Biophysical Properties, Thermal Stability, and Functional Impact of 8-oxo-7,8-dihydroguanine on Oligonucleotides of RNA. A Study of Duplex, Hairpins, and the Aptamer for preQ$_1$ as Models

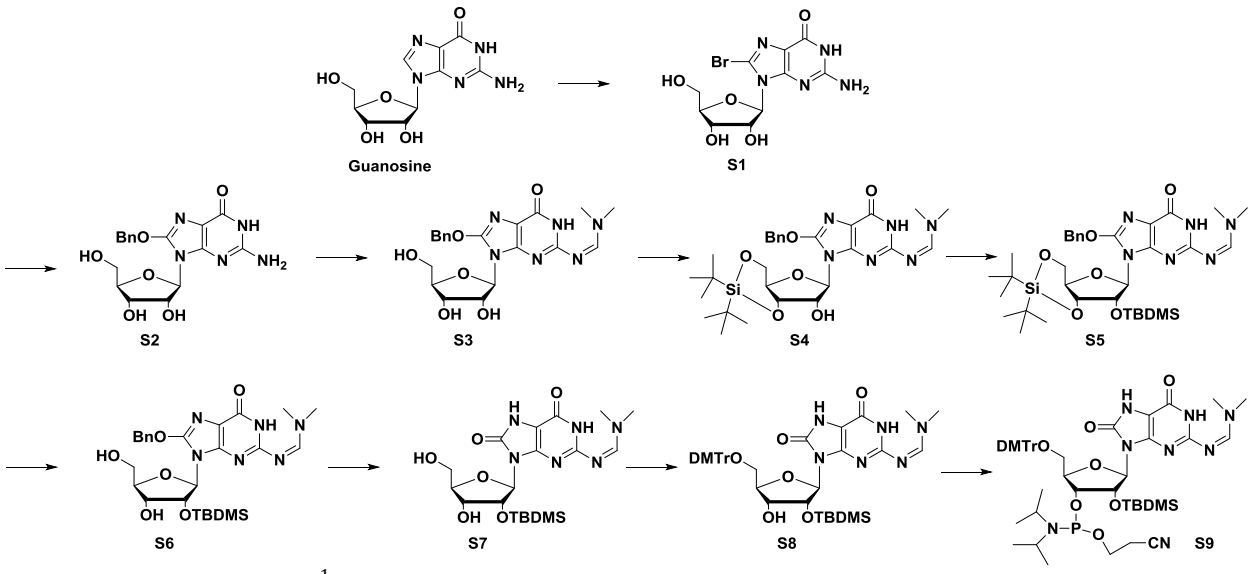
Yu Jung Choi,† Krzysztof S. Gibala,† Tewoderos Ayele, Katherine Van Deventer and Marino J. E. Resendiz*

Department of Chemistry, University of Colorado Denver, Science Building 1151 Arapahoe St, Denver, CO 80204

*Marino.resendiz@ucdenver.edu

† These authors contributed in the same amount to this work.

Supporting Information Index:



8-bromoguanosine (S1)[1]

Guanosine (13.0 g, 45.92 mmol) was dissolved in 350 mL H_2O and 2.6 mL (101.0 mmol) Br_2. The mixture was stirred for 2 h followed by addition of $NaHSO_3$ until a pale yellow suspension was obtained. Following vacuum filtration, the solid cake was recrystallized from a 1:1 MeOH/H_2O solution (400 mL). The mixture was filtered and washed with diethyl ether, yielding 15.0 g (90.2 %) of compound **S2**.

8-benzyloxyguanosine (S2)[1]

Sodium benzyloxide was prepared by adding sodium metal (4.6 g, 200.1 mmol) to benzyl alcohol (130 mL, 14058.2 mmol), and stirring for 12 h followed by addition of DMSO (85 mL). In a separate flask, **S2** (15 g, 42.8 mmol) was dissolved in 85 mL DMSO and stirred for 15 min. The two solutions were combined and placed in a 65 °C oil bath for 4.5 h. The reaction was neutralized using glacial acetic acid. The slurry was placed in diethyl ether and stirred for 5 min, then decanted. Acetone was placed into the slurry and stirred for 5 min. After vacuum filtration, the solid was stirred in DCM and filtered. The remaining solid was dried under reduced pressure and re-crystallized in 500 mL of a 1:1 EtOH/H_2O solution to yield **S2** in the form of a white solid (16.1 g, 41.1 mmol, 96.0 %) ^{1}H NMR(DMSO-d6) δ 7.47-7.38 (m, 5H), 5.62 (d, *J*= 6, 1H), 5.40 (s, 2H), 4.74 (t, *J*=12, 1H), 3.99 (t, *J*=12, 1H), 3.77 (q, *J*=12, 1H).

2-*N*- (dimethyl formamide)-8-benzyloxyguanosine (S3)

N,*N*-dimethylformamide dimethyl acetal (16.3 mL, 122.5 mmol) was added to a solution of **S3** (16.1 g, 41.1 mmol) in 161 mL DMF and stirred at 45 °C for 1.5 h. The mixture was concentrated under reduced pressure and re-dissolved in ethanol (20 mL). Following concentration under reduced pressure, **S3** was obtained in the form of a white foam (11.3 g, 25.4 mmol, 62.0 %). ^{1}H NMR(DMSO-d6) δ 8.46 (s, 1H), 7.51-7.37 (m, 5H), 5.71 (d, *J*=6, 1H), 5.45 (s, 2H), 4.78 (t, *J*=12, 1H), 4.07 (t, J=12, 1H), 3.81 (q, J=12, 1H), 3.53 (m, 1H), 3.14 (s, 3H), 3.02 (s, 3H); ^{13}C NMR (DMSO) δ 157.7, 156.8, 156.5, 151.7, 148.8, 135.8, 128.5, 128.3, 128.1, 114.3, 86.5, 85.2, 71.0, 70.8, 70.5, 62.0, 40.7, 34.6; IR 3234, 2926, 1674, 1331 cm^{-1}. HRMS m/z calculated for $C_{20}H_{25}N_6O_6$(M^++ H), 445.1830, observed m/z = 445.1845.

2-*N*- (dimethyl formamide)- 3',5'-*O*-bis-(*t*-butylsilyl)-8-benzyloxyguanosine (S4)

Nucleoside **S3** (1.2 g, 2.7 mmol) was azeotropically dried over pyridine (10 mL). The solid was then dissolved in DMF (10 mL) and cooled to 0 °C followed by addition of di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (1.75 mL, 5.4 mmol). The solution was stirred for 1 h and imidazole (0.9 g, 13.2 mmol) was added at once with further stirring (5 min). The solution was mixed with aq. NH_4Cl (30 mL, 0.01M) and extracted using ethyl acetate (10 mL x3). The organic residues were dried over brine and sodium sulfate, concentrated under reduced pressure and purified using column chromatography (1:1 DCM/EtOAc, 100 % DCM, 0-4 % MeOH/DCM) to afford nucleoside **S4** in the form of a white foam. (1.25 g, 2.1 mmol, 77 %). ^{1}H NMR(DMSO-d6) δ 11.37 (s, 1H), 8.47 (s, 1H), 7.39 (m, 5H), 5.73 (d, J=12, 1H), 5.43 (s, 2H), 4.65 (t, J=12, 1H), 4.27 (m, 1H), 4.21(m, 1H), 3.77 (m, 1H), 3.15 (s, 3H), 3.03 (s, 3H), 1.01-0.84 (m, 18H); ^{13}C NMR ($CDCl_3$) δ 157.8, 157.4, 156.2, 152.1, 149.3, 135.2, 128.9, 128.9, 128.8, 115.0, 88.2, 75.9, 74.4, 72.4, 72.3, 67.4, 41.5, 35.3, 27.4, 27.3, 22.6, 20.4; IR 2934, 2858, 1677, 1529,1338 cm^{-1}. HRMS m/z calculated for $C_{28}H_{41}N_6O_6Si(M^+ + H)$, 585.2851, observed m/z = 585.2849.

2-*N*- (dimethyl formamide)-2'-*O*-(t-butyldimethylsilyl)- 3',5'-*O*-bis-(*t*-butylsilyl)-8-benzyloxyguanosine (S5)

Nucleoside **S4** (1.12 g, 1.9 mmol) and imidazole (3.52 g, 51.7 mmol) were azeotropically dried over pyridine (3 mL). The obtained solids were then dissolved in DMF (10 mL) followed by addition of *tert*-butyldimethylsilyl trifluoromethanesulfonate (8.75 mL, 38.1 mmol) with stirring over 12 h at rt. Additional 4.35 mL (18.9 mmol) of the bis-triflate was added and stirred for additional 1 h. Saturated NH_4Cl was added and the organics were extracted over ethyl acetate (10 mL x3) with drying over brine and sodium sulfate. Purification using column chromatography (1:1 DCM/EtOAc, 100 % DCM, 0-2% MeOH/DCM) yielded **S5** in the form of a white foam (0.76 g, 1.1 mmol, 57 %). ^{1}H NMR(DMSO-d6) δ 11.41 (s, 1H), 8.42 (s, 1H), 7.43-7.40 (m, 5H), 5.74 (s, 1H), 5.44 (s, 2H), 4.65 (s, 1H), 4.29 (m, 2H), 3.80 (m, 1H), 3.11 (s, 3H), 3.02 (s, 3H), 1.02-0.80 (m, 27H), 0.03 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 157.7, 157.3, 156.0, 152.1, 149.1, 135.3, 128.8, 115.3, 90.3, 75.8, 74.3, 67.6, 41.4, 35.3, 27.5, 27.2, 25.9, 25.8, 22.6, 20.5, 18.1, -3.54, -4.30, -5.19; IR 2933, 2858, 1679, 1504,1336 cm^{-1}; HRMS m/z calculated for $C_{34}H_{55}N_6O_6Si_2(M^+ + H)$, 699.3716, observed m/z = 699.3726.

2-*N*- (dimethyl formamide)-2'-*O*-(t-butyldimethylsilyl)-8-benzyloxyguanosine (S6)

THF (8 mL) was added to a flask containing compound **S5** (0.45 g, 0.64 mmol) and cooled to 0 °C. Triethylamine trihydrofluoride (0.3 mL, 1.8 mmol) was dissolved in pyridine (0.5 mL) and added dropwise over a period of 20 min with stirring at 0° C. The reaction mixture was neutralized with slow addition of 15 % $NaHCO_3$ (10 mL) at 0 °C. Water (10 mL) was then added and the mixture was extracted with EtOAc (10 mL x3) and dried over brine and sodium sulfate. Column chromatography (0-5 % MeOH/EtOAc) afforded residue S**6** in the form of a white foam (0.17 g, 0.31 mmol, 48 %). ^{1}H NMR(DMSO-d6) δ 11.49 (s, 1H), 8.52 (s, 1H), 7.57-7.51 (m, 5H), 5.82 (d, J=6, 1H), 5.55 (s, 2H), 4.99 (m, 1H), 4.17 (m, 1H), 3.98 (m, 1H), 3.64 (m, 1H), 3.23 (s, 3H), 3.13 (s, 3H), 0.83 (s, 9H), 0.03 (m, 6H); ^{13}C NMR (CD_3OD) δ 159.3, 158.2, 154.1, 150.9, 136.5, 129.8, 129.7, 116.0, 88.5, 87.4, 74.6, 73.1, 72.6, 63.6, 61.5, 41.4, 35.2, 26.1,

20.9, 19.0, 14.5, -4.80, -5.09; IR 2928, 2856, 2357, 1662, 1619, 1339 cm^{-1}**;** HRMS m/z calculated for $C_{26}H_{39}N_6O_6Si(M^+ + H)$, 559.2695, observed m/z = 559.2718.

2-*N*-(dimethyl formamide)-2'-*O*-(t-butyldimethylsilyl)-8-hydroxy-7,8-dihydroguanosine (S7)

Residue **S6** (0.17 g, 0.31 mmol) and Pd/C (0.08 g, 0.75 mmol) were placed in a pressure chamber and suspended in EtOH (2.5 mL). The chamber was filled with H_2 (60 psi) and left stirring for 45 min. The mixture was then filtered through Celite® 545 and washed with EtOH (3 mL x3). Drying under reduced pressure yielded compound **S7** in the form of a white foam. (0.13 g, 0.27 mmol, 87 %). ^{1}H NMR(CD_3OD) δ 8.59 (s, 1H), 5.94 (d, 1H), 5.12 (, 1H), 4.33 (m, 1H), 4.11 (m, 1H), 3.88-3.71 (dd, 2H), 3.22 (s, 3H), 3.14 (s, 3H), 0.88 (s, 9H), 0.06 (s, 3H), -0.01 (s, 3H); ^{13}C NMR (CD_3OD) δ 159.5, 158.5, 154.2, 148.5, 103.9, 87.6, 87.1, 73.8, 72.8, 63.7, 41.5, 35.3, 26.2, 19.0, -4.75, -4.99; IR 2929, 2857, 1678, 1539,1342 cm^{-1}; HRMS m/z calculated for $C_{19}H_{55}N_6O_6Si(M^+ + H)$, 469.2225, observed m/z = 469.2215.

2-*N*-(dimethyl formamide)-2'-*O*-(t-butyldimethylsilyl)- 5'-*O*-(4,4'-dimethoxytrityl)-8-hydroxy-7,8-dihydroguanosine (S8)

Compound **S7** (0.20 g, 0.42 mmol) was azeotropically dried over pyridine (1 mL), and re-dissolved in pyridine (4mL). The solution was cooled to 0 °C followed by addition of 4,4'-dimethoxytriphenylmethyl chloride (0.166 g, 0.49 mmol) and stirring for 12 h with slow warming to r.t. The yellow solution was diluted with 15 % $NaHCO_3$ (10 mL) and extracted with ethyl acetate (7 mL x3) followed by drying over brine and sodium sulfate. Column chromatography (0- 5% MeOH/DCM) produced protected nucleoside **S8** in the form of a white foam (0.12 g, 0.16 mmol, 38 %). ^{1}H NMR($CDCl_3$) δ 8.34 (s, 1H), 7.38-6.72 (m, 13H), 5.82 (d, 1H), 5.02 (s, 1H), 4.42 (s, 1H), 3.94 (s, 1H), 3.68 (s, 6H), 3.34 (s, 2H), 2.92 (s, 3H), 2.79 (s, 3H), 0.84 (s, 9H), 0.01 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 158.4, 156.2, 153.1, 152.2, 149.7, 147.2, 145.0, 136.2, 136.1, 130.2, 128.3, 127.7, 113.1, 113.0, 103.2, 86.6, 86.0, 83.0, 72.7, 71.0, 55.2, 41.2, 35.0, 25.8, 18.1, -4.64, -5.01; IR 2857, 2361, 1673, 1627, 1508 cm^{-1}; HRMS m/z calculated for $C_{40}H_{50}N_6O_8NaSi(M^+ + H)$, 793.3352, observed m/z = 793.3346

2-*N*-(dimethyl formamide)-2'-*O*-(t-butyldimethylsilyl)- 3'-*O*-[(2-ethylcyano-*N,N*-diisopropylphosphoramidite)-5'-*O*-(4,4'-dimethoxytrityl)-8-hydroxy-7,8-dihydroguanosine (S9)

Nucleoside **S8** (0.12 g, 0.16 mmol) was azeotropically drived over pyridine (1 mL), and dissolved in anhydrous DCM (1 mL). Anhydrous DIPEA (0.08 mL, 0.47 mmol) and 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.07 mL, 0.31 mmol) were added and stirred for 1 hr. The solution was added to a flask containing 20% $NaHCO_3$ and extracted with DCM (2 mL x 3). The organics were dried over brine and sodium sulfate. Purification using column chromatography (0-3% MeOH/DCM) yielded phosphoramidite **S9** in the form of a white foam (0.12 g, 0.12 mmol, 77%). ^{1}H NMR($CDCl_3$) δ 8.46 (s, 1H), 7.46-6.77 (m, 13H), 5.91 (d, 1H), 5.15 (s, 1H), 4.50 (s, 1H), 3.23 (s, 1H), 3.76 (s, 6H), 3.74-2.61 (m, 8H), 3.03 (s, 3H), 2.93-2.82 (d, 3H), 2.61 (m, 1H), 1.15-1.04 (m, 12H), 0.84 (s, 9H), 0.05 (s, 3H), -0.03 (m, 3H); ^{31}P NMR($CDCl_3$) δ 149.7, 148.9. HRMS m/z calculated for $C_{49}H_{68}N_8O_9PSi$ ($M^+ + H$), 971.4616, observed m/z = 971.4567.

