

Supporting information for

Probing the binding requirements of modified nucleosides with the DNA nuclease SNM1A

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1. Additional concentration-dependence studies

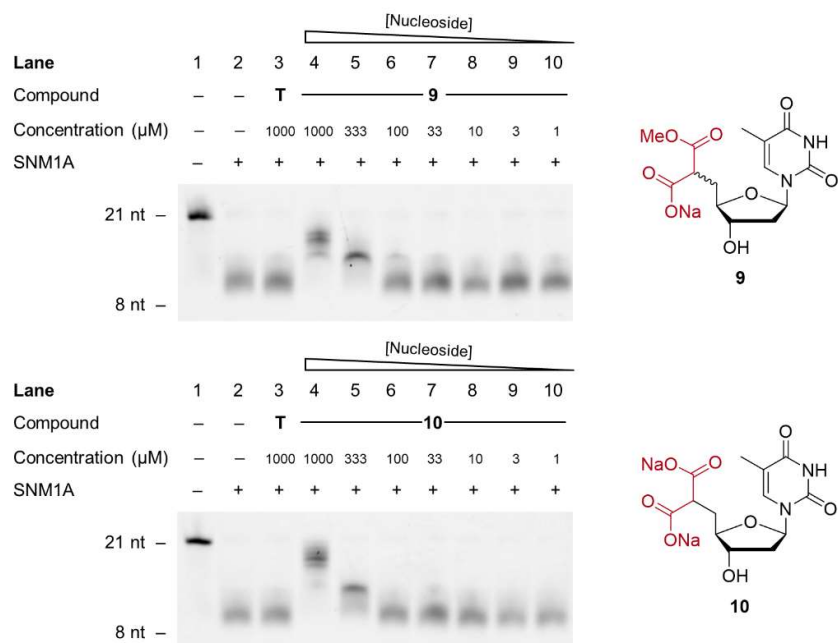


Figure S1: Concentration-dependence studies of 5'-C-linked malonates **9** and **10**. Digestion of the phosphorylated oligonucleotide (0.8 pmol, 80 nM) after incubation with SNM1A (25 fmol, 2.5 nM) and nucleosides **9** or **10** (1000-1 μM) or thymidine (T) (1 mM) for 60 min at 37 °C, analysed by denaturing PAGE. SNM1A was preincubated with the modified nucleoside for 5 min at 37 °C prior to the addition of the substrate. nt = nucleotides.

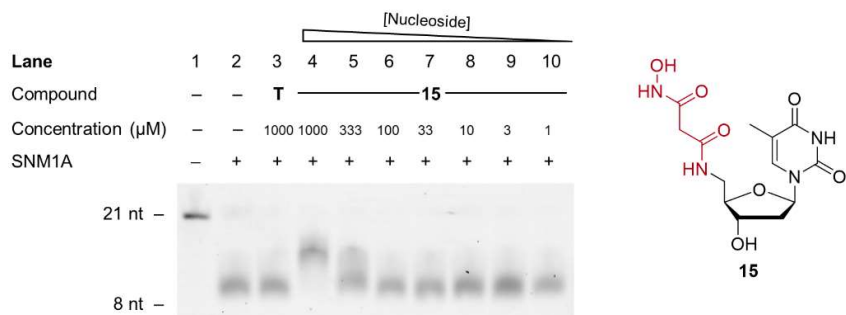


Figure S2: Concentration-dependence studies of 5'-N-linked malonate **15**. Digestion of the phosphorylated oligonucleotide (0.8 pmol, 80 nM) after incubation with SNM1A (25 fmol, 2.5 nM) and nucleosides **15** (1000-1 μM) or thymidine (T) (1 mM) for 60 min at 37 °C, analysed by denaturing PAGE. SNM1A was preincubated with the modified nucleoside for 5 min at 37 °C prior to the addition of the substrate. nt = nucleotides.

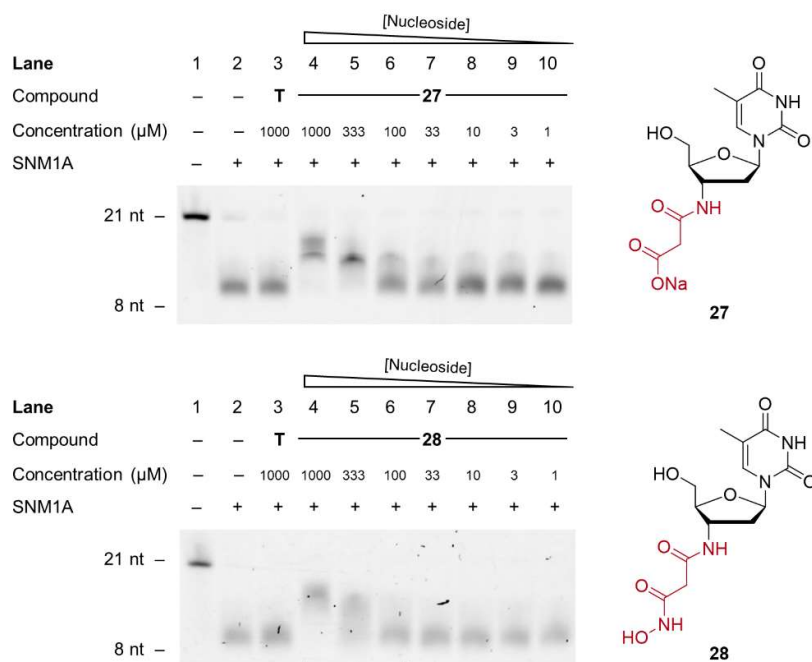


Figure S3: Concentration-dependence studies of 3'-N-linked malonates **27** and **28**. Digestion of the phosphorylated oligonucleotide (0.8 pmol, 80 nM) after incubation with SNM1A (25 fmol, 2.5 nM) and nucleosides **27** or **28** (1000-1 μM) or thymidine (**T**) (1 mM) for 60 min at 37 °C, analysed by denaturing PAGE. SNM1A was preincubated with the modified nucleoside for 5 min at 37 °C prior to the addition of the substrate. nt = nucleotides.

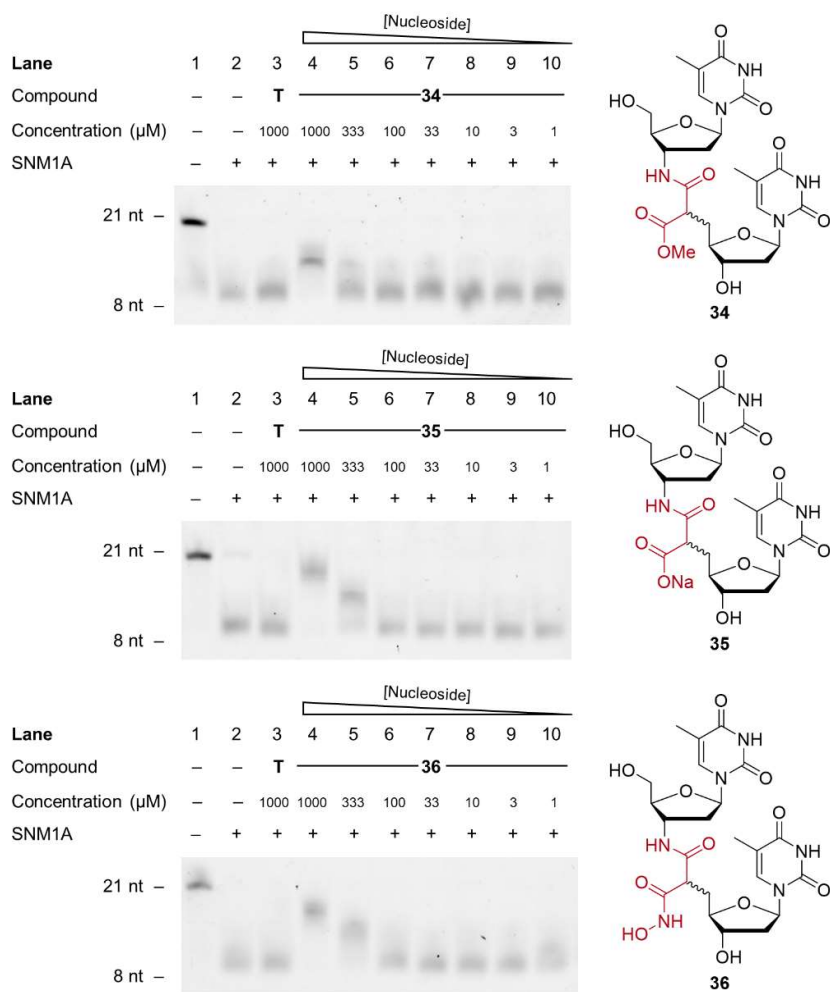
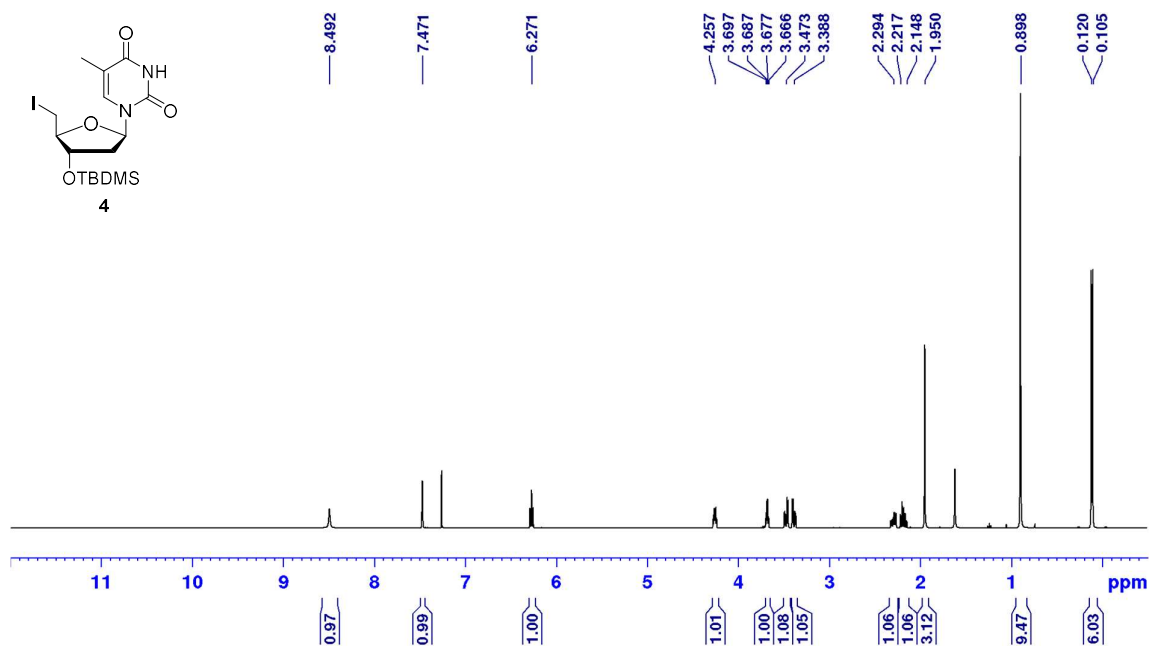


Figure S4: Concentration-dependence of dinucleosides **34-36**. Digestion of the phosphorylated oligonucleotide (0.8 pmol, 80 nM) after incubation with SNM1A (25 fmol, 2.5 nM) and nucleosides **34-36** (1000-1 μM) or thymidine (**T**) (1 mM) for 60 min at 37 °C, analysed by denaturing PAGE. SNM1A was preincubated with the modified nucleoside for 5 min at 37 °C prior to the addition of the substrate. nt = nucleotides.

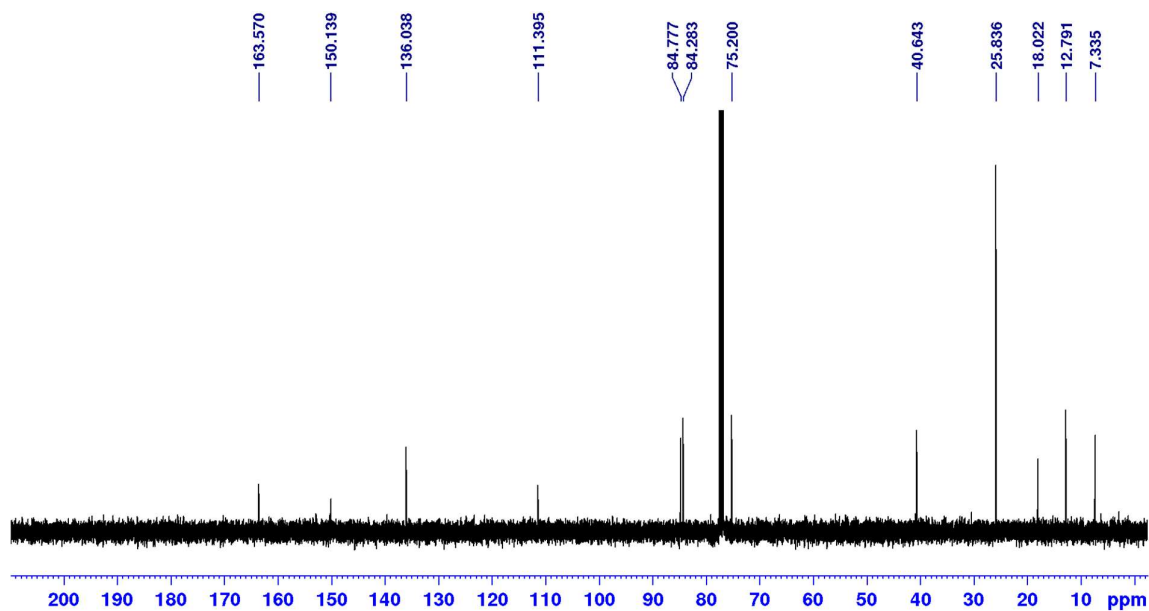
2. ^1H and ^{13}C NMR spectra

3'-O-(*tert*-Butyldimethylsilyl)-5'-deoxy-5'-iodothymidine (4)

^1H NMR (400 MHz, CDCl_3)

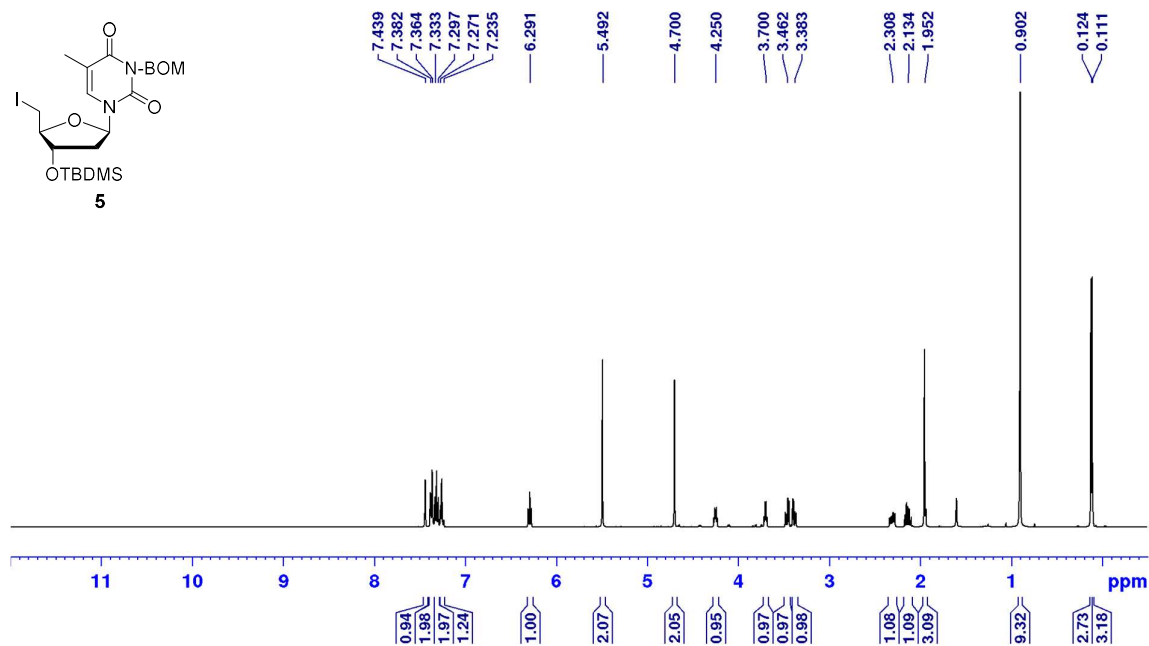


^{13}C NMR (100 MHz, CDCl_3)

