Supporting Information part III

Synthesis of the rosette-inducing factor RIF-1 and analogs

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Table of content: representative 1D and 2D NMR spectra of the following compounds

(2*R*,3*R*,5*R*)-3,5-Dihydroxy-2-((*R*)-2-hydroxy-13-methyltetradecanamido)-15-methyl-hexadecane-1-sulfonic acid (1)

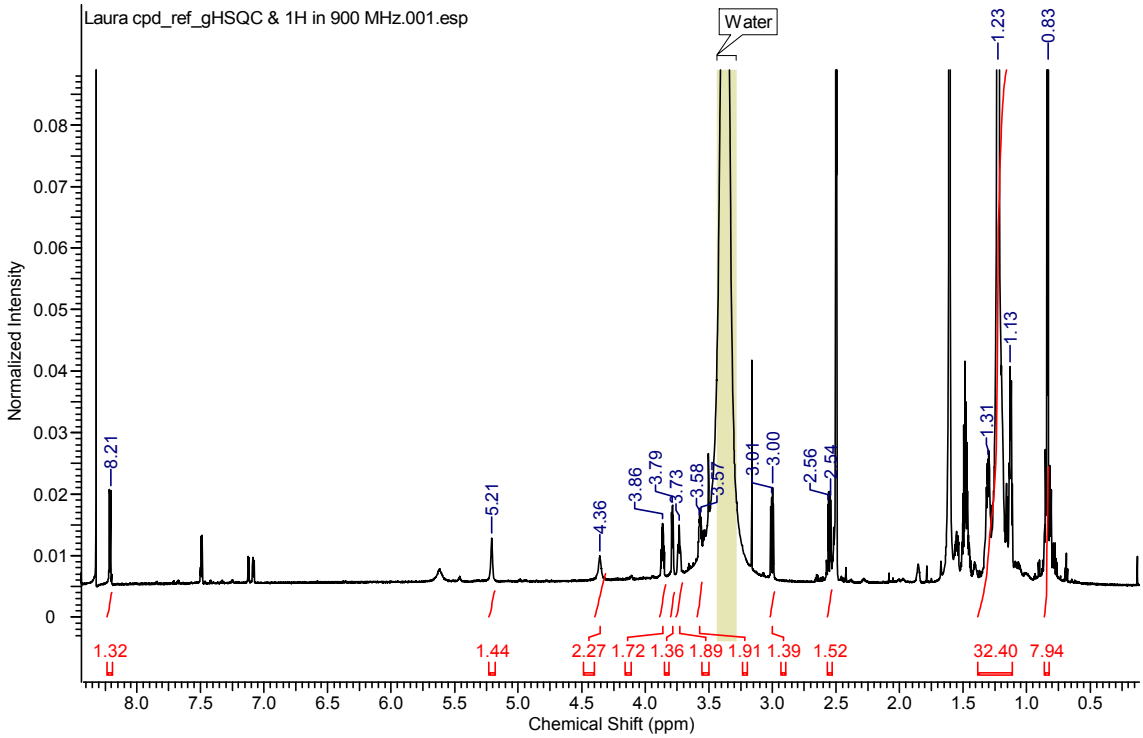

Figure S1. ^{1}H NMR spectrum of compound **1** (natural isolate, 900 MHz, d_6-DMSO).

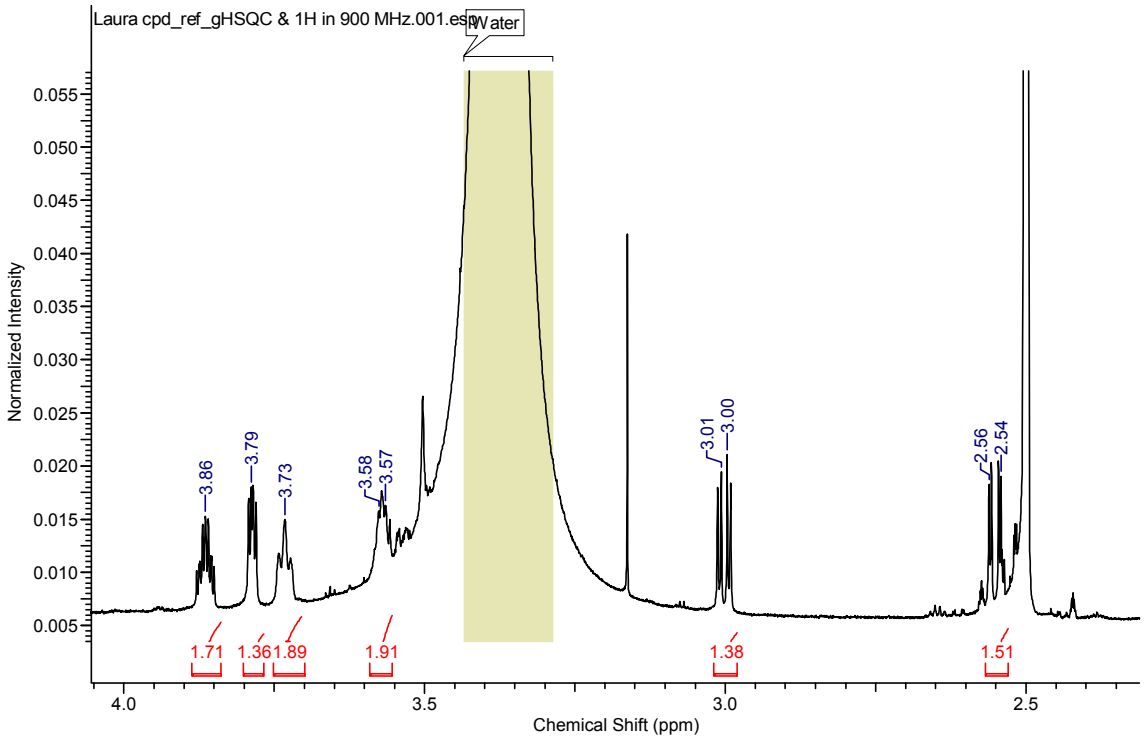

Figure S2. ^{1}H NMR spectrum of compound **1** (natural isolate, 900 MHz, d_6-DMSO).

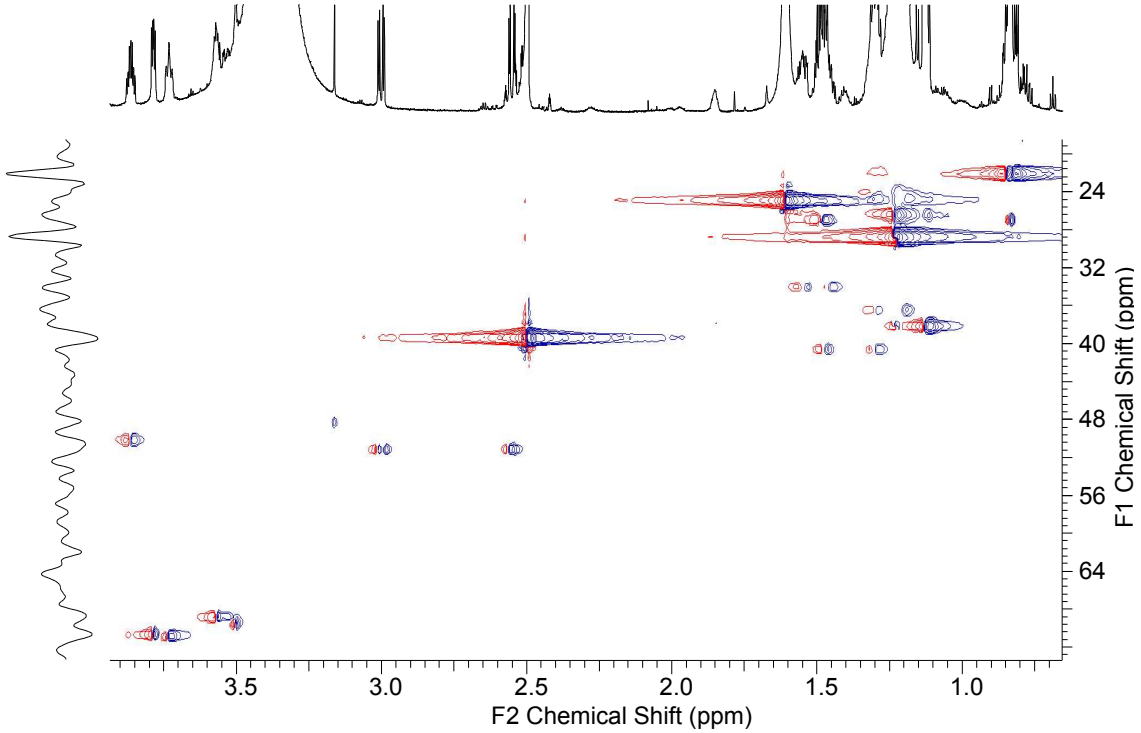

Figure S3. HSQC spectra of compound **1** (natural isolate, 900 MHz, d_6-DMSO).

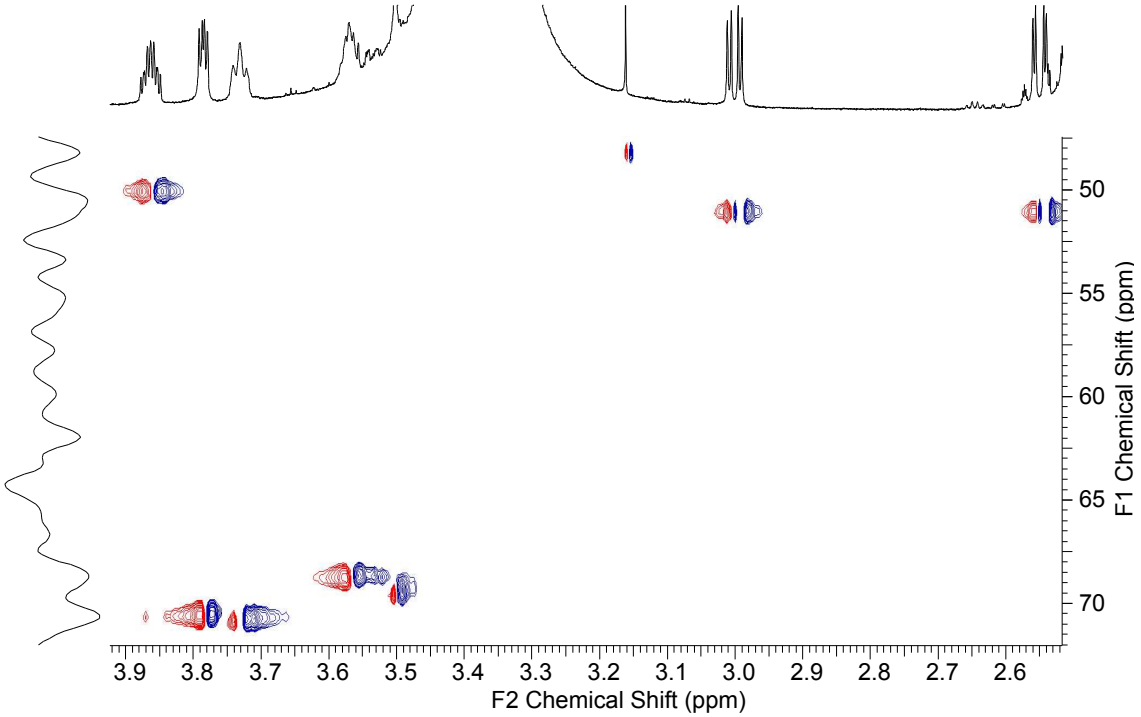

Figure S4. HSQC spectra of compound **1** (natural isolate, 900 MHz, d_6-DMSO).

(2*R*,3*R*,5*R*)-3,5-Dihydroxy-2-((*R*)-2-hydroxy-13-methyltetra-decanamido)-15-methyl-hexadecane-1-sulfonic acid (1)

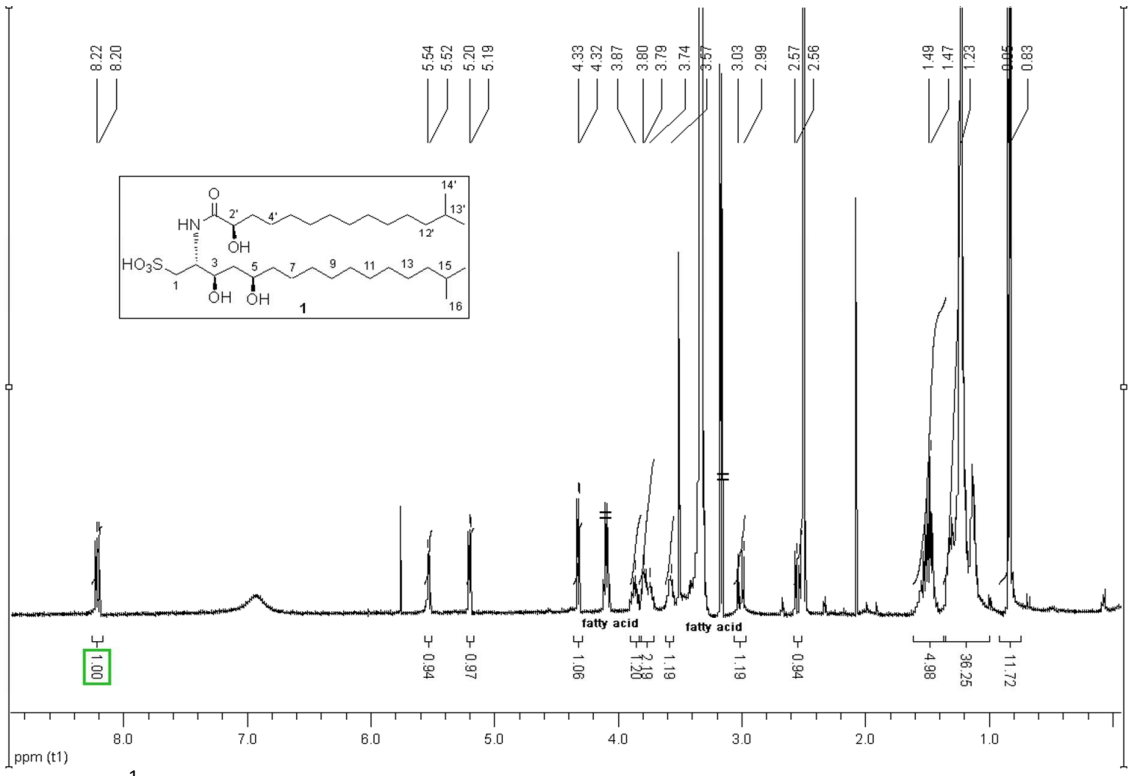

Figure S5. ^{1}H NMR spectrum of synthetic compound **1** (d_6-DMSO, 600 MHz).

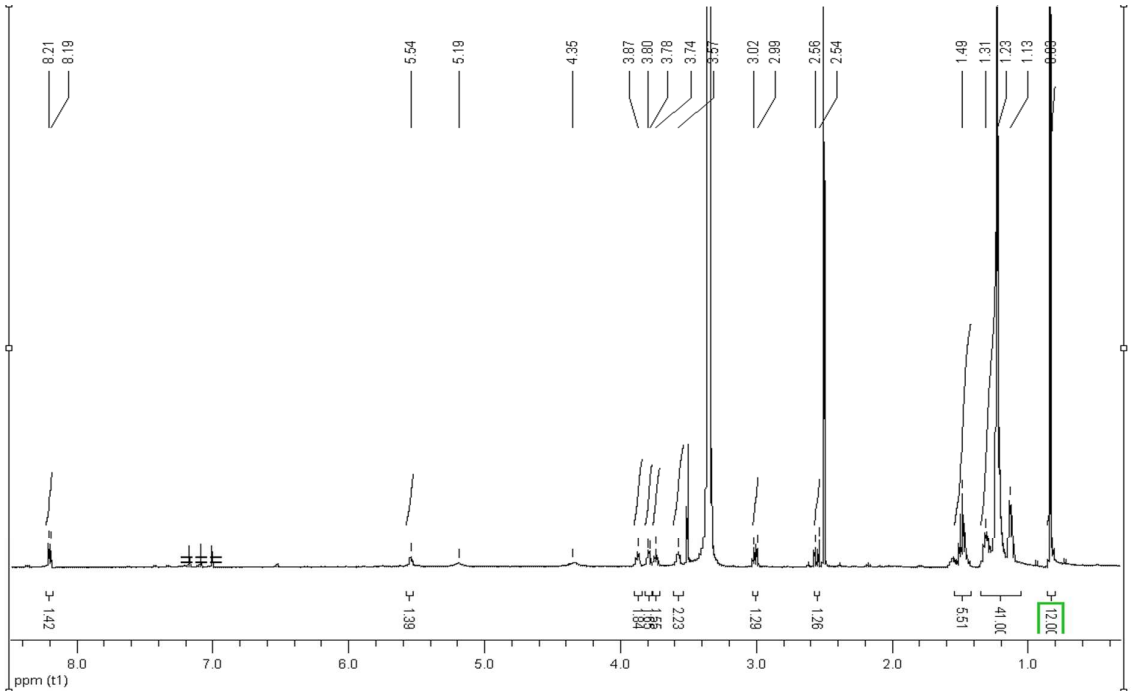

Figure S6. ^{1}H NMR spectrum of repurified synthetic compound **1** (d_6-DMSO, 600 MHz).

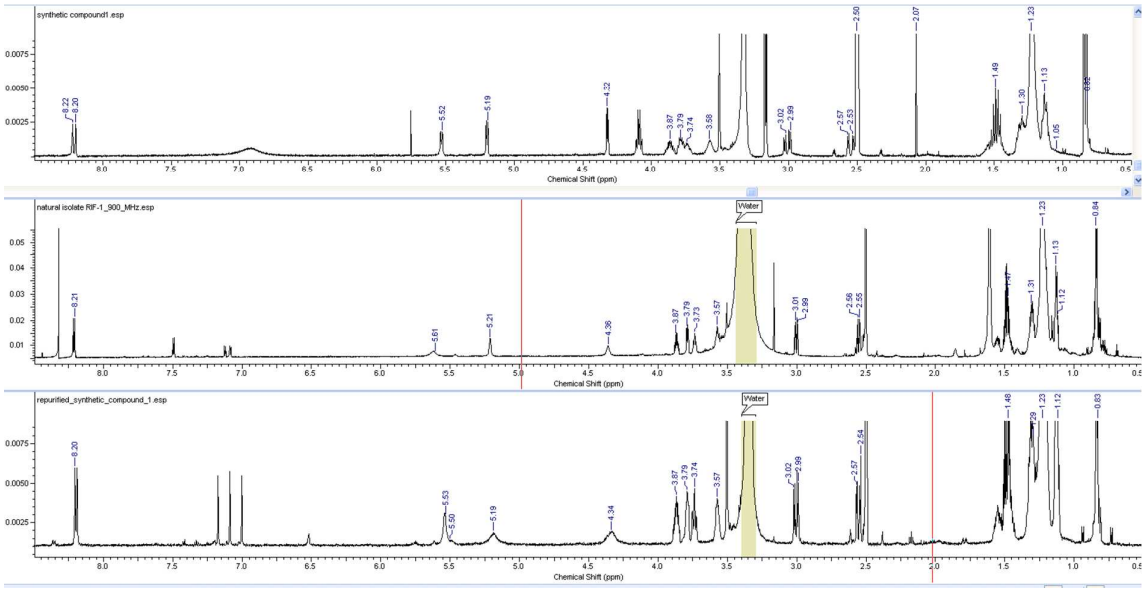

Figure S7. Comparison of ^{1}H NMR spectra: a) synthetic compound **1** (d_6-DMSO, 600 MHz), b) isolated RIF-1 (d_6-DMSO, 900 MHz); c) repurified synthetic compound **1** (d_6-DMSO, 600 MHz)

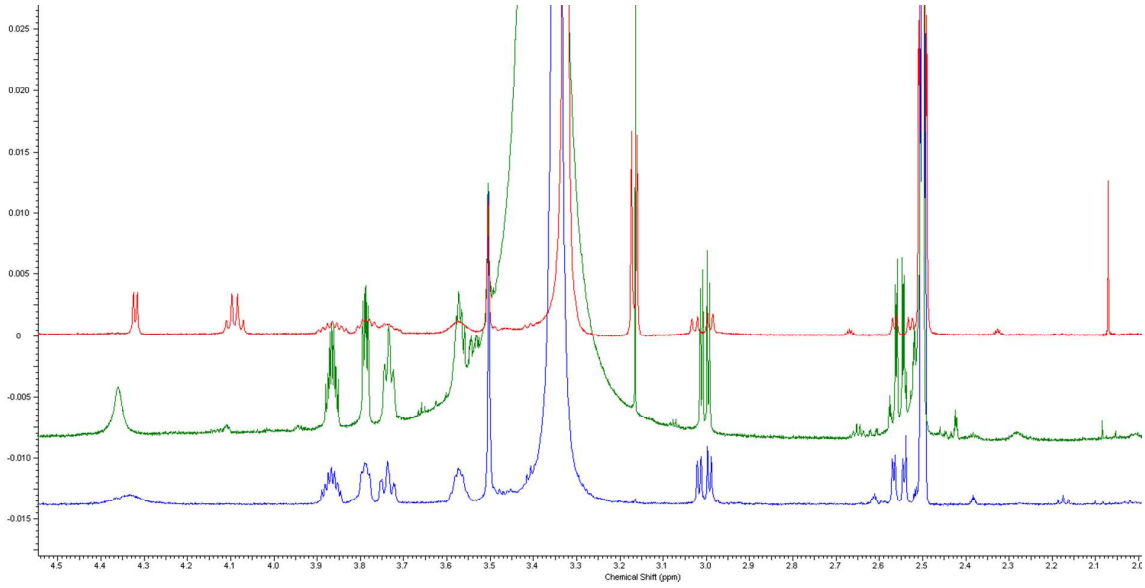

Figure S8. Comparison of ^{1}H NMR spectra: a) synthetic compound **1** (red, d_6-DMSO, 600 MHz), b) isolated RIF-1 (green, d_6-DMSO, 900 MHz); c) repurified synthetic compound **1** (blue, d_6-DMSO, 600 MHz)

