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Total Synthesis of Cytotoxic Macrolide Amphidinolide B_1 and the Proposed Structure of Amphidinolide B_2

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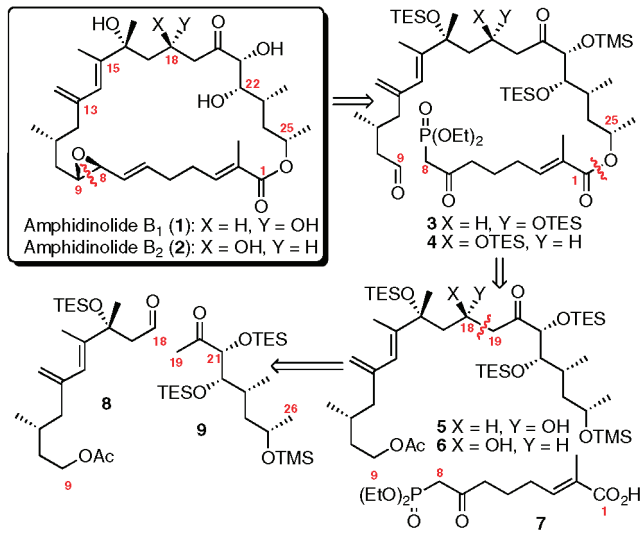
Amphidinolide B_1 (**1**) and its C_{18} epimer amphidinolide B_2 (**2**) have generated significant attention from the synthetic community[1,2] because of their potent cytotoxic activity against several cancer cell lines. In fact, macrolide **1** is the most potent member of this family with a reported IC_{50} value of 0.14 ng/mL against L1210 murine leukemia cell line.[3] Despite these efforts, their total syntheses remain elusive targets. Macrolides **1** and **2** represent considerable synthetic challenges because of their highly substituted $C_{13}-C_{15}$ diene functionality,[1c,4] the densely functionalized $C_{21}-C_{26}$ right-hand portion, the labile C_6-C_9 epoxy alkene, and a 26-membered lactone. Our retrosynthetic strategy starts by disconnection at $C_{8,9}$ to reveal the aldehydes **3** and **4** (Scheme 1). Further cleavage of the ester linkage at C_{25} would lead to the alcohols **5** and **6** and the keto-phosphonate **7**. The $C_{18}-C_{19}$ bond should be available via a diastereoselective aldol reaction with the aldehyde **8** and α-oxy ketone **9**. We have previously reported a chelation strategy for the synthesis of the 18*S* stereochemistry present in amphidinolide B_1[1b,c,5] and this approach has been used by others in the field,[6] including Fürstner's recent total syntheses of amphidinolide G and H.[6a] Herein, we report a nonchelation strategy for construction of the C_{18} stereochemistry and its application to the total syntheses of cytotoxic macrolide amphidinolide B_1 (**1**) and the proposed structure of amphidinolide B_2 (**2**).

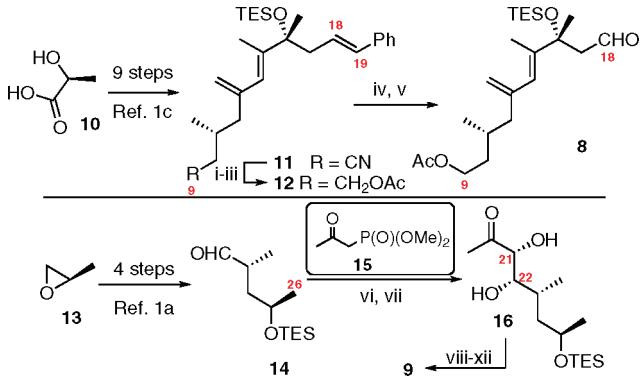
Our syntheses of the aldehyde and methyl ketone subunits commenced with the previously reported intermediates **11** and **14** (Scheme 2).[1a,c] Conversion of the C_9 nitrile **11** into the corresponding acetate **12** followed by selective functionalization of the $C_{18,19}$ alkene using AD mix β* provided the $C_{18,19}$ diol as an inconsequential 6:1 mixture of diastereomers.[1c] Cleavage of the resultant diol with sodium periodate provided the desired aldehyde **8**. For the synthesis of the Eastern subunit **9**, Horner–Wadsworth–Emmons olefination of aldehyde **14** followed by dihydroxylation yielded the diol **16**. Next, bis-silylation followed by selective C_{25} TES deprotection yielded the free alcohol at C_{25}. Finally, Mitsunobu inversion of the alcohol followed by saponification and TMS protection revealed the ketone **9**.

