Total Synthesis of Iejimalide A−D and Assessment of the Remarkable Actin-Depolymerizing Capacity of These Polyene Macrolides

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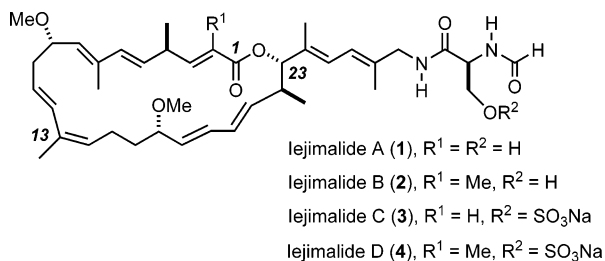
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Abstract: A concise and convergent total synthesis of the highly cytotoxic marine natural products iejimalide A−D (**1**−**4**) is reported, which relies on an effective ring-closing metathesis (RCM) reaction of a cyclization precursor containing no less than 10 double bonds. Because of the exceptional sensitivity of this polyunsaturated intermediate and its immediate precursors toward acid, base, and even gentle warming, the assembly process hinged upon the judicious choice of protecting groups and the careful optimization of all individual transformations. As a consequence, particularly mild protocols for Stille as well as Suzuki reactions of elaborate coupling partners have been developed that hold considerable promise for applications in other complex settings. Moreover, a series of non-natural "iejimalide-like" compounds has been prepared, differing from the natural lead in the polar head groups linked to the macrolide's N-terminus. With the aid of these compounds it was possible to uncover the hitherto unknown effect of iejimalide and analogues on the actin cytoskeleton. Their capacity to depolymerize this microfilament network rivals that of the latrunculins which constitute the standard in the field. Structural modifications of the peptidic terminus in **2** are thereby well accommodated, without compromising the biological effects. The iejimalides hence constitute an important new class of probe molecules for chemical biology in addition to their role as promising lead structures for the development of novel anticancer agents.

Introduction

Bioassay-guided fractionation of the methanol extracts of the tunicate *Eudistoma* cf. *rigida* collected off Ie island, Okinawa province, Japan, led to the discovery of a family of novel 24-membered polyene macrolides designated iejimalides A−D (**1**−**4**).[1] Because of their paucity (0.0003−0.0006% of the wet

Iejimalide A (**1**), R^1 = R^2 = H

Iejimalide B (**2**), R^1 = Me, R^2 = H

Iejimalide C (**3**), R^1 = H, R^2 = SO$_3$Na

Iejimalide D (**4**), R^1 = Me, R^2 = SO$_3$Na

weight of the tunicate), however, only the gross structure of these unusual secondary metabolites could be established during the original isolation campaign. It required a re-extraction from

a *Cystodytes* sp. to accumulate enough material for an unambiguous assignment of the relative and absolute stereochemistry by extensive 2D NMR investigations and degradation studies. It was during these investigations that the C.13−C.14 double-bond configuration was corrected to *Z* rather than *E* as originally proposed.[2]

Although the cytotoxicity of **1** and **2** was noticed early on, the initial assay solely surveyed two murine leukemia cell lines (iejimalide B: IC$_{50}$ = 1 ng/mL against L5178Y murine leukemia; IC$_{50}$ = 32 ng/mL against L1210 murine leukemia).[1] It was over a decade later that a more comprehensive data set was disclosed by the National Cancer Institute (NCI), showing the remarkable potency of **1** against 60 different human cancer cell lines, with GI$_{50}$ values as low as 13 nM (MDA-MB-231/ATCC breast cancer cell line) and total growth inhibition (TGI) values as low as 40 nM (M14 melanoma cell line).[3] Shortly thereafter, similar levels of activity were reported by Nozawa et al., who also demonstrated the in vivo activity of **3** and **4** against P388 leukemia at a dose of 200 μg·kg^{-1}·d^{-1}.[2a,4] A "COMPARE" analysis gave no significant correlation between

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(1) (a) Kobayashi, J.; Cheng, J.; Ohta, T.; Nakamura, H.; Nozoe, S.; Hirata, Y.; Ohizumi, Y.; Sasaki, T. *J. Org. Chem.* **1988**, *53*, 6147. (b) Kikuchi, Y.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. *Tetrahedron Lett.* **1991**, *32*, 797.

(2) (a) Nozawa, K.; Tsuda, M.; Ishiyama, H.; Sasaki, T.; Tsuruo, T.; Kobayashi, J. *Bioorg. Med. Chem.* **2006**, *14*, 1063. (b) Tsuda, M.; Nozawa, K.; Shimbo, K.; Ishiyama, H.; Fukushi, E.; Kawabata, J.; Kobayashi, J. *Tetrahedron Lett.* **2003**, *44*, 1395.

(3) The activity data are available from the NCI homepage. http://www.dtp.nci.nih.gov/docs/dtp_search.html (Feb 2007).

the activity profile of the iejimalides and that of the recorded anticancer agents, which might indicate an unprecedented mode of action.[2a] This remains speculative, however, as long as the molecular targets affected by the iejimalides are largely unknown. Only recently have vacuolar V-ATPases been identified as one such target,[5] but it remains unclear if inhibition of these proton pumps is the only and/or the decisive pathway responsible for the remarkable cytotoxicity of these marine natural products. The fact that **1** is more effective against purified yeast V-ATPase while **2** is more cytotoxic raises questions in this regard. Anyhow, the subtle influence of the methyl branch at C.2 is the only secured piece of information concerning structure/activity relationships (SAR), except for a recent disclosure on simple carbamate derivatives formed by derivatization of the natural product's serine −OH group, which retain activity even though they are 1−2 orders of magnitude less potent than the parent compounds against most of the tested cancer cell lines.[6]

Taken together, the available data suggest that the iejimalides deserve more comprehensive studies and might represent a novel lead in the quest for anticancer drugs. To this end, it is mandatory to solve the supply problem and to provide meaningful amounts of these enticing targets for further evaluation. We now present a full account of our work along these lines which led to the first and so far only total synthesis of all naturally occurring members of this series.[7] Moreover, it is shown that the actin cytoskeleton constitutes another cellular component that is strongly affected by these macrolides. The iejimalides therefore constitute a new class of probe molecules for chemical biology, irrespective of their potential relevance in a medicinal chemistry context.

Results and Discussion

Strategic Considerations. Although the structures of the iejimalides were disclosed as early as 1988,[1] the synthetic studies reported prior to our work were confined to the preparation of three subunits of moderate size, and no attempt for their assembly had been communicated.[8] A cursory inspection of **1−4** shows that five of the six chiral centers reside at allylic or even doubly allylic sites. Apprehensive that this feature likely potentiates the chemical lability of their polyunsaturated backbones, our first approach to **2** as the most active member of this series gravitated toward methodology that is believed to cope with such potentially fragile structural elements, notably in the late stages of the envisaged assembly process.

For the sake of flexibility it was planned to incorporate the peptide residue at the very end of the synthesis, only after cyclization of the macrolactone ring by a Yamaguchi lacton-

Scheme 1. Attempted Preparation of the Iejimalide Core by Yamaguchi Macrocyclization Triggered an Unprecedented Aromatization with Formation of the Polysubstituted Phenol Derivative **8**[a]
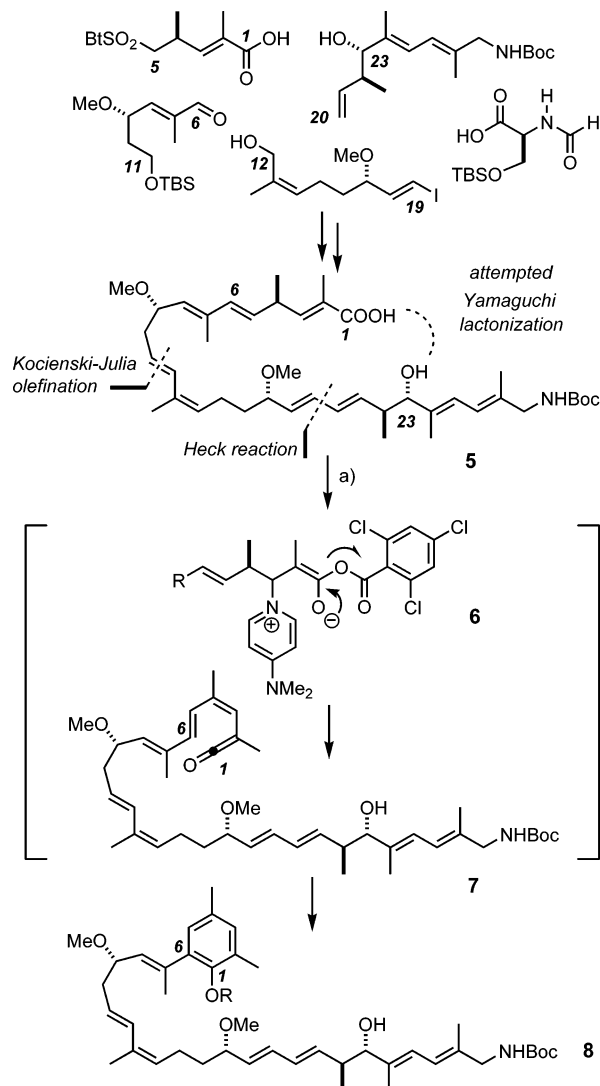
[a] (a) (i) 2,4,6-Trichlorobenzoyl chloride, Et₃N, THF; (ii) DMAP, cf., ref 7a.

ization or related methodology (Scheme 1).[9,10] Previous excellent experiences with the Julia−Kocienski olefination encouraged us to use this transformation in the present context as well.[11,12] Complemented by Pd-catalyzed C−C bond formations,[13] this ensemble of reliable and mild chemical transformations promised to open a flexible access route to **2** (and analogues) starting from five synthons of similar size and complexity.

(4) A preliminary report from the Walther Cancer Research Center claims spectacular effects and stunning morphological changes upon injection of iejimalides into tumor xenografts: http://www.nd.edu/~science/documents/waltherbrochure.pdf (Feb 2007).

(5) Kazami, S.; Muroi, M.; Kawatani, M.; Kubota, T.; Usui, T.; Kobayashi, J.; Osada, H. *Biosci., Biotechnol., Biochem.* **2006**, *70*, 1364.

(6) Schweitzer, D.; Zhu, J.; Jarori, G.; Tanaka, J.; Higa, T.; Davisson, V. J.; Helquist, P. *Bioorg. Med. Chem.* **2007**, *15*, 3208.

(7) Preliminary communications on the synthesis of iejimalide B: (a) Fürstner, A.; Aïssa, C.; Chevrier, C.; Teplý, F.; Nevado, C.; Tremblay, M. *Angew. Chem., Int. Ed.* **2006**, *45*, 5832. (b) Fürstner, A.; Nevado, C.; Tremblay, M.; Chevrier, C.; Teplý, F.; Aïssa, C.; Waser, M. *Angew. Chem., Int. Ed.* **2006**, *45*, 5837.

(8) (a) Cottard, M.; Kann, N.; Rein, T.; Åkermark, B.; Helquist, P. *Tetrahedron Lett.* **1995**, *36*, 3115. (b) Mendlik, M. T.; Cottard, M.; Rein, T.; Helquist, P. *Tetrahedron Lett.* **1997**, *38*, 6375. (c) Pedersen, T. M.; Hansen, E. L.; Kane, J.; Rein, T.; Helquist, P.; Norrby, P.-O.; Tanner, D. *J. Am. Chem. Soc.* **2001**, *123*, 9738.

