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Synthesis of (−)-Dactylolide and 13-Desmethylene-(−)-dactylolide and Their Effects on Tubulin

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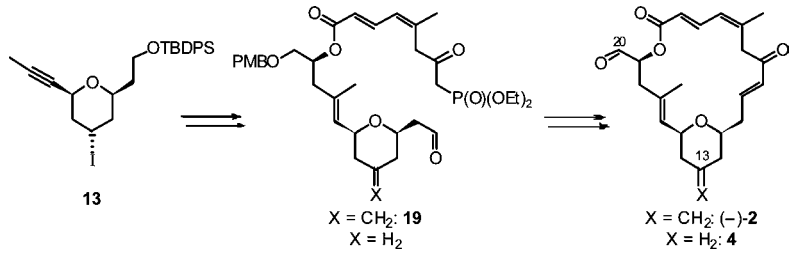
ABSTRACT



An efficient new synthesis has been elaborated for non-natural (−)-dactylolide ((−)-2) and its 13-desmethylene analogue 4, employing a HWE-based macrocyclization approach with *β*-keto-phosphonate/aldehyde 19 and the respective 13-desmethylene derivative as the key intermediates. Both (−)-2 and 4 as well as the corresponding C20 alcohols inhibit human cancer cell proliferation with IC_{50} values in the sub-micromolar range and induce the polymerization of tubulin in vitro.

(−)-Zampanolide ((−)-**1**) and (+)-dactylolide ((+)-**2**) are structurally related polyketide-based macrolides, which are characterized by a highly unsaturated 20-membered macrolactone core structure containing a *syn*-2,6-disubstituted tetrahydropyran ring with an exocyclic methylene group (Figure 1). Compound (−)-**1** was first isolated in 1996 by Tanaka and co-workers from the marine sponge *Fasciospongia rimosa* at Cape Zampa in Okinawa;[1] the compound was shown to exhibit high antiproliferative activity against different human cancer cell lines in vitro, with IC_{50}'s in the low nanomolar range (2−10 nM). Compound (−)-**1** features an unusual *N*-acylhemiaminal side chain that is not found in (+)-**2**. The latter was isolated in 2001 by Cutignano and co-workers from the sponge *Dactylospongia* sp. at the Vanuatu Islands.[2] In contrast to (−)-**1**, (+)-**2** is only a moderately potent inhibitor of human cancer cell growth with

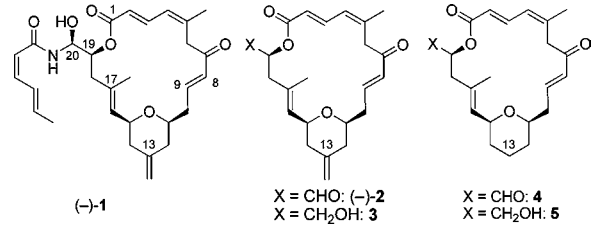


Figure 1. Structures of natural (−)-zampanolide ((−)-**1**), non-natural (−)-dactylolide ((−)-**2**), and analogue structures **3**−**5**.

IC_{50}'s in the low micromolar range.[2] Rather intriguingly, however, it was subsequently shown by Smith and co-workers[3] that the absolute configuration of the macrolactone core structure in (−)-**1** is opposite to that found in natural

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(1) Tanaka, J.; Higa, T. *Tetrahedron Lett.* **1996**, *37*, 5535.

(2) Cutignano, A.; Bruno, I.; Bifulco, G.; Caspullo, A.; Debitus, C.; Gomez-Paloma, L.; Riccio, R. *Eur. J. Org. Chem.* **2001**, 775.