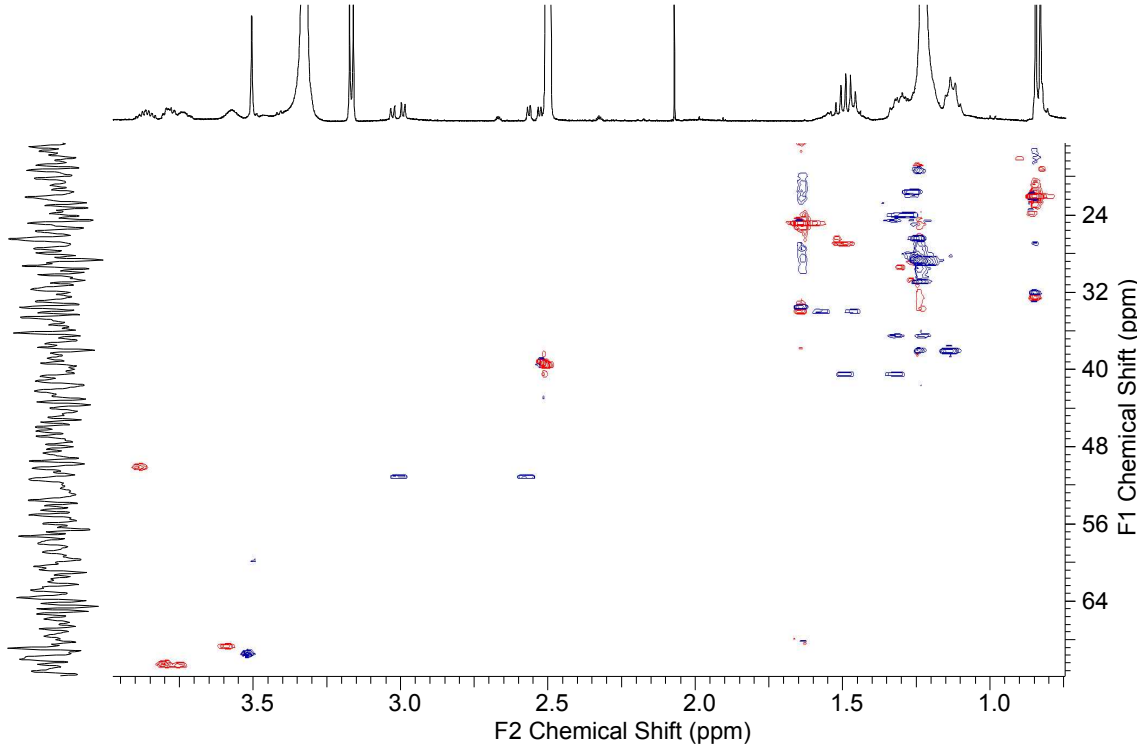

Figure S9. HSQC spectrum of compound **1** (synthetic compound, 600 MHz, d_6-DMSO).

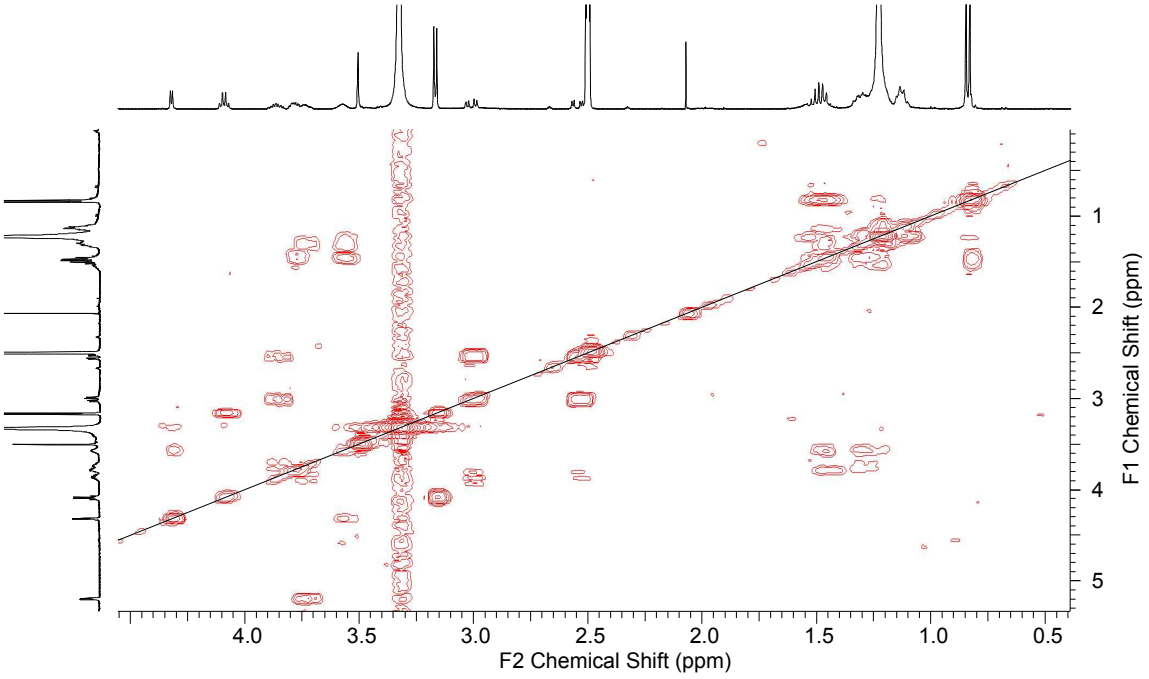

Figure S10. ^{1}H-^{1}H COSY spectrum of compound **1** (synthetic compound, 600 MHz, d_6-DMSO).

(2*R*,3*R*)-3-Hydroxy-15-methyl-2-(13-methyltetradecanamido)hexadecane-1-sulfonic acid (4)

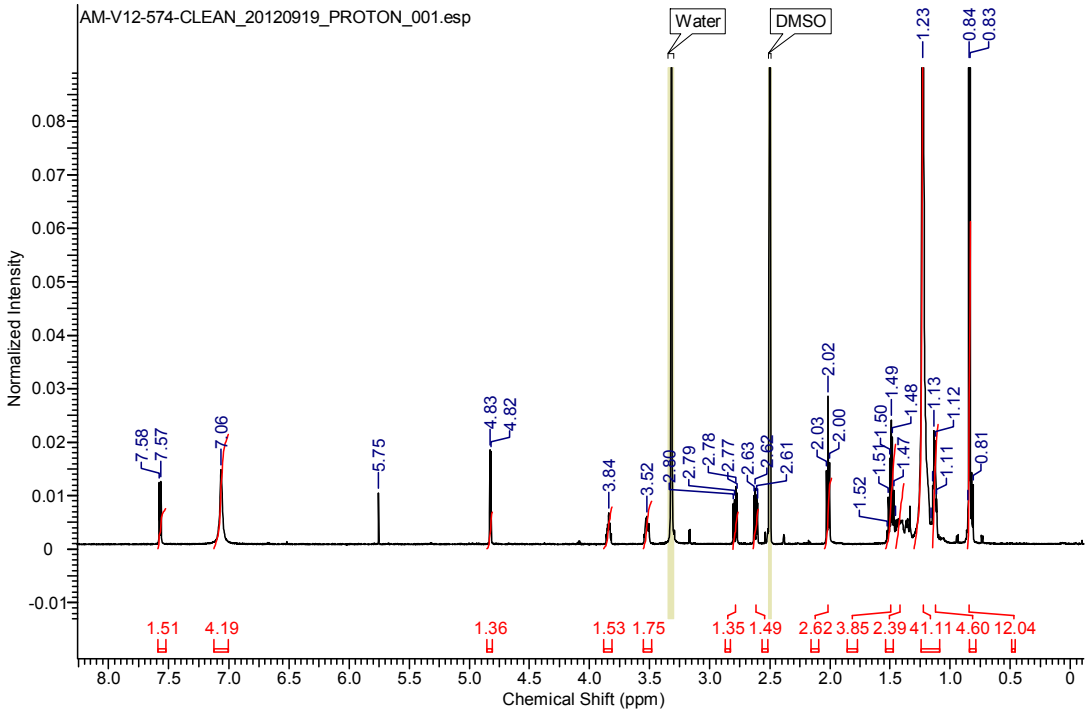

Figure S11. ^{1}H NMR spectrum of compound **4** (natural isolate, 600 MHz, d_6-DMSO).

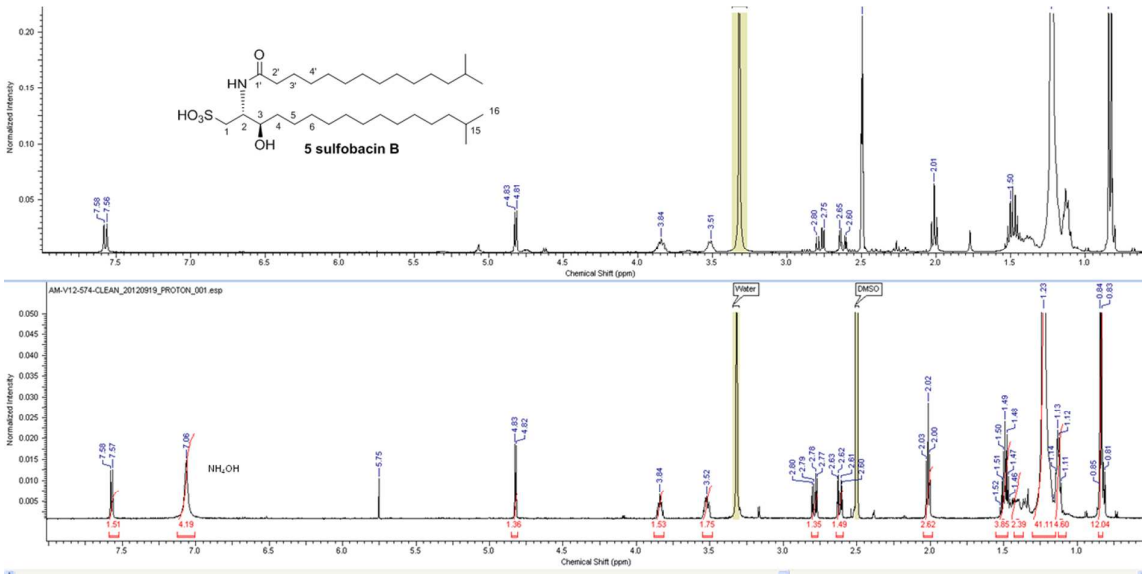

Figure S12. Comparison of ^{1}H NMR spectra of compound **4:** a) natural isolate without NH_4OH (d_6-DMSO, 600 MHz); b) natural isolate with NH_4OH (d_6-DMSO, 400 MHz); chemical shifts do not change upon presence of NH_4OH (NH_4^+, 7.07 ppm).

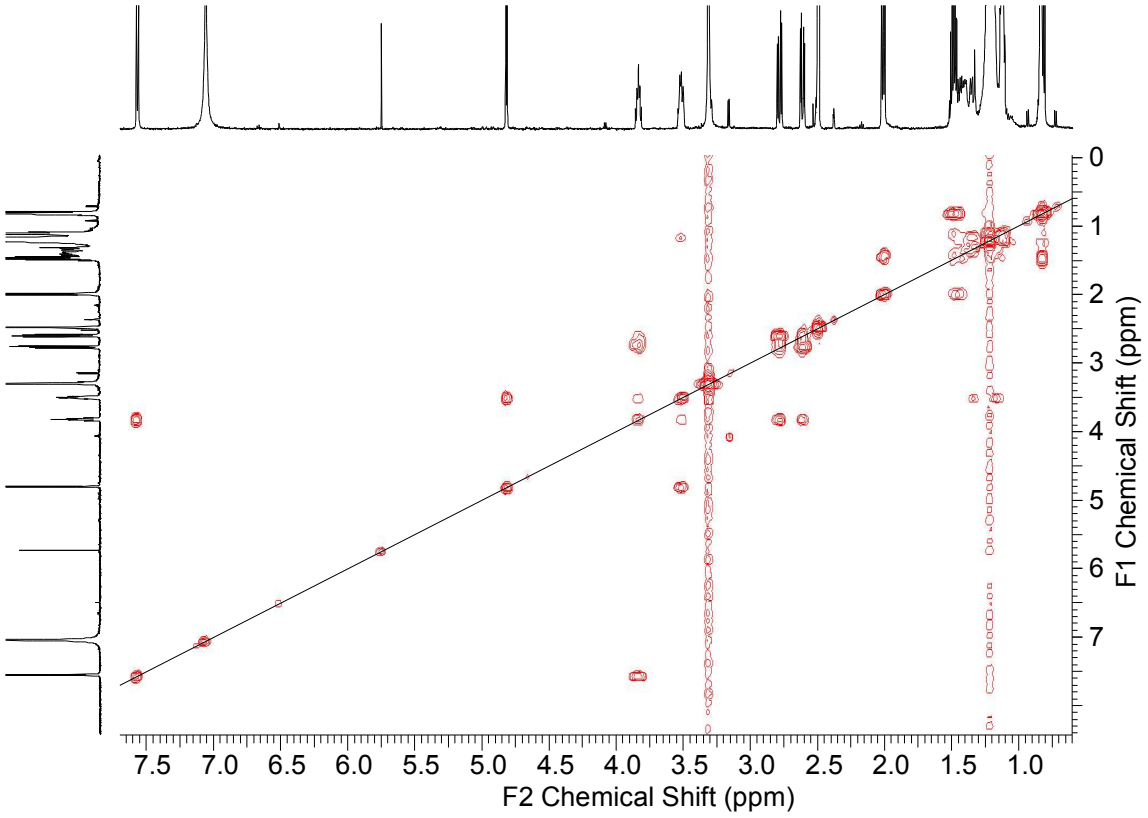

Figure S13. ^{1}H-^{1}H COSY spectrum of compound **4** (d_6-DMSO, 600 MHz).

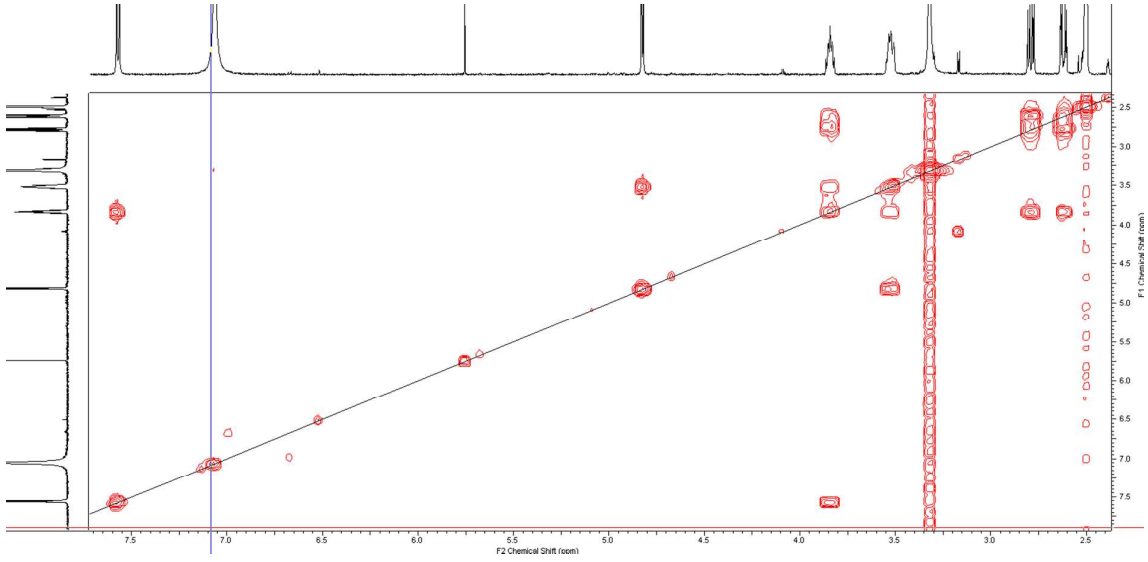

Figure S14. ^{1}H-^{1}H COSY spectrum of compound **4** (natural isolate, 600 MHz, d_6-DMSO): correlation between NH_4^+ (7.07 ppm) and H_20/OH^- (3.42 ppm).

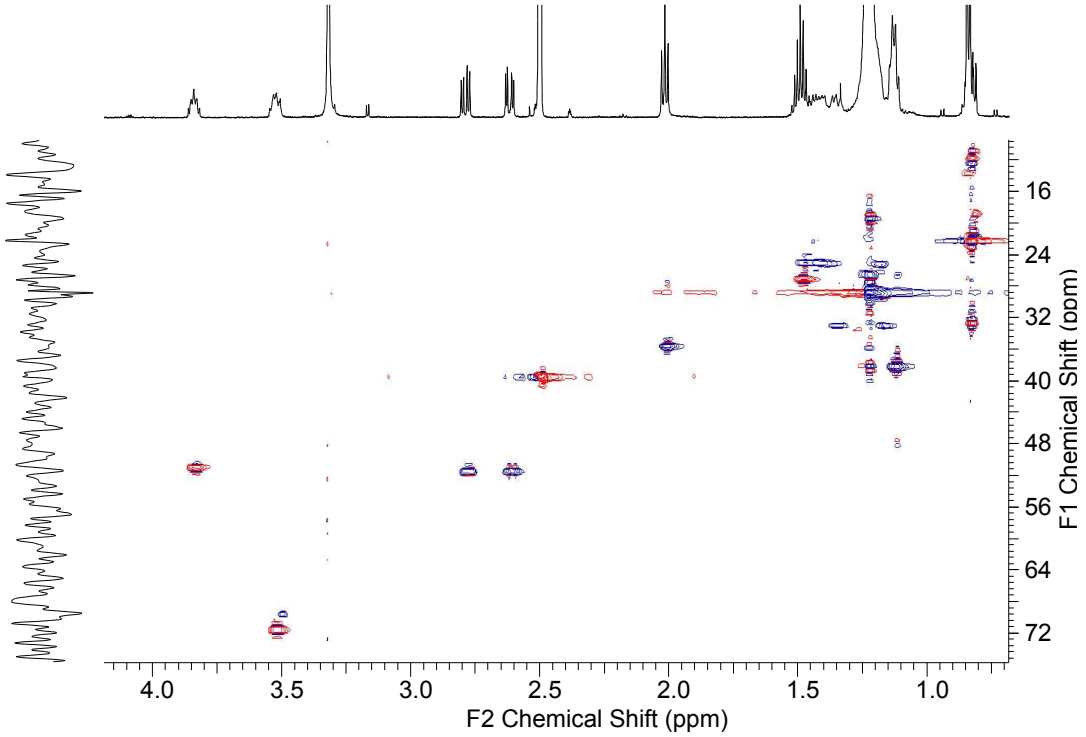

Figure S15. HSQC spectrum of compound **4** (natural isolate, 600 MHz, d_6-DMSO).

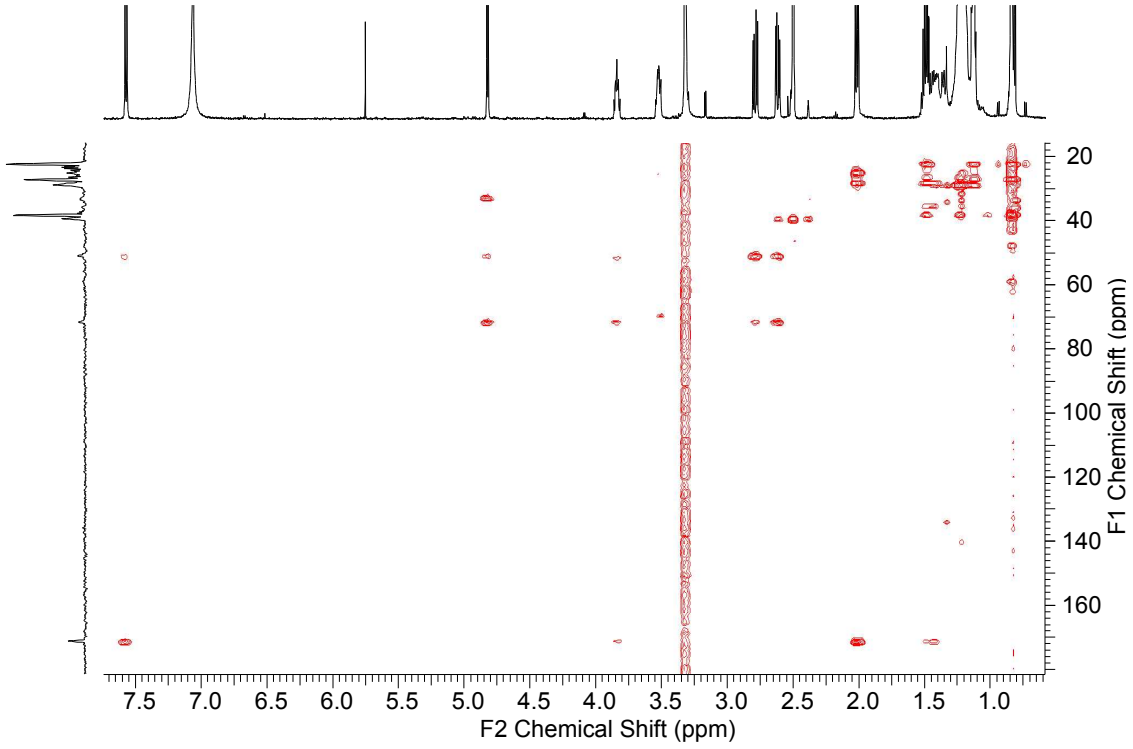

Figure S16. HMBC spectrum of compound **4** (natural isolate, 600 MHz, d_6-DMSO).

