Supporting Information for:

Bisubstrate Inhibitors of Nicotinamide *N*-Methyltransferase (NNMT) with Enhanced Activity

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S64 Supplementary Table S1: Methyltransferase selectivity study

S65 Supplementary Figure S1 and Supplementary Table S2: Modeling data

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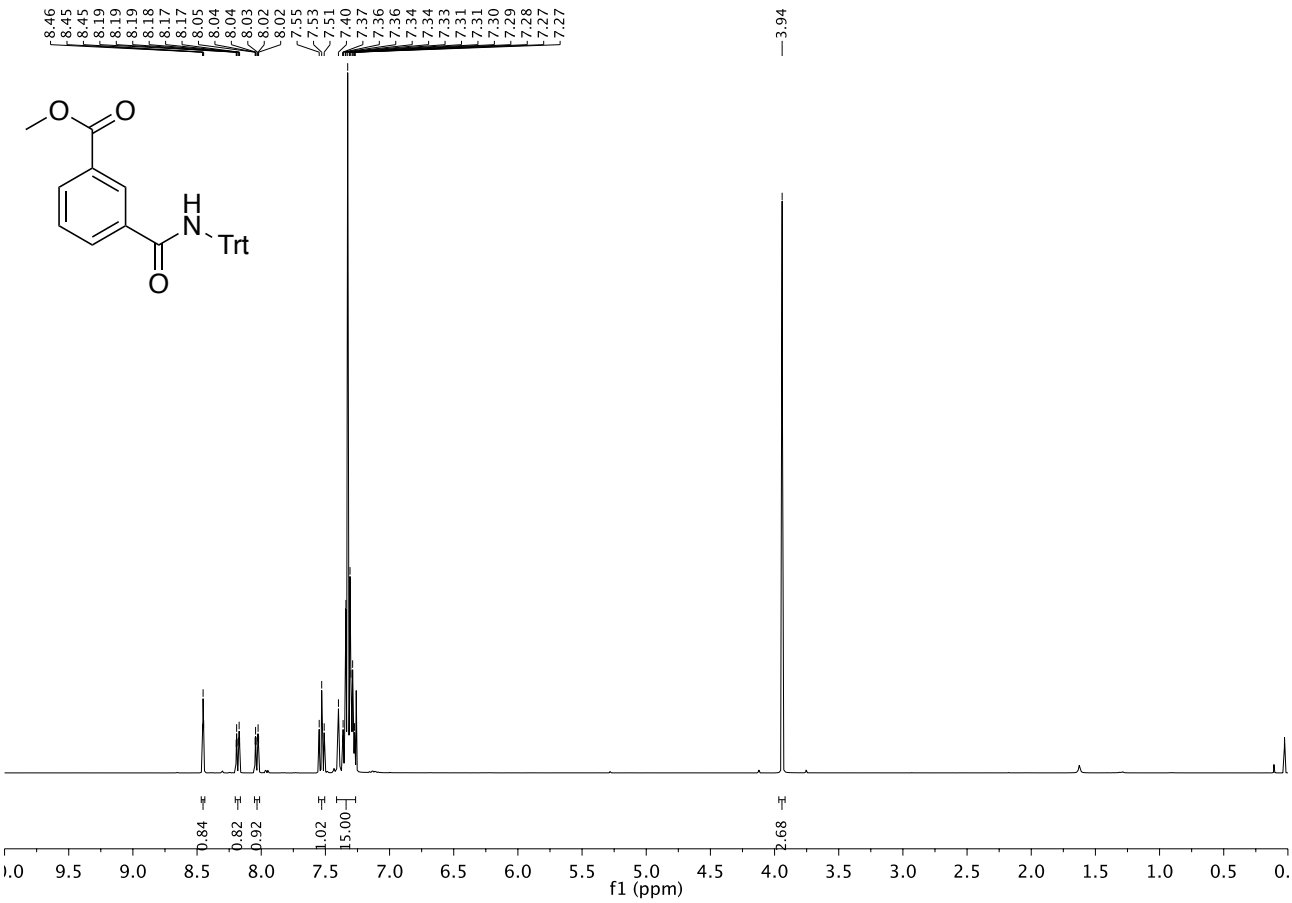
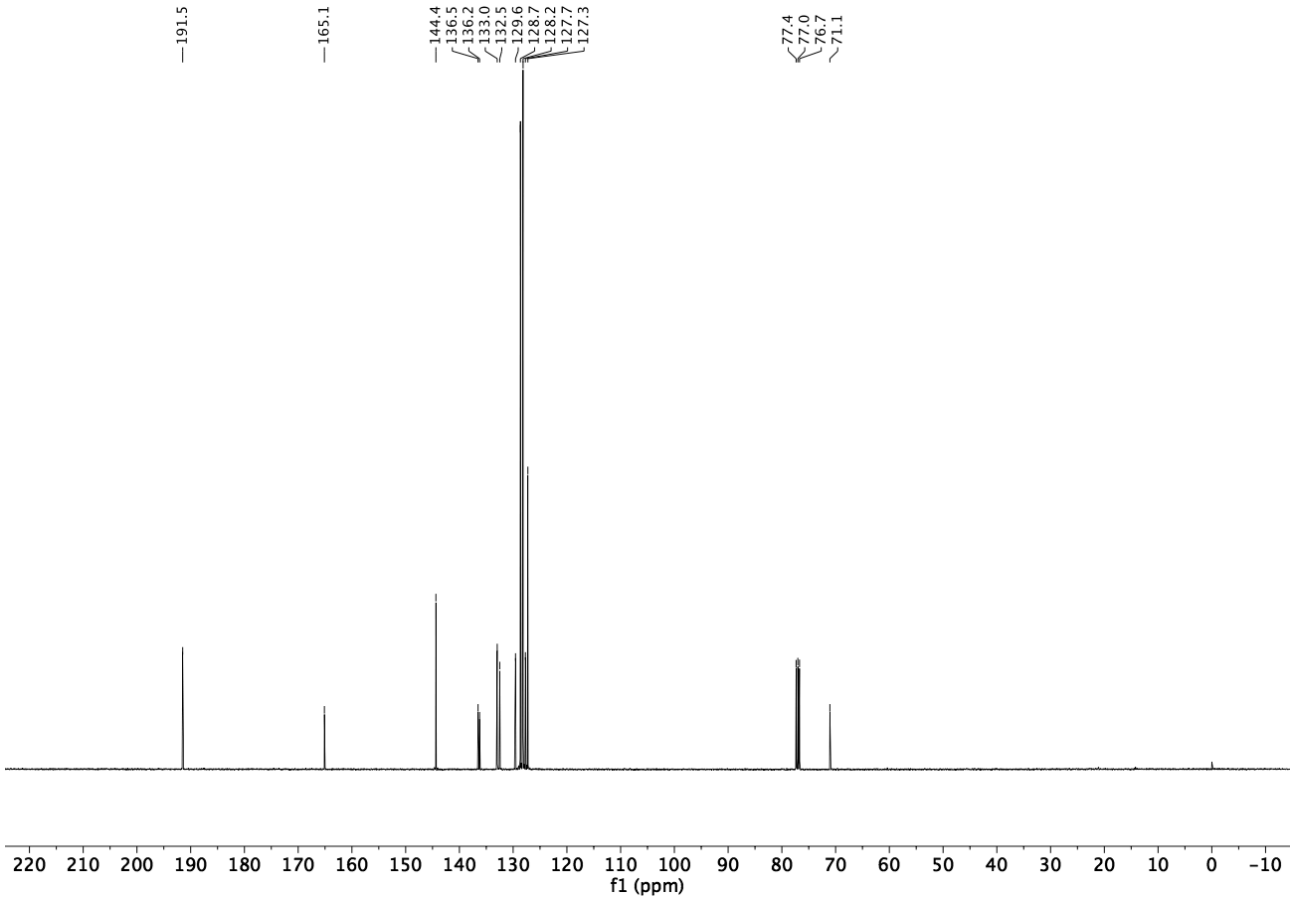
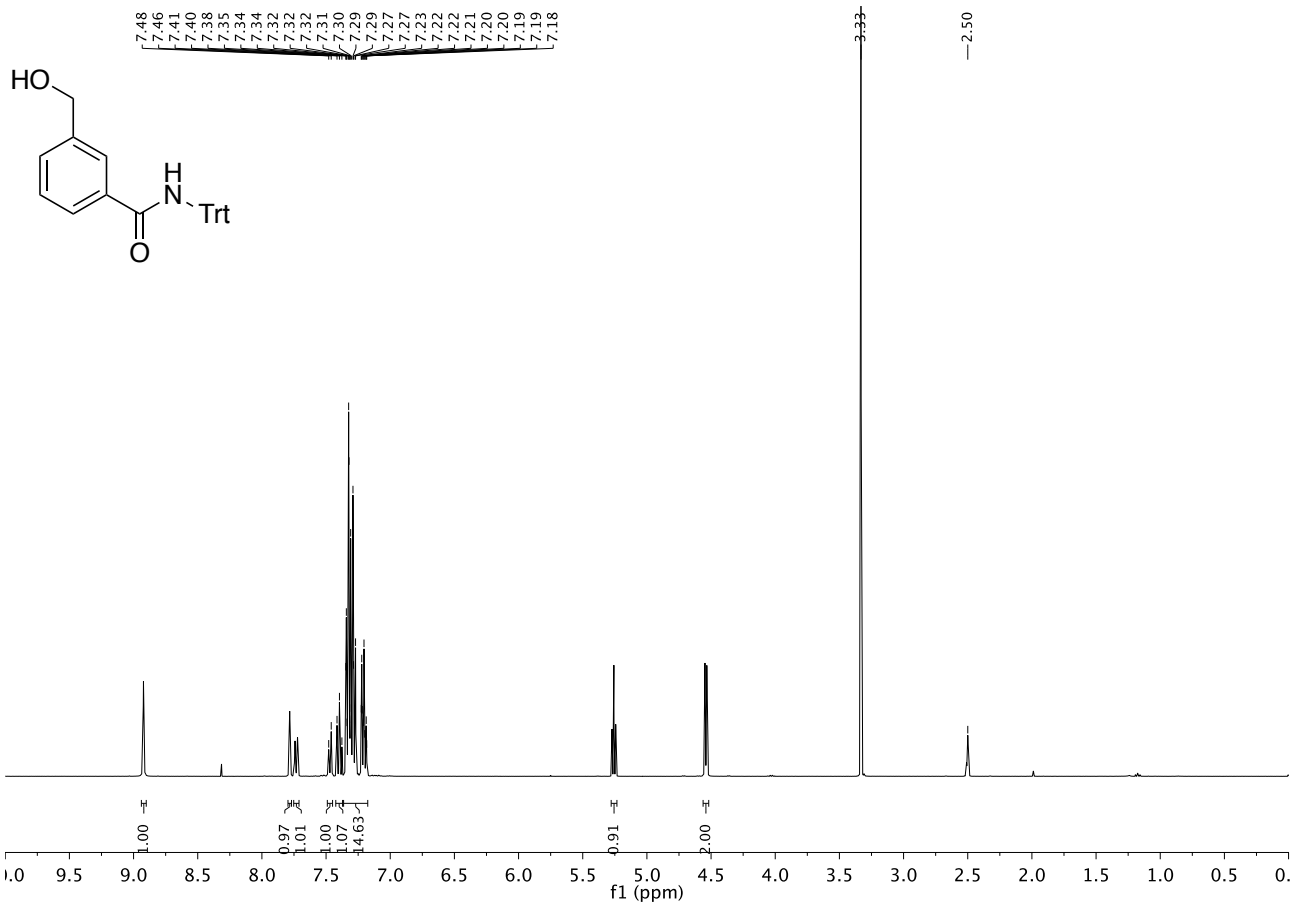
Compound **4** ^{1}H NMR ($CDCl_3$)



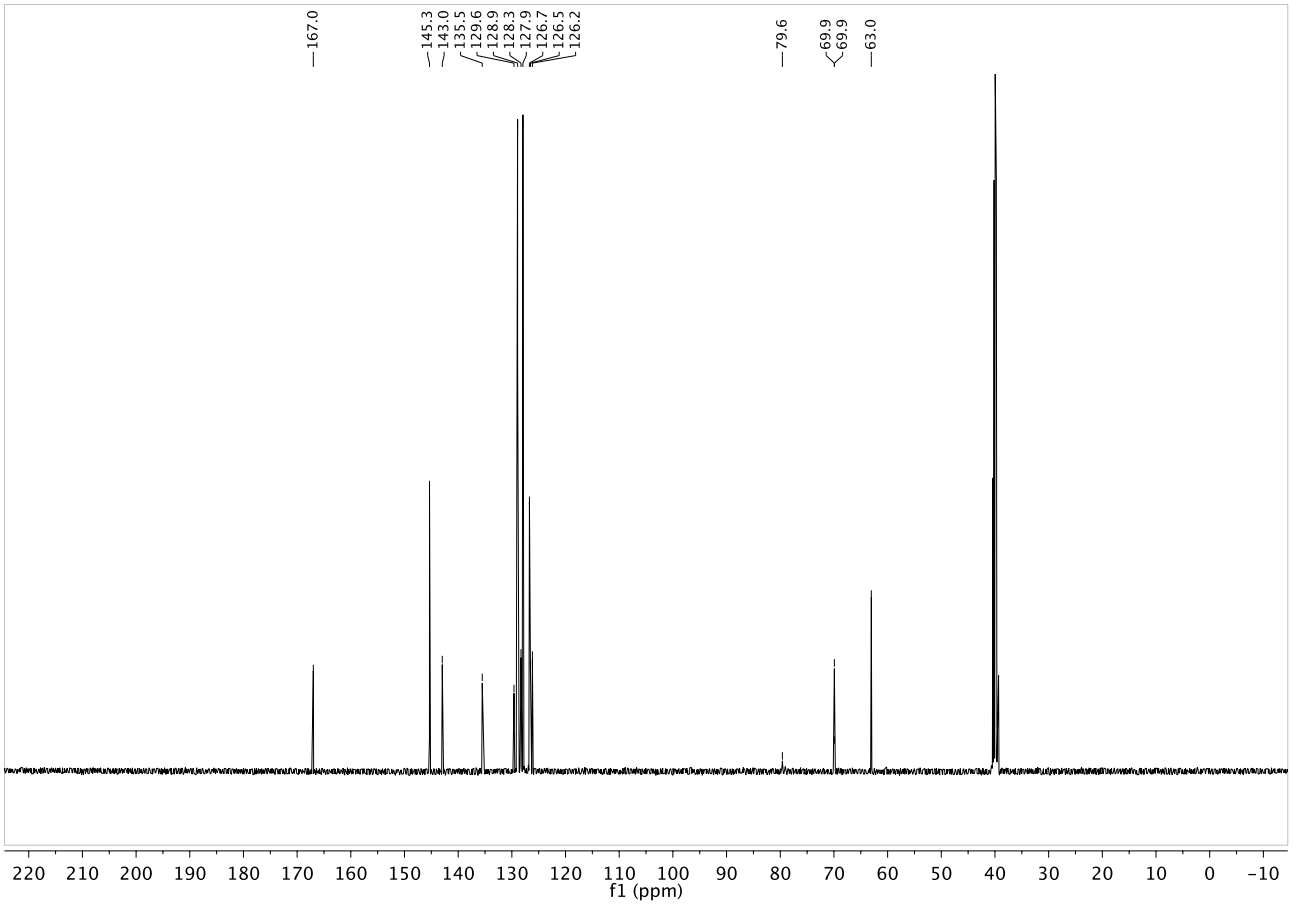
^{13}C NMR ($CDCl_3$)



Compound **5** ^{1}H NMR (DMSO-d_6)



^{13}C NMR (DMSO-d_6)



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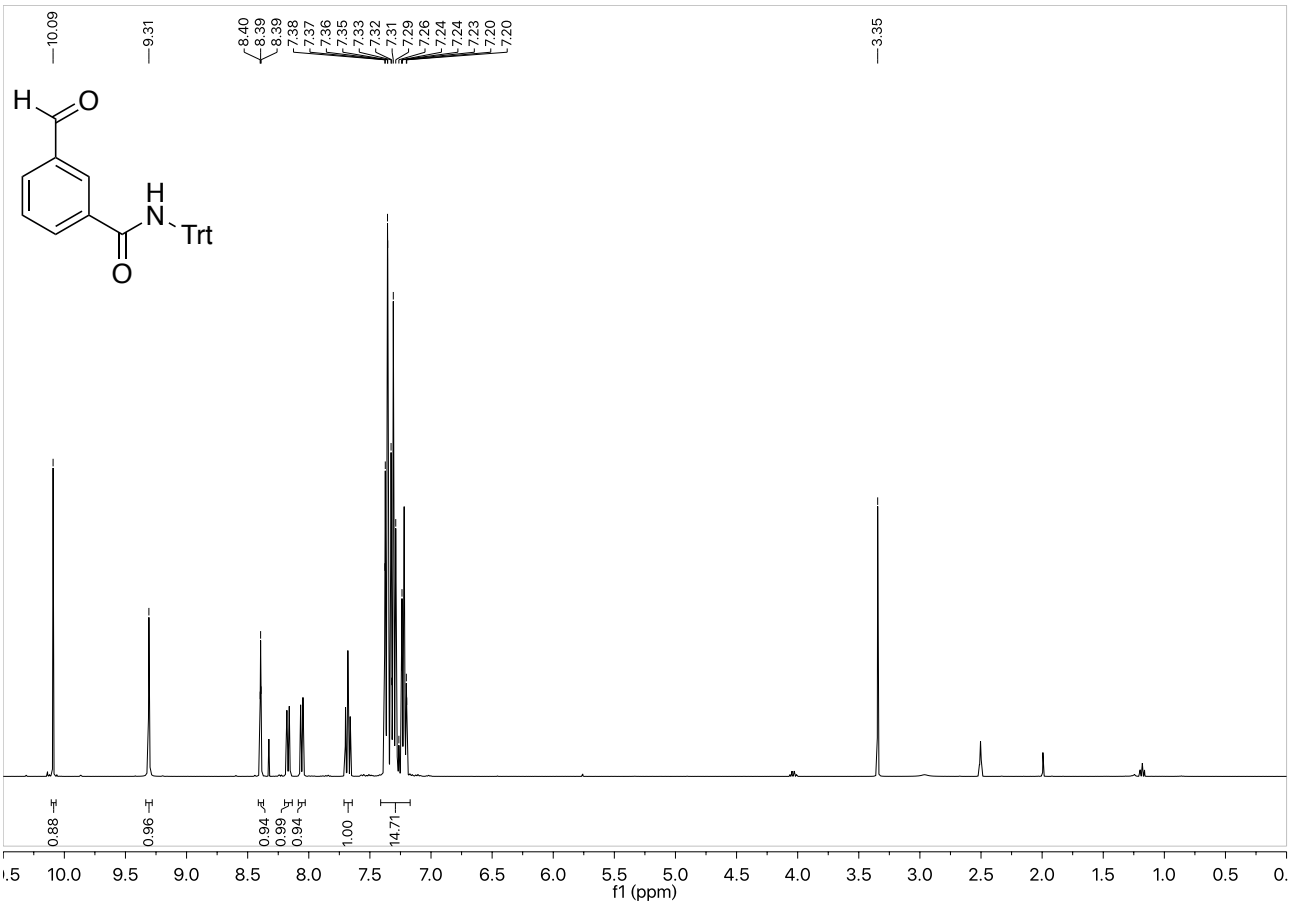
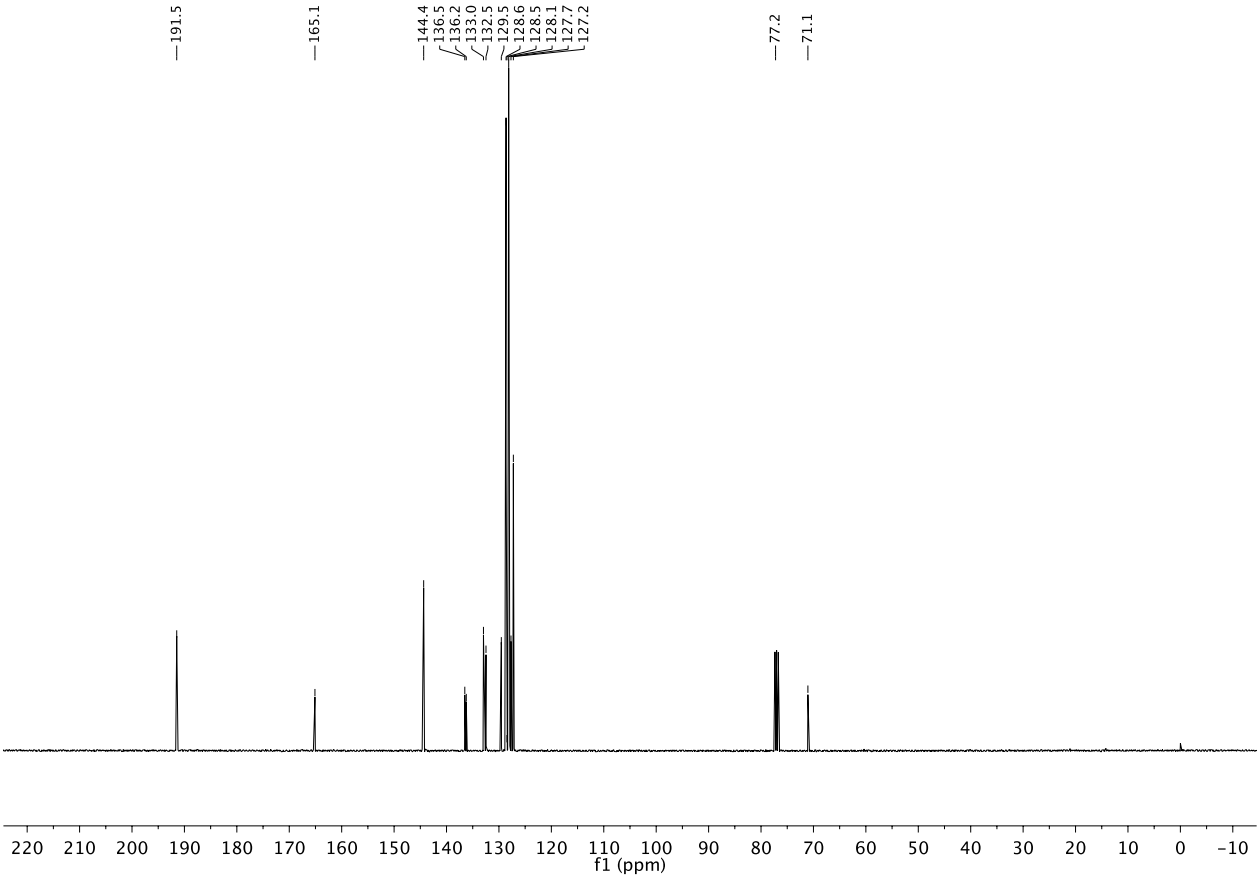
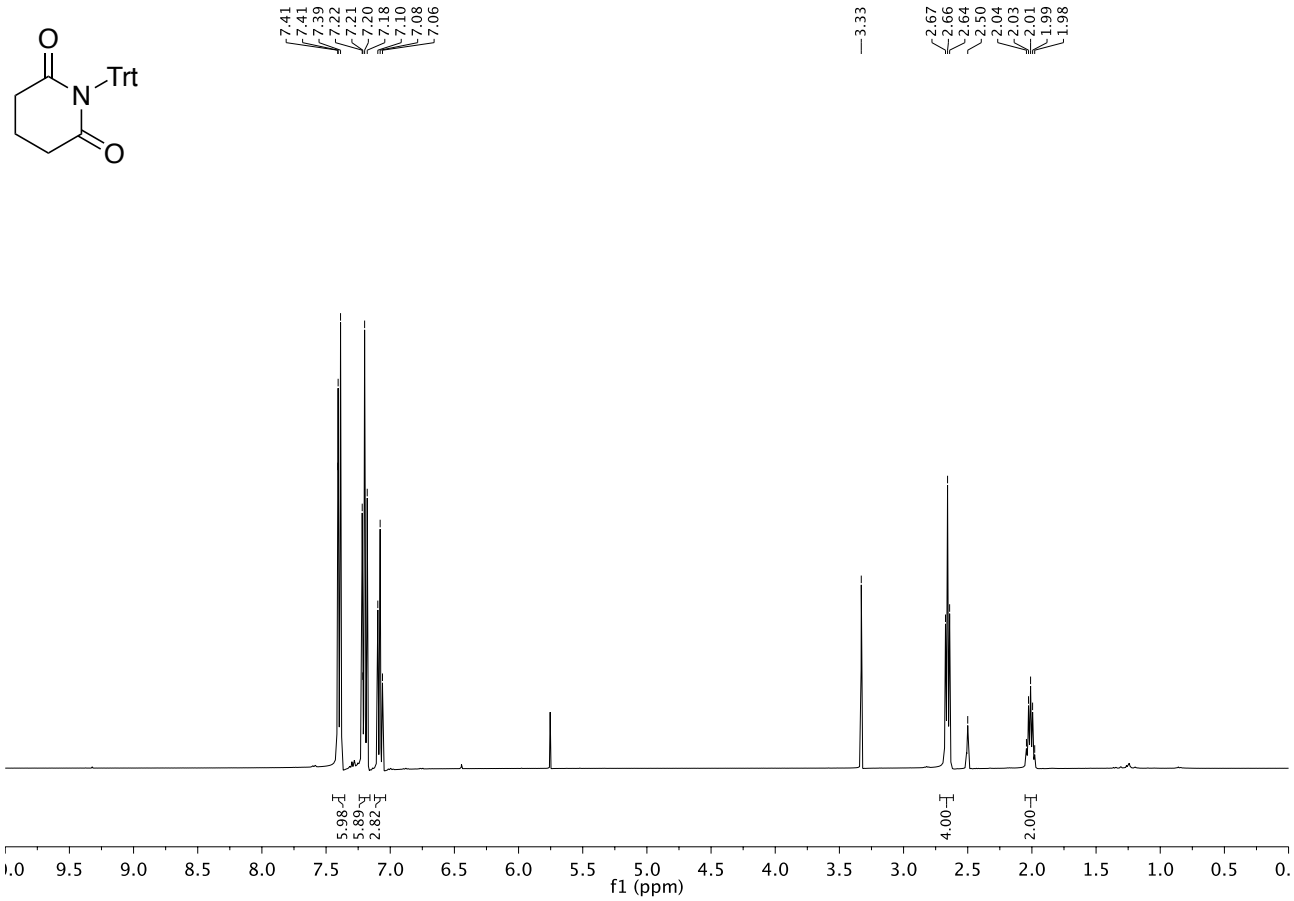


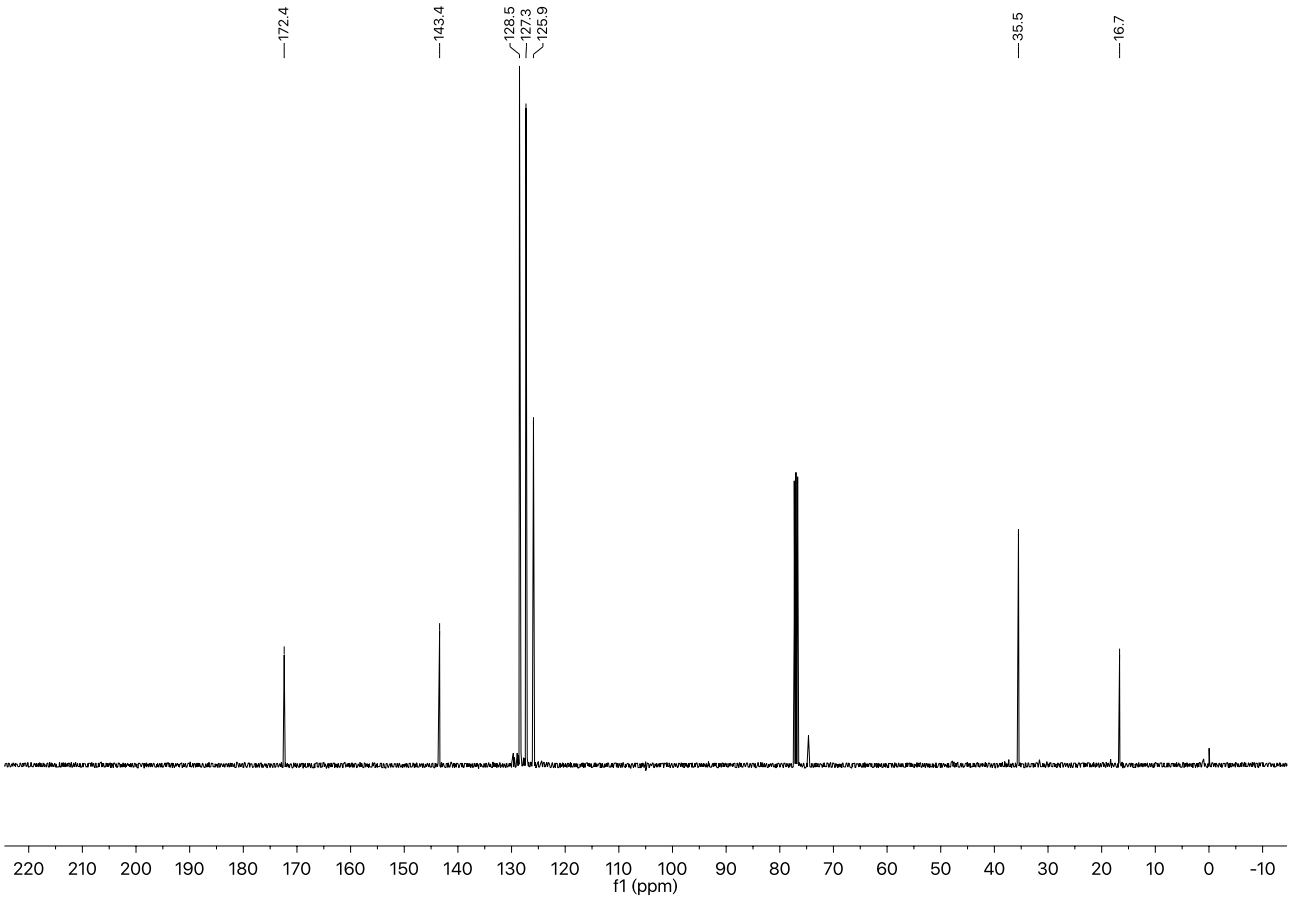
^{13}C NMR ($CDCl_3$)



Compound **11** ^{1}H NMR (DMSO-d_6)



^{13}C NMR ($CDCl_3$)



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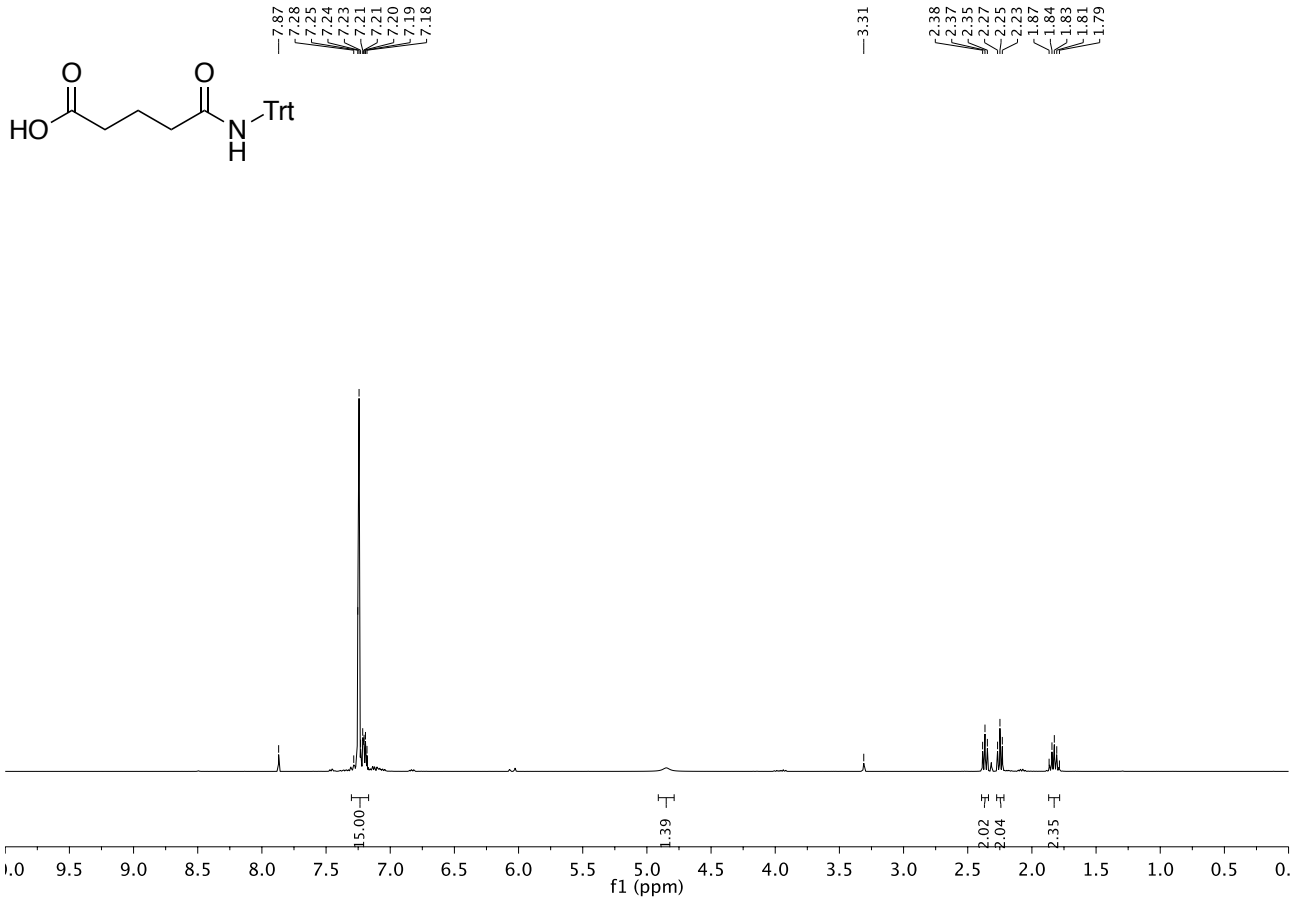
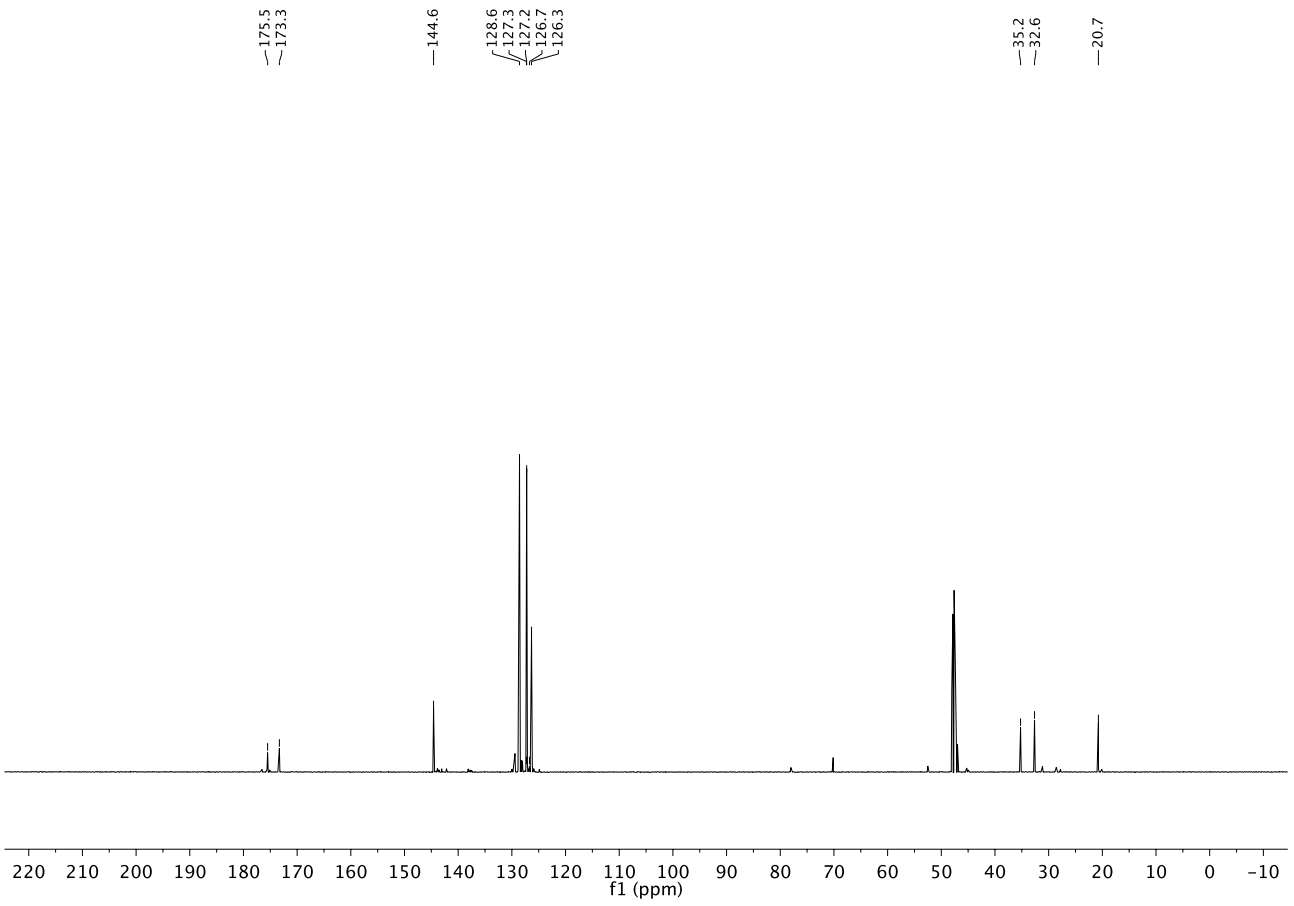


^{13}C NMR (Methanol-d_4)



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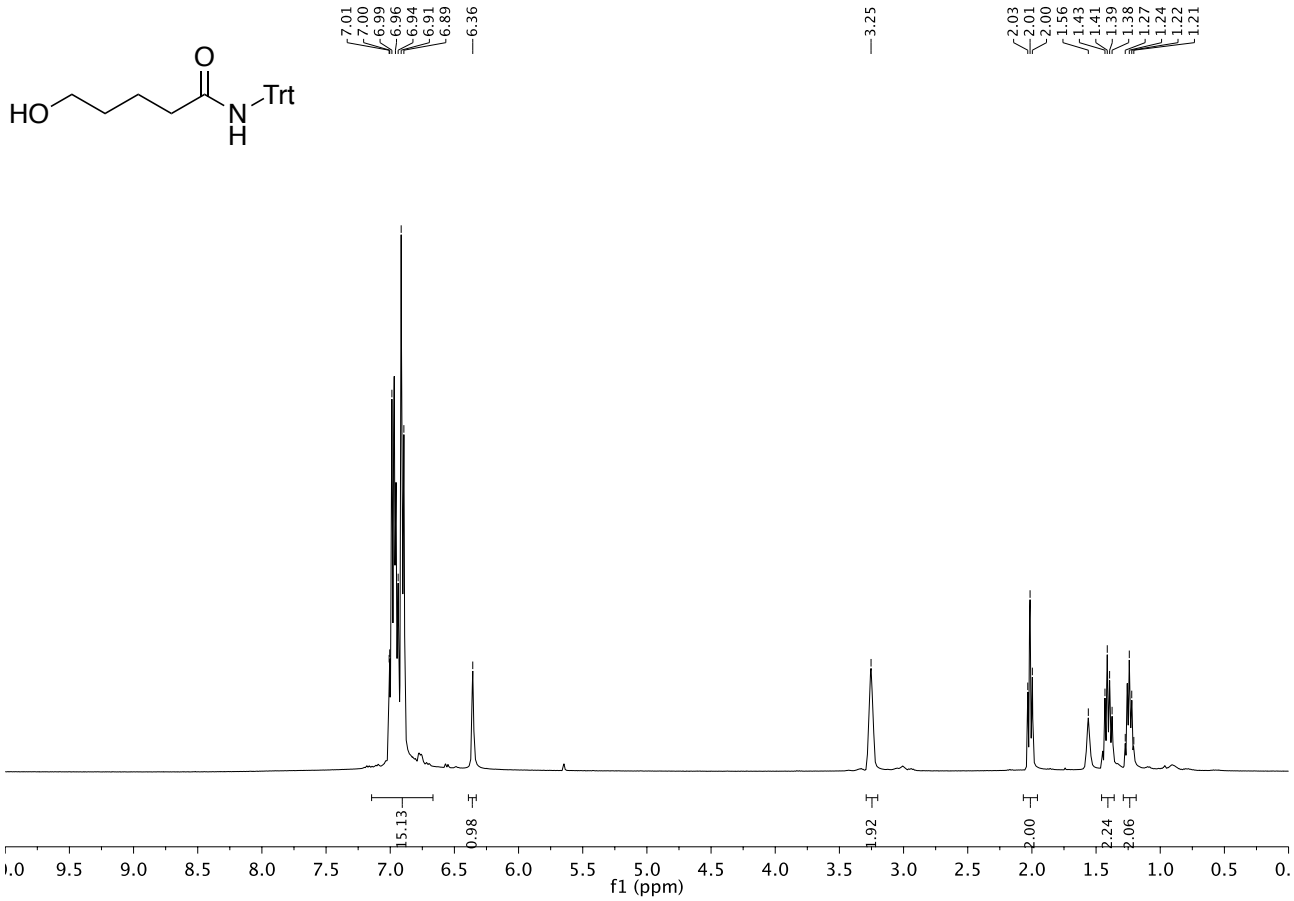
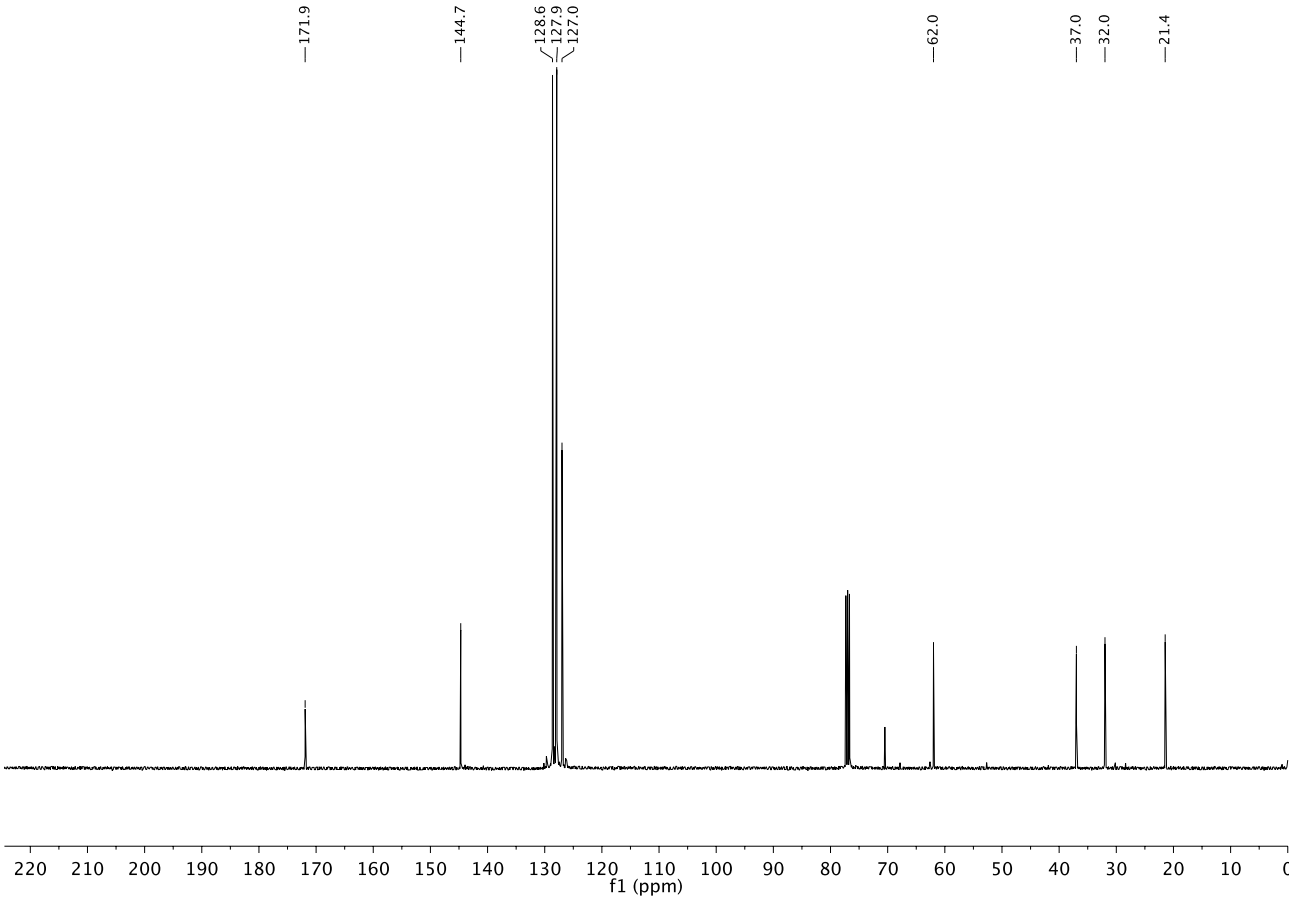


^{13}C NMR ($CDCl_3$)



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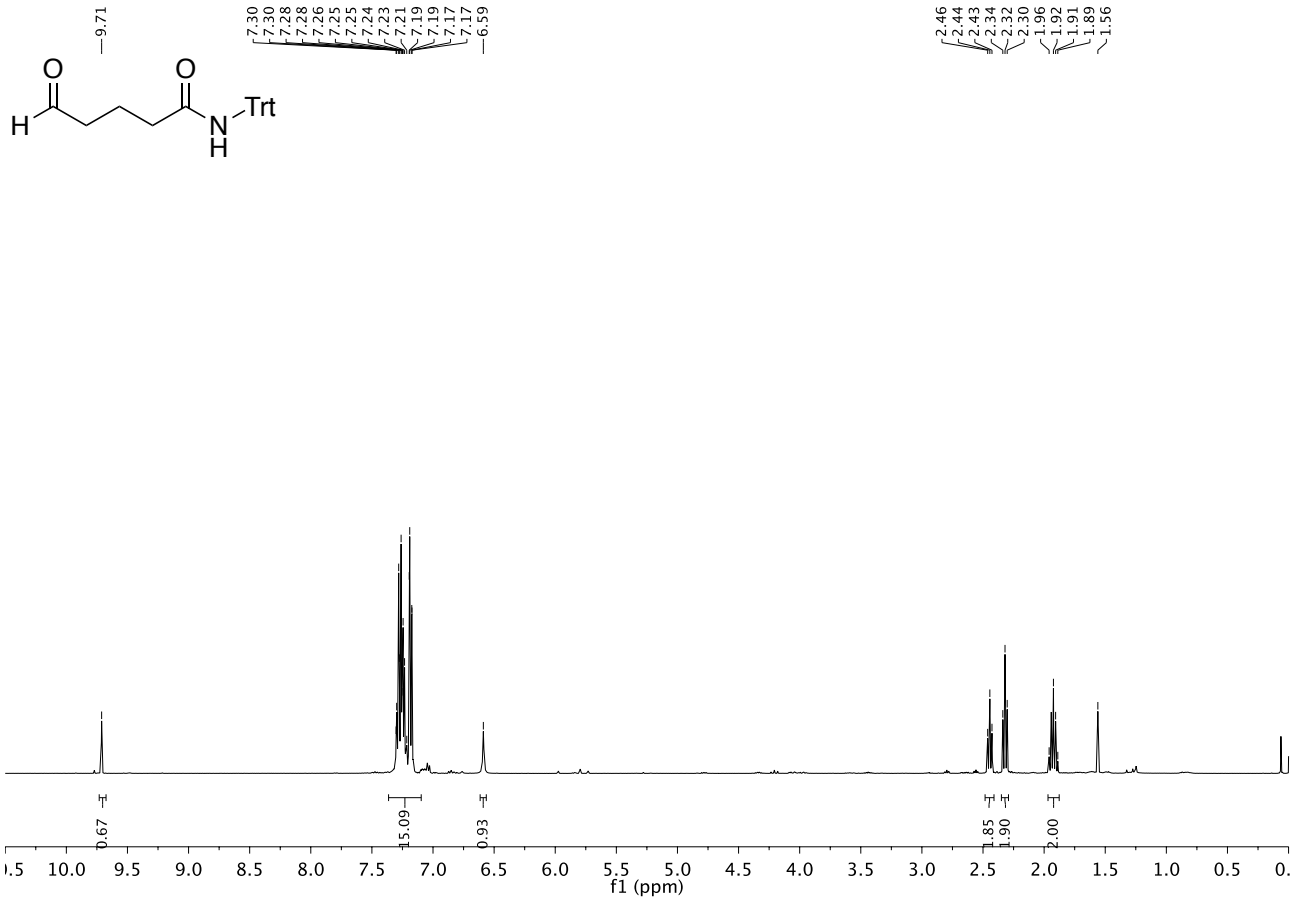
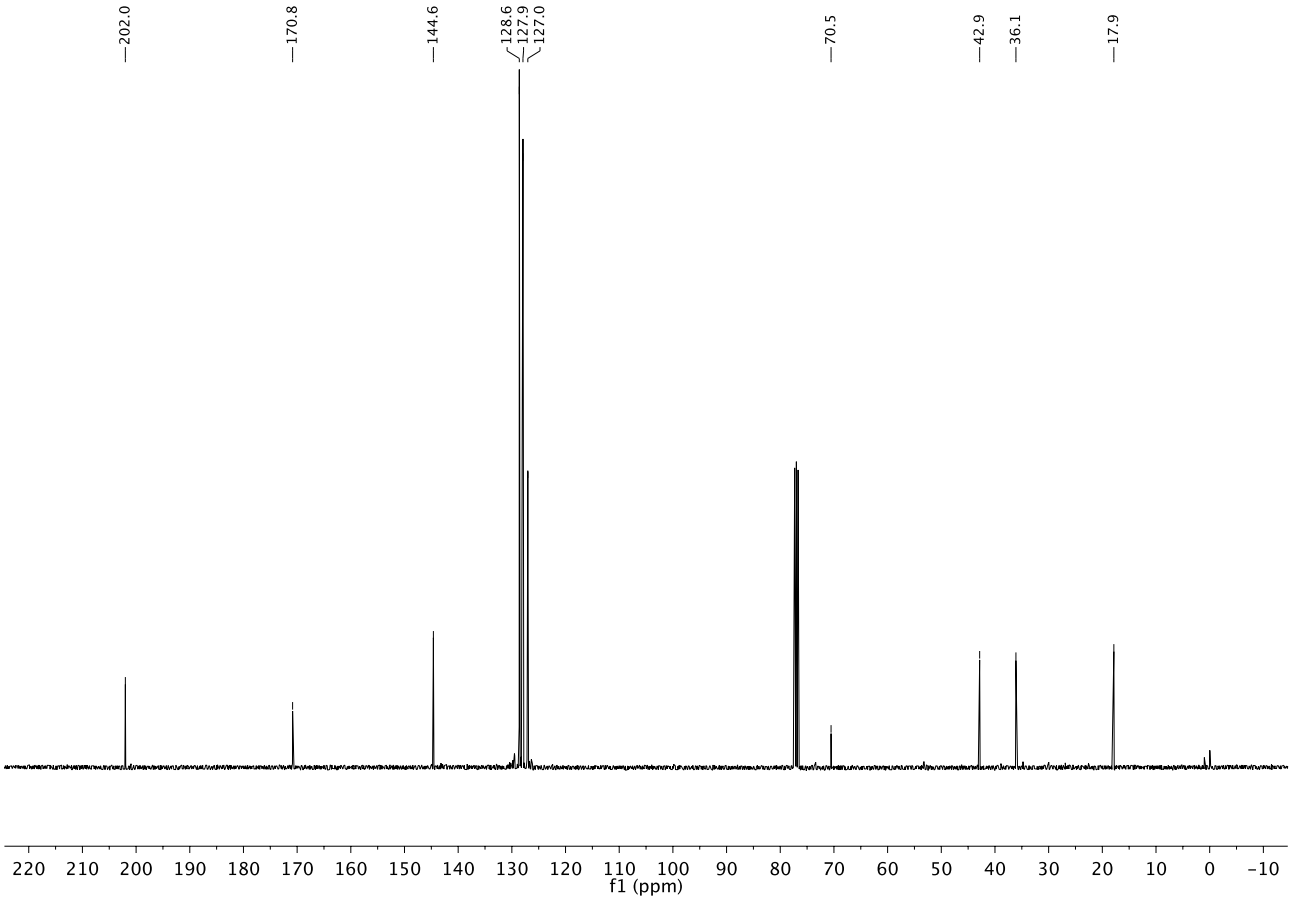
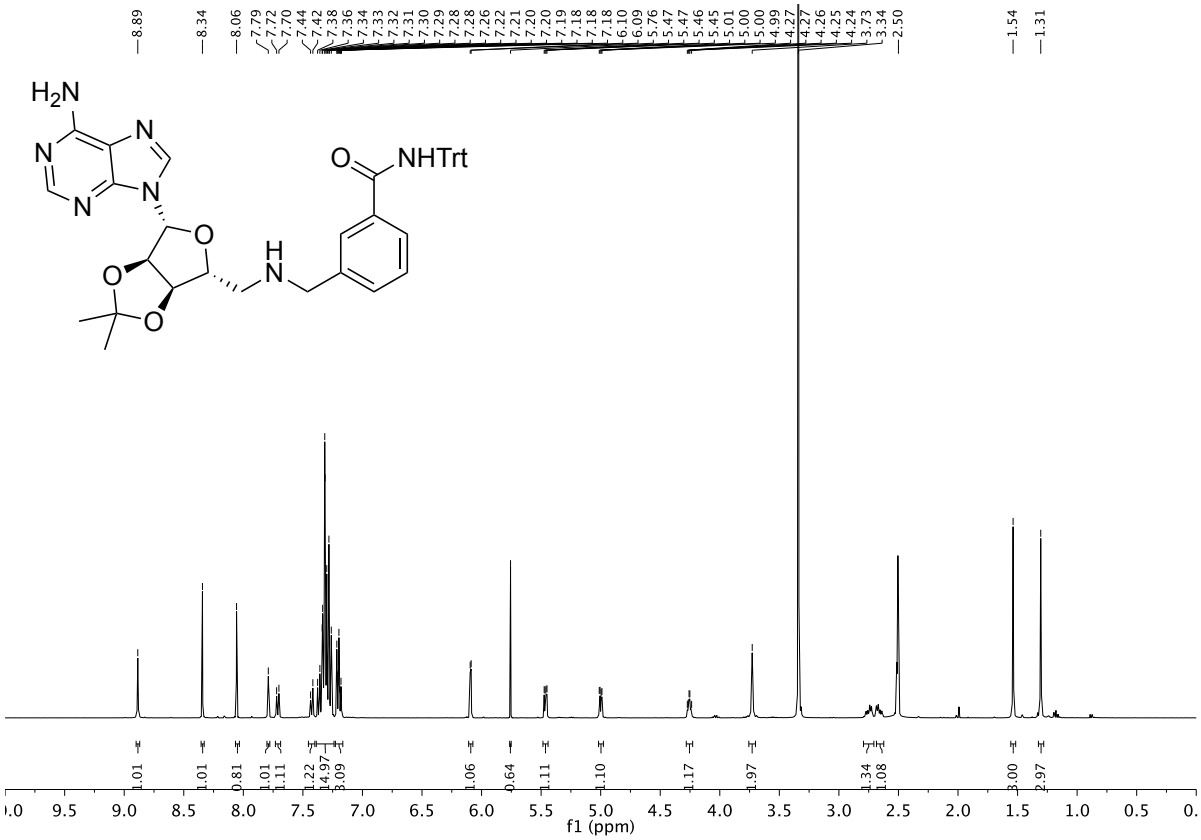


