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Synthesis of long chain β -lactones and their antibacterial activities against pathogenic mycobacteria

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Abstract: In the quest for new antibacterial agents, a series of novel long and medium chain mono- and disubstituted β -lactones has been developed. Their respective antibacterial activity using the resazurin microtiter assay (REMA) was further assessed against three pathogenic mycobacteria: *M. abscessus*, *M. marinum* and *M. tuberculosis*. Among the 16 β -lactones synthesized, only **VM005** exhibited promising activity against *M. abscessus*; while most of the β -lactones showed interesting activities against *M. marinum*, similar to those of classical antibiotic, isoniazid. Regarding *M. tuberculosis*, 6 compounds were found active against this mycobacterium, with β -lactone **VM008** being the best growth inhibitor. The promising antibacterial activities of the best compounds suggest that these molecules may serve as potential leads for the development of much more efficient anti-mycobacterial agents.

Introduction

Among the potent lipolytic enzyme inhibitors, β -lactones represent an important class of compounds bearing the strained 2-oxetanone 4-membered ring responsible for potent inhibitory activity against enzymes from the serine/cysteine hydrolase class.^[1] Chemical profiling studies have indeed revealed that β -lactones target more than 20 different enzymes of four classes comprising oxidoreductases, transferases, hydrolases, and ligases. The labelled enzymes contained either an active site serine or cysteine residue, and belonged to different classes, including lipases, proteases, esterases or thioesterases.^[1b, 1c] Most natural occurring and synthetic β -lactones that have been explored as antibacterials or enzyme inhibitors are *trans* diastereoisomers,^[2] however *cis*- β -lactones have been also shown to be as potent in some cases.^[2c, 2d] Recently, β -lactones have also emerged as attractive probes for activity-based protein profiling (ABPP) of the larger serine hydrolase family thus making them exemplary structures for the identification of novel biological targets.^[3] The most representative member of this family of inhibitors, is the FDA approved anti-obesity drug Orlistat (also known as tetrahydrolipstatin, THL, **1** – Figure 1), that inhibits the human digestive lipases by covalently binding to their active site.^[4] Functioning as a versatile serine/cysteine

hydrolase inhibitor, Orlistat, or its recent β -lactone EZ120 analog,^[5] has been reported to block *Mycobacterium tuberculosis* (*M. tb*) growth *via* covalent inhibition of various endogenous lipolytic enzymes involved in bacterial lipid metabolism.^[6]

Figure 1. Structure of Orlistat and related β -lactone analogs. The carbonyl reactive sites on the β -lactone ring, where nucleophilic attack by catalytic serine or cysteine residue occurs, are indicated by an arrow.^[1a, 1b]

The physician Robert Koch identified *M. tb* as the causative agent of tuberculosis (TB) in 1882. Today, more than a century later, TB remains the leading cause of death due to a single infectious agent according to the World Health Organization.^[7] Moreover, the continuous emergence of resistant strains highlights the pressing need for new therapeutic approaches.^[8] In this context, lipolytic enzymes represent novel and promising targets to combat the disease.^[9] Indeed, *M. tb* survival and persistence *in vivo* require the utilization of distinct host-derived lipids as energy source suggesting a major role of these enzymes in the tubercle bacilli life cycle.^[10] Many serine/cysteine hydrolases, which are involved in lipid metabolism, are playing an essential role in cell wall and mycolic acid biosynthesis / maintenance.^[11] Therefore, the use of inhibitors of these enzymes to impair such activity is becoming an interesting alternative strategy not only in the fight against TB,^[12] but also against other chronic mycobacterial infections like those caused by *M. marinum* and *M. abscessus*.^[13]

M. marinum is an opportunist pathogenic mycobacteria, typically found in aquatic environments where it causes a systemic tuberculosis-like infection and disease in ectotherms such as frogs and fish. In humans, this mycobacterium is typically limited to lesions of skin and soft tissues extremities, which established *M. marinum* as a relevant human pathogen albeit opportunistic.^[14] *M. abscessus* is responsible for cutaneous and

pulmonary infections, representing up to 90% of non-tuberculous mycobacteria (NTM) infections in cystic fibrosis (CF) populations.^[15] *M. abscessus* displays two distinct morphotypes: a smooth (S) and a rough (R) one.^[16] The major difference between both variants resides in the total loss of surface-associated glycopeptidolipids (GPL) in the R form.^[17] Moreover, several studies have clearly established the hypervirulence phenotype of the R variant in severe infection caused by *M. abscessus*.^[18] Accordingly, *M. abscessus* is intrinsically resistant to a broad range of antibiotics including most antitubercular drugs, and is considered the most pathogenic and chemotherapy-resistant rapidly growing mycobacterium.^[19]

In our quest for discovering novel anti-mycobacterial agents that might react covalently with catalytic serine/cysteine-based enzymes of the mycobacteria, we herein report the synthesis of a series of long and medium chain substituted β -lactones (*i.e.*, **VM** compounds, Figure 1) together with their antibacterial activities against the latter three pathogenic mycobacteria. The rationale behind the present design was to create structures which incorporate *i)* lipidic chains, such as the oleyl chain, that appear in naturally occurring lipids and resemble natural lipolytic enzyme substrates; but also *ii)* the β -lactone group which will interact with the catalytic serine or cysteine residue of hydrolases that might be essential for the growth and/or survival of the mycobacteria.^[6]

Results and Discussion

In order to investigate the effects of various α -substitutions on the β -lactone ring, we first used at the α -position either *i)* an unsaturated C16 alkenyl chain that derives from oleic acid, *ii)* a long saturated C16 alkyl chain, or *iii)* a medium saturated C6 alkyl chain. In each case, a C3 small chain was introduced at the β -position (*i.e.*, compounds **VM001**, **VM002** and **VM003**, respectively). The general route for the synthesis of the designed α,β -disubstituted β -lactones is depicted in Scheme 1. The appropriate carboxylic acids **2a-c** chosen as starting materials were first deprotonated by treatment with LDA. After reaction with commercial butyraldehyde, the corresponding β -hydroxy acids **3a-c** were obtained in 30-85% yields. Finally, cyclization of the intermediate α,β -disubstituted β -hydroxy acids **3a-c** by treatment with *p*-toluenesulfonyl chloride led to α,β -disubstituted β -lactones **VM001-3** in 42-67% yields (Scheme 1).^[20] Both β -hydroxy acids **3a-c** and β -lactones **VM001-3** were obtained as mixtures of diastereoisomers whose ratio was determined by ¹H NMR spectroscopy, and, in particular, by the relative peak integration of the characteristic α - and/or β -methinic protons (for a detailed analysis on the diastereomeric ratio, see Supplementary Material). According to the ¹H NMR spectra, the β -hydroxy acids **3a-c** were formed in an anti:syn isomer ratio varying from 6:4 to 7:3, while the corresponding isolated β -lactones **VM001-3** were in a *trans:cis* isomer ratio of around 7:3. At this initial stage of our research, it was preferred to synthesize the diastereoisomeric mixtures in order to identify potential leads that may later be prepared in diastereomerically or optically pure form. The chromatographic resolution of the diastereoisomers of the β -lactones is quite difficult as they present similar solubility and polarity.

Scheme 1. Synthesis of compounds **VM001-3**. Reagents and conditions: (i) a) LDA, dry THF, 0 °C, 1 h; b) butyraldehyde, dry THF, 0 °C, 1 h, then r.t. o.n.; (ii) *p*-TsCl, dry pyridine, 0 °C, 1 h, then 4 °C, 3 days.

These three β -lactones were tested for their anti-mycobacterial activity against three pathogenic mycobacterial strains as a preliminary test to identify a lead. The antibacterial activity was evaluated by determining their respective MIC using the resazurin microtiter assay (REMA).^[12b, 21] Therefore, susceptibility testing of the above molecules was performed towards *M. marinum* M; *M. tb* mc²6230 (H37Rv Δ RD1 Δ panCD) a derivative of H37Rv which contains a deletion of the RD1 region and *panCD*, resulting in a pan(-) phenotype strain;^[22] and both S and R variants of *M. abscessus* (Table 1).

First, in all cases, both S & R variants of *M. abscessus* remained unaffected by all three compounds up to a 100 μ g/mL concentration (Table 1); a result which proves that this bacteria well deserves its nickname of “antibiotics nightmare”.^[23] Regarding *M. marinum*, **VM003** exhibited moderate activity (MIC₅₀ = 15.6 μ g/mL), while **VM001** showed a good activity with a MIC₅₀ of 4.7 μ g/mL similar to that of isoniazid (Table 1). With regards to *M. tb* susceptibility, although Orlistat was able to block *M. tb* mc²6230 growth with quite good MIC₅₀ of 11.0 μ g/mL, only **VM001** displayed an interesting but moderate activity (MIC₅₀ = 31.8 μ g/mL) towards *M. tb*; whereas **VM003** appeared as a weak inhibitor (MIC₅₀ = 58.4 μ g/mL) and **VM002** was not active.

Table 1. Antibacterial activities of Orlistat and analogs **VM001-3** compared to standard antimicrobial agents against three pathogenic mycobacterial strains.^a

Cpds	MIC ₅₀ (μ g/mL) ^a			
	<i>M. abscessus</i> CIP 104536 ^T		<i>M. marinum</i> ATCC BAA-535/M	<i>M. tb</i> mc ² 6230
	Smooth	Rough		
Orlistat	44.4	57.0	6.8	11.0
VM001	>100	>100	4.7	31.8
VM002	>100	>100	>100	>100
VM003	>100	>100	15.6	58.4
INH	-	-	2.8	0.07
AMK	2.3	4.3	0.62	0.24

^a Minimum inhibitory concentration leading to 50% growth inhibition (MIC₅₀) as determined by the resazurin microtiter assay (REMA), are expressed as mean values of two independent assays performed in triplicate (CV% < 5%). INH: isoniazid; AMK: amikacin.

Taking into account these preliminary results and by identifying **VM001** with the oleyl chain at the α -position as the most promising one, we further investigated the effects of different

substituents on the β -position. Therefore, α,β -disubstituted β -lactones were synthesized (Scheme 2) in order to examine the influence of such side chains on the antibacterial activity of the corresponding compounds.

Scheme 2. A) Synthesis of compounds **VM009-12**. Reagents and conditions: (i) a) LDA, dry THF, 0 °C, 1 h; b) RCHO, dry THF, 0 °C, 1 h, then r.t. o.n.; (ii) *p*-TsCl, dry pyridine, 0 °C, 1 h, then 4 °C, 3 days. **B)** Racemic *cis* and *trans* pure stereoisomers obtained.

