Supporting Information

Metallo-β-lactamase inhibitor phosphonamidate monoesters

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Table of contents

1. Dose-response graphs

Table S1. The dose-response graphs.

Inhibitors	NDM-1	GIM-1	VIM-2
1a			
1b			

1c

191108 NDM-1 KAT-A-141-H1

191108 GIM-1 KAT-A-141-H1

KAT-A-141-H1 VIM-2

$IC_{50} = 500$ μM

1d

LOE-002-2-3 NDM-1

191105 GIM-1 LOE-002-2-3

191105 VIM-2 LOE-002-2-3

| **2** | 191030 NDM-1 KAT-A-053-H2 | 191030 GIM-1 KAT-A-053-H2 | 191030 VIM-2 KAT-A-053-H2 |
| **1e** | 191106 NDM-1 KAT-A-102-H1 | KAT-A-102-H1 GIM-1 | 191106 VIM-2 KAT-A-102-H1 |

IC_{50} = >2700 µM
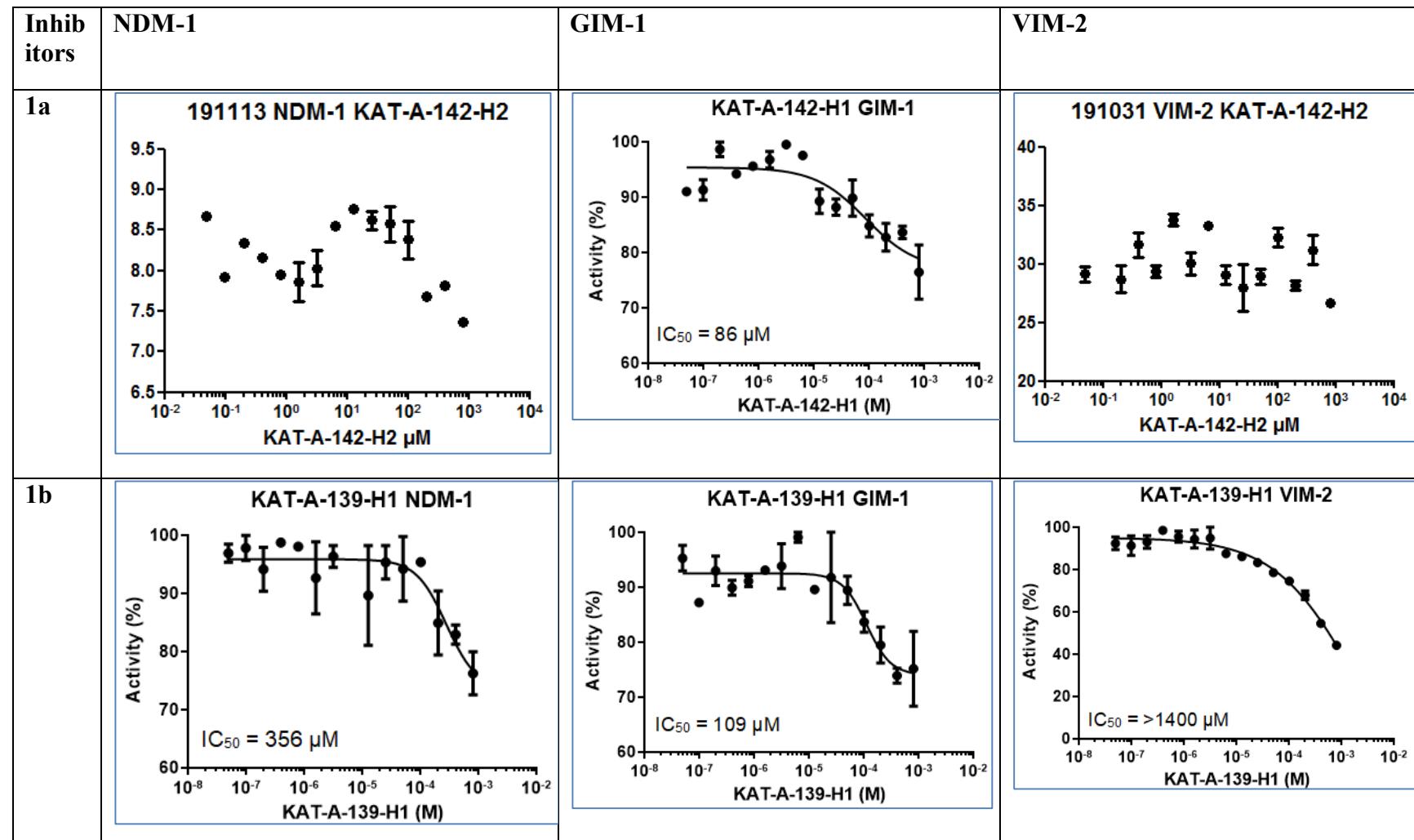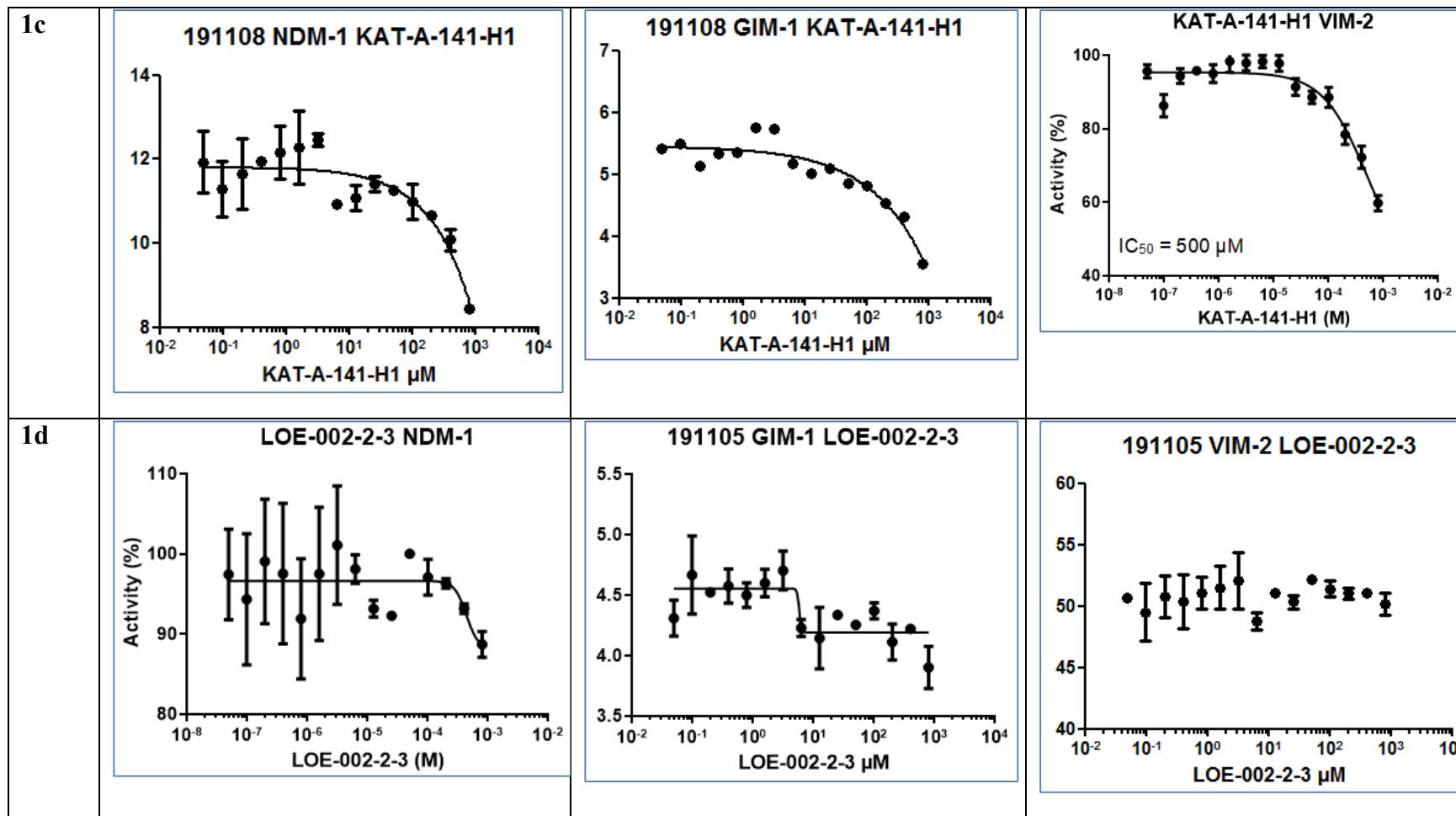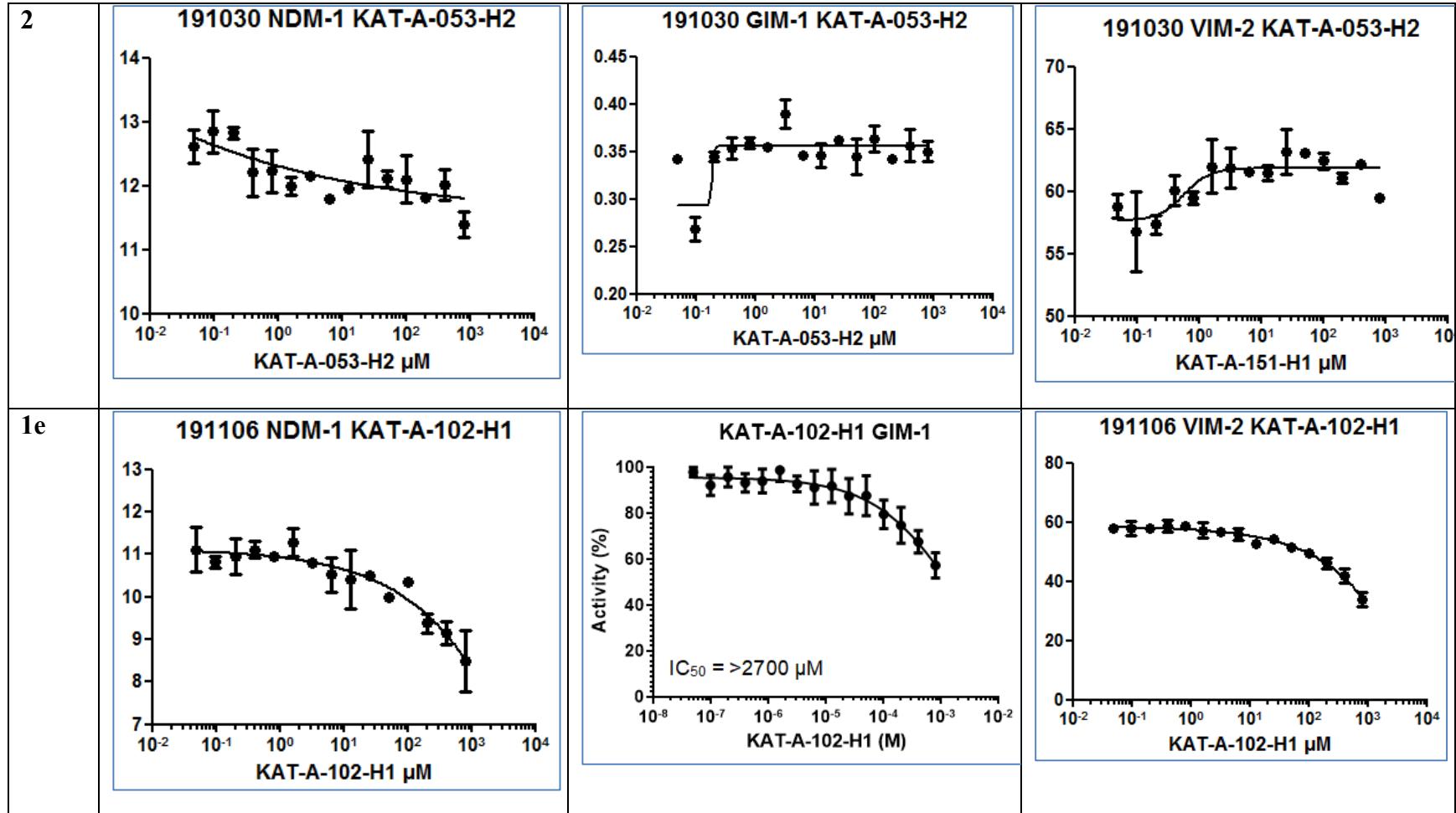
3. MIC studies for NDM-1

Table S2. Susceptibility assay with E. coli ATCC 25922 NDM-1

Compound	Minimum inhibitory concentration (mg/L)			
	Meropenem:compound ratio			Meropenem
No compound				32
EDTA 100 µM				< 2
1c	8:1024	4:1024	2:1024	
1b	8:1024	4:1024	2:1024	
1d	8:1024	4:1024	> 2:1024	
1a	8:1024	4:1024	2:1024	
1h	8:1024	4:1024	2:1024	

*__1e__, **1g** and **1f** could not be dissolved in MIC assay.

4. Cytotoxicity assay
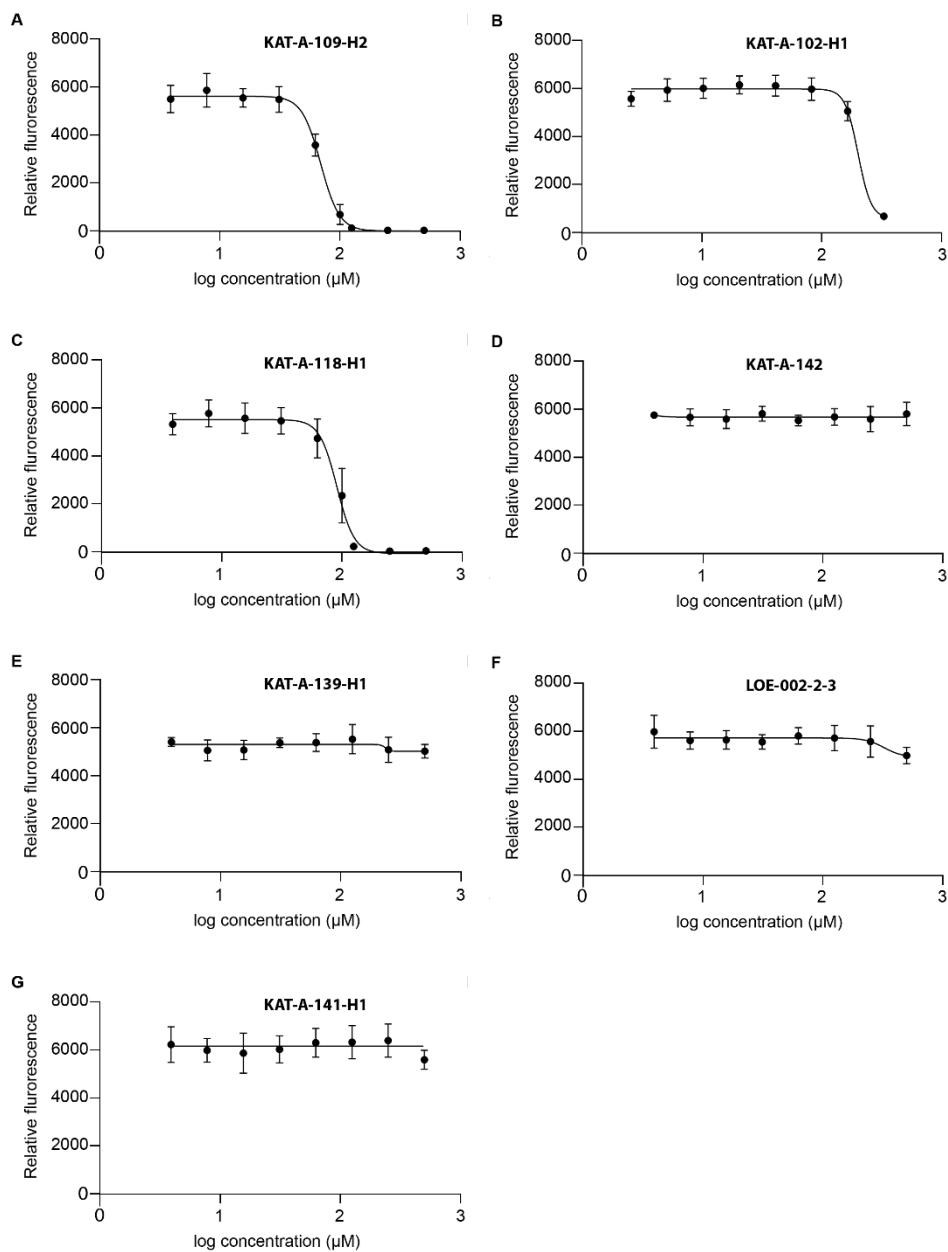
Figure S1. Cytotoxicity dose-response curves of phosphonamidate monoesters as determined in HeLa cells. The cells were treated with the compounds at the indicated concentrations, and cell viability was measured after 24 hours incubation. The following IC_{50} values were determined to be (A) 69.2 μM (SD = 1.0) for KAT-A-109-H2, (B) 201.4 μM (SD = 4.4) for KAT-A-102-H1 and (C) 90.7 μM (SD = 1.0) for KAT-A-118-H1. All remaining compounds (D-H) had IC_{50} values above 500 μM. Data is shown as mean ± SD of at least two independent experiments.

*A **1h**, B **1e**, C **1f**, D **1a**, E **1b**, F **1d**, G **1c**.