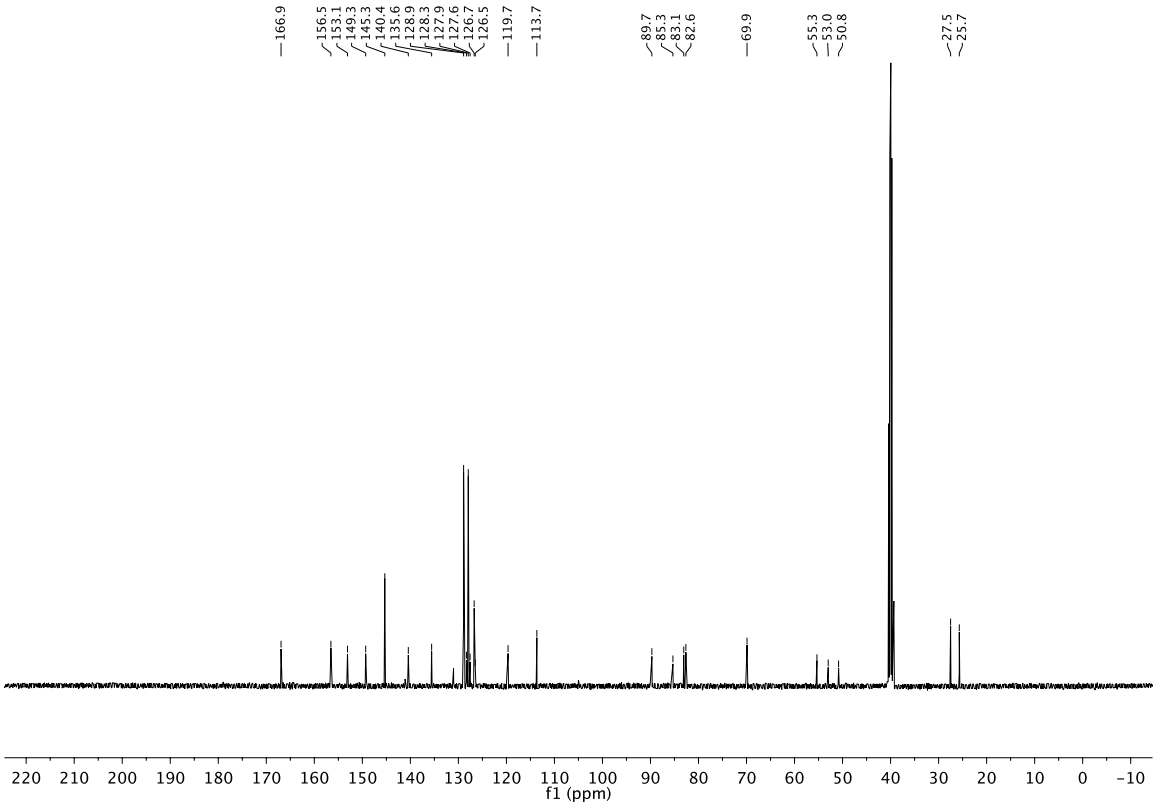
^{13}C NMR ($CDCl_3$)



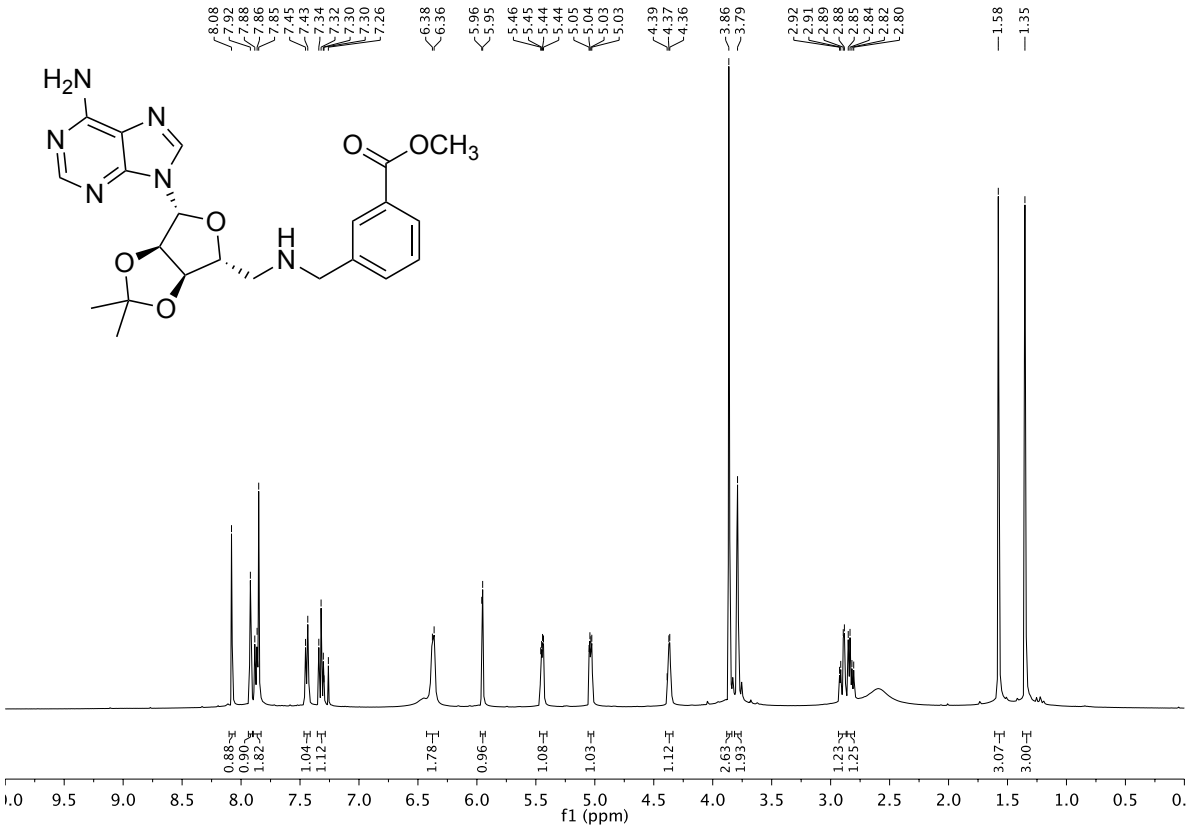
Compound **29** ^{1}H NMR (DMSO-d_6)



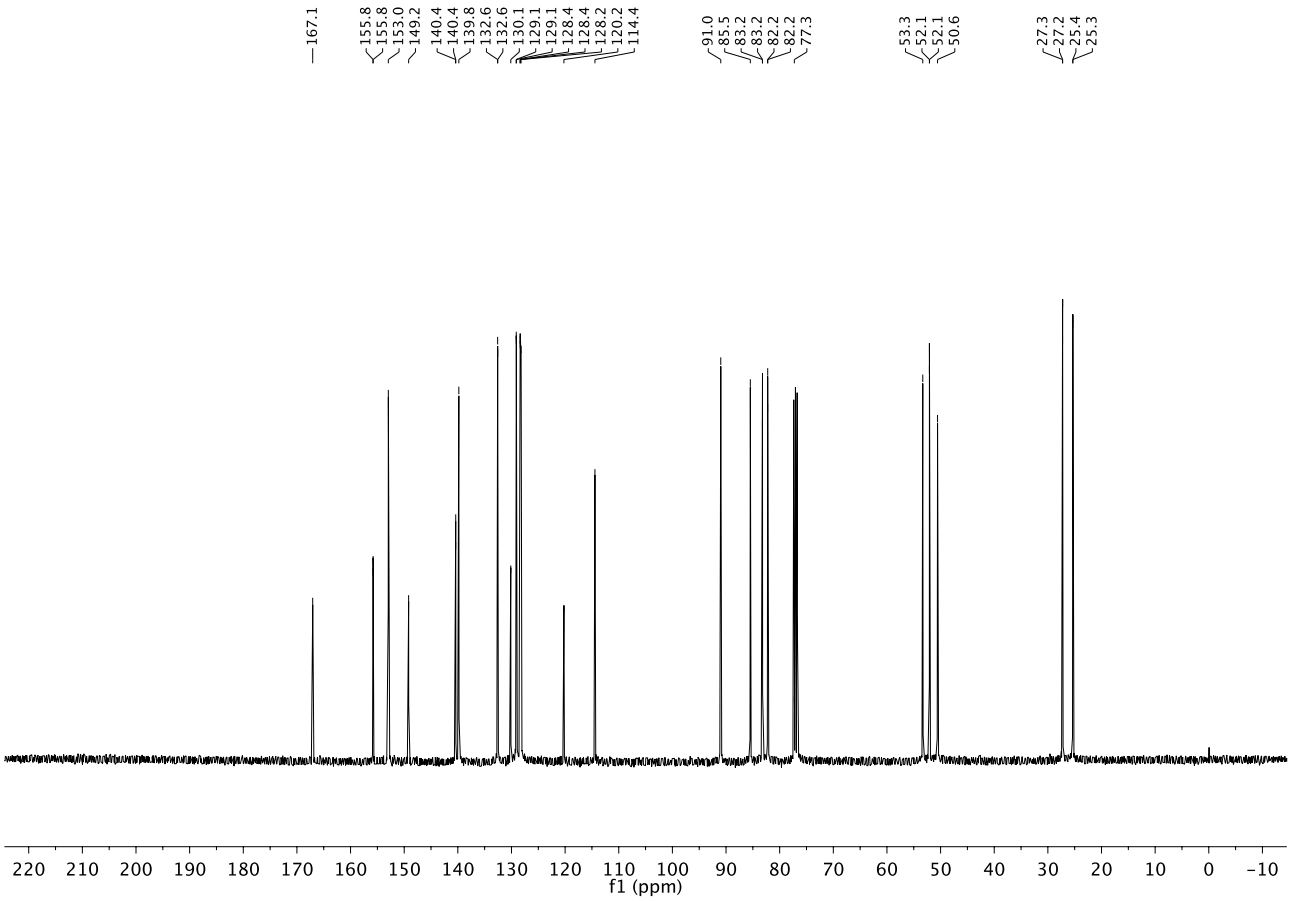
^{13}C NMR (DMSO-d_6)



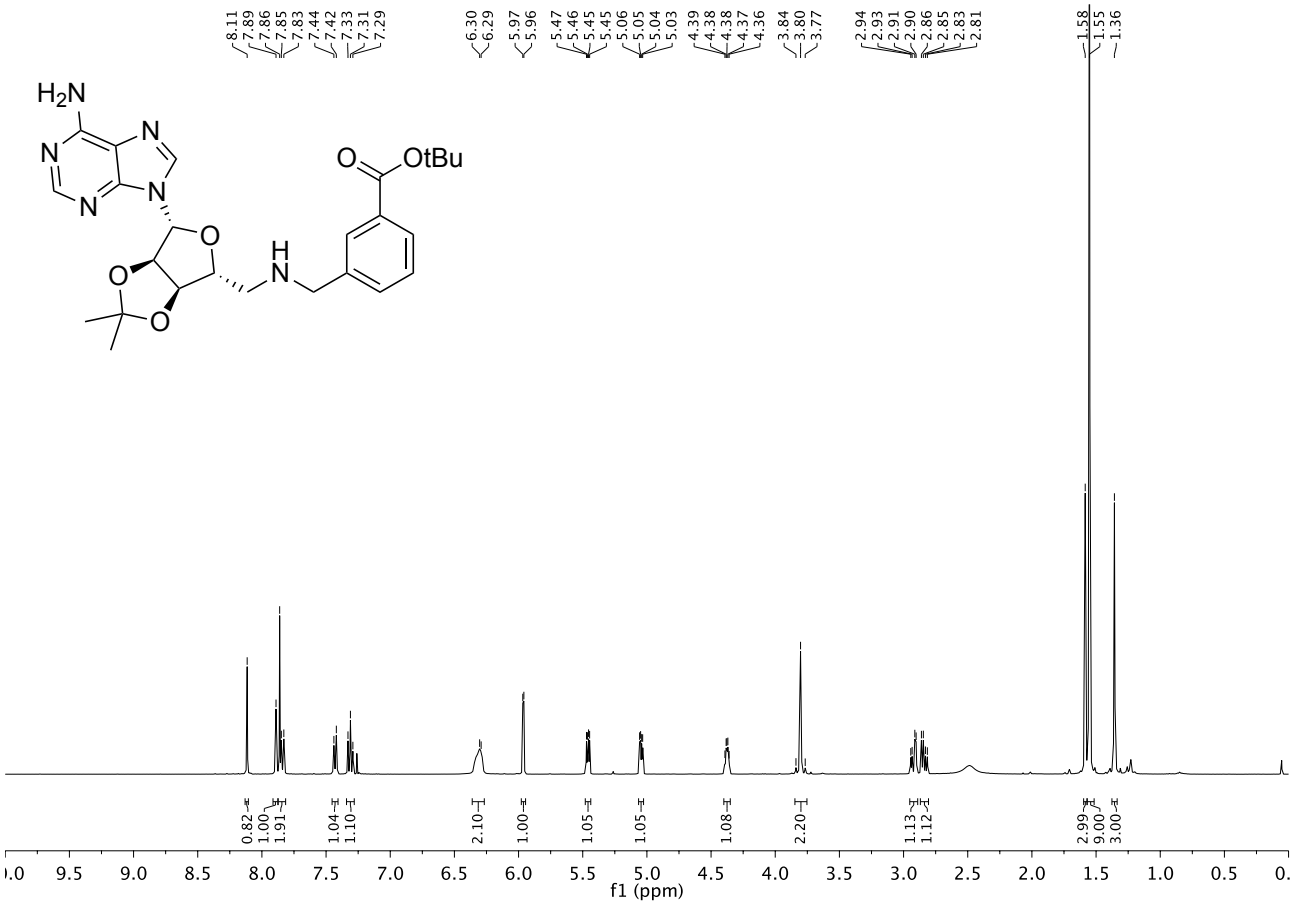
Compound **30** ^{1}H NMR ($CDCl_3$)



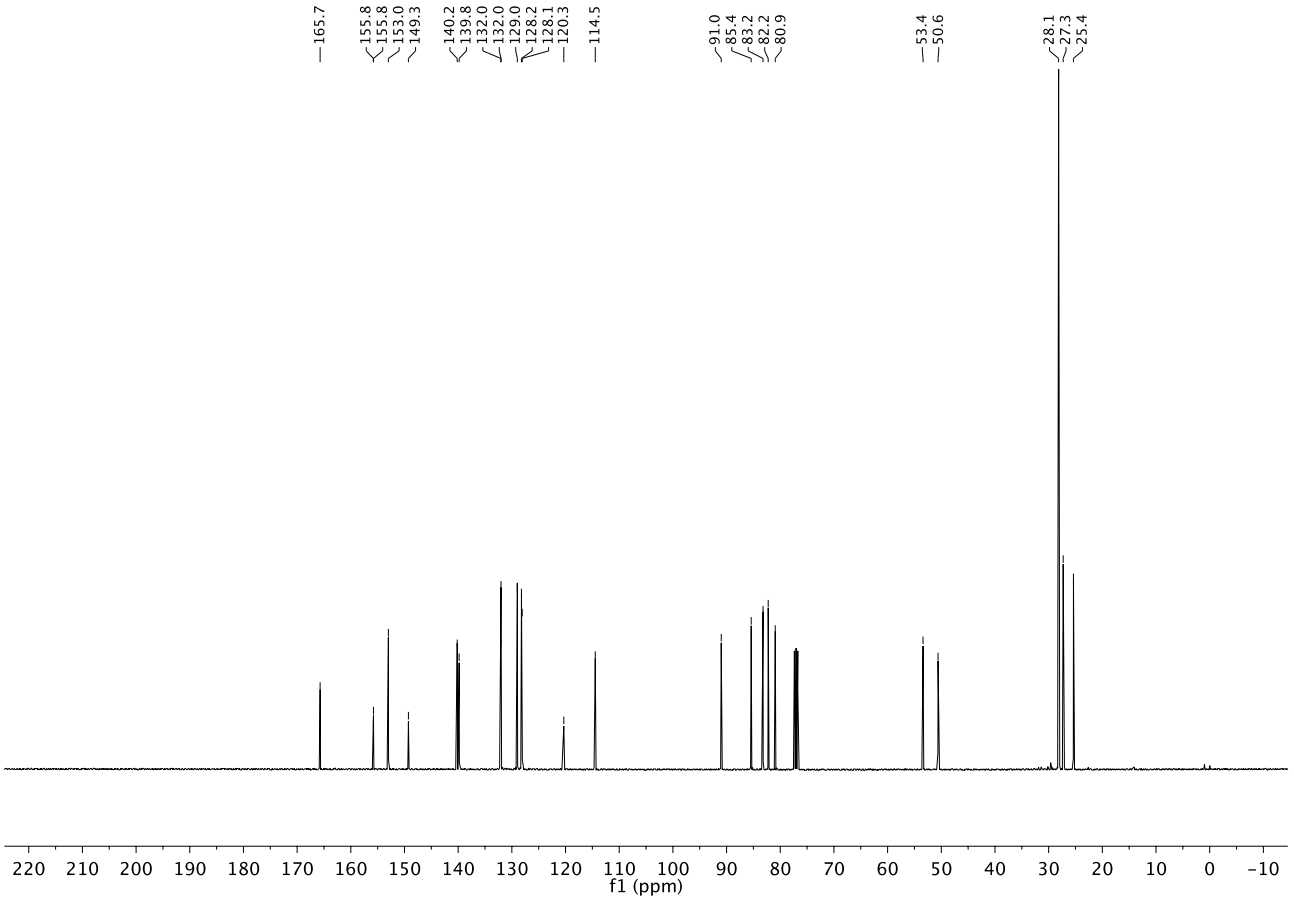
^{13}C NMR ($CDCl_3$)



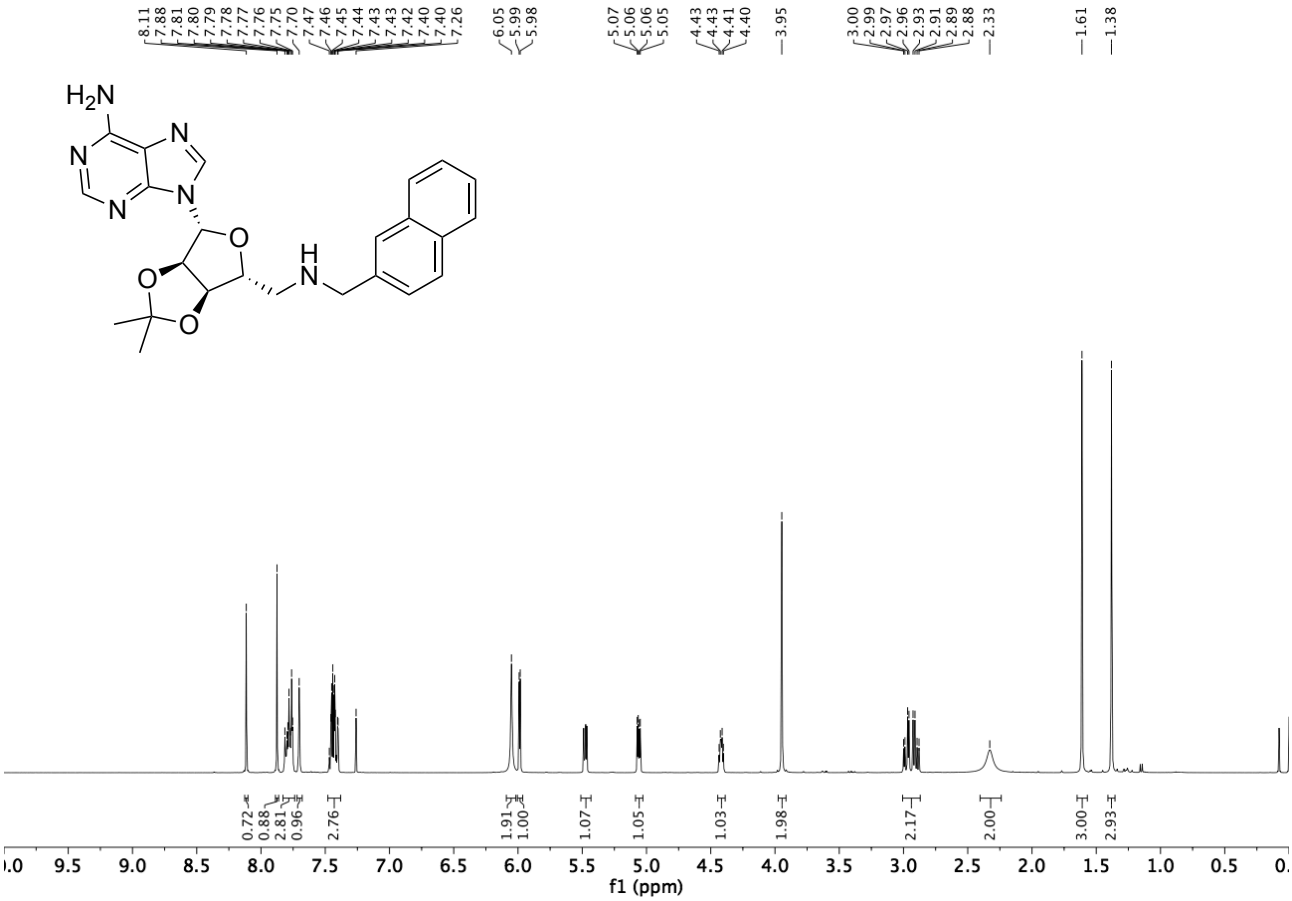
Compound **31** ^{1}H NMR ($CDCl_3$)



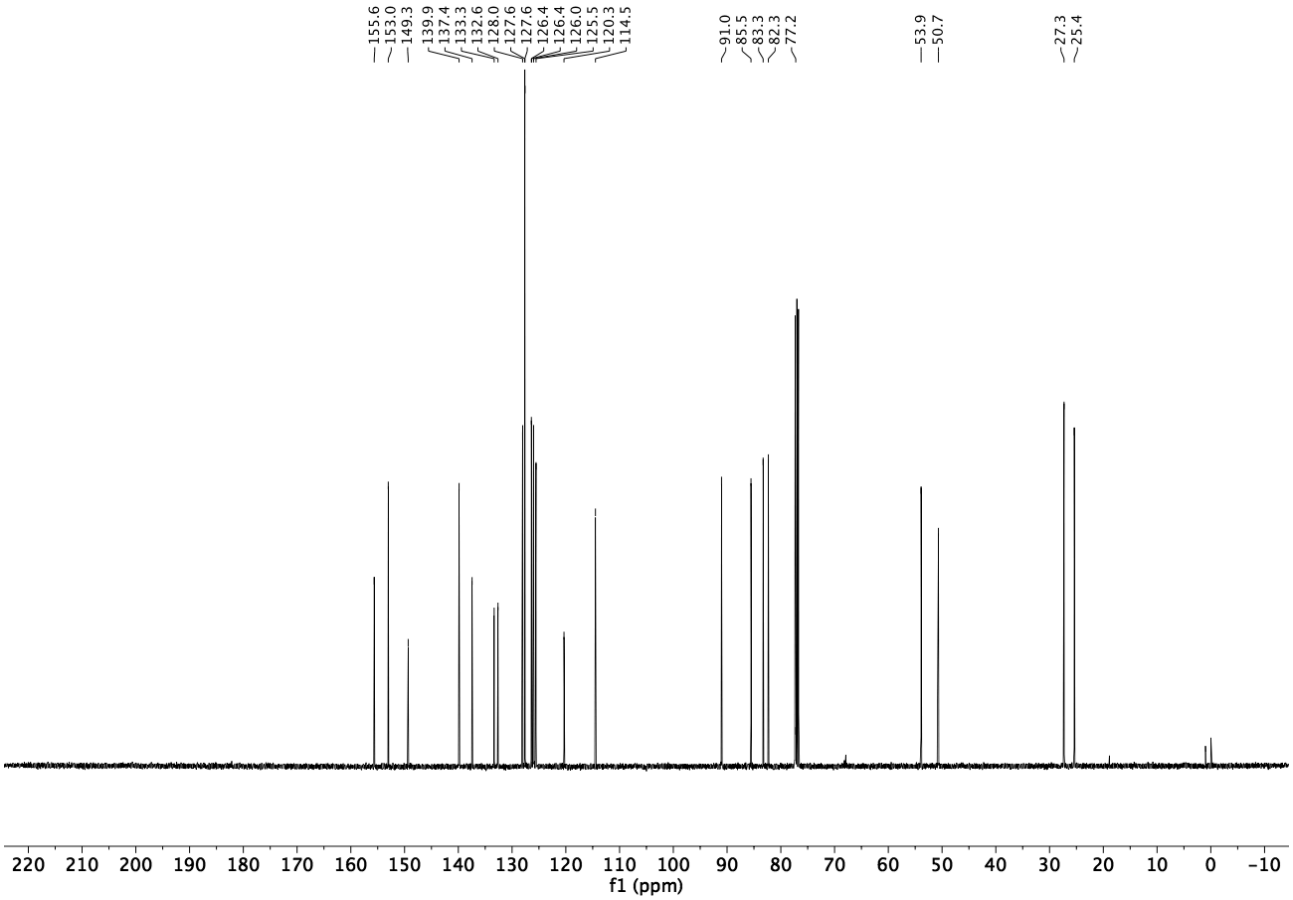
^{13}C NMR ($CDCl_3$)



Compound **32** ^{1}H NMR ($CDCl_3$)



^{13}C NMR ($CDCl_3$)



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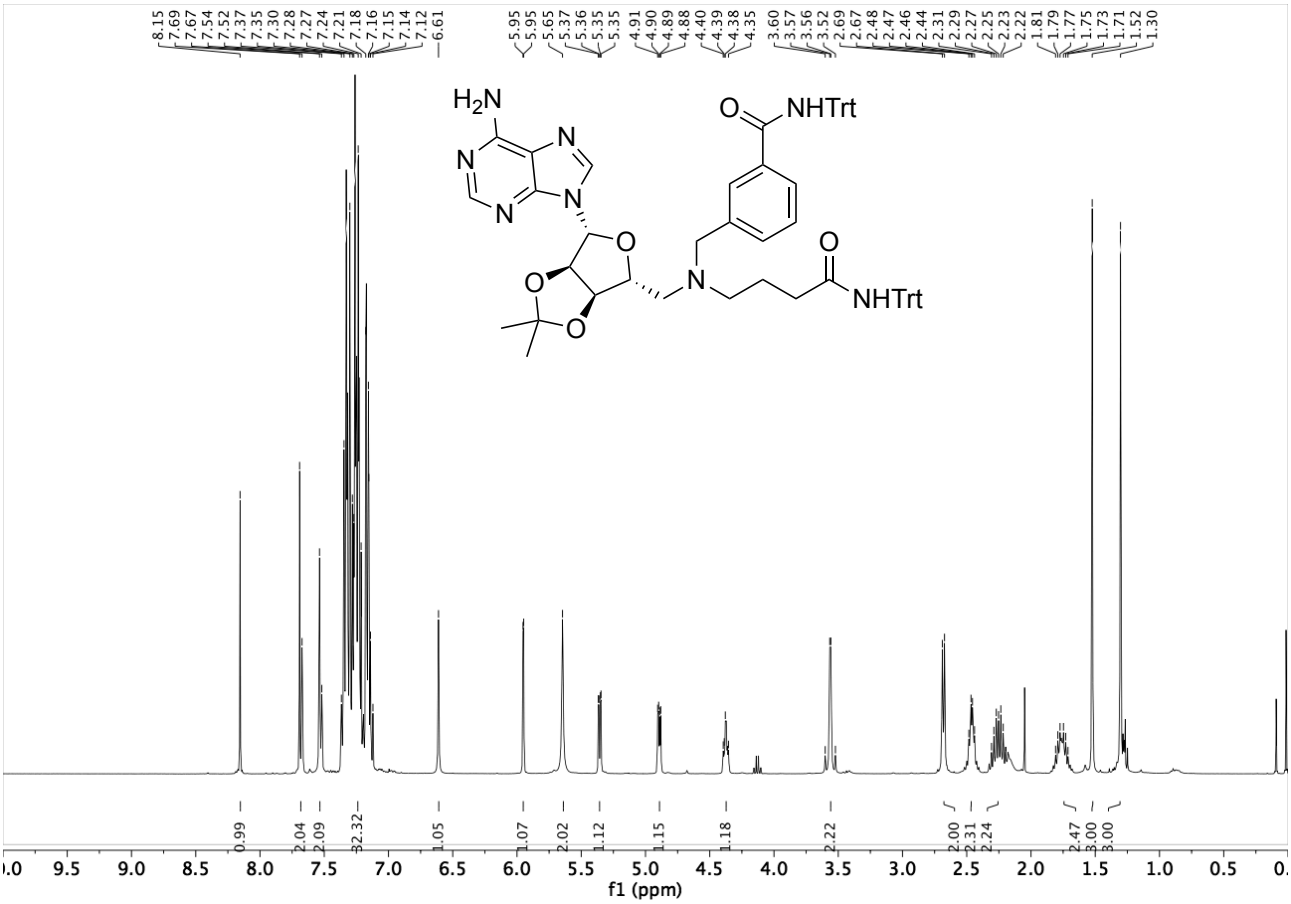
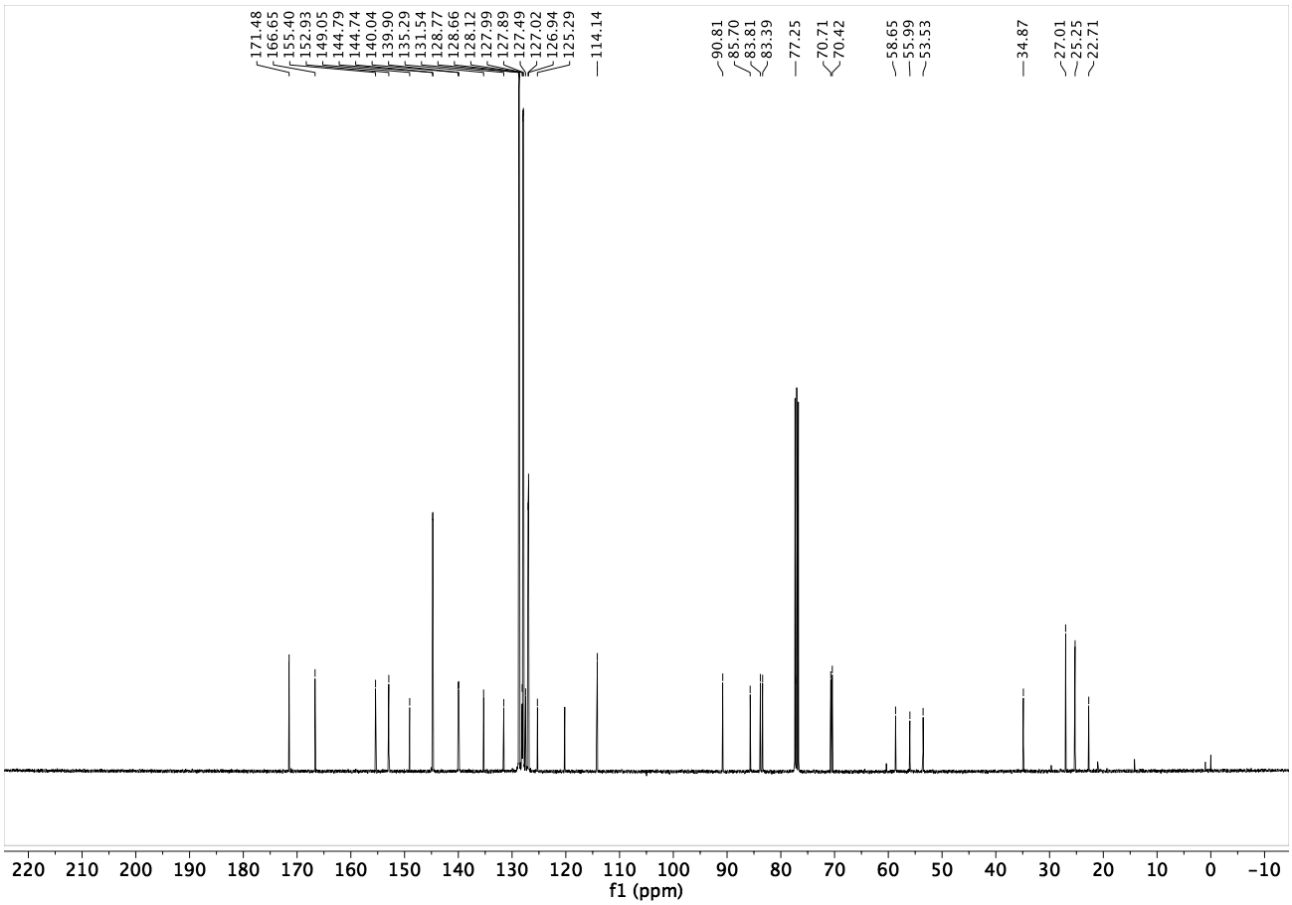
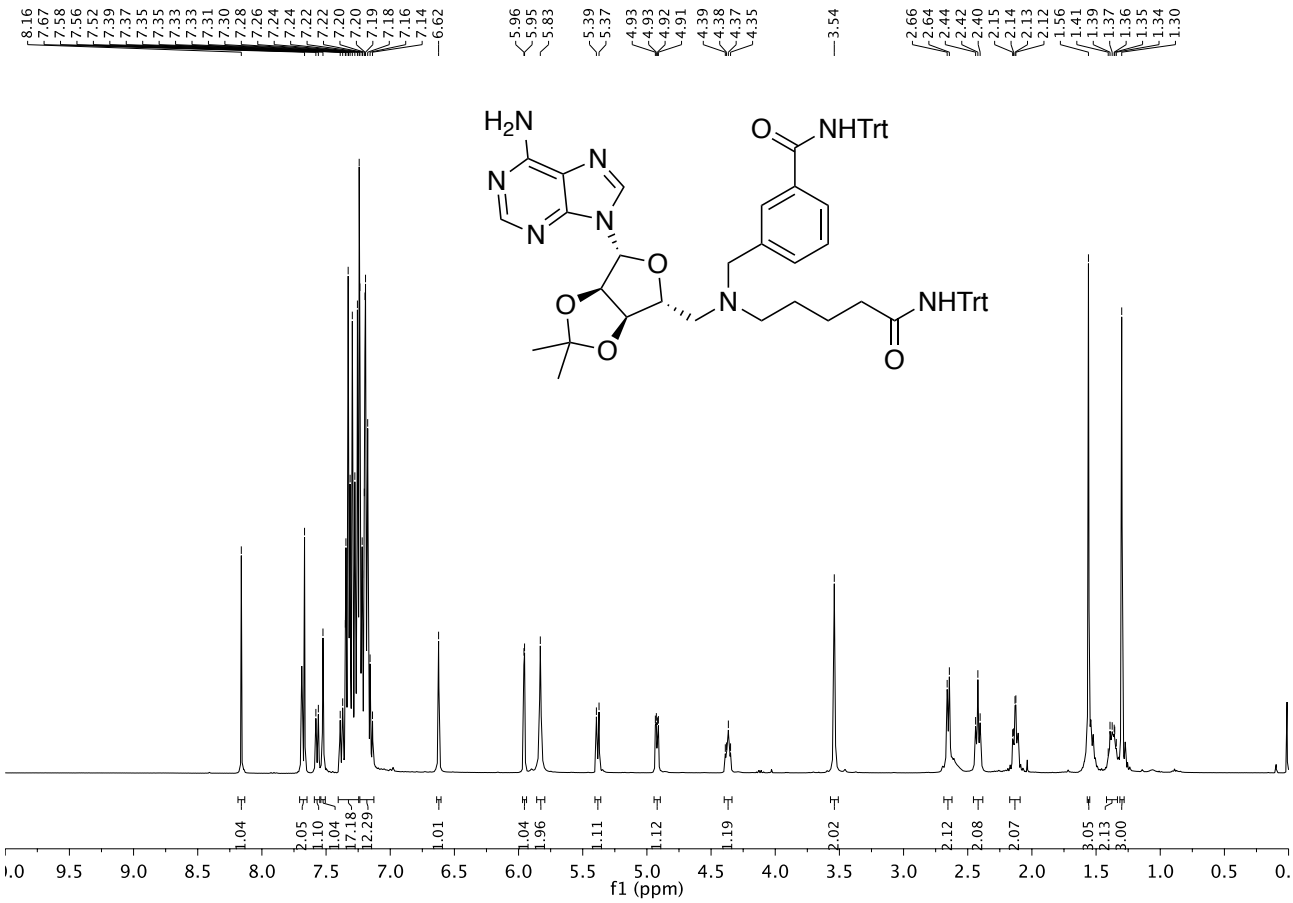


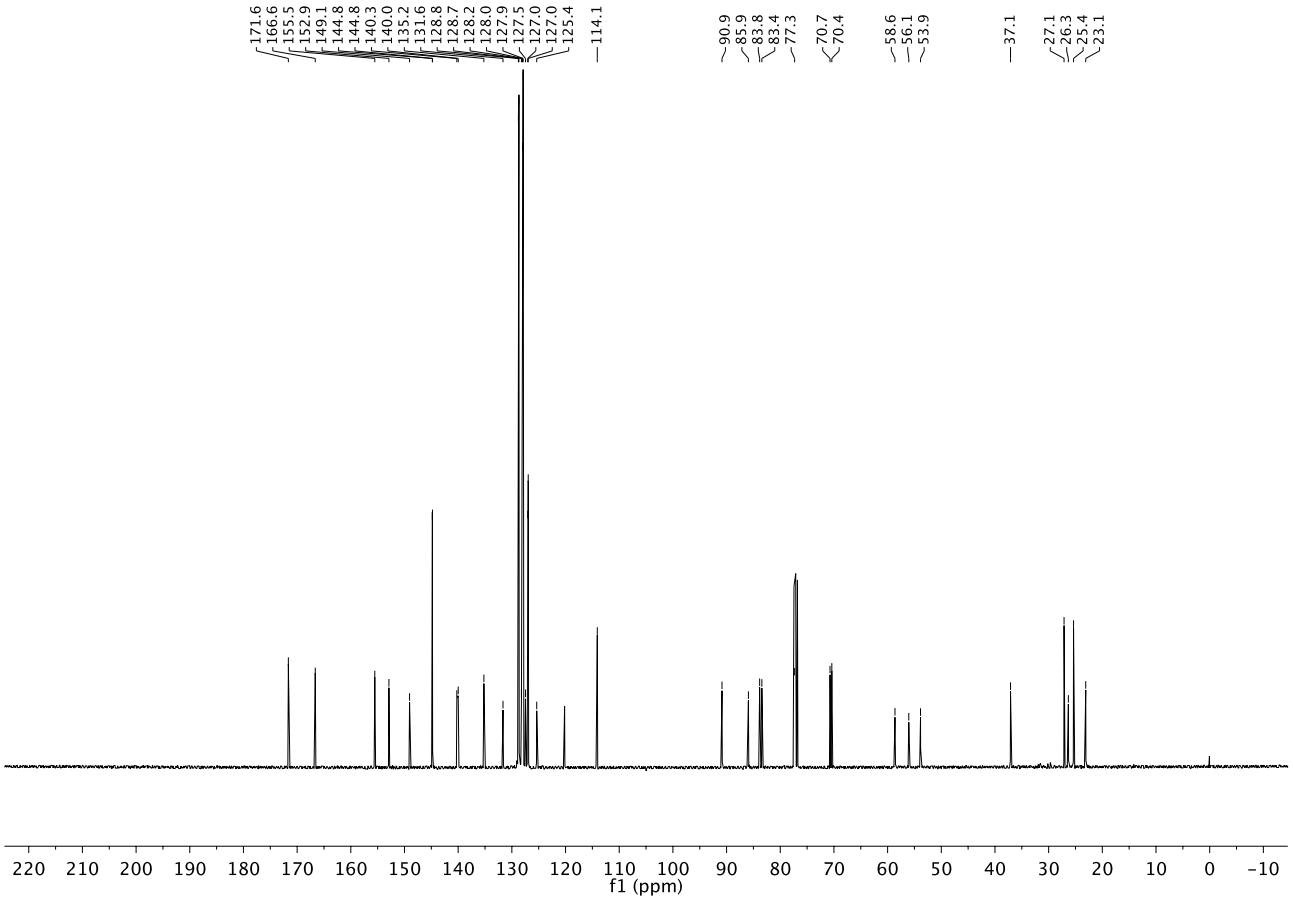
^{13}C NMR ($CDCl_3$)



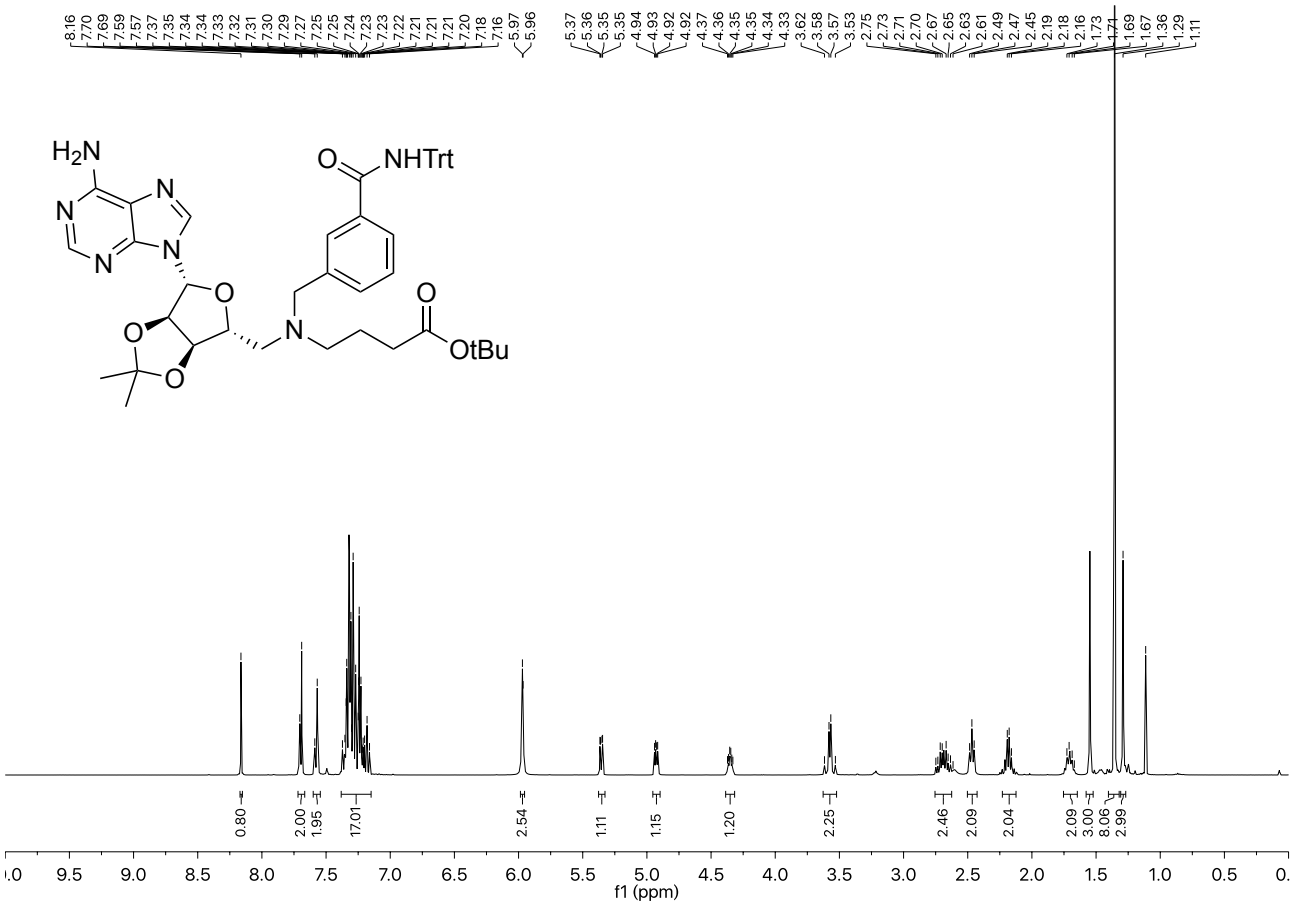
Compound **34** ^{1}H NMR ($CDCl_3$)



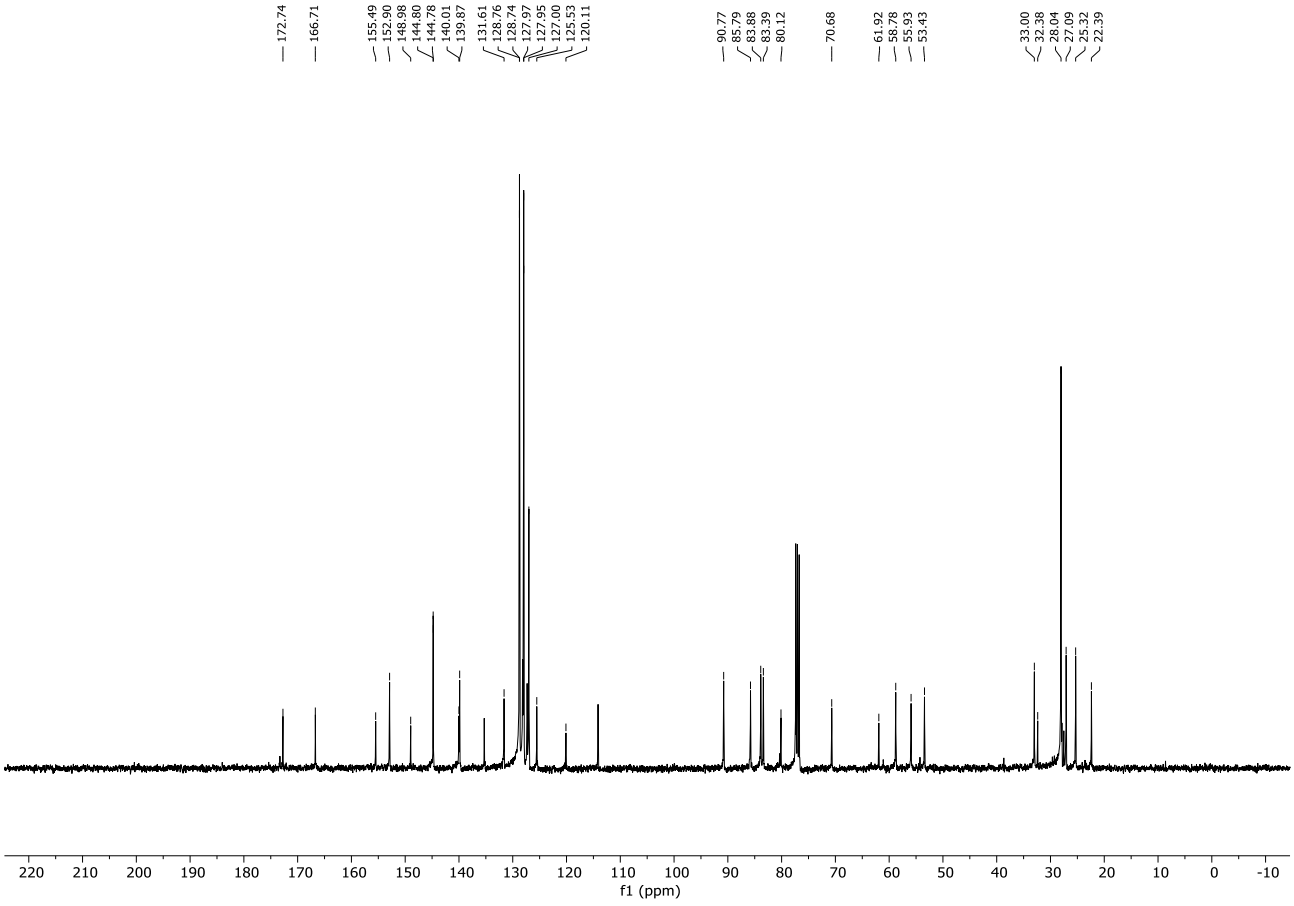
^{13}C NMR ($CDCl_3$)



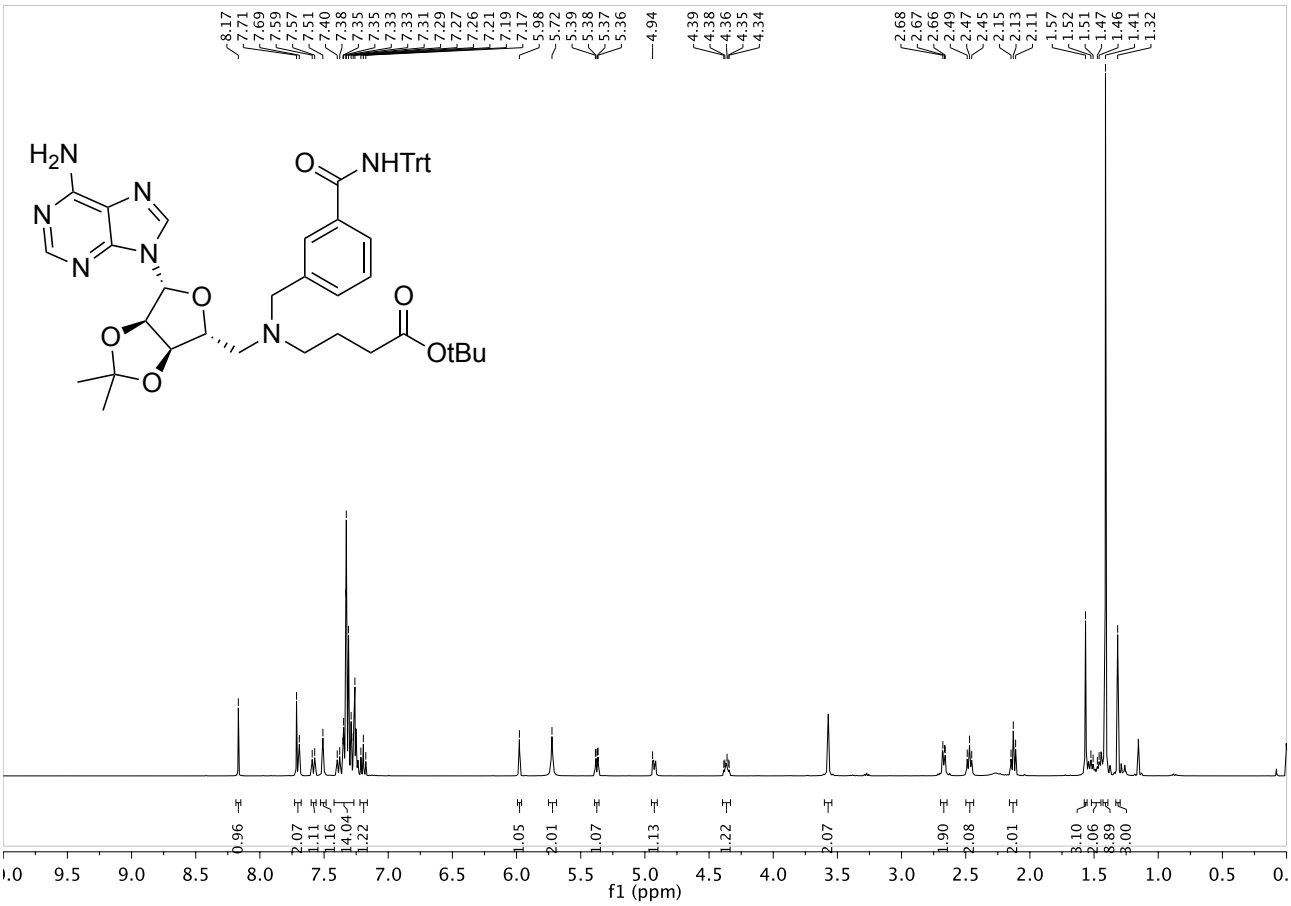
Compound **35** ^{1}H NMR ($CDCl_3$)



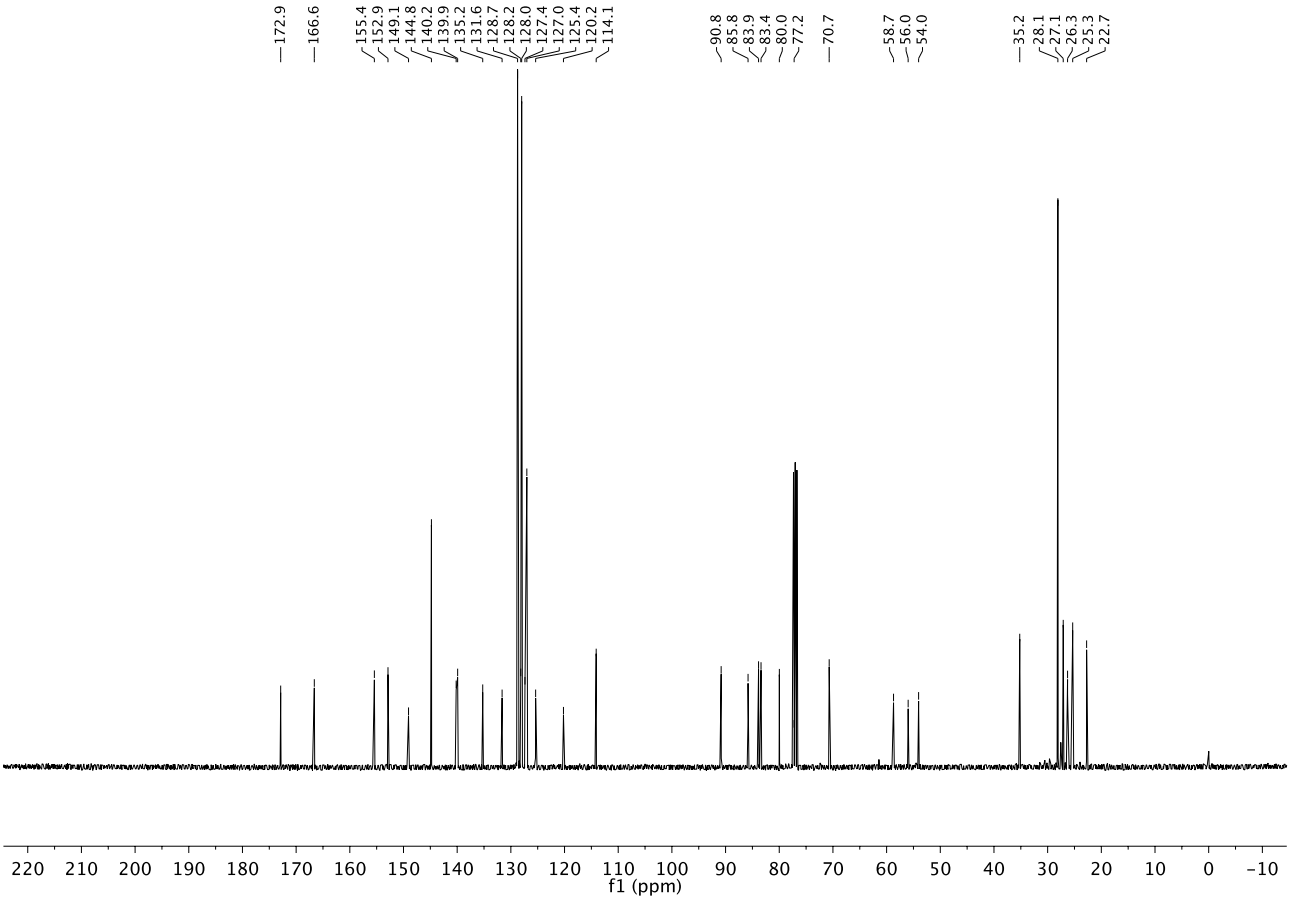
^{13}C NMR ($CDCl_3$)



Compound **36** ^{1}H NMR ($CDCl_3$)



^{13}C NMR ($CDCl_3$)



