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# Development of Cyclobutene- and Cyclobutane-Functionalized Fatty Acids with Inhibitory Activity against *Mycobacterium tuberculosis*

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Eleven fatty acid analogues incorporating four-membered carbocycles (cyclobutenes, cyclobutanes, cyclobutanones, and cyclobutanols) were investigated for the ability to inhibit the growth of *Mycobacterium smegmatis* (*Msm*) and *Mycobacterium tuberculosis* (*Mtb*). A number of the analogues displayed inhibitory activity against both mycobacterial species in minimal media. Several of the molecules displayed potent levels of inhibition against *Mtb*, with MIC values equal to or below those observed with the anti-tuberculosis drugs D-cycloserine and isoniazid. In contrast, two of the analogues that display the greatest activity against *Mtb* failed to inhibit *E. coli* growth under either set of conditions. Thus, the active molecules identified herein may provide the basis for the development of anti-mycobacterial agents against *Mtb*.

## Introduction

Tuberculosis (TB) resulting from *Mycobacterium tuberculosis* (*Mtb*) infection remains one of humankind's most significant disease threats. Based on skin test reactivity, it is estimated that one-third of the world's population has been exposed, resulting in approximately nine million incident cases annually and 1.4 million deaths (2010 data).[1] A number of therapeutic agents have been developed, but current treatment regimens require patients to take multiple drugs over a period of months and are associated with significant side effects.[2] The result is frequent patient noncompliance, leading to relapses and the emergence of drug resistance, with a high fraction of active cases now involving multidrug-resistant (MDR, XDR) strains.[1] There is, therefore, a need for new classes of therapeutics for TB.[3] Importantly, the lethal target and/or mechanism of action should also be novel to avoid established mechanisms of resistance and to provide synergy with current treatments.

Much of the hardiness and drug resistance of mycobacteria is due to an unusually thick lipid cell wall that contains a significant proportion of mycolic acids, a unique class of $C_{54}$–$C_{63}$ branched-chain fatty acids.[4] A number of existing treatments for *Mtb*, exemplified by isoniazid and ethionamide, disrupt mycolic acid biosynthesis by inhibiting important biosynthetic enzymes.[5] Mycobacteria incorporate intact $C_{16}$ and $C_{18}$ fatty acid skeletons as biosynthetic feed stocks, and given the prior evidence for the incorporation of modified fatty acids into mycolic acid biosynthesis,[6] we hypothesized that hijacking this pathway could provide a unique approach for the development of potential therapeutics.[4] Toward this end, we proposed to investigate specifically functionalized fatty acids for their ability to limit mycobacterial growth.[7] As our initial steps toward validating this hypothesis, we investigated two series of fatty acid analogues (Figure 1). One series (compounds **1**–**4**) is based on a decenoic acid (10:1) framework, and a second series (compounds **5**–**11**) is based on the frameworks of oleic or elaidic acids (18:1). The analogues preserve the approximate lengths and cross-sections of the "parent" fatty acids while incorporating four-membered carbocycles into the backbone. Herein we report that several of these analogues display significant anti-mycobacterial activity against *Mtb*.

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## Results and Discussion

### Substrate preparation

The only previous description of molecules included in this study was the preparation of a mixture of the methyl esters of **5** and **10** via a nonselective radical decarboxylation.[8] In search of an efficient, general, and stereospecific approach to incorporation of four-membered rings onto a fatty acid backbone, we were drawn to methodology for modification of dichlorocyclobutanones.[9] Our approach is illustrated in detail for the prepa-

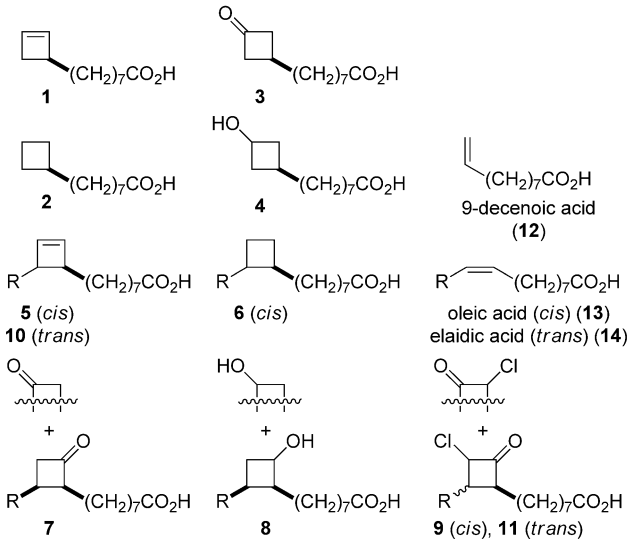
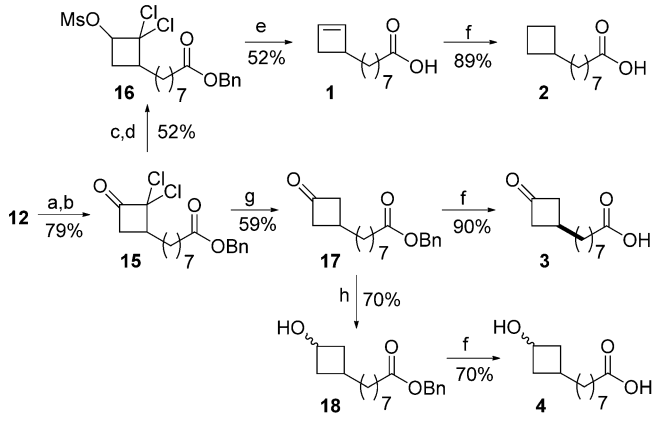



**Figure 1.** Analogues investigated, along with structures of oleic (*cis*-18:1), elaidic (*trans*-18:1) and decenoic (10:1) acids; R = octyl.

ration of analogues **1**–**4** (Scheme 1). The benzyl ester of 9-decenoic acid underwent cycloaddition with dichloroketene to afford one major dichlorobutanone, **15**,[10] which was only moderately stable toward purification, and was therefore directly reduced with sodium borohydride to furnish a mixture of stereoisomeric 2,2-dichloro-1-cyclobutanols. The choice of sodium borohydride was for convenience, as a similar product mixture is available using other reducing reagents such as $BH_3$·THF and $Na(OAc)_3BH$. The alcohols were converted into the corresponding methanesulfonate (mesylate) esters **16**. Reaction with sodium in ammonia resulted in simultaneous reductive cleavage of the benzyl ester and fragmentation/reduction of the β-chloro methanesulfonate to furnish cyclobutene **1**. Hydrogenation of **1** cleanly generated cyclobutane **2**. Alternatively, dehalogenation of the initial cycloadduct **15** with zinc




**Scheme 1.** Preparation of analogues **1**–**4** in the $C_{10}$ series. *Reagents and conditions*: a) DMAP, DCC, BnOH; b) Zn dust (5 equiv), $Cl_3CCOCl$ (2.5 equiv), RT, $Et_2O$; c) $NaBH_4$, 2-propanol; d) MsCl, $Et_3N$; e) $Na/NH_3$, −78 to −33 °C; f) $H_2$, Pd/C; g) Zn (5 equiv), AcOH; h) $NaBH_4$, MeOH. See Experimental Section for reaction details.

in acetic acid, followed by deprotection of the benzyl ester with $Pd(C)/H_2$, furnished cyclobutanone **3**. Hydride reduction of the intermediate ketone with hydride prior to hydrogenolysis generated cyclobutanol **4** as a mixture of diastereomers at the newly created alcohol stereocenter.

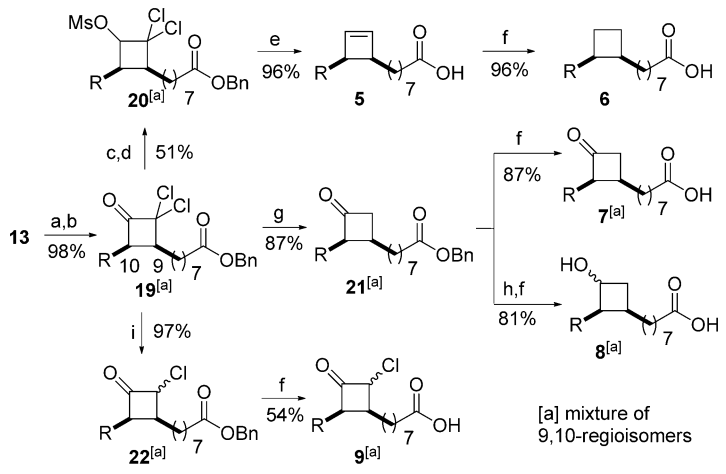
The preparation of analogues incorporating the four-membered ring carbocycles onto a *cis*-$C_{18}$ framework is illustrated in Scheme 2. In this case, the initial cycloaddition with benzyl oleate produced an inseparable mixture of regioisomeric dichlorocyclobutanones **19**. The cycloadducts, which were prone to decomposition during purification, were directly reduced to furnish a mixture of regioisomeric 2,2-dichloro-1-cyclobutanols. The corresponding methanesulfonate (mesylate) esters **20** underwent reduction/fragmentation as described above to furnish cyclobutene **5**,[8] which could be hydrogenated to cyclobutane **6**. The related cyclobutanone **7** and cyclobutanol **8** were prepared from the dichlorocyclobutanone in a similar manner as described earlier for the $C_{10}$ series. Alternatively, controlled dehalogenation of the dichlorocyclobutanone cycloadduct with stoichiometric Zn or Zn(Cu) in acetic acid furnished a mixture of regioisomeric monochlorocyclobutanones **22** contaminated by small amounts of dichloroketone (starting material) and cyclobutanone **3** (over-reduction).[11] Hydrogenolysis of the monochlorocyclobutanone as before, furnished analogue **9**.

The ready availability of elaidic acid, a $C_{18}$ fatty acid containing a *trans*-9,10 alkene, made it possible to investigate the influence of stereoisomers (Scheme 3). Beginning with elaidic acid (**14**) we were able to synthesize cyclobutene **10** and monochlorocyclobutanone **11** in a manner analogous to that described previously for oleate-derived substrates **5** and **9**. The only major difference from the route employed in Scheme 2 was the need to use activated Zn or Zn/Cu for the initial ketene generation/cycloaddition.[12]

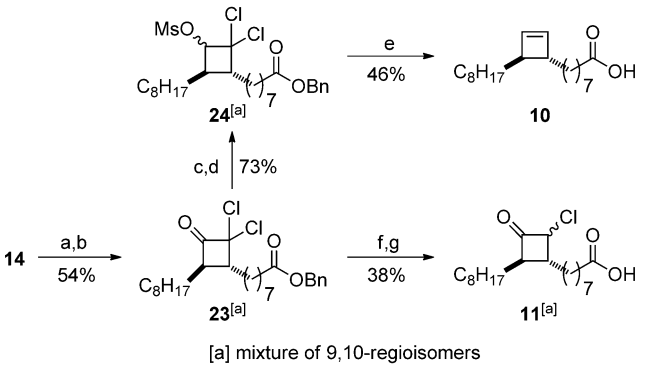
### Inhibitor stability and solubility

Analogues **1**–**11** were each tested for thermal stability using differential scanning calorimetry (DSC) in tandem with thermal gravimetric analysis (TGA); details are provided in the Supporting Information. Although we originally had concerns for the stability of the cyclobutene-containing analogues (**1**, **5**, and **10**) toward thermally induced cycloreversion,[13] we observed no decomposition of any analogue below 100 °C; most of the new analogues were stable to ≥150 °C.

One-dimensional (1D) $^1H$ NMR spectroscopy was used to verify the aqueous stability and solubility for each of the eleven analogues. An example series is illustrated in Figure 2; more detailed data are provided in the Supporting Information. The results demonstrate that the analogues are stable in aqueous buffer. All of the analogues based on a $C_{18}$ scaffold (compounds **5**–**11**) display evidence of aggregation at all concentrations tested (down to 100 μM). In contrast, analogues **1**–**4**, based on the $C_{10}$ backbone, were not observed to aggregate even at millimolar concentrations (Table 1).




**Scheme 2.** Synthesis of analogues **5**–**9**. *Reagents and conditions*: a) DMAP, DCC, BnOH; b) Zn dust (5 equiv), $Cl_3CCOCl$ (2.5 equiv), $Et_2O$; c) $NaBH_4$, 2-propanol; d) MsCl, $Et_3N$; e) $Na/NH_3$, −78 to −33 °C; f) $H_2$, Pd/C EtOAc; g) Zn (5 equiv), AcOH; h) $NaBH_4$, MeOH, −10 °C; i) Zn (1.1 equiv), AcOH. R = octyl. See Experimental Section for reaction details.




**Scheme 3.** Synthesis of analogues **10** and **11**. *Reagents and conditions*: a) DMAP, DCC, BnOH; b) Zn(Cu) (5 equiv), $Cl_3CCOCl$ (2.5 equiv), RT, $Et_2O$; c) $NaBH_4$, 2-propanol; d) MsCl, $Et_3N$; e) $Na/NH_3$, −78 to −33 °C; f) Zn (1.1 equiv), AcOH; g) $H_2$, Pd/C EtOAc. See Experimental Section for reaction details.

