

Asymmetric Synthesis of Both Enantiomers of Arteludovicinolide A

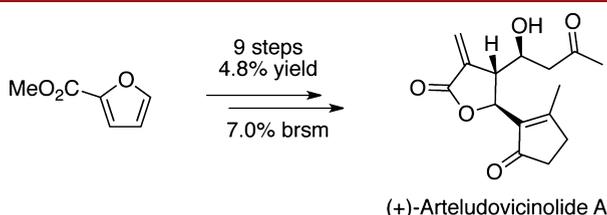
Andreas Kreuzer, Sabine Kerres, Thomas Ertl, Hannelore Rucker, Sabine Amslinger, and Oliver Reiser*

Institut für Organische Chemie, Universität Regensburg, Universitätsstr. 31, 93053 Regensburg, Germany

oliver.reiser@chemie.uni-regensburg.de

Received May 24, 2013

ABSTRACT



The first total synthesis of either enantiomer of Arteludovicinolide A and their biological evaluation is reported, featuring a new strategy for the asymmetric construction of γ -butyrolactones with stereogenic side chains in the 4-position. Starting from the renewable resource methyl 2-furoate, the sesquiterpene lactone was synthesized in 9 steps and 4.8% overall yield via an asymmetric cyclopropanation and two diastereoselective nucleophile additions making use of a donor-acceptor-cyclopropane-lactonization cascade. At noncytotoxic concentrations ($\leq 10 \mu\text{M}$) (+)-1 was found to have a 15 times higher *anti*-inflammatory activity ($4.87 \pm 1.1 \mu\text{M}$) than previously reported for concentrations of $\geq 45 \mu\text{M}$.

Artemisiae plants (Asteraceae) are widely distributed throughout the world, and many species have been used as medicinal plants especially in Chinese folk medicine.¹ The sesquiterpene lactone Arteludovicinolide A ((+)-1)² was first discovered in 1991 in the aerial parts of *Artemisia ludoviciana* and proved to possess anti-inflammatory activity (NO inhibition via iNOS pathway).^{2b} It belongs to the widely occurring class of *exo*-methylene- γ -butyrolactones,³ being *anti*-disubstituted in the 4- and 5-positions, as found for example in many paraconic acids⁴ or guaianolides.⁵ In the latter two classes of natural products, we have developed a methodology based on

cyclopropanecarbaldehyde **4** (Scheme 1), being readily available in either enantiomeric form by a Cu(I)-bis-(oxazoline) catalyzed asymmetric cyclopropanation of methyl 2-furoate (**7**) with ethyl diazoacetate followed by reductive ozonolysis.⁶ Upon diastereoselective addition of a nucleophile Nu¹ in agreement with the Felkin–Anh paradigm⁷ followed by base induced hydrolysis of the oxalic ester that triggers a retroaldol–lactonization cascade, lactone aldehyde **8** can be obtained with high *anti*-selectivity. Addition of a second nucleophile Nu² to **8** gives rise to lactones of type **9**. This strategy, however, is limited to the introduction of stabilized nucleophiles Nu¹ such as allyl, which precludes the synthesis of widely occurring γ -butyrolactones with alkyl or vinyl functionalities as found for example in (+)-arteludovicinolide A ((+)-1). We report here an alternative approach toward γ -butyrolactones **3** being identical to **9** in its relative substitution

(1) Huang, Z.-S.; Pei, Y.-H.; Liu, C.-M.; Lin, S.; Tang, J.; Huang, D.-S.; Song, T.-F.; Lu, L.-H.; Gao, Y.-P.; Zhang, W.-D. *Planta Med.* **2010**, *76*, 1710.