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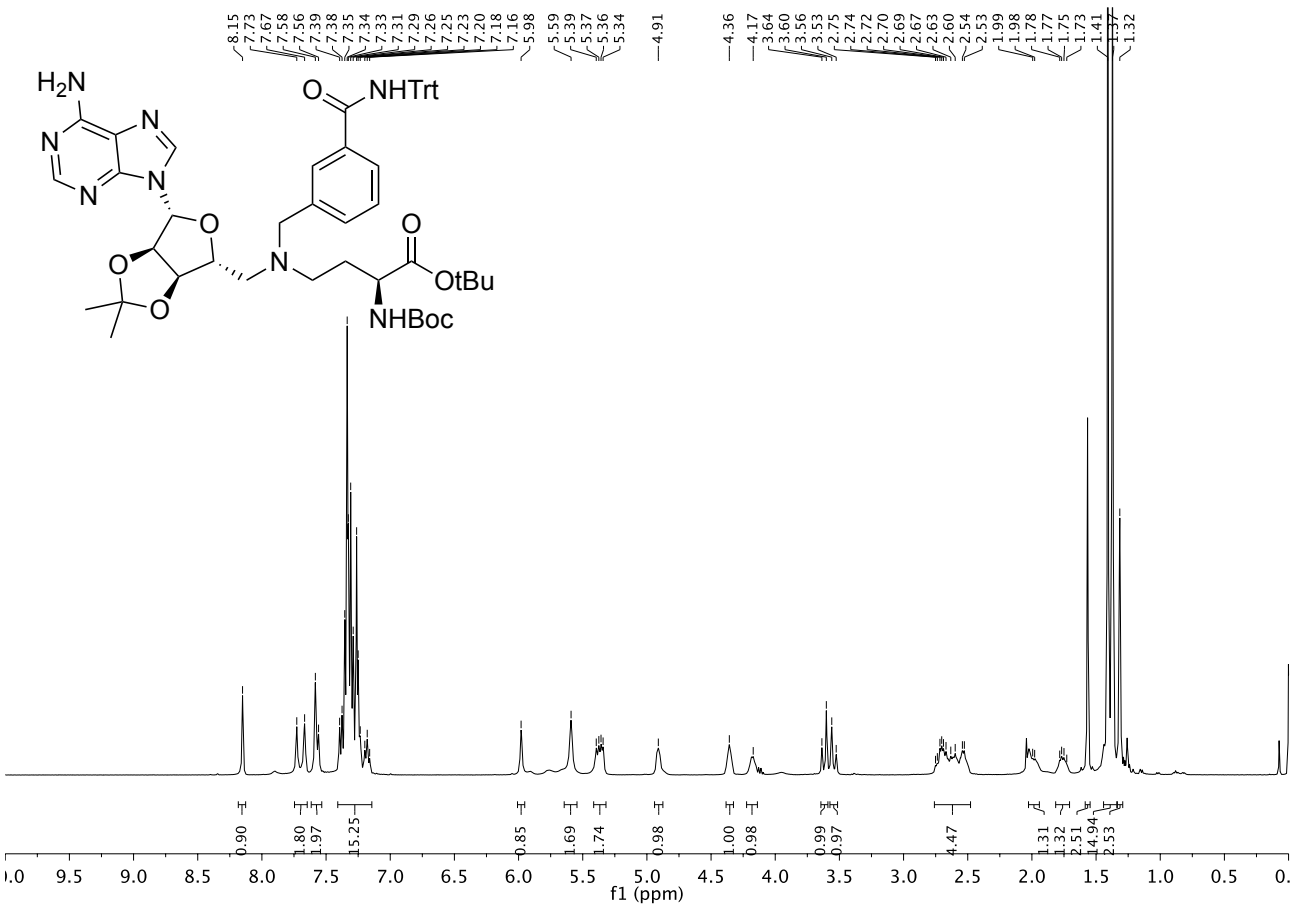

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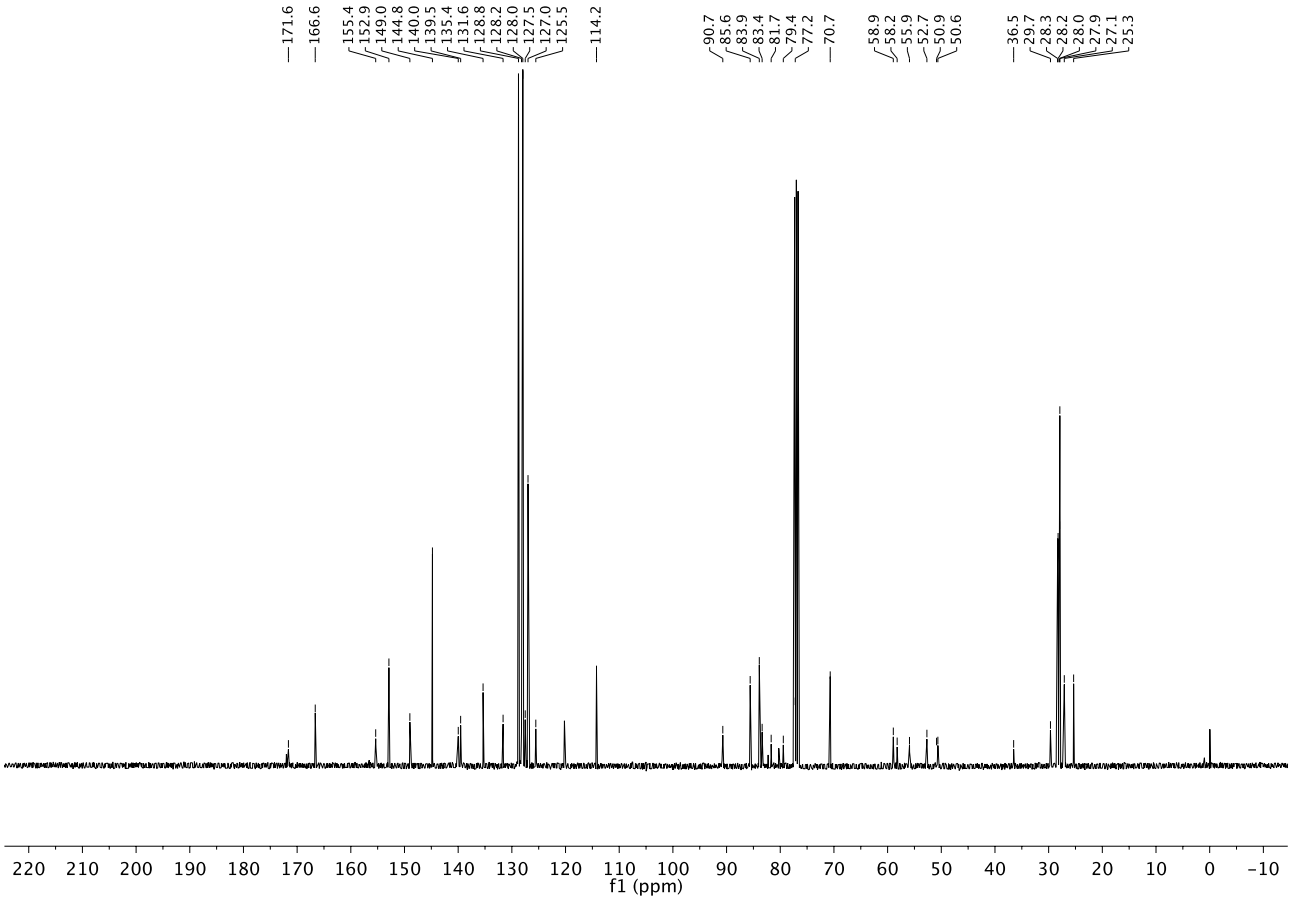

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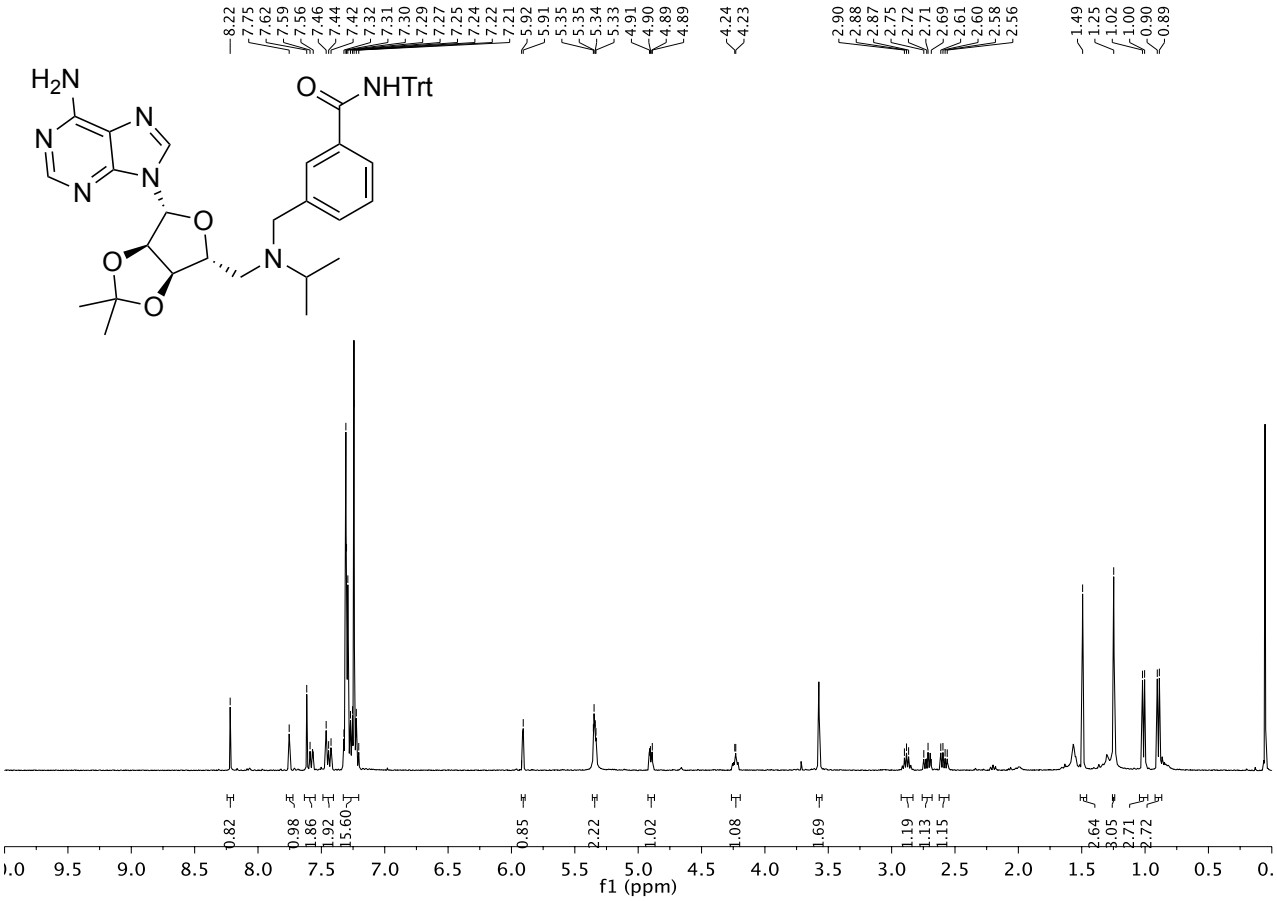
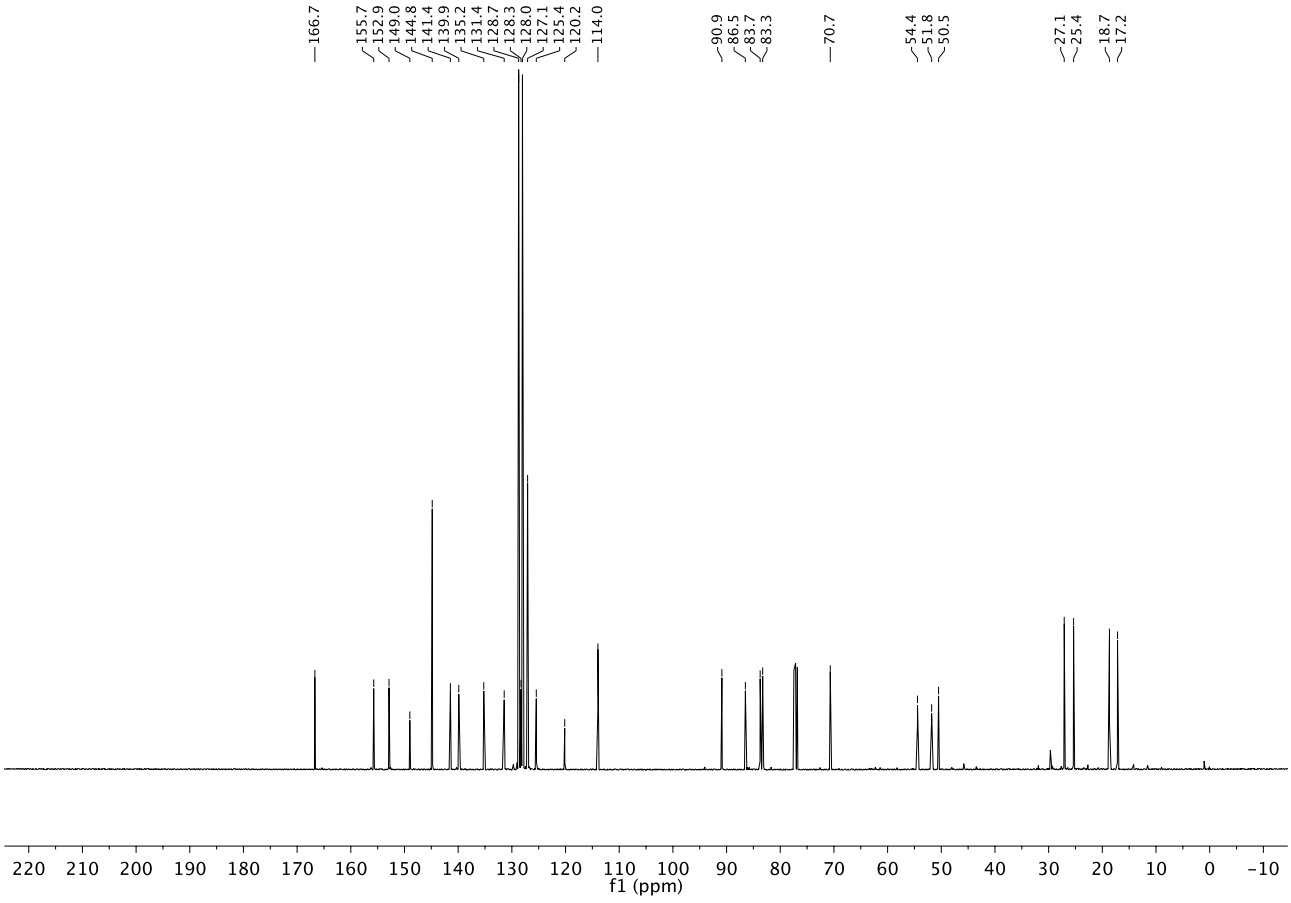


^{13}C NMR ($CDCl_3$)



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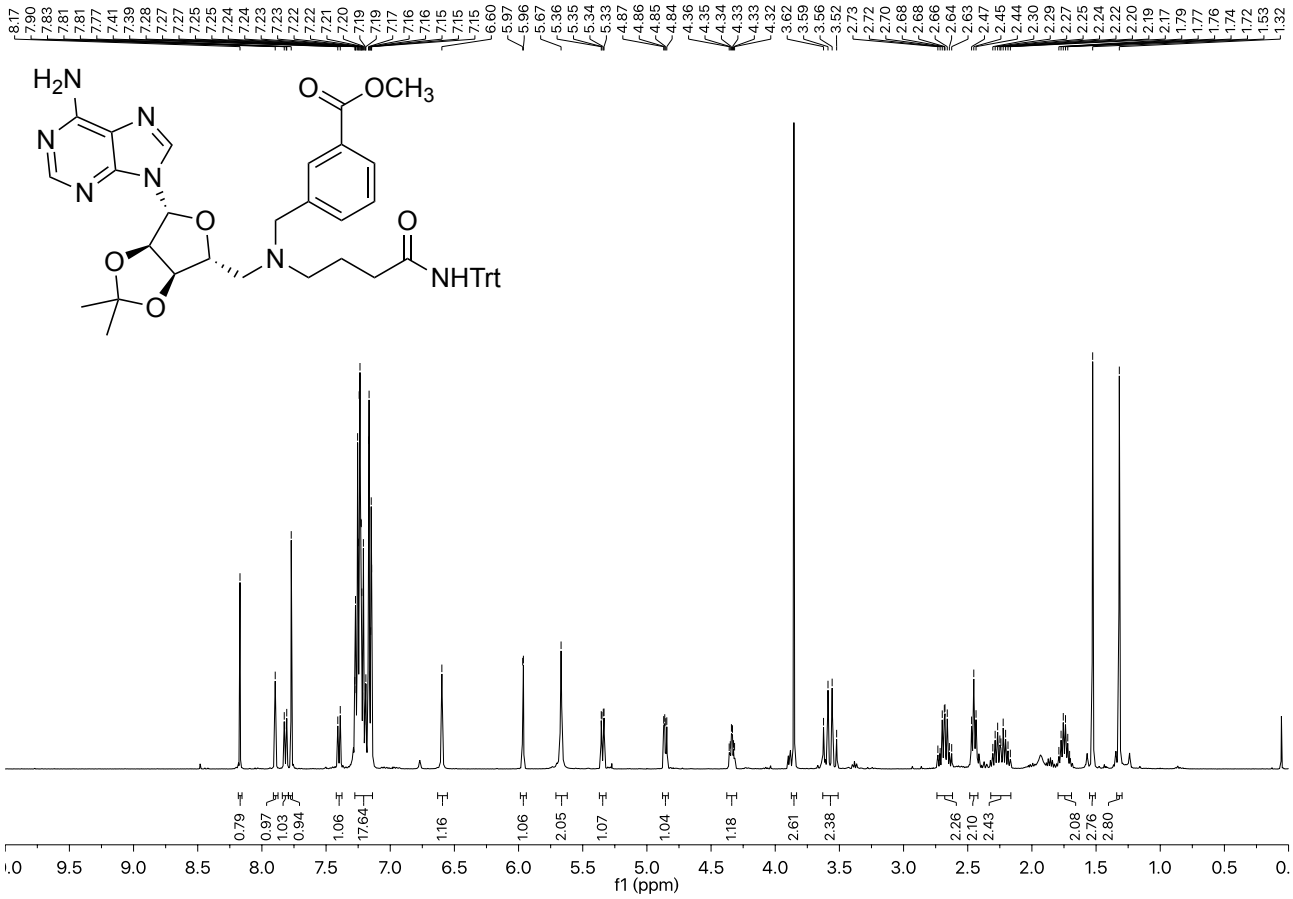
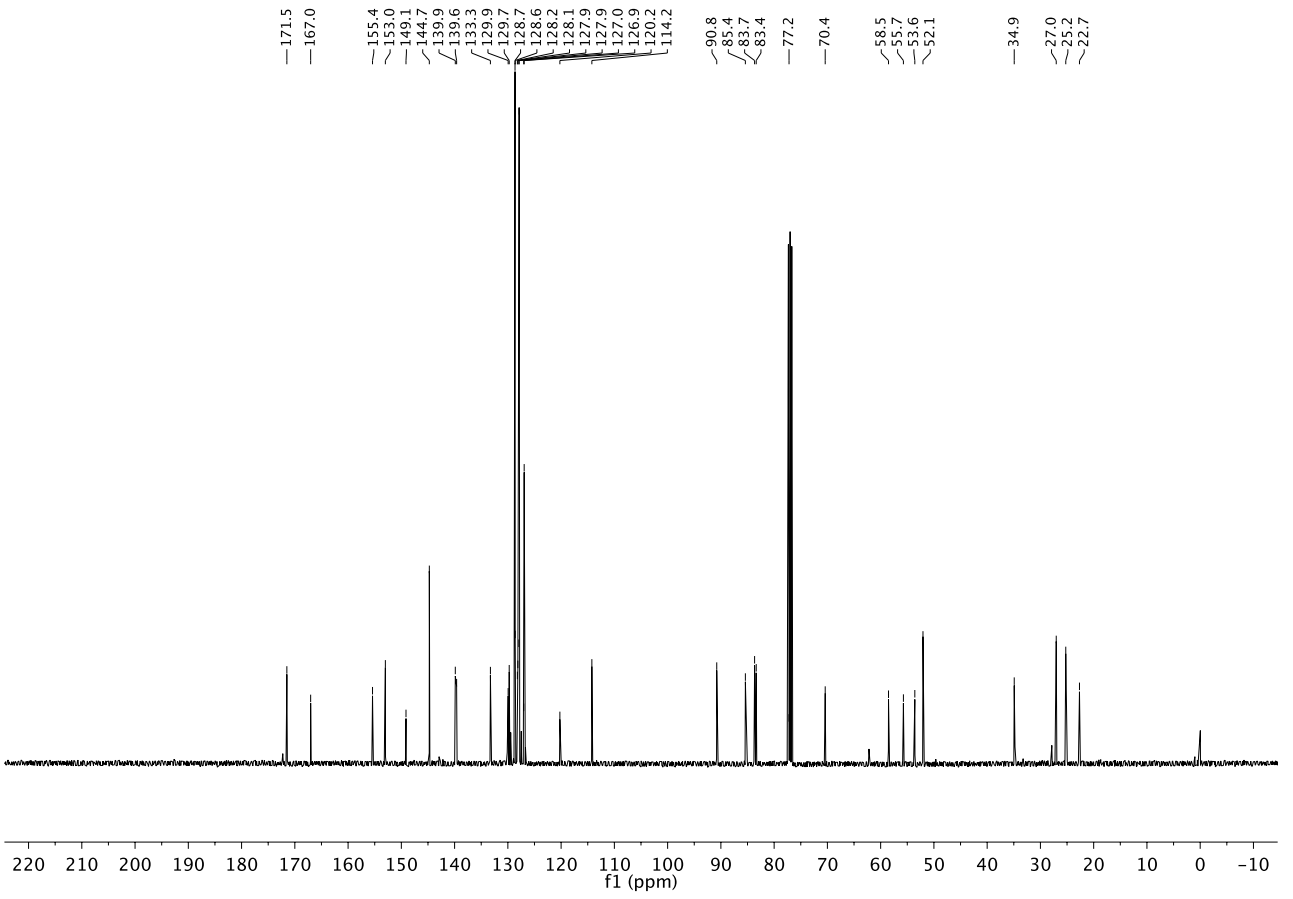
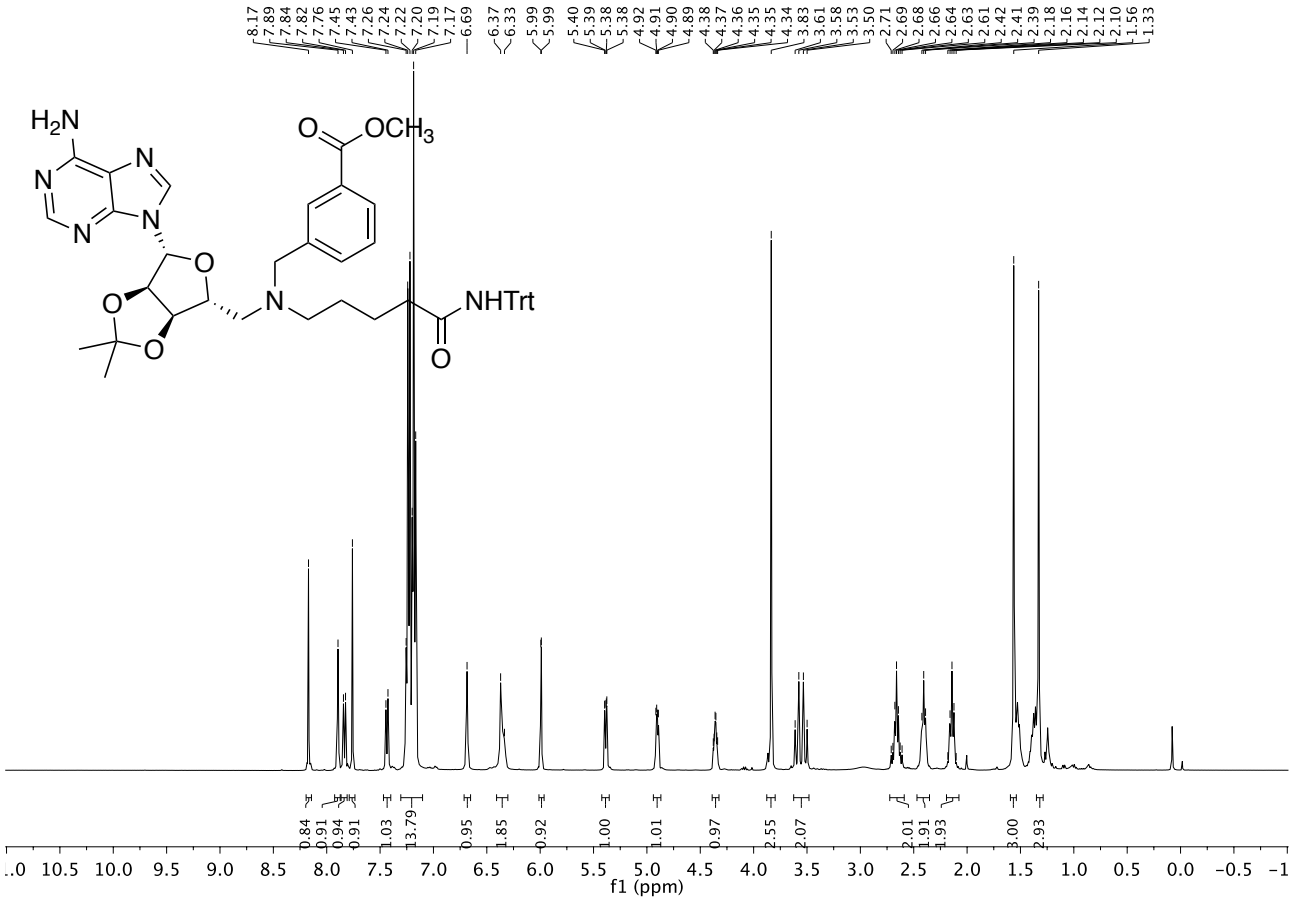


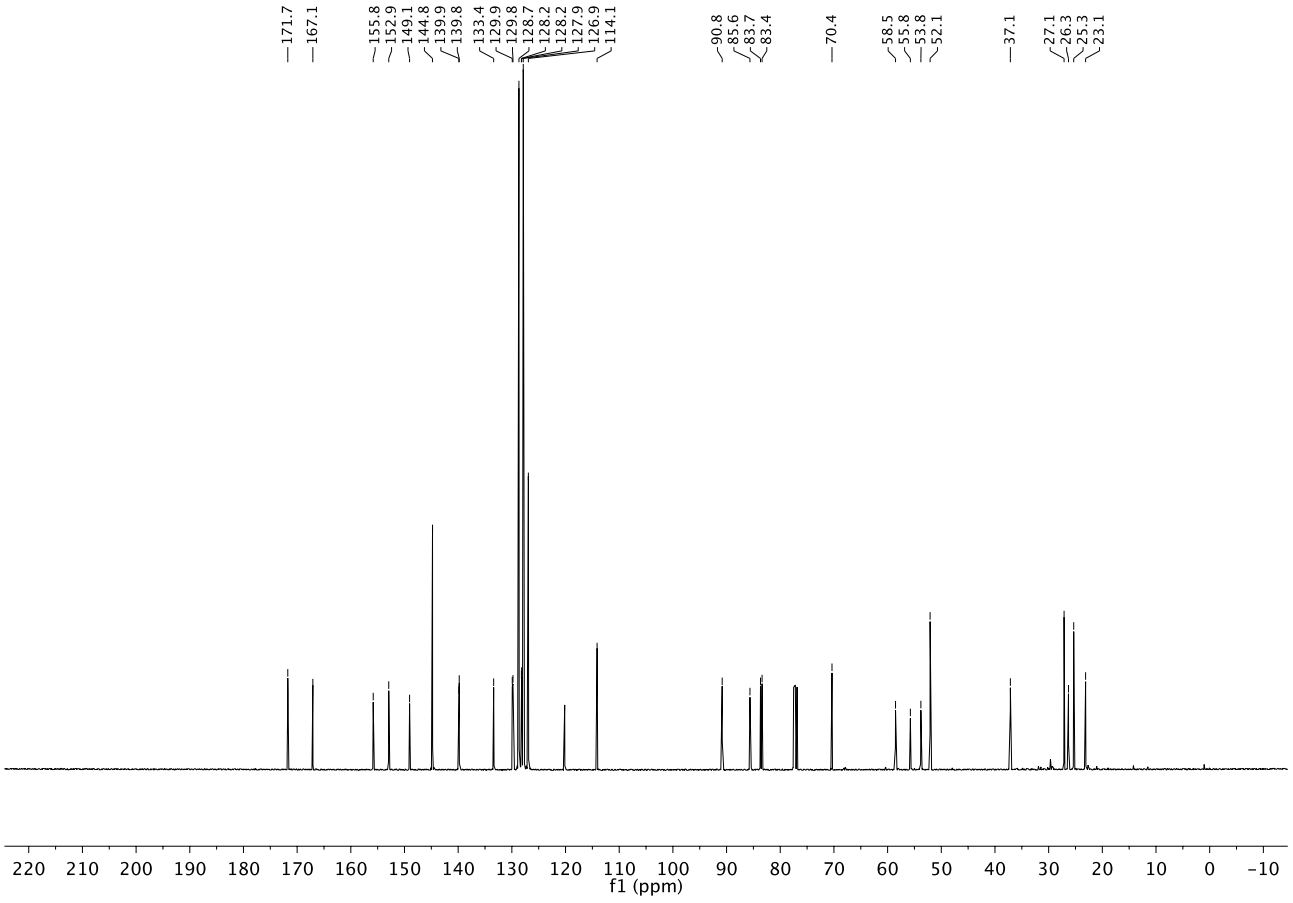
^{13}C NMR ($CDCl_3$)



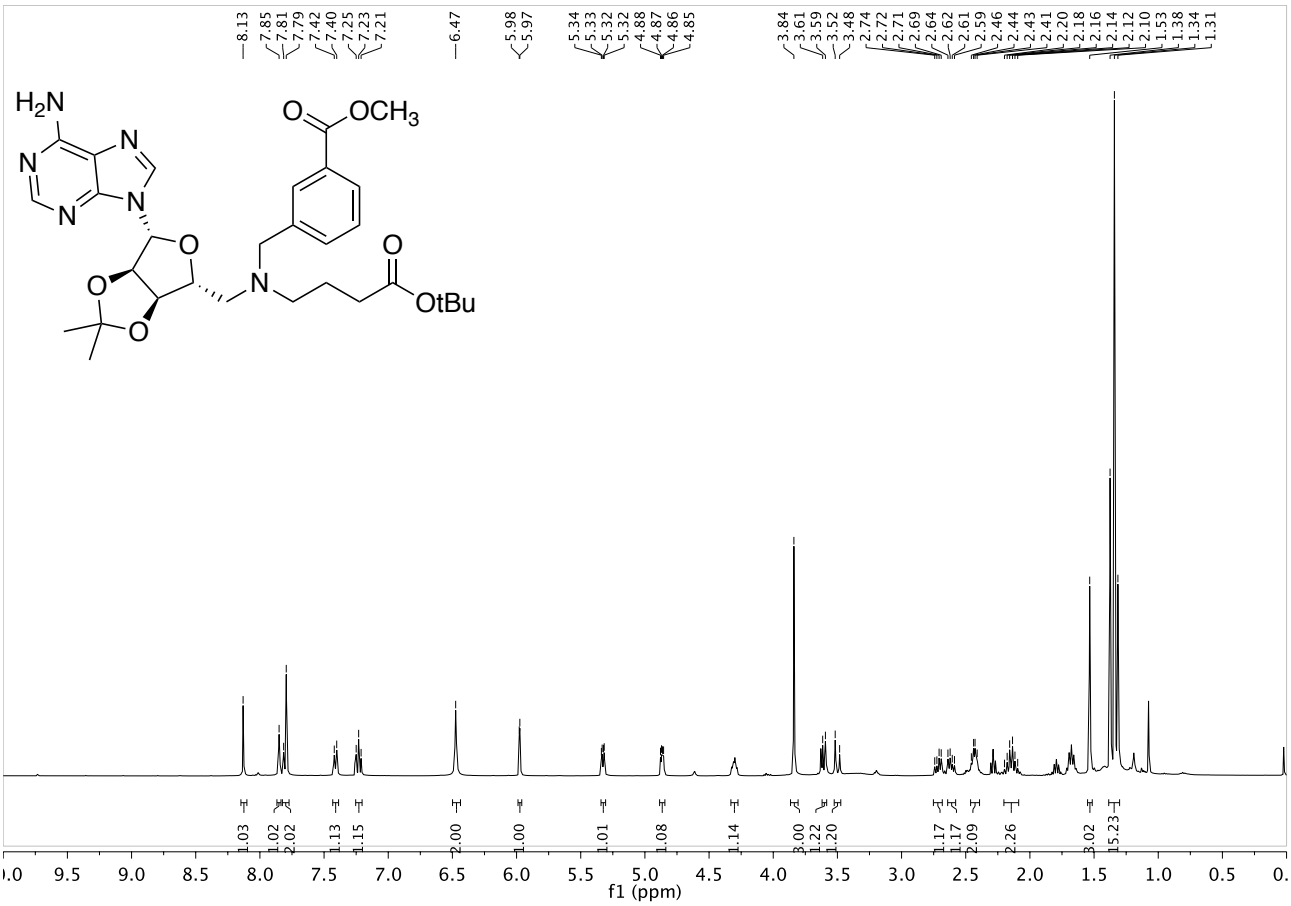
Compound **40** ^{1}H NMR ($CDCl_3$)



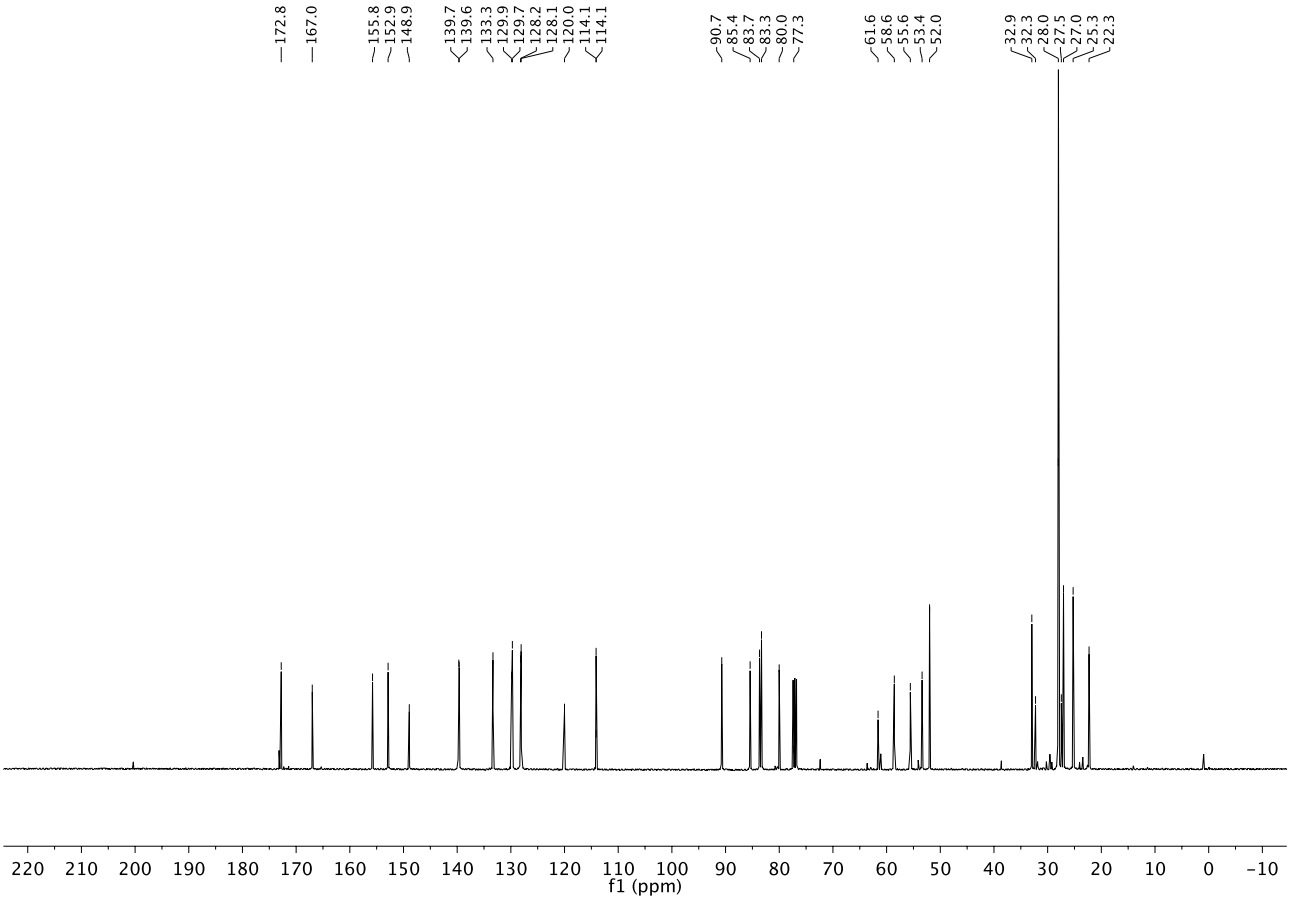
^{13}C NMR ($CDCl_3$)



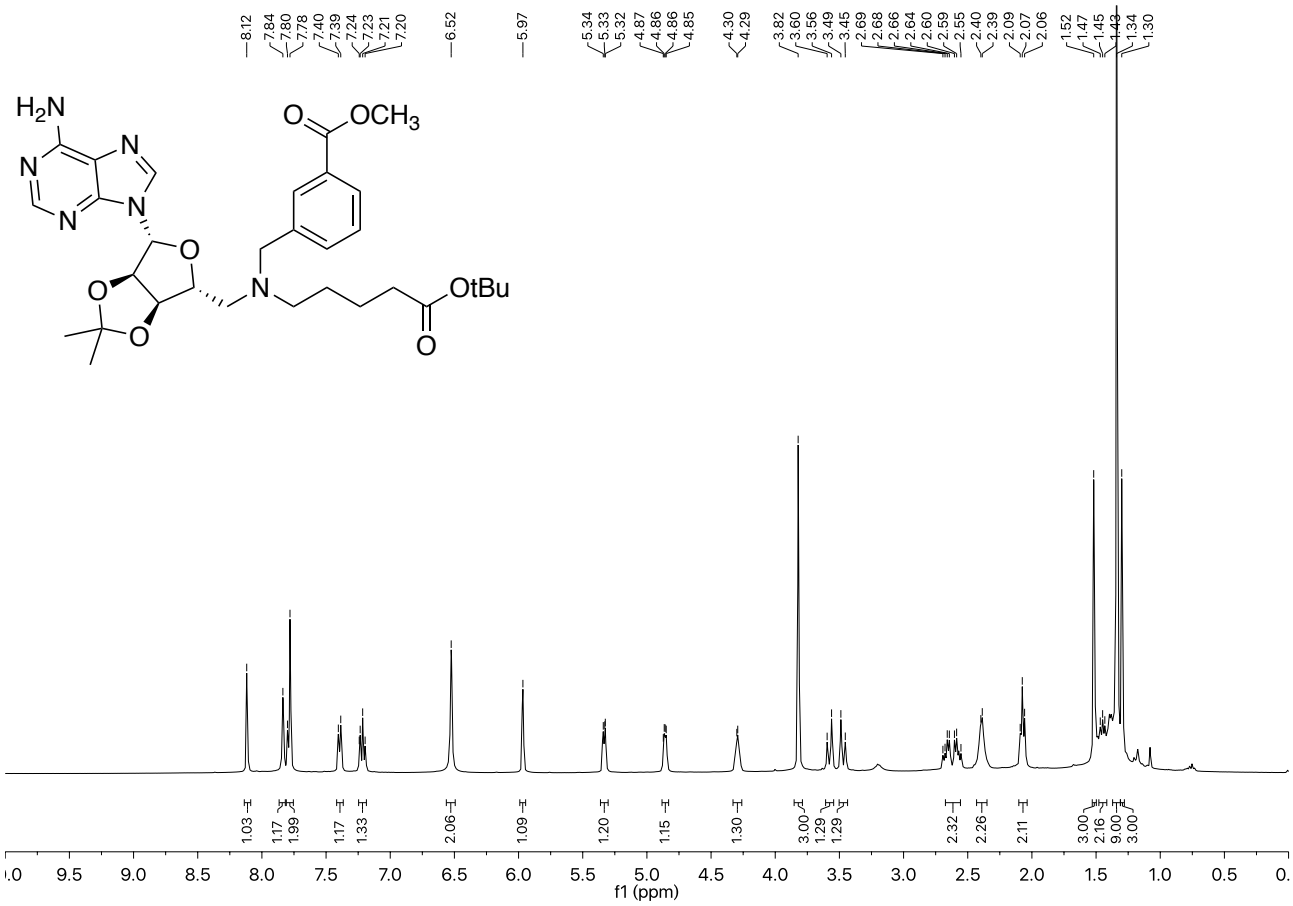
Compound **41** ^{1}H NMR ($CDCl_3$)



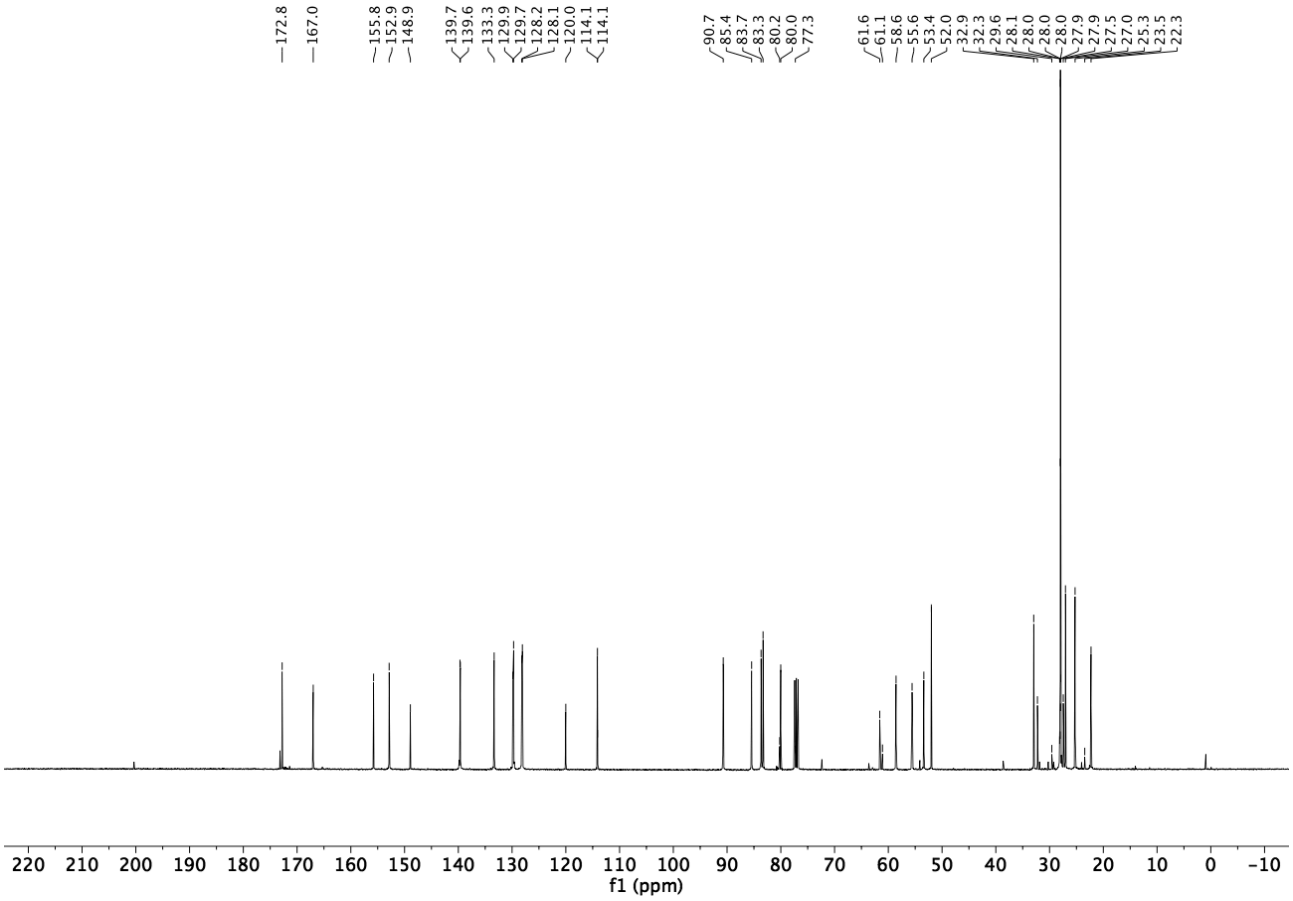
^{13}C NMR ($CDCl_3$)



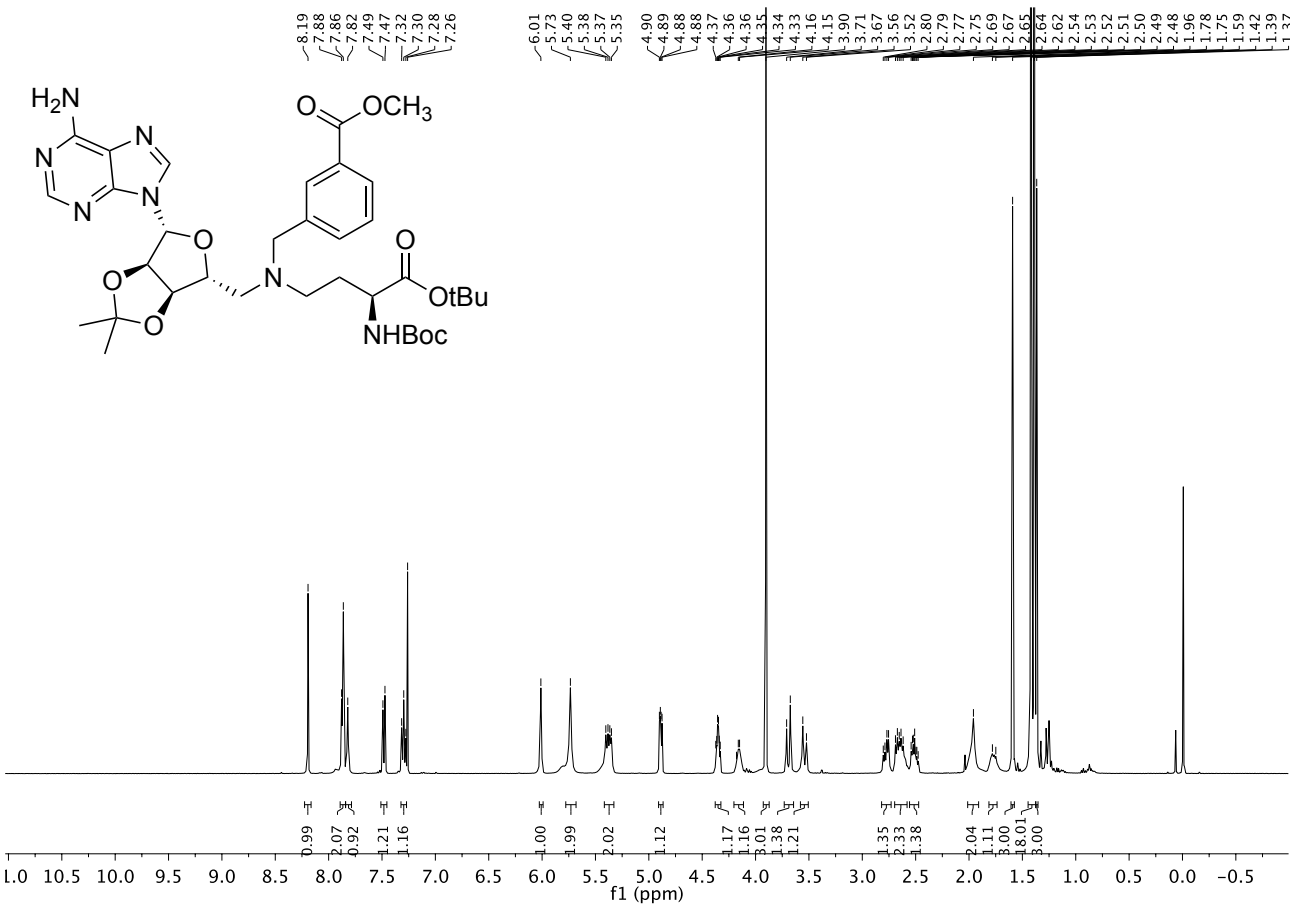
Compound **42** ^{1}H NMR ($CDCl_3$)



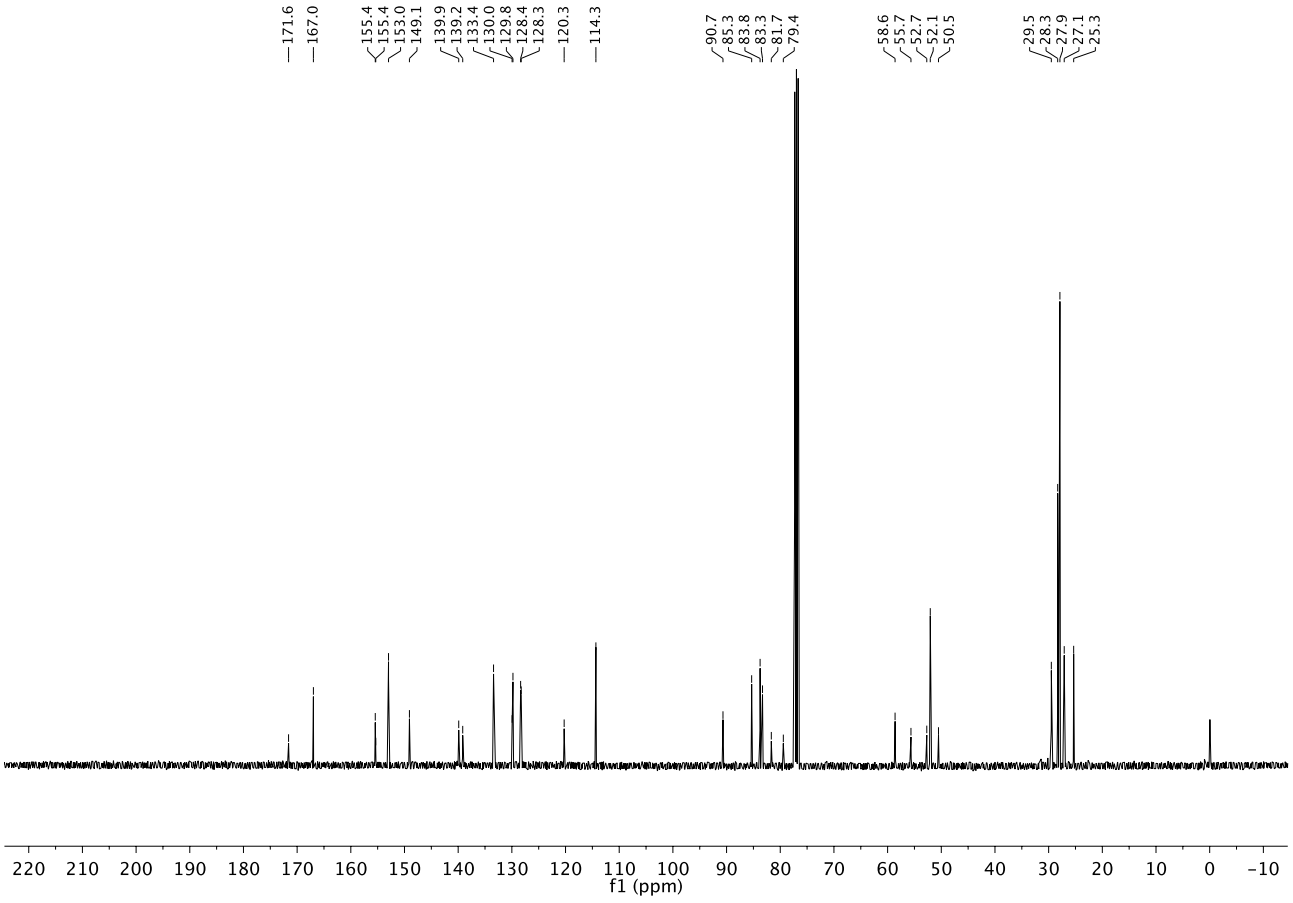
^{13}C NMR ($CDCl_3$)



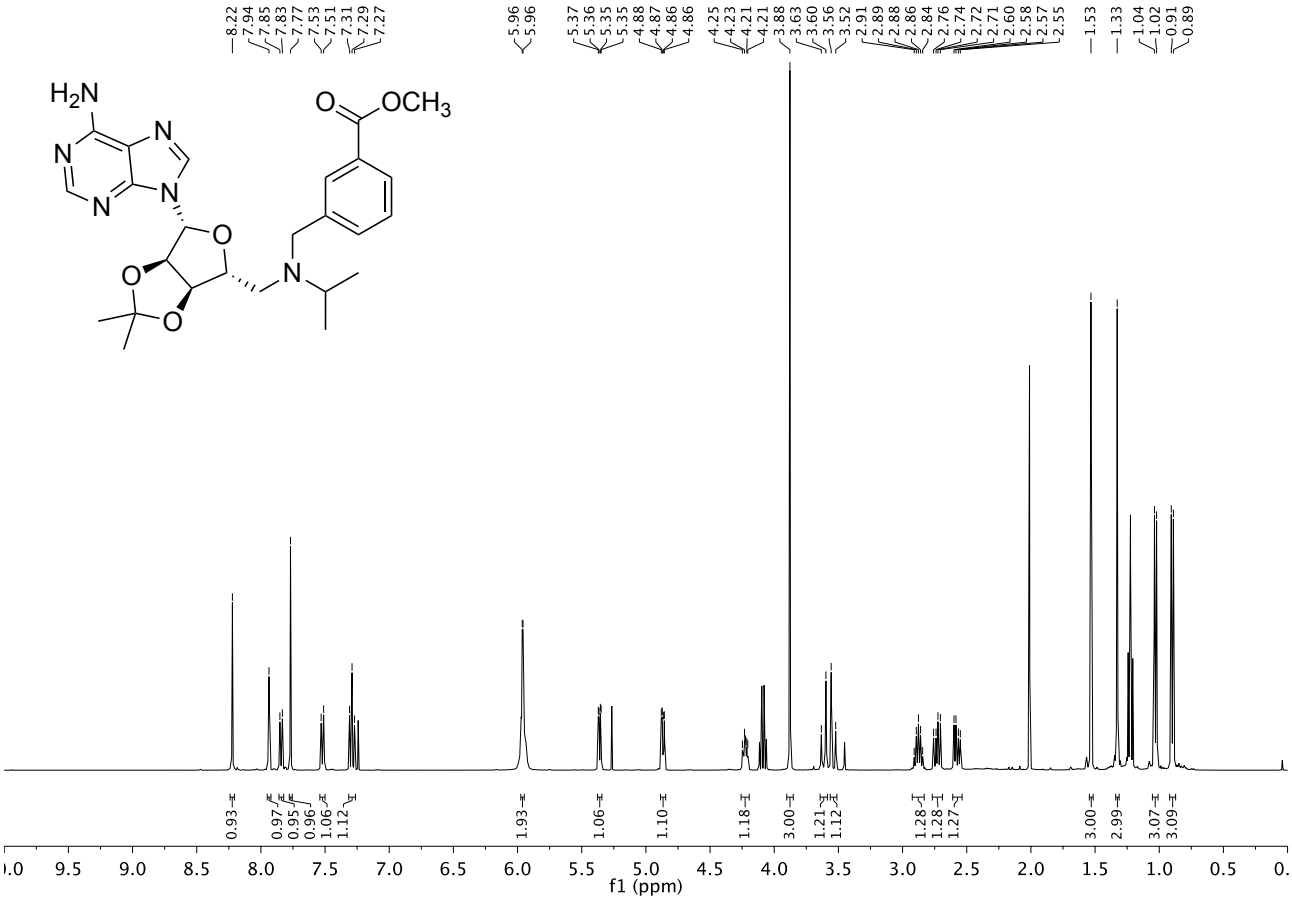
Compound **43** ^{1}H NMR ($CDCl_3$)



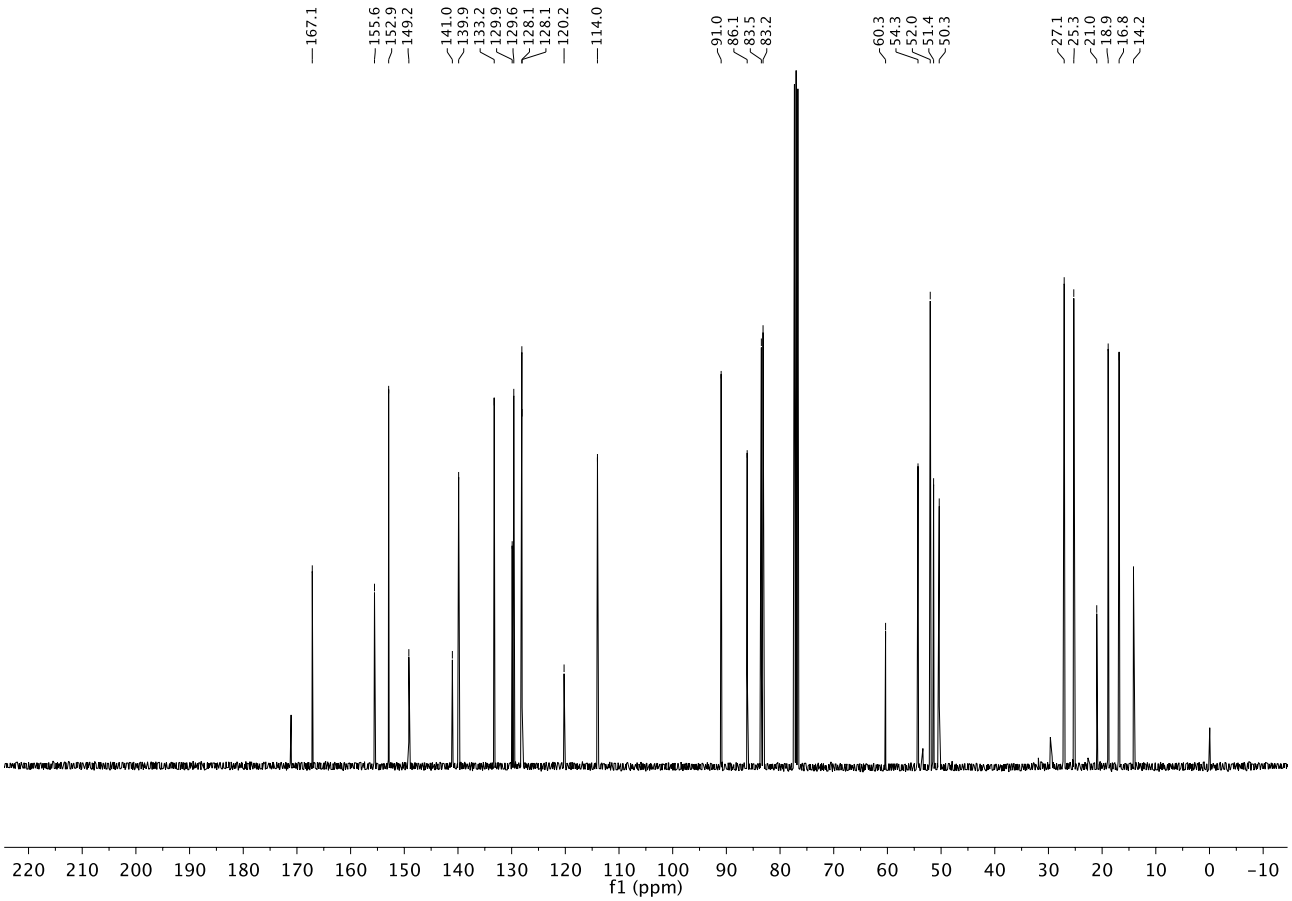
^{13}C NMR ($CDCl_3$)



