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Synthesis of the fungal macrolide berkeleylactone A and its inhibition of microbial biofilm formation†

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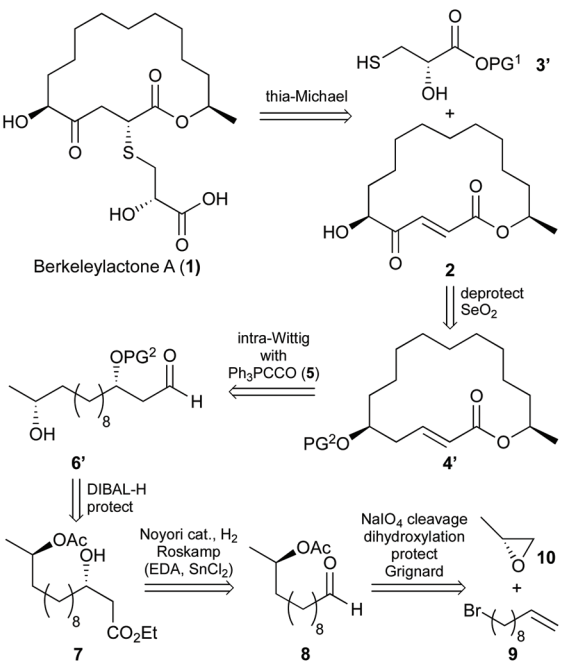
The fungal macrolide berkeleylactone A was synthesised in 13 steps and 24% yield using (*R*)-propylene oxide and an asymmetric Noyori hydrogenation of a β-ketoester to install the stereogenic centres. A domino addition–Wittig olefination of a 13-hydroxytetradecanal intermediate with the cumulated ylide Ph_3PCCO closed the macrocyle by establishing the α,β-unsaturated ester group, necessary for the attachment of the sidechain thiol *via* a thia-Michael reaction. The synthetic berkeleylactone A inhibited the formation of *Staphylococcus aureus* biofilms and showed significant dispersive effects on preformed biofilms of *Candida albicans* by at least 45% relative to untreated controls at concentrations as low as 1.3 μg mL^{-1}.

Introduction

The 16-membered macrolide berkeleylactone A (**1**, Scheme 1) was isolated in 2017 by Stierle *et al.* from a coculture fermentation of *Penicillium fuscum* and *P. camembertii*/*clavigerum*, aside of seven closely related berkeleylactones B–H sharing the same γ-oxopentadecanolide scaffold.[1] They also assigned the structure of macrolide **1**, including its absolute configuration, by ^{1}H NMR, ^{13}C NMR and HMBC spectra, as well as by a single-crystal X-ray diffraction analysis. Berkeleylactone A (**1**) was shown by this group to be the most and highly active congener of this series when tested in a broad screen against bacteria including various MRSA strains, and to operate by a novel mechanism of action not involving the bacterial ribosome. In 2019, Dixon, Caletková *et al.*[2] reported the first synthesis of berkeleylactone A, based upon a convergent approach to macrolide intermediate **2**, which had been employed previously by Chang *et al.*[3] for their formal synthesis of the related macrolide A26771B *via* a ring-closing metathesis (RCM). The product synthesised by Dixon, Caletková *et al.* matched the NMR and even X-ray diffraction analytical data published by Stierle *et al.*, yet differed conspicuously in the specific optical rotations, being $[\alpha]_D^{25}$ +0.5° (*c* 0.170, $CHCl_3$)[1] and $[\alpha]_D^{25}$ +101.0° (*c* 0.105, $CHCl_3$).[2] As a strategic alternative, we now developed a higher-yielding linear route to the target compound **1**. Its key step was a Wittig macrocyclisation of a 13-hydroxtetradecanal intermediate with the cumulated ylide Ph_3PCCO which proceeded in >60% yield and afforded, after allylic oxidation, the Michael system necessary for the attachment of the sidechain thiol. A similar approach had been previously applied by us for the syntheses of the macrolides



Scheme 1 Retrosynthesis of berkeleylactone A (**1**).

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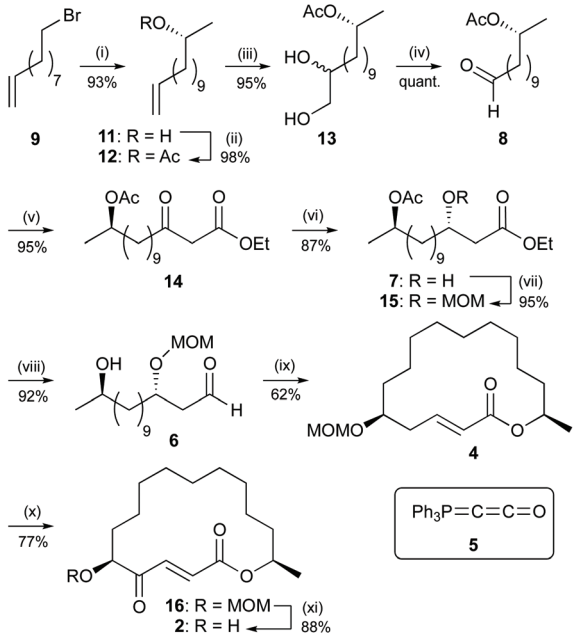
† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra of **1–4**, **6–9**, **11–16**, *rac*-**18**, (*R*)-**18**, **19–22**; HPLC chromatogrammes of **7**/*epi*-**7** and **1**; antimicrobial, antibiofilm, and cytotoxicity assays. Cell culture conditions and MTT-assay ^{1}H and ^{13}C NMR spectra of new compounds. See DOI: 10.1039/d1ob00717c

chloriolide (12-membered ring) and aspicillin (18-membered ring).[4]

Results and discussion

Our retrosynthetic approach is outlined in Scheme 1. The final steps of the synthesis of a suitably protected thiol sidechain **3′** and its attachment to the β-keto-2-enolide **2** by a thia-Michael addition were to follow Dixon's route with slight variations concerning the protecting group strategy. The enolide **2**, however, should be built up quite differently from the Chang/Dixon route. From our synthesis of the related macrolide A26771B,[5] we knew that 2-enoates can be easily oxidised with SeO_2 to the corresponding γ-keto-2-enoates, such as compound **4′** which should have its δ-hydroxy group protected to be on the safe side. Macrolide **4′** was to be obtained by a ring-closing domino addition–Wittig olefination of the secondary hydroxyaldehyde **6′**, carrying both stereogenic centres, with the cumulated phosphorus ylide Ph_3PCCO (**5**),[6] a reaction we had previously utilised to prepare macrolides of ring sizes ranging from 12 to 18.[7] Hydroxyaldehyde **6′** should be prepared by DIBAL-H reduction of a derivative of ester **7** having its second alcohol protected in a way to withstand these conditions. β-Hydroxyester **7** could be obtained by a Roskamp reaction[8] of aldehyde **8** with ethyl diazoacetate (EDA) and a stereoselective Noyori hydrogenation[9] of the resulting β-ketoester (not shown). Aldehyde **8** in turn should be accessible by copper(I) cyanide-catalysed ring opening[10] of epoxide **10** with the Grignard reagent prepared from commercial bromide **9**, followed by protection of the hydroxy group, dihydroxylation of the double bond, and periodate cleavage of the resulting diol.

