Supporting Information

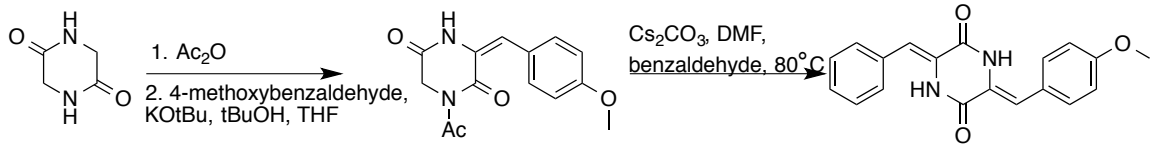
Phenotype-Guided Natural Products Discovery Using Cytological Profiling

*Jessica L. Ochoa, Walter M. Bray, R. Scott Lokey, Roger G. Linington**

Department of Chemistry and Biochemistry, University of California, Santa Cruz, 1156 High Street, Santa Cruz, California 95064, United States

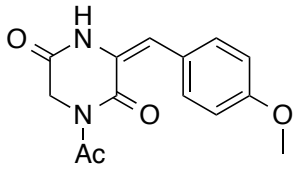
Table of contents

S1. Synthetic scheme for XR334 (**3**)



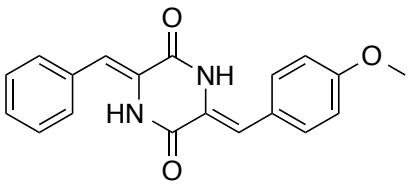
General Techniques

All reactions were performed in oven dried glassware under an inert atmosphere of N_2. Tetrahydrofuran (THF), was obtained from a Pur-Solv 400 solvent purification system manufactured by Innovative Technology. All reagents were used as purchased without further purification. Thin layer chromatography was performed with Merck Silica gel 60 F254 and visualized with a UV lamp at 254 nm. Crude reaction mixtures were purified using Silica Gel 60 (230 – 400 mesh ASTM). ^{1}H and ^{13}C NMR spectra were obtained in the UC Santa Cruz NMR facility on either 500 or 600 MHz spectrometers equipped with a 5 mm broadband probe and a 5 mm HCN triple resonance cryoprobe respectively. ^{1}H and ^{13}C NMRs are referenced to indicated solvent signals. High-resolution mass spectra were obtained using an Agilent 6230 ESI-TOF-MS. Optical rotation measurements were obtained using a Jasco P2000 digital polarimeter.



