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Synthesis of (−)-pregaliellalactone, conversion of (−)-pregaliellalactone to (−)-galiellalactone by mycelia of *Galiella rufa*

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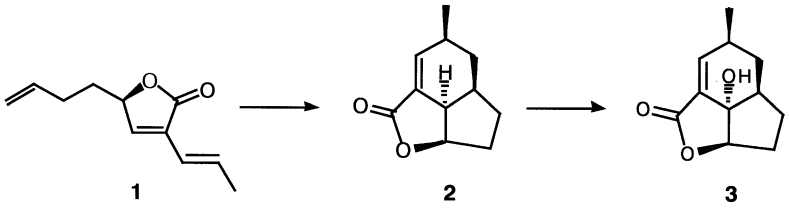
Abstract—An enantioselective synthesis of (−)-pregaliellalactone (**1**), a biosynthetic precursor of the potent fungal metabolite (−)-galiellalactone (**3**) produced by several ascomycetes, is reported. When fed to a culture of *Galiella rufa*, **1** was efficiently converted to **3**.

1. Introduction

The fungal metabolite (−)-galiellalactone (**3**), isolated from the ascomycetes *Galiella rufa* (strain A75-86) and A111-95,[1] has been shown to be a highly selective and potent inhibitor of interleukin-6 (IL-6) signalling in HepG2 cells.[2] Besides the biological activity, galiellalactone is also interesting because the biosynthesis is presumed to involve an intramolecular Diels–Alder cyclisation of (−)-pregaliellalactone (**1**) to (+)-desoxygaliellalactone (**2**), followed by an enzymatic hydroxylation to **3** (see Scheme 1).[3] Galiellalactone (**3**) and any analogues could be valuable tools for the investigation of the biochemical pathway of interleukin-6 signalling and may even serve as a lead structures when new drugs for the treatment of diseases linked to this pathway are developed, and there is a need for synthetic routes for such compounds. A synthesis of unnatural (+)-galiellalactone from commercially available *R*-(+)-pulegone has been described,[4] which made it possible to determine the absolute configuration of the natural product, and this could in principle be adopted also for the synthesis of (−)-**3**. However, a combined synthetic/biosynthetic approach to **3** would be attractive, if an effective synthesis of the precursor (−)-pregaliellalactone (**1**) was at hand and if (−)-**1** efficiently was converted to (−)-**3** by the fungus. Such an approach would combine the ability of synthesis to produce larger amounts of (−)-**1** as well as the selectivity of enzymatic conversions. If the enzymes in the biosynthetic pathway are active in the absence of another carbon source, only the desired metabolite would be produced and thereby easily isolated by a simple extraction. It might also be possible to feed the fungus with analogues of (−)-**1** and obtain the corresponding analogues of (−)-galiellalactone (**3**). A previous synthesis of (−)-**1** has been reported,[3c] starting from D-mannitol **1** was prepared in nine steps in an overall yield of less than 1%.

A wide variety of naturally occurring compounds possessing various biological activities contain a butenolide moiety,[5] and a number of synthetic routes to this type of compound have been developed. A few of these utilise propargylic alcohols as advanced intermediates[6] and since these are readily prepared in enatiomerically pure forms,



Scheme 1.

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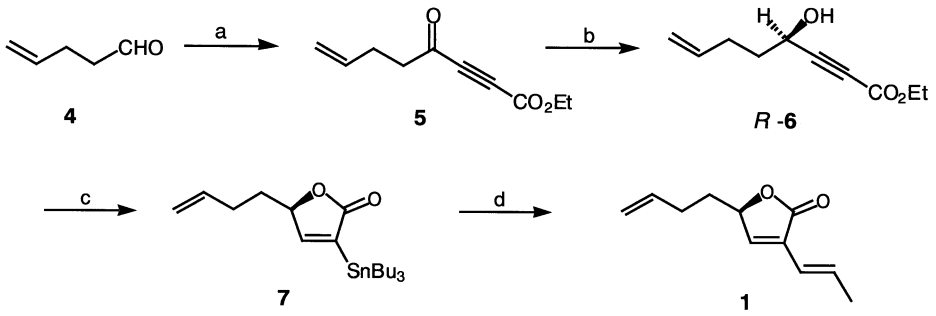


Scheme 2. (a) (i) *n*-BuLi, HMDS, ethylpropiolate, THF; (ii) Jones reagent, acetone, 58% (from **4**). (b) (*R*)-Alpine-borane, neat, 70, 83% ee. (c) Bu_3SnH, $Pd(Ph_3)_2Cl_2$, THF, 60%. (d) 1-Bromopropene, $Pd(PhCN)_2Cl_2$, CuI, $AsPh_3$, NMP, 50%.

e.g. through asymmetric reduction of the corresponding ketones,[7] this appeared to be an attractive approach for a short and efficient route towards (−)-pregaliellalactone (**1**). In the first part of this paper we describe the enantioselective synthesis of (−)-(**1**) (summarised in Scheme 2), while the second part discusses the conversion of (−)-**1** to (−)-**3** in vivo.

