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Chemoenzymatic synthesis and cytotoxicity of oenanthotoxin and analogues

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ABSTRACT

We developed a synthetic scheme for the synthesis of naturally occurring (14*R*)-oenanthotoxin and several analogs. Key-steps of this synthesis were an efficient homo-coupling of alkynes and a chemoenzymatic resolution of racemic oenanthotoxin using novozyme 435 and vinyl acetate. The compounds were screened for their cytotoxic activity using a photometric sulforhodamine B assays and several human tumor cell lines. Oenanthotoxin and many derivatives thereof were cytotoxic to tumor cell lines as well as to non-malignant mouse fibroblasts. The highest activity was determined for human ovarian cancer cells A2780 with EC_{50} = 3.8 μM.

1. Introduction

Recently the exceedingly poisonal plant *Oenathe crocata* (hemlock, water dropwort, dead man's finger) has been claimed[1] to be identical to herba Sardonica, a plant being common in Sardinia but also in western and southern Britain. It is regarded the most toxic plant in Britain to both humans and animals. Extracts of this plant have been used in pre-Roman Sardinia during the ritual killing of criminals or of people unable to support themselves.[1] Oenanthotoxin (**1**) and dihydro-oenanthotoxin (**2**, Fig. 1) were identified as the main toxic principles of this plant. Consumption of these compounds leads to a facial muscular contraction mimicking a sinister smile ('risus sardonicus' = sardonic smile),[1,2] a lockjaw, and small amounts are sufficient to cause death. Oenanthotoxin has also been 'discussed' as a chemistry tidbit for batman fans.[3] The most toxic part of the plant is its tuberous root (→ Dead man's fingers) but all parts of the plant are poisonous. The first report on poisonous constituents of this plant was given by J. Pohl as early as 1894.[4] On a molecular basis, it was the merit of Clarke et al.[5] and Anet et al.[6] to describe this toxic principle, while a first partial synthetic access to racemic oenanthotoxin has been established by Bohlmann et al. in 1968.[7,8] A median lethal dose (LD_{50}) is 2.94 mg/kg (rat) and 3.5 mg/kg (mouse), respectively.[5,9] Several cases of hemlock water dropwort poisoning of adults have been reported.[10–15]

The pro-convulsant effects of **1** are due to a down-regulation of GABAergic currents.[16,17] Compound **1** has been suggested for the treatment of excessive sleepiness[18] and for treating airway conditions.[19] In addition, polyacetylenes have been identified as new selective partial agonists of peroxisome proliferator activated receptor gamma (PPARγ).[17,20,21] Only a few analogues have been isolated from natural sources[22] so far and were investigated for their inhibitory effects on GABAergic currents in cultured rat hippocampal neurons.[17] Albeit there are several reports on the toxicity of these compounds, there are none on their cytotoxicity. For structurally related compounds, for example, falcarinols,[23] cicutoxin[24] or bupleurotoxin,[25] cytotoxic properties have been reported, but also their importance as health promoting compounds in food plants has been discussed.[23] Hence, we set out for a total synthesis of (14 *R*)-**1** and analogues, and to determine the cytotoxicity of these compounds employing several human tumor cell lines.

2. Results and discussion

2.1. Chemistry

Our synthetic approach started from 3,4-dihydro-2*H*-pyran (**3**, Scheme 1) whose treatment with NBS in MeOH[26,27] afforded 86% of *trans*-3-bromo-2-methoxytetrahydro-2*H*-pyran (**4**) that was reacted with methanolic KOH[28] to yield 2-methoxy-5,6-dihydropyran (**5**). This compound was also accessed in a more direct way from the reaction of **3** with NBS in MeOH followed by the addition of KOH in a one-pot reaction with an over-all yield of

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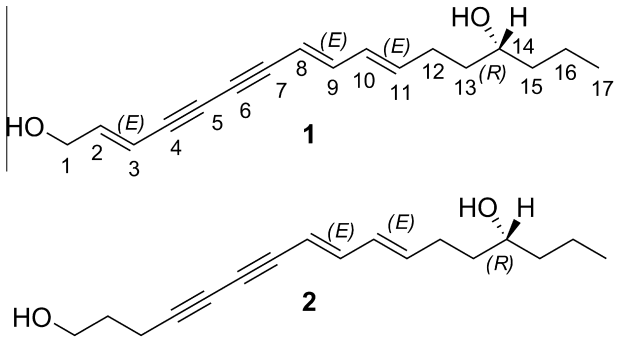


Figure 1. Structure of oenanthotoxin (**1**) and dihydro-oenanthotoxin (**2**) from *Oenanthe crocata* (hemlock, dead man's finger).

59%. Reaction of **5** with phosphoric acid[28] furnished 40% of (2*E*) penta-2,4-dienal (**6**), that could also be obtained—albeit in lower yields—from a similar reaction starting from **3**. Reaction of **6** with ethynylmagnesium bromide in the presence of lithium chloride[29] yielded 75% of the alkyne **7**[30,8] whose reaction with PBr_3 furnished 38% of **8**. This compound is very labile. It rapidly decomposes upon contact with silica gel. Purification by chromatography using basic Al_2O_3 followed by crystallization at 0 °C, however, gave pure **8**.

From the reaction of **8** with ethyl 3-oxo-hexanoate/sodium hydride compound **9** was obtained in 84% yield. This compound can be stored for several months at −20 °C in the dark without any significant decomposition. De-esterification of **9** (Scheme 2) followed by decarboxylation gave 51% of **10**. As an alternative to this classical synthesis a Weinreb–Nahm ketone synthesis was accomplished by converting **8** into its Weinreb amide **11** whose chain elongation reaction with propylmagnesium bromide in dry THF gave 96% of **10**. This compound was allowed to react in a Cadiot–Chodkiewicz coupling reaction[31] with (2*E*) 5-bromopent-2-en-4-yn-1-ol (**12**, obtained from the reaction of commercial (2*E*) pent-2-en-4-ynol with NBS in 55% yield)[32] to afford **13** but yields were low (18–29%), while the monocoupling of **10** with (2*E*) pent-2-en-4-ynol applying Li's modifications for the Glaser coupling[33] reaction gave **13** (50%) together with 25% of **14** as a side product.

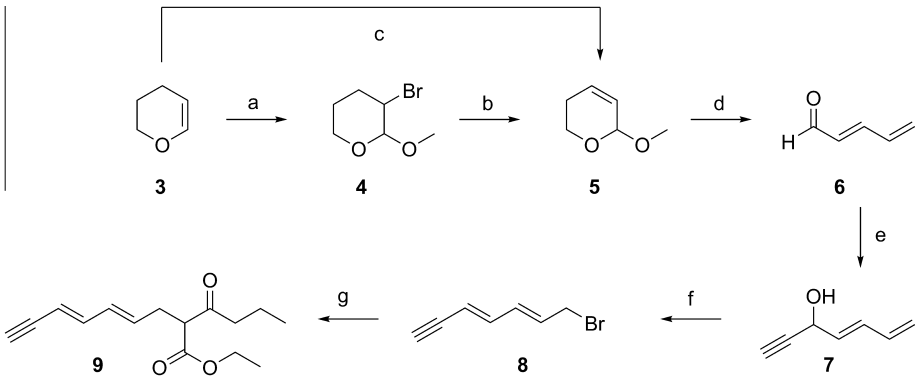
Reaction of **13** with $LiAlH_4$ gave racemic **1** in 83% isolated yield. Enantioselective reduction of **13** using (*R*)-(+) 2-methyl-CBS-oxazaborolidin[34,35] failed to give enantiomerically pure **1**. As an alternative a chemoenzymatic resolution was found to deliver better results. Thus, reaction of racemic **1** with vinyl acetate in the presence of the enzyme novozym 435[36] (Scheme 3) gave enantiomerically pure monoacetate **15** together with diacetate **16**. Reduction of **15** with $LiAlH_4$ gave optically pure (14*R*)-**1**. The optical purity was determined by a comparison of the optical rotation with data from the literature (found: $[\alpha]_D$ +30.3° (*c* 0.45, $CHCl_3$; reported:[1,6] $[\alpha]_D$ 34° (*c* 0.5, $CHCl_3$); its enantiomeric purity was determined by chiral HPLC using a 250 × 4.6 mm i.d. stainless-steel column packed with Daicel Chiralpak AD-H 5 μm material. Oenanthotoxin is photolabile. As determined by ^1H NMR spectroscopy, standing of a chloroform solution of **1** for 10 min in bright daylight, resulted in the formation of approximately 5% of a (2*E*,8*Z*,10*E*) stereoisomer. Compound **1** is characterized in its IR spectrum by the presence of bands at ν = 949 and 985 cm^{-1} being characteristic for *trans*-configurated alkenes (while signals typical for *cis*-alkenes between ν = 725–675 were missing at all). All signals of the ^1H and ^{13}C NMR spectrum could be assigned unambiguously, and the UV–vis spectrum was in full agreement with the spectrum as reported by Kite et al.[37] The resolution of **1** occurred with expected selectivity following the empirical rule proposed by Kazlauskas et al.[38]: high enantiomeric ratios are always found for long chain secondary alcohols with one substituent smaller than *n*-propyl. In addition, derivatization of **1** with (*S*) 2-methoxy-2-(1-naphthyl)-propanoic acid according to Kasai's procedure[39] and close inspection of the ^1H NMR spectra confirmed a (14*R*) configuration as previously deduced by Appendino et al. applying MTPA derivatization.[1]

Acetylation, benzoylation or chloro-acetylation of **13** (Scheme 4) furnished acylated 4-oxo analogues **17**–**19** in 85–93% yield, respectively. While the Jones oxidation of **13** at 0 °C using one equivalent of CrO_3 yielded aldehyde **20**, oxidation of **13** with an excess of CrO_3/H_2SO_4 afforded acid **21** in 53% isolated yield.

