The High-Intrinsic Diels–Alder Reactivity of (−)-Galiellalactone; Generating Four Quaternary Carbon Centers under Mild Conditions

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Dedicated to Prof. Wolfgang Steglich on the occasion of his 70th birthday

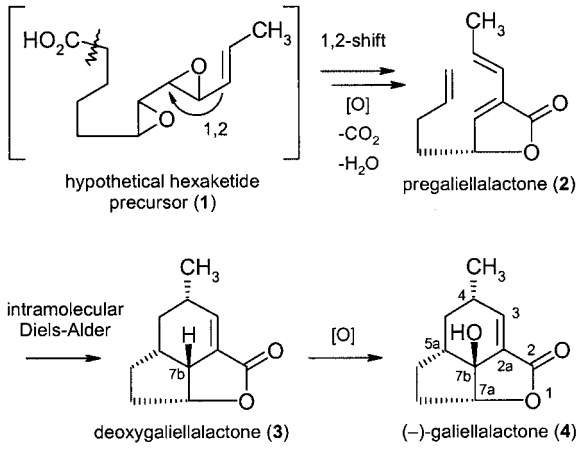
Keywords: Biological activity / Biosynthesis / Medicinal chemistry / Natural products / Polyketides / Signal transduction

The Interleukin-6 (IL-6) responsive JAK/STAT pathway seems to be correlated with major diseases such as chronic inflammation, arteriosclerosis, liver fibrosis, Parkinson and Alzheimer disease. In order to take a look at (–)-galiellalactone as a potential low-molecular-weight lead structure for non-proteinogenic IL-6 antagonists we carried out a microderivatization program that was aiming at bioactive congeners of the natural product and a preliminary structure-activity relationship. During this effort, galiellalactone showed reactivity generally governed by convex-concave face selectivity. *O*-Acyl derivatives of galiellalactone turned out to be precursors of bis(dehydratogaliellalactone), a novel oligocyclic Diels–Alder product of complicated architecture. During this extraordinary Diels–Alder-type conversion four quaternary carbon centers were generated at room temperature under mild conditions in a regio- and stereoselective fashion. The novel Diels–Alder transformation was used for a stereoselective hetero-derivatization of the natural product leading to an aza congener of altered IL-6 antagonistic activity in HepG2 hepatoma cells. The absolute (4*S*,5a*R*,7a*R*,7b*S*) configuration of natural (–)-galiellalactone was confirmed by X-ray crystallography with Cu-K_α radiation.

Introduction

(−)-Galiellalactone (**4**) was first isolated from cultures of the ascomycete *Galiella rufa* A75-86 by Anke and Hautzel,[1] and since then further wood-inhabiting fungi have been found to produce **4**.[2] The unusual tricyclic structure of galiellalactone (**4**) did not, however, automatically implicate a common biogenesis. Nonetheless, a biosynthesis that involves a 1,2-rearrangement of a hexaketide precursor (**1** → **2**) and an intramolecular Diels–Alder cyclization (**2** → **3**) as key steps has been proposed by Steglich (Scheme 1).[3] Feeding experiments[3] and recent microbiological investigations[4] support such an unusual pathway. The Diels–Alder step in particular is remarkable. To date, not many natural products are known that are biosynthesized by an enzyme-catalyzed [4+2] cycloaddition.[5]

The relative stereochemistry of galiellalactone was originally deduced by NMR spectroscopic[6] and X-ray diffraction[1] measurements. However, the absolute configuration of the natural product was only clarified recently by syntheses of (+)-galiellalactone (*ent*-**4**)[7,8] and (−)-galiellalactone (**4**).[7]



Scheme 1. Biosynthesis of (−)-galiellalactone (**4**)[3]

Recently, (−)-galiellalactone (**4**) was described as a selective, non-peptidic, low-molecular-weight inhibitor of the interleukin 6 (IL-6) responsive JAK/STAT pathway (Janus kinase/signal transducer and activator of transcription).[9] The activity of **4** was determined with a reporter gene assay to be submicromolar (IC_{50} = 0.25 μM) in HepG2 cells.[9] Systemic changes of the organism that are a consequence of

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inflammation are termed "acute-phase response" (APR).[10] The cytokine interleukin 6 (IL-6) acts as the major inducer of acute-phase proteins in the liver by the JAK/STAT pathway. Accordingly, IL-6 responsive genes (IL-6RE) play a key role within the acute-phase response and seem to be involved in many diseases such as liver fibrosis,[11] Alzheimer's disease,[12] Parkinson's disease,[13] arteriosclerosis,[14] and chronic inflammation.[15] Controlled regulation of the IL-6 induced cell signaling with low-molecular-weight drugs would therefore be a favorable starting point to develop new treatments for these diseases.

Results and Discussion

In order to obtain IL-6 antagonistic analogues of **4** that also inhibit JAK/STAT signaling we carried out a chemical derivatization program. Concurrently, a preliminary structure-activity relationship (SAR) should be established. A broad "decorating" derivatization approach, that uses the natural product more as a template, was avoided as it would most likely lead to inactive derivatives due to capping or "dilution" of the chemical functional groups which are often also the pharmacophores. Instead, we decided to focus on singular modifications of only individual functional groups within **4**. A luciferase reporter gene assay in HepG2 hepatoma cells permanently transfected with a respective reporter was used to monitor the bioactivity of the synthesized compounds. Within our assay system **4** showed single-digit micromolar activity (IC_{50} = 2.7 μM).

First, a new X-ray structure of natural **4** was obtained (Figure 1).[16] Although the natural product does not contain heavy hetero atoms, we were able to confirm[7] the absolute (4*S*,5a*R*,7a*R*,7b*S*) configuration of **4** by using Cu-K_α radiation as the X-ray source. The folded geometry of **4** implicates a convex side, bearing the tertiary alcohol at C7b at its very center, and a concave face that is fenced by the carbonyl group at C2, the methyl group at C4 and various hydrogen atoms (at C3, C5, C6 and C7). The central six-membered ring (C2a−C7b) is forced into the boat conformation by the annulated cyclopenta[*b*]furan-2-one system and the methyl substituent at C4.

