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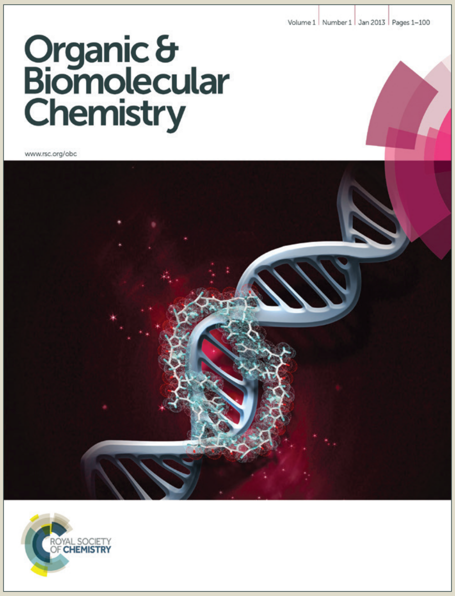
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Synthesis of Maculalactone A and Derivatives for Environmental Fate Tracking Studies

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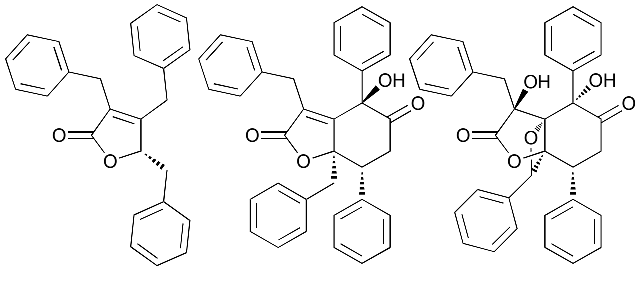
Maculalactone A (**1**) constitutes a promising antifouling agent inhibiting the formation of biofilms in marine and freshwater systems. In this study, we developed a new route based on a late-stage formation of the butenolide core leading to the total synthesis of maculalactone A (three steps, overall yield of 45%) and delivering material on gram scale. In addition, analogues of the title compound were assayed concerning their biological activity utilizing *Artemia franciscana* and *Thamnocephalus platyurus*. The most active analogue was functionalized with a rhodamine B fluorophore and was utilized in an *in vivo* staining experiment in *Artemia salina*. Two different tissues were found to accumulate this maculalactone A derivative.

Introduction

The accumulation of biological material on all surfaces immersed in biological fluids (referred to as biofouling), is a serious challenge for marine vessels, fresh water treatment and industrial plants.[1–5] The ban of previously employed tributyltin coatings by international organizations further aggravated this issue.[6] A bio-inspired approach to address this problem constitutes the use of natural products as antifouling agents.[7,8] In the recent years, marine butenolide natural products attracted interest, as they show good antifouling activity and quorum-sensing inhibition.[9–12] In the context of our own research, we have developed several natural product based approaches to prevent biofouling, ranging from novel algicidal natural products,[13–15] passivated metal oxide surfaces with antifouling properties[16–18] to active surfaces, functionalized with natural products, for controlling or inhibiting bacterial growth.[19,20]

The slowly growing cyanobacterium *Kyrtuthrix maculans* forms pure colonies on rocky shores, which, by the absence of other organisms in these colonies, suggests that some sort of chemical defence could be operating. Based on these observations, the group of Brown isolated the maculalactones, a family of interesting secondary metabolites, from this cyanobacterium (Fig. 1). While maculalactone A (**1**), B, C and L consist of a benzylated γ-lactone, maculalactone D, E, F (**2**), G, H, I, J and K (**3**) include an additional phenylpropanoid unit forming a six membered carbocycle.[21–24] Maculalactone A constitutes the most abundant congener in this series found in *K. maculans*. In addition, Brown *et al.* conducted preliminary antifouling experiments by submerging structures coated with a maculalactone A containing paint in the sea.[25] After 12 weeks, reduced area coverage with bivalves was observed. Although this reduction was partially compensated by an increased grow of algae, it demonstrated the potential of **1** as antifouling agent. However, little is known about the structural requirements or the mode of action of maculalactone A (**1**). In addition, the isolation of larger amounts of **1** from the slow-growing producer is unpractical and the three published total syntheses offer overall yields below 10%.[26–28] In this study, we have developed an efficient preparation of maculalactone A (**1**), have performed a preliminary structure–activity relationship study and have investigated the environmental fate of this compound in model crustaceans via labelled analogues.



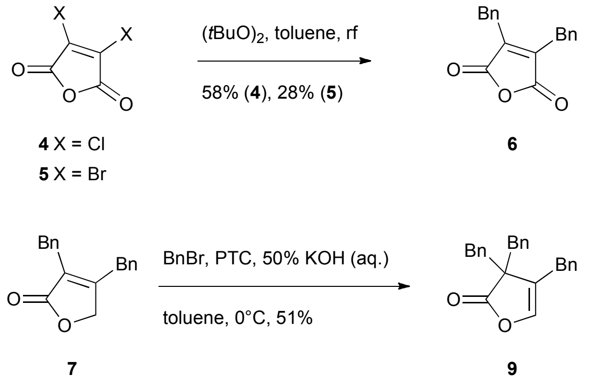
Maculalactone A (**1**) Maculalactone F (**2**) Maculalactone K (**3**)