(2) (a) Jakupovic, J.; Tan, R. X.; Bohlmann, F.; Boldt, P. E.; Jia, Z. J. *Phytochemistry* **1991**, *30*, 1573. (b) Lateff, A. A.; Gamal-Eldeen, A. M.; Turkey, F.; Hirata, T.; Paré, P. W.; Karchesy, J.; Kamel, M. S.; Ahmed, A. A.; Hegazy, M.-E. F. *Nat. Prod. Commun.* **2008**, *3*, 851. (c) Huang, Z.-S.; Pei, Y.-H.; Liu, V.; Lin, S.; Tang, J.; Huang, D.-S.; Song, T.-F.; Lu, L.-H.; Gao, Y.-P.; Zhang, W.-D. *Planta Med.* **2010**, *76*, 1710.

(3) Kitson, R. R. A.; Millemaggi, A.; Taylor, R. J. K. *Angew. Chem., Int. Ed.* **2009**, *48*, 9426.

(4) Bandichhor, R.; Nosse, B.; Reiser, O. *Top. Cur. Chem.* **2005**, *243*, 43.

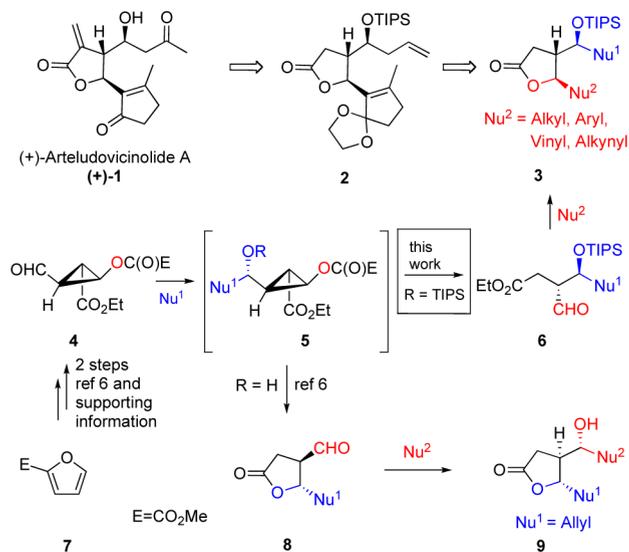
(5) Schall, A.; Reiser, O. *Eur. J. Org. Chem.* **2008**, 2353.

(6) (a) Kalidindi, S.; Jeong, W. B.; Schall, A.; Bandichhor, R.; Nosse, B.; Reiser, O. *Angew. Chem., Int. Ed.* **2007**, *45*, 6361. (b) Chhor, R. B.; Nosse, B.; Sörgel, S.; Böhm, C.; Seitz, M.; Reiser, O. *Chem.—Eur. J.* **2003**, *9*, 260. (c) Böhm, C.; Reiser, O. *Org. Lett.* **2001**, *3*, 1315. (d) Nosse, B.; Chhor, R. B.; Jeong, W. B.; Böhm, C.; Reiser, O. *Org. Lett.* **2003**, *5*, 941. (e) Böhm, C.; Schinnerl, M.; Bubert, C.; Zabel, M.; Labahn, T.; Parisini, E.; Reiser, O. *Eur. J. Org. Chem.* **2000**, 2955.

(7) Mengel, A.; Reiser, O. *Chem. Rev.* **1999**, *99*, 1191.

pattern but allowing the introduction of alkyl, aryl, vinyl, and alkynyl groups in the 5-position, thus greatly expanding the scope for the synthesis of the title compounds. As an application of this methodology, the enantioselective synthesis of either enantiomer of arteludovicinolide A ((+)-**1** and (–)-**1**) from commercial methyl 2-furoate (**7**), which is readily available on ton scale from hemicellulose, is demonstrated.

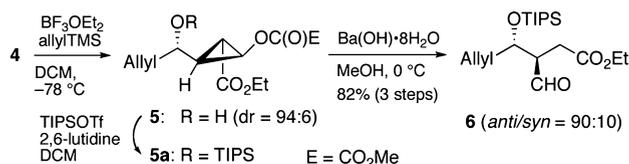
Scheme 1. Synthetic Strategies to Chiral γ -Butyrolactones



Aiming ultimately at the synthesis of naturally occurring (+)-**1**, cyclopropanecarbaldehyde **4** (90% ee) was synthesized in two steps from **7** in diastereomerically pure form on gram scale following the protocol developed by us (for detailed procedures, see Supporting Information).⁶ After having worked out the underlying methodology described below and the synthesis to (+)-**1**, the sequence was repeated starting with the analogous synthesis of (*ent*)-**4** (99% ee) to arrive at unnatural (–)-**1** to allow the biological evaluation of both enantiomers.