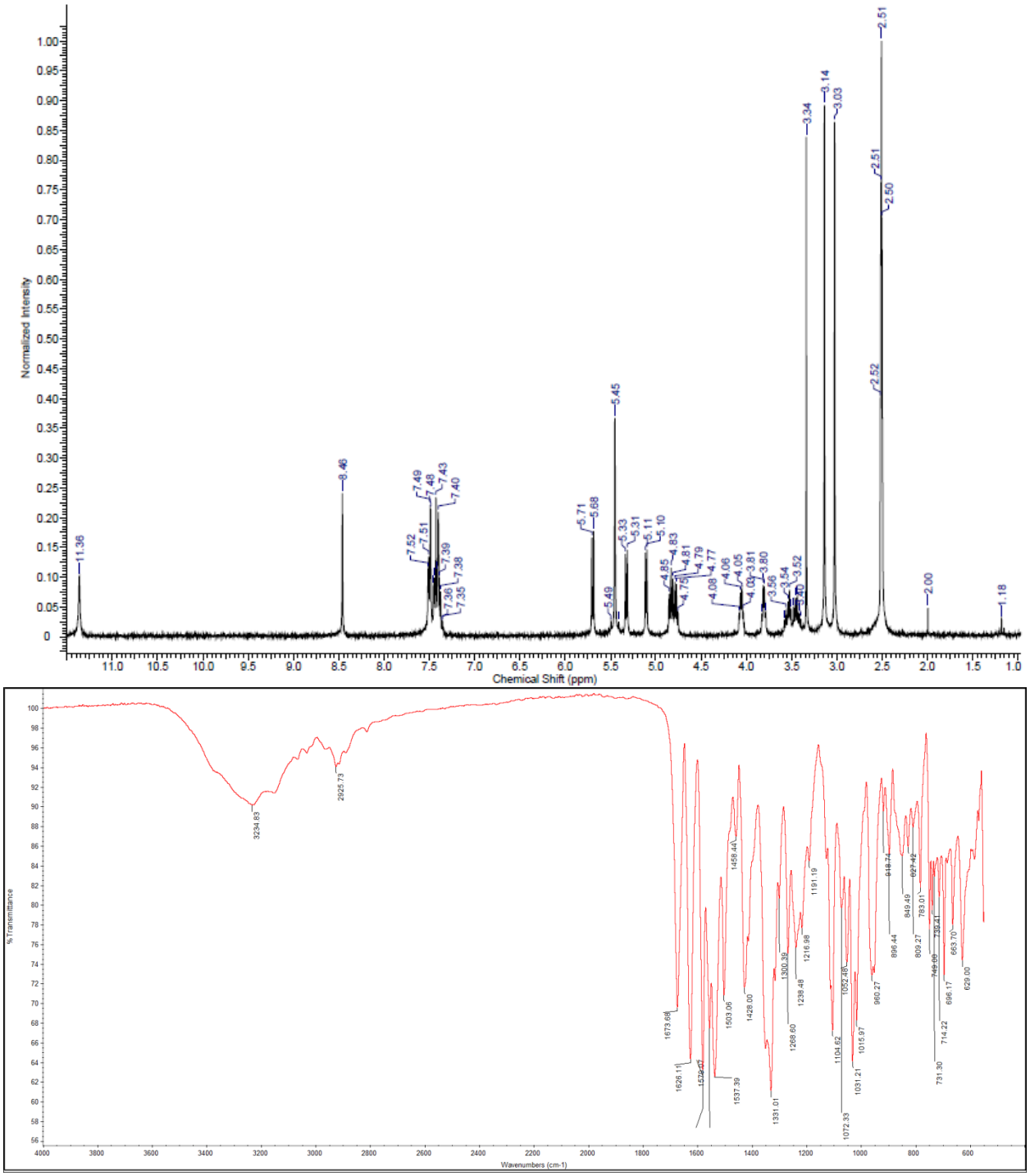


Figure **S1**. ^{1}H NMR (top) and IR (bottom) of compound **S3**.

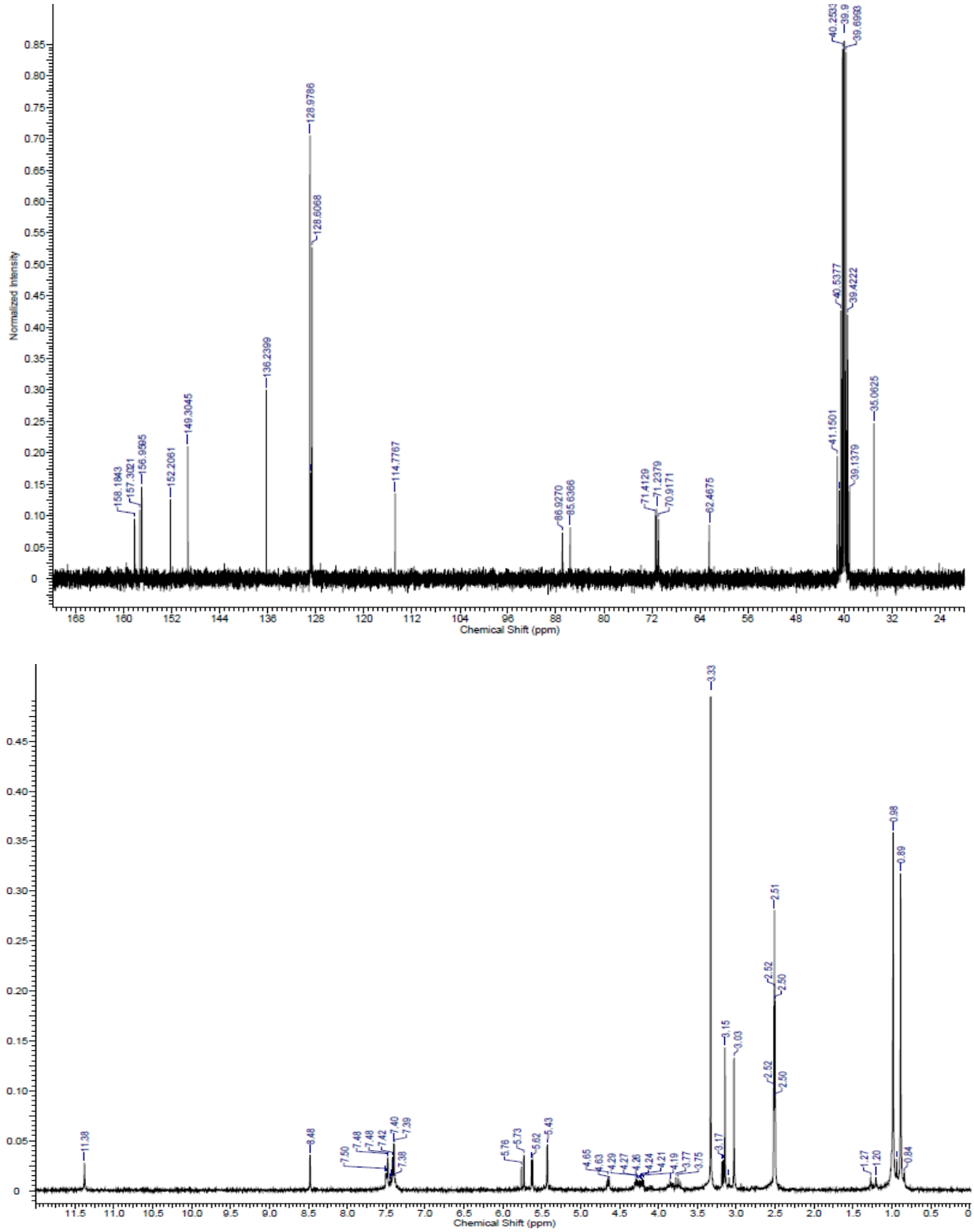


Figure **S2**. ^{13}C NMR of **S3** (top) and ^{1}H NMR (bottom) of compound **S4**.

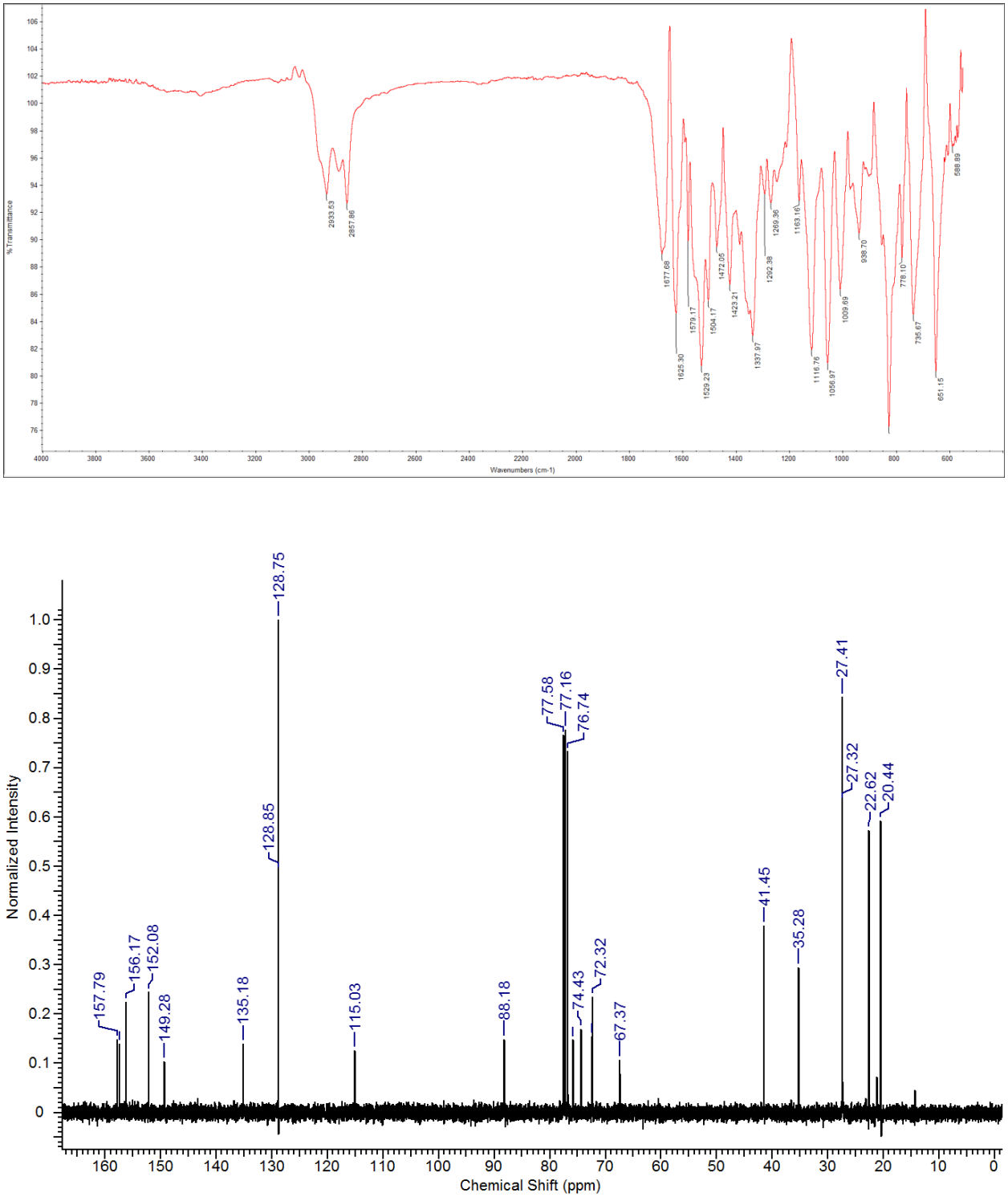


Figure **S3**. IR (top) and ^{13}C NMR(bottom) of compound **S4**.

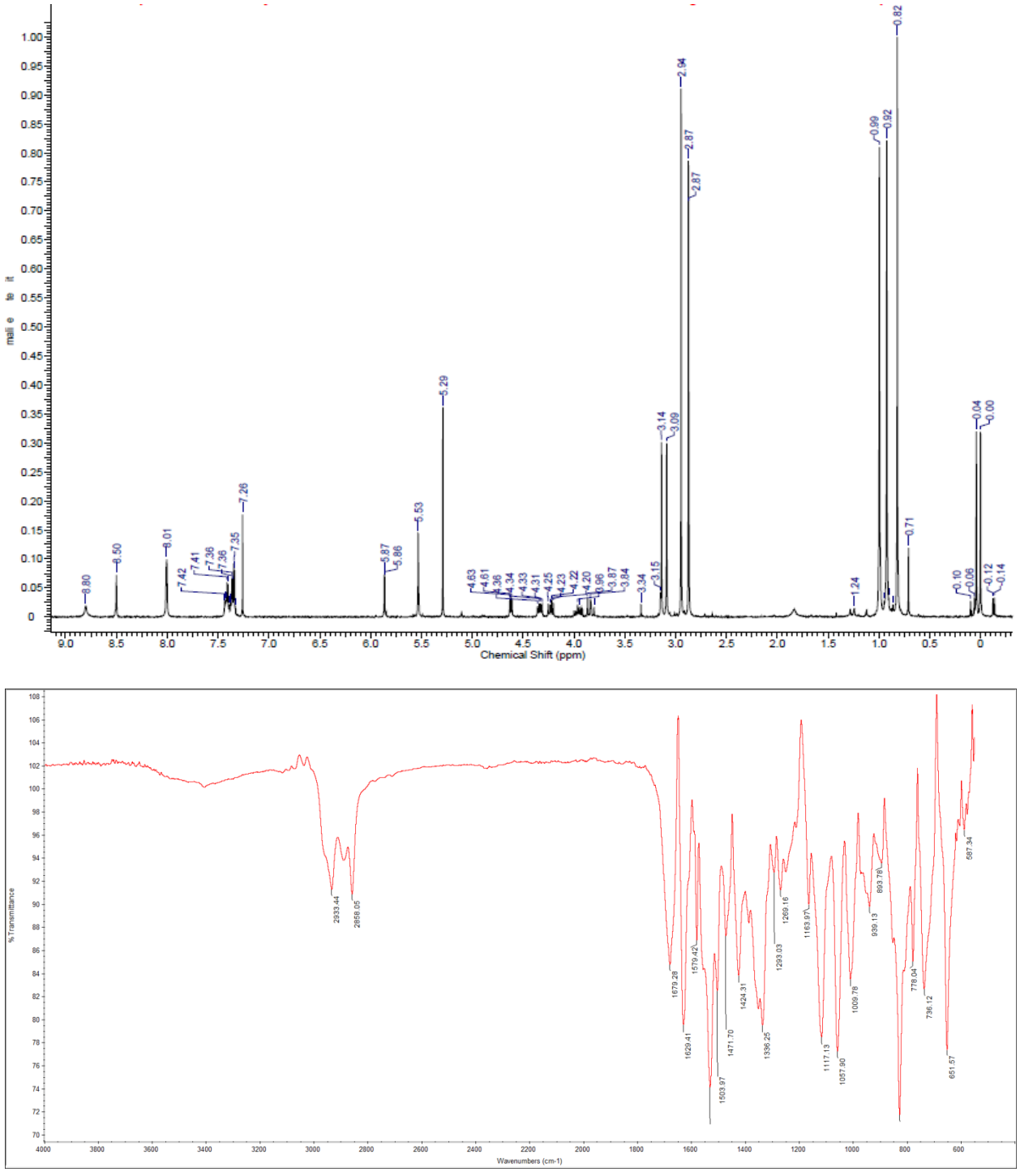


Figure **S4**. ^{1}H NMR (top) and IR of compound **S5**.

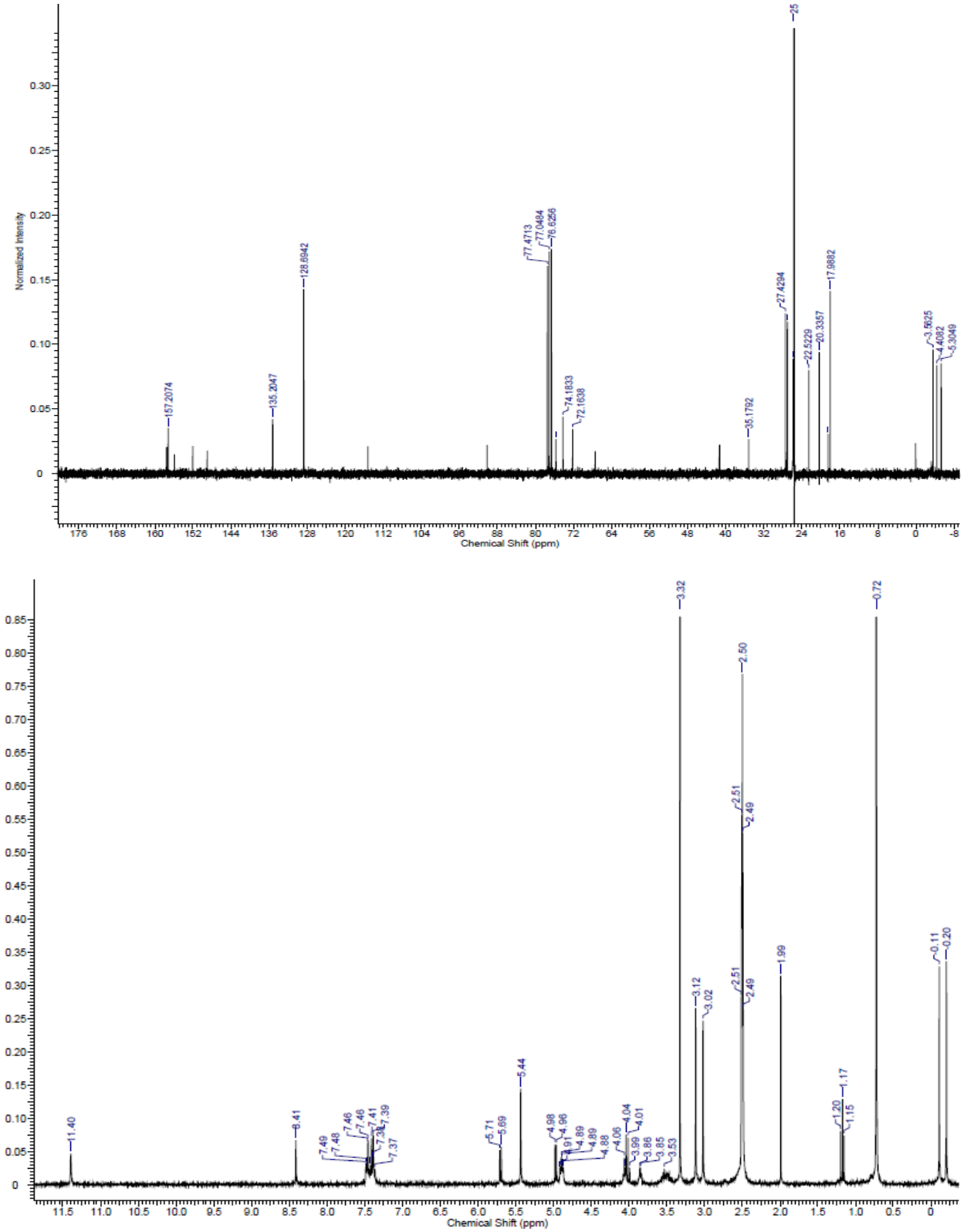


Figure **S5.** ^{13}C NMR of **S5** (top) and ^{1}H NMR (bottom) of compound **S6**.

