**Supplementary data content page**

**Title: The Isolation, Structure Elucidation and Bioactivity Study of Chilensosides A, $A_1$, B, C, and D, Holostane Triterpene Di-, Tri- and Tetrasulfated Pentaosides from the Sea Cucumber *Paracaudina chilensis* (Caudinidae, Molpadida)**

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**Content:**

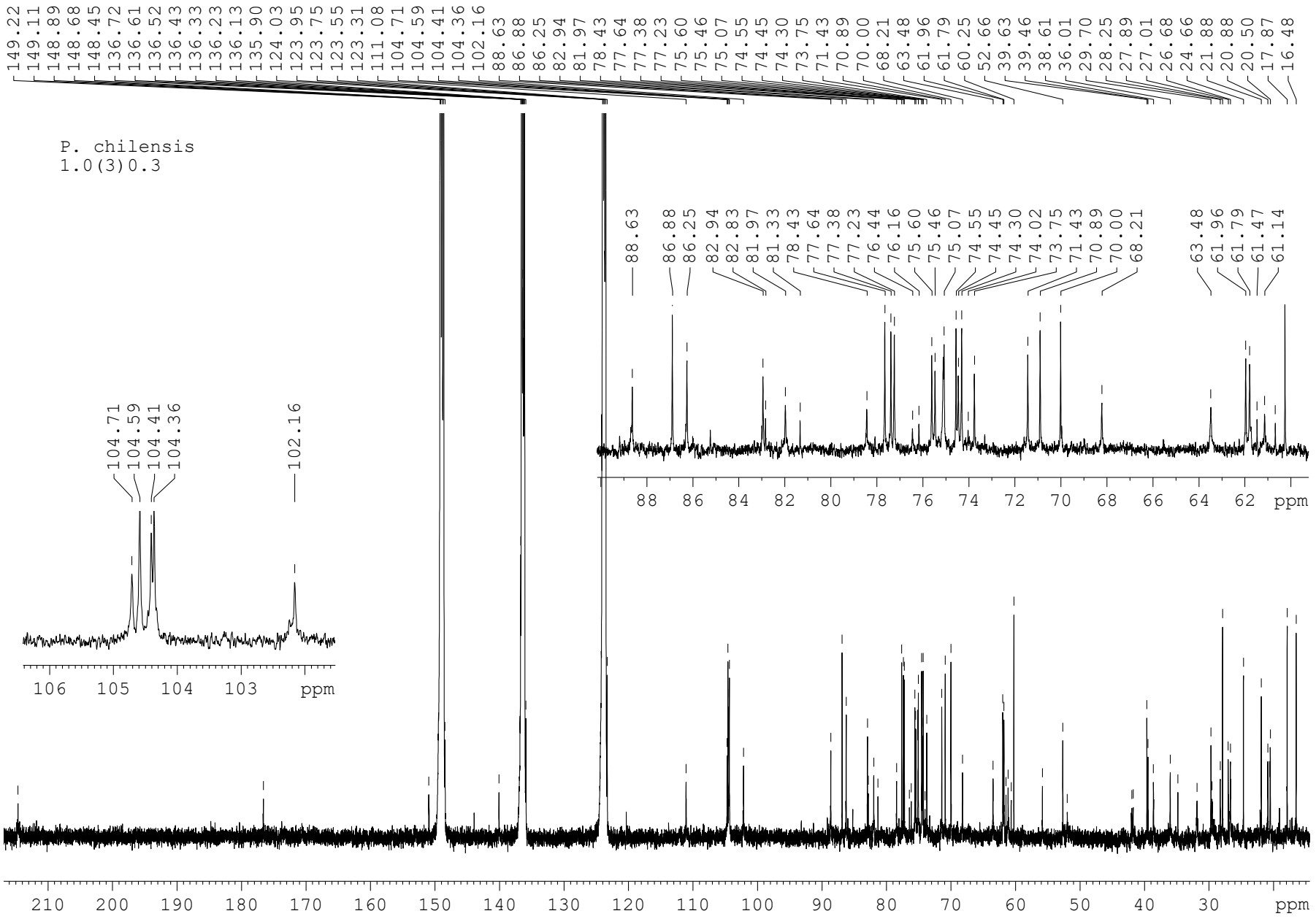



Figure S1. The $^{13}C$ NMR (176.04 MHz)spectrum of chilensosideA (**1**) in $C_5D_5N/D_2O$ (4/1)

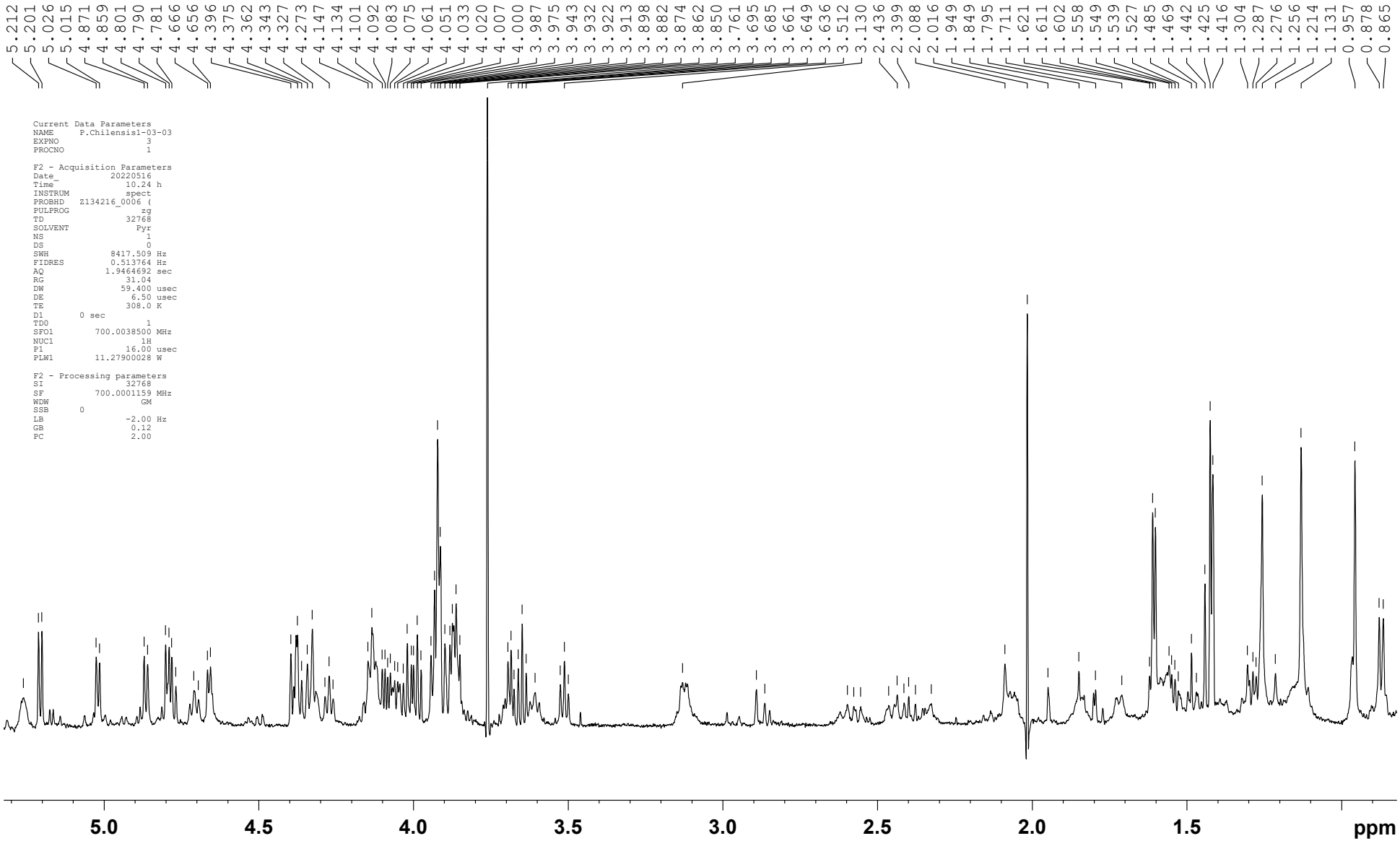



Figure S2. The $^{1}H$ NMR (700.13 MHz) spectrum of chilensoside A (**1**) in $C_5D_5N/D_2O$ (4/1)

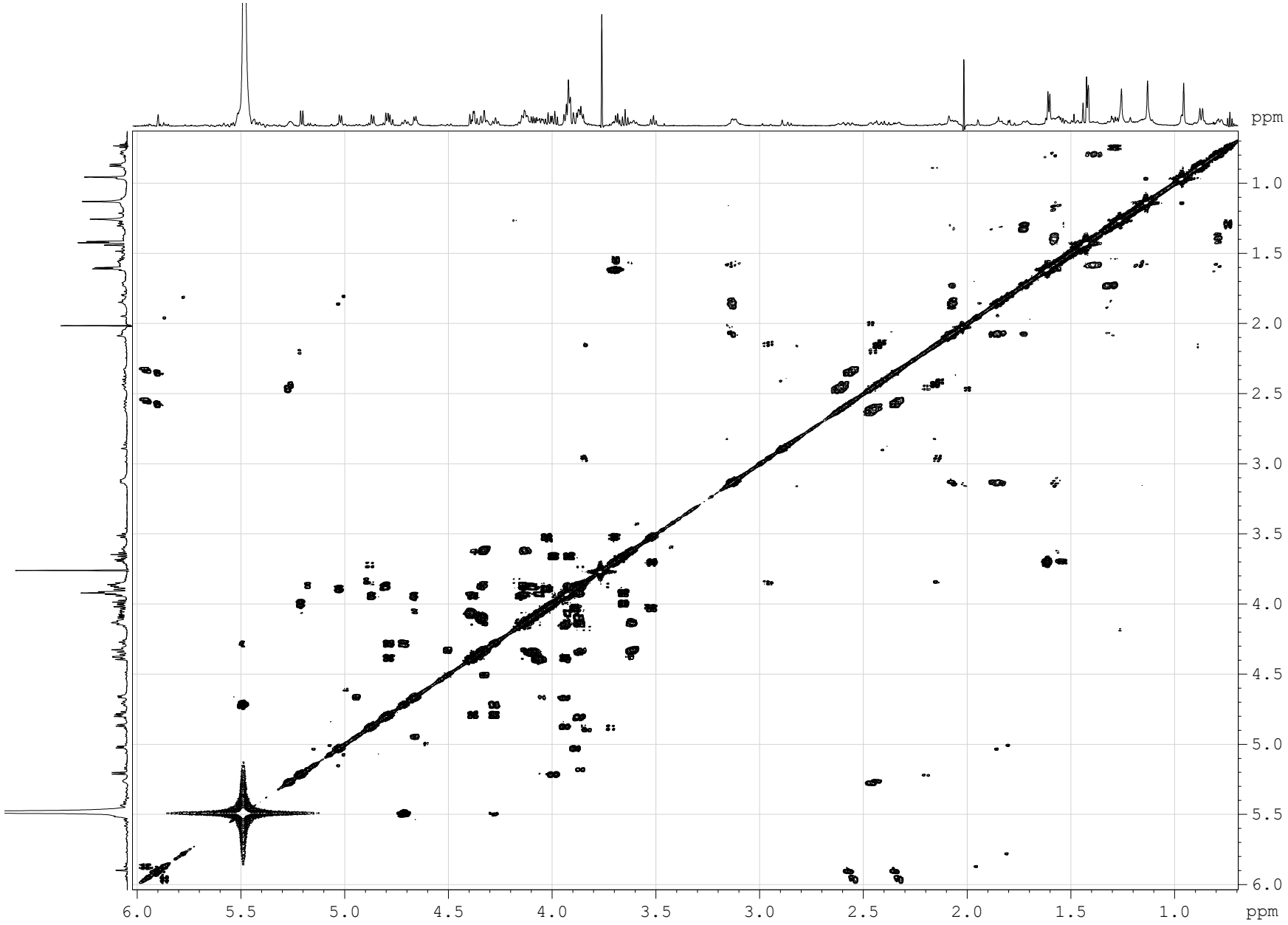



Figure S3. The COSY (700.13 MHz) spectrum of chilensoside A (**1**) in $C_5D_5N/D_2O$ (4/1)

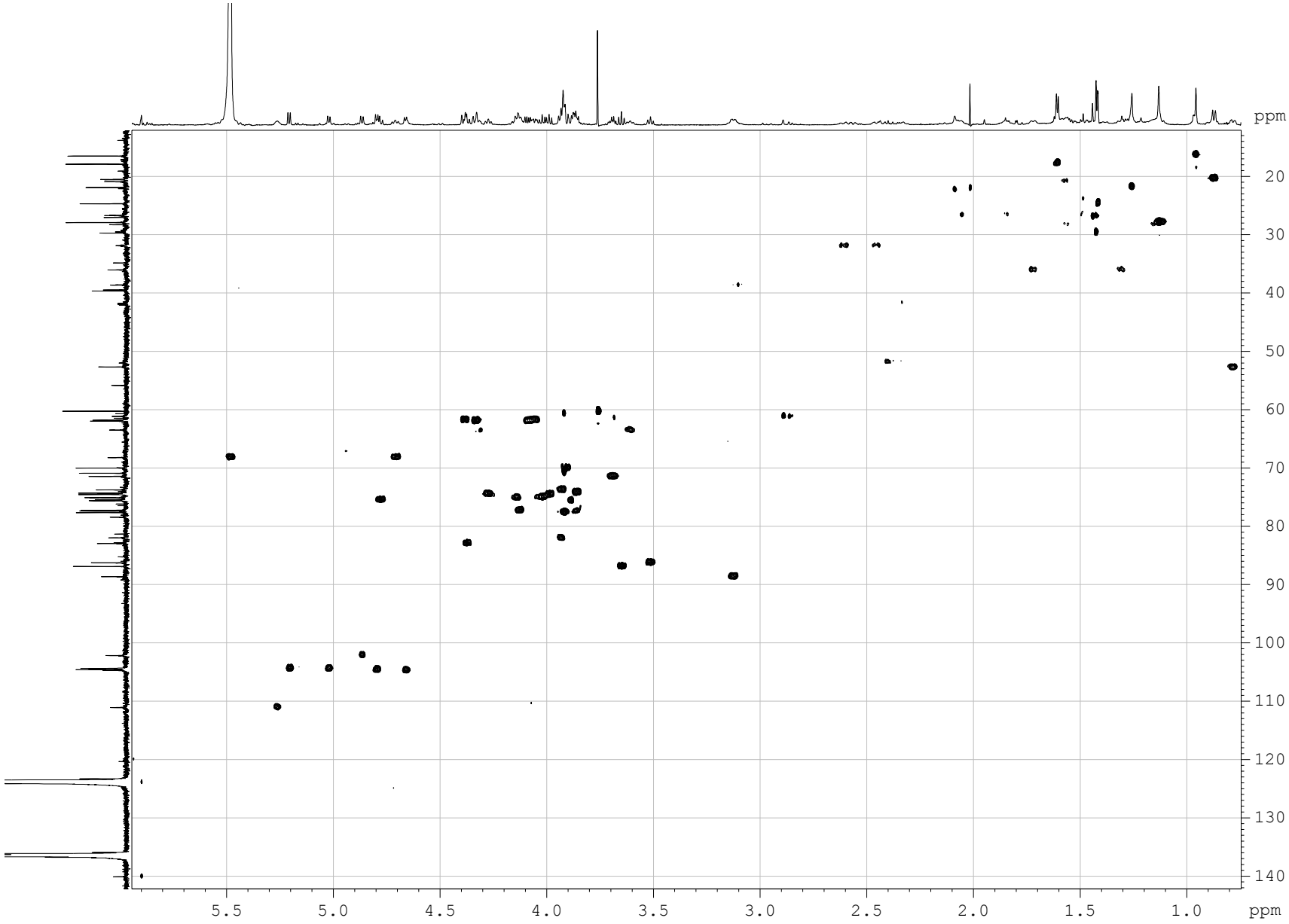



Figure S4. The HSQC (700.13 MHz) spectrum of chilensoside A (**1**) in $C_5D_5N/D_2O$ (4/1)

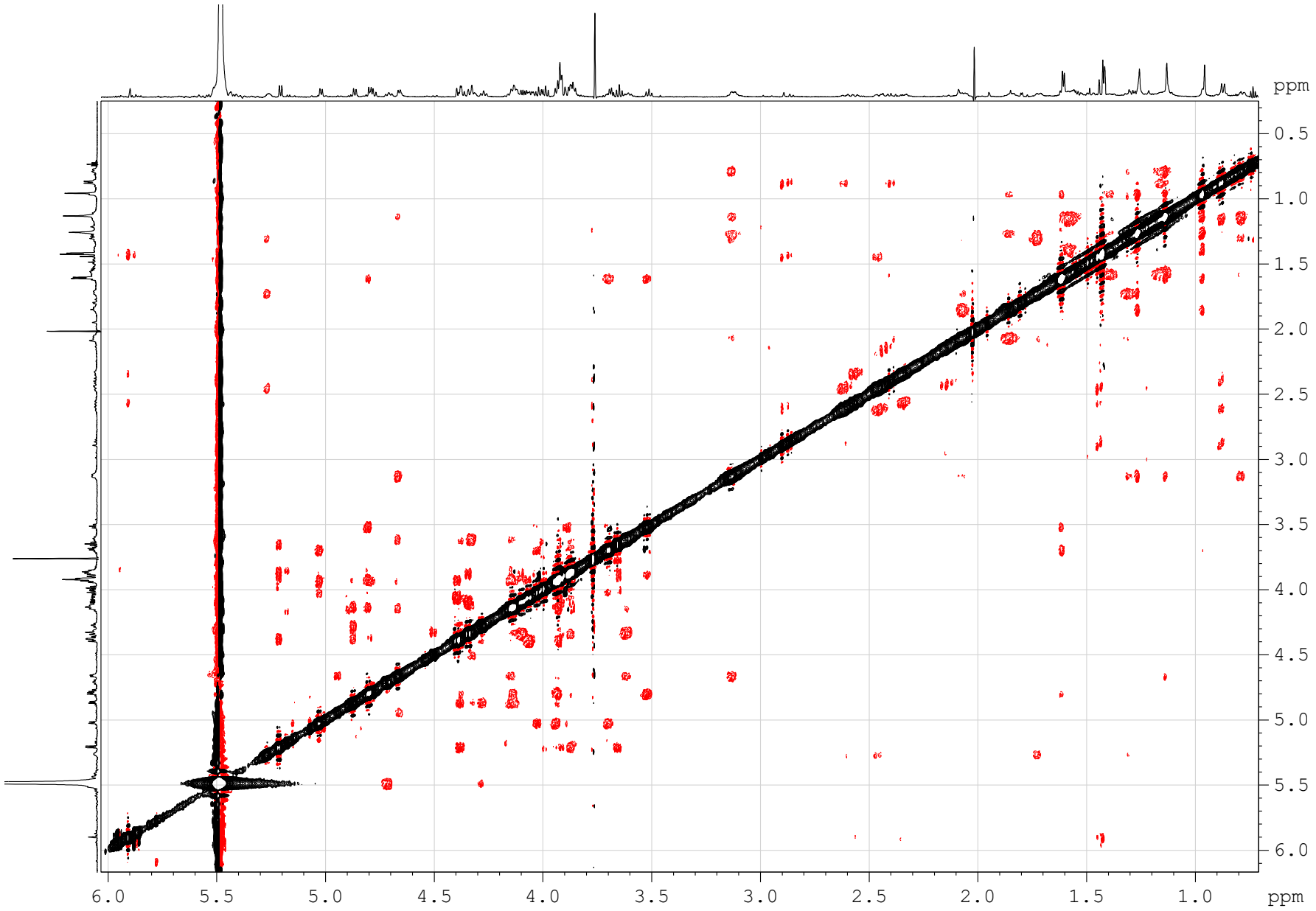



Figure S5. The ROESY (700.13 MHz) spectrum of chilensoside A (**1**) in $C_5D_5N/D_2O$ (4/1)