(3*Z*)-1-Acetyl-3-(4-methoxybenzylidene)-2,5-piperazinedione

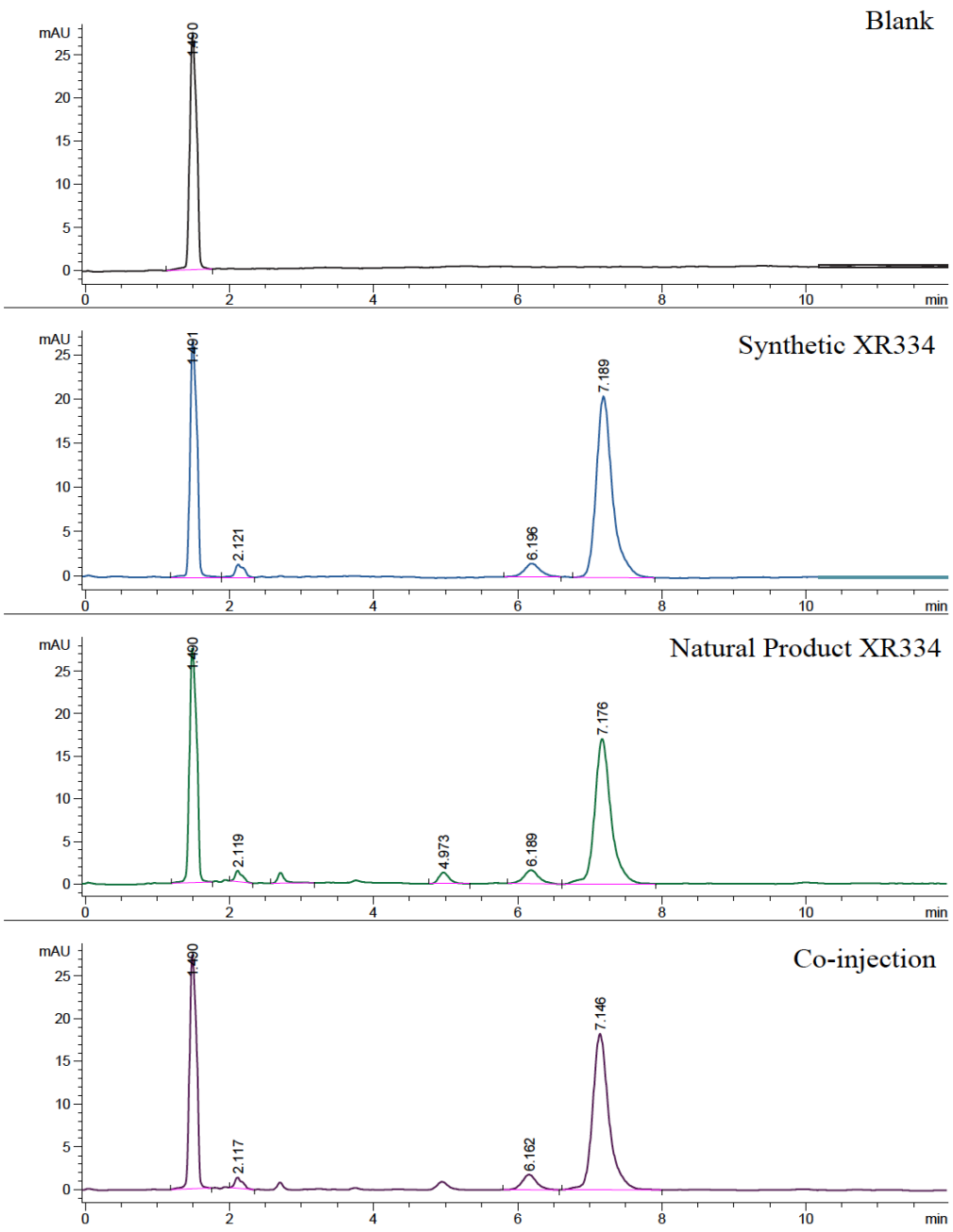
2,5-Piperazinedione (100 mg, 0.87 mmol) was heated in acetic anhydride (40 mL) at reflux for 4 hours. The crude reaction mixture was concentrated to dryness *in vacuo* and the product recrystalized from hexane-ethyl acetate to yield 1,4-diacetyl-2,5-piperazinedione as a tan solid. A solution of 1,4-diacetyl-2,5-piperazinedione (10 mg, 50.5 μmol) in dry THF (1 mL) was cooled to 0°C and a solution of potassium *t*-butoxide (5.67 mg, 50.5 μmol) in *t*-butanol (1 mL) added dropwise over 30 minutes and allowed to warm to room temperature overnight. The mixture was diluted with ethyl acetate (4 mL) and washed with water (2 x 2mL) and saturated brine (2 x 2mL) to afford (3*Z*)-1-acetyl-3-(4-methoxybenzylidene)-2,5-piperazinedione as a white precipitate. Physical properties and spectra were consistent with published data.