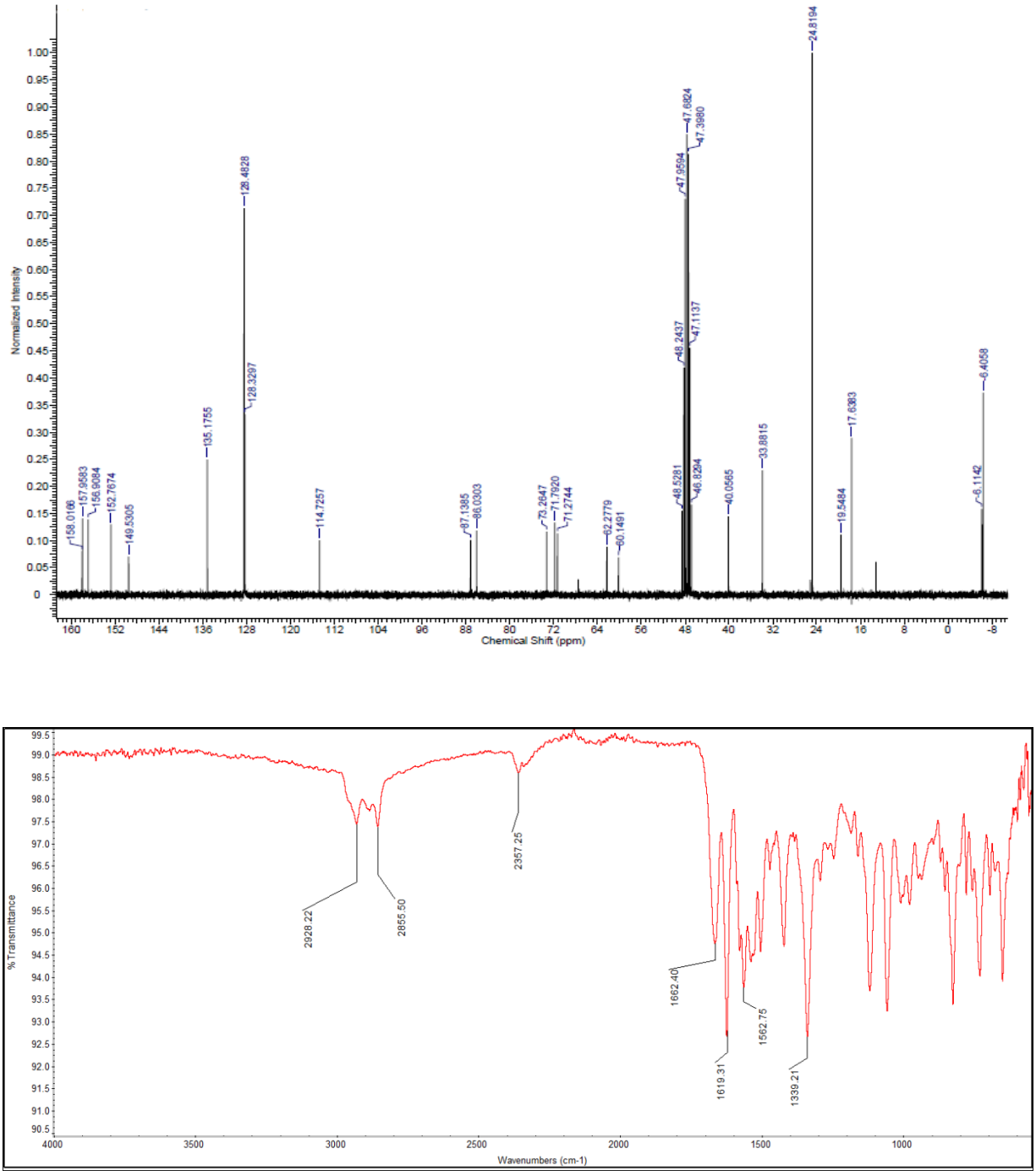


Figure **S6**.^{13}C NMR (top) and IR(bottom) of compound **S6**

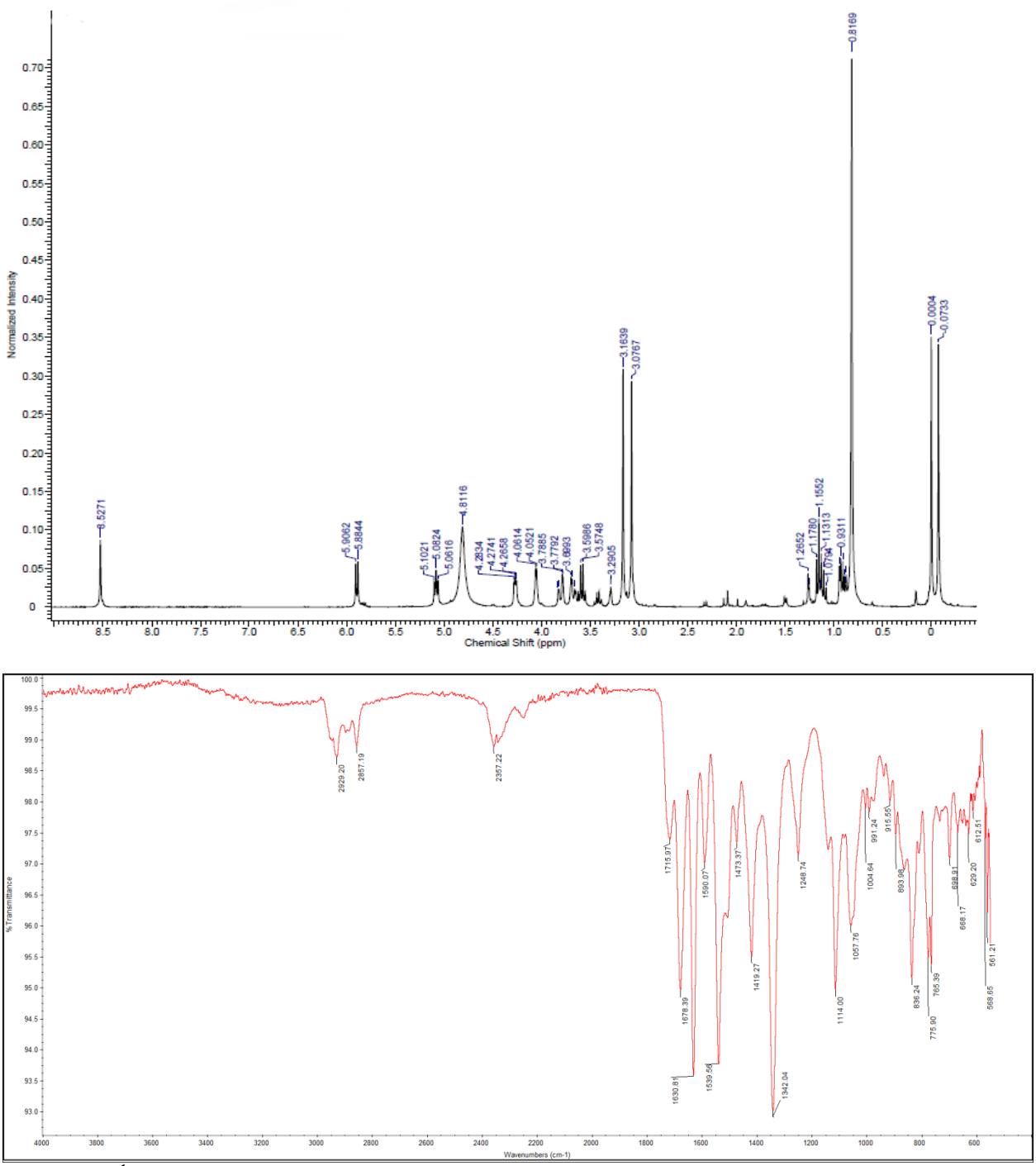


Figure **S7**. [1] H NMR(top) and IR(bottom) of compound **S7**.

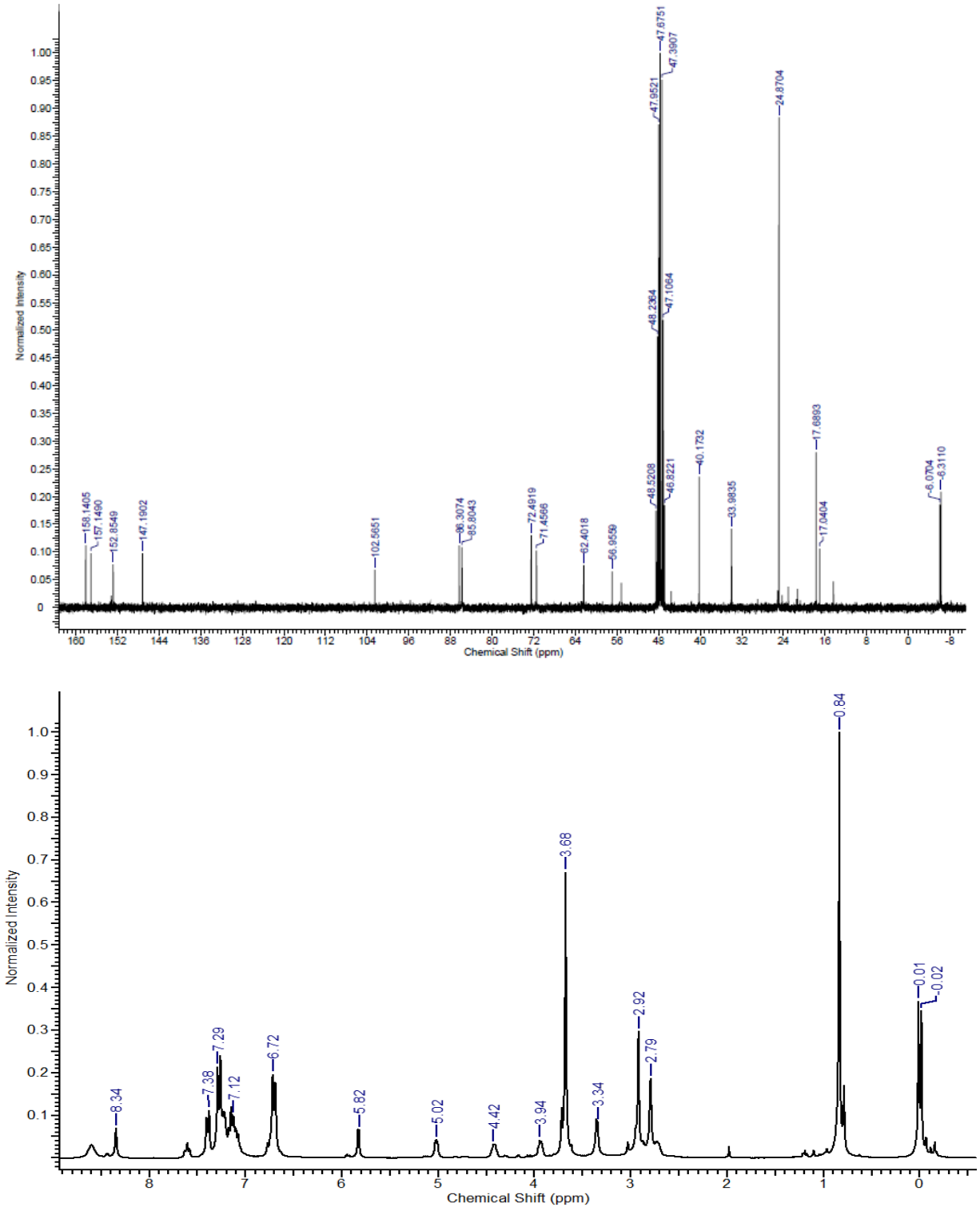


Figure **S8**. ^{13}C NMR (top) of **S7** and ^{1}H NMR (bottom) of compound **S8.**