A methyl, ethyl, hexyl or a nonyl chain was then incorporated as β -side chain (Scheme 2). The synthetic procedure was similar to that of the initial compounds. Preparation of the desired β -hydroxy acid using LDA and the appropriate aldehyde, was followed by cyclization using *p*-toluenesulfonyl chloride in pyridine. Thus, β -lactones **VM009-12** were obtained in 36-63% yields (Scheme 2A) with a *trans*:*cis* isomer ratio of 7:3.

Furthermore, from the lead of the initial screening, **VM001**, the two racemic mixtures of *cis*-**VM001** and *trans*-**VM001**, namely **VM007** and **VM008**, respectively, were successfully isolated *via* column chromatography in order to be tested (Scheme 2B). The geometry of these isolated stereoisomers was determined by ¹H NMR spectroscopy based on the chemical shifts of the characteristic peaks of α - and β -protons that were compared to the corresponding reference peaks in the literature.^[2d, 24] The racemic mixture of *trans*-**VM009** assigned as **VM013** was also chromatographically isolated in pure form and tested.

As we were also interested in investigating the influence of the absence of the β -side chain on the compound activity, racemic α -monosubstituted β -lactones, *i.e.* compounds **VM004** and **VM005**, were synthesized (Scheme 3). These two compounds were synthesized by using diethyl malonate as starting material. The appropriate alkyl or alkenyl bromide was used in order to prepare the substituted diethyl malonates **5a,b** that were then hydrolyzed under controlled conditions into the corresponding acids **6a,b** bearing one ester group. The ester groups were reduced using LiBH₄^[25] and finally the β -hydroxy acids **7a,b** were cyclized using DEAD/PPh₃^[26] to yield the desired β -lactones **VM004** and **VM005** in around 17% overall yield. In order to conclude whether the stereochemical hindrance and/or the absence of the α -proton will make a significant difference in the activity, we also prepared the α,α' -disubstituted β -lactone **VM006** bearing a second small C4 chain at the α -position. Here, diethyl butylmalonate **4c** was used as starting material and

similar reactions were performed, although stronger conditions (more equivalents of NaH and KOH, for **5c** and **6c**, respectively) and prolonged reaction times (overnight r.t. stirring for **6a,b** in comparison to 2 days reflux for **6c**) were applied.

Scheme 3. Synthesis of compounds **VM004-6**. Reagents and conditions: (i) a) NaH 60%, dry THF; b) RBr, reflux, o.n.; (ii) KOH, EtOH; (iii) LiBH₄ 2.0 M, *i*-PrOH/dry THF 1:2, 0 °C, 1 h, then r.t., o.n.; (iv) DEAD, PPh₃, 0 °C, 1 h, then r.t., o.n.

Finally, we also synthesized the racemic β -monosubstituted β -lactones **VM014-16** to study the effect of the lack of the α -side chain on the compound activity (Scheme 4). The appropriate carboxylic acids **2a, 8a,b** were converted to the corresponding β -keto esters **9a-c** using Masamune's method with 1,1'-carbonyl diimidazole (CDI) and monoethyl malonic acid magnesium salt.^[27] Reduction of the ketone using NaBH₄, followed by saponification yielded the β -monosubstituted β -hydroxy acids **11a-c**.

The cyclization into the β -lactone was not successful using either *p*-toluenesulfonyl chloride/pyridine or DEAD/PPh₃. So we explored other routes in order to achieve the cyclization and the most efficient reaction proved to be the use of 2,2'-dipyridyl disulphide with PPh₃ as a first step and then mercuric triflate at 50 °C as a second step, that produced β -lactones **VM014-16** in 28-66% yields.^[28]

Scheme 4. Synthesis of compounds **VM014-16**. Reagents and conditions: (i) a) CDI, dry THF, r.t., 6 h; b) MgCl₂, CH₃CH₂OCOCH₂COOK, r.t., 18 h; (ii) NaBH₄, EtOH, r.t.; (iii) NaOH, 1,4-dioxane, r.t., 16h; (iv) a) 2,2'-dipyridyl disulphide, PPh₃, dry CH₂Cl₂; b) Hg(CF₃SO₃)₂, dry MeCN, 50 °C, 20 min.

All these 12 new β -lactone derivatives were tested for their antibacterial activities against the three pathogenic

mycobacterial strains using the validated and routine REMA method with the use of resazurin as a marker for bacterial viability to determine their respective MIC₅₀^[12b, 13, 21] (Table 2).

Table 2. Antibacterial activities of Orlistat and its 16 analogs (**VM001-16**) compared to standard antimicrobial agents against three pathogenic mycobacterial strains.^a

Cpds	MIC ₅₀ (µg/mL) ^a				CC ₅₀ (µg/mL) ^b
	<i>M. abscessus</i> CIP 104536 ^T		<i>M. marinum</i> ATCC BAA-535/M	<i>M. tb</i> mc ² 6230	
	Smooth	Rough			
Orlistat	44.4	57.0	6.8	11.0	ND
VM001	>100	>100	4.7	31.8	>125
VM002	>100	>100	>100	>100	>125
VM003	>100	>100	15.6	58.4	113 ±1.5
VM004	>100	>100	18.4	>100	>125
VM005	>100	62.8	>100	>100	>125
VM006	>100	>100	>100	>100	114 ±1.8
VM007	>100	>100	4.4	>100	>125
VM008	>100	>100	5.6	19.7	>125
VM009	>100	>100	6.5	>100	>125
VM010	>100	>100	13.8	33.5	>125
VM011	>100	>100	7.6	>100	>125
VM012	>100	>100	16.4	>100	>125
VM013	>100	>100	13.8	33.6	>125
VM014	>100	>100	9.8	>100	>125
VM015	>100	>100	>100	64.6	>125
VM016	>100	>100	>100	>100	>125
INH	-	-	2.8	0.07	>20 ^c
AMK	2.3	4.3	0.62	0.24	>90 ^c

^a Minimum inhibitory concentration leading to 50% growth inhibition (MIC₅₀) as determined by the resazurin microtiter assay (REMA), are expressed as mean values of two independent assays performed in triplicate (CV% < 5%). The best MIC₅₀ obtained are highlighted in bold. ^b Cytotoxic concentration of **VM** compound leading to 50% macrophage (Raw264.7) cell death (CC₅₀) is the mean for a triplicated dose-response. ^c Data from^[29]. INH: isoniazid; AMK: amikacin.

Concerning *M. abscessus*, one interesting result was displayed by **VM005**, a α-monosubstituted β-lactone bearing a saturated long chain in the α position. This compound was found as active as Orlistat towards the R variant only, but with MIC₅₀ values around 14 times higher than that of amikacin (4.3 µg/mL) used as reference drug. **VM005** being the sole compound with antibacterial activity against *M. abscessus*, these data thus suggest more an isolated activity rather than a class effect.

Most of the β-lactones showed good activity against *M. marinum* (Table 2) with β-lactones **VM001** and **VM007** (*cis*-**VM001**) showing the most potent activity (MIC₅₀ of 4.7 and 4.4 µg/mL, respectively), similar to those of both Orlistat and INH. It should

be noted that the oleyl chain seems to be important in the β-lactone structure. Both α-monosubstituted (**VM004**) and β-monosubstituted (**VM014**) β-lactones bearing this side chain indeed displayed significant antibacterial activity against *M. marinum*, in contrast to other monosubstituted β-lactones **VM005** and **VM015-16**. Conversely, the second substituent was, however, not found to play a significant role in the activity.

Regarding *M. tb* susceptibility (Table 2), **VM008** (*trans*-**VM001**) displayed the most interesting antitubercular activity (MIC₅₀ = 19.7 µg/mL), of the same order of magnitude than that of Orlistat. **VM010** and **VM013** showed only low inhibitory activity against *M. tb* growth with similar MIC₅₀ of 33.5 and 33.6 µg/mL, respectively. The enhanced results of **VM008** and **VM013** (*trans*-**VM009**) on *M. tb* when compared to the *cis/trans* isomeric mixtures **VM001** (MIC₅₀ = 31.8 µg/mL) and **VM009** (MIC₅₀ >100 µg/mL), suggest that the *trans*-β-lactones are more active than the corresponding *cis* isomers. The replacement of the propionyl group at the β-position of **VM001** by the ethyl group (**VM010**) led to similar results. Conversely, the replacement by a methyl group (**VM009**) or by longer chains (**VM011-12**) resulted in inactive compounds. Finally, **VM015**, a β-monosubstituted β-lactone bearing a saturated C15 long chain, exhibited a modest but interesting MIC₅₀ value of 64.6 µg/mL, and could be used as a second lead in another series of β-lactones.

Finally, in view of testing our compounds in future *ex vivo* experiments, their toxicity against murine Raw264.7 macrophages was further investigated using a classical dose-response assay.^[30] The calculated response parameter was CC₅₀,^[12b, 21c, 29, 31] which corresponds to the concentration required for a 50% reduction of cell viability. Except for compounds **VM003** and **VM006**, in all other cases, no cytotoxicity against Raw264.7 macrophages was observed at the highest concentration tested (125 µg/mL); yielding a selectivity index (SI = CC₅₀/MIC₅₀) for **VM008** being in a range from >6.3 and up to >22.3 for *M. tb* and *M. marinum*, respectively (Table 2).

Conclusion

In conclusion, among the 16 β-lactones synthesized, only **VM005** exhibited promising activity against *M. abscessus*; while most of the β-lactones showed interesting activities against *M. marinum*, similar to those of both INH and Orlistat. Regarding *M. tb*, only 6 compounds were found active against this mycobacterium, with β-lactone **VM008** (*trans*-**VM001**) being the most promising one. Despite the fact that at this stage their exact mode of action (i.e., inhibition of serine hydrolases and/or unspecific mechanism of action) as well as their enzymatic targets remain unknown, the leads of these compounds may serve as potential scaffolds for the generation of new anti-mycobacterial agents. Moreover, based on their good antibacterial activities against *M. marinum* together with their low toxicity towards host cells, these compounds may represent potential promising candidates to treat cutaneous infections related to this extracellular bacteria. These various aspects are currently under study in our laboratories and will be reported in due course.

Experimental Section

Chemistry

General: Air- or moisture-sensitive reactions were carried out under an inert gas atmosphere. Thin-layer chromatography (TLC) was performed on Silica Gel 60 F254 aluminum plates. TLC spots were visualized with UV light and/or phosphomolybdic acid in EtOH and/or ninhydrin in EtOH. Chromatographic purification of products was accomplished using Merck Silica Gel 60 (230-400 mesh). Melting points were determined using a Büchi 530 apparatus and were uncorrected. ^1H and ^{13}C spectra were recorded on a Varian Mercury (200 MHz and 50 MHz respectively) in CDCl_3 . Chemical shifts are given in ppm using solvent as an internal standard and coupling constants (J) in Hz. Peak multiplicities are described as follows: s, singlet, d, doublet, t, triplet, q, quartet and m, multiplet. Electron spray ionization (ESI) mass spectra were recorded on a Finnigan, Surveyor MSQ Plus spectrometer. HRMS spectra were recorded on a Bruker Maxis Impact QTOF Spectrometer. Dichloromethane was dried by standard procedures and stored over molecular sieves. Extra dry THF over molecular sieves was purchased from Acros. All the products gave satisfactory elemental analysis. The diastereoisomeric ratio (d.r.) of the α,β -substituted β -hydroxy acids and β -lactones was determined by ^1H NMR spectroscopy. Compounds **5a**^[32], **5b**^[33], **6a**^[32], **6b**^[34], **9a**^[35], **9b**^[36], **9c**^[37], **10b**^[38] and **10c**^[39] were prepared according to the literature or to the methods described below and their spectroscopic data were in accordance with those in the literature.

