Supporting information (SI)

for:

Far-Red Molecular Rotor Fluorogenic Trehalose Probe for Live Mycobacteria Detection and Drug-Susceptibility Testing

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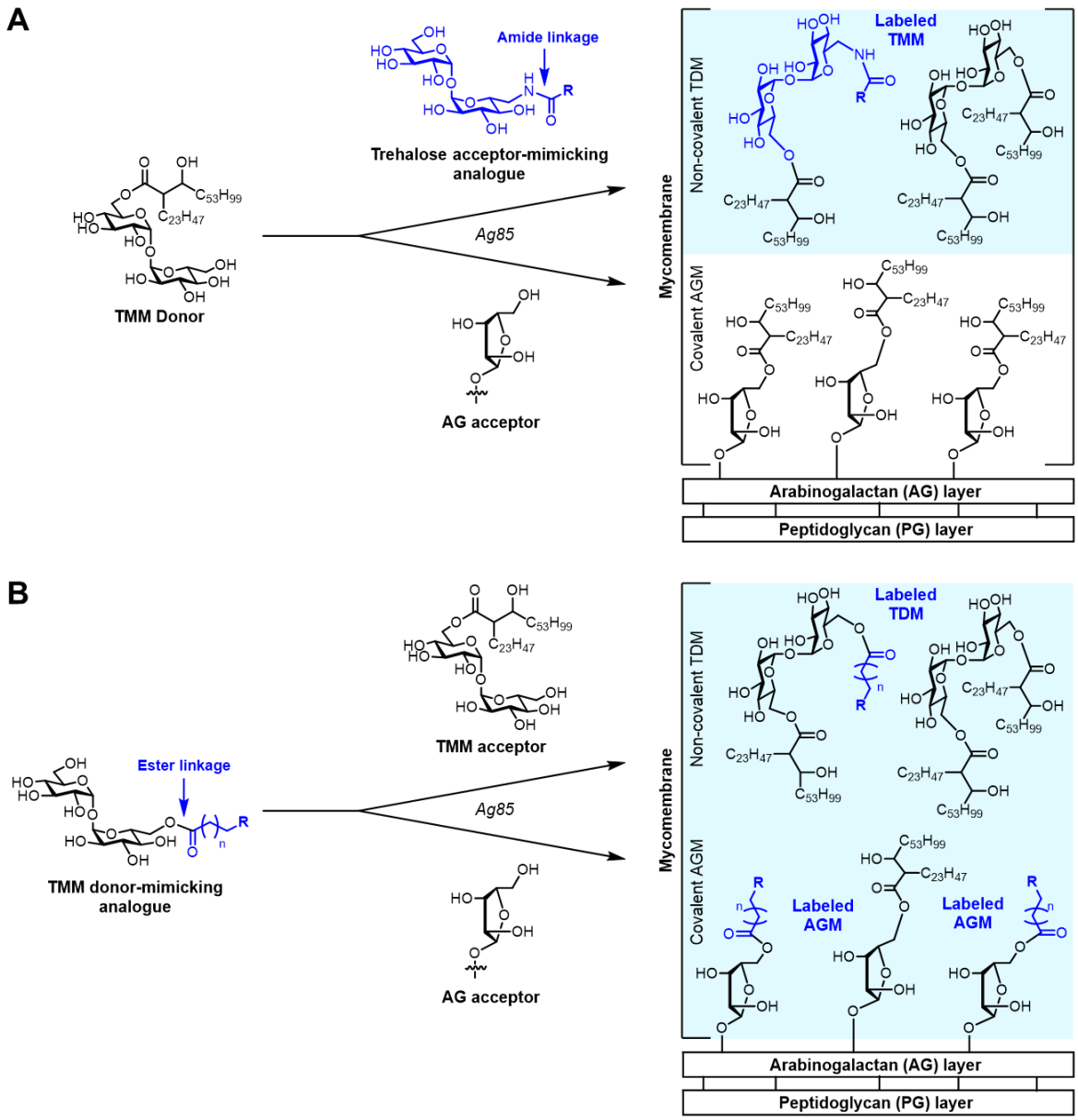
I. Supplementary figures and schemes



Scheme S1. Proposed metabolic labeling routes for (A) RMR-Tre and (B) RMR-TMM. Both probes exploit conserved Ag85 mycoloyltransferases. The linkage type (amide or ester) controls the incorporation route and target.[1] (A) Amide-linked trehalose analogues are incompetent to serve as a mycoloyl donors and only act as mycoloyl acceptors in Ag85-catalyzed reactions, thus it is proposed that RMR-Tre exclusively produces labeled TMM. (B) Trehalose analogues with ester-connecter linkers resemble and functionally mimic trehalose monomycolate (TMM). TMM donates its acyl chain to acceptor TMM and arabinogalactan (AG) molecules in extracellular reactions catalyzed by mycoloyltransferase enzymes (e.g., antigen 85 (Ag85) enzymes), producing mycomembrane glycolipids trehalose dimycolate (TDM) and arabinogalactan mycolate (AGM). Similar to several other TMM-mimicking trehalose analogues, it is proposed that RMR-TMM bearing ester-linked RMR groups would have their unnatural acyl chains transferred onto mycoloyl acceptors to produce RMR-modified TDM and AGM. R = RMR moiety.

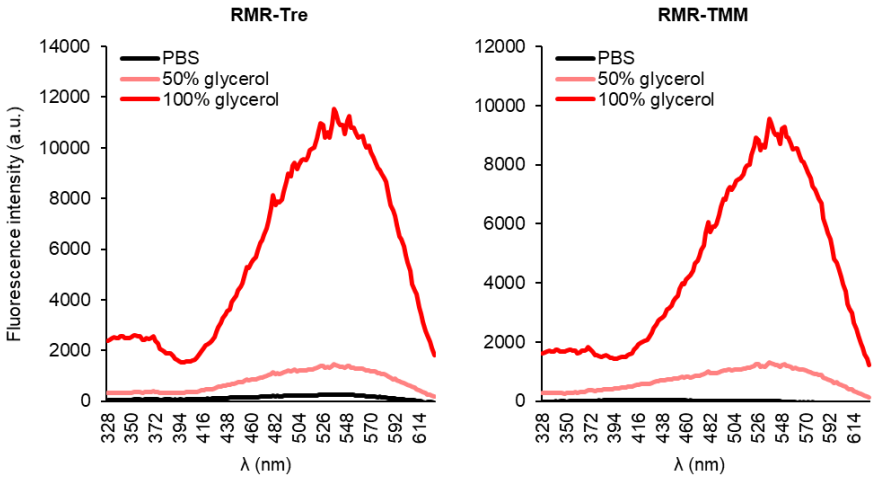


Figure S1. Excitation spectra for RMR-Tre and RMR-TMM measured in PBS (pH 7.4) containing 0–100% glycerol (emission measured at 660 nm). a.u., arbitrary units.

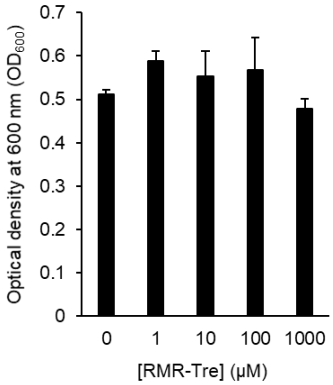


Figure S2. Growth inhibition evaluation of RMR-Tre in *M. smegmatis*. Bacteria were cultured in a plate reader for 4 h with shaking at 37 °C in the presence of 0–1000 μM of RMR-Tre, then optical density at 600 nm (OD_{600}) was measured. Error bars denote the standard deviation of three replicate experiments.