(9) (a) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989. (b) Review: Parenty, A.; Moreau, X.; Campagne, J.-M. *Chem. Rev.* **2006**, *106*, 911.

(10) For advanced applications from this laboratory see: (a) Lepage, O.; Kattnig, E.; Fürstner, A. *J. Am. Chem. Soc.* **2004**, *126*, 15970. (b) Mlynarski, J.; Ruiz-Caro, J.; Fürstner, A. *Chem. Eur. J.* **2004**, *10*, 2214.

(11) (a) Blakemore, P. R.; Cole, W. J.; Kocienski, P. J.; Morley, A. *Synlett* **1998**, 26. (b) Review: Blakemore, P. R. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2563.

(12) (a) Fürstner, A.; Albert, M.; Mlynarski, J.; Matheu, M.; DeClercq, E. *J. Am. Chem. Soc.* **2003**, *125*, 13132. (b) Fürstner, A.; Mlynarski, J.; Albert, M. *J. Am. Chem. Soc.* **2002**, *124*, 10274. (c) Fürstner, A.; Feyen, F.; Prinz, H.; Waldmann, H. *Tetrahedron* **2004**, *60*, 9543. (d) Fürstner, A.; Feyen, F.; Prinz, H.; Waldmann, H. *Angew. Chem., Int. Ed.* **2003**, *42*, 5361.

(13) Nicolaou, K. C.; Bulger, P. G.; Sarlah, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4442.

As has been reported recently, however, this somewhat conservative and seemingly secure blueprint could not be reduced to practice.[7a] Although the chosen strategy brought seco-acid **5** into reach, we were unable to effect its cyclization by any of the standard lactonization protocols. Most notably, the Yamaguchi method converted **5** into the phenol derivative **8** by an unprecedented aromatization process which is believed to involve ketene intermediates as shown in Scheme 1.[7a,14] This failure prompted a major revision of the original synthesis plan because the closure of the iejimalide's 24-membered ring needed to be relocated from the ester C−O bond to a distal C−C bond within the backbone.

Among the various conceivable scenarios, we opted for ring-closing metathesis (RCM).[15] Despite our excellent and long-lasting experiences with this transformation,[16,17] this strategic decision bore considerable risk and might even seem somewhat counterintuitive. Application of RCM to the iejimalide case demands for no less than the selective activation of 2 out of 10 double bonds in a suitable cyclization precursor. Furthermore, conjugated dienes in general are somewhat capricious substrates for metathetic transformations, not least because Grubbs-type carbene complexes can react either with the terminal or the internal double bond; in the latter case, cyclization affords undesired ring-contracted homologues, whereas insufficient regiocontrol over the site of attack leads to mixtures of limited synthetic utility.[18] Moreover, the still missing control over the stereochemical outcome of RCM-based macrocyclizations may increase the number of possible isomers even further.[19,20] Finally, one has to keep in mind that polar substituents in the substrate, when located at positions where they can chelate the incipient metal carbene intermediates, strongly impact the effectiveness of RCM-based macrocyclizations.[16,21] In consideration of these daunting issues, it appeared to us that the C.11−C.12 double bond in **2** might be the only promising site, if any, for an RCM-based approach toward this particular target.

Preparation of the Building Blocks. The synthetic route to iejimalide B commenced with the acylation of commercial bis-(trimethylsilyl)ethyne with 4-pentenoic acid chloride **9**, which

Scheme 2 [a]
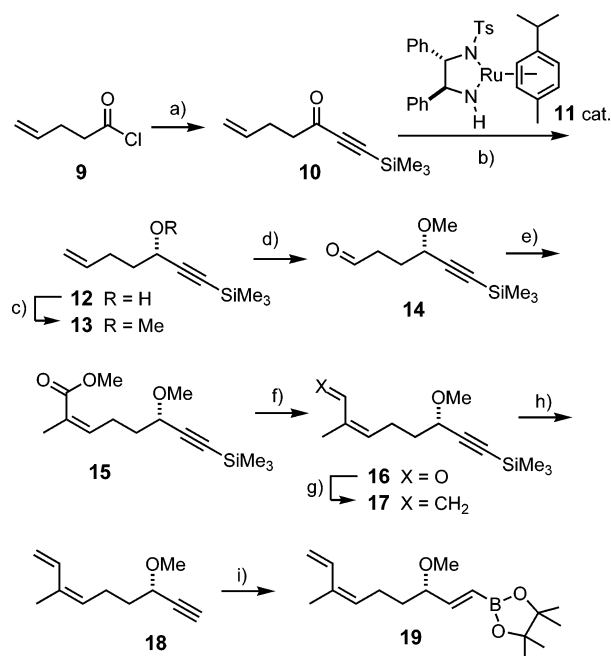
[a] Reagents and conditions: (a) bis(trimethylsilyl)acetylene, AlCl3, CH2Cl2, 0 °C, 83%; (b) complex **11** (0.6 mol %), i-PrOH, 98% (ee = 98.8%); (c) (i) n-BuLi, MeI, THF, −78 °C; (ii) DMSO, −25 °C → room temperature; (d) OsO4 cat., NaIO4, 2,6-lutidine, aqueous 1,4-dioxane, 74% (over both steps); (e) (CF3CH2O)2P(O)CH(Me)COOMe, KHMDS, 18-crown-6 (0.7 equiv), toluene, −20 °C, 87%; (f) (i) DIBAl−H, CH2Cl2, −78 °C; (ii) MnO2, CH2Cl2, 96% (over both steps); (g) [Ph3PCH3]+Br−, n-BuLi, THF, −78 °C → room temperature, quantitative; (h) K2CO3, MeOH, 83%; (i) pinacolborane, 9-BBN (10 mol %), THF, 45 °C, 56%.

gave the monosubstitution product **10** in 83% yield on a >16 g scale after convenient purification by Kugelrohr distillation (Scheme 2).[22] Asymmetric reduction of the ketone by transfer hydrogenation following Noyori's conditions worked admirably well, furnishing propargyl alcohol **12** in 98% yield in almost optically pure form (ee = 98.8%) without affecting the olefinic

(14) For another instructive example implying the formation of ketenes during Yamaguchi lactonization see: (a) Va, P.; Roush, W. R. *Org. Lett*. **2007**, *9*, 307. (b) Va, P.; Roush, W. R. *Tetrahedron* **2007**, *63*, 5768.

(15) (a) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res*. **2001**, *34*, 18. (b) Fürstner, A. *Angew. Chem., Int. Ed*. **2000**, *39*, 3013. (c) Schrock, R. R.; Hoveyda, A. H. *Angew. Chem., Int. Ed*. **2003**, *42*, 4592. (d) Connon, S. J.; Blechert, S. *Angew. Chem., Int. Ed*. **2003**, *42*, 1900. (e) Nicolaou, K. C.; Bulger, P. G.; Sarlah, D. *Angew. Chem., Int. Ed*. **2005**, *44*, 4490. (f) Deiters, A.; Martin, S. F. *Chem. Rev*. **2004**, *104*, 2199. (g) Fürstner, A. *Top. Catal*. **1997**, *4*, 285.

(16) (a) Fürstner, A.; Langemann, K. *J. Org. Chem*. **1996**, *61*, 3942. (b) Fürstner, A.; Kindler, N. *Tetrahedron Lett*. **1996**, *37*, 7005. (c) Fürstner, A.; Langemann, K. *Synthesis* **1997**, 792.

(17) Selected applications to the preparation of macrocyclic natural products from this group: (a) Fürstner, A.; Langemann, K. *J. Org. Chem*. **1996**, *61*, 8746. (b) Fürstner, A.; Langemann, K. *J. Am. Chem. Soc*. **1997**, *119*, 9130. (c) Fürstner, A.; Müller, T. *J. Org. Chem*. **1998**, *63*, 424. (d) Fürstner, A.; Gastner, T.; Weintritt, H. *J. Org. Chem*. **1999**, *64*, 2361. (e) Fürstner, A.; Seidel, G.; Kindler, N. *Tetrahedron* **1999**, *55*, 8215. (f) Fürstner, A.; Müller, T. *J. Am. Chem. Soc*. **1999**, *121*, 7814. (g) Fürstner, A.; Grabowski, J.; Lehmann, C. W. *J. Org. Chem*. **1999**, *64*, 8275. (h) Fürstner, A.; Thiel, O. R.; Kindler, N.; Bartkowska, B. *J. Org. Chem*. **2000**, *65*, 7990. (i) Fürstner, A.; Radkowski, K. *Chem. Commun*. **2001**, 671. (j) Fürstner, A.; Stelzer, F.; Rumbo, A.; Krause, H. *Chem. Eur. J*. **2002**, *8*, 1856. (k) Fürstner, A.; Jeanjean, F.; Razon, P. *Angew. Chem., Int. Ed*. **2002**, *41*, 2097. (l) Fürstner, A.; Jeanjean, F.; Razon, P.; Wirtz, C.; Mynott, R. *Chem. Eur. J*. **2003**, *9*, 320. (m) Fürstner, A.; Leitner, A. *Angew. Chem., Int. Ed*. **2003**, *42*, 308. (n) Aïssa, C.; Riveiros, R.; Ragot, J.; Fürstner, A. *J. Am. Chem. Soc*. **2003**, *125*, 15512. (o) Fürstner, A.; Aïssa, C.; Riveiros, R.; Ragot, J. *Angew. Chem., Int. Ed*. **2002**, *41*, 4763. (p) Scheiper, B.; Glorius, F.; Leitner, A.; Fürstner, A. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11960. (q) Fürstner, A.; Müller, C. *Chem. Commun*. **2005**, 5583. (r) Fürstner, A.; Nagano, T. *J. Am. Chem. Soc*. **2007**, *129*, 1906.

