Supporting Information

Structure and biosynthesis of the marine streptomycete ansamycin ansalactam A and its distinctive branched chain polyketide extender unit

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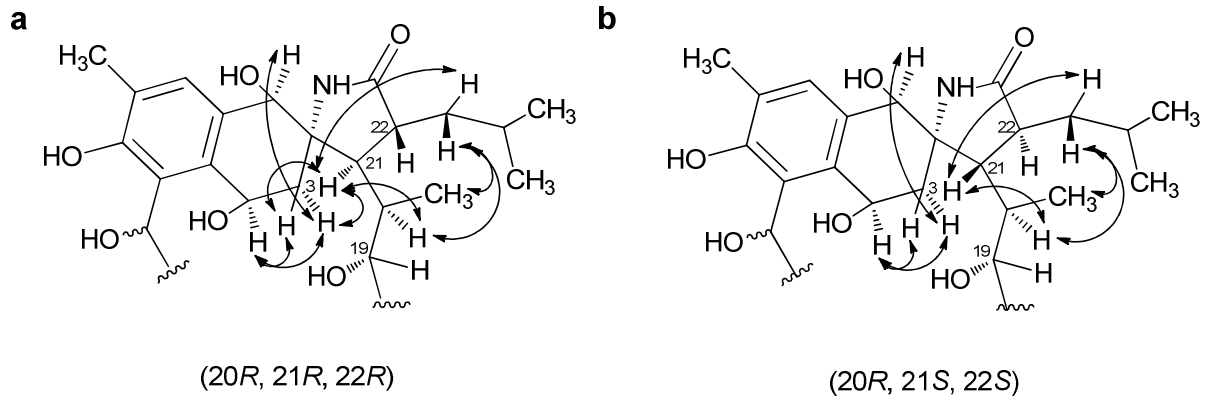

Figure S1. The two possible stereoisomers (a, b), and selected key ROESY correlations for determination of the configurations at C-21 and C-22 for the reduced ansalactam A alcohol (**5**).

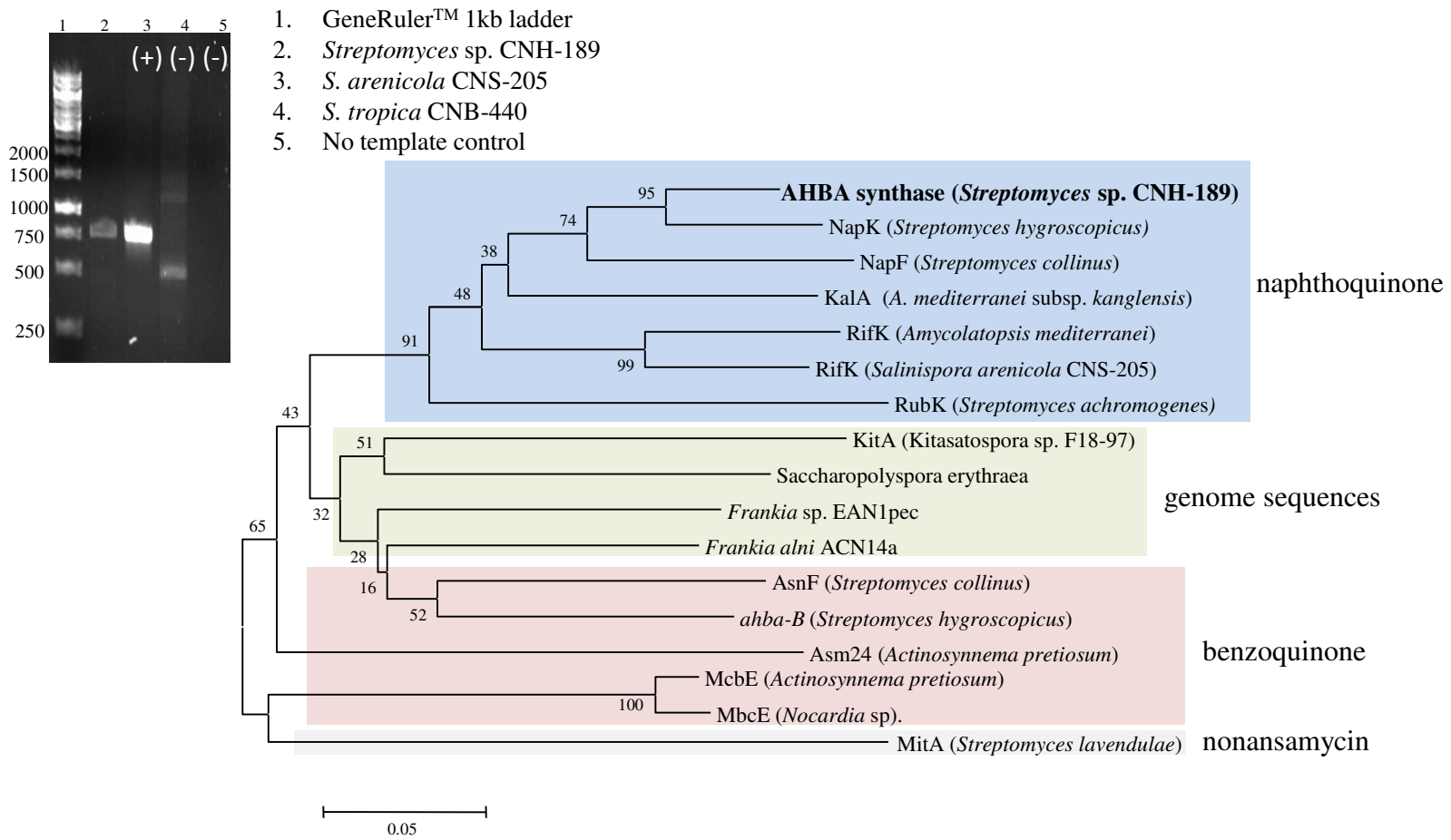

Figure S2. Indentification of a naphthoquinone-type AHBA synthase from *S*. sp. CNH-189. PCR amplificaiton of AHBA synthase genes from *Streptomyces* sp. strain CNH-189 (lane 2), *Salinispora arenicola* strain CNS-205 (lane 3, positive control), *S. tropica* strain CNB-440 (lane 4, negative control), and no template control (lane 5) using degenerate primers designed by Huitu et al. (see ref. 1). The 755 bp amplicon from CNH-189 was aligned in a neighbor-joining tree with AHBA synthases from characterized biosynthetic gene clusters of benzoquinone and naphthoquinone ansamycins as well as uncharacterized AHBA synthases from genome sequencing projects and the AHBA synthase involved in the biosynthesis of mitomycin, a nonansamycin compound derived from AHBA.

