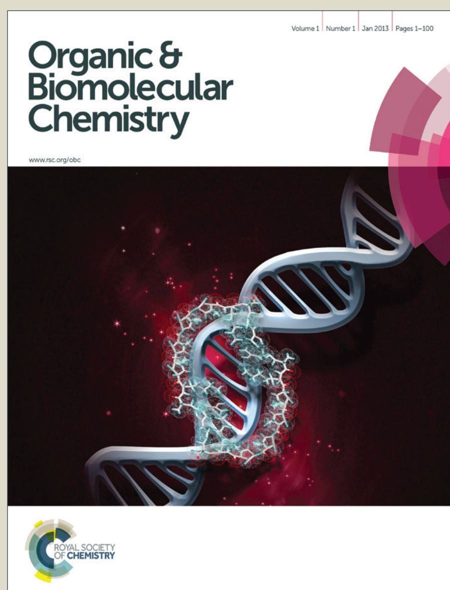
Organic & Biomolecular Chemistry

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/obc

Total Synthesis and Biological Evaluation of the Natural Product (–)-Cyclonerodiol, a New Inhibitor of IL-4 Signaling

Jens Langhanki [a†], Kristina Rudolph [b†], Gerhard Erkel [b], and Till Opatz [a*]

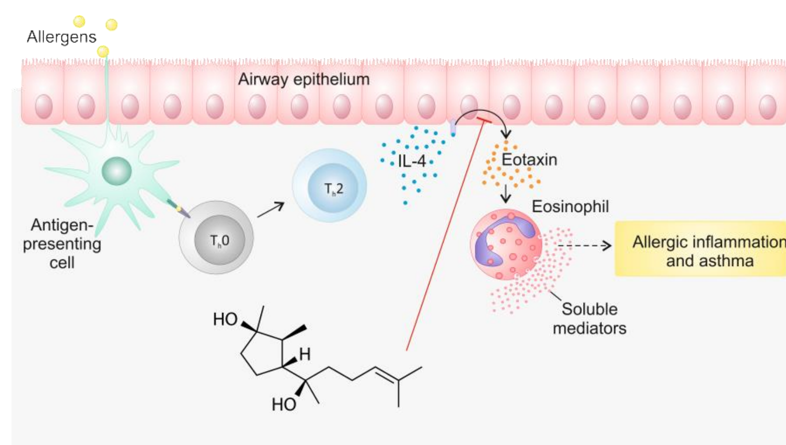
[a] Institute of Organic Chemistry, University of Mainz, Duesbergweg 10–14, D-55128 Mainz, Germany

[b] Department of Biotechnology, University of Kaiserslautern, Paul-Ehrlich-Str. 23, D-67663 Kaiserslautern, Germany

*Email: opatz@uni-mainz.de

†: authors contributed equally to this work.

Graphical Abstract



Organic & Biomolecular Chemistry Accepted Manuscript

Abstract

In a screening program of natural compounds from fungi, the known cyclopentanoid sesquiterpene (–)-cyclonerodiol was identified as a specific inhibitor of the IL-4 induced STAT6 signaling pathway (IC_{50} = 9.7 μM) which is required for the differentiation of naive CD4 T cells to T helper type 2 (Th2) lymphocytes. As many allergic conditions, including allergic asthma and atopic diseases, are driven by an excessive Th2 response, STAT6 is a promising target for the development of new therapeutics. The compound was synthesized in six steps from (–)-linalool using an epoxide radical cyclization as the key step.

Introduction

Asthma bronchiale is a chronic airway inflammation which affects about 300 million people worldwide making it one of the most common chronic diseases. Despite its widespread and increasing prevalence, only a few new drugs have been introduced during the last decades. While the gold standard of therapy, in form of inhaled corticosteroids, β_2-agonists and cholinergic antagonists, reduces symptoms and exacerbations in the majority of patients, 5–10% of asthmatics remain without effective control of their symptoms and account for ~50% of the health care costs of asthma which emphasizes the urgent need for novel effective therapies.[1],[2] The airway inflammation is orchestrated by numerous immune cells, which, upon activation, release various proinflammatory mediators and cytokines causing inflammatory cell infiltration, mucus hypersecretion, airway hyperresponsiveness (AHR), smooth muscle contraction and ultimately airway remodeling.[3]

In about 80% of asthmatic patients, chronic airway inflammation is driven by an immune response to inhaled allergens which is orchestrated by T helper type 2 (Th2) cells. The differentiation of naive T cells into Th2 cells is induced by antigen stimulation of T cell receptors in the presence of interleukin (IL)-4.[4],[5] Th2 lymphocytes express a distinct set of cytokines, such as IL-4 and IL-13, and thereby promote eosinophil inflammation, mucus production, AHR, immunoglobulin E (IgE) production and upregulation of high-affinity IgE

Organic & Biomolecular Chemistry Accepted Manuscript

receptor (FcεRI) which are all major components characteristic of an inflammatory asthma response.[6] One of the signaling cascades induced by IL-4 leads to the activation of the cytoplasmic transcription factor STAT6 which belongs to the family of signal transducers and activators of transcription (STAT). Although IL-4 also activates the IRS-1/2 signaling pathway, the majority of IL-4 responsive genes have been shown to be STAT6 dependent .[7] STAT6 is activated by binding to the phosphorylated IL-4 receptor through SH2 domains and is subsequently phosphorylated at C-terminal tyrosine residues. After phosphorylation, STAT6 disengages from the receptor and homodimerizes. Only STAT6 dimers are able to translocate to the nucleus where they bind to specific sequences in the promoter regions of IL-4 and IL-13 responsive genes.[8],[9] Elevated levels of Th2 lymphocytes, IL-4 and IL-13 have been found in the bronchoalveolar lavage (BAL) of asthmatic patients and allergic individuals and polymorphisms in the genes encoding STAT6 and the IL-4 receptor subunits have been genetically linked to asthma.[10] Therefore, disease progression in asthma should be positively affected by inhibiting the IL-4 signaling pathway as an early key regulatory point during the development of asthma.[5]

In a screening of fungal secondary metabolites, we identified cyclonerodiol (**1**), isolated from a *Fusarium* species, as an inhibitor of the IL-4 induced STAT6 signaling pathway in HepG2 and A549/8 cell lines (IC_{50} values: 10.5 μM and 9.7 μM, respectively). To date, cyclonerodiol has only been described as weak antifungal metabolite against *Cryphonectria parasitica*, while other tested fungi such as *Aspergillus flavus* and *Curvularia lunata* showed no growth inhibition.[6][10]

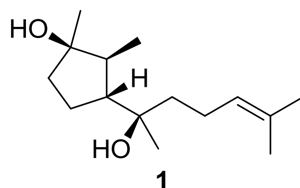


Figure 1. Structure of (–)-cyclonerodiol.

Organic & Biomolecular Chemistry Accepted Manuscript

Cyclonerodiol (**1**) belongs to the cyclopentanoid sesquiterpediols and was first described in 1970 by Nozoe et. al.[11]. It was isolated from *Trichothecium roseum*, a mould fungus that can be found worldwide on decaying crops. Over the years, cyclonerodiol was found in several other fungi, for instance *Gibberella*[12], *Fusarium*[13],[14], *Trichoderma*[15],[16],[17] *or Algicolorus* species.[18],[19] Its complete stereostructure was reported in 1990 by Laurent et al..[14]

