

Article

Profluorescent Fluoroquinolone-Nitroxides for Investigating Antibiotic–Bacterial Interactions

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Supplementary Material

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Methods

MIC susceptibility assay (in 96-well plate) for unfunctionalized nitroxides:

Nitroxides 2,2,6,6-tetramethylpiperidin-1-yloxy (TEMPO), 1,1,3,3-tetramethylisindolin-2-yloxy (TMIO), and 1,1,3,3-tetraethylisindolin-2-yloxy (TEIO) were also subjected to MIC susceptibility assays utilising the same methodology detailed above. TEMPO, TMIO, and TEIO were tested between the concentration range of 1200 to 2 μ M. MIC values (Table S1) were obtained from at least 3 biological replicates, each with at least 3 technical replicates.

Results

MIC susceptibility assay for unfunctionalized nitroxides:

Table 1. Measured MIC values for TEMPO, TMIO, and TEIO against Gram-negative *P. aeruginosa* and *E. coli*, and Gram-positive *S. aureus*, and *E. faecalis*.^[a]

Compound	<i>P. aeruginosa</i> ATCC 27853 MIC (μ M)	<i>E. coli</i> ATCC 25922 MIC (μ M)	<i>S. aureus</i> ATCC 29213 MIC (μ M)	<i>E. faecalis</i> ATCC 14933 MIC (μ M)
TEMPO	> 1200 ^[b]	> 1200 ^[b]	> 1200 ^[b]	> 1200 ^[b]
TMIO	> 1200 ^[b]	> 1200 ^[b]	> 1200 ^[b]	> 1200 ^[b]
TEIO	> 1200 ^[b]	> 1200 ^[b]	> 1200 ^[b]	> 1200 ^[b]

[a] All MICs were determined via broth microdilution method in accordance with CLSI standard; [b] Highest concentration tested.

Fluorescence microscopy images:

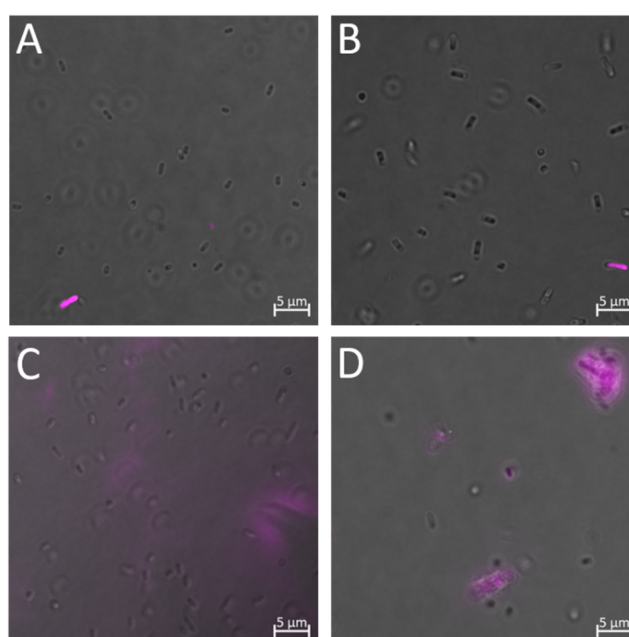
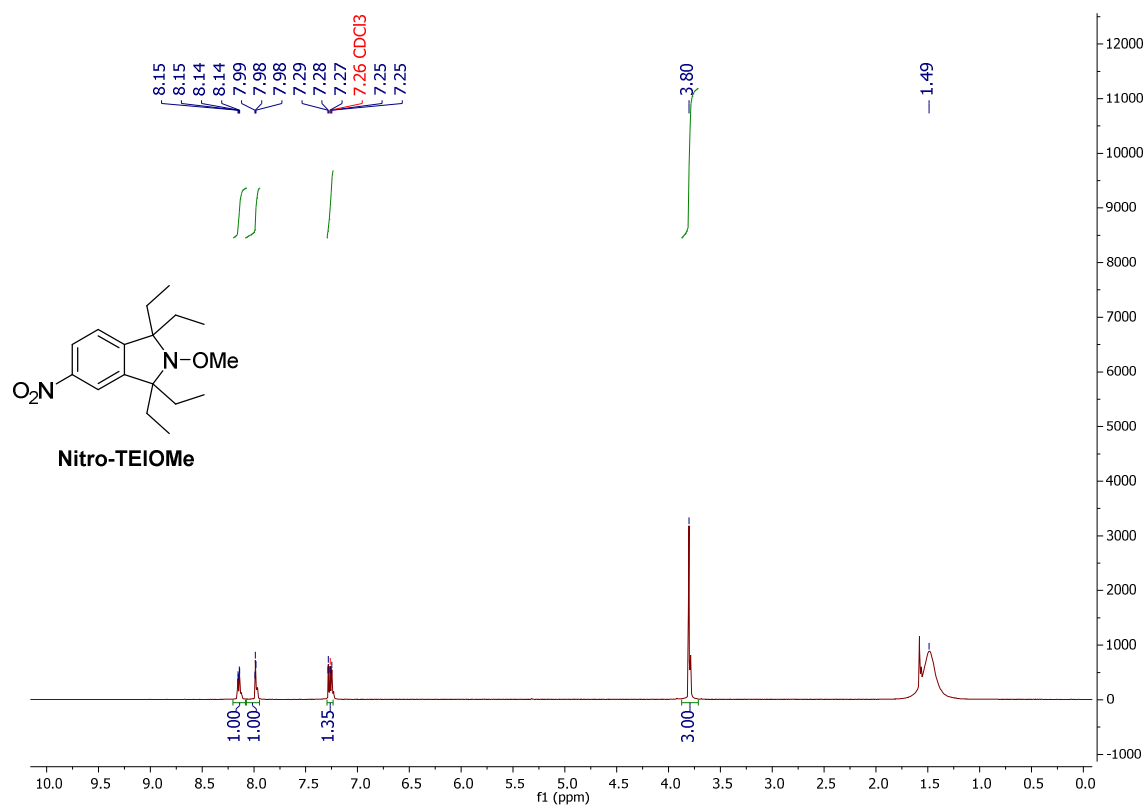
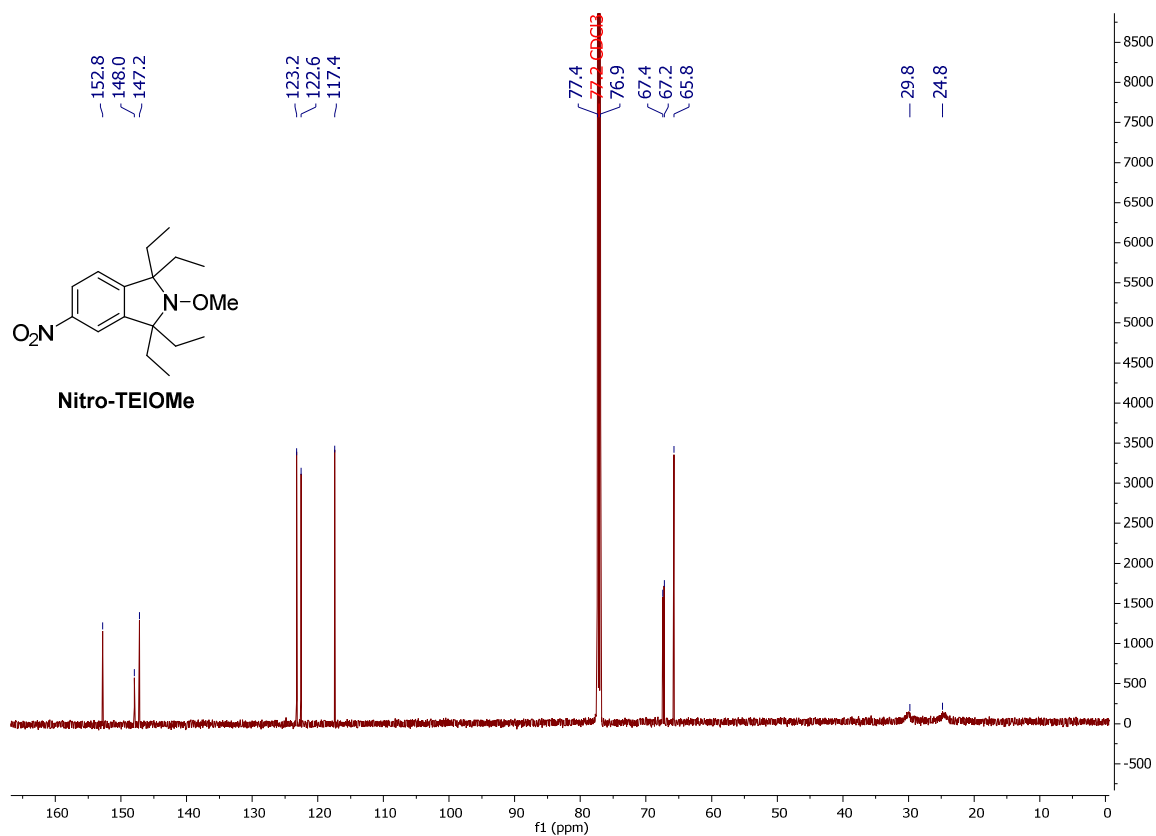
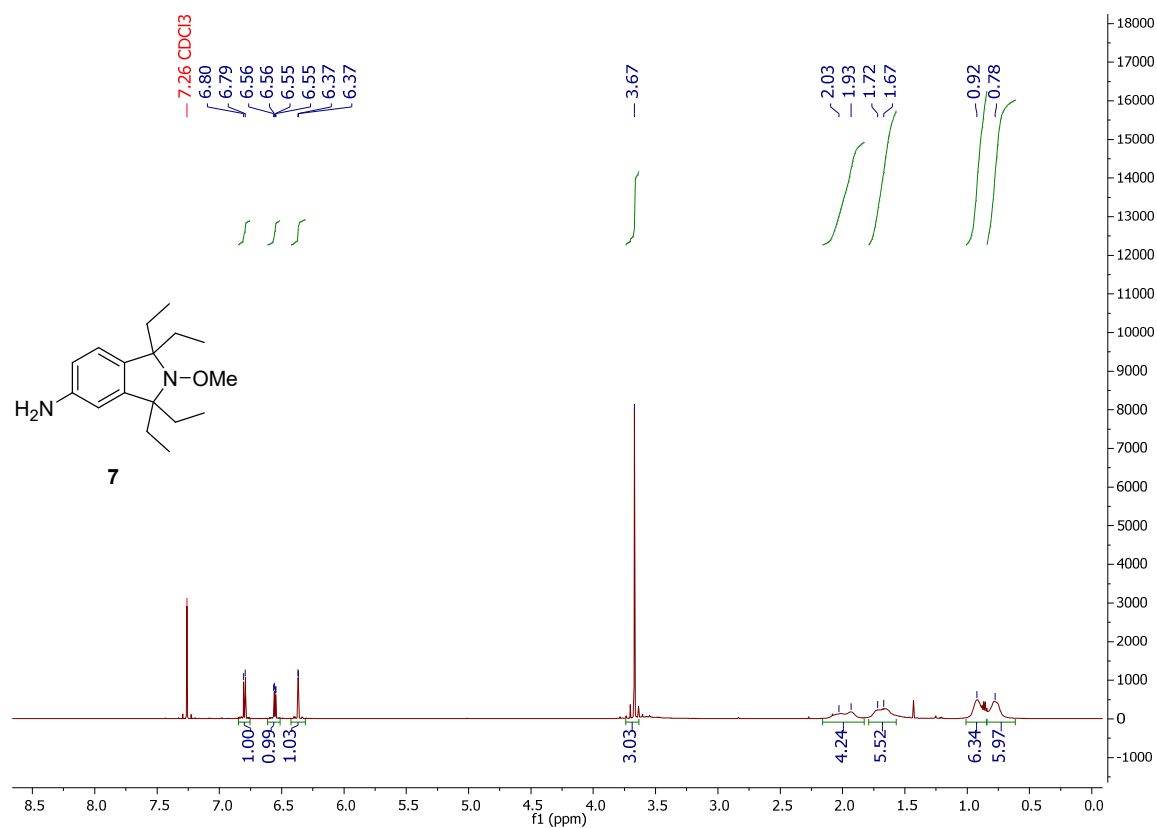
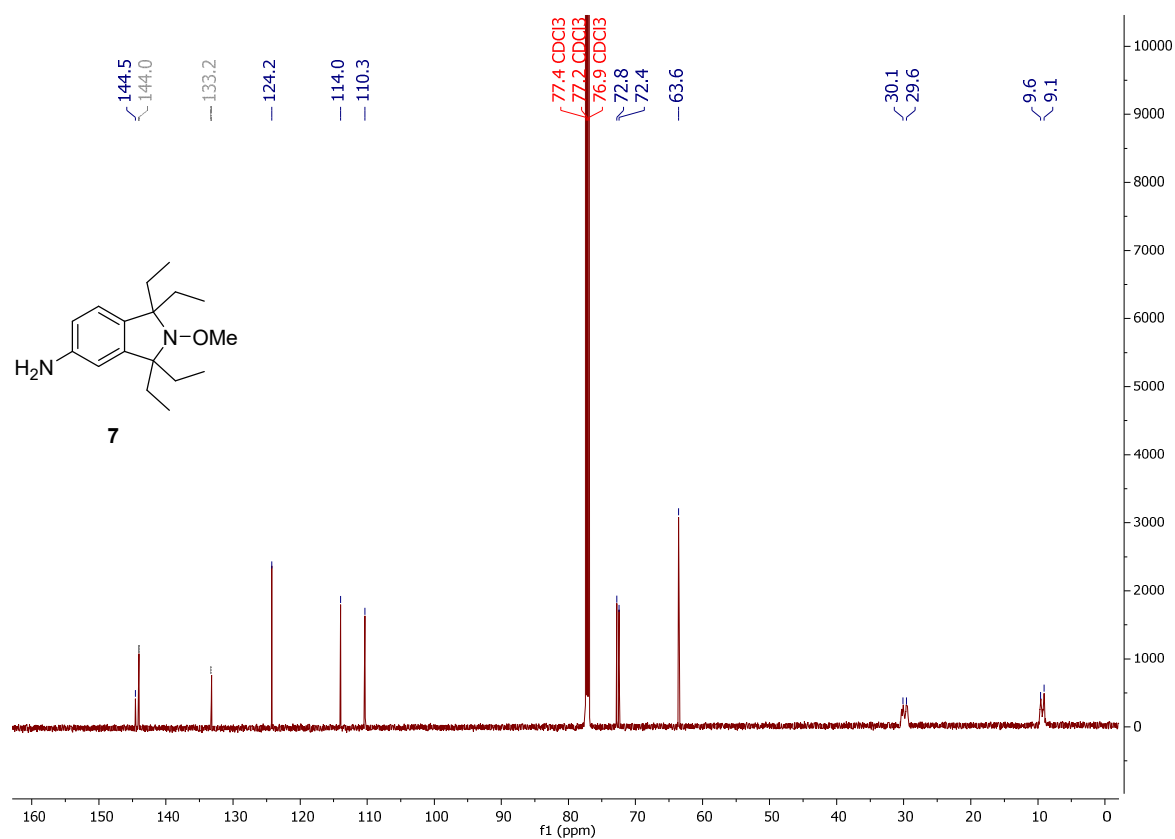
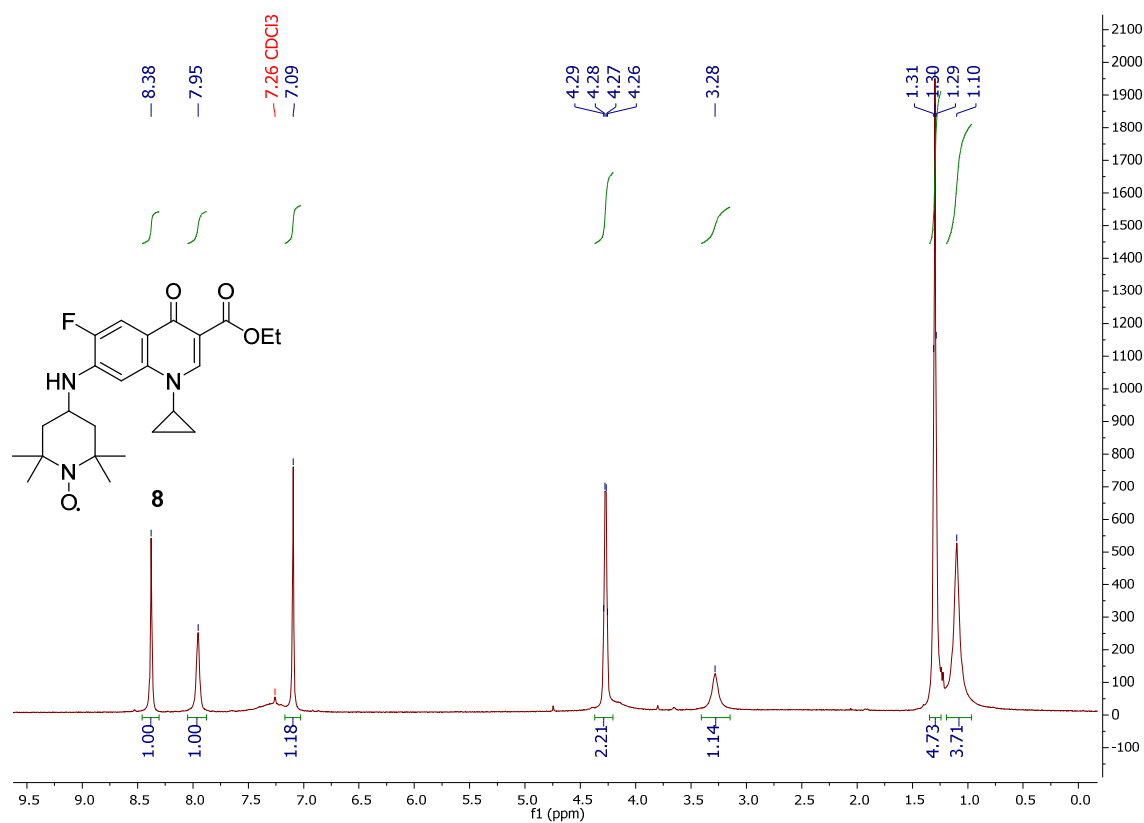
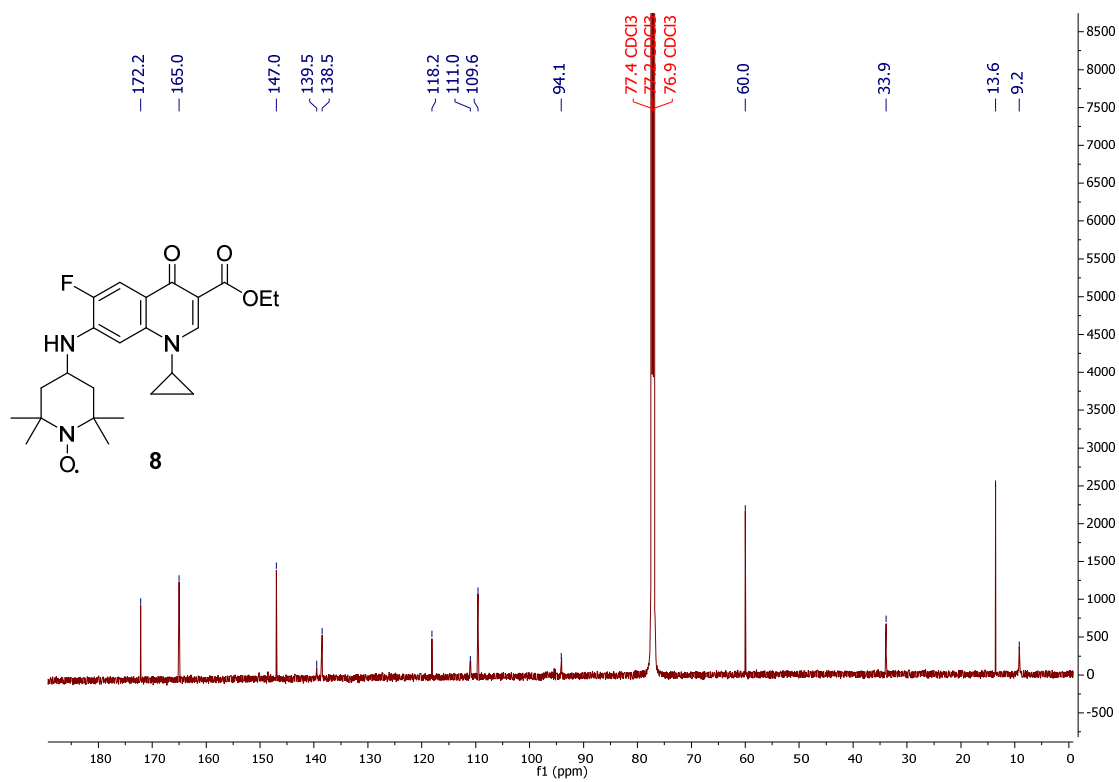
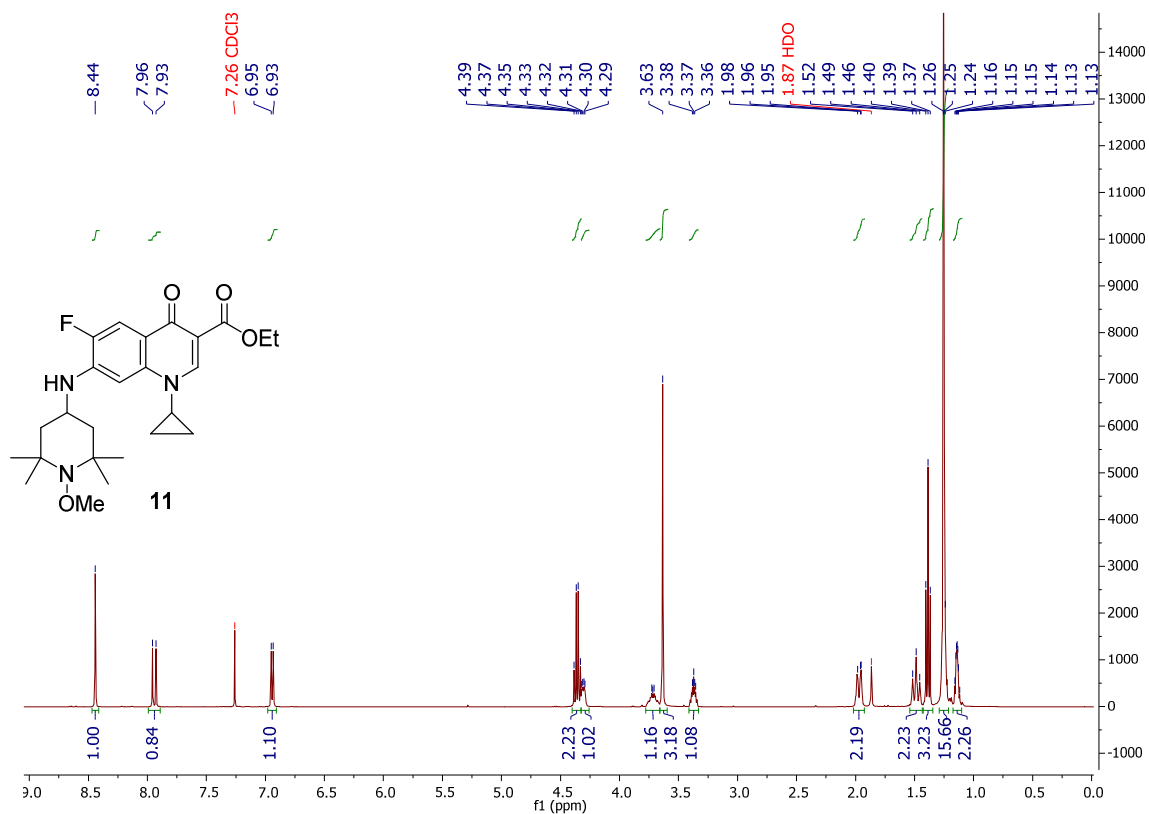
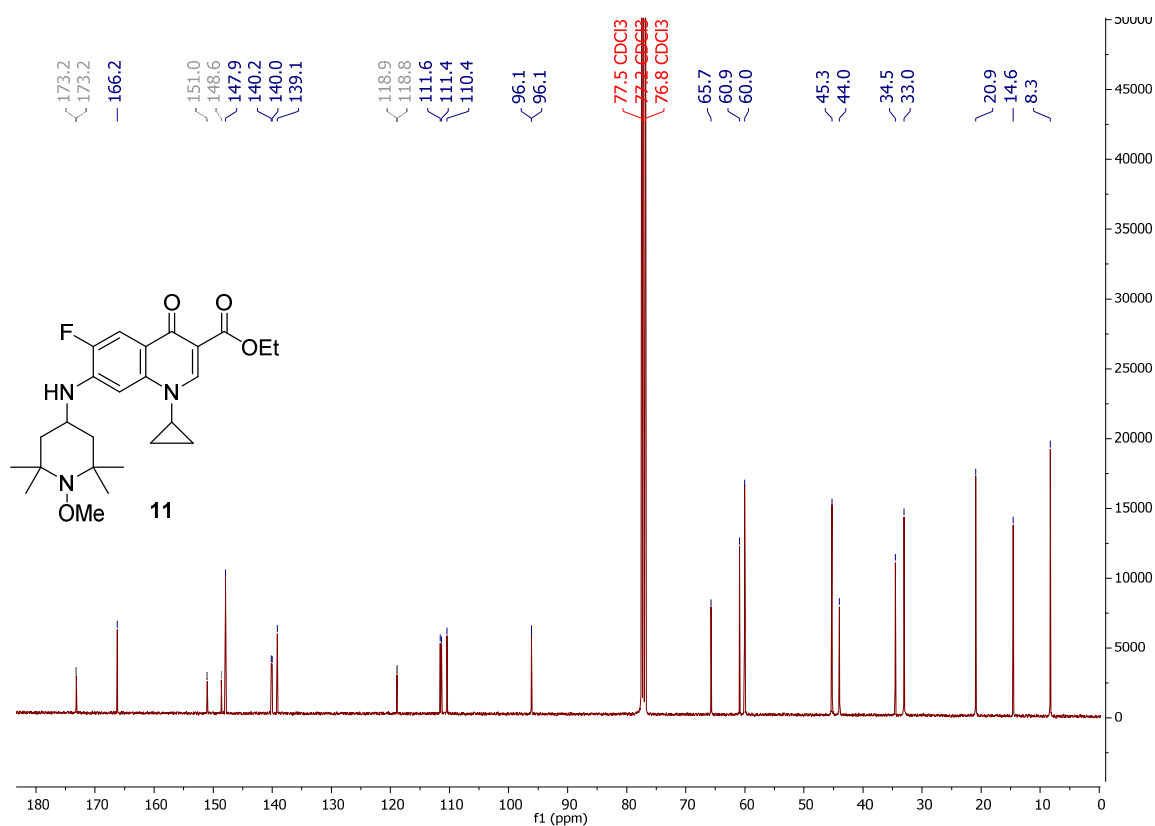


