OL | Organic Letters

pubs.acs.org/OrgLett Letter

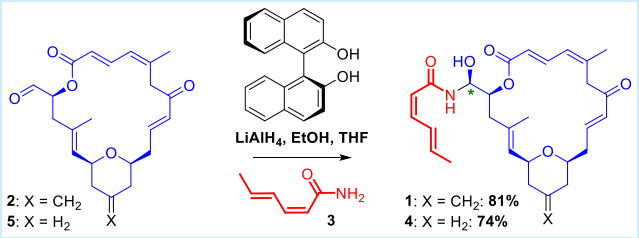
A Method for the Stereoselective Construction of the Hemiaminal Center in Zampanolides

Tobias M. Brütsch,[#] Simone Berardozzi,[#] Marlene L. Rothe, Mariano Redondo Horcajo, José Fernando Díaz, and Karl-Heinz Altmann*

Cite This: https://dx.doi.org/10.1021/acs.orglett.0c02974 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: We have developed a new method for the stereoselective establishment of the *N*-acyl hemiaminal moiety in zampanolide-type structures that involves the reaction of (*Z*,*E*)-sorbamide (**3**) with BINAL-H and subsequent amide transfer from a putative aluminum carboximidoate complex to the aldehyde moiety of a dactylolide precursor, such as **2** or **5**. The method has enabled the efficient synthesis of 13-desmethylene-(−)-zampanolide (**4**), which was found to be an equipotent cell growth inhibitor as the natural product (−)-zampanolide (**1**).



(−)-Zampanolide (**1**) (Figure 1) is a 20-membered, sponge-derived macrolide that was first isolated in 1996 by Tanaka and

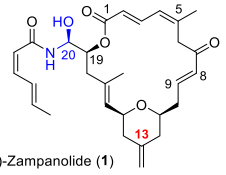


Figure 1. Structure of (−)-zampanolide (**1**).

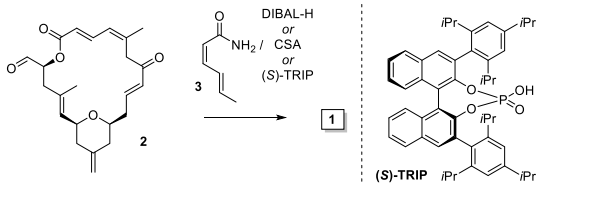
Higa from *Fasciospongia rimosa*.[1] The compound was found to inhibit the proliferation of human cancer cells *in vitro* with low nanomolar potency, but the mechanistic underpinnings of its cytotoxicity were only revealed in 2009, when **1** was reisolated from *Cacospongia mycofijiensis* by Northcote, Miller and co-workers and shown to be a potent microtubule-stabilizing agent.[2] Thus, (−)-zampanolide (**1**) exhibits the same mechanism of action as the clinical cancer drugs taxol, docetaxel, cabazitaxel, and ixabepilone,[3] but it is the only microtubule stabilizer known to date that binds to β-tubulin in a covalent fashion (by alkylation of βHis229 via 1,4-addition to the enone moiety in the macrocycle).[4]

Structurally, a particularly distinguishing characteristic of **1** is the C(20) hemiaminal unit that connects C(19) of the highly unsaturated macrolactone core to a (*Z*,*E*)-sorbamide moiety. Only a few other natural products are known that incorporate a hemiaminal structural motif, including a series of congeners of **1** that were isolated very recently by Northcote and co-workers (zampanolides B−E).[5] The latter differ from **1** in the geometry of individual double bonds, but they all retain nanomolar antiproliferative activity against HL-60 cells.[5]

In light of its intriguing structural features and its potentially medically relevant biological activity, it is not surprising that (−)-zampanolide (**1**) has been the target of several total syntheses;[6−11] in addition, a number of analogs have been prepared for SAR studies,[8,12−16] although the SAR of **1** is still underexplored. Different approaches toward the zampanolide macrocycle have been pursued as part of the different total syntheses of **1**, but the attachment of the sorbamide segment of the side chain in general has relied on the DIBALH[7,8] or Brønsted acid promoted[9−11] "aza-aldol" reaction between (−)-dactylolide (**2**) and (*Z*,*E*)-sorbamide (**3**) (Scheme 1).