2. Results and discussion

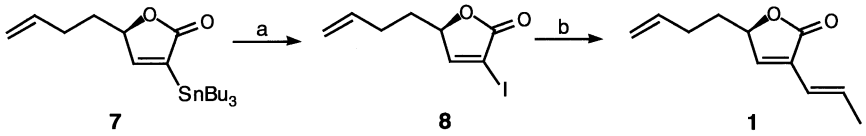
Ethylpropionate was lithiated with LiHMDS and added to 4-pentenal (**4**). LiHMDS was found to be a superior base compared to *n*-BuLi, resulting in a cleaner addition,[8] and the hydroxy propionate obtained was directly oxidised to the ketone **5** with Jones' reagent in acetone. The reduction of prochiral propargylic ketones with chiral reducing reagents or catalysts is an established way of making non-racemic secondary alcohols.[7] There are several reductive agents suitable for this, for example Corey's chiral (CBS)-oxazaborolidine as a catalyst together with BH_3 which reduced **5** with good ee values but with low yields,[9] presumably due to competing hydroboration of the terminal double bond. Instead, (*R*)-Alpine-borane was found to be the most efficient reagent,[10] giving good yield (70%) and selectivity (83% ee). Neat Alpine-borane was used for the reduction as the reagent is less reactive towards ketones than modified borane reagents and also required longer reaction times. An additional advantage with this reagent is that both enantiomers of the alcohol are accessible as both *R*- and *S*-Alpine borane are commercially available. The stereochemical outcome of the reduction is explained by the model proposed by Midland et al.[10a]

The hydroxy propionate ester (−)-**6** was consequently transformed to the stannyl butenolide **7** by palladium-catalyzed stannylation with tributylstannane hydride.[11] $Pd(PPh_3)_2Cl_2$ was found to be a good catalyst for this reaction, much better than tetrakis(triphenylphosphine)-palladium(0) which previously has been reported to be suitable for similar hydrostannylations.[12] The reaction was not entirely regioselective as the α/β isomers were formed in a 6:1 ratio,[13] but the two products were easily separated by chromatography. The use of the more sterically demanding catalyst $Pd[P(o\text{-}tol)_3]_2Cl_2$ did not change the yield or the regioselectivity of the reaction.[13b] To our knowledge this is the first direct synthesis of a 3-stannyl butenolide from a hydroxy propionate ester through a hydrostannylation reaction. Previous syntheses of 3-stannyl-2(5*H*)-furanones have used protected hydroxy propionates, but our results demonstrate that protection is not necessary for the stannylation.[14] Due to the many reactions available for such tin derivatives, this is an excellent method of making substituted butenolides.[15]

A direct cross-coupling of the stannyl butenolide **7** with 1-bromo-1-propene would afford us with the desired product **1**. The Stille cross-coupling reaction is a widely used and mild method for creating new carbon–carbon bonds,[16] it's usefulness stemming from the availability of organostannanes and coupling partners (halides and triflates) as well as the functional group tolerability. However, despite the many mechanistic studies that have been performed there are still no general conditions for the reaction. For the coupling of **7** with 1-bromopropene, $Pd(PhCN)_2Cl_2$ as a catalyst, triphenylarsine as a rate enhancing ligand,[17] and copper(I) iodide as co-catalyst in *N*-methylpyrollidinone (NMP) at 60°C proved to be the most efficient conditions,[18] providing the cross-coupled product **1** in 50% yield. The product was obtained in four steps with 16% overall yield.

Because the Stille coupling is not diastereoselective, both the *trans* and *cis* adducts can be formed if the stannylalkenyl or the alkenyl halide is not isomerically pure. 1-Bromopropene is commercially available both as a reasonably priced *E*/*Z*-mixture as well as the very expensive pure (*E*)-isomer. However, there is no need to chose the expensive alternative, if *E*/*Z*-1-bromopropene is used in the Stille coupling the resulting *E*/*Z*-mixture of **1** can easily be isomerised to pure *Z*-**1** by UV-light in the presence of a catalytic amount of iodine.[19] The pregaliellalactone (**1**) thus obtained had the identical spectroscopic properties as natural (−)-pregaliellalactone and an enantiomeric excess of 83% ($[\alpha]_D^{20}$(synth.)=−36.5, $[\alpha]_D^{20}$(nat.)=−40.8).