Most of the planned reactions proceeded with good to excellent yields. 10-Bromodec-1-ene (**9**), which is commercially available or readily accessible by nearly quantitative bromination of the respective alcohol with Ph_3P/Br_2/imidazole,[11] was converted to the Grignard reagent and then treated with (*R*)-propylene oxide ((*R*)-PPO, **10**) and catalytic copper(I) cyanide to afford the secondary alcohol **11** in 93% yield (Scheme 2). Its acetate **12** was dihydroxylated with $NMO/K_2OsO_4{\cdot}2H_2O$ (cat.) leaving diol **13** in 95% yield. It was cleaved with $NaIO_4/SiO_2$ [12] to give (*R*)-11-acetoxydodecanal (**8**) in quantitative yield. For the introduction of the second stereogenic centre, aldehyde **8** was converted to β-ketoester **14** *via* a high-yielding Roskamp extension reaction with EDA/tin(II) chloride. An enantioselective hydrogenation of the keto group of **14** with 1st generation Noyori catalyst and 40 bar H_2 afforded β-hydroxyester **7** in 87% yield and with >99% de. MOM-protection with P_2O_5/dimethoxymethane of the β-hydroxy group gave ester **15** (95%), which was reduced by DIBAL-H with concomitant acetyl deprotection of the 13-hydroxy group to afford hydroxyaldehyde **6** (92%), the immediate precursor for the macrocyclisation reaction with cumulated ylide Ph_3PCCO (**5**). Slow addition of **6** to a diluted solution of ylide **5** in toluene at 55 °C furnished macrolide **4** in 62% yield. Its oxidation with selenium dioxide in dry 1,4-dioxane at 155 °C left γ-keto-enoate **16** in 77% yield.

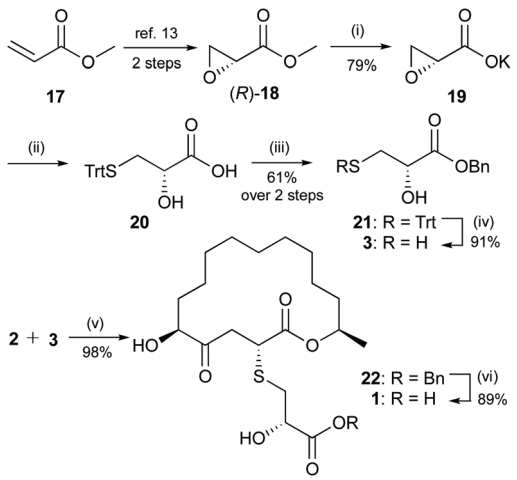


Scheme 2 Synthesis of δ-hydroxy-γ-keto-enolide **2**. Reagents and conditions: (i) Mg^0, THF, reflux, 3.5 h, then cat. CuCN, (*R*)-PPO (**10**), −40 °C (2 h) to −35 °C (18 h) to 0 °C; (ii) Ac_2O, pyridine, DMAP, CH_2Cl_2, rt, 19 h; (iii) $K_2OsO_4{\cdot}2H_2O$ (3 mol%), NMO, acetone/H_2O, 0 °C to rt, 28.5 h; (iv) $NaIO_4/SiO_2$, CH_2Cl_2, rt, 1.75 h; (v) EDA, $SnCl_2$ (12 mol%), CH_2Cl_2, 0 °C, rt, 3 h; (vi) 1st gen. Noyori catalyst (0.29 mol%) prepared from $[RuCl_2(benzene)]_2$ and (*S*)-BINAP, H_2 (40 bar), MeOH, 60 °C, 65 h; (vii) dimethoxymethane, P_2O_5, rt, 2 h; (viii) DIBAL-H, toluene, −78 °C, 90 min; (ix) Ph_3PCCO (**5**, 5 mM in toluene), 55 °C, 20 h; (x) SeO_2, 1,4-dioxane, 155 °C, 55 min; (xi) TFA, CH_2Cl_2, −10 °C, 9.5 h; MOM = methoxymethyl.

Deprotection with TFA gave δ-hydroxy-γ-keto-enolide **2** in 88% yield.

The sidechain was introduced as the (*S*)-benzyl 2-hydroxy-3-mercaptopropanoate (**3**, Scheme 3). It was synthesised from (*R*)-methyl glycidate *R*-(**18**) which is readily accessible in two steps from methyl acrylate (**17**) according to a protocol by Jacobsen.[13] Its saponification left the potassium salt **19**, whose ring was, as in the synthesis of berkeleylactone A by Dixon *et al.*, opened with sodium tritylthiolate to afford hydroxyacid **20**. Esterification with benzyl bromide/cesium carbonate and trityl deprotection gave thiol **3** which was reacted with δ-hydroxy-γ-keto-enolide **2** in the presence of triethylamine (cat.) to furnish **22**, the product of a thia-Michael addition, in 98% yield. The benzyl protecting group was removed by hydrogenolysis with Pd/C (cat.) which afforded berkeleylactone A (**1**) with a dr of 49 : 1 in 23.6% overall yield (longest linear sequence from bromide **9**). The specific optical rotation of our synthetic product was in agreement with that of Dixon's product, yet deviated considerably from that reported for the natural isolate.

The synthetic berkeleylactone A (**1**) was tested against selected bacteria, fungi, and cell lines for antimicrobial (including anti-biofilm) and cytotoxic effects. The minimum inhibitory concentrations (MIC) were assessed as described in



Scheme 3 Synthesis and attachment of sidechain **3**. Reagents and conditions: (i) KOH, MeOH, 0 °C to rt, 16 h; (ii) TrtSH, NaH, THF, then **19**, 0 °C to rt, 19 h; (iii) Cs_2CO_3, BnBr, DMF, rt, 16 h; (iv) TFA, iPr_3SiH, CH_2Cl_2, 0 °C (3.5 h) to rt (45 min); (v) NEt_3 (20 mol%), CH_2Cl_2, rt, 3 h; (vi) Pd/C (10 mol%), H_2, MeOH, rt, 1.75 h; Trt = Ph_3C (trityl).

the ESI† and are listed in Table 1. Compound **1** exhibited weak activities with MICs between 66.6 and 16.6 μg mL^{-1} against several filamentous fungi and yeasts. For the pathogenic yeast *Candida albicans*, weak effects with a MIC of 66.6 μg mL^{-1} were observed. In line with the results of the comprehensive biological studies by Stierle *et al.* and Dixon, Caletková *et al.*, compound **1** exhibited strong antibacterial effects against *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), and *Bacillus subtilis* (4.2 μg mL^{-1}). In contrast, no growth inhibition was observed of the Gram-negative bacteria *Acinetobacter baumannii*, *Chromobacterium violaceum*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Table 1 Antimicrobial activity of synthetic berkeleylactone A (**1**)

		MIC [μg mL^{-1}]	
Tested organisms	Strain no.	**1**	Reference
Bacteria			
Bacillus subtilis	DSM 10	4.2	16.6[a]
Staphylococcus aureus	DSM 346	4.2	0.2[a]
MRSA	DSM 11822	4.2	2.1[b]
Mycobacterium smegmatis	ATCC 700084	66.6	1.7[c]
Acinetobacter baumannii	DSM 30008	—	0.5[d]
Chromobacterium violaceum	DSM 30191	—	0.4[a]
Escherichia coli	DSM 1116	—	3.3[a]
Pseudomonas aeruginosa	PA14	—	0.8[e]
Fungi			
Mucor hiemalis	DSM 2656	16.6	4.2[f]
Pichia anomala	DSM 6766	—	4.2[f]
Rhodoturula glutinis	DSM 10134	33.3	2.1[f]
Candida albicans	DSM 1665	66.6	8.3[f]
Schizosaccharomyces pombe	DSM 70572	66.6	4.2[f]

References: [a] oxytetracycline, [b] vancomycin, [c] kanamycin, [d] ciprobay, [e] gentamicin, [f] nystatin.

