

SUPPLEMENTARY MATERIAL

New Antibacterial Chloro-Containing Polyketides from the Alga-Derived Fungus *Asteromyces cruciatus* KMM 4696

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New Antibacterial Chloro-Containing Polyketides from the Alga-Derived Fungus *Asteromyces cruciatus* KMM 4696

Abstract: Six new polyketides acrincipentyns A–F (**1–6**) were isolated from the alga-derived fungus *Asteromyces cruciatus* KMM 4696. Their structures were established based on spectroscopic methods. The absolute configurations of some compounds were assigned by the modified Mosher's method, ROESY data analysis as well as biogenetic considerations. Acrincipentyns A–E were identified to be the very first examples of chlorine-containing asperpentyn-like compounds. The cytotoxic and antimicrobial activities of the isolated compounds were examined. Acrincipentyns A–F were found as antimicrobial agents which inhibited sortase A enzyme activity, bacterial growth and biofilm formation of *Staphylococcus aureus* and decreased LDH release from human keratinocytes HaCaT in *S. aureus* skin infection *in vitro* model.

Keywords: *Asteromyces cruciatus*; marine fungi; secondary metabolites; polyketides; sortase A; chlore-containing metabolites; *Staphylococcus aureus*; antibacterial activity; biofilm formation.

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Experimental Section

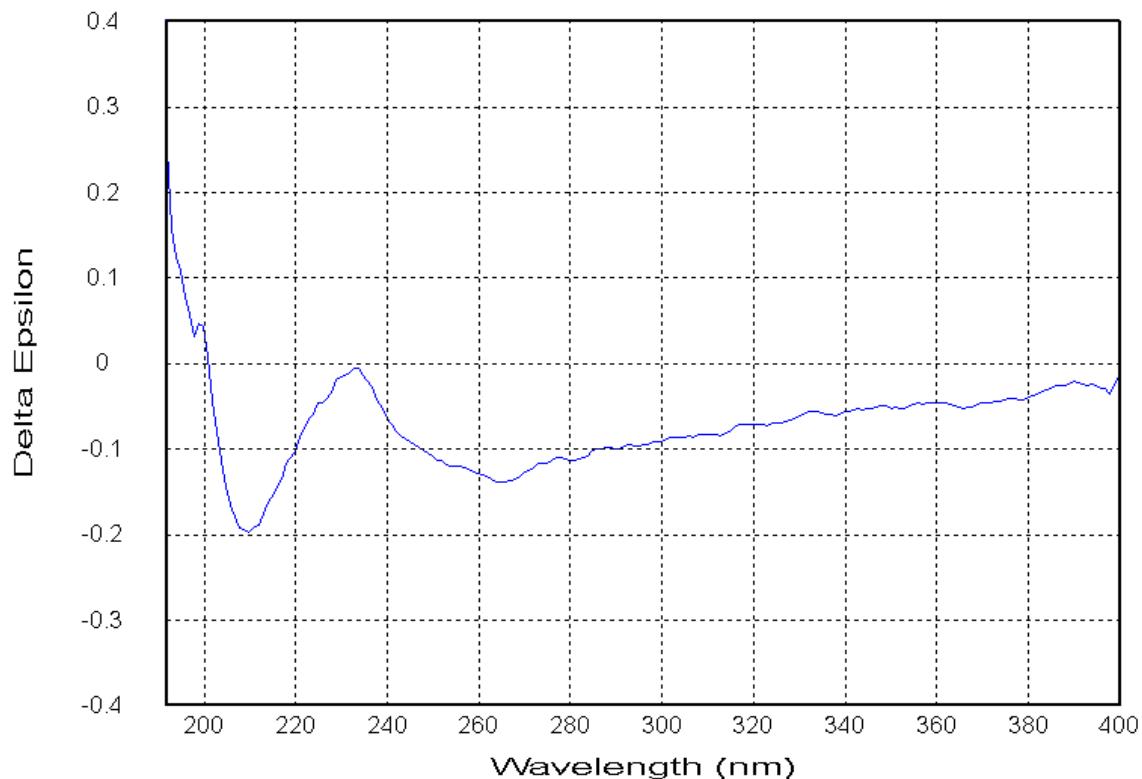


Figure S1. CD spectrum of **1**

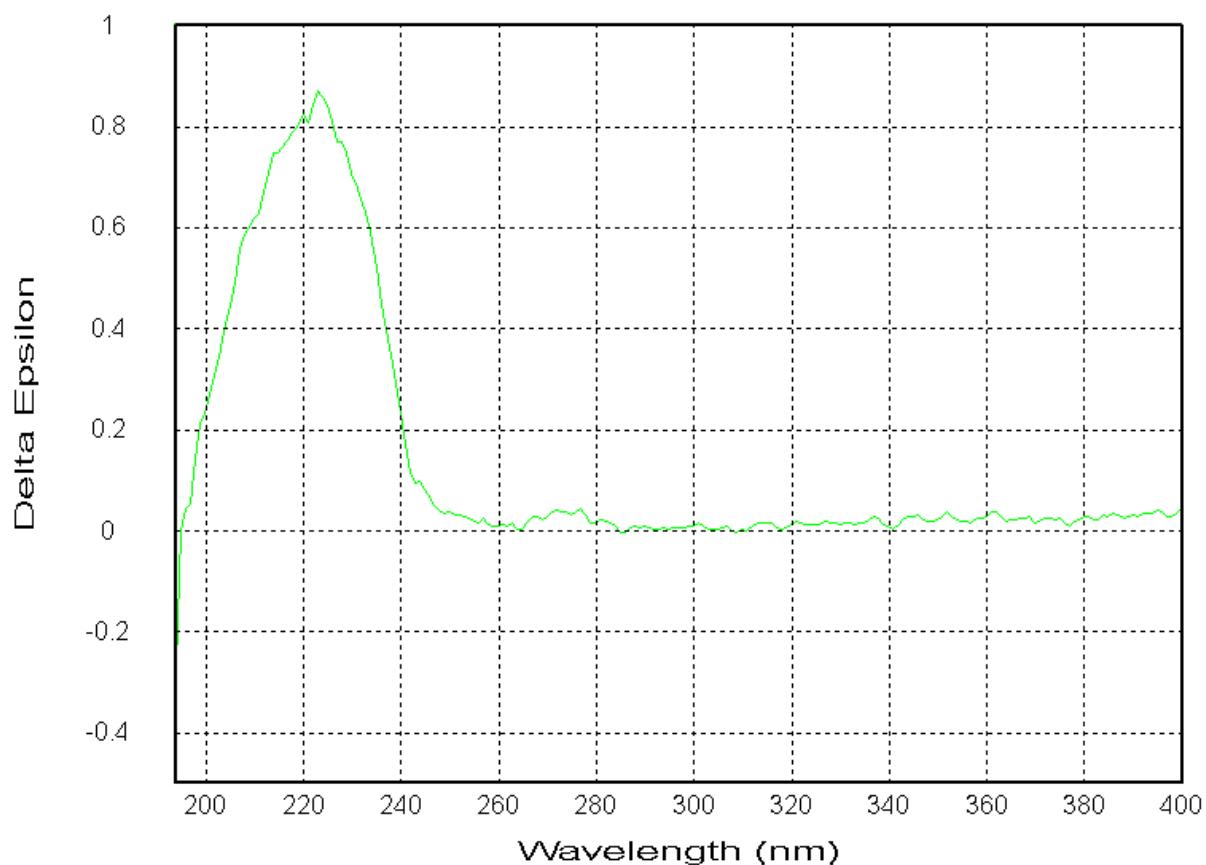


Figure S2. CD spectrum of **2**

