Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2015, **13**, 5139

Efficient synthesis of (*R*)-harmonine – the toxic principle of the multicolored Asian lady beetle (*Harmonia axyridis*)†

Nadja C. Nagel,[a] Anita Masic,[b] Uta Schurigt[b] and Wilhelm Boland*[a]

Received 7th March 2015,
Accepted 24th March 2015

DOI: 10.1039/c5ob00461f

www.rsc.org/obc

A flexible synthetic route to (*R*)-harmonine ((*R*)-**1**), the toxic principle of the Asian lady beetle *Harmonia axyridis* (*H. axyridis*), *via* reductive olefination of the macrocyclic lactone (*S*)-**5**, is reported. High enantiomeric purity is achieved by enantioselective saponification of the lactone *rac*-**5** with horse liver esterase. Minor modifications in the synthetic route give access to racemic and chiral harmonine (**1**), analogs and putative biosynthetic precursors. In addition, the antimicrobial activity of harmonine against *Leishmania major* (*L. major*) is demonstrated and provides the rationale for harmonine-based drug development against parasitic diseases.

Introduction

The multicolored Asian lady beetle *H. axyridis*, also known as the harlequin ladybird, is natively distributed from southern Siberia to southern China and from the Altai mountains to the Pacific coast.[1] This tree dwelling beetle, of the family Coccinellidae, is an important predator of aphids and scale insects and has been introduced for biological control in many countries. Over time populations began to establish and two decades ago *H. axyridis* became an invasive species in North America, Europe and South America threatening the native lady beetles.[1–3] The invasive success arises from intra-guild predation[2] and from *H. axyridis*' resistance to pathogens.[3] When a lady beetle is disturbed or attacked it releases droplets of hemolymph from the tibio-femoral joints of its legs. This behavior is referred to as reflex-bleeding.[4,5] The repellent and sometimes toxic properties of the hemolymph are due to some alkaloids, which are considered to be synthesized *de novo* by the beetles.[6] *H. axyridis* produces (*R*)-harmonine ((*R*)-**1**) ((17*R*,9*Z*)-octadec-9-ene-1,17-diamine) as the major defense compound (Fig. 1).[7]

(*R*)-Harmonine ((*R*)-**1**) displays antibacterial activity against fast-growing mycobacteria, *Mycobacterium tuberculosis*, and *Plasmodium falciparum* (*P. falciparum*), demonstrates multistage antimalarial activity,[7] and exhibits cytotoxicity against human tumor cell lines.[8] Recently microsporidia from the hemolymph of *H. axyridis* were shown to infect intra-guild predators.[9] In this context, (*R*)-harmonine ((*R*)-**1**) was postulated to protect the harlequin beetle against self-infection.[10] Currently, harmonine ((*R*)-**1**) is considered a promising lead for clinical and agricultural use (yellow biotechnology).[11] Although harmonine ((*R*)-**1**) has been isolated[12] and synthesized previously,[12–14] there is a need for rapid and efficient syntheses of harmonine ((*R*)-**1**) and related molecules, since the mode of action is still unknown. In particular, for bioassays larger quantities are needed.

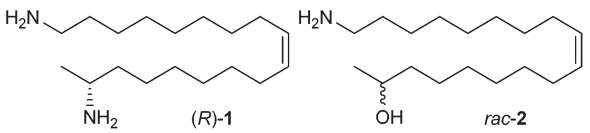


Fig. 1 (*R*)-Harmonine ((*R*)-1) and (9*Z*)-18-aminooctadec-9-en-2-ol (*rac*-**2**).

L. major is the causative agent of cutaneous leishmaniasis with an estimated annual incidence of 800 000–1.3 million new infections worldwide.[15,16] Current chemotherapy against leishmaniasis is limited due to the continuous development of drug resistance accompanied by severe side effects.[17] Therefore, naturally derived compounds like harmonine are investigated to identify and develop new therapies against leishmaniasis. The antiparasitic activity of harmonine against *P. falciparum* has already been reported and prompted the assessment of its activity against *L. major*.[7]

Here, we report a short and flexible synthetic route to harmonine ((*R*)-**1**) in only a few steps starting from the readily

[a]*Max Planck Institute for Chemical Ecology, Beutenberg Campus, Hans-Knoell-Straße 8, D-07745 Jena, Germany. E-mail: boland@ice.mpg.de*
[b]*Institute for Molecular Infection Biology, Josef-Schneider-Str. 2/D15, D-97080 Wuerzburg, Germany*
†Electronic supplementary information (ESI) available. See DOI: 10.1039/c5ob00461f

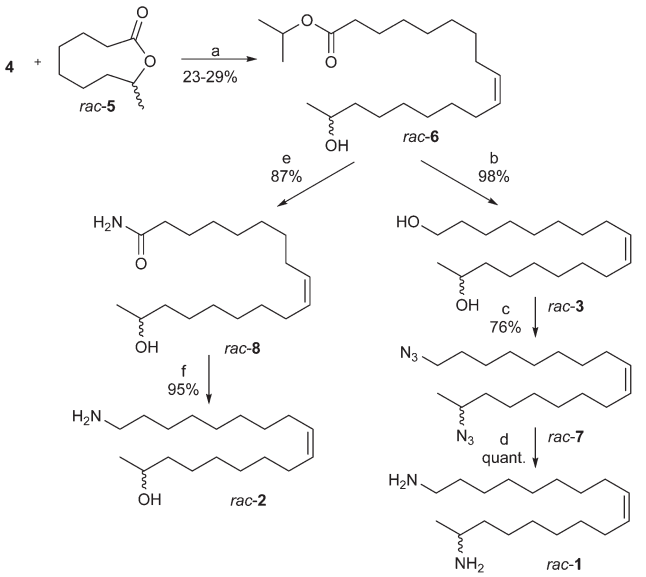
available macrocyclic lactone (*S*)-**5**. One-pot olefination of (*S*)-**5** directly provides the basic skeleton of harmonine ((*R*)-**1**) and allows the synthesis of biosynthetic precursors and structural analogs *via* functional group modifications. Furthermore, we report the antiparasitic activity of harmonine against the causative agent of cutaneous leishmaniasis, *L. major*.[18] Cultivation in the presence of (*R*)-**1** leads to the inhibition of parasitic proliferation with a consequent early necrotic cell death phenotype.

Results and discussion

According to Scheme 1 the harmonine backbone can be obtained from the lactone (*S*)-**5** and the phosphonium salt **4** by a Wittig-type reaction. The chiral lactone (*S*)-**5** is available with high ee and excellent yield by enantioselective hydrolysis with horse liver esterase. Furthermore, synthetic intermediates like ((*S*)-**3**) are promising candidates for biosynthetic and pharmaceutical studies.[19] By modifying the chain length of the phosphonium salt or the ring size of the lactone, analogs with different positions of the double bond and molecular size become available, thus opening a new synthetic route to harmonine-like compounds using a unified procedure.