Figure S3. ^{1}H NMR spectrum (500 MHz) of ansalactam A (**4**) in methanol-d_4

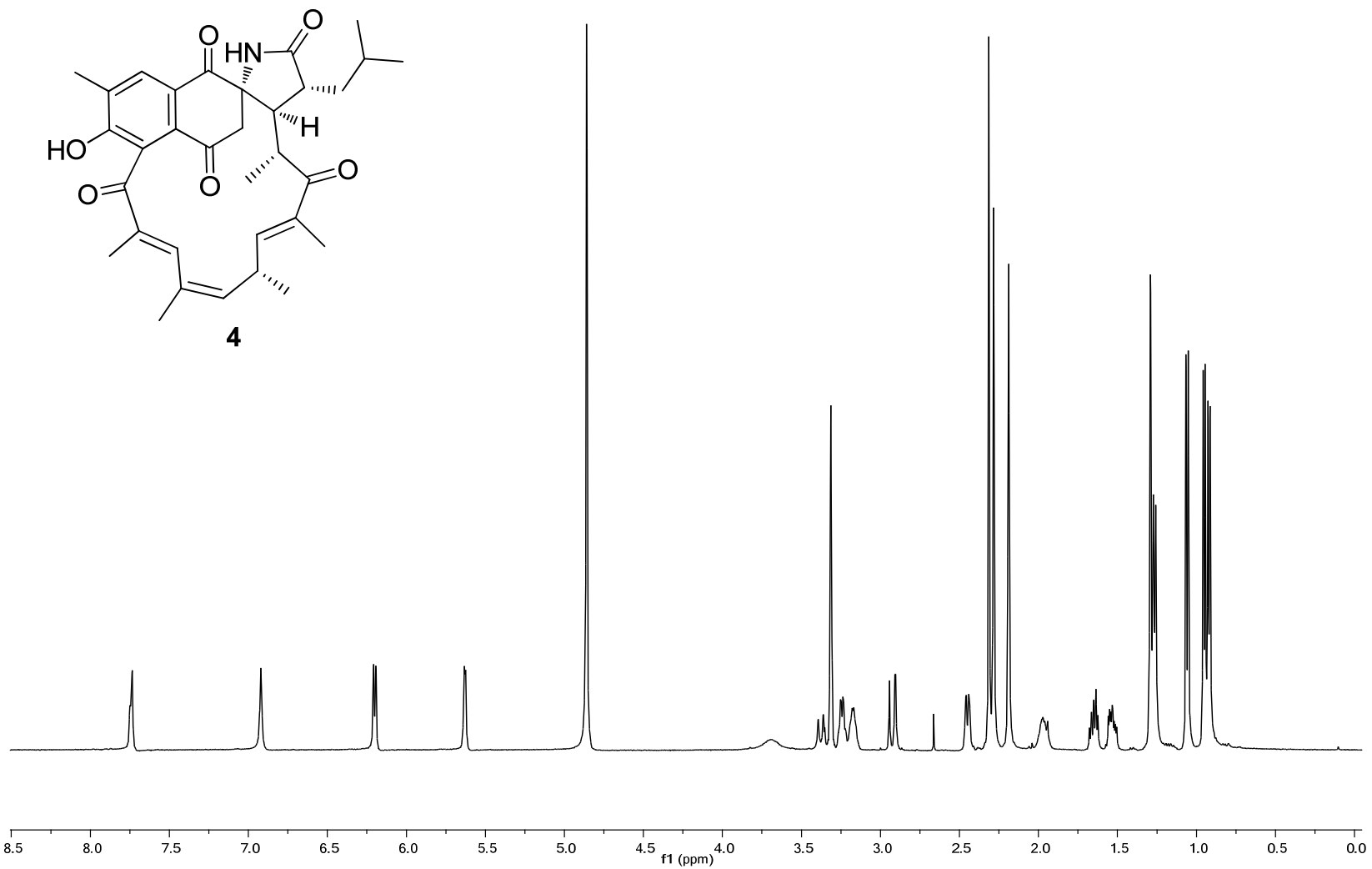

Figure S4. ^{13}C NMR spectrum (75 MHz) of ansalactam A (**4**) in methanol-d_4

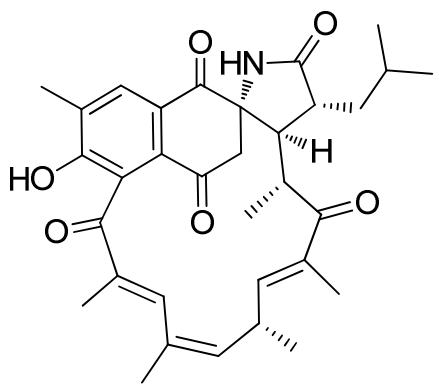

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Figure S5. gCOSY spectra (500 MHz) of ansalactam A (**4**) in methanol-d_4

