A Straightforward Diastereoselective Synthesis and Evaluation of Climacostol, A Natural Product with Anticancer Activities

Dennis Fiorini,[a] Sandra Giuli,[a] Enrico Marcantoni,*[a] Luana Quassinti,[b] Massimo Bramucci,[b] Consuelo Amantini,[c] Giorgio Santoni,[c] Federico Buonanno,[d] Claudio Ortenzi*[d]

[a] School of Sciences and Technologies, Section of Chemistry, University of Camerino, via S. Agostino 1, 62032 Camerino (MC), Italy
Fax +39(0737)402297; E-mail: enrico.marcantoni@unicam.it

[b] School of Pharmacy, Physiology, University of Camerino, via Gentile III da Varano, 62032 Camerino (MC), Italy

[c] School of Pharmacy, Anatomy Unit, University of Camerino, via Scalzino 3, 62032 Camerino (MC), Italy

[d] Department of Educational Sciences and Training, University of Macerata, P. le Bertelli 1, 62100 Macerata, Italy
E-mail: claudio.ortenzi@unimc.it

Received 28 December 2009; revised 30 December 2009

Dedicated to Professor Saverio Florio, University of Bari, on the occasion of his 70th birthday

Abstract: On the basis of continued interest in plant-derived natural products as anticancer agents, a shorter and more efficient synthesis of climacostol is reported. This compound showed an anticancer activity better than that of the natural product. The improved potency and selectivity can be due to the absence of traces of the undesired *E*-isomer present in the natural climacostol. Furthermore, the versatile strategy developed for this simple molecule such as climacostol could aid in the synthesis of other more complex natural products.

Key words: bioorganic chemistry, C–C bond formation, diastereoselectivity, natural products, protecting groups, Wittig reaction

To assure the quality of drugs, impurities must be monitored carefully. Impurities in an API (Active Pharmaceutical Ingredient) that include synthetic by-products must be limited to very small amounts, and are preferably substantially absent. Availability by synthesis of an authentic material can allow toxicological studies and provide a standard for routine monitoring of the drug product.[1]

The 5-alkenylresorcinols are a family of resorcinol lipids of remarkable interest because of their structure, biogenesis, mechanism of action, and potent biological activities,[2] which include antiparasitic, anticancer, anti-inflammatory,[3] and antioxidant properties.[4] Although resorcinolic lipids are being found in an increasing number of organisms, the study of their biological activity, physiological role, and participation in the regulation of metabolic processes has not resulted in a complete understanding of their biological importance.

Because of the ubiquitous occurrence of these alkenylresorcinols and their potential practical applications, there is an intrinsic interest in determining the role of these molecules in human life as well as in the environment. From the initial discovery of alkenylresorcinols and the subsequent determination of their alkenyl configuration, a plethora of studies exploring the structure-activity relationship (SAR) of alkenylresorcinol-based compounds have been reported.[2] The chemical structure of these compounds contains resorcinol (1,3-dihydroxybenzene) as the core, equipped with an odd-numbered carbon chain substituent, with various degrees of unsaturation (**1**, Figure 1). 1,3-Dihydroxy-5-[(*Z*)-non-2′-enyl]benzene [climacostol (**2**), Figure 1] is a rare example of a 5-alkenylresorcinol having a *Z*-configured carbon–carbon double bond[5] at the 2′-position.

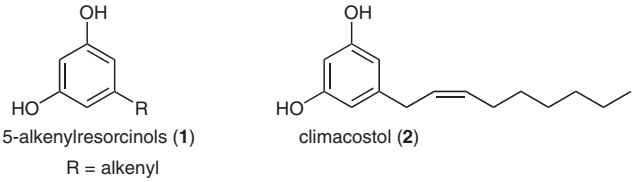


Figure 1 Structure of 5-alkenylresorcinols

Climacostol was isolated from the freshwater ciliated protozoan *Climacostomum virens*, which uses it as chemical weapon against unicellular and/or multicellular predators.[6] To date, this toxin represents the first example of a resorcinol isolated from protozoa, and its biological activity was investigated on free-living ciliates and invertebrates, and more recently, on cancer cell lines.[7] In particular, climacostol was preliminarily evaluated for its effect on human squamous carcinoma (A431) and human promyelocytic leukemia (HL60) cell lines. In this study, we extend the screening of the cytotoxic and pro-apoptotic effect of climacostol to additional cancer cell lines, in order to obtain a more exhaustive picture of the toxin global activity spectrum and, consequently, to better study its use in vivo research, and to evaluate its potential in cancer chemotherapy. Therefore, since large amounts of the toxin are necessary for biological test, and considering that the extant available synthetic methods have been poorly amenable to a large scale preparation of this compound, new synthetic strategies to the preparation of climacostol were required. To maintain the structural integrity of the compounds that display anticancer biological activities such as alkenylresorcinol **2**, prevention of isomerization of the *Z*-configuration is mandatory. To achieve this goal,

