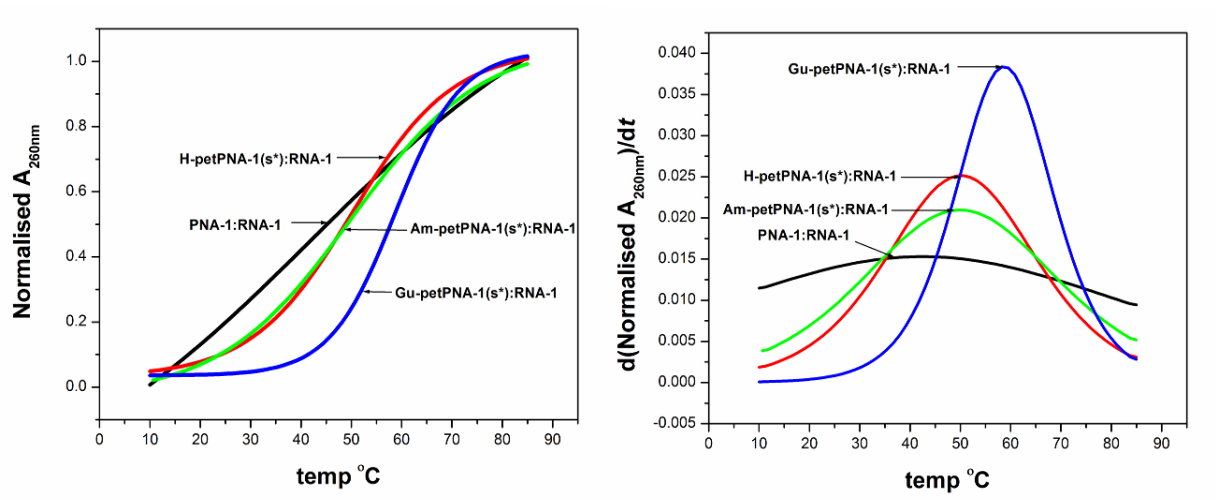


Fig. 64: A UV melting Curves of **PNA-1-Gu-petPNA-1(s*)** with **RNA-1** and **B** corresponding derivative graphs

Entry	Sequence	DNA-2 (DNA-4)	RNA-2 (RNA-4)	*p*DNA-5 (ΔT_m= *ap-p*)
PNA-2	H-TTACCTCAGT-Lys-NH_2	54.2 (47.5)	54.5(46.5)	53.9 (+0.3)
H-pet-PNA-2(s)	H-TTACCtCAGT-Lys-NH_2 R= H	57.6 (51.2)	52.6 (38.8)	55.4 (+1.8)
	H-TTACCt^{R}CAGt^R-Lys-NH_2			
H-pet-PNA-2(d)	R= H	63.2 (50.8)	48.1 (34.5)	59.3 (+3.9)
Am-pet-PNA-2(d)	R= NH_2	66.9 (58.1)	52.1 (43.8)	59.5 (+7.4)
Gu-pet-PNA-2(d)	R= NH-C(= NH) NH_2	68.9 (60.0)	54.2 (46.1)	59.9 (+9.0)

DNA-2: 5' ACTGAGGTAA3' RNA-2: 5' UGUAACUGAGGUAAGAGG 3'
DNA-4: 5' ACTG**T**GGTAA3' RNA-4: 5' UGUAACUGCGGUAAGAGG
DNA-5: 5' AATGGAGTCA 3'