(2*R*,3*R*,5*R*)-3,5-Dihydroxy-2-((*S*)-2-hydroxy-13-methyltetra-decanamido)-15-methyl-hexadecane-1-sulfonic acid (21)

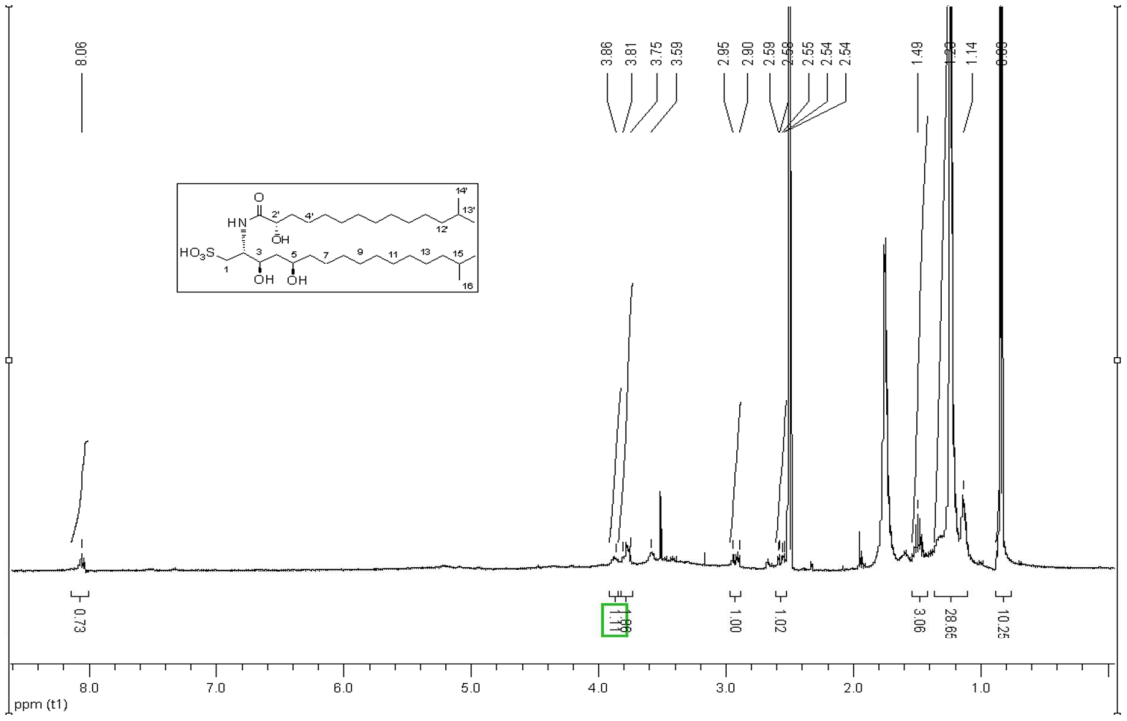

Figure S17. ^{1}H NMR spectra of synthetic compound **21** (600 MHz, d_6-DMSO).

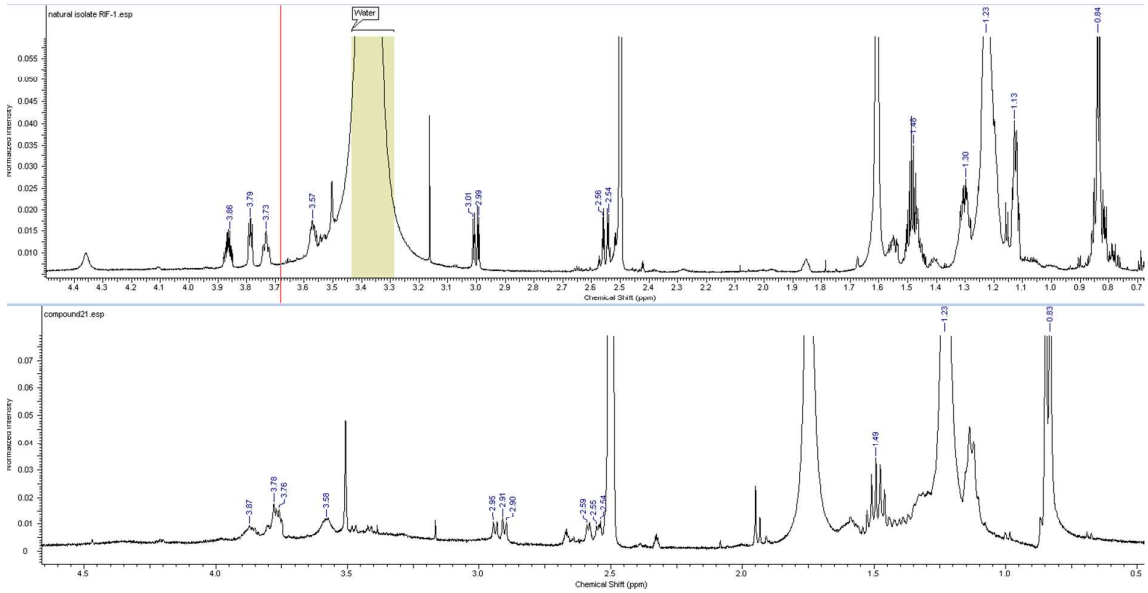

Figure S18. Comparison of ^{1}H NMR spectra: a) natural isolate of compound **1** (900 MHz, d_6-DMSO); b) synthetic compound **21** (600 MHz, d_6-DMSO).

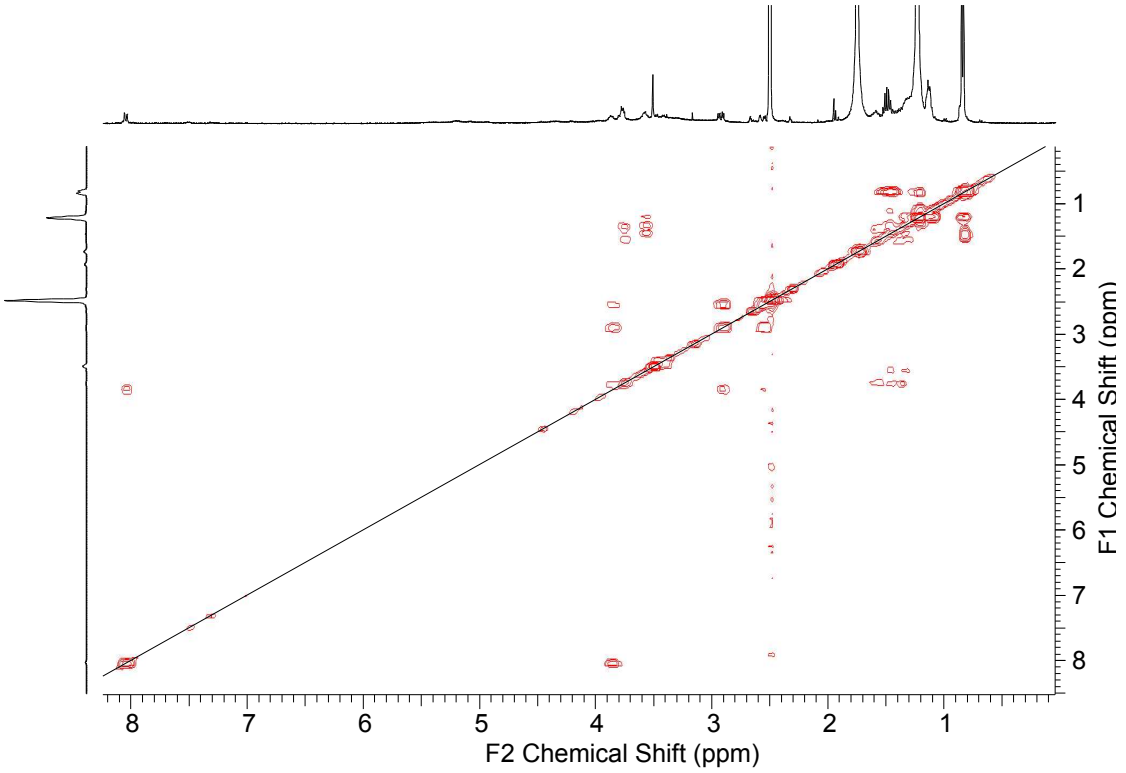

Figure S19. COSY spectrum of synthetic compound **21** (600 MHz, d_6-DMSO).

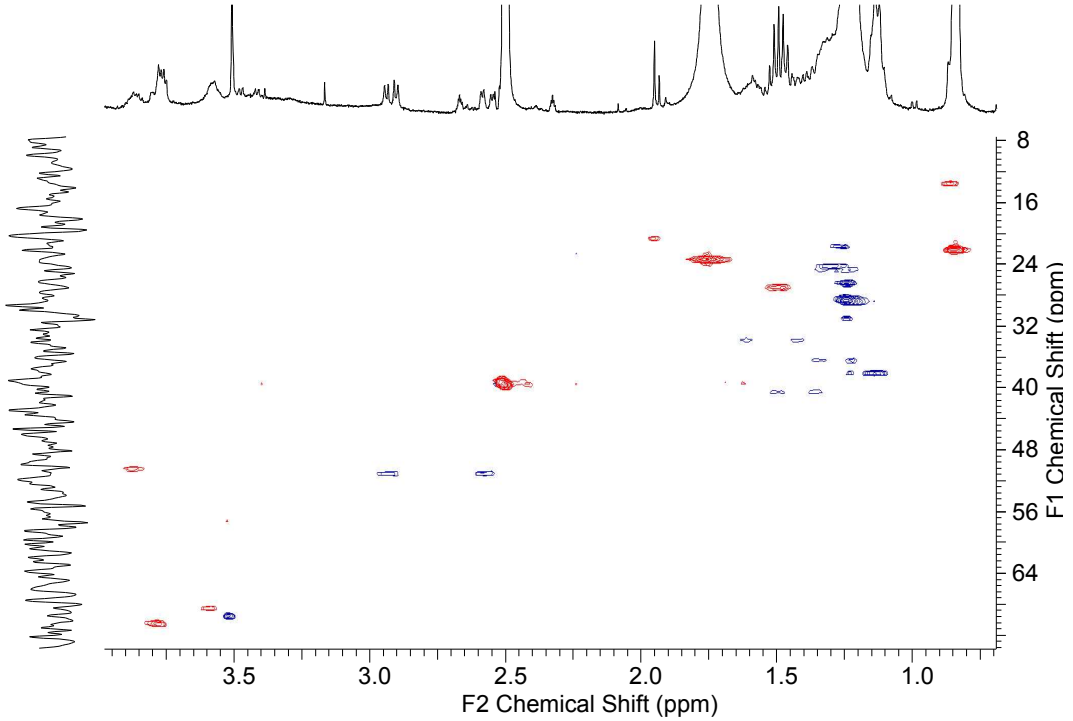

Figure S20. HSQC spectrum of synthetic compound **21** (600 MHz, d_6-DMSO).

(2*R*,3*R*,5*S*)-3,5-Dihydroxy-2-((*R*)-2-hydroxy-13-methyltetra-decanamido)-15-methyl-hexadecane-1-sulfonic acid (22)

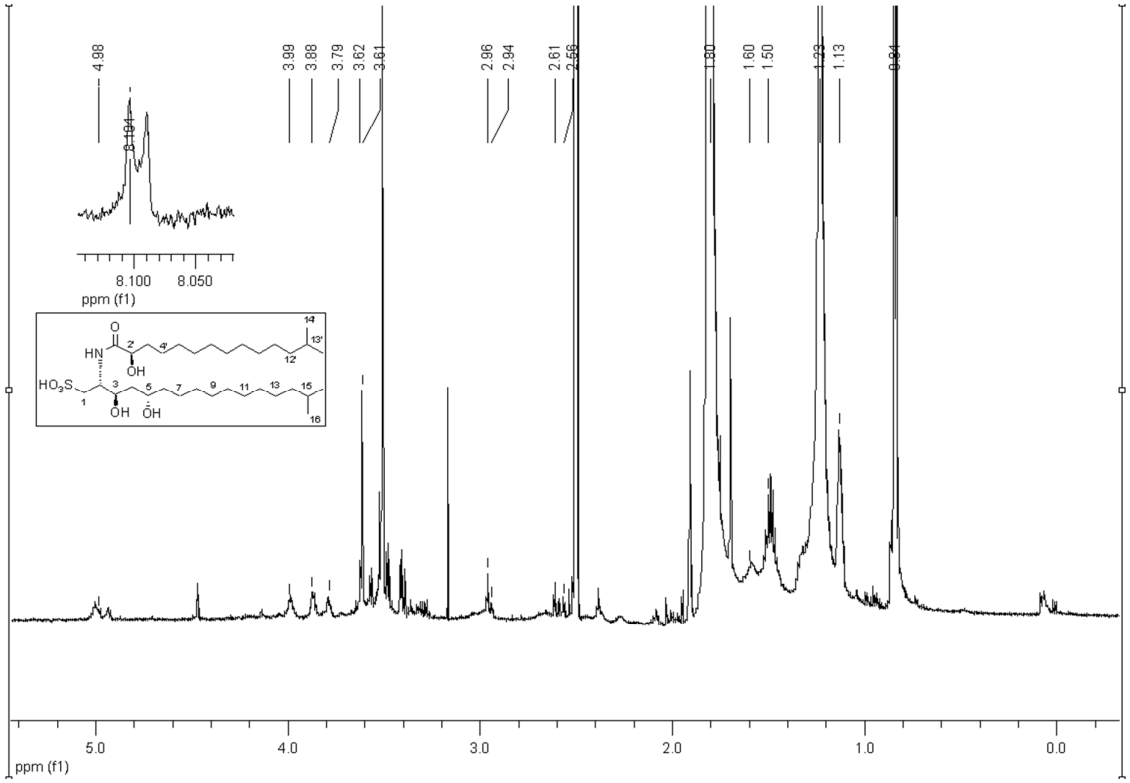

Figure S21. ^{1}H NMR spectrum of synthetic compound **22** (600 MHz, d_6-DMSO).

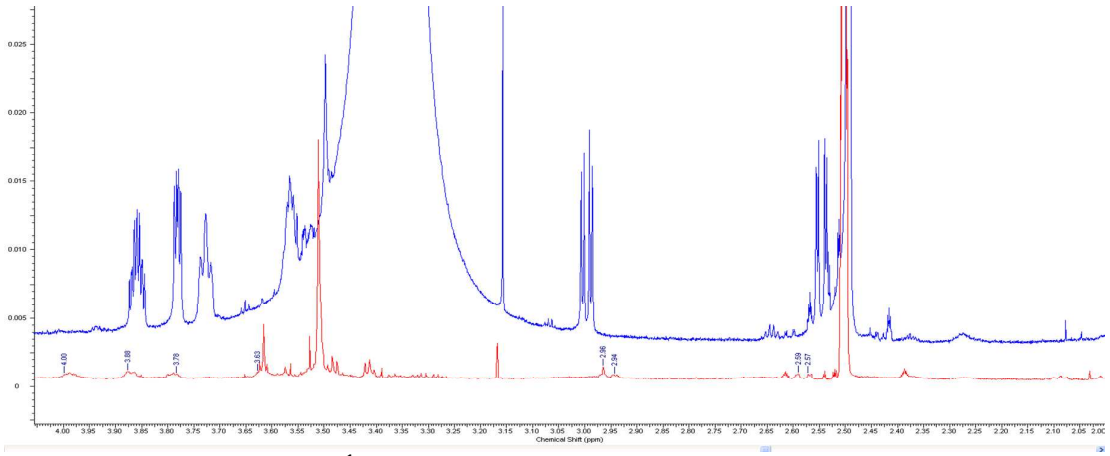

Figure S22. Comparison of ^{1}H NMR spectra: a) synthetic compound **22** (red, 600 MHz, d_6-DMSO), b) compound **1** (blue, natural isolate, 900 MHz, d_6-DMSO).

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(2*R*,3*R*,5*S*)-3,5-Dihydroxy-2-((*S*)-2-hydroxy-13-methyltetra-decanamido)-15-methyl-hexadecane-1-sulfonic acid (23)

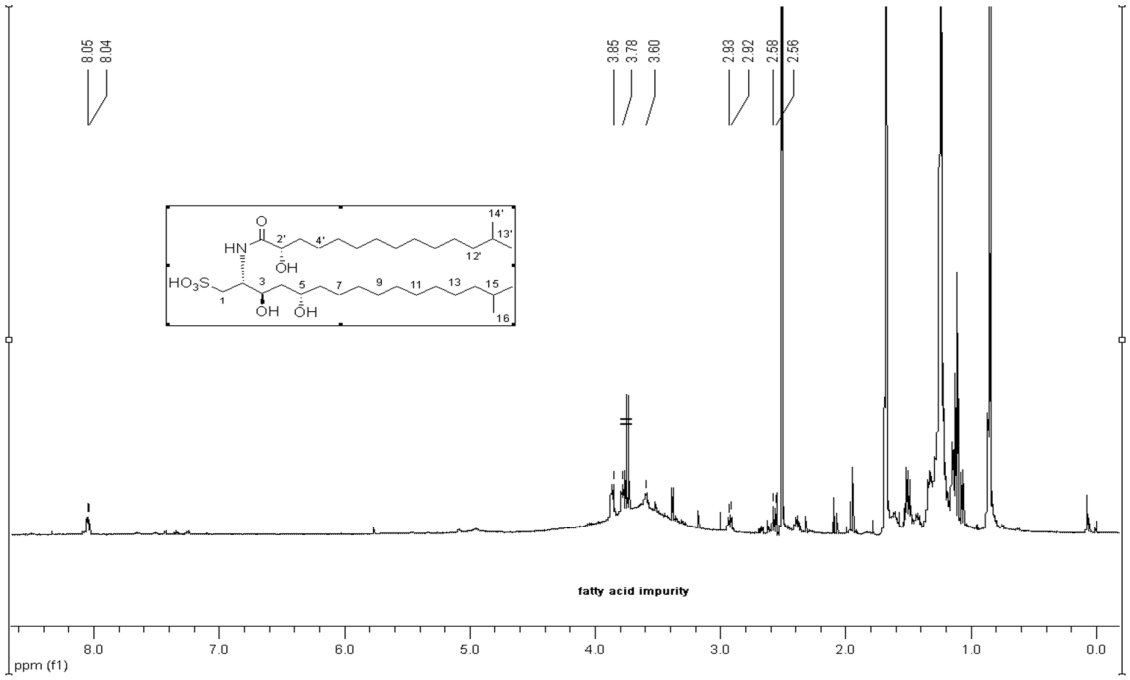

Figure S23. ^{1}H NMR spectrum of synthetic compound **23** (600 MHz, d_6-DMSO).

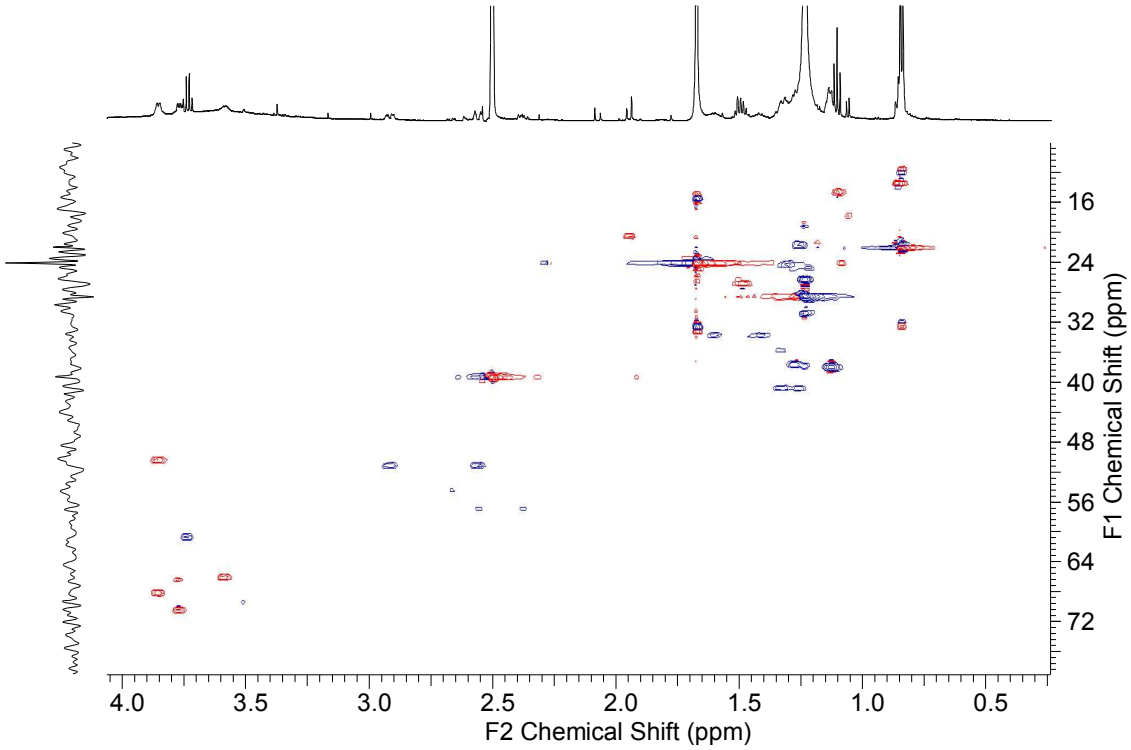

Figure S24. HSQC spectrum of synthetic compound **23** (600 MHz, d_6-DMSO).