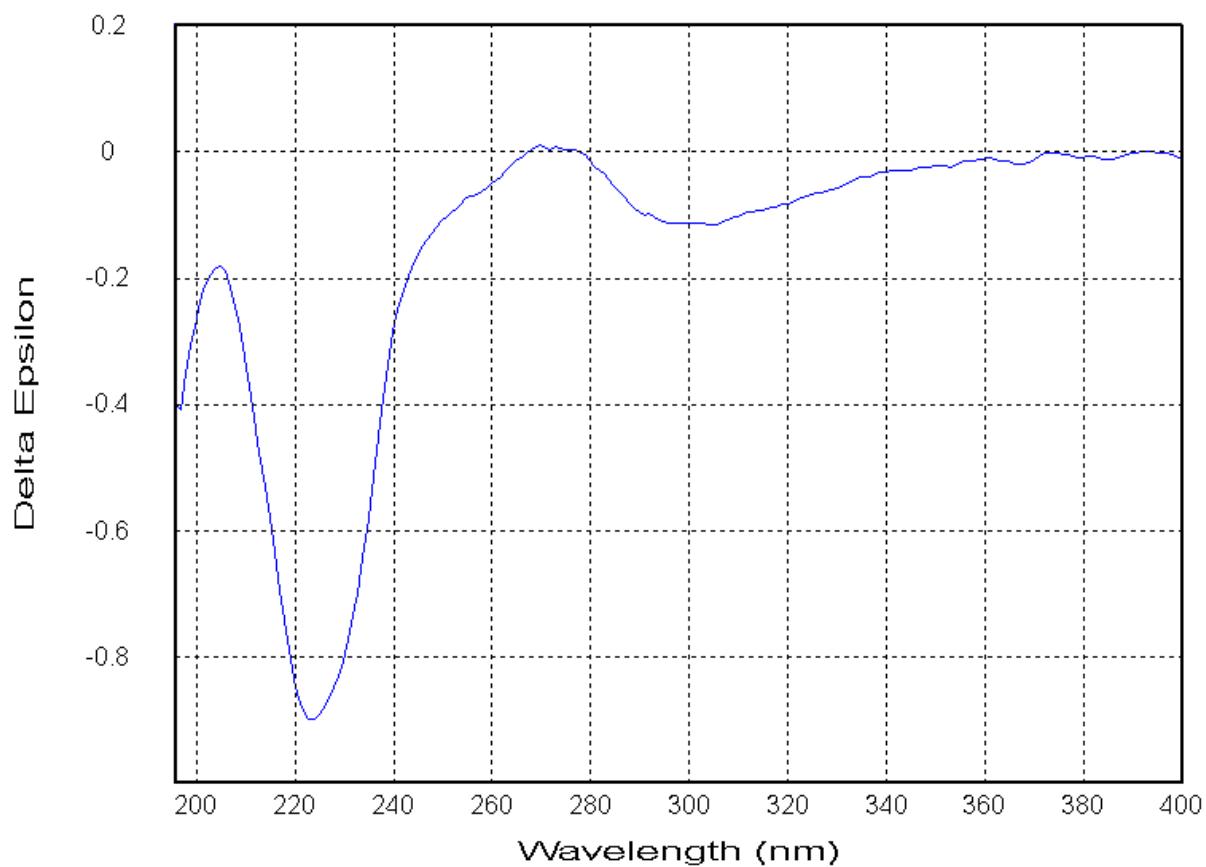


Figure S3. CD spectrum of 3

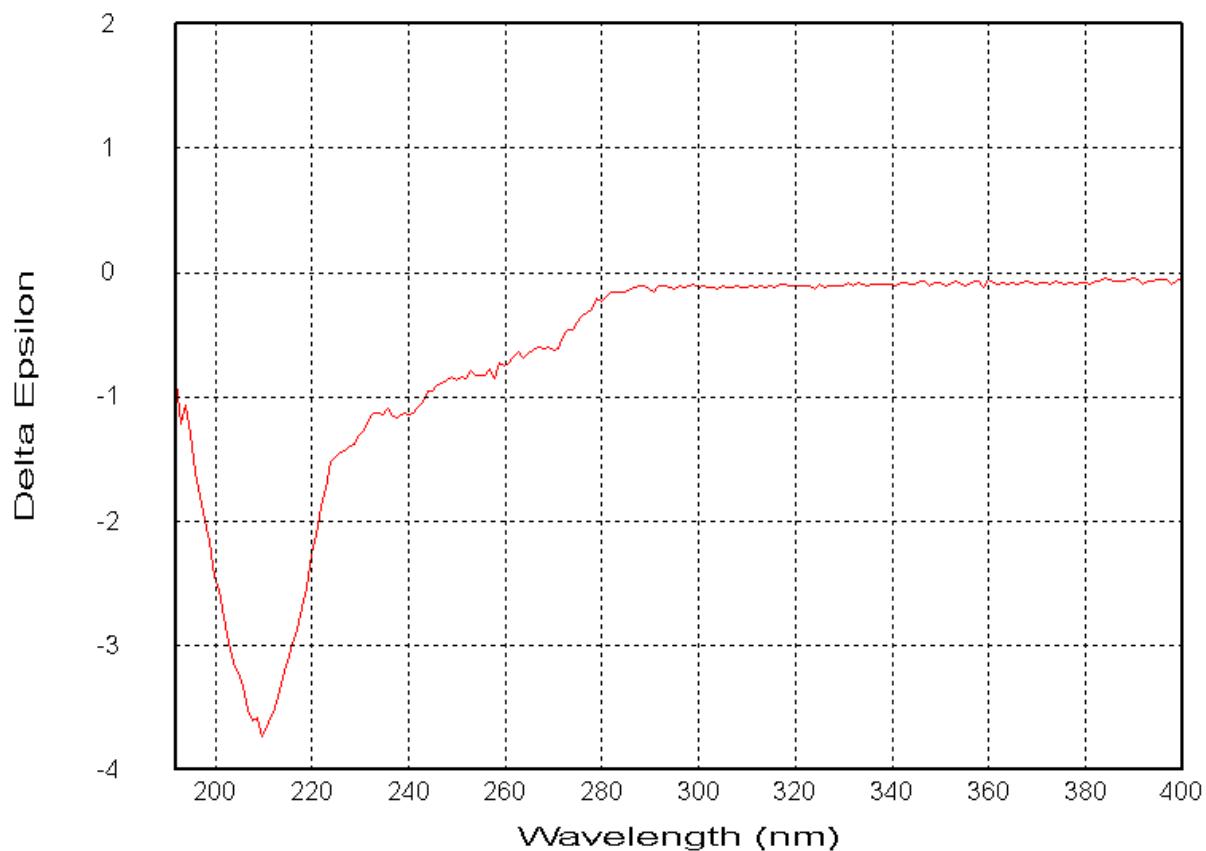


Figure S4. CD spectrum of 4

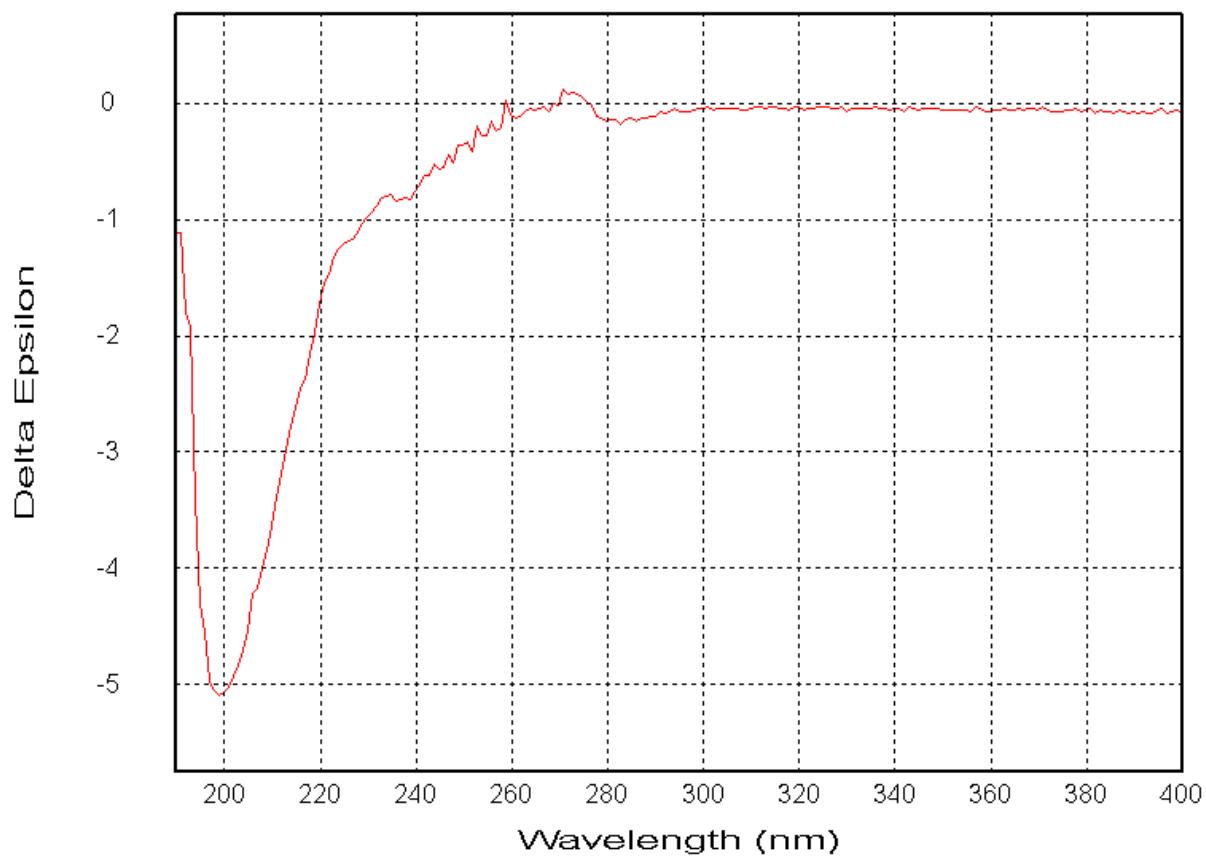


Figure S5. CD spectrum of **5**

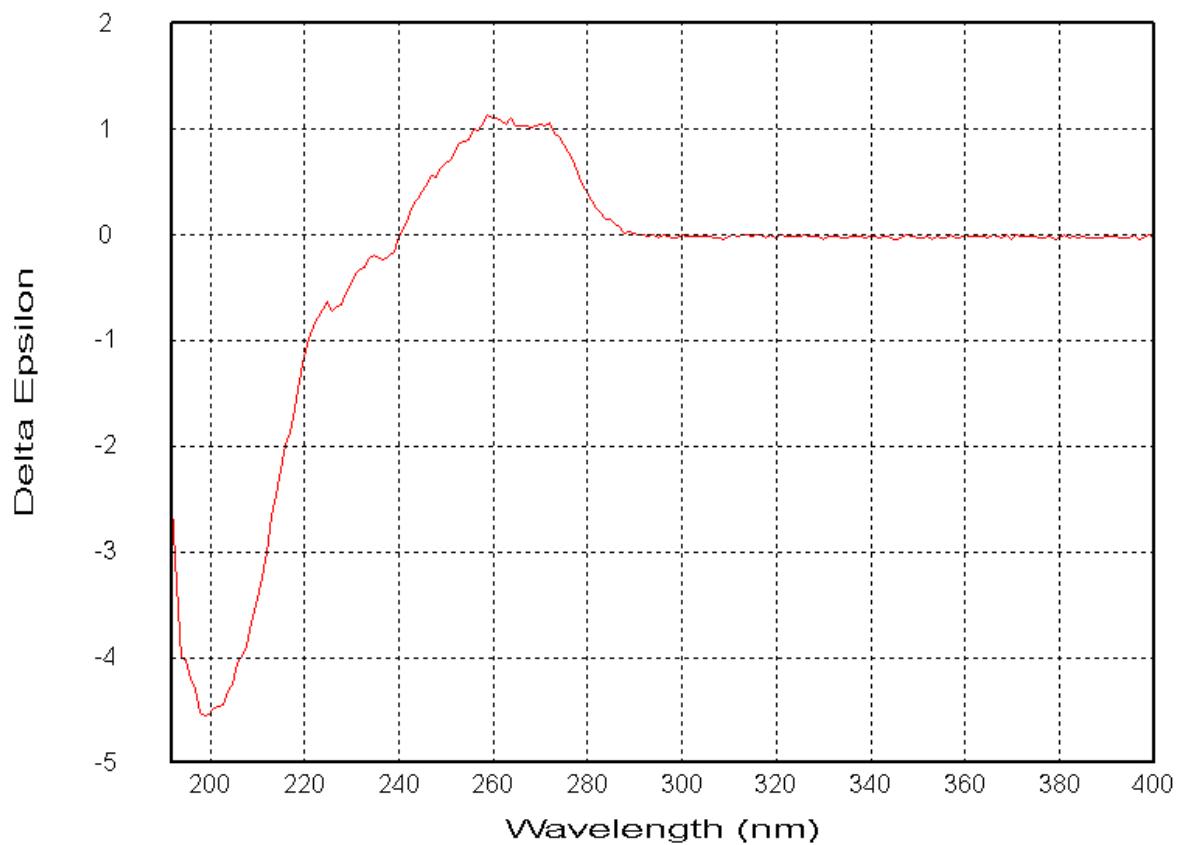


Figure S6. CD spectrum of **6**

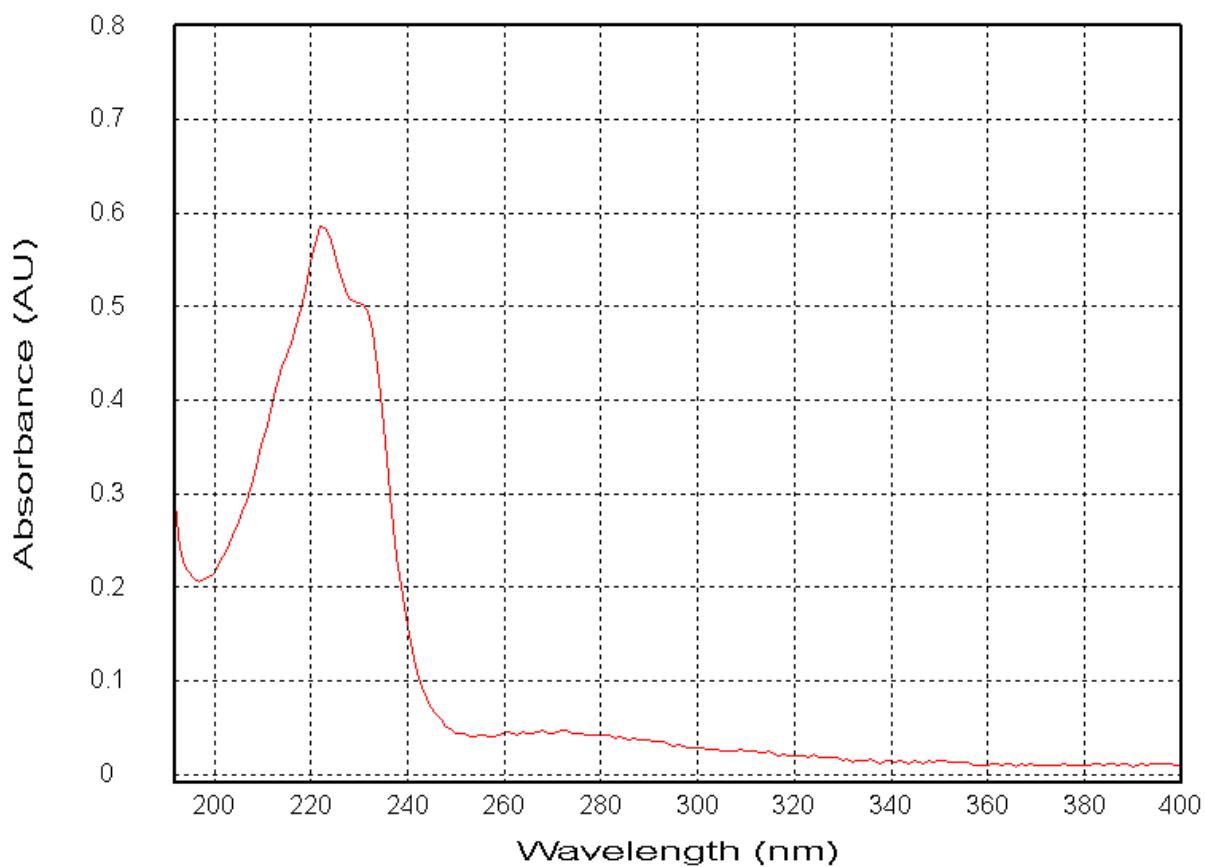


Figure S7. UV spectrum of **1**

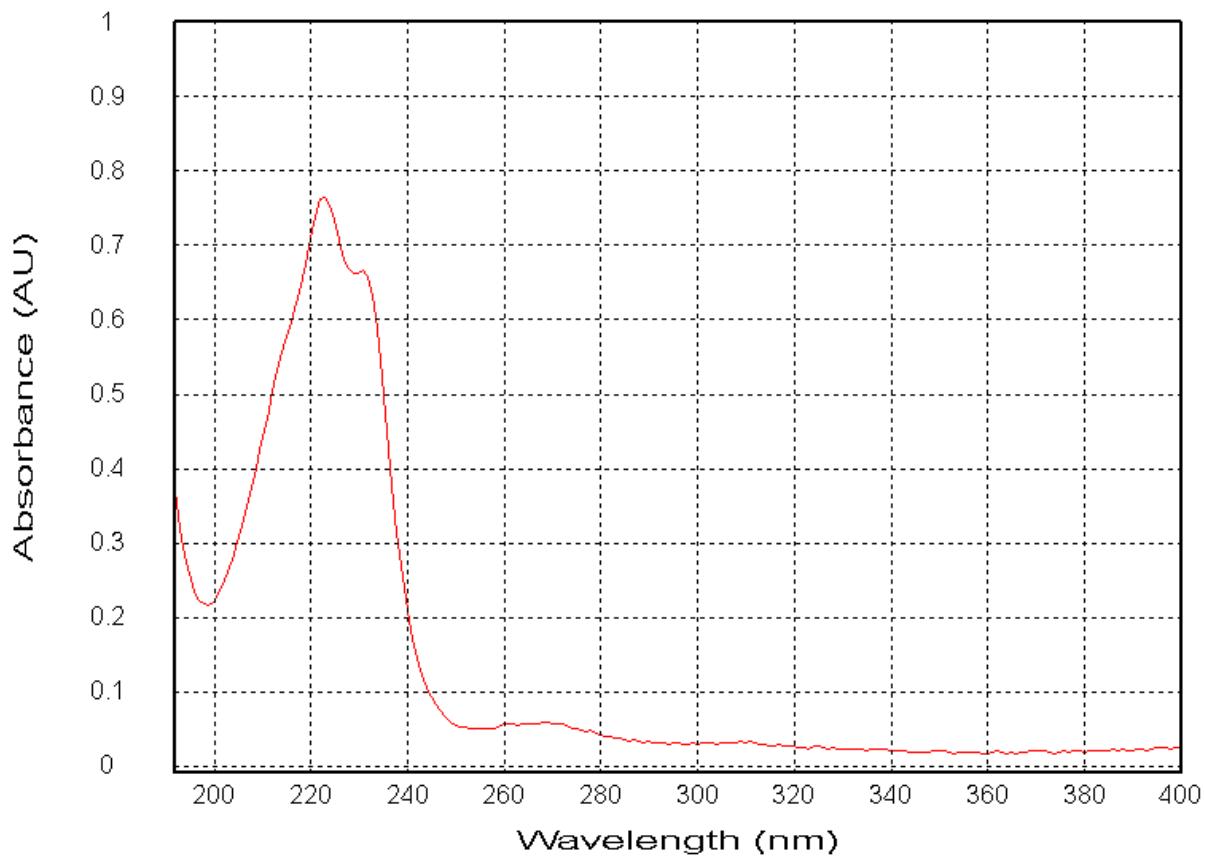


Figure S8. UV spectrum of **2**

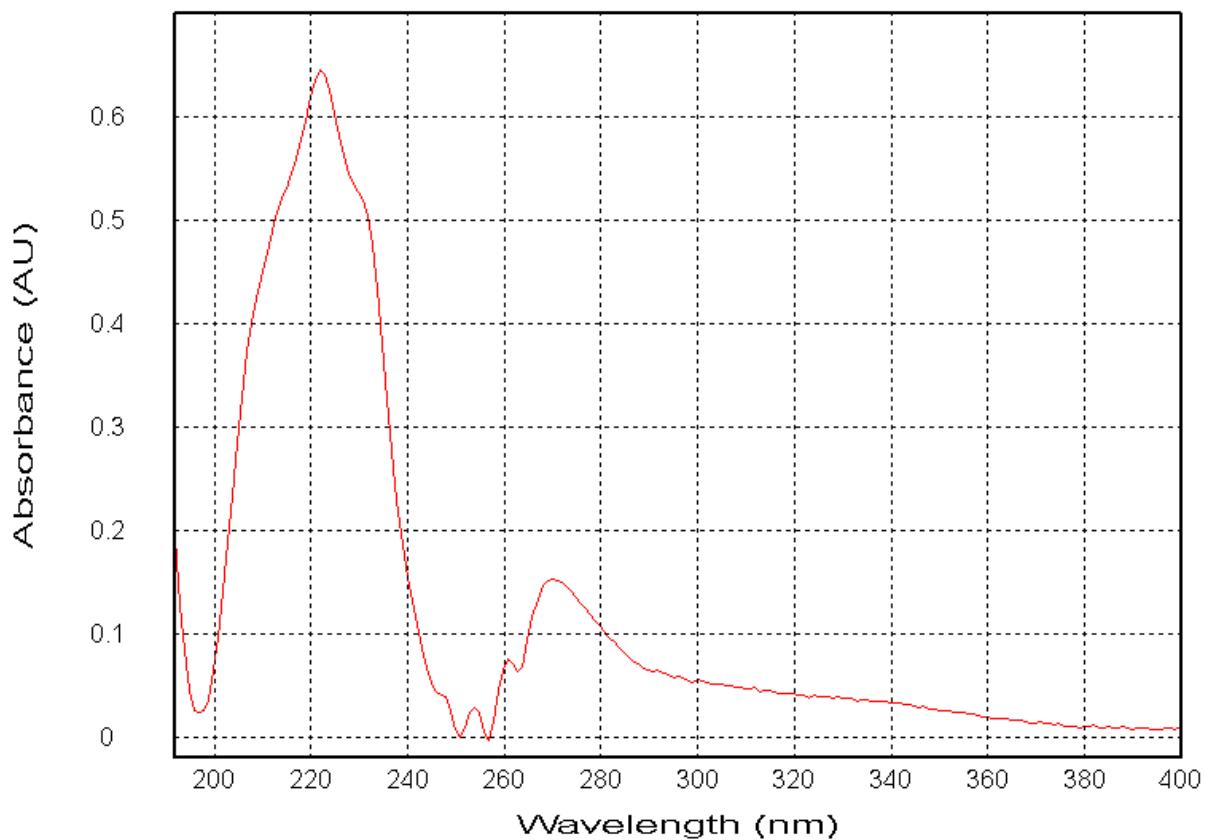


Figure S9. UV spectrum of **3**

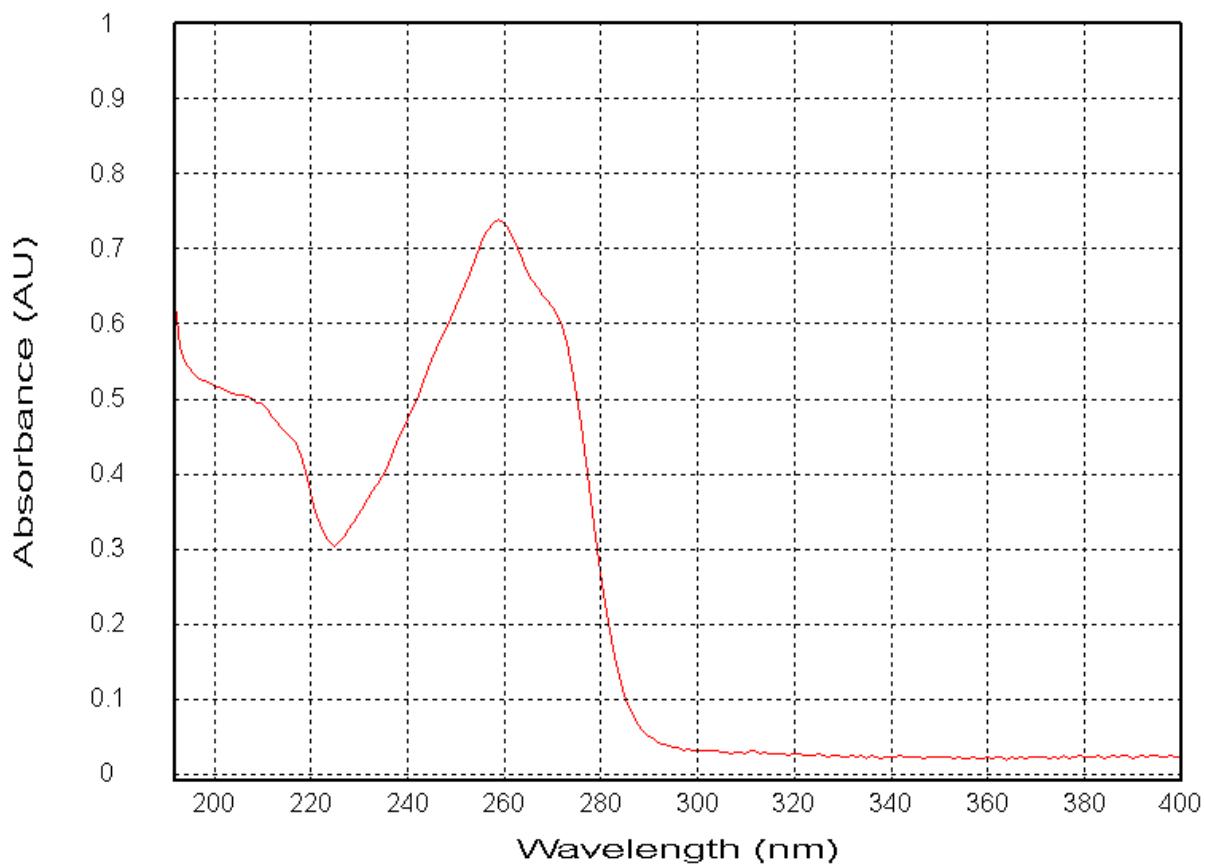


Figure S10. UV spectrum of **4**

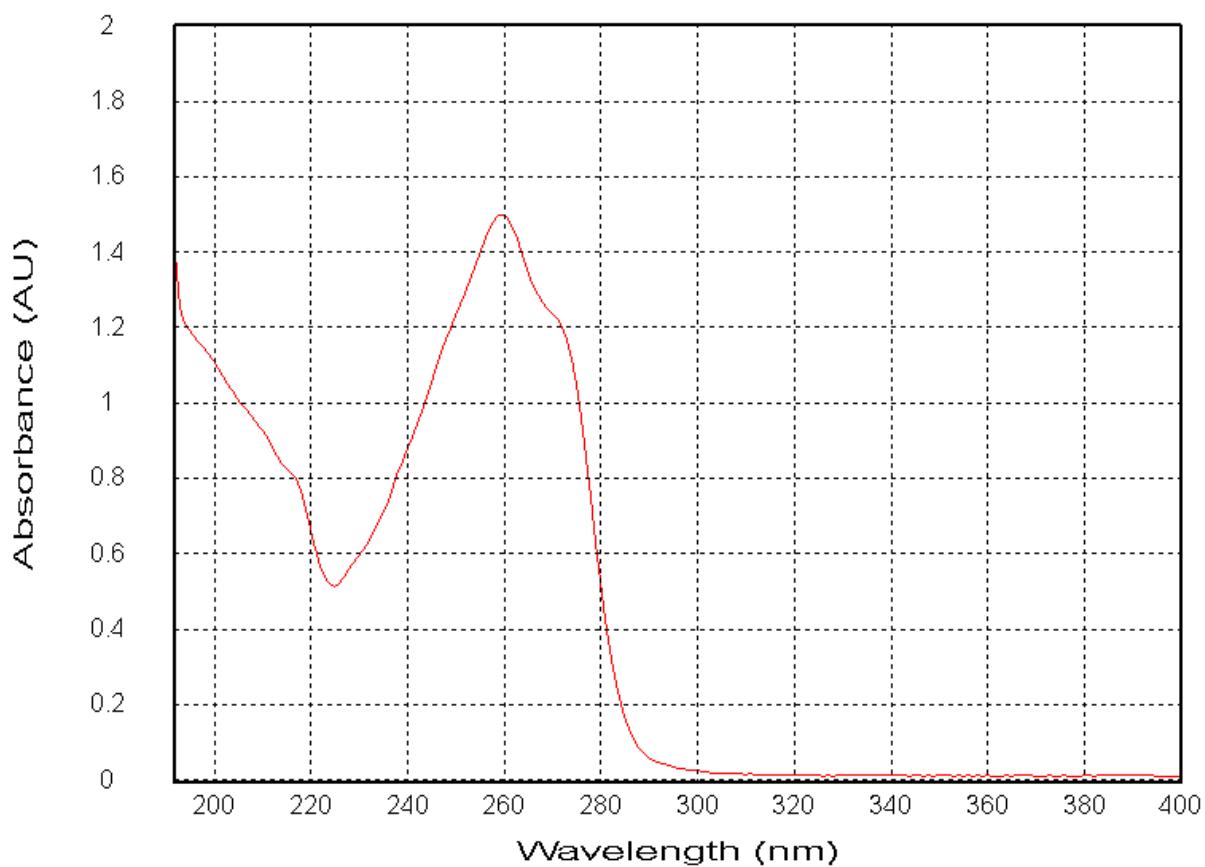


Figure S11. UV spectrum of **5**

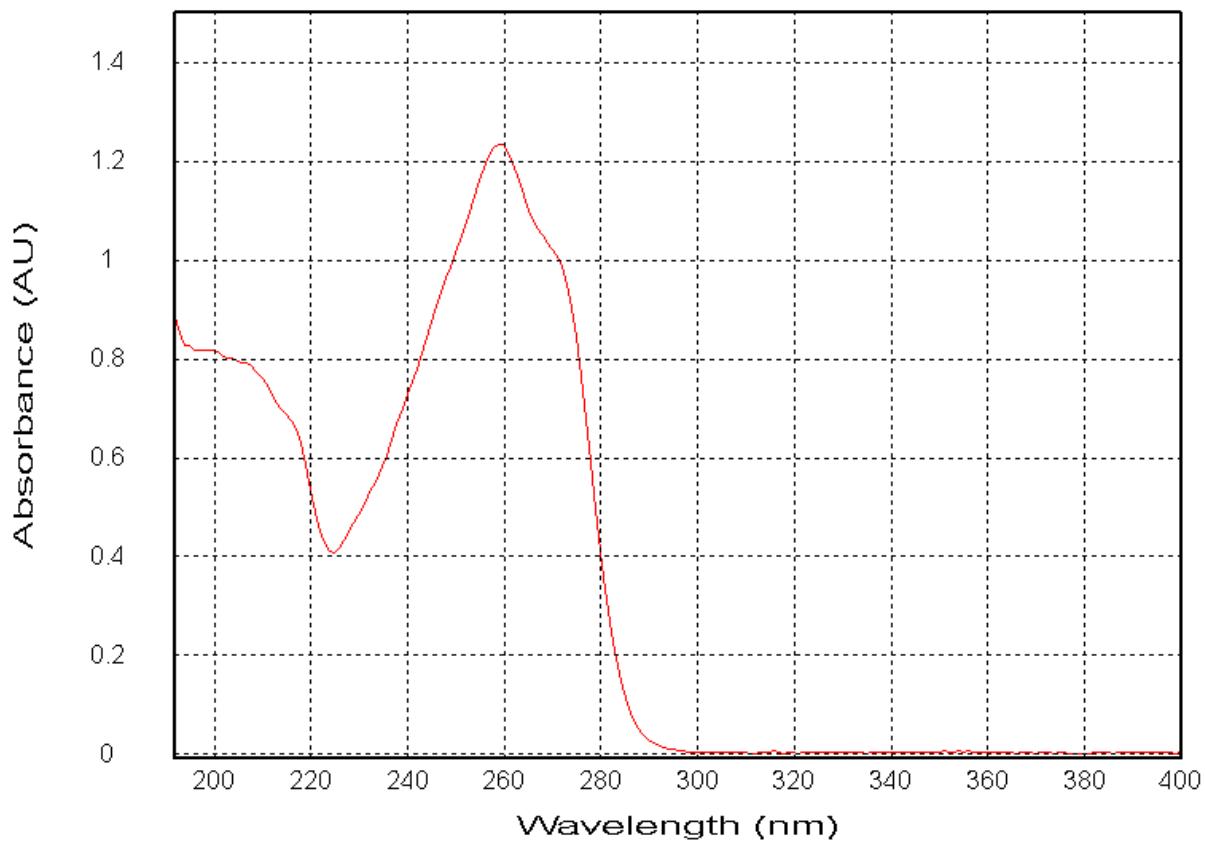


Figure S12. UV spectrum of **6**

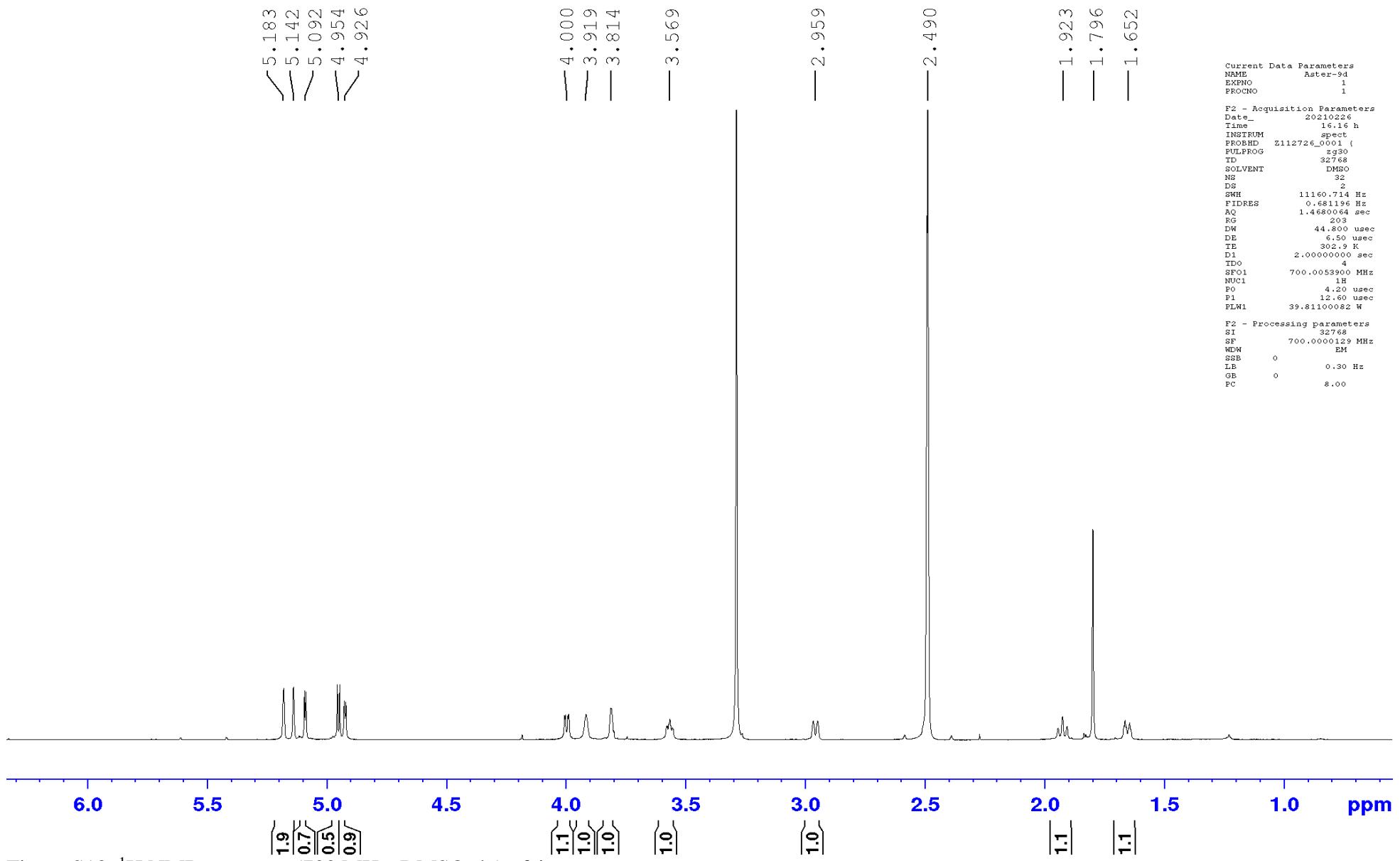


Figure S13. ^1H NMR spectrum (700 MHz, DMSO-d₆) of **1**

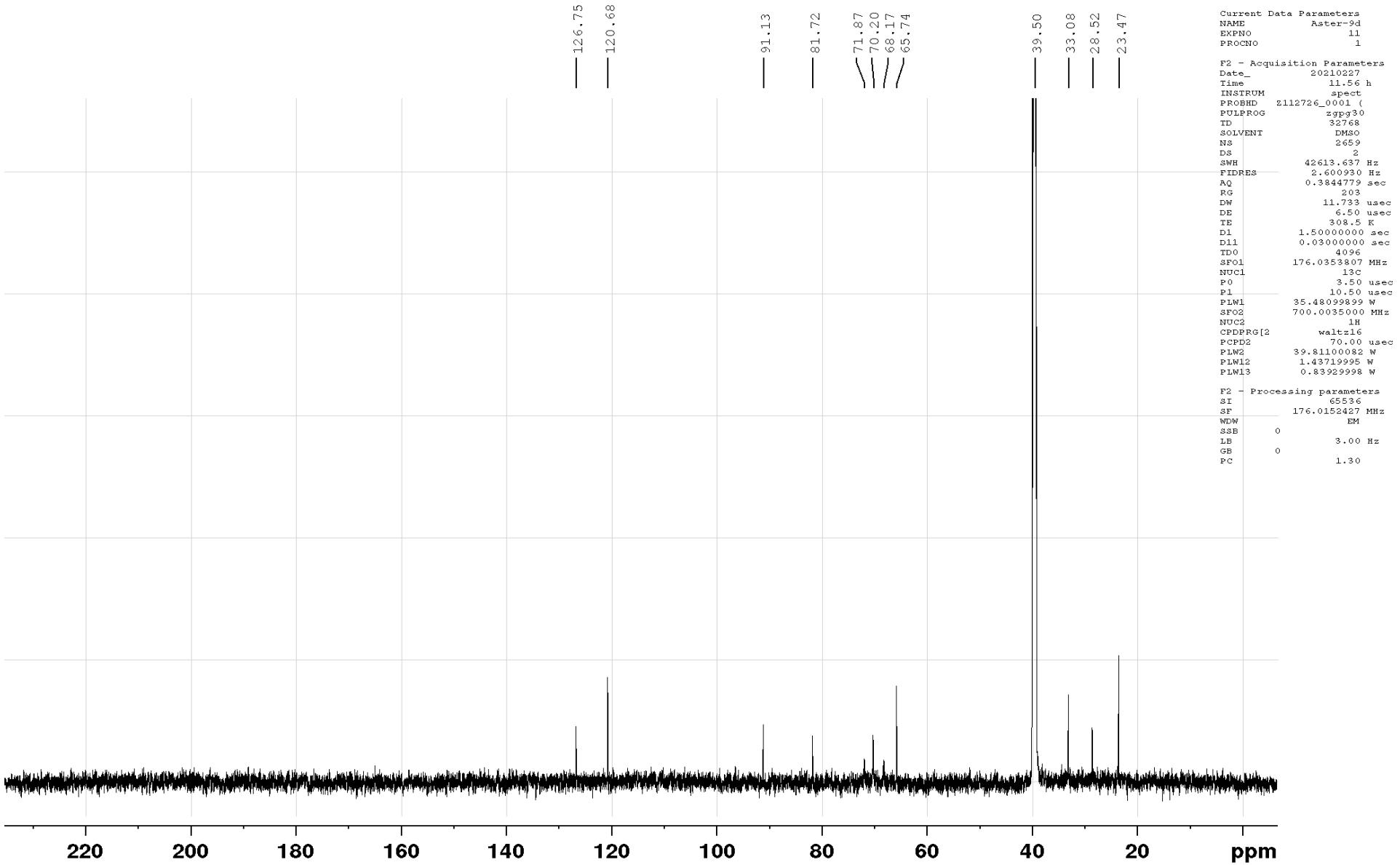


Figure S14. ^{13}C NMR spectrum (176 MHz, DMSO-d₆) of 1

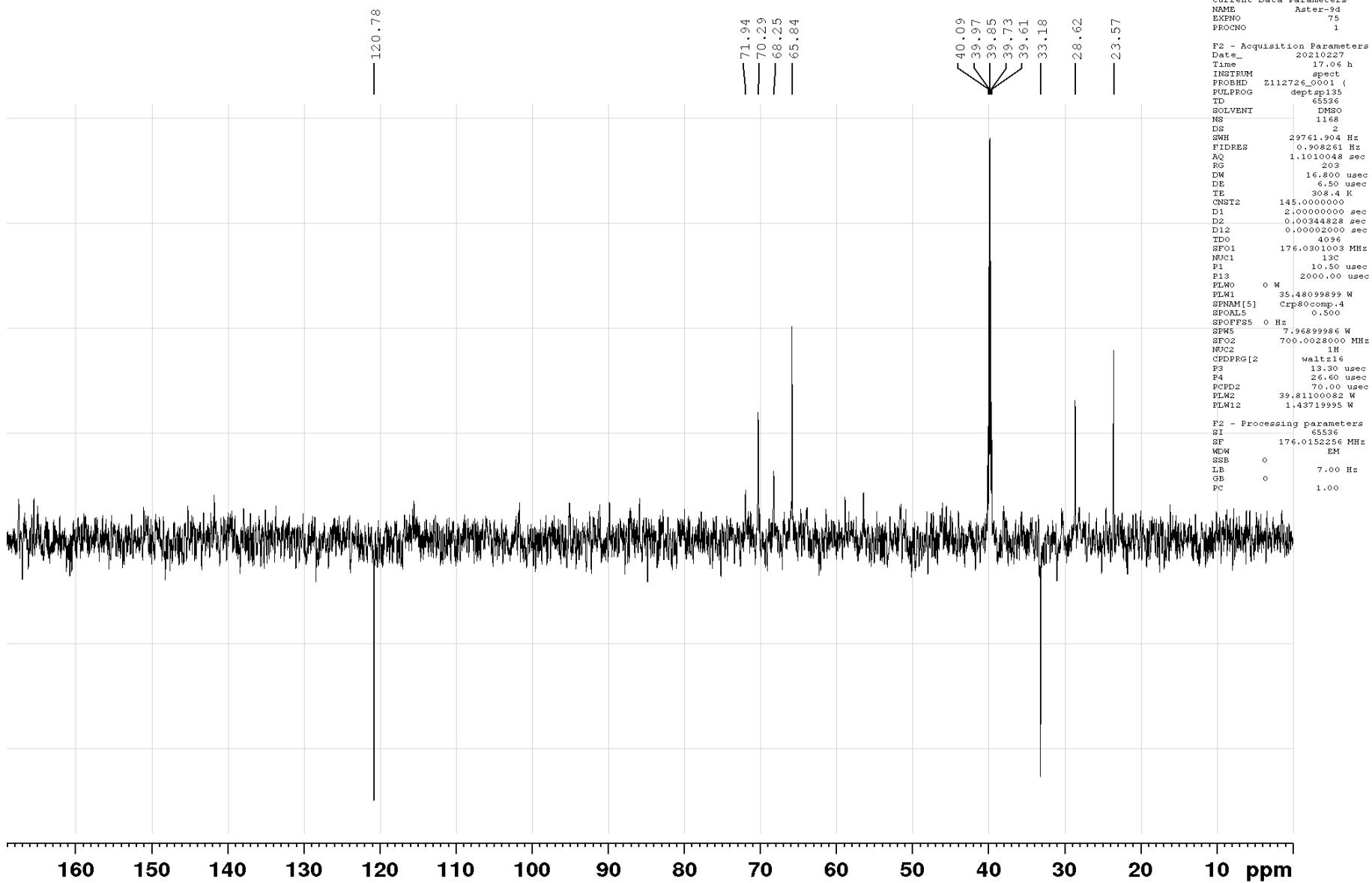


Figure S15. DEPT-135 NMR spectrum (176 MHz, DMSO-d₆) of **1**

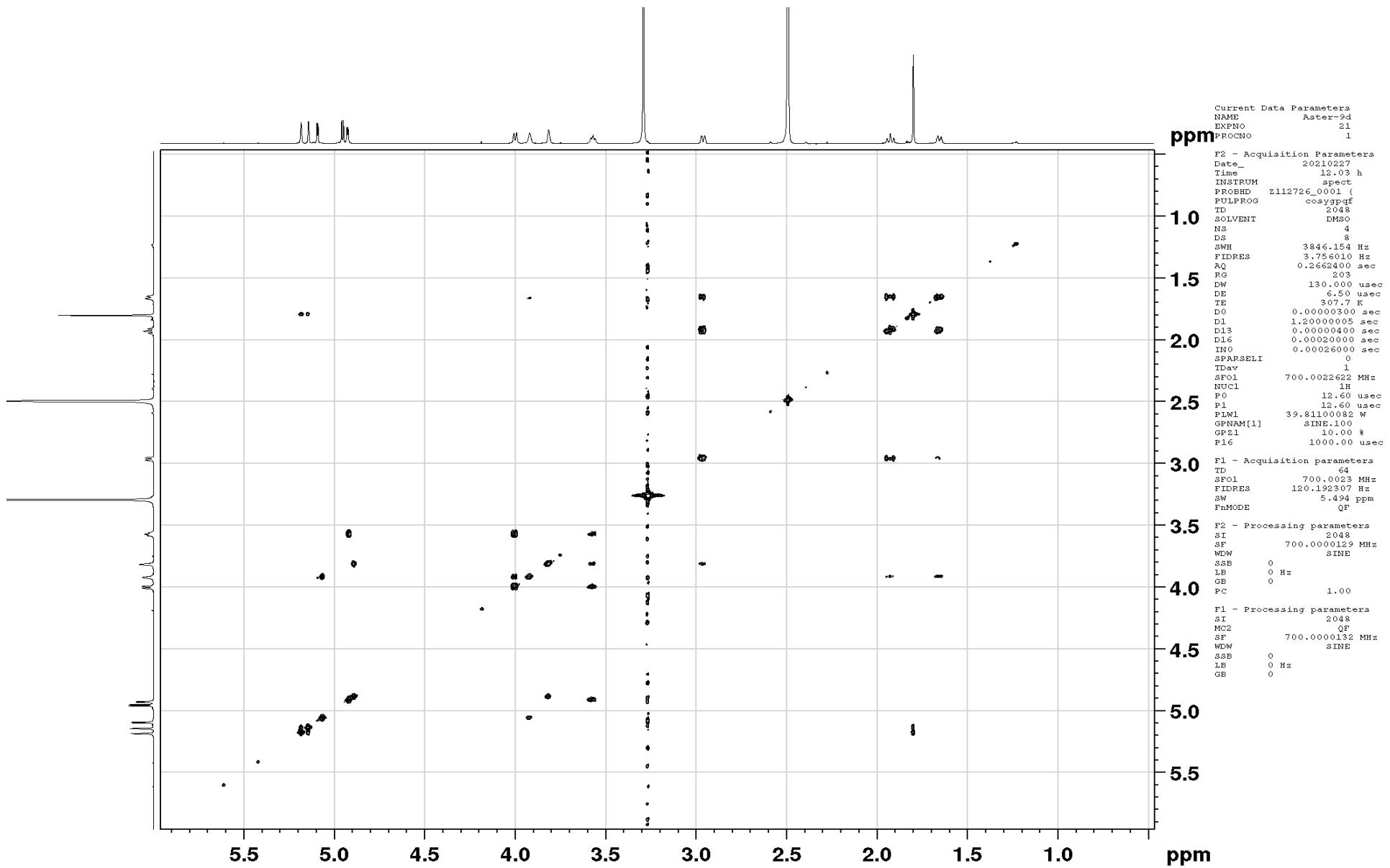


Figure S16. COSY-45 spectrum (700 MHz, DMSO-d₆) of **1**

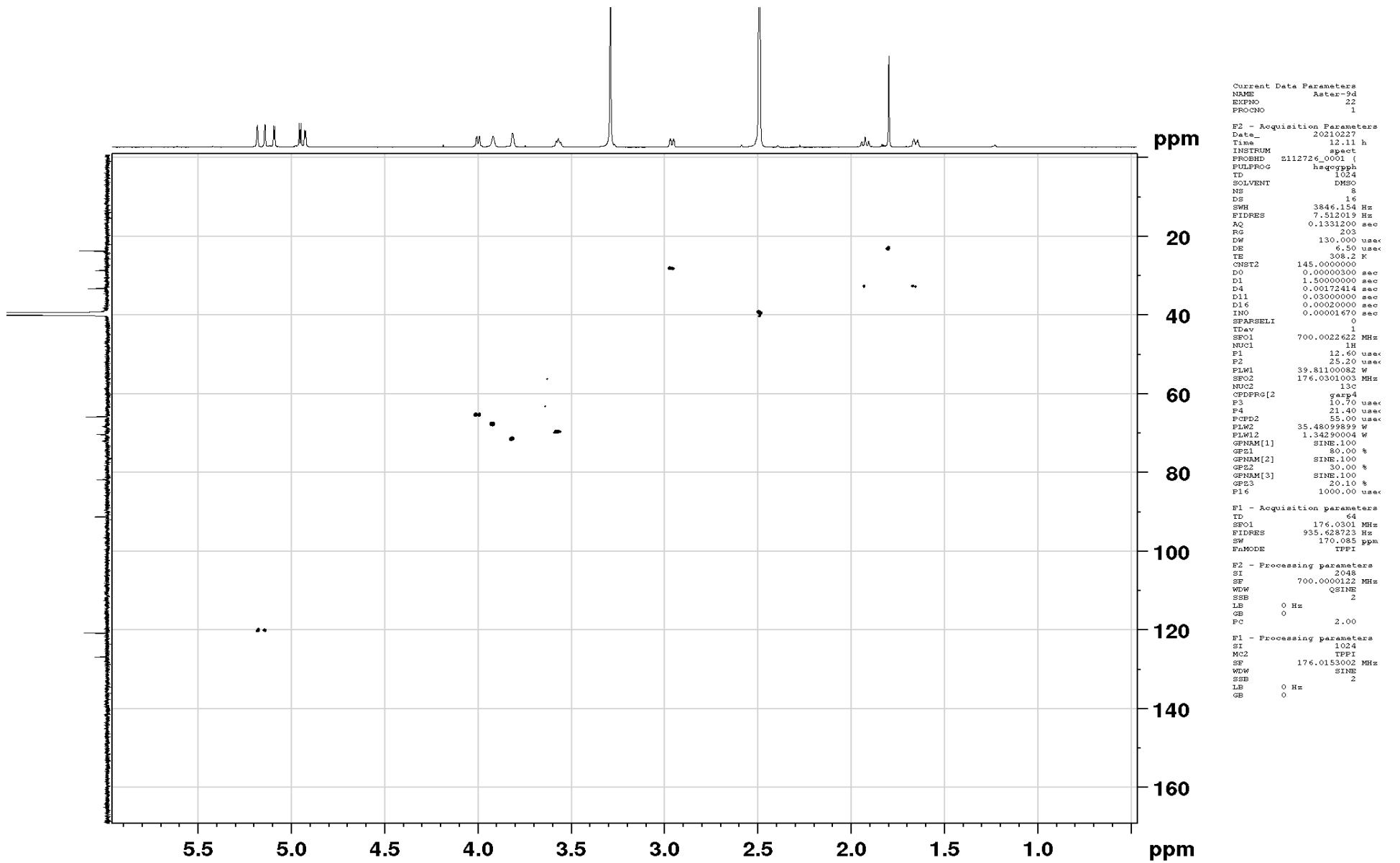


Figure S17. HSQC spectrum (700 MHz, DMSO-d₆) of **1**

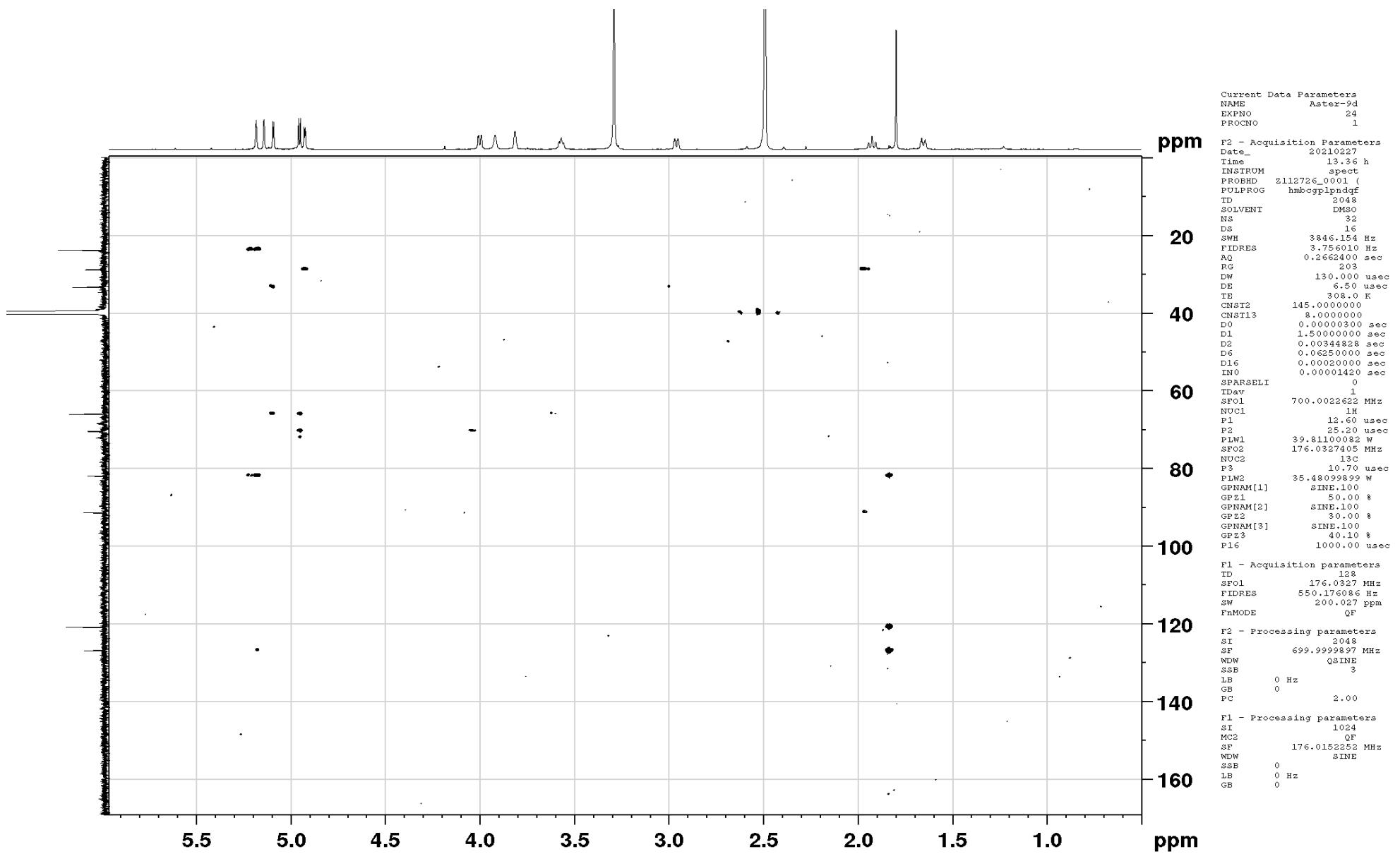


Figure S18. HMBC spectrum (700 MHz, DMSO-d₆) of **1**

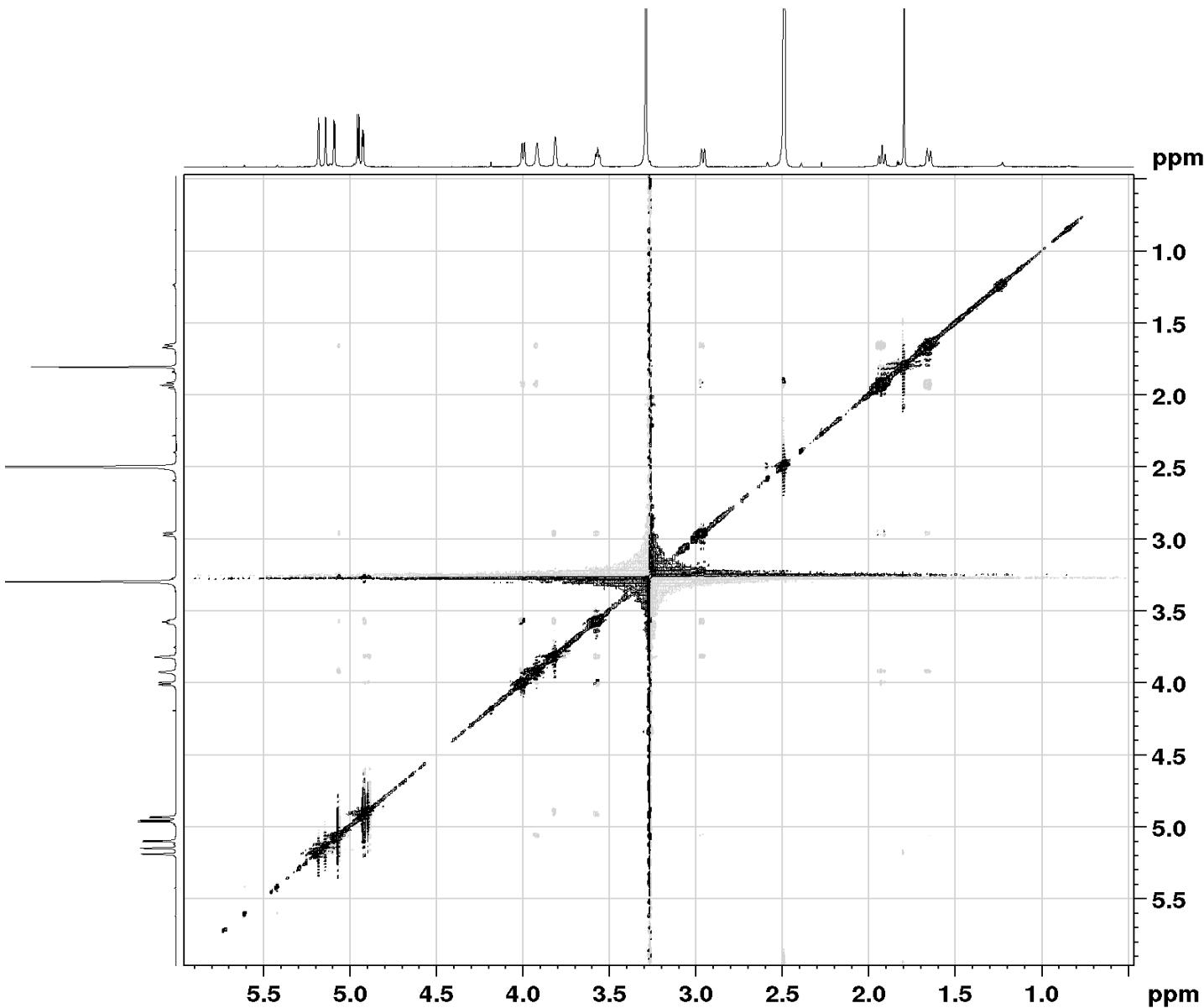


Figure S19. ROESY spectrum (700 MHz, DMSO-d₆) of **1**

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 PROCNO 1

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 PULPROG roesypnph.2
