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Enantioselective Total Synthesis of Mandelalide A and Isomandelalide A: Discovery of a Cytotoxic Ring-Expanded Isomer

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Supporting Information

ABSTRACT: The total synthesis of mandelalide A and its ring-expanded macrolide isomer isomandelalide A has been achieved. Unexpected high levels of cytotoxicity were observed with the ring-expanded isomandelalide A with a rank order of potency: mandelalide A > isomandelalide A > mandelalide B. Key aspects of the synthesis include Ag-catalyzed cyclizations (AgCC's) to construct both the THF and THP rings present in the macrocycle, diastereoselective Sharpless dihydroylation of a *cis*-enyne, and lithium acetylide coupling with a chiral epoxide.

In 2012, McPhail et al. reported the isolation of a series of novel macrolides in small quantities from a new species of *Lissoclinum* ascidian found in Algoa Bay, South Africa.[1] These compounds were named mandelalides A−D, and their structures were proposed by extensive spectroscopic analysis. Given the small quantities of material obtained from isolation, only preliminary evaluation of their biological properties could be performed. Mandelalides A and B showed low nanomolar cytotoxicity to lung (NCI-H460) and neuroblastoma (Neuro-2A) cancer cell lines. Not surprisingly, these structurally complex, cytotoxic compounds have attracted considerable synthetic attention from multiple laboratories. In 2014, Willwacher and Fürstner reported the total synthesis of the proposed structure **1**, which did not match the spectroscopic data of the natural product.[2] Shortly thereafter, Ghosh et al. reported the synthesis of the agylcone of the proposed structure of mandelalide A.[3] Ye et al. synthesized a series of structural variants of mandelalide A, which allowed them to unequivocally establish the absolute structure of mandelalide A as compound **2**.[4,5] Given the scarcity of **2** and our laboratory's long-standing interest in the synthesis of cytotoxic macrolides,[6] we sought to develop a robust synthesis of compound **2** to enable additional biological evaluation. Herein, we report the enantioselective total synthesis of mandelalide A (**2**).

As shown in Scheme 1, our synthesis strategy builds on the observation that both the THF and the THP rings present in macrolide **2** could be accessed by a silver-catalyzed cylization (AgCC) from the requisite propargylic benzoates. Our laboratory had previously demonstrated the utility of this general concept in our synthesis of amphidinolides C and F;[6b,d] however, we are unaware of any examples for accessing six-membered oxygenated heterocycles using AgCC.[7] Another intriguing strategic premise was the possibility that the macrocyclization event could be conducted with both the C_{23} and C_{24} alcohols exposed and governed by strong, preferential translactonization to the desired C_{23} macrolactone. Support for this premise can be seen in the observations that translactonization was operable with the originally proposed structure **1**[4] as well as with other macrolides such as amphidinolides G/H;[8] however, the revised structure **2** exists exclusively as the C_{23} macrolactone. If this hypothesis proved problematic, the C_{24} position could be masked prior to macrolactonization.

Synthesis of the northern C_{15}−C_{24} fragment **4** commenced from the alkyne **9**[6b,d] and iodide **10**[9] (Scheme 2). These building blocks were readily available from (*S*)-malic acid (**8**). Sonogashira coupling provided the *cis*-enyne in excellent yield using diisopropylamine as the solvent. Sharpless asymmetric dihydroxylation of *cis*-alkenes[10] has seen limited use in total synthesis[11] despite the widespread utility and prevalence of *anti*-diols. We were pleased to observe that Sharpless dihydroxylation using commercially available $(DHQD)_2PHAL$ provided the desired diastereomer **12** in good yield and reasonable diastereoselectivity (76%, 3.5:1 dr). Use of the pseudo-enantiomeric ligand $(DHQ)_2PHAL$ gave the opposite diastereomer in similar selectivity (1:3.6 dr). Interestingly, the Sharpless ligand specifically designed for *cis*-alkenes (DHQD-IND)[10] proved less effective (2.2:1 dr). Bisesterification using benzoyl chloride followed by desilylation generated the AgCC precursor. While our laboratory has explored the utility of diols as viable nucleophiles in the AgCC process,[6b,d] the alternate combination of using dibenzoates (e.g., **6**) has not been reported. We were pleased to discover that this substrate rapidly and smoothly undergoes cyclization to provide the unstable enol benzoate **13**. Subsequent *in situ* treatment with MeLi·LiBr revealed ketone **14** which also proved unstable until protection as its silyl ether **15** in good overall yield with excellent stereoselectivity (68%, > 10:1 dr). Ketone **15** was next olefinated with the Petasis reagent followed by diastereoselective reduction using Rh/Al_2O_3 to provide the 18*S* stereochemistry. Finally, removal of the Piv ester followed by conversion to Wittig salt **4** proceeded smoothly.

