Supporting Information

Synthesis and Characterization of Conformationally-Preorganized, MiniPEG-Containing γPNAs with Superior Hybridization Properties and Water Solubility

*Bichismita Sahu, Iulia Sacui, Srinivas Rapireddy, Kimberly J. Zanotti, Raman Bahal, Bruce A. Armitage and Danith H. Ly**

Department of Chemistry and Center for Nucleic Acids Science and Technology (CNAST),

Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15213.

Figure S1. UV-absorption profiles of saturated solutions of PNA11-13 after serial dilutions. The result shows that PNA12, which contained a single unit of L-alanine-derived γPNA, is more water soluble than the parent oligomer (PNA11). When fully-modified (PNA13), methylene group installed at every γ-backbone position, significantly decreased compared to PNA10 and PNA11.

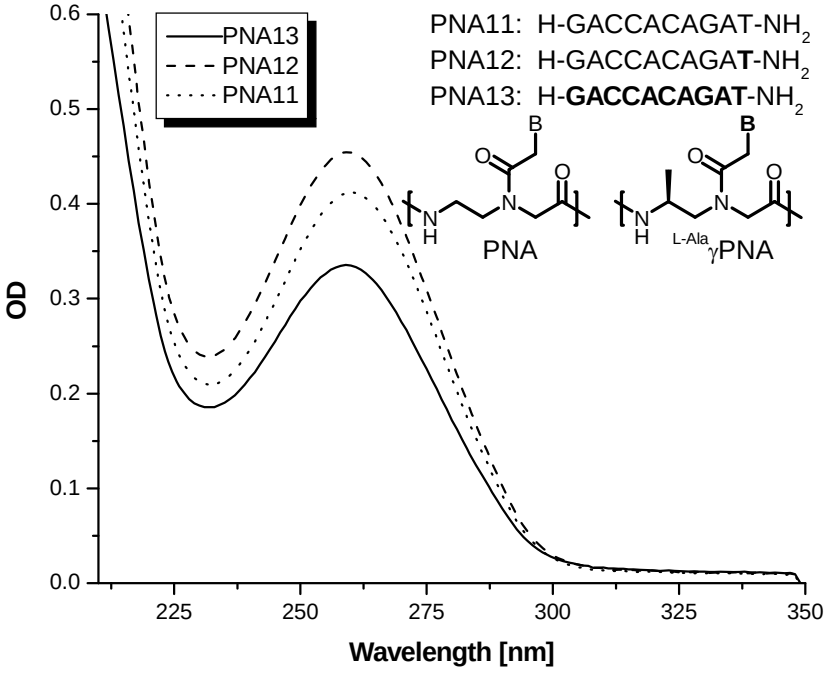


Figure S2. ^{1}H-NMR spectrum of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine (**2**)

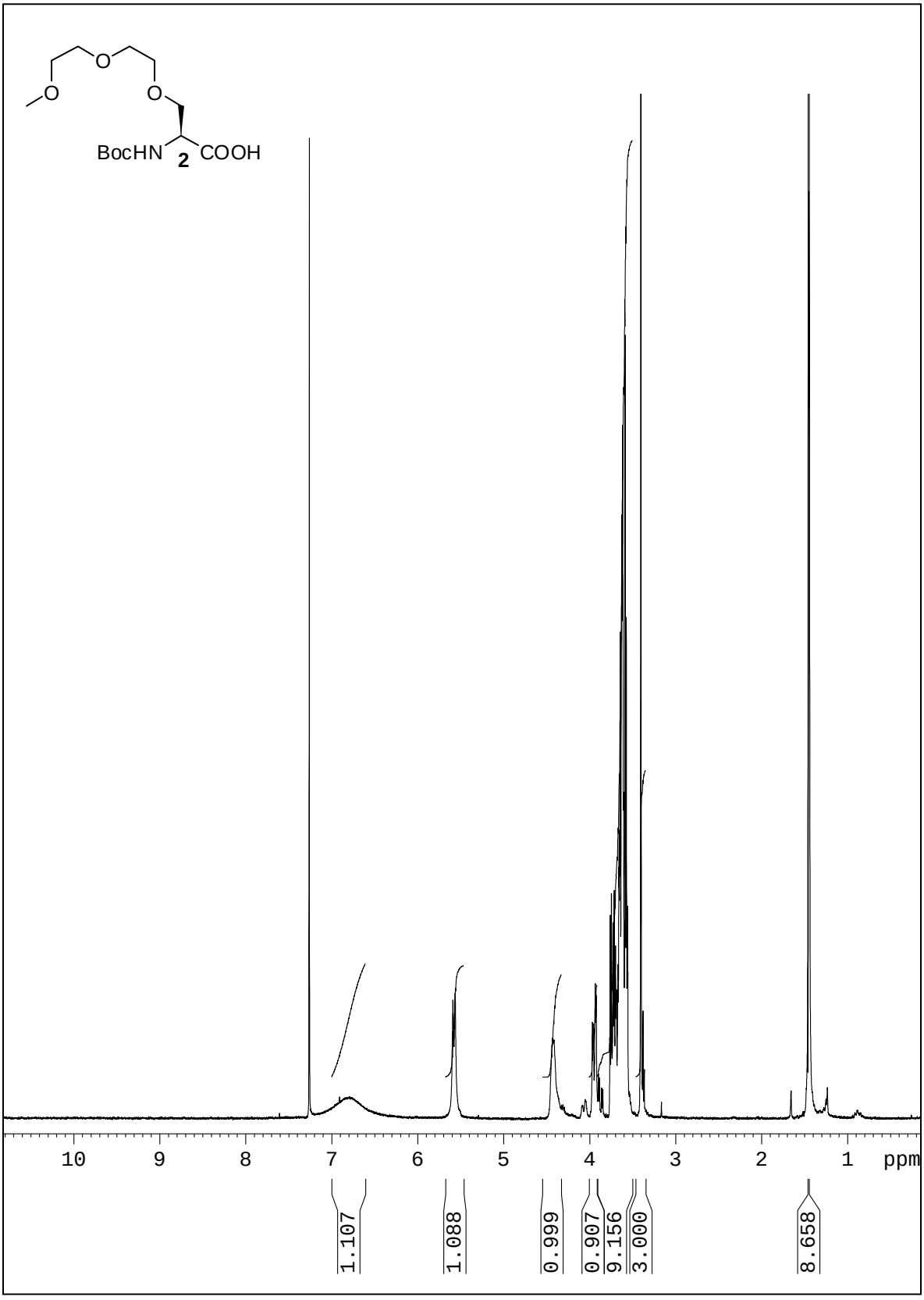
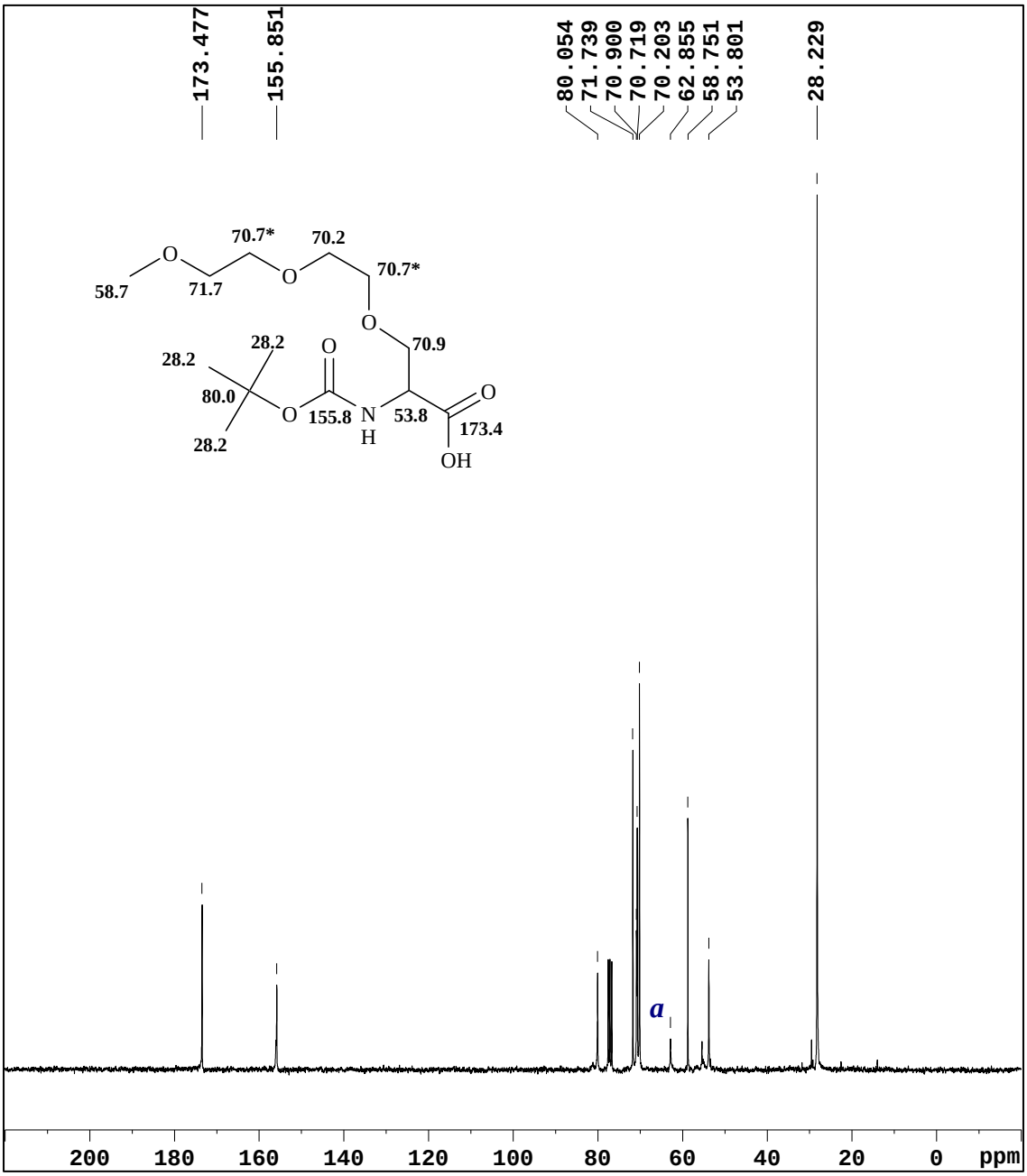


Figure S3. ^{13}C-NMR of Boc-(2(-2-methoxyethoxy)ethyl)-L-serine (**2**)



* Peaks are merged together

a Impurity peak

Figure S4. ^{1}H-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine-ol (**3**)

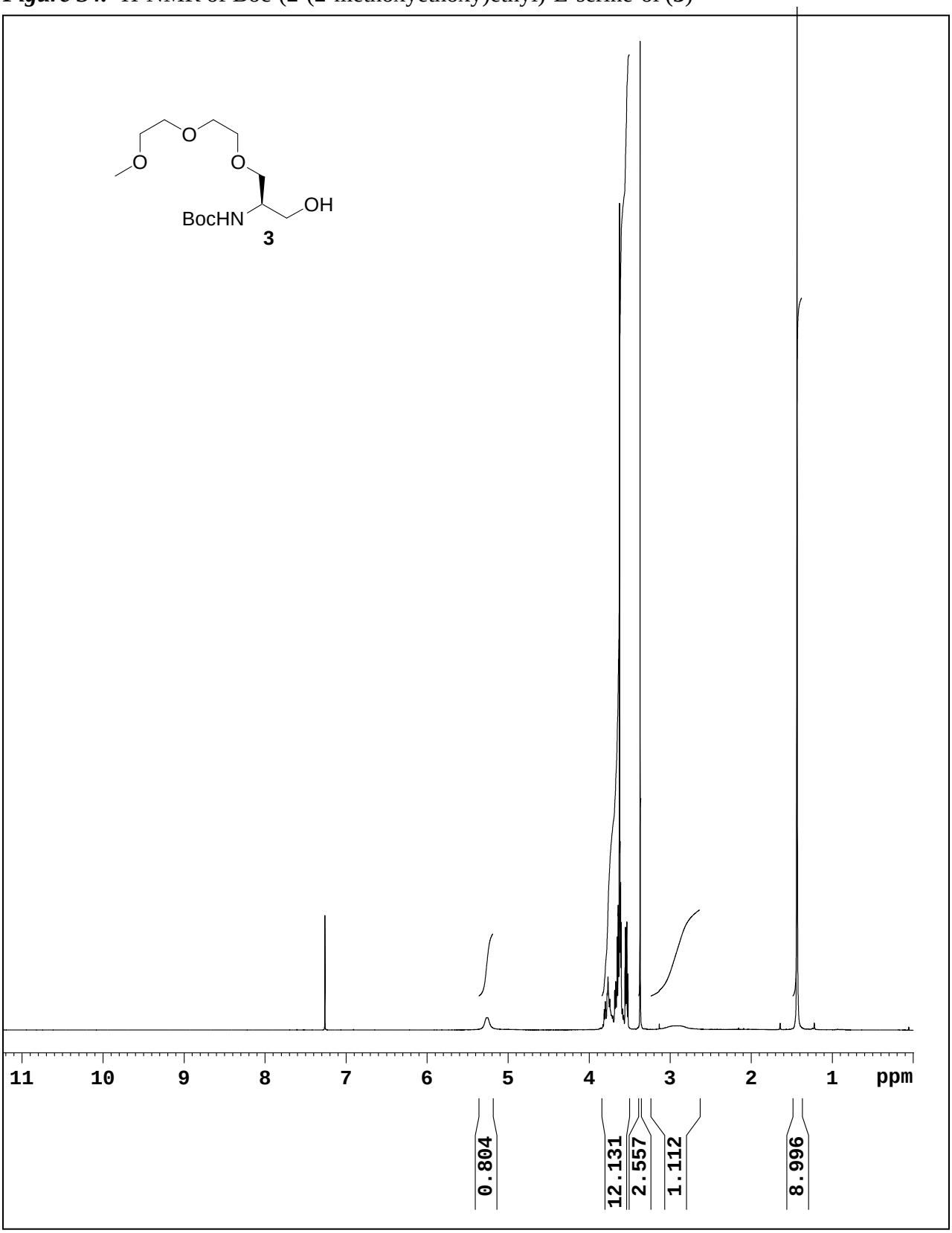
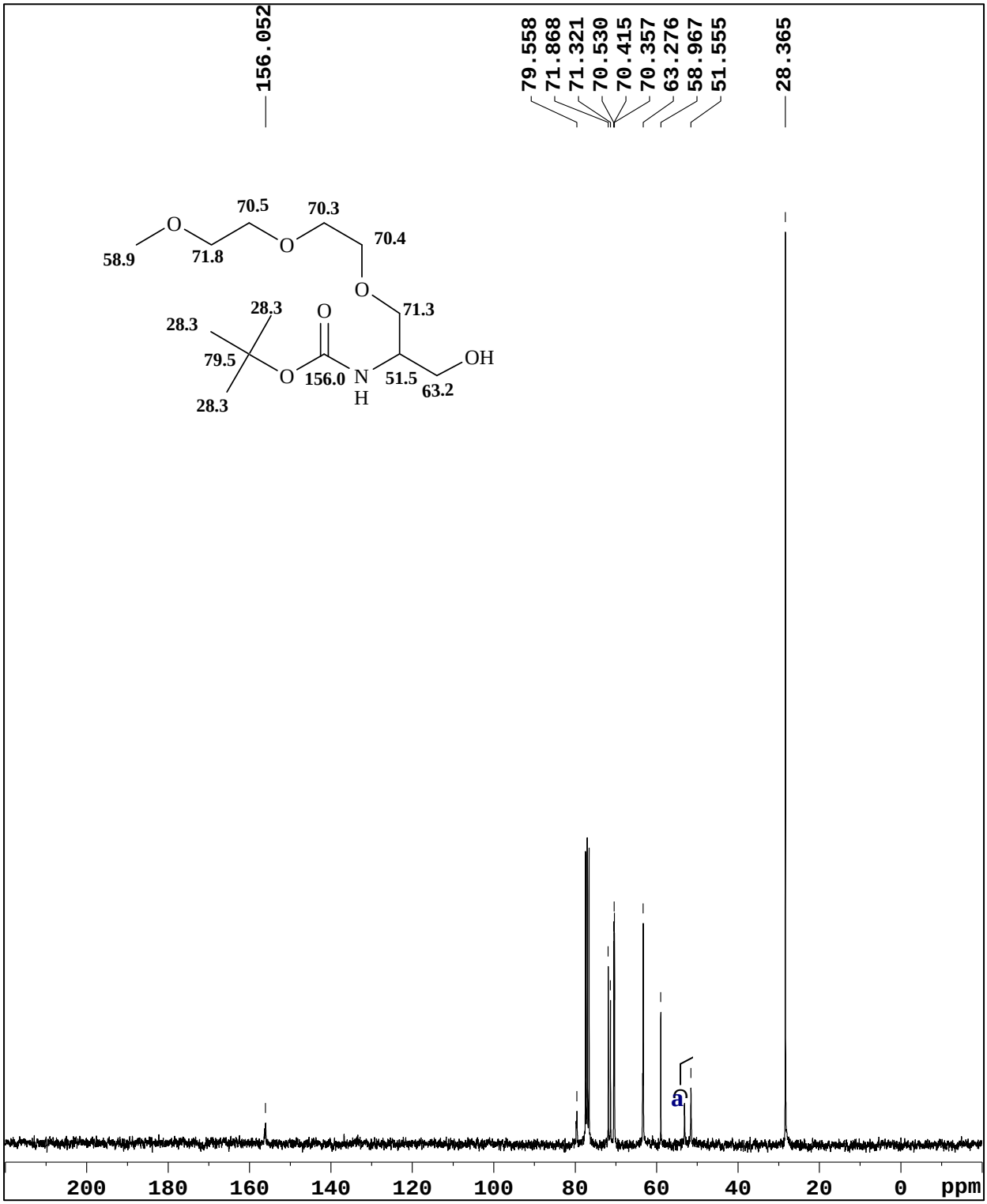


Figure S5. ^{13}C-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine-ol (**3**)



a Impurity peak

Figure S6. ^{1}H-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine -Ψ[CH_2N(o,p-diNBS)]Gly-OEt (**4**)

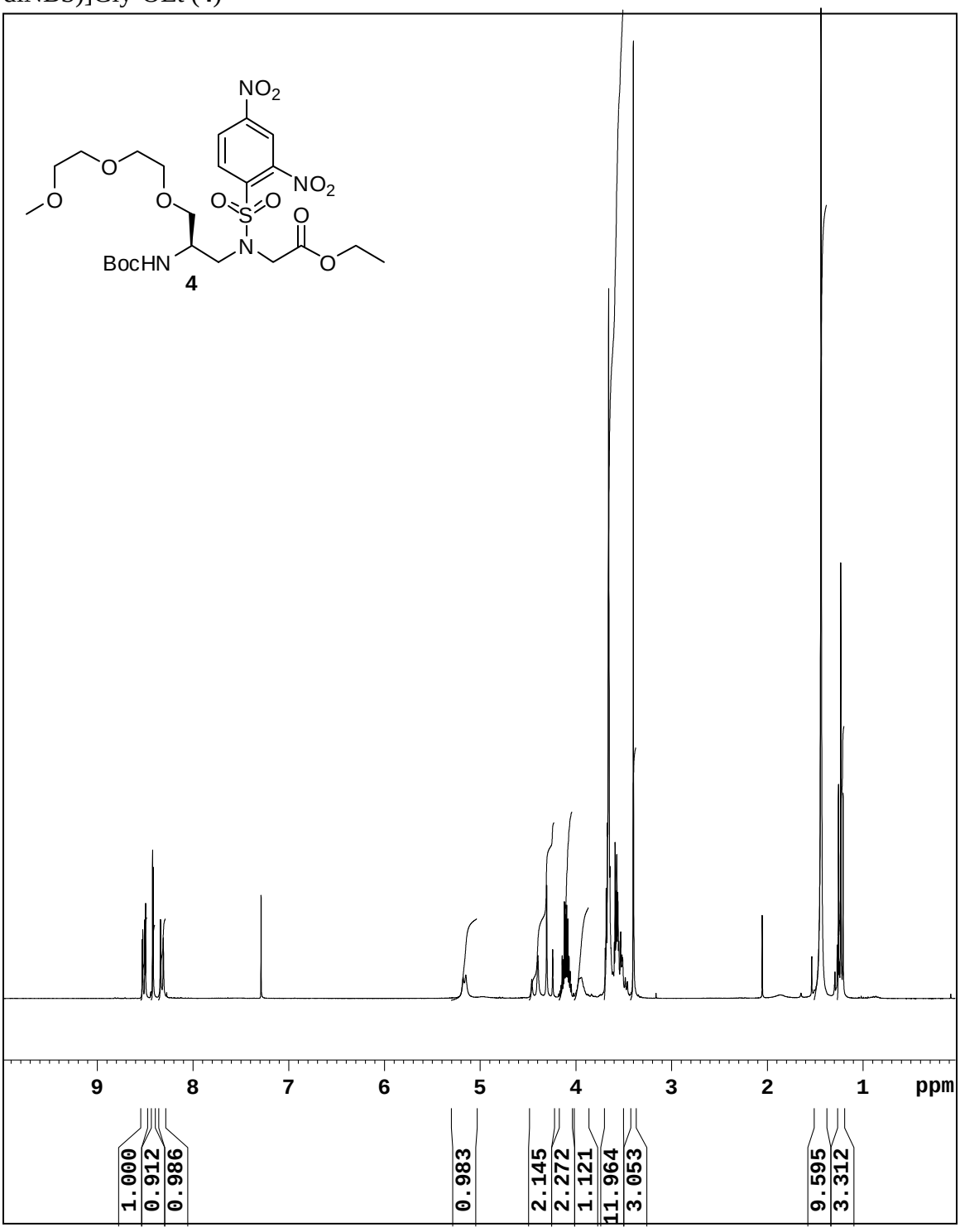


Figure S7. ^{13}C-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine -Ψ[CH_2N(o,p-diNBS)]Gly-OEt (**4**)

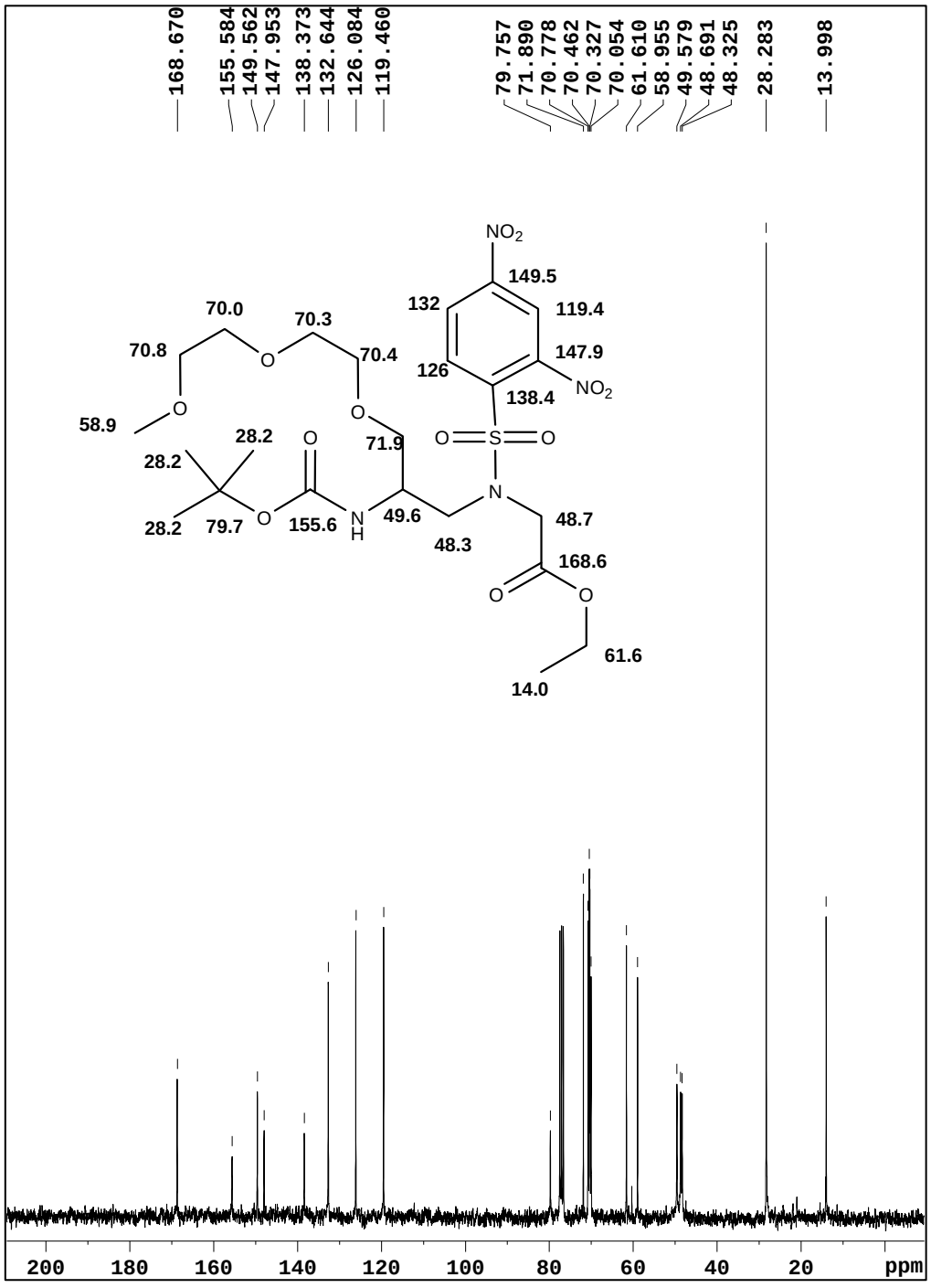


Figure S8. ^{1}H-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine backbone ethyl ester (**5**)