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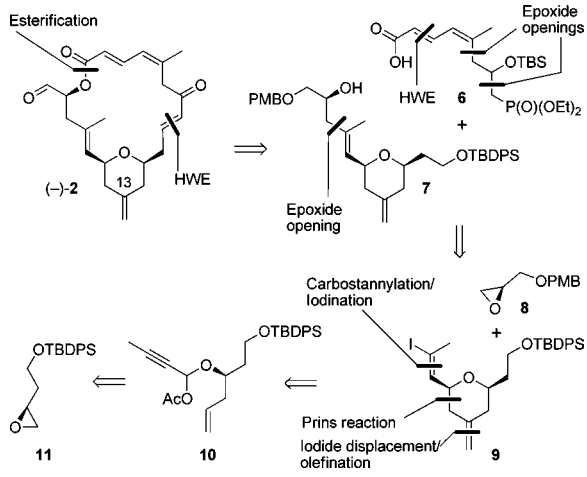
(+)-**2**. Interestingly, based on work by Ding and Jennings, synthetic (−)-**2** appears to be slightly more active than natural (+)-**2** (although a direct comparison is available only for a single cell line).[4] More recently, (−)-**1** was also isolated from the Togan sponge *Cacospongia mycofijiensis* by Northcote and co-workers, who demonstrated the compound to be an efficient promotor of tubulin assembly.[5] This suggests that (−)-**1** inhibits cancer cell growth through the same mechanism of action as Taxol or epothilones.[6]

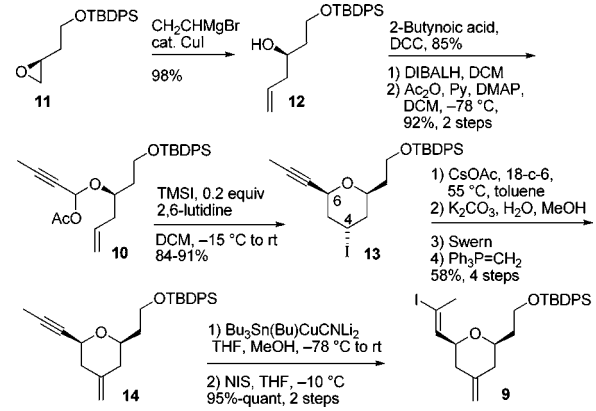
While a number of stereoselective syntheses of (−)-**1**[7] and (+)-**2**[8] have appeared in the literature, little work has been reported on analogue structures and their biological activity.[9] It also remains to be shown whether (−)-**2** or (+)-**2**, like (−)-**1**, may promote tubulin polymerization and stabilize microtubules. Intrigued by the divergent stereochemistry of natural (−)-**1** and (+)-**2** and in light of the distinct lack of SAR data for these structures, we had initiated a program on the synthesis of natural (−)-**1** and (+)-**2**, their non-natural counterparts (+)-**1** and (−)-**2**, and analogue structures for SAR studies, even before the recent discovery of the tubulin-polymerizing activity of (−)-**1**. In this paper, we now report on a new synthesis of (−)-**2**[10] and of dactylolide analogues **3**−**5** (Figure 1), all of which were found to have similar antiproliferative activity and to induce tubulin polymerization in vitro.

Scheme 1. Retrosynthetic Analysis for (−)-**2**



Our retrosynthesis for (−)-**2** is outlined in Scheme 1 and is centered around an intramolecular HWE reaction for the closure of the 20-membered macrolide ring. The requisite β-keto phosphonate/aldehyde percursor would be obtained via esterification of acid **6** and alcohol **7**, followed by silyl ether cleavage and oxidation. While HWE-based macrocyclizations involving the formation of the C=C double bond in α,β-unsaturated ketone units are well precedented in natural product synthesis, they have not been used extensively;[11] in particular, and quite surprisingly, this strategy has not been employed in the context of zampanolide or dactylolide syntheses. Alcohol **7** was envisioned to be accessible from protected (*R*)-glycidol **8** through regioselective epoxide opening with lithiated vinyl iodide **9**. The latter was to be obtained by Prins-type reaction of alkyne **10** to deliver a 4-iodotetrahydropyran derivative; the iodo substituent would then be elaborated into the desired methylene group. Finally, **10** would be derived from epoxide **11** which, in turn, is accessible from (*R*)-aspartic acid.[12] Acid **6** was envisaged to be accessed from a protected *Z*-vinyl iodide via reaction with epichlorohydrin followed by conversion of the resulting chlorohydrin to a new oxirane, epoxide opening with lithiated diethylphosphite, and finally, HWE reaction and ester hydrolysis.

Scheme 2. Synthesis of Vinyl Iodide **9**



The synthesis of vinyl iodide **9** started with the Cu-mediated regioselective epoxide opening of **11** (available from (*R*)-aspartic acid in three steps in 78% overall yield)[12] with vinyl-MgBr in excellent yield (98%; Scheme 2). Compound **12** was then elaborated into tetrahydropyran **13**

(3) Smith, A. B., III; Safonov, I. G.; Corbett, R. M. *J. Am. Chem. Soc.* **2002**, *124*, 11102.