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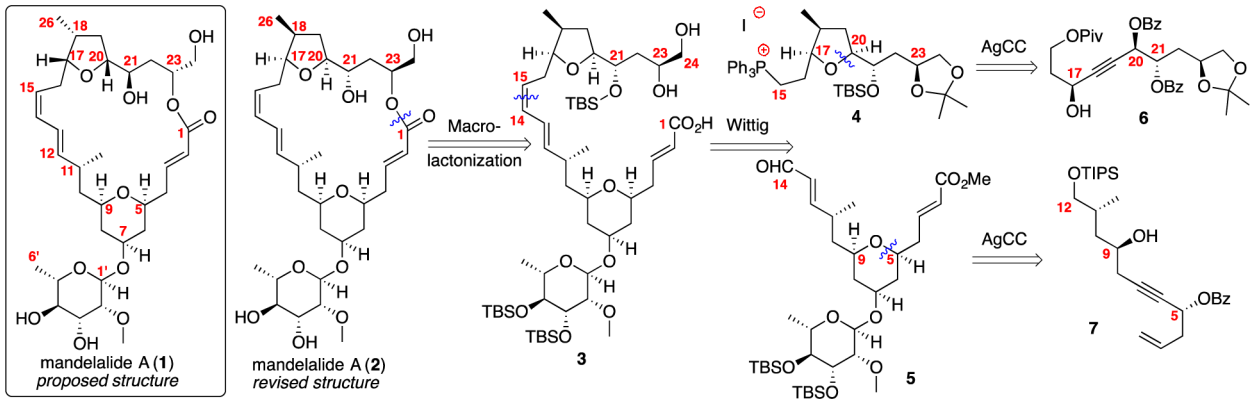
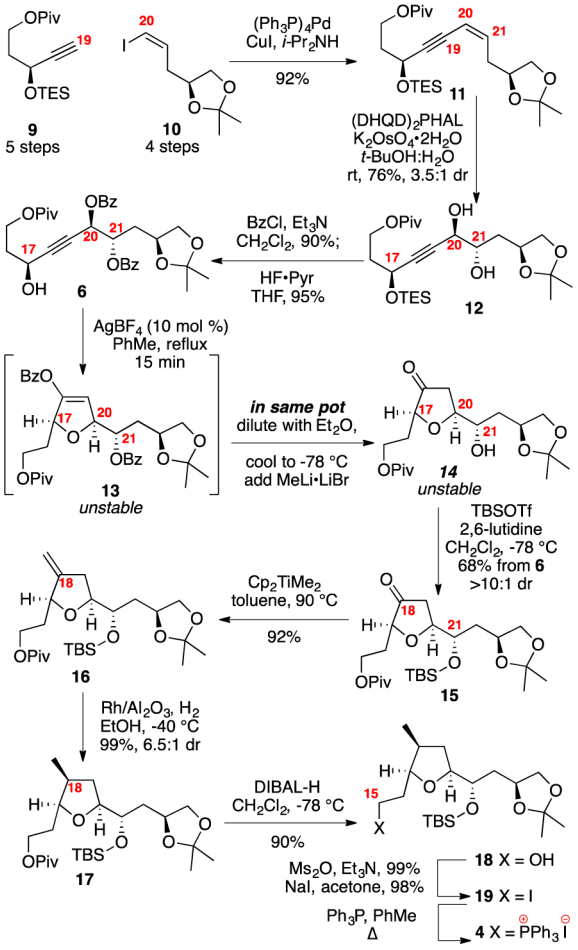
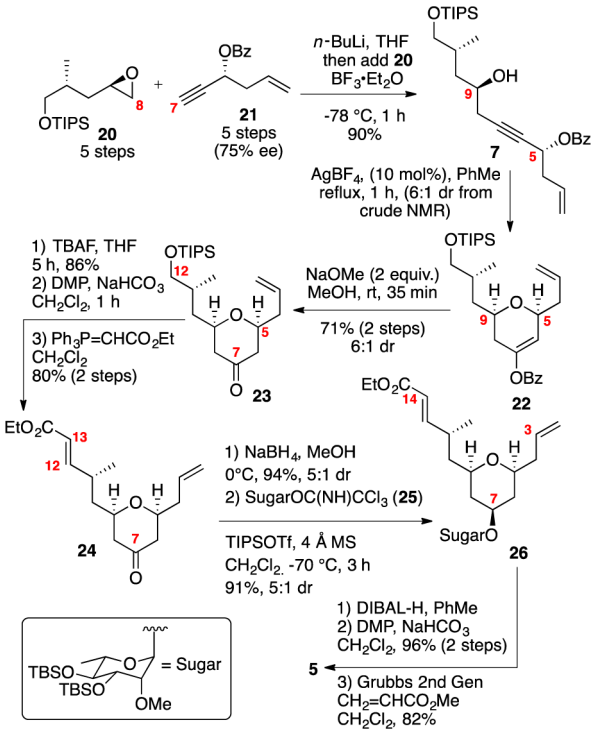
Scheme 1. Retrosynthetic Analysis of Mandelalide A (2)