The key diastereoselective aldol coupling is shown in Scheme 3. Treatment of ketone **9** under our standard LDA, Et_2O/THF conditions that proved effective with the C_{21} OPMB series[1c] resulted in low conversion and poor diastereoselectivity favoring the 18*S* stereochemistry [approximately 1.5:1 dr (**5**:**6**)]. Interestingly, addition of TMEDA led to a dramatic rate acceleration and a reversal of the selectivity [2:1 dr (**6**:**5**)]. Additional cooling of the reaction to −100 °C led to improved diastereoselectivity [8:1 dr (**6**:**5**)] in reasonable yield (65% overall). While we are still exploring the nature of the diastereoselectivity, one possible explanation could be a transition state which minimizes the dipoles of the C_{21} C−O σ bond and the enolate.[7] The alternate 18*S* diastereomer can be obtained by performing the reaction at higher temperatures (−40 °C,

Scheme 1. Retrosynthetic Analysis of Amphidinolide B_2



Scheme 2. Synthesis of Western and Eastern Subunits[a]

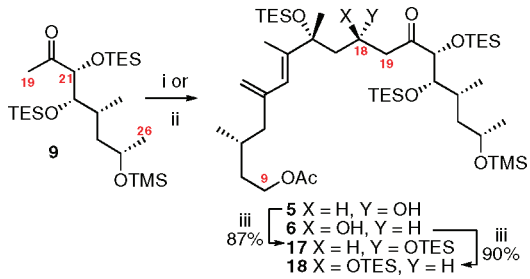


[a] (i) DIBAL-H, PhMe; (ii) $NaBH_4$, EtOH/CH_2Cl_2 (1:1), 85% (two steps); (iii) Ac_2O, pyr., CH_2Cl_2, 94%; (iv) AD Mix β*, *t*-BuOH, H_2O, 88%, 6:1 dr; (v) $NaIO_4$, THF, H_2O, 99%; (vi) **15**, NaH, DME, 76%; (vii) $K_2OsO_2 \cdot 4H_2O$, $(DHQ)_2PHAL$, $K_6Fe(CN)_3$, $MeSO_2NH_2$, K_2CO_3, *t*-BuOH, H_2O, 77%, 10:1 dr; (viii) TESOTf, 2,6-lut., CH_2Cl_2, −78 °C, 92%; (ix) HOAc: THF: H_2O (8:8:1), 0 °C, 89%; (x) DEAD, Ph_3P, 4-$NO_2-C_6H_4CO_2H$, THF, 82%; (xi) then $Ba(OH)_2 \cdot 8H_2O$, MeOH, 0 °C, 72%; (xii) TMSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C, 95%.

1.2:1 dr **5**:**6**). Silyl protection of alcohols **5** and **6** separately using TESCl/DMAP yielded the silyl ether compounds **17** and **18**, respectively.