Fig. 1 Selected maculalactones isolated from *Kyrtuthrix maculans*: Maculalactone A[21], F and K.[22]

Results and Discussion

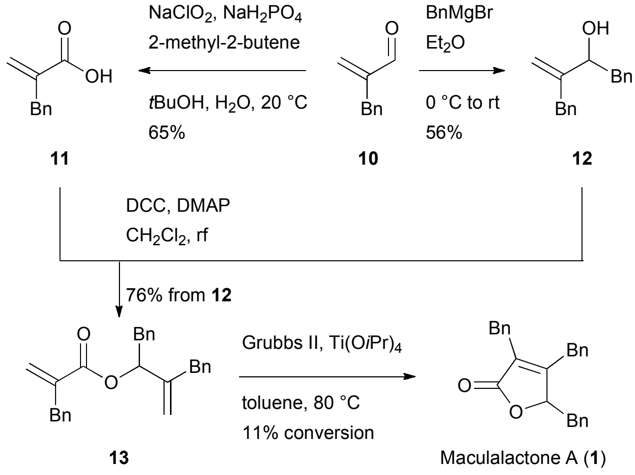
Improved Total Synthesis of Maculalactone A

To access gram-scale amounts of maculalactone A (**1**) and its structural analogues, an improved synthesis had to be developed. The new route should not only result in a higher overall yield, but should also allow for the modification of the different benzyl groups. We first targeted an optimization of the published routes to maculalactone A.[26–28] A survey of those revealed a general problem in the nucleophilic introduction of benzyl groups to the butenolide core. In a first phase, we tried to work around such a nucleophilic benzylation. Messorosh *et al.* recently described the radical benzylation of 2,3-dichloromaleic anhydride (**4**) in good yield.[29] The resulting anhydride **6** is a central intermediate in the synthesis of Brown and Argade.[26,27] However, the dichloride **4** is expensive and difficult to prepare in the laboratory. The corresponding dibromide **5** was synthesised[30] and subjected to the same conditions, giving **6** in 28% yield (Scheme 1). Overall, this route led to no improvement when compared with the known syntheses of **1**.



Scheme 1 Investigated shortcuts in the total syntheses of Brown and Argade *et al.* Reaction from **4** to **6** was previously published by Messorosh *et al.*[29] PTC = *N*-anthrylated quinine chloride synthesised as described by Hofstetter *et al.*[31]

Alternatively, a shortcut in the route of Argade[27] was investigated by an electrophlic substitution at the γ-position of intermediate **7** (Scheme 1). An enantioselective phase-transfer catalyst mediated procedure was expected to give optically enriched maculalactone A. However, the benzyl group was directed to the α-position giving the achiral lactone **9**.

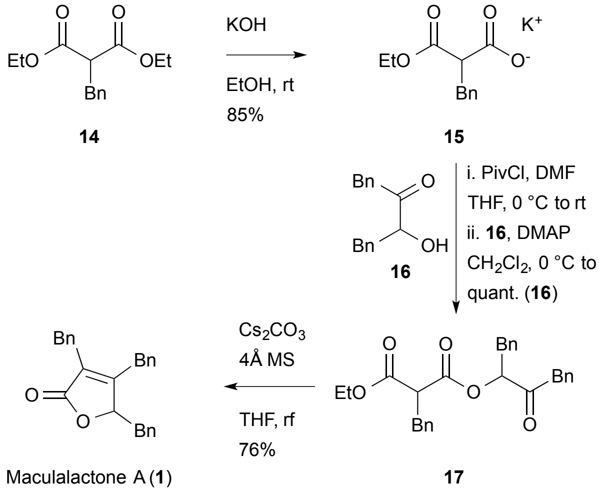


Scheme 2 Synthesis of maculalactone A by a late stage ring closing metathesis.

Since an improvement of the previously published routes did not appear feasible, new strategies were considered. Therefore, we focused on strategies with a late stage butenolide formation, thereby, avoiding benzylic disconnections. Among the various known methods to synthesise butenolides, only few approaches allow substituents in all three positions.[32–34]

Alkene metathesis has been applied for butenolide syntheses by different groups.[35–37] Diene **13** was therefore synthesised in a convergent fashion from the starting material **10**, which was accessed as described by Pihko *et al.*[38] The enal **10** was either converted to acid **11** applying a Pinnick oxidation or benzylated in a 1,2-addition to obtain alcohol **12**. The two parts were combined in a Steglich esterification giving diene **13** in an overall yield of 42 respectively 38%. However, the subsequent metathesis reaction under optimized conditions and in the presence of $Ti(O\textit{i}Pr)_4$[37] resulted in a low conversion of 11%. Despite this disappointing result, this route allowed for the first time the modification of a single benzyl moiety.

Another potentially rewarding strategy would constitute the late stage preparation of the olefinic bond through an elimination approach. To this goal, we investigated a procedure originally reported by Avetisyan *et al.*[39] in 1981 and later utilized in an industrial setting.[40] Key to this strategy would be the intramolecular cyclization of 3,6-dioxo-4-oxa-1-hexanoate derivative **17**, with subsequent loss of ethanol and CO_2 furnishing the desired butenolide (Scheme 3). In order to test this hypothesis, malonate **14** was saponified to the half-ester **15** in a single step[41] and converted to the corresponding anhydride with pivaloyl chloride. In the same pot, the anhydride intermediate was reacted with the known α-hydroxy ketone **16**, which itself is accessible via a Benzoin-type Umpolung reaction,[42] to the corresponding keto-diester **17**.