5. NMR assignment of NDM-1, chemical shift titration and NOE
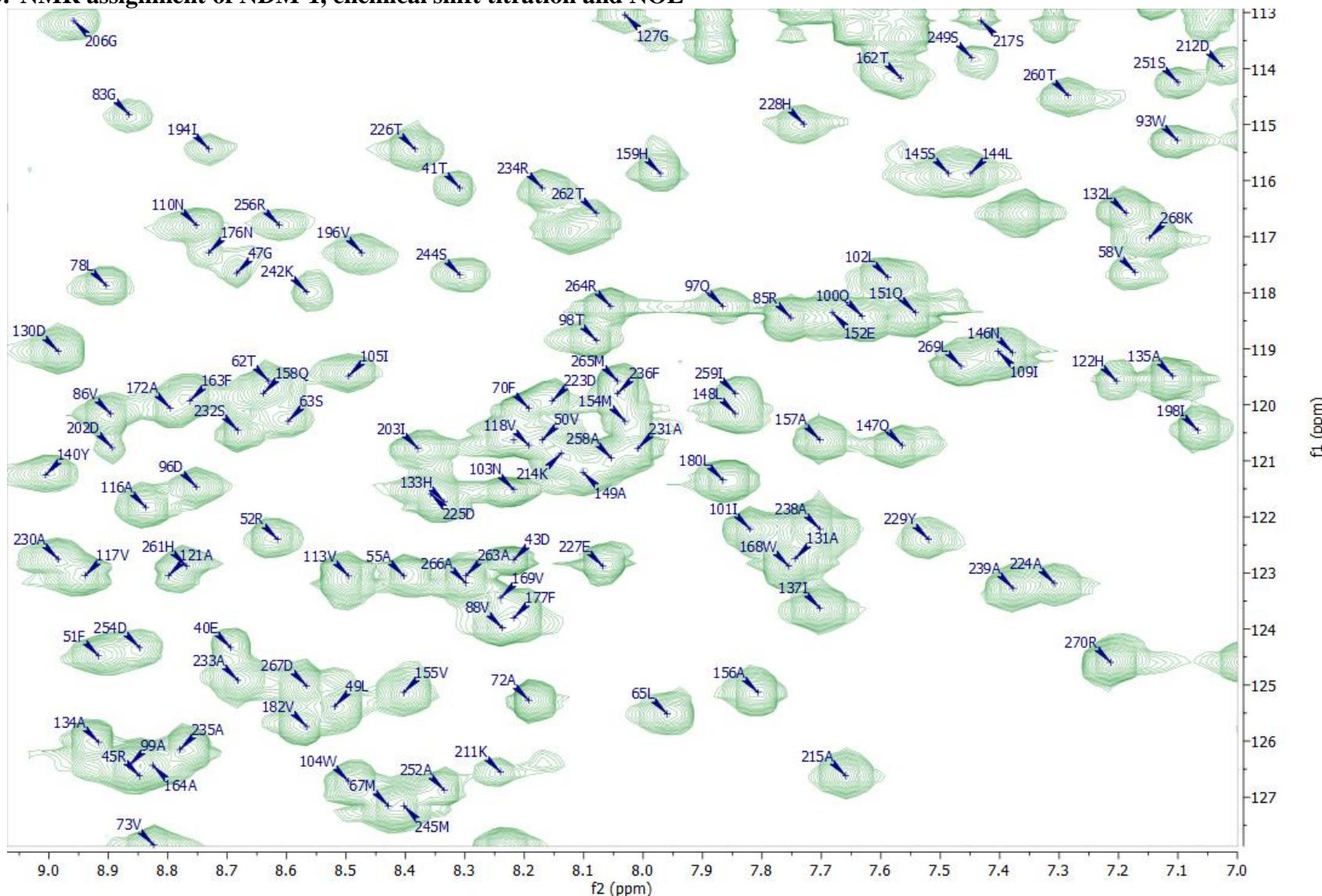
Figure S2. Expansion of the overlapping area of $^1H,^{15}N$ HSQC with assignment of chemical shift of NDM-1
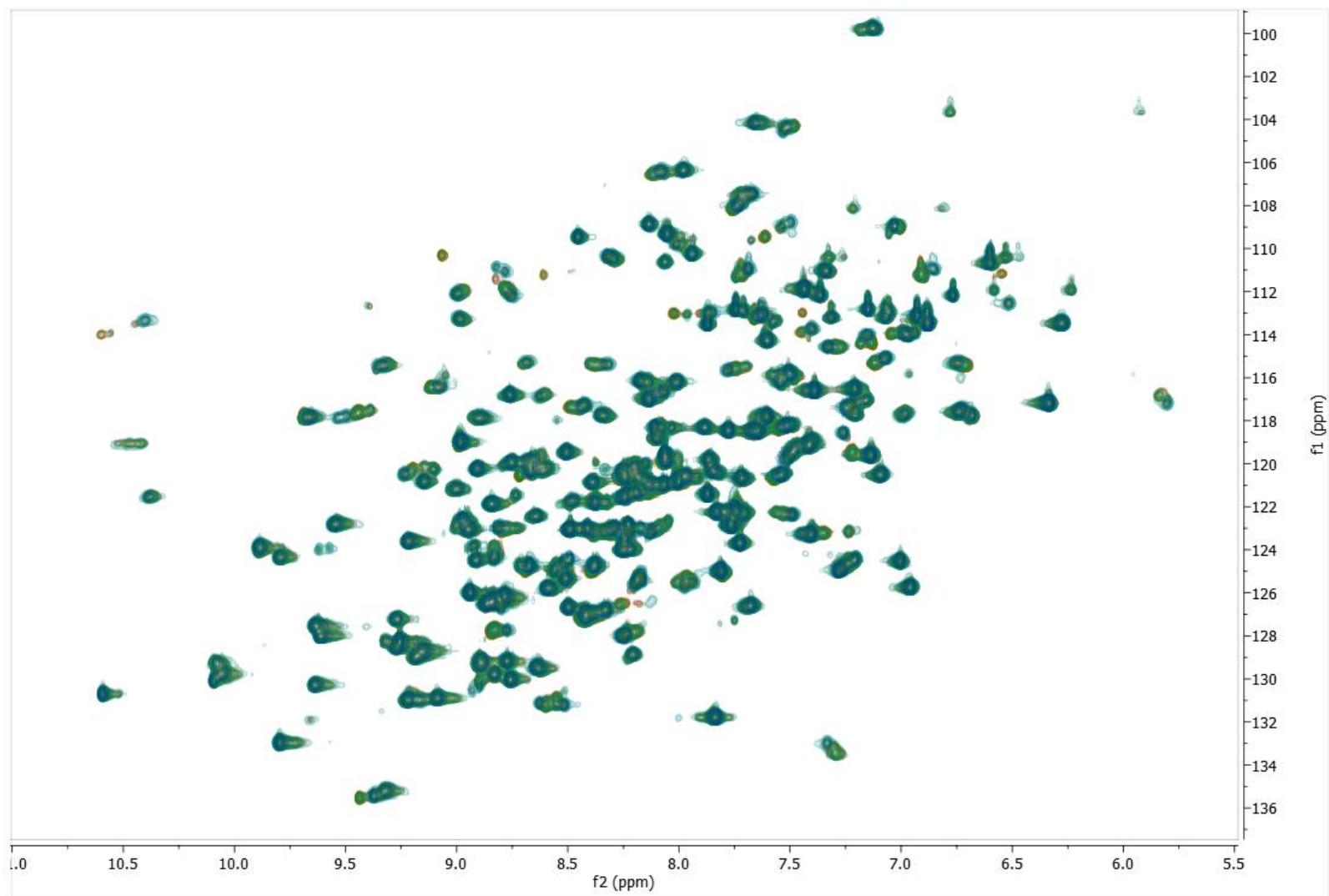
Figure S3. Superposition of the full ^{1}H,^{15}N HSQC spectrum of free NDM-1 (titration step 0 in red), and NDM-1 bound to **1d** (last step 10 eq blue)
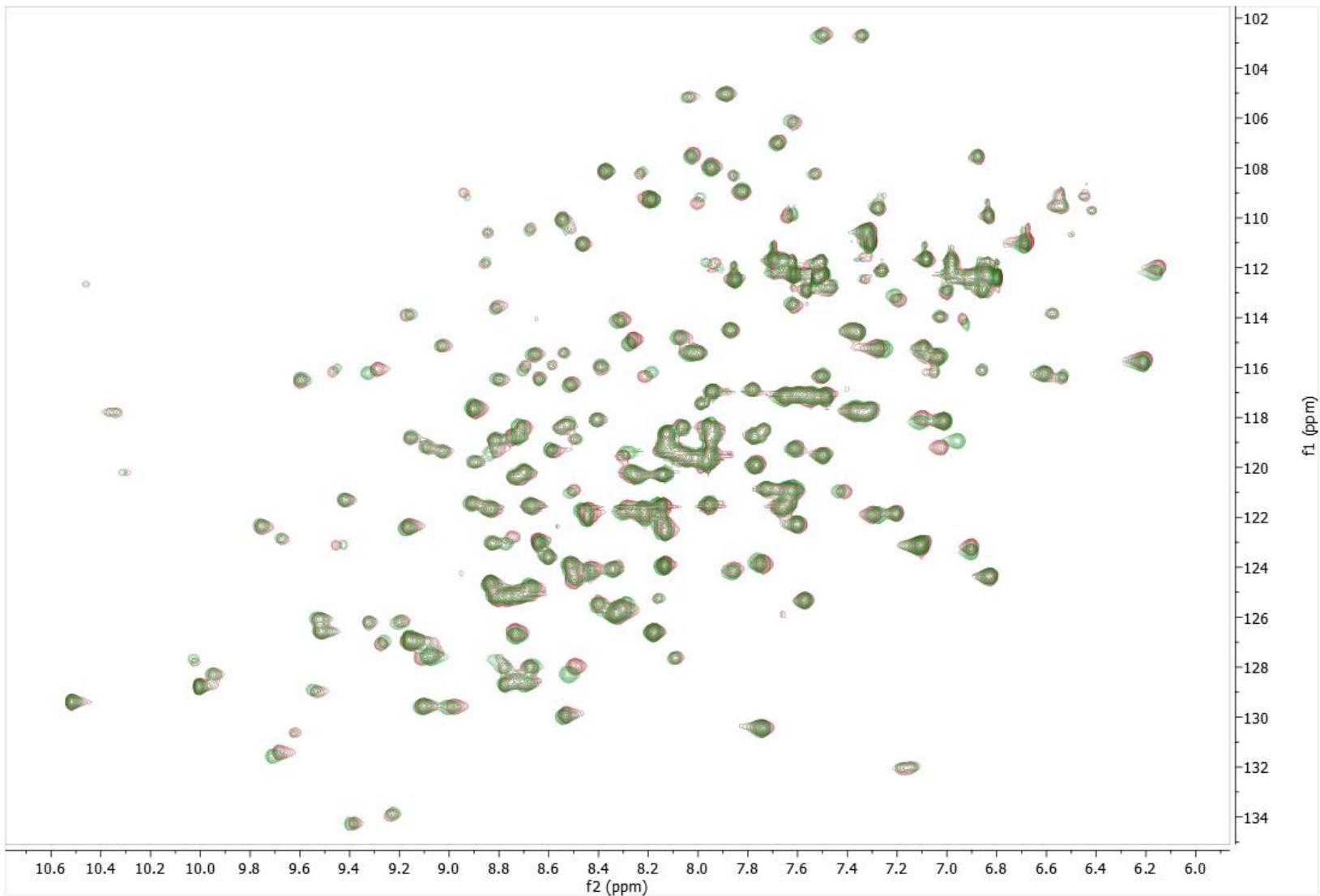
**Figure S4.** ^{1}H,^{15}N HSQC of NDM-1 (green) with 5% EtOH (red).
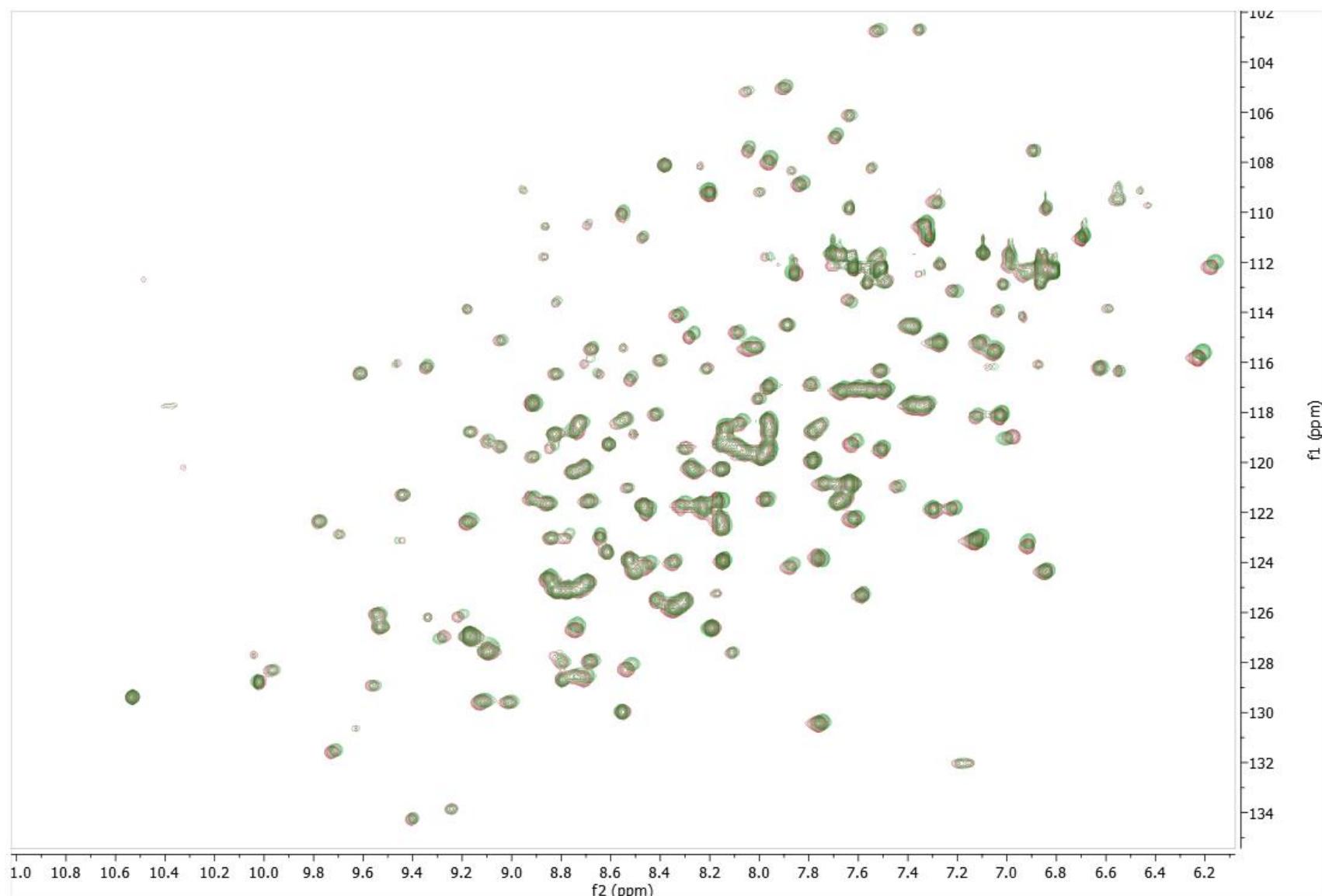
Figure S5. ^{1}H-^{15}N HSQC of NDM-1 (red) with 5% DMSO (green).
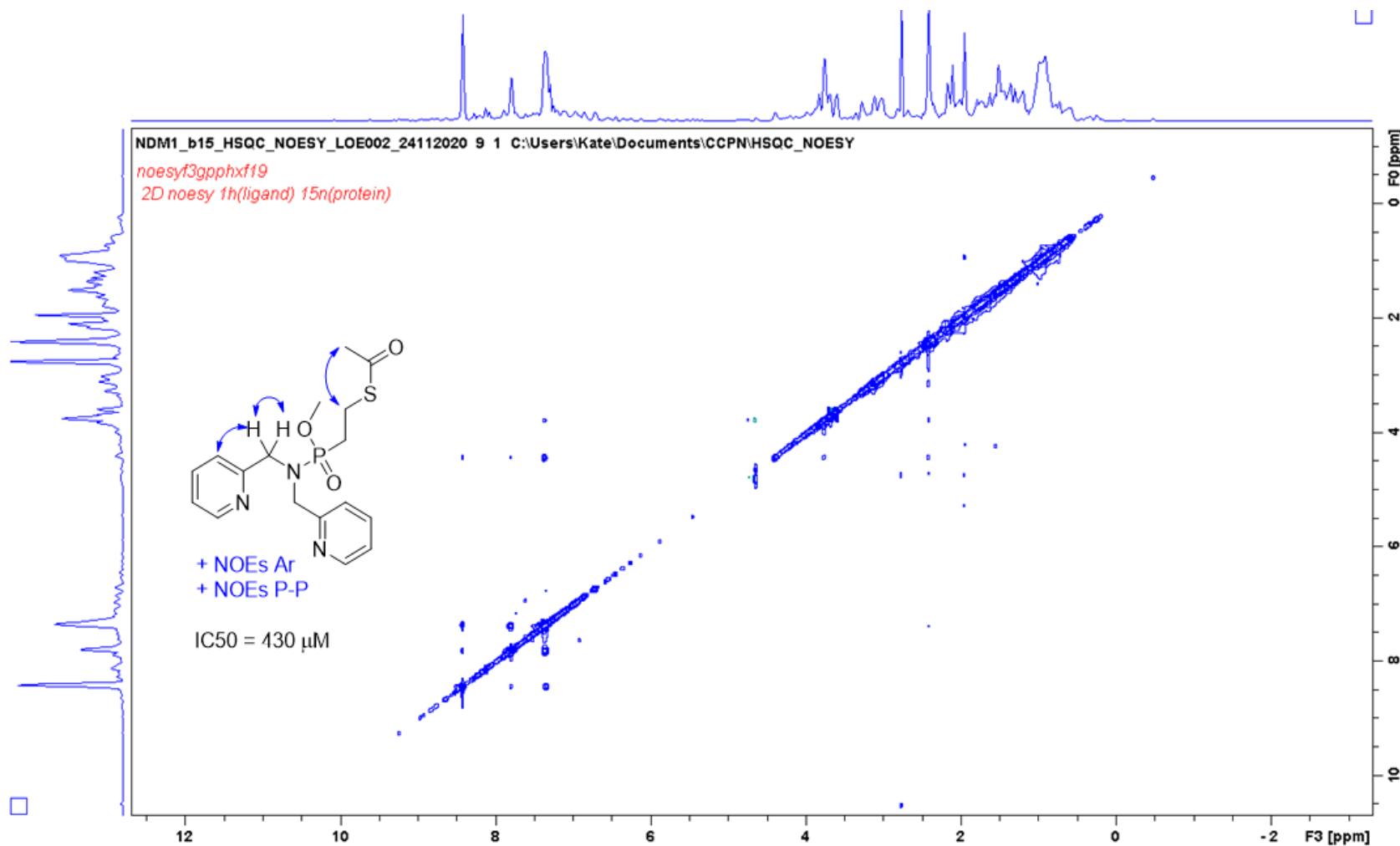
**Figure S6.** 2D ^{15}N-filtered NOESY for NDM-1 with **1d** (8 eq).
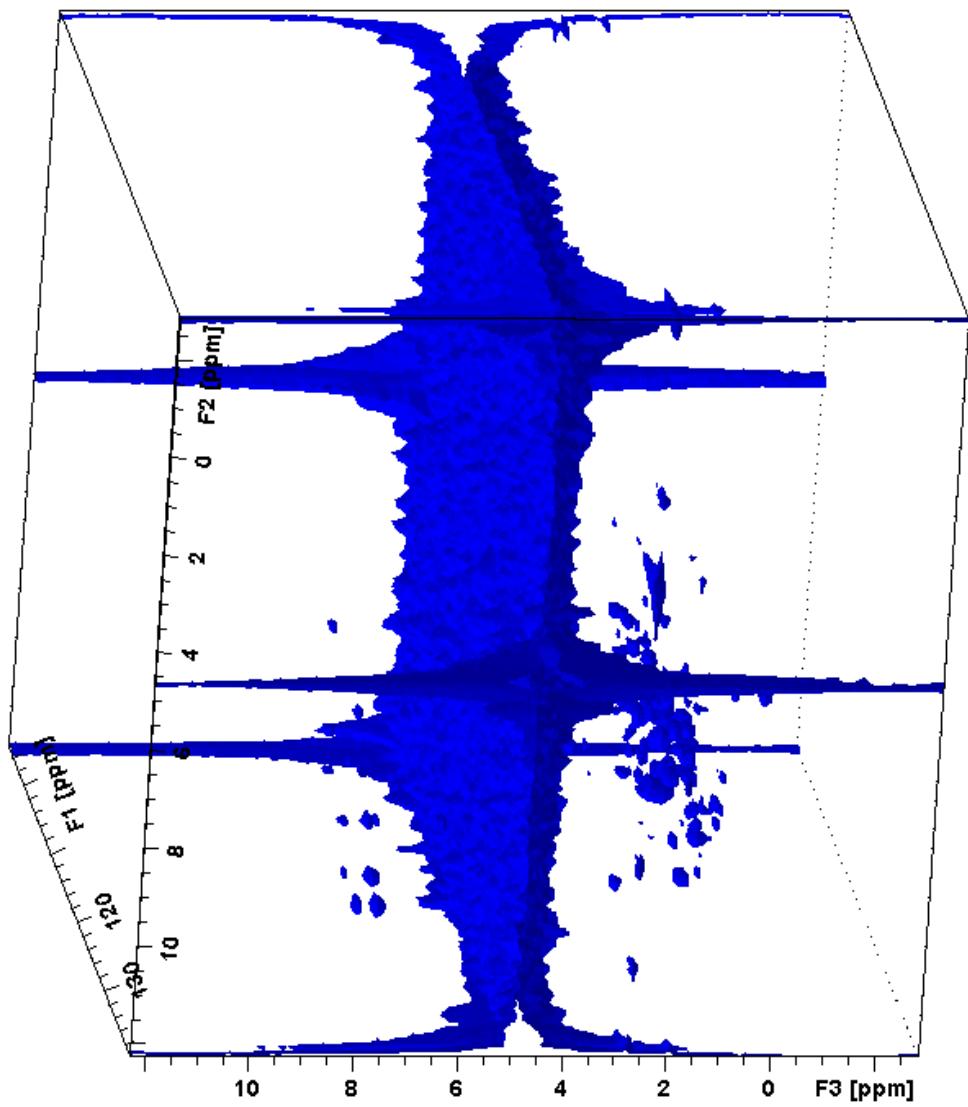
Figure S7. 3D ^{15}N-filtred HSQC-NOESY in 3D cube mode for NDM-1 and **1d** (8eq). Dimensions: F1 = chemical shift of ^{15}N protein; F2 = chemical shift of ^{1}H protein, F3 = chemical shift of ^{1}H ligand.
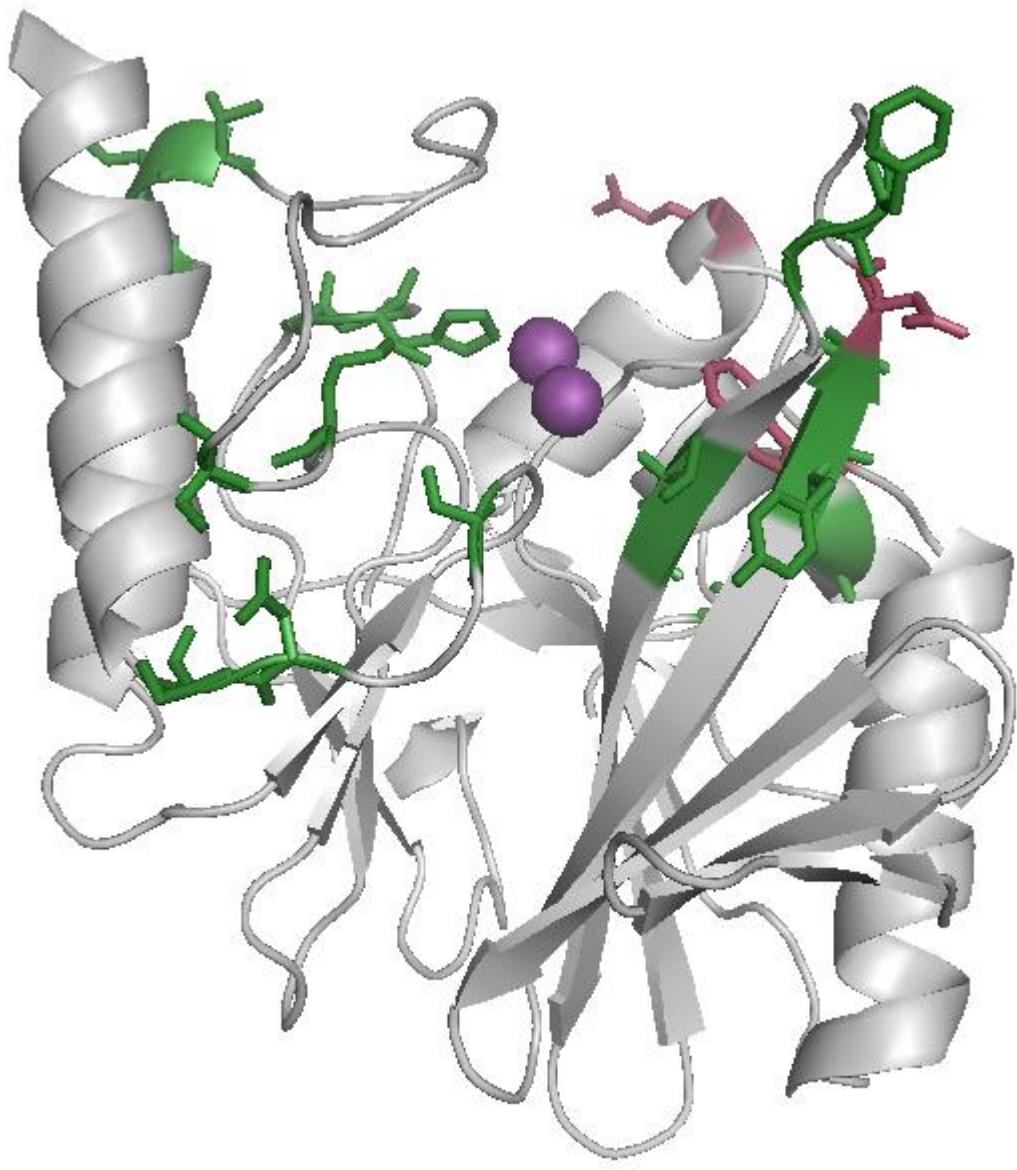
Figure S8. Highlighted green and pink amino acid residues on the NDM-1 (*PDB:4exs*) secondary structure with SSP corresponding to weighted average (^{1}H,^{15}N $\Delta\delta$) greater than the population mean plus standard deviation ($\mu+1\sigma$) . Pink colored residues are highlighted due to additional NOE correlations detected in the 3D ^{15}N-filtered HSQC-NOESY.

6. The protein sequence of NDM-1

Figure S9. Sequence of pET27b(+)_His-NDM-1

LacI

LacI promoter

T7 promoter

Lac operator

Ribosome binding site

PelB sequence

NDM-1 gene

TEV site encoding bases

His-tag

TGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTA
CGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTT
CCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTC
CCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAG
GGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACG
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GGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACC
TGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGT
GAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCA
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ACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAATCGATA
GATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATC
AGCATCCATGTTGGAATTTAATCGCGGCCTAGAGCAAGACGTTTCCCGTTGAATATG
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S15

CAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGAT
CAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAA
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GTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACG
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CAGTCGCGTACCGTCTTCATGGGAGAAATAATACTGTTGATGGGTGTCTGGTCAGA
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CCTGGTCATCCAGCGGATAGTTAATGATCAGCCCACTGACGCGTTGCGCGAGAAGAT
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GCGCCATTCGATGGTGTCCGGGATCTCGACGCTCTCCCTTATGCGACTCCTGCATTA
GGAAGCAGCCCAGTAGTAGGTTGAGGCCGTTGAGCACCGCCGCCGCAAGGAATGGT
GCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATACC
CACGCCGAAACAAGCGCTCATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGG
TGATGTCGGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCC
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TGTCGAACCAGCTTGCCCCGCAAGAGGGGATGGTTGCGGCGCAACACAGCCTGACT
TTCGCCGCCAATGGCTGGGTCGAACCAGCAACCGCGCCCAACTTTGGCCCGCTCAAG
GTATTTTACCCCGGCCCCGGCCACACCAGTGACAATATCACCGTTGGGATCGACGGC
ACCGACATCGCTTTTGGTGGCTGCCTGATCAAGGACAGCAAGGCCAAGTCGCTCGG
CAATCTCGGTGATGCCGACACTGAGCACTACGCCGCGTCAGCGCGCGCGTTTGGTGC
GGCGTTCCCCAAGGCCAGCATGATCGTGATGAGCCATTCCGCCCCCGATAGCCGCGC
CGCAATCACTCATACGGCCCGCATGGCCGACAAGCTGCGCTAATGAGGATCCGAAT
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AGAACTCGCCCCGGAAGACCCCGAGGATGTCGAGCACCACCACCACCACCACTGAG
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CAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTG
AAAGGAGGAACTATATCCGGAT

Figure S10. Protein sequence of the produced NDM-1 after TEV cleavage:

GQQMETGDQRFGDLVFRQLAPNVWQHTSYLDMPGFGAVASNGLIVRDGGRVLVVDTA
WTDDQTAQILNWIKQEINLPVALAVVTHAHQDKMGGMDALHAAGIATYANALSNQLA
PQEGMVAAQHSLTFAANGWVEPATAPNFGPLKVFYPGPGHTSDNITVGIDGTDIAFGGC
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DKLR

