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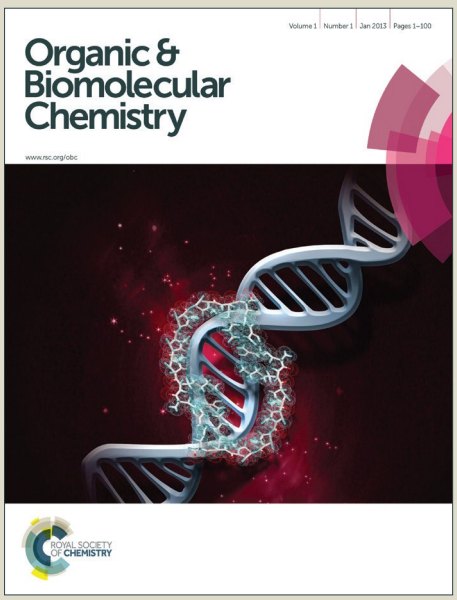

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ARTICLE

Total Synthesis and Preliminary SAR Study of (±)-Merochlorins A and B†

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A modular synthesis of merochlorins A and B, two naturally occurring antibiotics, has been achieved concisely from readily available building blocks in 4-6 steps. The key steps include the bio-inspired tandem phenol oxidative dearomatization/[5+2] and [3+2] cycloadditions to construct the tricyclic cores of the targets, and the intermolecular Diels-Alder reaction followed by dehydrogenative aromatization to assemble the remaining aromatic units. The antibacterial activities of merochlorins A, B and some advanced synthetic intermediates were also evaluated, which provided valuable information on the structure-activity relationship (SAR) of this class of new antibiotics.

Introduction

Due to the ever-increasing threat of antibiotic resistance, there is an urgent need to develop new antibacterial agents with novel chemical structures and mechanisms of action.[1] Natural products are arguably the most valuable resource for the discovery of new antibiotics.[2] A recent paradigm appeared in 2012, when a pair of chlorinated meroterpenoids, merochlorins A (**1**) and B (**2**) (Figure 1a), were identified from the marine bacterium *Streptomyces sp. strain* CNH-189 by Moore, Fenical and co-workers.[3] Both **1** and **2** display broad-spectrum antibacterial activity against various Gram-positive organisms, such as clinically relevant methicillin-resistant *Staphylococcus aureus strains* (MIC=2−4μg/mL) and vancomycin-resistant *Enterococcus faecium* (MIC=2μg/mL),[3-4] which renders them promising drug candidates for the development of new antibacterial agents. However, it was found that **1** was inactive (MIC>64μg/mL) against the examined bacterial strains when tested in Mueller-Hinton broth containing 10% human serum,[4] which somewhat compromised its potential as drug candidate. While further structural optimization may provide a solution to address this issue, the scarcity of **1** and **2** in natural resource makes it impractical to achieve such goal. Thus, the development of an efficient and modular synthetic approach to access ample supply of **1** and **2** as well as their analogues is in great demand.

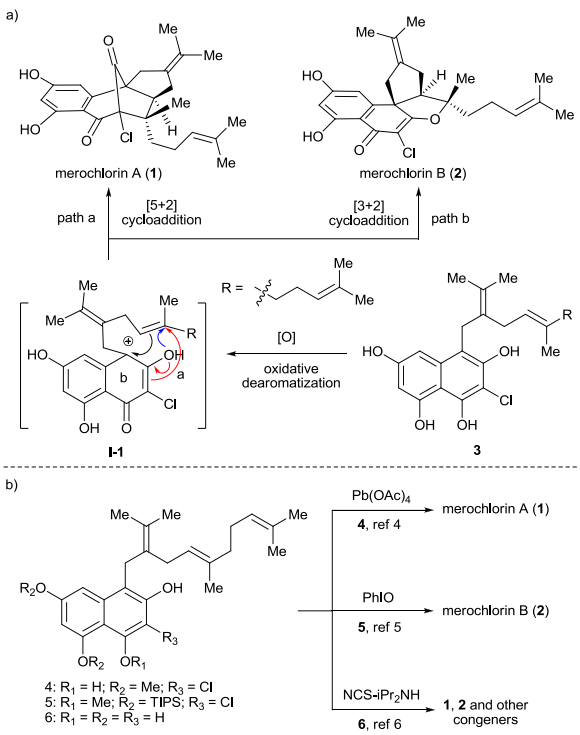

Figure 1. a) Proposed biosynthetic pathway and b) Reported biomimetic synthesis of merochlorins A and B.

While **1** and **2** bear different molecular skeletons, from the biosynthetic point of view they should be traced back to a common

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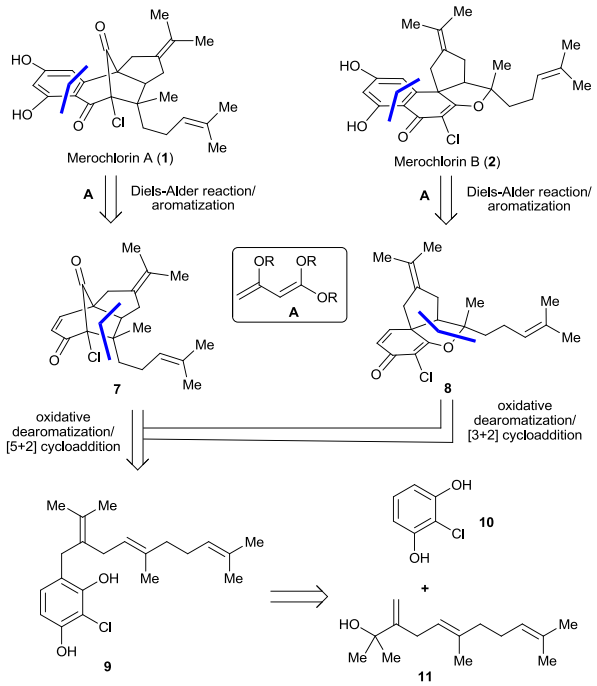
†Electronic Supplementary Information (ESI) available: The NMR spectra of compounds **1, 2, 7-9, 15, 16, 18** and **19**, and the CIF file of compounds **8** and **18**. See DOI: 10.1039/b000000x/