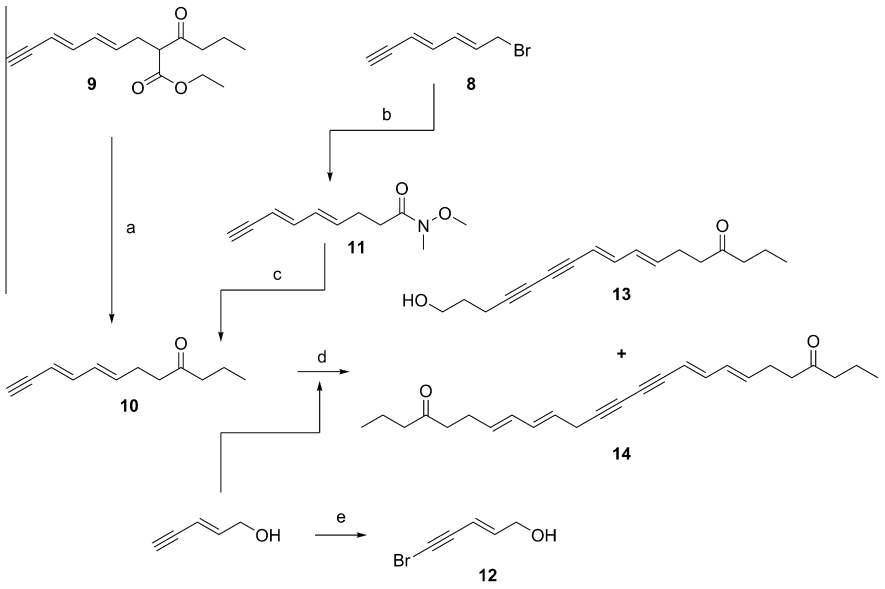
2.2. Biology

Cytotoxicity of oenanthotoxin (**1**) and derivatives **13**, **15**–**21** was determined using the photometric sulforhodamine B assay[40–43] employing six different human cancer cell lines and non-malignant mouse fibroblasts (NIH 3T3) for comparison. The results of these assays are compiled in the Table 1.

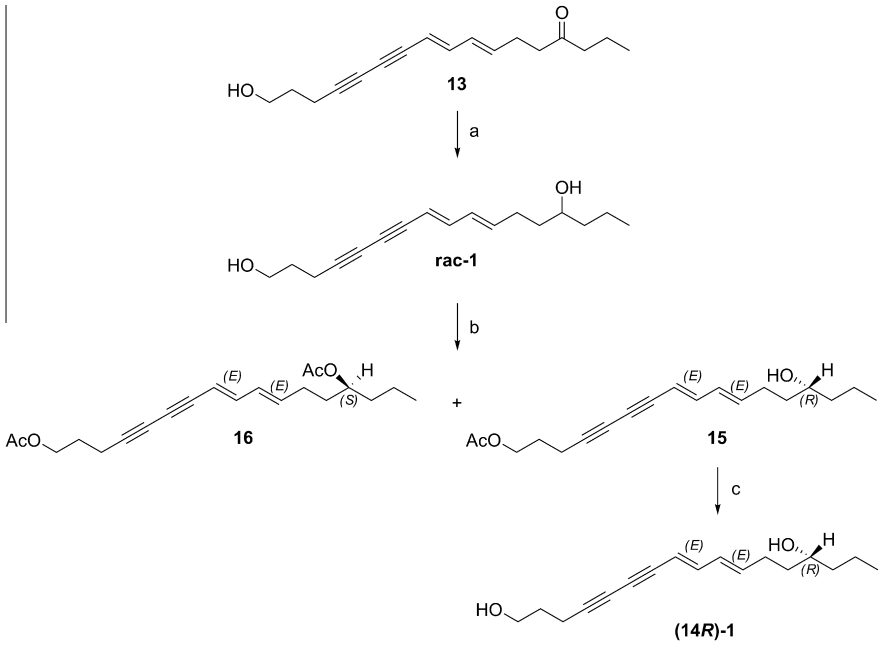
As a result, oenanthotoxin is cytotoxic to tumor cell lines as well as to non-malignant mouse fibroblast. This poly-acetylenic compound is as cytotoxic as standard compound betulinic acid.[44] The highest cytotoxicity was determined for ovarian cancer cells A2780 (EC_{50} = 3.8 μM) while for NIH 3T3 cells an EC_{50} = 10.1 μM was determined. Diacetylation as well as inversion of configuration at carbon C-14 had no significant influence onto the EC_{50} values, while mono-acetylation (as in **15**) significantly lowered cytotoxicity. Oxidation of the secondary hydroxyl group had only slight effects onto the EC_{50} values; the lowest EC_{50} value was determined for the 4-oxo-chloroacetate **19** with EC_{50} = 3.6 μM for A2780



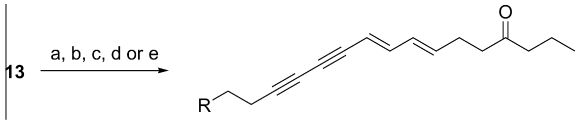
Scheme 1. Synthesis of precursors **4**–**9**: Reagents and conditions: (a) NBS, MeOH, 25 °C, 1 h, 86.2%; (b) KOH, MeOH, reflux, 6 h, 48.7%; (c) NBS, MeOH, 25 °C, 1 h; KOH, MeOH, reflux, 10 h, 59.2%; (d) H_3PO_4, 60 °C, hydrodistillation, 40.1%; (e) ethynylmagnesium bromide, lithium chloride, THF −20 °C → 25 °C, 3 h, 75%; (f) PBr_3, pyridine, reflux, 30 min, 37.9%; (g) ethyl 3-oxohexanoate, NaH, THF, 0 °C, 30 min, 83.7%.



Scheme 2. Synthesis of precursors **10**–**14**: Reagents and conditions: (a) NaOH, H_2O, 25 °C, 10 h → 50 °C, 4 h, hydrodistillation 51.1%; (b) *N*-Methoxy-*N*-methylacetamide, LDA, 25 °C, 1 h, 84%; (c) propylmagnesium chloride, ether, −15 °C → 25 °C, 1 h, 96%; (d) acetonitrile, $^{i}Pr_2NEt$, CuI, NBS, 25 °C, 12 h, 50% (of **13**) and 24.6% (of **14**); (e) NBS, $AgNO_3$, acetone, 0 °C, 2 h, 55.2%.



Scheme 3. Synthesis of oenanthotoxin **1**: Reagents and conditions: (a) $LiAlH_4$, ether, 25 °C, 2 h, 82.6%; (b) novozyme 435, vinyl acetate, $^{i}PrO^{i}Pr$, 40 °C, 15 h, 41.5% (of **16**) and 47.8% (of **15**); c) $LiAlH_4$, ether, 25 °C, 2 h, 79.4%.



17 R = CH_2-OAc
18 R = CH_2-OBz
19 R = CH_2-O-C(=O)-CH_2-Cl
20 R = CHO
21 R = CO_2H

Scheme 4. Synthesis of **14**–**21**: Reagents and conditions: (a) AcCl, NEt_3, DCM, 25 °C, 1 h, 93%; (b) BzCl, NEt_3, DCM, 25 °C, 1 h, 92%; (c) $ClCH_2C(=O)Cl$, NEt_3, DCM, 25 °C, 1 h, 85%; (d) CrO_3, H_2SO_4, 0 °C, 30 min, 89%; (e) CrO_3, H_2SO_4, 0 °C, 30 min, 53%.

ovarian cancer cells. Some of the compounds showed remarkable selectivity for the different cell lines. Parent compound **1** and the di-acetate **16** gave rather low EC_{50} values for A2780 cancer cells while being less cytotoxic to mouse fibroblasts. Benzoate **18** was not cytotoxic to NIH 3T3 cells (EC_{50} >30 μM, cut-off of the assay) but cytotoxic to undifferentiated human tyroid carcinoma cells 8505C and HT29 colorectal adenocarcinoma cells. Almost no cytotoxicity was established for acid **21** while aldehyde **20** gave low EC_{50} values for 8505C, A2780 and human breast carcinoma MCF-7 cells.

