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Graphical abstract

Asymmetric Synthesis and Structure-Activity Studies of the Fungal Metabolites Colletorin A, Colletochlorin A and their Halogenates Analogues

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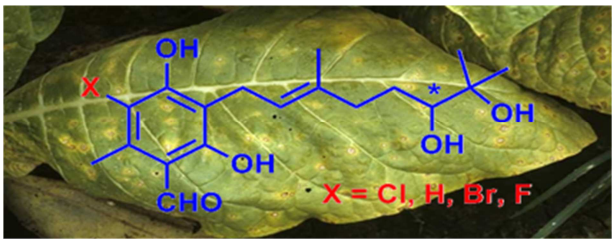

Asymmetric Synthesis and Structure-Activity Studies of the Fungal Metabolites Colletorin A, Colletochlorin A and their Halogenates Analogues

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Abstract

The first asymmetric total synthesis of both enantiomers of the natural products colletorin A and colletochlorin A is presented. The proposed methodology is based on the coupling reaction between highly substituted aromatic Gilman cuprates and optically active allyl bromides, in turn obtained by Sharpless asymmetric dihydroxylation. The latter ensured a high degree of regio- and stereocontrol in the enantioselective step of the synthesis. The same synthetic strategy has been also applied for the preparation of differently halogenated synthetic analogues of colletochlorin A in high enantiomeric purity. The enantioselective synthesis of colletorin A and colletochlorin A allows to reliably assign their absolute configuration. Preliminary assessment of their herbicidal and insecticidal properties evidence the possibility to modulate the bioactivity of these compounds, highlighting its dependence on both the absolute stereochemistry and the halogen nature.

Keywords: colletochlorin A, colletorin A, bioactive fungal metabolites, asymmetric dihydroxylation, coupling reactions, asymmetric synthesis.

Introduction

Fungi, being able to produce a large number of bioactive secondary metabolites, represent an excellent source of pharmaceuticals, antifungal, and herbicidal compounds.[1] Colletorins and colletochlorins are two groups of interesting bioactive metabolites isolated from *Colletotrichum nicotianae*, a fungus causing anthracnose disease of tobacco plants.[2] These compounds share as common structural feature a multi-substituted polyphenolic ring joined to a prenyl or diprenyl side chain. Some of them have this terpene moiety partly oxidized at the terminal double bond, displaying one or two hydroxylated stereogenic centers. This is the case of colletorin A (**1a**) and colletochlorin A (**1b**) (Figure 1), which possess a chiral diol moiety.[3] Phytotoxin **1b** has been also recently isolated from *Colletotrichum higginsianum*[4] and *Colletotrichum gloeosporioides*.[5] The structurally-related colletochlorin B (**2b**) (Figure 1) displays promising pharmaceutical activity being able to readily induce the differentiation of human promyelocytic cells (HL-60),[6] associated with the onset of diseases such as acute promyelocytic leukemia and neutrophilic leukopenia. Compound **2b** also shows inhibitory activity toward the enzymes acetylcholinesterase (AChE) and *β*-glucuronidase, as well as some toxicity toward human lung fibroblasts.[7] Moreover, a recent study has also revealed the phytotoxic activity of **1b** against *Ambrosia artemisiifolia*,[5] allowing to envisage its possible use as natural herbicidal for biocontrol of this weed responsible for serious allergies in humans. In this latter study, the (*R*) absolute configuration at the hydroxylated stereocenter of naturally occurring (+)-**1b** has been also determined by application of the modified Mosher's method,[5] while the absolute configuration of **1a** is still unknown. However, due to the scarce availability of these compounds from natural sources (indeed fungi can produce these metabolites in small amounts), only a few studies have been undertaken to investigate their biological properties. These reasons prompted us to attempt the development of a new stereoselective synthesis of both enantiomers of **1a** and **1b,** as well as their brominated and

fluorinated analogues **1c** and **1d**, with the aim to carry out a structure activity relationship study to define the role of both absolute stereochemistry and halogen nature on the biological properties of this class of phytotoxins.[8] Indeed, the role of the absolute configuration on the bioactive properties, well known for the majority of chiral compounds,[9] has been clearly recognized also in fungal metabolites,[10] and the influence of the presence and nature of halogen atoms on the same properties is well established too.[8,11]

Few approaches for the synthetic preparation of **1a** and **1b** are reported in the literature, none providing them in an optically active form. To the best of our knowledge, a single synthesis of racemic **1b** is described,[12] which employs, as a key step, a lithium-mediated C-alkylation of 1,5-dimethoxy-3-methyl-1,4-cyclohexadiene with the dihydroxylated side chain, in turn obtained from geranyl acetate. However, this strategy provided the desired compound in a very low yield and was not applicable neither to the synthesis of **1a** nor to the preparation of the halogenated analogues **1c** and **1d**.

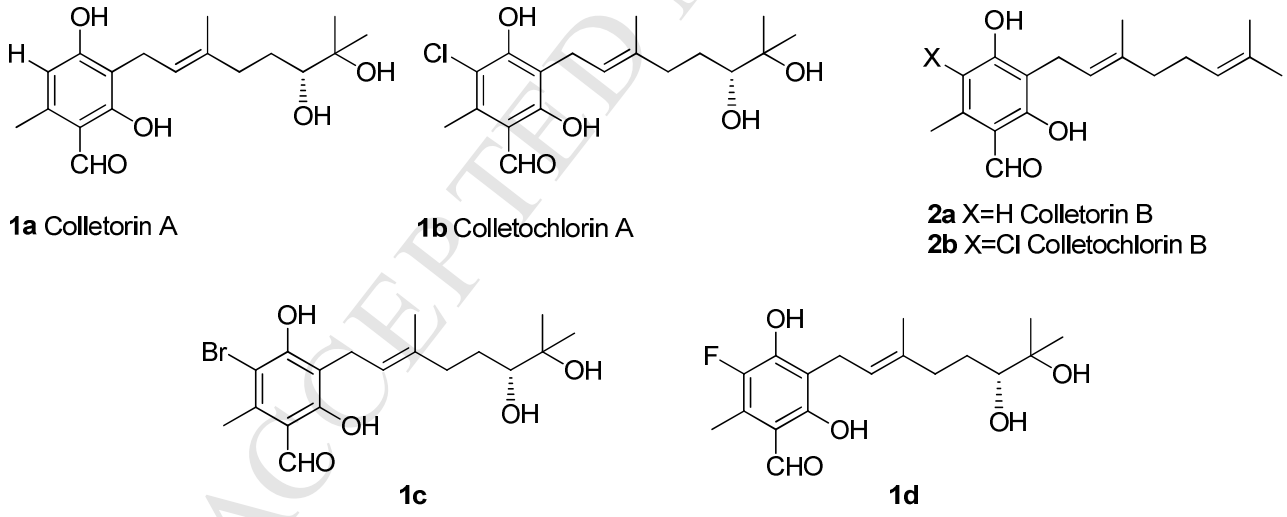
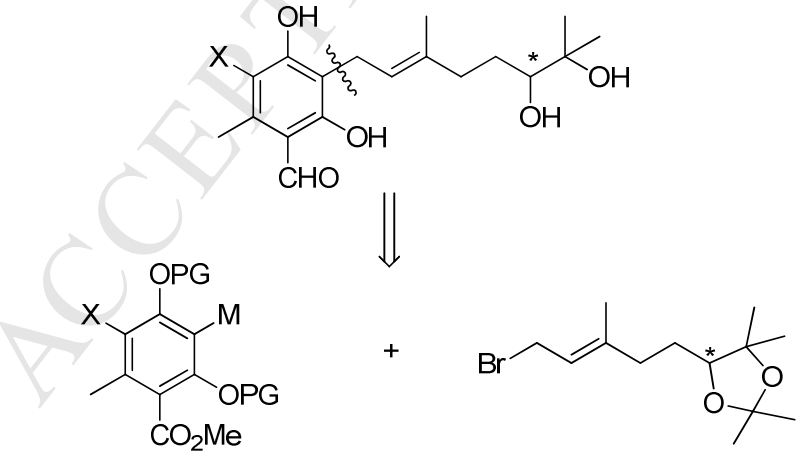


Fig. 1. Structure of colletorin A and B (**1a** and **2a**), colletochlorin A and B (**1b** and **2b**), and their synthetic analogues **1c** and **1d**.

We herein describe the first asymmetric synthesis of both enantiomers of **1a** and **1b** as well as that of the brominated and fluorinated unnatural analogues **1c** and **1d**. A preliminary investigation of the phytotoxic and insecticidal properties of the prepared compounds is also described.

Results and Discussion

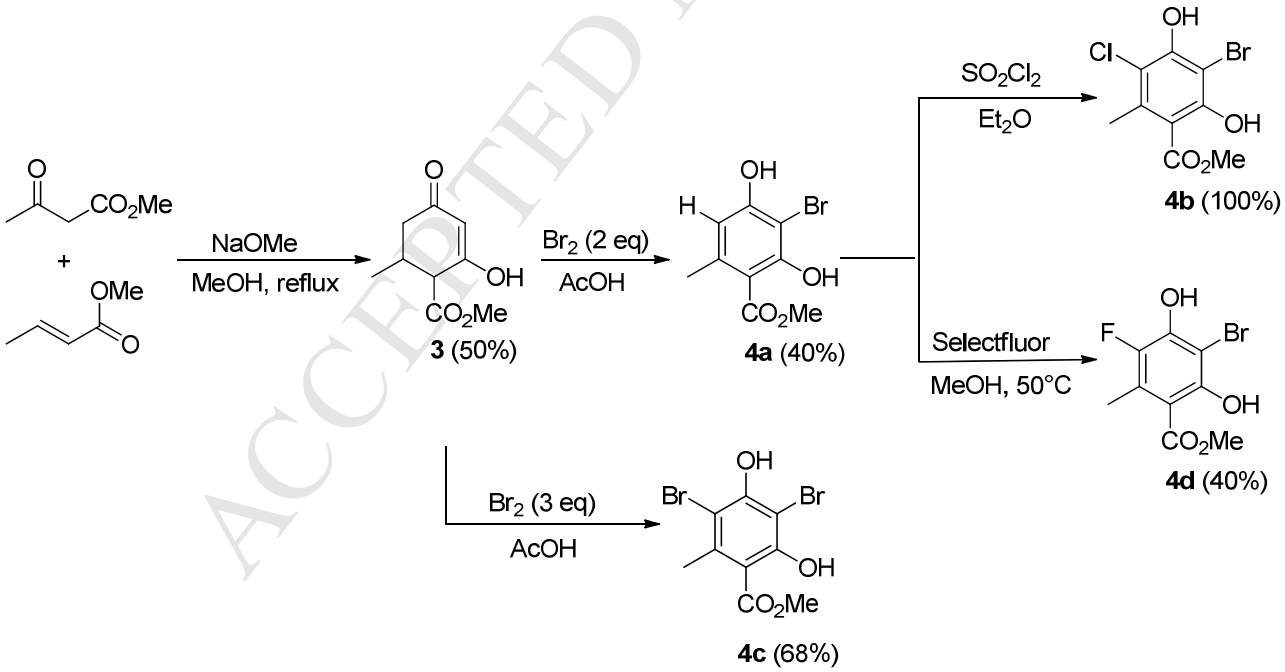
The synthetic approach described herein is based on the disconnection of the structures of **1a**-**1d** into an aromatic synthon and an optically active side chain (Scheme 1), having both all the functional groups protected (Scheme 1) and where the aromatic formyl group is replaced by a less reactive carbomethoxy precursor. A similar approach has been reported for the preparation of unsaturated analogues colletorin B (**2a**) and colletochlorin B (**2b**) (Figure 1), by alkylation of an aromatic mixed cuprate precursor with geranyl bromide.[13] In our case, we envisaged the possibility to obtain the chiral side chain in optically active form by a regio- and stereoselective Sharpless asymmetric dihydroxylation of geranyl acetate.[14] To avoid oxidizing conditions to the polyphenolic aromatic precursor, the diol moiety was introduced on the side chain before the cross-coupling reaction between the two moieties.



