Natural Products

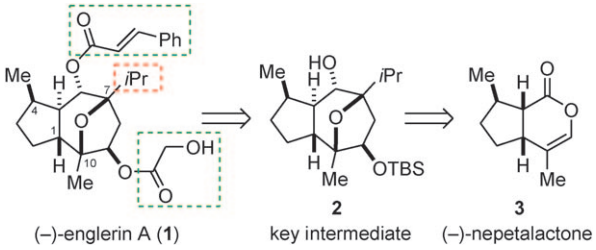
DOI: 10.1002/ange.201007790

# Total Synthesis and Biological Evaluation of (−)-Englerin A and B: Synthesis of Analogues with Improved Activity Profile**

*Lea Radtke, Matthieu Willot, Hongyan Sun, Slava Ziegler, Stephanie Sauerland, Carsten Strohmann, Roland Fröhlich, Peter Habenberger, Herbert Waldmann, and Mathias Christmann**

*Dedicated to Professor Dieter Enders on the occasion of his 65th birthday*

Although successful in many types of cancer, chemotherapy has shown at best moderate effectiveness in the treatment of renal cell carcinoma.[1] Currently approved therapies target the downstream signaling that leads to an over-expression of angiogenic factors such as VEGF and PDGF and include tyrosine kinase inhibitors (sorafenib, sutinib) as well as mTOR inhibitors (temsirolimus, everolimus). Owing to severe side effects and other drawbacks of the above-mentioned treatments, the identification of novel inhibitors of kidney cancer signaling pathways remains highly desirable. Toward this end, a collection of plant extracts was screened against an NCI 60-cell panel containing renal cancer cell lines along with eight other organ panels. Selecting for specific inhibitors of renal cancer cell lines led to the identification of englerin A and B, metabolites of *Phyllanthus engleri*, which is a plant indigenous to the East African countries of Tanzania and Zimbabwe. Englerin A (**1**) is a densely functionalized guaiane sesquiterpenoid with an oxatricyclic core flanked by two opposing ester side chains (Scheme 1). Following Beutler's initial report[2] on the strong and selective activity of **1** against six of eight renal cancer cell lines, both compounds




***Scheme 1.*** Strategy for the diverted synthesis of englerin A derivatives from intermediate **2** (green box) or earlier intermediates (red box). TBS = *tert*-butyldimethylsilyl.

received immediate attention from the synthetic community. In 2009, our research group established the previously unknown absolute configuration of englerin A by total synthesis of its (+)-enantiomer.[3] Shortly after, the groups of Ma[4] and Echavarren[5] reported independent total syntheses of (−)-**1** using elegant gold-catalyzed cyclization cascade reactions of open-chain precursors. A fourth synthesis of englerin was published by Nicolaou, Chen, and co-workers[6] and features an intermolecular rhodium-catalyzed [4+3] cycloaddition strategy and progress reports from other groups[7] are harbingers of ongoing interest.

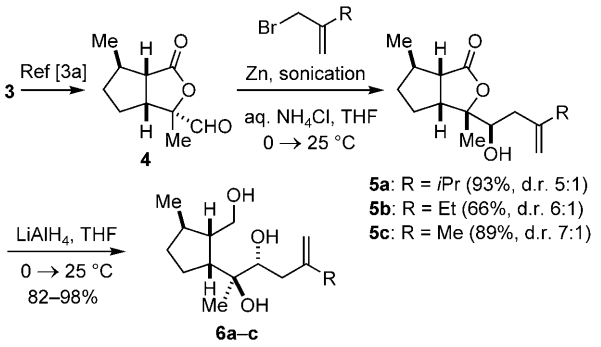
Herein, we report a reliable and scalable route to englerin's natural enantiomer (−)-**1** via intermediate **2**, encompassing englerin's complete guaiane core (**2**). This material fuelled an extensive SAR study[8] aimed to clarify the role of englerin's two ester side chains (green boxes). Diverting the synthesis[9] at an earlier intermediate allowed us to probe the influence of the isopropyl group (red box). When devising a strategy for a total synthesis program, it is mandatory to plan for the preparation of both enantiomers when the absolute configuration is unknown. The monoterpene nepetalactone possesses an iridoid substructure that is primed for olefin oxidation leading to englerin's oxidation pattern. While (+)-nepetalactone is readily available in bulk quantities from essential oil companies, the (−)-enantiomer **3** can be synthesized from (+)-citronellal on multigram scale following Schreiber's protocol.[10] In our initial total synthesis, the absolute configuration of englerin A was deduced from natural (+)-nepetalactone. For the biological evaluation of the natural product and key analogues, we had to start from synthetic nepetalactone **3** and thus taking advantage of the previously developed chemistry.[3a] This material was con-