Table 1
Cytotoxicity of selected compounds (EC_{50} values in μM from SRB assays after 96 h of treatment; the values are averaged from three independent experiments performed each in triplicate; confidence interval CI = 95%; cut-off 30 μM)

EC_{50}	518A2	8505C	A2780	A549	HT29	MCF7	NIH 3T3
Betulinic acid	11.9 ± 0.7	6.7 ± 0.1	11.0 ± 0.4	14.8 ± 0.4	13.1 ± 0.9	14.9 ± 0.9	10.0 ± 0.6
1	17.1 ± 1.1	10.5 ± 1.0	3.8 ± 0.1	10.9 ± 0.3	>30	20.6 ± 3.2	10.1 ± 1.6
13	19.1 ± 0.4	18.0 ± 2.6	4.9 ± 1.7	19.7 ± 0.5	21.2 ± 0.4	234.1 ± 0.4	11.1 ± 1.5
15	14.9 ± 0.6	20.3 ± 0.6	6.8 ± 0.3	20.5 ± 0.1	26.3 ± 0.5	21.3 ± 1.0	16.9 ± 1.2
16	14.7 ± 0.5	16.8 ± 1.1	3.9 ± 0.4	14.5 ± 2.0	17.9 ± 0.5	12.4 ± 0.5	11.8 ± 2.0
17	9.1 ± 0.1	15.6 ± 0.2	4.7 ± 0.4	56.4 ± 0.6	>30	21.6 ± 0.5	14.2 ± 0.1
18	20.5 ± 0.1	4.2 ± 0.7	>30	14.4 ± 1.2	7.9 ± 0.9	>30	26.7 ± 1.1
19	12.6 ± 0.5	19.3 ± 1.3	3.6 ± 0.1	5.1 ± 1.1	>30	26.9 ± 0.7	11.9 ± 0.7
20	>30	7.1 ± 0.5	7.0 ± 0.4	>30	22.2 ± 2.0	5.9 ± 1.9	13.2 ± 0.4
21	>30	19.3 ± 1.3	>30	>30	>30	>30	>30

Human cancer cell lines: 518A2 (melanoma), 8505C (thyroid carcinoma) A2780 (ovarian adenocarcinoma); A549 (alveolar basal epithelial adenocarcinoma) HT29 (colorectal adenocarcinoma), MCF7 (breast adenocarcinoma), and NIH 3T3 (non-malignant mouse fibroblasts).

3. Conclusions

We developed a synthetic scheme for the synthesis of naturally occurring (14*R*)-oenanthotoxin and several analogs. Key-steps of this synthesis are an efficient homo-coupling of alkynes and a chemoenzymatic resolution of racemic oenanthotoxin using novozyme 435 and vinyl acetate. The results from photometric SRB assays showed Oenanthotoxin and several of its derivatives as cytotoxic agents for a variety of different human tumor cell lines. Their good cytotoxicity qualifies some of these compounds (and derivatives thereof) for further studies.

4. Experimental

4.1. General – chemistry

Melting points are uncorrected (*Leica* hot stage microscope), NMR spectra were recorded using the Varian spectrometers Gemini 2000 or Unity 500 (δ given in ppm, J in Hz, internal Me_4Si; typical experiments: H–H-COSY, HMBC, HMQC, NOESY and DQF-COSY), MS spectra were taken on a Finnigan MAT LCQ 7000 (electrospray, voltage 4.1 kV, sheath gas nitrogen) instrument, IR and spectra were taken as KBR pills (or film) on a Perkin-Elmer Spectrum 1000 instrument. The optical rotation was measured on a Perkin-Elmer polarimeter at 20 °C; TLC was performed on silica gel (Merck 5554); elemental analyses were performed on a Vario EL (CHNS). The solvents were dried according to usual procedures. The purity of the compounds were determined by HPLC and found to be >98%. Betulinic acid (standard) and novozyme 435 were obtained from different commercial suppliers. Chiral HPLC separations were performed on 250 × 4.5 mm stainless-steel columns packed with Daicel Chiralpak AD-H 5 μm material using a gradient of *n*-hexane (A) and isopropanol (B) with a flow rate of 0.7 mL/min. The solvent gradient started with 7% B for 60 min, solvent B was then increased to 30% within 2 min, and then kept constant for 10 min.

4.2. General – biological screening

The SRB assay was performed as previously described.[40–43]

4.3. Syntheses

4.3.1. *Trans* 3-bromo-2-methoxy-tetrahydro-2*H*-pyran (4)

To a solution of NBS (150 g, 0.84 mol) in methanol (840 mL), at 0 °C a solution of 3,4-dihydro-2*H*-pyran (70.79 g, 0.84 mol) in methanol (210 mL) was slowly added, and the mixture was stirred for 1 h at 25 °C. Most of the solvent was removed under diminished pressure, ether (400 mL) was added, and the precipitate was filtered off and washed with ether (3 × 20 mL). The filtrate and washings were combined and distilled to yield **4** (141.44 g, 86.2%) as a colorless liquid; $b.p._{30}$ 91–92 °C (lit.: $b.p._{16}$ = 85–88 °C[26]; R_f = 0.51 (chloroform/hexane, 1:1); IR (film): ν = 2951s, 1740w, 1646w, 1440m, 1383m, 1356m, 1325w, 1310w, 1274w, 1221m, 1191m, 1116s, 1094s, 1071s, 1041s, 953s, 916m, 869m, 729m, 666m cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 4.49 (*d*, J = 4.6 Hz, 1H, H-1), 3.95–3.92 (m, 1H, H-2), 3.92–3.87 (m, 1H, H-5a), 3.54–3.59 (m, 1H, H-5b), 3.43 (s, 3H, CH_3), 2.39–2.33 (m, 1H, H-3a), 1.96–1.86 (m, 2H, H-3b, H-4a), 1.56–1.49 (m, 1H, H-4b) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 102.5 (O-*C*H-O, C-1), 62.7 (*C*H_2-O, C-5), 55.8 (*C*H_3-O, C-6), 49.3 (*C*H-Br, C-2), 30.4 (*C*H_2, C-3), 23.6 (*C*H_2, C-4) ppm.

4.3.2. 5,6-Dihydro-2-methoxy-2*H*-pyran (5)

From ***4****:* To a solution of potassium hydroxide (60.4 g, 1.07 mol) in methanol (140 mL), a solution of **4** (140.4 g, 0.72 mol) in methanol (47 mL) was slowly added, and the mixture was heated under reflux for 6 h. The mixture was diluted with ether (450 mL), and the precipitate was filtered off. The filtrate was distilled to yield **5** (40.0 g, 48.7%) as a colorless liquid.

From ***3****:* To a suspension of NBS (60.00 g, 0.34 mol) in methanol (120 mL) a solution of 3,4-dihydro-2*H*-pyran (**3**) (28.32 g, 0.34 mol) in methanol (30 mL) was slowly added at −10 °C. After stirring for 1 h at 25 °C, this reaction mixture was slowly added to a refluxing solution of potassium hydroxide (57.20 g, 1.02 mol) in methanol (150 mL). The mixture was heated under reflux for 10 h, diluted with ether (350 mL), and the precipitate was filtered off. The filtrate was washed with water (300 mL) and brine (200 mL), dried (Na_2SO_4) and distilled to yield **5** (22.75 g, 59.2%) as colorless liquid; $b.p._{48}$ = 58–59 °C (lit.: $b.p._{67}$ = 59–60 °C);[45] R_f = 0.36 (chloroform/hexane, 1:1); IR (film): ν = 3518w, 3045s, 2880s, 2826s, 2709w, 2654w, 2148w, 2069w, 2006w, 1656m, 1465s, 1426s, 1399s, 1351m, 1328s, 1266s, 1209s, 1107s, 1050s, 961s, 892s, 839s, 768s, 714s, 489s cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.99–5.95 (m, 1H, H-3), 5.68–5.65 (m, 1H, H-2), 4.74–4.72 (m, 1H, H-1), 3.84 (ddd, J = 11.3, 11.3, 3.7 Hz, 1H, H-5a), 3.66 (ddd, J = 11.2, 6.1, 2.2 Hz, 1H, H-5b), 3.35 (s, 3H, CH_3), 2.28–2.19 (m, 1H, H-4a), 1.87–1.80 (m, 1H, H-4b) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 129.0 (*C*H=CH, C-3), 125.8 (*C*H=CH, C-2), 94.9 (O-*C*H-O, C-1), 57.2 (*C*H_2-O, C-5), 55.0 (*C*H_3-O, C-6), 24.7 (*C*H_2, C-4) ppm.

4.3.3. (2*E*) 2,4-pentadienal (6)

A mixture of **5** (35 g, 0.31 mol) and phosphoric acid (240 mL, 2.38 m) was stirred at 60 °C until homogeneous. This solution was added dropwise to refluxing phosphoric acid (250 mL, 2.85 M) and subjected to a hydrodistillation (for 4 h). Distillation gave **6** (10.1 g, 40.1%) as an off-white liquid; $b.p._{27}$ = 35–37 °C (lit.: $b.p._{27}$ = 36–37 °C[27]); R_f = 0.51 (hexane/EtOAc, 8:2); IR (film): ν = 3348m, 2933m, 2820m, 2726m, 1682s, 1634s, 1421m, 1171s, 1109s, 1017s, 854m, 600m cm^{-1}; UV–vis (MeOH): λ_{max}

($\log\varepsilon$) = 265 (4.25) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.57 (*d*, *J* = 7.9 Hz, 1H, H-1), 7.08 (dddd, *J* = 15.4, 10.8, 0.8, 0.8 Hz, 1H, H-3), 6.58 (dddd, *J* = 16.9, 10.8, 10.0, 0.7 Hz, 1H, H-4), 6.16 (ddddd, *J* = 15.4, 7.9, 0.7, 0.7, 0.7 Hz, 1H, H-2), 5.73 (dddd, *J* = 16.7, 1.2, 0.7, 0.7 Hz, 1H, H-5$_{trans}$), 5.61 (dddd, *J* = 10.0, 1.2, 0.7, 0.7 Hz, 1H, H-5$_{cis}$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 193.4 (*C*=O, C-1), 151.7 (*C*H=CH, C-3), 134.7 (*C*H=CH_2, C-4), 132.2 (*C*H=CH, C-2), 127.3 (CH=*C*H_2, C-5) ppm.

