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Total Synthesis of Cytospolide D and Its Biomimetic Conversion to Cytospolides M, O, and Q

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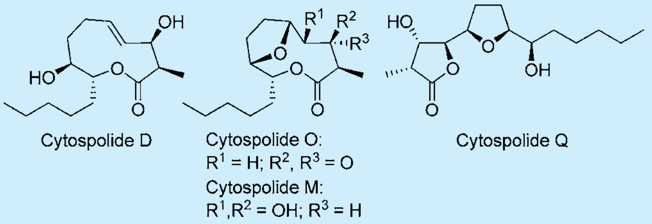
S *Supporting Information*

ABSTRACT: A total synthesis of cytospolide D, starting from L-glutamic acid, is described. The critical macrolactonization to the 10-membered lactone containing an (*E*)-configured double bond was successfully achieved by Shiina esterification. Conversion of cytospolide D to its bicyclic derivatives M, O, and Q was accomplished under mild conditions, lending support to the proposed biosynthetic hypothesis.



The first five members (**1**−**5** in Figure 1) of the group of cytospolides were isolated by Zhang et al. from extracts of the fungus *Cytospora* sp. growing on leaves of the evergreen shrub *Ilex canariensis*.[1] Cytospolides A−D (**1**−**4**) share the same carbon skeleton and only differ in the acetylation pattern of the two hydroxy groups (Figure 1). Interestingly, the C-2 epimer of **4**, cytospolide E (**5**), was also isolated from the same natural source. The inversion of the C-2 methyl group in cytospolide E (**5**) to the (2*S*)-configuration was found to significantly increase its activity against the A-549 tumor cell line. Shortly after the first publication, Zhang et al. reported on the discovery of 13 closely related natural products (cytospolides F−Q and decytospolides A and B) isolated from extracts of the same fungus cultivated on biomalt agar medium.[2] Here, cytospolides P (**8**) and Q (**9**) emerged as the most potent congeners with activity against different cancer cell lines.

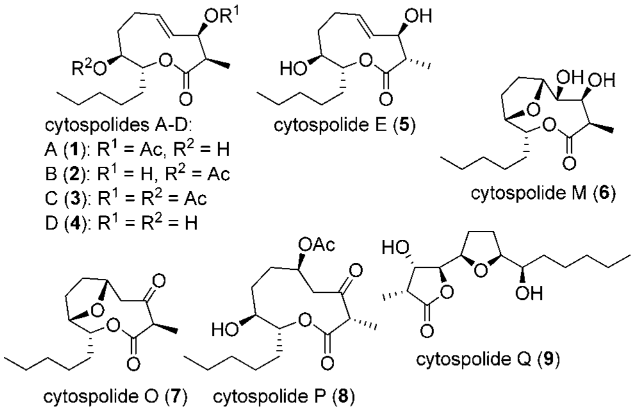
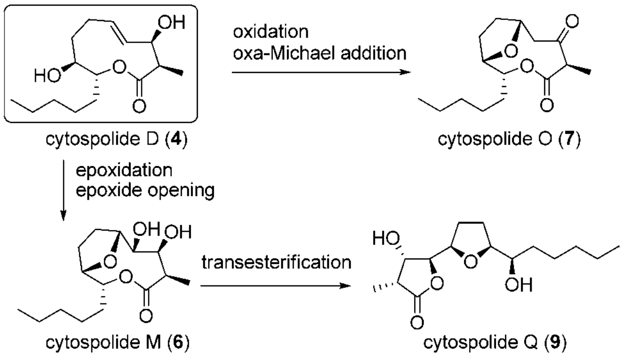


Figure 1. Selected structures of cytospolides.

