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Synthesis of a Cytotoxic Ansamycin Hybrid

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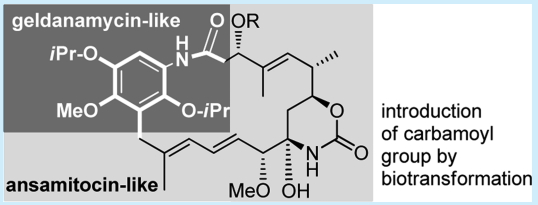
Supporting Information

ABSTRACT: The synthesis of a new ansamycin macrolactam derivative that contains an ansa chain based on ansamitocin and an aromatic core related to geldanamycin is reported. The selective introduction of the cyclic carbamoyl group at C7 and C9 relies on a biotransformation using a mutant strain of *S. hygroscopicus*, the geldanamycin producer. The ansamycin hybrid forms atropisomers that differ in their antiproliferative activity toward cancer cells.



The ansamycin antibiotics are a growing class of complex macrolactam antibiotics that are produced by various actinomycetes, but in the special case of maytansine (**4**) they can also occur in plants (Figure 1).[1] The ansamycin antibiotics

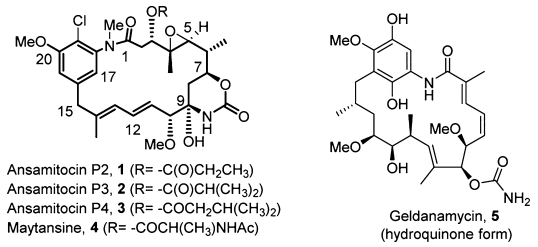


Figure 1. Structures of ansamycin antibiotics of the maytansinoid family **1**−**4**, geldanamycin.

possess a cyclic structure that is characterized by an aliphatic ansa chain that forms a bridge between two nonadjacent positions at the aromatic or quinone core. Important examples are the ansamitocins **1**−**3**, maytansine (**4**),[2,3] and geldanamycin (**5**).[4] The maytansinoids **1**−**4** exhibit strong antiproliferative activities in the lower nanomolar to subnanomolar range. A maytansin−antibody conjugate (trastuzumab emtansine; T-DM1, trade name Kadcyla) was approved as a chemotherapeutic against HER2-positive breast cancers early last year. While the maytansinoids bind to tubulin and inhibit its polymerization, the cytotoxicity of geldanamycin **5** is linked with the blocking of the ATP binding site of heat shock protein Hsp90α.[4]

The concept of hybrid architectures based on natural products has frequently been pursued in academia and the pharmaceutical industry.[5] The underlying idea is to develop a molecule that contains two different biological activities, and this is commonly achieved by linking two different small molecule drugs or natural products. The most prominent applications have been realized in conjugating antitumor drugs with tumor-specific ligands such as folic acid[6,7] or cyclic peptides.[8]

Although the maytansinoids and geldanamycin are structurally closely related as far as ring-size and position of functionalities are concerned, they target completely different proteins. Therefore, we pursued the idea to combine structural features recruited from both ansamycins antibiotics within one molecule. Since the exact mode of binding to Hsp90 is known for geldanamycin while unknown for the ansamitocins, we targeted a macrolactam composed of an ansamitocin backbone in order to maximize tubulin binding possibilities while the aromatic core resembles that of geldanamycin. The target hybrid ansamycins **6** and **7** would allow to participate in an H-bonding network with Hsp90 similar to geldanamycin[9] as assumed from SAR studies published for both biological targets.[10,11] We decided to pursue a total synthesis approach as we expected that late stage oxidation of the aromatic moiety of AP3 **2** would hardly be possible. Although supplementing the AHBA(−) mutant strain of *A. pretiosum* with a derivative of the biosynthetic starting building block 3,5-aminohydroxy benzoic acid is a powerful strategy in accessing new ansamitocins, it would fail here because substituents at C17 and C21 can not be incorporated by mutasynthesis.[12]