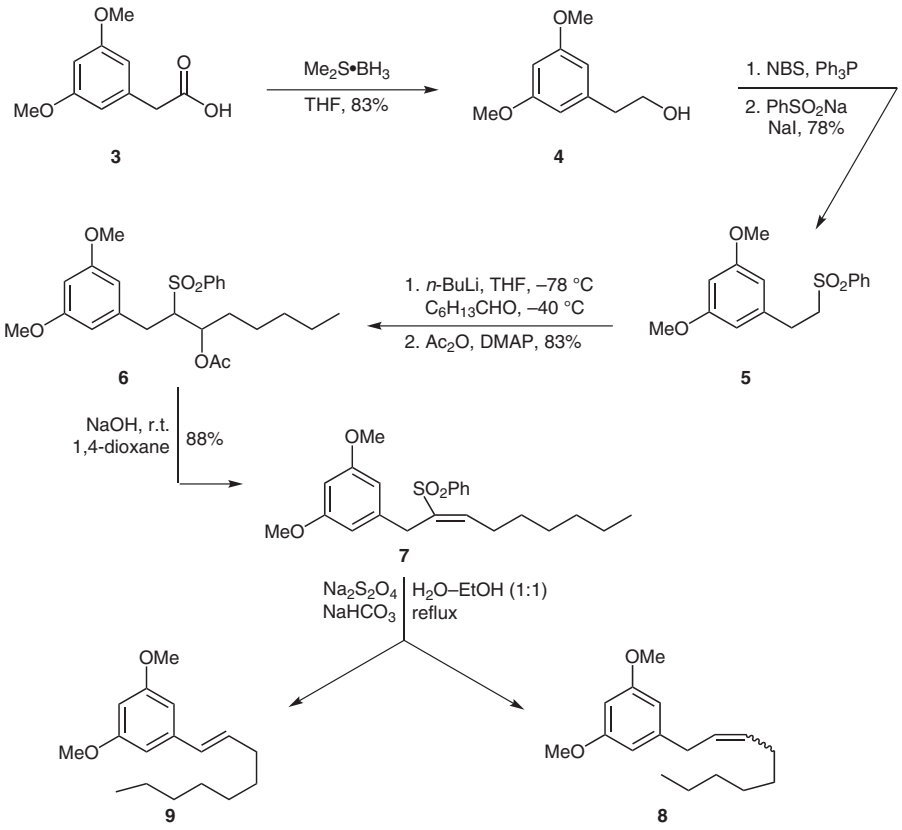
SYNTHESIS 2010, No. 9, pp 1550–1556
Advanced online publication: 09.03.2010
DOI: 10.1055/s-0029-1218695; Art ID: Z28309SS

various methodologies have been adopted for furnishing predominantly the *Z*-isomer of the final climacostol. However, none of the procedures displayed to date furnish exclusively the desired *Z*-isomer.

The carbon–carbon double bond is a structural feature on the border of framework and functionality, and the Wittig olefination along with the Diels–Alder and the aldol reactions is one of the most important reactions of all time. Generally, in Wittig reaction there is an equilibrium between reactants and products so that only the more stable of the two alkenes is produced. Thus, selectivity in the course of the construction of an organic molecule target can be achieved either by the choice of reagents (reagent control) or, less frequently, by the substrate (substrate control). One way to enforce the substrate–reagent interactions of a chemical transformation[8] is to employ substrate-bound reagent-directing groups.[9] Herein we report the use of this concept in the completion of a diastereoselective total synthesis of climacostol (**2**).

Despite there being a known procedure for the synthesis of **2**,[10] from a practical context there remained considerable room for improvement in the synthetic methodology. Drawbacks included in particular the length of the synthetic route, and the isomerization of the double bond ($\Delta^{2',3'} \rightarrow \Delta^{1',2'}$) during the removal of hydroxy protecting group of the 1,3-dihydroxybenzene moiety. Therefore, a new strategy for obtaining **2** was needed, that would incorporate an improved stereoselectivity. Within such a scheme, the development of an efficient choice of O-protecting group was an important objective for a convergent synthetic strategy of the climacostol. Thus, as a continuation of our work on the synthesis of molecule targets with biological activity,[11] we report herein a simple and convergent synthetic approach for climacostol (**2**).

A very broadly applicable strategy for the carbon–carbon double bond formation involves β-substituted sulfones, and the Julia–Kocienski olefination is operationally simple and enables for the straightforward assembly of advanced and functionalized intermediates in the course of total synthesis.[12] Thus, as an alternative route to acetylenic methods for deriving 5-alkenyl resocinol double bonds in the *Z*-configuration, we turned to the Julia–Kocienski protocol[13] for installing the *Z*-double bond in the alkenyl chain (Scheme 1). Sulfone **5**, required for coupling with heptanal was easily prepared by a one-pot reaction from the corresponding primary alcohol. Commercially available phenylacetic acid **3** was reduced to the corresponding alcohol **4** using $BH_3 \cdot SMe_2$,[14] and then converted to the primary sulfone **5** by treatment with *N*-bromosuccinimide, triphenylphosphine, and sodium phenylsulfinate under mild reaction conditions.[15] Addition of the lithium derivative of **5** to heptanal gave an adduct, which was acetylated in situ to afford a mixture of diastereomeric β-acetoxysulfone **6** in good yield. Compound **6** readily underwent β-elimination upon exposure to powdered NaOH in 1,4-dioxane at room temperature delivering vinyl sulfone **7** as a single stereoisomer.[16] Unfortunately, the last step of the Julia sequence, that is, the desulfination of compound **7** by exposure to $Na_2S_2O_4$ and $NaHCO_3$ in a mixture of water–

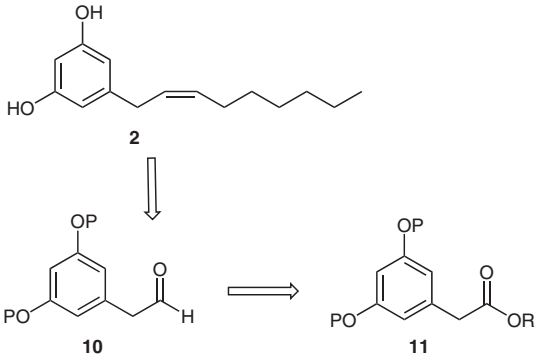


Scheme 1 Julia–Kocienski method for climacostol (**2**)