(18) For leading references on the synthesis of cyclic 1,3-dienes by RCM and illustrations of the associated selectivity issues, see: (a) Sedrani, R.; Kallen, J.; Martin Cabrejas, L. M.; Papageorgiou, C. D.; Senia, F.; Rohrbach, S.; Wagner, D.; Thai, B.; Eme, A.-M. J.; France, J.; Oberer, L.; Rihs, G.; Zenke, G.; Wagner, J. *J. Am. Chem. Soc*. **2003**, *125*, 3849. (b) Paquette, L. A.; Basu, K.; Eppich, J. C.; Hofferberth, J. E. *Helv. Chim. Acta* **2002**, *85*, 3033. (c) Garbaccio, R. M.; Stachel, S. J.; Baeschlin, D. K.; Danishefsky, S. J. *J. Am. Chem. Soc*. **2001**, *123*, 10903. (d) Yang, Z.-Q.; Geng, X.; Solit, D.; Pratislas, C. A.; Rosen, N.; Danishefsky, S. J. *J. Am. Chem. Soc*. **2004**, *126*, 7881. (e) Biswas, K.; Lin, H.; Njardarson, J. T.; Chappell, M. D.; Chou, T.-C.; Guan, Y.; Tong, W. P.; He, L.; Horwitz, S. B.; Danishefsky, S. J. *J. Am. Chem. Soc*. **2002**, *124*, 9825. (f) Dvorak, C. A.; Schmitz, W. D.; Poon, D. J.; Pryde, D. C.; Lawson, J. P.; Amos, R. A.; Meyers, A. I. *Angew. Chem., Int. Ed*. **2000**, *39*, 1664. (g) Bach, T.; Lemarchand, A. *Synlett* **2002**, 1302. (h) Lu, K.; Huang, M.; Xiang, Z.; Liu, Y.; Chen, J.; Yang, Z. *Org. Lett*. **2006**, *8*, 1193. (i) Wang, X.; Porco, J. A., Jr. *Angew. Chem., Int. Ed*. **2006**, *45*, 3067; Corrigenda *Angew. Chem., Int. Ed*. **2006**, *45*, 6607. (j) Rivkin, A.; Yoshimura, F.; Gabarda, A. E.; Cho, Y. S.; Chou, T.-C.; Dong, H.; Danishefsky, S. J. *J. Am. Chem. Soc*. **2004**, *126*, 10913. (k) Barluenga, S.; Lopez, P.; Moulin, E.; Winssinger, N. *Angew. Chem., Int. Ed*. **2004**, *43*, 3467. (l) Wang, X.; Bowman, E. J.; Bowman, B. J.; Porco, J. A., Jr. *Angew. Chem., Int. Ed*. **2004**, *43*, 3601. (m) Basu, S.; Waldmann, H. *J. Org. Chem*. **2006**, *71*, 3977. (n) Evano, G.; Schaus, J. V.; Panek, J. S. *Org. Lett*. **2004**, *6*, 525. (o) Wang, X.; Porco, J. A., Jr. *J. Am. Chem. Soc*. **2003**, *125*, 6040. (p) Ghosh, A. K.; Gong, G. *J. Org. Chem*. **2006**, *71*, 1085.

(19) Lacombe, F.; Radkowski, K.; Seidel, G.; Fürstner, A. *Tetrahedron* **2004**, *60*, 7315.

(20) For a discussion see: Fürstner, A.; Davies, P. W. *Chem. Commun*. **2005**, 2307.

(21) Fürstner, A.; Thiel, O. R.; Lehmann, C. W. *Organometallics* **2002**, *21*, 331.

(22) Suzuki, R.; Tsukuda, H.; Watanabe, N.; Kuwatani, Y.; Ueda, I. *Tetrahedron* **1998**, *54*, 2477.

and the acetylenic sites in vicinity.[23] In contrast, however, the seemingly trivial conversion of **12** into methyl ether **13** was surprisingly troublesome; only the procedure originally reported by Suzuki et al. using DMSO as dipolar cosolvent gave well-reproducible results upon up-scaling.[22]

The subsequent cleavage of the double bond in **13** in the presence of the alkyne unit was best performed with OsO_4 cat./ $NaIO_4$/2,6-lutidine,[24] whereas attempted ozonolysis gave a moderate yield of the desired aldehyde **14** (49%). A modified Still–Gennari olefination employing only substoichiometric amounts of 18-crown-6 as additive then afforded the required (*Z*)-configured enoate **15** as a single isomer,[25,26] which was subjected to a sequence of reduction/oxidation of the ester moiety, Wittig olefination of the resulting aldehyde **16**, and protodesilylation to unmask the terminal alkyne. Although the resulting (*Z*)-configured α,β-unsaturated aldehyde **16** is prone to isomerization and has to be handled with great care, this route required no chromatographic purification of any of the intermediates, afforded the desired product **18** in excellent overall yield, and was therefore deemed fully satisfactory. Compound **18** underwent chemoselective hydroboration with pinacolborane at the alkyne terminus without damaging the tethered 1,3-diene entity, provided that catalytic amounts of 9-BBN were present to promote the reaction.[27]

A suitable coupling partner **30** was obtained by adaptation of a literature route (Scheme 3).[8] Specifically, an effective Heck reaction[28] of **20** and **21** followed by routine protecting group and oxidation state management delivered multigram amounts of aldehyde **24** which was subjected to a palladium-catalyzed, Et_2Zn-mediated addition of propargyl mesylate **25**.[29,30] The best selectivity in favor of the required anti isomer was obtained with compound **25a** bearing a bulky TIPS group at the acetylene moiety (dr = 7.5:1), whereas the use of the terminal alkyne **25b** (R^1 = H) gave a less satisfactory outcome (dr = 3.5:1). This observation corroborates the conclusions reached by Marshall et al. in a concomitant study, emphasizing the superiority of propargyl mesylates terminated by sterically encumbered silyl substituents for reactions of this type.[31] After chromatographic purification, the major isomer **26** was obtained in 72% yield. Subsequent cleavage of the silyl moiety gave **27**, which was temporarily protected as the corresponding pivalate **28** prior to hydrozirconation/iodination.[32] Gratifyingly, the pivaloyl group could be selectively removed from the resulting

Scheme 3 *a*
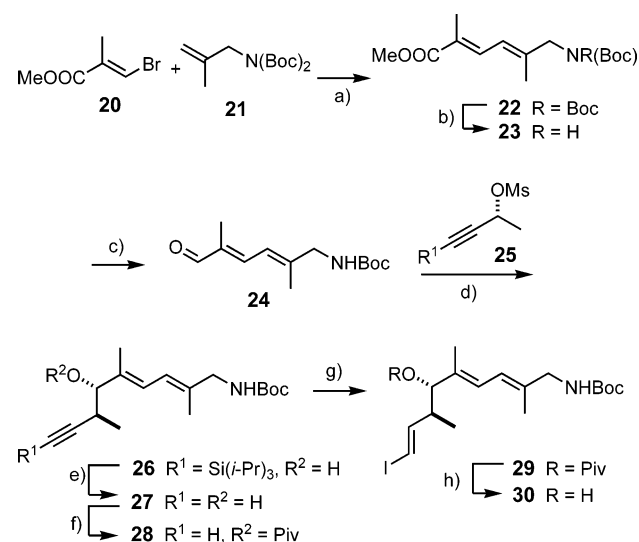
a Reagents and conditions: (a) Pd(OAc)$_2$ (3 mol %), P(*o*-tol)$_3$ (6 mol %), Et$_3$N, 100 °C, 84%; (b) trifluoroacetic acid, CH$_2$Cl$_2$, 87%; (c) (i) DIBAl–H, CH$_2$Cl$_2$, −78 °C, 97%; (ii) DMSO, (COCl)$_2$, Et$_3$N, CH$_2$Cl$_2$, −78 °C → room temperature, 79%; (d) **25**, Pd(OAc)$_2$ (5 mol %), PPh$_3$ (5 mol %), Et$_2$Zn (3 equiv), THF, −78 °C → −20 °C, 72%; (e) TBAF, THF, 94%; (f) pivaloyl chloride, pyridine, 0 °C → room temperature, 76%; (g) Cp$_2$Zr(H)Cl, THF, then I$_2$, 0 °C → room temperature, 85%; (h) DIBAl–H, CH$_2$Cl$_2$, −78 °C, 87%.
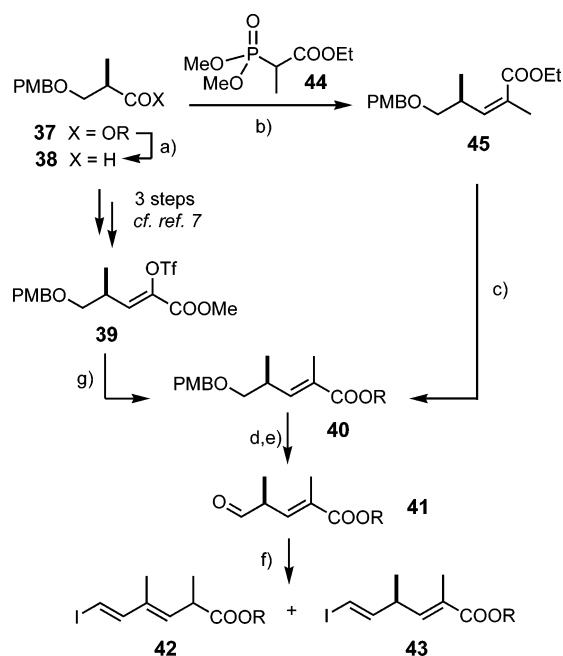
Scheme 4 *a*
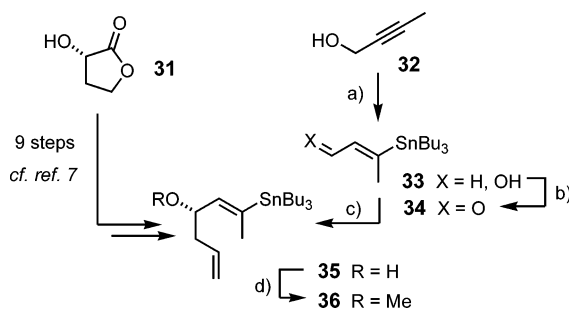
a Reagents and conditions: (a) Bu$_3$Sn(Bu)CuCNLi$_2$, THF, MeOH, −78 °C → −10 °C, ref 35; (b) MnO$_2$, CH$_2$Cl$_2$, 95%; (c) (−)-Ipc$_2$BOMe, allylMgBr, Et$_2$O, −78 °C, 75% (ee = 93%); (d) Me$_3$OBF$_4$, proton sponge, CH$_2$Cl$_2$, 88%.

alkenyl iodide **29** with the aid of DIBAl–H without affecting the terminal *N*-Boc moiety.

The original route to the northern hemisphere of **2** employed commercial lactone **31** (ee = 96%) as the starting material, which was converted into the required building block **36** in nine operations with 24% overall yield.[7,33] In an attempt to improve the overall "economy of steps",[34] however, the access route was redesigned once the total synthesis of **2** had been successfully completed, and the project entered the phase of optimization and up-scaling. The improved synthesis (Scheme 4) starts with a regioselective hydrostannation[35] of propargyl alcohol **32** followed by oxidation of **33** with MnO$_2$. Brown allylation[36] of

(23) (a) Matsumura, K.; Hashiguchi, S.; Ikariya, T.; Noyori, R. *J. Am. Chem. Soc.* **1997**, *119*, 8738. (b) Haack, K.-J.; Hashiguchi, S.; Fujii, A.; Ikariya, T.; Noyori, R. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 285. (c) Noyori, R.; Hashiguchi, S. *Acc. Chem. Res.* **1997**, *30*, 97. (d) For a recent application from this laboratory see: Fürstner, A.; Hannen, P. *Chem. Eur. J.* **2006**, *12*, 3006.
(24) Yu, W.; Mei, Y.; Kang, Y.; Hua, Z.; Jin, Z. *Org. Lett.* **2004**, *6*, 3217.
(25) Still, W. C.; Gennari, C. *Tetrahedron Lett.* **1983**, *24*, 4405.
(26) Mickel, S. J.; et al. *Org. Process Res. Dev.* **2004**, *8*, 113−121.
(27) (a) Arase, A.; Hoshi, H.; Mijin, A.; Nishi, K. *Synth. Commun.* **1995**, *25*, 1957. (b) Nicolaou, K. C.; Fylaktakidou, K. C.; Monenschein, H.; Li, Y.; Weyershausen, B.; Mitchell, H. J.; Wei, H.; Guntupalli, P.; Hepworth, D.; Sugita, K. *J. Am. Chem. Soc.* **2003**, *125*, 15433.
(28) (a) Bräse, S.; de Meijere, A. In *Metal-Catalyzed Cross-Coupling Reactions*, 2nd ed.; de Meijere, A., Diederich, F., Eds.; Wiley-VCH: Weinheim, Germany, 2004; Vol. 1; p 217. (b) Beletskaya, I. P.; Cheprakov, A. V. *Chem. Rev.* **2000**, *100*, 3009.
(29) (a) Marshall, J. A. *Chem. Rev.* **2000**, *100*, 3163. (b) Marshall, J. A.; Adams, N. D. *J. Org. Chem.* **1999**, *64*, 5201. (c) Marshall, J. A.; Schaaf, G. M. *J. Org. Chem.* **2001**, *66*, 7825.
(30) For recent applications from this group see: (a) Fürstner, A.; Kattnig, E.; Lepage, O. *J. Am. Chem. Soc.* **2006**, *128*, 9194. (b) Beşev, M.; Brehm, C.; Fürstner, A. *Collect. Czech. Chem. Commun.* **2005**, *70*, 1696.
(31) Marshall, J. A.; Eidam, P.; Eidam, H. S. *J. Org. Chem.* **2006**, *71*, 4840.
(32) Hart, D. W.; Schwartz, J. *J. Am. Chem. Soc.* **1974**, *96*, 8115−8116.

