Supporting information

Characterization of Acetylcholinesterase Inhibitory Constituents from *Annona glabra* Assisted by HPLC Micro-fractionation

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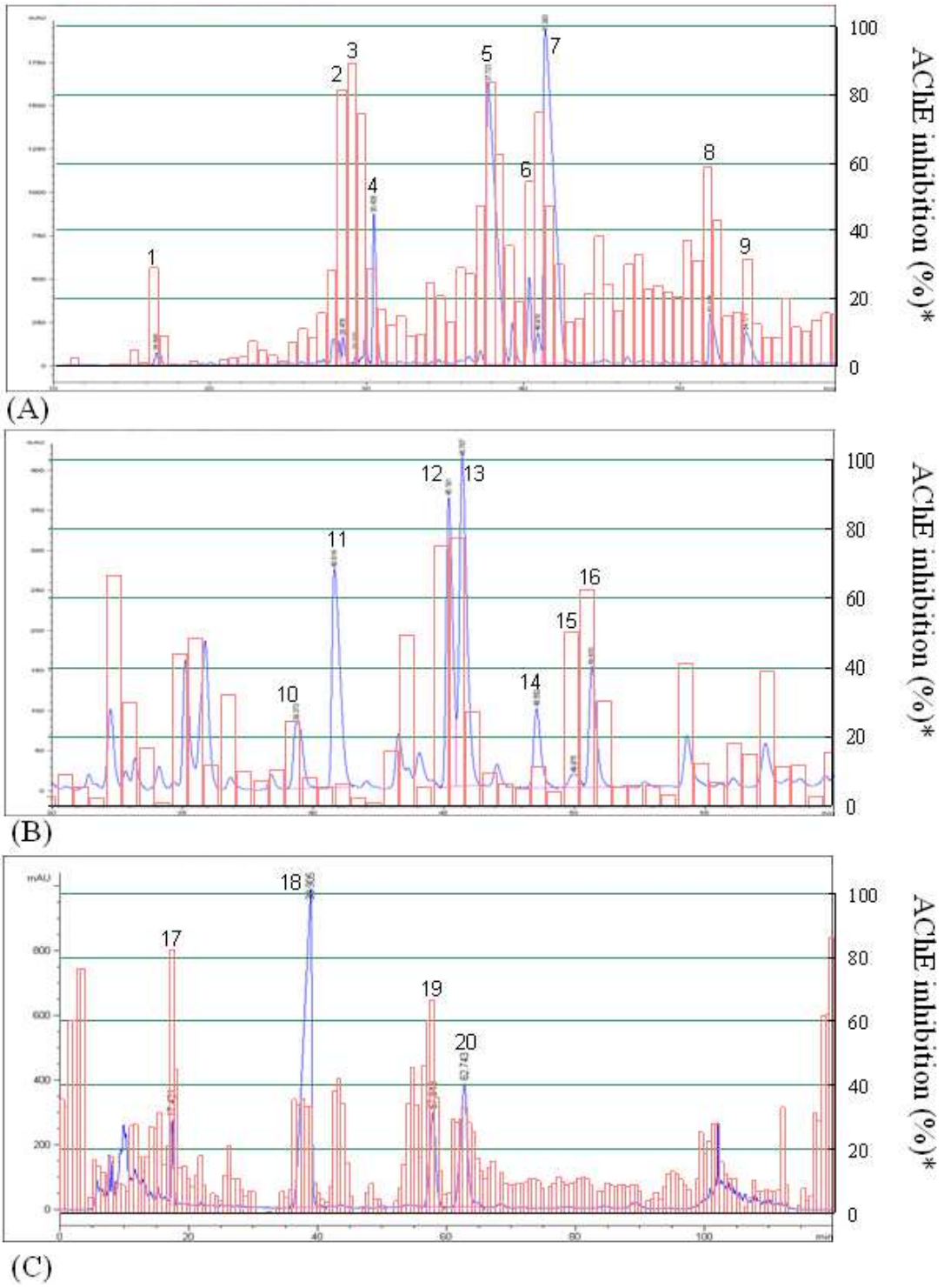
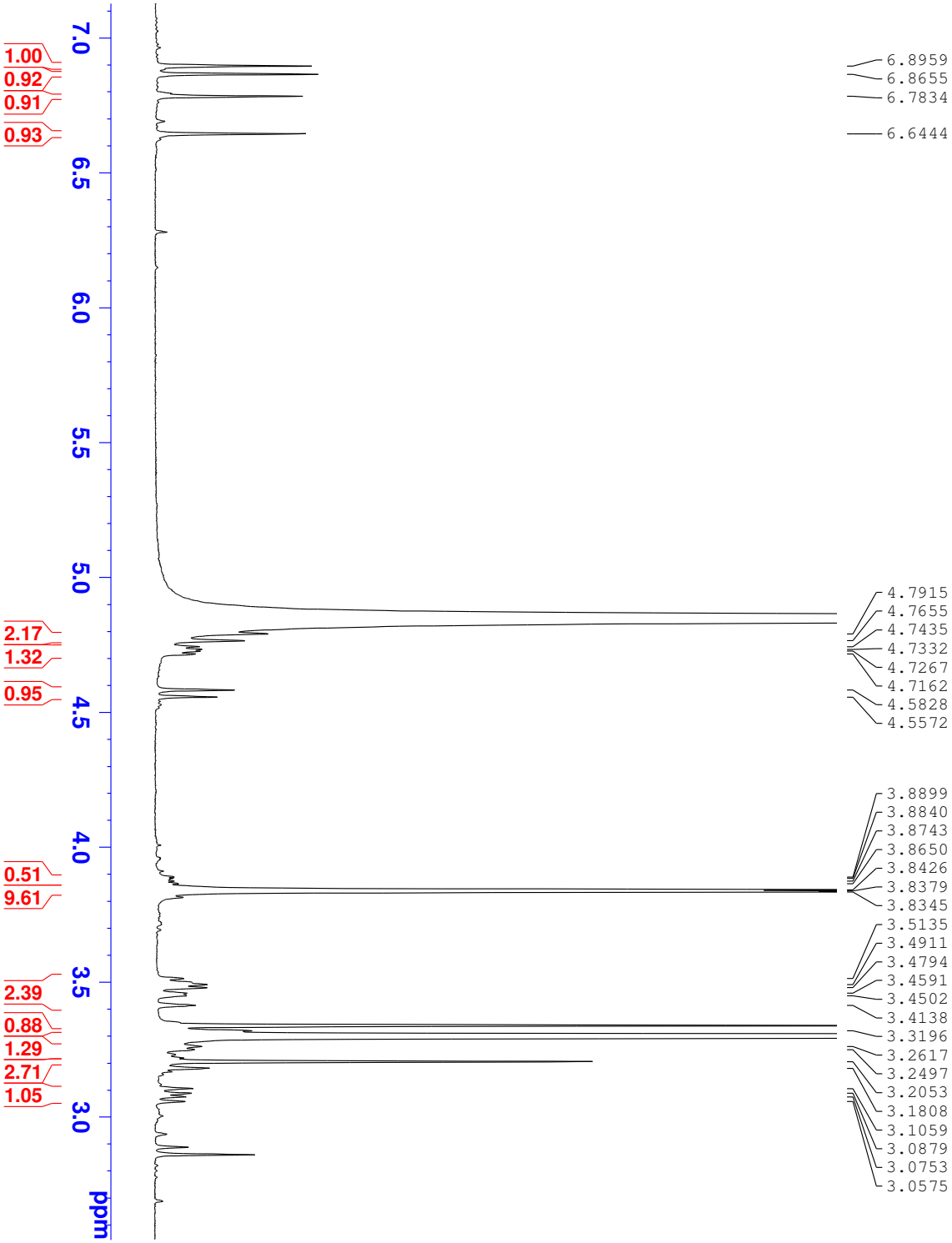
Content