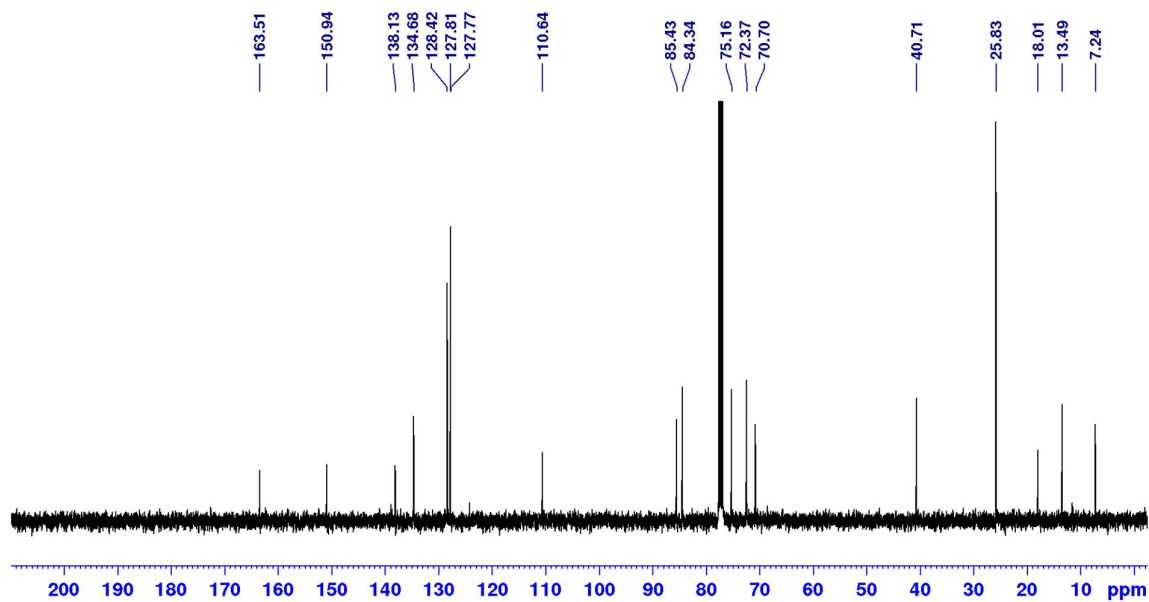


***N*-Benzyloxymethyl-3'-*O*-(*tert*-butyldimethylsilyl)-5'-deoxy-5'-iodothymidine (5)**

¹H NMR (400 MHz, CDCl₃)

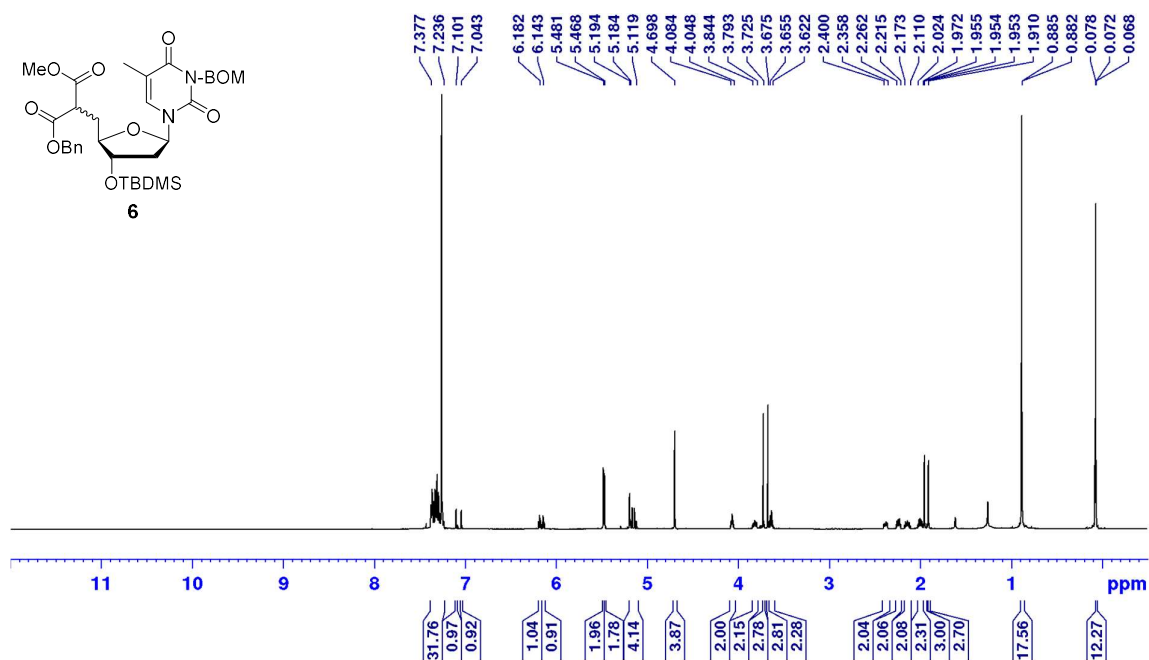


¹³C NMR (100 MHz, CDCl₃)

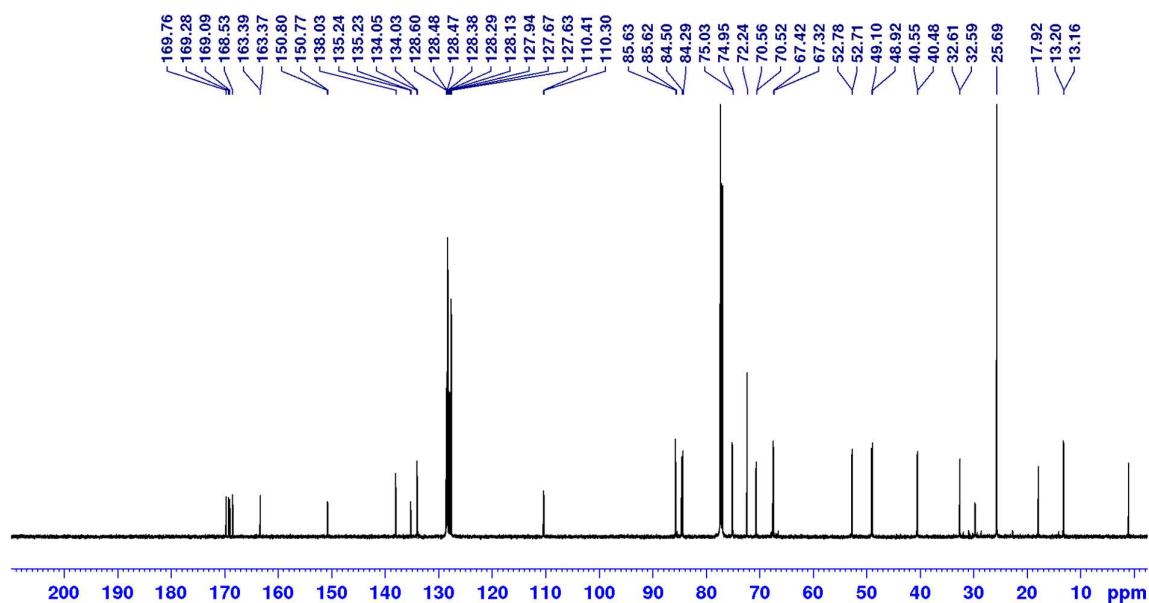


C-(N³-Benzyloxymethyl-3'-O-(*tert*-butyldimethylsilyl)-5'-deoxythymidin-5'-yl) benzyl methyl malonate (6):

¹H NMR (600 MHz, CDCl₃)

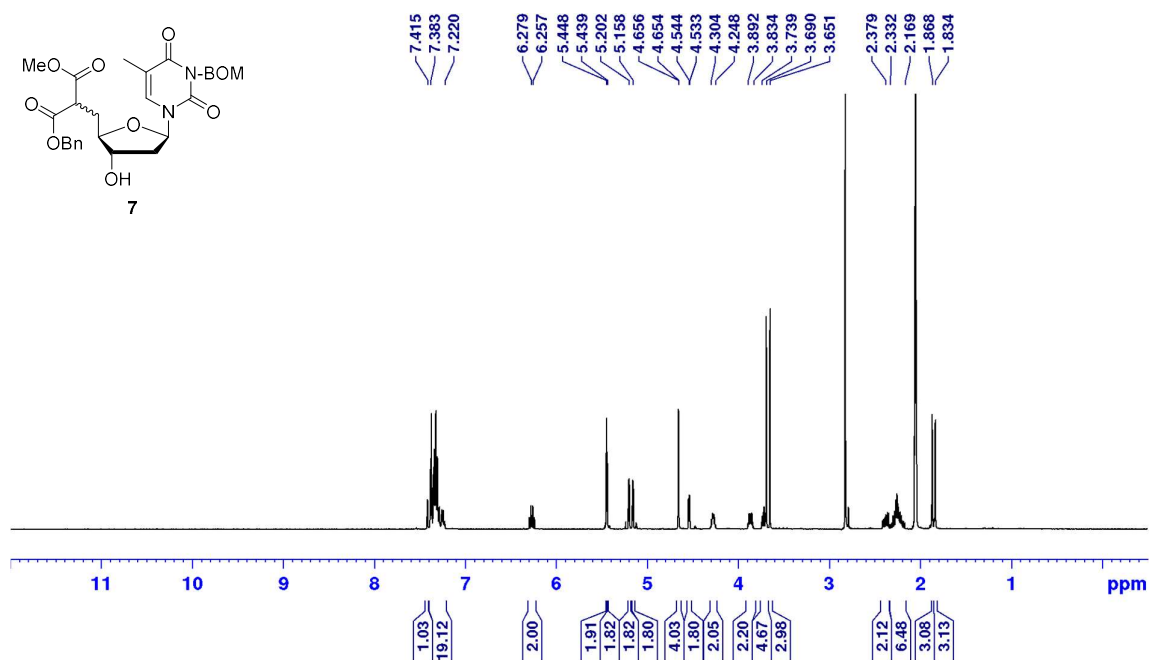


¹³C NMR (151 MHz, CDCl₃)

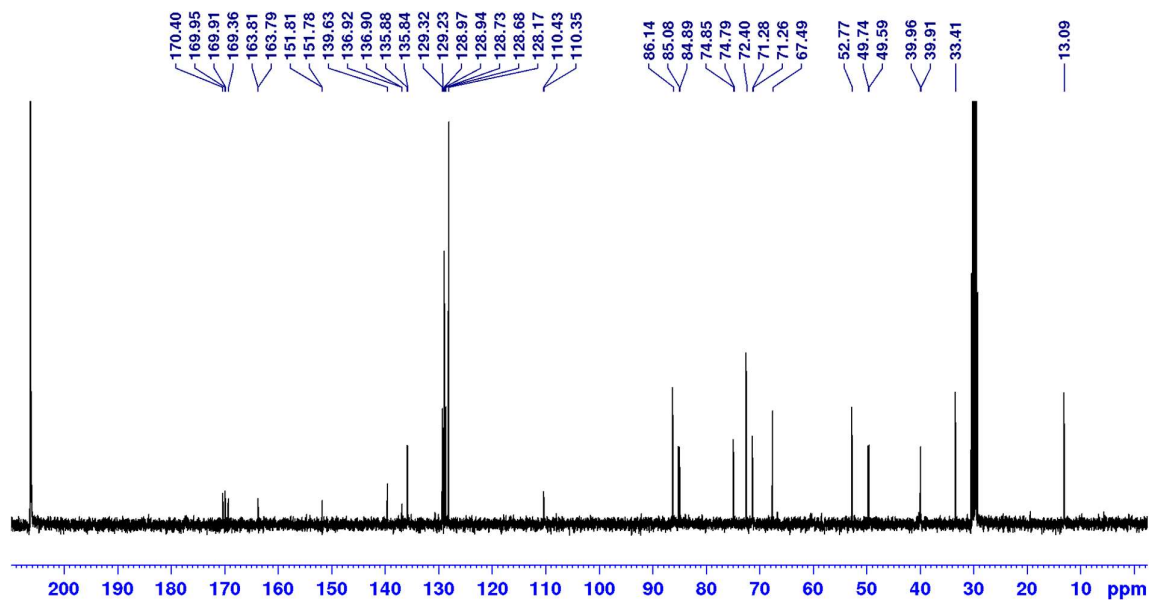


C-(N³-Benzyloxymethyl-5'-deoxythymidin-5'-yl) benzyl methyl malonate (7):

¹H NMR (400 MHz, acetone-d₆)

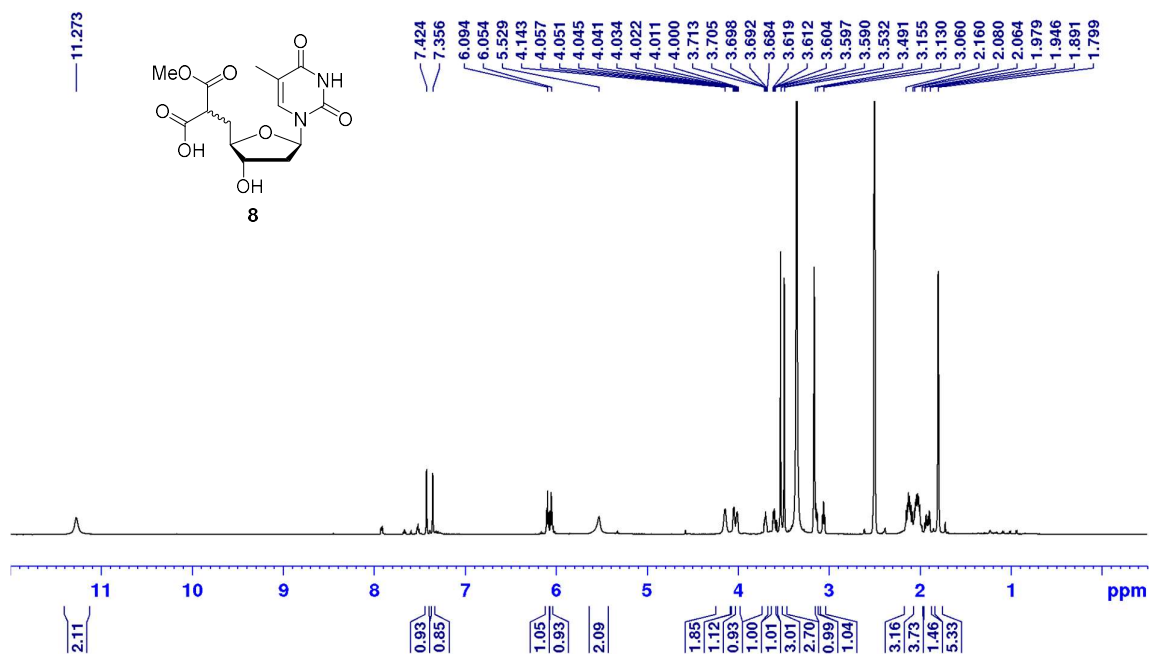


¹³C NMR (100 MHz, acetone-d₆)

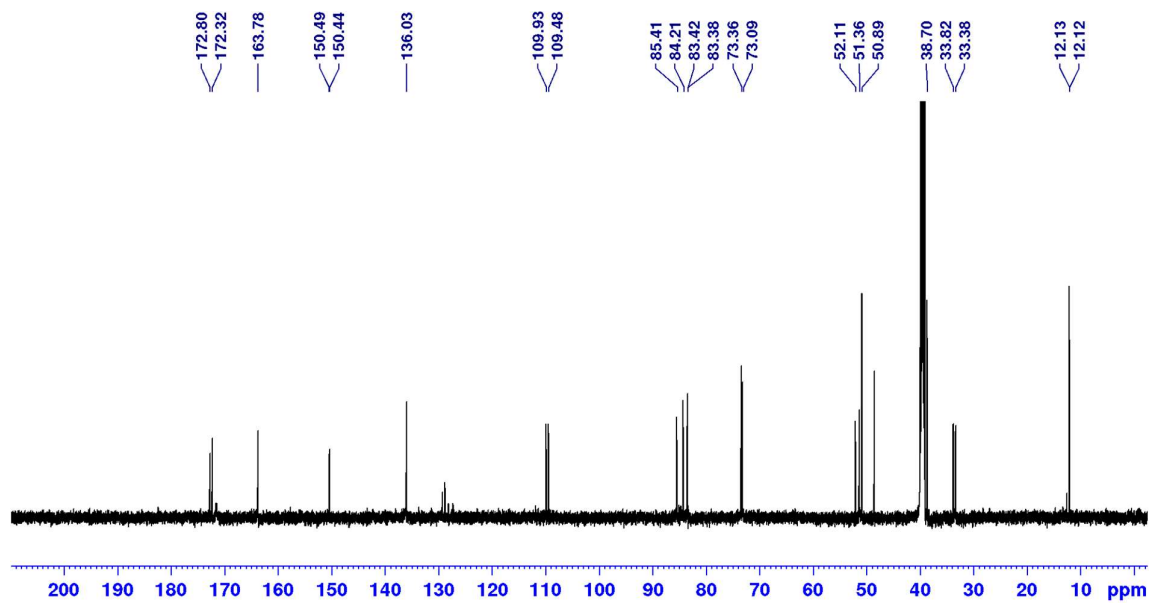


C-(5'-Deoxythymidin-5'-yl) malonic acid monomethyl ester (8):