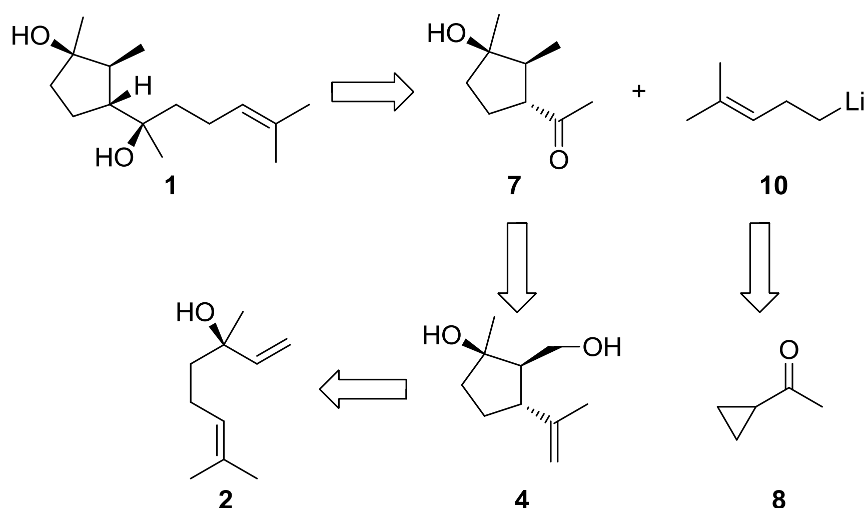
The biosynthesis was firstly published by Hanson et. al. in 1975.[13] Further investigations by Evans et. al. and Cane et. al. revealed the biosynthesis starting from mevalonate by ^{13}C isotopic labeling.[20]

In 1979, Matsuki et al. reported the first and hitherto only synthesis of cyclonerodiol which was prepared from nerolidol using a Hg^{2+}-mediated cyclohydratoization as part of an isomeric mixture.[21] As the use of mercury is problematic in conjunction with the preparation of compounds for biological testing or for human therapy, we initially attempted to substitute it for compounds of less toxic transition metals. However, experiments with [[1,2-bis(2,6-diisopropylphenyl)-4,5-dihydroimidazol-2-ylidene]chloro][3-phenylallyl] palladium(II), di-μ-chloro(η^4-1,5-cyclooctadien)ruthenium(II), chloro[1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene]gold (I) as well as iodocyclizations were unsuccessful.

Eventually, the target compound could be prepared by reaction of ketone **7** and homoprenyllithium (**10**) obtained in three steps from cyclopropyl methyl ketone (**8**) as outlined in Scheme 1. Ketone **7** could be obtained from diol **4** via Barton-Mc Combie desoxygenation and Lemieux-Johnson reaction. Compound **4** can be derived from

linalool epoxide by a reductive radical cyclisation with Cp_2TiCl according to a known proecedure. [22]

Organic & Biomolecular Chemistry Accepted Manuscript



Scheme 1. Retrosynthetic analysis of cyclonerodiol (**1**).

Results and Discussion

In order to search for compounds and to characterize their influence on IL-4 signaling, we used an IL-4 inducible transcriptional reporter in the hepatoma cell line HepG2 and the alveolar epithelial cell line A549/8, which drives luciferase expression under the control of multiple STAT6 binding elements together with an expression vector encoding the full length human STAT6 protein. Transient transfection of HepG2 cells or A549/8 cells and stimulation with 10 ng/mL IL-4 resulted in 120-140 fold activation over the basal level of luciferase expression in HepG2 cells and a ~ 20 fold activation of luciferase expression in A549/8 cells. In a screening of fungal secondary metabolites inhibiting the IL-4 inducible expression of the reporter gene, we identified **1** as selective inhibitor of STAT6 signaling. As exemplified in Fig. 1, **1** inhibited the IL-4 inducible STAT6-dependent promoter activity in a dose dependent manner with IC_{50}-values around 10 μM. The closely related IFN-γ induced Stat1/Stat2- and IL-6-induced Stat3-dependent reporter gene expression was not inhibited up to 40 μM **1**. To further test the specificity, we also determined the effect of **1** on NF-κB and TGF-β induced Smad2/3 driven luciferase expression. The NF-κB pathway has been shown to be an essential modulator of the transcription of pro-inflammatory genes and the TGF-β pathway has been implicated in the activation of pro-fibrotic gene expression in various diseases.

Organic & Biomolecular Chemistry Accepted Manuscript

Compound **1** did not affect both signaling pathways up to the highest concentrations tested (Figure 2).

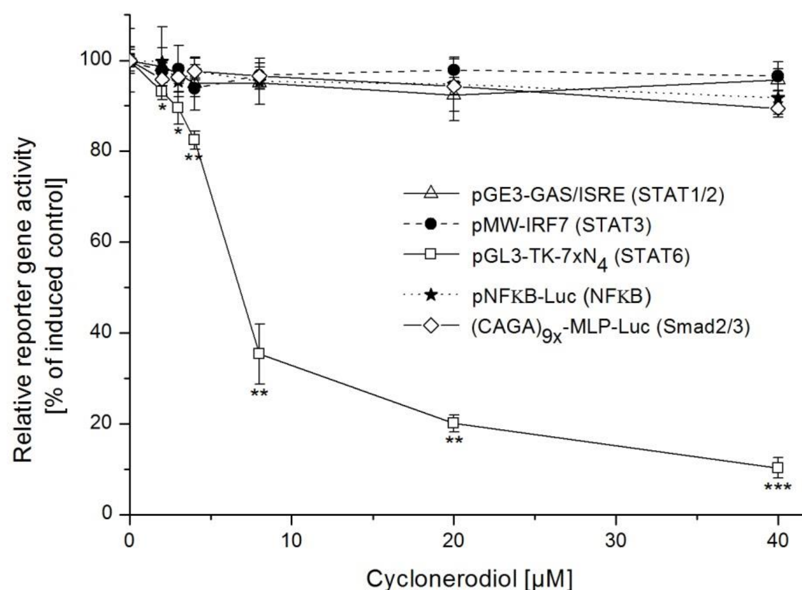


Figure 2. Effect of cyclonerodiol on the NFκB, Smad2/3 and various STAT dependent signaling pathways. A549/8 cells were transiently transfected with the indicated reporter gene construct and the constitutively active pRL-EF1α reporter gene. Cells were pretreated 1 h with or without test compound and stimulated with 5 ng/ml IL-4 (for Stat6), 10 ng/mL IFN-γ (for Stat1/2), 5 ng/mL TGF-β (for Smad2/3), 5 ng/mL TNF-α (for NF-κB) for 24 h. Control (100 %): only IL-4 stimulation. The expression of the reporter gene was determined and normalized as described in the experimental section. Results represent the mean ± SEM of at least three independent experiments (*: $p < 0.05$; **:$p < 0.01$; ***: $p < 0.001$ vs. induced, untreated cells).

To rule out cytotoxic effects, a XTT cell viability assay was performed measuring the reduction of 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT) into a colored formazan which is directly proportional to the amount of living cells. Up to the highest tested concentration of 100 µM **1**, no cytotoxic activity could be observed against the

Organic & Biomolecular Chemistry Accepted Manuscript

monolayer cell line A549/8 and the suspension cell line Jurkat during the 48 h incubation time (data not shown).

To examine the effect of **1** on the transcription of IL-4 inducible asthma relevant genes, quantitative real-time PCR experiments were performed using total RNA. Initial real-time experiments were carried out with RNA from different time points after IL-4 induction which revealed that asthma related marker genes as *ccl11*, *tnf-α* and *cxcl3* reached maximum levels in A549/8 cells after 6 h (data not shown) and therefore this induction time was chosen for further experiments. A549/8 cells stimulated with IL-4 for 6 h show a significant upregulation of mRNA levels of the cytokine *tnfa* and the chemokines *cxcl3* and *ccl11*. In accordance with the reporter gene assays, **1** significantly down-regulated the mRNA levels of the analyzed transcripts (Figure 3).

