Pseudotrienic Acid A and B, two Bioactive Metabolites from *Pseudomonas* sp. MF381-IODS

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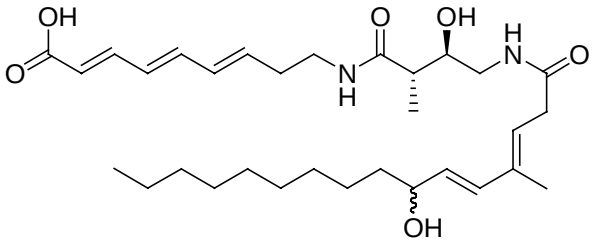
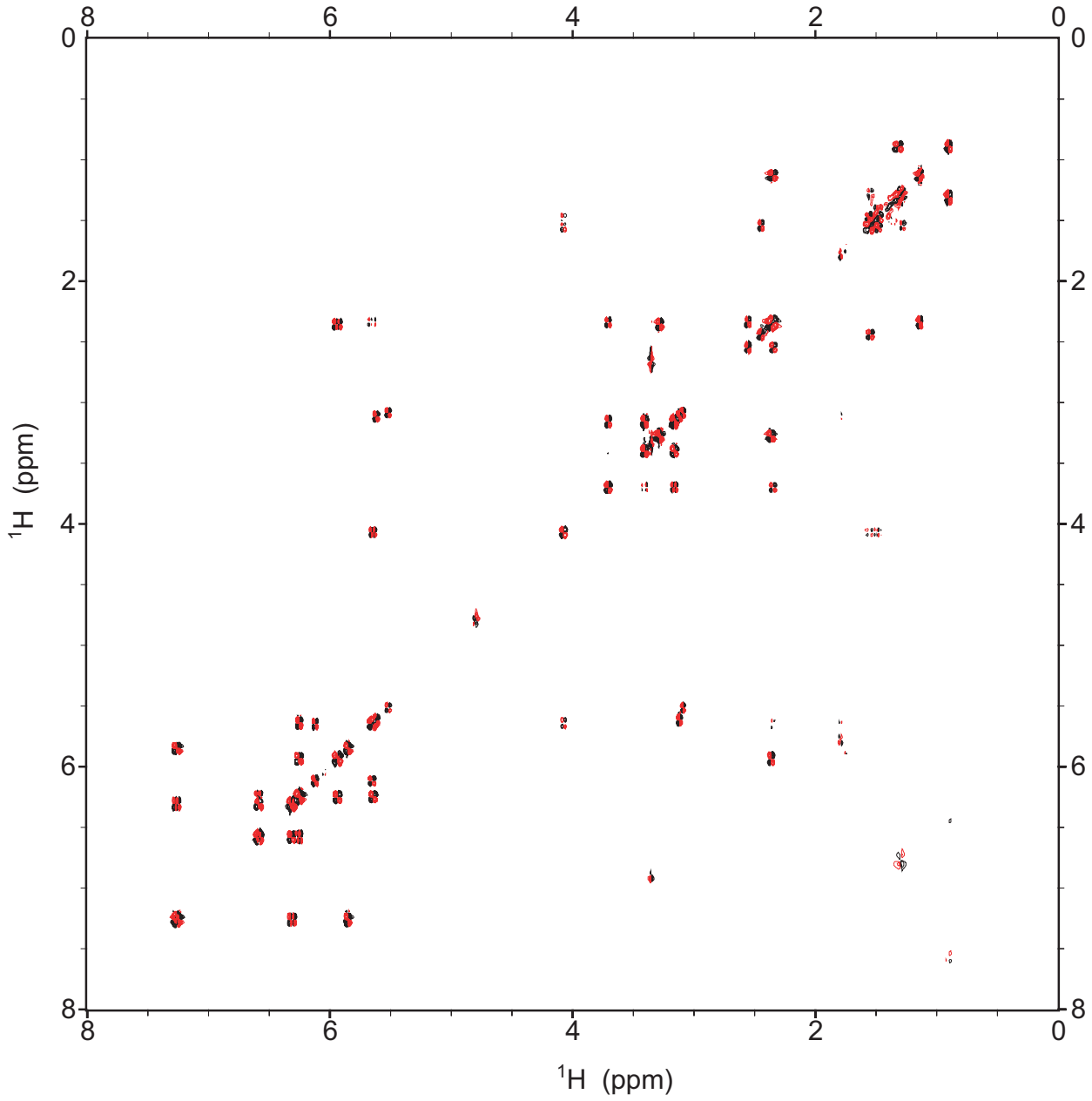
DDR methanol adduct (**3a**)**:** White powder; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.53 (1H, s, H-25), 6.77 (1H, ddd, *J* = 15.6, 6.5, 6.5 Hz, H-3), 6.60 (1H, dd, *J* = 15.0 Hz, 10.8, H-20), 6.38 (1H, d, *J* = 15.0 Hz, H-21), 6.26 (1H, s, H-23), 6.10 (1H, d, *J* = 10.8 Hz, H-19), 5.70 (1H, d, *J* = 15.6 Hz, H-2), 5.51 (1H, dd, *J* = 15.6, 9.0 Hz, H-9), 5.15 (1H, dd, *J* = 15.6, 8.4 Hz, H-10), 4.80 (1H, dd, *J* = 10.2, 3 Hz, H-15), 3.71 (3H, s, H-36), 3.25 (1H, d, *J* = 10.8 Hz, H-17), 3.18 (1H, m, H-7), 3.17 (3H, s, H-32), 3.14 (1H, d, *J* = 8.4 Hz, H-11), 3.04 (1H, dd, *J* = 9.6, ~1,5 Hz, H-13), 2.54 (1H, dd, *J* = 15.6, 7.2 Hz, H-27a), 2.46 (3H, s, H-35), 2.44 (1H, m, H-4a), 2.40 (1H, dd, *J* = 15.6, 7.2 Hz, H-27b), 2.23 (1H, bm, H-5), 