^1H NMR (600 MHz, DMSO- d_6)

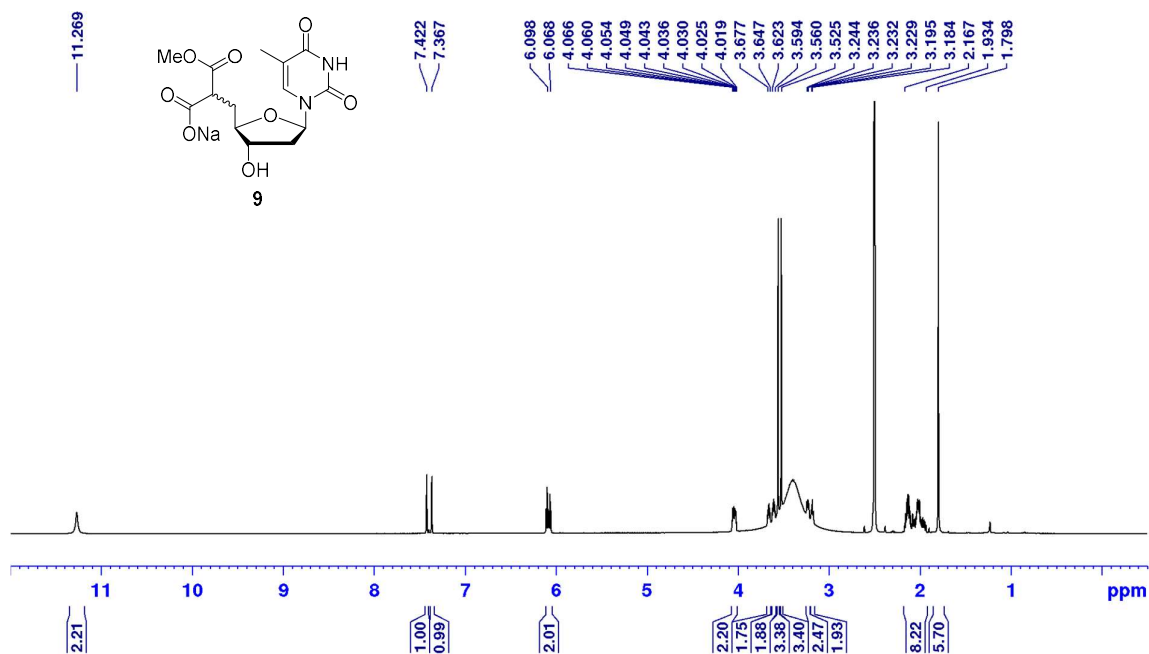


^{13}C NMR (150 MHz, DMSO- d_6)

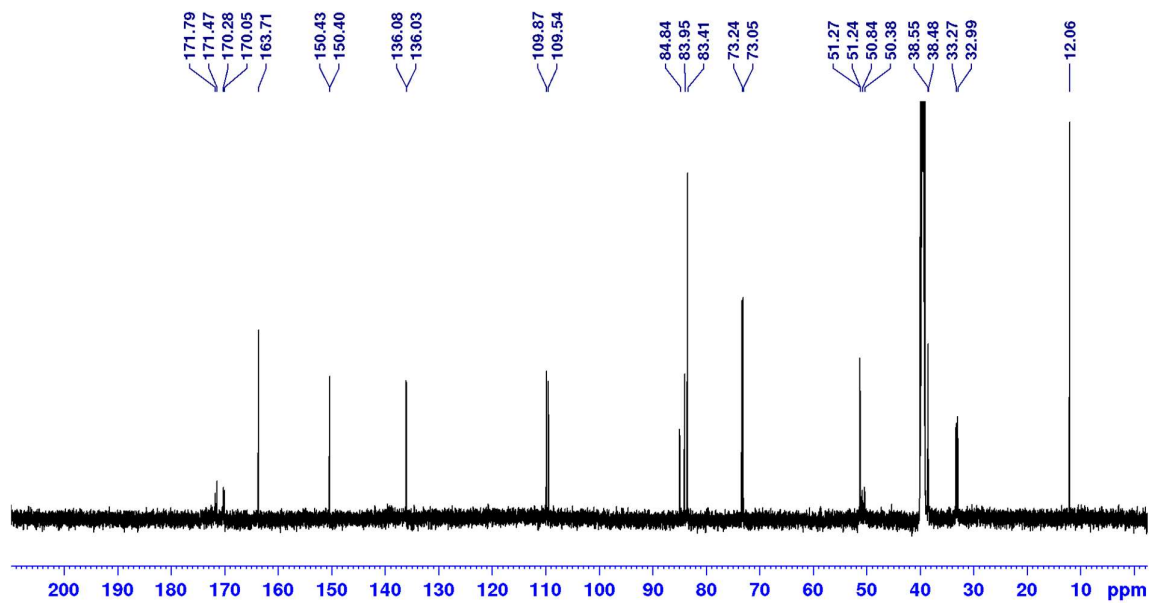


C-(5'-Deoxythymidin-5'-yl) monomethyl sodium malonate (9):

^1H NMR (600 MHz, DMSO- d_6)

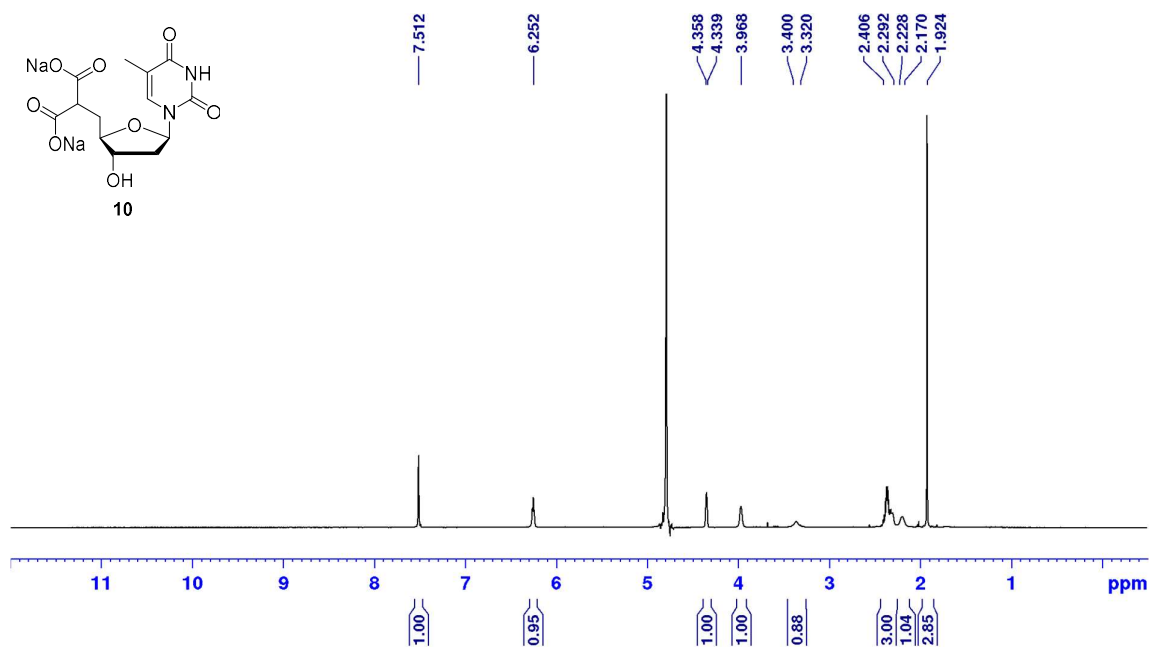


^{13}C NMR (150 MHz, DMSO- d_6)

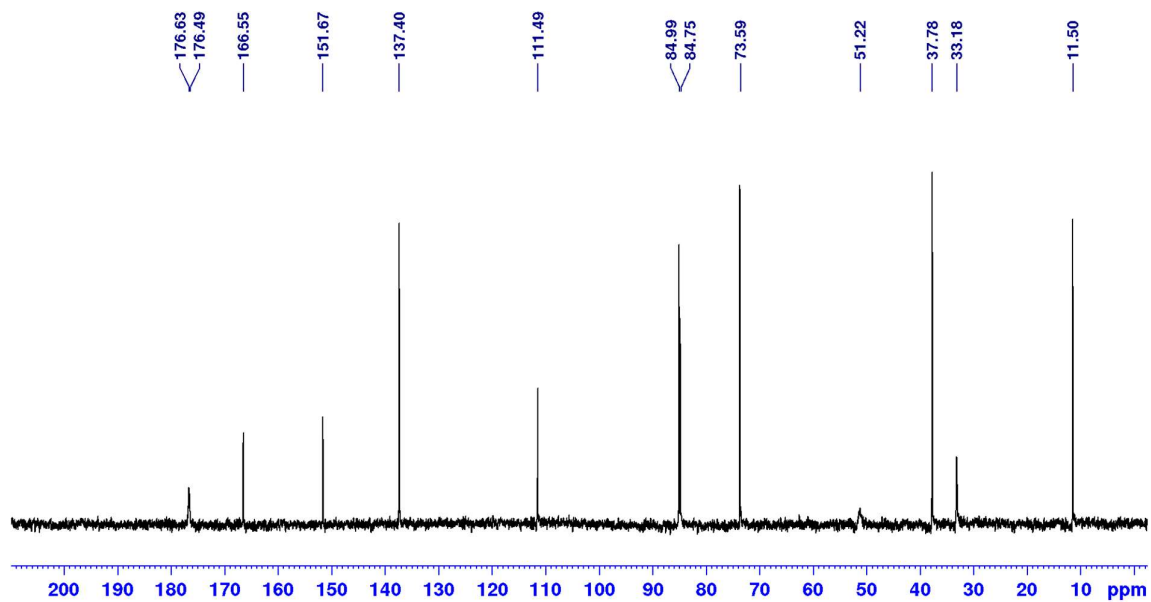


C-(5'-Deoxythymidin-5'-yl) disodium malonate (10):

^1H NMR (600 MHz, D_2O)

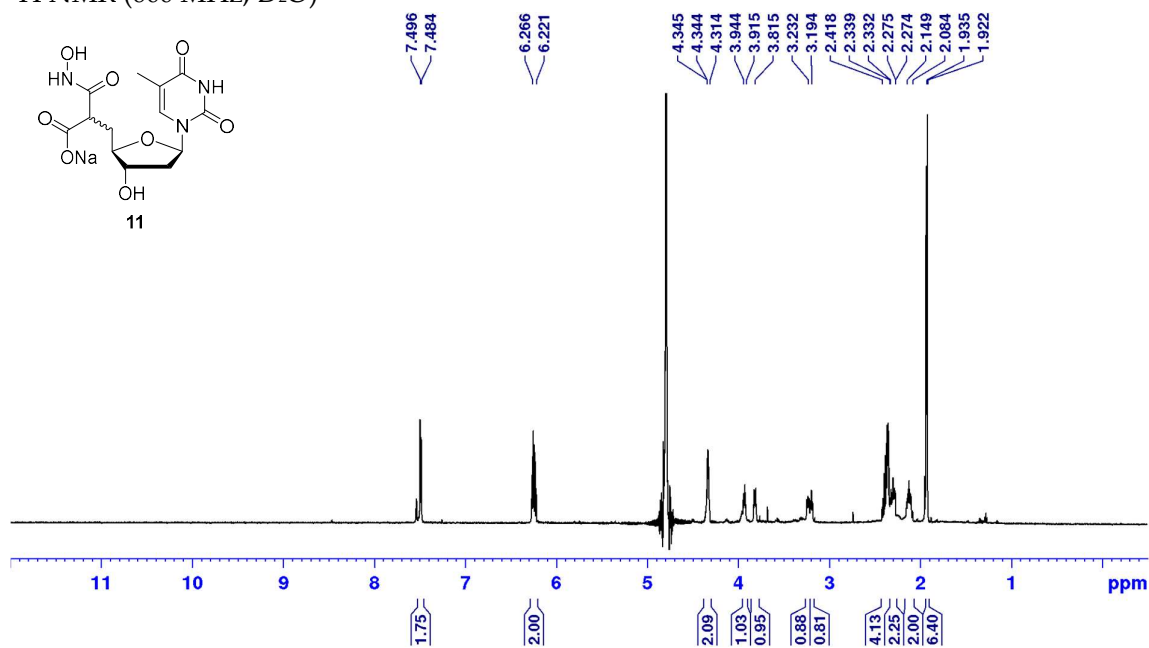


^{13}C NMR (150 MHz, D_2O)

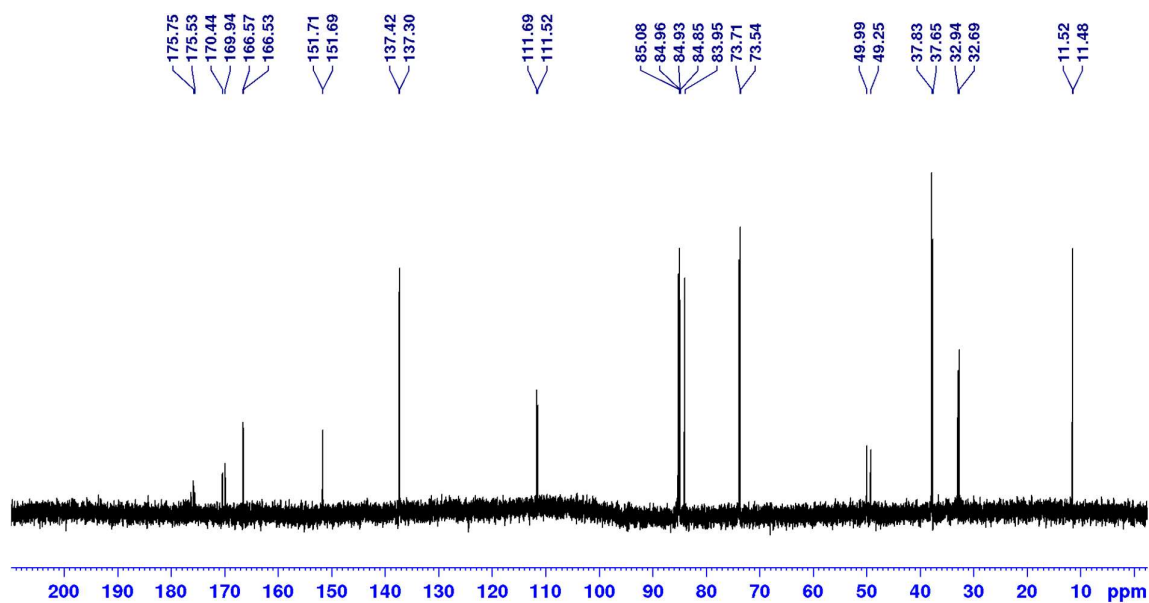


C-(5'-deoxythymidin-5'-yl) sodium N-hydroxymalonamide (11):

¹H NMR (600 MHz, D₂O)

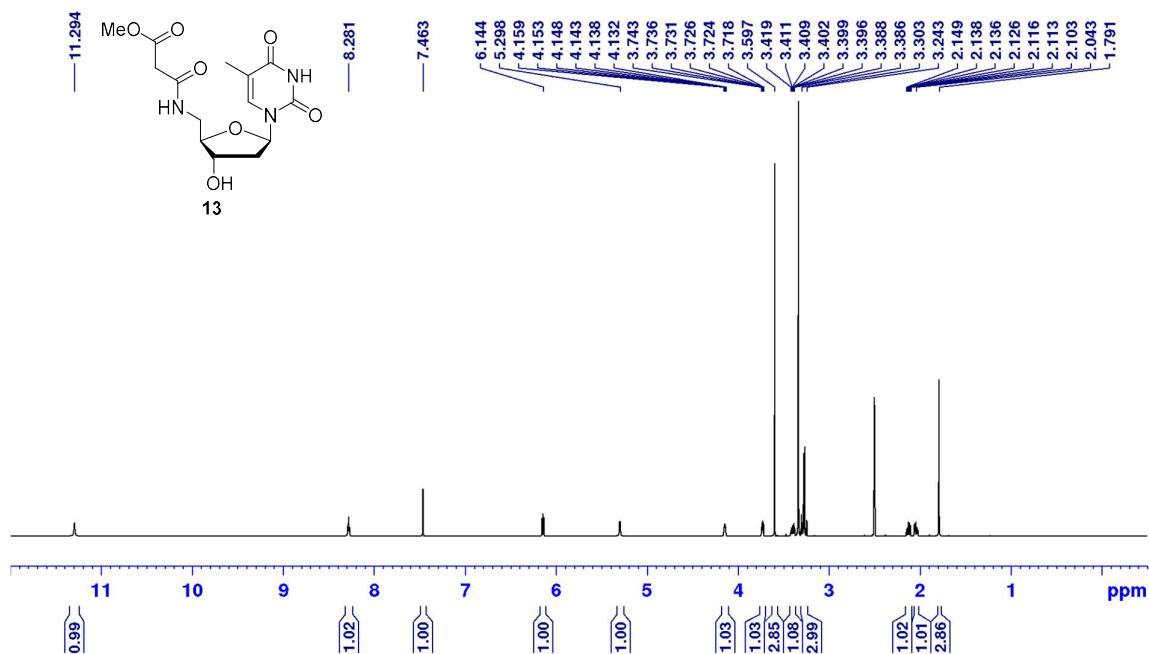


¹³C NMR (150 MHz, D₂O)