Compound **44** ^{1}H NMR ($CDCl_3$)



^{13}C NMR ($CDCl_3$)



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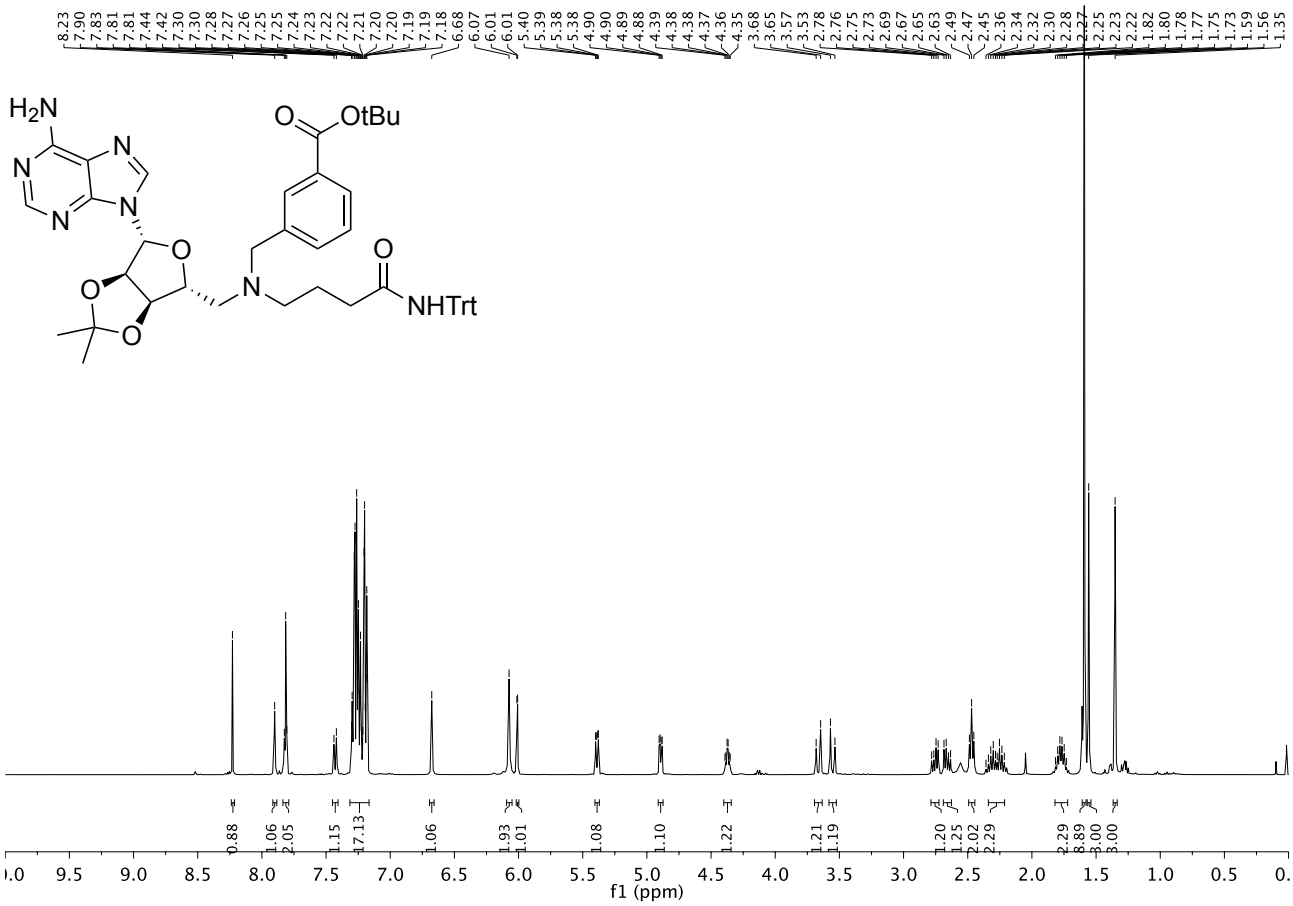
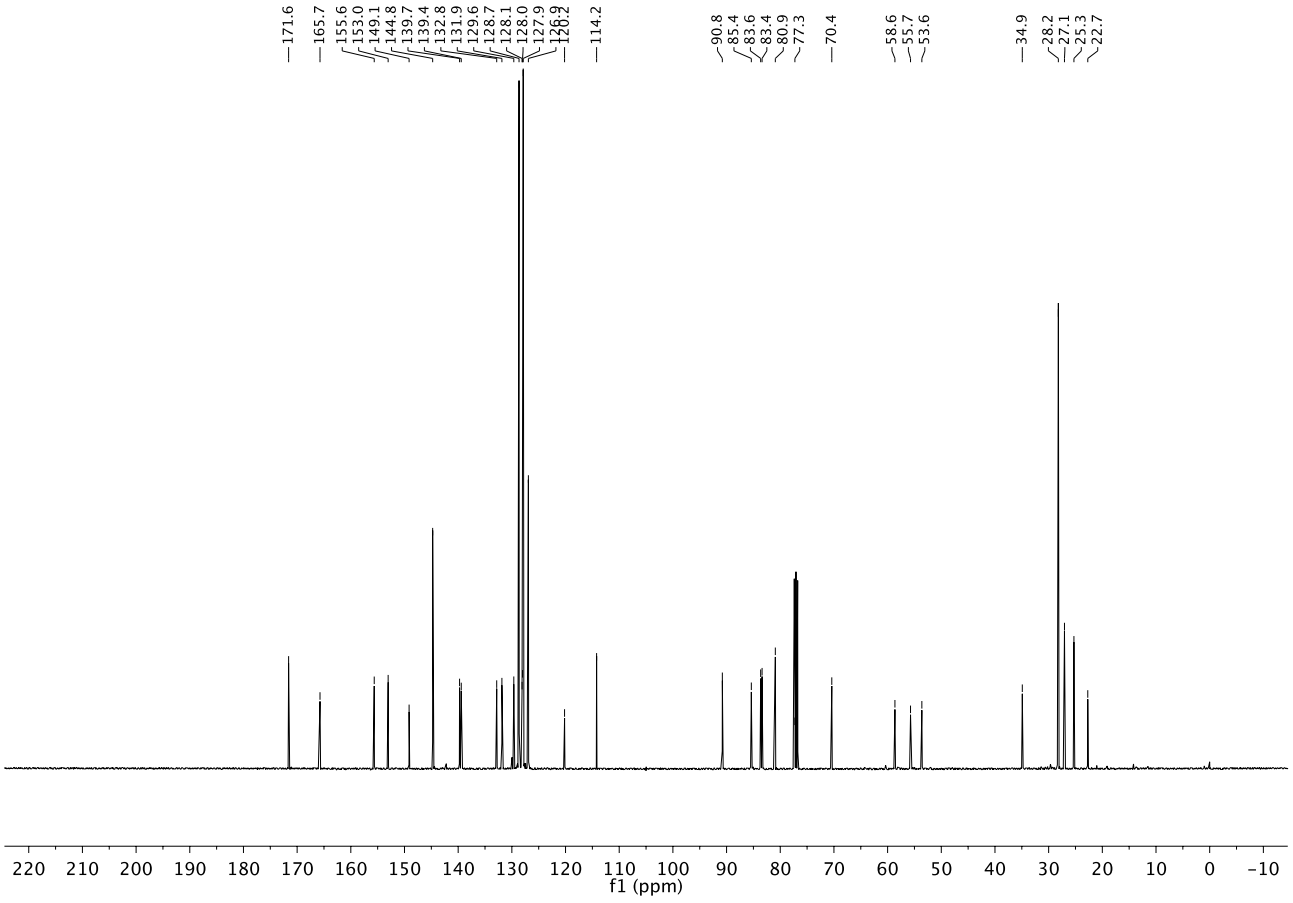
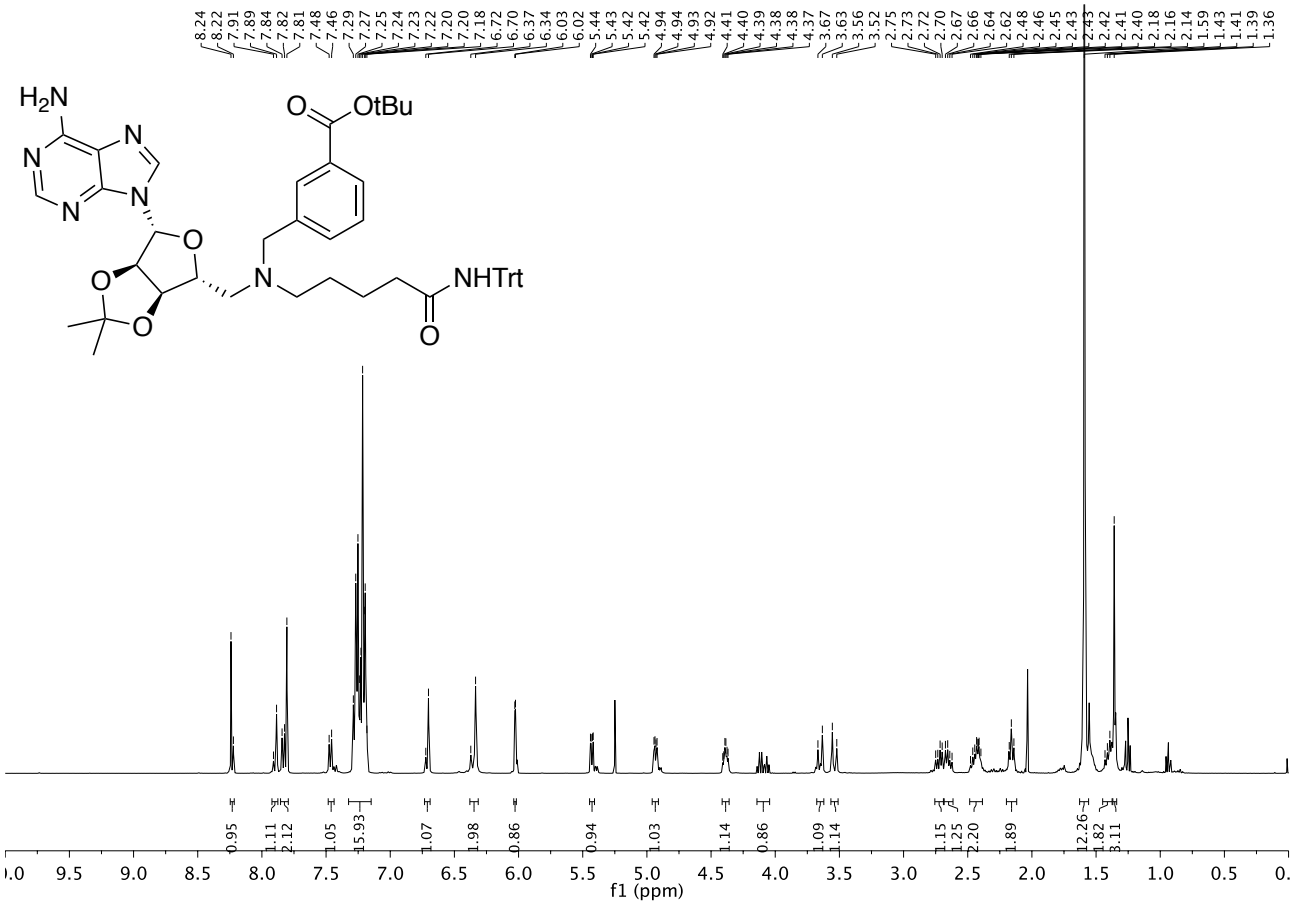


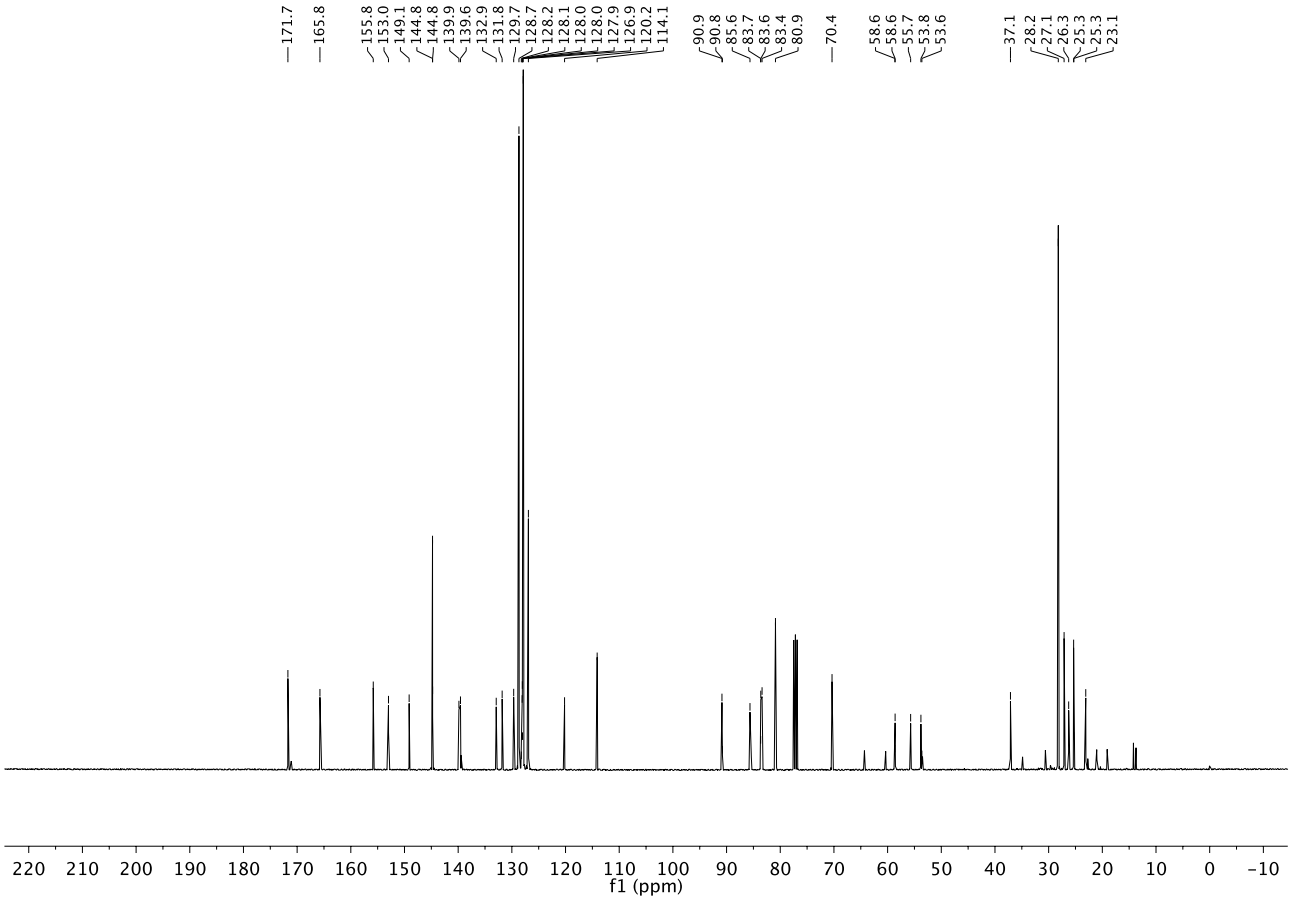
^{13}C NMR ($CDCl_3$)



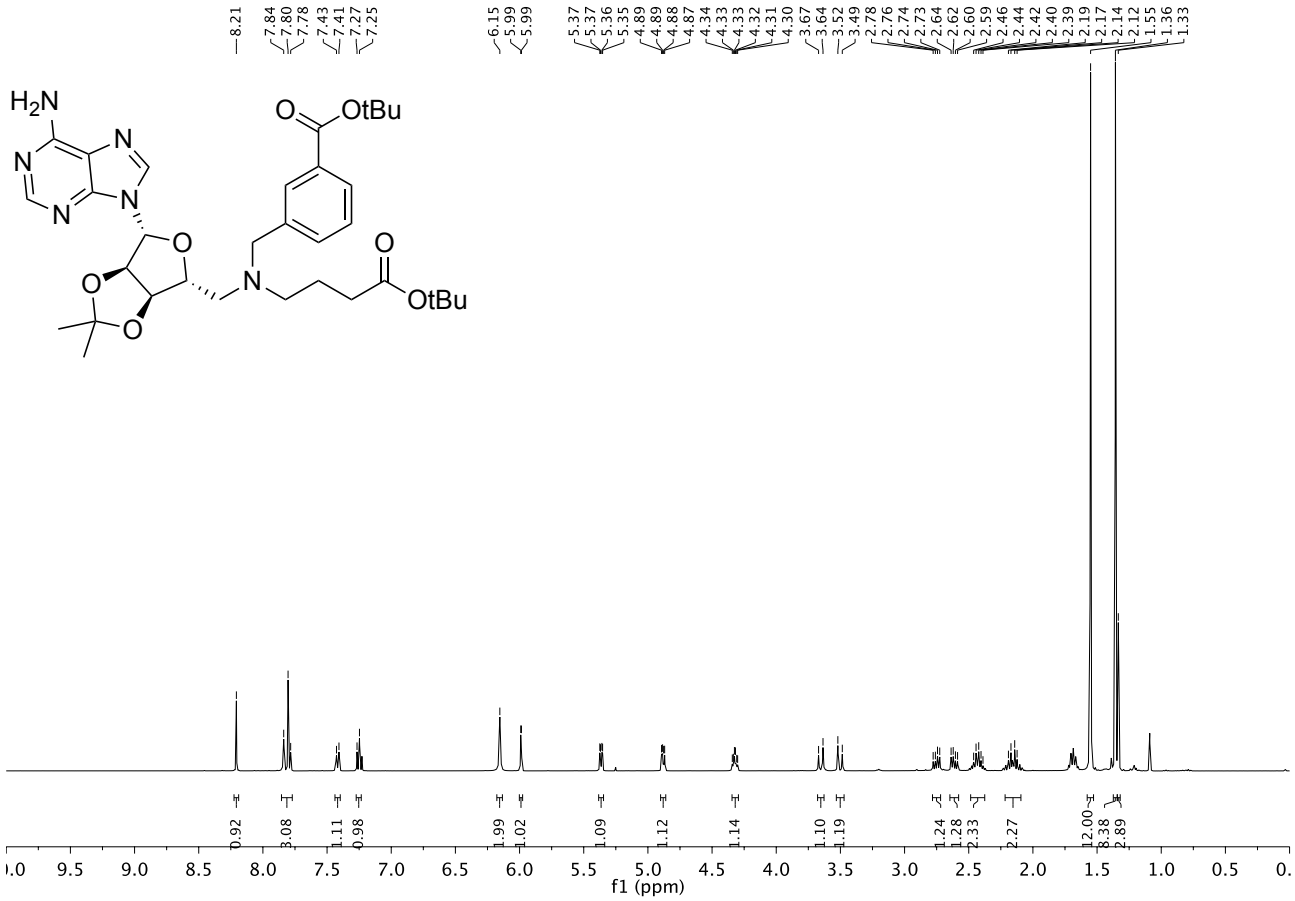
Compound **46** ^{1}H NMR ($CDCl_3$)



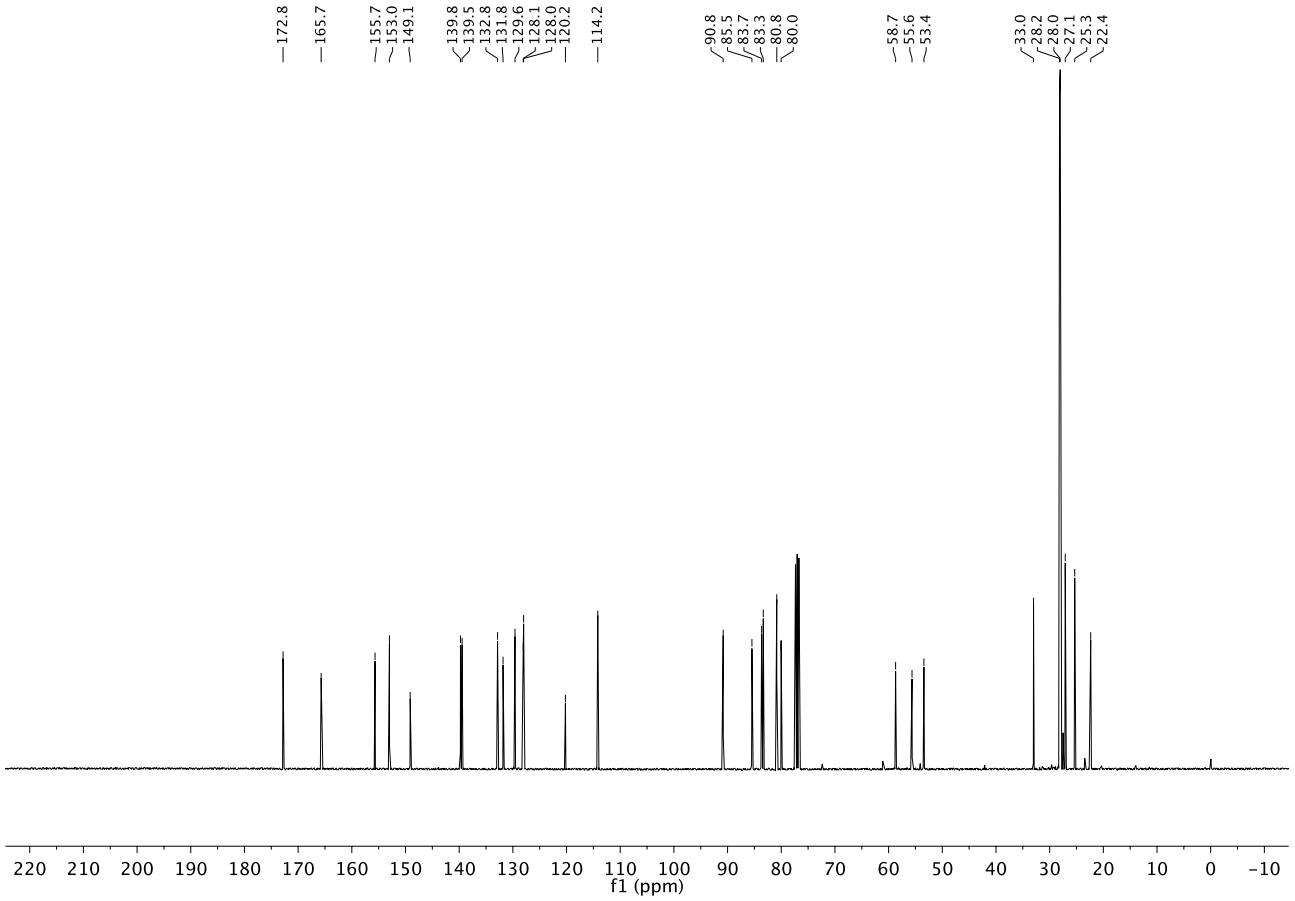
^{13}C NMR ($CDCl_3$)



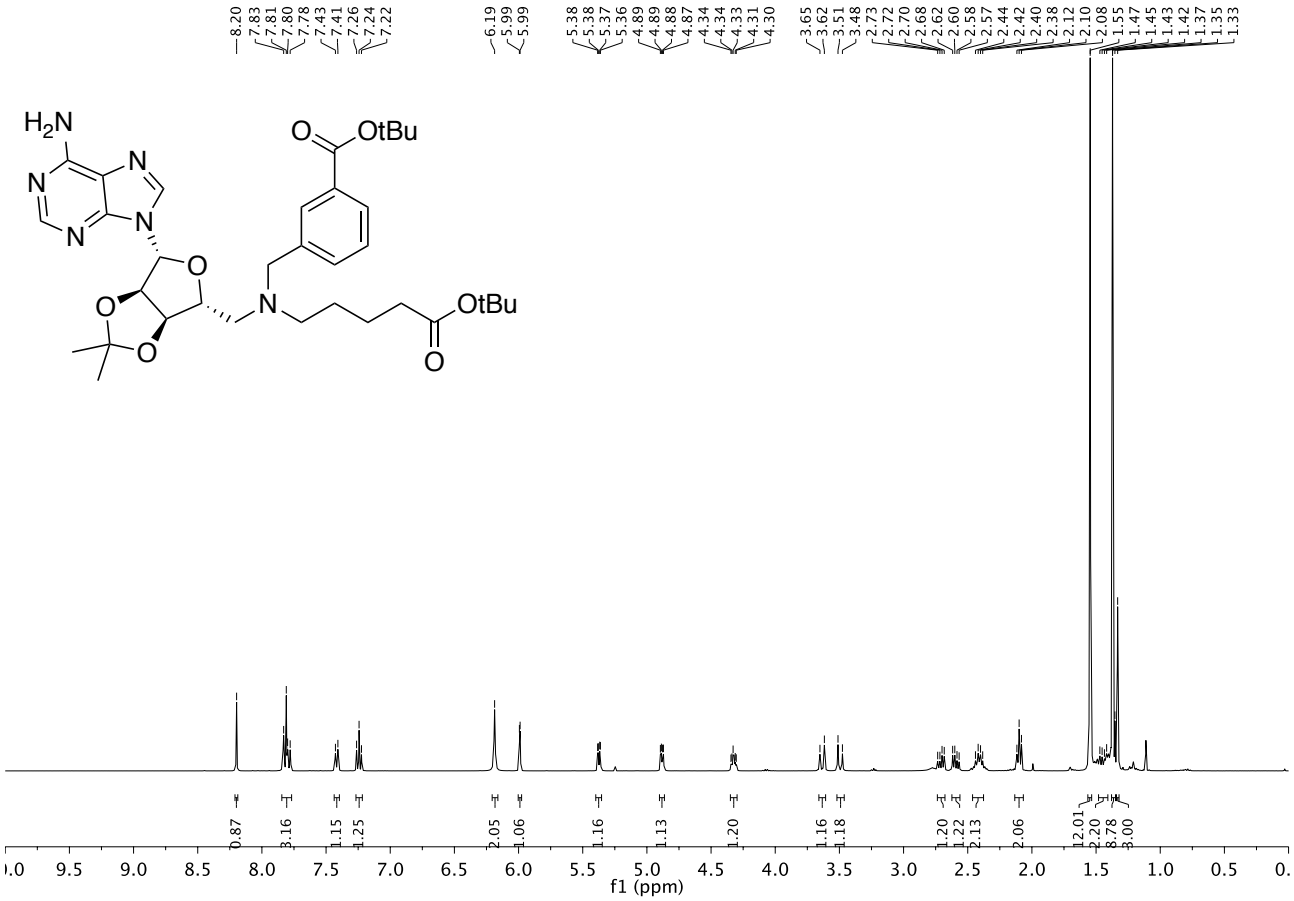
Compound **47** ^{1}H NMR ($CDCl_3$)



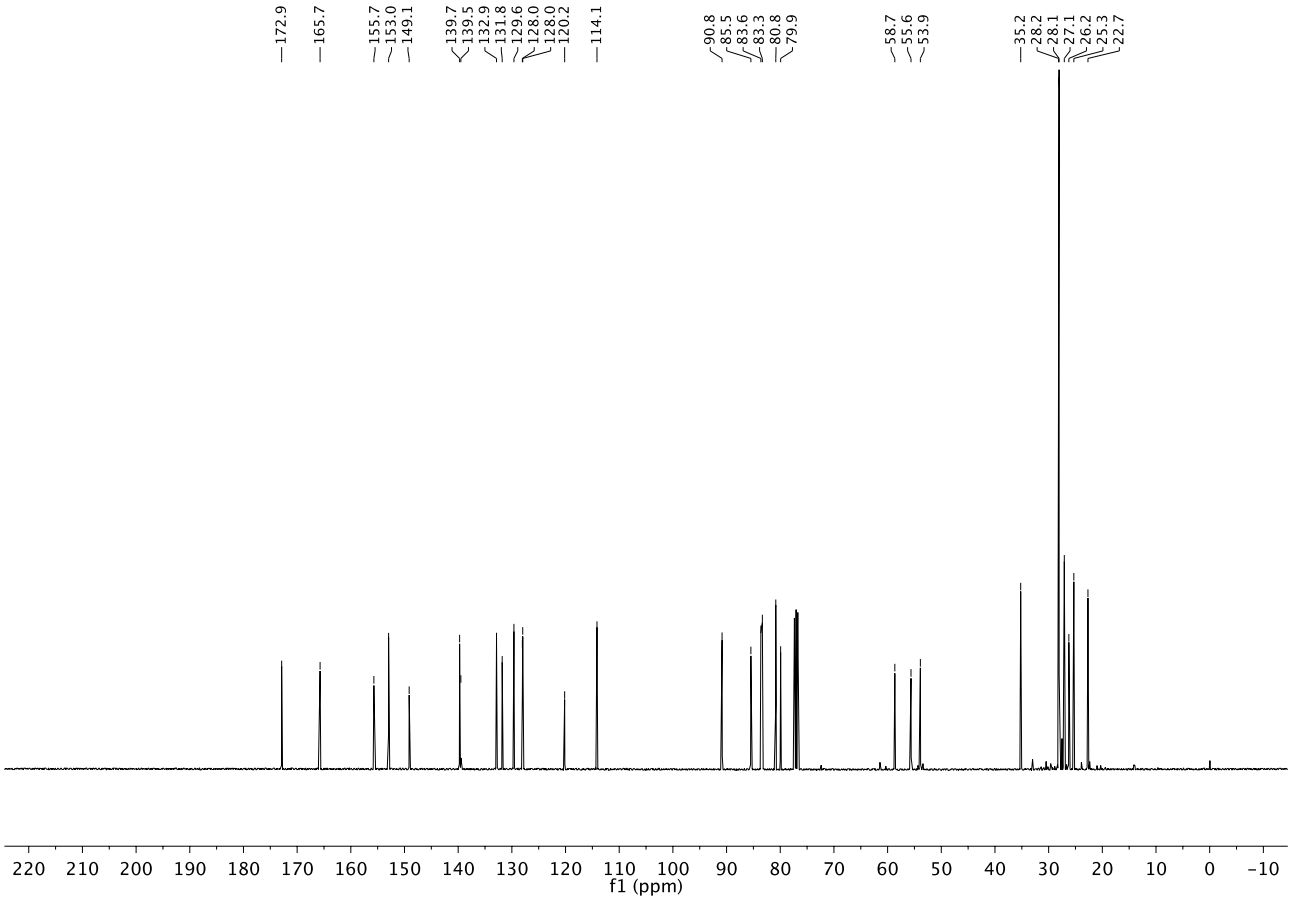
^{13}C NMR ($CDCl_3$)



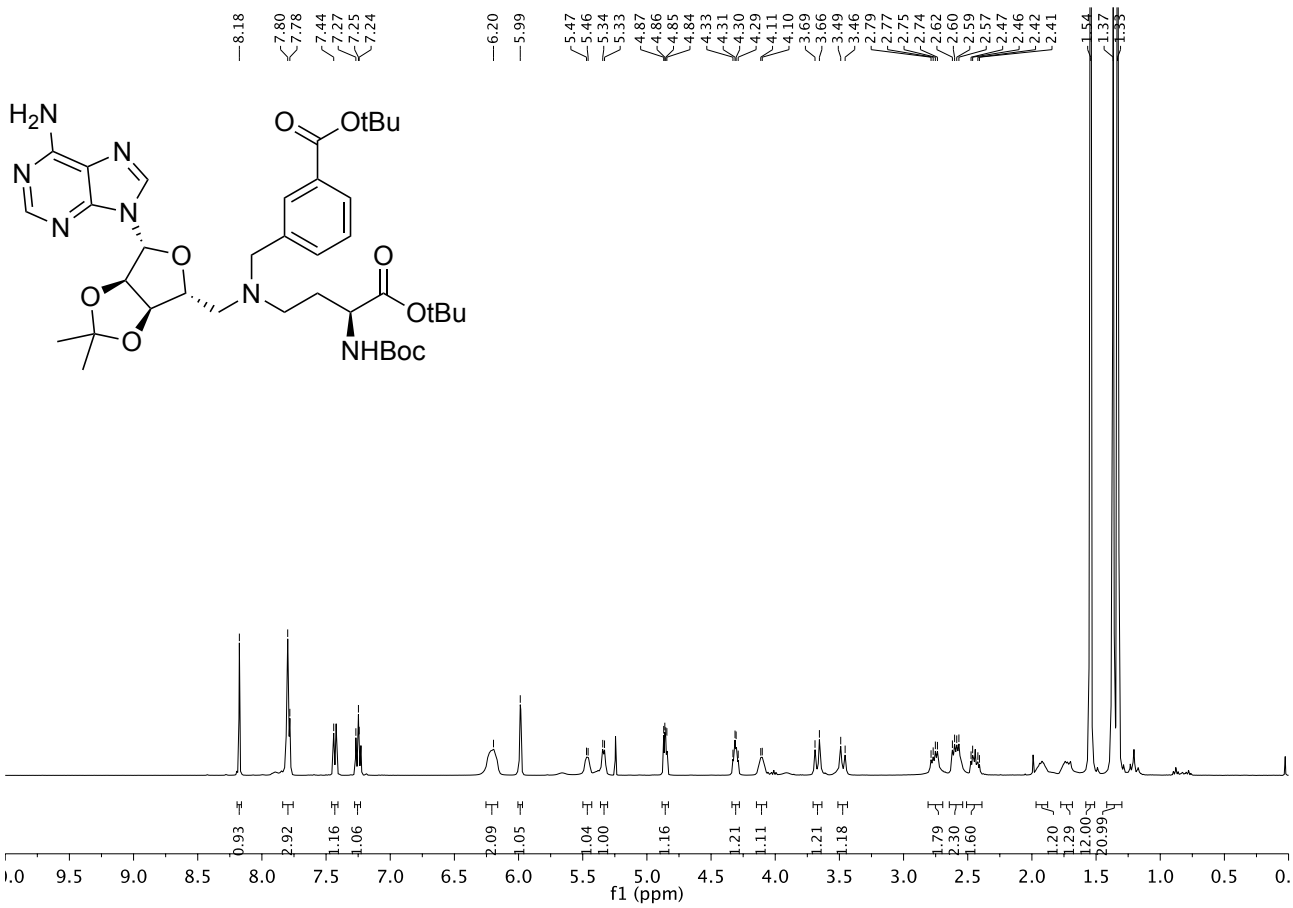
Compound **48** ^{1}H NMR ($CDCl_3$)



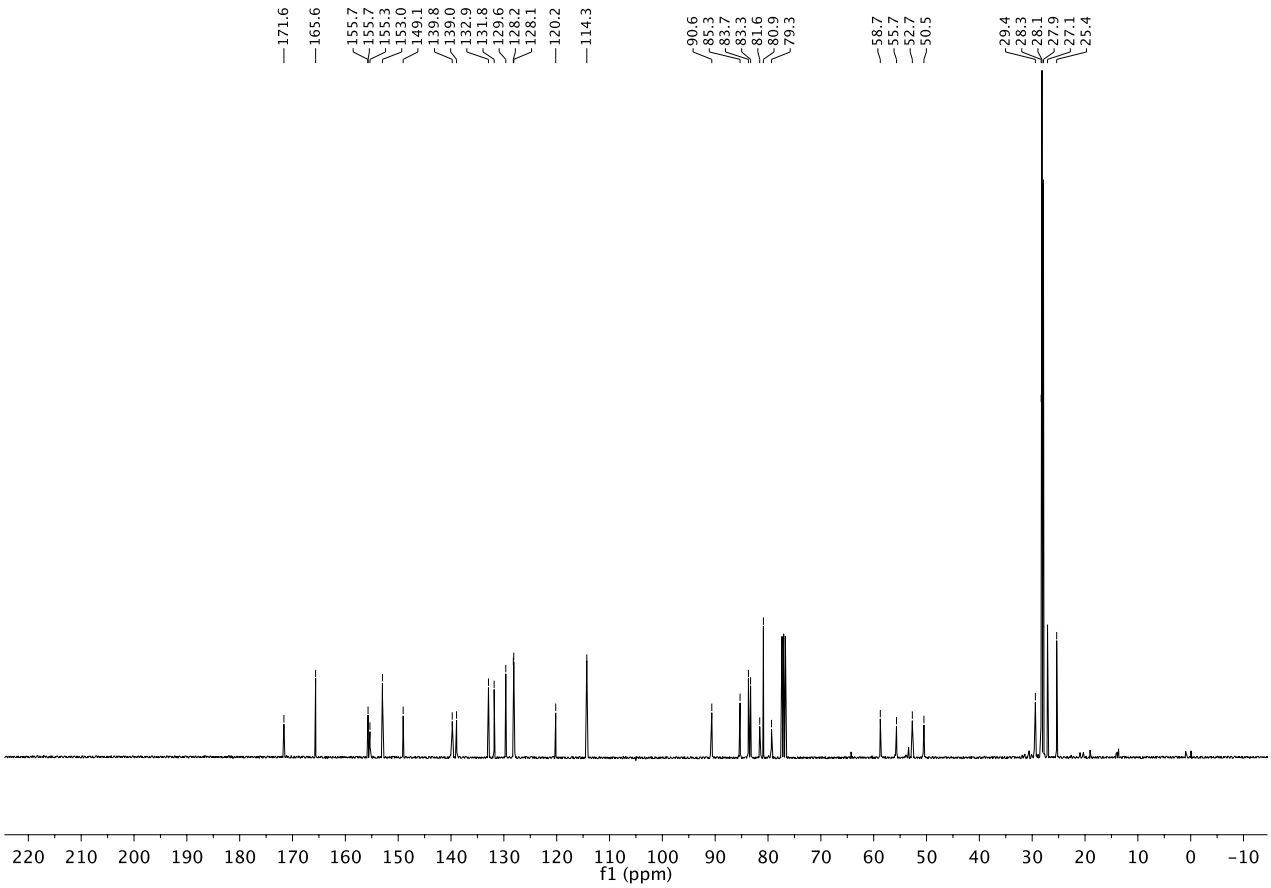
^{13}C NMR ($CDCl_3$)



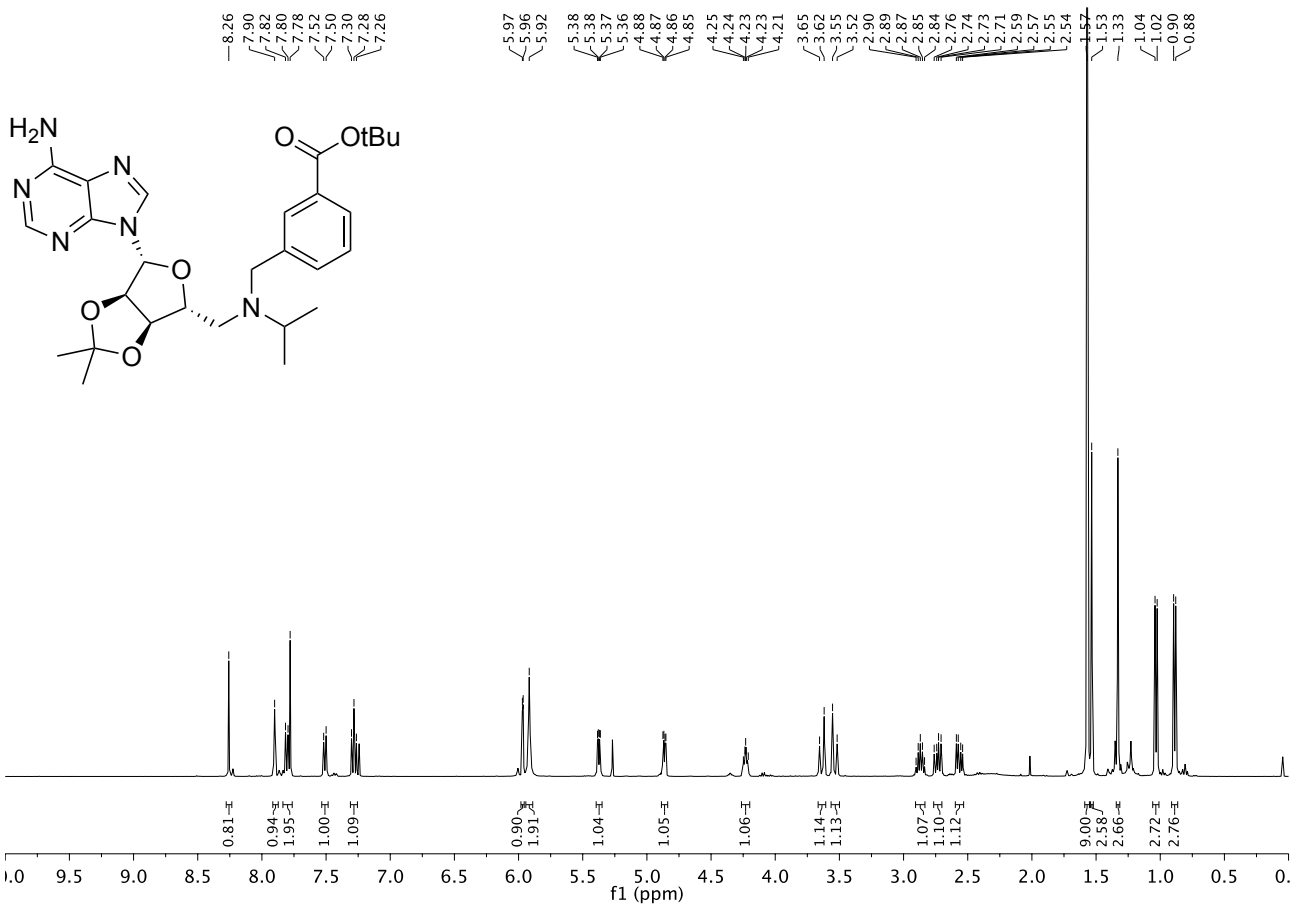
Compound **49** ^{1}H NMR ($CDCl_3$)



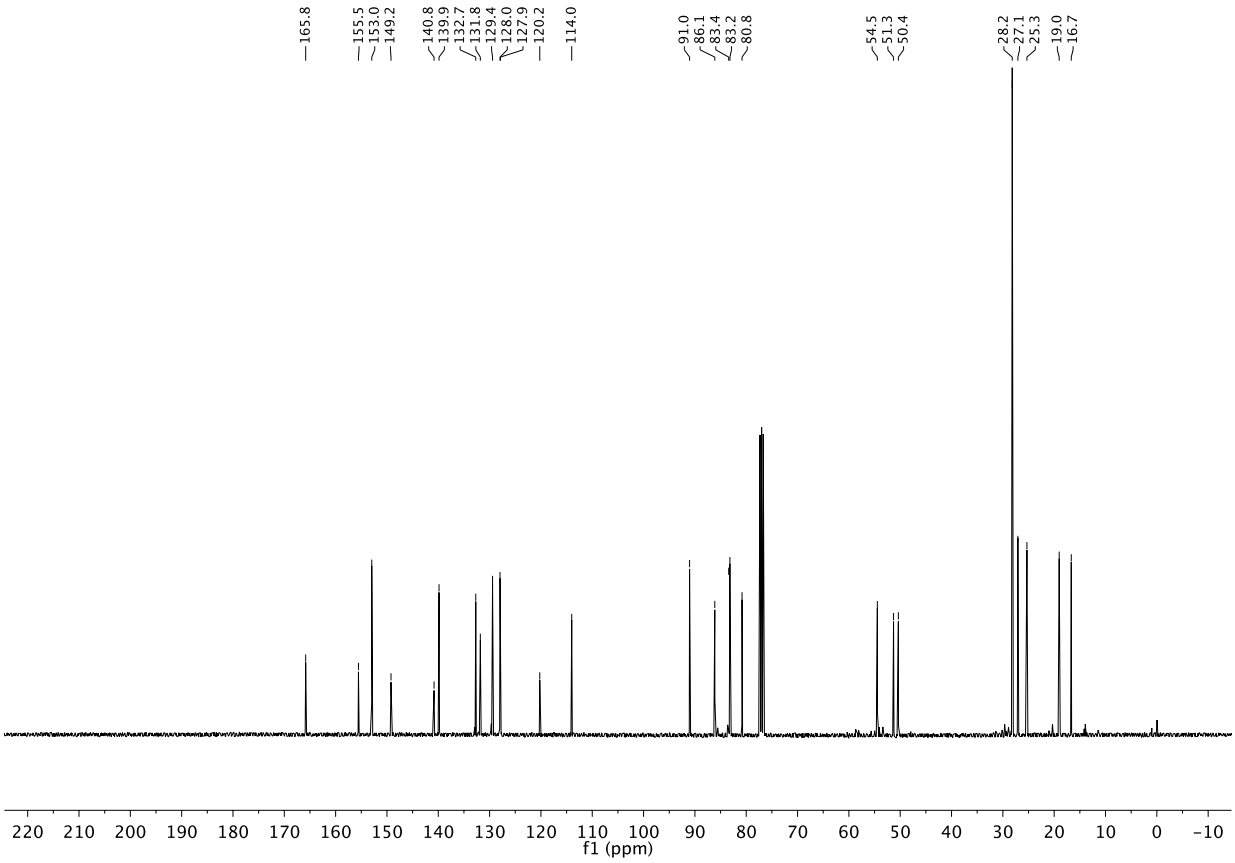
^{13}C NMR ($CDCl_3$)



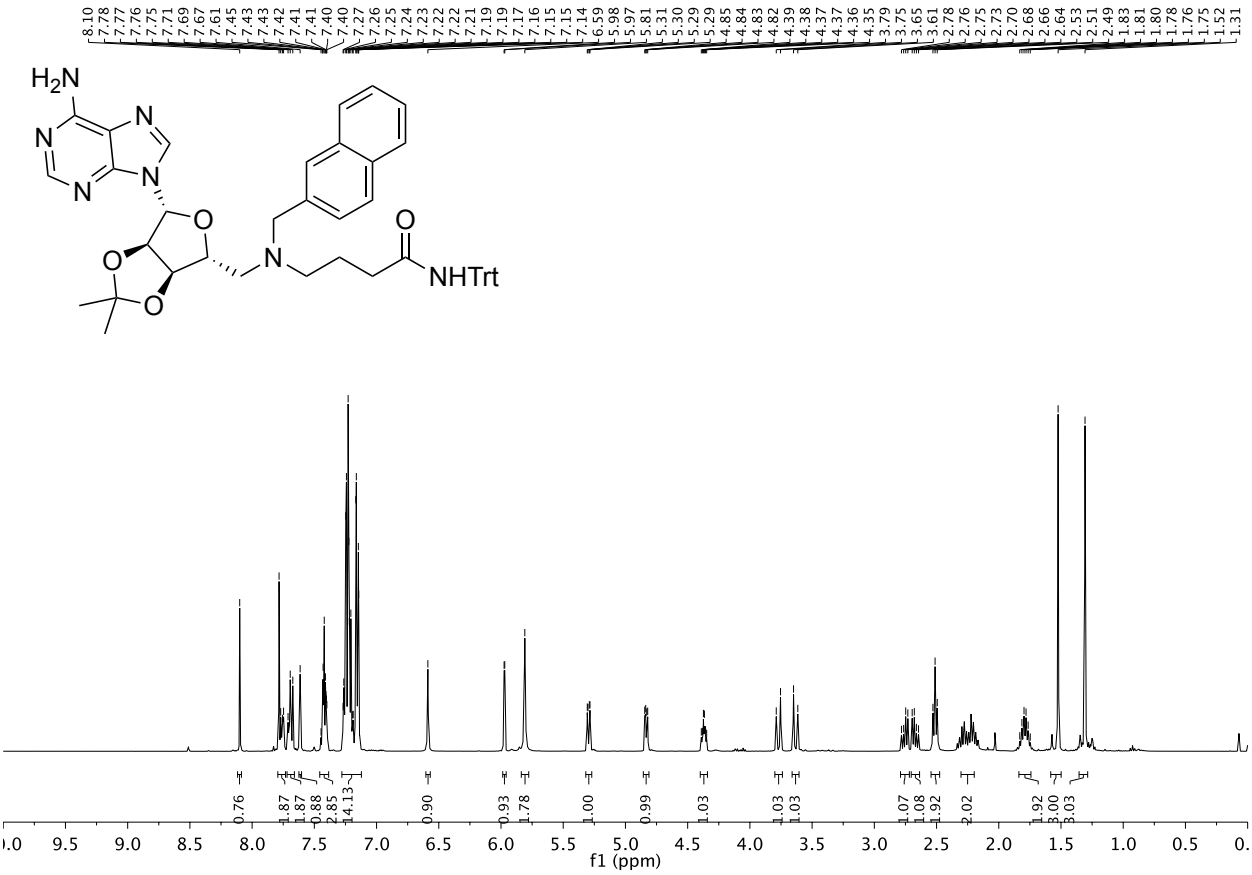
Compound **50** ^{1}H NMR ($CDCl_3$)



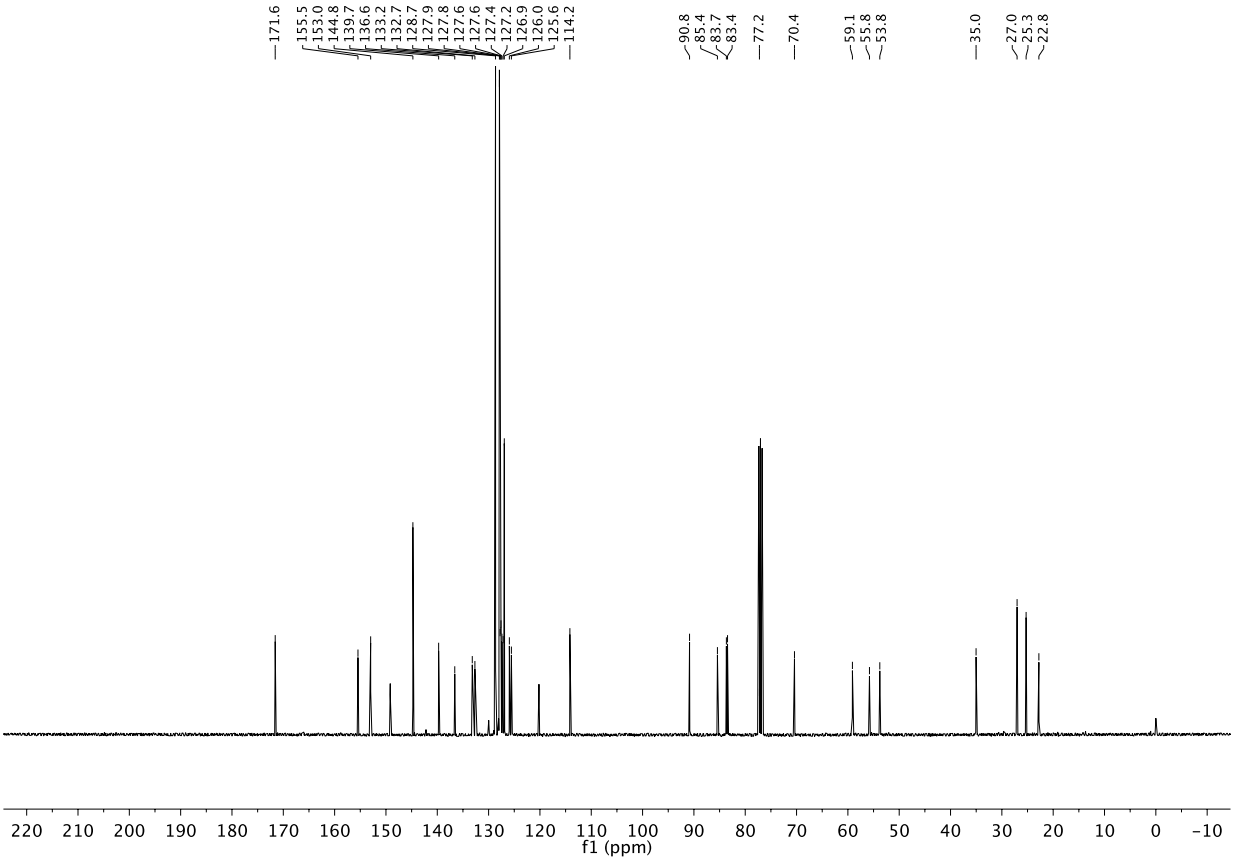
^{13}C NMR ($CDCl_3$)



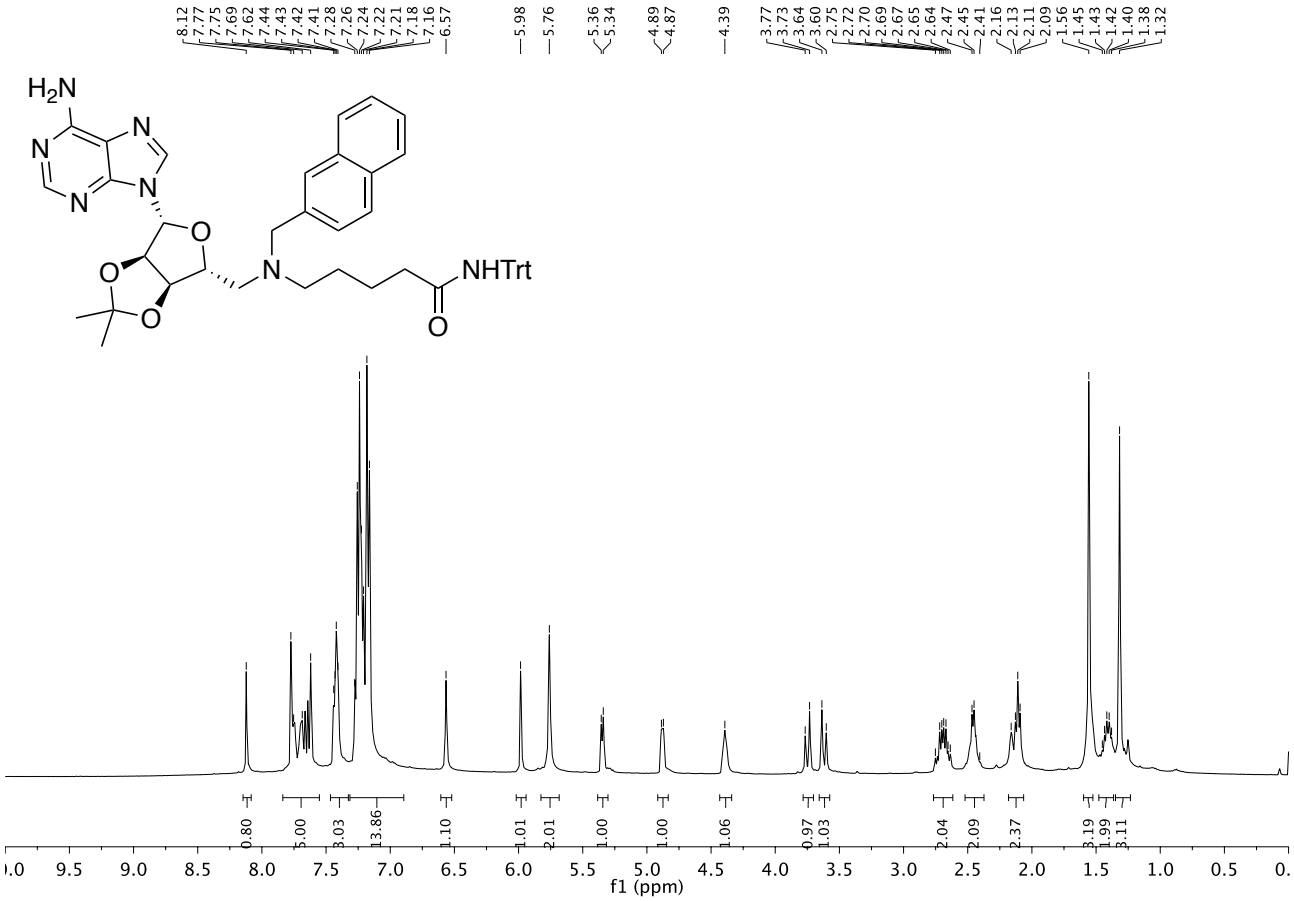
Compound **51** ^{1}H NMR ($CDCl_3$)