(4) Ding, F.; Jennings, M. P. *J. Org. Chem.* **2008**, *73*, 5965.

(5) Field, J. J.; Singh, A. J.; Kanakkanthara, A.; Halafihi, T.; Northcote, P. T.; Miller, J. H. *J. Med. Chem.* **2009**, *52*, 7328.

(6) For a review on microtubule-stabilizing natural products, see: Altmann, K.-H.; Gertsch, J. *Nat. Prod. Rep.* **2007**, *24*, 327.

(7) (a) Smith, A. B., III; Safonov, I. G.; Corbett, R. M. *J. Am. Chem. Soc.* **2001**, *123*, 12426. (b) Smith, A. B., III; Safonov, I. G.; Corbett, R. M. *J. Am. Chem. Soc.* **2002**, *124*, 11102. (c) Hoye, T. R.; Hu, M. *J. Am. Chem. Soc.* **2003**, *125*, 9576. (d) Uenishi, J.; Iwamoto, T.; Tanaka, J. *Org. Lett.* **2009**, *11*, 3262. Studies toward (−)-**1**: (e) Loh, T.-P.; Yang, J.-Y.; Feng, L.-C.; Zhou, Y. *Tetrahedron Lett.* **2002**, *43*, 7193. (f) Troast, D. M.; Porco, J. A., Jr. *Org. Lett.* **2002**, *4*, 991. (g) Troast, D. M.; Yuan, J.; Porco, J. A., Jr. *Adv. Synth. Catal.* **2008**, *350*, 1701.

(8) (a) Smith, A. B., III; Safonov, I. G. *Org. Lett.* **2002**, *4*, 635. (b) Aubele, D. L.; Wan, S. Y.; Floreancig, P. E. *Angew. Chem., Int. Ed.* **2005**, *44*, 3485. (c) Sanchez, C. C.; Keck, G. E. *Org. Lett.* **2005**, *7*, 3053.

(9) A notable exception is the work by Uenishi et al. (ref 7d), who have shown the C20 epimer of (−)-**1** to be ca. 10-fold less active than (−)-**1**.

(10) For previous syntheses of (−)-**2**, see: (a) Louis, I.; Hungerford, N. L.; Humphries, E. J.; McLeod, M. D. *Org. Lett.* **2006**, *8*, 1117. (b) Reference 4.

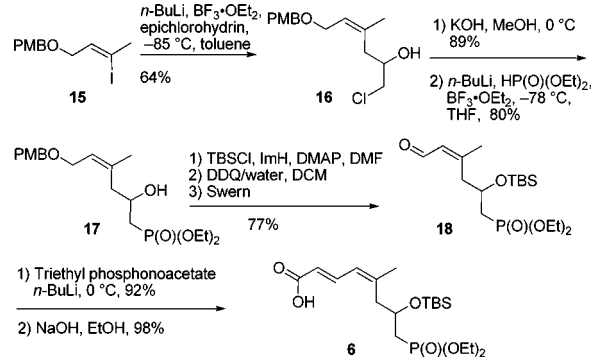
(11) For examples, see: (a) Nicolaou, K. C.; Seitz, S. P.; Pavia, M. R. *J. Am. Chem. Soc.* **1982**, *104*, 2030. (b) Paterson, I.; Yeung, K.-S. *Tetrahedron Lett.* **1993**, *34*, 5347. (c) Paterson, I.; Yeung, K.-S.; Watson, C.; Ward, R. A.; Wallace, P. A. *Tetrahedron* **1998**, *54*, 11935. (d) Kadota, I.; Hu, Y.; Packard, G. K.; Rychnovsky, S. D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11192. (e) Berger, G. O.; Tius, M. A. *J. Org. Chem.* **2007**, *72*, 6473.

