Supporting Information

α,β-Methylene-2'-deoxynucleoside 5'-triphosphates as non-cleavable substrates for DNA polymerases: Isolation, characterization, and stability studies of novel 2'-deoxycyclonucleosides, 3,5'-anhydro-dG and 2,5'-anhydro-dT

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Figure S16. TOCSY 1H NMR spectrum of cyclo-dG (**16**) recorded in DMSO-d6.

Figure S17. NOSEY 1H NMR spectrum of cyclo-dT(**17**) recorded in DMSO-d6. The cross-peaks circled in red indicate the close proximity of the H6 and H1' protons (i.e., *syn*-glycosidyl conformation).

Figure S18. Comparison of 13C NMR of dT and Cyclo-dT (**17**) recorded in DMSO-d6.

Figure S19. Comparison of UV of (a) α,β-methylene-dGDP (**11**)(green) and cyclo-dG (**16**) (blue); (b) α,β-methylene-dTDP (**10**)(green) and cyclo-dT (**17**)(blue).

Figure S20. The CD spectra of cyclo-dG (**16**) as a function of time (0 - 6 h) every hour at 90 °C in comparison with dG (dotted blue lines).

Figure S21. The CD spectra of cyclo-dT (**17**) as a function of time (0- 6 h) at 90 °C in comparison with dT (dotted blue lines).

Figure S22. pH dependence UV spectra of dG.

Figure S23. pH dependence UV spectra of cyclo-dG (**16**).

Figure S24. pH dependence CD spectra of cyclo-dG (**16**).

Figure S25. pH dependence UV spectra of dT.

Figure S26. pH dependence UV spectra of cyclo-dT(**17**).

Figure S27. pH dependence CD spectra of **18**.

Figure S28. pH dependence UV spectra of **18**.

Figure S29. 1H NMR spectrum of **18** in DMSO-d6.

Figure S30. 1H NMR spectrum of **18** in DMSO-d6 + D2O.

Figure S31. 13C NMR spectrum of **18** in D2O.

Figure S32. pH dependence CD spectra of cyclo-dX (**19**).

Figure S33. pH dependence UV spectra of cyclo-dX (**19**).

Figure S34. 1H NMR spectrum of cyclo-dX (**19**) in DMSO-d6**.**

Figure S36. 13C NMR spectrum of cyclo-dX (**19**) in DMSO-d6**.**

Figure S37. NOESY NMR spectrum of cyclo-dX (**19**) in DMSO-d6**.**

Figure S38. Cyclo-dX (**19**). The product ion spectrum of the $(M+H)^+$ ion acquired with LTQ-FTMS.

Figure S39. Comparison of UV of cyclo-dG (blue), acid intermediate **18** (pink), and cyclo-dX (**19**)(green).

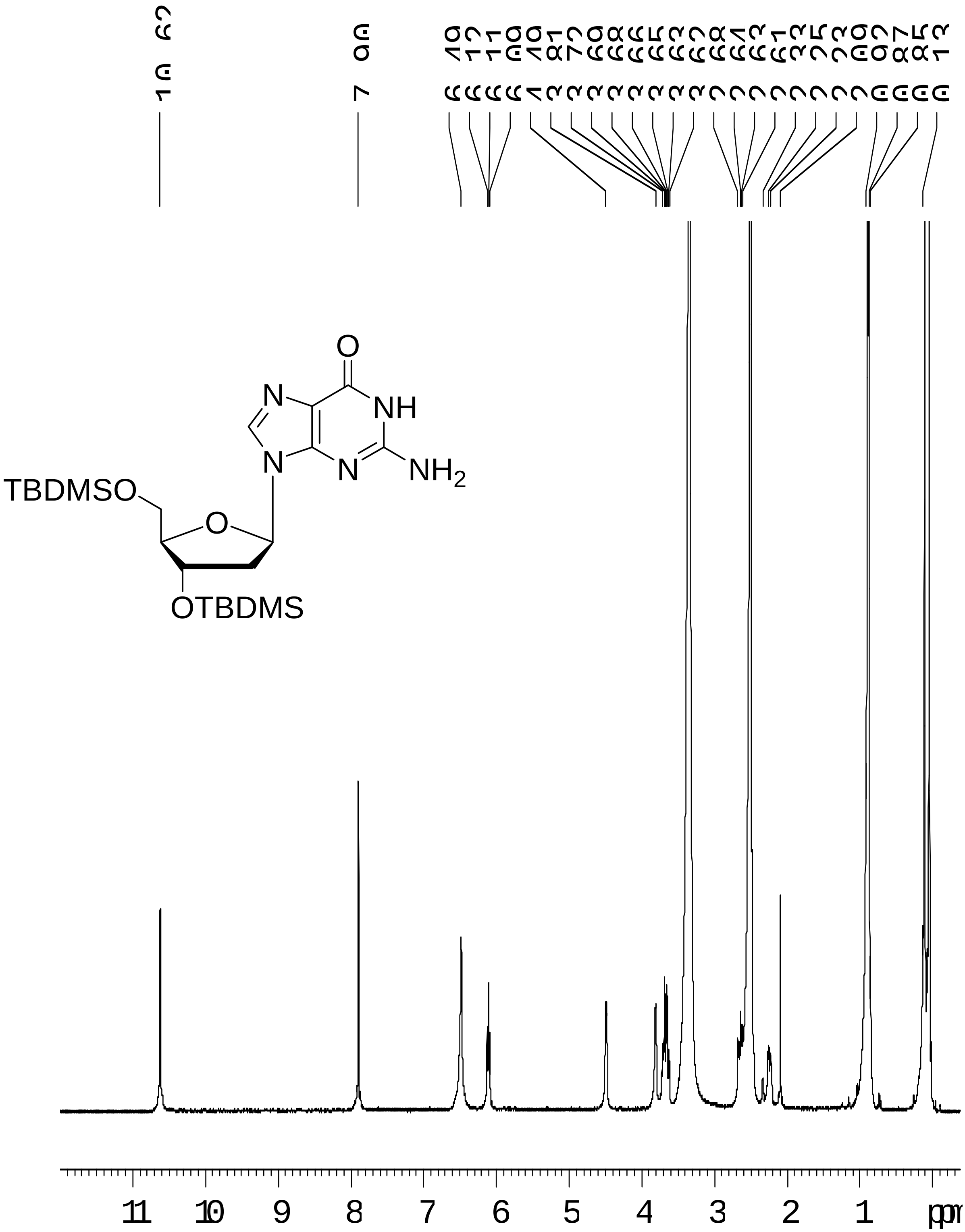


Figure S1: ^{1}H NMR spectrum of 5’,3’-*O*-*bis*-*tert*-butyl-dimethylsilyl-dG recorded in DMSO-d6

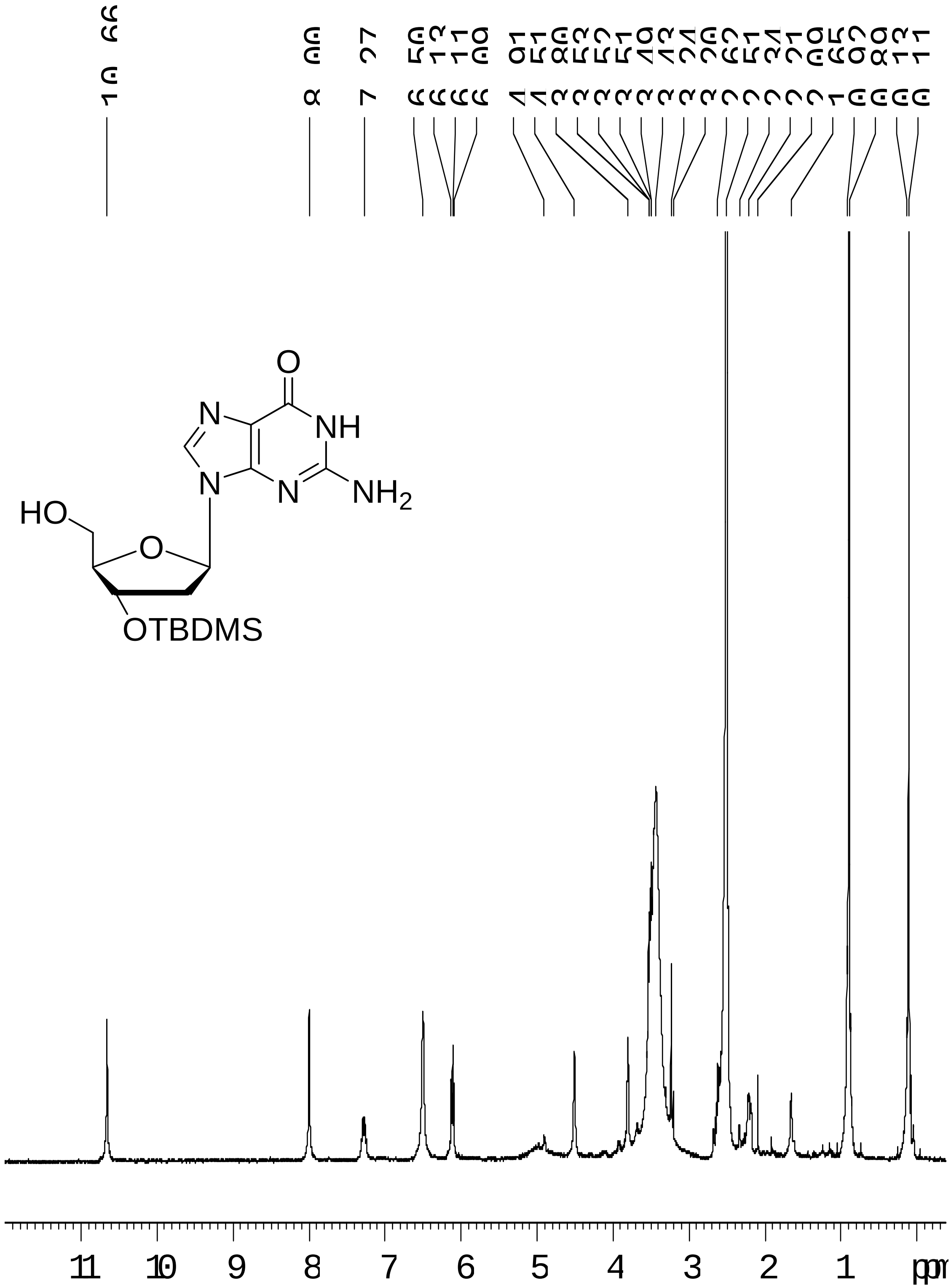


Figure S2: [1]H NMR spectrum of 3'-*O*-*tert*-butyl-dimethylsilyl-dG recorded in DMSO-d6

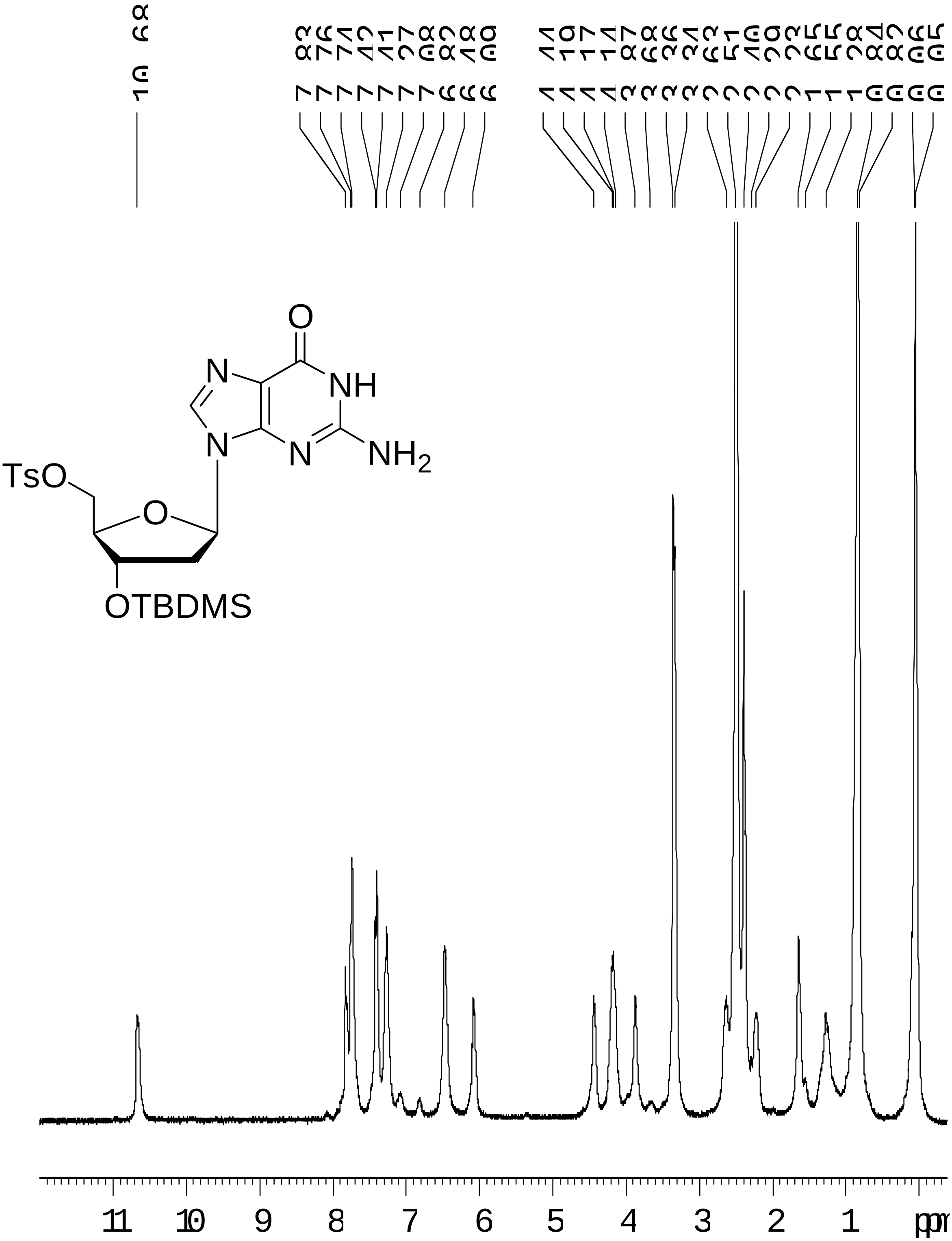


Figure S3: ^{1}H NMR spectrum of 5’-*O*-Tosyl-3’-*O*-*tert*-butyl-dimethylsilyl-dG recorded in DMSO-d6