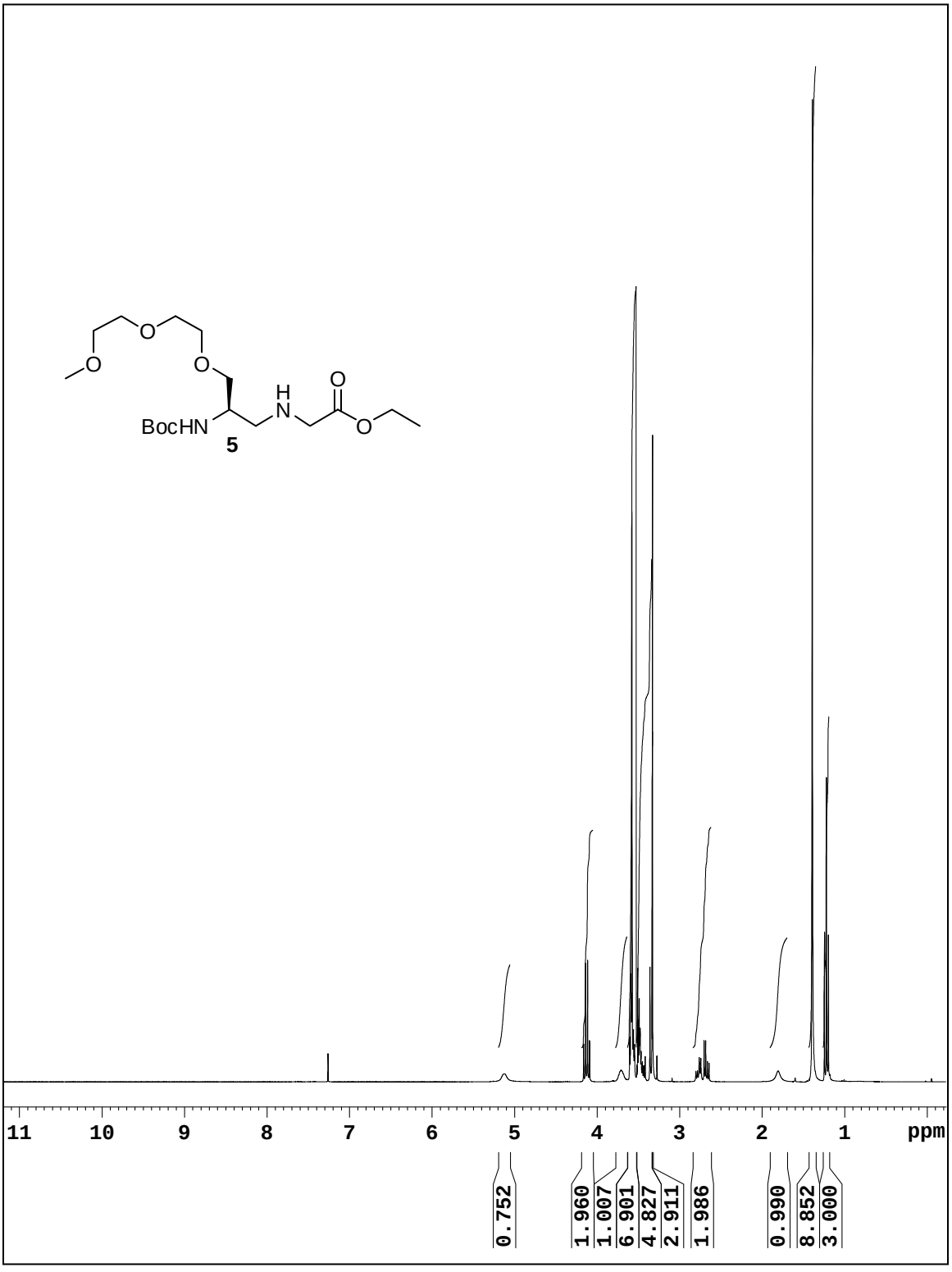
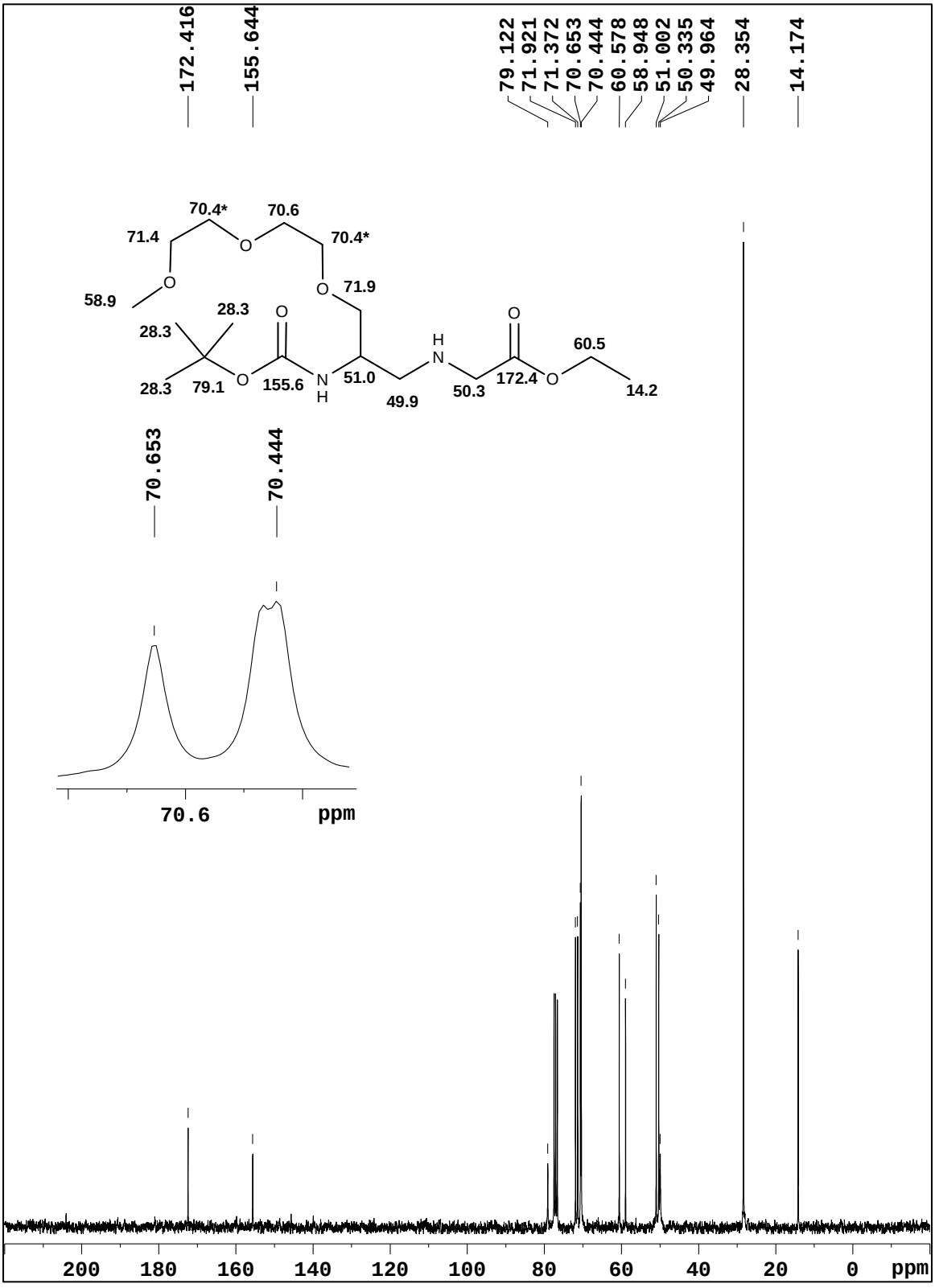


Figure S9. ^{13}C-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine backbone ethyl ester (**5**)



* Peaks are merged together

Figure S10. ^{1}H-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine thymine ethyl ester (**6a**)

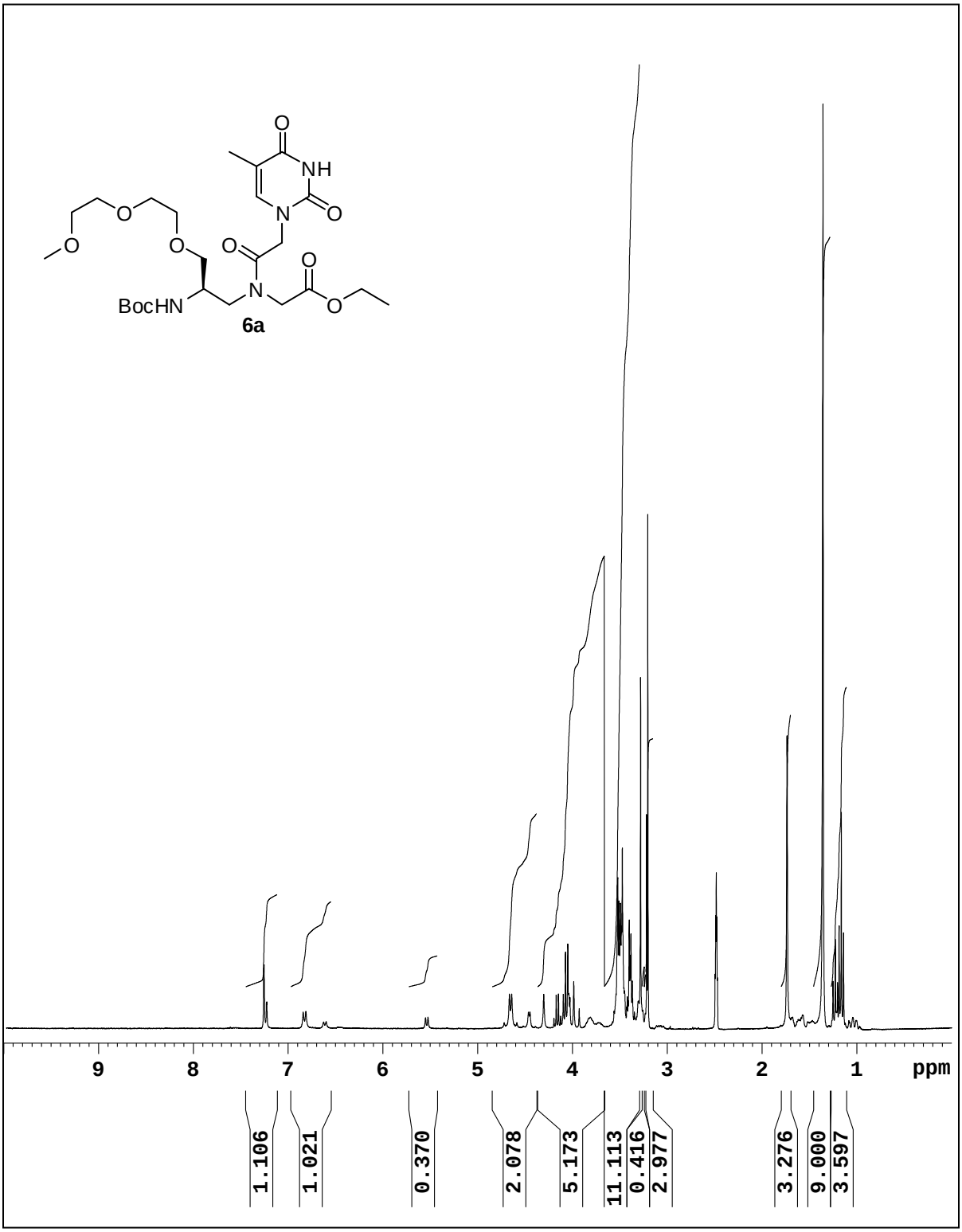
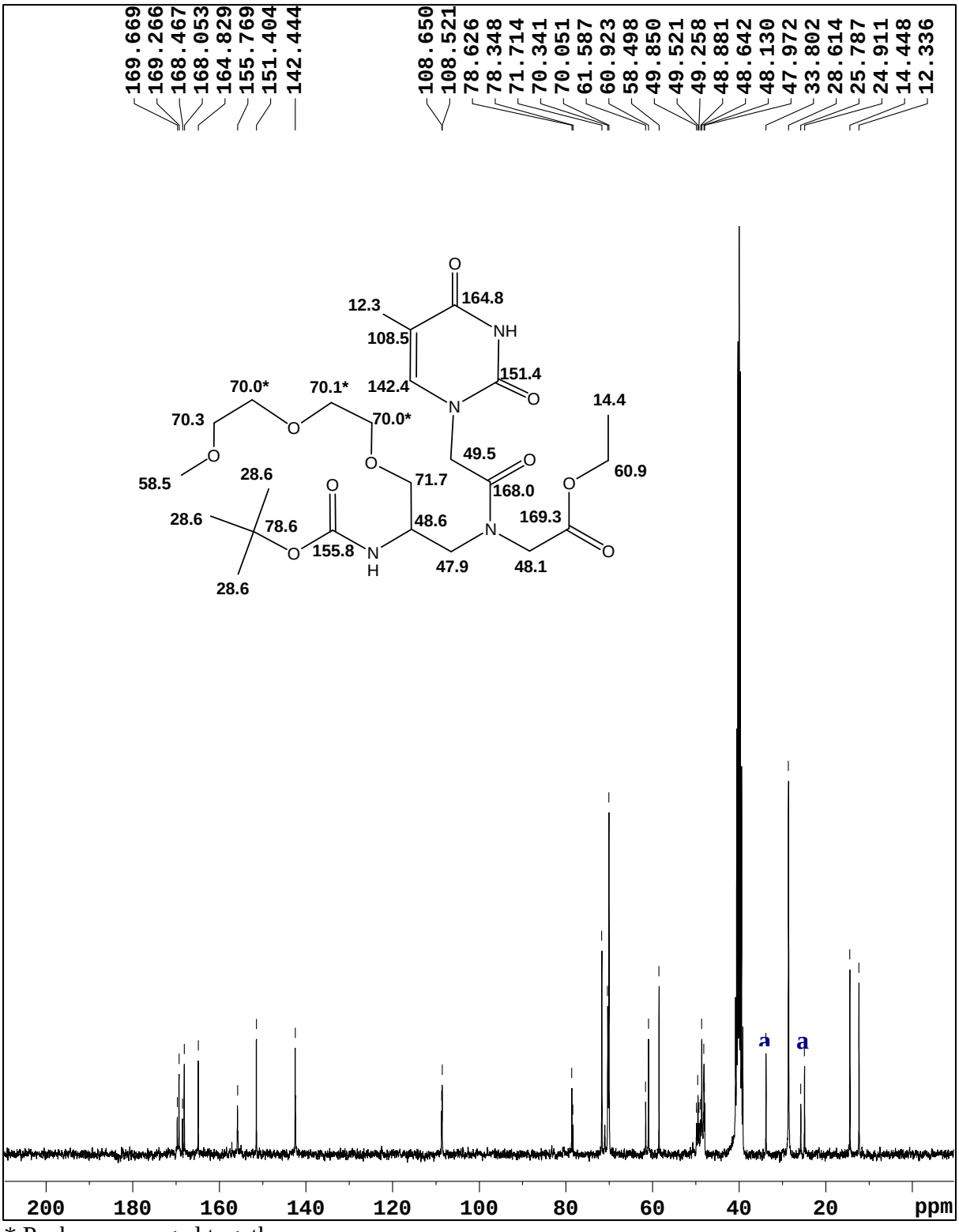


Figure S11. ^{13}C-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine thymine ethyl ester (**6a**)



* Peaks are merged together

a Impurity peak

Figure S12. ^{1}H-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine Adenine(Cbz) ethyl ester (**6b**)

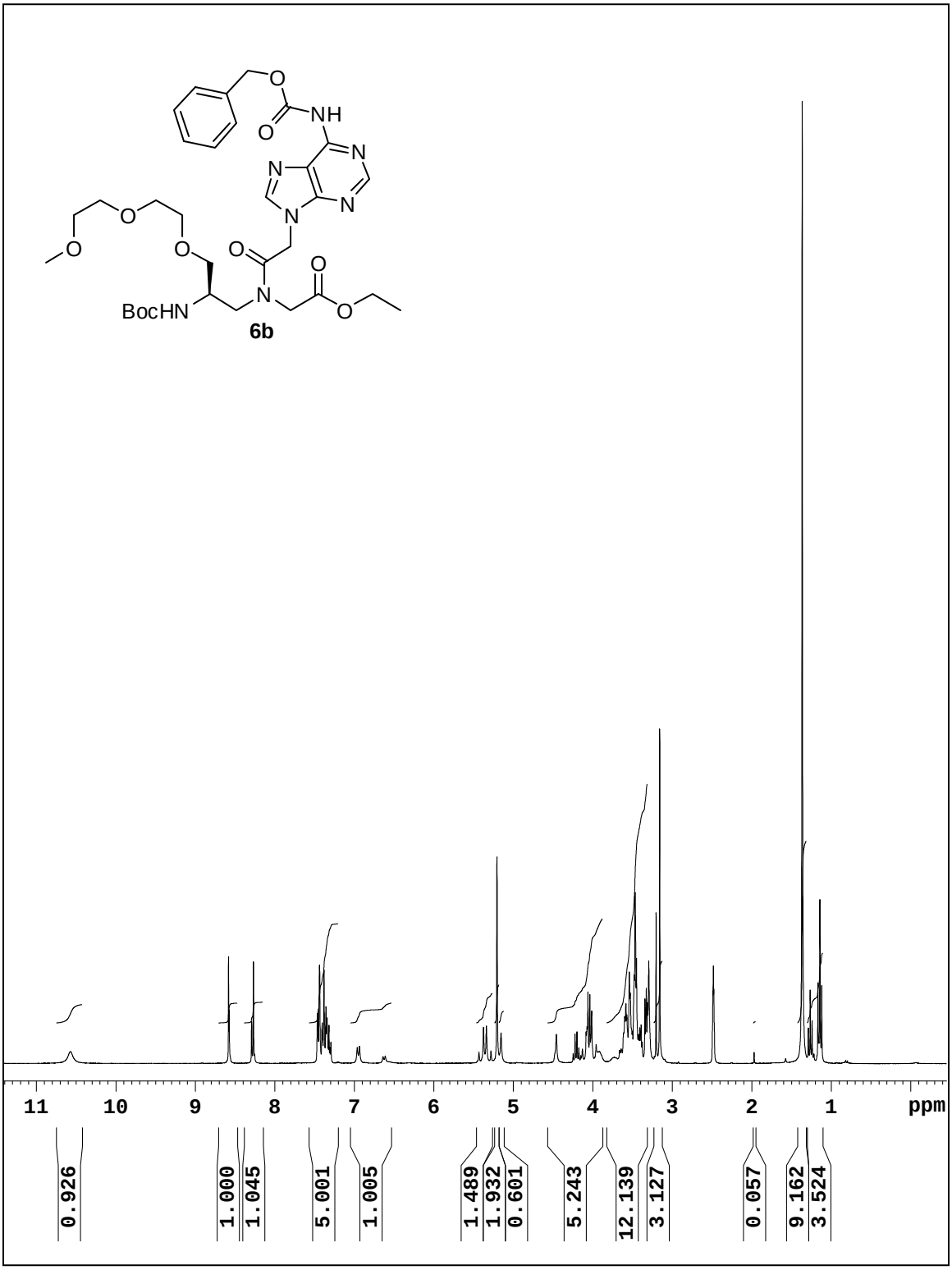
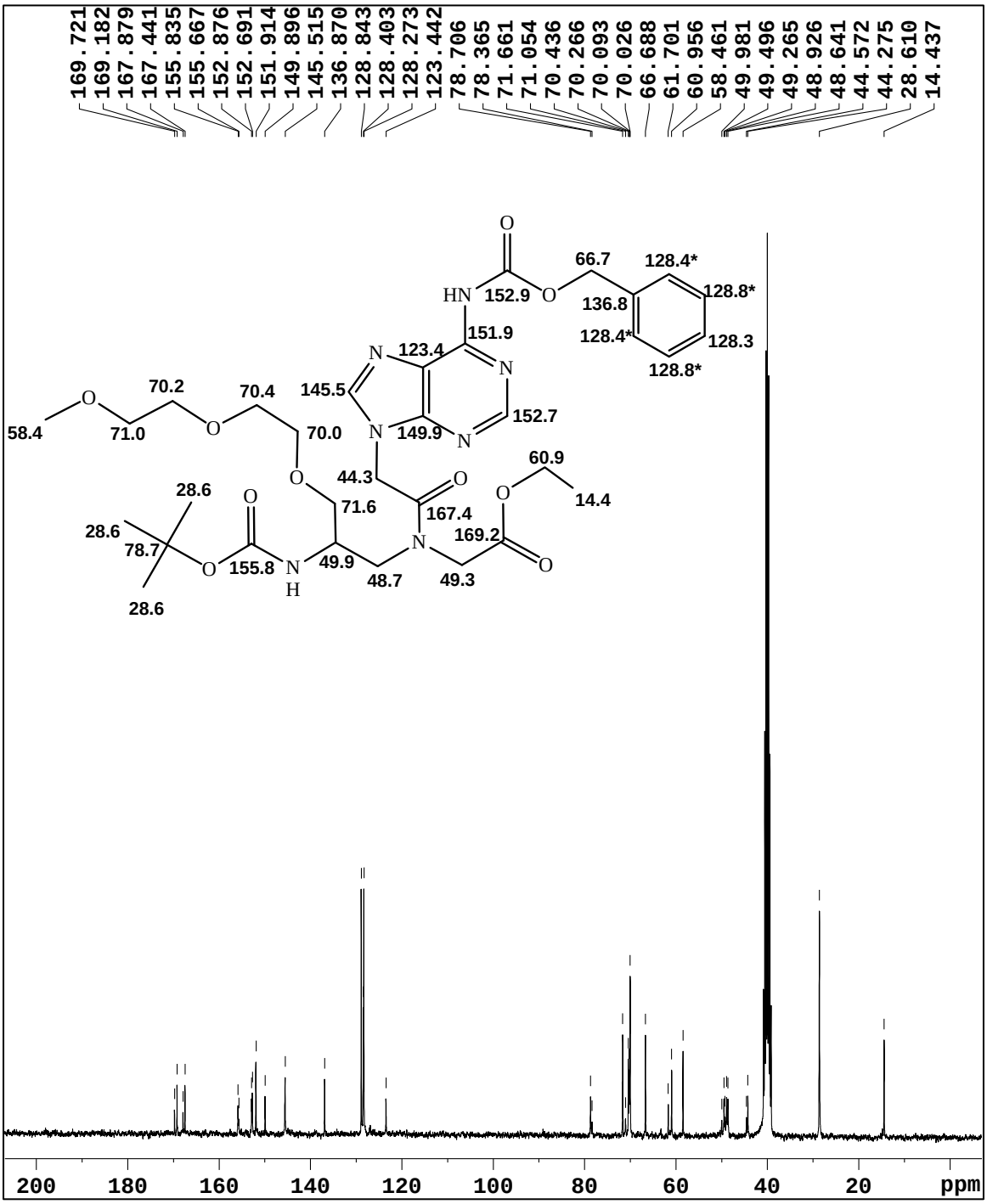


Figure S13. ^{13}C-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine Adenine(Cbz) ethyl ester (**6b**)



* Peaks are merged together

Figure S14. ^{1}H-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine Guanine(Cbz) ethyl ester (**6c**)

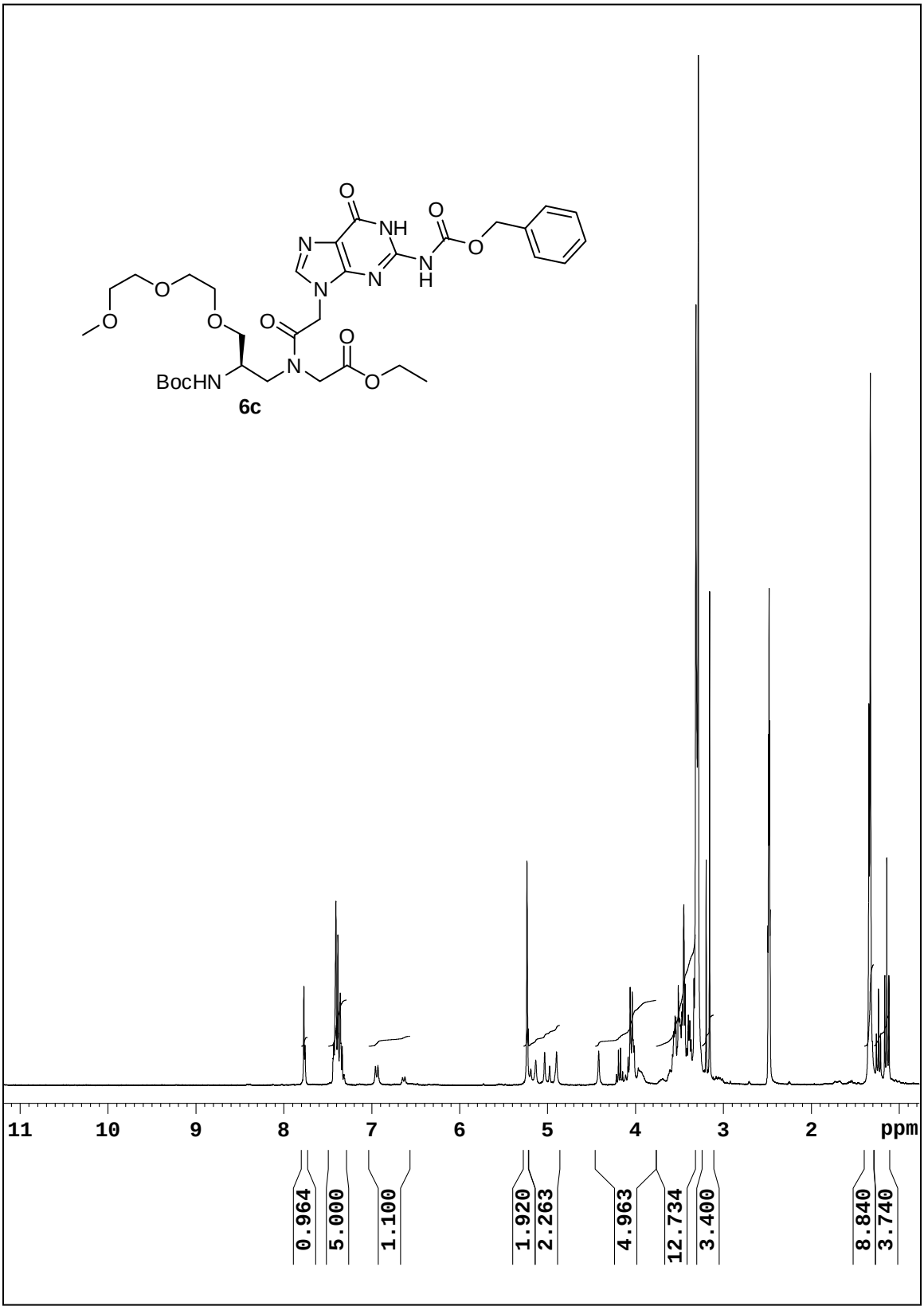
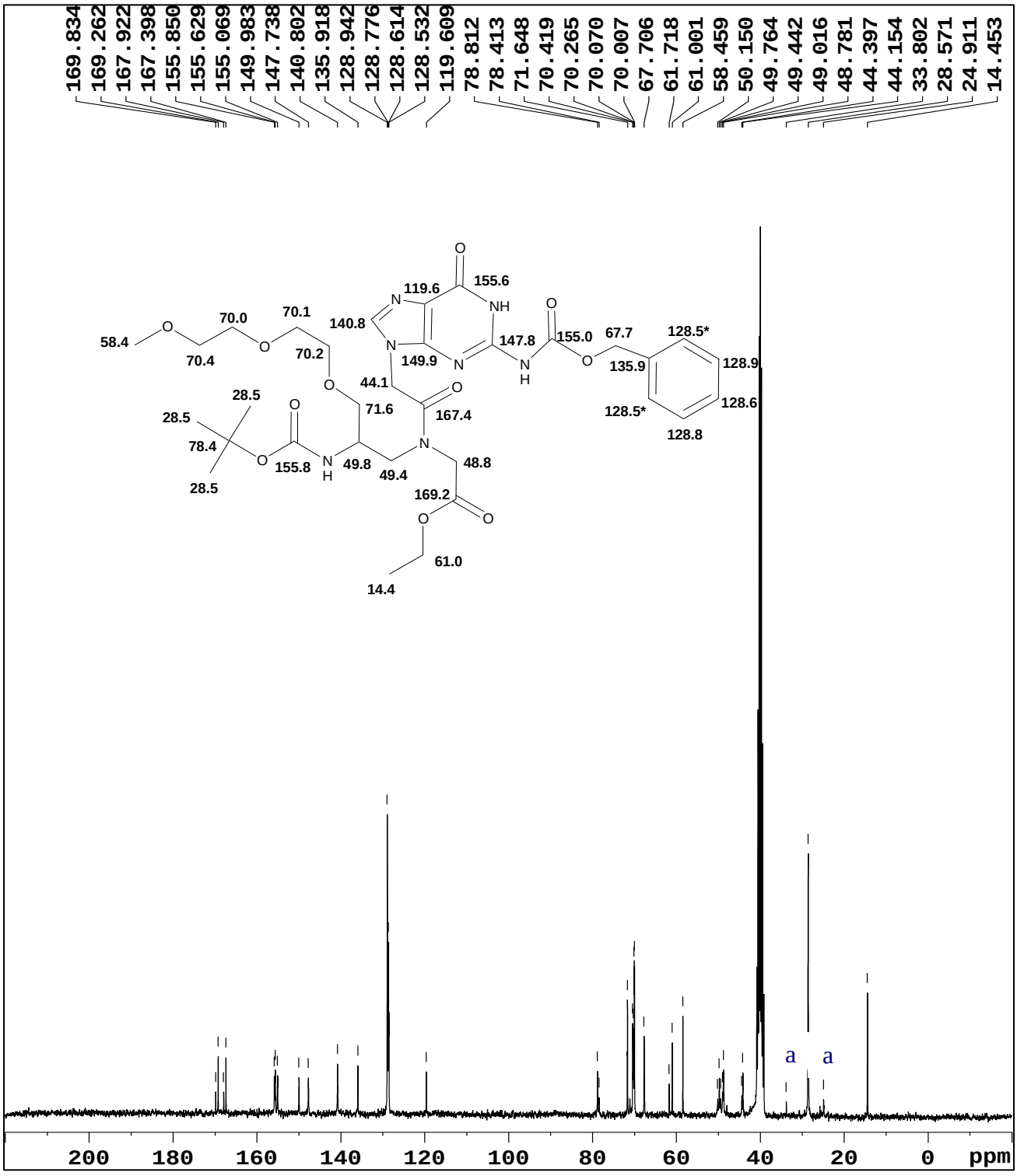