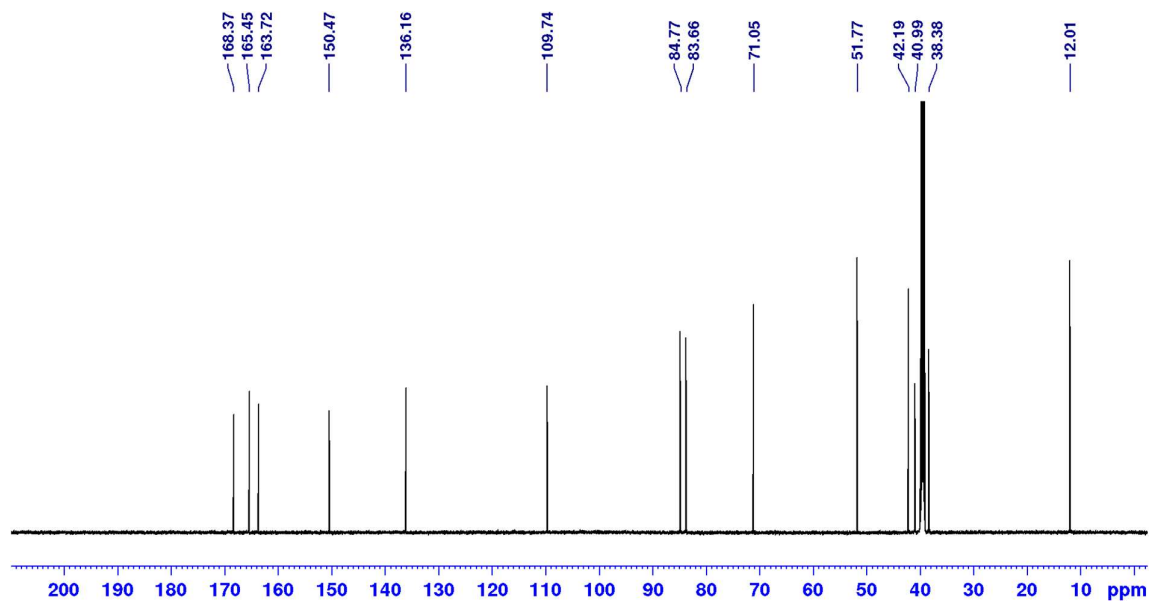


***N*-(5'-Deoxythymidin-5'-yl) amido methyl malonate (13):**

¹H NMR (600 MHz, DMSO-d₆)

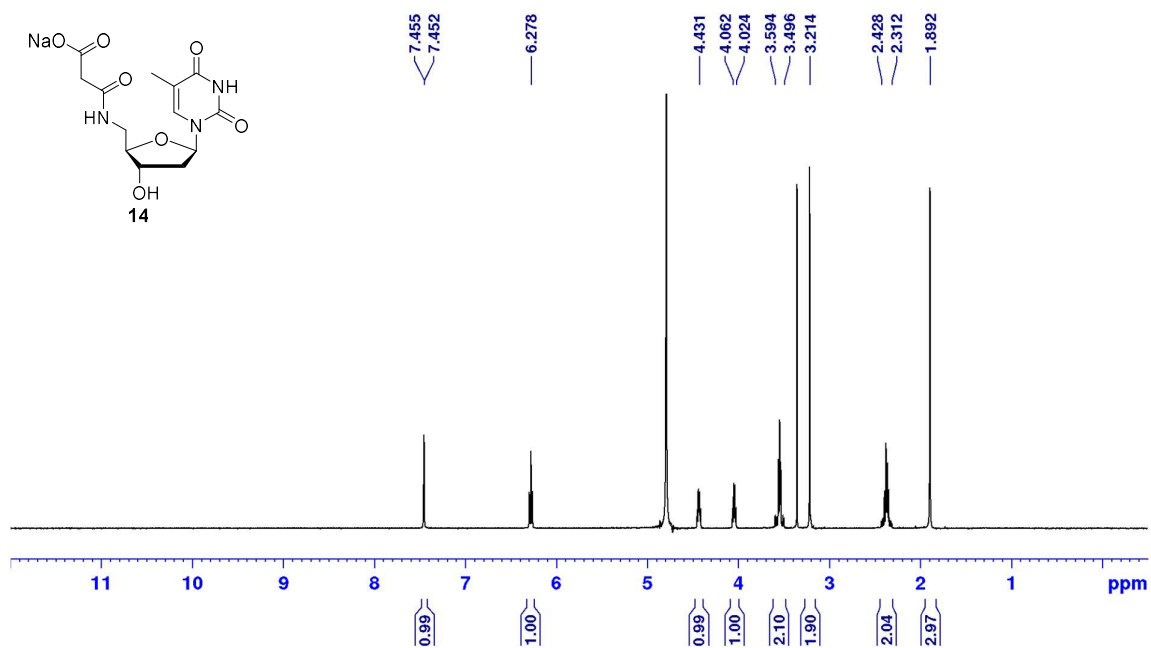


¹³C NMR (150 MHz, DMSO-d₆)

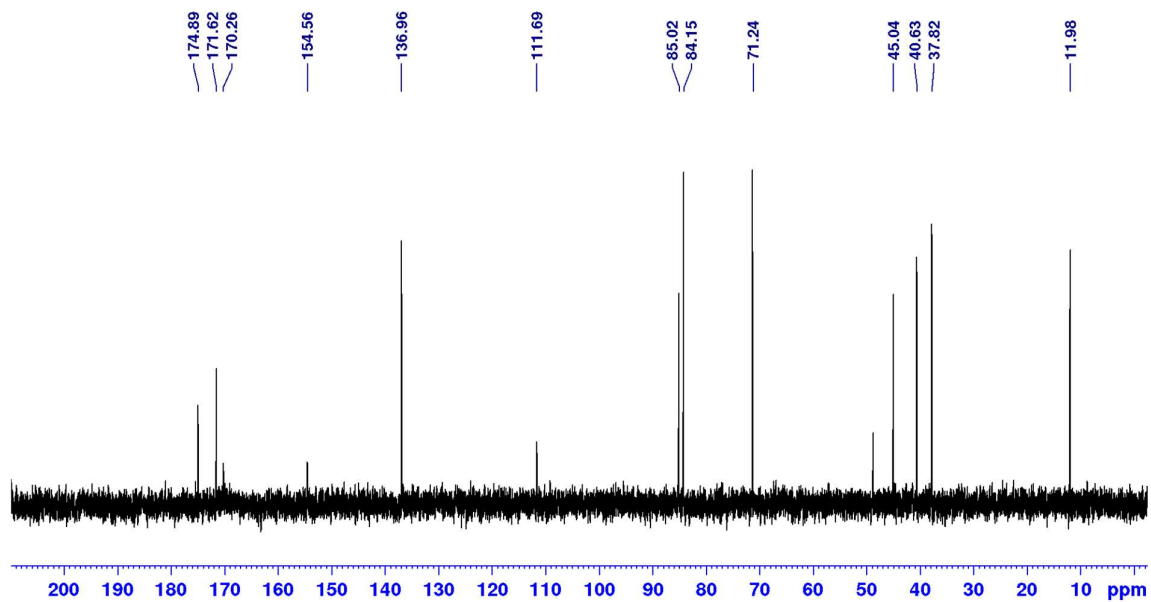


***N*-(5'-Deoxythymidin-5'-yl) amido sodium malonate (14):**

¹H NMR (400 MHz, D₂O)

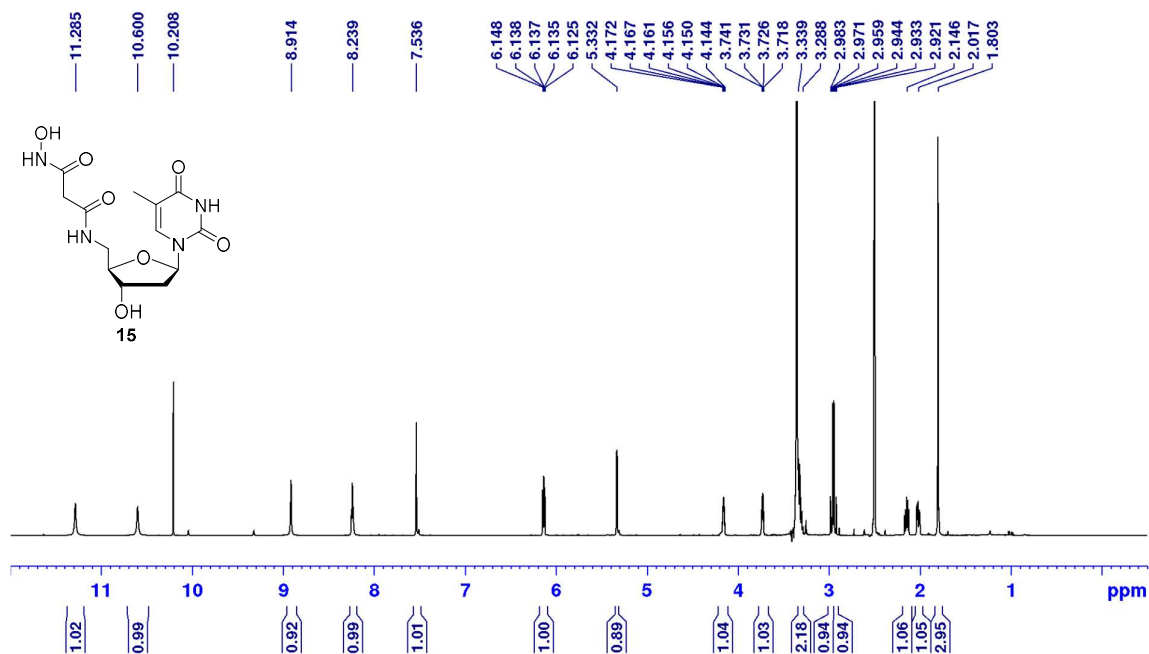


¹³C NMR (100 MHz, D₂O)

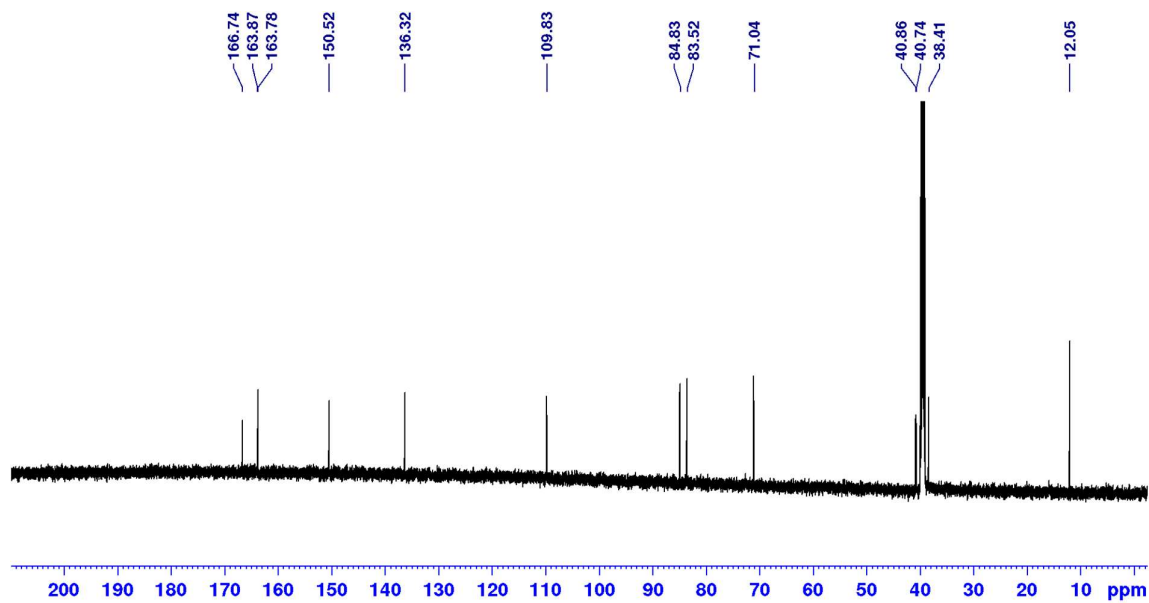


***N*-(5'-Deoxythymidin-5'-yl) amido *N*-hydroxymalonamide (15):**

¹H NMR (600 MHz, DMSO-d₆)

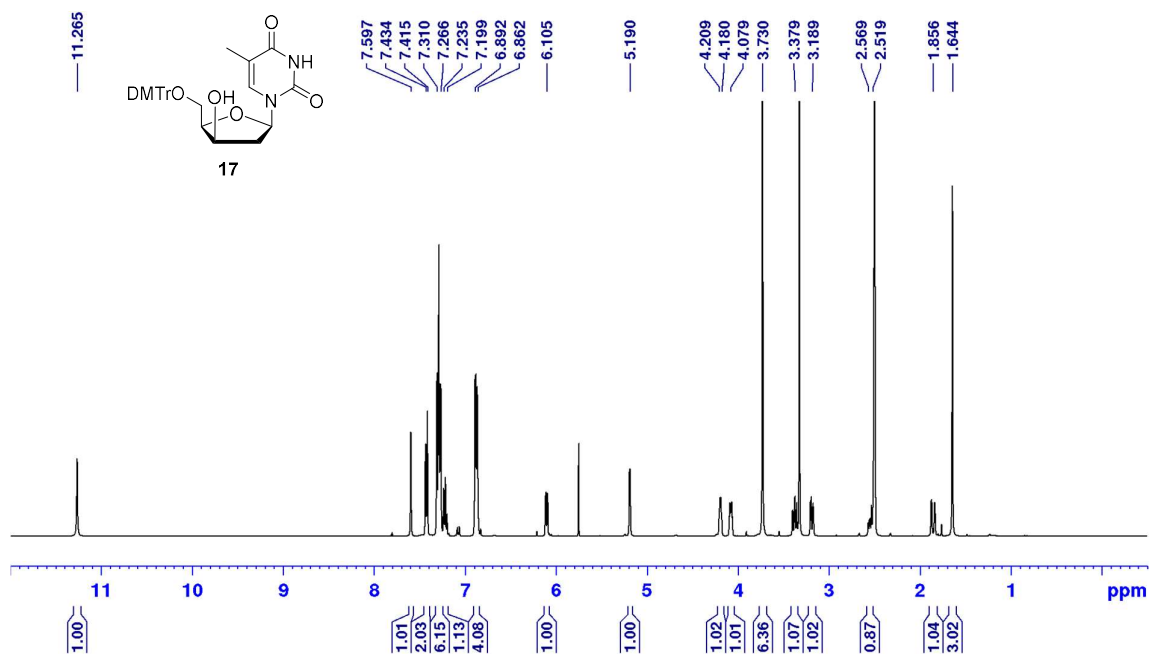


¹³C NMR (150 MHz, DMSO-d₆)

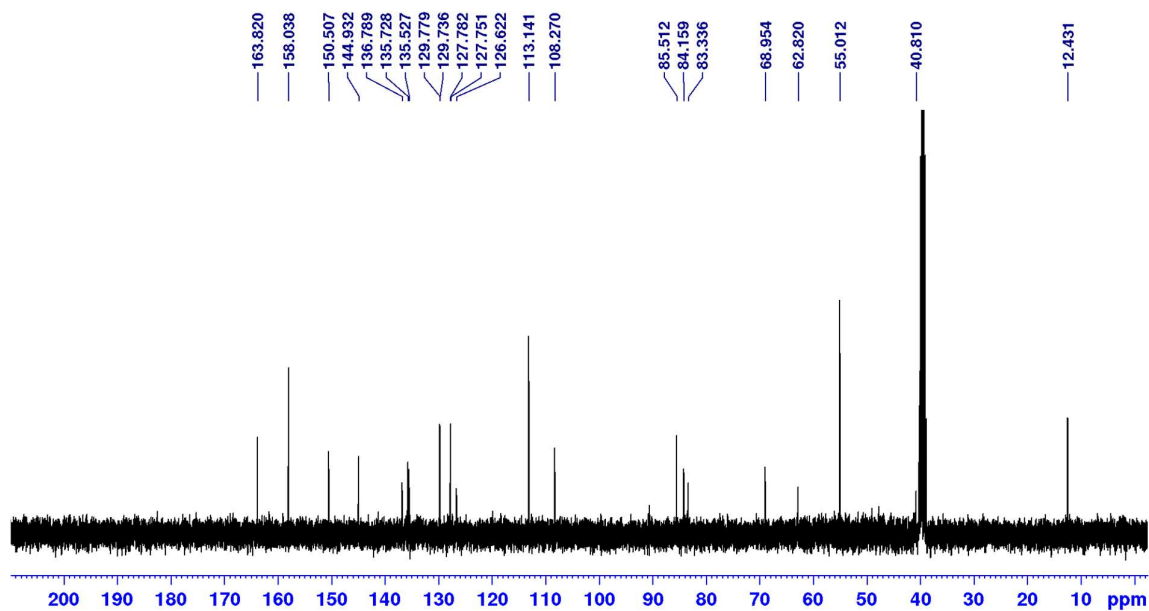


1-[5'-O-(4,4'-Dimethoxytrityl)-2'-deoxy- β -D-furanosyl]thymine (17):