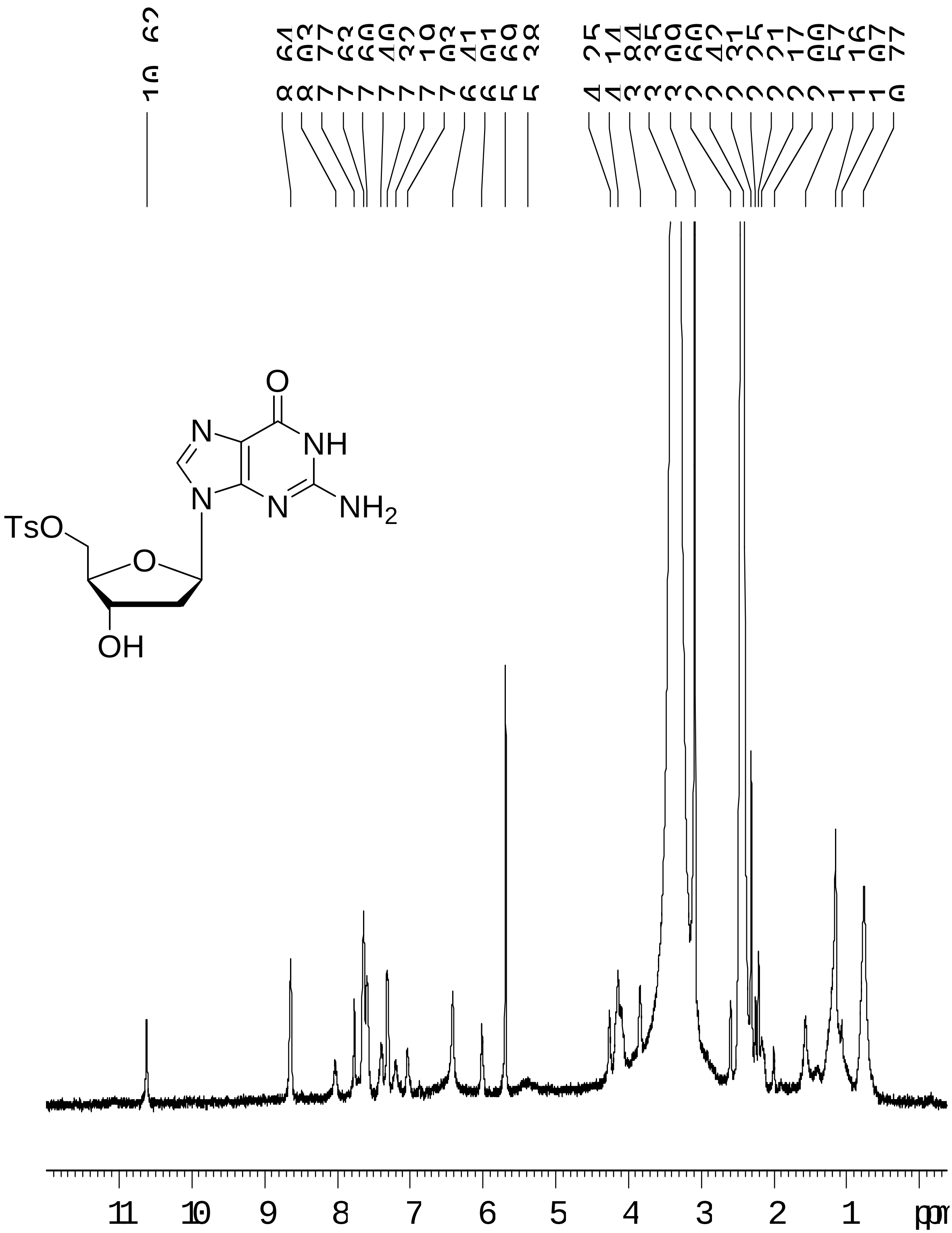


Figure S4: ^{1}H NMR spectrum of 5'-*O*-Tosyl-dG (**4**) recorded in DMSO-d6

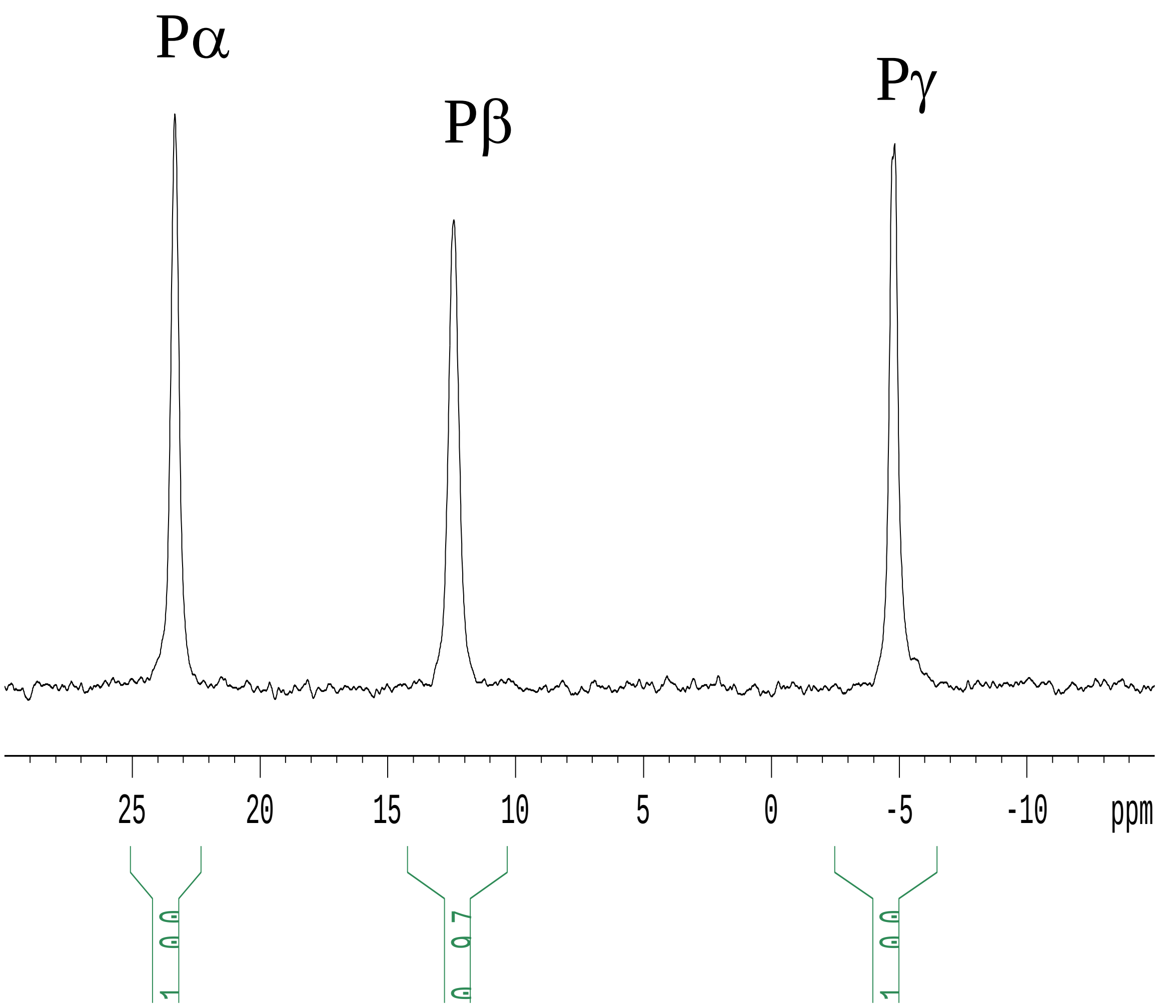


Figure S5: ^{31}P NMR spectrum of α,β-methylene-dATP (**12**) recorded in D_2O.

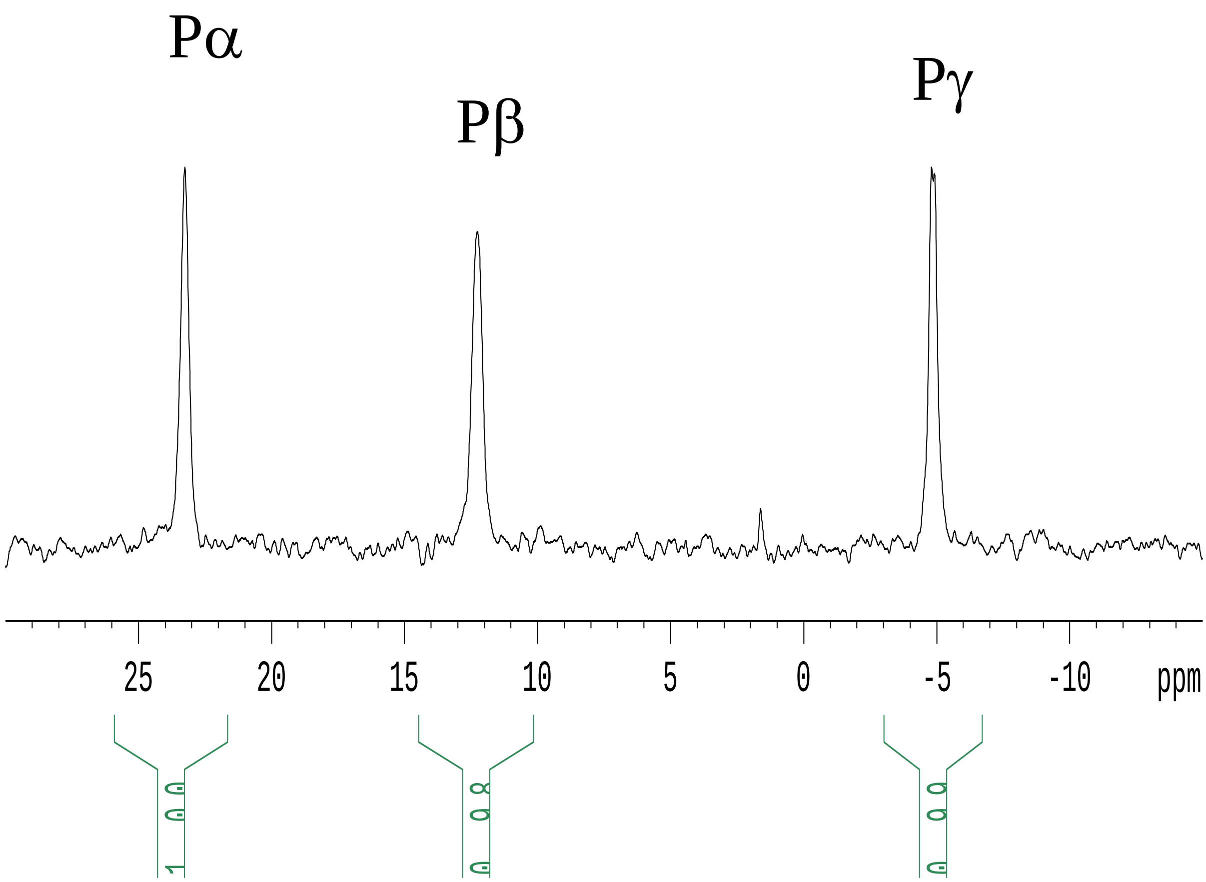


Figure S6: ^{31}P NMR spectrum of α,β-methylene-dCTP (**13**) recorded in D_2O.

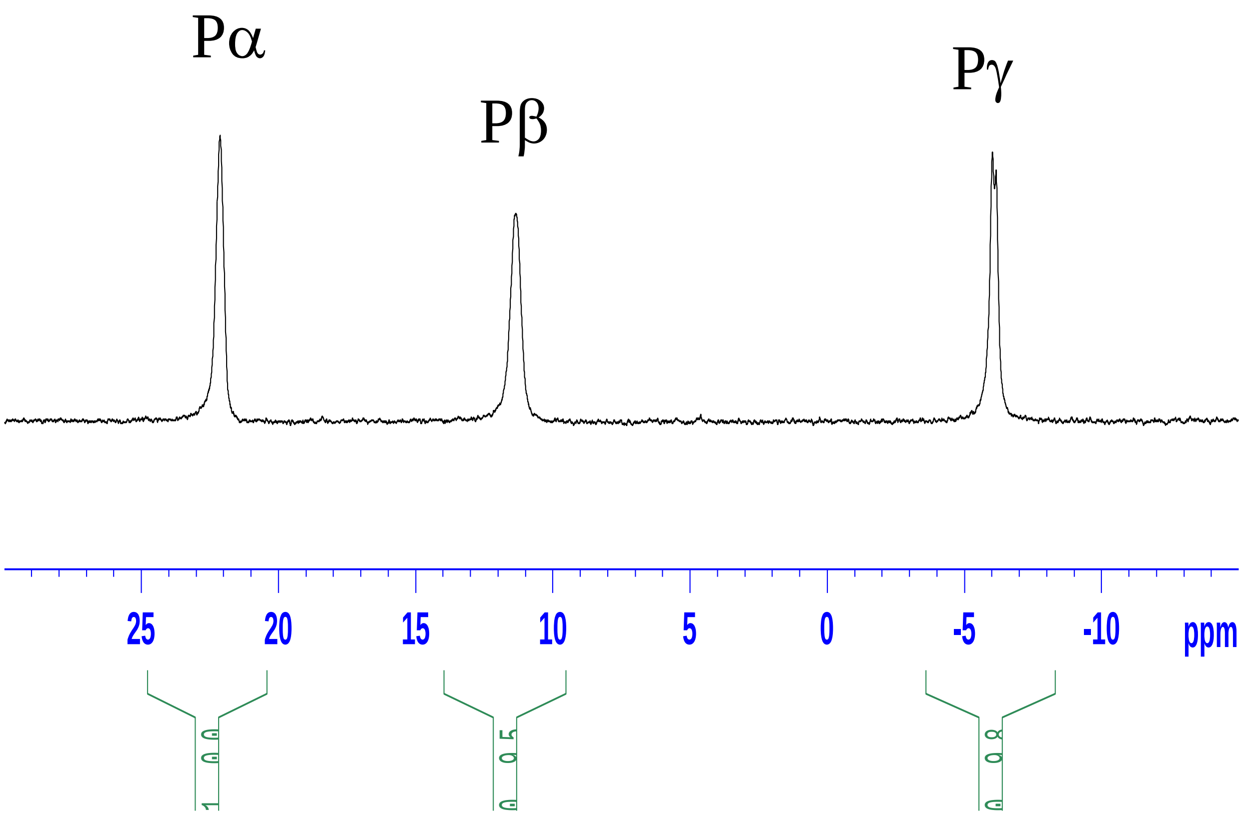


Figure S7: ^{31}P NMR spectrum of α,β-methylene-dGTP (**15**) recorded in D_2O.

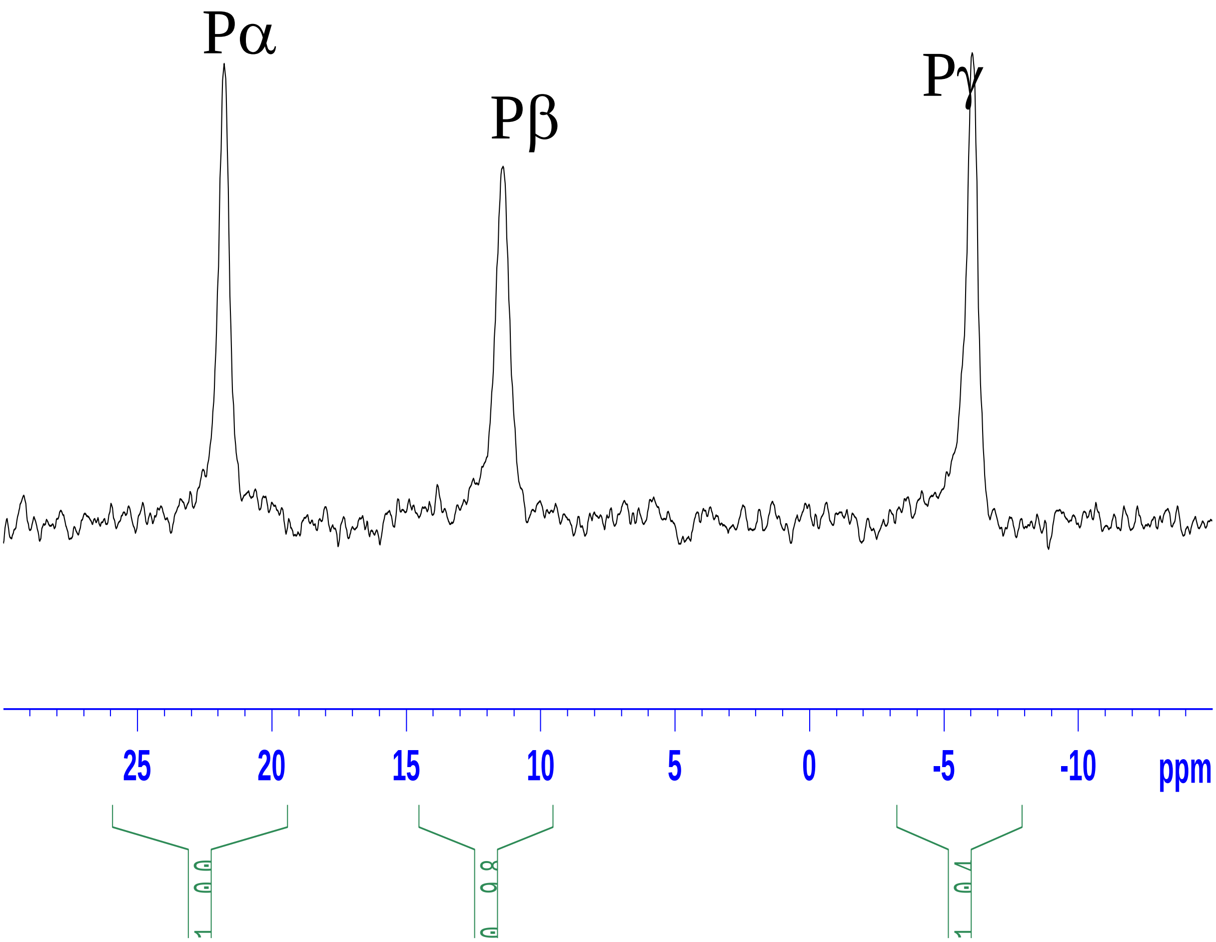


Figure S8: ^{31}P NMR spectrum of α,β-methylene-dTTP (**14**) recorded in D_2O.

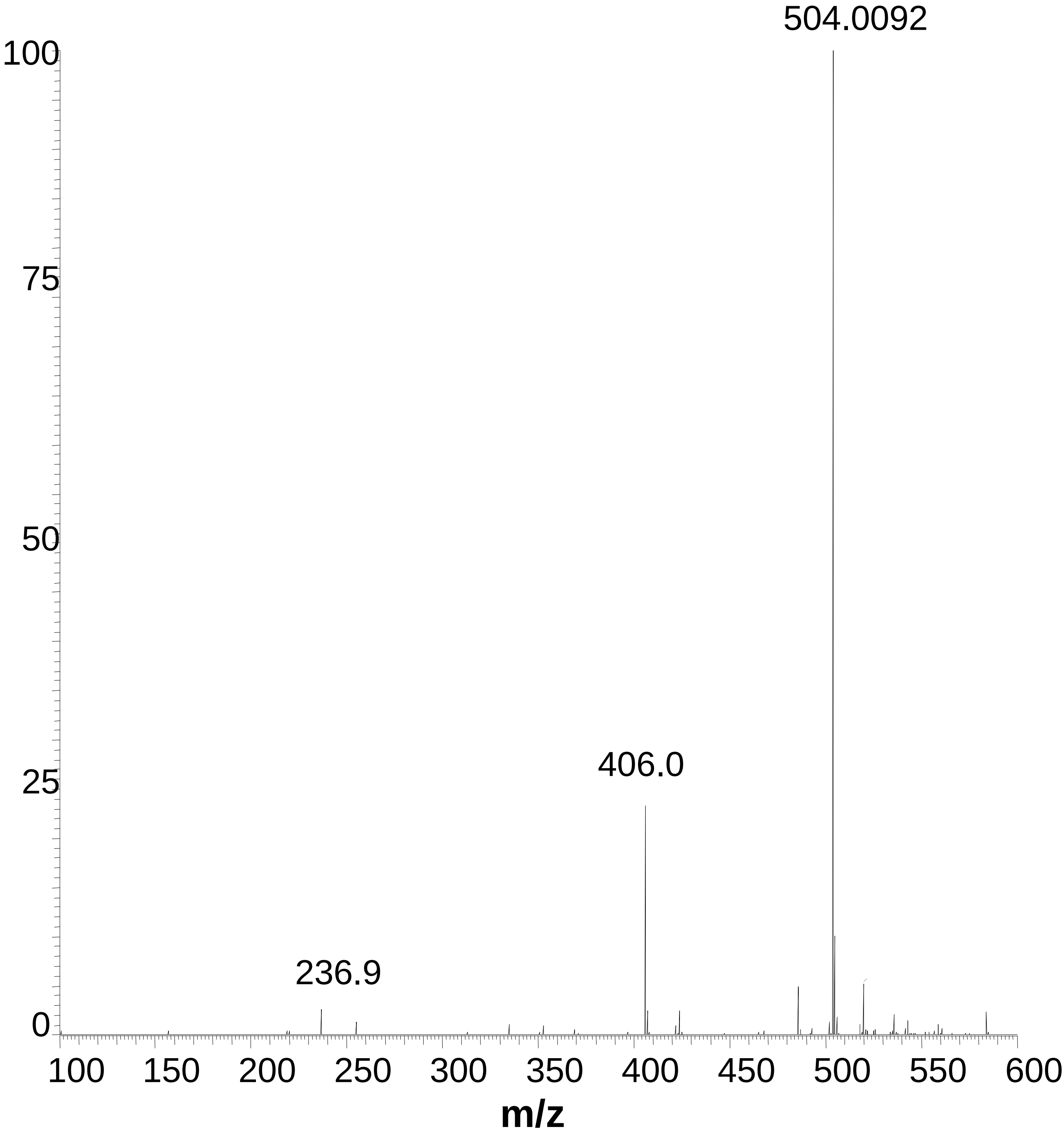


Figure S9: Negative ion ESI-LTQ-FTMS high resolution mass spectrum of α,β-methylene-dGTP (**15**). The theoretical mass of the $(M\text{-}H)^-$ ion is 504.0087. The measured mass is 504.0092