Following the retrosynthetic strategy the backbone of harmonine (*rac*-**1**) was assembled by reductive olefination[17] from *rac*-**5** and the ylide of (9-isopropoxy-9-oxononyl)triphenylphosphonium bromide (**4**) in a single operation. The resulting isopropylester *rac*-**6** was converted into the amide *rac*-**8** or directly reduced to the diol *rac*-**3** (Scheme 2). The lactone *rac*-**5** was obtained from commercial cyclooctanone[20] and the phosphonium salt **4** from 9-bromononanoic acid after esterification and reaction with triphenylphosphine.[21,22]

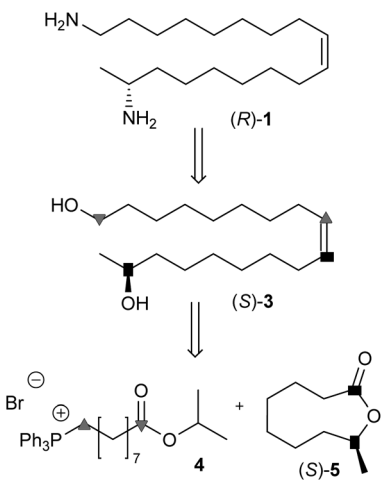
The one-pot "reductive olefination" generates from the lactone *rac*-**5** and DiBAlH at low temperatures an organo aluminium acetal that decomposes above −40 °C to a hydroxy aldehyde which reacts *in situ* with the phosphorane to the hydroxyester *rac*-**6** ((*Z*)/(*E*) > 98/2). The isopropylester was used to minimize side reactions during the Wittig olefination. Reduction of *rac*-**6** with lithium aluminum hydride afforded the diol *rac*-**3** in quantitative yield.[23] Following the Mitsunobu protocol the diol *rac*-**3** was converted into the diazide *rac*-**7** using triphenylphosphine (PPh_3), diphenyl phosphorazidate (DPPA) and diisopropyl azodicarboxylate (DIAD).[24] The final reduction of *rac*-**7** with an excess of $LiAlH_4$ afforded racemic harmonine (*rac*-**1**) in quantitative yield.[24] Overall, racemic harmonine (*rac*-**1**) could be prepared in only four steps and 22% overall yield starting from *rac*-**5** and **4**. Spectral data of *rac*-**1** were identical to literature values.[8,12]



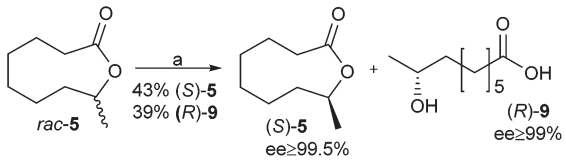
Scheme 2 Synthetic sequence to harmonine (*rac*-**1**) and a putative biosynthetic precursor *rac*-**2**. Reagents and conditions: (a) (i) DiBAlH, MeOH, toluene, −78 °C; (ii) KHMDS, THF, −78 °C; (iii) −78 °C to rt in 1 h, 1 h rt; (b) $LiAlH_4$, THF, rt; (c) PPh_3, DPPA, DIAD, THF, rt; (d) $LiAlH_4$, THF, rt; (e) Mg_3N_2, MeOH, 80 °C; (f) $LiAlH_4$, THF, 45 °C.

For biosynthetic studies[19] and pharmaceutical assays[7] the (*Z*)-18-aminooctadec-9-en-2-ol (*rac*-**2**) was of interest. Conversion of the isopropylester *rac*-**6** into the amide *rac*-**8** was readily achieved using magnesium nitride in refluxing methanol (Scheme 2).[23] Subsequent reduction with $LiAlH_4$ [25] provided the aminoalcohol *rac*-**2** in nearly quantitative yields over two steps.

The synthetic protocol was easily extended to chiral (17*R*,9*Z*)-1,17-diaminooctadec-9-ene ((*R*)-**1**). The key step was a kinetically controlled enantioselective saponification of the lactone *rac*-**5** which left behind unreacted (*S*)-lactone ((*S*)-**5**) (ee > 99.5%) in 43% yield (Scheme 3). For hydrolysis the lactone (*rac*-**5**) was suspended in a NaH_2PO_4-buffer (0.1 M, pH = 7.2) and horse liver esterase (HLE) was added as lyophilized powder.[26] The hydrolysis started immediately and the pH was kept constant by addition of dil. NaOH (0.5 M) to avoid spontaneous hydrolysis.



Scheme 1 Retrosynthesis of harmonine ((*R*)-**1**).



Scheme 3 Enantioselective, enzymatic saponification of *rac*-**5**. Reagents and conditions: (a) HLE, NaH_2PO_4, NaOH, rt.

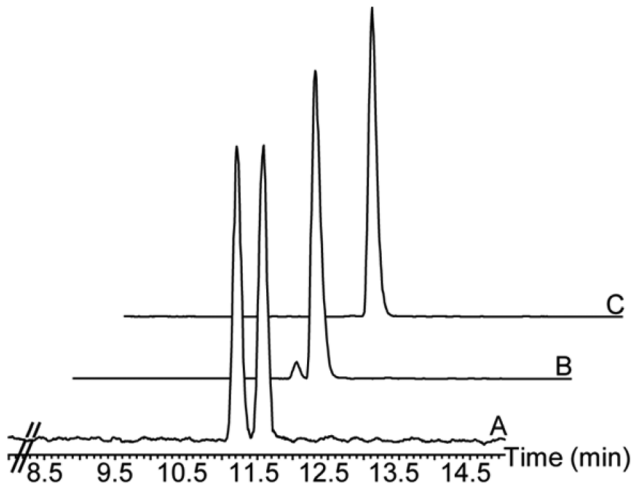


Fig. 2 Enantiomeric analysis on a β-6TBDM column. (A) Racemic lactone *rac*-**5**. (B) 98% hydrolysis of (*R*)-lactone. (C) Isolated lactone (*S*)-**5** (ee ≥ 99.5%).

The progress of the reaction was monitored using a GC-MS equipped with a chiral column (β-6TBDM) for resolution of the enantiomers (Fig. 2).

After hydrolysis of the (*R*)-lactone, the reaction stopped and both the remaining lactone (*S*)-**5** and the hydroxyacid (*R*)-**9** were obtained with good yield and excellent ee (*S*)-**5** (>99.5%), (*R*)-**9** (>99%).

The chiral lactone (*S*)-**5** was converted into (*R*)-harmonine ((*R*)-**1**) using the same sequence of reactions as shown in Scheme 2. The spectral data of (*R*)-**1** were identical to literature values.[8,12,13]

The ee of (*R*)-harmonine ((*R*)-**1**) was determined by ^{19}F NMR using (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA chloride) for derivatization.[27,28] Due to the large chemical shifts of the α-CF_3 groups of the MTPA-diastereomers the high ee of (*R*)-**1** was reliably confirmed (ee > 97%).

For the evaluation of the antileishmanial activity of harmonine, the AlamarBlue® assay with *L. major* promastigotes and the Britelite™ assay with Luciferase-transgenic *L. major* amastigotes were employed (Table 1). The cytotoxic effect of harmonine against host cells was investigated in bone marrow-derived macrophages (BMDM). The half maximal inhibitory concentration (IC_{50}) of harmonine was detected at 14.2 μM against *L. major* promastigotes and at 2.4 μM against the intracellular and clinically relevant *L. major* amastigote form. When compared to Miltefosine (1-hexadecylphosphocholine, positive control) with an IC_{50} value of 36.2 μM against *L. major* promastigotes and 33.0 μM against *L. major* amastigotes,[29] harmonine showed leishmanicidal activities at significantly lower concentrations. Miltefosine showed an IC_{50} value of 65.5 μM and harmonine showed a value of 36.5 μM against BMDM. The antileishmanial efficacy of a tested drug compared to its cytotoxicity against host cells is defined as the selectivity index (SI). SIs > 20 are considered excellent, as high antileishmanial activity and low cytotoxicity are desirable prerequisites for drug development.[30] Here, harmonine shows a very good SI of 15.2 towards *L. major* which is considerably higher than the SI of 2.0 of Miltefosine.