Table 2 Inhibition of biofilm formation of *S. aureus* and dispersion of preformed biofilms of *S. aureus* and *C. albicans* by berkeleylactone A (**1**) at various concentrations

		Biofilm inhibition [% ± SD]	Biofilm dispersion [% ± SD]
Tested organisms	Strain no.	**1**	
Staphylococcus aureus	DSM 1104	53 ± 10 (2 μg mL^{-1})[a]	55 ± 8 (250 μg mL^{-1})[b]
		20 ± 8 (0.3 μg mL^{-1})[a]	29 ± 7 (125 μg mL^{-1})[b]
Candida albicans	DSM 11225	—	79 ± 1 (31.3 μg mL^{-1})[c]
			45 ± 6 (1.3 μg mL^{-1})[c]
			17 ± 8 (0.17 μg mL^{-1})[c]

References [%]: [a] microporenic acid A (MAA): 83 (250 μg mL^{-1}), 77 (7.8 μg mL^{-1}), 40 (3.9 μg mL^{-1}); [b] MAA: 68 (250 μg mL^{-1}), 50 (62.5 μg mL^{-1}), 58 (31.3 μg mL^{-1}); [c] MAA: 33 (250 μg mL^{-1}); SD: standard deviation; – not tested.

Table 3 Cytotoxic activities of berkeleylactone A (**1**)

		IC_{50} [μM]	
Cell lines	Strain no.	**1**	Reference
L929	ACC 2	11.1	0.0006[a]
KB3.1	ACC 158	17.1	0.00006[a]

Reference: [a] epothilone B.

After establishing the MICs for *S. aureus*, *C. albicans* und *P. aeruginosa*, the effects of subtoxic concentrations of berkeleylactone A (**1**) on biofilms of these organisms were evaluated. More precisely, its inhibitory effects on the formation of biofilms of *S. aureus* and *P. aeruginosa*, and its dispersive effects on preformed biofilms of *S. aureus* and *C. albicans* were established (Table 2; *cf.* ESI† for details). Lactone **1** inhibited the formation of *S. aureus* biofilms by *ca.* 53% relative to untreated controls when applied at a concentration of 2 μg mL^{-1}, and by *ca.* 20% at a concentration of 0.3 μg mL^{-1}. No inhibitory effects were observed against *P. aeruginosa*. Moreover, lactone **1** exhibited significant dipersive effects on preformed biofilms of *C. albicans*, leading to a reduction of *ca.* 17% when applied at a concentration of 0.17 μg mL^{-1}, and of *ca.* 45% at a concentration of 1.3 μg mL^{-1}. Its dispersive effects on preformed biofilms of *S. aureus* were less pronounced. A reduction of the biofilm of *ca.* 55% required a dose of 250 μg mL^{-1}. When applied at 125 μg mL^{-1} it reduced the preformed biofilm by *ca.* 29%. In sum, synthetic berkeleylactone A (**1**) showed distinct effects on preformed biofilms of *C. albicans* at sub-MIC concentrations, a bioactivity that went unnoticed by Stierle *et al.* and Dixon, Caletková *et al.*

Synthetic berkeleylactone A (**1**) was also tested for cytotoxicity on mouse fibroblast cells (L929) and human cervix carcinoma cells (KB3.1) as described in the ESI.† Merely moderate cytotoxic activities were observed with half-maximum inhibitory concentrations (IC_{50}) of 11.1 μM (4.5 μg mL^{-1}) and 17.1 μM (6.9 μg mL^{-1}), respectively (Table 3).

Conclusions

Macrolide **1**, which was identified by Stierle *et al.* in a natural isolate and dubbed berkeleylactone A, was synthesised for the first time by Dixon, Caletková *et al.* in 10% yield, and now by us in 24% yield. Our synthesis has a linear rather than convergent character and uses a domino addition–Wittig olefination rather than an RCM reaction to close the macrocyclic ring. As to the identity of the natural product, there remains a shred of doubt, despite of matching single-crystal X-ray diffraction analyses of the isolated compound and the synthetic product of Dixon, Caletková *et al.* The NMR data of both synthetic products and of the isolated compound were consistent, apart from the acidic H-atoms not showing up in the ^{1}H NMR spectrum of the isolate, probably due to the sample preparation employing MeOD. However, the specific optical rotation of the natural isolate differed conspicuously from the similar values of the two synthetic samples. These findings point to a possible inhomogeneity of the natural isolate.

Our comprehensive biological characterisation of the synthetic berkeleylactone A (**1**) by antimicrobial, antibiofilm, and cytotoxicity assays confirmed its known strong antibiotic efficacy against *S. aureus* and MRSA, yet also revealed a distinct and hitherto unknown dispersive effect on preformed biofilms of *C. albicans* and an inhibitory effect on the formation of *S. aureus* biofilms, both at subtoxic concentrations. According to the National Institute of Health, biofilms cause more than 80% of microbial infections.[14,15] Pathogens, which are embedded in biofilms, are difficult to treat with antibiotics due to limits in drug penetration and increasing drug tolerance.[15,16] Strategies employing new agents that disperse preformed biofilms or combination regimens of biofilm inhibitors and established or new antibiotics have recently been recognised as promising and are being introduced in antibacterial drug discovery.[15]

Experimental section

General information

Melting points were determined with a Büchi M-565 melting point apparatus and are uncorrected. IR spectra were recorded with a PerkinElmer Spectrum 100 FT-IR spectrophotometer with ATR sampling unit. Optical rotations were measured at 589 nm (Na-D line) on a PerkinElmer 241 Polarimeter using solutions in chloroform, methanol or water. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker Avance III HD 500 spectrometer. Chemical shifts are given in parts per million using the residual solvent peak as an internal standard, *i.e.* 7.26 ppm (proton) and 77.16 (carbon) for $CDCl_3$, 3.31 ppm (proton) and 49.00 ppm (carbon) for CD_3OD, and 4.80 ppm for D_2O. Coupling constants (J) are quoted in Hz. Multiplicity abbreviations used: s singlet, d doublet, t triplet, qu quartet, qn quintet, sex sextet, m multiplet. High resolution mass spectra were obtained with a UPLC/Orbitrap MS system in ESI mode. The diastereomeric excess was determined by HPLC analysis (Waters Alliance HPLC; Waters 2695 Separation Module, Waters 2487 Dual λ Absorbance Detector) on chiral phase (Daicel Chiralpak AD-H), by RP-HPLC analysis (Shimadzu Nexera XR, SPD-M20A detector) on a C-18 column (Eurosphere II 100-3 C18 150 × 4 mm) or from ^{1}H NMR spectra.