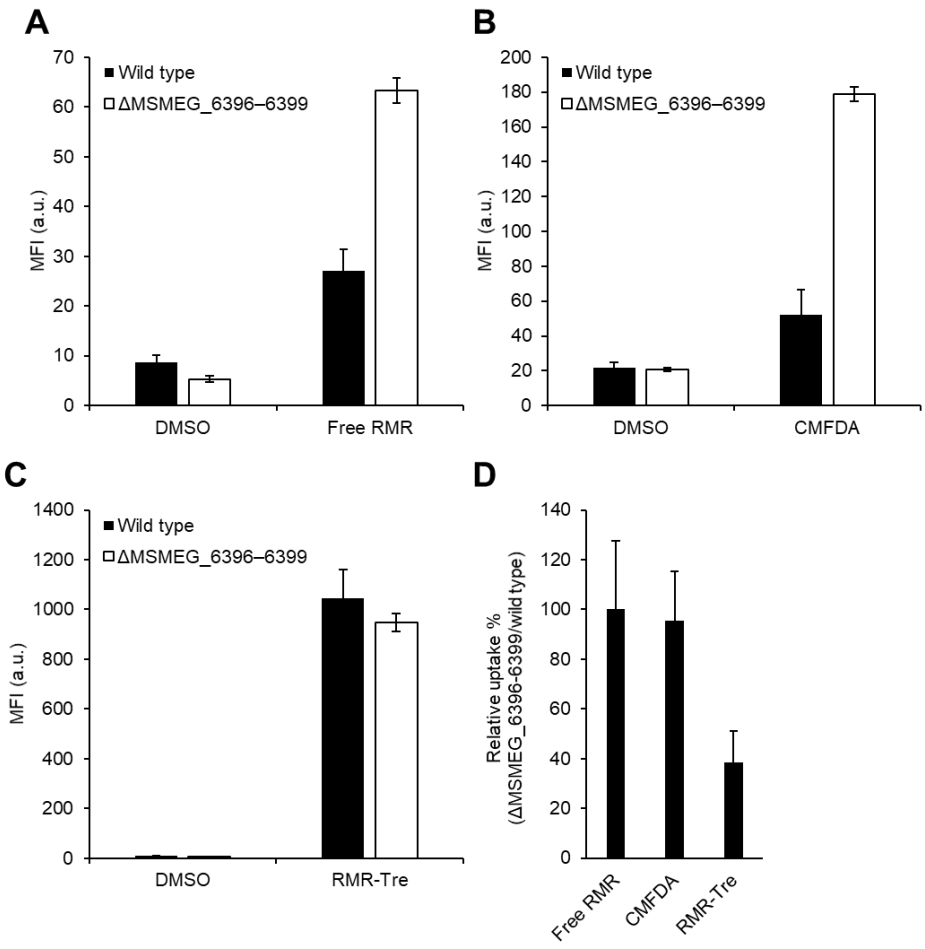


Figure S3. RMR-Tre labeling of *M. smegmatis* Ag85 triple knockout mutant *M. smegmatis* wild type and *M. smegmatis* ΔMSMEG_6396–6399[2] were incubated with shaking for 30 min in (A) free RMR (100 µM), (B) 5-chloromethylfluorescein diacetate (CMFDA, 5 µM), or (C) RMR-Tre (100 µM). Cells were washed thrice with PBSB, fixed with 4% formaldehyde, washed thrice with PBS, and analyzed by flow cytometry as described above. (A–C) Mean fluorescence intensity (MFI) in arbitrary units (a.u.) for each compound. (D) Percentage of MFI fold-change (relative uptake %) in mutant vs. wild-type bacteria, normalized to control compound free RMR (shown in manuscript).

Supplementary discussion: Ag85 activity is essential for viability in mycobacteria, so it is not possible to knock out all Ag85-encoding genes. Ag85 partial knockout mutants have increased mycomembrane permeability, while still retaining Ag85 incorporation machinery, which complicates their use to establish probe specificity.[3] To address this issue, we previously reported the use of control compounds to account for permeability effects in Ag85 partial knockout mutants.[4] Similarly, here we utilized both free RMR and CMFDA[5] as fluorescent permeability control compounds. As shown in (A) and (B), both of these control compounds were taken up more efficiently in *M. smegmatis* ΔMSMEG_6396–6399, presumably reflecting the increased permeability of this mutant. In (C), it is shown that RMR-Tre is taken up similarly in both strains. As shown in (D), when RMR-Tre labeling is expressed as relative uptake % normalized to control permeability probes, there was a ~60% reduction in the mutant compared to wild-type *M. smegmatis*. This result is consistent with our reported data utilizing this approach. Although this result does not completely rule out alternative incorporation route(s), taken together our data here and in Figure 3 are consistent with the proposed mechanism of Ag85-mediated incorporation.

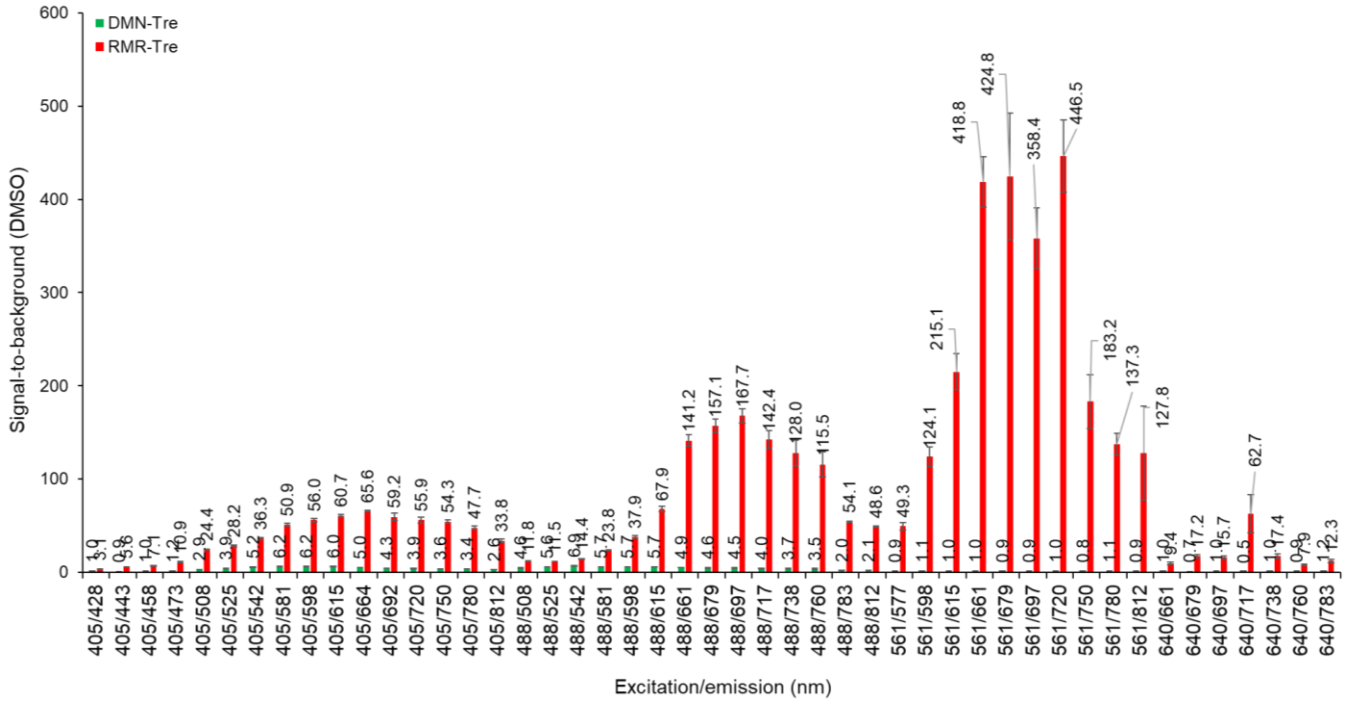


Figure S4. DMN-Tre and RMR-Tre labeling of *M. tuberculosis*. *M. tuberculosis* H37Rv was incubated for 18 h with shaking in DMN-Tre (100 µM), RMR-Tre (100 µM), or DMSO control, then cells were washed thrice with PBS containing 0.05% Tween-80, fixed with 4% formaldehyde, and analyzed by spectral flow cytometry. Signal-to-background values corresponding to each bar are shown above the bar. Green bars are DMN-Tre and red bars are RMR-Tre. Error bars denote the standard deviation of three replicate experiments.

II. Experimental procedures

General experimental for synthesis. Materials were obtained from commercial sources without further purification. Anhydrous solvents were obtained either commercially or from an alumina column solvent purification system. Analytical TLC was performed on glass-backed silica gel 60 Å plates (thickness 250 µm) and detected by UV or staining when appropriate. For compounds **1**–**3**, NMR spectra were obtained using 500 MHz or 600 MHz NMR systems and MS data were obtained using an Agilent 1200 HPLC-6130 MSD system. For RMR-Tre and RMR-TMM, NMR spectra were obtained using Varian Inova 500 or Bruker Avance Neo 500 systems and MS data were obtained using a Waters LCT premier XE system using reserpine as an internal standard for the lock mass. For free RMR, NMR spectra were obtained using a Varian Inova 600 and MS data were obtained using an Agilent 1200 HPLC-6130 MSD system. For NMR data, coupling constants (*J*) are reported in hertz (Hz) and chemical shifts are reported in ppm (δ) referenced to solvent peaks.

