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Total Synthesis and Structural Revision of (+)-Muironolide A

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Supporting Information

ABSTRACT: Muironolide A is a fascinating tetrachlorinated marine polyketide isolated from the sponge of *Phorbas* sp. Only 90 μg had been isolated, and the structure was established by nanoscale NMR techniques. Herein we report the total synthesis of the substance with the assigned structure of muironolide A, propose a revised structure based on NMR data, and complete the enantioselective total synthesis of muironolide A.

In 2009, Molinski and co-workers reported the structure of a remarkable marine natural product, muironolide A (**1**),[1] isolated from the sponge of the *Phorbas* species, the same specimen that earlier provided phorboxazoles A and B.[2] With only 90 μg (152 nmol) available, the complete structure of muironolide A was determined using recently developed nanoscale NMR techniques[3] as well as degradation chemistry with 30 μg of the material. The structure of muironolide A represents a new chemical entity, the main features of which include a 16-membered diester lactone with an unprecedented hexahydro-1*H*-isoindolone, a trichlorocarbinol ester, and chlorocyclopropane subunits. Given the extremely small amounts of this "nearly extinct" natural product, only fragmented studies of its bioactivity were possible, which, nevertheless, identified cytotoxic activity against the HCT-116 solid colon tumor cell line (IC_{50} 96.5 μg/mL) and antifungal activity against *Cryptococcus neoformans* (MIC 16 μg/mL).

Herein we report the enantioselective total synthesis of compound **1** with the originally assigned structure of muironolide A along with its C21,C22,C23-diastereomer **2** (Figure 1). Based on ^{1}H and ^{13}C NMR data from these two compounds, none of which matched those of the natural substance, we made a projection for the corrected structure. This structure was also accessed by total synthesis, and characterization data for this compound were found to be fully consistent with those of the natural product, resulting in the reassignment of stereochemistry at C21 and establishing the absolute stereochemistry as the enantiomer of **3**. Although the initially assigned structure did not fully match the data[1] for the natural product, inconsistencies appear to be confined to the fragment produced by the degradation chemistry, thus underscoring the power of the nanoscale NMR methods for determining the structure of this exceedingly rare complex natural product.

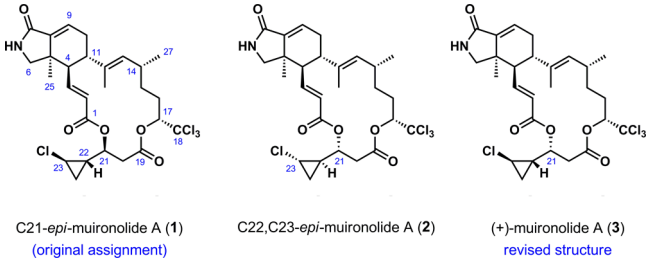
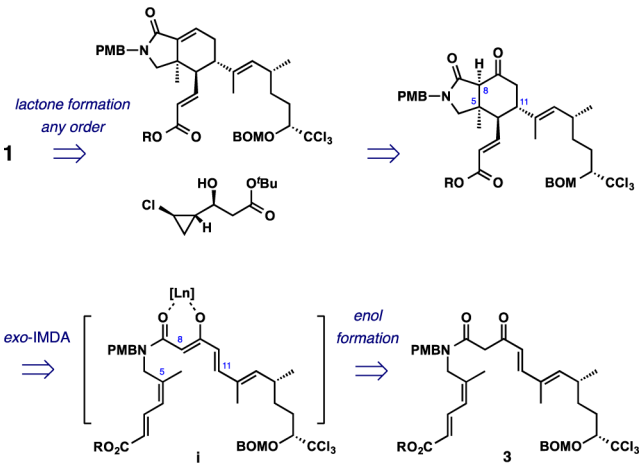


Figure 1. Original and revised structures of muironolide A.

The key features of our synthesis design are outlined in Scheme 1.[4] The design calls for a late stage macrolactone

Scheme 1



formation; the specific order of esterification was intentionally left flexible for optimization. A central transformation in this plan is the *exo*-selective lanthanide-catalyzed intramolecular Diels–Alder reaction for the construction of the hexahydro-1*H*-isoindolone subunit.[5,6] In this reaction, the enol form of the γ,δ-unsaturated β-keto amide precursor **3** serves as the dienophile, which is further activated by raising the HOMO of the diene after deprotonation in the presence of a lanthanide additive, giving rise to enolate chelate **i**.