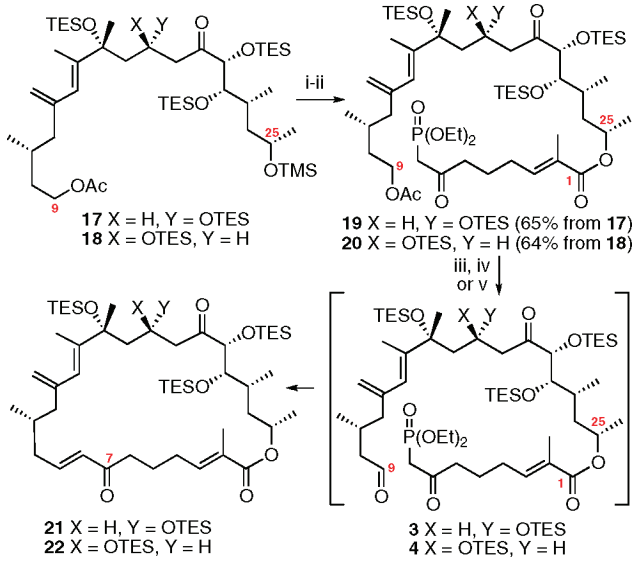
With an effective strategy for the coupling of the Eastern and Western subunits in hand, we turned our attention to the key macrocyclization event (Scheme 4). We chose to initially explore this sequence on the 18*R* compound **18**. TMS deprotection of silyl ether **18** at C_{25} and incorporation of the C_1-C_8 subunit provided

Scheme 3. Key Diastereoselective Aldol Coupling[a]



[a] (i) LDA, TMEDA, Et_2O, THF, −100 °C then add **8**, 65% (8:1 dr, **6**:**5**); (ii) LDA, TMEDA, Et_2O, THF, −40 °C then add **8**, 66% (1.2:1 dr, **5**:**6**); (iii) TESCl, DMAP, CH_2Cl_2, room temp.

Scheme 4. Closure of the Macrocycle[a]

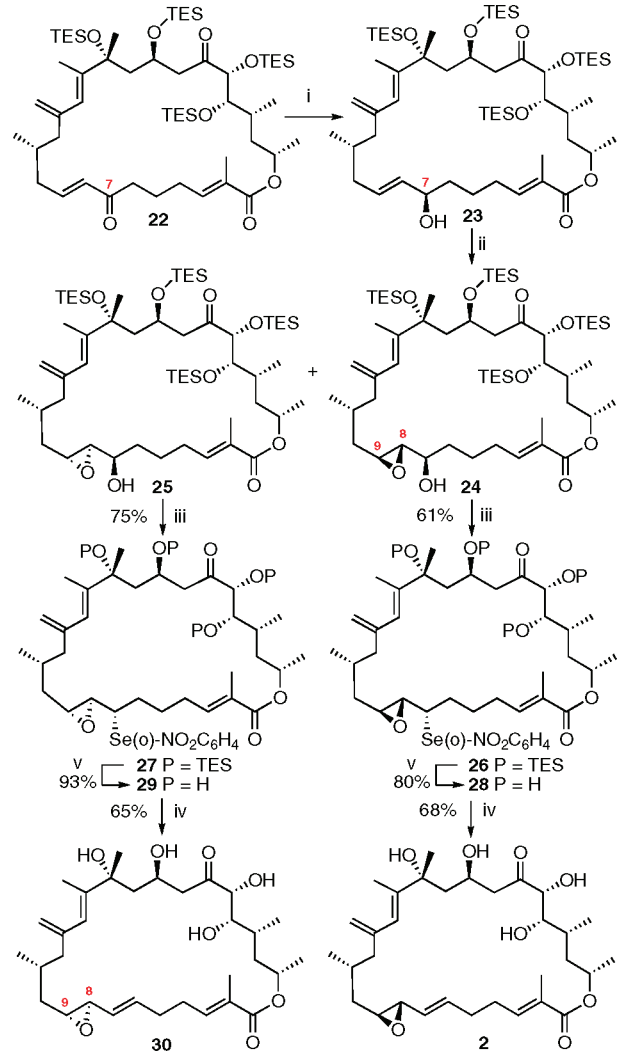


[a] (i) HOAc/THF/H_2O (8:8:1), −10°C; (ii) **5**, $Cl_3C_6H_2COCl$, Et_3N then DMAP, PhMe; (iii) $Ba(OH)_2 \cdot 8H_2O$, MeOH, H_2O, 89% for **19**, 91% for **20**; (iv) TPAP, CH_2Cl_2 then $Ba(OH)_2 \cdot H_2O$, THF, H_2O, 0.01 M final concentration, 51% of **21**; (v) TPAP, CH_2Cl_2 then LiCl, *i*-Pr_2NEt, MeCN, 0.01 M final concentration, 50% of **22**.