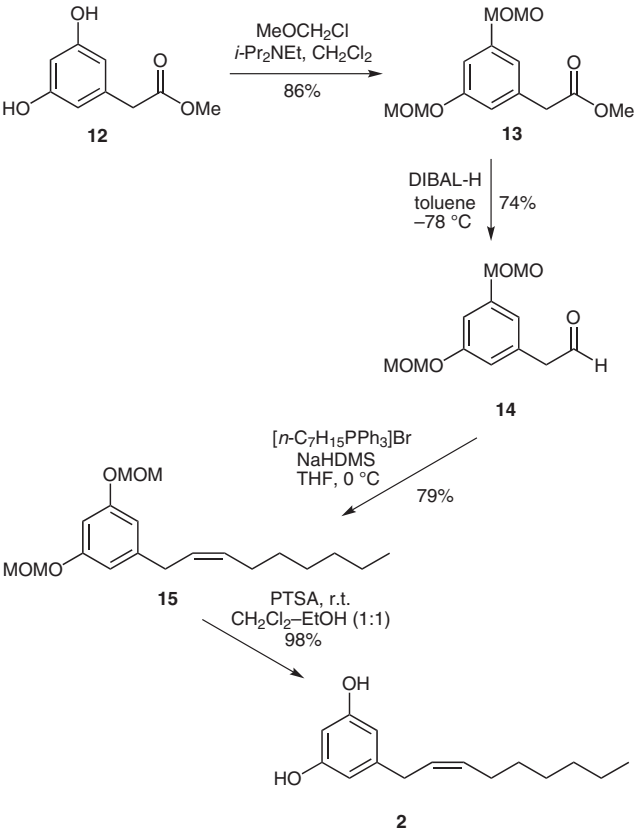
ethanol (1:1), gave a mixture of isomeric O-protected alkenylresorcinols **8** and **9**, which were inseparable by common chromatographic methods.[10d] After numerous unsuccessful attempts to selectively replace the phenylsulfonyl group with hydrogen, it became clear that this procedure would not be effective for producing climacostol. Thus, we proceeded to modify our synthetic strategy in order to furnish the molecular target without the unwanted isomerization of the carbon–carbon double bond.

Retrosynthetic analysis revealed a route based on the convergent approach outlined in Scheme 2. We surmised that the *Z*-configured carbon–carbon double bond could be introduced by a Wittig olefination from an aldehyde and an alkylphosphonium salt. Aldehyde **10** could be derived from commercially available methyl O-protected (3,5-dihydroxyphenyl)acetate **11**. Several years ago, Mori et al. reported a four-step synthesis of climacostol (**2**) based on a Wittig reaction using a *tert*-butyldimethylsilyl-protected 3,5-dihydroxyphenylacetaldehyde.[10b] However, our tentative silylalkyl deprotection furnished a mixture richer in the undesired *E*-isomer than the 5% reported by the authors, and indeed, it is known that tetrabutylammonium fluoride causes isomerization of the double bond geometry.[17] For this reason, we decided to utilize the knowledge acquired during our study of protecting groups in the chemoselective transformations of polyhydroxylated compounds,[18] toward the preparation of climacostol (**2**). After a number of unsuccessful attempts,[19] the methoxymethyl (MOM) group was found to be suitable for the synthetic problem in hand, and climacostol (**2**) was synthesized in four steps from commercially available methyl (3,5-dihydroxyphenyl)acetate (**12**). As outlined in Scheme 3, treatment of **12** with an excess of chloromethyl methyl ether,[20] afforded the bis-MOM protected 3,5-dihydroxyacetate **13** in 86% of yield after chromatography.[21] Subsequent reduction of the ester with diisobutylaluminum hydride in toluene at –78 °C released the corresponding deprotected aldehyde **14** in high yield. Next, the high reactivity of the aldehyde function was exploited in a Wittig olefination with *n*-heptylidenetriphenylphosphorane, giving the *Z*-alkene **15** as the only diastereoisomer (NMR spectra and direct HPLC analysis) in 79% yield. The ylide used in the Wittig reaction was obtained by reaction of sodium hexamethyldisilazide in THF with *n*-heptyltriphenylphosphonium bromide, which is not commercially available, but easily prepared by a known literature procedure.[22]

Having solved the olefin selectivity problem, and with the *Z*-isomer in hand, we turned our attention to the removal of the MOM-protecting group in order to obtain the alkenylresorcinol target **2**. Following the literature for selective MOM-deprotection in the presence of a *Z*-olefin moiety, adduct **15** was treated with $CeCl_3 \cdot 7H_2O$/NaI combination,[11d] and with TMSCl and Bu_4NBr in CH_2Cl_2.[23] However, in both instances the expected product was observed in low yield in addition to a mixture of products including some containing an isomerized double bond. Standard cleavage conditions of the MOM-protections



Scheme 2 Retrosynthesis of climacostol (**2**)



Scheme 3 Synthesis of climacostol (**2**)

(aq 6 M HCl)[24] led to decomposition of the resorcinol framework, while other methods commonly used for this deprotection were also unsuccessful ($LiBF_4$,[25] CBr_4/*i*-PrOH,[26] PPTS/*t*-BuOH,[27] 20% aq AcOH[28]). To overcome the problems encountered in the attempted MOM-deprotection,[20] we reasoned that the substrate could be unstable in hydrolytic conditions, and that *p*-toluenesulfonic acid (PTSA) in CH_2Cl_2–methanol solution[29] could represent an effective alternative to the commonly used trifluoroacetic acid (TFA) in CH_2Cl_2. It is known[30] that residual TFA in the deprotected product is rather difficult to completely remove, and its presence in a biology essay could interfere with the results, because of its toxicity against various cells. Therefore, nonaqueous conditions [excess PTSA, CH_2Cl_2–methanol (1:1), r.t.] successfully afforded target resorcinol-based climacostol (**2**) in quantitative yield. Pu-

