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Total synthesis of a dienynone from *Echinacea pallida*

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ABSTRACT

The first total synthesis of (8*Z*,13*Z*)-pentadeca-8,13-dien-11-yn-2-one is described. This dienynone was recently isolated from the *n*-hexane extract of *Echinacea pallida* roots and displayed a selective cytotoxic activity toward cancer cells, thus featuring as a potential anticancer lead. The product was obtained in 11 steps in 25% overall yield.

1. Introduction

The in vitro cytotoxic and pro-apoptotic activities of the *n*-hexane root extracts from the three medicinally important *Echinacea* species (*Echinacea purpurea*, *Echinacea angustifolia* var. *angustifolia*, and *Echinacea pallida*; Asteraceae family) have been recently demonstrated.[1] A more pronounced cytotoxic effect for *E. pallida* root extracts in comparison with the other two species was observed.[1] This is in agreement with the different chemical composition of this species with respect to the others belonging to the genus *Echinacea*: in fact, the typical hydrophobic constituents of *E. pallida* extracts were found to be polyacetylenes and polyenes, whereas the lipophilic extracts of *E. purpurea* and *E. angustifolia* mainly consist of alkamides.[2–7] More recently, the bioassay-guided isolation and characterization of most of the lipophilic constituents of *E. pallida* roots have been performed.[6,7] Among them, (8*Z*,13*Z*)-pentadeca-8,13-dien-11-yn-2-one (**1**) was found to be one of the major constituents (0.98 mg/g in the plant material and 0.19–1.90 mg/g in the herbal products)[6] as well as the most active compound.[7,8] Compound **1** displayed a very low IC_{50} value toward the colonic COLO320 cancer cell line (IC_{50}=2.34 μM) and a noteworthy activity toward pancreatic Mia PaCa-2 cancer cell line (IC_{50}=32.17 μM), the latter being a type with generally low sensitivity to therapeutic agents.[9]Apoptotic cell death was found to be involved in the cytotoxic activity of this molecule.[8] Dienynone **1** displayed a selective effect on cancer cells versus non-cancer cells, with an IC_{50} value higher than 100 μM against human embryonic kidney HEK-293 cell.[8] Furthermore, this compound was found to be able to cross the Caco-2 monolayer,[8] which is an accepted model of intestinal absorption,[10] indicating a potential good absorption in humans after oral administration.

Due to the difficulty in purifying this compound from *E. pallida* roots, whose extracts contain many other constituents of similar polarity, and owing to the need of higher amounts of **1** for biological assays, the total synthesis of this secondary metabolite was undertaken.

2. Discussion

2.1. Retrosynthesis

By analogy to the total synthesis of related structures,[11,12] our synthetic strategy hinged on a Sonogashira coupling reaction for the insertion of the terminal alkene moiety. Enyne precursor **A** can be disconnected by chain extension and selective reduction to alkyne **B**, which can be traced back to commercially available 1-hexyne and propylenoxide (Scheme 1).

2.2. Synthesis

Alkynol **3**[13] (Scheme 2) was synthesized in 72% overall yield by coupling of propyleneoxide and 1-hexyne (**2**) with *n*-butyllithium and boron trifluoride[14] followed by migration of the triple bond to

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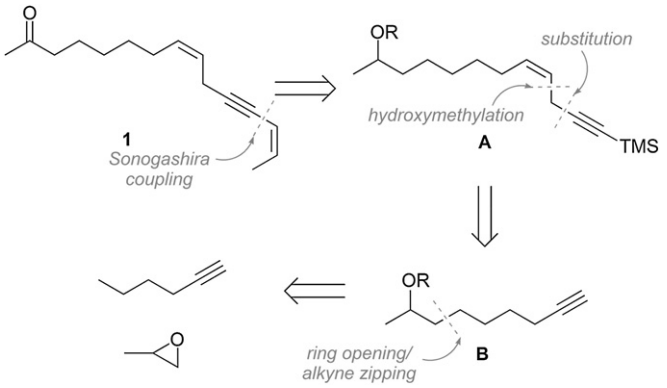


Scheme 1. Retrosynthetic analysis of dienynone **1**.

the chain terminus by means of the strong base KAPA (potassium hydride/1,3-diaminopropane).[15] The formation of the desired product **3** was confirmed by the presence of a diagnostic triplet in the ^{1}H NMR spectrum at 1.92 ppm ($^4J_{H\text{-}7\text{–}H\text{-}9}$=2.7 Hz, δ_C 68.2 ppm), referable to the terminal alkyne proton.

Finally, the resulting alcohol was protected as a THP ether (85% yield) following the classical protocol,[16] affording a mixture of stereoisomers clearly discernable by NMR analysis.

Protected alkynol **4** was converted into enyne **5** following the protocol described by Kraus,[11] which, in our hands, resulted in an improved 64% overall yield over four steps. In particular, hydroxymethylation of compound **4** was accomplished by reaction with ethylmagnesium bromide and formaldehyde. Subsequently, nucleophilic displacement of the hydroxy group with iodine followed by copper-catalyzed coupling with trimethylsilylacetylene allowed the insertion of the second acetylene moiety. Finally, compound **5** was obtained by regioselective reduction of the internal alkyne using nickel acetate and sodium borohydride.[17]