Table 1 Antileishmanial activity of harmonine

	IC_{50} (μM)[a]			
	L. major promastigotes	*L. major* amastigotes	BMDM[b]	SI[c]
(*R*)-Harmonine	14.2[d]	2.4	36.5	15.2
Miltefosine	36.2	33.0	65.5	2.0

[a] IC_{50}: half maximal inhibitory concentration. [b] BMDM: bone marrow-derived macrophages. [c] SI (Selectivity Index): IC_{50} for BMDM/IC_{50} for *L. major*. [d] IC_{50} synthetic *rac*-harmonine: 13.2.

The antileishmanial effect of harmonine as shown in Table 1 was further investigated. The cell morphology of *L. major* promastigotes was studied by means of transmitted light microscopy upon treatment with harmonine for 6 h, 10 h, and 24 h (Fig. 3). The visual examination of promastigotes incubated with harmonine showed rapid changes of the cell morphology. After 24 h only dead cells were observed upon harmonine-treatment whereas in the presence of dimethyl sulfoxide (DMSO) no significant changes in the cell morphology of the parasites were observed. Rounding of cells upon harmonine treatment was induced after 6 h of culture and dead cells were visible after 10 h of cultivation. DMSO-treated control *L. major* promastigotes show upon 6 h and 10 h of culture the characteristic flagellated and slender shape of the viable and unaffected parasite (Fig. 3).

The type of cell death induced by harmonine was investigated using flow cytometric approaches. The loss of membrane integrity in necrotic cells and the translocation of phosphatidylserine (PS) to the outside of the cellular membrane of apoptotic cells can be determined by Annexin V (AV, binds PS) and PI (DNA-binding) staining.[31] Double staining with AV-fluorescein isothiocyanate (FITC) and PI allows the discrimination between four *Leishmania* cell death phenotypes as described elsewhere: live (AV^-/PI^-), late necrotic/late apoptotic (AV^+/PI^+), early necrotic (AV^-/PI^+) and early apoptotic cells (AV^+/PI^-).[31,32]

Harmonine-treatment for 24 h induced early necrotic cell death in 31.6% and late necrotic/late apoptotic cell death in 51.2% of all the treated cells (Fig. 3). This means that after 24 h of harmonine-treatment a total of 82.8% of cells were

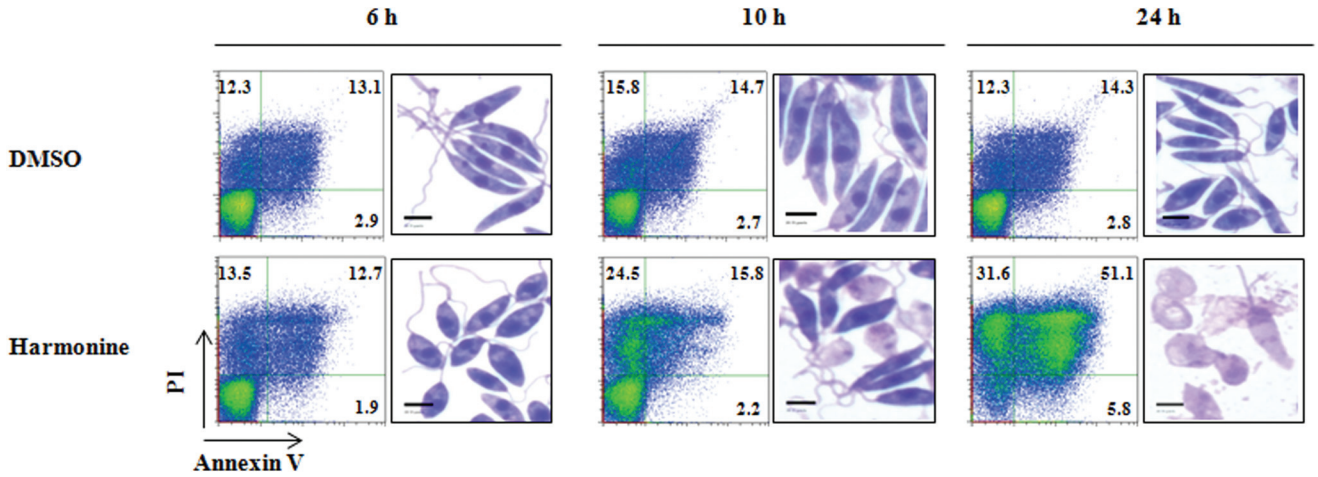


Fig. 3 Harmonine induced cell death in *L. major* promastigotes. *L. major* promastigotes were treated with 30 μM harmonine or 1% DMSO for 6 h, 10 h, and 24 h. Annexin V and PI double staining was performed for the determination of the cell death phenotype (dot-blots) and Diff-Quick staining was performed to investigate cell morphological changes *via* transmission light microscopy (pictures). Scale bars = 10 μm. Numbers in the quadrants represent cells (%) showing early necrotic, late necrotic, or apoptotic cell death.

dead. At the same time under normal growth conditions only 26.6% of cells were not viable (Fig. 3). Cell death during harmonine-treatment was observed to increase over time, as after 6 h a total of 26.2% and after 10 h a total of 40.3% of cells were dead, as indicated by PI staining. Early necrosis is characterized by PI-binding to the DNA of cells which have lost their cell membrane integrity. Harmonine was found to induce early necrotic cell death in *L. major*, as a clear population of 24.5% early necrotic cells was detected after 10 h of cultivation (Fig. 3). The increase of late necrotic/late apoptotic cells to 51.15% after 24 h is a consequence of an early necrotic cell death phenotype, as the rupture of the cell membrane allows AV to bind to PS in the dead parasite.

Conclusion

In conclusion a highly efficient and flexible synthetic route to chiral (*R*)-harmonine ((*R*)-**1**) is reported. A highly enantioselective hydrolysis of rac-**5** affords both the remaining lactone (*S*)-**5** and the hydroxy acid (*R*)-**9** in high yield and excellent enantiomeric purities. Reductive olefination of the lactone (*S*)-**5** with readily available phosphonium ylides gives direct access to the backbone of harmonine ((*R*)-**1**). Subsequent functional group modification provides derivatives and analogs of the natural product for structure–activity studies and mechanistic analyses to unravel the mode of action of the ladybeetle alkaloid harmonine ((*R*)-**1**). The pronounced activity of harmonine ((*R*)-**1**) against mycobacteria or the malaria parasite *P. falciparum* as is reported elsewhere,[7] and the activity against *L. major* as is shown for the first time in the present study are already encouraging observations for further investigations. Readily available synthetic harmonine and analogs may provide a base for the development of novel anti-parasitic drugs with both parasitocidal and transmission-blocking activities.[7]