^1H NMR (400 MHz, DMSO-d_6)

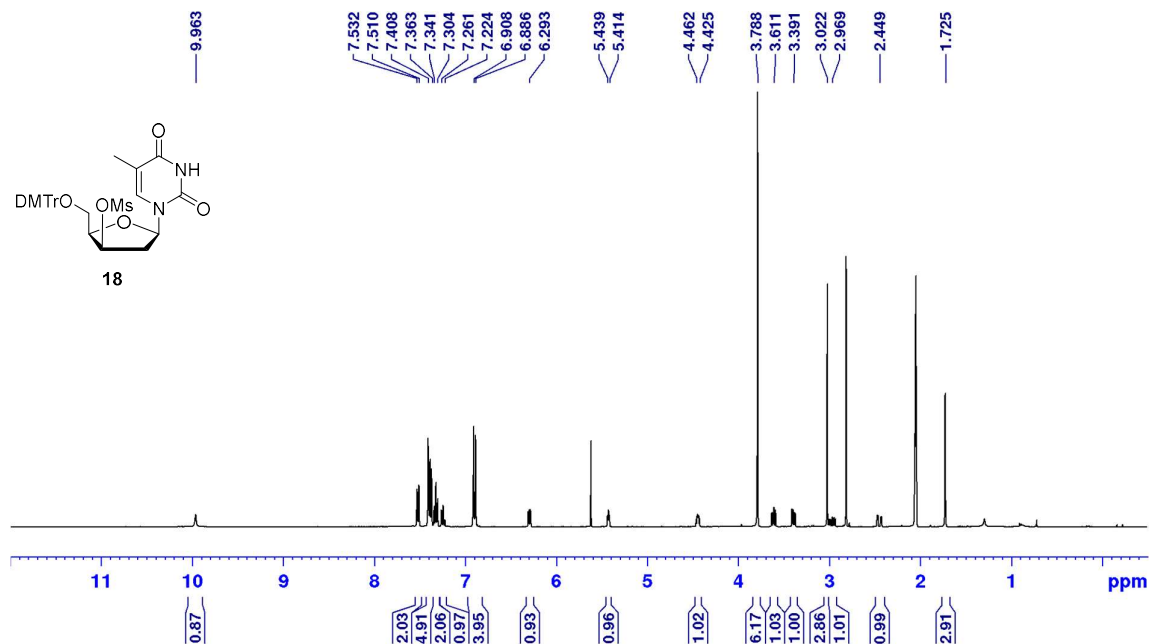


^{13}C NMR (100 MHz, DMSO-d_6)

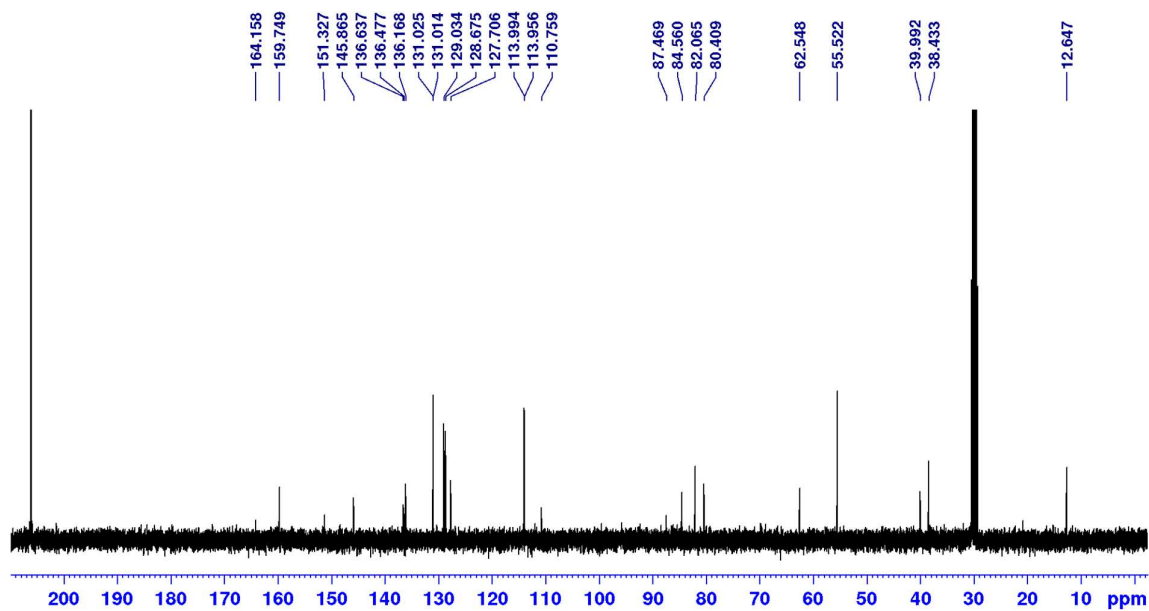


1-[5'-O-(4,4'-dimethoxytrityl)-3'-O-mesy1-2'-deoxy- β -D-furanosyl]thymine (18):

^1H NMR (400 MHz, acetone- d_6)

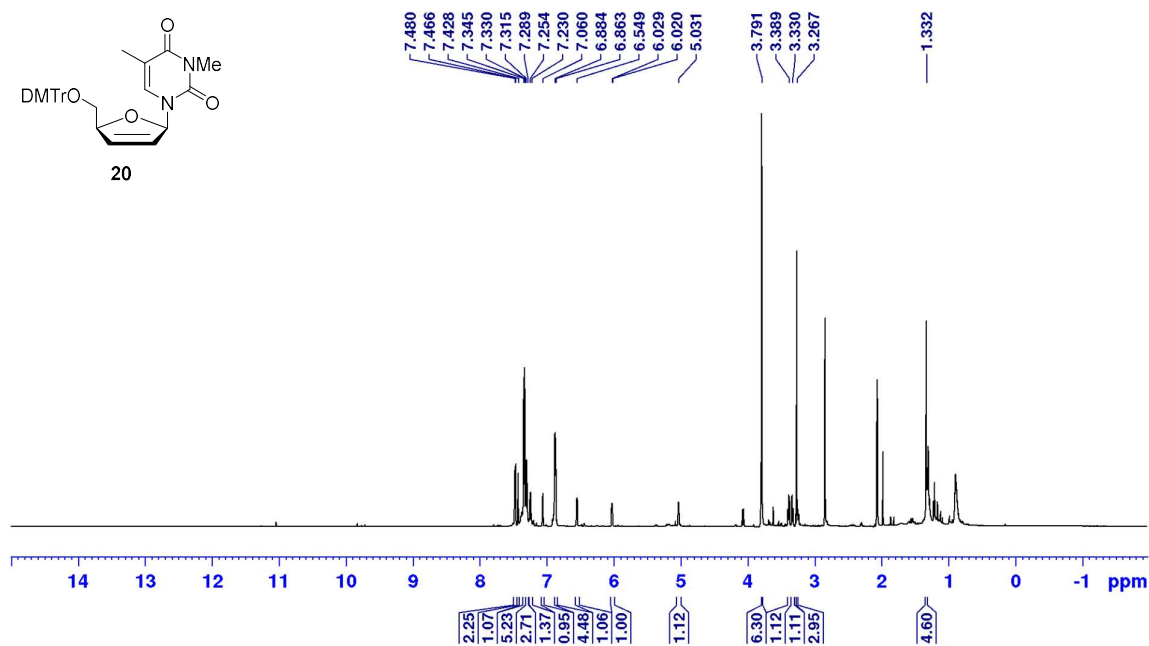


^{13}C NMR (100 MHz, acetone- d_6)

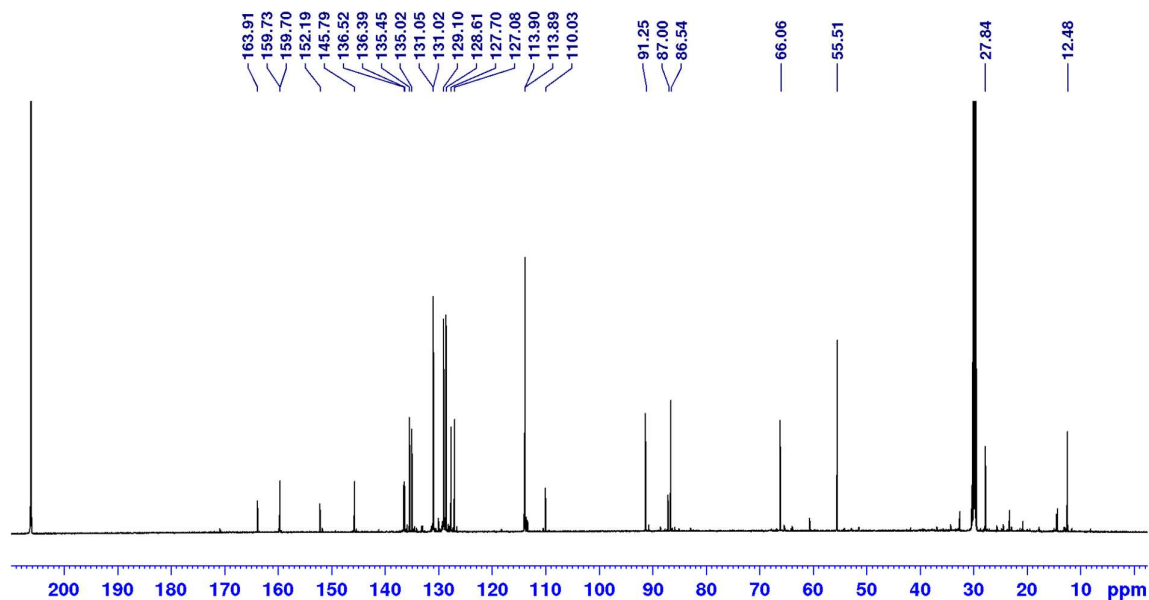


3'-Deoxy-2',3'-didehydro-5'-O-(4,4'-dimethoxytrityl)-N³-methylthymidine (20):

¹H NMR (600 MHz, acetone-d₆)

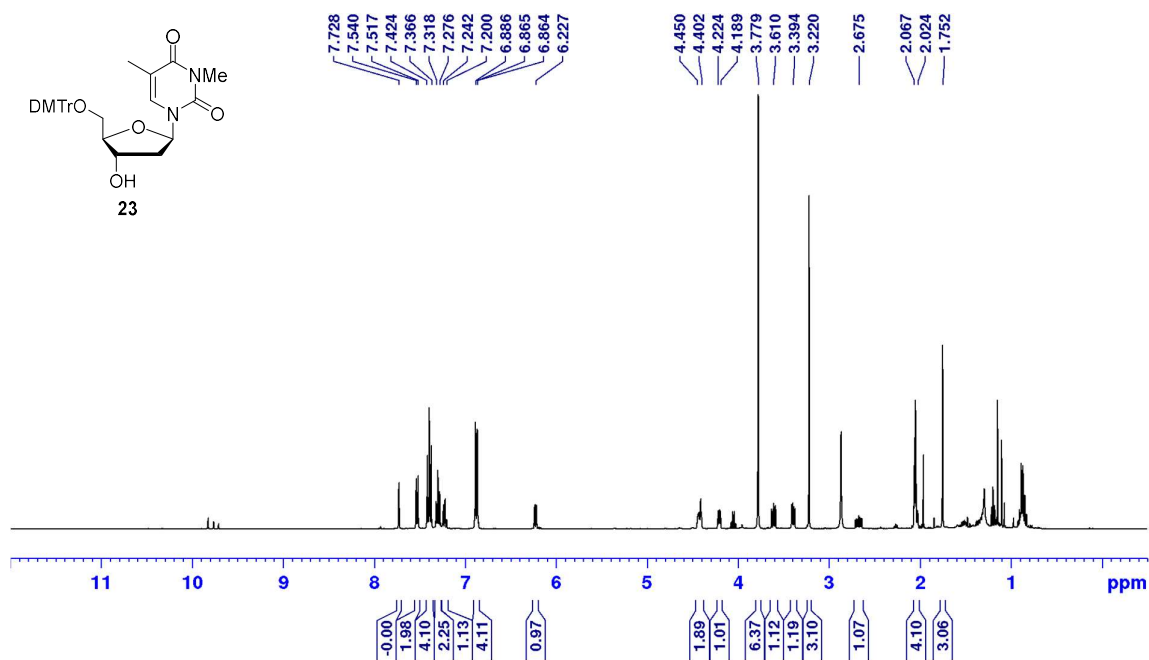


¹³C NMR (150 MHz, acetone-d₆):

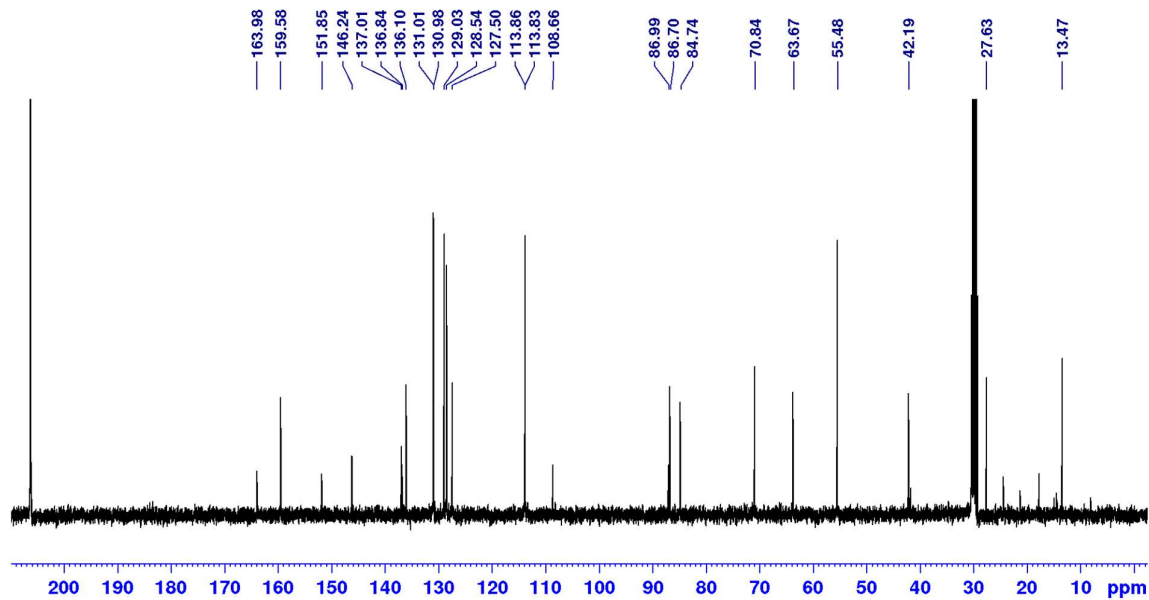


5'-O-(4,4'-Dimethoxytrityl)-N³-methyl thymidine (23):

¹H NMR (400 MHz, acetone-d₆)

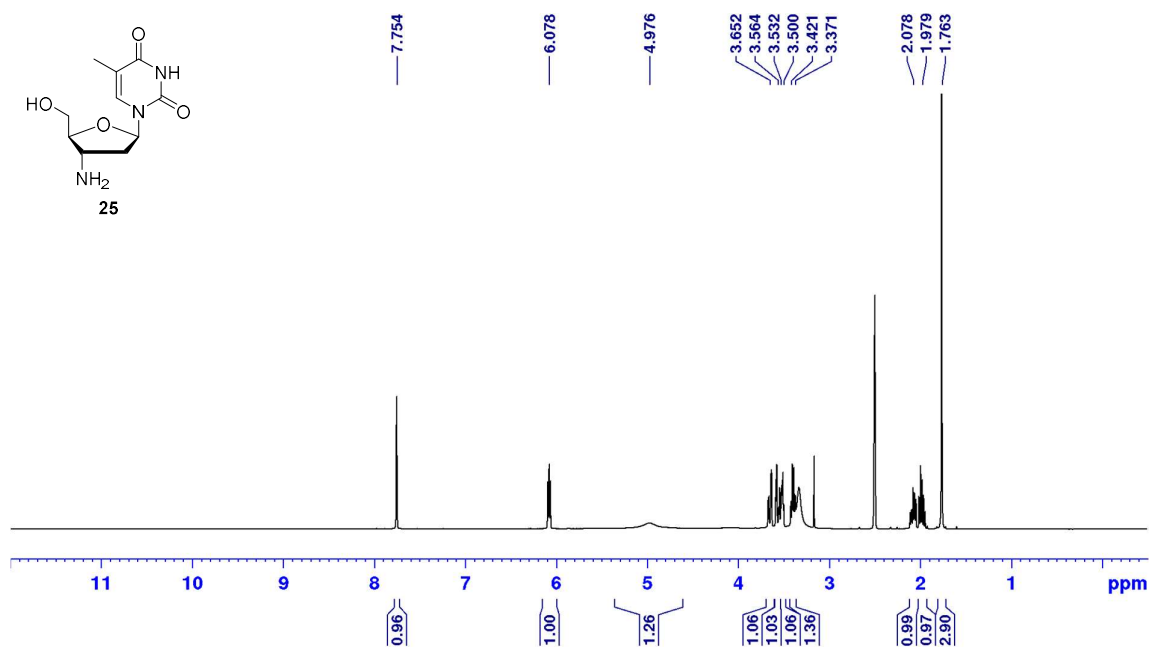


¹³C NMR (100 MHz, acetone-d₆)