rification of the crude product by normal phase column chromatography was not feasible, since the climacostol molecule tends to adsorb irreversibly to silica gel, and it could not be recovered even when using strong eluents.[31] Fortunately, our product did not require further purification, and the structure of 1,3-dihydroxy-5-[(*Z*)-non-2′-enyl]benzene (**2**) was established by spectral means. In particular, ^{1}H NMR spectrum showed that the proton signals H-2′ and H-3′ were not well separated. Thus, it was impossible to unambiguously assess the structure of **2** by conventional 2D NMR methods such as COSY and NOESY because it is known that they fail when key proton signals are poorly resolved.[32] The following stereochemical study of **2** was directed to confirm the stereochemistry at C-2′–C-3′ double bond. The absence of the strong 970 cm^{-1} band for the *E*-geometry in IR spectrum suggested the *Z*-geometry for the disubstituted double bond.[33] A characteristic electron ionization mass spectrum was observed with a notable molecular ion at *m*/*z* 234, and a base fragment at *m*/*z* 124, due to McLafferty rearrangement of the phenolic ring, and another minor fragment at *m*/*z* 123, due to the dihydroxytropylium ion formed by direct β-cleavage, and at *m*/*z* 137 due to γ-cleavage.[34] The *m*/*z* abundance ratio of 123/124 is ca 1:5, which is in agreement with a *meta*-dihydroxy substitution of the benzene ring.[35] But, above all, the odd-electron ion *m*/*z* 124 in conjunction with *m*/*z* 137 has been a good diagnostic for the presence of a double bond between carbons C-2′ and C-3′.[36] Thus, in our conditions for accomplishing the convergent synthesis of **2**, isomerization of the carbon–carbon double bond ($\Delta^{2',3'} \rightarrow \Delta^{1',2'}$) was not observed.

The cytotoxic effect of synthetic climacostol on prostatic adenocarcinoma (PC-3), glioblastoma (T98G and U87MG), and endothelial (EA.hy926) human cell lines was evaluated by MTT assay.[37] As shown in Table 1, a maximum cytotoxic effect was observed for cancer cell lines within the 40–60 μM range of climacostol, whereas no loss of viability was observed for EA.hy926 cells with toxin concentration of <100 μM. In particular, climacostol showed significant activity against PC-3, T98G, and U87MG cells, with IC_{50} values of 11.47, 15.14, and 19.74 μM, respectively, compared to calculated 213.20 μM for the EA.hy926 cells. Since climacostol displayed negligible cytotoxicity against EA.hy926 cells, no further investigations were performed on this cell line.

Table 1 Cytotoxic Activity Data for Synthetic Climacostol (**2**)

Cell line	IC_{50} (μM)	IC_{100} (μM)
PC-3	11.47	44.67
T98G	15.14	45.71
U87MG	19.74	56.23
EA.hy926	>200.00	400.00

Following the universal convergent scheme described in this work, we have found an efficient route to the synthesis of alkenylresorcinols, and this strategy has been efficiently applied to the preparation of climacostol (**2**), a natural and potential therapeutic agent for several types of cancers. The strategy introduced herein has three key points which make it considerably more useful than the previously reported sequences: (i) the synthetic route is brief and conveniently produces the target compound from easily accessible starting materials, (ii) the desired *Z*-configured natural product has been prepared in up to 63% overall yield, and (iii) without the presence of the *E*-isomer or the rearranged $\Delta^{1',2'}$-isomer.

In summary, a novel and significantly improved method for the convergent synthesis of climacostol (**2**) has been developed. An alternative, robust linear approach allowing selective formation of the desired molecule target **2** via aldehyde **14** has been described. Evaluation of these methods for the general synthesis of alkenylresorcinols is under way and will be presented elsewhere.

All air-sensitive reactions are carried out in flame dried glassware under an atmosphere of dry N_2. Solvents were distilled under N_2, THF, Et_2O, and pentane from sodium benzophenone ketyl and CH_2Cl_2 from CaH_2. Solutions were evaporated under reduced pressure with a rotary evaporator and the residue was chromatographed on a Baker silica gel (230–400 mesh) column using a 30% EtOAc in hexane as the eluent. Analytical TLC was performed using precoated glass-backed plates (Merck Kieselgel 60 F254) and visualized by UV light (254 nm) and/or by dipping the plates into Von's reagent [1.0 g of $Ce(SO_4)_2$ and 24.0 g of $(NH_4)_2MoO_4$ in 31 mL of H_2SO_4 and 470 mL of H_2O].

^{1}H NMR spectra were recorded in $CDCl_3$ on Varian Gemini 200 or Varian 400 spectrometers and are reported as follows: chemical shift, δ (ppm) [multiplicity, coupling constant *J* (Hz), and number of protons]. Residual protic solvent $CHCl_3$ (δ_H = 7.26) was used as the internal reference. ^{13}C NMR spectra were recorded in $CDCl_3$ at 50 MHz or 100 MHz on Varian Gemini 200 or Varian 400 spectrometers, using the central resonance of $CDCl_3$ (δ_C = 77.0) as the internal reference. IR spectra were recorded on PerkinElmer FTIR Paragon 500 spectrometer using thin films on NaCl plates. Only the characteristic peaks are quoted. Mass spectra were recorded on a Hewlett-Packard 5988 gas chromatograph with a mass-selective detector MSD HP 5790 MS, utilizing electron ionization (EI) at an ionizing energy of 70 eV. A fused silica column (30 m × 0.25 mm HP-5; cross-linked 5% PhMe siloxane, 0.10 HM film thickness) was used with a helium carrier flow of 30 mL/min. The temperature of the column was varied, after a delay of 3 min from the injection, from 65 to 300 °C with a slope of 15 °C min^{-1}.

Human T98G glioblastoma multiforme cell line (catalogue code CRL-1690) and human U87MG glioblastoma, astrocytoma cell line (catalogue code HTB-14) were purchased from the American Type Culture Collection (ATCC) and were maintained in Eagle's minimum essential medium (EMEM) with 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 100 IU/mL penicillin, 100 μg/mL streptomycin, and supplemented with 10% heat inactivated fetal bovine serum (HI-FBS). Human PC-3 prostatic adenocarcinoma cell line (catalogue number 90112714) was purchased from the European Collection of Animal Cell Culture (ECACC), maintained in Coon's modified Ham's F12 medium with 2 mM L-glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin, and supplemented with 7% HI-FBS. Human noncancer EA.hy926 umbilical vein endothelial cell line (ATCC; catalogue code CRL-2922) was maintained in Dulbecco modified Eagle's medium supplemented with 10% HI-FBS, 2% hypoxanthineaminopterin-thymi-

dine, 2 mM HEPES, 2 mM L-glutamine, 100 IU/mL penicillin, and 100 mg/mL streptomycin. Constituents and supplements of growth media were purchased from Sigma (St. Louis, MO, USA). All cells were grown in a humidifier of 5% CO_2 in air at 37 °C.