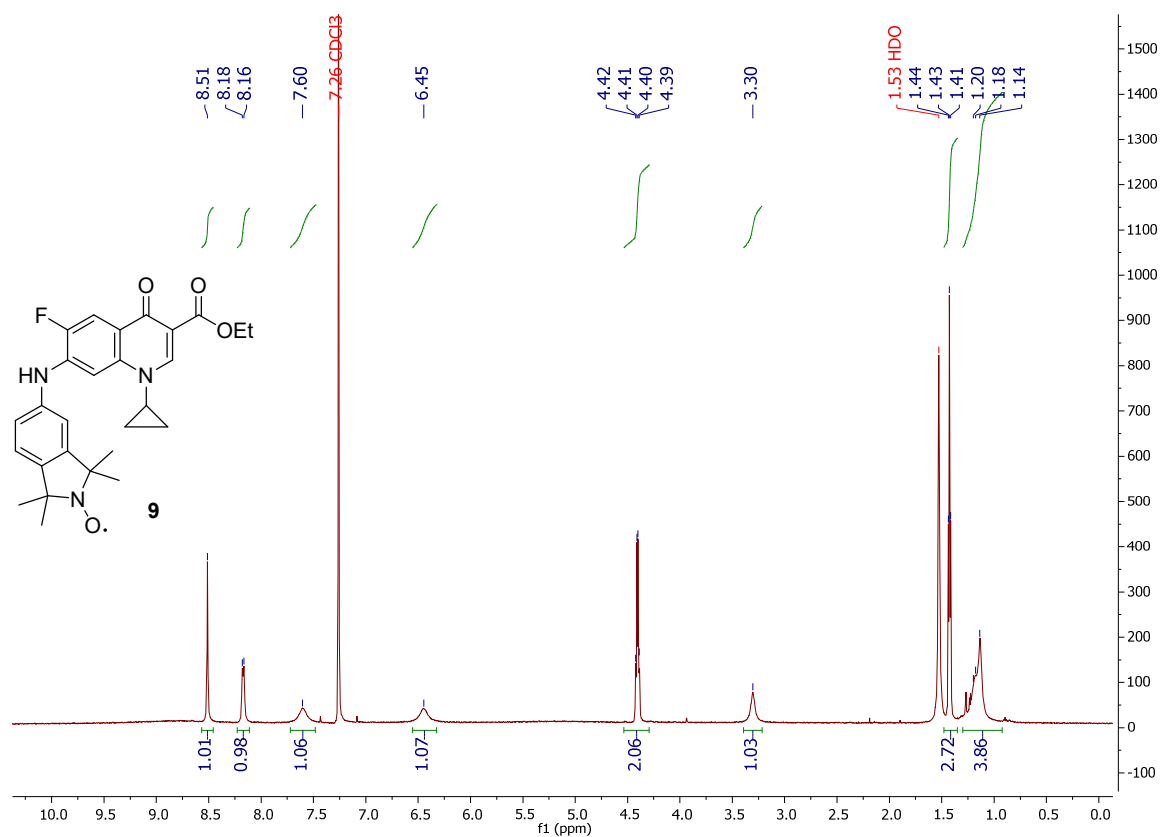
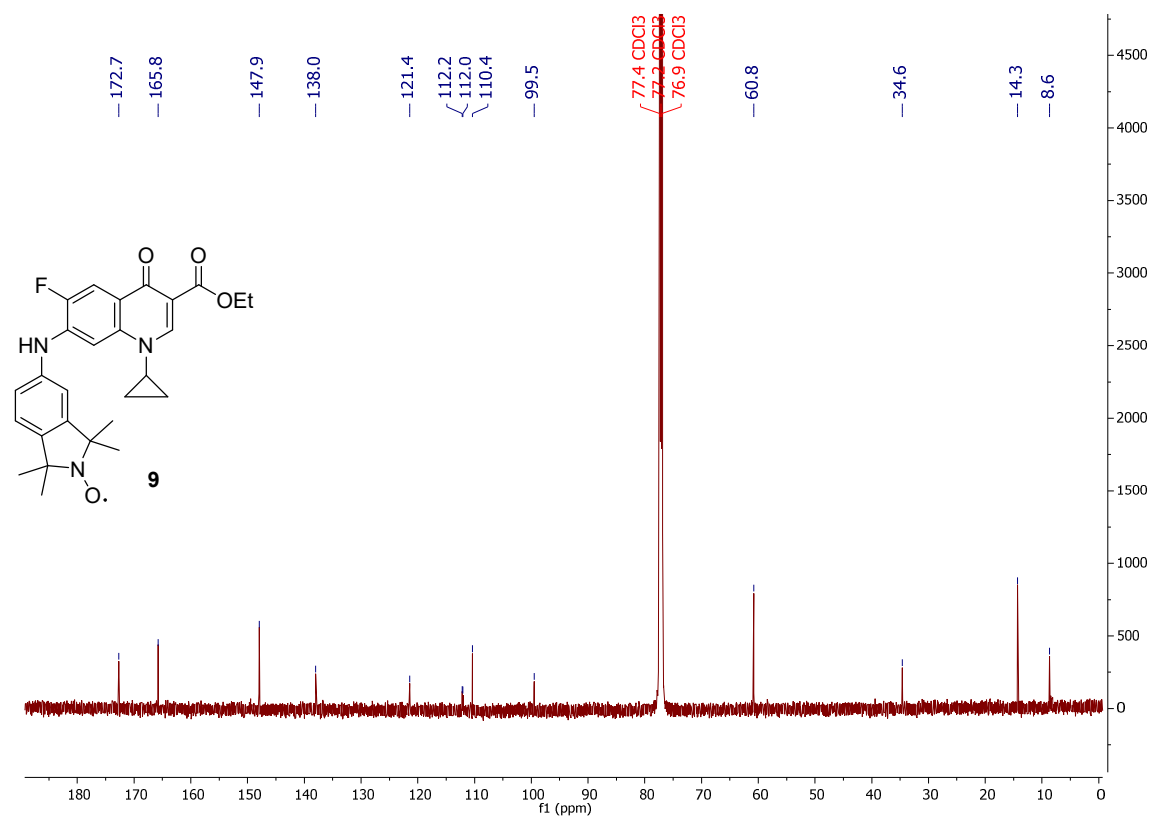
Figure S1. Fluorescent and brightfield overlay micrographs images of bacterial cells treated with FN 14 or FM 17. (A) FN 14 (150 μ M) and *P. aeruginosa*; (B) FN 14 (150 μ M) and *E. coli*; (C) FN 17 (150 μ M) and *P. aeruginosa*; (D) FN 17 (150 μ M) and *E. coli*. Scale bars are 5 μ M in length.

