Function-Oriented Studies Targeting Pectenotoxin 2: Synthesis of the GH-Ring System and a Structurally Simplified Macrolactone

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S *Supporting Information*

ABSTRACT: A chemical foundation for function-oriented studies of pectenotoxin 2 (PTX2) is described. A synthesis of the bicyclic GH-system, and the design and synthesis of a PTX2-analogue, is presented. While maintaining critical features for actin binding, and lacking the Achilles' heel for the natural product's anticancer activity (the AB-spiroketal), this first-generation analogue did not possess the anticancer properties of PTX2, an observation that indicates the molecular significance of features present in the natural product's CDEF-tetracycle.


pectenotoxin 2 (PTX2)
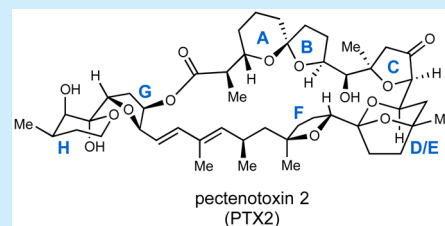
Pectenotoxin 2 (PTX2) is an exceedingly scarce marine-derived natural product that has become a target of interest for many chemical synthesis laboratories across the globe due to its potentially valuable anticancer activity and its daunting molecular structure (Figure 1).[1] While isolated over 30 years ago
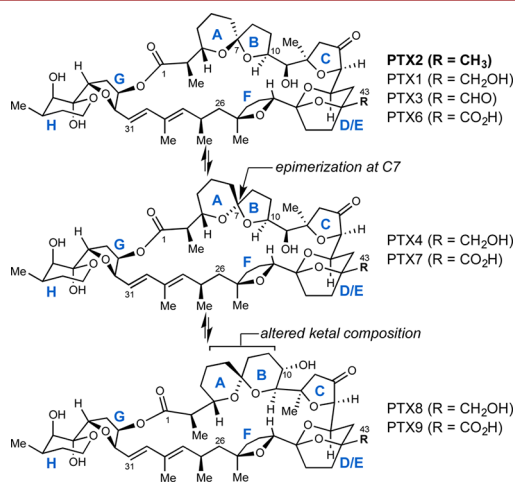
Figure 1. Introduction to the pectenotoxins.

in an attempt to identify causative agents of diarrhetic shellfish poisoning,[2] PTX2 has more recently been shown to possess affinity to G-actin, binding to a unique site with respect to other known marine-derived actin-targeting agents, and capping the barbed end without filament severing properties.[3] Distinct from other actin-disrupting agents, PTX2 has been claimed to possess selective toxicity toward p53 mutant and p53 null cancers (including variants that are highly chemoresistant).[4] While this natural product has been embraced as an intriguing challenge for numerous campaigns in total synthesis,[5] parts of its structure define significant liabilities for programs aimed at advancing it as a therapeutically relevant agent. For example, the AB-spiroketal
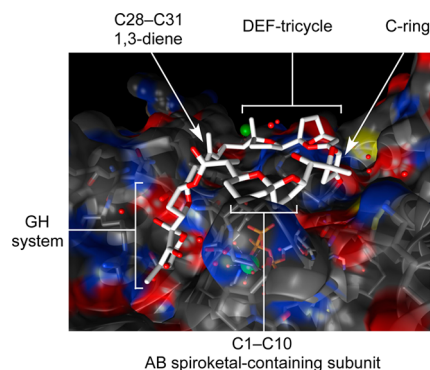
Figure 2. Structure of PTX2 bound to actin.[3]

system is known to undergo rapid isomerization to other PTX congeners that have markedly dampened anticancer activity (Figure 1). Further, it has been shown that oxidation at C43, or saponification of the macrolactone, results in family members that lack the toxicity profile of PTX2.[6]