**Table 1.** Critical micellar coefficient (CMC) of analogues under assay conditions.[a]

| CMC | ≤ 100 μM | ≥ 1000 μM |
|---|---|---|
| analogues | **5**–**11** | **1**–**4** |

[a] Designated concentration or range indicates onset of aggregation observed in $^1$H NMR spectra of buffer solutions; see Experimental Section for details.

### MIC determination

MICs were determined against the following bacteria: *Escherichia coli* (*E. coli*) wild-type strain G58-1; *M. smegmatis* (*Msm*) strain $mc^2155$, a non-pathogenic mycobacteria used as a model for *Mtb* to analyze processes that are likely to be conserved in the genus; and two *Mtb* strains (CDC1551 and H37Rv). Kanamycin (Kan) was used as a positive inhibition control for *E. coli*. Isoniazid (Inh), one of the main first-line drugs used to treat TB, and D-cycloserine (DCS), a second-line clinically used TB drug, were employed as controls that would allow benchmarking against other inhibitors. Results for the fatty acid analogues are listed in Table 2.

Neither of the two analogues, **1** (a $C_{10}$-cyclobutene) nor **6** (a $C_{18}$-cyclobutane), tested against *E. coli* were inhibitory in rich (LB broth) or minimal media. Interestingly, the susceptibility of *E. coli* toward the control drugs (DCS, Inh, and Kan) was greater in minimal media, as indicated by the significantly lower MIC values. Preliminary studies indicated that *Msm* was not susceptible to compounds **7** and **10** in complete Middlebrook 7H9 broth supplemented with either Tween or Tyloxapol (data not shown). In this context, a potential interfering compound is bovine serum albumin (BSA), which has been shown to bind to fatty acids.[14] Indeed, preliminary tests using *Msm* and compound **7** indicate that increasing concentrations of BSA significantly increase the MIC value (unpublished results). Thus, the entire set of compounds synthesized was tested in minimal media against *Msm* and the two *Mtb* strains. Under these conditions, none of the compounds demonstrated significant activity against *Msm*. However, four (**1**, **2**,

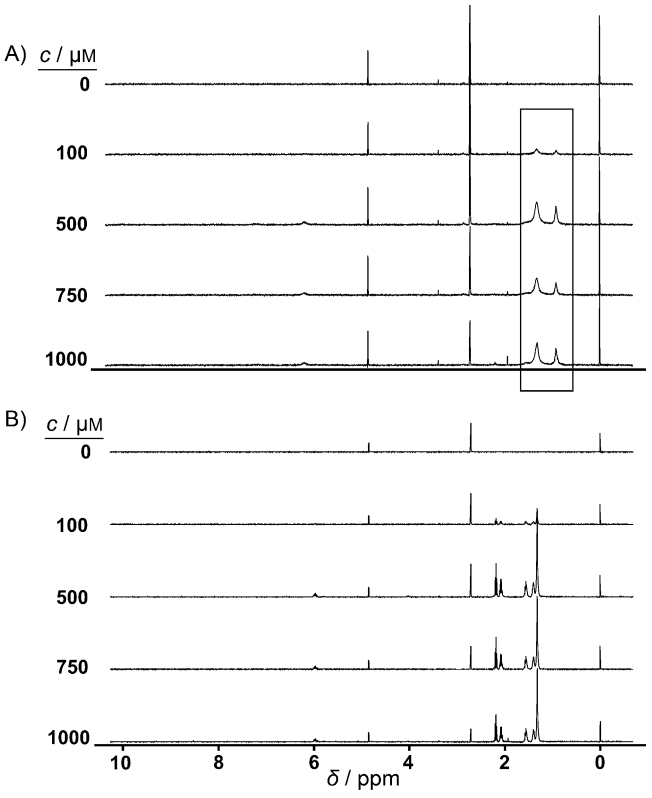



**Figure 2.** 1D $^1$H NMR spectra of compounds A) **5** and B) **1** were inspected for evidence of micelle formation (peak broadening) by comparing peak widths relative to TMSP (standard). Peak widths for **5** demonstrated potential micelle formation or aggregation over the range of tested concentrations (boxed region). No micelle formation was observed for compounds **1**–**4** between 100 and 1000 μM.

**Table 2.** MIC values against *E. coli*, *Msm*, and *Mtb*.

| Compd | Scaffold | Functionality | MIC [$\mu g\,mL^{-1}$/($\mu M$)][a] | | | | |
|---|---|---|---|---|---|---|---|
| | | | *E. coli*[b] | | *Msm*[c] | *Mtb* CDC1551 | *Mtb* H37Rv, week 7 |
| | | | LB[d] | MM[e] | MM[e] | MM[e] | MM[e] |
| **1** | $C_{10}$ | alkene | >512/(>2608) | >512/(2608) | 512/(2608) | 16/(82) | 16/(82) |
| **2** | $C_{10}$ | alkane | – | – | 512/(2582) | 4/(20) | 4/(20) |
| **3** | $C_{10}$ | ketone | – | – | 256/(1206) | 128/(603) | 256/(1206) |
| **4** | $C_{10}$ | alcohol | – | – | 128/(597) | 64/(299) | 128/(597) |
| **5** | *cis*-$C_{18}$ | alkene | – | – | 128/(415) | 128/(415) | 16/(52) |
| **6** | *cis*-$C_{18}$ | alkane | >512/(>1649) | >512/(1649) | 256/(824) | 8/(26) | 16/(52) |
| **7** | *cis*-$C_{18}$ | ketone | – | – | 128/(394) | 64/(197) | 32/(99) |
| **8** | *cis*-$C_{18}$ | alcohol | – | – | 256/(784) | 8/(24) | 2/(6) |
| **9** | *cis*-$C_{18}$ | chloroketone | – | – | 256/(713) | 256/(713) | 16/(45) |
| **10** | *trans*-$C_{18}$ | alkene | – | – | 256/(830) | 128/(415) | 16/(52) |
| **11** | *trans*-$C_{18}$ | chloroketone | – | – | 128/(357) | 256/(713) | 256/(713) |
| **12** | $C_{10}$ | alkene (acyclic) | – | – | 512/(3007) | 32/(188) | 32/(188) |
| DCS | | | 256/(2508) | 16/(157) | 64/(627) | 4/(39) | 8/(78) |
| isoniazid | | | >512/(>3733) | 16/(117) | 32/(233) | 4/(29) | 8/(58) |
| kanamycin | | | 64/(110) | <8/(14) | – | – | – |

[a] MIC values were determined as described in the Experimental Section. [b] G58-1, day 2. [c] $mc^2155$, day 4. [d] Luria–Bertani broth. [e] Minimal media.

**6**, and **8**) of the eleven compounds yielded MIC values $<100\ \mu M$ for both *Mtb* strains, with two of the compounds (**2** and **8**) proving superior to DCS on a molar basis. Importantly, compound **2** yielded MIC values equal (CDC1551 strain) or superior (H37Rv strain) to those obtained with Inh. In summary, these compounds were poor inhibitors of *Msm*, but quite effective against *Mtb* grown in minimal media.

### Nonspecific toxicity

A sampling of six analogues, along with decanoic acid (**12**) and the *Mtb* therapeutics Inh and DCS, were investigated for toxicity in RAW 264.7 macrophages. SDS was employed as a positive control for maximum toxic effect. As illustrated in Table S1 (Supporting Information), three of the tested analogues (**4**, **5**, and **6**), as well as DCS and Inh showed little or no toxicity at concentrations $<250\ \mu M$;[15] toxicity for **12** was only slightly greater. Modest or significant toxicity was observed for analogues **1**, **2**, and **8**.

## Discussion

The MIC results demonstrate promising levels of inhibition with a variety of four-membered-ring carbocycles (cyclobutanol, cyclobutanone, cyclobutene, and cyclobutane). A critically important component of evaluating new and potentially promising therapeutic entities is validation of a drug-like mode of action. Unfortunately, there are numerous undesirable mechanisms resulting from poor physical behavior of a compound that will result in a false positive in a biological assay. These poor properties may include insolubility, reactivity, micelle formation, impurities, aggregation, instability and nonspecific binding. This has resulted in numerous compounds erroneously reported as chemical leads that are not acceptable drug candidates because of these undesirable physiochemical properties.[16] The fatty acid analogues discussed herein are thermally stable. All of the analogues based on a $C_{18}$ backbone aggregate at concentrations overlapping the MIC values observed for *Mtb* or *Msm*. Correspondingly, the biological mechanism of action for analogues **5**–**11** is suspect, and these analogues are unlikely to be viable drug leads. The propensity for these compounds to form micelles or aggregate at concentrations lower than their MIC values must also be considered in any attempt to extract structure–activity relationships. In contrast, the analogues based on a $C_{10}$ backbone (compounds **1**–**4**) displayed no sign of aggregation at concentrations up to 1000 $\mu M$, levels well above the measured MICs (Table 1). The observation of significant and superior inhibition of *Mtb* with compounds from the shorter-chain series provides strong evidence that the inhibition is specific and not simply due to the general toxicity often observed with very hydrophobic substrates.[16] Although some short-chain fatty acids have also demonstrated limited antimicrobial activity,[7] *Mtb* is also reported to use decanoic acid (10:0) as a carbon source.[17] Moreover, decenoic acid (**12**), the parent 10:1 framework of our shorter-chain analogues, demonstrated very modest levels of inhibition in *Mtb*. Finally, we note that the extremely hydrophobic oleic acid (**13**), the parent 18:1 framework of analogues **5**–**9**, is used as a standard nutrient for *Mtb* at $0.05\ g\,L^{-1}$ ($177\ \mu M$), a concentration higher than the MICs of the best inhibitors. Nonetheless, oleic acid is always used in media containing BSA, which may ameliorate its toxicity.[14]

The nature of the functionality has a clear impact on activity. Within the less hydrophobic short-chain series (compounds **1**–**4**), the measured MICs vary by >60-fold against *Mtb* H37Rv and >30-fold against strain CDC1551. In contrast, a <10-fold difference is observed for MICs measured against *Msm*. Although the potential for micelle formation is cause for caution in making structure–activity comparisons involving the long-chain series, the different influences of a given functional group within the $C_{18}$ and $C_{10}$ series are interesting. For example, the short-chain cyclobutanone **3** and cyclobutanol **4** are

much less potent toward *Mtb* than the corresponding long-chain analogues **7** and **8**, whereas the values for the cyclobutenes (**1** versus **5** or **10**) are similar for *Mtb* (H37Rv). The influence of stereochemistry varies by analogue. The MICs for the *cis* and *trans* isomers of the $C_{18}$ monochloroketones (**9** and **11**) differ by $>10$-fold for *Mtb* (H37Rv); in contrast, no difference is observed between the *cis*- and *trans*-cyclobutenes **5** and **10** with either *Mtb* strain. Finally, we note that in both the $C_{18}$ and $C_{10}$ series, the saturated analogues (cyclobutanes **6** and **2**) are considerably more potent than the corresponding cyclobutenes **5** and **1**.

Comparing MIC values obtained against *Mtb* versus *Msm*, ten of eleven analogues demonstrate lower MIC values in at least one strain of *Mtb* compared with *Msm*; for six of the eleven, lower MIC values are obtained against both strains of *Mtb*. The ratio of MIC values (*Msm/Mtb*) ranges from less than one to $>600$; viewed across the eleven analogues, the average ratio of MICs for *Mtb* versus *Msm* is 68 (CDC1551) and nearly 90 (H37Rv). The different levels of inhibition produced in *Msm* and *Mtb* may result from a mechanism of action specific to *Mtb*. Isoniazid, for example, demonstrates 100-fold more potent inhibition of *Mtb* compared with *Msm* and possesses low toxicity toward other mycobacteria and prokaryotic pathogens.[18] The lack of inhibition observed in *E. coli* using two of the analogues (**1** and **6**) most potent against *Mtb* could indicate specific inhibition of a metabolic pathway unique to the latter.

The initial hypothesis behind the design of the molecules reported herein was that uptake of a fatty acid analogue bearing a reactive functional group (for example, cyclobutene, cyclobutanone, chloroketone) might disrupt an enzymatic process related to cell wall synthesis. We imagined that one such target might be methyltransferases, which are common in mycobacteria, but relatively uncommon in other organisms.[19] In an earlier example of a related approach, the inhibition of growth in *Mtb* observed in the presence of alkyne-containing fatty acids (MICs of 20–25 μM) was attributed to irreversible modification of an enoyl reductase within the mycobacterial FASII system.[4,7] However, the significant inhibition produced by cyclobutanes **2** and **6**, which lack a chemically reactive group, is more suggestive of a noncovalent mode of inhibition. The relatively low MIC values indicate our fatty acid analogues have promise as anti-mycobacterial agents, but there is still a need to confirm cellular uptake and to establish the in vivo target.