tetrahydroxynaphthalene (THN) precursor **3** (Figure 1a). It was believed that **3** could first undergo phenol oxidative dearomatization to form a carbocation intermediate **I-1**, which then diverts into **1** and **2** via [5+2] and [3+2] cycloadditions, respectively.[3] Interestingly, this hypothesis was quickly validated by several elegant synthetic studies. In 2013, George and co-workers reported the first biomimetic synthesis of **1** via tandem oxidative dearomatization/[5+2] cycloaddition (Figure 1b).[5] Subsequently, the first biomimetic synthesis of **2** was achieved by Trauner and co-workers via tandem oxidative dearomatization/[3+2] cycloaddition.[6] Of note, only a single and different target was obtained in each of the above works, probably due to the subtle difference between the substrates (**4** vs **5**) and oxidants [$Pd(OAc)_4$ vs PhIO] employed thereof. Another breakthrough appeared in 2014, when Moore and co-workers realized the enzymatic synthesis of **1** and **2** from pre-merochlorin **6** using a vanadium-dependent haloperoxidase.[7a] Meanwhile, they also accomplished the chemical synthesis of **1**, **2** and several other related congeners with the same precursor using NCS/iPr$_2$NH as both oxidant and chlorination agent.[7b] However, in this case both **1** and **2** generated only as minor components in relatively low yields. In this regard, a more efficient and practical approach enabling the diverted synthesis of merochlorins A and B remains to be established.

Results and discussion

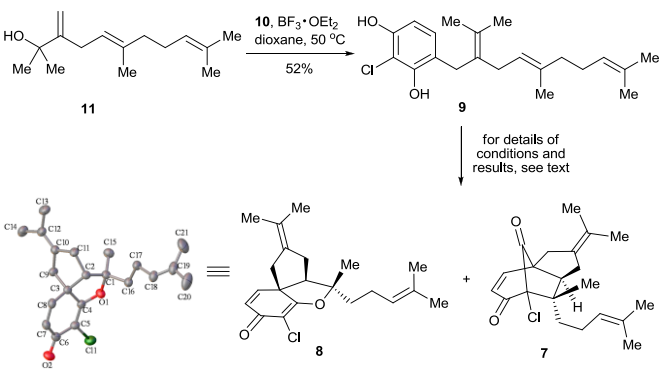
Attracted by the prominent biological profiles of merochlorins A and B, we launched a program toward their synthesis. Our strategic plan was depicted in Scheme 1. As shown, we envisioned that both **1** and **2** could be obtained from the corresponding enone precursors **7** and **8** via an intermolecular Diels-Alder reaction followed by dehydrogenative aromatization. Naturally, the key intermediates **7** and **8** could be accessed from the common precursor **9** via the bio-inspired tandem oxidative dearomatization/ [5+2] and [3+2] cycloadditions, respectively. In turn, **9** could be traced back to the readily accessible fragments **10**[8] and **11**[5,9] via Friedel-Crafts alkylation. Of note, different from the previously reported strategies,[5,6,7b] we projected to install the aromatic ring of the targets in the late stage of the synthesis, which not only avoids the tedious procedure for the preparation of fully functionalized THN derivatives (e.g. 5),[6] but also enables the access of other merochlorin analogues by using different diene components. The later feature is particularly important for the further biomedical studies. Herein we report the successful implementation of above strategy for the diverted total synthesis of merochlorins A and B. Moreover, we also evaluated the anti-bacterial activities of **1** and **2** as well as several advanced synthetic intermediates, which shed light on the preliminary structure-activity-relationship (SAR) of this new class of antibiotics.

Our studies commenced from the synthesis of the precursor **9**. To this end, the fragments **10**[8] and **11**[5,9] were first prepared following literature methods. Next, the assembly of **10** and **11** was achieved via a $BF_3{\cdot}OEt_2$-promoted Friedel-Crafts alkylation,[10] which led to the desired product **9** in 52% yield. With **9** secured in a scalable manner, we turned to explore the key step of our synthesis, the biomimetic oxidative dearomatization induced [5+2] and [3+2] cycloadditions.[11] Since hypervalent iodine(III) reagent-mediated phenol dearomatization followed by other transformations have been