the keto phosphonate **20**.[8] Removal of the C_9 acetate followed by Ley's TPAP oxidation revealed the target aldehyde **4**. The corresponding aldehyde **4** proved to be highly reactive as attempted isolation led to considerable loss of material. Interestingly, significant amounts of the aldehyde **4**, formed during the TPAP oxidation, appeared to undergo *spontaneous intramolecular Wadsworth*−*Emmons olefination* to provide the desired macrocycle **22**. The conversion could be driven to completion by the addition of LiCl and Hunig's base[9] in 51% yield. A similar sequence was followed for construction of the 18*S* macrocycle **21**. In this case, $Ba(OH)_2$ proved more effective for driving the macrocyclization to completion. Additionally, we were pleased to observe that macrocycle **21** crystallized upon standing—allowing us to confirm the stereochemistry in **21**.[10]

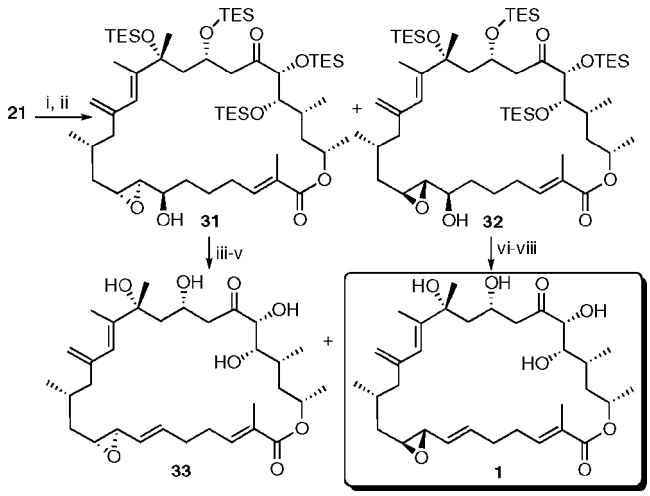
With an efficient route into the macrocycles **21** and **22**, the final challenges were the incorporation of the C_6−C_9 allylic epoxide moiety and deprotection. As before, we explored the initial chemistry on the 18*R* macrolactone **22** (Scheme 5). Our previous experiences with deprotection of late stage amphidinolide B intermediates made us mindful of the difficulty of the final deprotection sequence.[1d] Regioselective and stereoselective reduction of the C_7 carbonyl functionality could be accomplished with the (*S*)-CBS reagent.[11] We next had intended to epoxidize the alkene under Sharpless conditions;[12] however, the presumed steric congestion of the C_7 alcohol thwarted this approach. Fortunately, use of Ti(O*i*-Pr)$_4$ and TBHP in the *absence* of DIPT led to formation of both the *syn* and *anti* diastereomers—favoring the *syn* stereochemistry (2:1 dr).[13] As the *syn* diastereomer **24** contained the proposed stereochemistry in amphidinolide B_2, we initially proceeded forward with that diastereomer. Selenide incorporation[14] and elimination under our recently developed TPAP/NMO conditions[1d,15] yielded the fully functionalized macrocycle. Unfortunately, all attempts to remove the silyl protecting groups under fluoride or acidic conditions led to decomposition. Suspecting that the allylic epoxide might be the culpable functionality, we next explored global deprotection on the epoxy selenide **26**. We were quite pleased to find that treatment with TAS-F[6a] cleanly removed all silyl protecting groups to provide the polyol **28**. The final selenide oxidation/*syn* elimination proved problematic under standard (H_2O_2) conditions. This issue was not completely surprising as we have previously encountered this problem in our azaspiracid work[15] as well as a previous generation amphidinolide approach.[1d] We have attributed this deleterious reactivity to the α-hydroxy ketone moiety. Although our TPAP/NMO conditions[15] are not compatible with the polyol functionality of **28**, we were gratified to find that bistrimethylsi-