Scheme 2. Synthesis of Northern Fragment 4



Scheme 3. Synthesis of Southern Fragment 5



With the northern phosphonium salt in hand, we shifted our focus to the southern C_1–C_{14} subunit (Scheme 3). Starting from the readily available epoxide **20** and alkyne **21** (each accessible in 5 steps from commercially available sources), BF_3·Et_2O-mediated coupling under optimized conditions provided the homopropargylic alcohol **7**. While examples exist of alkyne/epoxide couplings in the presence of an ester,[12] propargylic benzoates have not been used before in the process. With the cyclization precursor **7** in hand, we explored the key AgCC to access the pyran **22**. While we are unaware of prior examples of related silver-catalyzed cyclizations for extending the AgCC process to larger ring systems,[7] we were gratified to discover that the transformation proceeded smoothly to yield **22** in a 6:1 dr at C_5 based on crude ^{1}H NMR. This level of diasteroselectivity may be explained by the modest level of enantiopurity (7:1 er) in the alkyne precursor **21**. The crude benzoate was immediately submitted to hydrolysis conditions (NaOMe, MeOH, rt) to generate the desired C_7 ketone **23** in good yield with no loss in stereochemical integrity. It is important to note that alternate protocols to convert the enol benzoate **22** into the ketone **23** (including MeLi·LiBr)[6b,d] led to inferior yields and/or reduced dr. Removal of the C_{12} silyl ether followed by oxidation and Wittig olefination generated the ester **24**. Borohydride reduction followed by Schmidt glycosidation[5] produced the target glycoside **26** in good yield and reasonable dr at $C_{1'}$. Finally, conversion of the C_{14} ester into its corresponding aldehyde and cross metathesis[13] generated the southern fragment **5**.