To access the β-keto amide precursor of the IMDA reaction, (+)-β-citronellene was first converted to trichlorocarbinol **4** by selective ozonolysis[7] and reaction of the resultant aldehyde with Me_3SiCCl_3 in the presence of sodium formate (Scheme 2).[8] To set the configuration at C17, an oxidation–reduction sequence was required. Oxidation of **4** with DMSO/$(CF_3CO)_2O$ (80% yield) followed by reduction of the trichloromethyl ketone with $[Ru(cymene)Cl]_2$ and (*R*,*R*)-TsDPEN under transfer hydrogenation conditions delivered **5** in 97% yield and 10:1

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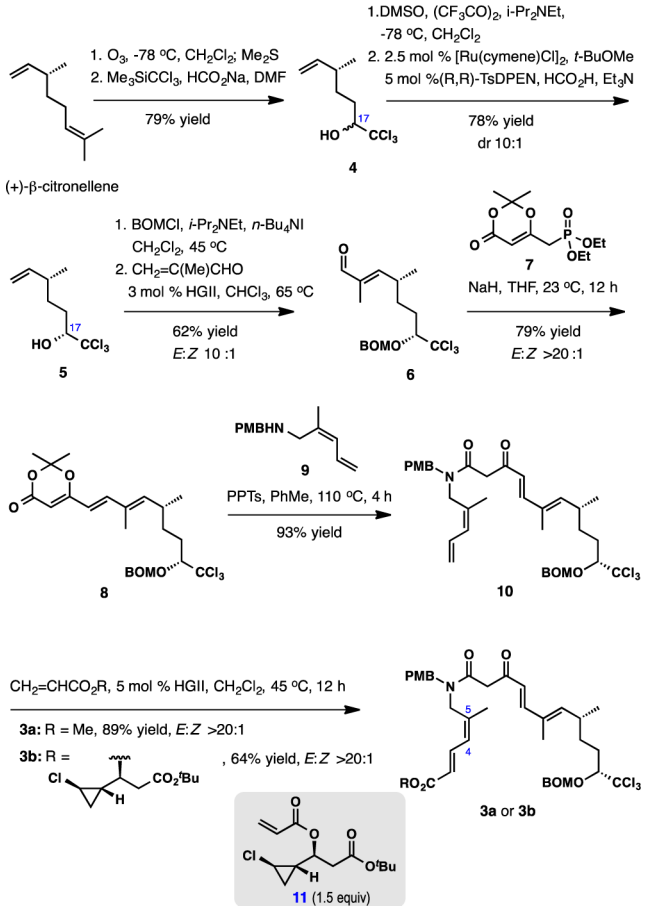
Scheme 2

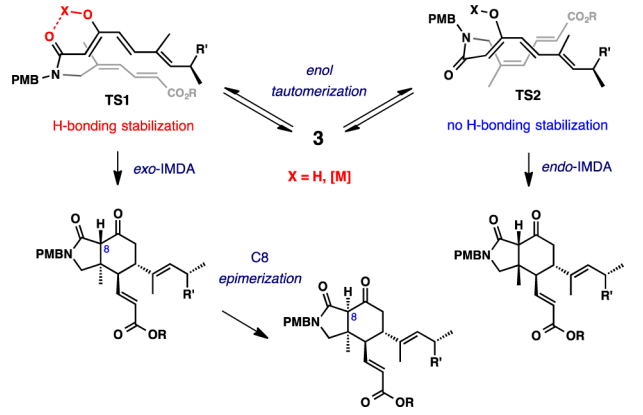


diastereoselectivity.[9] After benzyloxymethylation (BOMCl, *i*-Pr_2NEt, *n*-Bu_4NI, CH_2Cl_2, 45 °C, 12 h, 92% yield), cross-metathesis with methacrolein in the presence of 3 mol % of Hoveyda–Grubbs II catalysts (HGII) afforded aldehyde **6** in 67% yield as a 10:1 mixture of *E* and *Z* alkene isomers. The reaction was accompanied by the recovery of ∼20% of the starting material. Olefination with dioxinone phosphonate **7** readily afforded **8**, which, upon thermolysis with amine **9**[5] generated amide **10** in 93% yield. Cross-metathesis with methyl acrylate or functionalized acrylate **11**[10−12] (7 mol % HGII, CH_2Cl_2, 45 °C, 12 h) completed the synthesis of IMDA precursors **3a** and **3b**.