A variety of carbocyclic fatty acids including cyclopropyl, cyclopentyl, cyclohexyl, and cycloheptyl fatty acids are found in bacteria and plants.[20] Their roles, while not always completely understood, may be related to the control of membrane fluidity.[21] In the case of mycobacteria, the presence of cyclopropane subunits in mycolic acids is known to be associated with the structural integrity of the cell wall and the ability of the tubercle bacillus to resist oxidative stress inside macrophages. In contrast, cyclobutane fatty acids are rare. An unusual fatty acid incorporating a ladder-type structure of fused cyclobutanes, recently isolated from anammox bacteria found near undersea vents, is believed to contribute to the formation of dense and impermeable lipid membranes that protect the bacteria from harsh chemical environments.[20] Thus, as an alternative to disrupting mycolic acid biosynthesis, it is also plausible that our four-membered ring analogues are converted into analogues of mycolic acids and that the physical properties of these unnatural cell wall constituents results in cell death. However, it must be noted that while mycobacteria are known to directly incorporate structurally modified fatty acid feedstocks into mycolic acid biosynthesis,[6] there is as yet no evidence that the same is true for the specific fatty acid analogues described herein.[7] Indeed, further studies are required to determine an in vivo mechanism of inhibition for our compounds.

In this context, it is worth noting that our initial choice of molecular scaffold was based on the availability of low-cost precursors. If the modified fatty acid chains are indeed taken up into the mycobacterial biosynthetic pathways, greater activity or different specificities might be identified through the use of different molecular backbones or alternative positioning of the four-membered-ring carbocycles.

## Conclusions

Analogues of fatty acids incorporating four-membered-ring carbocycles may provide a starting point for the development of new anti-mycobacterials. Four of eleven analogues tested yielded MIC values $<100$ μM for both *Mtb* strains, with two of the analogues proving more active than DCS and one analogue proving equally potent or superior to isoniazid, one of the main first-line drugs used for the treatment of TB.

## Experimental Section

**General procedures**: All reagents and solvents were used as purchased except for pyridine and $CH_2Cl_2$ (distilled from $CaH_2$ and kept under $N_2$) and THF (distilled from Na/benzophenone under $N_2$). Reactions were performed under $N_2$ atmosphere unless stated otherwise. Thin-layer chromatography (TLC) was performed on 0.25 mm hard-layer silica G plates; developed plates were visualized with a hand-held UV lamp or by staining: 1% $Ce(SO_4)_2$ and 10% $(NH_4)_2MoO_4$ in 10% aq. $H_2SO_4$ (general stain, after heating); 1% aq. $KMnO_4$ (for unsaturated compounds); 3% vanillin in 3% $H_2SO_4$ in EtOH (general stain, after heating). Unless otherwise noted, NMR spectra were recorded at 400 ($^1$H) or 100 MHz ($^{13}$C) in $CDCl_3$; peaks are reported as: chemical shift (multiplicity, *J* couplings in Hz, number of protons); "app" and "br" refer to apparent and broad signals, respectively. IR spectra were recorded as neat films (ZnSe, ATR mode) with selected absorbances reported in wavenumbers ($cm^{-1}$). Flash chromatography was performed on 32–60 μm silica gel. Preparative HPLC was performed on a 21× 250 mm normal-phase Si (8 μm) column at a flow rate of 10 mL min$^{-1}$ of the indicated solvent, unless otherwise noted. Analytical purity of compounds was checked by using an analytical column (250 mm×4.6 mm; Microsorb) with 20% EtOAc/hexane at 1 mL min$^{-1}$; detection was carried out with a differential refractometer interfaced with a data module. All compounds tested for biological activity showed $>97$% purity by HPLC analysis except for **5** and **7**. Melting points are uncorrected.

**3-(Octanoic acid-8-yl-benzyl ester)-2,2-dichlorocyclobutanone (15)** was prepared similarly to compound **23** and was afforded (13.8 g, 80%) as a brown oil from benzyl 9-decenoate (12.4 g,

48 mmol), trichloroacetyl chloride (10.7 mL, 95 mmol), and Zn(Cu) (15.55 g, 238 mmol) in $Et_2O$ 0.1 M (340 mL). Consistent with a previous report, only one isomer was observed.[22] The assignment was confirmed by observation of the pair of geminal hydrogen atoms adjacent to the ketone at $\delta = 3.33$ and 2.94 ppm by $^1$H NMR and by the observation of 11 rather than 12 carbon atoms (symmetry) upon dechlorination to cyclobutanone **3**: $R_f = 0.34$ (10% EtOAc/Hex); IR: $\tilde{\nu} = 2935$, 2852, 1814, 1730, 905, 725 cm$^{-1}$; $^1$H NMR: $\delta =$ 7.39–7.29 (m, 5H), 5.12 (s, 2H), 3.33 (dd, $J = 9.4$, 17.2, Hz, 1H), 2.94 (dd, $J = 9.0$, 17.2 Hz, 1H), 2.88–2.81 (m, 1H), 2.37/2.36 (overlapping t, $J = 7.5$ Hz, 2H), 1.94–1.84 (m, 1H), 1.70–1.72 (m, 3H), 1.46–1.28 ppm (m, 8H); $^{13}$C NMR: $\delta = 192.8$,173.4, 136.0, 128.4, 128.0, 88.8, 65.9, 47.7, 45.8, 34.1, 31.2, 29.0, 28.9, 28.8, 27.2, 24.7 ppm; HREIMS calcd for $C_{19}H_{25}O_3Cl_2$: $[M+H]^+$ 371.1181, found: 371.1188.

**3-(Octanoic acid-8-yl-benzyl ester)-2,2-dichlorocyclobutanol methanesulfonate ester (16)** was prepared similarly to compound **20** from the previous dichloroketone **15** (10.0 g, 26.9 mmol), $NaBH_4$ (1.53 g, 40.4 mmol), and *i*PrOH (350 mL). The crude product was purified by flash column chromatography with 10% EtOAc/Hex to furnish the dichlorobutanol (5.76 g, 57%) as a colorless oil: $R_f = 0.39$ (20% EtOAc/Hex); IR: $\tilde{\nu} = 3440$ (br), 2927, 2854, 1733, 1164, 960, 697 cm$^{-1}$; $^1$H NMR: $\delta = 7.40$–7.30 (m, 5H), 5.12 (s, 2H), 4.73–4.64 (m, 0.1H) 4.34–4.27 (m, 0.9H), 2.97 (d, $J = 8.2$ Hz, 0.1H, O*H* is *trans* to backbone), 2.87 (d, $J = 10.3$ Hz, 0.9H, O*H* is *cis* to backbone), 2.48–2.34 (m, 1H), 2.36 (t, $J = 7.6$ Hz, 2H), 1.78–1.60 (m, 3H), 1.53–1.28 ppm (m, 11H); $^{13}$C NMR: $\delta = 173.7$, 136.0, 128.5, 128.1, 92.9, 75.4, 66.1, 45.6, 34.5, 34.2, 29.8, 29.2, 29.0, 28.9, 26.5, 24.8 ppm; HRESIMS calcd for $C_{19}H_{23}O_3Cl_2$ $[M+Na]^+$: 395.1157, found: 395.1151.

Conversion into the methanesulfonate was performed as described for compound **20**, using methanesulfonyl chloride (20 mL, 29.4 mmol), the dichlorobutanol (5.50 g, 14.7 mmol), and $Et_3N$ (9.20 mL, 66.3 mmol) in $CH_2Cl_2$ (70 mL). The crude product was purified by flash chromatography (10% EtOAc/Hex) to afford **16** as a light-yellow oil (6.13 g, 92%): $R_f = 0.36$ (20% EtOAc/Hex); IR: $\tilde{\nu} =$ 2927, 2858, 1731, 1364, 1179, 952 cm$^{-1}$; $^1$H NMR: $\delta = 7.36$–7.31 (m, 5H), 5.11 (s, 2H), 5.13–5.09 (m, 1H), 3.18 (s, 3H), 2.56–2.50 (m, 2H), 2.35 (t, $J = 7.6$ Hz, 2H), 1.93–1.28 ppm (m, 13H); $^{13}$C NMR: $\delta = 173.5$, 136.0, 128.4, 128.1, 88.4, 78.3, 66.0, 45.8, 39.3, 34.1, 31.6, 29.8, 29.1, 28.87, 28.85, 26.2, 24.8 ppm; HRESIMS calcd for $C_{20}H_{28}O_5Cl_2SNa$ $[M+Na]^+$: 473.0932, found: 473.0930.

**8-(2-Cyclobuten-1-yl)octanoic acid (1)** was prepared by a similar procedure as employed for **5** via reaction of Na metal (2.80 g, 120 mmol) with a solution of 5.40 g (12.0 mmol) of the mixture of methanesulfonate **16** in THF (30 mL) and $NH_3$ (~250 mL). The crude product was purified by flash column chromatography (2% EtOAc/Hex) to afford 9,10-ethenooctadecanoic acid **1** (1.64 g, 52%) as a low-melting white solid: $R_f = 0.47$ (Hex/EtOAc/AcOH 6:4:0.05); mp: 20–21 °C; IR: $\tilde{\nu} = 3112$ (br), 3043, 2920, 2853, 1705, 697 cm$^{-1}$; $^1$H NMR: $\delta = 10.27$ (br s, 1H, COO*H*), 6.11 (br d, $J = 2.4$ Hz, 1H), 6.04 (br dd, $J = 0.8$, 2.0 Hz, 1H), 2.77 (m, 1H), 2.66 (ddd, $J = 0.8$, 4, 13.2 Hz, 1H), 2.35 (t, $J = 7.2$ Hz, 2H), 2.04 (d, $J = 13.2$ Hz, 1H), 1.64 (quint., $J = 7.2$ Hz, 2H), 1.51–1.38 (br m, 2H), 1.38–1.23 ppm (br m, 8H); $^{13}$C NMR: $\delta = 180.0$, 141.1, 135.1, 44.2, 36.9, 34.6, 34.0, 29.5, 29.2, 29.0, 27.9, 24.7 ppm; HRESIMS calcd for $C_{12}H_{20}O_2Na$ $[M+Na]^+$: 219.1361, found: 219.1355; purity 100% by HPLC ($t_R$: 5.01 min).

**8-Cyclobutyloctanoic acid (2)** was prepared by a similar procedure as for **6** from the hydrogenation of cyclobutene **1** (0.034, 0.170 mmol), over 10% Pd/C (0.034 g, 0.030 mmol) in EtOAc (1.7 mL) to afford the unprotected acid **2** (0.030 g, 89%) as a low-melting white solid: $R_f = 0.47$ (Hex/EtOAc/AcOH 6:4:0.05); mp: 28–30 °C; IR: $\tilde{\nu} = 3035$ (br), 2916, 2849, 1695, 909, 733 cm$^{-1}$; $^1$H NMR: $\delta = 11.34$ (br s, 1H, COO*H*), 2.37 (t, $J = 7.2$, 2H), 2.25 (sept., $J = 7.2$, 1H), 2.10–1.98 (m, 2H), 1.90–1.75 (m, 2H), 1.70–1.52 (m, 2H), 1.40–1.12 ppm (br m, 10H); $^{13}$C NMR: $\delta = 180.3$, 37.0, 36.2, 34.1, 29.4, 29.2, 29.0, 28.4, 27.1, 24.7, 18.5 ppm; HRCIMS calcd for $C_{12}H_{22}O_2$: $[M+H]^+$ 199.1698, found: 199.1695; purity >99% by HPLC ($t_R$: 4.92 min).

**3-(Octanoic acid benzyl ester-8-yl)cyclobutanone (17)** was prepared in a similar manner as **21**, via reaction of dichloroketone **15** (1.05 g, 2.69 mmol), 6 mL glacial acetic acid, and Zn(Cu) (1.76 g, 26.9 mmol). The product was purified by flash chromatography (10% EtOAc/Hex) to afford a colorless oil (0.4859 g, 59%), which was a mixture of regioisomers: $R_f = 0.55$ (20% EtOAc/Hex); IR: $\tilde{\nu} =$ 2923, 2854, 2780, 2732, 1163, 730, 697 cm$^{-1}$; $^1$H NMR: $\delta = 7.35$–7.28 (br m, 5H), 5.09 (s, 2H), 3.13–3.03 (m, 2H), 2.65–2.57 (m, 2H), 2.33 (t, $J = 7.2$, 2H), 2.33–2.26 (m, 1H), 1.68–1.58 (br quint., $J = 6.4$, 2H), 1.58–1.48 (br q, $J = 7.2$, 2H), 1.29 ppm (br s, 8H); $^{13}$C NMR: $\delta = 208.2$, 173.3, 134.0, 128.3, 127.9, 65.8, 52.3, 36.1, 34.0, 28.9, 28.8, 28.0, 24.7, 23.6 ppm; HRESIMS calcd for $C_{19}H_{26}O_3Na$ $[M+Na]^+$: 325.1780, found: 325.1775.