Our first generation approach to coupling and macrocyclization is shown in Scheme 4. Treatment of phosphonium salt **4** with NaHMDS followed by addition of the aldehyde **5**

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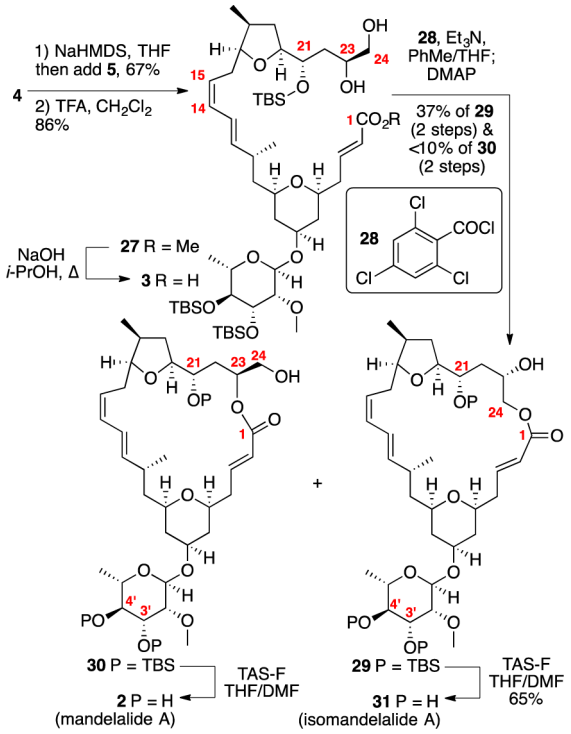
Scheme 4. Total Synthesis of Isomandelalide A (31)



Scheme 5. Total Synthesis of Mandelalide A (2)

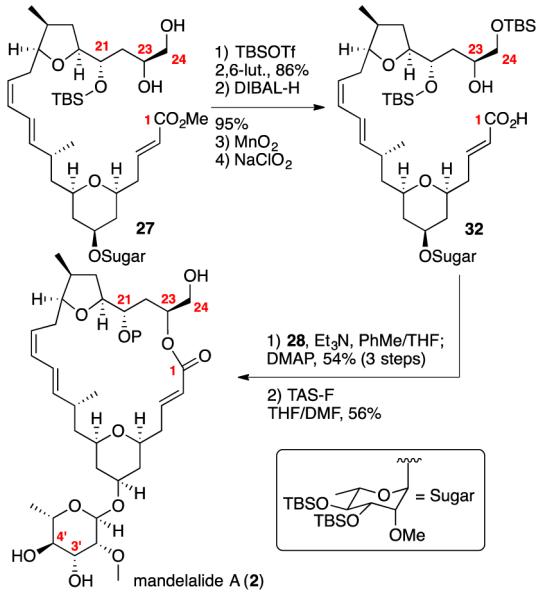


Table 1. Cytotoxic Effects of Compounds 2, 31, and 33 Against Human Cancer Cell Lines

	HeLa cervix		H460 lung	
compd. no.	EC50 (nM)	95% C.I. (nM)	EC50 (nM)	95% C.I. (nM)
2	10.5	8.6–12.2	13.2	8.6–18.0
31	17.1	13.1–21.7	30.3	15.0–42.5
33	35.5	16.1–52.4	35.5	26.3–61.2

cleanly provided the C_{12}–C_{15} *E,Z*-diene. Next, selective deprotection of the C_{23}–C_{24} acetonide in the presence of the C_7 glycoside and the acid-sensitive *E,Z*-diene motif was accomplished using TFA to generate the $C_{23,24}$ diol in excellent yield. Yamaguchi conditions smoothly induced macrolactonization; however, the ring-expanded, 25-membered isomer **29** was the primary isolable product from this reaction. Removal of the three silyl moieties in **29** using TAS-F provided the previously unknown, 25-membered macrolide called isomandelalide A (**31**). A second product **30** was also observed in the macrolactonization. Subsequent TAS-F treatment of the crude mixture from the macrolactonization revealed the desired mandelalide A (**2**); however, only minor amounts of **2** could be accessed through this route. The preference for **29** over **30** is surprising based on the observation by Ye et al. that **2** appeared to prefer the smaller, 24-membered macrolide.[4,14]