Figure S15. ^{13}C-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine Guanine(Cbz) ethyl ester (**6c**)



* Peaks are merged together

a Impurity peak

Figure S16. ^{1}H NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine Cytosine(Cbz) ethyl ester (**6d**)

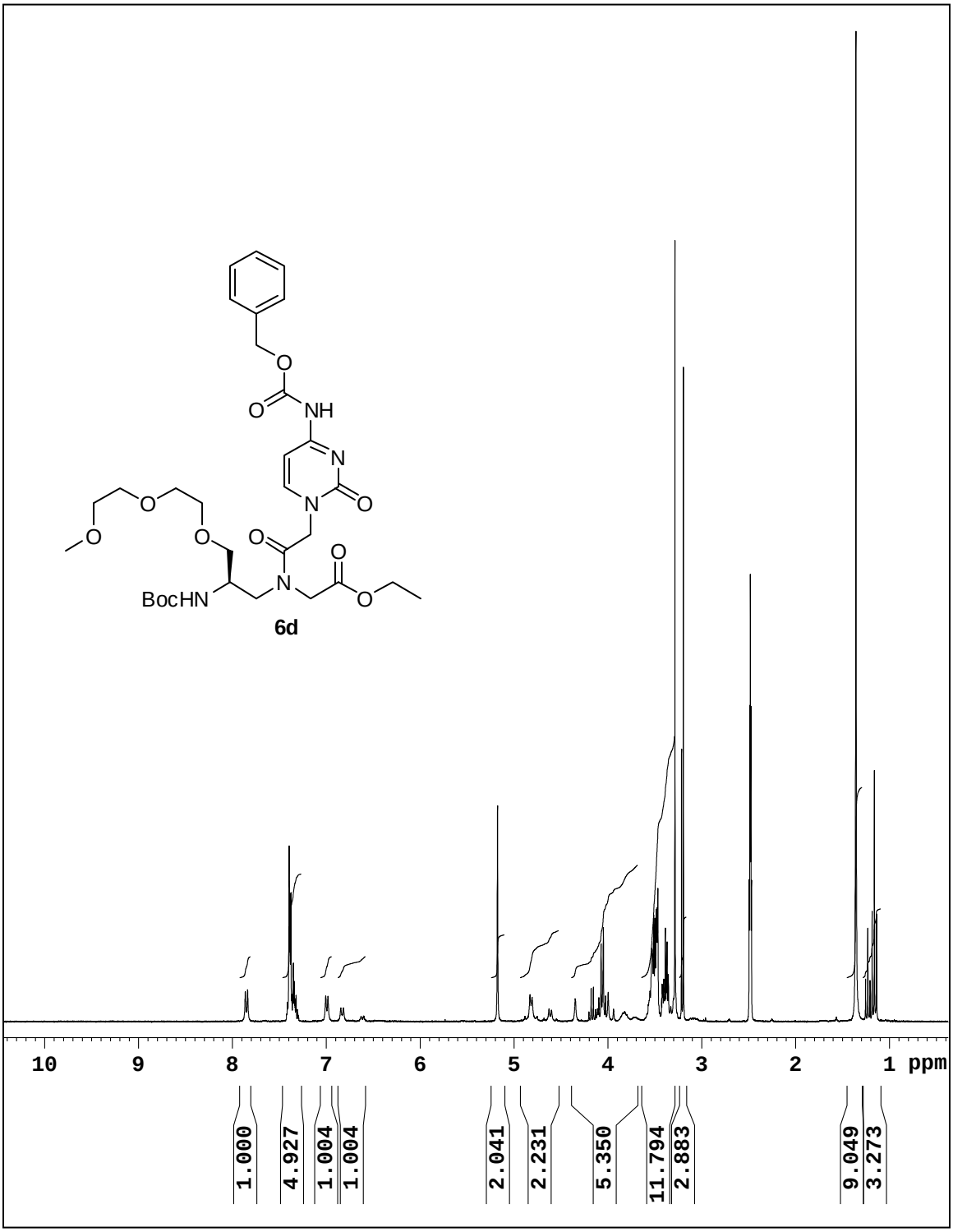
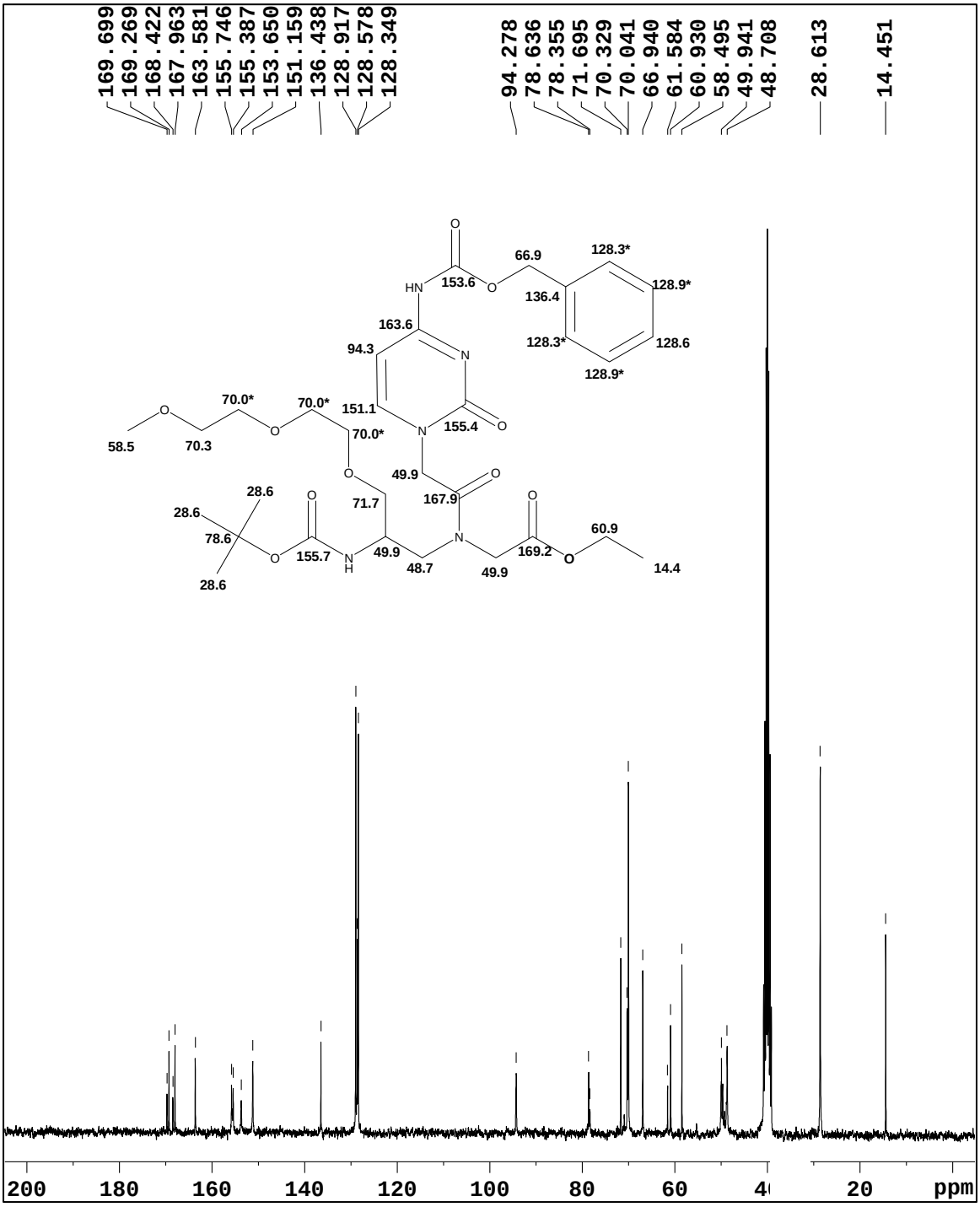


Figure S17. ^{13}C-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine Cytosine(Cbz) ethyl ester (**6d**)



* Peaks are merged together

Figure S18. ^{1}H-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine Thymine monomer (**7a**)

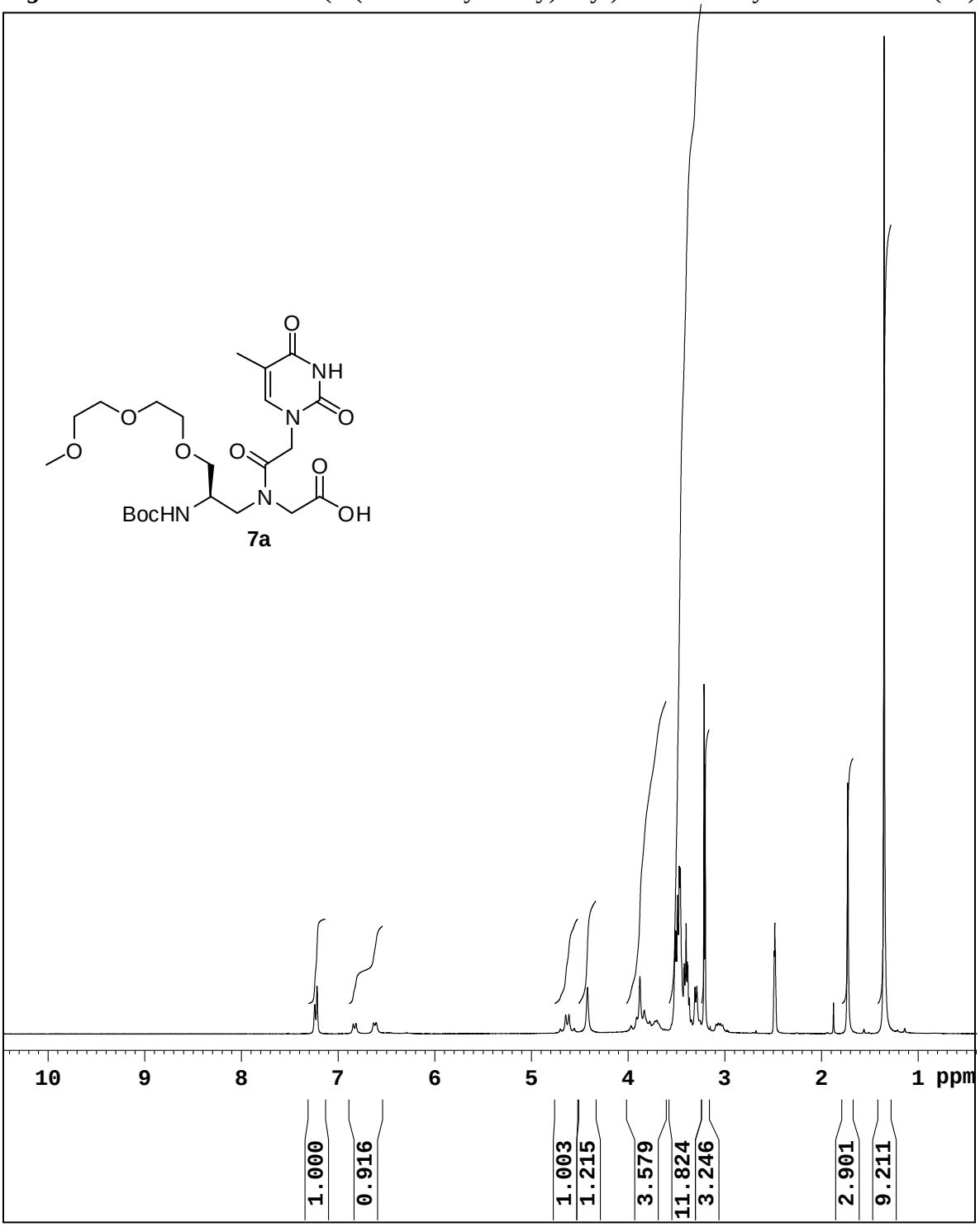


Figure S19. ^{13}C-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine Thymine monomer (**7a**)

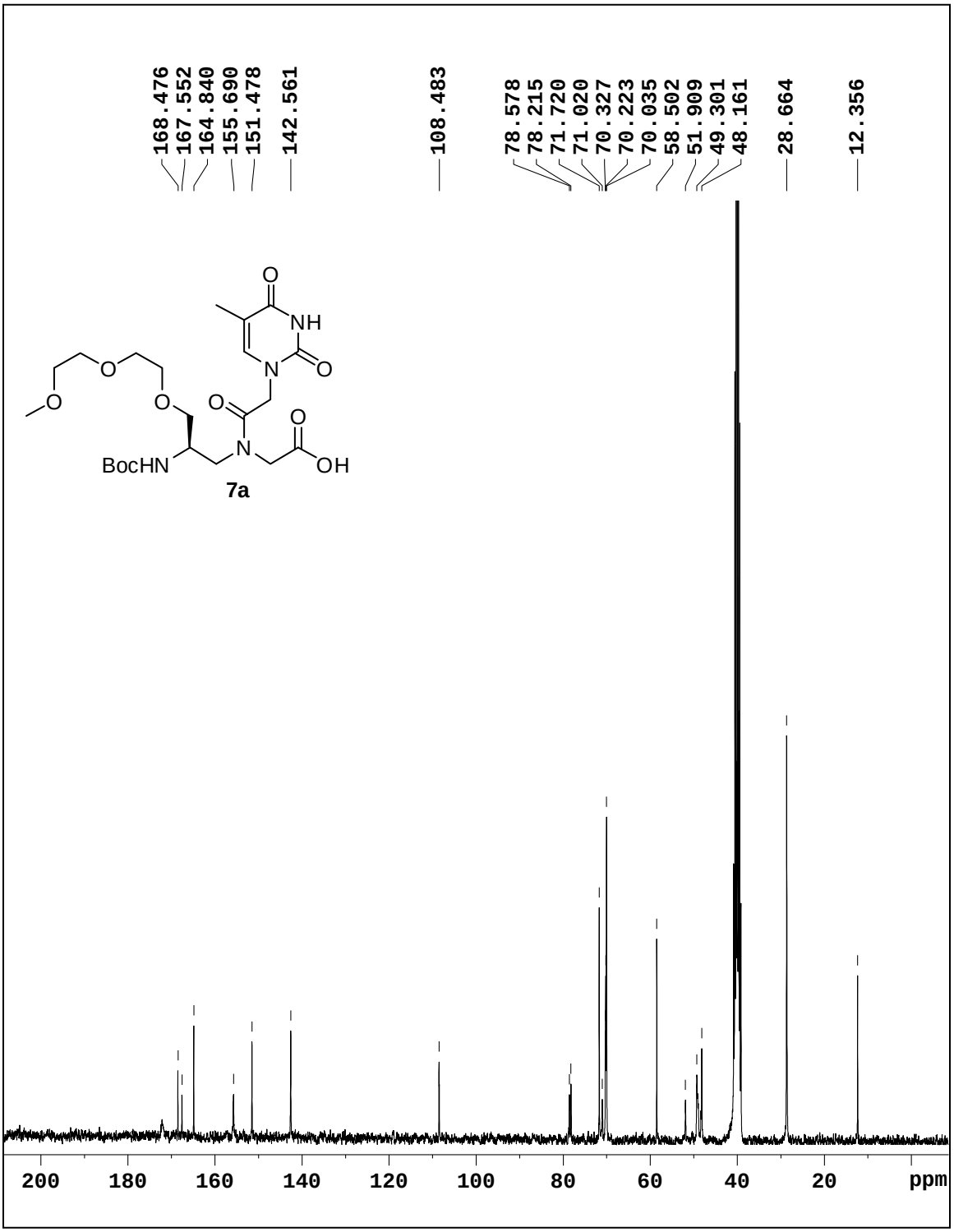


Figure S20. ^{1}H-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine Adenine(Cbz) monomer (**7b**)

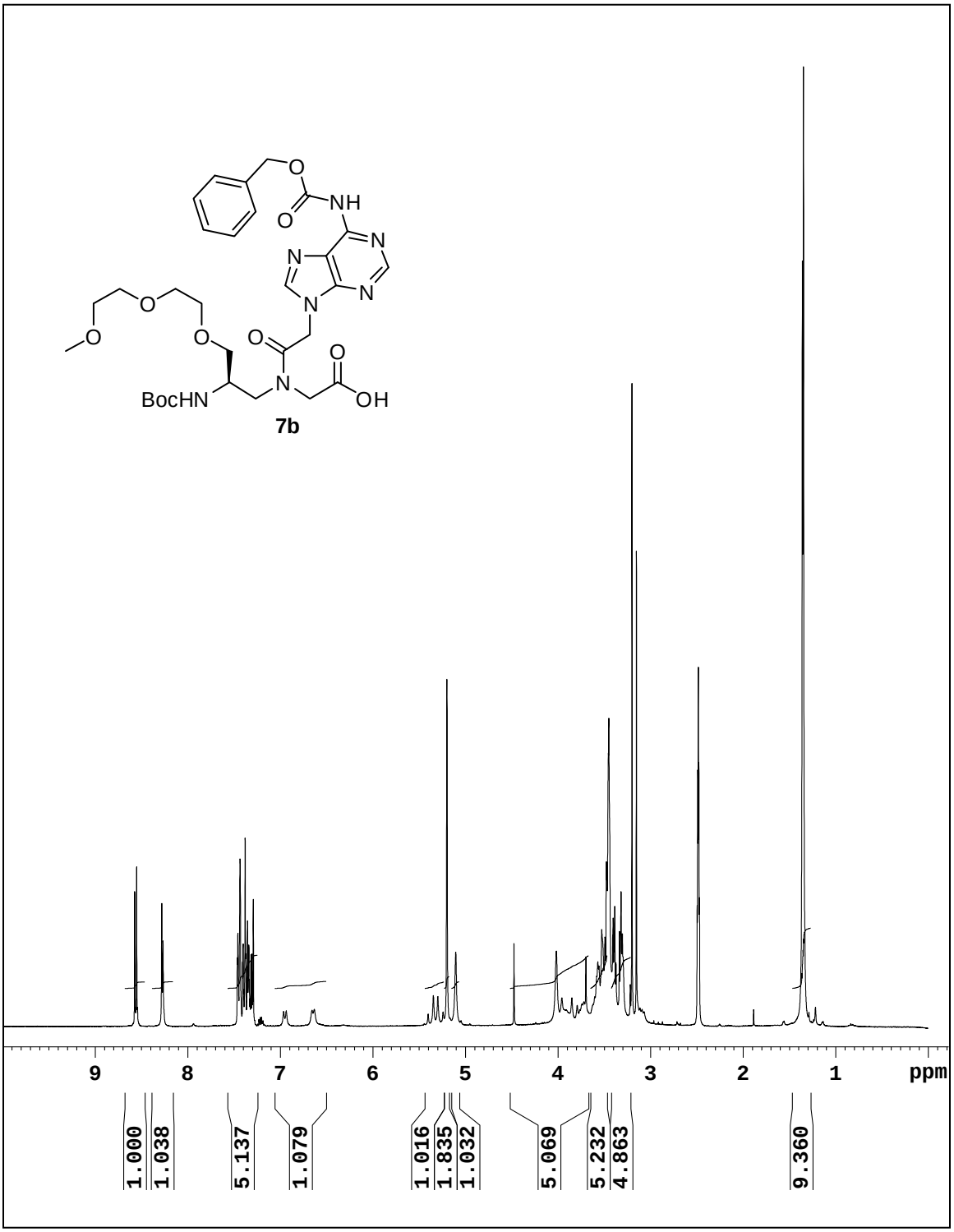


Figure S21. ^{13}C-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine Adenine(Cbz) monomer (**7b**)

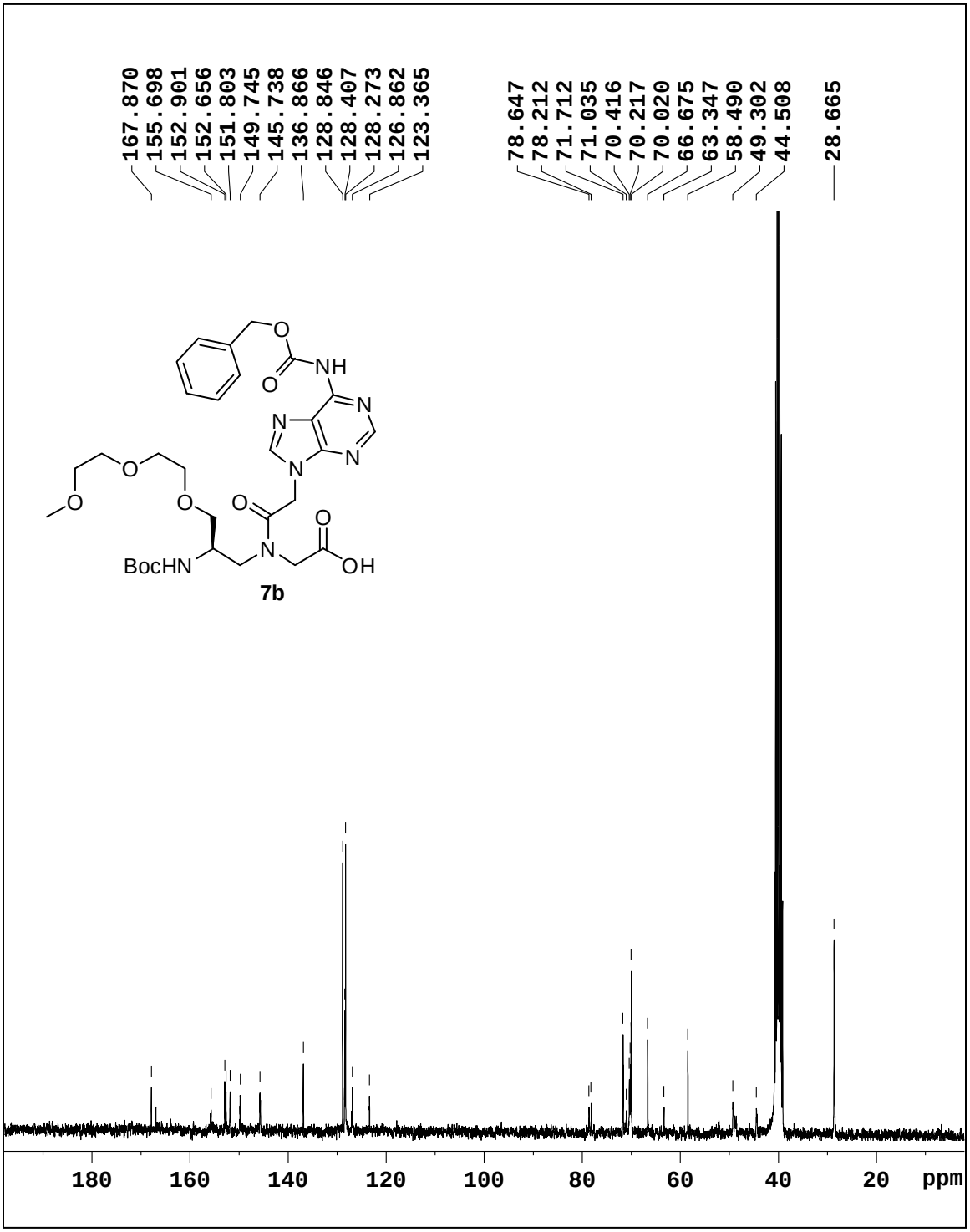


Figure S22. ^{1}H NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine Guanine(Cbz) monomer (**7c**)

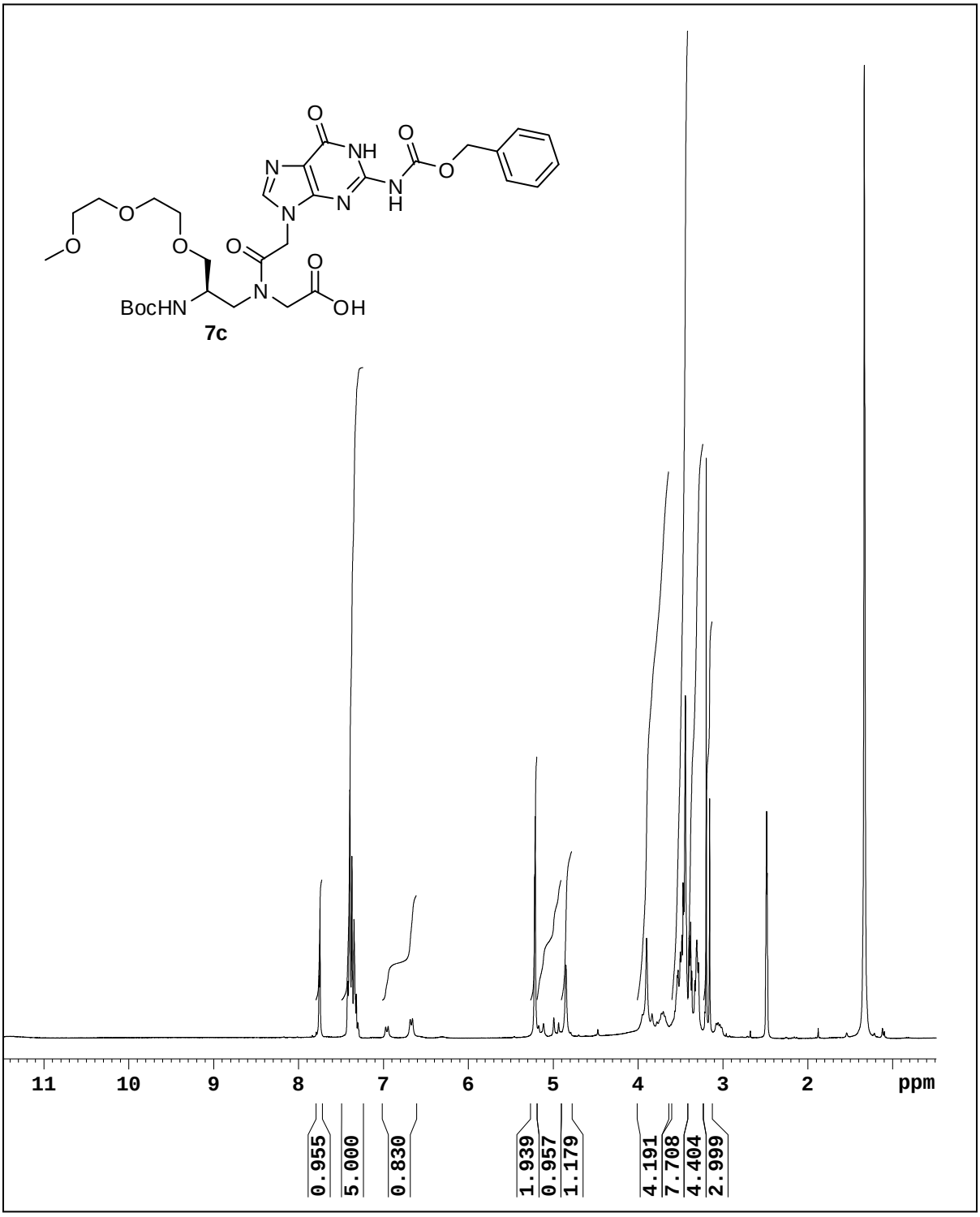


Figure S23. ^{13}C-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine Guanine(Cbz) monomer (**7c**)