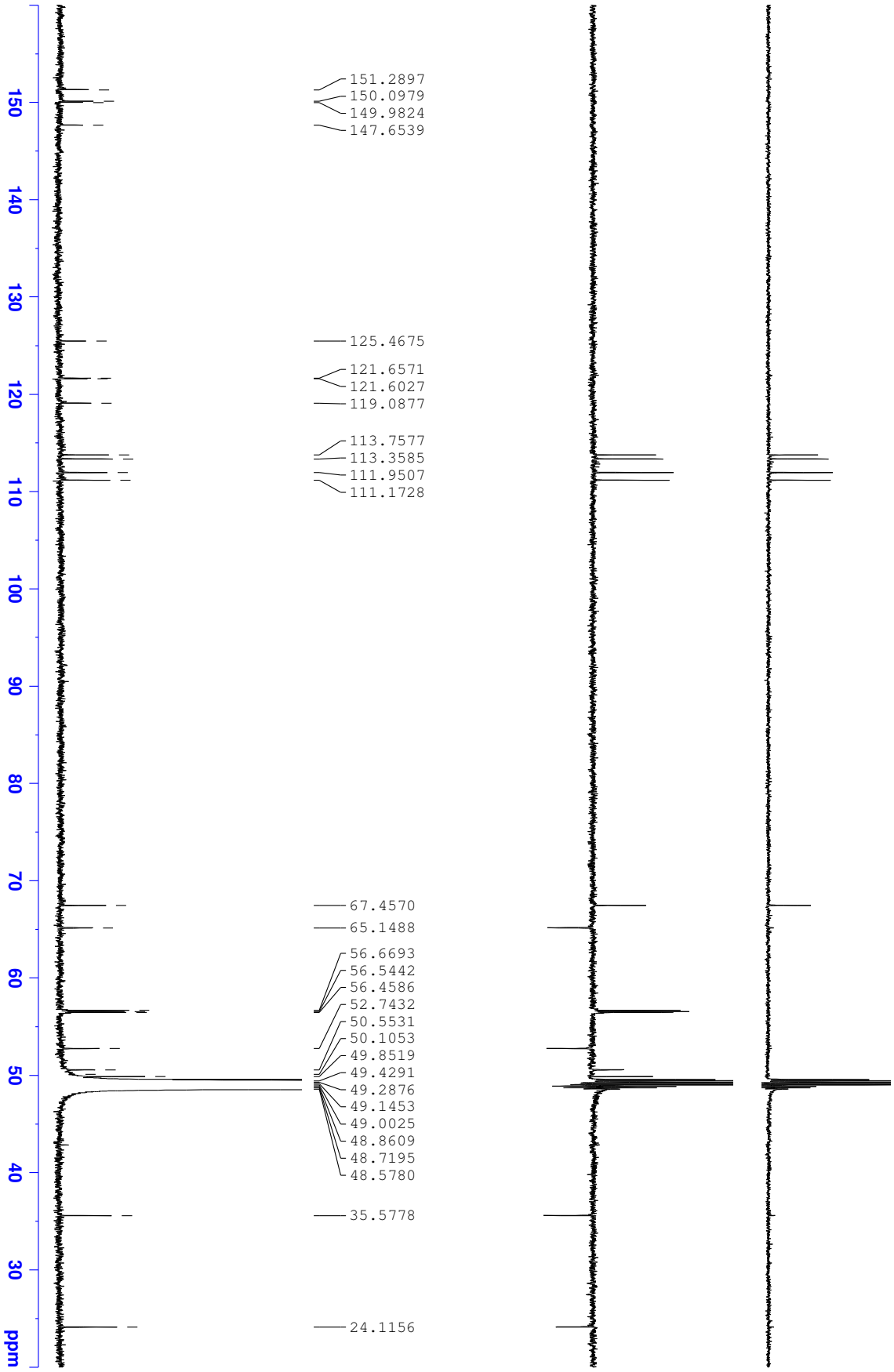


S1. Analytical-scale HPLC-microfractionation combined with AChE assay

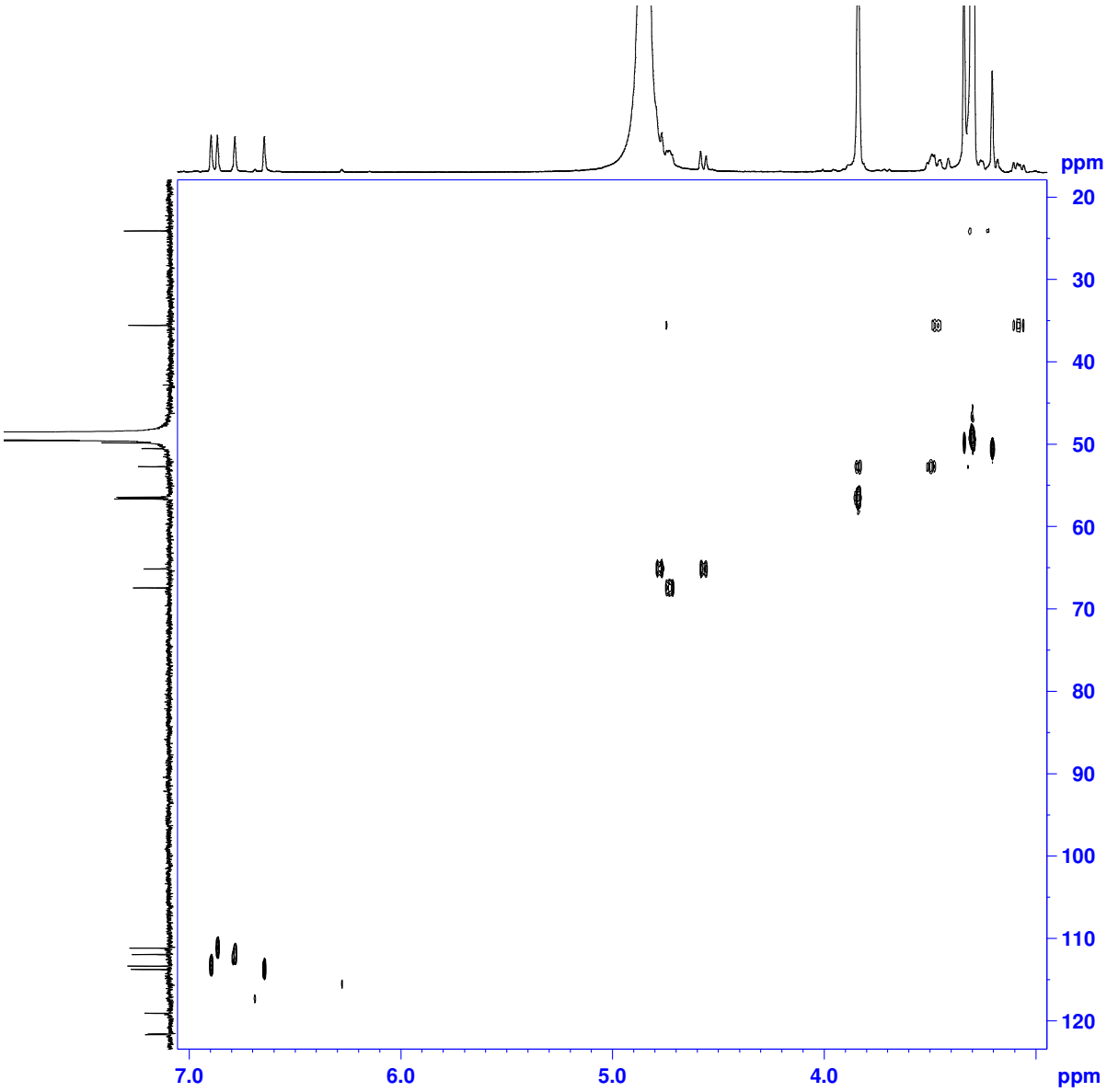
Samples A (fr.-I-2, 200 μg/10 μL), B (fr.-I-4-2, 100 μg/10 μL), and C (fr.-I-4-4-2m, 50 μg/10 μL) were fractionated by HPLC-DAD into 96-well microplates (A/C, monitored at 254 nm; B at 280 nm). The positive control, galanthmine in 10% MeOH (final conc. 0.04, 0.4 and 4 μM), was added to the wells 1-6 in Plate I and 91-96 in Plate II. *Each bar in the histogram corresponds to AChE inhibition (%) of each well.



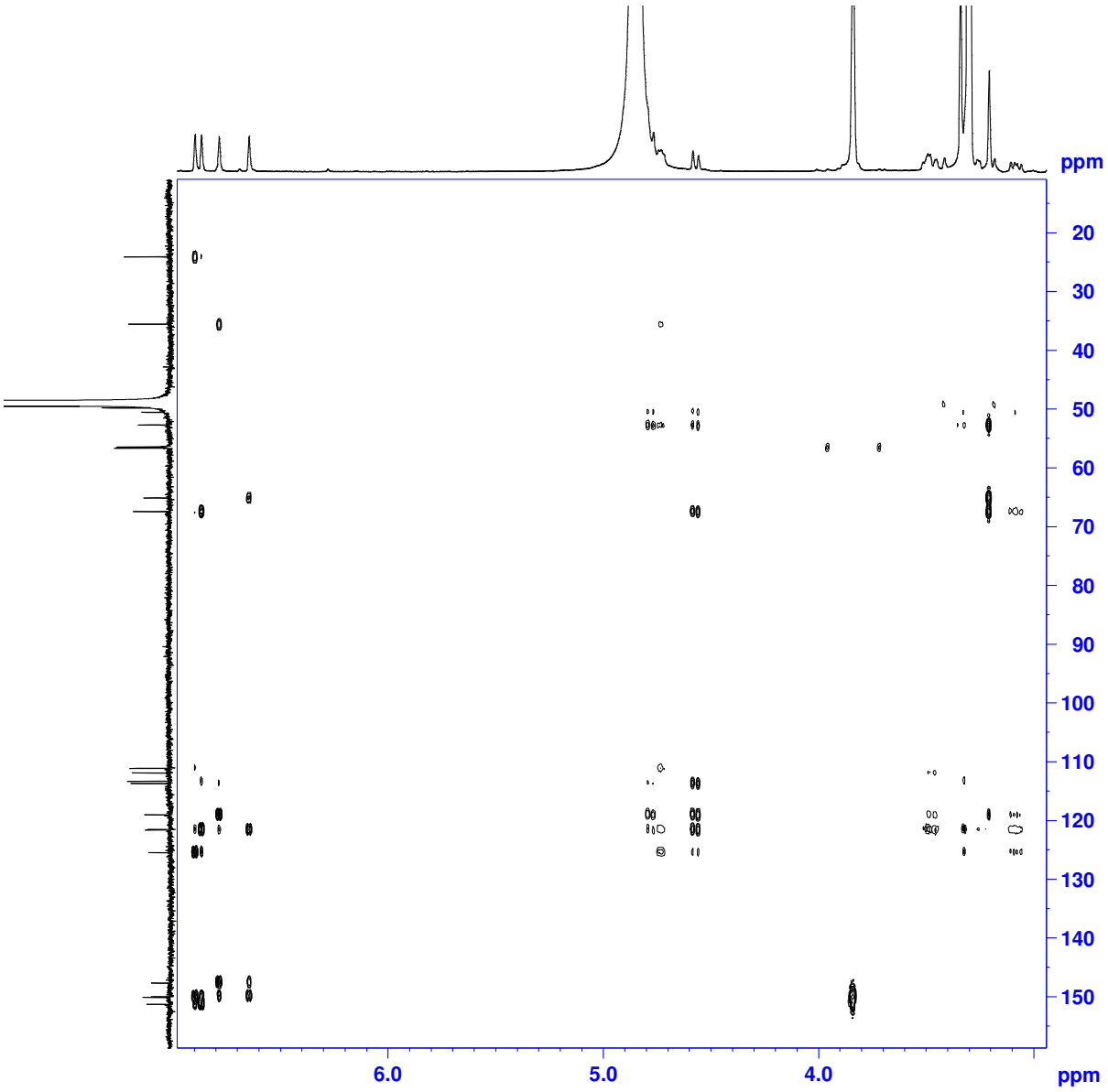
S2. ^{1}H NMR spectrum of **3** (600MHz, CD_3OD)