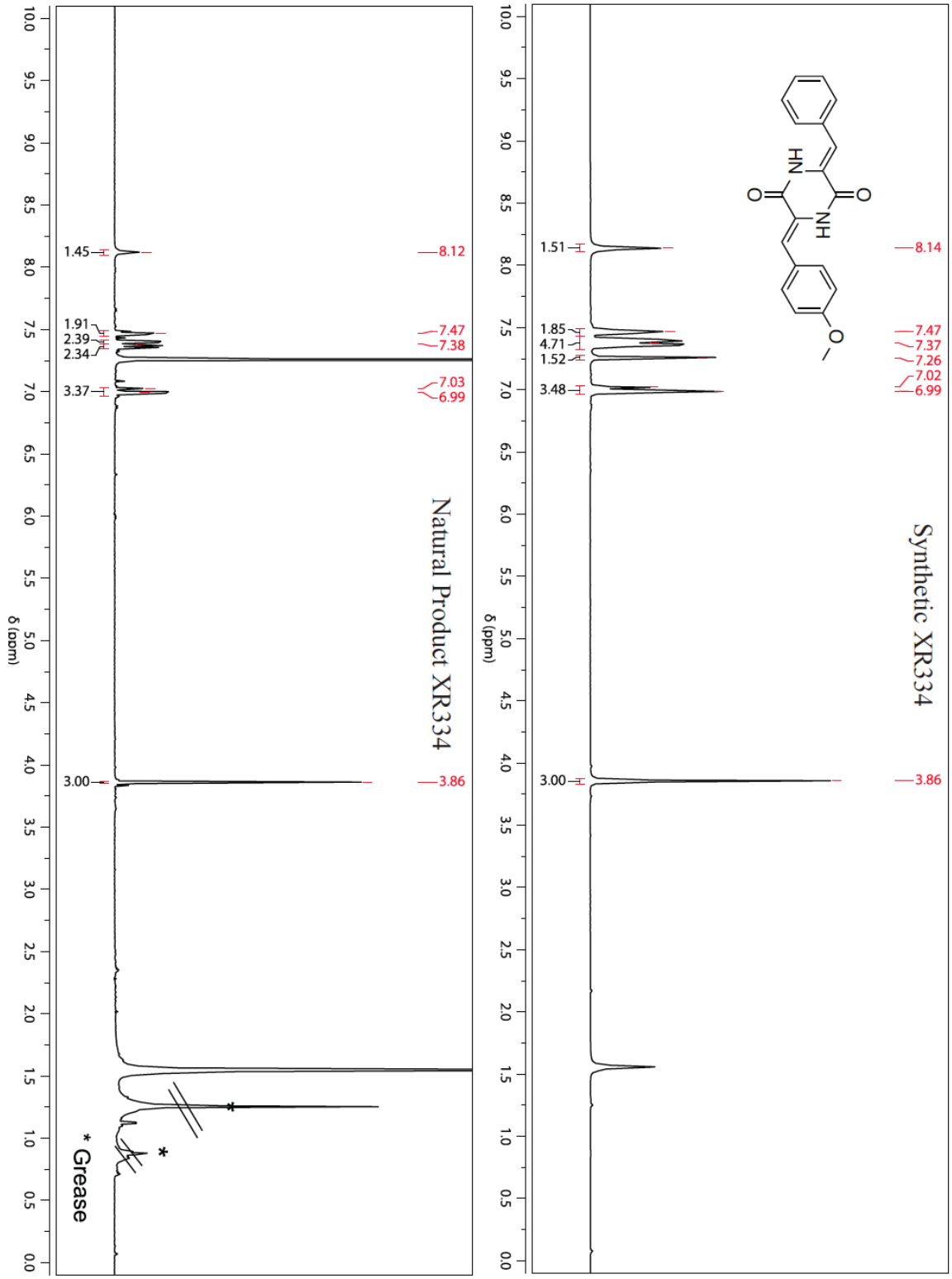
XR334

A mixture of (3*Z*)-1-acetyl-3-(4-methoxybenzylidene)-2,5-piperazinedione (5.00 mg, 18.25 μmol), caesium carbonate (5.95 mg, 18.25 μmol) and benzaldehyde (1.85 μL, 18.25 μmol) in dimethylformamide (5 mL) was heated at 90°C with stirring in air. After 2 hours the mixture was cooled to room temperature, diluted with ethyl acetate (1 mL) and washed with water (2 x 2mL) and saturated brine (2 x 2mL) to afford crude XR334, which was then subjected to RP-HPLC (Phenomenex Synergi Fusion-RP 10 micron, 80 Å, 250 x 4.6 mm, 65:35 MeOH/H_2O + 0.02% formic acid isocratic run over 10 min, 2 mL min^{-1} flow rate) to afford XR334 as a white solid with a retention time of 5.75 min. Physical properties and spectra were consistent with published data.