(2*R*,3*R*)-3-Hydroxy-2-((*R*)-2-hydroxy-13-methyltetradecanamido)-15-methylhexadecane-1-sulfonic acid (24)

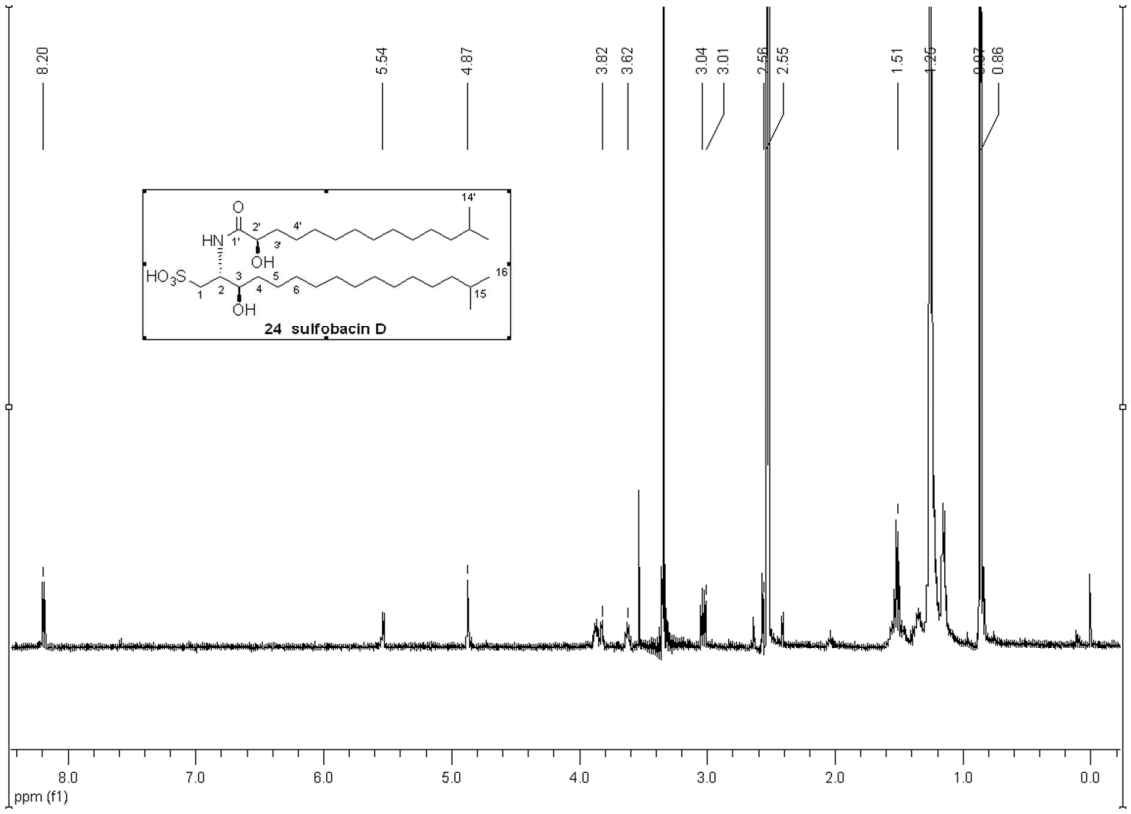

Figure S25. ^{1}H NMR spectrum of compound **24** (natural isolate, 600 MHz, d_6-DMSO).

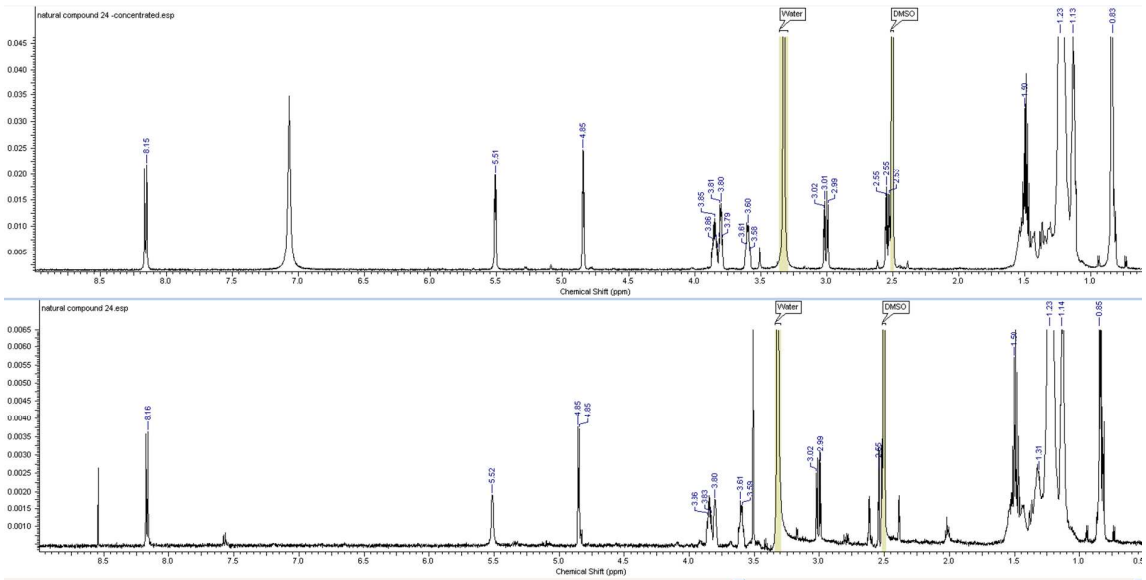

Figure S26. ^{1}H NMR spectrum of a) concentrated sample of compound **24** (natural isolate, 600 MHz, d_6-DMSO), b) repurified compound **24** (natural isolate, 600 MHz, d_6-DMSO).

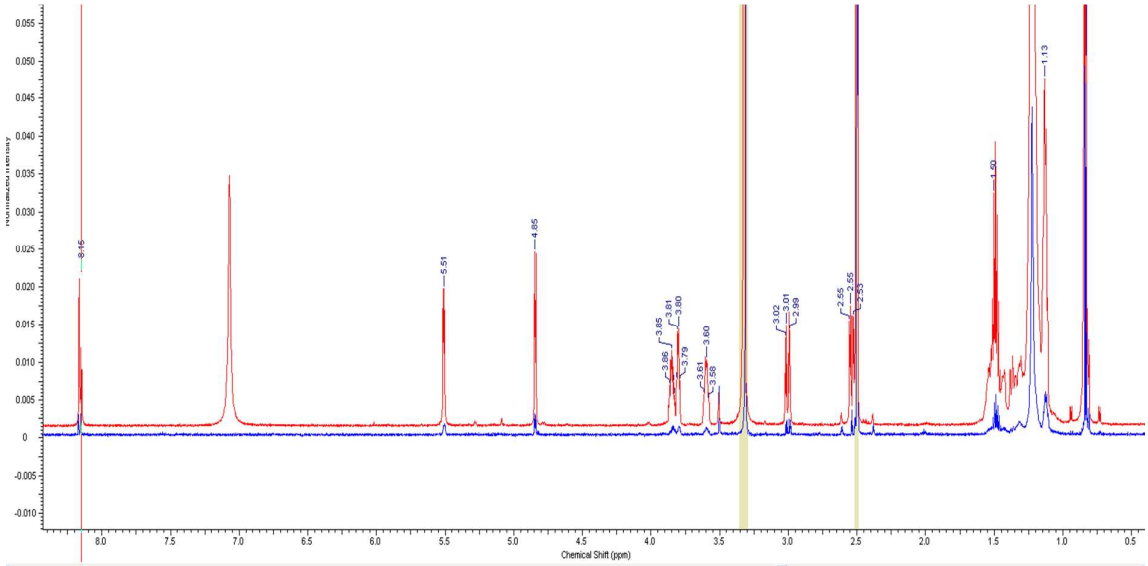

Figure S27. ^{1}H NMR spectrum of a) concentrated sample of compound **24** (red, natural isolate, 600 MHz, d_6-DMSO), b) repurified compound **24** (blue, natural isolate, 600 MHz, d_6-DMSO)

Figure S27. COSY spectrum of compound **24** (natural isolate, 600 MHz, d_6-DMSO).

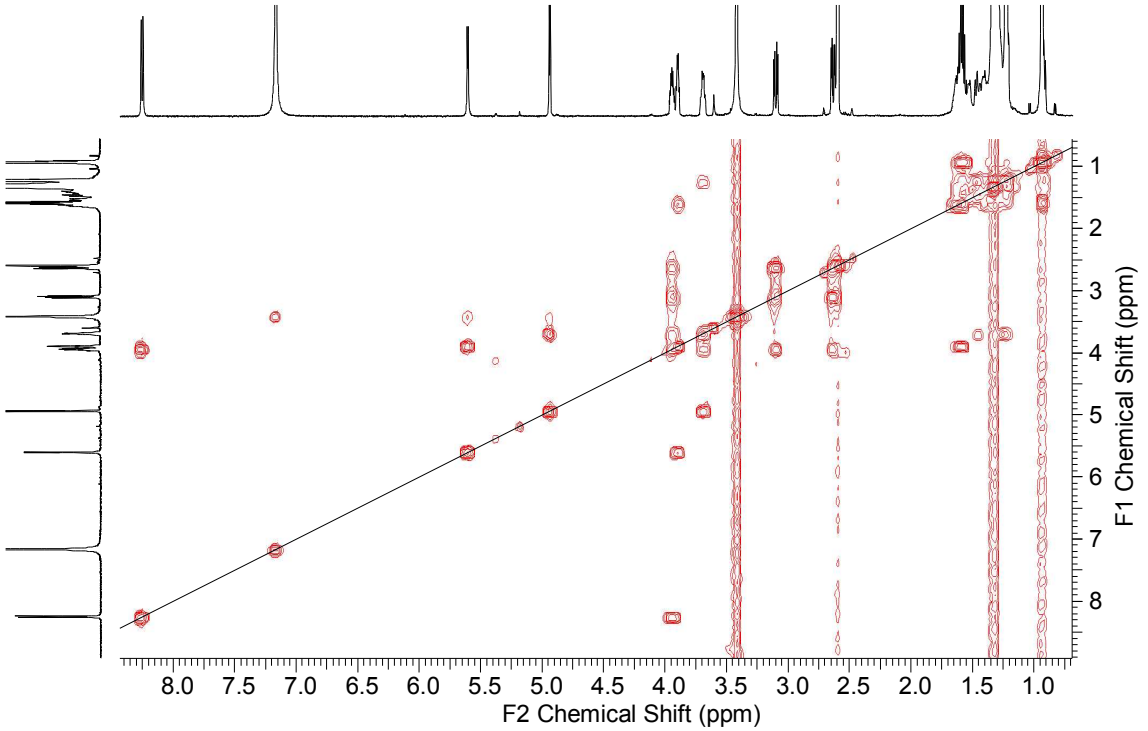

Figure S28. COSY spectrum of compound **24** (natural isolate, 600 MHz, d_6-DMSO).

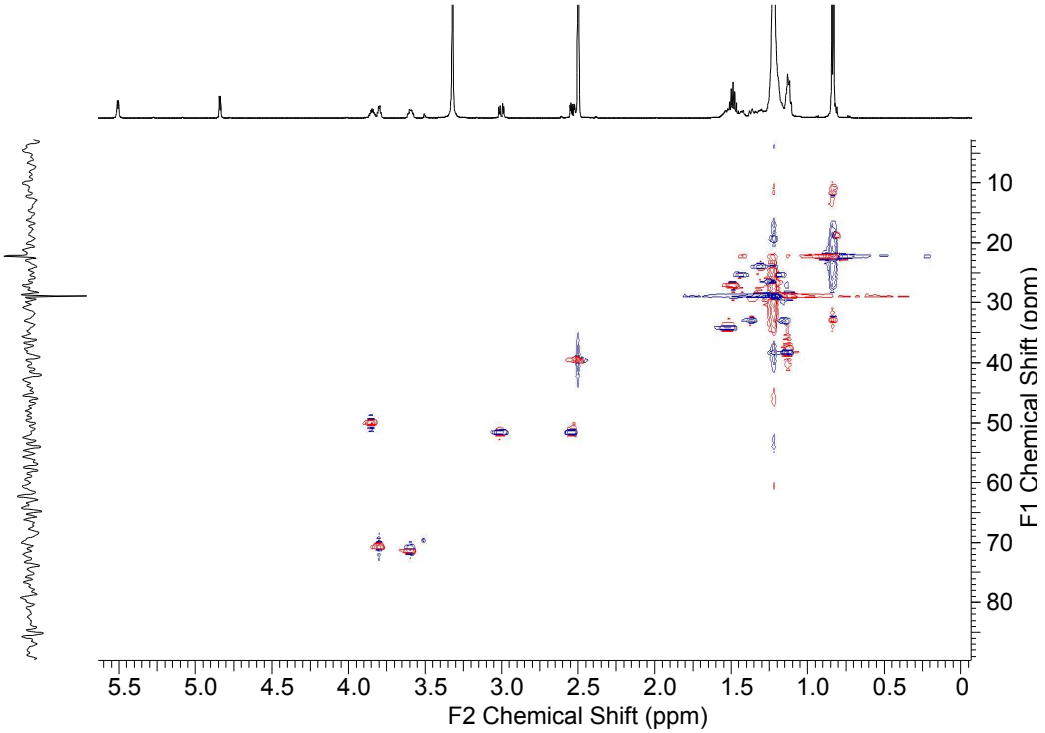

Figure S29. HSQC spectrum of compound **24** (natural isolate, 600 MHz, d_6-DMSO).

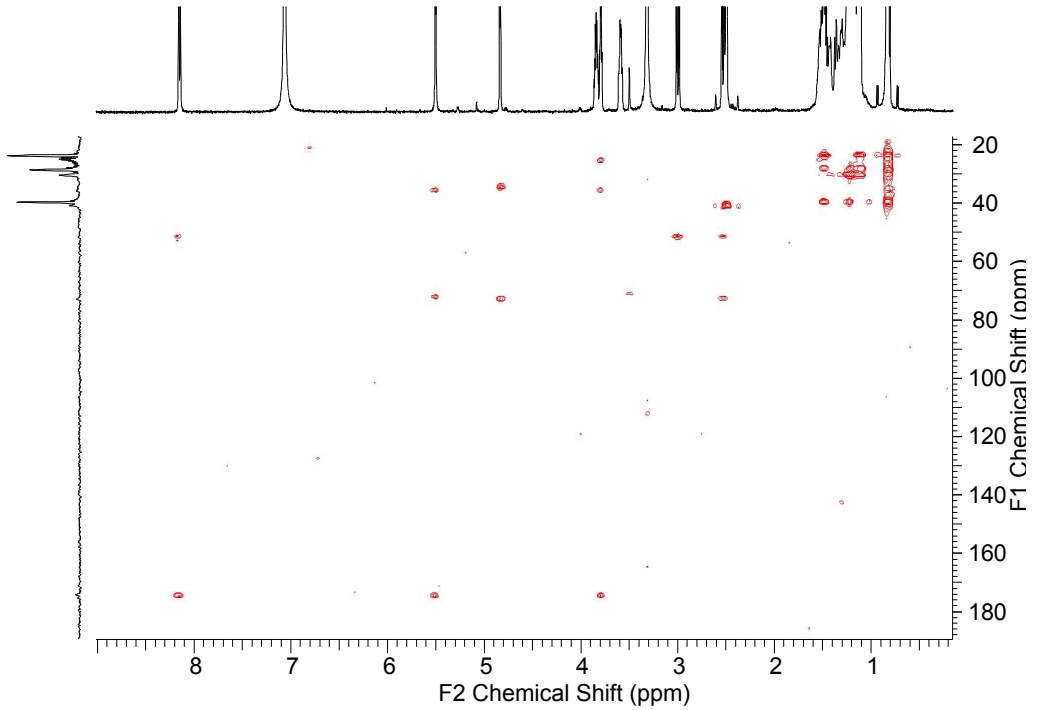

Figure S30. HMBC spectrum of compound **24** (natural isolate, 600 MHz, d_6-DMSO).

(2*R*,3*R*)-3-Hydroxy-2-((*R*)-2-hydroxy-13-methyltetradecanamido)-15-methylhexadecane-1-sulfonic acid (24)

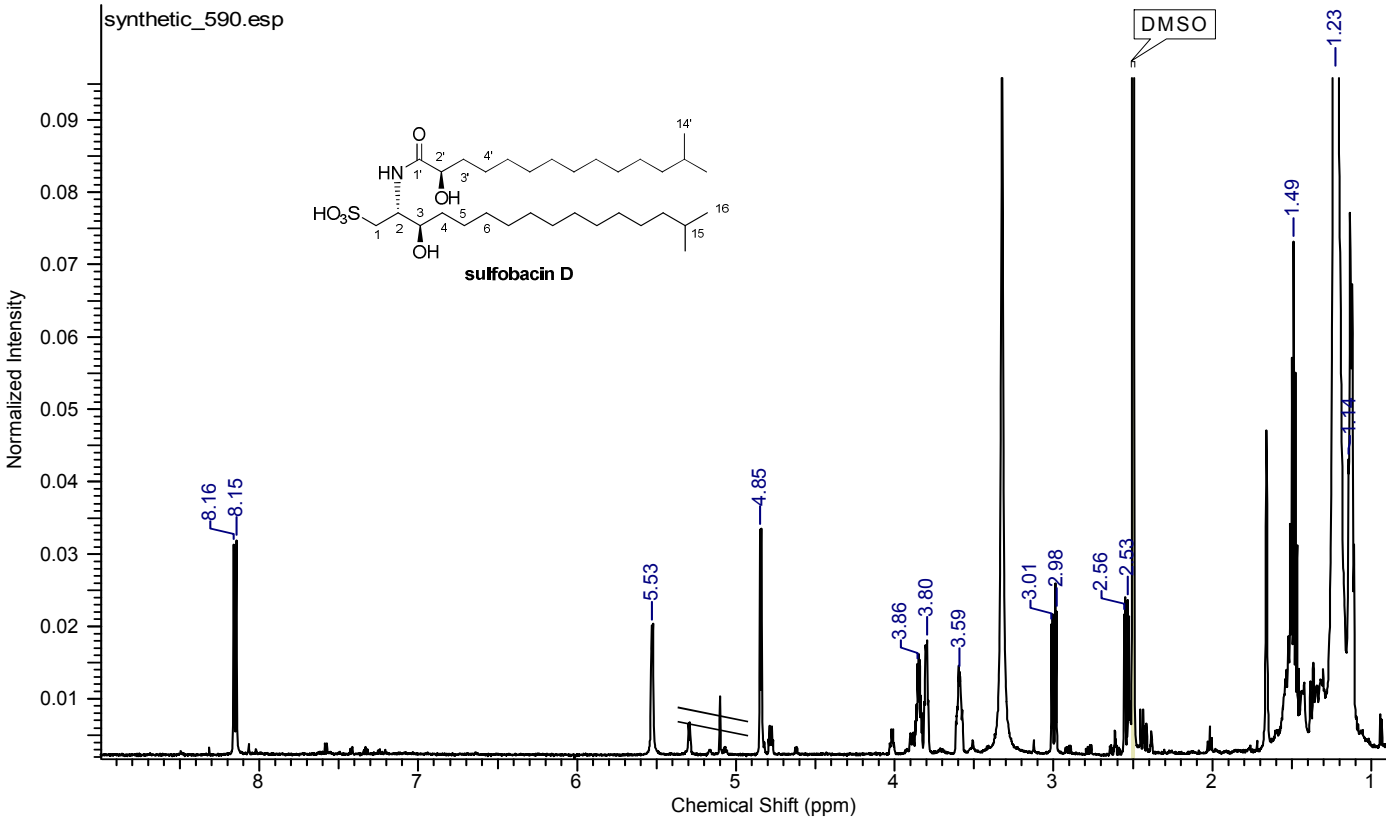

Figure 31. ^{1}H NMR spectrum of synthetic compound **24** (sulfobacin D, 600 MHz, d_6-DMSO).