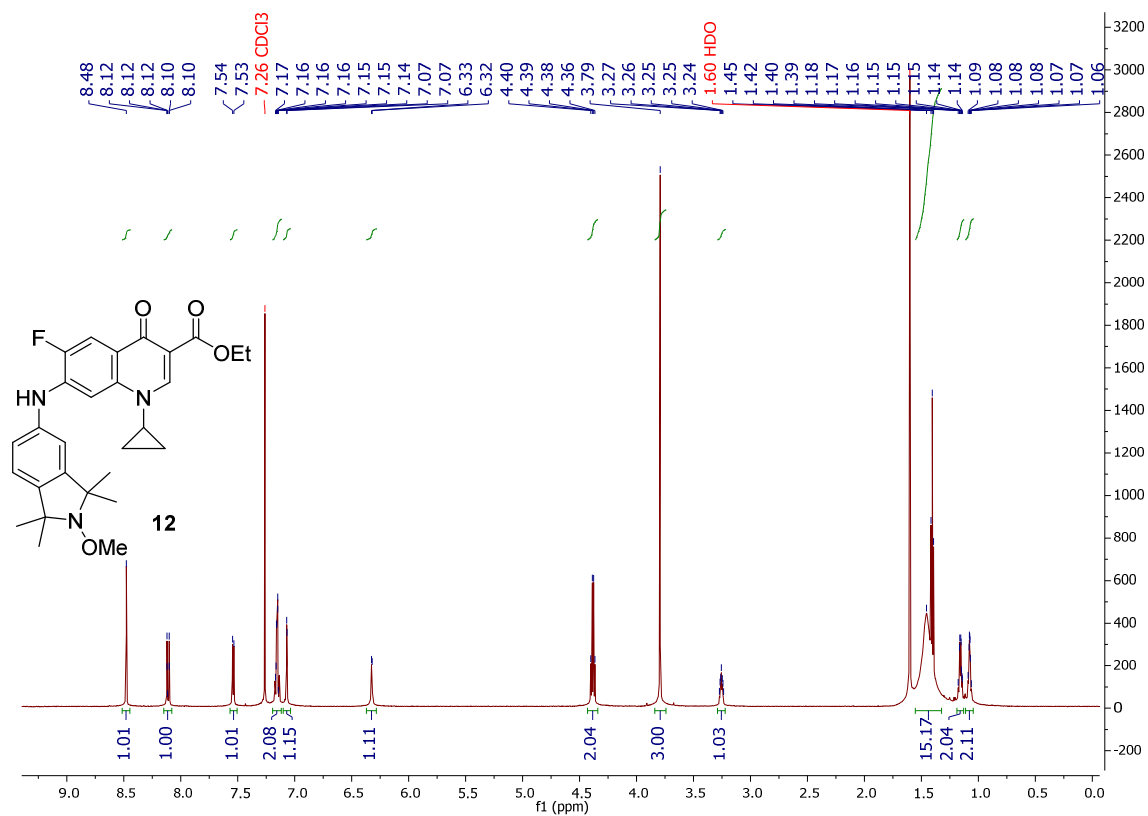
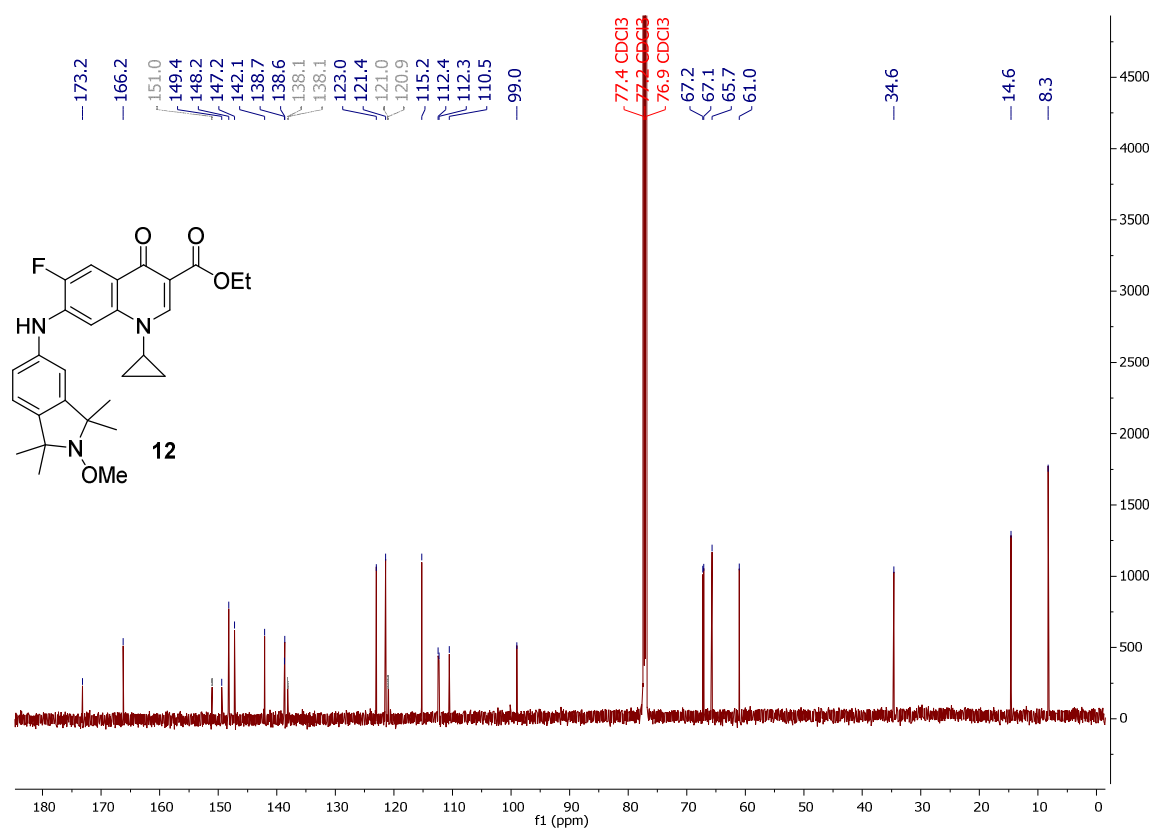
¹H NMR and ¹³C NMR SpectraFigure S2. ¹H NMR (CDCl₃, 600 MHz) spectrum of Nitro-TEIOMe.Figure S3. ¹³C NMR (CDCl₃, 150 MHz) spectrum of Nitro-TEIOMe.

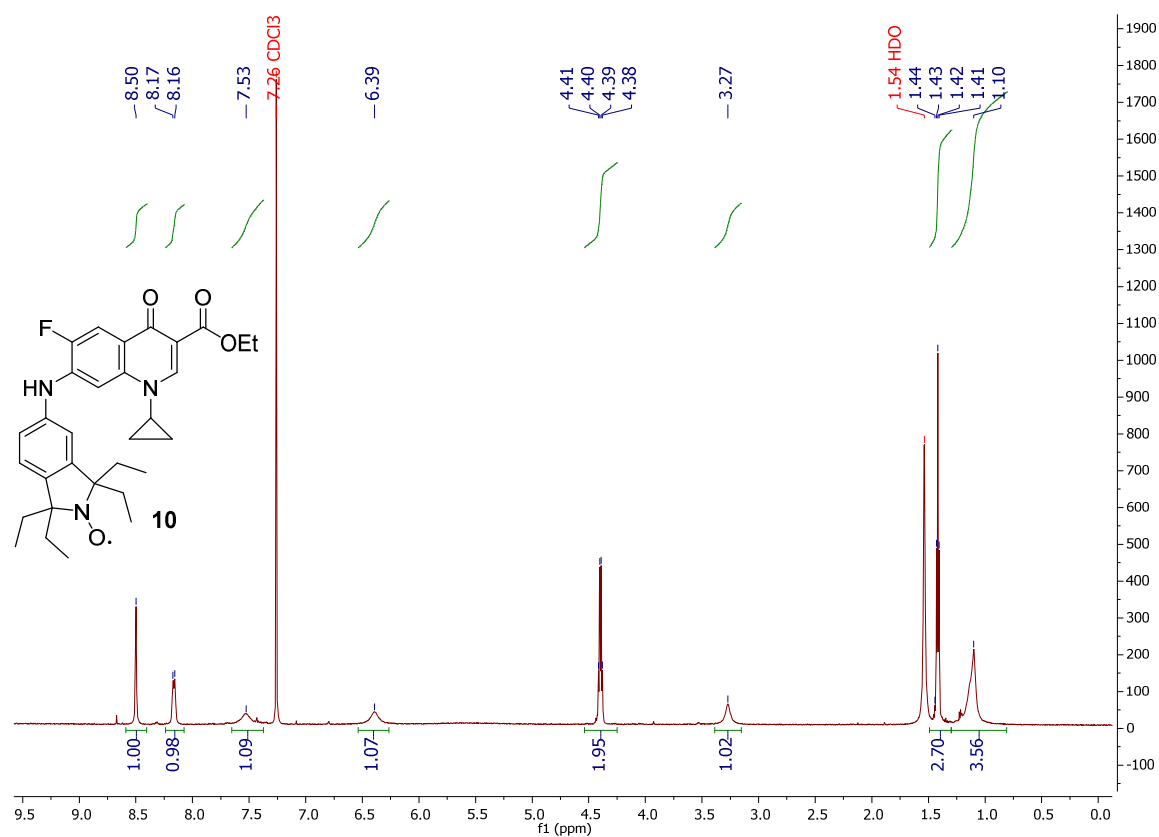
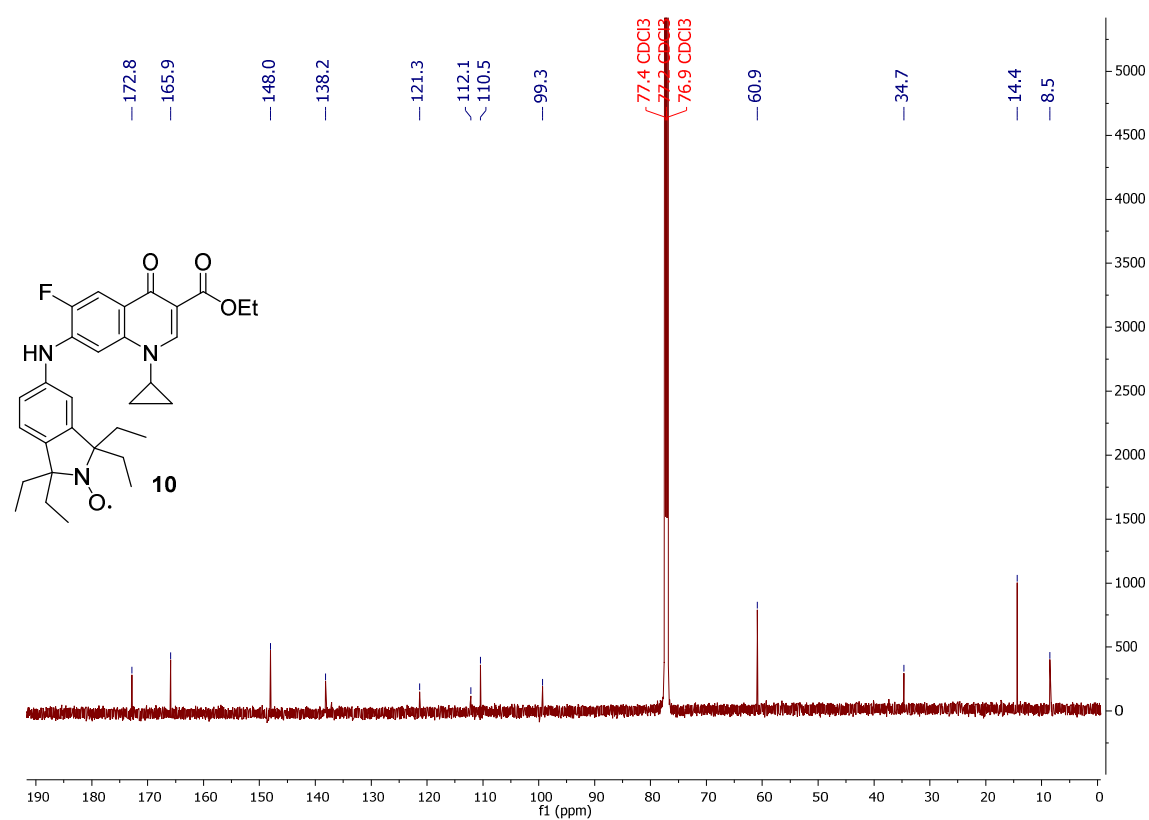
Figure S4. ¹H NMR (CDCl₃, 600 MHz) spectrum of **7**.Figure S5. ¹³C NMR (CDCl₃, 150 MHz) spectrum of **7**.