4.3.4. (4*E*) 4,6-heptadien-1-yn-3-ol (7)

A solution of ethynylmagnesium bromide (450 mL, 0.5 M in dry THF) containing lithium chloride (9.51 g, 0.225 mol) was cooled to −20 °C, and a solution of **6** (15.43 g, 0.188 mol) in dry THF (40 mL) was slowly added. Stirring at −15 °C was continued for 2 h, the mixture was allowed to warm to 25 °C and stirred for another hour. Usual aqueous work-up followed by column chromatography (silica gel, hexane/EtOAc, 8:2) gave **7** (15.25 g, 75.0%) as a colorless liquid; R_f = 0.41 (hexane/EtOAc, 8:2); IR (film): ν = 3377br s, 3298s, 3042m, 2117w, 1725m, 1605m, 1376m, 1267m, 1089m, 1003s, 953m, 913m, 656m, 561m cm^{-1}; UV–vis (MeOH): λ_{max} ($\log\varepsilon$) = 224 (4.36) nm; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.40 (dd, *J* = 14.7, 10.9 Hz, 1H, H-5), 6.28 (ddd, *J* = 16.6, 10.2, 10.2 Hz, 1H, H-6), 5.75 (dd, *J* = 14.7, 5.8 Hz, 1H, H-4), 5.23 (d, *J* = 16.1 Hz, 1H, H-7$_{trans}$), 5.12 (d, *J* = 9.6 Hz, 1H, H-7$_{cis}$), 4.92–4.88 (m, 1H, H-3), 2.57 (d, *J* = 2.2 Hz, 1H, H-1) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 135.5 (*C*H=CH, C-6), 132.4 (*C*H=CH, C-5), 131.4 (*C*H=CH, C-4), 118.8 (CH=CH_2, C-7), 82.8 (*C*≡CH, C-2), 74.0 (C≡*C*H, C-1), 61.9 (*C*HOH, C-3) ppm.

4.3.5. (3*E*,5*E*) 7-bromo-3,5-heptadien-1-yne (8)

To a solution of **7** (3.30 g, 30.52 mmol) in pyridine (0.28 g, 0.29 mL, 3.54 mmol) and dry ether (10 mL) at 0 °C, a solution of PBr_3 (4.13 g, 15.26 mmol) in dry ether (5 mL) was slowly added, and the mixture was stirred under reflux for 30 min. The mixture was poured onto crushed ice, extracted with ether (3 × 50 mL), and the extracts were washed with water (5 × 50 mL) and dried (Na_2SO_4). The solvent was removed and the residue subjected to column chromatography (Al_2O_3 basic, AS1, hexane) to yield a mixture of **8** and (3*Z*,5*E*)-7-bromohepta-3,5-dien-1-yne (20:3) that was crystallized at 0 °C. The crystals were collected and washed with cold hexane, and **8** (1.98 g, 37.9%) was obtained as an off-white solid; mp 14 °C (lit.: 0 °C[30]); R_f = 0.50 (hexane/EtOAc, 95:5); IR (film): ν = 3290s, 3031m, 2963w, 2095w, 1434m, 1200s, 982s, 614m, 562m cm^{-1}; UV–vis (MeOH): λ_{max} ($\log\varepsilon$) = 269 (4.07) nm;[1]H NMR (500 MHz, $CDCl_3$): δ = 6.65 (dddd, *J* = 15.7, 10.8, 0.7, 0.7 Hz, 1H, H-4), 6.31 (dddt, *J* = 15.0, 10.9, 1.0, 0.8 Hz, 1H, H-5), 5.99 (dddt, *J* = 15.0, 7.8, 0.8, 0.8 Hz, 1H, H-6), 5.64 (dd, *J* = 15.7, 2.1 Hz, 1H, H-3), 4.02 (dd, *J* = 7.9, 1.0 Hz, 2H, *C*H_2), 3.08 (dd, *J* = 2.4, 0.6 Hz, 1H, H-1) ppm;[13]C NMR (125 MHz, $CDCl_3$): δ = 141.7 (*C*H=CH, C-4), 138.5 (*C*H=CH, C-5), 131.8 (*C*H=CH, C-6), 112.3 (*C*H=CH, C-3), 82.5 (*C*≡CH, C-2), 80.9 (C≡*C*H, C-1), 32.3 (*C*H_2Br, C-7) ppm.

4.3.6. Ethyl (4*E*,6*E*) 2-butanoyl-4,6-nonadien-8-ynoate (9)

To a suspension of sodium hydride (62 mg, 2.60 mmol, hexane washed) in dry THF (3 mL) at 0 °C ethyl 3-oxo-hexanoate (411 mg, 2.60 mmol) was added, and the mixture was stirred for 30 min. A solution of **8** (450 mg, 2.63 mmol) in dry THF (2 mL) was added, and stirring at 25 °C was continued for 4 h. After dilution with ether and usual work-up followed by column chromatography (silica gel, hexane/EtOAc, 9:1) **9** (540 mg, 83.7%) was obtained as a colorless liquid; R_f = 0.49 (hexane/EtOAc, 9:1); IR (KBr): ν = 3287m, 2966m, 2096w, 1740s, 1715s, 1641m, 1465m, 1369m, 1204br s, 1031m, 989s, 638m cm^{-1}; UV–vis (MeOH): λ_{max} ($\log\varepsilon$) = 260 (4.28) nm; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.57 (dddd, *J* = 15.7, 10.8, 0.7, 0.7 Hz, 1H, H-6), 6.11 (dddt, *J* = 15.2, 10.8, 1.4, 0.8 Hz, 1H, H-5), 5.70 (dddt, *J* = 15.1, 7.3, 0.8, 0.8 Hz, 1H, H-4), 5.48 (dddd, *J* = 15.7, 2.4, 0.7, 0.7 Hz, 1H, H-7), 4.17 (qd, *J* = 7.1, 1.7 Hz, 2H, *C*H_2), 3.50 (t, *J* = 7.4 Hz, 1H, H-2), 2.99 (d, *J* = 2.3 Hz, 1H, H-9), 2.62 (ddd, *J* = 7.3, 7.3, 1.4 Hz, 2H, *C*H_2); 2.57–2.39 (m, 2H, *C*H_2), 1.63–1.55 (m, 2H, *C*H_2), 1.24 (t, *J* = 7.1 Hz, 3H, *C*H_3), 0.89 (t, *J* = 7.4 Hz, 3H, *C*H_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 204.3 (*C*=O, C-12), 169.1 (*C*=O, C-1), 143.0 (H*C*=CH, C-6), 133.3 (H*C*=CH, C-4), 132.1 (H*C*=CH, C-5), 109.4 (H*C*=CH, C-7), 82.9 (*C*≡CH, C-8), 79.5 (C≡*C*H, C-9), 61.6 (O-*C*H_2, C-10), 58.5 (*C*H, C-2), 44.2 (*C*H_2, C-13), 31.3 (*C*H_2, C-3), 17.0 (*C*H_2, C-14), 14.2 (*C*H_3, C-11), 13.7 (*C*H_3, C-15) ppm; MS (ESI, MeOH): *m*/*z* = 255.1 (8%, $[M+H]^+$), 271.1 (100%, $[M+Na]^+$); Anal. Calcd for $C_{15}H_{20}O_3$ (248.32): C 72.55, H 8.12; found: C 72.31, H 8.21.

4.3.7. (7*E*,9*E*) 7,9-dodecadien-11-yn-4-one (10)

*From **9**:* A mixture of **9** (540 mg, 2.17 mmol), sodium hydroxide (174 mg, 4.35 mmol) and water (3 mL) was stirred for 10 h at 25 °C and 4 h at 50 °C. The mixture was diluted with water (50 mL) and subjected to a hydrodistillation for 2 h. The organic layer was subjected to column chromatography (silica gel, hexane/EtOAc, 9:1) to afford **10**[8] (196 mg, 51.1%) as a colorless liquid.