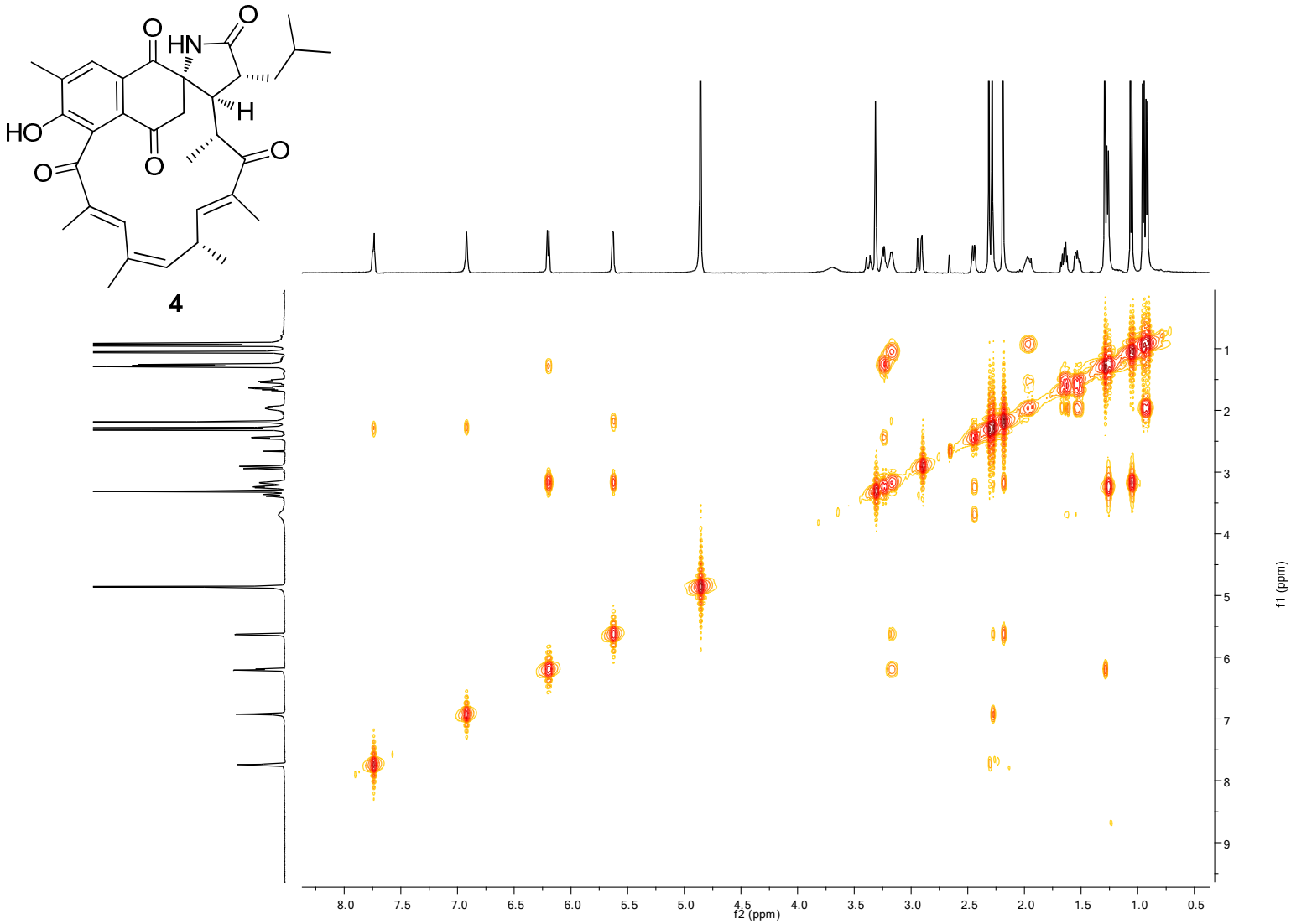

Figure S6. gHSQC spectra (500 MHz) of ansalactam A (**4**) in methanol-d_4

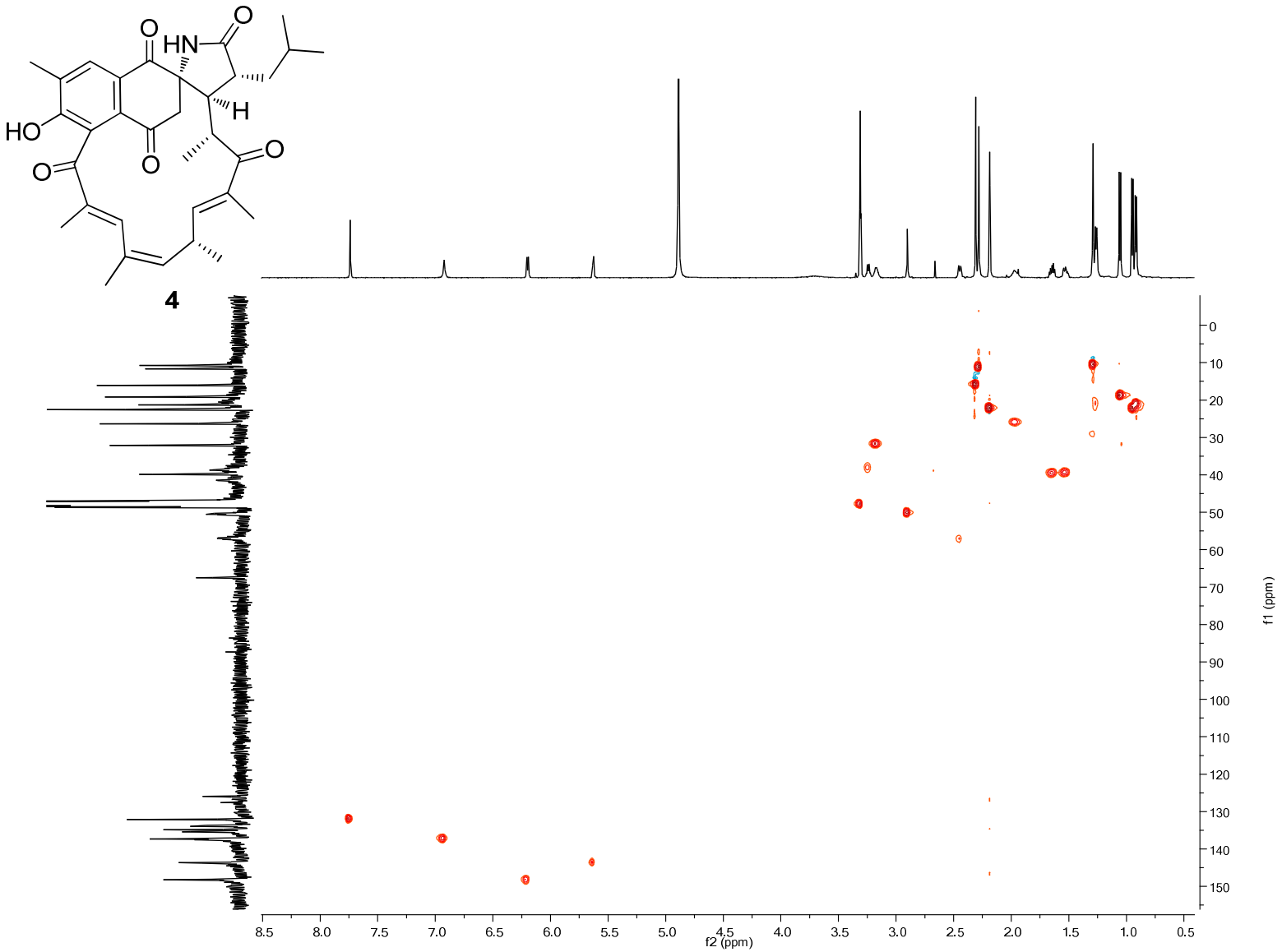

Figure S7. gHMBC spectra (500 MHz) of ansalactam A (**4**) in methanol-d_4

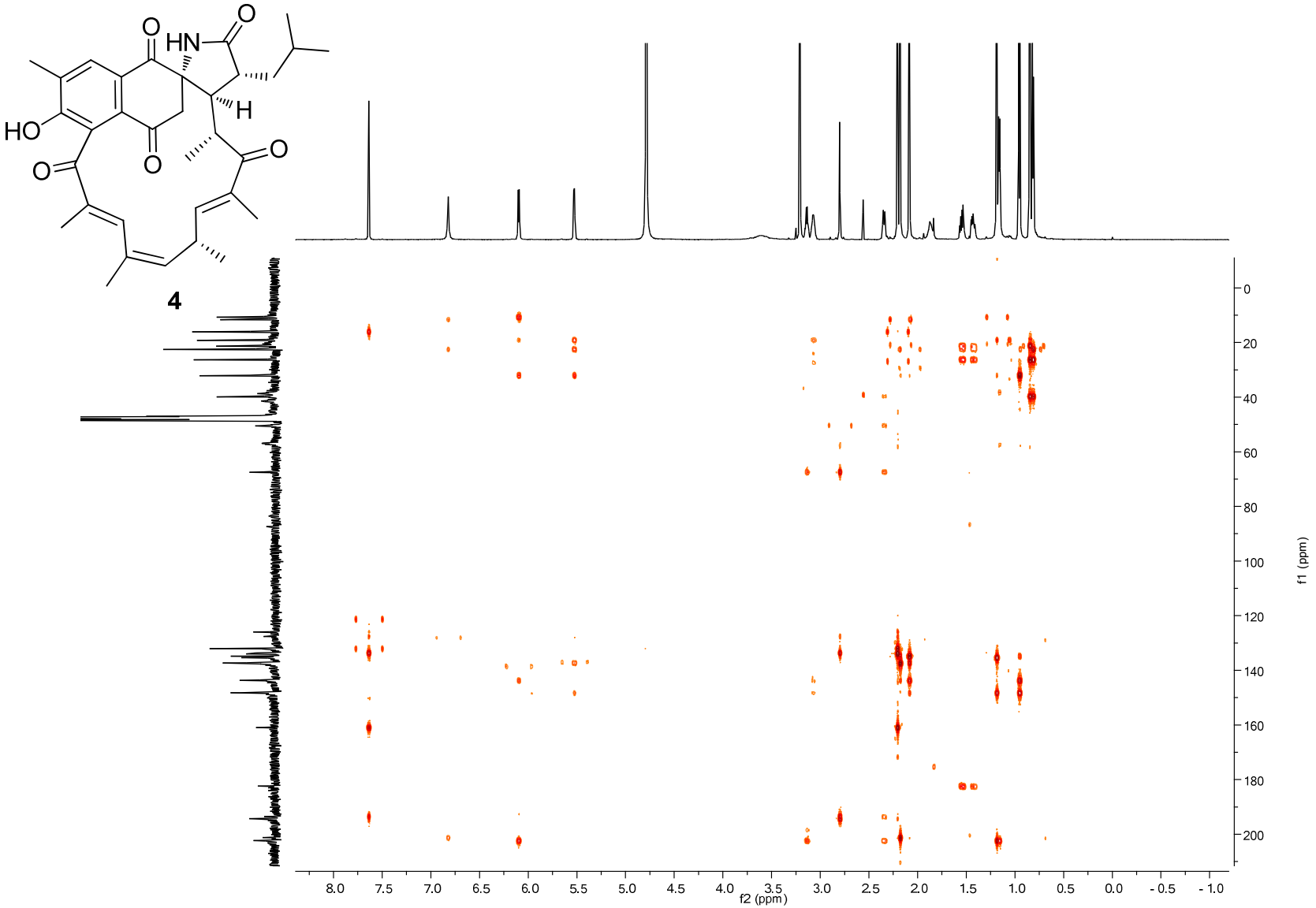

Figure S8. ROESY spectra (600 MHz) of ansalactam A (**4**) in methanol-d_4

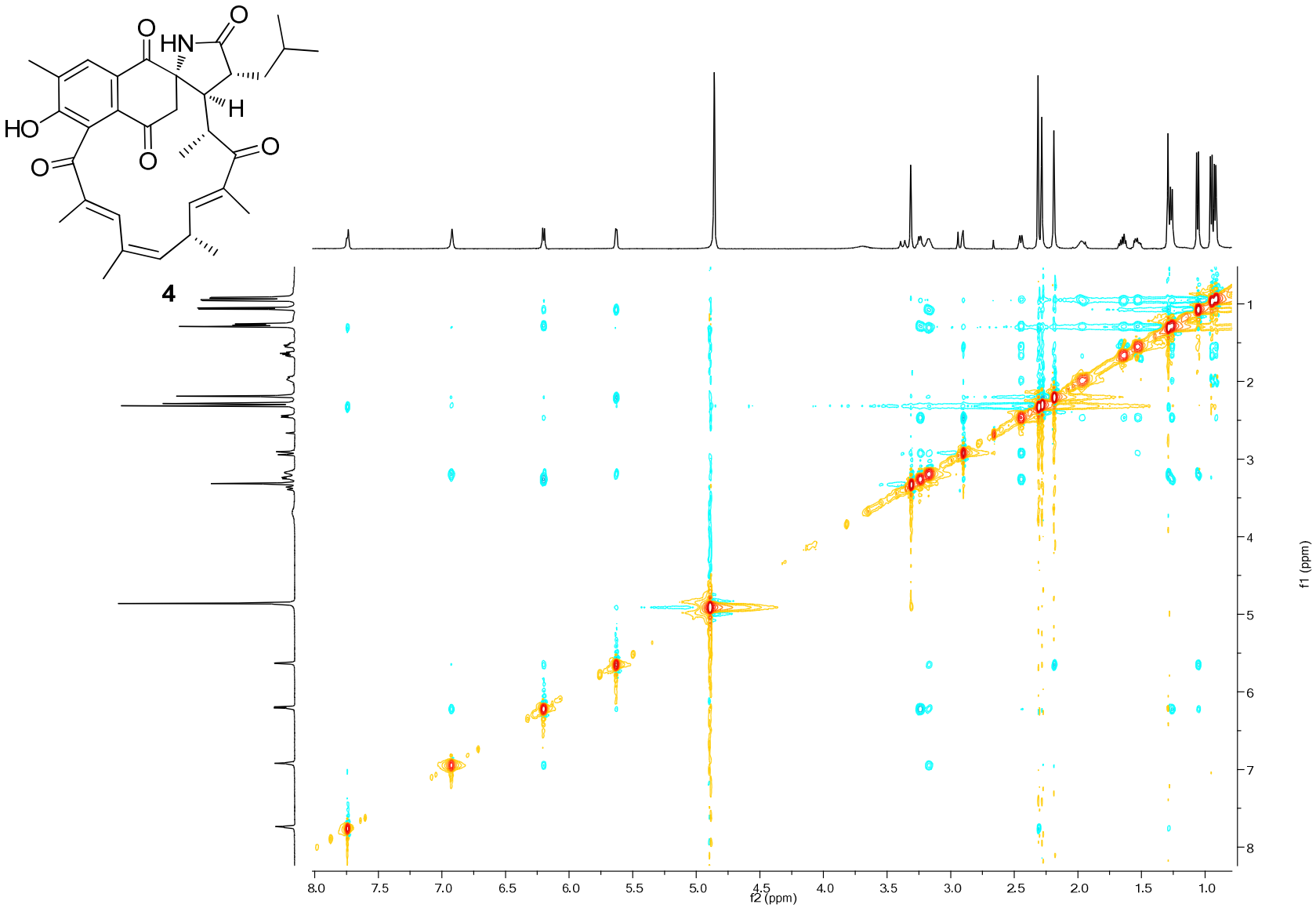

Figure S9. ^{1}H NMR spectrum (600 MHz) of polyol product **5** from ansalactam A (**4**) in methanol-d_4