As the sole exception, Smith's first total synthesis of (+)-zampanolide (*ent*-**1**) relied on a protected hemiaminal as a diastereomerically pure intermediate that was acylated on nitrogen with (*E*,*Z*)-sorbic acid (**3**).[6] Unfortunately, however, final deprotection of the hemiaminal hydroxy group was

Scheme 1. Establishment of the Hemiaminal Moiety and Elaboration of the C(19) Side Chain in Zampanolide[7−11]



Received: September 6, 2020

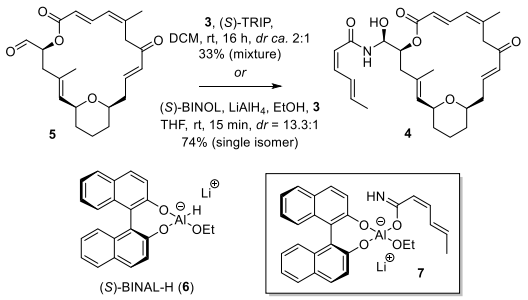
https://dx.doi.org/10.1021/acs.orglett.0c02974
Org. Lett. XXXX, XXX, XXX−XXX

accompanied by substantial epimerization of the C(20) stereocenter.[6]

While the DIBALH-based aza-aldol reaction proceeds without any meaningful selectivity,[7,8] Ghosh has shown that the use of the chiral Lewis acid (*S*)-TRIP (Scheme 1) gives **1** with ca. 3:1 selectivity.[10,11]

As part of an ongoing program on the synthesis and SAR investigations of analogs of **1**, we were interested in the biological activity of 13-desmethylene zampanolide (**4**) (see Scheme 2). If similarly active as **1**, **4** would represent a more

Scheme 2. Stereoselective Construction of the Hemiaminal Moiety in 4



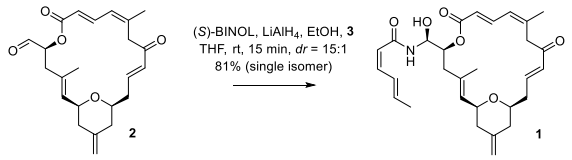
readily accessible template for SAR studies, at least within the framework of our own global strategy toward the synthesis of **1**.[8] Neither the synthesis of 13-desmethylene zampanolide (**4**) nor its biological activity has been reported; however, Taylor and co-workers have described the synthesis of 17-desmethyl-13-desmethylene zampanolide, which they found to be a 17- to 57-fold less potent cell growth inhibitor than **1** (for a 1.5:1 diastereomeric mixture at C(20); tested against three cancer cell lines).[16]

Initial attempts at the synthesis of **4** involved the addition of (*Z*,*E*)-sorbamide (**3**) to aldehyde **5**[8,12] with 10 mol % (*S*)-TRIP as a chiral Brønsted acid catalyst according to Ghosh.[10,11] The reaction proceeded with only moderate selectivity and gave **4** as a *ca.* 2:1 mixture of isomers in 33% yield after 16 h (with incomplete conversion).

Given the fact that we had planned to prepare a series of analogs of **4** for SAR studies, this level of selectivity was rather unsatisfactory and would lead to substantial losses of material in the purification process.[8] Trying to develop a solution to the selectivity problem, we took inspiration from Noyori's earlier work on the development of the chiral carbonyl reducing agents (*S*)- and (*R*)-BINAL-H (**6** and *ent*-**6**, Scheme 2).[17,18] The latter are generated from (*S*)- or (*R*)-BINOL, respectively, $LiAlH_4$, and ethanol. We hypothesized that the addition of (*Z*,*E*)-sorbamide (**3**) to a solution of (*S*)-BINAL-H could lead to the formation of the aluminum carboximidoate complex **7** (Scheme 2), which would then transfer the sorbamide moiety to **2** in a stereoselective fashion; based on Noyori's work on the BINAL-H reduction of aldehydes,[18] we predicted that the putative amide transfer reagent **7**, derived from (*S*)-BINOL, would give the desired C(20) *S* isomer **4** (Scheme 2). In the event, the addition of a solution of (*Z*,*E*)-sorbamide (**3**) in THF to a freshly prepared solution of (*S*)-BINAL-H (**6**) in THF at room temperature produced an almost clear solution of what we assumed to be BINAL-amide **7**. When this solution was added immediately to a solution of aldehyde **5** in THF at room temperature, full conversion of the latter was observed after 15 min. The reaction produced 13-desmethylene zampanolide (**4**) with an excellent *dr* of 13.3:1, which allowed the isolation of **4** as single isomer in 74% yield.[19] Lowering the reaction temperature to 0 °C improved the diastereomeric ratio further, but only low conversion was obtained, possibly due to decomposition of the BINAL-amide complex. The reaction of aldehyde **5** with the putative amide transfer reagent derived from (*R*)-BINOL (i.e., *ent*-**7**) gave the C(20)*R*-epimer of **4** as the major product in 61% isolated yield (the *dr* of the crude product before chromatography was 4.6:1). In contrast, Gosh and co-workers have reported that the (*R*)-TRIP-catalyzed reaction between **2** and **3** gave a 1:1 mixture of diastereomers at C(20).[11]