Except for the decytospolides, all newly isolated metabolites possess a more highly oxygenated carbon skeleton. Biosynthetically, cytospolides O (**7**) and P (**8**) are believed to be formed by an intra- or intermolecular oxa-Michael addition to the respective enones of **4** and **5**, while the bicyclic cytospolide M (**6**) is presumably derived from **4** by epoxidation and subsequent transannular epoxide opening. The *γ*-lactone **9** can be traced back to cytospolide M (**6**) via a transesterification (Scheme 1). Since the first publication, especially the more bioactive members of the cytospolide family, namely E (**5**) and P (**8**), have attracted significant research interest,[3−5] which has culminated in the publication of several total syntheses.[6−9]

Scheme 1. Proposed Biosynthetic Relationship between Cytospolides M, O, Q, and D



As part of our interest in biomimetic natural product chemistry,[10] we were aiming to establish a synthetic route toward cytospolide D (**4**) and to investigate its conversion into its higher oxygenated derivatives M (**6**), O (**7**), and Q (**9**), all of which have not been synthesized yet. Our retrosynthetic analysis of cytospolide D (**4**) is based on the retro-macrolactonization of **4** to give *seco*-acid **10** with a protected hydroxy group at C-3 as a precursor (Scheme 2). We envisaged the 2,3-*anti* relationship of the C-2 methyl group and the hydroxy group at C-3 to be

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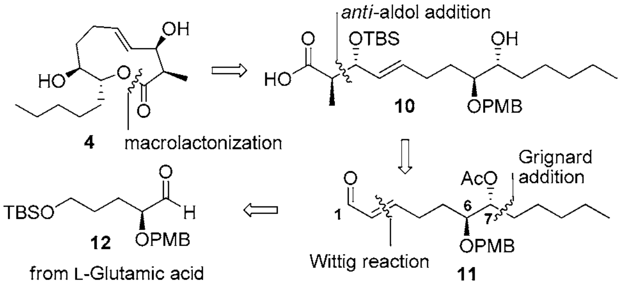
Scheme 2. Retrosynthetic Analysis of Cytospolide D



established by an *anti*-aldol addition of enal **11** using Paterson's method.[11] The enal moiety in **11** can be assembled by Wittig homologation using a stabilized ylide already bearing the aldehyde functionality.[12] For installation of the pentyl side chain we proposed a Grignard addition of pentylmagnesium bromide to α-chiral aldehyde **12**. In the case of a chelating protecting group such as PMB, the diastereoselectivity can be assumed according to the Cram-chelate model[13] which would give rise to the undesired 4,5-*syn* diol **13**. Conceivable means to invert the stereochemistry may be provided either by switching the mechanism to the polar Felkin model with nonchelating protection groups[14] or by a subsequent Mitsunobu reaction[15] of 4,5-*syn* diol **13**. Aldehyde **12** should be accessible from (2*S*)-1,2,5-pentanetriol (**16**).

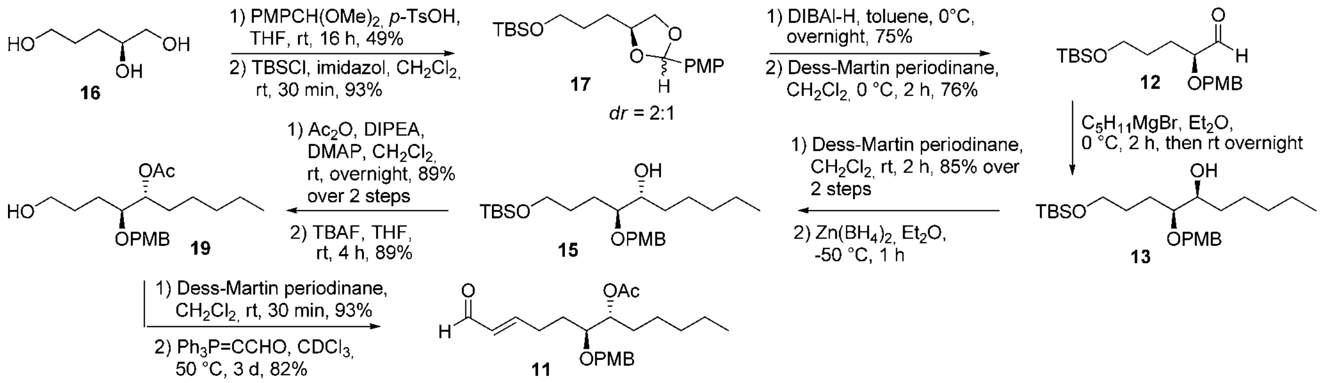
The synthesis commenced with (2*S*)-1,2,5-pentanetriol (**16**) obtained from L-glutamic acid.[16,17] In order to differentiate the hydroxy groups we installed the 1,2-*p*-methoxybenzylidene acetal, leaving the C-5 hydroxy group unprotected (Scheme 3). At C-5 we decided to introduce a TBS ether. Next PMP acetal **17** was opened with DIBAl-H at 0 °C in toluene.[18] Under these conditions the ratio of regioisomers was determined as 10:1 in favor of the desired secondary PMB ether **18**. Oxidation of **18** to the corresponding aldehyde **12** with Dess-Martin periodinane[19] proceeded with a 76% yield. When using basic reaction conditions as for instance with the Swern protocol,[20] diminished yields were obtained. Next, we investigated the diastereoselectivity of the Grignard addition. Thus, aldehyde **12** was reacted with pentylmagnesium bromide in diethyl ether, resulting in the exclusive formation of the 4,5-*syn* diol **13** in good yield. The configuration of diol **13** was assigned by NMR after deprotection of the PMB group and installation of an acetonide (see the Supporting Information for details).