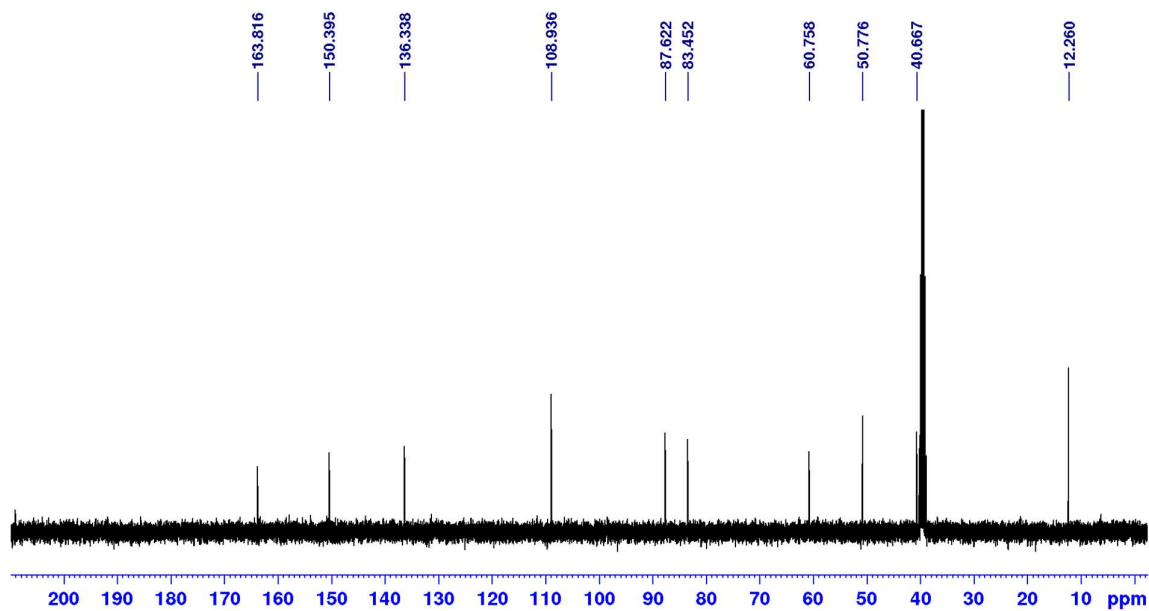


3'-Amino-3'-deoxythymidine (25):

^1H NMR (400 MHz, DMSO-d_6)

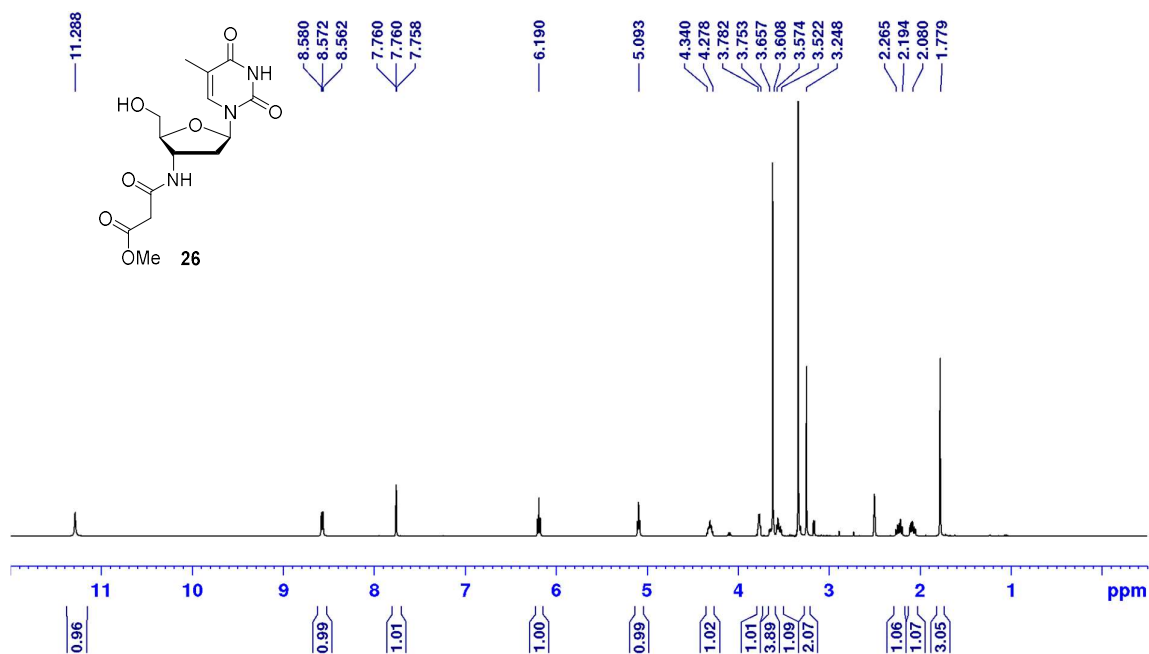


^{13}C NMR (100 MHz, DMSO-d_6)

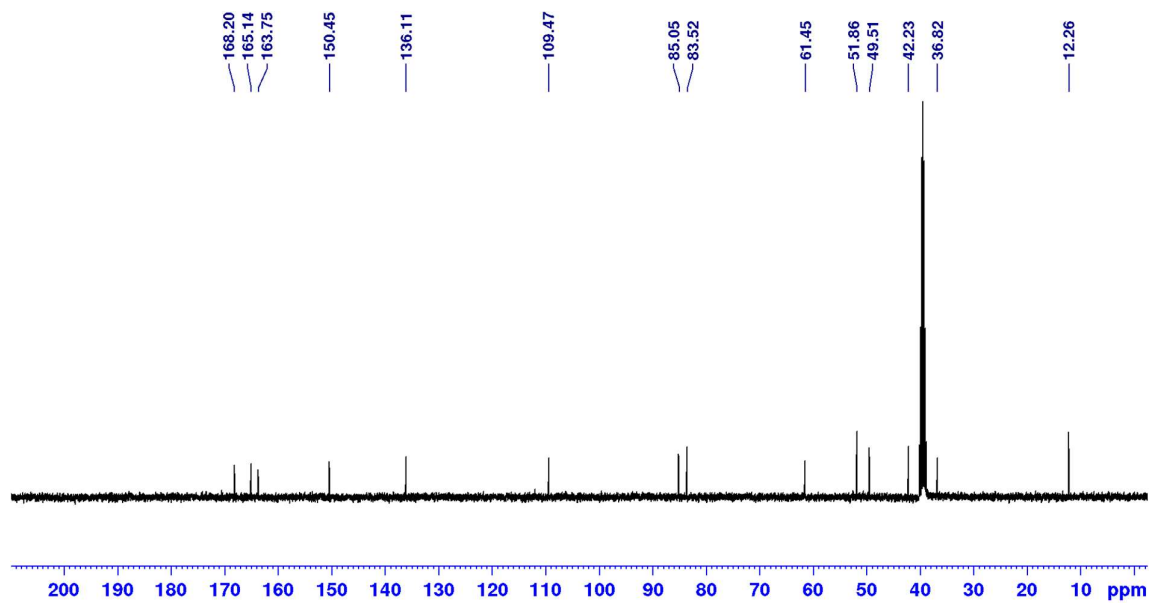


***N*-(3'-Deoxythymidin-3'-yl) amido methyl malonamide (26):**

¹H NMR (400 MHz, DMSO-d₆)

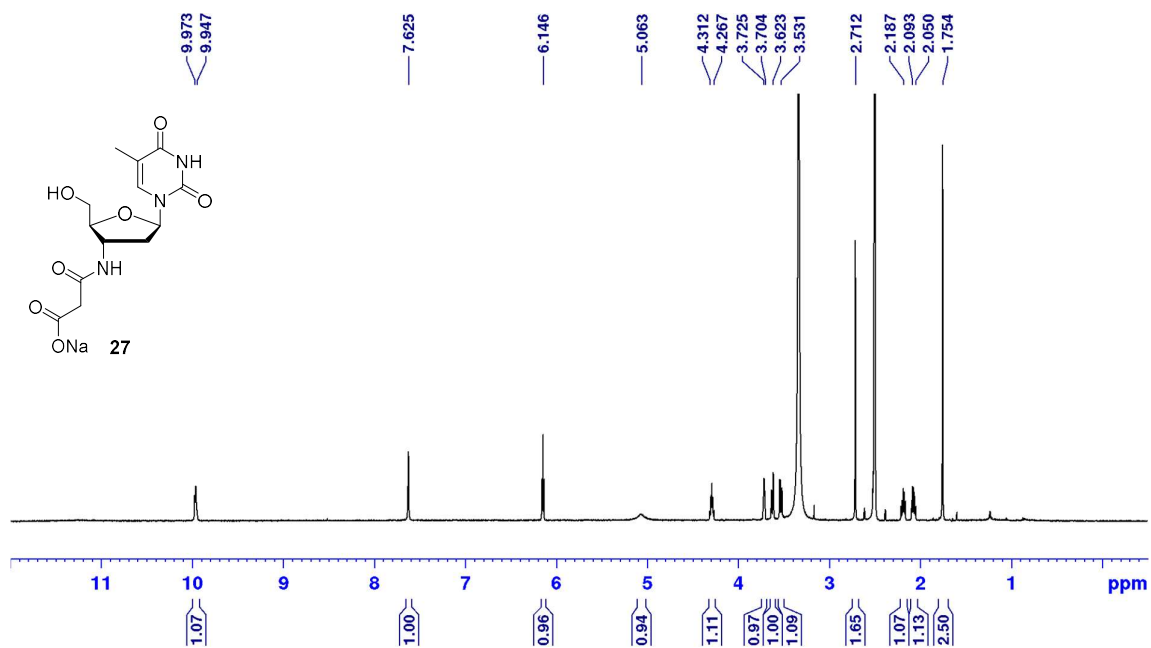


¹³C NMR (100 MHz, DMSO-d₆)

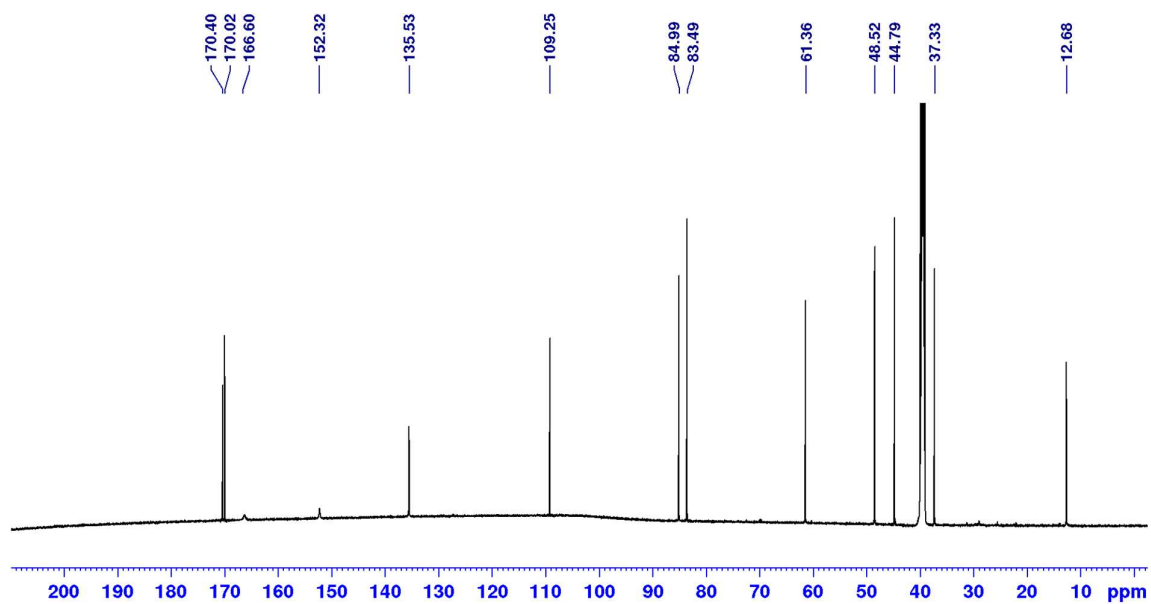


***N*-(3'-Deoxythymidin-3'-yl) amido sodium malonamide (27):**

¹H NMR (600 MHz, DMSO-d₆)

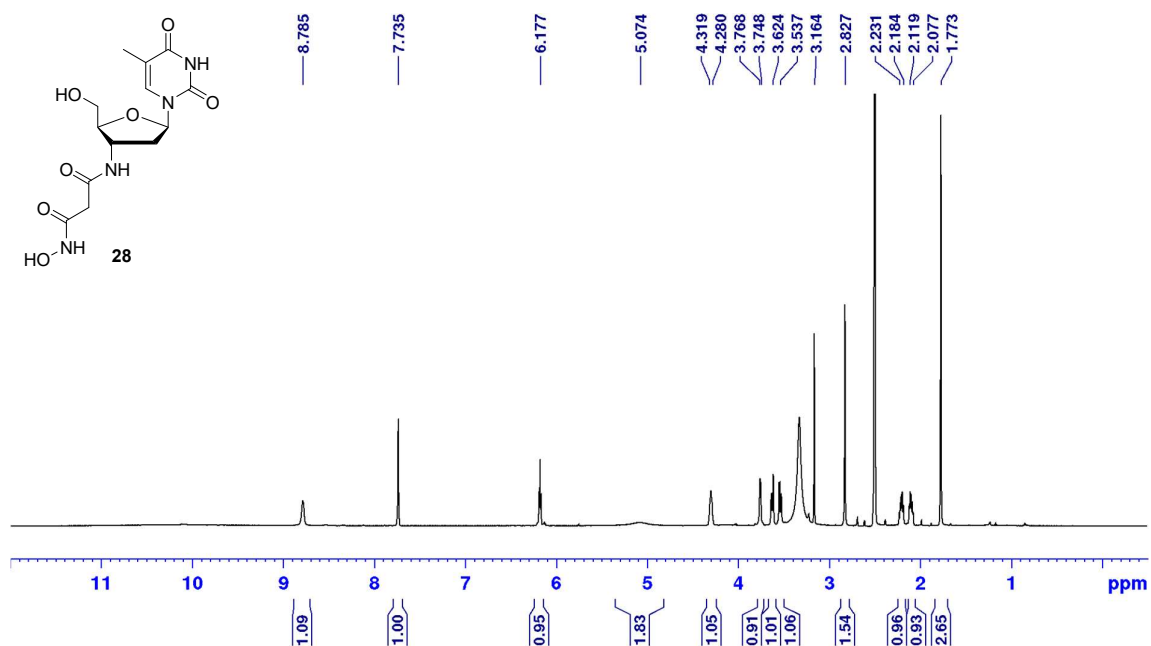


¹³C NMR (150 MHz, DMSO-d₆)