Borontrifluoride mediated allylation of **4** gives rise to **5** (94:6 dr, Scheme 2); however, rather than performing the direct base induced hydrolysis of the oxalylic ester functionality that would give rise to **8** (Nu¹ = allyl, Scheme 1), protection of the hydroxyl group with TIPS leading to **5a** was carried out. Hydrolysis of the oxalylic ester functionality now leads to the acyclic aldehyde **6**, which was investigated in reactions with nucleophiles (*vide infra*; Table 1).

Scheme 2. Synthesis of **6** via Sakurai Allylation, TIPS-Protection, and Saponification



Saponification of the labile oxalic ester in **5a** with Ba(OH)₂·8H₂O in MeOH gave rise to the α/β -chiral aldehyde **6** in 82% overall yield starting from **4**. The decrease in the diastereomeric ratio in the transformation from **5a** (94:6) to **6** (90:10) results from a epimerization on the aldehyde bearing carbon center most likely caused during saponification under the basic conditions employed. Importantly, the enantiopurity of **6** remained unchanged, ensuring that no erosion of stereochemistry by retroaldol/aldol processes had occurred (*vide infra*). Separation of the two diastereomers of **6** turned out to be impracticable; therefore **6** was used as the diastereomeric mixture.

The chemoselective addition of organolithium and organomagnesium reagents to the aldehyde functionality in **6** could be achieved in accordance with the Felkin–Anh paradigm⁷ with concurrent lactone formation to give rise to trans-substituted γ -butyrolactones **11** (Table 1). Additionally, chelation with the ester group is possible, which would further contribute to the formation of the major diastereomer **11a**. The stereocenter in the 3-position could be regarded as nonreinforcing according to the Evans model for 1,3-inductions;⁸ nevertheless, the stereocenter in the 2-position appears to be the dominating control element in the title transformation. High diastereoselectivity was observed especially when sterically demanding Grignard reagents were employed (entry 6), while the slim lithium(trimethylsilyl) acetylene (TMS-acetylene-Li) was unselective (entry 5). The good result from the introduction of a vinyl nucleophile is noteworthy (Table 1, entry 3), since it demonstrates the potential for the introduction of vinyl moieties at the lactone scaffold, which is a prevailing motive among the sesquiterpene lactones, particularly in the family of the germacranolides.⁹ Along with those, the arteludovicinolides (A–D)² and various closely related structures such as the *iso-seco*-tanapartolides¹⁰ appear to be accessible by the synthetic approach developed in this study.

Indeed, the synthesis of (+)-arteludovicinolide A (+)-**1**, and in parallel the synthesis of (–)-**1** starting from (*ent*)-**6** (99% ee) obtained from (*ent*)-**4** (*vide supra*), was readily accomplished: by reacting **6** (90% ee) with vinyl-lithium reagent **14** derived from hexane-2,5-dione,¹² the key structural element **2** was obtained in 67% yield along with *epi*-**2** in 5% yield,¹¹ the latter structure being confirmed by X-ray analysis (Scheme 3).

Because of the sensitive nature of α -methylene- γ -butyrolactones, we initially considered setting the methyl ketone functionality in the C-3 side chain of **1** via a Wacker–Tsuji oxidation prior to the final introduction of the

(8) Evans, D. A.; Dart, M. J.; Duffy, J. L.; Yang, M. G.; Livingston, A. B. *J. Am. Chem. Soc.* **1995**, *117*, 6619.