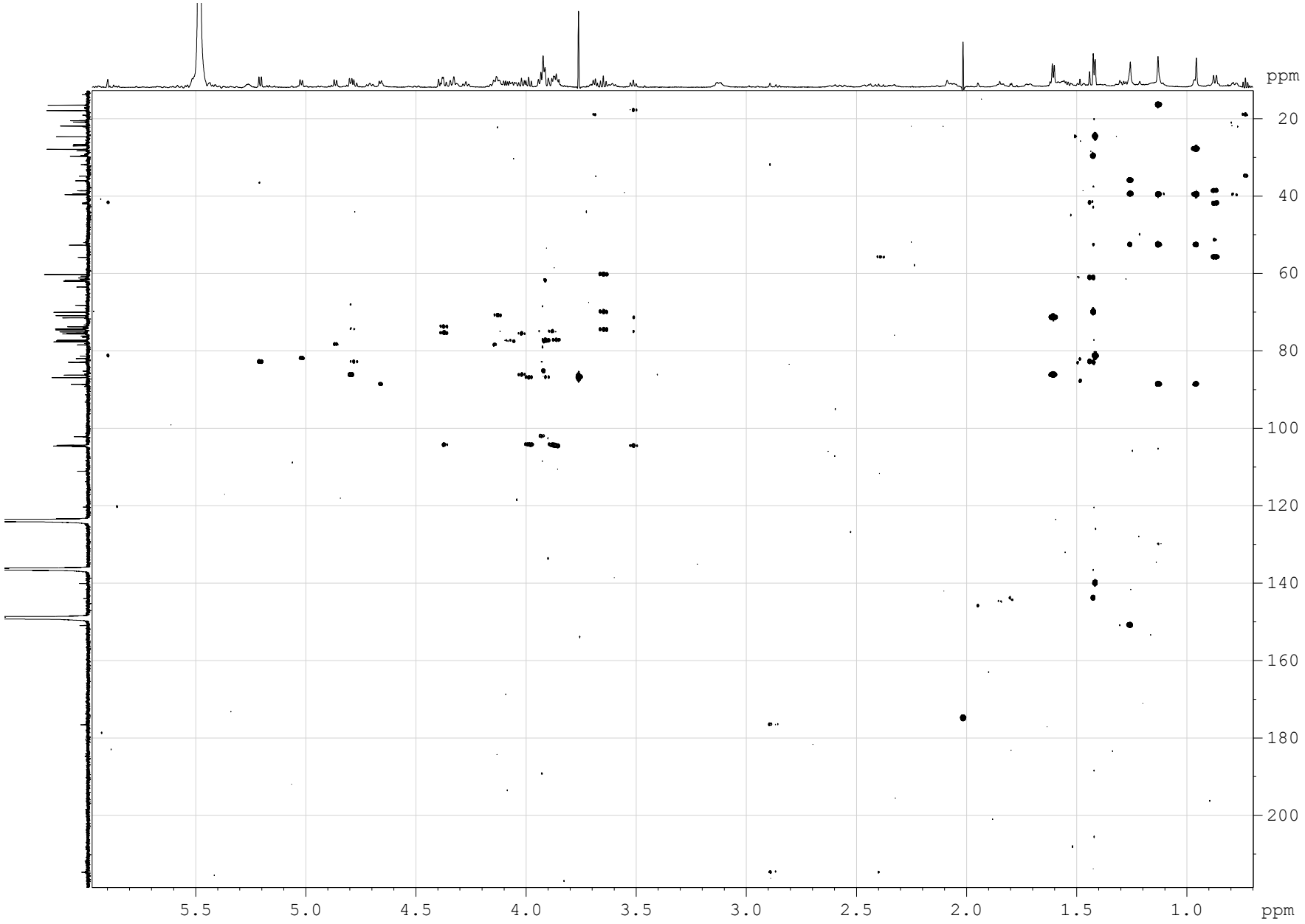



Figure S6. The HMBC (700.13 MHz) spectrum of chilensoside A (**1**) in $C_5D_5N/D_2O$ (4/1)

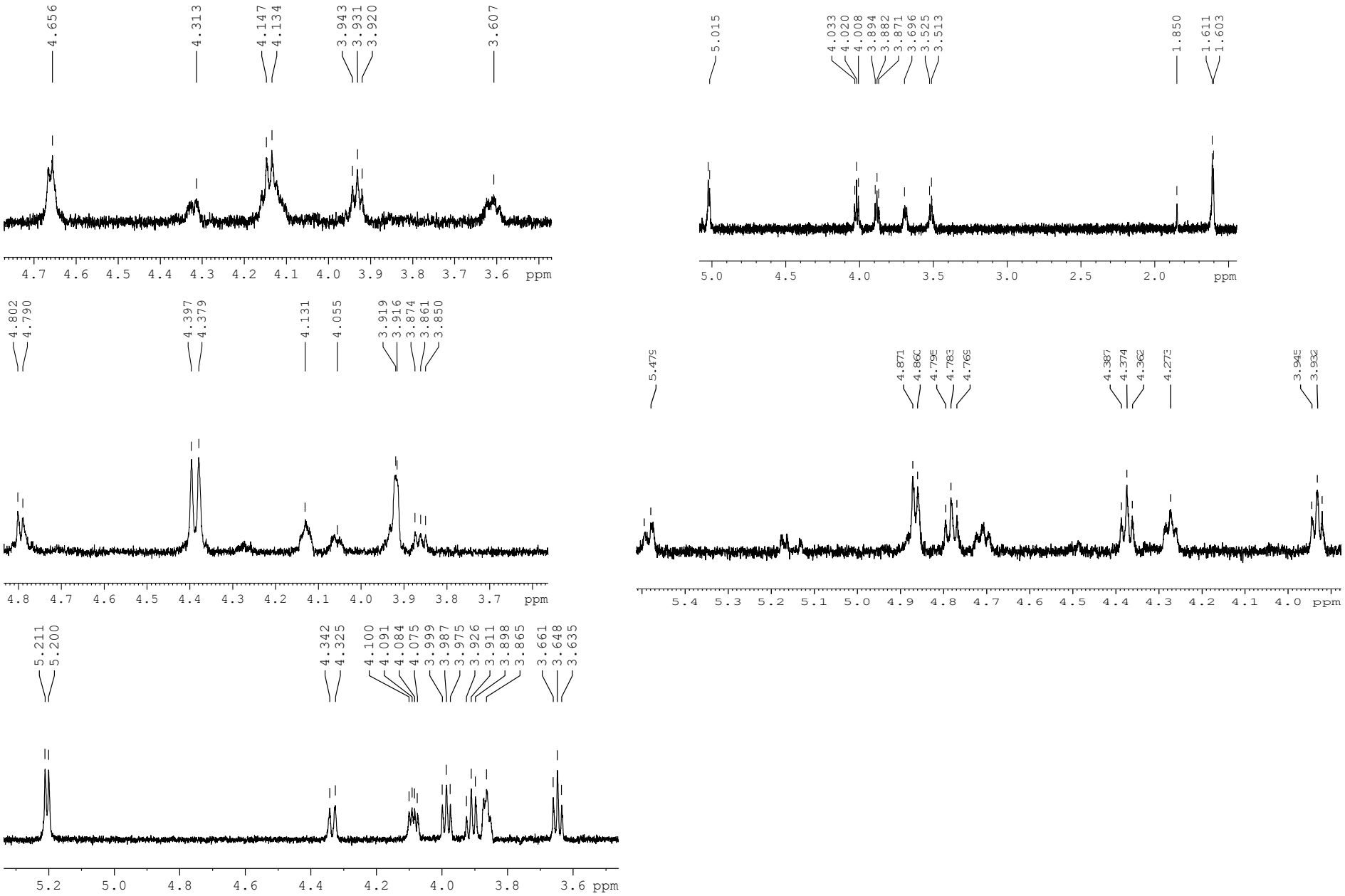
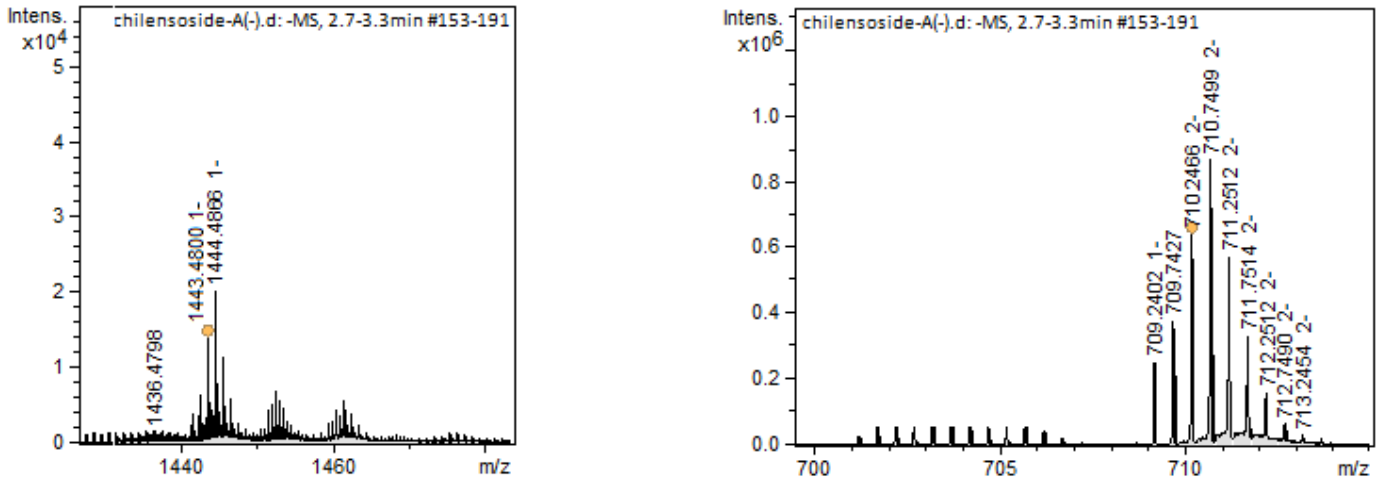



Figure S7. 1 D TOCSY (700.13 MHz) spectra of Xyl1, Qui2, Glc3, Glc4, MeGlc5 of chilensoside A (**1**) in $C_5D_5N/D_2O$ (4/1)




∗ The isotopic composition in the HR-ESI-MS of chilensosides A–B (**1**–**3**), where the ion peak of $[M_{Na}+1]^-$ is more intensive, than that of $[M_{Na}]^-$ is explained by the easy exchange of the protons at C-15, adjacent to 16-oxo-group, to deuterium during the forced long-term storage of the samples in $C_5D_5N/D_2O$ for the registration of the NMR spectra.

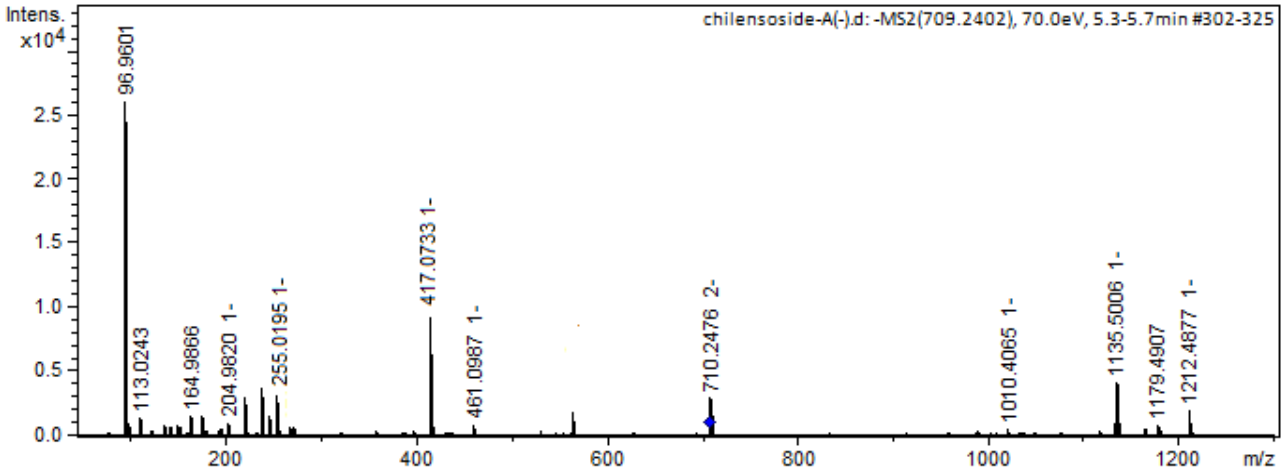



Figure S8. HR-ESI-MS and ESI-MS/MS spectra of chilensoside A (**1**)

**Table S1**: $^{13}C$ and $^{1}H$ NMR chemical shifts and HMBC and ROESY correlations of carbohydrate moiety of chilensoside $A_1$ (**2**)

| Atom | δcmult. [a, b, c] | $\delta_H$mult. (*J* in Hz) [d] | HMBC | ROESY |
|---|---|---|---|---|
| Xyl1 (1→C-3) | | | | |
| 1 | 104.7CH | 4.66d (6.6) | C: 3 | H-3; H-3, 5 Xyl1 |
| 2 | **82.1**CH | 3.90t (8.0) | C: 1 Qui2 | H-1Qui2 |
| 3 | 75.1CH | 4.12t (8.0) | | |
| 4 | **78.8** CH | 4.10 m | | H-1 Glc4 |
| 5 | 63.5 $CH_2$ | 4.32m | | |
| | | 3.60m | | H-1 Xyl1 |
| Qui2 (1→2Xyl1) | | | | |
| 1 | 104.4 CH | 4.98 d (8.2) | C: 2 Xyl1 | H-2 Xyl1; H-3, 5 Qui2 |
| 2 | 75.6 CH | 3.89 t (8.2) | | H-4 Qui2 |
| 3 | 74.9 CH | 4.04 t (8.2) | | H-1, 5 Qui2 |
| 4 | **86.1** CH | 3.50 t (8.2) | C: 1 Glc3 | H-1 Glc3 |
| 5 | 71.5 CH | 3.68 dd (5.7; 8.2) | | H-1 Qui2 |
| 6 | 17.8 $CH_3$ | 1.60 d (5.7) | C: 4, 5 Qui2 | H-4 Qui2 |
| Glc3 (1→4Qui2) | | | | |
| 1 | 104.6 CH | 4.79 d (8.5) | C: 4 Qui2 | H-4 Qui2; H-3, 5Glc3 |
| 2 | 74.4 CH | 3.85 t (8.5) | | |
| 3 | 77.2 CH | 4.13 t (8.5) | | H-1, 5Glc3 |
| 4 | 70.9 CH | 3.92 m | | H-6 Glc3 |
| 5 | 77.7 CH | 3.91 m | | H-1 Glc3 |
| 6 | 61.7$CH_2$ | 4.39d (11.6) | | |
| | | 4.05 dd (5.5; 11.6) | | |
| Glc4(1→4Xyl1) | | | | |
| 1 | 102.3 CH | 4.85 d (7.5) | C: 4 Xyl1 | H-4 Xyl1; H-3, 5 Glc4 |
| 2 | 73.7 CH | 3.93 t (8.6) | | |
| 3 | **83.0** CH | 4.37 t (8.6) | C: 1 MeGlc5 | H-1 MeGlc5; H-1 Glc4 |
| 4 | *75.5* CH | 4.78 t (8.6) | | |
| 5 | 74.3 CH | 4.26 t (9.7) | | H-1 Glc4 |
| 6 | *68.3* $CH_2$ | 5.50 m | | |
| | | 4.71 dd (9.7; 11.8) | | |
| MeGlc5(1→3Glc4) | | | | |
| 1 | 104.4 CH | 5.20 d (7.7) | C: 3 Glc4 | H-3 Glc4; H-3,5 MeGlc5 |
| 2 | 74.6 CH | 3.99 t (8.7) | | |
| 3 | 86.9 CH | 3.65 t (8.7) | | H-1 Me Glc5 |
| 4 | 70.0 CH | 3.91 t (8.7) | | |
| 5 | 77.4 CH | 3.86 t (8.7) | | H-1 MeGlc5 |
| 6 | 62.0 $CH_2$ | 4.34 d (10.6) | | |
| | | 4.09 dd (6.8; 11.6) | | H-4 MeGlc5 |
| OMe | 60.3 $CH_3$ | 3.76 s | C: 3 MeGlc5 | |

[a] Recorded at 125.67 MHz in $C_5D_5N/D_2O$ (4/1). [b] Bold = interglycosidic positions. [c] Italic = sulfate position. [d] Recorded at 500.12 MHz in $C_5D_5N/D_2O$ (4/1). Multiplicity by 1D TOCSY.

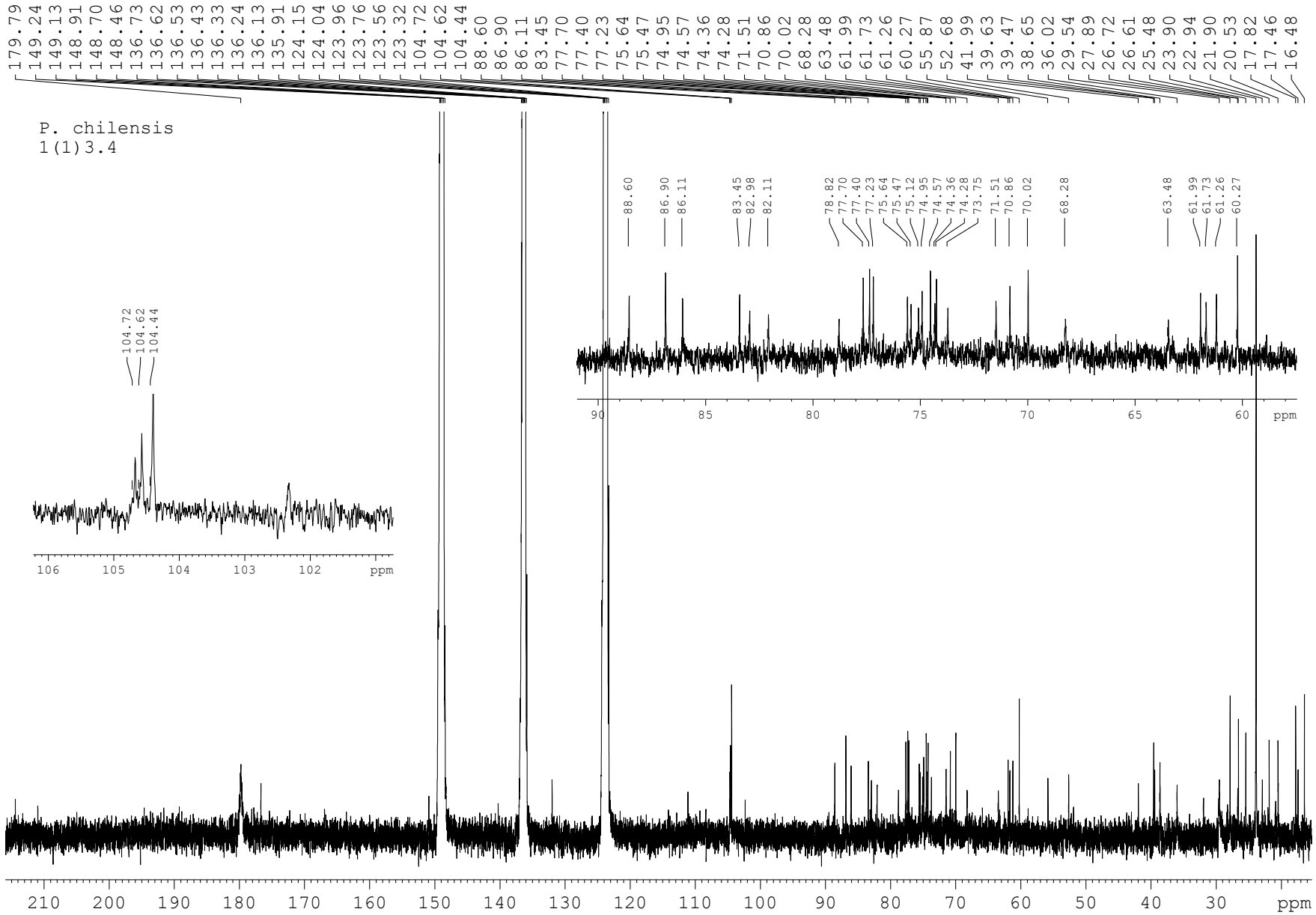



Figure S9. The $^{13}C$ NMR (125.67 MHz) spectrum of chilensoside $A_1$ (**2**) in $C_5D_5N/D_2O$ (4/1)

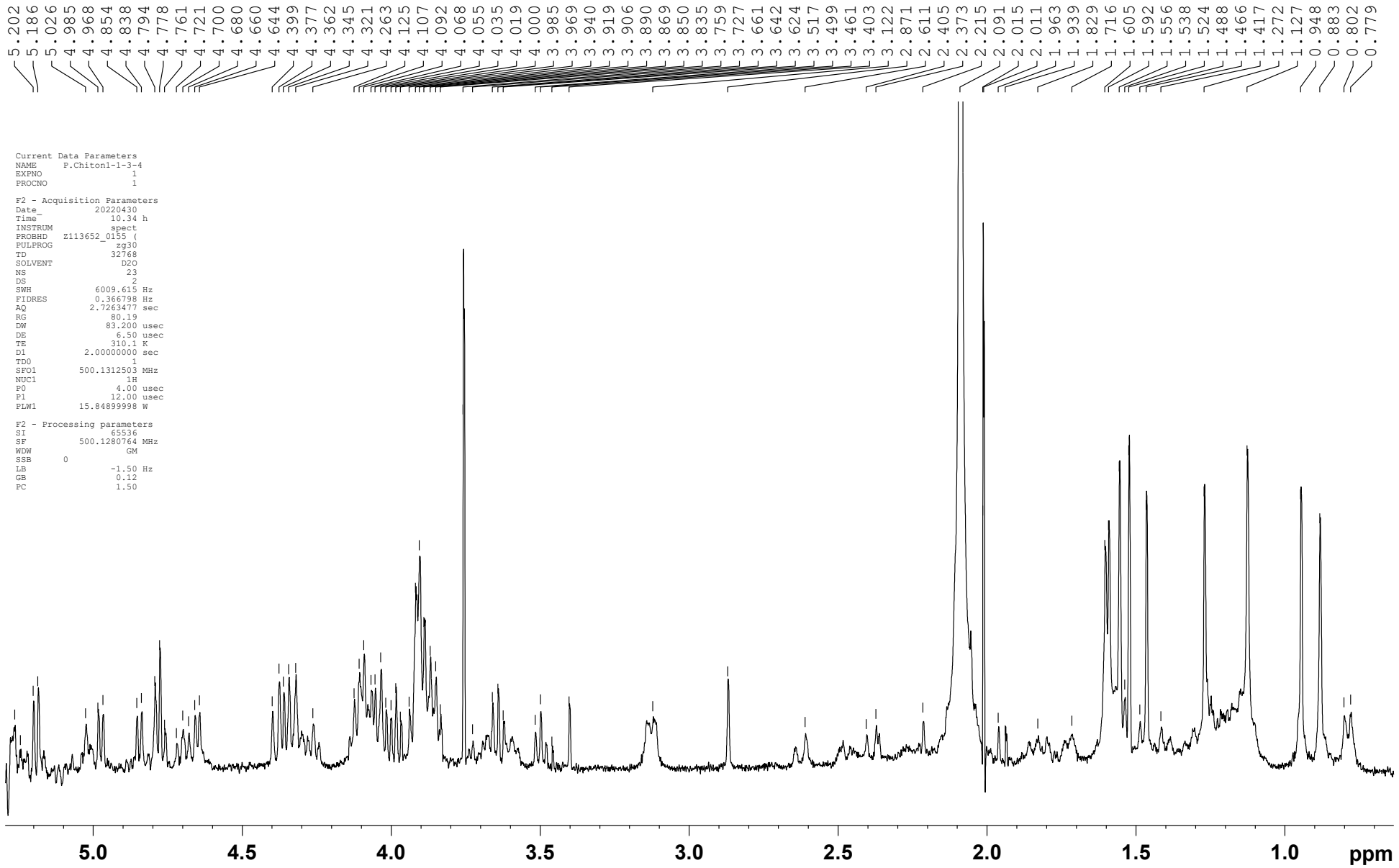



Figure S10. The $^{1}H$ NMR (500.12 MHz) spectrum of chilensoside $A_1$ (**2**) in $C_5D_5N/D_2O$ (4/1)