Scheme 1. Retrosynthetic Analysis of Merochlorins A and B

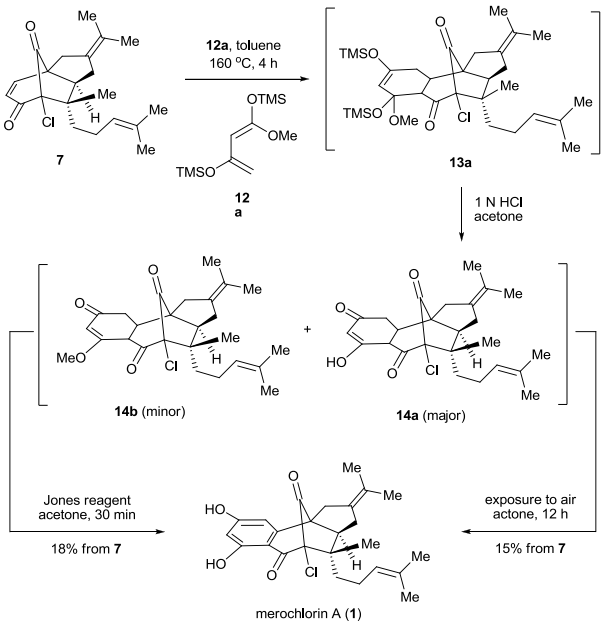
well documented,[12] we first evaluated some commonly used hypervalent iodine(III) reagents [e.g., $PhI(OAc)_2$ and $PhI(CF_3CO_2)_2$] in our scenario. To our delight, when the reaction was performed with $PhI(OAc)_2$ (PIDA) in CH_3CN at room temperature, the desired transformations did proceed to afford the tricyclic compounds **7** and **8** in 10% and 20% yields, respectively (condition 1, Scheme 2). A simple evaluation of the solvent effect revealed that comparable efficiency could be



Reaction conditions	Yield (%) 7	8
1: PIDA (1.2 equiv), CH_3CN, rt, 20 min	10	20
2: PIDA (1.2 equiv), CF_3CH_2OH, rt, 20 min	11	20
3: PIDA (1.2 equiv), DCM, rt, 20 min	9	16
4: PIDA (1.2 equiv), $(CF_3)_2CH_2OH$, rt, 20 min	7	18
5: PIFA (1.2 equiv), CH_3CN, rt, 20 min	6	24
6: PIDA (1.2 equiv), CF_3CH_2OH, 0 °C, 30 min	5	10
7: PIDA (1.2 equiv), CF_3CH_2OH, -20 °C, 30 min	trace	10
8: PIDA (1.2 equiv), K_2CO_3, CH_3CN, rt, 20 min	12	20
9: PIDA (1.2 equiv), $NaHCO_3$, CH_3CN, rt, 20 min	6	15
10: $Pb(OAc)_4$,$CHCl_3$, -40 °C-rt	17	23

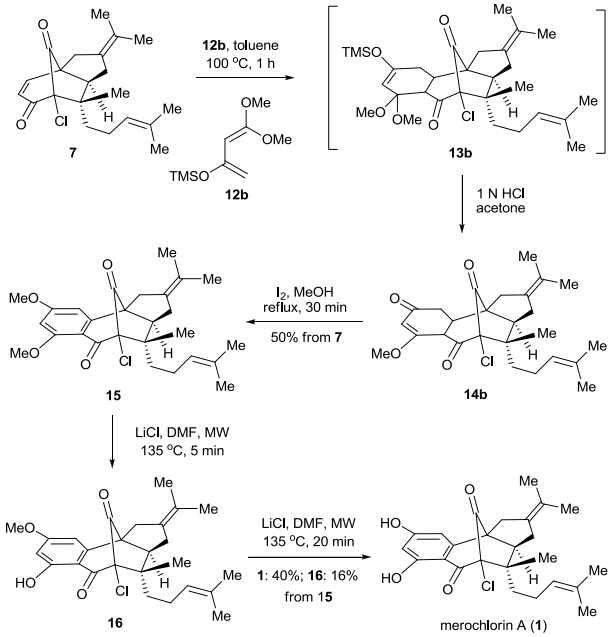
Scheme 2. Synthesis of Tricyclic Intermediates **7** and **8**

obtained with CF_3CH_2OH, while DCM and $(CF_3)_2CHOH$ afforded inferior results (conditions 2-4). Interestingly, while similar combined yield of **7** and **8** (30%) was obtained with $PhI(CF_3CO_2)_2$ (PIFA) employed as oxidant (condition 5), the selectivity of the products was notably improved (**7**:**8** = 1:4). To further improve the efficiency of the transformations, we also evaluated some other reaction parameters including the temperature and additives (condition 6-9), however, no significant improvement could be made. Eventually, we attempted some other oxidants (e.g., $Pb(OAc)_4$, DDQ and $FeCl_3$) in this reactions. It was found that while most of the reactions failed to give the desired products, $Pb(OAc)_4$ proved to the optimal choice (condition 10), which furnished **7** and **8** in 17% and 23% yields, respectively. It should be pointed out that no apparent side-products could be isolated in this reaction, implying that the moderate yields of the transformations mainly arouse from severe decomposition of the starting material. Although the efficiency of the above reaction remained to be improved, it enables the rapid access of the two key intermediates *en route* to **1** and **2** in one pot, thus differentiating itself from the previous studies.[5-6]



Scheme 3. One-Pot Synthesis of Merochlorin A

Having secured the synthesis of the tricyclic intermediates **7** and **8**, we then moved to complete the total synthesis of **1** and **2**. Based on the proposed strategic plan, the aromatic rings of **1** and **2** could be constructed via Diels-Alder reactions followed by aromatization. Of note, while similar strategy has been put into practice in many precedents,[13] most of them employed quinone derivatives as dienophiles, which are distinct from our case. To explore the feasibility of our design, we first attempted the Diels-Alder reaction between the dienophile **7** and diene **12a**.[14] To our delight, the reaction worked well under the thermal conditions (toluene, sealed tube, 160 °C, 4 h), providing **13a** as major product. It was found that **13a** was unstable and readily converted into a mixture of **14a** and **14b** upon chromatography. Besides, a small amount of **1** was alsodetected at this stage, which might be generated from **14a** via auto-oxidation with the action of air. Inspired by this observation, an operationally simple one-pot protocol was developed for the synthesis of **1**. Thus, the resulting mixtures of Diels-Alder reaction were directly treated with 1 N HCl and then allowed to exposed to air for 12 h, which finally afforded **1** in 15% overall yield for 3 steps. Alternatively, the dehydrogenative aromatization could also be accelerated by using the Jones reagent as extra oxidant,[13b] which gave a slightly improved overall yield (18%) in shorter reaction time (Scheme 3).