(33) Details concerning the original routes can also be found in the Supporting Information.
(34) For a discussion, see: Fürstner, A. *Synlett* **1999**, 1523.
(35) Betzer, J.-F.; Delaloge, F.; Muller, B.; Pancrazi, A.; Prunet, J. *J. Org. Chem.* **1997**, *62*, 7768.
(36) (a) Racherla, U. S.; Liao, Y.; Brown, H. C. *J. Org. Chem.* **1992**, *57*, 6614. (b) Brown, H. C.; Jadhav, P. K. *J. Am. Chem. Soc.* **1983**, *105*, 2092. (c) Recent large-scale application: Fürstner, A.; Fenster, M. D. B.; Fasching, B.; Godbout, C.; Radkowski, K. *Angew. Chem., Int. Ed.* **2006**, *45*, 5506.
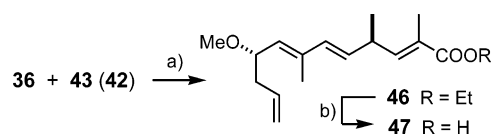
Scheme 5 [a]

Scheme 6 [a]

[a] Reagents and conditions: (a) Pd(PPh$_3$)$_4$ (5 mol %), CuTC, Ph$_2$PO$_2^-$NBu$_4^+$, DMF, room temperature, 80%; (b) aqueous LiOH, THF/MeOH, 85%.

[a] Reagents and conditions: (a) DIBAl−H, CH$_2$Cl$_2$, −78 °C; (b) compound **44**, LiHMDS, THF, −78 °C → −40 °C; (c) PhSSPh, AIBN, THF, reflux, 77% (R = Et, over three steps, *E/Z* = 20:1); (d) DDQ, CH$_2$Cl$_2$, H$_2$O (5% v/v), 83%; (e) Dess−Martin periodinane, CH$_2$Cl$_2$; (f) CHI$_3$, CrCl$_2$·1.8THF, 1,4-dioxane/THF (6:1), 51% (R = Et, over two steps); (g) MeZnCl, Pd(PPh$_3$)$_4$ (5 mol %), THF, 50 °C, 91% (R = Me).

the resulting crude aldehyde **34**[37] and subsequent O-methylation of alcohol **35** (ee = 93%) with Meerwein salt in the presence of proton sponge as the optimal base readily afforded product **36**. This "second generation" fragment synthesis represents a significant shortcut (four rather than nine steps), is significantly more productive (48% overall yield) and scaleable, and has the extra bonus of employing a much cheaper starting material.

Among the different building blocks required for the total synthesis of iejimalide B, the ester segment **43** was clearly most demanding. The original synthesis[7,33] was based on the conversion of Roche ester derivative **37**[38] into enol triflate **39** followed by cross-coupling with MeZnCl in the presence of catalytic amounts of Pd(PPh$_3$)$_4$.[39] The PMB−ether in the resulting enoate **40** was cleaved with DDQ, and the liberated alcohol was oxidized to the corresponding aldehyde **41** which underwent a Takai olefination[40] to give the desired alkenyl iodide **43** (R = Me) and the double-bond isomer **42** thereof (Scheme 5). Although these compounds were inseparable at that stage, a convenient purification could be achieved after fragment coupling with stannane **36** by a modified Stille reaction[41] (see below).

Although we were worried that the double-bond isomerization responsible for the formation of **42** might be reversible and hence could lead to racemization of the chiral center at C.4, the ee of product **43** was found largely unchanged and exceeded 90% in all samples investigated during the course of this project.

Triflate **39** has the distinct advantage of serving as a convenient springboard for systematic modifications at C.2 once the focus of the project shifts to the preparation of iejimalide analogues by "diverted total synthesis".[42] Such variations are desirable since iejimalide B (**2**) featuring a methyl branch at C.2 is reported to be more cytotoxic than iejimalide A (**1**) bearing a proton at that position.[1−3] From a mere efficiency viewpoint, however, a streamlined preparation of **43**, that reduces the number of steps in the longest linear sequence leading to iejimalide B, is an equally desirable task.

This goal might be achieved by routine olefination chemistry. However, various attempts to react aldehyde **38** with Ph$_3$P= C(Me)COOEt or phosphonate **44** were thwarted by partial racemization of the adjacent chiral center (ee < 55%). Only the Horner−Emmons reaction promoted by LiHMDS in THF at low temperature delivered an enoate in acceptable optical purity (ee ≥ 92%), albeit as the unwanted (*Z*)-isomer **45** (Scheme 5). The stereochemical outcome of the reaction could be rectified under free radical conditions (PhSSPh, AIBN), and the resulting ester **40** was elaborated as outlined above to give an inseparable mixture of vinyl iodide **43** (R = Et) and the conjugated diene **42** (R = Et).
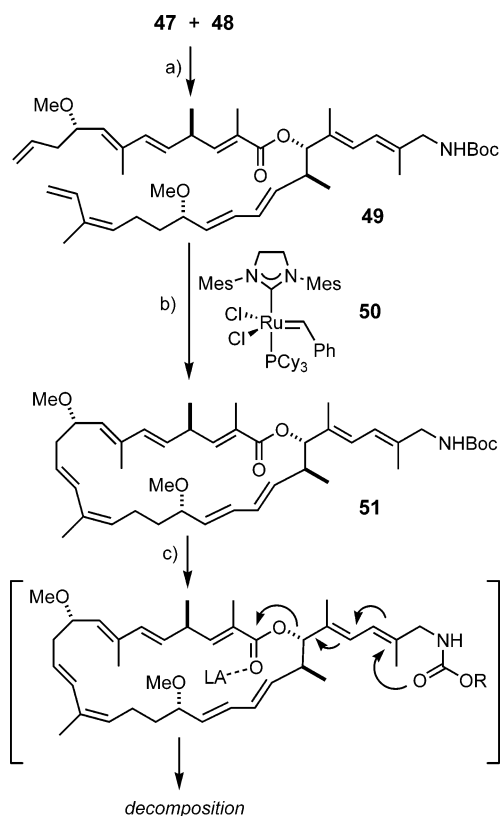
Fragment Coupling and Attempted Completion of the Synthesis. With all required components in hand, the project entered the next stage where effective fragment coupling processes had to be worked out. First and foremost, we focused on the elaboration of the "northern" segment by coupling of stannane **36** and iodide **43**, which was still contaminated by the undesired conjugated diene (**43**:**42** ≈ 6:1). Despite a most impressive track record, the projected Stille reaction[41] between these two partners required careful optimization. After considerable experimentation it was found that a cocktail containing Pd(PPh$_3$)$_4$, copper-2-thiophenecarboxylate (CuTC), and Ph$_2$PO$_2^-$NBu$_4^+$ in DMF promoted the reaction under notably mild conditions (Scheme 6).[43] Gratifyingly, the byproduct derived from cross-coupling of the isomeric iodide **42** could be removed by flash chromatography after saponification of the ester group, thus securing a productive entry into the required building block **47**.

At this juncture, we were facing the challenge of joining fragments **19** and **30** to the southern hemisphere of the iejimalides by a Suzuki reaction.[44] Having previously experienced the exceptional sensitivity of advanced iejimalide subunits toward base,[7a] much attention was paid to the reaction conditions (Scheme 7). Following a lead from the recent literature that

(37) Dominguez, B.; Pazos, Y.; de Lera, A. R. *J. Org. Chem.* **2000**, *65*, 5917.
(38) Heckrodt, T. J.; Mulzer, J. *Synthesis* **2002**, 1857.
(39) Negishi, E.; Zheng, X.; Tan, Z.; Qian, M.; Hu, Q.; Huang, Z. In *Metal-Catalyzed Cross-Coupling Reactions*, 2nd ed.; de Meijere, A., Diederich, F., Eds.; Wiley-VCH: Weinheim, Germany, 2004; Vol. 2; p 815.
(40) (a) Takai, K.; Nitta, K.; Utimoto, K. *J. Am. Chem. Soc.* **1986**, *108*, 7408. (b) Evans, D. A.; Black, W. C. *J. Am. Chem. Soc.* **1993**, *115*, 4497. (c) Review: Fürstner, A. *Chem. Rev.* **1999**, *99*, 991.
(41) Review: (a) Farina, V.; Krishnamurthy, V.; Scott, W. *Org. React.* **1997**, *50*, 1. (b) Espinet, P.; Echavarren, A. M. *Angew. Chem., Int. Ed.* **2004**, *43*, 4704.
(42) For a pertinent discussion of the concept of "diverted total synthesis", see: Wilson, R. M.; Danishefsky, S. J. *J. Org. Chem.* **2006**, *71*, 8329.
(43) (a) Fuwa, H.; Kainuma, N.; Tachibana, K.; Sasaki, M. *J. Am. Chem. Soc.* **2002**, *124*, 14983. (b) For the use of CuTC in Pd-free Stille reactions see: Allred, G. D.; Liebeskind, L. S. *J. Am. Chem. Soc.* **1996**, *118*, 2748. (c) For the use of Ph$_2$PO$_2^-$NBu$_4^+$, see: Durham, T. B.; Blanchard, N.; Savall, B. M.; Powell, N. A.; Roush, W. R. *J. Am. Chem. Soc.* **2004**, *126*, 9307.

(44) Reviews: (a) Suzuki, A. *J. Organomet. Chem.* **1999**, *576*, 147. (b) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.

Scheme 7 [a]

19 + 30 → (structure of **48**)
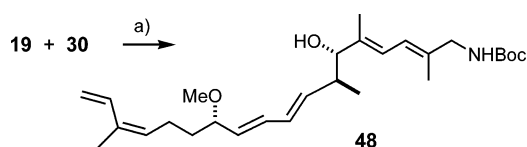
[a] Reagents and conditions: (a) (dppf)PdCl$_2$ (15 mol %), Ba(OH)$_2$·8H$_2$O (1.2 equiv), DMF, 40 °C, 66%.