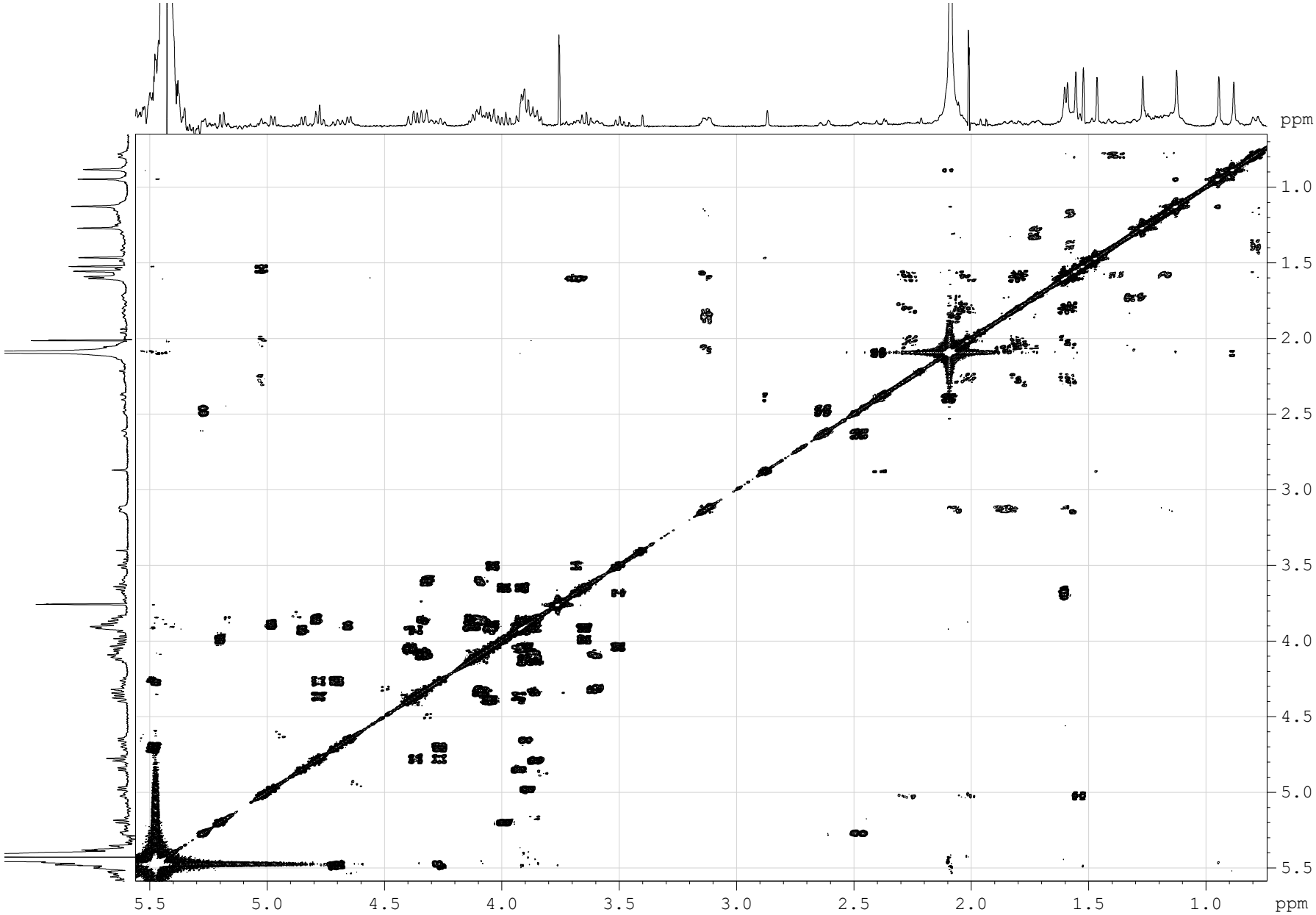



Figure S11. The COSY (500.12 MHz) spectrum of chilensoside $A_1$ (**2**) in $C_5D_5N/D_2O$ (4/1)

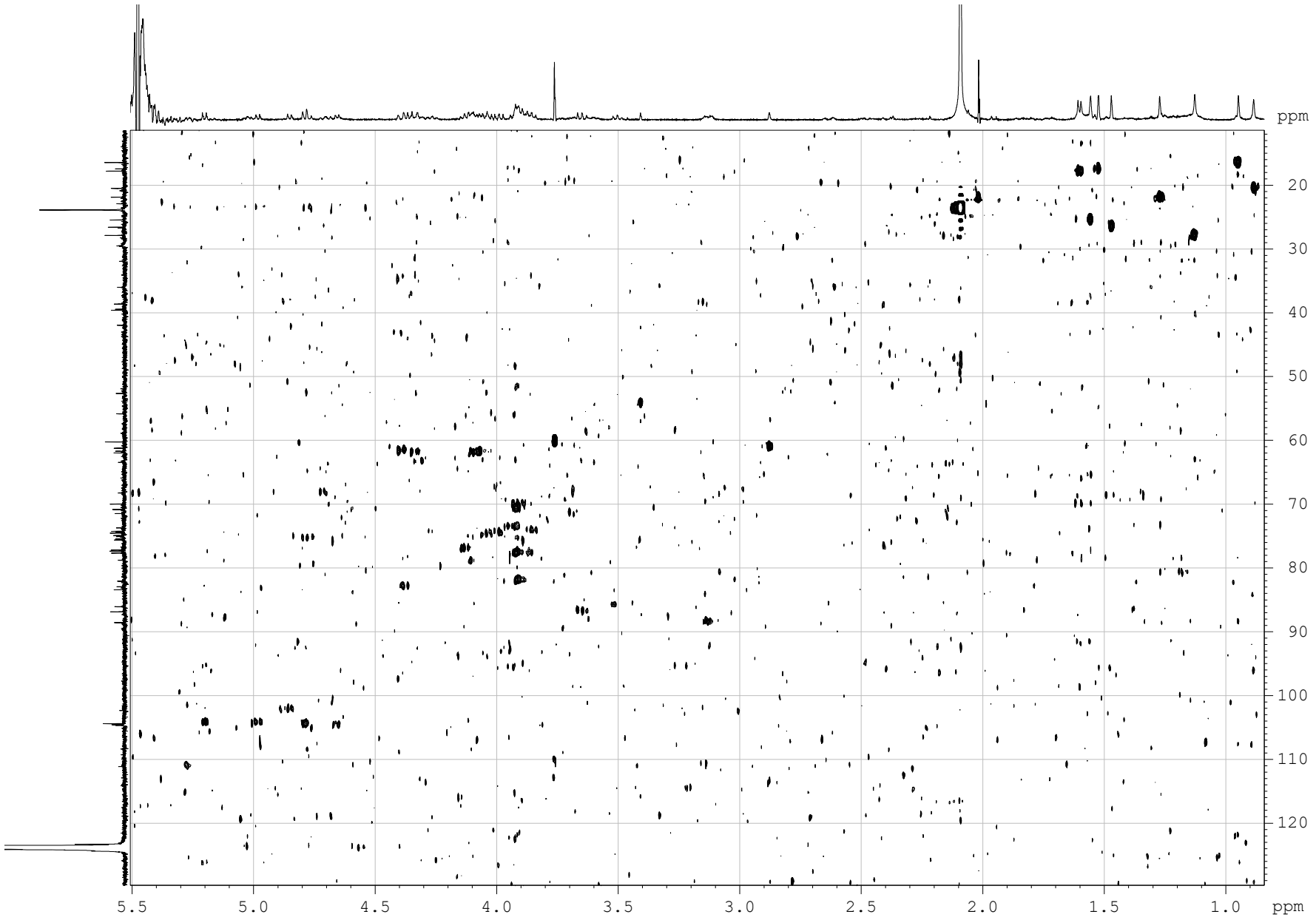



Figure S12. The HSQC (500.12 MHz) spectrum of chilensoside $A_1$ (**2**) in $C_5D_5N/D_2O$ (4/1)

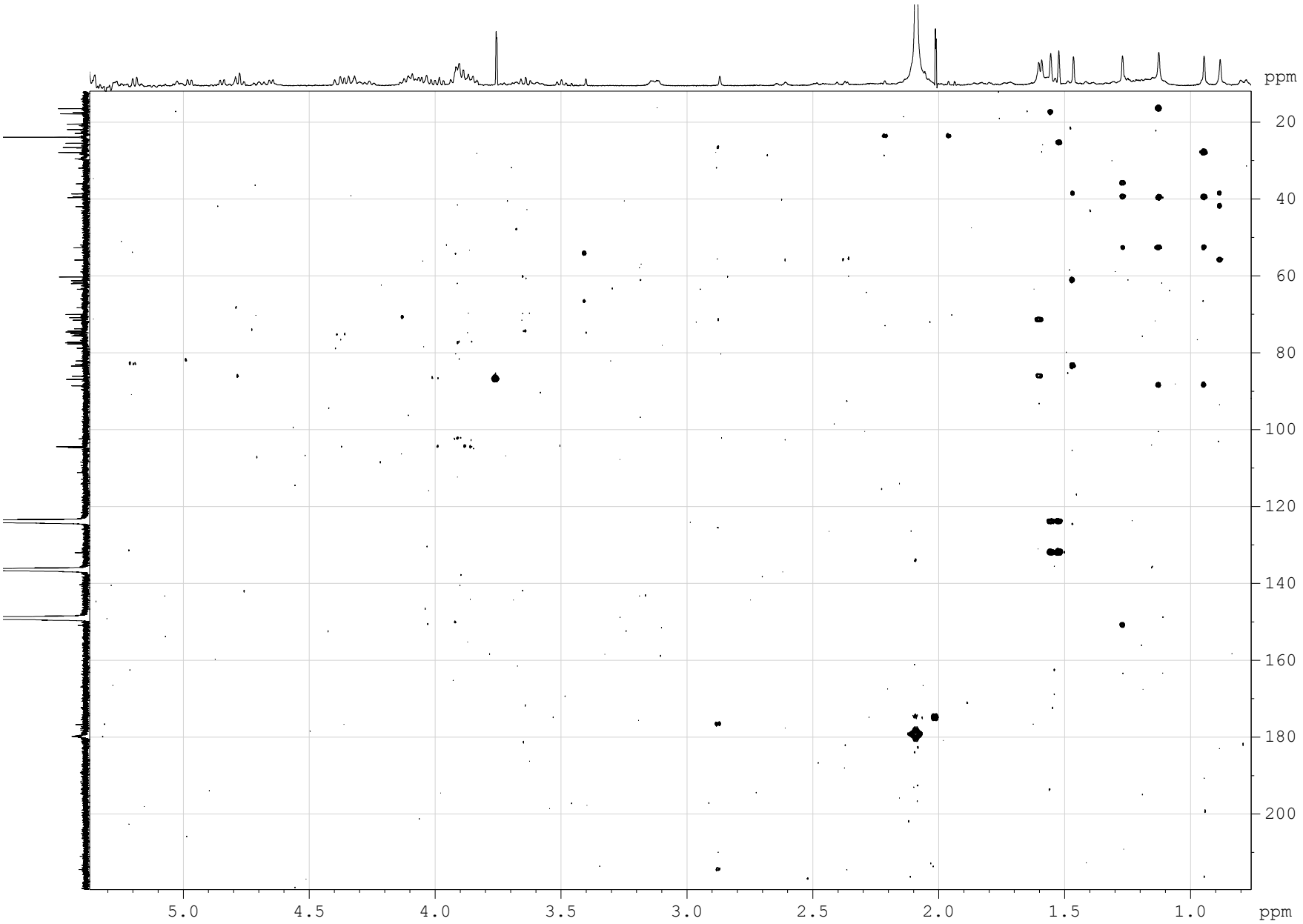



Figure S13. The HMBC (500.12 MHz) spectrum of chilensoside $A_1$ (**2**) in $C_5D_5N/D_2O$ (4/1)

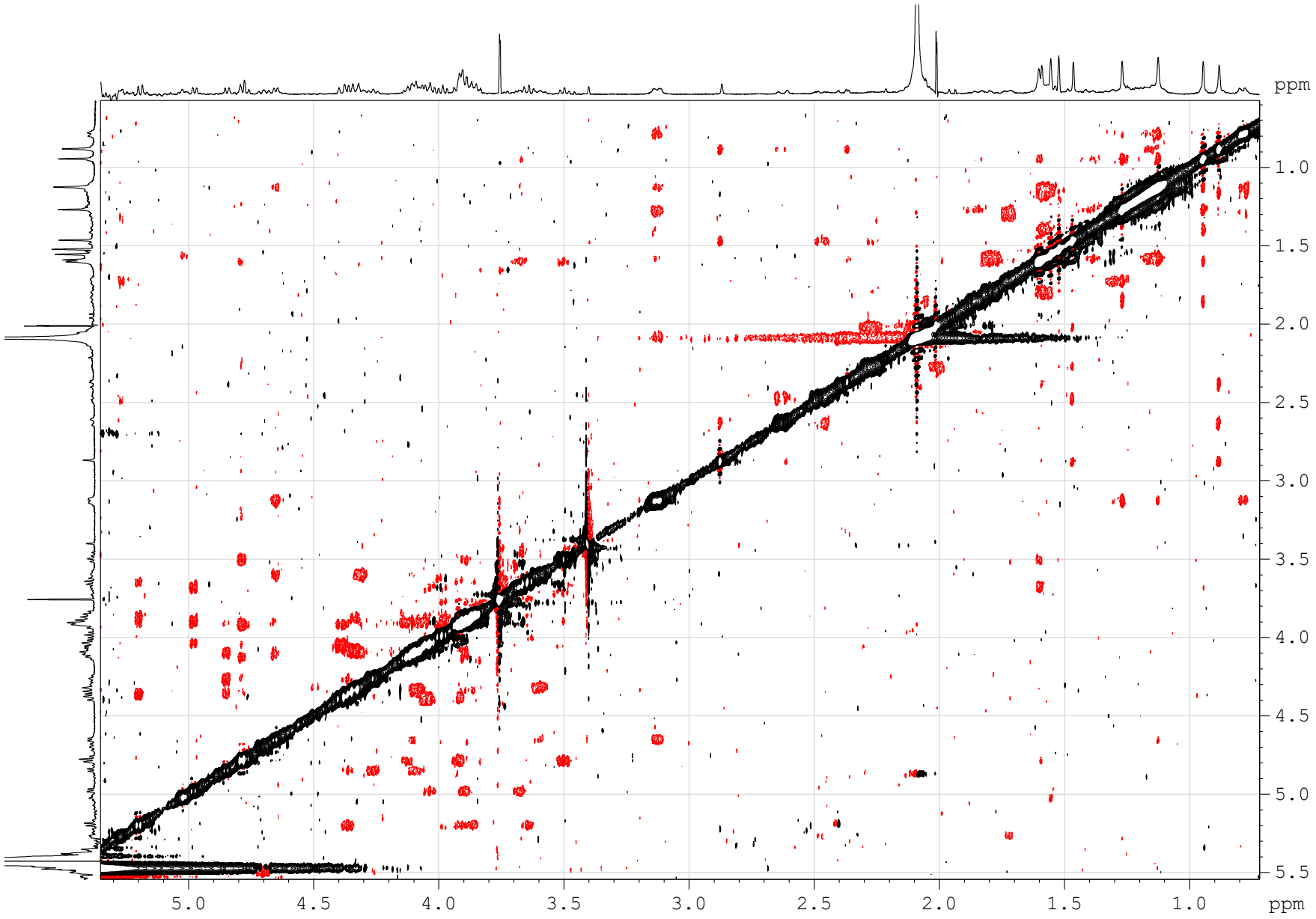



Figure S14. The ROESY (500.12 MHz) spectrum of chilensoside $A_1$ (**2**) in $C_5D_5N/D_2O$ (4/1)

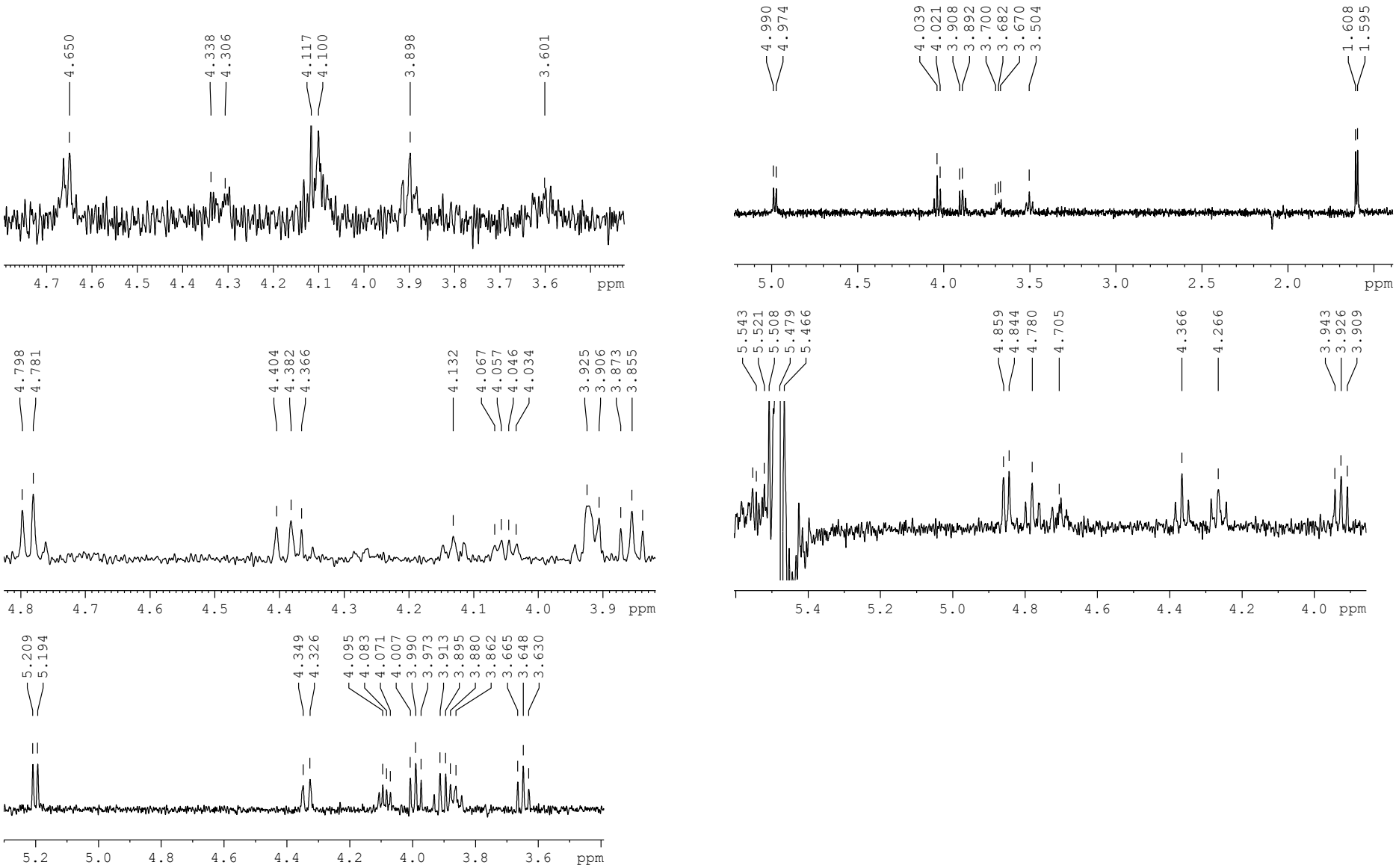
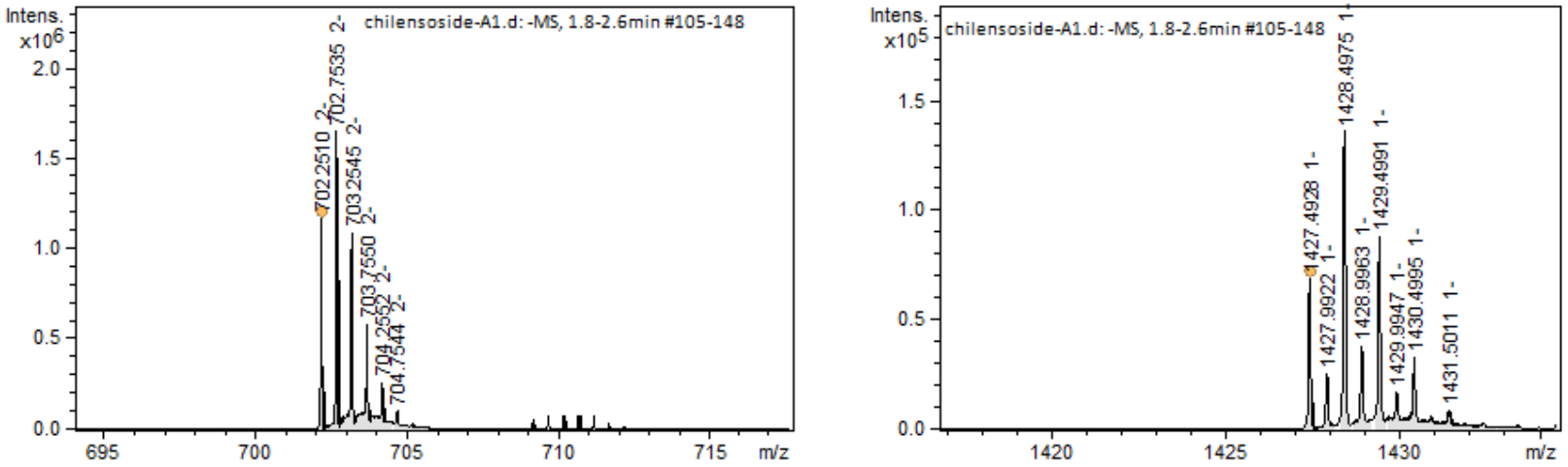



Figure S15. 1D TOCSY (500.12 MHz) spectra of Xyl1, Qui2, Glc3, Glc4, MeGlc5 of chilensoside $A_1$ (**2**) in $C_5D_5N/D_2O$ (4/1)




∗The isotopic composition in the HR-ESI-MS of chilensosides A–B (**1**–**3**), where the ion peak of $[M_{Na}+1]^-$ is more intensive, than that of $[M_{Na}]^-$ is explained by the easy exchange of the protons at C-15, adjacent to 16-oxo-group, to deuterium during the forced long-term storage of the samples in $C_5D_5N/D_2O$ for the registration of the NMR spectra.