General method for the synthesis of α,β -substituted β -hydroxy acids (3a-g): To a stirred solution of diisopropylamine (303 mg, 3 mmol) in dry THF (2 mL), under argon at 0 °C, a solution of 1.6M *n*-BuLi in hexane (1.9 mL, 3 mmol) was slowly added *via* syringe and the solution of LDA was stirred at 0 °C for 10 min. The carboxylic acid (**2a-c**) (1 mmol) in dry THF (3 mL) was then added and the solution was stirred at 0 °C for 1 h. Then, the appropriate aldehyde (1.3 mmol) in dry THF (2 mL) was added and the solution was stirred at 0 °C for 1 h and at room temperature overnight. The solvent was removed under reduced pressure. The reaction mixture was acidified with 1N HCl and extracted with Et₂O (3 x 30 mL). The organic layers were combined, washed with brine (30 mL) and dried. The solvent was removed and the product was purified by column chromatography eluting with a gradient of $\text{CHCl}_3/\text{MeOH}$ 97:3 to 95:5 (v/v).

(Z)-2-(1-Hydroxybutyl)octadec-9-enoic acid (3a): Use of butyraldehyde. Mixture of diastereoisomers (d.r. 6:4). Yield 85% (900 mg); Yellowish oil; ^1H NMR (200 MHz, CDCl_3): δ =5.44-5.23 (m, 2H), 3.95-3.80 (m, 0.4H), 3.80-3.61 (m, 0.6H), 2.54-2.36 (m, 1H), 2.14-1.85 (m, 4H), 1.83-1.07 (m, 26H), 1.03-0.77 (m, 6H); ^{13}C NMR (50 MHz, CDCl_3): δ =180.6, 129.9, 129.6, 71.9, 71.8, 51.2, 50.8, 37.3, 35.9, 31.8, 29.7, 29.6, 29.5, 29.3, 29.1, 27.8, 27.3, 27.1, 26.6, 22.6, 22.5, 19.1, 18.8, 18.1, 14.0, 13.9; HRMS m/z [$M+\text{Na}$]⁺ calcd for $\text{C}_{22}\text{H}_{42}\text{O}_3\text{Na}^+$: 377.3026, found: 377.3025; Anal. calcd for $\text{C}_{22}\text{H}_{42}\text{O}_3$: C, 74.52, H 11.94, found: C, 74.31, H 12.02.

2-(1-Hydroxybutyl)octadecanoic acid (3b): Use of butyraldehyde. Mixture of diastereoisomers (d.r. 6:4). Yield 30% (310 mg); White Solid; mp: 25-30 °C; ^1H NMR (200 MHz, CDCl_3): δ =3.94-3.81 (m, 0.4H), 3.81-3.63 (m, 0.6H), 2.54-2.36 (m, 1H), 1.80-1.17 (m, 34H), 1.03-0.80 (m, 6H); ^{13}C NMR (50 MHz, CDCl_3): δ =180.3, 72.0, 71.9, 51.3, 50.9, 37.3, 35.9, 31.9, 29.7, 29.5, 29.3, 27.8, 27.4, 26.6, 22.6, 19.1, 18.8, 14.0, 13.9; HRMS m/z [$M+\text{Na}$]⁺ calcd for $\text{C}_{22}\text{H}_{44}\text{O}_3\text{Na}^+$: 379.3183, found: 379.3181; Anal. calcd for $\text{C}_{22}\text{H}_{44}\text{O}_3$: C, 74.10, H 12.44, found: C, 73.98, H 12.51.

2-(1-Hydroxybutyl)octanoic acid (3c): Use of butyraldehyde. Mixture of diastereoisomers (d.r. 7:3). Yield 33% (200 mg); White solid; mp: 20-25 °C; ^1H NMR (200 MHz, CDCl_3): δ =3.94-3.80 (m, 0.3H), 3.80-3.63 (m, 0.7H), 2.53-2.35 (m, 1H), 1.85-1.15 (m, 14H), 1.03-0.77 (m, 6H); ^{13}C NMR (50 MHz, CDCl_3): δ 180.7, 72.0, 71.8, 51.1, 50.9, 37.4, 36.0, 31.6, 29.4, 29.3, 29.2, 27.7, 27.3, 26.6, 22.5, 19.1, 18.8, 14.0, 13.9; HRMS m/z [$M+\text{Na}$]⁺ calcd for $\text{C}_{12}\text{H}_{24}\text{O}_3\text{Na}^+$: 239.1618, found: 239.1619; Anal. calcd for $\text{C}_{12}\text{H}_{24}\text{O}_3$: C, 66.63, H 11.18, found: C, 66.45, H 11.27.

(Z)-2-(1-hydroxyethyl)octadec-9-enoic acid (3d): Use of acetaldehyde. Mixture of diastereoisomers (d.r. 7:3). Yield 52% (500 mg); Yellowish oil; ^1H NMR (200 MHz, CDCl_3): δ =5.43-5.23 (m, 2H), 4.12-3.99 (m, 0.3H), 3.99-3.85 (m, 0.7H), 2.52-2.25 (m, 1H), 2.11-1.85 (m, 4H), 1.77-1.55 (m, 2H), 1.55-1.03 (m, 23H), 0.87 (t, J = 7 Hz, 3H); ^{13}C NMR (50 MHz, CDCl_3): δ 179.9, 179.6, 129.9, 129.6, 68.5, 68.2, 52.9, 51.9, 34.1, 31.8, 29.7, 29.6, 29.5, 29.3, 29.2, 29.1, 29.0, 27.7, 27.1, 24.8, 22.6, 21.2, 19.8, 14.0; HRMS m/z [$M-\text{H}$]⁻ calcd for $\text{C}_{20}\text{H}_{37}\text{O}_3$: 325.2743, found: 325.2740; Anal. calcd for $\text{C}_{20}\text{H}_{38}\text{O}_3$: C, 73.57, H 11.73, found: C, 73.38; H 11.81.

(Z)-2-(1-hydroxypropyl)octadec-9-enoic acid (3e): Use of propanal. Mixture of diastereoisomers (d.r. 7:3). Yield 88% (900 mg); Yellowish oil; ^1H NMR (200 MHz, CDCl_3): δ =5.41-5.20 (m, 2H), 3.80-3.68 (m, 0.3H), 3.68-3.53 (m, 0.7H), 2.51-2.33 (m, 1H), 2.11-1.85 (m, 4H), 1.77-1.07 (m, 24H), 0.95 (t, J = 7 Hz, 3H), 0.85 (t, J = 7 Hz, 3H); ^{13}C NMR (50 MHz, CDCl_3): δ =179.8, 129.8, 129.5, 73.6, 50.7, 31.8, 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 29.0, 27.8, 27.7, 27.2, 27.1, 26.8, 22.6, 14.0, 10.2, 9.8; HRMS m/z [$M-\text{H}$]⁻ calcd for $\text{C}_{21}\text{H}_{39}\text{O}_3$: 339.2899, found: 339.2894; Anal. calcd for $\text{C}_{21}\text{H}_{40}\text{O}_3$: C, 74.07, H 11.84, found: C, 73.87, H 11.93.

(Z)-2-(1-hydroxyheptyl)octadec-9-enoic acid (3f): Use of heptanal. Mixture of diastereoisomers (d.r. 6:4). Yield 84% (1.1 g); Yellowish oil; ^1H NMR (200 MHz, CDCl_3): δ =5.44-5.23 (m, 2H), 3.92-3.77 (m, 0.4H), 3.77-3.58 (m, 0.6H), 2.54-2.30 (m, 1H), 2.14-1.85 (m, 4H), 1.82-1.09 (m, 32H), 0.95-0.75 (m, 6H); ^{13}C NMR (50 MHz, CDCl_3): δ =180.1, 129.8, 129.5, 72.2, 72.1, 51.2, 50.9, 35.1, 33.9, 31.8, 31.7, 29.7, 29.5, 29.4, 29.2, 29.1, 29.0, 27.7, 27.3, 27.1, 26.7, 25.9, 25.6, 22.6, 22.5, 14.0, 13.9; HRMS m/z [$M-\text{H}$]⁻ calcd for $\text{C}_{25}\text{H}_{47}\text{O}_3$: 395.3525, found: 395.3520; Anal. calcd for $\text{C}_{25}\text{H}_{48}\text{O}_3$: C, 75.70, H 12.20, found: C, 75.55, H 12.29.

(Z)-2-(1-hydroxydecyl)octadec-9-enoic acid (3g): Use of decanal. Mixture of diastereoisomers (d.r. 7:3). Yield 83% (1.10 g); Yellowish oil; ^1H NMR (200 MHz, CDCl_3): δ =5.44-5.23 (m, 2H), 3.90-3.76 (m, 0.3H), 3.76-3.55 (m, 0.7H), 2.54-2.31 (m, 1H), 2.14-1.85 (m, 4H), 1.80-1.03 (m, 38H), 0.95-0.75 (m, 6H); ^{13}C NMR (50 MHz, CDCl_3): δ =180.4, 129.9, 129.6, 72.2, 72.1, 51.2, 50.8, 35.2, 33.8, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 27.8, 27.3, 27.2, 26.5, 26.0, 25.7, 22.6, 14.0; HRMS m/z [$M-\text{H}$]⁻ calcd for $\text{C}_{28}\text{H}_{53}\text{O}_3$: 437.3995, found: 437.3994; Anal. calcd for $\text{C}_{28}\text{H}_{54}\text{O}_3$: C, 76.65, H 12.41, found: C, 76.47, H 12.52.

General method for the cyclization of α,β -substituted β -hydroxy acids to β -lactones (VM001-VM003, VM007-VM013): To a stirred solution of β -hydroxy acid (**3a-g**) (1 mmol) in dry pyridine (2 mL), under argon at 0 °C, *p*-toluenesulfonyl chloride (381 mg, 2 mmol) in dry pyridine (1 mL) was added slowly *via* syringe. The solution was stirred at 0 °C for 1 h and kept at 4 °C for 3 days. Then, Et₂O (30 mL) was added and the organic layer was washed with 10% Na_2CO_3 (2 x 30 mL), 1N HCl (2 x 30 mL) and brine (30 mL). The organic layer was dried and the solvent was removed *in vacuo*. The product was purified by column chromatography eluting with a gradient of petroleum ether (bp 40-60 °C) /AcOEt 97:3 to 95:5 (v/v).