Experimental

Synthesis

General methods. NMR spectra were recorded using a Bruker AV 400 spectrometer (Bruker, Rheinstetten/Karlsruhe, Germany) operating at 400 MHz (^{1}H), 100 MHz (^{13}C) and 376.5 MHz (^{19}F). Chemical shifts (δ) in ^{1}H, ^{13}C and ^{19}F NMR are given in ppm and are referenced to the residual solvent peak ($CDCl_3$: 7.27 (^{1}H NMR); 77.0 (^{13}C NMR)). GC-MS analysis was carried out using a Trace MS, 2000 Series (ThermoQuest, Engelsbach, Germany) equipped with an Alltech DB5 column (30 m × 0.25 m, 0.25 μm); carrier gas: helium. Enantiomers were separated on a Hydrodex-β-6TBDM column (25 m × 0.25 m) and analyzed with an ITQ 900 (Thermo Fischer Scientific, Bremen, Germany). HPLC-MS spectra were recorded using a Finnigan LTQ (Thermo Electron Company, Waltham, Massachusetts, USA) MS in APCI mode and a Purospher STAR RP18 column (250 mm × 2 mm, 5 μm). Infrared spectra were recorded using a Bruker Equinox 55 FTIR spectrophotometer over the 700–4000 cm^{-1} range with a spectral resolution of 2 cm^{-1} using the transmission mode. The optical rotation was measured with a JASCO P1030 polarimeter at 22–24 °C at a specific wavelength of 589 nm. High resolution mass spectra (HR-MS) were either obtained using a Hewlett Packgard 6890 GC operated with a Phenomenex ZBSHT column (30 m × 0.25 m, 0.25 μm) and connected to a Masspec MS02 (Mircomass, UK) (EI, 70 eV) or a QExactive Plus MS (Thermo Fischer Scientific, Bremen, Germany) equipped with an RP-18 column (150 mm × 2.1 mm, 120 Å) or *via* direct injection into the ESI source (capillary temp.: 275 °C). For preparative column chromatography silica gel (40–63 μm, Macherey-Nagel, Düren,

Germany) or Lichroprep RP-18 silica gel (40–63 μm, Merck, Darmstadt, Germany) was used. Thin layer chromatography was conducted on silica gel 60 F_{254} aluminum sheets (Merck, Darmstadt, Germany). The compounds were detected using Hanessian's stain, vanillin or potassium permanganate stain. Except for diethyl ether all solvents purchased were of HPLC grade and used without further purification. Diethyl ether was distilled prior to use. Anhydrous solvents were purchased as such. Horse liver esterase was obtained as lyophilized powder (0.5–1.0 U mg^{-1}, Sigma Aldrich, Saint Louis, MO, USA).

Isopropyl (9*Z*)-17-hydroxyoctadec-9-enoate (*rac*-6). A cold solution (−78 °C) of 9-methyloxonan-2-one (*rac*-**5**) (78 mg, 0.5 mmol) in dry toluene (2.7 ml) was treated within 10 min with DiBAlH (0.6 ml, 0.6 mmol, 1 M in hexane). Stirring was continued for an additional 40 minutes and the excess of DiBAlH was quenched by addition of dry MeOH (5 μl). Next, the cold solution was transferred into a cold solution (−78 °C) of the phosphorus ylide, prepared from 9-isopropoxy-9-oxononyl triphenylphosphonium bromide (**4**) (325 mg, 0.6 mmol) in dry THF (3.5 ml) and KHMDS (120 mg, 0.6 mmol). The reaction mixture was warmed to room temperature over 1 hour. Stirring was continued for 1 hour and the mixture was hydrolyzed by the addition of 5% HCl (2.6 ml). The layers were separated and the aqueous phase was extracted with Et_2O (2 × 5 ml). The combined organic extracts were washed with 5% HCl (20 ml), a saturated $NaHCO_3$ solution (20 ml) and water (20 ml). After removal of the solvents under reduced pressure, the crude product was purified by column chromatography on silica gel using hexane–ethyl acetate (5 : 1) for elution. Removal of the solvents yielded *rac*-**6** as a colorless oil (49 mg, 0.13 mmol, 26%, (*Z*)/(*E*) > 98/2; ylide preparation with *n*-BuLi: 29%, (*Z*)/(*E*) 80/20). R_f = 0.18 (hexane–ethyl acetate 5 : 1).

HRMS *m*/*z* calcd for $C_{21}H_{40}O_3$ 340.2978 $[M]^+$, found 340.2985; IR (thin film, cm^{-1}) ν 3437 (br, m), 2977 (m), 2964 (m), 2928 (s), 2855 (s), 1733 (s), 1655 (w), 1464 (m), 1374 (m), 1249 (m), 1181 (m), 1145 (m), 1110 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40–5.31 (m, 2 H, H-10/H-9), 5.01 (sp, $^3J_{1',2'} = {}^3J_{1',2''}$ = 6.2 Hz, 1 H, H-1′), 3.80 (tq, $^3J_{17,18} = {}^3J_{17,16}$ = 6.1 Hz, 1 H, H-17), 2.26 (t, $^3J_{2,3}$ = 7.7 Hz, 2 H, H-2), 2.04–1.96 (m, 4 H, H-11/H-8), 1.61 (m_c, 2 H, H-3), 1.48–1.38 (m, 2 H, H-16), 1.36–1.27 (m, 16 H, H-15–H-12/H-7–H-4), 1.23 (d, $^3J_{2',1'} = {}^3J_{2'',1'}$ = 6.3 Hz, 6 H, H-2′/H-2″), 1.19 (d, $^3J_{18,17}$ = 6.2 Hz, 3 H, H-18); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4 (C-1), 129.8 (C-10/C-9), 68.1 (C-17), 67.3 (C-1′), 39.4 (C-16), 34.7 (C-2), 29.7, 29.5, 29.2, 29.1, 29.1, 25.7 (C-15–C-12/C-7–C-4), 27.1 (C-11/C-8), 25.0 (C-3), 23.5 (C-18), 21.8 (C-2′/C-2″).

(17*S*,9*Z*)-17-Hydroxyoctadec-9-enoate ((*S*)-6). It is prepared from (9*S*)-9-methyloxonan-2-one ((*S*)-**5**) (750 mg, 4.80 mmol) as described. After purification, (*S*)-**6** was obtained as a colorless oil (366 mg, 1.08 mmol, 23%). $[\alpha]_D^{22}$ +2.9 (*c* 1.00, $CHCl_3$). All other spectral data were identical to *rac*-**6**.

(9*Z*)-Octadec-9-ene-1,17-diol (*rac*-3). $LiAlH_4$ (99 mg, 2.62 mmol) was suspended at 0 °C in dry THF (7.5 ml) and the ester *rac*-**6** (297 mg, 0.87 mmol) was added slowly. The reaction mixture was allowed to come to room temperature and stirred for 4.5 hours. Et_2O (9 ml) and water (10 ml) were added, the phases were separated and the aqueous layer was extracted with Et_2O (3 × 10 ml). The combined organic extracts were washed with water (30 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel using hexane–ethyl acetate (1 : 1) for elution. After removal of the solvents, *rac*-**3** was obtained as a slightly yellow oil (243 mg, 0.85 mmol, 98%). R_f = 0.35 (hexane–ethyl acetate 1 : 1).

HRMS *m*/*z* calcd for $C_{18}H_{36}O_2$ 284.2715 $[M]^+$, found 284.2705; IR (thin film, cm^{-1}) ν 3332 (br, m), 3004 (w), 2926 (s), 2854 (s), 1654 (w), 1462 (m), 1373 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40–5.30 (m, 2 H, H-10/H-9), 3.79 (tq, $^3J_{17,18} = {}^3J_{17,16}$ = 5.9 Hz, 1 H, H-17), 3.63 (t, $^3J_{1,2}$ = 6.6 Hz, 2 H, H-1), 2.06–1.95 (m, 4 H, H-11/H-8), 1.95–1.80 (br, 2 H, O–H), 1.56 (tt, $^3J_{2,3} = {}^3J_{2,1}$ = 6.7 Hz, 2 H, H-2), 1.49–1.38 (m, 2 H, H-16), 1.38–1.25 (m, 18 H, H-14–H-12/H-7–H-4), 1.18 (d, $^3J_{18,17}$ = 6.0 Hz, 3 H, H-18). ^{13}C NMR (100 MHz, $CDCl_3$) δ 129.9, 129.8 (C-10/C-9), 68.2 (C-17), 63.0 (C-1), 39.3 (C-16), 32.7 (C-2), 29.7, 29.7, 29.5, 29.4, 29.4, 29.2, 29.1 (C-14–C-12/C-7–C-4), 27.1 (C-11/C-8), 25.7 (C-15/C-3), 23.4 (C-18).