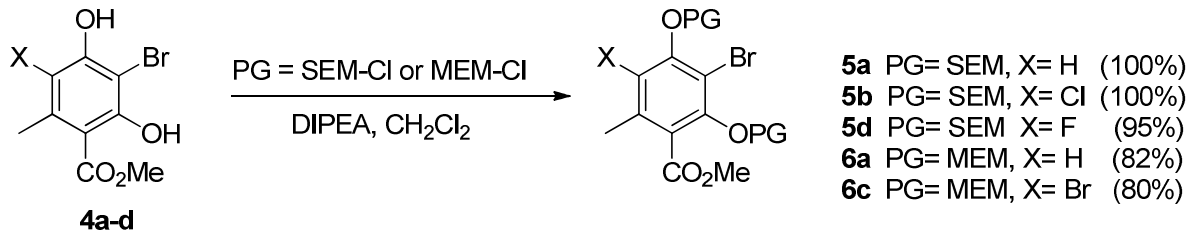
Scheme 1. Retrosynthetic approach

In Scheme 2 the synthetic steps for the preparation of the aromatic precursors are summarized. Cyclohexenone **3**, prepared by Robinson annulation,[15] was brominated and aromatized with either 2

or 3 equivalents of bromine to achieve benzoates **4a** and **4c**, respectively. Then, the unsubstituted position of **4a** was smoothly chlorinated by reaction with a sulfuryl chloride solution. Pure **4b** was obtained in quantitative yields after solvent removal under controlled pressure and temperature to prevent product sublimation. Conversely, the introduction of fluorine on the aromatic ring was rather difficult. Various unsuccessful attempts in different experimental conditions were made, by employing reagents already known for electrophilic fluorination of phenols, such as *N*-fluoropyridinium triflate[16] and *N*-chloromethyl-*N'*-fluorotriethylendiammonium-bis (tetrafluoborate) (Selectfluor®).[17] No conversion was obtained with the pyridinium salt even at high temperature and long reaction time. Finally, a moderate 50% conversion of **4a** in **4d** was obtained by reaction with Selectfluor® in methanol at 50°C. Higher temperatures and different solvents led to lower conversions and/or to the formation of unwanted quinones-type by-products. Through this synthetic sequence we then obtained all the aromatic precursors **4a-d** with all substituents in the correct position.

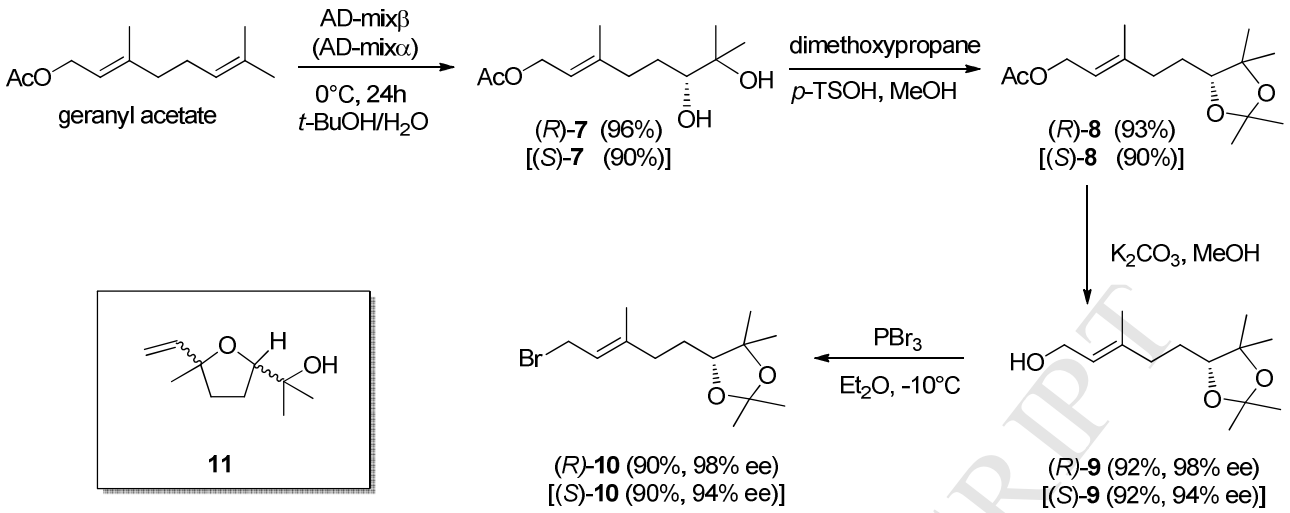


Scheme 2. Preparation of functionalized aromatic moieties **4a-d**.



Scheme 3. Protection of the phenolic moieties.

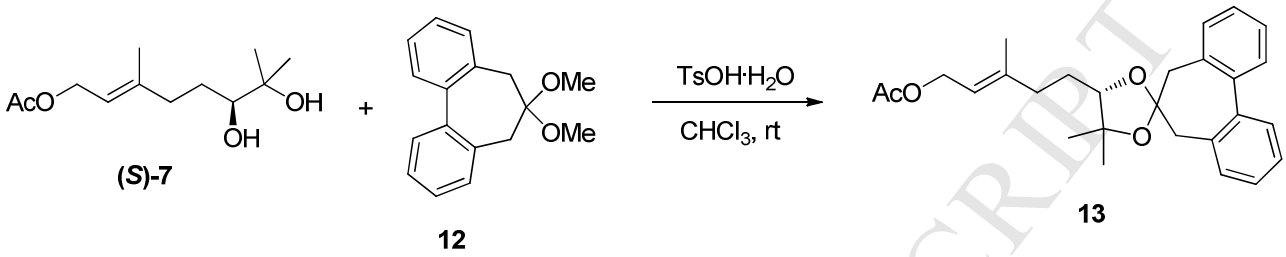
Finally, to compare the effect of protecting groups on the following synthetic steps, the phenolic groups of compounds **4a-d** were protected as either SEM- or MEM-ethers **5** and **6**, respectively (Scheme 3). The SEM-derivatives **5a**, **5b**, and **5d** were obtained in quantitative yield, while the protection with the cheaper MEM-chloride provided products **6a** and **6c** in a lower yield (80%). Both enantiomers of the side chain were prepared in high enantiomeric purity by Sharpless asymmetric dihydroxylation starting from geranyl acetate (Scheme 4). This procedure allows the formation of both enantiomers of the required diol **7** in high yield, high stereoselectivity (94-98% ee, see below), and full regioselectivity.[14] In this reaction, the temperature control is extremely important because at T < 0°C a very low conversion occurs, whereas at T > 4°C oxidation of both double bonds is observed. The absolute configuration of the obtained diols was preliminarily assigned[14a] on the basis of the predictions provided by the empirical Sharpless rule.[18] Accordingly, the oxidation reaction was expected to provide diol (*R*)-**7** when carried out with the ADmixβ reagent and diol (*S*)-**7** when ADmixα is employed. Diols (*R*)- and (*S*)-**7** were then transformed into ketals (*R*)-**8** and (*S*)-**8**, respectively, and the ester groups were hydrolyzed to give alcohols (*R*)- and (*S*)-**9**. Notably, all steps of the synthetic sequence, starting from geranyl acetate, provided very high yields (≥90%).



Scheme 4. Asymmetric synthesis of the side chain

Although the empirical Sharpless rule[18] is commonly employed to predict the absolute configuration of the diols obtained by alkene dihydroxylation with osmium tetraoxide as oxidant and either dihydroquinine or dihydroquinidine derivatives as chiral ligands, some cases have been described in which such a rule fails to predict the correct assignment.[19] Therefore, for a more sound configurational assignment, we turned out to the use of Electronic Circular Dichroism (ECD) spectroscopy. In particular, we employed flexible biphenyl chiroptical probes for this purpose, following an approach described by our group which proved to be very straightforward and reliable for the absolute configuration assignment to chiral diols,[20] acids,[21] and amines.[22] Following this approach, to assign the absolute configuration to a chiral diol it must be transformed in the corresponding biphenyl dioxolane and the ECD spectrum of the latter analyzed. A positive Cotton effect at 250 nm (A band) in the ECD spectrum reveals a (*R*) absolute configuration of the diol while a negative band is allied to an (*S*) configuration. Therefore, (−)-**7**, obtained from ADmixα, was transformed to the corresponding biphenyl dioxolane[20] **13** (Scheme 5) and ECD spectrum of the latter was recorded. The appearance of a clear negative Cotton effect at 250 nm in the ECD spectrum (Figure 2) allows us to assign (*S*) absolute configuration to the diol (−)-**7**, then reliably and independently confirming the tentative assignment based on the empirical Sharpless rule. This

also allowed to establish the absolute configuration of compounds **8**-**10** derived from **7**. Therefore, the (*R*)/(-), (*S*)/(+) relationship was established for the ketals **8** and **9**, while the instability of bromide **10** did not allow measurement of its optical rotation. Moreover, this result allows to correct the optical rotation sign of (*R*)-**9** erroneously reported in the literature.[23]



Scheme 5. Synthesis of the biphenyl dioxolane derivative **13**

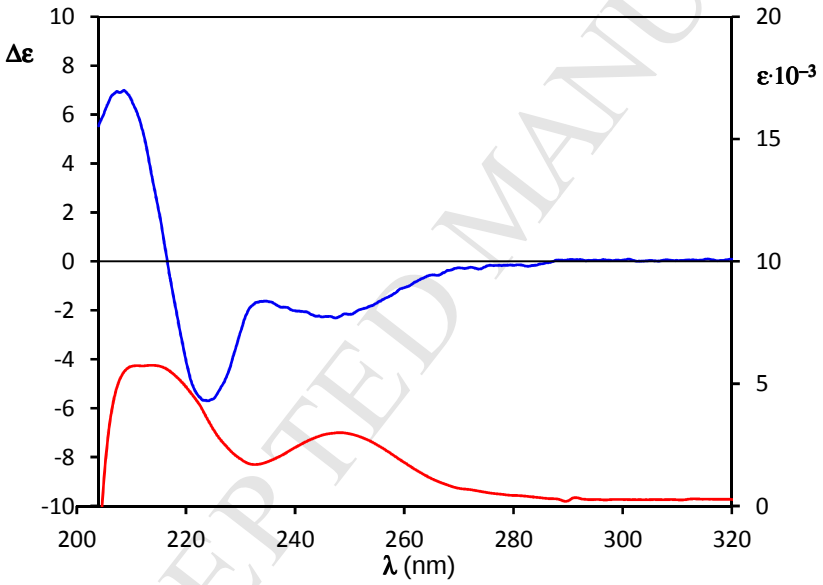


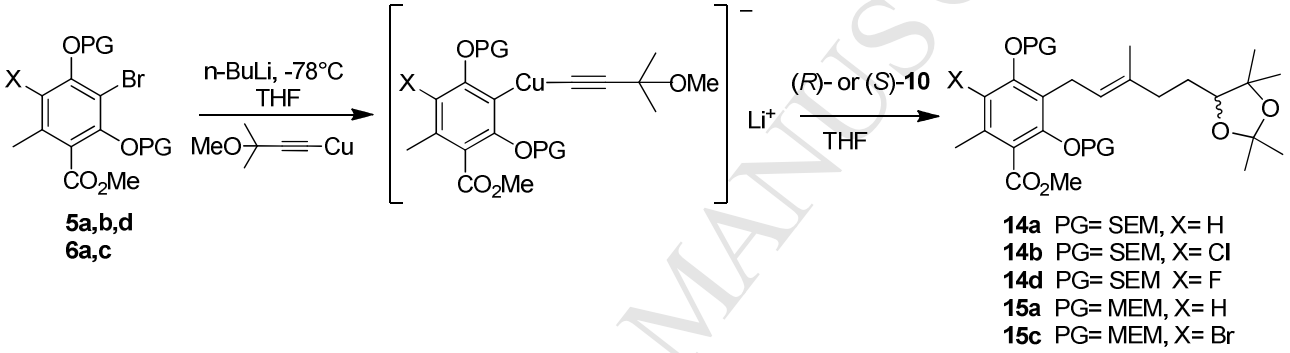
Fig. 2. ECD and UV spectra of biphenyldioxolane **13** in THF.