7. NMR spectra of the synthesized compounds
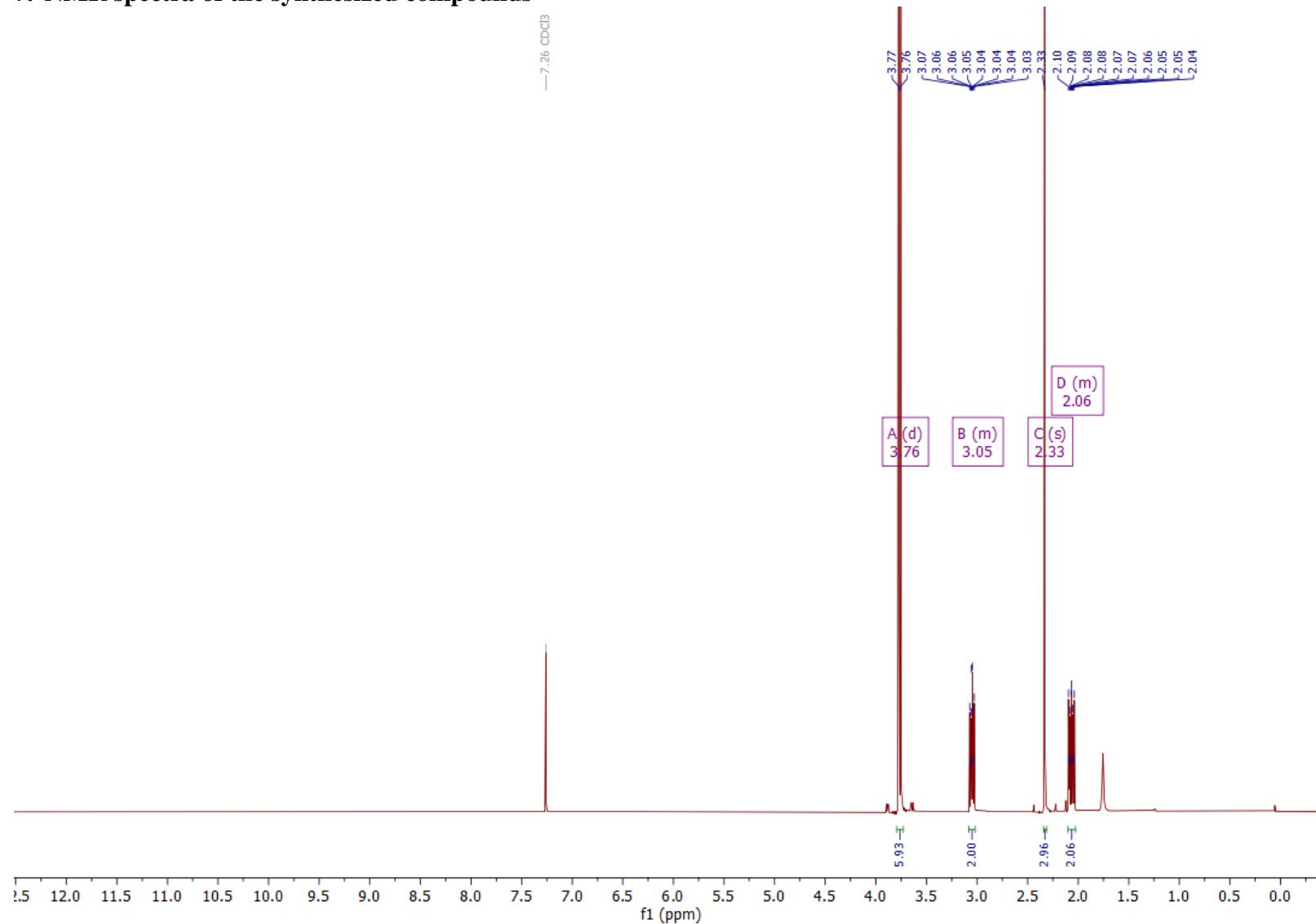
Figure S11. ^{1}H NMR spectrum of **4**.
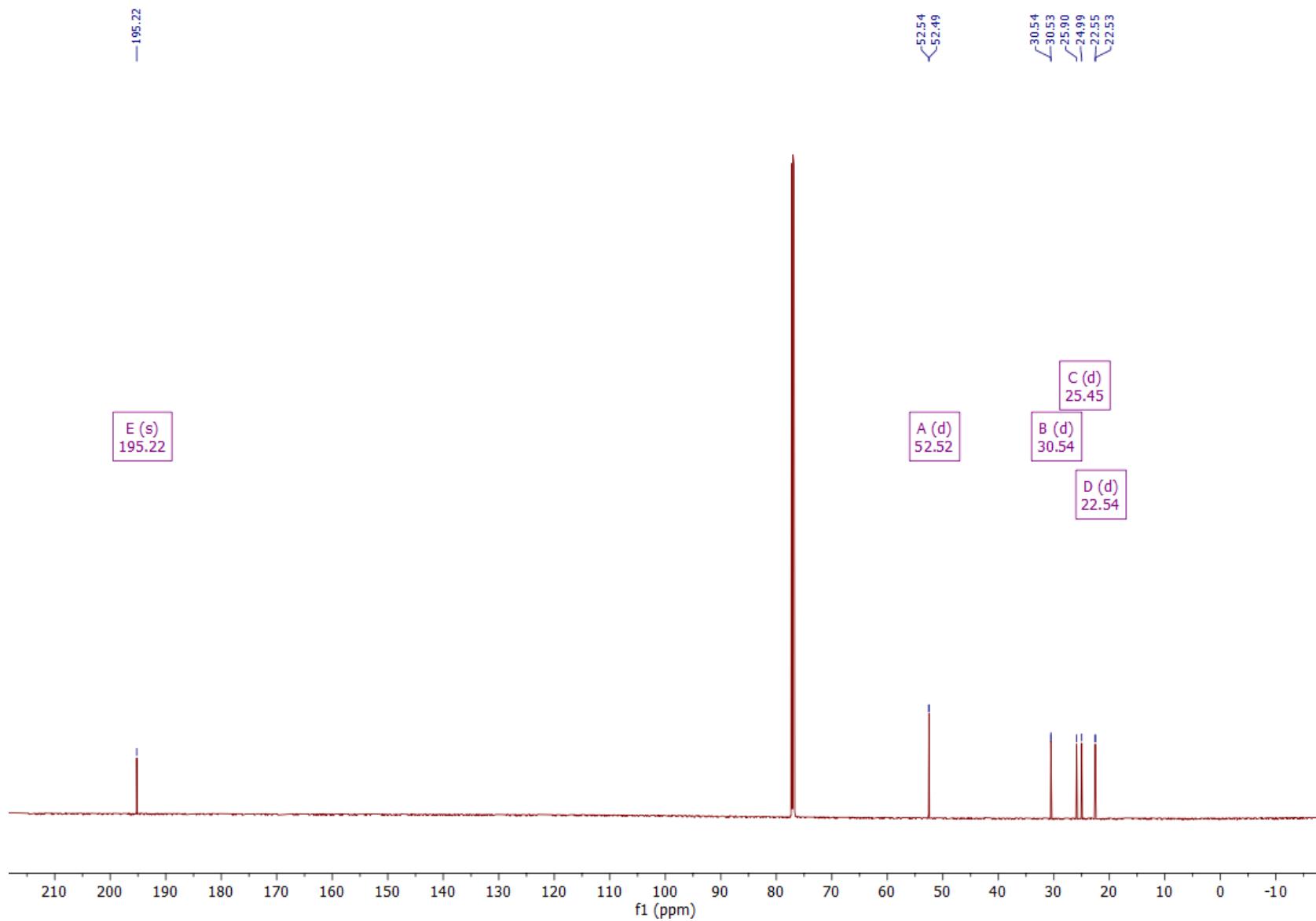
Figure S12. ^{13}C NMR spectrum of **4**.
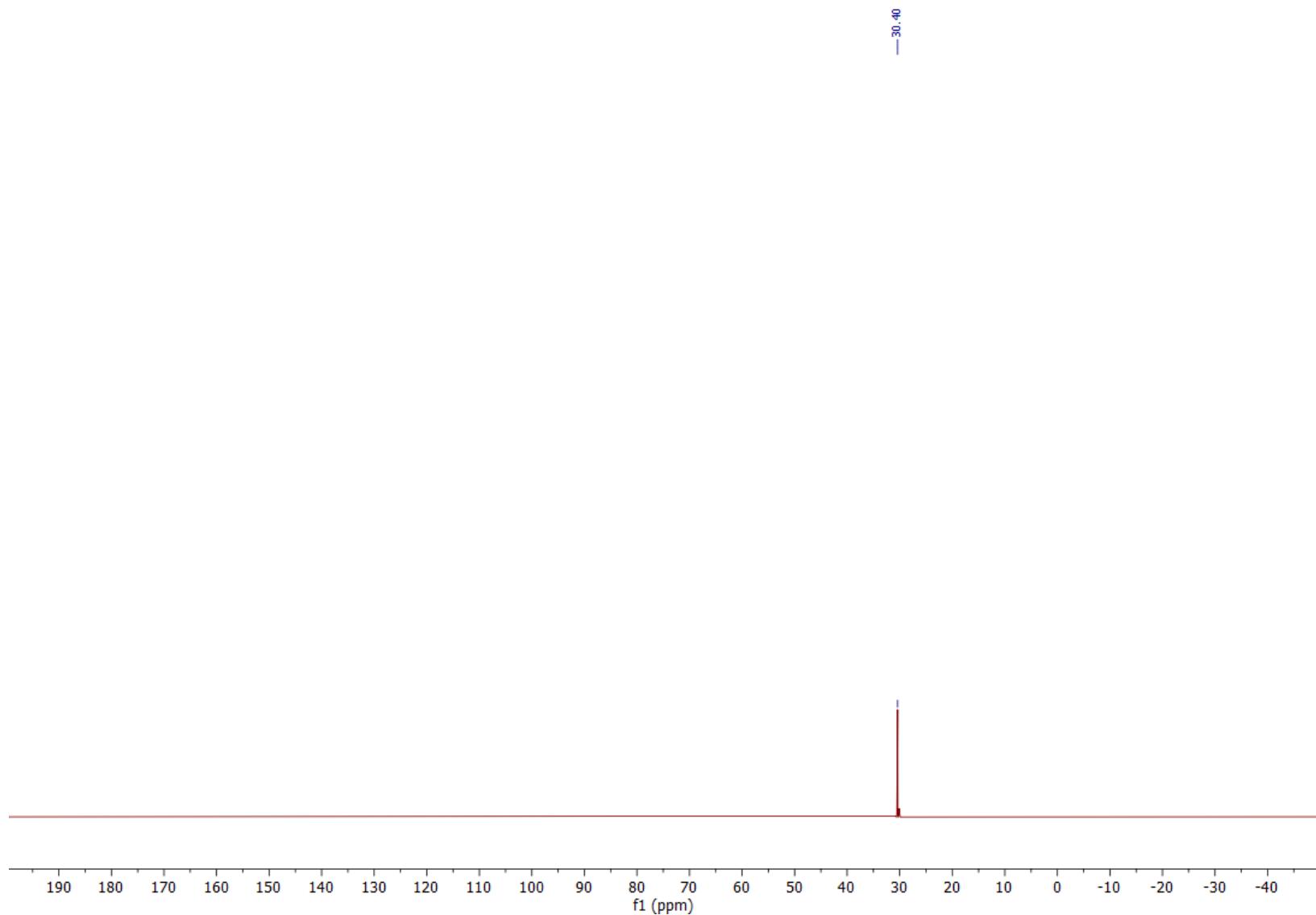
Figure S13. ^{31}P NMR spectrum of **4**.
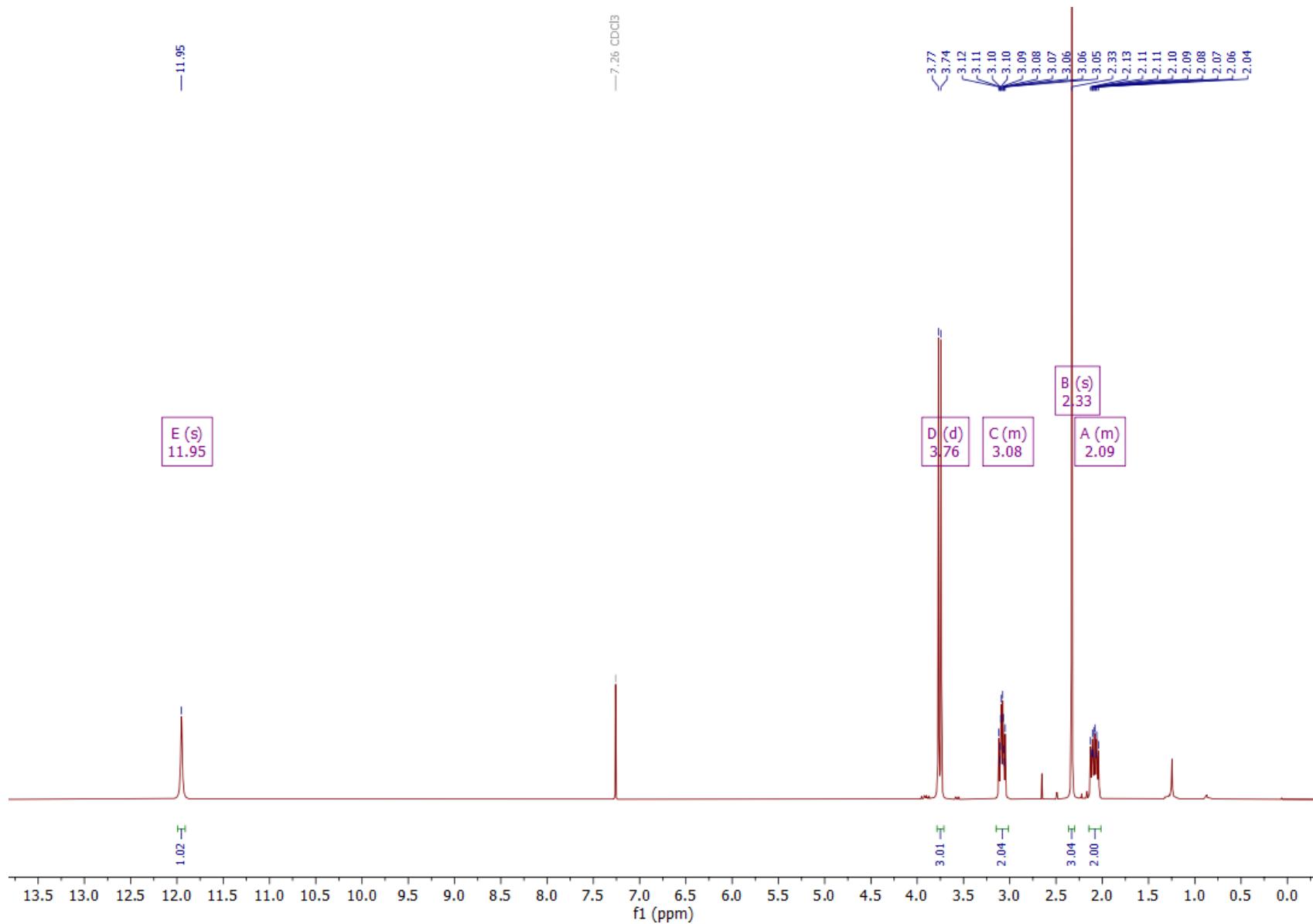
Figure S14. ^{1}H NMR spectrum of **5**.
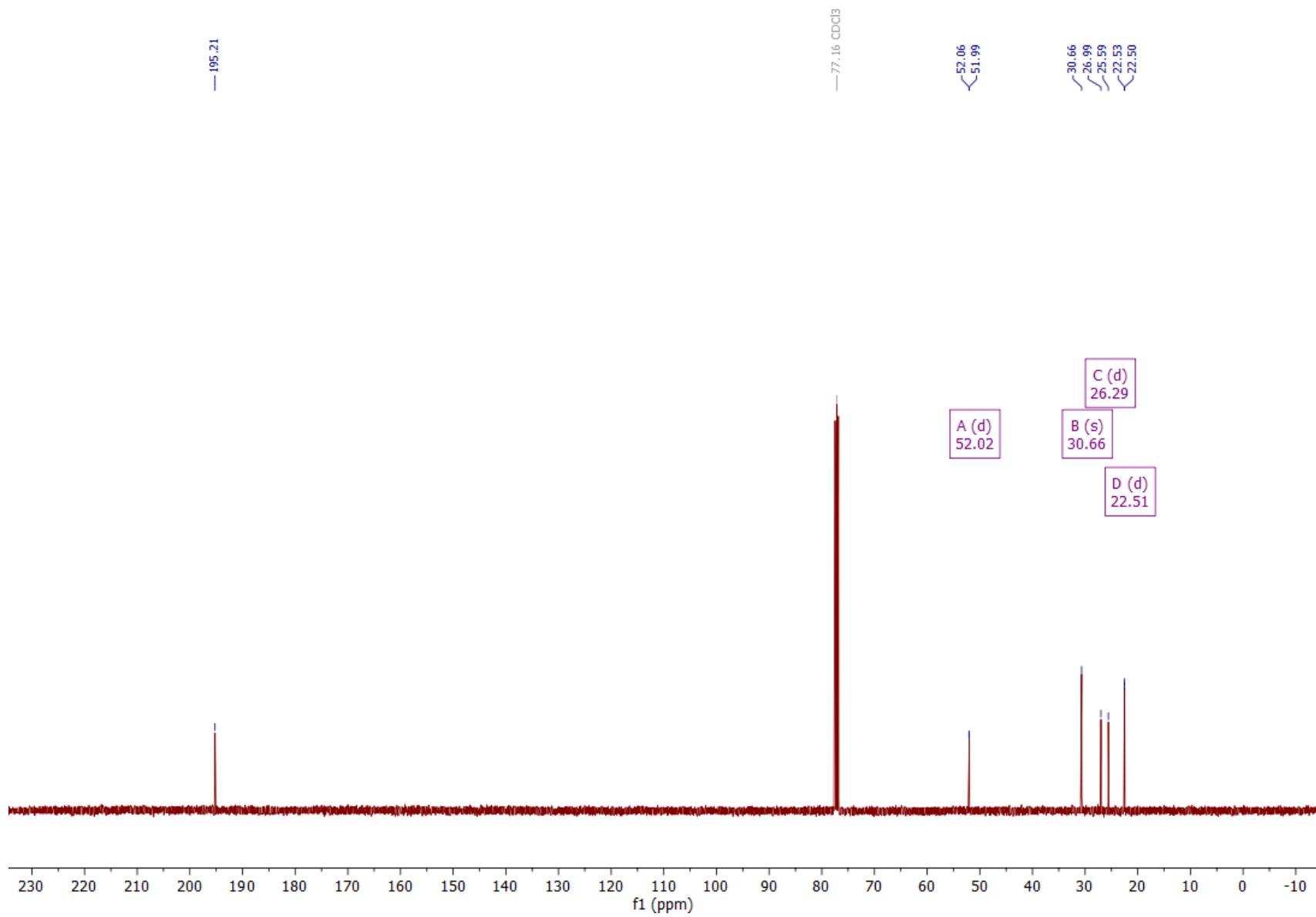
Figure S15. ^{13}C NMR spectrum of **5**.
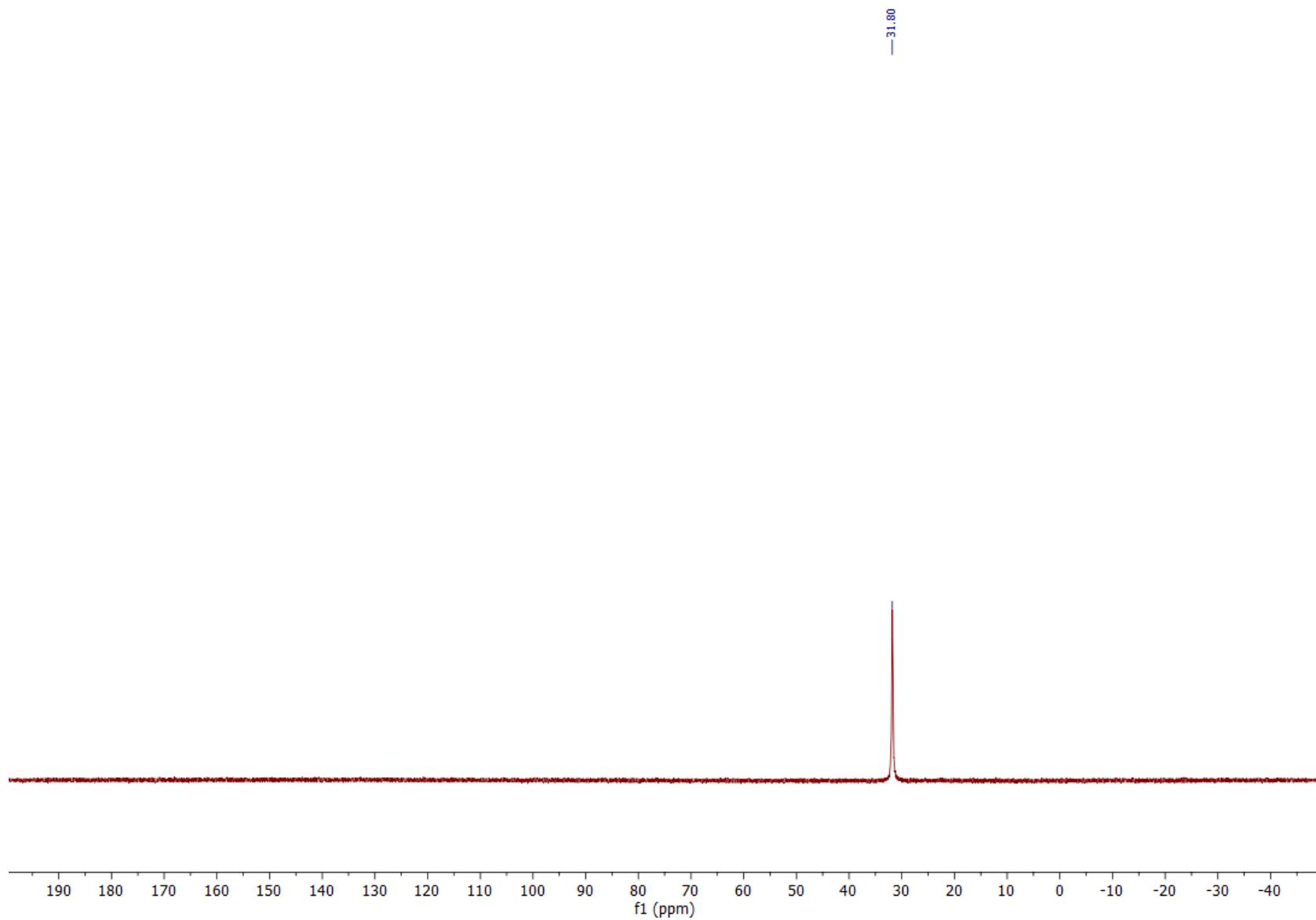
Figure S16. ^{31}P NMR spectrum of **5**.
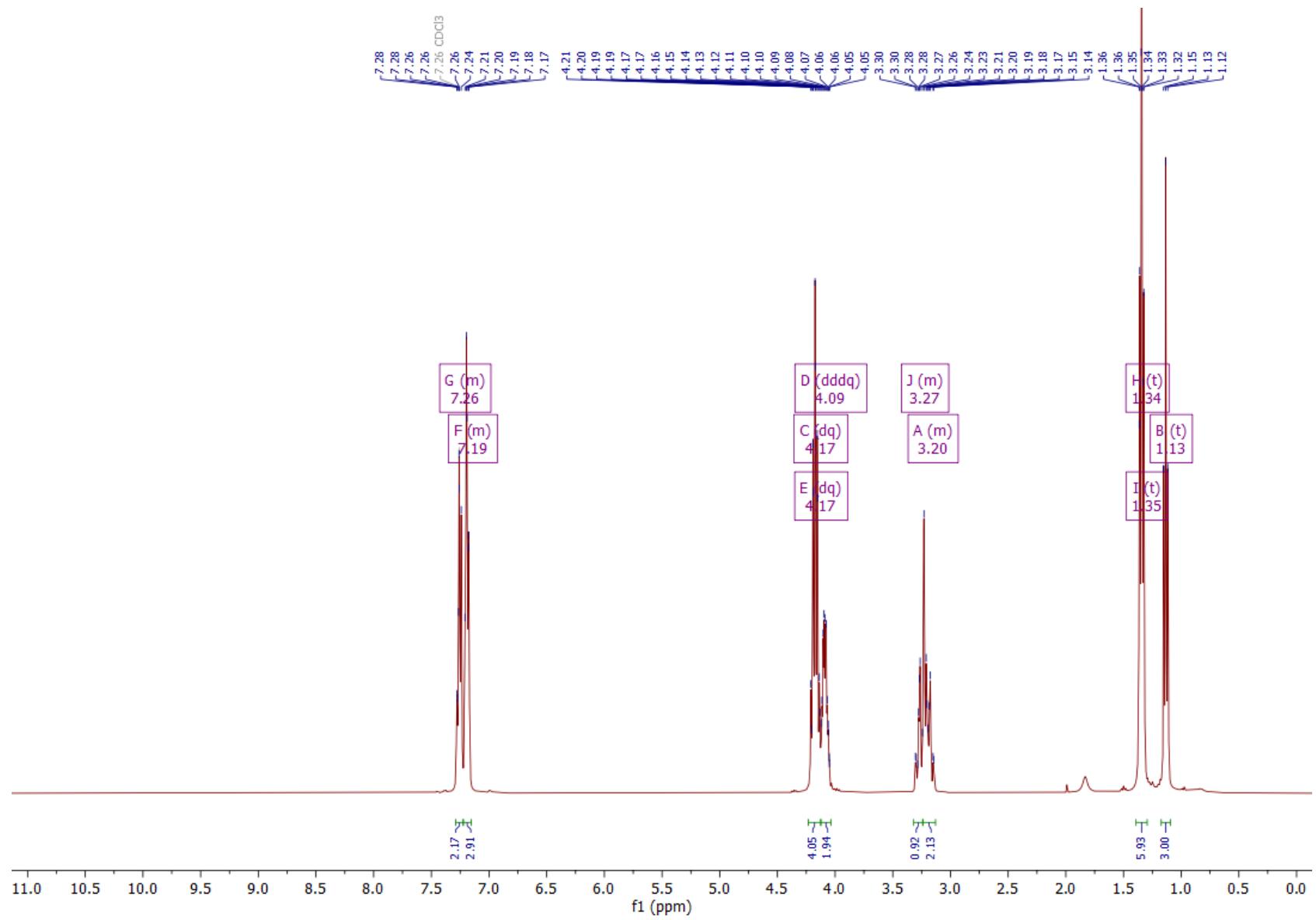
Figure S17. ^{1}H NMR spectrum of **7**.

Figure S18. ^{13}C NMR spectrum of **7**.
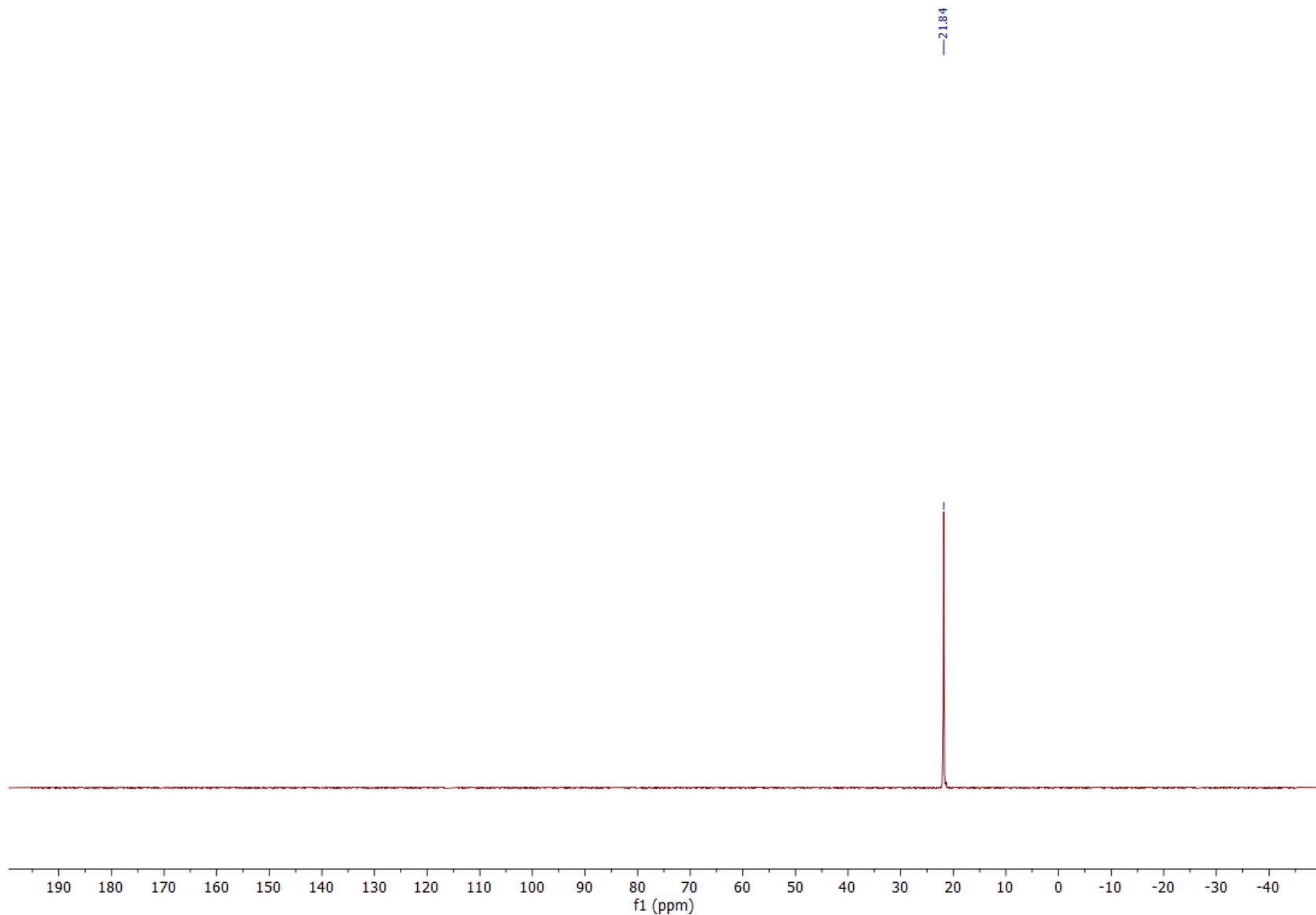
Figure S19. ^{31}P NMR spectrum of **7**.