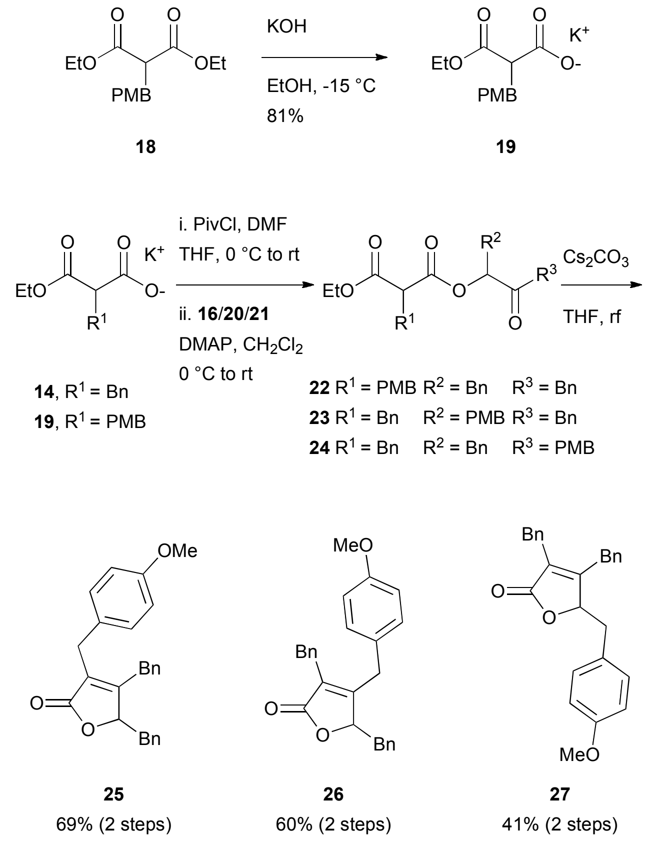


Scheme 3 Synthesis of maculalactone A by a late stage butenolide formation. Synthesis of **15** from **14** as published by Goel *et al.*[41]

The key step, *i.e.* the late stage cyclization of **17** to the butenolide **1**, was addressed next. After optimizing base, solvent and different additives, the cyclization was accomplished with caesium carbonate in refluxing THF, giving maculalactone A (**1**) in a yield of 76%. In order to the drive the reaction to completion, and to prevent alcoholysis of **17** with the formed ethanol, the presence of 4 Å molecular sieves was found to be crucial. Interestingly, the intermolecular version of this reaction led to the formation of product **1**, however, only little conversion could be observed.

We postulate, as a working hypothesis for this interesting reaction, the intramolecular attack of the deprotonated malonate to the ketone, followed by an intramolecular transesterification (liberation of EtOH) and formation of a β-lactone. Subsequent CO_2 release would then result in maculalactone A (**1**). The mechanism was studied by a series of experiments; however, direct mass spectrometric detection of intermediates was not possible. Interestingly, running the reaction in sealed tubes did not lead to product formation. Additional experiments will be necessary to study the mechanism of this transformation.

Based on this short approach, maculalactone A (**1**) was synthesised in an overall yield of 45% in three linear steps from the inexpensive, commercially available starting material **14**. In addition, this strategy allows for the differentiation of the benzyl groups via their sequential regioselective introduction. We took advantage of this approach to access analogues as described below.



Scheme 4 Synthesis of first generation maculalactone A analogues **25–27**. PMB = *para*-methoxybenzyl.

Syntheses of Maculalactone A Derivatives and SAR studies

For further investigations of the mode of action, environmental fate studies and potential applications in antifouling surface coatings, we were interested in modified maculalactone A derivatives. First, however, the appropriate position for modifications had to be identified in a structure–activity relationship study (SAR). We initially proposed the *para*-positions of the respective benzyl groups to have a minimal influence on the biological activity and, therefore, the three structural isomers **25**, **26** and **27**, each bearing a methoxy group in the *para*-position were synthesised (Scheme 4). Malonate **18** was synthesised based on a reported procedure[43] and converted to its potassium salt **19** at reduced temperature to prevent decarboxylation. The differently substituted α-hydroxy ketones Bn–CO–CH(OH)–PMB (**20**) and PMB–CO–CH(OH)–Bn (**21**) were prepared from the corresponding aldehydes via formation of the TMS protected cyanohydrins, followed by the addition of the respective Grignard reagent, as described in the literature.[44,45] The formation of ester **22**–**24** proceeded smoothly as did the ring closure (yields of 40 to 69% over two steps).