*From **11**:* To a solution of **11** (200 mg, 1.03 mmol) in THF (10 mL) at −15 °C a solution of *n*-propylmagnesium chloride (0.52 mL, 2 m in Et_2O, 1.04 mmol) was added, and stirring at −15 °C was continued for 1 h. The mixture was allowed to reach room temperature, poured into an aq. solution of NH_4Cl (satd.) and extracted with Et_2O (3 × 30 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$), filtered and evaporated to dryness. The residue was subjected to column chromatography (silica gel, hexane/ethyl acetate, 95:5) to yield **10** (175 mg, 96%) as colorless oil; R_f = 0.40 (hexane/EtOAc, 95:5); IR (KBr): ν = 3298m, 3025w, 2963m, 2095w, 1713s, 1640m, 1458m, 1411m, 1373m, 988s, 643m cm^{-1}; UV–vis (MeOH): λ_{max} ($\log\varepsilon$) = 259 (4.11) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.60 (dd, *J* = 15.7, 10.7 Hz, 1H, H-9), 6.09 (dddt, *J* = 15.2, 10.7, 1.4, 0.7 Hz, 1H, H-8), 5.79 (dddt, *J* = 14.8, 7.0, 0.8, 0.8 Hz, 1H, H-7), 5.47 (dddd, *J* = 15.7, 2.4, 0.7, 0.7 Hz, 1H, H-10), 2.98 (d, *J* = 2.4 Hz, 1H, H-12), 2.50 (t, *J* = 7.3 Hz, 2H, *C*H_2), 2.40–2.35 (m, 4H, 2 × *C*H_2), 1.64–1.56 (m, 2H, *C*H_2), 0.90 (t, *J* = 7.4 Hz, 3H, *C*H_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 210.0 (*C*=O, C-4), 143.5 (*C*H=CH, C-9), 136.7 (*C*H=CH, C-7), 130.3 (*C*H=CH, C-8), 108.5 (*C*H=CH, C-10), 83.1 (*C*≡CH, C-11), 79.1 (C≡*C*H, C-12), 45.0 (*C*H_2, C-6), 41.9 (*C*H_2, C-5), 26.9 (*C*H_2, C-3), 17.4 (*C*H_2, C-2), 13.9 (*C*H_3, C-1) ppm.

4.3.8. (4*E*,6*E*) *N*-methyl-*N*-methyloxy-4,6-nonadien-8-ynamide (11)

To a solution of *N*-methoxy-*N*-methylacetamide (66 mg, 0.64 mmol) in dry THF (2 mL) at −15 °C LDA [freshly prepared from diisopropylamine (65 mg, 0.64 mmol) and BuLi (0.45 mL, 1.6 m in hexane, 0.72 mmol) in dry THF (2 mL) at −15 °C was added, and the mixture was stirred for 10 min. A solution of **8** (100 mg, 0.58 mmol) in dry THF (5 mL) was added, and the mixture was stirred at room temperature for 1 h. Ether (50 mL) was added, and the organic layer was washed with water (3 × 50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and evaporated to dryness. The residue was subjected to column chromatography (silica gel, hexane/EtOAc, 6:4) to yield compound **11** (95 mg, 84%) as a colorless oil; R_f = 0.38 (hexane/ethyl acetate, 6:4); UV–vis ($CHCl_3$): λ_{max} ($\log\varepsilon$) = 263 nm (4.37); IR (KBr): ν = 3291m, 2936w, 2889vw, 1660vs, 1420m, 1386m, 1178w, 1108w, 990s cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.61 (dd, *J* = 15.7, 10.7 Hz, 1H, H-6), 6.12 (dddt, *J* = 15.2, 10.7, 1.4, 0.8 Hz, 1H, H-5), 5.85 (dt, *J* = 15.2, 6.9 Hz, 1H, H-4), 5.47 (dddd, *J* = 15.7, 2.4, 0.7, 0.7 Hz, 1H, H-7), 3.66 (s, 3H, *C*H_3 (11)), 3.16 (s, 3H, *C*H_3 (10)), 2.97 (d, *J* = 2.4 Hz, 1H, H-9), 2.54–2.40 (m, 4H, *C*H_2 (2) + *C*H_2 (3)) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 173.6 (*C*=O, C-1), 143.6 (*C*H=CH, C-6), 137.0 (*C*H=CH,

C-4), 130.2 (*C*H=CH, C-5), 108.4 (*C*H=CH, C-7), 83.1 (*C*≡CH, C-8), 79.0 (C≡*C*H, C-9), 61.4 (CH_3, C-11), 32.3 (CH_3, C-10), 31.3 (CH_2, C-2), 27.7 (CH_2, C-3) ppm; MS (ESI, MeOH): *m*/*z* = 194.0 (60%, $[M+H]^+$), 216.1 (50%, $[M+Na]^+$), 309.5 (22%, $[3M+K+H]^{2+}$), 386.8 (10%, $[2M+H]^+$), 406.0 (92%, $[4M+K+H]^{2+}$), 408.9 (100%, $[2M+Na]^+$); Anal. Calcd for $C_{11}H_{15}NO_2$ (193.24): C 68.37, H 7.82, N 7.25; found: C 68.19, H 7.99, N 7.11.

4.3.9. (2*E*) 5-bromo-2-penten-4-yn-1-ol (12)

To a suspension of NBS (1.04 g, 5.84 mmol), $AgNO_3$ (83 mg, 0.49 mmol) in acetone (10 mL) at 0 °C, a solution of (2*E*) 2-penten-4-yn-1-ol (0.40 g, 4.87 mmol) in acetone (8 mL) was added, and stirring at 0 °C was continued for another 2 h. Extraction with EtOAc followed by usual aqueous work-up and column chromatography (silica gel, DCM/ether, 95:5) gave **11** (433 mg, 55.2%) as a colorless solid; mp 28–29 °C (lit.: 34–36 °C)[46]; R_f = 0.47 (DCM/ether, 95:5); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.30 (dt, *J* = 15.9, 5.0 Hz, 1H, H-2), 5.72 (dt, *J* = 15.5, 2.0 Hz, 1H, H-3), 4.19 (dd, *J* = 5.0, 1.9 Hz, 2H, CH_2OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 143.5 (*C*H=CH, C-2), 109.8 (*C*H=CH, C-3), 78.1 (*C*≡CBr, C-4), 62.7 (CH_2OH, C-1), 50.1 (C≡*C*Br, C-5) ppm.

4.3.10. (7*E*,9*E*,15*E*) 17-hydroxy-7,9,15-heptadecatrien-11,13-diyn-4-one (13) and (7*E*,9*E*,15*E*,17*E*)-tetracosa-7,9,15,17-tetraen-11,13-diyne-4,21-dione (14)

To a solution of **10** (340 mg, 1.93 mmol) and (2*E*)-pent-2-en-4-yn-1-ol (317 mg, 3.86 mmol) in acetonitrile (55 mL), diisopropylethylamine (823 mg, 6.37 mmol), CuI (606 mg, 3.18 mmol) and NBS (566 mg, 3.18 mmol) were added, and the mixture was stirred at 25 °C for 12 h. The solvent was removed under diminished pressure, and the residue subjected to column chromatography (silica gel, hexane/EtOAc, 8:2 → 7:3) to yield **13** (245 mg, 50.0%) and **14** (83 mg, 24.6%).

Data for ***13****:* colorless solid; mp = 53–56 °C (lit.: 56–57 °C)[8]; R_f = 0.34 (hexane/EtOAc, 7:3); IR (KBr): ν = 2933m, 2198w, 2128w, 1707s, 1636m, 1432s, 1384s, 1095s, 1033s, 1010s, 988s, 949m, 876m, 539m, 470m cm^{-1}; UV–vis (MeOH): λ_{max} (log ε) = 212 (3.98), 248 (4.14), 266 (4.10), 278 (3.89), 294 (4.09), 314 (4.19), 336 (4.07) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.65 (dd, *J* = 15.5, 10.9 Hz, 1H, H-9), 6.39 (dt, *J* = 15.9, 4.9 Hz, 1H, H-16), 6.09 (dddt, *J* = 15.1, 10.8, 1.4, 0.7 Hz, 1H, H-8), 5.88–5.80 (m, 2H, H-7 + H-15), 5.56 (d, *J* = 15.1 Hz, 1H, H-10), 4.27–4.23 (m, 2H, CH_2 (17)), 2.50 (t, *J* = 7.2 Hz, 2H, CH_2 (5)), 2.41–2.35 (m, 4H, CH_2 (3) + CH_2 (6)), 1.63–1.56 (m, 2H, CH_2 (2)), 0.90 (t, *J* = 7.4 Hz, 3H, CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 210.0 (*C*=O, C-4), 145.2 (*C*H=CH, C-16), 145.0 (*C*H=CH, C-9), 137.8 (*C*H=CH, C-7), 130.4 (*C*H=CH, C-8), 109.3 (*C*H=CH, C-15), 108.3 (*C*H=CH, C-10), 81.4 (*C*≡C, C-11), 80.5 (*C*≡C, C-14), 76.0 (*C*≡C, C-12), 74.9 (*C*≡C, C-13), 62.9 (CH_2OH, C-17), 45.0 (CH_2, C-6), 41.8 (CH_2, C-5), 27.0 (CH_2, C-3), 17.4 (CH_2, C-2), 13.9 (CH_3, C-1) ppm; MS (ESI, MeOH): *m*/*z* = 263.1 (100%, $[M+Li]^+$), 298.8 (63%, $[M+Li+MeOH]^+$), 519.0 (18%, $[2M+Li]^+$).