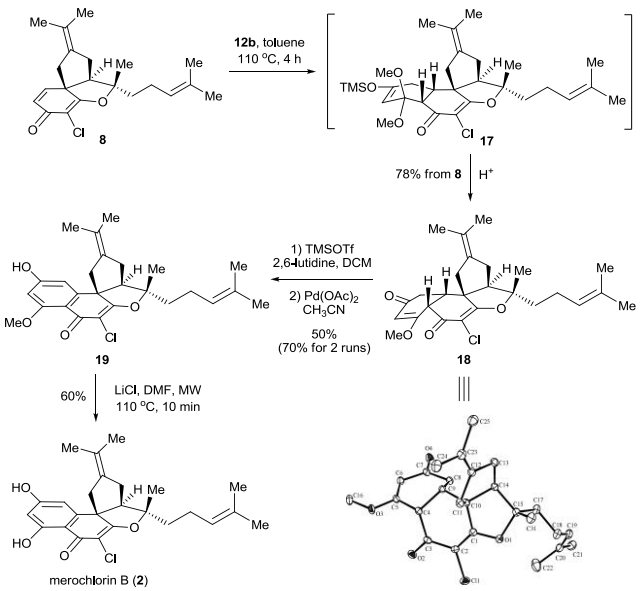


Scheme 4. Alternative Stepwise Synthesis of Merochlorin A

In parallel with the aforementioned work, an alternative and stepwise route towards **1** was also developed with **12b**[15] employed as the diene partner (Scheme 4). In this scenario, the Diels-Alder reaction could go to completion with relatively low temperature and short reaction time (toluene, 100 °C, 1 h). After the acidic work-up, **14b** was obtained as the predominant product,[16] which, upon the further treatment of I_2 in MeOH,[17] provided the di-methylated merochlorin A (**15)** in 50% overall yield. The endgame of the total synthesis of **1** was then completed by adopting a modified two-step procedure developed by George and co-workers.[5] Thus, upon treatment of **15** with LiCl in DMF at 135 °C **(**microwave irradiation) for 5 minutes led to the mono-methylated merochlorin A (**16**) as major product, along with a small amount of **1**. The resulting mixtures, after simple work-up, were further submitted to the same conditions as above, which finally afforded **1** and **16** in 40% and 16% yields, respectively. It should be pointed out that, while the further conversion of **16** to **1** could be achieved with the elongation of reaction time, severe decomposition of the final product was observed, resulting in decreased yield of **1**.

With the total synthesis of **1** achieved, we then moved to synthesize merochlorin B (**2**). Out of our expectation, the Diels-Alder reaction between **8** and **12a** failed to proceed under the conditions employed above (toluene, sealed tube, 160 °C, 4 h), indicating that the dienophile **8** displayed relatively lower reactivity than **7**. Gratifyingly, we quickly found that this problem could be resolved by using the less sterically hindered

and more reactive diene **12b** as reaction partner. Indeed, the Diels-Alder reaction of **8** with **12b** went to completion in refluxing toluene at 110 °C for 4 h, which, after simple acidic work-up, afforded the desired product **18** in 78% isolated yield. The structure of **18** was confirmed by the X-ray crystallography.[18] Next, the dehydrogenative aromatization of **18** was attempted with the same conditions (I_2, MeOH) used for the synthesis of **1**, however, only low yield of the corresponding product (structure not shown) was obtained. Thus, an alternative approach via Saegusa oxidation was employed, wherein **18** was first converted into the corresponding silyl enol ether, which then advanced to the mono-methylated merochlorins B (**19**) in 50% overall yield.[19] While the efficiency of this transformation appeared to be moderate, substantial amounts of **18** (ca. 40%) were recovered and could be recycled for the second time, thus improving overall yield to ca. 70%. Finally, demethylation of **19** was achieved under the same conditions as mentioned above, which gave merochlorin B (**2**) in 60% yield (Scheme 5).



Scheme 5. Total synthesis of merochlorin B

The accessibility of **1**, **2** and several advanced synthetic intermediates (e.g., **7**, **8**, **15**, **16**, **18**, and **19**) in the current work enabled us to perform the preliminary SAR study on this class of antibiotics. It should be pointed out that, although extensive effort has been devoted to the total synthesis of **1** and **2**,[5-7] little has been devoted to explore their SAR. As shown in Table 1, both **1** and **2** exhibited potent inhibitory activity against the examined bacterial strains including *E. faecalis*, *S. aureus*, *S. pneumonia* and MRSA, which was in good agreement with the previous results.[3-4] In comparison, all of the synthetic intermediates only showed weak or no inhibitory activity. These outcomes revealed that the aromatic domains of **1** and **2** played a very important role for their antibacterial activity (**7** vs **1**; **8** vs **2**). Particularly, it proved that both of the two free hydroxyl groups on the aromatic ring were essential for its antibacterial activity, since either the mono- or di-methylated merochlorins (**15**, **16** and **19**) turned out to be inactive in our test. The above information provides valuable information for the development of new merochlorin-derived antibiotics.

