SUPPORTING INFORMATION

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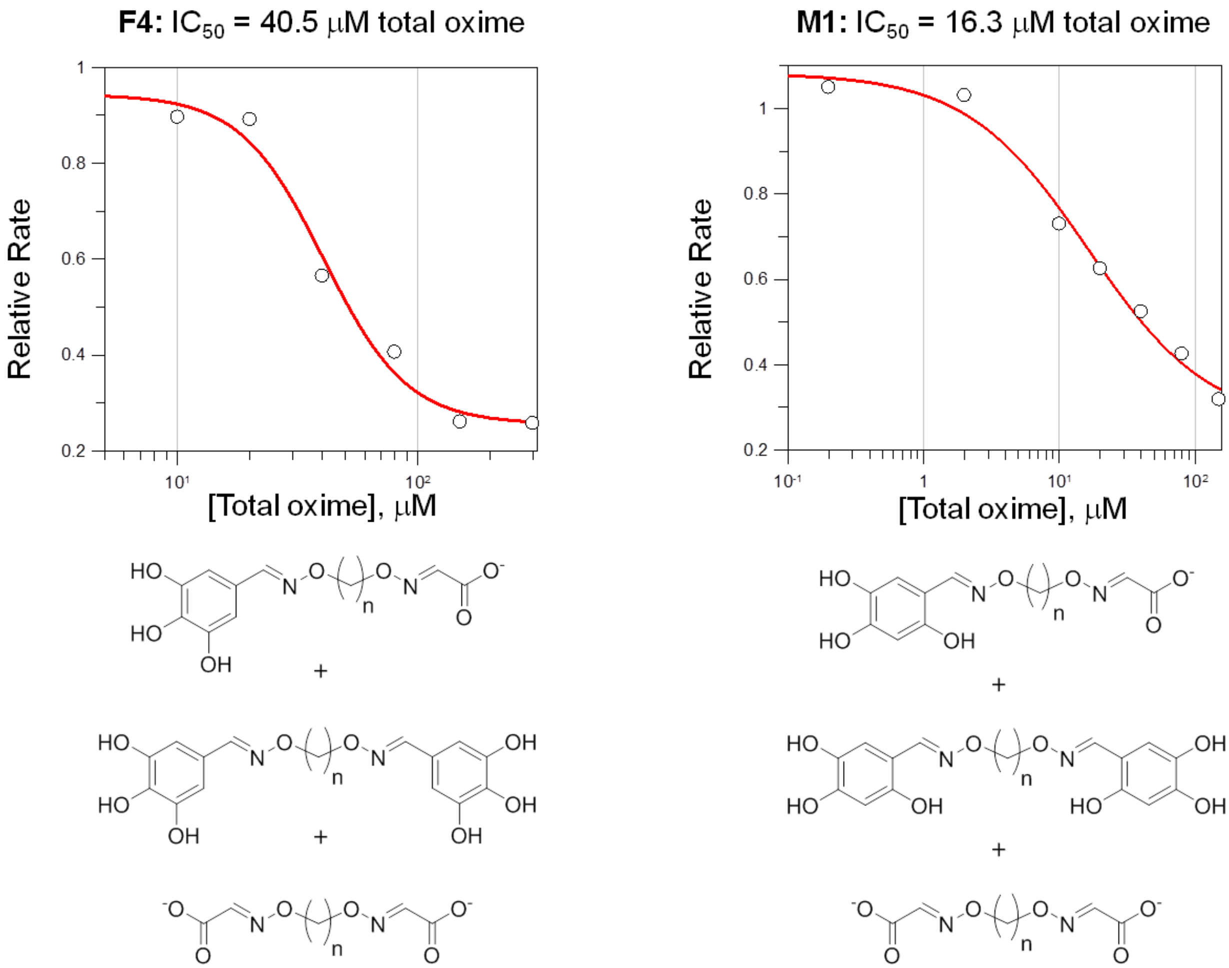


Figure S1. Dose-dependent inhibition of DXP synthase by oxime mixtures containing 2,4,5- and 3,4,5-trihydroxy scaffolds. The activity of each mixture was determined using the IspC-coupled assay described elsewhere.[1] Concentrations refer to the total concentration of dioxime products irrespective of oxime derivatization or length of the dialkyoxyamine portion. Alkoxyamine linkers with 2–5 methylenes were used.

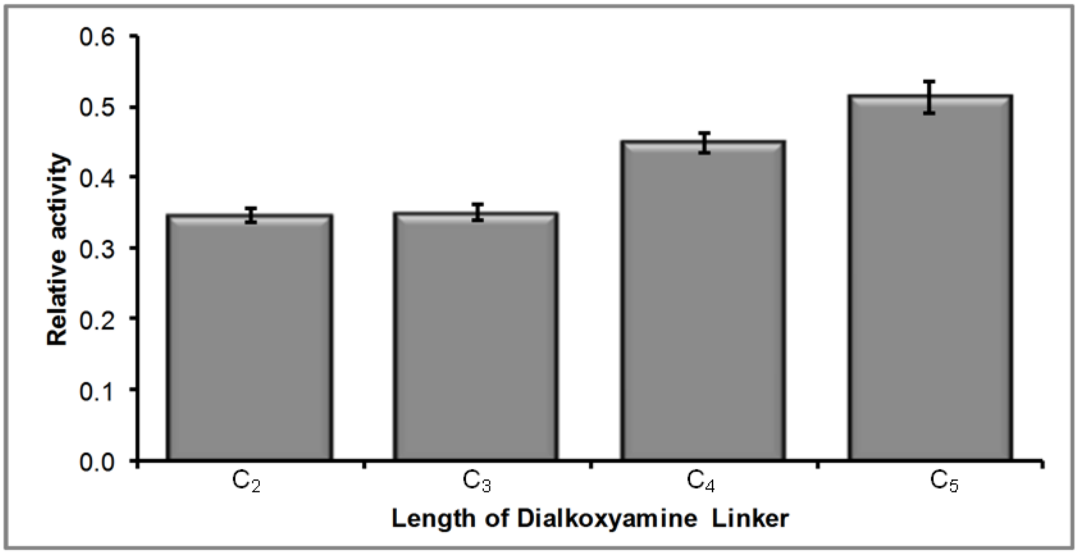


Figure S2. Determination of optimal linker length for 2,4,5-trihydroxybenzaloximes. Symmetrical oximes with varied dialkoxyamine linkers were synthesized by incubation the desired dialkoxyamine with 2 molar equivalents of 2,4,5-trihydroxybenzaldehyde overnight in DMSO. The resulting symmetrical oximes were tested directly for inhibition without purification.

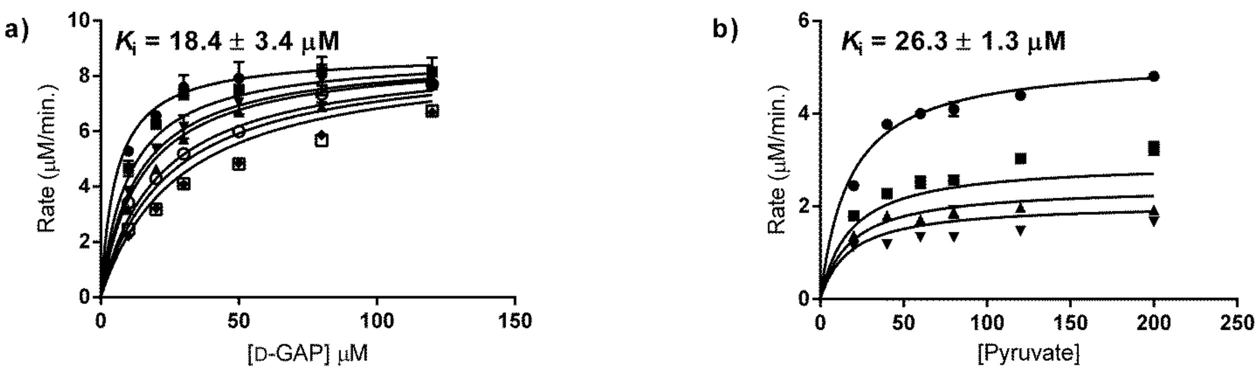


Figure S3. Inhibition of DXP synthase by mixed oxime **4**. a) Oxime **4** displays competitive inhibition against D-GAP as determined by model discrimination analysis (GraphPad Prism). Inhibitor concentrations: ● 0 μM, ■ 15 μM, ▲ 25 μM, ▼ 30 μM, ◆ 50 μM, ○ 60 μM, □ 75 μM. b) Oxime **4** displays noncompetitive inhibition against pyruvate as determined by model discrimination analysis (GraphPad Prism). Inhibitor concentrations: ● 0 μM, ■ 20 μM, ▲ 30 μM, ▼ 40 μM.