We were then interested in profiling the biological activity of maculalactone A and analogs. In the work of Brown *et al.*, the LC_{50} values of extracted maculalactone A were determined in ecotoxicity assays utilizing different stages of *Tetraclita japonica*, *Balanus amphitrite*, *Ibla cumingii* and *Artemia salina,*[25] of which the latter is often used for reference toxicity experiments. For comparison, we evaluated the compounds in the related *Artemia franciscana* as well as the sensitive freshwater crustacean *Thamnocephalus platyurus*, which is well-established in our group.[46,47]

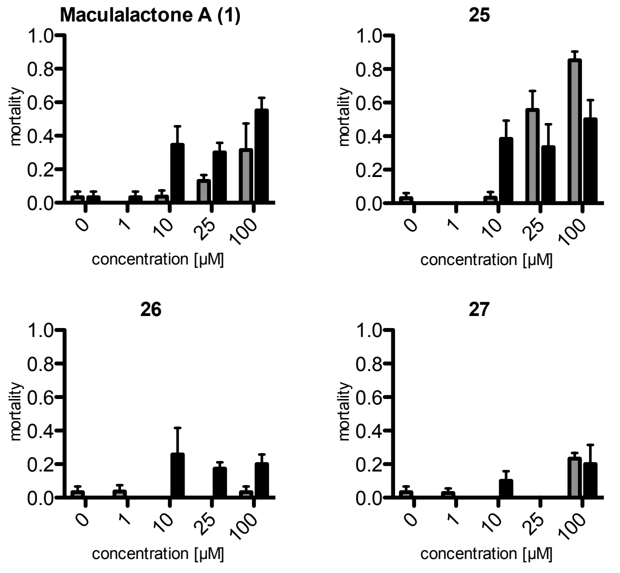
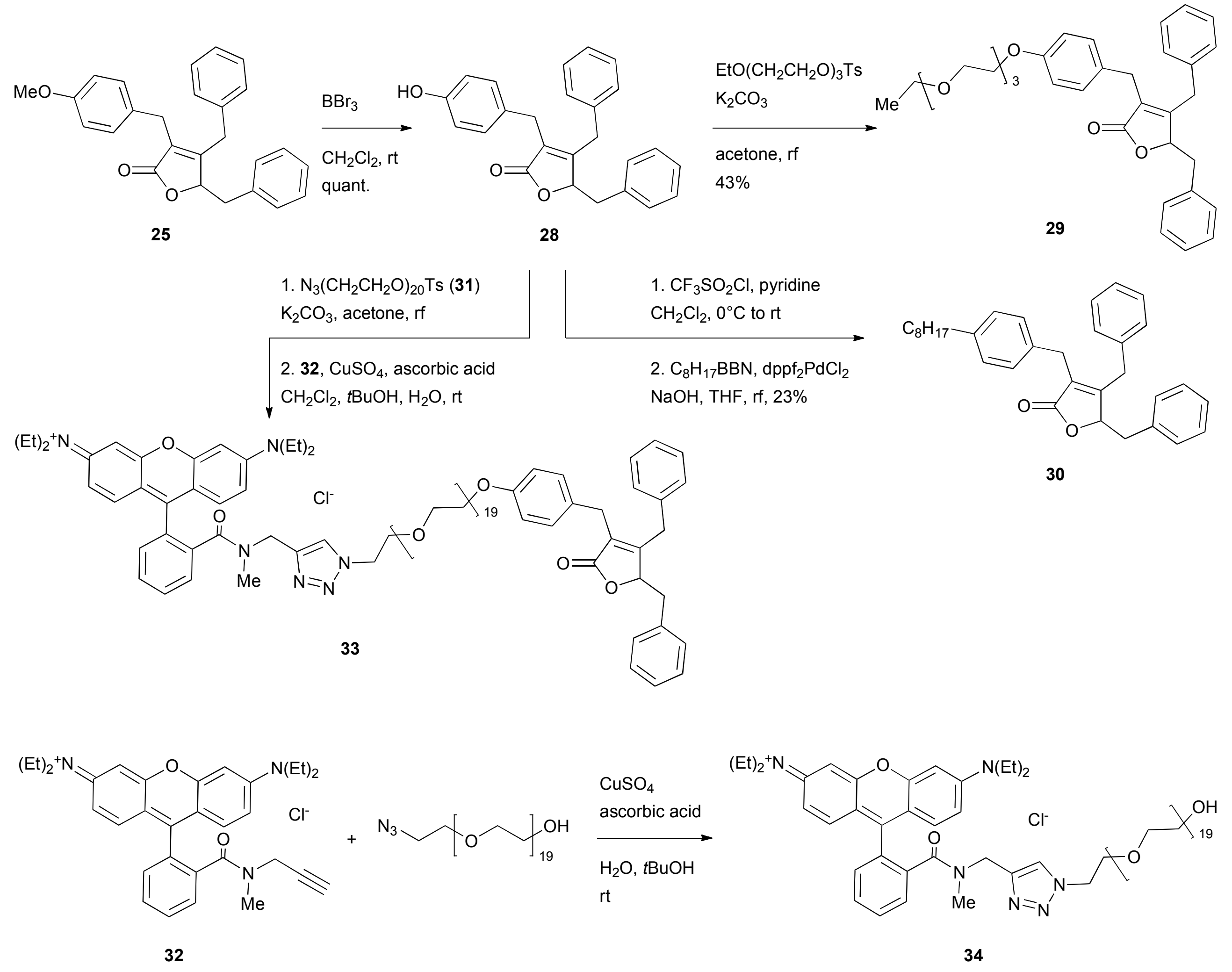


Fig 2 Biological evaluation of first generation maculalactone A analogues. Grey: *A. franciscana*, black: *T. platyurus*. Values are given as mean of three independent experiments with ten animals; error bars denote the standard error of the mean (SEM).