Enyne **5** was then desilylated using silver nitrate and potassium cyanide in methanol/water,[18] thus affording terminal alkyne **6** in 90% yield. Removal of the TMS group was easily confirmed by ^{1}H NMR analysis, which showed a highly diagnostic triplet at δ 1.94 ppm, corresponding to the terminal alkynyl proton (δ_C 67.5–67.8 ppm). Compound **6** was subjected to Sonogashira coupling[19] with commercial *cis*-bromopropene in the presence of $Pd(PPh_3)_4$ and copper iodide in piperidine[20] to afford the highly stable **7**[21] in 93% yield. The structure of **7** was confirmed by mass spectrometry and NMR spectroscopy. In particular, the ^{1}H NMR spectrum was characterized by the presence of a diagnostic doublet of doublet at δ 1.85 ppm, corresponding to the terminal allylic methyl group (δ_C 15.7 ppm). Due to the overlapping of olefinic proton signals in the ^{1}H spectrum, the configuration of the double bonds could not be assigned by direct measurement of coupling constants ($^3J_{H\text{–}H}$). For this purpose, a non-decoupled gHMBC experiment was run and accurate analysis of ^{13}C NMR satellites[22] allowed to assign the *Z* stereochemistry of both double bonds, ($^3J_{H\text{-}2\text{–}H\text{-}3}$=10.9 Hz, $^3J_{H\text{-}7\text{–}H\text{-}8}$= 5.7 Hz), thus confirming that the coupling reaction proceeded with retention of the cis-configuration.

Finally, the THP protecting group was quantitatively removed using catalytic *p*-toluenesulfonic acid in methanol at rt[23] and the resulting alcohol was converted to the corresponding ketone by Swern oxidation,[24] leading to compound **1** in 71% yield from enyne **5**. Spectroscopic data of compound **1** were in full agreement with those reported for the natural product isolated from *E. pallida* roots.[7] Dienynone **1** was found to be particularly prone to allylic oxidation, and required storage under inert atmosphere at −20 °C. In fact, NMR spectra of samples stored at higher temperatures (at 4 °C and at rt) for a few days showed a gradual increase of the oxidation products.[2,7] This is in agreement with what was observed in the course of the extraction and isolation of this compound from the natural matrix, whereby oxidized artifacts were detected upon exposure to atmospheric oxygen. At present, the problem can be circumvented by stocking the precursor **7** and proceeding with deprotection–oxidation steps on demand.

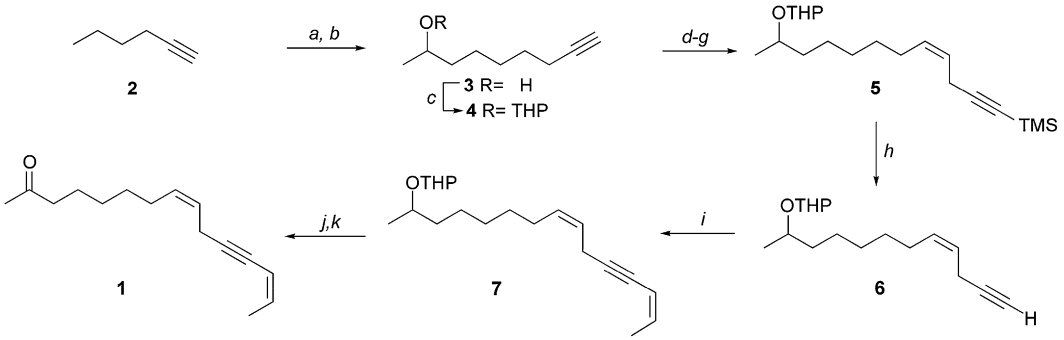
3. Conclusions

In conclusion, we have described the first total synthesis of dienynone **1**, originally isolated from *E. pallida* roots, which displays cytotoxic activity toward pancreatic and colonic cancer cell lines. Compound **1** was synthesized in 11 steps from 1-hexyne and propyleneoxide in 25% overall yield. Further studies on the biological activity of compound **1** are currently ongoing with the aim to shed light on the mechanism of action of this potential anticancer lead.

4. Experimental

4.1. General

All solvents used were anhydrous, unless stated otherwise, and all reactions requiring anhydrous conditions were performed using oven-dried and argon-flushed glassware. Anhydrous THF and diethyl ether were prepared by standard methods and freshly distilled from sodium benzophenone before use. Dichloromethane was dried according to standard procedures and stored upon 3 Å molecular sieves. All reagents were purchased from Aldrich. Chromatographic purification of compounds was carried out on silica gel (60–200 μm). Compounds were visualized by exposure to UV light and by dipping the plates in $KMnO_4$ stain followed by



Scheme 2. Synthesis of compound **1**. (a) *n*-BuLi, $BF_3{\cdot}Et_2O$, propylenoxide, THF, −78 °C, 3 h, 85%. (b) KAPA, rt overnight, 85%. (c) DHP, CH_2Cl_2, rt, 2 h, 85%. (d) EtMgBr, HCHO, THF, reflux 1 h, then rt overnight, 87%. (e) I_2, PPh_3, Et_2O/MeCN, 0 °C→rt, 4 h, 92%. (f) CuI, K_2CO_3, trimethylsilylacetylene, DMF, 0 °C→rt, then 35 °C, 6 h, 86%. (g) $NiAc_2{\cdot}4H_2O$, $NaBH_4$, ethylendiamine, H_2, EtOH, rt, 2 h, 93%. (h) $AgNO_3$, KCN, H_2O/MeOH, rt, 15 min, 86%. (i) *cis*-1-bromopropene, CuI, $Pd(PPh_3)_4$, piperidine, rt, overnight, 96%. (j) PTSA, MeOH, 40 °C, 1 h, 100%. (k) $(COCl)_2$, DMSO, TEA, CH_2Cl_2, −78 °C, 1.5 h, then rt, 1 h, 74%.

heating on a hot plate. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX200 spectrometer; chemical shifts (δ) are reported in parts per million downfield from TMS as internal standard (s singlet, d doublet, t triplet, q quartet, m multiplet, br s broad signal); coupling constants (J) are given in hertz. Two-dimensional NMR techniques (COSY, HMBC, HSQC, and NOESY) were utilized to aid the assignment of signals in ^{1}H and ^{13}C spectra. For mass spectral determinations a Finnigan MAT SSQ A and a Hewlett–Packard HP5972 spectrometer were used (EI, 70 eV). IR spectra were recorded on a Perkin–Elmer 1600 FTIR spectrophotometer; wavenumbers (ν_{max}) are in cm^{-1}. Elemental analyses were performed with a Carlo Erba Elemental Analyzer mod. 1110.