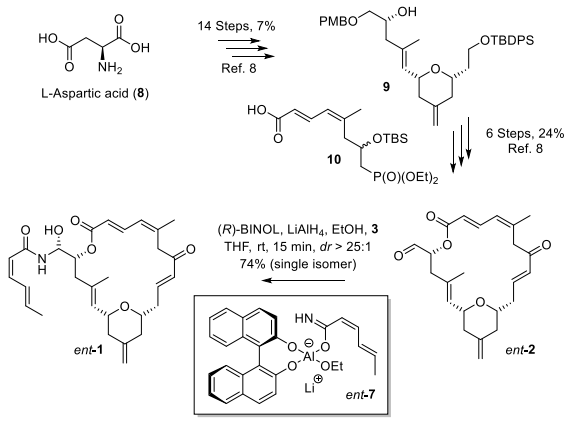
The above methodology was also successfully applied to the synthesis of natural (−)-zampanolide (**1**) from (−)-dactylolide (**2**) (Scheme 3); **1** was obtained in 81% yield, which makes this process more efficient than the (*S*)-TRIP-catalyzed aza-aldol reaction, which gave **1** in 51% yield.[10,11]

Scheme 3. Stereoselective Synthesis of (−)-Zampanolide (1) from (−)-Dactylolide (2)



Finally, we also prepared the enantiomer of **1**, i.e. *ent*-**1**, *via* aza-aldol reaction of *ent*-**2** with the amide transfer reagent formed from (*R*)-BINOL, $LiAlH_4$, ethanol, and **3** (Scheme 4).

Scheme 4. Stereoselective Synthesis of (+)-Zampanolide (*ent*-1)

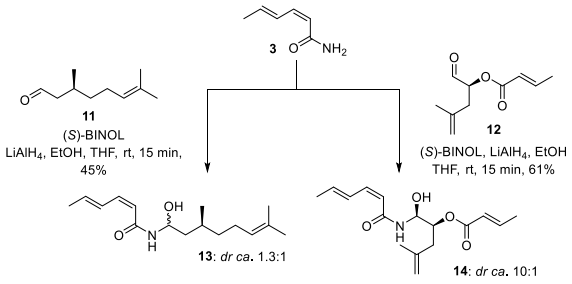


Aldehyde *ent*-**2** was prepared from L-aspartic acid in 14 steps and 7% overall yield according to the route that we had developed previously for the synthesis of **2**.[8,12] The aza-aldol reaction proceeded with a *dr* of >25:1 and delivered *ent*-**1** in 74% yield as a single isomer.[20]

After having established the utility of our stereoslective amide transfer method for the stereoselective installation of the C(20) stereocenter in zampanolide-type structures, we briefly investigated its applicability to other systems (Scheme 5). Somewhat disappointingly, the reaction of the amide transfer reagent obtained from (*S*)-BINOL and amide **3** with (*S*)-citronellal (**11**) gave *N*-acyl hemiaminal **13** essentially without selectivity; analogous results were obtained with benzamide instead of **3**. In contrast, reaction of (purported) **7** with

https://dx.doi.org/10.1021/acs.orglett.0c02974
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 5. (*Z*,*E*)-Sorbamide Transfer to Citronellal and Aldehyde 12



aldehyde **12** (prepared from PMB-protected (*S*)-glycidol in 4 steps and 53% overall yield; see the Supporting Information for details) gave **14** in 61% yield with a *dr* of 10:1. The selective formation of one *N*-acyl hemiaminal diastereomer from **12**, which retains the ester group and other structural features around the aldehyde moiety in **2**, suggests that (some of) these features are crucial for selective amide transfer.[21] At the same time, the preferential formation of C(20)-*epi*-**4** in the reaction of **2** with (purported) *ent*-**7** indicates a significant level of reagent control. Additional studies will be required to dissect reagent and substrate contributions to the selective formation of **1** and **4** from **2** and **5**, respectively.