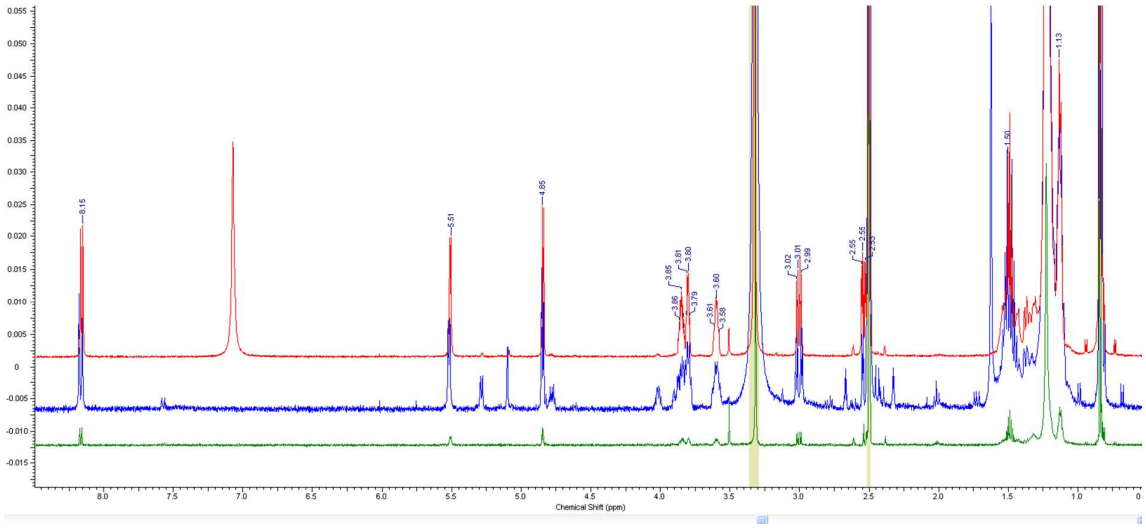

Figure 32. ^{1}H NMR spectra of a) concentrated sample of natural compound **24**, b) synthetic compound **24**, and c) repurified sample of natural isolate **24** (each 600 MHz, d_6-DMSO).

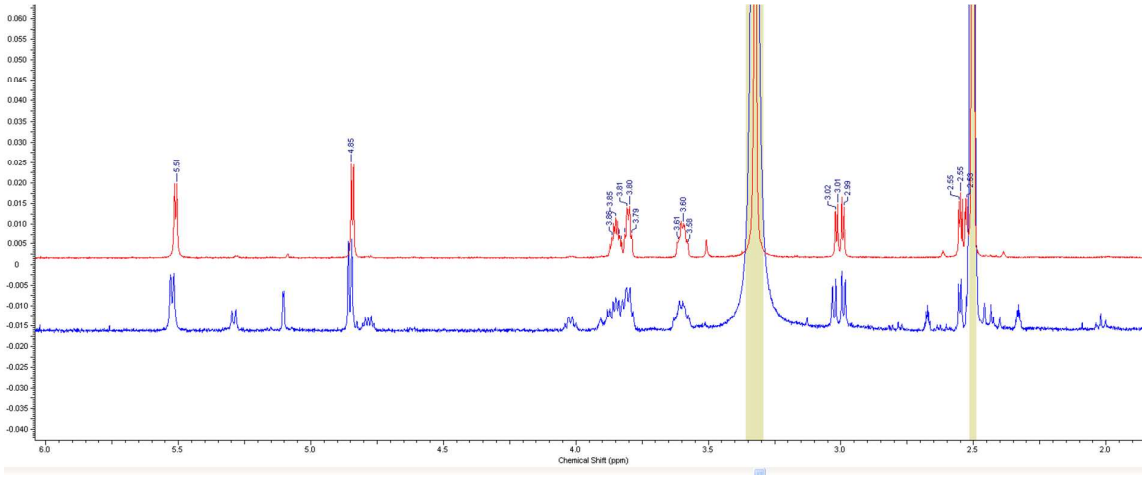

Figure 32. ^{1}H NMR spectra of a) concentrated sample of natural compound **24** (red), and b) **s**ynthetic compound **24** (blue) (each 600 MHz, d_6-DMSO).

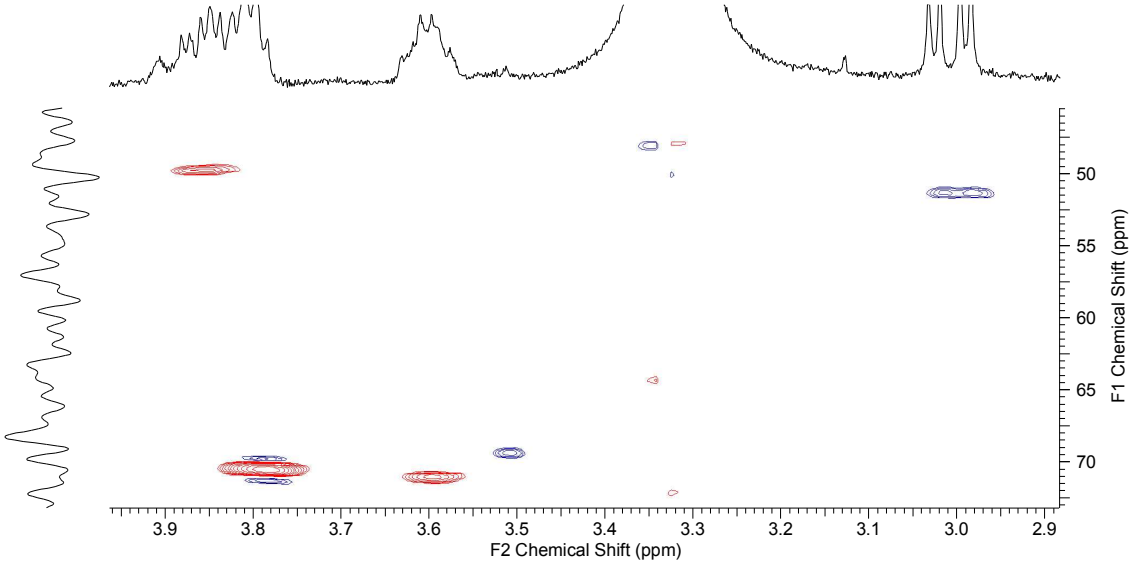

Figure 33. HSQC spectrum of synthetic compound **24** (sulfobacin D, 600 MHz, d_6-DMSO).

(2*R*,3*R*,5*R*)-3,5-Dihydroxy-15-methyl-2-(13-methyl-tetradecanamido)hexadecane-1-sulfonic acid (25)

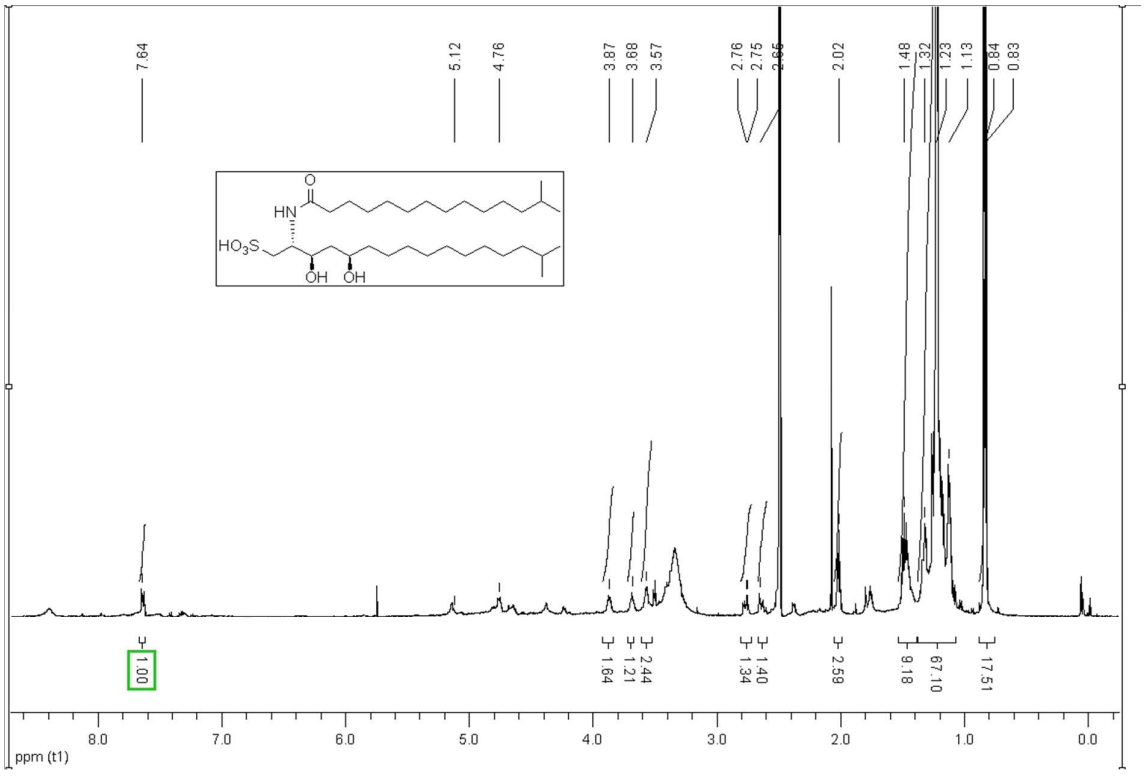

Figure 34. ^{1}H NMR spectrum of synthetic compound **25** (600 MHz, d_6-DMSO).

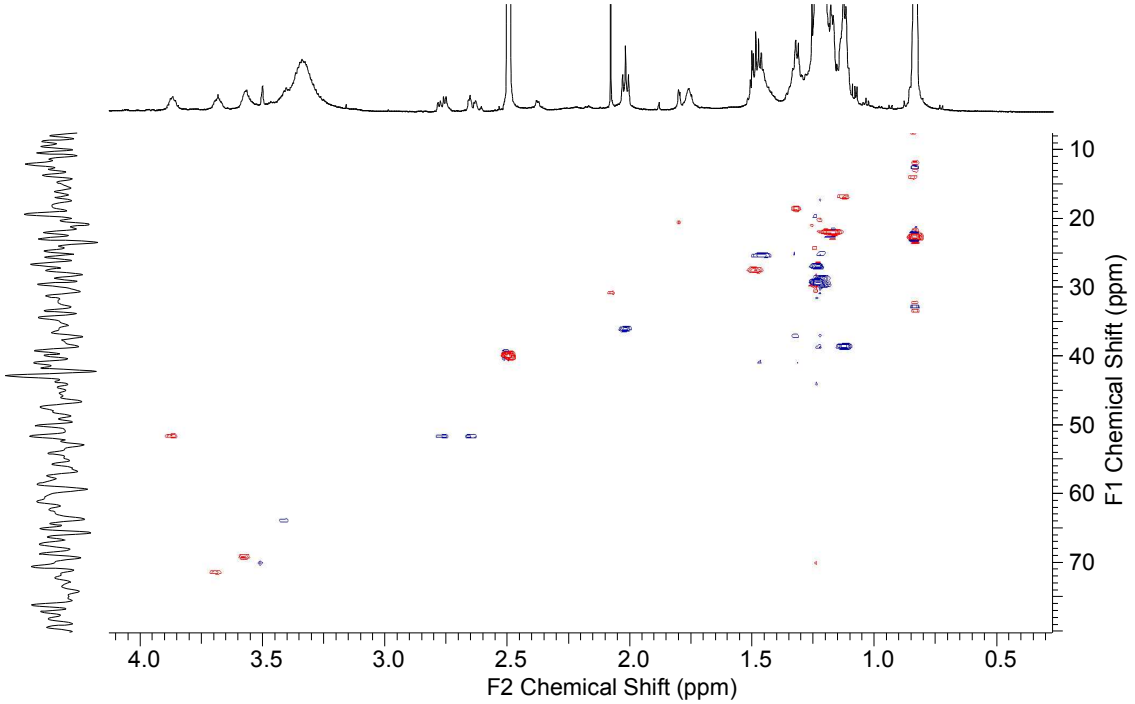

Figure 35. HSQC spectrum of synthetic compound **25** (600 MHz, d_6-DMSO).

(2*R*,3*R*,5*R*) 3,5-Dihydroxy-15-methyl-2-undec-10-ynamido-hexadecane-1-sulfonic acid (26)

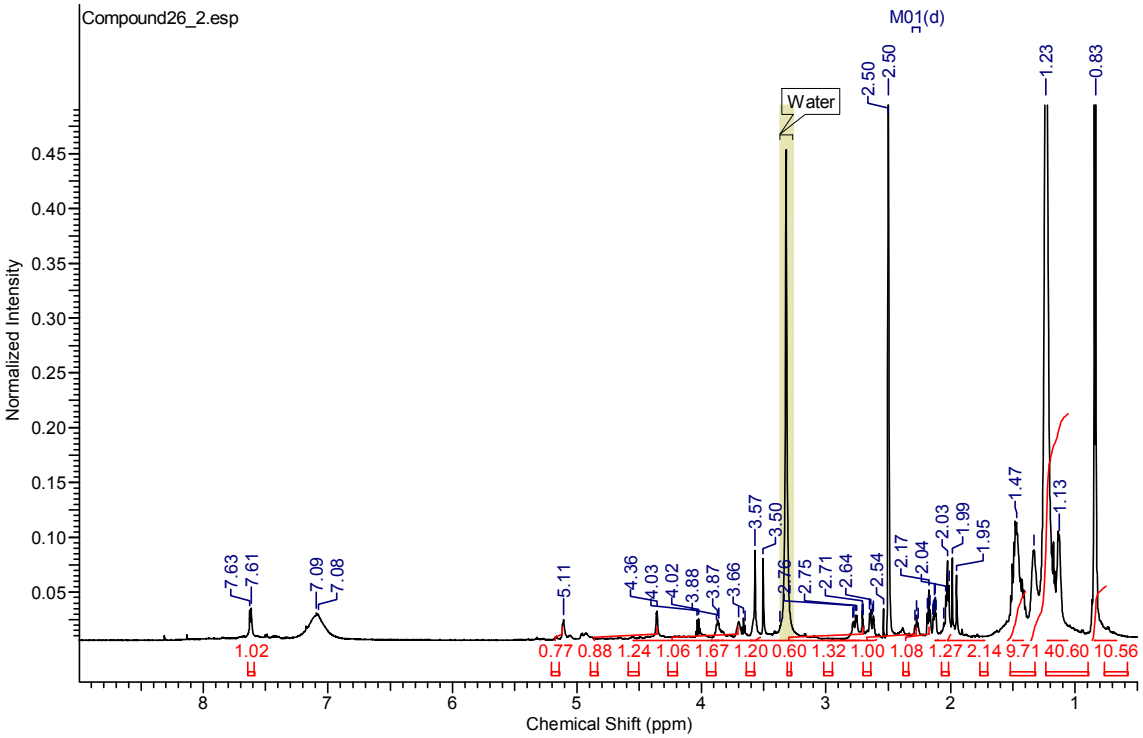

Figure 36. ^{1}H NMR of synthetic compound **26** (600 MHz, d_6-DMSO).

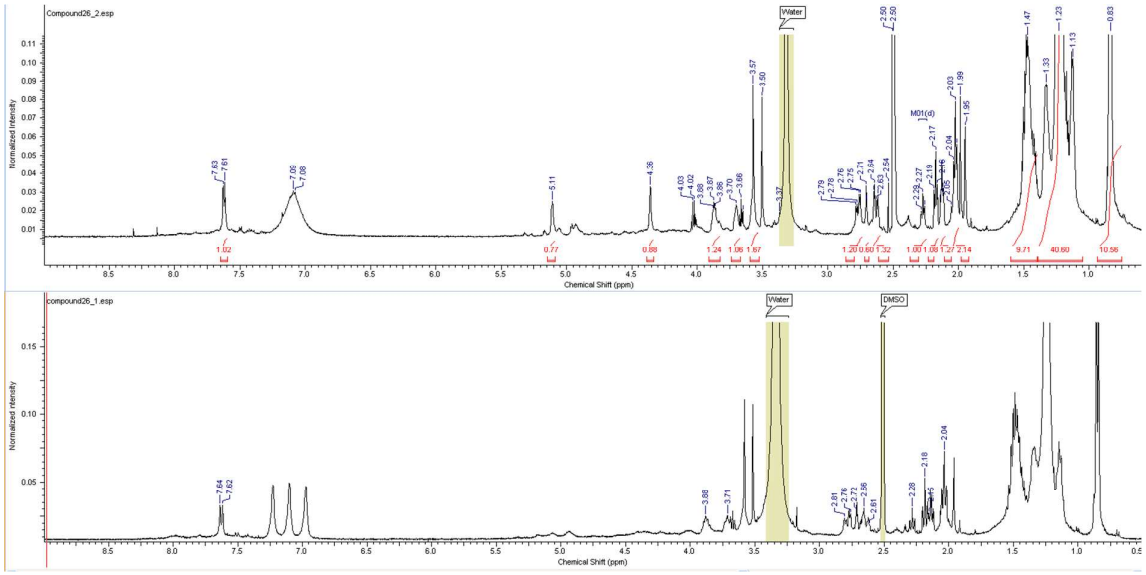

Figure 37. Comparison of ^{1}H NMR spectra of a) synthetic compound **26** with traces of NH_4OH, and b) after repurification. Chemical shifts did not change upon presence of NH_4OH.

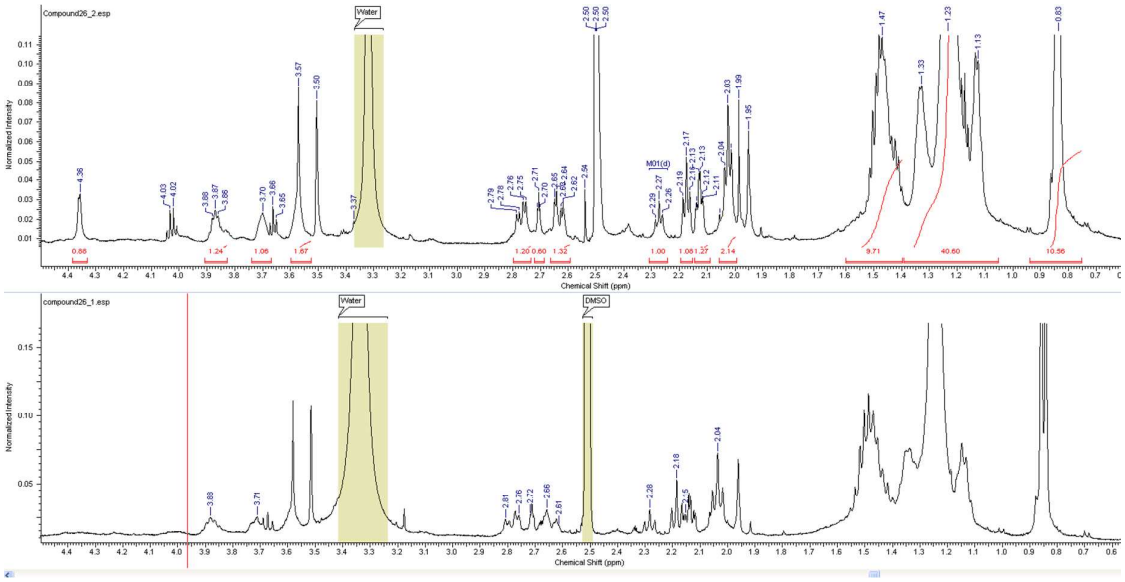

Figure 38. Comparison of ^{1}H NMR spectra of a) synthetic compound **26** with traces of NH_4OH, and b) synthetic compound **26** after repurification. Chemical shifts do not change upon presence of NH_4OH.

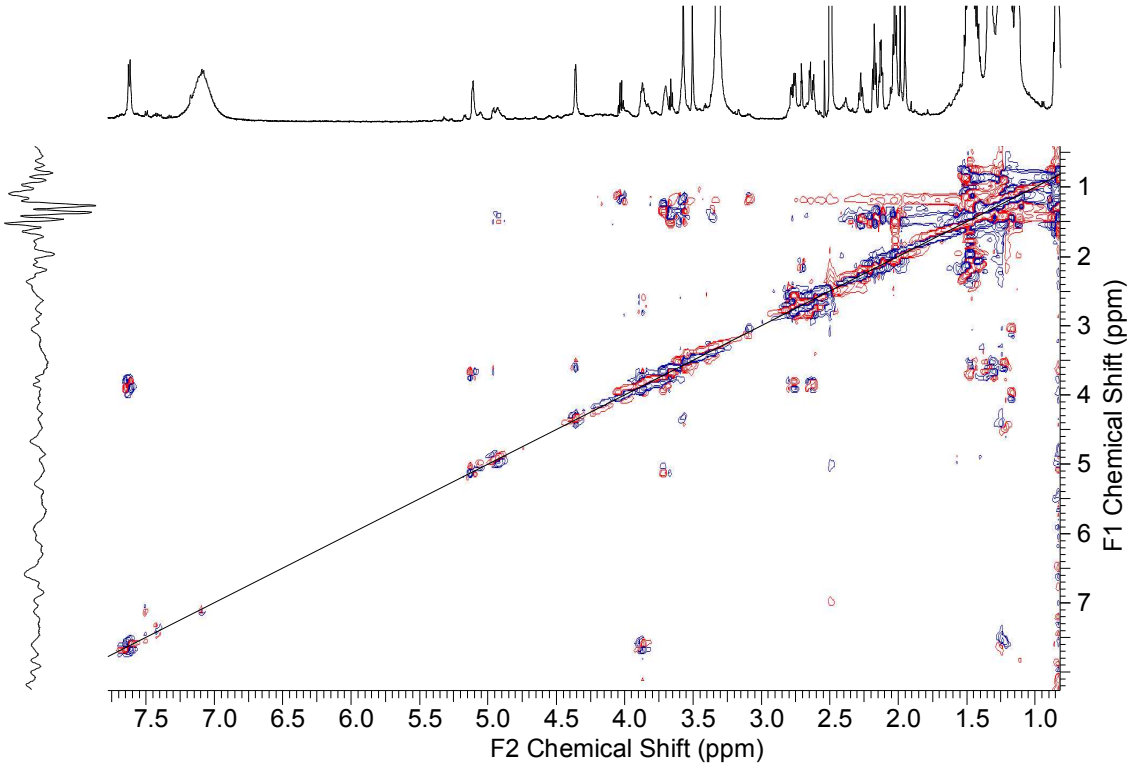

Figure 39. ^{1}H-^{1}H COSY spectrum of compound **26** (synthetic compound, 600 MHz, d_6-DMSO).