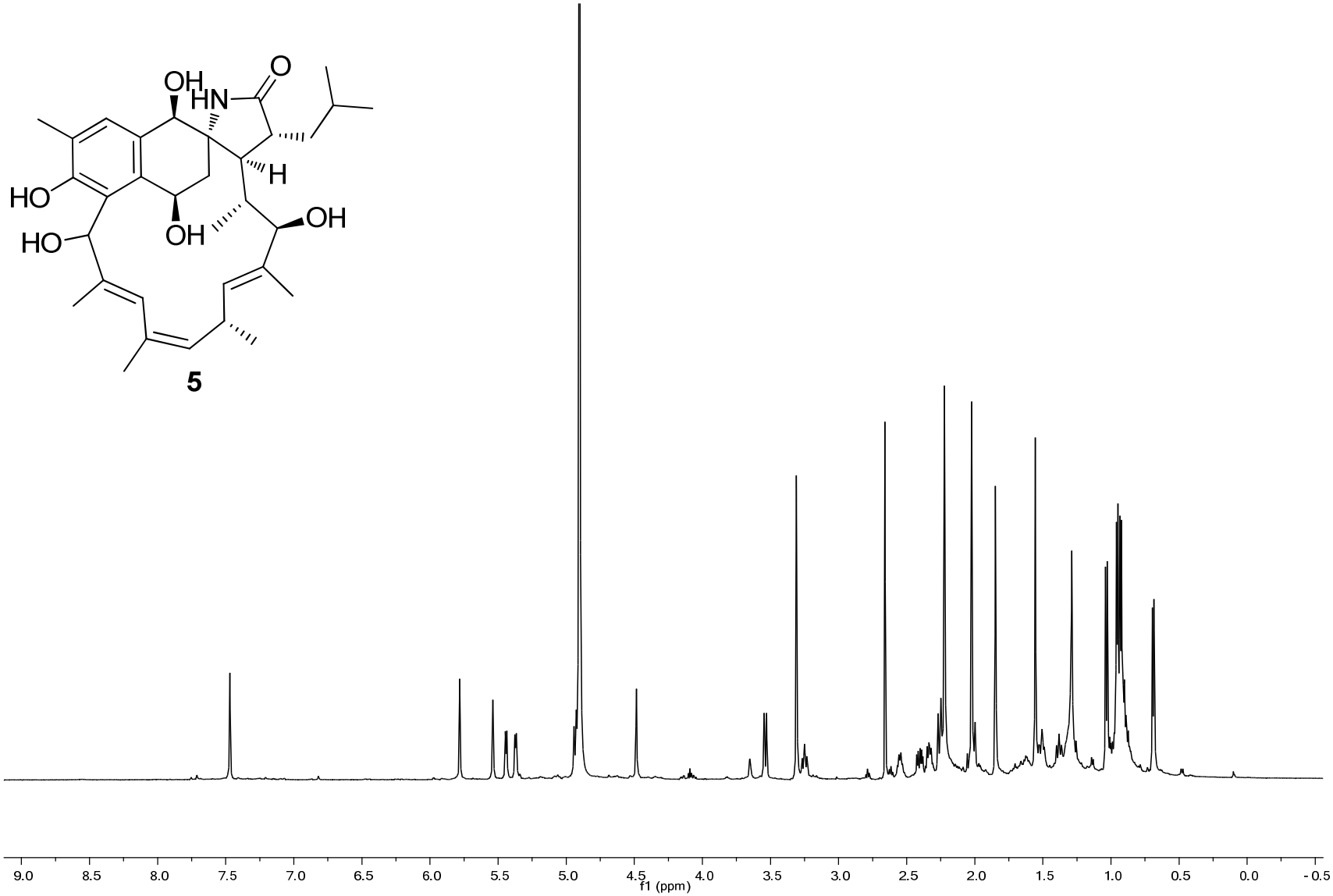

Figure S10. ^{13}C NMR spectrum (75 MHz) of polyol product **5** from ansalactam A (**4**) in methanol-d_4

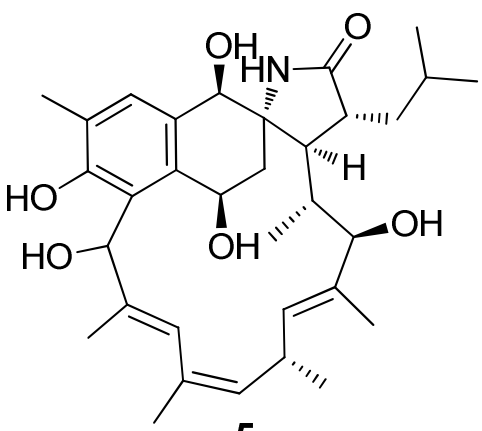

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Figure S11. ^{1}H NMR spectrum (600 MHz) of *bis*-*S*-MTPA ester (**6a**) of compound **5** in methanol-d_4

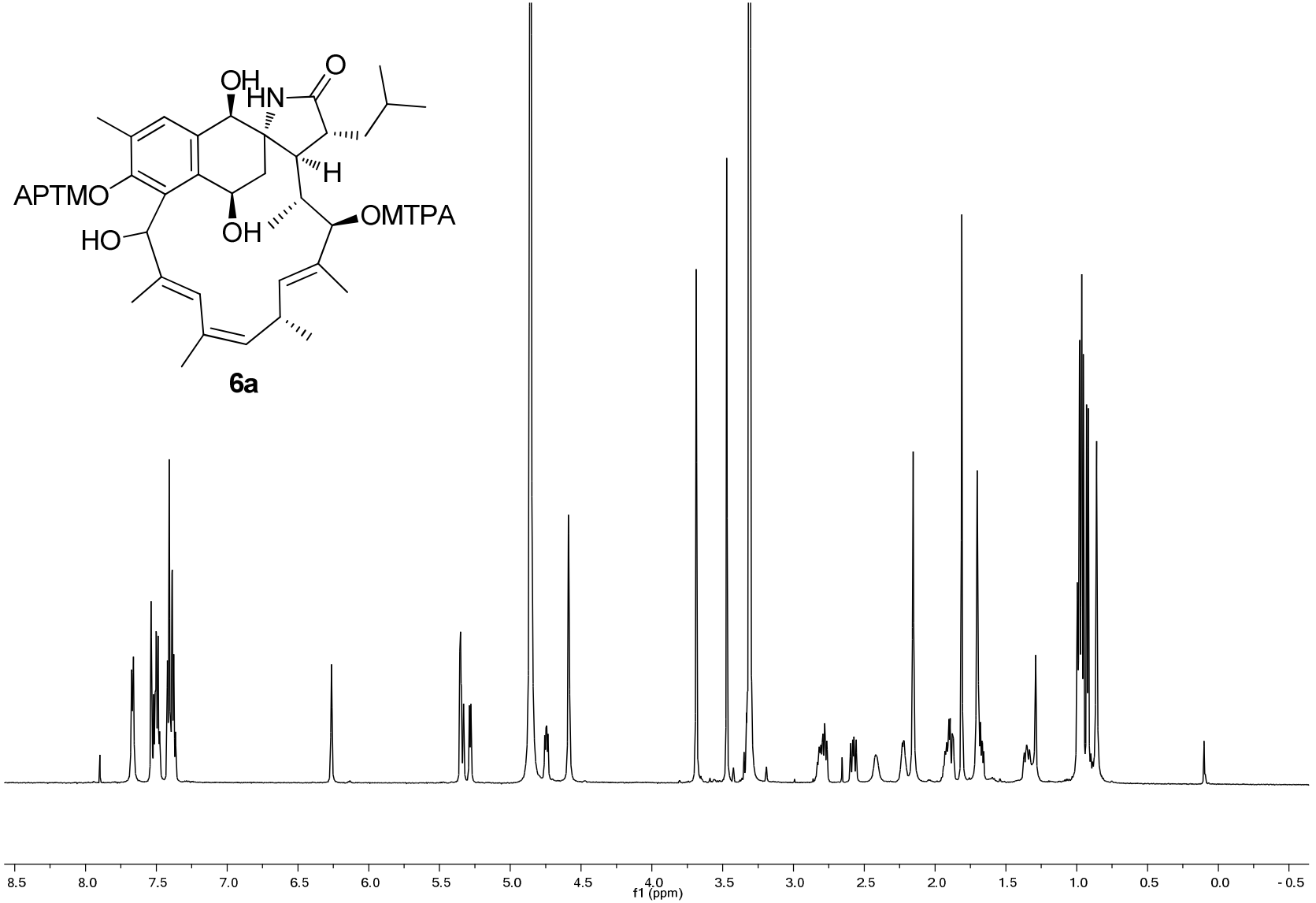


Figure S12. ^{1}H NMR spectrum (600 MHz) of *bis*-*R*-MTPA ester (**6b**) from compound **5** in methanol-d_4

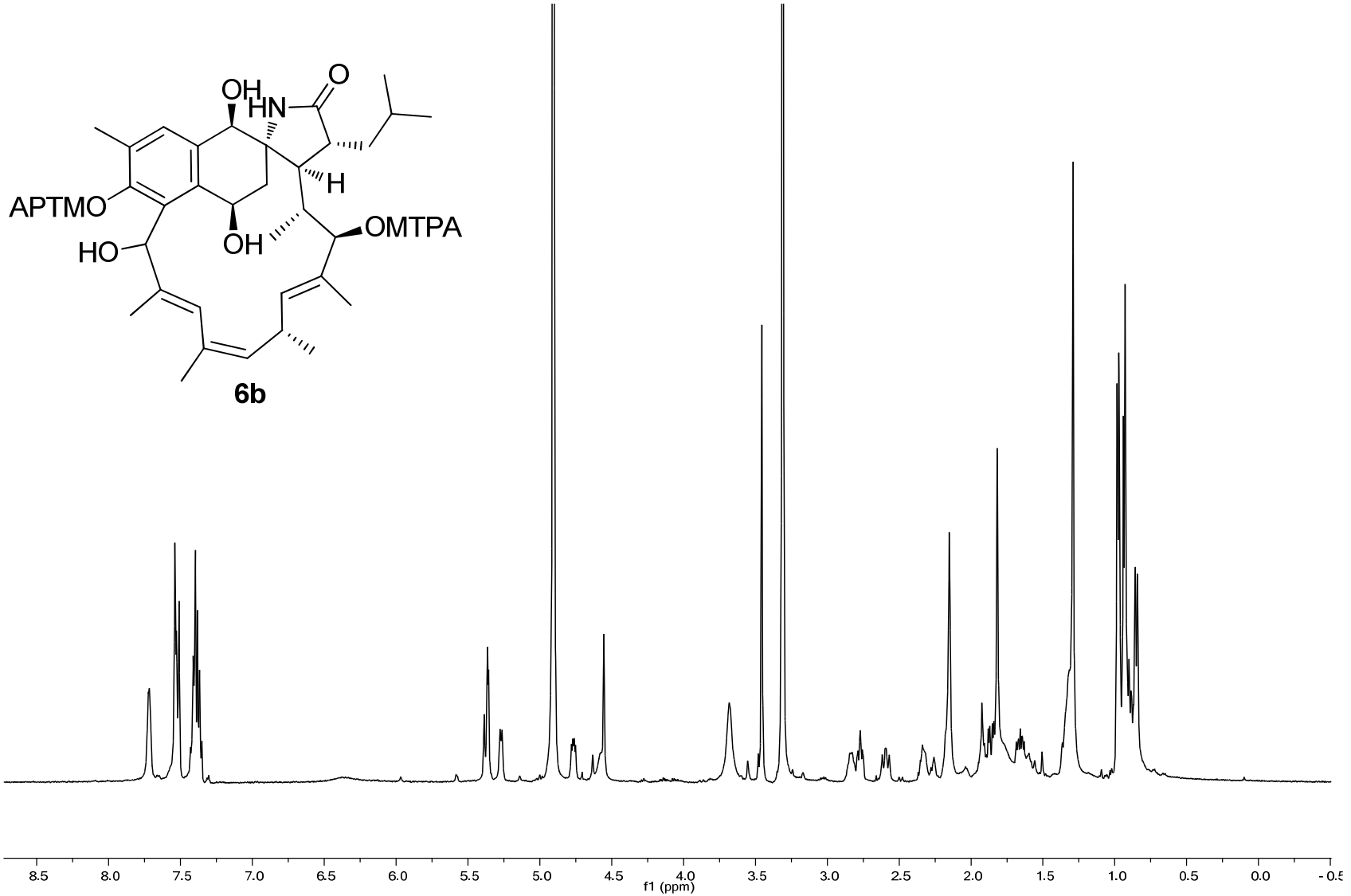


Figure S13. ^{1}H NMR spectrum (600 MHz) of vicinal diol **7** from ansalactam A (**4**) in methanol-d_4