21.84

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40
f1 (ppm)
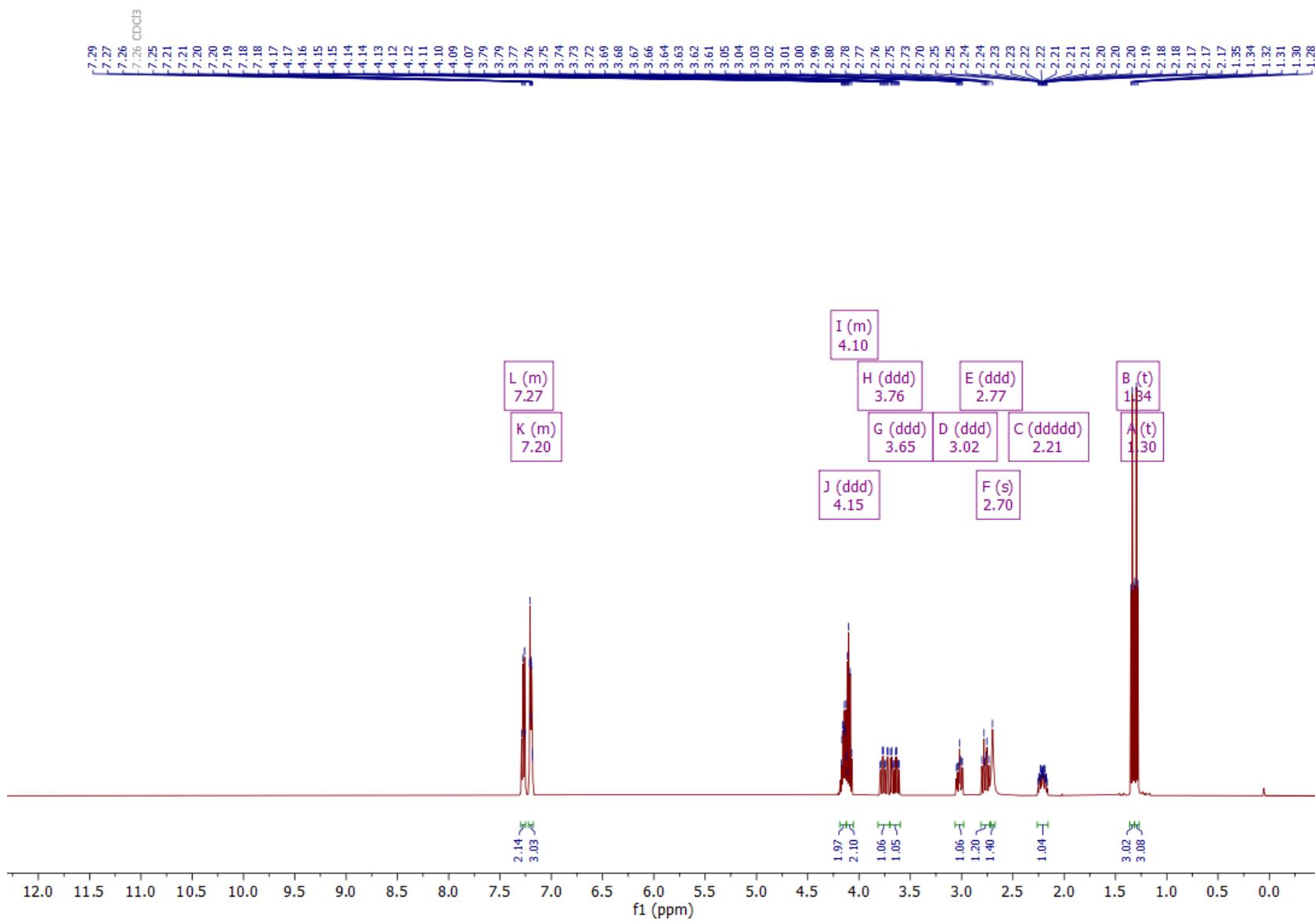
Figure S20. ^{1}H NMR spectrum of **8**.
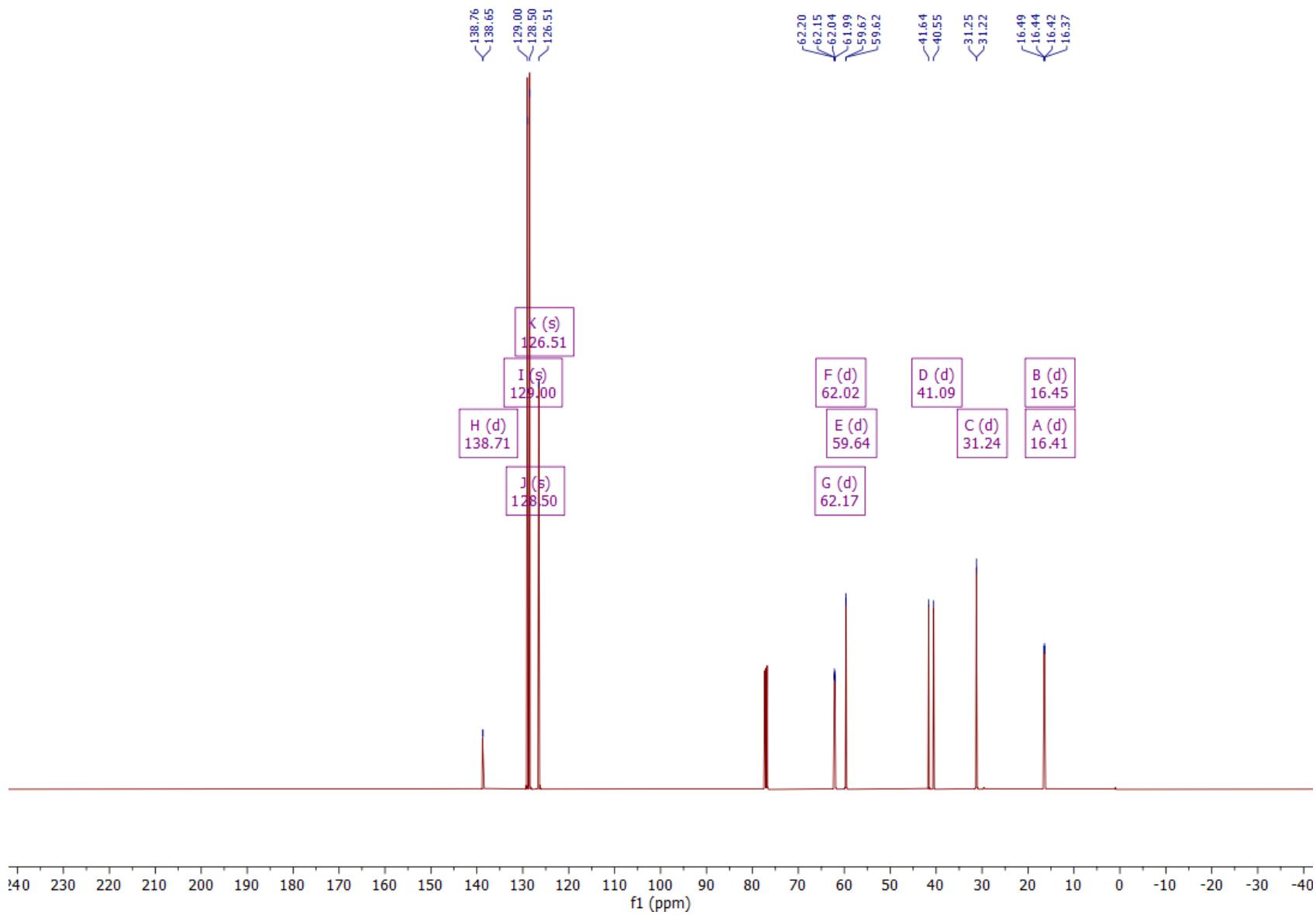
Figure S21. ^{13}C NMR spectrum of **8**.
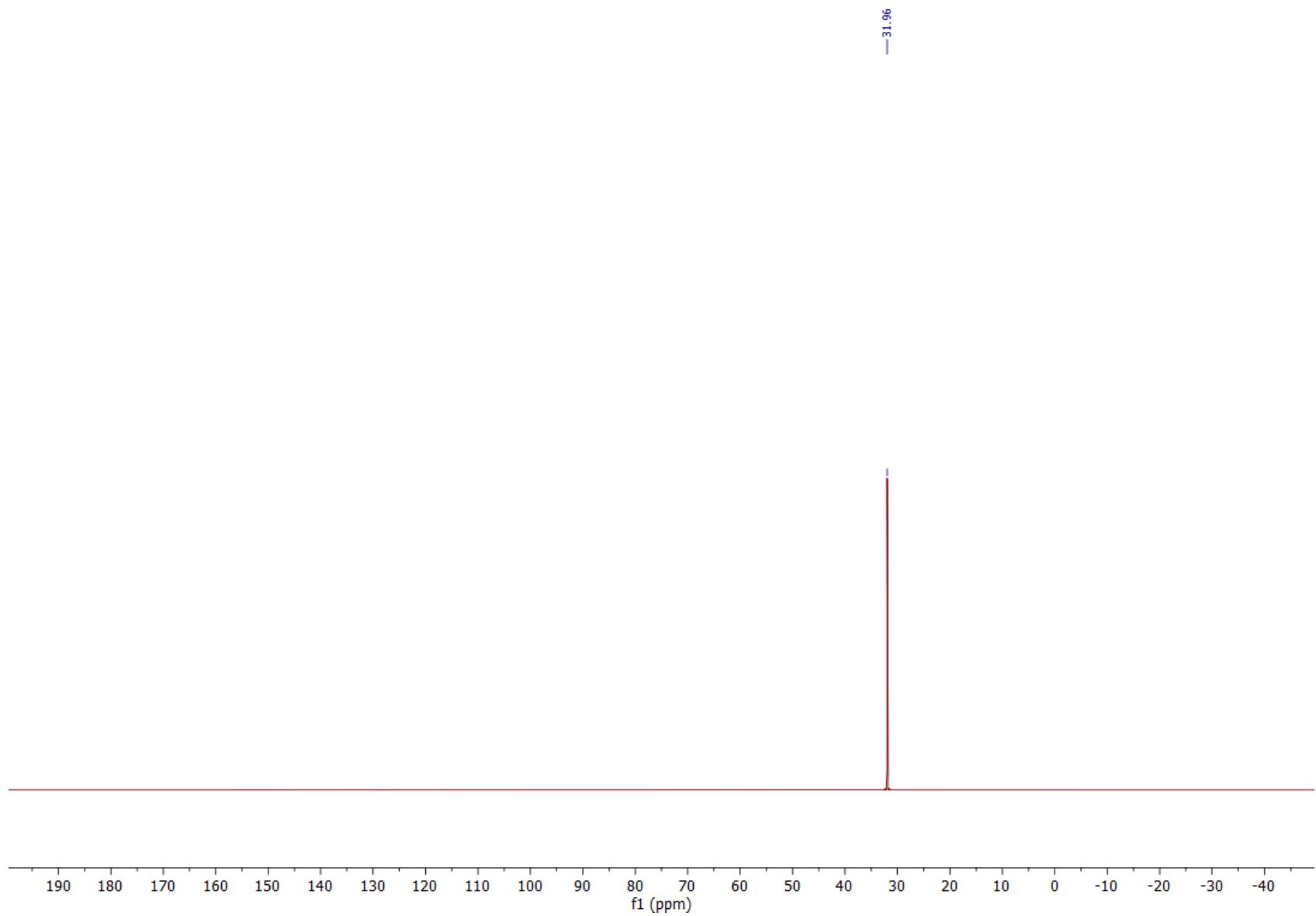
Figure S22. ^{31}P NMR spectrum of **8**.
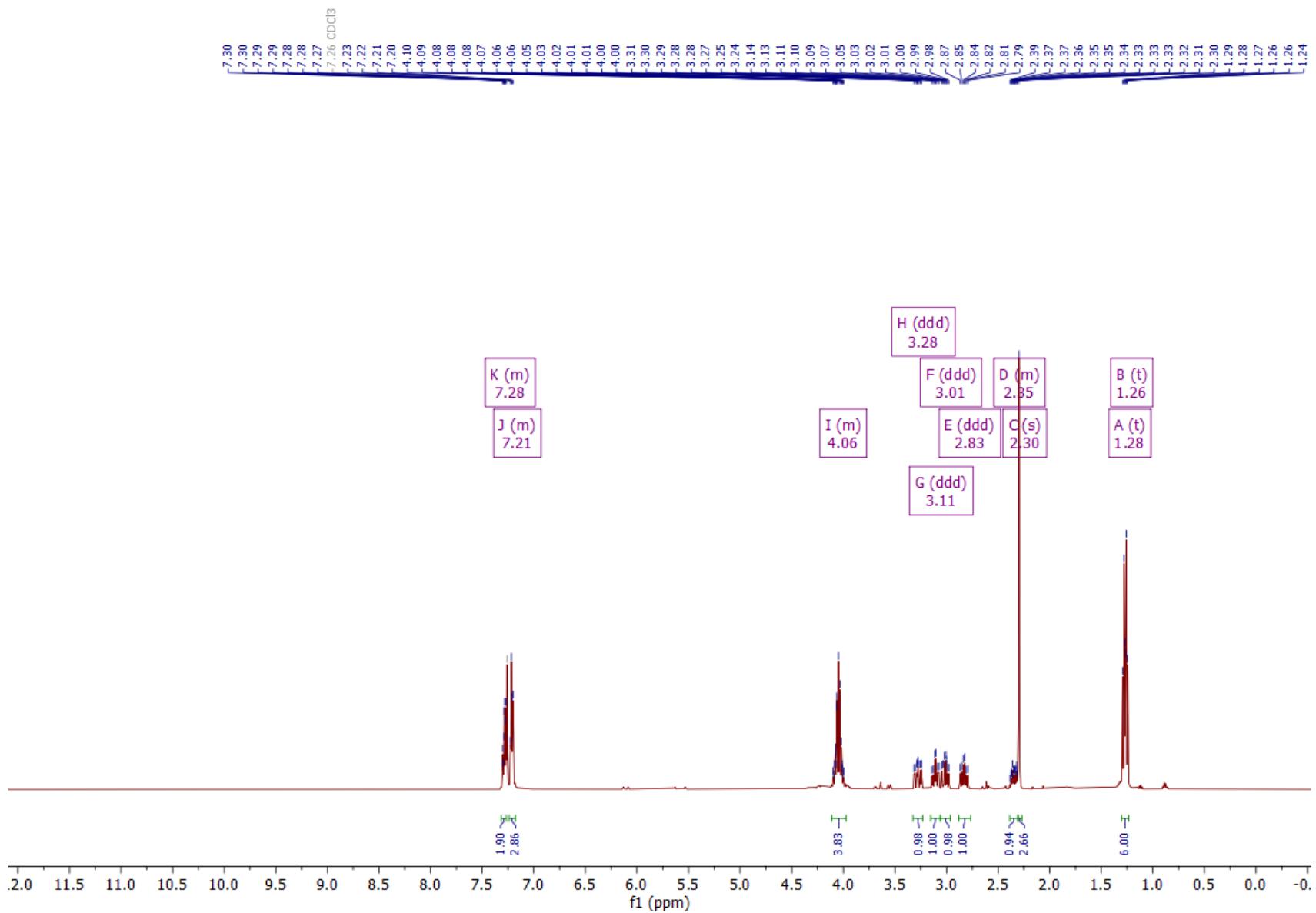
Figure S23. ^{1}H NMR spectrum of **9**.
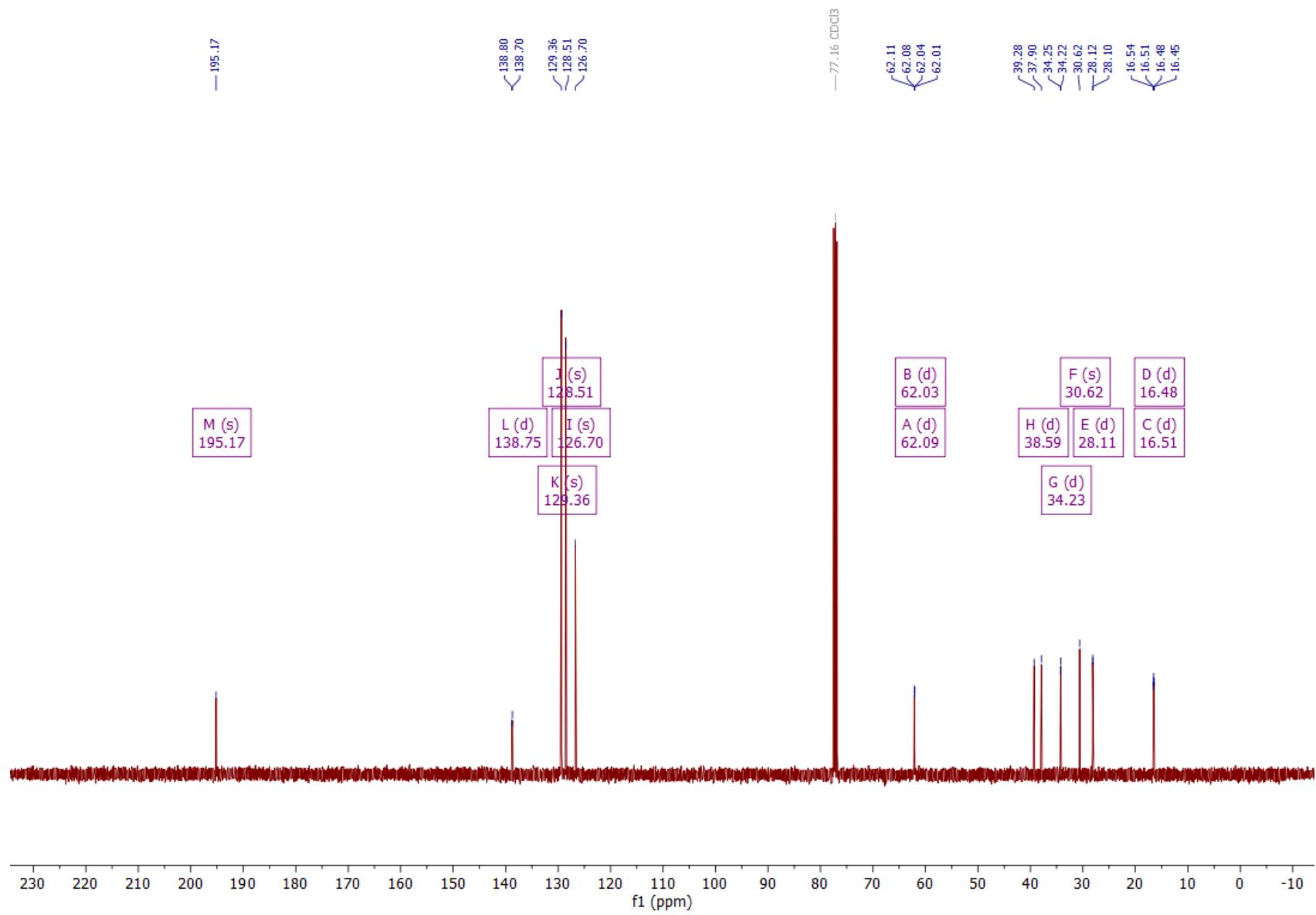
Figure S24. ^{13}C NMR spectrum of **9**.
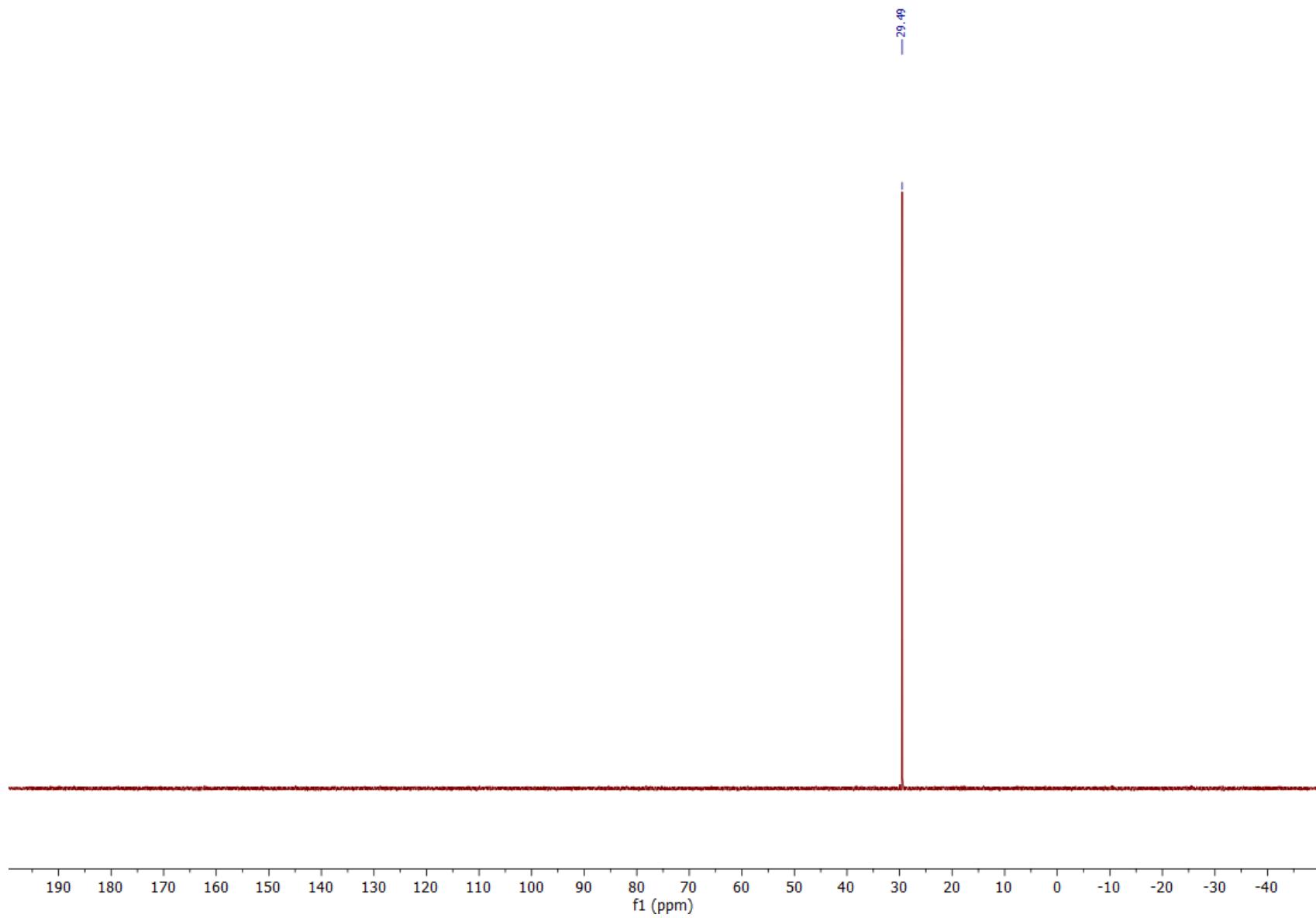
Figure S25. ^{31}P NMR spectrum of **9**.
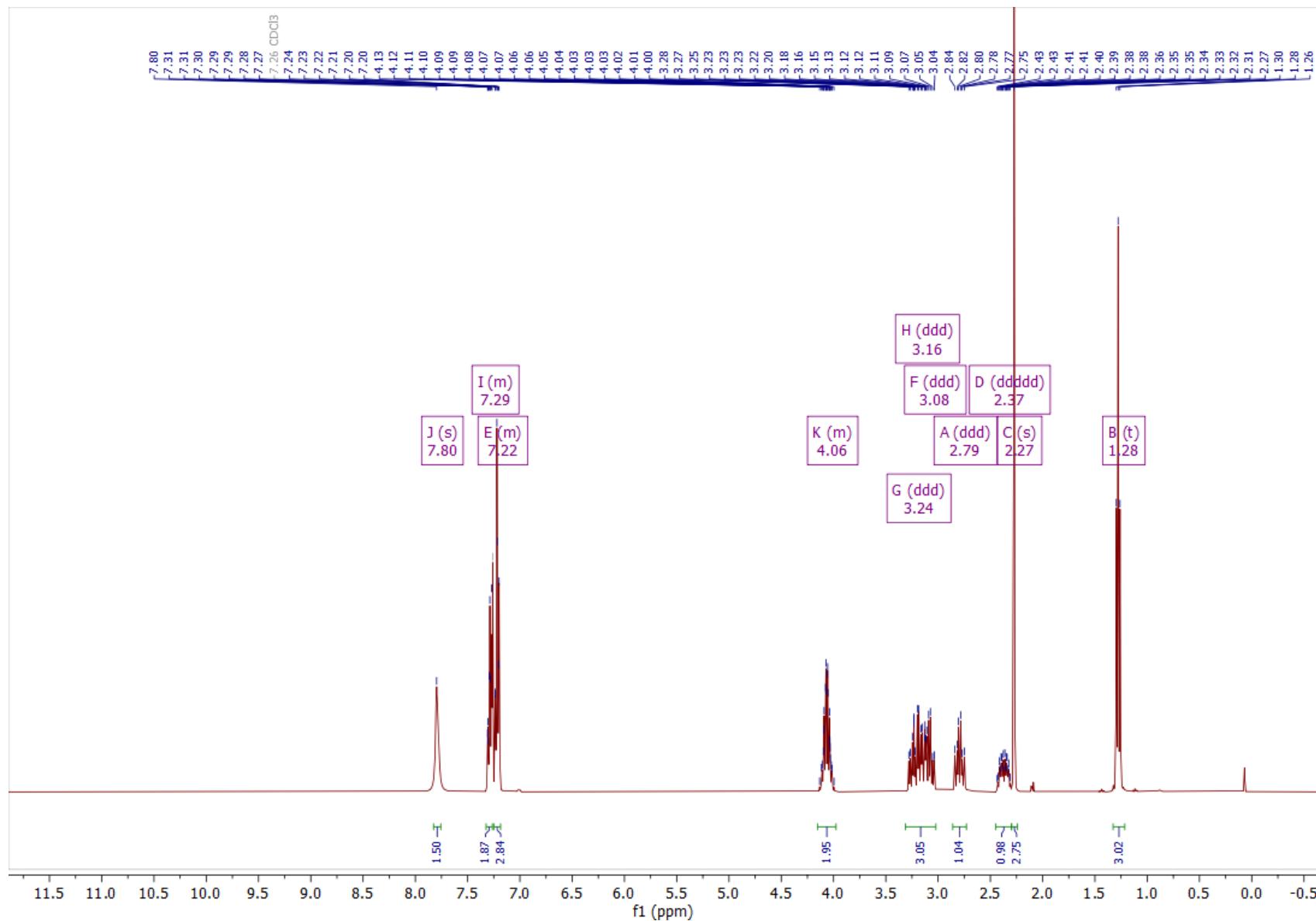
Figure S26. ^{1}H NMR spectrum of **10**.
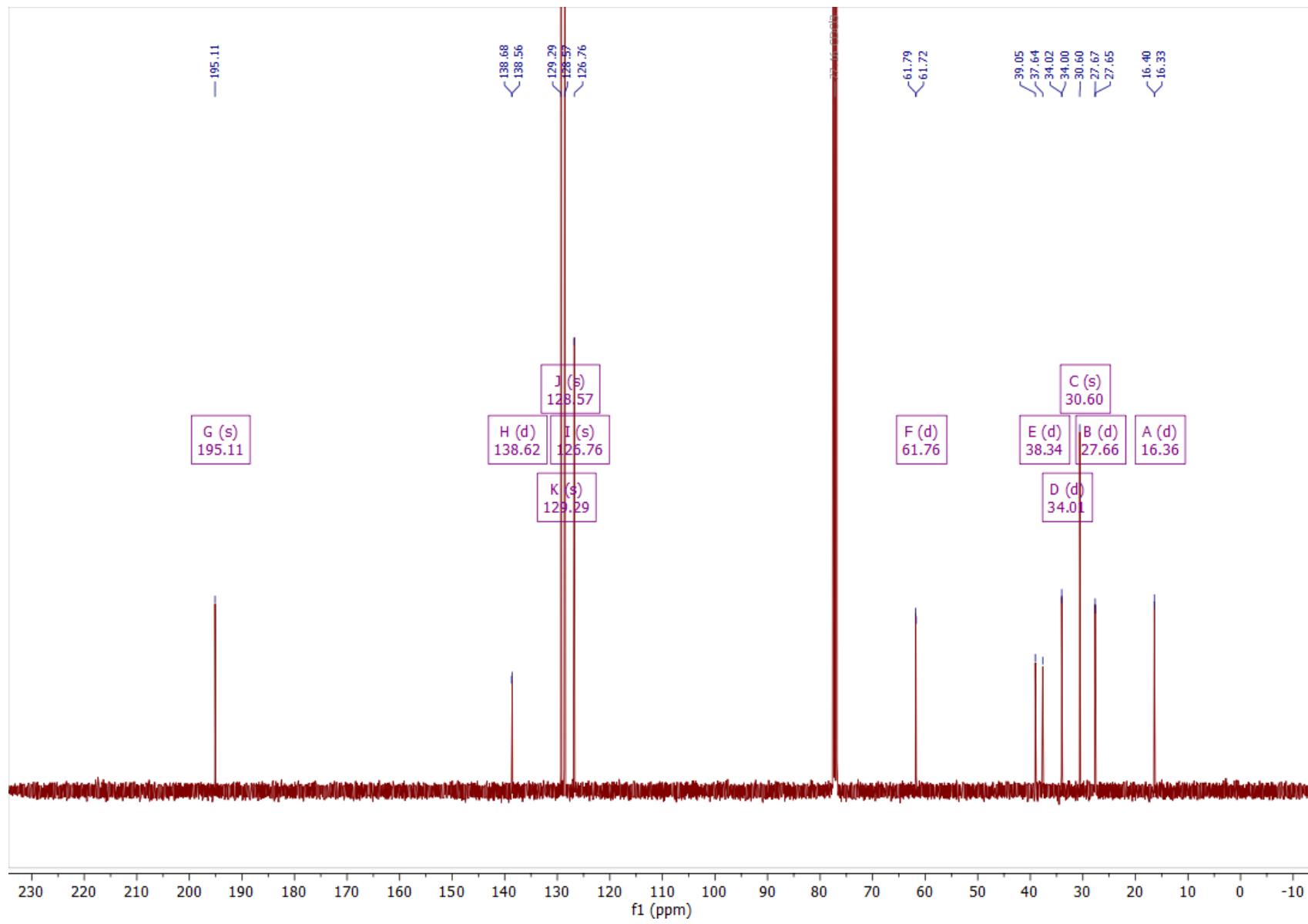