3-(6-(5-(2,2-Dicyanovinyl)thiophen-2-yl)-3,4-dihydroquinolin-1(2H)-yl)propanoic acid (free RMR, 2). To a 25 mL round bottom flask was added the known aldehyde 3-(6-(5-formylthiophen-2-yl)-3,4-dihydroquinolin-1(2H)-yl)propanoic acid (**1**) (589 mg, 1.867 mmol), malononitrile (296 mg, 4.48 mmol, 2.4 equiv), absolute ethanol (3.52 mL), and anhydrous pyridine (1 mL, dried over 4Å molecular sieves). The reaction was stirred at 50 °C overnight. The reaction was diluted with EtOAc (50 mL) and washed sequentially with 1N HCl (25 mL), H_2O (2 x 25 mL) and brine (25 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and the solvent was removed *in vacuo*. The product was purified via column chromatography (EtOAc:CH_2Cl_2:TFA/90:10:0.5) to yield pure **2** (free RMR, 632 mg, 93%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.68 (s, 1H), 7.60 (d, *J* = 4.1 Hz, 1H), 7.43 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.31 (d, *J* = 2.3 Hz, 1H), 7.25 (d, *J* = 4.2 Hz, 1H), 6.60 (d, *J* = 8.7 Hz, 1H), 3.70 (t, *J* = 7.1 Hz, 2H), 3.40 (t, *J* = 5.7 Hz, 2H), 2.79 (t, *J* = 6.3 Hz, 2H), 2.69 (t, *J* = 7.1 Hz, 2H), 1.98 (p, *J* = 6.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 176.84, 159.00, 150.25, 146.61, 141.12, 132.09, 127.89, 126.37, 123.40, 121.98, 120.20, 115.14, 114.26, 110.65, 73.30, 49.82, 46.95, 31.26, 28.03, 21.85. HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{20}H_{18}N_3O_2S$ 364.1114; Found 364.1115.

RMR-Tre. To a 5 mL round-bottom flask containing free RMR **2** (58.1 mg, 0.160 mmol) was added

anhydrous DMF (15 mL), TSTU (50.5 mg, 0.168 mmol), and DIEA (200 µL, 0.438 mmol). This mixture was stirred under argon for 30 min at room temperature to activate the acid. To the stirring mixture was added 6-amino-6-deoxy-α,α-D-trehalose (**3**, 53.5 mg, 0.157 mmol). After stirring at room temperature under nitrogen overnight, TLC indicated that the reaction was complete. The crude product was concentrated by rotary evaporation and purified by reverse phase chromatography on a Biotage Isolera One automated flash chromatography system (10 g C18 column; 5% CH_3CN in H_2O → 80% CH_3CN in H_2O; see gradient conditions and chromatogram below) to give RMR-Tre (65 mg, 60%) as a red solid. ^{1}H NMR (500 MHz, CD_3OD): δ 8.08 (s, 1 H), 7.71 (d, *J* = 4.5 Hz, 1 H), 7.45 (dd, *J* = 2.0, 8.5 Hz, 1 H), 7.37 (d, *J* = 4.0 Hz, 1 H), 7.33–7.32 (m, 1 H), 6.70 (d, *J* = 8.5 Hz, 1 H), 5.06 (d, *J* = 3.5 Hz, 1 H, H-1'), 4.99 (d, *J* = 3.5 Hz, 1 H, H-1), 3.87 (ddd, *J* = 2.6, 6.5, 10.0 Hz, 1 H, H-5'), 3.82–3.72 (m, 4 H, H-5, H-3, H-3', H-6a or H-6b), 3.70–3.61 (m, 3 H, RMR linker N-CH_2, H-6a or H-6b), 3.50 (dd. *J* = 2.5, 14 Hz, 1 H, H-6a' or H-6b'), 3.45 (dd, *J* = 3.8, 9.8 Hz, 1 H, H-2'), 3.42 (dd, *J* = 6.0, 14 Hz, 1 H, H-6a' or H-6b'), 3.40–3.36 (m, 3 H, H-2, RMR ring N-CH_2), 3.26 (t, *J* = 9.6 Hz, 1 H, H-4), 3.13 (t, *J* = 9.5 Hz, 1 H, H-4'), 2.76 (t, *J* = 6.0 Hz, 2 H, RMR benzylic CH_2), 2.59–2.47 (m, 2 H, RMR linker α-CH_2), 1.96–1.92 (m, 2 H, RMR ring CH_2). ^{13}C NMR (126 MHz, CD_3OD): 175.20, 160.94, 152.83, 148.66, 144.01, 133.59, 128.99, 127.80, 124.86, 123.47, 121.44, 116.77, 116.06, 112.39, 95.94, 95.86, 75.12, 74.70, 74.39, 73.77, 73.75, 73.70, 73.15, 72.47, 72.38, 63.19, 51.23, 50.35, 31.17, 29.47, 23.43. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{32}H_{39}N_4O_{11}S$ 687.2336; Found 687.2352.

RMR-TMM. To a 5 mL round-bottom flask containing free RMR **2** (14.2 mg, 0.0387 mmol) was added anhydrous DMF (1 mL), TSTU (11.7 mg, 0.0388 mmol), and DIEA (20 µL, 0.12 mmol). This mixture was stirred under nitrogen for 30 min at room temperature to activate the acid. To the stirring mixture was added dropwise a solution of 6-amino-6-deoxy-α,α-D-trehalose (**4**, 15.7 mg, 0.0307 mmol) in anhydrous DMF (1 mL). After stirring at room temperature under nitrogen overnight, TLC indicated that the reaction was complete. The crude product was concentrated by rotary evaporation and purified on a Biotage Isolera One automated flash chromatography system (10 g C18 column; 5% CH_3CN in H_2O → 80% CH_3CN in H_2O; see gradient conditions and chromatogram below) to give RMR-Tre (18.7 mg, 71%) as a red solid. ^{1}H NMR (500 MHz, CD_3OD): δ 8.09 (s, 1 H), 7.72 (s, *J* = 4.0 Hz, 1 H), 7.43 (dd, *J* = 2.3, 8.7 Hz,

1 H), 7.37 (d, *J* = 4.3 Hz, 1 H), 7.31 (d, *J* = 2.3 Hz, 1 H), 6.71 (d, *J* = 8.8 Hz, 1 H), 5.09 (d, *J* = 3.7 Hz, 1 H, H-1'), 5.07 (d, *J* = 3.7 Hz, 1 H, H-1), 4.36 (dd, *J* = 2.1, 12 Hz, 1 H, H-6a' or H-6b'), 4.19 (dd, *J* = 5.2, 12 Hz, 1 H, H-6a' or H-6b'), 4.01 (ddd, *J* = 1.9, 5.0, 10 Hz, 1 H, H-5'), 3.81 (ddd, *J* = 2.1, 5.2, 9.9 Hz, 1 H, H-5), 3.80–3.76 (m, 3 H, H-3', H-3, H-6a or H-6b), 3.68–3.64 (m, 3 H, RMR linker N-CH_2, H-6a or H-6b), 3.47 (dd, *J* = 3.8, 9.9 Hz, 1 H, H-2'), 3.46 dd, *J* = 3.8, 10 Hz, 1 H, H-2), 3.38 (t, *J* = 5.6 Hz, 2 H, RMR ring N-CH_2), 3.35–3.30 (m, 2 H, H-4', H-4), 3.11 (t, *J* = 7.0 Hz, 2 H, TMM acyl chain N-CH_2), 2.76 (t, *J* = 6.2 Hz, 2 H, RMR benzylic CH_2), 2.46 (t, *J* = 6.5 Hz, 2 H, RMR linker α-CH_2), 2.30 (t, *J* = 7.5 Hz, 2 H, TMM acyl chain α-CH_2), 1.94 (pent, *J* = 6.0 Hz, 2 H, RMR ring CH_2), 1.94 (pent, *J* = 6.0 Hz, 2 H, RMR ring CH_2), 1.56 (pent, *J* = 7.1 Hz, 2 H, TMM acyl chain β-CH_2), 1.42–1.34 (m, 2 H, TMM acyl chain N-$CH_2C\underline{H}_2$), 1.31–1.21 (m, 10 H, TMM acyl chain CH_2s). ^{13}C NMR (126 MHz, CD_3OD): δ 175.41, 174.04, 160.40, 152.35, 148.35, 143.59, 133.07, 128.41, 127.28, 124.19, 122.90, 120.90, 116.28, 115.55, 112.09, 95.19, 95.06, 74.63, 74.45, 73.89, 73.21, 73.18, 72.67, 71.91, 71.89, 71.41, 64.37, 62.63, 50.55, 49.85, 40.57, 35.01, 34.69, 30.50, 30.41, 30.36, 30.26, 30.16, 29.07, 27.99, 26.04, 22.93. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{42}H_{57}N_4O_{13}S$ 857.3643; Found 857.3626.