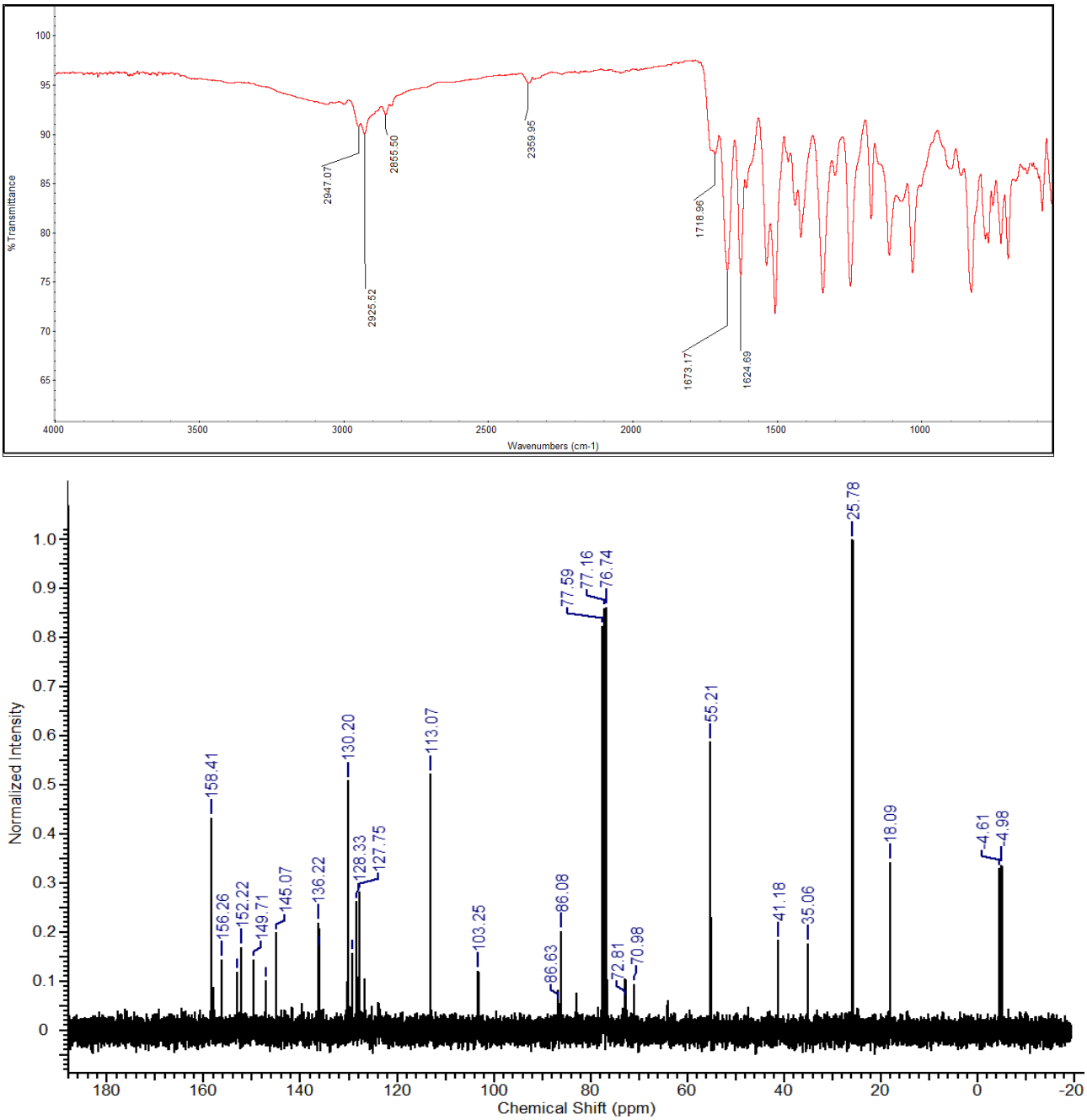


Figure **S9**. IR (top) and ^{13}C NMR (bottom) of compound **S8.**

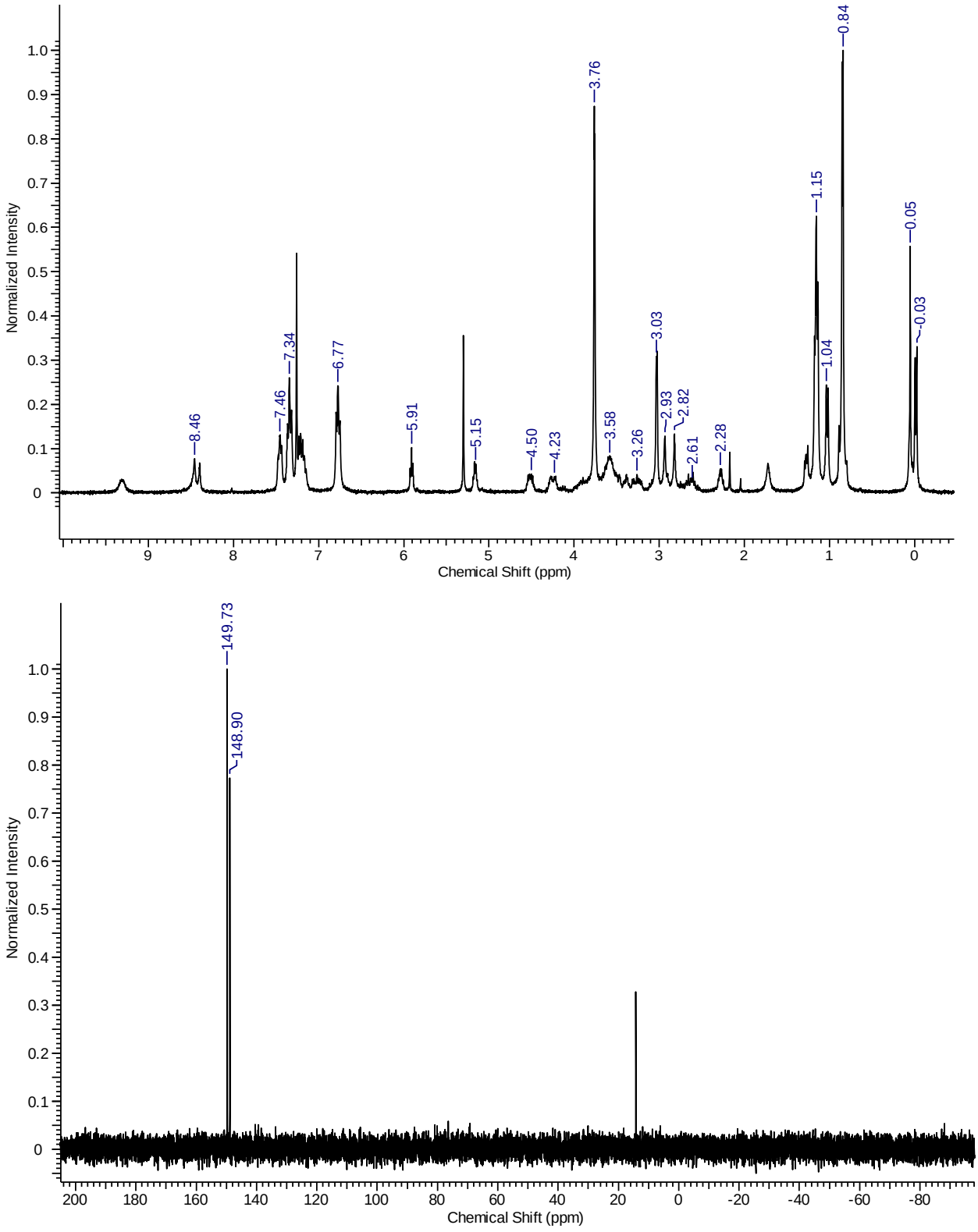


Figure **S10**. ^{1}H NMR (top) and ^{31} P NMR (bottom) of compound **S9.**

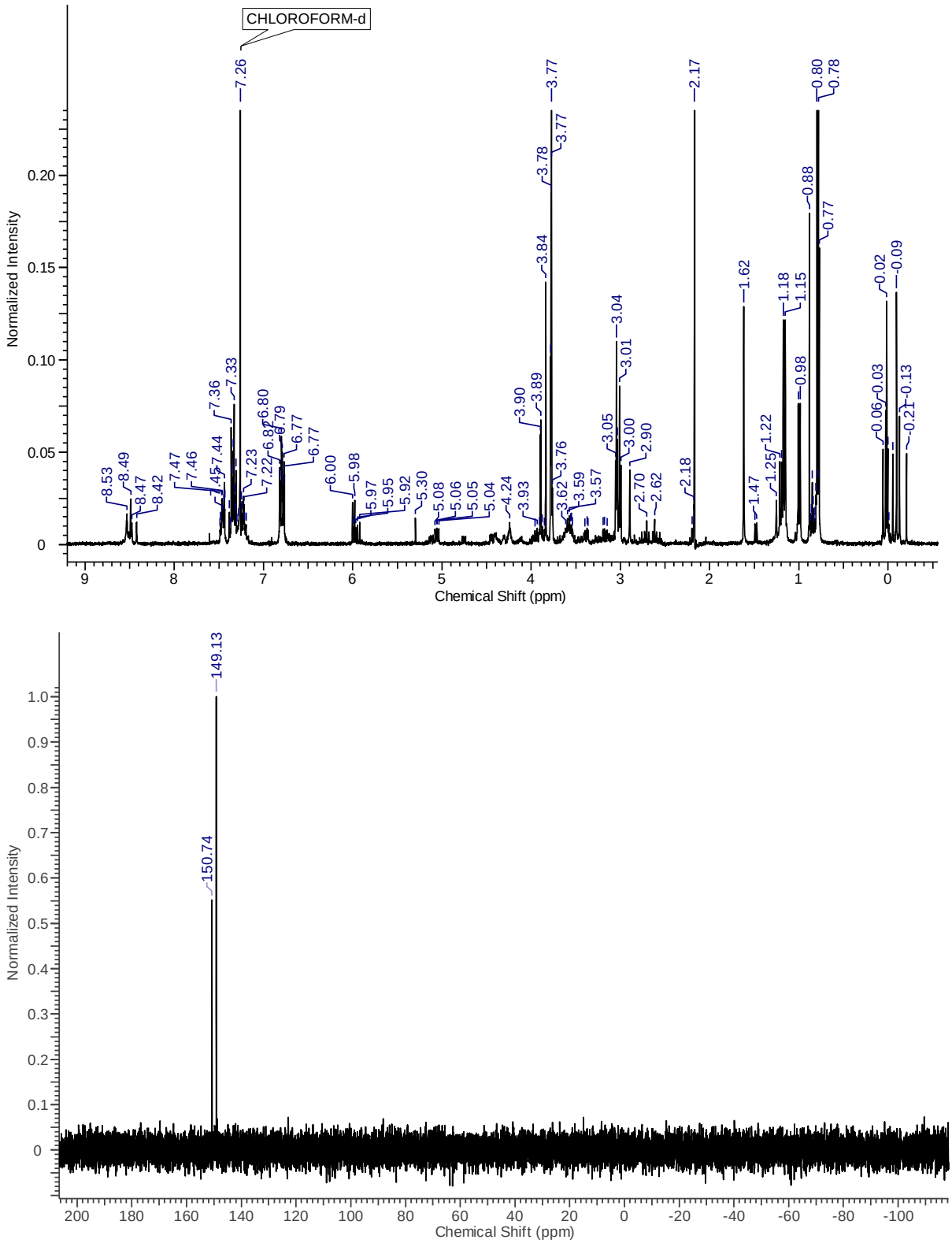


Figure **S11**. ^{1}H NMR (top) and ^{31} P NMR (bottom) of compound **S10**.

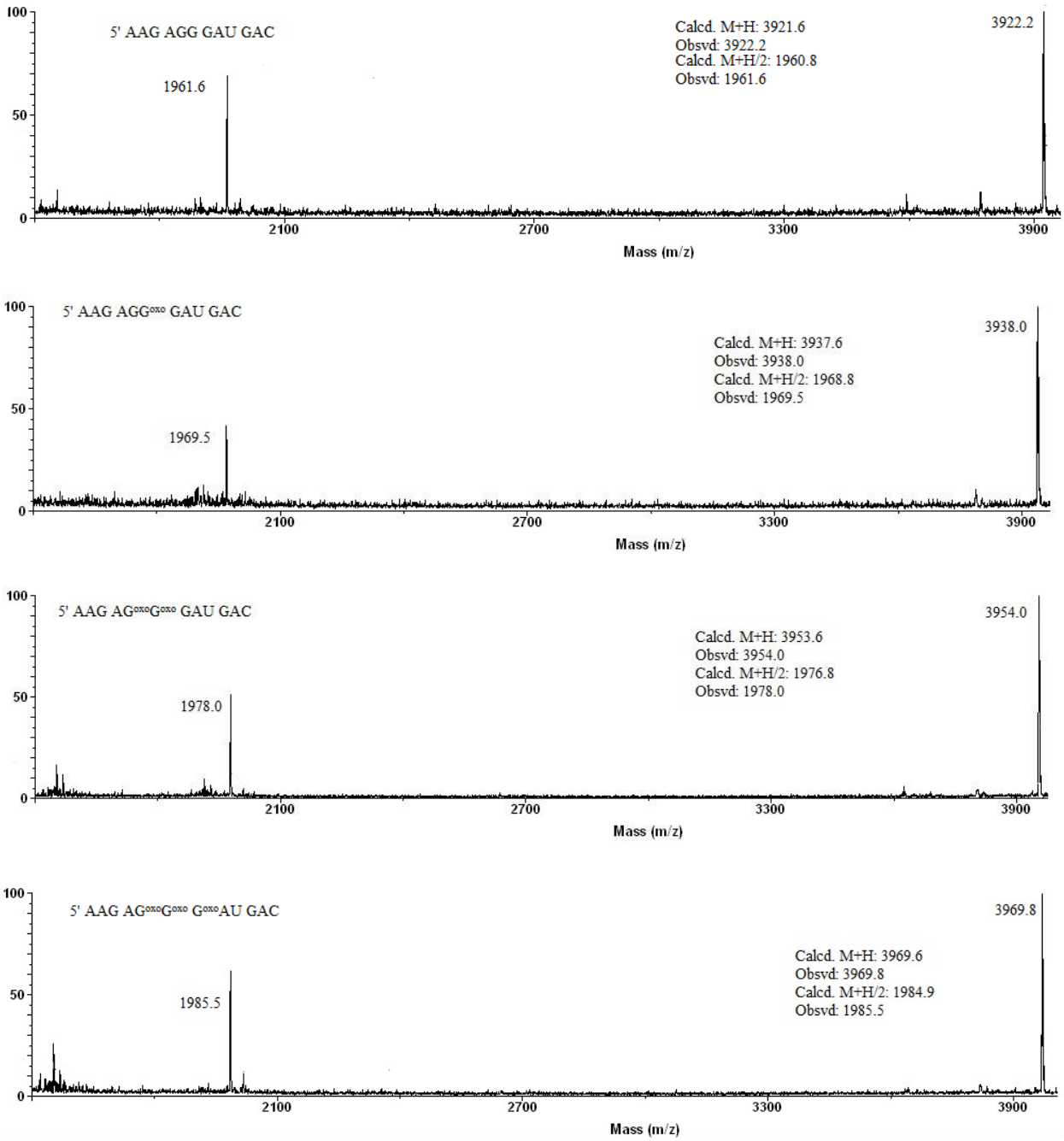


Figure S12. . MALDI-TOF MS of **1**, **2**, **3,** and **4** (from top to bottom).

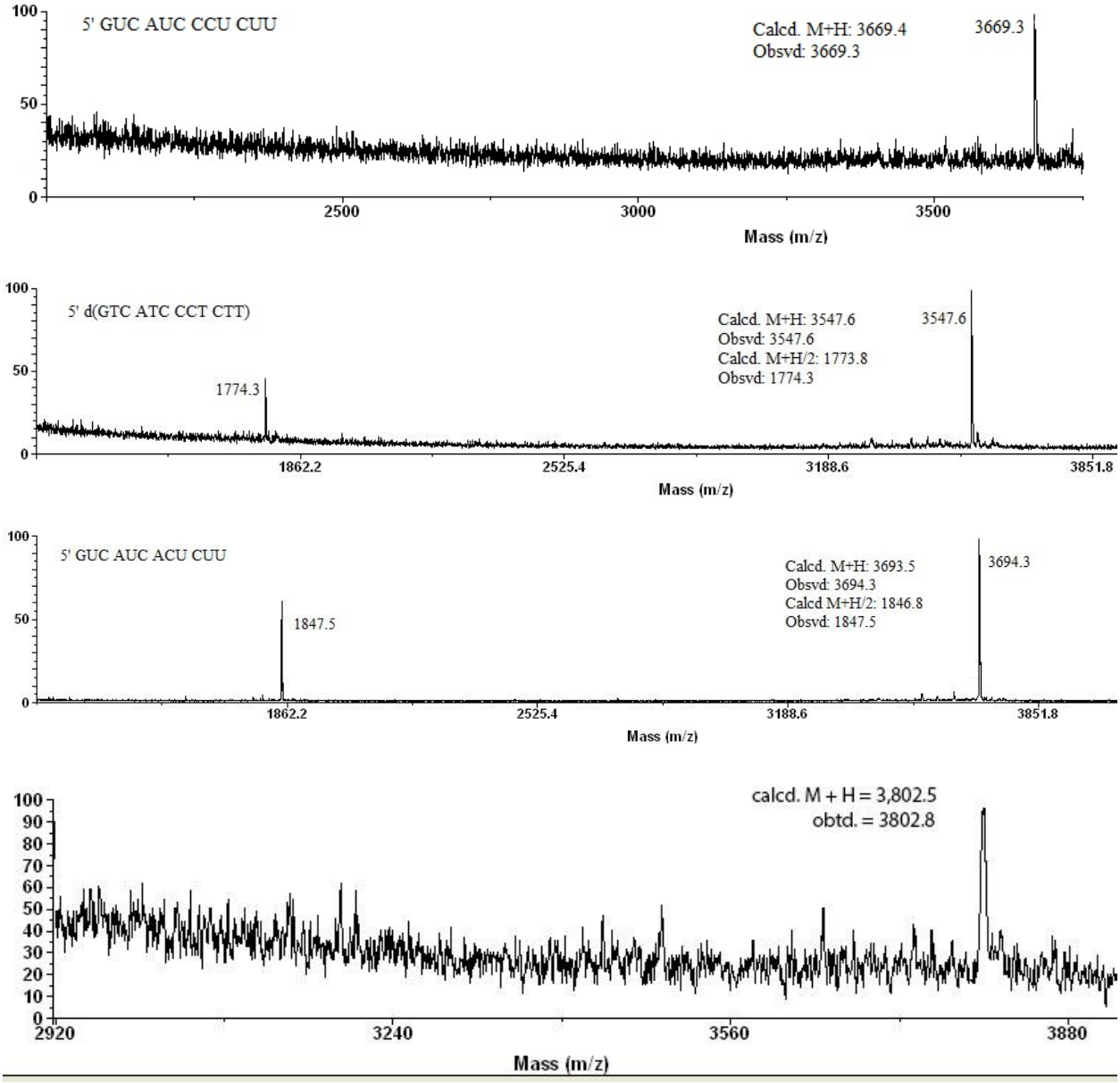


Figure S13. MALDI-TOF MS of **5**, **6, 7,** and **8** (from top to bottom).

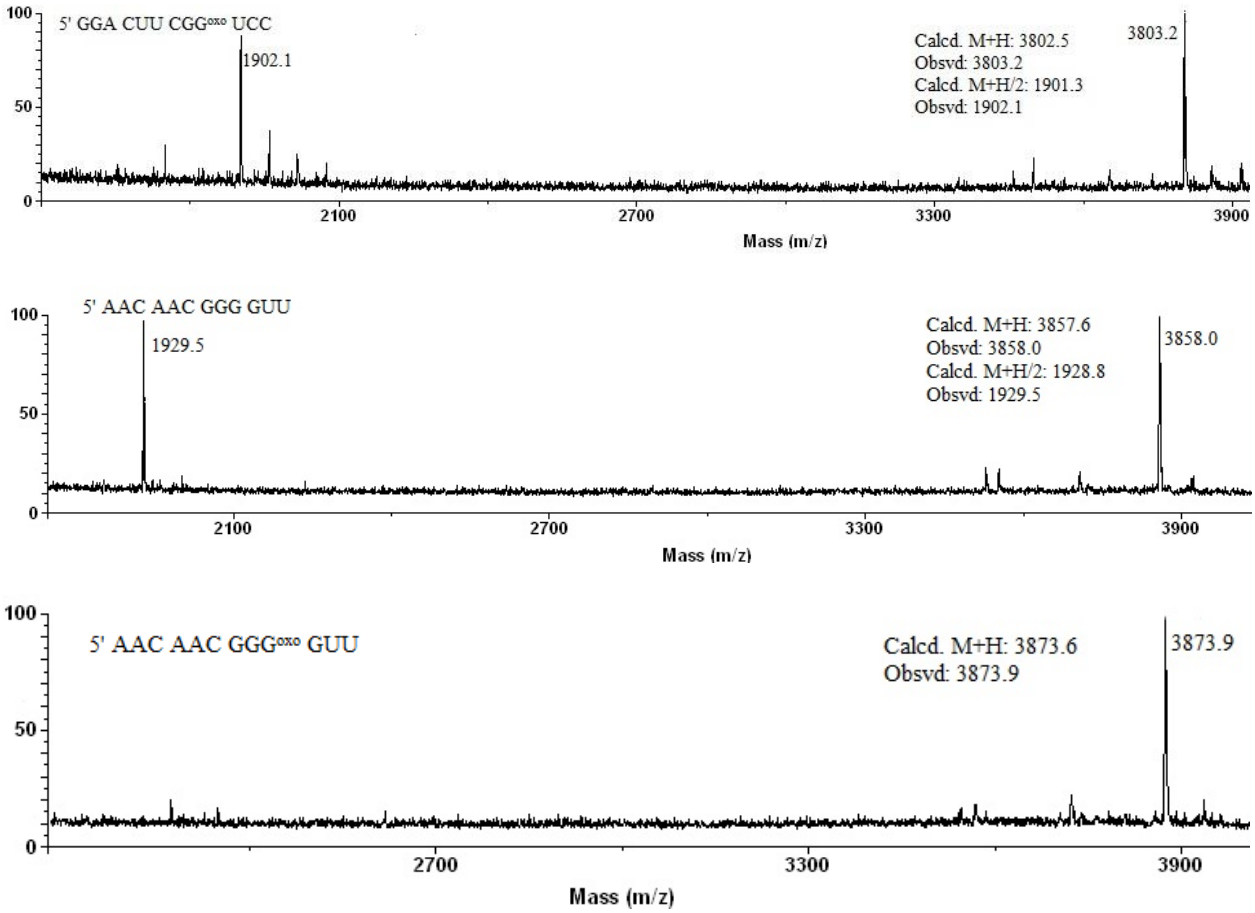


Figure S14. MALDI-TOF MS of **10, 11,** and **12** (from top to bottom).

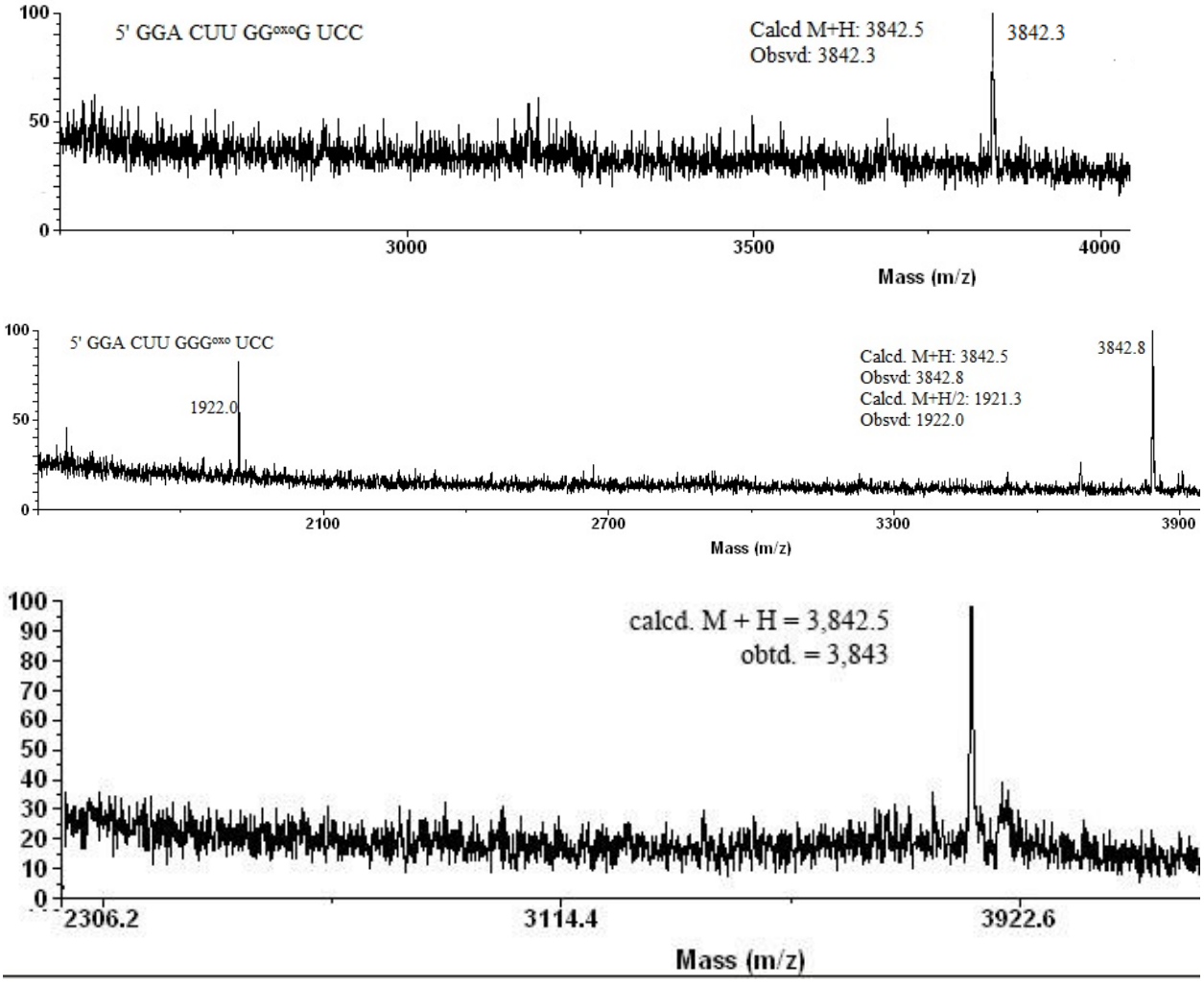


Figure S15. MALDI-TOF MS of **13, 15,** and **16** (from top to bottom).