[*] L. Radtke, Dr. M. Willot, S. Sauerland, Prof. C. Strohmann,[+] Prof. H. Waldmann, Prof. M. Christmann
TU Dortmund University, Faculty of Chemistry
Otto-Hahn-Strasse 6, 44227 Dortmund (Germany)
Fax: (+49) 231-755-5363
E-mail: mathias.christmann@tu-dortmund.de
Homepage: http://www.chemie.tu-dortmund.de/christmann
Dr. R. Fröhlich[+]
University of Münster, Institute of Organic Chemistry
Corrensstrasse 40, 48149 Münster (Germany)
Dr. H. Sun, Dr. S. Ziegler, Prof. H. Waldmann
Department of Chemical Biology
Max-Planck-Institute of Molecular Physiology
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany)
Dr. P. Habenberger
Lead Discovery Center GmbH
Emil-Figge-Strasse 76a, 44227 Dortmund (Germany)

[+] X-ray crystal structure analysis.

[**] We thank the Fonds der Chemischen Industrie (Dozentenstipendium to M.C.), the Alexander von Humboldt Foundation for postdoctoral fellowships (to M.W. and H.S.) and finally, we thank Takasago International Corporation for generous donation of (+)-citronellal.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201007790.

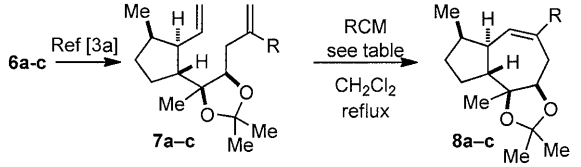



***Scheme 2.*** Diverting the synthesis from bicyclic aldehyde **4** using zinc-mediated allylation reactions. THF = tetrahydrofuran.

verted in decagram scale to the early key intermediate **4** and subjected to Barbier-type allylations (Scheme 2). At this juncture, we also opted for three different allyl bromides to investigate the role of the isopropyl substituent for the biological activity of the natural product. Reduction of the lactone (**5a–c**) with $LiAlH_4$ afforded the triols **6a–c** in excellent yields. The main portion of **4** was converted into the isopropyl derivative **5a**. Interestingly, the minor diastereomer of **5a** can be recycled in part by oxidation with IBX to the ketone and subsequent diastereoselective reduction of both the ketone and the lactone moiety ($LiAlH_4$, d.r. 3:1) to give crystalline triol intermediate **6a**.

In turn, the triols **6a–c** were converted into the dienes **7a–c**, which are precursors for the crucial ring-closing metathesis reaction. In the original total synthesis, we used 20 mol% of the second-generation Grubbs catalyst (Grubbs II) to achieve complete conversion of **7a** (R = *i*Pr) into the seven-membered ring with trisubstituted double bond. This amount could be lowered to 15 mol% without compromising the yield, whereas lower catalyst loadings led to incomplete conversions. Interestingly, when the size of the R group was decreased (*i*Pr > Et > Me), the rate of the ring-closing metathesis increased dramatically leading to excellent turnover and yields with as little as 5 mol% of catalyst (see Table 1).