Chemicals. All reagents were purchased from commercial sources and were used without further purification. All anhydrous solvents were used as supplied, except tetrahydrofuran, 1,4-dioxane and toluene which were freshly distilled over sodium/benzophenone, dichloromethane (CH_2Cl_2) which was freshly distilled over CaH_2, dimethylformamide (DMF) which was dried over molecular sieves (3 Å), and methanol (MeOH) which was freshly distilled over Mg. Moisture or air sensitive reactions were routinely carried out in oven-dried glassware under an argon atmosphere using standard Schlenk technique.

Chromatography. Analytical thin layer chromatography (TLC) was carried out using Merck silica gel 60 F_{254} pre-coated aluminum-backed plates. The compounds were visualized with UV light (254 nm) and/or ceric ammonium molybdate (CAM). Column chromatography was performed at medium pressure using wet-packed Macherey–Nagel silica gel 60, pore size 40–63 μm with the eluent specified.

Cell lines. The mouse fibroblasts L929 (DSMZ no. ACC 2) and the human cervix carcinoma cells KB3.1 (DSMZ no. ACC 158) were obtained from the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ), Braunschweig, Germany.

*(R)-Tridec-12-en-2-ol (**11**).*[10] A mixture of magnesium turnings (1.74 g, 71.5 mmol) and a catalytic amount of I_2 in dry THF (72 mL) under argon atmosphere was treated with 10-bromodec-1-ene (**9**, 13.1 mL, 65.0 mmol) while cooling with an ice bath. The resulting suspension was heated under reflux for 3.5 h. The supernatant solution of the Grignard reagent was added over 40 min to a solution of CuCN (537 mg, 6.00 mmol, 12 mol%) and (*R*)-PPO (*R*-**10**, 4.20 mL, 50.0 mmol) in dry THF (88 mL) at −40 °C. The solution was stirred at −40 °C for 2 h, then at −35 °C for 18 h. It was warmed to 0 °C and quenched with aqueous ammonia (50 mL) and sat. aqueous NH_4Cl-solution (100 mL). The aqueous solution was extracted with Et_2O (4 × 150 mL) and the combined organic phases were dried over Na_2SO_4. Removal of the solvent under reduced pressure gave the crude product, which was purified by column chromatography on silica gel (hexanes/EtOAc 9 : 1) to give alcohol **11** (9.22 g, 93%; loc. cit.:[14]: 85%) as a colourless oil. R_f = 0.33 (hexanes/EtOAc 4 : 1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.81 (ddt, J = 6.8, 10.1, 17.0 Hz, 1 H), 4.99 (dd, J = 1.6, 17.0 Hz, 1 H), 4.93 (m, 1 H), 3.79 (m, 1 H), 2.04 (m, 2 H), 1.51–1.22 (m, 17 H), 1.18 (d, J = 6.2 Hz, 3 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.4, 114.2, 68.3, 39.5, 34.0, 29.8, 29.74, 29.68, 29.6, 29.3, 29.1, 25.9, 23.6 ppm.

*(R)-Tridec-12-en-2-yl acetate (**12**).* A solution of alcohol **11** (7.99 g, 40.3 mmol), pyridine (11.2 mL, 137 mmol) and DMAP (98.5 mg, 806 μmol) in CH_2Cl_2 (20 mL) was treated with Ac_2O (11.4 mL, 121 mmol) at room temperature and stirred at room temperature for a further 19 h. It was poured into a mixture of

Et_2O (100 mL) and sat. aqueous NH_4Cl-solution (100 mL). The organic phase was separated and washed with sat. aqueous NH_4Cl-solution (100 mL). The combined aqueous phases were extracted with Et_2O (3 × 100 mL). The combined organic phases were washed with sat. aqueous $CuSO_4$-solution (100 mL) and brine (2 × 50 mL) and dried over Na_2SO_4. The solvent was evaporated, and the crude product was purified by filtration over a plug of silica with hexanes/EtOAc 4 : 1 (500 mL). After removal of the solvent, the ester **12** (9.51 g, 98%) was obtained as a colourless oil. R_f = 0.65 (hexanes/EtOAc 9 : 1); $[\alpha]_D^{23}$ −1.1° (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.81 (ddt, *J* = 6.7, 10.2, 17.0 Hz, 1 H), 4.99 (m, 1 H), 4.93 (m, 1 H), 4.88 (sex, *J* = 6.3 Hz, 1 H), 2.04 (m, 5 H), 1.57 (m, 1 H), 1.45 (m, 1 H), 1.41–1.22 (m, 14 H), 1.19 (d, *J* = 6.3 Hz, 3 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 139.4, 114.2, 71.2, 36.1, 34.0, 29.7 (2 C atoms), 29.60, 29.55, 29.3, 29.1, 25.5, 21.6, 20.1 ppm; IR ν_{max} 3080, 2979, 2927, 2855, 1739, 1640, 1464, 1372, 1243, 1128, 1020, 952, 909 cm^{-1}; HRMS (+ESI) *m*/*z* $[M + H]^+$ calcd for $C_{15}H_{29}O_2^+$ 241.21621, found 241.21602.

*(2R)-12,13-Dihydroxytridecan-2-yl acetate (**13**).* Ester **12** (4.81 g, 20.0 mmol) was dissolved in acetone (40 mL) and cooled to 0 °C. A solution of $K_2OsO_4{\cdot}2H_2O$ (221 mg, 600 μmol, 3 mol%) in H_2O (20 mL) and NMO (4.8 M in H_2O, 6.88 mL, 33.0 mmol) was added. The resulting mixture was allowed to warm to room temperature and stirred for 28.5 h. Na_2SO_3 (17.6 g, 140 mmol) was added and stirring was continued for a further 1.5 h. The solids were removed by filtration and the filter cake was washed wit EtOAc (150 mL). The volatile parts of the emulsion were removed by evaporation and the aqueous residue was extracted with EtOAc (4 × 200 mL). The combined organic phases were washed with brine (200 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (hexanes/EtOAc 1 : 6) to give diol **13** (5.20 g, 95%) as a colourless solid with a mp of 30–32 °C. R_f = 0.34 (hexanes/EtOAc 1 : 6); $[\alpha]_D^{20}$ −1.1° (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.88 (sex, *J* = 6.3 Hz, 1 H), 3.67 (m, 2 H), 3.43 (m, 1 H), 2.12 (s, 1 H), 2.01 (m, 4 H), 1.56 (m, 1 H), 1.43 (m, 4 H), 1.51–1.22 (m, 13 H), 1.20 (d, *J* = 6.3 Hz, 3 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 72.4, 71.2, 67.0, 36.0, 33.3, 29.7, 29.6, 29.55, 29.51, 25.6, 25.5, 21.6, 20.1 ppm; IR ν_{max} 3400, 2979, 2926, 2854, 1736, 1463, 1372, 1242, 1125, 1022, 953, 868, 723 cm^{-1}; HRMS (+ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{15}H_{30}O_4Na^+$ 297.20636, found 297.20319.