To improve the final step in Scheme 2, several alternative methods were investigated. By converting the stannyl butenolide **7** to the corresponding iodo compound **8**,[20] and preparing the stannylalkenyl derivative from 1-bromopropene,[21] the relationship of coupling partners in the Stille-reaction was reversed (see Scheme 3). This combination resulted in a faster coupling reaction due to the use of a sterically less hindered stannane, as the rate determining step of the Stille reaction is the Sn–Pd transmetallation.[22]



Scheme 3. *Reaction conditions*: (a) I_2, CH_2Cl_2, 82%; (b) 1-tributylstannylpropene, $Pd_2(dba)_3CHCl_3$, CuI, $AsPh_3$, NMP, 80% or BuLi, $ZnCl_2$, 1-tributylstannylpropene, $Pd(Ph_3P)_4$, THF, 73%.

With $Pd_2(dba)_3{\cdot}CHCl_3$ as a catalyst in NMP at room temperature, 1-tributylstannyl-1-propene and **8** gave the coupling product in 80% yield (see Scheme 3). An alternative to the Stille coupling with stannanes as the organometal partner, is the use of organozinc compounds in the palladium catalyzed coupling with halides developed by Negishi.[22] Changing the metal can accelerate the reaction more than changing the catalyst or ligand, and zinc has been shown to very effective in this sense.[23] Treatment of 1-tributylstannylpropene with *n*-BuLi followed by $ZnCl_2$ gives the corresponding organozinc derivative, which was coupled with **8** in the presence of $Pd(Ph_3P)_4$ to give **1** in 73% yield. The reaction is complete after 20 min and no remaining starting material was detected. Although this procedure increases the number of synthetic steps to 5, the total overall yield is higher (17%).

A preliminary report of a study of the biosynthesis of (−)-galiellalactone has appeared,[3a] in which pregaliellalactone (**1**) and desoxygaliellalactone (**2**) were key intermediates. Synthetic[3c] **1** was cyclised to desoxygaliellalactone (**2**) (at 9.8 kbar for 17 h at 45°C in toluene/benzene 9:1) with a yield of 58%, and attempts were made to hydroxylate[3c] **2** to galiellalactone (**3**) (see Scheme 1). The cyclisation would appear to be an intramolecular Diels–Alder reaction,[24] although this has not been confirmed, and it is still not clear why it proceeds so efficiently in vivo. However, the chemical hydroxylation of the central bridge head position would be expected to be a difficult reaction to perform,[25] and the attempt failed with SeO_2.[3c] Considering that enzymatic hydroxylations can be, and often are, highly selective and efficient,[26] contrary to synthetic hydroxylations, we instead wanted to make use of the biosynthetic potential of the fungus for converting **1** to **3**. *G. rufa*, (A75-85), was grown in a fermentor as described before.[2] After 7–10 days, when **3** or its precursor **1** could be detected in the culture, the mycelium was removed from the medium by filtration, washed thoroughly with saline before being suspended in a saline solution. **1**, dissolved in a minimal amount of MeOH, was added dropwise and the mixture was incubated at 24°C at 120 rpm. Aliquots were removed at regular intervals and analysed with HPLC, the conversion starts immediately and is almost complete after 40 h, and the disappearance of **1** is exactly followed by the appearance of **3**. This excludes the possibility that **1** first is degraded to something simple (e.g. acetate) which consequently is used by the fungus to make **3**.[3] Extraction with an organic solvent and a simple purification by chromatography to get rid of remaining starting material gave us galiellalactone (**3**) in 90% yield. If the mycelium was heated to 100°C prior to the incubation, no conversion of **1** to **3** was observed. The rate of conversion was 5 mg **1** per g biomass (d.w.) and hour, up to concentrations of 190 mg/l of **1**.

3. Conclusions

We have described a short and efficient way to produce (−)-pregaliellalactone (**1**), the biosynthetic precursors of (−)-galiellalactone (**3**), from 4-pentenal, and how **1** efficiently is converted to **3** by the mycelium of the ascomycete *G. rufa* strain A75-86. This will facilitate the production of (−)-galiellalactone (**3**), and possibly also of analogues of **3**, and thereby promote the study of its potent and selective IL-6 inhibitory effect.

4. Experimental

4.1. General

Materials were obtained from commercial suppliers and were used without further purification unless otherwise noted. THF was dried by refluxing over sodium/benzophenone ketyl immediately prior to use. CH_2Cl_2 and triethylamine were distilled from calcium hydride prior to use. All moisture and air-sensitive reactions were carried out under an atmosphere of dry nitrogen using oven-dried glassware. EIMS spectra (direct inlet, 70 eV) were recorded with a JEOL SX102 spectrometer, and the NMR spectra (in $CDCl_3$) with a Bruker DRX300 spectrometer at 300 MHz (^1H) and at 75 MHz (^{13}C), a Bruker DRX400 spectrometer (at 400/100 MHz) and a Bruker ARX500 spectrometer (at 500/125 MHz). IR spectra were recorded with a Perkin–Elmer 298 spectrometer. All flash chromatography was performed on 60 Å 35–70 μm Matrex silica gel (Grace Amicon). HPLC separation was conducted on a HP1090 series I (column: Merck LiChroCART® 125-4, LiChrospher® 100, RP-18, 5 μm; linear water/acetonitrile gradient). TLC analyses were made on Silica Gel 60 F_{254} (Merck) plates and visualized with anisaldehyde/sulphuric acid and heating. The ee values were determined by integrating the X–H signals in the ^1H NMR spectra of the corresponding Mosher (MTPA) esters.