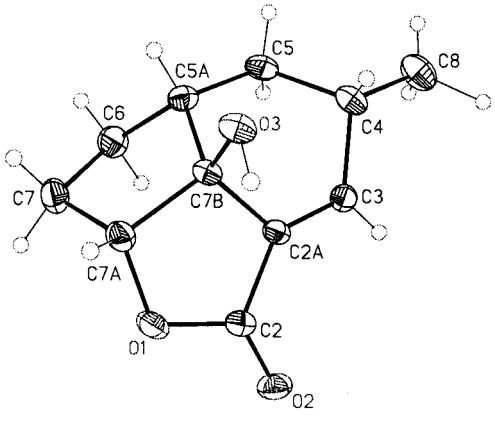
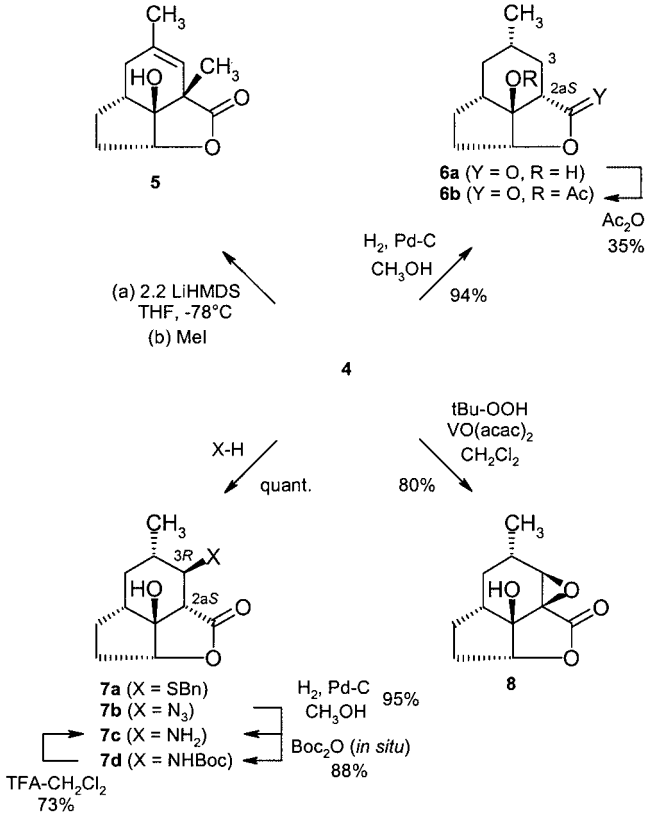


Figure 1. X-ray structure with the absolute configuration of natural (−)-galiellalactone (**4**)[16]

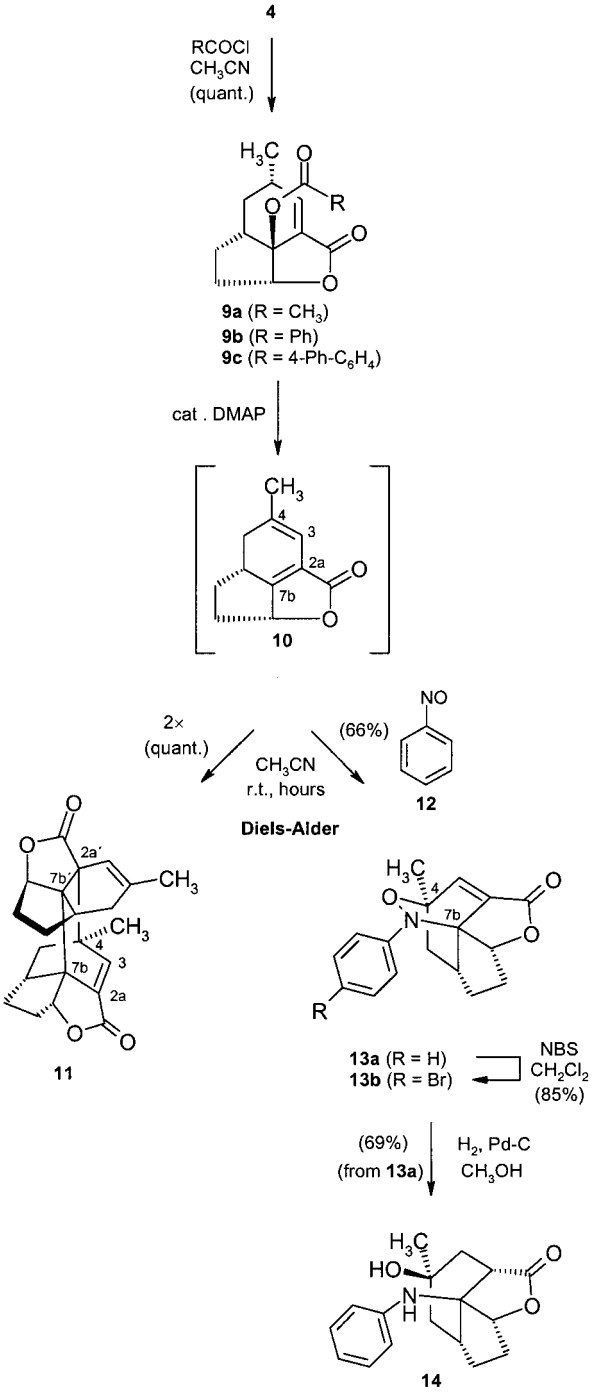
Clearly, the chemistry of galiellalactone (**4**) is governed by convex/concave face selectivity: catalytic hydrogenation of **4** yields (2a*S*)-2a,3-dihydrogaliellalactone (**6a**) exclusively (Scheme 2). Michael additions generally seem to take place at C3 from the *Re* face. Hence, the (3*R*) addition product was obtained by stirring **4** with phenylmethanethiol under basic conditions (**4** → **7a**), or with sodium azide under mildly acidic conditions (**4** → **7b**). Subsequent reduction yielded the corresponding amine (**7b** → **7c**) or the (*tert*-butoxycarbonyl)amine in the presence of di-*tert*-butyl dicarbonate (**7b** → **7d**). Epoxidation also took place from the convex face, yielding **8** in good yield under anhydrous conditions. When electrophiles like alkyl halides were allowed to react with the dianionic enolate of **4** at lower temperatures, C2a derivatives with a shifted double bond were obtained, as is exemplified by the methyl product **5**. Within our IL-6 responsive assay system, compounds **5**, **6**, **7** and **8** turned out to be inactive.



Scheme 2. Face selectivity governs the chemistry of (−)-galiellalactone (**4**)

A major synthetic aim of our derivatization efforts was to obtain hetero-congeners of **4** that bear a nitrogen functionality at C7b instead of the hydroxy group. One advantage of such a substitution would be the possibility to introduce further residues (*N*-alkyl, *N*-aryl), without abandonment of a hydrogen donor at this position. S_N2 reactions did not appear to be a promising strategy as inversion of stereochemistry is unfavorable at C7b. In order to evaluate S_N1 modifications at C7b that take place with retention of configuration, the tertiary alcohol was first activated by