(17*S*,9*Z*)-Octadec-9-ene-1,17-diol ((*S*)-3). It is prepared from (17*S*,9*Z*)-17-hydroxyoctadec-9-enoate ((*S*)-**6**) (295 mg, 0.87 mmol). After column chromatography (*S*)-**3** was obtained as a colorless oil (245 mg, 0.86 mmol, 98%). $[\alpha]_D^{22}$ +3.8 (*c* 1.00, $CHCl_3$). All other spectral data were identical to *rac*-**3**.

(9*Z*)-1,17-Diazidooctadec-9-ene (*rac*-7). (9*Z*)-Octadec-9-ene-1,17-diol (*rac*-**3**) (218 mg, 0.77 mmol) was dissolved in dry THF (13 ml) and cooled to 0 °C. PPh_3 (836 mg, 3.19 mmol), DPPA (0.97 ml, 4.51 mmol) and DIAD (0.85 ml, 4.29 mmol) were successively added with stirring and the reaction mixture was allowed to warm to room temperature. After 4 hours the solvent was removed and the reaction product was purified by column chromatography on silica gel using hexane–DCM (3 : 1) for elution. Removal of solvents afforded *rac*-**7** as a colorless oil (195 mg, 0.58 mmol, 76%). R_f = 0.30 (hexane–DCM 3 : 1).

HRMS *m*/*z* calcd for $C_{18}H_{34}N_6Na$ 357.2737 $[M + Na]^+$, found 357.2737; IR (thin film, cm^{-1}) ν 3004 (w), 2928 (s), 2855 (s), 2094 (s), 1653 (w), 1459 (m), 1378 (w), 1249 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.42–5.30 (m, 2 H, H-10/H-9), 3.42 (tq, $^3J_{17,18} = {}^3J_{17,16}$ = 6.5 Hz, 1 H, H-17), 3.26 (t, $^3J_{1,2}$ = 7.0 Hz, 2 H, H-1), 2.07–1.94 (m, 4 H, H-11/H-8), 1.61 (tt, $^3J_{2,3} = {}^3J_{2,1}$ = 7.0 Hz, 2 H, H-2), 1.57–1.28 (m, 20 H, H-16–H-12/H-7–H-3), 1.25 (d, $^3J_{18,17}$ = 6.6 Hz, 3 H, H-18). ^{13}C NMR (100 MHz, $CDCl_3$) δ 129.9, 129.8 (C-10/C-9), 58.0 (C-17), 51.5 (C-1), 36.2 (C-16), 29.7, 29.6, 29.4, 29.3, 29.2, 29.1, 29.1 (C-14–C-12/C-7–C-4), 28.8 (C-2), 27.2, 27.1 (C-11/C-8), 26.7 (C-3), 26.1 (C-15), 19.5 (C-18).

(17*R*,9*Z*)-1,17-Diazidooctadec-9-ene ((*R*)-7). It is prepared from (17*S*,9*Z*)-octadec-9-ene-1,17-diol ((*S*)-**3**) (200 mg, 0.70 mmol). Purification by column chromatography afforded (*R*)-**7** as a colorless oil (189 mg, 0.57 mmol, 81%). $[\alpha]_D^{22}$ −19.7 (*c* 1.06, $CHCl_3$). All other spectral data were identical to *rac*-**7**.

(9*Z*)-Octadec-9-ene-1,17-diamine (*rac*-1). $LiAlH_4$ (177 mg, 3.95 mmol) was suspended with stirring in dry THF (11 ml) and cooled to 0 °C. A solution of (9*Z*)-1,17-diazidooctadec-9-ene (*rac*-**7**) (165 mg, 0.49 mmol) in dry THF (5 ml) was added

dropwise and the suspension was warmed to room temperature. After stirring for 4 hours, water (30 ml) and $CHCl_3$ (30 ml) were added, the layers were separated and the aqueous phase was extracted with $CHCl_3$ (6 × 30 ml). The organic extracts were combined and solvents were removed under reduced pressure. The product was partitioned between MTBE (80 ml) and 2% HCl (80 ml). The layers were separated, the aqueous phase was carefully extracted with MTBE (3 × 20 ml) and the combined organic extracts were washed with 2% HCl (30 ml). The aqueous solutions were combined and MTBE (40 ml) and NH_3 (25% aqueous solution, 80 ml) were added. After separation of the two layers, the aqueous phase was extracted with MTBE (3 × 60 ml). The combined organic extracts were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure to yield *rac*-**1** as a colorless oil (139 mg, 0.49 mmol, quant.).

HRMS *m*/*z* calcd for $C_{18}H_{39}N_2$ 283.3108 $[M + H]^+$, found 283.3106; IR (thin film, cm^{-1}) ν 3320 (m), 3004 (w), 2921 (s), 2851 (s), 1639 (w), 1564 (m), 1468 (m), 1430 (w), 1390 (m), 1331 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.38–5.27 (m, 2 H, H-10/H-9), 2.85 (tq, $^3J_{17,18} = {}^3J_{17,16}$ = 6.0 Hz, 1 H, H-17), 2.66 (t, $^3J_{1,2}$ = 7.0 Hz, 2 H, H-1), 2.04–1.91 (m, 4 H, H-11/H-8), 1.50–1.38 (m, 6 H, H-2, N–H_2), 1.36–1.21 (m, 20 H, H-16–H-12/H-7–H-3), 1.03 (d, $^3J_{18,17}$ = 6.2 Hz, 3 H, H-18). ^{13}C NMR (100 MHz, $CDCl_3$) δ 129.8, 129.8 (C10/C-9), 46.9 (C-17), 42.1 (C-1), 40.1 (C-16), 33.7 (C-2), 29.7, 29.6, 29.6, 29.4, 29.4, 29.2, 29.2 (C-14–C-12/C-7–C-4), 27.1 (C11/C-8), 26.8 (C-3), 26.3 (C-15), 23.8 (C-18).

(17*R*,9*Z*)-Octadec-9-ene-1,17-diamine ((*R*)-1) (harmonine). It is prepared from (17*R*,9*Z*)-1,17-diazidooctadec-9-ene ((*R*)-**7**) (161 mg, 0.48 mmol). Harmonine ((*R*)-**1**) was obtained as a slightly yellow oil (135 mg, 0.48 mmol, 99%, ee > 97%). $[\alpha]_D^{24}$ −3.4 (*c* 1.04, C_6H_6). Spectroscopic data were identical to *rac*-**1**.