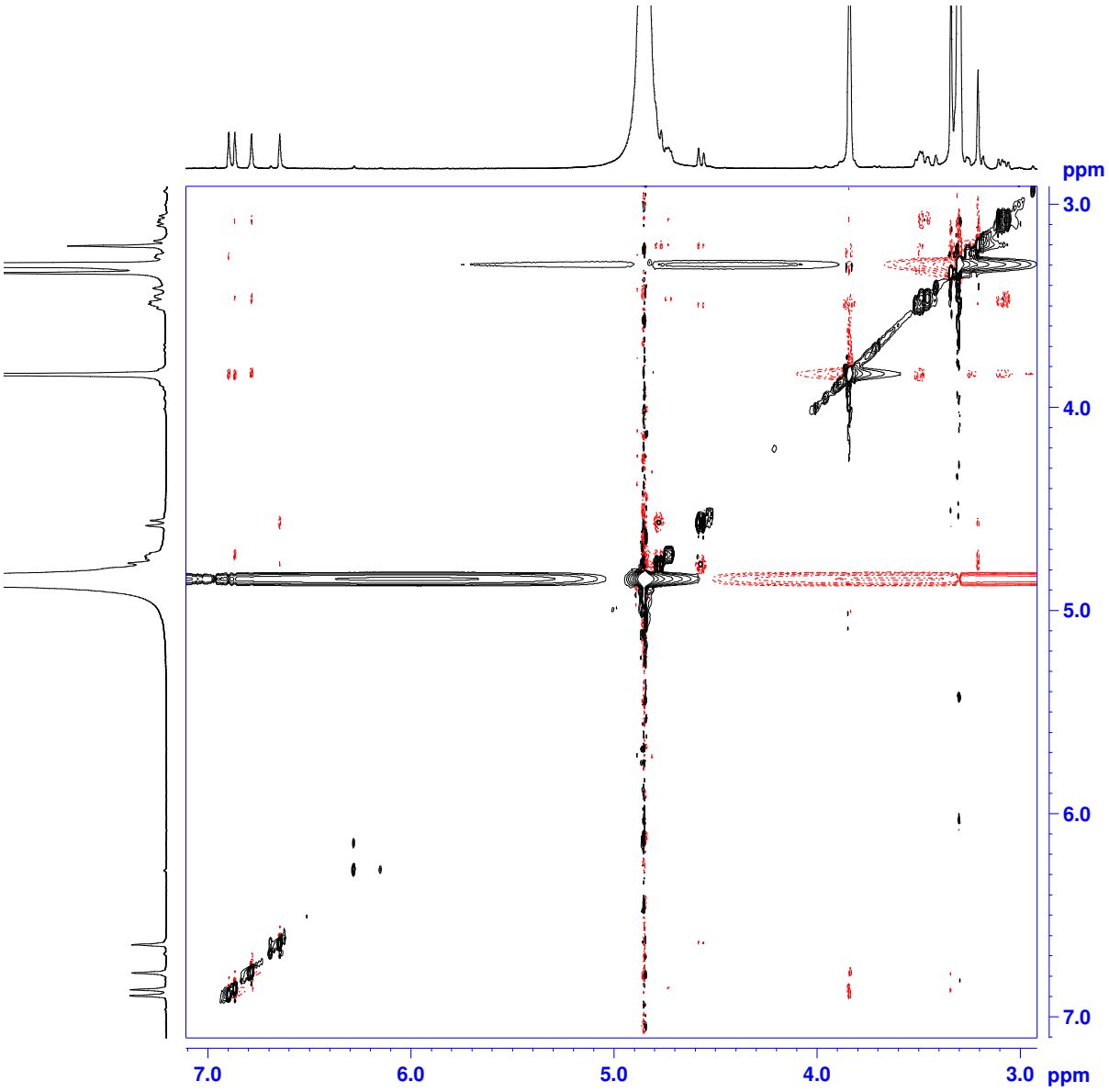
S3. ^{13}C NMR, DEPT-135 (mid.) and DEPT-90 (top) spectra of **3** (150MHz, CD_3OD).



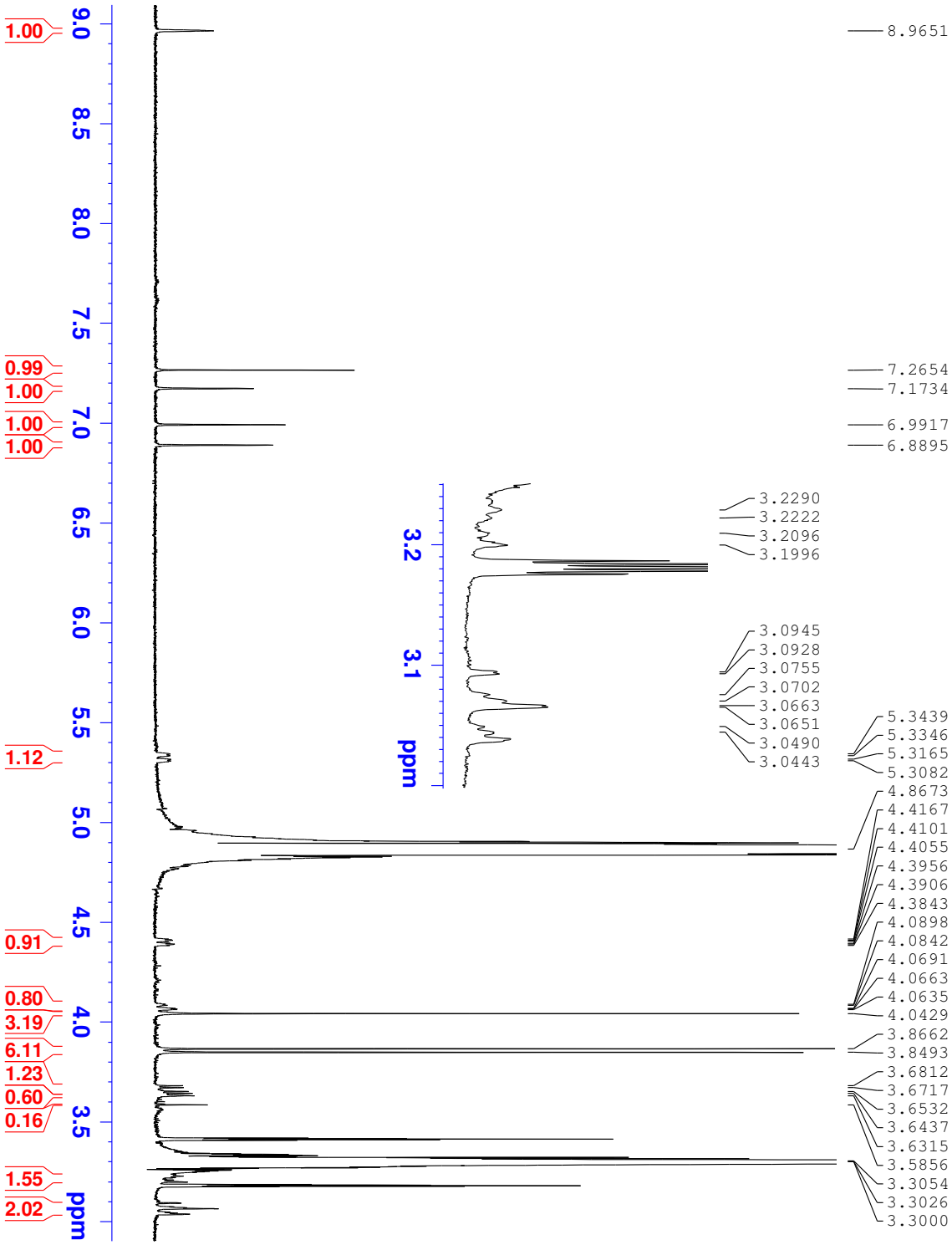
S4. HMQC spectrum of **3** (600MHz, CD_3OD)



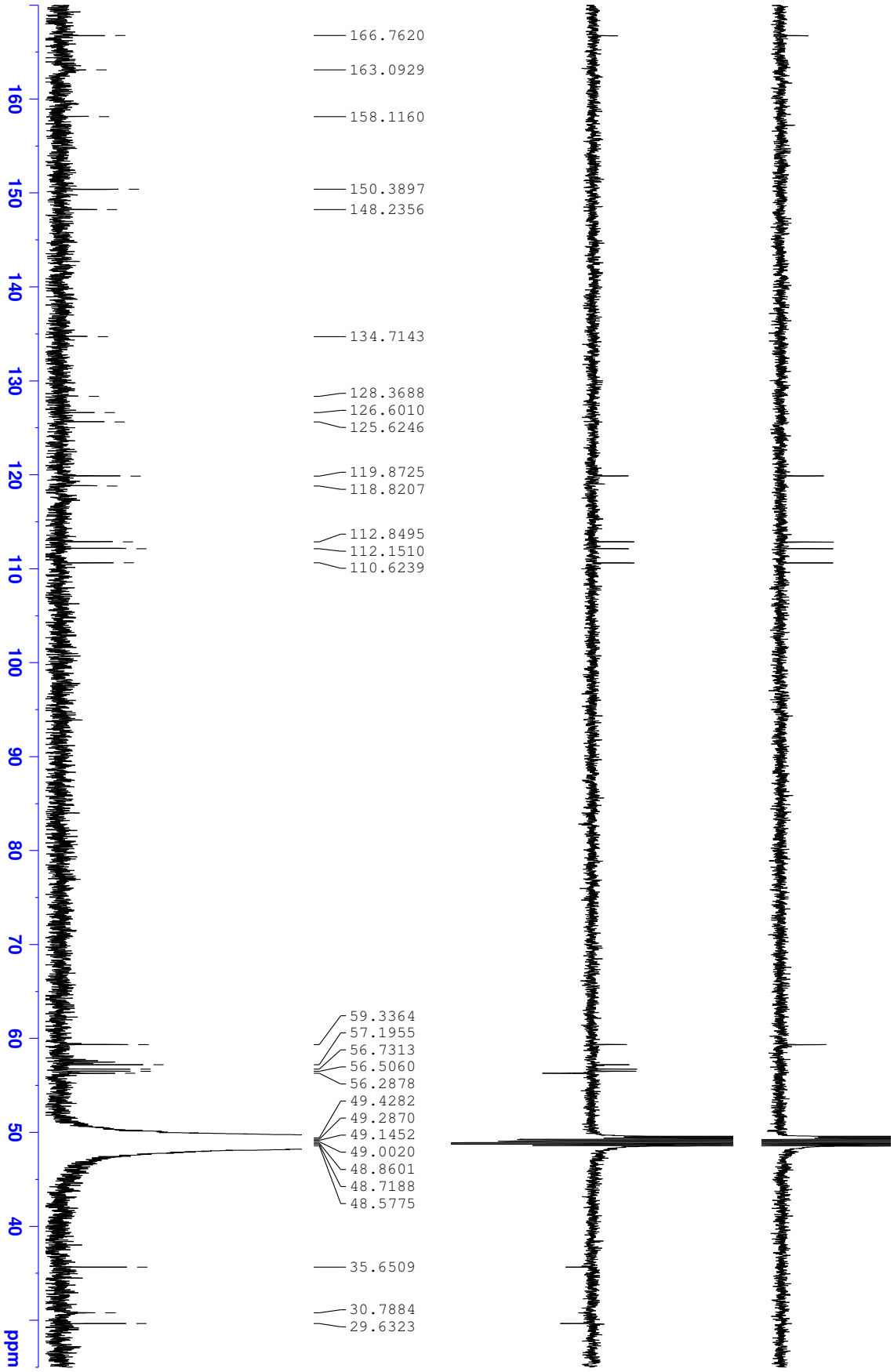
S5. HMBC spectrum of **3** (600MHz, CD_3OD)