acylation. Conversion of **4** by addition of a large excess of acyl chloride (e.g. AcCl, BzCl, 4-PhC$_6$H$_4$COCl) in aprotic solvents yielded products **9**, which are stable enough to be stored at −18 °C for months (Scheme 3). However, in solution, especially in the presence of strong bases (e.g. DBU), the esters **9** decomposed. To our surprise the nucleophilic base 4-(dimethylamino)pyridine (DMAP) triggered a high yielding conversion of **9** to a defined product which turned out to be a formal dimer of dehydratogaliellalactone (**10**) by LC-HR-MS. Extensive 2D NMR experiments revealed the unprecedented and complicated architecture of **11**. Structure **11** presents a new heptacyclic oxa-carbon ring system. Again, the absolute configuration was determined by an X-ray structure (2a′*R*,5a′*R*,7a′*R*,4*R*,7b′*S*,5a*R*,7a*R*,7b*S*) using Cu-K_α radiation (Figure 2). The connectivity between the two galiellalactone units over the central unsaturated six-membered ring system (C2a,3,4,2a′,7b′,7b) specified **11** as a Diels–Alder-type dimerization product of dehydratogaliellalactone (**10**). This high-yielding Diels–Alder reaction[17] is remarkable for several reasons: (a) four quaternary carbon centers are generated under mild conditions (room temperature, atmospheric pressure). Very few examples have been described[18] for Diels–Alder cyclizations of comparable steric demand, most of which were performed under harsh reaction conditions (elevated temperature,[19] high pressure[20]) or took advantage of highly electrophilic dienophiles like tetracyanoethylene (TCNE);[21] (b) the reaction proceeds with high regio- (> 95%) and diastereoselectivity (> 95%), and only one Diels–Alder product (**11**) was detected by HPLC-UV-MS; (c) tricyclic inner dienes like in situ intermediate **10** have not been described within the multitude of Diels–Alder-reactive cyclohexadienes.



Scheme 3. Diels–Alder dimerization and hetero-derivatization of **4**

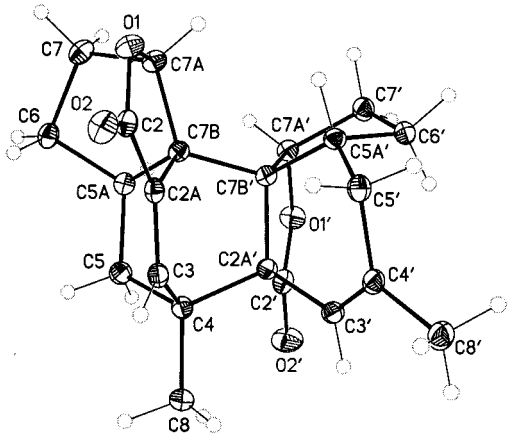
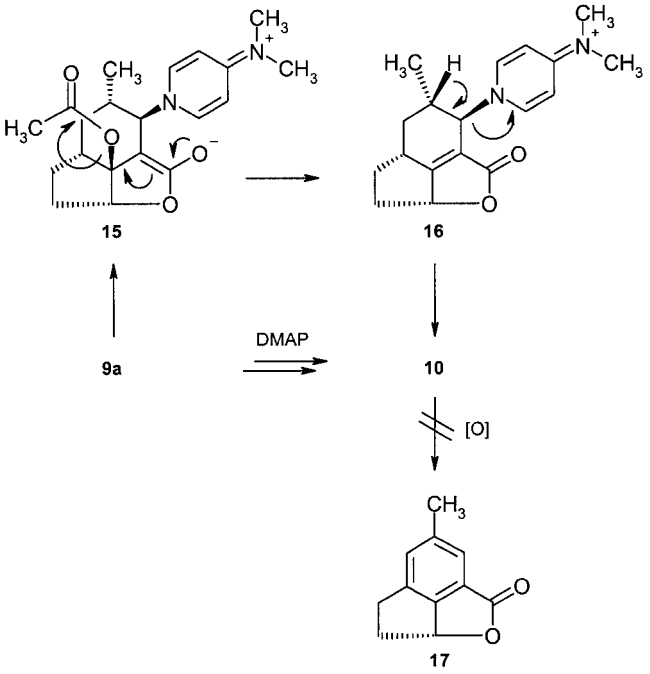


Figure 2. X-ray structure of **11** (ORTEP; 50% probability level)[16,19]

Strained diene **10** is postulated as an intermediate which is formed in situ[17,22] (additional Diels–Alder products of **10** are discussed below). However, ionic intermediates may also play a role in the conversion of **9** or **10** to **11**, as they do in many Diels–Alder-type reactions.[17,23] A possible mechanism for the DMAP-catalyzed in situ formation of **10** is depicted in Scheme 4. The overall 1,4-elimination of acetic acid (**9a** → **10**) is initiated by a Michael addition step (**9a** → **15**), followed by extrusion of the carboxylate (**15** → **16**)[24] and is finally completed with a 1,2-elimination of the dimethylpyridinium cation (**16** → **10**). Formation of **11** points at increased reactivity of **10** in comparison to open-chain diene analogues (e.g. 1,3-butadiene-2-carboxylic esters), that did not react under our reaction conditions. As expected, aromatizing oxidation of tricyclo[5.5.6] systems like **10** is unfavorable (**10** → **17**) due to increased ring strain[25] of the corresponding tricyclic cyclophanes **17**. Al-



Scheme 4. Possible mechanism for the DMAP-catalyzed formation of diene **10** from **9a** in situ

though conversion of **9** to **10** is accompanied by a flattening of the tricyclo[5.5.6] system, face selectivity is still high with regard to the diene and the dienophile. The convex/convex Diels−Alder product **11** is observed exclusively. Now, that the "lower" diene moiety of **11** (O1−C7b) showed the completely retained "natural" (4*S*,7b*S*) stereochemistry, a Diels−Alder strategy appeared promising to synthesize C7b-hetero-substituted congeners of **4**, and thereby circumvent our "S_N2 problem" at C7b.

Indeed, addition of DMAP to a stirred solution of **9** and nitrosobenzene (**12**) in dichloromethane at room temperature and atmospheric pressure triggered the anticipated Diels−Alder reaction in good yield with high regio- and diastereoselectivity (Scheme 3). Only the regioisomer **13a** with an oxygen atom at C4 and a nitrogen atom at C7b was obtained. X-ray structure analyses of **13a** and **13b** indicated that a selective cycloaddition of the nitroso dienophile had taken place at the convex face of the diene **10**, again yielding systems with the "natural" (7b*S*) configuration (Figure 3). The boat conformation of the central six-membered ring within **13a** strongly resembled the situation known from **4**. Our efforts were rewarded with the altered potency of **13a** (IC_{50} = 1.7 μM) in comparison with natural **4** (IC_{50} = 2.7 μM).

Hydrogenation (**13a** → **14**) not only opened the bicyclic cage by cleavage of the N−O bond (Scheme 3) but furthermore reduced the enone, thereby moving the system into the inactive dihydro series (cf. compound **6a**). Accordingly, no IL-6 antagonistic activity was observed for **14**.