In order to selectively access the desired natural product, the C_{24} position was masked as its TBS ether on diol **27** (Scheme 5). Subsequent conversion of the methyl ester into the acid **32** was accomplished by a redox process. Attempted direct saponification of the C_1 methyl ester on the C_{24} silylated product led to extensive silyl migration. We were pleased to see that Yamaguchi macrolactonization of the seco acid **32** proved effective on the C_{24} silyl ether series to give the known Ye intermediate.[4] Finally, global desilylation generated the natural product, the data for which matched with the literature values (^{1}H, ^{13}C, $[\alpha]_D$).[1] A modest amount of a second macrolide was observed under the cyclization process, which we hypothesize to be the C_{21} macrolactone (likely due to C_{21}/C_{23} silyl migration); however, this minor product decomposed under the TAS-F conditions.

Isomandelalide A (**31**) showed potent cytotoxicity in comparative testing with synthetic mandelalide A (**2**) and relative to the natural mandelalide B (**33**) (Table 1). Using assay conditions comparable to those used in the original bioassay-guided drug discovery screen,[1] synthetic **2** showed biological activity that was entirely consistent with the cytotoxicity observed in earlier testing of the natural product. Synthetic **2** was approximately 3-fold more potent than **33** against human HeLa cervical and H460 lung cancer cells. Although slightly less potent than **2**, isomandelalide A (**31**) was also a fully efficacious cytotoxin against both human cancer cell types with EC_{50} values of 17.1 and 30.3 nM for HeLa and NCI-H460 cells, respectively.

In conclusion, efficient total syntheses of mandelalide A (**2**) and its ring-expanded isomer isomandelalide A (**31**) have been completed. The unexpected potency of isomandelalide A raises intriguing questions about the biology of these natural products, given that variation in ring size of a macrocycle often leads to conformational changes that can have a notable impact on biological activity (e.g., amphidinolide G/H[15] and isoapoptolidin/apoptolidin[16]). The disclosed synthetic campaign demonstrated the utility of the AgCC for the construction of oxygenated heterocycles, including the first reported example for accessing a pyran ring system. The *E,Z*-diene motif was incorporated smoothly through a Wittig reaction. The macrocyclization preferences for the $C_{23,24}$ diol were explored. Further application of this methodology to access other members of the mandelalide family will be disclosed in due course.

■ ASSOCIATED CONTENT

Supporting Information

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Complete experimental details and biological protocols (PDF)
^{1}H and ^{13}C NMR spectra (PDF)

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Notes

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REFERENCES

(1) (a) Sikorska, J.; Hau, A. M.; Anklin, C.; Parker-Nance, S.; Davies-Coleman, M. T.; Ishmael, J. E.; McPhail, K. L. *J. Org. Chem.* **2012**, *77*, 6066−6075. (b) Sikorska, J.; Hau, A. M.; Anklin, C.; Parker-Nance, S.; Davies-Coleman, M. T.; Ishmael, J. E.; McPhail, K. L. *J. Org. Chem.* **2013**, *78*, 2812. (c) Davies-Coleman, M. T.; Veale, C. G. L. *Mar. Drugs* **2015**, *13*, 6366−6383.

(2) (a) Willwacher, J.; Fürstner, A. *Angew. Chem., Int. Ed.* **2014**, *53*, 4217−4221. See also: (b) Fürstner, A. *Angew. Chem., Int. Ed.* **2014**, *53*, 8587−8598.

(3) Reddy, K. M.; Yamini, V.; Singarapu, K. K.; Ghosh, S. *Org. Lett.* **2014**, *16*, 2658−2660.