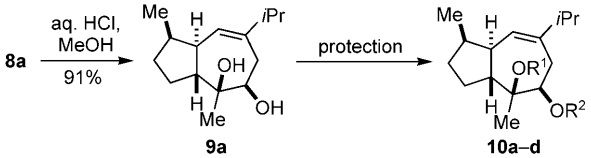
***Table 1:*** Synthesis of the englerin's guaiane core using a ring-closing metathesis (RCM) reaction.




| Entry | **7**: R | Catalyst (mol%) | *t* [h] | Yield [%][a] |
|---|---|---|---|---|
| 1 | **7a**: *i*Pr | Hoveyda–Grubbs (20) | 24 | 0 |
| 2 | **7a**: *i*Pr | Grubbs II (20) | 36 | 99 |
| 3 | **7a**: *i*Pr | Grubbs II (18) | 60 | 99 |
| 4 | **7a**: *i*Pr | Grubbs II (15) | 60 | 99 |
| 5 | **7a**: *i*Pr | Grubbs II (10) | 8 | 46[b] |
| 6 | **7a**: *i*Pr | Grubbs II (10) | 24 | 82[b] |
| 7 | **7b**: Et | Grubbs II (15) | 60 | 88 |
| 8 | **7b**: Et | Grubbs II (10) | 96 | 82[b] |
| 9 | **7c**: Me | Grubbs II (10) | 18 | 98 |
| 10 | **7c**: Me | Grubbs II (5) | 23 | 99 |

[a] Yield of isolated product. [b] Incomplete conversion.

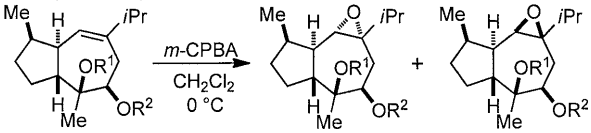
The major challenge in the remainder of the synthesis was the improvement of the moderate diastereoselectivity for the olefin epoxidation reported previously. Although the acetonide moiety exerted a very favorable influence on the desired facial selectivity (d.r. 11:1, see below), subsequent cleavage of the acetonide group in the presence of the epoxide group led to extensive decomposition. We therefore removed the acetonide unit prior to the epoxidation and studied the directing effect of several residues on the secondary alcohol (Scheme 3).




***Scheme 3.*** Removal of the acetonide group and protection of the hydroxy group(s).

Early introduction of the TBS-protected glycolate side (**10a**) led to a directed epoxidation in favor of desired diastereomer **11a** in good yield albeit with a moderate 2.3:1 ratio (Table 2, entry 1). Switching to the monoprotected TBS

***Table 2:*** Influence of the protecting group pattern on the diastereoselectivity of the epoxidation with *m*-CPBA.




| Entry | Alkene | $R^1$ | $R^2$ | Epoxide | **11**/**12** | Yield [%][a] |
|---|---|---|---|---|---|---|
| 1 | **10a** | H |  | **11a**,**12a** | 2.3:1 | 90 |
| 2 | **10b** | H | TBS | **11b**,**12b** | 5.4:1 | 91 |
| 3 | **10c** | TBS | TBS | **11c**,**12c** | 9:1 | 99 |
| 4 | **10d** | H | Troc | **11d**,**12d** | 1:20 | 73 |
| 5 | **8a** | 1,2-acetonide | | **11e**,**12e** | 11:1 | 78 |
| 6 | **9a** | H | H | **11f**,**12f** | 1:3 | 99 |

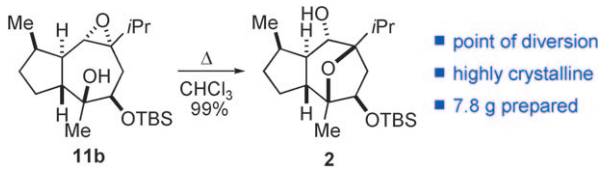
[a] Yield of isolated product. Troc = trichloroethoxycarbonyl.