4.2. Synthesis of 8-nonyn-2-ol (3)

4.2.1. 4-Nonyn-2-ol[13]

In a 100 mL three-necked flask 1-hexyne (1.7 mL, 24.0 mmol) was dissolved in anhydrous THF (10 mL) and cooled at −78 °C under an inert atmosphere. *n*-BuLi (1.6 M soln in hexanes, 16.5 mL, 26.4 mmol) was slowly added (30 min) and the resulting pale pink solution was stirred at −78 °C for 30 min. Afterward, neat $BF_3\cdot Et_2O$ (3.54 mL, 28.8 mmol) was added and after 15 min the turbid mixture was treated with a solution of freshly distilled propylenoxide (2.1 mL, 36.0 mmol) in THF (6 mL). The resulting clear solution was stirred for 2 h at −78 °C and the crude was then partitioned between satd NH_4Cl (100 mL) and Et_2O (3×100 mL). The collected organic layers were washed with water (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford a crude yellow oil, which was purified by column chromatography (eluant 9:1 Pet/EtOAc). 4-Nonyn-2-ol was isolated as a pale yellow oil (2.872 g, 85% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (3H, t, J=7.1 Hz, H-9), 1.22 (3H, d, J=5.9 Hz, H-1), 1.30–1.57 (4H, m, H-7, H-8), 2.00–2.10 (1H, br s, OH), 2.10–2.24 (2H, m, H-6), 2.25–2.36 (2H, m, H-3), 3.88 (1H, sex, J=6.1 Hz, H-2). ^{13}C NMR (200 MHz, $CDCl_3$): δ 13.5 (C-9), 18.3 (C-1), 21.9 (C-7/8), 22.1 (C-7/8), 29.4 (C-6), 31.0 (C-3), 66.5 (C-2), 76.0 (C-4/5), 83.1 (C-4/5). MS: m/z 140 (M$^+$, <1%), 139 (<1), 125 (<1), 111 (2), 107 (9), 97 (9), 93 (13), 81 (54), 79 (34), 67 (53), 55 (54), 54 (57), 45 (100). Anal. Calcd for $C_9H_{16}O$: C, 77.09; H, 11.50. Found: C, 77.23; H, 11.31.

4.2.2. 8-Nonyn-2-ol (**3**)[13]

In a 50 mL two-necked flask, dry potassium hydride (719 mg of a 35% suspension in mineral oil, 6.3 mmol, repeatedly washed with *n*-hexane then oven-dried) was treated with 1,3-diaminopropane (APA, 10.6 mL, 125 mmol) and heated at 40 °C for 1 h to give a brownish slurry (KAPA). 4-Nonyn-2-ol (352 mg, 2.5 mmol) dissolved in APA (1 mL) was then added at 0 °C and the resulting orange mixture was allowed to warm to rt overnight. The brown crude mixture was cautiously poured in a separatory funnel containing ice (100 g) and extracted with Et_2O (3×100 mL). The collected organic phases were washed with 4 M HCl (30 mL), dried over $MgSO_4$, filtered, and concentrated to afford compound **3** as a yellow oil (272 mg, 77% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.05 (3H, d, J=6.0 Hz, H-1), 1.30–1.65 (8H, m, H-3 to H-6), 1.86 (1H, br s, OH), 1.92 (1H, t, J=2.7 Hz, H-9), 2.16 (2H, dt, J=6.8, 2.7 Hz, H-7), 3.66–3.87 (1H, m, H-2). ^{13}C NMR (50 MHz, $CDCl_3$): δ 18.3 (C-1), 23.4 (C-6), 25.2 (C-4/5), 28.3 (C-4/5), 28.6 (C-3), 39.1 (C-7), 67.9 (C-2), 68.2 (C-9), 84.5 (C-8). MS: m/z 140 (M$^+$, <1%), 139 (<1), 125 (<1), 111 (2), 107 (9), 97 (9), 93 (13), 81 (54), 79 (34), 67 (53), 55 (54), 54 (57), 45 (100). Anal. Calcd for $C_9H_{16}O$: C, 77.09; H, 11.50. Found: C, 77.29; H, 11.33.

4.3. 8-(2-Oxacyclohexyl)oxy-1-nonyne (4)[11]