Preliminary investigations demonstrated the feasibility of the planned IMDA reaction for the assembly of the hexahydro-1*H*-isoindolone ring system of muironolide A.[5] When the substrate possesses the *Z* geometry at the C4–C5 double bond, as in **3**, the requisite relative stereochemistry of all newly formed stereogenic centers is achieved. The IMDA reaction can be performed directly by thermal activation or under catalytic conditions.[13] We found that the thermal cycloaddition can be performed simply by heating the substrate at 110 °C in toluene, with no base additive required. It was also characterized by a very high level of diastereocontrol favoring the *exo*-IMDA product (>30:1). Together, these observations are indicative of a polar concerted mechanism rather than a double Michael addition pathway.

The reactive hydroxy diene intermediate is accessed from the *β*-keto amide precursor through ketone–enol tautomerization (Scheme 3). We hypothesize that the *exo*-IMDA pathway is

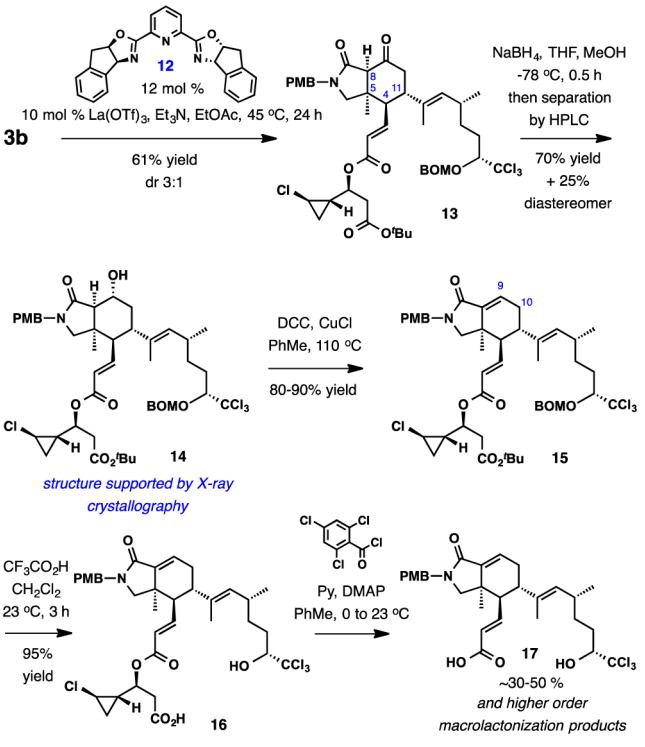
Scheme 3



strongly favored due to hydrogen-bonding stabilization in the transition structure **TS1** (X = H). In the transition structure for the disfavored *endo*-IMDA pathway the hydrogen bond is disrupted (**TS2**, X = H). Furthermore, replacing the hydrogen bond in **TS1** with a chelated metal enolate (**TS1**, X = M) was expected to increase electron density in the diene system, raising its HOMO and activating the diene for cycloaddition. As anticipated, such stabilized enolates could be generated catalytically via soft enolization, and a screen of Lewis acid additives revealed that lanthanide triflates or nitrates were effective catalysts.[13]

In the event, when **3b** was treated with 10 mol % of $La(OTf)_3$, 12 mol % of PYBOX ligand **12**, and triethylamine in ethyl acetate at 45 °C, *exo*-IMDA product **13** was obtained as an inseparable 3:1 mixture with its C4,C5,C8,C11-diastereomer in 61% combined yield (Scheme 4). Reduction of **13** with $NaBH_4$ afforded a mixture of two isomeric products which now could be readily separated by preparative HPLC. The major

Scheme 4



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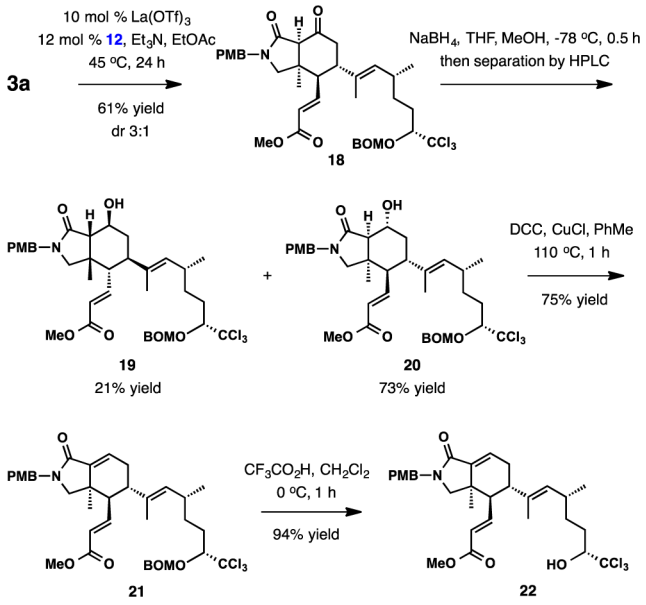
product (**14**) afforded high quality single crystals suitable for X-ray crystallographic analysis, which, critically, ascertained the configuration of *all* stereogenic centers of the target natural product. The functionalization of the hexahydro-1*H*-isoindolone ring system was concluded by dehydration with DCC and CuCl upon heating in toluene, giving **15** in high yield.[14] Of note, other methods (MsCl, Et_3N, then DBU; Burgess or Martin reagents) afforded the deconjugated C9−C10 alkene as the sole product.