derivative **10b** afforded a significant increase in selectivity (**11b**/**12b** = 5.4:1; Table 2, entry 2) without compromising the yield. This trend could be enhanced further with TBS groups on both alcohols (9:1, 99% yield; Table 2, entry 3). Most strikingly, protection of the secondary alcohol with the Troc group (Table 2, entry 4) resulted in a complete inversion of the facial selectivity. Despite the excellent selectivity of the acetonide derivative **8a** (Table 2, entry 5), problems with the subsequent conversions did not render this a viable alternative. Finally, epoxidation of the free diol **9a** (Table 2, entry 6) led to the preferred formation of the undesired diastereomer **12f**. The observed selectivities can be rational-

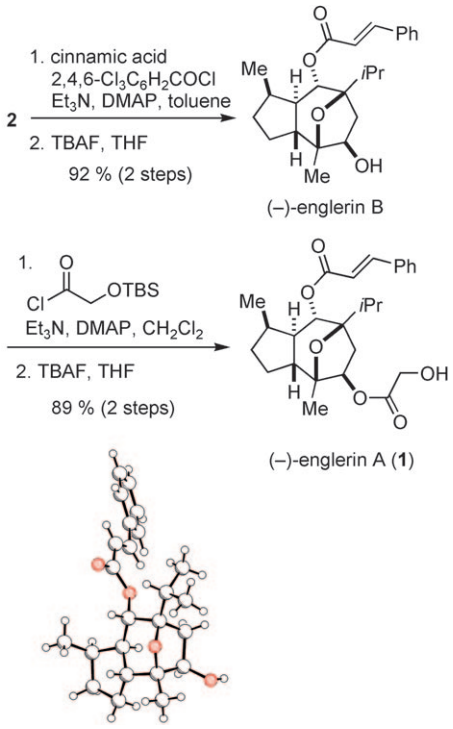
ized with the interplay of shielding one of the diastereotopic faces and the ability to coordinate the *m*-CPBA reagent and direct it to the same face.

For the completion of the synthesis we selected epoxide **11b** because it easily undergoes a transannular epoxide opening to form the highly crystalline key intermediate **2**, suitable for the subsequent introduction of different ester side chains (Scheme 4). In fact, we prepared 7.8 g of this material that served as base camp for the exploration of ester analogues of englerin A. As showcased below for the synthesis of the englerin A and B (see below), this robust and reliable route from **2** has allowed for the synthesis and evaluation of over 30 different ester derivatives.




***Scheme 4.*** Intramolecular epoxide opening to the key intermediate **2** for the synthesis of different diesters. DMAP = 4-dimethylaminopyridine, TBAF = tetra-*n*-butylammonium fluoride.

Esterification of **2** with cinnamic acid (or other acids for the synthesis of analogues) using a Yamaguchi esterification was followed by cleavage of the TBS ether mediated by TBAF to give englerin B as a crystalline solid (Scheme 5). In the enantiomeric series, we obtained single crystals that allowed for the unambiguous determination of the absolute




***Scheme 5.*** Completion of the synthesis of englerin A and B as blueprint for the synthesis of analogues.

configuration.[11] Finally, the glycolate ester side chain was introduced under standard conditions (DMAP, $Et_3N$, $CH_2Cl_2$), and subsequent removal of the TBS ether with TBAF concluding our second-generation synthesis of englerin A (**1**).