4.1.1. 4-Oxo-oct-7-en-2-ynoic acid ethyl ester (5). To *n*-BuLi (2.5 M in hexane) (16.28 ml, 40.7 mmol) was added hexamethyldisilazane (8.59 ml, 40.7 mmol) dropwise at 0°C. After the addition the cooling bath was removed and the solution was stirred for 15 min. The hexane was removed under reduced pressure and the remaining white crystals were dissolved in THF (68 ml). The solution was cooled to −78°C and ethyl propiolate (3.66 ml, 37.3 mmol) in THF (34 ml) was added dropwise over 30 min, whereafter the solution was stirred for an additional 20 min. 4-Pentenal (**4**) (3.35 ml, 33.9 mmol) in THF (17 ml) was added quickly but dropwise to the reaction mixture. The stirring at −78°C was continued for 20 min, after which the reaction was quenched with NH_4Cl(sat.) (4 ml) before

warming to room temperature. The reaction mixture was diluted with water and extracted with diethyl ether, the organic phase was washed with water and brine, dried ($MgSO_4$) and concentrated. The crude product was dissolved in acetone (100 ml) and Jones' reagent (9 ml) was added dropwise at 0°C. The dark brown solution was stirred for 2 h before 3.3 g of $NaHCO_3$ and $MgSO_4$ was added. The mixture was stirred for 15 min, filtered through silica which was washed with acetone and diethyl ether. The filtrate was concentrated and the crude product purified by flash chromatography (petroleum ether/ether 40:1). 3.52 g (58%) of the ketone was obtained, as a colourless oil. ν_{max} (film): 2985, 2925, 2220, 1720, 1690, 1365, 1250, 1015, 920 and 755 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.13 (3H, t, J=7.1 Hz, $-CH_2CH_3$), 2.23 (2H, m, 6-H), 2.54 (2H, t, J=7.2 Hz, 5-H), 4.10 (2H, q, J=7.1 Hz, $-CH_2CH_3$), 4.83 (1H, dq, J_1=11.1 Hz, J_2=1.5 Hz, 8-H), 4.87 (1H, dq, J_1=17.0 Hz, J_2=1.5 Hz, 8-H), 5.58 (1H, ddt, J_1=17.0 Hz, J_2=11.1 Hz, J_3=6.6 Hz, 7-H); ^{13}C NMR (CDCl3) δ 14.3, 27.7, 44.7, 63.4, 78.8, 80.8, 116.6. 136.0, 152.6, 184.6; HRMS (CI) calcd for $C_{10}H_{13}O_3$ (M+H) 181.0865, found 181.0853.

4.1.2. (*R*)-4-Hydroxy-oct-7-en-2-ynoic acid ethyl ester (6). To a round bottomed flask was added (*R*)-Alpine-borane (0.5 M in THF) (44.4 ml, 22.2 mmol). The THF was removed under reduced pressure, and the remaining yellow oil was cooled with an ice bath before ketone **5** (2.00 g, 11.09 mmol) was added dropwise. The reaction mixture was stirred overnight and allowed to reach room temperature. The solution was again cooled to 0°C and acetaldehyde (6.8 ml, 122 mmol) in THF (17 ml) was added dropwise, whereafter the solution was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the remaining oil was dissolved in diethyl ether (17 ml) and cooled to 0°C. 1,2-Aminoethanol (1.34 ml, 22.2 mmol) was added dropwise and a thick white precipitate was formed. The reaction mixture was filtered and the solids were washed with several portions of diethyl ether. The filtrate was concentrated, and flash chromatography (CH_2Cl_2/petroleum ether 1:1 to 100% CH_2Cl_2) of the crude product afforded 1.42 g (70%) of the alcohol, as a colourless oil. The enantiomeric excess was determined by ^1H NMR measurement of the corresponding Mosher ester. $[\alpha]_D^{20}=-10.3$ (c=8.2, $CHCl_3$); ν_{max} (film): 3020, 2945, 2235, 1705, 1255, 1220 and 770 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.31 (3H, t, J=7.1 Hz, $-CH_2CH_3$), 1.86 (2H, m, 6-H), 2.24 (2H, m, 5-H), 3.11 (1H, bd, J=4.1 Hz, –OH), 4.23 (2H, q, J=7.1 Hz, $-CH_2CH_3$), 4.50 (1H, m, -4H), 5.01 (1H, dq, J_1=10.2 Hz, J_2=1.5 Hz, -8H), 5.08 (1H, dq, J_1=17.1 Hz, J_2=1.5 Hz, -8H), 5.80 (1H, ddt, J_1=17.1 Hz, J_2=10.2 Hz, J_3=6.7 Hz, -7H); ^{13}C NMR ($CDCl_3$) δ 14.4, 29.5, 36.2, 61.7, 62.7, 77.0, 88.3, 116.1, 137.5, 154.0; HRMS (CI) calcd for $C_{10}H_{15}O_3$ (M+H) 183.1021, found 183.1014.