We have been fascinated by the chemical challenge that members of this natural product class present, and our early efforts established synthetic chemistry capable of addressing the C1−C26 hexacyclic subunit.[7] While we have found this domain to be appealing from an architectural standpoint, the published PTX2−actin structure (Figure 2) reveals that this region of the natural product appears to merely cap a shallow hydrophobic surface and engage in a hydrogen bond with actin through the C-ring ketone. In stark contrast, the C32−C40 domain that houses the GH-ring system projects into a narrow hydrophilic pocket and is secured by numerous hydrogen-bond interactions. Here, we describe a concise synthesis of the GH-bicyclic heterocycle of PTX2 and secure chemistry capable of fueling function-oriented

studies[8] in the area, delivering macrocyclic PTX2-analogues in ~20 linear steps from commercially available material.

At the outset of these studies, it was recognized that the shape of the hydrophobic capping region (C1−C26) is clearly important for toxicity, as acid-mediated equilibration of the C7 acetal results in isomers with markedly dampened toxicity profiles, and hydrolysis of the macrolactone abolishes activity. As a result, PTX2-analogues of interest would retain the critically important GH-ring system, yet offer different hydrophobic macrocycles that could serve to mitigate the acid-triggered deactivation of the natural product (i.e., be devoid of the AB-spiroketal system altogether). Unfortunately, the C31−C40 region of the pectenotoxin structure that contains the GH-bicyclic heterocycle has proven to be quite challenging to prepare, requiring longest linear sequences of ~21 to >30 chemical steps to establish only 10 backbone carbons.[9]

Due to this lack of step ecomony, efforts were first directed at defining a more concise approach to an intermediate containing the GH-bicyclic system. As illustrated in Figure 3, the substituted
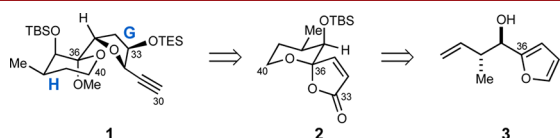

Figure 3. Retrosynthetic strategy for the C30−C40 subunit.

heterocycle **1** rose as an early target for synthesis. It was thought that this system could be accessed from substrate-directed functionalization of spiroketal **2**. The stereodefined spiroketal **2** was then reasoned to be readily available from the crotylation product of furfuraldehyde (**3**), through sequential silylation, oxidation, and cyclization processes.

The successful execution of this design is illustrated in Figure 4. Protection of the secondary alcohol of **3** (TBSCl, imidazole) was followed by regioselective hydroboration and oxidation to deliver **4**. Subsequent oxidation of the furan with m-CPBA, followed by oxidation with PDC, provided the spiroketal intermediate **2** (dr ≥20:1) in 78% yield for the two-step sequence.[10] With this intermediate in hand, conditions for substrate controlled stereoselective oxidation were explored. While initial attempts at conjugate borylation [B₂(pin)₂, (PPh₃)₃RhCl or a variety of copper catalysts] indicated a substantial lack of reactivity of the system, and subsequent attempts at dihydroxylation with OsO₄/NMO were not promising, it was later found that treatment with RuCl₃/NaIO₄ generated a valuable diol intermediate with exquisite levels of stereocontrol.[11] Finally, reductive deoxygena-

tion [SmI₂, (CH₂OH)₂, HMPA] provided the secondary alcohol **5** in 82% yield (dr ≥20:1).[12]

Installation of the tetrahydrofuran and alkyne motif of **1** was accomplished by semireduction of the lactone with DIBALH and Wittig reaction of the intermediate lactol with ylide **6**.[13] Enyne **7** was then stereoselectively epoxidized to furnish **9**,[14] which was immediately used in a stereoretentive ring-closing event via BF₃· OEt₂-mediated cyclization of the intermediate Co-complexed alkyne.[15] Finally, selective desilylation of the TMS-alkyne and protection of the G-ring secondary alcohol as its corresponding TES ether delivered the fully functionalized bicyclic target **1**. Notably, this reaction sequence proceeds in just 14 steps from furfural and has been employed to generate multigram quantities of this critically important PTX2-fragment.