A solution of **3** (498 mg, 3.6 mmol) and pyridinium *p*-toluenesulfonate (89 mg, 0.4 mmol) in dry CH_2Cl_2 (10 mL) was treated at 0 °C with dihydropyran (0.367 mL, 3.9 mmol). The solution was stirred for 1 h at 0 °C and for 1 h at rt, then it was partitioned between satd $NaHCO_3$ (20 mL) and CH_2Cl_2 (3×50 mL). The organic phases were dried over $MgSO_4$, filtered, and concentrated. The resulting brown oil was purified by column chromatography (eluant 9:1 Pet/EtOAc) to afford **4** as a yellow oil (209 mg, 80% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.09 (3H, d, J=6.1 Hz, H-9), 1.20 (3H, d, J=6.2 Hz, H-9), 1.28–1.88 (14H×2, m, H-4 to H-7, H-3′ to H-5′), 1.91 (1H, t, J=2.6 Hz, H-1), 1.92 (1H, t, J=2.6 Hz, H-1), 2.17 (2H×2, dt, J=6.9, 2.6 Hz, H-3), 3.40–3.54 (1H×2, m, H-6′), 3.60–3.98 (2H×2, m, H-6′, H-8), 4.58–4.65 (1H, m, H-2′), 4.65–4.73 (1H, m, H-2′). ^{13}C NMR (50 MHz, $CDCl_3$): δ 18.29 (C-3), 18.33 (C-3), 19.0 (C-9), 19.7 (C-4′), 20.0 (C-4′), 21.5 (C-9), 24.9 (C-6), 25.2 (C-6), 25.50 (C-5′), 25.55 (C-5′), 28.39 (C-5), 28.43 (C-5), 28.8 (C-4), 31.19 (C-3′), 31.22 (C-3′), 36.3 (C-7), 37.3 (C-7), 62.4 (C-6′), 62.7 (C-6′), 68.0 (C-1), 68.1 (C-1), 71.0 (C-8), 73.8 (C-8), 84.6 (C-2), 84.7 (C-2), 95.6 (C-2′), 98.6 (C-2′). MS: m/z 225 (M$^+$+1, <1%), 223 (<1), 209 (<1), 151 (<1), 129 (9), 123 (3), 107 (2), 101 (23), 93 (3), 85 (100), 81 (34), 79 (9), 77 (3), 67 (20), 57 (17), 56 (25), 55 (23). Anal. Calcd for $C_{14}H_{24}O_2$: C, 74.95; H, 10.78. Found: C, 75.09; H, 10.61.

4.4. Synthesis of 1-trimethylsilyl (4*Z*)-11-(2-oxacyclohexyl)oxydodeca-4-en-1-yne (5)

4.4.1. 9-(2-Oxacyclohexyl)oxy-2-decyn-1-ol[11]

Ethylmagnesium bromide was generated in situ from ethyl iodide (0.332 mL, 4.15 mmol) and magnesium turnings (12.4 mmol, 301 mg) in anhydrous Et_2O (4 mL) in the presence of a crystal of iodine. After refluxing for 20 min, the turbid gray solution was transferred via cannula into a 50 mL three-necked flask under argon, diluted with anhydrous THF (4 mL) and treated at rt with a solution of **4** (620 mg, 2.76 mmol) in THF (2 mL). A white precipitate was formed during the additions. The mixture was refluxed for 1 h at 70 °C and then cooled to 0 °C. Paraformaldehyde (125 mg, 4.2 mmol) suspended in THF (2 mL) was then added and the reaction was refluxed again at 70 °C for 1 h. The resulting yellow mixture was stirred at rt overnight and finally quenched with satd $NaHCO_3$ (100 mL), extracted with CH_2Cl_2 (2×100 mL), and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure and the crude residue was purified by column chromatography (eluant 2:1 Pet/EtOAc) to afford 9-(2-oxacyclohexyl)oxy-2-decyn-1-ol as a pale yellow liquid (504 mg, 87% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.08 (3H, d, J=6.2 Hz, H-10), 1.19 (3H, d, J=6.3 Hz, H-10), 1.28–1.93 (14H×2, m, H-5 to H-8, H-3′ to H-5′), 2.19 (2H×2, tt, J=6.8, 2.2 Hz, H-4), 2.20–2.35 (1H×2, br s, OH), 3.40–3.54 (1H×2, m, H-6′), 3.60–3.97 (2H×2, m, H-6′, H-9), 4.20 (2H×2, t, J=2.2 Hz, H-1), 4.59–4.72 (1H×2, m, H-2′). ^{13}C NMR (50 MHz, $CDCl_3$): δ 18.3 (C-4), 18.4 (C-4), 19.10 (C-10), 19.6 (C-4′), 19.9 (C-4′), 21.5 (C-10), 24.8 (C-7), 25.2 (C-7), 25.48 (C-5′), 25.52 (C-5′), 28.4 (C-6), 28.7 (C-5), 31.1 (C-3′), 31.2 (C-3′), 36.3 (C-8), 37.2 (C-8), 51.1 (C-1), 62.4 (C-6′), 62.6 (C-6′), 71.0 (C-9), 73.7 (C-9), 78.6 (C-2), 78.7 (C-2), 86.2 (C-3), 86.3 (C-3), 95.6 (C-2′), 98.4 (C-2′). MS: m/z 253 (M$^+$−1, <1%), 223 (<1), 165 (<1), 153 (<1), 135 (6), 129 (4), 107 (8), 101 (15), 93 (16), 85 (100), 81 (10), 79 (13), 77 (4), 67 (19), 57 (17), 56 (12), 55 (26). Anal. Calcd for $C_{15}H_{26}O_3$: C, 70.83; H, 10.30. Found: C, 70.67; H, 10.48.