The exclusive formation of the 4,5-*syn* diol **13** is indicative of the fact that the Grignard addition follows the Cram-chelate model. When switching to a nonchelating TBS group in the α-position or altering the reaction conditions (THF as solvent and pentyllithium as reagent) the yields markedly decreased and the selectivity only changed to 2:1 for the 4,5-*anti* diol **15**. We therefore investigated the inversion at C-5 by Mitsunobu esterification[15] with acetic acid.[21] Here, we observed a decline in the diastereomeric ratio from over 95:5 for the 4,5-*syn* diol **13** to 5:1 for the 4,5-*anti* product and a low yield of 33%. The decreased diastereomeric ratio can be explained by a partial direct esterification process by acyloxyphosphonium salts occurring in competition with the anticipated inversion.[22] As neither a change in selectivity in the Grignard addition nor the one-step inversion via Mitsunobu esterification led to an acceptable diastereoselectivity, we next tried to invert the C-5 hydroxy group by means of an oxidation−reduction sequence. Oxidation of **13** to α-alkoxy ketone **14** proceeded in high yields with Dess-Martin periodinane.[19] For the selective reduction of α-alkoxy ketones with chelating protecting groups, zinc borohydride has found ample use.[23] In our case, the reduction provided 4,5-*anti* diol **15** in a high diastereoselectivity of 94:6. The C-5 hydroxy group was then protected as acetate in order to allow for a concomitant cleavage of acetate and benzoate in the final stages of the synthesis prior to macrolactonization. Next, the primary TBS ether was cleaved and alcohol **19** was oxidized to aldehyde **20**. For the Wittig homologation we decided to use (triphenylphosphoranylidene)-acetaldehyde.[12] Although its reactivity with aldehyde **20** is quite low as compared to ethyl (triphenylphosphoranylidene)-acetate, the synthesis can be shortened by two subsequent transformations. The low reactivity of this stabilized ylide required prolonged heating up to 50 °C for 3 d. The conversion was monitored by running the reaction in $CDCl_3$ and ^1H NMR analysis at intervals.