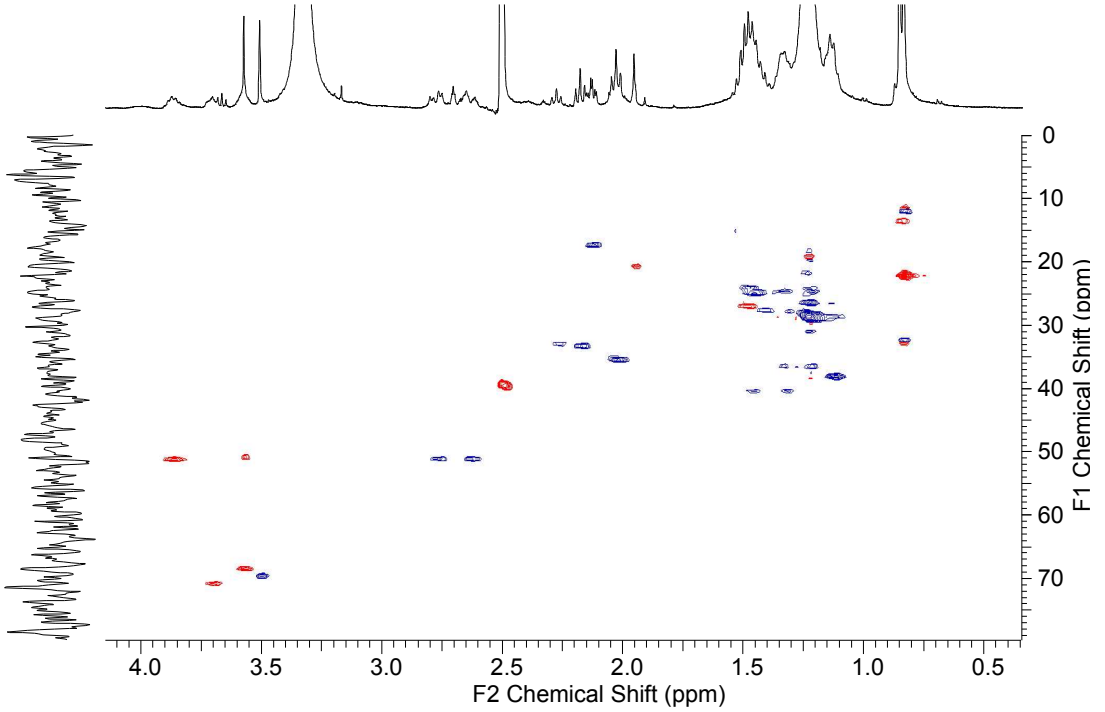

Figure 40. HSQC spectrum of synthetic compound **26** (600 MHz, d_6-DMSO).

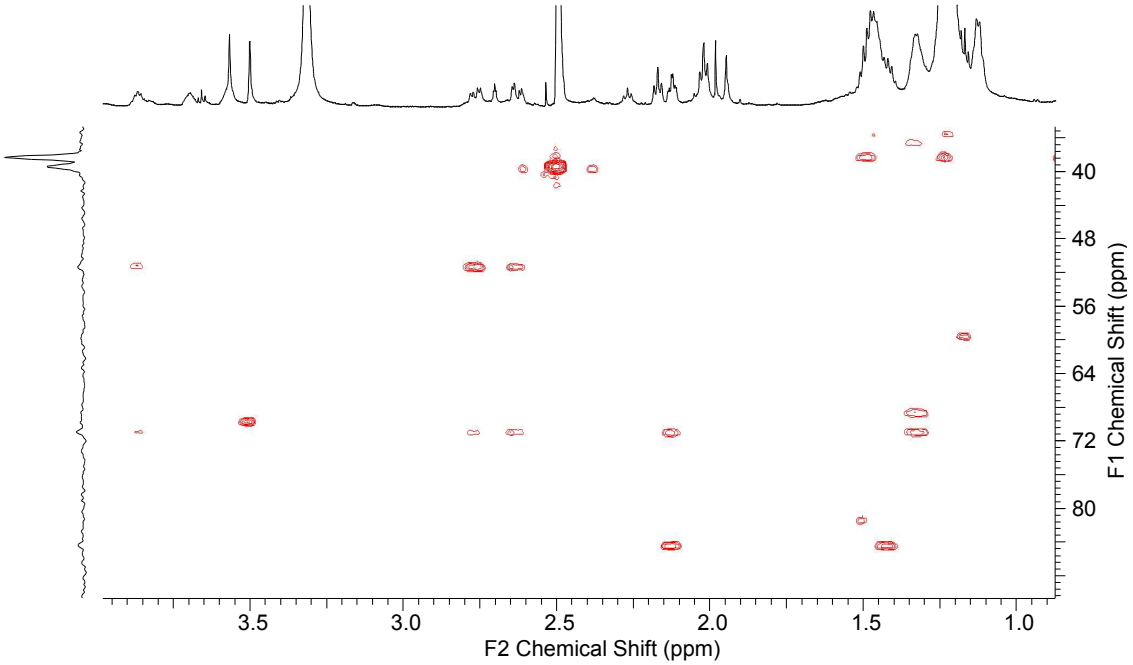

Figure 41. Representative HMBC spectrum of compound **26** (synthetic compound, 600 MHz, d_6-DMSO).

(2*R*,3*R*) 3-Hydroxy-2-stearamidooctadecane-1-sulfonic acid (27)

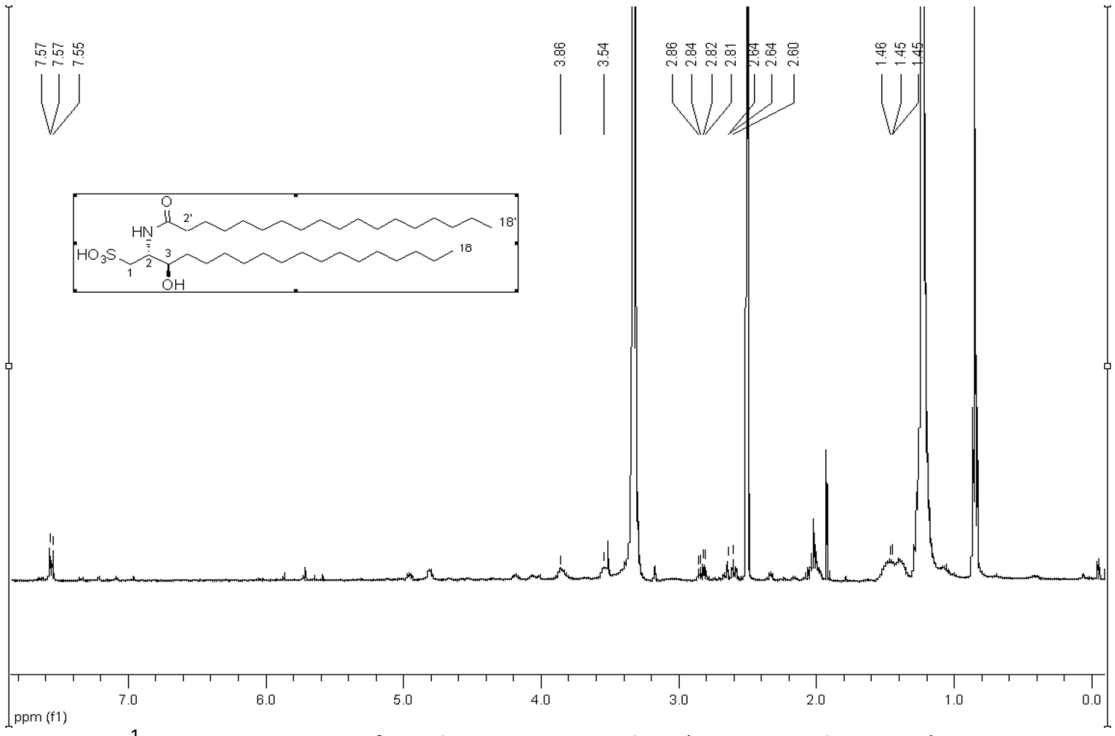

Figure 42. ^{1}H NMR spectrum of synthetic compound **27** (600 MHz, d_6-DMSO).

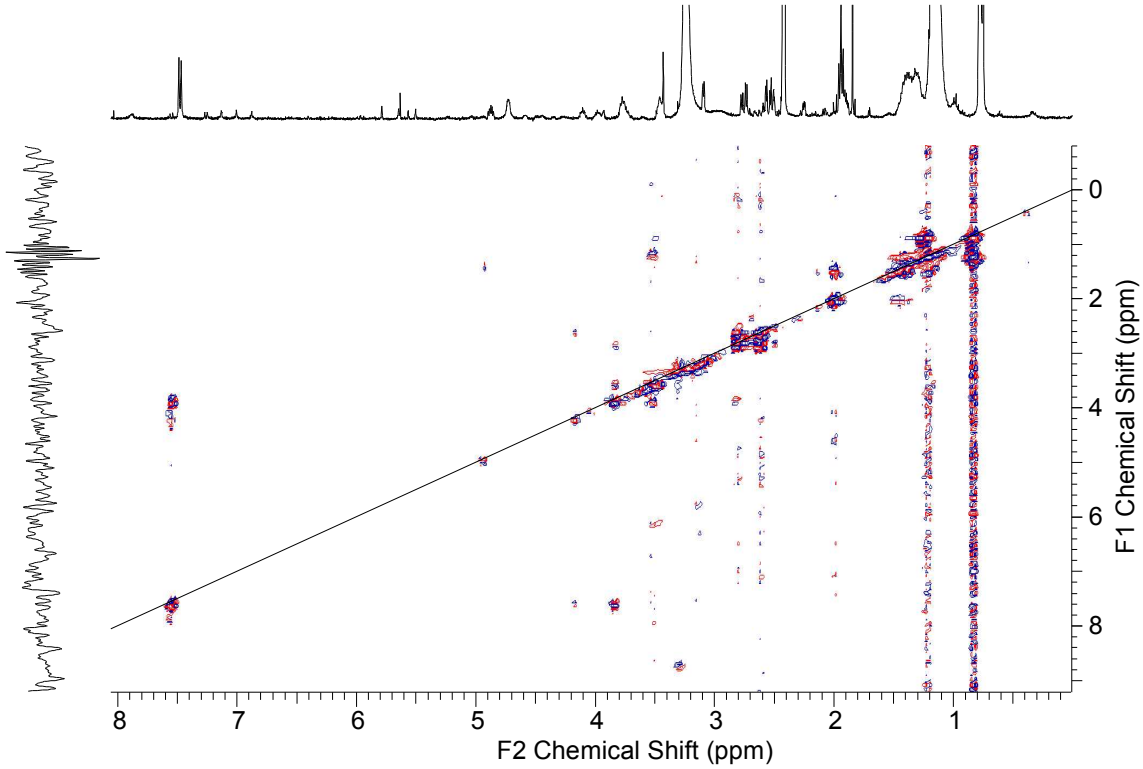

Figure 43. ^{1}H-^{1}H COSY spectrum of synthetic compound **27** (600 MHz, d_6-DMSO).

(2*R*,3*R*) 3-Hydroxy-2-tetracosanamidooctadecane-1-sulfonic acid (28)

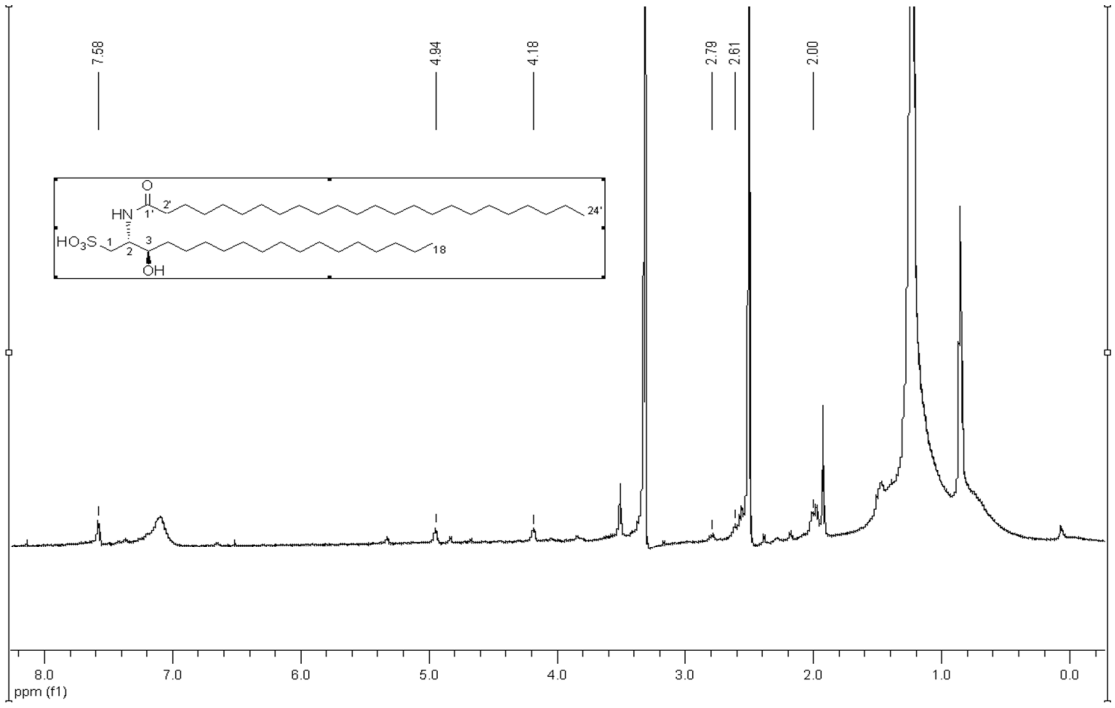

Figure 44. ^{1}H NMR spectrum of synthetic compound **28** (600 MHz, d_6-DMSO).

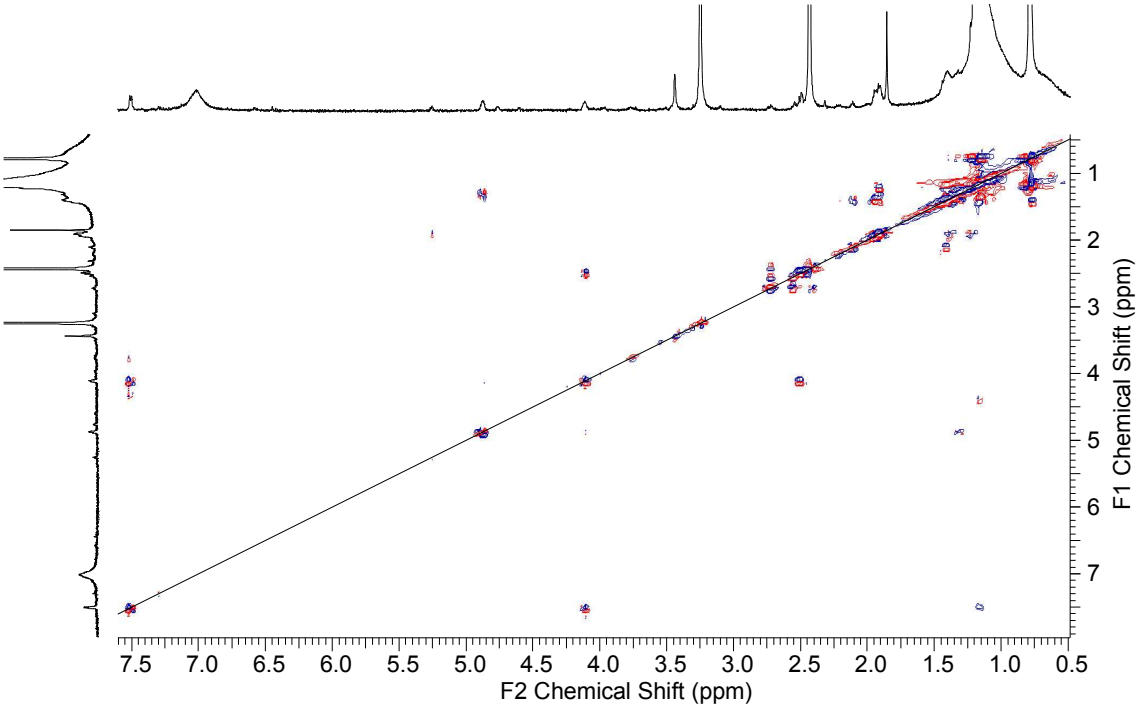

Figure 45. ^{1}H-^{1}H cosy spectrum of synthetic compound **28** (600 MHz, d_6-DMSO).

(2*R*,3*R*)-3-Hydroxy-15-methyl-2-(13-methyltetradecanamido)hexadecane-1-sulfonic acid (29)

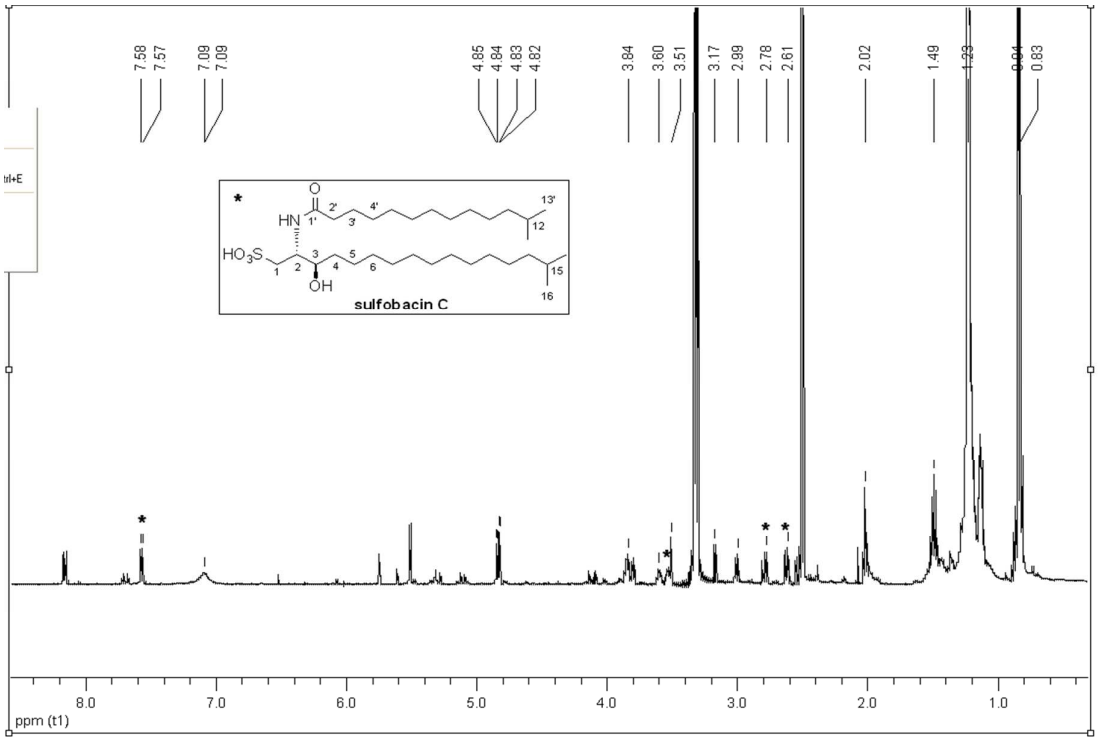

Figure 46. ^{1}H NMR spectrum of compound **29** (1:1 mixture of sulfobacin C (**29**) and D (**24**), natural isolate, 600 MHz, d_6-DMSO).

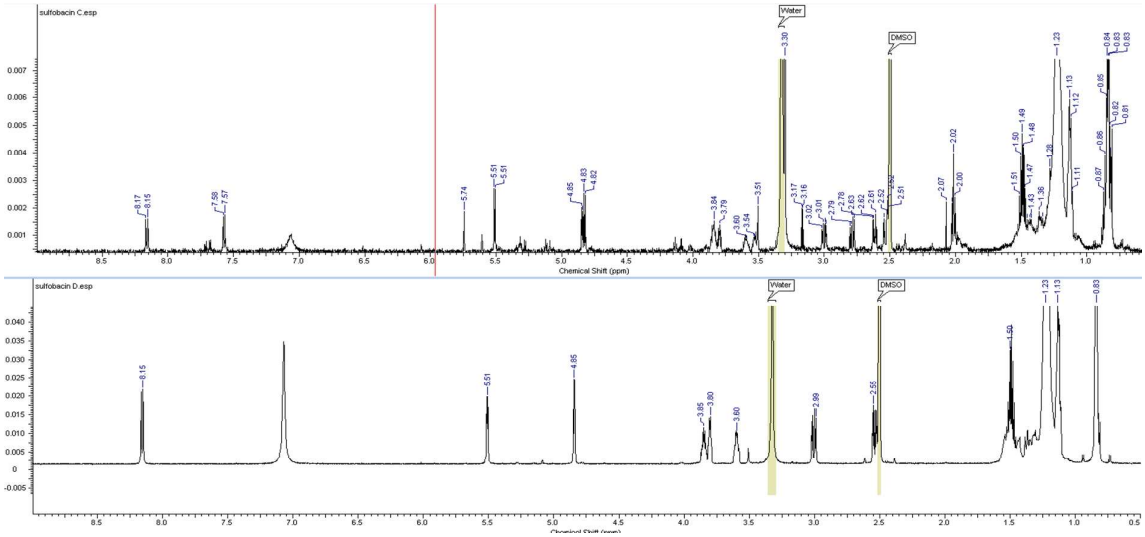

Figure 47. ^{1}H NMR spectra of a) compound **29** (sulfobacin C (**29**) as a 1:1 mixture of sulfobacin C (**29**) and sulfobacin D (**24**), and b) compound **24** (sulfobacin D).