*(R)-12-Oxododecan-2-yl acetate (**8**).* A solution of diol **13** (5.00 g, 18.2 mmol) in CH_2Cl_2 (182 mL) at room temperature was treated portionwise with $NaIO_4$-coated SiO_2 (approx. 0.7 mmol $NaIO_4$ per g reagent, 41.6 g, 29.1 mmol). The resulting suspension was vigorously stirred for 1.75 h and filtered. The filtrate was evaporated and the unstable aldehyde **8** (4.42 g, quant.) was obtained as a colourless oil. It was immediately used for the next step without further purification. R_f = 0.55 (hexanes/EtOAc 4 : 1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.76 (t, *J* = 1.8 Hz, 1 H), 4.88 (sex, *J* = 6.3 Hz, 1 H), 2.42 (dt, *J* = 1.8, 7.3 Hz, 2 H), 2.02 (s, 3 H), 1.59 (m, 3 H), 1.45 (m, 1 H), 1.36–1.22 (m, 12 H), 1.20 (d, *J* = 6.3 Hz, 3 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.1, 171.0, 71.2, 44.1, 36.0, 29.6, 29.54, 29.46, 29.3, 25.5, 22.2, 21.6, 20.1 ppm.

*Ethyl (R)-13-acetoxy-3-oxotetradecanoate (**14**).* A solution of aldehyde **8** (8.29 g, 34.2 mmol) and EDA (solution with 16 wt% CH_2Cl_2, 5.57 mL, 44.5 mmol) in dry CH_2Cl_2 (188 mL) under argon atmosphere was cooled to 0 °C and treated with dry $SnCl_2$ (777 mg, 4.10 mmol, 12 mol%). The mixture was stirred for 2.25 h at 0 °C, warmed to room temperature and stirred for a further 45 min. Brine (150 mL) was added and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic phases were washed with sat. aqueous $NaHCO_3$-solution (100 mL), dried over Na_2SO_4 and evaporated. The crude product was purified by silica gel column chromatography (hexanes/EtOAc 10 : 1 to 5 : 1). After removal of the solvent under reduced pressure, the remainder was redissolved in EtOAc (60 mL) and washed with 2 M aqueous HCl (50 mL) and sat. aqueous $NaHCO_3$-solution (50 mL). The organic phase was dried over Na_2SO_4 and removal of the solvent gave the β-ketoester **14** (11.0 g, 98%) as a colourless oil. R_f = 0.38 (hexanes/EtOAc 4 : 1); $[\alpha]_D^{20}$ −2.1° (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) *keto-form*: δ 4.88 (sex, *J* = 6.2 Hz, 1 H), 4.19 (q, *J* = 7.2 Hz, 2 H), 3.42 (s, 2 H), 2.52 (t, *J* = 7.4 Hz, 2 H), 2.02 (s, 3 H), 1.57 (m, 3 H), 1.46 (m, 1 H), 1.36–1.22 (m, 15 H), 1.19 (d, *J* = 6.2 Hz, 3 H) ppm; *enol-form*: δ 12.1 (s), 4.97 (s), 4.26 (dq, *J* = 4.4, 7.2 Hz), 3.16 (q, *J* = 5.5 Hz), 2.18 (t, *J* = 7.2 Hz) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.2, 171.0, 167.4, 71.2, 61.5, 49.5, 43.2, 36.0, 29.58, 29.55, 29.50 (2 C atoms), 29.1, 25.5, 23.6, 21.6, 20.1, 14.3 ppm; *enol-form* δ 89.1, 60.1, 35.2 ppm; IR ν_{max} 2979, 2929, 2856, 1735, 1644, 1464, 1371, 1312, 1245, 1028, 951; HRMS (+ESI) *m*/*z* $[M + Na]^+$ calcd for $C_{18}H_{32}O_5Na^+$ 351.21420, found 351.21313.

*Ethyl (3S,13R)-13-acetoxy-3-hydroxytetradecanoate (**7**).* The Noyori catalyst was synthesized as described.[9] $[RuCl_2(benzene)]_2$ (75.0 mg, 150 μmol) and (*S*)-BINAP (196 mg, 315 μmol) were dissolved in degassed dry DMF (5.25 mL) under an argon atmosphere. The solution was heated at 100 °C for 10 min, cooled down to 50 °C and the solvent was removed in high vacuum.

In a glove box the degassed β-ketoester **14** (10.3 g, 31.4 mmol) was dissolved in degassed dry MeOH (28 mL). Freshly prepared Noyori catalyst (85 mg, 90.7 μmol, 0.29 mol%) was added and the mixture was stirred until complete solution. It was transferred into a high-pressure autoclave which was purged five times with H_2 and filled with 40 bar H_2. The solution was stirred at 60 °C for 65 h. The pressure was released, and the solvent was evaporated. The crude product was purified by silica gel column chromatography (hexanes/EtOAc 7 : 1 to 4 : 1). Alcohol **7** (9.02 g, 87%) was obtained as a colourless oil with a de >99% (as to chiral HPLC). R_f = 0.23 (hexanes/EtOAc 4 : 1); $[\alpha]_D^{20}$ +13.8° (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.88 (sex, *J* = 6.2 Hz, 1 H), 4.17 (q, *J* = 7.2 Hz, 2 H), 3.99 (m, 1 H), 2.94 (d, *J* = 4.0 Hz, 1 H), 2.50 (dd, *J* = 3.0, 16.4 Hz, 1 H), 2.39 (dd, *J* = 9.2, 16.4 Hz, 1 H), 2.02 (s, 3 H), 1.61–1.23 (m, 21 H), 1.19 (d, *J* = 6.2 Hz, 3 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3, 171.0, 71.2, 68.1, 60.8, 41.4, 36.6, 36.0, 29.7, 29.62, 29.60, 29.57, 25.6, 25.5, 21.6, 20.1, 14.3 ppm;

IR ν_{max} 3471, 2980, 2927, 2855, 1733, 1464, 1371, 1242, 1023, 951; HRMS (+ESI) *m/z* $[M + H]^+$ calcd for $C_{18}H_{33}O_5^+$ 331.24790, found 331.24756.

*Ethyl (3S,13R)-13-acetoxy-3-(methoxymethoxy)tetradecanoate (**15**).* A solution of alcohol **7** (330 mg, 1.00 mmol) in dimethoxymethane (5 mL) under argon atmosphere was treated with P_2O_5 (355 mg, 2.50 mmol) at room temperature. The resulting suspension was stirred for 2 h and sat. aqueous $NaHCO_3$-solution (20 mL) was added. The solution was extracted with EtOAc (3 × 50 mL), the combined organic phases were washed with brine (50 mL) and dried over Na_2SO_4. Silica gel column chromatography (hexanes/EtOAc 8 : 1) gave MOM-ether **15** (354 mg, 95%) as a colourless oil. R_f = 0.58 (hexanes/EtOAc 3 : 1); $[\alpha]_D^{20}$ +2.4° (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.88 (sex, *J* = 6.2 Hz, 1 H), 4.66 (dd, *J* = 7.0, 16.4 Hz, 2 H), 4.14 (q, *J* = 7.1 Hz, 2 H), 3.98 (qn, *J* = 6.4 Hz, 1 H), 3.35 (s, 3 H), 2.55 (dd, *J* = 7.4, 15.2 Hz, 1 H), 2.45 (dd, *J* = 5.3, 15.2 Hz, 1 H), 2.02 (s, 3 H), 1.63–1.22 (m, 21H), 1.20 (d, *J* = 6.2 Hz, 3 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.7, 171.0, 96.1, 74.9, 71.2, 60.5, 55.7, 40.5, 36.1, 35.0, 29.72, 29.67, 29.64, 29.62, 29.58, 25.5, 25.3, 21.6, 20.1, 14.4 ppm; IR ν_{max} 2979, 2928, 2856, 1733, 1465, 1372, 1242, 1148, 1101, 1033, 918 cm^{-1}; HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{20}H_{38}O_6Na^+$ 397.25606, found 397.25546.