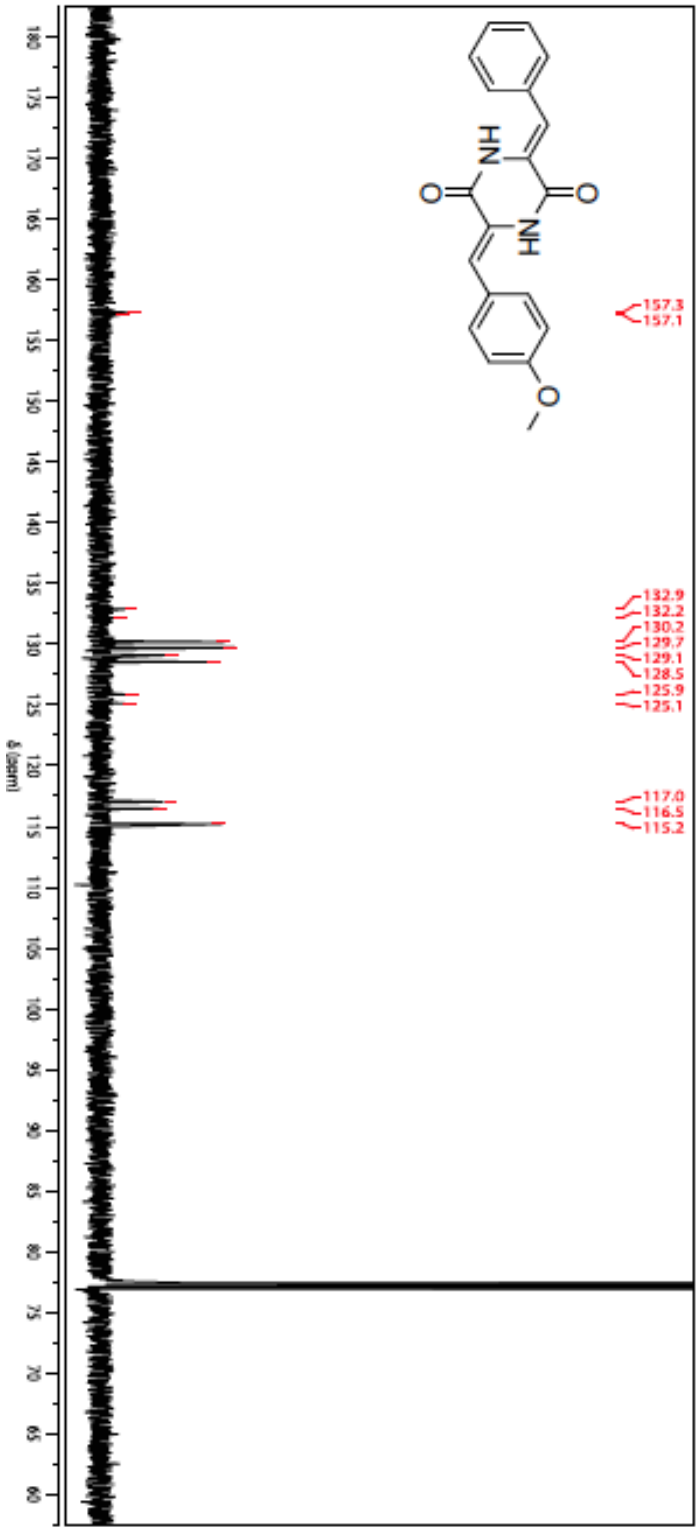
S2. Co-injection of synthetic and natural product XR334 (**3**)