The assays show comparable (same order of magnitude), but slightly lower toxicity of the synthetic maculalactone A (**1**) in *A. franciscana*, when compared to the report of Brown *et al.*[25] This difference is either caused by racemic **1** or by differences in the assay set-up, and should not be of concern. Interestingly, compound **25** featuring the PMB group in the α-position exhibits an increased toxicity in *A. franciscana*, whereas isomeric lactones **26** and **27** display lower or no toxic effects on the test organism. In addition to the toxicity, the assays exposed a strong effect on the swimming ability of the test organisms. While fast movement is observed in both the blank and in a 1 μM maculalactone A solution, upon increased concentration (10 μM), the ability of the animals to swim in a controlled manner is strongly impaired (for a video, see supplementary information).


MeO
25
BBr₃
CH₂Cl₂, rt
quant.
HO
28
EtO(CH₂CH₂O)₃Ts
K₂CO₃
acetone, rf
43%
Me
3
29
1. N₃(CH₂CH₂O)₂₀Ts (31)
K₂CO₃, acetone, rf
2. 32, CuSO₄, ascorbic acid
CH₂Cl₂, tBuOH, H₂O, rt
1. CF₃SO₂Cl, pyridine
CH₂Cl₂, 0°C to rt
2. C₈H₁₇BBN, dppf₂PdCl₂
NaOH, THF, rf, 23%
C₈H₁₇
30
(Et)₂⁺N
N(Et)₂
Cl⁻
19
Me
33
+
N₃
OH
19
CuSO₄
ascorbic acid
H₂O, tBuOH
rt
32
34

Scheme 5 Deprotection and modification of the most active maculalactone A analogue **25**. $EtO(CH_2CH_2O)_3Ts$ was prepared as described in literature.[48,49] Compound **32** was synthesised as described by Yan et al.[50]

As analogue **25** showed a similar or higher toxicity as the parent maculalactone A, it was used for further derivatisation studies. The methoxy group was quantitatively cleaved in the presence of boron tribromide to give phenol **28**, which was coupled with different residues. First, etherification with a triethylene glycol unit,[48,49] gave the short PEGylated lactone **29**. After mesitylation of phenol **28**, it was coupled with an octylborane derivative in a Suzuki reaction, affording compound **30**. Furthermore, phenol **28** was added to the activated PEG azide **31**. By a Click reaction, this intermediate was connected to the rhodamine B derivative **32**[50] giving the fluorescently labelled maculalactone A analogue **33**. Additionally, compound **34** with a free hydroxyl group instead of the maculalactone A moiety was synthesised as a negative control. However, this compound proved to be unstable (amide hydrolysis), and, after repeated purification, could only be obtained in very low yield.

Consequently, the PEGylated compound **29** and the fluorescently labelled, rhodamine B derivative **33** were subjected to the toxicity assay with *A. franciscana* (Fig. 3).

Surprisingly, compound **29**, with the short PEG residue, did not display any toxicity in this assay. However, the rhodamine B bearing analogue **33** exhibited toxicity comparable to the one observed for **1**. Therefore, this fluorescent analogue **33** was used as a model to examine the distribution of maculalactone A in crustaceans.

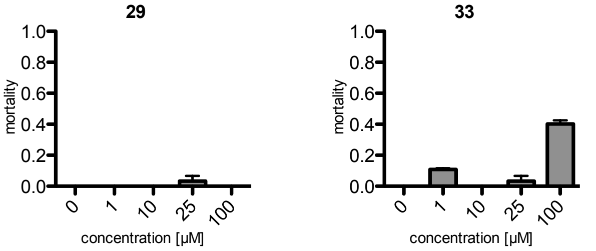


Fig 3 Toxicological tests of modificated maculalactone A analogues using *A. franciscana* as test organism. Values are given as mean of three independent experiments with ten animals, error bars denote the standard error of the mean (SEM).

Visualization of Maculalactone A Distribution in *Artemia salina*

The media of *A. franciscana* was found to be incompatible with subsequent visualization steps (fixation with formalin). Therefore, a more robust species of *A. salina* was employed for the *in vivo* experiments with the rhodamine B labelled macualalctone A derivative **33**.

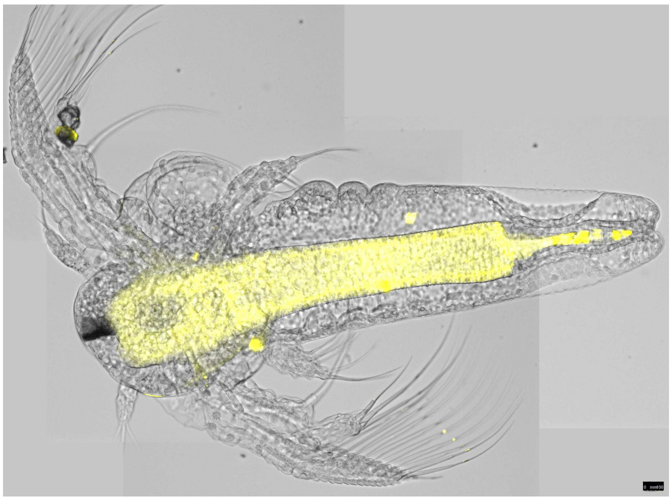


Fig 4 Overlay of four microscope pictures of *A. salina* nauplia stained with compound **33** (visual and fluorescence images). Scale bar = 100 µm.