Further synthetic work is underway in order to establish a first SAR for (−)-galiellalactone (**4**). However, three trends are already apparent for the IL-6 antagonistic activity within this series: (a) the alcohol functionality at C7b

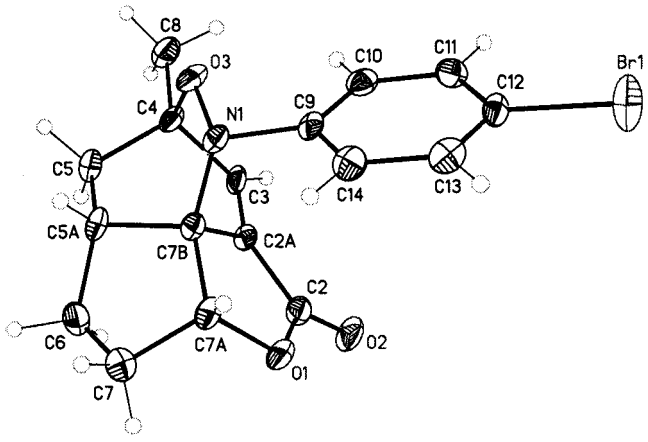


Figure 3. X-ray structure of **13b**[26]

may be replaced with anilines without loss of activity; (b) introduction of additional oxygen substituents is allowed at C4; (c) dihydro congeners like **6a**, devoid of the central Michael system and thereby conformationally distorted, do not show IL-6 antagonistic activity.

Conclusion

(−)-Galiellalactone (**4**) disclosed an intrinsic Diels−Alder principle that is as stimulating for further chemical and biological investigations as it is surprising due to the seemingly simple structure of this natural product, and clearly corresponds with the biogenesis of **4**. A new aspect is appended to a "Diels−Alder story" that originally started with Steglich's biosynthesis proposal[3] and very recently concluded with Sterner's[4] observation of a rate-enhanced cyclization of **2** within *G. rufa* cultures. It is the subject of ongoing experiments whether [4+2] cycloadditions of diene **10** also take place in a natural environment with dienophiles, present in vivo. Biological activity, correlated to the special Diels−Alder reactivity of galiellalactone, could be triggered in a targeted[27] fashion by activation at C7b through in vivo acylation.

Experimental Section

General Remarks: Chemicals of analytical grade were obtained from Merck KGaA (Germany) or Sigma Aldrich (Germany). Chromatography was carried out on silica gel 60 230−400 mesh (Merck KGaA, Germany) or Sephadex LH-20 (Pharmacia). Analytical HPLC was carried out with a WATERS 2690 unit with automated sample injector and Waters 996 diode array detector, employing the following instrumental conditions: column Xterra RP_8 5 μm (3.9 × 150 mm); mobile phase A: 0.01% TFA in water; B: 0.01% TFA in acetonitrile; flow: 1.2 mL/min; gradient: start 95% A; 12 min 5% A; 13 min 5% A; detection at 190−400 nm. HPLC-ESI-MS was performed with an HP 1100 HPLC system (Hewlett Packard GmbH, Germany) coupled to a Micromass-LCT mass spectrometer (Micromass, UK) using a Waters Symmetry 3 μm column as stationary phase (50 × 2.1 mm); mobile phase A: 0.1% formic acid in water; B: 0.1% formic acid in acetonitrile; gradient:

start 100% A; 1 min 100% A; 5 min 10% A; 6 min 10% A; 7 min 100% B. The FT-ICR instrument (HR-FT-ICR-MS) was an APEX II mass spectrometer (Bruker Daltonics, Billerica, MA, USA) equipped with a 160-mm room-temperature 7 Tesla actively shielded magnet, electrostatic ion transfer optics, octupole ion storage device and external off-axis electrospray ion source. HPLC-FT-ICR-MS was performed with an HP 1100 HPLC system synchronized to the FT-ICR spectrometer by contact closure. The whole effluent (250 μL/min) was directed to the external electrospray source. A heated nitrogen flow of 8 L/min with a temperature of 250 °C was used as both nebulizing and drying gas. TOF-HR-ESI-MS spectra were obtained with a Micromass-LCT mass spectrometer (capillary 3.2 kV, cone 42 V, source: 120 °C). Samples were injected with a syringe pump (Harvard Apparatus). Leucine-enkephaline was used as standard. NMR spectra were recorded with a Bruker Advance 400, a Bruker DRX 500 instrument with a TXI CryoProbe, or a Bruker DRX 700 spectrometer. ^{1}H and ^{13}C chemical shifts are given with respect to TMS or the solvent as internal standard ([D$_6$]DMSO: δ_H = 2.49, δ_C = 39.5 ppm; $CDCl_3$: δ_H = 7.25, δ_C = 77.0 ppm).

3β-(Benzylthio)-2aβ,3-dihydrogaliellalactone (7a): A solution of phenylmethanethiol (6 μL, 0.05 mmol) in EtOH (0.1 mL) was added slowly to a solution of galiellalactone (5 mg, 0.03 mmol) in ethanol (1 mL). The reaction mixture was stirred at room temp. for 12 h and concentrated in vacuo. Purification of the crude product was carried out by flash chromatography (SiO_2; EtOAc/cyclohexane, 1:3) yielding **7a** as a colorless solid (9.5 mg, quant.). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.74 (m, 1 H, 5-H), 1.02 (d, J = 6.8 Hz, 3 H, CH_3), 1.48 (m, 1 H, 6-H), 1.60 (m, 1 H, 4-H), 1.74 (ddd, J = 14.3, 6.1, 4.1 Hz, 1 H, 5-H), 1.99 (m, 1 H, 7-H), 2.06 (m, J = 8.5, 4.8 Hz, 1 H, 7-H), 2.09 (m, 1 H, 5a-H), 2.21 (m, 1 H, 6-H), 2.37 (s, 1 H, OH), 3.03 (dd, J = 7.2, 3.0 Hz, 1 H, 3-H), 3.16 (s, 1 H, 2a-H), 3.81 (s, 2 H, CH_2S), 4.54 (d, J = 4.5 Hz, 1 H, 7a-H), 3.25 (m, 1 H, ArH), 7.32 (m, 4 H, ArH) ppm. ^{13}C NMR (176 MHz, $CDCl_3$, TMS): δ = 21.43 (CH_3), 29.49 (C-7), 31.33 (C-6), 34.72 (C-4), 35.43 (C-5), 36.54 (SCH_2), 44.78 (C-3), 46.77 (C-5a), 51.72 (C-2a), 85.07 (C-7b), 88.89 (C-7a), 127.29 (C-4′), 128.68, 128.93 (2 C each, C-2′, C-3′, C-5′, C-6′), 137.56 (C-1′), 174.87 (C-2) ppm. HPLC-ESI-MS: m/z (%) = 319 (100) [M + H]$^+$. HR FT-ICR-MS: m/z = 319.13620 [M + H]$^+$ (calcd. for $C_{18}H_{23}O_3S$: 319.13624), 341.11812 [M + Na]$^+$ (calcd. for $C_{18}H_{22}O_3SNa$: 341.11819).