With a route to the GH-system secured, we focused our attention on establishing a general chemical synthesis pathway capable of fueling function-oriented[8] studies. These efforts began by defining a model substrate to guide our chemical efforts. For this, we employed a combination of molecular modeling and *in silico* docking studies to arrive at a reasonable first-generation synthetic target. First, PTX2 was docked to G-actin using AutoDock.[16] To our delight, this *in silico* experiment resulted in a predicted PTX2−actin structure that resembled the known PTX2−actin complex (Figure 5A vs Figure 2). Further *in silico* studies predicted that a simple enyne-derivative of PTX2 would associate with actin in a similar manner as the natural product (Figure 5B), and that the drastically simplified macrolactone **10** could be a good starting point for function-oriented pursuits (Figure 5C). In this latter case, however, notable differences in the "docked" structure include a flipping of the H-ring in the hydrophilic pocket to more closely resemble that seen in the actual PTX2−actin structure (Figure 2), and a lack of functionality in the C3−C26 PTX2-prosthetic capable of achieving a hydrogen bond similar to that seen with the natural product's C-ring ketone. Despite these variations, and an uncertainty regarding the significance of docking scores produced from AutoDock for the analysis of complex macrocyclic species like pectenotoxin 2, we moved forward with **10** as a target to drive the development of a general pathway to synthetic PTX2-inspired agents.