4.4.2. 1-Iodo-9-(2-oxacyclohexyl)oxy-2-decyne[11]

Imidazole (102 mg, 1.5 mmol) and triphenylphosphine (395 mg, 1.5 mmol) were dissolved in dry MeCN/Et_2O 3:1 (8 mL) and the solution was cooled at 0 °C. Iodine (382 mg, 1.5 mmol) was slowly added and the resulting orange slurry was stirred for 1 h at rt. The mixture was cooled to 0 °C, treated with a solution of 9-(2-oxacyclohexyl)oxy-2-decyn-1-olo (348 mg, 1.37 mmol) in Et_2O (4 mL), and finally warmed to rt and stirred for 1 h. The crude mixture was diluted with CH_2Cl_2 (100 mL), washed with satd $NaHCO_3$ (30 mL)

and brine (30 mL), dried over $MgSO_4$, filtered, and concentrated to afford an orange oil, which was purified by column chromatography (eluant 2:1 Pet/EtOAc). 1-Iodo-9-(2-oxacyclohexyl)oxy-2-decyne was recovered as a pale yellow oil (457 mg, 92% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.10 (3H, d, J=6.1 Hz, H-10), 1.22 (3H, d, J=6.5 Hz, H-10), 1.28–1.96 (14H×2, m, H-5 to H-8, H-3′ to H-5′), 2.13–2.26 (2H×2, m, H-4), 3.42–3.56 (1H×2, m, H-6′), 3.71 (2H×2, t, J=2.4 Hz, H-1), 3.63–4.00 (2H×2, m, H-6′, H-9), 4.60–4.74 (1H×2, m, H-2′). ^{13}C NMR (50 MHz, $CDCl_3$): δ −16.87 (C-1), −16.83 (C-1), 19.0 (C-4), 19.1 (C-10), 19.8 (C-4′), 20.0 (C-4′), 21.6 (C-10), 24.9 (C-7), 25.2 (C-7), 25.3 (C-5′), 25.5 (C-5′), 28.3 (C-6), 28.8 (C-5), 31.3 (C-3′), 36.4 (C-8), 37.3 (C-8), 62.5 (C-6′), 62.8 (C-6′), 68.0 (C-2), 71.0 (C-9), 73.8 (C-9), 85.0 (C-3), 95.7 (C-2′), 98.6 (C-2′). MS: m/z 363 (M^+−1, <1%), 263 (<1), 257 (<1), 237 (<1), 221 (<1), 193 (<1), 183 (<1), 136 (6), 135 (7), 129 (5), 107 (12), 101 (13), 94 (14), 93 (17), 85 (100), 81 (4), 79 (13), 77 (6), 67 (11), 57 (11), 56 (19), 55 (12). Anal. Calcd for $C_{15}H_{25}IO_2$: C, 49.46; H, 6.92. Found: C, 49.70; H, 7.13.

4.4.3. 1-Trimethylsilyl 11-(2-oxacyclohexyl)oxydodeca-1,4-diyne[11]

Oven-dried K_2CO_3 (192 mg, 1.38 mmol) and CuI (121 mg, 0.6 mmol) were suspended in anhydrous DMF (5 mL). The resulting slurry was cooled to 0 °C, and ethynyltrimethylsilane (0.695 mL, 5.0 mmol) and a solution of 1-iodo-9-(2-oxacyclohexyl)oxy-2-decyne (457 mg, 1.2 mmol) in DMF (2 mL) were added sequentially via syringe. The resulting mixture was warmed to rt and stirred overnight, whereupon the color turned from limpid yellow to turbid brick-red, and finally partitioned between satd NH_4Cl (50 mL) and CH_2Cl_2 (2×70 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude residue was purified by column chromatography (eluant 10:1 Pet/EtOAc) to afford 1-trimethylsilyl 11-(2-oxacyclohexyl)oxydodeca-1,4-diyne as a pale yellow oil (361 mg, 86% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.15 (9H×2, s, TMS), 1.09 (3H, d, J=6.1 Hz, H-12), 1.21 (3H, d, J=6.2 Hz, H-12), 1.22–1.96 (14H×2, m, H-7 to H-10, H-3′ to H-5′), 2.05–2.25 (2H×2, m, H-6), 3.17 (2H×2, t, J=2.4 Hz, H-3), 3.40–3.55 (1H×2, m, H-6′), 3.62–3.99 (2H×2, m, H-6′, H-11), 4.58–4.73 (1H×2, m, H-2′). ^{13}C NMR (50 MHz, $CDCl_3$): δ −0.1 (TMS), 10.8 (C-3), 18.6 (C-6), 18.7 (C-6), 19.1 (C-12), 19.7 (C-4′), 20.0 (C-4′), 21.5 (C-12), 24.9 (C-9), 25.3 (C-9), 25.5 (C-5′), 25.6 (C-5′), 28.6 (C-8), 28.9 (C-7), 31.2 (C-3′), 36.4 (C-10), 37.4 (C-10), 62.4 (C-6′), 62.7 (C-6′), 71.0 (C-11), 73.3 (C-4), 73.4 (C-4), 73.8 (C-11), 80.9 (C-5), 81.0 (C-5), 84.5 (C-1), 95.6 (C-2′), 98.6 (C-2′), 100.78 (C-2), 100.81 (C-2). MS: m/z 334 (M^+, <1%), 261 (<1), 233 (<1), 223 (<1), 203 (<1), 173 (6), 159 (20), 85 (100), 73 (66). Anal. Calcd for $C_{20}H_{34}O_2Si$: C, 71.80; H, 10.24. Found: C, 71.70; H, 10.43.