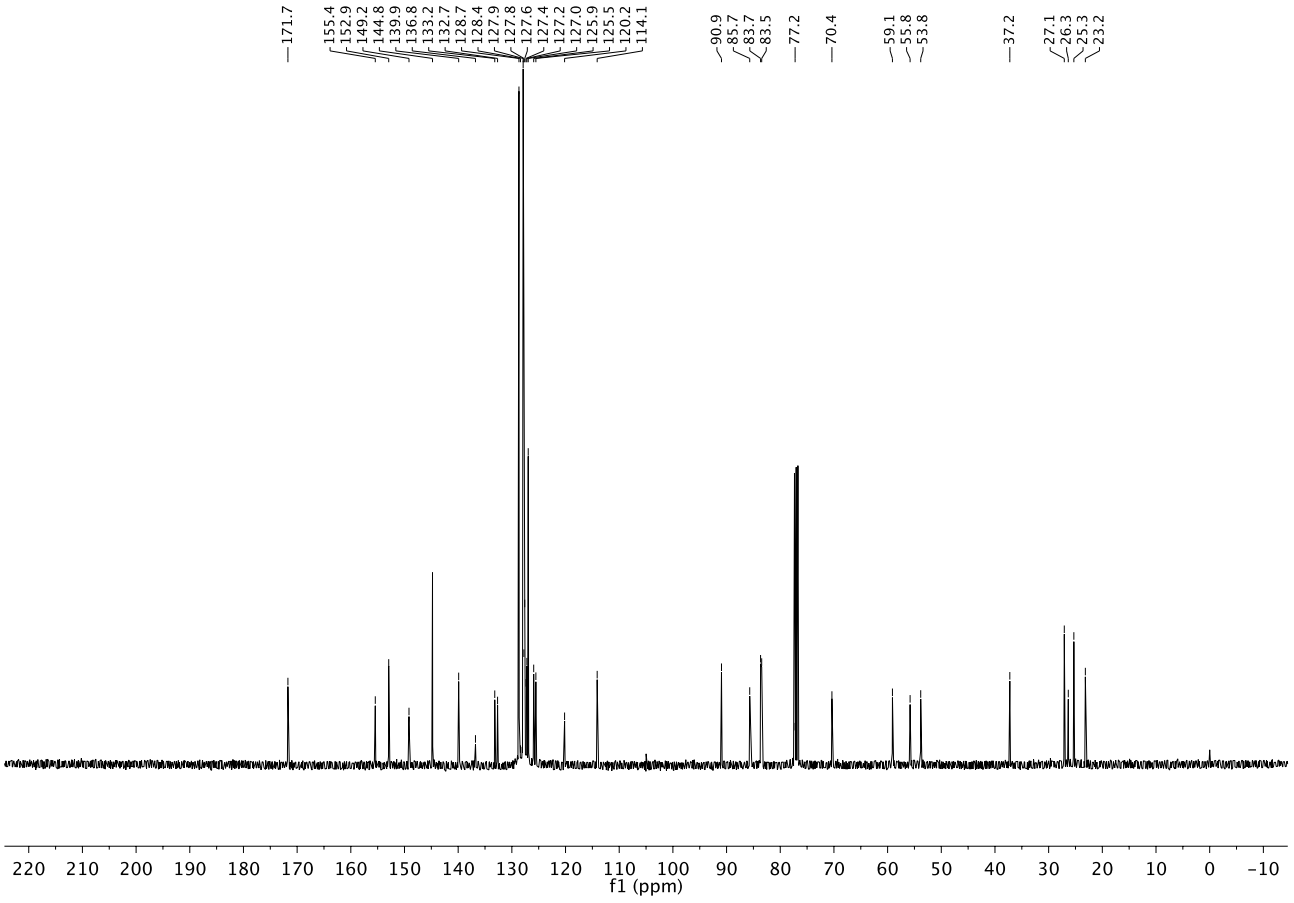
^{13}C NMR ($CDCl_3$)



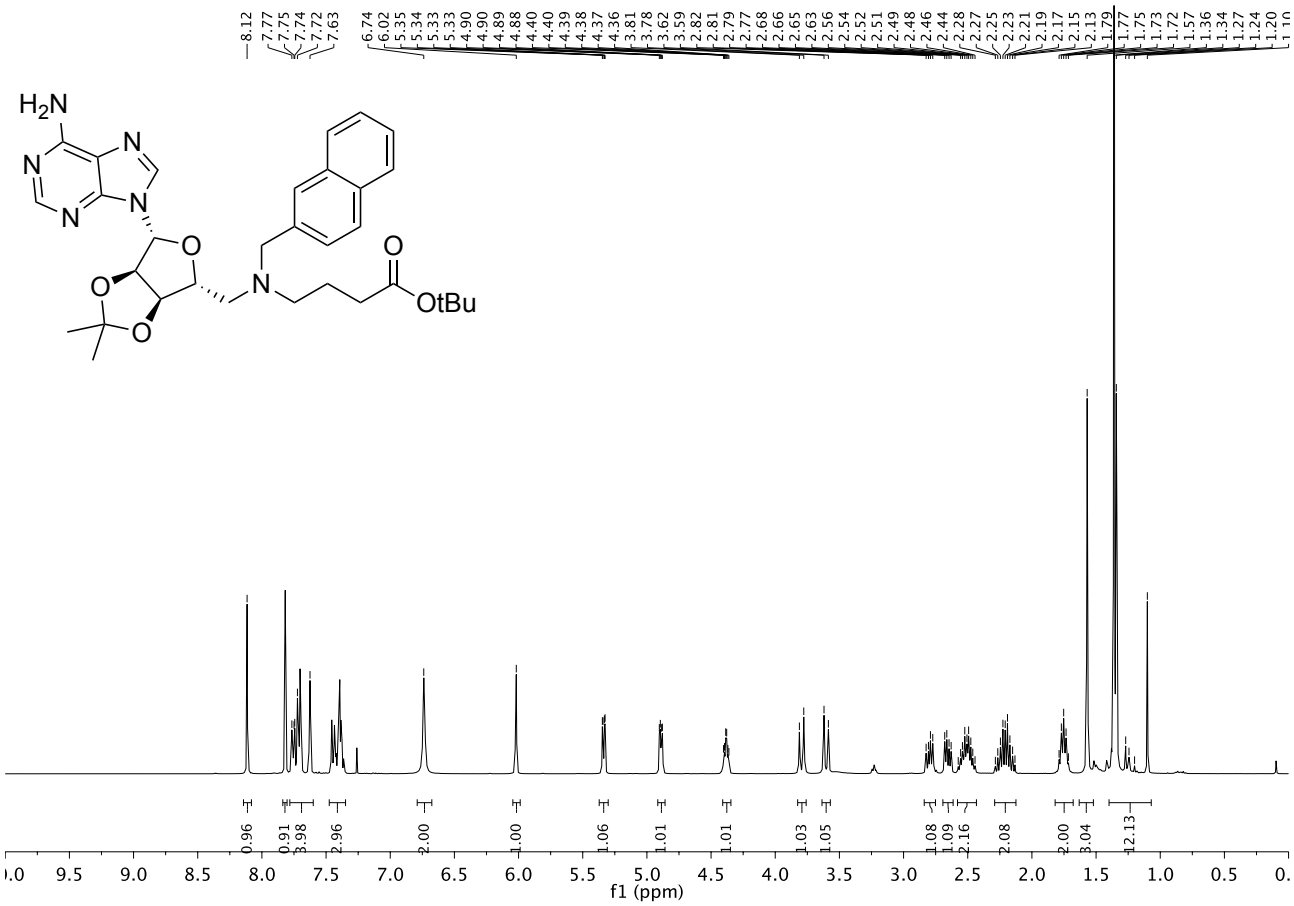
Compound **52** ^{1}H NMR ($CDCl_3$)



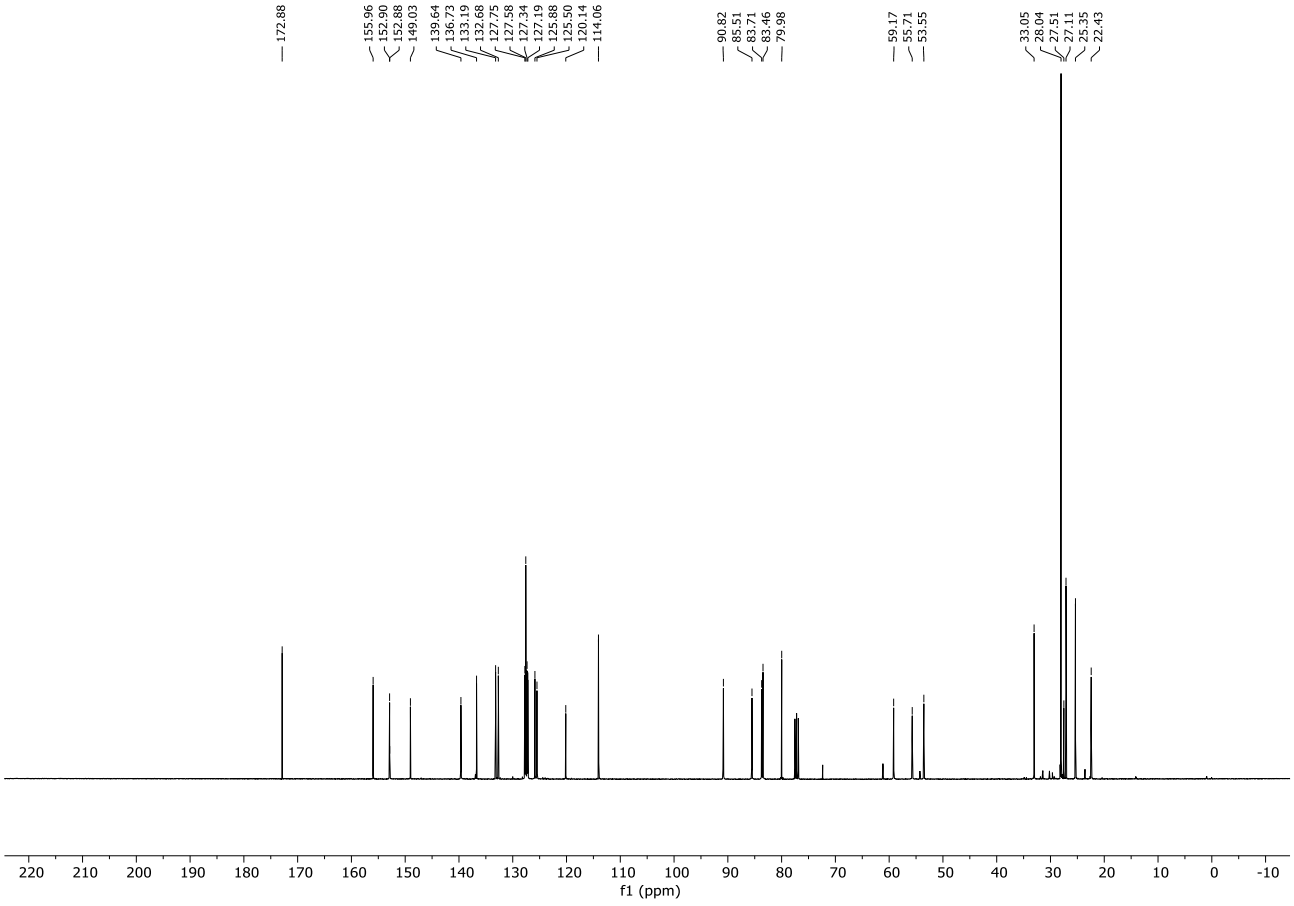
^{13}C NMR ($CDCl_3$)



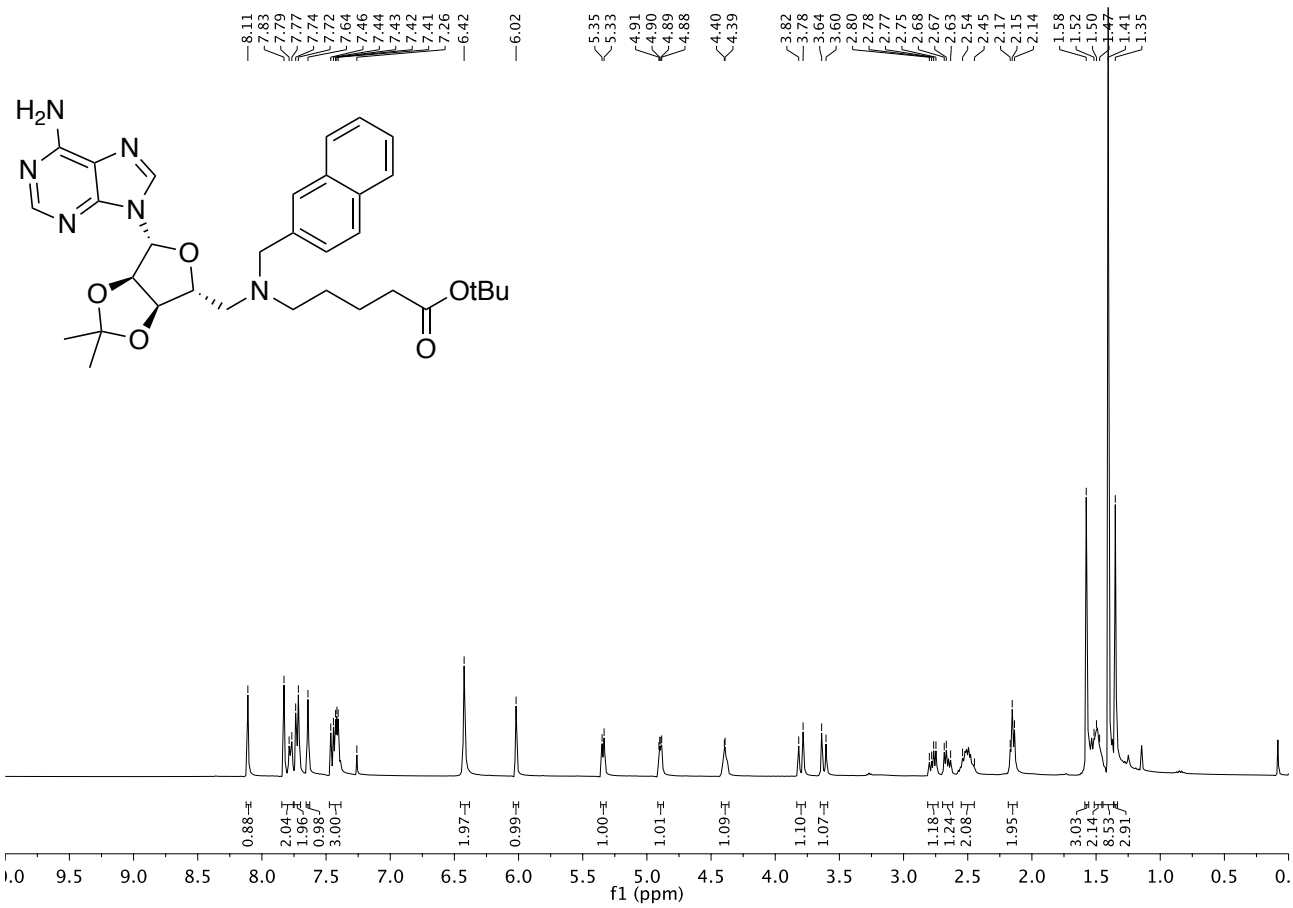
Compound **53** ^{1}H NMR ($CDCl_3$)



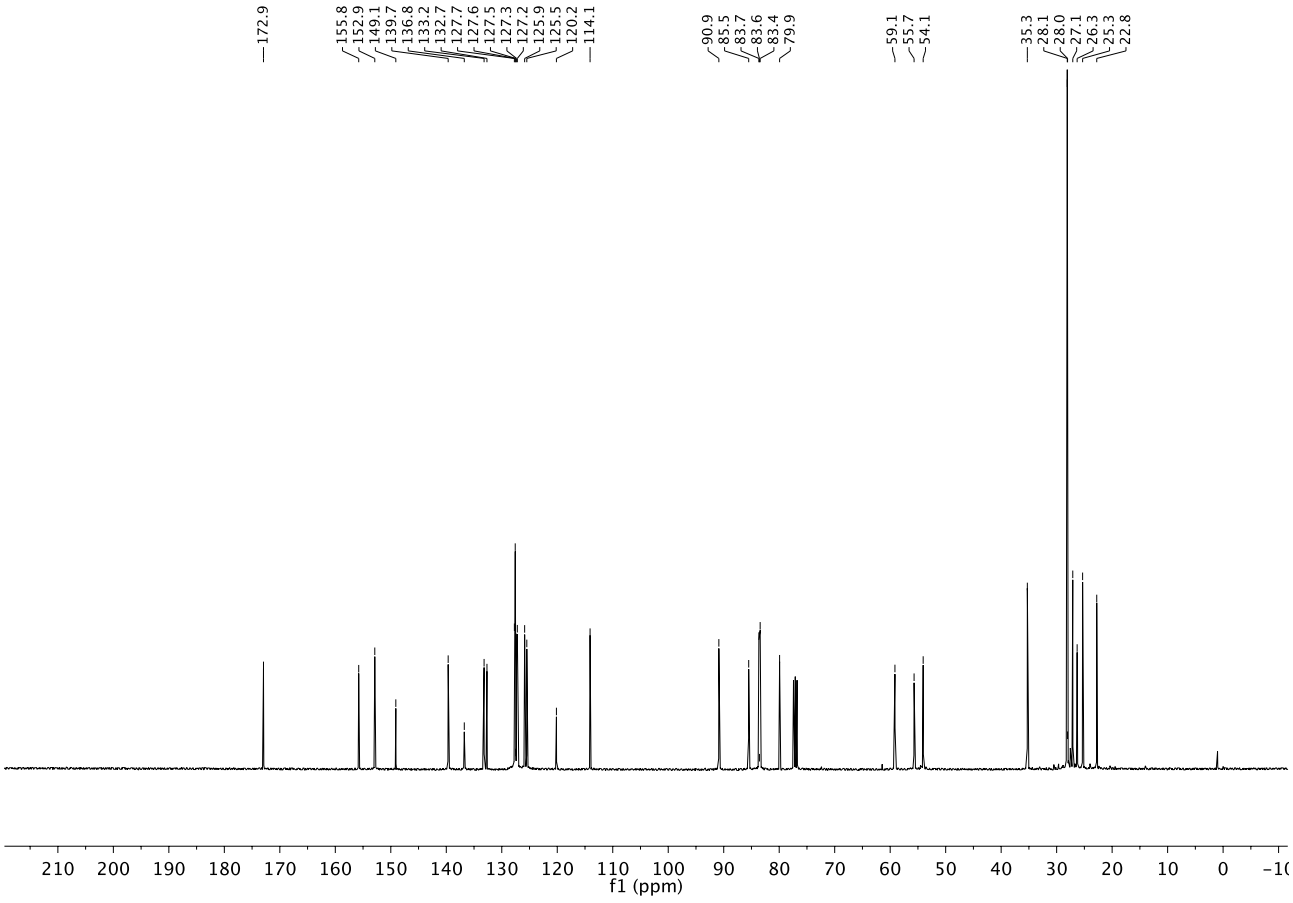
^{13}C NMR ($CDCl_3$)



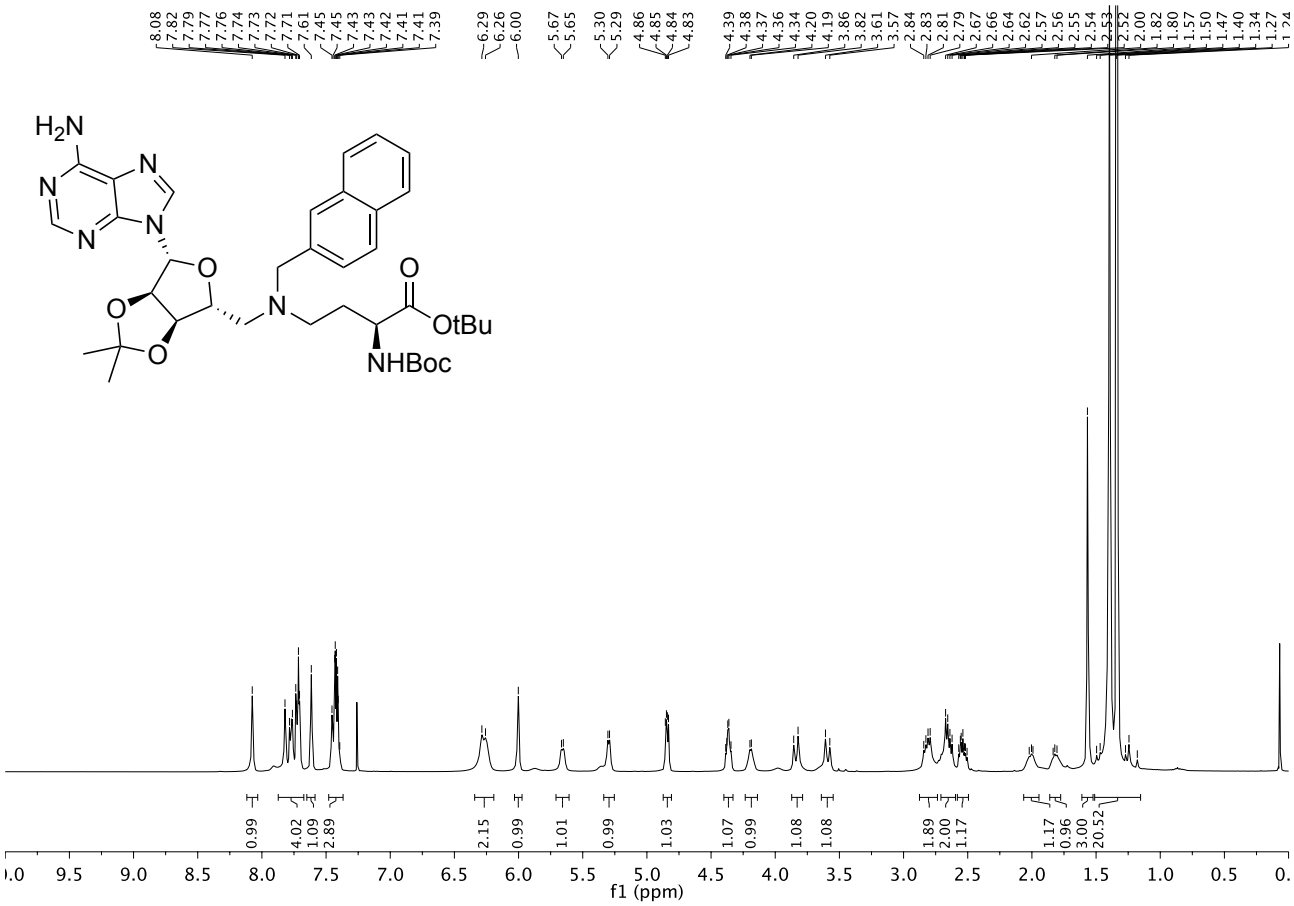
Compound **54** ^{1}H NMR ($CDCl_3$)



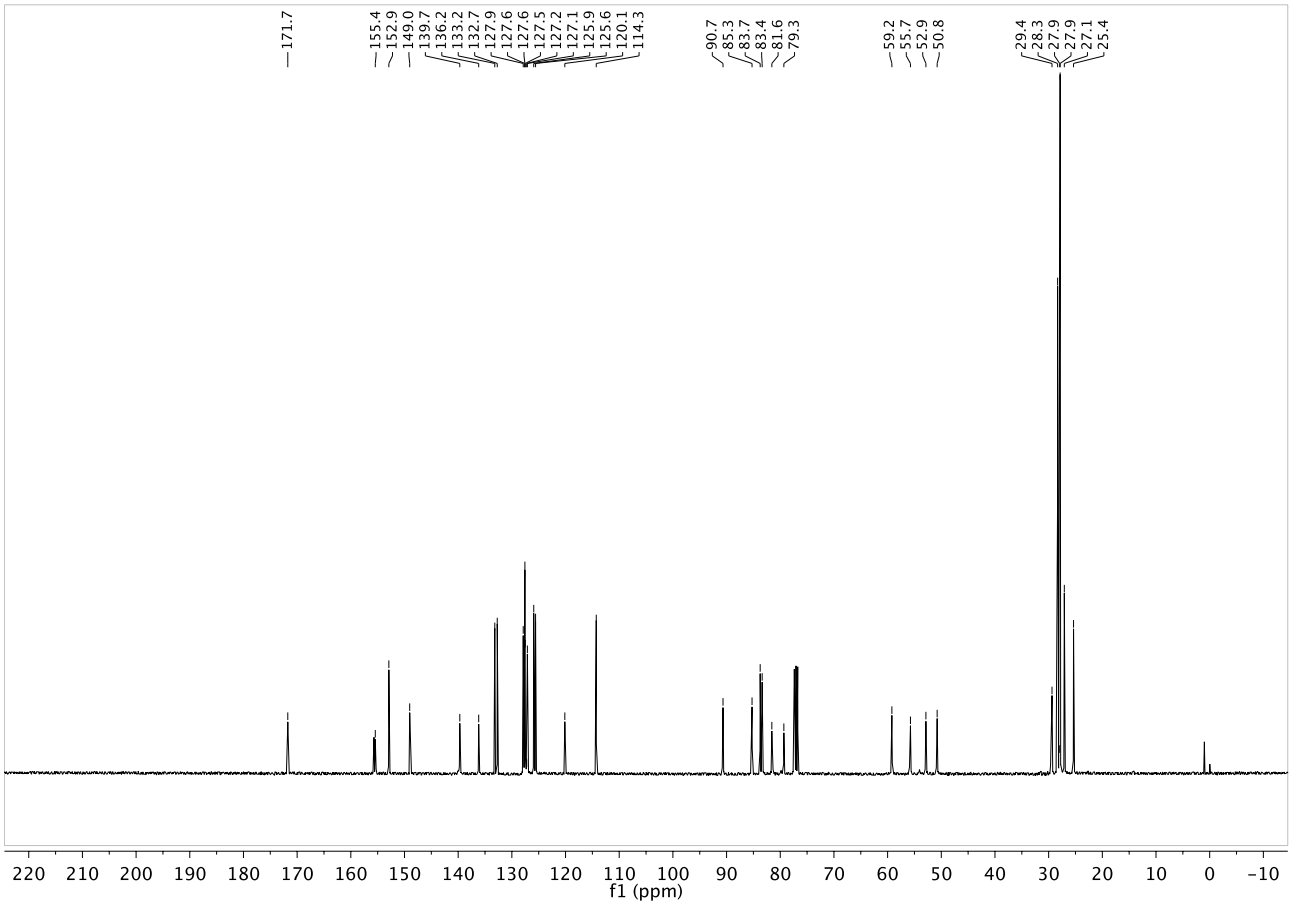
^{13}C NMR ($CDCl_3$)



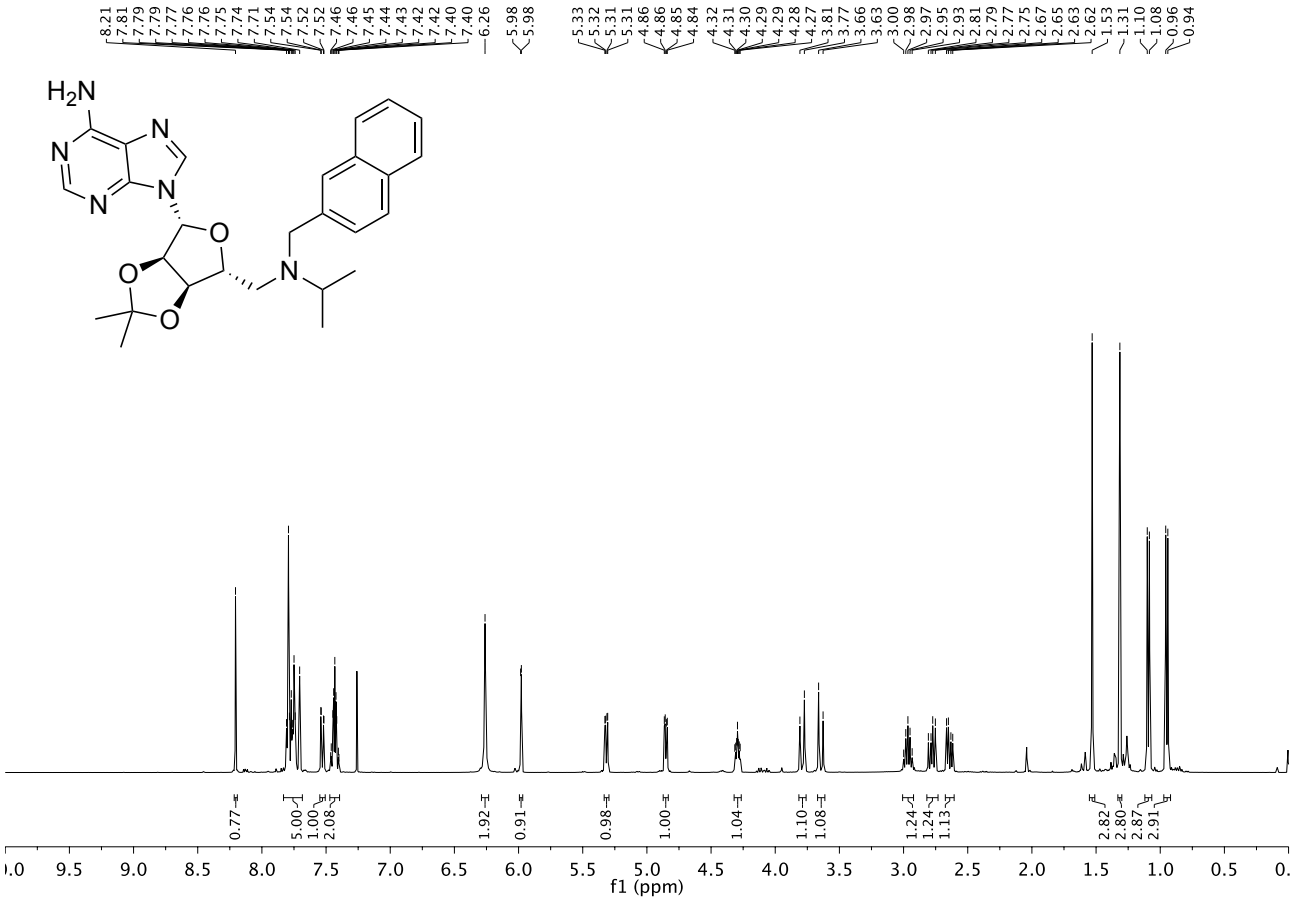
Compound **55** ^{1}H NMR ($CDCl_3$)



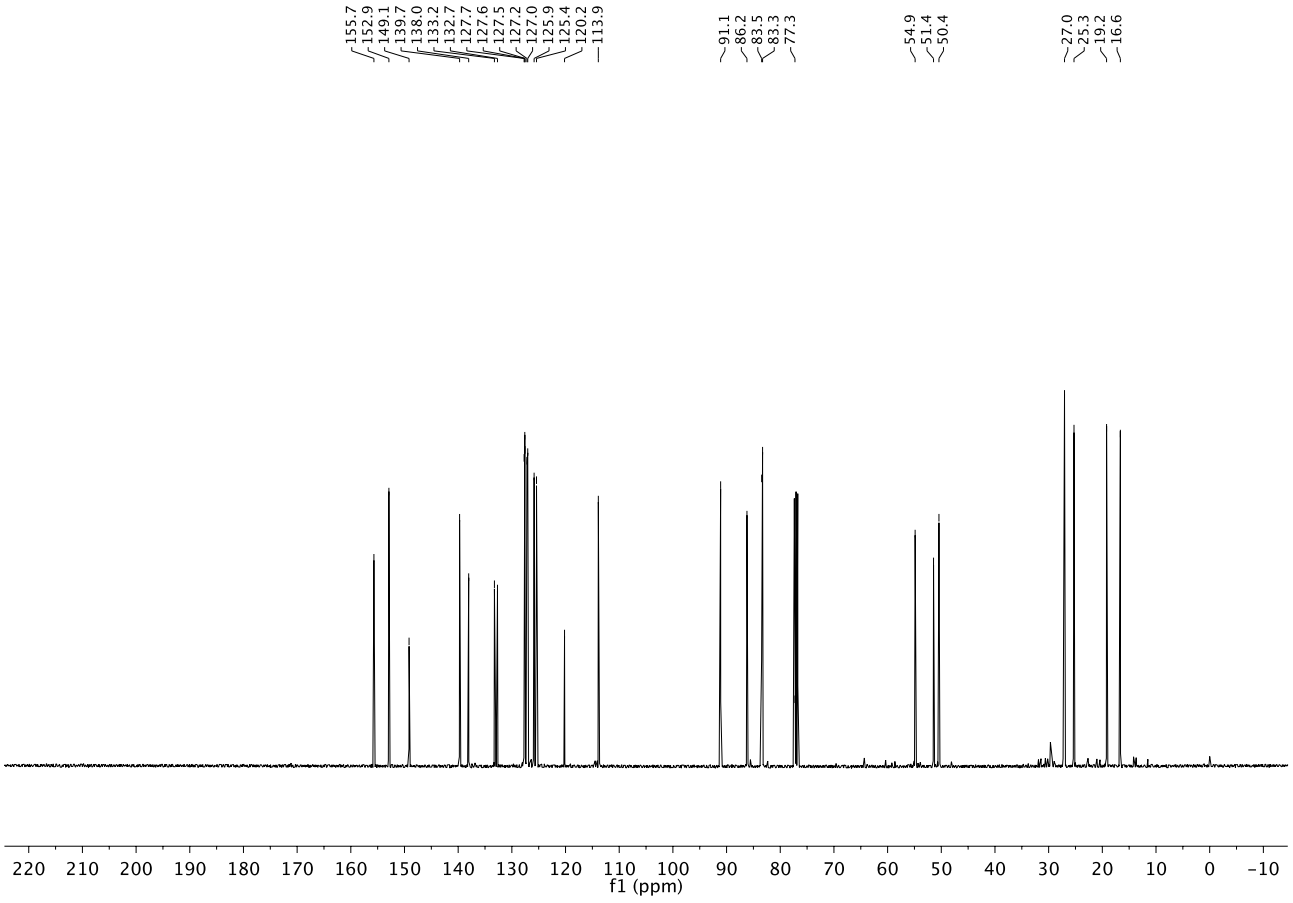
^{13}C NMR ($CDCl_3$)



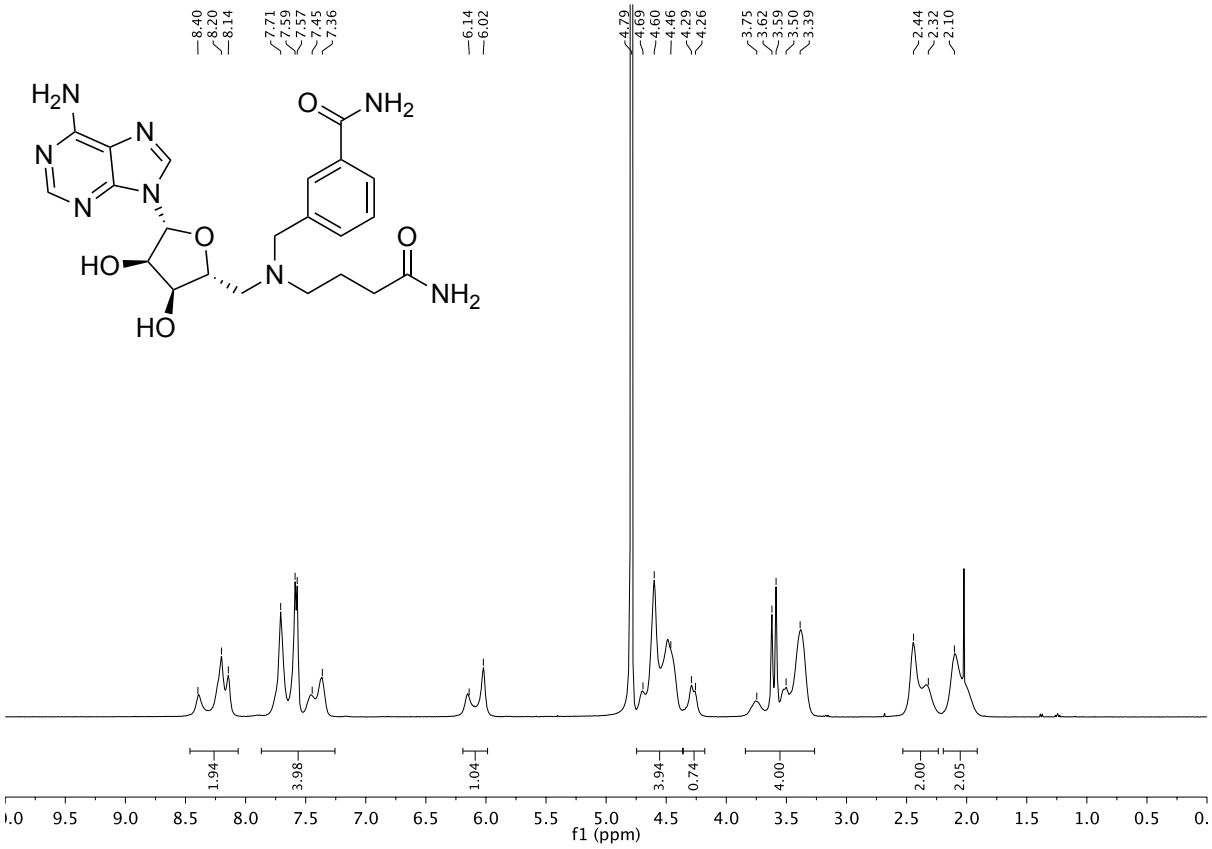
Compound **56** ^{1}H NMR ($CDCl_3$)



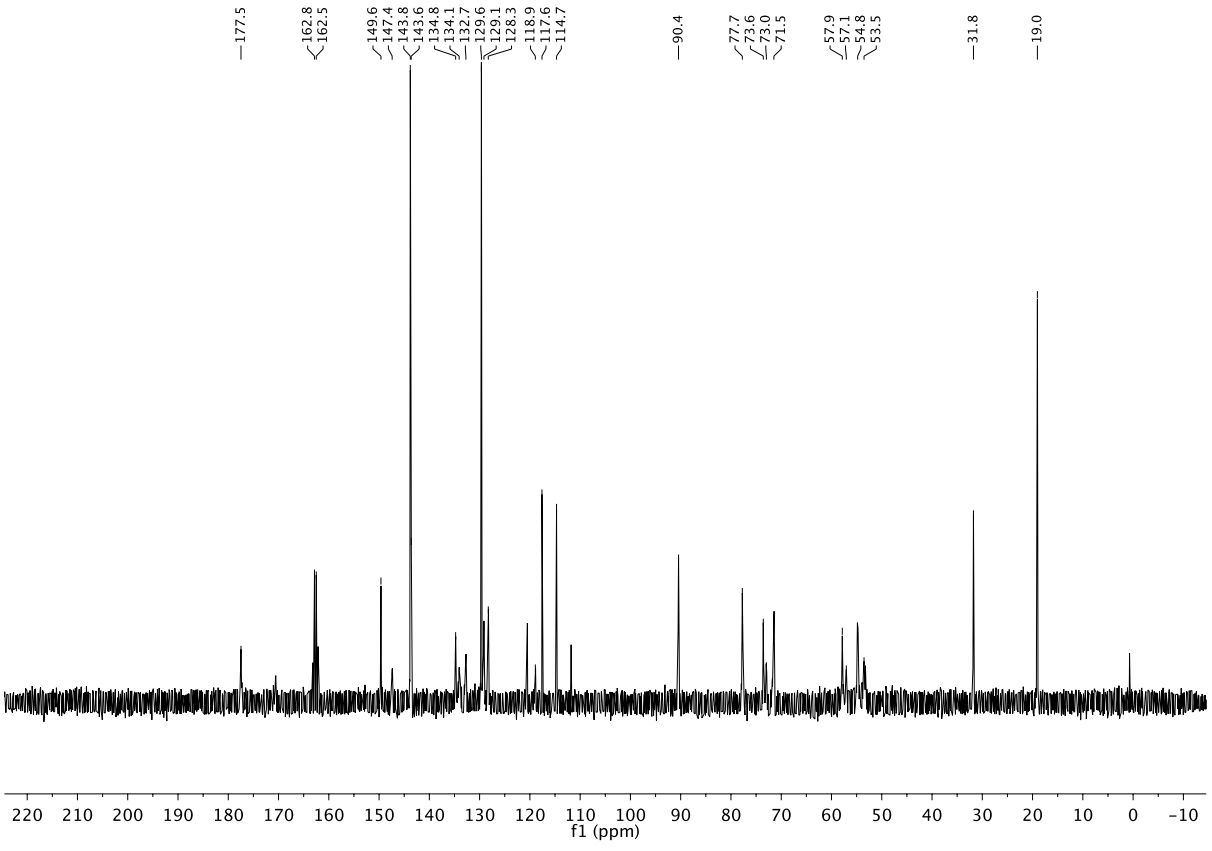
^{13}C NMR ($CDCl_3$)



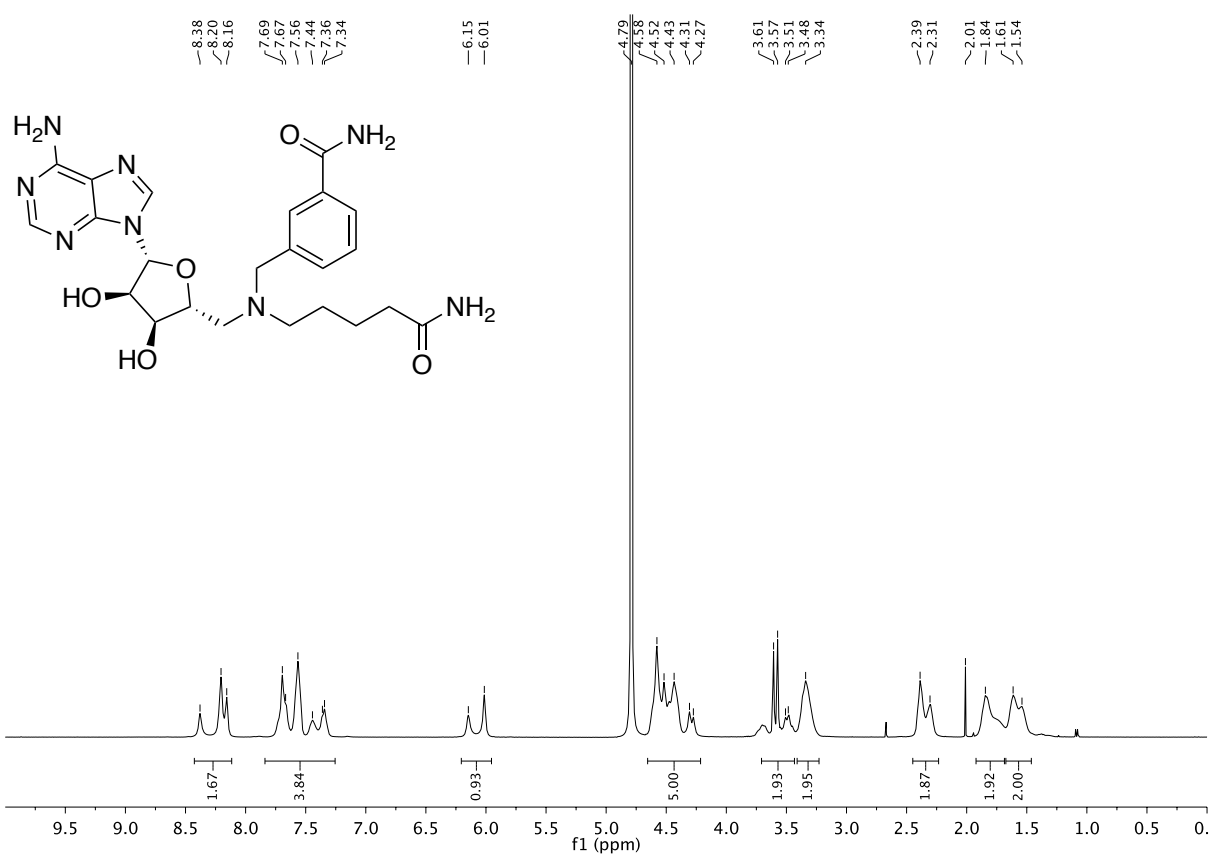
Compound **57** ^{1}H NMR (D_2O)



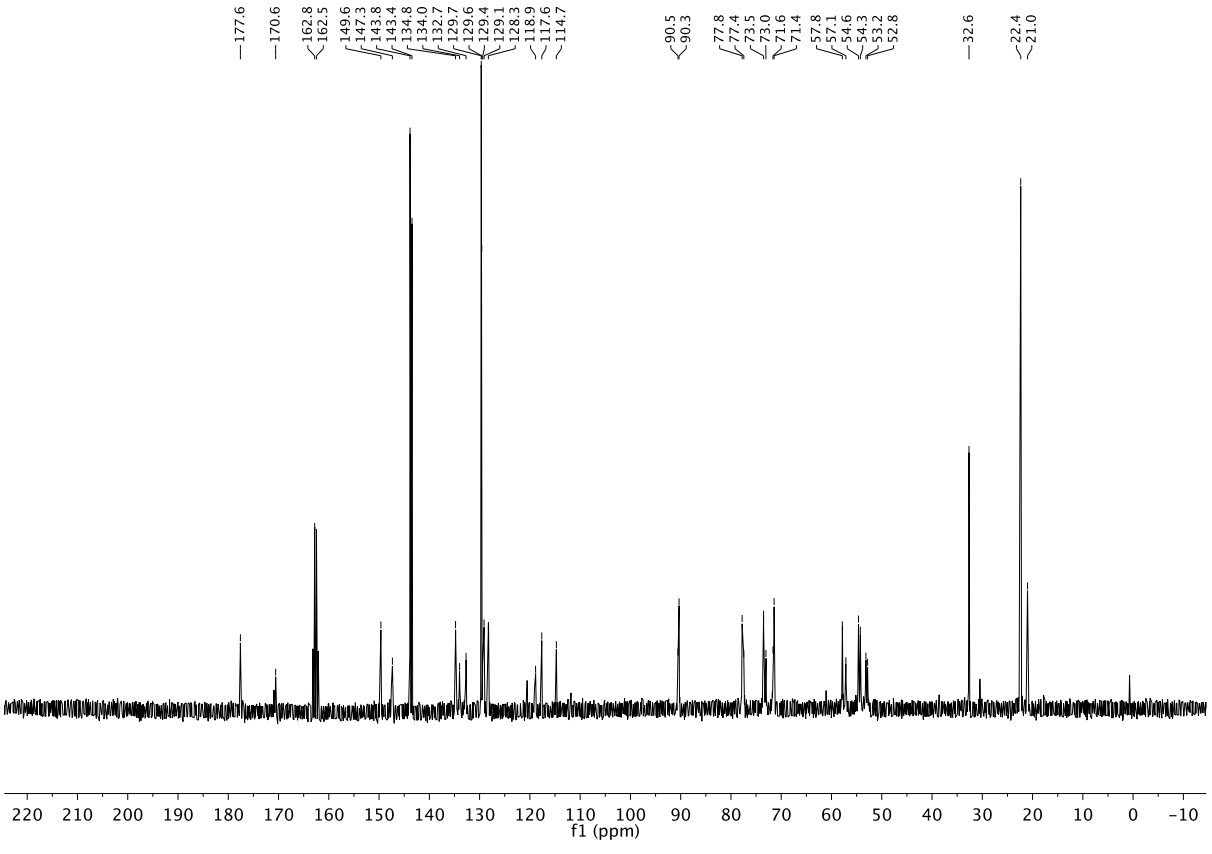
^{13}C NMR (D_2O)



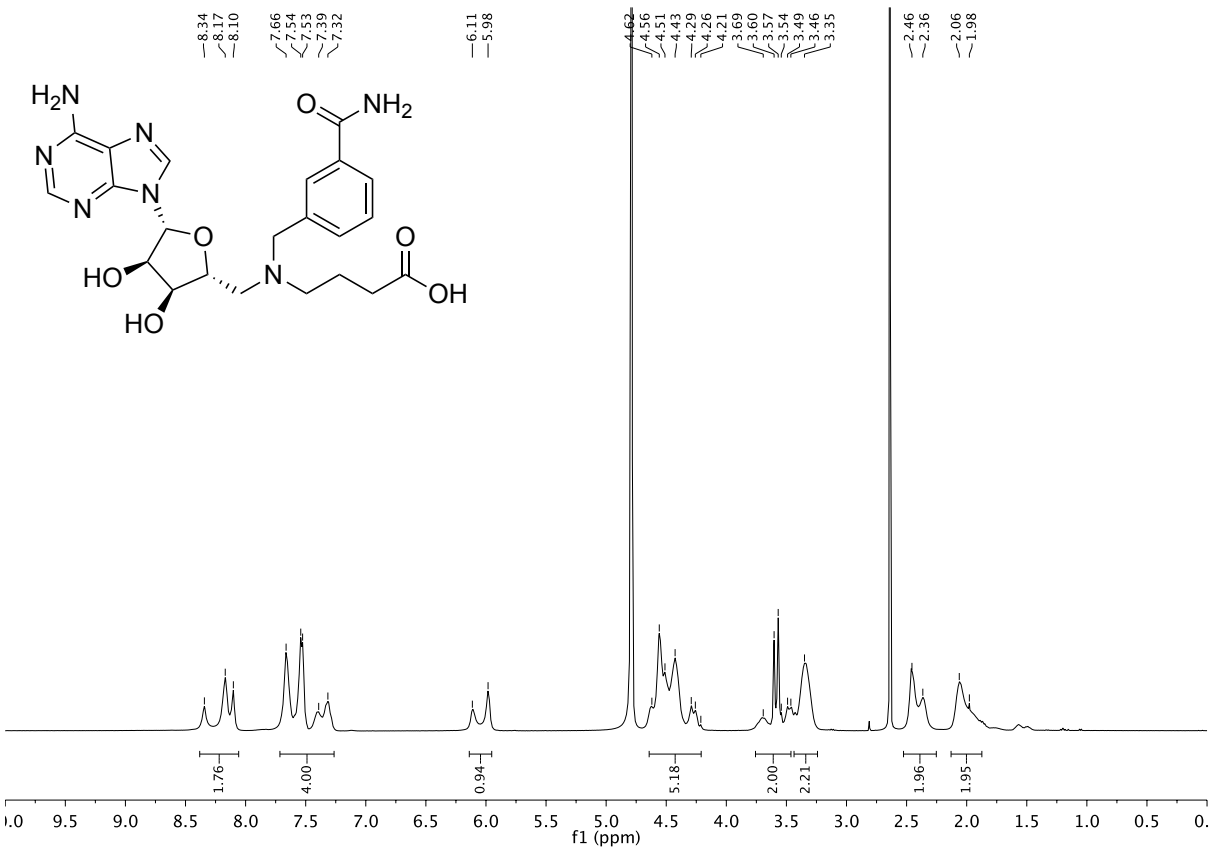
Compound **58** ^{1}H NMR (D_2O)



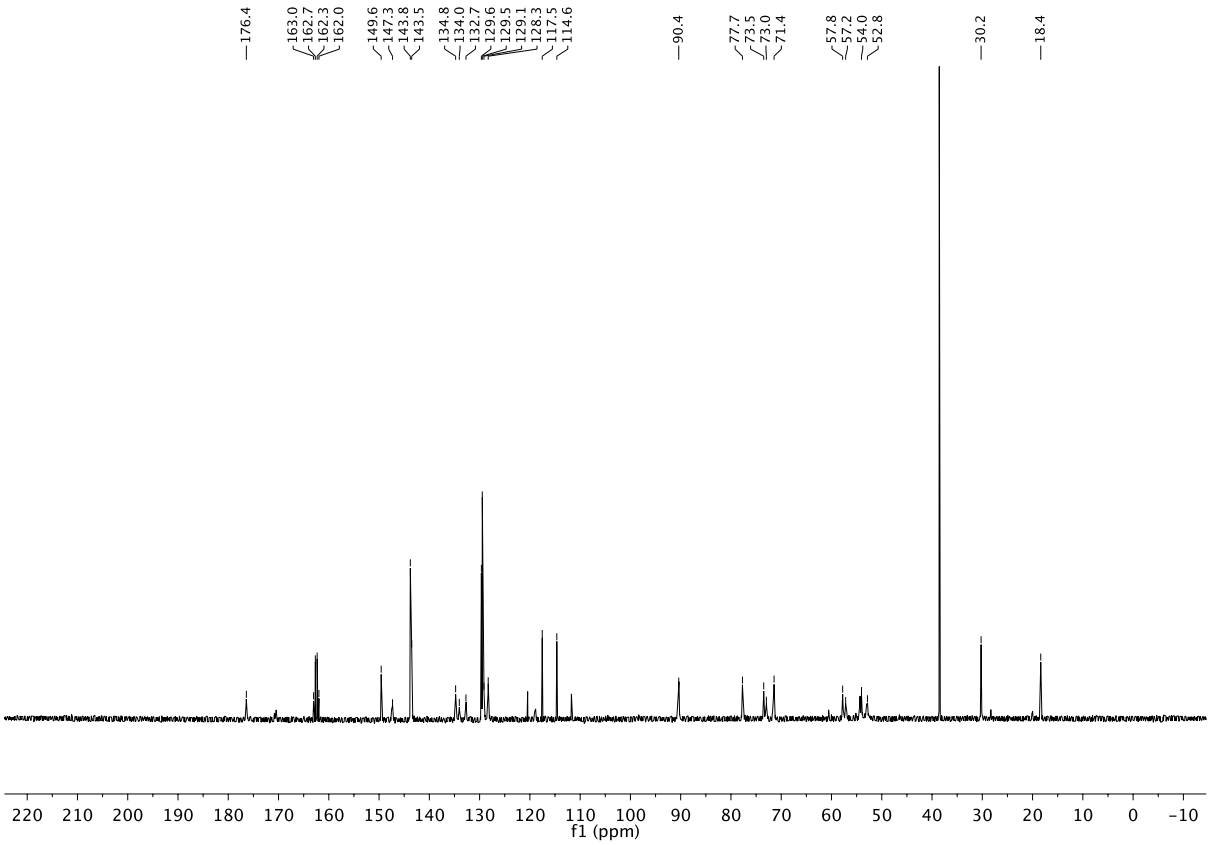
^{13}C NMR (D_2O)