(12) Frick, J. A.; Klassen, J. B.; Bathe, A.; Abramson, J. M.; Rapoport, H. *Synthesis* **1992**, 621.

in a highly stereoselective Prins-type reaction employing a segment coupling approach as developed by Rychnovsky.[13] In a first step, this involved esterification of **12** with 2-butynoic acid,[14] which was followed by DIBALH reduction of the ester and trapping of the aluminated intermediate at −78 °C with Ac_2O to furnish **10** in excellent yield (78% from **12**, dr ca. 1.6:1). Treatment of **10** with TMSI gave substituted tetrahydropyran **13** with the desired 2,6-*syn* relationship and the iodine located *anti* to the substituents in the 2- and 6-positions.[15] This is in line with previous observations by Rychnovsky for related transformations.[16] The use of other Lewis acids gave either lower conversion (TMSBr, BF_3•Et_2O, BF_3•$(HOAc)_2$, TFA) or led to partial epimerization ($SnBr_4$) at C6 (see numbering of **13** in Scheme 2). Attempts to conduct the Prins reaction directly with an appropriate aldehyde and a homoallylic alcohol failed completely. Iodide displacement from **13** with CsOAc/18-c-6 was accompanied by elimination, with both possible olefin products formed in significant amounts.[17] Elimination could be minimized, however, by conducting the reaction at lower temperature (55 °C instead of 90 °C), although extended reaction times (up to 3−4 days) were required under these conditions for full conversion. Base-induced cleavage of the acetate formed in the displacement step, Swern oxidation of the alcohol to the ketone, and Wittig reaction then served to install the exocyclic C═C double bond in **14** in good overall yield (58%, four steps from **13**). Conversion of **13** to the desired *E*-vinyl iodide **9** was achieved with in situ generated $Bu_3Sn(Bu)CuCNLi_2$[18] followed by Sn−I exchange with NIS. Alternative methods investigated for the formation of the metalated vinyl intermediate required for this transformation, such as Pd-mediated hydrostannylation,[18,19] silylcupration with Fleming's reagent,[20] or the use of the Schwartz reagent[21] afforded the desired product only in low yields and as mixtures of regioisomers (*E*/*Z* ratios between 5:1 and 1:1).

The preparation of unsaturated acid **6** started with BF_3•OEt_2-mediated coupling between lithiated *Z*-vinyl iodide **15** (obtained in two steps from 2-butynol in 79% yield)[22] and epichlorohydrin in toluene (Scheme 3).[23] No reaction was observed between epichlorohydrin and **15** in etheral solvents

Scheme 3. Synthesis of Unsaturated Acid **6**

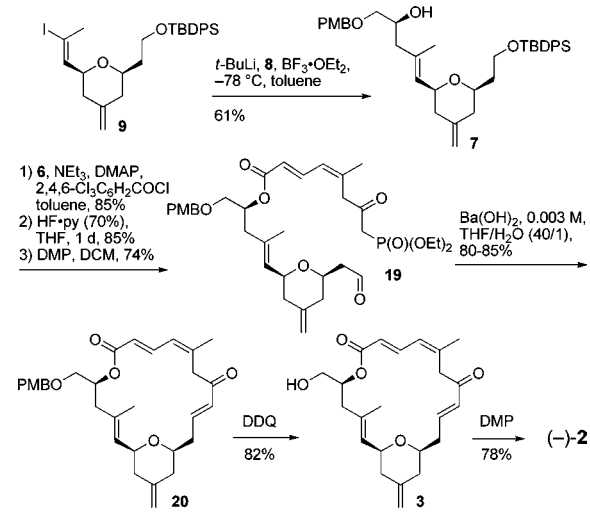


such as THF or Et_2O or mixtures thereof. This rather unexpected finding turned out to be crucial at a later stage of the synthesis.

Treatment of **16** with base followed by BF_3•OEt_2-mediated opening of the resulting epoxide with lithiated diethylphosphite gave *β*-hydroxy phosphonate **17**. TBS protection of **17** followed by PMB removal under oxidative conditions (DDQ) resulted in a mixture of aldehyde **18** and the corresponding allylic alcohol, which was oxidized under Swern conditions to deliver **18** as the sole product. HWE reaction followed by basic hydrolysis then afforded the desired acid **6**.