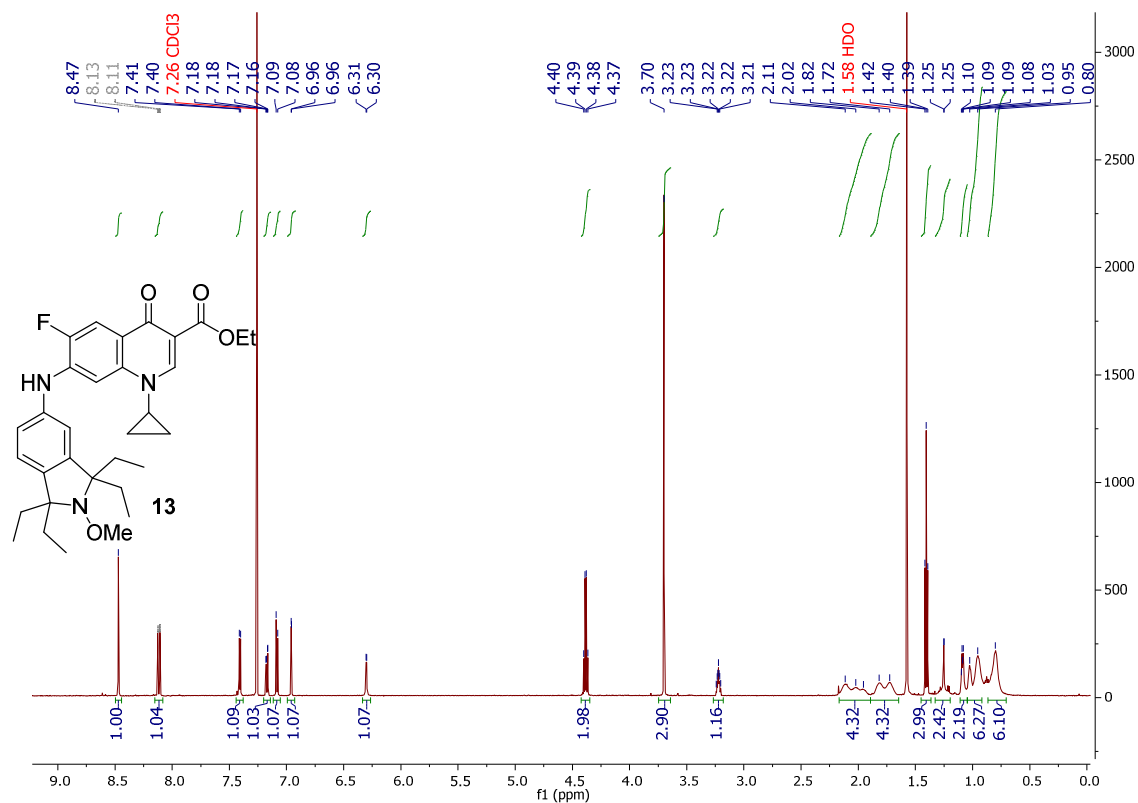
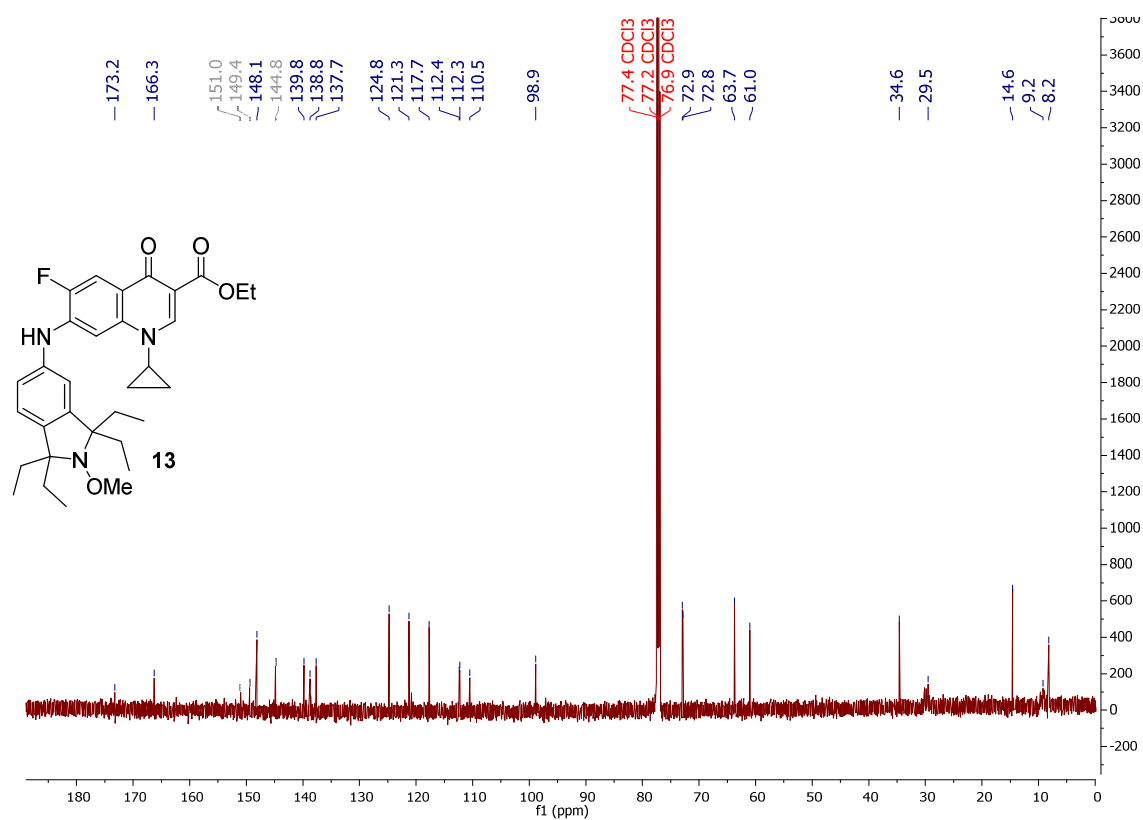
Figure 6. ^1H NMR (CDCl₃, 600 MHz) spectrum of **8**.Figure S7. ^{13}C NMR (CDCl₃, 150 MHz) spectrum of **8**.

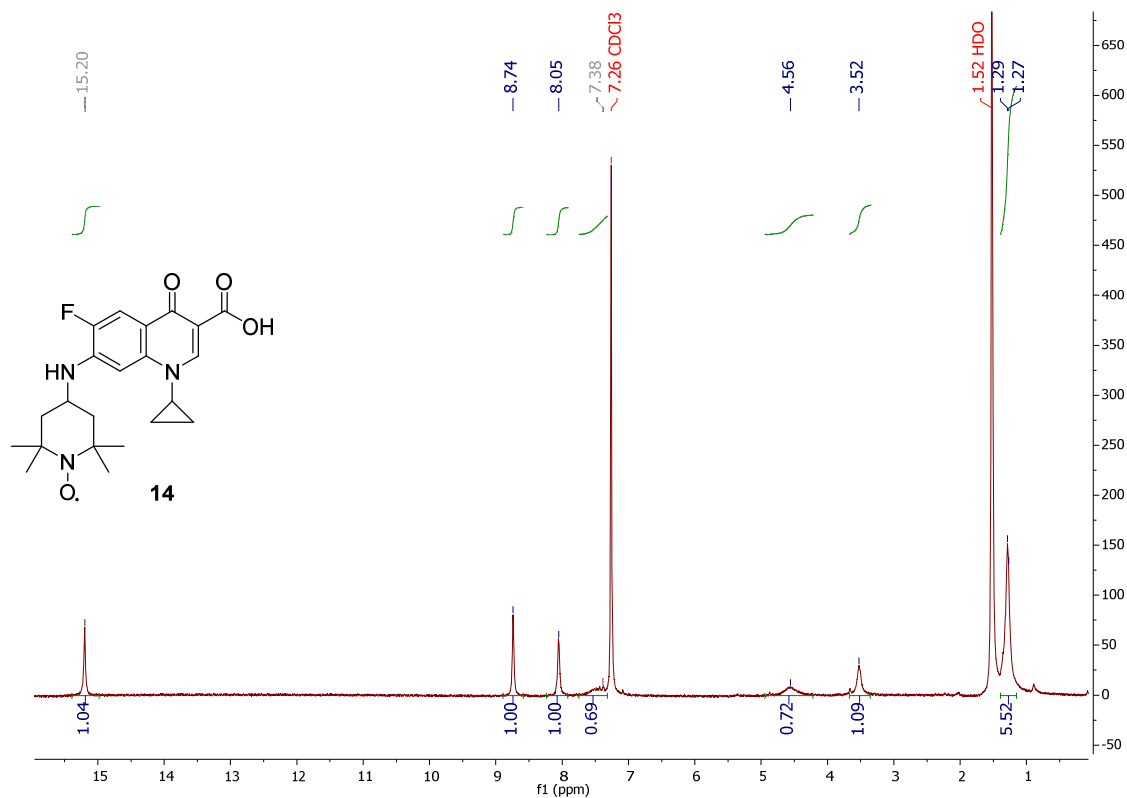
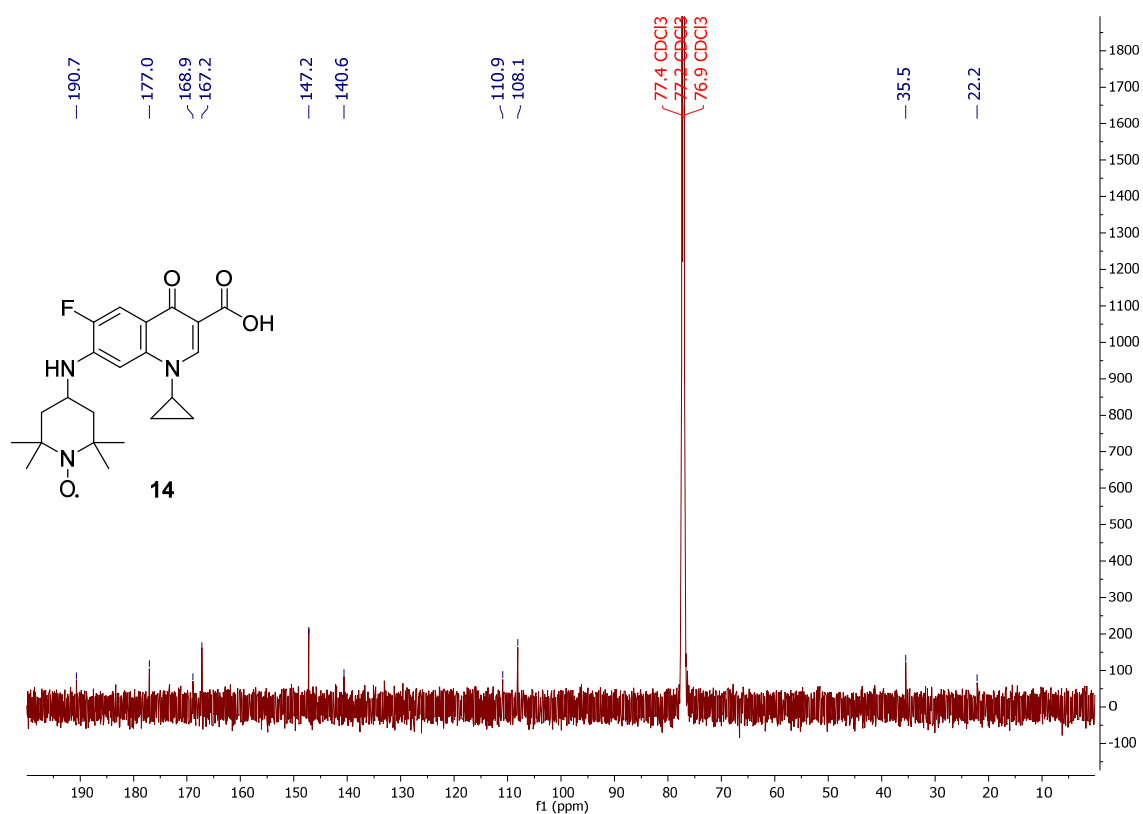
Figure 8. ¹H NMR (CDCl₃, 600 MHz) spectrum of **11**.Figure S9. ¹³C NMR (CDCl₃, 150 MHz) spectrum of **11**.