(4) Lei, H.; Yan, J.; Yu, J.; Liu, Y.; Wang, Z.; Xu, Z.; Ye, T. *Angew. Chem., Int. Ed.* **2014**, *53*, 6533−6537.

(5) (a) Wilwacher, J.; Heggen, D.-I. B.; Wirtz, C.; Thiel, W.; Fürtsner, A. *Chem. - Eur. J.* **2015**, *21*, 10416−10430. (c) Brütsch, T. M.; Bucher, P.; Altmann, K.-H. *Eur. J. Chem.* DOI: 10.1002/chem.201504918.

(6) (a) Lu, L.; Zhang, W.; Carter, R. G. *J. Am. Chem. Soc.* **2008**, *130*, 7253−7255. (b) Mahapatra, S.; Carter, R. G. *Angew. Chem., Int. Ed.* **2012**, *51*, 7948−7951. (c) Lu, L.; Zhang, W.; Nam, S.; Horne, D. A.; Jove, R.; Carter, R. G. *J. Org. Chem.* **2013**, *78*, 2213−2247. (d) Mahapatra, S.; Carter, R. G. *J. Am. Chem. Soc.* **2013**, *135*, 10792−10803.

(7) (a) Gockel, B.; Krause, N. *Org. Lett.* **2006**, *8*, 4485−4488. (b) Wang, Y.-M.; Kuzniewski, C. N.; Rauniyar, V.; Hoong, C.; Toste, F. D. *J. Am. Chem. Soc.* **2011**, *133*, 12972−12975. (c) Kotikalapudi, R.; Kumara Swamy, K. C. *Tetrahedron* **2013**, *69*, 8002−8012.

(8) Kobayashi, J.; Shimbo, K.; Sato, M.; Tsuda, M. *J. Org. Chem.* **2002**, *67*, 6585−6592.

(9) Taguchi, T.; Kitagawa, O.; Morikawa, T.; Nishiwaki, T.; Uehara, H.; Endo, H.; Kobayashi, Y. *Tetrahedron Lett.* **1986**, *27*, 6103−6106.

(10) Wang, L.; Sharpless, K. B. *J. Am. Chem. Soc.* **1992**, *114*, 7568−7570.

(11) (a) Dong, S.; Paquette, L. A. *J. Org. Chem.* **2005**, *70*, 1580−1596. (b) Halim, R.; Brimble, M. A.; Merten, J. *Org. Lett.* **2005**, *7*, 2659−2662. (c) Hicks, J. D.; Flamme, E. M.; Roush, W. R. *Org. Lett.* **2005**, *7*, 5509−5512. (d) Ermolenko, L.; Sasaki, N. A. *J. Org. Chem.* **2006**, *71*, 693−703. (e) Yuen, T.-Y.; Brimble, M. A. *Org. Lett.* **2012**, *14*, 5154−5157.

(12) (a) Ramana, C. V.; Raghupathi, N.; Gujar, M. K.; Chorghade, M. S. *Tetrahedron Lett.* **2005**, *23*, 4073−4075. (b) Tinsley, J. M.; Roush, W. R. *J. Am. Chem. Soc.* **2005**, *127*, 10818−10819.

(13) Carlson, E. C.; Rathbone, L. K.; Yang, H.; Collett, N. D.; Carter, R. G. *J. Org. Chem.* **2008**, *73*, 5155−5158.

(14) Blakemore, P. R.; Browder, C. C.; Hong, J.; Lincoln, C. M.; Nagorney, P. A.; Robarge, L. A.; Wardrop, D. J.; White, J. D. *J. Org. Chem.* **2005**, *70*, 5449−5460.

(15) Kobayashi, J.; Shigemori, H.; Ishibashi, M.; Yamasu, T.; Hirota, H.; Sasaki, T. *J. Org. Chem.* **1991**, *56*, 5221−5224.

(16) Wender, P. A.; Gulledge, A. V.; Jankowski, O. D.; Seto, H. *Org. Lett.* **2002**, *4*, 3819−3822.

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