The chemistry described above enables the preparation of multigram quantities of englerin A, thus making total synthesis a viable method for sustainable supply for our ongoing biological studies. As the next step, we initiated structure-activity relationship (SAR) studies with two goals: 1) to investigate whether the potent activity of englerin A could be further enhanced by modification of the ester side chains or the isopropyl group, and 2) to identify sites within the molecule that are less sensitive to loss of bioactivity upon modification with the intention of rendering englerin A a tool for chemical biology research. The cytotoxicity of 32 englerin A derivatives (Scheme 6) was tested with the A498 kidney cancer cell line. This cell line was selected because of its high sensitivity towards englerin A as described by Beutler and coworkers.[2]

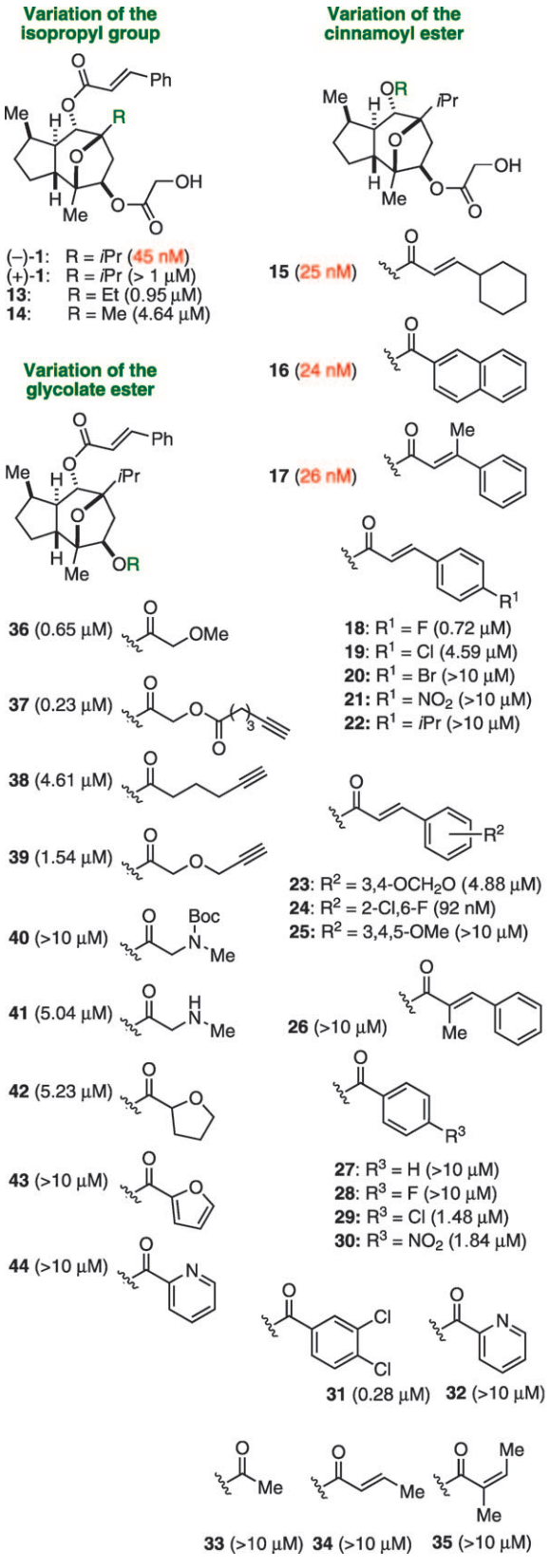
Comparison of the two synthesized enantiomers confirmed that (−)-**1** is highly cytotoxic with an $IC_{50}$ value of 45 nM while the enantiomer (+)-**1** is inactive up 1 μM. Replacing the isopropyl group with an ethyl group (**13**) or a methyl group (**14**) resulted in a 20-fold (Et) and 100-fold (Me) decrease of activity. We then turned our attention to the cinnamic ester side chain. Most strikingly, we were able to identify three derivatives with approximately twice the potency of the parent natural product englerin A (**1**). The 3-cyclohexyl acrylate **15**, the 3-naphthoate **16**, and the 3-methylcinnamate derivative **17** indicate that this part of the molecule is very responsive to structural changes in a positive sense. Substitutions in the aromatic ring of the cinnamate residue resulted in a decreased activity (**18**–**25**). Interestingly, benzoates were active down to the low micromolar range (**27**–**31**). The glycolate ester domain proved to be extremely sensitive toward modification. Methylation of the hydroxyl group (**36**) afforded a 14-fold decrease in activity. Esterification at the same position retains some activity; however it is unclear whether the ester **37** is rather a prodrug. Despite the diminished activity, derivatives **38** and **39** offer an alkyne handle for the future attachment of molecular probes. Other derivatives (**40**–**44**) did not show activities in the nanomolar range. The dose-response curves for the four most potent compounds including englerin A are shown in Figure 1.