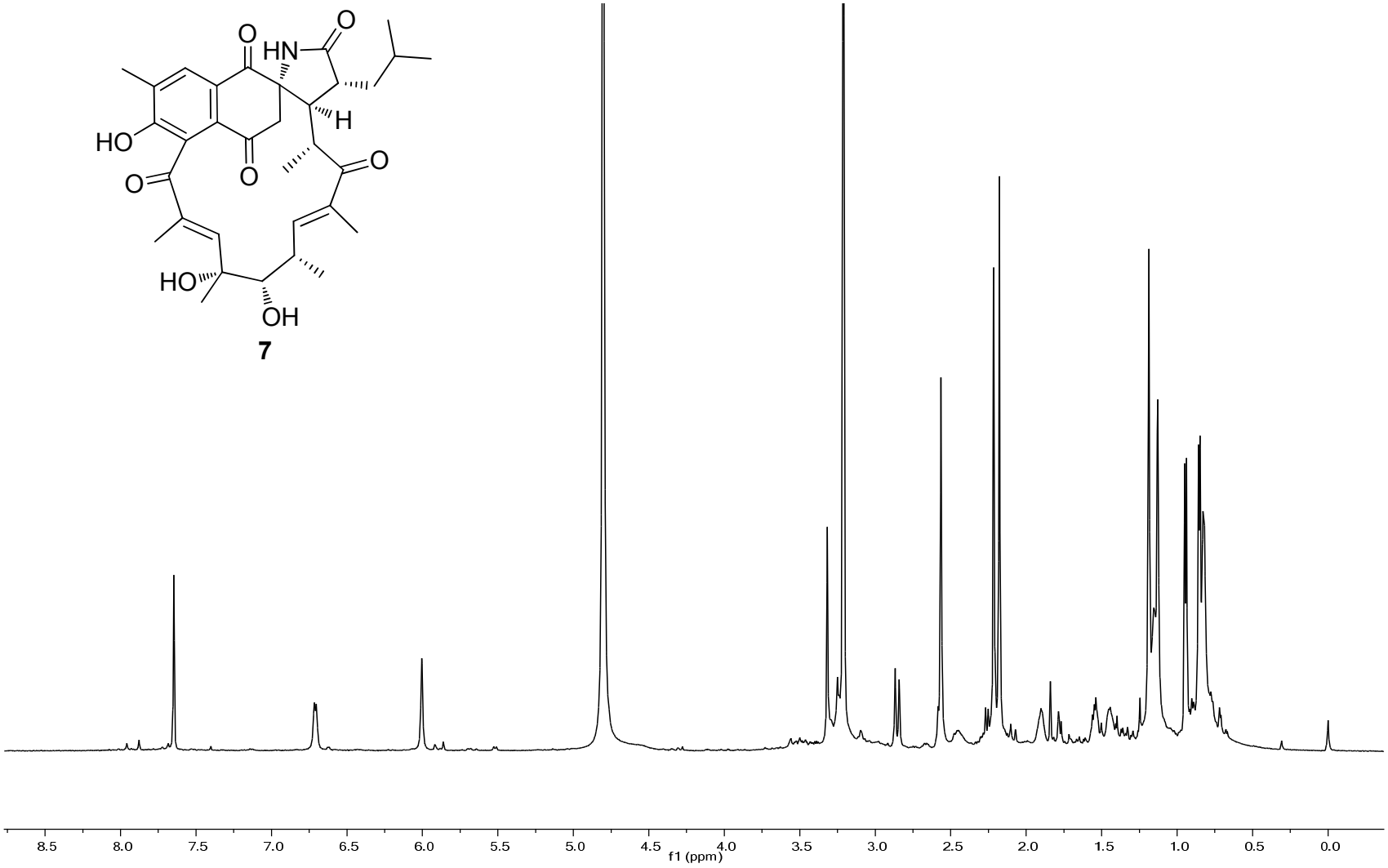


Figure S14. ^{1}H NMR spectrum (600 MHz) of *bis*-*S*-MTPA ester (**8a**) from compound **7** in DMSO-d_6.

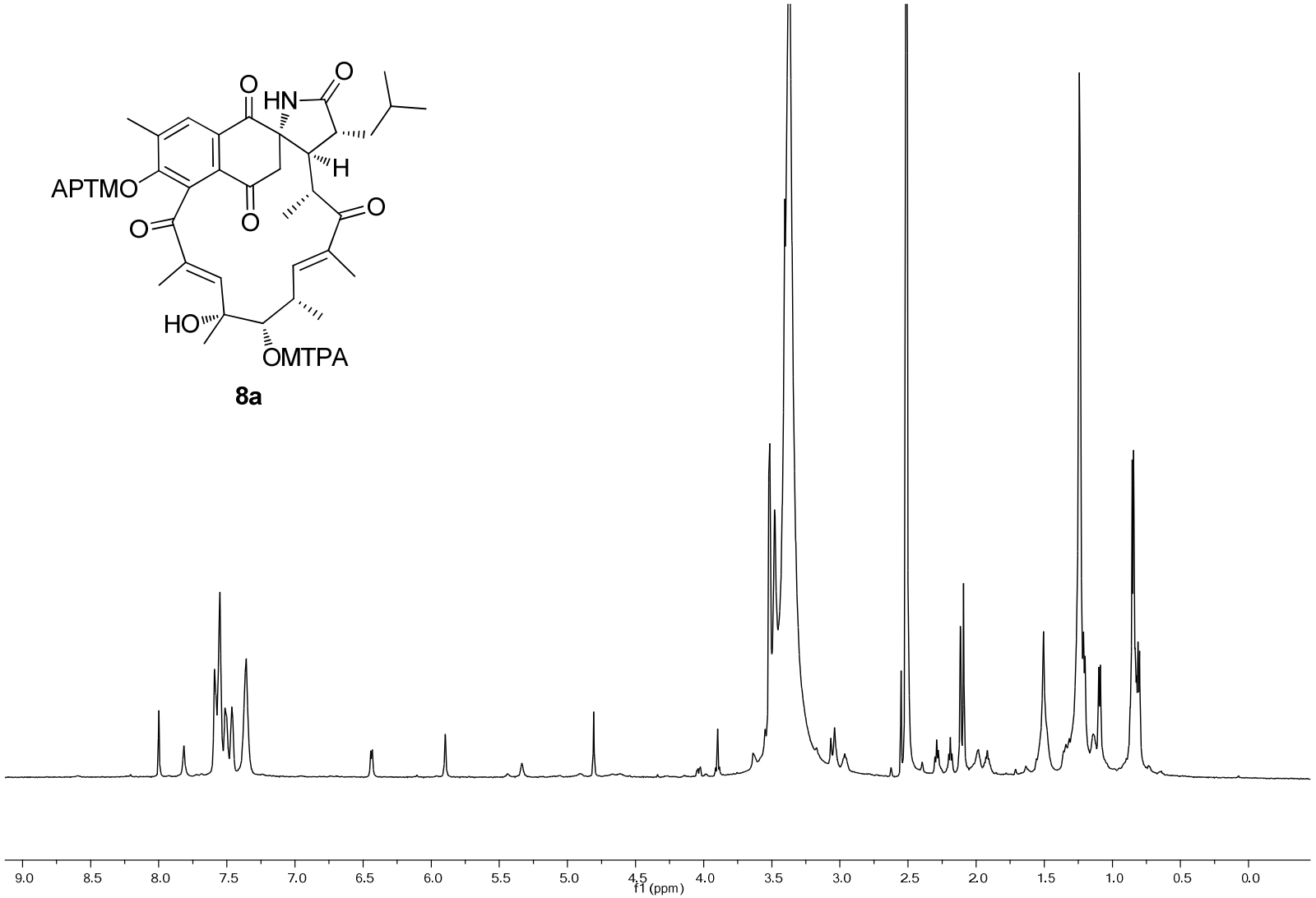
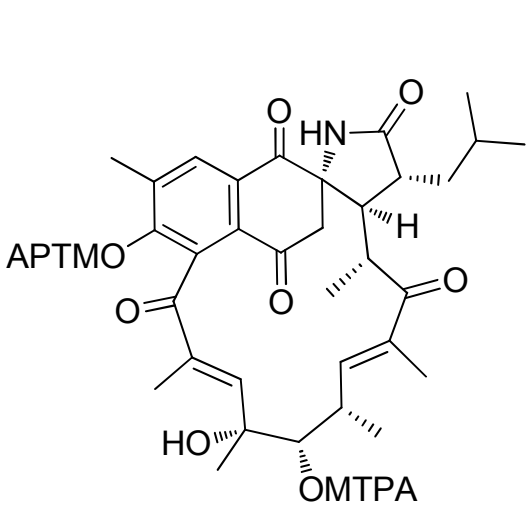