Figure S27. ^{13}C NMR spectrum of **10**.
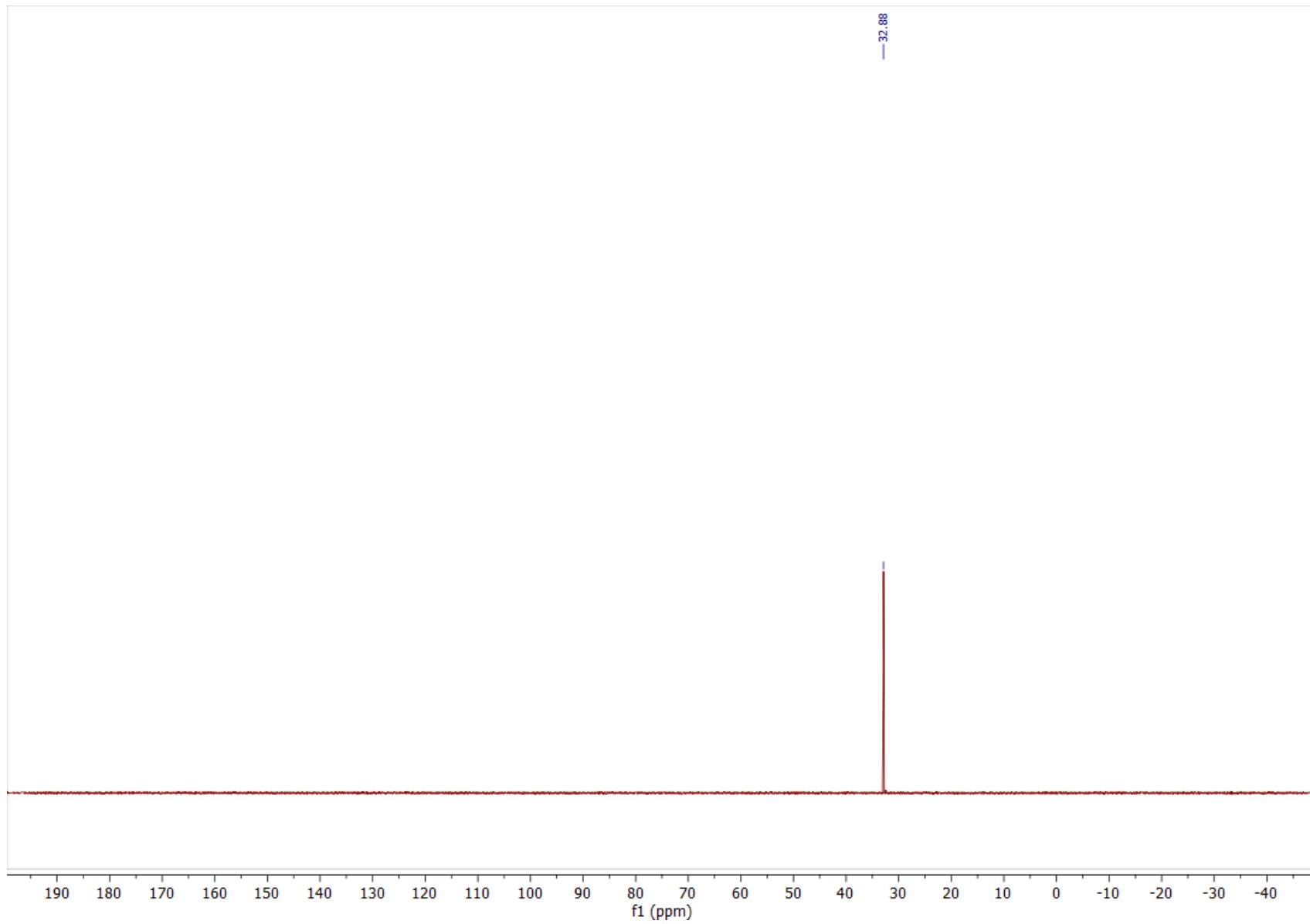
Figure S28. ^{31}P NMR spectrum of **10**.
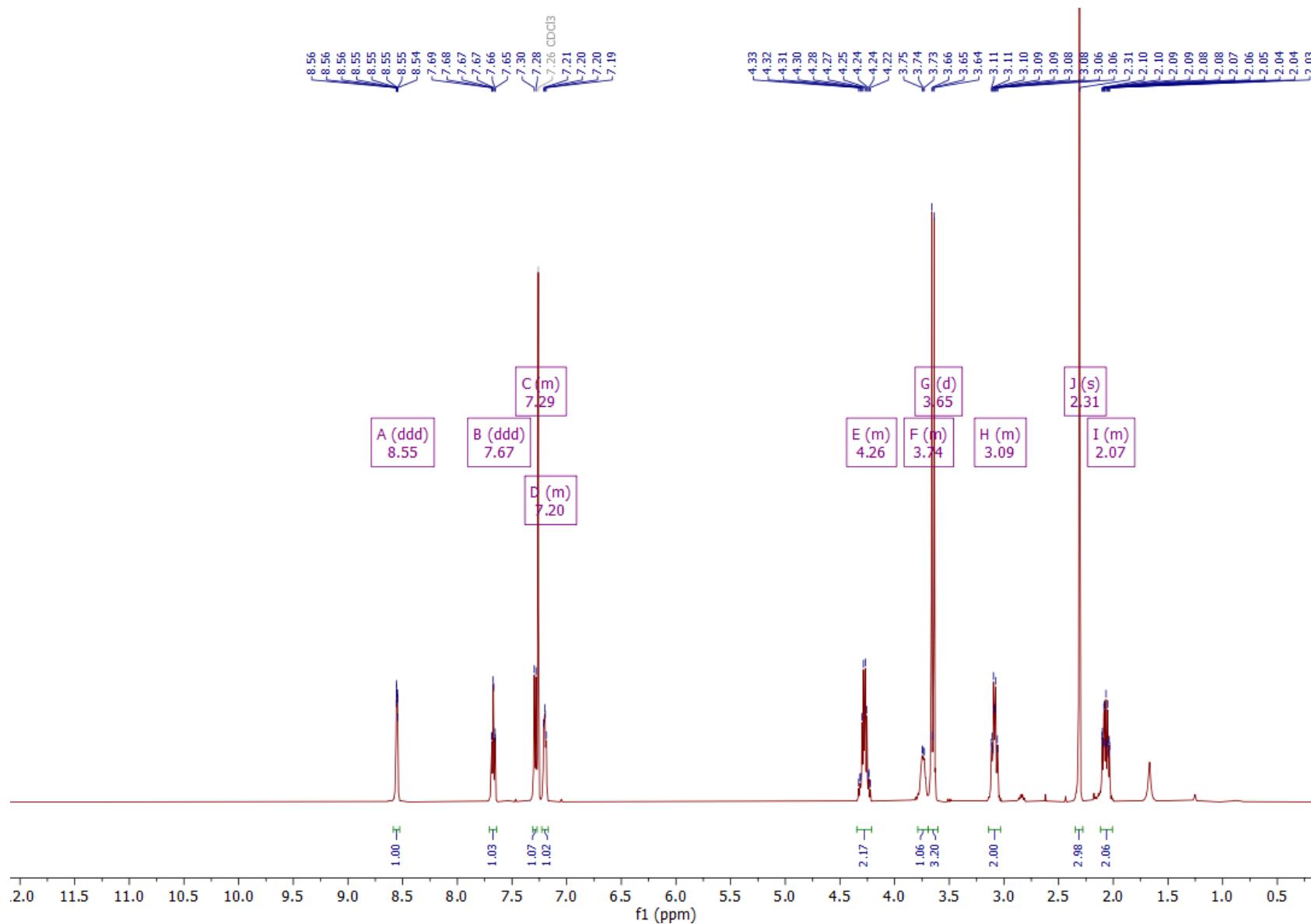
Figure S29. ^{1}H NMR spectrum of **1a**.
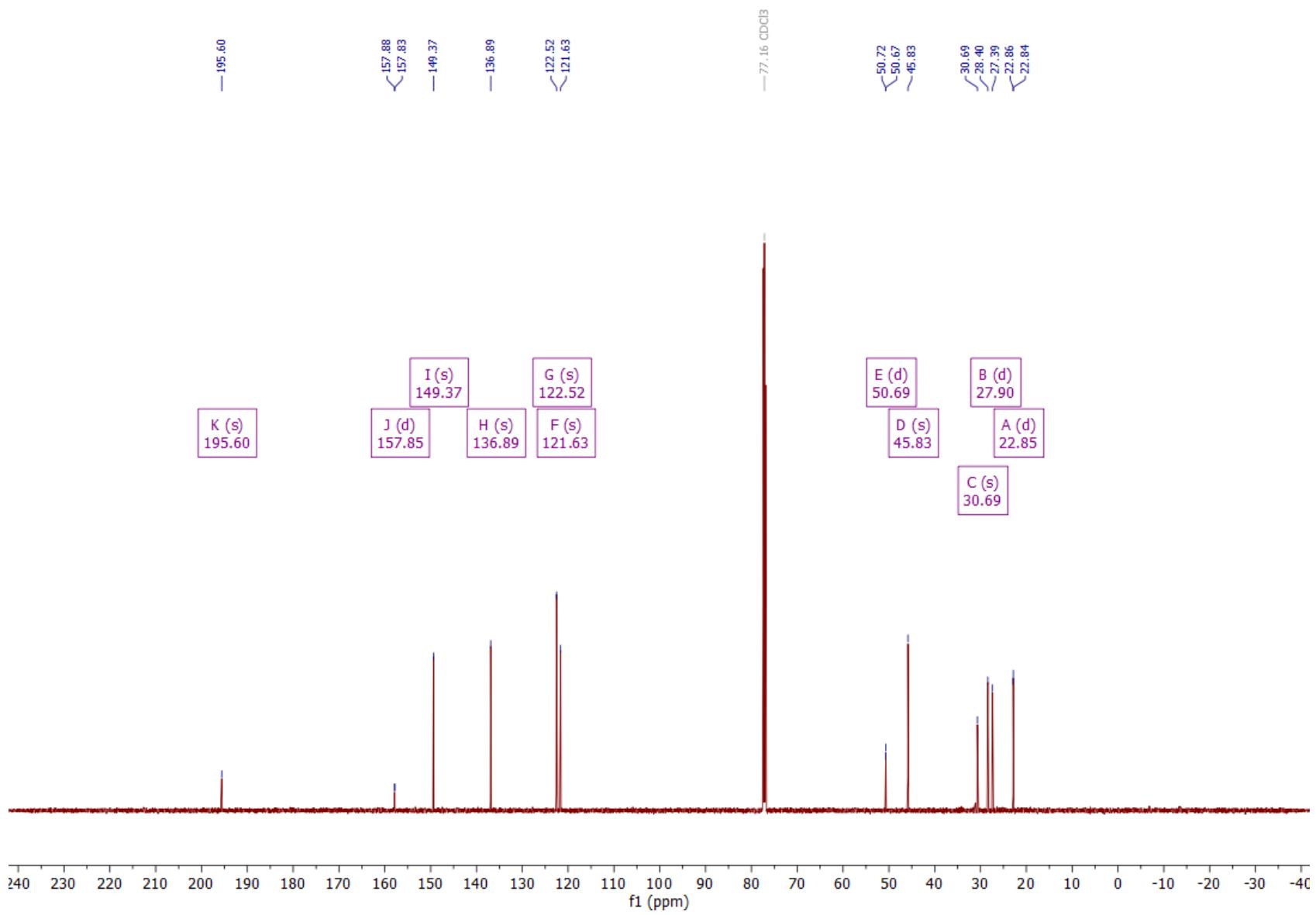
Figure S30. ^{13}C NMR spectrum of **1a**.
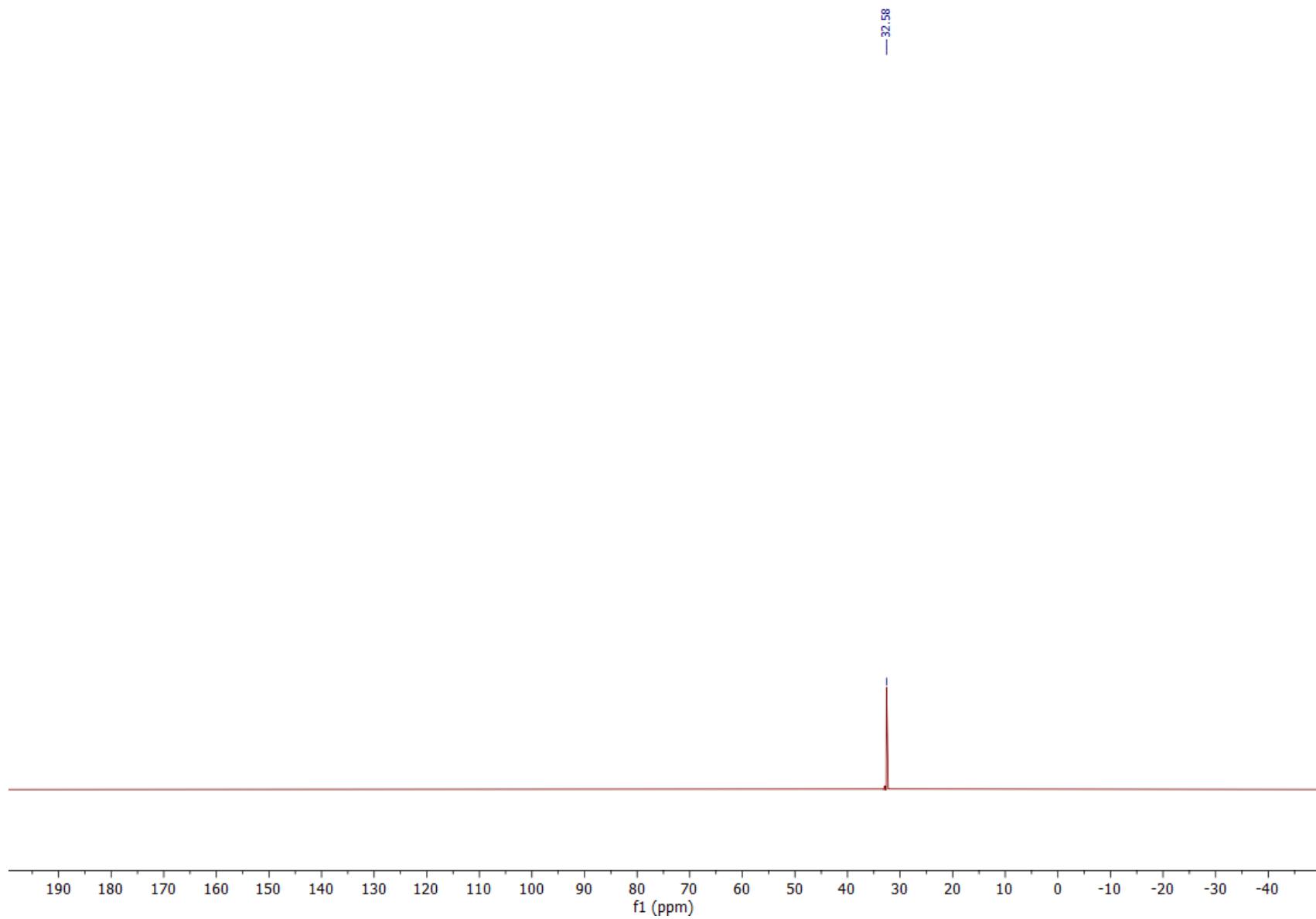
Figure S31. ^{31}P NMR spectrum of **1a**.
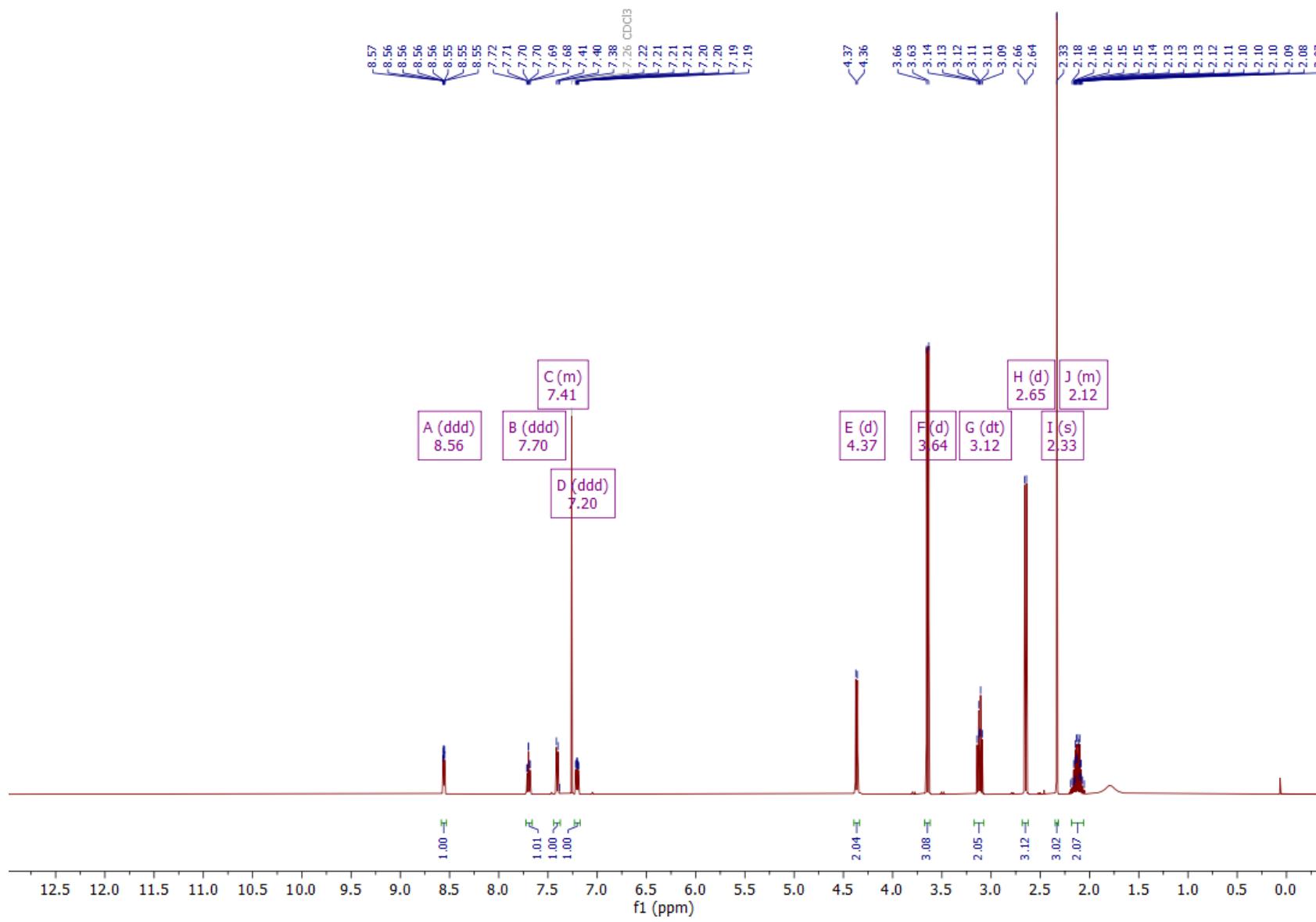
Figure S32. ^{1}H NMR spectrum of **1b**.
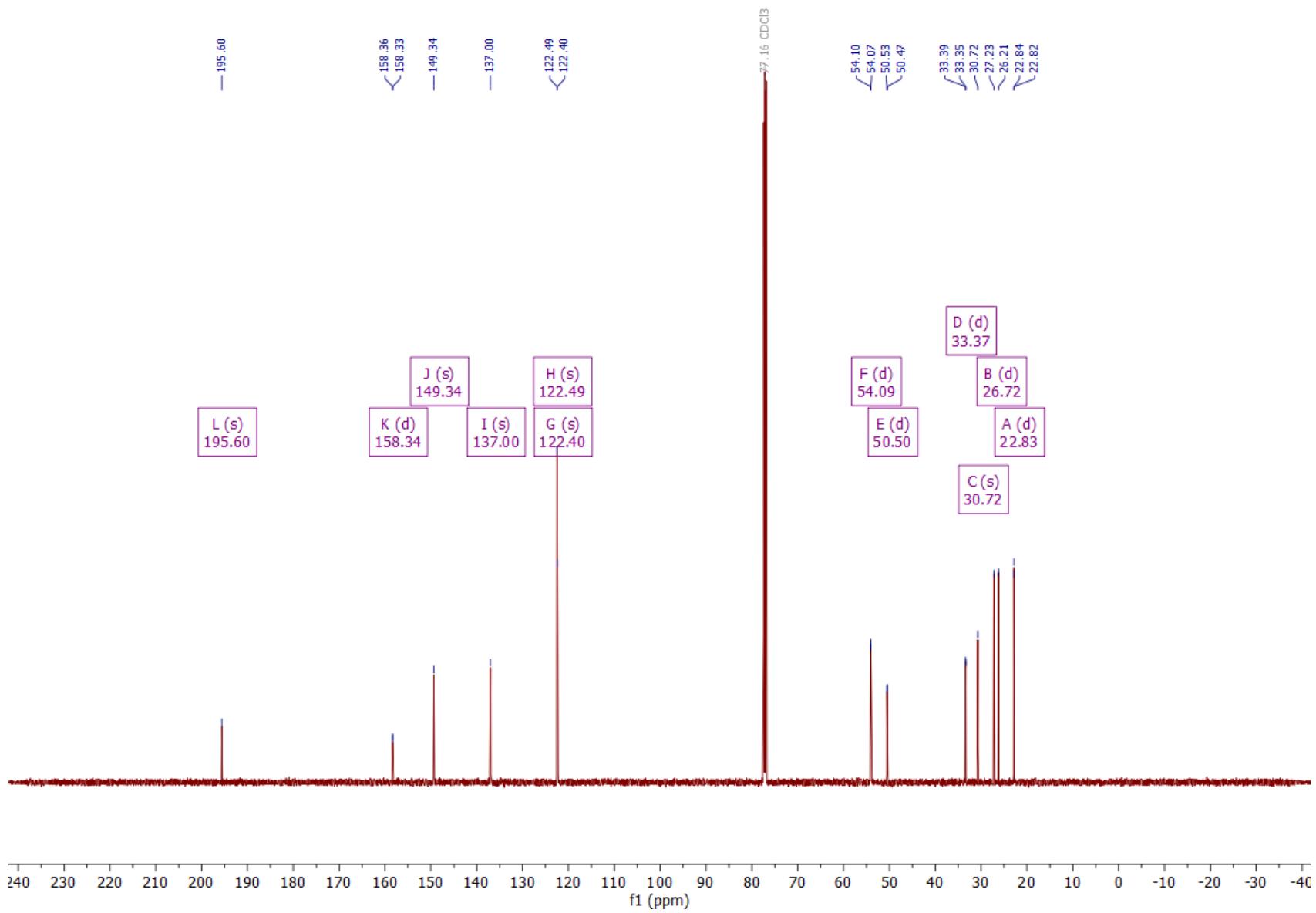
Figure S33. ^{13}C NMR spectrum of **1b**.
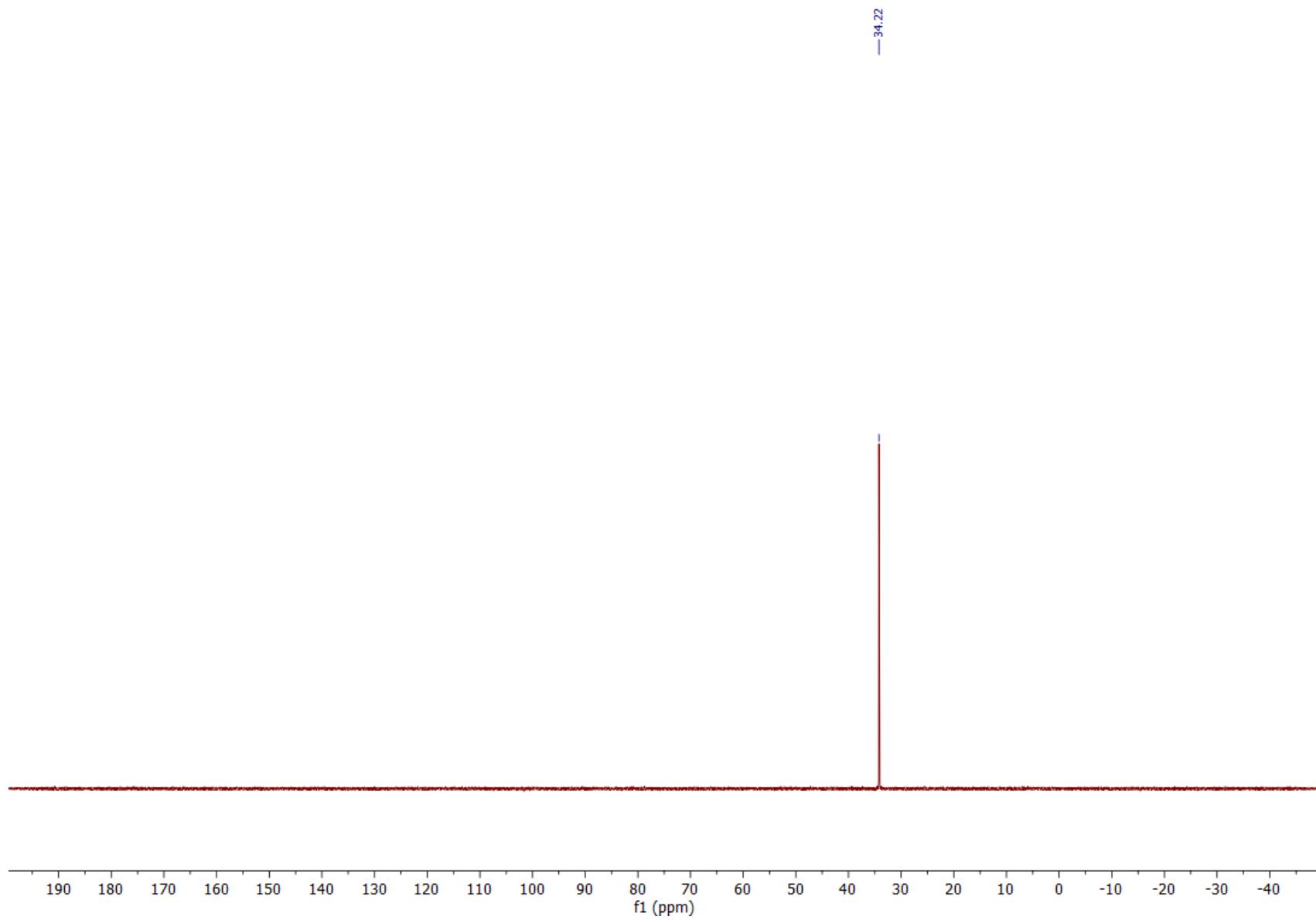
Figure S34. ^{31}P NMR spectrum of **1b**.
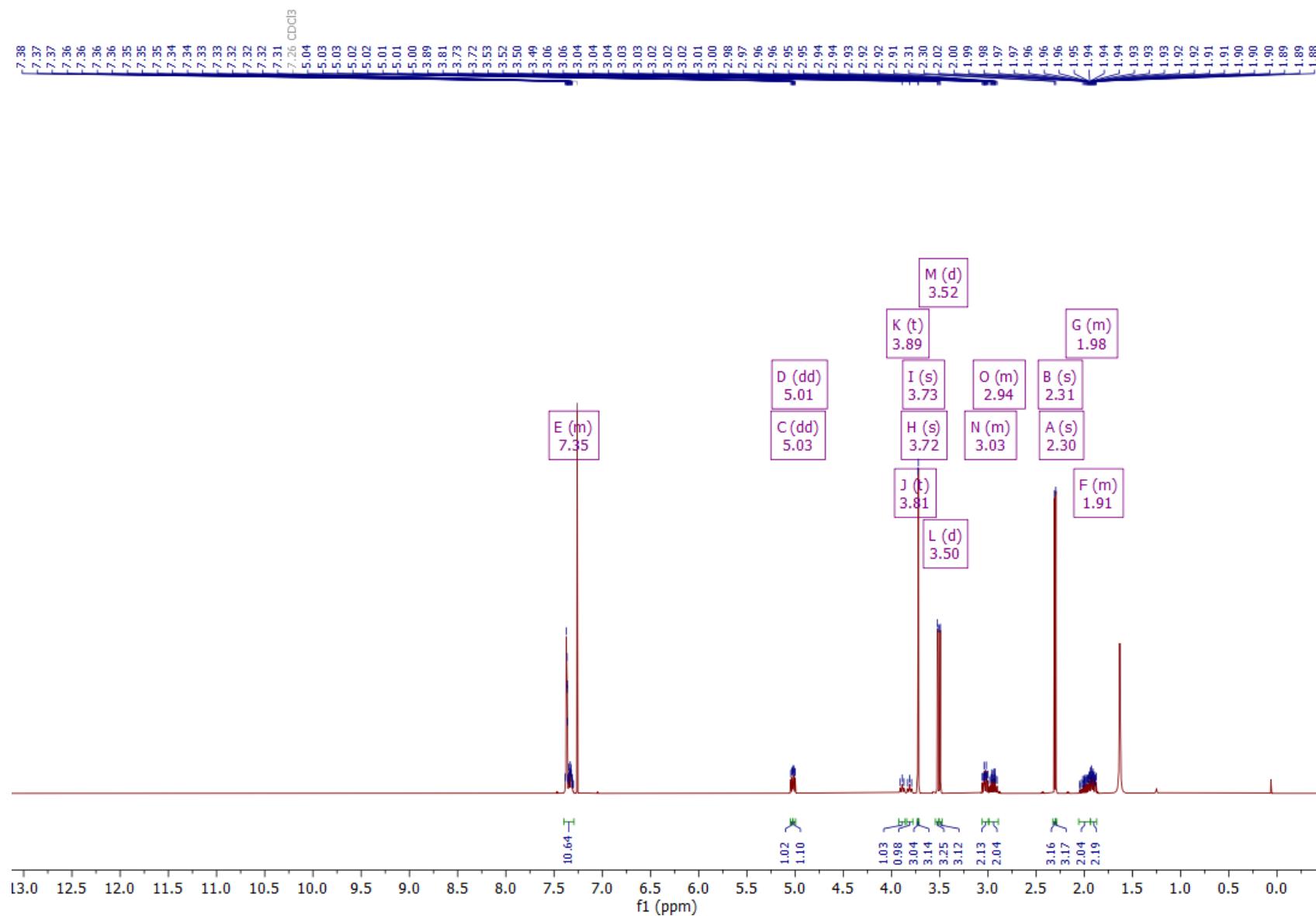