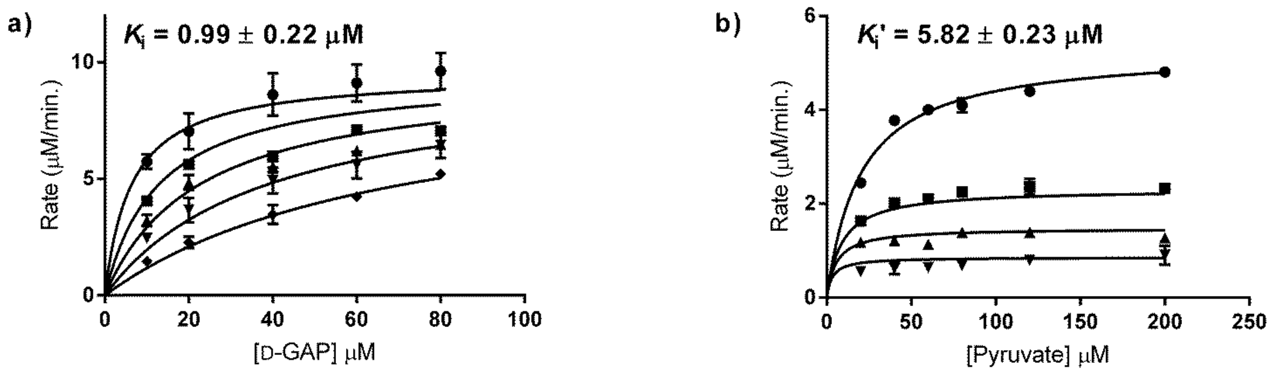


Figure S4. Inhibition of DXP synthase by symmetrical oxime **5**. a) Oxime **5** displays competitive inhibition against D-GAP as determined by model discrimination analysis (GraphPad Prism) Inhibitor concentrations: ● 0 µM, ■ 1.0 µM, ▲ 2.5 µM, ▼ 5.0 µM, ◆ 10 µM. b) Oxime **5** displays uncompetitive inhibition against pyruvate as determined by model discrimination analysis (GraphPad Prism) Inhibitor concentrations: ● 0 µM, ■ 7.5 µM, ▲ 15 µM, ▼ 30 µM

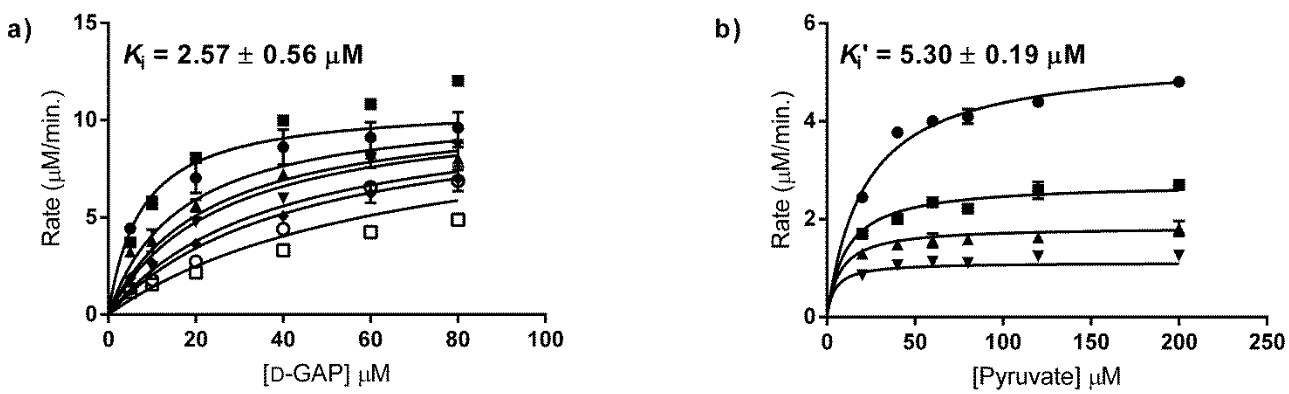


Figure S5. Inhibition of DXP synthase by 2,4,5-trihydroxybenzaldoximes **7**. a) Oxime **7** displays competitive inhibition against D-GAP as determined by model discrimination analysis (GraphPad Prism). Inhibitor concentrations: ● 0 µM, ■ 3 µM, ▲ 5 µM, ▼ 6 µM, ◆ 10 µM, ○ 12 µM, □ 20 µM. b) Oxime **7** displays uncompetitive inhibition against pyruvate as determined by model discrimination analysis (GraphPad Prism). Inhibitor concentrations: ● 0 µM, ■ 5 µM, ▲ 10 µM, ▼ 20 µM.

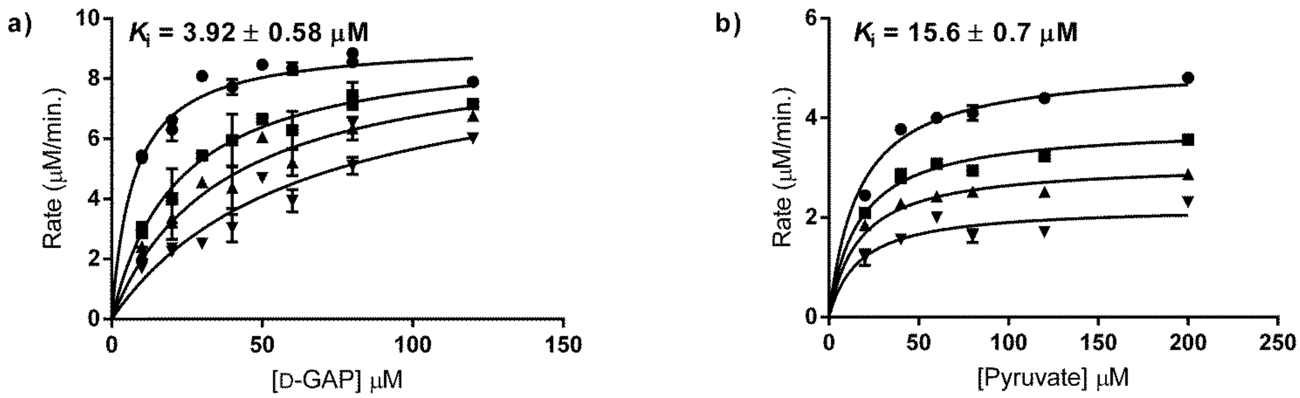


Figure S6. Inhibition of DXP synthase by 2,4,5-trihydroxybenzaldoxime **8.** a) Oxime **8** displays competitive inhibition against D-GAP as determined by model discrimination analysis (GraphPad Prism). Inhibitorconcentrations: • 0 μM, ▪ 8 μM, ▲ 16 μM, ▼ 30 μM. b) Oxime **8** displays noncompetitive inhibition against pyruvate as determined by model discrimination analysis (GraphPad Prism). Inhibitor concentrations: • 0 μM, ▪ 5 μM, ▲ 10 μM, ▼ 20 μM.

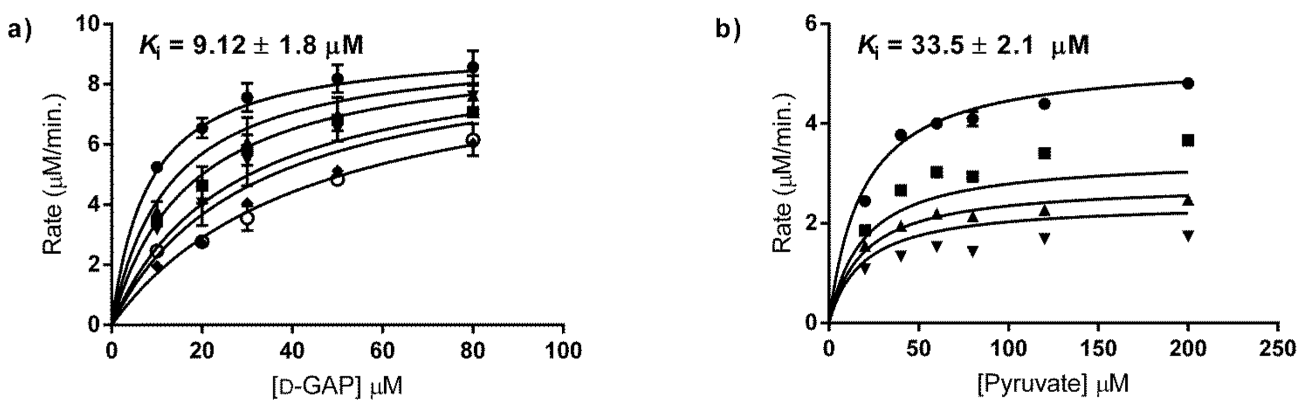


Figure S7. Inhibition of DXP synthase by 2,4,5-trihydroxybenzaldoxime **9.** a) Oxime **9** displays competitive inhibition against D-GAP as determined by model discrimination analysis (GraphPad Prism). Inhibitor concentrations: • 0 μM, ▪ 5 μM, ▲ 10 μM, ▼ 20 μM, ◆ 25 μM, ○ 40 μM. b) Oxime **9** displays noncompetitive inhibition against pyruvate as determined by model discrimination analysis (GraphPad Prism). Inhibitor concentrations: • 0 μM, ▪ 20 μM, ▲ 30 μM, ▼ 40 μM.

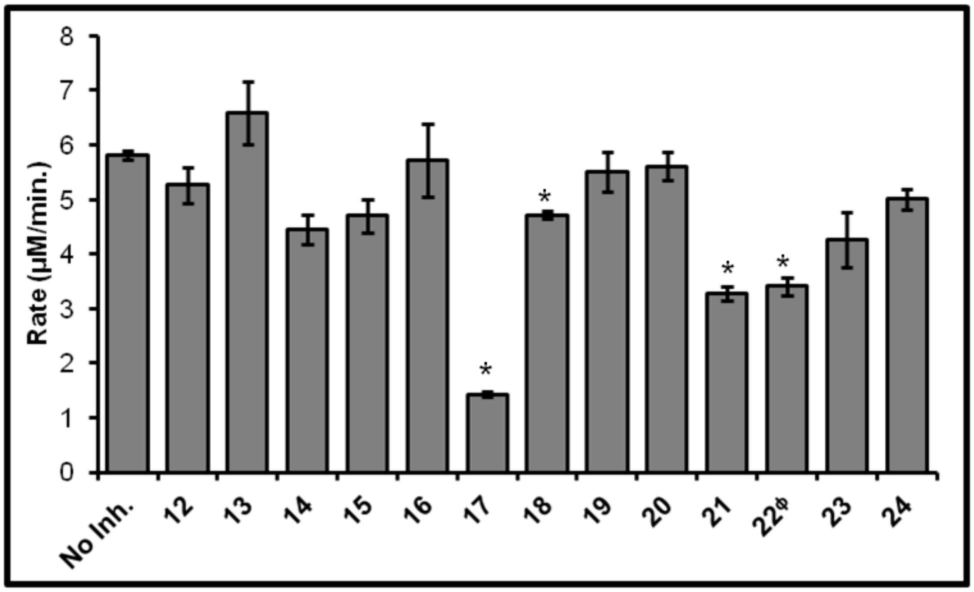


Figure S8. Inhibition of DXP synthase by methyloximes **12-24.** ᵠCompound was tested at 100 µM. *Significantly different from no inhibitor control ($p < 0.05$).