Bacterial strains, media, and reagents. The bacterial strains used in this work included *Mycobacterium smegmatis* mc^{2}155 wild type, *M. smegmatis* ΔMSMEG_6396–6399,[2] *M. tuberculosis* H37Rv, *M. tuberculosis* H37Rv mc^{2}6206 (Δ*panCD* Δ*leuCD*)[6] (an avirulent nutrient auxotroph that can be handled at biosafety level 2), *Corynebacterium glutamicum* 534, *Escherichia coli* K12 MG1655, and *Bacillus subtilis* 168. *M. smegmatis* and *M. tuberculosis* were cultured in Middlebrook 7H9 liquid medium supplemented with ADC (for *M. smegmatis*) or OADC (for *M. tuberculosis*), 0.5% glycerol, and 0.05% Tween-80. *M. tuberculosis* H37Rv mc^{2}6206 growth medium was additionally supplemented with 50 μg/mL leucine and 24 μg/mL pantothenate.[6] *C. glutamicum*, *E. coli*, and *B. subtilis* were cultured in LB liquid medium. All bacteria were cultured at 37 °C, except *C. glutamicum*, which was cultured at 30 °C. Stock solutions of free RMR and RMR-Tre were prepared in sterile in dimethyl sulfoxide (DMSO) at 10 mM and stored at –20 °C. Stock solutions of DMN-Tre and FITre (10 mM) and trehalose (1 M) were prepared in milli-Q water, sterile-filtered (0.2 μm), and stored at –20 °C unless otherwise noted. Prior to usage in labeling experiments, stock solutions were diluted to the desired concentration.

General procedure for bacterial labeling. Bacterial starter cultures were generated by inoculating a single isolated colony from a freshly streaked LB agar plate into 3 mL liquid medium in a culture tube. Starter cultures were incubated at 37 °C (or 30 °C for *C. glutamicum*) with shaking until reaching mid-logarithmic phase and then diluted with liquid medium to the desired density for initiating experiments. Labeling experiments were performed either in 96-well plate format or in aerated culture tubes. For experiments in 96-well plate format, bacteria were mixed with liquid medium and probe stock solution in sterile flat-bottom 96-well plates to achieve the desired cell density and probe concentration at a final volume of 200 µL. The final DMSO concentration for probes stocked in DMSO (and their corresponding controls) was 1%. Plates were incubated at 37 °C (or 30 °C for *C. glutamicum*) with shaking in a Tecan plate reader (Infinite F200 PRO operated by Tecan iControl software) until the desired endpoint (typical culture time was 4 h, unless otherwise stated). For wash steps, cells were transferred to a v-bottom 96-well plate, centrifuged (3 200 xg, 5 min, room temperature), and washed with PBS 1x containing 0.5% bovine serum albumin (PBSB) three times. For some experiments, cells were then fixed in 4% formaldehyde in PBS and washed thrice with PBS. Finally, cells were prepared for analysis using flow cytometry or fluorescence microscopy.

Flow cytometry. After fluorescent labeling of bacteria according to the above general procedure, cells were re-suspended in 200 µL of PBS, and 10–100 µL of sample were added to 5 mL polystyrene Falcon tubes (BD Biosciences) containing 500 µL of PBS, and analyzed using flow cytometry. Unless otherwise stated, flow cytometry was performed on a BD Biosciences FACSAria II flow cytometer. Fluorescence data were collected for 10,000 cells at an event rate of 500–1,000 events/sec and processed using BD FACSDIVA 8.0.1. All flow cytometry experiments were performed with three replicate samples, and the data shown are representative of at least two independent experiments. Scatter-gated fluorescence analysis was used to obtain mean fluorescence intensities with doublet discrimination. Spectral flow cytometry to evaluate *M. tuberculosis* labeling was performed similarly on a 4-laser Cytek Aurora and data was analyzed using FlowJo.

Concentration dependence of labeling. *M. smegmatis* cultures at an optical density at 600 nm (OD_{600nm}) of 0.5 in a 7H9 liquid medium were treated with increasing concentrations of RMR-Tre or 1%

DMSO and incubated with shaking for 4 h. The cells were pelleted by centrifugation, washed, re-suspended in PBS, and analyzed by flow cytometry.

Time dependence of labeling. *M. smegmatis* cultures at OD_{600nm} of 0.5 in a 7H9 liquid medium were treated with RMR-Tre to a final concentration of 100 μM and incubated with shaking. The cells were pelleted at the indicated time points by centrifugation, washed thrice with PBSB, fixed with 4% formaldehyde, washed thrice with PBS, and analyzed by flow cytometry.

Heat-killing. 1 mL of *M. smegmatis* culture in 7H9 liquid medium at OD_{600nm} of 0.5 in a 1.5 mL sterile microcentrifuge tube was heated at 95 °C with shaking for 30 min to heat-kill bacteria, or left unheated. The cells were pelleted and re-suspended in 1 mL of 7H9 liquid medium. Next, 200 μL aliquots of heat-killed and live bacteria were treated with RMR or RMR-Tre to a final concentration of 100 μM and incubated with shaking for 4 h. The cells were pelleted by centrifugation, washed thrice with PBSB, fixed with 4% formaldehyde, washed thrice with PBS, and analyzed by flow cytometry.

Trehalose competition. *M. smegmatis* cultures at OD_{600nm} of 0.5 in 7H9 liquid medium were treated with RMR-Tre to a final concentration of 100 μM and final trehalose concentrations of 0, 0.1, 1, 10, or 100 mM, and incubated with shaking for 1 h. Cells were washed thrice with PBSB, fixed with 4% formaldehyde, washed thrice with PBS, and analyzed by flow cytometry.

Ebselen inhibition. *M. smegmatis* cultures at OD_{600nm} of 0.5 in 7H9 liquid medium were treated with ebselen at a final concentration of 100 μg/mL and incubated with shaking for 3 h. Then RMR-Tre was added to a final concentration of 100 μM and incubated for an additional 1 h. Cells were washed thrice with PBSB, fixed with 4% formaldehyde, washed thrice with PBS, and analyzed by flow cytometry.

Labeling in Ag85 triple knockout mutant. *M. smegmatis* wild type and *M. smegmatis* ΔMSMEG_6396–6399 were incubated with shaking for 30 min in 100 μM RMR-Tre, or as controls, 100 μM free RMR or 5 μM 5-chloromethylfluorescein diacetate (CMFDA). Cells were washed thrice with PBSB, fixed with 4% formaldehyde, washed thrice with PBS, and analyzed by flow cytometry.

Bacterial species specificity of labeling. Separate cultures of *M. smegmatis* (in 7H9 liquid medium), *C. glutamicum, E. coli,* and *B. subtilis* (in LB liquid medium) at OD_{600nm} of 0.5 were treated with free RMR or RMR-Tre at a final concentration of 100 μM and incubated with shaking for 4 h. Cells were