 TD 2048
 SOLVENT DMSO
 NS 8
 DS 16
 SWH 3846.154 Hz
 FIDRES 3.756010 Hz
 AQ 0.2662400 sec
 RG 4
 DW 130.000 usec
 DE 6.50 usec
 TE 307.9 K
 D0 0.00011798 sec
 D1 1.5000000 sec
 D12 0.00002000 sec
 IN0 0.00026000 sec
 L4 649
 P15 200000.00 usec
 TDAv 1
 SP01 700.0022622 MHz
 NUCL1 1H
 F1 12.00 usec
 P25 154.0 usec
 P1M1 39.81100082 W
 P1W27 1.06599998 W

F1 - Acquisition parameters
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 SP01 700.0023 MHz
 FIDRES 30.048077 Hz
 SW 5.494 ppm
 FnMODE TPPI

F2 - Processing parameters
 SI 2048
 SF 700.0000124 MHz
 NDW SINE
 SSB 2
 LB 0 Hz
 GB 0
 PC 2.00

F1 - Processing parameters
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 MC2 TPPI
 SF 700.0000119 MHz
 NDW SINE
 SSB 2
 LB 0 Hz
 GB 0

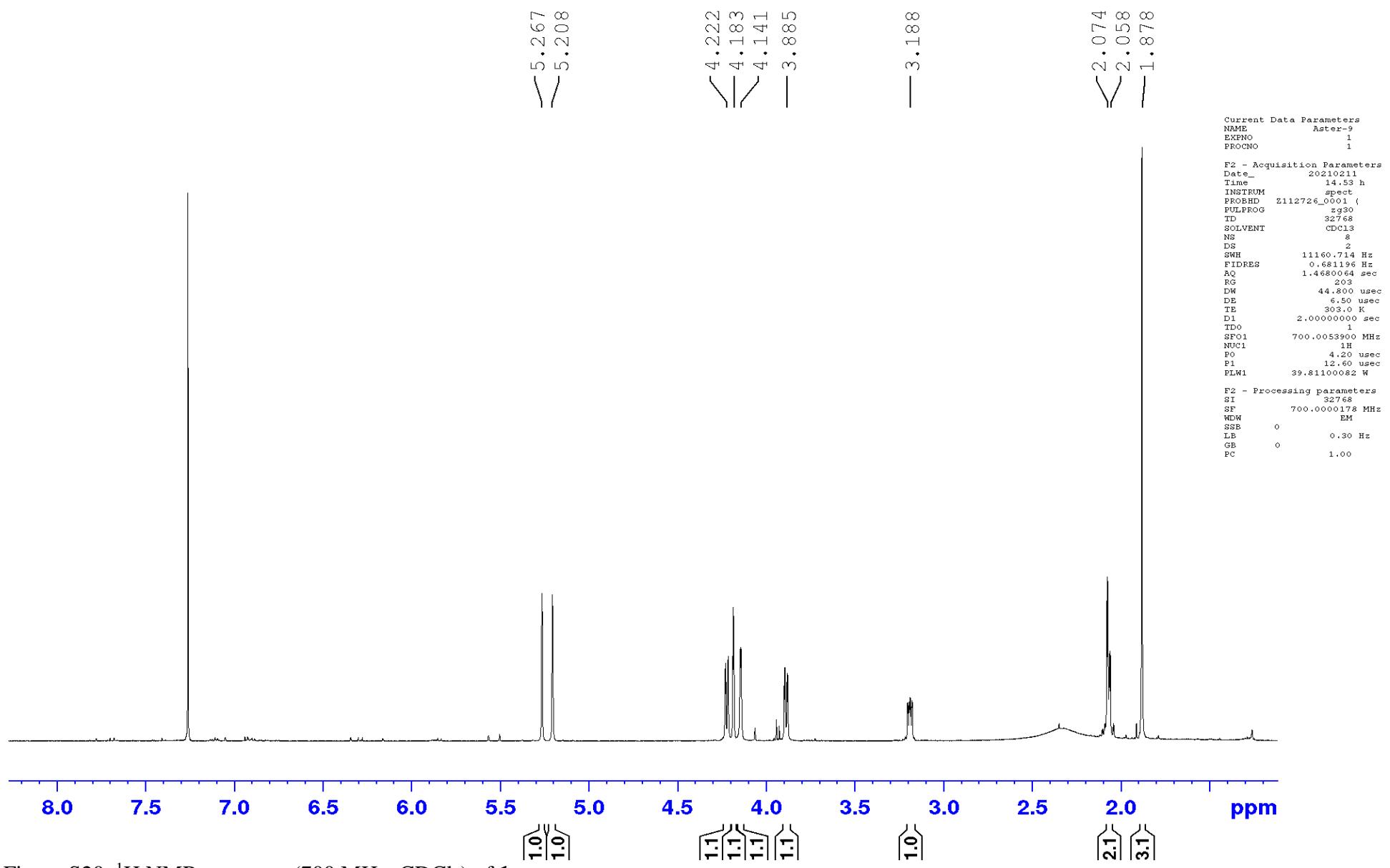


Figure S20. ¹H NMR spectrum (700 MHz, CDCl₃) of **1**

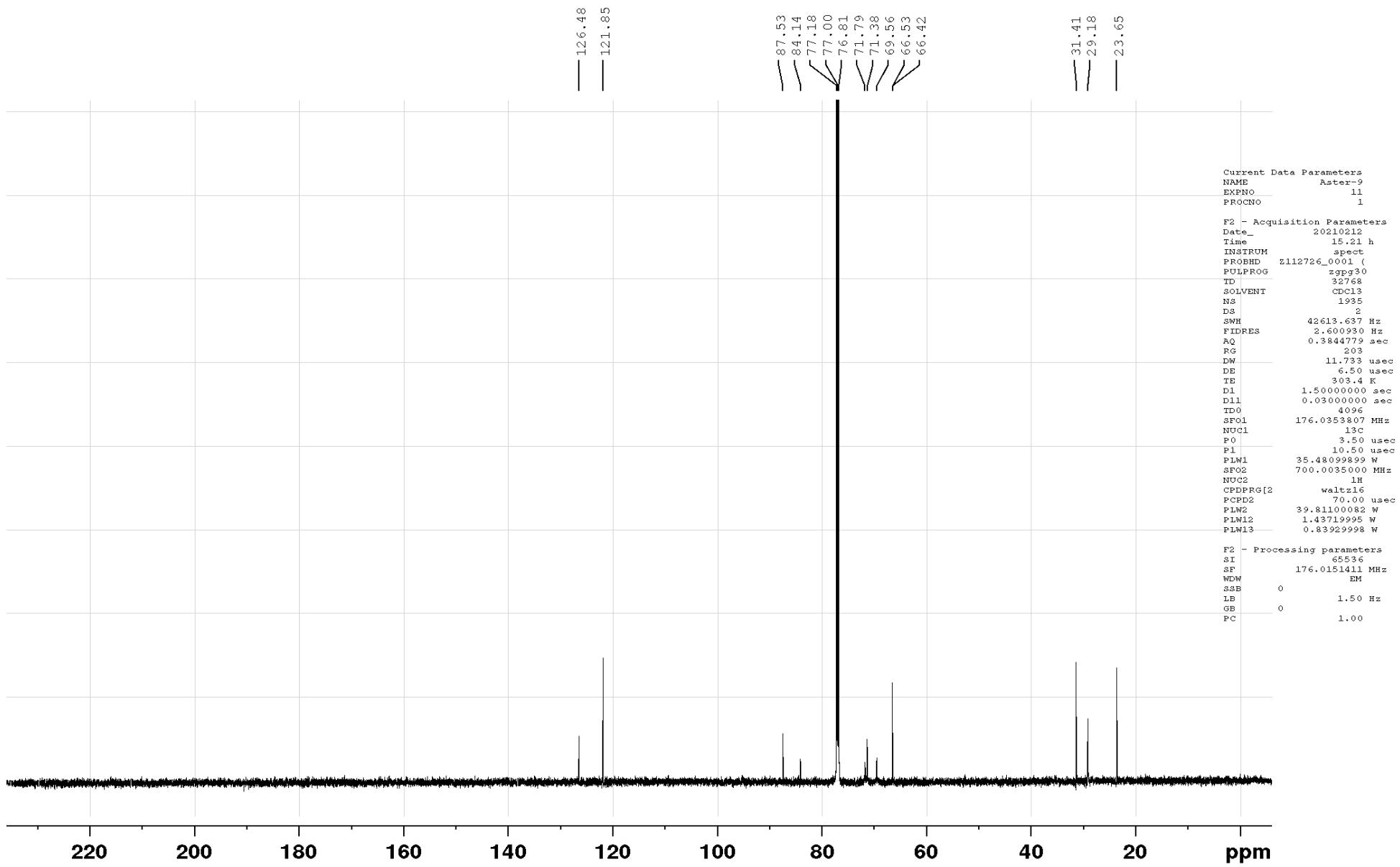


Figure S21. ^{13}C NMR spectrum (176 MHz, CDCl_3) of **1**

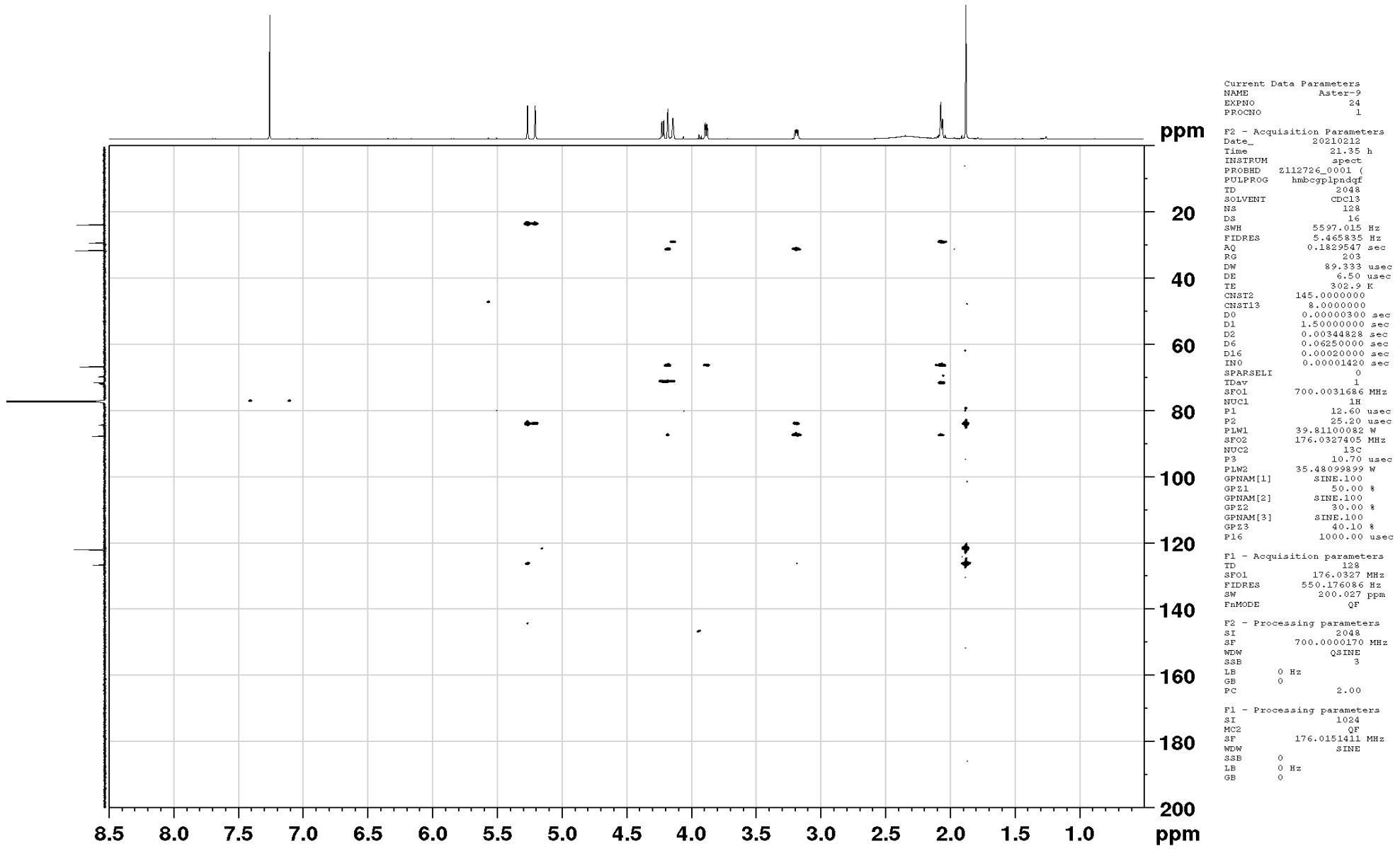


Figure S22. HMBC spectrum (700 MHz, CDCl_3) of **1**

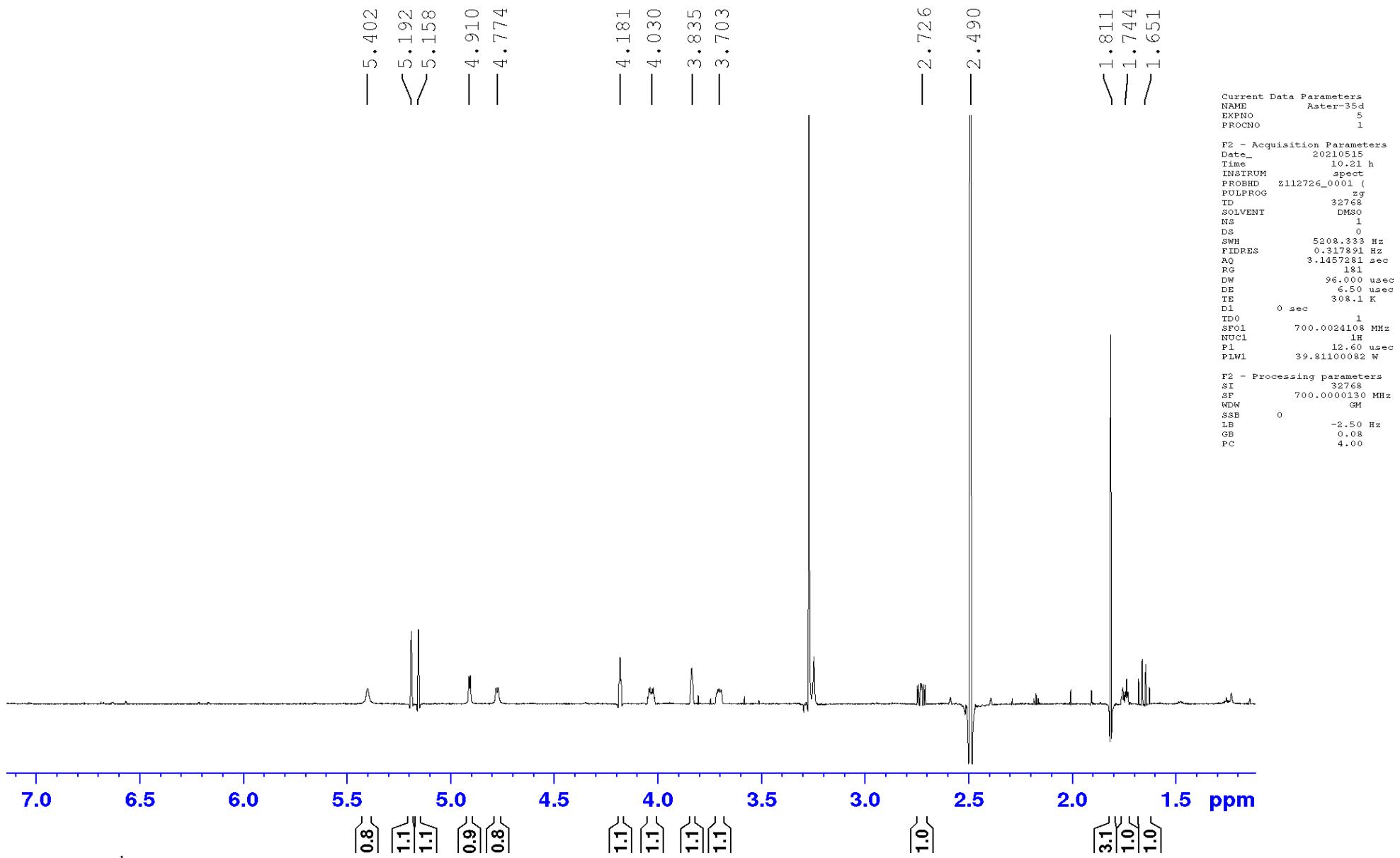


Figure S23. ^1H NMR spectrum (700 MHz, DMSO- d_6) of **2**

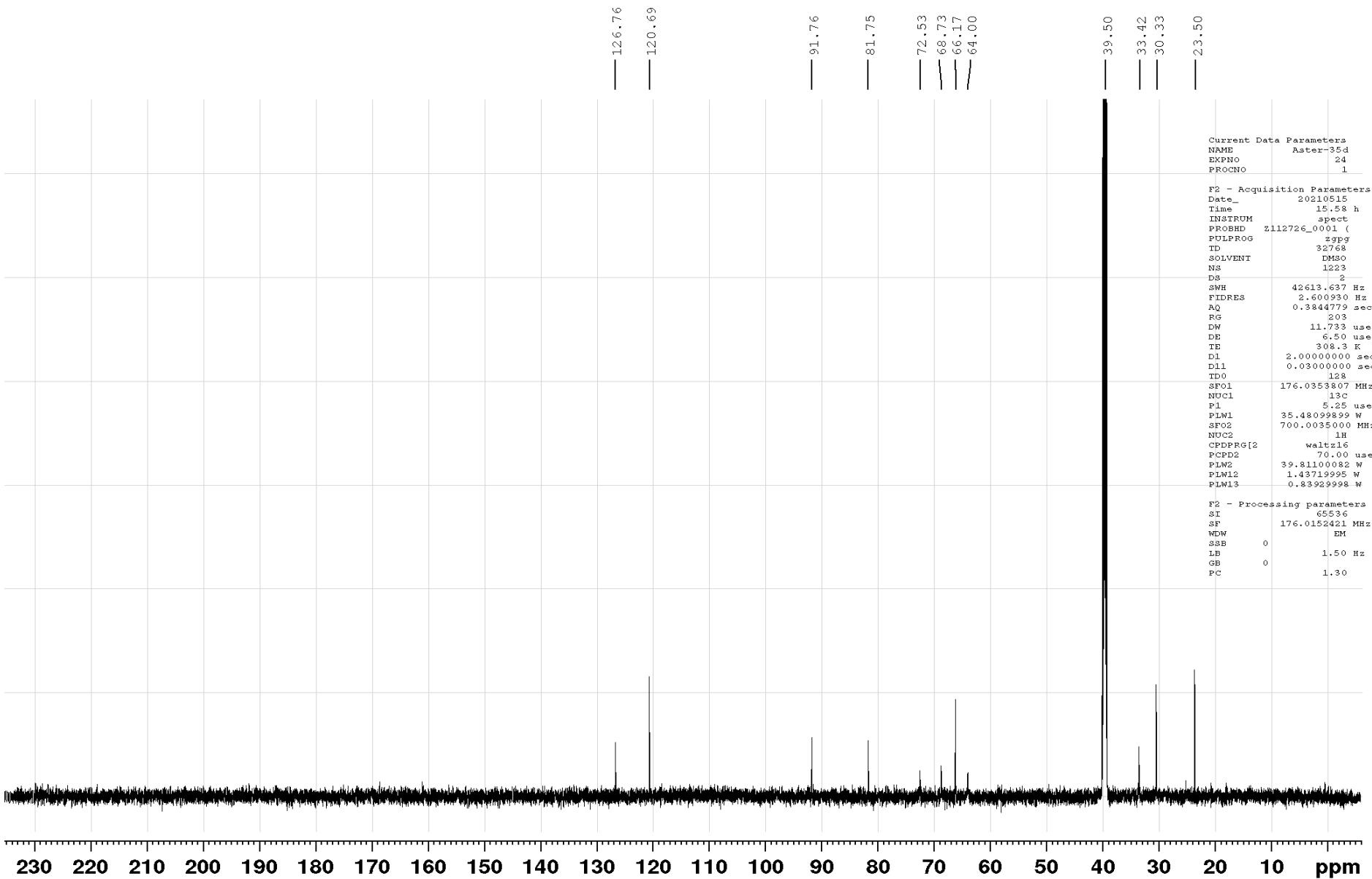


Figure S24. ^{13}C NMR spectrum (176 MHz, DMSO-d₆) of 2

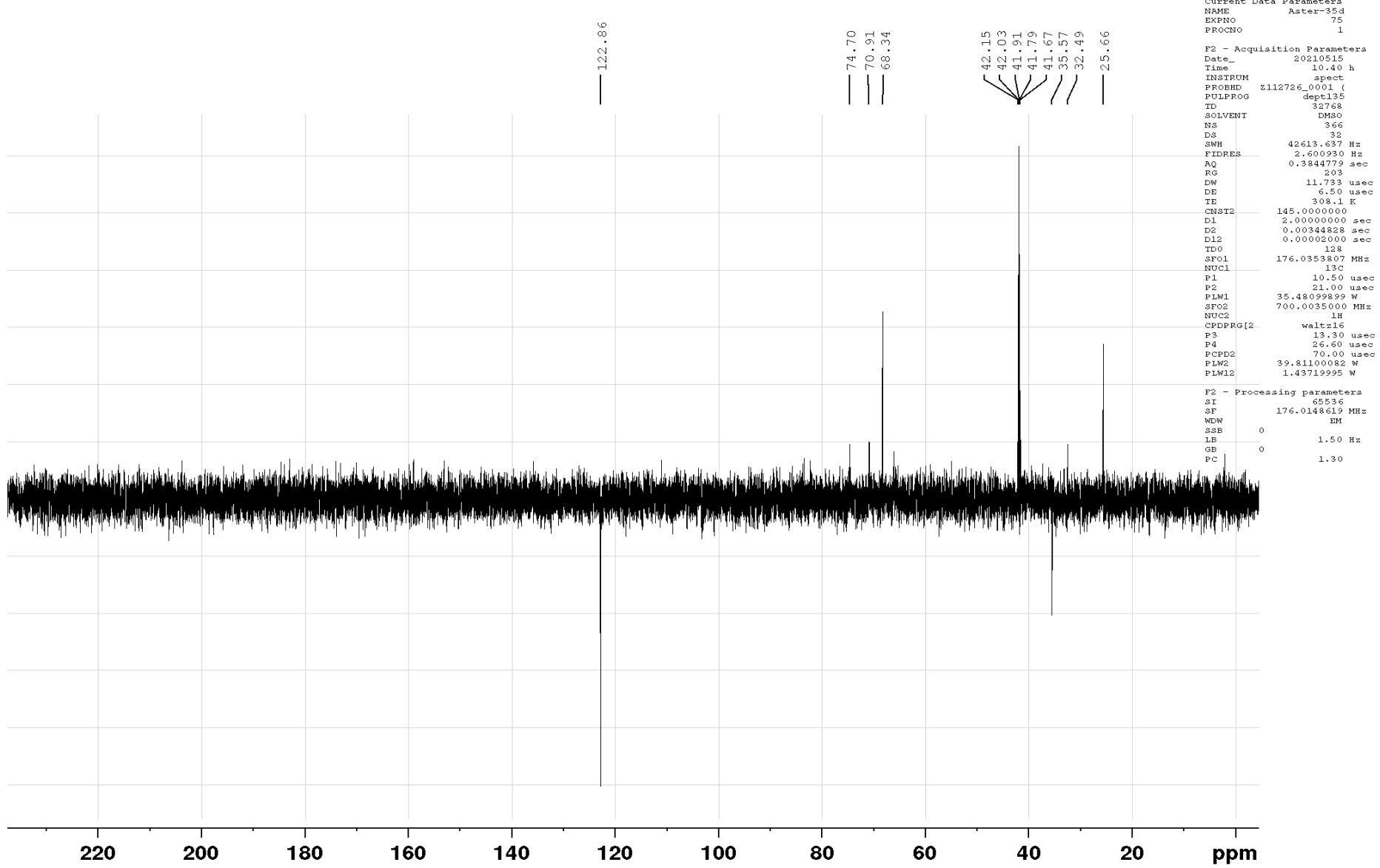


Figure S25. DEPT-135 NMR spectrum (176 MHz, DMSO-d₆) of 2

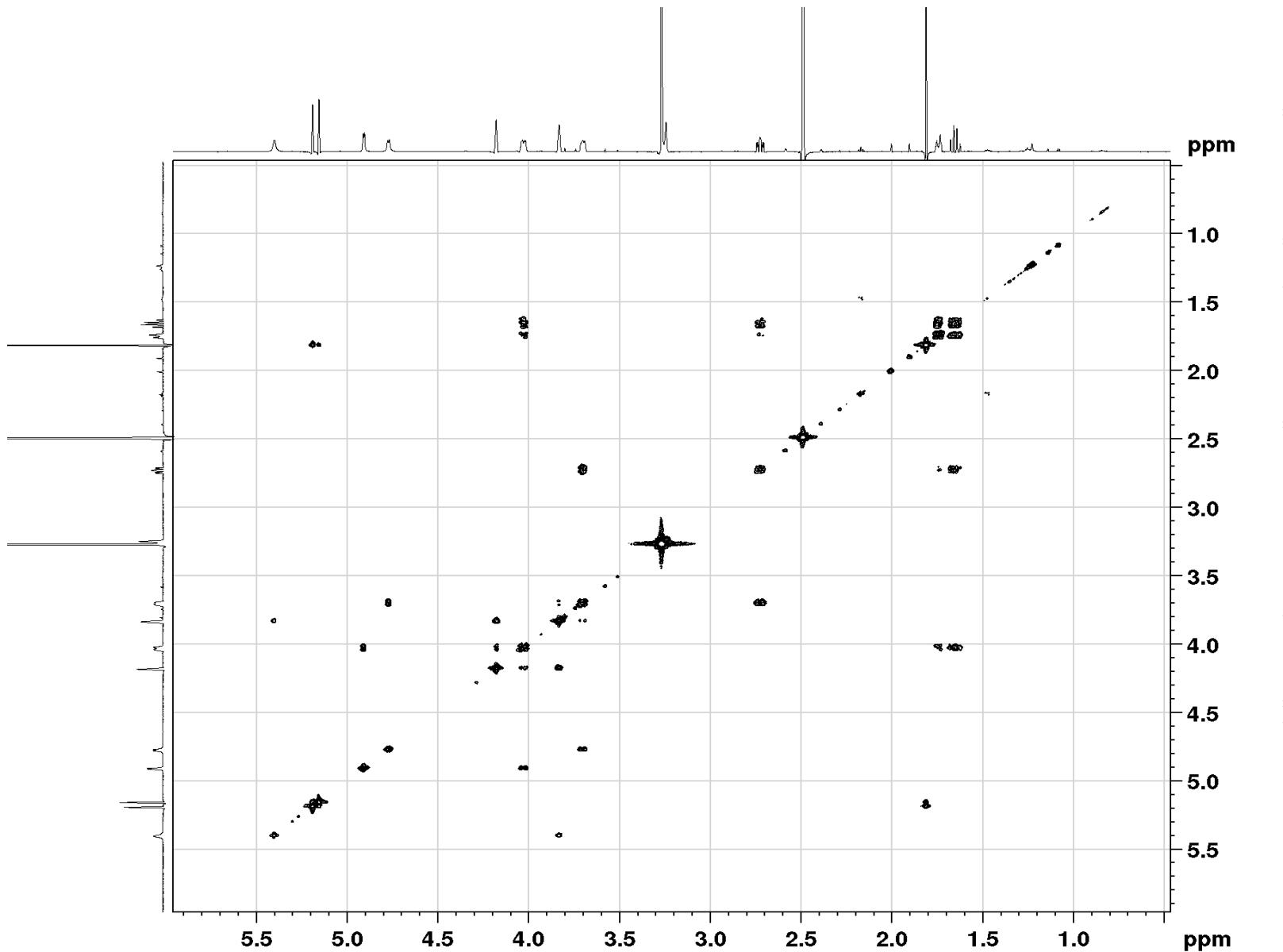


Figure S26. COSY-45 spectrum (700 MHz, DMSO-d₆) of 2

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Current Data Parameters
NAME Aster-35d
EXPNO 21
PROCNO 1