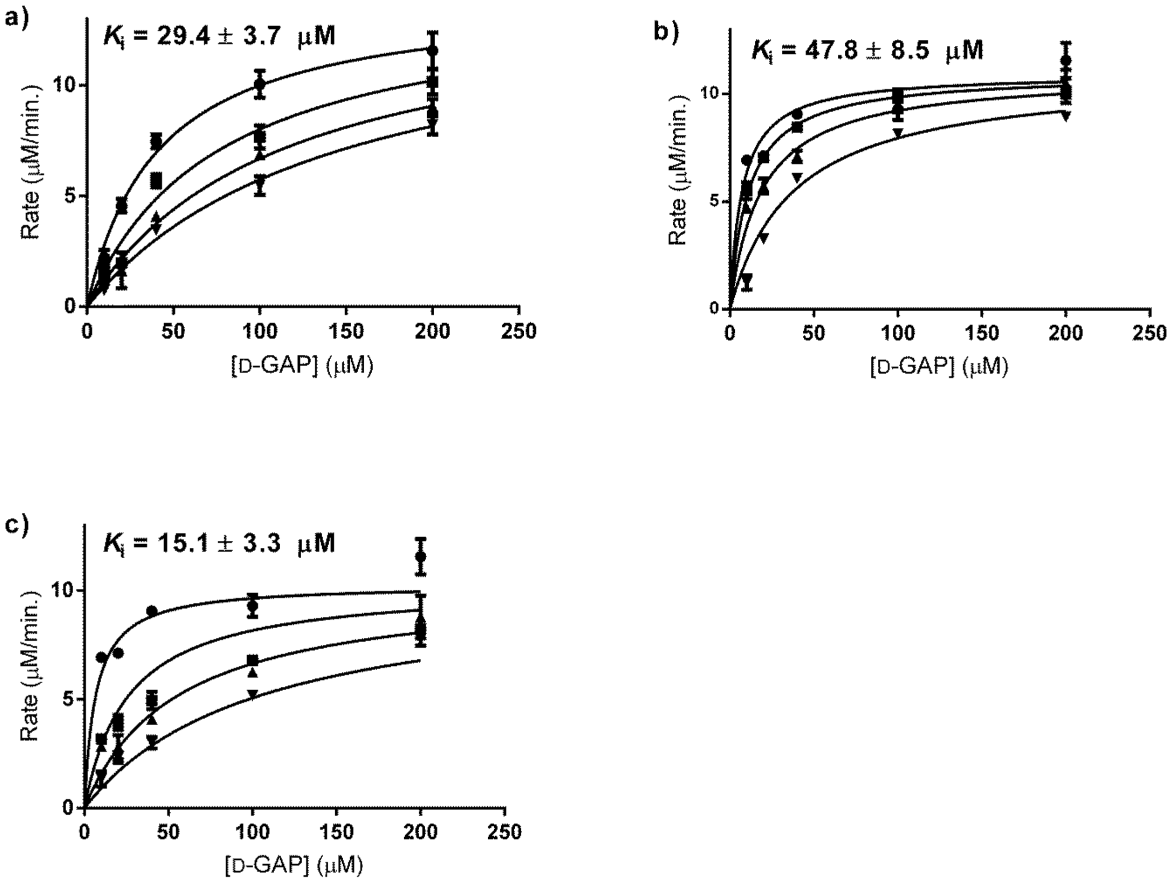


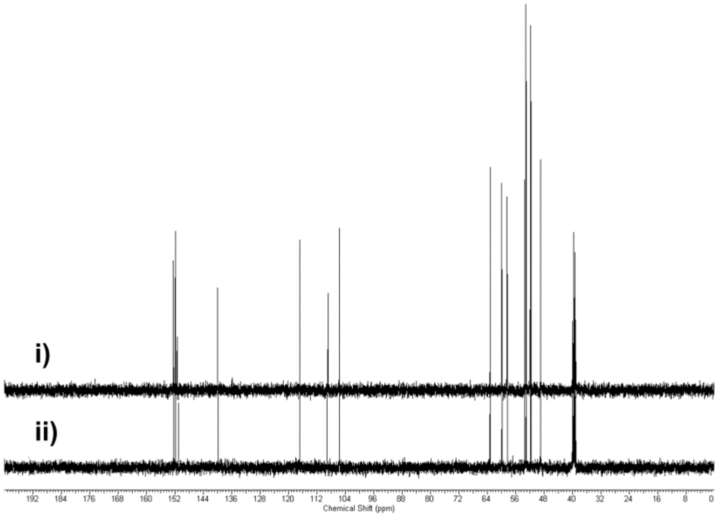
Figure S9. Inhibition of DXP synthase by oximes **17**, **21**, and **22**. Oximes **17, 21,** and **22** all display competitive inhibition against D-GAP as determined by model discrimination analysis (GraphPad Prism). a) Inhibition by **17**. Inhibitor concentrations: • 0 μM, ■ 25 μM, ▲ 50 μM, ▼ 75 μM. b) Inhibition by **21.** Inhibitor concentrations: • 0 μM, ■ 25 μM, ▲ 75 μM, ▼ 200 μM. c) Inhibition by **22**. Inhibitor concentrations: • 0 μM, ■ 20 μM, ▲ 50 μM, ▼ 100 μM.

Experimental Procedure for ^{13}C NMR analysis.

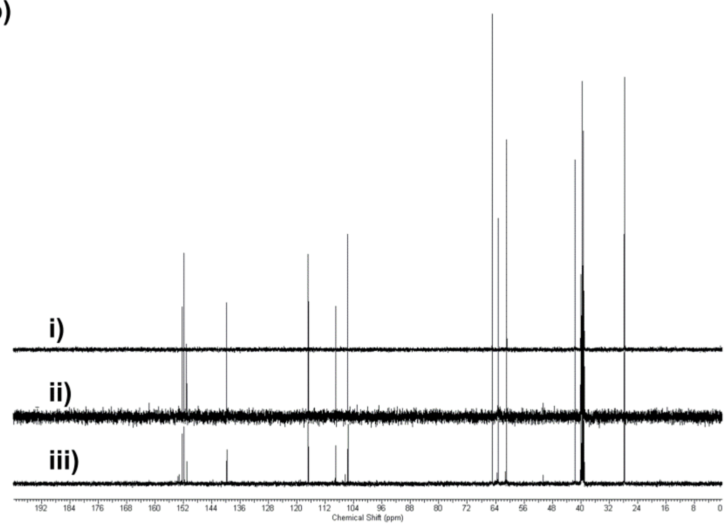
Analysis of Oxime 8 in aqueous solution. Oxime **8** (0.0182 g, 0.0994 mmol, 1 eq.) was dissolved in DMSO-d_6 (0.050 mL) and diluted into phosphate buffer (500 mM, pH 8, 0.351 mL) then HEPES (1M, 0.099 mL, 1 eq.). An initial spectrum was collected then another spectrum was collected after 24 hours.

Analysis of Oxime 8 in aqueous solution with β-mercaptoethanol (BME). A solution of oxime **8** (124 mM, 1 eq.) in phosphate buffer (500 mM, pH 8, 10% (v/v) DMSO-d_6) was prepared and a ^{13}C NMR spectrum was collected. BME (13.1 μL, 0.187 mmol, 3 eq.) was added to the oxime solution and the resulting solution was analyzed by ^{13}C NMR.

a)



b)



c)