**3-(Octanoic acid-8-yl)cyclobutanone (3)** was prepared by a similar procedure as for **7** through reaction of benzyl ester **17** (0.0346, 0.1 mmol), 10% Pd/C (0.020 g, 0.02 mmol) and EtOAc (1 mL). The product was purified by flash column chromatography on silica gel (step gradient of 20:40% EtOAc/Hex) to afford acid **3** (0.0189 g, 90%) as a low-melting white solid: $R_f = 0.3$ (Hex/EtOAc/AcOH 6:4:0.05); mp: 35–37 °C; IR: $\tilde{\nu} = 3094$ (br), 2923, 2853, 1780, 1705 cm$^{-1}$; $^1$H NMR: $\delta = 10.61$ (br s, 1H, COO*H*), 3.17–3.08 (m, 2H), 2.69–2.61 (m, 2H), 2.35 (br t, $J = 7.6$, 2H), 2.39–2.31 (br, 1H), 1.69–1.53 (br m, 4H), 1.32 ppm (br s, 8H); $^{13}$C NMR: $\delta = 208.8$, 179.9, 52.5, 36.3, 34.0, 29.2, 29.1, 28.9, 28.2, 24.6, 23.8 ppm; HRCIMS calcd for $C_{12}H_{20}O_3Na$ $[M+Na]^+$: 213.1491, found: 213.1490; purity >97% by HPLC ($t_R$: 10.75 min).

**3-(Octanoic acid benzyl ester)cyclobutanol (18)** was prepared by a similar procedure as compound **8** via reaction of cyclobutanone **17** (0.0509 g, 0.17 mmol) and $NaBH_4$ (0.013 g, 0.34 mmol) in anhydrous MeOH (2.5 mL). Compound **18** (after purification by flash column chromatography with 20% EtOAc/Hex) was isolated as a low-melting white solid (0.040 g, 76%). The product was a 9:1 mixture of *cis*/*trans* stereoisomers based on the previous assignments for cyclobutanol **8**; $R_f = 0.29$ (20% EtOAc/Hex); mp: 30–31 °C; IR: $\tilde{\nu} = 3356$ (br), 2922, 1734, 1154, 735, 696 cm$^{-1}$; $^1$H NMR: $\delta = 7.40$–7.30 (m, 5H), 5.11 (s, 2H), 4.42–4.34 (br m, 0.1H), 4.14–4.03 (br m, 0.9H), 2.48–2.40 (m, 0.18H), 2.35 (t, $J = 7.2$, 0.18H), 2.16–2.04 (m, 0.2H), 1.95 (t, $J = 6.6$, 0.2H), 1.70–1.56 (br m, 3H), 1.48–1.13 ppm (br m, 12H); $^{13}$C NMR: $\delta = 173.6$, 136.0, 128.4, 128.1, 66.0, 63.7, 39.7, 37.0, 34.2, 29.2, 29.1, 29.0, 27.3, 25.4, 24.82 ppm; HRCIMS calcd for $C_{19}H_{29}O_3Na$ $[M+Na]^+$: 305.2117, found: 305.2116.

**3-(Octanoic acid)cyclobutanol (4)** was prepared by a similar procedure as employed for **8** through reaction of benzyl ester **18** (0.034 g, 0.110 mmol), 10% Pd/C (0.024 g, 0.022 mmol) and EtOAc (1 mL). The crude product was purified by flash column chromatography (40% EtOAc/Hex) to afford acid **4** (0.013 g, 54%) as a white solid: $R_f = 0.24$ (Hex/EtOAc/AcOH 6:4:0.05); mp: 74–75 °C; IR: $\tilde{\nu} = 3341$ (br), 2916, 2849, 1695, 1064 cm$^{-1}$; $^1$H NMR (MeOD): $\delta =$ 4.30 (quint., $J = 6.8$, 0.1H), 4.01 (quint., $J = 6.4$, 0.9H), 2.45–2.35 (br m, 2H), 2.29 (t, $J = 7.2$, 2H), 1.75–1.55 (br m, 3H), 1.50–1.15 ppm (br m, 12H); $^{13}$C NMR (MeOD): $\delta = 176.3$, 62.8, 38.9, 36.9, 36.8, 33.6, 29.1, 29.0, 28.8, 27.1, 25.4, 24.7 ppm; HRESIMS calcd for $C_{12}H_{22}O_3Na$ $[M+Na]^+$: 237.1467 found: 237.1460; purity >97% by HPLC ($t_R$: 9.65 and 11.36 min).

**2,2-Dichloro-4-(octanoic acid benzyl ester-8-yl)-3-octylcyclobutanone-; 2,2-Dichloro-3-(octanoic acid benzyl ester-8-yl)-4-octylcyclobutanone (19)** was prepared by a modification of a known procedure.[22–24] To a mixture of zinc dust (0.879 g, 13.4 mmol) and benzyl oleate **19** (1.02 g, 2.73 mmol) in 20 mL anhydrous $Et_2O$ was slowly added a solution of trichloroacetyl chloride (0.75 mL, 6.8 mmol) in 7 mL $Et_2O$ via dropping funnel over a period of 45 min. The reaction was stirred until determined complete by the absence of starting material (4 h, TLC). The reaction mixture was filtered through a plug of Celite, and the residue was washed with $Et_2O$ (2×75 mL). The organic solution was stirred a minimum with 50% aq. $NaHCO_3$, and the separated aqueous layer back-extracted with $Et_2O$ (2×20 mL). The combined organic layers were dried over $Na_2SO_4$ and concentrated under vacuum to give the product (1.30 g, 99%) as a yellow oil. The unpurified material was generally used directly for the next reaction because of a tendency to decompose during chromatography. However, small quantities could be purified by rapid flash chromatography (5% EtOAc/Hex) to afford a 1:1 mixture of regioisomeric dichlorocyclobutanones **19** as a colorless oil: $R_f$ = 0.53 (10% EtOAc/Hex); IR: $\tilde{\nu}$ = 2926, 2855, 1800, 1735, 1456, 1162, 696 $cm^{-1}$; $^1H$ NMR: $\delta$ = 7.36–7.32 (5H), 5.120/5.116 (overlapping s, 2H), 3.55–3.49 (m, 1H) 2.97–2.93 (m, 1H), 2.37/2.35 (overlapping t, $J$ = 7.6 Hz, 2H), 1.74–1.62 (m, 4H), 1.66–1.48 (m, 4H), 1.45–1.27 (br m, 18H), 0.89/0.88 ppm (overlapping triplets, $J$ = 6.9 Hz, total 3H); $^{13}C$ NMR: $\delta$ = 197.5, 173.5, 136.1, 128.5, 128.1, 88.1, 66.0, 58.0, 57.9, 49.4, 49.4, 34.2, 31.8, 31.80, 29.60, 29.3, 29.3, 29.2, 29.18, 29.15, 29.12, 29.0, 28.9, 28.9, 28.04, 27.96, 26.72, 26.68, 26.3 ppm; HRFABMS (3-NBA + Li matrix): calcd for $C_{27}H_{40}O_3Cl_2Li$ $[M+Li]^+$: 489.2515, found: 489.2499.

**2,2-Dichloro-4-(octanoic acid benzyl ester-8-yl)-3-octylcyclobutanol methanesulfonate ester; 2,2-Dichloro-3-(octanoic acid benzyl ester-8-yl)-4-octylcyclobutanol methanesulfonate ester (20)**: Reduction of the dichlorocylobutanone **19** was achieved using a modification of a known procedure.[9,24] To a 0 °C solution of dichlorocyclobutanones **19** (1.5 g, 3.1 mmol) in *i*PrOH (45 mL) was added $NaBH_4$ (0.176 g, 4.65 mmol). The reaction was allowed to slowly warm to RT and stirred until starting material had disappeared (TLC, ~2.5 h; by-products tended to accumulate upon prolonged reaction). The reaction was then cooled to 0 °C and quenched with 1 N HCl (40 mL). The solution was stirred for an additional 30 min, concentration to one third the original volume, and then extracted with EtOAc (3×100 mL). The organic layer was washed sequentially with $H_2O$/sat. $NaHCO_3$/brine and then dried over $Na_2SO_4$. Evaporation of the organic solvent and flash chromatography of the residue on silica gel, using a step gradient of 5:10% EtOAc/Hex, gave (0.821 g, 55% yield) of a 1:1 mixture of regioisomeric cyclobutanols as a colorless oil. The products were predominantly a regioisomeric mixture of the *cis*,*cis* diastereomers[22] based on $^1H$ NMR, HSQC, 2D-COSY, and 2D-NOESY analysis:[25] $R_f$ (10% EtOAc/Hex) = 0.33 (major, OH is *cis* to backbone) 0.28 (minor, OH is *trans* to backbone), 0.2 (major, OH is *cis* to backbone) and 0.15 (minor, OH is *trans* to backbone); IR: $\tilde{\nu}$ = 3473, 3036, 2924, 2854, 1733, 1456, 1168, 696 $cm^{-1}$; $^1H$ NMR: $\delta$ = 7.36–7.32 (m, 5H), 5.116/5.112 (app s, total 2H), 4.55 (dd, $J$ = 10.8, 8.8 Hz, 0.92H, C*H*-OH, *cis* to the backbone assigned based on 2D-NOESY analysis and $^4J_{diagonal\,H}$ = 0), 3.99 (br t, $J$ = 10 Hz, 0.08H), 2.75–2.69 (m, 1H), 2.64/2.62 (overlapping d, $J$ = 10.8 Hz, total 1H, -O*H* proton assigned based on the disappearance of this peak upon $D_2O$ addition), 2.60–2.55 (m, 1H), 2.36/2.35 (overlapping t, $J$ = 7.6 Hz, total 2H), 1.67–1.62 (br m, 3H), 1.52–1.19 (24H), 0.89/0.88 ppm (overlapping t, $J$ = 6.8 Hz, total 3H). Small quantities of the individual isomers could be isolated by semipreparative HPLC (10% EtOAc/Hex, RI detection):

*First-eluting cis*: $R_f$ = 0.33; IR: 3457, 2924, 2854, 1736, 1456, 1170, 697 $cm^{-1}$; $^1H$ NMR: $\delta$ = 7.37–7.32 (m, 5H), 5.12 (s, 2H), 4.55 (dd, $J$ = 10.8, 8.8 Hz, 1H, C*H*-OH, *cis* to the backbone assigned based on 2D-NOESY analysis and $^4J_{diagonal\,H}$ = 0), 2.72 (dt, $J$ = 9.2, 5.6 Hz, 1H), 2.59 (d, $J$ = 10.8 Hz, 1H, -O*H*), 2.55 (app dq, $J$ = 9.2, 5.6 Hz, 1H), 2.36 (t, $J$ = 7.6 Hz, 2H), 1.67–1.51 (br m, 3H), 1.52–1.13 (m, 24H), 0.88 ppm (app t, $J$ = 6.8 Hz, 3H); $^{13}C$ NMR: $\delta$ = 173.6, 136.1, 128.5 (two overlapping signals), 128.2 (two overlapping signals), 93.5, 77.2, 66.1, 49.5, 40.9, 34.3, 31.8, 29.9, 29.4, 29.4, 29.3, 29.2, 29.0, 27.0, 25.7, 24.9, 23.2, 22.7, 14.1 ppm; HRFABMS (3-NBA matrix): calcd for $C_{27}H_{43}O_3Cl_2$ $[M+H]^+$: 485.2589, found: 485.2572.

*First-eluting trans*: $R_f$ = 0.28; IR: $\tilde{\nu}$ = 3449, 3028, 2920, 2850, 1736, 1456, 1157, 696 $cm^{-1}$; $^1H$ NMR: $\delta$ = 7.37–7.32 (5H), 5.12 (s, 2H), 3.99 (ddd, $J$ = 10.0, 8.4, 1.2 Hz, 1H, C*H*-OH, *trans* to the backbone assigned based on 2D-NOESY analysis and $^4J_{diagonal\,H}$ = 1.2 Hz), 2.68–2.64 (m, 1H), 2.52 (d, $J$ = 10.0 Hz, 1H, -O*H*), 2.34 (t, $J$ = 7.6 Hz, 1H), 2.29–2.20 (m, 1H), 1.67–1.26 (br m, 27H), 0.88 ppm (app t, $J$ = 6.8 Hz, 3H); $^{13}C$ NMR: $\delta$ = 173.6, 136.1, 128.5 (two overlapping signals), 128.2 (two overlapping signals), 91.1, 82.6, 66.1, 50.3, 44.4, 34.3, 31.9, 29.7, 29.5, 29.2, 29.04, 29.02, 28.4, 28.23, 28.15, 27.7, 24.9, 22.7, 14.1 ppm; ESIMS calcd for $C_{27}H_{42}O_3Cl_2Na$ $[M+Na]^+$: 507.2409, found: 507.2422.