P2 - Acquisition Parameters
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Time 10.47 h
INSTRUM spect
PROBHD 2112726_001 (
PULPROG cosyppqf
TD 2048
SOLVENT DMSO
NS 16
DS 8
SWH 5208.333 Hz
FIDRES 5.086263 Hz
AQ 0.1966080 sec
RG 203
DW 96.000 usec
DE 6.50 usec
TE 307.9 K
D0 0.00000300 sec
D1 1.20000005 sec
D13 0.00000400 sec
D16 0.00020000 sec
INO 0.00019200 sec
SPARSEL1 0
TDav 1
SF01 700.0024109 MHz
NUC1 1H
P0 12.60 usec
P1 12.60 usec
PLW1 39.81100082 W
GPNAME[1] SINE,100
GPZ1 10.00 %
P16 1000.00 usec

P1 - Acquisition parameters
TD 128
SF01 700.0024 MHz
FIDRES 81.380211 Hz
SW 7.440 ppm
FnMODE QF

P2 - Processing parameters
SI 2048
SF 700.0000130 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0
PC 1.00

P1 - Processing parameters
SI 2048
MC2 0
SF 700.0000130 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0

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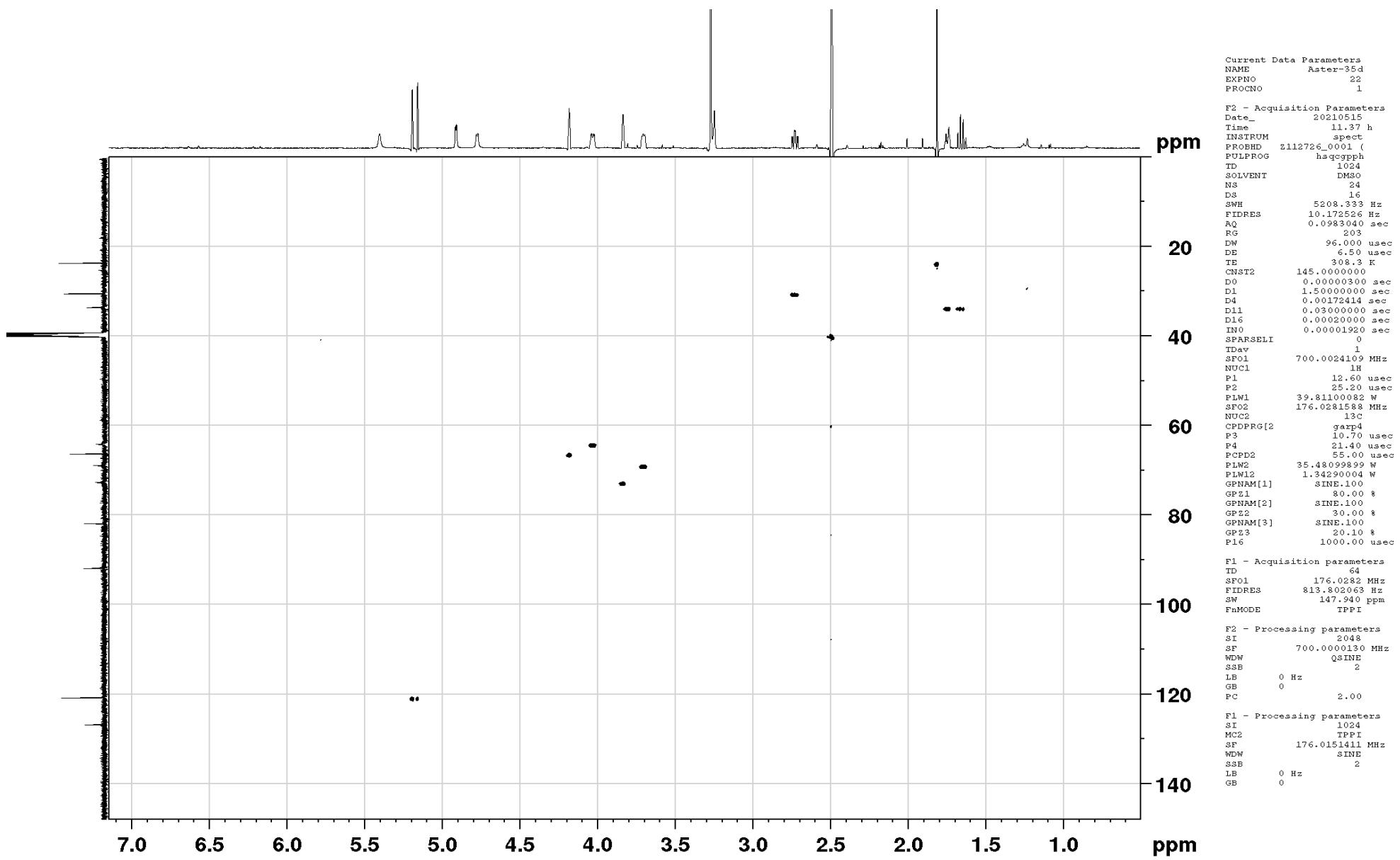


Figure S27. HSQC spectrum (700 MHz, DMSO-d₆) of **2**

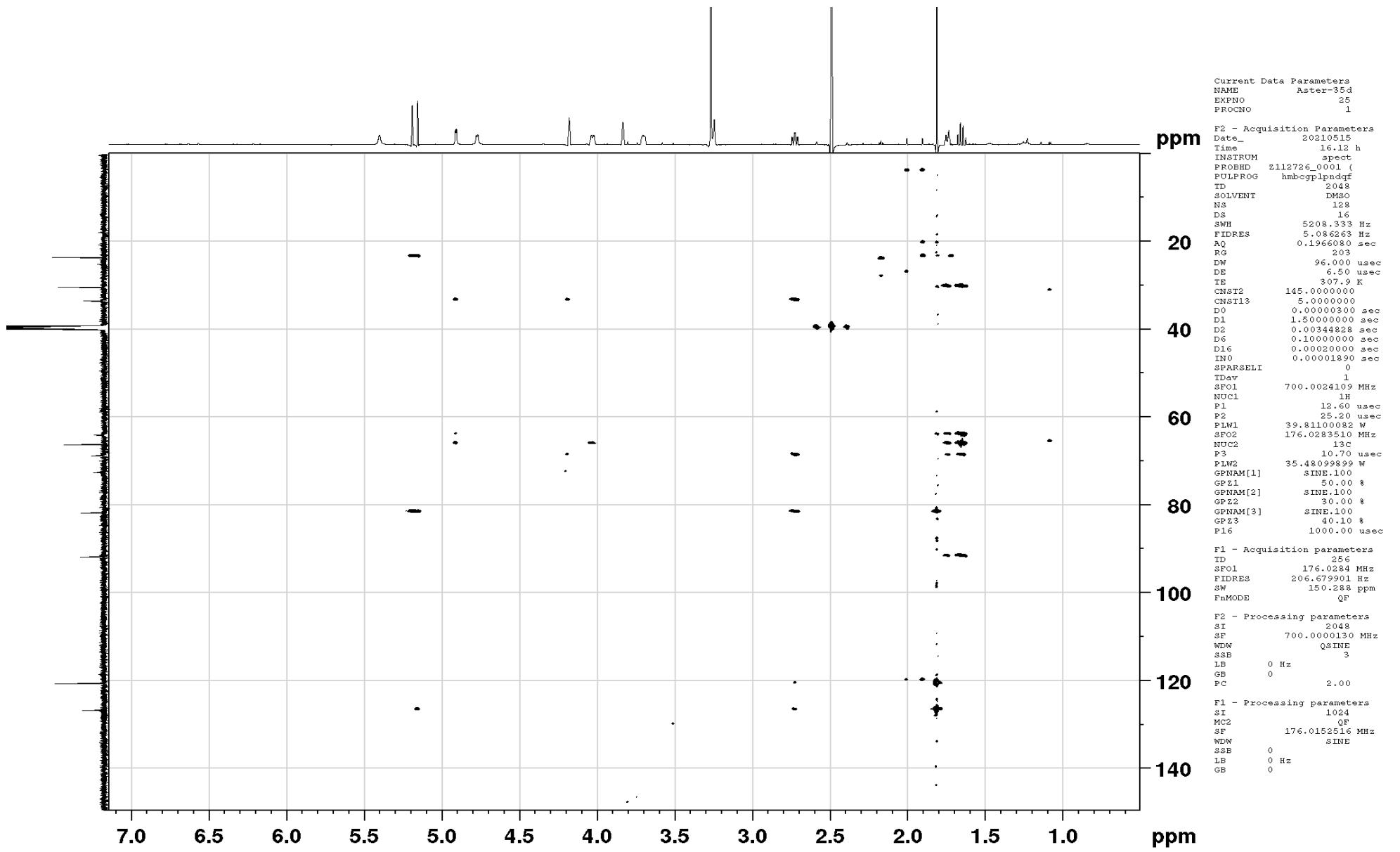


Figure S28. HMBC spectrum (700 MHz, DMSO-d₆) of **2**

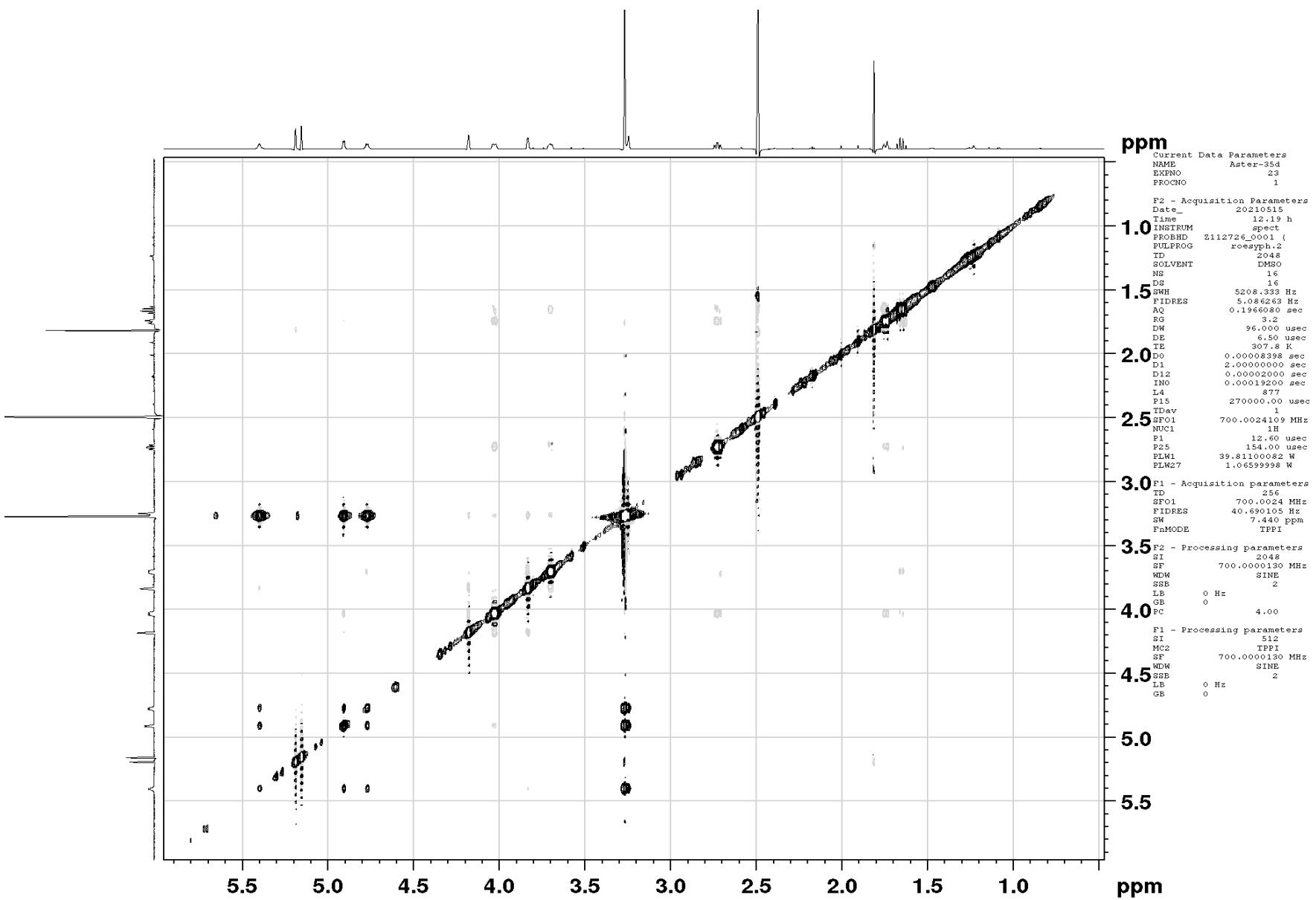


Figure S29. ROESY spectrum (700 MHz, DMSO-d₆) of 2

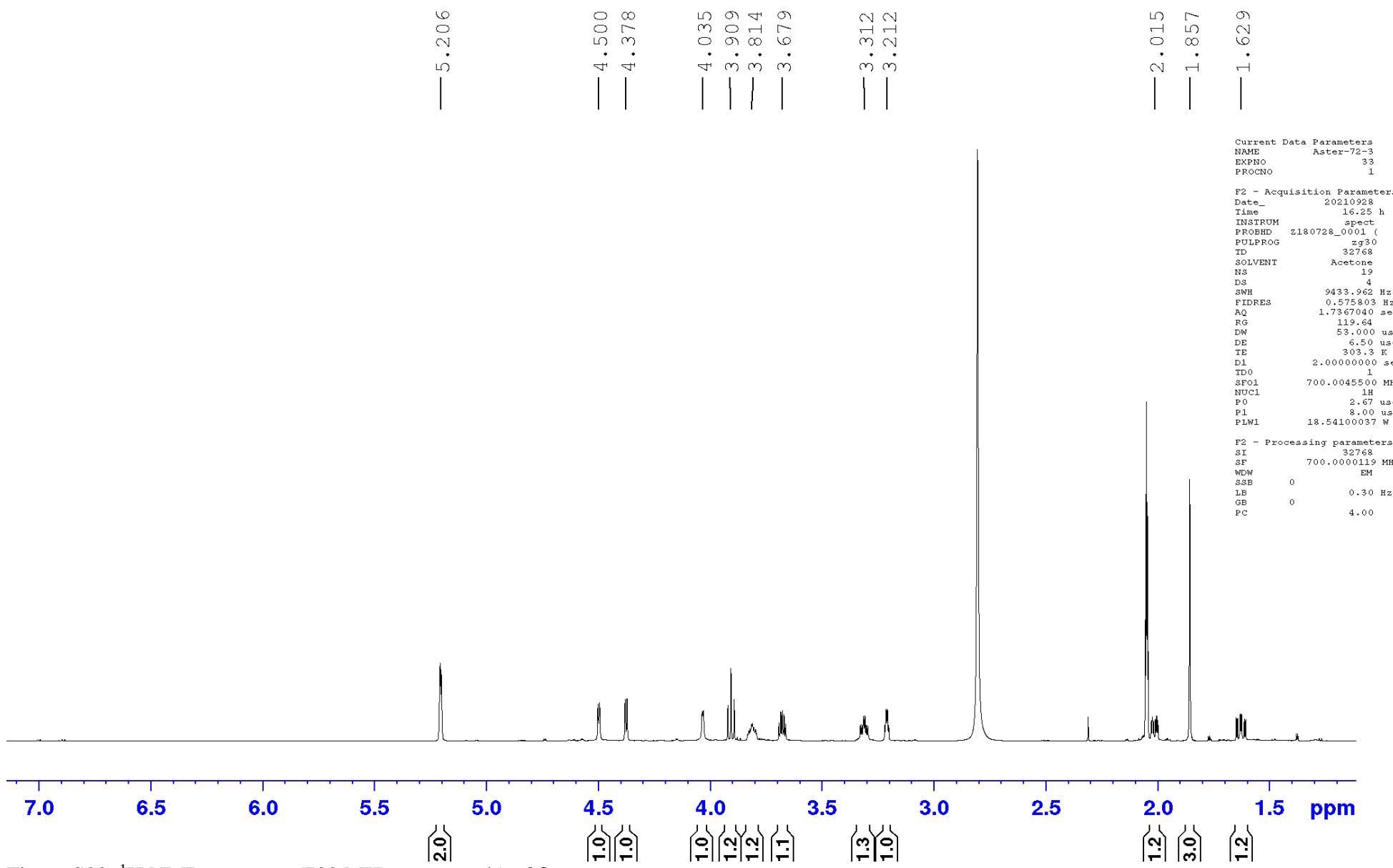


Figure S30. ^1H NMR spectrum (700 MHz, acetone- d_6) of **3**

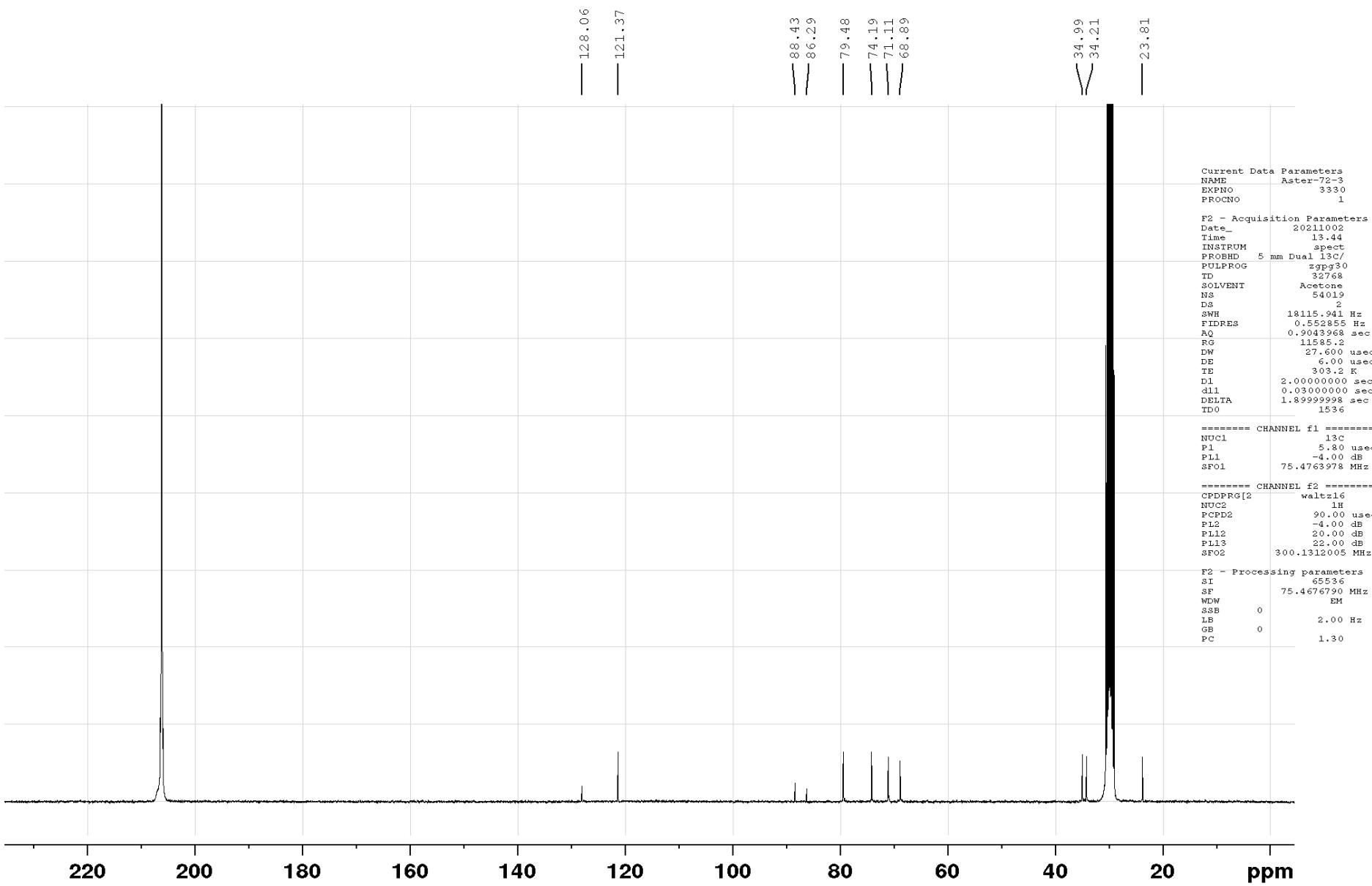


Figure S31. ^{13}C NMR spectrum (75 MHz, acetone- d_6) of **3**

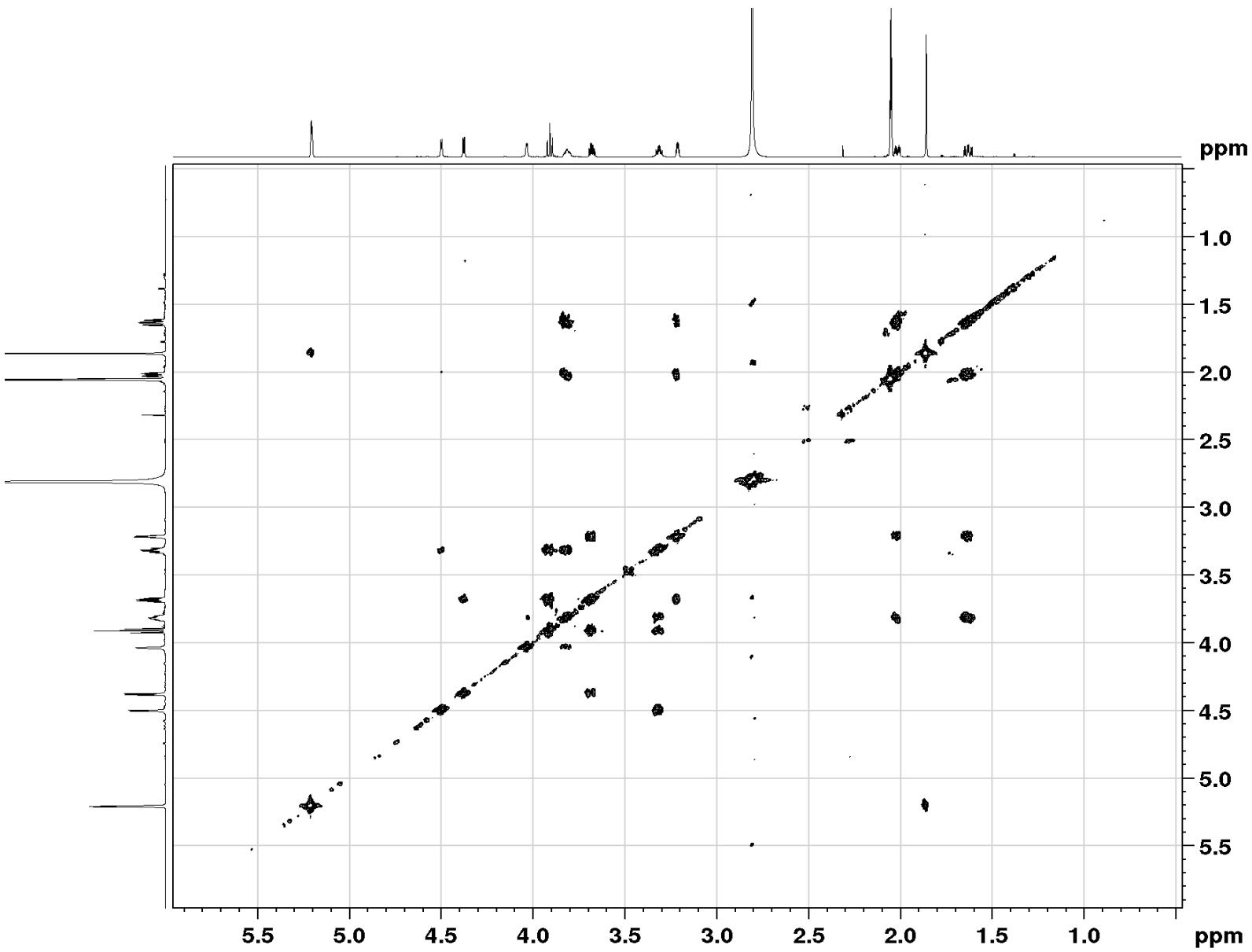


Figure S32. COSY-45 spectrum (700 MHz, acetone-d₆) of 3

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PROCNO    1

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PULPROG  cosyqwf
TD       4096
SOLVENT   Acetone
NS        16
DS        8
SWH      9435.062 Hz
FIDRES   4.60462 Hz
AQ       0.2170880 sec
RG       191.01
DW       53.000 usec
DE       6.50 usec
TE       30.00 K
DO       0.0000000 sec
D1       2.0000000 sec
D13      0.00000400 sec
D16      0.00020000 sec
INO      0.00010600 sec
SFAMSEL1
TD0      1
SFO1     700.0045500 MHz
NUC1     1H
P0       4.00 usec
P1       8.00 usec
P1W1    18.5410000 W
GENRAM[1] SINE,1.00
GPZ1    10.00 %
P1L6    1000.00 usec
P1 - Acquisition parameters
TD      128
SFO1   700.0045 MHz
FIDRES 147.405655 Hz
SW      13.477 ppm
FA MODE QF

E2 - Processing parameters
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SF      700.0000103 MHz
WDW     SINE
SSB      0
LB      0 Hz
GB      0
PC      1.00

P1 - Processing parameters
SI       2048
SF      700.0000104 MHz
WDW     SINE
SSB      0
LB      0 Hz
GB      0

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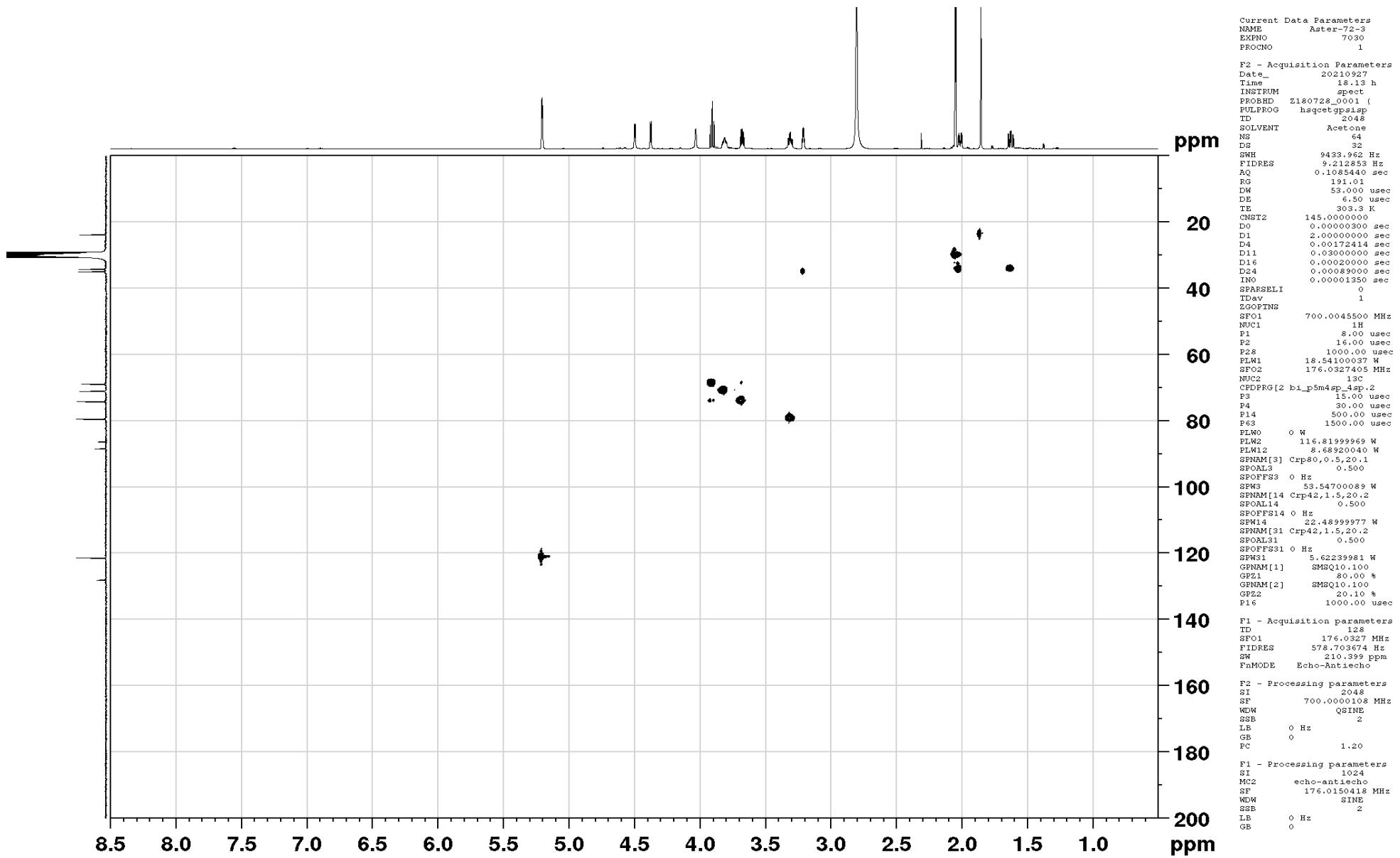