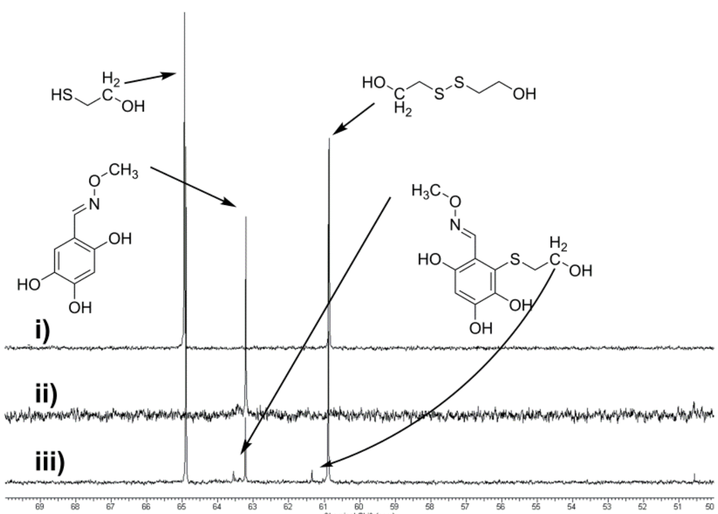
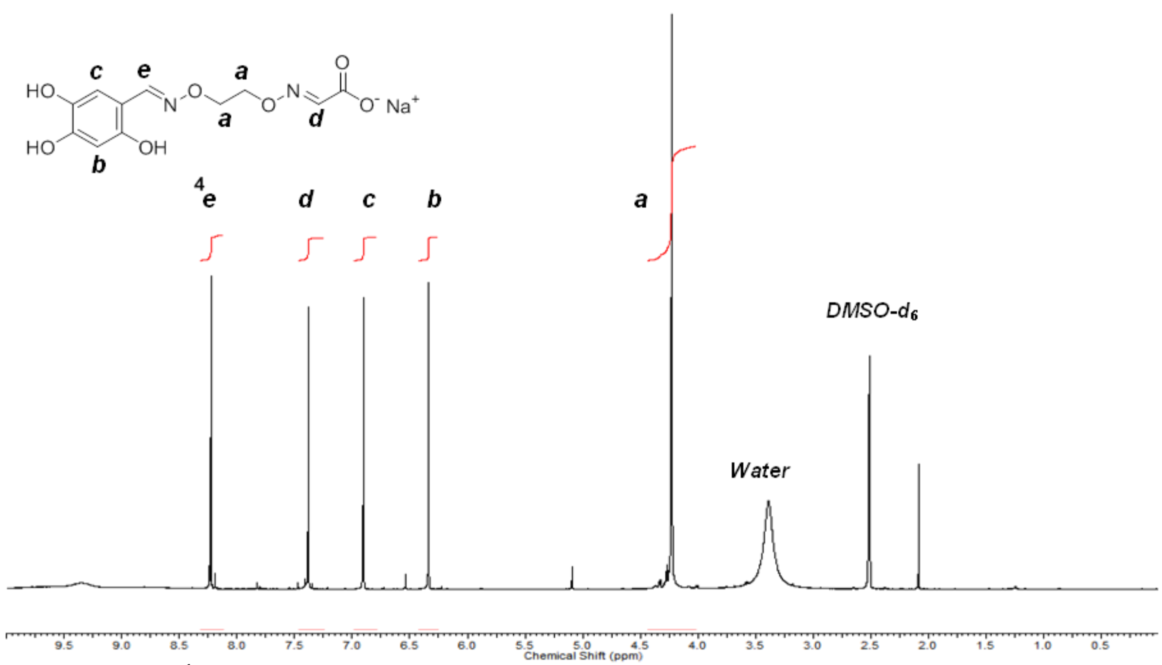
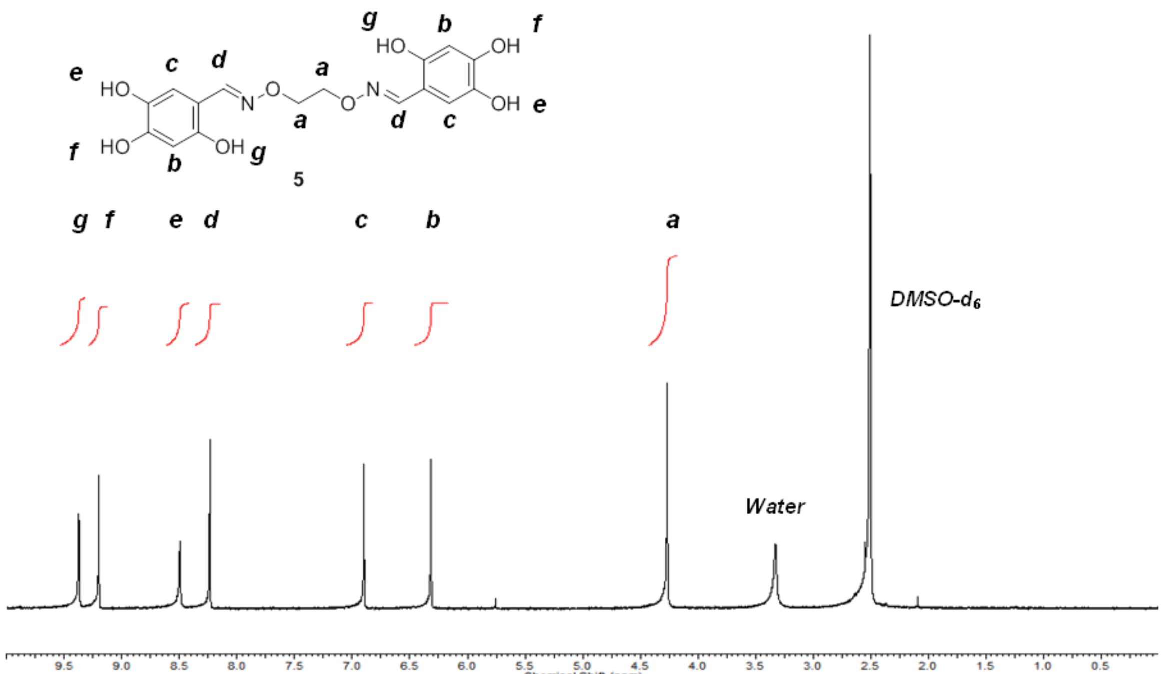


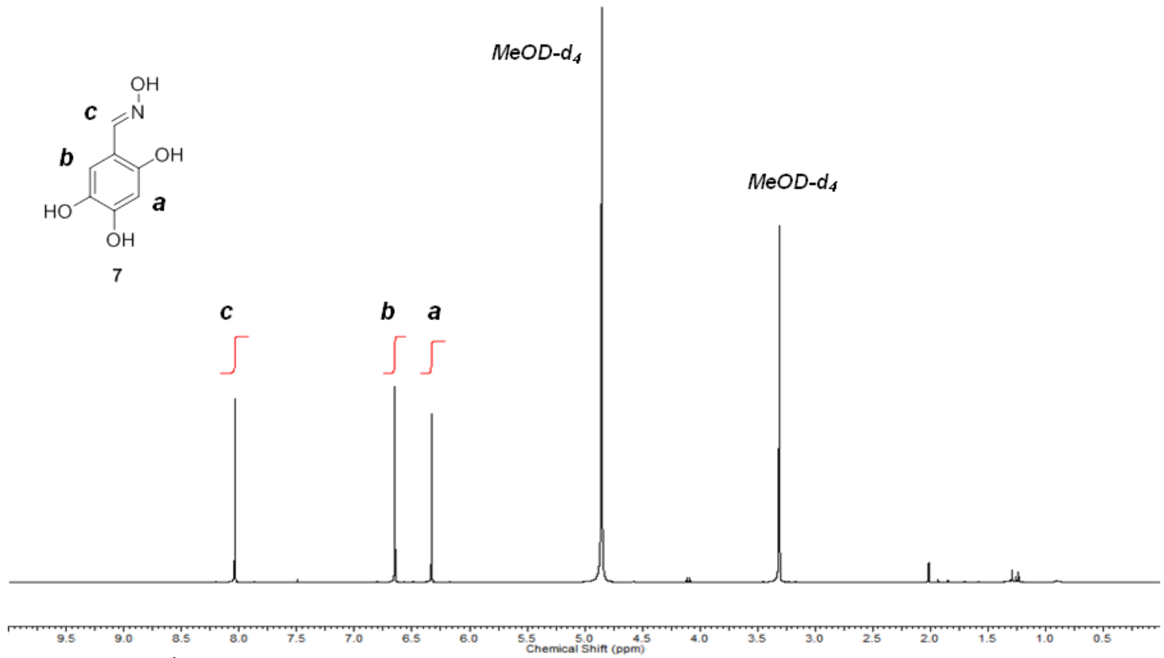
Figure S10. ^{13}C NMR of oxime **8** in the presence and absence of BME. a) No significant accumulation of quinone species in aqueous solution. ^{13}C NMR spectra of **8** are shown i) immediately after adding **8** to aqueous solution, ii) after incubation at ambient temperature for 24 hours in aqueous solution, b) BME adducts form upon addition of BME to **8**; i) Spectrum of BME alone in buffered aqueous solution with DMSO-d_6. ii) Spectrum of **8** in buffered aqueous solution with 1 molar equivalent of HEPES prior to addition of BME. iii) Spectrum of **8** immediately after adding BME (3 eq.) c) Enlarged view (50 – 70 ppm) of spectrum shown in b showing tentative peak assignments for BME adduct.



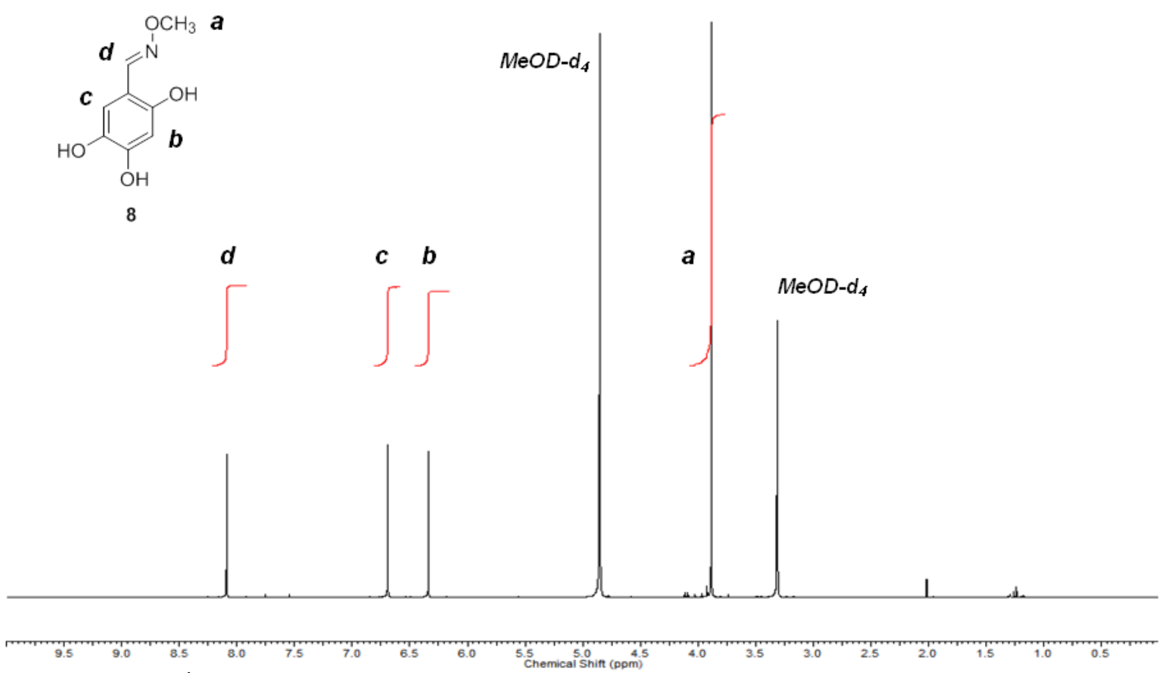
Figures S11. ^{1}H NMR spectra of **4**.