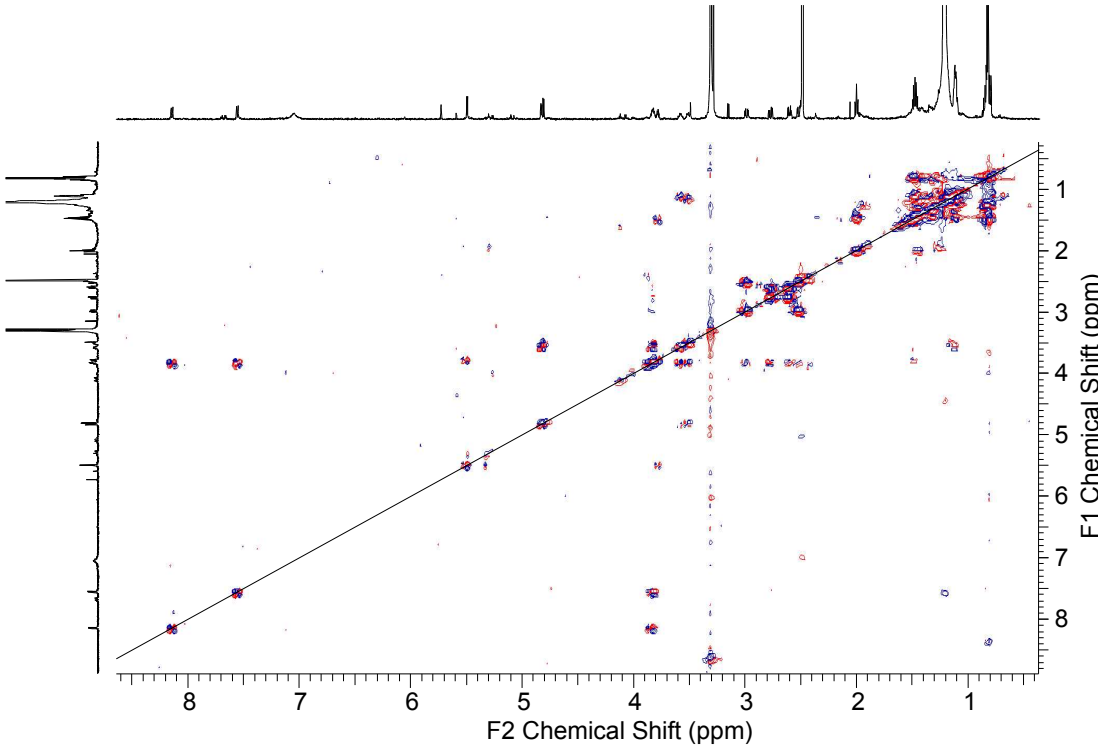

Figure 48. ^{1}H-^{1}H COSY spectrum of compound **29** (600 MHz, d_6-DMSO).

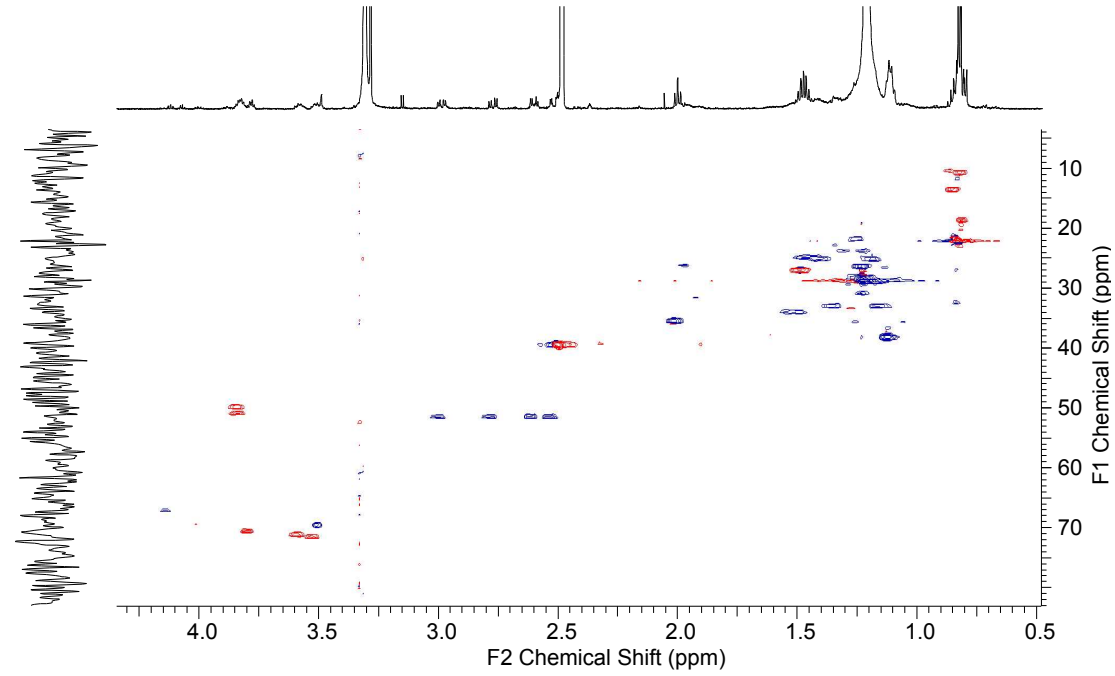

Figure 49. HSQC spectrum of compound **29** (600 MHz, d_6-DMSO).

(2*R*,3*R*)-3-Hydroxy-15-methyl-2-(13-methyltetradecanamido)hexadecane-1-sulfonic acid (30)

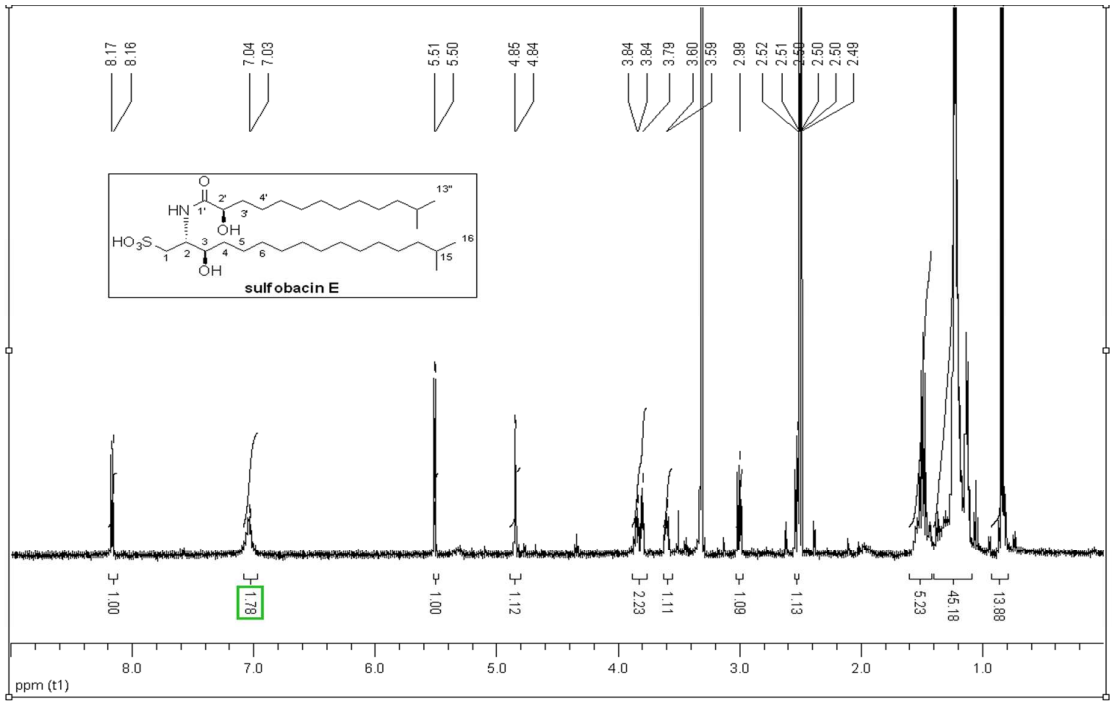

Figure 50. ^{1}H NMR spectrum of compound **30** (sulfobacin E, natural isolate, 600 MHz, d_6-DMSO).

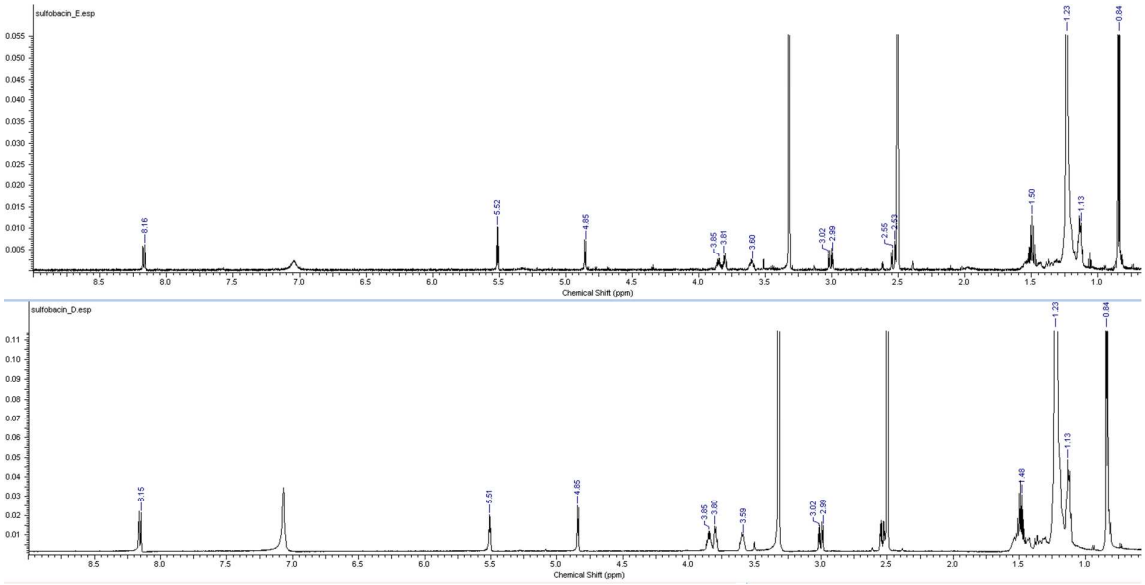

Figure 51. Comparison of ^{1}H NMR spectra of a) compound **24** (sulfobacin D, 600 MHz, d_6-DMSO), compound **30** (sulfobacin E, 600 MHz, d_6-DMSO).

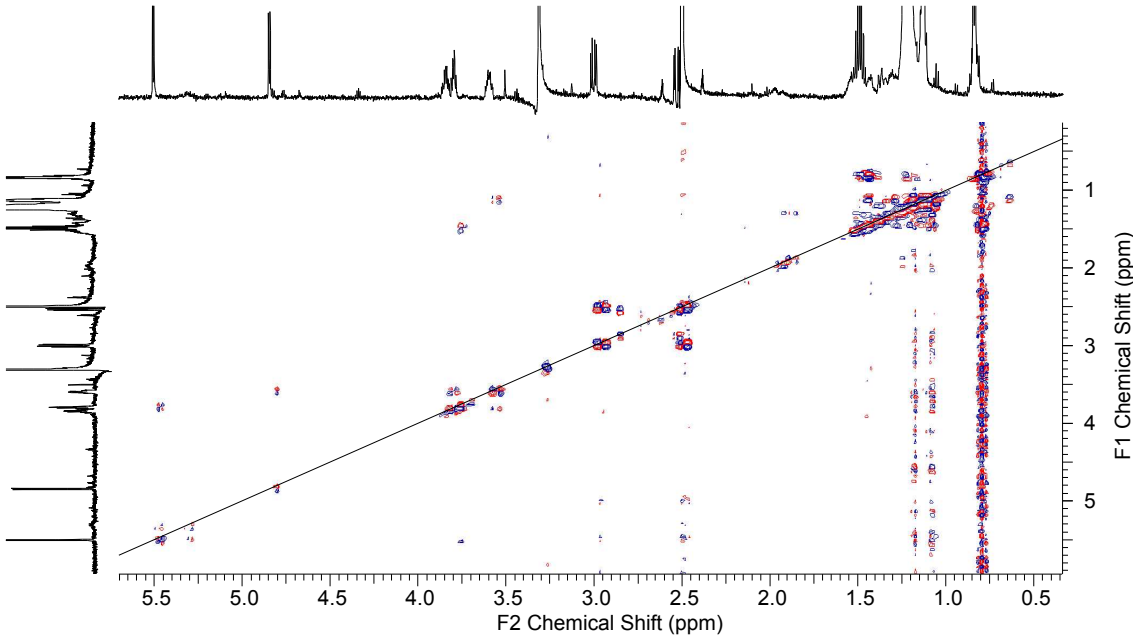

Figure 52. ^{1}H NMR spectrum of compound **30** (sulfobacin E, natural isolate, 600 MHz, d_6-DMSO).

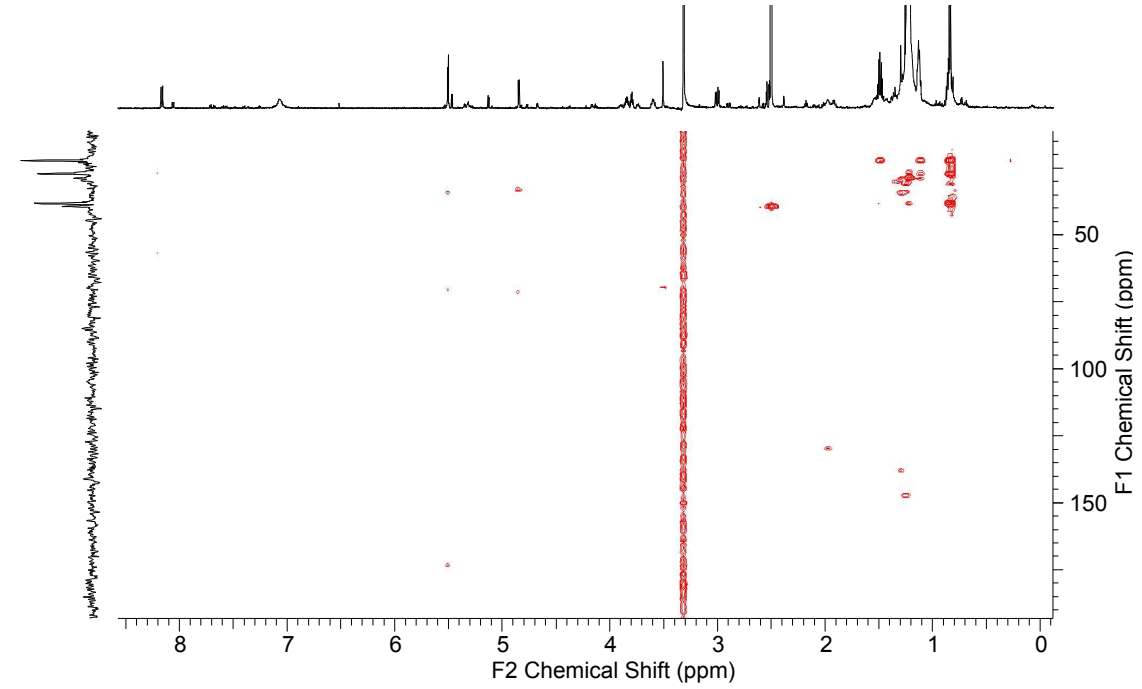

Figure 53. HMBC spectrum of compound **30** (synthetic compound, 600 MHz, d_6-DMSO).

(2*R*,3*R*,*E*)-3-Hydroxy-2-((*R*)-2-hydroxy-13-methyltetradecanamido)-15-methylhexadec-4-ene-1-sulfonic acid (31-*trans*)

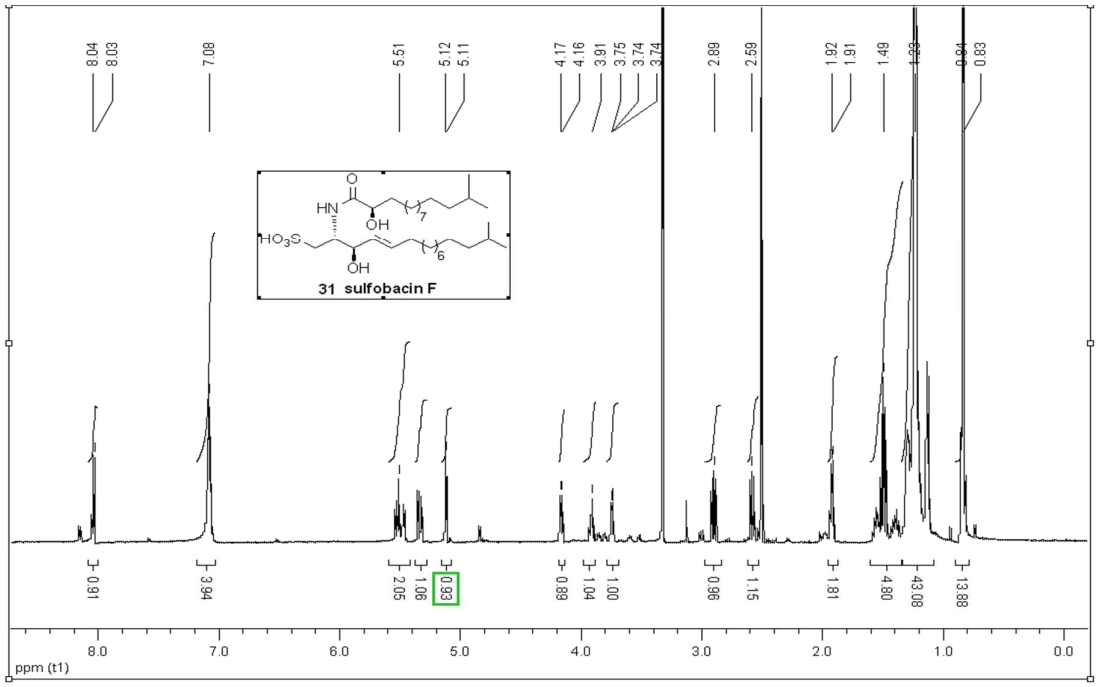

Figure 54. ^{1}H NMR spectrum of compound **31-*trans*** (natural isolate, 600 MHz, d_6-DMSO).

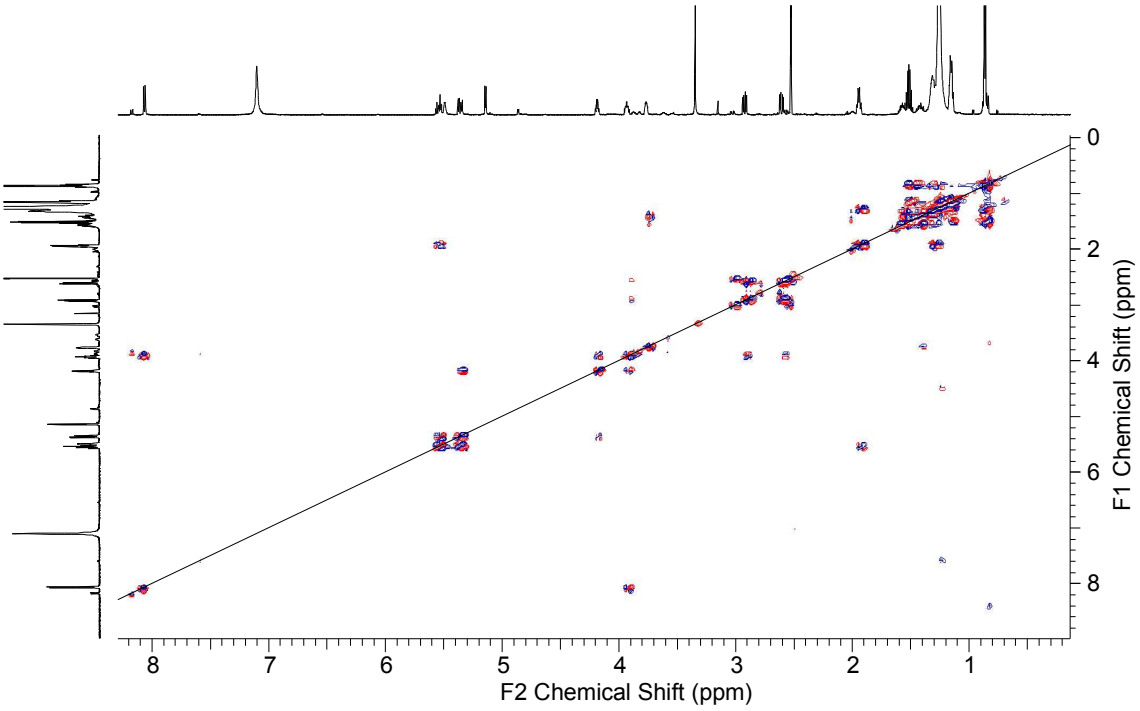

Figure 55. ^{1}H-^{1}H COSY spectrum of compound **31-*trans*** (natural isolate, 600 MHz, d_6-DMSO).