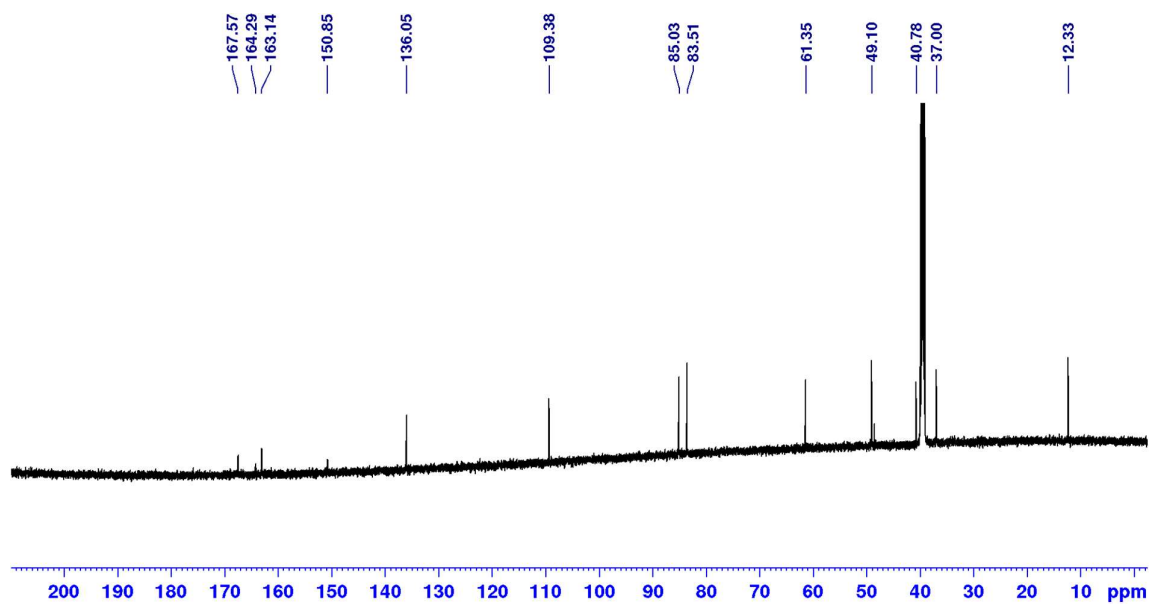


***N*-(3'-Deoxythymidin-3'-yl) amido *N*-hydroxymalonamide (28):**

¹H NMR (600 MHz, DMSO-d₆)

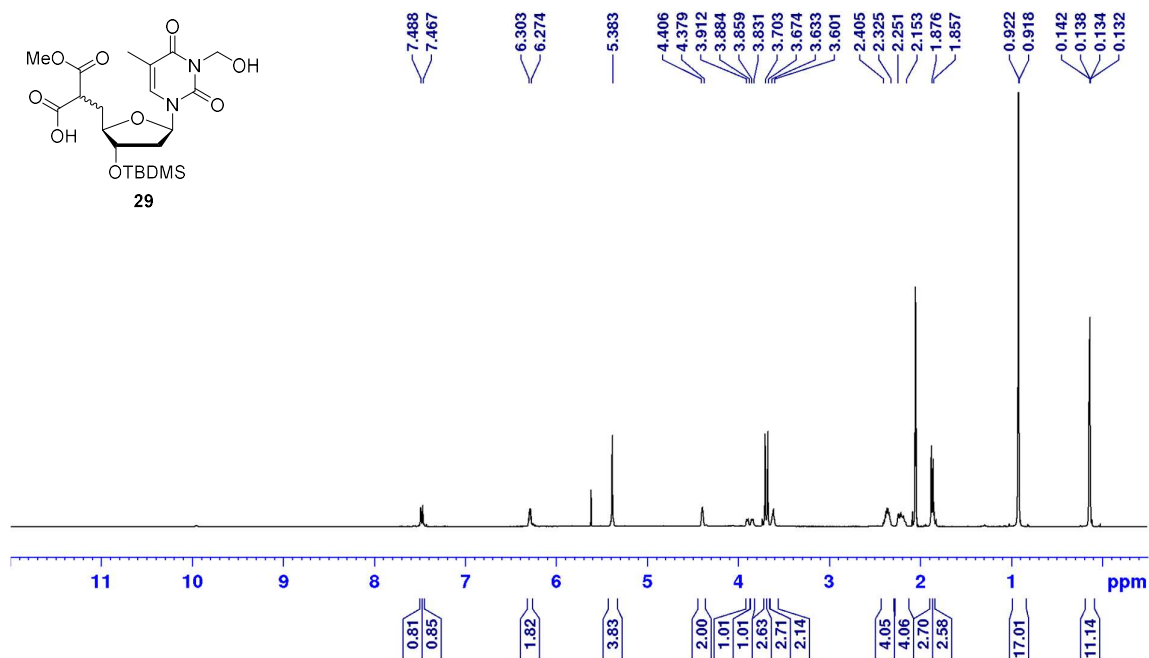


¹³C NMR (150 MHz, DMSO-d₆)

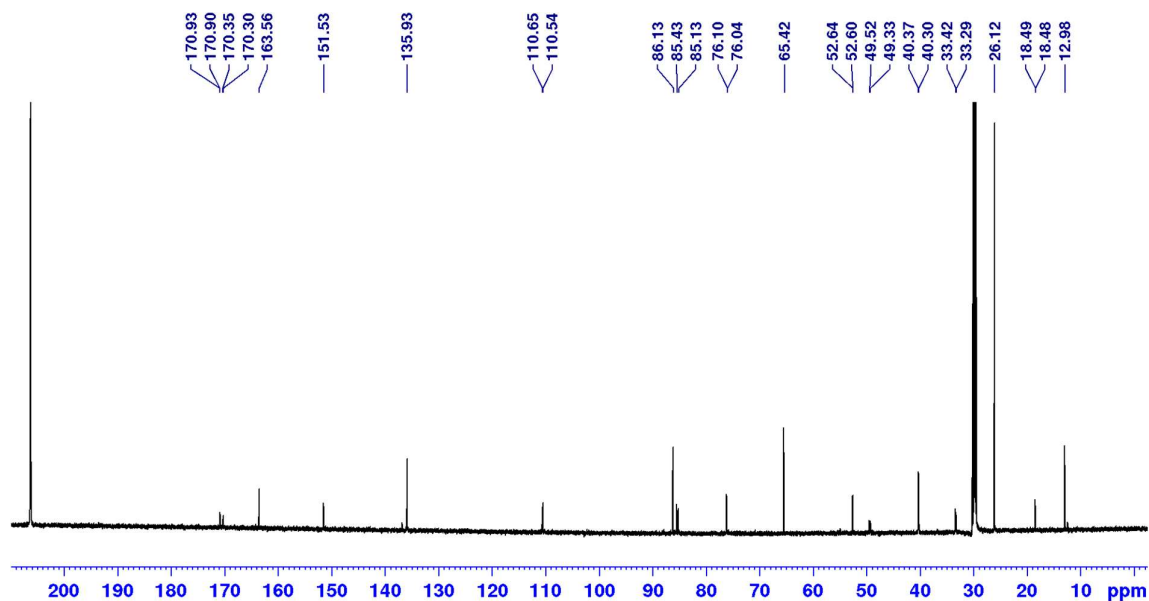


***C*-(3'-*O*-(*tert*-Butyldimethylsilyl)-5'-deoxy-*N*³-hydroxymethyl)thymidin-5'-yl) malonic acid monomethyl ester (29):**

¹H NMR (600 MHz, acetone-d₆)

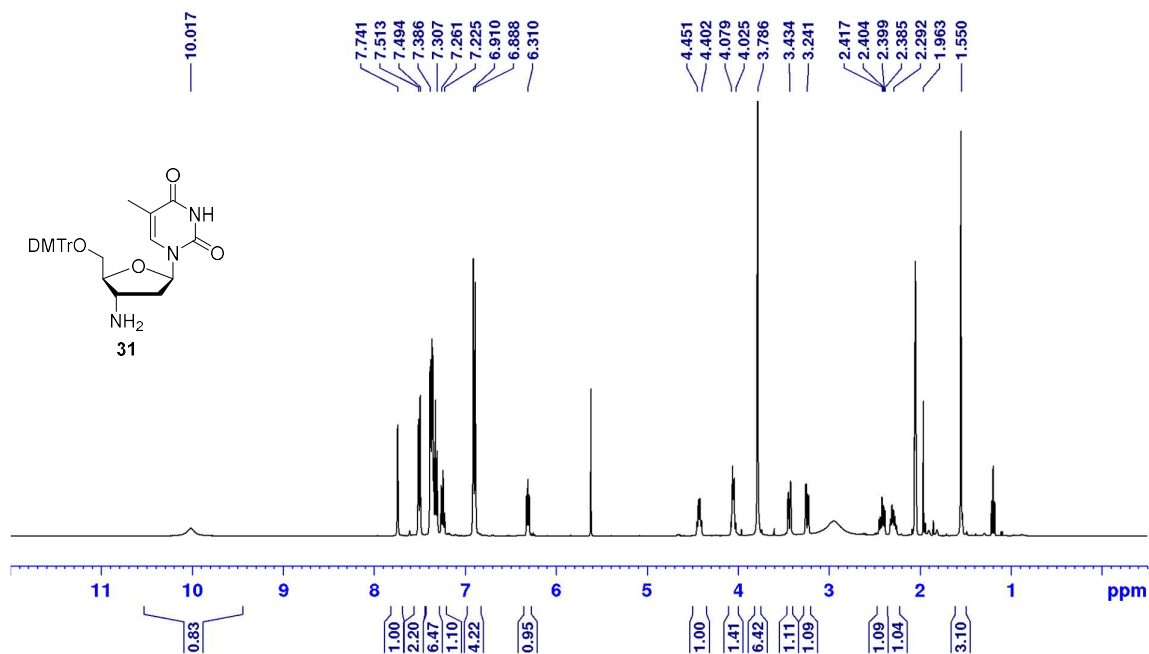


¹³C NMR (151 MHz, acetone-d₆)

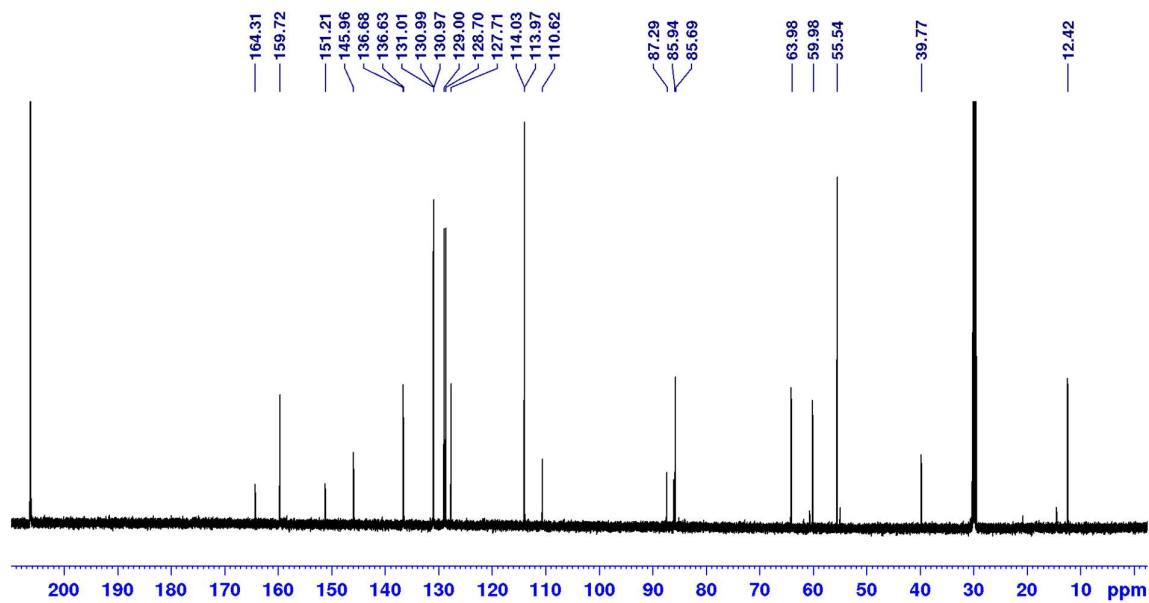


3'-Amino-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)thymidine (31):

^1H NMR (400 MHz, acetone- d_6)

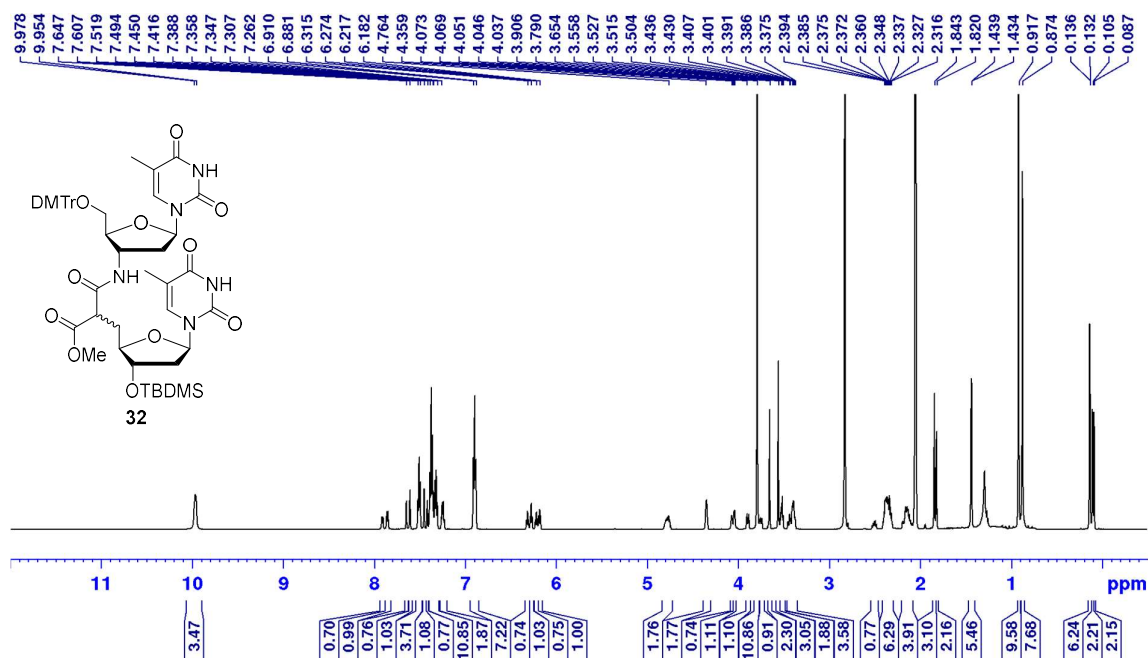


^{13}C NMR (151 MHz, acetone- d_6)

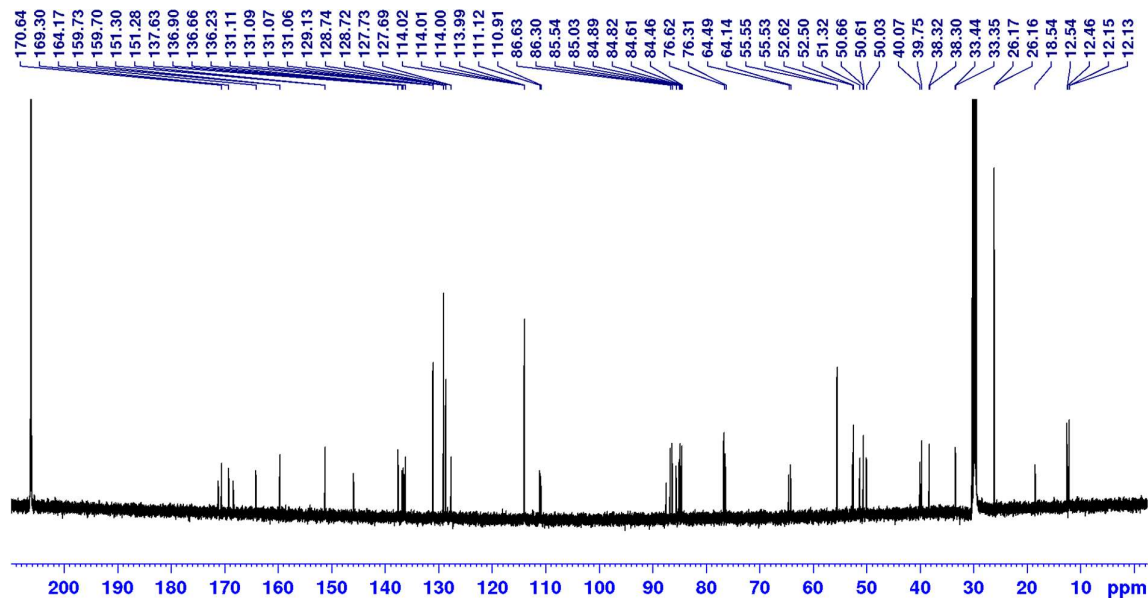


C-(3'-O-(*tert*-Butyldimethylsilyl)-5'-deoxythymidin-5'-yl)-N-(3'-deoxy-5'-O-(4,4'-dimethoxytrityl)thymidin-3'-yl) amido methyl malonate (32):

¹H NMR (600 MHz, acetone-d₆)