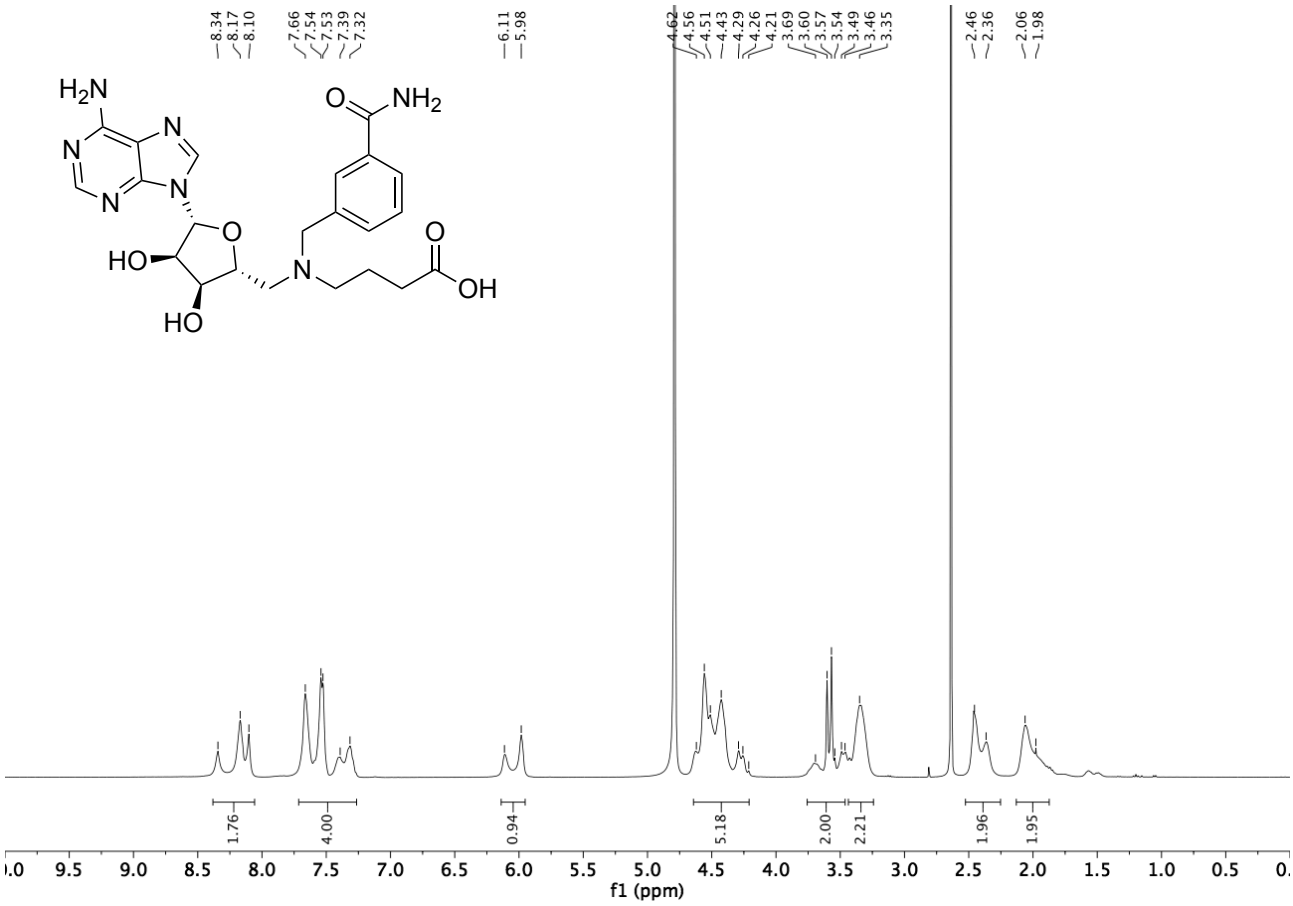
Compound **59** ^{1}H NMR (D_2O)



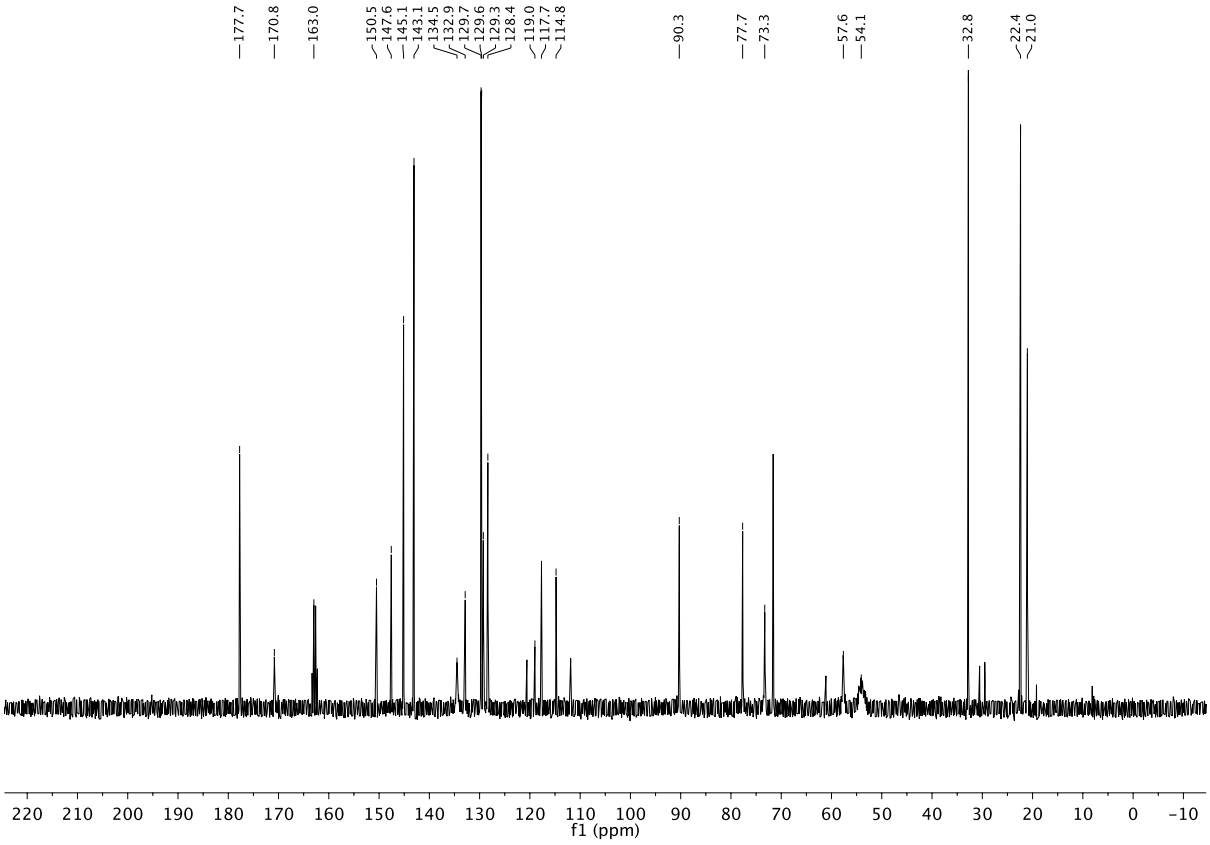
^{13}C NMR (D_2O)



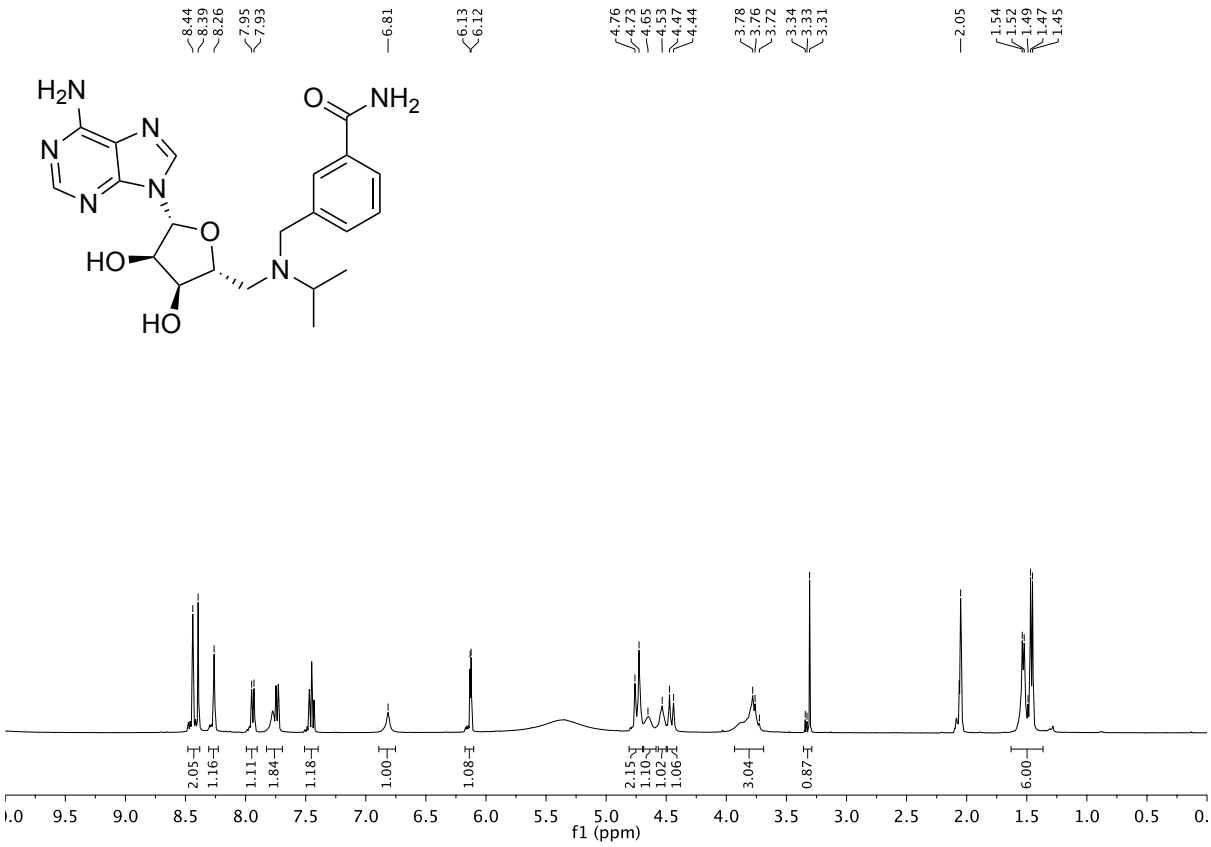
Compound **60** ^{1}H NMR (D_2O)



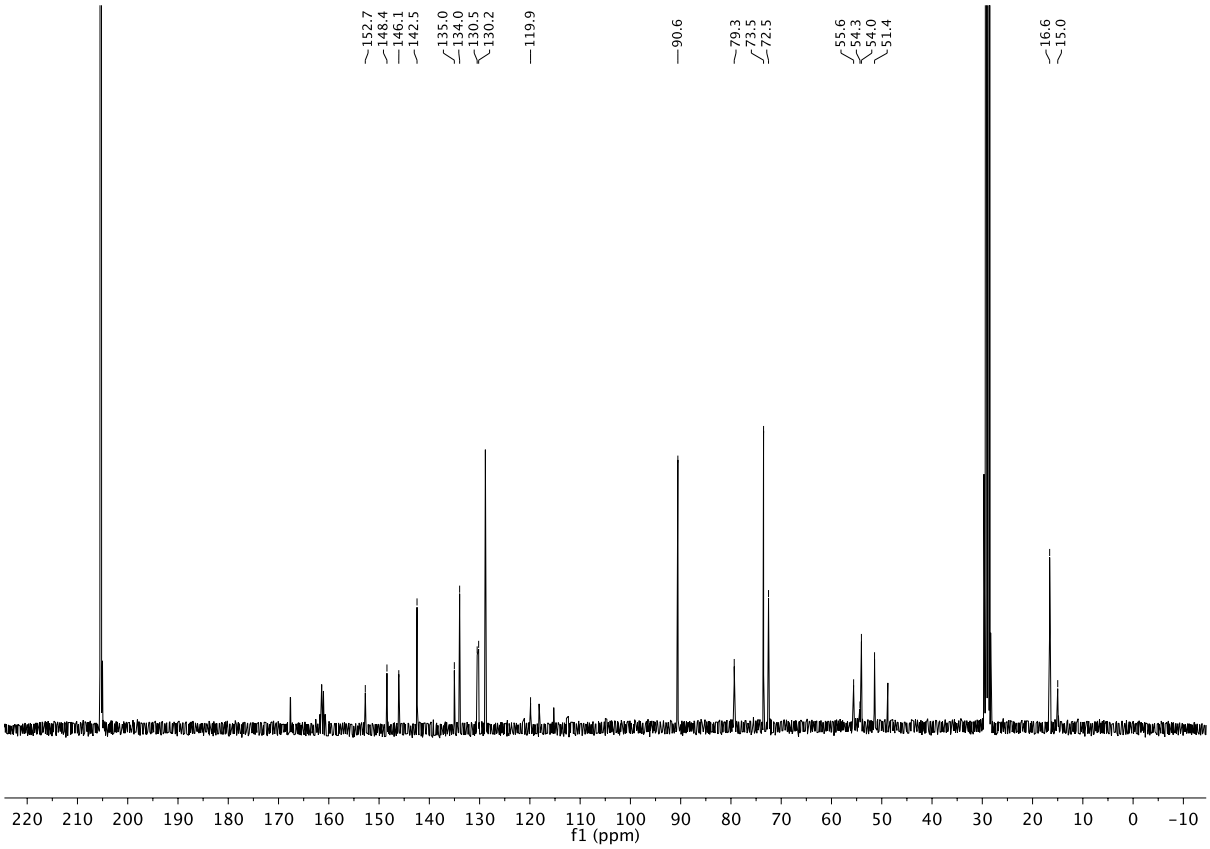
^{13}C NMR (D_2O)



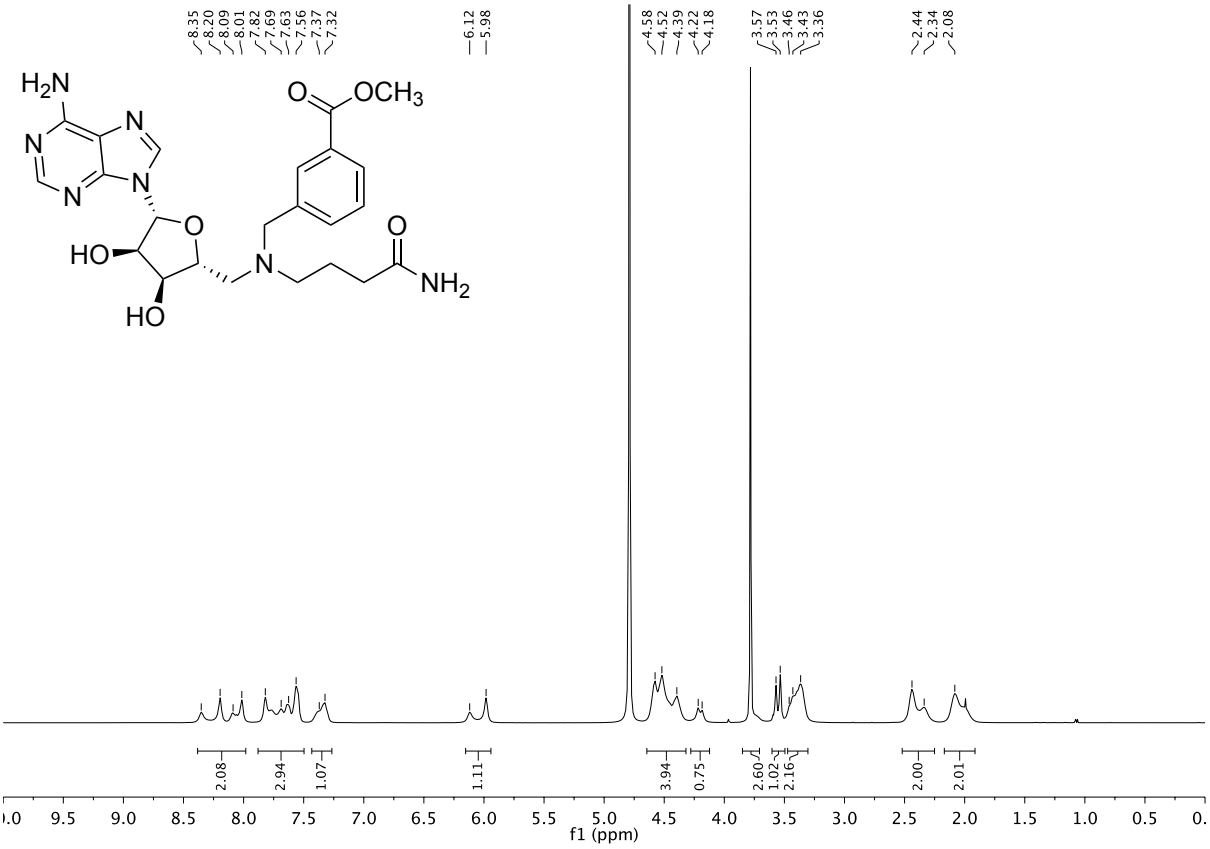
Compound **61** ^{1}H NMR (Acetone-d_6)



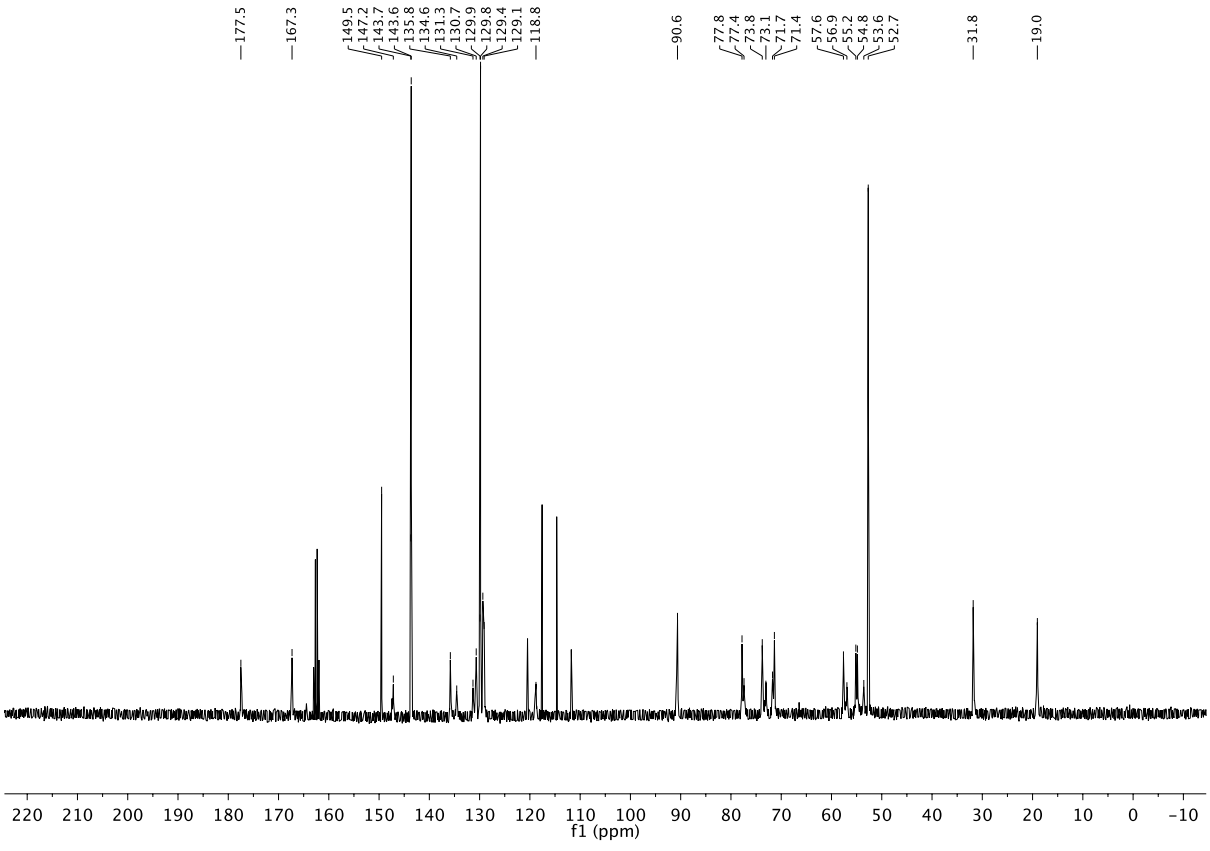
^{13}C NMR (Acetone-d_6)



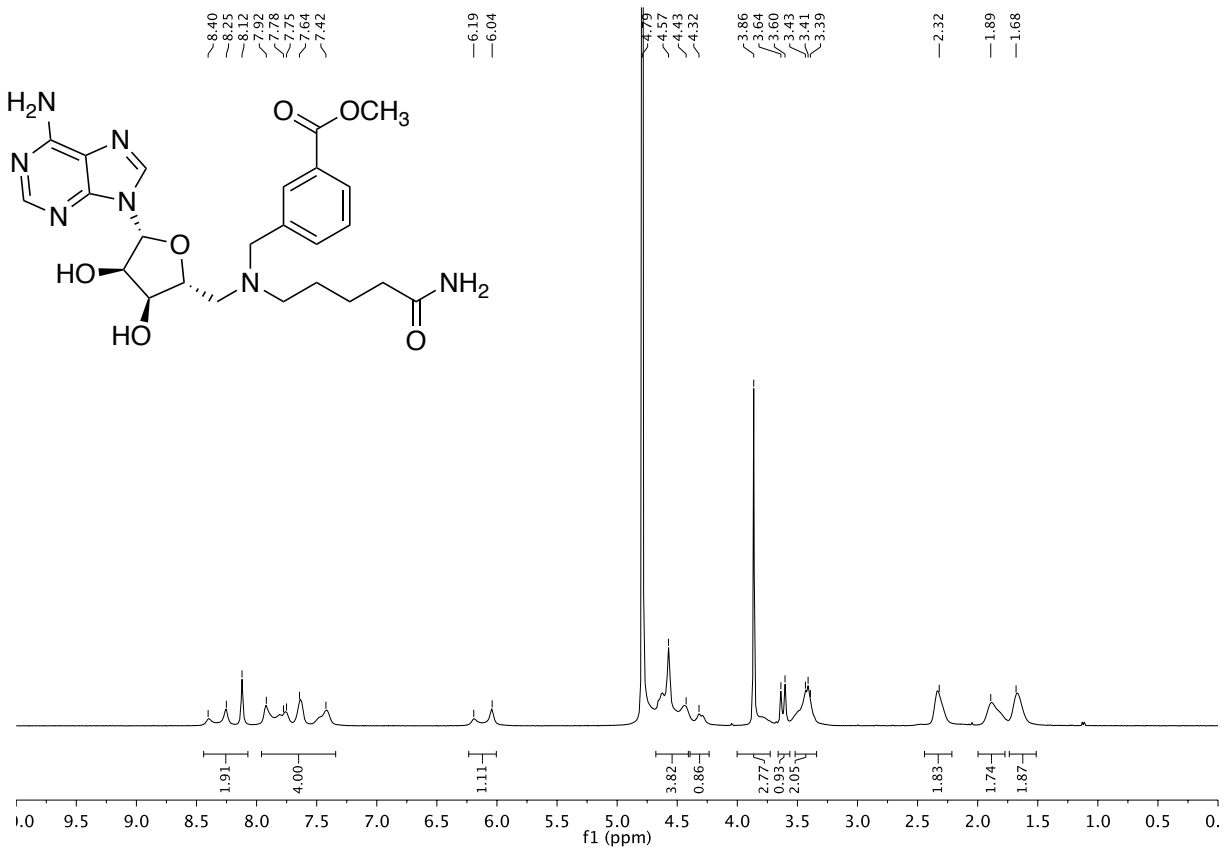
Compound **62** ^{1}H NMR (D_2O)



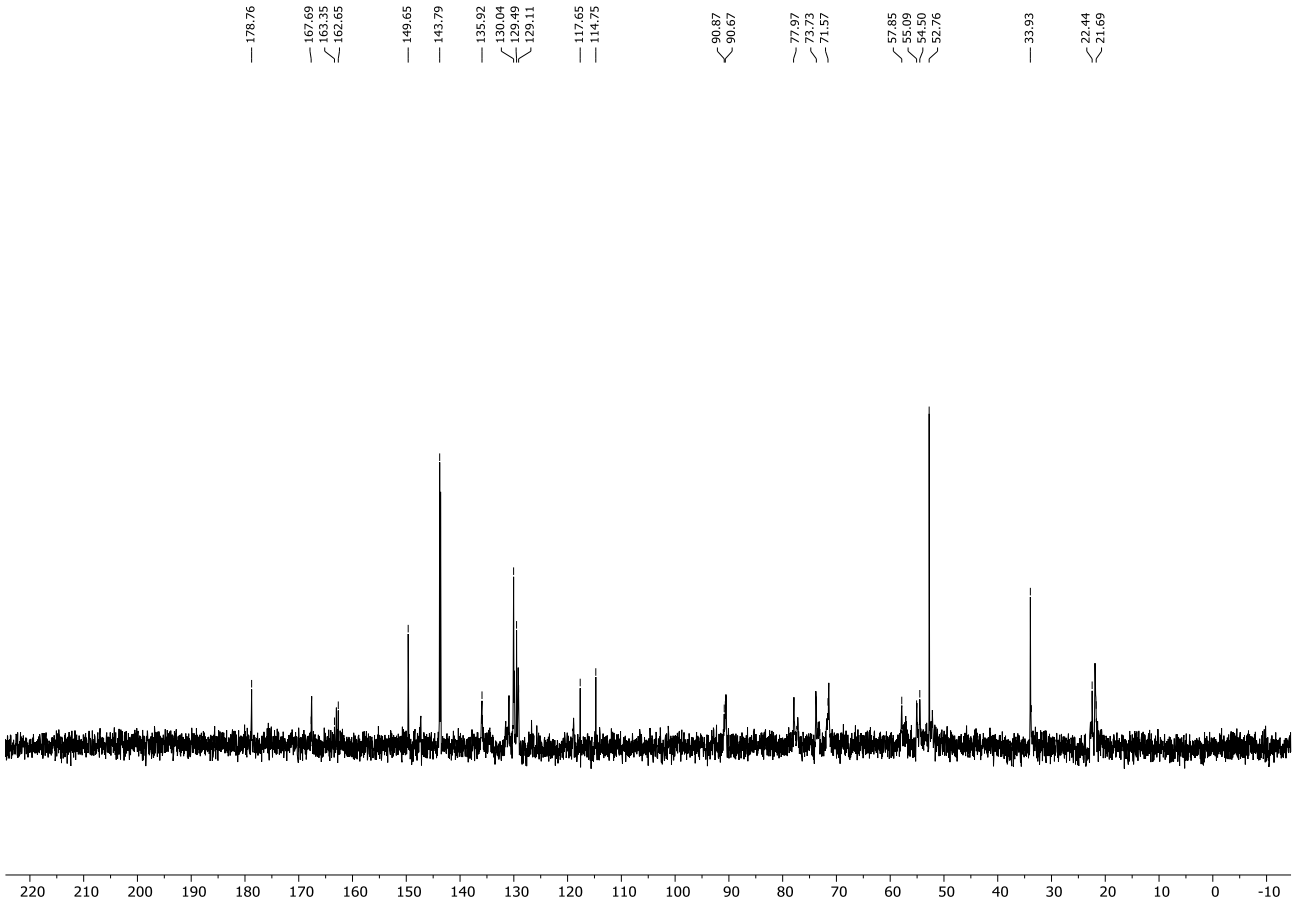
^{13}C NMR (D_2O)



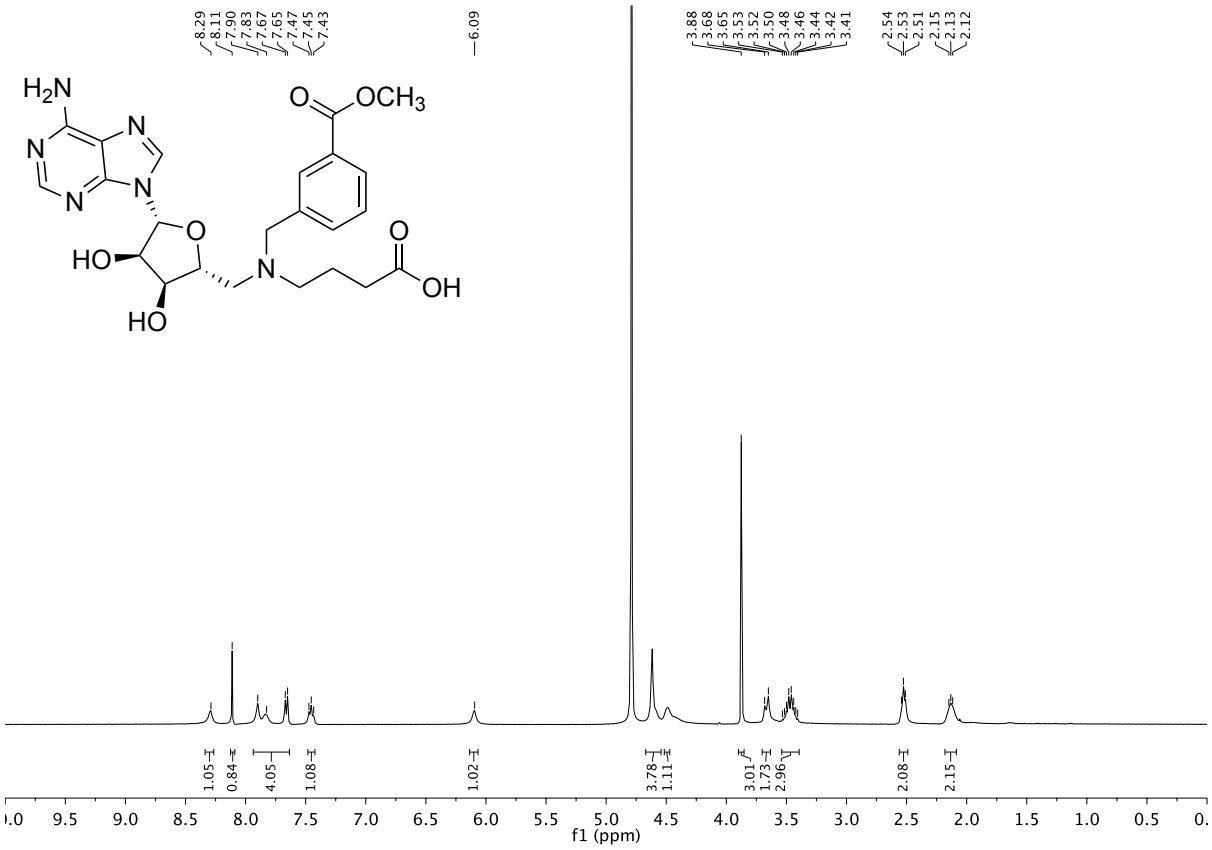
Compound **63** ^{1}H NMR (D_2O)



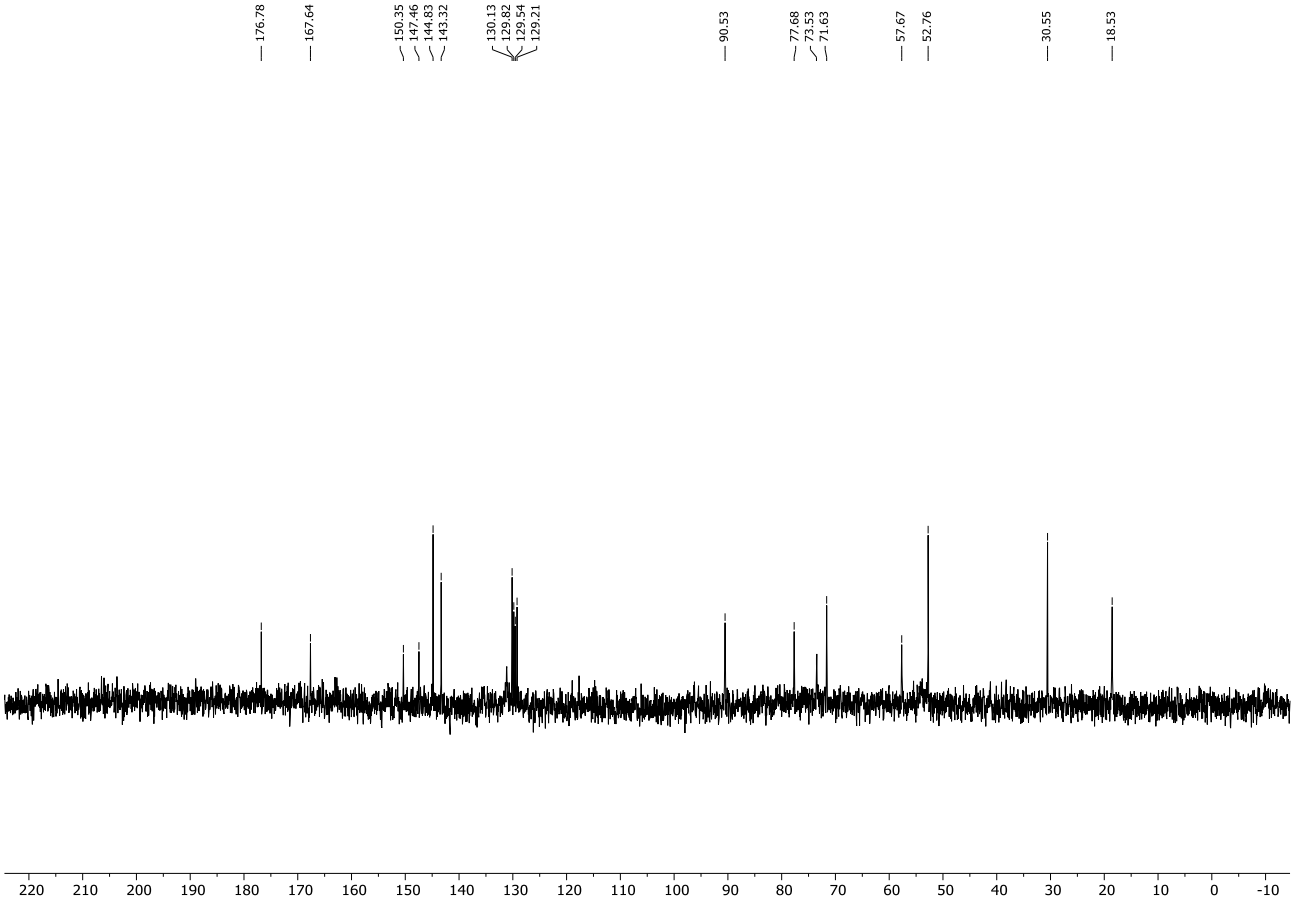
^{13}C NMR (D_2O)



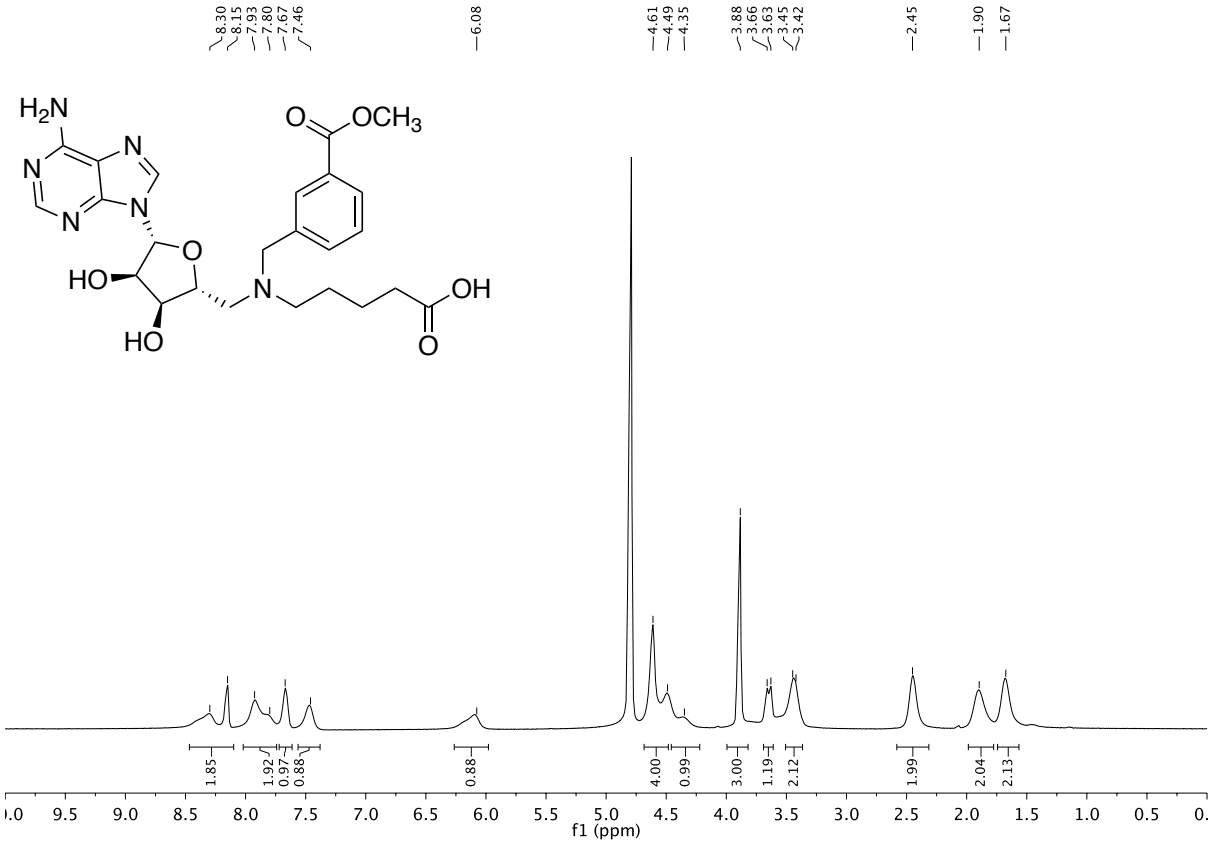
Compound **64** ^{1}H NMR (D_2O)



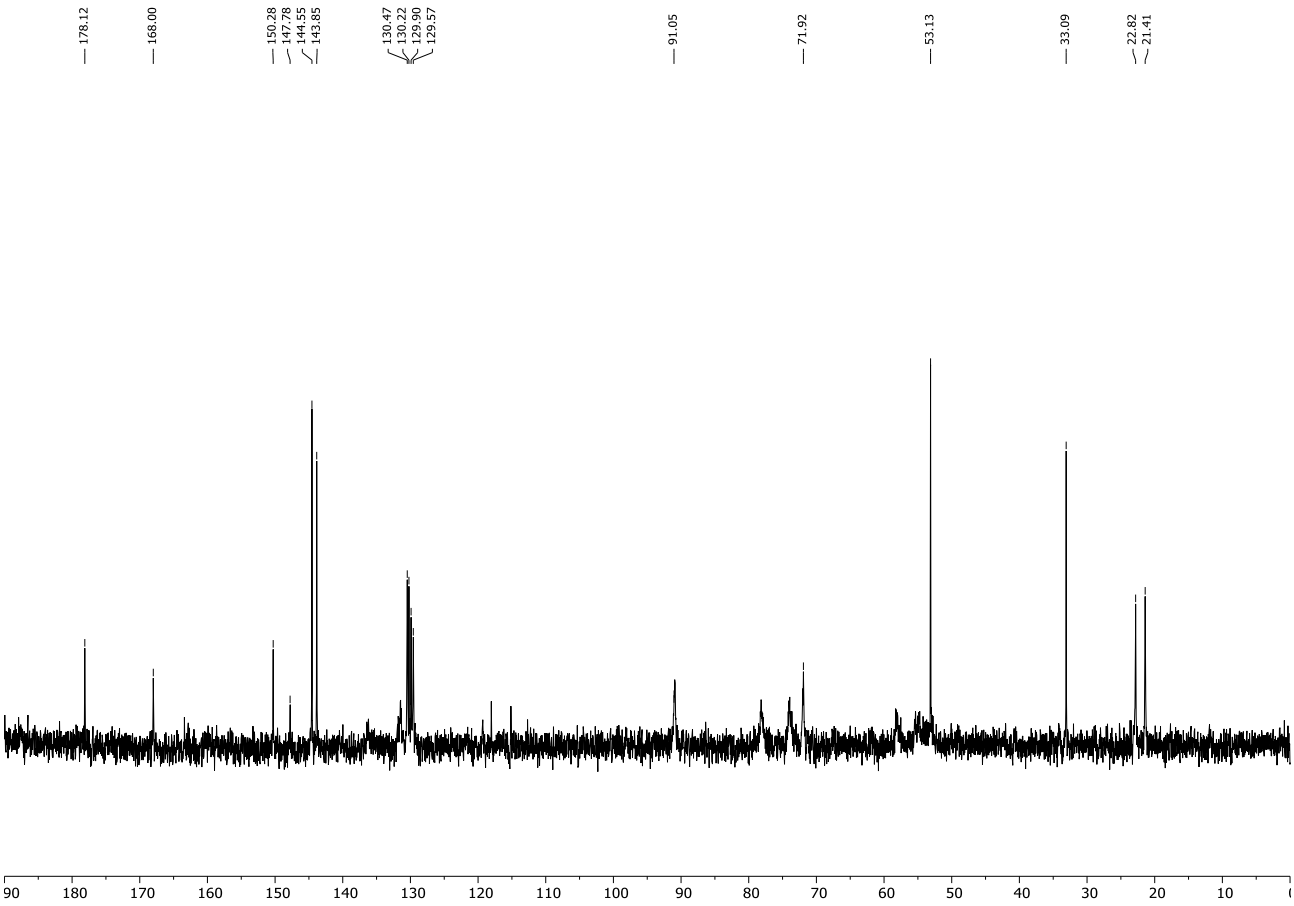
^{13}C NMR (D_2O)



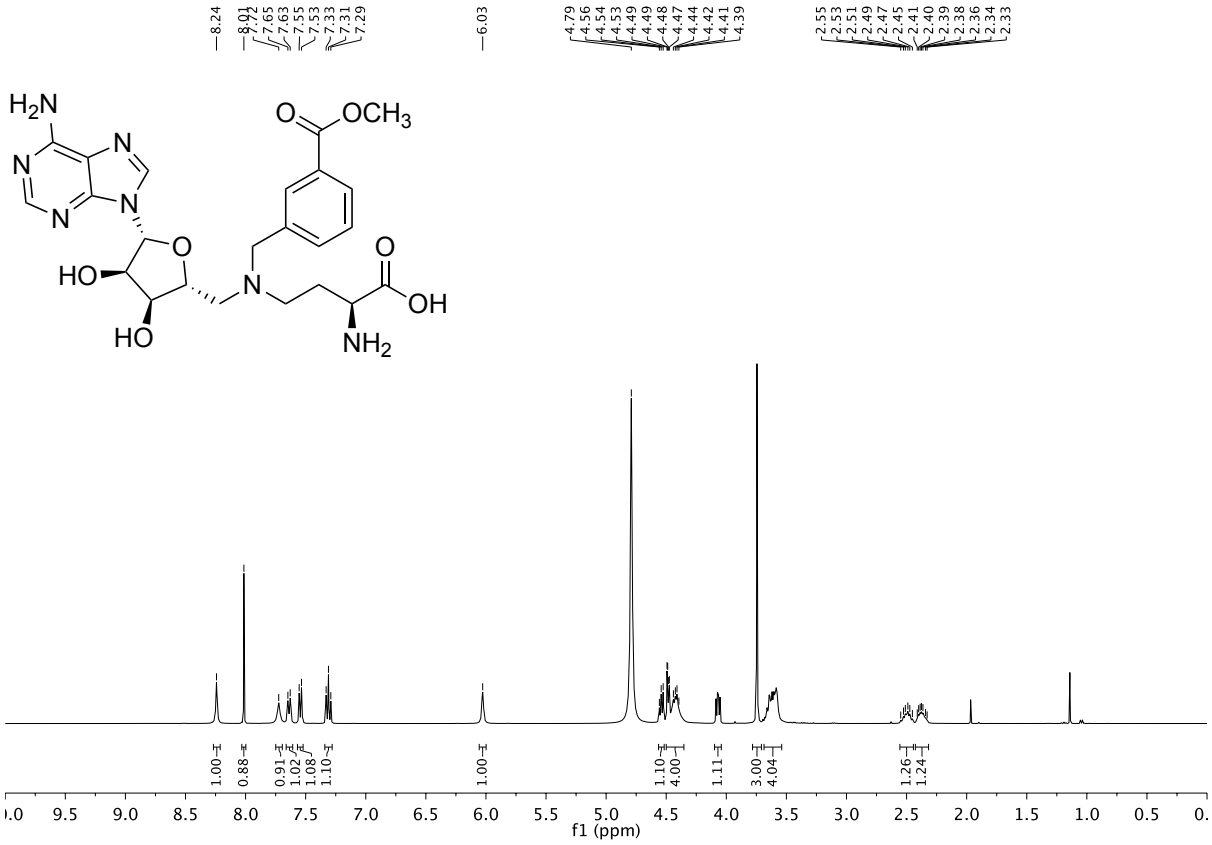
Compound **65** ^{1}H NMR (D_2O)



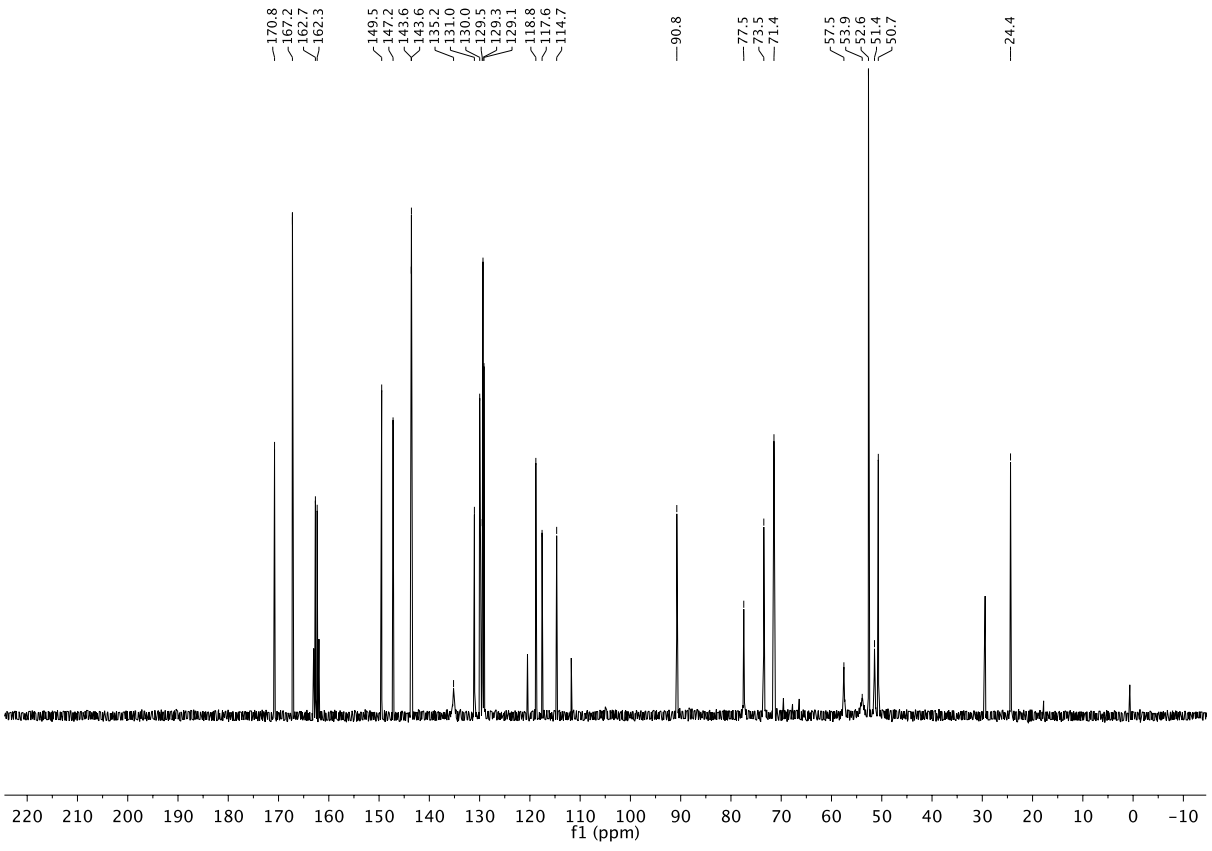
^{13}C NMR (D_2O)



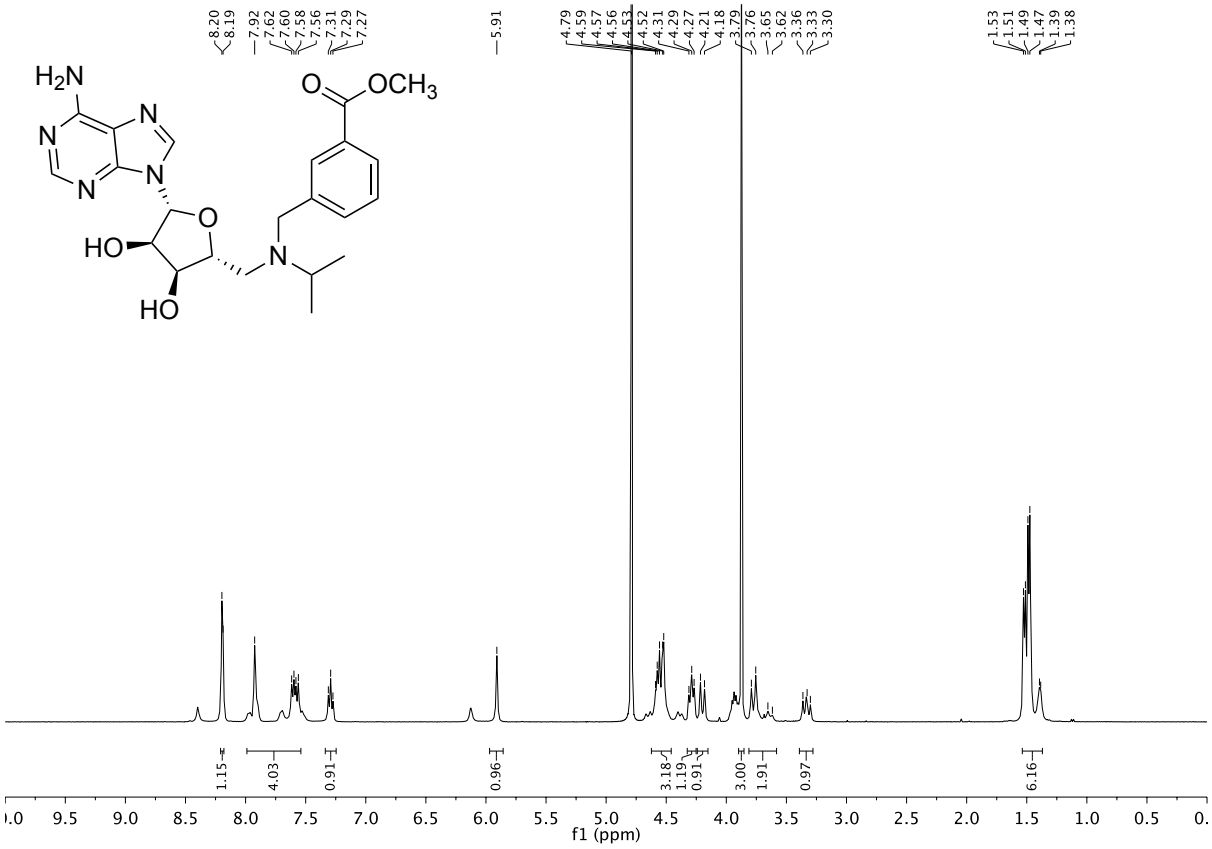
Compound **66** ^{1}H NMR (D_2O)



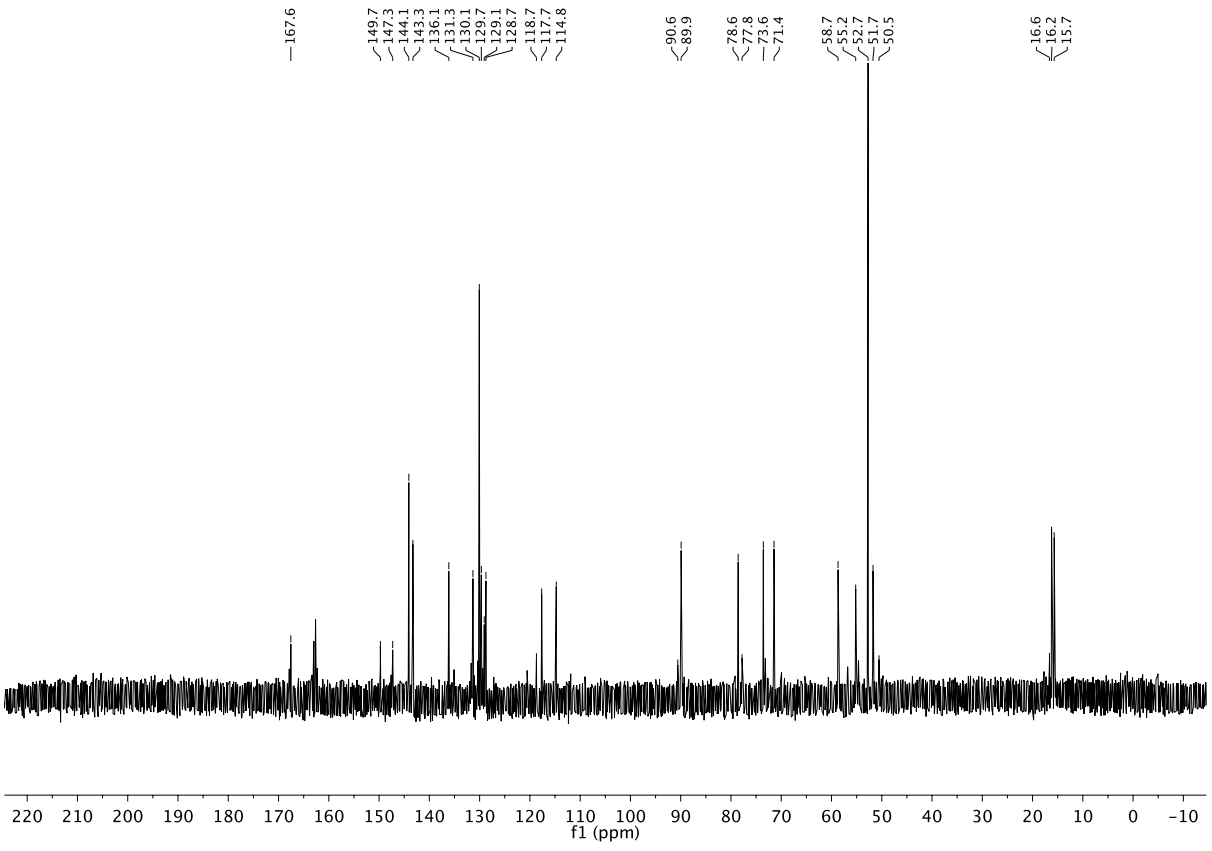
^{13}C NMR (D_2O)



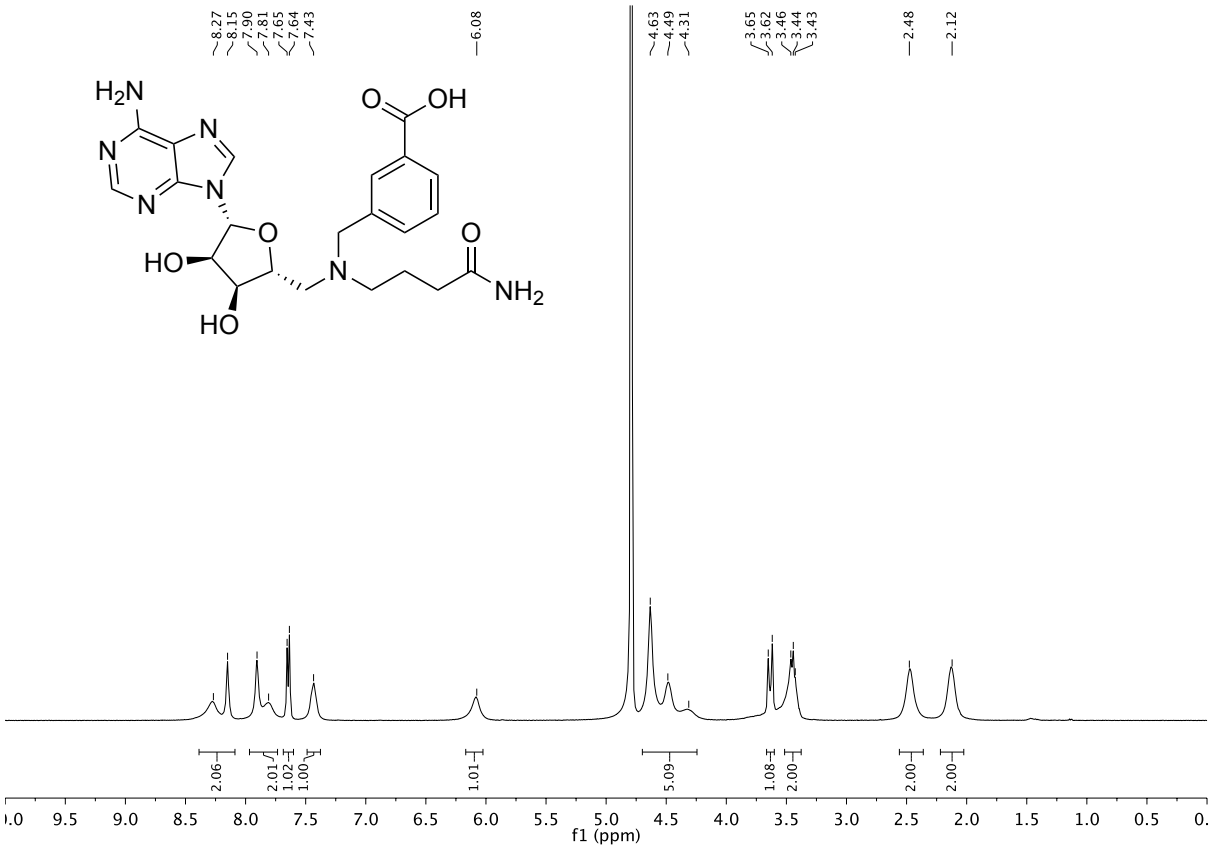
Compound **67** ^{1}H NMR (D_2O)