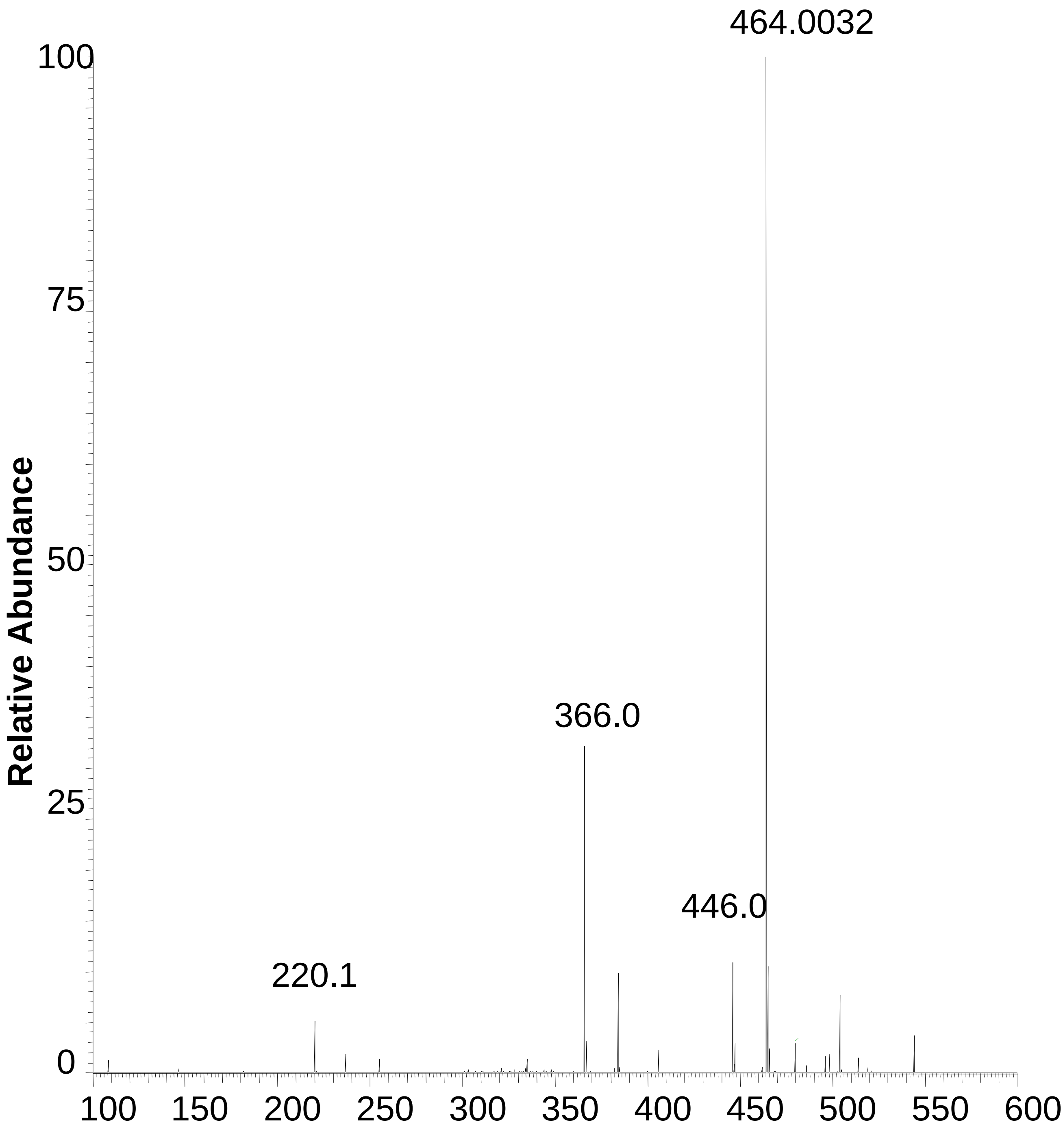


Figure S10: Negative ion ESI-LTQ-FTMS high resolution mass spectrum of α,β-methylene-dCTP (**13**). The theoretical mass of the $(M\text{-}H)^-$ ion is 464.0025. The measured mass is 464.0032.

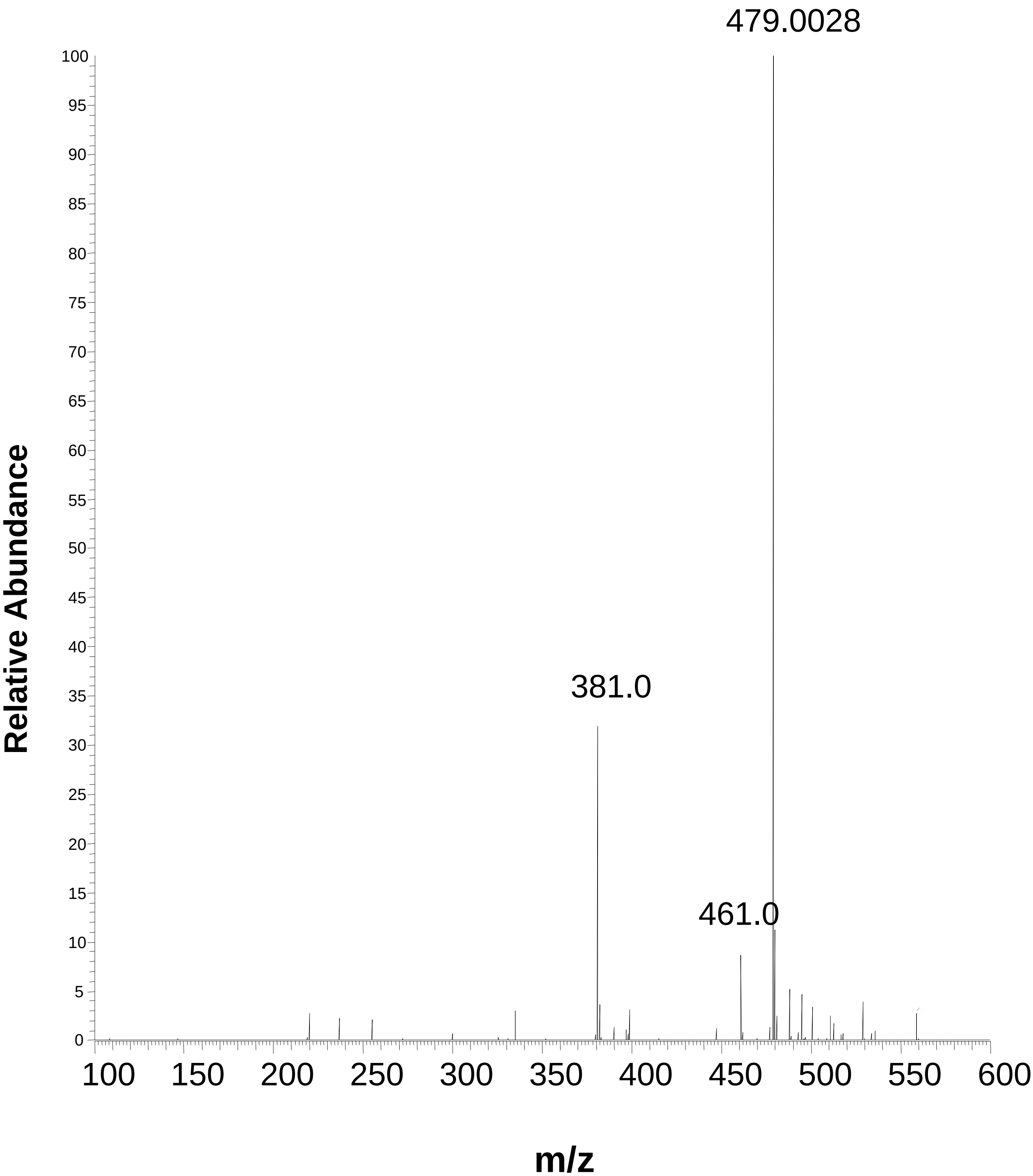


Figure S11: Negative ion ESI-LTQ-FTMS high resolution mass spectrum of α,β-methylene-dTTP (**14**). The theoretical mass of the $(M-H)^-$ ion is 479.0022. The measured mass is 479.0028.

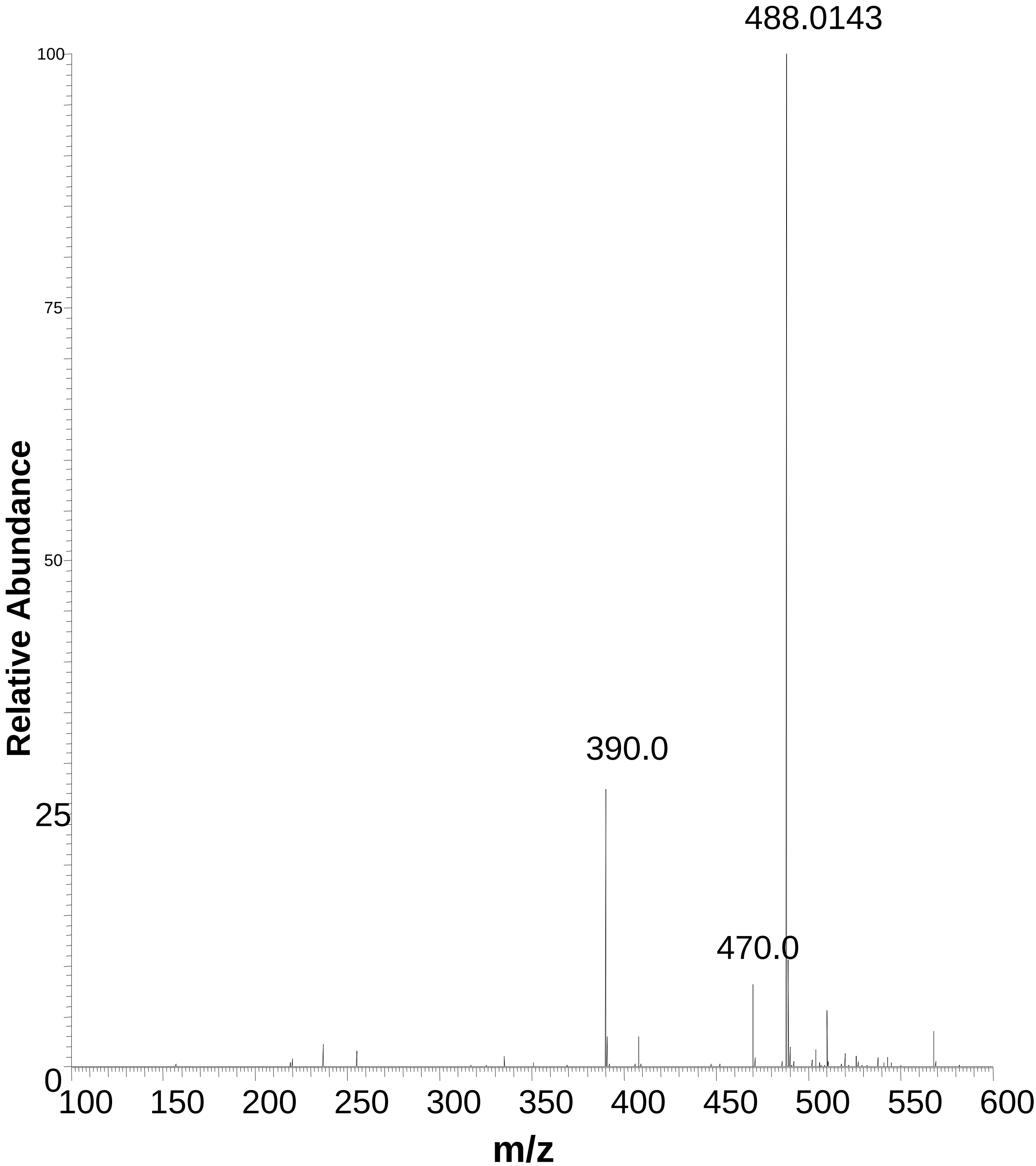


Figure S12: Negative ion ESI-LTQ-FTMS high resolution mass spectrum of α,β-methylene-dATP(**12**). The theoretical mass of the $(M-H)^-$ ion is 488.0137. The measured mass is 488.0143.

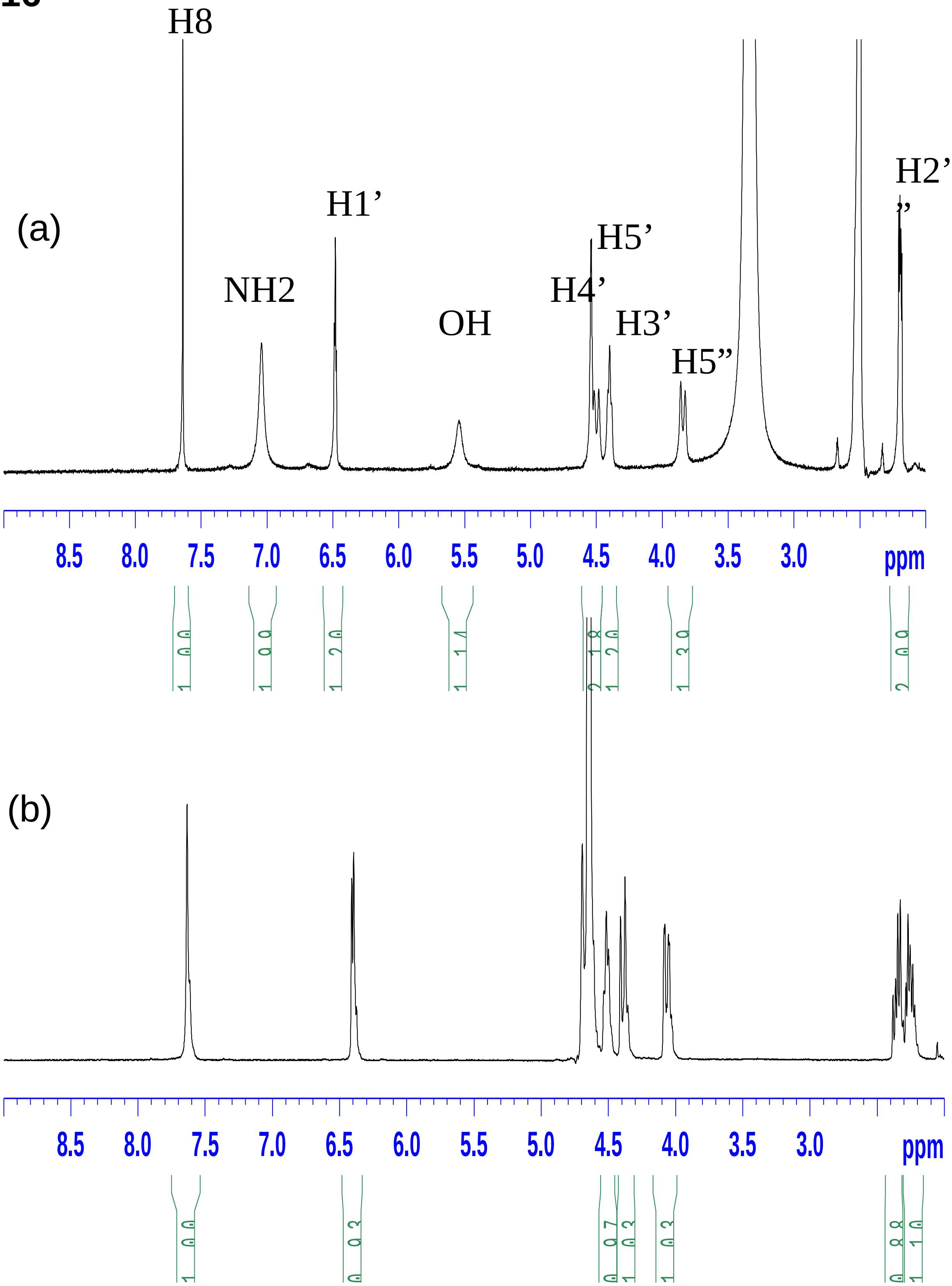


Figure S13: [1]H NMR spectrum of cyclo-dG (**16**) recorded in (a) DMSO-d6 and with D_2O added.

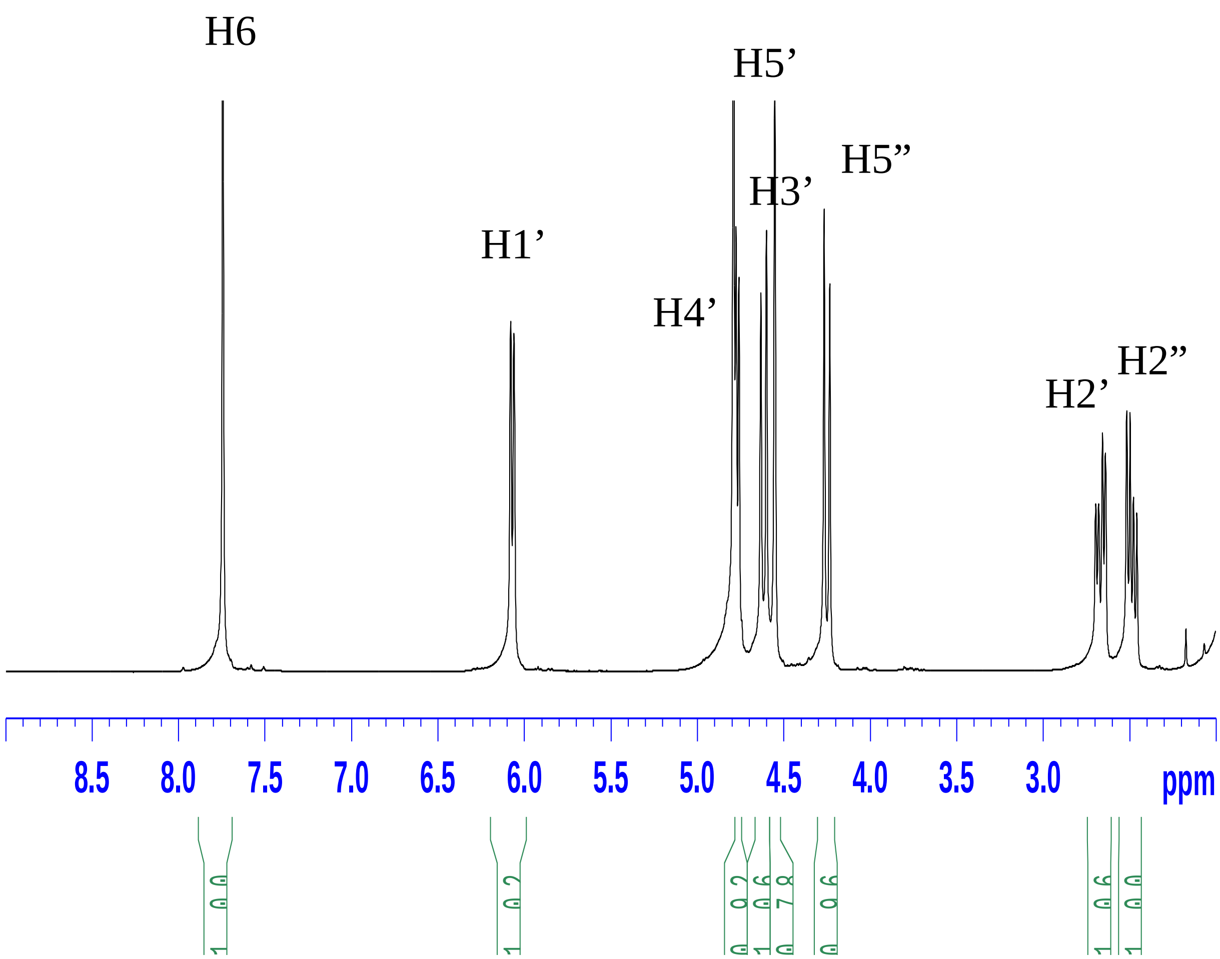


Figure S14: ^{1}H NMR spectrum of cyclo-dT (**17**) recorded in D_2O.