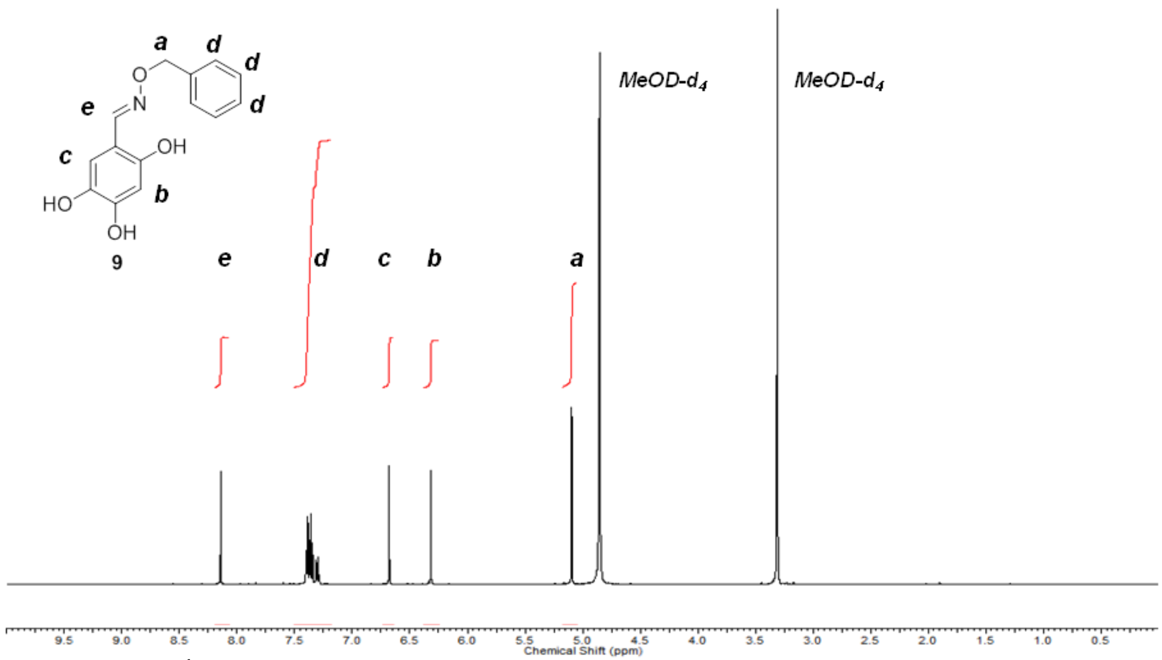
Figures S12. ^{1}H NMR spectra of **5**.



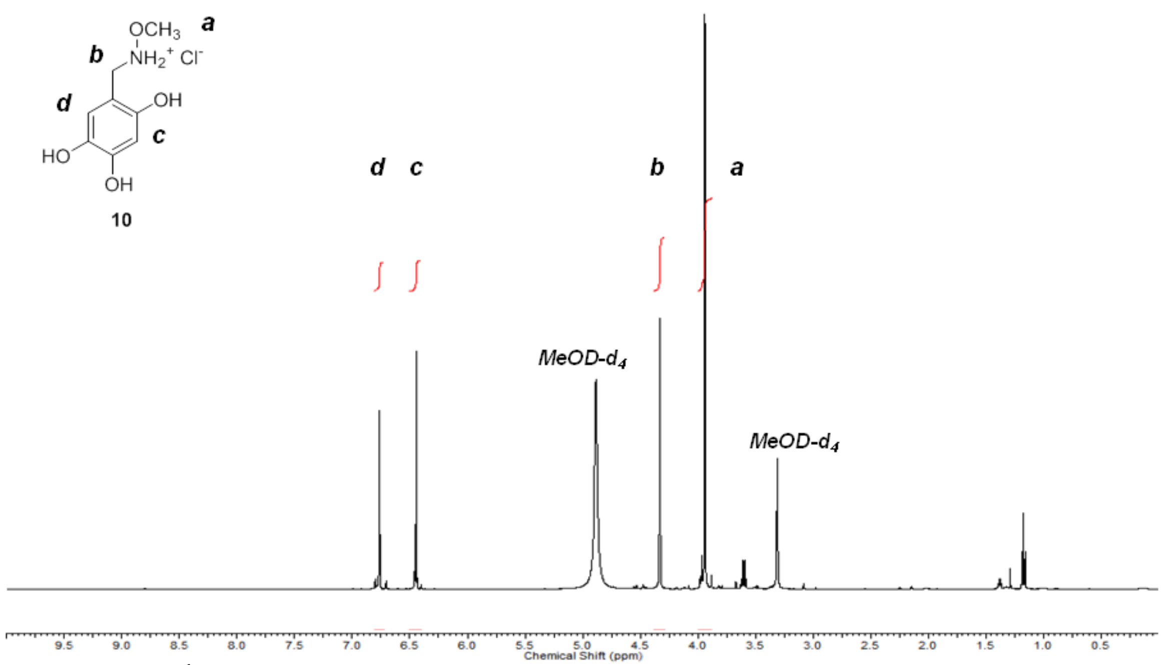
Figures S13. ^{1}H NMR spectra of **7**.



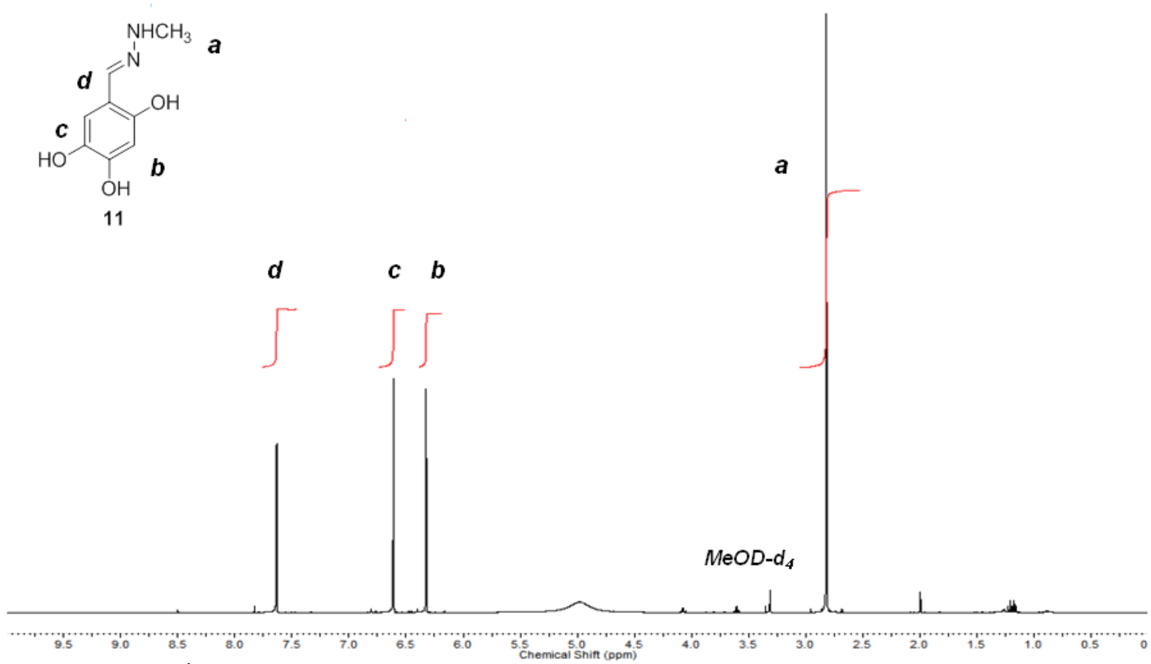
Figures S14. ^{1}H NMR spectra of **8**.



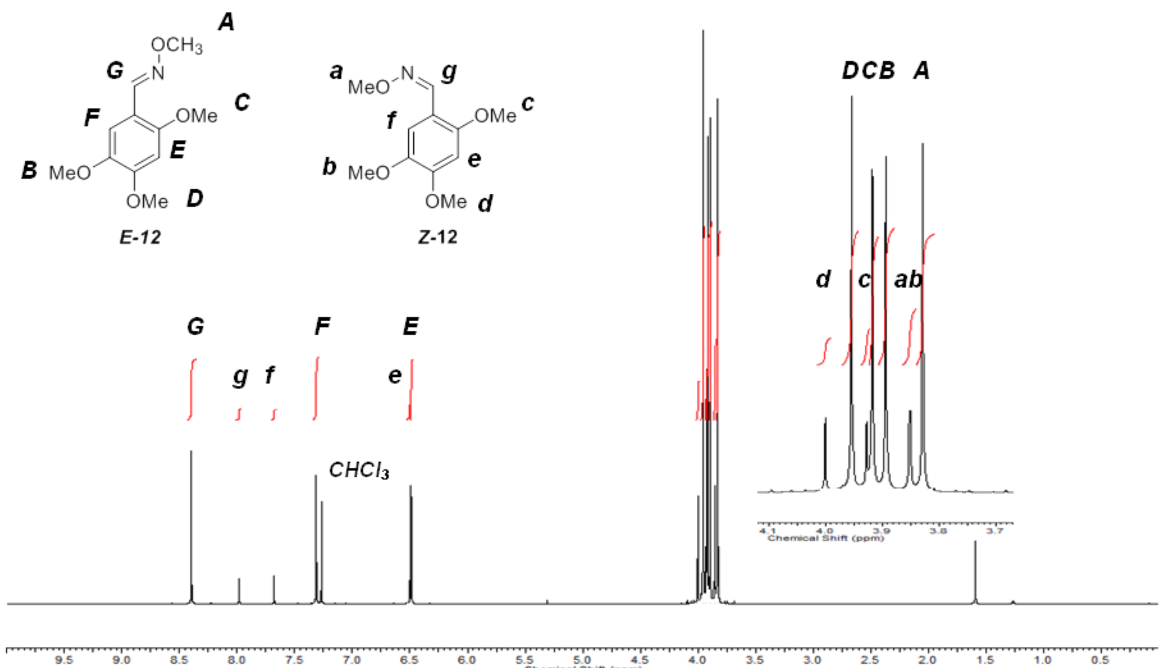
Figures S15. ^{1}H NMR spectra of **9**.