2-(3,5-Dimethoxyphenyl)-1-ethanol (4)

Caution! *Me_2S complexed with borane may release excess of Me_2S during the course of the reaction (stench!). This experiment should be carried out in a well-ventilated hood.*

In a 50 mL, two-necked, round-bottomed flask mounted with a short reflux condenser was placed a solution of 2-(3,5-dimethoxyphenyl)acetic acid (**3**; 0.47 g, 2.42 mmol) in anhyd THF (15 mL). To this was added $BH_3{\cdot}SMe_2$ (1.23 mL of a 2 M solution in THF) dropwise at r.t. The resulting mixture was stirred until the disappearance of acid (4 h) on TLC and GC diagnosis. The mixture was treated with anhyd MeOH (5.20 mL) and the resulting solution was concentrated to give a colorless oil, which was purified by column chromatography on silica gel (eluent: hexanes–EtOAc–MeOH, 6:3.5:0.5) affording alcohol **4** in 83% yield (0.36 g); colorless oil.

IR (neat): 3392, 1600, 1144 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.80 (br s, 1 H, OH), 2.80 (t, *J* = 6.60 Hz, 2 H,), 3.78 (s, 6 H), 3.83 (t, *J* = 6.60 Hz, 2 H), 6.33–6.40 (m, 3 H_{arom}).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 40.3, 56.2, 64.1, 98.7, 107.3, 143.2, 160.4.

MS (EI): *m*/*z* = 164 [$M^+ - H_2O$], 151 (100), 137, 112, 69, 51, 43, 39.

Anal. Calcd for $C_{10}H_{14}O_3$: C, 65.91; H, 7.74. Found: C, 65.88; H, 7.70.

1,3-Dimethoxy-5-[2-(phenylsulfonyl)ethyl]benzene (5)

To a stirred mixture of alcohol **4** (0.27 g, 1.52 mmol) and Ph_3P (0.64 g, 2.43 mmol) in anhyd DMF (7.6 mL) under argon was added at 0 °C *N*-bromosuccinimide (0.43 g, 2.43 mmol) in small portions over 10 min. The mixture was stirred for 2 h at r.t. To this mixture was added a mixture of $PhSO_2Na$ (0.5 g, 3.04 mmol) and NaI (0.023 g, 0.152 mmol) in 3 portions over 10 min. The resulting mixture was heated at 70 °C for 20 h, and then diluted with EtOAc (15.2 mL) and 3% aq $Na_2S_2O_3$ (15.2 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic extracts were successively washed with H_2O (2 × 20 mL) and brine (2 × 20 mL), and dried (Na_2SO_4). After filtration and concentration under reduced pressure, the residue was chromatographed on silica gel (hexanes–EtOAc, 6:4) to give 0.36 g (78%) of sulfone **5**; colorless oil.

IR (neat): 3090, 1440, 1150 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$): δ = 2.91–3.05 (m, 2 H), 3.29–3.78 (m, 2 H), 3.83 (s, 6 H), 6.22–6.30 (m, 3 H_{arom}), 7.50–7.95 (m, 5 H_{arom}).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 32.3, 50.2, 54.7, 100.7, 110.8, 128.4, 129.2, 133.3, 138.3, 140.4, 163.9.

Anal. Calcd for $C_{16}H_{18}O_4S$: C, 62.72; H, 5.92; S, 10.47. Found: C, 62.70; H, 5.88; S, 10.42.

1-[2-(3,5-Dimethoxyphenyl)-1-(phenylsulfonyl)ethyl]hexyl Acetate (6)

A 1.6 M solution of *n*-BuLi in hexane (0.72 mL, 1.15 mmol) was added slowly to a solution of phenyl sulfone **5** (0.29 g, 0.96 mmol) in anhyd THF (10 mL) at –78 °C. After stirring at the same temperature for 30 min, the reaction mixture was allowed to warm to –40 °C, while a yellow color developed. To this mixture was transferred by cannula a THF (5 mL) solution of heptanal (0.13 g, 1.140 mmol), followed after 2 h, by an excess of Ac_2O (0.16 mL, 1.71 mmol) and a catalytic amount of DMAP (7.36 mg, 0.06 mmol). The resulting mixture was warmed at r.t. and then left to stir until TLC indicated that no substrate **5** remained (2 h). The mixture was quenched by the addition of sat. aq NH_4Cl (10 mL). A standard workup by extraction with Et_2O (3 × 20 mL) and washing the combined Et_2O extracts with H_2O (2 × 20 mL) and brine (2 × 20 mL) gave a viscous oil in 83% crude yield, which was used directly without purification in the next step.

(*Z*)-1-(3,5-Dimethoxybenzyl)hept-1-enylphenylsulfone (7)

NaOH (35 mg, 0.88 mmol) was added to a solution of compound **6** (0.20 g, 0.44 mmol) in 1,4-dioxane (5 mL). The mixture was stirred at r.t. for 1 h (until no starting material remained, as monitored by TLC). The mixture was diluted with Et_2O (20 mL) and treated with H_2O (10 mL). The organic layer was separated, and the aqueous layer was extracted with Et_2O (3 × 25 mL). The combined organic extracts were washed with brine (2 × 20 mL), dried (Na_2SO_4), and evaporated to give the vinyl sulfone **7**, which was purified by flash chromatography (hexanes–EtOAc, 8:2); yield: 0.31 g (88%); pale yellow oil.

IR (neat): 3060, 1635, 1440, 1140, 1032 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$): δ = 0.82 (t, *J* = 6.60 Hz, 3 H), 1.20–1.28 (m, 8 H), 2.90–2.97 (m, 2 H), 3.15–3.29 (m, 2 H), 3.78 (s, 6 H), 6.90 (t, *J* = 7.0 Hz, 1 H), 6.30–6.39 (m, 3 H_{arom}), 7.40–7.82 (m, 5 H_{arom}).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 13.8, 22.0, 27.9, 28.5, 28.9, 31.4, 33.9, 54.2, 105.4, 113.0, 127.9, 129.3, 134.2, 140.5, 142.9, 145.2, 149.7.