(9*Z*)-17-Hydroxyoctadec-9-enamide (*rac*-8). Isopropyl (9*Z*)-17-hydroxyoctadec-9-enoate (*rac*-**6**) (237 mg, 0.70 mmol) was dissolved at 0 °C in MeOH (2.25 ml). Mg_3N_2 (353 mg, 3.48 mmol) was quickly added in one portion, the reaction vessel was thoroughly closed and the suspension was allowed to warm to room temperature. Stirring was continued for 1 hour. The reaction was heated to 80 °C and stirring was continued for 24.5 hours. After cooling to room temperature, $CHCl_3$ (25 ml) and water (25 ml) were added. The aqueous phase was neutralized with 3 M HCl, the layers were separated and the aqueous phase was extracted with $CHCl_3$ (2 × 25 ml). The organic layers were combined and the solvent was removed under reduced pressure. The residue was purified by column chromatography on RP-18 silica gel using H_2O–MeOH (1 : 9) for elution. $CHCl_3$ was added and the solvents were removed under reduced pressure. This procedure was repeated three times with $CHCl_3$ and three times with benzene to remove the last traces of water. The residue was concentrated under high vacuum to yield *rac*-**8** as a colorless, sticky oil. (182 mg, 0.61 mmol, 87%). R_f = 0.43 (H_2O–MeOH 1 : 9, RP-18).

HRMS *m*/*z* calcd for $C_{18}H_{36}NO_2$ 298.2741 $[M + H]^+$, found 298.2734; IR (thin film, cm^{-1}) ν 3358 (s), 3191 (br, m), 3003 (w), 2967 (w), 2924 (s), 2852 (s), 1703 (w), 1659 (s), 1633 (s), 1468 (m), 1423 (m), 1412 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.68 (d, $^2J_{NH,NH'}$ = 81.9 Hz, 2 H, N–H_2), 5.40–5.27 (m, 2 H, H-10/H-9), 3.78 (tq, $^3J_{17,18} = {}^3J_{17,16}$ = 5.7 Hz, 1 H, H-17), 2.20 (t, $^3J_{2,3}$ = 7.5 Hz, 2 H, H-2), 2.05–1.92 (m, 4 H, H-11/H-8), 1.84 (brs, 1 H, O–H), 1.62 (tt, $^3J_{3,4} = {}^3J_{3,2}$ = 7.1 Hz, 2 H, H-3), 1.51–1.37 (m, 2 H, H-16), 1.37–1.23 (m, 16 H, H-15–H-12/H-7–H-4), 1.17 (d, $^3J_{18,17}$ = 6.1 Hz, 3 H, H-18). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.8 (C-1), 130.3, 130.3 (C-10/C-9 (*E*)), 129.9, 129.8 (C-10/C-9), 68.0 (C-17), 39.3 (C-16), 35.9 (C-2), 32.5, 32.4 (C-11/C-8 (*E*)), 29.6, 29.6, 29.5, 29.5, 29.2, 29.2, 29.0 (C-14–C-12/C-7–C-4), 27.1, 27.1 (C-11/C-8), 25.7 (C-15), 25.5 (C-3), 23.4 (C-18).

(9*Z*)-18-Aminooctadec-9-en-2-ol (*rac*-2). $LiAlH_4$ (106 mg, 3.00 mmol) was suspended with stirring in dry THF (3 ml) and cooled to 0 °C. (9*Z*)-17-Hydroxyoctadec-9-enamide (*rac*-**8**) (168 mg, 0.56 mmol), dissolved in dry THF (2 ml), was added dropwise. The reaction was allowed to warm to room temperature, stirred for 4 hours at room temperature and for 19.5 hours at 45 °C. After cooling to room temperature, water (10 ml) and $CHCl_3$ (10 ml) were added. The layers were separated and the aqueous phase was extracted with $CHCl_3$ (5 × 10 ml). The organic extracts were combined and the solvents were removed under reduced pressure. The product was partitioned between MTBE (80 ml) and 2% HCl (80 ml). The aqueous phase was extracted with MTBE (3 × 20 ml) and the combined organic extracts were washed with 2% HCl (35 ml). Both aqueous solutions were combined and MTBE (50 ml) and NH_3 (25% aqueous solution, 80 ml) were added. The layers were separated and the aqueous phase was extracted with MTBE (3 × 80 ml). The combined organic extracts were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure yielding *rac*-**2** as oily, colorless wax (151 mg, 0.53 mmol, 95%).

HRMS *m*/*z* calcd for $C_{18}H_{38}NO$ 284.2948 $[M + H]^+$, found 284.2935; IR (thin film, cm^{-1}) ν 3332 (br, m), 3004 (m), 2925 (s), 2853 (s), 1632 (m), 1572 (m), 1464 (m), 1372 (m), 1315 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.38–5.28 (m, 2 H, H-10/H-9), 3.75 (tq, $^3J_{17,18} = {}^3J_{17,16}$ = 6.1 Hz, 1 H, H-17), 2.66 (t, $^3J_{1,2}$ = 7.0 Hz, 2 H, H-1), 2.04–1.91 (m, 4 H, H-11/H-8), 1.82–1.76 (m, 3 H, N–H_2, O–H), 1.48–1.35 (m, 5 H, H-16/H-15a/H-2), 1.35–1.23 (m, 17 H, H-15b/H-14–H-12/H-7–H-3), 1.15 (d, $^3J_{18,17}$ = 6.1 Hz, 3 H, H-18). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 130.3, 130.2 (C-10/C-9 (*E*)), 129.8, 129.8 (C-10/C-9), 67.7 (C-17 (*E*)), 67.7 (C-17), 42.1 (C-1), 39.4 (C-16), 33.6 (C-2 (*E*)), 33.6 (C-2), 32.5 (C-11/C-8 (*E*)), 29.6, 29.6, 29.5, 29.4, 29.4, 29.2, 29.1, (C-14–C-12/C-7–C-4), 27.1 (C-11/C-8), 26.8 (C-3), 26.8 (C-3 (*E*)), 25.7 (C-15), 23.4 (C-18).

Enzymatic hydrolysis of lactone (*rac*-5)

Racemic 9-methyloxonan-2-one (*rac*-**5**) (3.50 g, 22.4 mmol) was suspended in NaH_2PO_4 buffer (0.1 M, pH = 7.2, 100 ml) and stirred for 15 minutes. Then horse liver esterase (350 mg, lyophilized powder) was added and the pH was kept constant by dropwise addition of 0.5 M NaOH during the whole reaction time. After 12 hours, another portion of horse liver esterase (70 mg) was added and stirring was continued for 4 hours. Then Celite (3.50 g) and ice (7.00 g) were added, the suspension was stirred for 5 minutes and the solids were filtered off.

The filter cake was washed with Et_2O (2 × 50 ml). The layers of the filtrate were separated and the aqueous phase was extracted with Et_2O (3 × 50 ml). The combined organic extracts were washed with a saturated $NaHCO_3$ solution (100 ml) and dried over Na_2SO_4. Evaporation of the solvent yielded (9*S*)-9-methyloxonan-2-one ((*S*)-**5**) as a colorless liquid.

(9*S*)-9-Methyl-oxonan-2-one ((*S*)-5). Yield: 1.51 g (9.67 mmol, 86% of (*S*)-enantiomer, ee ≥ 99.5%). $[\alpha]_D^{23}$ +31.8 (*c* 1.00, THF).

HRMS *m*/*z* calcd for $C_9H_{16}O_2$ 156.1150 $[M]^+$, found 156.1152; IR (thin film, cm^{-1}) ν 2929 (s), 2857 (m), 1726 (s), 1575 (w), 1464 (w), 1427 (w), 1377 (w), 1286 (m), 1254 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.07 (m_c, 1 H, H-8), 2.25 (m_c, 2 H, H-2), 1.93 (m_c, 1 H, H-4a), 1.82–1.74 (m, 1 H, H-7a), 1.72–1.64 (m, 1 H, H-3a), 1.67–1.55 (m, 2 H, H-6a/H-3b), 1.55–1.43 (m, 3 H, H-7b/H-5a/H-4b), 1.37–1.27 (m, 2 H, H-6b/H-5b), 1.25 (d, $^3J_{9,8}$ = 6.5 Hz, 3 H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5 (C-1), 71.5 (C-8), 35.7 (C-2), 35.1 (C-7), 29.4 (C-5), 25.0 (C-4), 23.9 (C-3), 21.8 (C-6), 20.7 (C-9).