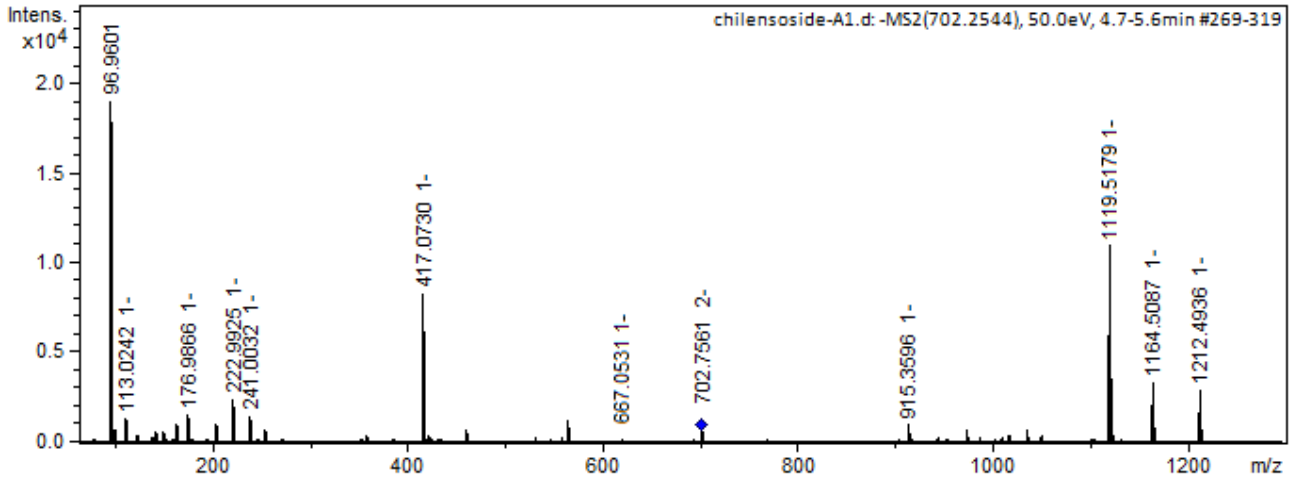



Figure S16. HR-ESI-MS and ESI-MS/MS spectra of chilensoside $A_1$ (**2**)

**Table S2.** $^{13}C$ and $^{1}H$ NMR chemical shifts, HMBC and ROESY correlations of the aglycone moiety of chilensoside B (**3**).

| Position | $\delta_C$ mult.[a] | $\delta_H$ mult. (*J* in Hz)[b] | HMBC | ROESY |
|---|---|---|---|---|
| 1 | 36.0 $CH_2$ | 1.73 m | | H-11 |
| | | 1.32 m | | H-3 |
| 2 | 26.6 $CH_2$ | 2.07 m | | |
| | | 1.86 m | | H-19, H-30 |
| 3 | 88.6 CH | 3.13 dd (4.7; 11.8) | | H-1, H-5, H-31, H1-Xyl1 |
| 4 | 39.4 C | | | |
| 5 | 52.6 CH | 0.79 brd (11.8) | C: 4, 19, 30 | H-1, H-3, H-7 |
| 6 | 20.8 $CH_2$ | 1.59 m | | |
| | | 1.39 m | | H-8, H-30 |
| 7 | 28.2 $CH_2$ | 1.58 m | | H-15 |
| | | 1.16 m | | H-5, H-32 |
| 8 | 38.6 CH | 3.13 m | | H-6 |
| 9 | 151.1 C | | | |
| 10 | 39.6 C | | | |
| 11 | 111.3 CH | 5.28 brs | C: 10, 13 | H-1 |
| 12 | 31.9 $CH_2$ | 2.64 brd (16.5) | C: 11, 18 | H-17 |
| | | 2.48 dd (5.9; 16.5) | C: 11, 14 | |
| 13 | 55.8 C | | | |
| 14 | 41.9 C | | | |
| 15 | 51.8 $CH_2$ | 2.40 d (16.0) | C: 13, 16, 17, 32 | |
| | | 2.10 d (16.0) | C: 14, 16, 32 | H-8 |
| 16 | 214.4 C | | | |
| 17 | 61.2 CH | 2.88 s | C: 12, 13, 16, 18, 20, 21 | H-12, H-23, H-32 |
| 18 | 176.6 C | | | |
| 19 | 21.8 $CH_3$ | 1.27 s | C: 1, 5, 9, 10 | H-1, H-2, H-8, H-30 |
| 20 | 83.4 C | | | |
| 21 | 26.5 $CH_3$ | 1.47 s | C: 17, 20, 22 | H-12, H-17, H-23 |
| 22 | 38.6 $CH_2$ | 1.80 m | | |
| | | 1.59 m | | |
| 23 | 22.9 $CH_2$ | 2.28 m | | |
| | | 2.04 m | | |
| 24 | 123.7 CH | 5.03 m | | H-22 |
| 25 | 132.0 C | | | |
| 26 | 25.4 $CH_3$ | 1.55 s | C: 24, 25, 27 | H-24 |
| 27 | 17.4 $CH_3$ | 1.52 s | C: 24, 25, 26 | H-23 |
| 30 | 16.4 $CH_3$ | 0.96 s | C: 3, 4, 5, 31 | H-2, H-6, H-19, H-31 |
| 31 | 27.8 $CH_3$ | 1.13 s | C: 3, 4, 5, 30 | H-3, H-5, H-6, H-30 |
| 32 | 20.5 $CH_3$ | 0.89 s | C: 8, 13, 14, 15 | H-7, H-12, H-15, H-17 |

[a] Recorded at 176.04 MHz in $C_5D_5N$. [b] Recorded at 700.13 MHz in $C_5D_5N$.

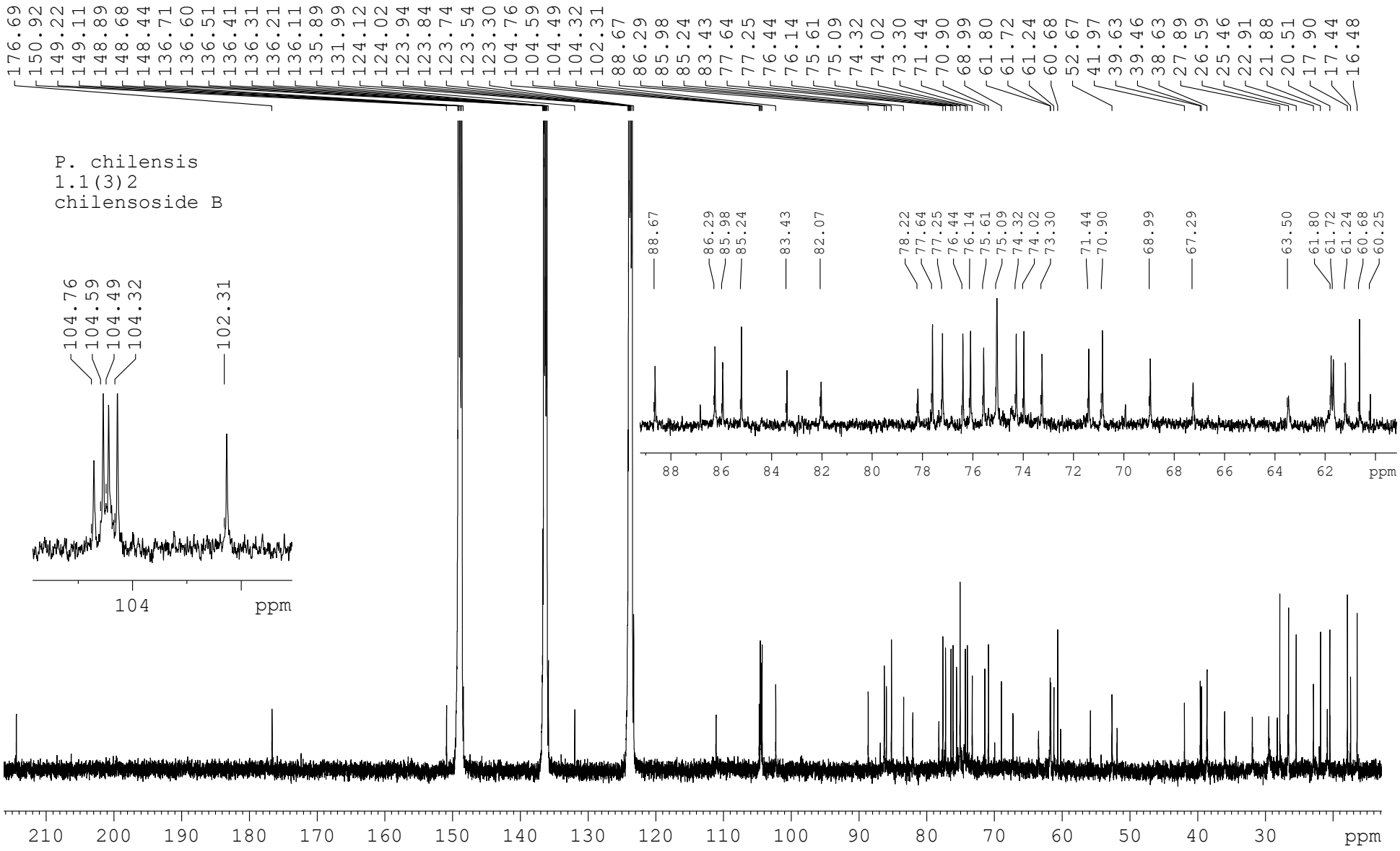



Figure S17. The $^{13}C$ NMR (176.04 MHz) spectrum of chilensoside B (**3**) in $C_5D_5N/D_2O$ (4/1)

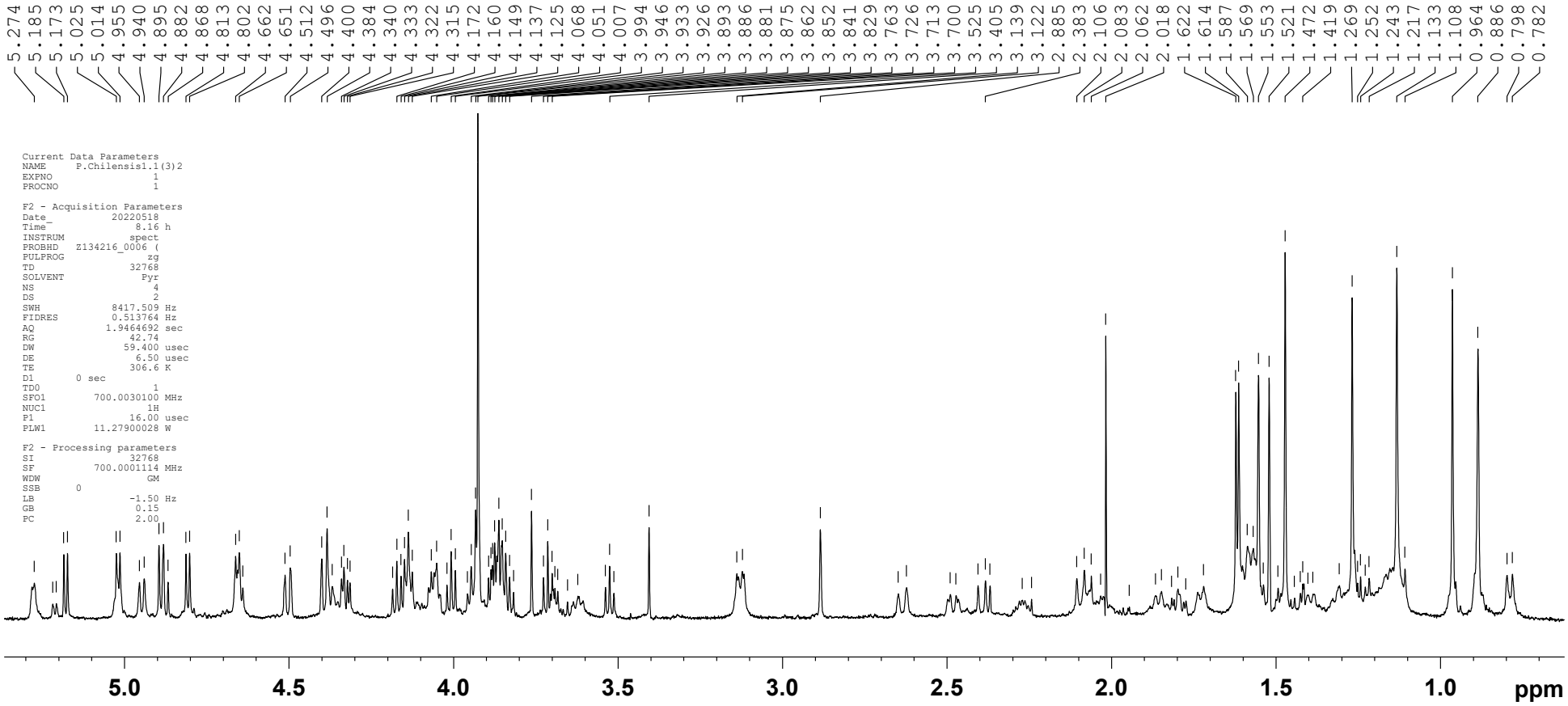



Figure S18. The $^{1}H$ NMR (700.13 MHz) spectrum of chilensoside B (**3**) in $C_5D_5N/D_2O$ (4/1)

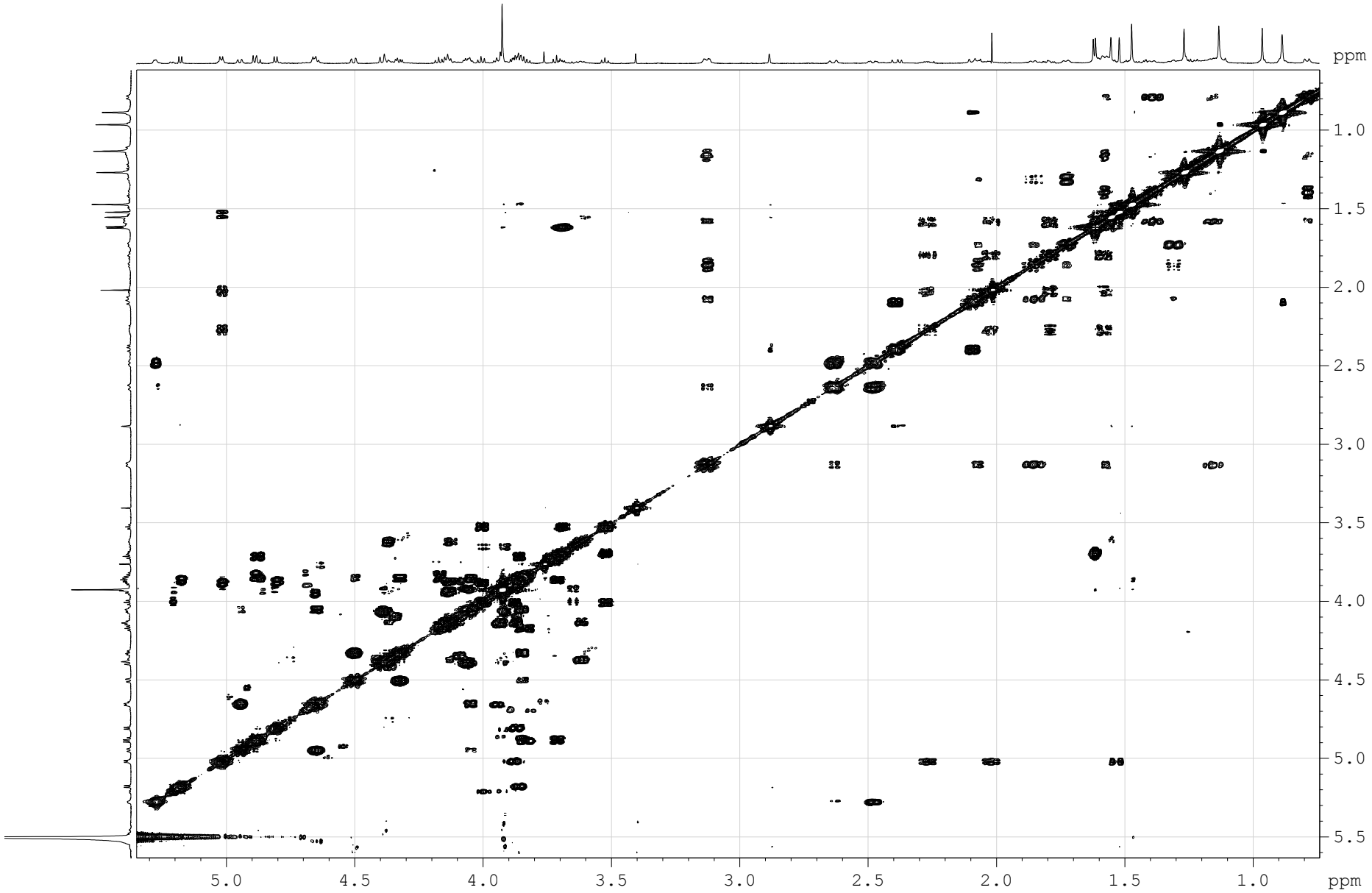



Figure S19. The COSY (700.13 MHz) spectrum of chilensoside B (**3**) in $C_5D_5N/D_2O$ (4/1)

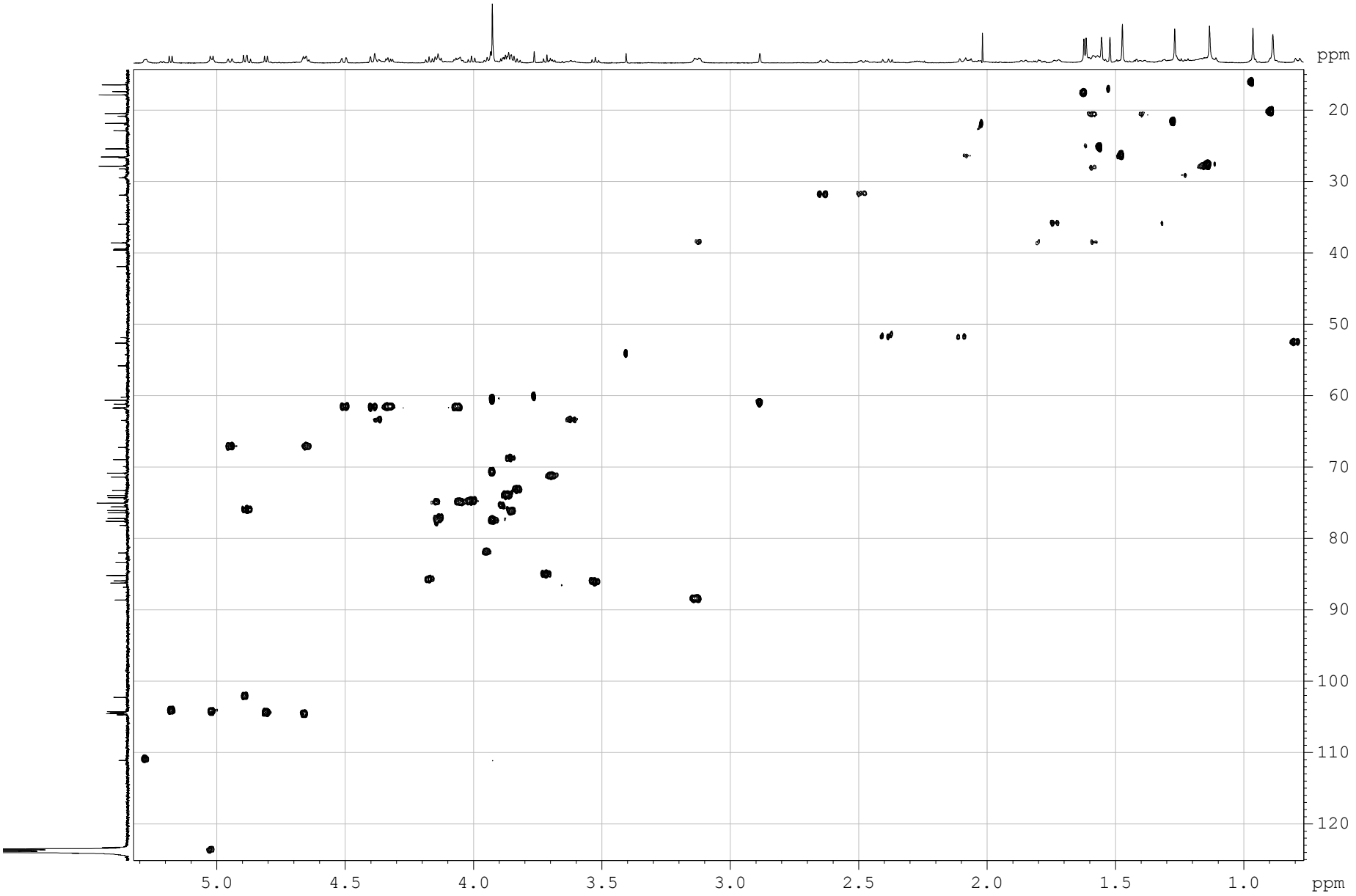



Figure S20. The HSQC (700.13 MHz) spectrum of chilensoside B (**3**) in $C_5D_5N/D_2O$ (4/1)