*Second-eluting cis*: $R_f$ = 0.20; IR: $\tilde{\nu}$ = 3464, 2924, 2854, 1736, 1456, 1169, 696 $cm^{-1}$; $^1H$ NMR: $\delta$ = 7.37–7.32 (m, 5H), 5.11 (s, 2H), 4.55 (dd, $J$ = 10.8, 8.8 Hz, 1H C*H*-OH, *cis* to the backbone assigned based on 2D-NOESY analysis and $^4J_{diagonal\,H}$ = 0), 2.72 (dt, $J$ = 9.2, 5.6 Hz, 1H), 2.65 (d, $J$ = 10.8 H z, 1H, -O*H*), 2.54 (app dq, $J$ = 9.2, 5.6 Hz, 1H), 2.35 (t, $J$ = 7.2 Hz, 2H), 1.68–1.60 (br m, 3H), 1.52–1.13 (m, 24H), 0.89 ppm (app t, $J$ = 6.8 Hz, 3H); $^{13}C$ NMR: $\delta$ = 173.7, 136.1, 128.5 (two overlapping signals), 128.2 (two overlapping signals), 93.5, 77.2, 66.1, 49.5, 40.9, 34.3, 31.8, 29.6, 29.4, 29.2, 29.1, 29.04, 29.00, 27.0, 25.8, 24.8, 23.2, 22.7, 14.1 ppm; HRFABMS (3-NBA matrix): calcd for $C_{27}H_{43}O_3Cl_2$ $[M+H]^+$: 485.2589, found: 485.2581

*Second-eluting trans*: $R_f$ = 0.15; IR: $\tilde{\nu}$ = 3464, 3033, 2925, 2854, 1738, 1498, 1160, 697 $cm^{-1}$; $^1H$ NMR: $\delta$ = 7.37–7.32 (m, 5H), 5.11 (s, 2H), 3.99 (ddd, $J$ = 10.0, 8.4, 1.2 Hz, 1H, C*H*-OH, *trans* to the backbone assigned based on 2D-NOESY analysis and $^4J_{diagonal\,H}$ = 1.2 Hz), 2.69–2.64 (m, 1H), 2.56 (d, $J$ = 10.0 Hz, 1H, -O*H*), 2.35 (t, $J$ = 7.6 Hz, 2H), 2.26–2.22 (m, 1H), 1.66–1.26 (m, 27H), 0.88 ppm (app t, $J$ = 6.8 Hz, 3H); $^{13}C$ NMR: $\delta$ = 173.7, 136.1, 128.5 (two overlapping signals), 128.2 (two overlapping signals), 91.1, 82.6, 66.1, 50.3, 44.3, 34.3, 31.8, 29.7, 29.4, 29.2, 29.04, 28.97, 28.5, 28.21, 28.18, 27.5, 24.8, 22.7, 14.1 ppm; HRESIMS calcd for $C_{27}H_{42}O_3Cl_2Na$ $[M+Na]^+$: 507.2409, found: 507.2426.

The mixture of regioisomeric cyclobutanols were converted into the methanesulfonate using a variant of published procedures.[9,24] Methanesulfonyl chloride (62 μL, 0.80 mmol) was slowly added to a stirred solution of the cyclobutanols at 0 °C (0.193 g, 0.398 mmol) and $Et_3N$ (0.25 mL, 8.0 mmol) in $CH_2Cl_2$ (2 mL) over 15 min. The reaction was allowed to slowly warm to RT and was stirred for 17 h or until no starting material remained (TLC). The reaction was diluted with $CH_2Cl_2$ (75 mL) and washed sequentially with 1 N HCl (2× 25 mL), sat. aq. $NaHCO_3$ (2×25 mL) and $H_2O$ (2×25 mL). The organic solution was then dried with $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography using 10% EtOAc/Hex to give **20** (0.206 g, 92%) as a light-yellow oil consisting of a 1:1 mixture of regioisomers, each a 92:8 mixture of *cis*/*trans* stereoisomers based on $^1H$ NMR: $R_f$ (10% EtOAc/Hex) = 0.31 (first *trans*), 0.26 (first *cis*/second *trans*), 0.21 (second *cis*); IR: $\tilde{\nu}$ = 3032, 2925, 2855, 1735, 1458, 1180, 696 $cm^{-1}$; $^1H$ NMR: $\delta$ =

7.36–7.33 (m, 5H), 5.33 (d, $J=9.6$ Hz, 0.92H), 5.114/5.109 (two s, total 2H), 4.84 (d, $J=7.6$ Hz, 0.08H), 3.208/3.205 (two s, total 3H), 2.76–2.74 (m, 2H), 2.36/2.34 (two overlapping t, $J=7.6$ Hz, total 2H), 1.66–1.60 (m, 4H), 1.46–1.26 (m, 22H), 0.88/0.87 ppm (overlapping t, $J=6.8$ Hz, total 3H). Small quantities of individual isomers could be separated for analysis by HPLC using 10% EtOAc/Hex. The stereochemical assignments were confirmed by 2D NMR experiments and by preparation of individual methanesulfonates from individual stereoisomers of alcohol **19**, prepared as described in the previous step.

*First eluting trans*: $R_f=0.31$; IR: $\tilde{\nu}=3032$, 2926, 2855, 1737, 1733, 1456, 1180, 697 $cm^{-1}$; $^1H$ NMR: $\delta=7.37$–7.32 (5H), 5.12 (s, 2H), 4.84 (d, $J=7.6$ Hz, 1H), 3.20 (s, 3H), 2.74–2.68 (m, 2H), 2.36 (t, $J=7.6$ Hz, 2H), 1.67–1.32 (br m, 26H), 0.88 ppm (t, $J=6.8$ Hz, 3H); $^{13}C$ NMR: $\delta=173.6$, 136.1, 128.5 (two overlapping signals), 128.2 (two overlapping signals), 86.4, 85.8, 66.1, 50.5, 41.1, 39.5, 34.3, 31.8, 29.5, 29.4, 29.3, 29.2, 29.01, 28.98, 28.3, 28.1, 27.6, 27.1, 24.9, 22.6, 14.1 ppm; HRESIMS calcd for $C_{28}H_{44}O_5Cl_2S$ $[M+Na]^+$: 585.2184, found: 585.2188.

*Second-eluting trans*: $R_f=0.26$; IR: $\tilde{\nu}=3040$, 2927, 2855, 1734, 1456, 1179, 697 $cm^{-1}$; $^1H$ NMR: $\delta=7.37$–7.32 (m, 5H), 5.11 (s, 2H), 4.84 (dd, $J=8.4$, 0.8 Hz, 1H), 3.19 (s, 3H), 2.76–2.65 (m, 2H), 2.35 (t, $J=7.6$ Hz, 2H), 1.67–1.32 (26H), 0.89 ppm (t, $J=6.8$ Hz, 3H); $^{13}C$ NMR $\delta=173.6$, 136.1, 128.5 (two overlapping signal), 128.2 (two overlapping signal), 86.4, 85.7, 66.1, 50.5, 41.1, 39.5, 34.3, 31.8, 29.6, 29.31, 29.25, 29.2, 29.0, 28.3, 28.1, 27.6, 27.0, 24.9, 22.7, 14.1 ppm: HRESIMS; calcd for $C_{28}H_{44}O_5Cl_2S$ $[M+Na]^+$: 585.2184, found: 585.2205.

*First-eluting cis*: $R_f=0.26$; IR: $\tilde{\nu}=2926$, 2855, 1736, 1456, 1182, 697 $cm^{-1}$; $^1H$ NMR: $\delta=7.37$–7.32 (m, 5H), 5.33 (app d, $J=9.6$ Hz, 1H), 5.11 (s, 2H), 3.19 (s, 3H), 2.80–2.72 (br m, 2H), 2.36 (app t, $J=7.6$ Hz, 2H), 1.70–1.63 (m, 4H), 0.87 ppm (t, $J=6.8$ Hz, 3H); $^{13}C$ NMR: $\delta=173.6$, 136.0, 128.5 (two overlapping signal), 128.1 (two overlapping signal), 88.9, 80.7, 66.0, 49.5, 40.2, 39.2, 34.2, 31.8, 29.7, 29.3, 29.3, 29.2, 29.0, 28.8, 26.7, 25.7, 24.8, 23.7, 22.6, 14.1 ppm: HRFABMS (3-NBA matrix): calcd for $C_{28}H_{45}O_5Cl_2S$ $[M+H]^+$: 563.2365, found: 563.2349.

*Second-eluting cis*: $R_f=0.21$; IR: $\tilde{\nu}=2925$, 2855, 1735, 1457, 1181, 697 $cm^{-1}$; $^1H$ NMR: $\delta=7.36$–7.32 (m, 5H), 5.33 (app d, $J=9.6$ Hz, 1H), 5.11 (s, 2H), 3.20 (s, 3H), 2.80–2.69 (br m, 2H), 2.34 (app t, $J=7.6$ Hz, 2H), 1.74–1.60 (4H), 1.50–1.19 ppm (22H); $^{13}C$ NMR: $\delta=173.6$, 136.1, 128.5 (two overlapping signal), 128.1 (two overlapping signal), 89.0, 80.7, 66.0, 49.5, 40.2, 39.3, 34.2, 31.8, 29.5, 29.5, 29.3, 29.2, 28.9, 28.9, 28.7, 26.8, 25.7, 24.8, 23.7, 22.6, 14.1 ppm; HRFABMS (3-NBA matrix): calcd for $C_{28}H_{45}O_5Cl_2S$ $[M+H]^+$: 563.2365, found: 563.2360.

**1-(Octanoic acid-8-yl)-2-octylcyclobutene (5)** was prepared from the regioisomeric mixture of dichloromethanesulfonates **20** using a reported procedure.[9,24] $NH_3$ (~15 mL, liquid) was condensed into a 100 mL three-necked round-bottom flask fitted with a dry ice/acetone condenser. Sodium (0.113 g, 4.90 mmol, sliced under Hex) was added in small pieces. The resulting deep-blue solution was stirred for 20 min. A solution of 0.276 g (0.49 mmol) of **20** in anhydrous THF (1.5 mL) was added over 10 min. The cooling bath was removed, and the reaction was allowed to stir at ~−35 °C ($NH_3$ at reflux) 5–30 min (depending on the scale of preparation). The reaction was then re-cooled to −78 °C (dry ice/acetone) and stirred for 2 h or until the starting material was consumed by TLC. Saturated aq. $NH_4Cl$ was slowly added until the blue color was no longer visible. The condenser was removed, and the reaction mixture was slowly allowed to warm to 0 °C with evaporation of $NH_3$. The remaining reaction mixture was diluted with 30 mL $H_2O$, and the suspension was extracted with $CH_2Cl_2$ (3×50 mL). The organic layers were combined and washed sequentially with 1 N aq. $HCl/H_2O$/saturated aq. $NaHCO_3/H_2O$/brine. The organic solution was then dried with $NaSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography using 10% EtOAc/Hex to afford the cyclobutene carboxylic acid **5** (0.131 g, 87%) as a low-melting white solid: $R_f=0.50$ (Hex/EtOAc/AcOH 80:20:1); mp: 33–35 °C; IR: $\tilde{\nu}=3493$, 3036, 2923, 2853, 1708, 1467, 1275, 728 $cm^{-1}$; $^1H$ NMR: $\delta=10.94$ (br s, 1H, COO*H*), 6.17–6.15 (br m, 2H), 2.83–2.75 (br s, 2H), 2.35 (t, $J=7.6$ Hz, 2H), 1.68–1.60 (br m, 2H), 1.52–1.40 (br m, 2H), 1.35–1.20 (23H), 0.88 ppm (t, $J=6.8$ Hz, 3H); $^{13}C$ NMR (found 18 C) $\delta=180.6$, 140.0, 139.9, 46.8, 46.7, 34.1, 31.9, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 29.2, 29.0, 28.3, 28.2, 24.6, 22.7, 14.1 ppm; HRFABMS (Gly matrix) calcd for $C_{20}H_{35}O_2$ $[M-H]^-$: 307.2637, found: 307.2636; purity >99% by HPLC ($t_R$: 4.63 min).

***cis*-9,10-Ethanooctadecanoic acid (6)**: A solution of cyclobutene **5** (0.031 g, 0.100 mmol) and 10% Pd/C (0.020 g, 0.020 mmol) in EtOAc (1 mL) was placed in a vial under an atmosphere of $H_2$ and stirred at RT overnight (16 h). The mixture was then filtered through a plug of Celite, which was washed with ~10 mL EtOAc. The solution was then evaporated under vacuum to afford acid **6** (0.030 g, 96%) as a colorless oil, which was sufficiently pure to use without chromatography: $R_f=0.50$ (Hex/EtOAc/AcOH 80:20:1); IR: $\tilde{\nu}=3051$ (br), 2921, 2852, 1708 $cm^{-1}$; $^1H$ NMR: $\delta=10.93$ (br s, 1H, COO*H*), 2.35 (t, $J=7.3$, 2H), 2.24 (br s, 2H), 2.00–1.90 (br m, 1H), 1.64 (quint., $J=7.3$ Hz, 2H), 1.60–1.50 (m, 2H), 1.45–1.10 (br m, 24H), 0.88 ppm (br t, $J=7.0$ Hz, 3H); $^{13}C$ NMR: $\delta=180.2$, 37.7, 37.6, 34.1, 31.9, 30.11, 30.05, 29.9, 29.7, 29.7, 29.4, 29.3, 29.1, 27.64, 27.55, 24.8, 24.7, 22.7, 14.1 ppm; HRCIMS calcd for $C_{20}H_{38}O_2$ $[M+H]^+$: 311.2950, found: 311.2949; purity 100% by HPLC ($t_R$: 4.56 min).