Scheme 8 [a]

47 + 48

a) ↓

(structure of **49**)

b) ↓ (complex **50** shown: Mes–N⌒N–Mes, Cl, Cl, Ru, PCy$_3$, Ph)

(structure of **51**)

c) ↓

(bracketed transition-state structure with LA···O, NH, OR)

↓

decomposition

[a] Reagents and conditions: (a) 2,4,6-trichlorobenzoyl chloride, Et$_3$N, DMAP cat., toluene, 73%; (b) complex **50** (2 × 10 mol %), CH$_2$Cl$_2$, room temperature, 2d, 96%; (c) TMSOTf, 2,6-lutidine, CH$_2$Cl$_2$, 0 °C, cf., text; LA = Lewis acid.

shows that Ba(OH)$_2$·8H$_2$O is a particularly suitable promoter for the formation of products embodying fragile diene units,[45] this protocol was adapted to the present case and found highly suitable. In fact, the amount of base could be reduced to 1.2 equiv and the reaction proceeded at or slightly above room temperature with acceptable rates, delivering product **48** in well-reproducible 66% isolated yield.

In contrast to the failed macrolactonization approaches to the iejimalides (see Scheme 1),[7a] the required ester could be installed without problems in an intermolecular setting. Thus, reaction of acid **47** with the sterically hindered alcohol **48** under Yonemitsu conditions[46] afforded product **49** in 73% yield (Scheme 8). The ring closure of this polyunsaturated compound with the aid of the "second generation" ruthenium alkylidene complex **50**[47,48] proceeded exquisitely well; though taking 2 days to reach complete conversion, the reaction could be performed at ambient temperature, thus minimizing the risk of decomposing

the thermally very sensitive polyene substrate. Under these conditions, the desired macrocycle **51** was obtained in almost quantitative yield as the required (*E*)-isomer only.

With this gratifying result, the completion of the total synthesis of iejimalide B seemed to be just a matter of two routine operations, i.e., cleavage of the Boc group followed by attachment of the serine terminus by standard peptide coupling. However, our hopes were dashed by the inability to remove the *N*-Boc substituent of **51** under a variety of experimental conditions. Even the use of TMSOTf buffered with 2,6-lutidine, reported to be a particularly mild method,[49,50] instantaneously decomposed the valuable product. This outcome was unexpected since scrupulous model studies performed at the outset of the project had unanimously encouraged the use of a Boc moiety for the protection of the amine terminus.[7a,33] It is believed that very subtle conformational effects in **51** enforced by the presence of the macrocyclic frame align the π-system of the diene with the lactone C–O σ-bond and hence render the latter a formidable leaving group upon activation with a Lewis acid (cf., Scheme 8).[7a,33]

Revised Strategy and Completion of the Total Synthesis. At this stage, we were facing two options for the completion of the first total synthesis of iejimalide B: on the one hand, a more dischargeable protecting group for the amine terminus might be found that can be removed in the penultimate step without deleterious crosstalk to the fragile ester moiety as observed in the −NBoc series. Alternatively, the required *N*-formyl serine residue could be installed early on, thus avoiding any late-stage protecting group manipulations at that site. Both routes were pursued in parallel and ultimately turned out to be successful; as will become evident from the results outlined below, however, they have clearly different preparative merits.

In striking contrast to the failed cleavage of the *N*-Boc group in **51** featuring an intact macrocycle, its removal from building block **29** proceeded readily and in high yield with the aid of TMSOTf/lutidine (Scheme 9). Although the coupling of the resulting amine with *O*-(*tert*-butyldimethylsilyl)-*N*-formyl-L-serine **57**[51] posed no problems, the presence of the peptide residue rendered the use of LiBEt$_3$H instead of DIBAl–H mandatory for the subsequent cleavage of the pivaloyl group in **52**. The Suzuki reaction of the resulting product **53** with boronate **19** was effected at room temperature under the previously optimized conditions. The seemingly trivial esterification of **54** with acid **47**, however, could only be effected with DCC and 4-pyrrolidinopyridine; in contrast, Yonemitsu conditions[46] were inappropriate, even though they had led to the closely related ester **49** without incident (cf., Scheme 8). RCM of the resulting polyene **55** delivered the 24-membered macrocycle **56** in 69% yield as a single (*E*)-isomer. In view of

(45) Gopalarathnam, A.; Nelson, S. G. *Org. Lett.* **2006**, *8*, 7.
(46) Hikota, M.; Sakurai, Y.; Horita, K.; Yonemitsu, O. *Tetrahedron Lett.* **1990**, *31*, 6367.
(47) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953.
(48) See also: (a) Ackermann, L.; Fürstner, A.; Weskamp, T.; Kohl, F. J.; Herrmann, W. A. *Tetrahedron Lett.* **1999**, *40*, 4787. (b) Fürstner, A.; Thiel, O. R.; Ackermann, L.; Schanz, H.-J.; Nolan, S. P. *J. Org. Chem.* **2000**, *65*, 2204. (c) Fürstner, A.; Ackermann, L.; Gabor, B.; Goddard, R.; Lehmann, C. W.; Mynott, R.; Stelzer, F.; Thiel, O. R. *Chem. Eur. J.* **2001**, *7*, 3236. (d) Fürstner, A.; Guth, O.; Düffels, A.; Seidel, G.; Liebl, M.; Gabor, B.; Mynott, R. *Chem. Eur. J.* **2001**, *7*, 4811.
(49) (a) Sakaitani, M.; Ohfune, Y. *J. Org. Chem.* **1990**, *55*, 870. (b) Nazaré, M.; Waldmann, H. *Chem. Eur. J.* **2001**, *7*, 3363. (c) Trost, B. M.; Fandrick, D. R. *Org. Lett.* **2005**, *7*, 823.
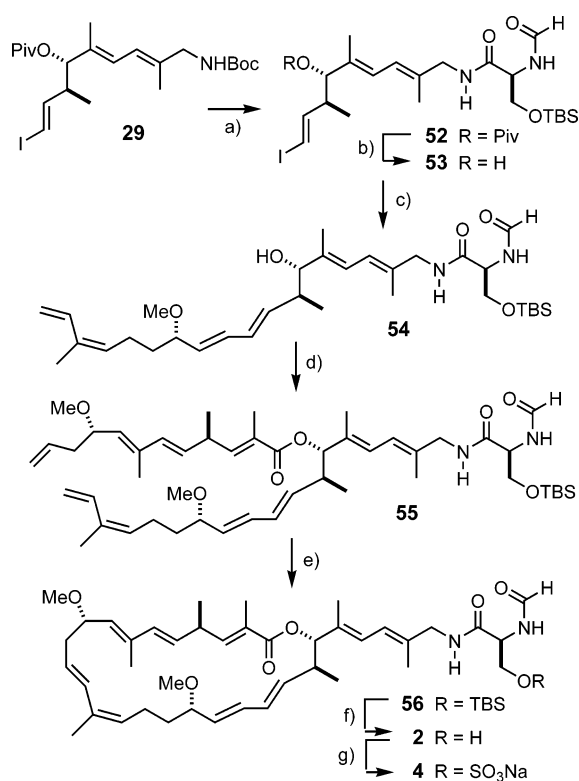(50) For an example, however, in which the use of TBSOTf/lutidine led to ester cleavage/elimination, see: Karama, U.; Höfle, G. *Eur. J. Org. Chem.* **2003**, 1042.
(51) Hill, D. R.; Hsiao, C.-N.; Kurukulasuriya, R.; Wittenberger, S. J. *Org. Lett.* **2002**, *4*, 111.

Scheme 9 [a]

[a] Reagents and conditions: (a) (i) TMSOTf, 2,6-lutidine, CH$_2$Cl$_2$, then CsF, 0 °C; (ii) *O*−TBS−*N*-formyl-L-serine (**57**), EDC, HOBt, *N*-methyl-morpholine (NMM), CH$_2$Cl$_2$, 85% (over both steps); (b) LiBEt$_3$H, THF, 0 °C, 70%; (c) boronate **19**, (dppf)PdCl$_2$ (15 mol %), Ba(OH)$_2$·8H$_2$O (1.2 equiv), DMF, room temperature, 70−80%; (d) acid **47**, DCC, 4-pyrrolidin-ylpyridine (30 mol %), CH$_2$Cl$_2$, 0 °C → room temperature, 85%; (e) complex **50** (15 mol %), CH$_2$Cl$_2$ (5 × 10^{-3} M), room temperature, 69%; (f) TBAF, THF, 0 °C, 80%; (g) SO$_3$·pyridine, ref 2a.

the exceptional thermal lability of the iejimalide precursors, it was once again instrumental that this key transformation required no heating. The outcome of this reaction is truly remarkable if interpreted in light of the implicit selectivity and stability issues. Although the strategic advantages and utilitarian potential of RCM need no further confirmation,[15,17] this specific case seems particularly instructive, shedding a radiant spotlight onto the superb application profile of Grubbs-type catalysts in general. The first total synthesis of iejimalide B was then completed by the cleavage of the only remaining TBS−ether in **56** with TBAF at low temperature. Its identity follows from the perfect congruence of the recorded data of the synthetic samples of **2** and those of the natural product.[1,2] Since the attachment of the sulfate ester to the serine −OH group of **2** had already been described using SO$_3$·pyridine,[2a] we could simply follow this protocol to reach iejimalide D (**4**) as well. The ^{1}H NMR spectra of natural **4** and the synthetic material recorded at 600 MHz are also superimposable and in full agreement with the proposed structure.[2a]

Alternative Total Synthesis and Late-Stage Diversification. Once a concise and productive route to the parent compound had been established, systematic structural editing of the natural product became our next immediate goal. As iejimalide B is assembled in a convergent fashion from five building blocks of similar size and complexity, the underlying synthesis blueprint should qualify very well for a "diverted total synthesis" campaign.[42] Although this is indeed the case and a first round

of synthesis-driven mapping of the macrocyclic frame of the natural lead is well advanced, the desirable variations of the peptide moiety remain the only quite laborious part of such an endeavor. This is caused by the fact that the peptide group is introduced at a rather early phase of the successful assembly process, whereas attempted installation in the final stages had failed due to our inability to remove the −NBoc moiety from the advanced intermediate **51** as described above. This might be rectified, however, with the aid of a more suitable protecting group that allows unmasking of the N-terminus without destroying the unusually sensitive compound.

Since the successful cleavage of the −OTBS ether in the final step of the total synthesis shows that iejimalide B withstands exposure to TBAF in THF at 0 °C, we opted for a −NTeoc group that might be discharged under similarly mild conditions. The required starting material **58** was best prepared from **23** by protecting group interchange.[52] From there on, the sequence essentially follows the route previously described, although some modifications were necessary (Scheme 10). One such adaptation concerns the oxidation of alcohol **59** to the corresponding aldehyde **60**, which had to be performed with MnO$_2$ as the oxidant, whereas the Swern procedure,[53] though highly productive in the −NBoc series, would not work satisfactorily in this case. The excellent diastereo- and enantioselectivity garnered in the subsequent Marshall reaction[29−31] of **60** with the TIPS-protected propargyl mesylate **25a** (R^1 = TIPS) is also worth noting. The ensuing problem of removing the TIPS group from the alkyne without touching the −NTeoc moiety could be conveniently solved with TBAF, upon strictly limiting the amount of the reagent to 1 equiv and keeping the temperature at 0 °C. Like in the previous series, temporary protection of the alcohol as the corresponding pivalate was a necessary prelude for the hydrozirconation/iodination tandem, after which the pivalate ester was removed on exposure to LiBEt$_3$H to give product **62** in good overall yield.