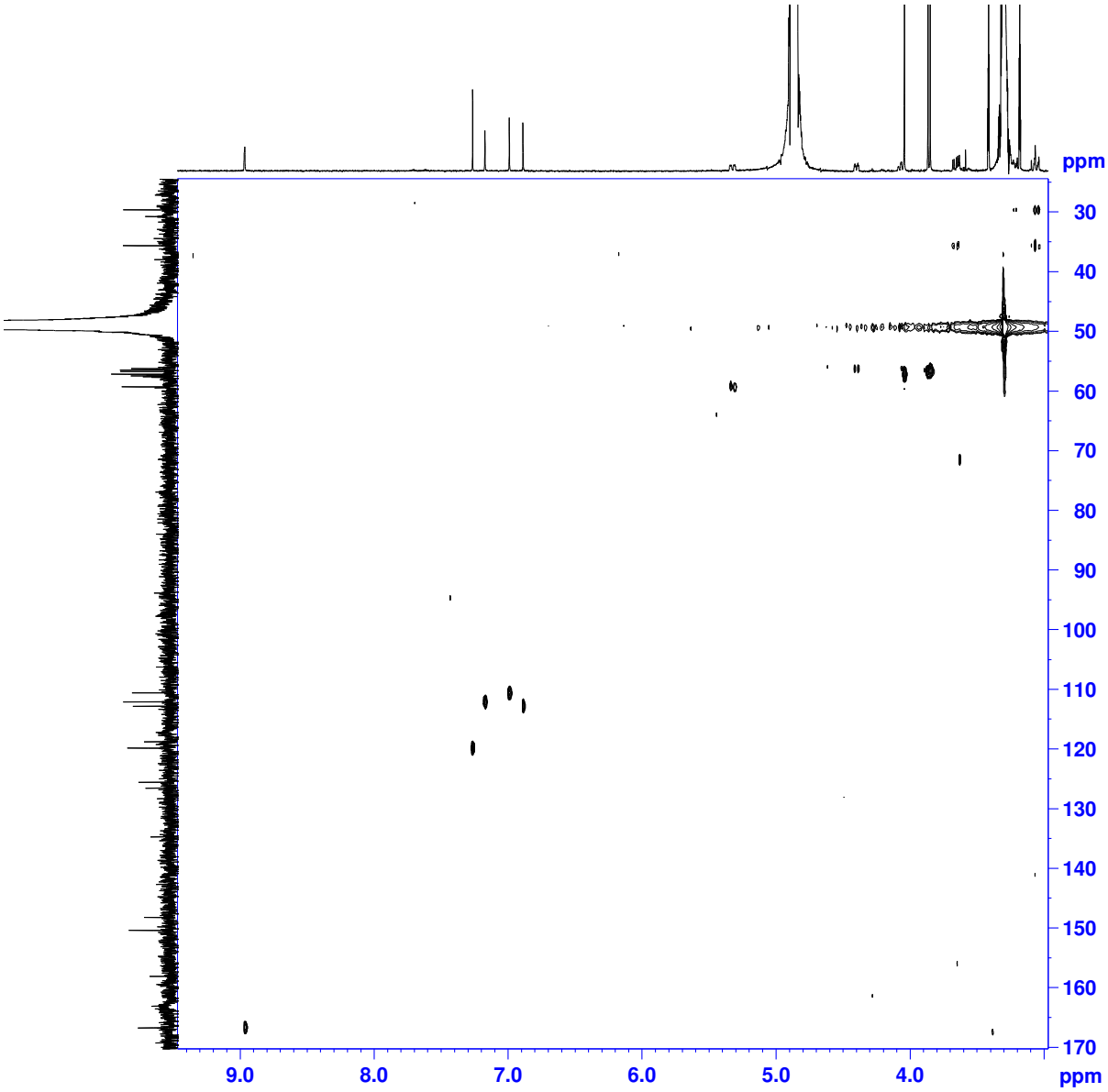
S6. NOESY spectrum of **3** (600MHz, CD_3OD)



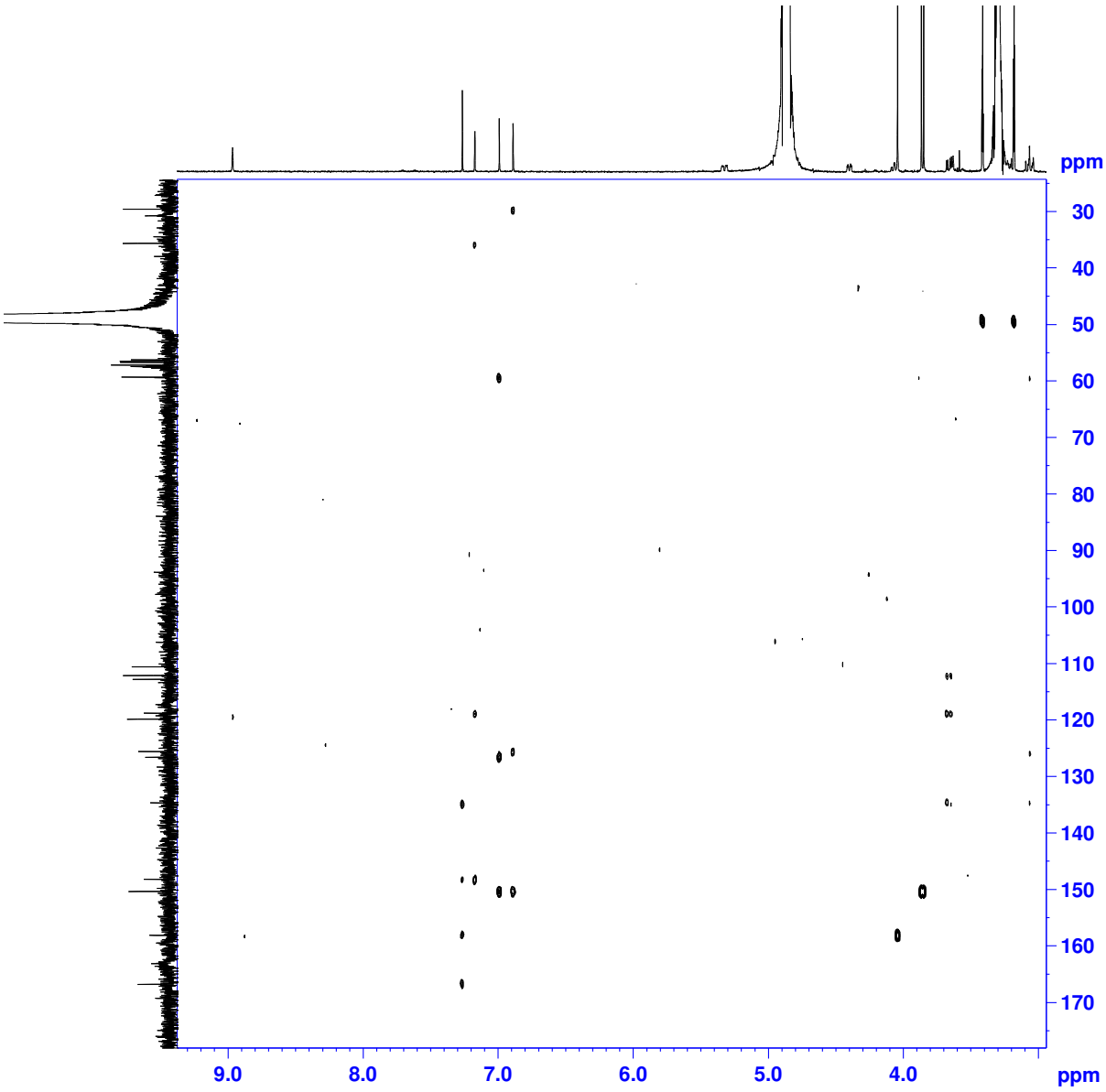
S7. ^{1}H NMR spectrum of **10** (600MHz, CD_3OD)