Figure S33. HSQC spectrum (700 MHz, acetone-d₆) of 3

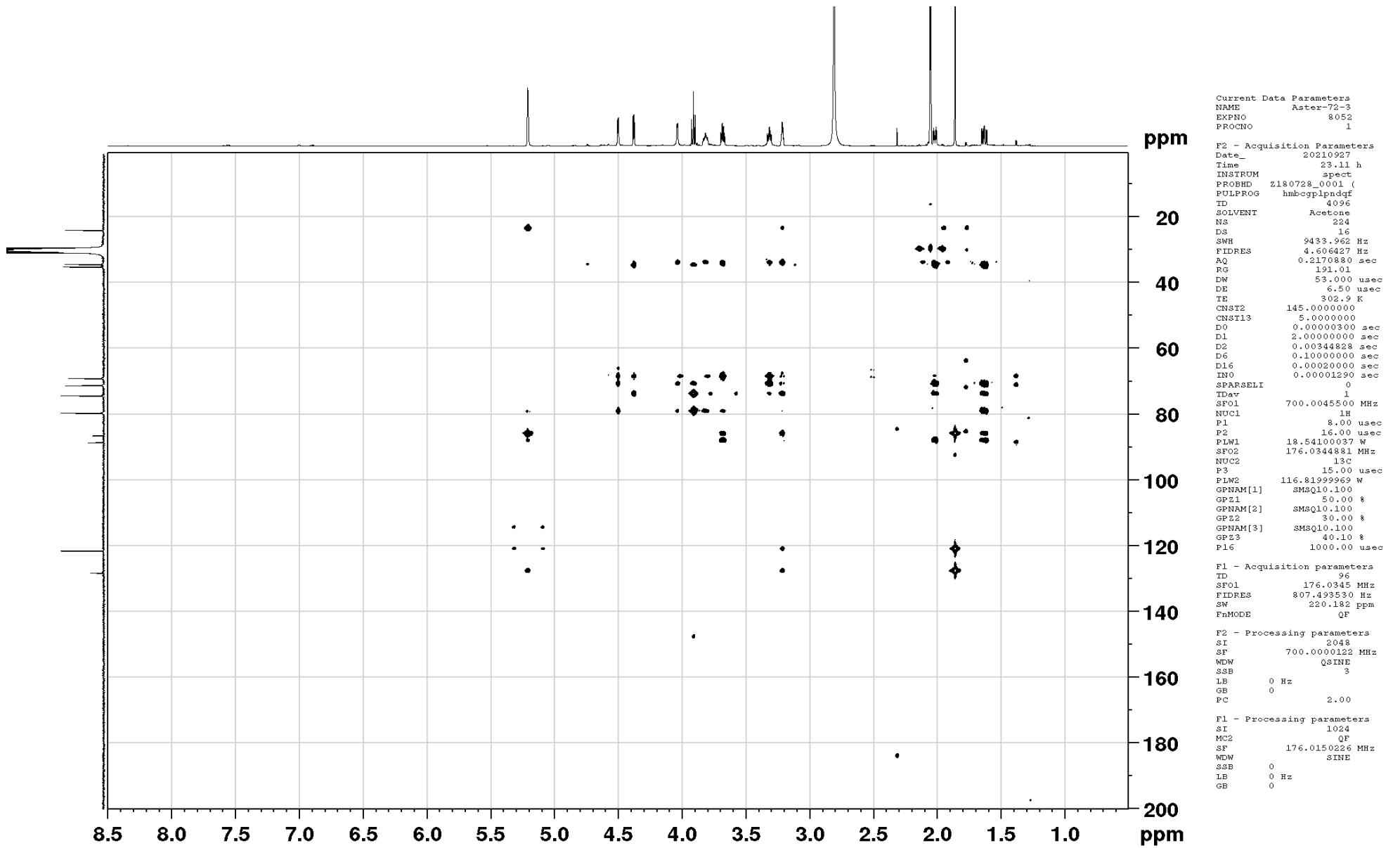


Figure S34. ^1H - ^{13}C HMBC spectrum (700 MHz, acetone- d_6) of 3

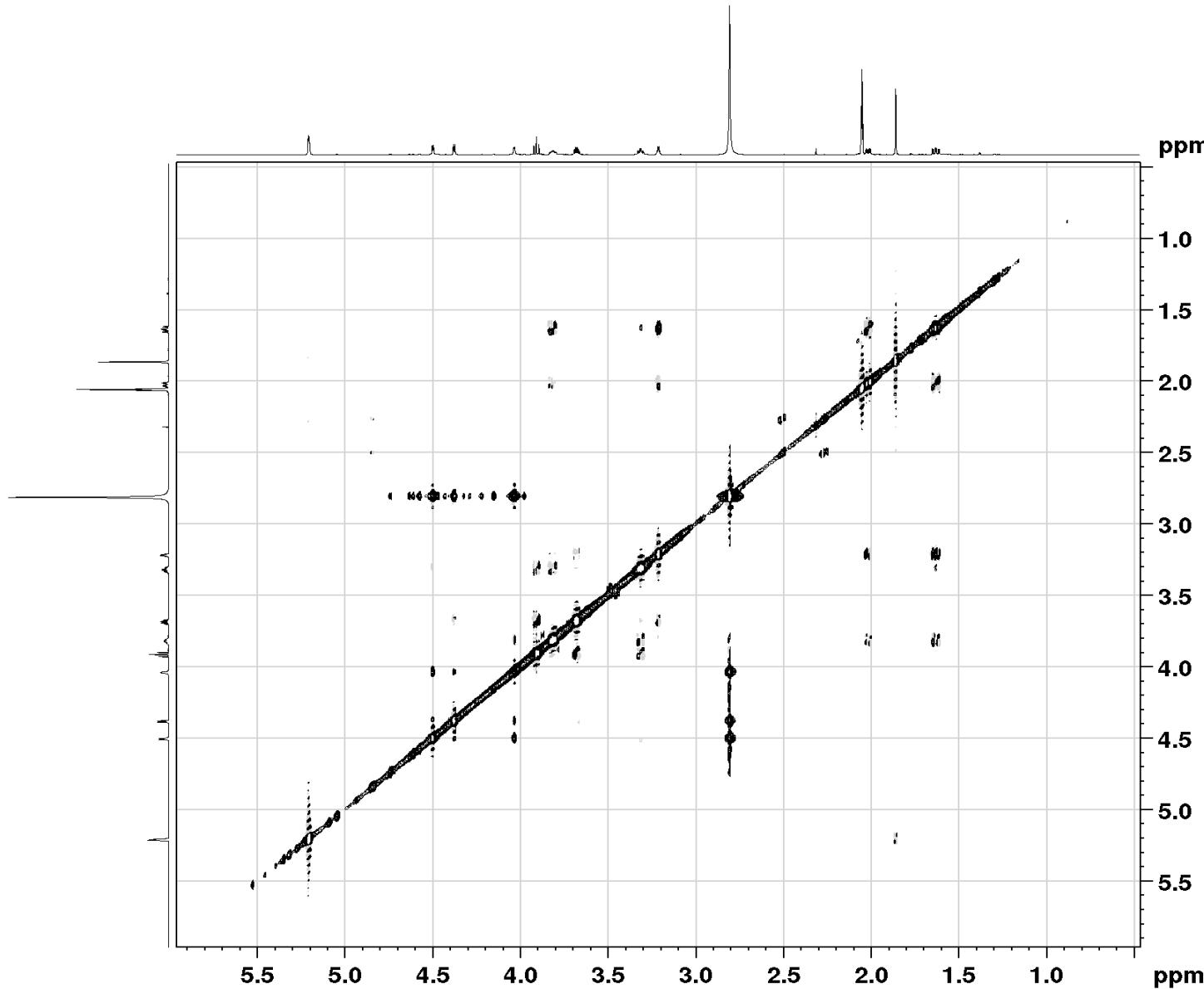


Figure S35. ROESY spectrum (700 MHz, acetone-d₆) of 3

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PROCNO    1

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TD      4096
SOLVENT Acetone
NS      16
DS      16
SWH    9433.962 Hz
FIDRES 4.606427 Hz
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RG      2
DW     53.000 usec
DE     5.50 usec
TE     302.7 K
D0     0.00004391 sec
D1     2.79999995 sec
D12    0.00002000 sec
D10    0.000010600 sec
L4      877
P15    270000.00 usec
TDav   1
SF01  700.0045500 MHz
NUC1   1H
P1     8.00 usec
P25    154.00 usec
PLW1  18.54100037 W
PLW27  0.20014000 W

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FIDRES 36.851414 Hz
SW     13.477 ppm
FnMODE TPPI

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PC     4.00

F1 - Processing parameters
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SF     700.0000102 MHz
WDW   SINE
SSB      2
LB      0 Hz
GB      0

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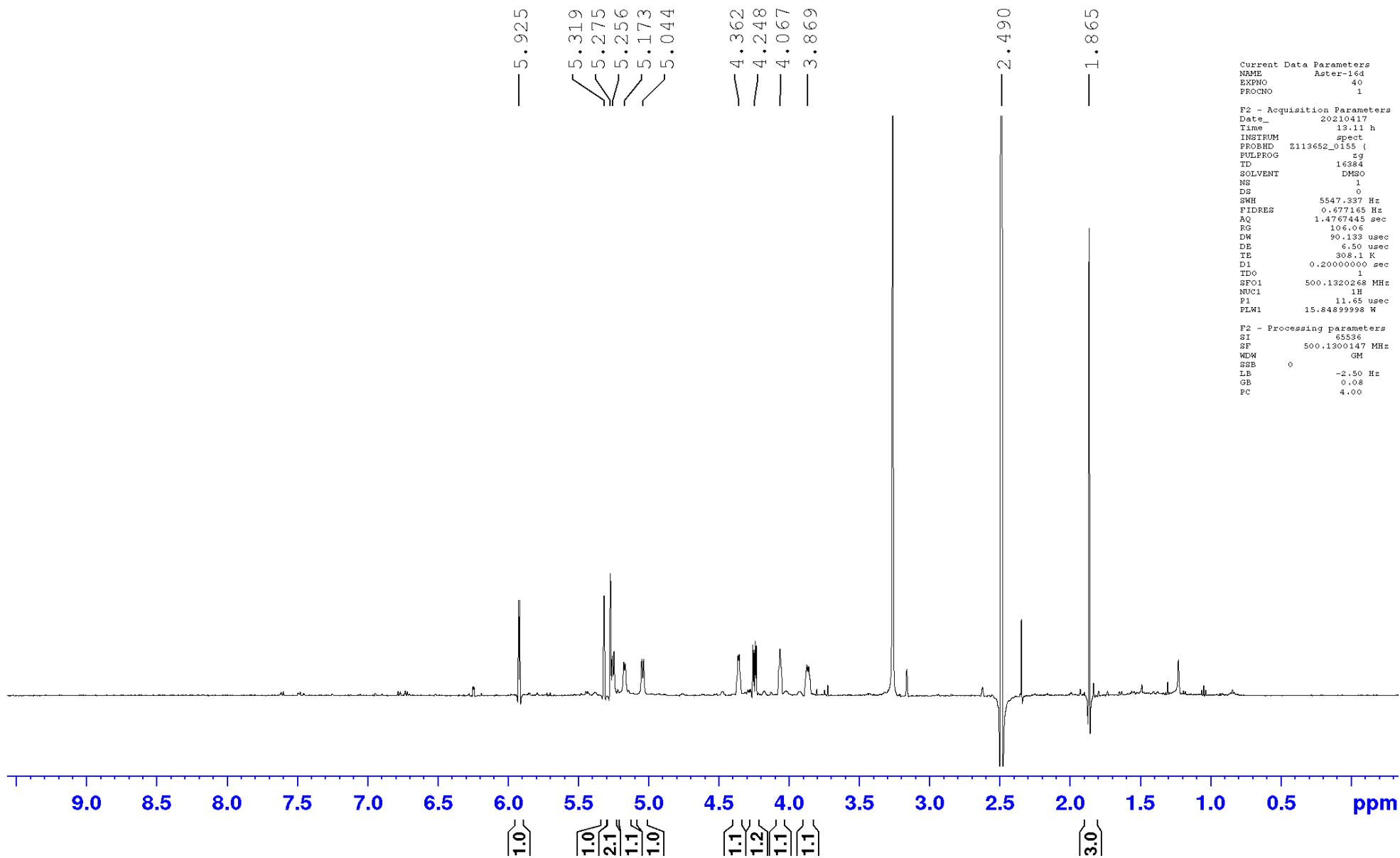