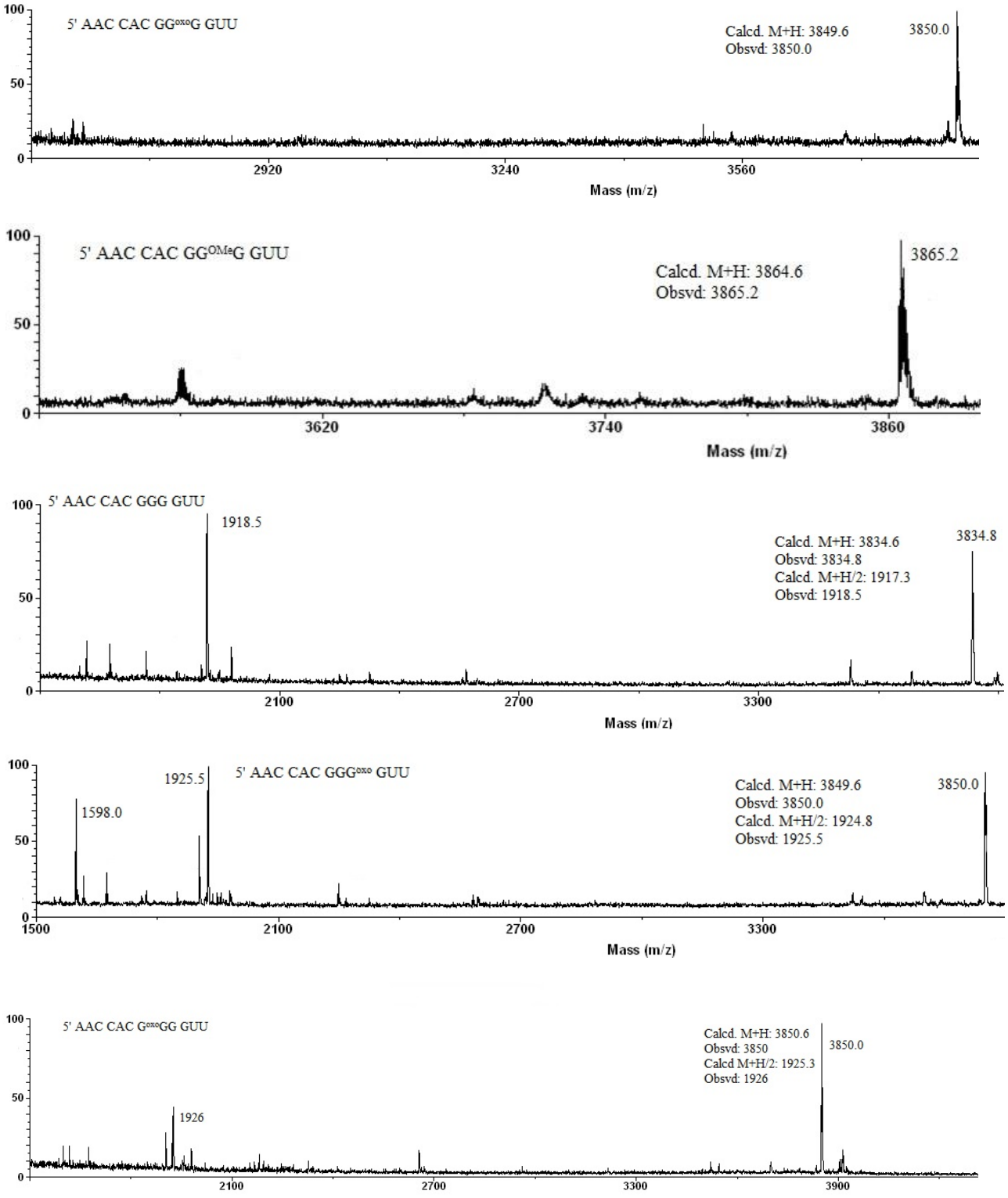


Figure S16. MALDI-TOF MS of **17, 17-OMe, 18**, **19,** and **20** (from top to bottom).

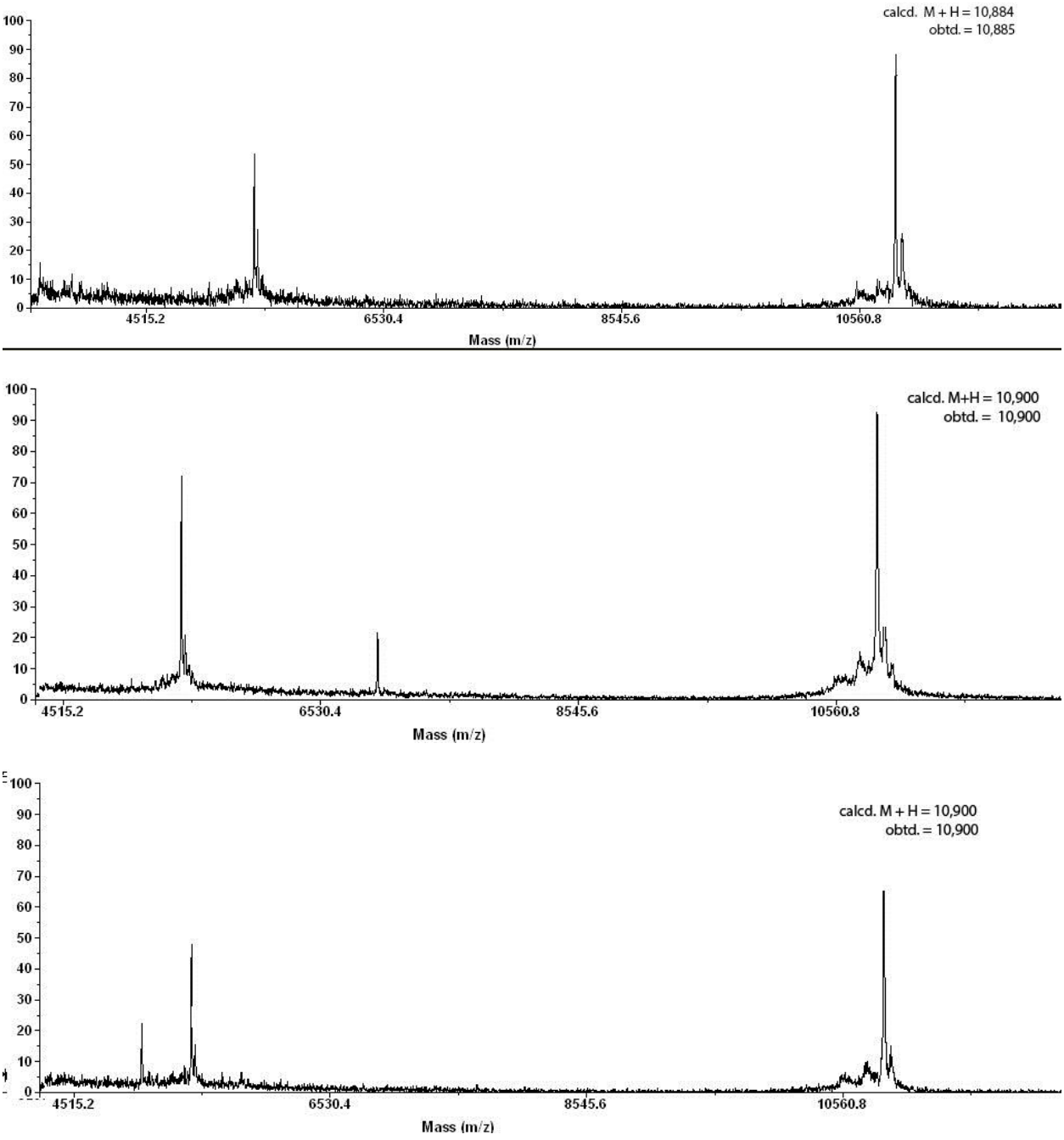


Figure S17. MALDI-TOF MS of **21, 22,** and **23** (from top to bottom)

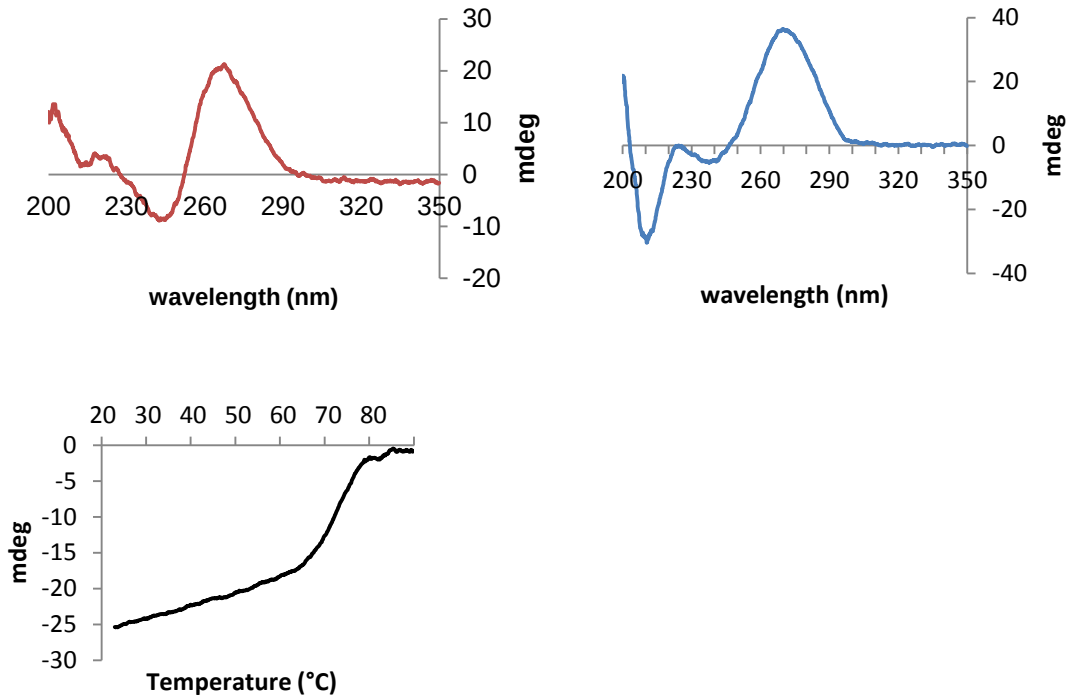


Figure S18. CDs of strands **1** (top left), **1**:**5** (top right), and melt of **1**:**5** (bottom).

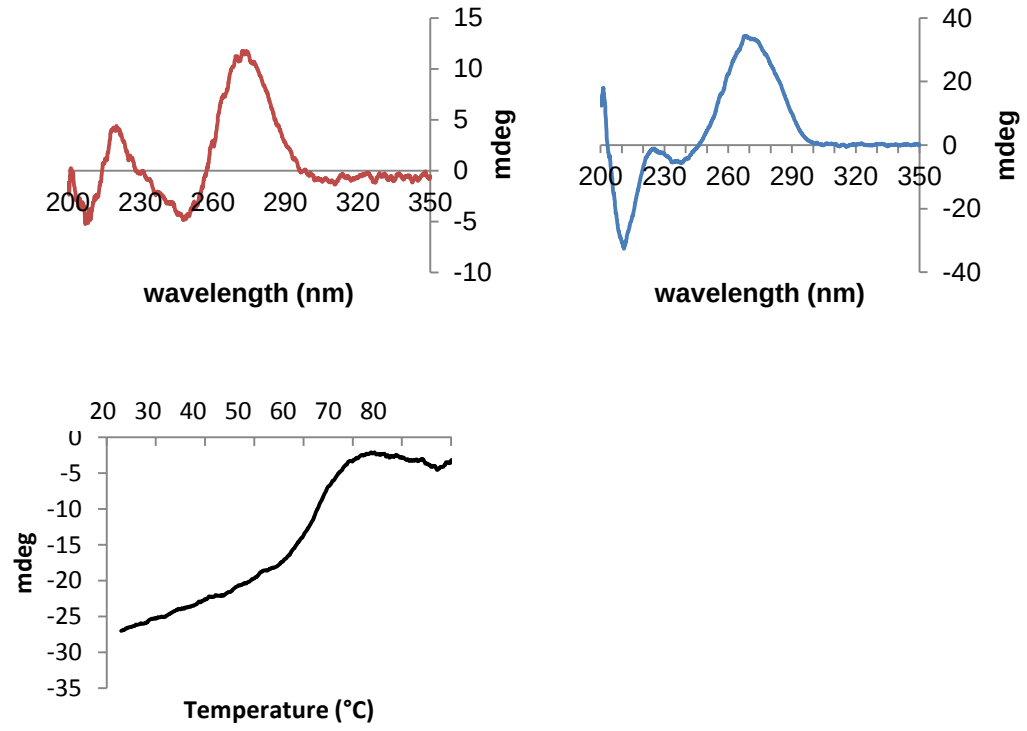


Figure S19. CDs of strand **2** (top left), **2**:**5** (top right), and melt of **2**:**5** (bottom).

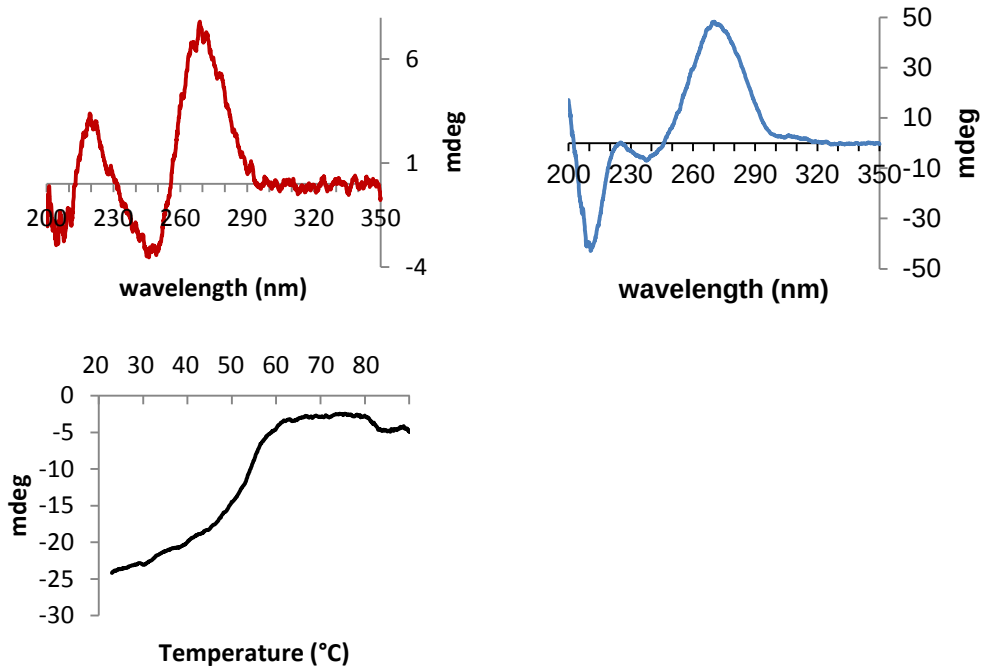


Figure S20. CDs of strand **3** (top left), **3**:**5** (top right), and melt of **3**:**5** (bottom).

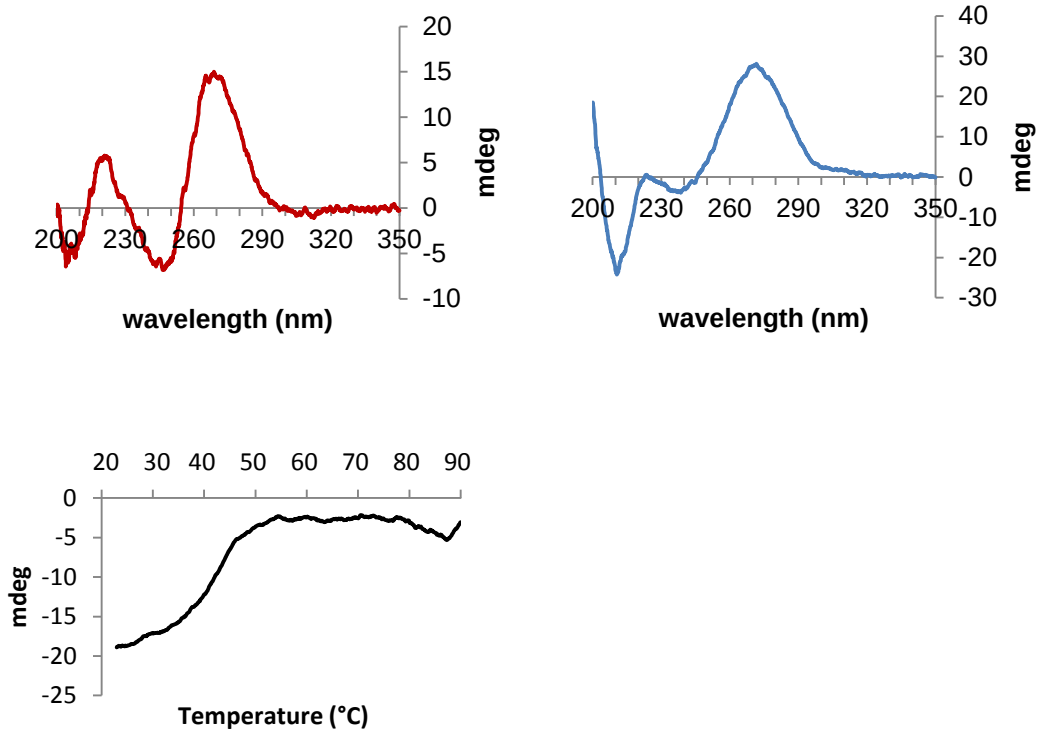


Figure S21. CDs of strand **4** (top left), **4**:**5** (top right), and melt of **4**:**5** (bottom).