4.1.3. (*R*)-5-But-3-enyl-3-(tri-*n*-butyl-stannanyl)-5*H*-furan-2-one (7). $Pd(Ph_3)_2Cl_2$ (47 mg, 0.07 mmol) was added to a solution of **6** (606 mg, 3.32 mmol) in THF (6.6 ml). The reaction vessel was flushed with nitrogen and Bu_3SnH (1.01 ml, 3.65 mmol) was added dropwise over 30 min to the reaction solution. The solution was stirred for an additional 10 min whereafter the solvent was removed under reduced pressure. The residue was purified by flash chromatography (hexane/triethylamine 96:4). 830 mg (60%) **7** and 144 mg (10%) of the β isomer were isolated, as colourless oils. $[\alpha]_D^{20}=-20.9$ (c=2.2, $CHCl_3$); ν_{max} (film): 2960, 925, 1735, 1215, 1145, 955, 920 and 750 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.90 (9H, t, J=7.2 Hz, $-CH_2CH_3$), 1.10 (6H, m, $-CH_2-$), 1.33 (6H, m, $-CH_2-$), 1.54 (6H, m, $-CH_2$), 1.71 (1H, q, J=7.5 Hz, -6H), 1.85 (1H, m, -6H), 2.22 (2H, m, -7H), 5.03 (1H, m, -9H), 5.05 (1H, m, -5H), 5.08 (1H, m, -9H), 5.81 (1H, ddt, J_1=17.0 Hz, J_2=10.3 Hz, J_3=6.6 Hz, -8H), 7.46 (1H, dt, J_1=11.1 Hz, J_2=1.4 Hz, -4H); ^{13}C NMR ($CDCl_3$) δ 9.7, 13.6, 27.1, 28.7, 32.7, 84.3, 115.8, 135.5, 137.0, 165.2, 177.8; HRMS (EI) calcd for $C_{16}H_{27}O_2Sn$ ($M-C_4H_9$) 371.1033, found 371.1030.

4.1.4. (*R*)-5-But-3-enyl-3-iodo-5*H*-furan-2-one (8). Iodine (228 mg, 0.90 mmol) in CH_2Cl_2 (10 ml) was added dropwise over 40 min to of the stannanyl-butenolide **7** (383 mg, 0.90 mmol) in CH_2Cl_2 (4.5 ml) at room temperature. The reaction mixture was then stirred for 3 h. The solvent was removed and the crude product was dissolved in 3 ml of diethyl ether. To this solution was added 50% KF (aq.) (3 ml) and the resulting mixture was stirred for 2 h. The mixture was filtered through celite which was washed with three portions of diethyl ether. The phases were separated, and the ether phase was dried ($MgSO_4$) and concentrated. Purification by flash chromatography (hexane/diethyl ether 10:1) gave 195 mg (82%) **8**, as a colourless oil, and 26 mg (7%) of recovered starting material **7**. $[\alpha]_D^{20}=-17.5$ (c=2.0, $CHCl_3$); ν_{max} (film): 3020, 2920, 1760, 1220, 970, and 770 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.75–1.90 (2H, m), 2.20–2.30 (2H, m), 5.00–5.15 (3H, m), 5.80 (1H, ddt, J_1=16.9 Hz, J_2=10.2 Hz, J_3=6.7 Hz, -8H), 7.75 (1H, d, J=1.7 Hz, -4H); ^{13}C NMR ($CDCl_3$) δ 28.9, 32.2, 84.7, 85.5, 116.5, 136.3, 161.1, 170.0; HRMS (EI) calcd for $C_8H_9O_2I$ 263.9647, found 263.9638.