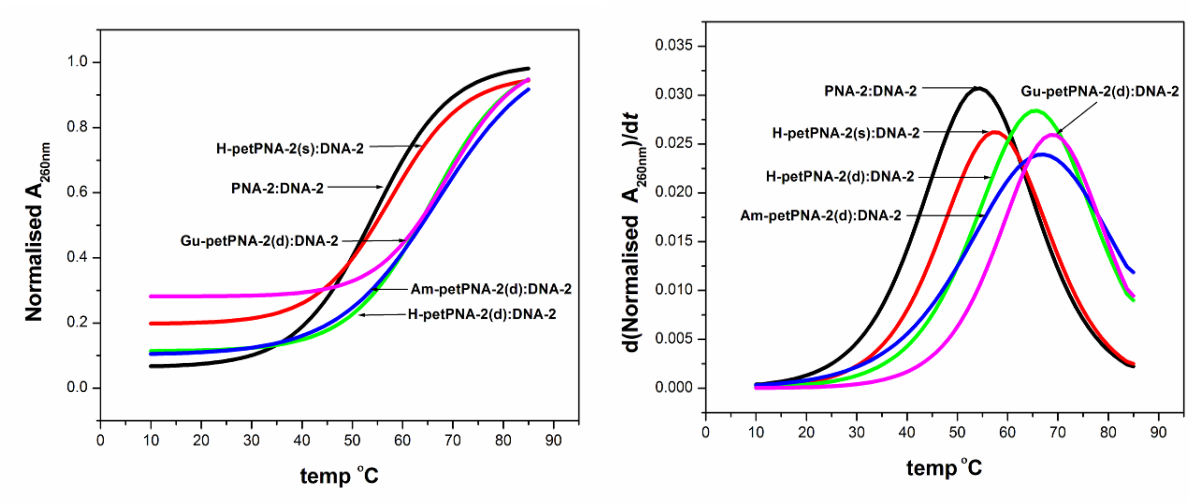


Fig. 65: **A** UV melting Curves of **PNA-2-Gu-petPNA-2(d)** with **DNA-2** and **B** corresponding derivative graphs

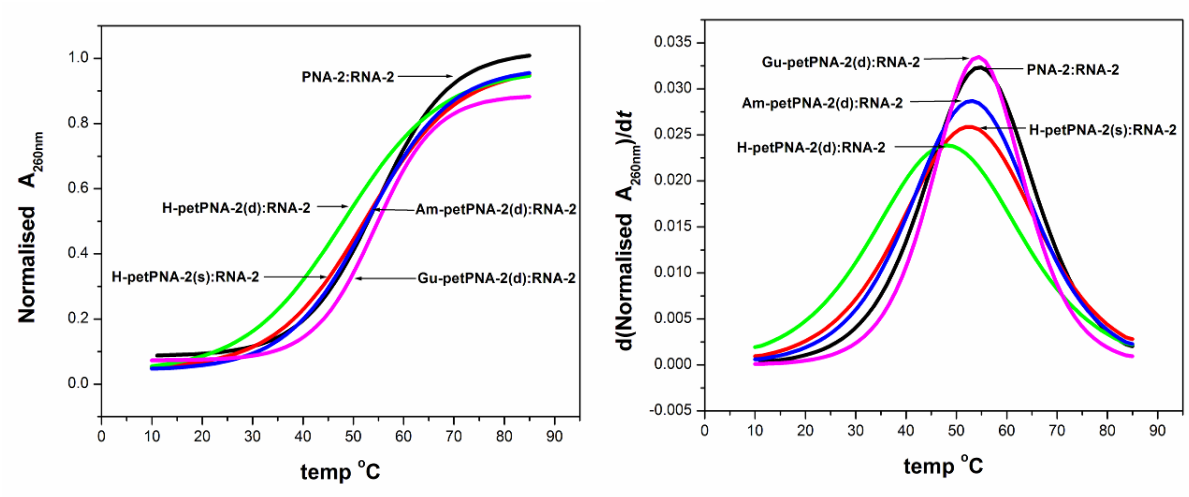


Fig. 66: **A** UV melting Curves of **PNA-2-Gu-petPNA-2(d)** with **RNA-2** and **B** corresponding derivative graphs

Entry	Sequence	DNA-1 (DNA-3)	RNA-1 (RNA-3)
PNA-3	H-CTTCTTCCTT-Lys-NH_2	52.0 (42.6)	55.6 (45.2)
Am-petPNA-3(t)	H- $CTt^{R}CTt^{R}CCt^{R}t^{R}$-Lys-$NH_2$ R= NH_2	63.7(49.9)	65.5(57.6)
Gu-petPNA-3(t)	R= NH-C(= NH) NH_2	64.8 (54.5)	68.3(59.2)

DNA -6: 5'AAGGAAGAAG 3' RNA-6: 5' AAGGAAGAAG 3'
DNA 7: 5' AAGGTAGAAG 3' RNA 7: AAGGUAGAAG 3'