To determine the enantiomeric excess of alcohol **9** by chiral HPLC analysis, both enantiomers were transformed into the corresponding benzoates and separated on Chiralcel OD chiral stationary phase, providing 98% ee for (*R*)-**9** and 94% ee for the (*S*)-**9** enantiomer. Alcohols (*R*)-**9** and (*S*)-**9** were then transformed, by treatment with 0.5 equivalent of PBr_3 at −7°C in anhydrous diethyl ether, into the corresponding bromides (*R*)-**10** and (*S*)-**10**, respectively. After work-up, pure bromides (*R*)- and (*S*)-**10** were isolated in 90% yield, as confirmed by their ^{1}HNMR spectra. Bromides **10** were

used within 3-4 days of their preparation because they turned out to be very light sensitive slowly transforming, even if stored in the dark at low temperature and under inert atmosphere, into a diastereoisomeric mixture of tetrahydrofuran **11** (Scheme 4).[14a] The same behavior was shown by the triflates of alcohols (*R*)- and (*S*)-**9**. In fact, by treating the alcohols **9** with triflic anhydride at −78°C, the corresponding triflate was obtained in only 43% yield and a significant amount of tetrahydrofuran **11** was isolated.

The coupling between 3-bromo-benzoates **5** and **6** and the optically active bromides **10** was first attempted by Suzuki-Miyaura cross-coupling. Initially, various attempts were carried out to transform **5** and **6** or even the unprotected analogues **4a-d** into the boronic esters required for the Suzuki-Miyaura coupling, but these reactions failed, probably as a consequence of the high steric hindrance at the halogenated position on the aromatic moieties. Therefore, we turned to employ mixed cuprate chemistry for the coupling. Accordingly, SEM-protected benzoates **5** were lithiated at C-3 with *n*-butyllithium at −78°C in THF and treated at the same temperature with a freshly prepared solution of (3-methyl-3-methoxy-1-butynyl)copper in THF and HMPA to provide the mixed cuprate.[24] Then, (*R*)- or (*S*)-bromide **10** in THF was added to this solution and the mixture was slowly warmed at room temperature stirring for 18-20 h (Scheme 6). After quenching with a pH 8 solution of ammonia/ammonium chloride the crude product was purified by column chromatography affording benzoates **14** in 40-72% yield (Scheme 6). The same procedure provided benzoate **15** starting from the MEM-protected bromoesters **6**. As shown in Table 1, the reactions do not appear to be influenced by the nature of the protecting group on the aromatic ring, since the SEM-benzoate **5a** and its MEM-analogue **6a** gave the coupling products **14a** and **15a**, respectively, in comparable yields (entry 1 vs 2). By working in a slight excess of bromides **10** the yield is increased. In fact, in this case the coupling provided the 5-haloesters **15c** and **14b** in 70% yield, starting from **6c** and **5b**, respectively (entries 3 and 4). No significant influence by the nature of the halogen atom on the aromatic ring was found. A larger amount of electrophile did not improve the yield but made the cross-coupling side reaction between the butynyl copper and bromide **10** more

significant. Notably, in these reaction conditions the metalation of 3,5-dibromobenzoate **6c** is completely regioselective, since no trace of products resulting from either lithiation at C-5 position of the substrate or from a double coupling reaction was detected. Conversely, a lower scale seems to have negative effects on yield, because the same substrate **5b** provided the product **14b** in only 40% yield when the reaction was carried out on 0.3 mmol solution (entry 5). From the coupling of 5-chlorobenzoate **5b** with both bromides (*R*)- and (*S*)-**10**, both enantiomers of the precursor **14b** of colletochlorin A (**1b**) were prepared, while the fluorinated analogue was also obtained in moderate yield (entry 6) from 5-fluorobenzoate **5d.**



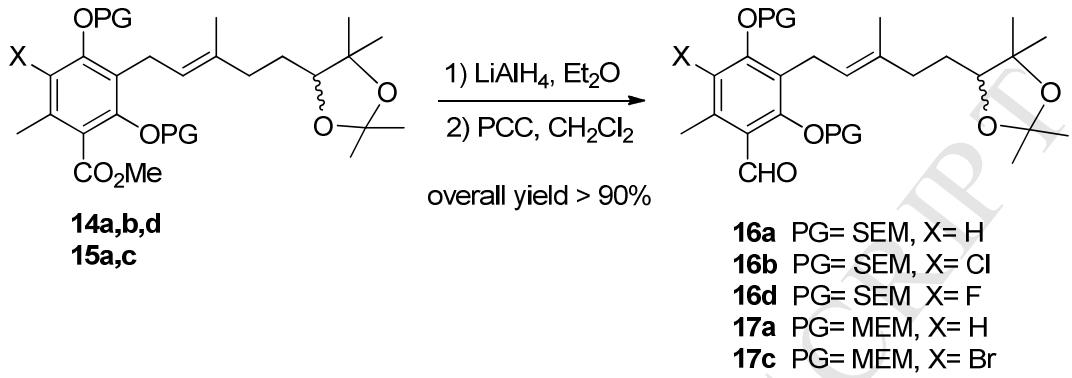
Scheme 6. Cross-coupling reaction. See Table 1 for reaction yields.

Table 1. Coupling reactions**:** synthesis of benzoates **14-15**

Entry[a]	Substrate	X	PG	10 (eq.)	Product	Yield[b] (%)
1	**5a**	H	SEM	(*S*)-**10** (1.0)	(*S*)-**14a**	42
2	**6a**	H	MEM	(*R*)-**10** (1.0)	(*R*)-**15a**	40
3	**6c**	Br	MEM	(*R*)-**10** (1.2)	(*R*)-**15c**	70
4	**5b**	Cl	SEM	(*S*)-**10** (1.2)	(*S*)-**14b**	72
5[c]	**5b**	Cl	SEM	(*R*)-**10** (1.2)	(*R*)-**14b**	40
6	**5d**	F	SEM	(*R*)-**10** (1.2)	(*R*)-**14d**	46

a) All reactions were carried out using 0.5 mmol of substrate. b) Isolated yield. c) Performed with 0.3 mmol of **5b**.

The ester moiety of all compounds **14** and **15** was then transformed into the aldehyde in high overall yields by means of a $LiAlH_4$ reduction followed by oxidation of the resulting alcohol with pyridinium chlorochromate (PCC) (Scheme 7).



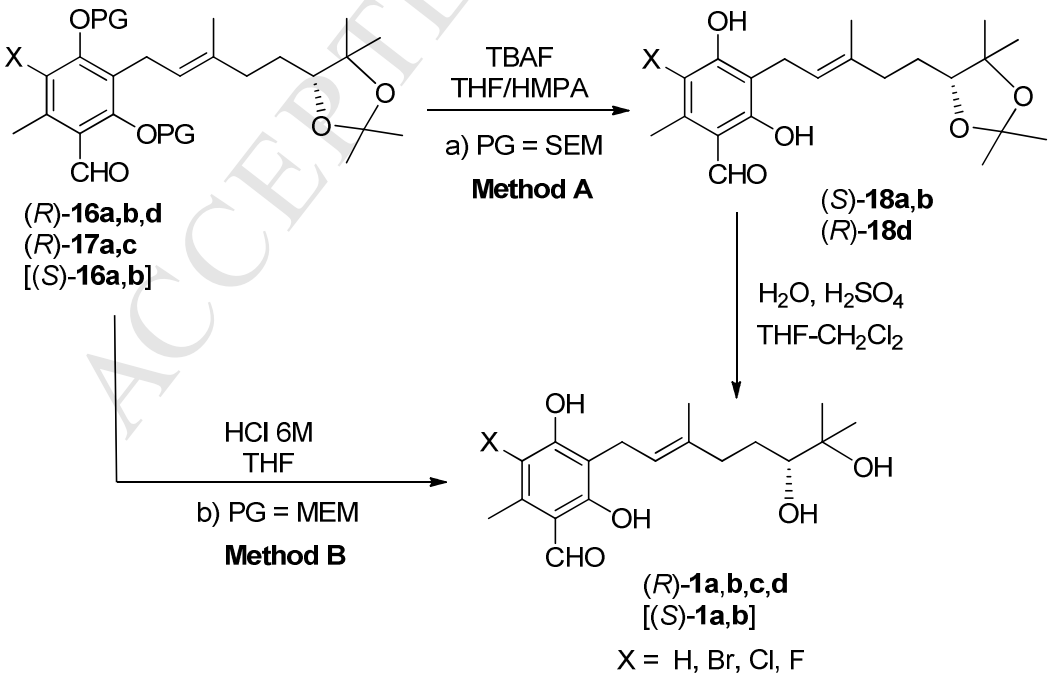
14a,b,d
15a,c

16a PG= SEM, X= H
16b PG= SEM, X= Cl
16d PG= SEM X= F
17a PG= MEM, X= H
17c PG= MEM, X= Br

Scheme 7.