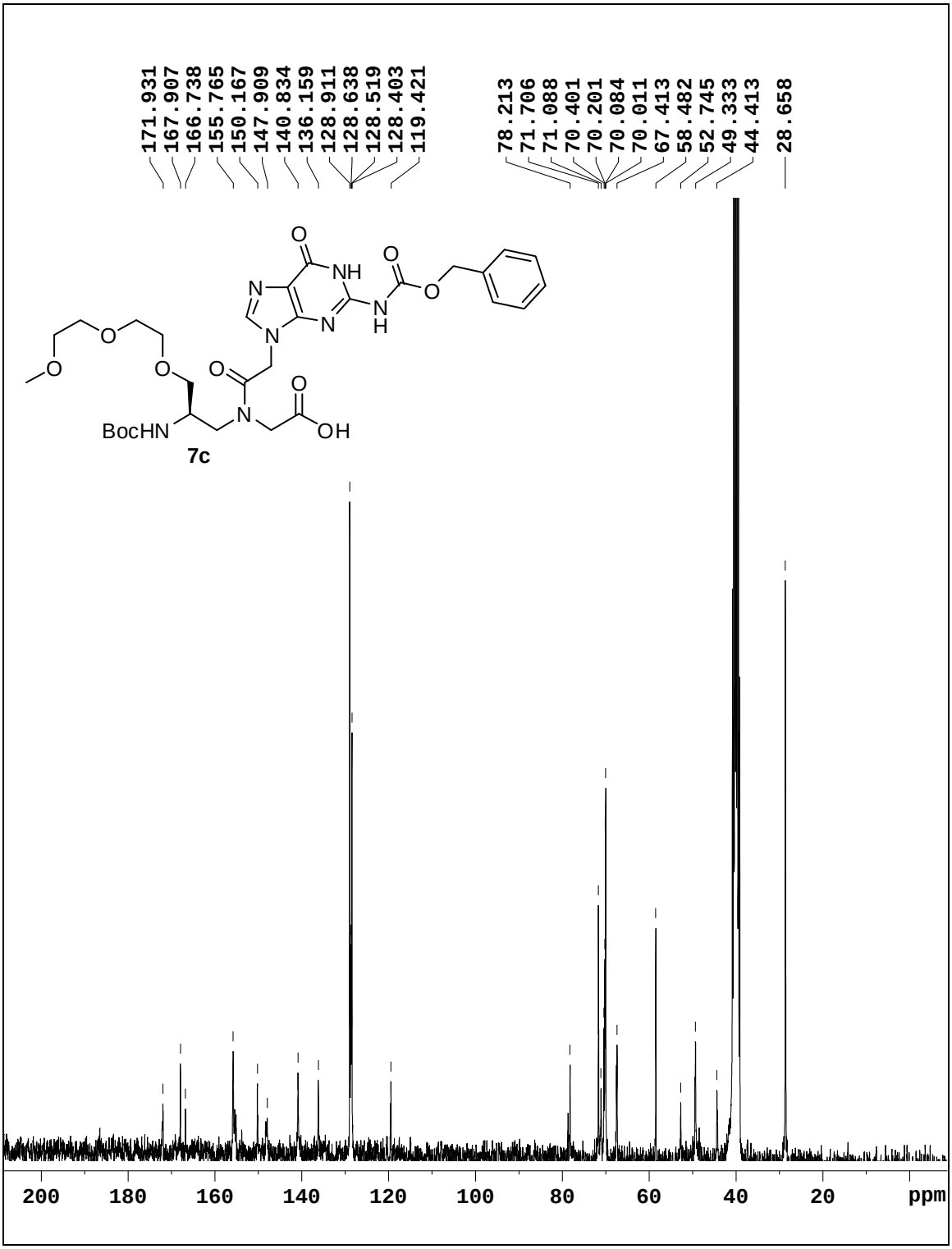


Figure S24. [1]H-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine Cytosine(Cbz) monomer (**7d**)

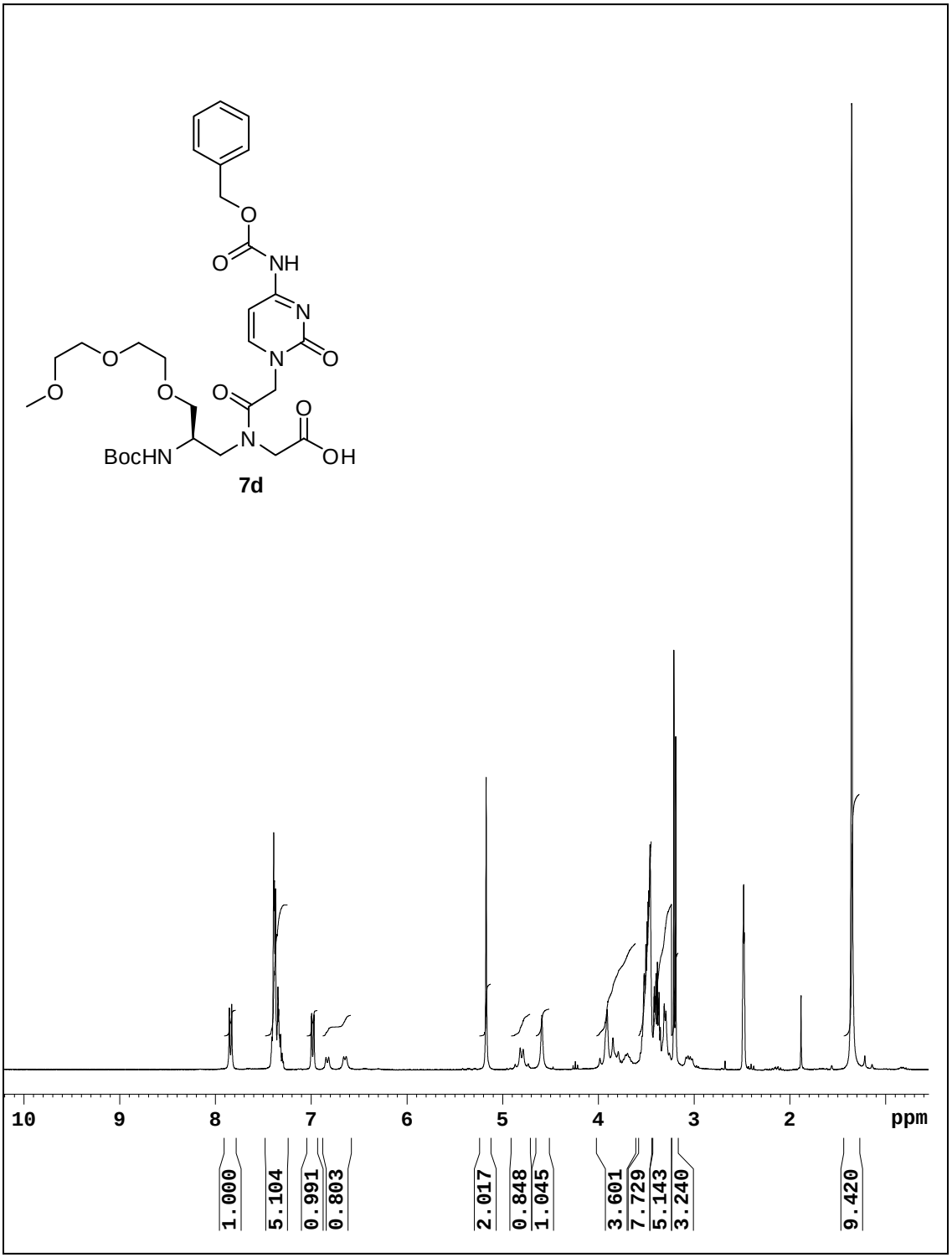
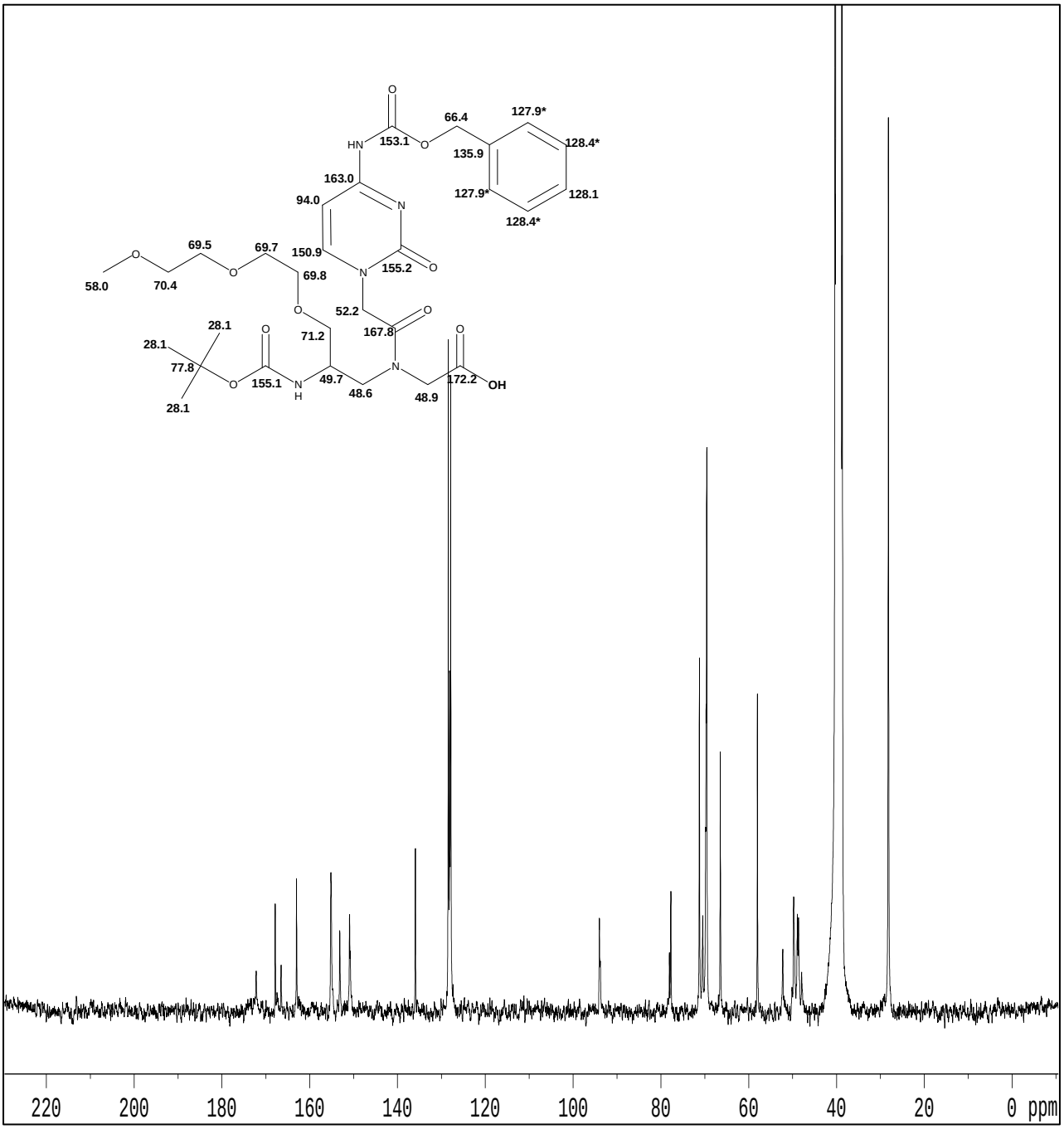
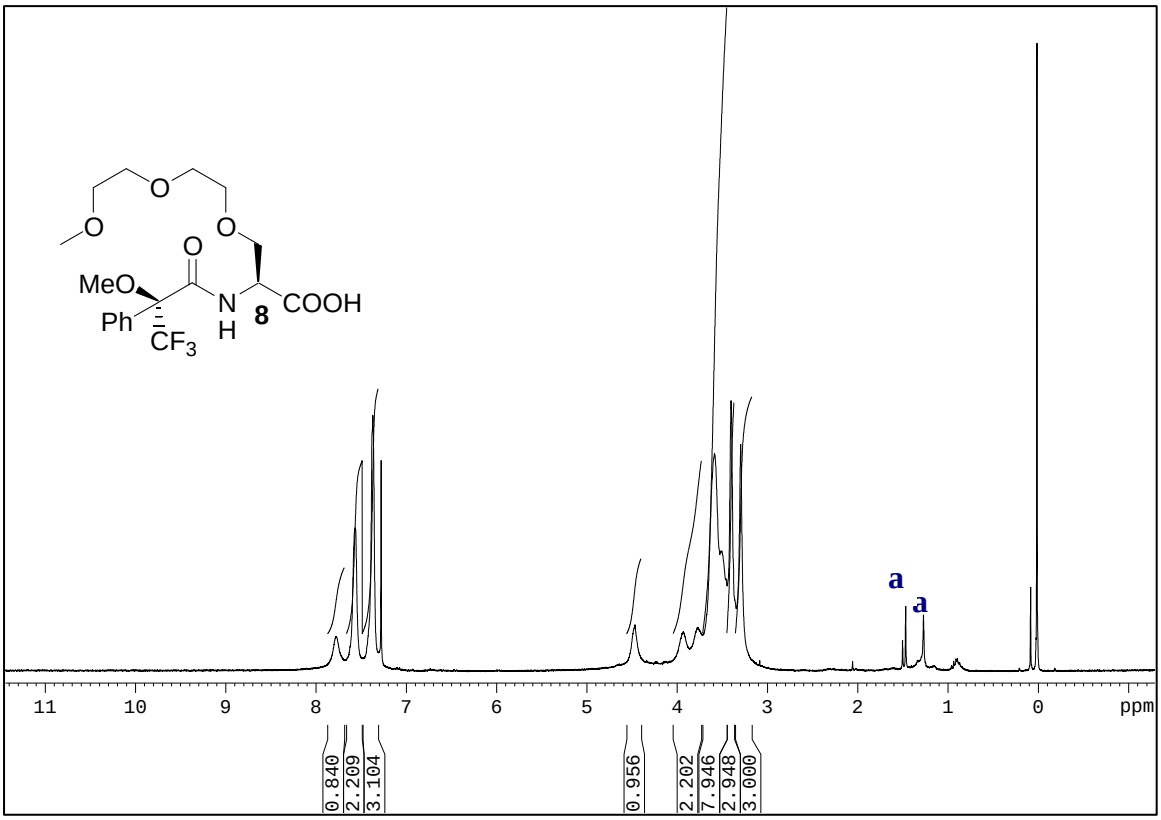


Figure S25. ^{13}C-NMR of Boc-(2-(2-methoxyethoxy)ethyl)-L-serine Cytosine(Cbz) monomer (**7d**)



* Peaks are merged together

Figure S26. ^{1}H-NMR of 3-[2-(2-methoxy-ethoxy)-ethoxy]-2-(3,3,3-trifluoro-2-methoxy-2-phenyl-propionylamino)-propionic acid (**8**).



a Impurity peaks

Figure S27. ^{13}C-NMR of 3-[2-(2-methoxy-ethoxy)-ethoxy]-2-(3,3,3-trifluoro-2-methoxy-2-phenyl-propionylamino)-propionic acid (**8**).

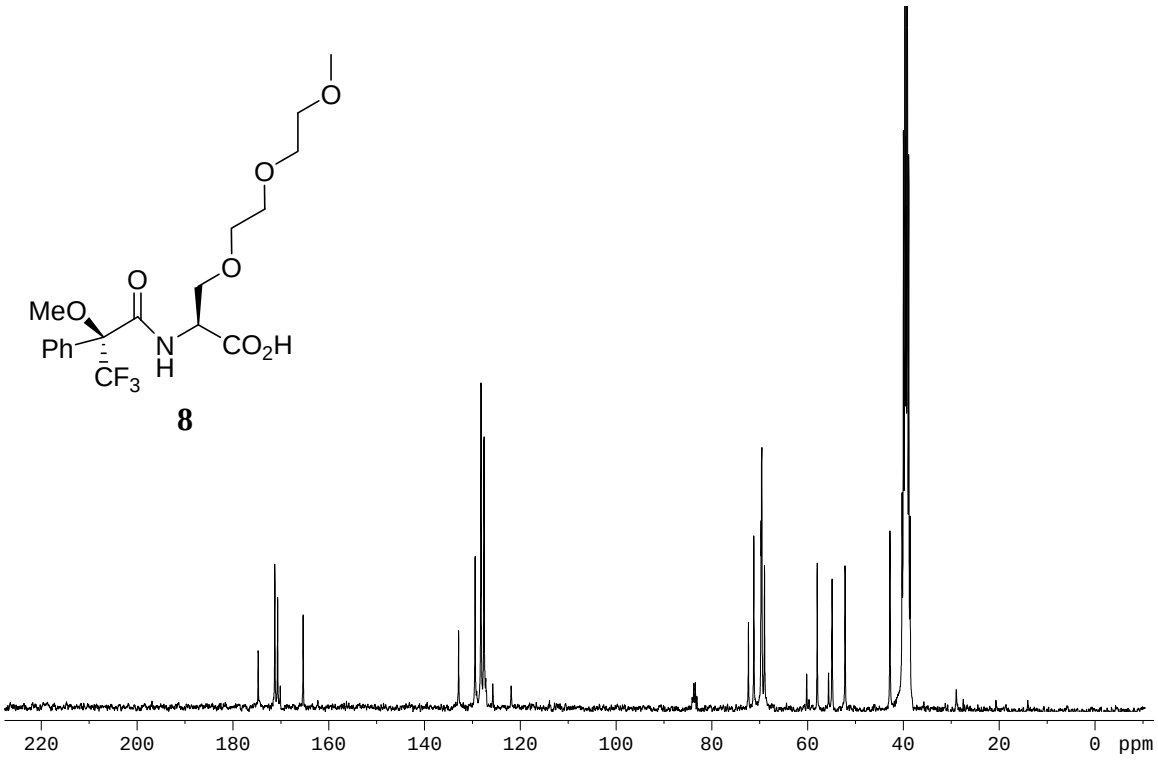


Figure S28. ^{1}H-NMR of 3,3,3-trifluoro-*N*-{2-hydroxy-1-[2-(2-methoxy-ethoxy)-ethoymethyl]-ethyl}-2-methoxy-2-phenyl-propionamide (**9**).

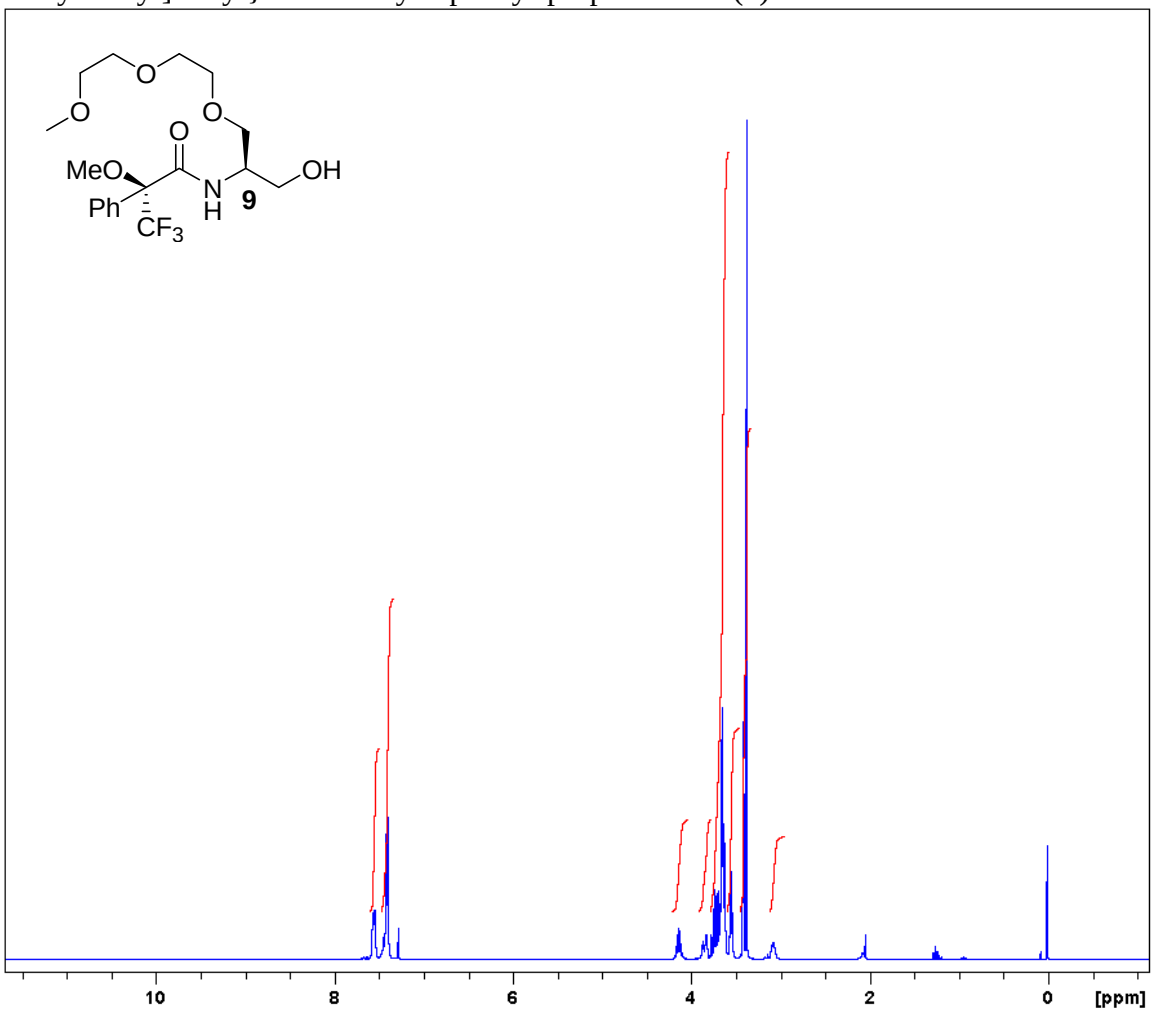


Figure S29. ^{13}C-NMR of 3,3,3-trifluoro-N-{2-hydroxy-1-[2-(2-methoxy-ethoxy)-ethoymethyl]-ethyl}-2-methoxy-2-phenyl-propionamide (**9**).

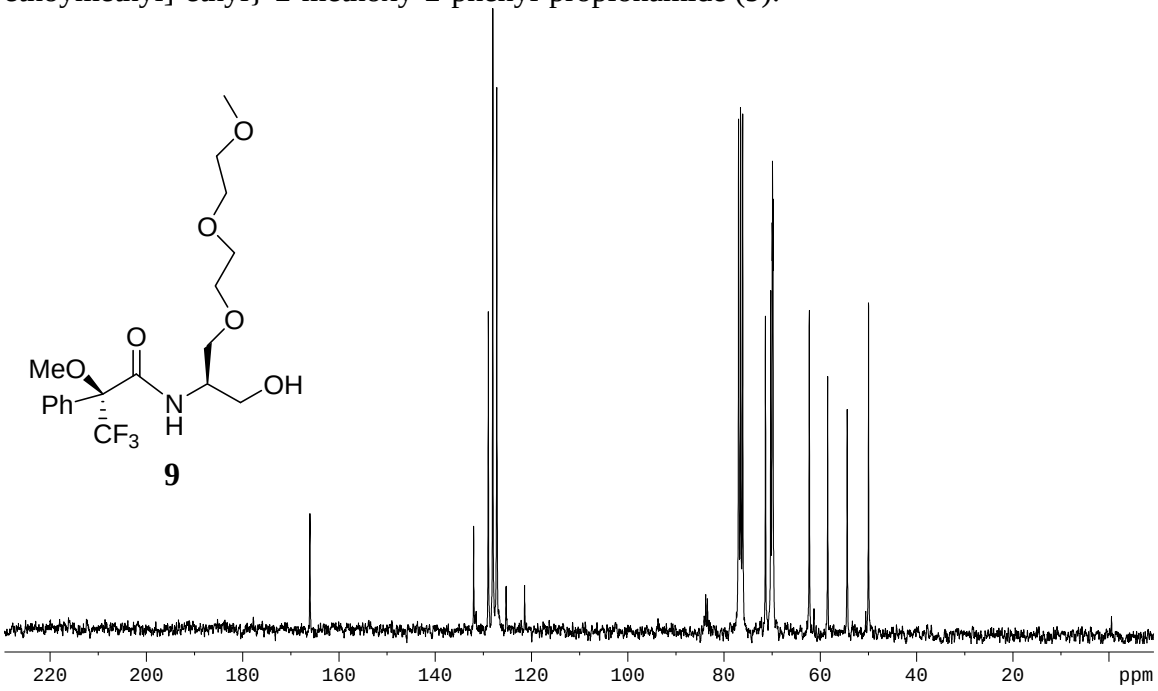


Figure S30. [1]H-NMR of {[3-[2-(2-Methoxy-ethoxy)-ethoxy]-2-(3,3,3-trifluoro-2-methoxy-2-phenyl-propionylamino)-propyl]-[2-thymine-acetyl]-amino}-acetic acid (**10**).