Anal. Calcd for $C_{23}H_{30}O_4S$: C, 68.62; H, 7.51; S, 7.97. Found: C, 68.59; H, 7.54; S, 7.95.

Methyl 2-[3,5-Di(methoxymethoxy)phenyl]acetate (13)

To a stirred solution of methyl (3,5-dihydroxyphenyl)acetate (**12**; 0.50 g, 2.94 mmol) in CH_2Cl_2 (60 mL) were added *i*-Pr_2NEt (20 mL, 11.5 mmol) and chloromethyl methyl ether (8.95 mL, 11.76 mmol) dropwise at 0 °C under N_2. The mixture was warmed to r.t. and stirred overnight. CH_2Cl_2 was removed in vacuum, the residue taken up in Et_2O (45 mL), washed with 10% aq HCl (2 × 30 mL), H_2O (2 × 30 mL), aq 10% NaOH (2 × 30 mL), and brine (45 mL), dried ($MgSO_4$), and concentrated at reduced pressure. The residue was purified by silica gel column chromatography (hexanes–EtOAc, 8:2) to give **13** (0.68 g, 86%) as a colorless oil.

IR (neat): 3005, 1730, 1605, 1200, 840 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 3.40 (s, 6 H), 3.62 (s, 2 H), 3.69 (s, 3 H), 5.15 (s, 4 H), 6.60–6.65 (m, 3 H_{arom}).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 41.0, 51.8, 57.2, 92.3, 98.7, 105.5, 139.0, 157.9, 172.6.

MS (EI): *m*/*z* (%) = 270 [M^+], 238, 211, 121, 45 (100).

Anal. Calcd for $C_{13}H_{18}O_6$: C, 57.77; H, 6.71. Found: C, 57.75; H, 6.68.

2-[3,5-Di(Methoxymethoxy)phenyl]acetaldehyde (14)

A solution of DIBAL-H in toluene (1.0 M, 2.21 mL, 2.21 mmol) was added to a stirred and cooled solution of **13** (0.50 g, 1.85 mmol) in anhyd toluene (10 mL) at –78 °C under N_2. The reaction mixture was allowed to warm to –60 °C while stirring for 3 h, then cooled to –78 °C again, and afterwards quenched with MeOH (5 mL). The mixture was filtered through Celite, and the resulting solid was washed with Et_2O (75 mL). The filtrate and washings were successively washed with H_2O (2 × 20 mL) and brine (2 × 20 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc–hexanes, 8:2) to give the title compound **14** (0.42 g, 94%) as a pale yellow oil.

IR (neat): 3006, 2822, 1735, 1598, 1190, 880 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.60 (d, *J* = 2.1 Hz, 2 H), 5.75 (s, 4 H), 6.30–6.35 (m, 3 H_{arom}), 9.57 (d, *J* = 2.1 Hz, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 52.6, 56.6, 92.7, 99.3, 104.6, 138.7, 158.6, 200.0.

MS (EI): *m*/*z* (%) = 240 [M$^+$], 180, 152, 123, 45 (100).

Anal. Calcd for $C_{12}H_{16}O_5$: C, 59.99; H, 6.71. Found: C, 59.97; H, 6.70.

***n*-Heptyltriphenylphosphonium Bromide**

To a solution of 1-bromoheptane (25 mmol) in toluene (50 mL) was added Ph_3P (7.20 g, 27.5 mmol). After refluxing for 48 h, the reaction mixture was cooled to r.t. and the solvent was removed under reduced pressure. The crude product was dissolved in CH_2Cl_2 (15 mL), then added dropwise to Et_2O (75 mL). After stirring for 1 h, the precipitate was filtered and dried under vacuum affording the title compound in pure form (10.35 g, 96%); white crystals; mp 165 °C.

IR (neat): 3050, 1424, 1265, 895 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.80 (t, *J* = 6.7 Hz, 3 H), 1.15–1.21 (m, 8 H), 1.59–1.61 (m, 2 H), 3.80–3.86 (m, 2 H), 7.60–7.65 (m, 15 H_{arom}), 9.57 (d, *J* = 2.1 Hz, 1 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.5, 22.0 (d, $J_{C,P}$ = 4.9 Hz), 22.9 (d, $J_{C,P}$ = 51.0 Hz), 28.7, 30.5, 31.1, 31.7, 119.0 (3 C, d, $J_{C,P}$ = 83.0 Hz), 130.7 (6 C, d, $J_{C,P}$ = 11.5 Hz), 133.9 (6 C, d, $J_{C,P}$ = 9.0 Hz), 134.9 (3 C, d, $J_{C,P}$ = 4.0 Hz).

(*Z*)-1-[3,5-Dimethoxymethoxy)phenyl]non-2′-ene (15)

To a suspension of *n*-heptyltriphenylphosphonium bromide, previously dried by three azeotropic distillations with anhyd benzene, (1.92 g, 4.34 mmol) in anhyd THF (20 mL) was added a solution of sodium hexamethyldisilazide (NaHDMS) in THF (1.0 M, 4.64 mL, 4.64 mmol) at 0 °C, and the mixture was stirred at r.t. for 1 h. A solution of **14** (0.30 g, 1.24 mmol) in THF (15 mL) was added to the ylide at –10 °C and the resulting mixture was stirred at 0 °C for 4 h, and finally stirred at r.t. for 1 h. Sat. aq NH_4Cl (10 mL) was added to the mixture and extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with brine (2 × 20 mL) and dried ($MgSO_4$). Filtration and concentration of the mixture under reduced pressure gave the crude material, which was purified by chromatography on silica gel (EtOAc–hexanes, 1:9) to give 0.31 g (79%) of **15** as a colorless oil.