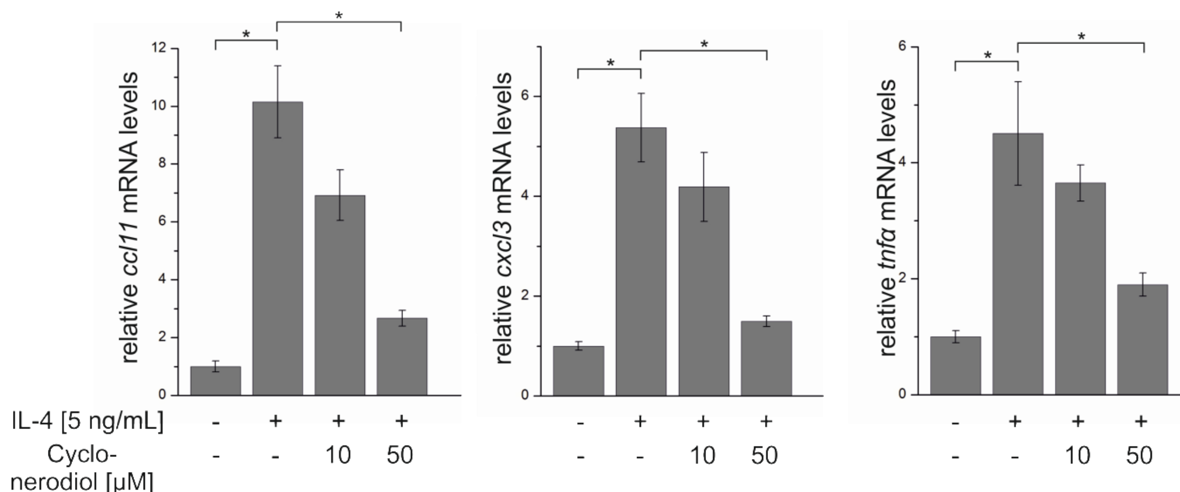


Figure 3. Effect of cyclonerodiol on mRNA levels of selected IL-4 inducible genes in A549/8 cells. Fold change (relative to uninduced control) of mRNA levels of *ccl11, cxcl3 and tnfa* in A549/8 cells induced by 5 ng/mL IL-4 for 6 h. Cells were pretreated with different cyclonerodiol concentrations for 1 h prior to IL-4 stimulation. Total RNA was reverse transcribed and cDNAs measured with a Step-One real time PCR. Values were expressed as relative mRNA content of IL-4 induced versus unstimulated cells (*: $p < 0.05$ vs. uninduced cells) and IL-4 stimulated and cyclonerodiol treated cells versus unstimulated cells, corrected for *gapdh* as reference determined in the same sample in parallel. Data are

Organic & Biomolecular Chemistry Accepted Manuscript

shown as mean values ± SEM of three independent experiments (*: $p < 0.05$ vs. induced cells).

To confirm the data obtained by real-time experiments, western blot experiments were performed for eotaxin-1 (ccl11) protein expression. Compound **1** significantly reduced IL-4 induced eotaxin-1 protein expression in A549/8 cells in a dose-dependent manner (Figure 4).

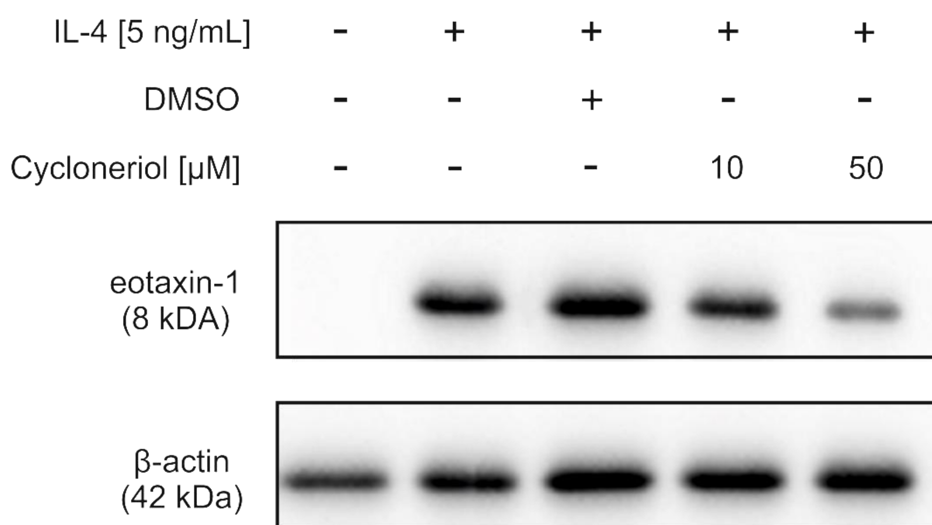
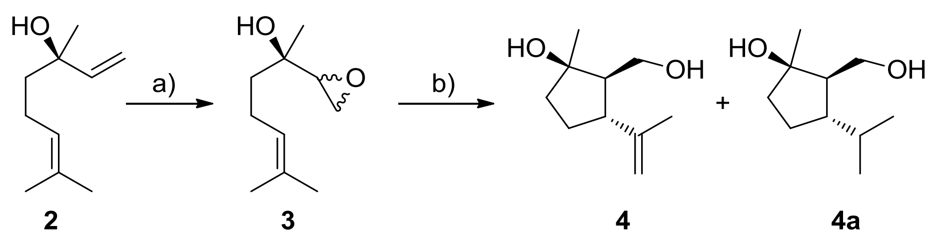


Figure 4. Effect of cyclonerodiol on eotaxin-1 (ccl11) expression. A549/8 cells were pretreated with cyclonerodiol for 1 h and stimulated with 5 ng/ml IL-4 for 24 h. Total cell lysates were analyzed by western blotting for eotaxin-1. The same blot was stripped and reprobed using a β-actin antibody as loading control. Results were confirmed by repeated experiments.

To prepare cyclic diol **4** from commercial (*R*)-linalool (**2**), the 1,2 double bond in **2** was epoxidized regioselective according to the Sharpless protocol using VO(acac)$_2$ and ^{t}BuOOH in benzene.[23] Both diastereomers **3** were obtained in a ratio of *(R,S)*:*(R,R)* = 4 : 3. A separation of the diastereomers is not required since they can be cyclized to known diol **4** in a stereoconvergent manner as demonstrated by Morales et al. in their protecting group-free synthesis of the chokols (Scheme 2).[22]

Organic & Biomolecular Chemistry Accepted Manuscript



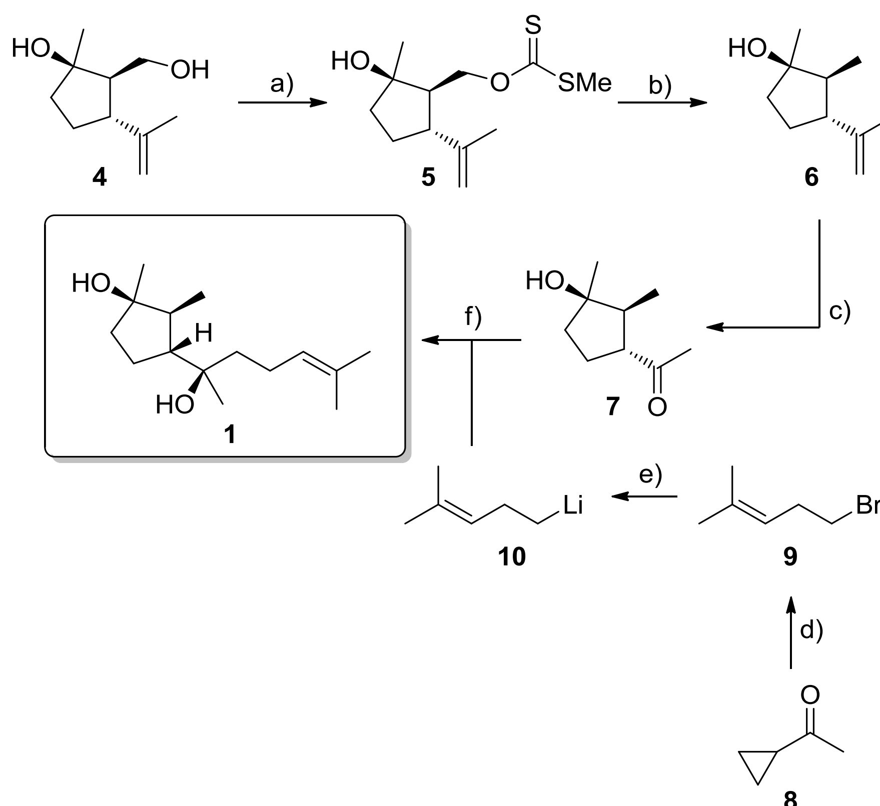
Scheme 2: Synthesis of diol **4**. Reagents and conditions: a) $VO(acac)_2$, ^tBuOOH, benzene, 80 °C, 3 h, 80%; b) Cp_2TiCl_2 (2.1 eq.), Mn (8 eq.), NEt_3 (2 eq.), THF, RT, 20 min, 71%.