Retrosynthetically, we planned to chemoselectively introduce the cyclic carbamoyl group and the isobutyrate side chain at the very end of the synthesis via a mutasynthetic operation using our mutant strains of *A. pretiosum* or *S. hygroscopicus*. For carrying out this mutasynthesis, proansamitocin derivative **8** became one key intermediate (Scheme 1).[21f] Macrolactam **8** is further dissected into vinyl iodide **9** and the eastern ansachain **10** which could be merged by an intermolecular Heck reaction[13] followed by a Goldberg macrolactamization.[14] Importantly, Panek and co-workers demonstrated the feasibility of the Goldberg macrolactamization with electron rich aromatic systems in their total synthesis of geldanamycin.[15] We planned to access pentasubstituted arene **9** from compound **11**. We also planned to employ a vinylogous aldol reaction as key step for the synthesis of the ansa chain **10**.[16] For achieving this highly

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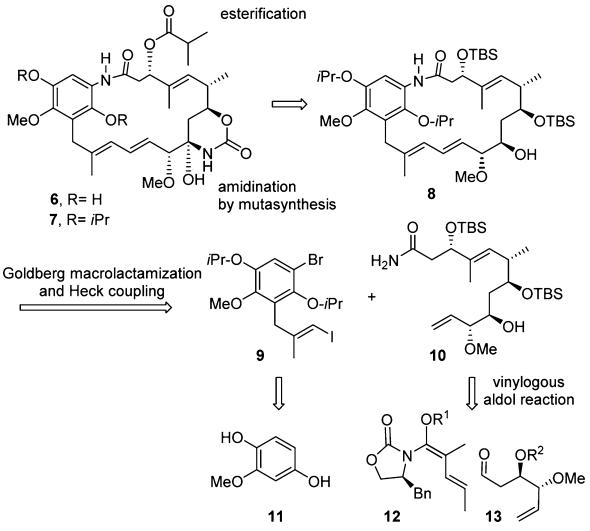
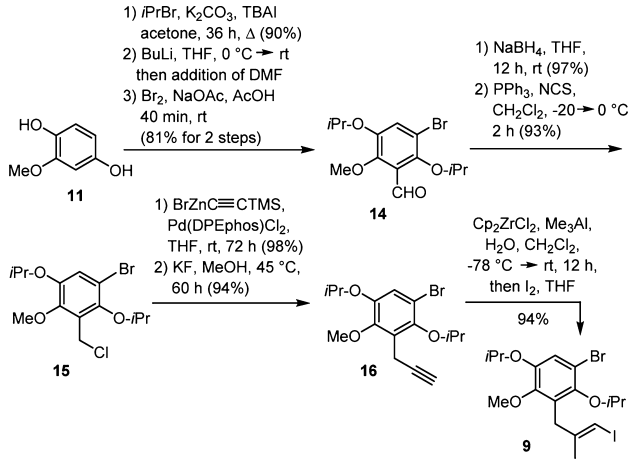
Scheme 1. Retrosynthetic Plan



convergent step, facile access to enol **12** and aldehyde **13** was necessary. Aldehyde **13** was chosen as a key building block because alternative synthetic routes could be envisaged from **13** if the vinylogous aldol reaction would turn out to be problematic.

The synthesis of the western fragment commenced from hydroquinone **11** (Scheme 2). A sequence of protection,

Scheme 2. Synthesis of the Aromatic Fragment 9

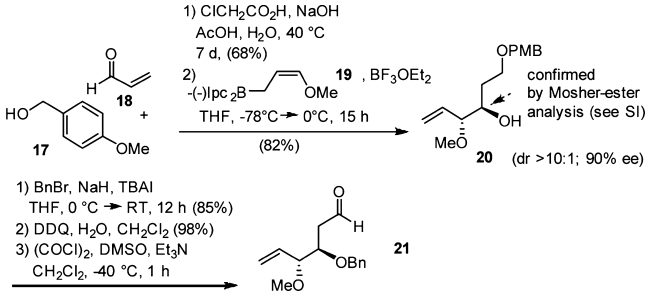


formylation, and bromination yielded the pentasubstituted aromatic core **14**.[15] Reduction followed by Appel-type reaction then provided compound **15**. Palladium-catalyzed coupling of **15** with an alkynyl zinc reagent,[17] followed by mild desilylation, provided compound **16**. Finally, zirconium-mediated carboalumination[18] followed by iodination provided vinyl iodide **9**.