IR (neat): 3003, 1590, 1155, 885 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.94 (t, *J* = 6.5 Hz, 3 H), 1.28–1.50 (m, 8 H), 6.50–6.55 (m, 3 H_{arom}).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 14.3, 22.9, 27.5, 29.3, 29.9, 32.1, 33.8, 56.2, 94.6, 102.5, 110.0, 127.7, 131.6, 144.0, 158.6.

MS (EI): *m*/*z* (%) = 322 [M$^+$], 245, 212, 137, 123, 45 (100).

Anal. Calcd for $C_{19}H_{30}O_4$: C, 70.77; H, 9.38. Found: C, 70.74; H, 9.35.

1,3-Dihydroxy-5-[(*Z*)-non-2′-enyl]benzene (Climacostol, 2)

p-Toluenesulfonic acid (4.2 g, 21 mmol) was added to a solution of **15** (0.28 g, 0.87 mmol) in a mixture of CH_2Cl_2–MeOH (30 mL, 1:1). The reaction mixture was stirred overnight at r.t. Subsequently, solid $NaHCO_3$ (0.17 g) and H_2O (18 mL) were added until pH 6. The aqueous layer was extracted with EtOAc (3 × 20 mL). The organic layer was washed with H_2O (2 × 20 mL) and brine (2 × 20 mL), dried ($MgSO_4$), and concentrated to give 0.2 g (98%) of climacostol (**2**), which did not require further purification; yellow oil.

IR (neat): 3339, 3011, 1600, 1467, 1154, 835 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.90 (t, *J* = 6.6 Hz, 3 H), 1.26–1.36 (m, 8 H), 2.09 (q, *J* = 6.6 Hz, 2 H), 3.25 (d, *J* = 5.9 Hz, 2 H), 5.38–5.58 (m, 2 H), 6.11 (br s, 2 H), 6.19 (t, *J* = 2.2 Hz, 1 H), 6.29 (d, *J* = 2.1 Hz, 2 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 14.3, 22.8, 27.4, 29.2, 29.8, 31.9, 33.5, 100.7, 108.2, 127.57, 131.6, 144.6, 156.9.

MS (EI): *m*/*z* (%) = 234 [M$^+$], 163, 137, 124 (100), 123, 107, 91, 69.

Anal. Calcd for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46. Found: C, 76.86; H, 9.45.

Acknowledgment

This work was supported by funds from the Universities of Camerino and Macerata, and from the MIUR, Rome (PRIN 2006).

References

(1) (a) Hengtrakul, P.; Mathias, M.; Lorenz, K. *J. Nutr. Biochem.* **1991**, *2*, 20. (b) Arisawa, M.; Ohmura, K.; Kobayashi, A.; Morita, N. *Chem. Pharm. Bull.* **1989**, *37*, 2431. (c) Marumo, S.; Hattori, H.; Katayama, M. *Agric. Biol. Chem.* **1985**, *49*, 1521.
(2) Kozubek, A.; Tyman, J. H. P. *Chem. Rev.* **1999**, *99*, 1.
(3) (a) Parikka, K.; Rowland, I. R.; Welch, R. W.; Wähälä, K. *J. Agric. Food Chem.* **2006**, *54*, 1646. (b) Winata, A.; Lorenz, K. *J. Food Process Preserv.* **1996**, *20*, 417. (c) Shobha, S. V.; Ramadoss, C. S.; Ravindranath, B. *J. Nat. Prod.* **1994**, *57*, 1755.
(4) (a) Kociubinska, A.; Godlewska, M.; Stasiuk, M.; Kozubek, A.; Peczynska-Czoch, W.; Opolski, A.; Kaczmarek, L.; Gubernator, J. *Cell. Mol. Biol. Lett.* **2002**, *7*, 289. (b) Barrow, R. A.; Capon, R. J. *Aust. J. Chem.* **1991**, *44*, 1393.
(5) Generally the *E*-derivative is less cytotoxic than its *Z*-stereoisomer, see: Cushman, M.; Nagarathanam, D.; Gopal, D.; Chakraborti, A. K.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1991**, *34*, 2579.
(6) Miyake, A.; Buonanno, F.; Saltalamacchia, P.; Masaki, M. E.; Iio, H. *Eur. J. Protistol.* **2003**, *39*, 25.
(7) (a) Buonanno, F. *Ital. J. Zool.* **2005**, *72*, 293. (b) Buonanno, F.; Quassinti, L.; Bramucci, M.; Amantini, C.; Lucciarini, R.; Santoni, G.; Iio, H.; Ortenzi, C. *Chem. Biol. Interact.* **2008**, *176*, 151.
(8) Hoveyda, A. H.; Evans, D. A.; Fu, G. C. *Chem. Rev.* **1993**, *93*, 1307.
(9) Rein, C.; Demel, P.; Outten, R. A.; Netscher, T.; Breit, B. *Angew. Chem. Int. Ed.* **2007**, *46*, 8670.
(10) (a) Masaki, M. E.; Hiro, S.; Usiki, Y.; Harumoto, T.; Terazima, M. N.; Buonanno, F.; Miyake, A.; Iio, H. *Tetrahedron* **2004**, *60*, 7041. (b) Abe, Y.; Mori, K. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 2110. (c) Masaki, M. E.; Harumoto, T.; Terazima, M. N.; Miyake, A.; Usuki, Y.; Iio, H. *Tetrahedron Lett.* **1999**, *40*, 8227. (d) Trivedi, S. V.; Subbaraman, A. S.; Chattopadhyay, S.; Mamdapur, V. R. *Molecules* **1999**, *4*, 245.
(11) (a) Bartoli, G.; Bosco, M.; Giuli, S.; Giuliani, A.; Lucarelli, L.; Marcantoni, E.; Sambri, L.; Torregiani, E. *J. Org. Chem.* **2005**, *70*, 1941. (b) Bartoli, G.; Bosco, M.; Dalpozzo, R.; Giuliani, A.; Marcantoni, E.; Mecozzi, T.; Sambri, L.; Torregiani, E. *J. Org. Chem.* **2002**, *67*, 9111. (c) Bartoli, G.; Bellucci, M. C.; Bosco, M.; Marcantoni, E.; Massaccesi, M.; Petrini, M.; Sambri, L. *J. Org. Chem.* **2000**, *65*, 4553. (d) Bartoli, G.; Bosco, M.; Marcantoni, E.; Sambri, L.; Torregiani, E. *Synlett* **1998**, 209. (e) Bartoli, G.; Bosco, M.; Dalpozzo, R.; De Nino, A.; Marcantoni, E.; Sambri, L. *J. Org. Chem.* **1998**, *63*, 3745. (f) Giovannini, R.; Marcantoni, E.; Petrini, M. *J. Org. Chem.* **1995**, *60*, 5706. (g) Ballini,