With 13-desmethylene zampanolide (**4**) in hand, we went on to address our original question of the effects of the compound on cancer cell growth. As can be seen from the data summarized in Table 1, 13-desmethylene zampanolide (**4**) inhibits the proliferation of human cancer cell lines *in vitro* with nanomolar IC_{50} values and, thus, exhibits essentially the same antiproliferative activity as the natural product **1**.

Table 1. Inhibition of Cancer Cell Growth by 1, 4, and *ent*-1 (IC_{50} Values [nM])[a]

Cell line	1	4	*ent*-1
A2780 (ovarian)	1.9 ± 0.01[c,d]	1.7 ± 0.4	10500 ± 1138
A2780 AD (ovarian)[b]	2.2 ± 0.21[c,d]	3.53	11445 ± 70
A549 (lung)	3.2 ± 0.4[c]	1.0 ± 0.2	n.d.[e]

[a]Cells were exposed to compounds for 72 h. Values are means of two (A2780 AD) or four (A2780, A549) independent experiments ± SD. [b]Pgp-overexpressing multidrug-resistant variant of the A2780 parental line. [c]Values from ref 22 (A2780 and A278 AD) and ref 8 (A549). [d]Values obtained when **1** was used as positive control for *ent*-**1**: A2780 = 0.8 ± 0.01; A2780 AD = 2.7 ± 0.21. [e]n.d. = not determined.

These results are in agreement with previous findings for 13-desmethylene (−)-dactylolide (**5**), and they indicate that the C(13)-exomethylene group is not essential for the biological activity of zampanolide-type structures. We have also compared the activity of (−)-zampanolide (**1**) and its enantiomer *ent*-**1** against the two ovarian carcinoma cell lines A2780 and A2780 AD. Very intriguingly, while the addition of the (*Z*,*E*)-sorbamide moiety to **2** leads to a >100-fold increase in potency for **1**,[8] the elaboration of *ent*-**2** into *ent*-**1** does not produce any change in activity, making *ent*-**1** > 1000-fold less potent than the natural product **1**.

In summary, we have developed a new approach for the stereoselective transfer of (*Z*,*E*)-sorbamide (**3**) onto the aldehyde group of (−)-dactylolide (**2**), 13-desmethylene dactylolide (**5**), and (+)-dactylolide (*ent*-**2**). The method has allowed the synthesis of **1**, *ent*-**1**, and also of **4** and its C(20) *R* epimer from the respective aldehydes with unprecedented efficiency. At the biological level, 13-desmethylene zampanolide (**4**) was found to be equipotent with the natural product **1**. This finding has been exploited in the synthesis of analogs of **4** with modified macrocycles, which was greatly aided by the methodology developed for the stereoselective elaboration of the hemiaminal-linked side chain. The corresponding SAR study will be published in a separate paper.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c02974.

Experimental details; details of the experiments (PDF)
NMR spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Karl-Heinz Altmann − *Department of Chemistry and Applied Biosciences, ETH Zürich, 8093 Zürich, Switzerland;* orcid.org/0000-0002-0747-9734; Email: karl-heinz.altmann@pharma.ethz.ch

Authors

Tobias M. Brütsch − *Department of Chemistry and Applied Biosciences, ETH Zürich, 8093 Zürich, Switzerland*

Simone Berardozzi − *Department of Chemistry and Applied Biosciences, ETH Zürich, 8093 Zürich, Switzerland;* orcid.org/0000-0002-3352-9811

Marlene L. Rothe − *Department of Chemistry and Applied Biosciences, ETH Zürich, 8093 Zürich, Switzerland*

Mariano Redondo Horcajo − *Centro de Investigaciones Biológicas Margarita Salas, 28040 Madrid, Spain*