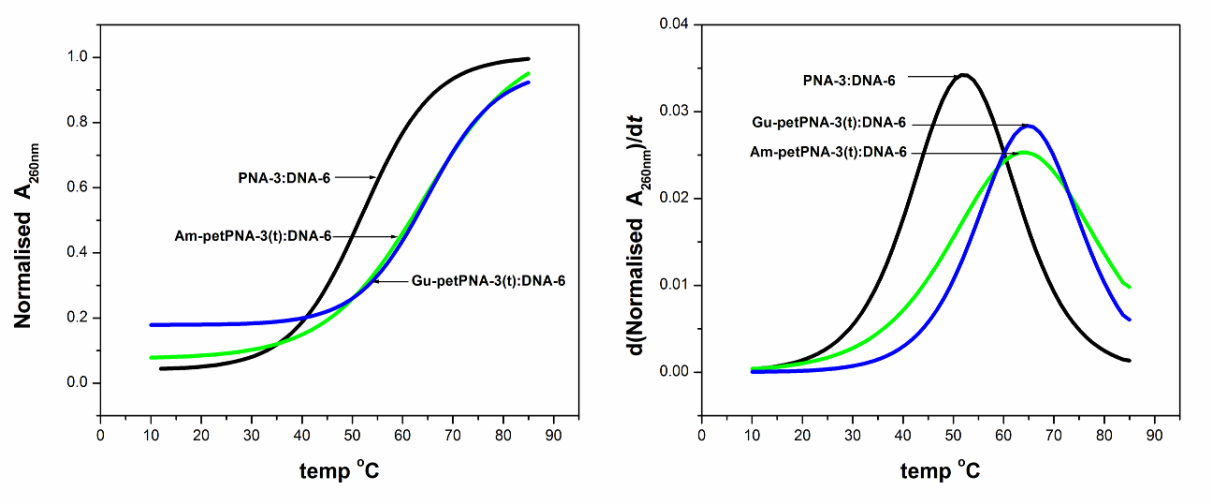


Fig. 67: **A** UV melting Curves of **PNA-3-Gu-petPNA-3(t)** with **DNA-6** and **B** corresponding derivative graphs

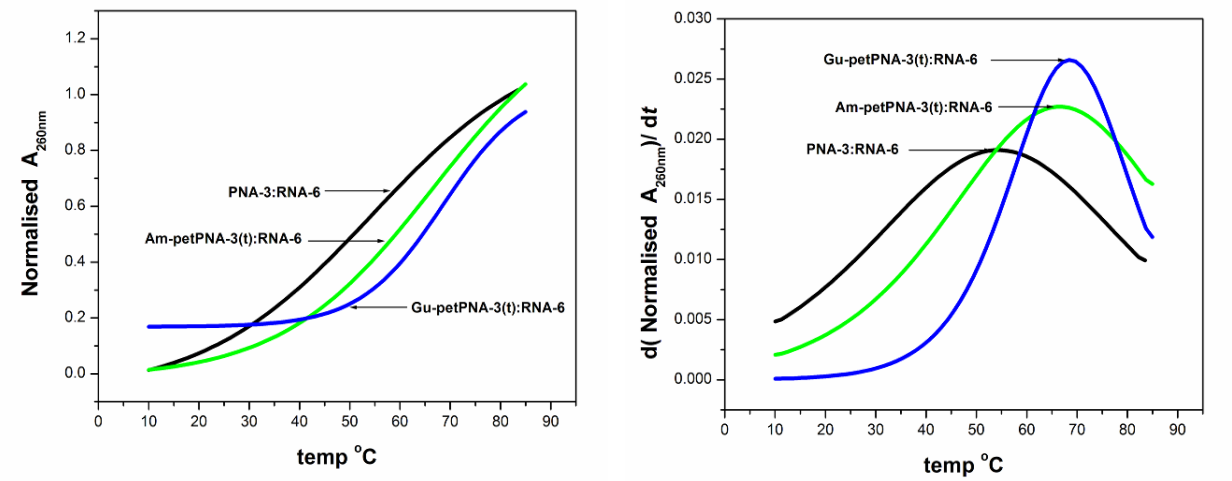


Fig. 68: **A** UV melting Curves of **PNA-3-Gu-petPNA-3(t)** with **RNA-6** and **B** corresponding derivative graphs