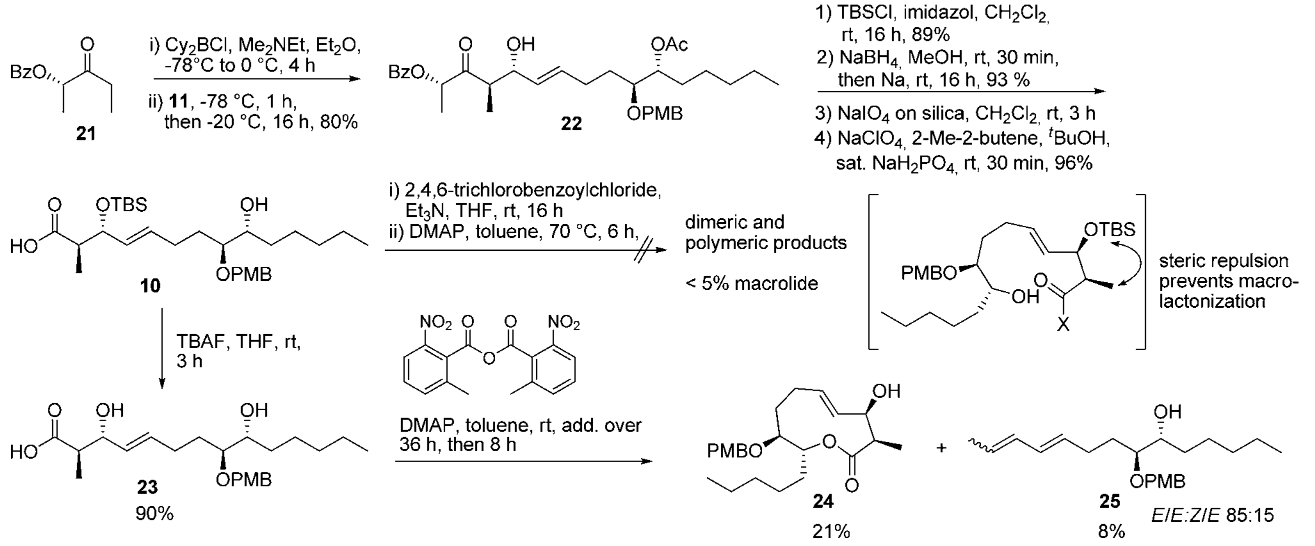
To complete the assembly of the carbon skeleton we applied the Paterson *anti*-aldol addition with (2*S*)-benzoyloxy-3-pentanone (**21**)[11] to establish the desired (2*R*,3*R*) configuration of carboxylic acid **10** (Scheme 4). The C-3 hydroxy group of aldol product **22** was protected as TBS ether. The chiral auxiliary was then cleaved by reduction of the ketone with sodium borohydride, saponification of the benzoate as well as the acetate, and subsequent glycol cleavage[24] of the resulting 2,3-diol. The ensuing aldehyde was directly oxidized to *seco*-acid **10** using sodium chlorite.[25,26]

Having synthesized this macrolide precursor **10**, we next investigated the macrolactonization. A similar *seco*-acid with a different set of protecting groups (PMB at C-3 and Bn at C-8) had been cyclized in 40% yield by Kamal et al.[9] using the Yamaguchi

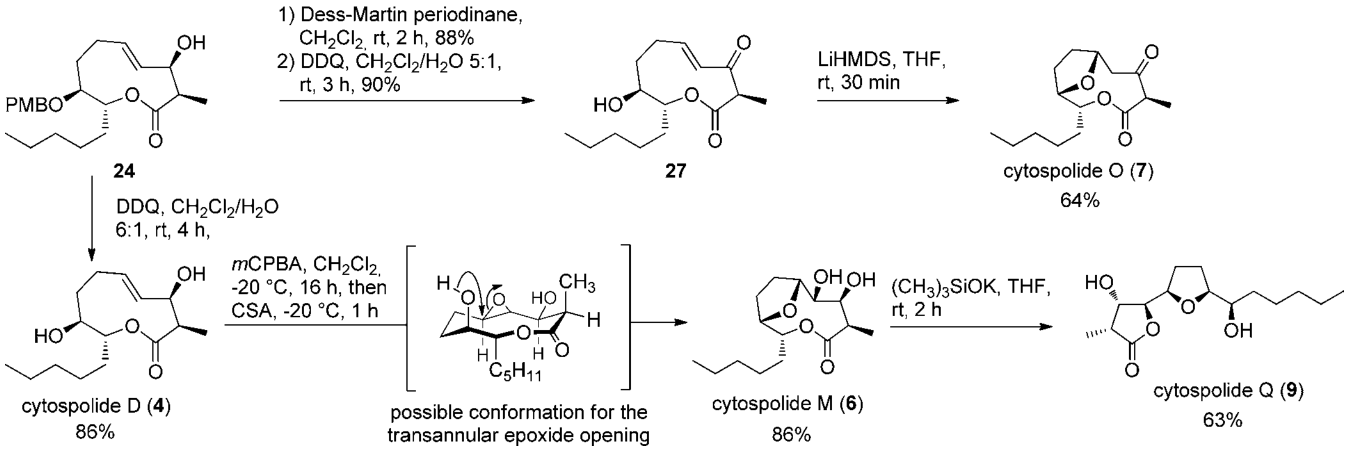
Scheme 3. Synthesis of Aldehyde 11



Scheme 4. Synthesis of *seco*-Acids 10 and 23 and Investigations into the Macrolactonization