José Fernando Díaz − *Centro de Investigaciones Biológicas Margarita Salas, 28040 Madrid, Spain;* orcid.org/0000-0003-2743-3319

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.0c02974

Author Contributions

[#]T.M.B. and S.B. contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Swiss National Science Foundation (Projects 200021_149253 and 200020_175744 (KHA)). Funding was also obtained from the Ministerio de Ciencia e Innovación PID2019-104545RB-I00 and H2020-MSCA-ITN-2019 860070 TUBINTRAIN (JFD). We are grateful to Dr. Bernhard Pfeiffer, Dr. Leo Betschart, and Philipp Waser for the NMR support, Kurt Hauenstein for crucial technical advice (all ETH Zurich), and to the MoBiAS platform at ETH Zurich for HRMS spectra.

■ REFERENCES

(1) Tanaka, J.; Higa, T. Zampanolide, a New Cytotoxic Marcrolide from a Marine Sponge. *Tetrahedron Lett.* **1996**, *37*, 5535−5538.

(2) Field, J. J.; Singh, A. J.; Kanakkanthara, A.; Halafihi, T.; Northcote, P. T.; Miller, J. H. Microtubule-Stabilizing Activity of Zampanolide, a Potent Macrolide Isolated from the Tongan Marine

https://dx.doi.org/10.1021/acs.orglett.0c02974
Org. Lett. XXXX, XXX, XXX−XXX

Sponge *Cacospongia Mycofijiensis*. *J. Med. Chem.* **2009**, *52*, 7328−7332.
(3) Cao, Y. N.; Zheng, L. L.; Wang, D.; Liang, X. X.; Gao, F.; Zhou, X. L. Recent advances in microtubule-stabilizing agents. *Eur. J. Med. Chem.* **2018**, *143*, 806−828.
(4) Prota, A. E.; Bargsten, K.; Zurwerra, D.; Field, J. J.; Díaz, J. F.; Altmann, K.-H.; Steinmetz, M. O. Molecular Mechanism of Action of Microtubule-Stabilizing Anticancer Agents. *Science* **2013**, *339*, 587−590.
(5) Taufa, T.; Singh, A. J.; Harland, C. R.; Patel, V.; Jones, B.; Halafihi, T.; Miller, J. H.; Keyzers, R. A.; Northcote, P. T. Zampanolides B − E from the Marine Sponge *Cacospongia Mycofijiensis*: Potent Cytotoxic Macrolides with Microtubule-Stabilizing Activity. *J. Nat. Prod.* **2018**, *81*, 2539−2544.
(6) Smith, A. B.; Safonov, I. G.; Corbett, R. M. Total Syntheses of (+)-Zampanolide and (+)-Dactylolide Exploiting a Unified Strategy. *J. Am. Chem. Soc.* **2002**, *124*, 11102−11113.
(7) Hoye, T. R.; Hu, M. Macrolactonization via Ti(IV)-Mediated Epoxy-Acid Coupling: A Total Synthesis of (−)-Dactylolide [and Zampanolide]. *J. Am. Chem. Soc.* **2003**, *125*, 9576−9577.
(8) Zurwerra, D.; Glaus, F.; Betschart, L.; Schuster, J.; Gertsch, J.; Ganci, W.; Altmann, K.-H. Total Synthesis of (−)-Zampanolide and Structure-Activity Relationship Studies on (−)-Dactylolide Derivatives. *Chem. Eur. J.* **2012**, *18*, 16868−16883.
(9) Uenishi, J.; Iwamoto, T.; Tanaka, J. Total Synthesis of (−)-Zampanolide and Questionable Existence of (−)-Dactylolide as the Elusive Biosynthetic Precursor of (−)-Zampanolide in an Okinawan Sponge. *Org. Lett.* **2009**, *11*, 3262−32655.
(10) Ghosh, A. K.; Cheng, X. Enantioselective Total Synthesis of (−)-Zampanolide, a Potent Microtubule-Stabilizing Agent. *Org. Lett.* **2011**, *13*, 4108−4111.
(11) Ghosh, A. K.; Cheng, X.; Bai, R.; Hamel, E. Total Synthesis of Potent Antitumor Macrolide (−)-Zampanolide: An Oxidative Intramolecular Cyclization-Based Strategy. *Eur. J. Org. Chem.* **2012**, *2012*, 4130−4139.
(12) Zurwerra, D.; Gertsch, J.; Altmann, K.-H. Synthesis of (−)-Dactylolide and 13-Desmethylene-(−)-Dactylolide and Their Effects on Tubulin. *Org. Lett.* **2010**, *12*, 2302−2305.