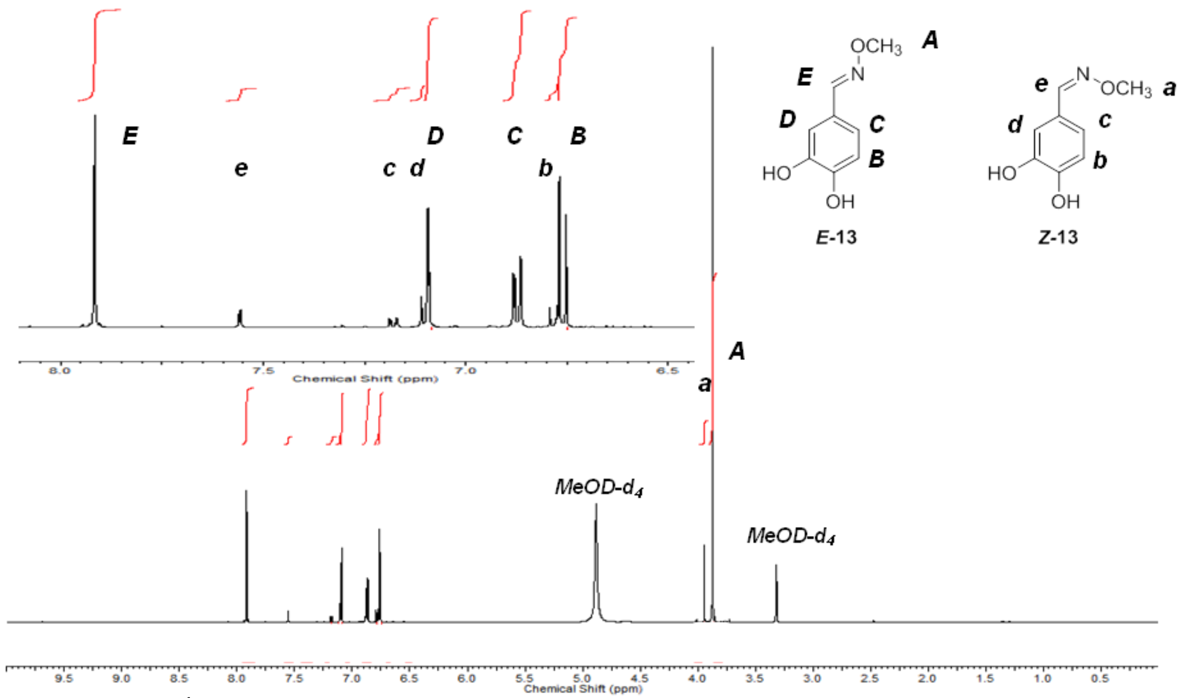
Figures S16. ^{1}H NMR spectra of **10**.



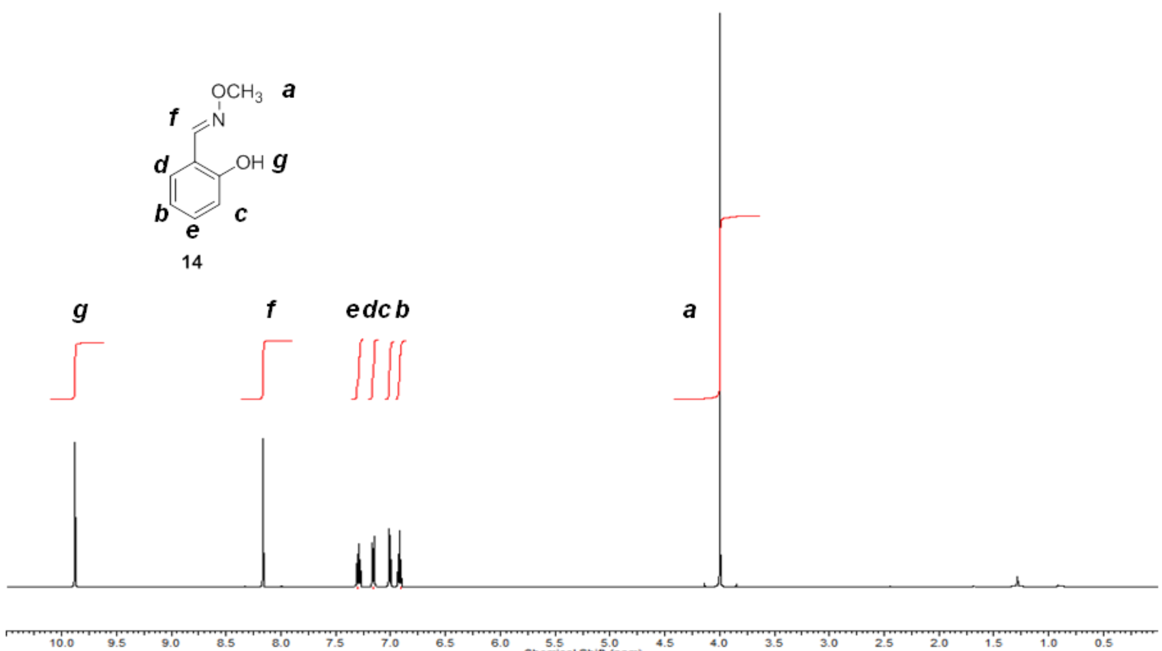
Figures S17. ^{1}H NMR spectra of **11**.



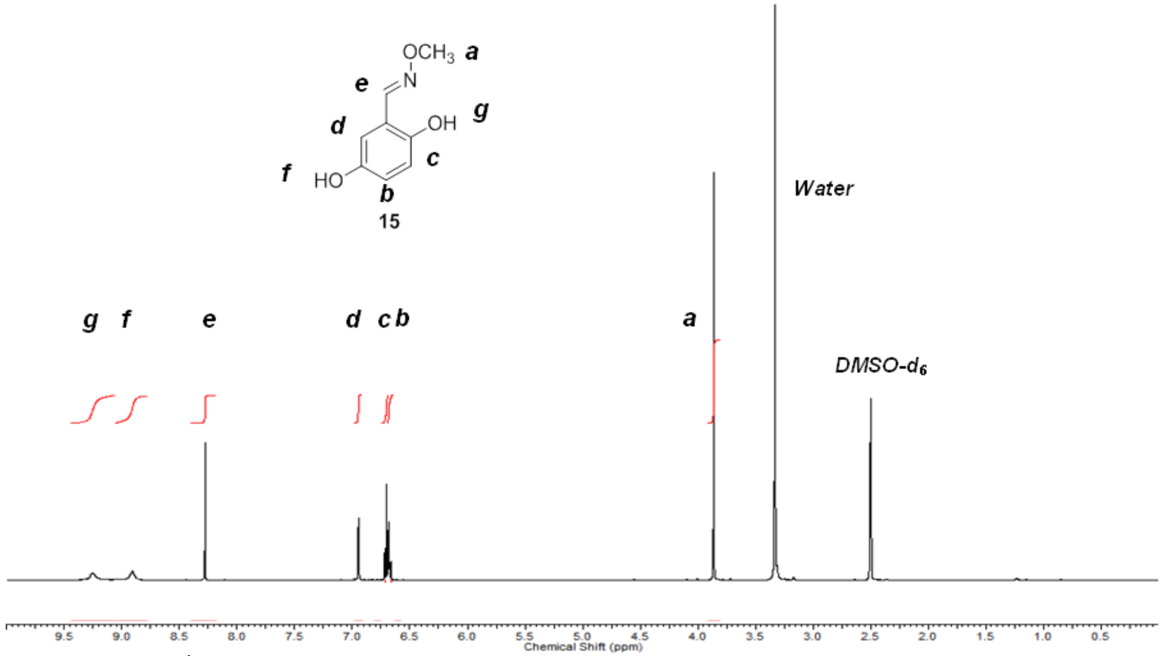
Figures S18. ^{1}H NMR spectra of **12**.



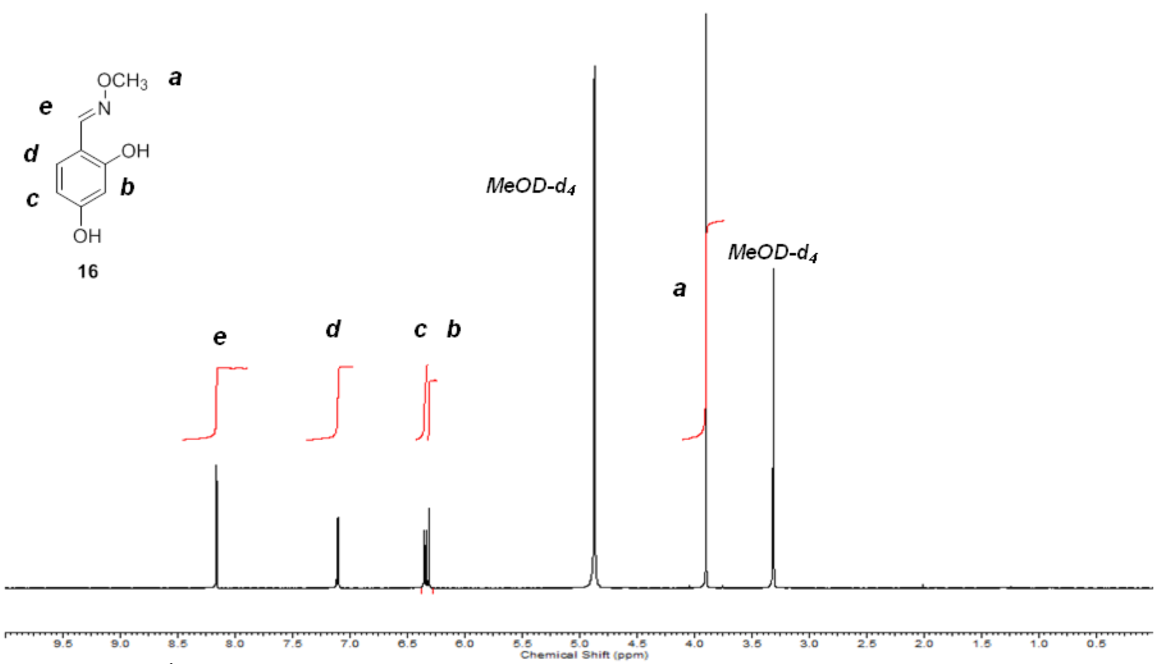
Figures S19. ^{1}H NMR spectra of **13**.