Scheme 5. Synthesis of Cytospolide D and its Biomimetic Conversion to Cytospolides M, O, and Q



esterification.[27] However, when we screened these conditions with our substrate, the desired 10-membered macrolide was only formed in very low yields of less than 5%. Neither prolonging the period of addition up to 16 h nor variation of the temperature or concentrations significantly improved the outcome. The low tendency of the mixed anhydride to form the 10-membered macrolide may, in addition to the obvious ring strain, be attributed to an unfavorable steric repulsion between the C-2 methyl group and the large TBS ether at C-3 (Scheme 4). In this respect it is worth noting that during the synthesis of cytospolide P (**8**) by Raju et al.[6] the C-2 epimeric *seco*-acid with TBS at C-3 and lacking the double bond was cyclized under Yamaguchi conditions in a good yield of 76%. In order to decrease steric congestion and increase the conformational flexibility of the *seco*-acid, we screened smaller protecting groups at C-3. Notably, even small protecting groups such as MOM or acetate did not lead to significant amounts of the 10-membered macrolide. Therefore, we next investigated the cyclization of the unprotected dihydroxy acid **23**. When we applied the Yamaguchi protocol to dihydroxy acid **23**, macrolactone **24** was not produced at all. Instead, **23** appears to undergo an acylation of the allylic alcohol. To suppress this undesired esterification, we probed the Shiina lactonization using 2-methyl-6-nitrobenzoic anhydride.[28] After optimization we found that addition of dihydroxy acid **23** over 36 h to a solution of 2-methyl-6-nitrobenzoic anhydride and DMAP at 20 °C led to formation of the desired macrolide **24** in 21% yield, a reaction which may actually proceed through a β-lactone intermediate. Notably, in all these experiments, diene **25**, formed by dehydrative decarboxylation,[29] was observed as a byproduct. The predominant formation of the (E/E)-isomer of this side product leads to a *syn*-elimination as a mechanistic rationale which is again indicative of the formation of a β-lactone intermediate.[30] When dichloromethane was used as the solvent instead of toluene, **25** was produced exclusively and no macrolide **24** was detected. Similarly, the use of Mukaiyama's reagent[31] in dichloromethane gave diene **25** as the only product. Finally, the PMB group in **24** was deprotected with DDQ to give cytospolide D (**4**) in a good yield (Scheme 5). The ^{1}H NMR spectrum of synthetic cytospolide D (**4**) showed a second set of signals with about 15% intensity. In the course of the isolation of the natural product Zhang et al. noted the same additional set of signals at almost identical relative intensity and were able to establish the existence of two conformers.

For the biomimetic conversion of cytospolide D (**4**) to cytospolide M (**6**) we investigated a diastereoselective epoxidation.[32] When **4** was reacted with *m*CPBA at −20 °C overnight, a clean and highly diastereoselective conversion to the epoxide was observed. Under these slightly acidic reaction conditions the epoxide instantaneously underwent partial cyclization[33] to give cytospolide M (**6**), though the reactive conformer presumably has to accommodate several pseudoaxial substituents. Addition of a catalytic amount of CSA completed the formation of the natural [6.2.1]-bicycle. To further convert the 10-membered lactone **6** into the 5-membered γ-lactone **9**, we tried different conditions for saponification. Lactone **6** showed remarkable stability toward 1 N HCl and 1 N NaOH, but finally with potassium trimethylsilanolate[34] lactone **6** could be opened to the carboxylate. After aqueous workup and concentration *in vacuo*, a spontaneous recyclization to cytospolide Q (**9**) occurred. However, under these basic reaction conditions, we also observed partial epimerization. For the synthesis of cytospolide O (**7**) we utilized an oxa-Michael addition strategy. Macrolide **24** was oxidized to enone **26** and then deprotected to give **27**. Contrary to the epoxide of **4**, enone **27** did not undergo a spontaneous oxa-Micheal addition, but after formation of the alkoxide with LiHMDS, cytospolide O (**7**) was produced in 63% yield.