As depicted in Figure 6A, synthesis of **10** was reasoned to be possible by macrolactonization of seco-acid **11**, a compound deemed to be readily available from the coupling of **1** to the triaryl-ether fragment **12**. Synthesis of **12** was accomplished as depicted in Figure 6B and proceeded by stepwise homologation of 1,4-bis(bromomethyl)benzene **13**. Sequential nucleophilic displacement, first with the anion of 4-hydroxybenzyl alcohol and then with the phenoxide of **14**, was effective for generating gram
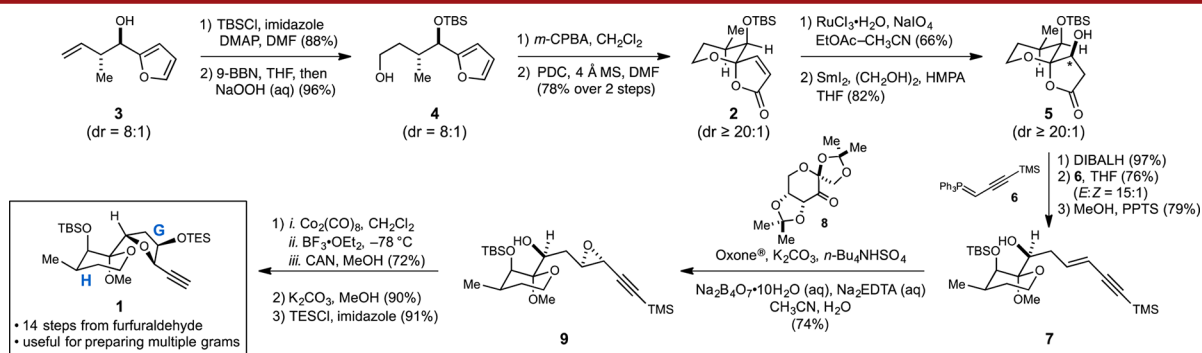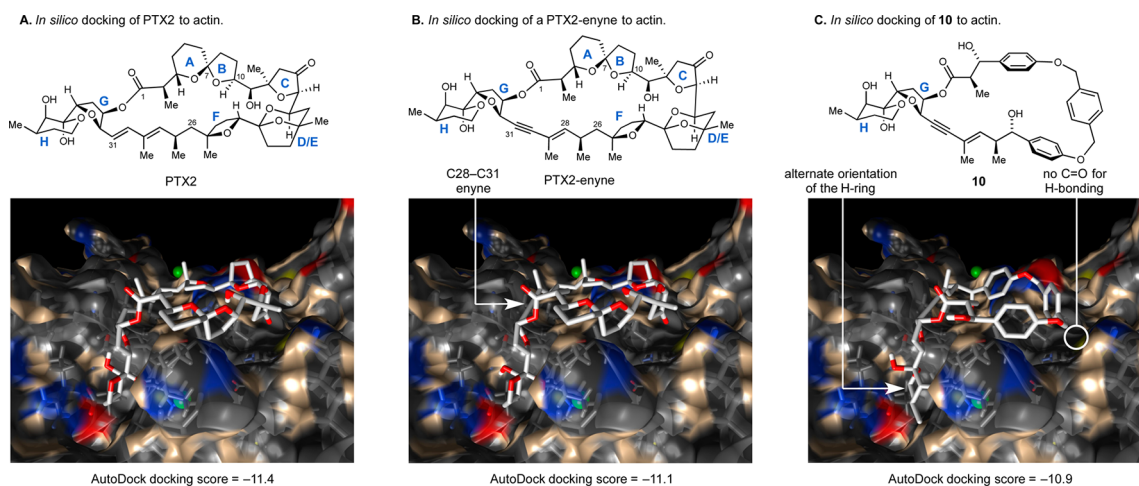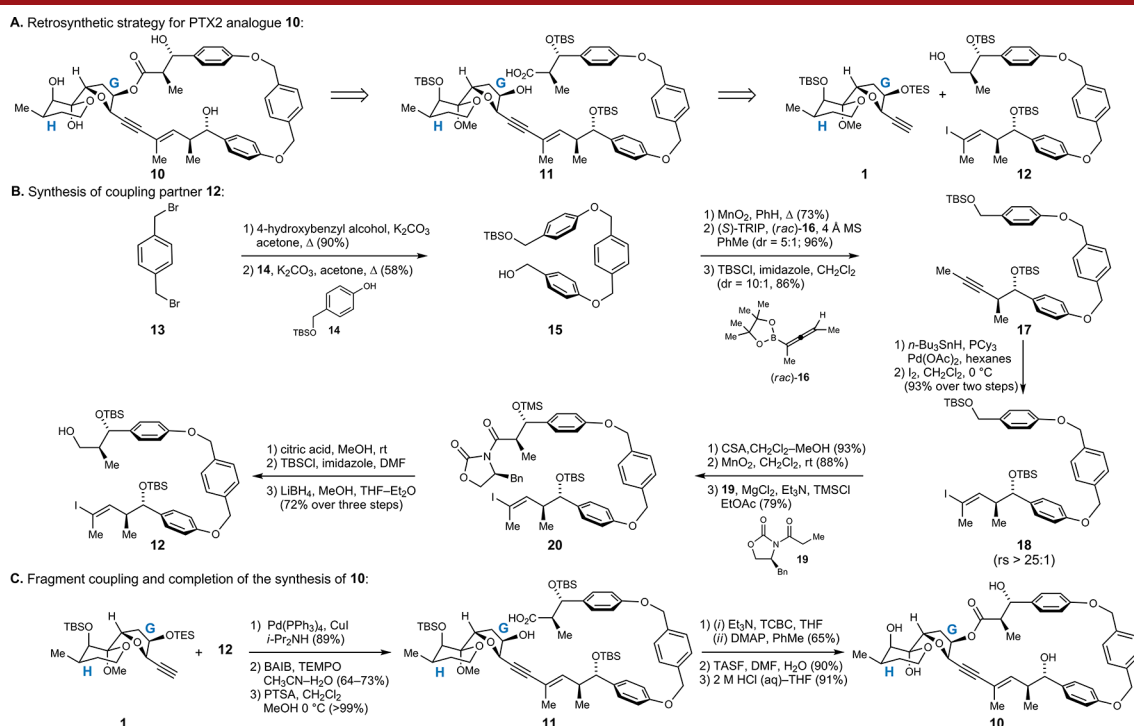

Figure 4. Synthesis of the GH-bicyclic domain of the pectenotoxins from the crotylation product of furfuraldehyde.