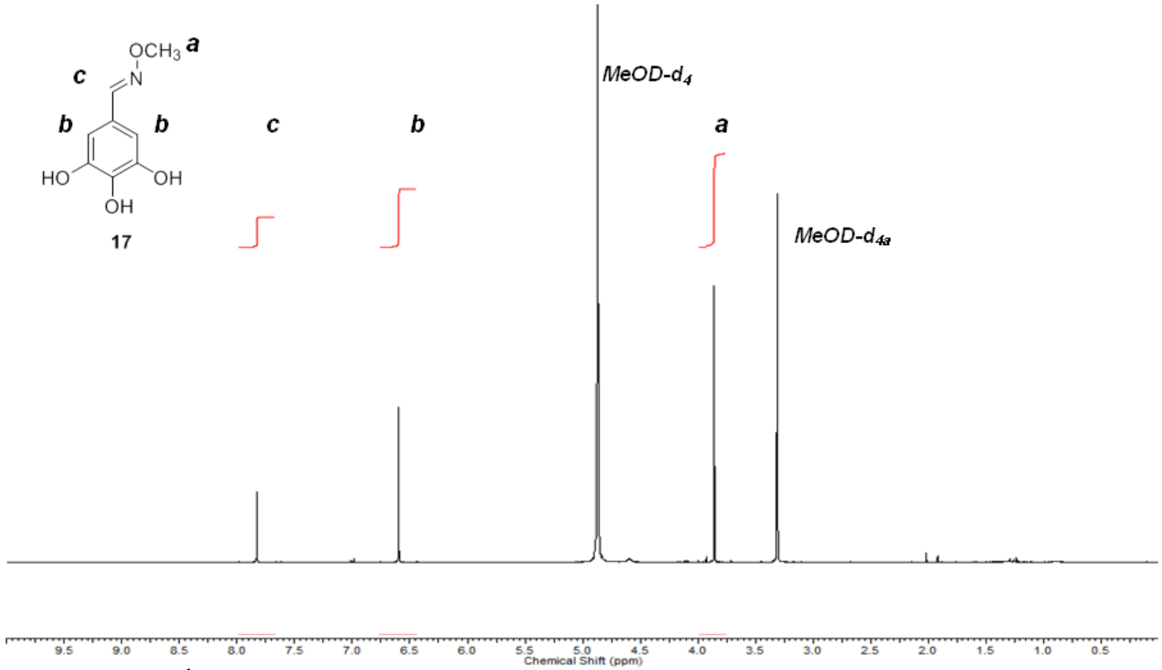
Figures S20. ^{1}H NMR spectra of **14**.



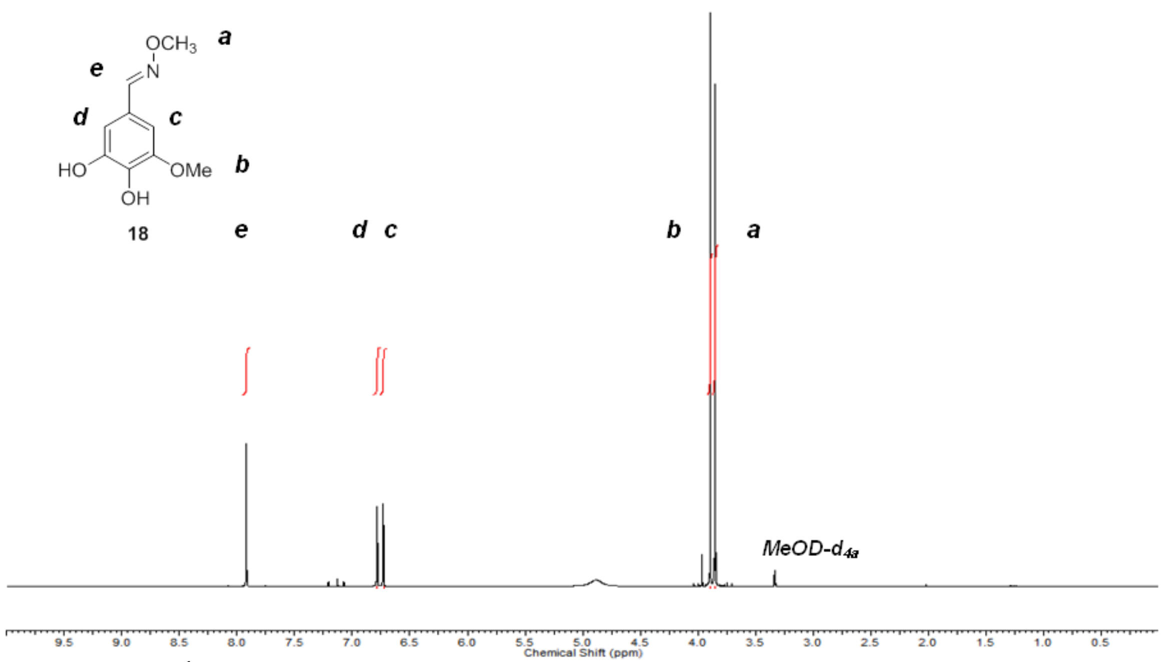
Figures S21. ^{1}H NMR spectra of **15**.



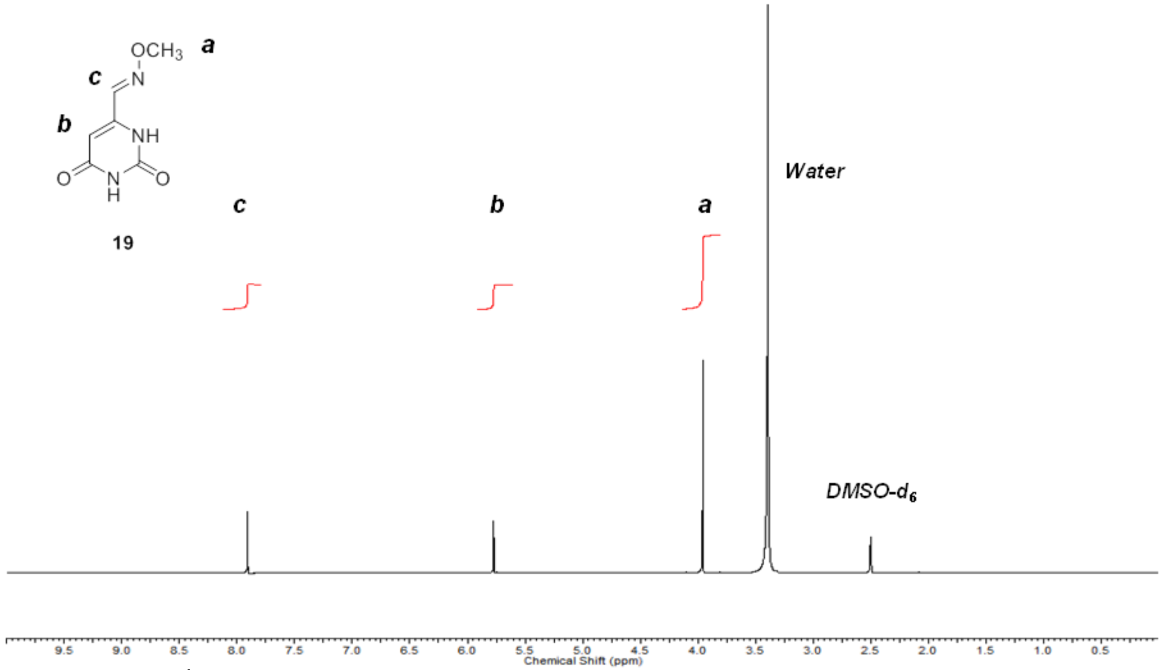
Figures S22. ^{1}H NMR spectra of **16**.



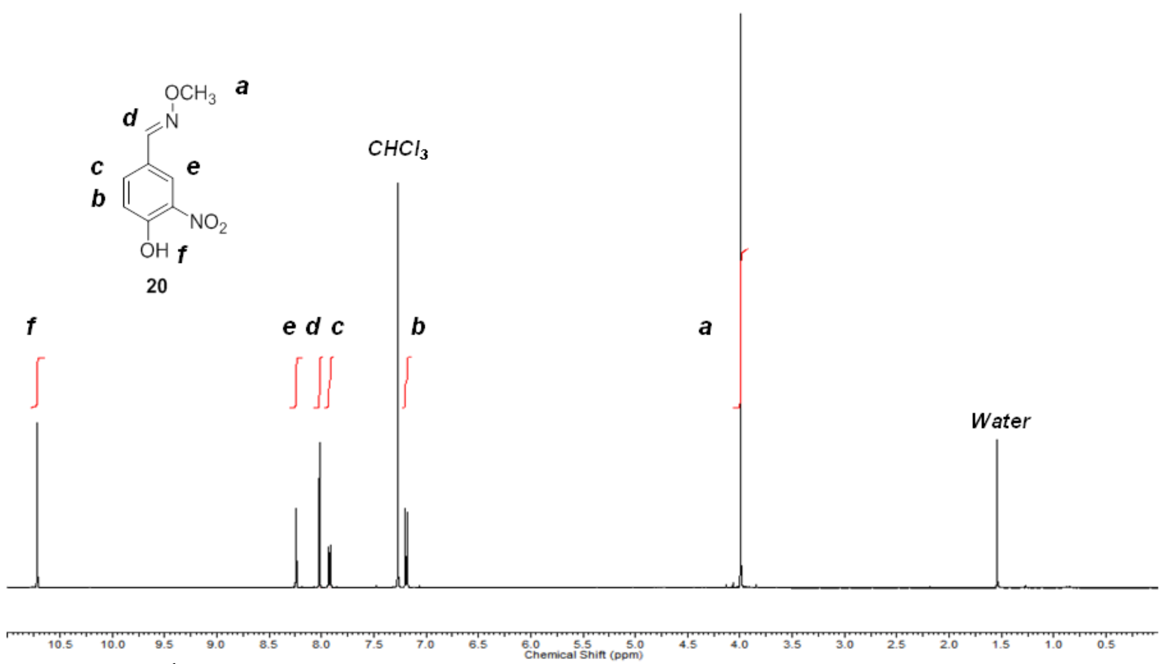
Figures S23. ^{1}H NMR spectra of **17**.