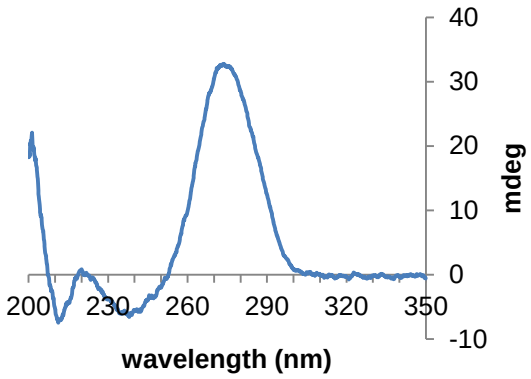
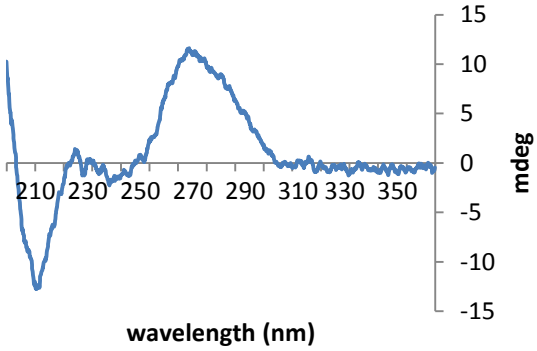


Figure S22. CD of **10**.



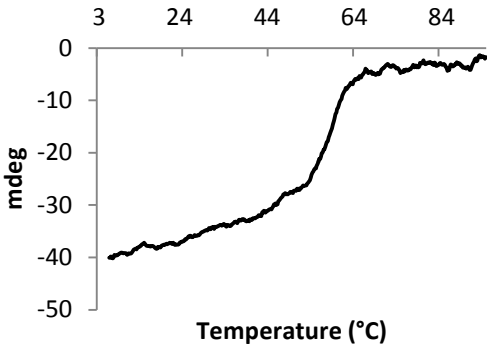
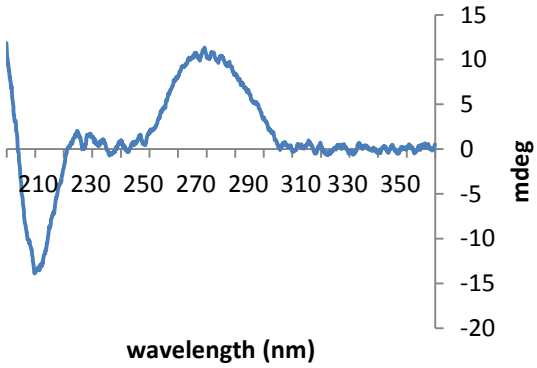


Figure S23. CD (left) and melt (right) of **1**:**7**.



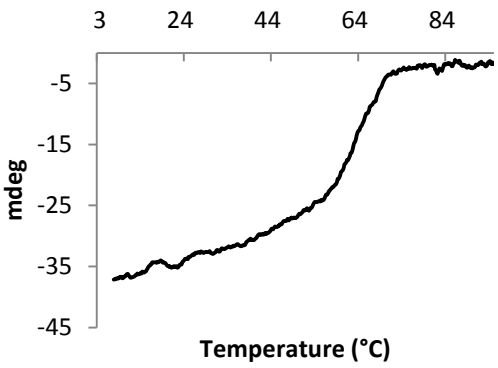


Figure S24. CD (left) and melt (right) of **2**:**7**.

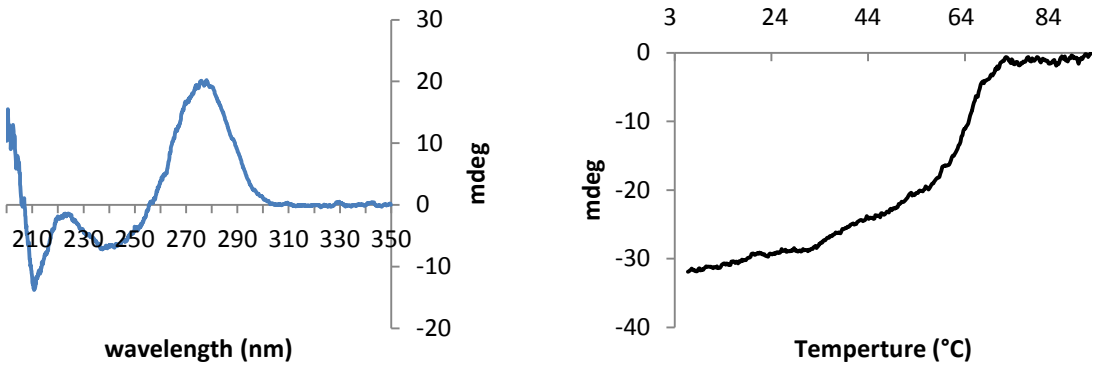


Figure S25. CD (left) and melt (right) of **1**:**6**.

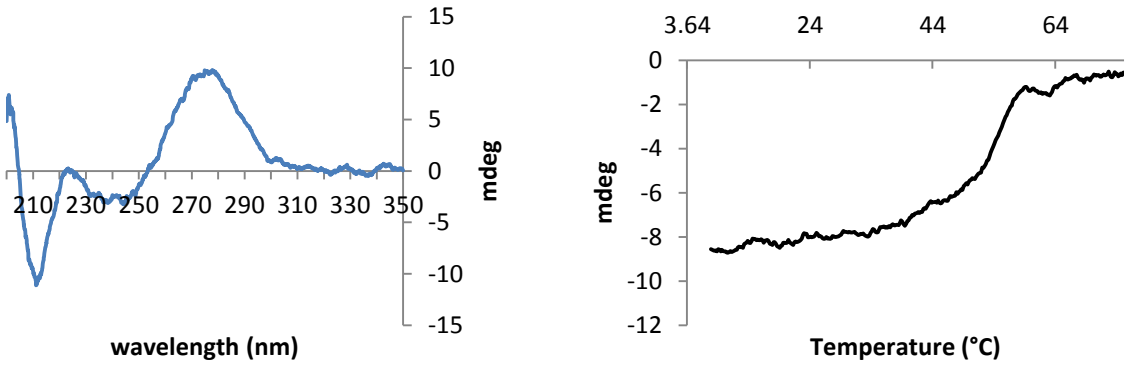


Figure S26. CD (left) and melt (right) of **2**:**6**.

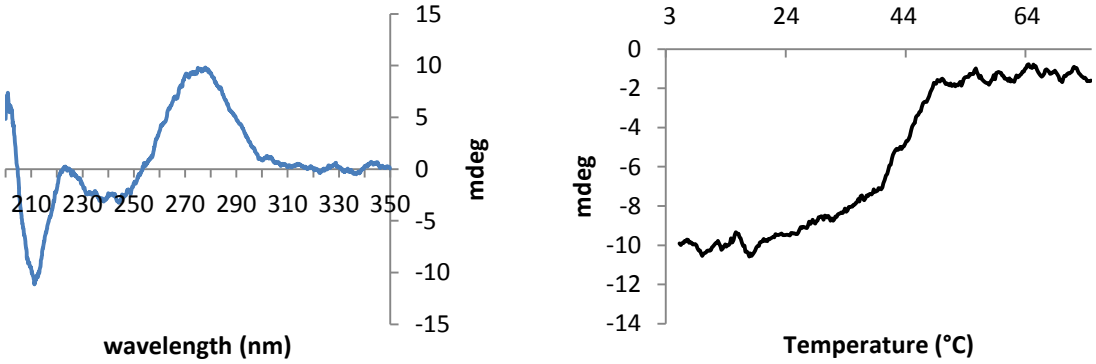


Figure S27. CD (left) and melt (right) of **3**:**6**.

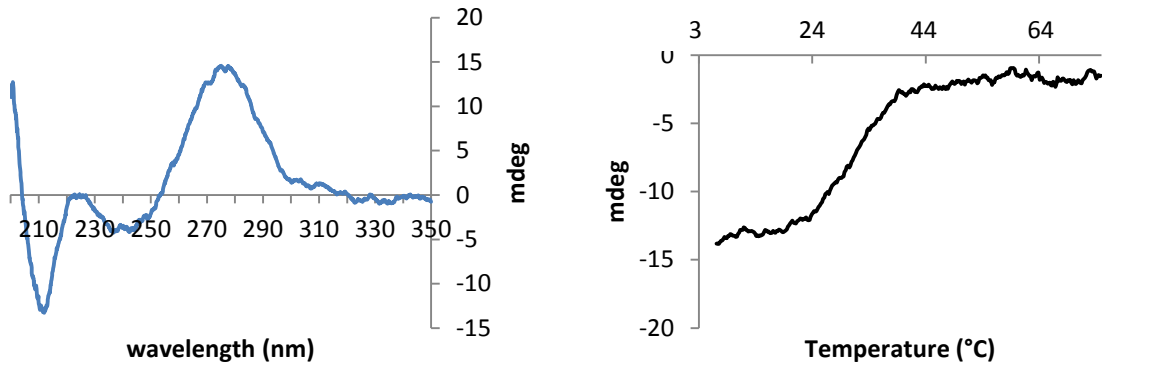


Figure S28. CD (left) and melt (right) of **4**:**6**.

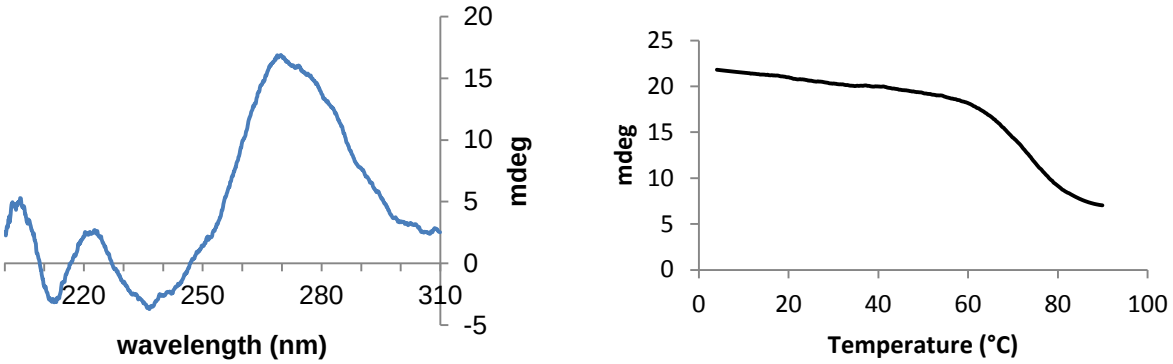


Figure S29. CD (left) and melt (right) of **8**.

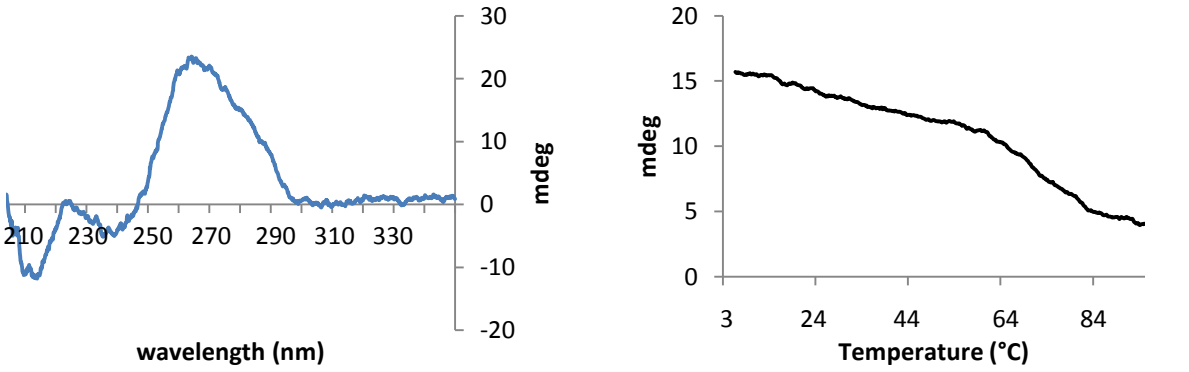


Figure S30. CD (left) and melt (right) of **9**.

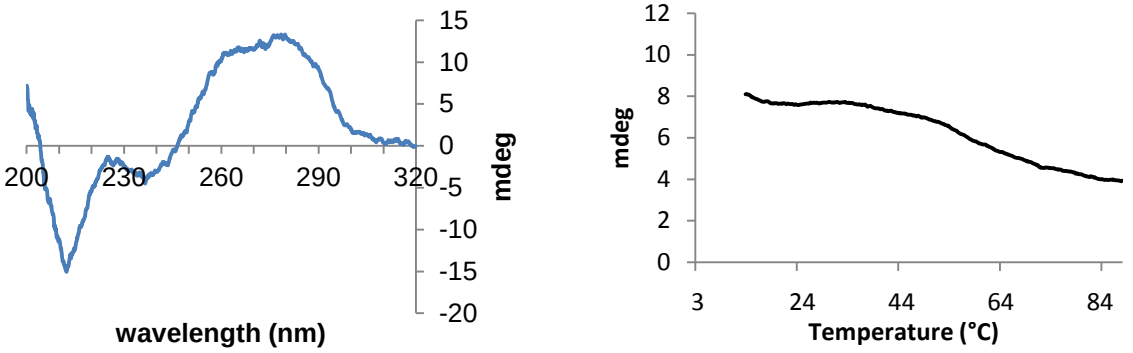


Figure S31. CD (left) and melt (right) of **10**.

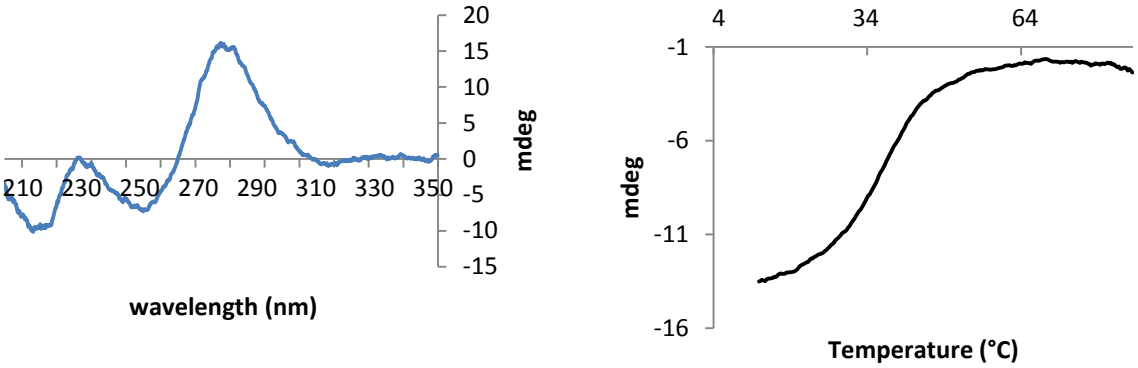


Figure S32. CD (left) and melt (right) of **11**.

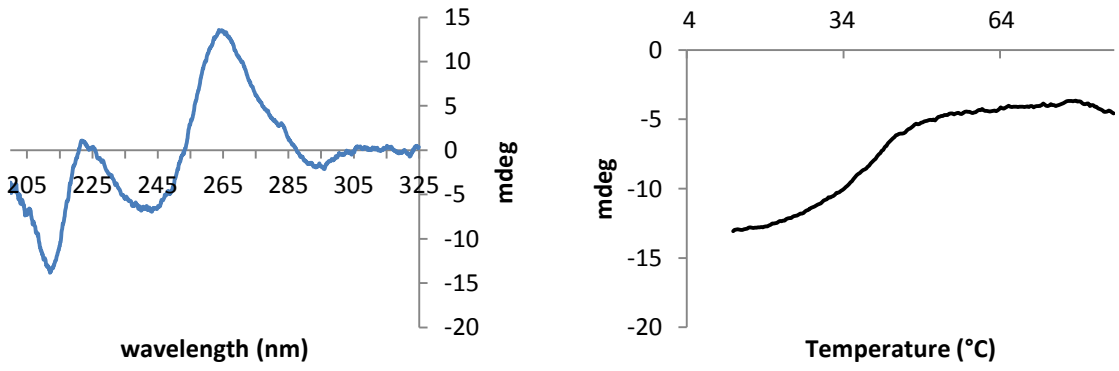


Figure S33. CD (left) and melt (right) of **12**.

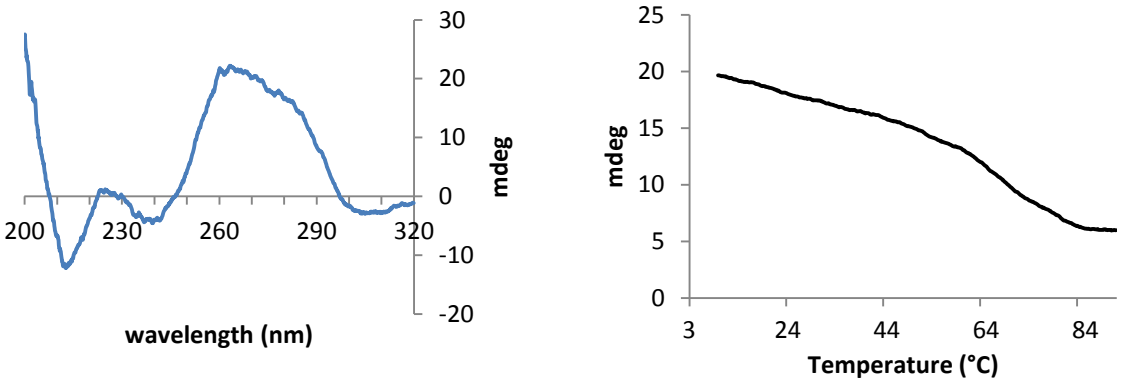


Figure S34. CD (left) and melt (right) of **13**.