The final step of the total synthesis involves the deprotection of the phenolic and diol moieties (Scheme 8). The procedure reported in the literature to cleave SEM and MEM protecting groups by P_2I_4 in dichloromethane which, thanks to the mild conditions employed, is claimed to be the best for resorcinols, was instead unsuccessful in our case, providing no reaction at room temperature and a mixture of unidentified products by treatment at higher temperature or for longer reaction time. Instead, reaction of **16a** with TBAF in THF/HMPA provided the removal of both SEM groups, giving ketal **18a** (Scheme 8). The latter was then hydrolized with H_2SO_4/H_2O providing colletorin A (*S*)-**1a** in 49% overall yield (Table 2, entry 1, method A). As regards to the deprotection of MEM ethers we discarded the use of the most common reagents, namely Lewis acids such as zinc or magnesium bromide, as it is known that acidic conditions induce ring closing and chroman or chromene formation and are not applicable to highly functionalized phenol ethers like **17**.[13a,25] Therefore, we decided to test the same procedure used above to cleave the SEM groups, even if only a single example reported its use to remove MEM.[26] We however observed that TBAF was usuitable to remove both MEM ethers, providing only the 4-monoprotected 2-hydroxybenzaldehyde. After several attempts, it was found that treatment of the solution of MEM

ethers **17a**,**c** in THF with 6M HCl provides the simultaneous deprotection of both the phenol and diol moieties, giving colletorin (*R*)-**1a** as well as the brominated analogue (*R*)-**1c** in moderate to good yields (Table 2, entries 2-3, method B). For comparison, the two enantiomeric benzaldehydes (*S*)-**16b** and (*R*)-**16b** were deprotected according to the method A and B, respectively, to provide both enantiomers of colletochlorin A, (*S*)-**1b** and (*R*)-**1b,** (entries 4-5). Although the procedure with HCl (method B) was simpler and shorter, while providing the simultaneous cleavage of both SEM protecting group and ketal moiety, a better overall yield was achieved using the two-step sequence based on the selective deprotection of phenolic and diol moieties (method A). This protocol was also used as final step in the total synthesis of the fluorinated analogue (*R*)-**1d** (entry 6). The enantioselective synthesis of **1a-d** allows to establish to all these compounds the (R)/(+), (S)/(-) relationship between the absolute configuration and the optical rotation sign, then providing a reliable absolute configuration assignment to the naturally occurring colletorin A (**1a**) and colletochlorin A (**1b**). In the latter case such assignment agrees with that carried out by application of the Mosher's method.[5]



Scheme 8. Final steps of total synthesis. See Table 2 for reaction yields.

Table 2: Deprotection of phenol and diol moieties.

Entry	Substrate	X	PG	Method	Product	Total Yield (%)
1	(*S*)-**16a**	H	SEM	**A**	(*S*)-**1a**	49
2	(*R*)-**17a**	H	MEM	**B**	(*R*)-**1a**	30
3	(*R*)-**17c**	Br	MEM	**B**	(*R*)-**1c**	55
4	(*S*)-**16b**	Cl	SEM	**A**	(*S*)-**1b**	46
5	(*R*)-**16b**	Cl	SEM	**B**	(*R*)-**1b**	37
6	(*R*)-**16d**	F	SEM	**A**	(*R*)-**1d**	30

Finally, the synthesized compounds were subjected to bioactivity assays in order to investigate their biological properties. In particular, we wanted to evaluate their phytotoxicity and insecticidal activity. The phytotoxic activity was assayed on *Ambrosia artemisifolia* and *Sonchus arvensis*, two weedy plants, on which the toxicity of the naturally occurring **1b** was recently reported with comparable results,[4,5] by using a leaf puncture assay. Droplets of solutions (10 µl) containing the metabolites (20 µg) were added to detached leaves previously wounded with a needle, kept in moistened chambers, thus observing the eventual appearance of necrotic lesions. The effect on chlorophyll content was tested on an aquatic model plant, *Lemna minor*, by adding the test solutions to wells containing fronds of the plant, and then determining by spectrophotometer the eventual reduction of the chlorophyll content in comparison with an untreated control. The results, summarized in Table 3, indicate that both enantiomers of **1b** exhibit the strongest phytotoxicity on *S. arvensis* and, among thm, the natural enantiomer (*R*)-**1b** shows a slightly higher toxicity than its enantiomer on *A. artemisifolia* plants. Conversely, the other tested compounds namely, both (*R*)- and (*S*)-**1a** and the halogenated unnatural analogues (*R*)-**1c** and (*R*)-**1d** cause only weak effects. Moreover on the *L. minor* assay, all the tested molecules cause a reduction in chlorophyll content, even though to a different extent, with (*S*)-**1a** being the most active compound and the fluorinated analogue (*R*)-**1d** the least. In conclusion, the presence of a chlorine atom seems to impart to these

componds higher phytotoxicity against weeds, in respect to the other halogens. The same enhanced phytotoxicity is observed with the naturally occurring (*R*) absolute configuration at the hydroxylated stereocenter. Conversely, halogenated compounds displayed lower phytotoxicity against *Lemna minor*. These results highlight the relevant effects that both the halogen nature and the absolute configuration have on the bioactivity of these fungal metabolites.

Table 3. Phytotoxic activity assays

Compound	*Sonchus arvensis*[a]	*Ambrosia artemisiifolia*	*Lemna minor*[b]
(*R*)-**1a**	++	+	62.7
(*S*)-**1a**	+	+	71.6
(*R*)-**1b**	+++	++++	59.1
(*S*)-**1b**	+++	+++	43.5
(*R*)-**1c**	++	+	59.5
(*R*)-**1d**	++	+	16.0

[a]Necrotic symptoms expressed as diameter of the necrosis in the leaf puncture assay, by using a visual scale from "-" = no symptoms, to "++++" = necrosis around 1 cm diameter. [b]Reduction of chlorophyll content expressed as percentage in comparison with the untreated control.

Insecticidal activity of both enantiomers of **1b** and the brominated analogue (*R*)-**1c** was examined *via* topical application and injection on *Aedes aegypti*, the yellow fever mosquito, which is a vector for transmitting several tropical fevers.[27] The results (Table 4) show that all three compounds exhibit negligible insecticidal activity against the adult mosquitos in both test methods. Except for (*R*)-**1b** at 2 μg/mosquito dose (3.3 ± 3.3% mortality), none of the compounds shows any mortality *via* topical application. Moreover, (*R*)-**1b** shows greater mortality in the injection assay (13 ± 3.3%), but this value is not statistically significant compared to controls. A pre-treatment with piperonyl butoxide (PBO) slightly increases toxicity of all three tested compounds, but again the effect is not statistically significant in a t-test compared to compound injected alone. Thus, mixed-function oxidases do not seem to be a major route of metabolism in the tested compounds in *A. aegypti*, and the result suggests that the observed low toxicity is due to limited intrinsic insecticidal potency.

Table 4. Insecticidal activity assays *Aeades aegypti*

Compd	Topical application			Injection		
	dose (μg/insect)	knock-down (% ± SE)[a]	mortality (% ± SE)[a]	dose (μg/insect)	knock-down (% ± SE)	mortality (% ± SE)
(*R*)-**1b**	2	0.0 ± 0.0	3.3 ± 3.3	0.1	6.7 ± 3.3	13.3 ± 3.3
	1	0.0 ± 0.0	0.0 ± 0.0	0.1 + PBO[b]	20.0 ± 5.8	33.3 ± 8.8
(*S*)-**1b**	2	0.0 ± 0.0	0.0 ± 0.0	0.1	10.0 ± 5.8	6.7 ± 3.3
	1	0.0 ± 0.0	0.0 ± 0.0	0.1 + PBO	16.7 ± 8.8	30.0 ± 10.0
(*R*)-**1c**	2	0.0 ± 0.0	0.0 ± 0.0	0.1	6.7 ± 3.3	10.0 ± 5.8
	1	0.0 ± 0.0	0.0 ± 0.0	0.1 + PBO	13.3 ± 3.3	23.3 ± 3.3
control		0.0 ± 0.0	0.0 ± 0.0	without PBO	10.0 ± 5.8	10.0 ± 5.8
				with PBO	6.7 ± 3.3	10.0 ± 5.8

[a]knock-down effect was recorded at 1h post-treatment, and 24h for mortality.
[b] For assay with a mono-oxygenase synergist, mosquitoes were pre-treated with piperonyl butoxide (PBO, at 0.5 μg/insect) *via* a topical application, and left for 4 hr.

Conclusions

Herein we have shown an efficient method for the highly enantioselective synthesis of colletorin A (**1a**) and colletochlorin A (**1b**) and their brominated (**1c**) and fluorinated (**1d**) synthetic analogues. This protocol allows to carry out the most critical steps of the whole synthetic pathway, namely the coupling and deprotection reactions, in medium to good yields. Indeed it was possible to obtain the precursors of such compounds in 40-72% yields by coupling reactions between highly substituted aromatic mixed cuprates and optically active allylic bromides. Moreover, two different procedures for the deprotection of both phenolic and ketal moieties have been tested, depending on the nature of the phenolic protecting group. Finally, a high regio- and stereocontrol is also guaranteed by the Sharpless asymmetric dihydroxylation of geranyl acetate, which constitutes the enantioselective step of the total synthesis. We believe that this strategy could be successfully applied also to other highly functionalized phenolic compounds bearing terpenoid side chains. The enantioselective synthesis of **1a-d** allows to reliably assign the absolute configuration to the natural compounds **1a**

and **1b**. The preliminary tests of biological activity as herbicides and insecticides carried out highlight the potential bioactivity of these compounds and allow sorting out the dependence on the absolute stereochemistry and on the halogen nature on the compound bioactivity.

Experimental Section

General Information

^{1}H (500 or 400 MHz) and ^{13}C (125 or 100 MHz) NMR spectra were recorded in $CDCl_3$ on a Varian INOVA 500 or a Varian INOVA 400 spectrometer, using tetramethylsilane (TMS) as an internal standard. ^{19}F (376 MHz) NMR spectra were recorded in $CDCl_3$ on a Varian INOVA 400 spectrometer, using $CFCl_3$ as an internal standard. GC–MS spectra were recorded on a Hewlett Packard 6890 gas chromatograph, equipped with a mass spectrometric detector HP-5973 type and a capillary column HP-5MS 30 m x 0.25 mm. Optical rotations were measured on a JASCO DIP-370 polarimeter, with α values expressed in $deg \cdot cm^3 \cdot g^{-1} \cdot dm^{-1}$ and concentration *c* in $g \cdot (100\ mL)^{-1}$. HPLC analyses were performed on a JASCO PU-1580 intelligent HPLC pump equipped with a Varian 2550 UV detector and a CHIRALCEL OD column. Analytical thin layer chromatography (TLC) was performed using silica gel 60 Macherey-Nagel sheets and visualized by ultraviolet radiation and/or spraying the plates with a potassium permanganate solution; column chromatography separations were carried out using silica gel 60 (70–230 mesh). Et_2O and THF were freshly distilled before their use on sodium benzophenone ketyl under nitrogen atmosphere. *N*,*N*-diisopropylethylamine (DIPEA), trimethylamine, and CH_2Cl_2 were dried by distillation over calcium hydride and stored under a nitrogen atmosphere. Commercially available *n*-butyllithium (Aldrich) was a 1.6 M solution in hexane. The other analytical grade solvents and commercially available reagents were used without further purification. 3-Hydroxy-4-carbomethoxy-5-methylcyclohexenone **3** was prepared starting from methyl crotonate and methyl acetoacetate as described.[15] The ketal **12** was prepared as described[20] and (3-methyl-3-methoxy-1-butynyl)copper

was obtained starting from the commercially available (Aldrich) cuprous acetylide (2-methylbut-3-yn-2-ol) as already described elsewere.[24]

*Synthesis of the aromatic precursors (**4a-d**)*

*Methyl 3-bromo-2,4-dihydroxy-6-methylbenzoate (**4a**)*

To a solution of cyclohexenone **3** (1.0 g, 5.4 mmol) in acetic acid (10 mL) 2 equivalents of bromine (0.56 mL, 10.8 mmol) were added. The mixture was first stirred at room temperature for 30 min and then heated at 100 °C for further 30 min. After cooling to rt, the mixture was poured into water/ice and extracted with ethyl acetate. The organic layer was washed with a saturated sodium metabisulphite solution and brine. The crude product was purified by column chromatography on silica gel (hexane:diethyl ether 7:3) (772 mg, 55%).^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 2.49 (s, 3H); 3.96 (s, 3H); 6.00 (bs 1H); 6.47 (s, 1H); 12.56 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 24.2; 52.3; 96.6; 106.2; 110.7; 142.6; 156.8; 161.1; 172.0. MS (EI): m/z 262 (M^+, 30); 260 (M^+, 30); 230 (98); 228 (100); 149 (20).