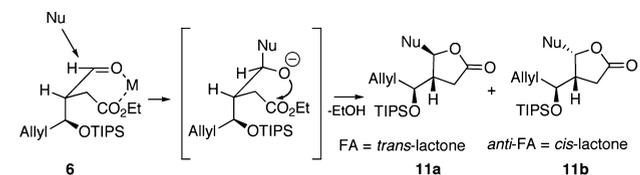
(9) Zidorn, C. *Phytochemistry* **2008**, *69*, 2270.

(10) (a) Huneck, S.; Zdero, C.; Bohlmann, F. *Phytochemistry* **1986**, *25*, 883. (b) Tan, R. X.; Jakupovic, J.; Bohlmann, F.; Jia, Z. J.; Huneck, S. *Phytochemistry* **1991**, *30*, 583.

(11) **2** and **11** were formed along with minor amounts of epimers reflecting the diastereomeric *anti/syn* 9:1 ratio of starting material **6**. These epimers could be removed in the following transformations; see Supporting Information for a detailed analysis.

(12) See Supporting Information for the preparation of **13**.

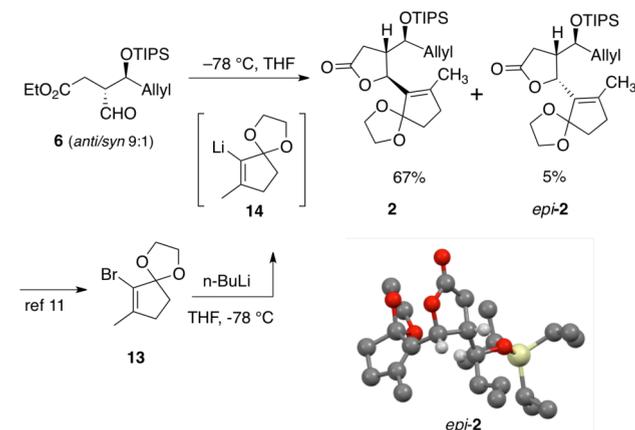
Table 1. Formation of the Major γ -Lactone **11a** from **6**, Being Rationalized via a Combined Felkin–Anh (FA)/Chelation Pathway



entry	nucleophile	yield [%] ^a	<i>trans/cis</i> ^b
1	Me-MgBr	68	84:16
2	Me-Li	62	79:21
3	vinyl-MgBr	52	89:11
4	Ph-MgBr	53	71:29
5	TMS-acetylene-Li	57	52:48
6	ⁱ Pr-MgBr	46	96:04

^a Isolated yields. ^b Based on ¹H NMR of the crude.¹¹

Scheme 3. Synthesis of the Key Structural Element of **1** via Lithium Organyl **14** Addition to **6**