(Z)-3-(Hexadec-7-en-1-yl)-4-propyloxetan-2-one (VM001): Mixture of diastereoisomers (d.r. 7:3). Yield 67% (330 mg); Colorless oil; ^1H NMR (200 MHz, CDCl_3): δ =5.45-5.24 (m, 2H), 4.63-4.48 (m, 0.3H), 4.30-4.15 (m, 0.7H), 3.67-3.52 (m, 0.3H), 3.24-3.09 (m, 0.7H), 2.14-1.90 (m, 4H), 1.90-1.55 (m, 4H), 1.55-1.20 (m, 22H), 0.99 (t, J = 7 Hz, 3H), 0.88 (t, J = 7 Hz, 3H); ^{13}C NMR (50 MHz, CDCl_3): δ =172.3, 171.6, 130.1, 130.0, 129.6, 129.5, 77.9, 75.4, 56.1, 52.6, 36.4, 32.1, 31.9, 29.7, 29.5, 29.4, 29.3, 29.2, 28.9, 27.8, 27.5, 27.2, 27.0, 26.9, 23.9, 22.6, 18.9, 18.4, 14.1, 13.7; HRMS m/z [$M+\text{Na}$]⁺ calcd for $\text{C}_{22}\text{H}_{40}\text{O}_2\text{Na}^+$: 359.2921, found: 359.2927; Anal. calcd for $\text{C}_{22}\text{H}_{40}\text{O}_2$: C, 78.51, H 11.98, found: C, 78.30, H 12.09.

3-Hexadecyl-4-propyloxetan-2-one (VM002): Mixture of diastereoisomers (d.r. 7:3). Yield 42% (100 mg); White solid; mp: 41-44 °C; ^1H NMR (200 MHz, CDCl_3): δ =4.61-4.47 (m, 0.3H), 4.29-4.15 (m, 0.7H), 3.67-3.50 (m, 0.3H), 3.24-3.08 (m, 0.7H), 1.96-1.13 (m, 34H), 0.98

(t, $J = 7$ Hz, 3H), 0.87 (t, $J = 7$ Hz, 3H); ^{13}C NMR (50 MHz, CDCl_3): δ =172.3, 171.6, 77.9, 75.4, 56.1, 52.6, 36.4, 32.1, 31.9, 29.6, 29.5, 29.4, 29.3, 29.2, 27.8, 27.5, 26.9, 23.9, 22.6, 18.9, 18.4, 14.1, 13.7; HRMS m/z [$M+\text{Na}$] $^+$ calcd for $\text{C}_{22}\text{H}_{42}\text{O}_2\text{Na}^+$: 361.3077, found: 361.3079; Anal. calcd for $\text{C}_{22}\text{H}_{42}\text{O}_2$: C, 78.05, H 12.50, found: C, 77.89, H 12.60.

3-Hexyl-4-propyloxetan-2-one (VM003):^[20] Mixture of diastereoisomers (d.r. 7:3). Yield 60% (70 mg); Colorless oil; ^1H NMR (200 MHz, CDCl_3): δ =4.62-4.48 (m, 0.3H), 4.29-4.17 (m, 0.7H), 3.68-3.53 (m, 0.3H), 3.24-3.11 (m, 0.7H), 1.97-1.17 (m, 14H), 0.99 (t, $J = 7$ Hz, 3H), 0.89 (t, $J = 7$ Hz, 3H); ^{13}C NMR (50 MHz, CDCl_3): δ =172.3, 171.6, 77.9, 75.5, 56.1, 52.6, 36.4, 32.2, 31.4, 29.0, 28.9, 27.8, 27.5, 26.9, 23.9, 22.5, 22.4, 18.9, 18.4, 14.0, 13.8, 13.7; HRMS m/z [$M+\text{Na}$] $^+$ calcd for $\text{C}_{12}\text{H}_{22}\text{O}_2\text{Na}^+$: 221.1512, found: 221.1513; Anal. calcd for $\text{C}_{12}\text{H}_{22}\text{O}_2$: C, 72.68, H 11.18, found: C, 72.50, H 11.31.

cis-(Z)-3-(Hexadec-7-en-1-yl)-4-propyloxetan-2-one (VM007): Yield 10% (10 mg); Colorless oil; ^1H NMR (200 MHz, CDCl_3): δ =5.45-5.24 (m, 2H), 4.63-4.48 (m, 1H), 3.67-3.52 (m, 1H), 2.14-1.90 (m, 4H), 1.90-1.42 (m, 6H), 1.55-1.20 (m, 20H), 0.99 (t, $J = 7$ Hz, 3H), 0.88 (t, $J = 7$ Hz, 3H); ^{13}C NMR (50 MHz, CDCl_3): δ =172.3, 130.0, 129.6, 75.4, 52.6, 32.1, 31.9, 29.7, 29.5, 29.4, 29.3, 28.9, 27.5, 27.2, 27.0, 23.9, 22.6, 18.9, 14.1, 13.7; HRMS m/z [$M+\text{Na}$] $^+$ calcd for $\text{C}_{22}\text{H}_{40}\text{O}_2\text{Na}^+$: 359.2921, found: 359.2927; Anal. calcd for $\text{C}_{22}\text{H}_{40}\text{O}_2$: C, 78.51, H 11.98, found: C, 78.32, H 12.08.

trans-(Z)-3-(Hexadec-7-en-1-yl)-4-propyloxetan-2-one (VM008): Yield 37% (50 mg); Colorless oil; ^1H NMR (200 MHz, CDCl_3): δ =5.45-5.24 (m, 2H), 4.23 (dd, $J = 7$ Hz, 6 Hz, 4 Hz, 1H), 3.17 (ddd, $J = 9$ Hz, 7 Hz, 4 Hz, 1H), 2.14-1.90 (m, 4H), 1.90-1.65 (m, 4H), 1.55-1.20 (m, 22H), 0.99 (t, $J = 7$ Hz, 3H), 0.88 (t, $J = 7$ Hz, 3H); ^{13}C NMR (50 MHz, CDCl_3): δ =171.6, 130.1, 129.5, 77.9, 56.1, 36.4, 31.9, 29.7, 29.5, 29.4, 29.3, 29.2, 28.9, 27.8, 27.2, 27.0, 26.9, 22.6, 18.4, 14.1, 13.7; HRMS m/z [$M+\text{Na}$] $^+$ calcd for $\text{C}_{22}\text{H}_{40}\text{O}_2\text{Na}^+$: 359.2921, found: 359.2927; Anal. calcd for $\text{C}_{22}\text{H}_{40}\text{O}_2$: C, 78.51, H 11.98, found: C, 78.35, H 12.11.

(Z)-3-(hexadec-7-en-1-yl)-4-methyloxetan-2-one (VM009): Mixture of diastereoisomers (d.r. 7:3). Yield 36% (110 mg); Colorless oil; ^1H NMR (200 MHz, CDCl_3): δ =5.44-5.23 (m, 2H), 4.81-4.66 (m, 0.3H), 4.46-4.33 (m, 0.7H), 3.67-3.52 (m, 0.3H), 3.22-3.10 (m, 0.7H), 2.11-1.90 (m, 4H), 1.89-1.60 (m, 2H), 1.56 (d, $J = 6$ Hz, 2.1H), 1.46 (d, $J = 6$ Hz, 0.9H), 1.42-1.13 (m, 20H), 0.89 (t, $J = 7$ Hz, 3H); ^{13}C NMR (50 MHz, CDCl_3): δ =172.0, 171.3, 130.1, 130.0, 129.6, 129.5, 74.6, 71.7, 57.6, 52.8, 31.9, 29.7, 29.6, 29.5, 29.3, 29.2, 29.1, 28.9, 27.7, 27.3, 27.2, 27.1, 26.8, 23.9, 22.7, 20.3, 15.6, 14.1; HRMS m/z [$M+\text{Na}$] $^+$ calcd for $\text{C}_{20}\text{H}_{36}\text{O}_2\text{Na}^+$: 331.2607, found: 331.2611; Anal. calcd for $\text{C}_{20}\text{H}_{36}\text{O}_2$: C, 77.87, H 11.76, found: C, 77.62, H 11.85.

(Z)-4-ethyl-3-(hexadec-7-en-1-yl)oxetan-2-one (VM010): Mixture of diastereoisomers (d.r. 7:3). Yield 52% (420 mg); Colorless oil; ^1H NMR (200 MHz, CDCl_3): δ =5.42-5.20 (m, 2H), 4.49-4.35 (m, 0.3H), 4.19-4.06 (m, 0.7H), 3.64-3.47 (m, 0.3H), 3.20-3.05 (m, 0.7H), 2.09-1.89 (m, 4H), 1.89-1.55 (m, 4H), 1.53-1.10 (m, 20H), 1.08-0.92 (m, 3H), 0.85 (t, $J = 7$ Hz, 3H); ^{13}C NMR (50 MHz, CDCl_3): δ =172.1, 171.4, 130.0, 129.9, 129.5, 129.4, 79.0, 76.8, 55.6, 52.4, 31.8, 29.7, 29.5, 29.4, 29.2, 29.1, 28.8, 27.8, 27.5, 27.4, 27.1, 27.0, 26.9, 23.8, 23.4, 22.6, 14.0, 9.8, 9.0; HRMS m/z [$M+\text{Na}$] $^+$ calcd for $\text{C}_{21}\text{H}_{38}\text{O}_2\text{Na}^+$: 345.2764, found: 345.2764; Anal. calcd for $\text{C}_{21}\text{H}_{38}\text{O}_2$: C, 78.20, H 11.88, found: C, 78.01, H 11.98.

(Z)-3-(hexadec-7-en-1-yl)-4-hexyloxetan-2-one (VM011): Mixture of diastereoisomers (d.r. 7:3). Yield 56% (530 mg); Colorless oil; ^1H NMR (200 MHz, CDCl_3): δ =5.43-5.22 (m, 2H), 4.57-4.42 (m, 0.3H), 4.25-4.12 (m, 0.7H), 3.64-3.48 (m, 0.3H), 3.21-3.07 (m, 0.7H), 2.11-1.89 (m, 4H), 1.89-1.58 (m, 4H), 1.58-1.10 (m, 28H), 0.95-0.78 (m, 6H); ^{13}C NMR (50 MHz, CDCl_3): δ =172.1, 171.4, 130.0, 129.9, 129.5, 129.4, 78.0, 75.6, 56.0, 52.5, 34.4, 31.8, 31.5, 30.1, 29.7, 29.5, 29.4, 29.2, 29.1, 28.9, 28.8, 27.8, 27.5, 27.1, 27.0, 26.9, 25.4, 24.9, 23.8, 22.6, 22.4, 14.0, 13.9; HRMS m/z [$M+\text{Na}$] $^+$ calcd for $\text{C}_{25}\text{H}_{46}\text{O}_2\text{Na}^+$: 401.3390, found: 401.3396; Anal. calcd for $\text{C}_{25}\text{H}_{46}\text{O}_2$: C, 79.30, H 12.25, found: C, 79.11, H 12.35.