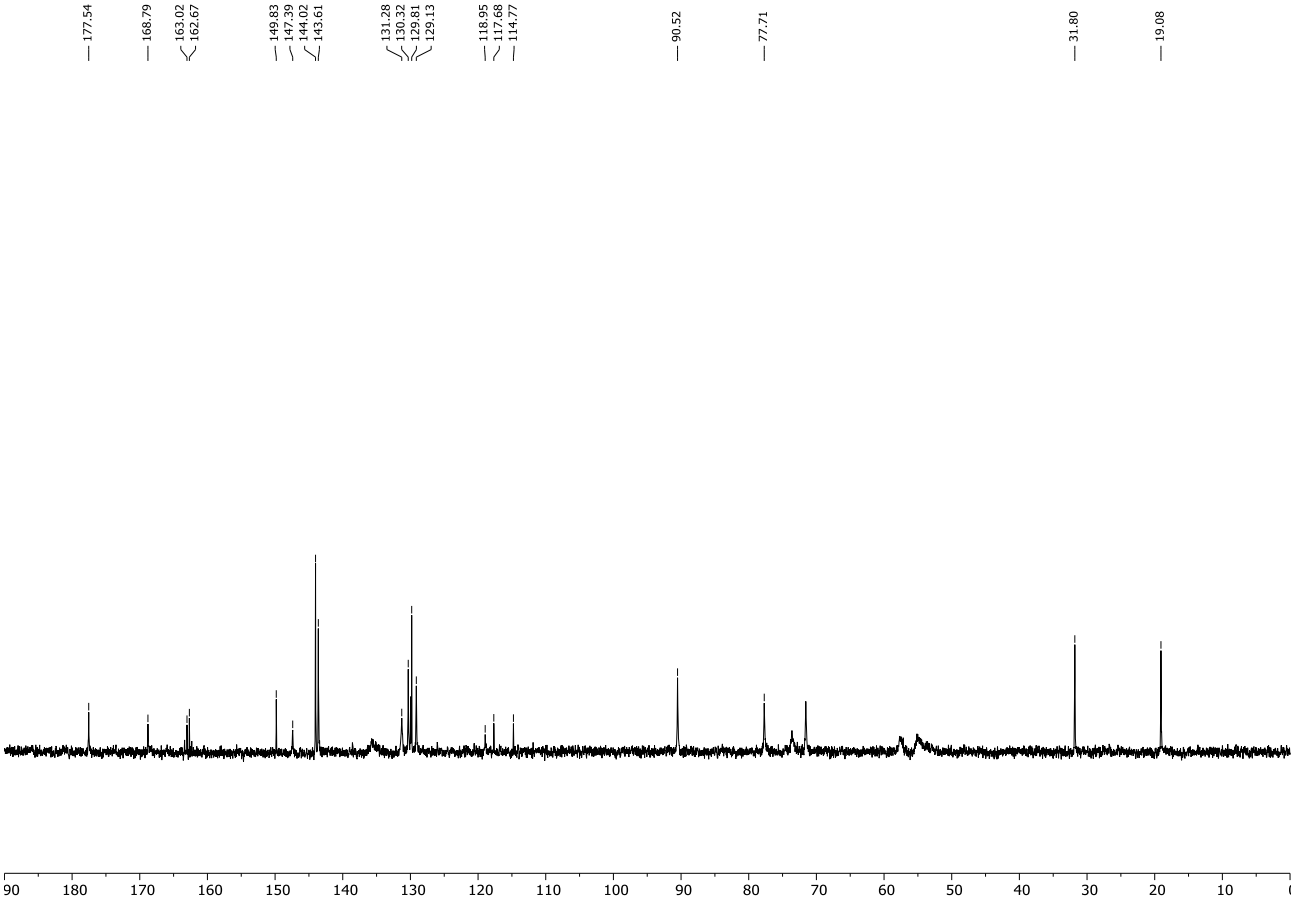
^{13}C NMR (D_2O)



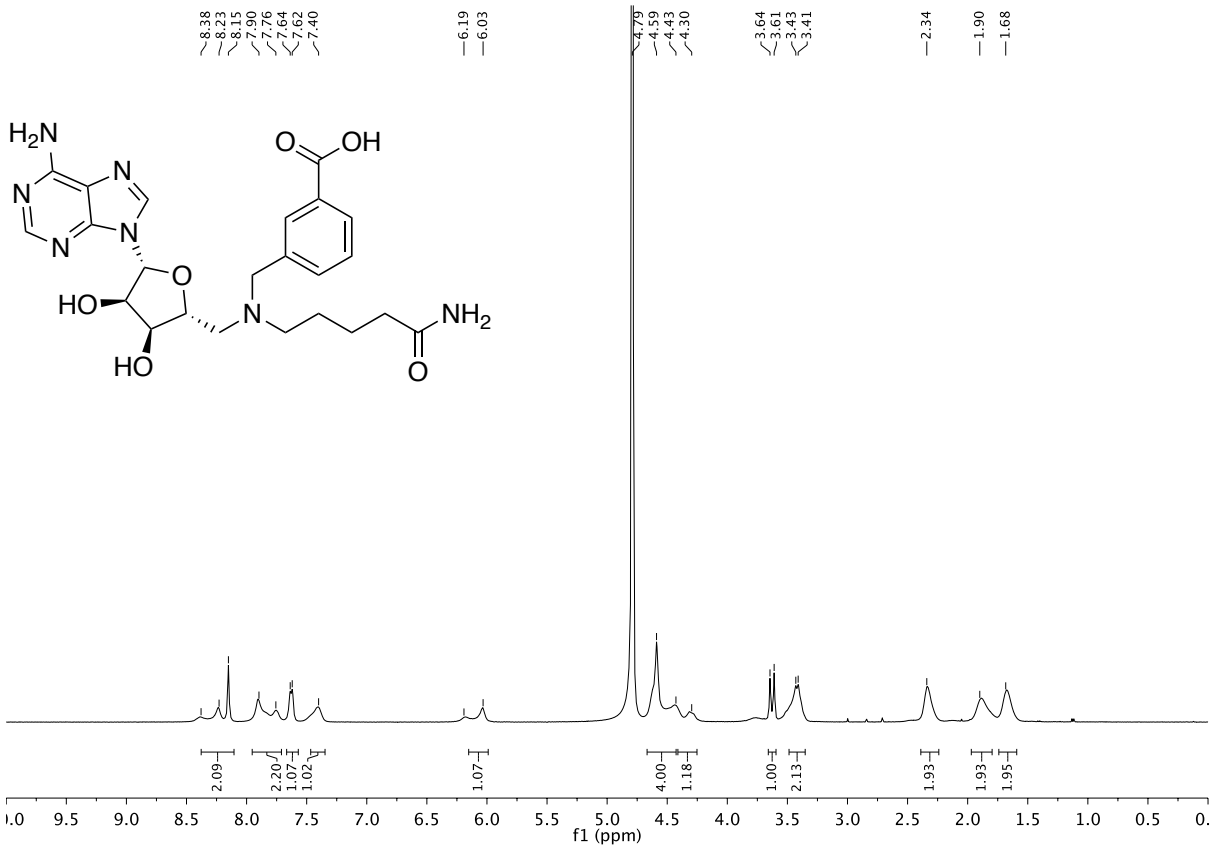
Compound **68** ^{1}H NMR (D_2O)



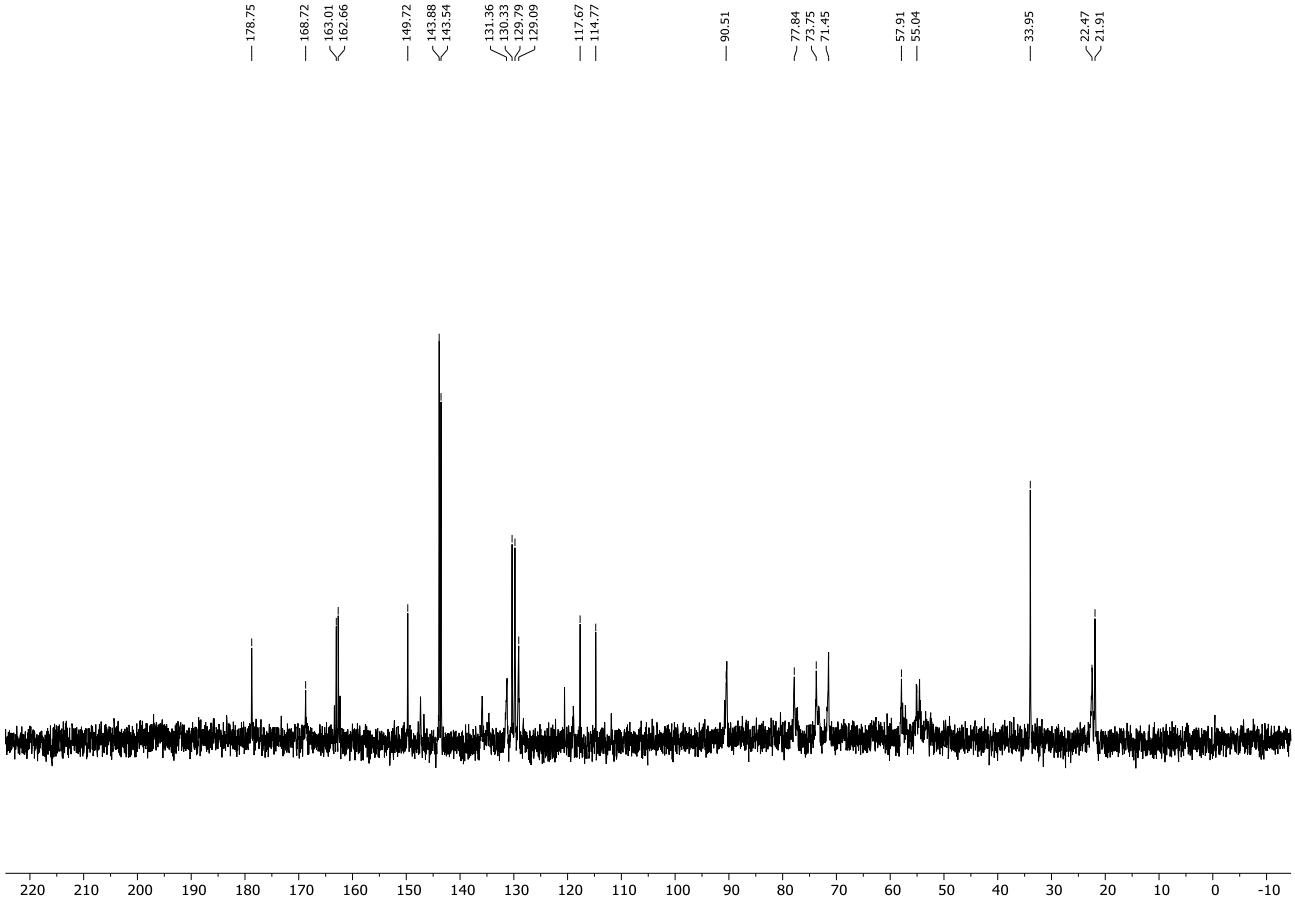
^{13}C NMR (D_2O)



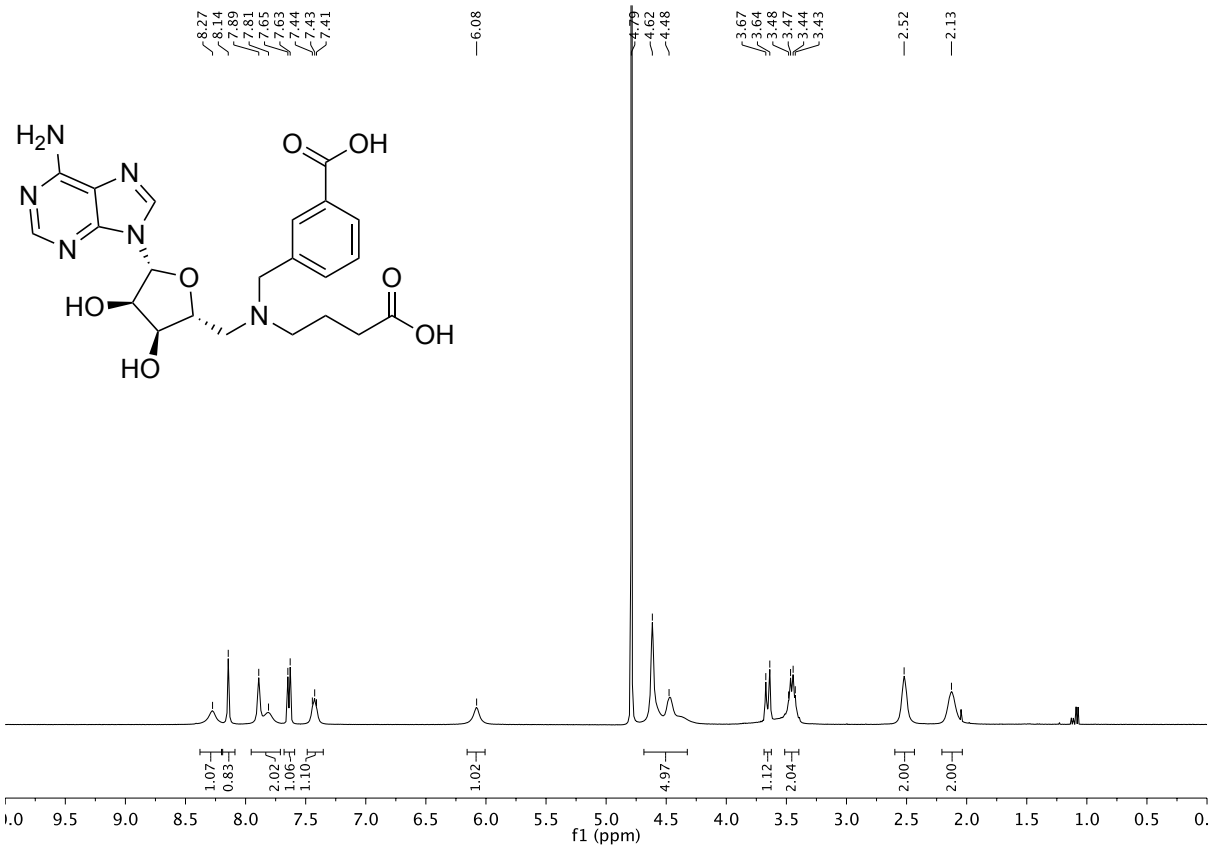
Compound **69** ^{1}H NMR (D_2O)



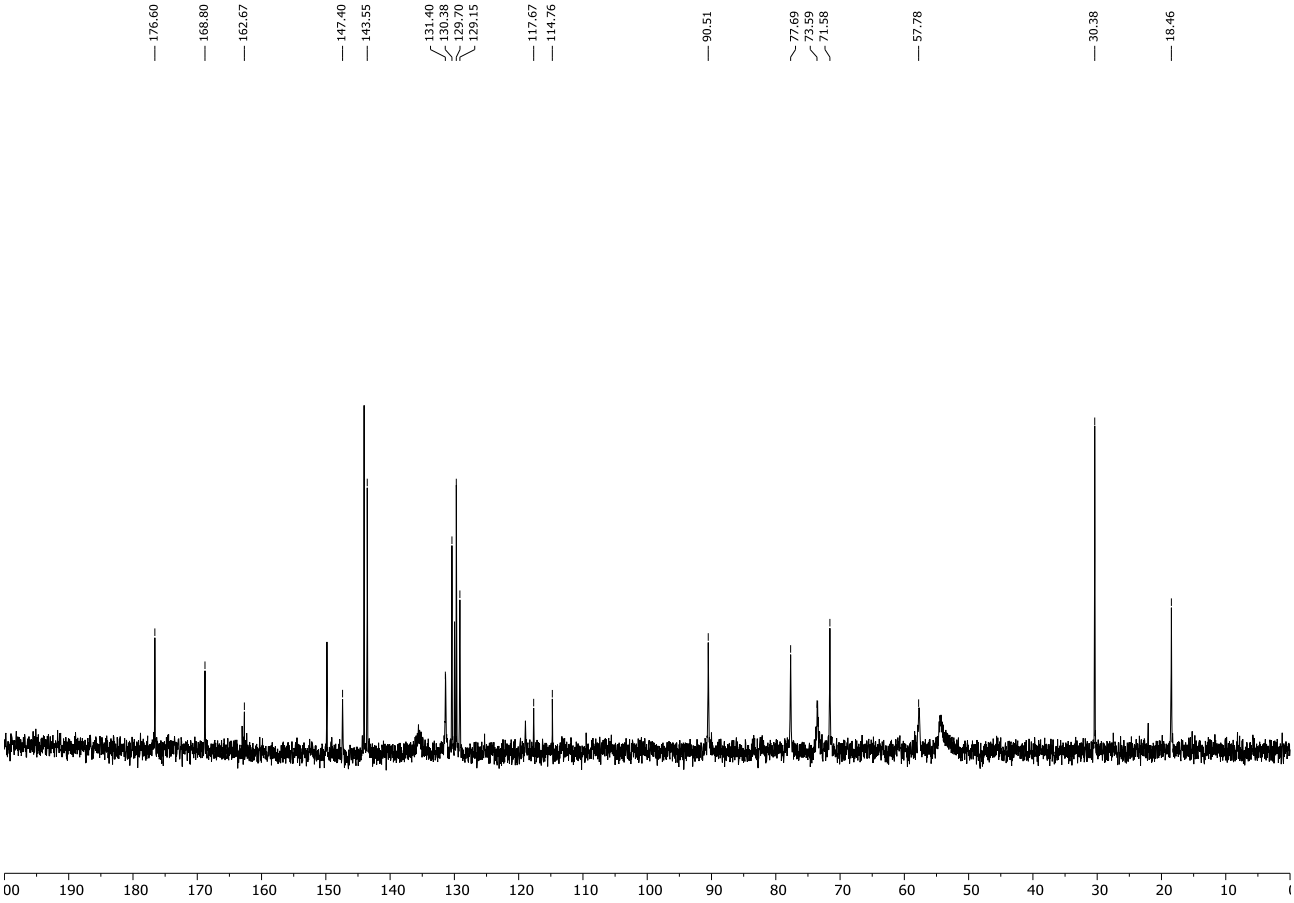
^{13}C NMR (D_2O)



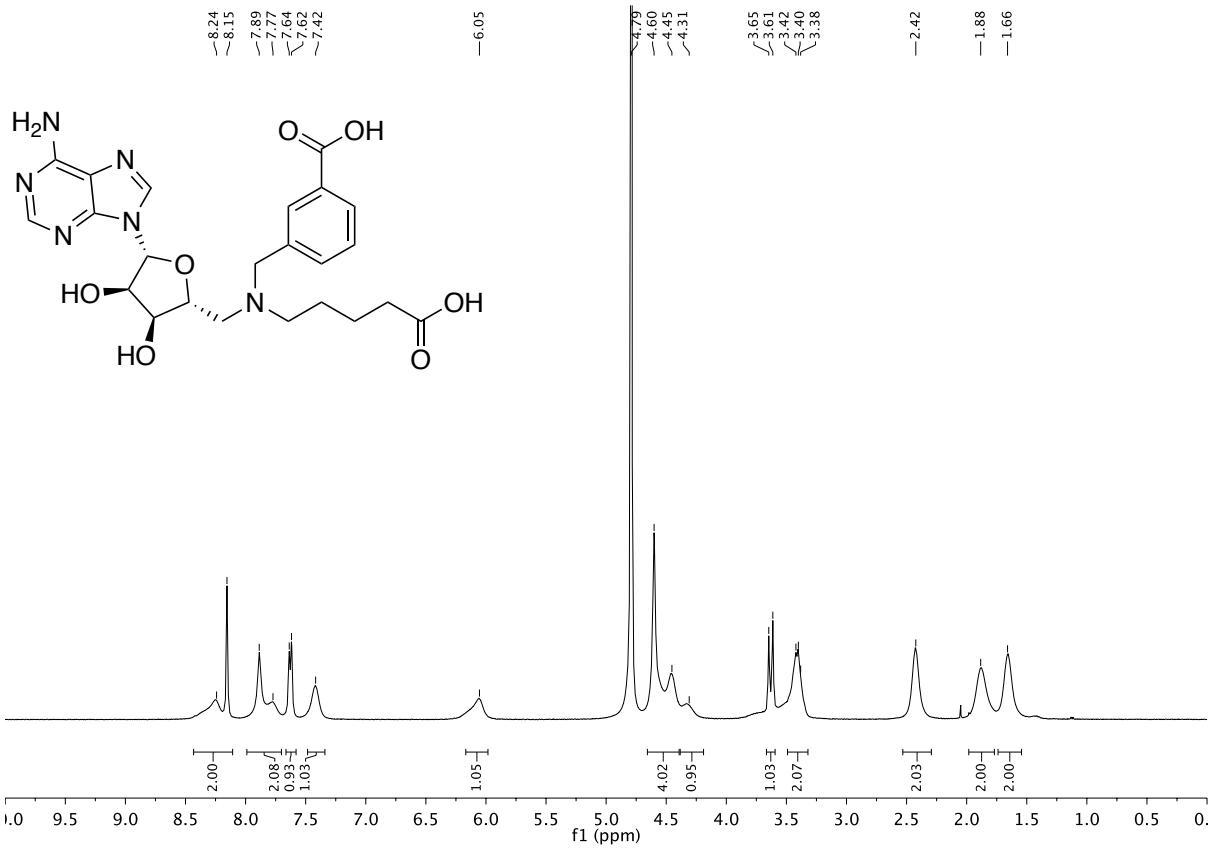
Compound **70** ^{1}H NMR (D_2O)



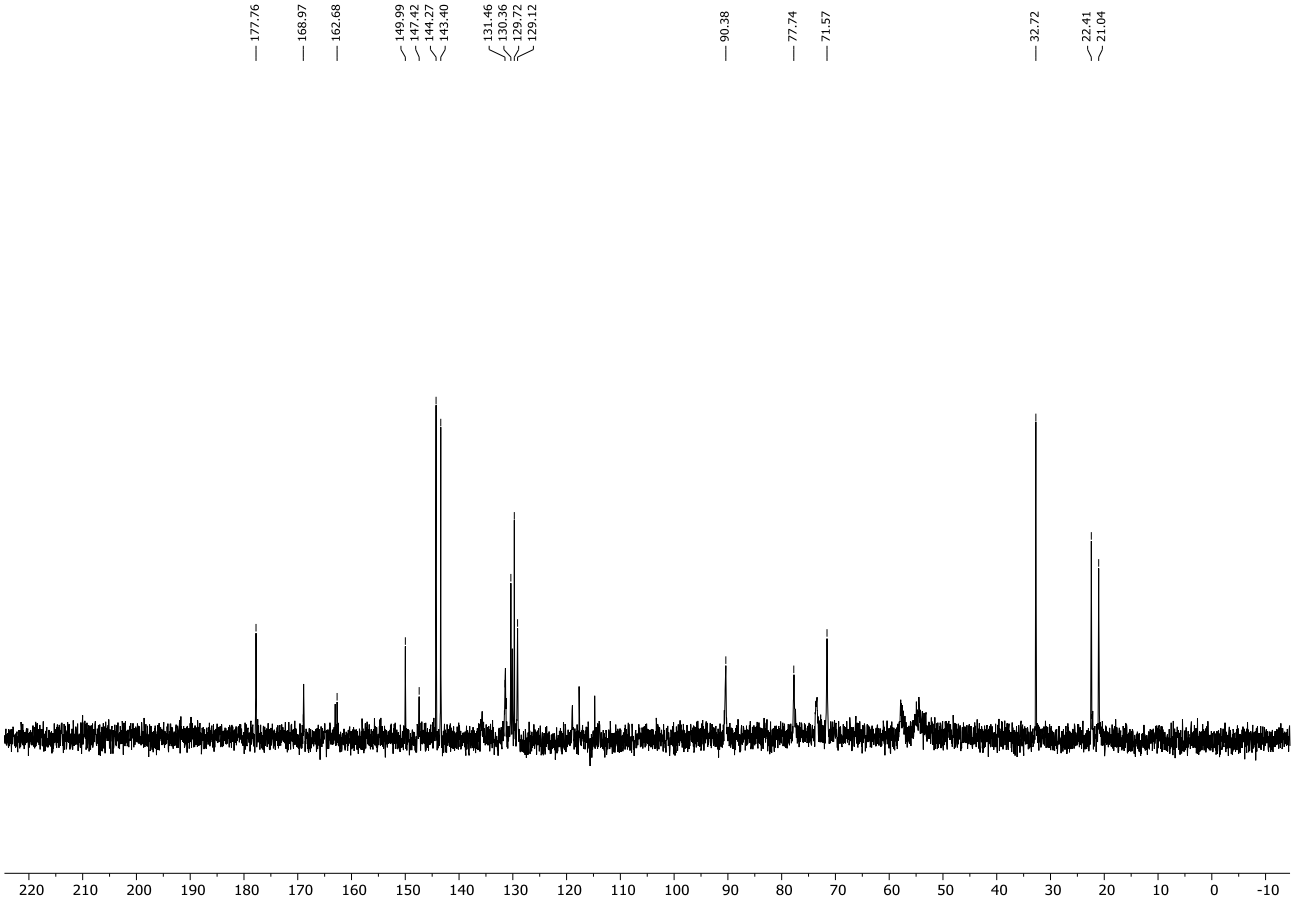
^{13}C NMR (D_2O)



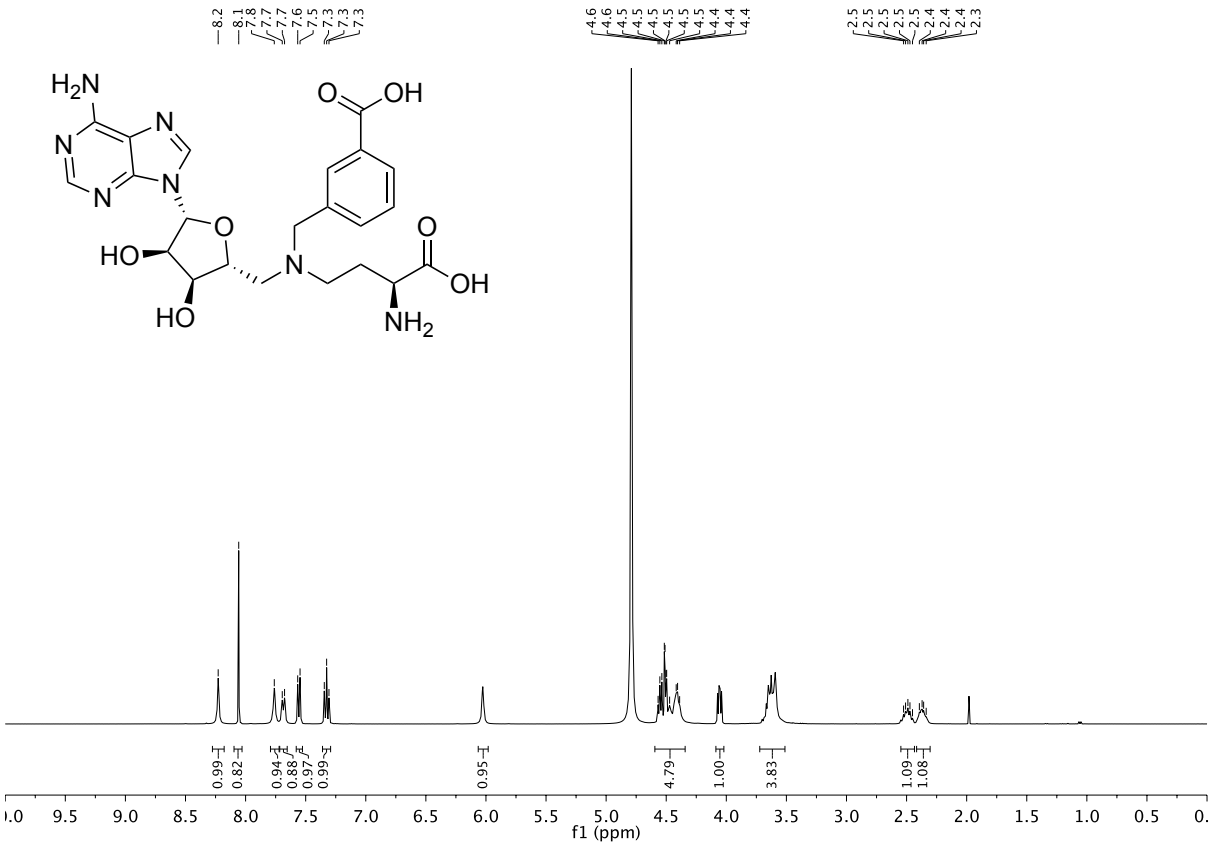
Compound **71** ^{1}H NMR (D_2O)



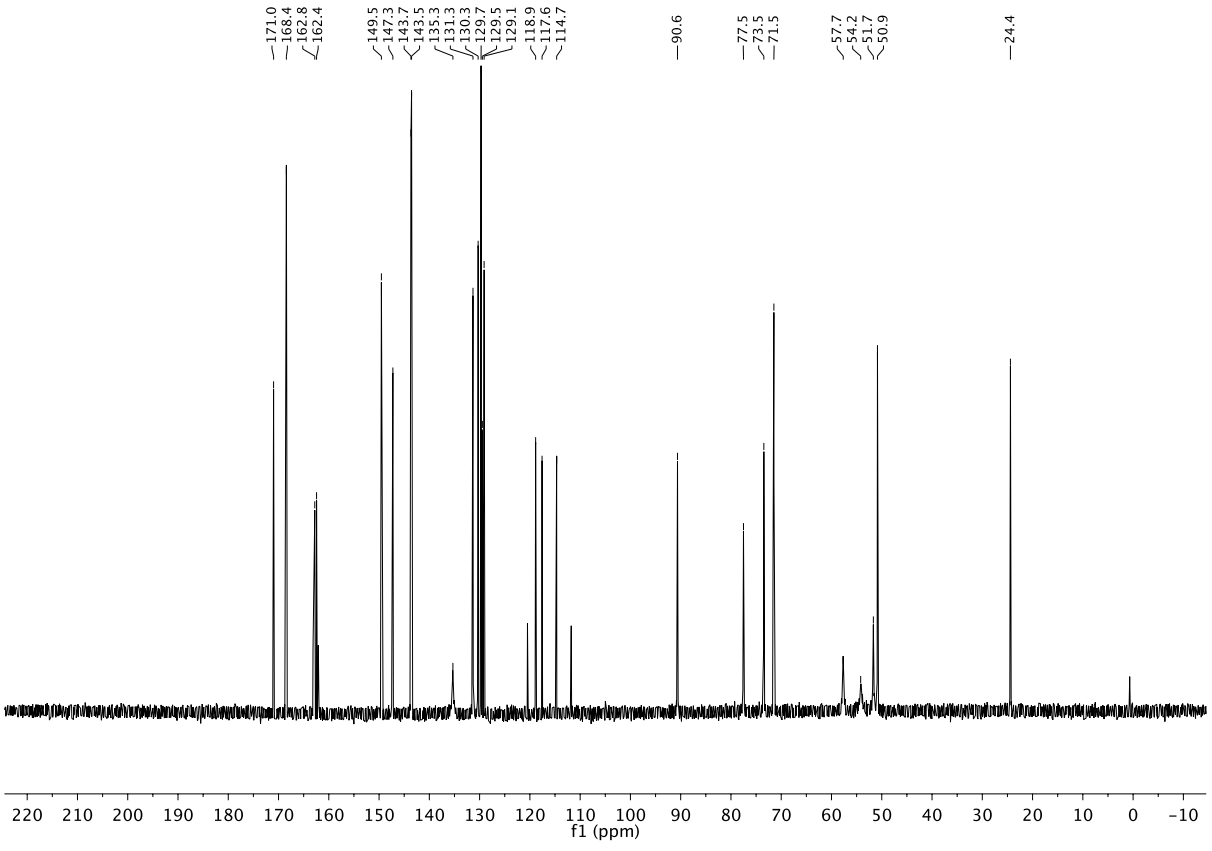
^{13}C NMR (D_2O)



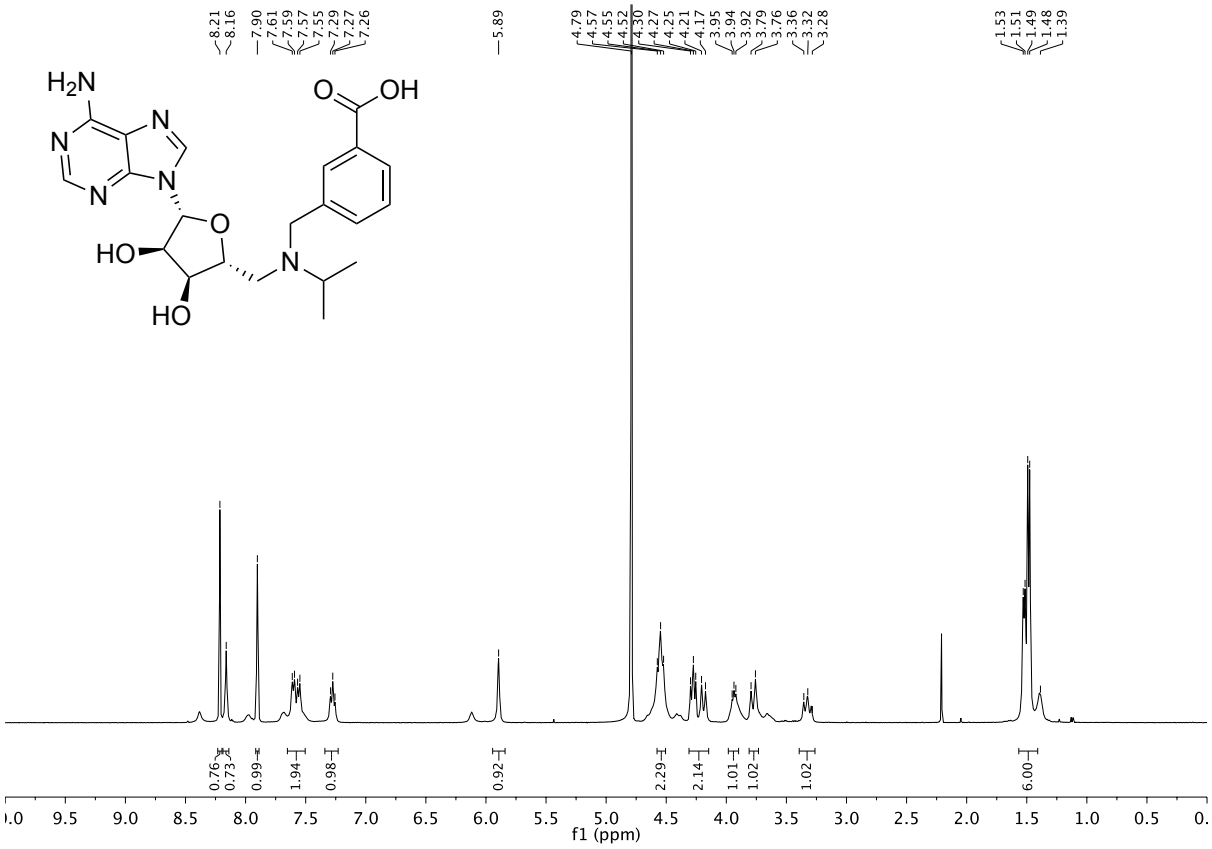
Compound **72** ^{1}H NMR (D_2O)



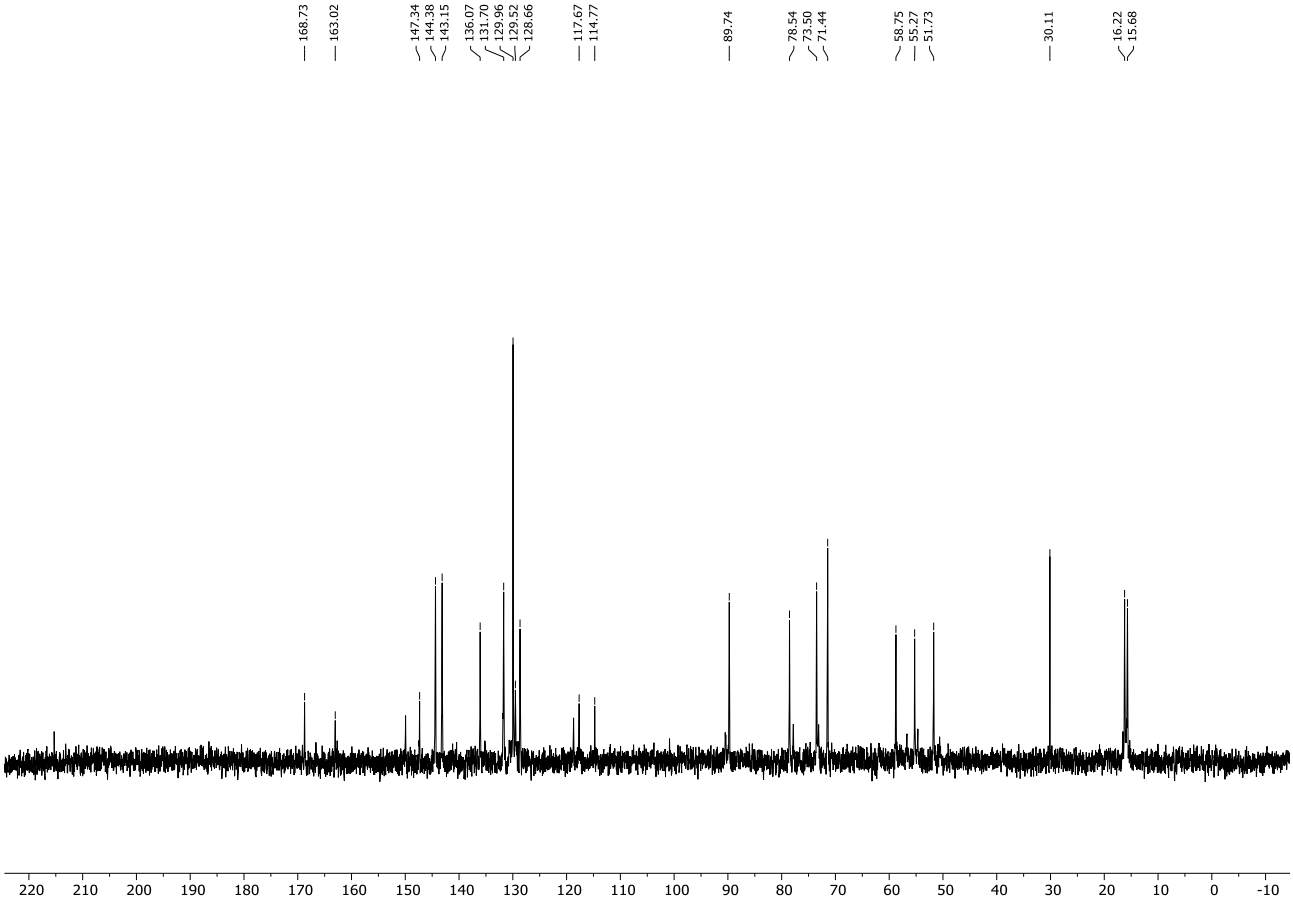
^{13}C NMR (D_2O)



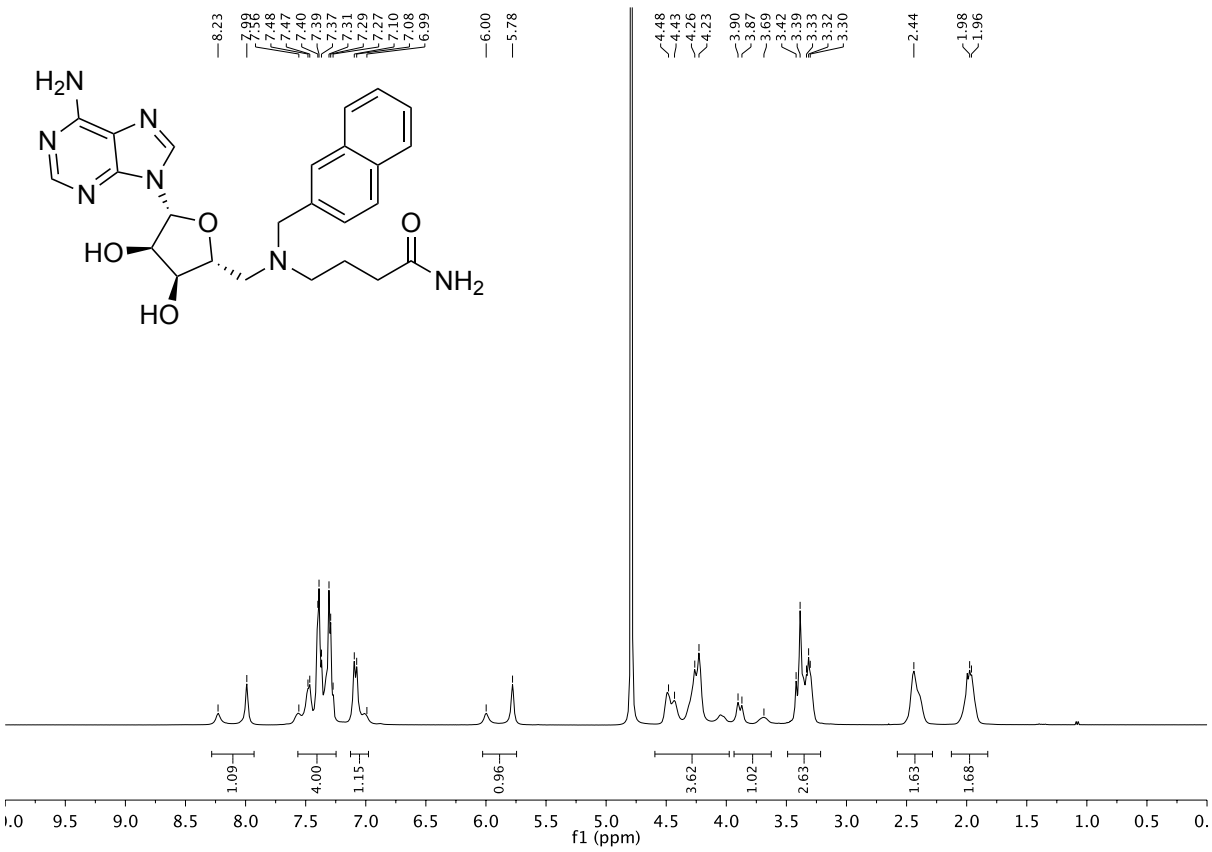
Compound **73** ^{1}H NMR (D_2O)



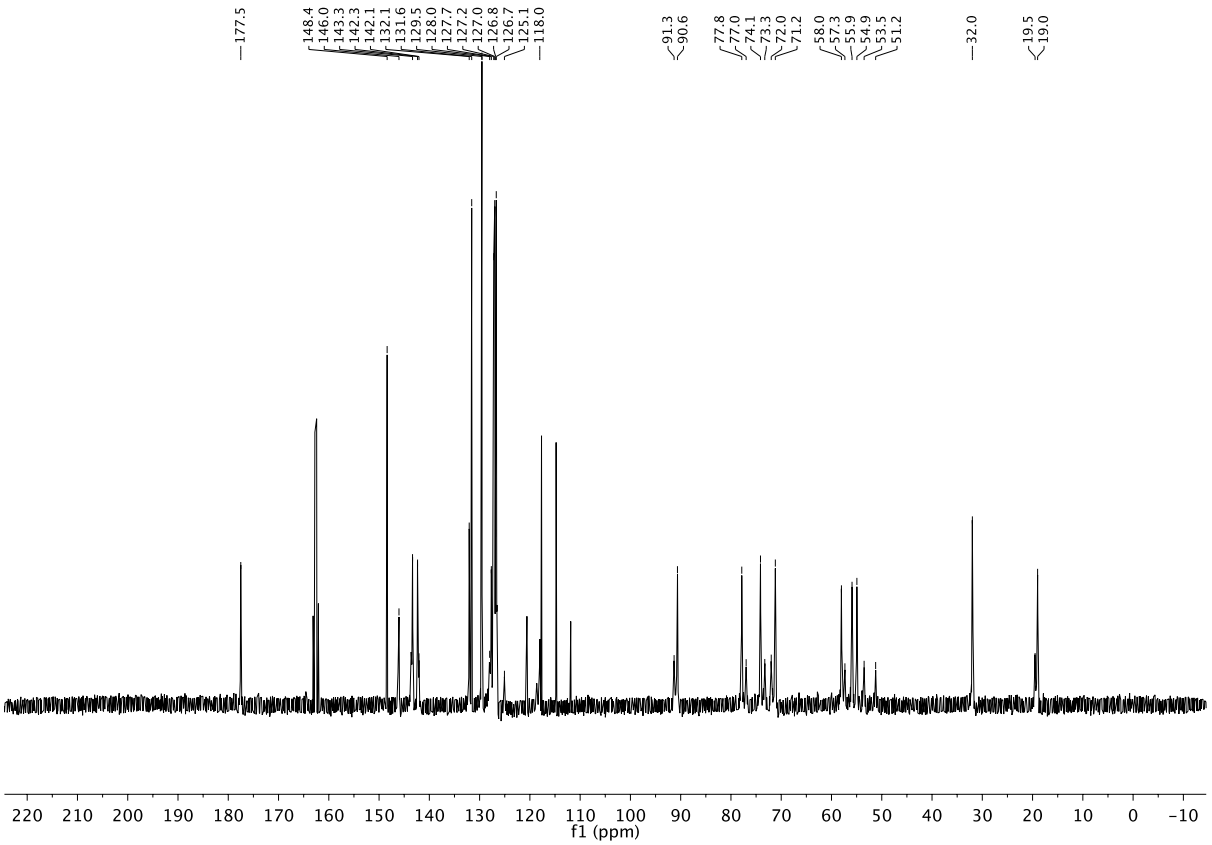
^{13}C NMR (D_2O)



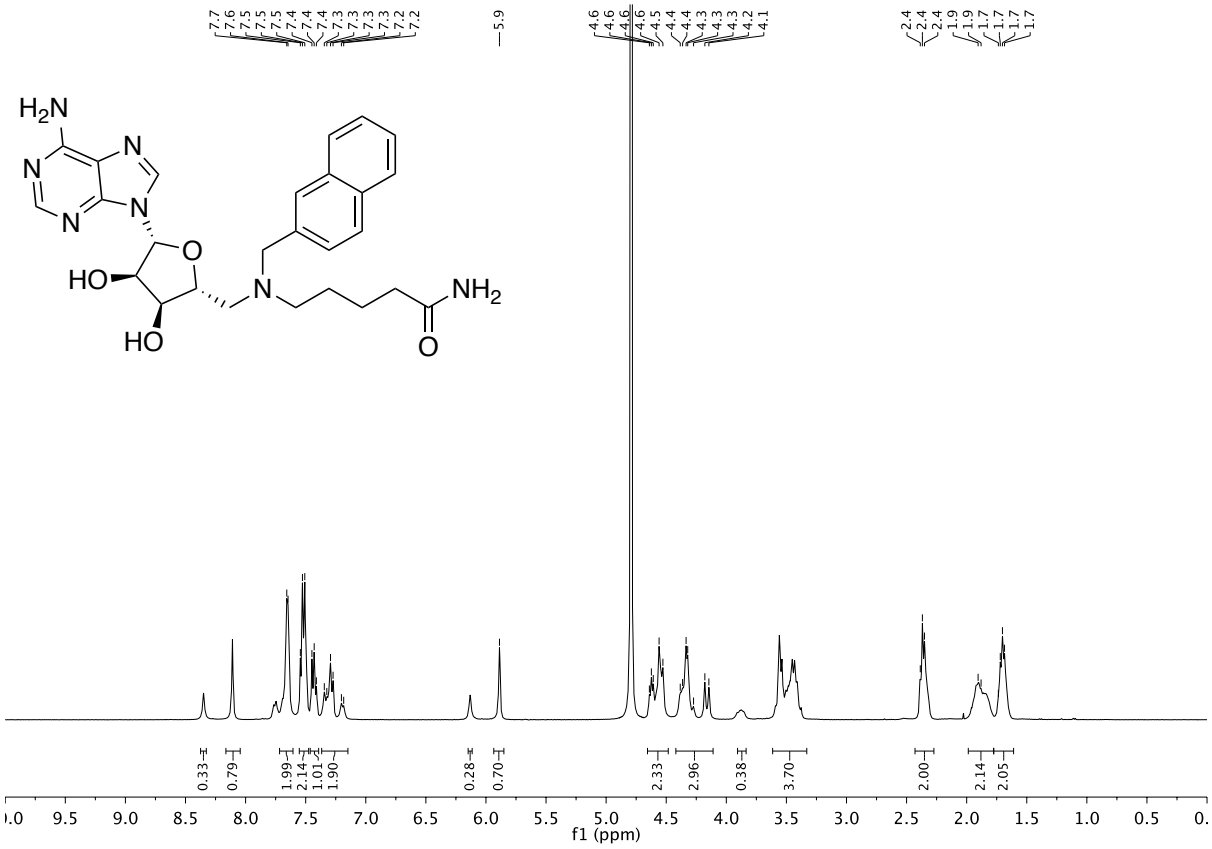
Compound **74** ^{1}H NMR (D_2O)



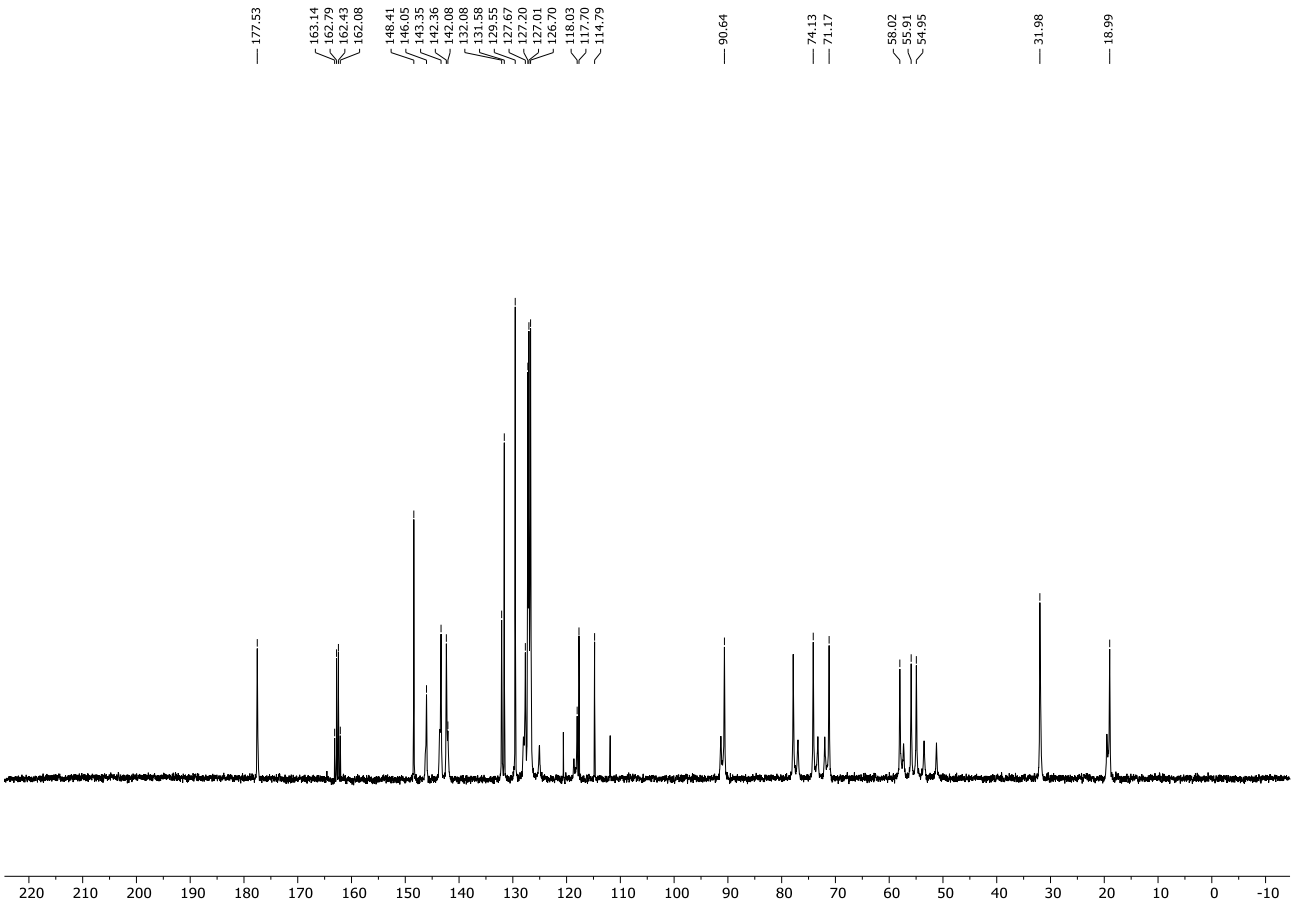
^{13}C NMR (D_2O)



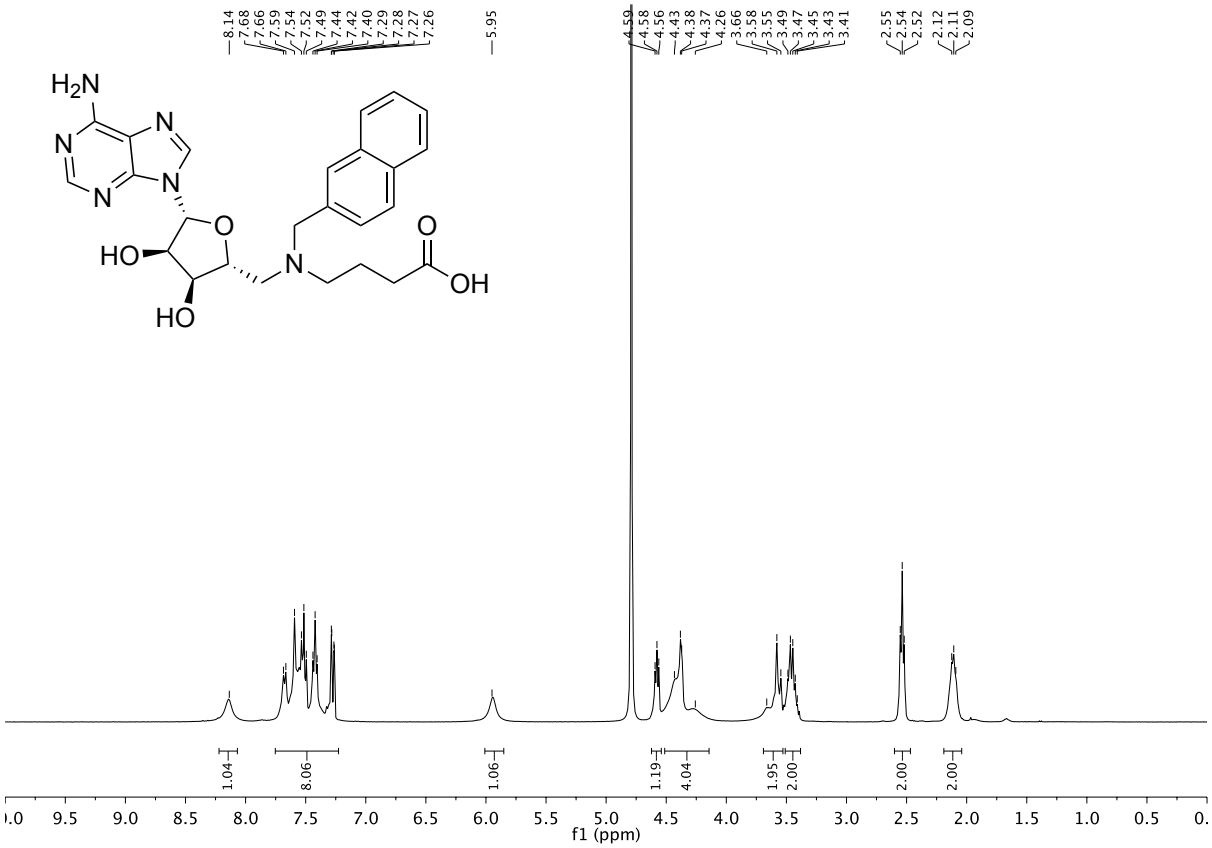
Compound **75** ^{1}H NMR (D_2O)