In summary we established a synthetic route toward cytospolide D (**4**). Significant hurdles emerging during the macrolactonization could be circumvented by omitting a protecting group at C-3 and through use of Shiina's method. However, the low yield of 21% underlines the difficulties in closing a 10-membered ring containing an *E*-double bond. The first total syntheses of the higher oxygenated cytospolides M (**6**), O (**7**), and Q (**9**) were successfully achieved using a strategy reflecting the putative biosynthetic pathways based on cytospolide D (**4**).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02193.

General experimental procedures, spectroscopic data (^{1}H, ^{13}C, IR) and HRMS of all compounds (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Lu, S.; Kurtán, T.; Yang, G.; Sun, P.; Mándi, A.; Krohn, K.; Draeger, S.; Schulz, B.; Yi, Y.; Li, L.; Zhang, W. *Eur. J. Org. Chem.* **2011**, *2011*, 5452–5459.

(2) Lu, S.; Sun, P.; Li, T.; Kurtán, T.; Mándi, A.; Antus, S.; Krohn, K.; Draeger, S.; Schulz, B.; Yi, Y.; Li, L.; Zhang, W. *J. Org. Chem.* **2011**, *76*, 9699–9710.

(3) Vadhadiya, P. M.; Rout, J. K.; Ramana, C. V. *Tetrahedron* **2015**, *71*, 9088–9094.

(4) Yadav, J. S.; Pandurangam, T.; Suman Kumar, A.; Adi Narayana Reddy, P.; Prasad, A. R.; Subba Reddy, B. V.; Rajendraprasad, K.; Kunwar, A. C. *Tetrahedron Lett.* **2012**, *53*, 6048–6050.

(5) Rej, R. K.; Nanda, S. *Eur. J. Org. Chem.* **2014**, *2014*, 860–871.

(6) Suman, P.; Raju, B. C. *Org. Biomol. Chem.* **2014**, *12*, 3358–3361.

(7) Chatterjee, S.; Guchhait, S.; Goswami, R. K. *J. Org. Chem.* **2014**, *79*, 7689–7695.

(8) Rej, R. K.; Kumar, R.; Nanda, S. *Tetrahedron* **2015**, *71*, 3185–3194.

(9) Kamal, A.; Balakrishna, M.; Venkat Reddy, P.; Rahim, A. *Tetrahedron: Asymmetry* **2014**, *25*, 148–155.

(10) (a) Schmidt, J.; Stark, C. B. W. *Org. Lett.* **2012**, *14*, 4042–4045. (b) Giera, D. S.; Stark, C. B. W. *RSC Adv.* **2013**, *3*, 21280–21284. (c) Schmidt, J.; Stark, C. B. W. *J. Org. Chem.* **2014**, *79*, 1920–1928. (d) Schmidt, J.; Khalil, Z.; Capon, R. J.; Stark, C. B. W. *Beilstein J. Org. Chem.* **2014**, *10*, 1228–1232. (e) Müller, J. M.; Stark, C. B. W. *Angew. Chem., Int. Ed.* **2016**, *55*, 4798–4802.

(11) (a) Paterson, I.; Wallace, D. J.; Velazquez, S. M. *Tetrahedron Lett.* **1994**, *35*, 9083–9086. (b) Paterson, I.; Wallace, D. J.; Cowden, C. J. *Synthesis* **1998**, *1998*, 639–652.

(12) (a) Staab, H. A.; Sommer, N. *Angew. Chem.* **1962**, *74*, 294. (b) Schobert, R.; Gordon, G. J. *Science of Synthesis* **2004**, *27*, 973–1070.