(Z)-3-(hexadec-7-en-1-yl)-4-nonyloxetan-2-one (VM012): Mixture of diastereoisomers (d.r. 7:3). Yield 63% (600 mg); Colorless oil; ^1H NMR (200 MHz, CDCl_3): δ =5.44-5.23 (m, 2H), 4.59-4.45 (m, 0.3H), 4.26-4.13 (m, 0.7H), 3.65-3.50 (m, 0.3H), 3.22-3.07 (m, 0.7H), 2.14-1.91 (m, 4H), 1.91-1.56 (m, 4H), 1.56-1.10 (m, 34H), 0.89 (t, $J = 7$ Hz, 6H); ^{13}C NMR (50 MHz, CDCl_3): δ =172.2, 171.5, 130.0, 129.9, 129.5, 129.4, 78.0, 75.6, 56.0, 52.5, 34.4, 31.8, 31.7, 30.1, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 28.9, 27.8, 27.5, 27.1, 27.0, 26.9, 25.5, 25.0, 23.9, 22.6, 14.0; HRMS m/z [$M+\text{Na}$] $^+$ calcd for $\text{C}_{28}\text{H}_{52}\text{O}_2\text{Na}^+$: 443.3859, found: 443.3861; Anal. calcd for $\text{C}_{28}\text{H}_{52}\text{O}_2$: C, 79.94, H 12.46, found: C, 79.77, H 12.57.

trans-(Z)-3-(hexadec-7-en-1-yl)-4-nonyloxetan-2-one (VM013): Yield 20% (20 mg); Colorless oil; ^1H NMR (200 MHz, CDCl_3): δ =5.44-5.23 (m, 2H), 4.40 (dq, $J = 6$ Hz, 4 Hz, 1H), 3.16 (ddd, $J = 9$ Hz, 7 Hz, 4 Hz, 1H), 2.11-1.90 (m, 4H), 1.89-1.60 (m, 2H), 1.56 (d, $J = 6$ Hz, 3H), 1.50-1.13 (m, 20H), 0.89 (t, $J = 7$ Hz, 3H); ^{13}C NMR (50 MHz, CDCl_3): δ =171.3, 130.1, 129.6, 74.6, 57.6, 31.9, 29.7, 29.5, 29.3, 29.2, 29.1, 28.9, 27.7, 27.2, 27.1, 26.8, 22.7, 20.3, 14.1; HRMS m/z [$M+\text{Na}$] $^+$ calcd for $\text{C}_{20}\text{H}_{36}\text{O}_2\text{Na}^+$: 331.2607, found: 331.2611; Anal. calcd for $\text{C}_{20}\text{H}_{36}\text{O}_2$: C, 77.87, H 11.76, found: C, 77.65, H 11.87.

Diethyl (Z)-2-butyl-2-(octadec-9-en-1-yl)malonate (5c): A solution of diethyl butyl malonate (583 mg, 2.7 mmol) in dry THF (2.7 mL) was slowly added *via* syringe to a stirred mixture of 60% NaH (140 mg, 3.5 mmol) in dry THF (5.4 mL) and the solution was stirred under argon at room temperature for 10 min. Then, a solution of the oleyl bromide (894 mg, 2.7 mmol) in dry THF (1.6 mL) was slowly added *via* syringe, and the mixture was stirred at room temperature for 10 min and then refluxed overnight. The solvent was removed under reduced pressure, water (80 mL) was added to the reaction mixture which was extracted with Et_2O (3 x 80 mL). The organic layers were combined, washed with brine and dried. The solvent was removed and the product was purified by column chromatography eluting with a gradient of petroleum ether (bp 40-60 $^\circ\text{C}$)/AcOEt 97:3 to 90:10 (v/v). Yield 58% (730 mg); Colorless oil; ^1H NMR (200 MHz, CDCl_3): δ =5.43-5.23 (m, 2H), 4.17 (q, $J = 7$ Hz, 4H), 2.10-1.93 (m, 4H), 1.93-1.76 (m, 4H), 1.45-1.01 (m, 34H), 0.96-0.80 (m, 6H); ^{13}C NMR (50 MHz, CDCl_3): δ =171.6, 129.6, 129.5, 60.5, 57.2, 31.9, 31.7, 31.6, 29.7, 29.6, 29.3, 29.2, 29.1, 29.0, 27.0, 25.9, 23.7, 22.8, 22.5, 13.9, 13.6; MS (ESI) m/z (%): 484.5 [($M+\text{NH}_4^+$), 100]; Anal. calcd for $\text{C}_{29}\text{H}_{54}\text{O}_4$: C, 74.63, H 11.66, found: C, 74.44, H 11.75.

(Z)-2-Butyl-2-(ethoxycarbonyl)icos-11-enoic acid (6c): To a stirred solution of substituted malonate **5c** (700 mg, 1.5 mmol) in EtOH (3 mL), a solution of KOH (2.1 mmol) in EtOH (3 mL) was added and the solution was refluxed for 2 days. The solvent was removed under reduced pressure, water (45 mL) was then added to the reaction mixture which was extracted with CH_2Cl_2 (45 mL). The aqueous layer was acidified with 1N HCl and extracted with CH_2Cl_2 (3 x 45 mL). The organic layers were combined, washed with brine and dried. The solvent was removed and the product was purified by column chromatography eluting with a gradient of $\text{CHCl}_3/\text{MeOH}$ 98:2 to 95:5 (v/v). Yield 40% (260 mg); Yellowish oil; ^1H NMR (200 MHz, CDCl_3): δ =5.44-5.25 (m, 2H), 4.31 (q, $J = 7$ Hz, 2H), 2.12-1.73 (m, 8H), 1.45-1.00 (m, 31H), 0.96-0.81 (m, 6H); ^{13}C NMR (50 MHz, CDCl_3): δ =176.7, 173.6, 129.9, 129.7, 61.7, 57.6, 34.1, 33.9, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 27.0, 26.6, 24.4, 22.8, 22.6, 14.1, 14.0, 13.8; MS (ESI) m/z (%): 437.4 [($M-\text{H}$), 100]; Anal. calcd for $\text{C}_{27}\text{H}_{50}\text{O}_4$: C, 73.92, H 11.49, found: C, 73.78, H 11.61.

Synthesis of α -substituted β -hydroxy acids (7a-c): To a stirred solution of ethoxycarbonyl acids (**6a-c**) (1 mmol) in iPrOH/dry THF 1:2 (2.4 mL), a solution of LiBH_4 2.0 M in THF (2 mL, 4 mmol) was added under argon at 0 $^\circ\text{C}$ and was stirred at 0 $^\circ\text{C}$ for 1 h and at room temperature overnight. The solvents were removed under reduced pressure, water (30 mL) was added to the reaction mixture and was extracted with Et_2O (30 mL). The aqueous layer was acidified with HCl 1N and extracted with CH_2Cl_2 (3 x 30 mL). The organic layers were combined, washed with brine and dried. The solvent was removed and

the product was purified by column chromatography eluting with a gradient of CHCl₃/MeOH 95:5 to 90:10 (v/v).

(Z)-2-(hydroxymethyl)icos-11-enoic acid (7a): Yield 71% (300 mg); Colorless oil; ¹H NMR (200 MHz, CDCl₃): δ=5.44-5.24 (m, 2H), 3.77 (d, *J* = 6 Hz, 2H), 2.68-2.49 (m, 1H), 2.14-1.88 (m, 4H), 1.78-1.13 (m, 26H), 0.88 (t, *J* = 7 Hz, 3H); ¹³C NMR (50 MHz, CDCl₃): δ=180.4, 129.9, 129.8, 62.9, 47.5, 31.9, 29.7, 29.5, 29.4, 29.3, 28.2, 27.2, 22.7, 14.1; HRMS *m/z* [*M*+Na]⁺ calcd for C₂₁H₄₀O₃Na⁺: 363.2870, found: 363.2868; Anal. calcd for C₂₁H₄₀O₃: C, 74.07, H 11.84, found: C, 73.90, H 11.95.

2-(Hydroxymethyl)octadecanoic acid (7b)^[40]: Yield 50% (120 mg); White solid; mp: 78-81 °C; mp of literature: 85.5-87 °C;^[40] ¹H NMR (200 MHz, CDCl₃): δ=3.80 (d, *J* = 6 Hz, 2H), 2.71-2.52 (m, 1H), 1.79-1.47 (m, 2H), 1.47-1.03 (m, 28H), 0.88 (t, *J* = 7 Hz, 3H); ¹³C NMR (50 MHz, CDCl₃): δ=179.4, 62.8, 47.1, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 28.2, 27.2, 22.7, 14.1; HRMS *m/z* [*M*+Na]⁺ calcd for C₁₉H₃₈O₃Na⁺: 337.2713, found: 337.2711.

(Z)-2-Butyl-2-(hydroxymethyl)icos-11-enoic acid (7c): Yield 73% (150 mg); Colorless oil; ¹H NMR (200 MHz, CDCl₃): δ=5.44-5.25 (m, 2H), 3.68 (s, 2H), 2.17-1.84 (m, 4H), 1.77-1.45 (m, 4H), 1.45-1.07 (m, 28H), 0.99-0.78 (m, 6H); ¹³C NMR (50 MHz, CDCl₃): δ=183.1, 129.9, 129.8, 64.9, 50.6, 33.1, 32.8, 31.9, 30.3, 29.8, 29.6, 29.5, 29.3, 27.2, 26.1, 24.0, 23.3, 22.7, 14.1, 14.0; HRMS *m/z* [*M*+Na]⁺ calcd for C₂₅H₄₈O₃Na⁺: 419.3496, found: 419.3493; Anal. calcd for C₂₅H₄₈O₃: C, 75.50, H 12.20, found: C, 75.39, H 12.28.

Cyclization of α-substituted β-hydroxy acids to β-lactones (VM004-VM006): To a stirred solution of PPh₃ (525 mg, 2 mmol) in dry THF (10 mL), DEAD (348 mg, 2 mmol) was slowly added under argon at 0 °C and the solution was stirred at 0 °C for 1 h. Then, solution of β-hydroxy acid **7a-c** (1 mmol) in dry THF (8 mL) was added at 0 °C and the solution was stirred at 0 °C for 1 h and at room temperature overnight. The solvent was removed under reduced pressure and the product was purified by column chromatography eluting with a gradient of petroleum ether (bp 40-60 °C)/AcOEt 95:5 to 90:10 (v/v).