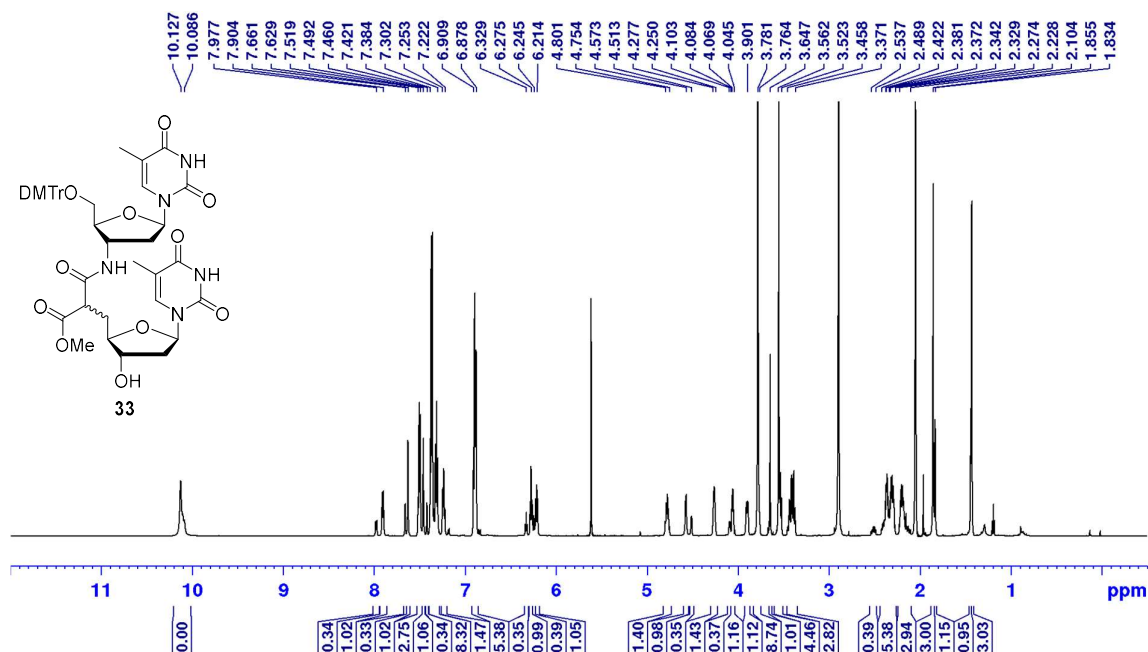


¹³C NMR (151 MHz, acetone-d₆)

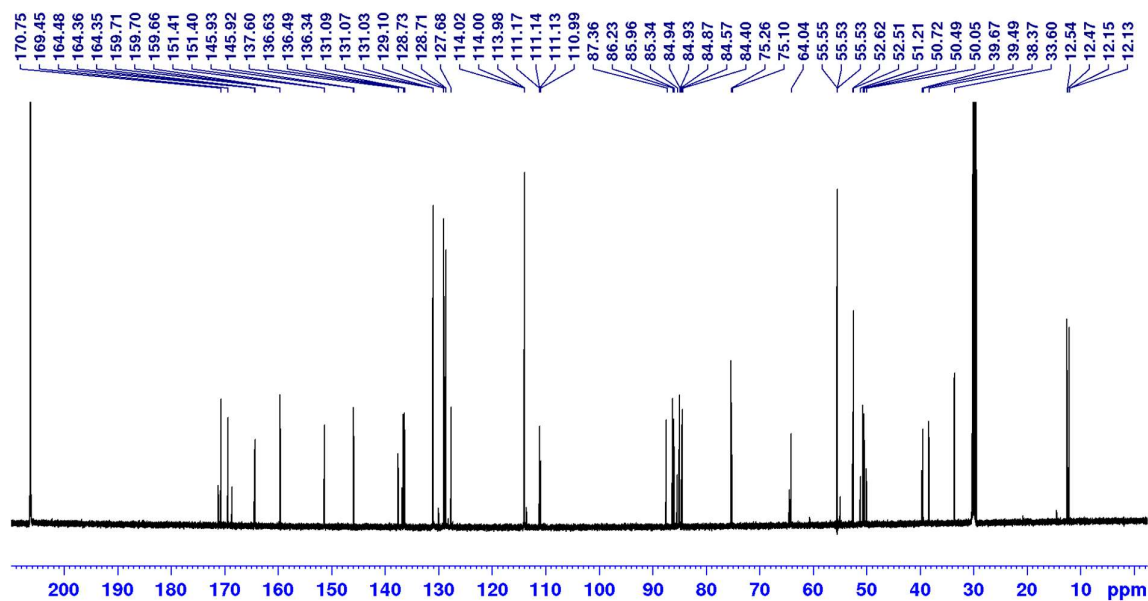


***N*-(3'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)thymidin-3'-yl)-*C*-(5'-deoxythymidin-5'-yl) amido methyl malonate (33):**

¹H NMR (600 MHz, acetone-d₆)

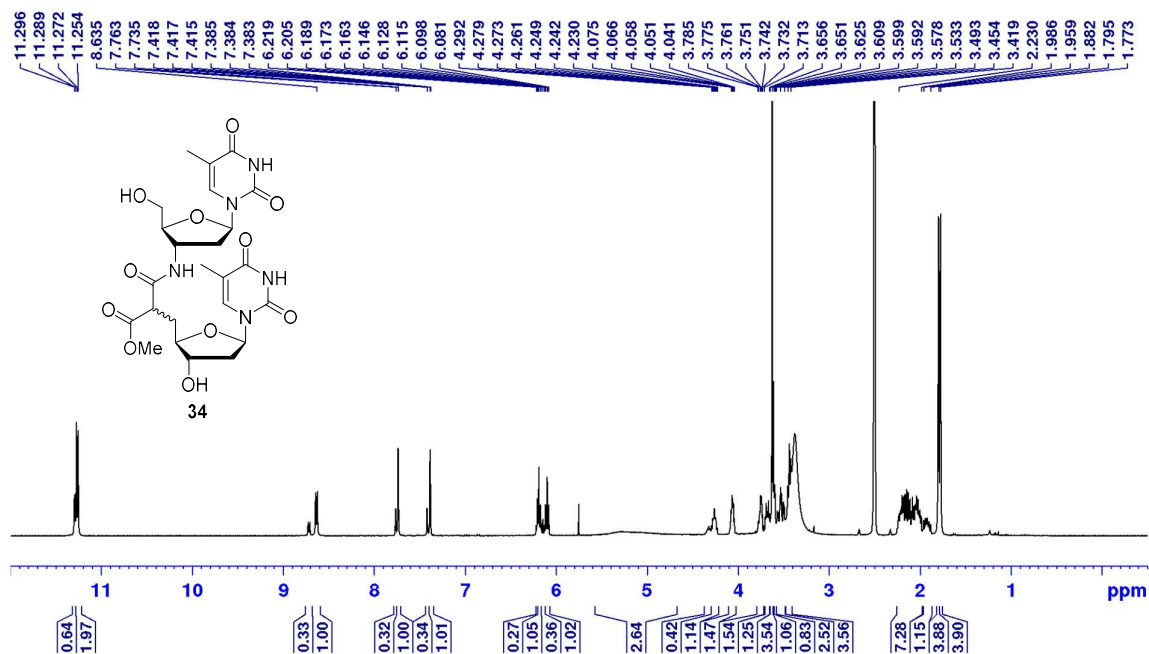


¹³C NMR (151 MHz, acetone-d₆)

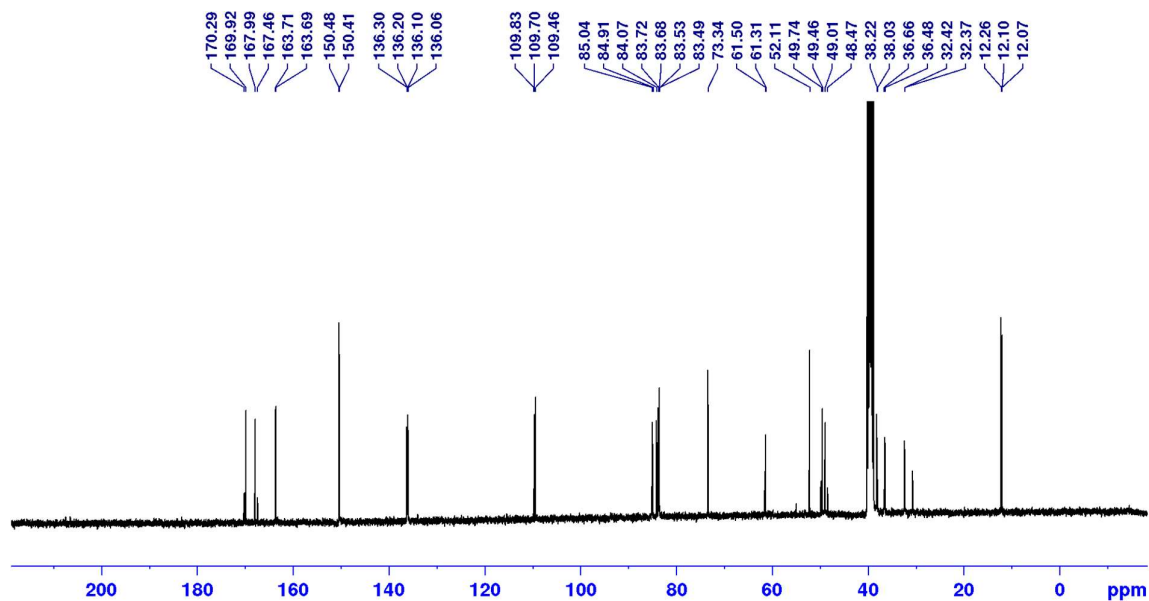


***N*-(3'-Deoxythymidin-3'-yl)-C-(5'-deoxythymidin-5'-yl) amido methyl malonate (34):**

¹H NMR (400 MHz, DMSO-d₆)

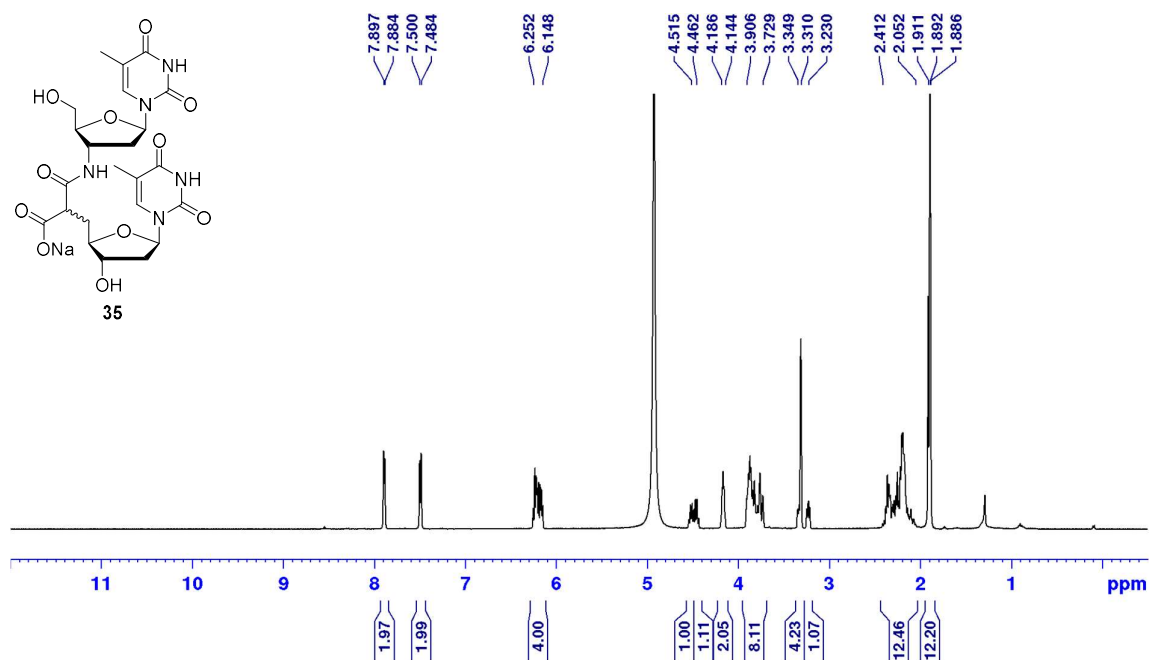


¹³C NMR (100 MHz, DMSO-d₆)

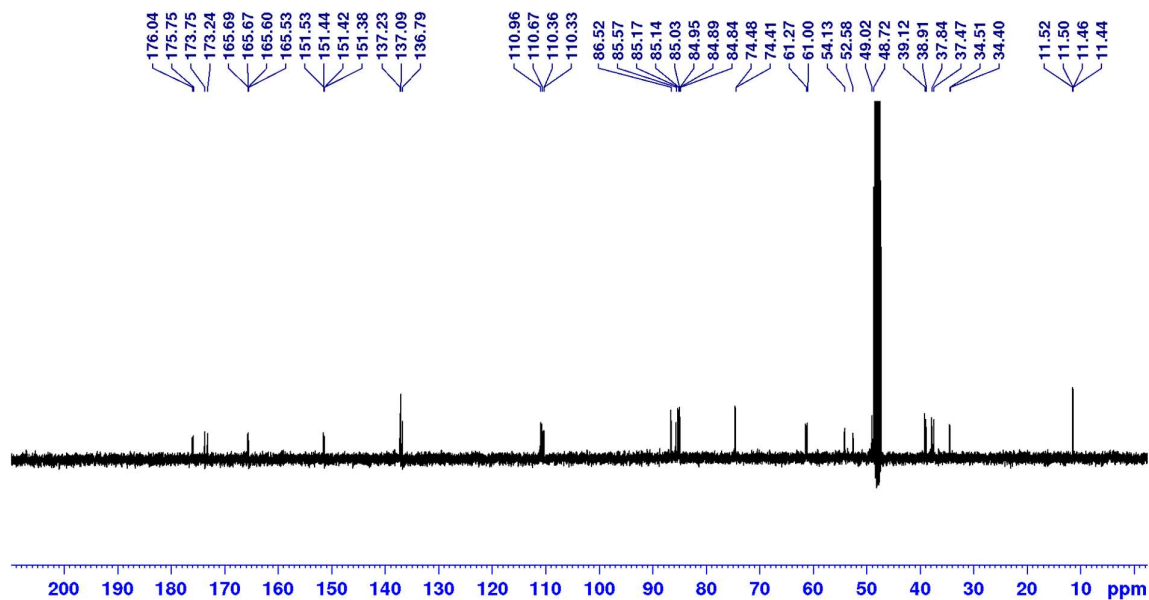


***N*-(3'-Deoxythymidin-3'-yl)-C-(5'-deoxythymidin-5'-yl) amido sodium malonate (35):**

¹H NMR (600 MHz, CD₃OD)

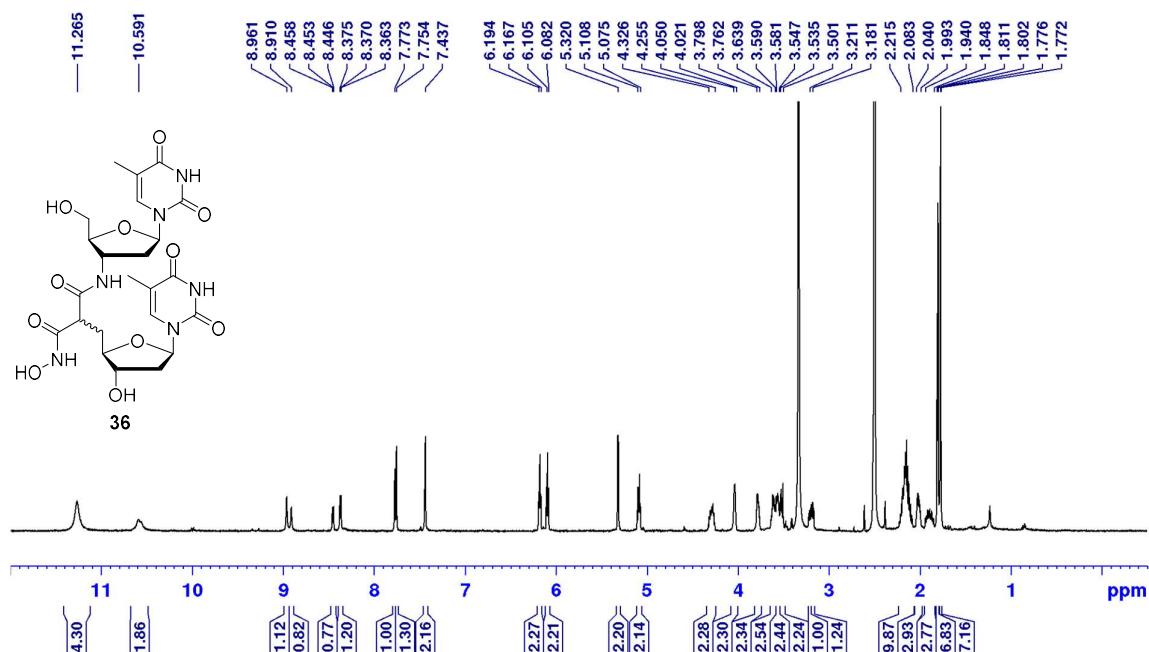


¹³C NMR (151 MHz, CD₃OD)



***N*-(3'-Deoxythymidin-3'-yl)-C-(5'-deoxythymidin-5'-yl) amido *N*-hydroxy malonamide (36):**

¹H NMR (600 MHz, DMSO-d₆)



¹³C NMR (151 MHz, DMSO-d₆)