*(3S,13R)-13-Hydroxy-3-(methoxymethoxy)tetradecanal (**6**).* A solution of MOM-ether **15** (5.71 g, 15.2 mmol) in dry toluene (100 mL) under an argon atmosphere was cooled down to −78 °C and treated with DIBAL (1 M in hexanes, 33.5 mL, 33.5 mmol) while stirring over a period of 15 min. Stirring was continued for a further 75 min at −78 °C. Acetone (500 µL) was added and the solution was stirred for 15 min. The mixture was poured into sat. aqueous Na-K-tartrate solution (300 mL) and stirred for 1.5 h. The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic phases were washed with brine (200 mL), dried over Na_2SO_4 and evaporated. After silica gel column chromatography (petrol ether/EtOAc 2 : 1 to 1 : 1) hydroxyaldehyde **6** (4.03 g, 92%) was obtained as a colourless resin. R_f = 0.50 (hexanes/EtOAc 1 : 1); $[\alpha]_D^{20}$ +9.0° (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.80 (dd, *J* = 1.8, 2.8 Hz, 1 H), 4.66 (dd, *J* = 7.0, 16.1 Hz, 2 H), 4.07 (qn, *J* = 6.5 Hz, 1 H), 3.79 (sex, *J* = 5.9 Hz, 1 H), 3.35 (s, 3 H), 2.64 (ddd, *J* = 2.8, 7.1, 16.3 Hz, 1 H), 2.56 (ddd, *J* = 1.8, 4.7, 16.3 Hz, 1 H), 1.61 (m, 1 H), 1.53 (m, 1 H), 1.47–1.24 (m, 17 H), 1.20 (d, *J* = 6.2 Hz, 3 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.7, 95.9, 73.3, 68.3, 55.8, 48.9, 39.5, 35.1, 29.74, 29.69, 29.68, 29.64, 29.60, 25.9, 25.3, 23.7 ppm; IR ν_{max} 3404, 2925, 2854, 1725, 1465, 1373, 1149, 1101, 1031, 918 cm^{-1}; HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{16}H_{32}O_4Na^+$ 311.21928, found 311.21878.

*(6S,16R,E)-6-(Methoxymethoxy)-16-methyloxacyclohexadec-3-en-2-one (**4**).* A solution of Ph_3PCCO (**5**, 199 mg, 659 µmol) in dry toluene (55 mL) under an argon atmosphere was warmed to 55 °C and treated dropwise with a solution of hydroxyaldehyde **6** (95 mg, 329 µmol) in dry toluene (10 mL) over a period of 15 h. The resulting solution was stirred for a further 5 h before the solvent was removed under reduced pressure. Silica gel column chromatography (hexanes 100% to hexanes/EtOAc 5 : 1) afforded macrolide **4** (64 mg, 62%) as a colourless oil. R_f = 0.60 (hexanes/EtOAc 4 : 1); $[\alpha]_D^{20}$ −43.7° (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.89 (dt, *J* = 7.5, 15.6 Hz, 1 H), 5.87 (d, *J* = 15.6 Hz, 1 H), 5.01 (ddq, *J* = 2.7, 6.3, 9.1 Hz, 1 H), 4.70 (d, *J* = 7.0 Hz, 1 H), 4.65 (d, *J* = 7.0 Hz, 1 H), 3.68 (m, 1 H), 3.38 (s, 3 H), 2.58 (m, 1 H), 2.38 (m, 1 H), 1.59 (m, 2 H), 1.53 (m, 1 H), 1.63–1.16 (m, 18 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1, 144.2, 124.8, 95.3, 75.9, 71.1, 55.6, 36.8, 35.3, 31.8, 28.0, 27.7, 27.4, 26.5, 26.4, 23.8, 22.0, 20.5 ppm; IR ν_{max} 2927, 2857, 1715, 1656, 1457, 1355, 1318, 1264, 1147, 1098, 1033, 917 cm^{-1}; HRMS (+ESI) *m/z* $[M + H]^+$ calcd for $C_{18}H_{33}O_4^+$ 313.23734, found 313.23682.

*(6S,16R,E)-6-(Methoxymethoxy)-16-methyloxacyclohexadec-3-ene-2,5-dione (**16**).* Macrolide **4** (40 mg, 128 µmol) and SeO_2 (42.6 mg, 384 µmol) were suspended in dry 1,4-dioxane (2 mL) in a sealed vessel under argon atmosphere and heated at 155 °C for 55 min. The mixture was filtered over a plug of Celite® and the filtrate was evaporated. After silica gel column chromatography (hexanes/EtOAc 8 : 1) ketone **16** (32 mg, 77%) was obtained as yellowish solid of mp 44–47 °C. R_f = 0.63 (hexanes/EtOAc 4 : 1); $[\alpha]_D^{20}$ −56° (*c* 1.00, $CHCl_3$), lit[17] $[\alpha]_D^{27}$ −49° (*c* 0.282, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (d, *J* = 15.8 Hz, 1 H), 6.77 (d, *J* = 15.8 Hz, 1 H), 5.09 (ddq, *J* = 2.9, 6.3, 9.1 Hz, 1 H), 4.68 (d, *J* = 6.8 Hz, 1 H), 4.65 (d, *J* = 6.8 Hz, 1 H), 4.23 (dd, *J* = 5.1, 7.2 Hz, 1 H), 3.35 (s, 3 H), 1.80 (m, 2 H), 1.59 (m, 2 H), 1.44–1.12 (m, 17 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.2, 165.1, 135.0, 132.1, 96.3, 82.0, 72.7, 56.2, 34.9, 30.6, 27.82, 27.76, 27.7, 26.8, 26.6, 23.7, 22.0, 20.3 ppm; IR ν_{max} 2929, 2857, 1721, 1704, 1623, 1460, 1267, 1031, 951, 919 cm^{-1}; HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{18}H_{30}O_5Na^+$ 349.19855, found 349.19806.

*(6S,16R,E)-6-Hydroxy-16-methyloxacyclohexadec-3-ene-2,5-dione (**2**).* A solution of ketone **16** (50 mg, 153 µmol) in dry CH_2Cl_2 (2 mL) was kept under argon atmosphere at −10 °C and treated with TFA (1 mL). The mixture was stirred for 9.5 h at −10 °C, then treated with sat. aqueous $NaHCO_3$-solution (40 mL), and the aqueous phase was finally extracted with CH_2Cl_2 (3 × 40 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. After silica gel column chromatography (hexanes/EtOAc 8 : 1 to 6 : 1) alcohol **2** (38 mg, 88%) was obtained as a colourless crystalline solid of mp 83–85 °C, lit[10] 84–85 °C. R_f = 0.38 (hexanes/EtOAc 4 : 1); $[\alpha]_D^{20}$ +35.8° (*c* 1.00, $CHCl_3$), lit[10] $[\alpha]_D^{20}$ +22.4° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 (d, *J* = 15.9 Hz, 1 H), 6.80 (d, *J* = 15.9 Hz, 1 H), 5.18 (m, 1 H), 4.55 (q, *J* = 4.5 Hz, 1 H), 3.45 (d, *J* = 4.5 Hz, 1 H), 1.85 (m, 2 H), 1.73 (m, 1 H), 1.50 (m, 2 H), 1.46–1.01 (m, 15 H), 0.96 (m, 1 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.6, 165.2, 135.0, 132.7, 76.6, 72.8, 34.3, 31.2, 28.22, 28.17, 27.4, 27.2, 26.9, 23.5, 20.7, 19.8 ppm; IR ν_{max} 3456, 3066, 2923, 2854, 1714, 1696, 1644, 1459, 1353, 1286, 1190, 1057, 984 cm^{-1}; HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{16}H_{26}O_4Na^+$ 305.17233, found 305.17117.