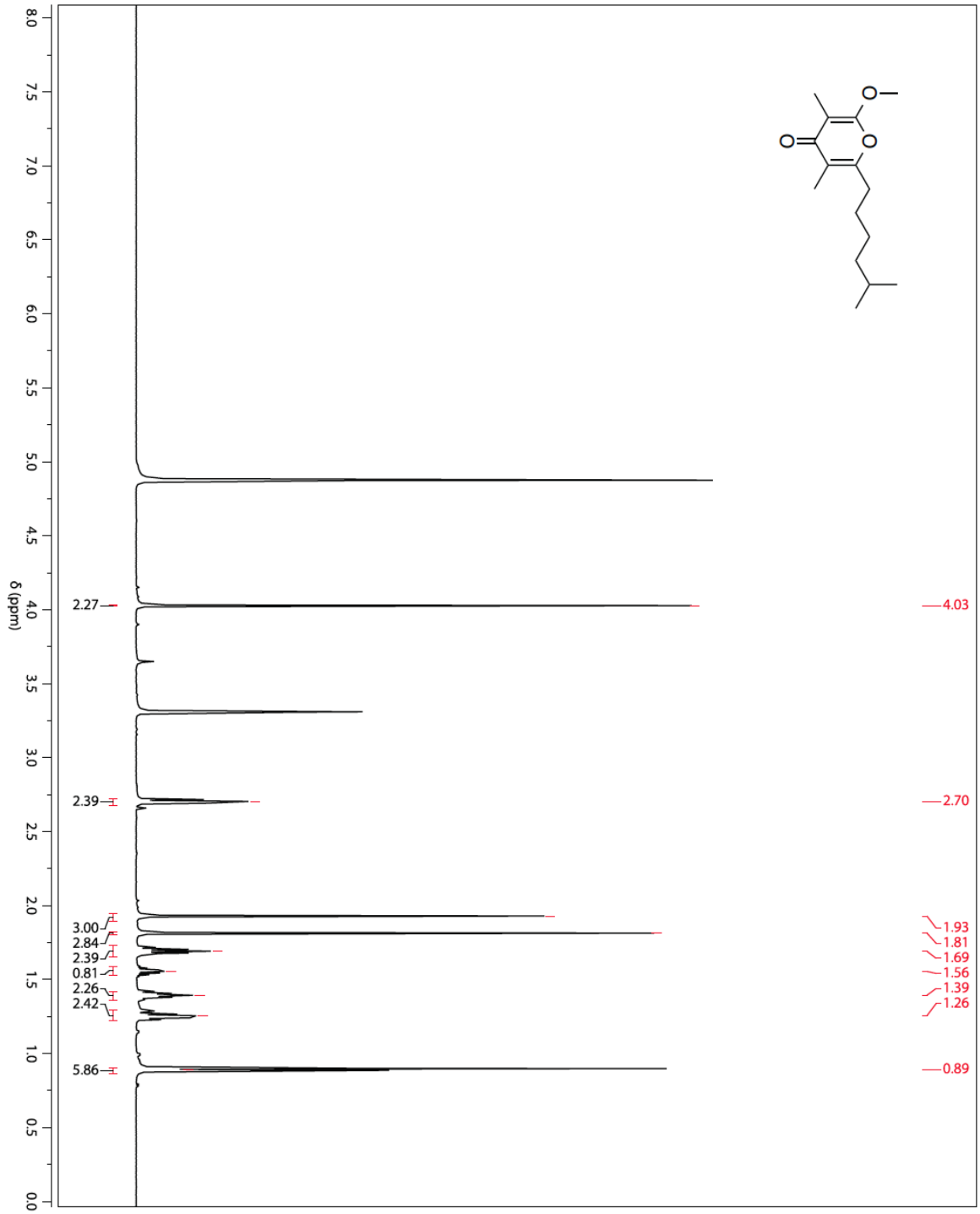
S3. ^{1}H NMR (600MHz, $CDCl_3$) spectrum of synthetic and natural product XR334 (**3**);



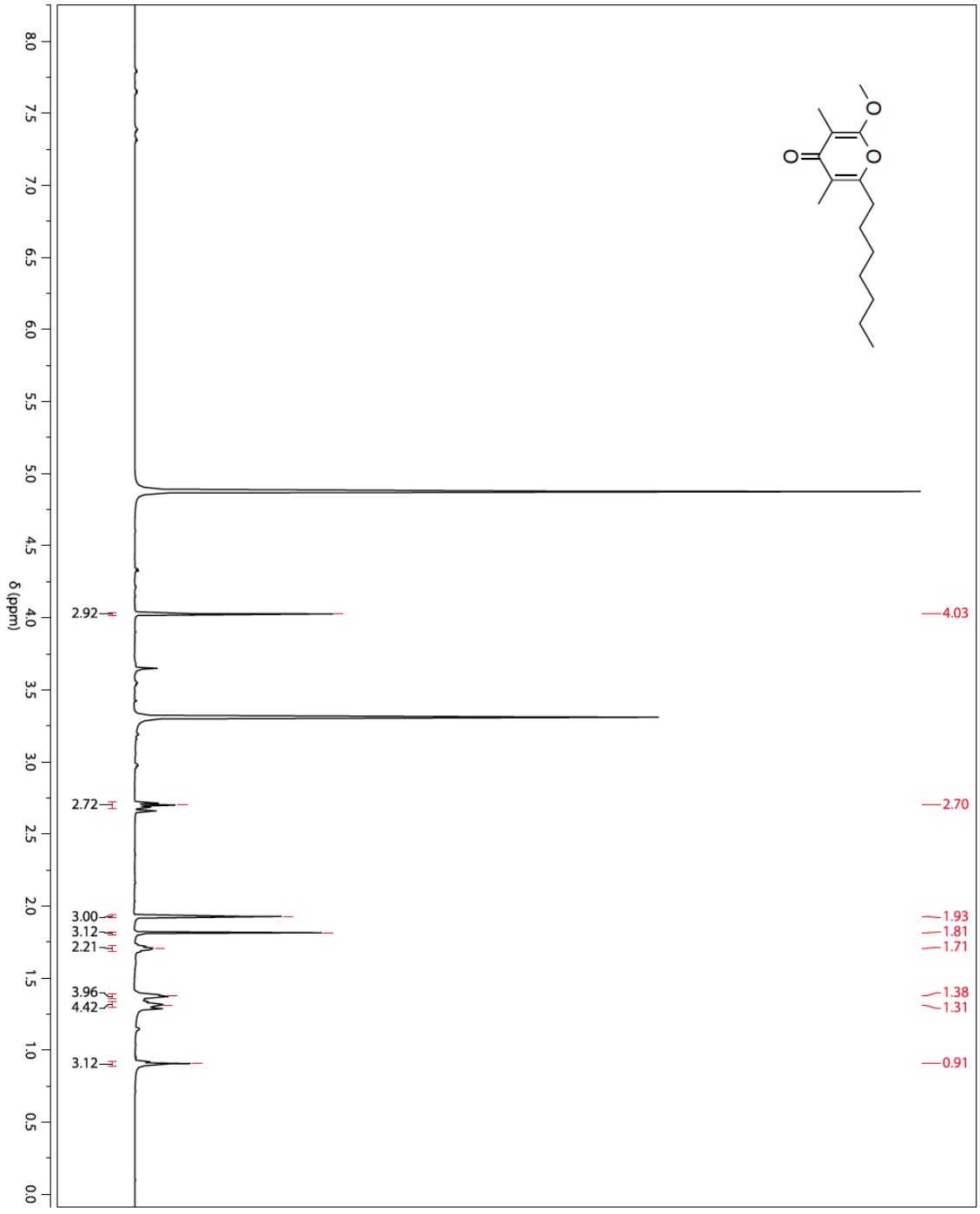
S4. ^{13}C NMR (150 MHz, CD_3OD) spectrum of XR334 (**3**)