Simultaneous removal of the *tert*-butyl ester and benzyloxymethyl groups was achieved effectively by exposure of **15** to trifluoroacetic acid in CH_2Cl_2 at room temperature. Of various attempted methods for macrolactonization of *seco*-acid **16**, the Yamaguchi reagent[15] appeared most promising. However, even under optimal conditions, hydroxyl acid **17** was isolated as a major product, apparently arising from β-elimination in the chlorocyclopropyl acid subunit. Other products resulted from higher order macrolactonizations, and the amount of the intended product was low at best. Therefore, the alternative order of ester installations for the assembly of the 16-membered macrolactone was tested.

Using the same conditions for the IMDA reaction of **3a** (10 mol % $La(OTf)_3$, 12 mol % ligand **12**, Et_3N, EtOAc, 45 °C, 24 h), isoindolone **18** was isolated in 61% yield, again as a 3:1 mixture with its diastereomer (Scheme 5). After reduction with

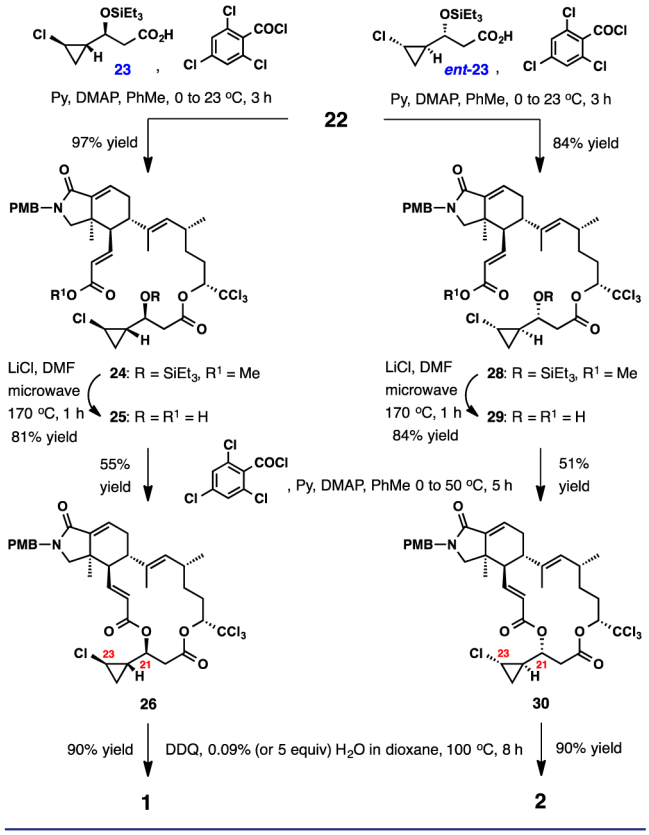
Scheme 5



sodium borohydride and purification by preparative HPLC, dehydration of **20**[16] followed by removal of the BOM group (trifluoroacetic acid, CH_2Cl_2, 0 °C, 1 h) readily afforded trichlorocarbinol **22**.

Esterification of **22** with carboxylic acid **23**[10] to ester **24** was best achieved using the Yamaguchi conditions (97% yield) (Scheme 6). Both the methyl ester and the triethylsilyl ether in **24** could be cleaved in one step under nucleophilic conditions with microwave irradiation. In contrast to intermediate **16**, the resulting *seco* acid **25** underwent a rather clean macrolactonization upon mild heating in toluene with 2,4,6-trichlorobenzoyl chloride, DMAP, and pyridine, affording **26** in 55% yield. After careful optimization, we found that the final amide deprotection is ideally performed under oxidative

Scheme 6



conditions upon heating at 100 °C with DDQ in dioxane with a controlled water content of 0.09% (90% yield).[17] The ^1H and ^{13}C NMR data of the resulting compound did not match those reported for the natural product.