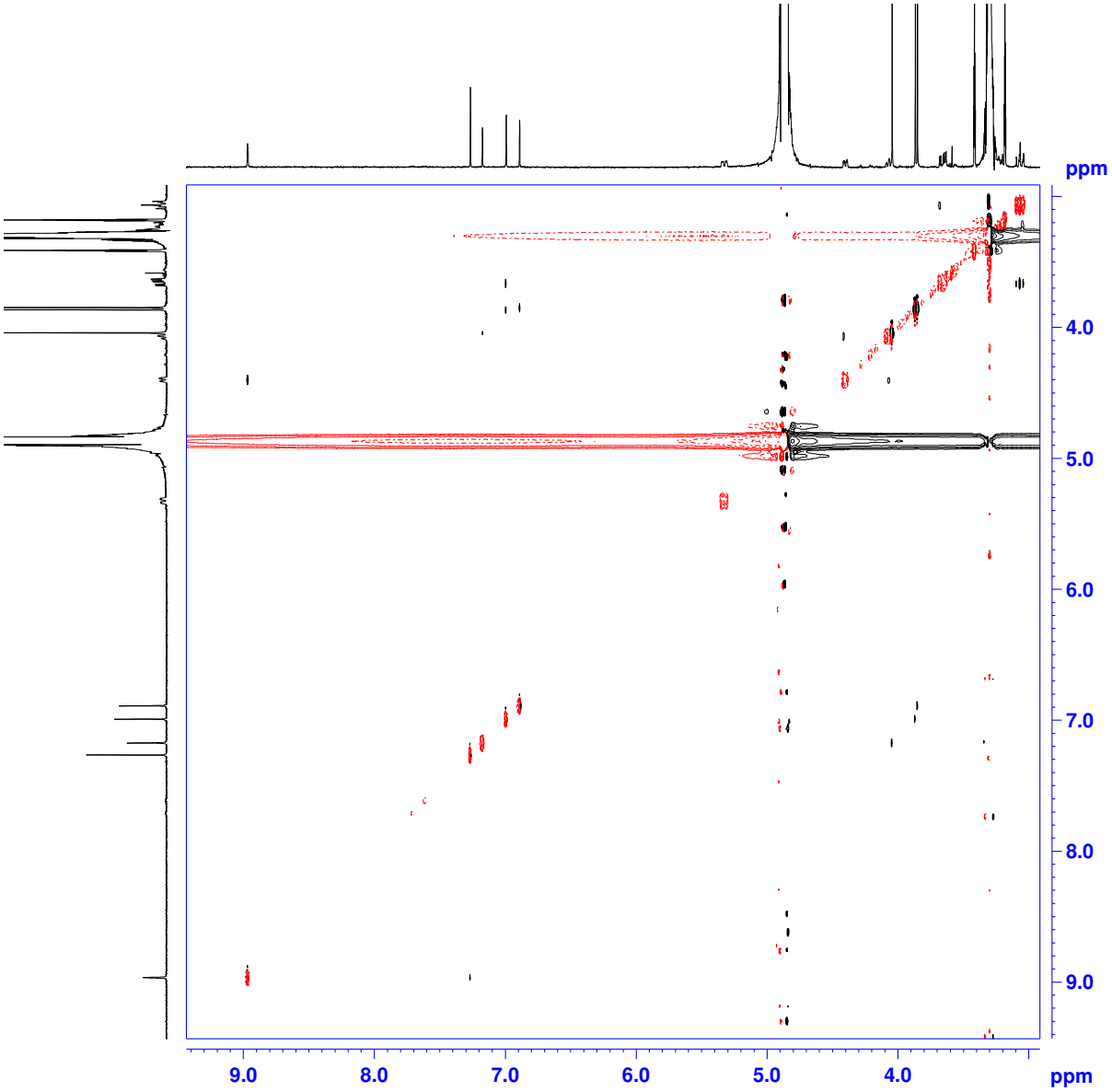
S8. ^{13}C NMR, DEPT-135 (mid.) and DEPT-90 (top) spectra of **10** (150MHz, CD_3OD)



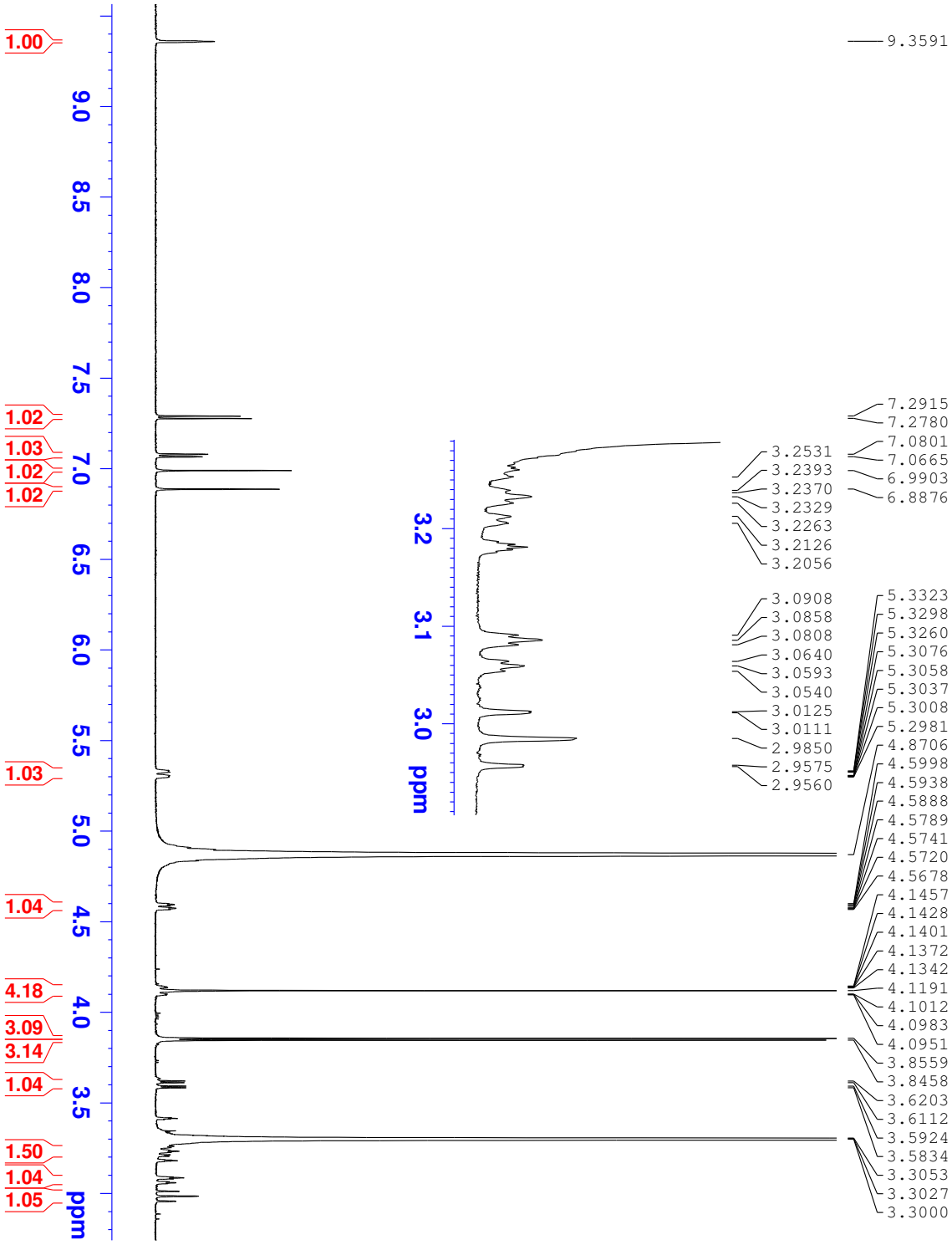
S9. HSQC spectrum of **10** (600MHz, CD_3OD)



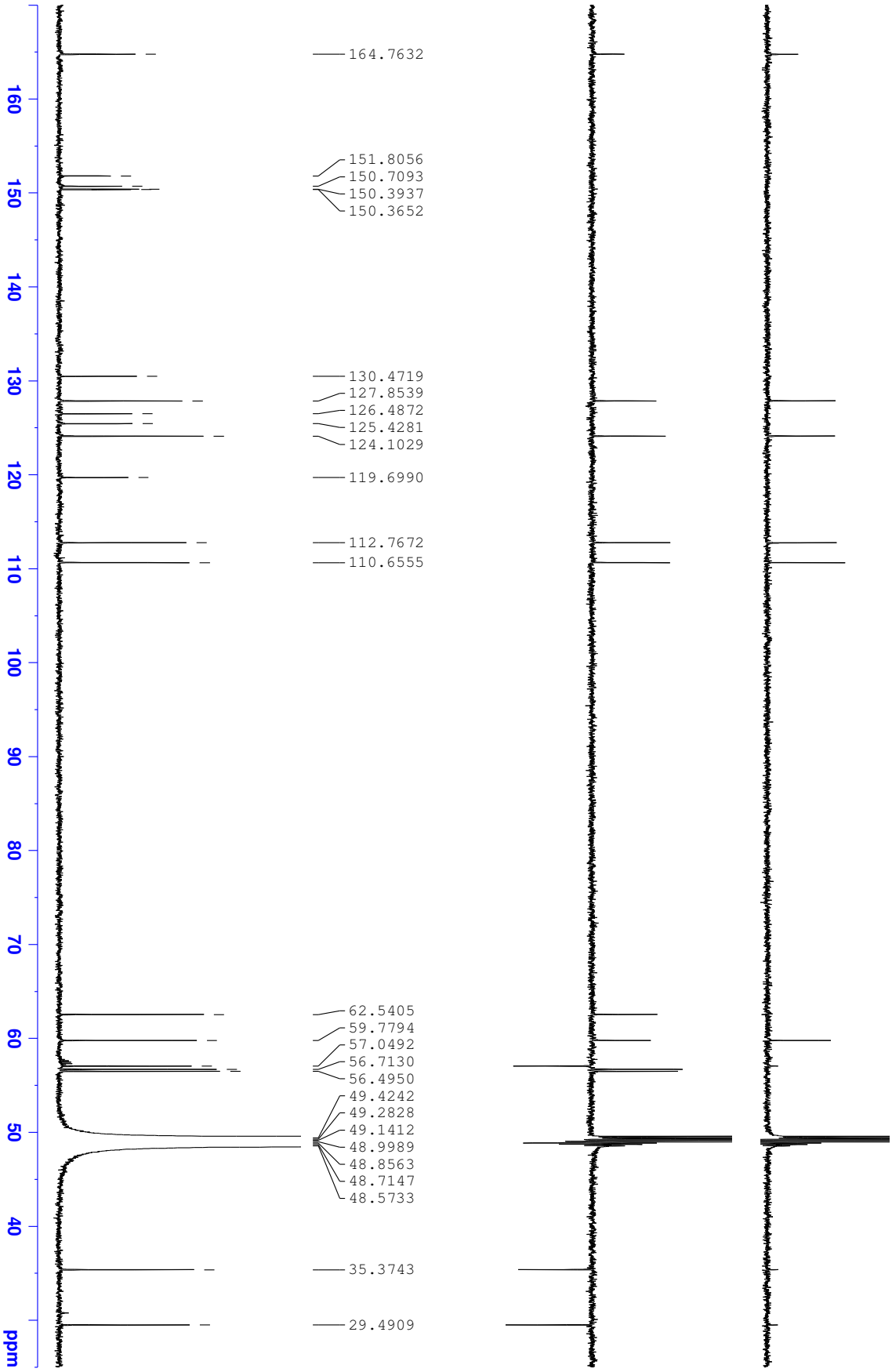
S10. HMBC spectrum of **10** (600MHz, CD_3OD)