Table 1. Antibacterial activity of **1**, **2** and their analogues

compound	MIC(μg/mL)			
	E. faecalis[a]	*S. aureus*[b]	*S. pneumoniae*[c]	MRSA[d]
1	1-2	1-2	8-16	0.5-1
2	1-2	1-2	8-16	1-2
7	32	16-32	16-32	N.D.
8	>64	>64	>64	N.D
15	>64	>64	>64	N.D
16	>64	>64	>64	N.D
18	>64	>64	>64	N.D
19	>64	>64	>64	N.D
Vancomycin	1-2	0.5-1	0.25-0.5	0.25-0.5
Ampicillin	1-2	1-2	0.125-0.25	>32

Notes: [a]Enterococcus faecalis ATCC29212; [b]Staphylococcus aureus ATCC29213; [c]Streptococcus pneumoniae NCTC 7466; [d]Methicillin resistant Staphylococcus aureus BAA-1695

Conclusions

In summary, we have achieved a concise and modular synthesis of merochlorins A and B, two naturally occurring antibiotics, from the readily accessible fragments. The key elements of our synthesis include the bio-inspired tandem phenol oxidative dearomatization/ [5+2] and [3+2] cycloadditions to access the tricyclic cores of the targets, and the intermolecular Diels-Alder reactions followed by aromatization to assemble the remaining aromatic units. Moreover, the antibacterial activities of **1**, **2** and some advanced synthetic intermediates were also evaluated, and the outcomes shed light on the preliminary SAR of this class of new antibiotics. We are now working on the development of new merochlorin analogs with simplified structures and improved drug properties.

Experimental section

General information

Unless otherwise mentioned, all reactions were carried out under a nitrogen atmosphere and anhydrous conditions and all reagents were purchased from commercial suppliers without further purification. Solvent purification was conducted according to Purification of Laboratory Chemicals (Peerrin, D. D.; Armarego, W. L. and Perrins, D. R., Pergamon Press: Oxford, 1980). Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. Reactions were monitored by Thin Layer Chromatography on plates (GF254) supplied by Yantai Chemicals (China) using UV light as visualizing agent and an ethanolic solution of phosphomolybdic acid and cerium sulfate, and heat as developing agents. If not specially mentioned, flash column chromatography uses silica gel (200-300 mesh) supplied by Tsingtao Haiyang Chemicals (China). NMR spectra were recorded on Bruker AV400 instrument. TMS was used as internal standard for ^{1}H NMR (0 ppm), and solvent signal was used as reference for ^{13}C NMR ($CDCl_3$, 77.16 ppm). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, br= broad, td =

triple doublet, qd= quarter doublet, m = multiplet. Infrared (IR) spectra were recorded on a Thermo Nicolet Avatar 330 FT-IR spectrometer. High-resolution mass spectra (HRMS) were recorded on a Bruker ESI-Q/TOF MS, Low-resolution mass spectral analyses were performed with a Waters AQUITY UPLCTM/MS. The microwave reactions were conducted with a CEM microwave reactor (model: DISCOVERY-SP, 300W).

(*E*)-2-chloro-4-(5,9-dimethyl-2-(propan-2-ylidene)deca-4,8-dien-1-yl)benzene-1,3-diol (9). To a solution of **10** (1.98 g, 18 mmol) in dioxane (21 mL) at 50 ℃ was added $BF_3{\cdot}Et_2O$ (0.888 mL, 7.19 mmol), and then a solution of **11** (2.0 g, 9.0 mmol) in dioxane (2.8 mL) was added over 1 h. The resulting solution was allowed to stir at room temperature for 30 min, and then quenched by addition of water (20 mL). The product was extracted with diethyl ether (15 mL × 3) and the combined organic layers were washed with dilute NaOH (0.1 N), water, and brine. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. Purification by flash column chromatography on silica gel (PE/EtOAc = 6:1) afforded compound **9** (1.63 g, 52%) as a thick oil. R_f = 0.38 (silica gel, PE/EtOAc = 6:1); IR (film): 3460, 2966, 2913, 2856, 1612, 1493, 1440, 1165, 869, 802, 610; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (d, *J* = 8.4 Hz, 1H), 6.54 (d, *J* = 8.4 Hz, 1H), 5.80 (s, 1H), 5.34 (s, 1H), 5.08 (t, *J* = 6.0 Hz, 1H), 4.99 (t, *J* = 6.7 Hz, 1H), 3.37 (s, 2H), 2.68 (d, *J* = 6.8 Hz, 2H), 2.06-2.03 (m, 2H), 1.97-1.93 (m, 2H), 1.80 (s, 3H), 1.79 (s, 3H), 1.68 (s, 3H), 1.60 (s, 3H), 1.51 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.4, 150.1, 135.5, 131.3, 129.6, 128.4, 128.3, 124.4, 122.3, 119.0, 107.3, 107.2, 39.7, 32.3, 30.6, 26.6, 25.7, 20.8, 20.6, 17.7, 16.0 ppm; HRMS (ESI) m/z $[M-H]^-$ calcd for $C_{21}H_{29}ClO_2$ 347.1787, found 347.1787.