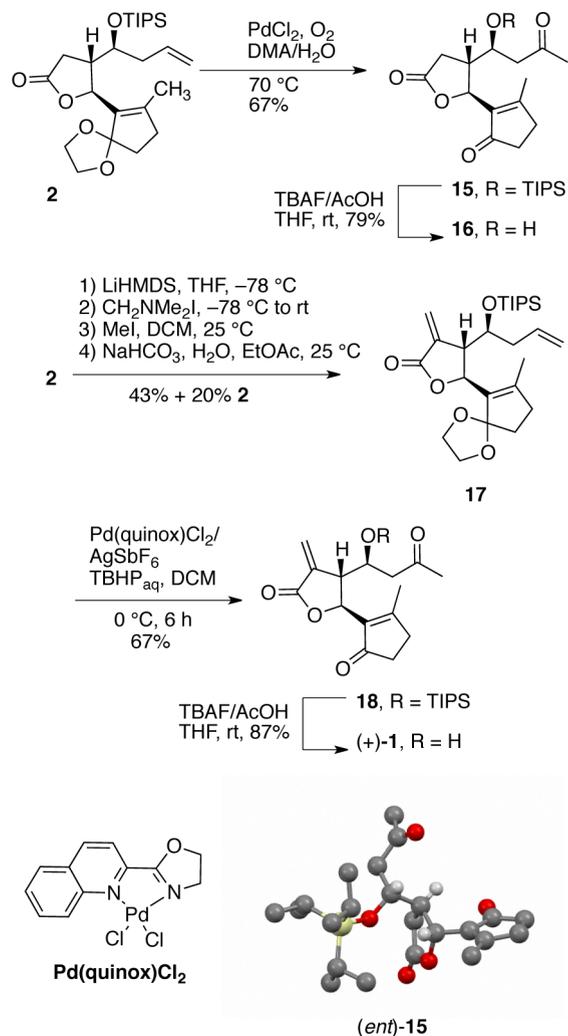


exo-methylene group (Scheme 4). Unfortunately, under various conditions tried for the oxidation of **2**, the preservation of the ketal moiety was not possible, which is necessary for the subsequently intended deprotonation at the lactone α -position to introduce the *exo*-methylene group. Several attempts to reprotect the ketone moieties in **15** were unsuccessful: either no reaction or complete decomposition was observed. Nevertheless, at this stage we were able to obtain an X-ray structure of *ent*-**15** synthesized from (*ent*)-**2**, which confirmed its relative and absolute configuration.

Also the direct conversion of the terminal olefin in **2** into a ketal with Hg(OAc)₂ and PdCl₂ in the presence of ethylenglycol¹³ was not successful (not shown). However, **15** could be desilylated to **16** for biological evaluation (vide infra). We therefore investigated the possibility of a

(13) Hunt, D. F.; Rodeheaver, G. T. *Tetrahedron Lett.* **1972**, *34*, 3595.

Scheme 4



Wacker–Tsuji oxidation in the presence of an *exo*-methylene group, as it was to the best of our knowledge unexplored. Starting from **2**, the introduction of the *exo*-methylene group *via* dimethylmethylideneammonium iodide (Eschenmoser's salt)¹⁴ was determined to be the most convenient and afforded the desired product **17** in 43% yield over three steps along with 20% of recovered starting material. The application of copper-free Wacker–Tsuji oxidation conditions¹⁵ (PdCl₂, O₂, dimethylacetamide/H₂O 8:1) to **2** afforded **18** in 52% yield along with approximately 7% of inseparable aldehyde contaminations arising from double bond cleavage. Fortunately, the mild Pd(quinox)Cl₂ catalyzed TBHP mediated Wacker oxidation¹⁶ gave **18** in 67% yield without byproduct formation. Final deprotection of **18** with buffered TBAF in THF¹⁷ afforded (+)-**1**, with spectroscopic data in accordance

(14) Schreiber, J.; Maag, H.; Hashimoto, N.; Eschenmoser, A. *Angew. Chem., Int. Ed.* **1971**, *10*, 330.

(15) Mitsudome, T.; Umetani, T.; Nosaka, N.; Mori, K.; Mizugaki, T.; Ebitani, K.; Kaneda, K. *Angew. Chem., Int. Ed.* **2006**, *45*, 481.

(16) Michel, B. W.; Camelio, A. M.; Cornell, C. N.; Sigman, M. S. *J. Am. Chem. Soc.* **2009**, *131*, 6076.