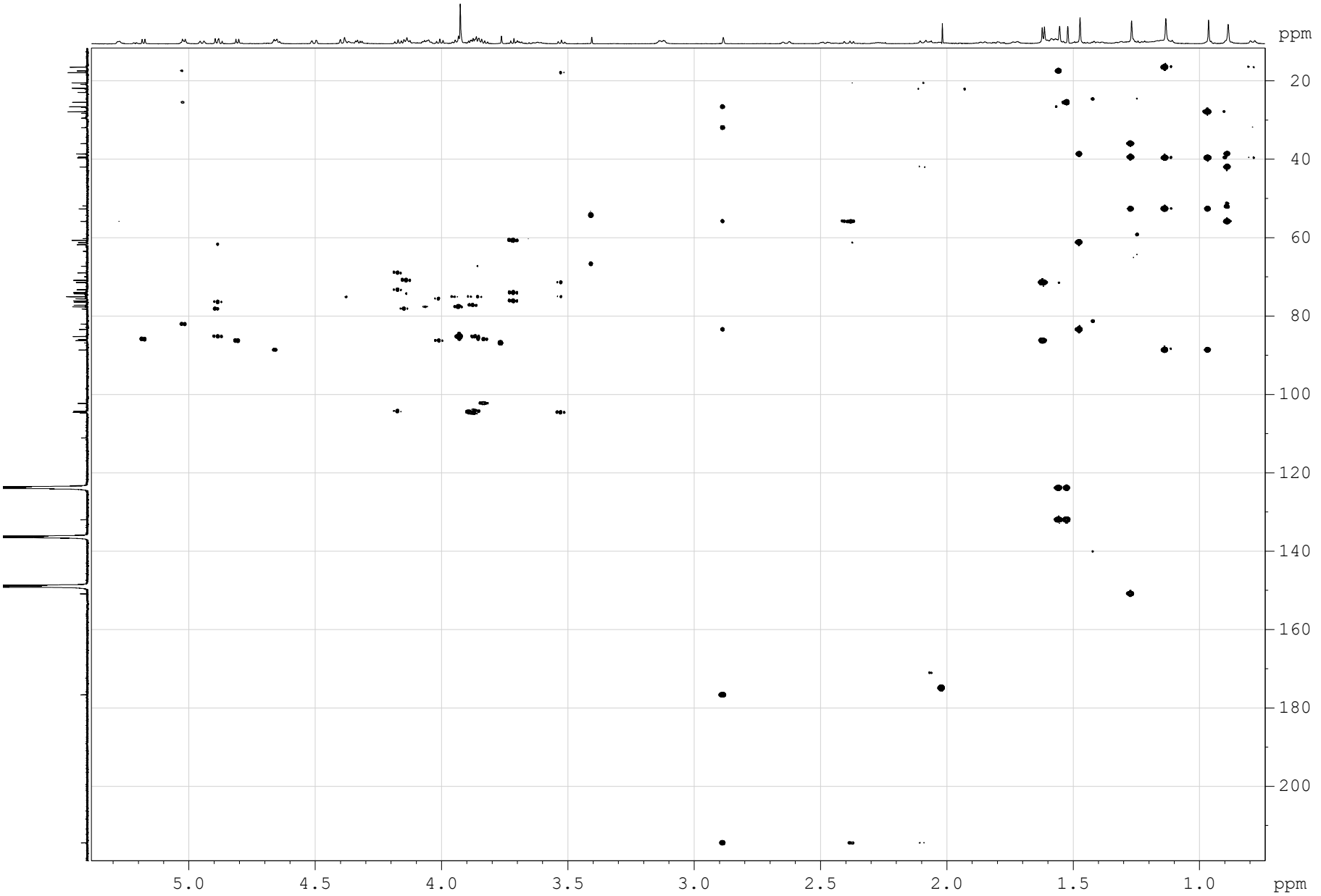



Figure S21. The HMBC (700.13 MHz) spectrum of chilensoside B (**3**) in $C_5D_5N/D_2O$ (4/1)

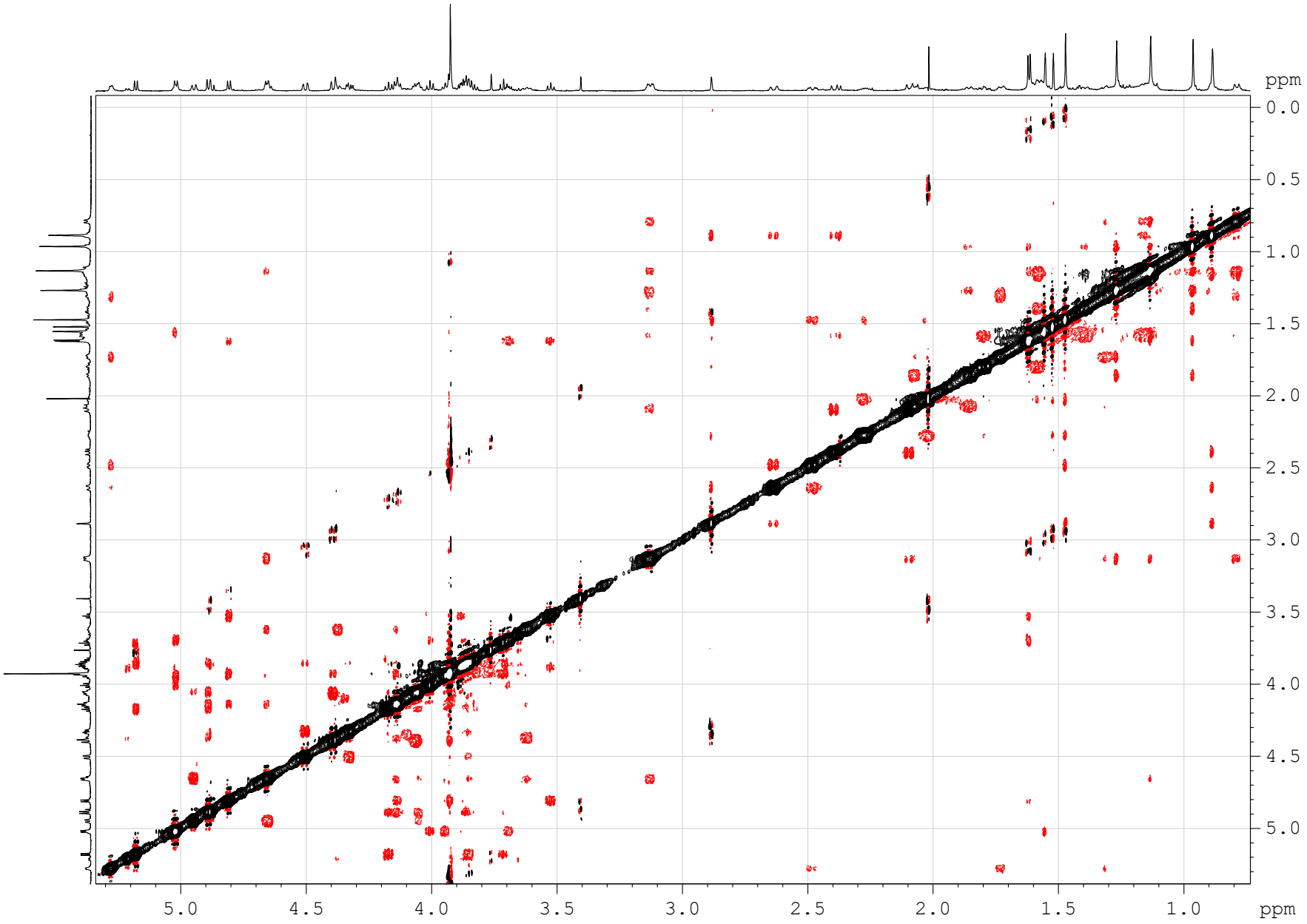



Figure S22. The ROESY (700.13 MHz) spectrum of chilensoside B (**3**) in $C_5D_5N/D_2O$ (4/1)

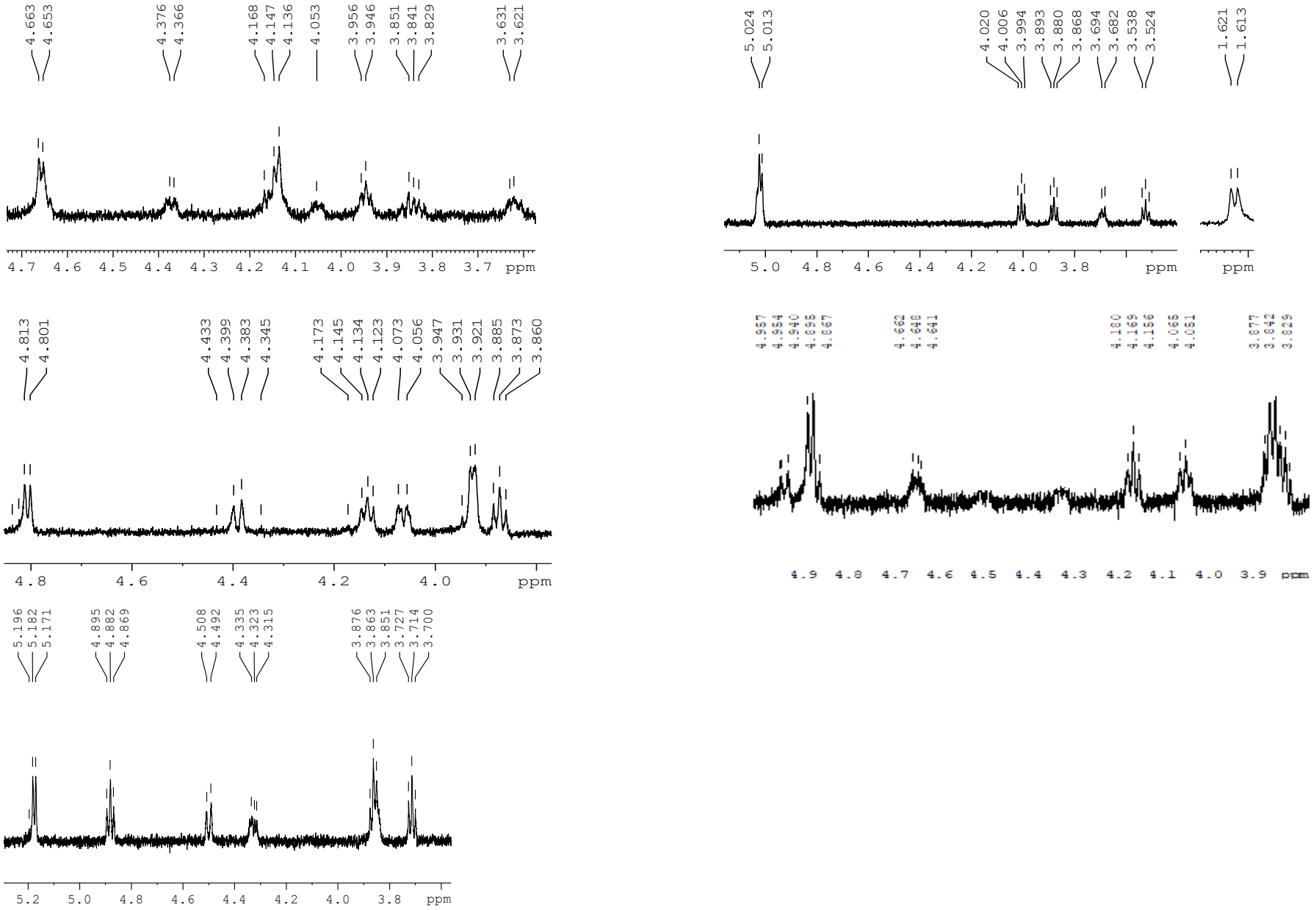
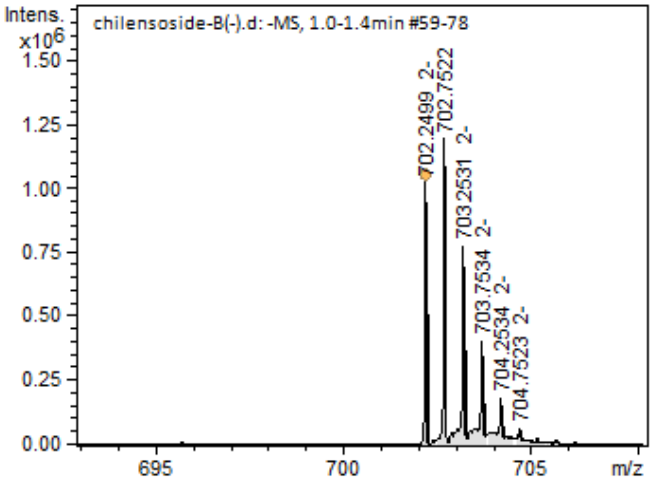



Figure S23. 1 D TOCSY (700.13 MHz) spectra of Xyl1, Qui2, Glc3, Glc4, MeGlc5 of chilensoside B (**3**) in $C_5D_5N/D_2O$ (4/1)




∗ The isotopic composition in the HR-ESI-MS of chilensosides A–B (**1**–**3**), where the ion peak of $[M_{Na}+1]^-$ is more intensive, than that of $[M_{Na}]^-$ is explained by the easy exchange of the protons at C-15, adjacent to 16-oxo-group, to deuterium during the forced long-term storage of the samples in $C_5D_5N/D_2O$ for the registration of the NMR spectra.

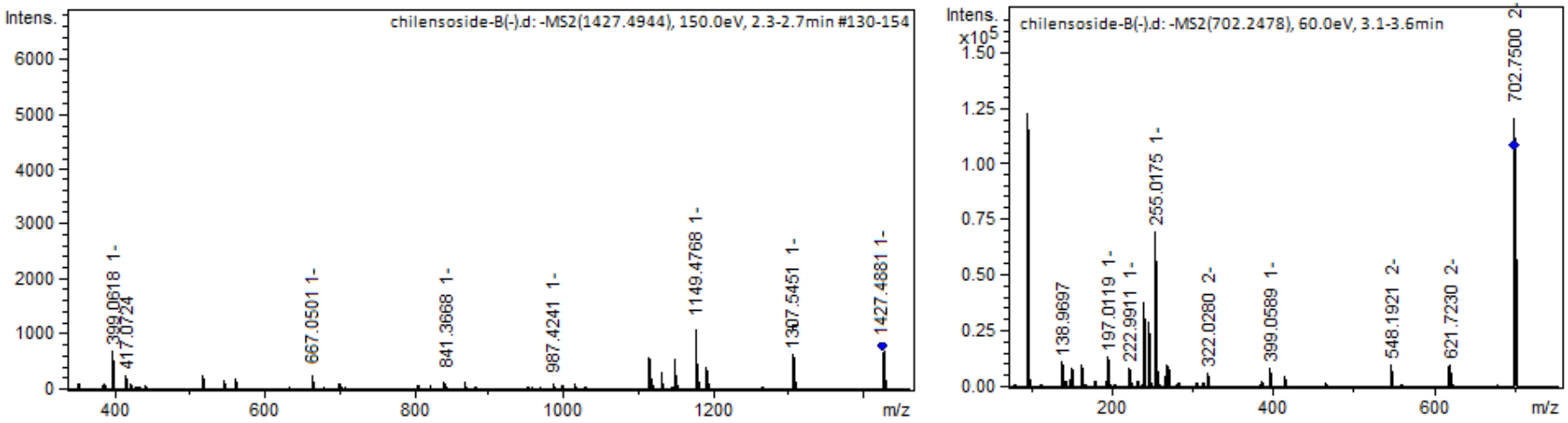



Figure S24. HR-ESI-MS and ESI-MS/MS spectra of chilensoside B (**3**)

**Table S3.** $^{13}C$ and $^{1}H$ NMR chemical shifts, HMBC and ROESY correlations of the aglycone moiety of chilensoside C (**4**).

| Position | $\delta_C$ mult.[a] | $\delta_H$ mult. (*J* in Hz)[b] | HMBC | ROESY |
|---|---|---|---|---|
| 1 | 36.0 $CH_2$ | 1.73 m | | H-11 |
| | | 1.31 m | | H-3 |
| 2 | 26.7 $CH_2$ | 2.06 m | | |
| | | 1.85 m | | H-19, H-30 |
| 3 | 88.6 CH | 3.13 dd (5.4; 10.9) | | H-1, H-5, H-31, H1-Xyl1 |
| 4 | 39.4 C | | | |
| 5 | 52.7 CH | 0.79 brd (10.9) | C: 4, 6, 19, 30 | H-1, H-3, H-7 |
| 6 | 20.9 $CH_2$ | 1.61 m | | |
| | | 1.41 m | | H-8, H-30 |
| 7 | 28.3 $CH_2$ | 1.60 m | | H-15 |
| | | 1.17 m | | H-5, H-32 |
| 8 | 38.6 CH | 3.13 m | | H-6, H-15, H-19 |
| 9 | 151.0 C | | | |
| 10 | 39.6 C | | | |
| 11 | 111.2 CH | 5.27 brd (5.8) | C: 10, 13 | H-1 |
| 12 | 31.9 $CH_2$ | 2.63 brd (16.8) | C: 11, 18 | H-17, H-32 |
| | | 2.47 dd (5.8; 16.8) | C: 11, 14 | H-17, H-21 |
| 13 | 55.8 C | | | |
| 14 | 42.0 C | | | |
| 15 | 51.9 $CH_2$ | 2.40 d (15.4) | C: 13, 16, 17, 32 | |
| | | 2.11 d (15.4) | C: 14, 16, 32 | H-8 |
| 16 | 214.4 C | | | |
| 17 | 61.2 CH | 2.87 s | C: 12, 13, 16, 18, 20, 21 | H-12, H-23, H-32 |
| 18 | 176.7 C | | | |
| 19 | 21.9 $CH_3$ | 1.28 s | C: 1, 5, 9, 10 | H-1, H-2, H-8, H-30 |
| 20 | 83.4 C | | | |
| 21 | 26.6 $CH_3$ | 1.47 s | C: 17, 20, 22 | H-12, H-17, H-23 |
| 22 | 38.6 $CH_2$ | 1.80 m | | |
| | | 1.59 m | | |
| 23 | 22.9 $CH_2$ | 2.28 m | | H-21 |
| | | 2.04 m | | |
| 24 | 123.8 CH | 5.03 m | | H-22 |
| 25 | 132.0 C | | | |
| 26 | 25.5 $CH_3$ | 1.55 s | C: 24, 25, 27 | H-24 |
| 27 | 17.4 $CH_3$ | 1.52 s | C: 24, 25, 26 | H-23 |
| 30 | 16.4 $CH_3$ | 0.95 s | C: 3, 4, 5, 31 | H-2, H-6, H-19, H-31 |
| 31 | 27.9 $CH_3$ | 1.13 s | C: 3, 4, 5, 30 | H-3, H-5, H-6, H-30 |
| 32 | 20.5 $CH_3$ | 0.88 s | C: 8, 13, 14, 15 | H-7, H-12, H-15, H-17 |

[a] Recorded at 176.04 MHz in $C_5D_5N$. [b] Recorded at 700.13 MHz in $C_5D_5N$.