(8*R*)-8-Hydroxynonanoic acid ((*R*)-9). The water phase was covered with Et_2O (100 ml) and 2 M HCl (60 ml) was added. The layers were separated and the aqueous phase was extracted with Et_2O (3 × 100 ml). The combined organic extracts were washed with a saturated NaCl solution (150 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure to yield (8*R*)-8-hydroxynonanoic acid ((*R*)-**9**) as colorless wax (1.52 g, 8.72 mmol, 78% of (*R*)-enantiomer, ee ≥ 99%). $[\alpha]_D^{23}$ −8.5 (*c* 1.00, THF).

HRMS *m*/*z* calcd for $C_9H_{18}O_3Na$ 197.1148 $[M + Na]^+$, found 197.1146; IR (thin film, cm^{-1}) ν 3391 (br, s), 2933 (s), 2858 (s), 1711 (s), 1462 (m), 1410 (m), 1376 (m), 1261 (m), 1127 (m), 1103 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.25 (br, 2 H, O–H, COO–H), 3.81 (tq, $^3J_{8,9}$ = $^3J_{8,7}$ = 6.1 Hz, 1 H, H-8), 2.36 (t, $^3J_{2,3}$ = 7.5 Hz, 2 H, H-2), 1.65 (tt, $^3J_{3,4}$ = $^3J_{3,2}$ = 7.5 Hz, 2 H, H-3), 1.50–1.40 (m, 3 H, H-7/H-6a), 1.40–1.30 (m, 5 H, H-6b/H-5/H-4), 1.20 (d, $^3J_{9,8}$ = 6.1 Hz, 3 H, H-9); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 179.0 (C-1), 68.2 (C-8), 39.2 (C-7), 33.9 (C-2), 29.2, 29.0 (C-5/C-4), 25.5 (C-6), 24.6 (C-3), 23.5 (C-9).

Isopropyl 9-bromononanoate (10). 9-Bromononanoic acid (5.2 g, 21.92 mmol) was dissolved in dry 2-propanol (50 ml) and sulfuric acid (1 ml) was added. The flask was equipped with Dean-Stark apparatus and the solution was heated to reflux for 19 hours. After the solution was cooled down to room temperature, 2-propanol was removed under reduced pressure, the residue was dissolved in Et_2O (20 ml) and the organic solution was washed with a saturated Na_2CO_3 solution (20 ml), distilled water (20 ml) and a saturated NaCl solution (20 ml). The ethereal solution was dried over $MgSO_4$, the solvent was removed and the crude product was purified by column chromatography on silica gel (DCM). After removal of the solvent, **10** was obtained as a colorless oil (5.23 g, 18.73 mmol, 86%). R_f = 0.70 (DCM).

HRMS *m*/*z* calcd for $C_{12}H_{23}O_2BrNa$, $C_{12}H_{23}O_2{}^{81}BrNa$ 301.0774, 303.0753 $[M + Na]^+$, found 301.0776, 303.0753; IR (thin film, cm^{-1}) ν 2979 (m), 2932 (s), 2857 (m), 1731 (s), 1466 (m), 1374 (m), 1256 (m), 1181 (m), 1145 (w), 1110 (s), 964 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.00 (sp, $^3J_{1',2'}$ = $^3J_{1',2''}$ = 6.2 Hz, 1 H, H-1′), 3.40 (t, $^3J_{9,8}$ = 6.9 Hz, 2 H, H-9), 2.26 (t, $^3J_{2,3}$ = 7.6 Hz, 2 H, H-2), 1.85 (m_c, 2 H, H-8), 1.61 (m_c, 2 H, H-3), 1.42 (m_c, 2 H, H-7), 1.34–1.28 (m, 6 H, H-6–H-4), 1.22 (d, $^3J_{2',1'}$ = $^3J_{2'',1'}$ = 6.2 Hz, 6 H, H-2′/H-2″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3 (C-1), 67.3 (C-1′), 34.6 (C-2), 33.9 (C-9), 32.7 (C-8), 29.0, 29.0 (C-5/C-4), 28.5 (C-6), 28.1 (C-7), 24.9 (C-3), 21.8 (C-2′, C-2″).

(9-Isopropoxy-9-oxononyl)triphenylphosphonium bromide (4). Isopropyl 9-bromononanoate (**10**) (2.89 g, 10.35 mmol) was dissolved in dry toluene (65 ml) and PPh_3 (2.71 g, 10.35 mmol) was added. The solution was refluxed for 24 hours under argon, cooled to room temperature and the supernatant was transferred to a new flask. There again PPh_3 (1.36 g, 5.18 mmol) was added and the solution was stirred under reflux for 3 days. After cooling to room temperature, the supernatant was discarded and both bottom layers were combined and purified by column chromatography on silica gel (DCM–MeOH 14 : 1). The solvents were removed under reduced pressure and **4** was obtained as a sticky syrup (4.33 g, 8.00 mmol, 77%). R_f = 0.51 (DCM–MeOH 14 : 1).

HRMS *m*/*z* calcd for $C_{30}H_{38}O_2P$ 461.2604 $[M]^+$, found 461.2601; IR (thin film, cm^{-1}) ν 3057 (w), 2977 (w), 2855 (s), 2802 (w), 1720 (s), 1587 (w), 1522 (w), 1485 (m), 1104 (m), 856 (s), 842 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88–7.76 (m, 9 H, H_{ar}-6/H_{ar}-2/H_{ar}-4), 7.74–7.67 (m, H_{ar}-5/H_{ar}-3), 4.96 (sp, $^3J_{1',2'}$ = $^3J_{1',2''}$ = 6.2 Hz, 1 H, H-1′), 3.82–3.71 (m, 2 H, H-9), 2.20 (t, $^3J_{2,3}$ = 7.5 Hz, 2 H, H-2), 1.68–1.56 (m, 4 H, H-8/H-7), 1.53 (m_c, 2 H, H-3), 1.30–1.21 (m, 6 H, H-6–H-4), 1.20 (d, $^3J_{2',1'}$ = $^3J_{2'',1'}$ = 6.2 Hz, 6 H, H-2″/H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3 (C-1), 135.0, 135.0 (C_{ar}-4), 133.7, 133.6 (C_{ar}-6/C_{ar}-2), 130.5, 130.4 (C_{ar}-5/C_{ar}-3), 118.8, 118.0 (C_{ar}-1), 67.3 (C-1′), 34.6 (C-2), 30.3, 30.2 (C-7), 28.9, 28.9, 28.7 (C-6–C-4), 24.8 (C-3), 23.0, 22.6 (C-9), 22.6, 22.5 (C-8), 21.8 (C-2′/C-2″).