Figure S15. ^{1}H NMR spectrum (600 MHz) of *bis*-*R*-MTPA ester (**8b**) from compound **7** in DMSO-d_6



8b

Figure S16. ^{13}C NMR spectrum (125 MHz) of **5** **not** enriched from [1-^{13}C]leucine feeding study in methanol-d_4

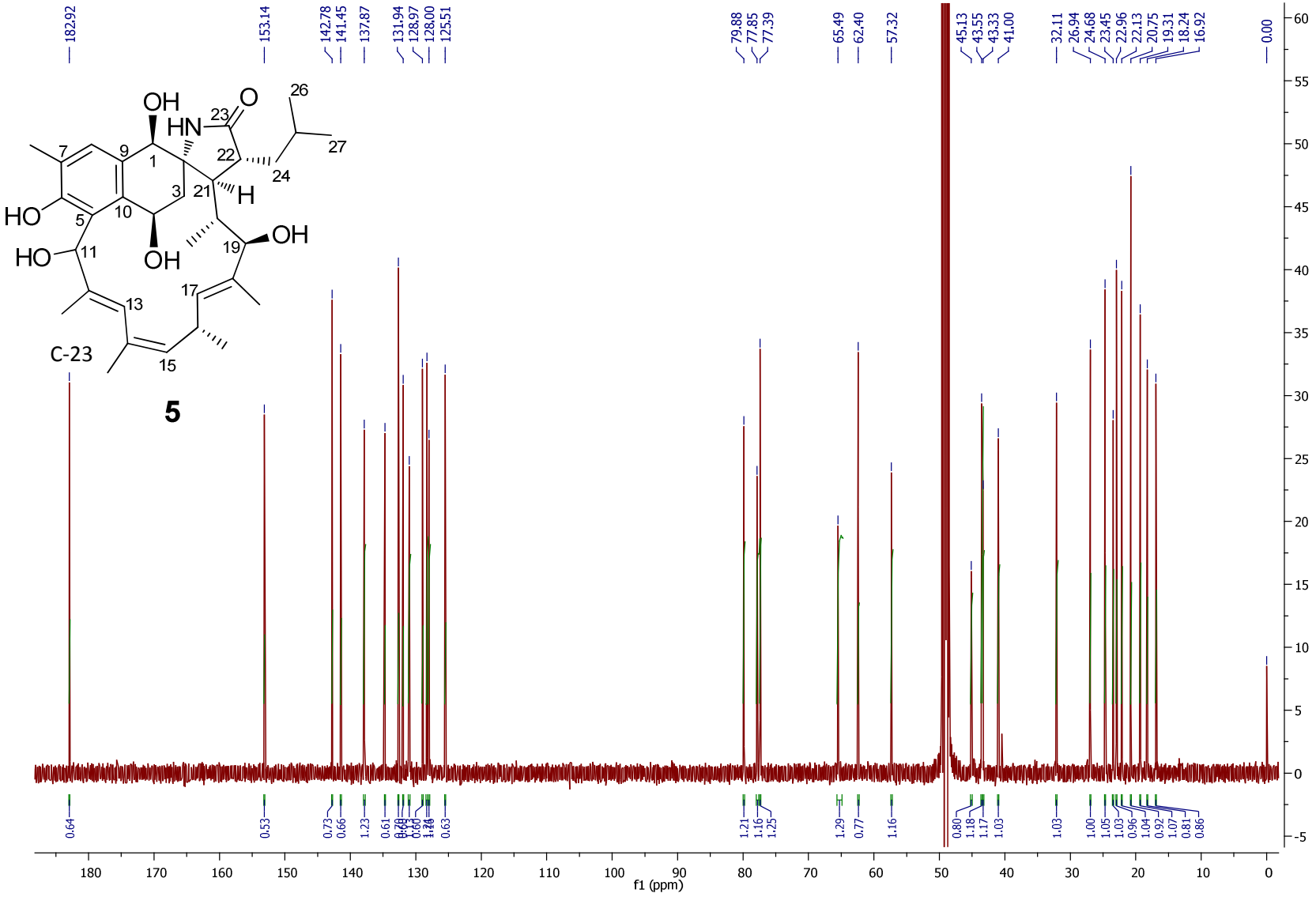


Figure S17. ^{13}C NMR spectrum (125 MHz) of **5** from [1-^{13}C]propionate feeding study in methanol-d_4

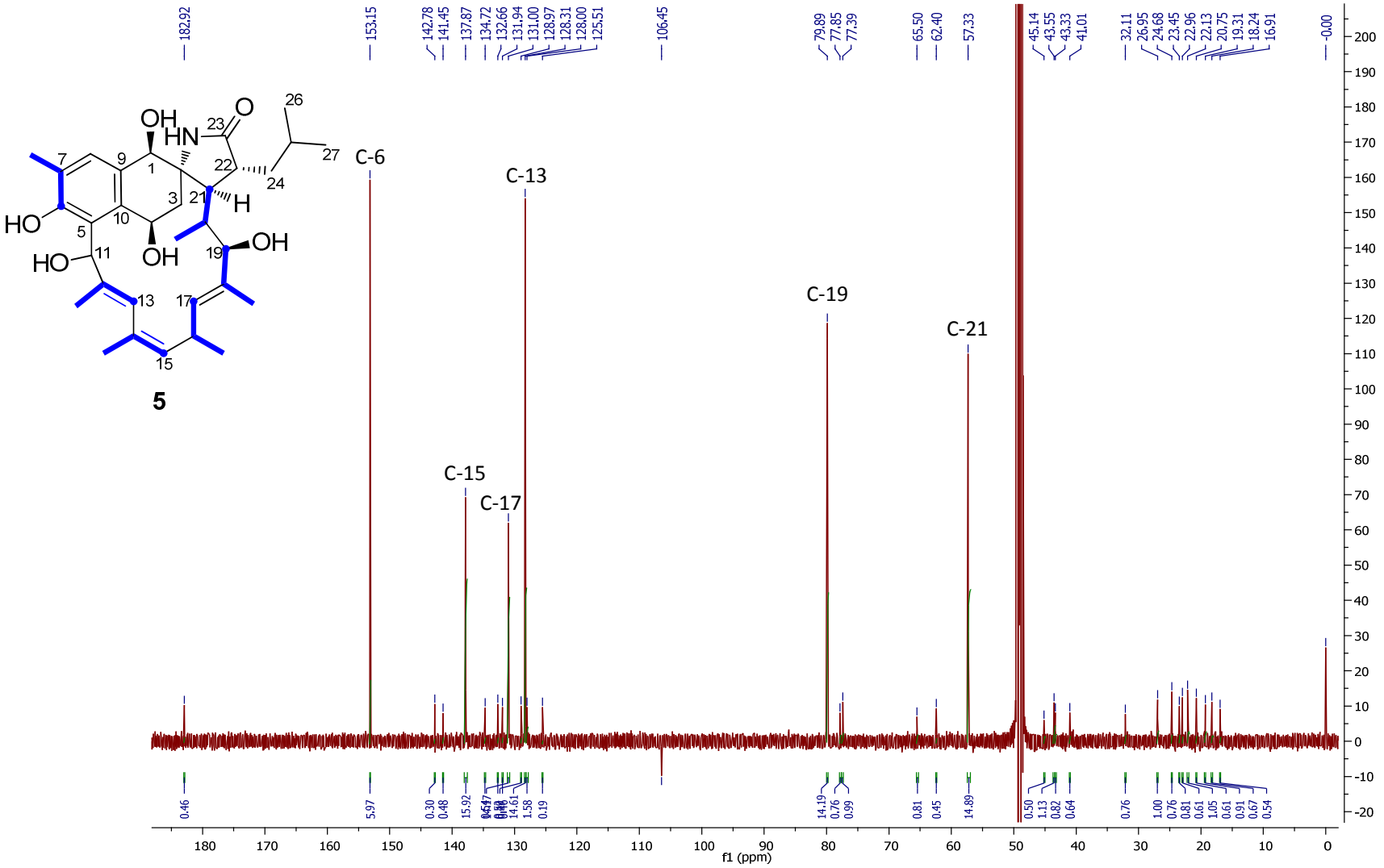


Figure S18. ^{13}C NMR spectrum (125 MHz) of **5** from [1,2-$^{13}C_2$]acetate feeding study in methanol-d_4