Figure 5. *In silico* docking experiments for design of a first-generation PTX2-inspired agent.

Figure 6. Establishment of a synthesis pathway capable of fueling function-oriented studies: *convergent assembly of a PTX2-inspired agent.*

quantities of the desymmetrized benzylic alcohol **15**. Oxidation to the corresponding aldehyde (MnO₂, PhH), followed by stereoselective propargylation[17] and silylation of the resulting homopropargylic alcohol, gave **17** (dr = 10:1). Regioselective hydrostannylation[18] of the internal alkyne (*n*-Bu₃SnH, PCy₃, Pd(OAc)₂) was followed by conversion to the vinyl iodide **18** (I₂, CH₂Cl₂).[19] Selective desilylation of the primary silyl ether (CSA, CH₂Cl₂−MeOH), oxidation to the aldehyde (MnO₂, CH₂Cl₂), and subsequent catalytic *anti*-Evans' aldol reaction with **19** generated **20** in 65% yield over the three steps.[20] Finally, conversion of **20** to the planned coupling partner **12** was accomplished by cleavage of the TMS ether with citric acid in methanol, protection of the resulting secondary alcohol as a TBS ether, and reduction to the primary alcohol with LiBH₄ (72% overall yield for this final three-step sequence).

With gram quantities of **1** and **12** in hand, effort was directed toward completing the synthesis of PTX2-analogue **10**. As

illustrated in Figure 6C, Sonogashira coupling[21] was highly effective for uniting the two fragments (89% yield). Sequential oxidation to the carboxylic acid and selective deprotection of the TES ether then delivered *seco*-acid **11**. To our delight, cyclization under the conditions of Yamaguchi[22] was effective for generating the macrolactone, and a simple two-step deprotection sequence converted the intermediate macrolactone to **10** in 82% yield.

With chemistry established to advance the synthetic GH-system **1** to a macrocyclic analogue of PTX2 in just six additional linear transformations, we elected to explore the anticancer and actin-perturbing properties of this first-generation analogue. While docking studies suggested that **10** was capable of mimicking the binding of PTX2 to actin, dosing of this compound in four different human cancer cell lines (MDA-MB-231, MALME, HT29, and H522) at concentrations up to 10 μM led to no observable inhibition of growth. While this lack of cellular activity could be related to distinct permeability

characteristics of the synthetic ligand, biochemical assessment of macrolactone **10**'s action on actin polymerization led to similar results (no effect was seen up to a concentration of 10 μM). At this early stage, it is apparent that the drastic alteration of the natural product's C1−C26 domain in **10** abolishes actin-targeting properties and that future analogue design should target congeners that retain additional natural product-inspired complexity in this region. Given our earlier success in preparing PTX2's CDEF-tetracycle, we look forward to integrating this subunit into second-generation analogues.

Overall, a step-economical pathway for synthesis of the GH-bicyclic heterocycle of the pectenotoxins has been established that proceeds in 33% to >53% fewer steps than previously described synthetic routes. This new synthetic pathway was used to prepare multiple grams of the GH-system (**1**), and a simple six-step sequence was identified for advancing this intermediate to a fully functionalized PTX2-analogue. While this chemical achievement delivered the first PTX2-analogue (**10**) for evaluation in function-oriented studies, this agent was found to possess diminished activities in comparison to the natural product (actin depolymerization and cytotoxicity). In short, preliminary docking studies proved ineffective as a tool to guide the design of a functional first-generation analogue, and it has been concluded that additional molecular complexity to mimic the C1−C26 region of the natural product may be useful in identifying synthetic analogues with natural product-like activity. Studies along these lines are currently underway.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b02435.

Procedures and spectroscopic data (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) (a) Kim, G.-Y.; Kim, W.-J.; Choi, Y. H. *Mar. Drugs* **2011**, *9*, 2176. (b) Dominguez, H. J.; Paz, B.; Daranas, A. H.; Norte, M.; Franco, J. M.; Fernández, J. J. *Toxicon* **2010**, *56*, 191.