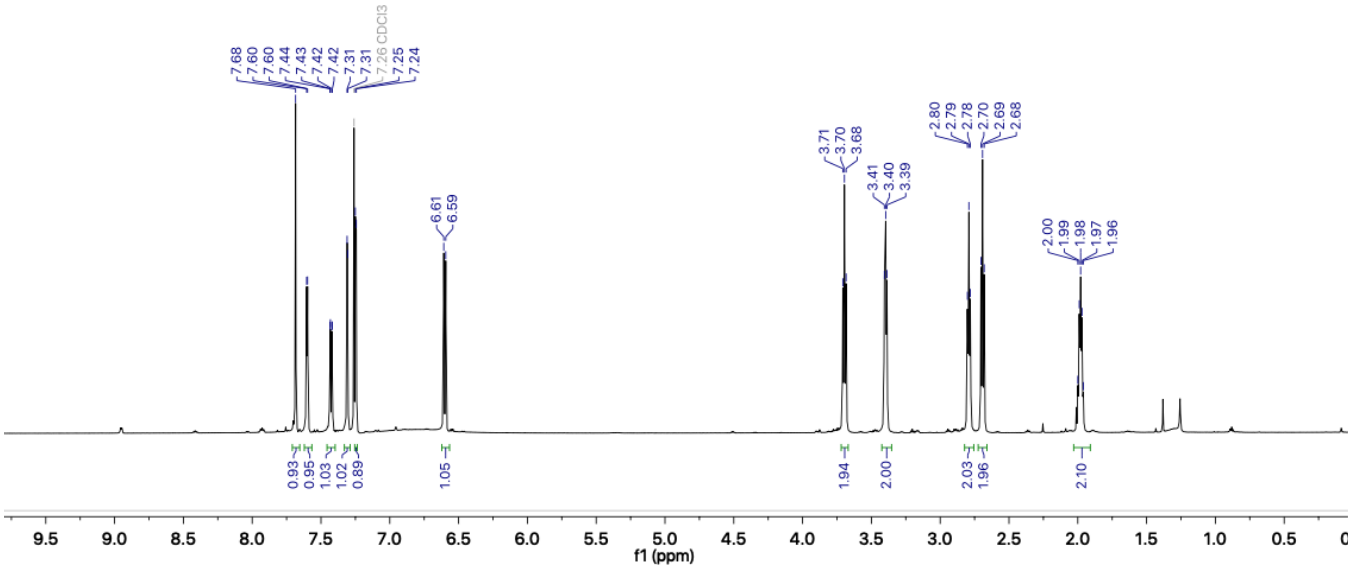
washed thrice with PBSB, fixed with 4% formaldehyde, washed thrice with PBS, and analyzed by flow cytometry.

No-wash fluorescence microscopy. 500 µL cultures of *M. smegmatis* in 7H9 growing in liquid medium were treated with free RMR, RMR-Tre, DMN-Tre, or FITre at final concentrations of 100 µM, or left untreated as a control, and incubated at 37 °C with shaking. At time points of 10, 20, 40, and 60 min, 10 µL aliquots were placed directly onto a microscope slide, lightly spread into a thin layer using the edge of a coverslip and allowed to air dry briefly in the dark. Fluoromount-G mounting medium (SouthernBiotech) was applied, then coverslips were placed over the sample and immobilized with adhesive. Microscopy was immediately carried out using an EVOS FL (Life Technologies) inverted microscope equipped with a 100 × 1.4 numerical aperture Plan-Apochromat oil immersion lens. Fluorescence imaging was performed using GFP (maximum excitation/emission = 470/510 nm) and RFP (maximum excitation/emission = 531/593 nm) LED light cubes. Images were captured with a Sony ICX445 CCD camera. Image acquisition and processing were performed identically for all test and control samples being compared. Imaging data shown are representative of at least two independent experiments.

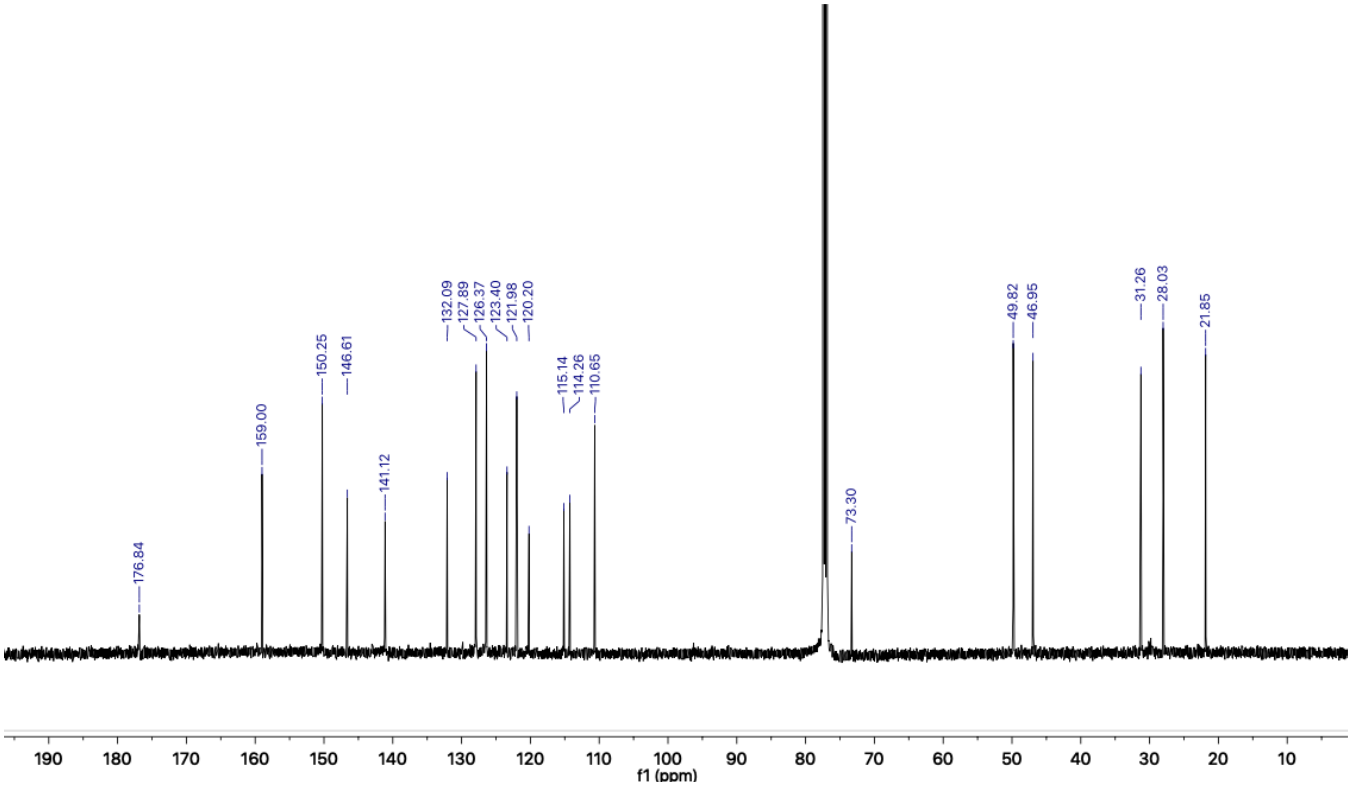
M. tuberculosis **drug-susceptibility evaluation.** *M. tuberculosis* H37Rv $mc^2$6206 strains transformed with or without an integrating, kanamycin-resistant plasmid were grown to early-log phase then incubated or not with rifampicin or kanamycin at the World Health Organization (WHO)-defined critical concentrations (1 µg/mL and 2.5 µg/mL respectively) for 24 hours followed by incubation in 100 µM RMR-Tre for an additional 18 hours. *M. tuberculosis* was inactivated by direct 1:1 addition of 10% formalin. Without washing, fixed *M. tuberculosis* were analyzed by flow cytometry.