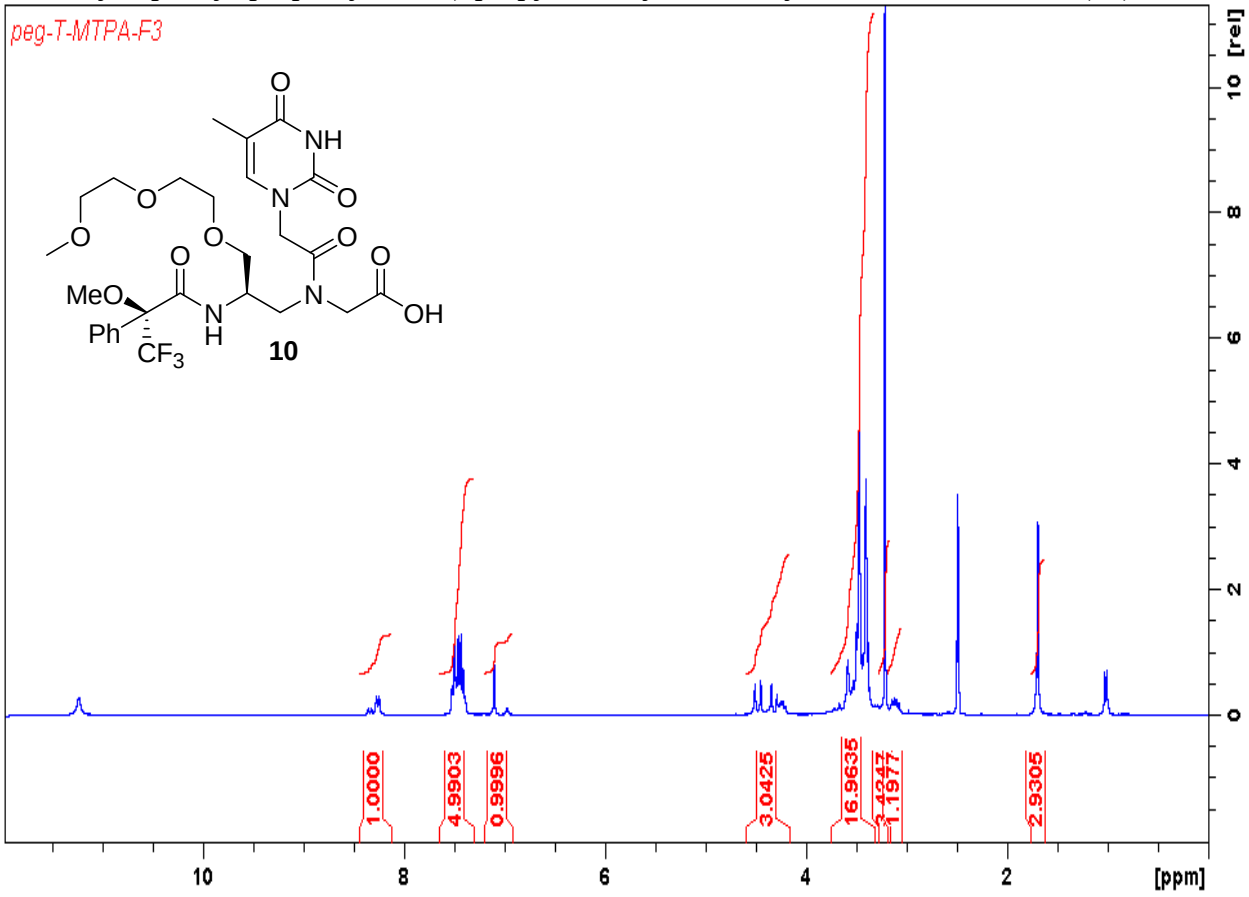
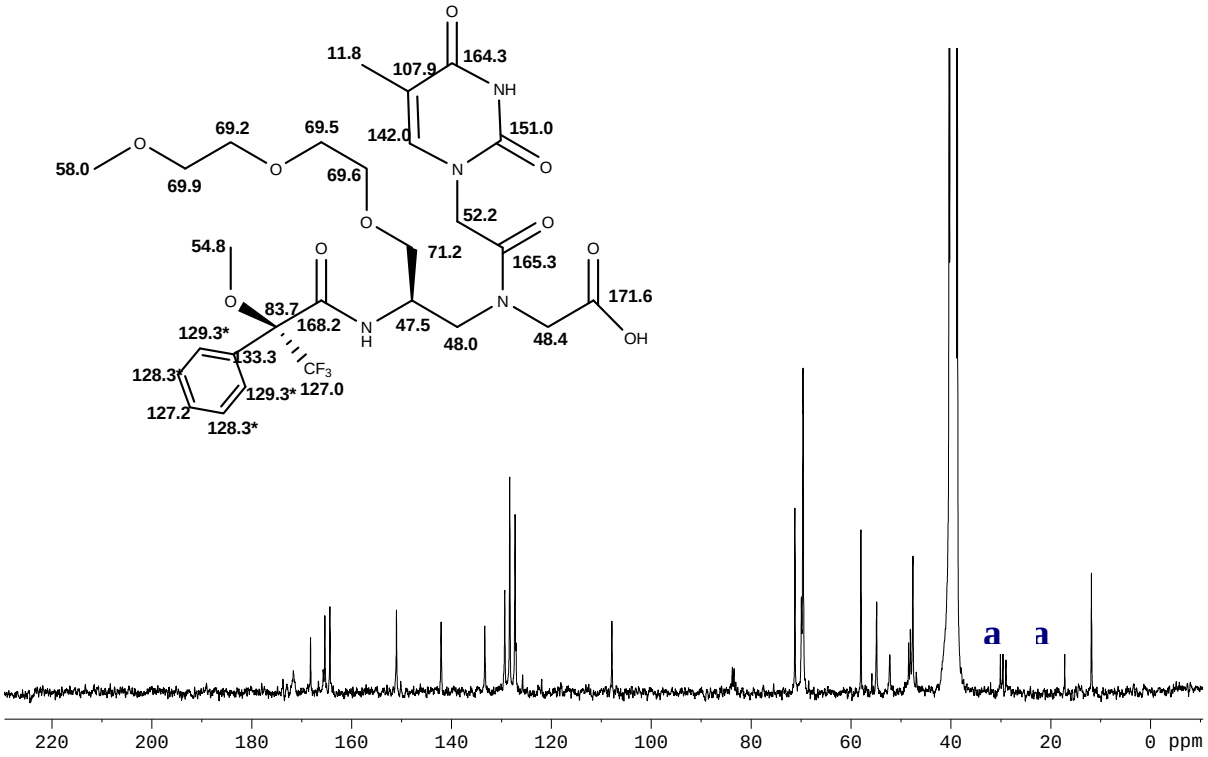


Figure S31. ^{13}C-NMR of {[3-[2-(2-Methoxy-ethoxy)-ethoxy]-2-(3,3,3-trifluoro-2-methoxy-2-phenyl-propionylamino)-propyl]-[2-thymine-acetyl]-amino}-acetic acid (**10**)

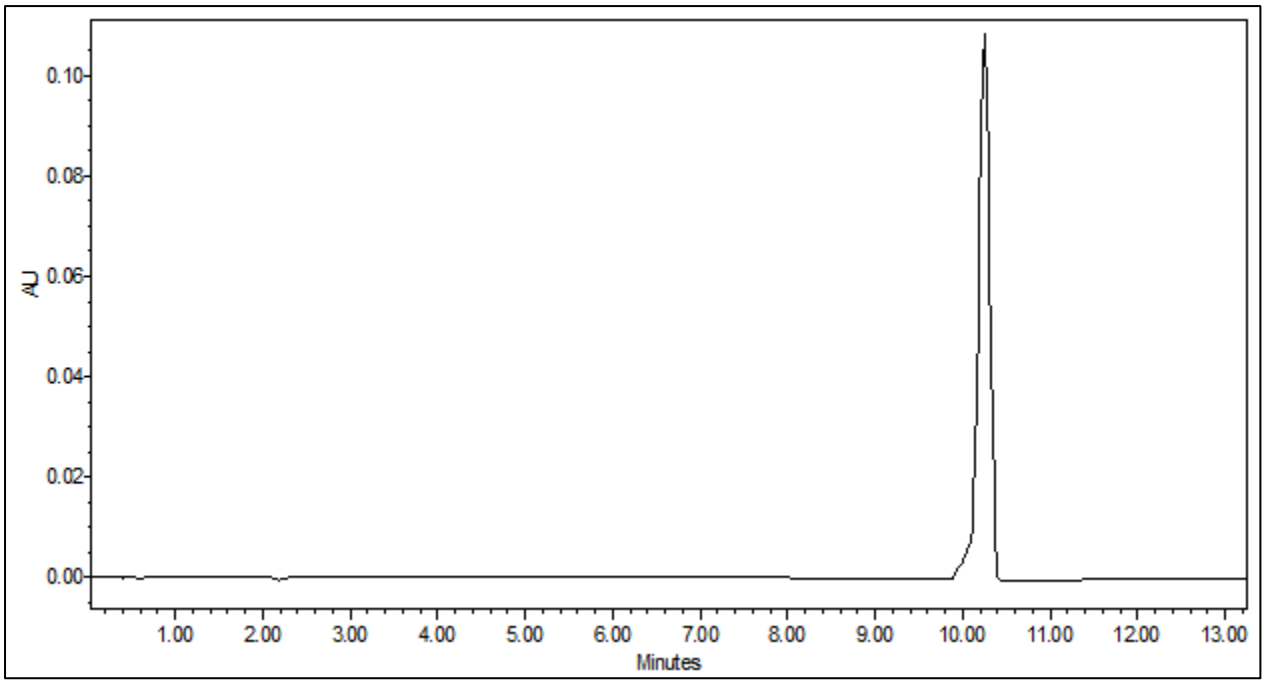


* Peaks are merged together

a Impurity peaks

Figure S32. Reinjected HPLC trace of PNA1.

PNA1: H-GCATGTTTGA-NH2 (Unmodified PNA)



Eluent A: 0.1% TFA in water, and eluent B: 0.1% TFA in ACN. The gradient was 0-40% of eluent B in 50 minutes at 40°C with a flow rate of 3.0 mL/min.