Tricyclic intermediates 7 and 8. To a solution of **9** (500 mg, 1.43 mmol) in $CHCl_3$ (145 mL) at -40 ℃ was added $Pb(OAc)_4$ (762 mg, 1.72 mmol) portion wise. The reaction mixture was stirred at -40 ℃ for 5 min before gradually warming to room temperature over 30 min. The mixture was filtered through a short pad of SiO_2 and the filtrate was concentrated under vacuum. The residue was purified by flash column chromatography on silica gel (PE/EtOAc = 20:1, 10:1, 6:1) to give compound **7** (84 mg, 17%) as a yellow oil and compound **8** (113 mg, 23%) as a yellow solid. **7**: R_f = 0.45 (silica gel, PE/EtOAc = 6:1); IR (film): 2971, 1914, 1854, 1775, 1685, 1601, 1446, 1374, 810, 737; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 9.2 Hz, 1H), 6.33 (d, *J* = 9.2 Hz, 1H), 4.97 (t, *J* = 6.9 Hz, 1H), 3.21 (d, *J* = 16.5 Hz, 1H), 2.53-2.35 (m, 3H), 2.21-2.15 (m, 1H), 2.08-1.99 (m, 2H), 1.90-1.83 (m, 1H), 1.67 (s, 3H), 1.63 (s, 3H), 1.62 (s, 3H), 1.57 (s, 3H), 1.17-1.10 (m, 1H), 0.99 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.8, 191.9, 159.6, 132.3, 131.6, 129.2, 124.1, 123.2, 93.9, 60.8, 57.8, 43.4, 40.6, 33.4, 32.6, 25.6, 22.6, 21.0, 20.9, 19.7, 17.6 ppm; HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{21}H_{27}ClO_2$ 347.1772, found 347.3043. **8**: R_f = 0.24 (silica gel, PE/EtOAc = 6:1); IR (film): 2971, 2916, 2860, 1651, 1596, 1385, 1297, 1063, 820, 737; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.74 (d, *J* = 9.6 Hz, 1H), 6.16 (d, *J* = 9.6 Hz, 1H), 5.03 (t, *J* = 6.5 Hz, 1H), 2.85 (d, *J* = 17.2 Hz, 1H), 2.73-2.62 (m, 1H), 2.57-2.48 (m, 3H), 2.03-1.95 (m, 2H), 1.69-1.68 (m, 2H), 1.67 (s, 3H), 1.63 (s, 3H), 1.56 (s, 6H), 1.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.6, 174.5, 143.8, 132.5, 130.4, 127.0, 125.8, 122.8, 105.0, 97.1, 59.1, 51.3, 44.9, 44.6, 32.5, 25.6, 22.3, 22.1, 21.5, 21.3, 17.6 ppm; HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{21}H_{27}ClO_2$ 347.1772, found 347.3031.

Dimethylated merochlorin A (15). A solution of **7** (200 mg, 0.58 mmol) and **12a** (351.5 mg, 1.74 mmol) in toluene (1.5 mL) was stirred in a sealed tube at 110 °C for 1 h. After cooling to room temperature, the toluene was removed under vacuum. The resulting residue was dissolved in acetone (5 mL), and then an aqueous solution of HCl (1 N, 2 mL) was added. The reaction mixtures were stirred at room temperature for 30 min, and then poured into water (10 ml). The mixtures were extracted with DCM (3 × 10 mL), the combined organic layer was sequentially washed with a saturated solution of NH_4Cl and brine. After the removal of organic solvent, the residue was dissolved in MeOH (16 mL), followed by addition of I_2 (220.8 mg, 0.87 mmol). The reaction mixture was heated at reflux for 30 min before quenching by saturated sodium thiosulfate solution (6 mL). The mixture was extracted with ethyl acetate (3 × 15 mL) and the organic layers were dried over Na_2SO_4. The solvents were removed under vacuum, and the residue was purified by flash column chromatography on silica gel (PE/EA = 10:1) to give **15** (138 mg, 50%) as a white solid. R_f = 0.3 (silica gel, PE/EtOAc = 2:1); IR (film): 2954, 2920, 2850, 1595, 1462, 1259, 1092, 1013, 795, 733; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.51 (d, *J* = 1.7 Hz, 1H), 6.39 (d, *J* = 1.8 Hz, 1H), 5.00 (t, *J* = 6.7 Hz, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.15 (d, *J* = 15.2 Hz, 1H), 2.74 (d, *J* = 15.2 Hz, 1H), 2.46-2.31 (m, 3H), 2.16-2.07 (m, 1H), 1.85-1.80 (m, 1H), 1.77 (s, 3H), 1.74-1.67 (m, 1H), 1.64 (s, 3H), 1.62 (s, 3H), 1.56 (s, 3H), 1.33-1.24 (m, 1H), 0.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.6, 187.3, 165.2, 164.0, 151.6, 131.7, 131.6, 124.0, 123.7, 114.8, 100.0, 97.1, 91.1, 61.2, 58.9, 56.3, 55.7, 44.3, 38.4, 32.1, 29.7, 25.6, 22.8, 21.1, 20.8, 17.7, 16.0 ppm; HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{27}H_{33}ClO_4$ 457.2146, found 457.2141.