4.1.5. (*R*)-5-But-3-enyl-3-(*E*)-propenyl-5*H*-furan-2-one, (−)-pregaliellalactone (1). *Stille coupling with **13** and bromopropene*. 1-Bromopropene (*cis/trans* mixture) (69 μl, 0.80 mmol) was added to degassed NMP (2.6 ml) followed by the sequential addition of CuI (16 mg, 0.08 mmol), AsPh3 (50 mg, 0.17 mmol) and $Pd(PhCN)_2Cl_2$ (13 mg, 0.03 mmol). The solution was stirred for 10 min before the stannanyl butenolide **7** (280 mg, 0.66 mmol) in degassed NMP (2.6 ml) was added dropwise at room temperature. The reaction solution was heated to 60°C and stirred for 19 h. After cooling to room temperature NH_4Cl (sat.) was added and stirring continued for 10 min. The solution was extracted with diethyl ether, the organic phase was washed with water, dried ($MgSO_4$) and concentrated. Flash chromatography (hexane/ether 10:1) afforded 59 mg (50%) of a *cis/trans* mixture. The mixture was dissolved in carbon tetrachloride (5 ml) and a crystal of iodine was added to the solution, The solution was then irradiated with a UV lamp for 1.5 h at room temperature. The solution was shaken with $Na_2S_2O_3$(sat) and the phases were separated. The organic phase was dried ($MgSO_4$) and concentrated to give 59 mg of the pure trans isomer, as a colourless oil.

*Stille coupling with **7** and 1-tributylstannanyl-propenyl*. To a degassed solution of iodo butenolide **8** (345 mg, 1.31 mmol) in NMP (5.3 ml) was added in sequential

order CuI (25 mg, 0.13 mmol), $AsPh_3$ (80 mg, 0.26 mmol) and $Pd_2(dba)_3{\cdot}CHCl_3$ (13.6 mg, 0.01 mmol). The dark colour of the catalyst disappeared upon stirring giving a yellow solution. After stirring for 10 min at room temperature a degassed solution of 1-tributylstannanyl-propenyl (520 mg, 1.57 mmol) in NMP (5.3 ml) was added dropwise. The solution was heated to 65°C and stirred for 8 h. NH_4Cl (sat.) was added and stirring continued for 10 min. The solution was extracted with diethyl ether, the organic phase was washed with water, dried ($MgSO_4$) and the concentrated. Flash chromatography (hexane/ether 10:1) afforded 190 mg (80%) of coupled *cis* and *trans* product. This was isomerised (vide supra) to yield 190 mg (80%) **1**, as a colourless oil.

Negishi coupling of ***7*** *and 1-tributylstannanyl-propenyl*. To a solution of 1-tributylstannanyl-propenyl (616 mg, 1.86 mmol) in THF (2.0 ml) was added *n*-BuLi (2.5 M in hexane) (0.82 ml, 2.04 mmol) at −78°C. The solution was stirred for 15 min whereafter $ZnCl_2$ (278 mg, 2.04 mmol) in THF (2.0 ml) was added dropwise. The yellow colour disappeared. The resulting solution was warmed to −20°C and then stirred at that temperature for 10 min. During this time $Pd(Ph_3P)_4$ (92 mg, 0.08 mmol) in THF (2.0 ml) was added to the iodo-butenolide **8** (342 mg, 1.30 mmol) at room temperature and then cooled to 0°C. The resulting orange solution was then added to the organozinc solution, and the mixture was stirred at 0°C for 20 min. The reaction mixture was allowed to reach room temperature, quenched with NH_4Cl (sat.) and extracted with diethyl ether. The organic phase was dried ($MgSO_4$) and the solvent was evaporated. The crude product was triturated with 1 ml of diethyl ether and then filtered through SiO_2 which was washed with 10 ml of petroleum ether/diethyl ether 10:1. The filtrate was concentrated and then purified with flash chromatography (petroleum ether/diethyl ether 6:1) affording 169 mg (73%) of *cis*/*trans* mixture, as a colourless oil, which was isomerised (vide supra) to 169 mg pure **1**. $[\alpha]_D^{20}=-36.5$ (c=1.4, $CHCl_3$); ν_{max} (film): 3020, 2920, 1755, 1450, 1215, 1115, 970, 920 and 760 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.73 (1H, m, -6H), 1.79 (1H, m, -6H), 1.83 (3H, dd, J_1=6.8 Hz, J_2=0.8 Hz, 7′H), 2.21 (2H, m, -7H), 4.93 (1H, dd, J_1=6.1 Hz, J_2=6.1 Hz, -5H), 5.01 (1H, dq, J_1=1.6 Hz, J_2=17.1 Hz, -9H), 5.06 (1H, dq, J_1=1.6 Hz, J_2=10.2 Hz, -9H), 5.78 (1H, ddt, J_1=17.1 Hz, J_2=10.2 Hz, J_3=6.8 Hz, -8H), 6.10 (1H, dd, J_1=15.8 Hz, J_2=0.8 Hz, -5′H), 6.79 (1H, dq, J_1=6.8 Hz, J_2=15.8 Hz, -6′H), 7.02 (1H, d, J=0.8 Hz, -4H); ^{13}C NMR ($CDCl_3$) δ 18.8, 29.1, 32.8, 79.9, 115.9, 119.7, 129.7, 133.5, 136.8, 145.4, 171.9; HRMS (EI) calcd for $C_{11}H_{14}O_2$ 178.0994, found 178.0989.