4.4.4. 1-Trimethylsilyl (4Z)-11-(2-oxacyclohexyl)oxydodeca-4-en-1-yne (**5**)[11]

$NiAc_2\cdot 4H_2O$ (252 mg, 1.0 mmol) was dissolved in EtOH (10 mL) and treated with a suspension of $NaBH_4$ (38 mg, 1.0 mmol) in EtOH (3 mL) under H_2. The green solution turned immediately black due to the formation of the catalyst, which was then poisoned with ethylendiamine (3.2 mL of a 0.623 M soln in EtOH, 2.0 mmol). A solution of 1-trimethylsilyl-11-(2-oxacyclohexyl)oxydodeca-1,4-diyne (1.693 g, 5.1 mmol) in EtOH (6 mL) was then added at rt and the mixture was stirred for 2 h. The crude was filtered through a short Celite pad and the solvent was removed in vacuo to afford a brown oil, which was purified by column chromatography (eluant 10:1 Pet/EtOAc). The title compound **5** was isolated as a pale yellow oil (1.582 g, 93%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.17 (9H×2, s, TMS), 1.08 (3H, d, J=6.1 Hz, H-12), 1.19 (3H, d, J=6.1 Hz, H-12), 1.22–1.95 (14H×2, m, H-7 to H-10, H-3′ to H-5′), 1.94–2.13 (2H×2, m, H-6), 2.85–3.05 (2H×2, m, H-3), 3.39–3.56 (1H×2, m, H-6′), 3.60–4.00 (2H×2, m, H-6′, H-11), 4.57–4.75 (1H×2, m, H-2′), 5.25–5.60 (2H×2, m, H-4, H-5). ^{13}C NMR (50 MHz, $CDCl_3$): δ 0.06 (TMS), 18.3 (C-3), 19.0 (C-12), 19.7 (C-4′), 20.0 (C-4′), 21.5 (C-12), 25.3 (C-9), 25.5 (C-9), 25.58 (C-5′), 25.63 (C-5′), 27.08 (C-6), 27.11 (C-6), 29.22 (C-8), 29.28 (C-7), 29.31 (C-7), 31.18 (C-3′), 31.20 (C-3′), 36.4 (C-10), 37.4 (C-10), 62.4 (C-6′), 62.7 (C-6′), 71.0 (C-11), 73.8 (C-11), 83.8 (C-1), 95.6 (C-2′), 98.6 (C-2′), 105.4 (C-2), 123.82 (C-4), 123.88 (C-4), 132.79 (C-5), 131.87 (C-5). MS: m/z 336 (M^+, <1%), 321 (<1), 263 (1), 235 (<1), 219 (2), 205 (1), 191 (1), 173 (4), 161 (8), 159 (12), 135 (4), 119 (5), 101 (5), 85 (100), 73 (62), 59 (10). Anal. Calcd for $C_{20}H_{36}O_2Si$: C,71.37; H, 10.78. Found: C, 71.30; H, 10.89.

4.5. (4Z)-11-(2-Oxacyclohexyl)oxydodeca-4-en-1-yne (6)[11]

To a solution of **5** (1.011 g, 3.0 mmol) in MeOH (20 mL) at rt a solution of $AgNO_3$ (657 mg, 3.9 mmol) in 3:1 MeOH/H_2O (8 mL) was slowly added. The resulting mixture was stirred for 15 min at rt, then a solution of KCN (1.160 g, 1.8 mmol) in water (10 mL) was added dropwise. After 20 min at rt, TLC showed complete conversion and the mixture was quenched with brine (50 mL), extracted with Et_2O (3×150 mL), dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by column chromatography (eluant 10:1 Pet/EtOAc) to afford **6** as a pale yellow liquid (561 mg). Variable quantities of the corresponding deprotected alcohol were also isolated (107 mg) (86% total yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.08 (3H, d, J=6.1 Hz, H-12), 1.19 (3H, d, J=6.3 Hz, H-12), 1.20–1.90 (14H×2, m, H-7 to H-10, H-3′ to H-5′), 1.94 (2H×2, t, J=2.7 Hz, H-1), 1.95–2.10 (2H×2, m, H-6), 2.80–3.00 (2H×2, m, H-3), 3.37–3.55 (1H×2, m, H-6′), 3.60–4.00 (2H×2, m, H-6′, H-11), 4.55–4.75 (1H×2, m, H-2′), 5.30–5.56 (2H×2, m, H-4, H-5). ^{13}C NMR (50 MHz, $CDCl_3$): δ 16.8 (C-3), 19.0 (C-12), 19.7 (C-4′), 20.0 (C-4′), 21.5 (C-12), 25.2, 25.5, 25.57, 25.63, 27.01 (C-6), 27.05 (C-6), 29.19 (C-8), 29.24 (C-8), 29.27 (C-7), 29.4 (C-7), 31.18 (C-3′), 31.2 (C-3′), 36.4 (C-10), 37.4 (C-10), 62.3 (C-6′), 62.7 (C-6′), 67.6 (C-1), 67.8 (C-1), 71.0 (C-11), 73.8 (C-11), 95.6 (C-2′), 98.5 (C-2′), 114.1 (C-2), 123.5 (C-4), 123.6 (C-4), 132.0 (C-5), 132.1 (C-5). GC–MS: m/z 264 (M^+, <1%), 263 (<1), 249 (<1), 191 (1), 163 (2), 147 (3), 129 (9), 121 (9), 107 (9), 101 (20), 93 (12), 91 (12), 85 (100), 79 (11), 67 (9). Anal. Calcd for $C_{17}H_{28}O_2$: C, 77.22; H, 10.67. Found: C, 77.09; H, 10.84.

4.6. (2Z,7Z)-14-(2-Oxacyclohexyl)oxypentadeca-2,7-dien-4-yne (7)

To a solution of *cis*-1-bromopropene (0.262 mL, 3.0 mmol), $Pd(PPh_3)_4$ (10 mg, 0.01 mmol), and **6** (564 mg, 2.1 mmol) in freshly distilled piperidine (3.5 mL), CuI (33 mg, 0.17 mmol) was added at rt under argon. The yellow reaction mixture was stirred for 1.5 h at rt and then an additional portion of Pd catalyst (10 mg, 0.01 mmol) was added. After stirring overnight at rt the mixture turned dark green and became turbid. The mixture was quenched with brine (20 mL), extracted with Et_2O (5×50 mL), and the organic layers were washed with 5% aq HCl (5 mL). The first aqueous phase was acidified to pH 6 with 10% aq HCl and extracted with CH_2Cl_2 (2×50 mL). The collected organic phases were dried over $MgSO_4$, filtered, and concentrated to afford **7** as a brownish liquid, which was used as such for subjection to the following reaction.