A marker concentration of 1 µM was found to be optimal, as no toxic effects were determined at this level. The two days old animals were incubated for 24 hours in 1 µM solutions of the marked maculalactone A **33**, the negative control **34**, rhodamine B and a blank solution. This was followed by a washing procedure were the crustaceans were twice transferred in fresh media and incubated for three hours. Finally, the larvae were fixed in a formalin solution in PBS puffer. The stained and fixed organisms were examined by fluorescence microscopy under identical lighting conditions (see supporting information for micrographs). While rhodamine B led to a general staining of the entire body, the negative control **34** did not show any fluorescence at all. In contrast, the macualalactone A marker **33** led to a well defined distribution in the animal with a selective staining of certain organs (Fig 4). A highly stained rod-shaped tissue in the centre of the body is observed, which can be attributed to the intestine,[51] and is sharply terminated towards the tail region.

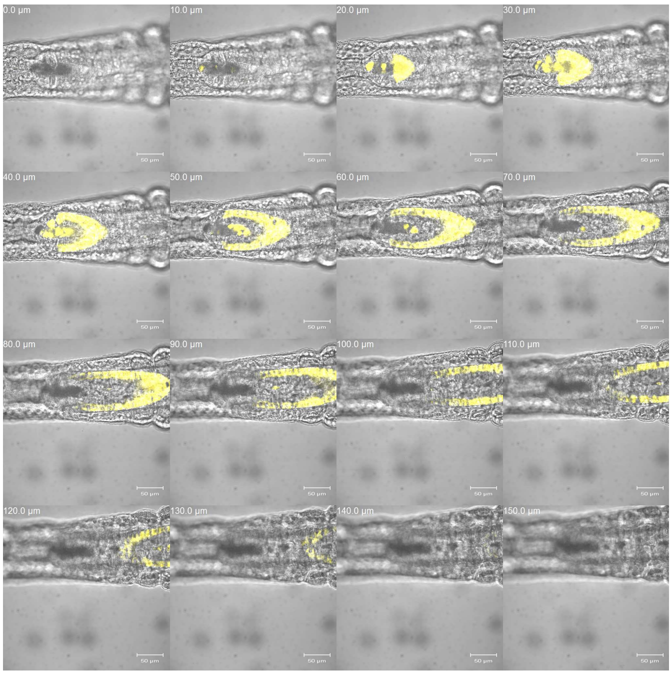


Fig 5 Tail region of *Artemia salina* nauplia stained with compound **33**. Visual and fluorescence images, 10 µm slides in the *z*-axis, scale bar = 50 µm.

Further support for this observation stems from examination by a confocal microscope, which disclosed more details of this region. Slices through the animal (Figure 5) reveal a tube-like structure, which is in agreement with the hypothesis that the intestine, but not its content, is stained by the marker **33**. Further evidence is presented by the well-defined localization of the fluorescently labelled natural product **33**, which can be rationalized by a selective staining and little or no diffusion. A similar, well-defined staining is observed towards the head region (see supporting information for details). In summary, the evidence presented supports the notion that the labelled maculalactone A derivative is accumulated in the animal in well-defined tissue. However, as little is known about the anatomy of *A. salina* in larvae stage, more detailed assignment of the involved tissues will require further experiments based on the preliminary successful use of the fluorescently labelled probe **33**.

Conclusions

In conclusion, we have developed a new and efficient route to maculalactone A (**1**) that delivered gram-amounts of materials in three steps and 45 % overall yield. The key step of the synthesis employed a late stage butenolide formation involving an intramolecular malonate addition with subsequent extrusion of CO_2. This route enabled the preparation of macualalcatone analogues **25**, **26** and **27**, of which their biological activity was compared in a toxicity assay using *A. franciscana* and *T. platyurus*. Significant activity was observed with compound **25**, which was further modified. The rhodamine B labelled derivative **33** proved to be the most interesting for tissue distribution and environmental fate studies in *A. salina*. Staining of specific tissues of the organisms supported the hypothesis that selective targets can be reached by maculalactone in the animal. However, further investigations are required to understand the distribution of maculalactone A (**1**) on living organisms, which should be enabled by the probe reported herein.