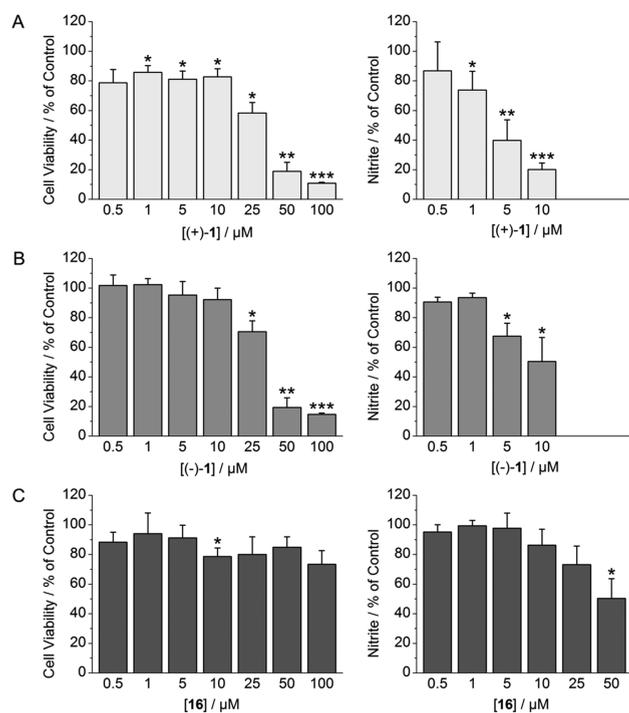


Figure 1. Results of in vitro assays performed with RAW264.7 cells stimulated with 10 ng/mL of LPS. Charts on the left side refer to MTT tests of (+)-**1**, (–)-**1**, and **16** after an incubation time of 24 h at different concentrations. Charts on the right display the influence of (+)-**1**, (–)-**1**, and **16** on NO-production (Griess assay). Data represent at least three independent experiments performed in quadruplicates. Levels of significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

with the literature and molecular mass confirmed by HRMS analysis. Furthermore, the enantiomer (–)-**1** was prepared in an analogous way starting from enantiopure *ent*-**4**. Chiral HPLC analysis of **1** was not met with success due to the sensitive nature of **1**, whereas the separation of **15** and **18** was possible and revealed an enantiomeric purity

(17) Debenham, J. S.; Rodebaugh, R.; Fraser-Reid, B. *J. Org. Chem.* **1997**, *62*, 4591.

(18) See Supporting Information for further details.

(19) Reference 2b reports an IC_{50} of 70.4 μM , taken however at a much higher concentration range (> 45 μM), which is according to our measurements already in the cytotoxic region.

of 90% ee and 91% ee respectively (employed starting material **4**: 90% ee). HPLC analysis of *ent*-**15** and *ent*-**18** (starting from enantiopure *ent*-**4**) showed enantiopure compounds (> 99% ee), indicating that no racemization occurs in the course of the entire synthesis.

(+)-**1**, (–)-**1**, and **16** were tested for cytotoxicity (MTT cell viability test, Figure 1 left charts) and anti-inflammatory activity (iNOS-inhibition via Griess assay, Figure 1 right charts) in the noncytotoxic concentration range.¹⁸

In the cell viability assay, (+)-**1** and (–)-**1** exhibited similar IC_{50} values of 45.3 ± 2.6 and 49.3 ± 2.7 μM respectively (Figure 1A,B left charts). In contrast to that (+)-**1** showed significantly higher iNOS inhibition with an IC_{50} value of 4.87 ± 1.1 μM ¹⁹ compared to the unnatural (–)-**1** ($IC_{50} = 10.3 \pm 5.7$ μM). Since it is known for sesquiterpene lactones that, besides the α -methylene group, a second Michael acceptor like the cyclopentenone moiety can play an important role in their biological activity,²⁰ we also tested **16**. However, **16** had no significant cytotoxic activity ($c < 100$ μM) and only weak anti-inflammatory properties ($IC_{50} = 50.2 \pm 18.3$ μM).

In conclusion we have developed the first enantioselective syntheses of (+)- and (–)-arteludovicinolide A, which were obtained in 9 steps and 4.8% overall yield (7.0% brsm) starting from 2-furoic ester **7**. Moreover, we could show that at noncytotoxic concentrations (≤ 10 μM) (+)-**1** has a 15 times higher anti-inflammatory activity than previously reported for concentrations of ≥ 45 μM .^{2b} Finally, the *exo*-methylene group in the lactone moiety was determined to be decisive for the biological activity of the natural product.³

Acknowledgment. We thank Sabine Stempfhuber and Dr. Michael Bodensteiner, Department of Crystallography, University of Regensburg, for X-ray structure analysis.

Supporting Information Available. Experimental procedures, NMR spectra for all compounds, and CIF-data for *epi*-**2** and *ent*-**15**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

(20) Schmidt, T. J.; Lyss, G.; Pahl, H. L.; Merfort, I. *Bioorg. Med. Chem.* **1999**, *7*, 2849.

The authors declare no competing financial interest.