For characterization purposes the crude material was purified by column chromatography (eluant 10:1 Pet/EtOAc) to afford **7** as a yellow liquid (613 mg, 96% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.10 (3H, d, J=6.1 Hz, H-15), 1.22 (3H, d, J=6.3 Hz, H-15), 1.25–1.95 (14H×2, m, H-10 to H-13, H-3′ to H-5′), 1.85 (3H, dd, J=6.7, 1.7 Hz, H-1), 2.00–2.20 (2H×2, m, H-9), 3.00–3.20 (2H×2, m, H-6), 3.40–3.60 (1H×2, m, H-6′), 3.63–4.05 (2H×2, m, H-6′, H-14), 4.60–4.78 (1H×2, m, H-2′), 5.35–5.60 (3H×2, m, H-3, H-7, H-8), 5.79–6.05 (1H×2, m, H-2). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.7 (C-1), 17.9 (C-6), 19.1 (C-15), 19.7 (C-4′), 20.1 (C-4′), 21.5 (C-15), 25.3 (C-12), 25.5 (C-12), 25.6 (C-5′), 25.7 (C-5′), 27.07 (C-9), 27.11 (C-9), 29.3 (C-10, C-11), 31.2 (C-3′), 36.4 (C-13), 37.5 (C-13), 62.4 (C-6′), 62.8 (C-6′), 71.1 (C-14), 73.8 (C-14), 77.0 (C-4), 93.0 (C-5), 95.6 (C-2′), 98.6 (C-2′),

110.28 (C-3), 110.29 (C-3), 124.28 (C-7), 124.35 (C-7), 131.6 (C-8), 131.7 (C-8), 137.11 (C-2), 137.13 (C-2). MS: *m*/*z* 304 (M^+, 2%), 289 (<1), 275 (<1), 231 (1), 220 (2), 202 (2), 187 (5), 173 (7), 159 (5), 145 (7), 131 (10), 117 (20), 105 (13), 91 (25), 85 (100), 79 (11), 67 (10). IR (neat): ν_{max} 2927, 2855, 2198, 1620, 1465, 1134, 1021, 992, 866. Anal. Calcd for $C_{20}H_{32}O_2$: C, 78.90; H, 10.59. Found: C, 78.81; H, 10.44.

4.7. Synthesis of (8*Z*,13*Z*)-pentadeca-8,13-dien-11-yn-2-one (1)

4.7.1. (8Z,13Z)-Pentadeca-8,13-dien-11-yn-2-ol

A solution of **7** (108 mg, 0.4 mmol) and *p*-toluenesulfonic acid (4 mg, 0.2 mmol) in MeOH (2.5 mL) was stirred for 1 h at 40 °C, and then the solvent was evaporated under reduced pressure. The crude residue was partitioned between water (5 mL) and Et_2O (3×30 mL), and the collected organic phases were washed with satd $NaHCO_3$ (20 mL), dried over $MgSO_4$, filtered, and concentrated to afford pure (8*Z*,13*Z*)-pentadeca-8,13-dien-11-yn-2-ol as a yellow liquid (77 mg, 100% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.18 (3H, d, *J*=6.2 Hz, H-1), 1.20–1.53 (8H, m, H-3 to H-6), 1.59 (1H, br s, OH), 1.84 (3H, ddt, *J*=6.8, 1.8, 0.5 Hz, H-15), 1.97–2.20 (2H, m, H-7), 3.00–3.18 (2H, m, H-10), 3.65–3.90 (1H, m, H-2), 5.36–5.53 (3H, m, H-8, H-9, H-13), 5.77–6.01 (1H, m, H-14). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.6 (C-15), 17.9 (C-10), 23.4 (C-1), 25.6 (C-4), 27.0 (C-7), 29.2 (C-5), 29.3 (C-6), 39.3 (C-3), 68.0 (C-2), 77.0 (C-12), 92.9 (C-11), 110.3 (C-13), 124.4 (C-9), 131.6 (C-8), 137.1 (C-14). MS: *m*/*z* 220 (M^+, 2%), 187 (5), 173 (12), 159 (16), 145 (34), 131 (55), 120 (94), 105 (100), 91 (94), 77 (40), 65 (15). IR (neat): ν_{max} 3420 (broad), 2925, 2853, 2182, 1667, 1463, 1376, 1024, 962. Anal. Calcd for $C_{15}H_{24}O$: C, 81.76; H, 10.98. Found: C, 81.90; H, 11.12.