(Z)-3-(Octadec-9-en-1-yl)oxetan-2-one (VM004): Yield 52% (55 mg); Colorless oil; ¹H NMR (200 MHz, CDCl₃): δ=5.44-5.24 (m, 2H), 4.36 (t, *J* = 6 Hz, 1H), 4.01 (t, *J* = 6 Hz, 1H), 3.78-3.61 (m, 1H), 2.13-1.91 (m, 4H), 1.91-1.63 (m, 2H), 1.55-1.16 (m, 24H), 0.88 (t, *J* = 7 Hz, 3H); ¹³C NMR (50 MHz, CDCl₃): δ=171.8, 129.9, 129.7, 65.0, 52.0, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.1, 27.2, 27.1, 26.8, 22.6, 14.1; HRMS *m/z* [*M*+Na]⁺ calcd for C₂₁H₃₈O₂Na⁺: 345.2764, found: 345.2767; Anal. calcd for C₂₁H₃₈O₂: C, 78.20, H 11.88, found: C, 78.02, H 11.99.

3-Hexadecyloxetan-2-one (VM005): Yield 74% (20 mg); White solid; mp: 48-50 °C; ¹H NMR (200 MHz, CDCl₃): δ=4.37 (t, *J* = 6 Hz, 1H), 4.01 (t, *J* = 6 Hz, 1H), 3.79-3.63 (m, 1H), 1.99-1.65 (m, 2H), 1.54-1.15 (m, 28H), 0.89 (t, *J* = 7 Hz, 3H); ¹³C NMR (50 MHz, CDCl₃): δ=171.8, 65.0, 52.1, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.1, 26.8, 22.7, 14.1; HRMS *m/z* [*M*+Na]⁺ calcd for C₁₉H₃₆O₂Na⁺: 319.2608, found: 319.2612; Anal. calcd for C₁₉H₃₆O₂: C, 76.97, H 12.24, found: C, 76.78, H 12.32.

(Z)-3-Butyl-3-(octadec-9-en-1-yl)oxetan-2-one (VM006): Yield 70% (50 mg); Colorless oil; ¹H NMR (200 MHz, CDCl₃): δ=5.45-5.25 (m, 2H), 4.10 (s, 2H), 2.12-1.89 (m, 4H), 1.80-1.61 (m, 4H), 1.54-1.15 (m, 28H), 1.02-0.78 (m, 6H); ¹³C NMR (50 MHz, CDCl₃): δ=174.6, 130.0, 129.8, 69.2, 61.5, 32.5, 32.2, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 27.2, 27.1, 26.5, 24.3, 22.9, 22.7, 14.1, 13.9; HRMS *m/z* [*M*+Na]⁺ calcd for C₂₅H₄₆O₂Na⁺: 401.3390, found: 401.3389; Anal. calcd for C₂₅H₄₆O₂: C, 79.30, H 12.25, found: C, 79.19, H 12.33.

Ethyl (Z)-3-hydroxyicos-11-enoate (10a): NaBH₄ (38 mg, 1 mmol) was added to a solution of β-keto ester **9a** (704 mg, 2 mmol) in EtOH (8 mL) under argon. The mixture was stirred at room temperature until complete

conversion. 1N HCl (10 mL) was then added at 0 °C and the mixture was extracted with CH₂Cl₂ (3 x 30 mL). The organic layer was washed with brine, dried and concentrated under vacuum. The product was purified by column chromatography eluting with a gradient of petroleum ether (bp 40-60 °C)/AcOEt 90:10 to 80:20 (v/v). Yield 78% (276 mg); Colorless oil; ¹H NMR (200 MHz, CDCl₃): δ=5.41-5.27 (m, 2H), 4.17 (q, *J* = 7 Hz, 2H), 4.07-3.92 (m, 1H), 2.82-2.60 (m, 1H), 2.60-2.30 (m, 2H), 2.10-1.90 (m, 4H), 1.55-1.15 (m, 27H), 0.88 (t, *J* = 7 Hz, 3H); ¹³C NMR (50 MHz, CDCl₃): δ=173.1, 129.9, 129.8, 68.0, 60.6, 41.3, 36.5, 31.9, 29.7, 29.5, 29.3, 29.2, 27.2, 25.4, 14.2, 14.1; Anal. calcd for C₂₂H₄₂O₃: C, 74.52, H 11.94, found: C, 74.38, H 12.05.

General method for the synthesis of β-hydroxy acids (11a-c): Aqueous solution of 1N NaOH (2 mL, 2 mmol) was added to a solution of β-hydroxy ester **12a-c** (1 mmol) in 1,4-dioxane (2 mL). The reaction mixture was stirred at room temperature overnight. 1N HCl (3 mL, 3 mmol) and H₂O (15 mL) was then added and the mixture was extracted with Et₂O (3 x 30 mL). The organic layer was washed with brine, dried and concentrated under vacuum.

(Z)-3-Hydroxyicos-11-enoic acid (11a): Yield 84% (411 mg); White solid; mp: 58-60 °C; ¹H NMR (200 MHz, CDCl₃): δ=5.44-5.28 (m, 2H), 4.12-3.96 (m, 1H), 2.65-2.37 (m, 2H), 2.12-1.87 (m, 4H), 1.70-1.00 (m, 24H), 0.88 (t, *J* = 6 Hz, 3H); ¹³C NMR (50 MHz, CDCl₃): δ=178.0, 130.0, 129.8, 68.0, 41.1, 36.4, 31.9, 29.7, 29.5, 29.4, 29.3, 29.2, 27.2, 25.4, 22.6, 14.1; Anal. calcd for C₂₀H₃₈O₃: C, 73.57, H 11.73, found: C, 73.35, H 11.86.

3-Hydroxyoctadecanoic acid (11b)^[41]: Yield 75% (338 mg); White solid; mp: 89-90 °C; mp of literature: 88-90 °C;^[42] ¹H NMR (200 MHz, CDCl₃): δ=4.05-3.87 (m, 1H), 2.60-2.25 (m, 4H), 1.60-1.00 (m, 26H), 0.85 (t, *J* = 7 Hz, 3H); ¹³C NMR (50 MHz, CDCl₃): δ=176.0, 68.3, 41.2, 36.7, 32.1, 29.9, 29.8, 29.7, 29.6, 25.7, 22.9, 14.3.

3-Hydroxyhexanoic acid (11c)^[43]: Yield 81% (160 mg); Colorless oil; ¹H NMR (200 MHz, CDCl₃): δ=7.00 (s, 1H), 4.20-3.90 (m, 1H), 2.65-2.32 (m, 2H), 1.65-1.20 (m, 4H), 0.93 (t, *J* = 7 Hz, 3H); ¹³C NMR (50 MHz, CDCl₃): δ=177.7, 67.8, 41.1, 38.4, 18.6, 13.9.

General method for the synthesis of β-substituted β-lactones (VM014-VM016): In a dry flask 2,2'-dipyridyl disulphide (330 mg, 1.5 mmol) and triphenylphosphine (420 mg, 1.6 mmol) were dissolved in dry CH₂Cl₂ (10 mL) at room temperature under argon. The hydroxy acid **11a-c** (1 mmol) was slowly added. After 30 min, the resulting yellow solution was added dropwise to a vigorously stirred solution of mercury(II) trifluoromethanesulfonate (2 mmol) in dry MeCN (24 mL) at 50 °C under argon. After 20 min at 50 °C, the reaction mixture was filtered and the precipitate was washed several times with CH₂Cl₂. The solvent was removed under reduced pressure and the product was purified by column chromatography eluting with a gradient of petroleum ether (bp 40-60 °C)/AcOEt 85:15 to 80:20 (v/v) to give the β-lactone.

(Z)-4-(Heptadec-8-en-1-yl)oxetan-2-one (VM014): Yield 28% (87 mg); Yellowish oil; ¹H NMR (200 MHz, CDCl₃): δ=5.44-5.26 (m, 2H), 4.60-4.42 (m, 1H), 3.51 (dd, *J* = 16 Hz, 6 Hz, 1H), 3.06 (dd, *J* = 16 Hz, 6 Hz, 1H), 2.10-1.95 (m, 4H), 1.90-1.65 (m, 2H), 1.55-1.15 (m, 22H), 0.88 (t, *J* = 7 Hz, 3H); ¹³C NMR (50 MHz, CDCl₃): δ=168.4, 130.0, 129.7, 71.3, 42.8, 29.7, 29.6, 29.5, 29.3, 29.1, 29.0, 27.2, 24.9, 22.7, 14.1; HRMS *m/z* [*M*+Na]⁺ calcd for C₂₀H₃₆O₂Na⁺: 331.2607, found: 331.2609; Anal. calcd for C₂₀H₃₆O₂: C, 77.87, H 11.76, found: C, 77.68, H 11.89.

4-Pentadecyloxetan-2-one (VM015): Yield 66% (187 mg); White solid; mp: 47.5-49 °C; ¹H NMR (200 MHz, CDCl₃): δ=4.58-4.40 (m, 1H), 3.51 (dd, *J* = 16 Hz, 6 Hz, 1H), 3.06 (dd, *J* = 16 Hz, 6 Hz, 1H), 1.95-1.65 (m, 2H), 1.64-1.10 (m, 26H), 0.88 (t, *J* = 7 Hz, 3H); ¹³C NMR (50 MHz, CDCl₃): δ=168.4, 71.3, 42.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 14.1; HRMS *m/z* [*M*+Na]⁺ calcd for C₁₈H₃₄O₂Na⁺: 305.2451, found: 305.2450; Anal. calcd for C₁₈H₃₄O₂: C, 76.54, H 12.13, found: C, 76.32, H 12.19.

4-Propyloxetan-2-one (VM016)^[44]: Yield 42% (48 mg); Colorless oil; ¹H NMR (200 MHz, CDCl₃): δ=4.60-4.45 (m, 1H), 3.52 (dd, *J* = 16 Hz, 6 Hz, 1H), 3.06 (dd, *J* = 16 Hz, 6 Hz, 1H), 2.00-1.65 (m, 2H), 1.64-1.32 (m, 2H), 0.99 (t, *J* = 7 Hz, 3H); ¹³C NMR (50 MHz, CDCl₃): δ=168.4, 71.1, 42.9, 36.7, 18.3, 13.7; HRMS *m/z* [M+Na]⁺ calcd for C₆H₁₀O₂Na⁺: 137.0573, found: 137.0576; Anal. calcd for C₆H₁₀O₂: C, 63.14, H 8.83, found: C, 62.97, H 8.92.

In vitro anti-mycobacterial activity and cytotoxicity

Biological assay for antibacterial activity: Stock solutions of Orlistat (10 mg/mL) or VM molecules (5 mg/mL), in which the compounds were found to be completely soluble in dimethyl sulfoxide (DMSO), were prepared prior to susceptibility testing.