R.; Marcantoni, E.; Petrini, M. *J. Org. Chem.* **1992**, *57*, 1316.
(12) Ballini, R.; Bosica, G.; Marcantoni, E. *Tetrahedron* **1996**, *52*, 10705.
(13) Aïssa, C. *Eur. J. Org. Chem.* **2009**, 1831; and references cited therein.
(14) Lanni, T. B.; Greene, K. L.; Kolz, C. N.; Para, K. S.; Visnick, T.; Mobley, J. L.; Dudley, D. T.; Baginski, T. J.; Liimatta, M. B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 756.
(15) Murakami, T.; Furusawa, K. *Synthesis* **2002**, 479.
(16) The *Z*-stereochemistry was assigned on the basis of the ^{13}C chemical shifts of the allylic methylene carbons, see: Porta, A.; Re, S.; Zanoni, G.; Vidari, G. *Tetrahedron* **2007**, *63*, 3989; and confirmed by spectroscopic correlation with compound **2**.
(17) Hayami, J.; Ono, N.; Kaji, A. *Tetrahedron Lett.* **1968**, 1385.
(18) (a) Bartoli, G.; Giovannini, R.; G iuliani, A.; Marcantoni, E.; Massaccesi, M.; Melchiorre, P.; Paoletti, M.; Sambri, L. *Eur. J. Org. Chem.* **2006**, 1476. (b) Bartoli, G.; Bellucci, M. C.; Bosco, M.; Cappa, A.; Marcantoni, E.; Sambri, L.; Torregiani, E. *J. Org. Chem.* **1999**, *64*, 5696.
(19) (a) Kocienski, P. J. *Protecting Groups*; Georg Thieme Verlag: Stuttgart, **2005**, 285–308. (b) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; Wiley-Interscience: New York, **1999**, 23–148.
(20) Although this reagent itself is only mildly carcinogenic, it is usually contaminated with small amounts of bis(chloromethyl) ether, which is strongly carcinogenic. For this reason the literature contains a variety of methods to reduce laboratory hazards, see: Berliner, M.; Belecki, K. *Org. Synth.* **2007**, *84*, 102; and references cited therein.
(21) Kitamura, Y.; Nara, S.; Nakagawa, H.; Nakatsu, R.; Soga, S.; Kajita, J.; Shiotsu, Y.; Kanda, Y. Patent EP 1 704 856, **2005**; *Chem. Abstr.* **2005**, *143*,146661.
(22) Dickschat, J. S.; Helmuke, E.; Schultz, S. *Chemistry & Biodiversity* **2005**, *2*, 318.
(23) Hermann, C.; Pais, G. C. G.; Geyer, A.; Kühnert, S. M.; Maier, M. E. *Tetrahedron* **2000**, *56*, 8461.
(24) Boger, D. L.; Brunette, S. R.; Garbaccio, R. M. *J. Org. Chem.* **2001**, *66*, 5163.
(25) Ireland, R. E.; Varney, M. D. *J. Org. Chem.* **1986**, *51*, 635.
(26) Lee, A. S.-Y.; Hu, T.-J.; Chu, S.-F. *Tetrahedron* **2001**, *57*, 2121.
(27) Monti, H.; Leandri, G.; Klas-Ringuet, M.; Corriol, C. *Synth. Commun.* **1983**, *13*, 1021.
(28) Chan, K.-F.; Zhao, Y.; Chow, L. M. C.; Chan, T. H. *Tetrahedron* **2005**, *61*, 4149.
(29) (a) Lélias-Vanderperre, A.; Chambran, J.-C.; Espinosa, E.; Terrier, P.; Leize-Wagner, E. *Org. Lett.* **2007**, *9*, 2961. (b) Kunnagaiu, N.; Matsunaga, S.; Kinoshita, T.; Harada, S.; Okada, S.; Sakamoto, S.; Yamaguchi, K.; Shibosaki, M. J. *J. Am. Chem. Soc.* **2003**, *125*, 2169.
(30) Yan, B.; Shi, R.; Zhang, B.; Kishirsagar, T. *J. Comb. Chem.* **2007**, *9*, 684.
(31) Ross, A. B.; Aman, P.; Andersson, R.; Kamal-Eldin, A. *J. Chromatogr., A* **2004**, *1054*, 157.
(32) Crouch, R. C.; McFayden, R. B.; Daluge, S. M.; Martin, G. E. *Magn. Reson. Chem.* **1990**, *28*, 792.
(33) Van Aller, R. M.; Clark, L. R.; Pessoney, G. F.; van Rogers, A. A. *Lipids* **1983**, *18*, 617.
(34) Suzuki, Y.; Esumi, Y.; Uramoto, M.; Kono, Y. A. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 480.
(35) Zarnowski, R.; Suzuki, Y.; Esumi, Y.; Pieter, S. J. *Phytochemistry* **2000**, *55* , 975.
(36) Knodler, M.; Berardini, N.; Kanmerer, D. R.; Carle, R.; Schieber, A. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 945.
(37) Page, M.; Bejaoui, N.; Cinq-Mars, B.; Lemieux, P. *Int. J. Immunopharm.* **1988**, 785.