The radical cyclisation of **3** is induced by SET (single electron transfer) from Cp_2TiCl prepared by in situ-reduction of titanocene dichloride by manganese powder, a procedure which was developed by Nugent and RajanBabu and was e.g. successfully used for the synthesis of furanolignans by Roy et al. or the synthesis of puupehedione by Gansäuer et al.[24],[25],[26] Morales et al. showed in theoretical studies that the cyclisation of (*R*)-linalool epoxide by this method preferably leads to the formation of (1*R*,2*R*,3*R*)-2-(hydroxymethyl)-1-methyl-3-prop-1-en-2-yl)cyclopentanol.[22] This prediction was based on DFT calculations (UM05/Ahlrichs-p VDZ) of the transition state for the 5-*exo*-trig-cyclization of the C-centered radical. This cyclization method is highly attractive for the preparation of **4** since the two newly created stereocenters have the correct configuration.

Along with the desired product **4**, the reduced byproduct 2-(hydroxymethyl)-3-isopropyl-1-methylcyclopentanol was formed in a 9:1 ratio and could not be separated by column chromatography. Pure diol **4** could instead be obtained by recrystallization in diethyl ether and *n*-hexane at the expense of the yield. The 9:1-mixture was used for subsequent transformations as the reduced side product could be conveniently removed at a later stage. Attempts to deoxygenate the primary alcohol via formation of a monoalkyldiimine with NBSH (o–nitrobenzenesulfonylchloride) and hydrazine as demonstrated by Myers et al. met with little success.[27] In contrast, the method of Barton and Mc Combie furnished the desired reduced product **6** via *S*-methyl xanthogenate **5**.[28] Next, the olefin was transformed to a

Organic & Biomolecular Chemistry Accepted Manuscript

ketone. Here, Lemieux-Johnson oxidation was superior to ozonolysis. The best results were obtained with K_2OsO_4/$NaIO_4$ in a mixture of THF/H_2O/^{t}BuOH (3:1:0.4). The last step was the nucleophilic addition of a homoprenyl unit to the ketone carbonyl. The corresponding bromide **9** is commercially available but can be conveniently prepared by acidic opening of cyclopropyl methyl ketone **8** with methylmagnesium bromide (Scheme 3). [29]



Scheme 3. Synthesis of **1**. Reagents and conditions: a) CS_2 (4 eq.), NaH (1.2 eq.), MeI (8 eq.), THF, RT, 30 min, 82%; b) $^n Bu_3SnH$ (3 eq.), AIBN (0.1 eq.), toluene, 110 °C, 15 min, 72%; c) $K_2Os_4{\cdot}H_2O$ (0.2 eq.), $NaIO_4$ (10 eq.), THF, H_2O, ^{t}BuOH (3:1:0.5), RT, 14h, 47%; d) MeMgBr (1.2 eq.),Et_2O, H_2SO_4, 1h, 85% ; e) Li (1.1 eq.), Et_2O, 50 min, –10 °C; f) **7**, THF, -78 °C -> RT, 72% of diasteromeric mixture, 33% after preparative HPLC.

Attempted halogen/lithium exchange on homoprenyl iodide only furnished the Wurtz product 2,9-dimethyldeca-2,8-diene. When the corresponding bromide was used in combination with magnesium turnings to prepare the Grignard compound, again a large amount of the

Organic & Biomolecular Chemistry Accepted Manuscript

dimerization products was observed. When homoprenyl bromide was lithiated with ^{t}BuLi, (1*R*,2*S*,3*R*)-3-(2-hydroxy-3,3-dimethylbutan-2-yl)-1,2-dimethylcyclopentanol could be isolated exclusively upon reaction of the organolithium reagent with ketone **7**, showing that halogen/lithium exchange had not taken place and that ^{t}BuLi had attacked the carbonyl carbon instead. Eventually, treatment of homoprenyl bromide with elemental lithium in diethyl ether at –10 °C and addition of ketone **7** in THF at –78 °C led to the desired product **1** in 72% yield. The diastereoselectivity of the addition steps was 10:1 and the minor diastereomer could be removed by preparative HPLC which was unfortunately associated with significant losses in yield. The major diastereomer turned out to be identical in all respects to the natural product (see supporting information for a comparison of the NMR data) and inhibited the IL-4 induced signaling pathway with the same potency. The optical rotation of synthetic **1** ($[\alpha]_D^{22}$ = – 19.6° (c = 1.00, $CHCl_3$)) was in good accordance with the reported value for natural cyclonerodiol ($[\alpha]_D^{24}$ = – 21.0° (c = 1.04, $CHCl_3$)). The relative configuration of natural **1** was deduced from NMR experiments by Laurent at al. and is in accordance with the Cram and the Felkin-Anh-model for the addition of homoprenyl lithium to ketone **7**.[14]

Conclusion

In summary, a total synthesis of (–)-cyclonerodiol was achieved in six linear steps and 5% yield from (–)-linalool. The stereoconvergent reductive radical cyclization of linalool epoxide served as the key step. Cyclonerodiol was found to be a specific inhibitor of the IL-4 induced STAT6 signaling which mediates allergic diseases such as asthma. Further work will focus on the exact molecular target.

Experimental

General Experimental Procedures.

Organic & Biomolecular Chemistry Accepted Manuscript

Fungal Material. The fungal strain E01031 was derived from the culture collection belonging to the Institute of Biotechnology and Drug Research (IBWF e.V.), Kaiserslautern, Germany. By microscopic examination of the fungal morphology, it was identified as a member of the genus *Fusarium*. The fungus was grown for long term maintenance on agar slants consisting of malt extract medium (4% malt extract, 2% agar).

Fermentation, Extraction and Purification. Fermentation was performed in a Biolafitte C-6 fermenter with 20 L of malt extract medium at room temperature with agitation (120 rpm) and aeration (3 L of air/min). Samples of the culture fluid were taken on a daily basis to determine the inhibitory effect of the crude extract in a STAT6 dependent reporter gene assay as described below. Fermentation was stopped after 9 days, when the glucose in the medium was depleted and the pH had risen over a value of 4.5. At this point, the inhibitory effect on the STAT6 dependent reporter gene assay had reached a maximum. After separating the culture fluid from the mycelium by filtration, the fluid was extracted twice with an equal volume of ethyl acetate. The solvent was dried over Na_2SO_4 and evaporated *in vacuo*. Cyclonerodiol was isolated by bioactivity-guided fractionation of the crude extract (0.9 g) which was separated by chromatography on silica gel (Merck 60). By elution with cyclohexane/EtOAc (70:30), 60.6 mg of an enriched fraction were obtained and further purification by preparative HPLC (Macherey–Nagel, Düren, Germany; Nucleosil RP18; column 21 × 250 mm, flow 20 mL/min) with MeCN/H_2O (55:45) as eluent yielded 3.6 mg cyclonerodiol (t_R: 12.6 min). The purity of the isolated compound as estimated by HPLC-DAD/MS analysis was greater than 98%.

All following experiments were performed using the compound obtained by isolation as well as the compound obtained by chemical synthesis.