Figure S35. ^{1}H NMR spectrum of **1c**.
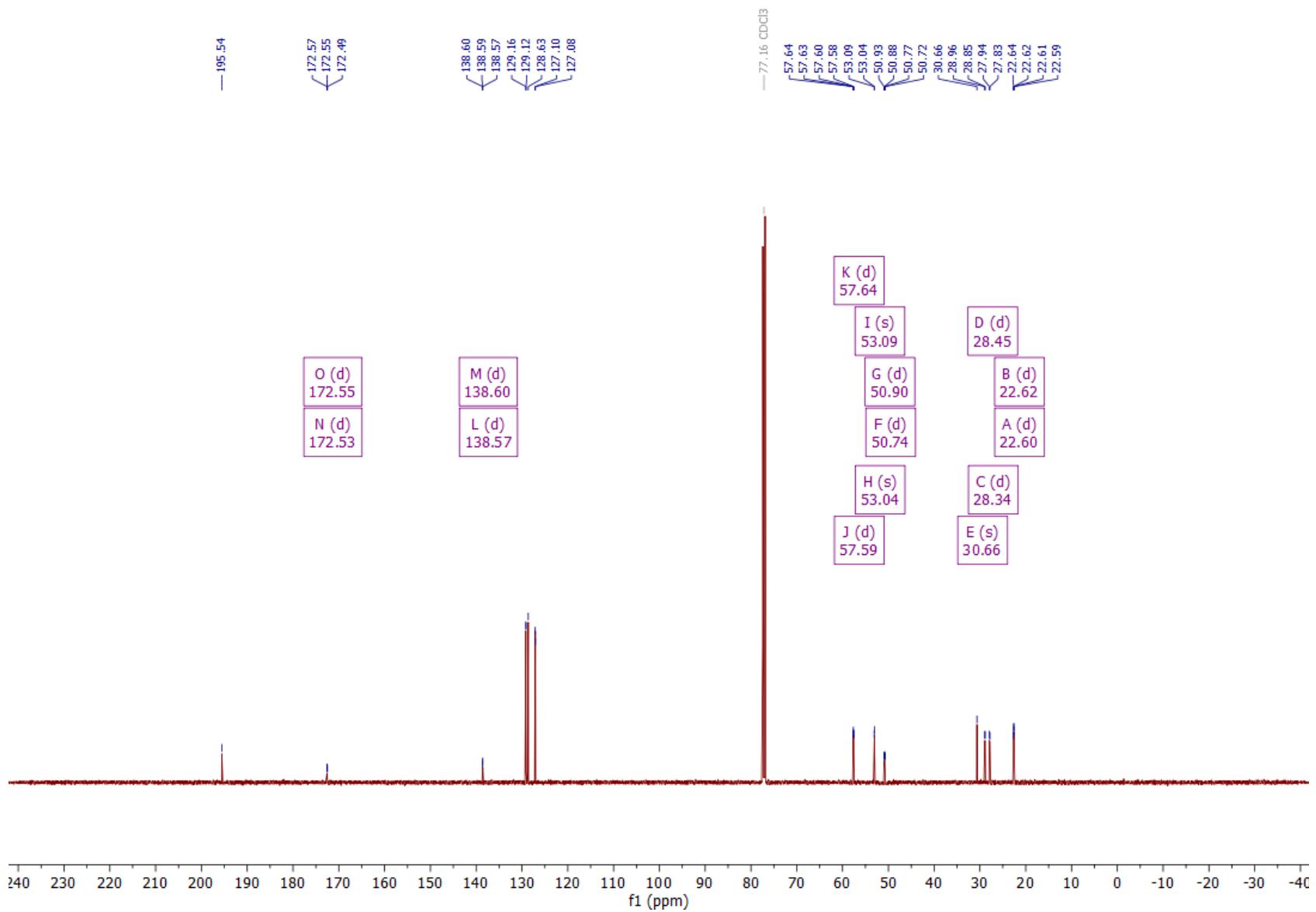
Figure S36. ^{13}C NMR spectrum of **1c**.
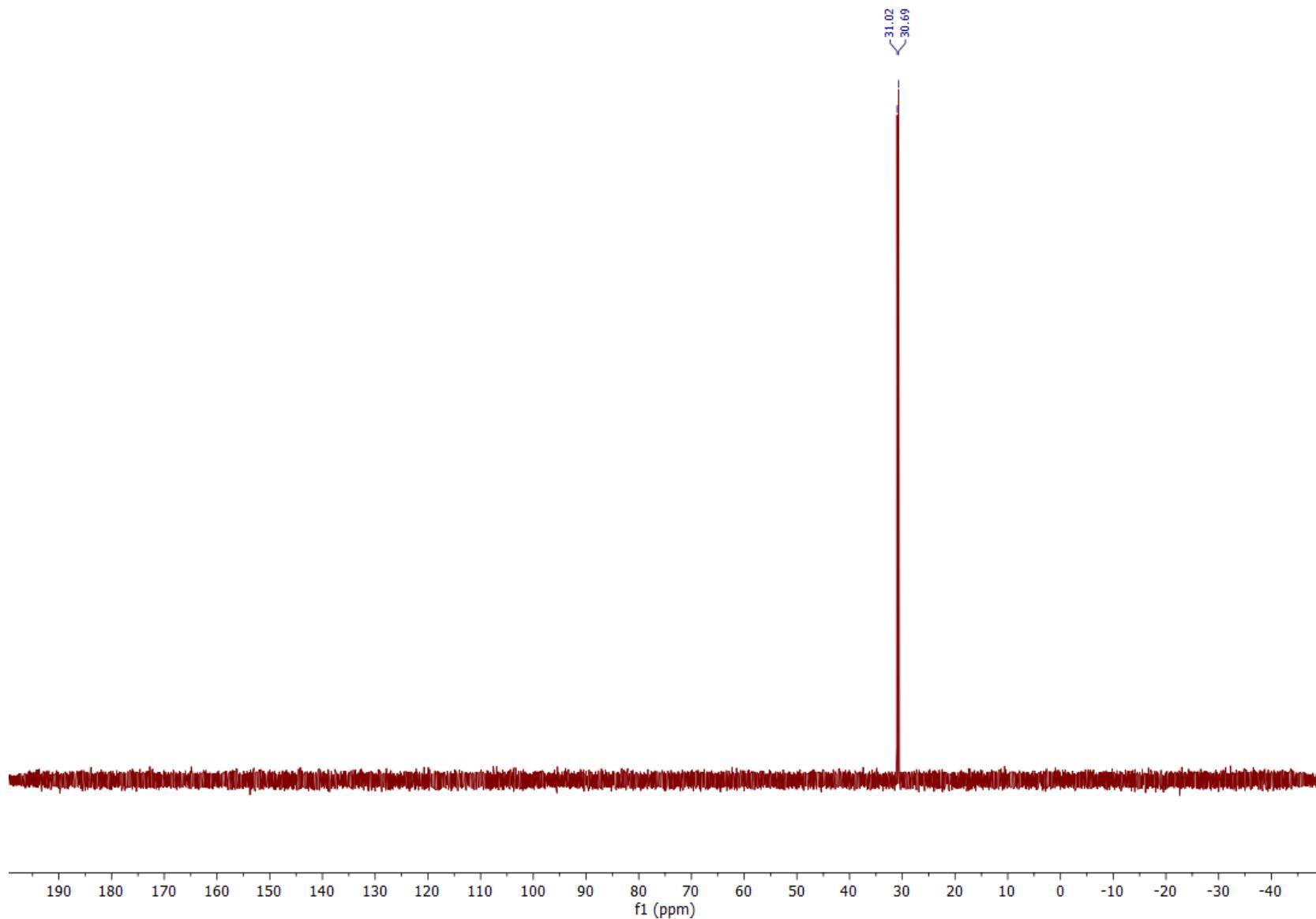
Figure S37. ^{31}P NMR spectrum of **1c**.
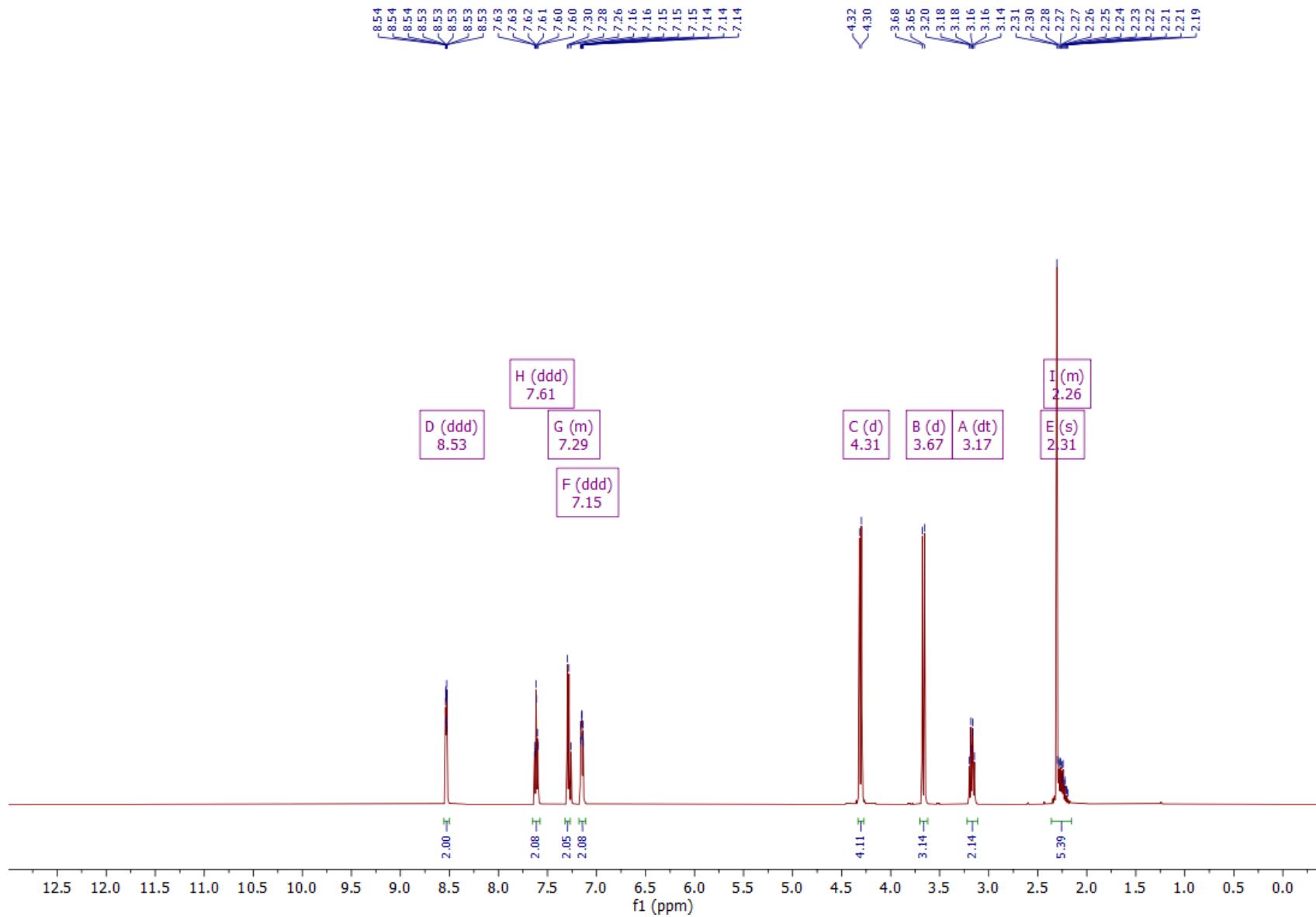
Figure S38. ^{1}H NMR spectrum of **1d**.
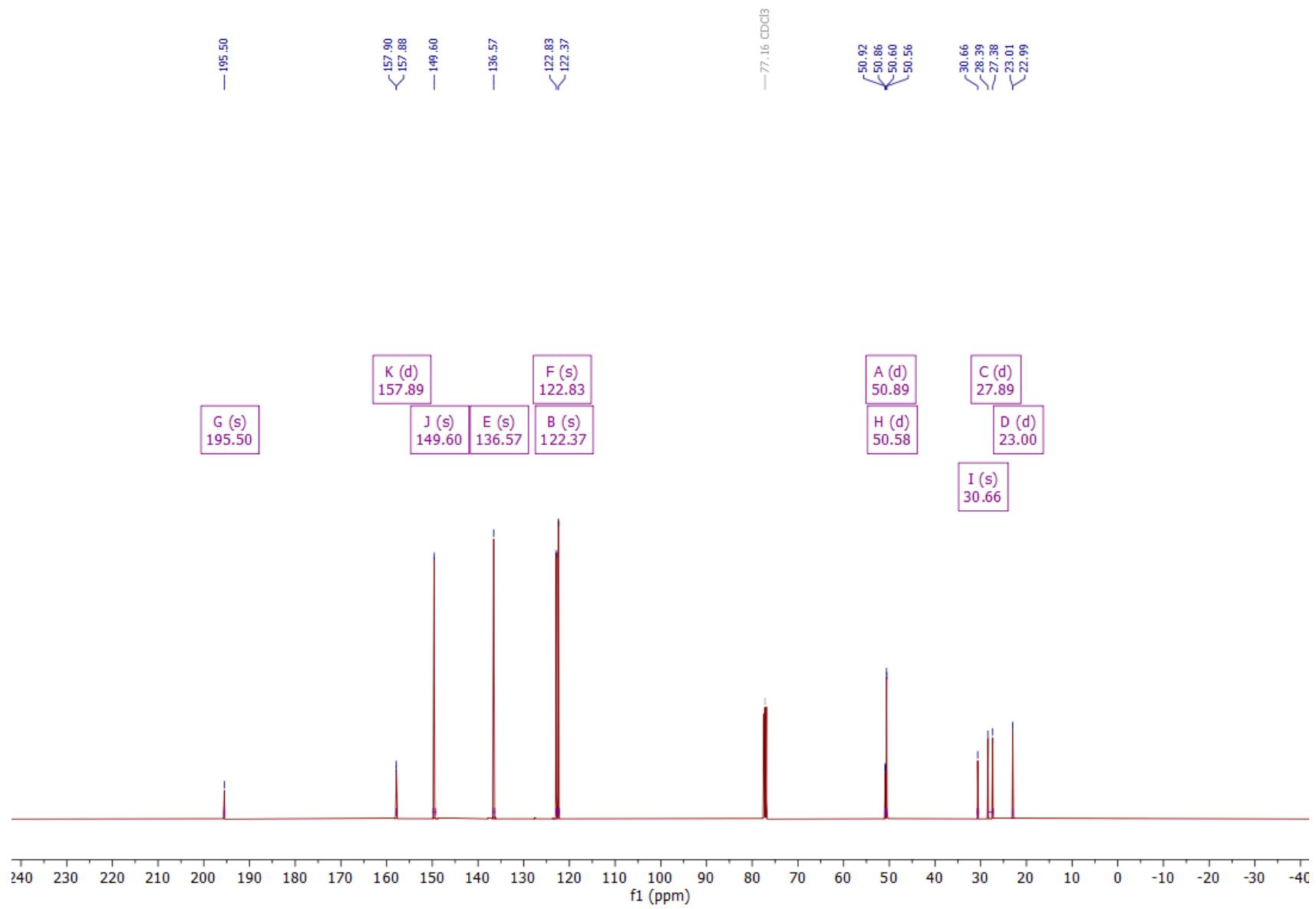
Figure S39. ^{13}C NMR spectrum of **1d**.
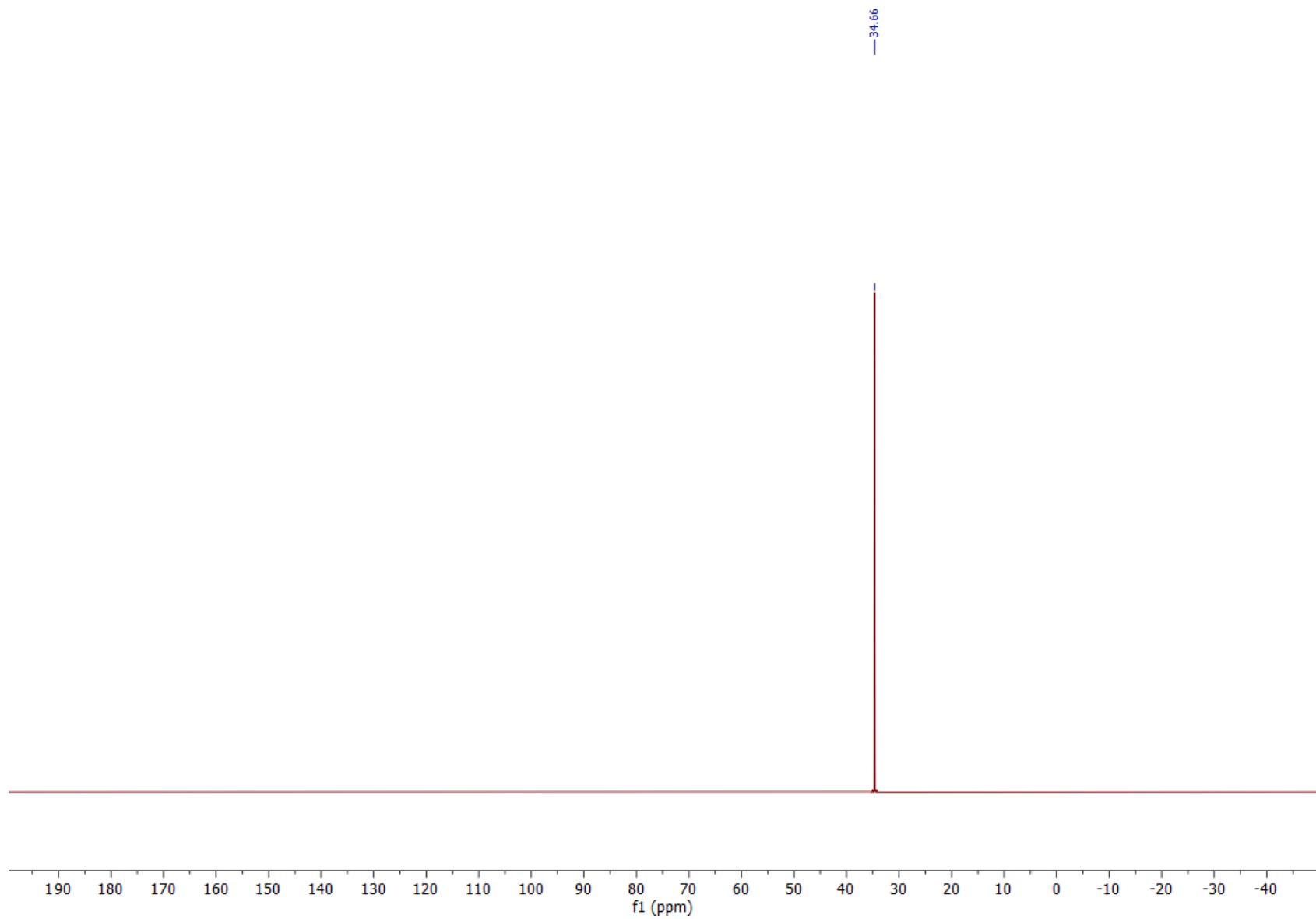
Figure S40. ^{31}P NMR spectrum of **1d**.
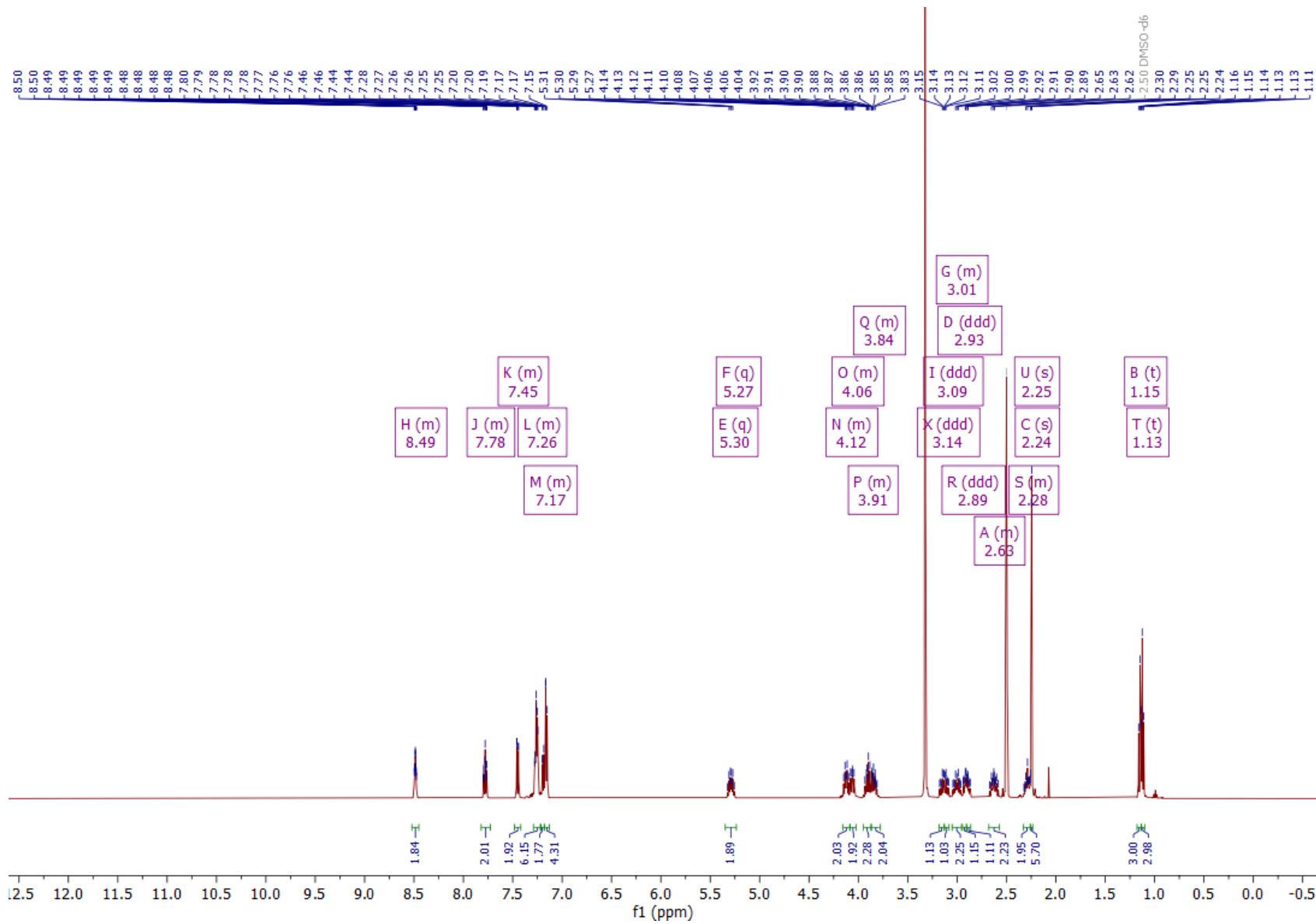
Figure S41. ¹H NMR spectrum of **1e**.
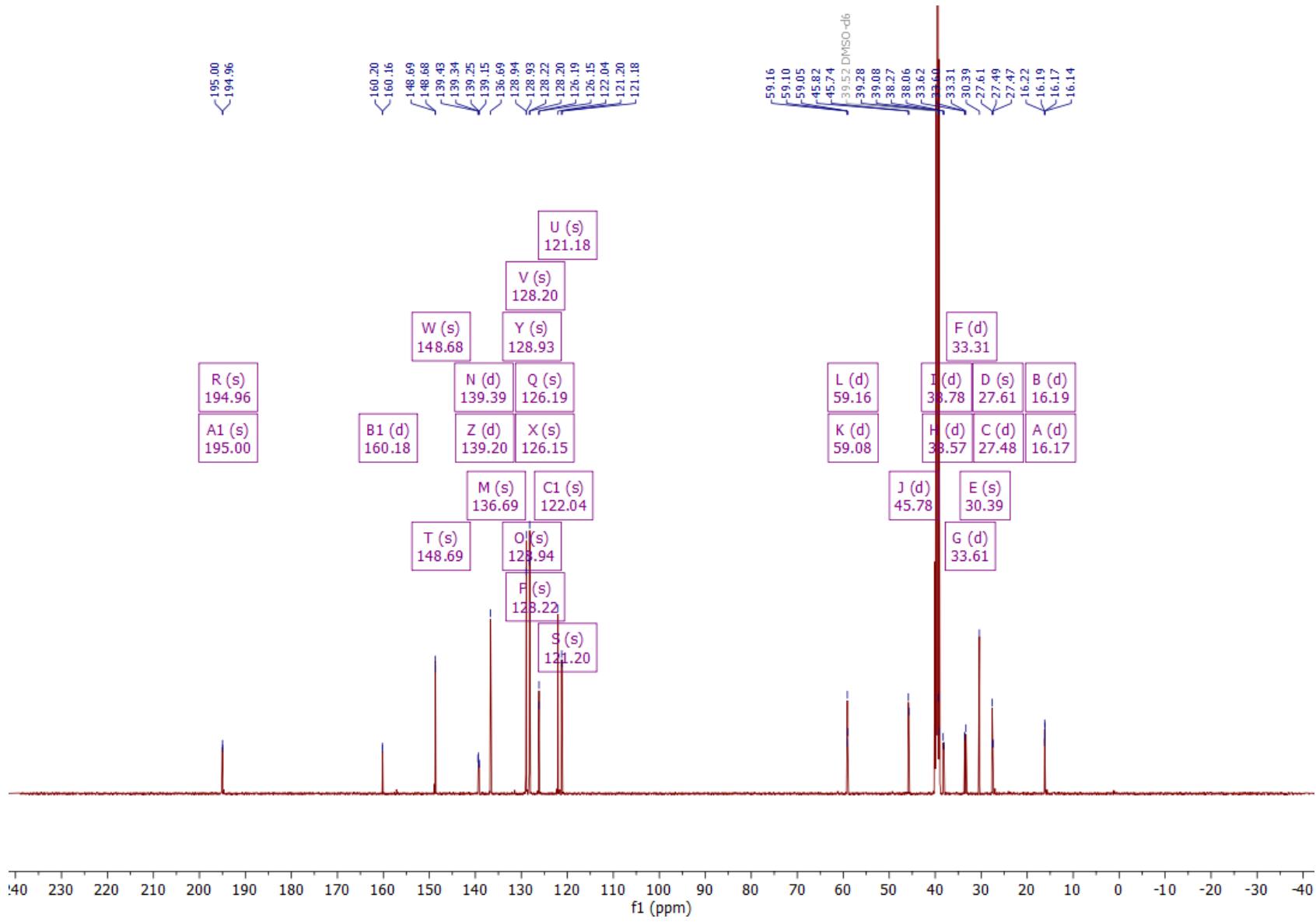
Figure S42. ^{13}C NMR spectrum of **1e**.
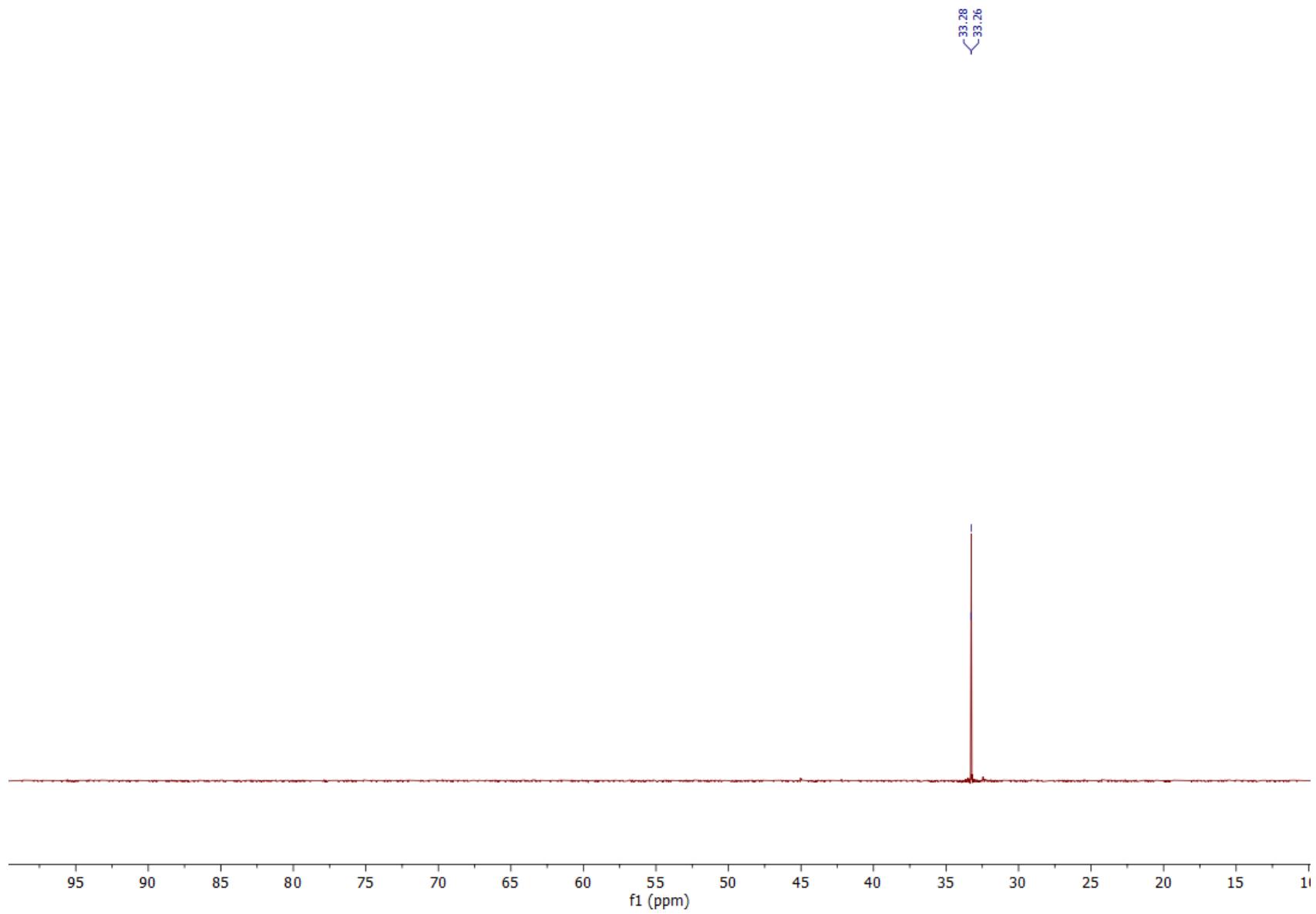
Figure S43. ^{31}P NMR spectrum of **1e**.