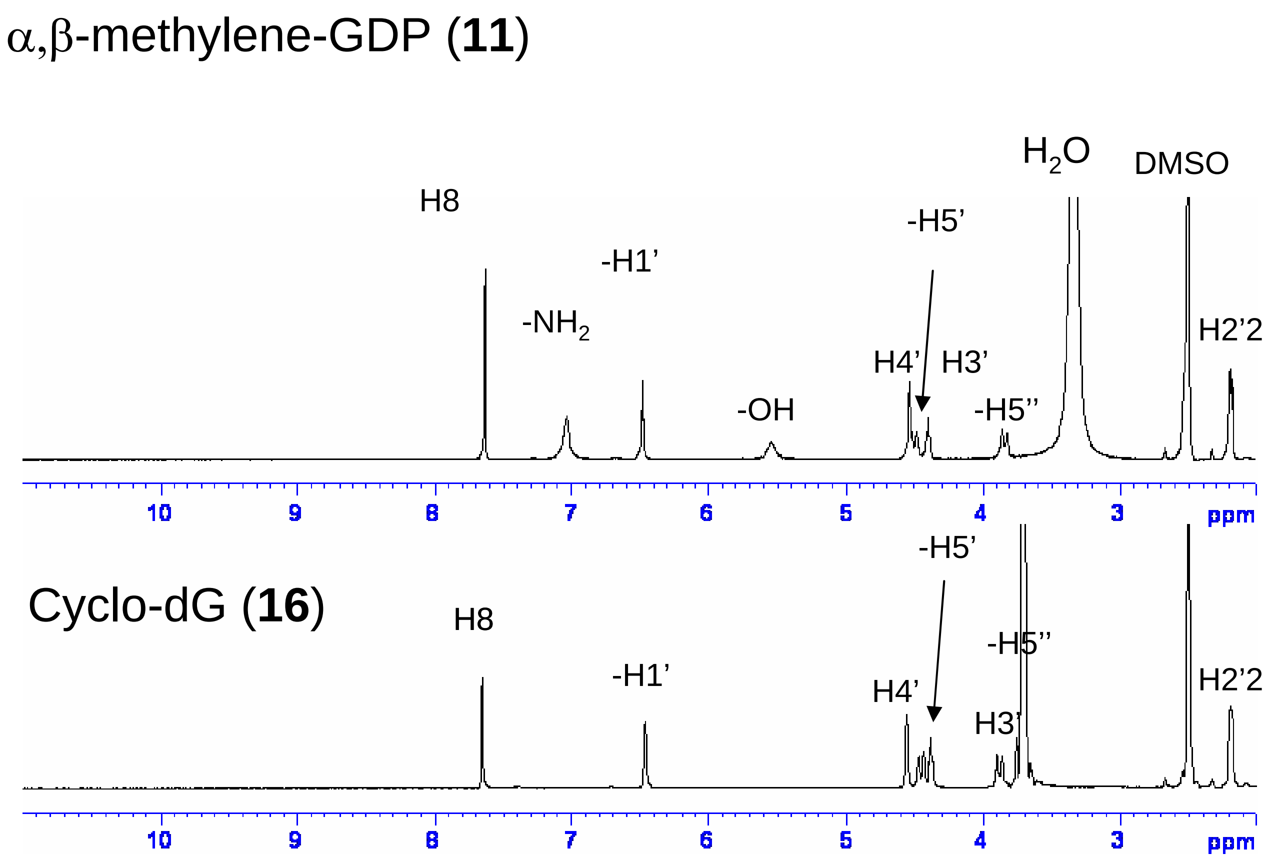


Figure S15. Comparison of 1H NMR spectra of α,β–methylene-dGDP (**11**) and cyclo-dG (**16**) recorded in DMSO-d6.

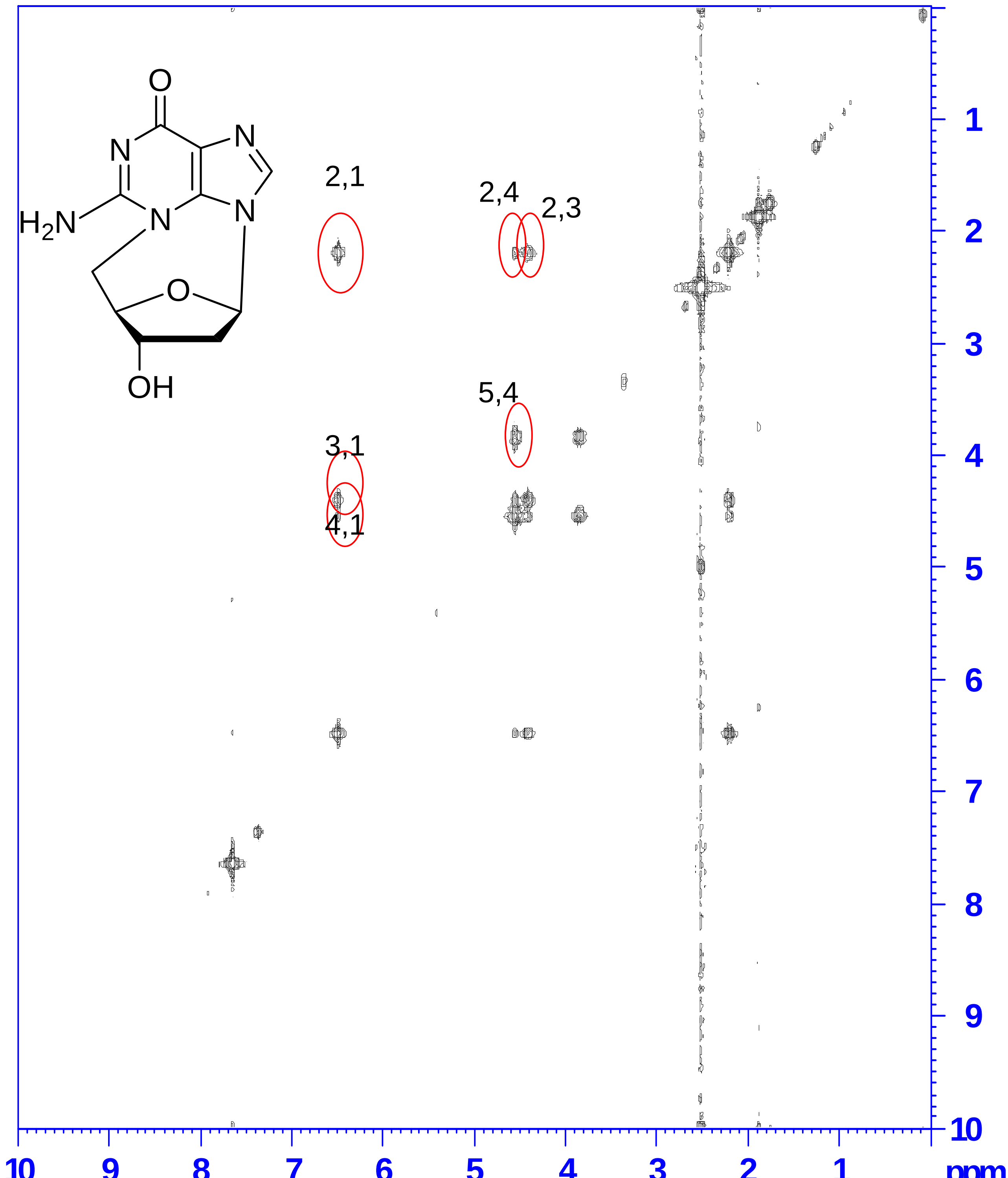


Figure S16. TOCSY [1]H NMR spectrum of cyclo-dG (**16**) recorded in DMSO-d6.

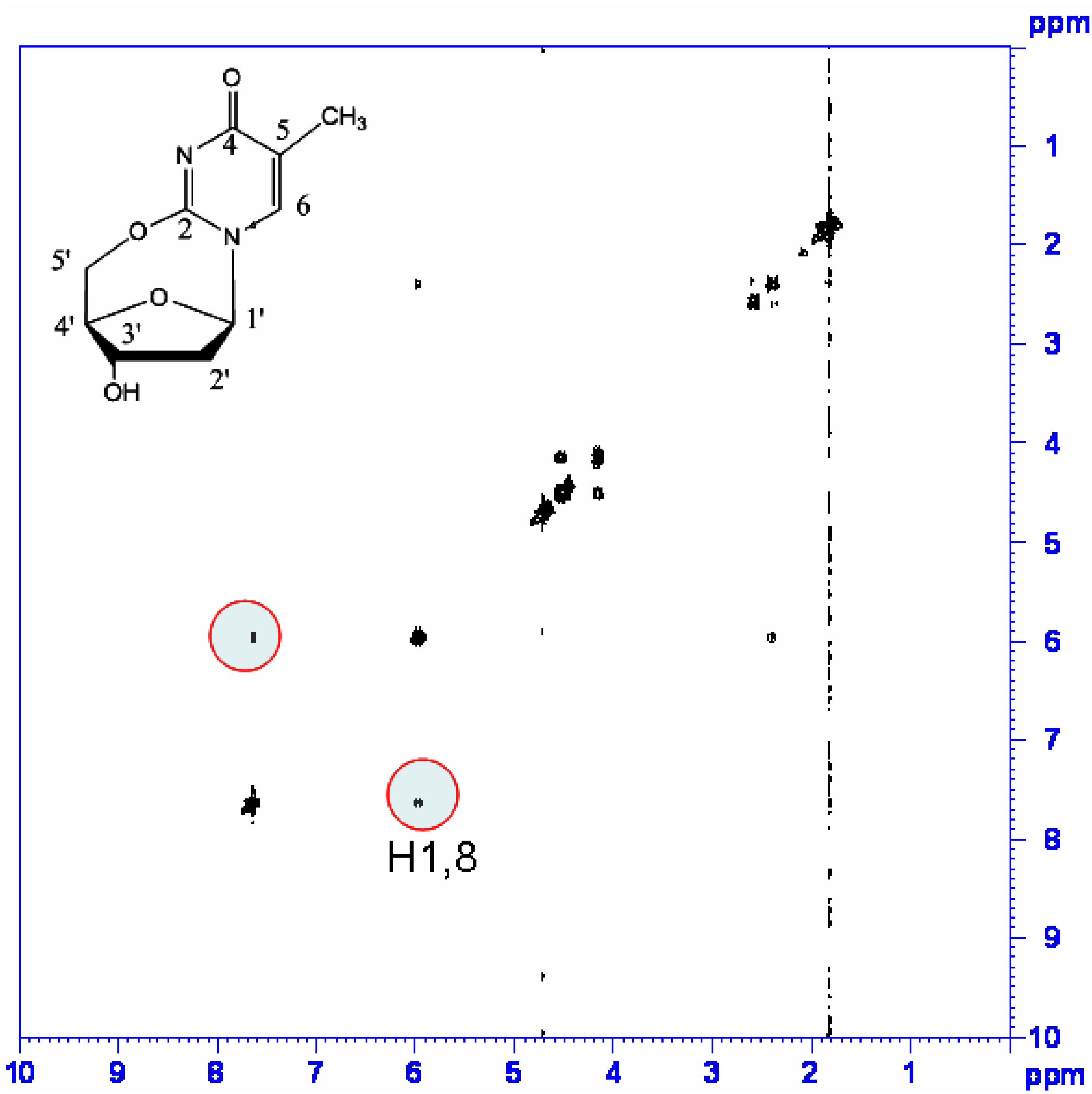


Figure S17. NOSEY ^{1}H NMR spectrum of cyclo-dT(**17**) recorded in DMSO-d_6. The cross-peaks circled in red indicate the close proximity of the H6 and H1' protons (i.e., *syn*-glycosidyl conformation).

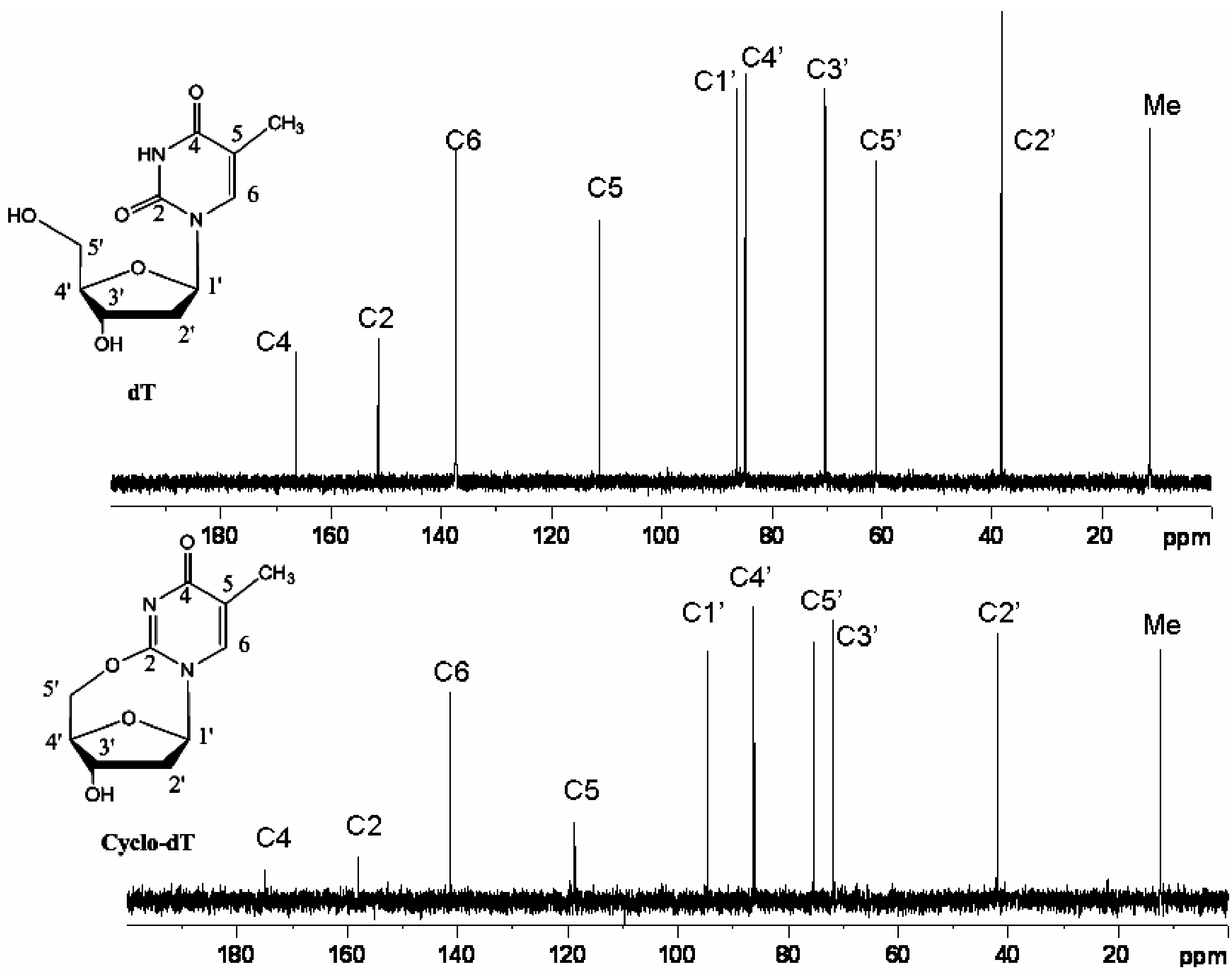


Figure S18. Comparison of ^{13}C NMR of dT and cyclo-dT (**17**) recorded in DMSO-d_6.

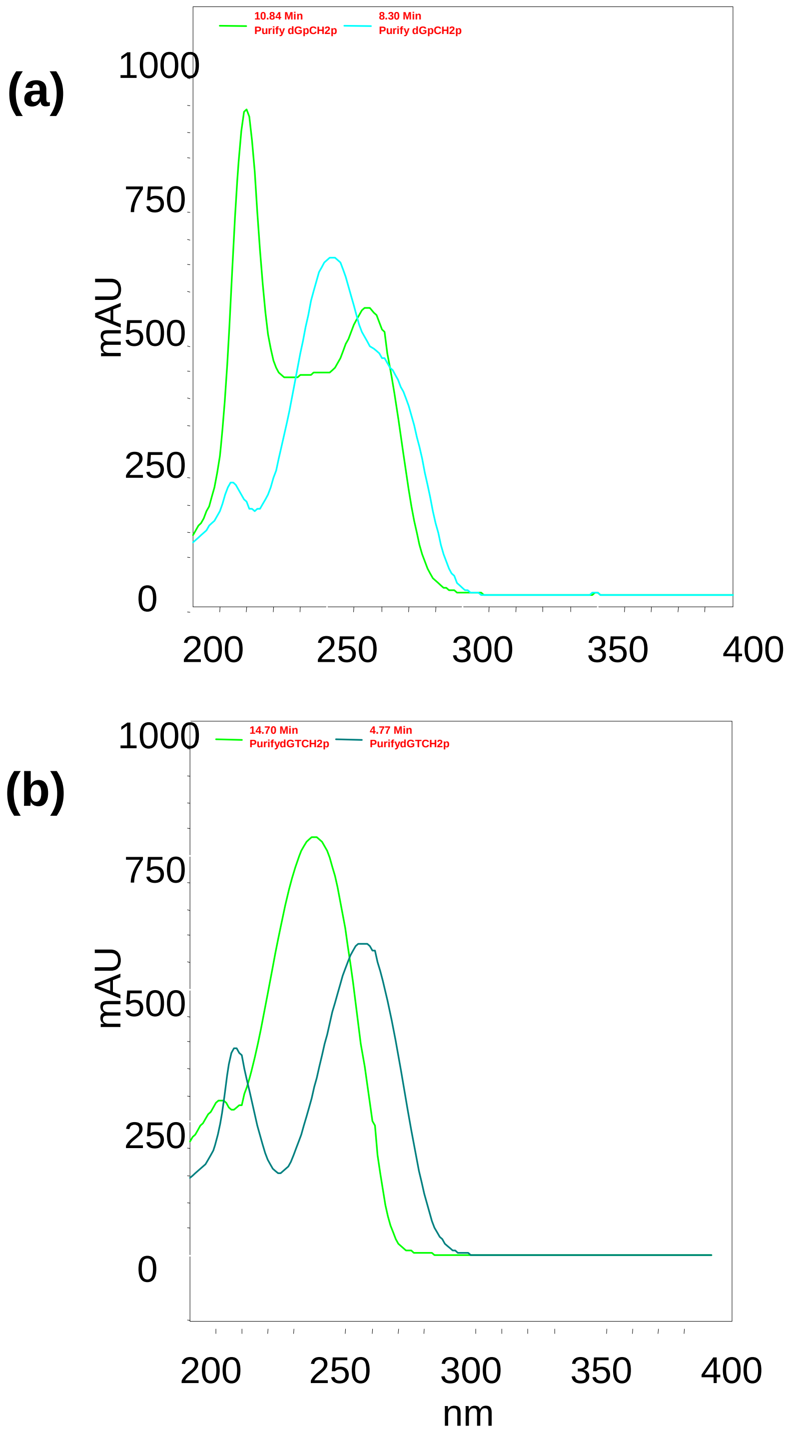


Figure S19. Comparison of UV of (a) α,β-methylene-dGDP (**11**)(green) and cyclo-dG (**16**) (blue); (b) α,β-methylene-dTDP (**10**)(green) and cyclo-dT (**17**)(blue).