3β-Azido-2aβ,3-dihydrogaliellalactone (7b): Sodium azide (200 mg, 3.1 mmol) was added to a stirred solution of galiellalactone (**4**; 50.0 mg, 0.26 mmol) in acetic acid (1.5 mL). When HPLC monitoring indicated total conversion of the starting material (ca. 1 h), the reaction mixture was diluted with *tert*-butyl methyl ether (40 mL), then washed with saturated aqueous $NaHCO_3$ (2 × 10 mL), as well as brine (10 mL), dried (Na_2SO_4), filtered through a silica cartridge and concentrated under reduced pressure. The title compound was obtained as a colorless liquid (61 mg, quant.). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.70 (m, 1 H, 5-H), 1.08 (d, J = 6.6 Hz, 3 H, CH_3), 1.34 (m, 1 H), 1.52 (br. s, 1 H, OH), 1.70–1.86 (m, 2 H), 1.87–2.05 (m, 3 H), 2.07–2.18 (m, 1 H), 3.03 (d, J = 1.1 Hz, 1 H, 2a-H), 3.65 (dd, J = 8.3, 2.1 Hz, 1 H, 3-H), 4.54 (d, J = 3.0 Hz, 1 H, 7a-H) ppm. HPLC-(neg.)ESI-MS: m/z (%) = 236 (100) [M − H]$^-$. Due to low stability, as a result of HN_3 elimination, azide **7b** was immediately hydrogenated (**7b** → **7c**) or converted into the (*tert*-butoxycarbonyl)amine (**7b** → **7d**).

3β-[(*tert*-Butoxycarbonyl)amino]-2aβ,3-dihydrogaliellalactone (7d): A solution of freshly prepared azide **7b** (10.0 mg, 0.04 mmol) and Boc_2O (18.4 mg, 0.08 mmol) in EtOAc (1.5 mL) was stirred with 10% Pd/C (2 mg) under hydrogen at normal pressure for about 3 h. Filtration through Celite, followed by evaporation of the solvent under reduced pressure yielded the crude product, which was further purified by flash chromatography (SiO_2; EtOAc/cyclohexane, 1:2). The title compound was obtained as a colorless solid (11.6 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.49 (m, 1 H, 5-H), 0.94 (d, J = 6.6 Hz, 3 H, CH_3), 1.25 (m, 1 H), 1.34 (s, 9 H, *t*Bu), 1.47 (br. s, 1 H, OH), 1.77 (m, 1 H), 1.85–2.00 (m, 2 H), 2.00–2.24 (m, 3 H), 3.04 (br. "s", 1 H, 2a-H), 3.15 (m, 1 H, 3-H), 4.54 (d, J = 3.0 Hz, 1 H, 7a-H), 5.44 (br. d, J = 6.7 Hz, 1 H, NH) ppm. HPLC-ESI-MS: m/z (%) = 334 (100) [M + Na]$^+$, 312 (20) [M + H]$^+$. FT-ICR-MS: m/z = 334.16255 [M + Na]$^+$ (calcd. for $C_{16}H_{25}NNaO_5$: 334.16249).

3β-Amino-2aβ,3-dihydrogaliellalactone (7c). Method A: A solution of freshly prepared azide **7b** (10.4 mg, 0.04 mmol) in EtOAc (1.5 mL) was stirred with 10% Pd/C (2 mg) under hydrogen at normal pressure for 3 h. After filtration through Celite, and evaporation of the solvent under reduced pressure, the title compound was obtained as the free base as a colorless oil (9.2 mg, quant., > 95% pure by HPLC). **Method B:** TFA (0.4 mL) was added dropwise to solution of **7d** (21 mg, 0.07 mmol) and the mixture stirred until HPLC monitoring indicated total conversion (ca. 3 h). After evaporation of the solvent, supported by sequential trituration with heptane (twice) and methanol, the crude product was purified by gel chromatography (Sephadex LH-20; MeOH/TFA, 1:0.001). After evaporation of the solvent, the title compound was obtained as the trifluoroacetate as a colorless solid (16 mg, 73%). ^{1}H NMR (400 MHz, CD_3OH): δ = 0.73 (m, 1 H, 5-H), 1.14 (d, J = 6.6 Hz, 3 H, CH_3), 1.31 (m, 1 H), 1.88 (m, 1 H, 4-H), 1.95–2.04 (m, 3 H), 2.20 (m, 2 H), 3.13 (d, J = 1.6 Hz, 1 H, 2a-H), 3.37 (dd, J = 9.5, 2.5 Hz, 1 H, 3-H), 4.61 (br. t, 1 H, 7a-H) ppm. HPLC-ESI-MS: m/z (%) = 212 (100) [M + H]$^+$. HR-FT-ICR-MS: m/z = 212.12803 [M + H]$^+$ (calcd. for $C_{11}H_{18}NO_3$ 212.12812).

2aβ,3-Dihydrogaliellalactone (6a): A solution of galiellalactone (**4**; 100 mg, 0.51 mmol) in methanol (2.0 mL) was stirred with 10% Pd/C (200 mg) under hydrogen at normal pressure for 3 h. After filtration through Celite and evaporation of the solvent under reduced pressure, the title compound was obtained as a colorless solid (95 mg, 94%, > 95% pure by HPLC). ^{1}H NMR (700 MHz, $CDCl_3$): δ = 0.67 (m, 1 H, 5-H), 0.92 (d, J = 6.7 Hz, 3 H, CH_3), 1.37 (m, 1 H, 6-H), 1.52 (ddd, J = 14.0, 10.5, 3.5 Hz, 1 H, 3-H), 1.66 (m, 1 H, 4-H), 1.79 (m, 1 H, 5-H), 1.89 (m, 1 H, 7-H), 1.92–1.97 (m, 2 H, 7-H, 5a-H), 2.02–2.09 (m, 2 H, 3-H, 6-H), 2.84 (dd, J = 10.0, 3.8 Hz, 1 H, 2a-H), 4.49 (d, J = 4.1 Hz, 1 H, 7a-H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$, TMS): δ = 22.12 (CH_3), 26.64 (C4), 28.29 (C-3), 29.77 (C-7), 32.05 (C-6), 36.00 (C-5), 45.29 (C-2a), 47.46 (C-5a), 84.24 (C-7b), 89.57 (C-7a), 177.68 (C-2) ppm. IR (KBr): $\tilde{\nu}$ = 3431 (m, br.), 2951 (m), 2927 (m), 1744 (s), 1459 (m), 1184 (s), 1088 (s), 981 (s). HR-ESI-MS: m/z = 238.1432 [M + CH_3CN + H]$^+$ (calcd. for $C_{13}H_{20}NO_3$: 238.1443). HR-FT-ICR-MS: m/z = 415.20886 [2 M + Na]$^+$ (calcd. for $C_{22}H_{32}NaO_6$: 415.20911).

2aβ,3-Dihydrogaliellalacton-7b-yl Acetate (6b): A solution of acetic anhydride (10.4 mg, 0.1 mmol) in dichloromethane (200 μL) was added at 0 °C to a solution of dihydrogaliellalactone (**6a**; 10 mg, 0.05 mmol), triethylamine (16 μL, 0.1 mmol), and DMAP (1.3 mg, 10 μmol) in dichloromethane (1 mL) and stirred at room temperature for 12 h. The reaction mixture was diluted with EtOAc (50 mL) and washed with 2 N aqueous citric acid (2 × 10 mL), saturated aqueous $NaHCO_3$ (2 × 10 mL), and brine (10 mL). It was then dried (Na_2SO_4), filtered and concentrated under reduced pressure. Flash chromatography (SiO_2; EtOAc/cyclohexane, 1:2) of the crude product afforded **6b** (4.5 mg, 35%) as a colorless solid.