*4.7.2. (8Z,13Z)-Pentadeca-8,13-dien-11-yn-2-one (**1**)*

To a solution of oxalyl chloride (0.196 mL, 2.2 mmol) in CH_2Cl_2 (4 mL) at −78 °C anhydrous DMSO (0.317 mL, 4.5 mmol) in CH_2Cl_2 (2 mL) was slowly added dropwise under argon flow. After stirring for 30 min, a solution of (8*Z*,13*Z*)-pentadeca-8,13-dien-11-yn-2-ol (447 mg, 2.0 mmol) in CH_2Cl_2 (3 mL) was added. The resulting orange solution was stirred for 50 min at −78 °C and finally treated with anhydrous triethylamine (1.43 mL, 10.1 mmol). The mixture was allowed to warm to rt over 2 h and then stirred for an additional 1 h. The turbid orange mixture was quenched with water (30 mL) and extracted with CH_2Cl_2 (2×60 mL). The combined organic phases were washed with NH_4Cl (100 mL, to pH 7) and brine (50 mL), then dried over $MgSO_4$, filtered, and concentrated to afford an oily residue, which was purified by column chromatography (eluant 10:1 Pet/EtOAc). Dienone **1** was isolated as a pale yellow liquid (327 mg, 74% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.20–1.48 (4H, m, H-5, H-6), 1.48–1.68 (2H, m, H-4), 1.84 (3H, dd, *J*=6.8, 1.7 Hz, H-15), 1.98–2.17 (2H, m, H-7), 2.30 (3H, s, H-1), 2.41 (2H, t, *J*=7.4 Hz, H-3), 3.00–3.18 (2H, m, H-10), 5.33–5.79 (3H, m, H-8, H-9, H-13), 5.89 (1H, dq, *J*=10.7, 6.8 Hz, H-14). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.6 (C-15), 17.9 (C-10), 23.6 (C-4), 26.9 (C-7), 28–7 (C-5), 29.1 (C-6), 29.8 (C-1), 43.8 (C-3), 77.2 (C-12), 92.8 (C-11), 110.3 (C-13), 124.5 (C-9), 131.3 (C-8), 137.1 (C-14), 208.9 (C-2). MS: *m*/*z* 218 (M^+, 16%), 203 (4), 185 (5), 175 (9), 160 (26), 145 (40), 131 (46), 120 (74), 105 (100), 91 (68), 79 (19), 77 (27), 65 (10). IR (neat): ν_{max} 3020, 2933, 2857, 2182, 1720, 1434, 1361, 1162, 1079, 954. Anal. Calcd for $C_{15}H_{22}O$: C, 82.52; H, 10.16. Found: C, 82.34; H, 10.29.

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References and notes

1. Chicca, A.; Adinolfi, B.; Martinotti, E.; Fogli, S.; Breschi, M. C.; Pellati, F.; Benvenuti, S.; Nieri, P. *J. Ethnopharmacol.* **2007**, *110*, 148–153.
2. Bauer, R.; Khan, I. A.; Wagner, H. *Planta Med.* **1988**, *54*, 426–430.
3. Bauer, R.; Remiger, P.; Wagner, H. *Phytochemistry* **1988**, *27*, 2339–2342.
4. Bauer, R.; Remiger, P. *Planta Med.* **1989**, *55*, 367–371.
5. Bauer, R.; Remiger, P.; Wagner, H. *Phytochemistry* **1989**, *28*, 505–508.
6. Pellati, F.; Calò, S.; Benvenuti, S.; Adinolfi, B.; Nieri, P.; Melegari, M. *Phytochemistry* **2006**, *67*, 1359–1364.
7. Pellati, F.; Calò, S.; Benvenuti, S. *J. Chromatogr., A* **2007**, *1149*, 56–65.
8. Chicca, A.; Pellati, F.; Adinolfi, B.; Matthias, A.; Massarelli, I.; Benvenuti, S.; Martinotti, E.; Bianucci, A. M.; Bone, K.; Lehmann, R.; Nieri, P. *Br. J. Pharmacol.* **2008**, *153*, 879–885.
9. Yang, L.; Cao, Z.; Yan, H.; Wood, W. C. *Cancer Res.* **2003**, *63*, 6815–6824.
10. Matthias, A.; Blanchfield, J. T.; Penman, K. G.; Toth, I.; Lang, C. S.; De Voss, J. J.; Lehmann, R. P. *J. Clin. Pharm. Ther.* **2004**, *29*, 7–13.
11. Kraus, G. A.; Bae, J.; Schuster, J. *Synthesis* **2005**, *20*, 3502–3504.
12. Nicolaou, K. C.; Ladduwahetty, T.; Taffer, I. M.; Zipkin, R. E. *Synthesis* **1986**, *4*, 344–347.
13. Dobbs, A. P.; Venturelli, A.; Butler, L. A.; Parker, R. J. *Synlett* **2005**, 1–3.
14. Zhou, Y.; Porco, J. A., Jr.; Snyder, J. K. *Org. Lett.* **2007**, *9*, 393–396.
15. (a) Brown, C. A. *J. Org. Chem* **1974**, *39*, 3913–3918; (b) Brown, C. A.; Yamashita, A. *J. Am. Chem. Soc.* **1975**, *97*, 891–892.
16. Green, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; Wiley-Interscience: New York, NY, 1999; Vols. 49–54, pp 708–711.
17. Brown, A. C.; Ahuja, V. K. *J. Org. Chem.* **1973**, *38*, 2226–2230.
18. Crouch, R. D. *Tetrahedron* **2004**, *60*, 5833–5871.
19. (a) Sonogashira, K. *J. Organomet. Chem.* **2002**, *653*, 46–49; (b) Chinchilla, R.; Nájera, C. *Chem. Rev.* **2007**, *107*, 874–922.
20. Alami, M.; Ferri, F.; Linstrumelle, G. *Tetrahedron Lett.* **1993**, *34*, 6403–6406.
21. Compound **7** showed high stability toward oxidation, even if stored in $CDCl_3$ solution at rt for long periods.
22. (a) Buevich, A. V.; Chan, T.-M.; Wang, C. H.; McPhail, A. T.; Ganguly, A. K. *Magn. Reson. Chem.* **2005**, *43*, 187–199; (b) Kamiensk-Trela, K.; Wojcik, J. *Nucl. Magn. Reson.* **2006**, *35*, 152–198.
23. Kiyota, H.; Nakashima, D.; Oritani, T. *Biosci. Biotechnol. Biochem.* **1999**, *63*, 2110–2117.
24. Mancuso, A. J.; Huang, S. L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480–2482.