Data for ***14****:* off-white solid; mp = 75−76 °C (lit.: 76 °C)[8]; R_f = 0.55 (hexane/EtOAc, 8:2); IR (KBr): ν = 2959m, 2187w, 2124w, 1702s, 1636m, 1420m, 1377m, 1126m, 1077m, 1032m, 982s cm^{-1}; UV–vis (MeOH): λ_{max} (log ε) = 259 (3.94), 279 (3.96), 294 (3.98), 317 (3.99), 338 (4.11), 363 (4.07) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.64 (dd, *J* = 15.5, 10.9 Hz, 2H, H-9 + H-16), 6.12 (dddt, *J* = 15.0, 10.9, 1.5, 0.5 Hz, 2H, H-8 + H-17), 5.83 (dt, *J* = 15.1, 7.0 Hz, 2H, H-7 + H-18), 5.58 (d, *J* = 15.5 Hz, 2H, H-10 + H-15), 2.50 (t, *J* = 7.3 Hz, 4H, CH_2 (5) + CH_2 (20)), 2.41–2.35 (m, 8H, CH_2 (3) + CH_2 (6) + CH_2 (19) + CH_2 (22)), 1.64–1.56 (m, 4H, CH_2 (2) + CH_2 (23)), 0.90 (t, *J* = 7.4 Hz, 6H, CH_3 (1) + CH_3 (24)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 209.9 (*C*=O, C-4 + C-21), 144.7 (*C*H=CH, C-9 + C-16), 137.7 (*C*H=CH, C-7 + C-18), 130.5 (*C*H=CH, C-8 + C-17), 108.5 (*C*H=CH, C-10 + C-15), 82.3 (*C*≡CH, C-11 + C-14), 76.4 (C≡*C*H, C-12 + C-13), 45.0 (CH_2, C-6 + C-19), 41.8 (CH_2, C-5 + C-20), 27.0 (CH_2, C-3 + C-22), 17.4 (CH_2, C-2 + C-23), 13.9 (CH_3, C-1 + C-24) ppm; MS (ESI, MeOH): *m*/*z* = 351.1 (60%, $[M+H]^+$), 368.1 (28%, $[M+NH_4]^+$), 373.1 (42%, $[M+Na]^+$), 701.4 (100%, $[2M+H]^+$), 723.1 (30%, $[2M+Na]^+$), 733.3 (22%, $[2M+H+MeOH]^+$).

4.3.11. (2*E*,8*E*,10*E*) 2,8,10-Heptadecatrien-4,6-diyne-1,14-diol (rac-1)

$LiAlH_4$ (23 mg, 0.61 mmol) was added to a solution of **13** (154 mg, 0.60 mmol) in dry ether (5 mL), and the mixture was stirred at 25 °C for 2 h. Usual aqueous workup followed by column chromatography (silica gel, hexane/EtOAc, 7:3) gave **rac-1** (128 mg, 82.6%) as a colorless solid; mp = 78–81 °C (lit.: 68 °C)[8]; R_f = 0.20 (hexane/EtOAc, 7:3); IR (KBr): ν = 3380br s, 3284br s, 2962m, 2936m, 2870m, 2196w, 2126w, 1632m, 1440m, 1368m, 1090s, 986s, 948s cm^{-1}; UV–vis (MeOH): λ_{max} (log ε) = 208 (3.74), 250 (3.94), 266 (3.90), 281 (3.68), 296 (3.89), 315 (4.00), 336 (3.88) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.69 (dd, *J* = 15.5, 10.8 Hz, 1H, H-9), 6.39 (dt, *J* = 15.9, 5.0 Hz, 1H, H-2), 6.14 (dd, *J* = 14.9, 10.9 Hz, 1H, H-10), 5.89 (dt, *J* = 14.9, 7.3 Hz, 1H, H-11), 5.86 (ddt, *J* = 15.9, 1.9, 1.0 Hz, 1H, H-3), 5.56 (d, *J* = 15.6 Hz, 1H, H-8), 4.25 (dd, *J* = 4.9, 2.0 Hz, 2H, CH_2 (1)), 3.64–3.59 (m, 1H, H-14), 2.34–2.24 (m, 1H, H-12a), 2.26–2.16 (m, 1H, H-12b), 1.61–1.48 (m, 2H, CH_2 (13)), 1.49–1.30 (m, 2H, CH_2 (16)), 1.46–1.40 (m, 2H, CH_2 (15)), 0.93 (t, *J* = 7.0 Hz, 3H, CH_3 (17)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 145.3 (*C*H=CH, C-9), 144.9 (*C*H=CH, C-2), 139.5 (*C*H=CH, C-11), 130.0 (*C*H=CH, C-10), 109.3 (*C*H=CH, C-3), 107.8 (*C*H=CH, C-8), 81.6 (*C*≡C, C-7), 80.4 (*C*≡C, C-4), 75.8 (*C*≡C, C-6), 75.0 (*C*≡C, C-5), 71.2 (*C*HOH, C-14), 62.9 (CH_2OH, C-1), 39.9 (CH_2, C-15), 36.6 (CH_2, C-13), 29.3 (CH_2, C-12), 18.9 (CH_2, C-16), 14.2 (CH_3, C-17) ppm; Anal. Calcd for $C_{17}H_{22}O_2$ (258.36): C 79.03, H 8.58; found: C 78.77, H 8.64.

4.3.12. (2*E*,8*E*,10*E*,14*R*) 2,8,10-Heptadecatrien-4,6-diyne-1,14-diol (oenanthotoxin, (14*R*)-1)

Following the procedure given for *rac*-**1**, from **15** (24.0 mg, 0.08 mmol) and $LiAlH_4$ (3.0 mg, 0.08 mmol) followed by column chromatography (silica gel, hexane/EtOAc, 7:3) **1** (16.2 mg, 79.4%) was obtained as a colorless solid; mp 84–86 °C (lit.: 87 °C)[47]; $[\alpha]_D$ +30.3° (*c* 0.45, chloroform) [lit.: $[\alpha]_D$ +30.5° (chloroform), $[\alpha]_D$ 34° (*c* 0.5, chloroform)];[1,6,48] Anal. Calcd for $C_{17}H_{22}O_2$ (258.36): C 79.03, H 8.58; found: C 78.87, H 8.71.

4.3.13. (2*E*,8*E*,10*E*,14*R*) 1-*O*-Acetyl-2,8,10-heptadecatrien-4,6-diyne-1,14-diol (15) and (2*E*,8*E*,10*E*,14*S*) 1,14-di-*O*-acetyl-2,8,10-heptadecatrien-4,6-diyne-1,14-diol (16)

To a solution of rac-**1** (29 mg, 0.11 mmol) in diisopropylether (8 mL) and vinyl acetate (1 mL) novozyme® 435 (93 mg) was added, and the mixture was stirred at 40 °C for 15 h. The mixture diluted with diisopropylether (10 mL), and the enzyme was filtered off. The solvent was removed under diminished pressure, and the residue subjected to column chromatography (silica gel, hexane/EtOAc, 7:3) to yield **15** (14.0 mg, 41.5%) and **16** (18.4 mg, 47.8%).

Data for ***15****:* colorless solid; mp 69 °C; R_f = 0.45 (hexane/EtOAc, 7:3); IR (KBr): ν = 3364br s, 2958m, 2936m, 2198w, 2128w, 1741s, 1636m, 1444m, 1363m, 1245s, 992m, 944s cm^{-1}; UV–vis (MeOH): λ_{max} (log ε) = 208 (3.70), 253 (3.82), 267 (3.76), 280 (3.55), 297 (3.74), 314 (3.85), 336 (3.73) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.68 (ddd, *J* = 15.6, 10.8, 0.5 Hz, 1H, H-9), 6.27 (dt, *J* = 15.9, 5.8 Hz, 1H, H-2), 6.13 (dddt, *J* = 15.1, 10.8, 1.4. 0.7 Hz, 1H, H-10), 5.88 (dt, *J* = 15.1, 7.2 Hz, 1H, H-11), 5.82 (ddt, *J* = 15.9, 1.7, 1.1 Hz, 1H, H-3), 5.54 (dd, *J* = 15.6, 0.5 Hz, 1H, H-8), 4.61 (dd, *J* = 5.8, 1.7 Hz, 2H, CH_2 (1)), 3.62–3.56 (m, 1H, H-14), 2.34–2.14 (m, 2H, CH_2 (12)), 2.07 (s, 3H, CH_3 (19)), 1.63–1.44 (m, 2H, CH_2

(13)), 1.47–1.28 (m, 2H, CH_2 (16)), 1.44–1.38 (m, 2H, CH_2 (15)), 0.91 (t, J = 6.9 Hz, 3H, CH_3 (17)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.6 (*C*=O, C-18), 145.5 (*C*H=CH, C-9), 139.7 (*C*H=CH, C-11), 139.5 (*C*H=CH, C-2), 129.9 (*C*H=CH, C-10), 112.3 (*C*H=CH, C-3), 107.6 (*C*H=CH, C-8), 82.0 (*C*≡C, C-7), 79.7 (*C*≡C, C-4), 75.8 (*C*≡C, C-6), 75.6 (*C*≡C, C-5), 71.1 (*C*HOH, C-14), 63.8 (*C*H_2OAc, C-1), 39.9 (*C*H_2, C-15), 36.6 (*C*H_2, C-13), 29.2 (*C*H_2, C-12), 20.9 (*C*H_3, C-19), 18.9 (*C*H_2, C-16), 14.2 (*C*H_3, C-17) ppm; MS (ESI, MeOH): m/z = 318.1 (20%, $[M+NH_4]^+$), 323.1 (100%, $[M+Na]^+$), 623.0 (24%, $[2M+Na]^+$).