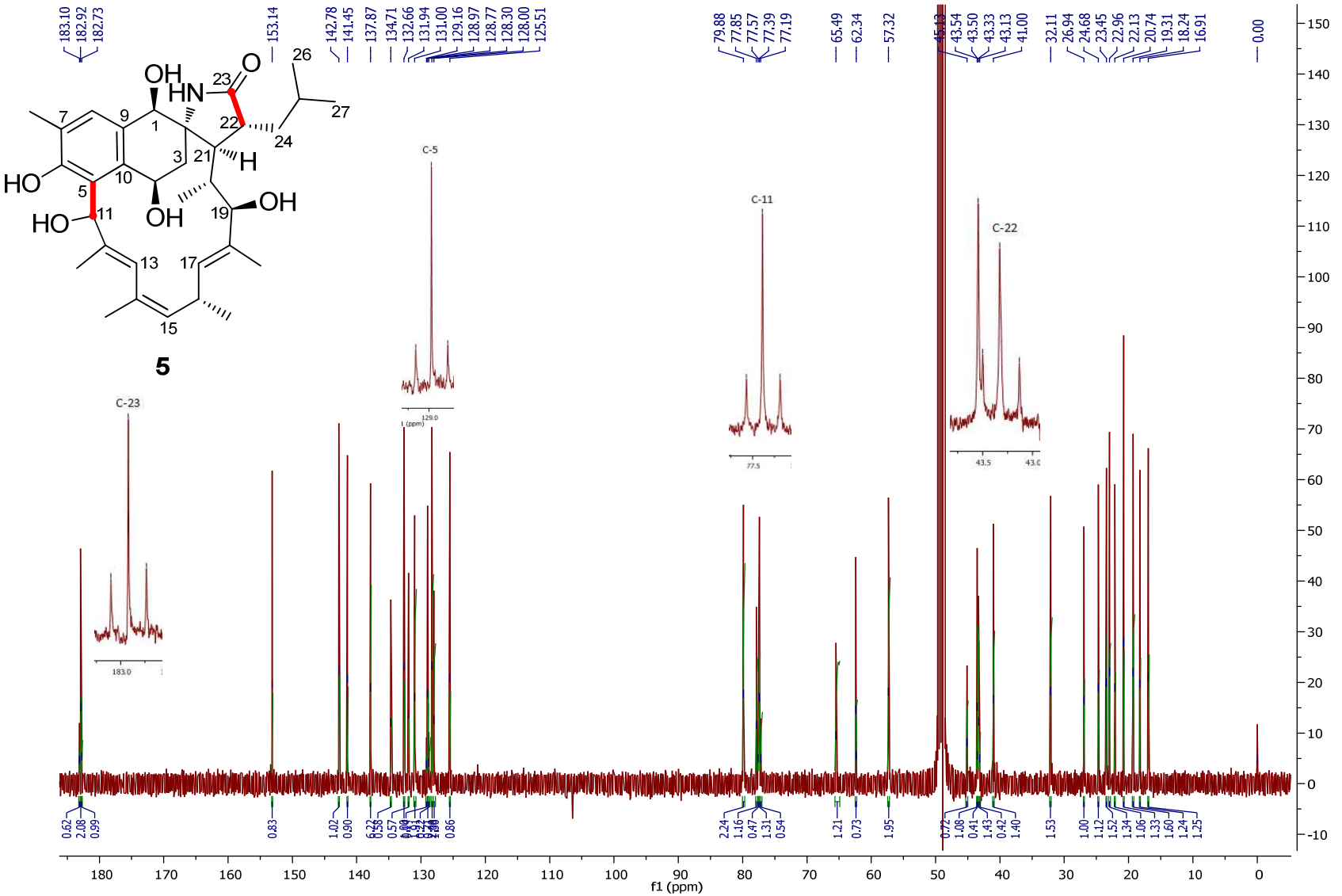


Figure S19. ^{13}C NMR spectrum (125 MHz) of **5** from [1-^{13}C]isobutyrate feeding study in methanol-d_4

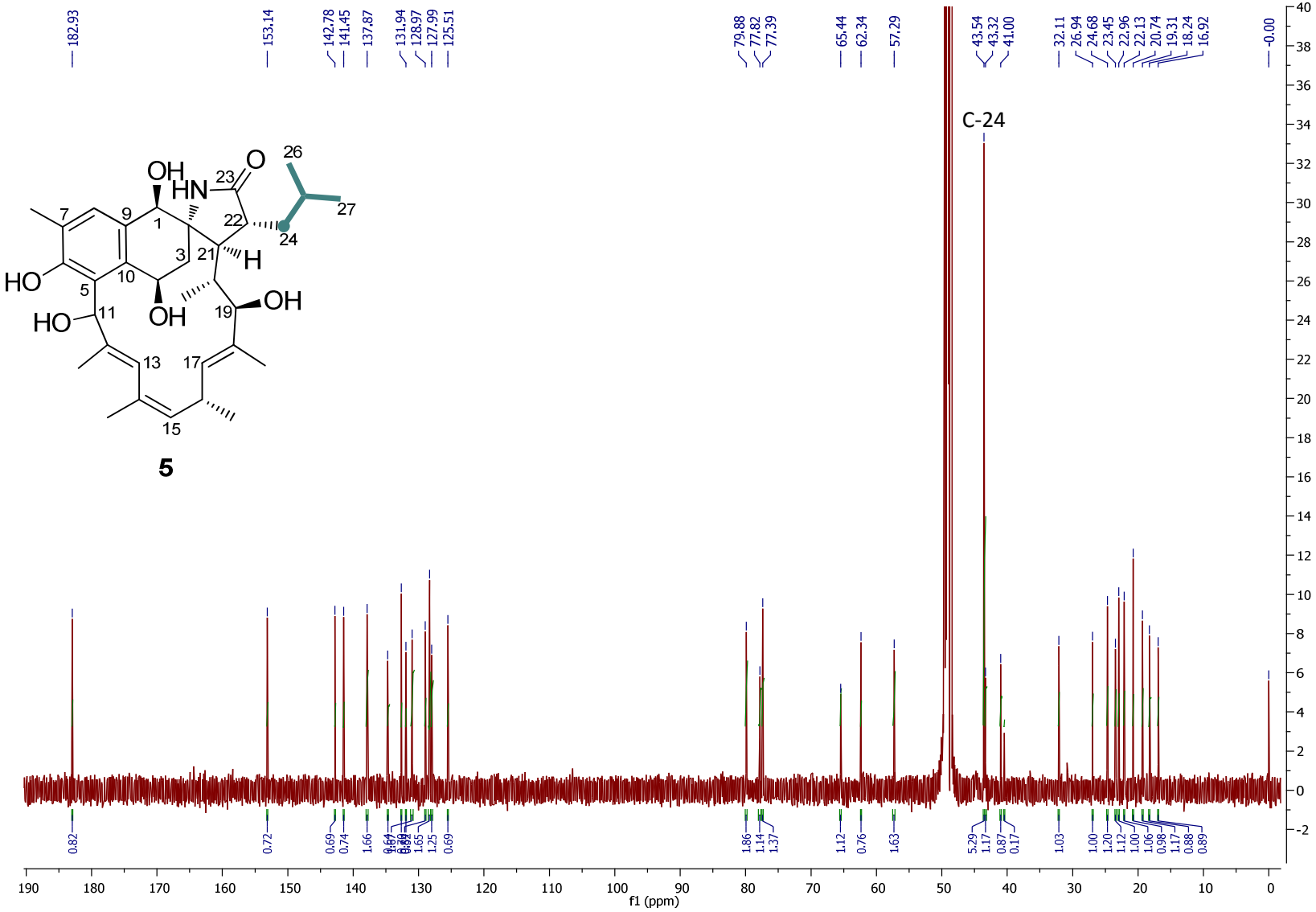
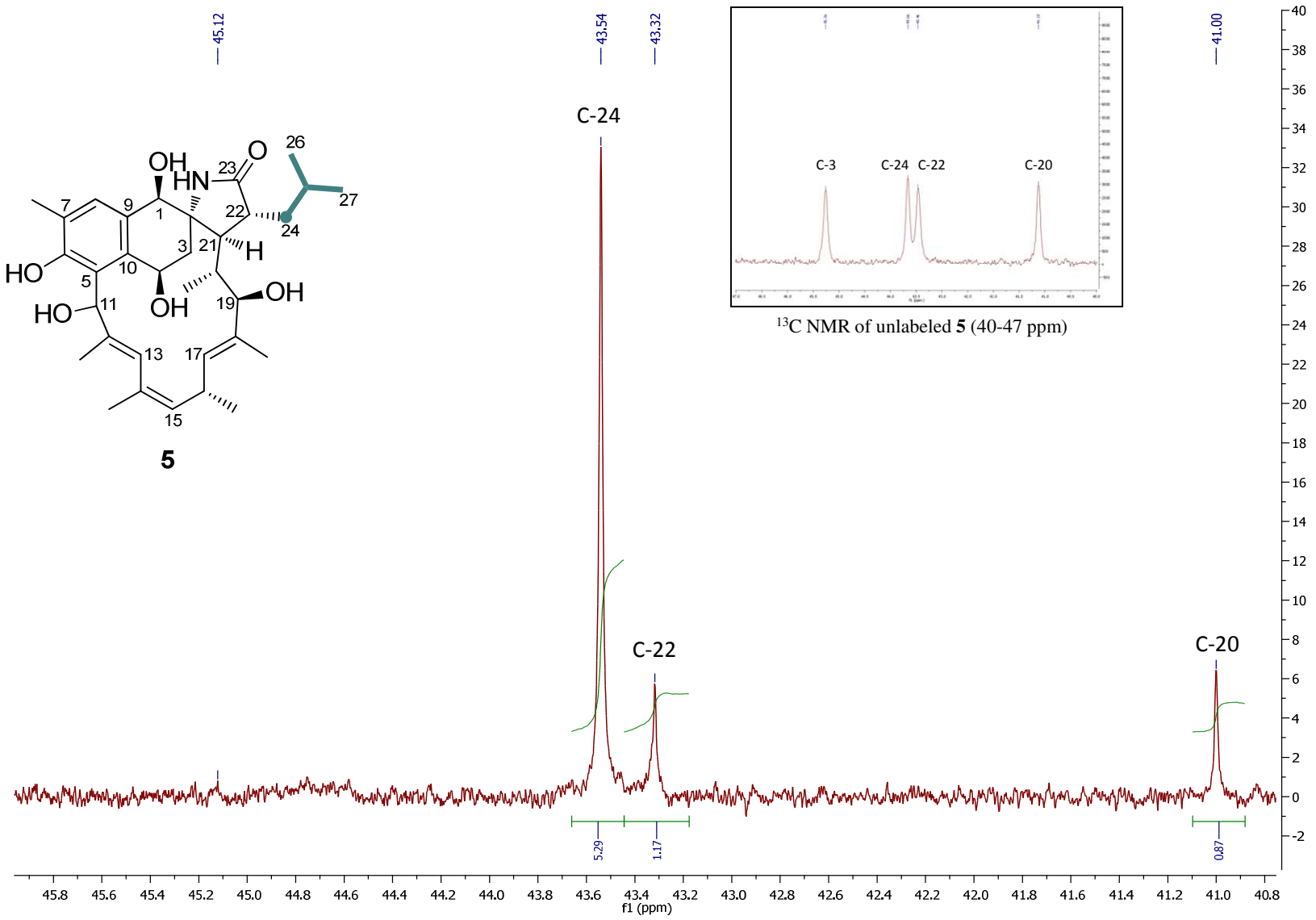


Figure S20. ^{13}C NMR spectrum (125 MHz) of **5** from [1-^{13}C]isobutyrate feeding study in methanol-d_4 (expanded).