Monomethylated merochlorin A (16). To a solution of **15** (50 mg, 0.11 mmol) in DMF (1.2 mL) was added LiCl (70 mg, 1.65 mmol). The mixture was heated at 135 ℃ under microwave irradiation for 5 min. After cooling to room temperature, the reaction was quenched with 1 N HCl (0.1 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The resulting crude material was used in next step without further purification. Pure sample of **16** could be obtained by flash column chromatography on silica gel (PE/EA = 15:1-4:1) as a while solid. R_f = 0.5 (silica gel, PE/EtOAc = 4:1); IR (film): 2954, 2920, 2849, 1623, 1462, 1377, 1264, 1202, 1021, 800; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.33 (s, 1H), 6.43 (d, *J* = 2.0 Hz, 1H), 6.36 (d, *J* = 2.1 Hz, 1H), 5.00 (t, *J* = 6.5 Hz, 1H), 3.86 (s, 3H), 3.14 (d, *J* = 15.4 Hz, 1H), 2.71 (d, *J* = 15.5 Hz, 1H), 2.46-2.30 (m, 3H), 2.14-2.10 (m, 1H), 1.86-1.81 (m, 1H), 1.77 (s, 3H), 1.72-1.67 (m, 1H), 1.65 (s, 6H), 1.58 (s, 3H), 1.23-1.19 (m, 1H), 0.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.3, 194.2, 166.8, 166.6, 149.6, 132.2, 131.4, 124.0, 123.4, 110.7, 102.5, 99.2, 90.6, 61.4, 58.6, 55.9, 45.3, 39.4, 31.9, 29.1, 25.6, 22.7, 21.1, 20.9, 17.7, 16.7 ppm; HRMS (ESI) m/z $[M-H]^-$ calcd for $C_{26}H_{31}ClO_4$ 443.1989, found 443.1976.

Merochlorin A (1). To a solution of the crude material obtained above in DMF (1.2 mL) was added LiCl (70 mg, 1.65 mmol). The mixture was heated at 135 ℃ under microwave irradiation for 20 min. After cooling to room temperature, the reaction was quenched with 1 N HCl (0.1 mL) and then extracted with EtOAc (3 × 2 mL). The combined organic layers were washed with brine (3 × 5 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by flash column chromatography on silica gel (PE/EA = 15:1-4:1) to give merochlorin A (**1**) (18.8 mg, 40%) as a yellow solid, together with substantial amount of **16** (7.8

mg, 16%). 1: R_f = 0.4 (silica gel, PE/EtOAc = 2:1); IR (film): 2960, 2923, 2853, 1625, 1583, 1453, 1255, 1168; ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.99 (s, 1H), 11.36 (s, 1H), 6.46 (d, *J* = 2.0 Hz, 1H), 6.28 (d, *J* = 2.0 Hz, 1H), 5.01 (t, *J* = 7.1 Hz, 1H), 2.95 (d, *J* = 15.2 Hz, 1H), 2.73 (d, *J* = 15.2 Hz, 1H), 2.46-2.38 (m, 2H), 2.13-2.09 (m, 1H), 1.86-1.80 (m, 1H), 1.73 (s, 3H), 1.63 (s, 3H), 1.61 (s, 3H), 1.54 (s, 3H), 1.46 (dt, *J* = 13.4, 4.9 Hz, 1H), 1.20 (dt, *J* = 12.9, 4.1 Hz), 0.88 (s, 3H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 199.8, 193.0, 166.6, 165.8, 150.3, 132.4, 131.6, 124.3, 123.0, 109.6, 103.3, 101.8, 91.0, 61.3, 58.1, 45.1, 39.4, 31.7, 29.0, 25.8, 22.5, 21.3, 21.0, 17.9, 16.5 ppm; HRMS (ESI) m/z [M-H]$^-$calcd for $C_{25}H_{29}ClO_4$ 427.1682, found 427.1679.

Tetracyclic intermediate 18. A solution of **8** (80 mg, 0.23 mmol) and **12b** (186.8 mg, 0.92 mmol) in toluene (1.0 mL) was stirred in a sealed tube at 110 ℃ for 4 h. After that, another portion of **12b** (200 mg) was added, and the reaction mixtures were allowed to stirred for another 6 h before cooling to room temperature. The solvent was evaporated, and the residue was dissolved in acetone (5 mL). An aqueous solution of HCl (1 N, 1 mL) was added, and the resulting mixtures were stirred at room temperature for 30 min. After that, the mixtures were poured into water (10 mL), and extracted with DCM (3 × 10 mL). The combined organic layers were sequentially washed with a saturated solution of NH_4Cl and brine, and then dried over Na_2SO_4. The solvent was removed under vacuum, and the residue was purified by flash column chromatography on silica gel (EtOAc/PE = 3:1) to give **18** (78 mg, 78%) as a yellow solid. R_f = 0.2 (silica gel, PE/EtOAc = 2/1); IR (film): 2954, 2926, 2849, 1612, 1213, 1170, 1066, 981, 823, 730; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.41 (s, 1H), 5.05 (t, *J* = 6.9 Hz, 1H), 3.80 (s, 3H), 3.58 (d, *J* = 4.1 Hz, 2.89 (d, *J* = 17.3 Hz, 1H), 2.69 (t, *J* = 3.8, 3.9 Hz, 1H), 2.62-2.48 (m, 3H), 2.46-2.41 (m, 2H), 2.33-2.26 (m, 1H), 2.04-1.99 (m, 2H), 1.72-1.67 (m, 2H), 1.66 (s, 3H), 1.64 (s, 3H), 1.61 (s, 3H), 1.58 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.2, 184.9, 176.1, 174.6, 132.8, 130.3, 126.0, 122.6, 103.3, 102.8, 96.8, 59.3, 56.5, 49.9, 48.8, 44.2, 40.7, 40.5, 36.1, 31.4, 25.6, 22.0, 21.5, 21.3, 17.7 ppm; HRMS (ESI) m/z [M+H]$^+$calcd for $C_{26}H_{33}ClO_4$ 445.2140, found 445.1841.