Figure S36. ^1H NMR spectrum (500 MHz, DMSO-d_6) of 4

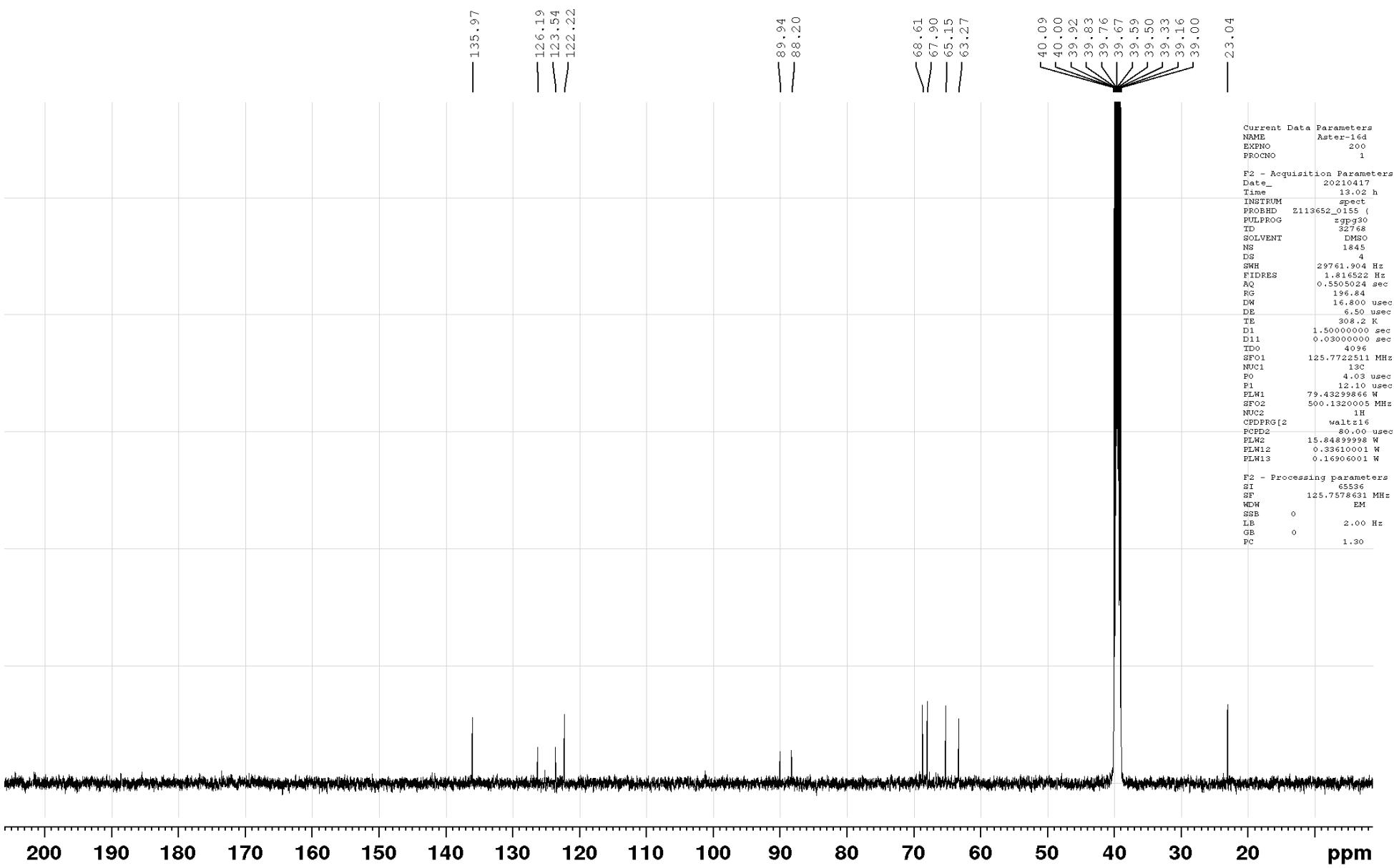


Figure S37. ^{13}C NMR spectrum (125 MHz, DMSO- d_6) of 4

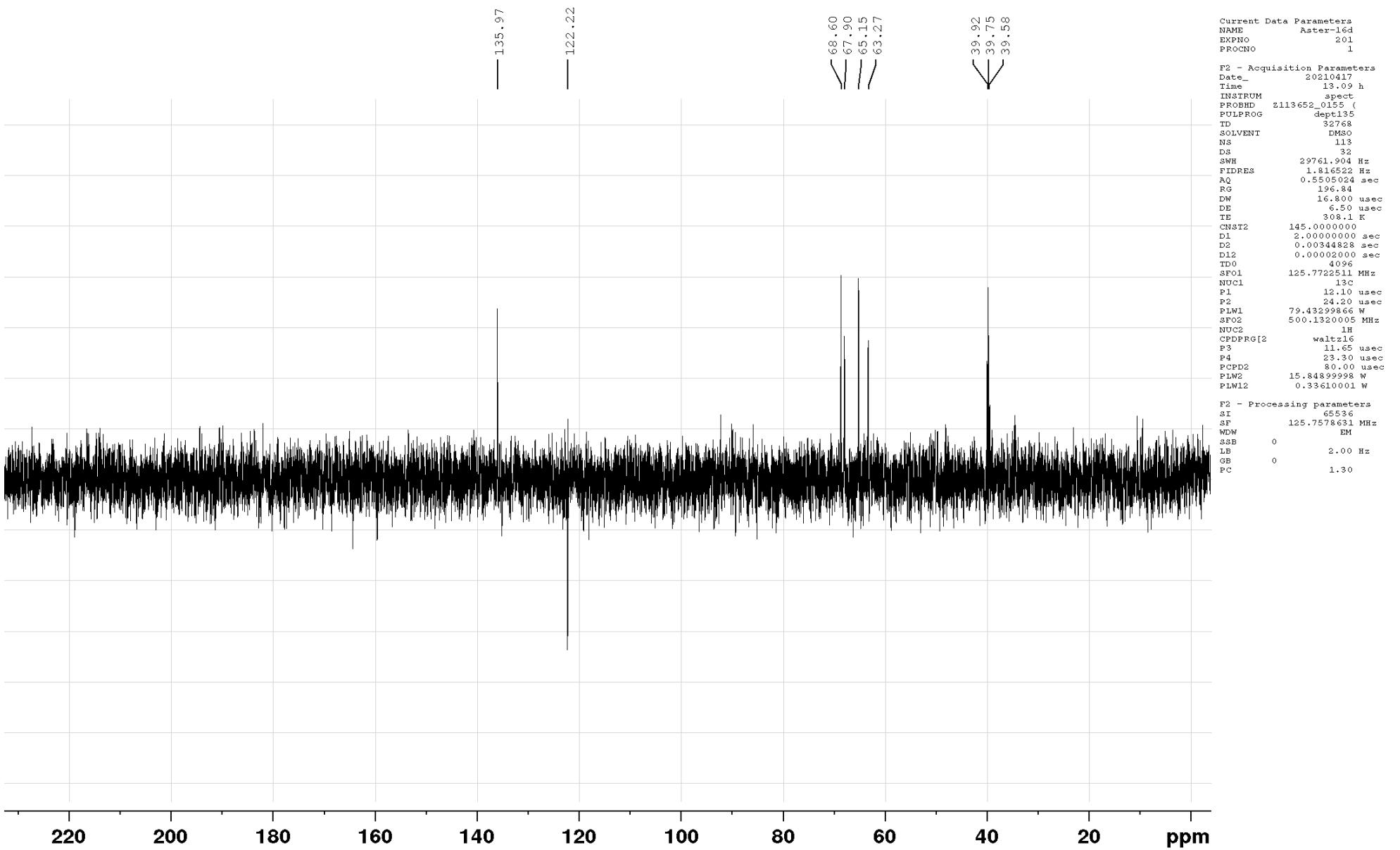


Figure S38. DEPT-135 NMR spectrum (125 MHz, DMSO- d_6) of 4

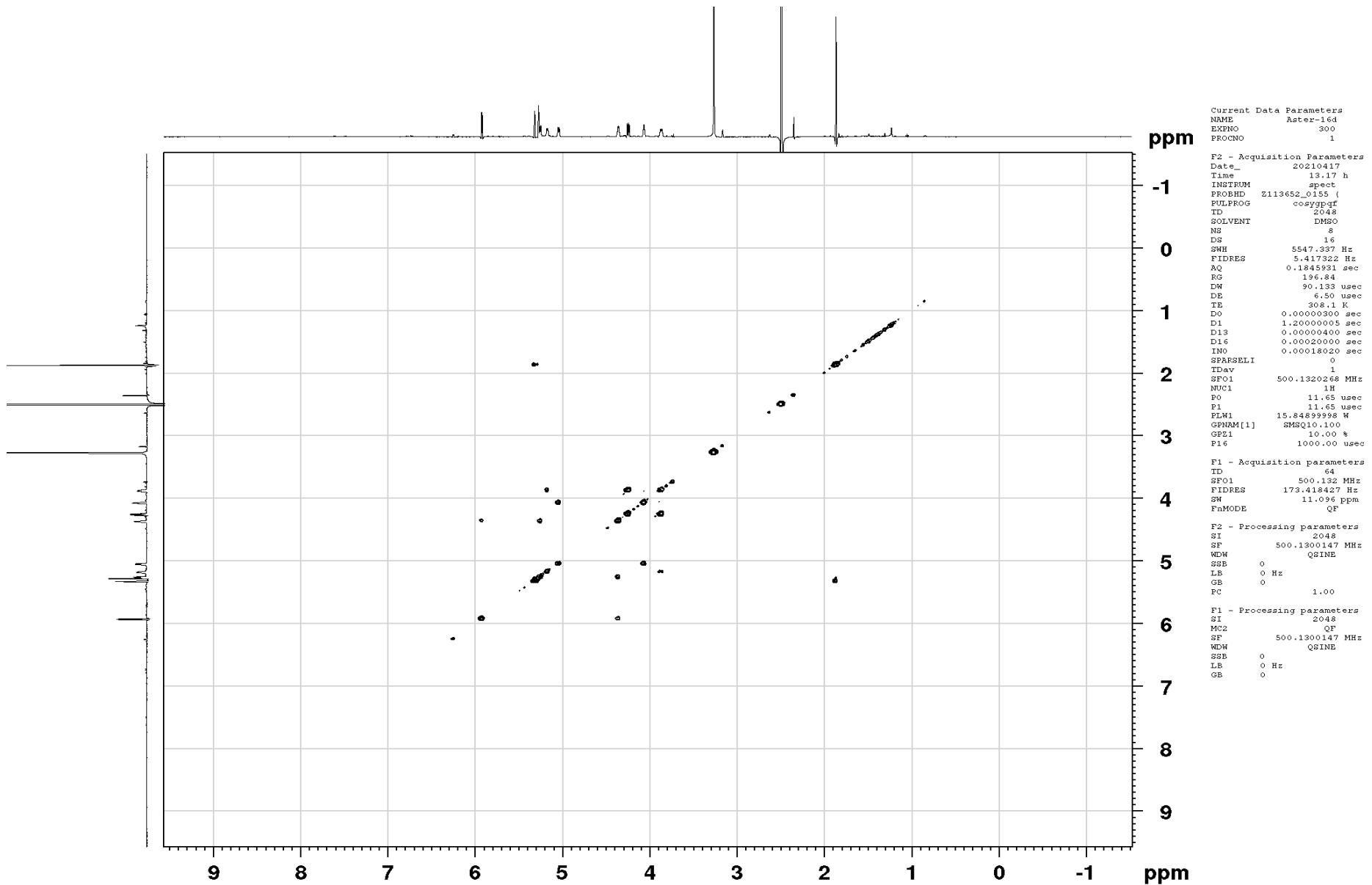


Figure S39. COSY-45 spectrum (500 MHz, DMSO-d₆) of 4

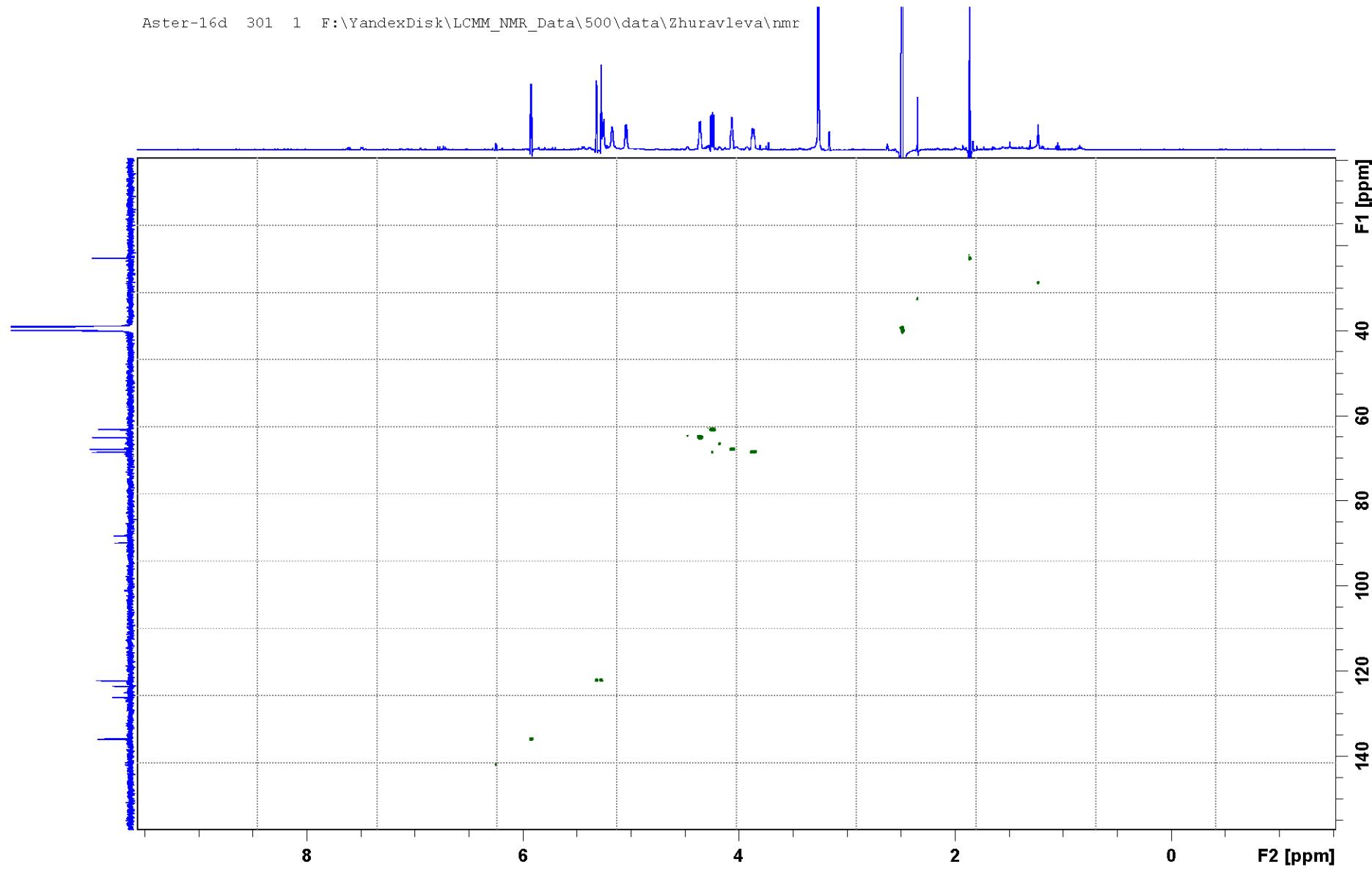


Figure S40. HSQC spectrum (500 MHz, DMSO-d_6) of **4**

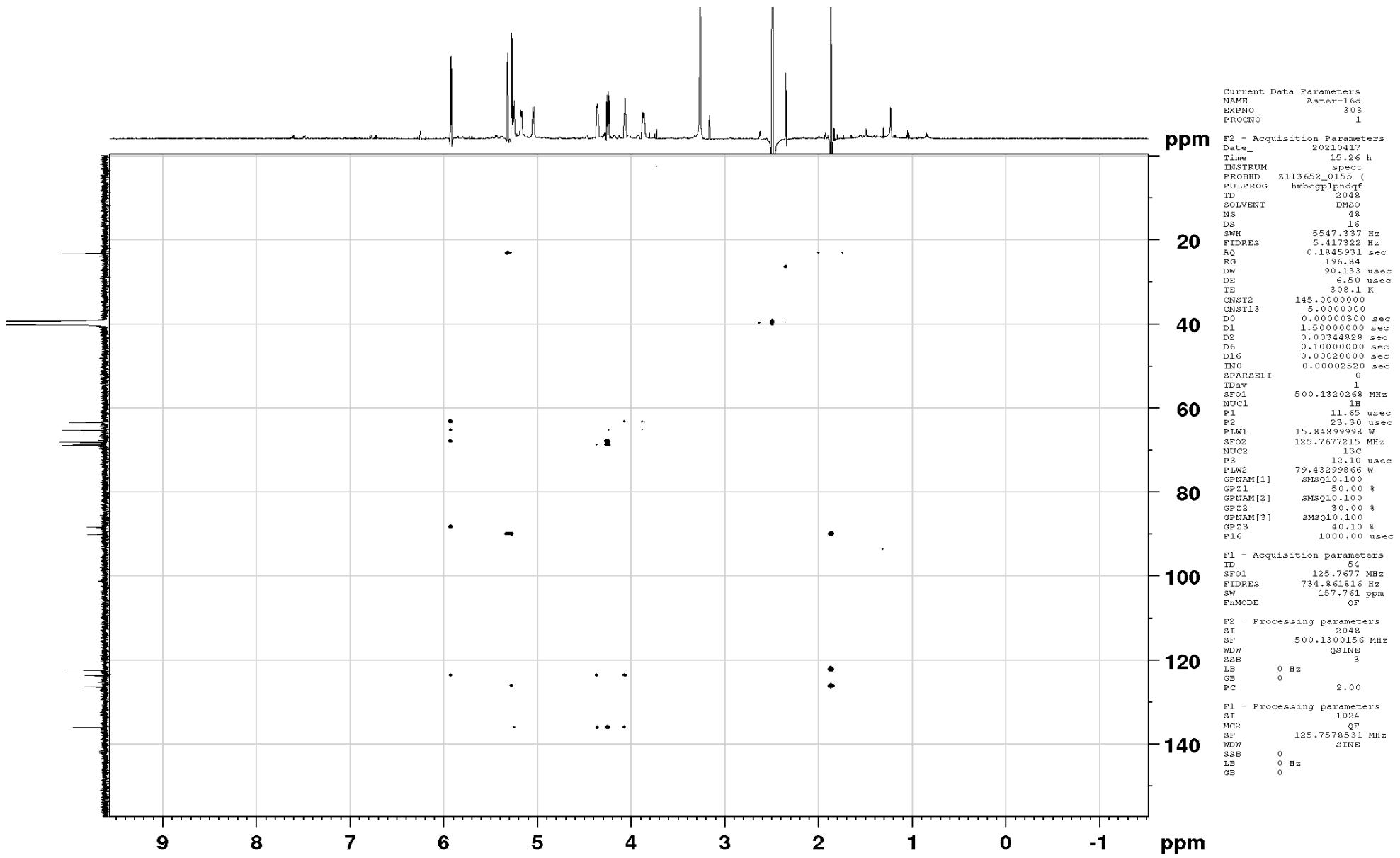


Figure S41. HMBC spectrum (500 MHz, DMSO-d₆) of **4**

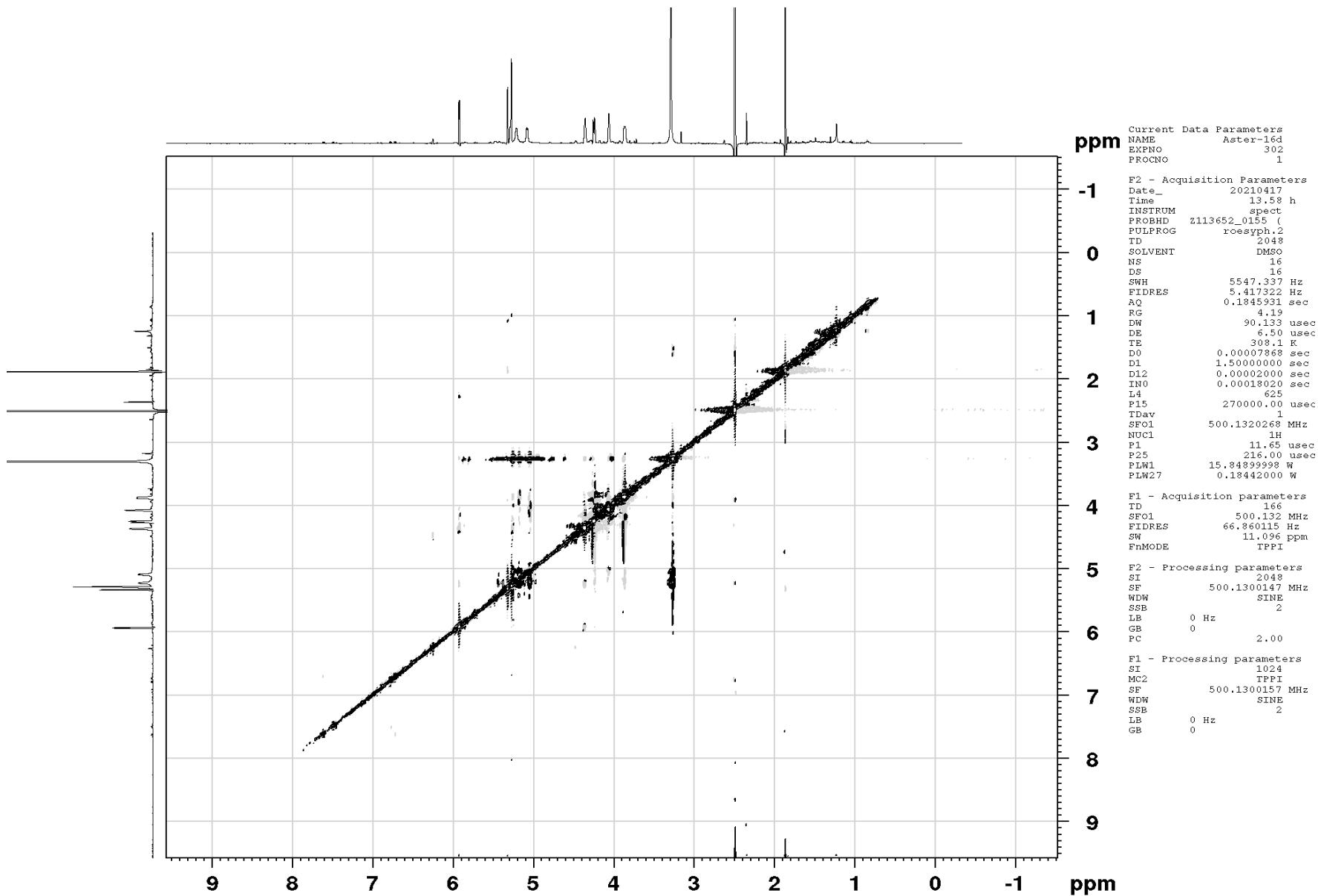


Figure S42. ROESY spectrum (500 MHz, DMSO- d_6) of **4**

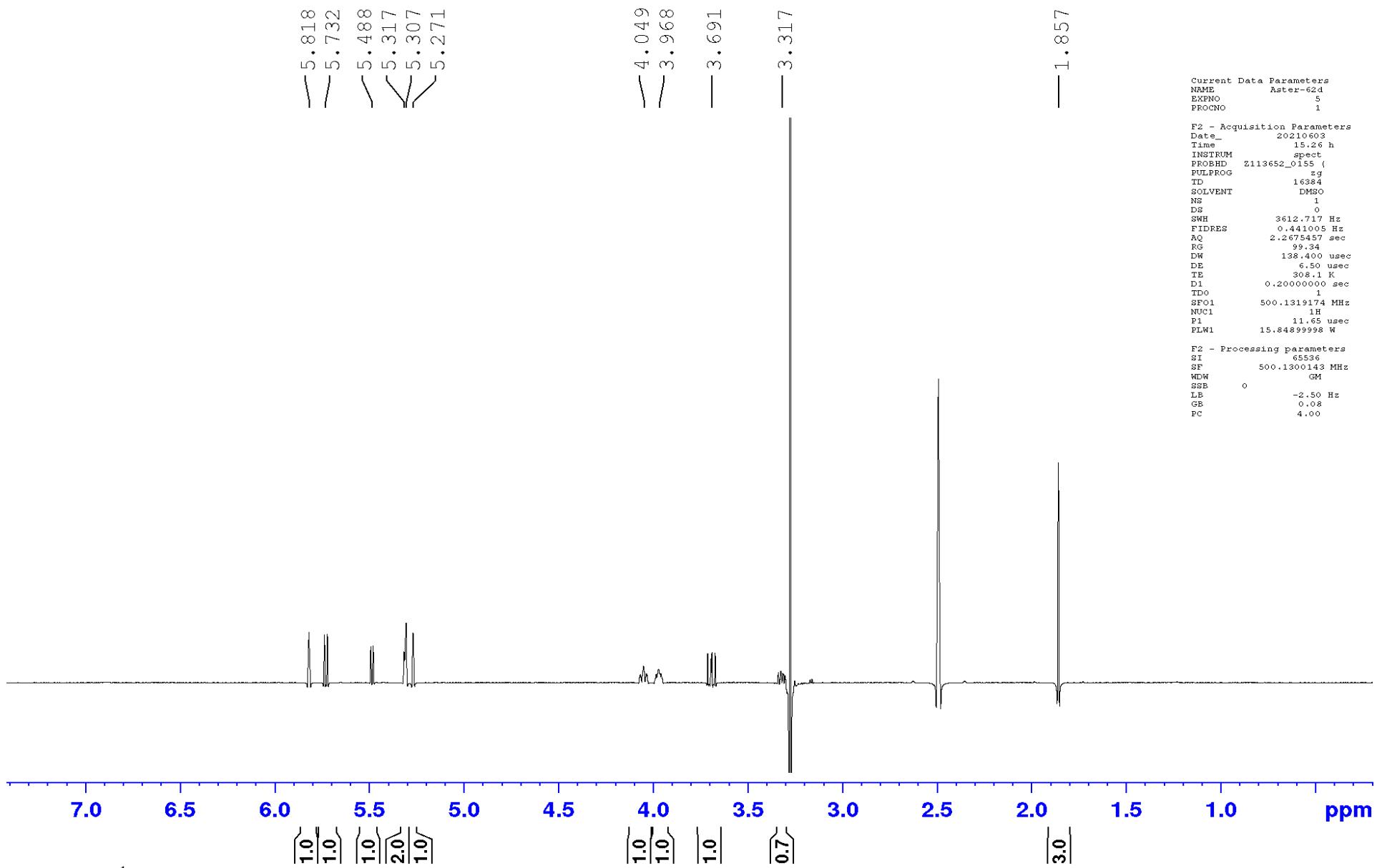


Figure S43. ^1H NMR spectrum (500 MHz, DMSO-d_6) of **5**

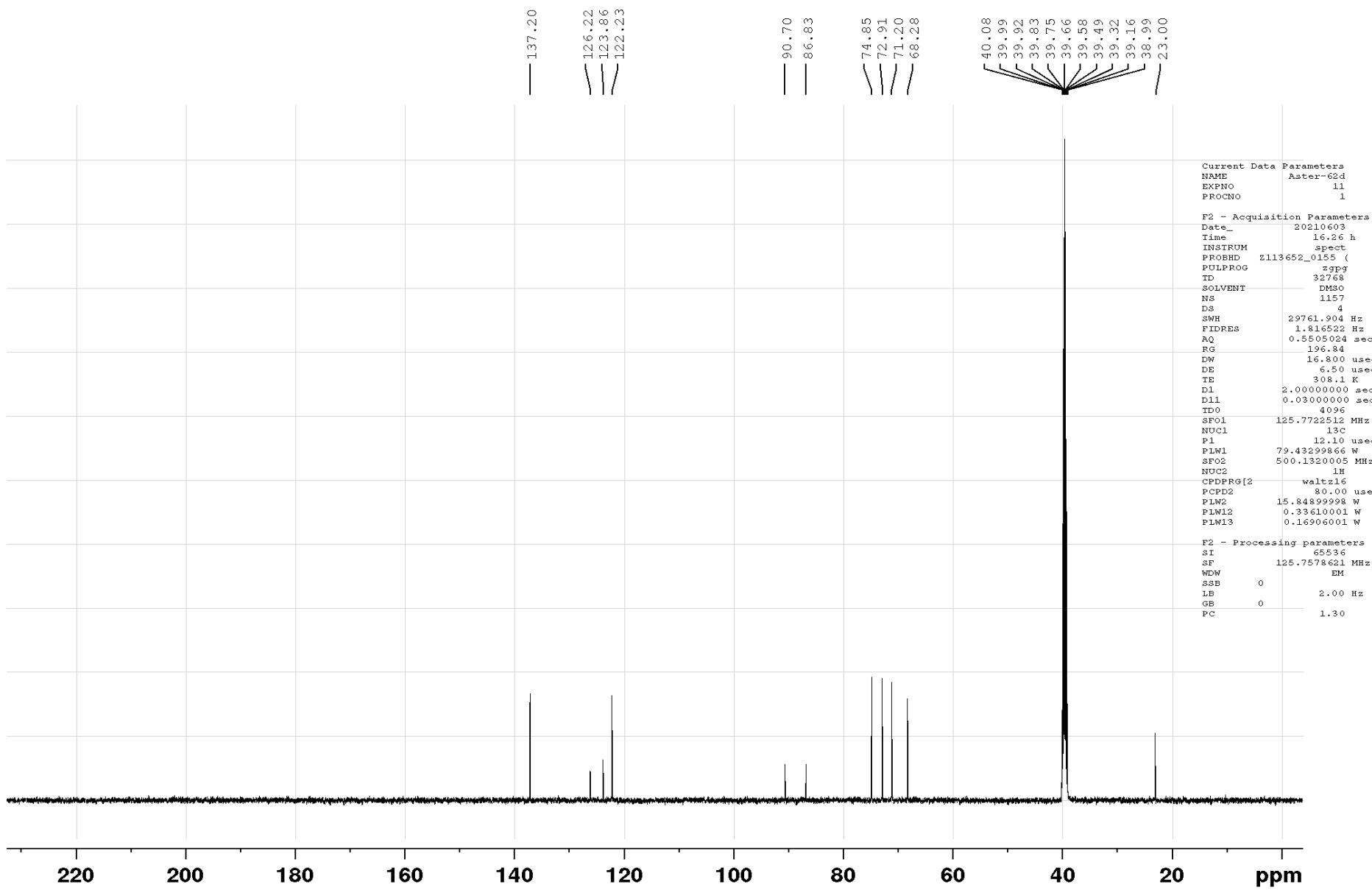


Figure S44. ^{13}C NMR spectrum (125 MHz, DMSO- d_6) of **5**

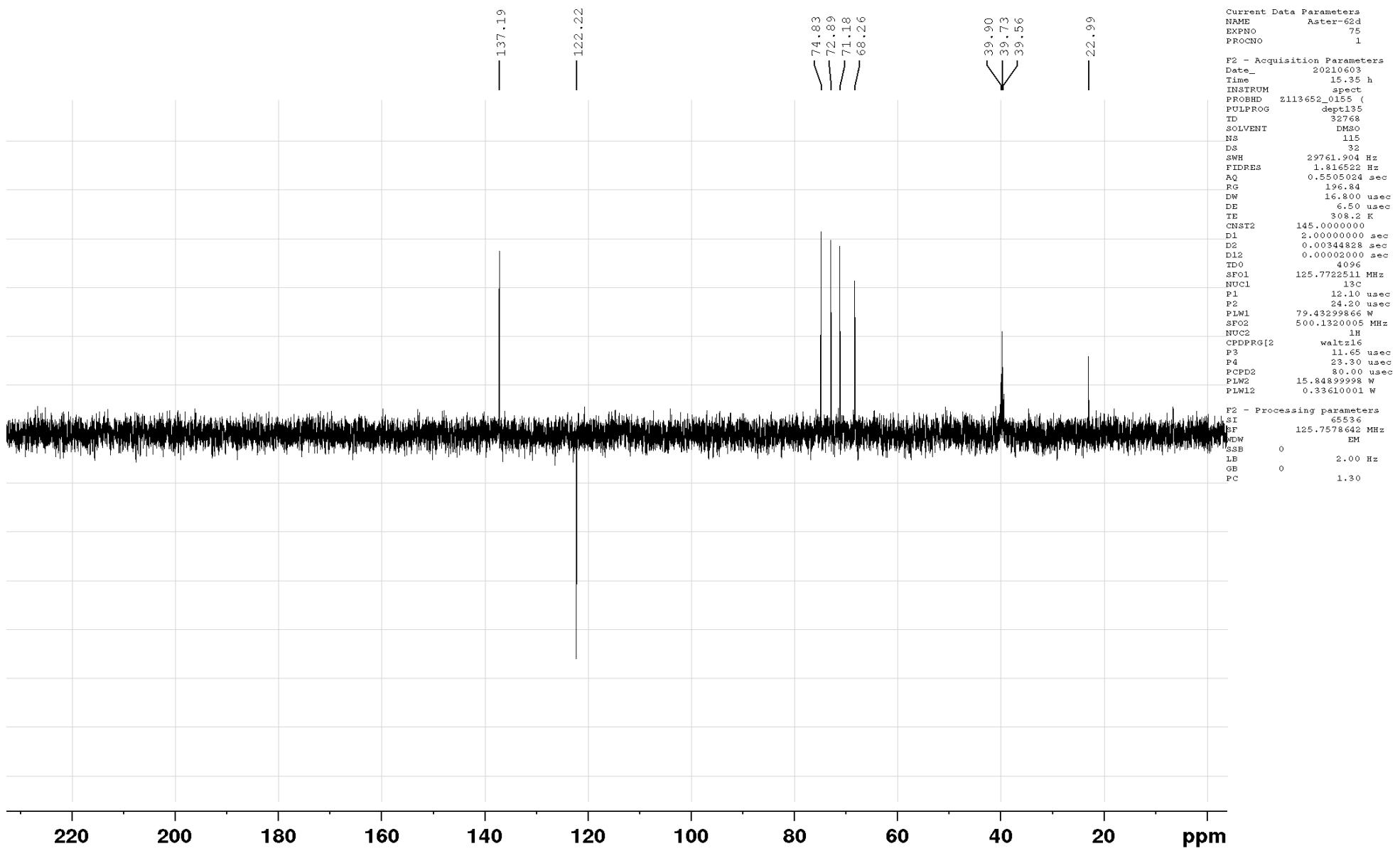


Figure S45. DEPT-135 NMR spectrum (125 MHz, DMSO-d₆) of **5**

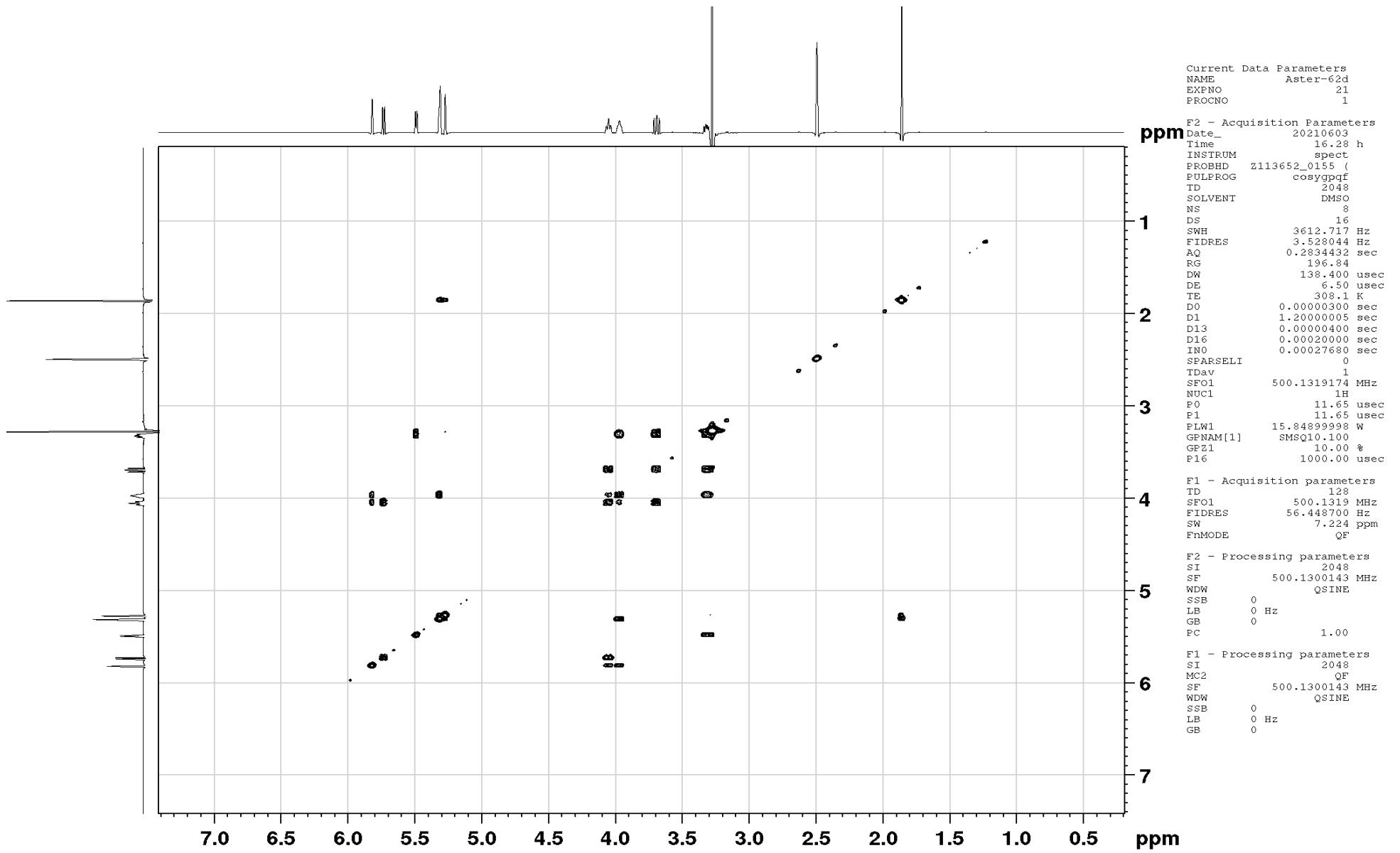


Figure S46. COSY-45 spectrum (500 MHz, DMSO-d₆) of **5**

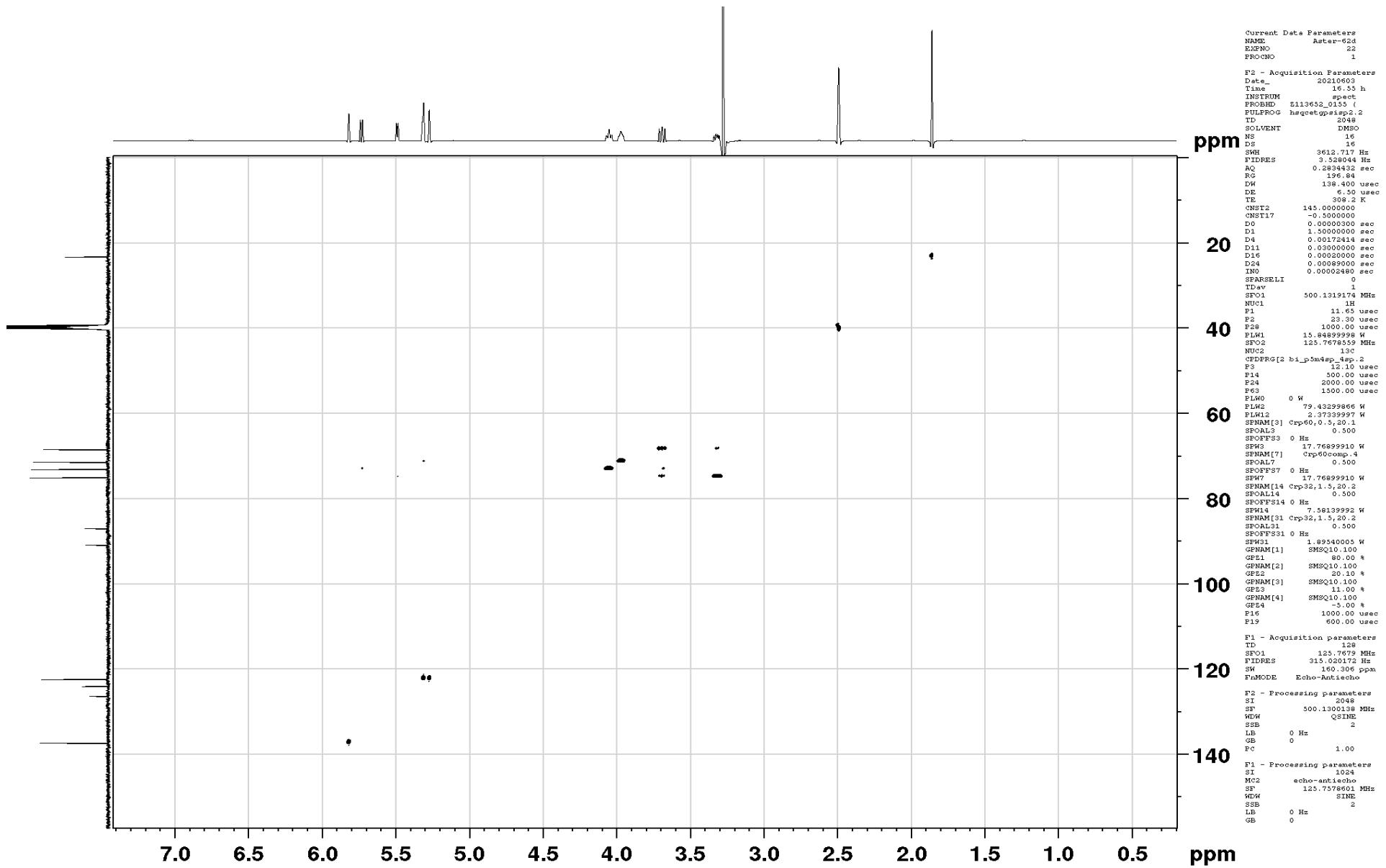