5

^{13}C NMR of unlabeled **5** (40-47 ppm)

Figure S21. ^{13}C NMR spectrum (125 MHz) of **5** from [2-^{13}C]4-Me-2-pentenoic acid feeding study in methanol-d_4

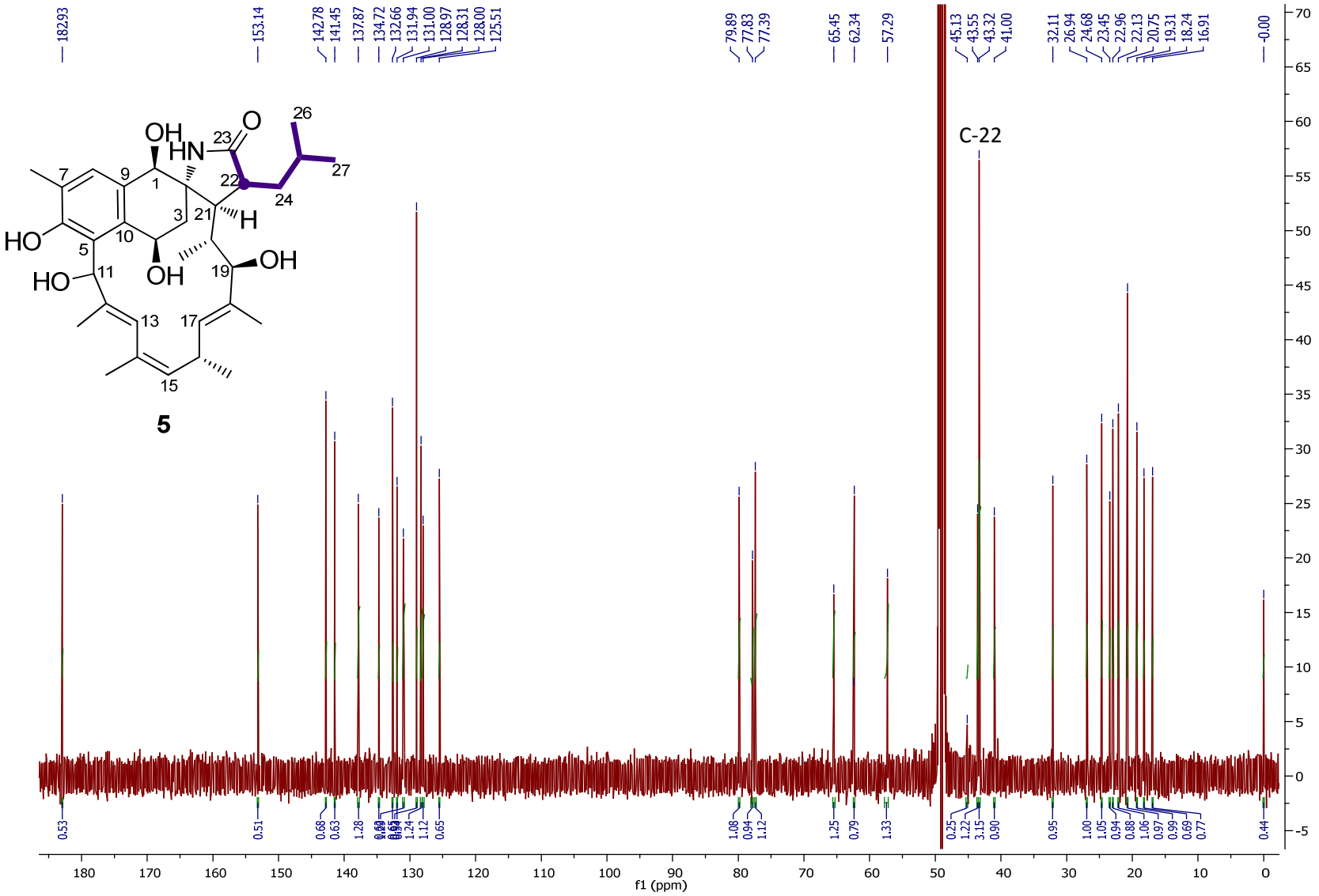
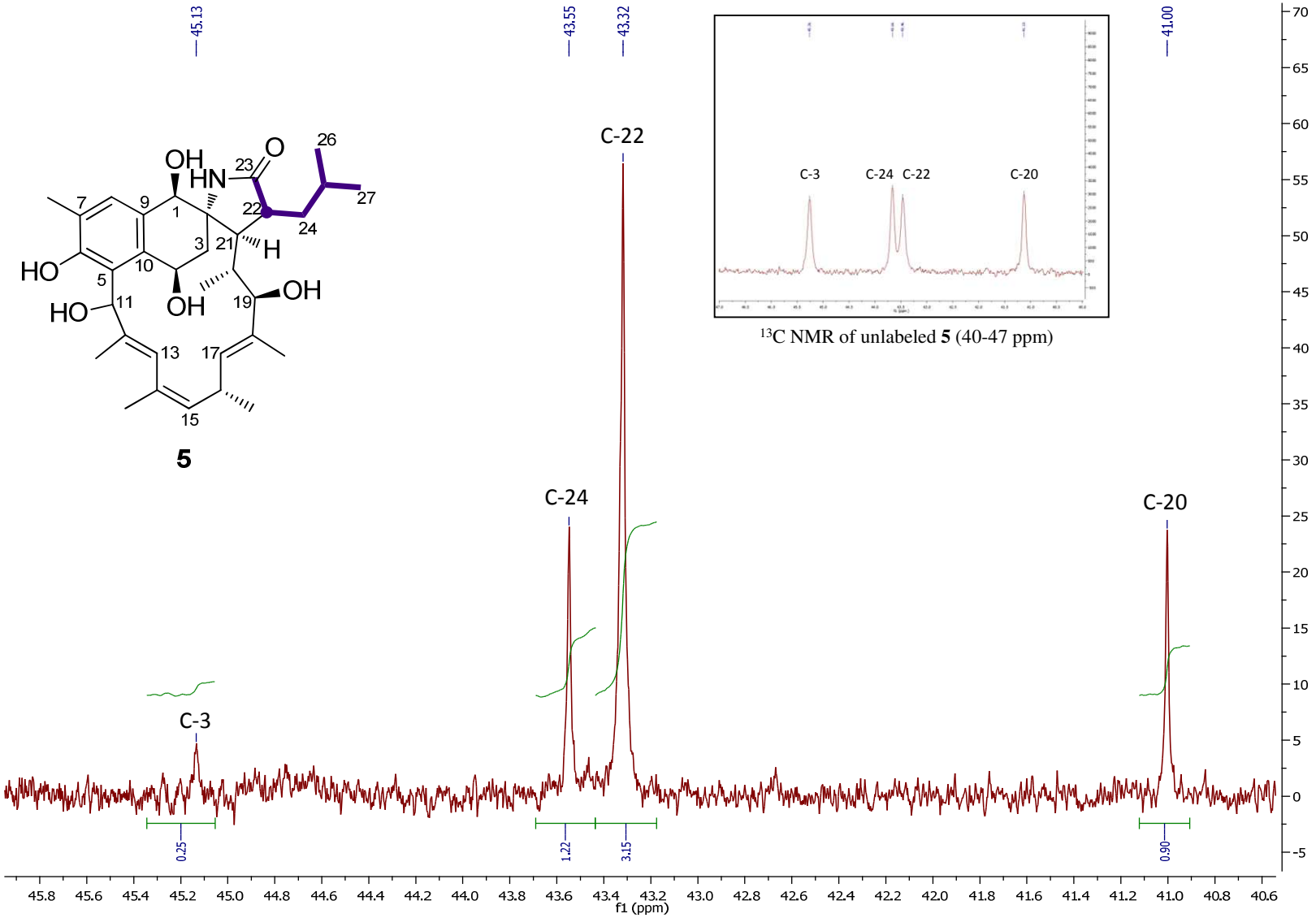


Figure S22. ^{13}C NMR spectrum (125 MHz) of **5** from [2-^{13}C]4-Me-2-pentenoic acid feeding study in methanol-d_4 (expanded).



References

(1) Huitu, Z.; Linzhuan, W.; Aiming, L.; Guizhi, S.; Feng, H.; Qiuping, L.; Yuzhen, W.; Huanzhang, X.; Qunjie, G.; Yiguang, W. *J. Appl. Microbiol.* **2009**, *106*, 755.

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Mo, S.; Kim, D. H.; Lee, J. H.; Park, J. W.; Basnet, D. B.; Ban, Y. H.; Yoo, Y. J.; Chen, S.-w.; Park, S. R.; Choi, E. A.; Kim, E.; Jin, Y.-Y.; Lee, S.-K.; Park, J. Y.; Liu, Y.; Lee, M. O.; Lee, K. S.; Kim, S. J.; Kim, D.; Park, B. C.; Lee, S.-g.; Kwon, H. J.; Suh, J.-W.; Moore, B. S.; Lim, S.-K.; Yoon, Y. J. *J. Am. Chem. Soc.*, accepted.