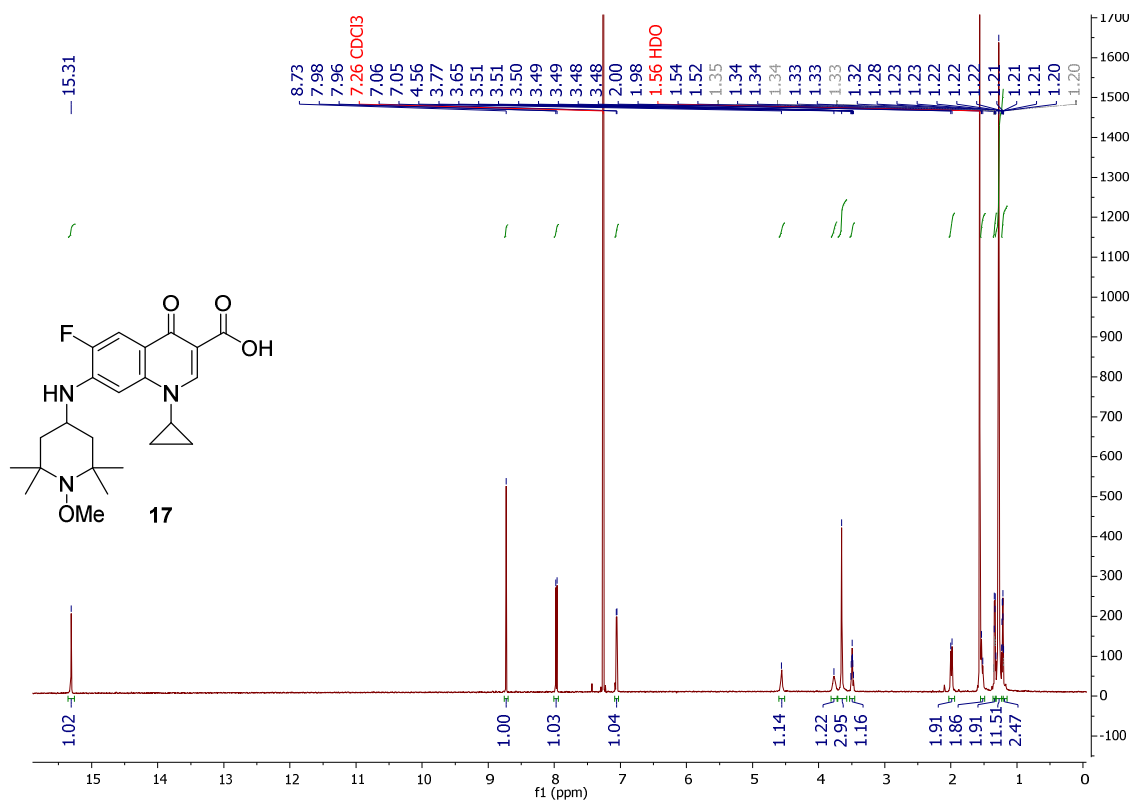
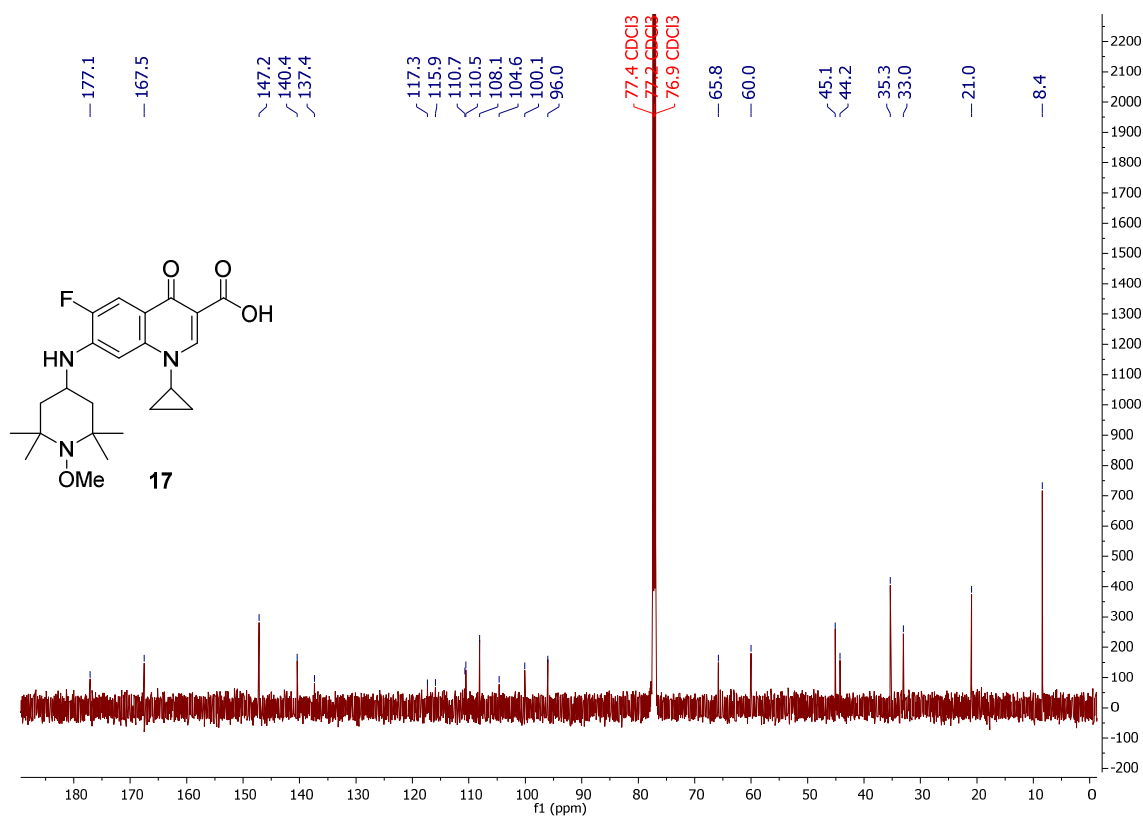
Figure S10. ^1H NMR (CDCl₃, 600 MHz) spectrum of **9**.Figure S11. ^{13}C NMR (CDCl₃, 150 MHz) spectrum of **9**.

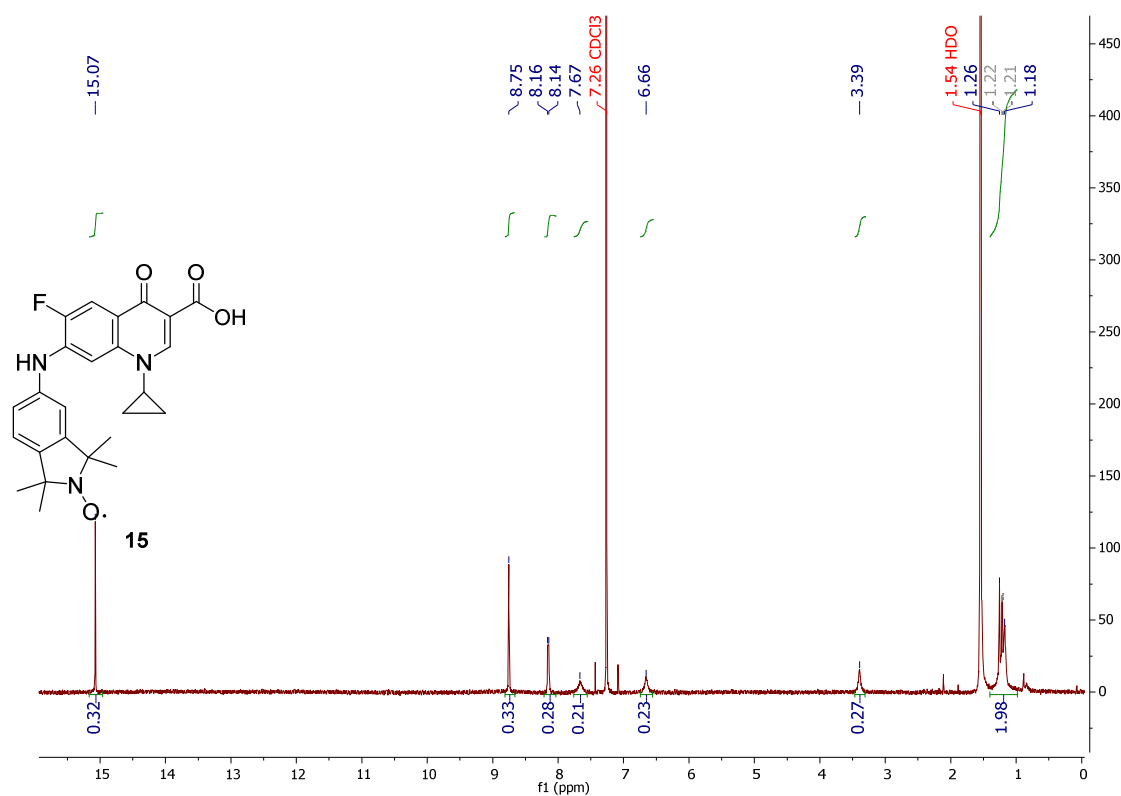
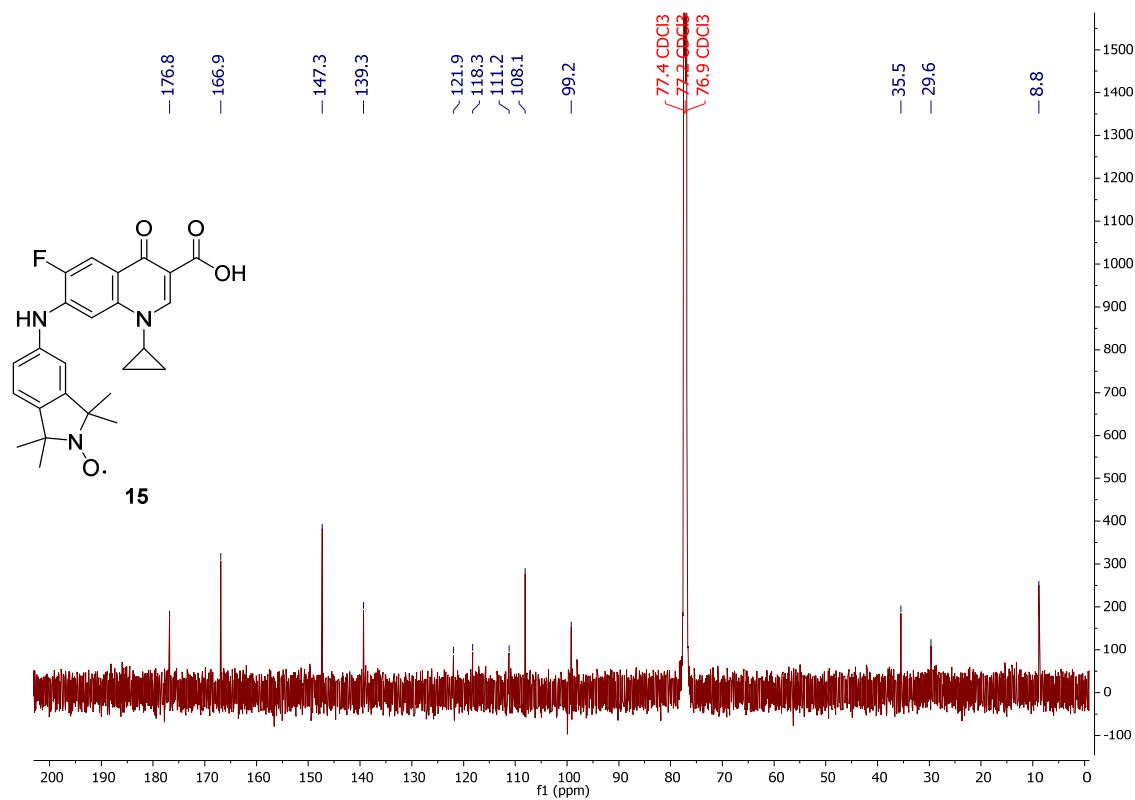
Figure S12. ¹H NMR (CDCl₃, 600 MHz) spectrum of 12.Figure S13. ¹³C NMR (CDCl₃, 150 MHz) spectrum of 12.

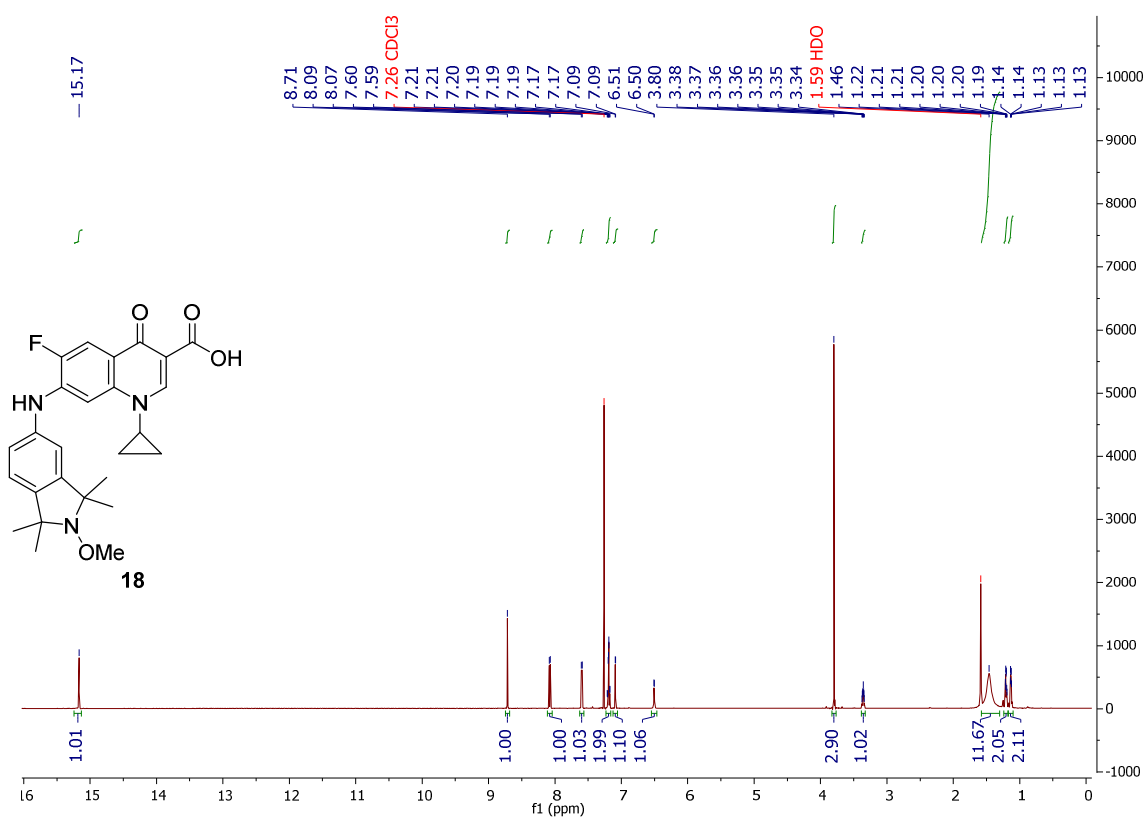
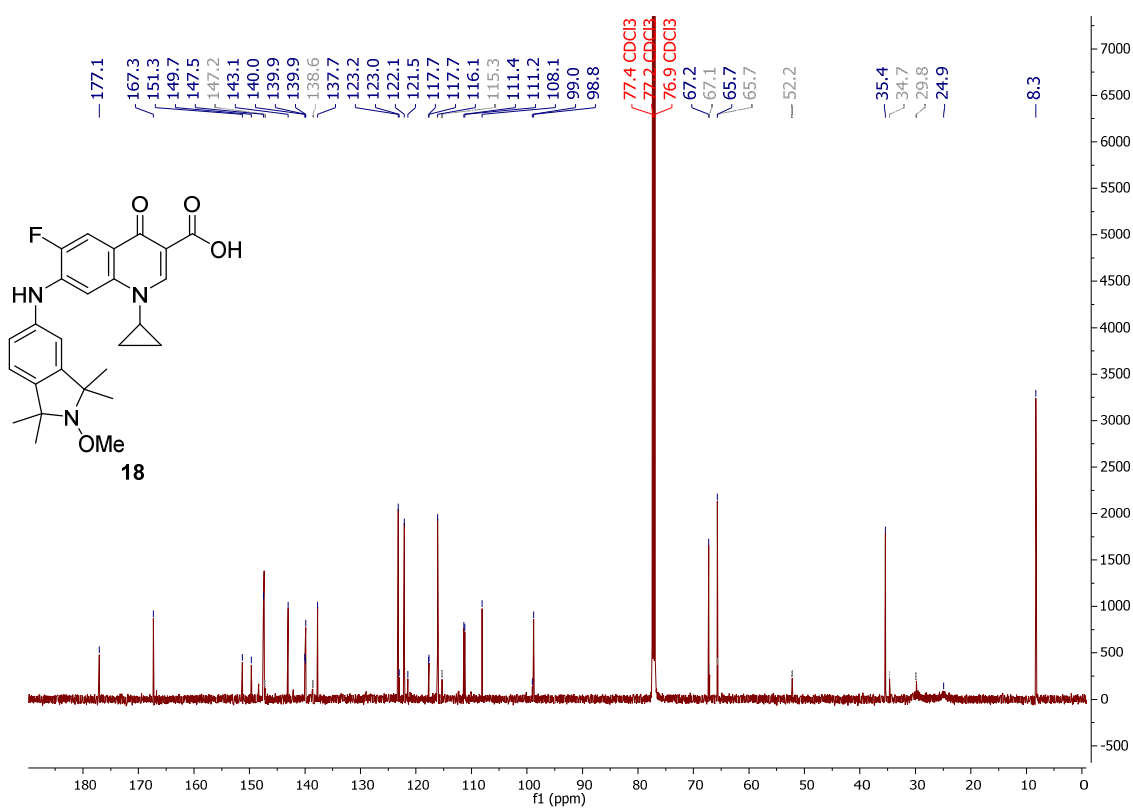
Figure S14. ¹H NMR (CDCl₃, 600 MHz) spectrum of 10.Figure S15. ¹³C NMR (CDCl₃, 150 MHz) spectrum of 10.