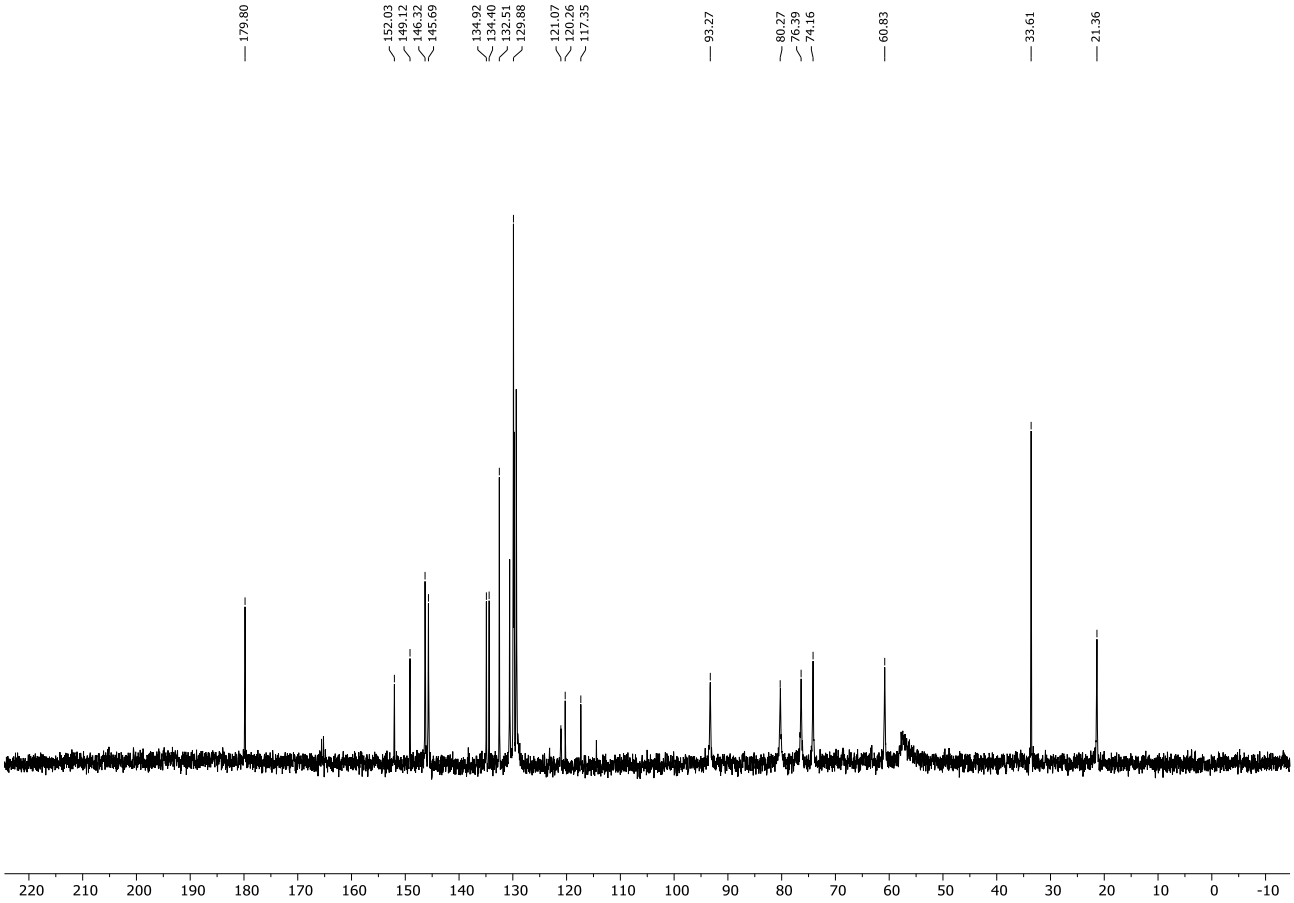
^{13}C NMR (D_2O)



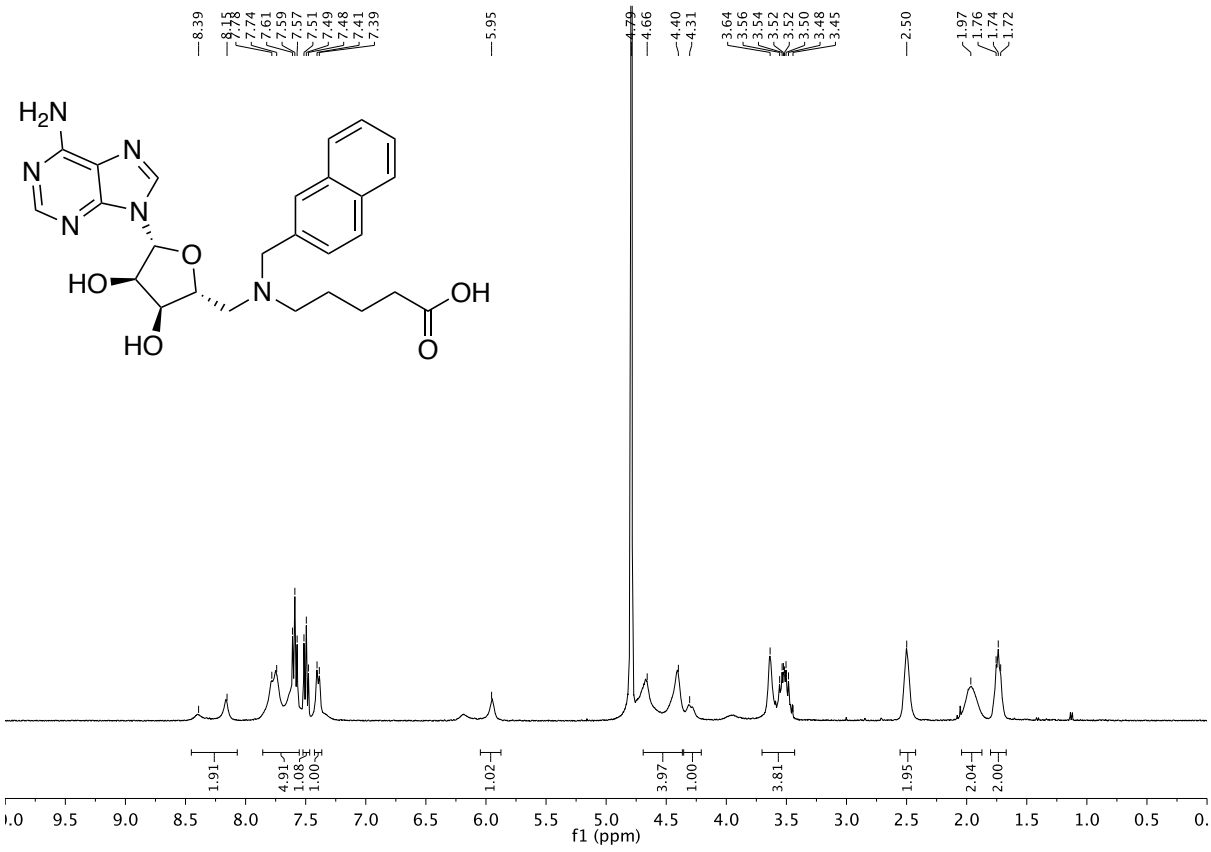
Compound **76** ^{1}H NMR (D_2O)



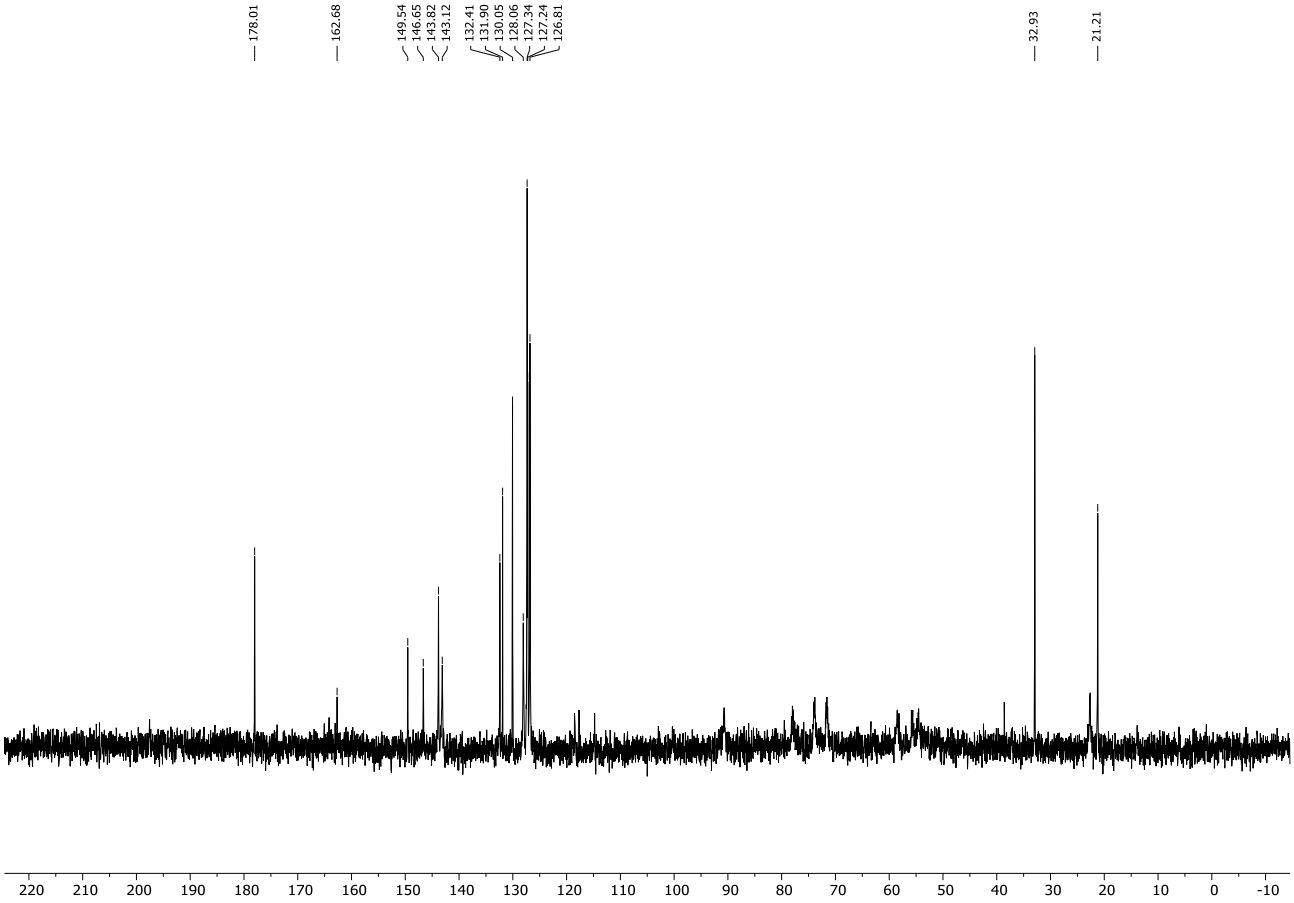
^{13}C NMR (D_2O)



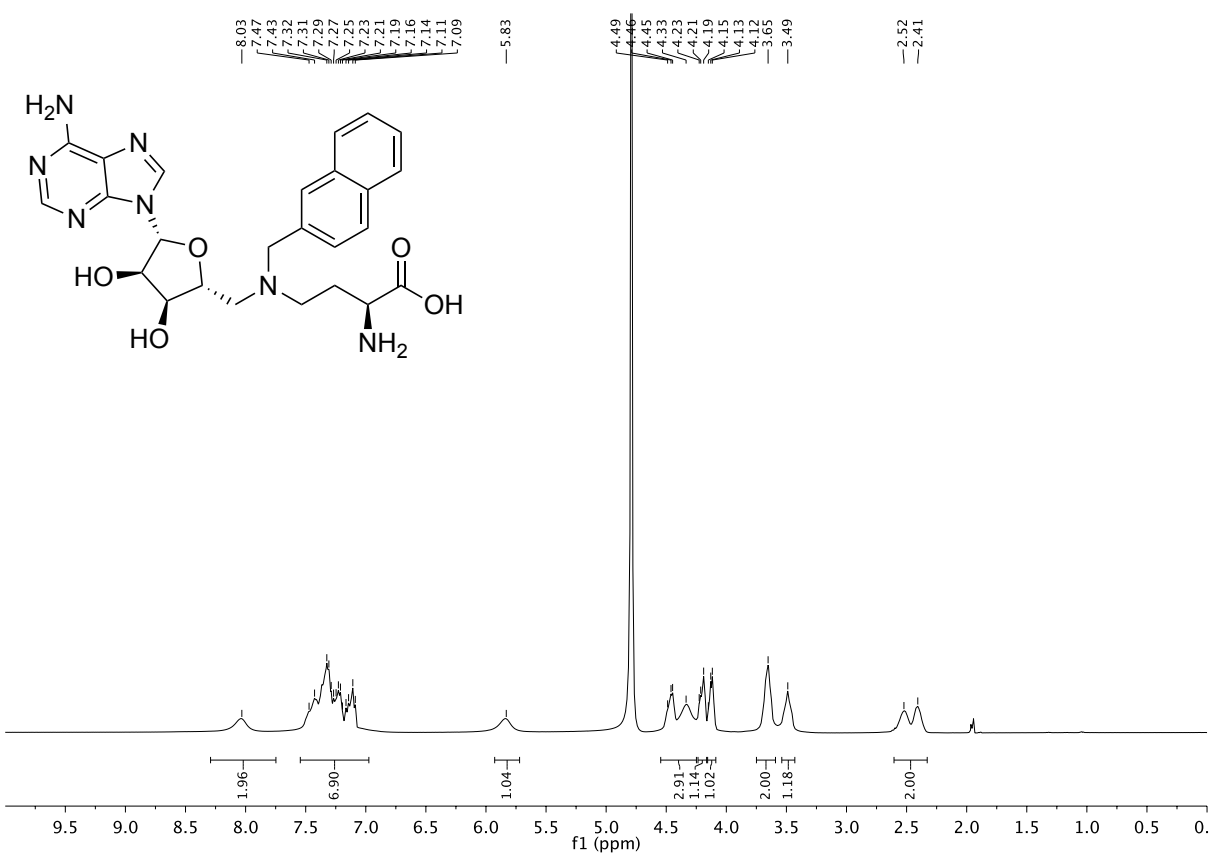
Compound **77** ^{1}H NMR (D_2O)



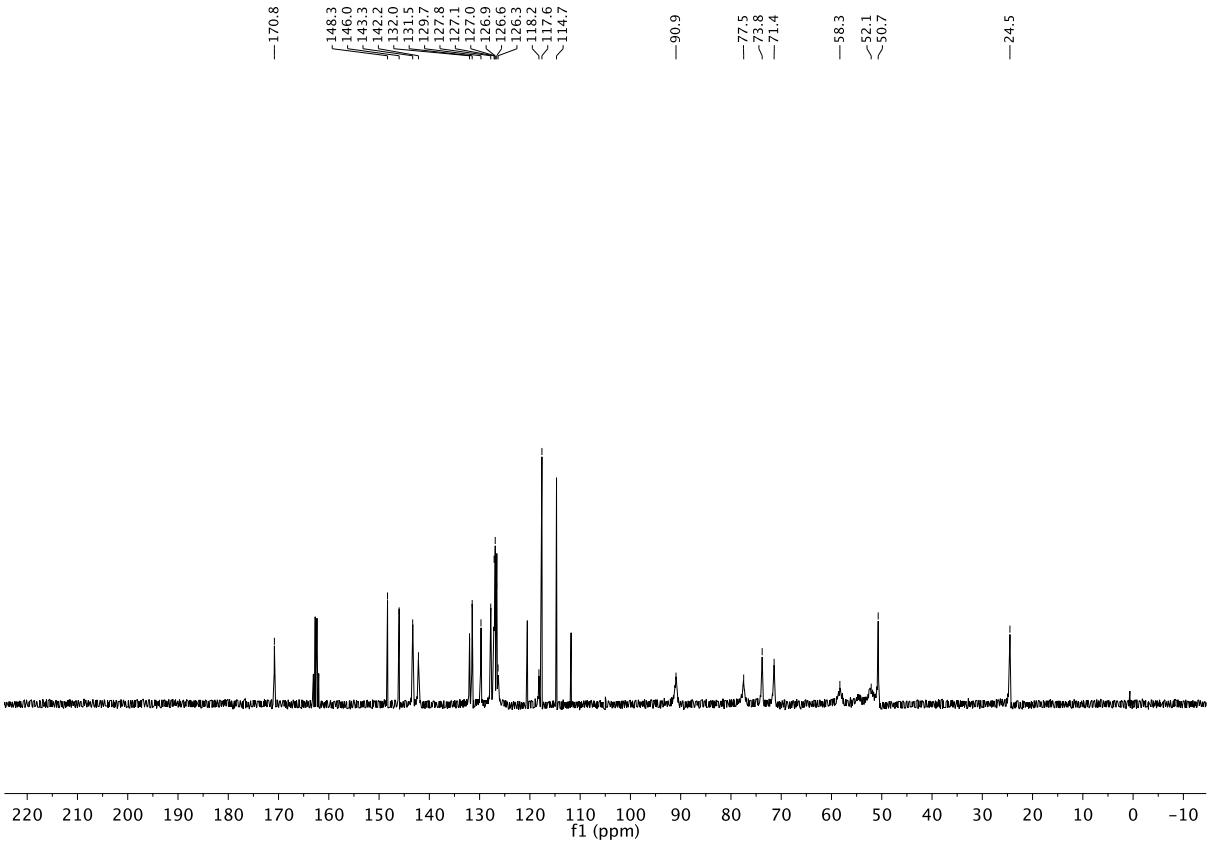
^{13}C NMR (D_2O)



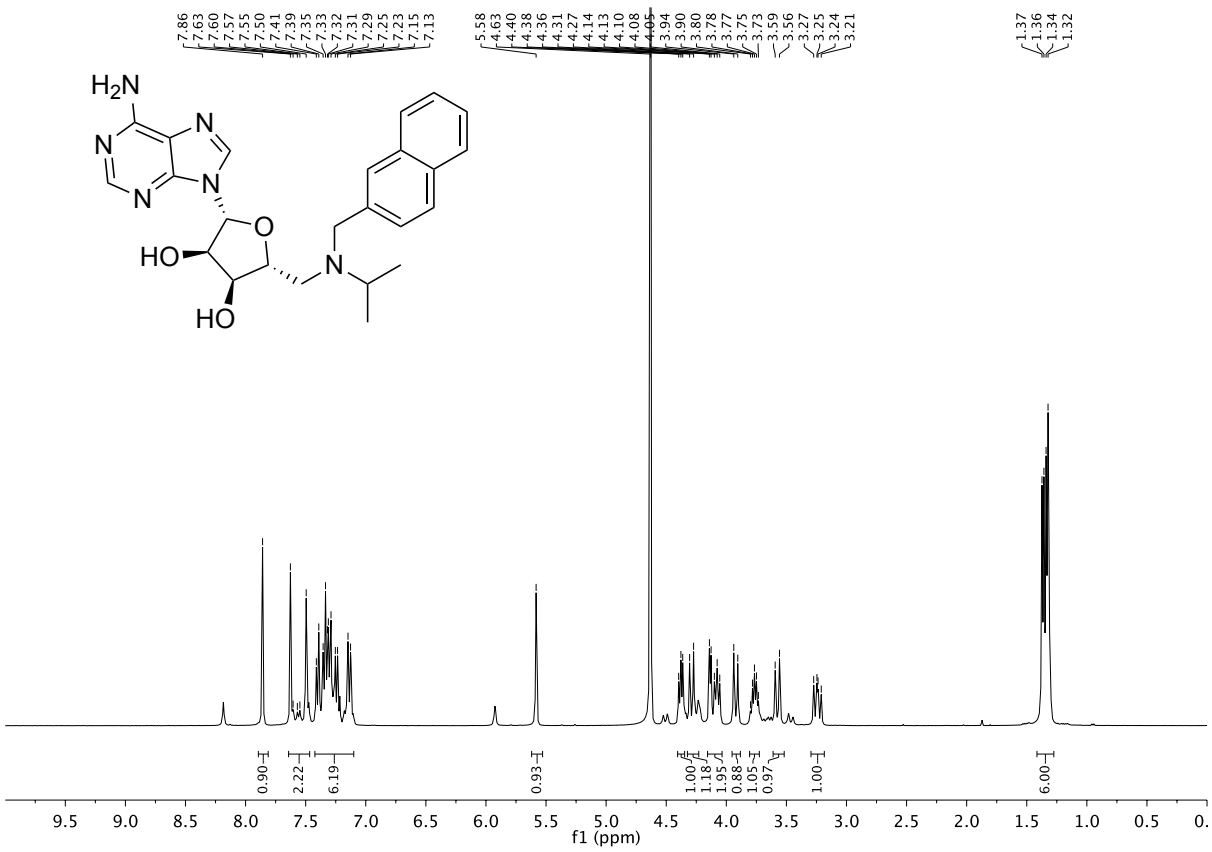
Compound **78** ^{1}H NMR (D_2O)



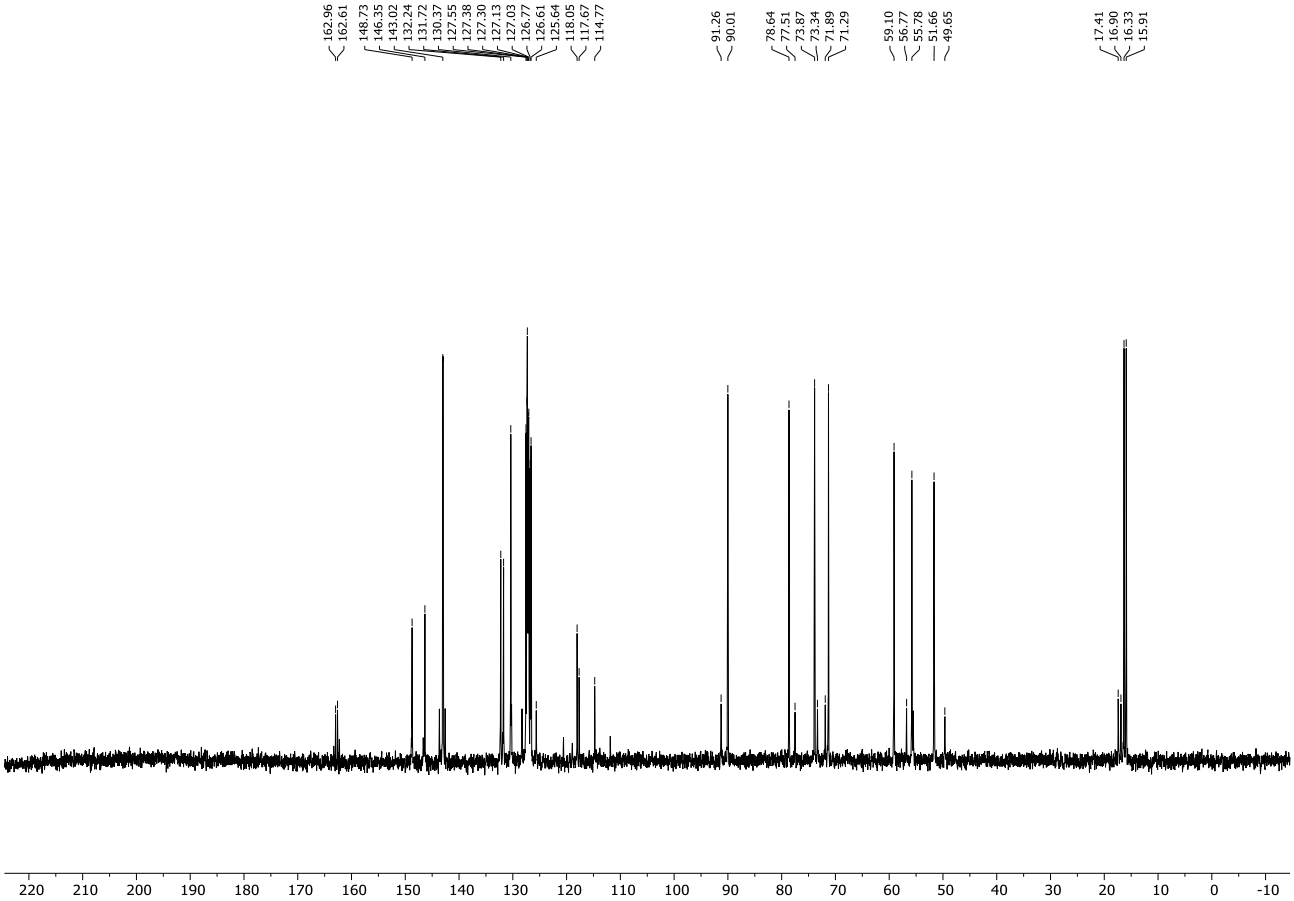
^{13}C NMR (D_2O)



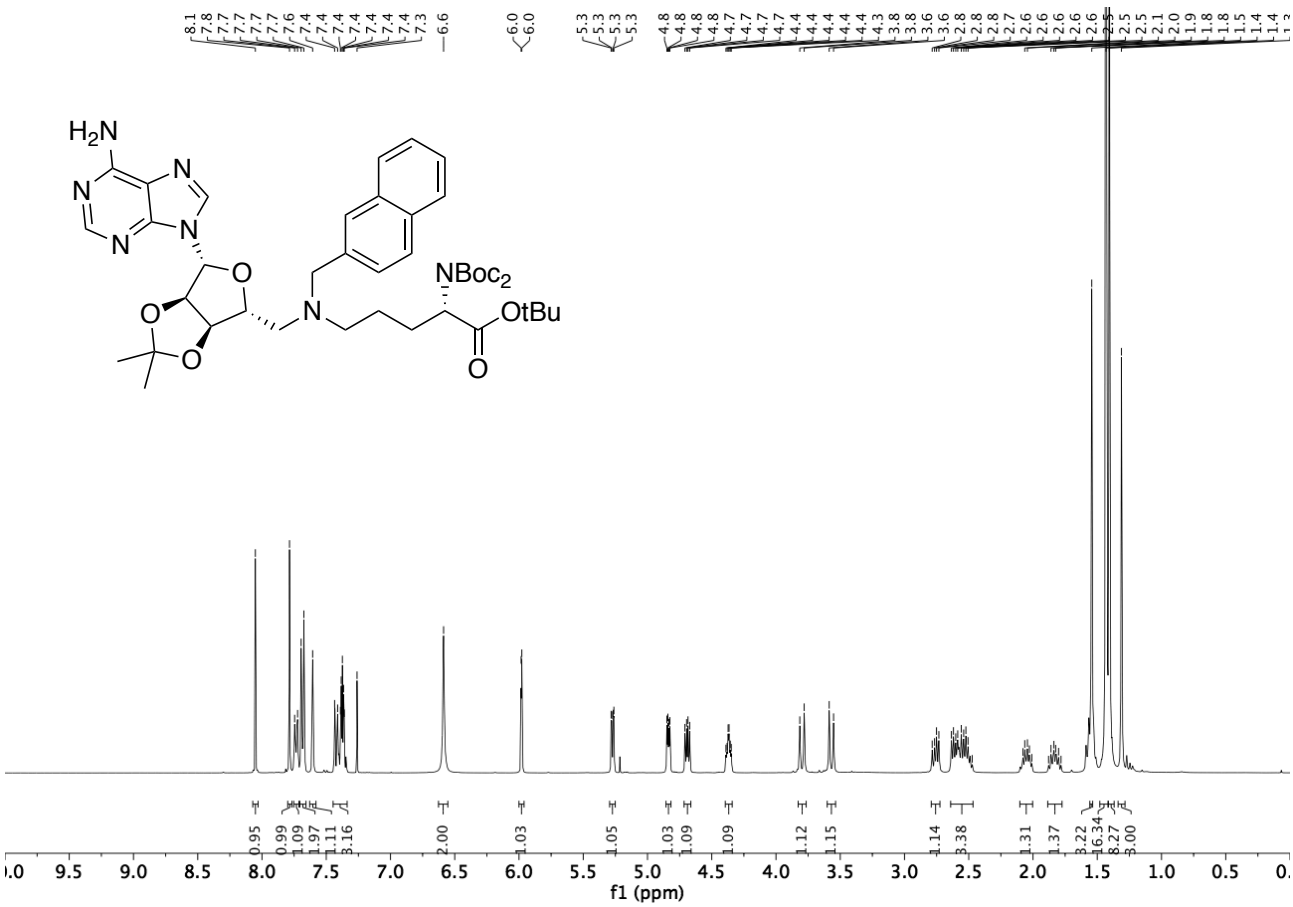
Compound **79** ^{1}H NMR (D_2O)



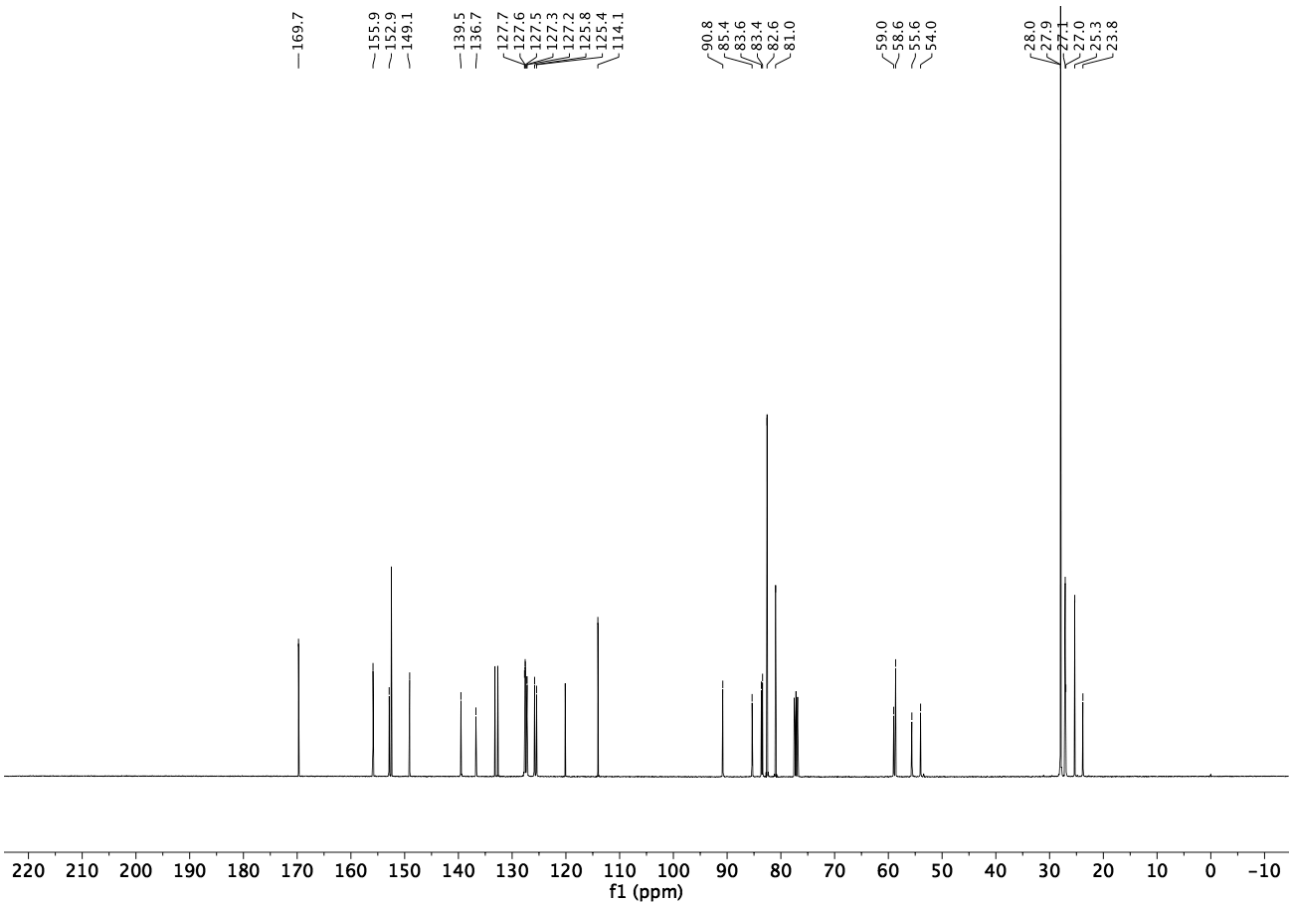
^{13}C NMR (D_2O)



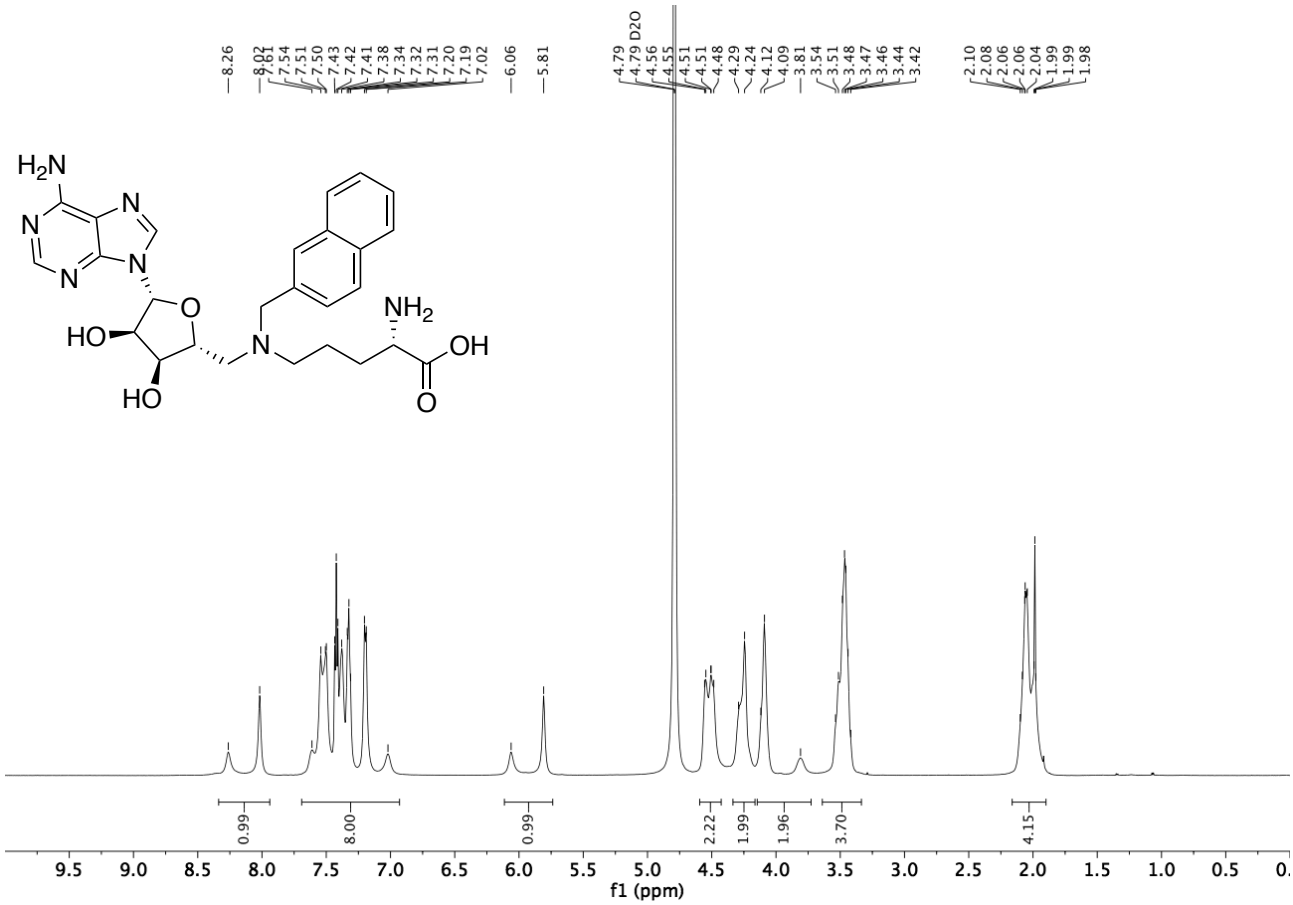
Compound **80** ^{1}H NMR ($CDCl_3$)



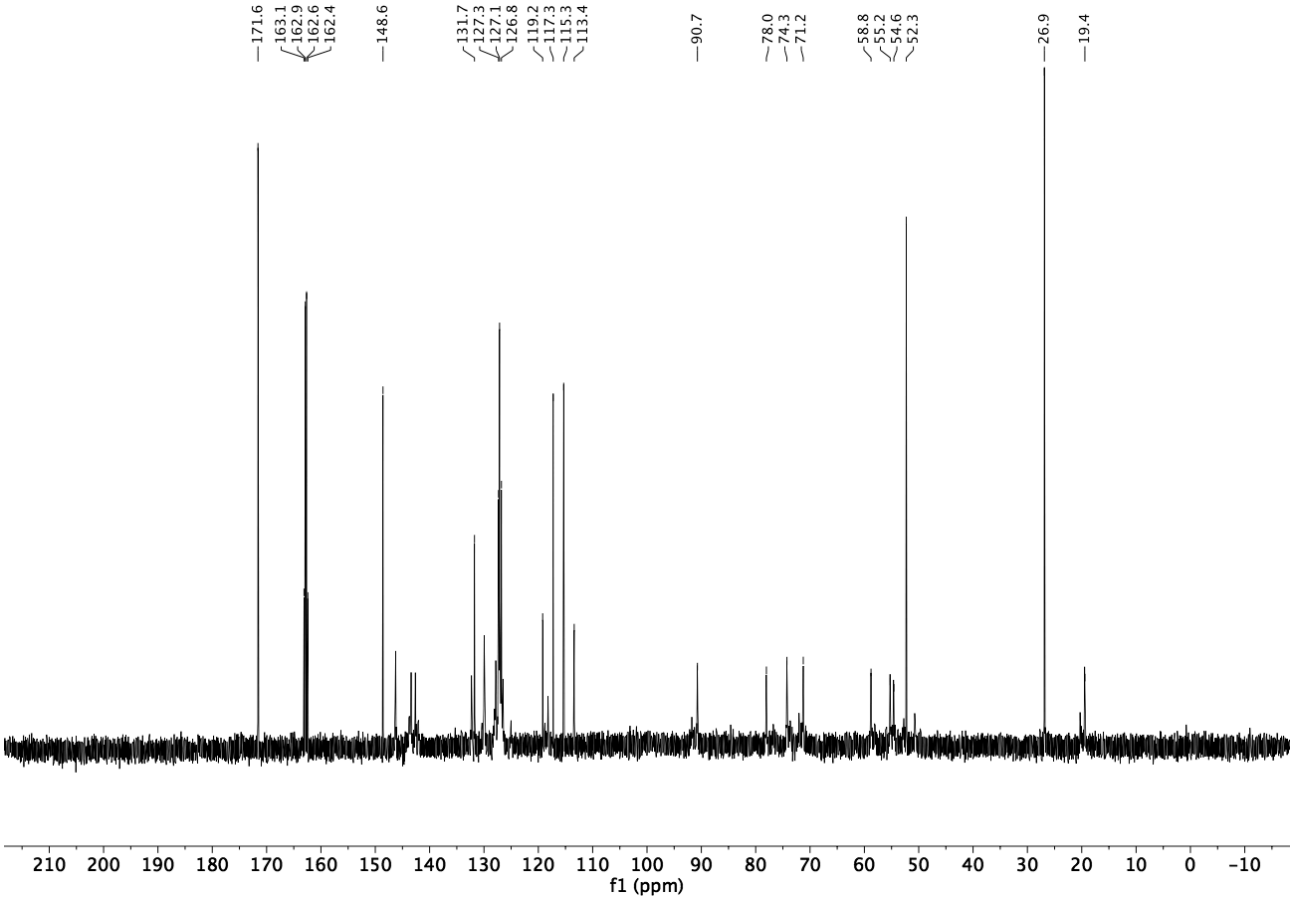
^{13}C NMR ($CDCl_3$)



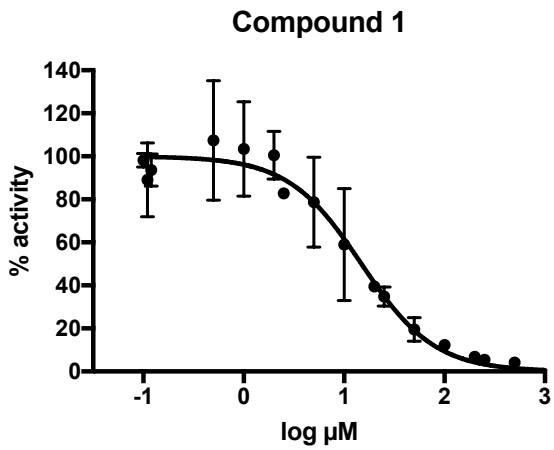
Compound **81** ^{1}H NMR (D_2O)

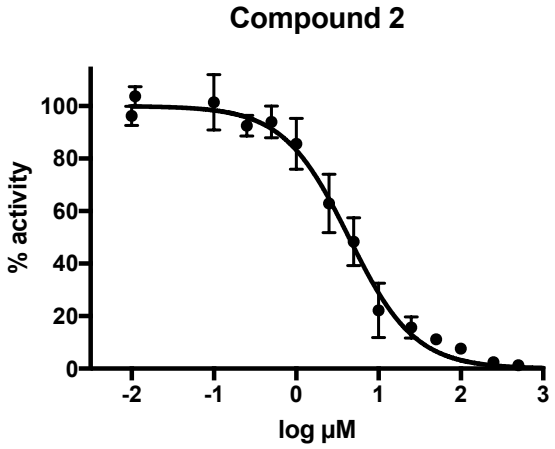


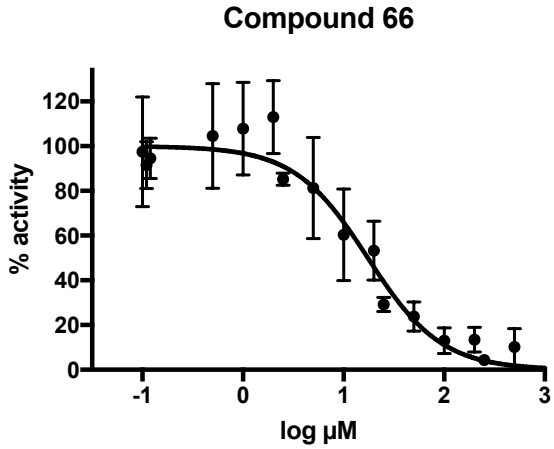
^{13}C NMR (D_2O)

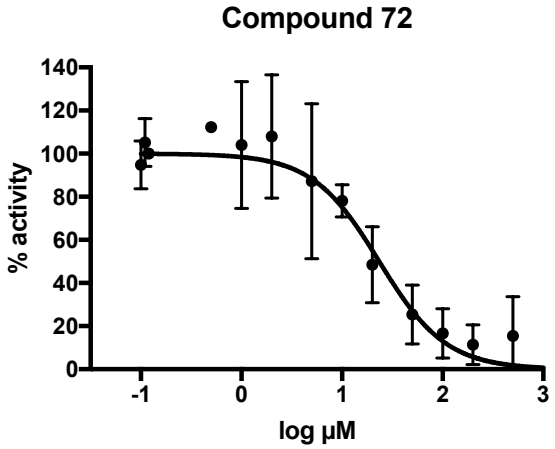


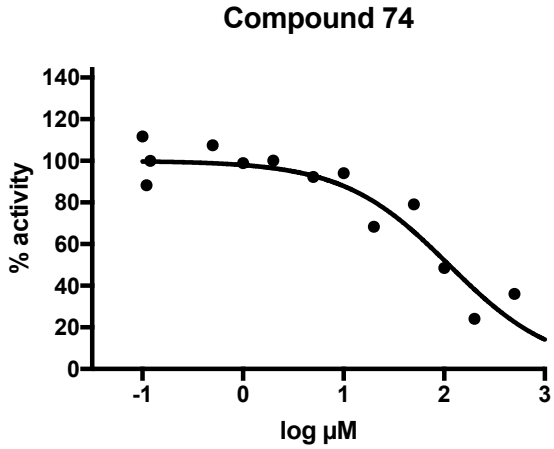
Inhibition curves

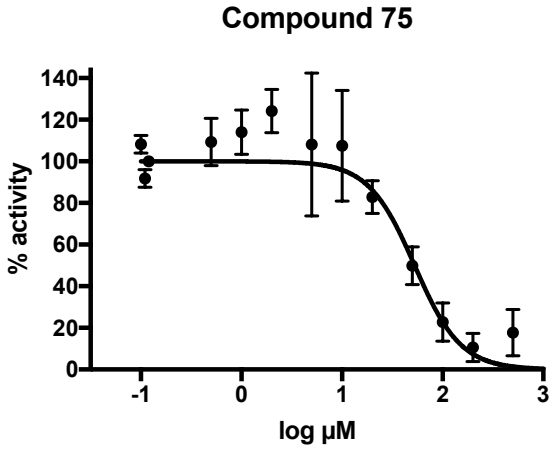


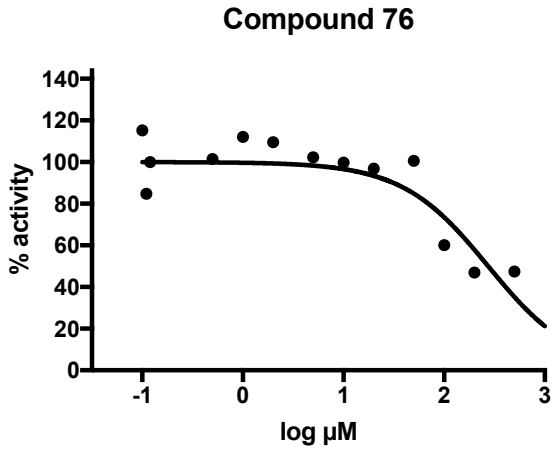


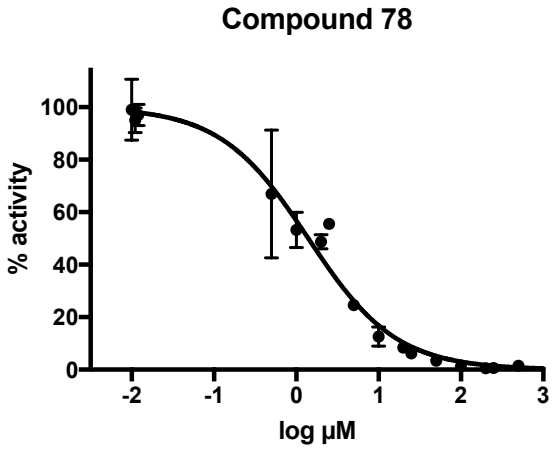









Compound 76
% activity
log μM


Compound 78
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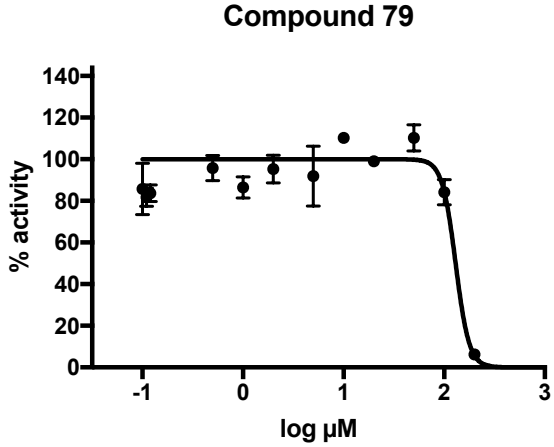
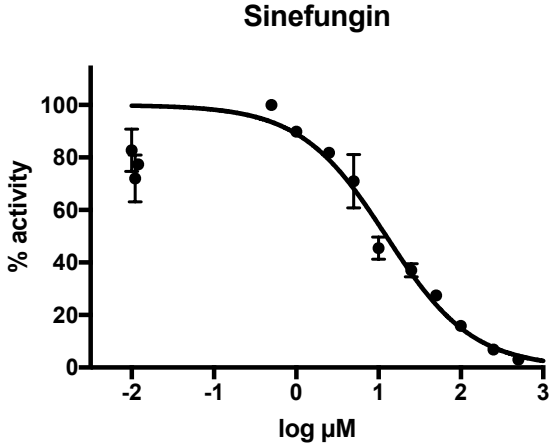
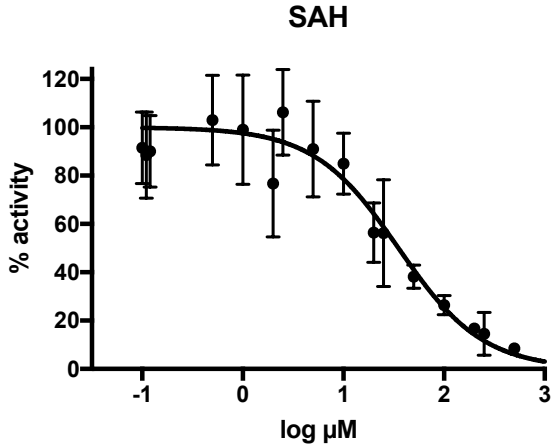
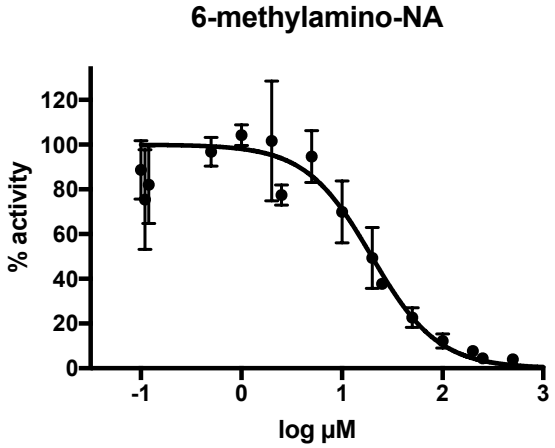
log μM


Compound 79
% activity
log μM

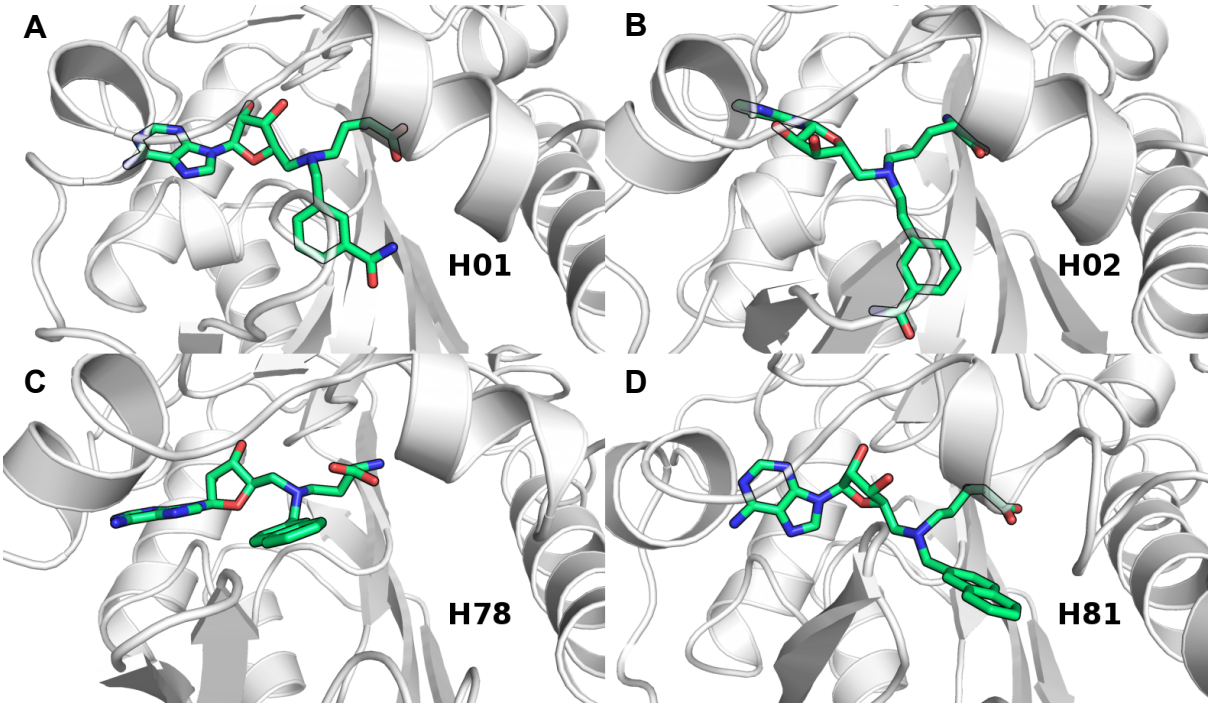

Sinefungin
% activity
log μM


SAH
% activity
log μM


6-methylamino-NA
% activity
log μM

Supplemental Table S1. Methyltransferase selectivity screen with compound **78**

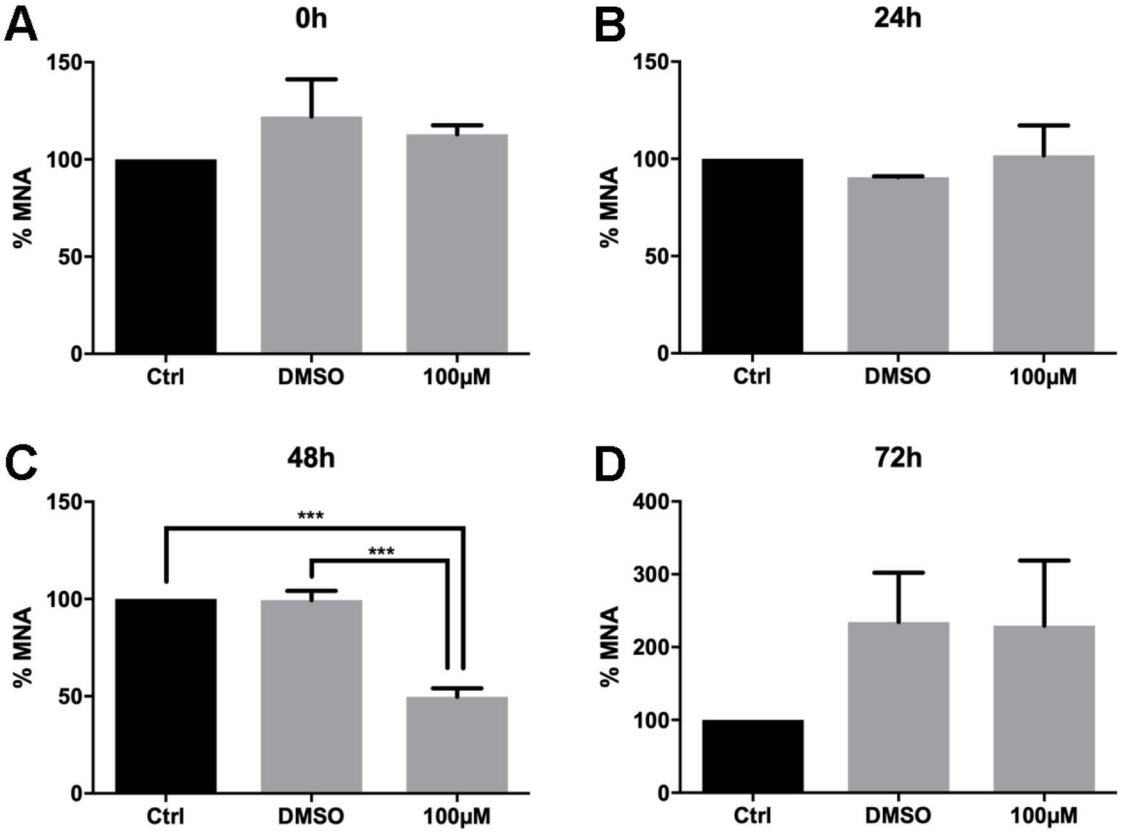
Enzyme	**IC_{50}**
NNMT	1.41 μM
NSD2	> 50 μM
PRMT1	> 50 μM



Supplemental Figure S1. Predicted binding of compounds **1 (A)**, **2 (B)**, **78 (C)**, and **81 (D)** in the active site of NNMT (PDB ID: 3ROD). All ligands position their three branches in a similar space in the catalytic pocket. However, branch orientations and interactions are different.

Supplemental Table S2. Estimated protein/ligand binding energies between NNMT (PDB ID: 3ROD) and compounds **1**, **2**, **78**, and **81**

Compound	**Calculated Binding energy**	**Experimental IC_{50} value**
1	-95.3 +/- 6.8 kcal/mol	14.9 µM
2	-87.2 +/- 7.3 kcal/mol	4.4 µM
78	-91.0 +/- 6.3 kcal/mol	1.4 µM
81	-89.7 +/- 7.2 kcal/mol	> 250 µM



Supplemental Figure S2. Cellular MNA content in HSC-2 cells. Human oral cancer cells (HSC-2) were treated with 100µM compound **78** and MNA levels were measured after 0, 24, 48 and 72 hours (**A-D** respectively). Results were compared with untreated cells and cells treated with DMSO (0.1% final concentration). The 48-hour time-point shows a significant reduction in MNA levels of the treated cells compared to the untreated cells and DMSO control. After 72 hours an increase in MNA levels is observed in both the DMSO control and compound **78**, but not for the untreated cells, suggesting the effect is attributable to DMSO.