^{1}H NMR (700 MHz, $CDCl_3$): δ = 0.81 (m, 1 H, 5-H), 0.96 (d, J = 6.7 Hz, 3 H, CH_3), 1.34 (m, 1 H, 3-H), 1.45 (m, 1 H, 6-H), 1.77 (m, 1 H, 4-H), 1.88 (m, 2 H, 7-H, 5-H), 2.01 (m, 1 H, 6-H), 2.03 (s, 3 H, CH_3CO), 2.18 (m, 2 H, 7-H, 3-H), 2.49 (m, 1 H, 5a-H), 3.19 ("t", J = 8.6 Hz, 1 H, 2a-H), 5.02 (dd, J = 5.9, 2.8 Hz, 1 H, 7a-H) ppm. ^{13}C NMR (176 MHz, $CDCl_3$, TMS): δ = 21.86 (CH_3CO), 22.13 (CH_3), 26.09 (C-4), 30.36 (C-3), 30.67 (C-7), 31.99 (C-6), 34.57 (C-5), 42.57 (C-5a), 43.76 (C-2a), 87.48 (C-7a), 90.68 (C-7b), 170.21 (CH_3CO), 177.44 (C-2) ppm. HPLC-ESI-MS: m/z (%) = 239 (100) $[M + H]^+$.

2aβ,3β-Epoxy-2aβ,3-dihydrogaliellalactone (8): Solid vanadyl acetylacetonate (4.1 mg, 0.02 mmol) was added at 0 °C to a solution of galiellalactone (**4**; 30.0 mg, 0.15 mmol) in dry CH_2Cl_2 (1.0 mL), followed 5 min later by a 3 M solution of dry *tert*-butyl hydroperoxide (139.2 mg, 1.54 mmol) in dry toluene (0.515 mL), which was added dropwise with vigorous stirring. Subsequently, the reaction mixture was allowed to warm up to room temperature under argon. When total consumption of the starting material was observed (ca. 16 h, HPLC monitoring), a stock aqueous solution of Na_2SO_3 (2 mL) was added and the mixture stirred for a further 30 min. Afterwards, the reaction mixture was diluted with EtOAc (50 mL) and washed with water (3 × 10 mL) and brine (10 mL). It was then dried (Na_2SO_4), filtered and concentrated under reduced pressure. Flash chromatography (SiO_2; EtOAc/cyclohexane, 1:1) of the crude product afforded **8** (26.1 mg, 80%) as a colorless solid. ^{1}H NMR (700 MHz, $CDCl_3$): δ = 1.15 (d, J = 7.8 Hz, 3 H, CH_3), 1.28 (dt, J = 14.9, 4.2 Hz, 1 H, 5-H), 1.45 (m, 1 H, 6-H), 1.72 (ddd, J = 14.9, 6.9, 4.8 Hz, 1 H, 5-H), 1.77 (m, 1 H, 6-H), 1.83 (m, 1 H, 7-H), 1.98-2.05 (m, 2 H, 5a-H, 7-H), 2.09 (m, 1 H, 4-H), 2.55 (br. s, 1 H, OH), 3.47 (s, 1 H, 3-H), 4.92 (d, J = 7.2 Hz, 1 H, 7a-H) ppm. ^{13}C NMR (176 MHz, $CDCl_3$): δ = 20.40 (CH_3), 25.87, 26.10, 28.34, 30.68, 42.13, 57.48, 64.73, 77.28 (C-7b), 89.84 (C-7a), 172.98 (C-2) ppm. HR-ESI-MS: m/z = 443.1696 $[2 M + Na]^+$ (calcd. for $C_{22}H_{28}O_8Na$: 443.1682).

2aβ-Methyl-3,4-didehydro-2a,3-dihydrogaliellalactone (5): A 1 M solution of LiHMDS in THF (645 μL) was added dropwise at −78 °C to a solution of galiellalactone (**4**; 50 mg, 0.26 mmol) in dry THF (1 mL). After 20 min, iodomethane (160 μL, 2.57 mmol) was added with vigorous stirring. The reaction mixture was allowed to warm up to room temperature over about 16 h, then quenched at 0 °C with methanol (1 mL) and saturated aqueous $NaHCO_3$ (1 mL), stirred for another 5 min and diluted with EtOAc (50 mL). The organic phase was then washed with saturated aqueous $NaHCO_3$ (10 mL), 5% aqueous citric acid (10 mL), and brine (10 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. Flash chromatography (SiO_2; EtOAc/cyclohexane 1:1) of the crude product and subsequent purification by preparative HPLC (RP18; acetonitrile/water/0.1% TFA) afforded the title compound (20.2 mg, 38%) as a colorless solid. ^{1}H (400 MHz, $CDCl_3$): δ = 1.33 (s, 3 H, CH_3), 1.51 (m, 1 H), 1.73−1.80 (m, 1 H), 1.76 (s, 3 H, CH_3), 1.86 (d, J = 17.3 Hz, 1 H), 1.93−2.05 (m, 2 H), 2.18 (s, 1 H, OH), 2.22 (m, 1 H), 2.34 (dd, J = 17.4, 6.5 Hz, 1 H), 4.70 (d, J = 4.6 Hz, 1 H, 7a-H), 5.44 (s, 1 H, 3-H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 21.64, 23.67, 28.98, 29.33, 31.13, 46.51, 47.22, 83.63, 89.87, 121.48, 133.18, 179.88 ppm. HPLC-ESI-MS: m/z (%) = 209 (100) $[M + H]^+$. HR-ESI-MS: m/z = 439.2090 $[2 M + Na]^+$ (calcd. for $C_{24}H_{32}O_6Na$: 439.2097).

Galiellalacton-7b-yl Acetate (9a): Acetyl chloride (1.5 mL, 35.2 mmol) was added dropwise to a solution of **4** (200 mg, 1.03 mmol) in dry acetonitrile (5 mL) at room temperature (water bath). The reaction mixture was stirred for an additional 2 h and the solvent was then removed under reduced pressure. Careful drying in vacuo yielded **9a** as a colorless solid (236 mg, 97%, > 95% pure by HPLC): ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 0.99 (m, 1 H, 5-H), 1.18 (m, 1 H, 6-H), 1.21 (d, J = 7.9 Hz, 3 H, CH_3), 1.88−1.97 (m, 2 H, 7-H, 6-H), 2.05 (s, 3 H, $COCH_3$), 2.10 (m, 1 H, 7-H), 2.25 (m, 1 H, 5-H), 2.55 (m, 1 H, 4-H), 2.75 (m, 1 H, 5a-H), 5.08 (d, J = 6.9 Hz, 1 H, 7a-H), 6.97 (d, J = 3.3 Hz, 1 H, 3-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, TMS): δ = 20.36 (CH_3), 21.08 (CH_3CO), 28.97 (C-4), 31.34 (C-6), 31.84 (C-7), 32.87 (C-5), 41.68 (C-5a), 85.61 (C-7a), 87.13 (C-7b), 129.10 (C-2a), 148.24 (C-11), 169.00 (C-2), 170.45 (CH_3CO) ppm. HPLC-ESI-MS: m/z (%) = 177 (100) $[M - AcOH + H]^+$ = [**10** + H]$^+$, 237 (40) $[M + H]^+$. HR-FT-ICR MS: m/z = 237.11224 $[M + H]^+$ (calcd. for $C_{13}H_{17}O_4$ 237.11214).