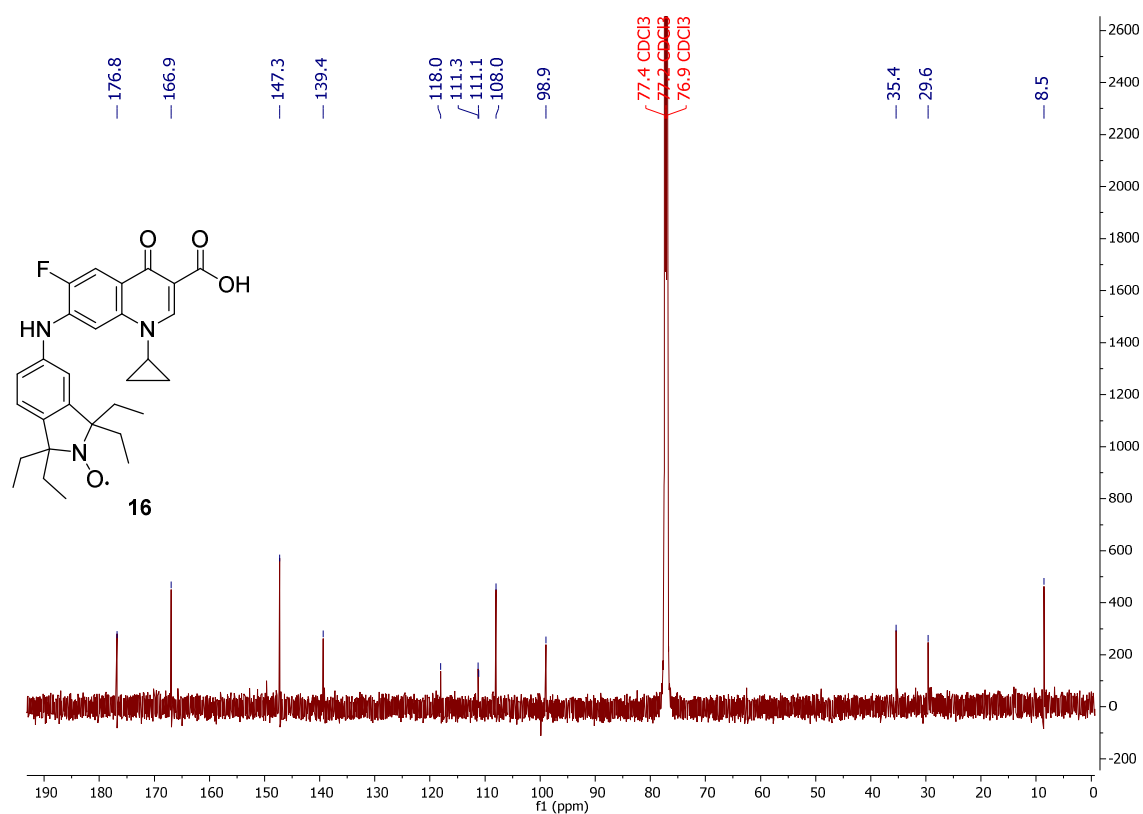
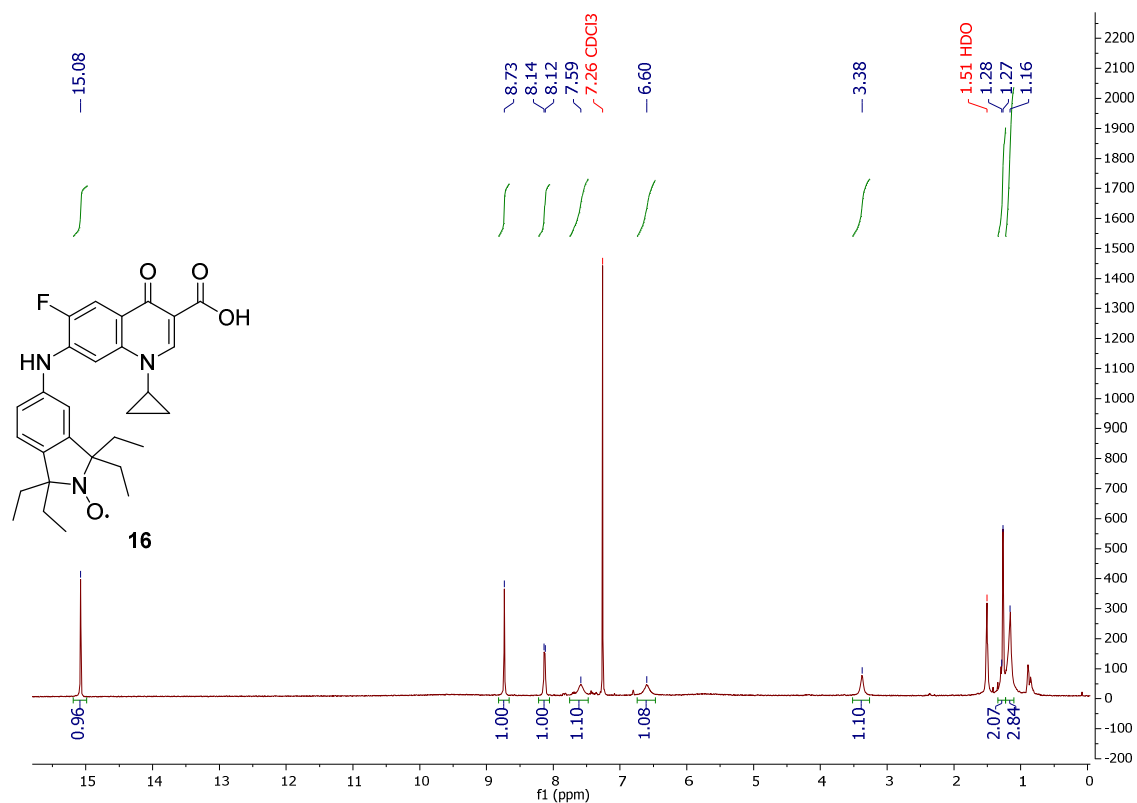
Figure S16. ¹H NMR (CDCl₃, 600 MHz) spectrum of 13.Figure S17. ¹³C NMR (CDCl₃, 150 MHz) spectrum of 13.

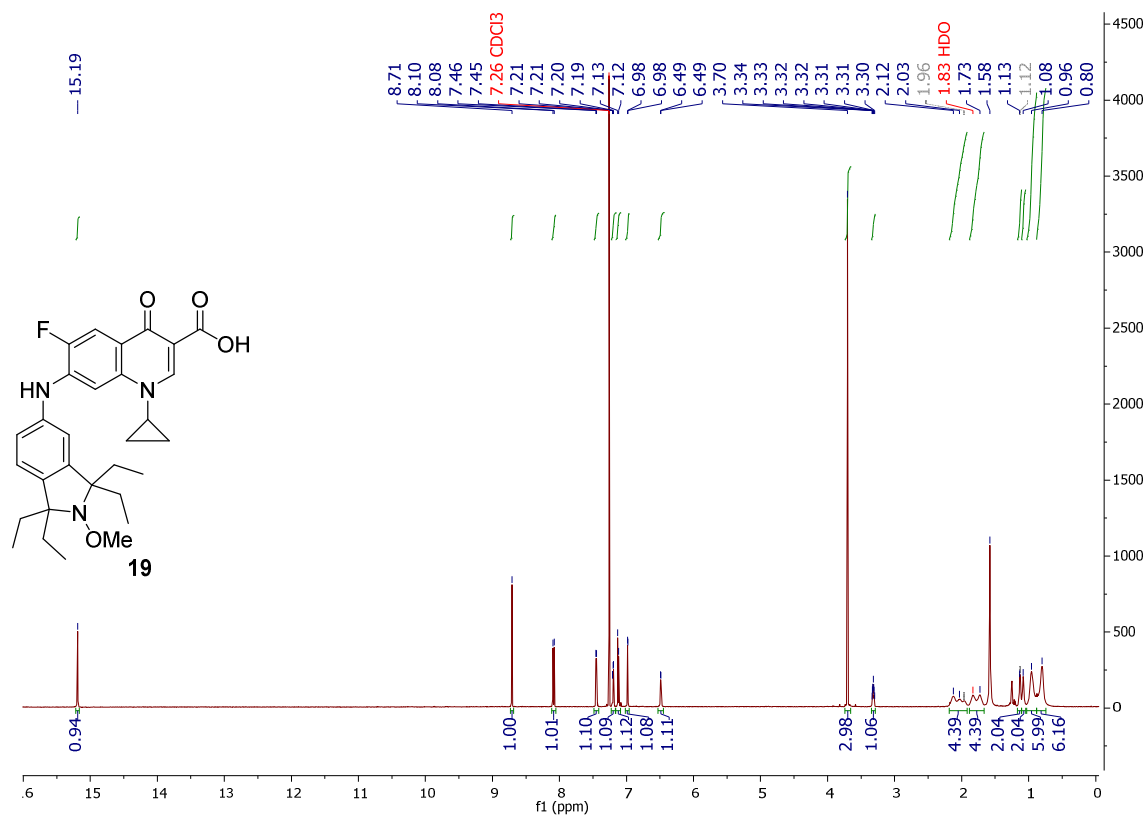
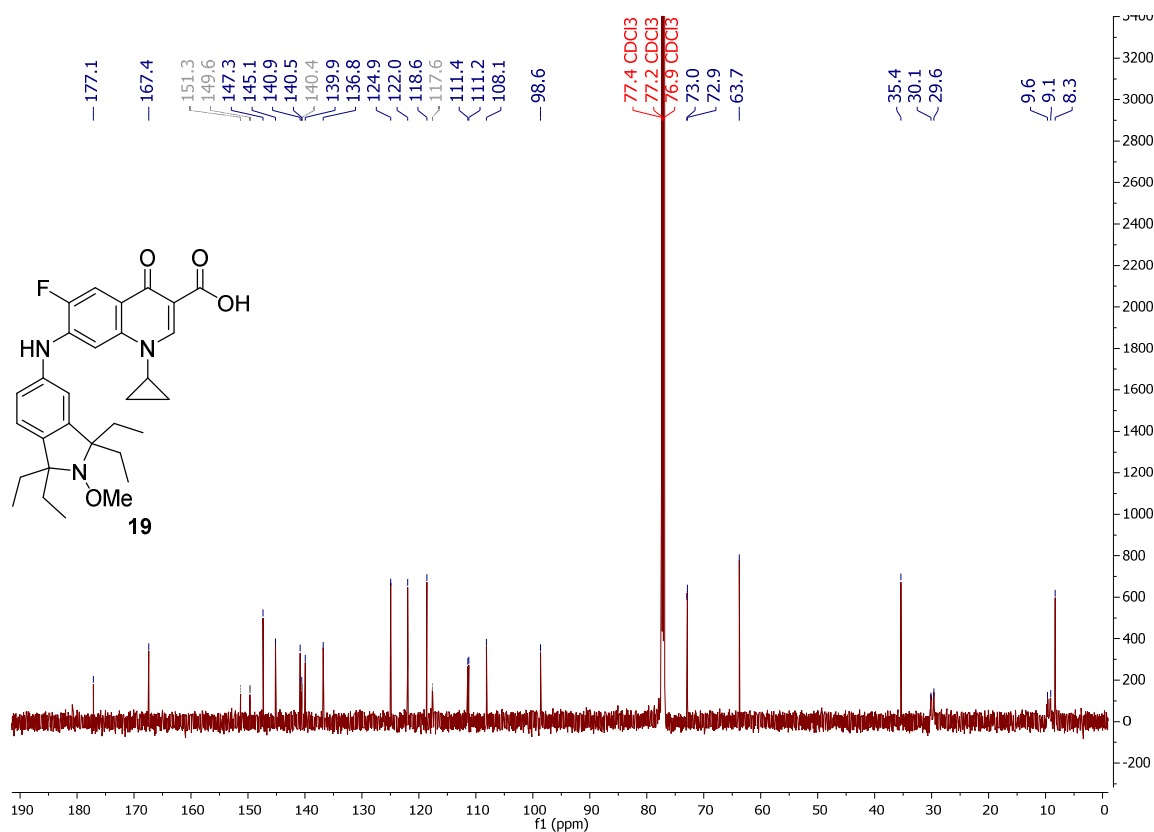
Figure S18. ¹H NMR (CDCl₃, 600 MHz) spectrum of 14.Figure S19. ¹³C NMR (CDCl₃, 150 MHz) spectrum of 14.

Figure S20. ¹H NMR (CDCl₃, 600 MHz) spectrum of 17.Figure S21. ¹³C NMR (CDCl₃, 150 MHz) spectrum of 17.

Figure S22. ¹H NMR (CDCl₃, 600 MHz) spectrum of 15.Figure S23. ¹³C NMR (CDCl₃, 150 MHz) spectrum of 15.

Figure S24. ¹H NMR (CDCl₃, 600 MHz) spectrum of 18.Figure S25. ¹³C NMR (CDCl₃, 150 MHz) spectrum of 18.



Figure S28. ¹H NMR (CDCl₃, 600 MHz) spectrum of 19.Figure S29. ¹³C NMR (CDCl₃, 150 MHz) spectrum of 19.

LCMS Chromatograms and HRMS Spectra

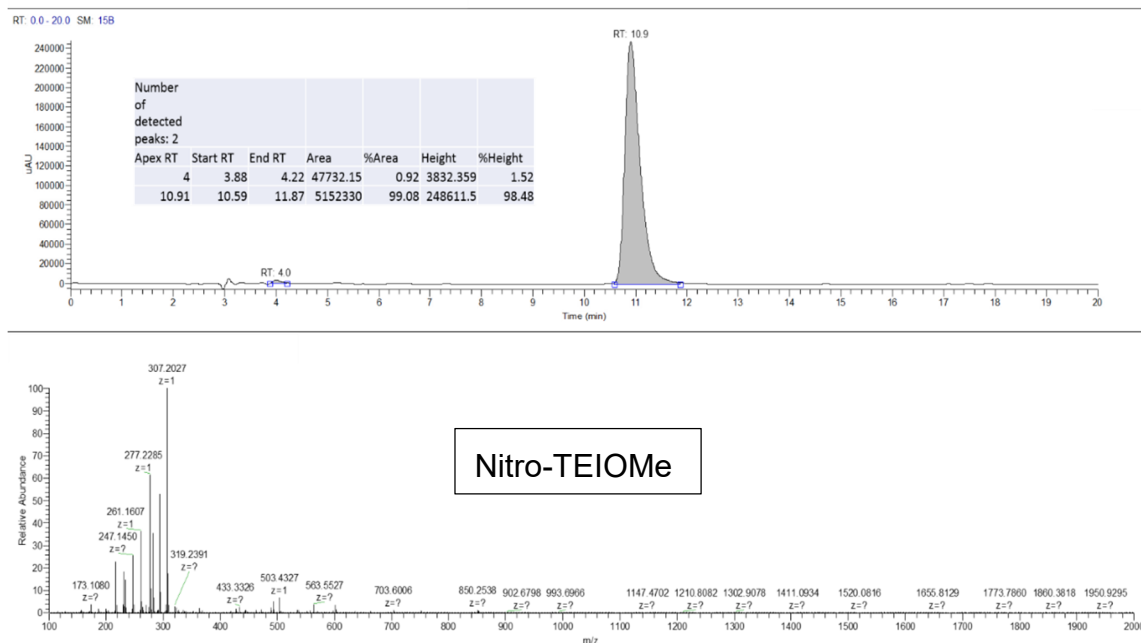


Figure S30. LCMS chromatogram and HRMS spectrum of Nitro-TEIOMe.