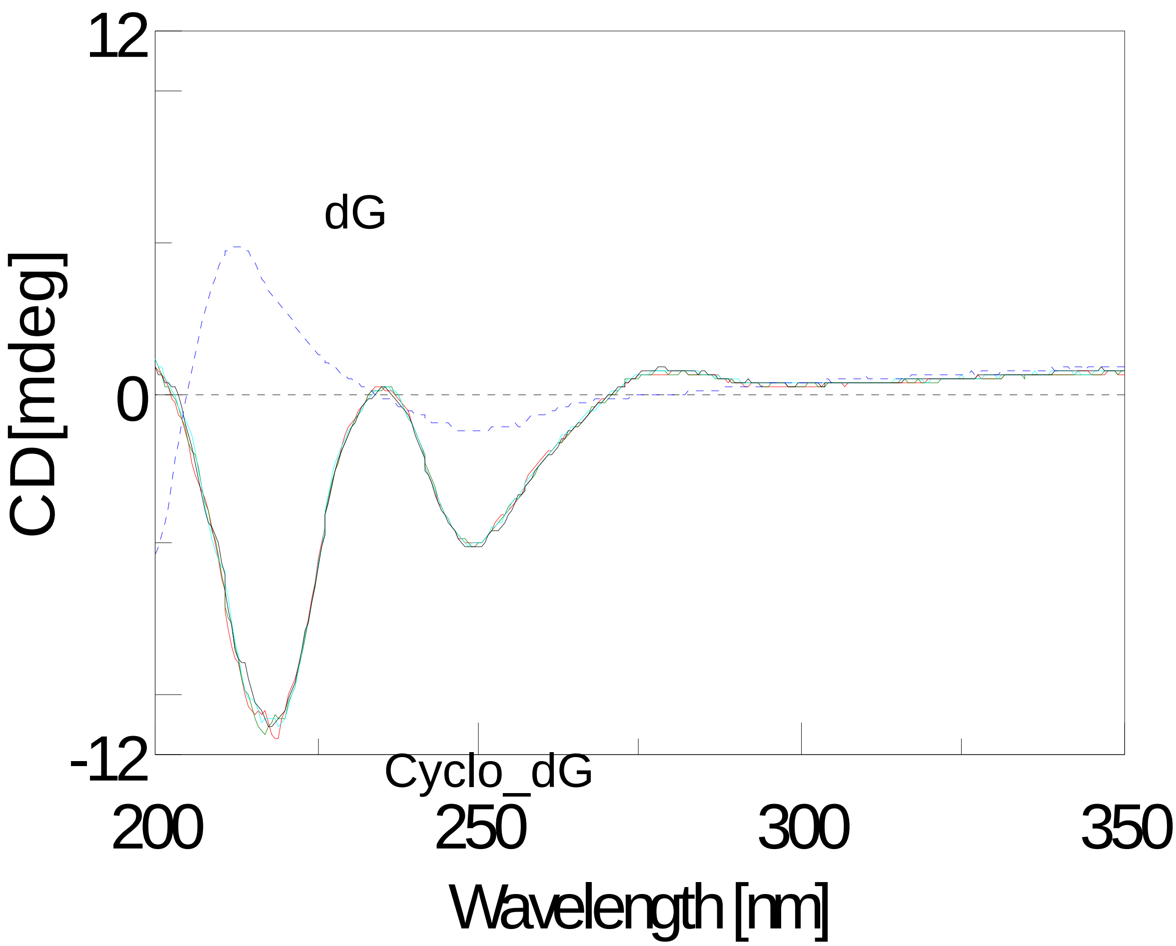


Figure S20. The CD spectra of cyclo-dG (**16**) as a function of time (0 - 6 h) every hour at 90 °C in comparison with dG (dotted blue lines)..

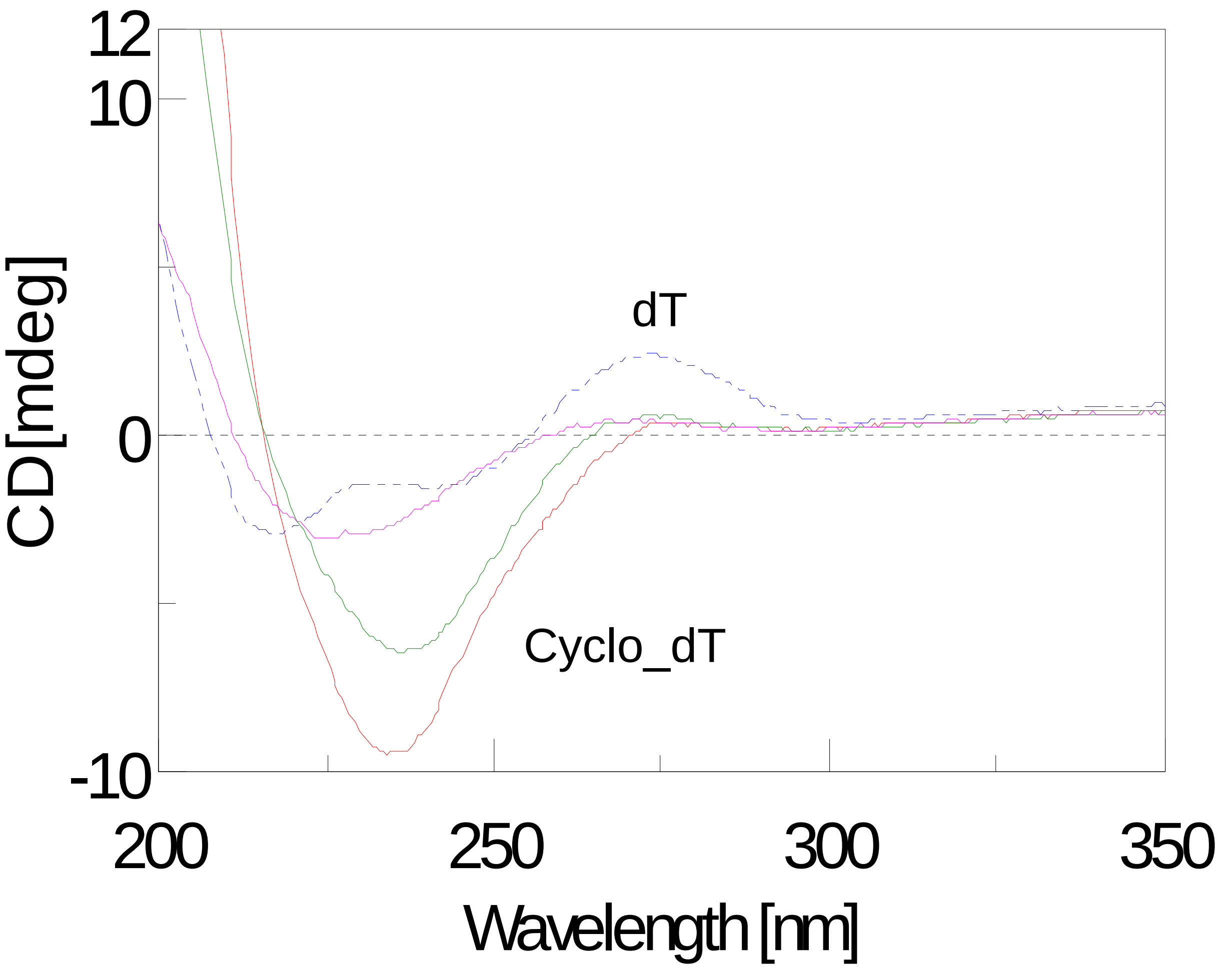


Figure S21. The CD spectra of cyclo-dT (**17**) as a function of time (0- 6 h) at 90 °C in comparison with dT (dotted blue lines).

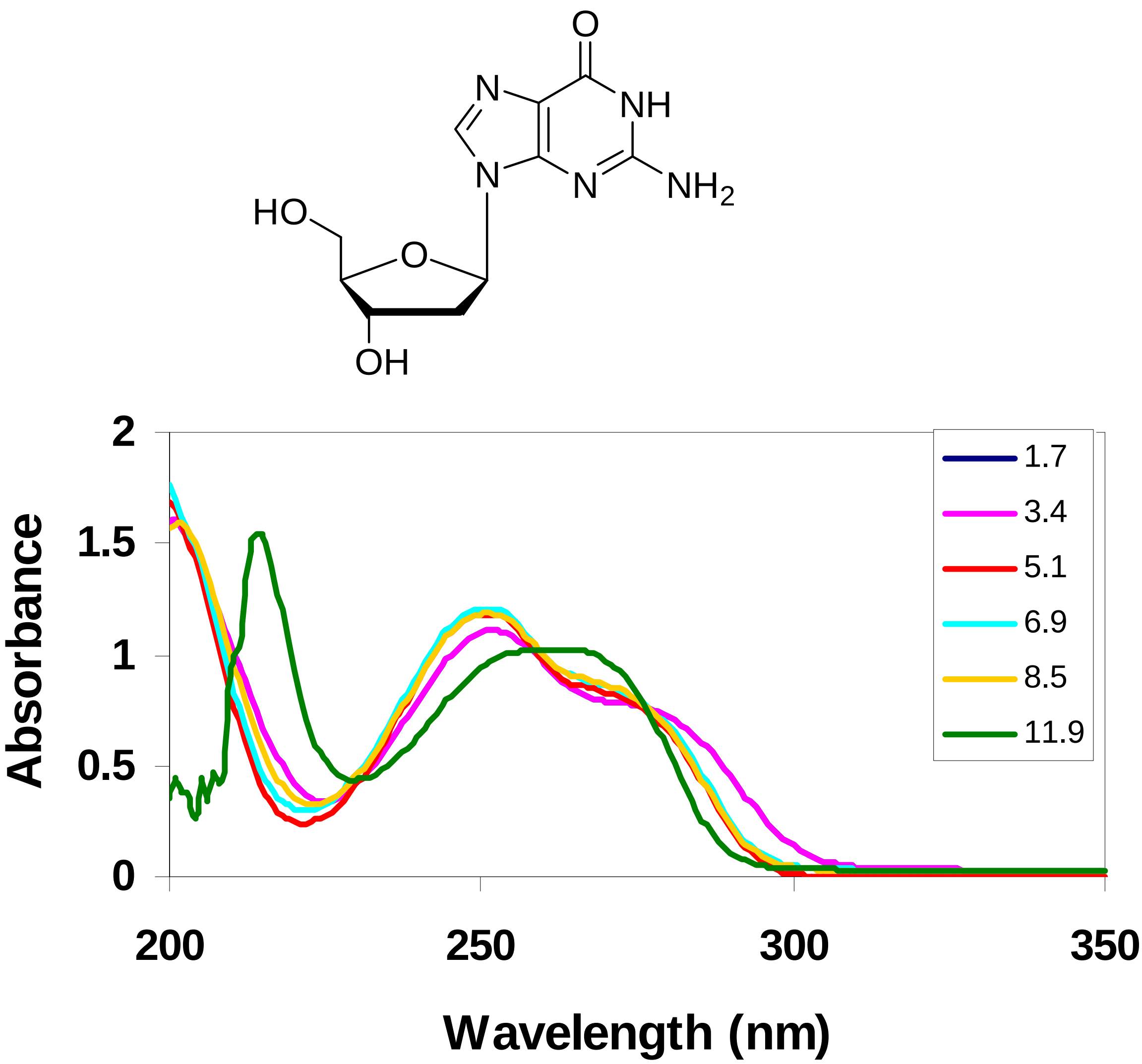
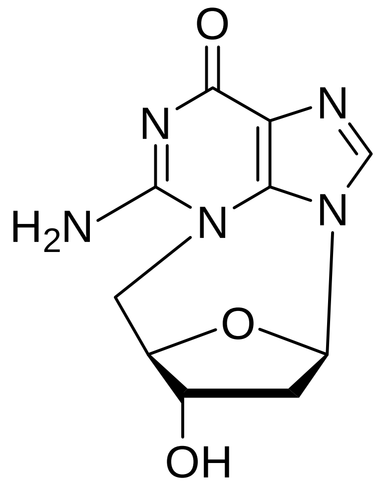


Figure S22. pH dependence UV spectra of dG.



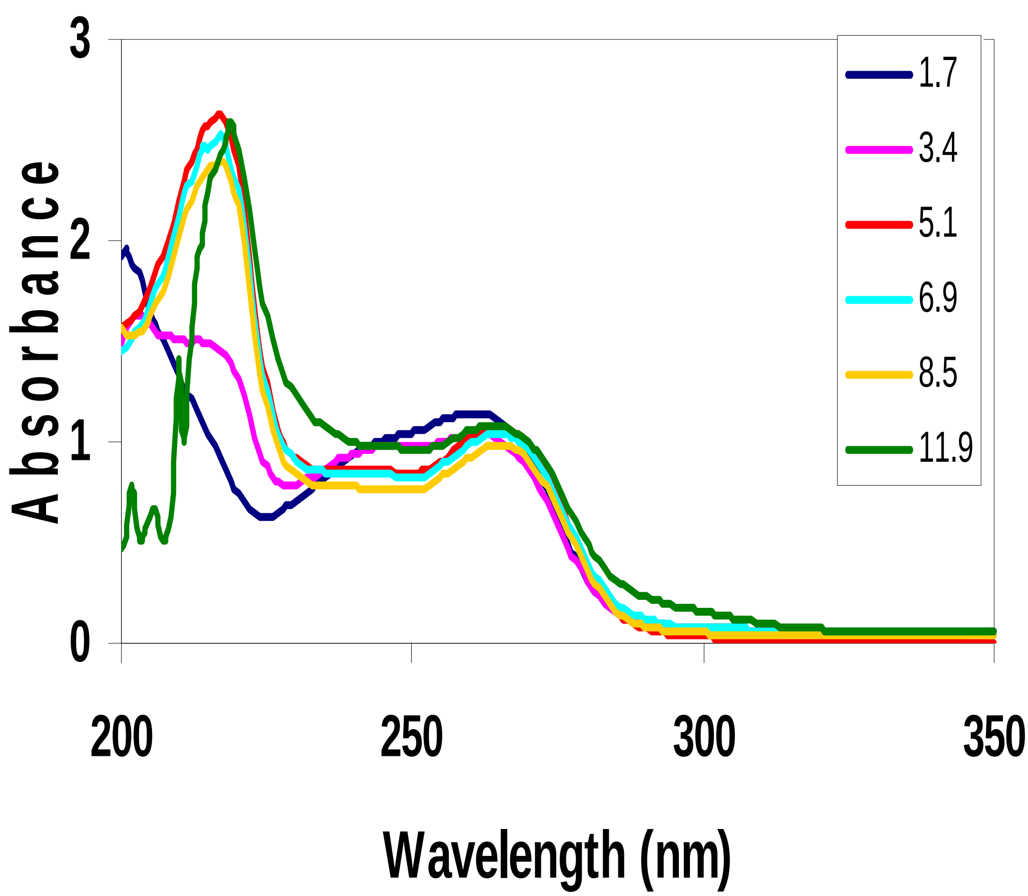
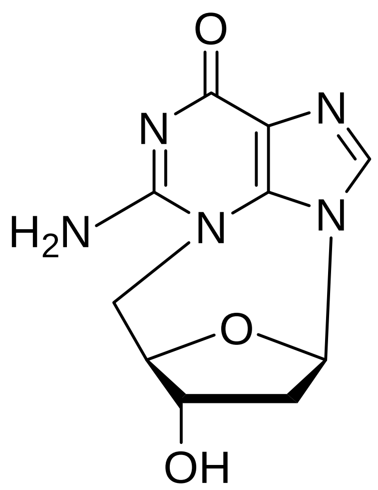


Figure S23. pH dependence UV spectra of cyclo-dG (**16**).



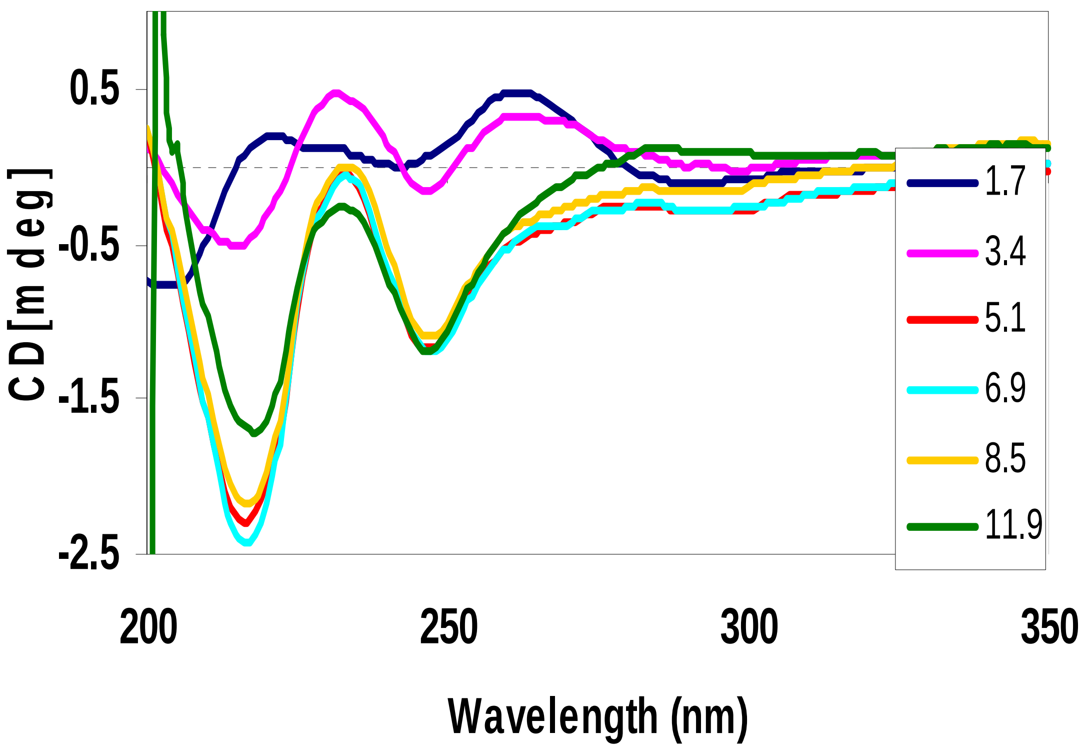


Figure S24. pH dependence CD spectra of cyclo-dG (**16**).