Bacterial strains and growth condition: *M. marinum* ATCC BAA-535/M, *M. abscessus* CIP104536^T with either a smooth (S) or rough (R) morphotype, and *M. tuberculosis* mc²6230 (H37Rv Δ RD1 Δ panCD)^[22] strains were routinely grown in Middlebrook 7H9 broth (BD Difco) supplemented with 0.2% glycerol, 0.05% Tween 80 (Sigma-Aldrich) and 10% oleic acid, albumin, dextrose, catalase (OADC enrichment; BD Difco) (7H9-S^{OADC}). In the case of *M. tuberculosis* mc²6230, 24 µg/mL D-panthothenate (Sigma-Aldrich) was also added in the 7H9-S^{OADC} medium. All cultures were kept at 37 °C without shaking, except *M. marinum* which was cultured at 32 °C.

Resazurin microtiter assay (REMA) for MICs determination: Susceptibility testing was performed using the Middlebrook 7H9 broth microdilution method. All assays were carried out at least in duplicate. MICs were determined in 96-well flat-bottom Nunclon Delta Surface microplates with lid (Thermo-Fisher Scientific, ref. 167008) using the resazurin microtiter assay (REMA).^[21a] Briefly, log-phase bacteria were diluted to a cell density of 5 × 10⁶ cells/mL in 7H9-S^{OADC} (7H9 broth + 10% OADC + 0.2% glycerol + 0.05% Tween 80, and 24 µg/mL D-panthothenate when needed). Then 100 µL of the above inoculum was added to each well containing 100 µL 7H9-S^{OADC} medium, serial two-fold dilutions of the selected inhibitor or controls to a final volume of 200 µL (final bacterial charge of 2.5 × 10⁶ cells/mL per well). The final volume of DMSO was kept under 3%. Growth controls containing no inhibitor but with 3% DMSO (*i.e.*, bacteria only = B), inhibition controls containing 50 µg/mL kanamycin and sterility controls (*i.e.*, medium only = M) without inoculation were also included. Plates were incubated at 37 °C (32 °C for *M. marinum*) in a humidity chamber^[45] to prevent evaporation for 3-5 days (*M. smegmatis*, *M. abscessus*) or 10-14 days (*M. marinum*, *M. bovis* BCG, *M. tuberculosis* mc²6230). Then, 20 µL of a 0.025% (*w/v*) resazurin solution was added to each well, and the plates were incubated at 37 °C (or 32 °C) for color change from blue to pink or violet and for a reading of fluorescence units (FU). Fluorescence corresponding to the resazurin reduction was quantified using a Tecan Spark 10M multimode microplate reader (Tecan Group Ltd, France) with excitation at 530 nm and emission at 590 nm. For fluorometric MIC determinations, a background subtraction was performed on all wells with a mean of M wells. Relative fluorescence units were defined as: RFU% = (test well FU/mean FU of B wells) × 100. MIC values were determined by fitting the RFU% sigmoidal dose-response curves in Kaleidagraph 4.2 software (Synergy Software) as previously reported.^[12b, 13, 21c] The lowest drug concentrations inhibiting 50% of bacterial growth was defined as the MIC₅₀. Isoniazid (INH) and amikacin (AMK) were used as reference drugs.

Determination of cytotoxicity (resazurin assay): The cytotoxicity of compounds against eukaryotic cells was measured based on the reduction of resazurin^[30] as a value of cellular viability by metabolic activity. Murine (Raw264.7) macrophages (American Type Culture Collection TIB-71) were cultured from a freezer stock in Dulbecco's modified Eagle medium (DMEM; Gibco) supplemented with 10% heat-inactivated fetal calf serum (FBS, Invitrogen). Cells were grown at 37 °C and 5% CO₂ to subconfluent concentrations, then 1 × 10⁵ cells/well were

seeded in 96-well flat-bottom Nunclon Delta Surface microplates with lid (ThermoFisher Scientific, ref. 167008) in a final volume of 200 µL per well and cultured for additional 24 h. The medium was removed by aspiration, and 200 µL of serial two-fold dilution of each compound in DMEM-10% FBS were then added to each well. In each case, the final volume of DMSO was kept strictly under 3%. After 24 h incubation, 20 µL of a 0.025% (*w/v*) resazurin solution was added to each well. Fluorescence was measured following a 4-h incubation at 37 °C and 5% CO₂ in the dark, by excitation at 530 nm and emission at 590 nm as described above, leading to relative metabolic activities. Addition of DMSO was used as 100% viability reference and addition of 0.2% Triton X100 solution served as negative standard (0% viability). The compound concentration leading to 50% macrophage cell death was defined as the CC₅₀.^[12b, 21c, 29, 31] All experiments were performed as two independent triplicates.

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References

- [1] a) S. L. Robinson, J. K. Christenson, L. P. Wackett, *Nat. Prod. Rep.* **2018**; b) T. Böttcher, S. A. Sieber, *Angew. Chem. Int. Ed.* **2008**, *47*, 4600-4603; c) P. C. Nguyen, V. S. Nguyen, B. P. Martin, P. Fourquet, L. Camoin, C. D. Spilling, J.-F. Cavalier, C. Cambillau, S. Canaan, *J. Mol. Biol.* **2018**, <https://doi.org/10.1016/j.jmb.2018.1009.1017>.
- [2] a) R. D. Richardson, G. Ma, Y. Oyola, M. Zancanella, L. M. Knowles, P. Cieplak, D. Romo, J. W. Smith, *J. Med. Chem.* **2008**, *51*, 5285-5296; b) F. Weinandy, K. Lorenz-Baath, V. S. Korotkov, T. Böttcher, S. Sethi, T. Chakraborty, S. A. Sieber, *ChemMedChem* **2014**, *9*, 710-713; c) S. Ponzano, F. Bertozzi, L. Mengatto, M. Dionisi, A. Armirotti, E. Romeo, A. Berteotti, C. Fiorelli, G. Tarozzo, A. Reggiani, A. Duranti, G. Tarzia, M. Mor, A. Cavalli, D. Piomelli, T. Bandiera, *J. Med. Chem.* **2013**, *56*, 6917-6934; d) V. C. Purohit, R. D. Richardson, J. W. Smith, D. Romo, *J. Org. Chem.* **2006**, *71*, 4549-4558.
- [3] a) T. Böttcher, S. A. Sieber, *MedChemComm* **2012**, *3*, 408-417; b) K. Camara, S. S. Kamat, C. C. Lasota, B. F. Cravatt, A. R. Howell, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 317-321.
- [4] a) E. K. Weibel, P. Hadvary, E. Hochuli, E. Kupfer, H. Lengsfeld, *J. Antibiotics* **1987**, *40*, 1081-1085; b) B. Borgstrom, *Biochim. Biophys. Acta* **1988**, *962*, 308-316; c) A. Bénarouche, V. Point, F. Carrière, J.-F. Cavalier, *Biochimie* **2014**, *101*, 221-231.
- [5] J. Lehmann, T.-Y. Cheng, A. Aggarwal, A. S. Park, E. Zeiler, R. M. Raju, T. Akopian, O. Kandror, J. C. Sacchetti, D. B. Moody, E. J. Rubin, S. A. Sieber, *Angew. Chem. Int. Ed.* **2018**, *57*, 348-353.
- [6] a) P. K. Crellin, J. P. Vivian, J. Scoble, F. M. Chow, N. P. West, R. Brammananth, N. I. Proellocks, A. Shahine, J. Le Nours, M. C. J. Wilce, W. J. Britton, R. L. Coppel, J. Rossjohn, T. Beddoe, *J. Biol. Chem.* **2010**, *285*, 30050-30060; b) V. Delorme, S. V. Diomandé, L. Dedieu, J.-F. Cavalier, F. Carrière, L. Kremer, J. Leclaire, F. Fotiadu, S. Canaan, *PLoS ONE* **2012**, *7*, e46493; c) M. S. Ravindran, S. P. Rao, X. Cheng, A. Shukla, A. Cazenave-Gassiot, S. Q. Yao, M. R. Wenk, *Mol. Cell. Proteomics* **2014**, *13*, 435-448; d) C. Rens, F. Laval, M. Daffe, O. Denis, R. Frita, A. Baulard, R. Wattiez, P. Lefevre, V. Fontaine, *Antimicrob.*