The synthesis of aldehyde **21** started with the Michael addition of *p*-methoxybenzyl acohol **17** to acroleine **18** (Scheme 3).[19] The resulting aldehyde was subjected to a Brown allylation[20] using the *Z*-configured allyl borane **19**, which furnished alcohol **20** with high stereocontrol. Mosher ester analysis confirmed the absolute configuration of the carbinol moiety.[21]

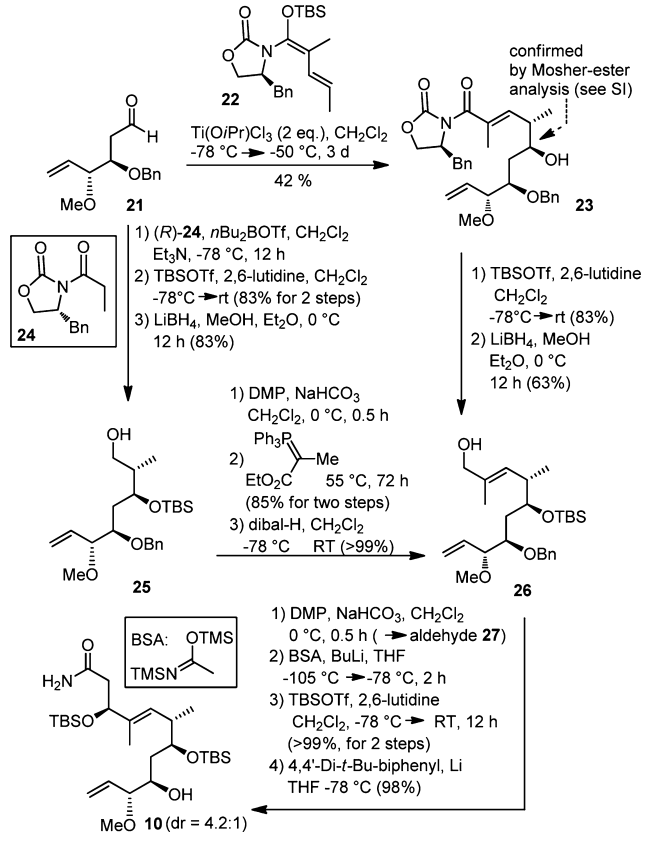
For assembling the carbon backbone of C3−C10[22] aldehyde **21** was subjected to a vinylogous aldol protocol[16] based on Kobayashi's work.[23] Thus, formation of amide **23** proceeded in

Scheme 3. Synthesis of the C7−C12 Fragment[22]



moderate yield when aldehyde **21** was coupled with vinylogous silyl enol ether **22** (Scheme 4). We faced difficulties with

Scheme 4. Two Synthetic Approaches toward the C3−C12 Fragment



reproducibility on large scale, and substantial optimization (e.g., Lewis acids, choice of silyl group) did not improve the results. The configuration of the newly formed carbinol moiety was confirmed by Mosher ester analysis and through chemical correlation using material obtained through the independent route (Scheme 4). This second route involved a *syn* Evans aldol reaction followed by protection of the secondary alcohol and reductive cleavage of the auxiliary to provide alcohol **25**. A sequence of oxidation, Wittig olefination, and reduction then furnished allylic alcohol **26**.

Concerning the vinylogous aldol reaction, we found that there the choice of Lewis acid is delicate. Strong Lewis acids such as $TiCl_4$ enforce degradation, while milder Lewis acids did not lead to full conversion and reactants **21** and **22** were recovered in high yield. The synthesis of the polyketide

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ansachain was finalized by oxidation of the allylic alcohol **26** to the aldehyde **27** followed by an aldol reaction using a BSA-derived lithium enolate.[24] The diasterofacial selectivity of this substrate-controlled process was good at low temperatures and gave the desired 3*S*-configured diastereoisomer. The preference for *si*-face attack can be rationalized from the preferred conformation of aldehyde **27** (Figure 2), which minimizes

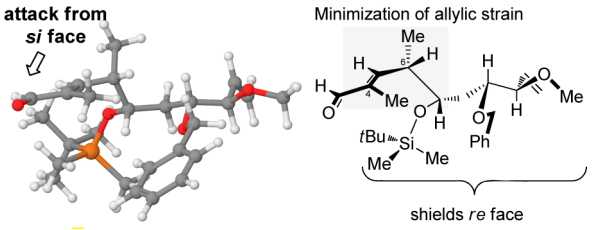


Figure 2. Lowest energy conformation of **27**.[25]

the allylic strain of the methyl groups in C4 and C6.[22] TBS-protection and radical-initiated removal of the benzyl protecting group yielded the C1−C11 fragment **10** (Scheme 4).