*Methyl 3,5-dibromo-2,4-dihydroxy-6-methylbenzoate (**4c**)*

To a solution of cyclohexenone **3** (1.0 g, 5.4 mmol) in acetic acid (10 mL) 3 equivalents of bromine (0.84 mL, 16.2 mmol) were added. The mixture was first stirred at room temperature for 30 min and then heated at 100°C for further 30 min. After cooling to rt, the mixture was poured into water/ice and extracted with ethyl acetate. The organic layer was washed with a saturated sodium metabisulphite solution and brine. The crude product was purified by crystallization from hexane (826 mg, 45%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.66 (s, 3H); 3.98 (s, 3H); 6.48 (s 1H); 12.19 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 23.3; 52.8; 96.3; 105.5; 107.7; 140.5; 153.9; 159.4; 171.2. MS (EI): m/z 342 (M^+, 12); 340 (M^+, 24); 338 (M^+, 12); 310 (50); 308 (100); 306 (50).

*Methyl 3-bromo-5-chloro-2,4-dihydroxy-6-methylbenzoate (**4b**)*

To a solution of the monobromide **4a** (0.524 g, 2.0 mmol) in diethyl ether (4 mL) a solution of sulfuryl chloride (1 M in dichloromethane, 4 mL) was added and the mixture was stirred at room temperature for 3 h. By solvent removal under reduced pressure (300-400 mbar, 40°C) the pure product was obtained (560.5 mg, 95%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.61 (s, 3H); 3.98 (s, 3H); 6.46 (bs 1H); 12.23 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 19.8; 52.7; 96.6; 107.3; 113.8; 138.7; 153.3; 159.0; 171.3. MS (EI): m/z 298 (M^+, 6); 296 (M^+, 24); 294 (M^+, 18); 266 (26); 364 (100); 262 (78).

*Methyl 3-bromo-2,4-dihydroxy-5-fluoro-6-methylbenzoate (**4d**)*

To a solution of the monobromide **4a** (0.930 g, 3.58 mmol) in methanol (25 mL) Selectfluor® (1.65 g, 1.3 eq.) was added and the mixture was heated to 50°C for 24 h. The reaction was monitored by GC-MS analysis and quenched, when no further conversion occurred (about 50%), by cooling at room temperature and removing the solvent (300 mbar, 40°C). Then the residue was treated with water and extracted with diethyl ether. The pure product was obtained by column chromatography on silica gel (dichloromethane) in 45% yield (448 mg). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 2.68 (s, 3H); 3.98 (s, 3H); 6.48 (s 1H); 12.19 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 13.6 (d, J = 7.5 Hz); 52.5; 96.8 (d, J = 2.2 Hz); 104.9; 126.7 (d, J = 14.4 Hz); 143.2 (d, J = 231.4 Hz); 147.0 (d, J = 21.7 Hz); 157.0 (d, J = 2.3 Hz); 171.6 (d, J = 3.0 Hz). ^{19}F (376 MHz, $CDCl_3$) δ (ppm): -145.4. MS (EI): m/z 280 (M^+, 26); 278 (M^+, 27); 248 (98); 246 (100).

General procedure for the protection of phenolic groups

2,4-dihydroxybenzoates **4a-d** (1.0 mmol) were dissolved in 4 mL of dry dichloromethane and DIPEA (5.0 mmol, 1.04 mL) was added, followed by the slow addition of the SEM-Cl or MEM-Cl (4.0 mmol). The mixture was stirred at room temperature for 2-4 h and then poured into water/ice and extracted with diethyl ether. The crude products were purified by column chromatography on silica gel (hexane:diethyl ether 7:3 for SEM-ethers and 1:1 for MEM-protected compounds).

*Methyl 2,4-bis[2-(trimethylsilyl)ethoxymethoxy]-3-bromo-6-methylbenzoate (**5a**)*

Following the general procedure the product was isolated in quantitative yield (521 mg) as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.01 (s, 9H); 0.04 (s, 9H); 0.96 (t, *J* = 8.5 Hz, 2H); 1.01 (t, *J* = 8.5 Hz, 2H); 2.29 (s, 3H); 3.79 (t, *J* = 8.5 Hz, 2H); 3.85 (t, *J* = 8.5 Hz, 2H); 3.90 (s, 3H); 5.15 (s, 2H); 5.30 (s, 2H); 6.83 (s 1H).

*Methyl 2,4-bis[2-(trimethylsilyl)ethoxymethoxy]-3-bromo-5-chloro-6-methylbenzoate (**5b**)*

Following the general procedure the product was isolated in quantitative yield (556 mg) as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.04 (s, 18H); 1.01 (m, 4H); 2.30 (s, 3H); 3.83 (t, *J* = 8.5, Hz, 2H); 3.92 (s, 3H); 4.00 (t, *J* = 8.5 Hz, 2H); 5.14 (s, 2H); 5.21 (s, 2H).

*Methyl 2,4-bis[2-(trimethylsilyl)ethoxymethoxy]-3-bromo-5-fluoro-6-methylbenzoate (**5d**)*

Following the general procedure the product was isolated in 95% yield (512 mg) as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.04 (s, 18H); 1.19 (m, 4H); 2.34 (s, 3H); 3.85 (t, *J* = 8.5 Hz, 2H); 3.92 (s, 3H); 4.02 (t, *J* = 8.5 Hz, 2H); 5.15 (s, 2H); 5.21 (s, 2H).

*Methyl 2,4-bis[(2-methoxyethoxy)methoxy]-3-bromo-6-methylbenzoate (**6a**)*

Following the general procedure the product was isolated in 82% yield (357 mg) as colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 2.34 (s, 3H); 3.37 (s, 3H); 3.40 (s, 3H); 3.72 (m, 4H); 3.79 (m, 2H); 3.85 (m, 2H); 3.89 (s, 3H); 5.24 (s, 2H); 5.33 (s, 2H); 6.93 (s, 1H).

*Methyl 2,4-bis[(2-methoxyethoxy)methoxy]-3,5-dibromo-6-methylbenzoate (**6c**)*

Following the general procedure the product was isolated in 80% yield (413 mg) as colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.32 (s, 3H); 3.38 (s, 3H); 3.39 (s, 3H); 3.58 (m, 4H); 3.70 (m, 2H); 3.91 (s, 3H); 4.09 (m, 2H); 5.18 (s, 2H); 5.24 (s, 2H).

Asymmetric synthesis of the side chain

*(R,E)-6,7-Dihydroxy-3,7-dimethyloct-2-en-1-yl acetate ((R)-**7**)*

AD-mix-β (14 g, 10 mmol) and methanesulfonamide (951 mg, 10 mmol) were added to a 1:1 mixture of *t*-BuOH:H_2O (100 mL) and vigorously stirred for 15 min at room temperature. After cooling to 0 °C, geranyl acetate (2.13 mL, 10 mmol) was added, the mixture was stirred at that temperature for 24 h, then quenched with solid sodium metabisulfite (13.5 g). The mixture was extracted several times with dichloromethane and the combined organic layers were washed with a 10% aq. NaOH solution and brine. The crude product was purified by column chromatography on silica gel (hexane:ethyl acetate 7:3) to afford the diol (*R*)-**7** in 96% yield (2.20 g) as a colorless oil. $[\alpha]_D^{25}$ = +26.1 (c = 0.7, $CHCl_3$), lit.[28] $[\alpha]_D^{23}$ = +26.8 (c = 1.0, $CHCl_3$). Following the same procedure with AD-mix-α, the enantiomeric diol (*S*)-**7** was isolated in 90% yield (2.07 g). $[\alpha]_D^{25}$ = −23.9 (c = 0.9, $CHCl_3$), lit.[29] $[\alpha]_D^{20}$ = −25.1 (c = 0.7, EtOH). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.17 (s, 3H); 1.22 (s, 3H); 1.45 (m, 1H); 1.62 (m, 1H); 1.73 (s, 3H); 1.91 (br s, 2H); 2.06 (s, 3H); 2.12 (m, 1H); 2.32 (m, 1H); 3.45 (dd, *J* = 10.5, 2.0 Hz, 1H) 4.59 (d, *J* = 7.0 Hz, 2H); 5.40 (br t, *J* = 7.0 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 16.4; 21.01; 23.2; 26.5; 29.4; 35.6; 61.3; 73.0;

78.0; 118.7; 142.0; 171.1. MS (EI): m/z 170 (M^+ - AcOH, 1); 152 (3); 111 (18); 94 (32); 81 (27); 68 (72); 59 (100); 43 (73).

*(R,E)-3-Methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl acetate ((R)-**8**)*

To a solution of the diol (*R*)-**7** (2.06 g, 9 mmol) in methanol (5 mL) dimethoxypropane (15 mL) and *p*-toluensulfonic acid monohydrate (10 mg) were sequentially added and the mixture was stirred at room temperature for 3 h. The mixture was quenched with pyridine (0.051 mL) and concentrated under reduced pressure. The residue was dissolved in ethyl acetate and filtered on a silica gel pad to give the pure ketal in 93% yield (2.26 g). $[\alpha]_D^{25}$ = −2.0 (c = 1, $CHCl_3$). Following the same procedure, the ketal (*S*)-**8** was isolated in 90% yield (2.19 g). $[\alpha]_D^{25}$ = +2.0 (c = 1, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.10 (s, 3H); 1.24 (s, 3H); 1.33 (s, 3H); 1.42 (s, 3H); 1.50 (m, 1H); 1.65 (m, 1H); 1.73 (s, 3H); 2.06 (s, 3H); 2.10 (m, 1H); 2.29 (m, 1H); 3.65 (dd, *J* = 9.5, 3.0 Hz, 1H) 4.59 (d, *J* = 7.5 Hz, 2H); 5.39 (dt, *J* = 7.0, 1.5 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 16.4; 20.9; 22.7; 25.8; 26.7; 27.1; 28.3; 36.4; 61.1; 79.9; 82.5; 106.4; 118.6; 141.3; 170.8. MS (EI): m/z 255 (M^+ - 15, 52); 153 (54); 135 (60); 85 (50); 81 (65); 71 (84); 43 (100).