(2) Yasumoto, T.; Murata, M.; Oshima, Y.; Sano, M.; Matsumoto, G. K.; Clardy, J. *Tetrahedron* **1985**, *41*, 1019.

(3) Allingham, J. S.; Miles, C. O.; Rayment, I. *J. Mol. Biol.* **2007**, *371*, 959.

(4) (a) Chae, H.-D.; Choi, T.-S.; Kim, B.-M.; Jung, J. H.; Bang, Y.-J.; Shin, D. Y. *Oncogene* **2005**, *24*, 4813. (b) Jung, J. H.; Sim, C. J.; Lee, C.-O. *J. Nat. Prod.* **1995**, *58*, 1722. (c) Shin, D. Y.; Kim, G. Y.; Kim, N. D.; Jung, J. H.; Kim, S.-K.; Hang, H. S.; Choi, Y. H. *Oncol. Rep.* **2008**, *19*, 517. (d) Chae, H. D.; Kim, B. M.; Yun, U. J.; Shin, D. Y. *Oncogene* **2008**, *27*, 4115. For action of other actin-targeting cytotoxic agents, see: (e) Lee, Y.-J.; Tsai, C.-H.; Hwang, J.-J.; Chiu, S.-J.; Sheu, T.-J.; Keng, P. C. *Int. J. Oncol.* **2009**, *34*, 581.

(5) For the total synthesis of PTX2, see: (a) Fujiwara, K.; Suzuki, Y.; Koseki, N.; Aki, Y.; Kikuchi, Y.; Murata, S.; Yamamoto, F.; Kawamura, M.; Norikura, R.; Matsue, H.; Murai, A.; Katoono, R.; Kawai, H.; Suzuki, T. *Angew. Chem., Int. Ed.* **2014**, *53*, 780. For total syntheses of PTX4 and PTX8, see: (b) Evans, D. A.; Rajapakse, H. A.; Stenkamp, D. *Angew. Chem., Int. Ed.* **2002**, *41*, 4569. (c) Evans, D. A.; Rajapakse, H. A.; Chiu, A.; Stenkamp, D. *Angew. Chem., Int. Ed.* **2002**, *41*, 4573.

(6) (a) Espiña, B.; Louzao, M. C.; Ares, I. R.; Fonfría, E. S.; Vilariño, N.; Vieytes, M. R.; Yasumoto, R.; Botana, L. M. *Chem. Res. Toxicol.* **2010**, *23*, 504. (b) Ares, I. R.; Louzao, M. C.; Espiña, B.; Vieytes, M. R.; Miles, C. O.; Yasumoto, T.; Botana, L. M. *Cell. Physiol. Biochem.* **2007**, *19*, 283. (c) Sasaki, K.; Wright, J. L. C.; Yasumoto, T. *J. Org. Chem.* **1998**, *63*, 2475.

(7) (a) Canterbury, D. P.; Micalizio, G. C. *Org. Lett.* **2011**, *13*, 2384. (b) Kubo, O.; Canterbury, D. P.; Micalizio, G. C. *Org. Lett.* **2012**, *14*, 5748.

(8) Wender, P. A. *Nat. Prod. Rep.* **2014**, *31*, 433.