The diasterofacial selectivity of this substrate-controlled process was good at low temperatures and gave the desired 3*S*-configured diastereoisomer. The preference for *si*-face attack can be rationalized from the preferred conformation of aldehyde **27** (Figure 3), which minimizes the allylic strain of

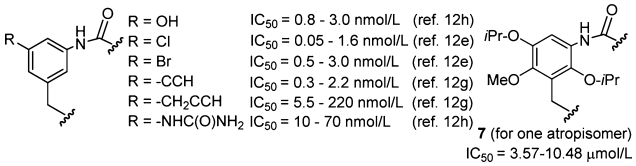


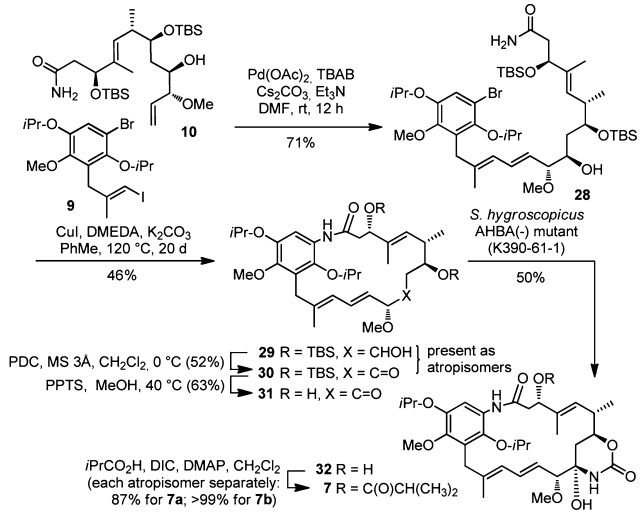
Figure 3. Antiproliferative activities of **7** and of ansamitocin derivatives structurally related to derivative **7**.

the methyl groups in C4 and C6.[25] TBS-protection and radical-initiated removal of the benzyl protecting group yielded C1−C11 fragment **10** (Scheme 4).

Heck coupling under Jeffrey conditions yielded seco carboxamide **28**, which was cyclized under Goldberg conditions. Surprisingly, the resulting macrolactam **29** was present as two atropisomers. These could be separated chromatographically, and the thermodynamically less favored could be converted into the other one when being heated in DMF at 145 °C.

We had to test a series of oxidants (e.g., DMP, Swern, TPAP, Oppenauer) to establish the keto functionality at C9 before PDC and molecular sieves (3 Å) were found to be best suited to deliver compound **30**.[26] During these trials, we observed decomposition or oxidation of the electron-rich aromatic moiety. Ketone **30** was desilylated using PPTS, which provided macrolactam **31**. The removal of the bulky silyl groups provided rotational flexibility in the macrocycle and no conformationally stable atropisomers could be detected anymore. The carbamoyl group was selectively introduced by a mutasynthetic biotransformation[27] using an aminohydroxybenzoic acid blocked mutant of the geldanamycin producer *S. hygroscopicus*[12f] which provided ansamycin derivative **32**. Surprisingly, the corresponding AHBA(−) mutant of the ansamitocin producer *A. pretiosum* did not perform any tailoring modifications on macrolactam **31** despite the fact that the ansachain in **31** closely resembles proansamitocin.[12f] Finally, introduction of the ester side chain provided ansamycin derivative **7** (Scheme 5), but all attempts to remove the isopropyl groups and generate hydroquinone **6** failed, an observation that Bach et al. also noted recently.[28,29]

Scheme 5. Chemoenzymatic Endgame of Ansamycin Synthesis (7a and 7b Refer to Atropisomers)



Compounds **32** and **7** demonstrated atropisomerism for which the two isopropoxy groups can be made responsible. One of the two atropisomer of **32** and **37** was conformationally stable and could be separated via HLPC and spectroscopically characterized. In contrast, the other atropisomers showed broad lines in the ^{1}H NMR spectra likely due to the presence of several conformers.