Figure S33. MALDI-TOF MS of PNA1

Calculated Mass = 2764.9

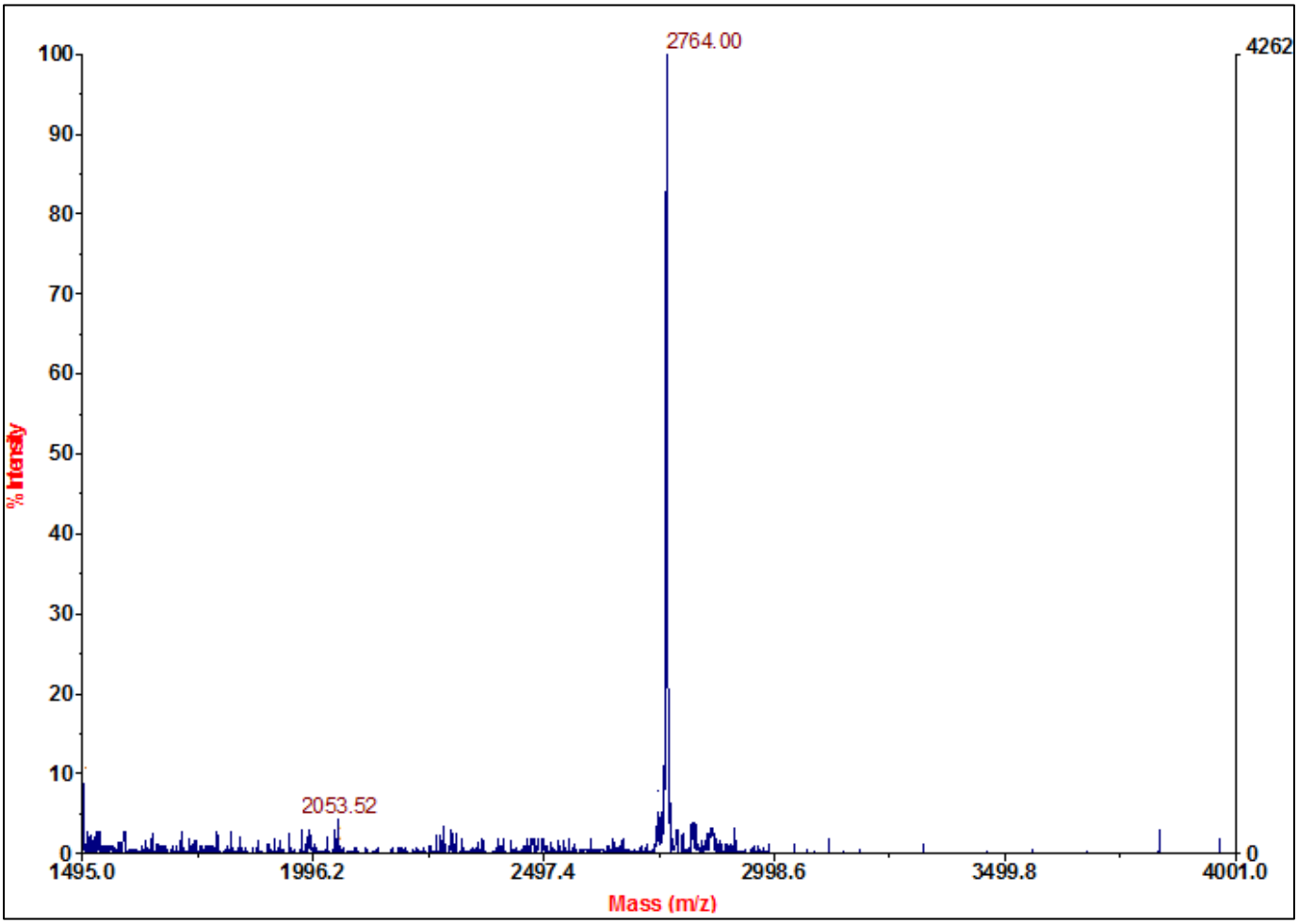


Figure S34. Reinjected HPLC trace of PNA2

PNA2: H-GCATGTTTGA-NH2

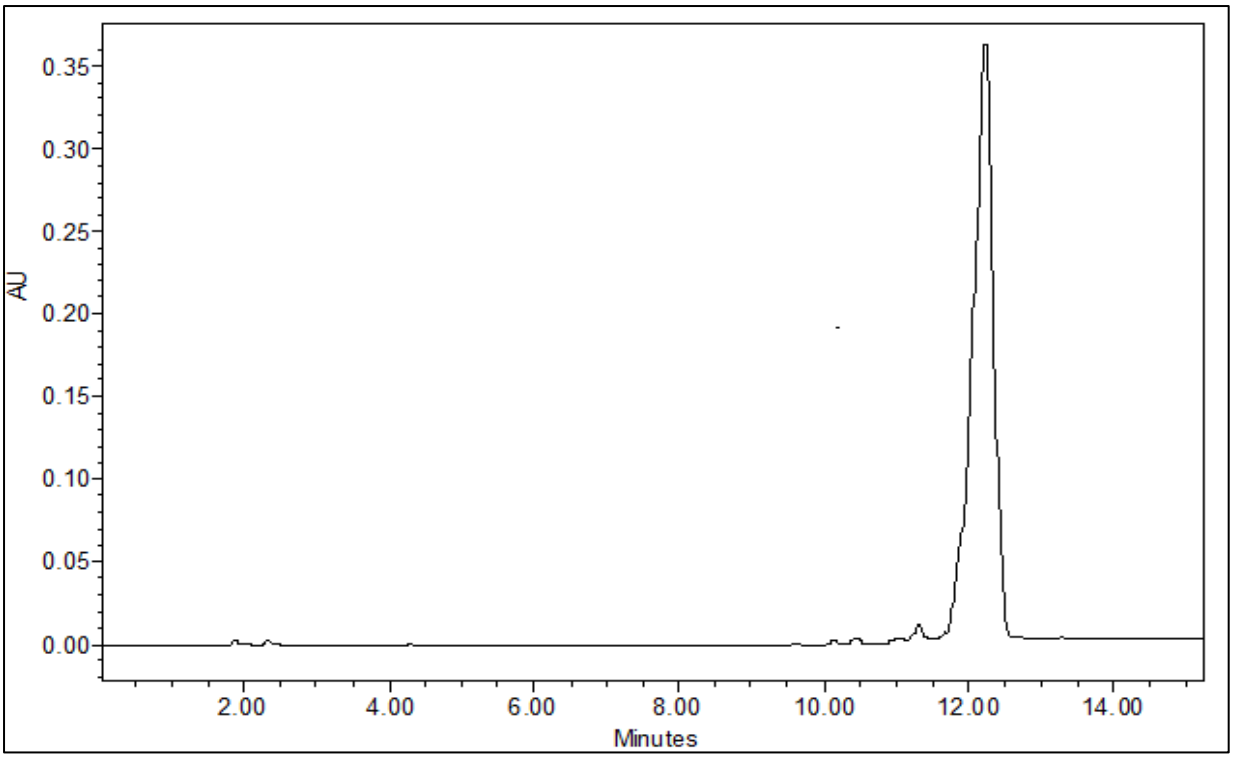


Figure S35. MALDI-TOF MS of PNA2

Calculated Mass = 2883.1

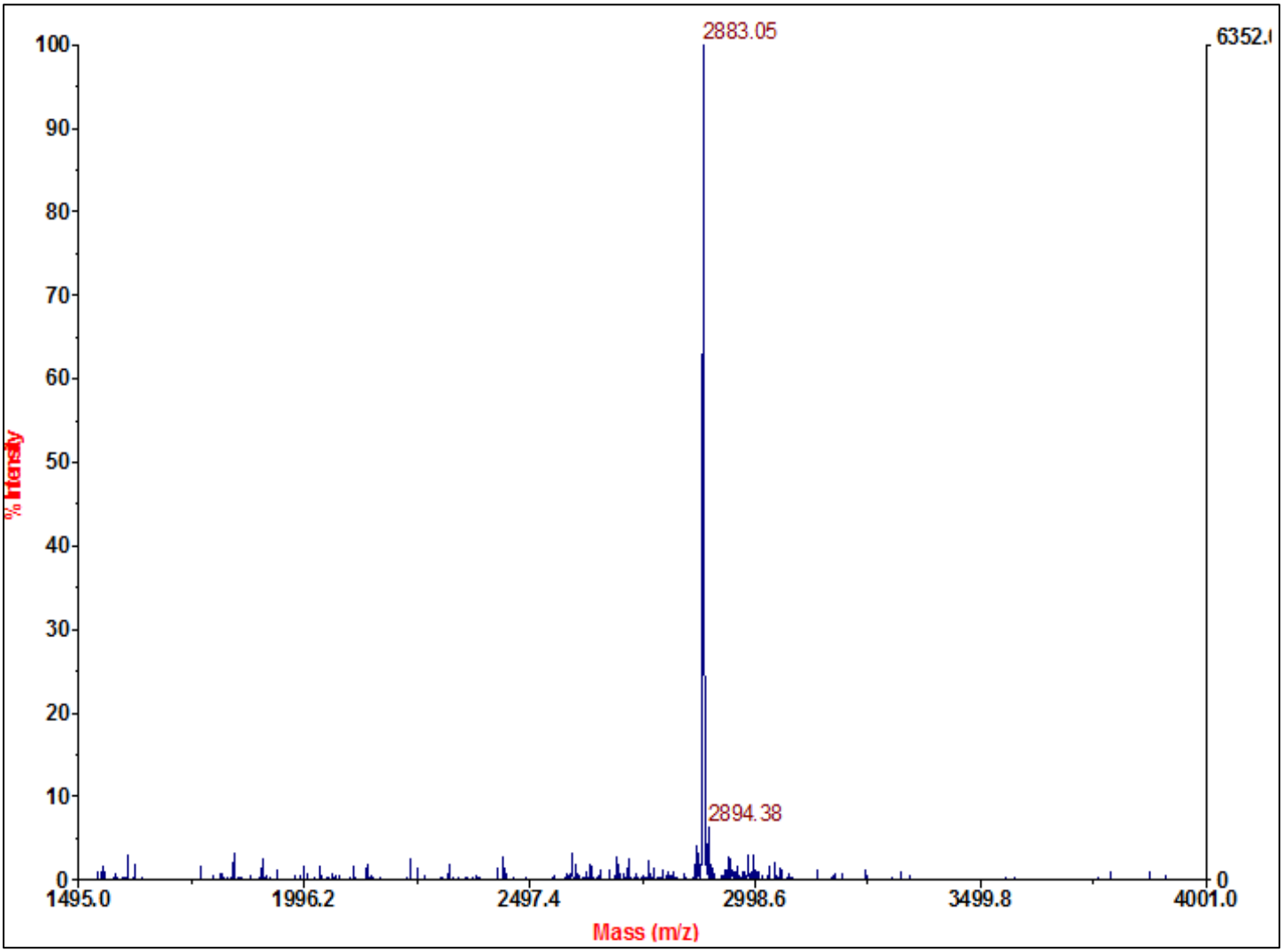


Figure S36. Reinjected HPLC trace of PNA3

PNA3: H-GCA<u>T</u>G<u>T</u>T<u>T</u>GA-NH_2

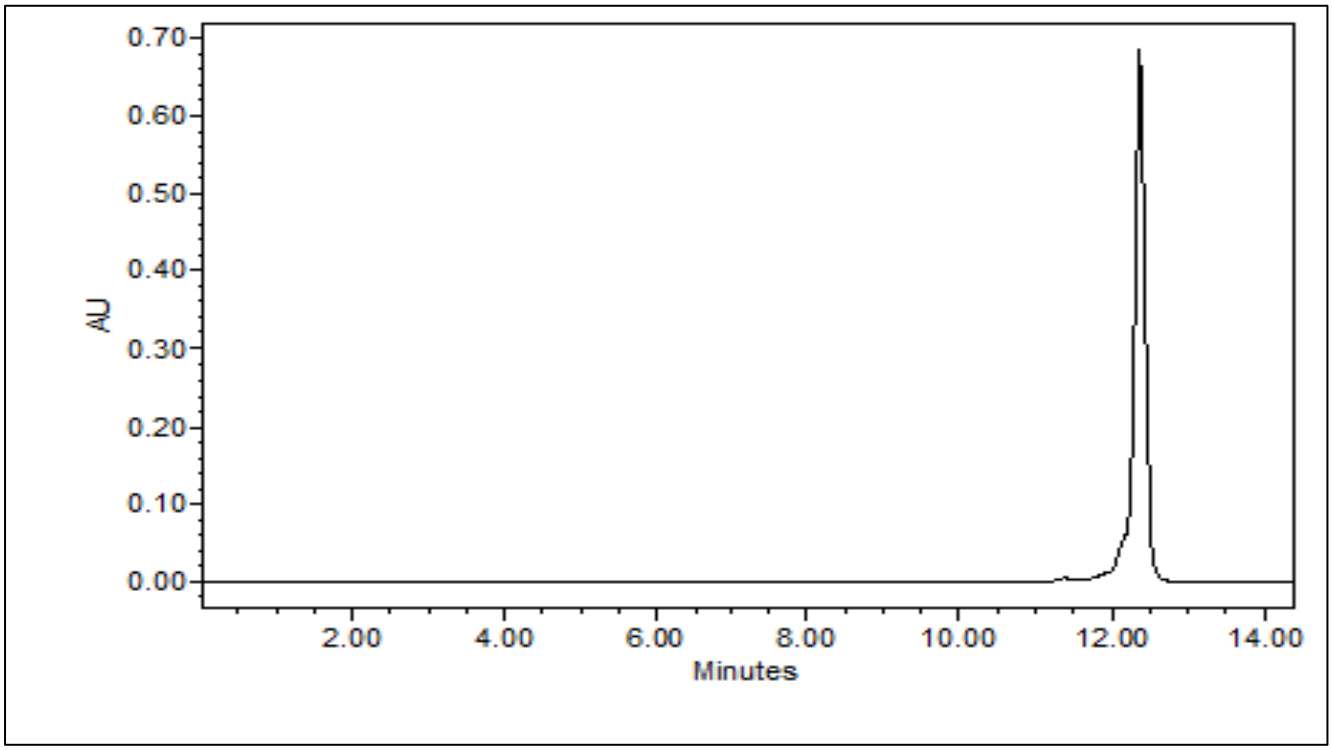


Figure S37. MALDI-TOF MS of PNA3

Calculated Mass = 3119.1

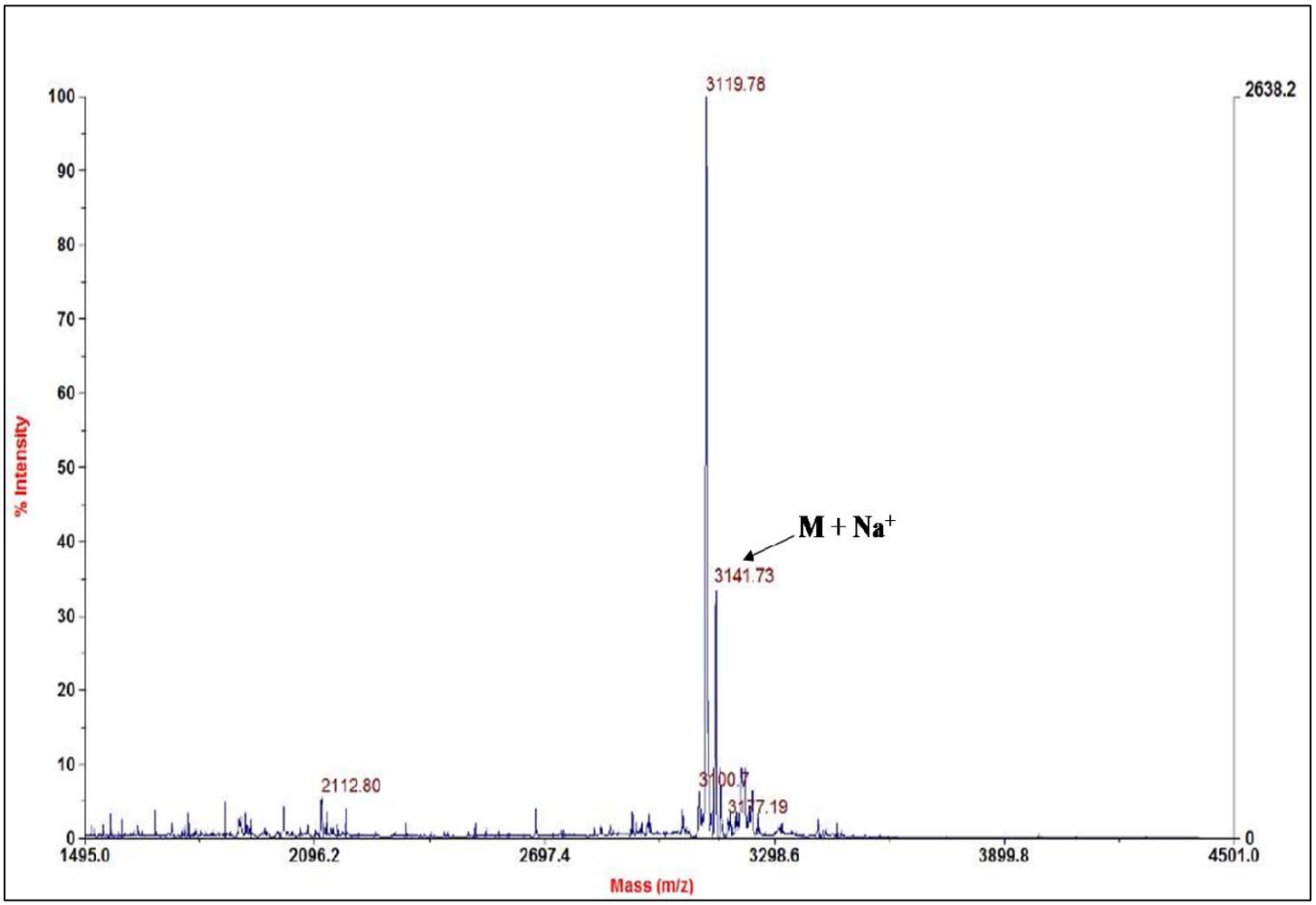


Figure S38. Reinjected HPLC trace of PNA4

PNA 4: H-G<u>C</u>A<u>T</u>G<u>TT</u>GA-NH_2

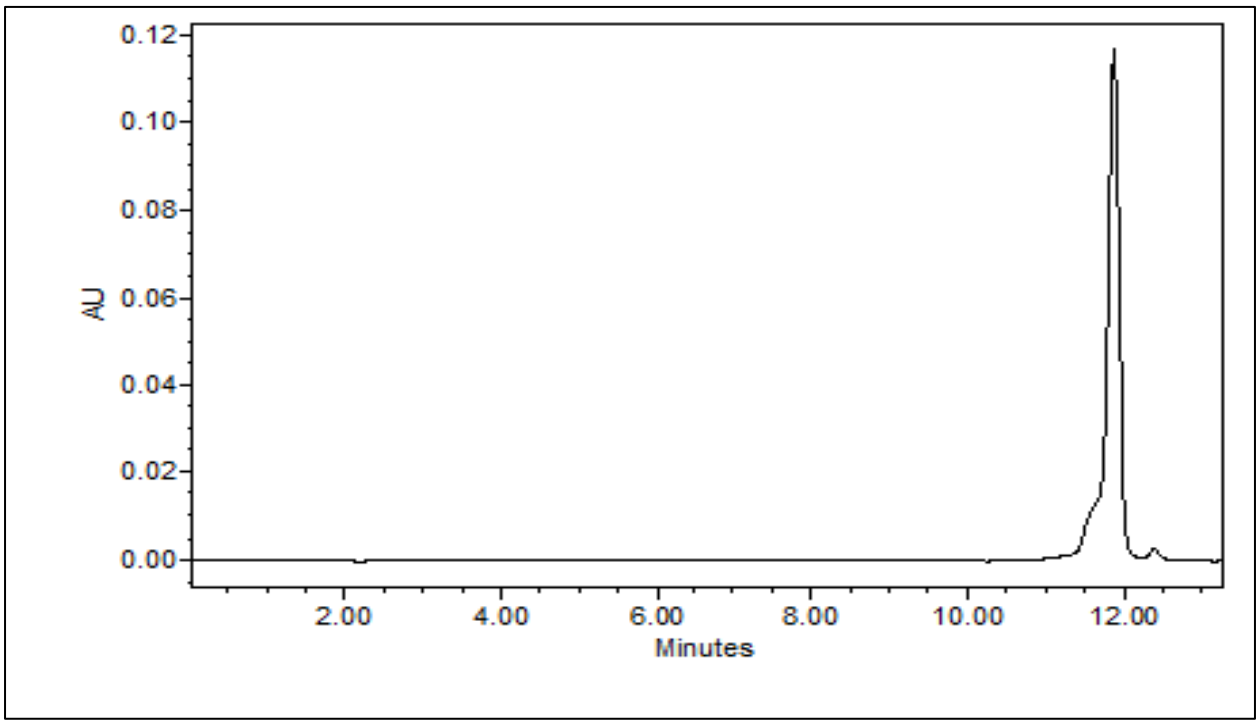


Figure S39. MALDI-TOF MS of PNA4

Calculated Mass = 3201.1

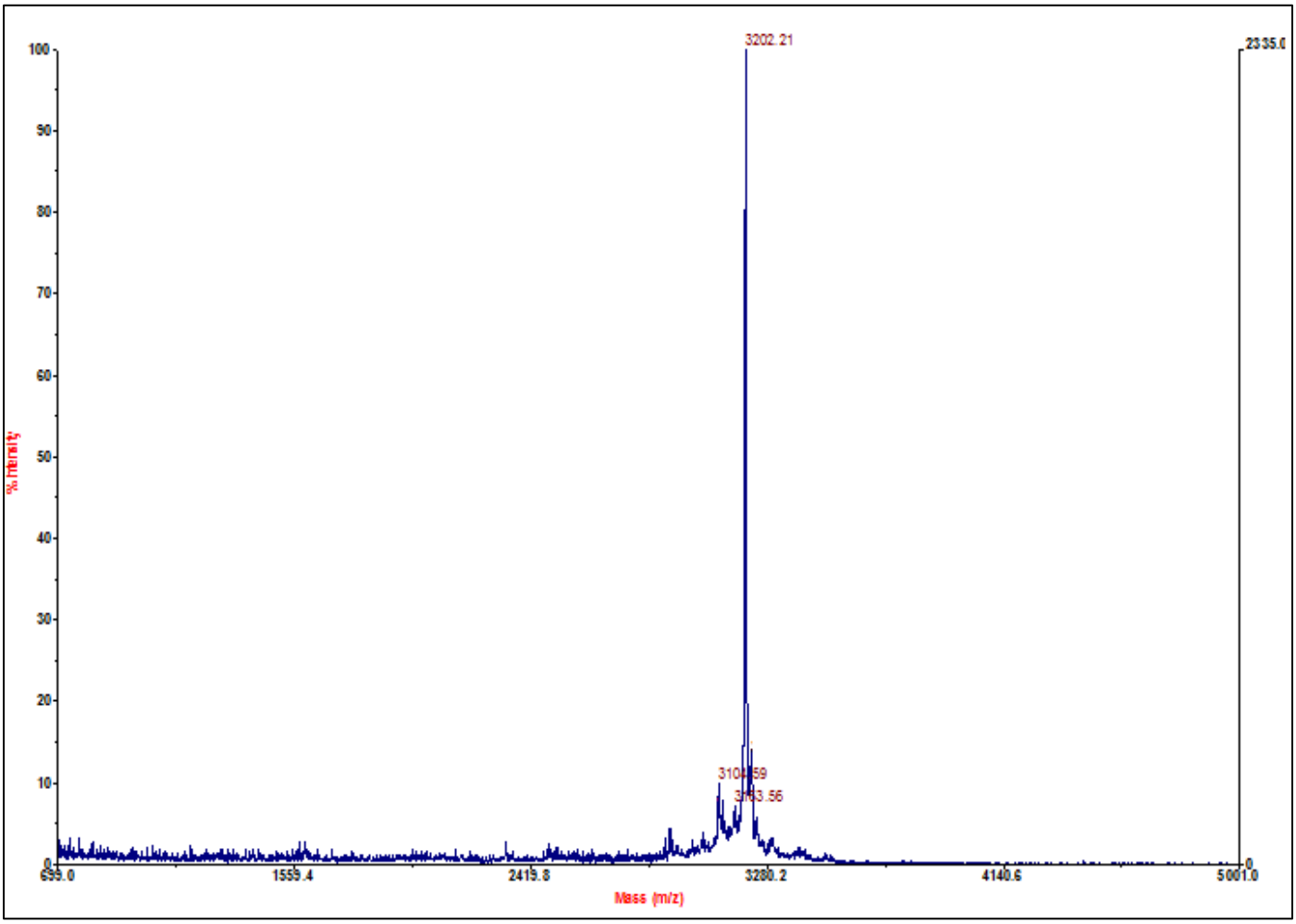


Figure S40. Reinjected HPLC trace of PNA5

PNA 5: H-GCATGTTTGA-NH_2

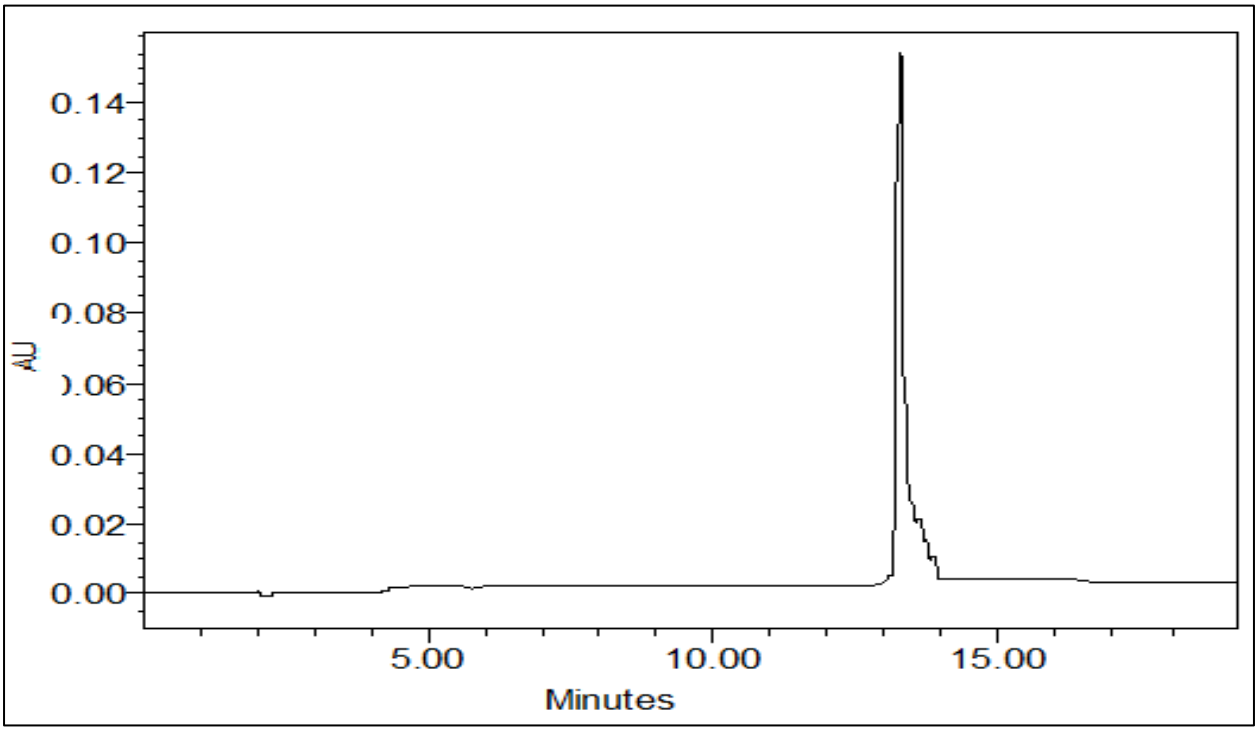


Figure S41. MALDI-TOF MS of PNA5

Calculated Mass = 3945.2

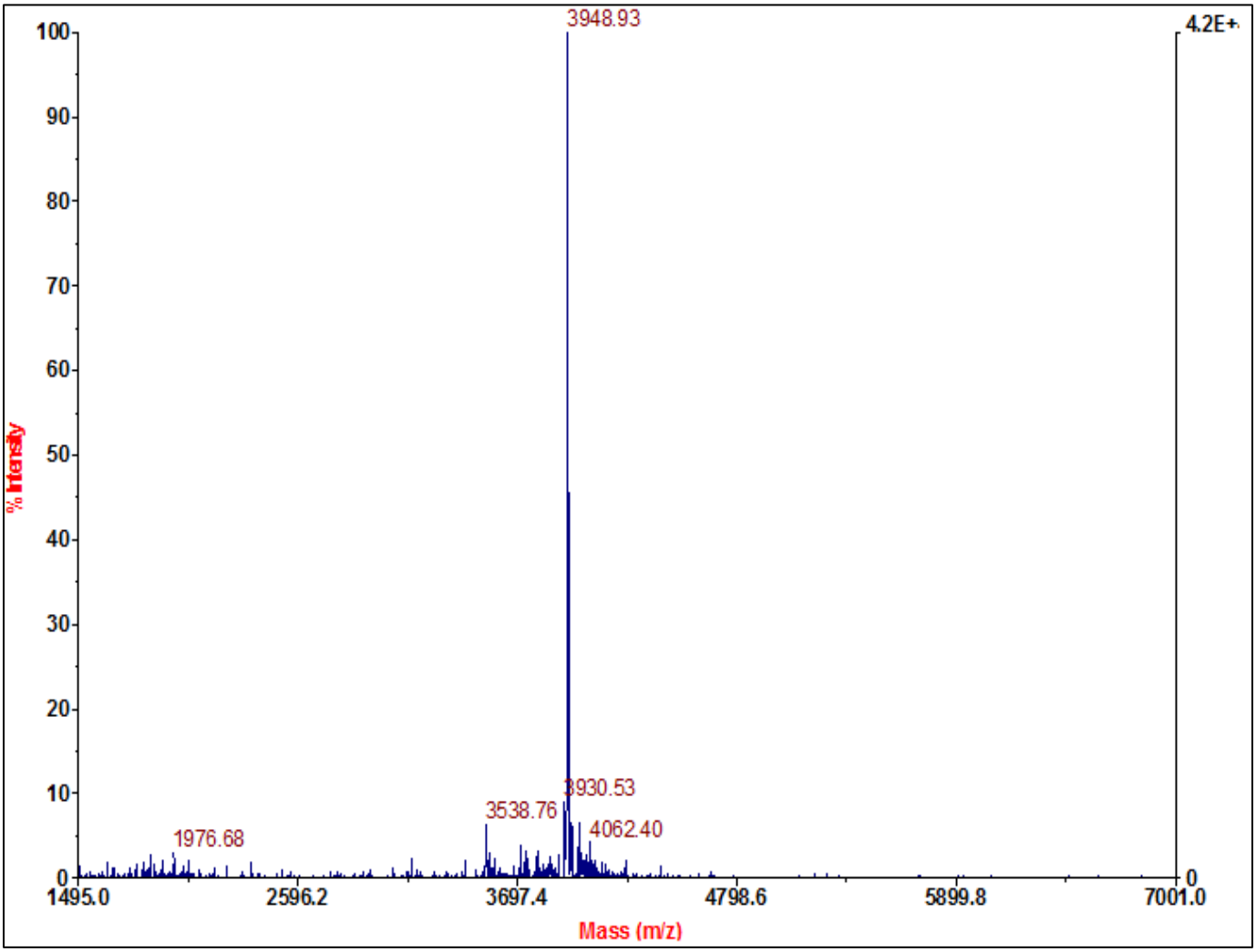