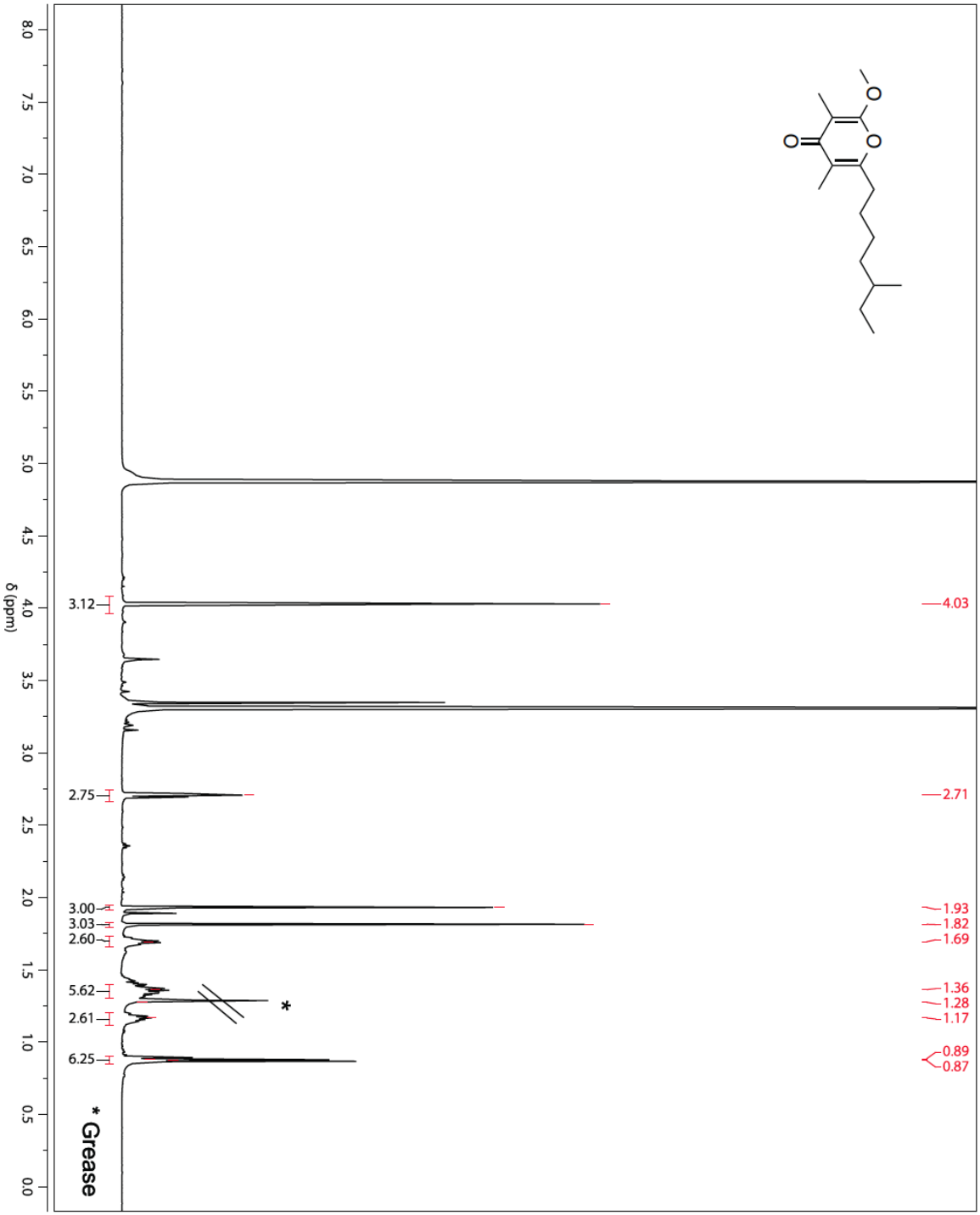
S5. ^{1}H NMR (600MHz, CD_3OD) spectrum of nocapyrone B (**4**)