Comparison of the NMR data suggest that the stable atropisomers exhibit a similar conformation to ansamitocin P3 (**2**) in solution.

Biological evaluation conducted with the conformationally stable atropisomers of **7** and **32** revealed no antiproliferative activity toward mouse fibroblasts L929 and selected cancer lines, which contrasts other very potent ansamitocin derivatives that also lack the *N*-methyl group and the oxirane ring (for selected data, see Figure 3). To our surprise, the minor atropisomers of **7** and **32** exhibit a pronounced acitivity against cervix carcinoma cells KB-3−1 (2.2 and 5.0 μg mL^{-1}, respectively) as well as against ovary adenocarcinoma cells SKOV-3 (4.5 and 7.2 μg mL^{-1}, respectively). Since these isomers conformtionally do not resemble ansamitocin it is likely that the antiproliferative activity is based on a completely different biological mechanism, particularly as they also showed no inhibitory activity against Hsp90α in a competition assay with fluorescent-labeled ATP.[25]

In conclusion, we accomplished the synthesis of the first ansamitocin hybrid derivative **7** that resembles the aromatic substitution pattern of geldanamycin. Key steps are a highly selective mutabiosynthetic step, a Goldman cyclization, and a Heck coupling of the two main fragments. The biological evaluation unexpectedly revealed that one of two atropisomers irrespective whether acylated at C3 or not shows antiproliferative activity against selected cancer cell lines but does not target Hsp90α. As all tubulin-addressing ansamitocins require

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esterification, one can speculate whether **7** is an ansamycin derivative addressing a new biological target. It needs to be noted that the conformational flexibility of these atropisomers are important for being able to adapt to a biological target including a new one. Our current studies are directed toward elucidating the biological target of macrolactam **7** and analogues.