General Procedure for Diels−Alder Reactions with Diene Precursors 9: DMAP (2 mg, 0.01−0.1 mmol) was added to a solution of **9** (0.1 mmol) and the dienophile (0.2−1.0 mmol) in dry acetonitrile or dichloromethane (2−10 mL). The reaction mixture was stirred at room temperature until HPLC showed no more starting material (reaction time: several hours at 1 equiv. DMAP to several days at 0.1 equiv. DMAP). The reaction mixture was concentrated in vacuo followed by dilution with EtOAc (20 mL). The organic layer was washed with 5% aqueous citric acid (1 mL) and saturated aqueous $NaHCO_3$ (4 × 100 mL), dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification of the crude product was carried out by flash chromatography (SiO_2; EtOAc/cyclohexane, 1:2) or preparative HPLC (RP18; acetonitrile/water/0.1% TFA).

Bis(dehydratogaliellalactone) (11): This compound was prepared according to the General Procedure, from either **9a** (25 mg, 0.1 mmol), **9b** or **9c** and DMAP (0.1 mmol). It was obtained as a colorless solid after preparative HPLC purification (19.5 mg, quant.). $[\alpha]_D^{20}$ = +108 (c = 0.075, $CHCl_3$). NMR: see Table 2 (Supporting Information). HR-FT-ICR-MS: m/z = 353.17479 $[M + H]^+$ (calcd. for $C_{22}H_{25}O_4$: 353.17474), 727.32414 $[2 M + Na]^+$ (calcd. for $C_{44}H_{48}O_8Na$: 727.32414). X-ray: see Table 1. Compound **11** was also obtained in a one-pot procedure starting from **4**. After acylation with acetyl chloride (1.1 equiv.) DMAP (1 equiv.) was added to the reaction mixture.

Aza Derivative 13a: This compound was prepared according to the General Procedure from **9a** or **9b** (0.1 mmol) and nitrosobenzene (**12**; 1.0 mmol). Yield: 18.5 mg (66%). HPLC-UV (CH_3CN/H_2O + 0.1% TFA, 6:4) λ_{max} = 219 (s), 239 (sh), 332 (m) nm. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.04 (m, J = 12.9, 6.4 Hz, 1 H, 6-H), 1.33 (dd, J = 12.9, 5.3 Hz, 1 H, 5-H), 1.69 (s, 3 H, CH_3), 1.92 (d"t", J = 12.8, 6.4 Hz, 1 H, 6-H), 2.09 (dd, J = 14.8, 6.4 Hz, 1 H, 7-H), 2.18 (m, 1 H, 7-H), 2.32 (dd, J = 12.9, 8.9 Hz, 1 H, 5-H), 2.64 (m, 1 H, 5a-H), 5.24 (d, J = 6.8 Hz, 1 H, 7a-H), 6.94 (m, 2 H, 2′-H, 6′-H), 7.07 (m, 1 H, 4′-H), 7.21 (m, 2 H, 3′-H, 5′-H), 7.33 (s, 1 H, 3-H) ppm. ^{13}C NMR (176 MHz, $CDCl_3$): δ = 21.24 (CH_3), 29.71 (C-6), 34.36 (C-7), 39.00 (C-5), 40.97 (C-5a), 75.99 (C-7b), 77.09 (C-4), 82.45 (C-7a), 121.02 (2 C, C-2′, C-6′), 125.63 (C-4′), 128.50 (2 C, C-3′, C-5′), 130.32 (C-2a), 140.00 (C-3), 148.00 (C-1′), 165.57 (C-2) ppm. HPLC-ESI-MS: m/z (%) = 284 (100) $[M + H]^+$. HR-ESI-MS: m/z = 284.1257 $[M + H]^+$ (calcd. for $C_{17}H_{18}NO_3$: 284.1287). X-ray: see Table 1.

Bromo Aza Derivative 13b: Solid NBS (3.8 mg, 21 μmol) was added to a solution of **13a** (5 mg, 18 μmol) in dichloromethane (500 μL). The reaction mixture was stirred at room temperature overnight, concentrated under reduced pressure, diluted with acetonitrile (2 mL) and purified by preparative HPLC (RP18; acetonitrile/water/0.1% TFA). The title compound was obtained as a colorless

Table 1. Crystallographic data as well as details of the structure solution and refinement procedures for **4**, **11**, **13a** and **13b**

	4	**11**	**13a**	**13b**
Empirical formula	$C_{11}H_{14}O_3$	$C_{22}H_{24}O_4$	$C_{17}H_{17}NO_3$	$C_{17}H_{16}Br_1NO_3$
Formula mass [g mol^{-1}]	194.22	352.41	283.32	362.22
T [K]	90	90	153	153
λ [Å]	1.54178	1.54178	0.71073	0.71073
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a [Å]	7.2434(2)	7.46630(10)	8.9290(2)	9.4343(5)
b [Å])	9.2195(2)	12.5508(2)	9.0771(2)	9.4343(5)
c [Å]	14.2172(3)	18.1910(2)	17.1453(4)	9.4343(5)
V [Å^3]	949.43(4)	1704.64(4)	1389.62(5)	1509.03(15)
Z	4	4	4	4
$d_{calcd.}$ [Mg·m^{-3}]	1.359	1.373	1.354	1.594
μ [mm^{-1}]	0.805	0.753	0.093	2.736
F(000)	416	752	600	736
Crystal size [mm]	0.5 × 0.5 × 0.5	0.60 × 0.10 × 0.04	0.40 × 0.40 × 0.03	1.50 × 0.30 × 0.02
θ range [°]	5.72–71.00	4.28–72.40	2.38–30.53	2.42–28.50
Reflections collected	9554	17997	16139	17948
Independent reflections [R(int)]	1764 [0.0343]	3251 [0.0482]	4159 [0.0463]	3822 [0.0960]
Observed reflections [$F_o > 4\sigma(F_o)$]	1762	2977	3631	2587
Data/restraints/parameters	1764/0/184	3251/1/331	4159/1/258	3822/1/216
Absolute structure parameter[29]	0.01(19)	−0.04(13)	−	−0.009(14)
Basf [with Twin (σ)]	0.01(19)	0.00(14)	−	0.000(14)
Extinction coefficient	0.055(3)	−	−	−
Goodness-of-fit on F^2	1.101	1.067	1.009	0.990
Final $R1$, $wR2$ [$I > 2\sigma(I)$][a,b]	0.0345, 0.0844	0.0345, 0.0881	0.0359, 0.0881	0.0492, 0.0957
Final $R1$, $wR2$ (all data)[a,b]	0.0345, 0.0844	0.0348, 0.0885	0.0439, 0.0934	0.0909, 0.1097
Max./min. diff. [e·Å^{-3}]	0.208/−0.326	0.195/−0.324	0.289/−0.206	0.650/−0.808
a/b[b]	0.582/0.1529	0.551/0.2430	0.551/0	0.609/0
Absorption correction max./min.	1.000000/0.852724	1.000000/0.854653	1.000000/0.557525	1.000000/0.364383