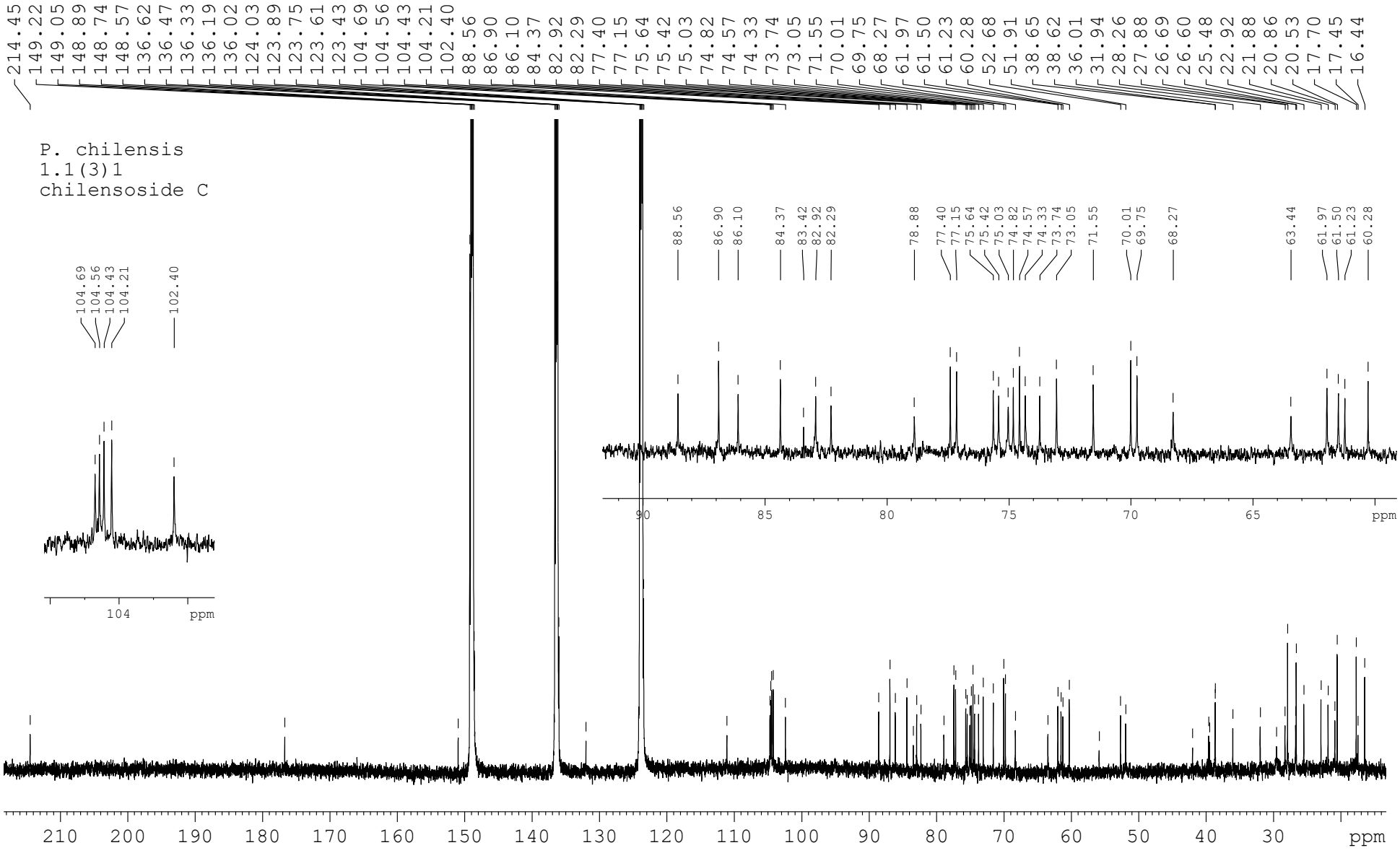



Figure S25. The $^{13}C$ NMR (176.04 MHz) spectrum of chilensoside C (**4**) in $C_5D_5N/D_2O$ (4/1)

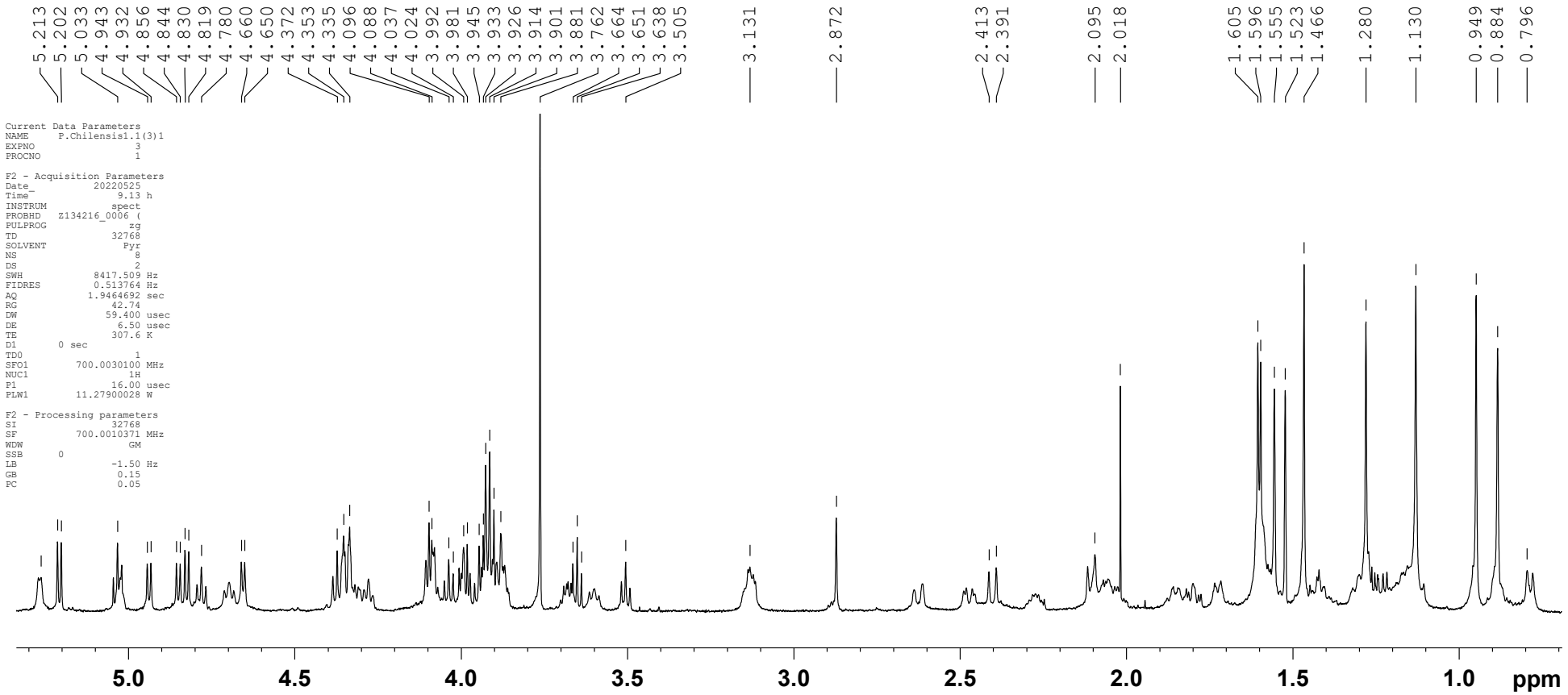



Figure S26. The $^{1}$H NMR (700.13 MHz) spectrum of chilensoside C (**4**) in $C_5D_5N/D_2O$ (4/1)

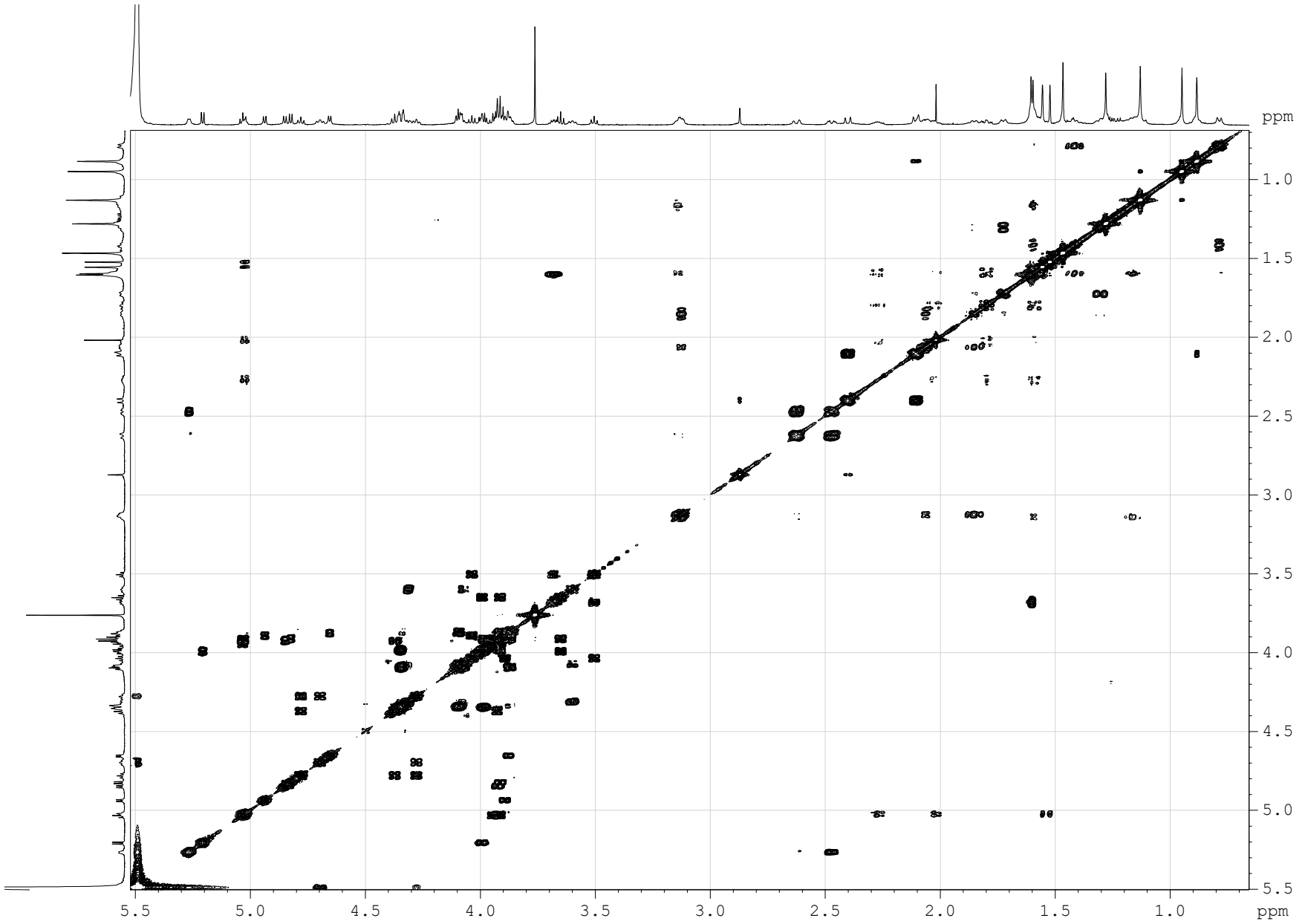



Figure S27. The COSY (700.13 MHz) spectrum of chilensoside C (**4**) in $C_5D_5N/D_2O$ (4/1)

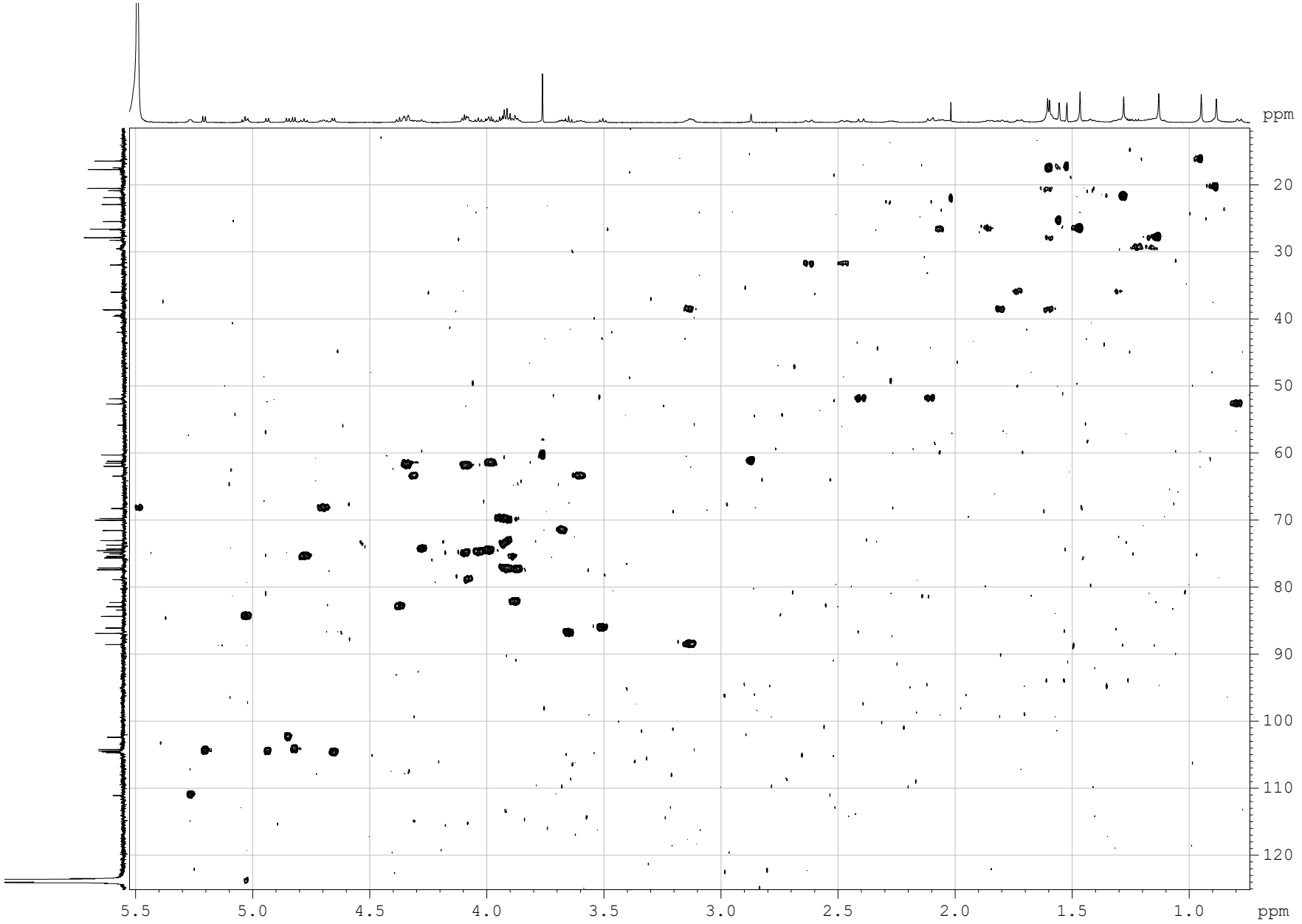



Figure S28. The HSQC (700.13 MHz) spectrum of chilensoside C (**4**) in $C_5D_5N/D_2O$ (4/1)

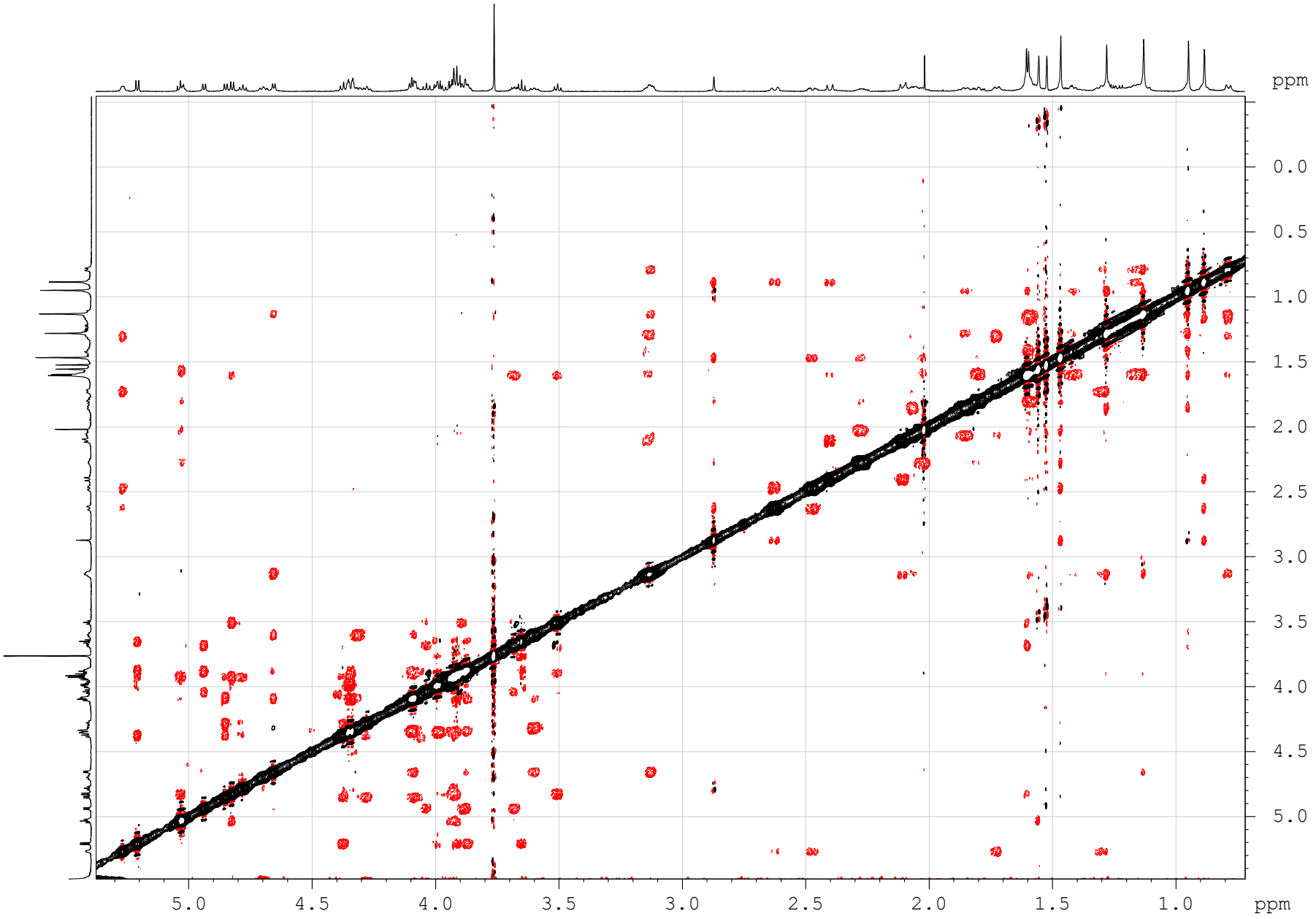



Figure S29. The ROESY (700.13 MHz) spectrum of chilensoside C (**4**) in $C_5D_5N/D_2O$ (4/1)

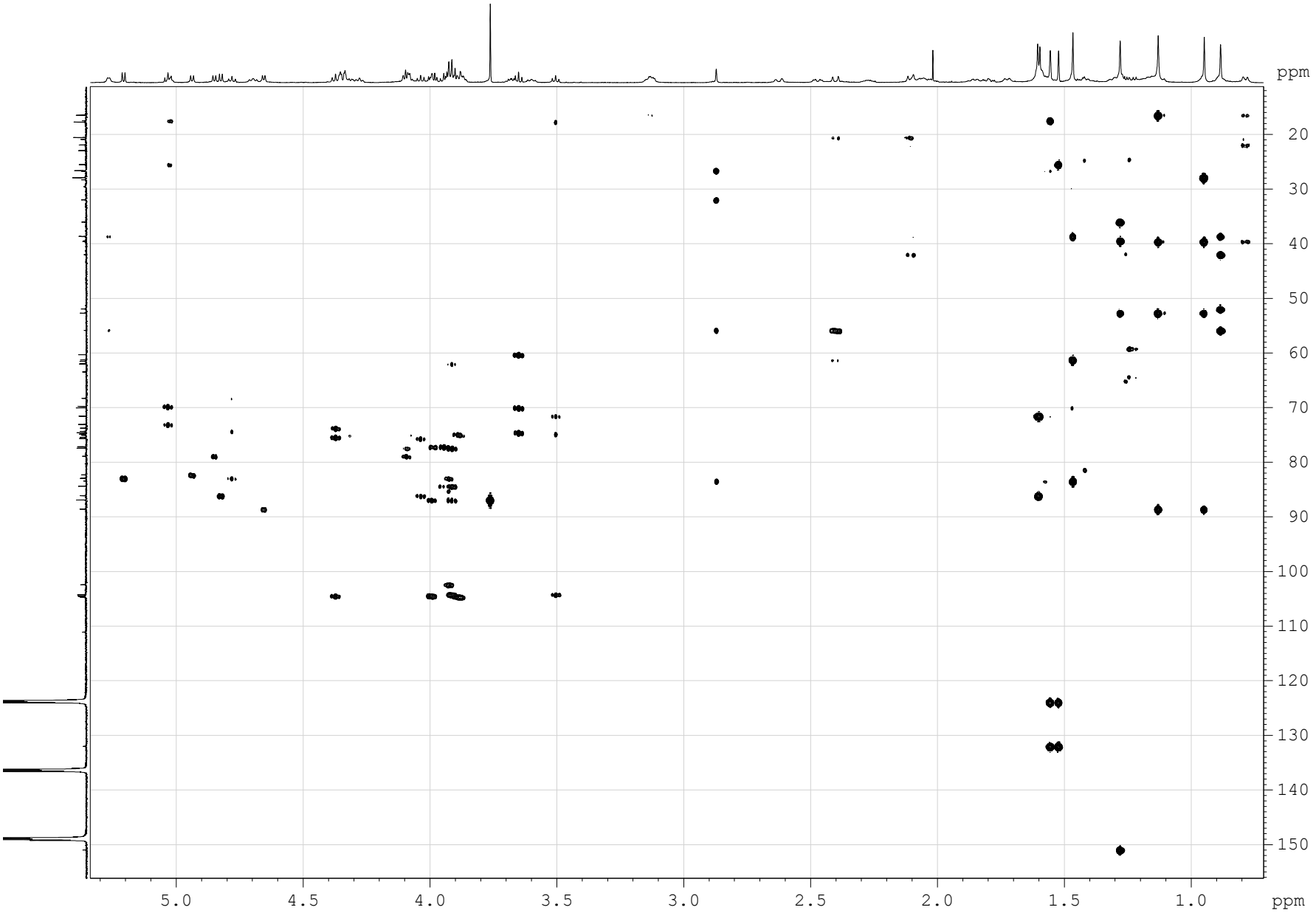



Figure S30. The HMBC (500.12 MHz) spectrum of chilensoside C (**4**) in $C_5D_5N/D_2O$ (4/1)