2.15 (3H, s, H-34), 2.10 (1H, m, H-16), 2.08 (1H, m, H-4b), 2.05 (1H, m, H-8), 1.98 (1H, m, H-14a), 1.86 (3H, s, H-33), 1.80 (1H, m, H-14b), 1.78 (1H, M, H-6a), 1.35 (3H, s, H-30), 1.07 (1H, m, H-6b), 1.05 (3H, d, *J* = 6.6 Hz, H-29), 1.00 (3H, d, *J* = 6.6 Hz, H-31); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.9 (C, C-28), 165.5 (C, C-1), 161.4 (C, C-26), 146.5 (CH, C-3), 141.3 (CH, C-9), 138.4 (C, C-24), 137.6 (CH, C-21), 137.0 (C, C-22), 136.7 (C, C-18), 135 (CH, C-25), 129.7 (CH, C-19), 125.6 (CH, C-10), 124.9 (CH, C-2), 124.3 (CH, C-20), 120.7 (CH, C-23), 89.4 (CH, C-17), 78.7 (CH, C-13), 74.4 (CH, C-7), 73.5 (CH, C-15), 66.0 (C, C-12), 63.9 (CH, C-11), 56.8 (CH_3, C-32), 51.8 (CH_3, C-36), 45.7 (CH, C-8), 40.8 (CH_2, C-27), 39.5 (CH, C-16), 38.0 (CH_2, C-6), 37.7 (CH_2, C-4), 32.2 (CH, C-5), 32.0 (CH_2, C-14), 17.3 (CH_3, C-29), 14.6 (CH_3, C-34), 13.9 (CH_3, C-35), 11.7 (CH_3, C-33), 11.4 (CH_3, C-30), 10.4 (CH_3, C-31); ESIMS *m/z* 664.3 $[M+Na]^+$.