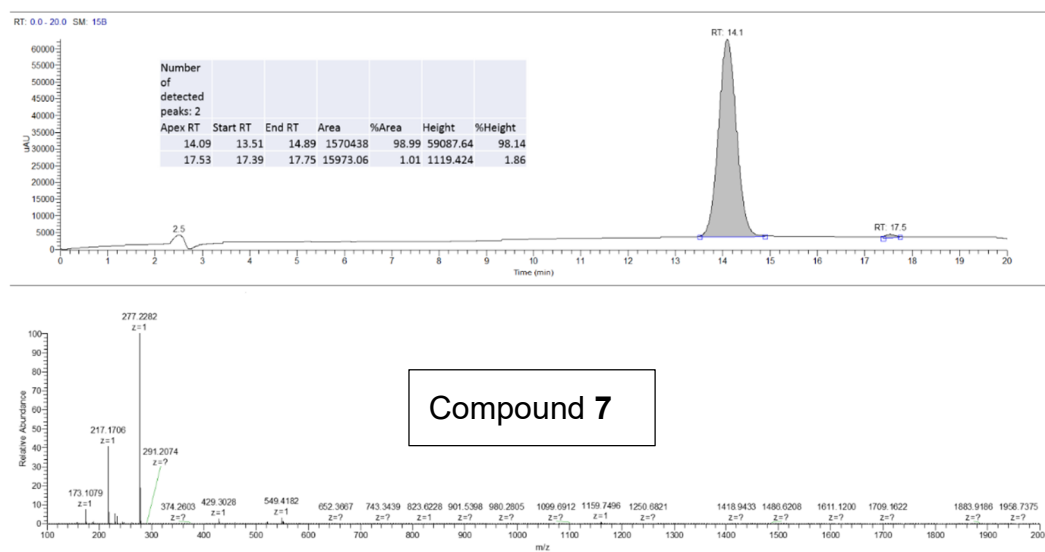


Figure S31. LCMS chromatogram and HRMS spectrum of 7.

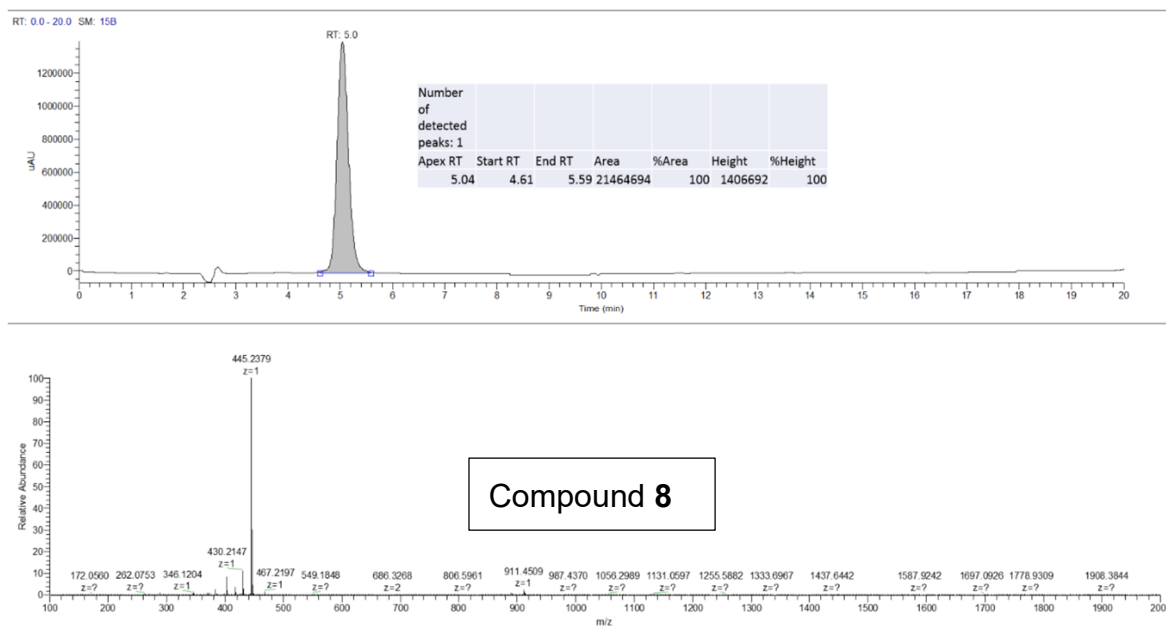


Figure S32. LCMS chromatogram and HRMS spectrum of 8.

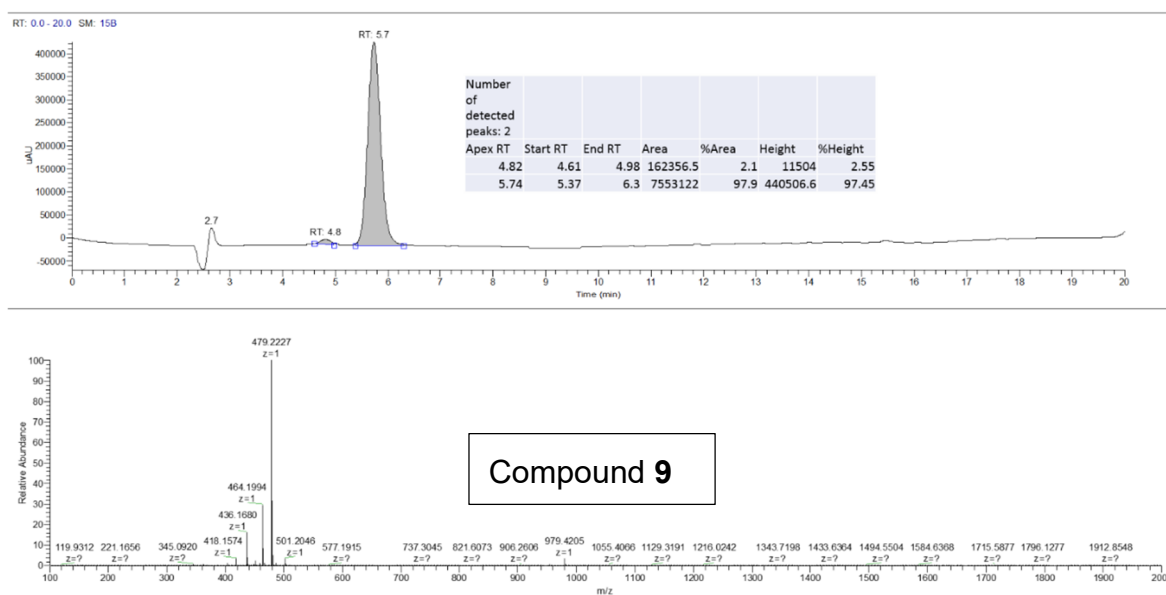


Figure S33. LCMS chromatogram and HRMS spectrum of 9.

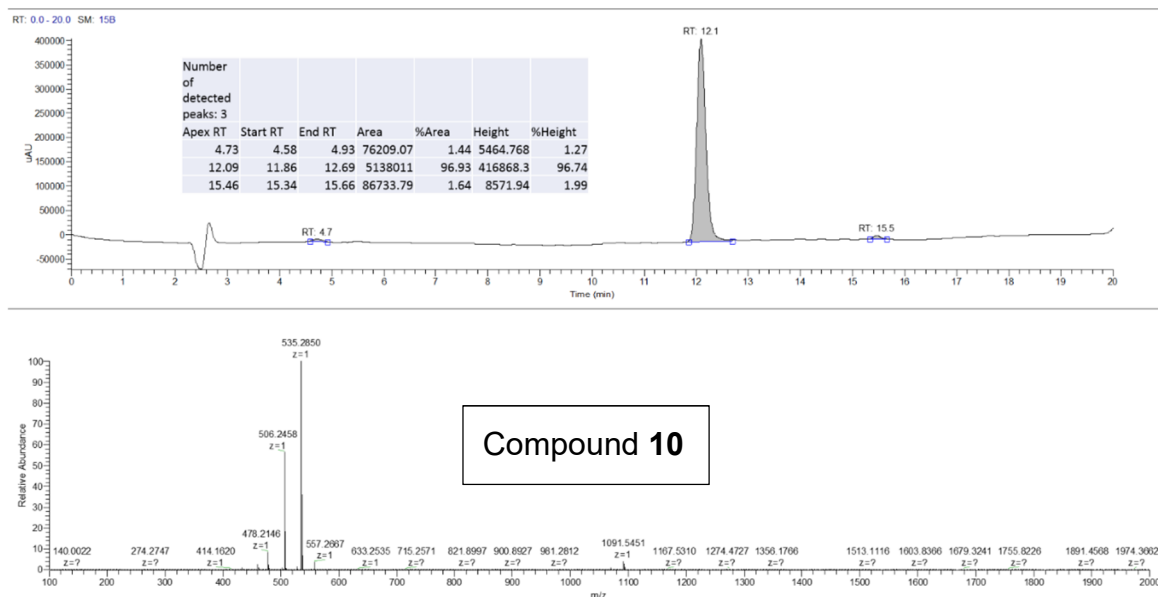


Figure S34. LCMS chromatogram and HRMS spectrum of 10.

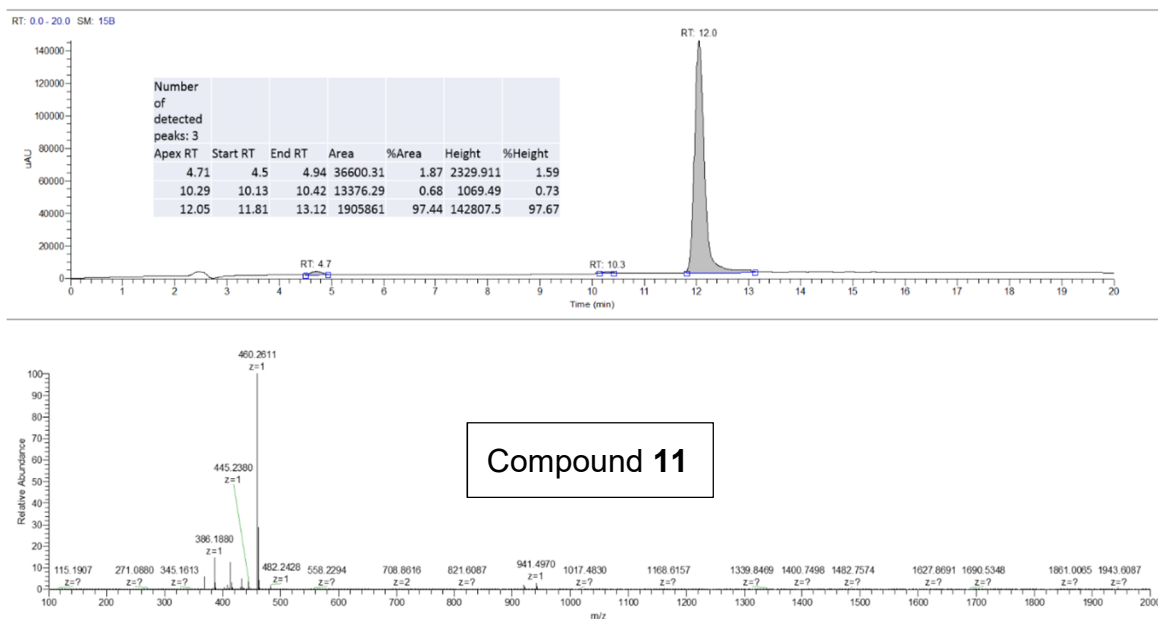


Figure S35. LCMS chromatogram and HRMS spectrum of 11.

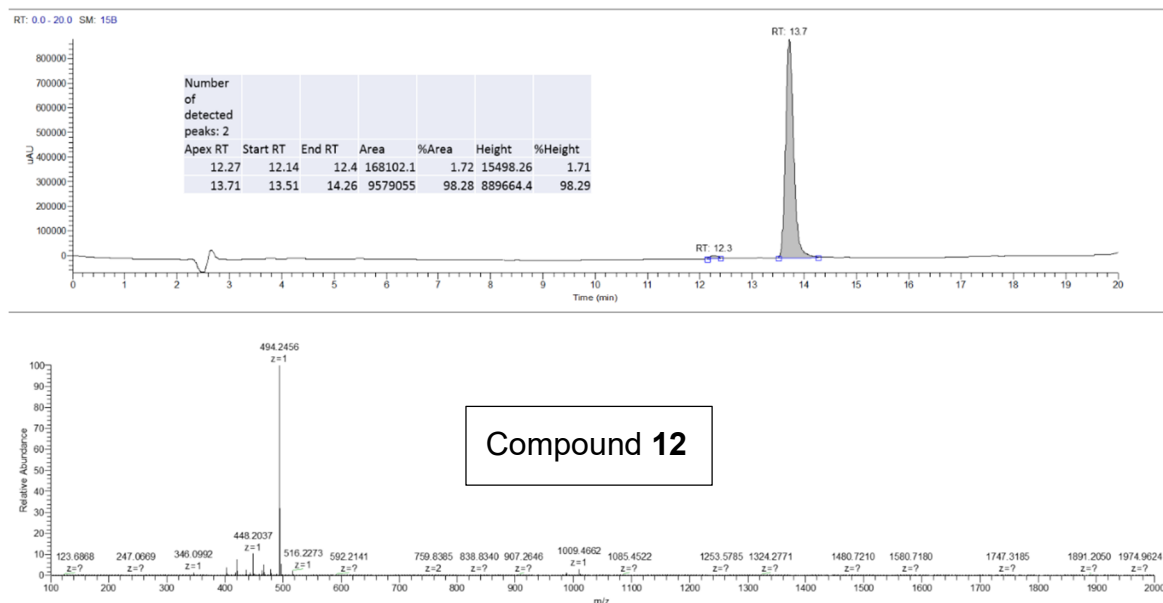


Figure S36. LCMS chromatogram and HRMS spectrum of 12.

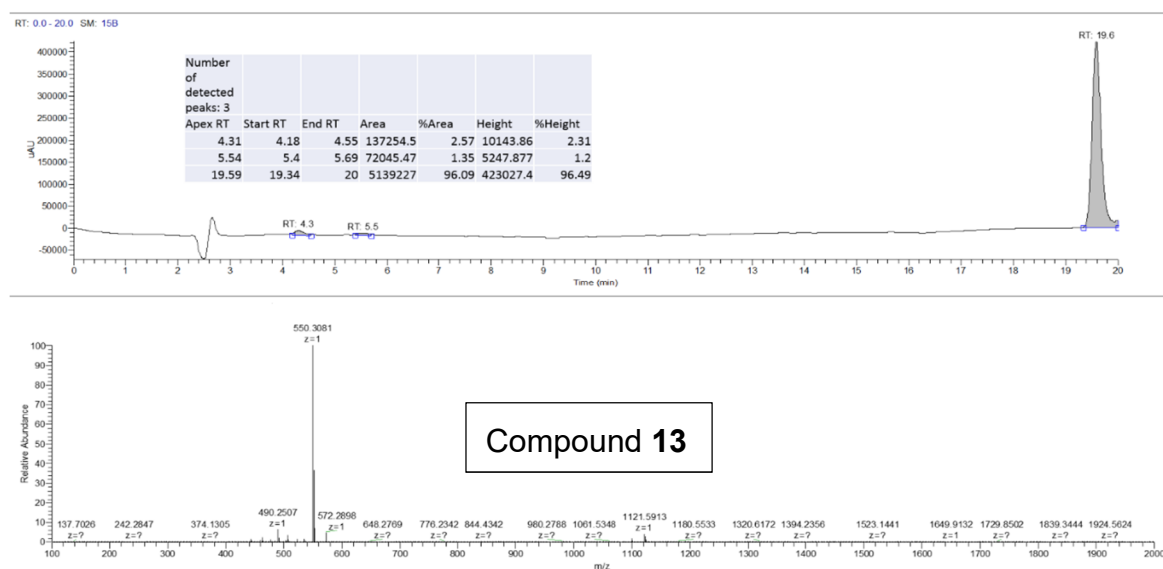


Figure 37. LCMS chromatogram and HRMS spectrum of 13.