Cell Culture and Cytotoxicity Assay. The human alveolar type II epithelial cell line A549/8 (DSMZ ACC107) and the hepatocellular cell line HepG2 were maintained in DMEM medium supplemented with 5% (A549/8) or 10% (HepG2) fetal calf serum (FCS), 65 µg/ml penicillin and 100 µg/mL streptomycin sulfate at 37°C and 5% CO_2. Jurkat cells were kept in RPMI-

Organic & Biomolecular Chemistry Accepted Manuscript

1640 medium with 10% FCS, penicillin (65 µg/mL) and streptomycin (100 µg/mL). To evaluate the effect of cyclonerodiol on cellular proliferation and viability, an XTT based cell viability assay was performed after 24 and 48 h as previously described using the monolayer cell line A549/8 and the suspension cell line Jurkat.[30]

Reporter Gene Assays. Screening of fungal extracts was performed using the hepatocellular cell line HepG2, while the biological characterization of cyclonerodiol was conducted using the airway epithelial cell line A549/8. The STAT6 driven reporter plasmid pGL3-TK-7xN_4 contains the herpes simplex virus thymidine kinase promoter under the control of 7 copies of the palindromic sequence TTC$(N)_4$GAA.[31] The STAT6 expression plasmid (TOPO-Stat6), the Stat3 reporter gene (pMV-IRF7) and the Smad2/3 driven reporter gene ($(CAGA)_{9x}$-MLP-Luc) have been previously described.[30],[31],[32] The NFκB driven reporter plasmid pNFκB-Luc and the Stat1/2 driven reporter plasmid (pGE3-GAS/ISRE) were obtained from Clontech (Saint-Germain-en-Laye, France). The control reporter vector pRL-EF1α for data normalization was purchased from Promega (Dual-Luciferase-Reporter-Assay). Luciferase-based reporter gene expression was thereby normalized for transfection variability and cytotoxicity against renilla expression of the constitutively active vector control (pRL-EF1α) assayed in the same sample.

Quantitative Real-Time PCR Analysis. A549/8 cells were seeded in 6 well plates at a cell density of 1 x 10^4 cells/mL and allowed to grow for 24h. After starving the cells for 24 h in DMEM with 0.5% FCS, cells were treated for 1 h in the absence or presence of cyclonerodiol and induced with 5 ng/mL IL-4 for additional 6 h. Unstimulated and untreated samples served as controls. For RNA isolation, cells were lysed in RNeasy lysis buffer (Qiagen, Hilden, Germany) and using the Qiagen RNeasy mini kit, total cellular RNA was prepared. The RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas, St. Leon-Roth, Germany) was used to generate first strand complementary DNA (cDNA) from total cellular RNA. To quantify gene expression, 50 ng cDNA were used in a qRT-PCR using the SYBR Green Mastermix with the following gene-specific primers: *CCL-11* (Genbank Accesion

Organic & Biomolecular Chemistry Accepted Manuscript

NM_002986) 5’-TACCCCTTCAGCGACTAGAGAG-3’ and 5’-GAGTTGGAGATTTTTGGTCCAG-3', *CXCL-3* (Genbank Accesion NM_002090) 5’-TGGTCACTGAACTGCGCT-3’ and 5’-ATGCGGGGTTGAGACAAG -3' (PCR product size: 159 bp), glyceraldehyde-3-phosphate dehydrogenase *GAPDH* (Genbank Accession NM_002046) 5’-CCTCCGGGAAACTGTGG-3’ and 5’-AGTG-GGGACACGGAAG-3’ (PCR product size: 140 bp), *TNFα* (Genbank Accession NM_000594) 5’-CACAGTGAAGTGCTGGGAAC-3’ and 5’-GGCTCCGTGTCTCAAGGGAAG-3’ (PCR product size: 224 bp). All primers were manufactured by MWG-Biotech AG (Ebersberg, Germany). Measurements were performed using a StepOne Real-time PCR system with the following protocol: initial activation of HotStar Taq DNA polymerase for 15 min at 95°C, 45 cycles comprised of 15 s denaturation at 94°C, 30 s annealing at 56°C and 30 s extension/detection at 72°C.

Relative mRNA amounts were determined using the relative quantification model with kinetic PCR efficiency correction according to Pfaffl here the relative expression ratio of a target gene is calculated based on the crossing point difference of a sample (treatment) versus control and the real-time PCR efficiency.[33] The target-gene expression is then normalized by the expression of a reference gene, here *gapdh*.

Total Cell Extraction and Western Blot Analysis. A549/8 cells were seeded in 6 well plates at 1 x 10^4 cells/mL. After 24 h, the cells were starved for additional 24 h in DMEM containing 0.5% FCS. Following a 60 min pretreatment with or without cyclonerodiol, cells were induced with 5 ng/mL IL-4 for 30 min. Using Totex detergent buffer (20 mm HEPES (pH 7.9), 350 mm NaCl, 20% (v/v) glycerol, 1% (v/v) NP40, 1 mm MgCl2, 0.5 mm EDTA, 0.1 mm EGTA, 10 mm NaF, 0.5 mm Na_3VO_4, protease-inhibitor cocktail), cells were lysed and cellular debris removed by centrifugation (1000 x g, 10 min, 4°C). Same amounts of proteins were separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane which was probed with an antibody for eotaxin-1 (sc-374233, Santa Cruz, Dallas, Texas) and thereafter with the. Signals were visualized by the enhanced chemoluminescence detection system

Organic & Biomolecular Chemistry Accepted Manuscript

(New England Biolabs, Beverly, USA). After stripping the nitrocellulose membrane in stripping buffer (0.5 M Tris (pH 6.8), 10% (w/v) SDS, 0.8% 2-mercaptoethanol) under agitation for 45 min at 50°C, the nitrocellulose membrane was reprobed with a β-actin antibody (4967, New England Biolabs, Beverly, USA) followed by an incubation with the appropriate horseradish peroxidase conjugated secondary antibody and signal detection.

Statistical analysis. Shown data respresents means ± SEM. Statistical differences were determined by Student's t test for comparison of multiple means (*: $p < 0.05$; **:$p < 0.01$; ***: $p < 0.001$).

General Procedures:

All reagents were reagent grade and used without further purification unless otherwise noted. All reactions involving air or moisture sensitive reagents or intermediates were performed under an inert atmosphere of argon in glassware that was oven dried. Reaction temperatures referred to the temperature of the particular cooling/heating bath. Chromatography was performed using flash chromatography of the indicated solvent system on 35-70 µm silica gel (*Acros Organics*) unless otherwise noted. Alternatively the purifications were performed on an Isolera™ Flash Purification System (*Biotage*®) with an integrated diode array detector. Preparative HPLC separation were carried out on a Nucleosil 50/5 column (*Macherey& Nagel*), 32 mm X 250 mm at a flow rate of 64 mL/min. HPLC with refractive index detection was used. ^{1}H NMR and ^{13}C NMR spectra were recorded ona*Bruker Avance-III HD 300* MHz or a *Bruker Avance-II 400* MHz. Chemical shifts were referenced to the deuterated solvent (e.g., for $CDCl_3$, δ= 7.26 ppm and 77.16 ppm for ^{1}H and ^{13}C NMR, respectively) and reported in parts per million (ppm, δ) relative to tetramethylsilane (TMS, δ = 0.00 ppm).[34] Coupling constants (*J*) were reported in Hz and the splitting abbreviations used were: s, singlet; d, doublet; t, triplet; m, multiplet;br, broad. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates ($60F_{254}$) using an aqueous solution of sulfuric acid, $Ce(SO_4)_2 \cdot 4H_2O$ and $(NH_4)_6Mo_7O_{24}.4H_2O$ and heat as

Organic & Biomolecular Chemistry Accepted Manuscript

developing agents. Specific reactions were monitored by LC-MS on a 1200 HPLC-unit from *Agilent Technologies* with binary pump and integrated diode array detector coupled to a LC/MSD-Trap-mass-spectrometer from *Bruker*. Ionization was achieved by an electron-spray-ionization source (ESI) or an atmospheric-pressure-chemical-ionization source (APCI). High-resolution masses were recorded on a *Waters QTof-Ultima 3*-Instrument with *Lockspray*-Interface and a suitable external calibrant. Infrared spectra were recorded as FT-IR spectra using a diamond ATR unit and are reported in terms of frequency of absorption (ν, cm^{-1}). Tetrahydrofuran, benzene, toluene and diethylether was distilled from sodium and benzophenone.