■ ASSOCIATED CONTENT

Supporting Information

Descriptions of experimental procedures for compounds, analytical characterization and description of biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Funayama, S.; Cordell, G. A. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: New York, 2000; Vol. *23*, pp 51−106.
(2) Cassady, J. M.; Chan, K. K.; Floss, H. G.; Leistner, E. *Chem. Pharm. Bull.* **2004**, *52*, 1−26.
(3) Kirschning, A.; Harmrolfs, K.; Knobloch, T. *C. R. Chim.* **2008**, *11*, 1523−1543.
(4) Franke, J.; Eichner, S.; Zeilinger, C.; Kirschning, A. *Nat. Prod. Rep.* **2013**, *30*, 1299−1323.
(5) Tietze, L. F.; Bell, H. P.; Chandrasekhar, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 3996−4028; *Angew. Chem.* **2003**, *42*, 3996−4028.
(6) Taft, F.; Harmrolfs, K.; Nickeleit, I.; Heutling, A.; Kiene, M.; Malek, N.; Sasse, F.; Kirschning, A. *Chem.—Eur. J.* **2012**, *18*, 880−886.
(7) Salazar, M. D.; Ratnam, M. *Cancer Metastasis Rev.* **2007**, *26*, 141−152.
(8) Haubner, R.; Wester, H. J.; Burkhart, F.; Senekowitsch-Schmidtke, R.; Weber, W.; Goodman, S. L.; Kessler, H.; Schwaiger, M. *Bioconjugate Chem.* **2001**, *12*, 84−91.
(9) Stebbins, C. E.; Russo, A. A.; Schneider, C.; Rosen, N.; Hartl, F. U.; Pavletich, N. P. *Cell* **1997**, *89*, 239−250.
(10) Kawai, A.; Akimoto, H.; Kozai, Y.; Ootsu, K.; Tanida, S.; Hashimoto, N.; Nomura, H. *Chem. Pharm. Bull.* **1984**, *32*, 3441−3451.
(11) Janin, J. L. *J. Med. Chem.* **2005**, *48*, 7503−7510.
(12) (a) Kubota, T.; Brünjes, M.; Frenzel, T.; Xu, J.; Kirschning, A.; Floss, H. G. *ChemBioChem* **2006**, *7*, 1221−1225. (b) Taft, F.; Brünjes, M.; Floss, H. G.; Czempinski, N.; Grond, S.; Sasse, F.; Kirschning, A. *ChemBioChem* **2008**, *7*, 1057−1060. (c) Taft, F.; Brünjes, M.; Knobloch, T.; Floss, H. G.; Kirschning, A. *J. Am. Chem. Soc.* **2009**, *131*, 3812−3813. (d) Harmrolfs, K.; Brünjes, M.; Dräger, G.; Floss, H. G.; Sasse, F.; Taft, F.; Kirschning, A. *ChemBioChem* **2010**, *11*, 2517−2520. (e) Knobloch, T.; Floss, H. G.; Harmrolfs, K.; Knobloch, T.; Sasse, F.; Taft, F.; Thomaszewski, B.; Kirschning, A. *ChemBioChem* **2011**, *12*, 540−547. (f) Eichner, S.; Knobloch, T.; Floss, H. G.; Fohrer, J.; Harmrolfs, K.; Hermane, J.; Schulz, A.; Sasse, F.; Spiteller, P.; Taft, F.; Kirschning, A. *Angew. Chem.* **2012**, *124*, 776−781; *Angew. Chem., Int. Ed.* **2012**, *51*, 752−757. (g) Harmrolfs, K.; Mancuso, L.; Thomaszewski, B.; Sasse, S.; Kirschning, A. *Beilstein J. Org. Chem.* **2014**, *10*, 535−543. (h) Mancuso, L.; Jürjens, G.; Hermane, J.; Harmrolfs, K.; Eichner, S.; Fohrer, J.; Sasse, F.; Colisi, V.; Kirschning, A. *Org. Lett.* **2013**, *15*, 4442−4445.
(13) (a) Jeffrey, T. *Tetrahedron Lett.* **1985**, *26*, 2667−2670. (b) Jeffrey, T. *Tetrahedron* **1996**, *52*, 10113−10130.
(14) Goldberg, I. *Chem. Ber.* **1906**, *39*, 1691−1692.
(15) Qin, H.-L.; Panek, S. *Org. Lett.* **2008**, *10*, 2477−2479.
(16) Kalesse, M.; Cordes, M.; Symkenberg, G.; Lu, H.-H. *Nat. Prod. Rep.* **2014**, *31*, 563−594.
(17) Qian, M.; Negishi, E. *Tetrahedron Lett.* **2005**, *46*, 2927−2930.
(18) (a) Wipf, P.; Lim, S. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1068−1071. (b) Zhu, G.; Negishi, E. *Chem.—Eur. J.* **2008**, *14*, 311−318.
(19) Herb, C.; Meier, M. E. *J. Org. Chem.* **2003**, *68*, 8129−8135.
(20) (a) Brown, H. C.; Jadhav, P. K.; Bhat, K. S. *J. Am. Chem. Soc.* **1988**, *110*, 1535−1538. (b) Brown, H. C.; Jadhav, P. K. *J. Org. Chem.* **1984**, *49*, 4091−4092.
(21) (a) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543−2549. (b) Ohtani, I.; Kasumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096. (c) Hoye, T. R.; Jeffrey, C. S.; Shao, F. *Nature Prot.* **2007**, *2*, 2451−2454.
(22) Throughout the text we use numbering of the ansamitocins.
(23) Shirokawa, S.-i.; Kamiyama, M.; Nakamura, T.; Okada, M.; Nakazaki, T.; Hosokawa, S.; Kobayashi, S. *J. Am. Chem. Soc.* **2004**, *126*, 13604−13605.
(24) Morwick, T. *Tetrahedron Lett.* **1980**, *21*, 3227−3230.
(25) Details are provided in the Supporting Information.
(26) Herscovici, J.; Egron, M.-J.; Antonakis, K. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1967−1973.
(27) (a) Kirschning, A.; Hahn, F. *Angew. Chem.* **2012**, *124*, 4086−4096; *Angew. Chem., Int. Ed.* **2012**, *51*, 4012−4022. (b) Kirschning, A.; Taft, F.; Knobloch, T. *Org. Biomol. Chem.* **2007**, 3245−3295.
(28) Hampel, T.; Neubauer, T.; van Leeuwen, T.; Bach, T. *Chem.—Eur. J.* **2012**, *18*, 10382−10392.
(29) Protecting group strategies for phenolic analogues of geldanamycin are reported by: Wrona, I. E.; Gozman, A.; Taldone, T.; Chiosis, G.; Panek, J. S. *J. Org. Chem.* **2010**, *75*, 2820−2835.