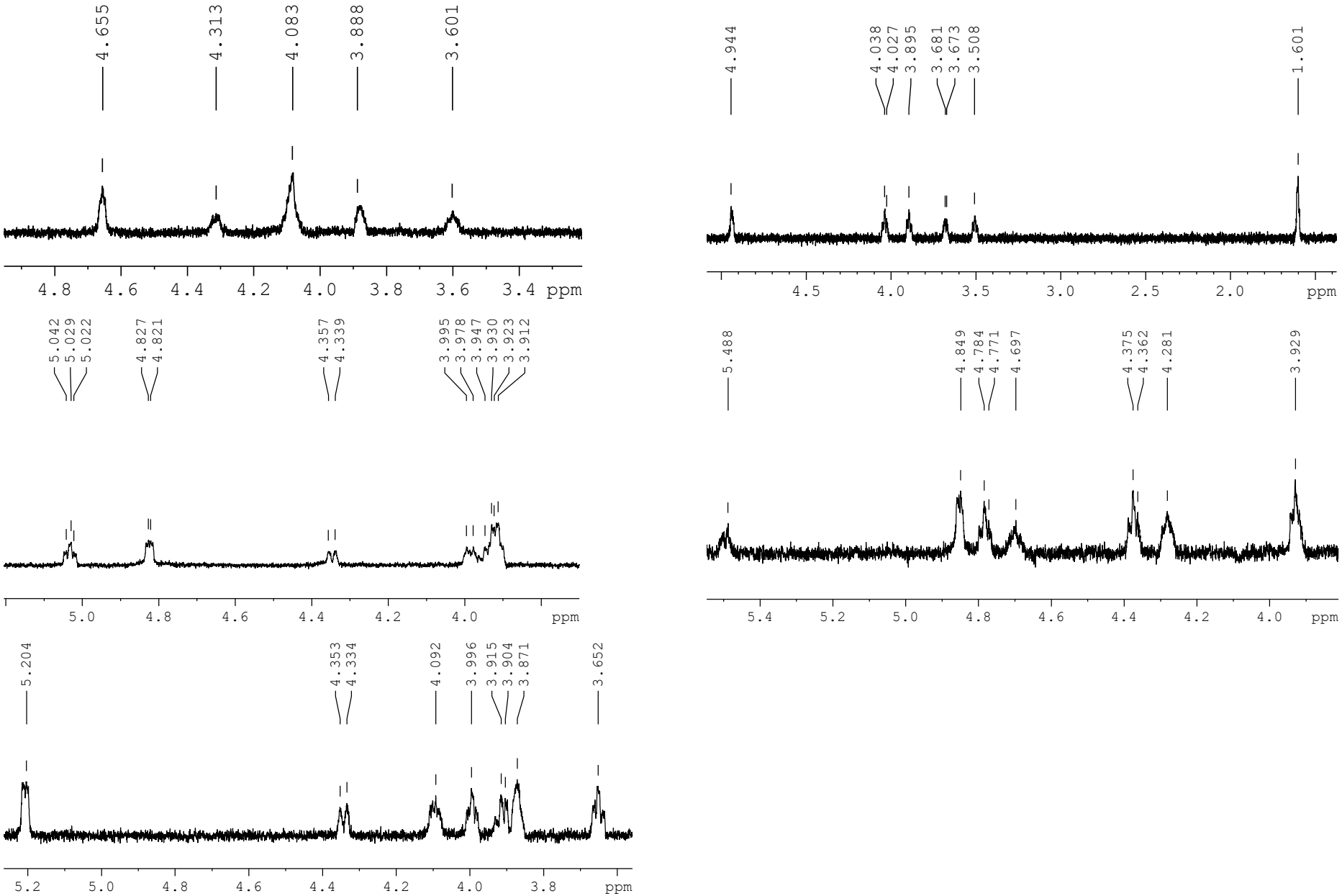



Figure S31. 1D TOCSY (700.13 MHz) spectra of Xyl1, Qui2, Glc3, Glc4, MeGlc5 of chilensosideC (**4**) in $C_5D_5N/D_2O$ (4/1)

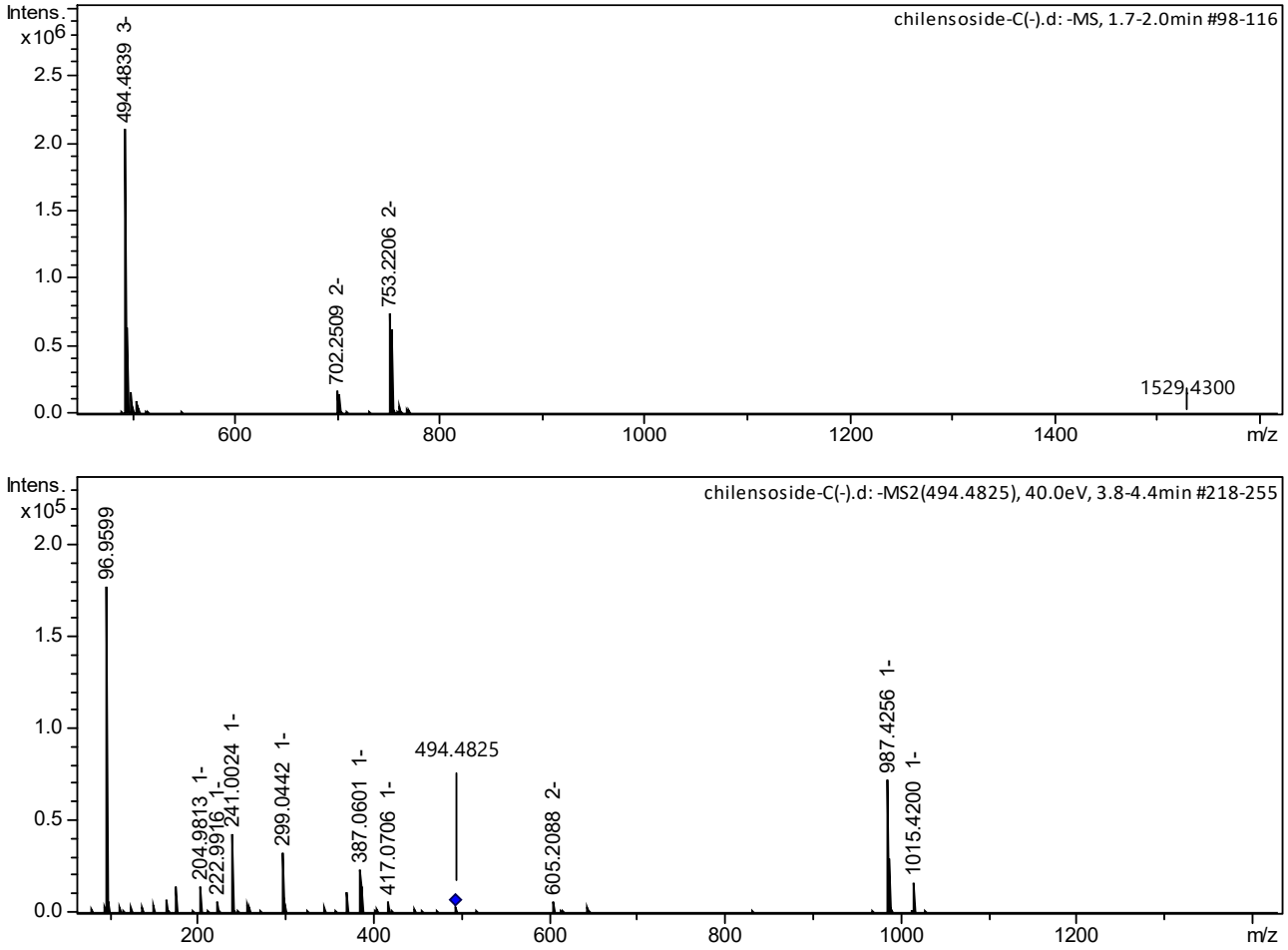



Figure S32. HR-ESI-MS and ESI-MS/MS spectra of chilensosideC (**4**)

**Table S4.** $^{13}C$ and $^{1}H$ NMR chemical shifts, HMBC and ROESY correlations of the aglycone moiety of chilensoside D (**5**).

| Position | $\delta_C$ mult.[a] | $\delta_H$ mult. (*J* in Hz)[b] | HMBC | ROESY |
|---|---|---|---|---|
| 1 | 36.0 $CH_2$ | 1.73 m | | H-11 |
| | | 1.32 m | | H-3 |
| 2 | 26.7 $CH_2$ | 2.06 m | | |
| | | 1.83 m | | H-19, H-30 |
| 3 | 88.3 CH | 3.11 dd (4.4; 11.5) | | H-1, H-5, H-31, H1-Xyl1 |
| 4 | 39.4 C | | | |
| 5 | 52.5 CH | 0.78 brd (11.5) | C: 4, 6, 19, 30 | H-1, H-3, H-7 |
| 6 | 20.9 $CH_2$ | 1.59 m | | |
| | | 1.40 m | | H-8, H-30 |
| 7 | 28.3 $CH_2$ | 1.60 m | | H-15 |
| | | 1.17 m | | H-5, H-32 |
| 8 | 38.7 CH | 3.13 m | | H-6, H-15, H-19 |
| 9 | 150.8 C | | | |
| 10 | 39.4 C | | | |
| 11 | 111.2 CH | 5.29 brd (5.2) | C: 10, 13 | H-1 |
| 12 | 31.9 $CH_2$ | 2.65 brd (17.1) | C: 11, 18 | H-17, H-32 |
| | | 2.49 dd (6.0; 17.1) | C: 11, 14 | H-17, H-21 |
| 13 | 55.8 C | | | |
| 14 | 42.0 C | | | |
| 15 | 51.9 $CH_2$ | 2.41 d (15.6) | C: 13, 16, 17, 32 | |
| | | 2.10 d (15.6) | C: 14, 16, 32 | H-8 |
| 16 | 214.6 C | | | |
| 17 | 61.2 CH | 2.90 s | C: 12, 13, 16, 18, 20, 21 | H-12, H-23, H-32 |
| 18 | 176.7 C | | | |
| 19 | 21.9 $CH_3$ | 1.29 s | C: 1, 5, 9, 10 | H-1, H-2, H-8, H-30 |
| 20 | 83.5 C | | | |
| 21 | 26.6 $CH_3$ | 1.48 s | C: 17, 20, 22 | H-12, H-17, H-23 |
| 22 | 38.6 $CH_2$ | 1.80 m | | |
| | | 1.60 m | | |
| 23 | 22.9 $CH_2$ | 2.29 m | | H-21 |
| | | 2.04 m | | |
| 24 | 124.0 CH | 5.03 m | | H-22 |
| 25 | 132.1 C | | | |
| 26 | 25.5 $CH_3$ | 1.55 s | C: 24, 25, 27 | H-24 |
| 27 | 17.4 $CH_3$ | 1.52 s | C: 24, 25, 26 | H-23 |
| 30 | 16.4 $CH_3$ | 0.90 s | C: 3, 4, 5, 31 | H-2, H-6, H-19, H-31 |
| 31 | 27.9 $CH_3$ | 1.10 s | C: 3, 4, 5, 30 | H-3, H-5, H-6, H-30 |
| 32 | 20.5 $CH_3$ | 0.89 s | C: 8, 13, 14, 15 | H-7, H-12, H-15, H-17 |

[a] Recorded at 125.67 MHz in $C_5D_5N$. [b] Recorded at 500.12 MHz in $C_5D_5N$.

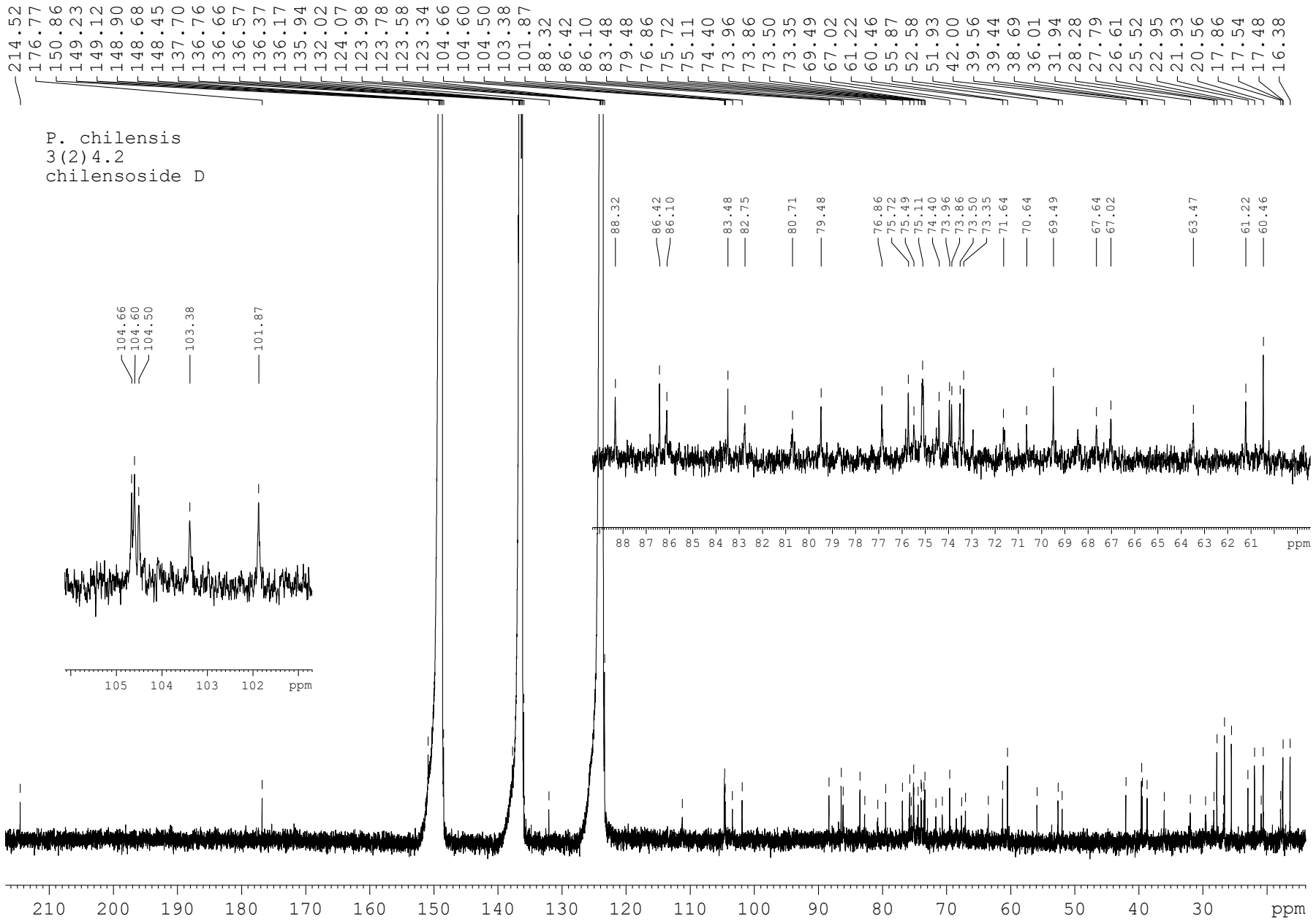



Figure S33. The $^{13}C$ NMR (125.67 MHz) spectrum of chilensoside D (**5**) in $C_5D_5N/D_2O$ (4/1)

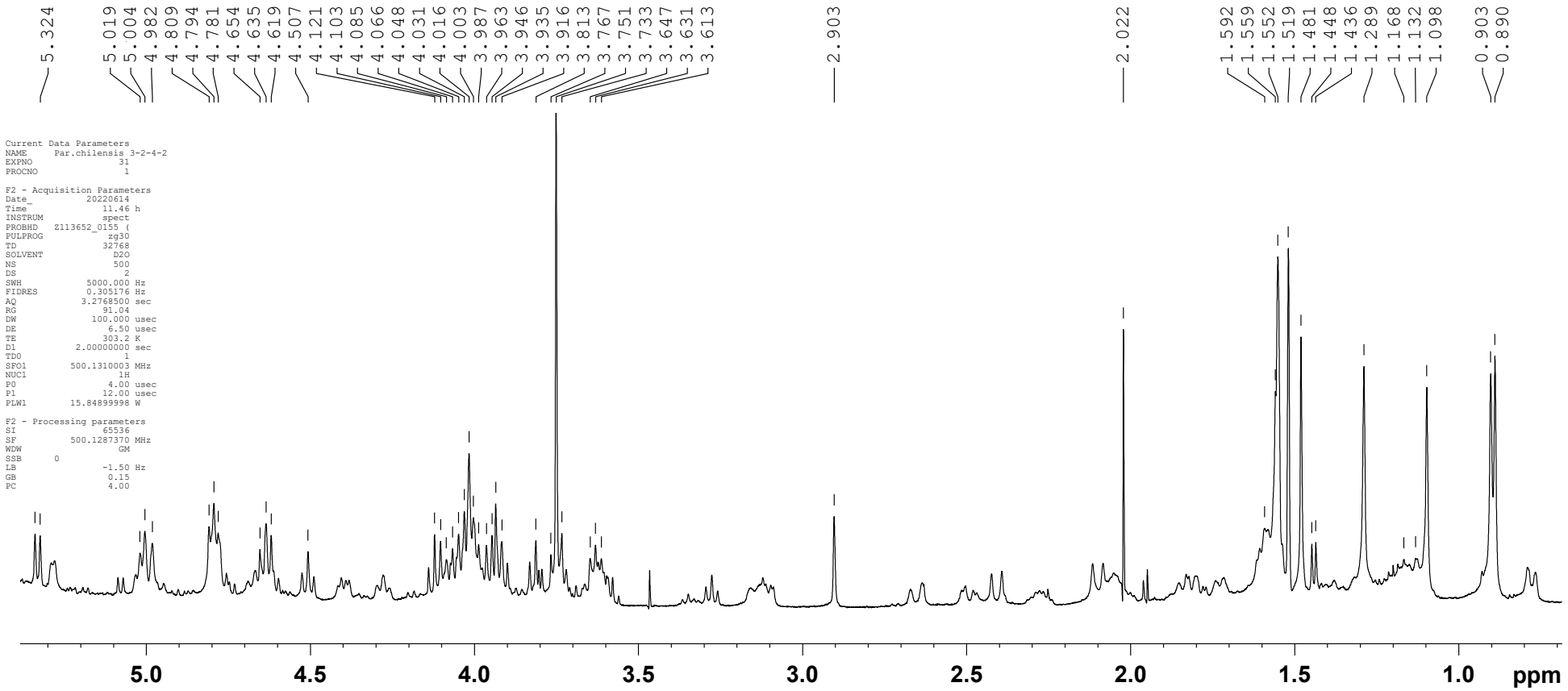



Figure S34. The $^{1}$H NMR (500.12 MHz) spectrum of chilensoside D (**5**) in $C_5D_5N/D_2O$ (4/1)

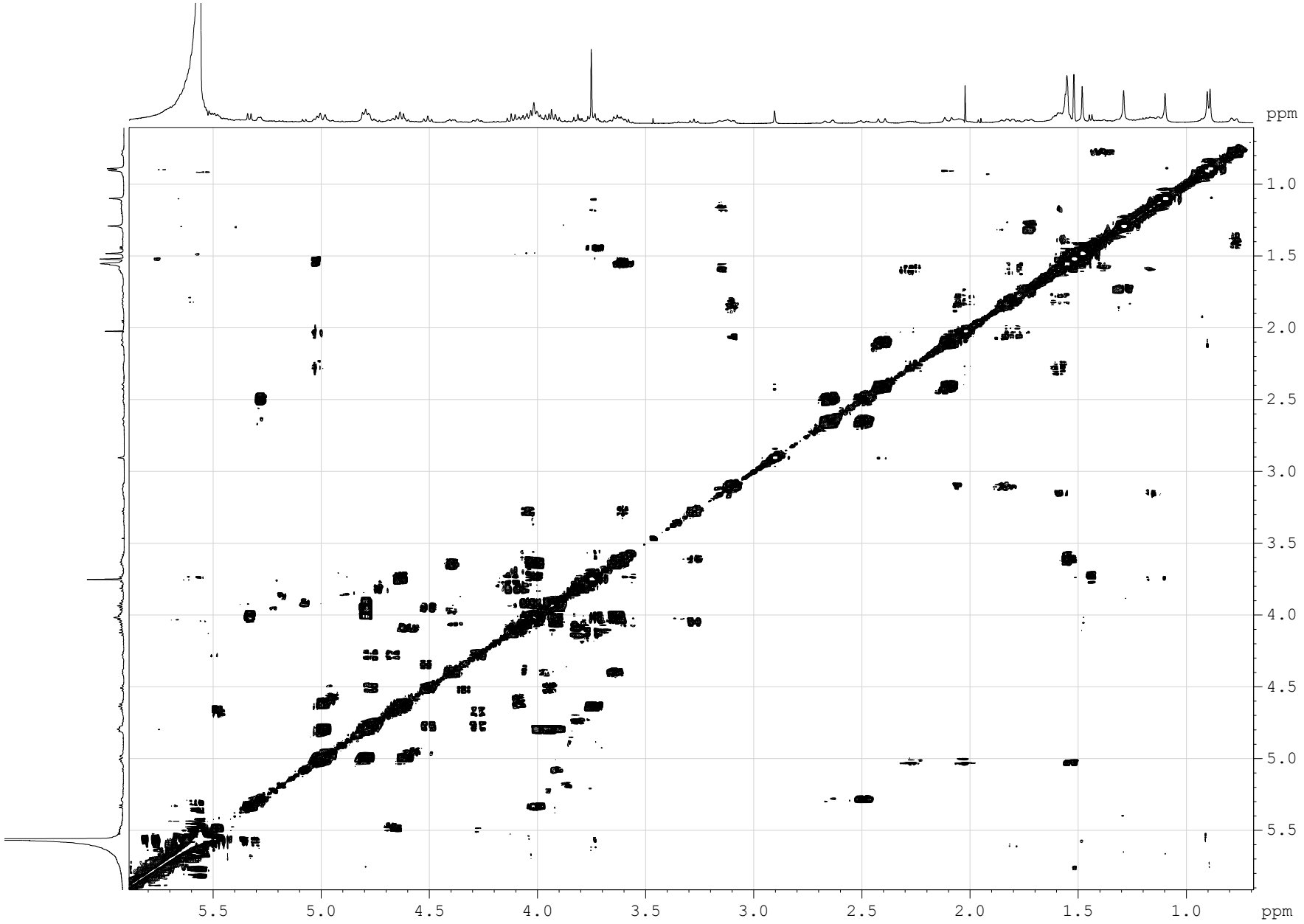



Figure S35. The COSY (500.12 MHz) spectrum of chilensoside D (**5**) in $C_5D_5N/D_2O$ (4/1)