(13) (a) Cram, D. J.; Elhafez, F. A. A. *J. Am. Chem. Soc.* **1952**, *74*, 5828–5835. (b) Cram, D. J.; Wilson, D. R. *J. Am. Chem. Soc.* **1963**, *85*, 1245–1249. (c) Reetz, M. T. *Acc. Chem. Res.* **1993**, *26*, 462–468. (d) Mengel, A.; Reiser, O. *Chem. Rev.* **1999**, *99*, 1191–1223.

(14) (a) Chérest, M.; Felkin, H.; Prudent, N. *Tetrahedron Lett.* **1968**, *9*, 2199–2204. (b) Anh, N. T.; Eisenstein, O. *Tetrahedron Lett.* **1976**, *17*, 155–158. (c) Anh, N. T. *Top. Curr. Chem.* **1980**, *88*, 145–162.

(15) (a) Kurihara, T.; Nakajima, Y.; Mitsunobu, O. *Tetrahedron Lett.* **1976**, *17*, 2455–2458. (b) Mitsunobu, O. *Synthesis* **1981**, *1981*, 1–28.

(16) Larcheveque, M.; Lalande, J. *Tetrahedron* **1984**, *40*, 1061–1065.

(17) Kuehnert, S. M.; Maier, M. E. *Org. Lett.* **2002**, *4*, 643–646.

(18) Takano, S.; Akiyama, M.; Sato, S.; Ogasawara, K. *Chem. Lett.* **1983**, *12*, 1593–1596.

(19) (a) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155–4156. (b) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277–7287.

(20) Mancuso, A. J.; Swern, D. *Synthesis* **1981**, *1981*, 165–185.

(21) Initially, we intended to make use of the good dr of **13** and synthesized the C-9 epimer of *seco*-acid **10** aiming at a late stage inversion via Mitsunobu macrolactonization. Unfortunately, the desired macrolide was not formed under these conditions.

(22) Ahn, C.; Correia, R.; DeShong, P. *J. Org. Chem.* **2002**, *67*, 1751–1753.

(23) (a) Nakata, T.; Tanaka, T.; Oishi, T. *Tetrahedron Lett.* **1983**, *24*, 2653–2656. (b) Takahashi, T.; Miyazawa, M.; Tsuji, J. *Tetrahedron Lett.* **1985**, *26*, 5139–5142.

(24) Schmidt, A.-K. C.; Stark, C. B. W. *Synthesis* **2014**, *46*, 3283–3308.

(25) Lindgren, B. O.; Nilsson, T. *Acta Chem. Scand.* **1973**, *27*, 888–890.

(26) Bal, B. S.; Childers, W. E., Jr.; Pinnick, H. W. *Tetrahedron* **1981**, *37*, 2091–2096.

(27) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989–1993.

(28) Shiina, I.; Kubota, M.; Oshiume, H.; Hashizume, M. *J. Org. Chem.* **2004**, *69*, 1822–1830.

(29) Mulzer, J.; Brüntrup, G. *Chem. Ber.* **1982**, *115*, 2057–2075.

(30) Luengo, J. I.; Koreeda, M. *Tetrahedron Lett.* **1984**, *25*, 4881–4884.

(31) (a) Mukaiyama, T.; Usui, M.; Saigo, K. *Chem. Lett.* **1976**, *5*, 49–50. (b) Mukaiyama, T. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 707–721.

(32) For early work on diastereoselective epoxidations using macrocyclic stereocontrol, see: (a) Still, W. C.; Romero, A. G. *J. Am. Chem. Soc.* **1986**, *108*, 2105–2106. (b) Vedejs, E.; Buchanan, R. A.; Watanabe, Y. *J. Am. Chem. Soc.* **1989**, *111*, 8430–8438.

(33) A similar instantaneous transannular epoxide opening was observed during the synthesis of Englerin A: Radtke, L.; Willot, M.; Sun, H.; Ziegler, S.; Sauerland, S.; Strohmann, C.; Fröhlich, R.; Habenberger, P.; Waldmann, H.; Christmann, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 3998–4002.

(34) Laganis, E. D.; Chenard, B. L. *Tetrahedron Lett.* **1984**, *25*, 5831–5834.