*(R,E)-3-Methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-ol ((R)-**9**)*

To a solution of the ketal (*R*)-**8** (2.16 g, 8 mmol) in methanol (27 mL) potassium carbonate (510 mg) was added and the mixture was stirred at room temperature. After 24 h the mixture was quenched with 2 mL of a saturated aq. solution of NH_4Cl and methanol was removed under reduced pressure. The residue was dissolved in ethyl acetate and the organic phase was washed with brine. The pure alcohol (*R*)-**9** was isolated by evaporation of the solvent in 92% yield (1.68 g) as a colorless oil. $[\alpha]_D^{25}$ = −2.8 (c = 1, $CHCl_3$) . Following the same procedure, the alcohol (*S*)-**9** was obtained in 92% yield (1.68 g). $[\alpha]_D^{25}$ = +3.1 (c = 1, $CHCl_3$). Both alcohols **9** were stored at - 20 °C under argon atmosphere. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.10 (s, 3H); 1.25 (s, 3H); 1.33 (s,

3H); 1.42 (s, 3H); 1.52 (m, 1H); 1.64 (m, 1H); 1.70 (s, 3H); 2.04 (s, 1H); 2.07 (m, 1H); 2.27 (m, 1H); 3.65 (dd, *J* = 9.2, 3.2 Hz, 1H) 4.17 (d, *J* = 7.0 Hz, 2H); 5.46 (br t, *J* = 7.0 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 16.3; 22.9; 26.0; 26.9; 27.5; 28.6; 36.6; 59.3; 80.1; 82.9; 106.6; 123.6; 139.1. MS (EI): m/z 213 (M^+ - 15, 46); 142 (48); 109 (22); 81 (100); 71 (92); 59 (60); 43 (88).

In order to determine the enantiomeric excesses by chiral HPLC analysis, a small portion of both alcohols **9** was transformed into the corresponding benzoate as follows: anhydrous triethylamine (21 μL, 0.15 mmol) and benzoyl chloride (12 μL, 0.1 mmol) were sequentially added to a solution of the alcohol (23 mg, 0.1 mmol) in anhydrous diethyl ether at 0°C. The mixture was then stirred at room temperature for 16 h, poured into icy water and extracted with ethyl acetate to give the benzoate in 80% yield (25.8 mg). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.12 (s, 3H); 1.25 (s, 3H); 1.33 (s, 3H); 1.43 (s, 3H); 1.55 (m, 1H); 1.68 (m, 1H); 1.81 (s, 3H); 2.14 (m, 1H); 2.33 (m, 1H); 3.68 (dd, *J* = 9.5, 3.5 Hz, 1H) 4.87 (d, *J* = 7.5 Hz, 2H); 5.54 (br t, *J* = 7.5 Hz, 1H); 7.44 (t, *J* = 8.0 Hz, 2H); 7.56 (t, *J* = 8.0 Hz, 1H); 8.06 (d, *J* = 8.0 Hz, 2H). The enantiomers were separated by HPLC on Chiralcel-OD column, hexane:2-propanol 98:2, 254 nm: t_R(*S*) = 9.6 min, t_R(*R*) = 11.7 min. The alcohol (*R*)-**9** was found to possess 98% ee, while its enantiomer (*S*)-**9** gave 94% ee.

*(R,E)-5-(5-bromo-3-methylpent-3-en-1-yl)-2,2,4,4-tetramethyl-1,3-dioxolane ((R)-**10**)*

To a solution of the alcohol (*R*)-**9** (230 mg, 1.0 mmol) in anhydrous diethyl ether PBr_3 (48 μL, 0.51 mmol) was slowly added at −7°C. After 20 min the mixture was diluted with diethyl ether and poured into a cooled 10% aq. $NaHCO_3$ solution. The separated organic phase was washed with $NaHCO_3$ and brine and the solvent removed under reduced pressure to give the pure bromide (*R*)-**10** in 90% yield (262 mg). Bromide (*S*)-**10** was obtained by the same procedure, starting from alcohol (*S*)-**9**, in the same 90% yield (262 mg). These compounds are stable for about one week only if stored at −20 °C under argon atmosphere. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.10 (s, 3H); 1.25

(s, 3H); 1.33 (s, 3H); 1.42 (s, 3H); 1.50 (m, 1H); 1.63 (m, 1H); 1.75 (s, 3H); 2.12 (m, 1H); 2.31 (m, 1H); 3.66 (dd, *J* = 9.5, 2.5 Hz, 1H) 4.03 (d, *J* = 8.3 Hz, 2H); 5.59 (br t, *J* = 8.3 Hz, 1H).

*(E)-5-(4',4'-dimethyl-5,7-dihydrospiro[dibenzo[a,c][7]annulene-6,2'-[1,3]dioxolan]-5'-yl)-3-methylpent-2-en-1-yl acetate (**13**)*

To a solution of the ketal **12** (91.6 mg, 0.36 mmol) in anhydrous $CHCl_3$ (3 mL) the diol (*S*)-**7** (83 mg, 0.36mmol) and traces of *p*-toluenesulfonic acid mono-hydrate were added in the presence of 4-Å molecular sieves. After 8 h of stirring at room temperature, the reaction mixture was filtered and the solvent removed under reduced pressure. The crude product was purified by column chromatography on silica gel (diethyl ether:petroleum ether 1:3) giving the biphenyl dioxolane **13** as a yellow oil (43.8 mg, 29% yield). $[\alpha]_D^{25}$ = −70.0 (c = 1.75, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.22 (s, 3H); 1.28 (s, 3H); 1.59 (m, 1H); 1.69 (m, 1H); 1.75 (s, 3H); 2.06 (s, 3H); 2.18 (m, 1H); 2.32 (m, 1H); 2.65 (m, 1H); 2.72 (m, 2H); 2.86 (m, 1H); 3.75 (br s, 1H); 4.64 (br s, 2H); 5.46 (br s, 1H); 7.24 (t, *J* = 7 Hz, 1H); 7.30 (m, 3H); 7.36 (t, *J* = 7 Hz, 2H); 7.44 (d, *J* = 7.0 Hz, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 10.9; 20.0; 21.1; 26.8; 35.9; 36.6; 43.9; 50.4; 61.4; 80.3; 82.5; 116.2; 119.2; 126.9; 127.3; 128.1; 128.5; 136.1; 140.2; 145.7; 171.1.

*General procedure for coupling reactions: synthesis of benzoates **14** and **15***

A solution of 3-methoxy-3-methyl-1-butyne (57 mg, 0.57 mmol) in THF (0.5 mL) was treated with *n*-BuLi (1.6 M in hexane, 356 μL, 0.57 mmol) at 0°C, stirred for 5 min and added to a suspension of copper iodide (109 mg, 0.57 mmol) in THF (0.5 mL), precooled to 0°C, giving a red-orange solution which was stirred at that temperature for 30 min. Then HMPA (175 μL) was added and the resulting solution of the cuprous acetylide was transferred to a solution of the desired lithium-aryl reagent previously prepared as follows: the benzoates **5** (or **6**) (0.5 mmol) were dissolved in THF (3 mL), the solution was cooled at −78°C, treated with *n*-BuLi (0.57mmol) and, after 15 min, with the

solution of cuprous acetylide. The resulting clear solution was stirred at −78°C for 30 min and the optically active bromide **10** (0.57 mmol) solution in THF (0.7 mL) was added. The mixture was slowly warmed to room temperature and stirred for 16-20 h. The mixture was poured in a pH 8 aqueous ammonia/ saturated ammonium chloride solution and extracted with diethyl ether. The organic phase was filtered on Celite and the crude products were purified by column chromatography on silica gel.

*(S,E)-methyl 6-methyl-3-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)-2,4-bis((2-(trimethylsilyl)ethoxy)methoxy)benzoate ((S)-**14a**)*

The product was isolated by column chromatography on silica gel (hexane:diethyl ether 3:1) in 42% yield (137 mg). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 0.00 (s, 9H); 0.02 (s, 9H); 0.97 (m, 4H); 1.06 (s, 3H); 1.21(s, 3H); 1.25 (m, 1H); 1.27 (s, 3H); 1.39 (s, 3H); 1.58 (m, 1H); 1.77 (s, 3H); 1.98 (m, 1H); 2.18 (m, 1H); 2.28 (s, 3H); 3.37 (d, *J* = 6.8 Hz, 2H); 3.61 (dd, *J* = 9.4, 3.4 Hz, 1H); 3.75 (m, 4H); 3.88 (s, 3H); 4.99 (s, 2H); 5.20 (br t, *J* = 7.0 Hz, 1H); 5.22 (s, 2H); 6.75 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): -1.4; 16.3; 18.0;18.1; 19.9; 22.9; 23.2; 26.0; 26.7; 27.7; 28.5; 36.7; 52.0; 66.3; 67.5; 80.0; 82.9; 92.6; 99.0; 106.4; 111.8; 122.0; 123.2; 134.3; 135.2; 153.6; 157.0; 168.7.

*(R,E)-methyl 2,4-bis((2-methoxyethoxy)methoxy)-6-methyl-3-(3-methyl-5-(2,2,5,5tetramethyl- 1,3-dioxolan-4-yl)pent-2-en-1-yl)benzoate ((R)-**15a**)*

The product was purified by column chromatography on silica gel (hexane:ethyl acetate 1:1). In this case a further chromatographic purification was necessary (silica gel, chloroform) affording the pure product in 40% yield (114 mg). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.08 (s, 3H); 1.21(s, 3H); 1.27 (s, 3H); 1.30 (m, 1H); 1.40 (s, 3H); 1.44 (m, 1H); 1.58 (m, 1H); 1.77 (s, 3H); 2.00 (m, 1H); 2.18 (s, 3H); 3.32 (d, *J* = 7.0 Hz, 2H); 3.37 (s, 3H); 3.39 (s, 3H); 3.55 (m, 4H); 3.62 (dd, *J* =

9.5, 3.0 Hz, 1H); 3.72 (m, 2H); 3.79 (m, 2H); 3.88 (s, 3H); 4.83 (s, 2H); 5.03 (br t, *J* = 6.8 Hz, 1H); 5.22 (s, 2H); 6.84 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 16.2; 16.5; 22.9; 24.9; 26.0; 26.7; 27.6; 28.5; 36.6; 52.1; 59.0; 67.4; 67.7; 71.4; 71.5; 71.7; 80.0; 82.8; 92.3; 93.5; 94.0; 100.1; 106.4; 122.7; 123.4; 134.2; 135.2; 152.7; 156.1; 169.1.

*(R,E)-methyl 3-bromo-4,6-bis((2-methoxyethoxy)methoxy)-2-methyl-5-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)benzoate ((R)-**15c**)*

The product was isolated in 70% yield (226 mg) by column chromatography on silica gel (hexane:diethyl ether 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.07 (s, 3H); 1.21(s, 3H); 1.27 (s, 3H); 1.39 (s, 3H); 1.43 (m, 1H); 1.57 (m, 1H); 1.74 (s, 3H); 1.99 (m, 1H); 2.18 (dt, *J* = 10.0, 4.5 Hz, 1H); 2.32 (s, 3H); 3.38 (s, 3H); 3.39 (s, 3H); 3.48 (d, *J* = 6.0 Hz, 2H); 3.58 (m, 4H); 3.60 (dd, *J* = 10, 3.2 Hz, 1H); 3.83 (m, 2H); 3.90 (s, 3H); 3.97 (m, 2H); 5.04 (s, 2H); 5.14 (s, 2H); 5.22 (br t, *J* = 6.4 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 16.4; 20.8; 22.9; 24.6; 26.0; 26.7; 27.6; 28.5; 36.5; 52.4; 59.1; 66.7; 69.3; 69.6; 71.6; 71.7; 80.0; 82.7; 95.6; 98.7; 99.7; 106.4; 122.7; 127.1; 129.2; 134.5; 135.1; 152.3; 154.6; 167.9.