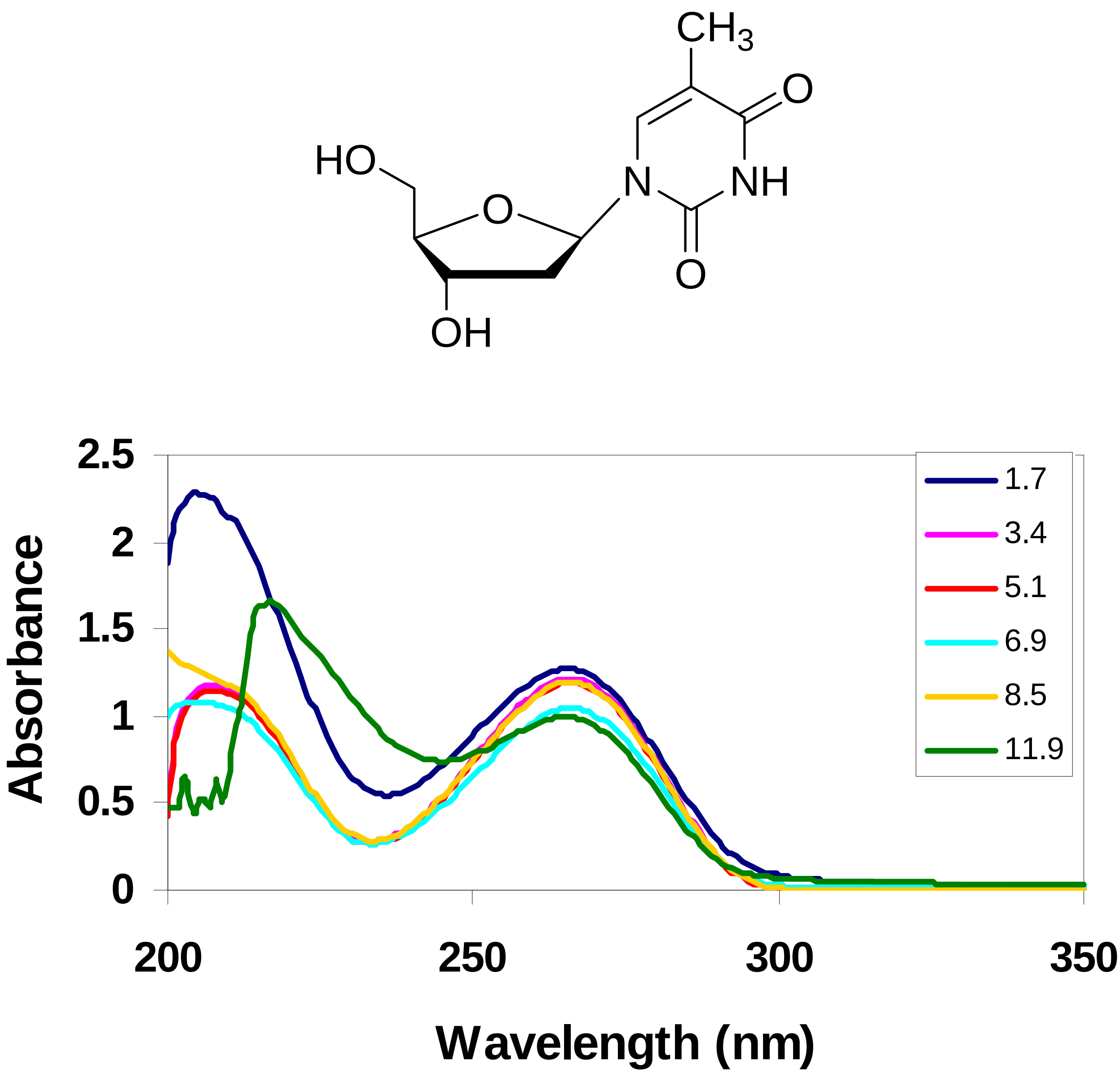


Figure S25. pH dependence UV spectra of dT.

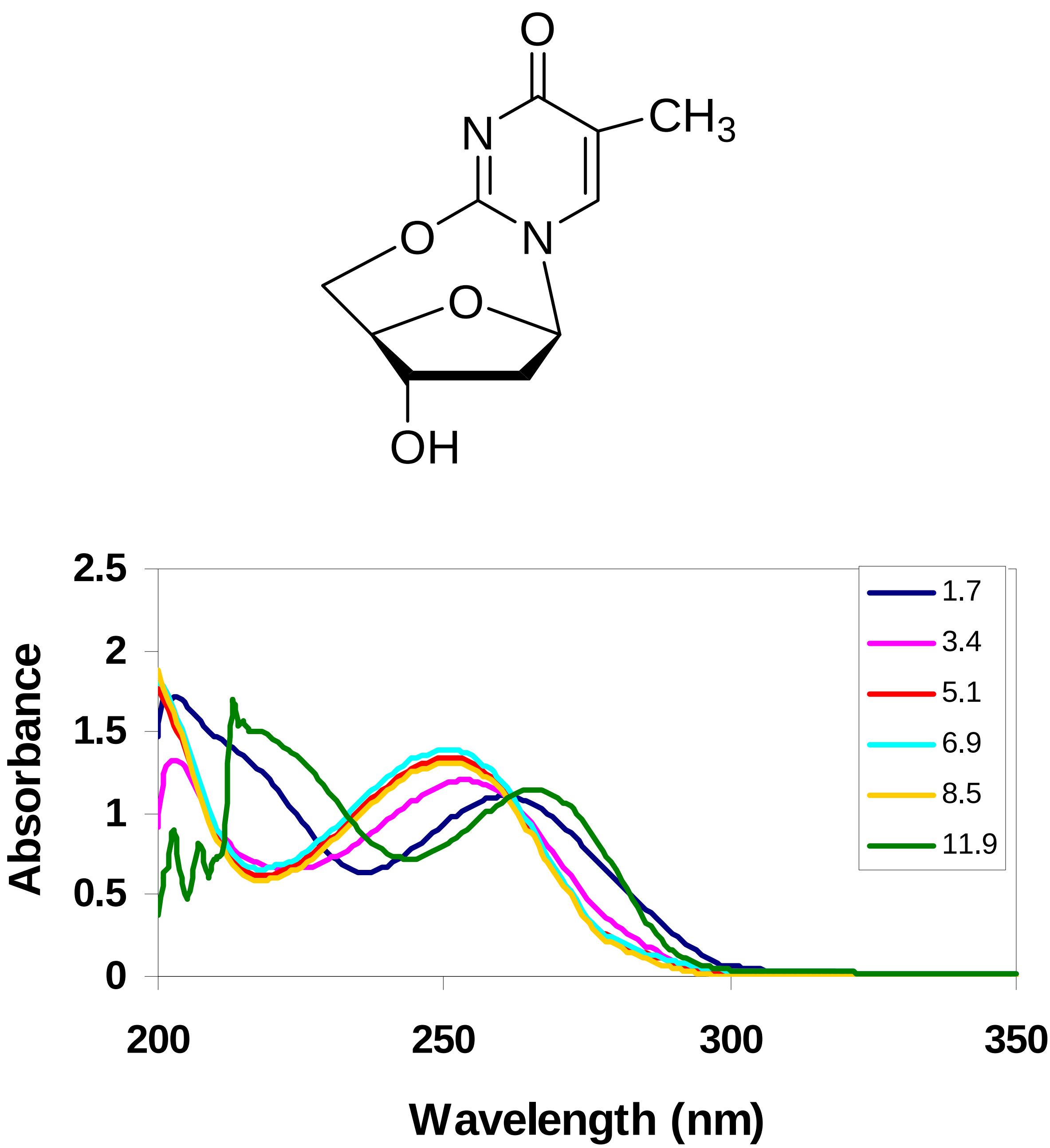
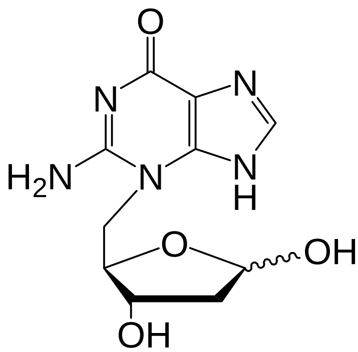


Figure S26. pH dependence UV spectra of cyclo-dT(**17**).



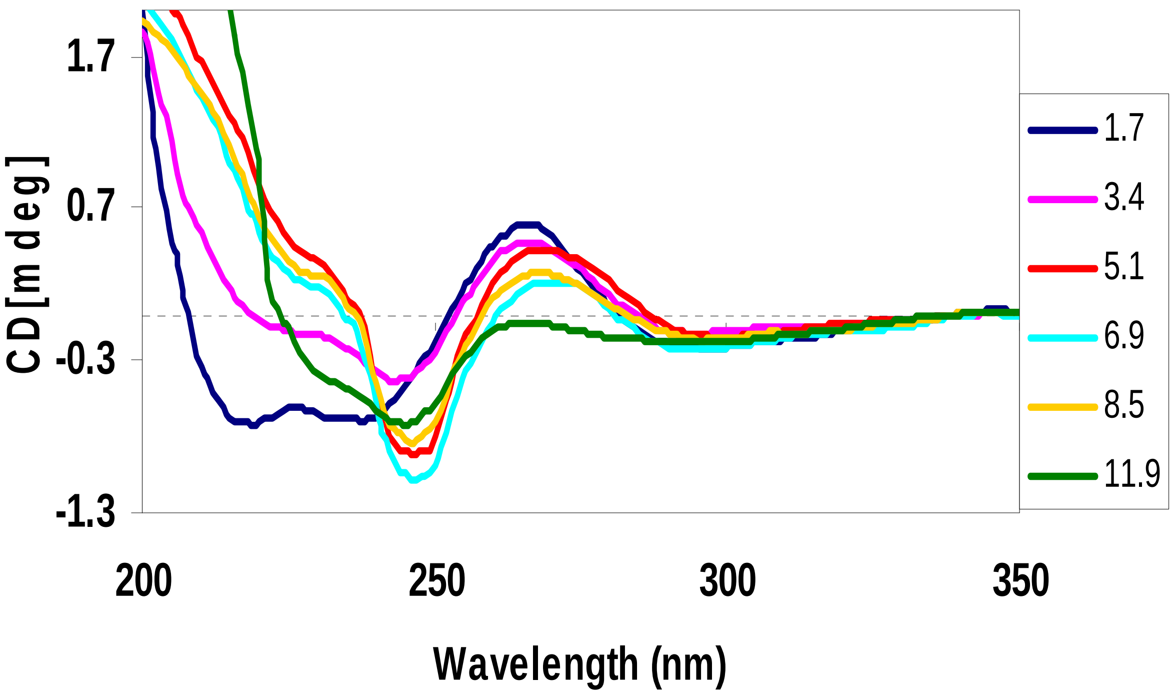


Figure S27. pH dependence CD spectra of **18**.

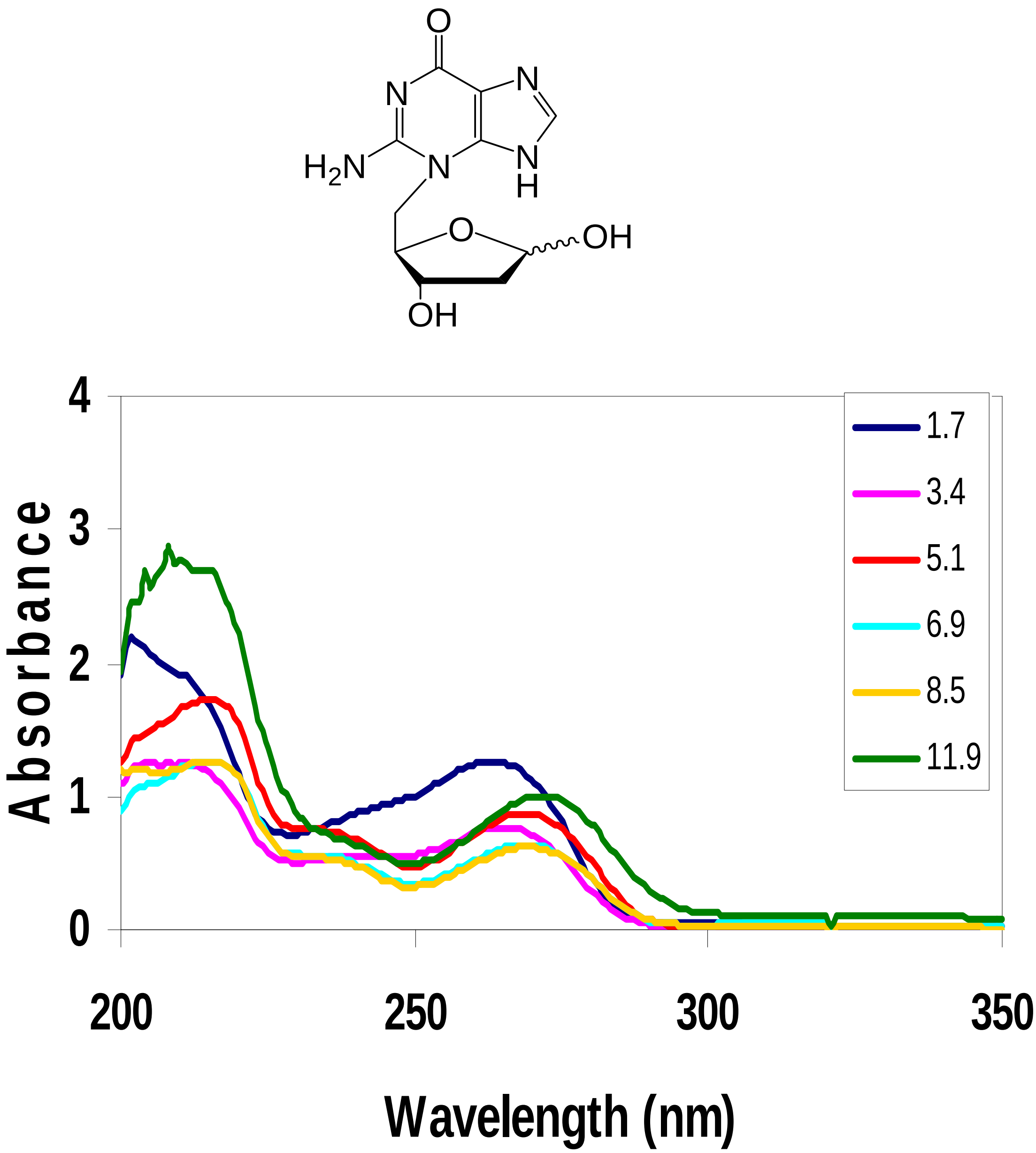


Figure S28. pH dependence UV spectra of **18**.

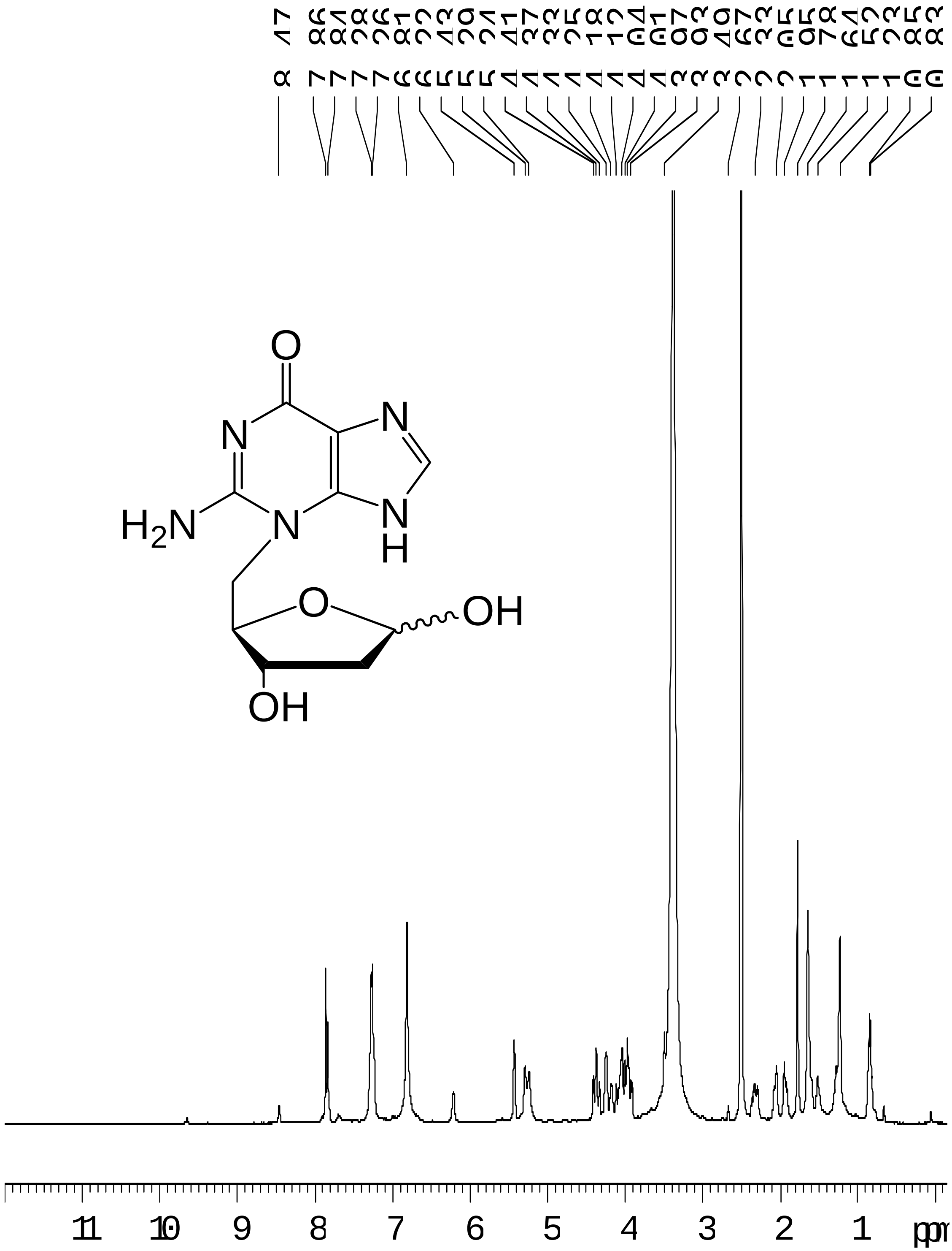


Figure S29. ^{1}H NMR spectrum of **18** in DMSO-d6.