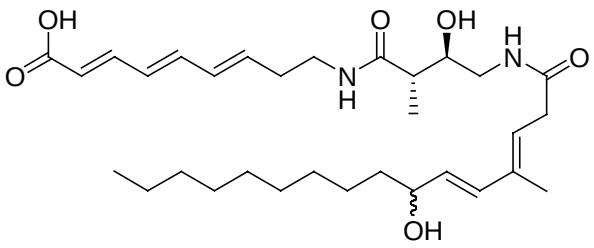
Pyrrolnitrine (**4**)**:** White powder; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.29 (1H, br s, NH), 7.53 (1H, m, H-4'), 7.45 (1H, m, H-5), 7.43 (1H, m, H-6'), 6.85 (1H, m, H-2), 6.84 (1H, m, H-5); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 149.6 (C, C-2'), 130.6 (CH, C-4'), 130.2 (CH, C-5'), 129.0 (CH, C-6'), 128.2 (C, C-1'), 125.3 (C, C-3'), 117.5 (CH, C-5), 116.7 (CH, C-2), 115.7 (C, C-4), 112.5 (C, C-3); ESIMS *m/z* 279 $[M+Na]^+$.

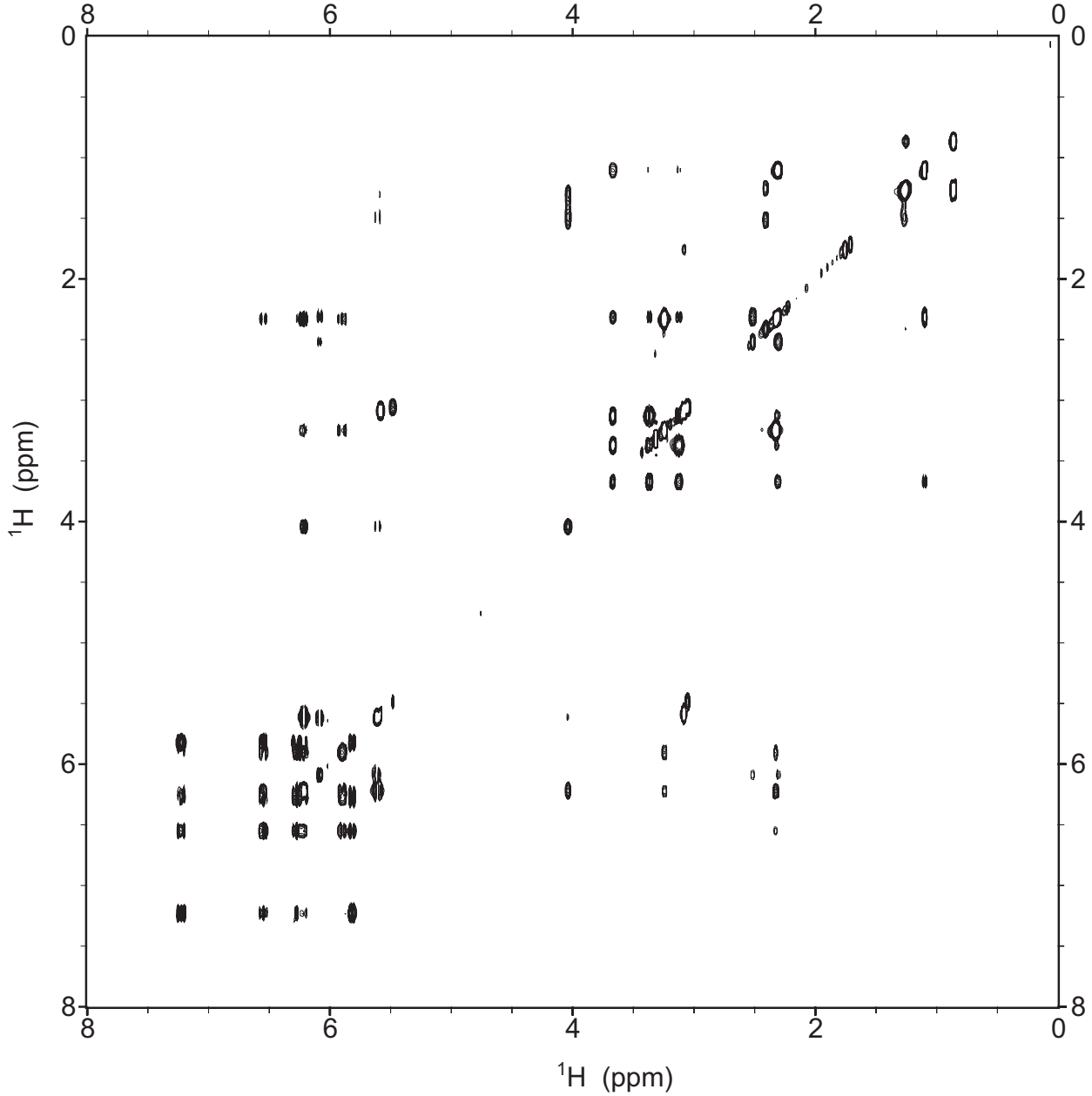
DQFCOSY spectrum of **1** (CD_3OD, 600 MHz)