- Agents Chemother.* **2016**, *60*, 6193-6199; e) C. M. Goins, T. D. Sudasinghe, X. Liu, Y. Wang, G. A. O'Doherty, D. R. Ronning, *Biochemistry* **2018**, *57*, 2383-2393; f) C. M. Goins, S. Dajnowicz, M. D. Smith, J. M. Parks, D. R. Ronning, *J. Biol. Chem.* **2018**, *293*, 3651-3662.
- [7] WHO, **2018**, http://www.who.int/tb/publications/global_report/en/.
- [8] M. Pai, M. A. Behr, D. Dowdy, K. Dheda, M. Divangahi, C. C. Boehme, A. Ginsberg, S. Swaminathan, M. Spigelman, H. Getahun, D. Menzies, M. Raviglione, *Nat. Rev. Dis. Primers* **2016**, *2*, 16076.
- [9] L. Dedieu, C. Serveau-Avesque, L. Kremer, S. Canaan, *Biochimie* **2013**, *95*, 66-73.
- [10] a) H. Bloch, W. Segal, *J. Bacteriol.* **1956**, *72*, 132-141; b) J. D. McKinney, K. Honer zu Bentrup, E. J. Munoz-Elias, A. Miczak, B. Chen, W. T. Chan, D. Swenson, J. C. Sacchettini, W. R. Jacobs, Jr., D. G. Russell, *Nature* **2000**, *406*, 735-738; c) A. K. Pandey, C. M. Sassetti, *Proc. Natl. Acad. Sci. U S A* **2008**, *105*, 4376-4380.
- [11] a) J. T. Belisle, V. D. Vissa, T. Sievert, K. Takayama, P. J. Brennan, G. S. Besra, *Science* **1997**, *276*, 1420-1422; b) G. Johnson, *Curr. Protein Pept. Sci.* **2017**, *18*, 190-210; c) P. Santucci, S. Diomande, I. Poncin, L. Alibaud, A. Viljoen, L. Kremer, C. de Chastellier, S. Canaan, *Infect. Immun.* **2018**, *86*, e00394-00318.
- [12] a) K. R. Tallman, S. R. Levine, K. E. Beatty, *ACS Infect. Dis.* **2016**, *2*, 936-944; b) P. C. Nguyen, V. Delorme, A. Bénarouche, B. P. Martin, R. Paudel, G. R. Gnawali, A. Madani, R. Puppo, V. Landry, L. Kremer, P. Brodin, C. D. Spilling, J.-F. Cavalier, S. Canaan, *Sci. Rep.* **2017**, *7*, 11751.
- [13] P. C. Nguyen, A. Madani, P. Santucci, B. P. Martin, R. R. Paudel, S. Delattre, J.-L. Herrmann, C. D. Spilling, L. Kremer, S. Canaan, J.-F. Cavalier, *Int. J. Antimicrob. Agents* **2018**, *51*, 651-654.
- [14] a) L. Ramakrishnan, *Curr. Sci.* **2004**, *86*, 82-92; b) T. P. Stinear, T. Seemann, P. F. Harrison, G. A. Jenkin, J. K. Davies, P. D. Johnson, Z. Abdellah, C. Arrowsmith, T. Chillingworth, C. Churcher, K. Clarke, A. Cronin, P. Davis, I. Goodhead, N. Holroyd, K. Jagels, A. Lord, S. Moule, K. Mungall, H. Norbertczak, M. A. Quail, E. Rabinowitsch, D. Walker, B. White, S. Whitehead, P. L. Small, R. Brosch, L. Ramakrishnan, M. A. Fischbach, J. Parkhill, S. T. Cole, *Genome Res.* **2008**, *18*, 729-741.
- [15] A. L. Roux, E. Catherinot, F. Ripoll, N. Soismier, E. Macheras, S. Ravilly, G. Bellis, M. A. Vibet, E. Le Roux, L. Lemonnier, C. Gutierrez, V. Vincent, B. Fauroux, M. Rottman, D. Guillemot, J. L. Gaillard, *J. Clin. Microbiol.* **2009**, *47*, 4124-4128.
- [16] a) S. T. Howard, E. Rhoades, J. Recht, X. Pang, A. Alsup, R. Kolter, C. R. Lyons, T. F. Byrd, *Microbiology* **2006**, *152*, 1581-1590; b) B. E. Jönsson, M. Gilljam, A. Lindblad, M. Ridell, A. E. Wold, C. Welinder-Olsson, *J. Clin. Microbiol.* **2007**, *45*, 1497-1504.
- [17] A. L. Roux, A. Viljoen, A. Bah, R. Simeone, A. Bernut, L. Laencina, T. Deramautd, M. Rottman, J. L. Gaillard, L. Majlessi, R. Brosch, F. Girard-Misguich, I. Vergne, C. de Chastellier, L. Kremer, J. L. Herrmann, *Open biology* **2016**, *6*, 160185.
- [18] a) A. Bernut, J. L. Herrmann, K. Kissa, J. F. Dubremetz, J. L. Gaillard, G. Lutfalla, L. Kremer, *Proc. Natl. Acad. Sci. U S A* **2014**, *111*, E943-952; b) E. Catherinot, J. Clarissou, E. Etienne, F. Ripoll, J. F. Emile, M. Daffe, C. Perronne, C. Soudais, J. L. Gaillard, M. Rottman, *Infect. Immun.* **2007**, *75*, 1055-1058.
- [19] P. H. Candido, S. Nunes Lde, E. A. Marques, T. W. Folescu, F. S. Coelho, V. C. de Moura, M. G. da Silva, K. M. Gomes, M. C. Lourenco, F. S. Aguiar, F. Chitolina, D. T. Armstrong, S. C. Leao, F. P. Neves, F. C. Mello, R. S. Duarte, *J. Clin. Microbiol.* **2014**, *52*, 2990-2997.
- [20] C. Wedler, K. Kleiner, A. Kunath, H. Schick, *Liebig's Annalen* **1996**, 881-885.
- [21] a) J. C. Palomino, A. Martin, M. Camacho, H. Guerra, J. Swings, F. Portaels, *Antimicrob. Agents Chemother.* **2002**, *46*, 2720-2722; b) J. Rybníček, A. Vocat, C. Sala, P. Busso, F. Pojer, A. Benjak, S. T. Cole, *Nat. Commun.* **2015**, *6*, 7659; c) P. C. Nguyen, V. Delorme, A. Bénarouche, A. Guy, V. Landry, S. Audebert, M. Pophillat, L. Camoin, C. Crauste, J.-M. Galano, T. Durand, P. Brodin, S. Canaan, J.-F. Cavalier, *Bioorg. Chem.* **2018**, *81*, 414-424.
- [22] V. K. Sambandamurthy, S. C. Derrick, T. Hsu, B. Chen, M. H. Larsen, K. V. Jalapathy, M. Chen, J. Kim, S. A. Porcelli, J. Chan, S. L. Morris, W. R. Jacobs, Jr., *Vaccine* **2006**, *24*, 6309-6320.
- [23] R. Nessar, E. Cambau, J. M. Reytrat, A. Murray, B. Gicquel, *J. Antimicrob. Chemother.* **2012**, *67*, 810-818.
- [24] P. Meier, F. Broghammer, K. Latendorf, G. Rauhut, R. Peters, *Molecules* **2012**, *17*, 7121.
- [25] B. Hu, M. Prashad, D. Har, K. Prasad, O. Repič, T. J. Blacklock, *Org. Process Res. Dev.* **2007**, *11*, 90-93.
- [26] D. H. Kim, J.-i. Park, S. J. Chung, J. D. Park, N.-K. Park, J. H. Han, *Bioorg. Med. Chem.* **2002**, *10*, 2553-2560.
- [27] D. W. Brooks, L. D.-L. Lu, S. Masamune, *Angew. Chem. Int. Ed.* **1979**, *18*, 72-74.
- [28] M. C. Pirrung, F. Zhang, S. Ambadi, Y. Gangadhara Rao, *Org. Biomol. Chem.* **2016**, *14*, 8367-8375.
- [29] T. Christophe, M. Jackson, H. K. Jeon, D. Fenistein, M. Contreras-Dominguez, J. Kim, A. Genovesio, J. P. Carralot, F. Ewann, E. H. Kim, S. Y. Lee, S. Kang, M. J. Seo, E. J. Park, H. Skovierova, H. Pham, G. Riccardi, J. Y. Nam, L. Marsollier, M. Kempf, M. L. Joly-Guillou, T. Oh, W. K. Shin, Z. No, U. Nehrass, R. Brosch, S. T. Cole, P. Brodin, *PLoS Pathog.* **2009**, *5*, e1000645.
- [30] a) J. O'Brien, I. Wilson, T. Orton, F. Pognan, *Eur. J. Biochem.* **2000**, *267*, 5421-5426; b) C. Rodrigues Felix, R. Gupta, S. Geden, J. Roberts, P. Winder, S. A. Pomponi, M. C. Diaz, J. K. Reed, A. E. Wright, K. H. Rohde, *Antimicrob. Agents Chemother.* **2017**, *61*, e00743-00717; c) C. S. Foo, A. Lupien, M. Kienle, A. Vocat, A. Benjak, R. Sommer, D. A. Lamprecht, A. J. C. Steyn, K. Pethe, J. Piton, K.-H. Altmann, S. T. Cole, *mBio* **2018**, *9*, e01276-01218.
- [31] a) M. Pitucha, M. Woś, M. Miazga-Karska, K. Klimek, B. Miroslaw, A. Pachuta-Stec, A. Gladysz, G. Ginalska, *Med. Chem. Res.* **2016**, *25*, 1666-1677; b) D. Inoyama, S. D. Paget, R. Russo, S. Kandasamy, P. Kumar, E. Singleton, J. Occi, M. Tuckman, M. D. Zimmerman, H. P. Ho, A. L. Perryman, V. Dartois, N. Connell, J. S. Freundlich, *Antimicrob. Agents Chemother.* **2018**, *62*, 02063-02017.
- [32] C. Woelk, M. Heinze, P. Kreideweiss, M. Dittrich, G. Brezesinski, A. Langner, B. Dobner, *Int. J. Pharm.* **2011**, *409*, 46-56.
- [33] Brigl, *Hoppe Seyler's Z. Physiol. Chem.* **1915**, *95*, 161-194.
- [34] M. Heinze, G. Brezesinski, B. Dobner, A. Langner, *Bioconjug. Chem.* **2010**, *21*, 696-708.
- [35] H. A. Abd El Salam, N. O. Shaker, E. M. El-Telbani, G. A. M. Nawwar, *J. Chem. Res.* **2009**, *2009*, 400-404.
- [36] G. Sbardella, S. Castellano, C. Viciodini, D. Rotili, A. Nebbioso, M. Miceli, L. Altucci, A. Mai, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2788-2792.
- [37] R. A. Maurya, K.-I. Min, D.-P. Kim, *Green Chem.* **2014**, *16*, 116-120.
- [38] E. Santaniello, A. Manzocchi, *Synthesis* **1977**, *1977*, 698-699.
- [39] P. H. Lee, K. Bang, K. Lee, S.-y. Sung, S. Chang, *Synth. Commun.* **2001**, *31*, 3781-3789.
- [40] P. E. Pfeffer, E. Kinsel, L. S. Silbert, *J. Org. Chem.* **1972**, *37*, 1256-1258.
- [41] S.-H. Chen, X. Sun, R. Boyer, J. Paschal, D. Zeckner, W. Current, M. Miceli, M. Rodriguez, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2107-2110.
- [42] L. Negelmann, S. Pisch, U. Bornscheuer, R. D. Schmid, *Chem. Phys. Lipids* **1997**, *90*, 117-134.
- [43] M. Sailer, K. I. Dubicki, J. L. Sorensen, *Synthesis* **2015**, *47*, 79-82.
- [44] J. W. Amoroso, L. S. Borketey, G. Prasad, N. A. Schnarr, *Org. Lett.* **2010**, *12*, 2330-2333.
- [45] a) V. Makarov, B. Lechartier, M. Zhang, J. Neres, A. M. van der Sar, S. A. Raadsen, R. C. Hartkoorn, O. B. Ryabova, A. Vocat, L. A. Decosterd, N. Widmer, T. Buclin, W. Bitter, K. Andries, F. Pojer, P. J. Dyson, S. T. Cole, *EMBO Mol. Med.* **2014**, *6*, 372-383; b) A. Walzl, N. Kramer, M. R. Mazza, D. Falkenhagen, M. Hengstschläger, D. Schwanzer-Pfeiffer, H. Dolznig, *Int. J. Appl. Sci. Technol.* **2012**, *2*, 17-21.

Entry for the Table of Contents

R¹, R² : H, alkyl, alkenyl

A series of long and medium chain mono- and disubstituted β -lactones was designed, synthesized and tested for their anti-mycobacterial activities against three pathogenic mycobacteria: *M. abscessus*, *M. marinum* and *M. tuberculosis*. Several β -lactones bearing an unsaturated oleyl chain exhibited promising anti-mycobacterial activities against *M. tuberculosis* and *M. marinum*, while only one α -monosubstituted β -lactone bearing a long saturated chain exhibited promising activity against *M. abscessus*.