The three most active derivatives of englerin (**15**–**17**) show activity within the same magnitude. To select for the most promising candidate for future derivatizations, we ran viability assays with four other cell lines (Table 3). While none of

***Table 3:*** Assessing the viability (μM) in four cell lines.

| Entry | Compound | Cell types | | | |
|---|---|---|---|---|---|
| | | Caki-1 | HEK293 | HeLa | MDA-MB-468 |
| 1 | **1** | –[a] | 24.8 | 15.9 | 17.7 |
| 2 | **15** | –[a] | 29.2 | >30 | >30 |
| 3 | **16** | –[a] | >30 | >30 | >30 |
| 4 | **17** | –[a] | 13.9 | 15.2 | 19.4 |

[a] Inactive, no $IC_{50}$ value was determined.




***Scheme 6.*** Structure-activity relationship (SAR) of the englerin analogues. Boc = *tert*-butoxycarbonyl.

the tested compounds showed potent activity in these assays, it turned out that compound **16** is virtually inactive in these

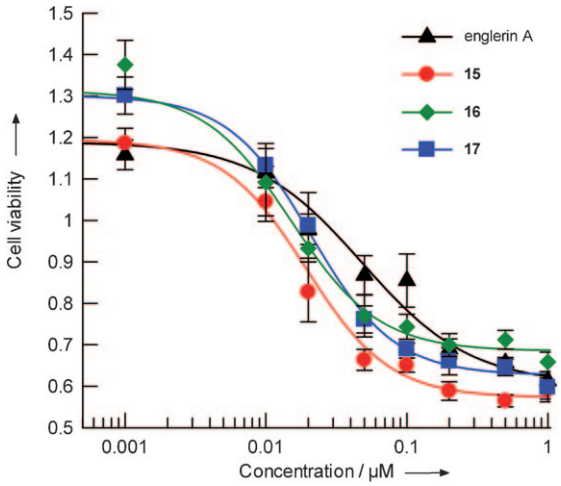



***Figure 1.*** Cytotoxicity dose-response curves are shown for **15**, **16**, and **17** in comparison to englerin A (–)-**1**. A498 cells were treated in four replicates with different concentration of the compounds for 48 h. Cytotoxic activity was determined using the WST-1 reagent. Data are shown as mean ($n = 4$) ± SD and were fitted using GraFit 5.0 software.

cell lines, rendering it a highly potent and selective inhibitor of the A498 kidney cancer cell line.

To investigate whether englerin and its active analogues are able to selectively target kidney cancer cell lines we investigated the cytotoxicity of compounds **1** and **15**–**17** in several normal kidney cell lines of different origin (MDCK, BSC-1, RC-124). To our delight the tested compounds differentiate between them and renal cancer cell lines and are more active on the cancer cell lines by at least a factor of 100 (with $IC_{50} > 5$ μM). Such selectivity is rare among potential candidate compounds for drug discovery and deserves particular attention. This finding highlights that englerin A and derivatives may be promising starting points for the development of novel agents for specific therapy of renal cell cancer by possibly avoiding common chemotherapy related side effects.

Here, we have disclosed our second-generation approach enabling the synthesis of an advanced englerin precursor **2** on multigram scale. From that point, thorough SAR studies of the two ester side chains have been conducted culminating in the discovery of derivatives with significantly improved activity over the natural product. Compound **16** shows improved cytotoxicity along with increased selectivity. In addition, we identified derivatives of the glycolate ester that allow for the attachment of molecular handles for future chemical biology studies. Diverting the synthesis at an earlier stage afforded analogues with a truncated isopropyl chain, which would be inaccessible by semisynthetic methods. Future studies will be concerned with understanding the molecular basis of englerin's extraordinary biological profile.