The final assembly of (−)-**2** commenced with the reaction of vinyl iodide **9** with PMB-protected (*R*)-glycidol **8** (Scheme 4). As for the reaction of **15** with epichlorohydrin, and in

Scheme 4. Final Steps in the Total Synthesis of (−)-**2**



spite of literature precedence for related transformation (see, e.g., refs 7b and 7d), **7** was only formed when toluene was used as the solvent. No traces of product were observed if THF, Et_2O, or mixtures thereof were used, independent of the precursor for the lithiated vinyl nucleophile (e.g., vinyl

(13) (a) Rychnovsky, S. D.; Hu, Y.; Ellsworth, B. *Tetrahedron Lett.* **1998**, *39*, 7271. (b) Rychnovsky, S. D.; Kopecky, D. J. *J. Org. Chem.* **2000**, *65*, 191. (c) Jaber, J. J.; Mitsui, K.; Rychnovsky, S. D. *J. Org. Chem.* **2001**, *65*, 4679. (d) Rychnovsky, S. D.; Marumoto, S.; Jaber, J. *J. Org. Lett.* **2001**, *3*, 3815. (e) Marumoto, S.; Jaber, J. J.; Vitale, J. P.; Rychnovsky, S. D. *Org. Lett.* **2002**, *4*, 3919. (f) Jasti, R.; Vitale, J.; Rychnovsky, S. D. *J. Am. Chem. Soc.* **2004**, *126*, 9904. (g) Vitale, J. P.; Wolckenhauer, S. A.; Nga, M. D.; Rychnovsky, S. D. *Org. Lett.* **2005**, *7*, 3255. (h) Jasti, R.; Rychnovsky, S. D. *J. Am. Chem. Soc.* **2006**, *128*, 13640.

(14) Trost, B. M.; Rudd, M. T. *J. Am. Chem. Soc.* **2005**, *127*, 4763.

(15) No NOEs were observed between C(4)H (see numbering of **13** in Scheme 2) with the protons α to the tetrahydropyran oxygen.

(16) For a previous example of an axial-selective Prins reaction, see ref 13f.

(17) See also ref 13g. In our case, the use of $AgOCOCF_3$ only led to elimination of HI.

(18) Betzer, J.-F.; Delaloge, F.; Muller, B.; Pancrazi, A.; Prunet, J. *J. Org. Chem.* **1997**, *62*, 7768.

(19) Liron, F.; Knochel, P. *Chem. Commun.* **2004**, 304.

(20) Fleming, I.; Newton, T. W.; Roessler, F. *J. Chem. Soc., Perkin Trans. 1* **1981**, 2527.

(21) Schwartz, J.; Labinger, J. A. *Angew. Chem., Int. Ed.* **1976**, *15*, 333.

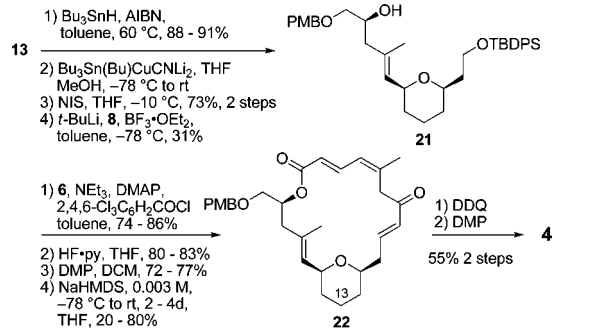
(22) Chau, A.; Paquin, J.-F.; Lautens, M. *J. Org. Chem.* **2006**, *71*, 1924.

iodide, vinyl bromide, vinyl tributylstannane); likewise, the use of cuprates, variations in Lewis acid ($SnCl_4$, $MgBr_2$•OEt_2, TMSOTf), or the addition of HMPA or NMP did not result in any improvement. Gratifyingly, the reaction between **8** and **9** under optimized conditions in toluene delivered alcohol **7** as a single isomer in yields of up to 61%. Esterification of **7** with acid **6** under Yamaguchi conditions (DCC or EDCI were less effective) followed by global desilylation (HF•pyridine) and a one-step oxidation of the resulting monoprotected triol afforded the desired HWE substrate **19** in good yields. Initial attempts at base-induced macrocyclization of **19** involved the use of NaHMDS in THF as the solvent, and while **20** was indeed obtained under these conditions, extended reaction times were required (2−4 d) and yields were highly variable (20−80%). After significant experimentation it was finally discovered that the use of activated $Ba(OH)_2$[11b,c] not only substantially reduced the reaction time (to 0.5−1 h at 0 °C) but, importantly, also led to increased and reproducible yields in the range of 80−85%. Under all conditions investigated, the macrolactone with the desired C8−C9 *E* configuration was the only detectable isomer.

After the successful implementation of the crucial ring-closure step, the synthesis of (−)-**2** was completed by PMB removal, to furnish **3**, and DMP oxidation of the primary alcohol in 64% yield for the two steps from **20**.