(*R*)-6-Methyl-2-((*S*)oxiran-2-yl)hept-5-en-2-ol) (3),

A solution of (–)-linalool (20.0 g, 130 mmol, $[\alpha]_D^{22}$ = – 23.3° (c = 1.00, $CHCl_3$)) and $VO(acac)_2$ (1.72 g, 6.50 mmol, 5 mol%.) was refluxed in benzene (500 mL) for 10 minutes under argon atmosphere. A solution of ^{t}BuOOH in decane (5.5 M, 26.0 ml, 143 mmol, 1.10 eq.) was slowly added to the reaction mixture. During the addition the color changed from dark blue to bright yellow. After 2.5 h the green reaction mixture was cooled to room temperature, washed with saturated aqueous $NaHCO_3$ solution (250 mL) and brine (250 mL) and dried over anhydrous $Mg(SO_4)_2$. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (cyclohexane/ethyl acetate, 5:1) to give the title compound (17.7 g, 104 mmol, 80%, diastereomeric mixture) as a yellowish oil.

R_f 0.30 (silica gel, cyclohexane/ethyl acetate, 5:1);

Signals assignable to (*R*,*S*)-**3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.15–5.08 (m, 1H, H-5), 2.97 (dd, ^2J = 4.1 Hz, ^3J = 2.8 Hz, 1H, H-2'), 2.77 (dd, ^3J = 5.1 Hz, ^3J = 2.8 Hz, 1H, H_{trans}-3'), 2.69 (dd, ^2J = 4.0 Hz, ^3J = 5.1 Hz, 1H, H_{cis}-3'), 2.17–2.05 (m, 2H, H-4), 1.69 (s, 3H, H-7), 1.65–1.53 (m, 2H, H-3), 1.59 (s, 3H, H-7'),1.31 (s, 3H, H-1); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm) 131.9 (C-6), 124.0 (C-5), 69.3 (C-2), 57.7 (C-2'), 43.3 (C-3'), 38.7 (C-3), 26.0 (C-1), 25.7 (C-7'), 22.2 (C-4), 17.6 (C-7).

Organic & Biomolecular Chemistry Accepted Manuscript

Signals assignable to (*R*,*R*)-**3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.15–5.08 (m, 1H, H-5), 2.92 (dd, ^{2}J = 4.0 Hz, ^{3}J = 2.8 Hz, 1H, H-2'), 2.85 (dd, ^{3}J = 5.1 Hz, ^{3}J = 2.9 Hz, 1H, H_{trans}-3'), 2.73 (dd, ^{2}J = 4.0 Hz, ^{3}J = 5.1 Hz, 1H, H_{cis}-3'), 2.17–2.05 (m, 2H, H-4), 1.69 (s, 3H, H-7), 1.65–1.53 (m, 2H, H-3), 1.60 (s, 3H, H-7‘), 1.20 (s, 3H, H-1); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm) 131.8 (C-6), 124.2 (C-5), 69.2 (C-2), 57.8 (C-2'), 44.3 (C-3'), 41.2 (C-3), 25.7 (C-7‘), 22.8 (C-1), 22.0 (C-4), 17.6 (C-7).

The spectral data are in accordance with literature.[22]

(1*R*,2*R*,3*R*)-2-(Hydroxymethyl)-1-methyl-3-(prop-1-en-2-yl)cyclopentanol (4)

A mixture of Cp_2TiCl_2 (30.7 g, 123 mmol, 2.10 eq.) and manganese dust (25.8 g, 470 mmol, 8.00 eq., 325 mesh) was stirred at room temperature in deoxygenated THF (300 mL). The initially red mixture became green within 30 min. Epoxide **3** (10.0 g, 58.7 mmol) and Et_3N (15.1 mL, 117.5 mmol, 2.00 eq.) was dissolved in deoxygenated THF (200 mL) and subsequently transferred *via cannula* to the Cp_2TiCl solution. The reaction mixture was stirred at room temperature until consumption of the starting material as indicated by TLC (20 min). The mixture was filtered and concentrated in *vacuo* to a volume of approx. (100 mL) were obtained. Then the mixture was diluted with MTBE (100 mL) and 2N hydrochloric acid (200 mL) was added. The yellowish precipitate thus formed was filtered.

The organic phase was separated and concentrated to half of its original volume in *vacuo*. Hydrochloric acid (2N, 100 mL) was added and the mixture was stirred for 10 min. The separated organic phase was washed with brine (100 mL), dried with anhydrous $MgSO_4$ and filtered. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (cyclohexane/ethyl acetate, 2:1) to afford the title compound (7.06 g, 41.4 mmol, 71%) as an inseparable 90:10-mixture with the reduced compound **4a** in form of a yellowish waxy solid which was used for subsequent transformations without further purification.

Organic & Biomolecular Chemistry Accepted Manuscript

Organic & Biomolecular Chemistry Accepted Manuscript

R_f = 0.18 (silica gel, cyclohexane/ethyl acetate, 2:1);

Purification on an analytical scale: A small amount of the mixture **4** and **4a** (170 mg, 1.00 mmol) was dissolved in diethyl ether (2 mL) and ^{n}hexane (2 mL) and cooled to –30 °C. After 1 h, the formed precipitate was filtered off, washed three times with cold ^{n}hexane (–30°C, 5 mL) and dried *in vacuo* to afford pure compound **4** (90 mg, 0.53 mmol, 53%) as a colorless solid.

IR (ATR): ν_{max}/cm^{-1} 3340, 2958, 2928, 2872, 1643, 1455, 1372, 1295, 1151, 1058, 1023, 888;

$[\alpha]_D^{22}$ – 24.3° (c 0.57, $CHCl_3$);

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.79–4.77 (m, 1H, H-7), 4.76–4.73 (m, 1H, H-7), 3.90 (dd, ^2J = 11.4 Hz, ^3J = 3.2 Hz, 1H, H-9), 3.71 (dd, ^2J = 11.4 Hz, ^3J = 5.1 Hz, 1H, H-9), 2.86 (dt, ^3J = 10.8 Hz, ^3J = 8.7 Hz, 1H, H-3), 1.98–1.88 (m, 1H, H-4), 1.80–1.71 (m, 2H, H-5), 1.70 (s, 3H, H-8), 1.64–1.59 (m, 1H, H-2), 1.54–1.49 (m, 1H, H-4), 1.39 (s, 3H, H-10); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm) 131.9 (C-6), 124.0 (C-5), 69.3 (C-2), 57.7 (C-2‘), 43.3 (C-3‘), 38.7 (C-3), 26.0 (C-1), 25.7 (C-7‘), 22.2 (C-4), 17.6 (C-7).

(1*R*,2*R*,3*S*)-2-(hydroxymethyl)-3-isopropyl-1-methylcyclopentanol (reduced side product)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.85 (dd, ^2J = 11.4 Hz, ^3J = 3.0 Hz, 1H, H-9), 3.71 (dd, ^2J = 11.3 Hz, ^3J = 6.4 Hz, 1H, H-9), 1.84 (m, 1H, H-3), 1.83–1.75 (m, 1H, H-4), 1.72–1.65 (m, 2H, H-5), 1.65–1.58 (m, 1H, H-6), 1.52–1.46 (m, 1H, H-2), 1.35 (s, 3H, H-10), 1.34–1.29 (m, 1H, H-4), 0.93 (d, ^3J = 6.8 Hz, 3H, H-7), 0.84 (d, ^3J = 6.7 Hz, 3H, H-8); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm) 131.9 (C-6), 124.0 (C-5), 69.3 (C-2), 57.7 (C-2‘), 43.3 (C-3‘), 38.7 (C-3), 26.0 (C-1), 25.7 (C-7‘), 22.2 (C-4), 17.6 (C-7).