Antileishmanial activity

The virulent *L. major* isolate (MHOM/IL/81/FE/BNI) and Luciferase-transgenic (Luc-tg.) *L. major* were maintained by continuous passage in female BALB/c mice. *L. major* amastigotes were isolated from lesions as described previously[33,34] and promastigotes were grown *in vitro* in blood-agar cultures at 27 °C, 5% CO2, and 95% humidity. AlamarBlue® assays for the determination of antileishmanial activities against *L. major* promastigotes and BMDM cytotoxicity, and Britelite™ plus (PerkinElmer, Waltham, MA, USA) assays against intracellular Luc-tg. *L. major* amastigotes were performed as previously reported.[34]

Diff-Quick staining for transmitted light microscopy

After incubation for 6 h, 10 h, and 24 h in the presence of 30 μM harmonine or 1% DMSO as the solvent control, *L. major* promastigotes were harvested and centrifuged using a Cytospin 3 centrifuge (Shandon, Frankfurt, Germany) on microscopic slides. Cytospin preparations were stained using the Differential Quick stain (Diff-Quick) dye (Medion Diagnostics AG, Duedingen, Switzerland), according to the manufacturer's protocol. Diff-Quik stains the leishmanial nuclei, the kinetoplasts dark purple and the cytoplasm light purple allowing the

observation of phenotypic changes within the parasite. The stained cells were analyzed by transmitted light microscopy under a 50× objective on a Nikon ECLIPSE 50*i* microscope equipped with a digital camera (Nikon, Tokyo, Japan). The images were processed using NIS Elements D software (Nikon).

Determination of the cell death phenotype by flow cytometric analysis

L. major promastigotes were either treated for 6 h, 10 h, and 24 h with 30 μM harmonine or 1% DMSO as the solvent control. Cell staining was performed using an Annexin V-FITC Apoptosis detection kit (Sigma-Aldrich, Saint Louis, MO, USA) according to the manufacturer's protocol. The stained samples were immediately analyzed by flow cytometry using a MACS Quant Analyzer (Miltenyi Biotech, Bergisch Gladbach, Germany).

Acknowledgements

We thank Dr Christian Paetz (MPI CE, Jena) for recording the ^{19}F-NMR spectra and Sybille Lorenz (MPI CE, Jena) for HR-MS measurements. Furthermore, the authors thank Martina Schultheis (Institute for Molecular Infection Biology, Wuerzburg) for the determination of IC_{50} and cytotoxicity values, and excellent technical support. This work was supported and financed by the Max Planck Society and by a grant of the Deutsche Forschungsgemeinschaft (DFG), Collaborative Research Center 630 (SFB 630/TP B3), "Recognition, Preparation and Functional Analysis of Agents against Infectious Diseases".

References

1 R. L. Koch, *J. Insect Sci.*, 2003, **3**, 32.
2 H. Roy and E. Wajnberg, *BioControl*, 2008, **53**, 1.
3 H. Roy, P. J. Brown, P. Rothery, R. Ware and M. N. Majerus, *BioControl*, 2008, **53**, 265.
4 S. Al Abassi, M. A. Birkett, J. Pettersson, J. A. Pickett and C. M. Woodcock, *CMLS, Cell. Mol. Life Sci.*, 1998, **54**, 876.
5 T. L. Galvan, S. Kells and W. D. Hutchison, *J. Agric. Food Chem.*, 2008, **56**, 1065.
6 D. Daloze, J.-C. Braekman and J. Pasteels, *Chemoecology*, 1994, **5–6**, 173.
7 C. R. Röhrich, C. J. Ngwa, J. Wiesner, H. Schmidtberg, T. Degenkolb, C. Kollewe, R. Fischer, G. Pradel and A. Vilcinskas, *Biol. Lett.*, 2012, **8**, 308.
8 N. C. Alam, I. S. Choi, K.-S. Song, J.-K. Hong, C. O. Lee and J. H. Jung, *Bull. Korean Chem. Soc.*, 2002, **23**, 497.
9 A. Vilcinskas, K. Stoecker, H. Schmidtberg, C. R. Röhrich and H. Vogel, *Science*, 2013, **340**, 862.
10 A. Vilcinskas, H. Schmidtberg, A. Estoup, A. Tayeh, B. Facon and H. Vogel, *Insect Sci.*, 2014, DOI: 10.1111/1744-7917.12159.
11 A. Vilcinskas and H. Schmidtberg, *Biol. Unserer Zeit*, 2014, **44**, 386.
12 M. F. Braconnier, J. C. Braekman and D. Daloze, *Bull. Soc. Chim. Belg.*, 1985, **94**, 605.
13 D. Enders and D. Bartzen, *Liebigs Ann. Chem.*, 1991, **1991**, 569.
14 S. Chandra Philkhana, P. Dhasaiyan, B. L. V. Prasad and D. S. Reddy, *RSC Adv.*, 2014, **4**, 30923.
15 J. Alvar, I. D. Velez, C. Bern, M. Herrero, P. Desjeux, J. Cano, J. Jannin and M. den Boer, *PLoS One*, 2012, **7**, e35671.
16 World Health Organization, 2014.
17 S. L. Croft, S. Sundar and A. H. Fairlamb, *Clin. Microbiol. Rev.*, 2006, **19**, 111.
18 H. J. de Vries, S. H. Reedijk and H. D. Schallig, *Am. J. Clin. Dermatol.*, 2015, **16**, 99–109.
19 E. Haulotte, P. Laurent and J.-C. Braekman, *Eur. J. Org. Chem.*, 2012, **2012**, 1907.
20 J. van Buijtenen, B. A. C. van As, M. Verbruggen, L. Roumen, J. A. J. M. Vekemans, K. Pieterse, P. A. J. Hilbers, L. A. Hulshof, A. R. A. Palmans and E. W. Meijer, *J. Am. Chem. Soc.*, 2007, **129**, 7393.
21 Y. Yang, M. R. Mannion, L. N. Dawe, C. M. Kraml, R. A. Pascal and G. J. Bodwell, *J. Org. Chem.*, 2011, **77**, 57.
22 S. H. Woo, S. Frechette, E. A. Khalil, G. Bouchain, A. Vaisburg, N. Bernstein, O. Moradei, S. Leit, M. Allan, M. Fournel, M.-C. Trachy-Bourget, Z. Li, J. M. Besterman and D. Delorme, *J. Med. Chem.*, 2002, **45**, 2877.
23 T. Ooi, Y. Uematsu and K. Maruoka, *J. Org. Chem.*, 2003, **68**, 4576.
24 L. Jiao, E. Herdtweck and T. Bach, *J. Am. Chem. Soc.*, 2012, **134**, 14563.
25 Z. Csíki and P. Fügedi, *Tetrahedron*, 2010, **66**, 7821.
26 E. Fouque and G. Rousseau, *Synthesis*, 1989, 661.
27 J. A. Dale and H. S. Mosher, *J. Am. Chem. Soc.*, 1973, **95**, 512.
28 G. R. Sullivan, J. A. Dale and H. S. Mosher, *J. Org. Chem.*, 1973, **38**, 2143.
29 J. Glaser, M. Schultheis, S. Hazra, B. Hazra, H. Moll, U. Schurigt and U. Holzgrabe, *Molecules*, 2014, **19**, 1394.
30 S. Nwaka and A. Hudson, *Nat. Rev. Drug Discovery*, 2006, **5**, 941.
31 I. Vermes, C. Haanen, H. Steffens-Nakken and C. Reutelingsperger, *J. Immunol. Methods*, 1995, **184**, 39.
32 G. Zhang, V. Gurtu, S. R. Kain and G. Yan, *BioTechniques*, 1997, **23**, 525.
33 C. Bogdan, H. Moll, W. Solbach and M. Rollinghoff, *Eur. J. Immunol.*, 1990, **20**, 1131.
34 G. Bringmann, K. Thomale, S. Bischof, C. Schneider, M. Schultheis, T. Schwarz, H. Moll and U. Schurigt, *Antimicrob. Agents Chemother.*, 2013, **57**, 3003.