4.1.6. (4*R*,5a*S*,7a*S*,7b*R*)-5,5a,6,7,7a,7b-Hydro-7b-hydroxy-4-methyl-indeno[1,7-*bc*]furan-2(4*H*)-one, (−)-galiellalactone (3). Mycelium from the culture broth (0.5–1 l, equivalent to 2 g/l mycelium dry weight) was separated by filtration and washed thoroughly with sterile saline (2 l) The mycelium was then transferred to a 2 l Erlenmeyer flask containing sterile saline (1 l). (−)-Pregaliellalactone (190 mg, 1.06 mmol) was dissolved in methanol (1 ml) and added to the flask. The flask was incubated on a rotary shaker at 120 rpm and 24°C. Aliquots were removed at regular intervals and analysed by HPLC. The incubation was terminated when the conversion of **1** was finished. The saline was filtrated and extracted twice with ethyl acetate. The organic phase was dried (Na_2SO_4), and after evaporation of the solvent **3** was isolated from the extract by preparative HPLC (Merck LiChrosorb Diol®, 7 μm, column 250–25 mm) using a linear cyclohexane/*tert*-butyl methyl ether gradient. 186 mg (90%) **3** was obtained as white crystals, mp 53–56°C. $[\alpha]_D^{20}=-52.8°$ (c=0.46, $CHCl_3$); ν_{max} (film): 2960, 2925, 1755, 1215, 1190, 1020, 970, 900 and 755 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.06 (1H, ddd, J_1=4.6 Hz, J_2=8.0 Hz, J_3=13.9 Hz, –CH(CH_3)CH_2–), 1.16 (1H, m, –CH(O)CH_2CH_2–), 1.18 (3H, d, J=7.3 Hz, –CH_3), 1.73 (1H, dddd, J_1=2 Hz, J_2=3 Hz, J_3=7.0 Hz, J_4=14.7 Hz, –CH(O)CH_2CH_2–), 1.85 (1H, dddd, J_1=13.4 Hz, J_2=7.2 Hz, J_3=7.2 Hz, J_4=3.0 Hz, –CH(O)CH_2CH_2–), 2.07 (1H, dddd, J_1=7.2 Hz, J_2=7.5 Hz, J_3=11.0 Hz, J_4=14.7 Hz, –CH(O)CH_2CH_2–), 2.24 (1H, dt, J_1=7.4 Hz, J_2=7.4 Hz, J_3=13.9 Hz, –CH(CH_3)CH_2–), 2.43 (1H, dddd, J_1=4.6 Hz, J_2=7 Hz, J_3=7.4 Hz, J_4=10.6 Hz, –$CH_2CH$$CH_2$–), 2.63 (1H, dqdd, J_1=3.1 Hz, J_2=7.3 Hz, J_3=7.4 Hz, J_4=8.0 Hz, –CHCH_3), 3.43 (1H, s, OH), 4.77 (1H, dd, J_1=2.0 Hz, J_2=7.5 Hz, –CH(O)CH_2CH_2–), 7.01 (1H, d, J=3.1 Hz, –CH(CH_3)CH–); ^{13}C NMR ($CDCl_3$) δ 20.9, 29.0, 31.4, 33.0, 43.1, 81.8, 89.8, 130.7, 150.1, 169.7; HRMS (EI) calcd for $C_{11}H_{14}O_3$ 194.0943, found 194.0945.

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References

1. (a) Köpcke, B.; Johansson, M.; Sterner, O.; Anke, H. *J. Antibiot.* **2002**, *55*, 104. (b) Hautzel, R.; Anke, H. *Z. Naturforsch.* **1990**, *45c*, 68.
2. Weidler, M.; Rether, J.; Anke, T.; Erkel, G. *FEBS Lett.* **2000**, *484*, 1.
3. (a) Steglich, W.; Eizenhöfer, Th.; Casser, B.; Steffan, U.; Rabe, R.; Böker, H. J.; Knerr, H.; Anke, H.; Anke, T. *Dechema-Monographien* **1993**, *129*, 3. (b) Anke, H.; Hautzel, R.; Sheldrick, W. S.; Casser, I.; Steglich, W. *Abstracts of the Seventh International Congress of Pesticide Chemistry*, 1990; Vol. 1, p 1. (c) Schneider, M. PhD Dissertation, Ludwig-Maximilians-Universität Munich, 1996. (d) Knerr, H. J. PhD Dissertation, University of Kaiserslautern, 1995.
4. Johansson, M.; Sterner, O. *Org. Lett.* **2001**, *19*, 2843.
5. Knight, D. W. *Contemp. Org. Synth.* **1994**, *60*, 287.
6. (a) Trost, B. M.; Müller, T. J. J.; Martinez, J. *J. Am. Chem. Soc.* **1995**, *117*, 1888. (b) Marshall, J. A.; Wolf, M. A.; Wallace, E. M. *J. Org. Chem.* **1997**, *62*, 367. (c) Cowell, A.; Stille, J. K. *Tetrahedron Lett.* **1979**, *2*, 133. (d) Yu, W.; Alper, H. *J. Org. Chem.* **1997**, *62*, 5684.
7. (a) Brown, H. C.; Park, W. S.; Cho, B. T.; Ramachandran, P. V. *J. Org. Chem.* **1987**, *52*, 5406. (b) Singh, V. K. *Synthesis* **1992**, *7*, 605.
8. Crimmins, M. T.; Nantermet, P. G.; Trotter, B. W.; Vallin, I. M.; Watson, P. S.; McKerlie, L. A.; Rheinhold, T. L.; Cheung, A. W.-H.; Stetson, A. S.; Dedopoulou, D.; Gray, J. L. *J. Org. Chem.* **1993**, *58*, 1038.
9. (a) Corey, E. J.; Bakshi, R. K.; Shibata, S. *J. Am. Chem. Soc.*