III. NMR spectra, mass spectra, and LC chromatograms

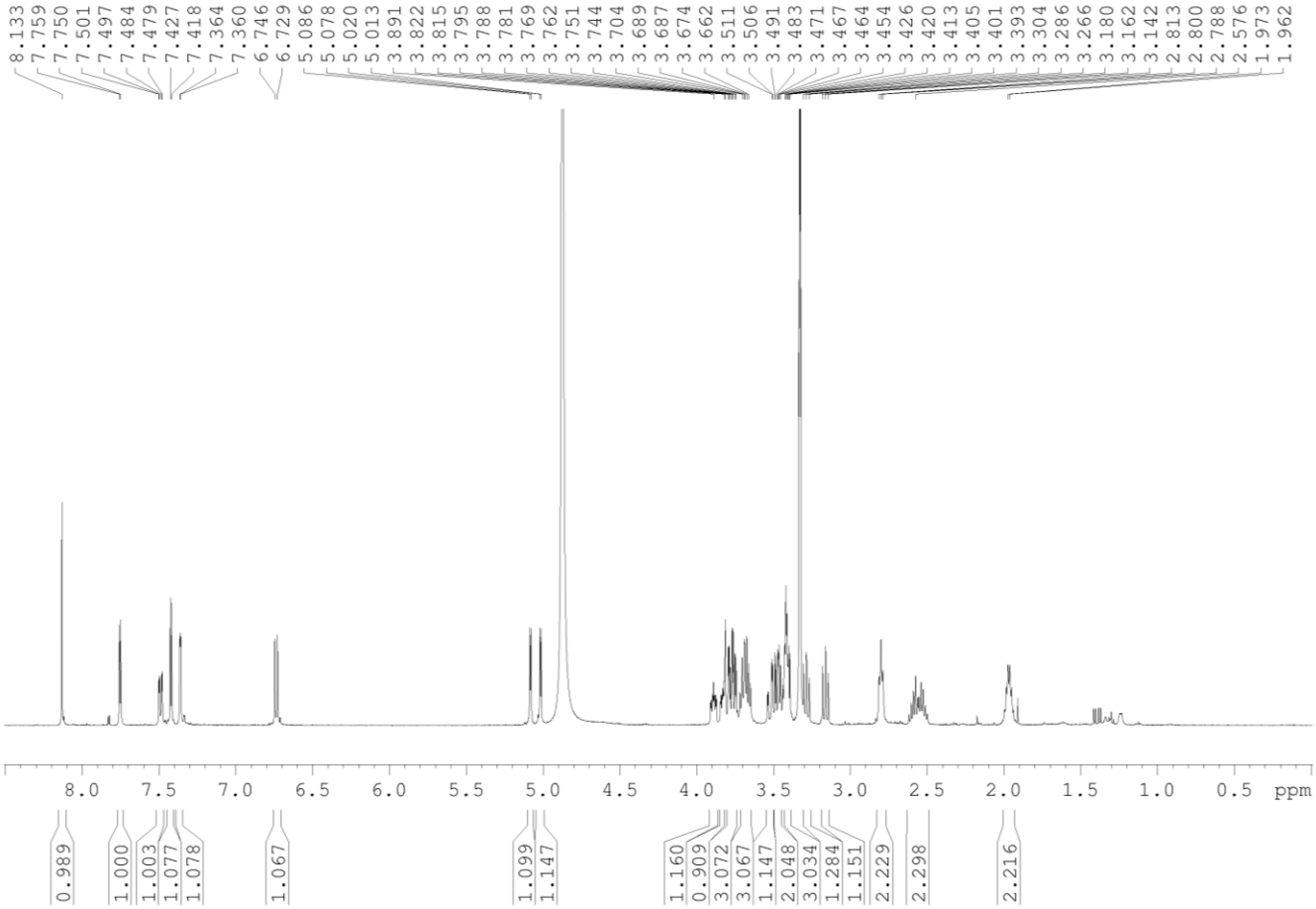
Free RMR (2) ^{1}H NMR (600 MHz, $CDCl_3$)



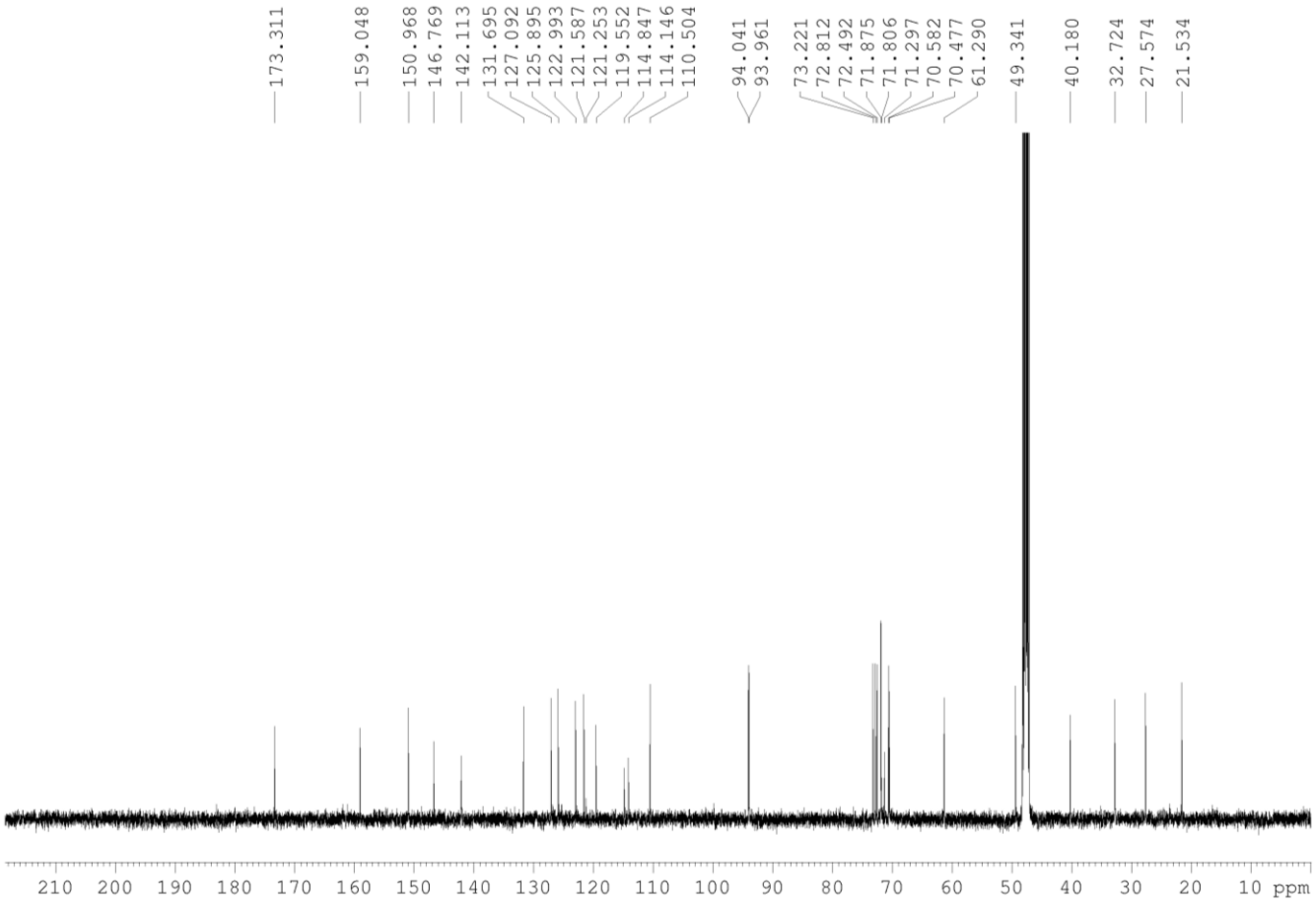
Free RMR (2) ^{13}C NMR (150 MHz, $CDCl_3$)



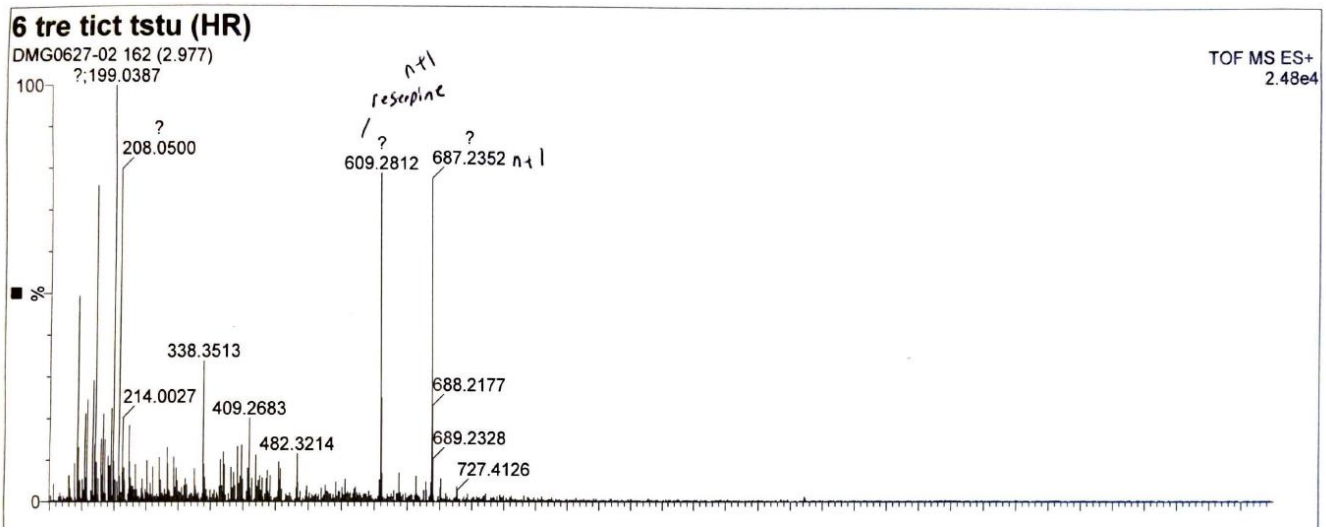
RMR-Tre ^{1}H NMR (500 MHz, CD_3OD)



RMR-Tre ^{13}C NMR (126 MHz, CD_3OD)