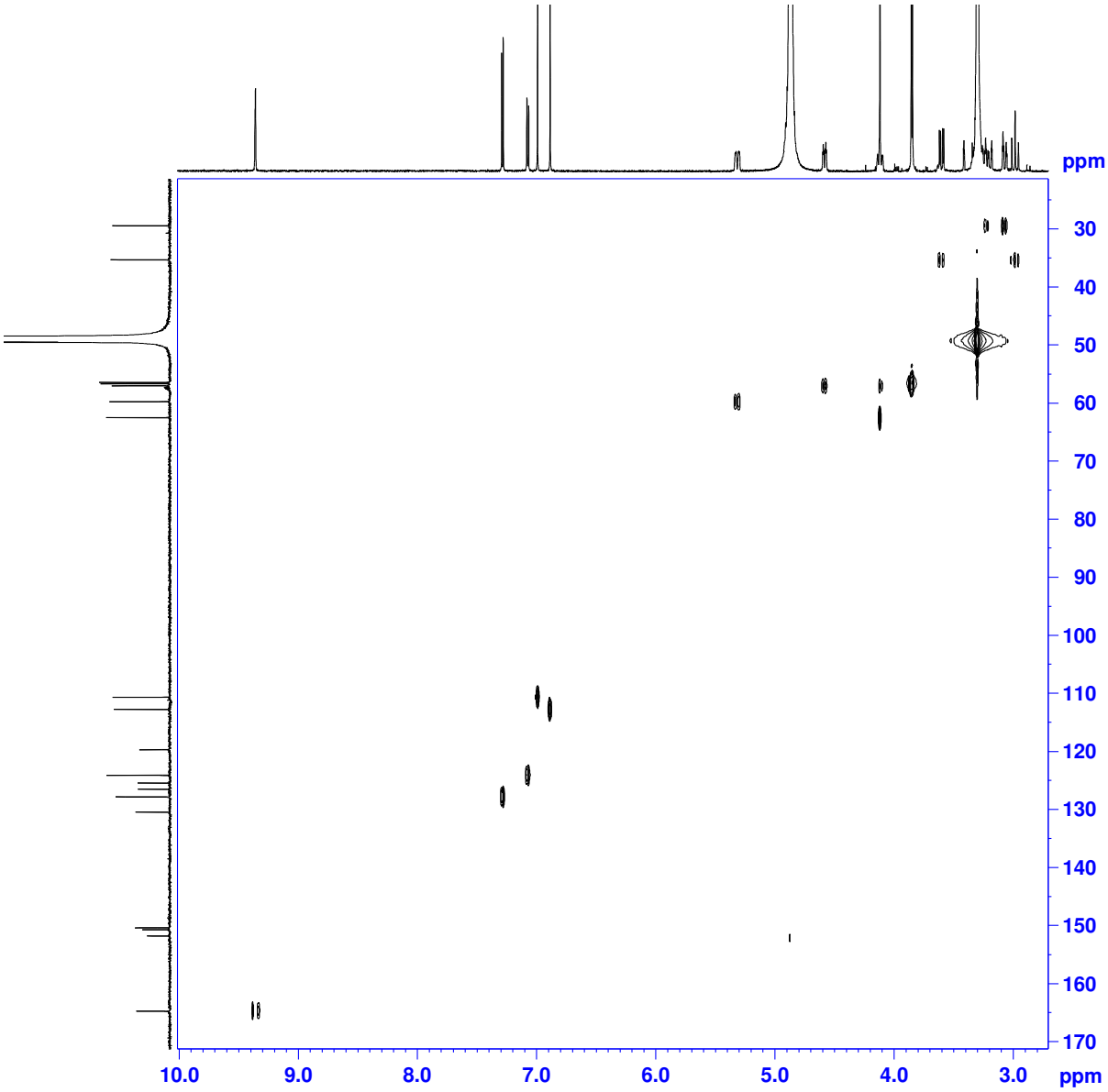
S11. NOESY spectrum of **10** (600MHz, CD_3OD)



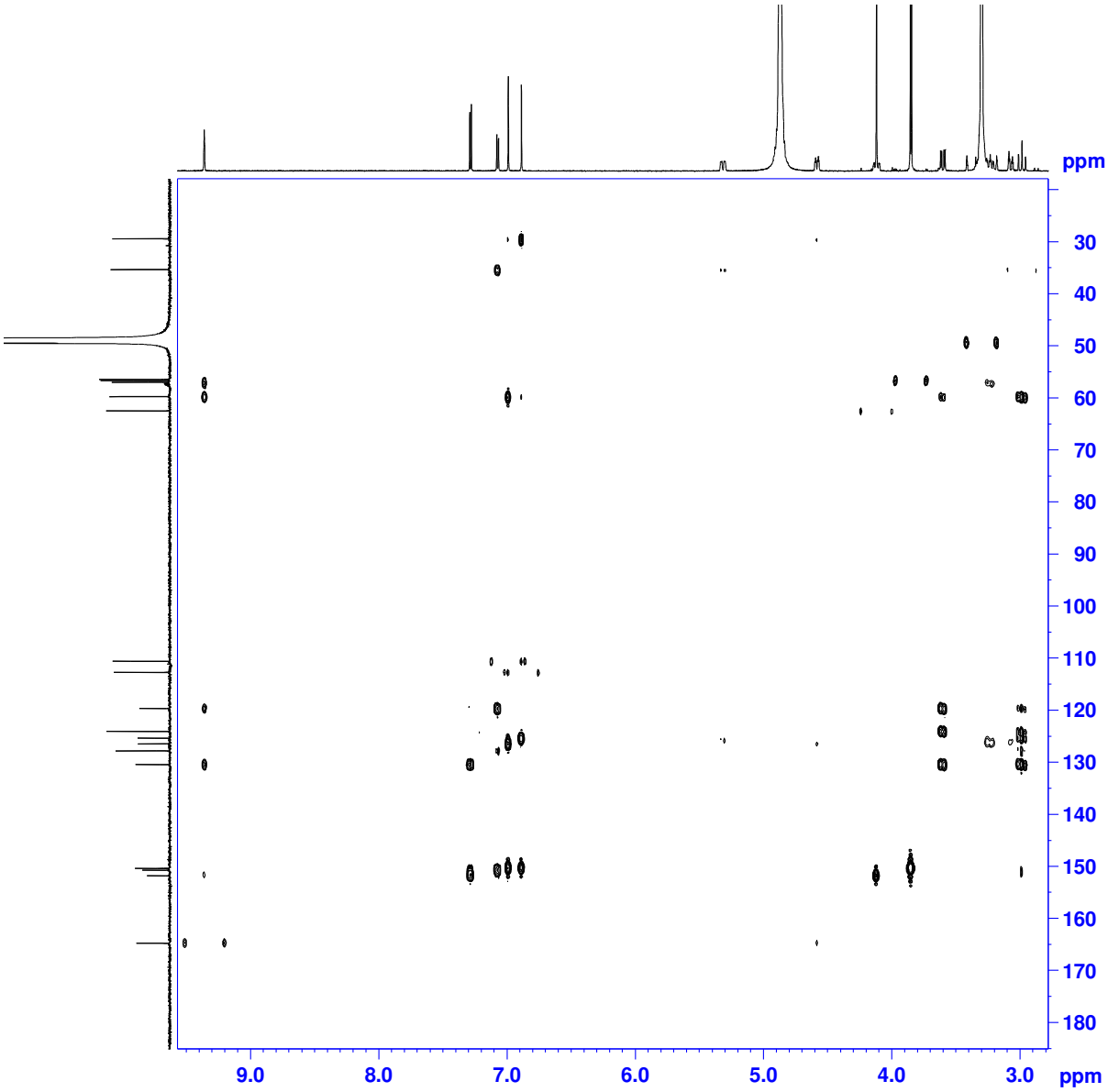
S12. ^{1}H NMR spectrum of **11** (600MHz, CD_3OD)



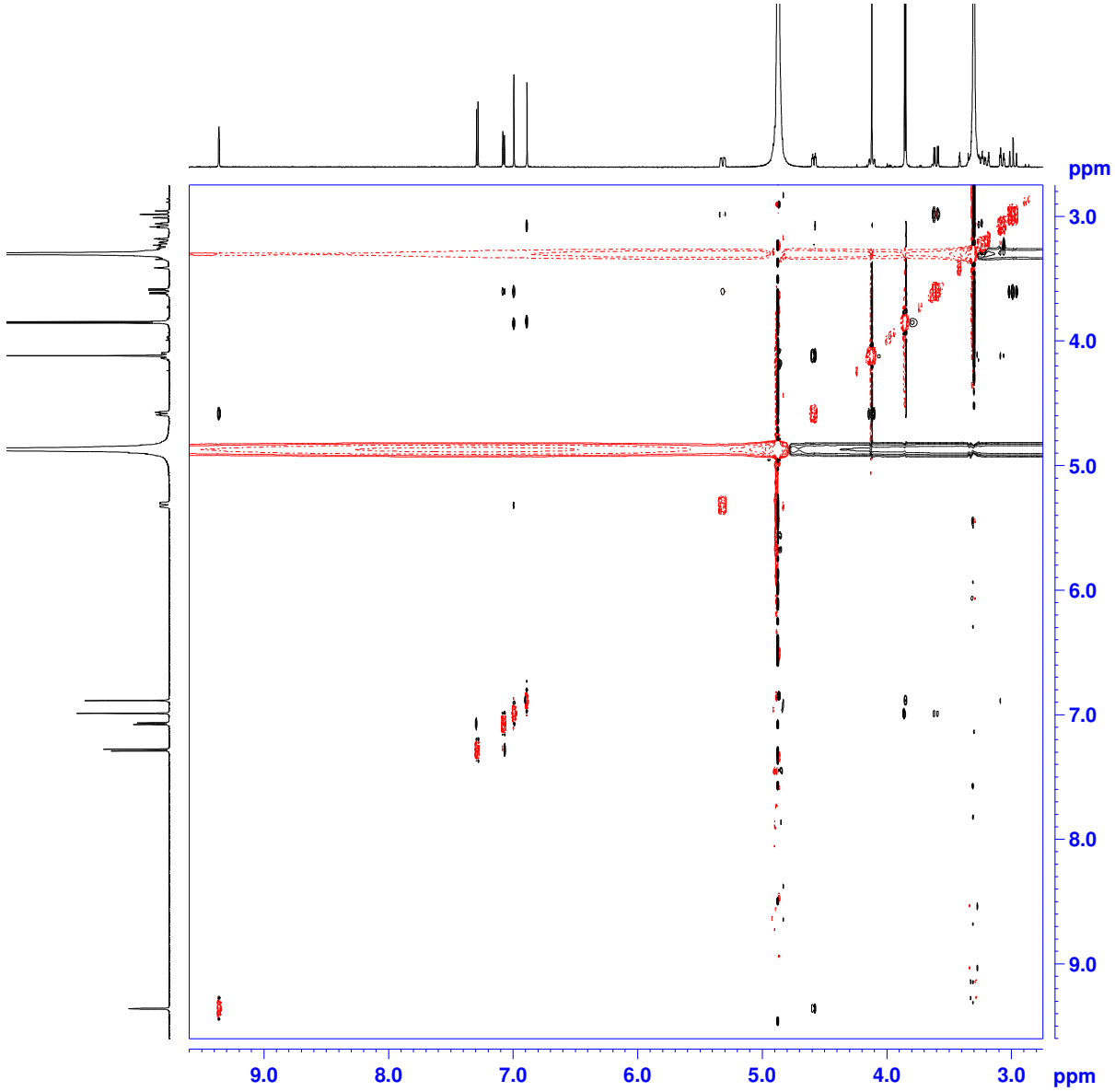
S13. ^{13}C NMR, DEPT-135 (mid.)/-90 (top) spectra of **11** (150MHz, CD_3OD)