**2-(Octanoic acid benzyl ester-8-yl)-3-octylcyclobutanone and 3-(Octanoic acid benzyl ester-8-yl)-2-octylcyclobutanone (21)** was prepared by a modification of a known procedure.[22–24] To a solution of the regioisomeric dichloroketones **19** (0.670 g, 1.40 mmol) in glacial acetic acid (3 mL) was added Zn(Cu) (0.450 g, 6.90 mmol). The mixture was stirred at RT under $N_2$ until TLC showed complete disappearance of starting material (~5 h). The reaction mixture was then filtered through a plug of Celite and washed with $Et_2O$. The filtrate was washed sequentially with saturated aq. $NaHCO_3$ and $H_2O$, and dried over $Na_2SO_4$. The residue obtained upon concentration was purified by flash chromatography on silica gel (5% EtOAc in Hex) to afford (0.388 g, 87%) of a mixture of cyclobutanones **21** as a colorless oil: $R_f=0.41$ (10% EtOAc/Hex); IR: $\tilde{\nu}=3034$, 2923, 2853, 1774, 1735, 1161, 696 $cm^{-1}$; $^1H$ NMR: $\delta=7.36$–7.26 (br m, 5H), 5.115 (s, 2H), 3.28–3.17 (br m, 1H), 3.15–3.05 (br m, 1H), 2.51–2.43 (m, 1H), 2.42–2.32 (br m, 1H), 2.35/2.34 (overlapping t, $J=7.53$ Hz, 2H), 1.70–1.50 (br m, 4H), 1.45–1.14 (br m, 22H), 0.88/0.87 ppm (overlapping t, $J=7.19$ Hz, 3H); $^{13}C$ NMR: $\delta=212.0$, 173.6, 136.1, 128.5, 128.1, 66.0 (br s), 61.9/61.8 (two app s), 50.1/50.1 (two app s), 34.2, 31.8, 30.1, 30.0, 29.6, 29.6, 29.5, 29.4, 29.4, 29.23, 29.17, 29.0, 28.2, 28.08, 28.05, 27.98, 27.6, 24.9, 24.3, 24.3, 22.6, 14.1 ppm; HREIMS calcd for $C_{27}H_{41}O_3Na$ $[M+Na]^+$: 437.3032, found: 437.3029.

**2-(Octanoic acid-8-yl)-3-octylcyclobutanone and 3-(octanoic acid-8-yl)-2-octylcyclobutanone (7)**: A solution of cyclobutanones **21** (0.039 g, 0.09 mmol) and 10% Pd/C (0.020 g, 0.18 mmol) in anhydrous MeOH (1 mL) in a vial was placed under an atmosphere of $H_2$ and stirred at RT overnight (16 h). The mixture was then concentrated under vacuum. The residue was resuspended in EtOAc (~20 mL) and filtered through a plug of Celite. The residue obtained

upon concentration was purified by flash column chromatography on silica gel (10:25% EtOAc/Hex) to afford the regioisomeric mixture of carboxylic acids **7** (0.027 g, 87%) as a colorless oil: $R_f = 0.16$ (25% EtOAc/Hex); IR: $\tilde{\nu} = 3322$ (br), 2926, 2856, 1776, 1709, 913, 749 $cm^{-1}$; $^1H$ NMR: $\delta = 3.28–3.18$ (br m, 1H), 3.13/3.09 (overlapping dd, $J = 9.6$, 3.1 and 9.0, 3.5, 0.56H/0.44H), 2.50/2.46 (two app abnormal-shape quints., 0.55H/0.52H), 2.42 (br dd, $J = 9.6$, 4.5 Hz, 1H), 2.35/2.34 (two app t, $J = 7.45$ Hz, 2H), 1.63–1.54 (br m, 4H), 1.43–1.18 (br m, 22H), 0.88/0.87 ppm (overlapping br t, $J = 7.0$ Hz, 3H); $^{13}C$ NMR: $\delta = 212.1$, 179.9, 62.0/61.9 (two app s), 50.20/50.17 (two app s), 34.0, 31.9, 30.1, 30.1, 29.7, 29.6, 29.6, 29.4, 29.3, 29.2, 29.02, 28.98, 28.2, 28.1, 28.0, 27.6, 24.6, 24.3, 24.3, 22.7, 14.1 ppm; HREIMS calcd for $C_{20}H_{36}O_3Na$ $[M+Na]^+$: 347.2557, found: 347.2557; purity >97% by HPLC ($t_R$: 6.82 min).

**2-(Octanoic acid-8-yl)-3-octylcyclobutanol and 3-(Octanoic acid-8-yl)-2-octylcyclobutanol (8)**: To a −10 °C (ice/NaCl bath) solution of the cyclobutanone **21** (0.037 g, 0.070 mmol) in anhydrous MeOH (1.5 mL) was added $NaBH_4$ (0.005 g, 0.150 mmol) under $N_2$. The reaction mixture was stirred at −10 °C until no starting material remained on TLC (~20 min). The reaction was diluted with $CH_2Cl_2$ (~5 mL) and quenched with sat. $NaHCO_3$ (2 mL). The mixture was stirred for 10 min. The organic layer was removed and the aqueous layer was extracted with $CH_2Cl_2$ (3×5 mL). The organic layers were combined, dried over with $Na_2SO_4$, and evaporated to afford the alcohol as a colorless oil (0.029 g, 99%). Although the crude product appears pure by $^1H$ NMR, it is actually a mixture of two regioisomers, each a 70:30 mixture of the 1,2,3-*cis*/*cis* and the 1,2-*trans*-2,3-*cis* isomers (identities established through 2D NMR). The individual isomers can be detected by TLC in 20% EtOAc/Hex: $R_f = 0.50$ (major, all *cis*), 0.45 (major, all *cis*), 0.39 (minor, OH is *trans* to backbone), and 0.37 (minor, OH is *trans* to backbone); IR: $\tilde{\nu} =$ 3396 (br), 2922, 2852, 1737, 1156, 732, 696 $cm^{-1}$; $^1H$ NMR: $\delta = 7.39–7.28$ (br m, 5H), 5.14 (s, 2H), 4.23 br q, $J = 7.3$, 0.7H), 3.93 (br q, $J =$ 7.3, 0.3H), 2.42–2.36 (overlapping signal, 1H), 2.37 (t, $J = 7.6$, 2H), 2.15–1.95 (br m, 1H), 1.90–1.78 (br m, 1H), 1.70–1.60 (br m, 2H), 1.60–1.48 br m, 2H), 1.45–1.20 (br m, 22H), 0.90 ppm (br t, $J = 7.6$, 2H); $^{13}C$ NMR: $\delta = 173.68/173.65$ (overlapping), 136.1, 128.5, 128.1, 72.2, 66.09/66.06 (two overlapping s), 66.0, 43.97/43.95 (overlapping), 37.3, 34.3, 31.9, 30.7, 30.6, 30.2, 29.94, 29.87, 29.8, 29.7, 29.62, 29.58, 29.5, 29.33, 29.29, 29.2, 29.14, 29.08, 29.0, 27.7, 27.6, 24.90, 24.88, 23.4, 23.3, 22.7, 14.1 ppm; HRESIMS calcd for $C_{27}H_{44}O_3Na$ $[M+Na]^+$: 439.3188, found: 439.3174.

Using a procedure similar to that described for preparation of **6**, the cyclobutanol benzyl esters (0.025 g, 0.060 mmol) were subjected to hydrogenation in the presence of 10% Pd/C (0.013 g, 0.012 mmol) and EtOAc (0.6 mL) to afford cyclobutanoic acid **8** (0.016 g, 81%) as a colorless oil. Although the product is assumed to consist of a similar mixture of regio- and stereoisomers as was present in the benzyl ester precursor, the individual isomers are not separable: $R_f = 0.32$ (EtOAc/Hex/AcOH 4:6:0.05); IR: $\tilde{\nu} = 3309$ (br), 2922, 2853, 1709 $cm^{-1}$; $^1H$ NMR: $\delta = 4.22$ (q, $J = 7.6$, 0.7H, assumed -OH is *cis* to backbone from the characterization of starting material), 3.92 (q, $J = 7.6$, 0.3H, assumed OH is *trans* to backbone), 2.43–2.33 (br m, 1H), 2.34 (t, $J = 7.3$, 2H), 2.18–1.92 (m), 1.84 (br sept., $J = 8.0$, 1H), 1.68–1.58 (br quint., $J = 7.0$, 2H), 1.58–1.48 (br m, 2H), 1.43–1.18 (br m, 22H), 0.88 ppm (br t, $J = 7.0$ Hz, 3H); $^{13}C$ NMR: $\delta = 179.50/179.46$ (two app s), 72.28, 66.16/66.12 (two app s), 48.87/48.79 (two app s), 43.97, 37.26, 35.06, 33.98, 31.89, 30.69/30.62, 30.24, 30.18, 29.90, 29.87, 29.79, 29.74, 29.63, 29.60, 29.51, 29.46, 29.34, 29.31, 29.21, 29.11, 29.06, 29.01, 28.99, 28.97, 28.67, 28.60, 28.05, 27.95, 27.92, 27.73, 27.60, 24.66/24.63, 23.38/23.34, 22.67, 14.10 ppm; HRESIMS calcd for $C_{20}H_{38}O_3Na$ $[M+Na]^+$: 349.2719, found 349.2715; purity >99% by HPLC ($t_R$: 11.8 and 15.9 min).

**2-Chloro-4-(octanoic acid benzyl ester-8-yl)-3-octylcyclobutanone; 2-Chloro-3-(octanoic acid benzyl ester-8-yl)-4-octylcyclobutanone (22)** was prepared based on a modification of a known procedure.[9,22,24] To a solution of dichloroketone **19** (0.992 g, 2.100 mmol) in 4 mL glacial acetic acid was added Zn dust (0.148 g, 2.30 mmol). [Note: The amount of Zn required depends on the purity of the dichlorocyclobutanone; it was generally necessary to add a second equivalent after ~12 h of reaction.] The mixture was stirred at RT until complete disappearance of the starting material was observed (16 h, TLC). The reaction mixture was then cooled in an ice bath and diluted with $H_2O$ (20 mL). The resulting solution was extracted with EtOAc (3×50 mL) and the combined organic layers were washed sequentially with $H_2O$ (2×100 mL) and saturated $NaHCO_3$ solution (2×50 mL). The resulting organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure to furnish a colorless oil (0.895 g, 97% crude yield) as a 1:1 mixture of regioisomers, each a 9:1 mixture of *cis*/*trans* stereoisomers ($^1H$ NMR); the mixture was used directly in the next step. Analysis of the $^1H$ NMR indicated that the expected product was accompanied by recovered starting material **19** (~14%) and the cyclobutanone **21** (over-reduction product, ~10%). These by-products could not be removed easily by flash chromatography. $R_f = 0.58/0.53$ (major), 0.48/0.43 (minor) (10% EtOAc in Hex); IR: $\tilde{\nu} = 3036$, 2925, 2854, 1789, 1735, 1456, 1162, 696 $cm^{-1}$; $^1H$ NMR: $\delta = 7.36–7.25$ (m, 5H), 5.12/5.11 (overlapping s, 2H), 4.97 (dd, $J = 2.6$, 9.5, 0.1H), 4.51 (dd, $J = 2.8$, 7.6 Hz, 0.9H), 3.35–3.20 (m, 1.1H), 2.75–2.65 (m, 0.1H), 2.53–2.40 (m, 0.9H), 2.36/2.35 (overlapping t, $J = 7.2$ Hz, 2H), 1.75–1.20 (m, 26H), 0.92–0.85 ppm (m, 3H); $^{13}C$ NMR: $\delta = 203.48/203.45$, 173.5, 136.1, 128.5, 128.1, 66.0, 65.7, 58.3, 58.2, 40.74/40.72, 34.18, 31.77/31.75, 29.5, 29.3, 29.24, 29.16, 29.14, 29.13, 29.02, 28.97, 28.90, 28.86, 28.02, 27.96, 27.92, 27.87, 25.64/25.57, 24.80/24.78, 22.59/22.58, 14.0 ppm; HRESIMS calcd for $C_{27}H_{41}O_3ClNa$ $[M+Na]^+$: 471.2642, found 471.2635.