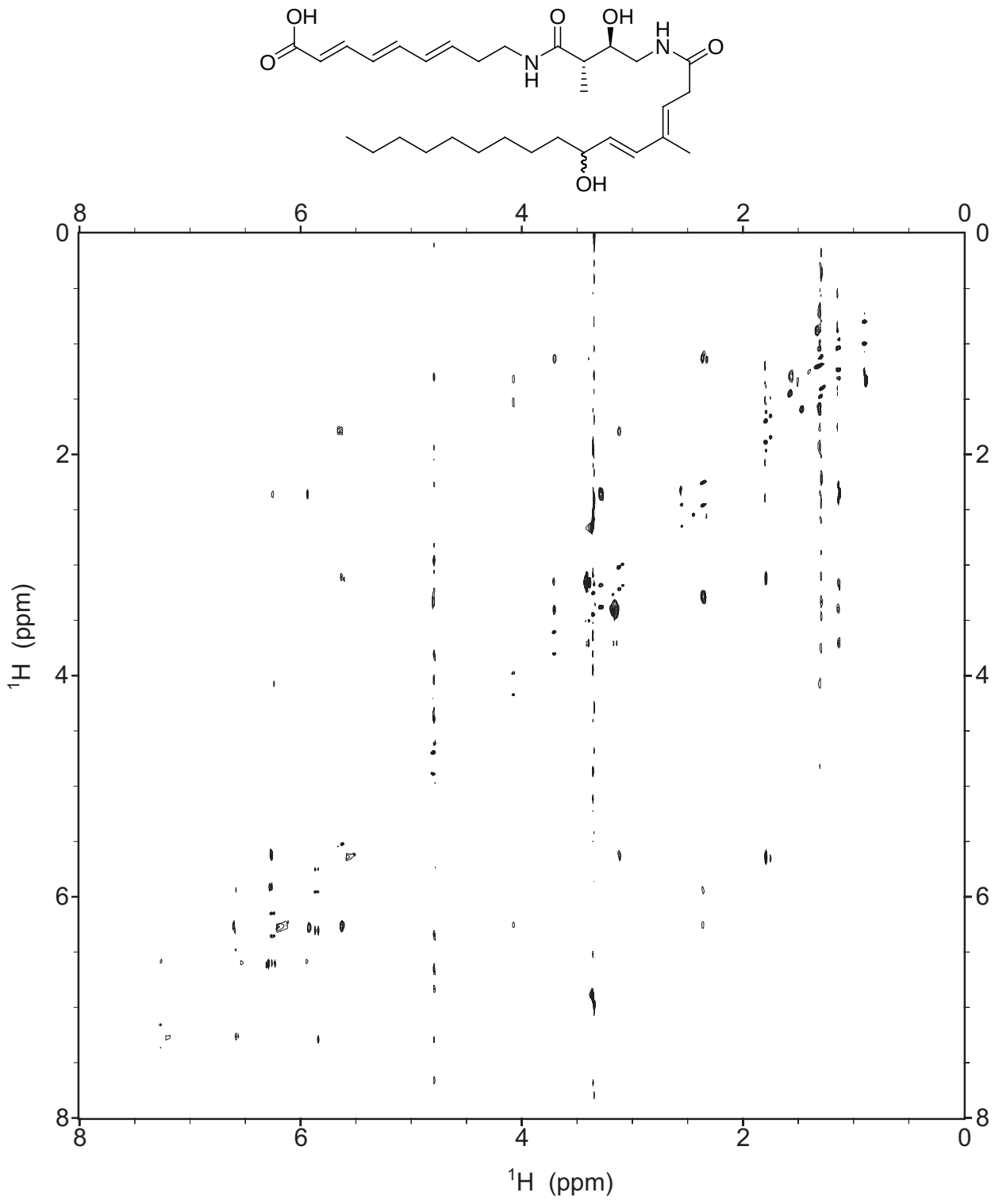


TOCSY spectrum of **1** (CD_3OD, 600 MHz)