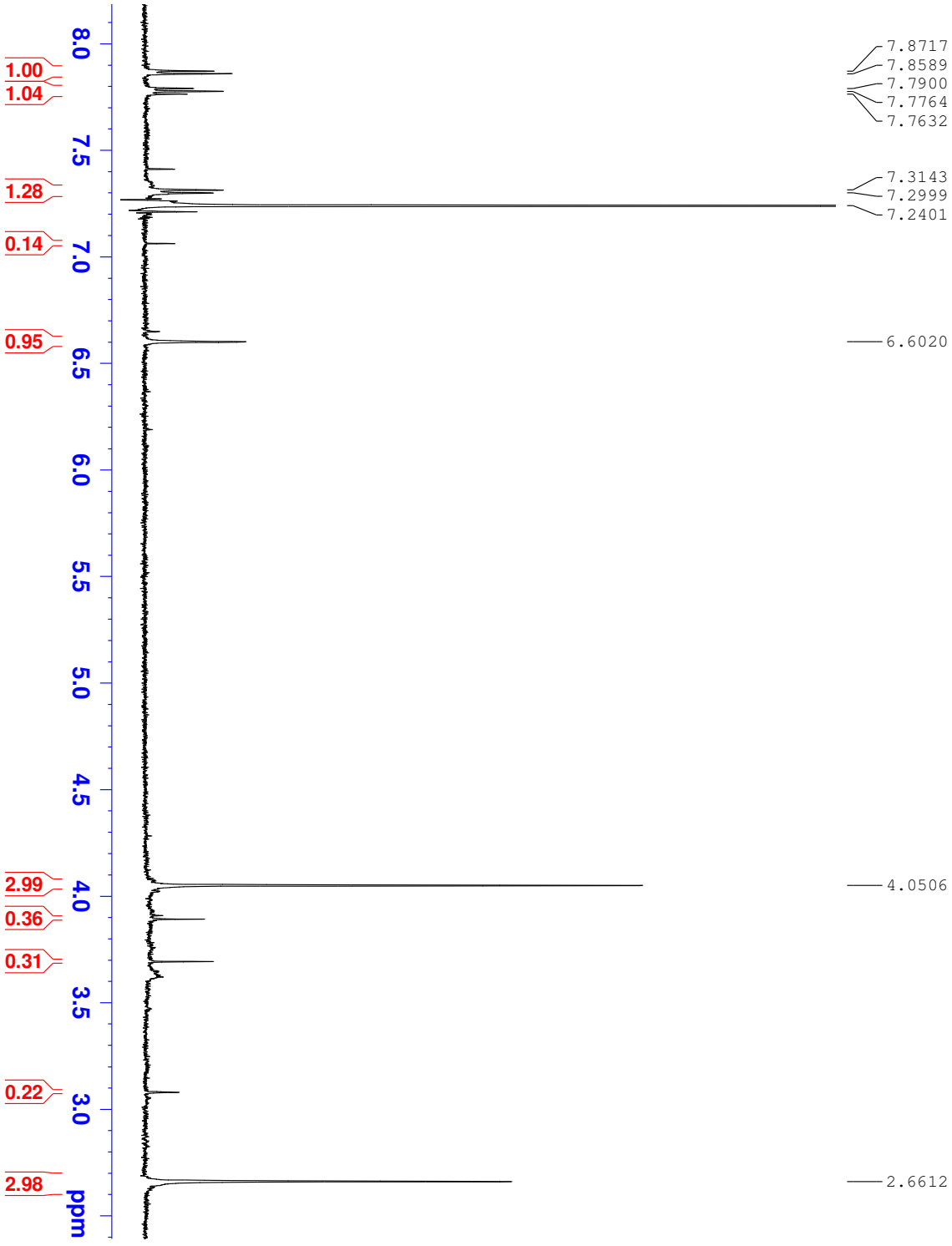
S14. HSQC spectrum of **11** (600MHz, CD_3OD)



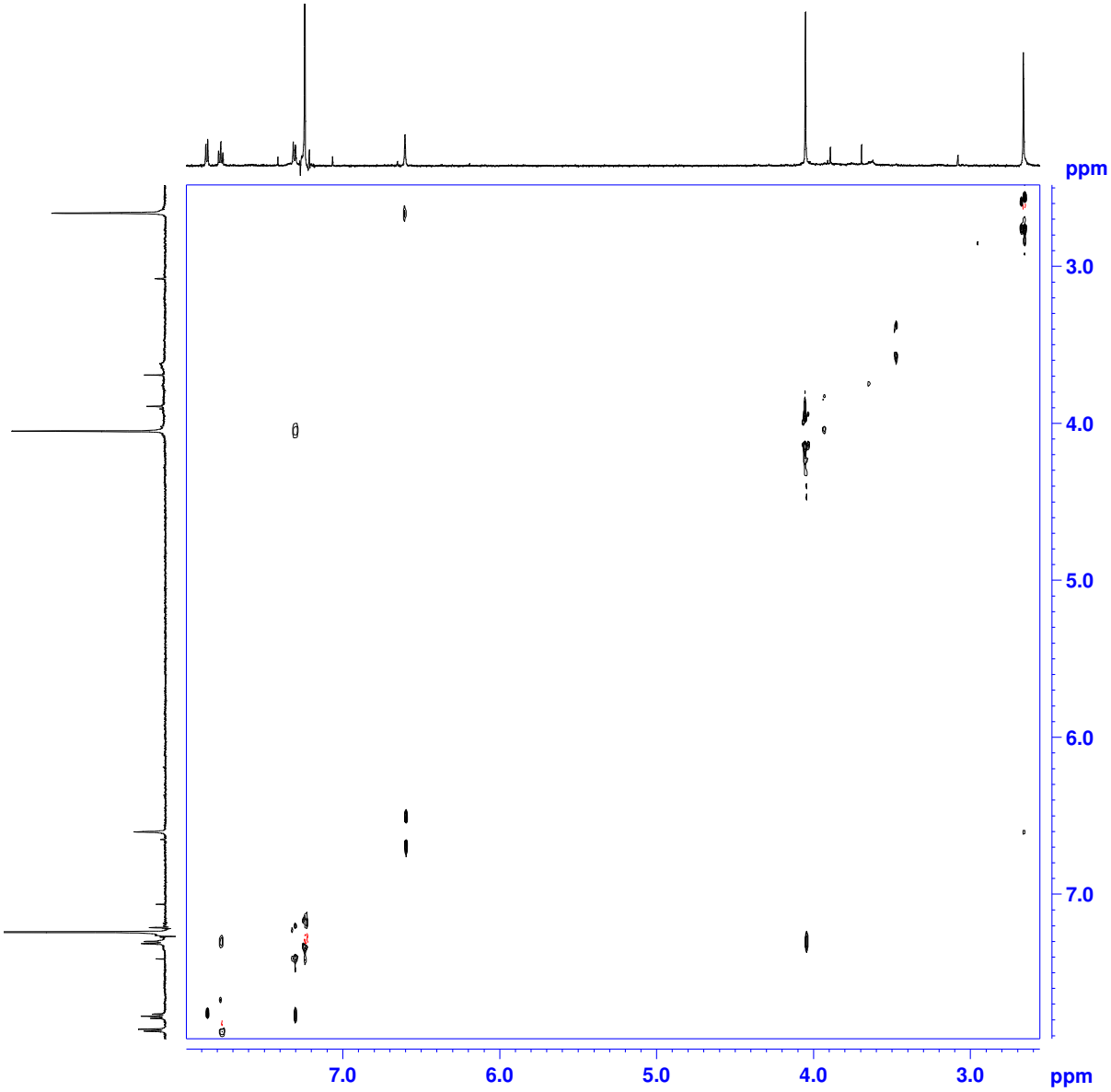
S15. HMBC spectrum of **11** (600MHz, CD_3OD)



S16. NOESY spectrum of **11** (600MHz, CD_3OD)



S17. ^{1}H NMR spectrum of **17** (600MHz, $CDCl_3$)



S18. NOESY spectrum of **17** (600MHz, $CDCl_3$)

S19. UV, ^{1}H NMR and ESIMS Data of **1**, **2**, **4–9**, **12-16**, **and 18-20**

Pycnarrhine trifluoroacetate (TFA) (1): UV λ_{max} (MeOH) 250, 310 nm; ^{1}H NMR (CD_3OD, 600 MHz): δ3.21 (2H, t, *J* = 8.3 Hz), 3.69 (3H, s), 3.96 (2H, t, *J* = 8.3 Hz), 4.00 (3H, s), 7.07 (1H, s), 7.15 (1H, s); (+)ESIMS: *m/z* 192 $[M-TFA]^+$.

1*S*-(+)-Reticuline Trifluroacetic acid (TFAH) salt (2): $[\alpha]^{25}_D$ +22.6 (*c* 0.27, MeOH); UV λ_{max} (MeOH) 227, 283 nm; CD (MeOH) (CE) 290 (+), 235(+); ^{1}H NMR (CD_3OD, 400 MHz): δ2.89 (3H, s), 3.84 (6H, s), 4.51 (1H, m) 6.27 (1H, s), 6.3 (1H, d, *J* = 8.2 Hz), 6.69 (1H, bs), 6.79 (1H, s), 6.90 (1H, d, *J* = 8.2 Hz); (+)ESIMS: *m/z* 330 $[M-TFA]^+$.