**2-Chloro-4-(octanoic acid-8-yl)-3-octylcyclobutanone-; 2-Chloro-3-(octanoic acid-8-yl)-4-octylcyclobutanone (9)** was prepared in a similar manner as compound **6** from reaction of benzyl ester **22** (0.085 g, 0.19 mmol), 10% Pd/C (0.040 g, 0.038 mmol) and EtOAc (1.9 mL). The product, acid **9** (0.048 g, 70%), was obtained as a colorless oil after purification by flash column chromatography (20% EtOAc/Hex). The product, which was predominantly the *cis*,*cis* stereoisomer on the cyclobutanone, included ~5% of an inseparable *trans*-chlorocyclobutanone **11** ($^1H$ NMR signal at $\delta = 4.37$ ppm), probably arising from the epimerization of **9**: $R_f = 0.19$ (Hex/EtOAc 4:1); IR: $\tilde{\nu} = 3036$, 2924, 2854, 1788, 1706, 1461 $cm^{-1}$; $^1H$ NMR: $\delta =$ 10.74 (br s, 1H, COO*H*), 4.96 (dd, $J = 9.4$, 2.6, 0.05H), 4.50 (dd, $J =$ 7.6, 2.8, 0.95H), 3.35–3.20 (m, 1.05H), 2.46 (quint., $J = 8.1$, 0.95H), 2.74–2.62 (m, 0.05H), 2.35/2.33 (overlapping t, $J = 7.3$, 2H), 1.80–1.15 (m, 22H), 0.88/0.87 ppm (overlapping t, $J = 6.9$, 3H); $^{13}C$ NMR: $\delta = 203.62/203.56$, 180.1, 65.7, 58.30/58.26, 401.8, 34.0, 31.8, 29.5, 29.4, 29.3, 29.18/29.15, 29.04/29.00, 28.9, 28.04/27.98, 27.95/27.90, 25.67/25.59, 24.5, 22.6, 14.1 ppm; HRESIMS calcd for $C_{20}H_{35}O_3ClNa$ $[M+Na]^+$: 381.2172, found 381.2191.

**2,2-Dichloro-4-(octanoic acid benzyl ester-8-yl)-3-octylcyclobutanone-; 2,2-Dichloro-3-(octanoic acid benzyl ester-8-yl)-4-octylcyclobutanone (23)** was prepared based on a known procedure.[22–24] To a mixture of Zn(Cu) (1.84 g, 28.0 mmol) and benzyl elaidate (2.07 g, 5.60 mmol) in 11 mL of anhydrous $Et_2O$ was added over 2 h (syringe pump) trichloroacetyl chloride (2.0 mL, 18 mmol). The reaction mixture was stirred at RT for 5 h (or until the starting material is consumed by TLC) and filtered through a plug of Celite.

The residue was washed with $Et_2O$ (20 mL). The combined ether layers were transferred to a round-bottom flask and cooled over ice. The black solution was then diluted with 40 mL $H_2O$ and 40 mL sat. $NaHCO_3$. The mixture was allowed to warm to RT and stirred overnight. The aqueous layer was extracted with $Et_2O$ (3× 50 mL), and the combined organic layers were dried over $Na_2SO_4$ and evaporated to dryness. The residue obtained upon concentration could be used for the next step without further purification or, alternatively, could be purified by rapid flash column chromatography through a short plug of silica (2.5%/5% EtOAc/Hex) to afford a yellow oil (1.69 g, 63%) consisting of a 1:1 mixture of regioisomers: $R_f$ = 0.63 (20% EtOAc/Hex); IR: $\tilde{\nu}$ = 2926, 2855, 1802, 1735, 1456, 1162, 696 $cm^{-1}$; $^1H$ NMR: $\delta$ = 7.47–7.23 (m, 5H), 5.120/5.117 (overlapping s, 2H), 3.158/3.126 (overlapping dt, $J$ = 7.2, 1.5 Hz, 1H), 2.56–2.46 (m, 1H), 2.37/2.34 (overlapping t, $J$ = 7.6 Hz, 2H), 1.96–1.18 (m, 26H), 0.90/0.87 ppm (overlapping t, $J$ = 7.2 Hz, 3H); $^{13}C$ NMR, 75 MHz: $\delta$ = 196.3, 173.5, 136.0, 128.4, 128.1, 87.1, 66.0, 60.7, 60.6, 52.3, 34.2, 34.1, 31.8, 31.7, 31.37, 31.36, 29.42, 29.40, 29.3, 29.23, 29.20, 29.14, 29.12, 29.08, 28.93, 28.90, 28.85, 27.5, 27.4, 27.12, 27.05, 24.80, 24.78, 22.59, 22.57, 14.04, 14.03 ppm; HRESIMS calcd for $C_{27}H_{40}O_3Cl_2Na$ $[M+Na]^+$: 505.2252, found: 505.2248.

**2,2-Dichloro-4-(octanoic acid benzyl ester-8-yl)-3-octylcyclobutanol, methanesulfonate ester; 2,2-Dichloro-3-(octanoic acid benzyl ester-8-yl)-4-octylcyclobutanol, methanesulfonate ester (24)** was prepared by a similar procedure as **20** through reaction of dichlorocyclobutanone **23** (0.210 g, 0.400 mmol), $NaBH_4$ (0.055 g, 1.50 mmol), and *i*PrOH (6 mL). The 1:1 mixture of regioisomeric dichlorocyclobutanols (0.166 g, 85%) was obtained as a colorless oil following flash chromatography (5% EtOAc/Hex): $R_f$ = 0.66(minor)/0.61(major)/0.56(minor)/0.51(major) (10% EtOAc/Hex); IR: $\tilde{\nu}$ = 3459 (br), 2924, 2853, 1736, 1456, 1161, 734, 696 $cm^{-1}$; $^1H$ NMR: $\delta$ = 7.36–7.31 (m, 5H), 5.12 (s, 2H), 4.37 (brd, $J$ = 6.4 Hz, 0.4H), 3.91 (brd, $J$ = 8.0 Hz, 0.6H), 2.80–2.45 (brm, 2H), 2.37/2.36 (overlapping t, $J$ = 7.6 Hz, 2H), 1.80–1.05 (brm, 26H), 0.89/0.88 ppm (overlapping t, $J$ = 6.7 Hz, 3H). Small quantities of individual isomers could be separated for analysis by flash column chromatography using 10% EtOAc/Hex. Stereochemical assignments are based on 2D NMR experiments (COSY, NOESY, and HSQC).

*First-eluting $H_1$-$H_3$ trans*: $R_f$ = 0.66; IR: $\tilde{\nu}$ = 3463 (br), 2927, 2855, 1738, 1453, 1158 $cm^{-1}$; $^1H$ NMR: $\delta$ = 7.37–7.31 (m, 5H), 5.12 (s, 2H), 4.38 (ddd, $^3J_{H1,H4}$ = 6.8 Hz, $^3J_{H1,OH}$ = 5.1 Hz, $^4J_{H1,H3(trans)}$ = 1.3 Hz, 1H, C*H*-OH), 2.62–2.55 (m, 1H, C*H*-$CCl_2$), 2.44 (d, $^3J_{H1,OH}$ = 5.1 Hz, -O*H*), 2.36 (t, $J$ = 7.6 Hz, 2H), 2.18–2.08 (m, 1H, C*H*-CHOH), 1.80–1.10 (brm, 26H), 0.89 ppm (t, $J$ = 6.7 Hz, 3H); $^{13}C$ NMR (NOESY): $\delta$ = 173.6, 136.1, 128.5, 128.2, 90.2, 78.5, 66.1, 57.0, 42.0, 34.3, 31.9, 31.3, 29.7, 29.5, 29.4, 29.3, 29.0, 27.7, 27.5, 26.6, 24.9, 22.7, 14.1 ppm; HRESIMS calcd for $C_{27}H_{40}O_3Cl_2$ $[M+Na]^+$: 507.2409, found: 507.2409.

*First-eluting $H_1$-$H_3$ cis*: $R_f$ = 0.61; IR: $\tilde{\nu}$ = 3448 (br), 2925, 2854, 1737, 1456, 1163, 734, 697 $cm^{-1}$; $^1H$ NMR: $\delta$ = 7.37–7.31 (m, 5H), 5.12 (s, 2H), 3.91 (dd, $^3J_{H1,OH}$ = 10.8 Hz, $^3J_{H1,H4(trans)}$ = 8.0 Hz, $^4J_{H1,H3(cis)}$ = 0 Hz, 1H, C*H*-OH), 2.46 (d, $J$ = 10.8 Hz, 2H, O*H*), 2.36 (t, $J$ = 7.6 Hz, 2H), 2.11–2.04 (m, 1H, C*H*-$CCl_2$), 1.75–1.18 (m, 27H), 0.88 ppm (t, $J$ = 6.8 Hz, 3H); $^{13}C$ NMR, 75 MHz (NOESY): $\delta$ = 173.6, 136.1, 128.5, 128.2, 91.0, 81.6, 66.1, 51.9, 48.1, 34.3, 33.0, 31.8, 30.0, 29.6, 29.41, 29.38, 29.2, 29.0, 27.3, 26.8, 24.9, 22.6, 14.1 ppm; HRESIMS calcd for $C_{27}H_{40}O_3Cl_2$ $[M+Na]^+$: 507.2409, found: 507.2415.

*Second-eluting $H_1$-$H_3$ trans*: $R_f$ = 0.56; IR: $\tilde{\nu}$ = 3463 (br), 2925, 2854, 1736, 1456, 1163, 696 $cm^{-1}$; $^1H$ NMR: $\delta$ = 7.36–7.31 (m, 5H), 5.11 (s, 2H), 4.40–4.34 (m, 1H), 2.64–2.55 (m, 1H, C*H*-OH), 2.64–2.55 (m, 1H, C*H*-$CCl_2$), 2.46 (d, $J$ = 4.8 Hz, 1H, O*H*), 2.35 (t, $J$ = 7.6 Hz, 2H), 2.20–2.03 (m, 1H, C*H*-CHOH), 1.80–1.10 (brm, 26H), 0.88 ppm (t, $J$ = 6.4 Hz, 3H); $^{13}C$ NMR, 75 MHz: $\delta$ = 173.7, 136.1, 128.5, 128.2, 90.2, 78.4, 66.1, 57.0, 42.0, 34.3, 31.9, 31.3, 29.6, 29.5, 29.41, 29.39, 29.2, 29.1, 29.0, 27.6, 27.5, 26.7, 24.9, 22.7, 14.1 ppm; HRESIMS calcd for $C_{27}H_{40}O_3Cl_2$ $[M+Na]^+$: 507.2409, found: 507.2398.

*Second-eluting $H_1$-$H_3$ cis*: $R_f$ = 0.51; IR: $\tilde{\nu}$ = 3444 (br), 2924, 2854, 1734, 1162, 734, 696 $cm^{-1}$; $^1H$ NMR: $\delta$ = 7.37–7.31 (m, 5H), 5.11 (s, 2H), 3.90 (dd, $^3J_{H1,OH}$ = 10.8 Hz, $^3J_{H1,H4(trans)}$ = 8.0 Hz, $^4J_{H1,H3(cis)}$ = 0 Hz, 1H, C*H*-OH), 2.52 (O*H*, d, $J$ = 10.8 Hz, 2H), 2.35 (t, $J$ = 7.6 Hz, 2H), 2.13–2.02 (m, 1H, C*H*-$CCl_2$), 1.75–1.18 (m, 27H), 0.89 ppm (t, $J$ = 6.8 Hz, 3H); $^{13}C$ NMR: (75 MHz): $\delta$ = 173.7, 136.1, 128.5, 128.1, 91.0, 81.6, 66.1, 51.9, 48.0, 34.2, 33.0, 31.8, 30.0, 29.6, 29.4, 29.3, 29.2, 29.02, 28.97, 28.9, 27.2, 26.8, 24.9, 24.8, 22.6, 14.1 ppm; HRESIMS calcd for $C_{27}H_{40}O_3Cl_2$: $[M+Na]^+$ 507.2409, found:507.2409.

The mixture of dichlorocyclobutanols was converted into the methanesulfonates (mesylate) in a similar manner as for **20**. Reaction of methanesulfonyl chloride (0.05 mL, 0.70 mmol), the dichlorocyclobutanols (0.166 g, 0.300 mmol), and $Et_3N$ (0.20 mL, 1.7 mmol) in $CH_2Cl_2$ (2 mL), followed by purification using flash chromatography (5% EtOAc/Hex) provided dichloromesylate **24** as a light-yellow oil (0.146 g, 86%) in a mixture of inseparable regio- and stereoisomers: $R_f$ = 0.40/0.38 (20% EtOAc/Hex); IR: $\tilde{\nu}$ = 2925, 2851, 1734, 1368, 1180, 964, 697 $cm^{-1}$; $^1H$ NMR: $\delta$ = 7.38–7.30 (m, 5H), 5.20 (dd, $J$ = 7.1, 1.2 Hz, 0.4H), 5.115/5.112 (two overlapping s, total 2H), 4.84 (d, $J$ = 8.8 Hz, 0.6H), 3.202/3.199 (two overlapping s, 1.8H), 3.166/3.163 (two overlapping s, total 1.2H), 2.66–2.58 (m, 0.4H), 2.40–2.30 (m, 2H), 2.21–2.12 (m, 0.6H), 2.10–1.98 (m, 0.6H), 1.80–1.15 (m, 26.4H), 0.91–0.86 ppm (m, 3H); $^{13}C$ NMR: $\delta$ = 173.6, 136.10/136.08, 128.5/128.1, 86.45/86.38, 84.09, 83.86/83.82, 66.03/66.01, 57.18, 51.81/51.80, 44.51/44.50, 41.71, 39.5, 39.0, 34.2, 32.21/32.18, 31.78/31.77, 31.41/31.36, 30.1, 29.49 29.46, 29.42, 29.34, 29.32, 29.27, 29.22, 29.16, 28.95, 28.90, 28.2, 27.0, 26.9, 26.8, 26.7, 26.6, 26.5, 26.4, 26.3, 24.8, 22.6, 14.1 ppm; HRESIMS $C_{28}H_{44}O_5Cl_2SNa$ $[M+Na]^+$: 585.2184, found: 585.2186.