(9) (a) Heapy, A. M.; Wagner, T. W.; Brimble, M. A. *Synlett* **2007**, 2359. (b) Heapy, A. M.; Brimble, M. A. *Tetrahedron* **2010**, *66*, 5424. For other synthetic studies targeting the pectenotoxins, see: (c) Kouridaki, A.; Sofiadis, M.; Montagnon, T.; Vassilikogiannakis, G. *Eur. J. Org. Chem.* **2015**, 7240. (d) Donohoe, T. J.; Lipinski, R. M. *Angew. Chem., Int. Ed.* **2013**, *52*, 2491. (e) Kemppainen, E. K.; Sahoo, G.; Valkonen, A.; Pihko, P. M. *Org. Lett.* **2012**, *14*, 1086. (f) Fujiwara, K.; Suzuki, Y.; Koseki, N.; Murata, S.; Murai, A.; Kawai, H.; Suzuki, T. *Tetrahedron Lett.* **2011**, *52*, 5589. (g) Aho, J. E.; Piisola, A.; Syam Krishnan, K.; Pihko, P. M. *Eur. J. Org. Chem.* **2011**, 1682. (h) Joyasawal, S.; Lotesta, S. D.; Akhmedov, N. G.; Williams, L. J. *Org. Lett.* **2010**, *12*, 988. (i) Carley, S.; Brimble, M. A. *Org. Lett.* **2009**, *11*, 563. (j) Aho, J. E.; Salomäki, E.; Rissanen, K.; Pihko, P. M. *Org. Lett.* **2008**, *10*, 4179. (k) Helmboldt, H.; Aho, J. E.; Pihko, P. M. *Org. Lett.* **2008**, *10*, 4183. (l) Kolakowski, R. V.; Williams, L. J. *Tetrahedron Lett.* **2007**, *48*, 4761. (m) Fujiwara, K.; Aki, Y.; Yamamoto, F.; Kawamura, M.; Kobayashi, M.; Okano, A.; Awakura, D.; Shiga, S.; Murai, A.; Kawai, H.; Suzuki, T. *Tetrahedron Lett.* **2007**, *48*, 4523. (n) Lotesta, S. D.; Hou, Y.; Williams, L. J. *Org. Lett.* **2007**, *9*, 869−872. (o) Vellucci, D.; Rychnovsky, S. D. *Org. Lett.* **2007**, *9*, 711. (p) Brimble, M. A.; Halim, R. *Pure Appl. Chem.* **2007**, *79*, 153. (q) Bondar, D.; Liu, J.; Müller, R.; Paquette, L. A. *Org. Lett.* **2005**, *7*, 1813. (r) Halim, R.; Brimble, M. A. *Org. Biomol. Chem.* **2006**, *4*, 4048−4058 and references cited therein.

(10) Robertson, J.; Meo, P.; Dallimore, J. W. P.; Doyle, B. M.; Hoarau, C. *Org. Lett.* **2004**, *6*, 3861.

(11) (a) Shing, T. K. M.; Tai, V. W.-F.; Tam, E. K. W. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2312. (b) Plietker, B.; Niggemann, M. *Org. Biomol. Chem.* **2004**, *2*, 2403.

(12) Hanessian, S.; Girard, C.; Chiara, J. L. *Tetrahedron Lett.* **1992**, *33*, 573.

(13) Corey, E. J.; Fleet, G. W. J.; Kato, M. *Tetrahedron Lett.* **1973**, *14*, 3963.

(14) (a) Cao, G.-A.; Wang, Z.-X.; Tu, Y.; Shi, Y. *Tetrahedron Lett.* **1998**, *39*, 4425. (b) Wang, Z.-X.; Cao, G.-A.; Shi, Y. *J. Org. Chem.* **1999**, *64*, 7646.

(15) Smith, A. B., III; Fox, R. J. *Org. Lett.* **2004**, *6*, 1477.

(16) Trott, O.; Olson, A. J. *J. Comput. Chem.* **2010**, *31*, 455.

(17) Tsai, A. S.; Chen, M.; Roush, W. R. *Org. Lett.* **2013**, *15*, 1568.

(18) Semmelhack, M. F.; Hooley, R. J. *Tetrahedron Lett.* **2003**, *44*, 5737.

(19) Cornil, J.; Echeverria, P.-G.; Reymond, S.; Phansavath, P.; Ratovelomanana-Vidal, V.; Guérinot, A.; Cossy, J. *Org. Lett.* **2016**, *18*, 4534.

(20) Evans, D. A.; Tedrow, J. S.; Shaw, J. T.; Downey, C. W. *J. Am. Chem. Soc.* **2002**, *124*, 392.

(21) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *16*, 4467.

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