Based on the chemistry developed for the synthesis of (−)-**2**, we were then able to efficiently address the construction of 13-desmethylene-(−)-dactylolide (**4**), again building on Prins product **13** as a key intermediate (Scheme 5). In spite

Scheme 5. Synthesis of 13-Desmethylene-(−)-dactylolide (**4**)



of the potential susceptibility of the alkyne moiety toward radical hydrostannylation, **13** could be selectively dehalogenated with Bu_3SnH (1.1 equiv) and AIBN. In analogy to the synthesis of (−)-**2**, carbostannylation/iodination and reaction with epoxide **8** delivered homoallylic alcohol **21**. Yamaguchi esterification of **21** with acid **6**, global desilylation with HF•pyridine, oxidation with DMP, and HWE-based macrocyclization then afforded macrolactone **22**.[24] PMB removal and oxidation completed the synthesis of target structure **4**, which is the first backbone-modified zampanolide/dactylolide analogue reported so far.

Compound (−)-**2**, its 13-desmethylene derivative **4**, as well as alcohols **3** and **5** were assessed for their antiproliferative and potential tubulin-polymerizing effects. All four compounds inhibit human cancer cell growth with comparable potency and IC_{50} values in the sub-micromolar range (Table 1). In addition, (−)-**2**, **3**, **4**, and **5** all show significant tubulin-

Table 1. Antiproliferative Activity of (−)-**2**, **3**, **4**, and **5** (IC_{50} values (nM)[a] after 72 h Exposure Time)[25]

cell line	(−)-**2**	**4**	**3**	**5**
A549	301.5 ± 4.3	149.0 ± 12.8	127.5 ± 2.9	189.0 ± 19.3
MCF-7	247.6 ± 2.6	68.0 ± 5.6	106.0 ± 3.6	114.4 ± 10.2
HCT116	210.4 ± 4.7	249.5 ± 28.2	155.8 ± 2.1	74.1 ± 1.5

[a] GI_{50} values of 198 and 346 nM have been reported for (−)-**2** against the MCF-7 and HCT116 cell lines, respectively.[4] No data are available in ref 4 for A549 cells.

polymerizing activity (41%−74% induction of tubulin polymerization relative to the effect of 25 μM of epothilone B (10 μM tubulin, 2 μM test compound) vs 82% for epothilone A). Thus, (−)-**2**, like natural (−)-zampanolide, is a new microtubule stabilizer, with neither the methylene group at C13 nor the aldehyde functionality at C20 being essential for antiproliferative and tubulin-polymerizing activity.

In conclusion, we have established an efficient new synthesis of (−)-dactylolide ((−)-**2**). Utilizing an advanced intermediate from this synthesis, we have then followed the same HWE-based macrocyclization strategy to prepare 13-desmethylene-(−)-dactylolide (**4**). Both compounds as well as the corresponding alcohols **3** and **5** are potent inhibitors of human cancer cell growth and promote tubulin polymerization in vitro. Efforts to exploit these findings in the design and synthesis of new potent analogues are currently ongoing in our laboratory.

Acknowledgment. We thank the Roche Research Foundation for a Ph.D. fellowship to D.Z. We are indebted to Dr. Bernhard Pfeiffer and Fabienne Gaugaz (ETHZ) for NMR support, to Kurt Hauenstein (ETHZ) for technical advice, and to Louis Bertschi (ETHZ, LOC MS-Service) for HRMS spectra acquisition.

Supporting Information Available: Synthetic procedures, complete spectroscopic data, ^1H and ^{13}C NMR data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) For literature precedence for the coupling of a lithiated aromatic compound with epichlorohydrin in toluene, see: Okano, K.; Tokuyama, H.; Fukuyama, T. *J. Am. Chem. Soc.* **2006**, *128*, 7136.

(24) For **22**, macrocyclization so far has only been performed with NaHMDS as a base; the reaction was hampered by the same reproducibility problems as the cyclization of **19**. Due to a lack of material, the use of $Ba(OH)_2$ has not yet been investigated in this case.

(25) For experimental details, see: Dietrich, S. A.; Lindauer, R.; Stierlin, C.; Gertsch, J.; Matesanz, R.; Notararigo, S.; Díaz, J. F.; Altmann, K.-H. *Chem.—Eur. J.* **2009**, *15*, 10144.