Figure S47. HSQC spectrum (500 MHz, DMSO-d₆) of **5**

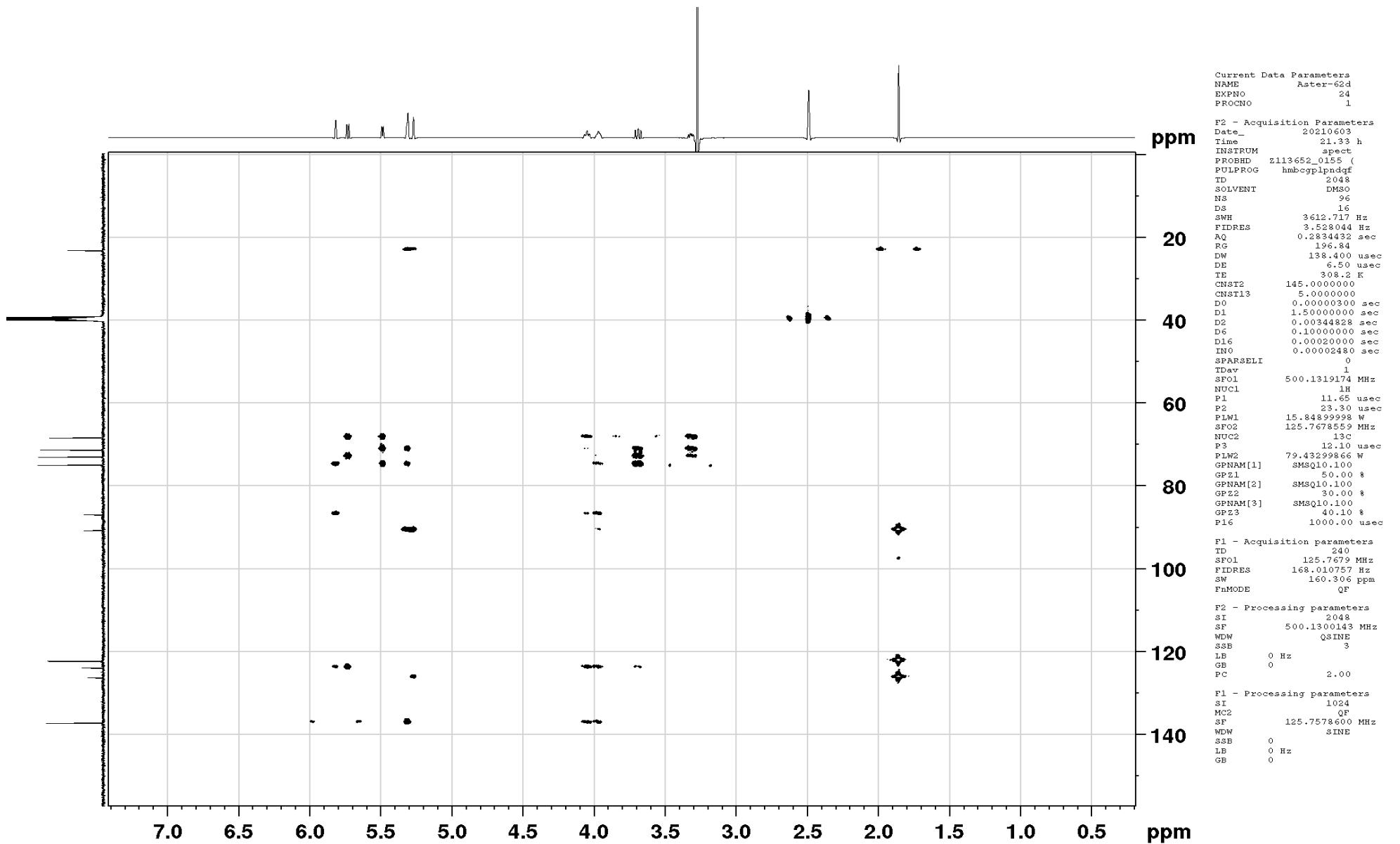


Figure S48. HMBC spectrum (500 MHz, DMSO- d_6) of **5**

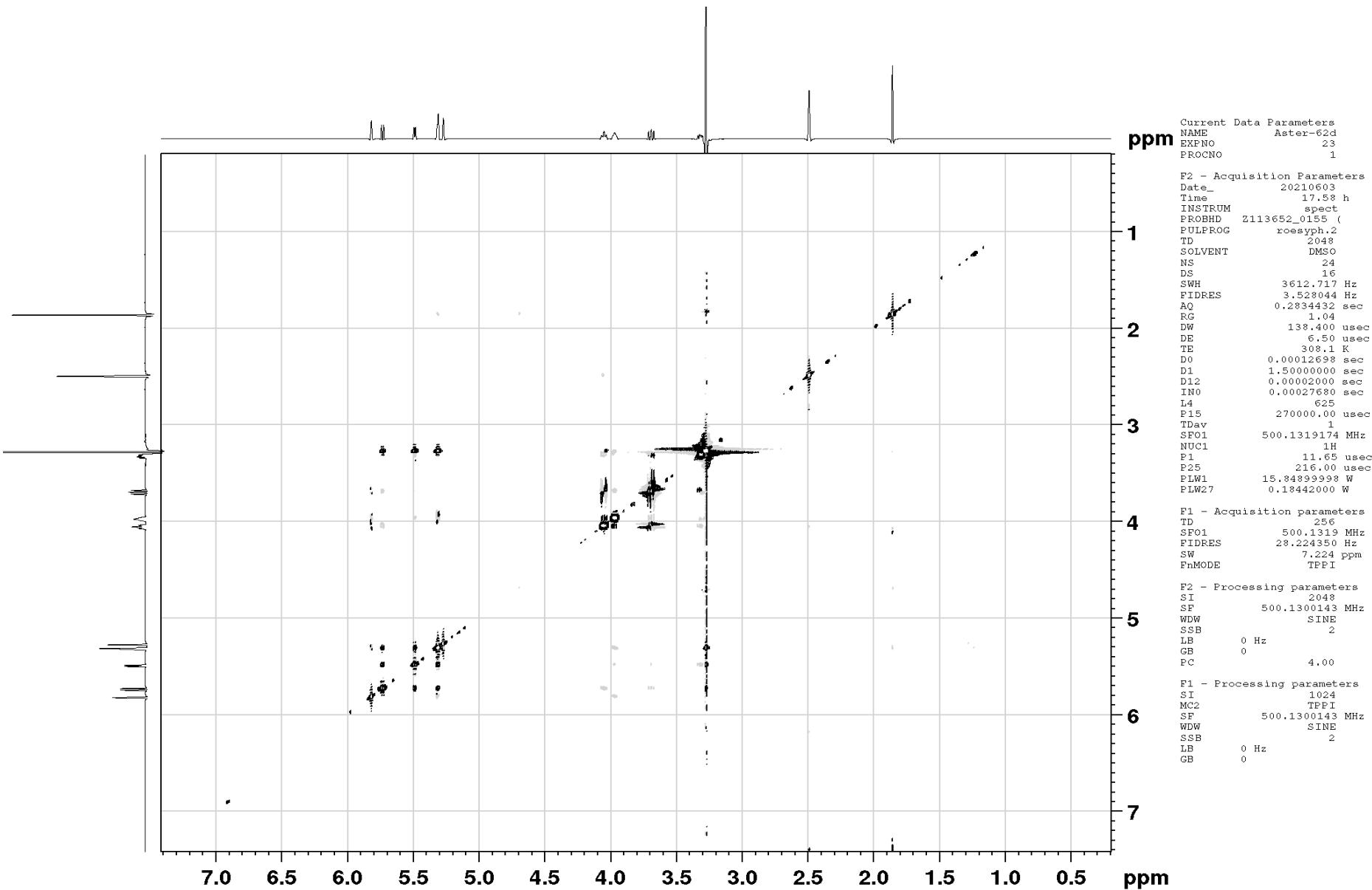


Figure S49. ROESY spectrum (500 MHz, DMSO-d₆) of **5**

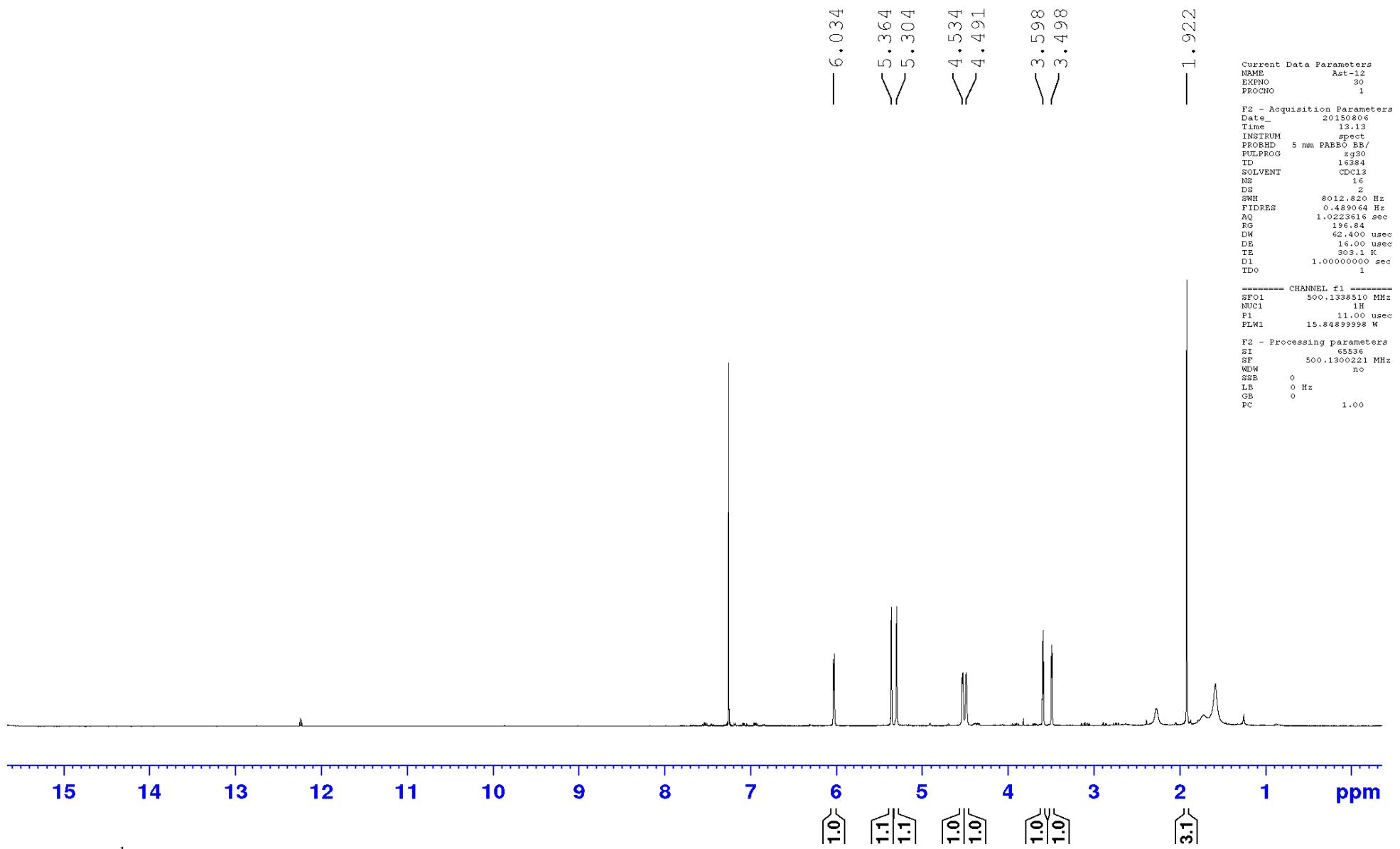


Figure S50. ^1H NMR spectrum (500 MHz, CDCl_3) of **6**

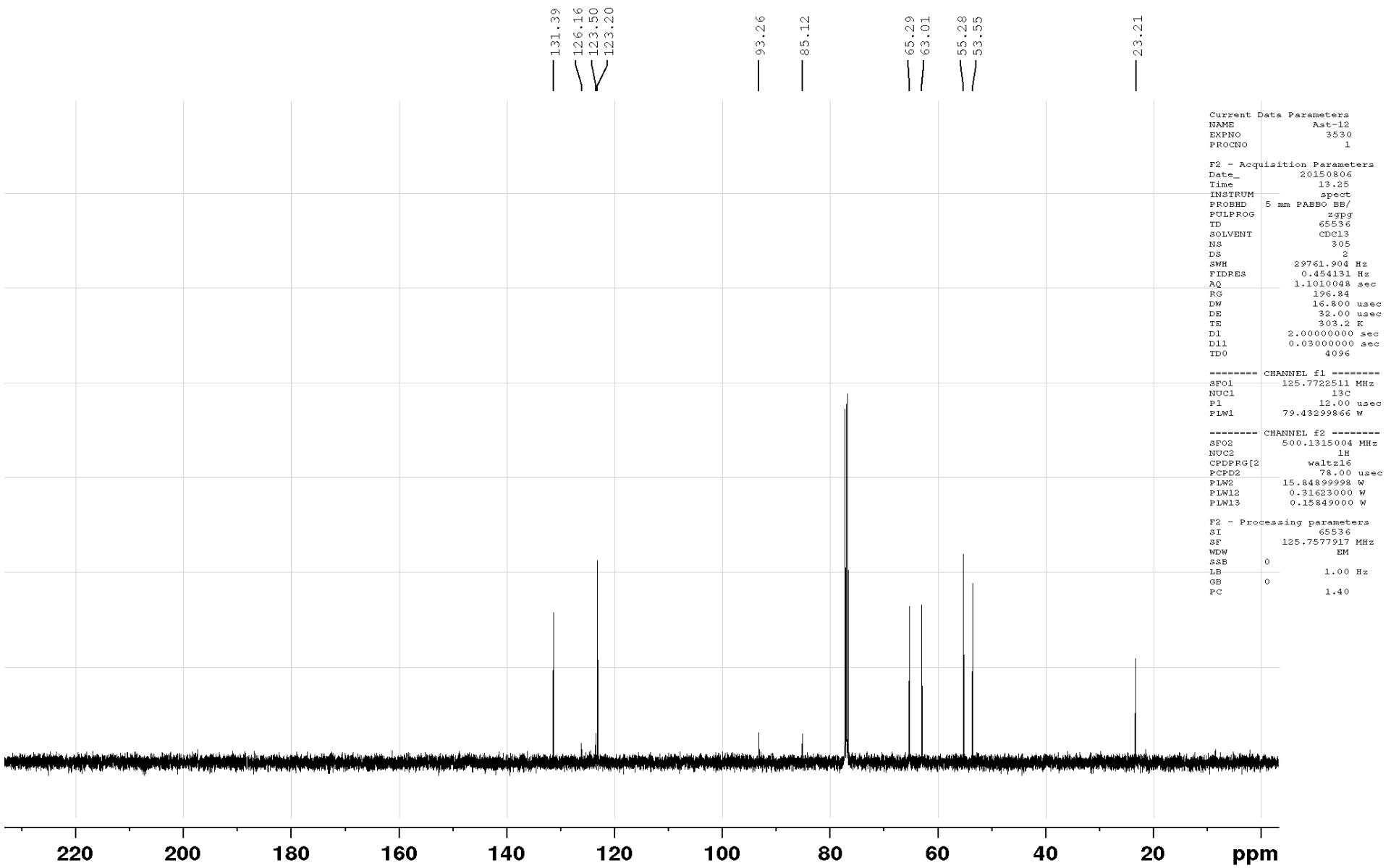


Figure S51. ¹³C NMR spectrum (125 MHz, CDCl₃) of **6**

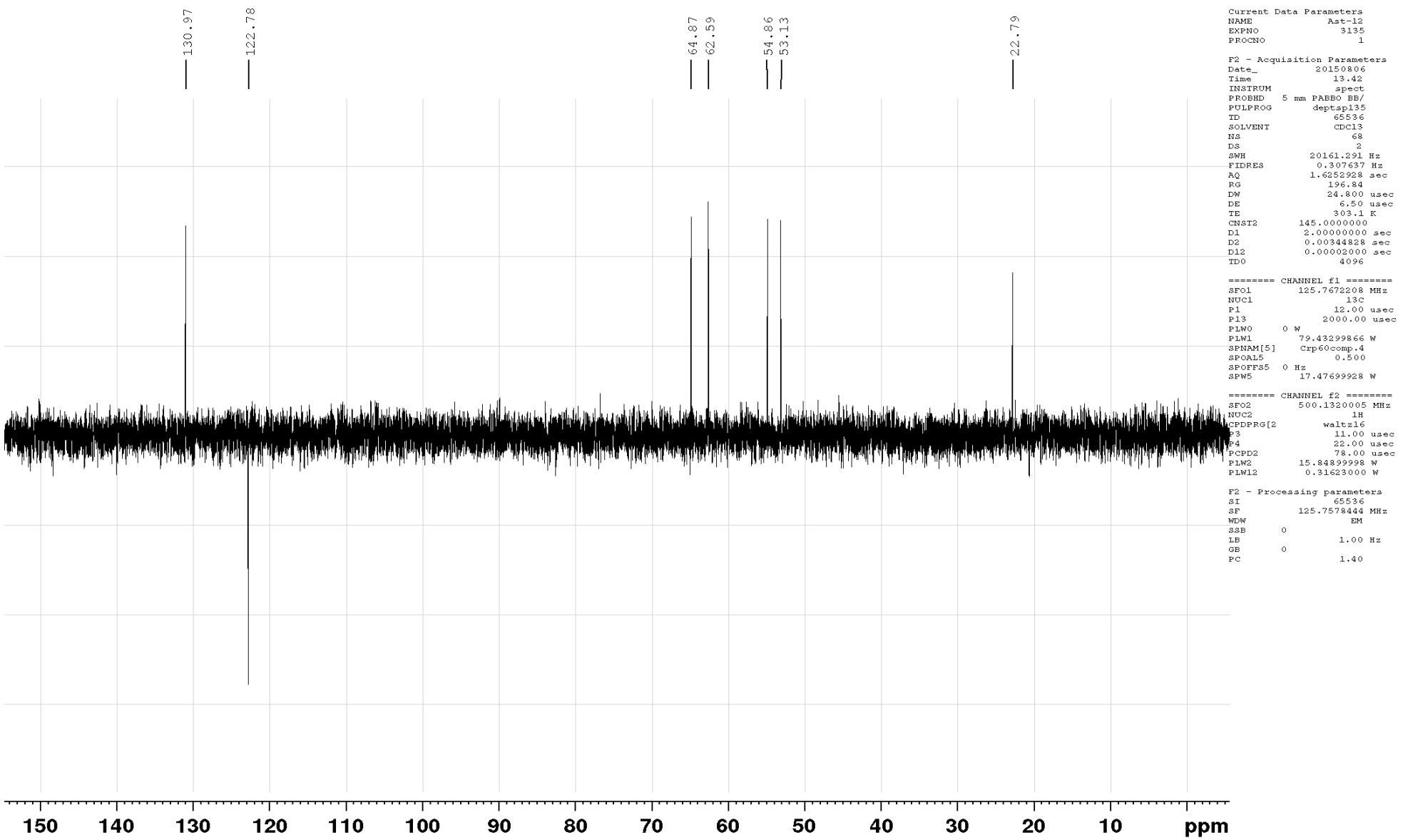


Figure S52. DEPT-135 NMR spectrum (125MHz, CDCl₃) of **6**

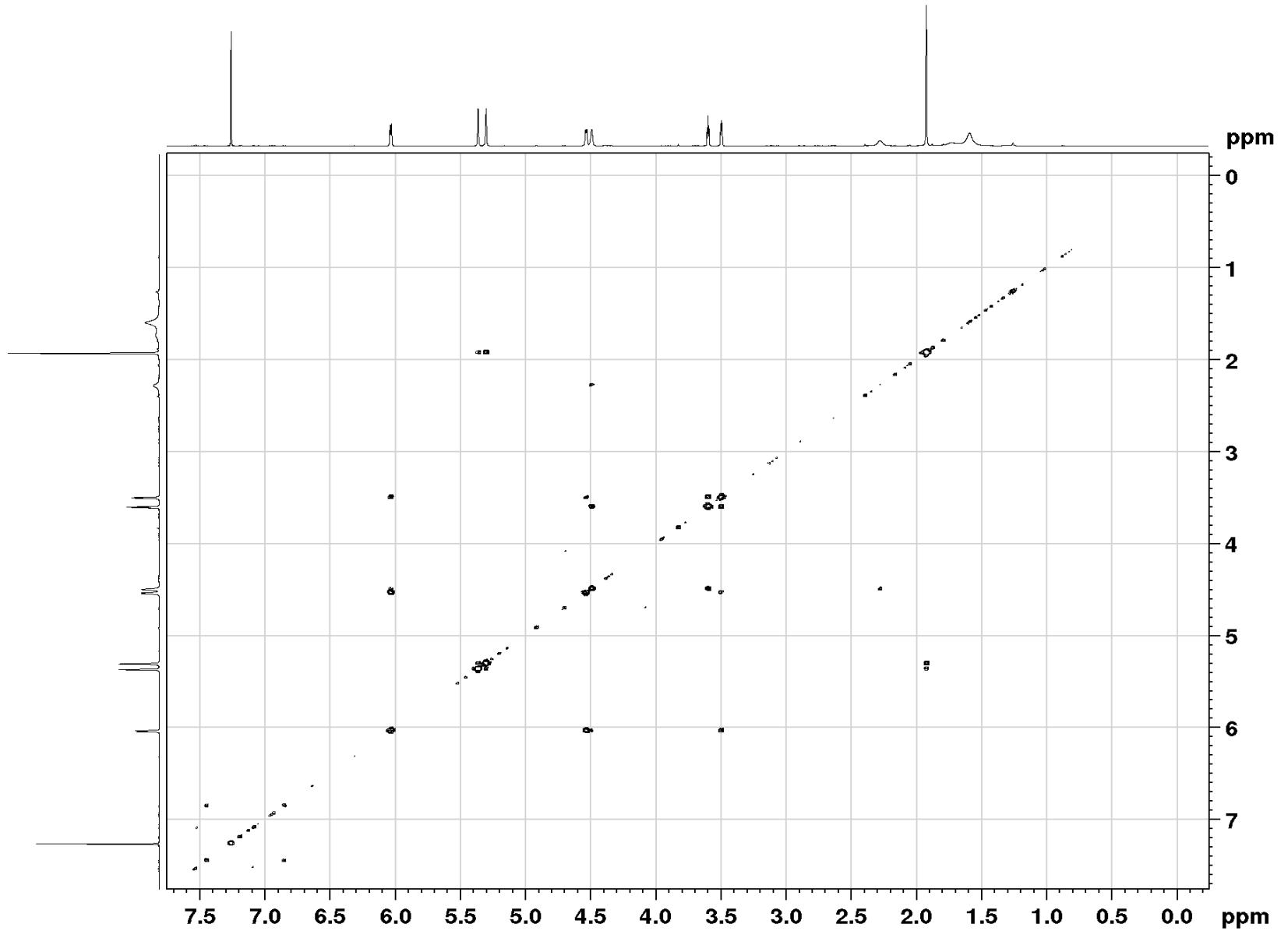


Figure S53. COSY-45 spectrum (500 MHz, CDCl_3) of **6**

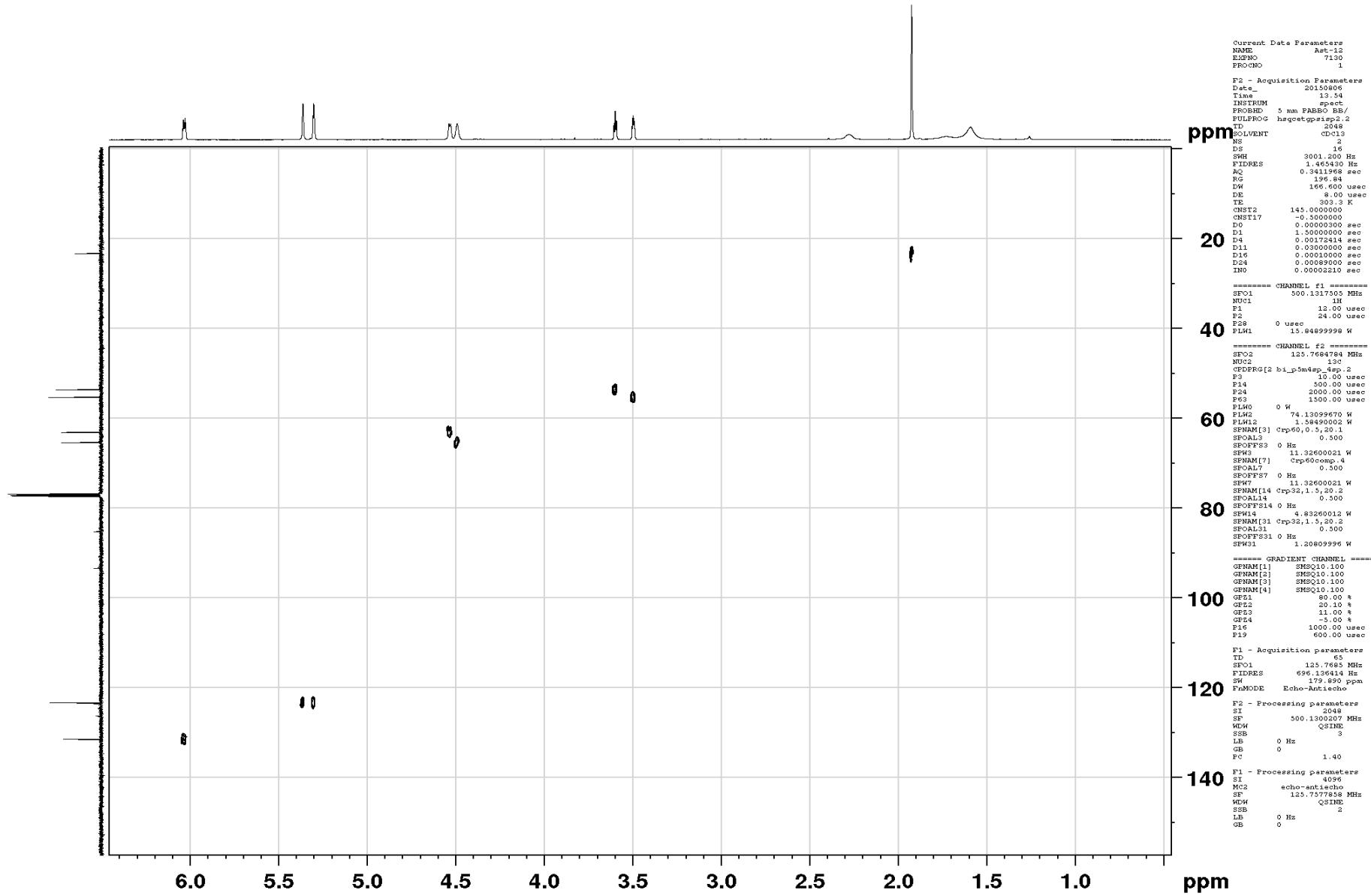


Figure S54. HSQC spectrum (500 MHz, CDCl₃) of **6**

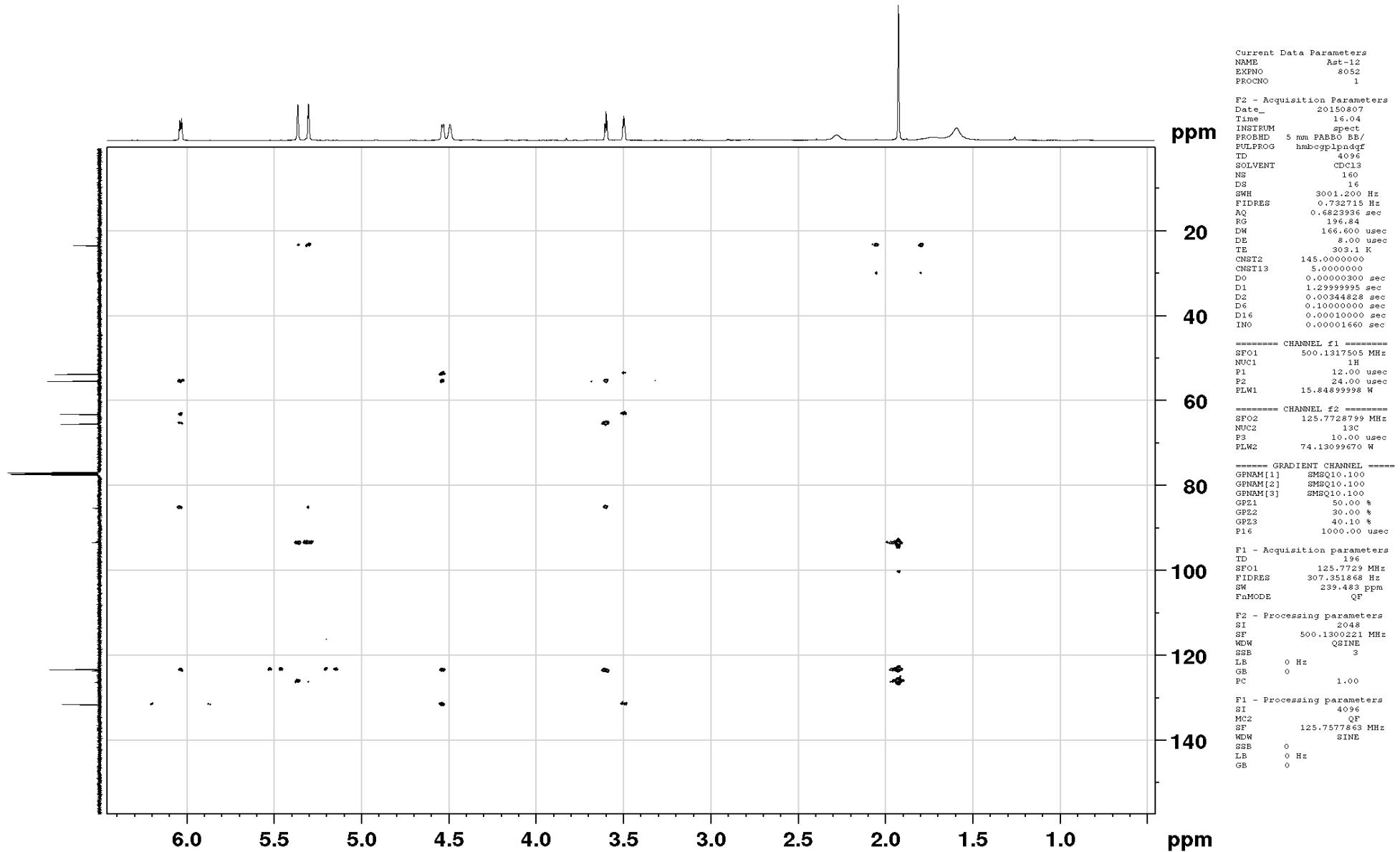


Figure S55. HMBC spectrum (500 MHz, CDCl₃) of **6**

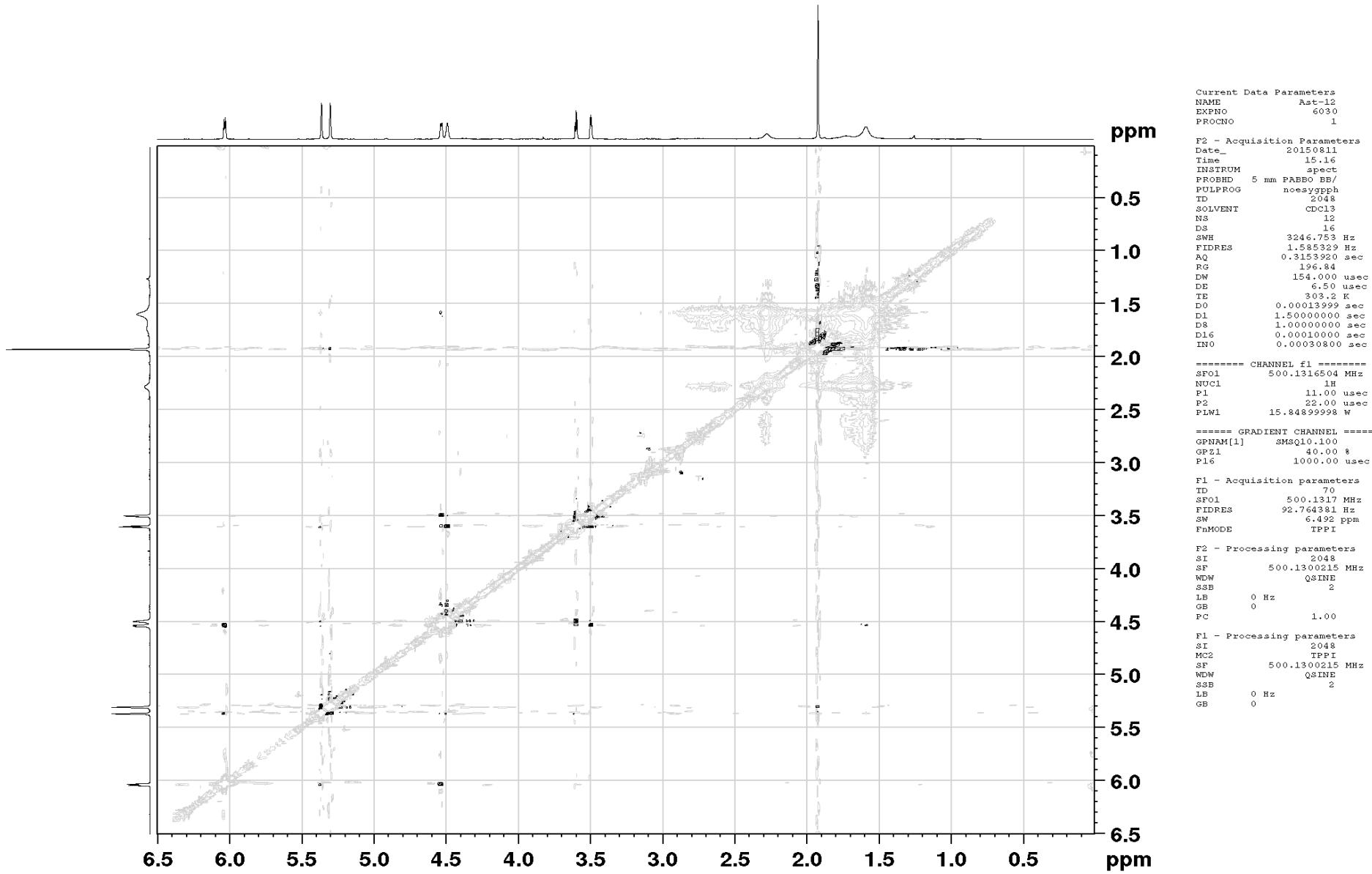


Figure S56. NOESY spectrum (500 MHz, CDCl₃) of **6**

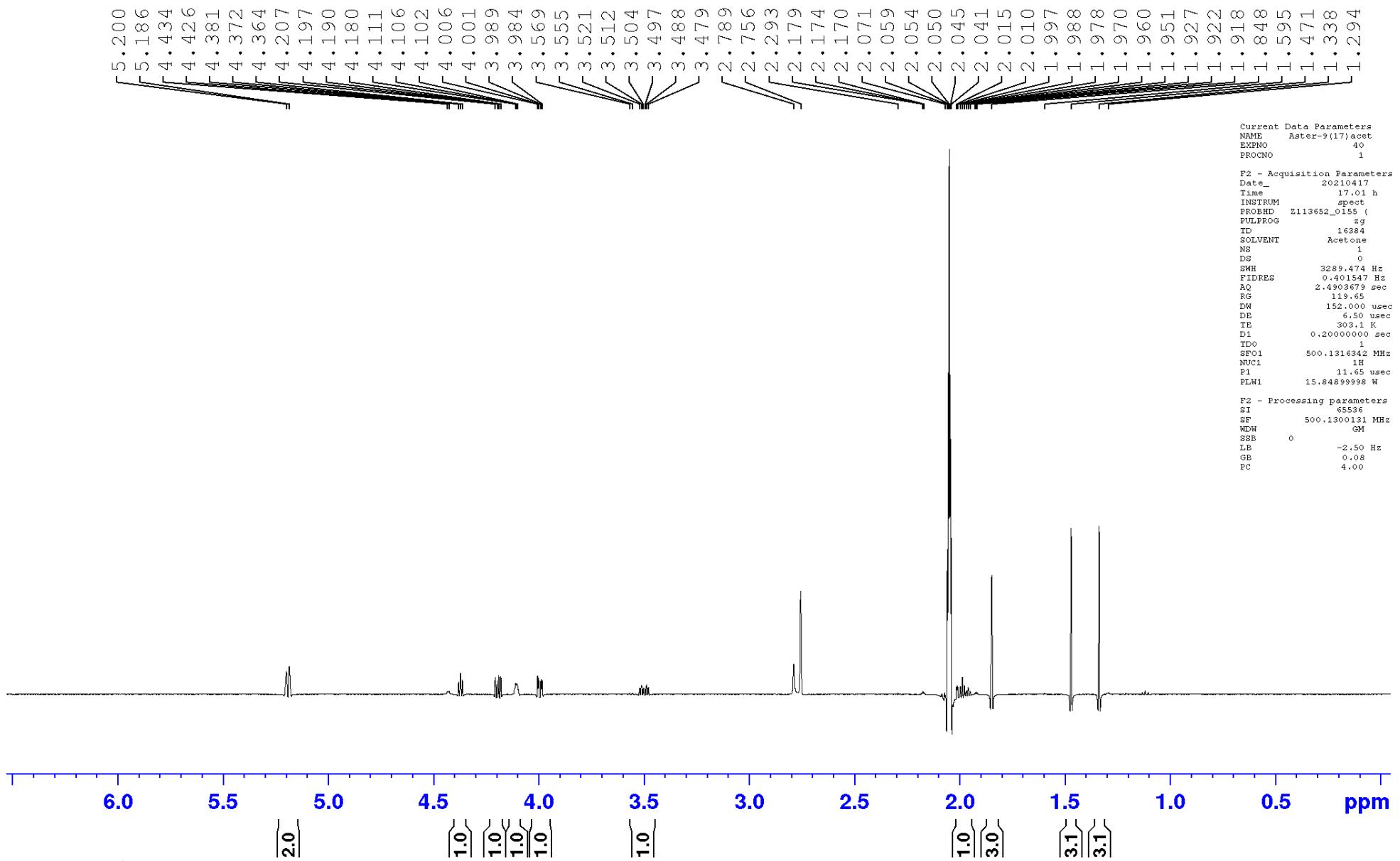


Figure S57. ^1H NMR spectrum (500 MHz, acetone- d_6) of **1a**

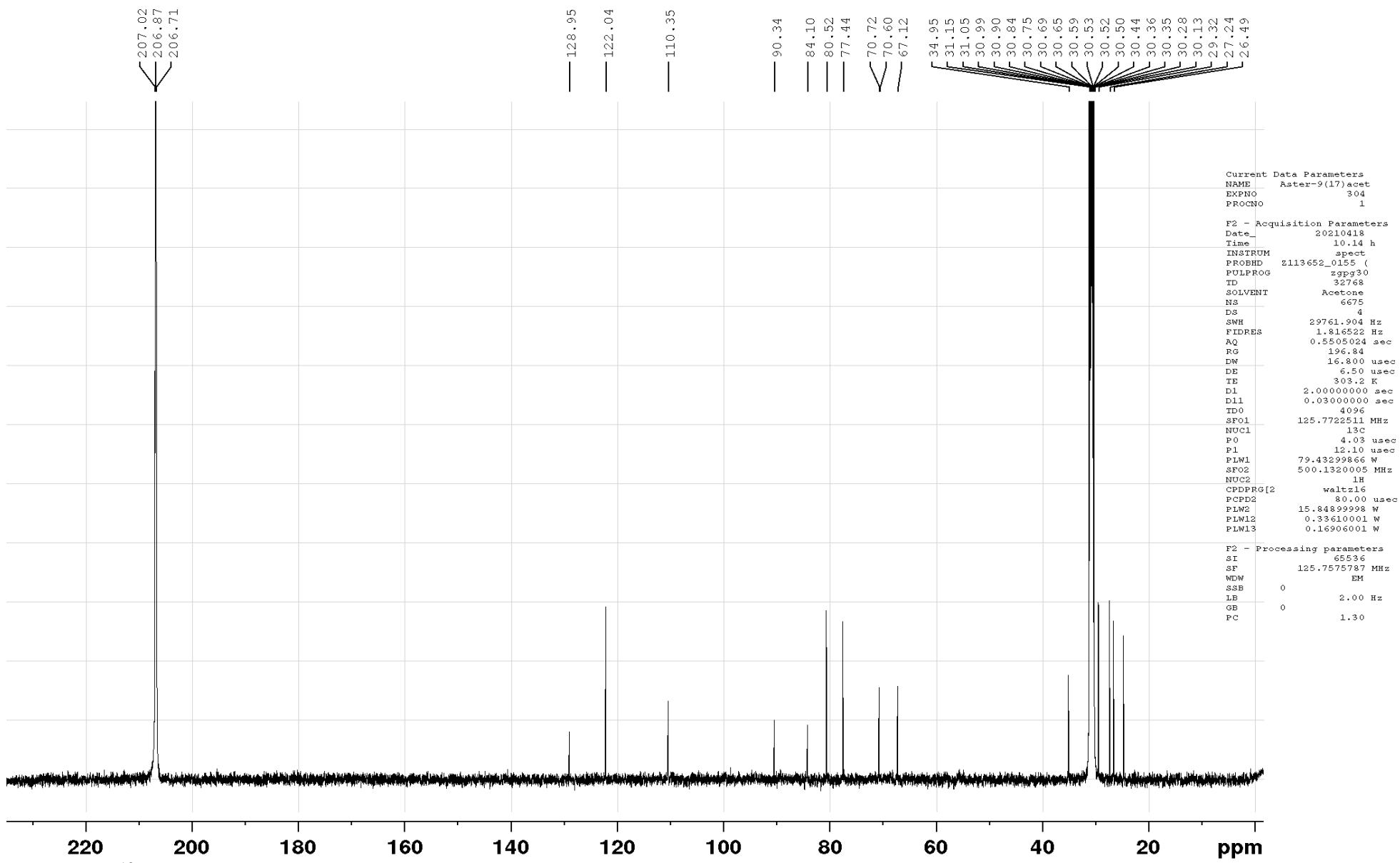