*(S,E)-methyl 3-chloro-2-methyl-5-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)-4,6-bis((2-(trimethylsilyl)ethoxy)methoxy)benzoate ((S)-**14b**)*

The product was isolated in 72% yield (247 mg) by column chromatography on silica gel (hexane: diethyl ether 3:1). By the same procedure, starting from 0.3 mmol of substrate, the enantiomer (*R*)-**14b** was obtained in 40% yield (82 mg). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.02 (s, 9H); 0.03 (s, 9H); 0.98 (m, 4H); 1.06 (s, 3H); 1.21 (s, 3H); 1.27 (s, 3H); 1.39 (s, 3H); 1.43 (m, 1H); 1.58 (m, 1H); 1.77 (s, 3H); 2.00 (m, 1H); 2.19 (dt, *J* = 10.5, 4.5 Hz, 1H); 2.29 (s, 3H); 3.37 (d, *J* = 6.8 Hz, 2H); 3.61 (dd, *J* = 9.5, 3.5 Hz, 1H); 3.76 (m, 2H); 3.89 (m, 2H); 3.91 (s, 3H); 4.99 (s, 2H); 5.09 (s, 2H); 5.25 (br t, *J* = 6.0 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): −1.4; 16.5; 17.6; 18.1; 22.9;

24.4; 26.0; 26.7; 27.7; 28.5; 36.6; 52.4; 67.6; 67.8; 80.0; 82.8; 97.8; 99.1; 106.4; 122.8; 124.9; 126.9; 129.2; 132.7; 135.0; 151.6; 153.6; 168.0.

*(R,E)-methyl 3-fluoro-2-methyl-5-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en -1-yl)-4,6-bis((2-(trimethylsilyl)ethoxy)methoxy)benzoate ((R)-**14d**)*

The product was isolated in 46% yield (154 mg) by column chromatography on silica gel (hexane:diethyl ether 3:1) followed by further column chromatography on silica gel (dichloromethane). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.03 (s, 9H); 0.04 (s, 9H); 0.99 (m, 4H); 1.08 (s, 3H); 1.22 (s, 3H); 1.29 (s, 3H); 1.41 (s, 3H); 1.43 (m, 1H); 1.61 (m, 1H); 1.78 (s, 3H); 2.02 (m, 1H); 2.20 (s, 3H); 2.21 (m, 1H); 3.44 (d, *J* = 6.0 Hz, 2H); 3.63 (br d, *J* = 9.5 Hz, 1H); 3.78 (t, *J* = 8.5 Hz, 2H); 3.85 (t, *J* = 9.0 Hz, 2H); 3.91 (s, 3H); 4.98 (s, 2H); 5.18 (s, 2H); 5.24 (m, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): −1.4; 18.0; 18.1; 22.9 (d, *J* = 12.5 Hz); 24.0; 26.0 (d, *J* = 11.5 Hz); 26.7 (d, *J* = 9.5 Hz); 27.7; 28.5 (d, *J* = 7.6 Hz); 30.9; 31.0; 36.6; 52.3 (d, *J* = 20.0 Hz); 67.5; 67.6; 80.0; 82.9 (d, *J* = 19.0 Hz); 97.3 (d, *J* = 7.6 Hz); 99.2; 106.4; 121.9 (d, *J* = 19.5 Hz); 122.8 (d, *J* = 24.0 Hz); 125.0; 128.4; 134.9; 149.2 (d, *J* = 119.3 Hz); 151.6; 167.5; 206.9.

*General procedure for the synthesis of aldehydes **16** and **17***

To a suspension of $LiAlH_4$ (18 mg, 0.47 mmol) in diethyl ether (4 mL) a solution of benzoates **14** (or **15**, 0.2 mmol) in diethyl ether (5 mL) was added at 0°C and the mixture was stirred at room temperature for 2 h. Then it was diluted with diethyl ether (15-20 mL), treated with an aqueous saturated solution of sodium sulfate (167 μL), filtered and the solvent removed under reduced pressure. The crude alcohol was dissolved in dichloromethane (7 mL), treated with PCC (2 eq.) and stirred at room temperature for 1h. Then, 2 more equivalents of PCC were added and the mixture was stirred for further 2-3 hours, when a complete conversion to aldehyde was detected by TLC

analysis. The mixture was diluted with diethyl ether, filtered on celite and subsequently on a silica gel pad, eluting with diethyl ether. By removing the solvent at reduced pressure all pure aldehydes were recovered in about 90% overall yield.

*(S,E)-(6-methyl-3-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)-2,4-bis ((2-(trimethylsilyl)ethoxy)methoxy)benzaldehyde (**16a**)*

(111 mg, 89%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): −0.03 (s, 9H); 0.02 (s, 9H); 0.97 (m, 4H); 1.07 (s, 3H); 1.20(s, 3H); 1.24 (m, 1H); 1.27 (s, 3H); 1.39 (s, 3H); 1.59 (m, 1H); 1.79 (s, 3H); 2.01 (m, 1H); 2.21 (m, 1H); 2.59 (s, 3H); 3.39 (d, *J* = 6.5 Hz, 2H); 3.63 (dd, *J* = 9.5, 4.0 Hz, 1H); 3.76 (m, 2H); 3.84 (m, 2H); 5.06 (s, 2H); 5.22 (br t, *J* = 6.8 Hz, 1H); 5.30 (s, 2H); 6.78 (s, 1H); 10.36 (s, 1H).

*(R,E)-(2,4-bis((2-methoxyethoxy)methoxy)-6-methyl-3-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)benzaldehyde (**17a**)*

(112.5 mg, 88%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.07 (s, 3H); 1.20 (s, 3H); 1.25 (s, 3H); 1.39 (s, 3H); 1.45 (m,1H); 1.58 (m, 1H); 1.78 (s, 3H); 2.01 (m, 1H); 2.18 (dt, *J* = 10.0, 4.5 Hz, 1H); 2.53 (s, 3H); 3.36 (d, *J* = 7.0 Hz, 2H); 3.38 (s, 3H); 3.39 (s, 3H); 3.57 (m, 4H); 3.61 (dd, *J* = 9.0, 3.5 Hz, 1H); 3.79 (m, 2H); 3.84 (m, 2H); 4.83 (s, 2H); 5.02 (br t, *J* = 6.8 Hz, 1H); 5.33 (s, 2H); 6.85 (s, 1H); 10.54 (s, 1H).

*(R,E)-(3-bromo-4,6-bis((2-methoxyethoxy)methoxy)-2-methyl-5-(3-methyl-5-(2,2,5,5-tetra methyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)benzaldehyde (**17c**)*

(111 mg, 90%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.07 (s, 3H); 1.21 (s, 3H); 1.27 (s, 3H); 1.39 (s, 3H); 1.44 (m,1H); 1.59 (m, 1H); 1.77 (s, 3H); 2.01 (m, 1H); 2.18 (m, 1H); 2.67 (s, 3H); 3.37 (s, 3H); 3.39 (s, 3H); 3.48 (d, *J* = 6.0 Hz, 2H); 3.55 (m, 2H); 3.60 (m, 2H); 3.63 (dd, *J* = 10.0, 3.6 Hz,

1H); 3.86 (m, 2H); 3.98 (m, 2H); 5.09 (s, 2H); 5.21 (s, 2H); 5.23 (br t, *J* = 6.5 Hz, 1H); 10.34 (s, 1H).

*(S,E)-(3-chloro-2-methyl-5-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)-4,6-bis((2-(trimethylsilyl)ethoxy)methoxy)benzaldehyde (**16b**)*

(121 mg, 92%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.03 (s, 18H); 0.99 (m, 4H); 1.07 (s, 3H); 1.21(s, 3H); 1.27 (s, 3H); 1.40 (s, 3H); 1.44 (m, 1H); 1.59 (m, 1H); 1.79 (s, 3H); 2.02 (m, 1H); 2.21 (m, 1H); 2.63 (s, 3H); 3.47 (d, *J* = 6.5 Hz, 2H); 3.63 (dd, *J* = 9.2, 3.2 Hz, 1H); 3.81 (m, 2H); 3.91 (m, 2H); 5.02 (s, 2H); 5.18 (s, 2H); 5.26 (br t, *J* = 7.0 Hz, 1H); 10.36 (s, 1H).

*(R,E)-(3-fluoro-2-methyl-5-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)-4,6-bis((2-(trimethylsilyl)ethoxy)methoxy)benzaldehyde (**16d**)*

(113 mg, 88%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 0.07 (s, 18H); 0.97 (m, 4H); 1.07 (s, 3H); 1.20 (s, 3H); 1.26 (s, 3H); 1.39 (s, 3H); 1.43 (m, 1H); 1.60 (m, 1H); 1.79 (s, 3H); 2.02 (m, 1H); 2.20 (m, 1H); 2.47 (s, 3H); 3.43 (d, *J* = 5.5 Hz, 2H); 3.62 (dd, *J* = 9.5, 3.5 Hz, 1H); 3.81 (m, 4H); 5.01 (s, 2H); 5.23 (br t, *J* = 6.0 Hz, 1H); 5.26 (s, 2H); 10.34 (s, 1H).

Deprotection reactions: synthesis of colletorin A, colletochlorin A and analogues

Method A: step 1 (general procedure)

To a solution of SEM-protected 2,4-dihydroxybenzaldehydes **16** (0.16 mmol) in HMPA (1,6 mL) a TBAF solution (1M in THF, 10 eq., 1.6 mL) was added at room temperature. Then the reaction mixture was warmed up to 70°C and stirred for 4-5 h. After removal of THF, the residue was poured in a mixture of ethyl acetate and brine. The solution was extracted with ethyl acetate and the

solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (hexane: ethyl acetate 3:1) affording the corresponding ketals **18**.

*(R,E)-2,4-dihydroxy-6-methyl-3-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)pent-2-en-1-yl)benzaldehyde ((S)-**18a**)*

Following the general procedure the product was isolated in 70% yield (70.8 mg) as a pale yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.08 (s, 3H); 1.22 (s, 3H); 1.28 (s, 3H); 1.39 (s, 3H); 1.50 (m,1H); 1.63 (m, 1H); 1.84 (s, 3H); 2.11 (m, 1H); 2.26 (m, 1H); 2.49 (s, 3H); 3.40 (d, *J* = 7.0 Hz, 2H); 3.63 (dd, *J* = 10.0, 3.5 Hz, 1H); 5.30 (br t, *J* = 7.0 Hz, 1H); 6.05 (br s, 1H); 6.21 (s, 1H); 10.08 (s, 1H); 12.74 (s, 1H).

*(S,E)-3-chloro-4,6-dihydroxy-2-methyl-5-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl) pent-2-en-1-yl)benzaldehyde ((S)-**18b**)*

Following the general procedure the product was isolated in 70% yield (44.5 mg) as a pale yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.07 (s, 3H); 1.19 (s, 3H); 1.23 (s, 3H); 1.39 (s, 3H); 1.47 (m,1H); 1.61 (m, 1H); 1.80 (s, 3H); 2.03 (m, 1H); 2.19 (dt, *J* = 12.5, 5.5 Hz, 1H); 2.60 (s, 3H); 3.40 (d, *J* = 9.0 Hz, 2H); 3.61 (dd, *J* = 11.5, 4.5 Hz, 1H); 5.26 (br t, *J* = 9.0 Hz, 1H); 6.40 (br s, 1H); 10.13 (s, 1H); 12.69 (s, 1H).