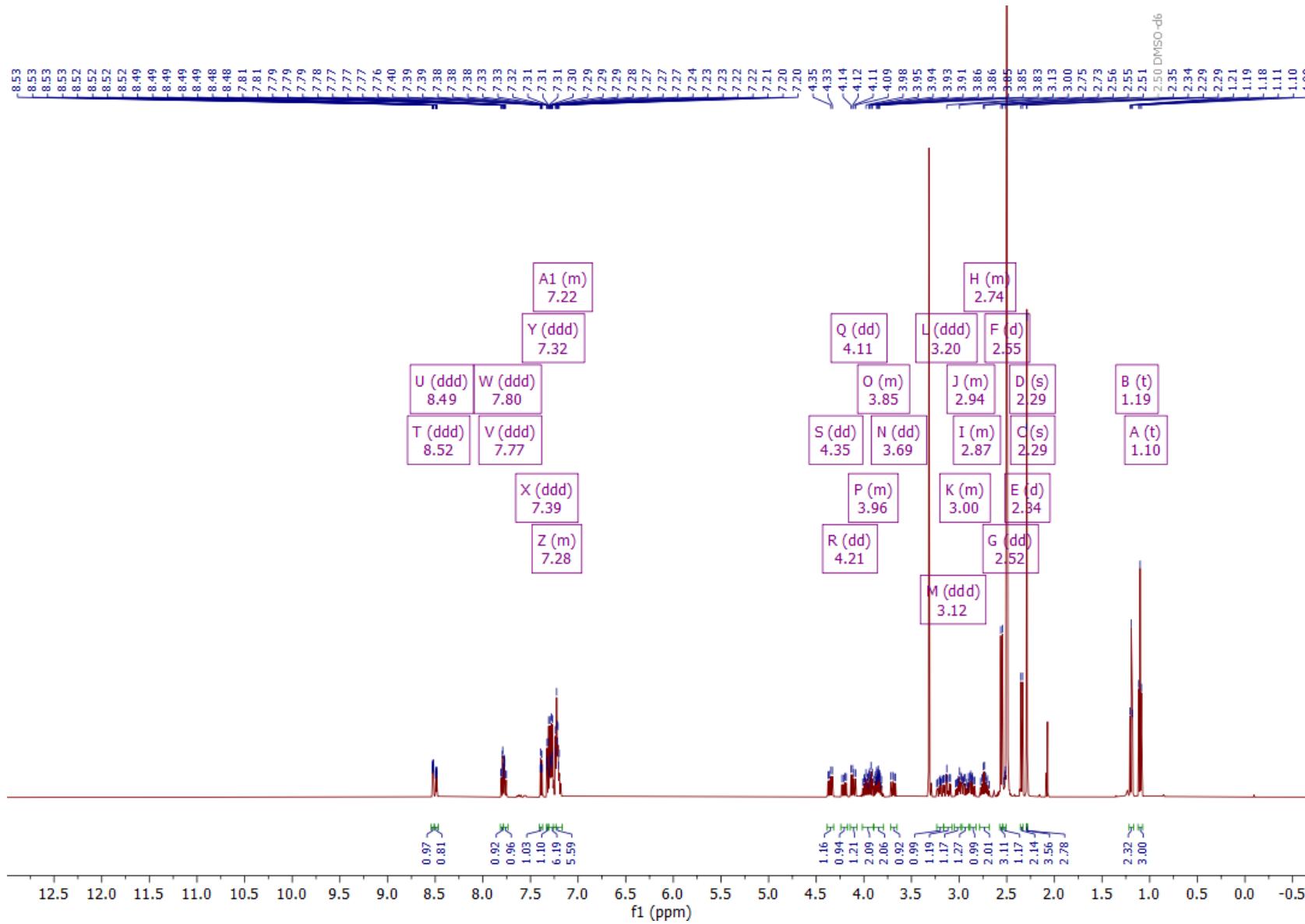
Figure S44. ^{1}H NMR spectrum of **1f**.
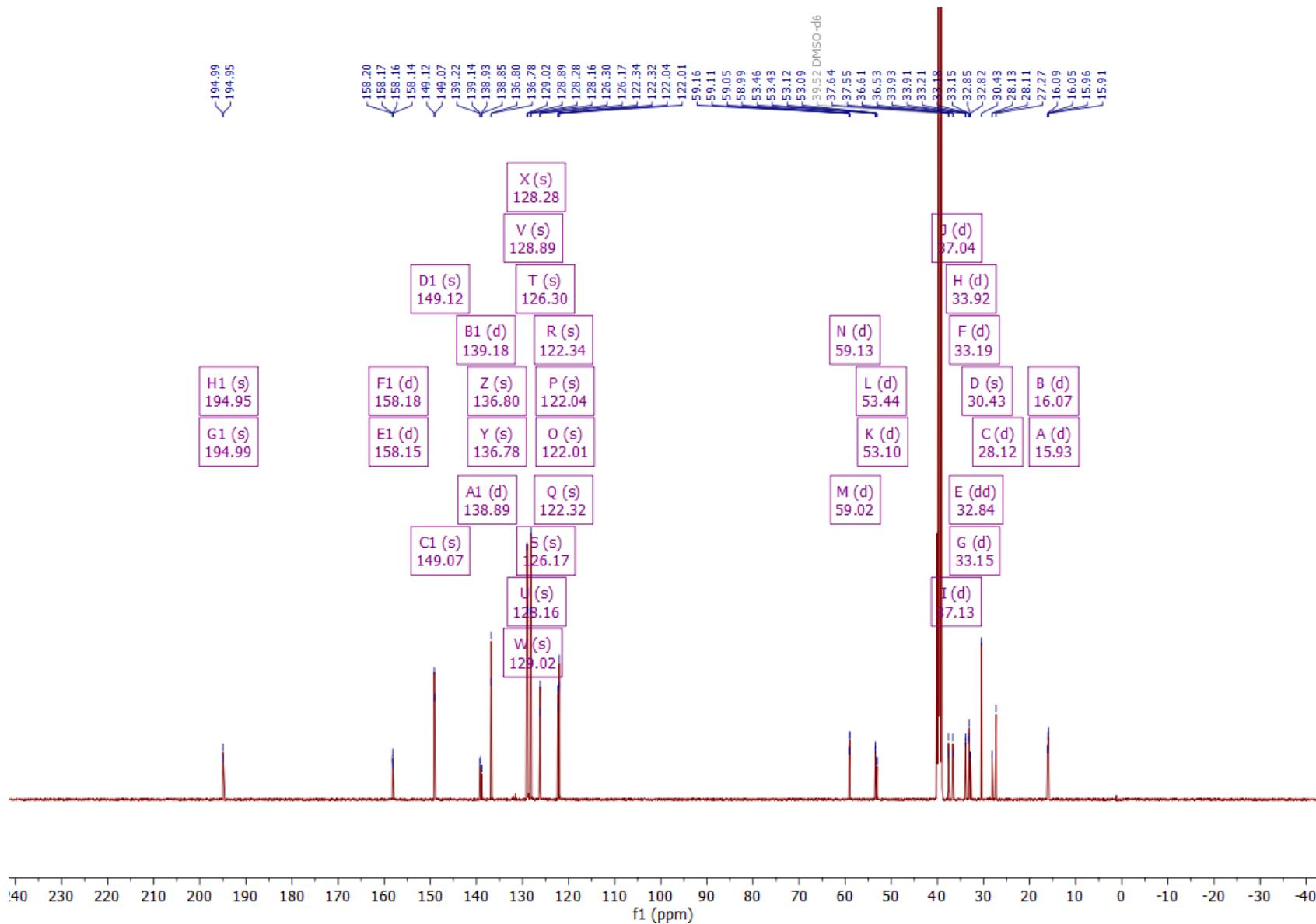
Figure S45. ^{13}C NMR spectrum of **1f**.
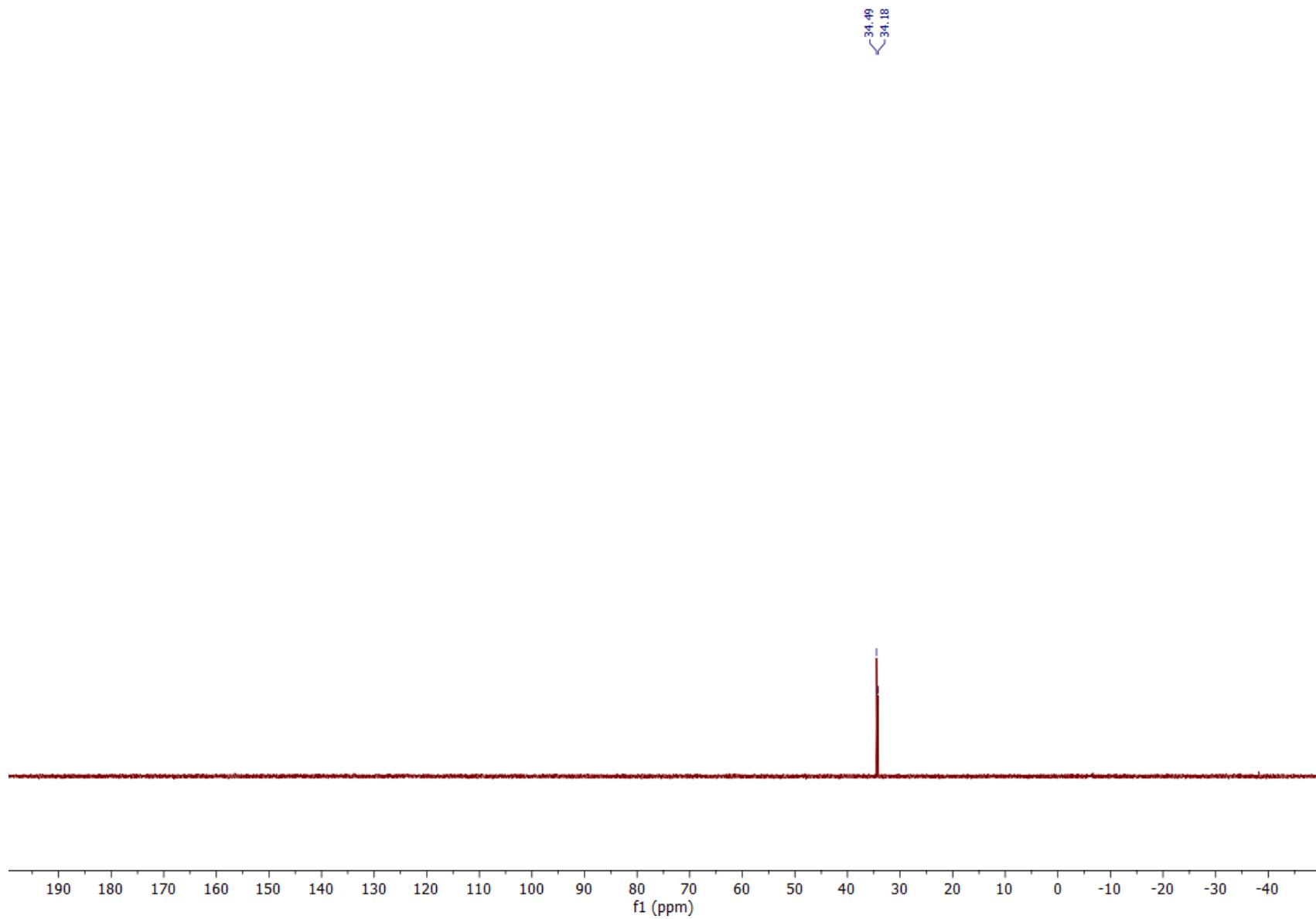
Figure S46. ^{31}P NMR spectrum of **1f**.
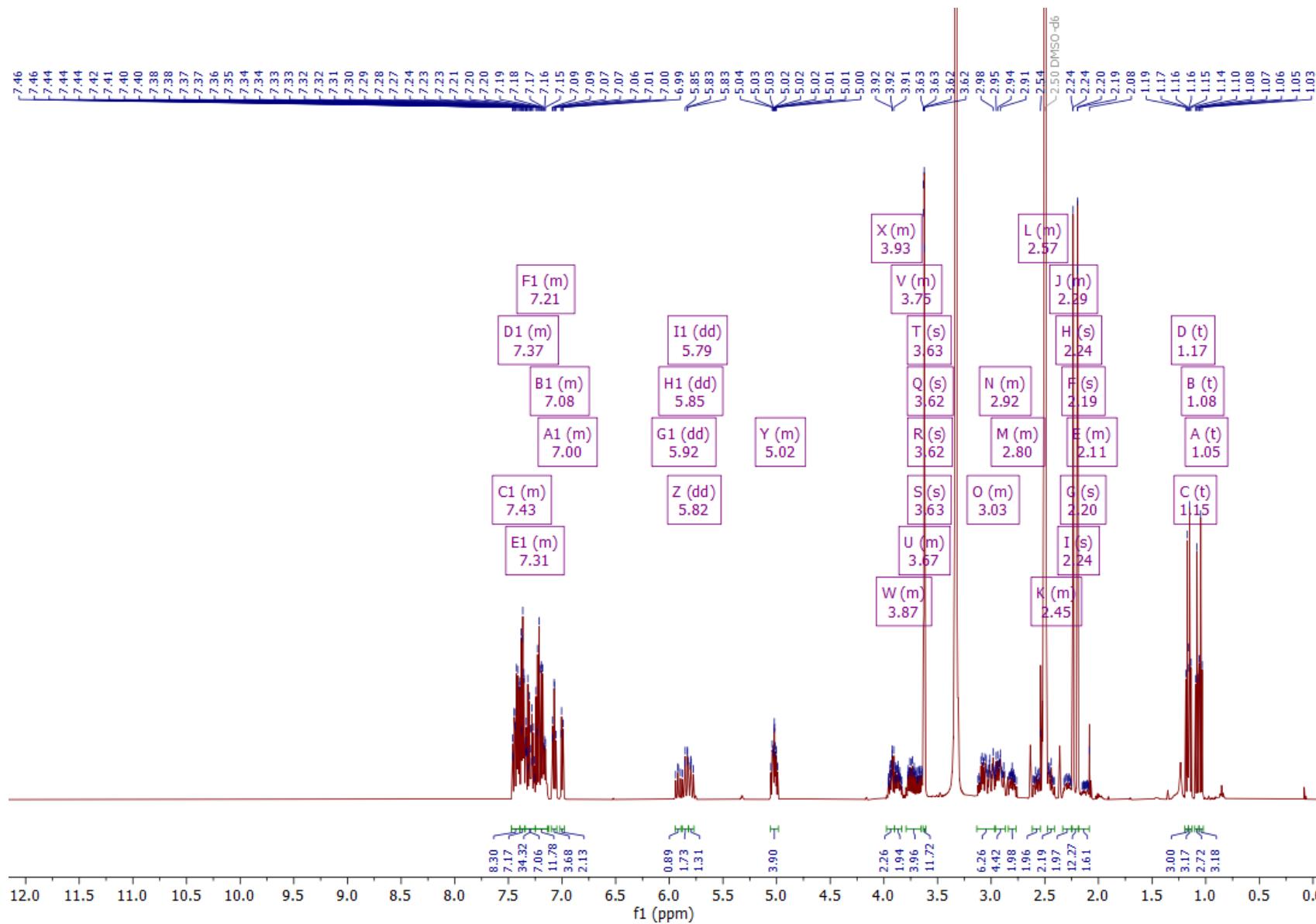
Figure S47. ^{1}H NMR spectrum of **1g**.
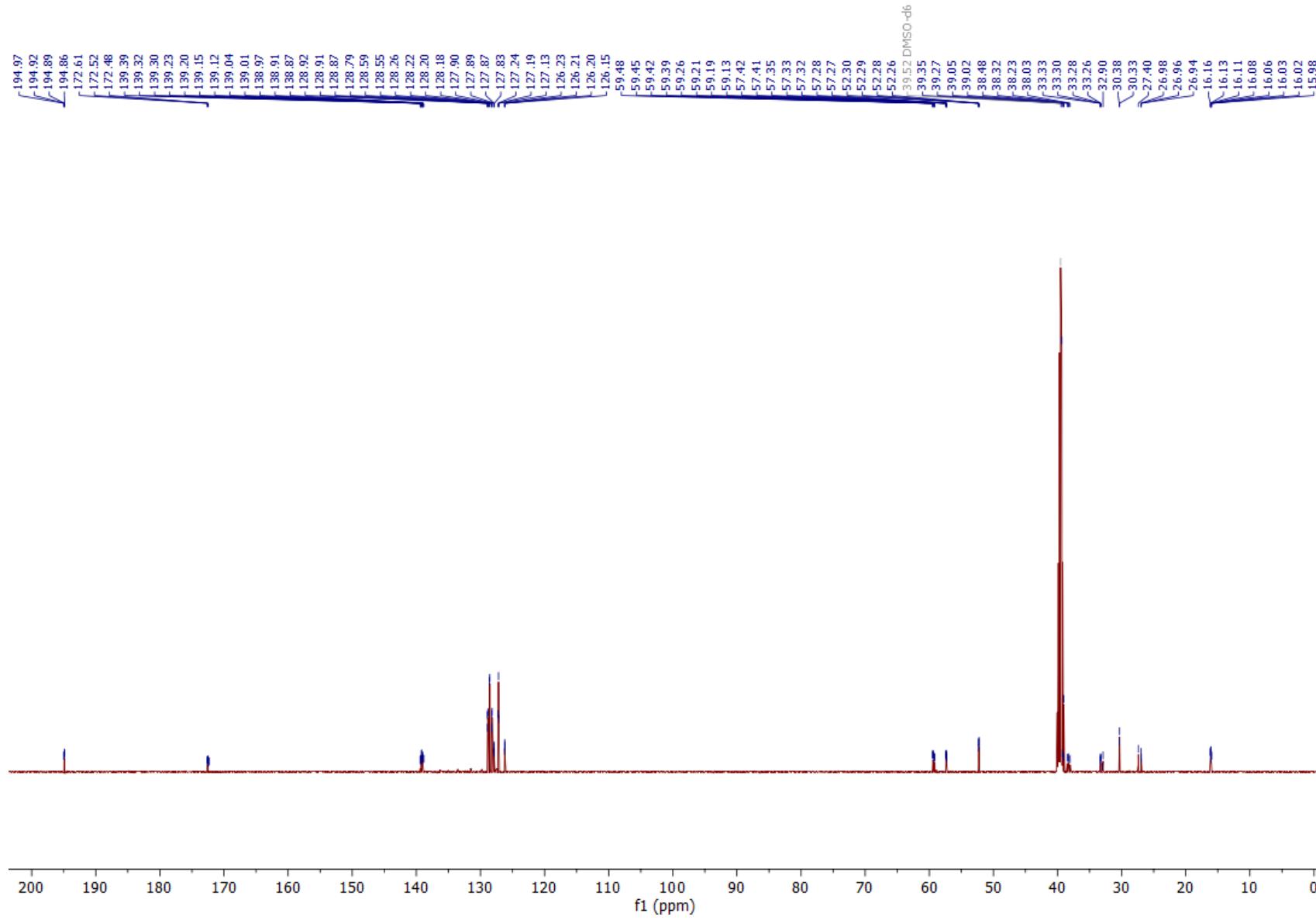
Figure S48. ^{13}C NMR spectrum of **1g**.
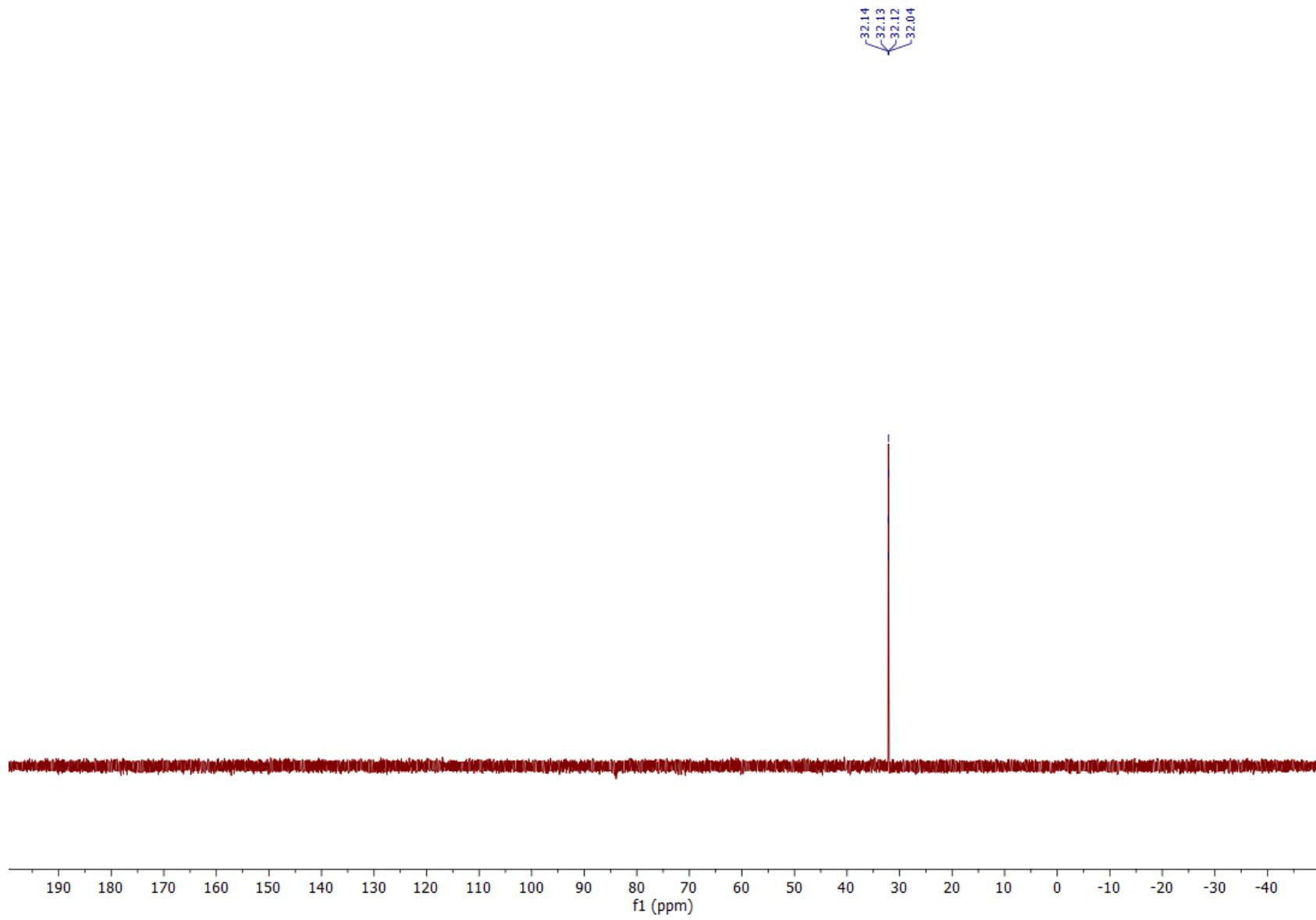
Figure S49. ^{31}P NMR spectrum of **1g**.
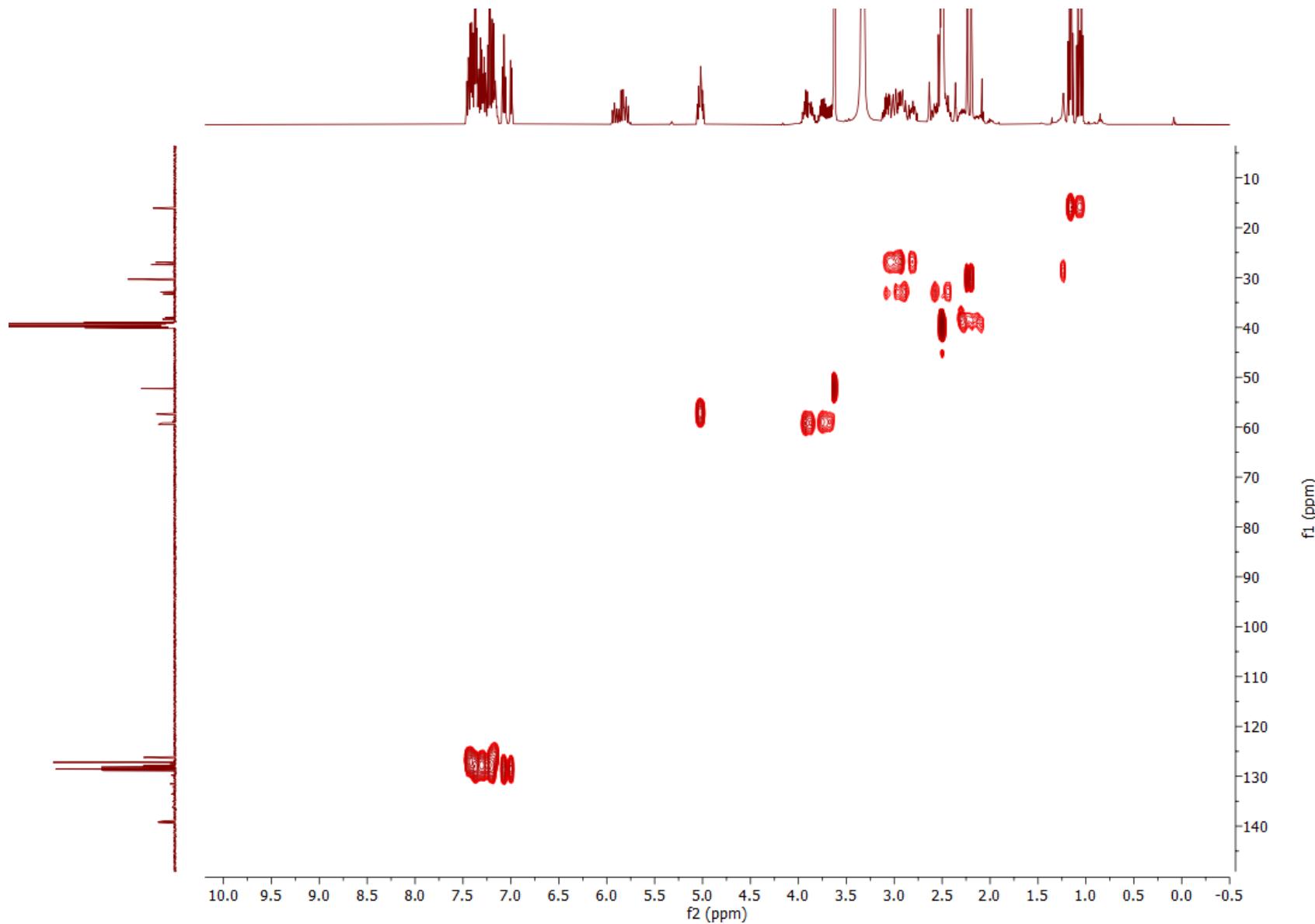
Figure S50. ^{1}H-^{13}C HSQC NMR spectrum of **1g**.
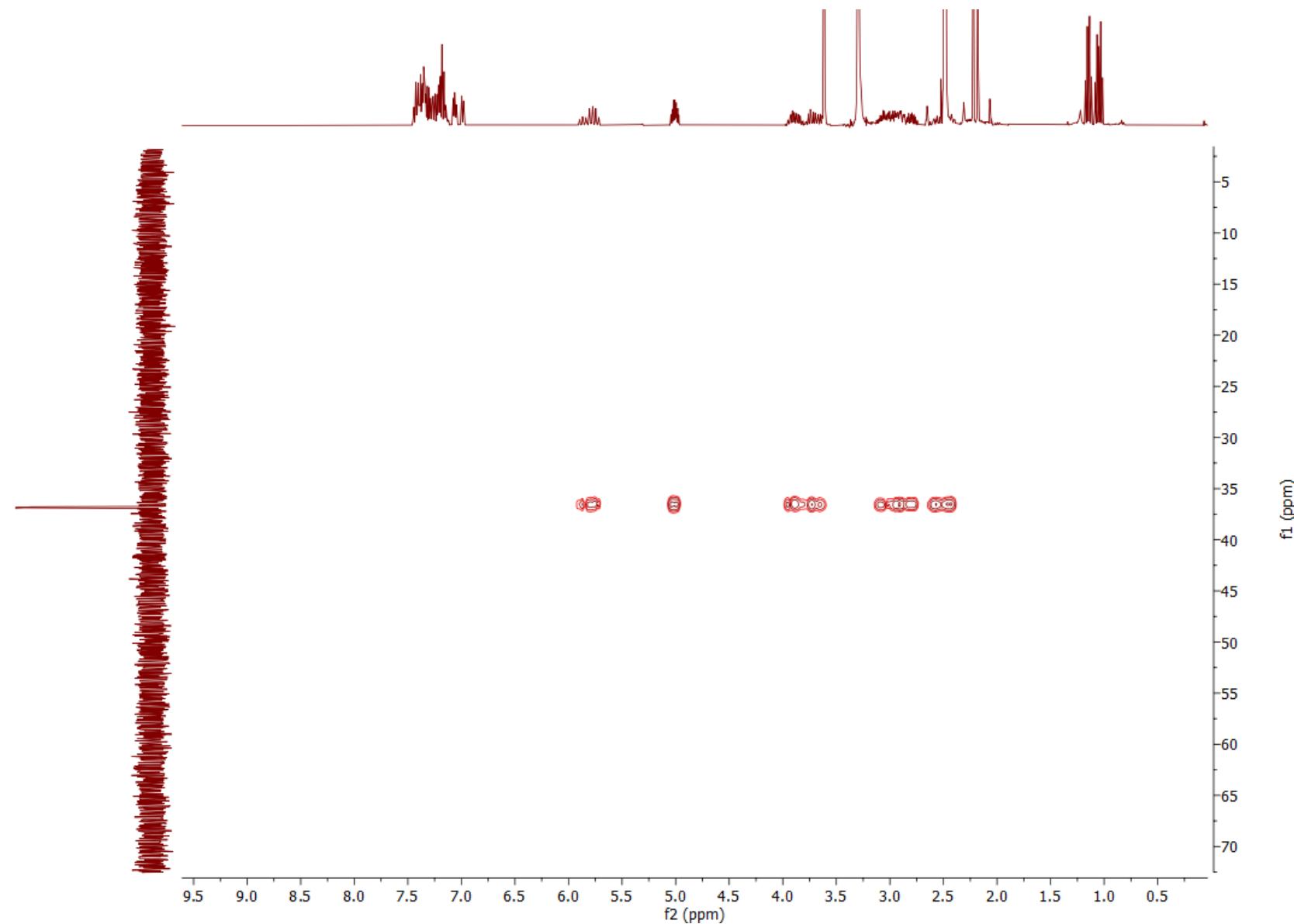
Figure S51. ^{1}H-^{31}P HMBC NMR spectrum of **1g**.