**1-(Octanoic acid-8-yl)-2-octylcyclobutene (10)** was prepared from reductive fragmentation and deprotection of dichloromethansulfonate **24** (0.143 g, 0.250 mmol) in THF (1 mL), $NH_3$ (~10 mL) and Na (~0.090 g, 4.10 mmol) by a procedure similar to that employed for **5**. The crude product was purified by flash chromatography (10% EtOAc/Hex) to afford the acid **10** (0.035 g, 46%) as a colorless oil: $R_f$ = 0.35 (Hex/EtOAc 60:40); IR: $\tilde{\nu}$ = 3124, 2923, 2852, 1708, 910, 736 $cm^{-1}$; $^1H$ NMR: $\delta$ = 6.12 (brs, 2H), 2.35 (t, $J$ = 7.2 Hz, 2H), 2.26 (t, $J$ = 7.2 Hz, 2H), 1.64 (quint., $J$ = 7.2 Hz, 2H), 1.44 (brm, 4H), 1.35–1.20 (23H), 0.88 ppm (t, $J$ = 6.8 Hz, 3H); $^{13}C$ NMR: $\delta$ = 179.7, 139.2, 139.1, 50.4, 50.3, 34.02, 33.97, 31.9, 29.9, 29.6, 29.3, 29.2, 29.0, 28.4, 28.3, 24.7, 22.7, 14.1 ppm; HRFABMS (3-NBA matrix): calcd for $C_{20}H_{35}O_2Li_2$ $[M-H+2Li]^+$: 321.2957, found: 321.2969; purity 100% by HPLC ($t_R$: 4.73 min).

**2-Chloro-4-(octanoic acid-8-yl)-3-octylcyclobutanone-; 2-Chloro-3-(octanoic acid-8-yl)-4-octylcyclobutanone (11)** was prepared by a similar procedure as described for compound **9**. Reaction of benzyl ester **23** (0.500 g, 1.000 mmol), Zn(Cu) (0.074 g, 1.10 mmol) and glacial acetic acid (2 mL) furnished, after flash chromatography (5% EtOAc/Hex), *trans*-monochlorocyclobutanone (0.313 g, 70%) as a colorless oil, which included an equal mix of the 2-chloro-4-octyl and 2-chloro-3-octyl regioisomers, each of which included both epimers at the chloride-bearing carbon: $R_f$ = 0.42/0.39 (minor) and 0.33 (major) (10% EtOAc/Hex). The amount of Zn dust varied from 1.1 to 2.2 equivalents; generally, a second equivalent of zinc dust was added to the incomplete reaction mixture after stirring for ~12 h. The residual dichlorocyclobutanone was easily removed by flash column chromatography. However, the cyclobutanone

product of over-reduction was formed in significant amounts (28% based on $^1$H NMR and was nearly inseparable by flash column chromatography: IR: $\tilde{\nu}$ = 2924, 2854, 1788, 1735, 1457, 1161, 733, 696 cm$^{-1}$; $^1$H NMR: $\delta$ = 7.40–7.32 (m, 5H), 5.113/5.110 (overlapping s, 2H), 4.95 (dd, $J$ = 3.1, 9.4, 0.3H), 4.37 (dt, $J$ = 2.7, 7.8 Hz, 0.7H), 2.97–2.90 (m, 0.3H), 2.84–2.74 (m, 0.7H), 2.40–2.30 (overlapping m, 2.3H), 2.08–1.97 (m, 0.7H), 1.85–1.10 (m, 26H), 0.92–0.84 ppm (m, 3H); $^{13}$C NMR: $\delta$ = 204.6, 202.0, 173.5, 136.1, 128.5, 128.1, 66.0, 65.52/65.46, 63.98, 63.78/63.75, 63.57/63.52, 60.74/60.69, 49.8, 43.7, 37.4, 36.63/36.59, 35.12/35.10, 34.2, 31.8, 31.3, 30.3, 30.1, 29.5, 29.42, 29.38,29.32, 29.25, 29.19, 29.03, 29.00, 28.96, 28.93, 28.88, 28.2, 27.67, 27.60, 27.55, 27.48, 27.38, 27.3, 27.2, 27.1, 24.83/24.80, 22.6, 14.0 ppm; HRESIMS calcd for $C_{27}H_{41}O_3ClNa$ $[M+Na]^+$: 471.2642, found: 471.2630.

The resulting mixture of monochloroketone mixtures was subjected to debenzylation described in the preparation of compound **6**. Reaction of the benzyl ester mixture (0.034 g, 0.11 mmol), with 10% Pd/C (0.024 g, 0.022 mmol) and EtOAc (1 mL) afforded the unprotected acid (0.013 g, 54%) as a white solid: $R_f$ = 0.24 (Hex/EtOAc/AcOH 6:4:0.05); mp: 74–75 °C; IR: $\tilde{\nu}$ = 3062, 2924, 2854, 1789, 1708, 1464 cm$^{-1}$; $^1$H NMR: $\delta$ = 8.70 (br s, 1H), 4.95 (dd, $J$ = 2.9, 9.2, 0.3H), 4.37 (ddd, $J$ = 7.8, 2.5, 1.3, 0.7H), 2.97–2.91 (m, 0.3H), 2.85–2.73 (m, 0.7H), 2.40–2.22 (m, 22H), 2.11–2.98 (m, 0.7H), 1.85–1.10 (m, 26H), 0.92–0.82 ppm (m, 3H); $^{13}$C NMR $\delta$ = 211.7, 204.7, 202.2, 179.9, 65.6/65.5, 64.0, 63.85/63.82, 63.64/63.58, 60.82/60.77, 49.81, 43.8, 37.5, 36.69/36.65, 35.2, 34.0, 31.8, 31.3, 30.4, 30.2, 29.55, 29.48, 29.44, 29.38, 29.31, 29.25, 29.2, 29.06, 29.06, 28.9, 28.3, 28.2, 27.73, 27.67, 27.62, 27.44, 27.37, 27.26, 27.20, 27.17, 24.63/24.60, 22.7, 14.1 ppm; HRESIMS calcd for $C_{20}H_{35}O_3ClNa$ $[M+Na]^+$: 381.2172, found: 381.2170.

**Determination of inhibitor aqueous stability and solubility**: 1D $^1$H NMR spectroscopy was used to verify the chemical purity, aqueous stability, and concentration-dependent micelle formation of eleven fatty acid analogues. Each compound was dissolved in [$D_6$]DMSO to obtain a stock concentration of 20 mM. Six different concentrations were prepared from the stock solutions for NMR analysis: 1.00 mM, 750 μM, 500 μM, 100 μM, and 0 μM. All NMR samples consisted of 600 μL of a deuterated 50 mM potassium phosphate buffer at pH 7.2 with 50 μM of 3-(trimethylsilyl)propionic-2,2,3,3-$d_4$ acid sodium salt (TMSP). Each 600 μL NMR sample contains 30 μL (5%) of [$D_6$]DMSO and was transferred to a 5 mm NMR tube for analysis.

A Bruker Avance DRX 500 MHz spectrometer equipped with a 5 mm triple-resonance cryoprobe ($^1$H, $^{13}$C, $^{15}$N) with a $z$-axis gradient was used for all 1D $^1$H NMR experiments. Acquisition of NMR spectra was automated using a BACS-120 sample changer and Icon NMR software. All spectra were acquired at 298.15 K with 16 dummy scans, 64 scans, 32 K data points, a spectral width of 5482.46 Hz, and a relaxation delay of 1.5 s. The NMR spectra were processed and analyzed using ACD/1D NMR Manager (Advanced Chemistry Development). The resulting 1D $^1$H NMR spectra were visually inspected for evidence of micelle formation (peak broadening), or chemical instability/impurities (additional peaks).

**Measurement of nonspecific cytotoxicity**: RAW 264.7 macrophages were incubated with 0–100 μM of oleic acid or the $C_{18}$ cyclobutene fatty acid **2**, each delivered as bovine serum albumin complexes. After 24 h, cell viability was relative to untreated controls was assessed using an IN CTYOTOX-CVDE Crystal violet Dye Elution Kit. The values shown in Table S1 are means ± SEM based on measurement in triplicate.

**Bacterial strains and culture conditions**: Bacterial strains used in this study are the *E. coli* wild-type strain G58–1, the genome sequencing *Msm* strain mc$^2$155, and the *Mtb* strains CDC1551 and H37Rv. Cells were grown shaking at 37 °C in LB broth for *E. coli* or complete Middlebrook 7H9 broth supplemented with 0.05% *v/v* Tween 80 to $OD_{600}$ 0.6–1.2 for mycobacteria. For MIC determinations, cells were inoculated into LB for *E. coli* or a modified previously reported minimal medium for both *E. coli* and mycobacteria.[26] The minimal media components and final concentrations are as follows: 22 mM dibasic potassium phosphate, 16 mM monobasic potassium phosphate, $2.8\times10^{-5}$ mM ferric chloride, $8.7\times10^{-3}$ mM zinc sulfate, $8.4\times10^{-4}$ mM cobalt(II) chloride, $1.0\times10^{-2}$ mM manganese chloride, $6.8\times10^{-2}$ mM calcium chloride, 2.4 mM magnesium sulfate, 5.0 mM ammonium chloride, 25 mM glycerol, and 0.02% *v/v* Tyloxapol.

**Drug susceptibility assays**: MICs were determined by a 96-well microplate method, as described by Chacon et al.[26] Bacteria were harvested, washed 2× with minimal media, and inoculated to an initial concentration of ~$1.0\times10^5$ colony forming units (CFU) per well. The initial inoculum was plated to verify retrospectively the desired CFU mL$^{-1}$ for each strain. Stocks of fatty acid analogue compounds were prepared in 100% [$D_6$]DMSO, as this solvent was chosen to provide consistency with the NMR studies. Appropriate doubling dilution gradients (μg mL$^{-1}$ compound dissolved in the corresponding percent *v/v* [$D_6$]DMSO) were prepared in the following ranges for each compound tested: *E. coli*, 8–512; *Msm*, 16–1024; and *Mtb*, 0.25–256 (e.g., 64 μg mL$^{-1}$ of compound corresponded to 0.064% *v/v* of [$D_6$]DMSO). Alternatively, the concentration of [$D_6$]DMSO was adjusted in all wells to its maximum concentration in the corresponding gradient (e.g., 64 μg mL$^{-1}$ of compound corresponded to 0.256% *v/v* of [$D_6$]DMSO). We tested cells in the absence of compound and [$D_6$]DMSO to verify cell viability. The effect of [$D_6$]DMSO was also tested by growing cells in minimal media with [$D_6$]DMSO and no compound. We observed no significant effect of [$D_6$]DMSO on cell viability for *E. coli* and *Msm*. However for *Mtb*, we observed some variability with anomalous inhibitory results in a few technical replicate wells containing [$D_6$]DMSO alone at higher concentrations. Nonetheless, most of the replicate wells displayed no inhibition, allowing us to eliminate these anomalous results. Moreover, the effect of the compound was observed at [$D_6$]DMSO concentrations with no inhibitory action. Plates were incubated at 37 °C for 2 days (*E. coli*), 4 days (*Msm*), or 7 weeks (*Mtb*). MIC values were determined by the consistent results of three biological and three technical replicates. The MIC was defined by taking the mode of three independent cultures where the MIC did not differ by more than one doubling dilution, discarding any results that are two doubling dilutions away from the mode.

**Abbreviations**: attenuated total reflection (ATR), correlation spectroscopy (COSY), dicyclohexyl carbodiimide (DCC), D-cycloserine (DCS), *N*,*N*′-dimethylformamide (DMF), hexadeutero dimethyl sulfoxide ([$D_6$]DMSO), differential scanning calorimetry/thermal gravimetric analysis (DSC/TGA), ethyl acetate (EtOAc), hexane (Hex), high-performance liquid chromatography (HPLC), high-resolution mass spectrometry via electrospray ionization (HRESIMS), high-resolution mass spectrometry via fast atom bombardment (HRFABMS), heteronuclear single quantum coherence spectroscopy (HSQC), 4-dimethylaminopyridine (DMAP), isopropanol (*i*PrOH), multidrug resistant (MDR), minimum inhibitory concentration (MIC), *Mycobacterium smegmatis* (*Msm*), *Mycobacterium tuberculosis* (*Mtb*), nuclear Overhauser effect spectroscopy (NOESY), nuclear magnetic resonance (NMR), tetrahydrofuran (THF), thin-layer chromatography (TLC), ultraviolet (UV).

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