Monomethylated merochlorin B (19). To a solution of **18** (39 mg, 0.088 mmol) in dry DCM (1.1 mL) at 0 ℃ was added 2,6-lutidine (0.036 ml, 0.307 mmol) and $Me_3SiOSO_2CF_3$ (0.048 ml, 0.2635 mmol) sequentially. The reaction mixtures kept stirring at 0 ℃ for 1 h before quenched with saturated aqueous $NaHCO_3$. The mixtures were extracted with DCM, and the combined organic phase was dried over Na_2SO_4. The solvent was removed under vacuum and the residue was further evaporated under oil pump vacuum to remove the left lutidine. The resulting enol silane was resolved in anhydrous MeCN (2.3 mL), to which a portion of $Pd(OAc)_2$ (20.58 mg, 0.092 mmol) was added. The mixtures were stirred at room temperature under N_2 atmosphere protection for 12 h, and then filtered through a pad of Celite using DCM as eluent. After removing the filtrate, the residue was purified by flash column chromatography on silica gel (EtOAc/PE = 3:1) to give **19** (19.4 mg, 50%) a a white solid, together with recovered **18** (15.6 mg, 40%). The recovered **18** could be recycled for the second time, thus the overall yield could be improved to 70% after two runs. **19**: R_f = 0.0.3 (PE/EtOAc = 1:2); IR (film): 2957, 2926, 2855, 1600, 1575, 1453, 1320, 1267, 1069, 730; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 (s, 1H), 6.59 (d, *J* = 1.4 Hz, 1H), 6.44 (d, *J* = 1.4 Hz, 1H), 5.04 (t, *J* = 6.9 Hz, 1H), 3.72 (s, 3H), 2.96-2.90 (m, 2H), 2.82 (d, *J* = 16.6 Hz, 1H), 2.64-2.60 (m, 1H), 2.52 (d, *J* = 16.6 Hz, 1H), 2.04-1.99 (m, 2H), 1.73-1.69 (m, 2H), 1.62 (s, 3H), 1.59 (s, 3H), 1.54 (s, 3H), 1.45 (s, 3H), 1.42 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.7, 173.0, 162.5, 162.3, 149.5, 132.5, 130.6, 126.1, 122.9, 111.1, 104.3, 103.1, 98.7, 96.5, 60.0, 55.7, 53.4, 49.2, 44.4, 34.9, 25.6, 22.4, 22.3, 21.4, 21.3, 17.6 ppm; HRMS (ESI) m/z [M+H]$^+$ calcd for $C_{26}H_{31}ClO_4$ 443.1984, found 443.1981.

Merochlorin B (2). To a solution of **19** (40 mg, 0.09 mmol) in DMF (0.9 mL) was added LiCl (56.7 mg, 1.35 mmol). The mixture was heated at 135 ℃ under microwave irradiation for 10 min. After cooling to room temperature, the reaction was quenched with 1 N HCl (0.1 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by flash column chromatography on silica gel (PE/EtOAc = 1:1) to give merochlorin B (**2**) (23.2 mg, 60%) as a white solid. R_f = 0.17 (PE/EtOAc = 1:1); IR (film): 2960, 2926, 2855, 1600, 1450, 1261, 1089, 1058, 1021, 798; ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.91 (s, 1H), 10.55 (s, 1H), 6.18 (d, *J* = 2.0 Hz, 1H), 6.16 (d, *J* = 2.0 Hz, 1H), 5.05 (t, *J* = 6.6 Hz, 1H), 2.99 (d, *J* = 7.6 Hz, 1H), 2.85 (d, *J* = 18.6 Hz, 1H), 2.81 (d, *J* = 18.4 Hz, 1H), 2.72 (d, *J* = 17.9 Hz, 1H), 2.47 (d, *J* = 18.3 Hz, 1H), 2.04-1.93 (m, 2H), 1.76-1.71 (m, 2H), 1.70 (s, 3H), 1.56 (s, 3H), 1.48 (s, 6H), 1.41 (s, 3H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 184.1, 176.0, 163.4, 163.3, 147.7, 131.3, 130.6, 125.5, 123.3, 105.9, 103.6, 101.0, 99.7, 97.9, 60.1, 52.0, 49.1, 43.2, 34.4, 25.4, 22.1, 21.9, 21.3, 21.2, 17.4 ppm; HRMS (ESI) m/z [M-H]$^-$ calcd for $C_{25}H_{29}ClO_4$ 427.1682, found 427.1680.

Materials for Biological Assays : *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* BAA-1695 (MRSA), *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* D39, NCTC 7466 were included in this study. MHB broth was used for the MIC determination for all the bacteria. Dimethyl sulfoxide (DMSO) was used as the solvent for all the compounds, and the final concentration in the test solution is less than 1%. What's more, DMSO at the test concentration was used as vehicle control in the determination of MIC, and vancomycin and ampicillin were also used as quality control.

Methods for Biological Assay : Minimum inhibitory concentration is the lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism under a preset condition. In this study, MIC of each compound was determined using broth microdilution methods according to guidelines described by the Clinical and Laboratory Standards Institue (CLSI). Bacteria were incubated to mid-log phase and diluted to a final concentration 106 CFU/ml according to a previously determined conversion factor between OD and CFU. Choose a suitable range of antimicrobial concentrations, for this study the range is 1 to 128 μg/mL, and then dilute the antimicrobial stock solutions to 2× concentrates relative to the final concentration in MHB (with 5% horse sheep for S. pneumoniae). Mix the bacterial culture and antimicrobial solutions in a 1:1 volume ratio, so the final bacterial concentration is 5 × 105 CFU/mL. Culture the bacteria under 37 ^{o}C for 20 to 24 hours. Check the growth of the bacterial cultures by detecting change in culture turbidity or OD at the end of incubation, MIC is between the lowest concentration without bacterial growth and the highest concentration with bacterial growth.

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