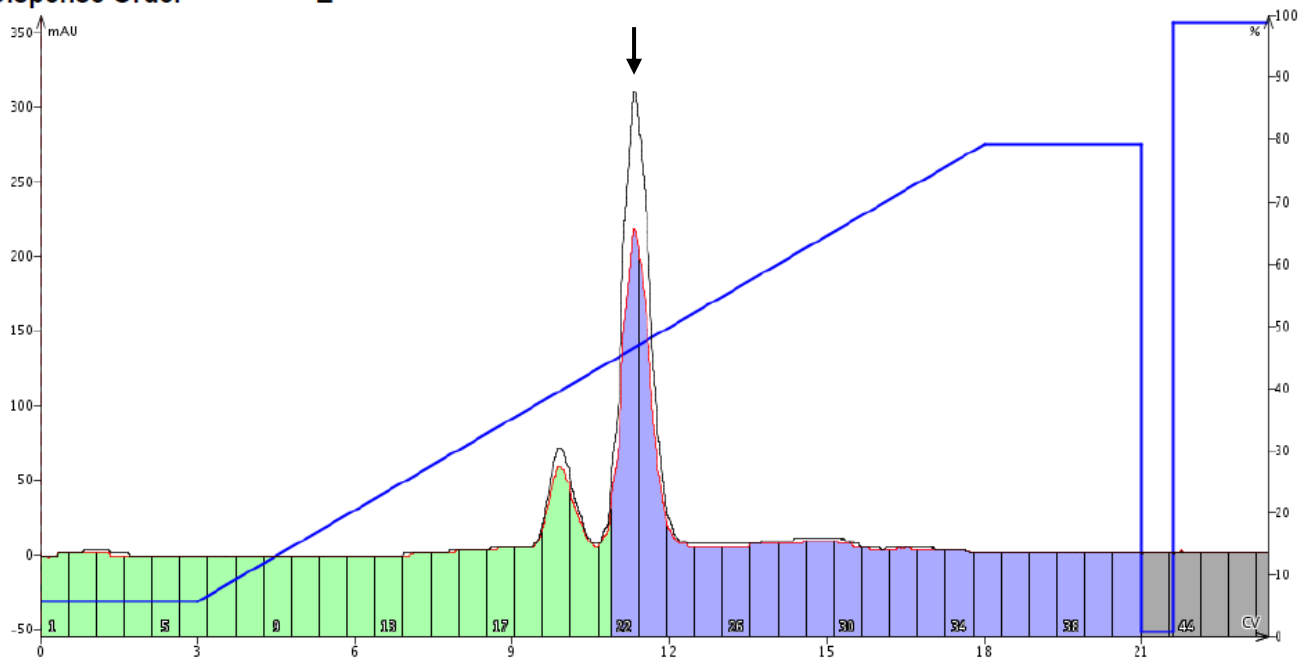


RMR-Tre HR ESI MS



RMR-Tre LC purification chromatogram

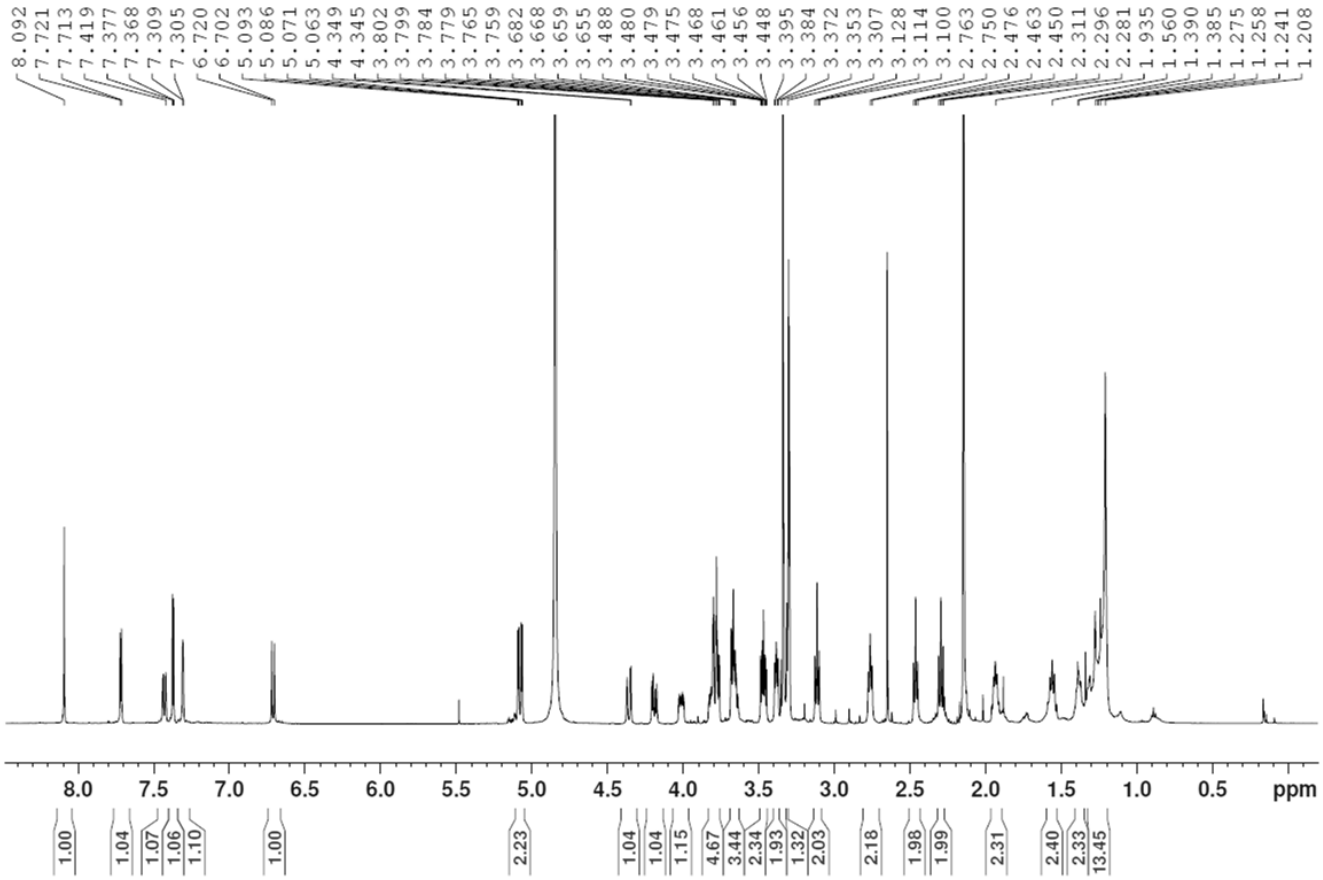
Cartridge	SNAP 10g	**Detection Mode**	UV1
Flowrate	12 ml/min	**UV1 (Collection)**	254 nm
Solvent A	Water	**UV2 (Monitor)**	280 nm
Solvent B	Acetonitrile	**Collect All**	On
Rack Type	13x100 mm		
Max Fraction Volume	8 ml		
Dispense Order	Z		