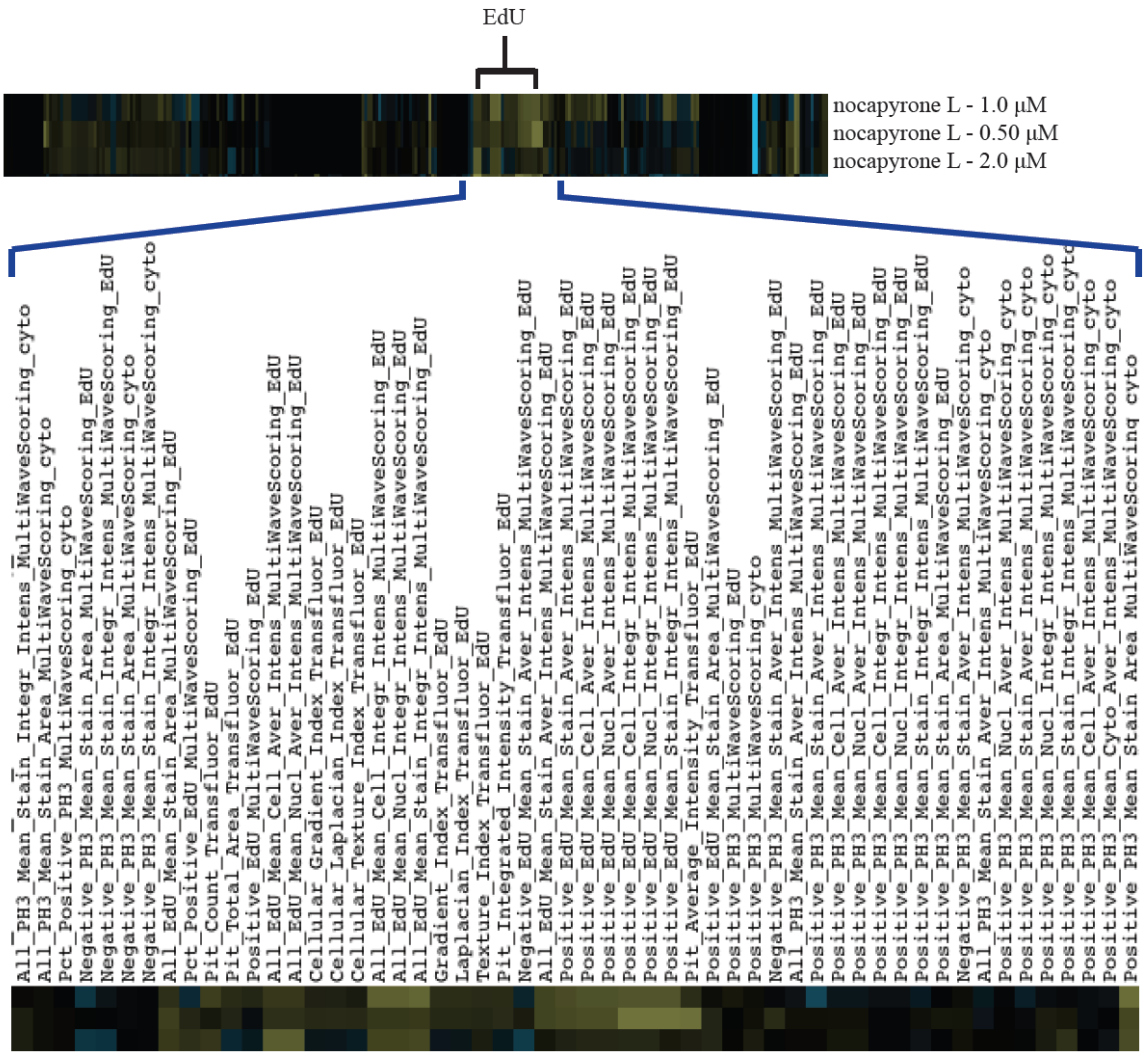
S6. ^{1}H NMR (600MHz, CD_3OD) spectrum of nocapyrone H (**5**)