This compound qualifies as a key building block en route to the iejimalides. Its preparation was not only highly productive but also found nicely scalable. Moreover, the *N*-Teoc group could be cleaved off with the aid of TBAF, a fact that augured well for the envisaged end game. The resulting amine **63** was then coupled with *N*-formyl serine **57** to give product **53** previously employed en route to **2** (cf., Scheme 9). Thereby the *N*-Teoc-based strategy not only converges with the already established total synthesis (see above) but actually turned out to be superior in terms of practicality and scalability.

Alternatively, the *N*-Teoc group can be taken further through the assembly process. In doing so, the foregoing optimization of the crucial transformations paid off, most notably in the hydrozirconation/iodination process and the Suzuki coupling. Esterification of the resulting compound **64** with acid **47** was best mediated by EDC·HCl (EDC = *N*-3-(dimethylaminopro-pyl)-*N*′-ethyl-carbodiimide), and the crucial ring closure of polyene **65** by RCM could once more be effected in good yield with the aid of the ruthenium carbene **50** at ambient temperature, thus setting the stage for the removal of the Teoc group and introduction of an assortment of amino acid residues at the released amine terminus.

(52) The direct formation of **58** by a Heck reaction of bromide **20** and *N*-Teoc-2-methylallylamine gave only ca. 55% yield, required a tedious purification, and was therefore less practical and productive.

(53) Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165.

Scheme 10 [a]
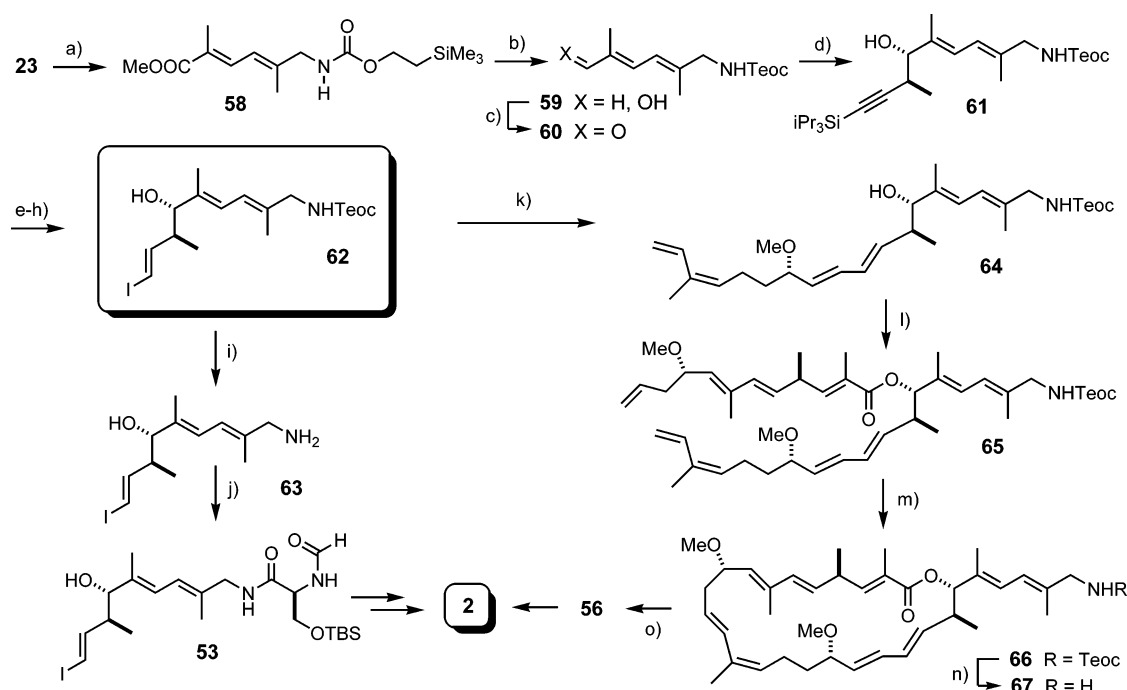
[a] Reagents and conditions: (a) (i) trifluoroacetic acid, CH_2Cl_2; (ii) 4-nitrophenyl-2-trimethylsilylethyl-carbonate, Et_3N, CH_2Cl_2, 96%; (b) DIBAl−H, CH_2Cl_2, −78 °C, 95%; (c) MnO_2, CH_2Cl_2, quantitative; (d) compound **25a** (R^1 = TIPS), Pd(OAc)$_2$ (5 mol %), PPh$_3$ (5 mol %), Et$_2$Zn (3 equiv), THF, −78 °C → −20 °C, 71% (ee > 96%); (e) TBAF (1.0 equiv), THF, 0 °C, 89%; (f) pivaloyl chloride, pyridine, DMAP cat., 94%; (g) Cp$_2$Zr(H)Cl, THF, then I$_2$, 0 °C → room temperature, 81%; (h) LiBEt$_3$H, THF, 82%; (i) TBAF (4 equiv), 0 °C → room temperature, THF; (j) compound **57**, EDC, HOBt, NMM, CH_2Cl_2, 80% (over both steps); (k) boronate **19**, (dppf)PdCl$_2$ (15 mol %), Ba(OH)$_2$·8H$_2$O (1.2 equiv), DMF, room temperature, 70%; (l) acid **47**, EDC·HCl, 4-pyrrolidinylpyridine (30 mol %), CH_2Cl_2, 0 °C → room temperature, 78%; (m) complex **50** (20 mol %), CH_2Cl_2 (5 × 10^{-3} M), room temperature, 78%; (n) TBAF (4 equiv), THF, 0 °C → room temperature; (o) compound **57**, EDC, HOBt, NMM, 30% (over both steps).

Even though −NTeoc cleavage in segment **62** had been straightforward, the analogous manipulation of the elaborate macrocycle **66** was considerably more delicate. Thus, exposure of **66** to an excess of TBAF in THF at 0 °C gave amine **67** which was found unusually sensitive and had to be processed without delay (Scheme 10). Coupling with the serine derivative **57** was effected with EDC and HOBt (1-hydroxy-benzotriazole), providing iejimalide B (**2**) via the already known precursor **56**. However, the crude material contained traces of a second unidentified isomer, which had to be removed by preparative HPLC.

Next, the route was extended to the preparation of an assortment of iejimalide analogues, in which the naturally occurring *N*-formyl serine residue is replaced by other amide moieties (Scheme 11). Specifically, product **68** incorporates *N*-formyl-L-valine instead of the serine group in **2**, whereas in **69** the *N*-formamide entity has been replaced by an *N*-benzoyl group. The structural modification in compound **70** is more profound, as the entire N-acylated amino acid segment is exchanged for a dansyl label. Together with the *N*-Boc and *N*-Teoc carbamate derivatives **51** and **66**, respectively, this panel of non-natural "iejimalide-like" molecules allowed for a first round of biological screening that unraveled the hitherto unknown actin-binding properties of macrolides of this type (see below).

Total Synthesis of Iejimalide A. After having established two successful routes to iejimalide B (**2**) differing in the stage at which the conspicuous *N*-formyl serine terminus is attached to the macrocyclic core, we embarked on a total synthesis of the companion natural product iejimalide A (**1**) devoid of the methyl branch adjacent to the lactone moiety.

Scheme 11 [a]
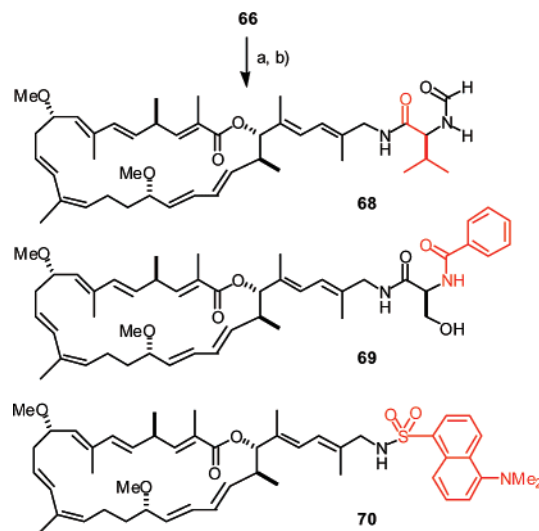
[a] Reagents and conditions: (a) TBAF (4 equiv), THF, 0 °C → room temperature; (b) *N*-formyl-L-valine, EDC·HCl, 1-hydroxy-7-azabenzotriazole (HOAt), collidine, 75% (**68**, over two steps); analogously 31% (**69**, over two steps); 45% (**70**, over two steps).

This goal seemed easily attainable by replacement of a single building block by its des-methyl analogue. However, the exceptionally low level of homology in the behavior of seemingly closely related compounds as well as the unusual sensitivity of many polyunsaturated intermediates belonging to this series surfaced once again during this venture. It was only after a surprisingly profound strategic change that iejimalide A came into reach.

Scheme 12 [a]
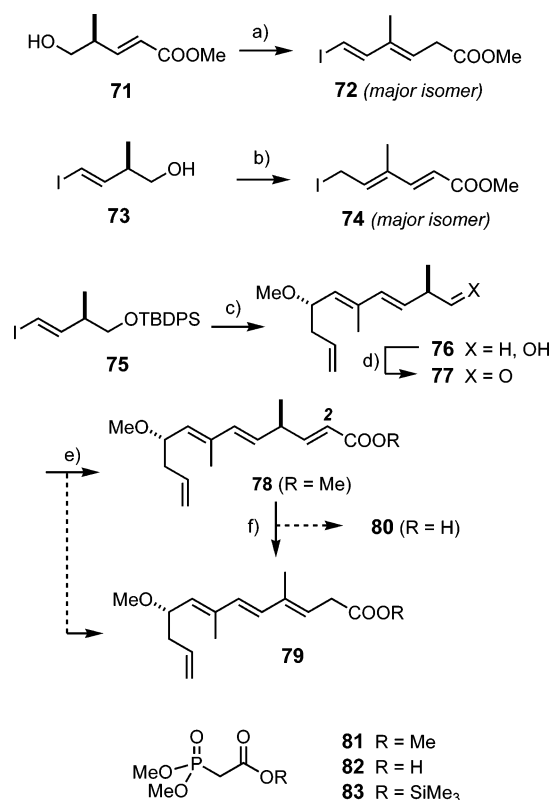
[a] Reagents and conditions: (a) (i) Dess−Martin periodinane, CH₂Cl₂; (ii) CHI₃, CrCl₂·1.8THF, 1,4-dioxane, 49% (dr ≈ 4:1); (b) (i) Dess−Martin periodinane, CH₂Cl₂; (ii) Me₃SiCH₂COOEt, LiHMDS, THF, −78 °C → room temperature; <50%; (c) (i) stannane **36**, Pd(PPh₃)₄ (5 mol %), CuTC, Ph₂PO₂⁻NBu₄⁺, DMF, room temperature, 82%; (ii) TBAF, THF, 0 °C → room temperature, 94%; (d) Dess−Martin periodinane, CH₂Cl₂; (e) phosphonate **81** (1.05 equiv), LiCl, *i*-Pr₂NEt, MeCN, 30% (**78:79** ≈ 9:1); (f) aqueous LiOH, THF/MeOH, *or* Me₃SnOH, 1,2-dichloroethane, 80 °C, see text.