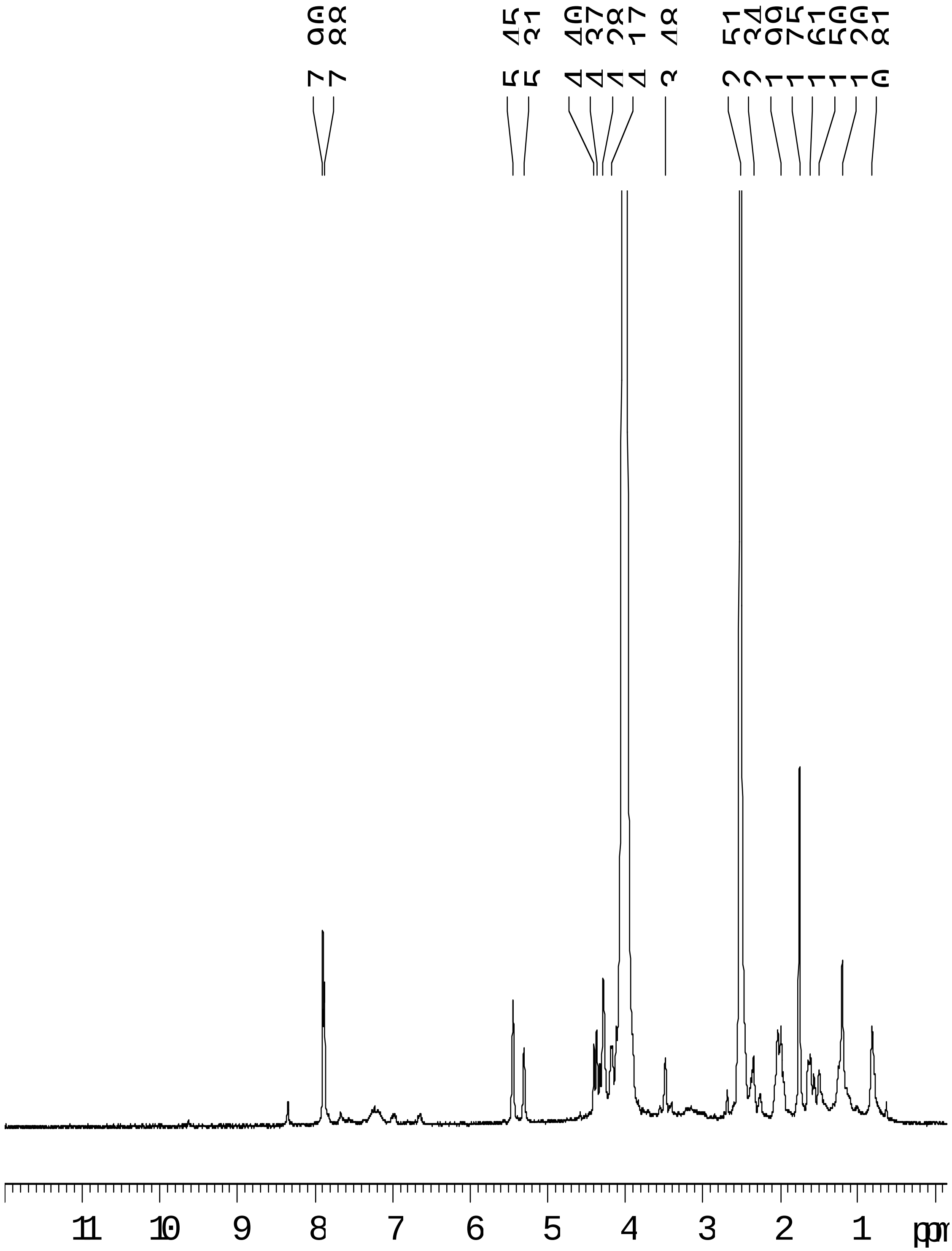


Figure S30. ^{1}H NMR spectrum of **18** in DMSO-d6 + D_2O.

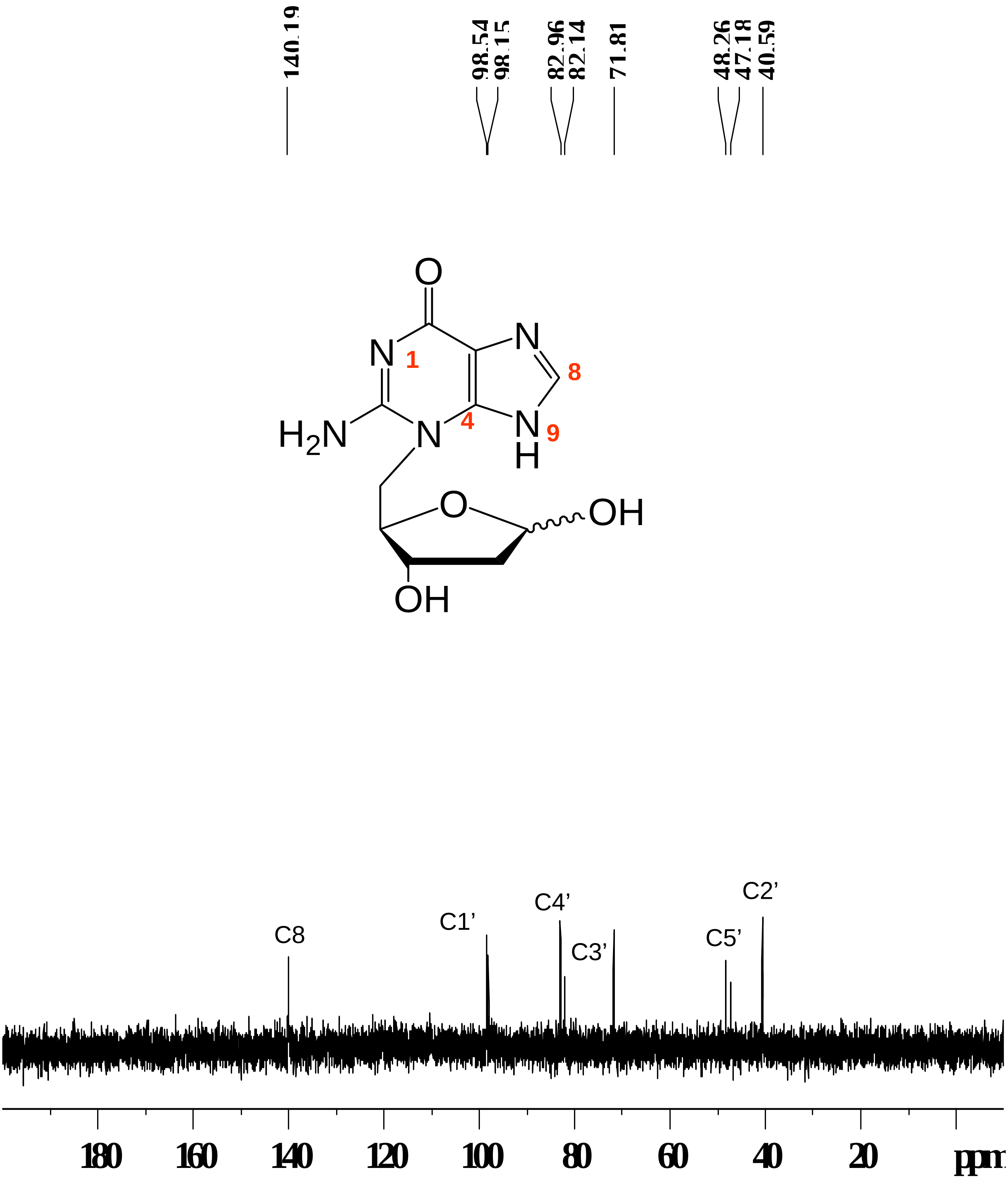
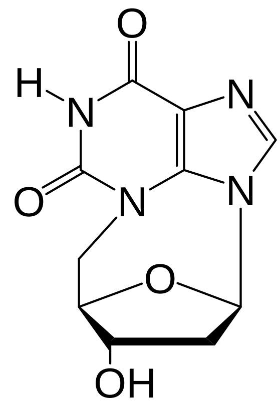


Figure S31. ^{13}C NMR spectrum of **18** in D_2O.



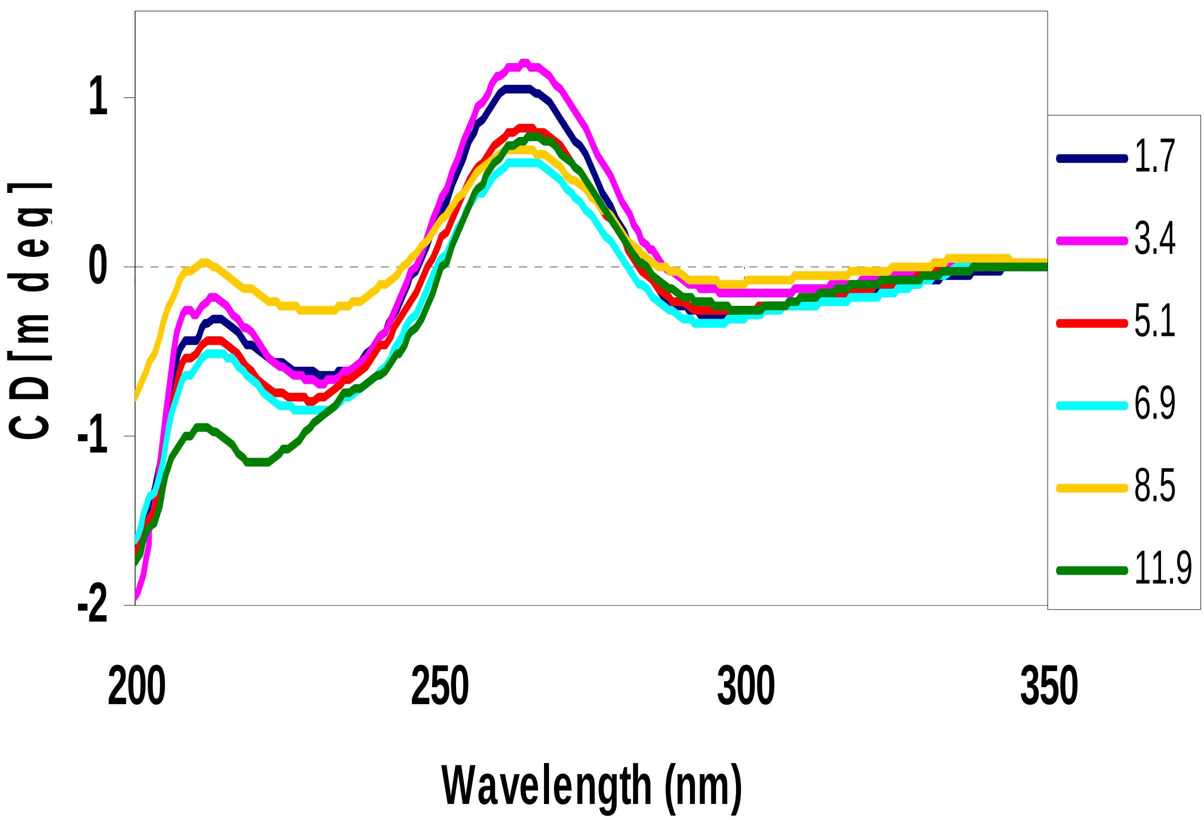
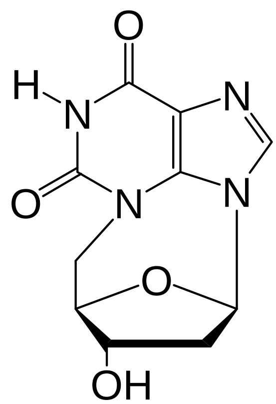


Figure S32. pH dependence CD spectra of cyclo-dX (**19**).



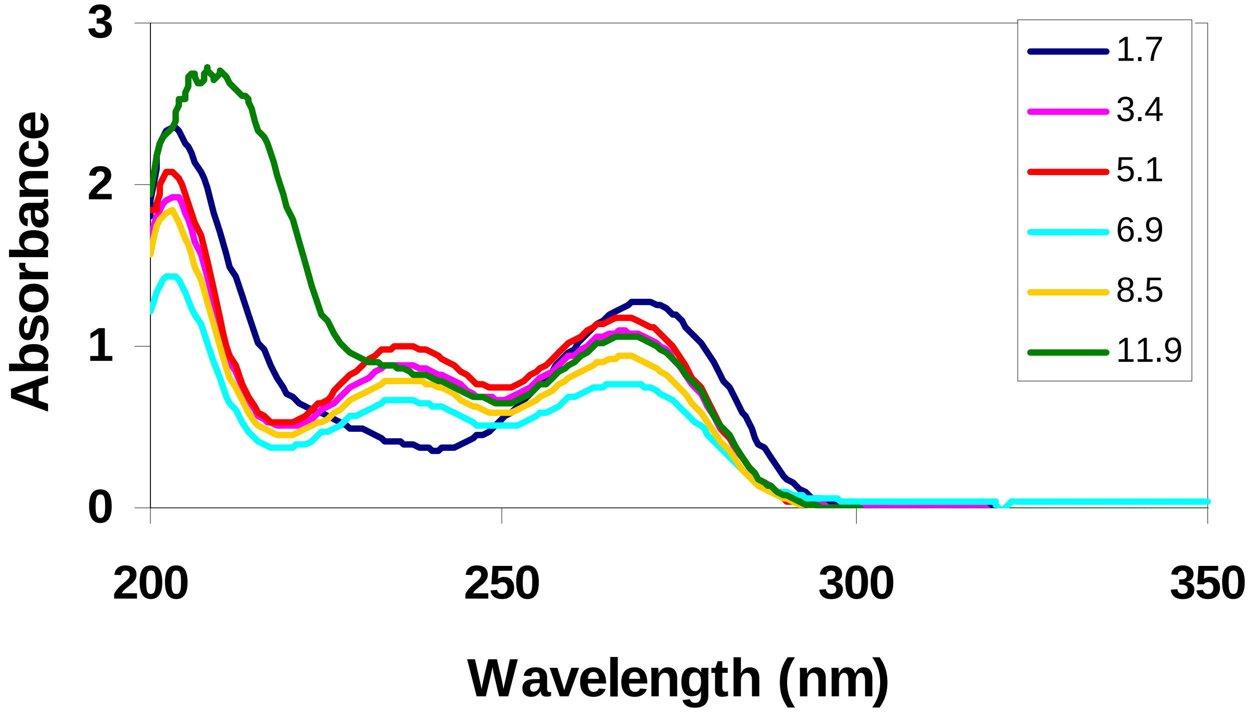


Figure S33. pH dependence UV spectra of cyclo-dX (**19**).

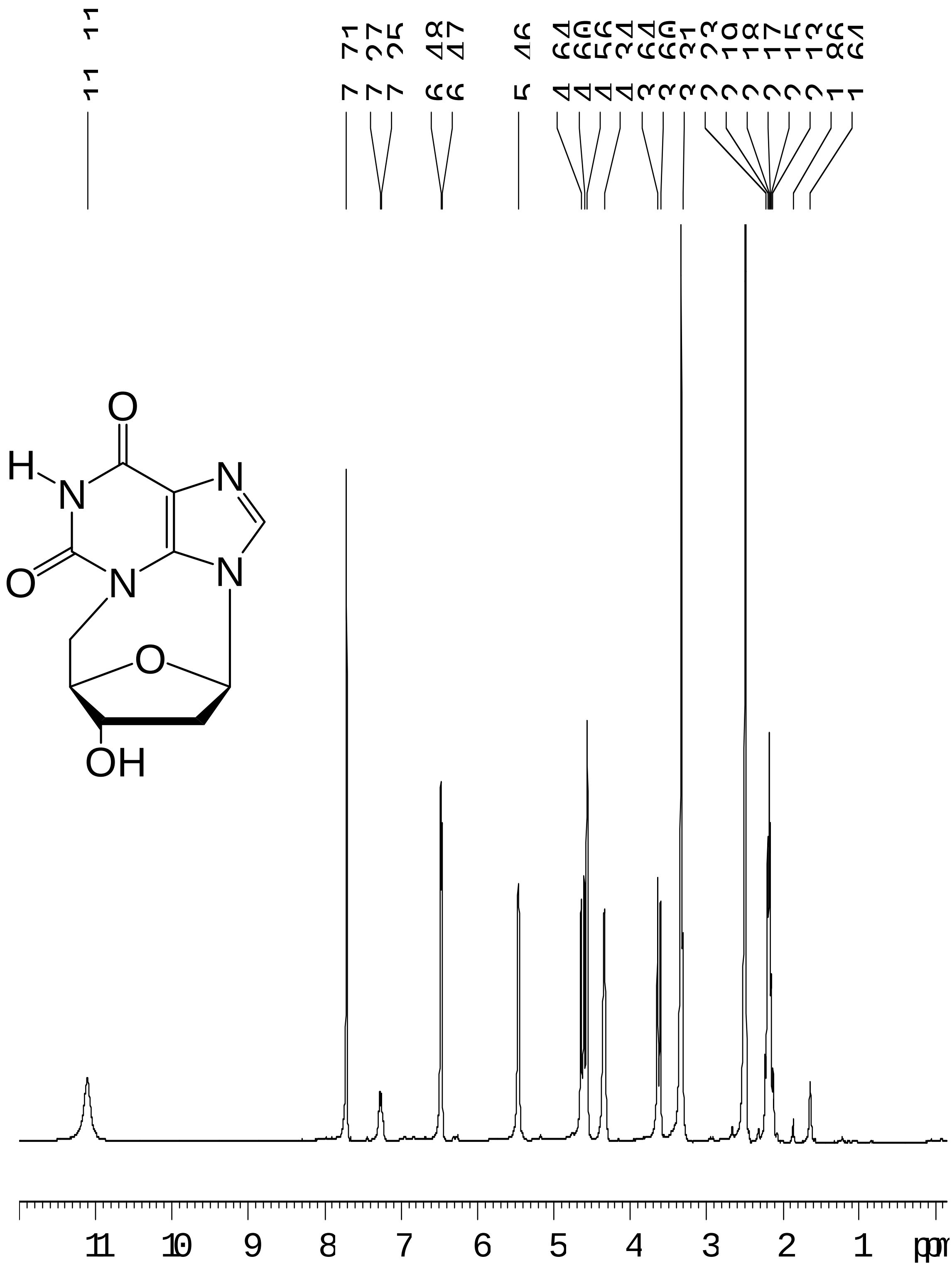


Figure S34. [1]H NMR spectrum of cyclo-dX (**19**) in DMSO-d_6.