*Methyl oxirane-2-carboxylate rac-(**18**).* According to the protocol of Jacobsen[13] aqueous NaOCl (6 wt%, 588 mL, 480 mmol) was cooled to 0 °C and methyl acrylate (**17**, 31.6 mL,

348 mmol) was added. The emulsion was stirred for 30 min at 0 °C and the ice bath was removed. The aqueous solution was stirred for another 1.5 h, cooled again by an ice bath, and extracted with CH_2Cl_2 (4 × 100 mL). The combined organic phases were dried over Na_2SO_4 and evaporated at 30 °C (100 mbar). The remaining solution was distilled (85 mbar, 85 °C) which gave *rac*-methyl glycidate (*rac*-**18**, 11.4 g, 32%) as a colourless liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.79 (s, 3 H), 3.45 (dd, J = 2.5, 4.1 Hz, 1 H), 2.97 (dd, J = 2.5, 6.5 Hz, 1 H), 2.94 (dd, J = 4.1, 6.5 Hz, 1 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.8, 52.6, 47.3, 46.4 ppm.

*Methyl (R)-oxirane-2-carboxylate R-(**18**).* (*S*,*S*)-*N*,*N*'-Bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II) (408 mg, 675 μmol, 0.75 mol%) and *p*TsOH (136 mg, 716 μmol, 0.795 mol%) were dissolved in CH_2Cl_2 (9 mL) and stirred, open to air, for 1 h. The solvent was removed under reduced pressure. *rac*-Methyl glycidate (*rac*-**18**, 7.88 mL, 90.0 mmol) and H_2O (1.14 mL, 63.0 mmol) were added and the solution was stirred at room temperature for 21 h and then at 85 °C for 2 h. The precipitate was filtered off and washed with H_2O (3 × 20 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL) and the combined organic phases were dried over Na_2SO_4. The solvent was removed by rotary evaporation (30 °C, 100 mbar) and the remainder distilled under vacuum (80 °C, 70 mbar). *R*-Methyl glycidate (*R*-**18**, 2.99 g, 33%) was obtained as a colourless liquid. $[\alpha]_D^{25}$ +8.9° (*c* 5.34, MeOH), lit[13] $[\alpha]_D^{26}$ −10.3° (*c* 5.34, MeOH) for the *S*-enantiomer; ^{1}H NMR (500 MHz, $CDCl_3$) 3.79 (s, 3 H), 3.45 (dd, J = 2.5, 4.1 Hz, 1 H), 2.97 (dd, J = 2.5, 6.5 Hz, 1 H), 2.94 (dd, J = 4.1, 6.5 Hz, 1 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8, 52.7, 47.4, 46.5 ppm.

*Potassium (R)-oxirane-2-carboxylate (**19**).* KOH (524 mg, 9.33 mmol) was dissolved in MeOH (18 mL) at 0 °C and (*R*)-methyl glycidate (*R*-**18**, 1.00 g, 9.80 mmol) was added. The solution was warmed to room temperature and stirred for 16 h. The solvent was evaporated, and the crude product recrystallized from MeOH/Et_2O. (*R*)-Potassium glycidate (**19**, 930 mg, 79%) was obtained as a colourless solid which showed decomposition at 141 °C. $[\alpha]_D^{26}$ +31.1° (*c* 1.05, H_2O), lit[18] $[\alpha]_D^{20}$ +31.8° (*c* 1.05, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 3.35 (m, 1 H), 2.93 (m, 1 H), 2.79 (m, 1 H) ppm; ^{1}H NMR (500 MHz, CD_3OD) δ 3.23 (dd, J = 2.6, 4.4 Hz, 1 H), 2.81 (dd, J = 4.6, 6.4 Hz, 1 H), 2.72 (m, J = 2.6, 6.4 Hz, 1 H) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ 176.8, 50.4, 46.3 ppm.

*(S)-2-Hydroxy-3-(tritylthio)propanoic acid (**20**).* A solution of triphenylmethanethiol (1.32 g, 4.76 mmol) in dry THF (30 mL) was kept under an argon atmosphere at 0 °C and treated portionwise with NaH (60 wt% in mineral oil, 89 mg, 2.22 mmol). The resulting mixture was treated with (*R*)-potassium glycidate (**19**, 400 mg, 3.17 mmol), then warmed to room temperature, stirred for 19 h, and finally poured into H_2O (100 mL). The aqueous phase was extracted with Et_2O (2 × 50 mL), adjusted to pH = 4 and extracted with Et_2O (3 × 70 mL) and EtOAc (70 mL). The combined organic phases were dried over Na_2SO_4 and evaporated. The crude carboxylic acid **20** (1.00 g) was used for the next step without further purification. R_f = 0.69 (CH_2Cl_2/MeOH 9 : 1 + 1% HCOOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, J = 7.6 Hz, 6 H), 7.29 (t, J = 7.6 Hz, 6 H), 7.23 (t, J = 7.6 Hz, 3 H), 3.82 (dd, J = 4.2, 7.2 Hz, 1 H), 2.77 (dd, J = 4.2, 13.2 Hz, 1 H), 2.67 (dd, J = 7.2, 13.2 Hz, 1 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.7, 144.4, 129.6, 128.2, 127.1, 69.0, 67.2, 36.2 ppm; IR ν_{max} 3348, 3054, 3031, 2930, 1733, 2605, 1717, 1594, 1488, 1444, 1240, 1183, 1083, 1033, 741, 696 cm^{-1}; HRMS (−ESI) m/z $[M - H]^-$ calcd for $C_{22}H_{19}O_3S^-$ 363.10494, found 363.10468.

*Benzyl (S)-2-hydroxy-3-(tritylthio)propanoate (**21**).* To a solution of crude carboxylic acid **20** (450 mg, 1.23 mmol) in DMF (10 mL) was added Cs_2CO_3 (483 mg, 1.48 mmol). After 30 min of stirring at room temperature, benzyl bromide (584 μL, 4.92 mmol) was added and the suspension was stirred for a further 16 h. The mixture was poured into sat. aqueous NH_4Cl-solution (100 mL) and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with H_2O (2 × 100 mL), dried over Na_2SO_4, and evaporated. Purification by silica gel column chromatography (petrol ether/EtOAc 5 : 1 to 1 : 1) gave benzyl ester **21** (276 mg, 61% over two steps) as a colourless resin. R_f = 0.66 (hexanes/EtOAc 2 : 1); $[\alpha]_D^{20}$ −48.3° (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45–7.15 (m, 20 H), 5.15 (q, J = 12.2 Hz, 2 H), 4.04 (q, J = 5.6 Hz, 1 H), 2.82 (d, J = 6.0 Hz, 1 H), 2.58 (d, J = 5.6 Hz, 2 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 144.5, 135.1, 129.7, 128.72, 128.66, 128.4, 128.1, 126.9, 69.6, 67.6, 66.8, 36.6 ppm; IR ν_{max} 3472, 3057, 3032, 2930, 1733, 1594, 1489, 1444, 1173, 1082, 741, 695 cm^{-1}; HRMS (+ESI) m/z $[M + Na]^+$ calcd for $C_{29}H_{26}O_3SNa^+$ 477.14949, found 477.14865.