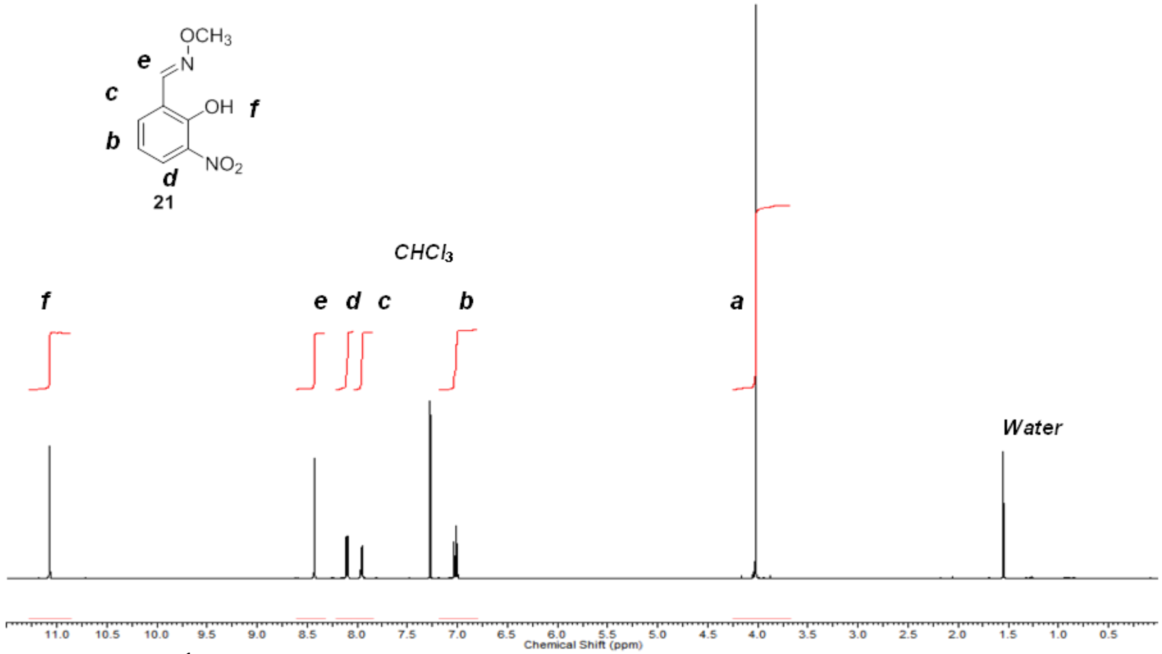
Figures S24. ^{1}H NMR spectra of **18**.



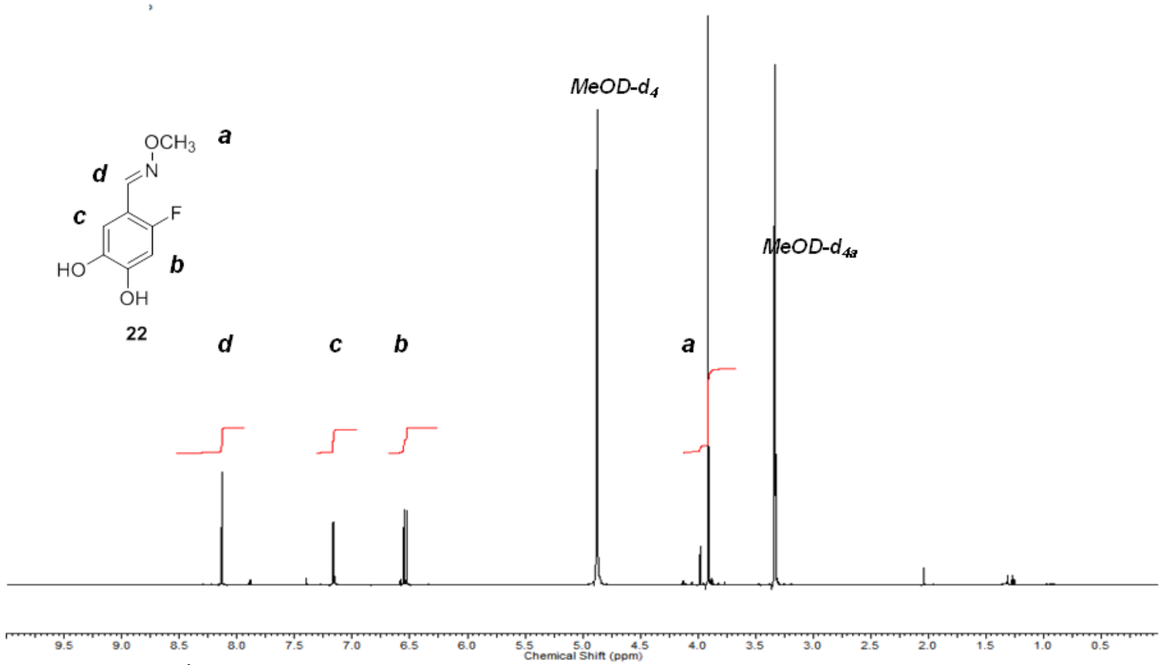
Figures S25. ^{1}H NMR spectra of **19**.



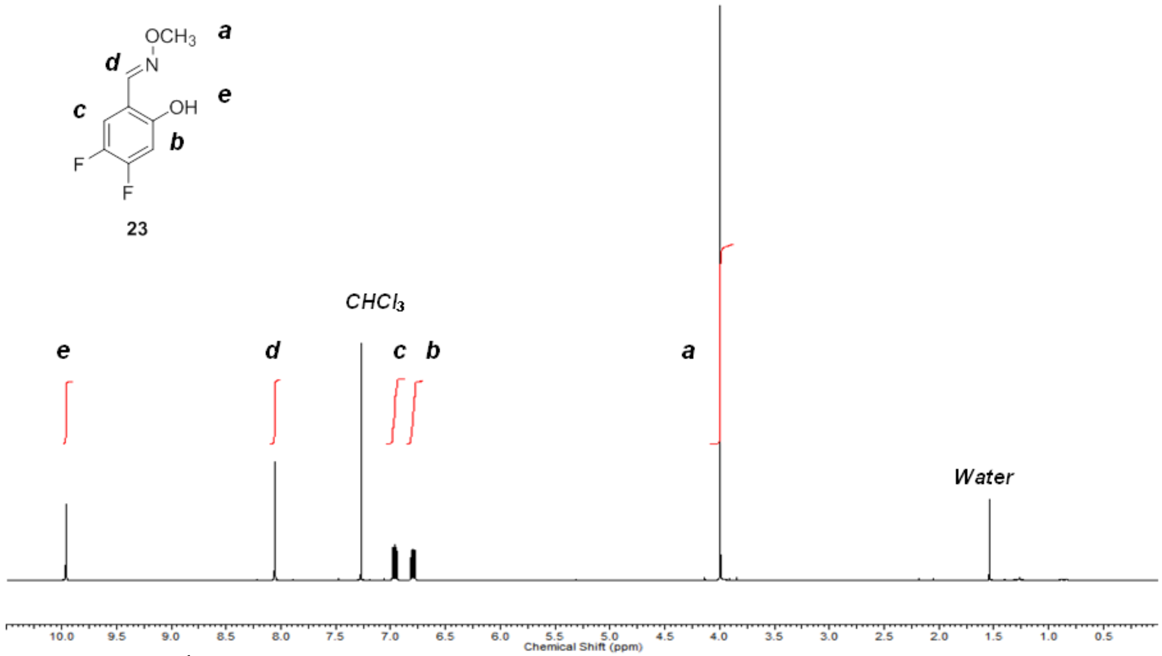
Figures S26. ^{1}H NMR spectra of **20**.



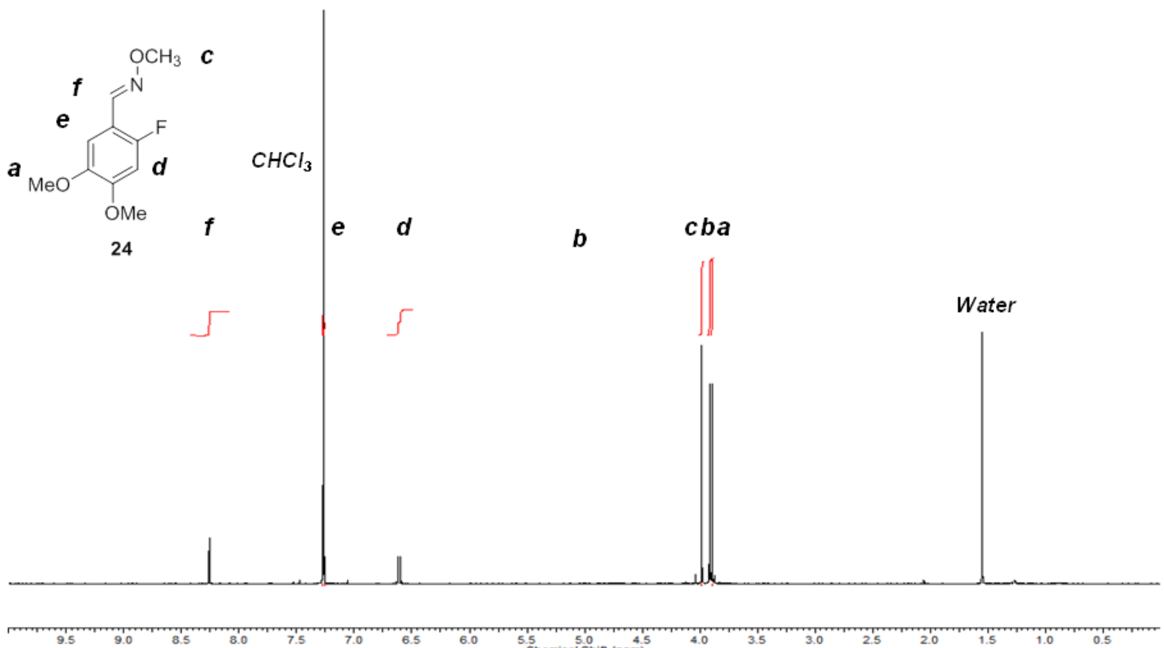
Figures S27. ^{1}H NMR spectra of **21**.



Figures S28. ^{1}H NMR spectra of **22**.



Figures S29. ^{1}H NMR spectra of **23**.



Figures S30. ^{1}H NMR spectra of **24**.

Reference:

1. Brammer LA, Smith JM, Wade H, Meyers CF. 1-deoxy-D-xylulose 5-phosphate synthase catalyzes a novel random sequential mechanism. *Journal of Biological Chemistry*. 2011;286(42):36522-36531.