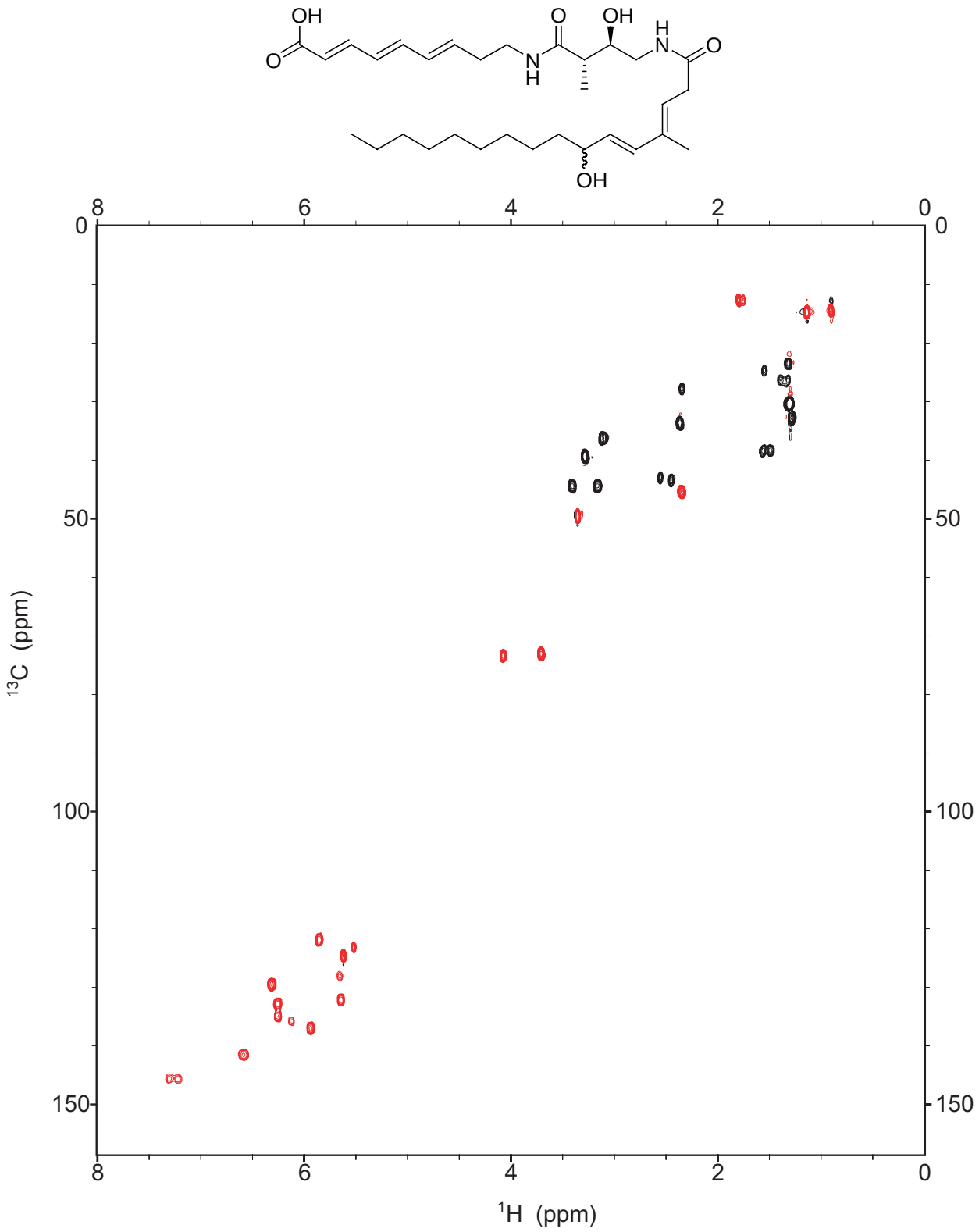




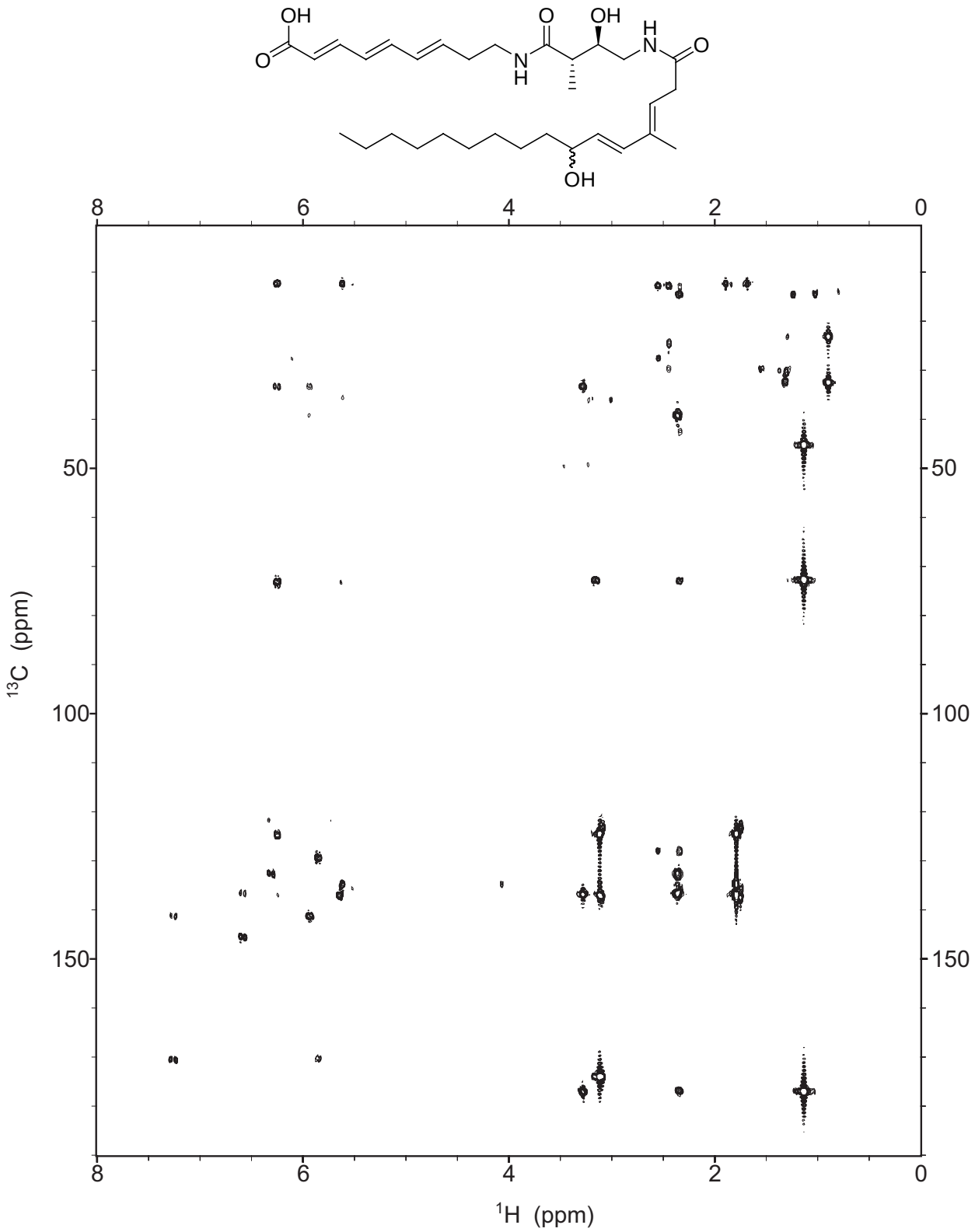
NOESY spectrum of **1** (CD_3OD, 600 MHz)



DEPTHSQC spectrum of **1** (CD_3OD, 150/600 MHz)



HMBC spectrum of **1** (CD_3OD, 150/600 MHz)



Lactone formation of 2 to 2a:

About 1 mg of **2** was dissolved in 950 μL 1% TFA in 50/50 MeCN/H_2O and analyzed at 30 min intervals by RP-HPLC with UV-detection at 310 nm (41% A for 15 min, 41% to 100% A in 5 min, 100% A for 10 min; A: aqueous 10 mM ammonium acetate at pH 4.5, B: CH_3CN). Compound **2** eluted at t_R=14.1 min and a peak at t_R=22.7 min appeared immediately at t=0 and reached maximum area after 60 min. This also correlated with disappearance of the peak at t_R=14.1 min. When subjected to LC-ESIMS in negative mode, the peak at t_R=22.7 min correlated to *m/z* of 499.5 $[M-H]^-$, *i.e.* corresponding to the lactone **2a.** The peak of **2a** was isolated and subjected to ^1H NMR and COSY in CD_3CN/D_2O 50/50 (see page S9, spectrum C). To verify the lactone formation, ^1H NMR and COSY was run on 0.5 mg of **2** dissolved in 500 μL CD_3CN/D_2O 50/50 (see page S9, spectrum A). Then TFA was added to a concentration of 5% and the mixture was monitored by ^1H NMR and COSY. After five min about 60% of the material had formed lactones (see page S9, spectrum B). Although the high TFA content influenced the HDO resonance the chemical shifts and connectivities could be verified by comparison with the isolated lactone sample from the HPLC experiment and the control spectra acquired prior to addition of TFA.

^{1}H NMR spectra (600 MHz) of lactone formation analysis with the H-20 (**2**) and H-16 (**2a**) resonance marked. A: ^{1}H NMR of **2** (CD_3CN/D_2O 50/50); B: ^{1}H NMR of **2** five min after addition of 5% TFA (CD_3CN/D_2O 50/50, 5% TFA); C: ^{1}H NMR of **2a** (CD_3CN/D_2O 50/50)