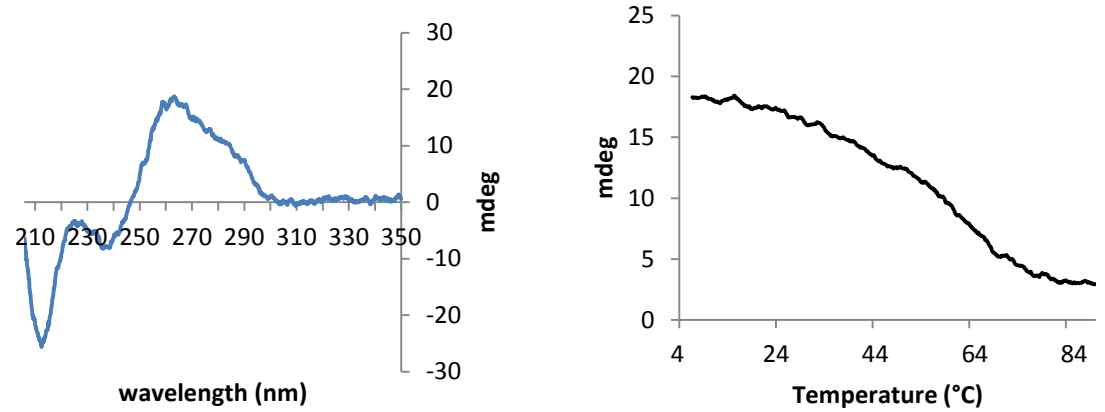


Figure S35. CD (left) and melt (right) of **14**.

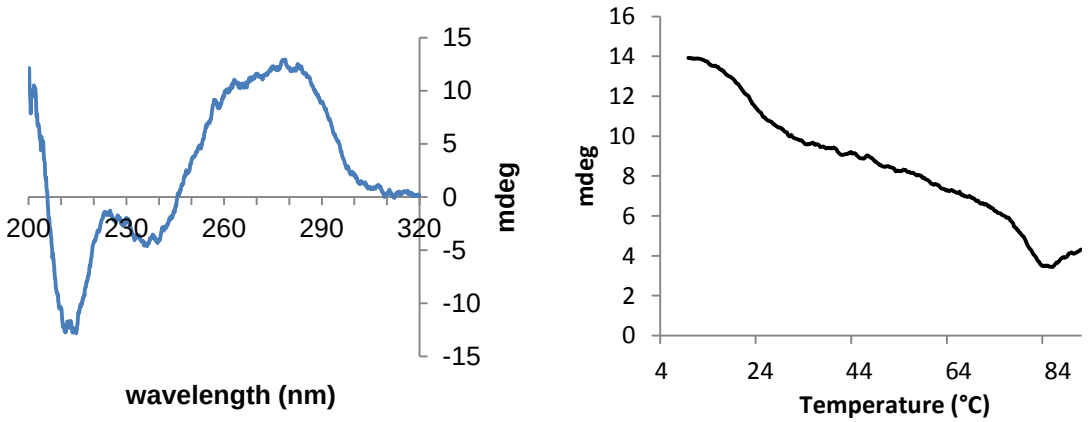


Figure S36. CD (left) and melt (right) of **15**.

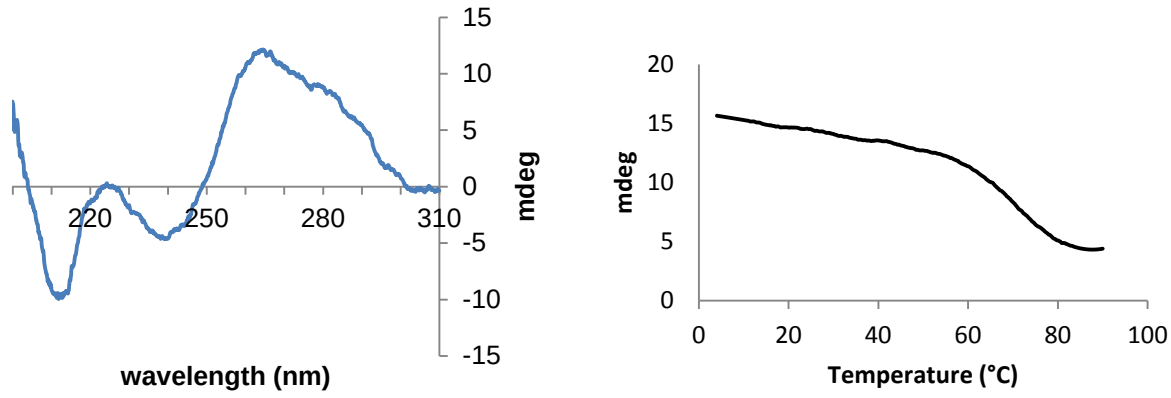


Figure S37. CD (left) and melt (right) of **16**.

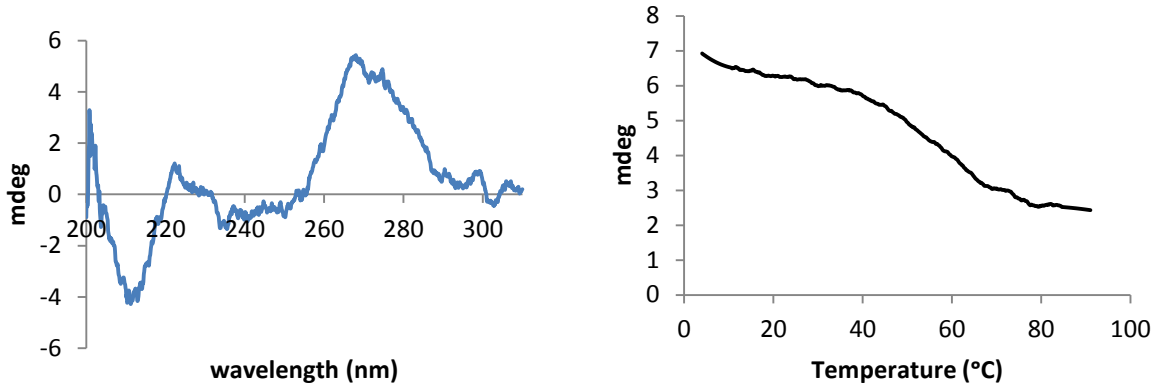


Figure S38. CD (left) and melt (right) of **17'**.

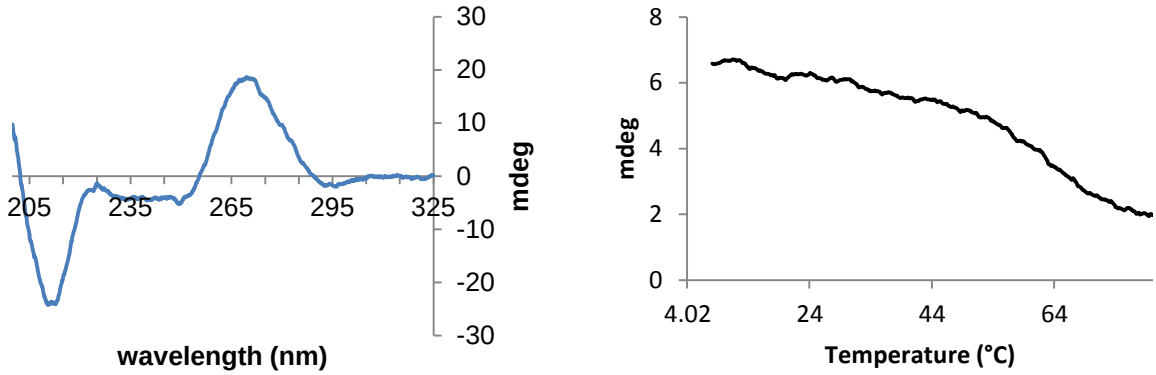


Figure S39. CD (left) and melt (right) of **17**.

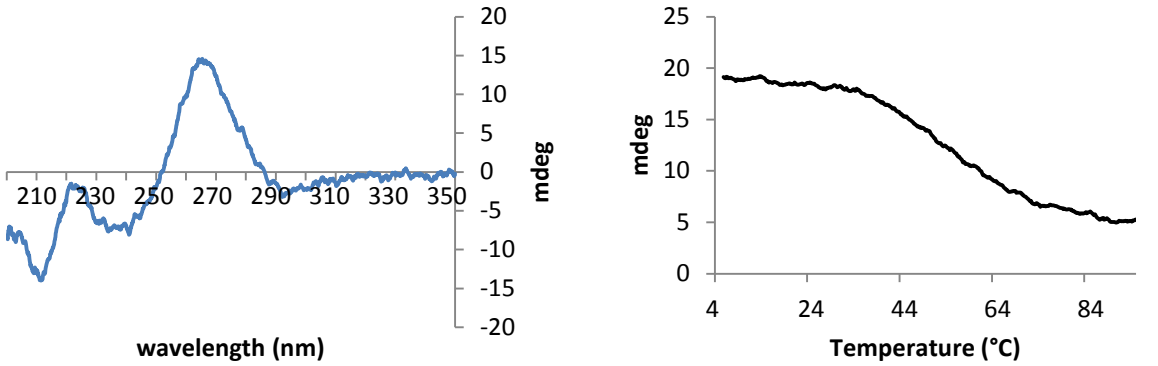


Figure S40. CD (left) and melt (right) of **17-OMe**.

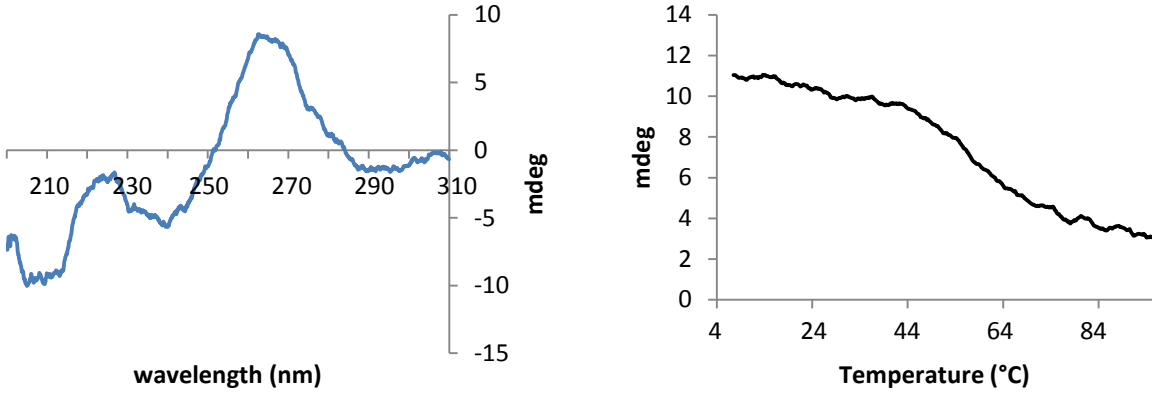


Figure S41. CD (left) and melt (right) of **18**.

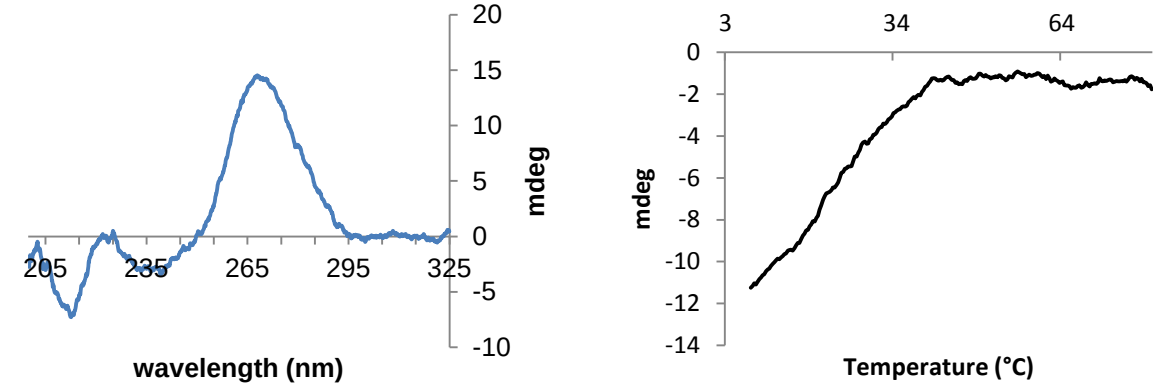


Figure S42. CD (left) and melt (right) of **19**.

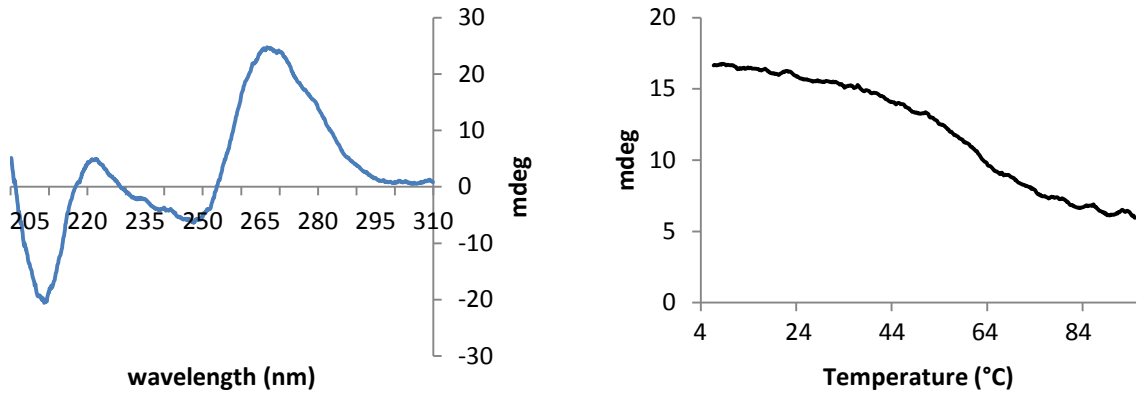


Figure S43. CD (left) and melt (right) of **20**.

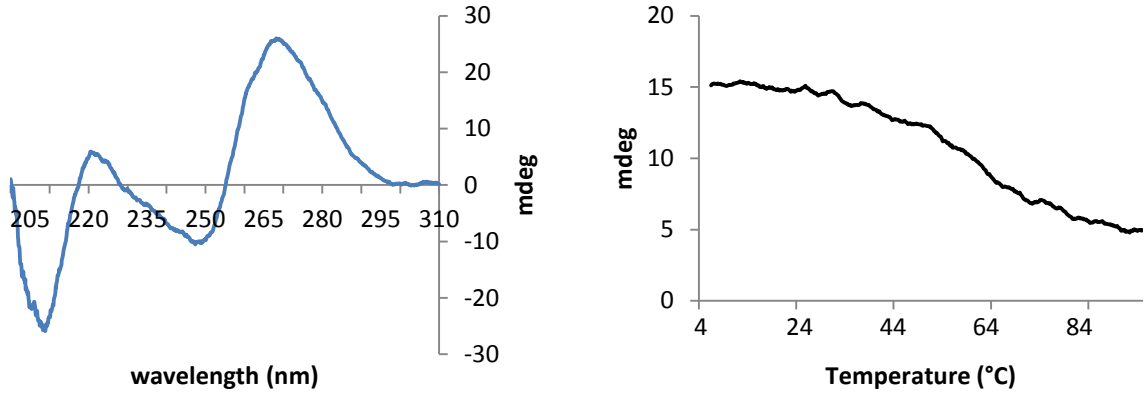


Figure S44. CD (left) and melt (right) of **21**.

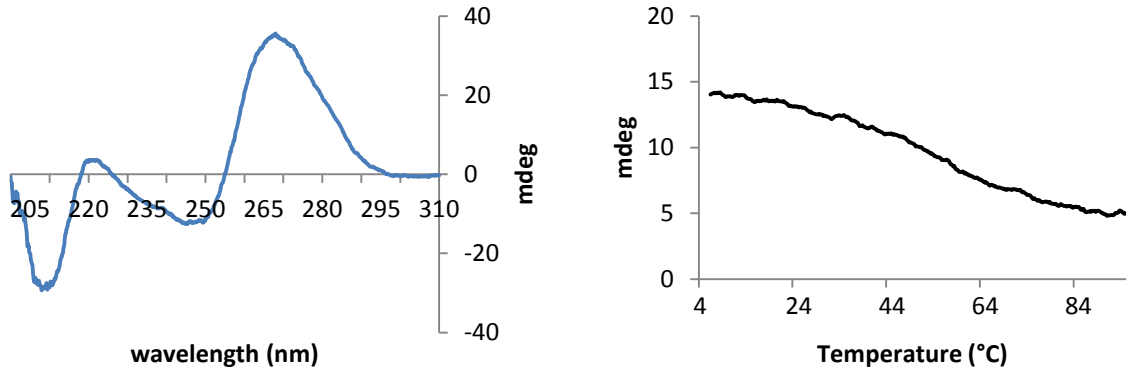


Figure S45. CD (left) and melt (right) of **22**.

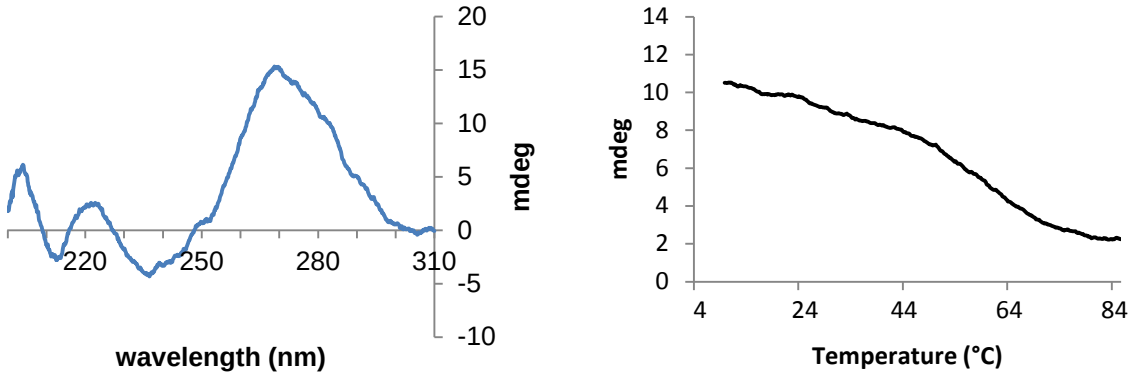


Figure S46. CD (left) and melt (right) of **23**.