Data for ***16****:* colorless liquid; R_f = 0.45 (hexane/EtOAc 8:2); IR (KBr): ν = 2933m, 2199w, 2120w, 1737s, 1636m, 1438m, 1384m, 1242s, 1027s, 986m cm^{-1}; UV–vis (MeOH): λ_{max} (log ε) = 208 (3.68), 250 (3.83), 267 (3.78), 280 (3.55), 297 (3.77), 314 (3.89), 336 (3.76) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.67 (ddd, J = 15.6, 10.7, 0.5 Hz, 1H, H-9), 6.27 (dt, J = 15.9, 5.8 Hz, 1H, H-2), 6.10 (dddt, J = 15.2, 10.9, 1.5. 0.7 Hz, 1H, H-10), 5.88–5.80 (m, 2H, H-3 + H-11), 5.55 (d, J = 15.6 Hz, 1H, H-8), 4.92–4.84 (m, 1H, H-14), 4.61 (dd, J = 5.8, 1.7 Hz, 2H, CH_2 (1)), 2.17–2.10 (m, 2H, CH_2 (12)), 2.07 (s, 3H, CH_3 (19)), 2.02 (s, 3H, CH_3 (21)), 1.68–1.59 (m, 2H, CH_2 (13)), 1.58–1.41 (m, 2H, CH_2 (15)), 1.40–1.21 (m, 2H, CH_2 (16)), 0.89 (t, J = 7.3 Hz, 3H, CH_3 (17)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.9 (*C*=O, C-20), 170.5 (*C*=O, C-18), 145.4 (*C*H=CH, C-9), 139.5 (*C*H=CH, C-2), 138.9 (*C*H=CH, C-11), 130.0 (*C*H=CH, C-10), 112.3 (*C*H=CH, C-3), 107.9 (*C*H=CH, C-8), 81.9 (*C*≡C, C-7), 79.7 (*C*≡C, C-4), 75.7 (*C*≡C, C5, C-6) 73.6 (*C*HOAc, C-14), 63.8 (*C*H_2OAc, C-1), 36.4 (*C*H_2, C-15), 33.4 (*C*H_2, C-13), 28.9 (*C*H_2, C-12), 21.3 (*C*H_3, C-21), 20.9 (*C*H_3, C-19), 18.6 (*C*H_2, C-16), 14.1 (*C*H_3, C-17) ppm; MS (ESI, MeOH): m/z = 365.1 (100%, $[M+Na]^+$), 706.9 (13%, $[2\ M+Na]^+$).

4.3.14. (2*E*,8*E*,10*E*) 14-Oxo-2,8,10-heptadecatriene-4,6-diyn-1-yl-acetate (17)

To a solution of **13** (50 mg, 0.20 mmol) and triethylamine (25 mg, 0.25 mmol) in dry DCM (5 mL) acetyl chloride (20 mg, 0.25 mmol) was added. The mixture was stirred for 1 h, diluted with ether (30 mL), and the organic layer was washed with water (3 × 30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and evaporated to dryness. The residue was subjected to column chromatography (silica gel, hexane/EtOAc, 9:1) to yield **17** (54 mg, 93%) as a colorless oil, R_f = 0.39 (hexane/ethyl acetate, 8:2); UV–vis (MeOH): λ_{max} (log ε) = 252 (3.81), 266 (3.77), 296 (3.75), 314 (3.85), 337 nm (3.72); IR (film): ν = 2962w, 2928w, 2201vw, 2125vw, 1743vs, 1714m, 1636m, 1585w, 1383w, 1364w, 1237s, 1076m, 1027m, 987m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.66 (dd, J = 15.4, 11.0 Hz, 1H, H-9), 6.28 (dt, J = 15.9, 5.8 Hz, 1H, H-16), 6.12 (dddt, J = 15.0, 10.7, 1.4, 0.7 Hz, 1H, H-8), 5.88–5.81 (m, 2H, H-15 + H-7), 5.56 (d, J = 15.6 Hz, 1H, H-10), 4.62 (dd, J = 5.8, 1.7 Hz, 2H, CH_2 (17)), 2.51 (t, J = 7.1 Hz, 2H, CH_2 (5)), 2.42–2.36 (m, 4H, CH_2 (3) + CH_2 (6)), 2.08 (s, 3H, CH_3 (19)), 1.64–1.56 (m, 2H, CH_2 (2)), 0.91 (t, J = 7.4 Hz, 3H, CH_3 (1)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 209.9 (*C*=O, C-4), 170.5 (*C*=O, C-18), 145.2 (*C*H=CH, C-9), 139.6 (*C*H=CH, C-16), 138.1 (*C*H=CH, C-7), 130.4 (*C*H=CH, C-8), 112.3 (*C*H=CH, C-15), 108.2 (*C*H=CH, C-10), 81.8 (*C*≡C, C-11), 79.8 (*C*≡C, C-14), 75.8 (*C*≡C, C-12), 75.7 (*C*≡C, C-13), 63.8 (*C*H_2, C-17), 45.0 (*C*H_2, C-6), 41.8 (*C*H_2, C-5), 27.0 (*C*H_2, C-3), 20.9 (*C*H_3, C-19), 17.4 (*C*H_2, C-2), 13.9 (*C*H_3, C-1) ppm; MS (ESI, MeOH): m/z = 316.0 (100%, $[M+NH_4]^+$), 321.1 (46%, $[M+Na]^+$), 337.0 (10%, $[M+K]^+$), 618.9 (12%, $[2M+Na]^+$); Anal. Calcd for $C_{19}H_{22}O_3$ (298.38): C 76.48, H 7.43; found: C 76.31, H 7.55.

4.3.15. (2*E*,8*E*,10*E*) 14-Oxo-2,8,10-heptadecatriene-4,6-diyn-1-yl-benzoate (18)

To a solution of (**13** (50 mg, 0.20 mmol) and triethylamine (25 mg, 0.25 mmol) in dry DCM (5 mL) benzoyl chloride (35 mg, 0.25 mmol) was added. After 1 h the reaction was diluted with ether (30 mL) and the organic layer was washed with water (3 × 30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and evaporated to dryness. The residue was subjected to column chromatography (silica gel, hexane/EtOAc, 9:1) to yield **18** (65 mg, 92%) as a colorless solid; mp = 45–46 °C; R_f = 0.55 (hexane/ethyl acetate, 9:1); UV–vis (MeOH): λ_{max} (log ε) = 252 (3.61), 267 (3.57), 280 (3.35), 296 (3.54), 315 (3.67), 337 nm (3.54); IR (KBr): ν = 2959w, 2927w, 2196vw, 2125vw, 1716vs, 1635m, 1452w, 1382w, 1277s, 1123m, 1071m, 1026m, 988m, 711m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.06–8.03 (m, 2H, C$H_{aromat.}$), 7.59–7.56 (m, 1H, C$H_{aromat.}$), 7.47–7.43 (m, 2H, C$H_{aromat.}$), 6.65 (dd, J = 15.3, 11.0 Hz, 1H, H-9), 6.41 (dt, J = 15.9, 5.7 Hz, 1H, H-16), 6.12 (dddt, J = 15.1, 10.8, 1.4, 0.7 Hz, 1H, H-8), 5.94 (ddt, J = 15.9, 2.8, 1.7 Hz, 1H, H-15), 5.84 (dt, J = 15.1, 7.0 Hz, 1H, H-7), 5.57 (d, J = 15.4 Hz, 1H, H-10), 4.89 (dd, J = 5.7, 1.7 Hz, 2H, CH_2 (17)), 2.51 (t, J = 7.1 Hz, 2H, CH_2 (5)), 2.42–2.36 (m, 4H, CH_2 (3) + CH_2 (6)), 1.64–1.56 (m, 2H, CH_2 (2)), 0.91 (t, J = 7.4 Hz, 3H, CH_3 (1)) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 209.9 (*C*=O, C-4), 166.1 (*C*=O, C-18), 145.2 (*C*H=CH, C-9), 139.6 (*C*H=CH, C-16), 138.1 (*C*H=CH, C-7), 133.4 (*C*H$_{aromat.}$, C22), 130.4 (*C*H=CH, C-8), 129.9 (*C*$_{aromat.}$), 129.8 (*C*H$_{aromat}$), 128.6 (*C*H$_{aromat.}$, C21), 112.4 (*C*H=CH, C-15), 108.2 (*C*H = CH, C-10), 81.9 (*C*≡C, C-11), 79.8 (*C*≡C, C-14), 75.8 (*C*≡C, C-12), 75.8 (*C*≡C, C-13), 64.2 (*C*H_2, C-17), 45.0 (*C*H_2, C-6), 41.8 (*C*H_2, C-5), 27.0 (*C*H_2, C-3), 17.4 (*C*H_2, C-2), 13.9 (*C*H_3, C-1) ppm; MS (ESI, MeOH): m/z = 377.8 (54%, $[M+NH_4]^+$), 383.1 (78%, $[M+Na]^+$), 563.1 72%, ($[3M+2Na]^{2+}$), 742.9 (100%, $[2M+Na]^+$); Anal. Calcd for $C_{24}H_{24}O_3$ (360.45): C 79.97, H 6.71; found: C 79.63, H 6.95.