Figure S58. ^{13}C NMR spectrum (125 MHz, acetone-d₆) of **1a**

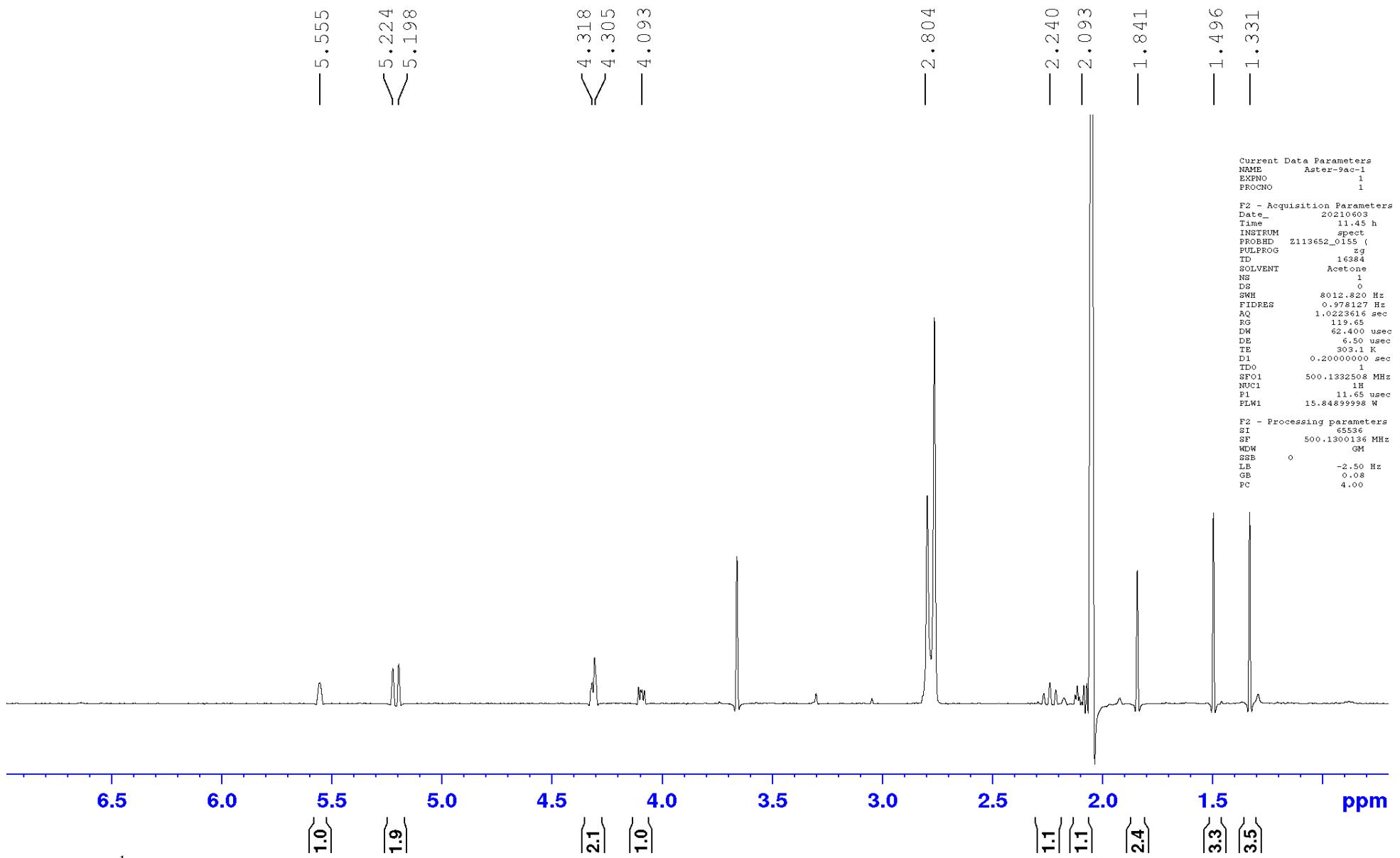


Figure S59. ^1H NMR spectrum (500 MHz, acetone- d_6) of **1a-1**

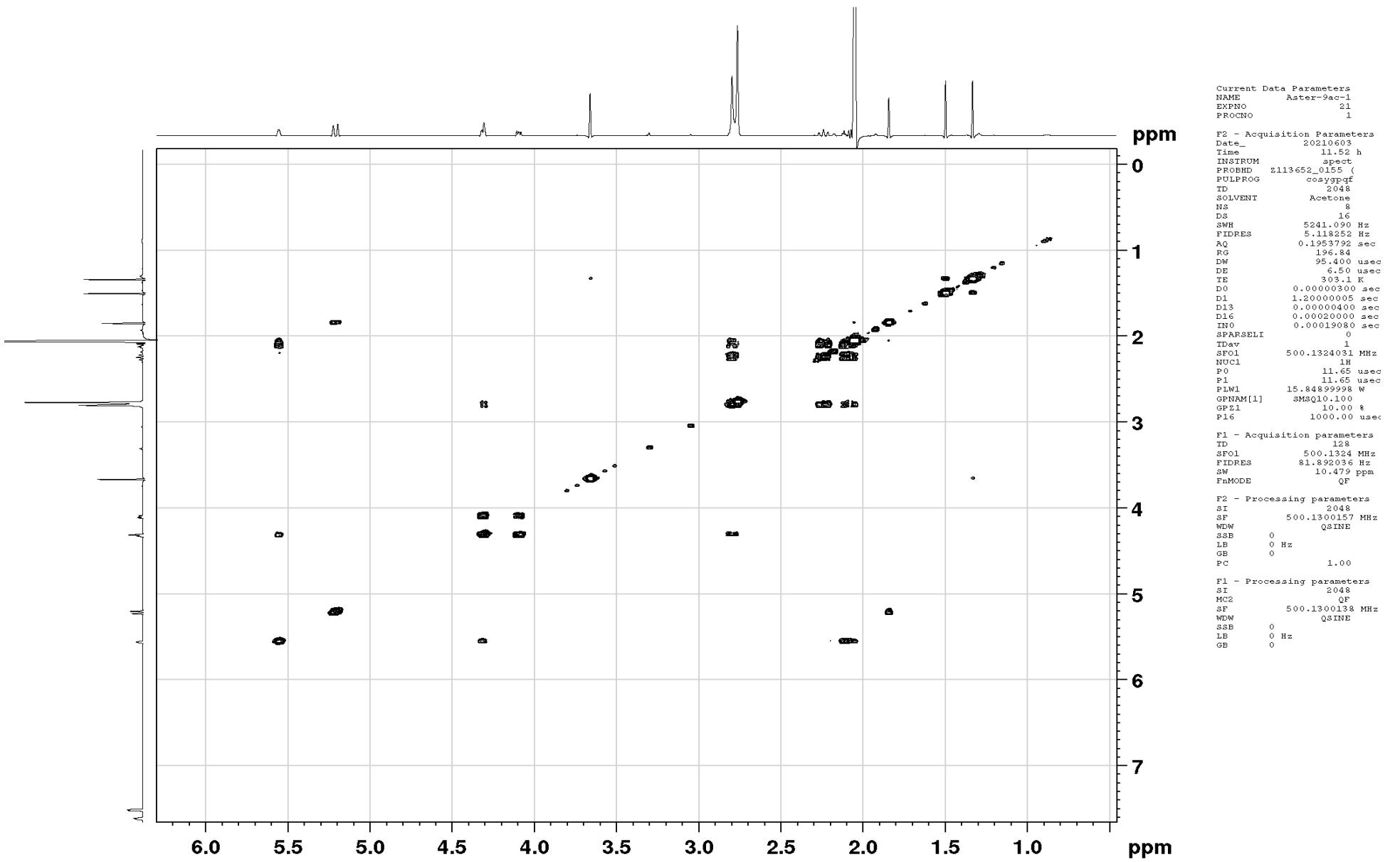


Figure S60. COSY-45 spectrum (500 MHz, acetone-d₆) of 1a-1

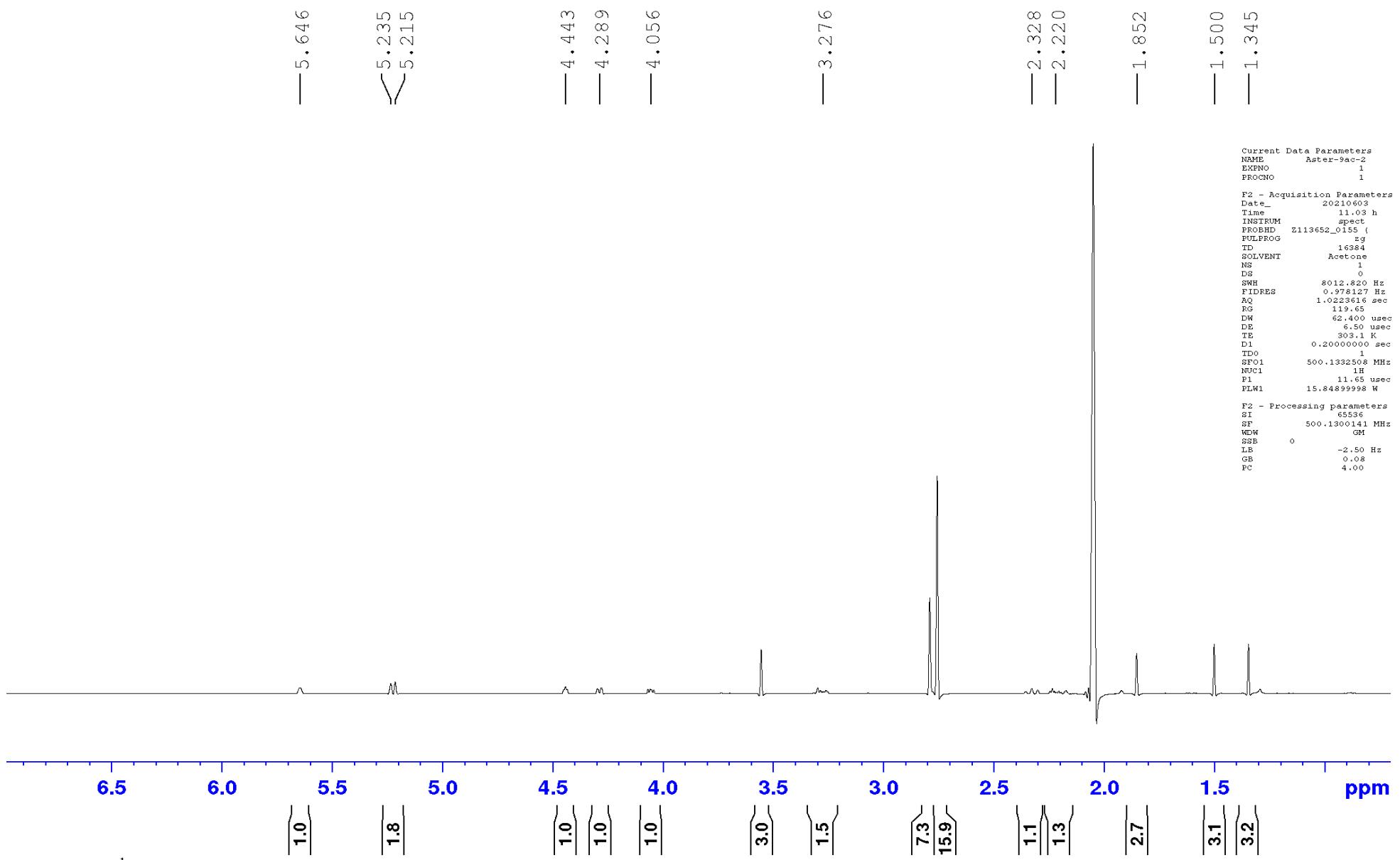


Figure S61. ^1H NMR spectrum (500 MHz, acetone- d_6) of **1a-2**

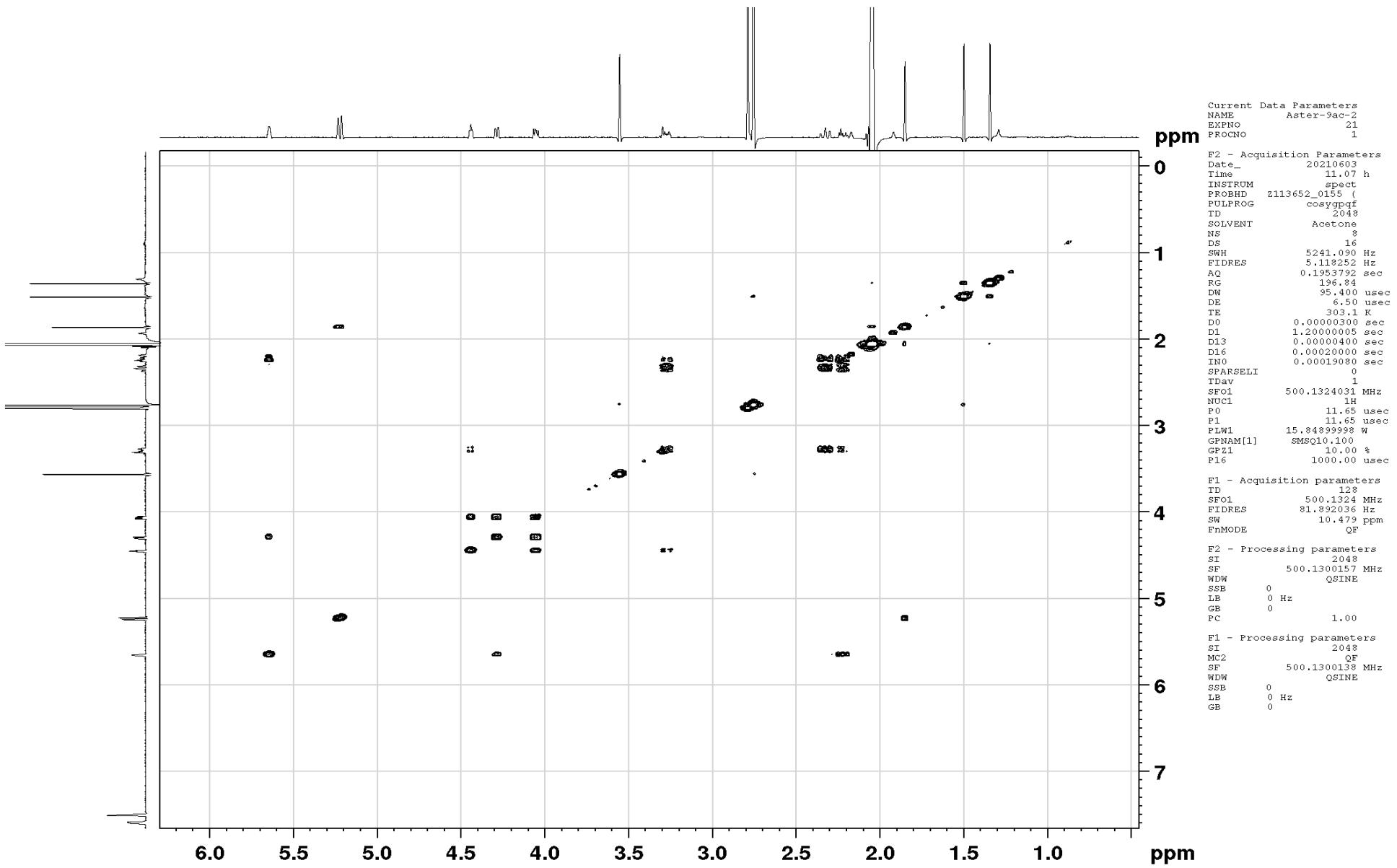


Figure S62. COSY-45 spectrum (500 MHz, acetone-d₆) of 1a-2

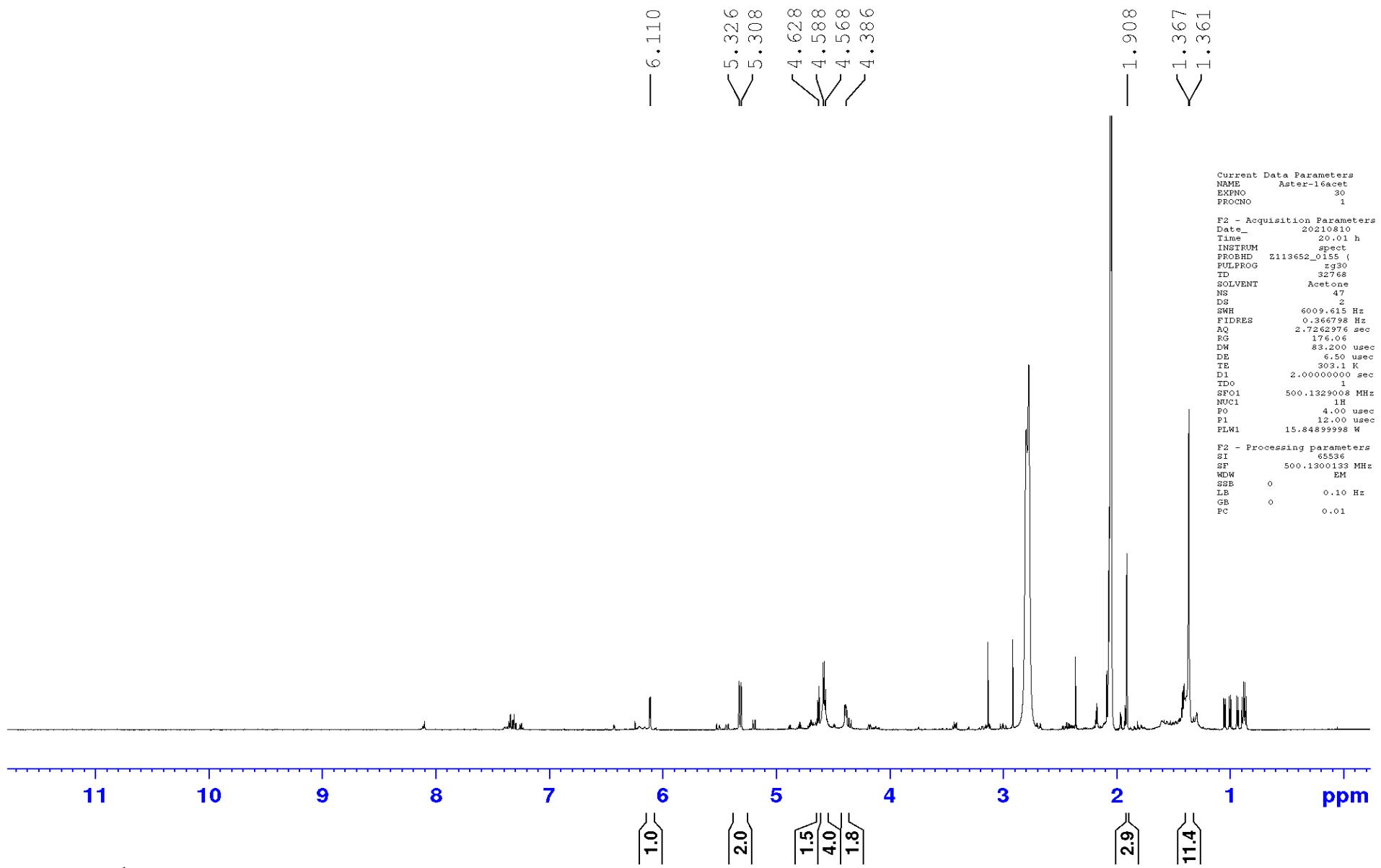


Figure S63. ^1H NMR spectrum (700 MHz, acetone- d_6) of **4a**

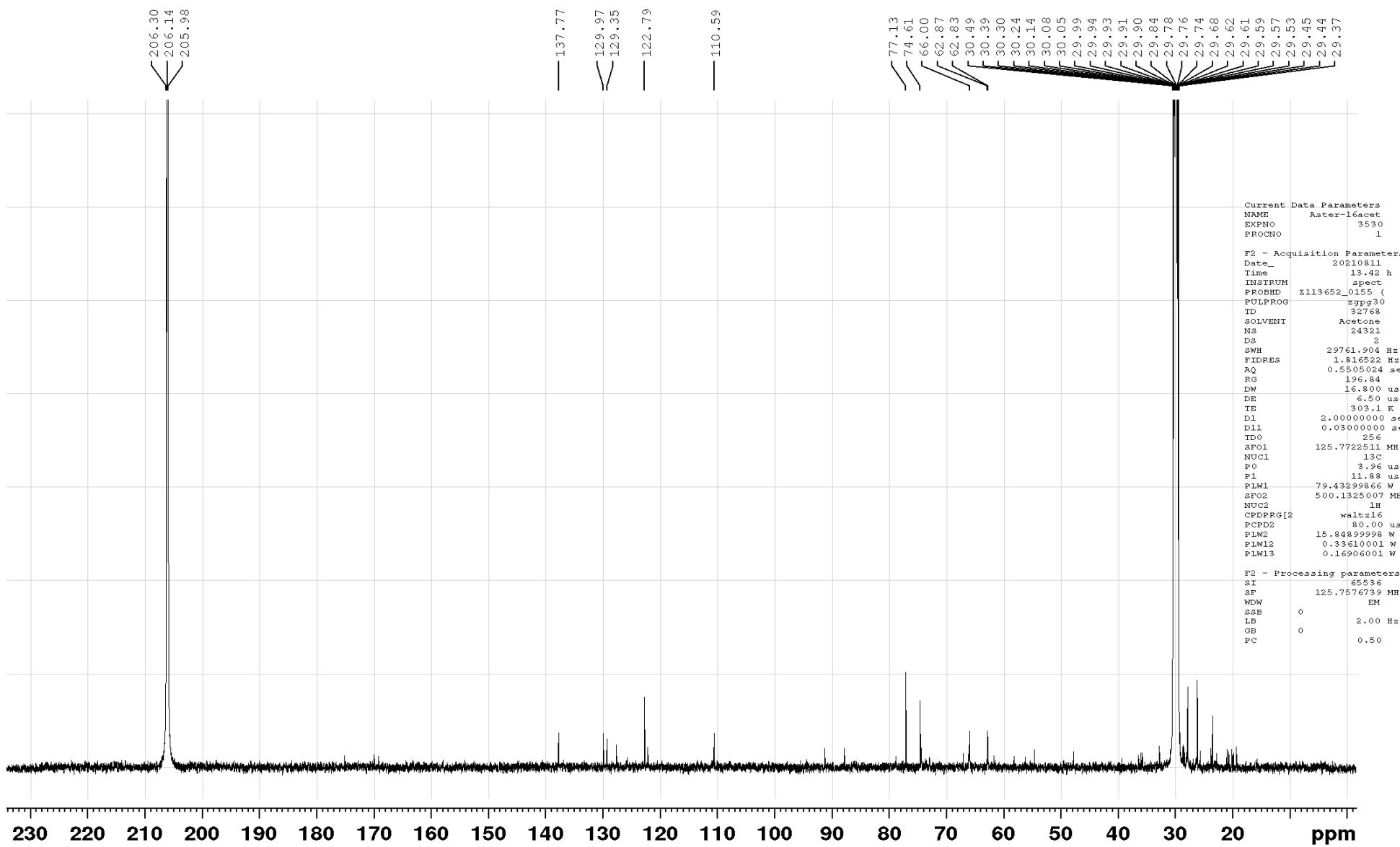


Figure S64. ¹³C NMR spectrum (125 MHz, acetone-d₆) of **4a**

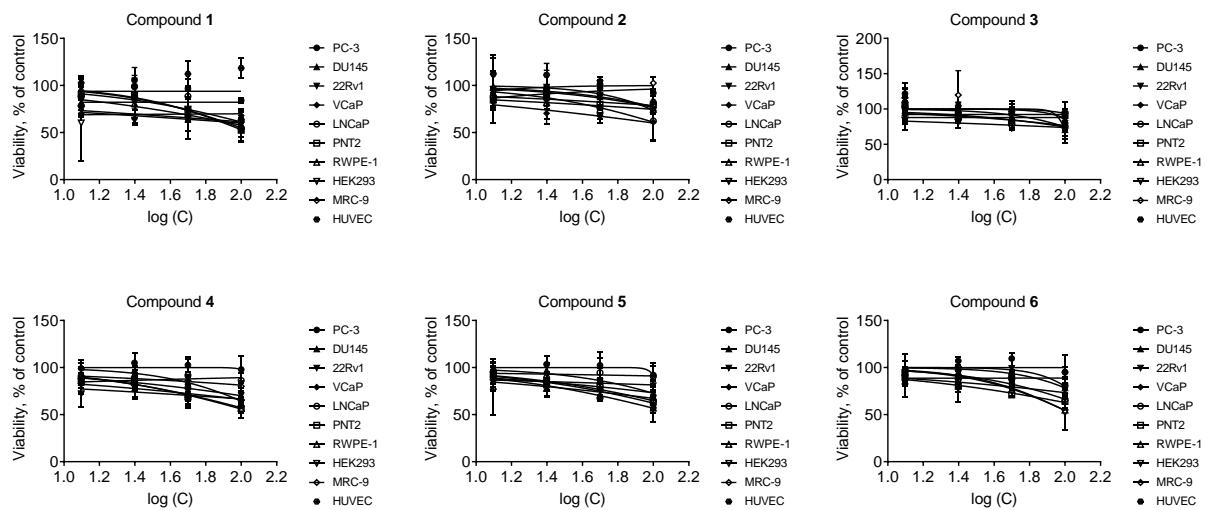


Figure S65. Viability of the cells exposed to the investigated compounds for 72 h. Viability was measured using MTT assay and normalized to control.

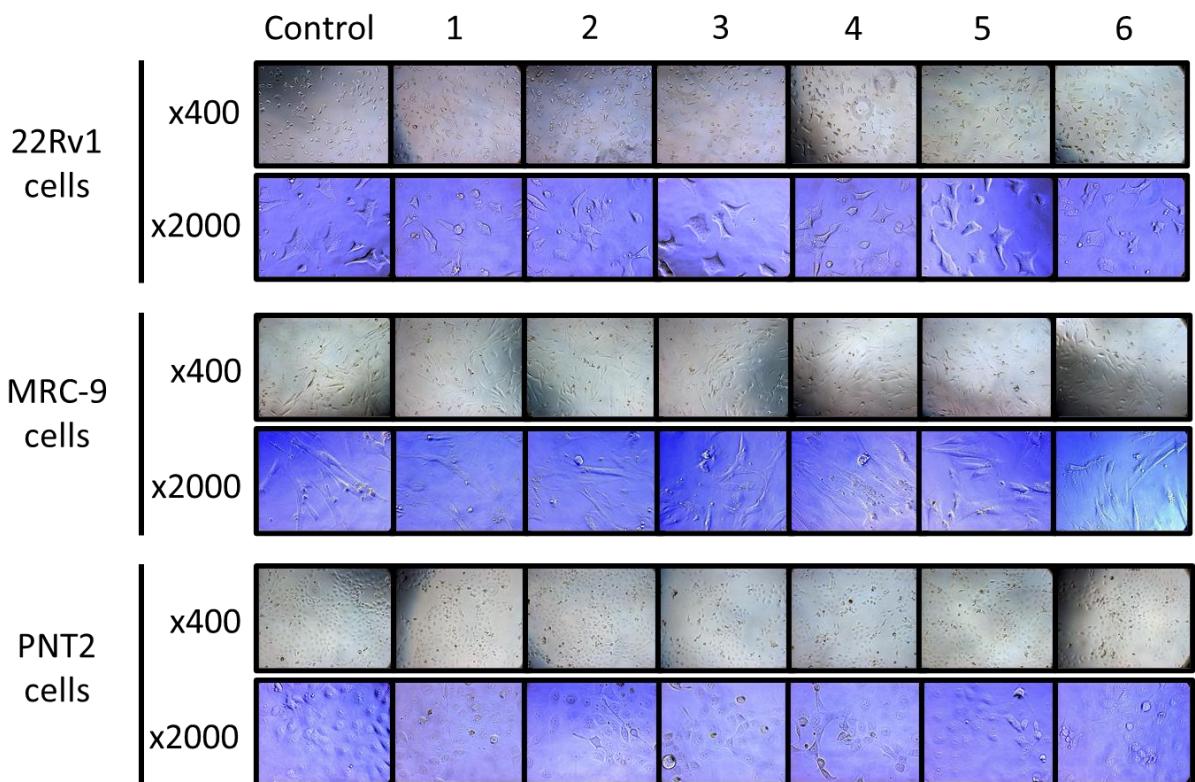


Figure S66. Microphotographies of the cells exposed to the investigated compounds for 72 h. Viability was measured using MTT assay and normalized to control.