*Benzyl (S)-2-hydroxy-3-mercaptopropanoate (**3**).* A solution of benzyl ester **21** (190 mg, 418 μmol,) and *i*Pr$_3$SiH (103 μL, 502 μmol) in CH_2Cl_2 (10 mL) at 0 °C was treated with TFA (200 μL) and stirred for 3.5 h at 0 °C and for a further 45 min at room temperature. Toluene (2 × 10 mL) was added and the volatiles were removed by rotary evaporation. After silica gel column chromatography (petrol ether/EtOAc 5 : 1 to 3 : 1) thiol **3** (80 mg, 91%) was obtained as a colourless resin. R_f = 0.67 (hexanes/EtOAc 1 : 1); $[\alpha]_D^{20}$ +43.2° (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (m, 5 H), 5.25 (q, J = 12.1 Hz, 2 H), 4.47 (m, 1 H), 3.17 (d, J = 5.8 Hz, 1 H), 2.96 (ddd, J = 3.8, 8.0, 14.0 Hz, 1 H), 2.87 (ddd, J = 4.4, 9.5, 14.0 Hz, 1 H), 1.56 (dd, J = 8.0, 9.5 Hz, 1 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8, 135.0, 128.90, 128.86, 128.7, 70.9, 68.0, 29.0 ppm; IR ν_{max} 3471, 3069, 3035, 2946, 2574, 1733, 1498, 1455, 1256, 1187, 1095 cm^{-1}; HRMS (+ESI) m/z $[M + H]^+$ calcd for $C_{10}H_{13}O_3S$ 213.05799, found 213.05783.

*Benzyl (S)-2-hydroxy-3-(((3R,6S,16R)-6-hydroxy-16-methyl-2,5-dioxooxacyclohexadecan-3-yl)thio)propanoate (**22**).* Macrolide **2** (59 mg, 209 μmol) and thiol **3** (51 mg, 240 μmol) were dissolved at room temperature in CH_2Cl_2 (2 mL) and NEt_3 (4.23 μL, 4.8 μmol, 20 mol%) was added. The solution was stirred for 3 h and the solvent was removed by rotary evaporation. Silica gel column chromatography (CH_2Cl_2/EtOAc 10 : 1 to 4 : 1) afforded thioether **22** (101 mg, 98%) as a colourless oil with a diastereomeric ratio of 15 : 1. R_f = 0.47 (hexanes/EtOAc 1 : 1); $[\alpha]_D^{20}$ +99.0° (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (m, 5 H), 5.22 (s, 2 H), 4.94 (m, 1 H), 4.53 (m, 1 H), 4.33

(m, 1 H), 4.02 (dd, J = 6.1, 8.3 Hz, 1 H), 3.52 (s, 1 H), 3.33 (d, J = 4.5 Hz, 1 H), 3.24 (dd, J = 3.7, 14.5 Hz, 1 H), 3.22 (dd, J = 8.3, 18.4 Hz, 1 H), 2.98 (dd, J = 5.7, 14.4 Hz, 1 H), 2.69 (dd, J = 6.1, 18.4 Hz, 1 H), 1.83 (m, 2 H), 1.55 (m, 1 H), 1.47–1.34 (m, 17 H), 0.96 (m, 1 H) ppm; *epimer* δ 3.80 (t, J = 6.9 Hz), 3.10 (m) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.0, 172.7, 172.0, 135.0, 128.8, 128.6, 76.2, 72.7, 70.7, 67.9, 41.2, 41.0, 35.8, 34.6, 32.6, 26.9, 26.8, 26.7, 26.1, 25.4, 23.1, 20.9, 19.9 ppm; *epimer* δ 76.6, 73.5, 71.3, 67.7, 43.6, 40.7, 37.3, 35.1, 27.3, 26.4, 26.3, 25.9, 23.3, 21.9, 19.5 ppm; IR ν_{max} 3472, 2929, 2858, 1717, 1456, 1263, 1172, 1092, 1005, 734, 697 cm^{-1}; HRMS (+ESI) m/z $[M + H]^+$ calcd for $C_{26}H_{39}O_7S^+$ 495.24110, found 495.24069.

*Berkeleylactone A (**1**).* To a solution of thioether **22** (22 mg, 44.4 μmol) in MeOH (6 mL) under argon atmosphere was added Pd/C (10 wt%, 4.8 mg, 4.44 μmol, 10 mol%). The reaction flask and the suspension were purged with H_2. The mixture was stirred for 1.75 h at room temperature under an atmosphere of H_2 (1 atm), filtered over a plug of Celite®, and washed with MeOH (30 mL). Rotary evaporation of the filtrate gave the crude product which was purified by column chromatography on silica gel (CH_2Cl_2/MeOH + 0.5% HCOOH 30 : 1 to 25 : 1) to afford berkeleylactone A (**1**, 16 mg, 89%) as a colourless crystalline solid of mp 110–113 °C, lit[2] 119–121 °C. R_f = 0.38 (CH_2Cl_2/MeOH 10 : 1 + 1% HCOOH); $[\alpha]_D^{20}$ +94.5° (*c* 1.00, $CHCl_3$), lit[1] $[\alpha]_D^{25}$ +0.5° (*c* 0.17, $CHCl_3$), lit[2] $[\alpha]_D^{25}$ +101.0° (*c* 0.105, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.14–4.83 (m, 4 H), 4.55 (m, 1 H), 4.40 (m, 1 H), 4.03 (t, J = 7.0 Hz, 1 H), 3.28 (m, 1 H), 3.22 (dd, J = 7.7, 18.5 Hz, 1 H), 3.00 (dd, J = 5.5, 14.5 Hz, 1 H), 2.80 (dd, J = 6.3, 18.5 Hz, 1 H), 1.85 (m, 2 H), 1.56 (m, 1 H), 1.51–1.13 (m, 17 H), 0.98 (m, 1 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.0, 175.2, 172.4, 76.3, 73.3, 70.5, 41.4, 41.0, 35.8, 34.6, 32.4, 26.8, 26.75, 26.7, 26.1, 25.3, 23.0, 20.8, 19.9 ppm; IR ν_{max} 3433, 2929, 2858, 1716, 1458, 1261, 1170, 1092, 908, 729 cm^{-1}; HRMS (+ESI) m/z $[M + H]^+$ calcd for $C_{19}H_{33}O_7S^+$ 405.19415, found 405.19345.

Author contributions

MGS planned the chemical synthesis, carried out all chemical reactions, and isolated, purified and analysed all reaction products, and wrote the experimental part of the manuscript. HS and HZ conducted and evaluated all biological assays. MS supervised the biological studies, RS supervised the chemical part and wrote the manuscript.

Conflicts of interest

There are no conflicts to declare.

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