4.3.16. (2*E*,8*E*,10*E*) 14-Oxo-2,8,10-heptadecatriene-4,6-diyn-1-yl-chloroacetate (19)

To a solution of **13** (50 mg, 0.20 mmol) and triethylamine (25 mg, 0.25 mmol) in dry DCM (5 mL) chloroacetyl chloride (28 mg, 0.25 mmol) was added. Reaction and workup as described above followed by column chromatography (silica gel, hexane/EtOAc, 9:1) gave **19** (55 mg, 85%) as a colorless solid; mp = 64–65 °C; R_f = 0.39 (silica gel, hexane/ethyl acetate, 8:2); UV–vis (MeOH): λ_{max} (log ε) = 211 (3.82), 252 (4.01), 267 (3.97), 280 (3.74), 296 (3.95), 315 (4.07), 338 nm (3.94); IR (KBr): ν = 2966m, 2936w, 2195vw, 2125vw, 1742vs, 1700s, 1631w, 1414m, 1381w, 1318m, 1196vs, 1128w, 1078w, 977s cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.67 (dd, J = 15.5, 10.9 Hz, 1H, H-9), 6.28 (dt, J = 15.9, 6.0 Hz, 1H, H-16), 6.12 (dddt, J = 15.1, 10.8, 1.4, 0.7 Hz, 1H, H-8), 5.90–5.81 (m, 2H, H-15 + H-7), 5.56 (d, J = 15.5 Hz, 1H, H-10), 4.74 (dd, J = 6.0, 1.6 Hz, 2H, CH_2 (17)), 4.08 (s, 2H, CH_2 (19)), 2.51 (t, J = 7.1 Hz, 2H, CH_2 (5)), 2.42–2.35 (m, 4H, CH_2 (3) + CH_2 (6)), 1.64–1.56 (m, 2H, CH_2 (2)), 0.91 (t, J = 7.4 Hz, 3H, CH_3 (1)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 209.9 (*C*=O, C-4), 166.9 (*C*=O, C-18), 145.4 (*C*H=CH, C-9), 138.2 (*C*H=CH, C-16), 138.1 (*C*H=CH, C-7), 130.4 (*C*H=CH, C-8), 113.5 (*C*H=CH, C-15), 108.1 (*C*H=CH, C-10), 82.2 (*C*≡C, C-11), 79.4 (*C*≡C, C-14), 76.3 (*C*≡C, C-12), 75.7 (*C*≡C, C-13), 65.3 (*C*H_2, C-17), 45.0 (*C*H_2, C-6), 41.7 (*C*H_2, C-5), 40.8 (*C*H_2, C-19), 27.0 (*C*H_2, C-3), 17.4 (*C*H_2, C-2), 13.9 (*C*H_3, C-1) ppm; MS (ESI, MeOH): m/z = 333.0 (18%, $[M+H]^+$), 350.1 (56%, $[M+NH^4]^+$), 355.0 (82%, $[M+Na]^+$), 521.9 (66%, $[3M+2Na]^{2+}$), 686.7 (30%, $[2M+Na]^+$); Anal. Calcd for $C_{19}H_{21}ClO_3$ (332.82): C 68.57, H 6.36; found: C 68.45, H 6.54.

4.3.17. (2*E*,8*E*,10*E*) 14-Oxo-2,8,10-heptadecatriene-4,6-diynal (20)

To a solution of **13** (50 mg, 0.20 mmol) in acetone (4 mL) at 0 °C Jones reagent [freshly prepared from CrO_3 (20 mg, 0.20 mmol), H_2O (65 mg) and H_2SO_4 (20 mg)] was added. The mixture was stirred for 30 min at 0 °C; MeOH (0.5 mL) was added. After stirring for additional 10 min, the reaction was diluted with ether (30 mL), and the organic layer was washed with water (3 × 30 mL) and

brine (30 mL), dried ($MgSO_4$), filtered and evaporated to dryness. The residue was subjected to column chromatography (silica gel, hexane/EtOAc, 8:2) to yield **20** (44 mg, 89%) as an off-white solid; mp = 34–37 °C; R_f = 0.81 (hexane/EtOAc, 6:4); UV–vis (MeOH): λ_{max} (log ε) = 220 (4.06), 257 (4.16), 266 (4.18), 295 (4.18), 314 (4.18), 339 (4.13), 364 nm (3.98); IR (KBr): ν = 2961w, 2935w, 2187m, 1702s, 1683vs, 1634m, 1597m, 1417w, 1379w, 1288w, 1121s, 982m, 956m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 9.58 (*d*, *J* = 7.6 Hz, 1H, H-1), 6.74 (dd, *J* = 15.6, 10.8 Hz, 1H, H-9), 6.66 (dd, *J* = 15.9, 1.1 Hz, 1H, H-3), 6.53 (dd, *J* = 15.9, 7.6 Hz, 1H, H-2), 6.15 (dddt, *J* = 15.1, 10.9, 1.4, 0.7 Hz, 1H, H-10), 5.92 (dt, *J* = 15.0, 7.0 Hz, 1H, H-11), 5.62 (d, *J* = 15.6 Hz, 1H, H-8), 2.52 (t, *J* = 7.5 Hz, 2H, CH_2 (13)), 2.44–2.36 (m, 4H, CH_2 (15) + CH_2 (12)), 1.65–1.56 (m, 2H, CH_2 (16)), 0.91 (*t*, *J* = 7.4 Hz, 3H, CH_3 (17)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 209.8 (C=O, C-14), 192.8 (C=O, C-1), 146.8 (CH=CH, C-9), 141.2 (CH=CH, C-2), 139.6 (CH=CH, C-11), 131.2 (CH=CH, C-10), 130.3 (CH=CH, C-3), 107.5 (CH=CH, C-8), 88.6 (C≡C, C-7), 88.4 (C≡C, C-5), 78.0 (C≡C, C-4), 75.2 (C≡C, C-6), 45.0 (CH_2, C-12), 41.6 (CH_2, C-13), 27.0 (CH_2, C-15), 17.4 (CH_2, C-16), 13.9 (CH_3, C-17) ppm; MS (ESI, MeOH): *m*/*z* = 255.3 (98%, $[M+H]^+$), 272.1 (34%, $[M+NH_4]^+$), 277.2 (14%, $[M+Na]^+$), 287.2 20%, ($[M+H+MeOH]^+$), 309.3 (96%, $[M+Na+MeOH]^+$); Anal. Calcd for C17H18O2 (254.32): C 80.28, H 7.13; found: C 79.97, H 7.22.

4.3.18. (2*E*,8*E*,10*E*)-14-Oxo-2,8,10-heptadecatriene-4,6-diynoic acid (21)

A solution of **13** (50 mg, 0.20 mmol) in acetone (4 mL) was cooled to 0 °C and Jones reagent [freshly prepared from CrO_3 (40 mg, 0.40 mmol), H_2O (130 mg) and H_2SO_4 (40 mg)] was added. The mixture was stirred for 30 min at 0 °C; MeOH (0.5 mL) was added. After stirring for an additional 10 min, the reaction was diluted with ether (30 mL), and the organic layer was washed with water (3 × 30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and evaporated to dryness. The residue was subjected to column chromatography (silica gel, EtOAc) to yield **21** (28 mg, 53%) as an off-white solid; mp = 128–131 °C; R_f = 0.10 (hexane/EtOAc, 6:4); UV–vis (MeOH): λ_{max} (log ε) = 215 (4.10), 265 (4.21), 282 (4.23), 297 (4.16), 315 (4.16), 336 (4.16), 351 nm (4.11); IR (KBr): ν = 2960m, 2930m, 2192vw, 1703vs, 1680s, 1613m, 1417m, 1382w, 1266m, 1202w, 1128w, 982m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 6.89 (dd, *J* = 15.8, 1.0 Hz, 1H, H-3), 6.72 (dd, *J* = 15.4, 10.9 Hz, 1H, H-9), 6.30 (d, *J* = 15.8 Hz, 1H, H-2), 6.14 (dd, *J* = 15.1, 10.9 Hz, 1H, H-10), 5.90 (dt, *J* = 14.4, 7.0 Hz, 1H, H-11), 5.60 (d, *J* = 15.6 Hz, 1H, H-8), 2.52 (t, *J* = 7.4 Hz, 2H, CH_2 (13)), 2.45–2.36 (*m*, 4H, CH_2 (15) + CH_2 (12)), 1.66–1.57 (m, 2H, CH_2 (16)), 0.91 (t, *J* = 7.4 Hz, 3H, CH_3 (17)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 210.0 (C=O, C-14), 170.2 (C=O, C-1), 146.5 (CH=CH, C-9), 139.2 (CH=CH, C-11), 131.6 (CH=CH, C-2), 130.3 (CH=CH, C-10), 126.6 (CH=CH, C-3), 107.7 (CH=CH, C-8), 86.4 (C≡C, C-7), 84.5 (C≡C, C-5), 78.4 (C≡C, C-4), 75.3 (C≡C, C-6), 44.8 (CH_2, C-12), 41.7 (CH_2, C-13), 27.0 (CH_2, C-15), 17.4 (CH_2, C-16), 13.9 (CH_3, C-17) ppm; MS (ESI, MeOH): *m*/*z* = 269.0 100%, ($[M-H]^-$), 314.8 (12%, $[M+HCO_2]^-$); Anal. Calcd for $C_{17}H_{18}O_3$ (270.32): C 75.53, H 6.71; found: C 75.41, H 6.89.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.07.031.

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