1987, *109*, 5551. (b). Corey, E. J.; Bakshi, R. K.; Shibata, S.; Chen, C.; Singh, V. K. *J. Am. Chem. Soc.* **1987**, *109*, 7925.
10. (a) Midland, M. M.; Tramontano, A.; Kazubski, A.; Graham, R. S.; Tsai, D. J. S.; Cardin, D. B. *Tetrahedron* **1984**, *40*, 1371. (b) Garcia, J.; López, M.; Romeu, J. *Tetrahedron: Asymmetry* **1999**, *40*, 2617.
11. (a) Smith, N. D.; Mancuso, J.; Lautens, M. *Chem. Rev.* **2000**, *100*, 3257. (b) Miyake, H.; Yamamura, K. *Chem. Lett.* **1989**, 1371.
12. Rossi, R.; Carpita, A.; Cossi, P. *Tetrahedron* **1992**, *40*, 8801.
13. (a) Zhang, H. X.; Guibé, F.; Balavoine, G. *J. Org. Chem.* **1990**, *55*, 1857. (b) Greeves, N.; Torode, J. S. *Synlett* **1994**, 537. (c) Betzer, J.; Delaloge, F.; Muller, B.; Pancrazi, A.; Prunet, J. *J. Org. Chem.* **1997**, *62*, 7768.
14. Mabon, R.; Richecœur, A. M. E.; Sweeney, J. B. *J. Org. Chem.* **1999**, *64*, 328.
15. Richecœur, A. M. E.; Sweeney, J. B. *Teterahedron* **2000**, *56*, 389.
16. (a) Stille, J. K. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 508. (b) Mitchell, T. N. *Synthesis* **1992**, 803. (c) Farina, V. *Pure Appl. Chem.* **1996**, *68*, 73.
17. (a) Farina, V.; Krishnan, B. *J. Am. Chem. Soc.* **1991**, *113*, 9585. (b) Farina, V.; Krishnan, B.; Marshall, D. R.; Roth, G. P. *J. Org. Chem.* **1993**, *58*, 5434.
18. Farina, V.; Kapadia, S.; Krishnan, B.; Wang, C.; Liebeskind, L. S. *J. Org. Chem.* **1994**, *59*, 5905.
19. Engler, T. A.; Chai, W. *Tetrahedron Lett.* **1996**, *37*, 6969.
20. Bellina, F.; Carpita, A.; De Santis, M.; Rossi, R. *Tetrahedron* **1994**, *50*, 12029.
21. Lee, A. S.; Dai, W. *Tetrahedron* **1997**, *53*, 859.
22. Negishi, E.; Takahashi, T.; Baba, S.; Van Horn, D. E.; Okukado *J. Am. Chem. Soc.* **1987**, *109*, 2393.
23. Pihko, P. M.; Koskinen, A. M. P. *Synlett* **1999**, *12*, 1966.
24. Ichihara, A.; Oikawa, H. *Curr. Org. Chem.* **1998**, *2*, 365.
25. (a) Trost, B. M., Fleming, I., Eds.; *Comprehensive Organic Synthesis*, Pergamon: Oxford, 1991; Vol. 7 Chapter 1.4, Brown, S. M.; Chapter 2.1, Bulman, P. C.; McCarthy, T. J.. (b) Nicolaou, K. C.; Sorensen, E. *Classics in Total Synthesis*; VCH: Weinheim, 1996; pp 461, and references there cited.
26. Garcia-Granados, A.; Parra, A.; Simeó, Y.; Extremera, A. L. *Tetrahedron* **1998**, *54*, 14421.