***S*-(+)-*N*-methylcorydine.TFA (4)**: $[\alpha]^{25}_D$ +88.0 (*c* 0.25, MeOH); UV λ_{max} (MeOH) 221, 265, 305 nm; CD (MeOH) (CE) 305 (+), 270 (-), 236 (+); ^{1}H NMR (CD_3OD, 400 MHz): δ2.80 (1H, t, 12.8), 3.03 (3H, s), 3.09 (1H, m), 3.36 (2H, m), 3.41 (3H, s), 3.69 (3H, s), 3.75 (2H, m), 3.91 (6H, s), 4.49 (1H, dd, *J* = 3.0, 13.0 Hz), 6.93 (1H, s), 7.09 (1H, d, *J* = 8.3 Hz), 7.23 (1H, d, *J* = 8.3 Hz); (+)ESIMS: *m/z* 356 $[M-TFA]^+$.

***R*-(-)-Asimilobine.TFAH (5)**: $[\alpha]^{25}_D$ -87.0 (*c* 1.0, MeOH); UV λ_{max} (MeOH) 228, 271, 307 nm; CD (MeOH) (CE) 271 (+), 233 (-); ^{1}H NMR (CD_3OD, 400 MHz): δ 2.95 (1H, t, 13.9), 2.99 (1H, dd, *J* = 5.0, 10.8 Hz), 3.08 (1H, dd, *J* = 4.6, 13.9 Hz), 3.21 (1H, ddt, *J* = 1.4, .6.8, 12.8 Hz), 3.39 (1H, dt, *J* = 5.0, 12.8 Hz), 3.59 (3H, s), 3.73 (1H, dd, *J* = 1.4, 6.8 Hz), 4.27 (1H, dd, *J* = 4.6, 13.9 Hz), 6.74 (1H, s), 7.26-7.37 (3H, m), 8.36 (1H, d, *J* = 8.0 Hz); (+)ESIMS: *m/z* 268 $[M-TFA]^+$.

***R*-(-)-Actinodaphnine.TFAH (6)**: $[\alpha]^{25}_D$ -60.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} 218, 282, 306 nm; CD (MeOH) (CE) 315 (+), 279 (+), 238 (-), 217(+); ^{1}H NMR (CD_3OD, 400 MHz): δ2.89 (1H, t, 13.6), 3.00 (1H, dd, *J* = 5.2, 13.6 Hz), 3.15-3.68 (3H, m), 3.72 (1H, dd, *J* = 5.4, 12.4 Hz), 3.88 (3H, s), 4.39 (1H, dd, *J* = 4.6, 13.8 Hz),

6.00(1H, d, *J* = 0.9 Hz), 6.14 (1H, d, *J* = 0.9 Hz), 6.66 (1H, s), 6.77 (1H, s), 7.71 (1H, s); (+)ESIMS: *m/z* 312 $[M-TFA]^+$.

***R*-(-)-Norushinsunine.TFAH (7)**: $[\alpha]^{25}_D$ -62.0 (*c* 1.0, MeOH); UV λ_{max} (MeOH) 209, 271, 317 nm; CD (MeOH) (CE) 273 (+), 233 (-); ^{1}H NMR (CD_3OD, 400 MHz): δ2.98 (1H, dd, *J* = 4.8, 17.2 Hz), 3.23 (1H, m), 3.51 (1H, dt, *J* = 4.8, 12.4 Hz), 3.68 (1H, dd, *J* =1.6, 6.4 Hz), 4.56 (1H, bd), 4.8 (1H, d, *J* = 3.6 Hz), 6.02(1H, d, *J* = 0.9 Hz), 6.15 (1H, d, *J* = 0.9 Hz), 6.72 (1H, s), 7.34-7.48 (3H, m),8.21 (1H, s); (+)ESIMS: *m/z* 282 $[M-TFA]^+$.

***S*-(+)-3-Hydroxynornantenine.TFAH (8)**: $[\alpha]^{25}_D$ + (MeOH); UV λ_{max} (MeOH) 217, 283, 307 nm; CD (MeOH) (CE) 283 (-), 242 (+); ^{1}H NMR (CD_3OD, 400 MHz): δ2.80 (1H, t, 13.6), 2.94 (1H, dd, *J* =4.8, 13.6 Hz), 2.97 (1H, m), 3.05 (1H, dt, *J* = 1.6, 5.6, 17.6 Hz), 3.71 (3H, s), 3.77 (1H, dd, *J* =0.6, 6.0 Hz), 3.89 (3H, s), 4.24 (1H, dd, *J* = 4.4, 14.0 Hz), 5.96(2H, d, *J* = 1.2 Hz), 6.81 (1H, s), 7.76 (1H, s); (+)ESIMS: *m/z* 342 $[M-TFA]^+$.

***R*-(-)-3-Hydroxynornuciferine.TFAH (9)**: $[\alpha]^{25}_D$ -54.5(*c* 0.11, MeOH); UV λ_{max} (MeOH) 279 nm; CD (MeOH) (CE) 281 (+), 238 (-), 218 (+); ^{1}H NMR (CD_3OD, 400 MHz): δ2.91 (1H, t, 13.6), 3.05 (1H, dd, *J* = 4.4, 13.6 Hz), 3.71 (3H, s), 3.77 (1H, ddd, *J* = 1.2, 6.4, 12.8 Hz), , 3.90 (3H, s), 4.29 (1H, dd, *J* = 4.8, 14.0 Hz), 7.20-7.34 (3H, m),8.21 (1H, d, *J* = 8.0 Hz); (+)ESIMS: *m/z* 298 $[M-TFA]^+$.

Pseudocolumbamine.TFA (12): UV λ_{max} (MeOH) 240, 288, 311sh, 340, 380 nm; ^{1}H NMR (CD_3OD, 600 MHz): δ3.24 (2H, t, *J* = 6.5 Hz), 3.97 (3H, s), 4.05 (3H, s), 4.12 (3H, s), 4.79 (2H, t, *J* = 6.6 Hz), 7.02 (1H, s), 7.55 (1H, s), 7.60 (2H, s), 8.50 (1H, s), 9.29 (1H, s); (+)ESIMS: *m/z* 338.1 $[M-TFA]^+$.

Dehydrocorydalmine.TFA (13): UV λ_{max} (MeOH) 226, 272, 344, 420 nm; ^{1}H NMR (CD_3OD, 600 MHz): δ3.29 (2H, t, *J* = 6.0 Hz), 3.94 (3H, s), 3.99 (3H, s), 4.16 (3H, s), 4.92 (2H, t, *J* = 6.0 Hz), 7.05 (1H, s), 7.66 (1H, s), 7.8 (H, d, *J* = 9.0 Hz),

7.91 (H, d, *J* = 9.0 Hz), 8.77 (1H, s), 9.66 (1H, s); (+)ESIMS: *m/z* 338.1 $[M-TFA]^+$.

Dehydrocorytenchine.TFA (14): UV λ_{max} (MeOH) 240, 287, 310sh, 342, 380 nm; ^{1}H NMR (CD_3OD, 600 MHz): δ3.25 (2H, t, *J* = 6.5 Hz), 3.93 (3H, s), 3.98(3H, s), 4.10 (3H, s), 4.76 (2H, t, *J* = 6.6 Hz), 7.04 (1H, s), 7.44 (1H, s), 7.61 (1H, s), 7.64 (1H, s), 8.54 (1H, s), 9.24 (1H, s); (+)ESIMS: *m/z* 338.1 $[M-TFA]^+$.

Palmatine.TFA (15): UV λ_{max} (MeOH) 227, 266, 347, 429 nm; ^{1}H NMR (CD_3OD, 600 MHz): δ3.27 (2H, t, *J* = 6.4 Hz), 3.94 (3H, s), 3.99 (3H, s), 4.11 (3H, s), 4.21 (3H, s), 4.93 (2H, t, *J* = 6.4 Hz), 7.05 (1H, s), 7.67 (1H, s), 8.01 (H, d, *J* = 9.0 Hz), 8.12 (H, d, *J* = 9.0 Hz), 8.80 (1H, s), 9.76 (1H, s); (+)ESIMS: *m/z* 352.1 $[M-TFA]^+$.

Pseudopalmatine.TFA (16): UV λ_{max} (MeOH) 240, 288, 309sh, 338 376 nm; ^{1}H NMR (CD_3OD, 600 MHz): δ3.94 (2H, t, *J* = 6.5 Hz), 3.94 (3H, s), 3.99 (3H, s), 4.06 (3H, s), 4.13 (3H, s), 4.82 (2H, t, *J* = 6.4 Hz), 7.05 (1H, s), 7.60 (1H, s), 7.61 (1H, s), 7.64 (1H, s), 8.65 (1H, s), 9.30 (1H, s); (+)ESIMS: *m/z* 352.1 $[M-TFA]^+$.

Liriodenine (18): UV λ_{max} (MeOH) 245, 270, 312, 418 nm; ^{1}H NMR (CD_3OD, 400 MHz): δ6.28 (2H, s), 7.00 (1H, s), 7.42 (1H, t, *J* = 7.2 Hz), 7.58 (1H, t, *J* = 7.2 Hz), 7.68 (1H, br. s), 8.18 (1H, br. d, *J* = 7.7 Hz), 8.25 (1H, br. d, *J* = 7.7 Hz), 8.51 (1H, br. s); (+)ESIMS: *m/z* 298 $[M + Na]^+$.

Lysicamine (Oxonuciferine) (19): UV λ_{max} (MeOH) 238, 270, 318, 329, 415 nm; ^{1}H NMR ($CDCl_3$, 600 MHz): δ4.00 (3H, s), 4.05 (3H, s), 7.21 (1H, s) 7.57 (1H, dt, *J* = 0.8, 7.2 Hz), 7.76 (1H, ddd, *J* = 1.7, 7.2, 8.4 Hz), 7.79 (1H, d, *J* = 5.1 Hz), 8.58 (1H, dd, *J* = 1.7, 8.4 Hz), 8.90 (1H, d, *J* = 5.1 Hz), 9.17 (1H, br. d, *J* = 8.4 Hz); (+)ESIMS: *m/z* 314 $[M + Na]^+$.

Oxonantenine (20): UV λ_{max} (MeOH) 242, 273, 322, 361, 445 nm; ^{1}H NMR ($CDCl_3$, 600 MHz): δ3.98 (3H, s), 4.07 (3H, s), 6.12 (2H, s), 7.17 (1H, s), 7.75 (1H, d, *J* = 4.8 Hz), 7.98 (1H, s), 8.66 (1H, s), 8.87 (1H, d, *J* = 5.1 Hz); (+)ESIMS: *m/z* 358

$[M + Na]^+$.