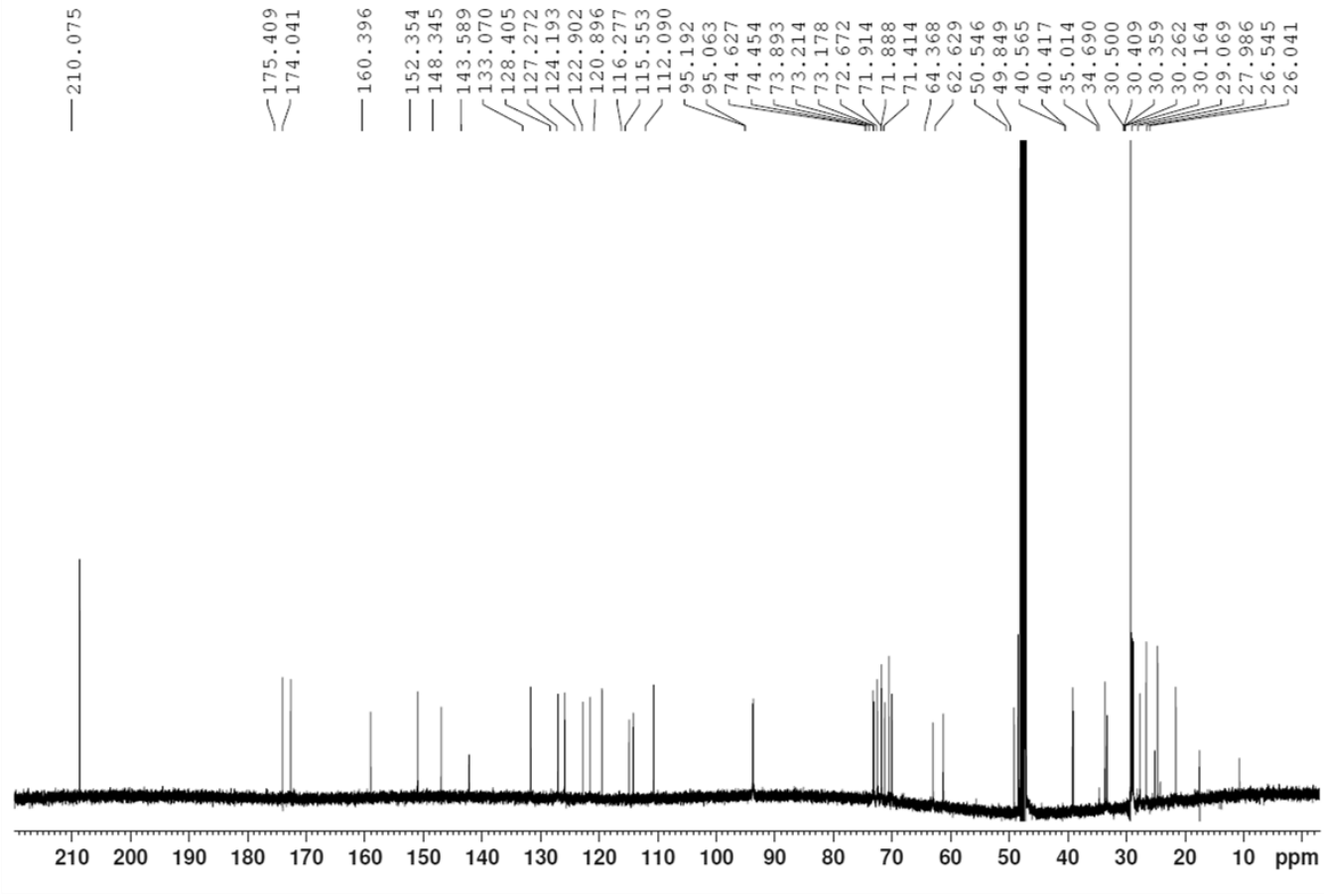
Gradient

	Solvents	Mix	Length (CV)	
Equil.	A/B	5%	3.0	flowrate 12 ml/min
1	A/B	5%	3.0	
2	A/B	5% - 80%	15.0	
3	A/B	80%	3.0	

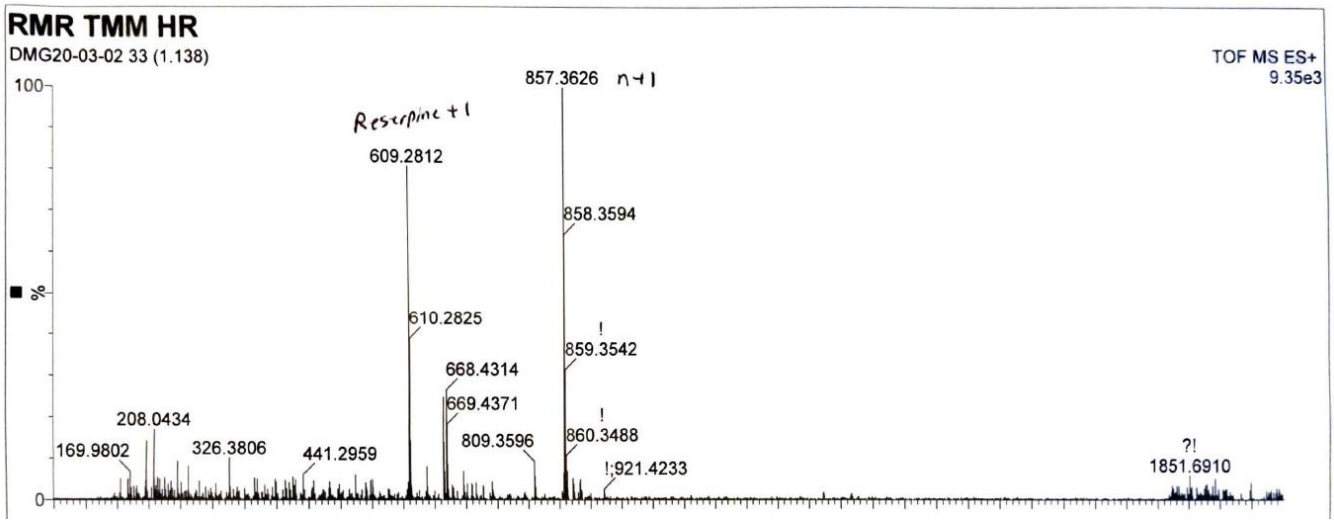
RMR-TMM ^{1}H NMR (500 MHz, CD_3OD)



RMR-TMM ^{13}C NMR (126 MHz, CD_3OD)

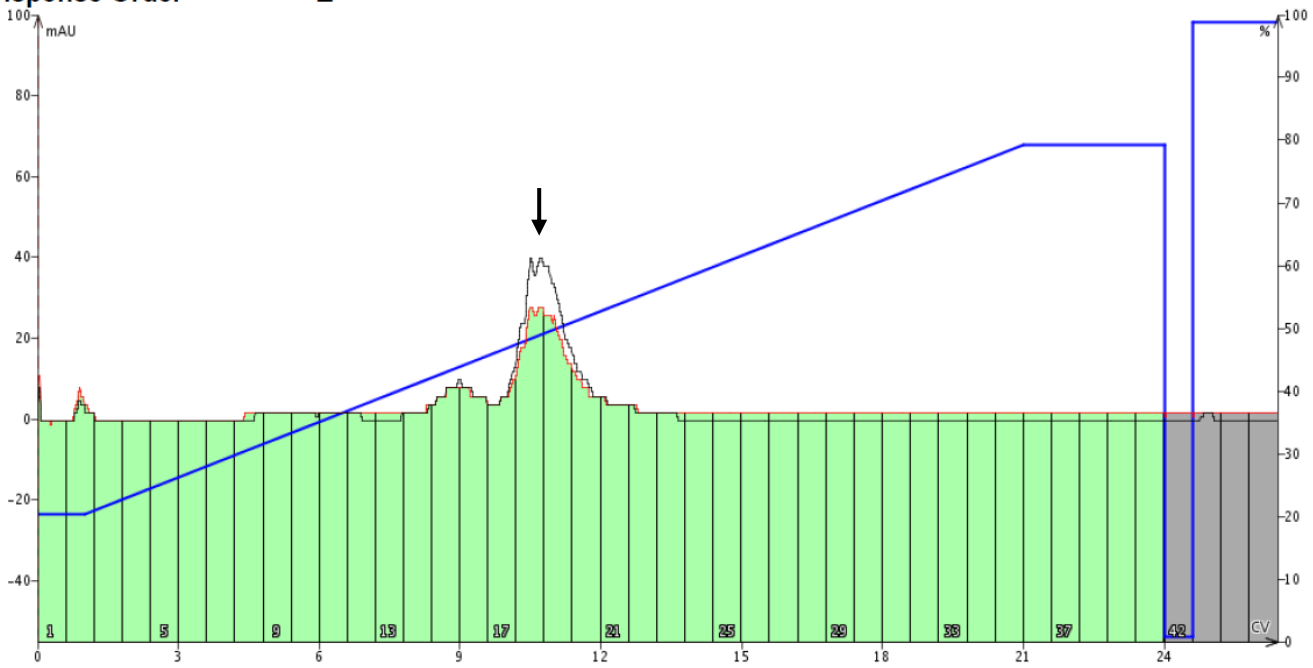


RMR-TMM HR ESI MS



RMR-TMM HPLC purification chromatogram

Cartridge	SNAP 10g	**Detection Mode**	UV1
Flowrate	12 ml/min	**UV1 (Collection)**	254 nm
Solvent A	Water	**UV2 (Monitor)**	280 nm
Solvent B	Acetonitrile	**Collect All**	On
Rack Type	13x100 mm		
Max Fraction Volume	9 ml		
Dispense Order	Z		



Gradient

	Solvents	Mix	Length (CV)	
Equil.	A/B	20%	3.0	flowrate 12 ml/min
1	A/B	20%	1.0	
2	A/B	20% - 80%	20.0	
3	A/B	80%	3.0	

IV. References

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