[a] $R_1 = \Sigma(F_o - F_c)/\Sigma F_o$. [b] $wR_2 = [\Sigma\{w(F_o^2 - F_c^2)^2\}/\Sigma\{w(F_o^2)^2\}]^{1/2}$, $w = 1/[\sigma^2 F_o^2 + (aP)^2 + bP]$, $P = (F_o^2 + 2F_c^2)/3$.

solid (5.4 mg, 85%). HPLC-UV (CH_3CN/H_2O + 0.1% TFA, 7:3) λ_{max} = 217 (s), 252 (s), 333 (m) nm. HPLC-ESI-MS: m/z (%) = 362 (100) [M (^{79}Br) + H]$^+$. HR-ESI-MS: m/z = 362.0365 [M (^{79}Br) + H]$^+$ (calcd. for $C_{17}H_{17}NO_3Br$: 362.0392). X-ray: see Table 1.

4β-Hydroxy-7bβ-(phenylamino)-2aβ,3-dihydrogaliellalactone (14): A solution of **13a** (20 mg, 71 μmol) in methanol (2.0 mL) was stirred with 10% Pd/C (10 mg) under hydrogen at normal pressure for 3 h. After filtration, evaporation of the solvent under reduced pressure and further purification by preparative HPLC (RP18; acetonitrile/water/0.1% TFA), the title compound was obtained as a colorless solid (14 mg, 69%). ^{1}H NMR (700 MHz, $CDCl_3$): δ = 1.24 (dd, J = 14.4, 9.2 Hz, 1 H), 1.31 (s, 3 H, CH_3), 1.46 (m, 1 H), 1.92–1.99 (m, 2 H), 2.01–2.13 (m, 4 H), 2.27 (m, 1 H), 3.39 (dd, J = 9.6, 4.2 Hz, 1 H, 2a-H), 5.10 (d, J = 4.1 Hz, 1 H, 7a-H), 6.75 (d, J = 7.6 Hz, 2 H, ArH), 6.92 ("t", J = 6.9 Hz, 1 H, ArH), 7.23 ("t", J = 8.4 Hz, 2 H) ppm. ^{13}C NMR (176 MHz, $CDCl_3$): δ = 30.40, 30.86, 31.20, 34.80, 37.97, 40.35, 44.38, 68.37, 69.38, 85.50, 119.45 (2 C), 121.37, 129.55 (2 C), 144.36, 178.08 ppm. HR-FT-ICR-MS: m/z = 288.15938 [M + H]$^+$ (calcd. for $C_{17}H_{22}NO_3$: 288.15942).

Assay

Plasmids and Transfections: Three copies of the SIF-RE (serum-inducible factor-response element) from the published c-fos promoter were cloned into a pcDNA1-CMV-promoter luciferase reporter vector (Promega). The identity of the promoter insert was confirmed by sequencing. Transfection of the SIF-RE-luciferase construct in combination with pRSVneo gene was performed using Lipofectamine (Life Technologies). Cells stably expressing the transgene were selected by addition of G418 (Life Technologies) to the growth medium and single IL-6 responsive clones were isolated by limiting dilution. The clone showing the best induction of luciferase activity in response to IL-6 was used for all subsequent experiments.

Reporter-Gene Assay: Cells were maintained in RPMI 1640 supplemented with 25 mM HEPES, 10% FCS, penicillin/streptomycin, and 0.5 mg/mL G418 (Life Technologies). After reaching confluency, cells were seeded in 384-well microlite F plates at a density of 1 × 10^{-4} cells/well in 25 μL of RPMI 1640 supplemented with 1 mg/mL BSA and cultured for 24 h. Stock solutions of test compounds dissolved in DMSO were diluted in RPMI 1640, and 1 mg/mL BSA (final DMSO concentration in the assay 0.1% DMSO) and 5 μL of this test-compound solution was added to each well; 16 h after test-compound addition, the cells were stimulated with 1 ng/mL (final concentration) of human recombinant IL-6 (Sigma) diluted in RPMI 1640 and 1 mg/mL BSA. Following cell lysis and substrate addition, luciferase activity was determined 4 h after stimulation by means of a luminometer (Hamamatsu).

X-ray Crystallography: Measurements for **4** and **11** were carried out with a Bruker-Nonius diffractometer equipped with a Proteum CCD area detector, an FR591 rotating anode with Cu-K_α radiation, Montel mirrors as monochromator and a Kryoflex low-temperature device. Software: Data collection Proteum version 1.37 (Bruker-Nonius 2002), data reduction Saint Plus Version 6.22 (Bruker-Nonius 2002), absorption correction SADABS version 2.03 (2002) and structure solution and refinement SHELXTL version 6.12 (Sheldrick, 2000). Measurements for **13a** and **13b** were performed with a Siemens P4 diffractometer equipped with a SMART-CCD-1000 area detector, a MACScience Co. rotating anode with Mo-K_α radiation, a graphite monochromator and a Sie-

mens low-temperature device LT2. Software: Data collection Smart version 5.060 (BrukerAXS 1999), data reduction Saint+ version 6.02 (Bruker AXS 1999), absorption correction SADABS (Bruker AXS 1999) and structure solution and refinement SHELXTL version 6.12 (Sheldrick, 2000).[28] The crystallographic data as well as details of the structure solution and refinement procedures for **4**, **11**, **13a** and **13b** are reported in Table 1. CCDC-206595 (**4**), -206596 (**11**), -206598 (**13a**) and -206597 (**13b**) contain the supplementary crystallographic data for this paper. This data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: (internat.) + 44-1223-336033; E-mail: deposit@ccdc.cam.ac.uk].

Acknowledgments

We thank Prof. Heidrun Anke, Prof. Timm Anke and Dr. Gerhard Erkel (IBWF, Kaiserslautern, Germany) for the delivery of several grams of natural (−)-galiellalactone. The help of Dr. Joachim Wesener, Heinz Musche (MS), Dr. Daniel Gondol (NMR) and Dr. Oliver Gutbrod (molecular modeling) is also gratefully acknowledged. Furthermore, we are grateful to Drs. Markus Weidler, Robert Velten, and Martin Hendrix for helpful discussions, as well as to Dr. Timo Flessner for proof reading of the manuscript.

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Received March 1, 2004
Early View Article
Published Online May 26, 2004