Originally, it was planned to prepare **1** by esterification of **54** with acid **80** lacking the methyl branch at C.2 (relative to acid **47** used en route to **2**) and elaboration of the resulting product according to the established route. Despite considerable experimentation, however, compound **80** could never be obtained in pure form due to the very pronounced migratory aptitude of its skipped double bonds. The reactions depicted in Scheme 12 are representative. Thus, oxidation of **71** followed by a Takai olefination[40] of the resulting aldehyde mainly afforded the conjugated iodo-1,3-diene derivative **72**, whereas the inverse strategy starting from **73** with the vinyl iodide already in place gave the "shifted" diene **74** in poor yield. Analogous chain extension of the very labile aldehyde **77** was similarly unrewarding, affording the conjugated triene **79** under a variety of experimental conditions. All in all, the outcome was largely independent of the chosen oxidants (Dess−Martin periodinane, TPAP/NMO, Swern) and the reagents used for the olefination step (Wittig, Horner−Emmons, Peterson, Julia). Only the Masamune−Roush conditions[54] were found to deliver the desired enoate **78** in workable isomeric purity (dr ≈ 9:1), albeit in low yield. However, all attempts to saponify this product scrambled its double bonds even before hydrolysis took place, although some of the mildest methods for ester cleavage known

(54) Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. *Tetrahedron Lett.* **1984**, *25*, 2183.

Scheme 13 [a]
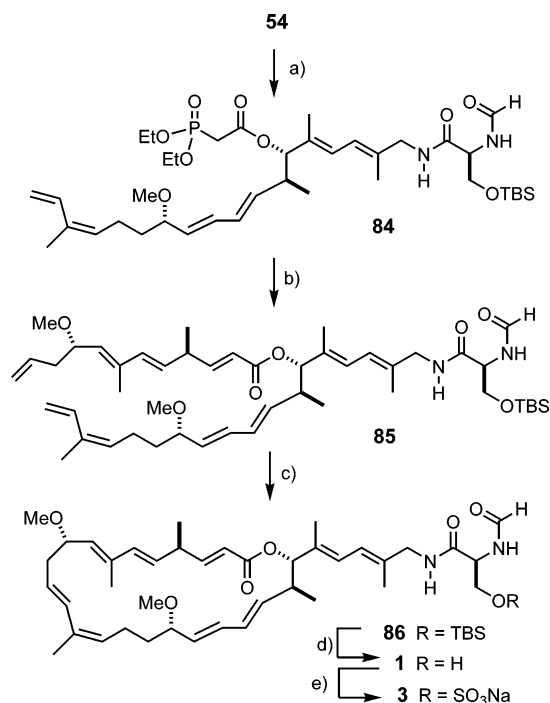
[a] Reagents and conditions: (a) (EtO)₂P(O)CH₂COOH, DCC, 4-pyrrolidinylpyridine (30 mol %), CH₂Cl₂, 0 °C → room temperature, 82%; (b) aldehyde **77** (2−2.2 equiv), LiCl, *i*-Pr₂NEt, MeCN, room temperature, 36% (65% based on recovered starting material); (c) complex **50** (20 mol %), CH₂Cl₂ (5 × 10⁻³ M), room temperature, 55%; (d) TBAF, THF, 0 °C, 67%; (e) SO₃·pyridine, ref 2a.

to date have been applied.[55] Attempts to produce acid **80** directly by olefination of **77** with **82** or **83** as the prenucleophiles were equally unsuccessful.

Since all attempts to manipulate the ester bond in **78** were to no avail, it was decided to graft the ketophosphate entity onto the hindered allylic alcohol **54** and to perform a Masamune−Roush olefination with this much more elaborate donor (Scheme 13). Gratifyingly, reaction of compound **84** with an excess of freshly prepared aldehyde **77** (2−2.2 equiv) in the presence of LiCl and Hünig base in MeCN at ambient temperature mainly gave the desired product **85**, although the reaction never went to completion. All attempts to drive the conversion by extending the reaction time, increasing the temperature, using a larger excess of **77**, or dosing the aldehyde to the mixture via syringe pump resulted in extensive decomposition and made tedious purification necessary.[56] Therefore, the olefination was stopped at fairly low conversions (ca. 40−45%) and unreacted ketophosphonate recovered, furnishing ca. 35% isolated yield of compound **85** per round (65% based on recovered starting material). This batchwise approach gave sufficient quantities of this highly sensitive polyene as the required cyclization precursor to explore the final stages of the total synthesis. To this end, **85** was subjected to RCM which provided macrocycle **86** in respectable 55% yield. Some isomeric impurities could be removed after cleavage of the −OTBS ether, thus furnishing iejimalide A (**1**) in analytically pure form. The spectroscopic data of the synthetic samples matched those of the natural

(55) Nicolaou, K. C.; Estrada, A. A.; Zak, M.; Lee, S. H.; Safina, B. S. *Angew. Chem., Int. Ed.* **2005**, *44*, 1378.

(56) Attempts to effect the Horner−Emmons reaction with LiOCH(CF₃)₂ as the base, which is claimed to be particularly mild, only led to extensive decomposition; Blasdel, L. K.; Myers, A. G. *Org. Lett.* **2005**, *7*, 4281.

product in every regard.[1,2] Because the one-step conversion of **1** into its sulfated congener iejimalide C (**3**) on treatment with SO$_3$·pyridine has already been reported,[2a] our venture also represents a formal total synthesis of this companion macrolide.

Assessment of the Actin-Binding Properties of the Iejimalides and Analogues. Actin is the most abundant protein in eukaryotic cells and as such is involved in many fundamental cellular functions.[57] Monomeric G-actin (globular actin) is capable of reversibly assembling into long and flexible polymers (F-actin, filamentous actin); the ensuing cytoskeleton plays an essential role, inter alia, in basic cellular motility processes such as cytokinesis, exocytosis, and endocytosis, accounts for the shape and mechanical properties of the cells, effects cell locomotion, and is responsible for strength development in muscles.[57]

Over the years, many small molecules have been discovered that interfere with the highly regulated processes of actin polymerization/depolymerization.[58] Such compounds usually play a role in the chemical defense of organisms under the evolutionary pressure of densely populated habitats. According to their mode of action, they can be classified as either actin stabilizers or, conversely, as components that induce disassembly of the microfilament and/or prevent its formation.[58] In the latter case, the agents either interfere with the ATP-binding cleft or target the "barbed end" of actin monomers, thereby preventing their aggregation.

"Barbed end" binding natural products include aplyronine, mycalolide, ulapualide, scytophycin, swinholide, reidispongiolide, sphinxolide, lobophorolide, and others.[58] Although diverse in structural detail, these secondary metabolites feature a conspicuous similarity in global terms (Figure 1).[58] Specifically, they consist of a large hydrophobic ring (color coded in green) that binds to a shallow hydrophobic patch on the actin surface as evident from pertinent X-ray crystal structure analyses.[59] The protein's hydrophobic area extends into a fairly narrow cleft that is nicely complementary to the "tail" region of the bound small molecule (red), which invariably terminates in a polar head group (blue) entertaining strong hydrogen bonds to the protein host. Remarkably, the individual "tails" exhibit a highly conserved substitution pattern that enforces an extended conformation and thereby helps to reduce the entropic cost upon binding. Equally striking is the preeminence of form(en)amide termini in compounds of this type.[58]

Due to our longstanding interest in the synthesis, molecular editing, and evaluation of actin-binding small molecules,[60–62]
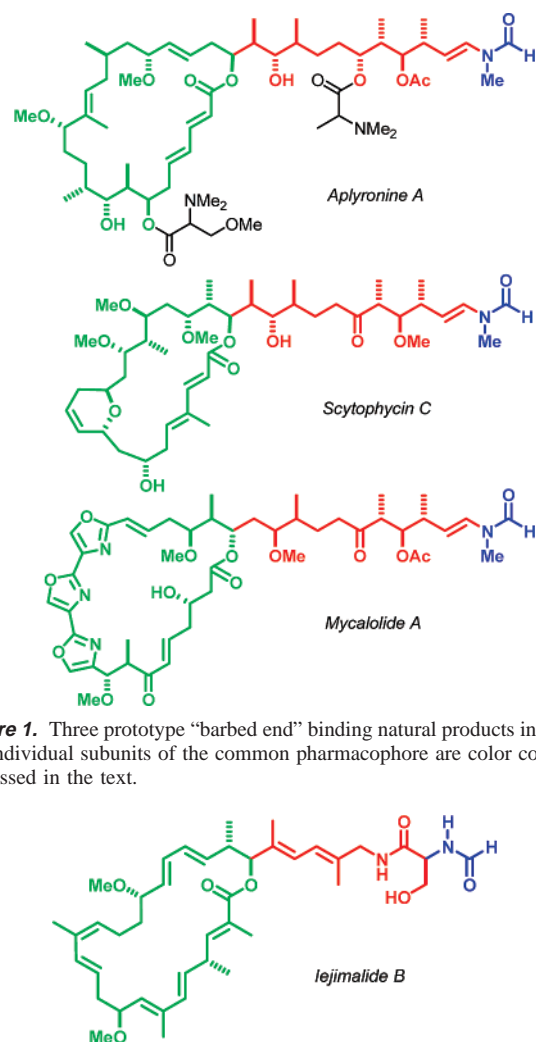
Figure 1. Three prototype "barbed end" binding natural products in which the individual subunits of the common pharmacophore are color coded as discussed in the text.


Figure 2. Representation of iejimalide B (**2**) in a way that highlights the distinct homology to the common "pharmacophore" of known "barbed end" binding small molecules.

we noticed a discrete homology between this common "pharmacophore" of the known barbed end binders and the overall constitution of the iejimalides (Figure 2). The latter also consist of a very hydrophobic macrocycle (green), an extended side chain (red), rigified by the presence of a 1,3-diene moiety, and a polar or even very polar (if the sulfate is in place) head group. The presence of a formamide (blue) in **1–4**, even though peptidic rather than part of an enamide, further corroborates this view. Therefore, we speculated that the iejimalides might interact with actin as one of their cellular targets.

As a consequence, iejimalide B and its non-natural analogues were subjected to a well-established phenomenological assay calibrated with latrunculin A as a positive control.[63] Specifically, NIH/3T3 fibroblasts were incubated with DMSO solutions of the respective compound at different concentrations and the induced morphological changes were visualized by staining the

(57) (a) Sheterline, P.; Clayton, J.; Sparrow, J. C. *Actin*, 4th ed.; Oxford University Press: New York, 1999. (b) Pollard, T. D.; Blanchoin, L.; Mullins, R. D. *Annu. Rev. Biophys. Biomol. Struct.* **2000**, *29*, 545. (c) Lodish, H.; Baltimore, D.; Berk, A.; Zipursky, S. L.; Matsudaira, P.; Darnell, J. *Molecular Cell Biology*, 3rd ed.; Scientific American Books: New York, 1995.

(58) Reviews: (a) Yeung, K.-S.; Paterson, I. *Angew. Chem., Int. Ed.* **2002**, *41*, 4632. (b) Peterson, J. R.; Mitchison, T. J. *Chem. Biol.* **2002**, *9*, 1275. (c) Giganti, A.; Friederich, E. In *Progress in Cell Cycle Research*; Meijer, L., Jézéquel, A., Roberge, M., Eds.; Springer: Berlin, 2003; Vol. 5; p 511. (d) Fenteany, G.; Zhu, S. *Curr. Top. Med. Chem.* **2003**, *3*, 593. (e) Jordan, M. A.; Wilson, L. *Curr. Opin. Cell Biol.* **1998**, *10*, 123. (f) Allingham, J. S.; Klenchin, V. A.; Rayment, I. *Cell. Mol. Life Sci.* **2006**, *63*, 2119.