Figure S42. Reinjected HPLC trace of PNA6

H-ACGGGTAGAATAACAT-NH_2

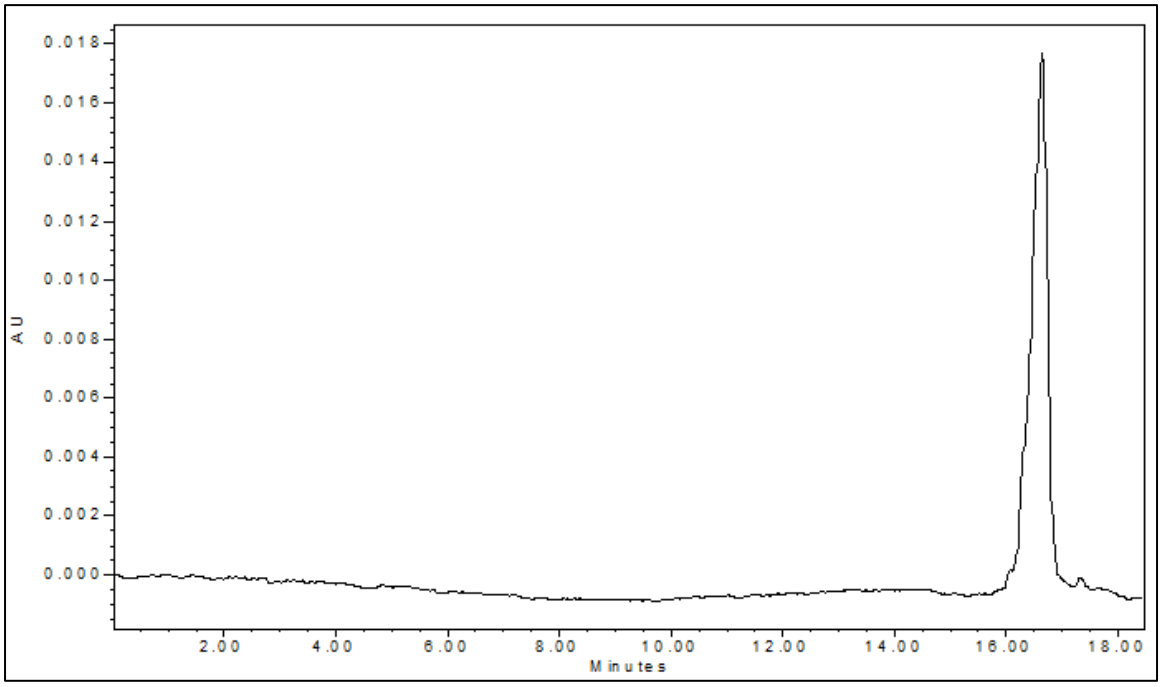


Figure S43. MALDI-TOF MS of PNA6

Calculated Mass: 4421.12

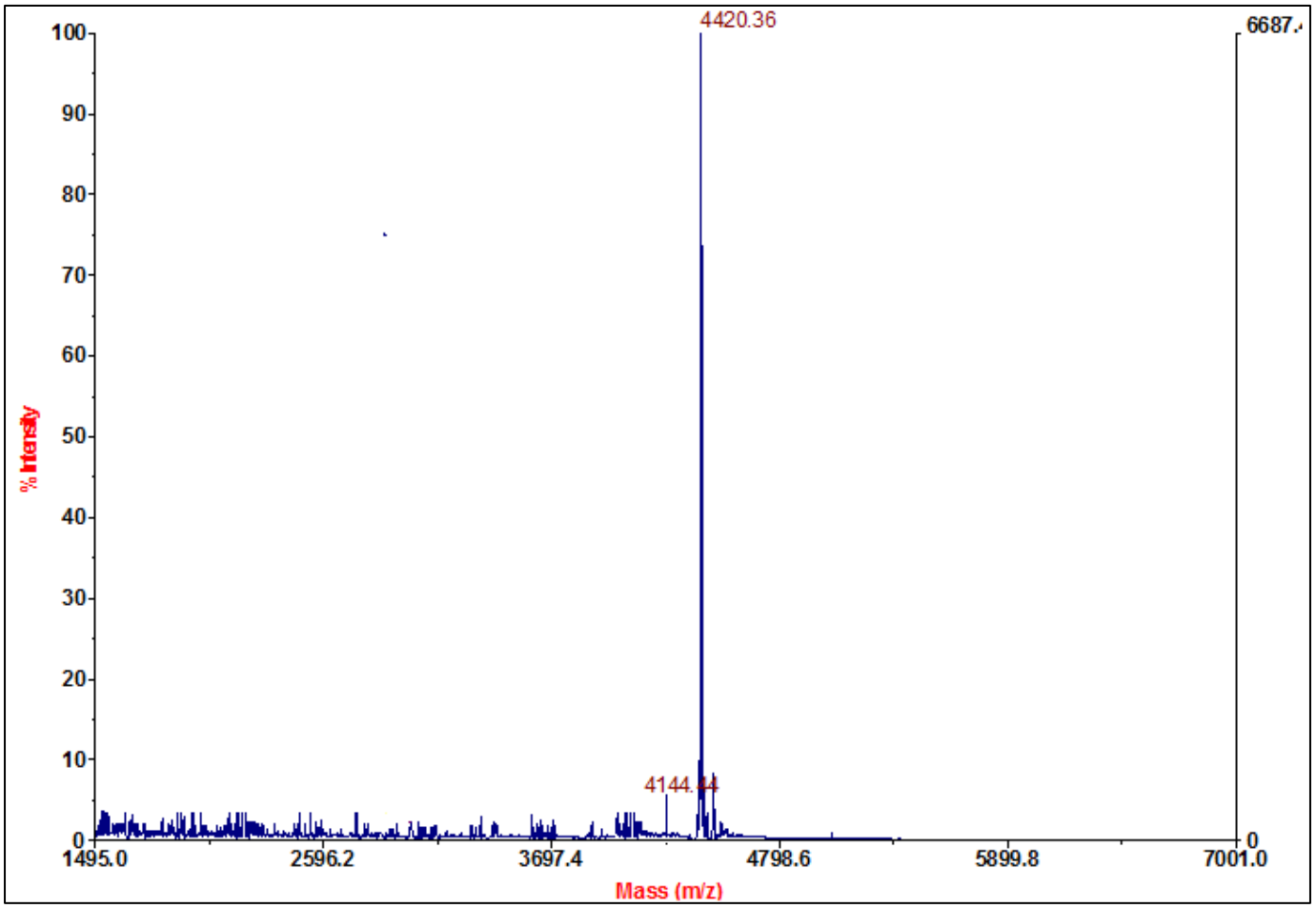


Figure S44. Reinjected HPLC trace of PNA7

PNA 7. H-ACGGGTAGAATAACAT-NH_2

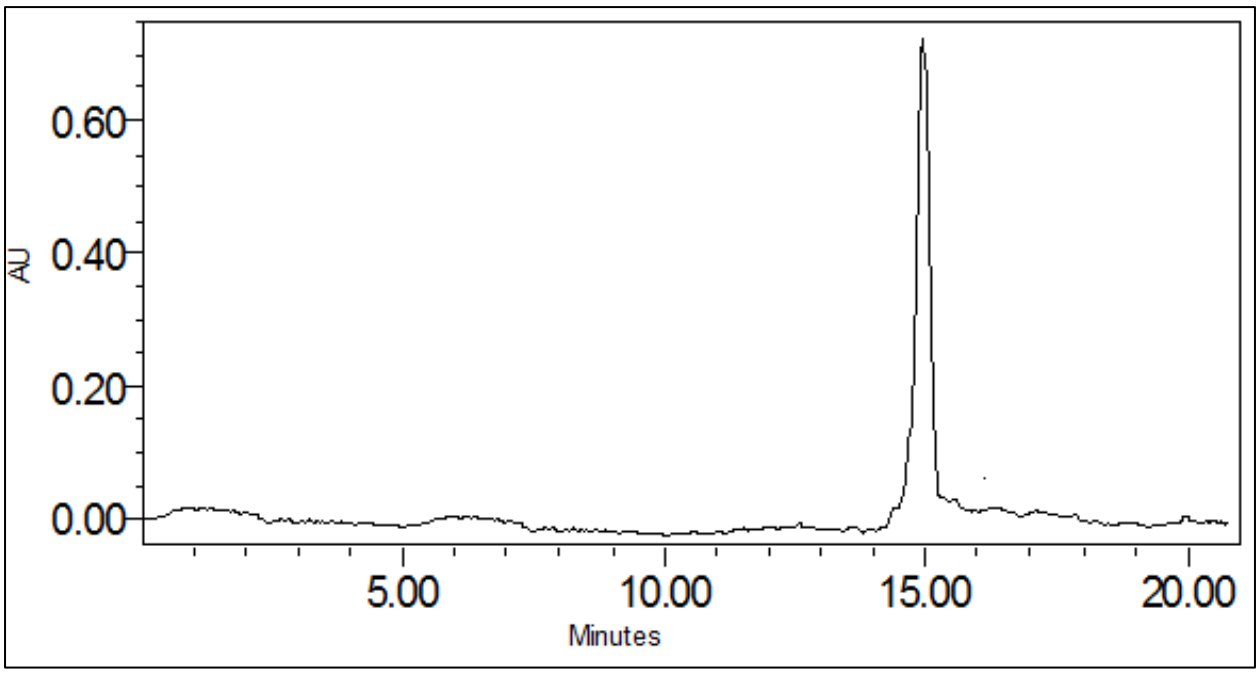


Figure S45. MALDI-TOF MS of PNA7

Calculated Mass = 4539.1

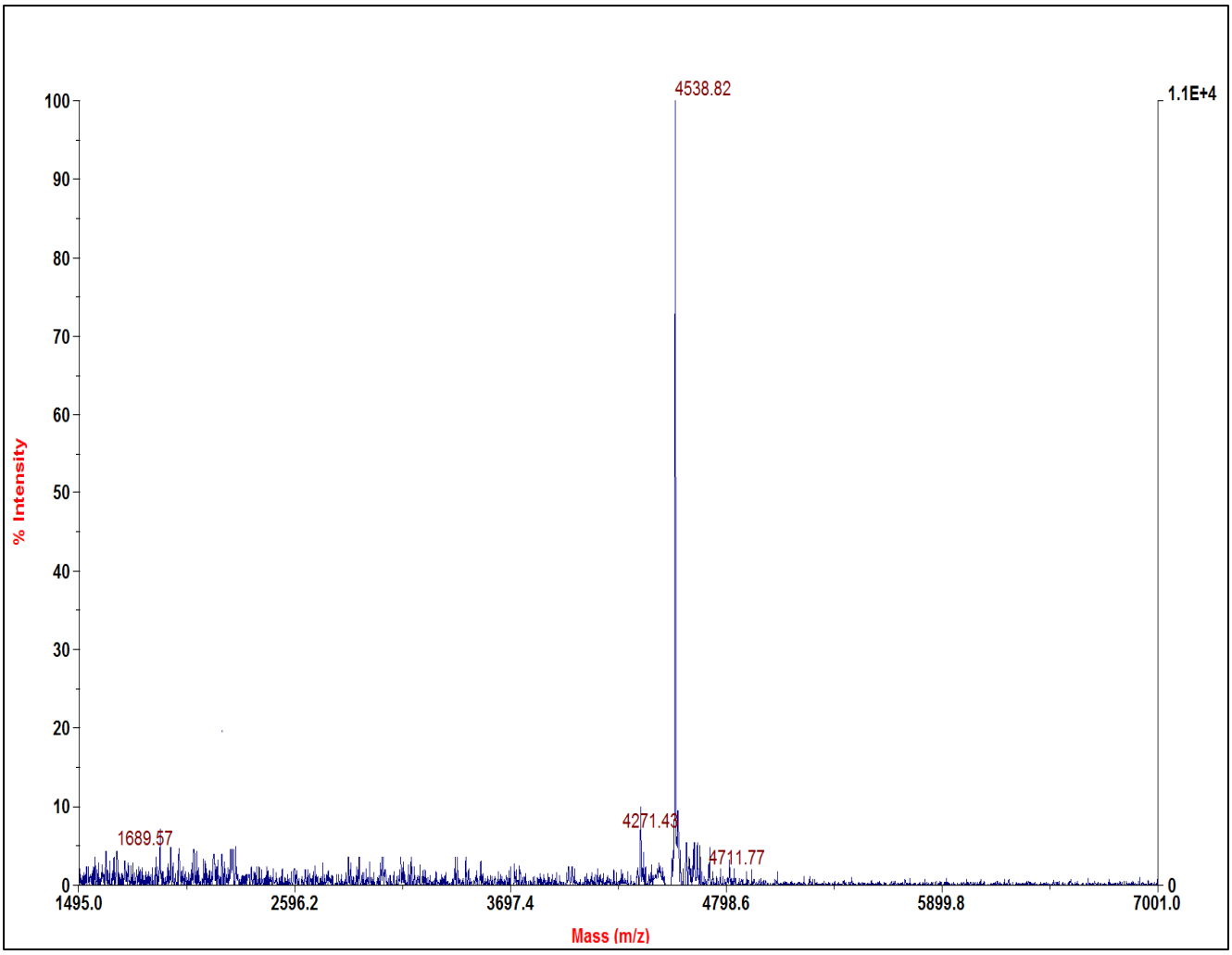


Figure S46. Reinjected HPLC trace of PNA8

PNA 8. H-ACGGGTAGAATAACAT-NH_2

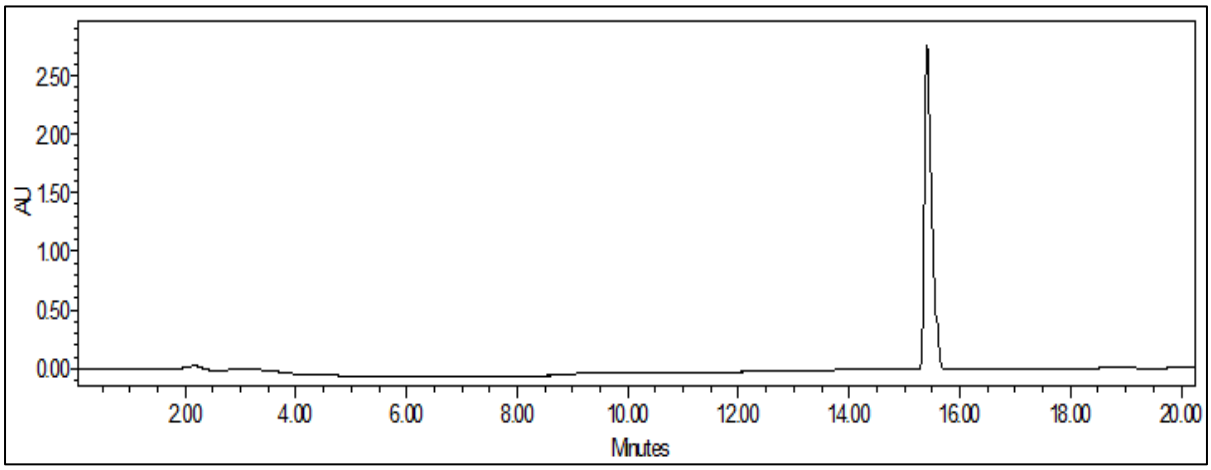


Figure S47. MALDI-TOF MS of PNA8

Calculated Mass = 4783.1

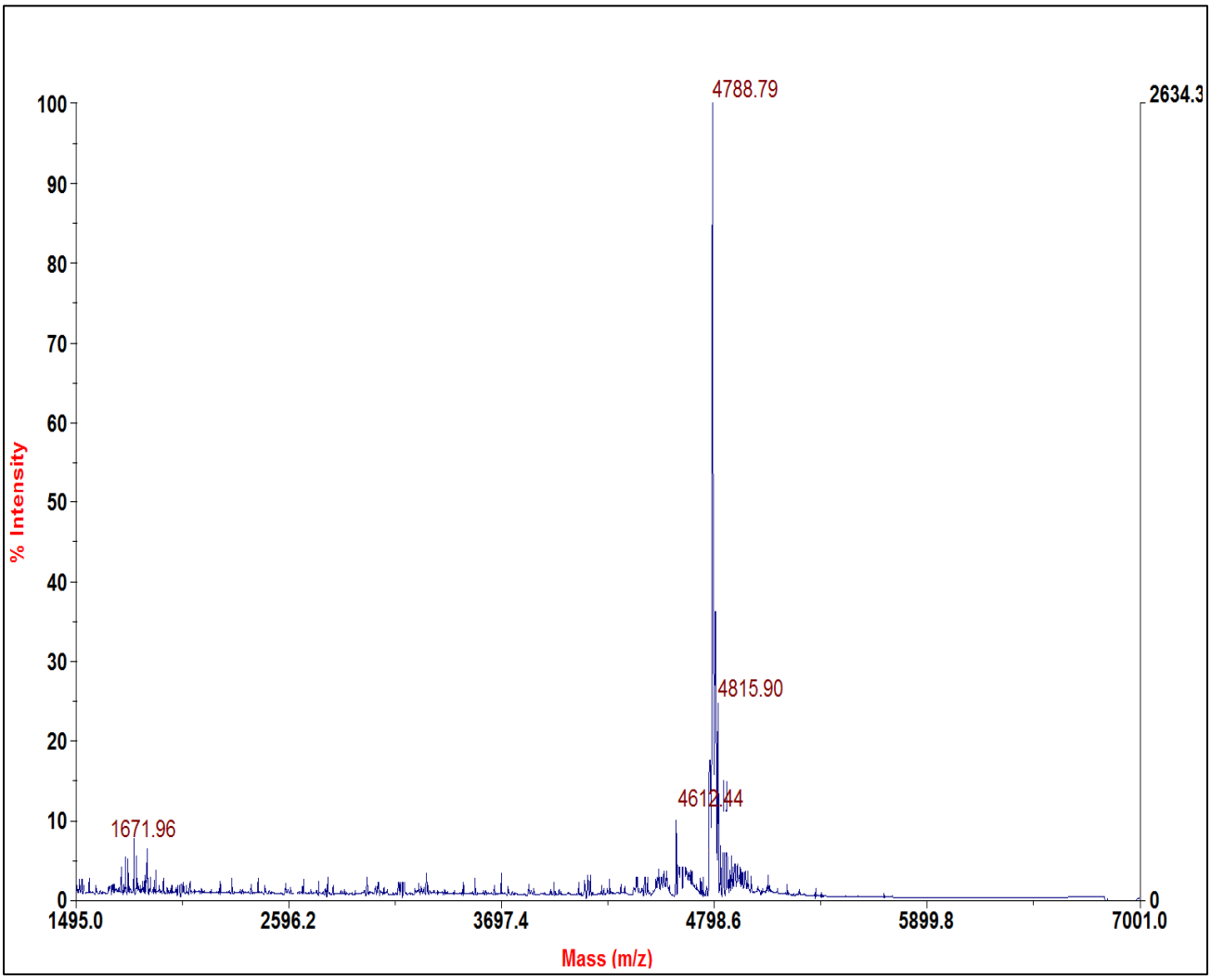


Figure S48. Reinjected HPLC trace of PNA9

H-AC<u>G</u>GG<u>T</u>AG<u>A</u>AT<u>A</u>AC<u>A</u>T-NH_2

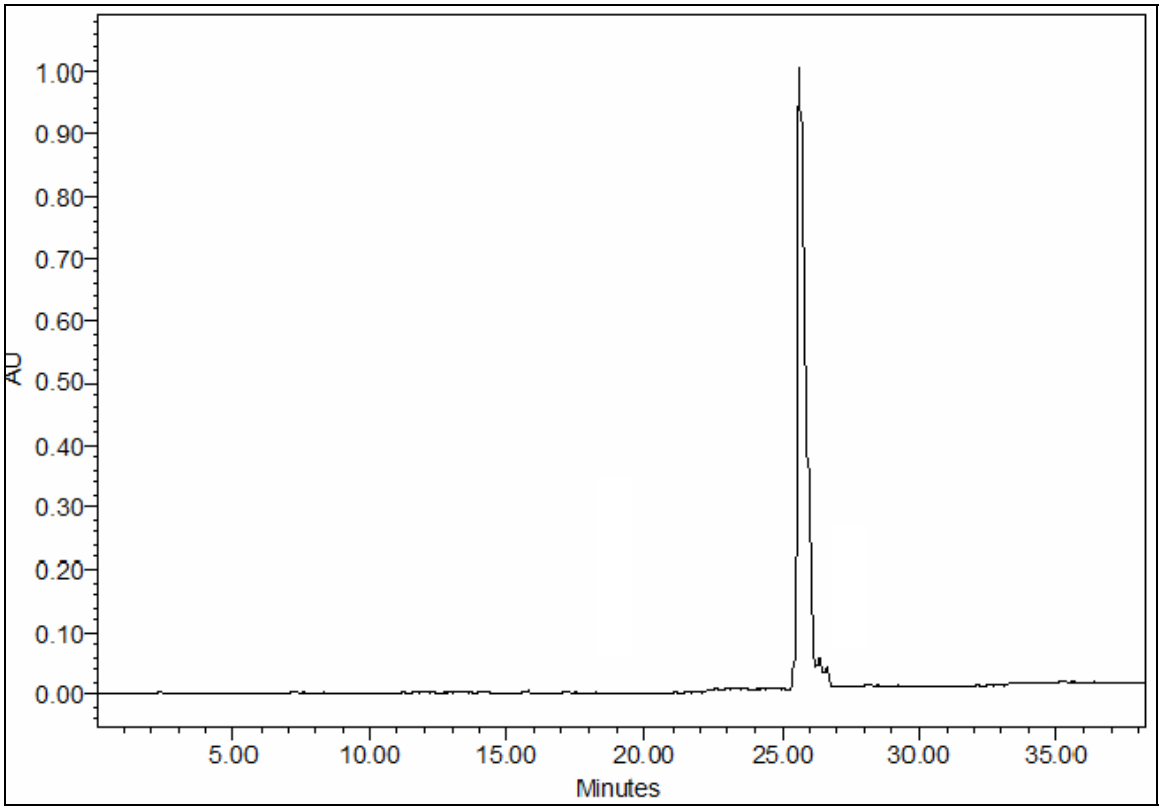


Figure S49. MALDI-TOF MS of PNA9

Calculated Mass = 5055.1

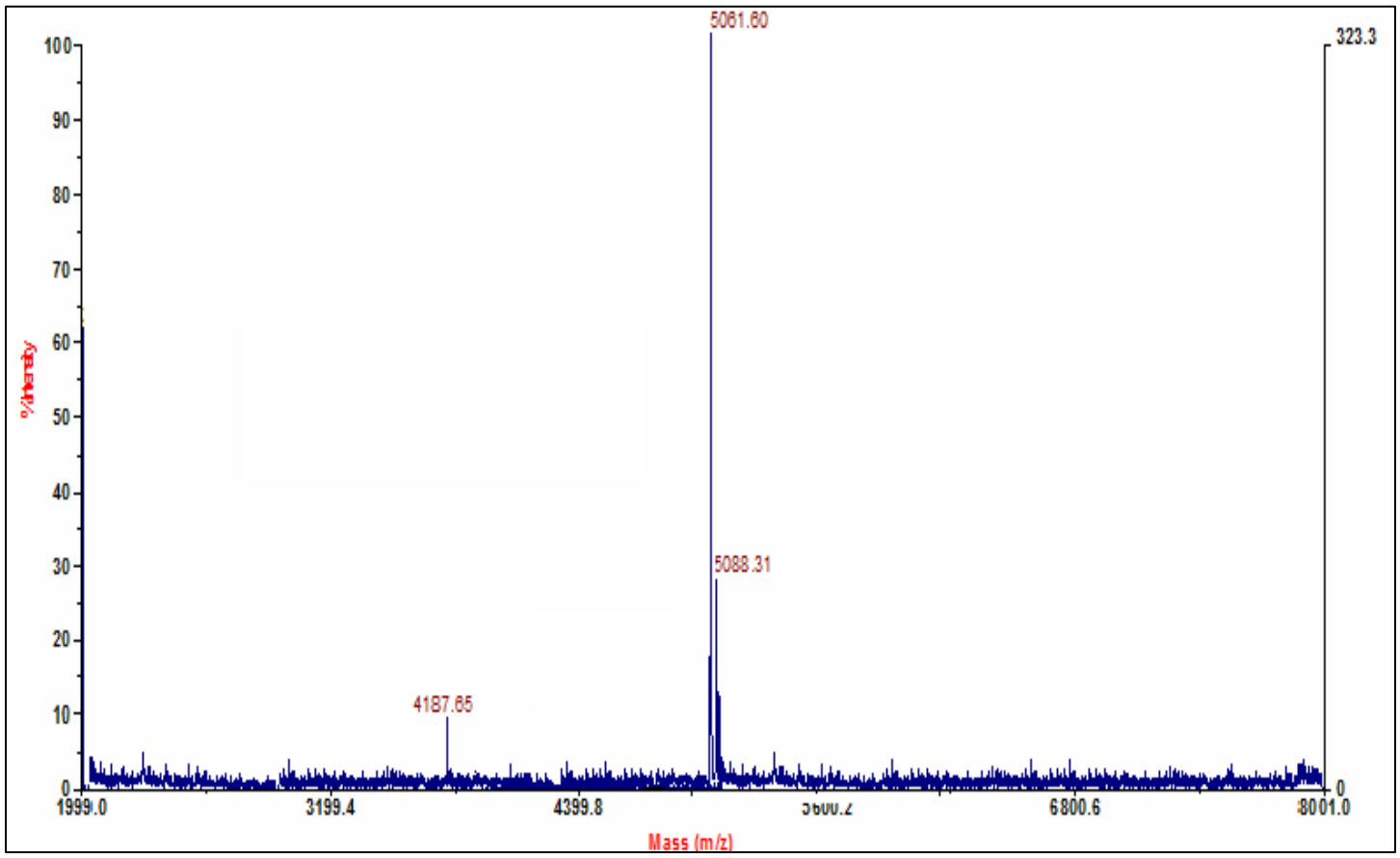


Figure S50. Reinjected HPLC trace of PNA10

H-A<u>C</u><u>G</u><u>G</u>T<u>A</u>G<u>A</u><u>A</u>T<u>A</u><u>A</u><u>C</u>A<u>T</u>-NH_2