The data are in accordance with the literature.[22]

Organic & Biomolecular Chemistry Accepted Manuscript

O-[(1R,2R,5R)(2-Hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclopentyl)methyl]S-methylcarbonodithioate (5)

Compound **4** (7.00 g, 41.1 mmol, containing 10% of the reduced byproduct **4a**) and CS_2 (9.94 mL, 165 mmol, 4.00 eq.) was dissolved in THF (300 mL). NaH (1.97 g, 49.3 mmol, 1.20 eq., 60% dispersion in mineral oil) was suspended in THF (200 mL), combined with the alcohol solution at 0 °C and was stirred at this temperature for 30 min. The reaction mixture was stirred at room temperature for 60 min while its changed color from yellowish to brown. Iodomethane (20.6 mL, 329 mmol, 8.00 eq.) was added slowly (CAUTION!) and stirring was continued for 15 min. The mixture was diluted with MTBE (200 mL) and washed two times with brine (250 mL). The organic layer was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (cyclohexane/ethyl acetate, 10:1) to afford 8.80 g (33.8 mmol, 82%) of the title compound as a yellowish oil. The material still contained the saturated analogue but was used without further purification for the next step.

R_f 0.25 (silica gel, cyclohexane/ethyl acetate, 10:1).

IR (ATR) ν_{max}/cm^{-1} 3456, 2959, 2872, 1643, 1455, 1375, 1216, 1054, 965, 890;

$[\alpha]_D^{22}$ – 13.4° (c 0.53, $CHCl_3$);

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.83 (dd, ^2J = 11.2 Hz, ^3J = 8.7 Hz, 1H, H-9), 4.80–4.78 (m, 1H, H-7), 4.77–4.74 (m, 1H, H-7), 4.65 (dd, ^2J = 11.2 Hz, ^3J = 4.4 Hz, 1H, H-9)2.66–2.58 (m, 1H, H-5), 2.56 (s, 3H, H-11), 2.13 (ddd,^2J = 11.1 Hz, ^3J = 8.4 Hz,^3J = 4.4 Hz, 1H, H-1), 2.02–1.92 (m,1H, H-4), 1.84–1.76 (m, 2H, H-3), 1.74 (s, 3H, H-8), 1.58–1.51 (m, 1H, H-4), 1.41 (s,3H, H-12);^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm) 216.0 (C-10), 146.0 (C-6), 111.3 (C-7), 79.7 (C-2), 73.0 (C-9), 50.5 (C-1), 49.0 (C-5), 41.3 (C-3), 28.2 (C-12), 28.1 (C-4), 19.0 (C-11).

The data are in accordance with the literature.[22]

Organic & Biomolecular Chemistry Accepted Manuscript

(1*R*,2*S*,3*R*)-1,2-Dimethyl-3-(prop-1-en-2-yl)cyclopentanol (6)

Methyl xanthogenate **5** (6.00 g, 23.0 mmol) and AIBN (378 mg, 2.30 mmol, 0.10 eq.) were dissolved in deoxygenated toluene (500 mL) under argon atmosphere. $^{n}Bu_3SnH$ (12.2 mL, 46.1 mmol, 2 eq.) was added at room temperature. Subsequently the mixture was heated to reflux for 15 minutes and the reaction mixture rapidly turned dark. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (cyclohexane/ethyl acetate, 8:1) to afford the title compound (2.56 g, 16.6 mmol, 72%) as a colorless oil. The material contained 13% (NMR) of the saturated analogue but was used without further purification for the next step.

R_f 0.24 (silica gel, cyclohexane/ethyl acetate, 8:1).

$[\alpha]_D^{22}$ – 13.8° (c = 1.00, $CHCl_3$);

^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.73–4.71 (m, 2H, H-7), 2.43 (dt, ^3J = 11.3 Hz, ^3J = 8.9 Hz, 1H, H-3),1.92 (ddt, ^2J = 12.7 Hz, ^3J = 8.9 Hz, ^3J = 7.5 Hz, 1H, H-4), 1.79–1.72 (m, 2H, H-5)1.69 (t, ^4J = 1.1 Hz,3H, H-8), 1.55–1.49 (m, 1H, H-2), 1.49–1.42 (m, 1H, H-4'), 1.28 (s,3H, H-10), 0.86 (d, ^3J = 6.8 Hz,3H, H-9); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm) 147.1 (C-6), 110.3 (C-7), 80.3 (C-1), 52.7 (C-3), 47.0 (C-2), 40.0 (C-5), 27.5 (C-4), 26.6 (C-10), 19.0 (C-8), 10.6 (C-9).

The data are in accordance with the literature.[22]

1-((1*R*,2*S*,3*R*)-3-Hydroxy-2,3-dimethylcyclopentyl)ethanone (7)

Olefin **6** (1.00 g, 6.49 mmol) and $K_2OsO_4.2H_2O$ (47.9 mg, 0.130 mmol, 2 mol%) were dissolved in THF (100 mL), H_2O (33 mL) and $^{t}BuOH$ (10 mL) under stirring at room temperature. Solid $NaIO_4$ (13.9 g, 64.9 mmol, 10 eq.) was added within 5 minutes in two portions. The reaction mixture was filtered after 16 h, diluted with H_2O (50 mL) and extracted

with EtOAc (200 mL). The separated aqueous layer was extracted twice with EtOAc (100 mL each). The combined organic layers were washed with cold saturated aqueous $Na_2S_2O_3$ solution (150 mL), dried over anhydrous $MgSO_4$ and filtered. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (cyclohexane/ethyl acetate, 3:1) to afford the title compound (474 mg, 3.04 mmol, 47%) as a light yellowish oil which became a waxy solid at room temperature.

R_f 0.16 (silica gel, cyclohexane/ethyl acetate, 3:1).

IR (ATR) ν_{max}/cm^{-1} 3438, 2971, 2933, 2874, 1694, 1454, 1426, 1370, 1350, 1204, 1024, 567;

$[\alpha]_D^{22}$ – 52.0° (c = 1.00, $CHCl_3$);

^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.83 (td, ^3J = 10.4 Hz, ^3J = 6.9 Hz, 1H, H-1), 2.17 (s, 3H, H-7), 2.16–2.11 (m, 1H, H-5), 1.92 (dq, ^3J = 10.3 Hz, ^3J = 6.8 Hz, 1H, H-2), 1.83–1.71 (m, 2H, H-4), 1.71–1.61 (m, 1H, H-5), 1.28 (s, 3H, H-9), 0.96 (d, ^3J = 6.8 Hz, 3H, H-8); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm) 211.4 (C-6), 80.7 (C-3), 57.7 (C-1), 46.0 (C-2), 40.0 (C-4), 29.9 (C-7), 25.7 (C-5), 25.7 (C-9), 11.6 (C-8);

FD-MS: *m/z* calculated for $[C_9H_{16}O_2]^+$: 156.12, found: 156.27 $[M]^+$. No ionization could be achieved in ESI-HRMS.

(1*R*,2*S*,3*R*)-3-(2-Hydroxy-6-methylhept-5-en-2-yl)-1,2-dimethylcyclopentanol, cyclonerodiol (1)

Lithium wire (50.0 mg, 7.00 mmol, 11.0 eq.) was freshly cut and added to meticulously deoxygenated diethyl ether. A portion (25 drops) of homoprenyl bromide (1.04 g, 6.40 mmol, 10.0 eq.) was added under stirring at room temperature. After the reaction has started the mixture was cooled to 10 °C. Within 5 min., three quarters of the homoprenyl bromide was added dropwise, after a further 10 min., the rest was added in one portion. After 50 min., almost all the lithium was consumed. The mixture was cooled to 78 °C and a solution of

Organic & Biomolecular Chemistry Accepted Manuscript

ketone **7** (100 mg, 0.64 mmol, 1.00 eq.) in dry THF (4 mL) was added continuously. The reaction mixture was stirred at this temperature for 5 h and then allowed to warm up to room temperature before saturated NH_4Cl solution (10 mL) and EtOAc (25 mL) were added. The aqueous layer was extracted twice with EtOAc (25 mL each) and the combined organic layers were washed with water (20 mL), dried over $MgSO_4$ and filtered. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (cyclohexane/ethyl acetate, gradient 5% to 50% ethyl acetate, Isolera™ Flash Purification System) to afford the title compound (110 mg, 0.46 mmol, 72%) as mixture of diastereomers (d.r. = 10:1) in form of a colorless viscous oil. The mixture was separated by preparative HPLC (^{c}Hex/EtOAc, 3:1, R_t 7 min) to give pure cyclonerodiol (**1**, 50 mg, 33%).