(59) (a) Allingham, J. S.; Zampella, A.; D'Auria, M. V.; Rayment, I. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 14527. (b) Klenchin, V. A.; Allingham, J. S.; King, R.; Tanaka, J.; Marriott, G.; Rayment, I. *Nat. Struct. Biol.* **2003**, *10*, 1058. (c) Allingham, J. S.; Tanaka, J.; Marriott, G.; Rayment, I. *Org. Lett.* **2004**, *6*, 597.

(60) (a) Fürstner, A.; Kirk, D.; Fenster, M. D. B.; Aïssa, C.; De Souza, D.; Müller, O. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 8103. (b) Fürstner, A.; Kirk, D.; Fenster, M. D. B.; Aïssa, C.; De Souza, D.; Nevado, C.; Tuttle, C. T. T.; Thiel, W.; Müller, O. *Chem. Eur. J.* **2007**, *13*, 135.

(61) (a) Fürstner, A.; Turet, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 3462. (b) Fürstner, A.; De Souza, D.; Parra-Rapado, L.; Jensen, J. T. *Angew. Chem., Int. Ed.* **2003**, *42*, 5358. (c) Fürstner, A.; De Souza, D.; Turet, L.; Fenster, M. D. B.; Parra-Rapado, L.; Wirtz, C.; Mynott, R.; Lehmann, C. W. *Chem. Eur. J.* **2007**, *13*, 115.

(62) Fürstner, A.; Nagano, T.; Müller, C.; Seidel, G.; Müller, O. *Chem. Eur. J.* **2007**, *13*, 1452.

(63) (a) Spector, I.; Shochet, N. R.; Kashman, Y.; Groweiss, A. *Science* **1983**, *219*, 493. (b) Spector, I.; Shochet, N. R.; Blasberger, D.; Kashman, Y. *Cell Motil. Cytoskeleton* **1989**, *13*, 127.
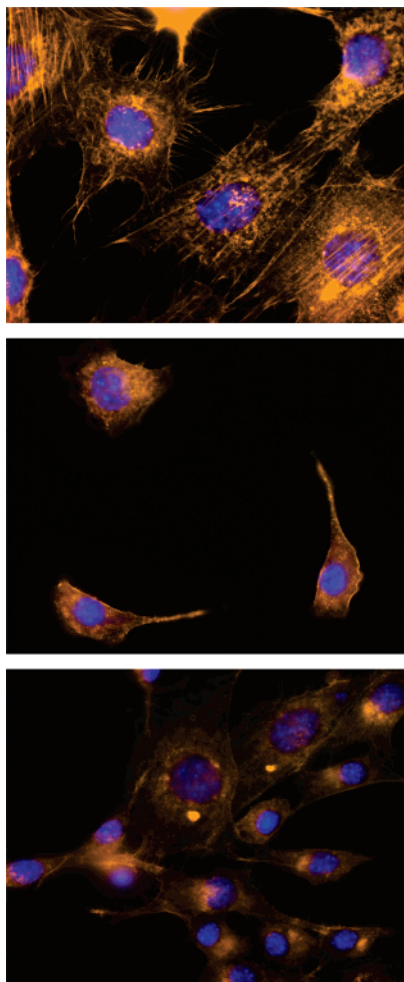
Figure 3. Fluorescence micrographs (250×) of NIH3T3 fibroblast showing the actin depolymerization capacity of iejimalide B (**2**) and the synthetic analogue **51**. The actin filament is stained with fluorescence-marked phalloidin, the nuclei with 2-(4-amidinophenyl)-6-indolecarbamidine hydrochloride (DAPI): top, untreated cells; middle, after incubation with **2** (1 μM); bottom, after incubation with **51** (5 μM).

Table 1. Actin Microfilament Disrupting Activity of Iejimalide B (**2**) and Fully Synthetic Analogues[a,b]

	1 μM		5 μM		10 μM	
compd	actin	MTT	actin	MTT	actin	MTT
2	++	90%	++	80%	++	nr
68	+	80%	+	75%	++	67%
69	+	70%	+	70%	++	70%
51	±	nr	+	110%	++	nr
66	±	nr	±	nr	±	95%

[a] "Actin": fluorescence microscopic assessment of the effect of the compounds on the actin filament and the cell morphology. "MTT": colorimetric cytotoxicity assay, allowing one to estimate the number of living cells (in percent) after 24 h of incubation time. [b] Abbreviations: ±, weak effect—largely intact cell morphology, filamentous and slightly disrupted actin; +, significant effect—filamentous and disrupted actin, visible changes in cell morphology; ++, very strong effect—disrupted actin, advanced cell rounding (compare representative micrographs in Figure 3); nr = not recorded.

Figure 4. Concentration-dependent up-regulation of caspase 3 activity by **2** and analogue **51**.

actin cytoskeleton with fluorescence-marked phalloidin. In parallel, the number of living cells was counted after 24 h by the MTT protocol[64] as a rough estimate for the toxicity of the compounds at a given concentration.
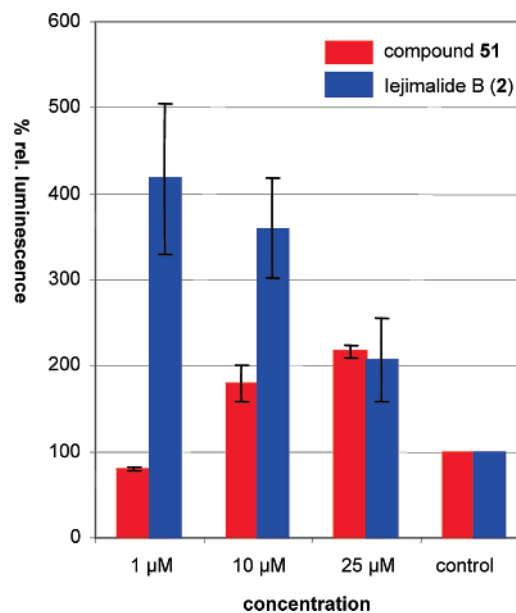
As can be seen from the selected micrographs in Figure 3, iejimalide B in fact induces severe changes of the actin cytoskeleton even at 1 μM concentration and is therefore *at least equipotent to latrunculin A and the best latrunculin analogues which constitute the standard in the field*.[60,63] At the same time, the number of living NIH/3T3 cells, after an incubation time of 24 h, was only slightly reduced even upon increasing the initial concentration of **2** (Table 1). Importantly, no effect on the tubulin cytoskeleton could be noticed in a parallel assay.

Equally remarkable is the fact that this pronounced activity is largely retained upon formal substitution of the *N*-formyl serine terminus in **2** by other head groups. All compounds investigated affect the actin filament to a significant degree. This does not only include compound **68** featuring an *N*-formyl valine residue instead of the serine moiety of **2** but also

compound **69** in which the formamide of **2** had been formally replaced by a more bulky *N*-benzoyl group; even the −NBoc derivative **51** retains significant activity (Figure 3, bottom). Only compound **66** terminating in a *N*-Teoc residue is somewhat less potent, whereas the *N*-dansyl derivative **70** was insoluble in the medium and could not be assessed in detail.

Moreover, preliminary data indicate that **2** as well as its unnatural −NBoc congener **51** up-regulate the activity of caspase 3, a key enzyme involved in a signal transduction cascade leading to apoptosis (Figure 4).[65] Caspases 3 and 7 are members of the cysteine aspartic acid-specific protease (caspase) family and play key effector roles in apoptosis in mammalian cells.[66] Together with caspase 6, these proteases form the execution level of apoptosis, which is activated by the initiator caspase 9. Curiously, however, the effect of iejimalide B on caspase 3 activity *diminishes* with increased concentration, whereas compound **51** shows a normal correlation. Although the phenomenon of inverse dose dependence is not unprec-

(64) Atta-ur-Rahman; Choudhary, M. I.; Thomsen, W. J. *Bioassay Techniques for Drug Development*; Harwood Academic Publishers: Amsterdam, 2001.

(65) (a) Cohen, G. M. *Biochem. J.* **1997**, *326*, 1. (b) Cory, S. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12077. (c) Thompsen, C. B. *Science* **1995**, *267*, 1456.
(66) Budihardjo, I.; Oliver, H.; Lutter, M.; Luo, X.; Wang, X. *Annu. Rev. Cell Dev. Biol.* **1999**, *15*, 269.

edented, further studies are necessary to better understand this peculiar observation.[67]

Overall, this study shows that the iejimalides interfere with actin, causing depolymerization of this basic cytoskeleton at least as potently as the reference compounds commonly employed. Therefore, they constitute highly valuable probe molecules in the realm of chemical biology, irrespective of their potential role as leads for medicinal chemistry purposes. Although a more detailed investigation is warranted to address the question if "barbed end" binding truly is the cause for the strong effect on actin, our data show that the peptide residue of **2** is a permissive site for significant structural modifications without compromising or annihilating the actin depolymerization capacity. Ongoing studies in this laboratory aim at shedding further light into this remarkable biological effect by a second round of synthesis-driven molecular editing of the iejimalide's molecular frame.

Conclusions

Though counterintuitive at first sight, application of RCM[15] to appropriate cyclization precursors containing no less than 10 double bonds opened the door to a concise total synthesis of the highly cytotoxic marine natural products iejimalide A−D. This result must be interpreted in the light of an alternative approach based on conventional macrocyclization techniques which failed to afford the polyene macrolide core of these demanding targets.[7a] The blueprint underlying the successful RCM approach has been reduced to practice in two different formats, one of which was optimized for maximum efficiency, whereas the other one allows for late-stage diversity, thus enabling not only the preparation of the parent compound itself but also of a set of analogues differing in the polar head group attached to the macrolide's N-terminus. This panel of compounds was instrumental for the discovery of the potent actin-depolymerizing capacity of the iejimalides, which rivals the activity of the latrunculins constituting the standard in the field.[60,63] Thereby, the conspicuous *N*-formyl serine moiety decorating all naturally occurring iejimalides is a permissive site for substantial structural modification.

The synthetic venture also revealed the exceptional sensitivity of many of the required building blocks toward acid, base, and even gentle warming, which rendered the optimization of the key fragment coupling events imperative. In response to these stringent requirements, particularly mild protocols for Suzuki as well as Stille reactions of elaborate coupling partners have been developed that hold considerable promise for applications in other demanding settings too. Further work along these lines, including a more profound synthesis-driven editing of the iejimalide framework as well as studies into the cytotoxicity profile of designed "iejimalide-like" compounds, is underway and will be reported in due course.

Supporting Information Available: Complete ref 26, full experimental details including spectroscopic and analytical data of all new compounds, and description of the "first generation" syntheses of various building blocks. This material is available free of charge via the Internet at http://pubs.acs.org.

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(67) At this point, we can only speculate about the possible cause of this inverse concentration dependence. It is important to note that the luminescence assay determines the absolute activity of the enzyme. Although the data summarized in Table 1 show that the number of living cells is only slightly reduced upon increasing the concentration of **2**, any such reduction will necessarily affect the measured activity. Moreover, solubility issues within the cell have to be taken into consideration.