**Figure S52.** ^{1}H NMR spectrum of **1h**.

Figure S53. ^{13}C NMR spectrum of **1h**.
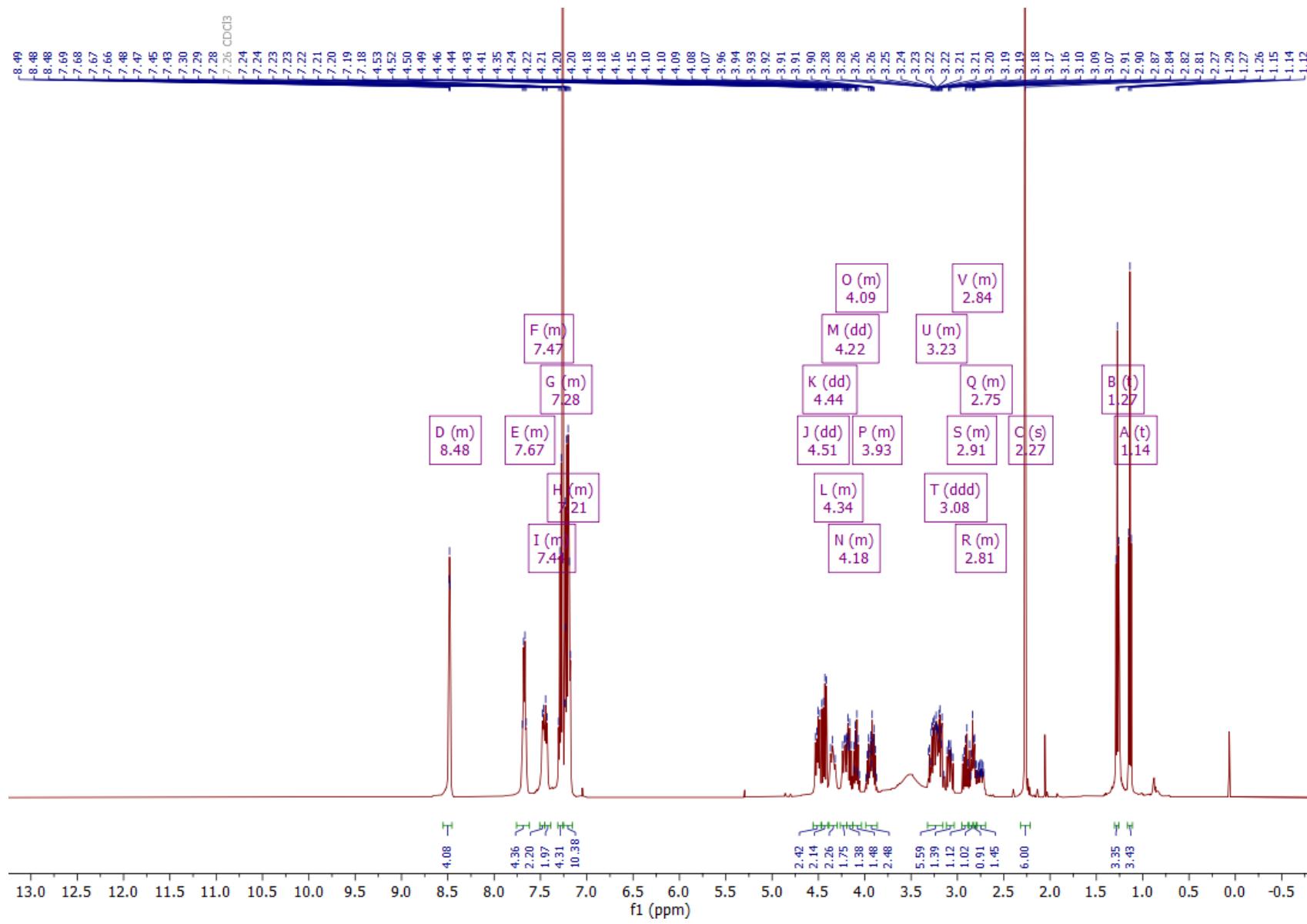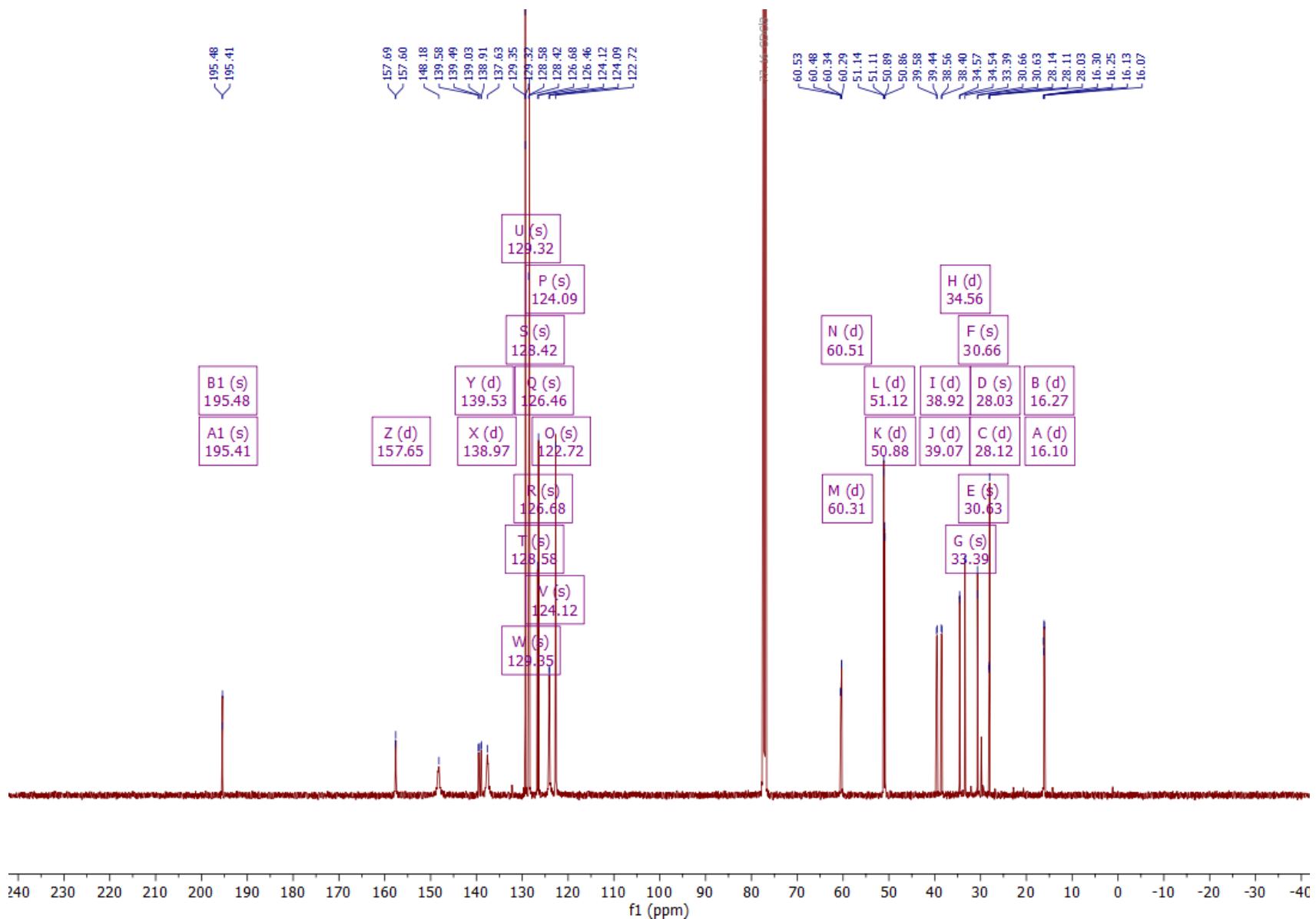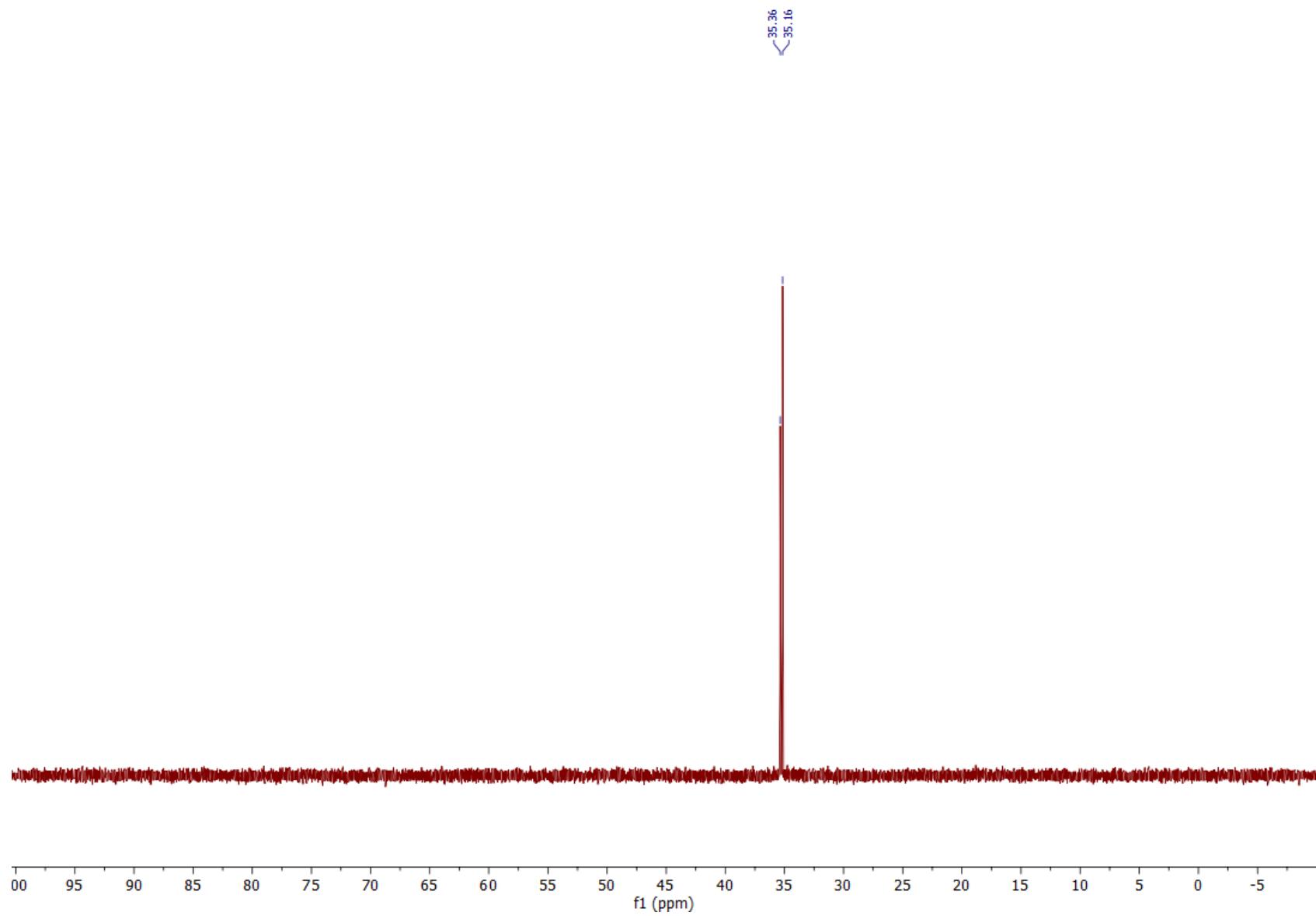
Figure S54. ^{31}P NMR spectrum of **1h**.