*(R,E)-3-fluoro-4,6-dihydroxy-2-methyl-5-(3-methyl-5-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl) pent-2-en-1-yl)benzaldehyde ((R)-**18d**)*

Following the general procedure the product was isolated in 70% yield (42.6 mg) as pale yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.06 (s, 3H); 1.20 (s, 3H); 1.25 (s, 3H); 1.38 (s, 3H); 1.44 (m,1H); 1.60 (m, 1H); 1.79 (s, 3H); 2.01 (m, 1H); 2.18 (dt, *J* = 10.0, 3.5 Hz, 1H); 2.40 (s, 3H); 3.35

(d, *J* = 7.0 Hz, 2H); 3.62 (dd, *J* = 10.5, 3.0 Hz, 1H); 4.16 (br s, 1H); 5.28 (br t, *J* = 7.0 Hz, 1H); 9.91 (s, 1H); 12.61 (s, 1H).

Method A: step 2 (general procedure)

To a solution of ketals **18** (0,11 mmol) in THF: dichloromethane 1:1 mixture (3 mL) were added in sequence H_2O (123 μL) and conc. H_2SO_4 (84 μL). The reaction mixture was stirred at room temperature for 30 min. and then was quenched adding dropwise saturated aqueous $NaHCO_3$ up to pH = 7. The resulting mixture was extracted with ethyl acetate and the organic layer was washed with brine. The solvent was evaporated under reduced pressure and the obtained residue was purified by column chromatography on silica gel (hexane:ethyl acetate 1:1) affording the desired products (*S*)-**1a**, (*S*)-**1b** and (*R*)-**1d**.

*(S)-Colletorin A ((S)-**1a**)*

Following the general procedure, starting from (*S*)-**18a**, the product was isolated in 70% yield (24.8 mg) as a white foamy solid. $[\alpha]_D^{25}$ = -8.7 (c 0.3; $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.15 (s, 3H); 1.19 (s, 3H); 1.43 (m,1H); 1.69 (m, 1H); 1.82 (s, 3H); 2.04 (bs, 2H); 2.14 (m, 1H); 2.28 (m, 1H); 2.49 (s, 3H); 3.34 (d, *J* = 10.0 Hz, 1H); 3.39 (d, *J* = 6.8 Hz, 2H); 5.30 (br t, *J* = 6.8 Hz, 1H); 6.21 (s, 1H); 10.07 (s, 1H); 12.74 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 16.1; 18.0; 21.1; 23.2; 26.4; 29.3; 36.9; 73.2; 78.3; 110.6; 111.9; 113.2; 121.9; 138.3; 141.9; 162.2; 163.6; 192.9. Elemental analysis: found: C, 67.15; H, 8.06; O, 24.79. Calc. for $C_{18}H_{26}O_5$: C, 67.06; H, 8.13; O 24.81%.

*(S)-Colletochlorin A ((S)-**1b**)*

Following the general procedure, starting from (*S*)-**18b**, the product was isolated in 65% yield (25.5 mg) as a white foamy solid. $[\alpha]_D^{25}$ = −18.6 (c 0.5; $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm):

1.13 (s, 3H); 1.17 (s, 3H); 1.41 (m,1H); 1.58 (m, 1H); 1.79 (s, 3H); 2.02 (bs, 2H); 2.07 (m, 1H); 2.22 (m, 1H); 2.59 (s, 3H); 3.32 (dd, *J* = 12.0, 1.6 Hz, 1H); 3.39 (d, *J* = 6.7 Hz, 2H); 5.27 (br t, *J* = 6.7 Hz, 1H); 10.12 (s, 1H); 12.68 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 14.4; 16.0; 22.0; 23.2; 26.3; 29.5; 36.9; 73.0; 78.3; 113.2; 113.5; 114.2; 121.6; 136.4; 137.7; 156.4; 162.1; 193.2. Elemental analysis: found: C, 60.50; H, 7.09; Cl, 9.87; O, 22.54. Calc. for $C_{18}H_{25}ClO_5$: C, 60.59; H, 7.06; Cl, 9.94; O, 22.42%.

*(R)-Colletofluorin ((R)-**1d**)*

Following the general procedure, starting from (*R*)-**18d**, the product was isolated in 43% yield (16.1 mg) as a white foamy solid. $[\alpha]_D^{25}$ = +14.5 (c 0.4; $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.14 (s, 3H); 1.18 (s, 3H); 1.43 (m,1H); 1.59 (m, 1H); 1.80 (s, 3H); 2.05 (bs, 2H); 2.10 (m, 1H); 2.21 (m, 1H); 2.45 (s, 3H); 3.34 (d, *J* = 10.5 Hz, 1H); 3.37 (d, *J* = 6.5 Hz, 2H); 5.29 (br t, *J* = 6.5 Hz, 1H); 10.02 (s, 1H); 12.53 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 16.0; 21.4 (d, *J* = 1.5 Hz); 23.2; 26.3; 29.4; 29.7; 36.9; 73.0; 78.3; 111.2; 114.4; 121.8; 124.2 (d, *J* = 14.4 Hz); 136.5; 143.2 (d, *J* = 227.6 Hz); 150.5 (d, *J* = 16.7 Hz); 159.8; 192.7. ^{19}F (376 MHz, $CDCl_3$) δ (ppm): −152.3. Elemental analysis: found: C, 63.48; H, 7.45; F, 5.85; O, 23.22. Calc. for $C_{18}H_{25}FO_5$: C, 63.51; H, 7.40; F, 5.58; O, 23.50%.

Method B (general procedure)

6M HCl (24,4 eq., 407 μL) was slowly added to a stirred solution of **17a**, **17c** or **16b** (0,10 mmol) in THF (500 μL) at room temperature. After 16-18 h, the reaction was quenched adding dropwise saturated aqueous $NaHCO_3$ up to pH = 7. Then the solution was diluted with ethyl acetate and the organic layer was washed with brine. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography on silica gel (hexane:ethyl acetate 1:1) giving the pure compounds (*R*)-**1a**, (*R*)-**1b** and (*R*)-**1c**.

*(R)-Colletorin A ((R)-**1a**)*

Following the general procedure, starting from (*R*)-**17a**, the product was isolated in 30% yield (9.7 mg) as a white foamy solid. $[\alpha]_D^{25}$ = +9.8 (c 0.4; $CHCl_3$). ^{1}H- and ^{13}C-NMR spectra were identical to those of (*S*)-**1a**. Elemental analysis: found: C, 67.02; H, 8.15; O, 24.83. Calc. for $C_{18}H_{26}O_5$: C, 67.06; H, 8.13; O, 24.81%.

*(R)-Colletochlorin A ((R)-**1b**)*

Following the general procedure, starting from (*R*)-**16b**, the product was isolated in 37% yield (13.2 mg) as a white foamy solid. $[\alpha]_D^{25}$ = +13.0 (c 0.4; $CHCl_3$), lit.[3a] $[\alpha]_D^{25}$ = +11.6 (c 10; MeOH) . ^{1}H- and ^{13}C-NMR spectra were identical to those of (*S*)-**1b**. Elemental analysis: found: C, 60.62; H, 7.01; Cl, 9.97; O, 22.40. Calc. for $C_{18}H_{25}ClO_5$: C, 60.59; H, 7.06; Cl, 9.94; O, 22.42%.

*(R)-Colletobromin ((R)-**1c**)*

Following the general procedure, starting from (*R*)-**17c**, the product was isolated in 55% yield (22.0 mg) as a pale yellow foamy solid. $[\alpha]_D^{25}$ = +10.9 (c 0.5; $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.14 (s, 3H); 1.18 (s, 3H); 1.42 (m,1H); 1.59 (m, 1H); 1.81 (s, 3H); 2.05 (bs, 2H); 2.09 (m, 1H); 2.24 (m, 1H); 2.65 (s, 3H); 3.36 (br d, *J* = 10.0 Hz, 1H); 3.43 (d, *J* = 7.2 Hz, 2H); 5.28 (br t, *J* = 7.0 Hz, 1H); 6.42 (bs, 1H); 10.16 (s, 1H); 12.73 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 16.1; 17.6; 22.2; 23.3; 26.3; 29.5; 36.9; 72.9; 78.3; 106.1; 114.2; 114.3; 121.6; 136.6; 139.7; 156.9; 162.7; 193.5. Elemental analysis: found: C, 53.83; H, 6.32; Br, 19.89; O, 19.96. Calc. for $C_{18}H_{25}FO_5$: C, 53.87; H, 6.28; Br, 19.91; O, 19.93%.

Plant biological assays

Leaf puncture assay. The metabolites were tested at 2 µg/µl concentration on *Ambrosia artemisiifolia* and *Sonchus arvensis* (family: Asteraceae). Droplets (20 µL) of the solution

containing the compound were applied to detached leaves previously punctured with a needle. Five replications were used for each plant species tested. Leaves were kept in a moistened chamber under continuous fluorescent lights at 25°C. The eventual appearance of symptoms, consisting in circular necrosis, was observed three days after droplet application. Control treatments were carried out by applying droplets of a methanol solution (MeOH 1%). Effects were expressed on a visual scale from "-" = no symptoms, to "++++" = necrosis around 1 cm diameter.

Lemna minor assay. Pure compounds were tested against *Lemna minor* at 2 µg/µl concentration, by adapting a protocol already described.[30] Briefly, the wells of sterile, polystyrene 96-well microtiter plates were filled with a 100 µL aliquot of solutions containing the metabolites to be tested at 2 µg/µl concentration. One frond of an actively growing axenic *L. minor* plant was placed into each well. Control wells were included in each plate. Four replications were prepared for each compound. The plates were incubated in a growth chamber with 12/24 h fluorescent lights and observed daily up to 7 days. One day after the application of the test solution, 100 µL of distilled H_2O was added to each well. The chlorophyll contents of the fronds were determined by extracting the fronds from each well with 95% ethanol. A 2.0 ml aliquot of ethanol was added to the test tube containing the fronds, and incubated in the dark for 6 h. This aliquot was removed, and a second 1.5 ml aliquot was added and incubated in the dark overnight. The ethanol extracts were combined and the chlorophyll content in each sample was determined spectrophotometrically from the absorbance at 649 and 665 nm by using the equation by Marr et al.[31]

Mosquitos biological assays

Topical and injection bioassays were performed essentially as described.[32] Adult females were reared from *Aedes aegypti* larvae provided by the United States Department of Agriculture, Agricultural Research Service (USDA, ARS, Gainesville, FL, USA). After emergence, adult female mosquitoes were maintained at 28°C under a 12 h light/dark cycle, and fed a diet of 10% sucrose solution. Compounds were dissolved in ethanol. Mosquitoes (2-7 days old) were briefly

anaesthetized on ice, and 200 nL of the treatment solution applied to the dorsal thorax with a microdispenser. Control treatments were vehicle alone. Mosquitoes were then held for 24 h in paper cups with 10% sucrose solution for sustenance. For toxicity by injection, each compound was dissolved in ethanol and diluted into mosquito saline (5% ethanol final concentration). After cold anesthesia, mosquitoes were placed on their sides, and a 0.2 µL aliquot was injected into the thorax with a glass capillary needle attached to a micro-syringe. After treatment, the mosquitoes were held in paper cups and fed sugar water, with mortality recorded at 24 h (n = 10/dose, replicated three times) in all cases. For synergism against monooxygenase metabolism, 500 ng of PBO dissolved in ethanol was applied topically to the mosquitoes 4 h before topical or injected treatment with test material.

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