Because carboxylic acid **23** has its origin in an enzymatic resolution process,[10] its enantiomer ***ent*-23** was also readily available. Replicating the same sequence of reactions that delivered **1** with ***ent*-23** afforded another isomer of muironolide A, compound **2**. Its NMR data also did not match the natural substance; however, chemical shift analysis of ^1H and ^{13}C NMR spectra revealed a markedly closer correlation (Figure 2). As can be seen from the analysis of ^{13}C NMR spectra, the major differences are confined to the C21−C26 region, i.e. mostly to the *trans*-chlorocyclopropyl ketide (CCK) unit. Originally, the configuration of this section of the muironolide A could not be determined spectroscopically and required an audacious effort that combined degradation chemistry with 30 μg of the natural sample, chemical correlation, and Mosher ester analysis. In our work, we ascribed a much closer correlation of NMR data between the natural product and **2**, vs **1**, to the inverted configuration at C21, which we presumed to have a larger impact on the macrocycle conformation vis-a-vis the exocyclic CCK unit. On the basis of these conjectures, as well as an alternative interpretation of HPLC correlation data in the original report, we hypothesized that muironolide A is the C21 epimer of **1**.

This hypothesis was put to test when **22** had been converted to the macrolactone using CCK acid **24**[10] and the same four-step sequence of reactions (Scheme 7). The physical data for the resulting compound were in full agreement with those reported for natural muironolide A; however, circular dichroism (CD) spectroscopy demonstrated that it is enantiomeric to the

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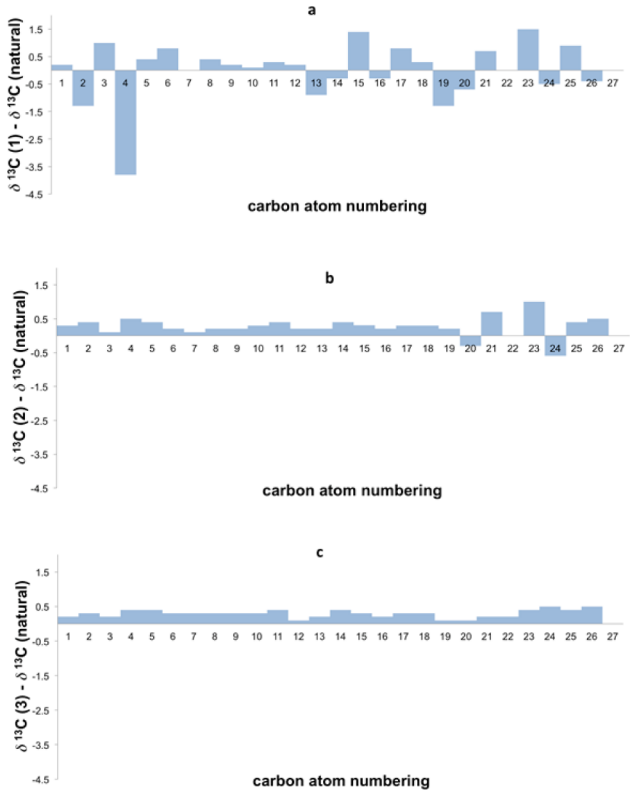
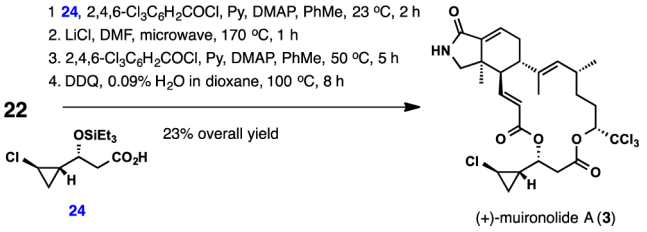


Figure 2. Differences in the ^{13}C NMR chemical shifts between (a) **1**, (b) **2**, and (c) **3** (synthetic) and natural muironolide A.

Scheme 7



natural sample,[10] thus establishing its absolute configuration as *opposite* to that of **3**.

The enantioselective total synthesis of (+)-muironolide A resulted in the adjustment of configuration at C21 and reassignment of the absolute configuration of the natural product. By delivering 25 mg of the natural product in the first unoptimized effort, over 250 times the amount isolated from the natural sources, it paves the way for a more systematic evaluation of the biological profile of muironolide A. Perhaps more importantly, this work highlights the powerful combination of nanoscale NMR analysis with total synthesis for uncovering the full potential of "nearly extinct", exceedingly rare components of natural product extracts.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures, copies of NMR spectra, CD measurements. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b03531.

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Notes

The authors declare no competing financial interest.

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