Experimental section

All chemicals have been purchased from Acros, Alfa Aesar, Fluka or Sigma-Aldrich and were used without further purification if not otherwise reported. Solvents applied for chemical transformations were either puriss quality or HPLC grade solvents. Dry solvents were purchased from Sigma-Aldrich. Technical grade solvents for extractions and chromatography were distilled before usage. Air or moisture sensitive reactions were set up in dry glassware under nitrogen atmosphere. All synthetic transformations have been monitored by either thin layer chromatography (TLC) or ^{1}H-NMR spectroscopy. Yields refer to purified, dried and spectroscopically pure compounds. TLC was performed on Merck silica gel 60 F254 plates. The developed plates were examined under UV light at 254 nm and stained with an aqueous $KMnO_4/Na_2CO_3$ solution. Concentration was performed by rotary evaporation at 40 °C under reduced pressure (unless otherwise stated). Flash chromatography was performed using silica gel 60 (230–400 mesh) from Merck or Silicycle with a forced flow eluent at 0.1–0.3 bar pressure. All ^{1}H- and ^{13}C-NMR spectra were recorded using a Bruker DPX-NMR 400 MHz (^{1}H) and 100 MHz (^{13}C) or a Bruker Avance DRX 500 MHz (^{1}H) and 125 MHz (^{13}C) spectrometer at room temperature. Chemical shifts (δ-values) are reported in ppm, spectra were calibrate related to solvents' residual proton chemical shifts, multiplicity is reported as follows: s = singlet, d = doublet, t = triplet or m = multiplet and coupling constant J in Hz. IR spectra were recorded on a Varian 800 FT-IR ATR spectrophotometer. The absorptions are reported in cm^{-1}. Mass spectra were recorded either on a high-resolution Bruker maXis 4G spectrometer using elecrospray ionization (HR-MS) or a Finnigan MAT 95Q spectrometer using electronic inonization (EI-MS). Elementary analysis (EA) was measured on a Perkin-Elmer Analysator 240. HPLC analysis was performed with a Dionex-Chromatography system using a P680 pump and a PDA-100 detector. Preparative reversed-phase HPLC was performed using Varian Prep Star pumps and a Dionex UltiMate 3000 RS detector or a Dionex P680 pump and a Dionex PDA-100 detector. Melting points (Mp) were determined using a Büchi B545 apparatus in open capillaries and are uncorrected. Absorptions spectra (UV/VIS) were recorded on a Shimadzu UV-1650 PC instrument. Emission spectra were recorded on a Shimadzu RF-5301 PC instrument. Additional procedures and characterization data can be found in the electronic supplementary information (ESI), see DOI: 10.1039/b000000x

Potassium 2-benzyl-3-ethoxy-3-oxopropanoate (15)

A published procedure[41] was modified as follows: To a solution of diethyl benzylmalonate (**14**, 5.11 g, 20.0 mmol, 1.00 eq.) in EtOH (20 ml) was added a freshly filtered solution of potassium hydroxide (1.39 g, 21.0 mmol, 1.05 eq.) in EtOH (20 ml) at room temperature. The reaction was stirred for six hours at room temperature. The precipitate was filtered off and the filtrate was concentrated to a yellowish foam. The residue was suspended twice in Et_2O and again concentrated. After drying at high vacuum, the foam was triturated overnight in Et_2O (50 ml). The precipitate was isolated by filtration to give the title compound **15** (4.44 g, 17 mmol, 85%) as white solid. **^{1}H-NMR** (400 MHz, D_2O): 7.30–7.22 (m, 2H), 7.22–7.15 (m, 3H), 4.06–3.96 (m, 2H), 3.51 (dd, J = 8.8, 7.4 Hz, 1H), 3.05–3.00 (m, 2H), 1.07 (t, J = 7.1 Hz, 3H). **^{13}C-NMR** (101 MHz, D_2O): 176.0, 173.3, 139.1, 128.7, 128.6, 126.6, 62.0, 57.3, 35.0, 13.2. **IR** (neat): 3396, 2980, 1710, 1598, 1496, 1454, 1359, 1321, 1234, 1150, 1095, 1061, 1028, 950, 914, 858, 830, 745, 698. **HRMS**: Calculated for $C_{12}H_{13}O_4$ $[M\text{-}K]^-$: 221.0819, found: 221.0822. **Mp:** 120 °C (decomposition). The analytical data match those reported in the literature.[41]

3-Hydroxy-1,4-diphenylbutan-2-one (16)

A published procedure[42] was modified as follows: To a solution of sodium acetate (328 mg, 4.00 mmol, 0.200 eq.) and freshly distilled phenyl acetaldehyde (4.77 ml, 40.0 mmol, 2.00 eq.) in EtOH (20 ml) was added 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride (540 mg, 2.00 mmol, 0.100 eq.). The mixture was heated to reflux for three hours, cooled down and mixed with ice water (20 ml). After stirring for another hour, the mixture was saturated with NaCl and extracted three times with EtOAc. The org. layers were washed with brine, combined, dried with Na_2SO_4 and concentrated. The crude product was purified by column chromatography (SiO_2, pentane/EtOAc (7:1 to 5:1)) to give the title compound **16** (2.9 g, 12 mmol, 61%) as yellow liquid, which crystallized after several days. **^{1}H-NMR** (500 MHz, $CDCl_3$): 7.39–7.26 (m, 6H), 7.25–7.12 (m, 4H), 4.51 (ddd, J = 7.4, 5.5, 4.9 Hz, 1H), 3.81 (d, J = 15.9 Hz, 1H), 3.75 (d, J = 15.9 Hz, 1H), 3.22 (d, J = 5.6 Hz, 1H), 3.15 (dd, J = 14.1, 4.7 Hz, 1H), 2.90 (dd, J = 14.1, 7.4 Hz, 1H). **^{13}C-NMR** (126 MHz, $CDCl_3$): 209.2, 136.5, 133.1, 129.6, 129.5, 128.9, 128.8, 127.5, 127.2, 76.8, 45.9, 40.3. The analytical data match those reported in the literature.[42,44]

1-Ethyl 3-(3-oxo-1,4-diphenylbutan-2-yl) 2-benzylmalonate (17) as a mixture of diastereomers

To a suspension of potassium 2-benzyl-3-ethoxy-3-oxopropanoate (19.0 g, 72.8 mmol, **15**, 1.40 eq.) in dry THF