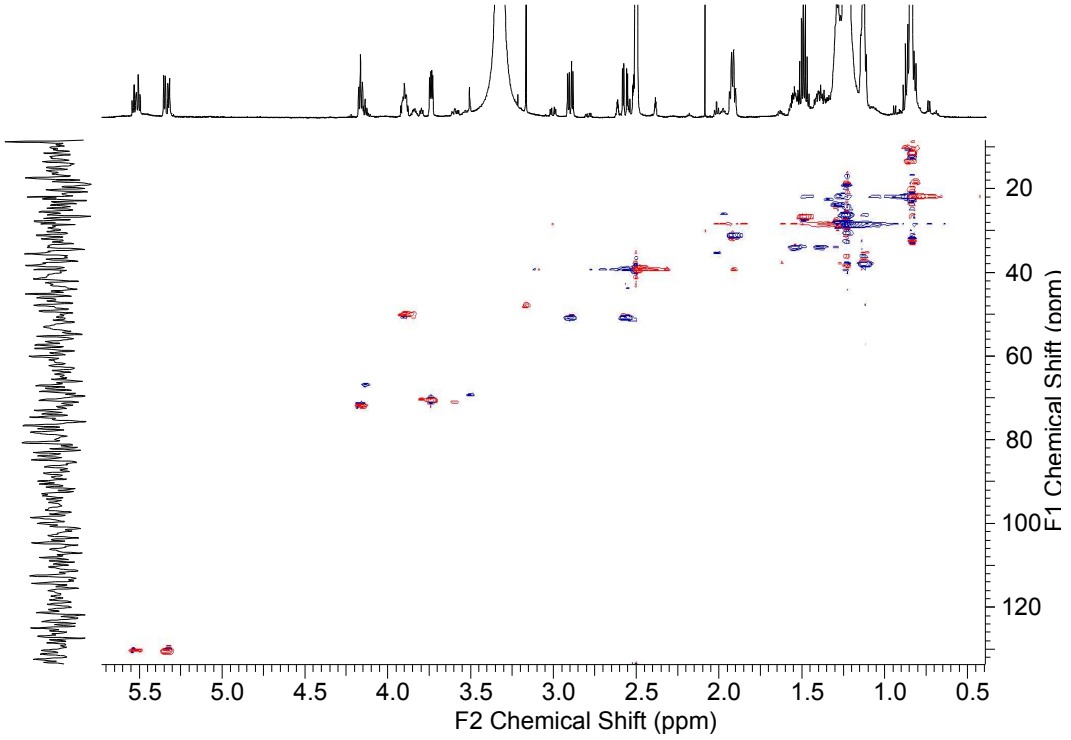

Figure 56. HSQC spectrum of compound **31-*trans*** (600 MHz, d_6-DMSO).

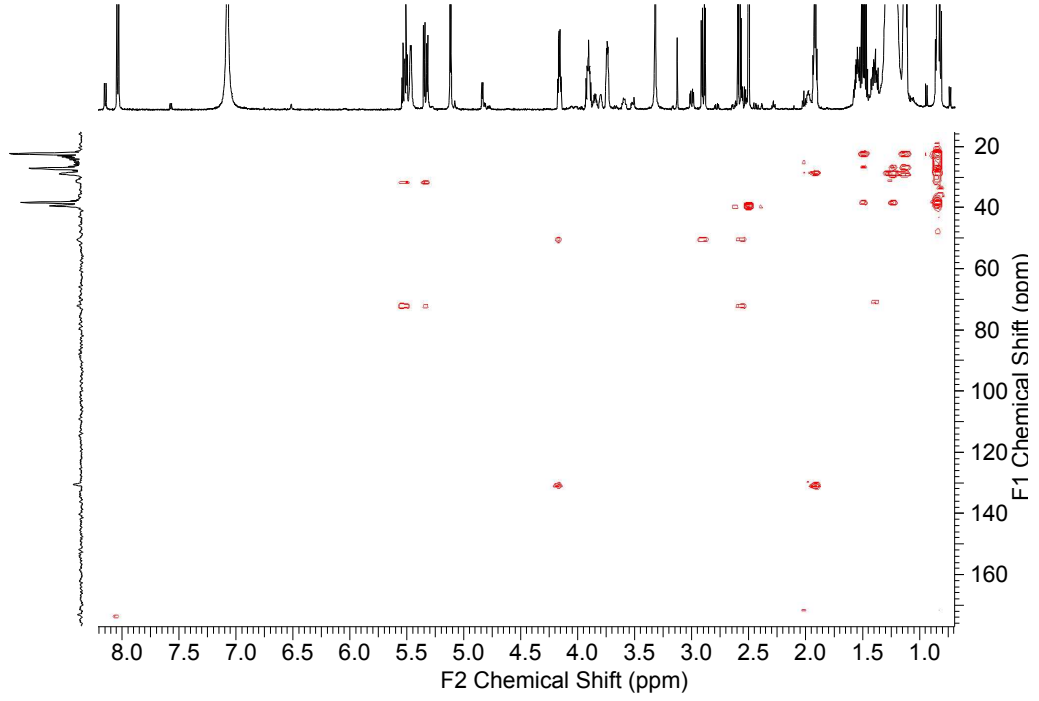

Figure 57. HMBC spectrum of compound **31-*trans*** (600 MHz, d_6-DMSO).

(2*R*,3*R*,*Z*)-3-Hydroxy-2-((*R*)-2-hydroxy-13-methyltetradecanamido)-15-methylhexadec-4-ene-1-sulfonic acid (31-*cis*)

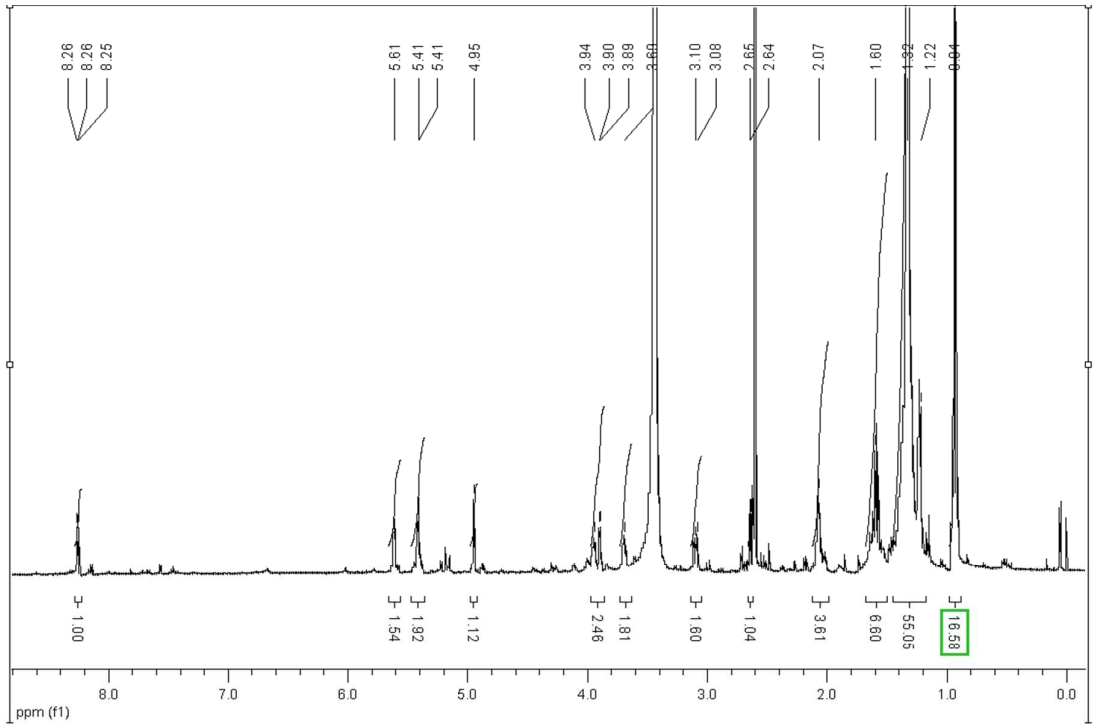

Figure 58 ^{1}H NMR spectrum of compound **31-*cis*** (sulfobacin F-*cis*, natural isolate, 600 MHz, d_6-DMSO).

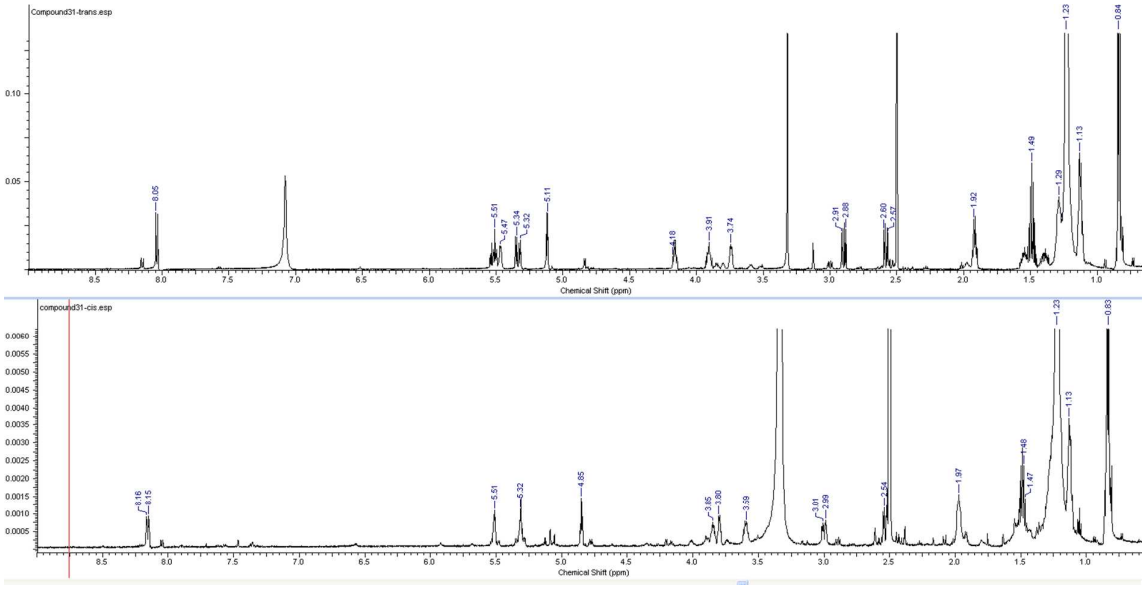

Figure 59. Comparison of ^{1}H NMR spectra of a) compound **31-*trans*** (sulfobacin F-*trans*, natural isolate, 600 MHz, d_6-DMSO), and compound **31-*cis*** (sulfobacin F-*cis*, natural isolate, 600 MHz, d_6-DMSO).

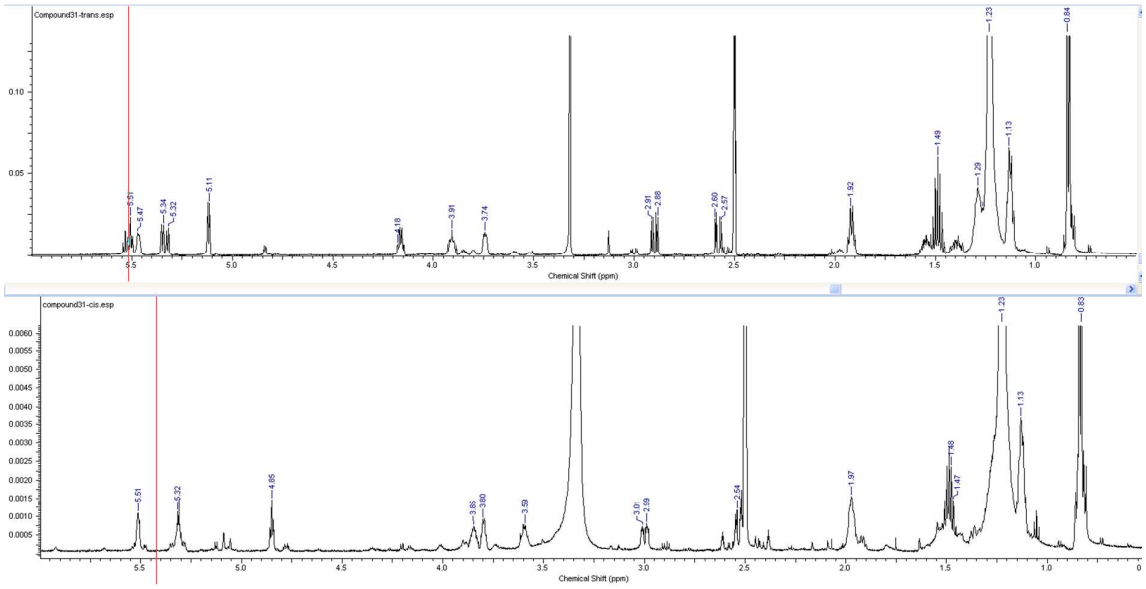

Figure 60. Comparison of ^{1}H NMR spectra of a) compound **31-*trans*** (sulfobacin F-*trans*, natural isolate, 600 MHz, d_6-DMSO), and compound **31-*cis*** (sulfobacin F-*cis*, natural isolate, 600 MHz, d_6-DMSO).

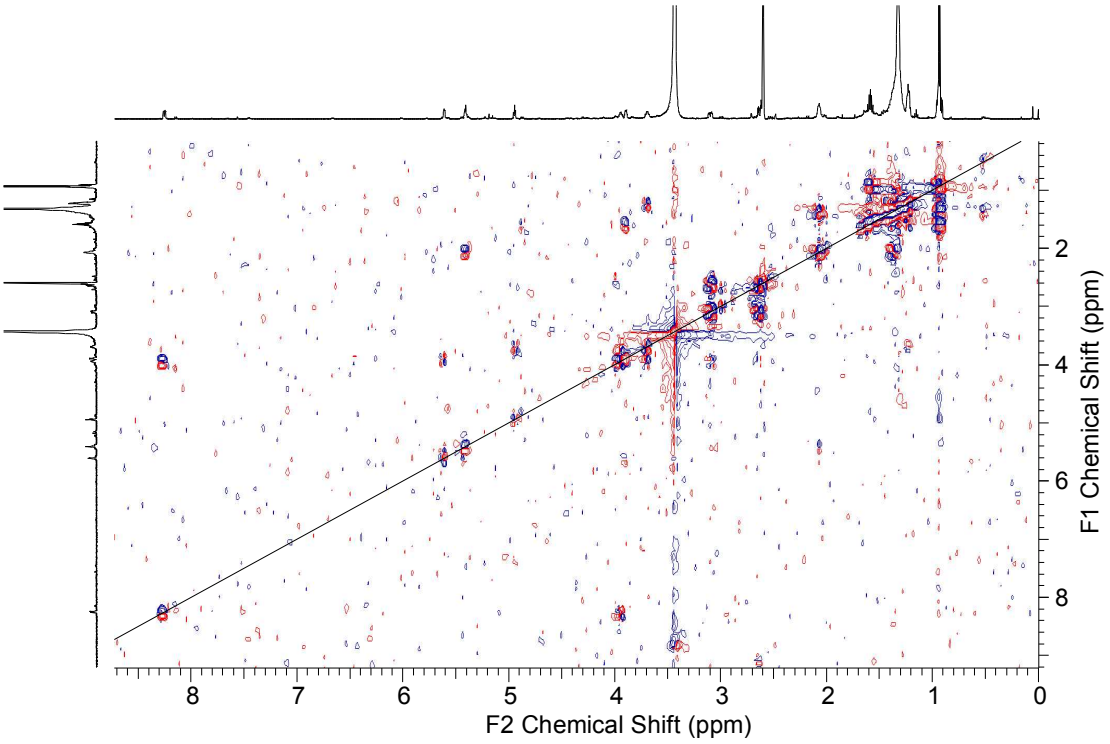

Figure 61. ^{1}H-^{1}H COSY spectrum of compound **31-*cis*** (sulfobacin F-*cis*, natural isolate, 600 MHz, d_6-DMSO).

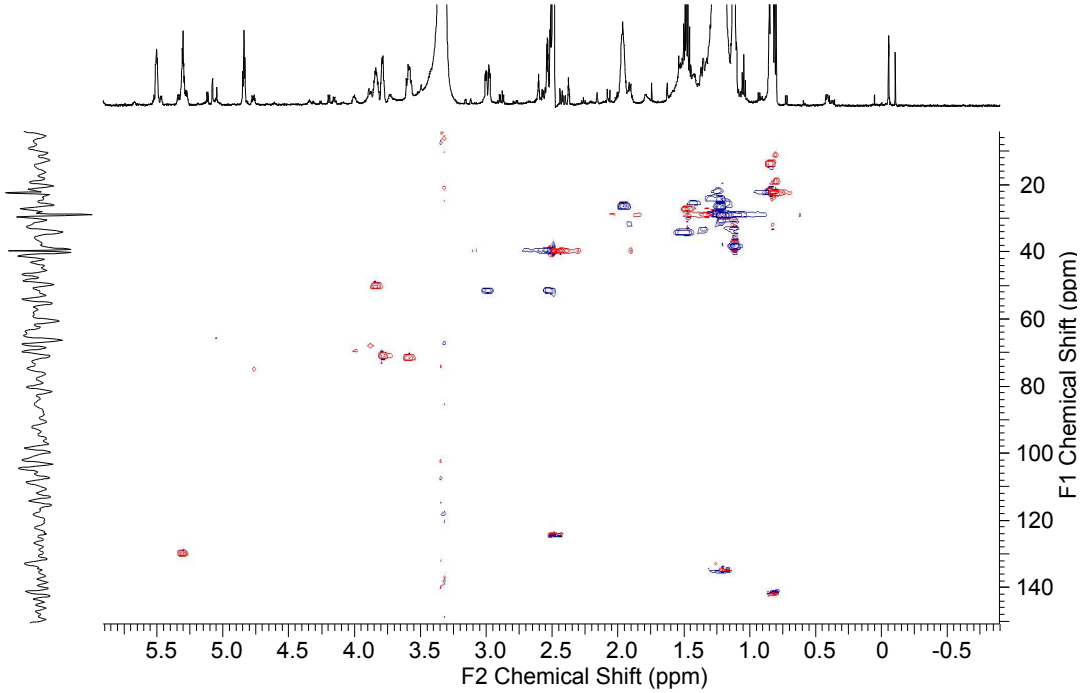

Figure 62. HSQC spectrum of compound **31-*cis*** (sulfobacin F-*cis*, natural isolate, 600 MHz, d_6-DMSO).

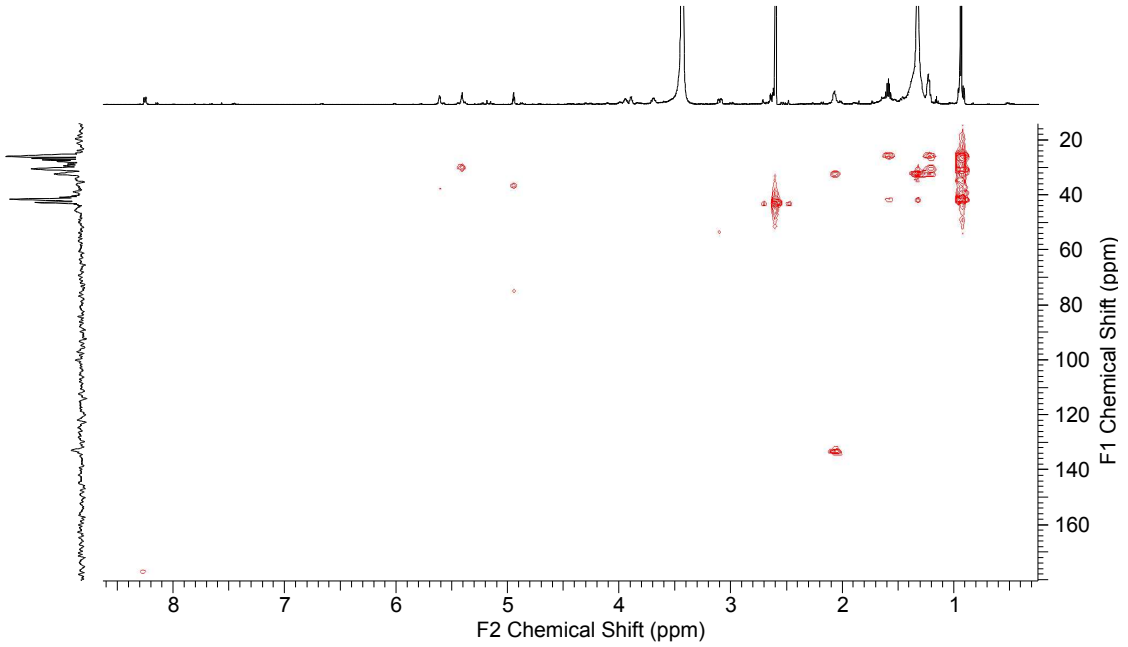

Figure 63. HMBC spectrum of compound **31-*cis*** (600 MHz, d_6-DMSO).