R_f 0.20 (silica gel, cyclohexane/ethyl acetate, 5:1).

IR (ATR) ν_{max}/cm^{-1} 3442, 2965, 2929, 2876, 1453, 1375, 1284, 1204, 1148, 1117, 919, 885;

$[a]_D^{22}$ – 19.6° (c 1.00, $CHCl_3$); Lit. $[a]_D^{24}$ – 21.0° (c 1.04, $CDCl_3$);

^{1}H NMR, COSY, HSQC. HMBC (400 MHz, $CDCl_3$) δ (ppm) 5.16–5.10 (m, 1H, H-5'), 2.12–2.02 (m, 2H, H-4'), 1.90–1.82 (m, 2H, H-3, H-4), 1.73–1.45 (m, 6H, 2x H-5, H-2,H-4, 2x H-3'), 1.67 (br s, 3H, H-8'), 1.61 (br s, 3H, H-7'), 1.27 (s, 3H, C1-Me), 1.17 (s, 3H, C1'), 1.05 (d, ^3J = 6.8 Hz, 3H, C2-Me); ^{13}C NMR, HSQC, HMBC (100.6 MHz, $CDCl_3$) δ (ppm) 131.8 (C-6'), 124.5 (C-5'), 81.3 (C-1), 74.9 (C-2'), 54.3 (C-3), 44.2 (C-2), 40.4 (C-5, C3'),26.1 (C1-Me), 25.7 (C-8'), 25.1 (C-4), 24.3 (C-1'), 22.7 (C-4'), 17.7 (C-7'), 14.6 (C-2-Me);

HRMS (ESI): calculated for $[C_{15}H_{28}O_2 + Na]^+$: 263.1987, found: 263.1992.

Acknowledgments

This work was supported by the Zeiss-foundation. We thank Dr. J. C. Liermann (Mainz) for NMR spectroscopy, Dr. N. Hanold (Mainz) for mass spectrometry as well as Andrea Frank and Prof. U. Nubbemeyer (Mainz) for preparative HPLC.

Organic & Biomolecular Chemistry Accepted Manuscript

References

(1] G. Caramori, D. Groneberg, K. Ito, P. Casolari, I. Adcock, A. Papi, *J. Occ. Med. Tox.* 2008, **3**, S6.

(2] K. Mullane, *Biochem. Pharmacol.* 2011, **82**, 586-599.

(3] N. A. Hanania, *Chest* 2008, **133**, 989-998.

(4] J. R. Murdoch, C. M. Lloyd, *Mutat. Res.* 2010, **690**, 24-39.

(5] D. Gibeon, A. Menzies-Gow, *Clin. Med.* 2013, **13**, 477-481.

(6] T.T. Hansel, P. J. Barnes, *Prog. Respir. Res.* 2010, **39**, 1 - 14.

(7] H. Tozawa, Y. Kanki, J.-i. Suehiro, S. Tsutsumi, T. Kohro, Y. Wada, H. Aburatani, W. C. Aird, T. Kodama, T. Minami, *Mol. Cell. Biol.* 2011, **31**, 2196-2209.

(8] K. Stokes, N. LaMarche, A. August, *J. Immunol.* 2014, **192**, 119.119.

(9] H.H. Walford, T. A. Doherty, *JAK-STAT* 2013, **2**, e25301.

(10] G. Damera, J. R. A. Panettieri, *Br. J. Pharmacol.* 2011, **163**, 68-80.

(11] S. Nozoe, M. Goi, N. Morisaki, *Tetrahedron Lett.* 1970, **11**, 1293-1296.

(12] B. E. Cross, R. E. Markwell, J. C. Stewart, *Tetrahedron* 1971, **27**, 1663-1667.

(13] J. R. Hanson, P. B. Hitchcock, R. Nyfeler, *J. Chem. Soc., Perkin Trans. 1* 1975, **0**, 1586-1590.

(14] D. Laurent, N. Goasdoue, F. Kohler, F. Pellegrin, N. Platzer, *Magn. Reson. Chem.* 1990, **28**, 662-664.

(15] T. Fujita, Y. Takaishi, Y. Takeda, T. Fujiyama, T. Nishi, *Chem. Pharm. Bull.* 1984, **32**, 4419-4425.

(16] H. G. Cutler, J. M. Jacyno, R. S. Phillips, R. L. Vontersch, P. D. Cole, N. Montemurro, *Agricultural and Biological Chemistry* 1991, **55**, 243-244.

Organic & Biomolecular Chemistry Accepted Manuscript

(17] Q. Huang, Y. Tezuka, Y. Hatanaka, T. Kikuchi, A. Nishi, K. Tubaki, *Chem. Pharm. Bull.* 1995, **43**, 1035-1038.

(18] X. Li, M. K. Kim, U. Lee, S.-K. Kim, J. S. Kang, H. D. Choi, B. W. Son, *Chem. Pharm. Bull.* 2005, **53**, 453-455.

(19] X. Li, D. Zhang, U. Lee, X. Li, J. Cheng, W. Zhu, J. H. Jung, H. D. Choi, B. W. Son, *J. Nat. Prod.* 2007, **70**, 307-309.

(20] a) R. Evans, J. R. Hanson, R. Nyfeler, *J. Chem. Soc., Perkin Trans. 1* 1976, **0**, 1214-1217; b) D. E. Cane, R. Iyengar, M.-S. Shiao, *J. Am. Chem. Soc.* 1981, **103**, 914-931; c) D. E. Cane, R. Iyengar, *J. Am. Chem. Soc.* 1978, **100**, 3256-3258; d) D. E. Cane, M.-S. Shiao, *J. Am. Chem. Soc.* 1978, **100**, 3203-3207.

(21] Y. Matsuki, M. Kodama, S. Itô, *Tetrahedron Lett.* 1979, **20**, 2901-2904.

(22] C. P. r. Morales, J. Catalán, V. Domingo, J. A. González Delgado, J. A. Dobado, M. M. Herrador, J. F. Quílez del Moral, A. F. Barrero, *J. Org. Chem.* 2011, **76**, 2494-2501.

(23] K. B. Sharpless, R. C. Michaelson, *J. Am. Chem. Soc.* 1973, **95**, 6136-6137.

(24] T. V. RajanBabu, W. A. Nugent, *J. Am. Chem. Soc.* 1989, **111**, 4525-4527.

(25] S. C. Roy, K. K. Rana, C. Guin, *J. Org. Chem.* 2002, **67**, 3242-3248.

(26] A. Gansäuer, A. Rosales, J. Justicia, *Synlett* 2006, **2006**, 927-929.

(27] A. G. Myers, M. Movassaghi, B. Zheng, *J. Am. Chem. Soc.* 1997, **119**, 8572-8573.

(28] D. H. R. Barton, S. W. McCombie, *J. Chem. Soc., Perkin Trans. 1* 1975, **0**, 1574-1585.

(29] W. Biernacki, A. Gdula, *Synthesis* 1979, **1979**, 37-38.

(30] K. Rudolph, A. Serwe, G. Erkel, *Cytokine* 2013, **61**, 285-296.

(31] G. Erkel, H. Belahmer, A. Serwe, T. Anke, H. Kunz, H. Kolshorn, J. Liermann, T. Opatz, *J. Antibiot.* 2008, **61**, 285-290.

Organic & Biomolecular Chemistry Accepted Manuscript

(32] M. Weidler, J. Rether, T. Anke, G. Erkel, *Biochem. Biophys. Res. Commun.* 2000, **276**, 447-453.

(33] M. W. Pfaffl, in *Real-time PCR*, 1 ed. (Ed.: M. T. Dorak), Taylor & Francis Group, Abingdon, **2006**, pp. 63-82.

(34] G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw, K. I. Goldberg, *Organometallics* 2010, **29**, 2176-2179.

Organic & Biomolecular Chemistry Accepted Manuscript