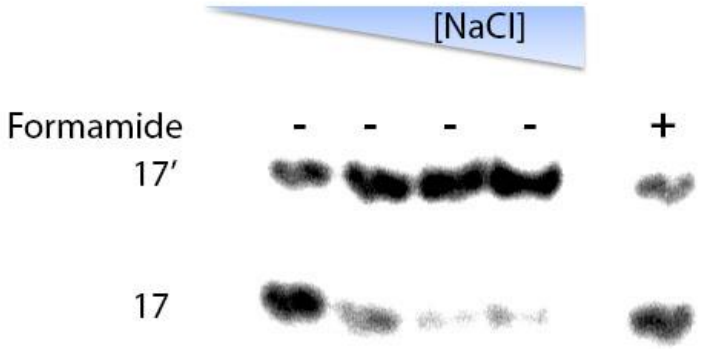


Figure S47. Mobility shift assays for: **17** with increasing [NaCl] at 50, 100, 500, and 1000 mM

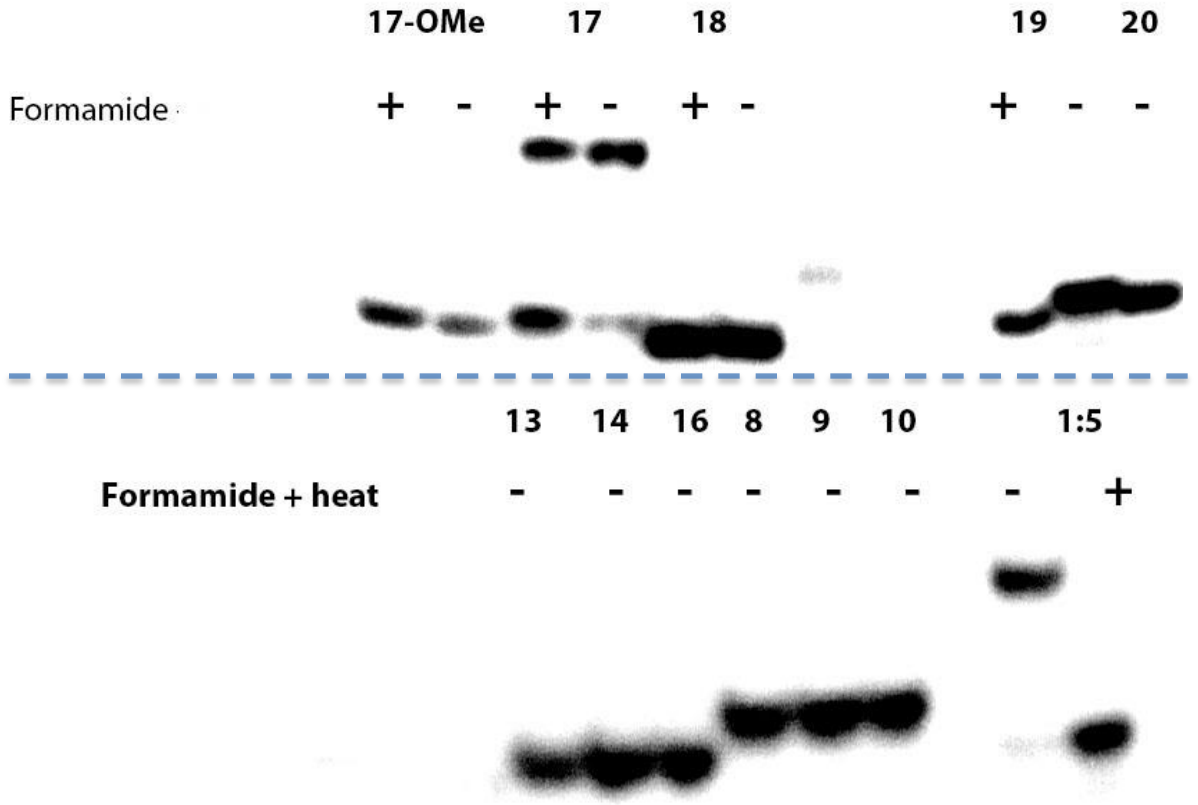


Figure S48. Mobility shift assays for: **17-OMe**, **17**, **18**, **19**, **20** w/wo addn. of formamide (middle); and **13**, **14**, **16**, **8-10** wo addn. of formamide and control duplex **1**:**5** w/wo formamide + heat 75° C (bottom). All gels were carried out in 20 % non-denaturing PAGE.

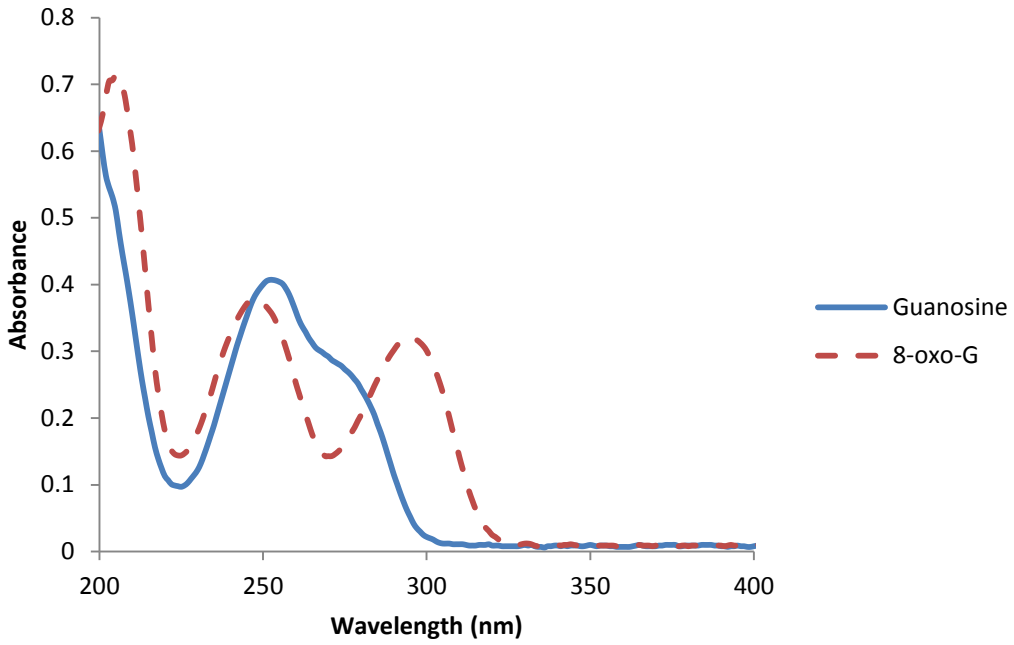
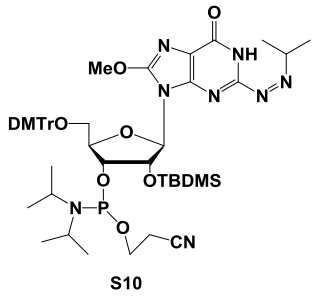


Figure **S49**. UV-Vis of Guanosine and 8-oxo-G.



S10

Figure **S50**. Synthesis of **S10** was accomplished by following the known procedures, and the ^{1}H NMR and ^{31}P NMR chemical shifts matched reference[2].

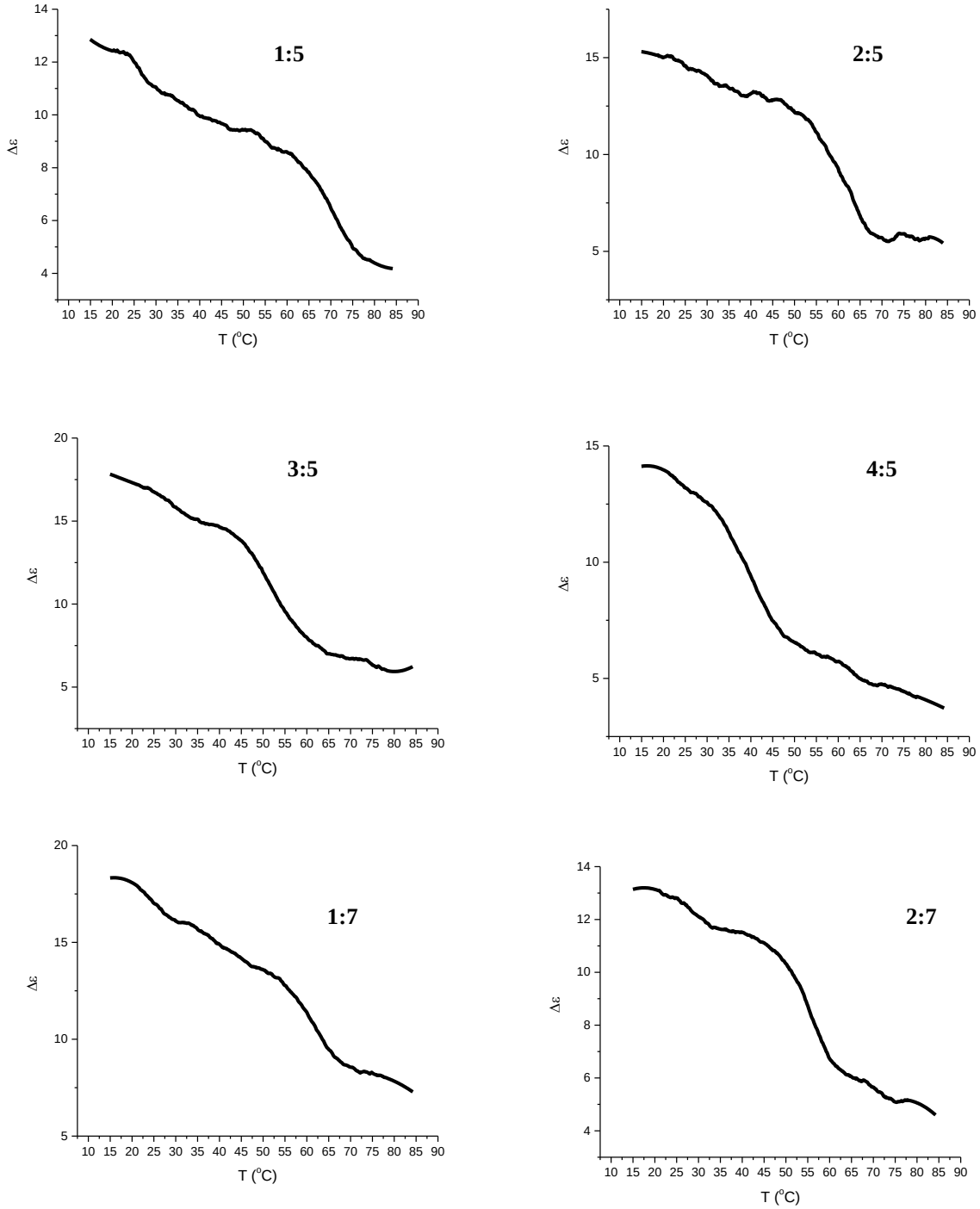


Figure 51. T_m measurements of strands **1:5, 2:5, 3:5, 4:5, 1:7** and **2:7**, CD recorded at 270 nm

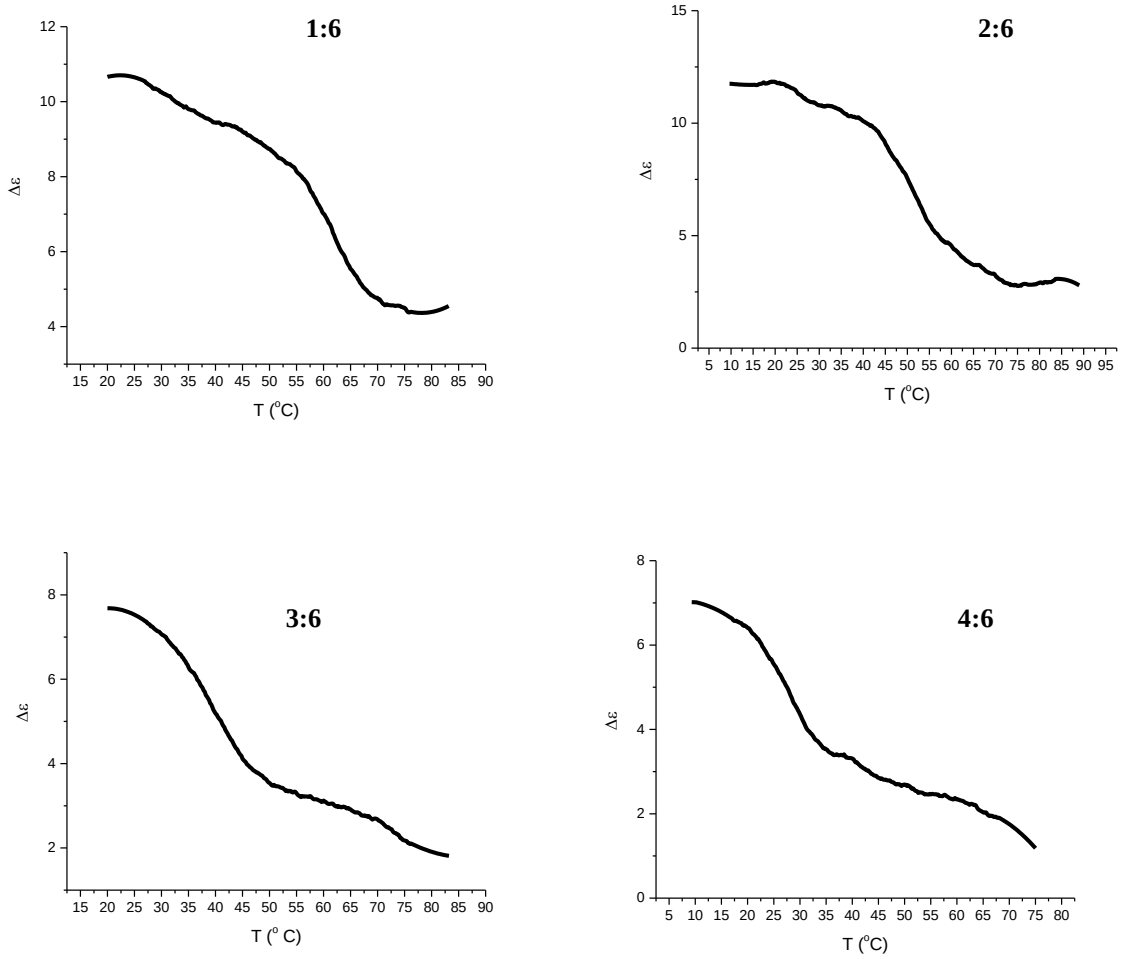


Figure 52. T_m measurements of strands **1:6, 2:6, 3:6,** and **4:6**, CD recorded at 270 nm

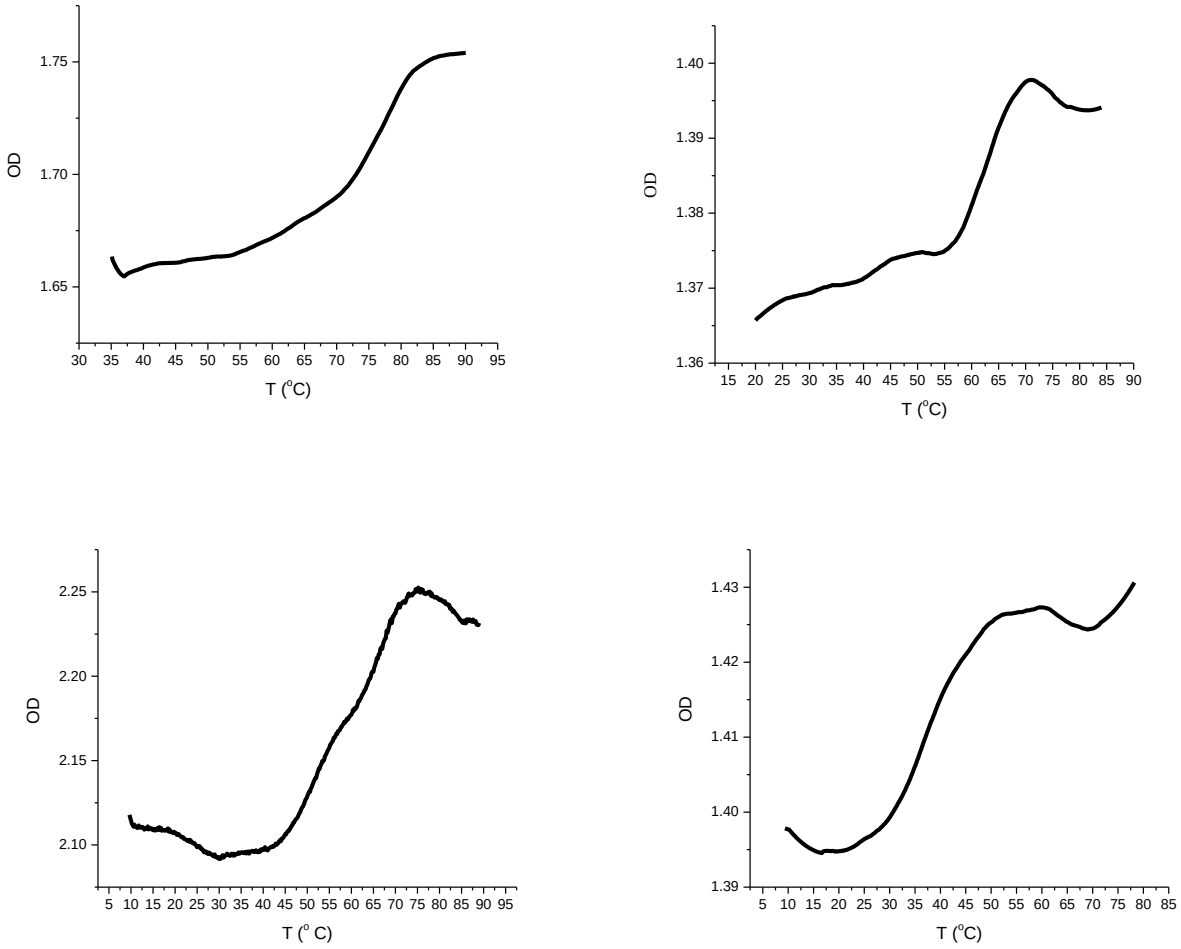


Figure 53. T_m measurements of strands **1:5, 2:5, 3:5,** and **4:5, 1:7** OD recorded

REFERENCES

1. Lin, T.S.; Chen, J.C., Ishiguro, K.; Sartorelli, A.C. (1985) 8-Substituted Guanosine and 2'-Deoxyguanosine Derivatives as Potential Inducers of the Differentiation of Friend Erythroleukemia Cells.*J. Med. Chem.*, **28**, 1194-1198.

2. Baranowski, D.S.; Kotkowiak, W.; Kierzek, R.; Pasternak, A. (2015) Hybridization Properties of RNA Containing 8-Methoxyguanosine and 8-Benzyloxyguanosine. *PLoS ONE* **10**(9). doi: 10.1371/journal.pone.0137674.