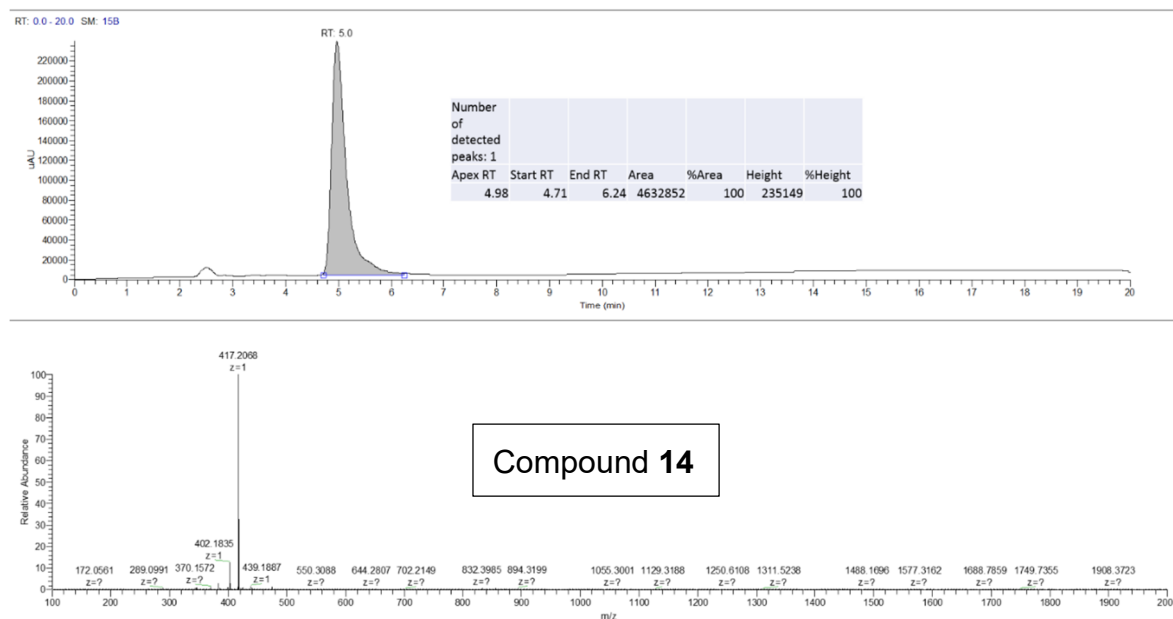


Figure S38. LCMS chromatogram and HRMS spectrum of 14.

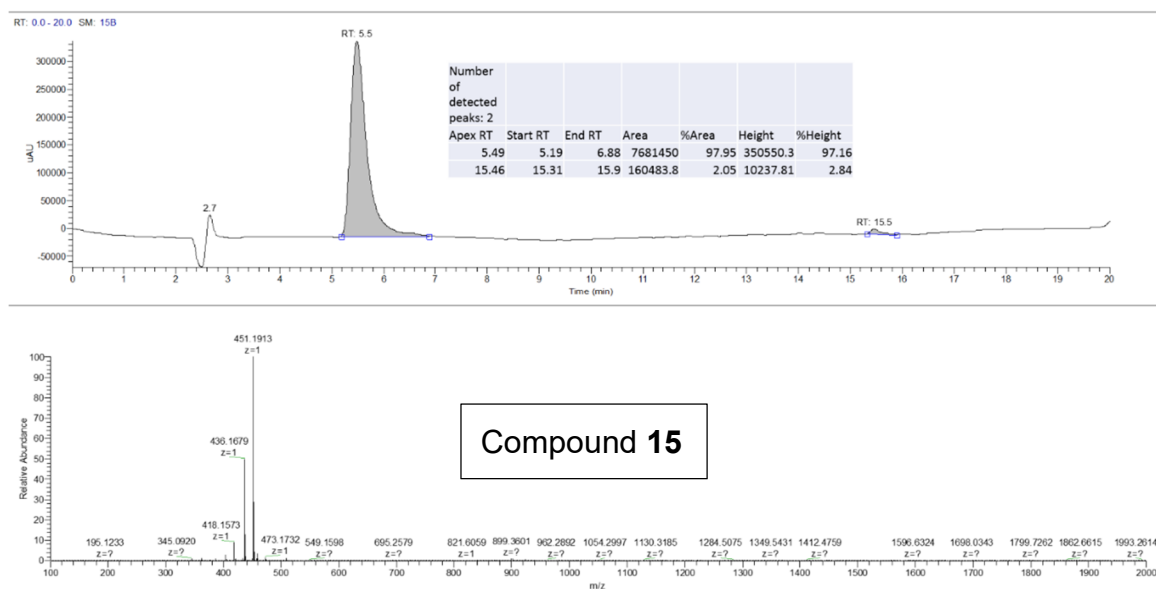


Figure S39. LCMS chromatogram and HRMS spectrum of 15.

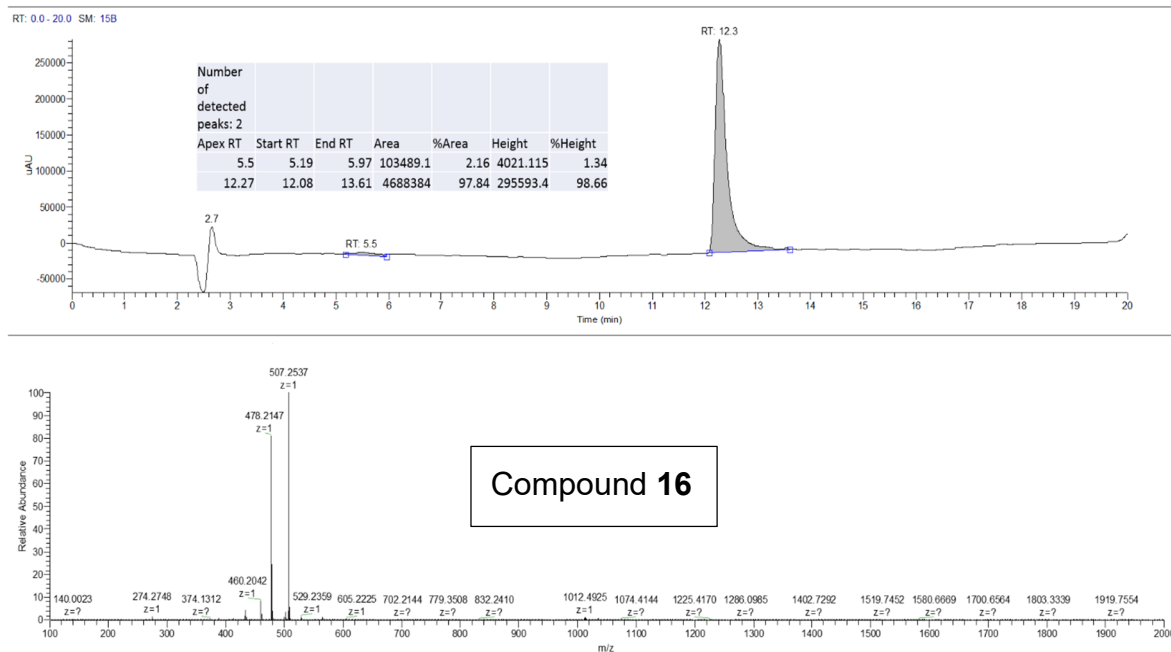


Figure S40. LCMS chromatogram and HRMS spectrum of 16.

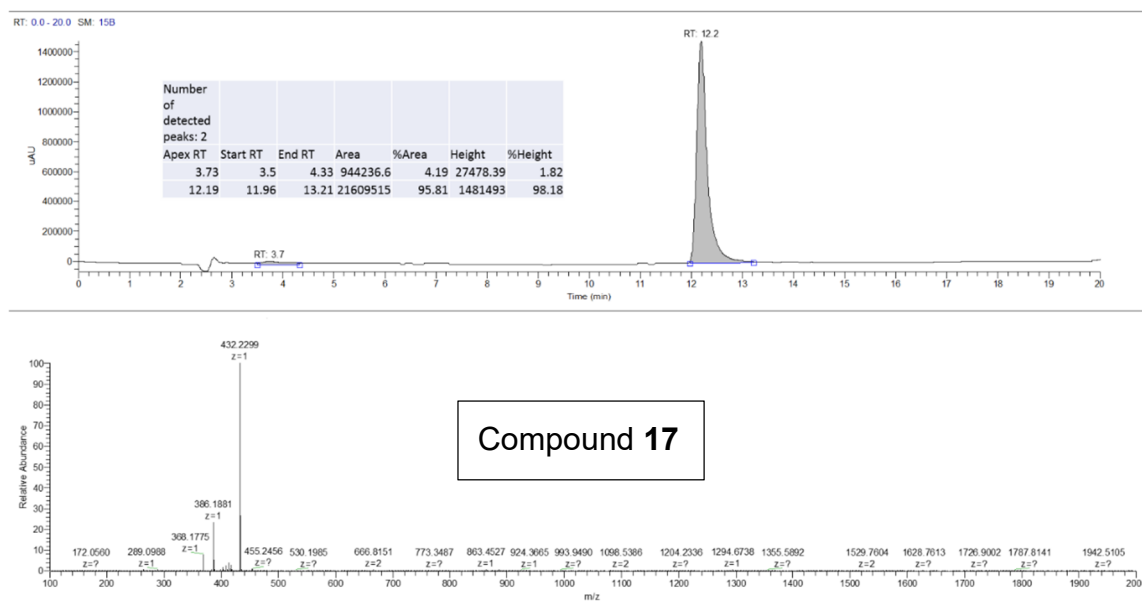


Figure S41. LCMS chromatogram and HRMS spectrum of 17.

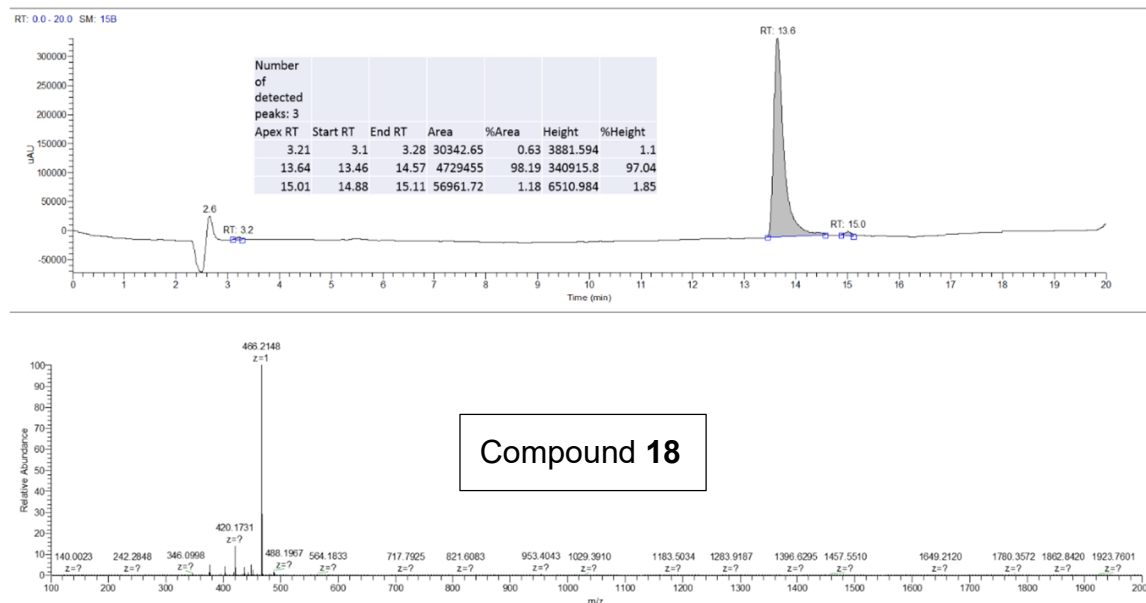


Figure S42. LCMS chromatogram and HRMS spectrum of 18.

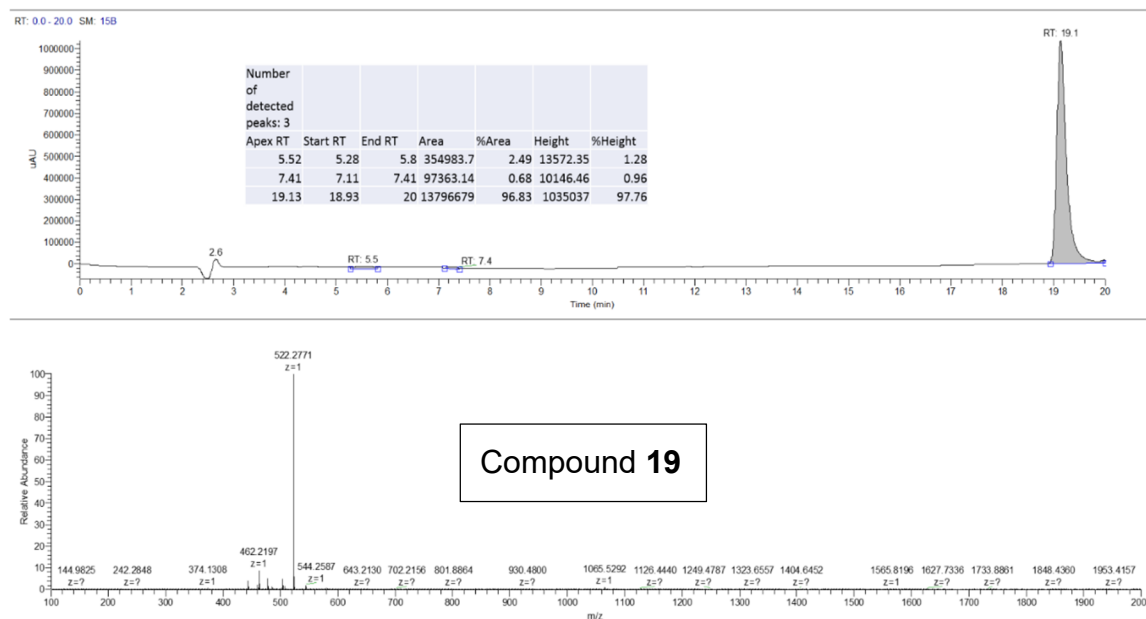


Figure S43. LCMS chromatogram and HRMS spectrum of 19.