Scheme 5. Synthesis of Proposed Structure of Amphidinolide B_2 and its $C_{8,9}$ Diastereomer[a]



[a] (i) (*S*)-CBS, $BH_3 \cdot DMS$, THF, −20 °C, 3 h, >10:1 dr, 67%; (ii) Ti(O*i*-Pr)$_4$, TBHP, CH_2Cl_2, −20 °C, 5 h, mol. sieves, 74%, 2:1 dr (**24**:**25**); (iii) Bu_3P, *o*-$NO_2C_6H_4SeCN$, THF; (iv) TAS-F, DMF/THF/H_2O (10/1/0.02), 0 °C; (v) TMSOOTMS, $NaHCO_3$, CH_2Cl_2.

Scheme 6. Total Synthesis of Amphidinolide B_1[a]



[a] (i) (*S*)-CBS, BH_3·DMS, THF, −30 °C, 3 h, 3.6:1 dr, 74%; (ii) Ti(O*i*-Pr)$_4$, TBHP, CH_2Cl_2, −20 °C, 5 h, mol. sieves, 77%, 1.5:1 dr (**31**:**32**); (iii) Bu_3P, *o*-$NO_2C_6H_4SeCN$, THF, 89%; (iv) TAS-F, DMF/THF/H_2O (10/1/0.02), 0 °C, 98%; (v) TMSOOTMS, $NaHCO_3$, CH_2Cl_2, 65%; (vi) Bu_3P, *o*-$NO_2C_6H_4SeCN$, THF, 45%; (vii) TAS-F, DMF/THF/H_2O (10/1/0.02), 0 °C, 73%; (viii) TMSOOTMS, $NaHCO_3$, CH_2Cl_2, 68%.

lylperoxide (TMSOOTMS) cleanly facilitated selenide oxidation with in situ syn elimination to reveal product **2**. Surprisingly, this compound **2** *did not match* with the spectra data provided for the natural product amphidinolide B_2.[3] We followed a similar sequence to the $C_{8,9}$ epoxide diastereomer **30** which too did not correspond with the reported data for amphidinolide B_2. In both cases, the ^{1}H NMR shift for the H_{14} alkene was shifted significantly downfield as compared to the natural product data. Careful inspection of the isolation paper revealed that the stereochemical analysis of amphidinolide B_2 was based primarily on the differences in the ^{1}H NMR in the C_{17}−C_{19} region of the natural product as compared to amphidinolide B_1 (**1**).[3b] It is important to note that Shimizu and Clardy obtained X-ray crystallographic structure of natural product **1**.[3b] It is clear from our work that the structural differences between amphidinolide B_1 and B_2 are more complicated than initially expected. *On the basis of this information, we have concluded that the proposed structure of amphidinolide B_2 is incorrect*.

Next, we shifted our focus to the total synthesis of amphidinolide B_1 (**1**) (Scheme 6). We applied an analogous strategy for the synthesis of **1** as was described for the 18*R* series. It appears that a slight reversal in selectivity in the epoxidation occurs with the 18*S* stereochemistry—now with a modest preference for the undesired $C_{8,9}$ epoxide. Fortunately, these diastereomers are chromatographically separable. Conversion of syn epoxide **32** to the selenide followed by TAS-F deprotection yielded the penultimate intermediate. Finally, we were grateful to find that tandem selenide oxidation/elimination using our bis-TMS peroxide conditions yielded the natural product amphidinolide B_1 (**1**). The synthesized material **1** matched with the spectra data reported by Kobayashi and co-workers for amphidinolide B_1.[3c]

In summary, the first total syntheses of amphidinolide B_1 and the proposed structure of amphidinolide B_2 have been achieved. The longest linear sequence is 25 steps from commercially available lactic acid. Determination of the actual structure of amphidinolide B_2 will be reported in due course.

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Supporting Information Available: Complete experimental procedures are provided, including ^{1}H and ^{13}C spectra, of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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