Received: December 10, 2010
Published online: March 30, 2011

**Keywords:** guaianes · renal cancer · structure-activity relationships · terpenes · total synthesis

[1] K. A. Furge, J. P. MacKeigan B. T. Teh, *Lancet Oncol.* **2010**, *11*, 571 – 578.
[2] a) R. Ratnayake, D. Covell, T. T. Ransom, K. R. Gustafson, J. A. Beutler, *Org. Lett.* **2009**, *11*, 57 – 60; b) J. A. Beutler, R. Ratnayake, D. Covell, T. R. Johnson, WO 2009/088854, **2009**.
[3] a) M. Willot, L. Radtke, D. Könning, R. Fröhlich, V. H. Gessner, C. Strohmann, M. Christmann, *Angew. Chem.* **2009**, *121*, 9269 – 9272; *Angew. Chem. Int. Ed.* **2009**, *48*, 9105 – 9108; b) M. Willot, M. Christmann, *Nat. Chem.* **2010**, *2*, 519 – 520.
[4] Q. Zhou, X. Chen, D. Ma, *Angew. Chem.* **2010**, *122*, 3591 – 3594; *Angew. Chem. Int. Ed.* **2010**, *49*, 3513 – 3516.
[5] K. Molawi, N. Delpont, A. M. Echavarren, *Angew. Chem.* **2010**, *122*, 3595 – 3597; *Angew. Chem. Int. Ed.* **2010**, *49*, 3517 – 3519.
[6] K. C. Nicolaou, Q. Kang, S. Y. Ng, D. Y.-K. Chen, *J. Am. Chem. Soc.* **2010**, *132*, 3517 – 3519.
[7] a) V. Navickas, D. B. Ushakov, M. E. Maier, M. Ströbele, H.-J. Meyer, *Org. Lett.* **2010**, *12*, 3418 – 3421; b) J. Xu, E. J. E. Caro-Diaz, E. A. Theodorakis, *Org. Lett.* **2010**, *12*, 3708 – 3711; c) K. Ishida, H. Kusama, N. Iwasawac, *J. Am. Chem. Soc.* **2010**, *132*, 8842 – 8843; d) B.-F. Sun, C.-L. Wang, R. Ding, J.-Y. Xu, G.-Q. Lin, *Tetrahedron Lett.* **2010**, DOI: 10.1016/j.tetlet.2010.11.087; e) D. Parmar, K. Price, M. Spain, H. Matsubara, P. A. Bradley, D. J. Procter, *J. Am. Chem. Soc.* **2011**, *133*, 2418 – 2420.
[8] For independent SAR studies, see: a) K. P. Chan, D. Y.-K. Chen, *ChemMedChem* **2011**, *6*, 420 – 423; b) D. B. Ushakov, V. Navickas, M. Ströbele, C. Maichle-Mössmer, F. Sasse, M. E. Maier, *Org. Lett.* **2011**, DOI: 10.1021/ol200499t.
[9] a) A. M. Szpilman, E. M. Carreira, *Angew. Chem.* **2010**, *122*, 9786 – 9823; *Angew. Chem. Int. Ed.* **2010**, *49*, 9592 – 9628; b) R. M. Wilson, S. J. Danishefsky, *J. Org. Chem.* **2006**, *71*, 8329 – 8351.
[10] S. L. Schreiber, H. V. Meyers, K. B. Wiberg, *J. Am. Chem. Soc.* **1986**, *108*, 8274 – 8277.
[11] We show (−)-englerin B in the picture although the crystal structure was determined for the (+)-enantiomer. CCDC 755644 ((+)-englerin B) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.