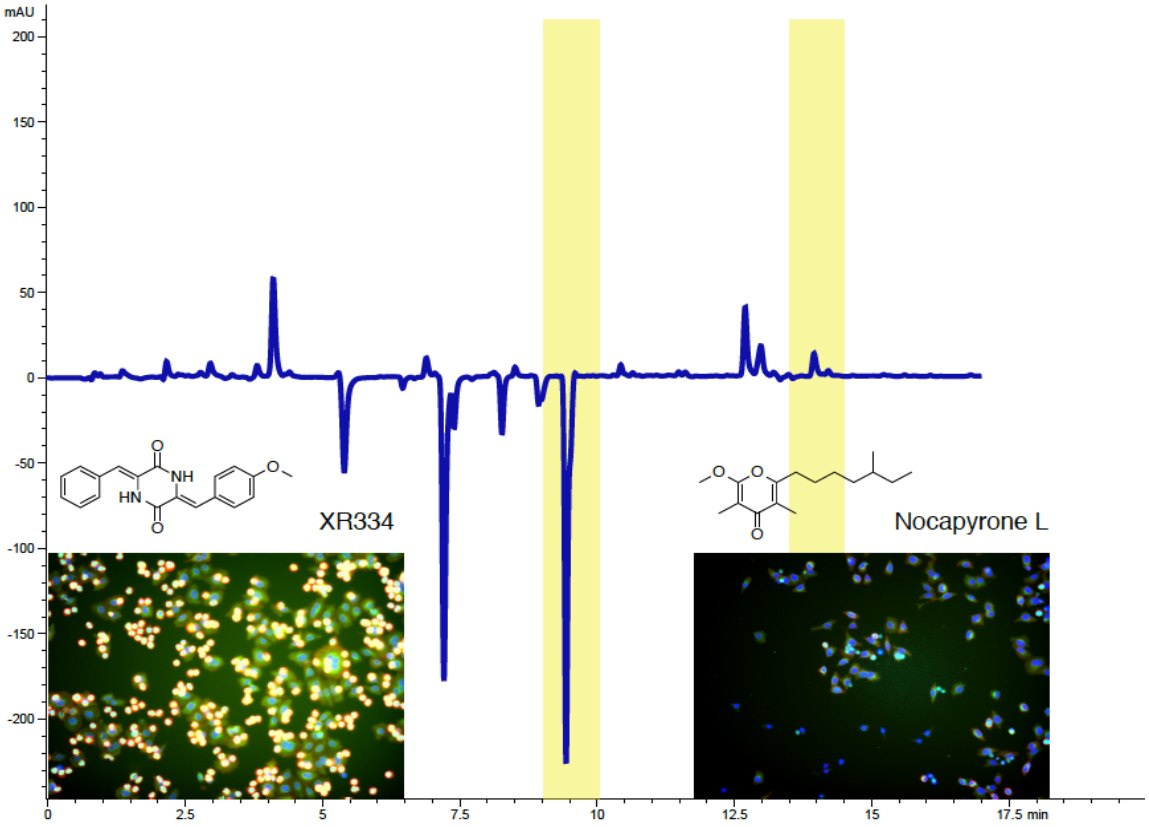
S7. ^{1}H NMR (600MHz, CD_3OD) spectrum of nocapyrone L (**6**)



S8. Expanded CP fingerprint of nocapyrone L (**6**)



S9. HPLC trace for reverse phase stage of fractionation for RLUS1665D extract



S10. Dilution Series of XR334 and Nocapyrone L