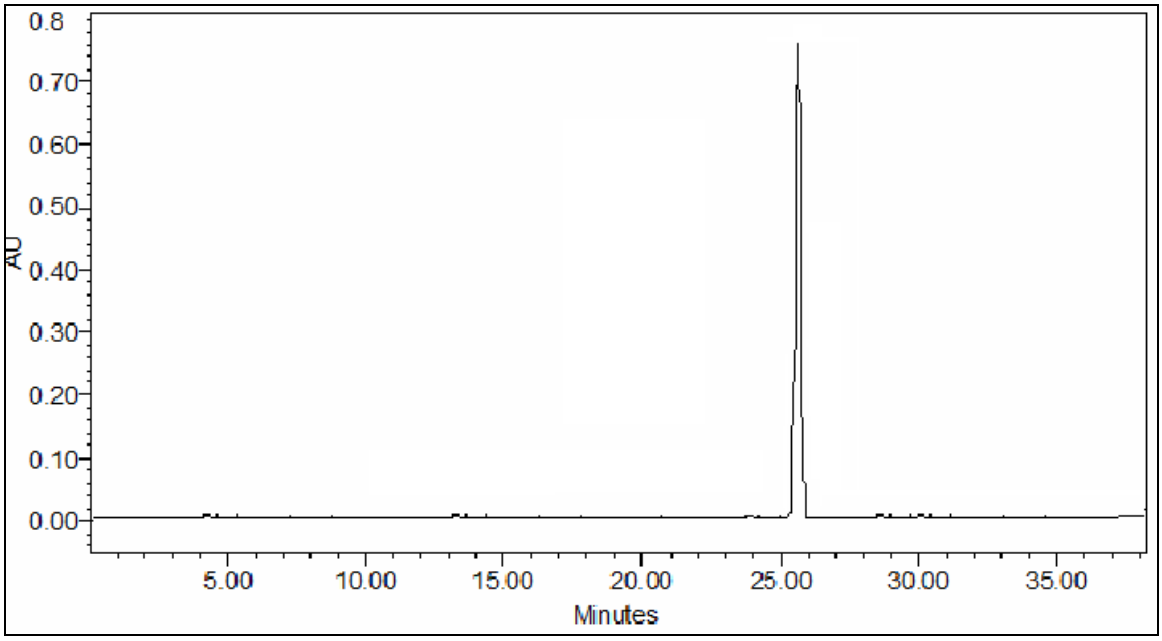


Figure S51. MALDI-TOF MS of PNA10

Calculated Mass = 5428.1

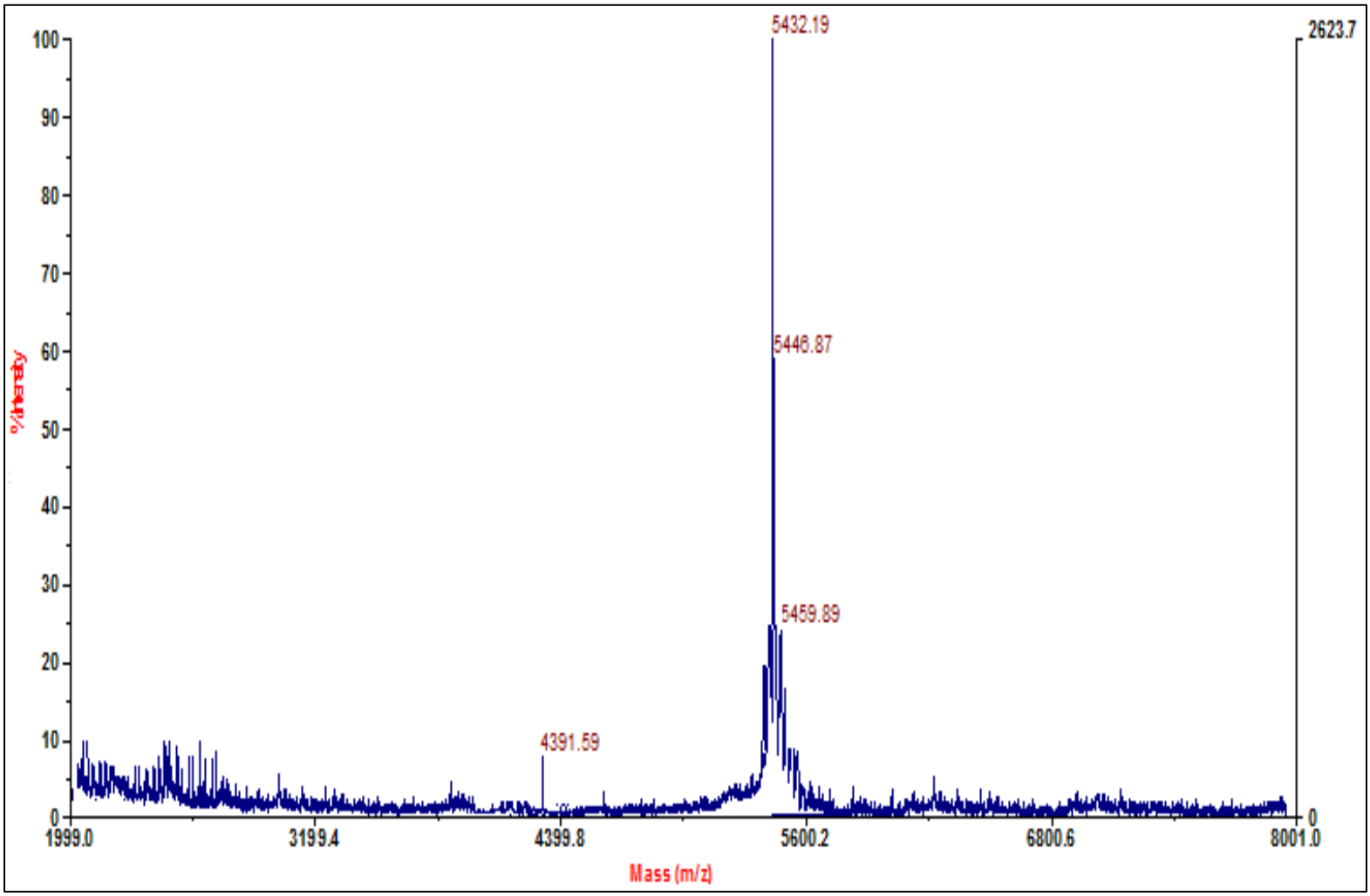


Figure S52. Reinjected HPLC trace of PNA1X

PNA 1X: H-^{L}Orn(X)-^{L}Lys-GCATGTTTGA-NH_2

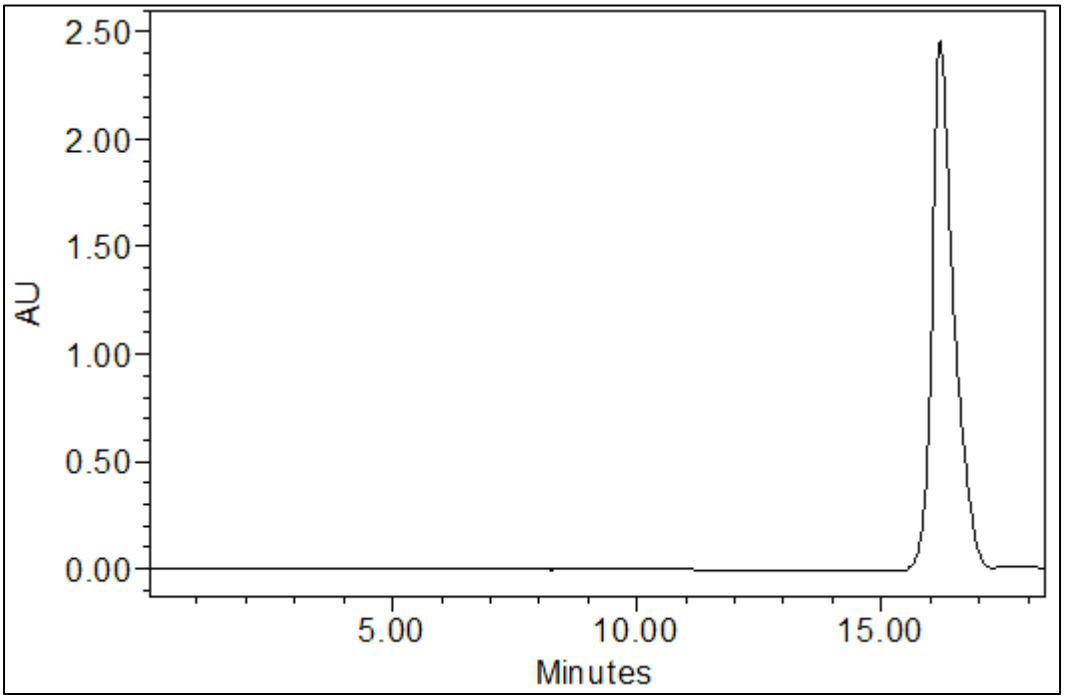


Figure S53. MALDI-TOF MS of PNA1X

Calculated Mass = 3360.1

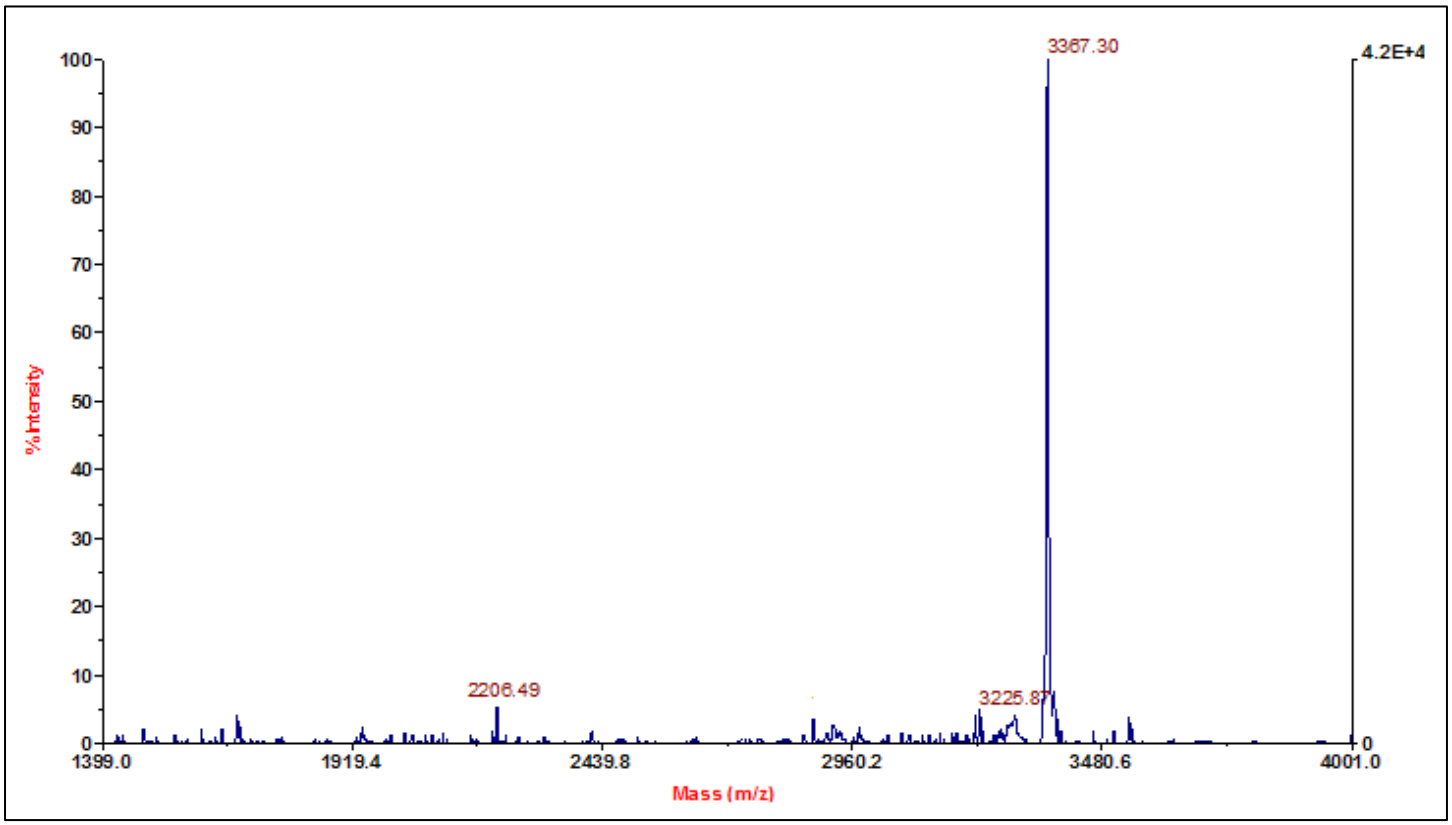


Figure S54. Reinjected HPLC trace of PNA1Y

H-^{L}Lys-GCATGTTTGA-^{L}Orn(Y)-NH_2

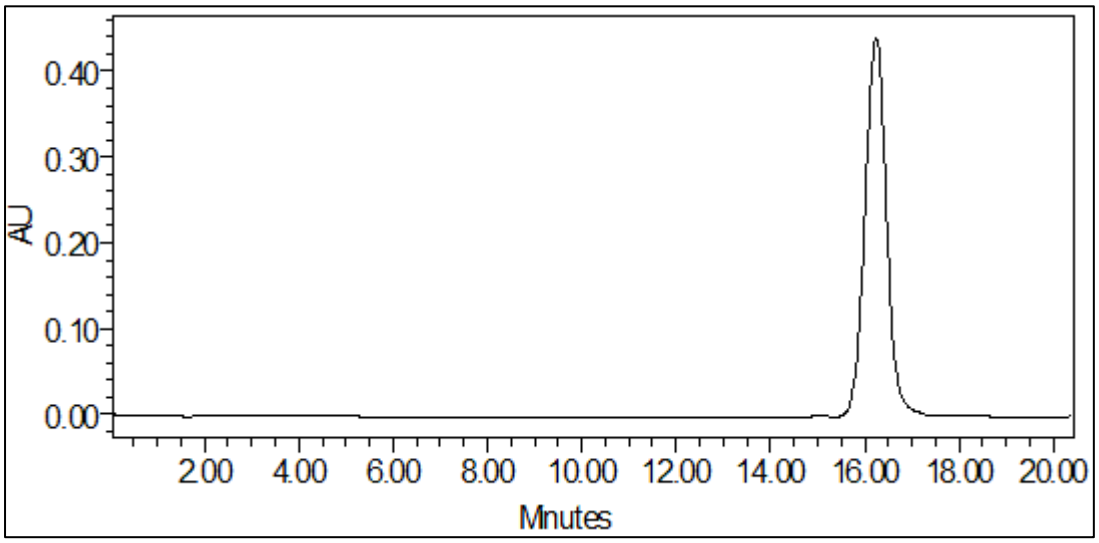


Figure S55. MALDI-TOF MS of PNA1Y

Calculated Mass = 3413.2

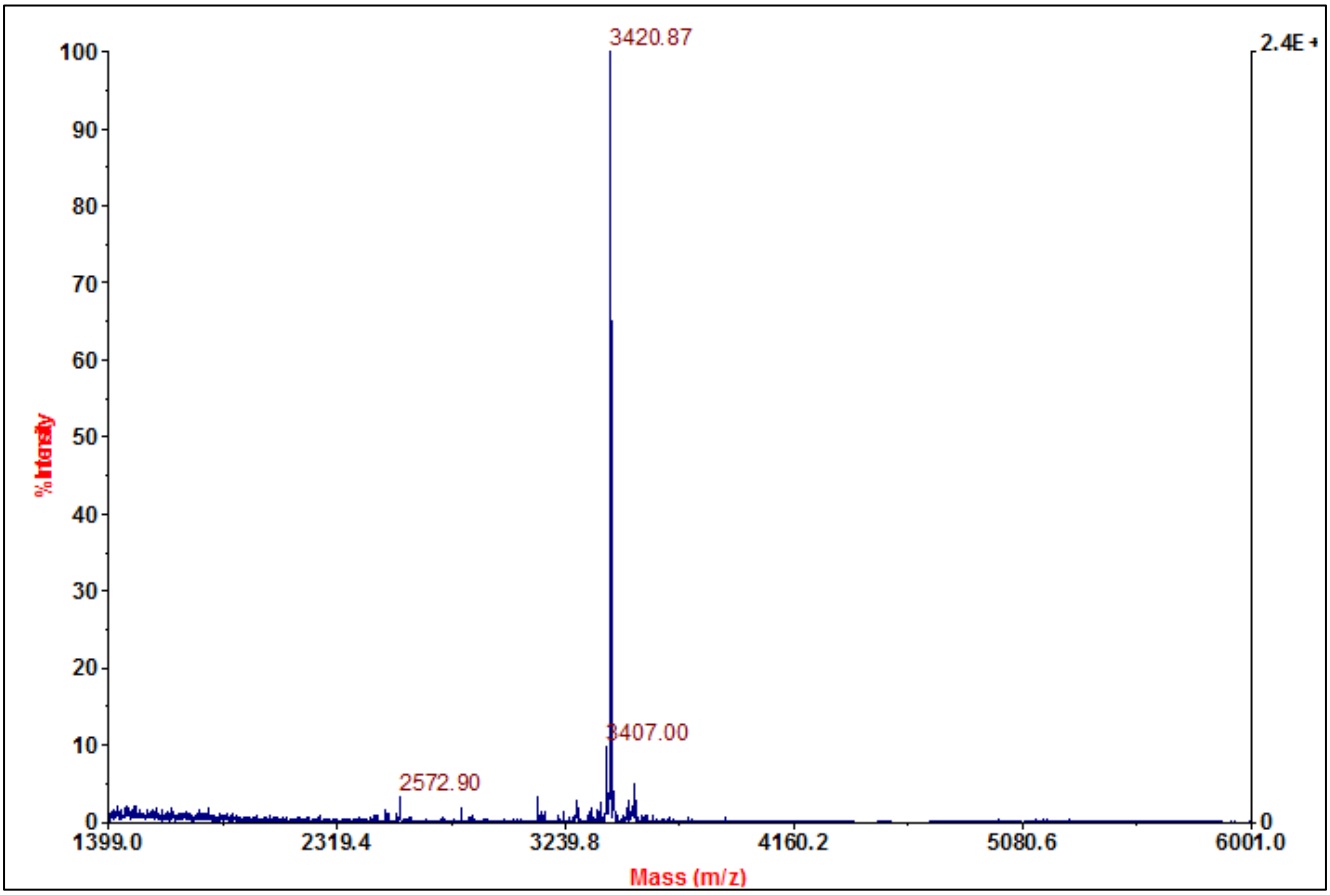


Figure S56. Reinjected HPLC trace of PNA4X

H-^{L}Orn(X)-^{L}Lys-G<u>C</u>A<u>T</u>G<u>T</u>T<u>T</u>G<u>A</u>-NH_2

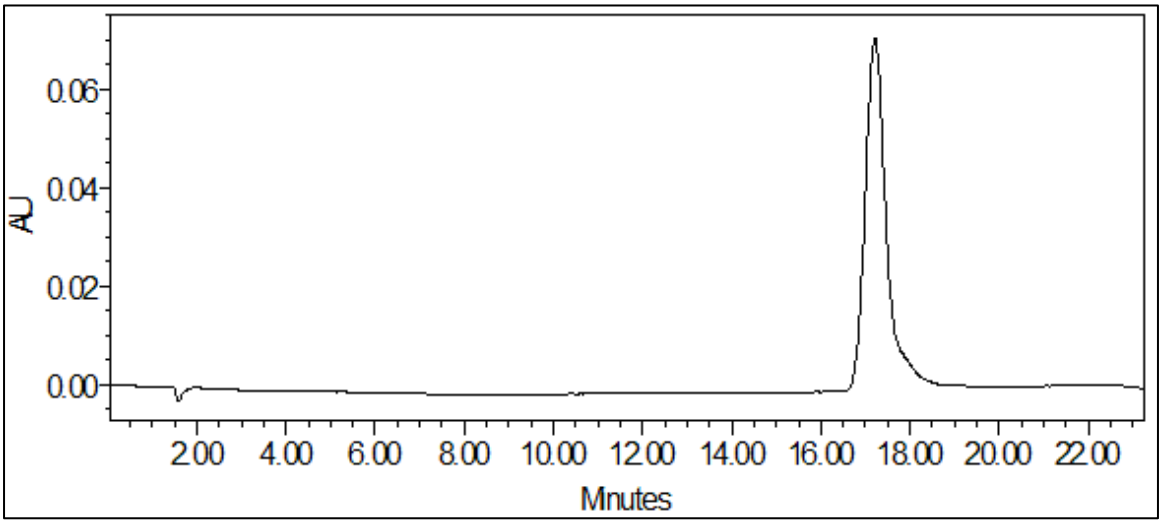


Figure S57. MALDI-TOF MS of PNA4X

Calculated Mass = 3964.1

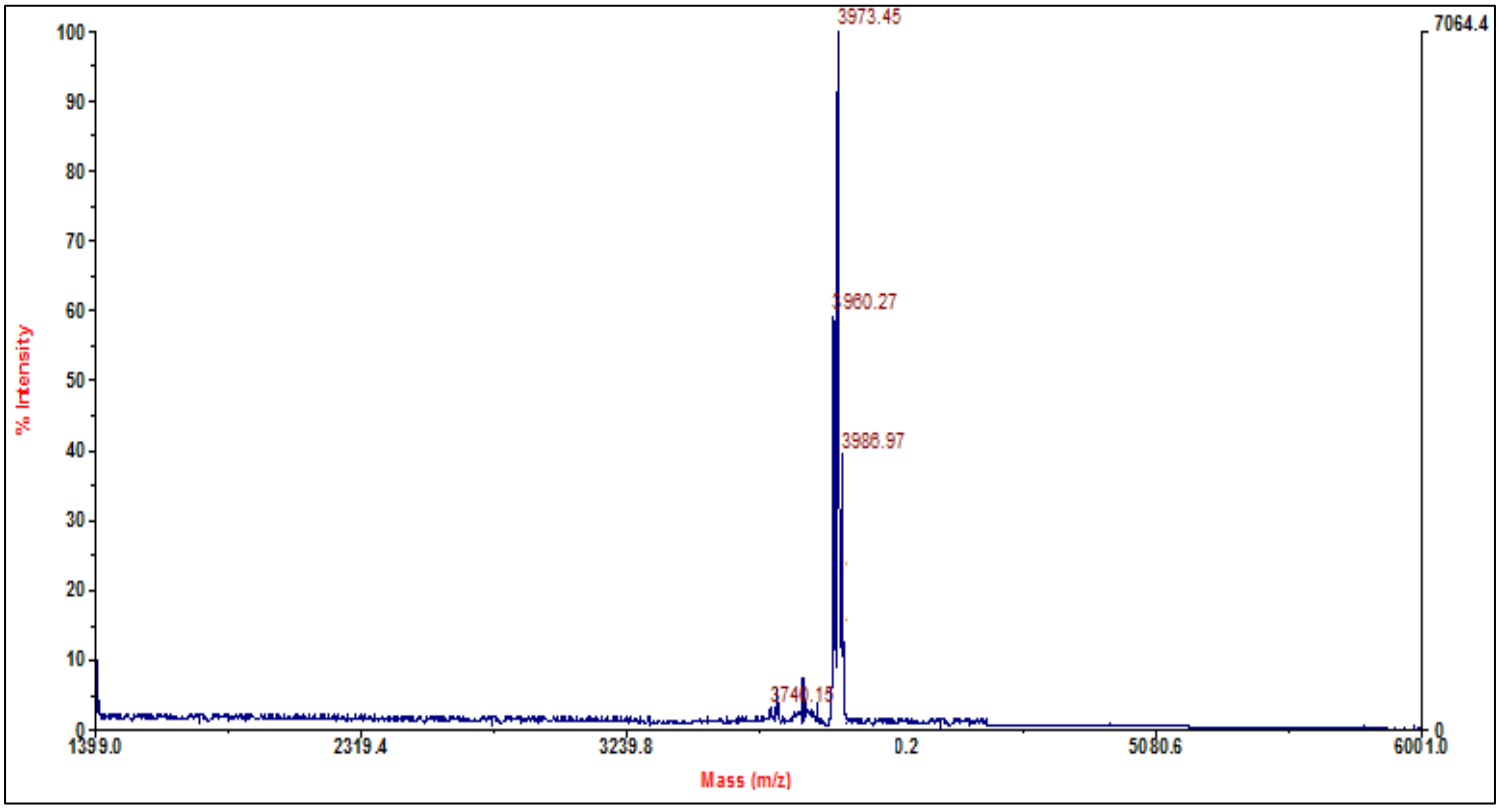


Figure S58. Reinjected HPLC trace of PNA4Y

H-^{L}Lys-G<u>C</u>A<u>T</u>G<u>T</u>T<u>T</u>G<u>A</u>-^{L}Orn(Y)-NH_2

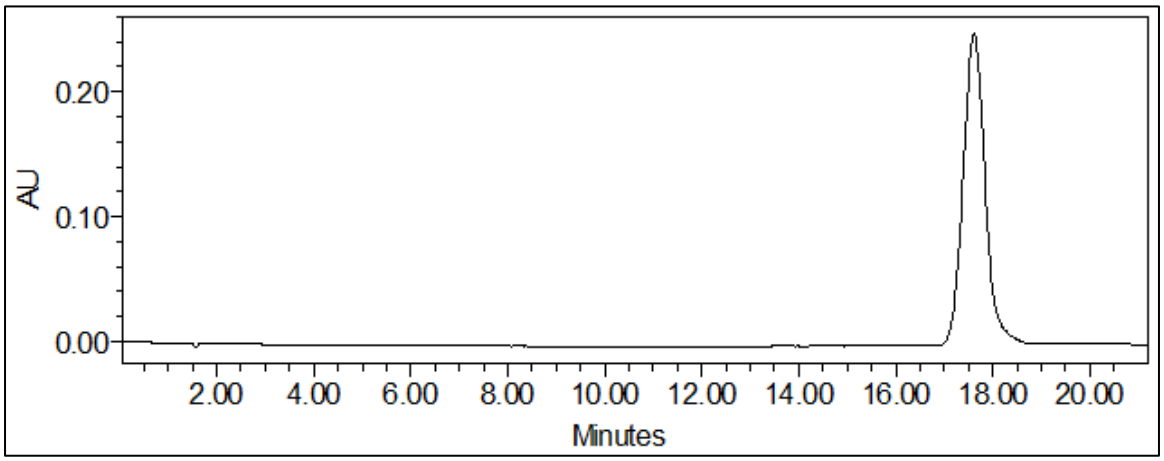


Figure S59. MALDI-TOF MS of PNA4Y

Calculated Mass = 4010.2

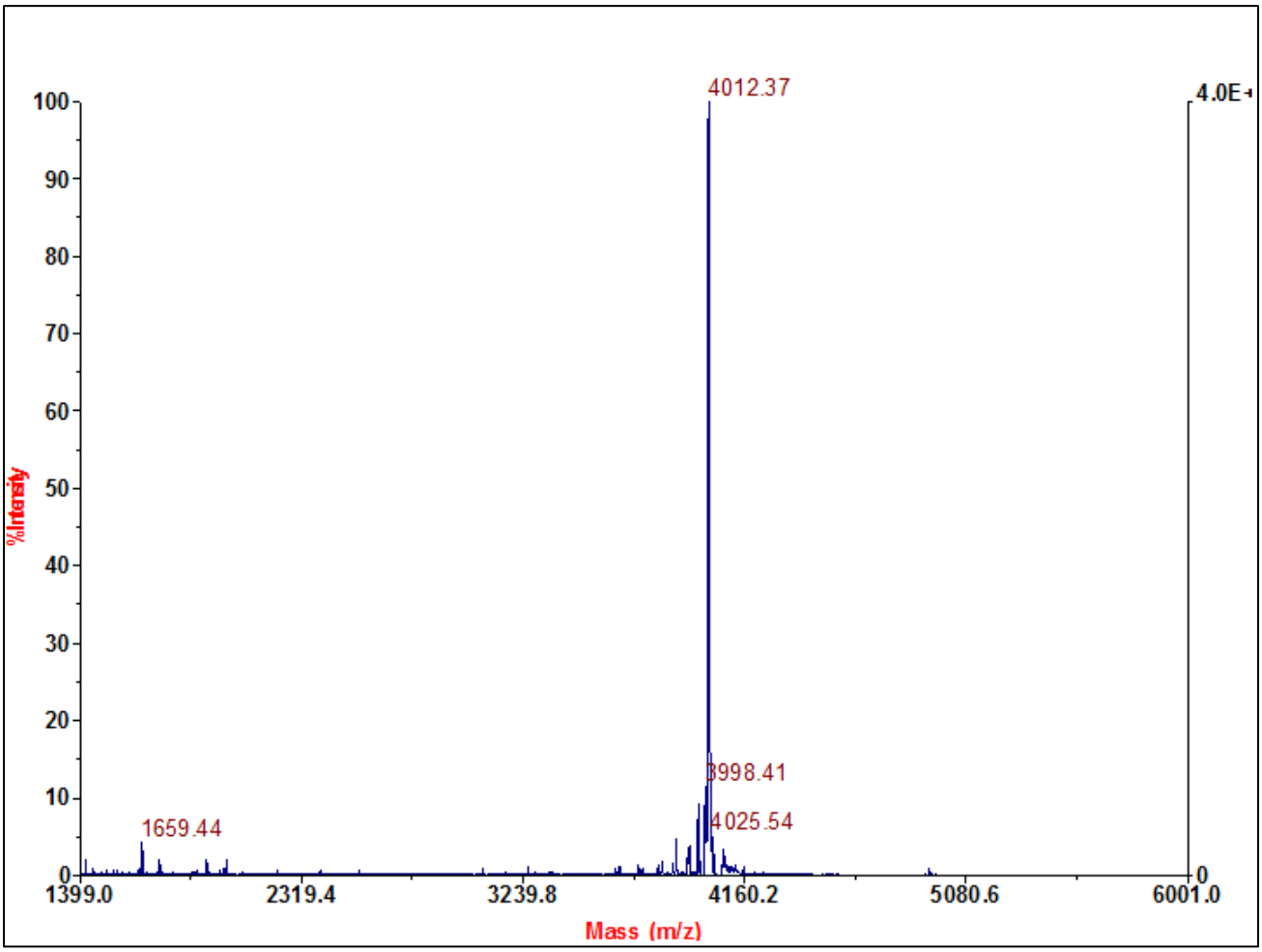


Figure S60. UV-melting curves of PNA5 with perfect match and mismatch DNA

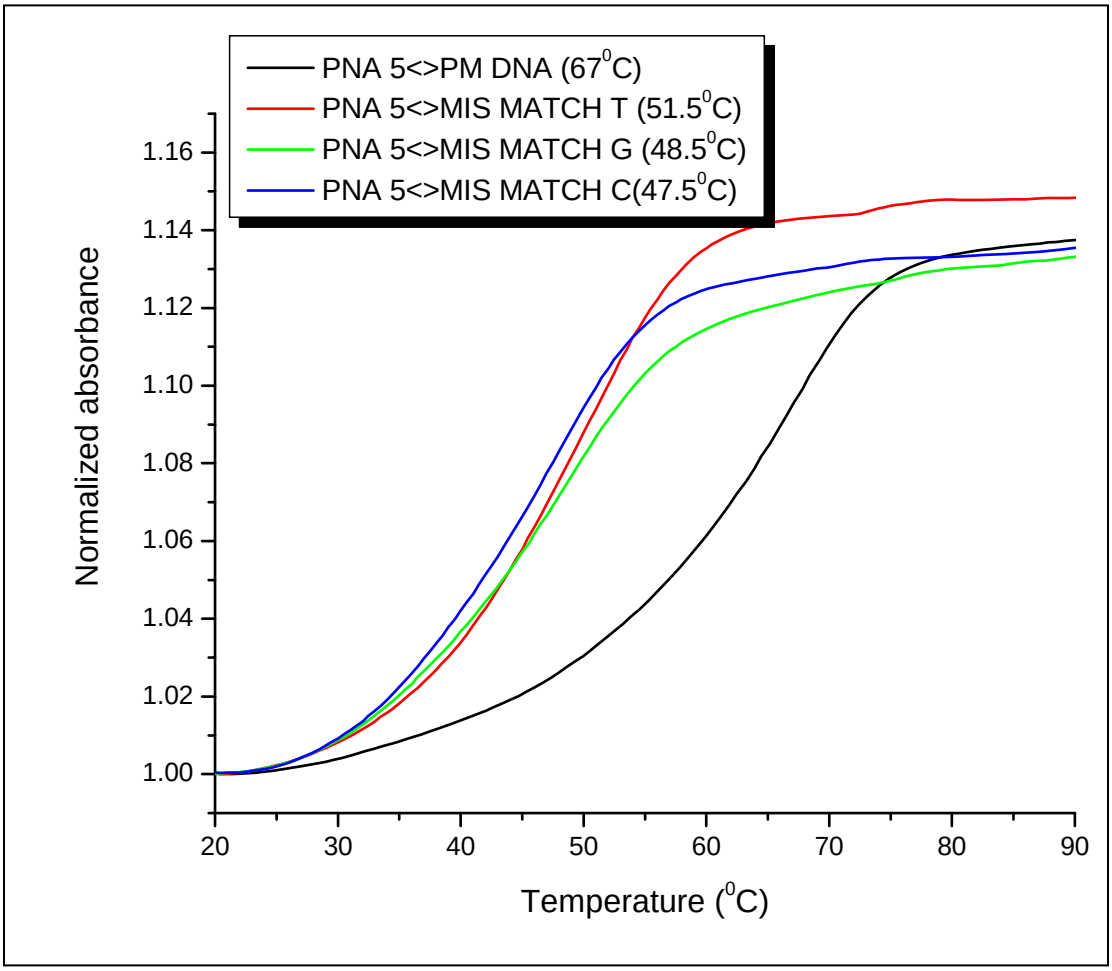


Figure S61. UV-melting curves of PNA5 with perfect match and mismatch RNA

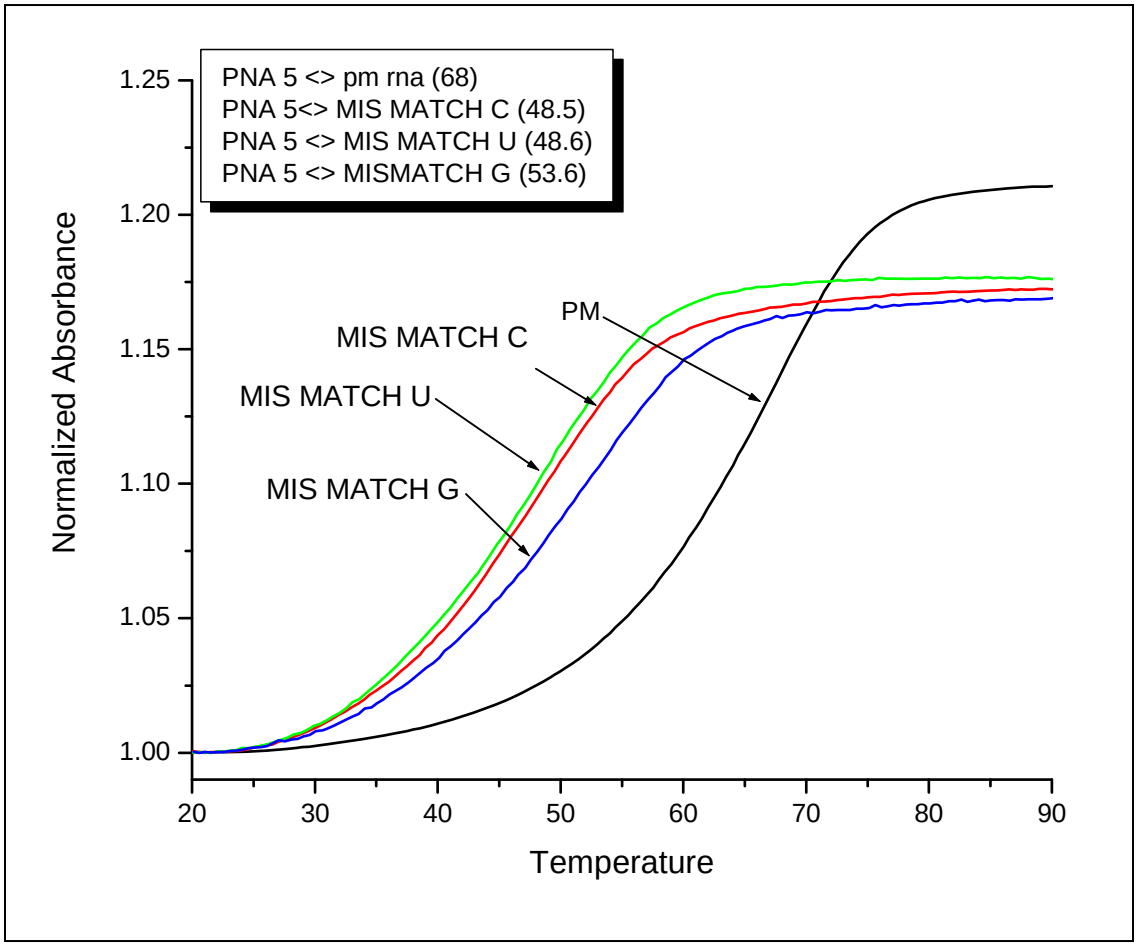


Figure S62. CD-melting curve of PNA2 (monitored at 260 nm, 5 μM strand concentration, prepared in 10 mM NaPi buffer at pH 7.4)

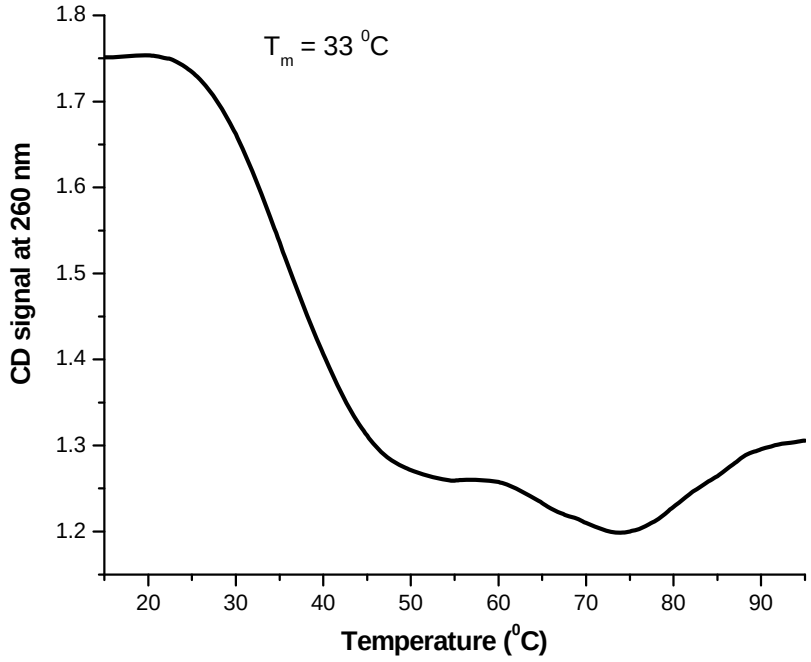


Figure S63. CD-melting curve of PNA3 (monitored at 260 nm, 5 μM strand concentration, prepared in 10 mM NaPi buffer at pH 7.4)

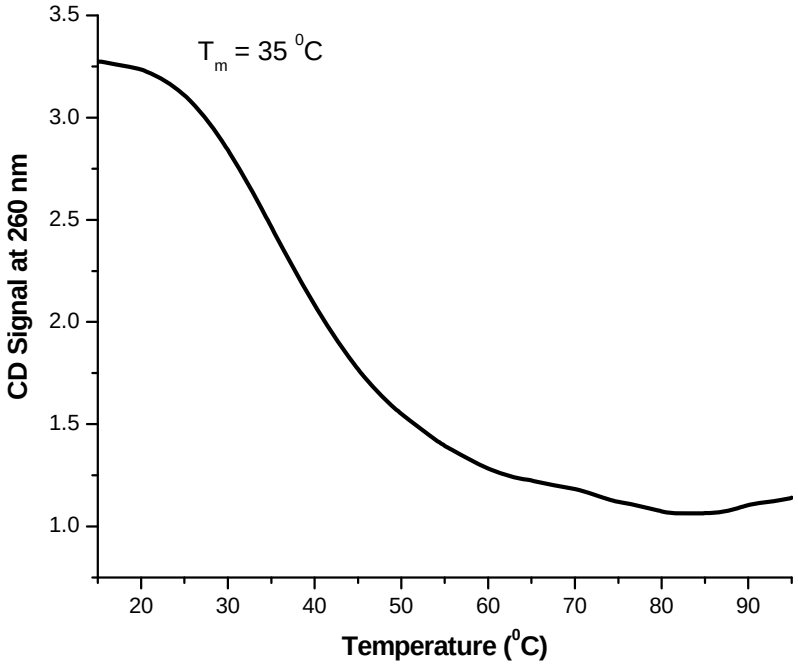


Figure S64. CD-melting curve of PNA4 (monitored at 260 nm, 5 μM strand concentration, prepared in 10 mM NaPi buffer at pH 7.4)

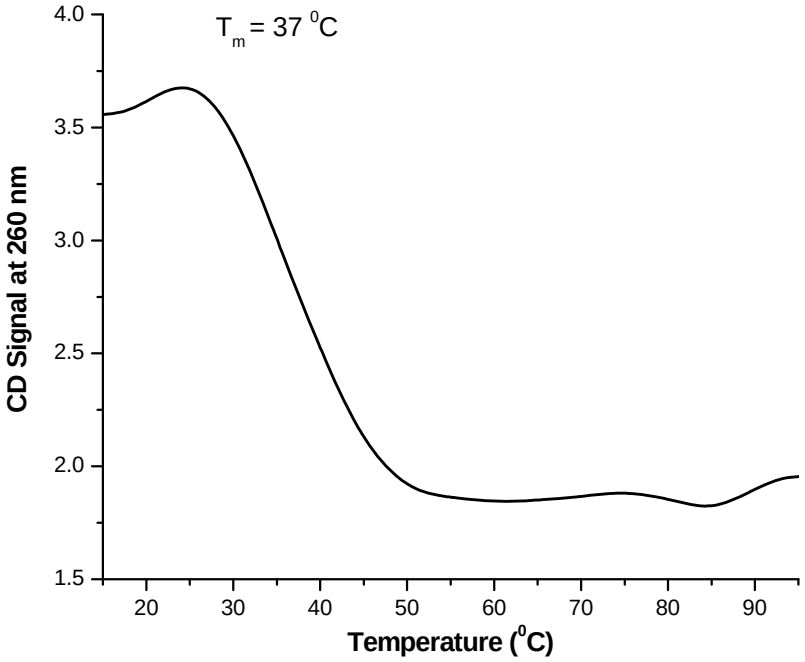


Figure S65. CD-melting curve of PNA5 (monitored at 260 nm, 5 μM strand concentration, prepared in 10 mM NaPi buffer at pH 7.4)

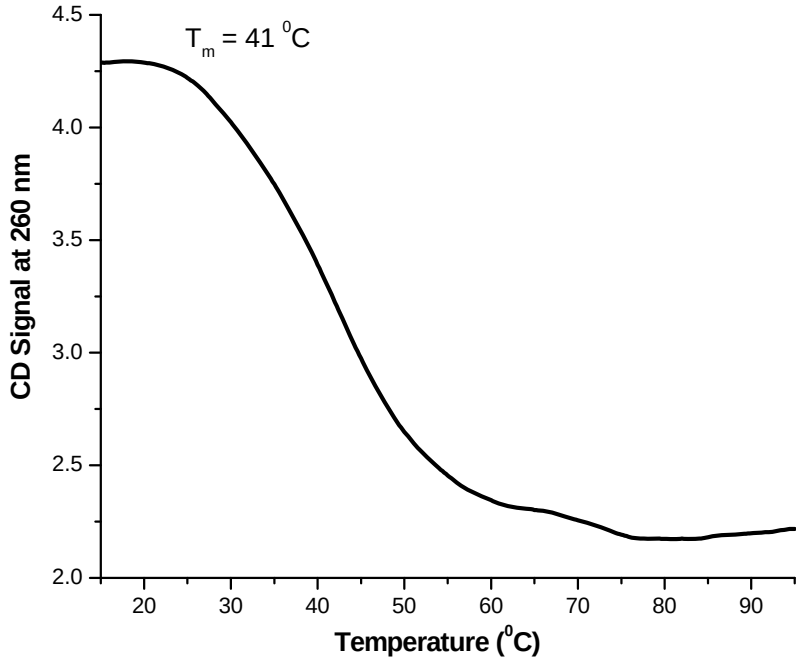


Figure S66. SPR sensorgrams (solid black lines) and fits (red dotted lines) for hybridization of PNA probes to immobilized perfect match DNA target. Solutions contained 10-50 nM PNA in 10 nM increments. Error bars at t = 420 sec illustrate standard deviations for three separate trials.