(13) Chen, G.; Wang, R.; Vue, B.; Patanapongpibul, M.; Zhang, Q.; Zheng, S.; Wang, G.; White, J. D.; Chen, Q.-H. Optimized synthesis and antiproliferative activity of desTHPdactylolides. *Bioorg. Med. Chem.* **2018**, *26*, 3514−3520.
(14) Chen, G.; Patanapongpibul, M.; Jiang, Z.; Zhang, Q.; Zheng, S.; Wang, G.; White, J. D.; Chen, Q.-H. Synthesis and antiproliferative evaluation of new zampanolide mimics. *Org. Biomol. Chem.* **2019**, *17*, 3830−3844.
(15) Chen, G.; Jiang, Z.; Zhang, Q.; Wang, G.; Chen, Q.-H. New Zampanolide Mimics: Design, Synthesis, and Antiproliferative Evaluation. *Molecules* **2020**, *25*, 362.
(16) Henry, J. L.; Wilson, M. R.; Mulligan, M. P.; Quinn, T. R.; Sackett, D. L.; Taylor, R. E. Synthesis, Conformational Preferences, and Biological Activity of Conformational Analogues of the Microtubule-Stabilizing Agents, (−)-Zampanolide and (−)-Dactylolide. *MedChemComm* **2019**, *10*, 800−805.
(17) Noyori, R.; Tomino, I.; Tanimoto, Y.; Nishizawa, M. Rational Designing of Efficient Chiral Reducing Agents. Highly Enantioselective Reduction of Aromatic Ketones by Binaphthol-Modified Lithium Aluminum Hydride Reagents. *J. Am. Chem. Soc.* **1984**, *106*, 6709−6716.
(18) Noyori, R.; Tomino, I.; Yamada, M.; Nishizawa, M. Synthetic Applications of the Enantioselective Reduction by Binaphthol-Modified Lithium Aluminum Hydride Reagents. *J. Am. Chem. Soc.* **1984**, *106*, 6717−6725.
(19) The configurational assignment of the C(20) stereocenter in **2** as *S* was based on the fact that the compound was identical with the major isomer obtained in the (*S*)-TRIP-catalyzed reaction between **5** and **3**. This assignment was further supported by the data obtained with **1** and ***ent*-1**, as well as by the X-ray crystal structure of an analog of **4** that was obtained as the major isomer in the reaction of an aldehyde closely related to **5** with the putative **7**.
(20) As pointed out by one of the reviewers, the yield of ***ent*-1** from ***ent*-2** (74%) in principle should be the same as that of **1** from **2** (81%). The observed difference in isolated yields can be ascribed to different reaction scales and, in particular, slightly different losses of material during HPLC purification.
(21) One of the reviewers suggested that the apparent lack of selectivity in the formation of **13** might be caused by the greater susceptibility of this compound to epimerization (*via* fragmentation/ionization) compared to **14**, due to the absence of an electron-withdrawing β-acyloxy group. While we cannot rule out possible epimerization of **13**, it would appear that a β-acyloxy group could also *promote* ionization at the hemiaminal center by neighbouring group participation through the carbonyl oxygen.
(22) Field, J. J.; Pera, B.; Calvo, E.; Canales, A.; Zurwerra, D.; Trigili, C.; Rodríguez-Salarichs, J.; Matesanz, R.; Kanakkanthara, A.; Wakefield, S. J.; Singh, A. J.; Jiménez-Barbero, J.; Northcote, P.; Miller, J. H.; López, J. A.; Hamel, E.; Barasoain, I.; Altmann, K.-H.; Díaz, J. F. Zampanolide, a Potent New Microtubule-Stabilizing Agent, Covalently Reacts with the Taxane Luminal Site in Tubulin α,β-Heterodimers and Microtubules. *Chem. Biol.* **2012**, *19*, 686−698.

https://dx.doi.org/10.1021/acs.orglett.0c02974
Org. Lett. XXXX, XXX, XXX−XXX