(250 ml) at 0 °C was added DMF (201 µl, 5.20 mmol, 0.050 eq.) followed by the dropwise addition of pivaloyl chloride (11.0 ml, 88.4 mmol, 1.70 eq.) over five minutes. During the addition of pivaloyl chloride, the reaction mixture turned to a solution and after another 10 minutes to a gel. This gel was then kept for one hour at 0 °C and for 2.5 hours at room temperature, during which the gel turned back into a solution. The resulting solution was concentrated, dried in high vacuum, again dissolved in dry CH_2Cl_2 (250 ml), concentrated and dried again in high vacuum without exceeding a temperature of 25 °C. The residue was dissolved in dry CH_2Cl_2 (250 ml) and cooled to 0 °C. The addition of 4-dimethylaminopyridine (635 mg, 5.20 mmol, 0.100 eq.) to the reaction mixture was followed by the addition of a solution of 3-hydroxy-1,4-diphenylbutan-2-one (**16**, 12.5 g, 52.0 mmol, 1.00 eq.) in dry CH_2Cl_2 (50 ml) over 10 minutes. The reaction was allowed to reach room temperature overnight before it was quenched at 0 °C with a mixture of water and a sat. aq. $NaHCO_3$ solution (1:1 v/v). The layers were separated and the aq. layer was extracted three times with CH_2Cl_2. The org. layers were washed with a sat. aq. NH_4Cl solution, combined, dried with Na_2SO_4 and concentrated. The crude product was purified by column chromatography (SiO_2, pentane/EtOAc (6:1)) to give the title compound **17** (23.1 g, 52 mmol, quantitative) as yellowish liquid. **Rf** (SiO_2, pentane/Et_2O (2:1)): 0.7. **^{1}H-NMR** (500 MHz, $CDCl_3$): 7.32–7.20 (m, 8H), 7.20–7.15 (m, 2H), 7.14–7.09 (m, 2H), 7.06–6.99 (m, 3H), 5.35–5.25 (m, 1H), 4.21–4.04 (m, 2H), 3.76–3.69 (m, 1H), 3.58 (s, 1H), 3.50 (d, J = 16.9 Hz, 0.5H), 3.38 (d, J = 16.9 Hz, 0.5H), 3.26–3.11 (m, 2H), 3.06–2.93 (m, 2H), 1.19–1.11 (m, 3H). **^{13}C-NMR** (126 MHz, $CDCl_3$): 204.4, 204.3, 168.5, 168.4, 168.4, 168.3, 137.7, 137.6, 135.7, 135.5, 132.9, 132.9, 129.9, 129.9, 129.6, 129.6, 129.0, 129.0, 128.7, 128.7, 128.7, 127.3, 127.3, 127.2, 127.1, 127.1, 79.6, 79.4, 61.9, 61.9, 53.8, 53.7, 46.5, 46.4, 37.1, 34.7, 34.7, 14.1, 14.1. **IR** (neat): 3031, 2982, 2936, 1732, 1497, 1454, 1369, 1276, 1229, 1149, 701, 633. **HRMS**: Calculated for $C_{28}H_{32}NO_5$ $[M+NH_4]^+$: 462.2275, found: 462.2274. **EI-MS** (70 eV) m/z (%): 444(3, $[M]^+$), 353 (9), 205 (43), 160 (10), 159 (100), 131 (68), 91 (94).

Maculalactone A (1)

A two neck round bottom flask was equipped with a condenser and a device to run the condensed solvent over a bed of 4 Å molecular sieves. In the flask, a mixture of cesium carbonate (1.23 g, 3.78 mmol, 0.200 eq.) and THF (400 ml) was heated until the suspension was strongly refluxing. After refluxing for 30 minutes, a solution of 1-ethyl 3-(3-oxo-1,4-diphenylbutan-2-yl) 2-benzylmalonate (**17**, 8.40 g, 18.9 mmol, 1.00 eq.) in THF (20 ml) was added over 25 minutes. The reaction was further stirred under strong reflux. After 3.5 hours, the finished reaction was cooled to room temperature. The mixture was filtrated over a bed of SiO_2 and the solids were washed with TBME. The filtrate was concentrated. The crude product was purified by recrystallization from water and methanol to give the title compound **1** (5.1 g, 14 mmol, 76%) as off-white crystals. **^{1}H-NMR** (400 MHz, $CDCl_3$): 7.34–7.23 (m, 6H), 7.22–7.10 (m, 5H), 7.03 (d, J = 6.5 Hz, 2H), 6.92–6.85 (m, 2H), 4.99–4.89 (m, 1H), 3.92 (d, J = 15.6 Hz, 1H), 3.63 (d, J = 15.3 Hz, 1H), 3.57 (d, J = 15.3 Hz, 1H), 3.48 (d, J = 15.6 Hz, 1H), 3.23 (dd, J = 14.5, 3.9 Hz, 1H), 2.82 (dd, J = 14.6, 6.1 Hz, 1H). **^{13}C-NMR** (101 MHz, $CDCl_3$): 173.7, 161.8, 137.8, 136.1, 135.0, 129.6, 129.2, 128.8, 128.7, 128.3, 127.5, 127.3, 126.5, 81.7, 77.4, 38.1, 33.4, 29.5. The analytical data match those reported in the literature.[26]

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Notes and references

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