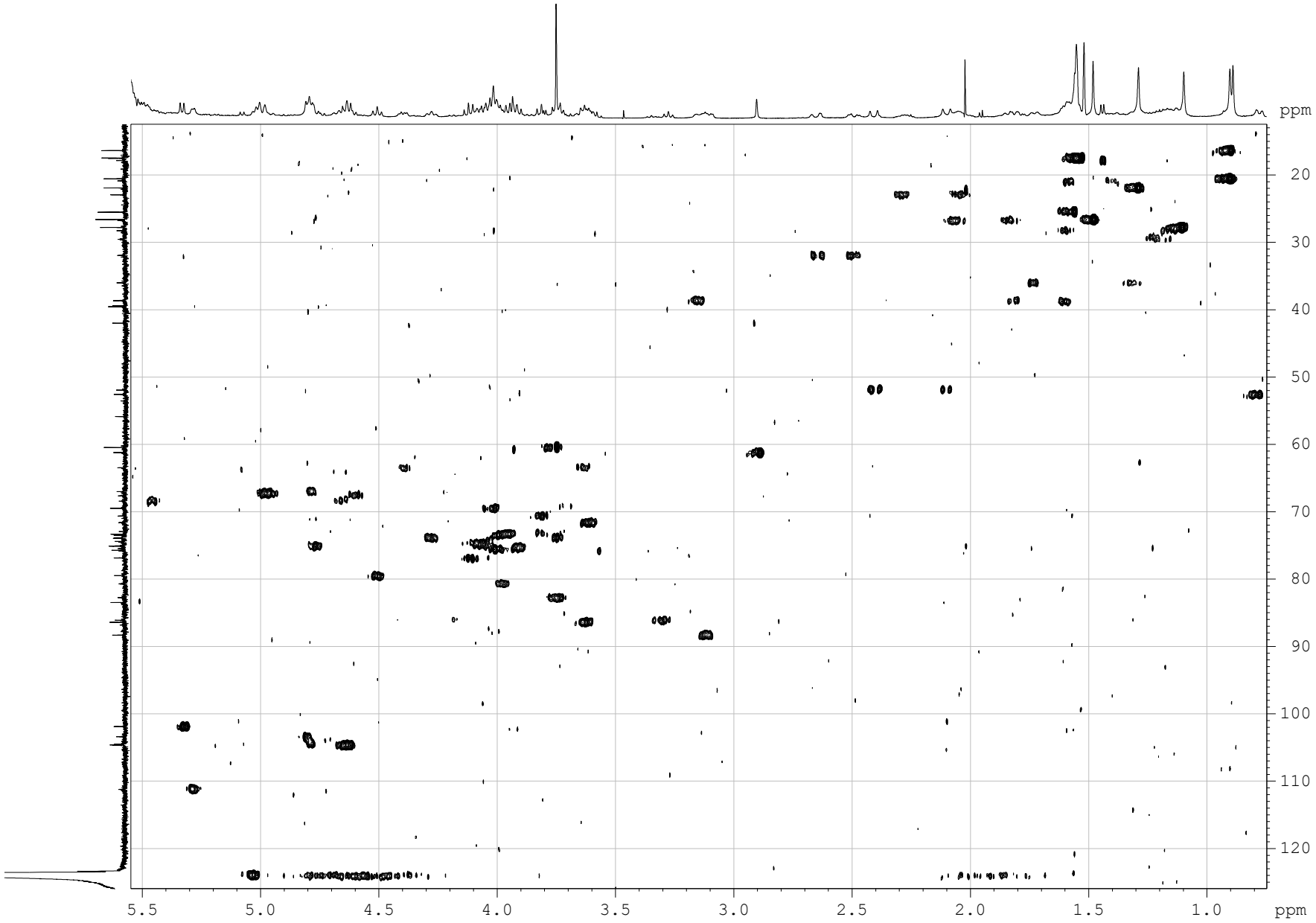



Figure S36. The HSQC (500.12 MHz) spectrum of chilensoside D (**5**) in $C_5D_5N/D_2O$ (4/1)

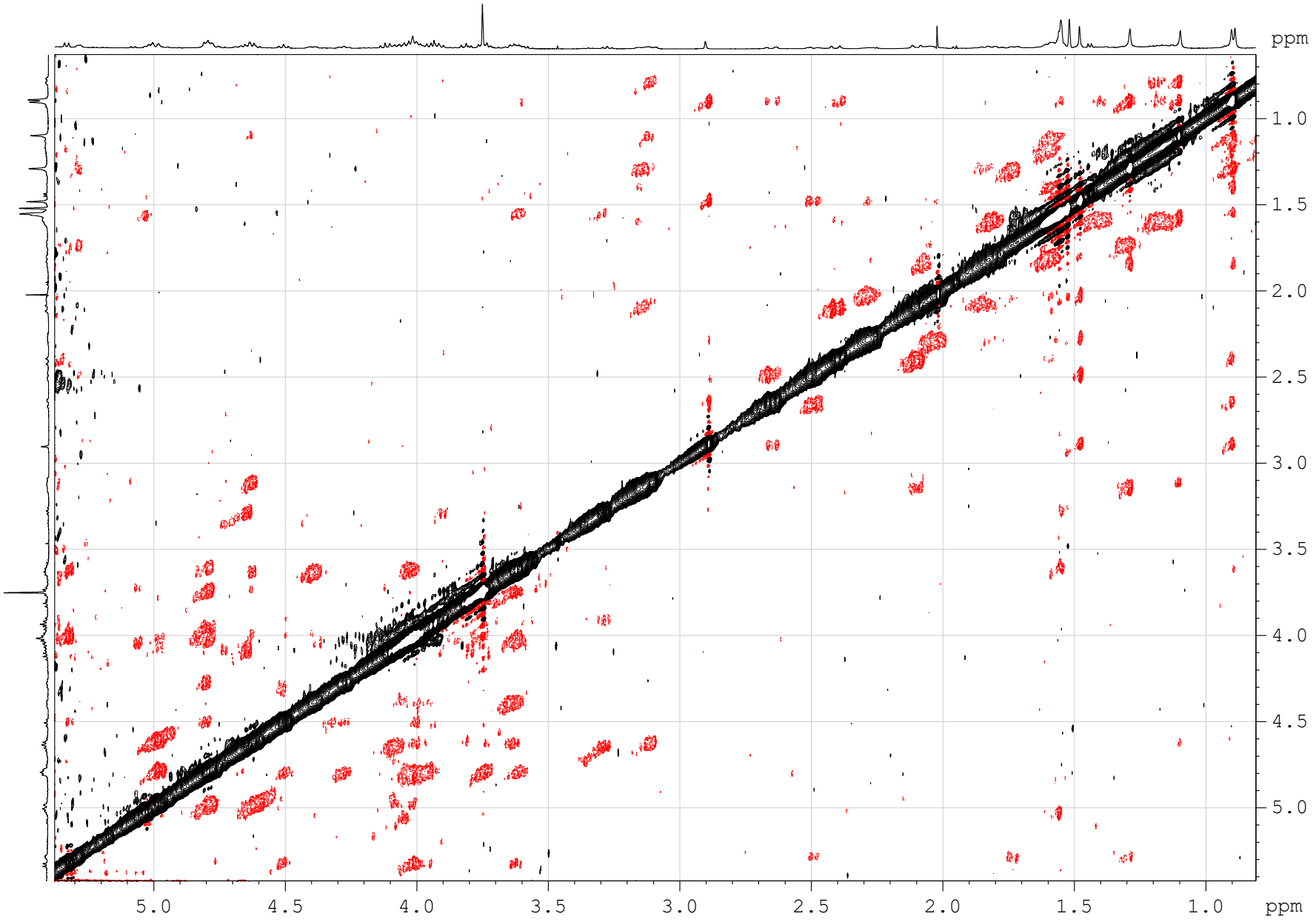



Figure S37. The ROESY (500.12 MHz) spectrum of chilensoside D (**5**) in $C_5D_5N/D_2O$ (4/1)

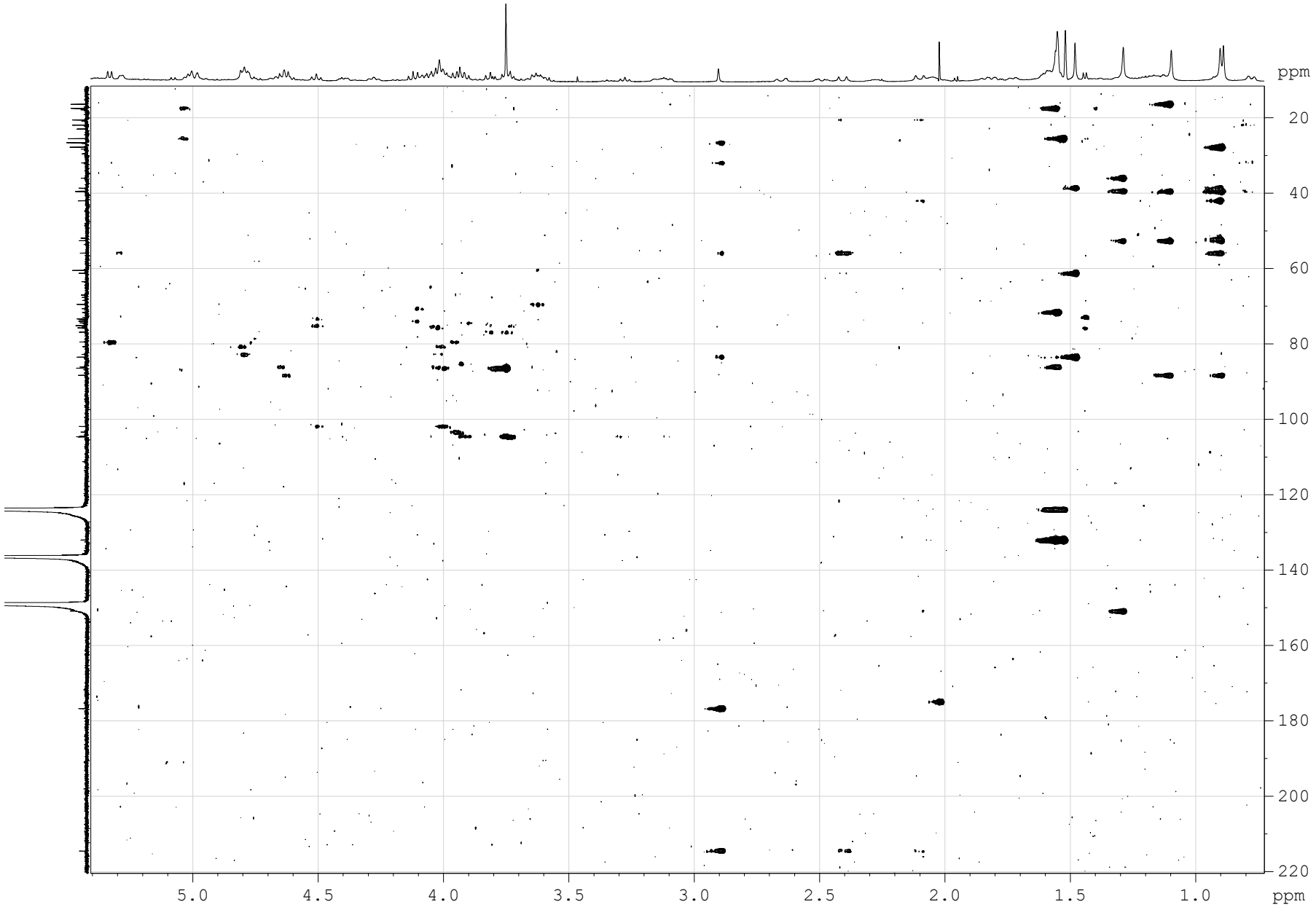



Figure S38. The HMBC (500.12 MHz) spectrum of chilensoside D (**5**) in $C_5D_5N/D_2O$ (4/1)

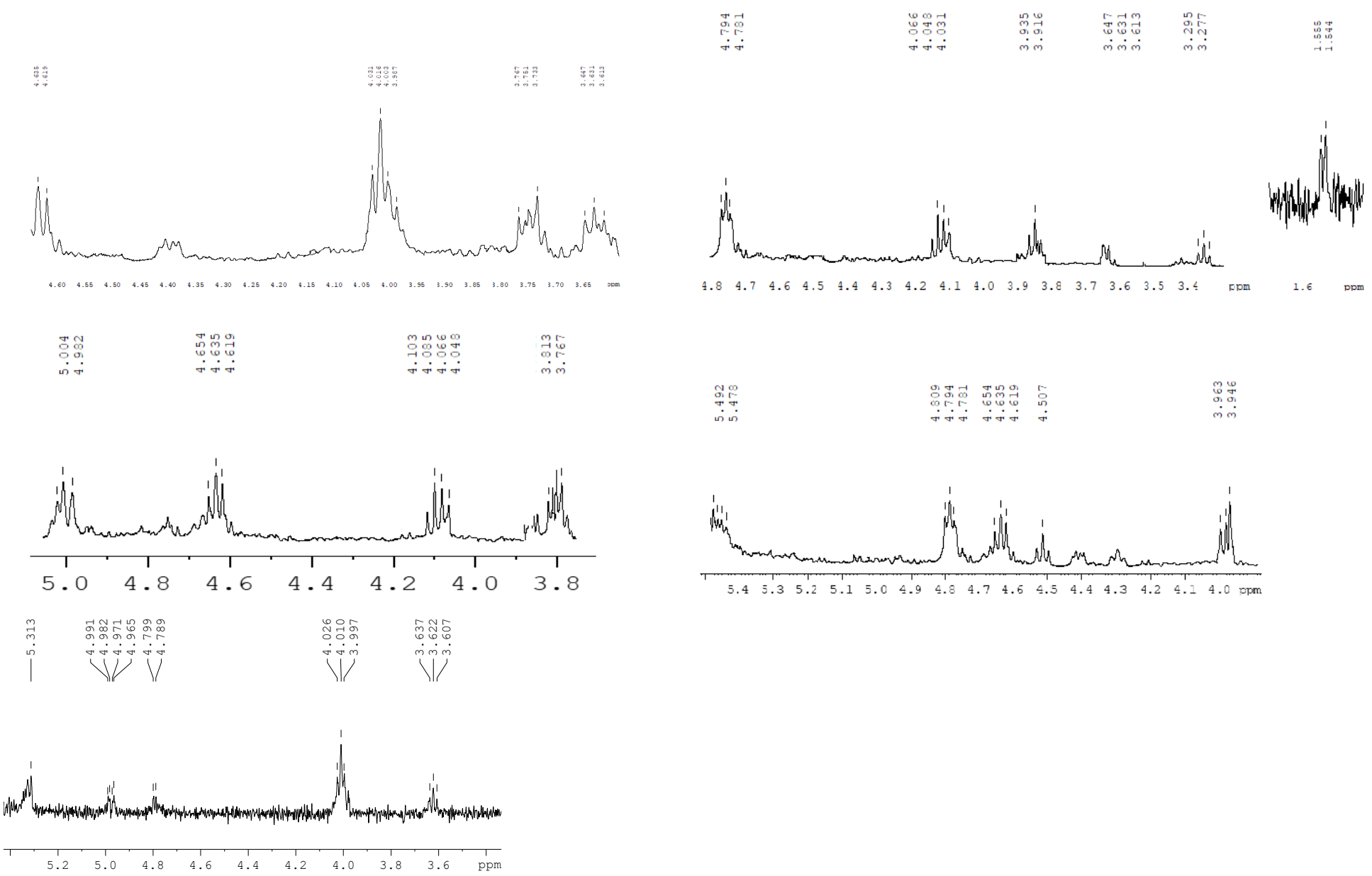


Figure S39. 1D TOCSY (500.12 MHz) spectra of Xyl1, Qui2, Glc3, Glc4, MeGlc5of chilensoside D (**5**) in $C_5D_5N/D_2O$ (4/1)

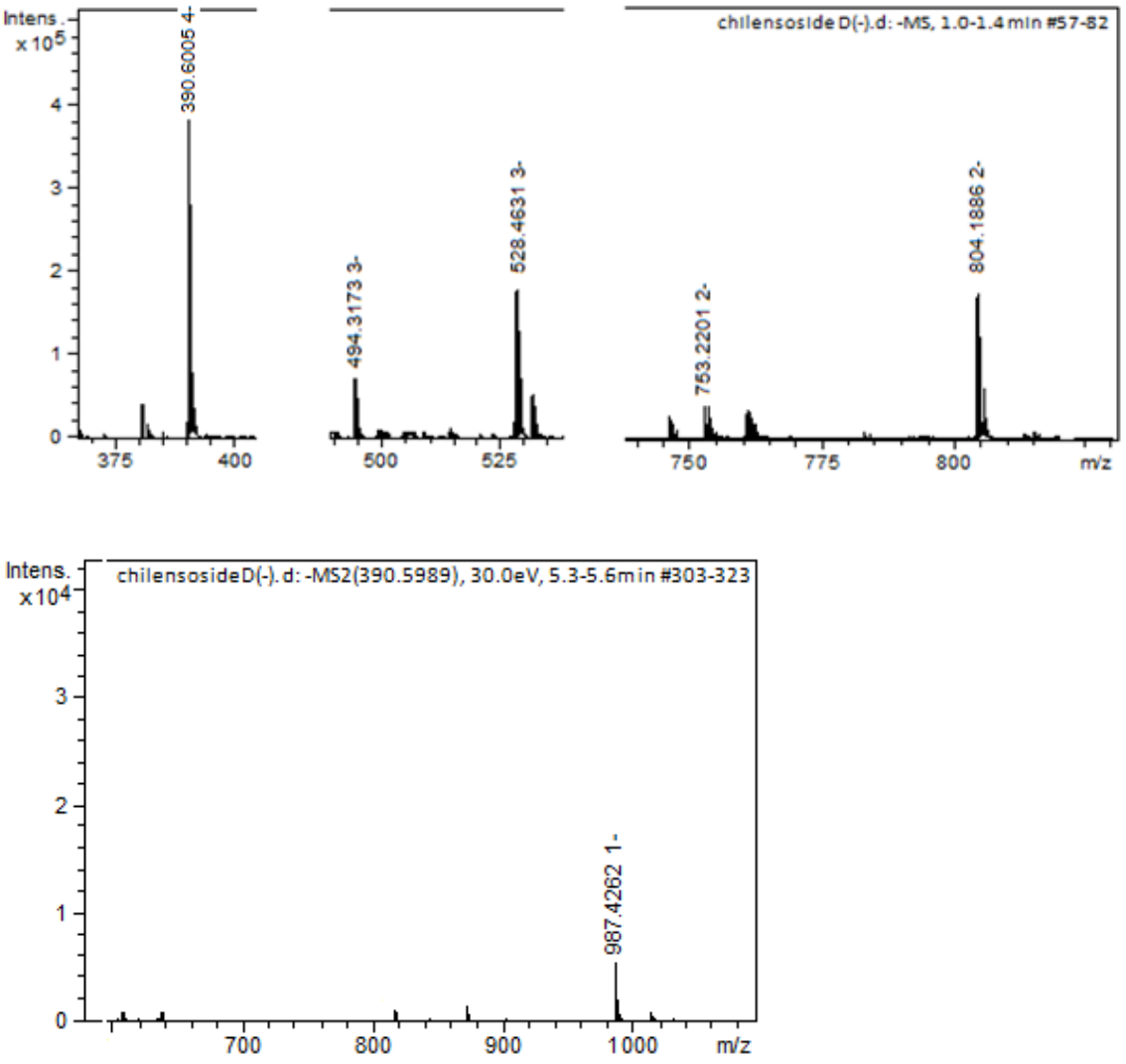



Figure S40. HR-ESI-MS and ESI-MS/MS spectra of chilensoside D (**5**)