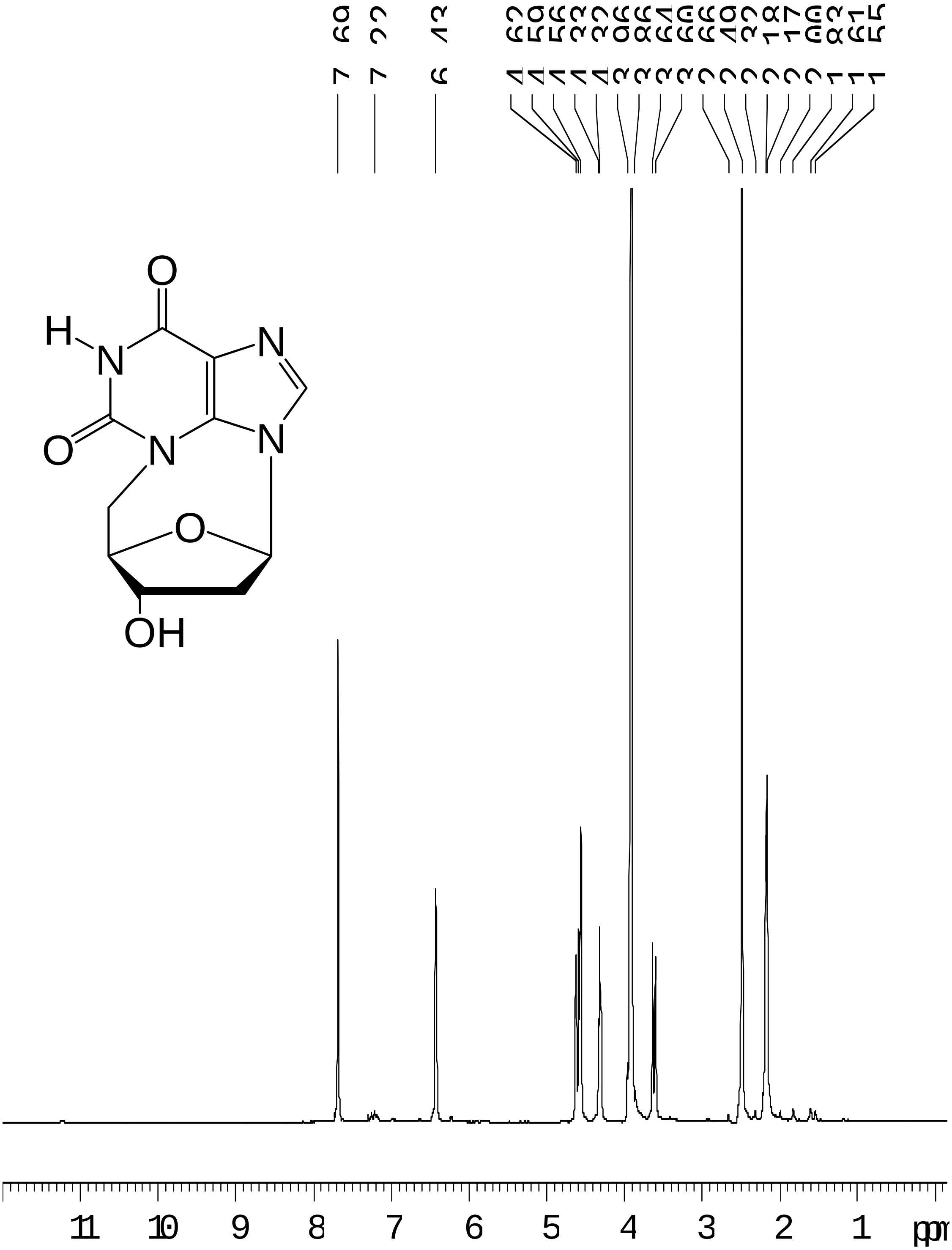


Figure S35. [1]H NMR spectrum of cyclo-dX (**19**) in DMSO-d6 + D_2O

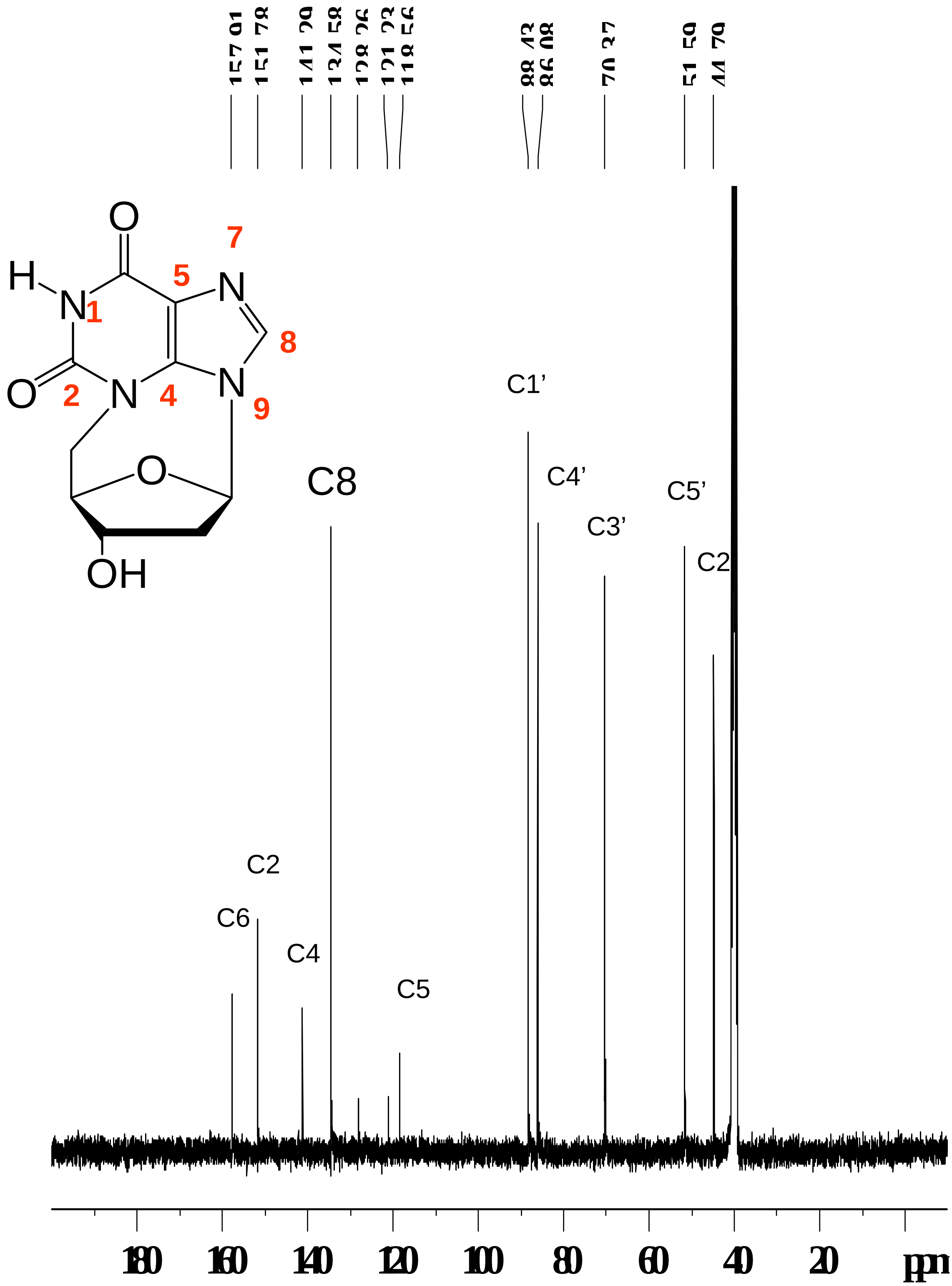


Figure S36. ^{13}C NMR spectrum of cyclo-dX (**19**) in DMSO-d6**.**

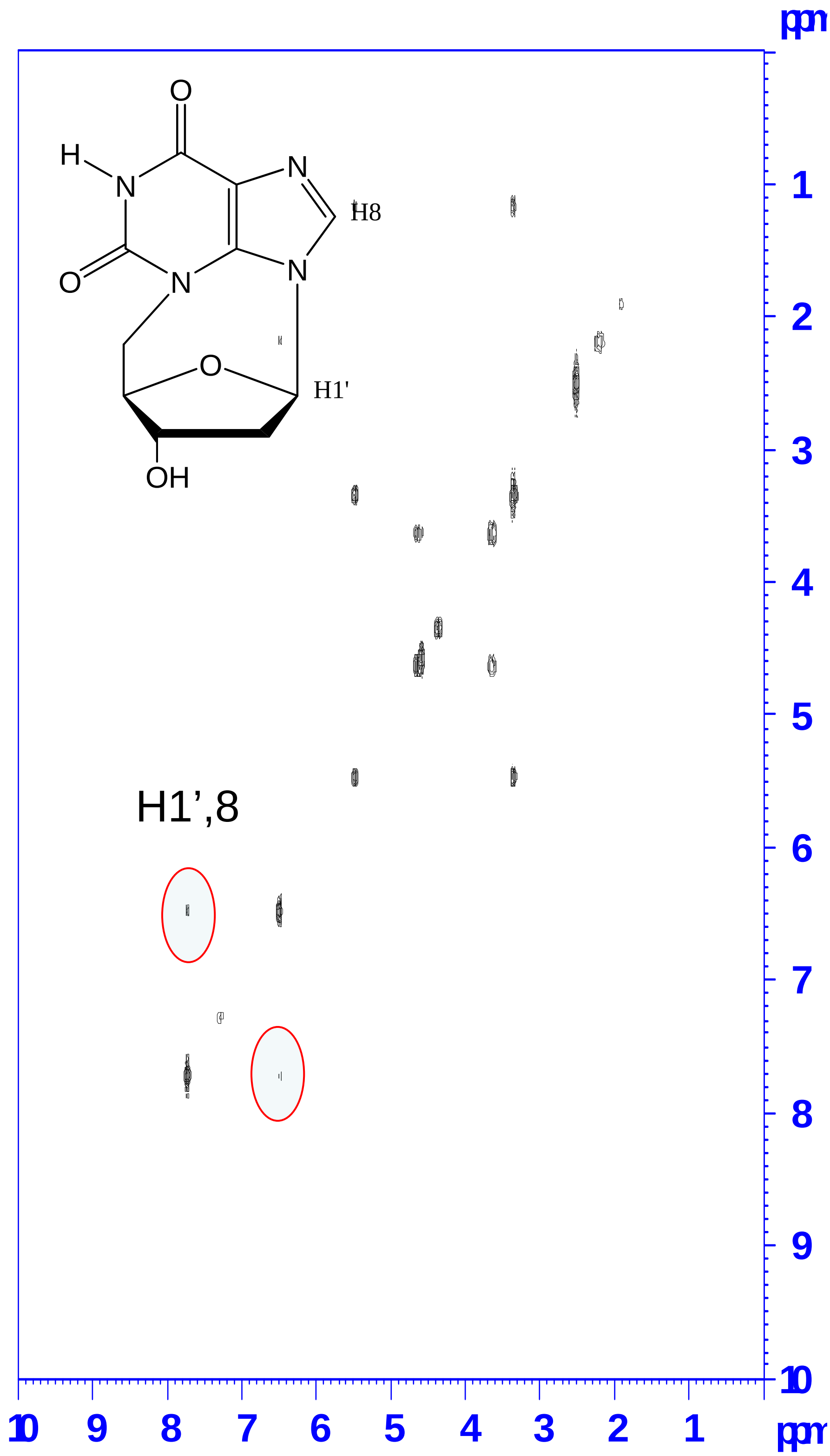


Figure S37. NOESY NMR spectrum of cyclo-dX (**19**) in DMSO-d6**.**

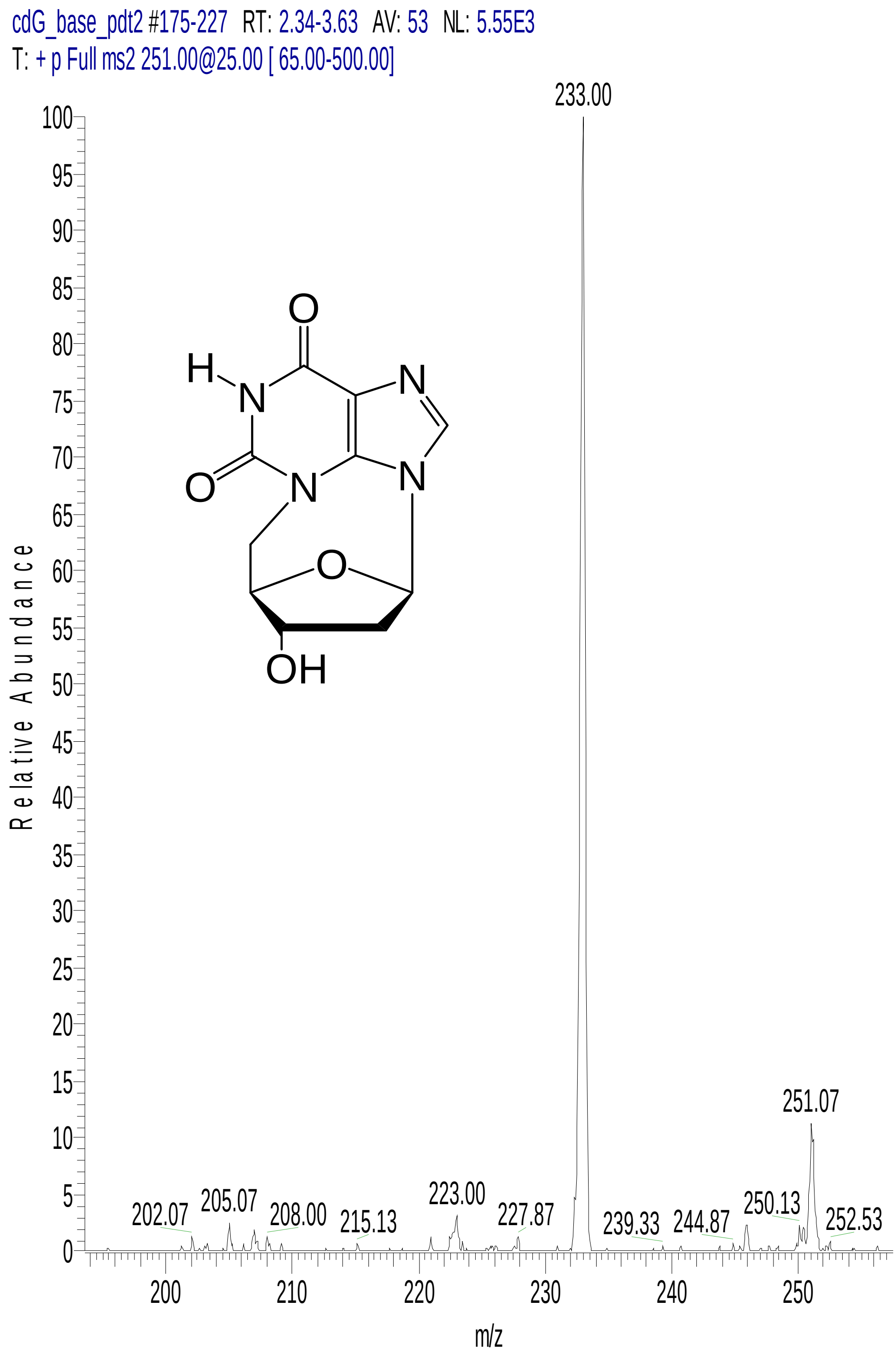


Figure S38. Cyclo-dX (**19**). The product ion spectrum of the (M+H)$^+$ ion acquired with LTQ-FTMS.

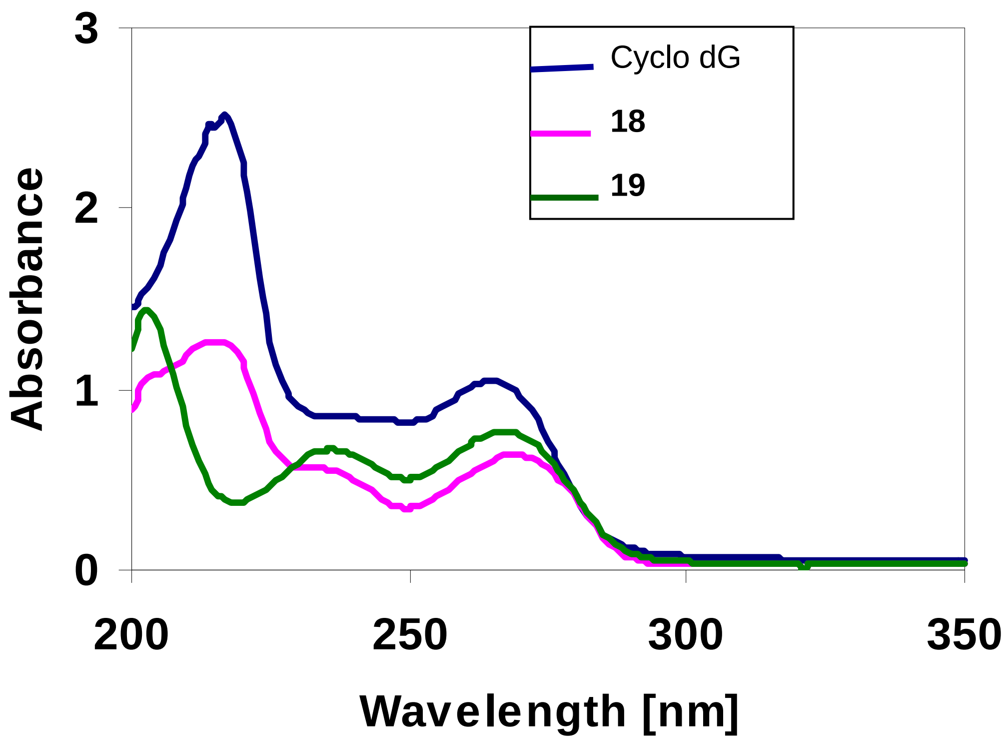


Figure S39. Comparison of UV of cyclo-dG (blue), acid intermediate **18** (pink), and cyclo-dX (**19**)(green).