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Concise total synthesis and structure revision of metacridamides A and B†

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Concise total synthesis of metacridamides A and B was accomplished through repetitive vinylogous Mukaiyama aldol reactions and ynamide-mediated macrolactonization. Spectral data of both synthetic products were identical to those of the natural products, resulting in the revision of the absolute configuration of the C-9 position to be *S*.

Metacridamides A (**1a**) and B (**1b**) were isolated from the conidia of *Metarhizium acridum* by Krasnoff *et al.* in 2012 as cytotoxic natural products that form a 17-membered macrocycle consisting of a nonaketide chain and a Phe residue[1] (Fig. 1). Structure elucidation of **1a** has been performed by mass spectrometric and NMR spectroscopic analyses, and the absolute configuration has also been determined by X-ray single crystal analysis to be 3*S*, 8*S*, 9*R*, 12*S*, 13*S*, 16*S*, 17*R*. Notably, **1a** exhibits moderate cytotoxicity against Caco-2, MCF-7, and HepG2/C3A (IC$_{50}$ = 6.2, 11.0, and 10.8 μM, respectively), while **1b** shows cytotoxicity only against HepG2/C3A (IC$_{50}$ = 18.2 μM) of the three cancer cells. Thus, the acetyl group at the C-13 position in **1a** should be important for inducing the cytotoxic effect. However, the role of the acetyl group in the mechanism of action has been unclear, for example, an improvement of cell permeability or contribution to the binding affinity. Therefore, synthetic studies of metacridamides A (**1a**) and B (**1b**) should be an important and interesting route to achieve elucidation of the mechanism of action. We now report the first total synthesis and structure revision of metacridamides A (**1a**) and B (**1b**) by the repetitive vinylogous Mukaiyama aldol reaction and ynamide-mediated macrolactonization.

During our synthetic efforts for the metacridamides, Ghosh, S. *et al.* reported the total synthesis of the proposed structure of metacridamide B (**1b**), in which the absolute configuration of the C-9 carbon was suggested to be revised as the *S* configuration.[2] Additionally, we carefully validated the X-ray structure of metacridamide A (**1a**) (CCDC 840273†) at the beginning of our synthetic study of **1a**, resulting in the absolute configuration of the C-9 carbon that was found to be *S*. Based on these considerations, the structure of metacridamide A (**1a**) should be revised to **1a′**. Therefore, we designed our synthetic strategy for **1a′** as illustrated in Scheme 1. The desired structure of **1a′** can be synthesized by macrolactonization of the cyclization precursor **2**, which can be simply prepared by coupling the acid **4** with a phenylalanine derivative **3**. Notably, the acid **4** possesses the repeated structure as highlighted, therefore, we planned the synthesis of **4** using the repetitive vinylogous Mukaiyama aldol reaction (VMAR)[3,4] from the aldehyde **8**. Repetitive VMAR has been previously attempted for the synthesis of (+)-TMC-151C reported by Kobayashi *et al.*[5] Elongation of the polyketide chain by VMAR using an α,β-unsaturated aldehyde was problematic due to the low reactivity of the α,β-unsaturated aldehyde,[6] therefore, the reaction was investigated in the presence of excess amount of a Lewis acid. However, the decomposition of the polyketide structure was observed due to the removal of protecting groups under strong Lewis acidic conditions. According to the previous report, we designed the intermediate **5** possessing Lewis acid-tolerant protecting
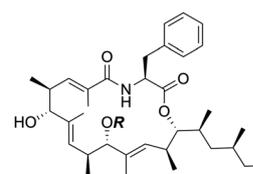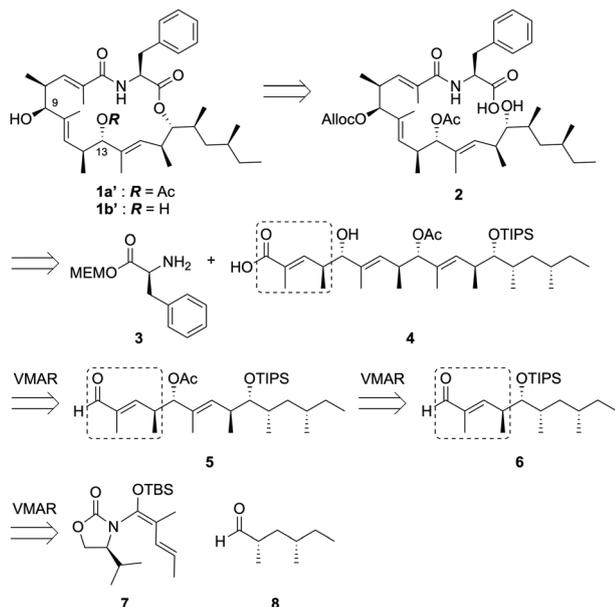
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Metacridamide A (**1a**) : ***R*** = Ac
Metacridamide B (**1b**) : ***R*** = H

Fig. 1 Reported structures of metacridamides A (**1a**) and B (**1b**).

Scheme 1 Retrosynthesis of **1a′** and **1b′**.

groups. The intermediate **5** can be prepared using the α,β-unsaturated aldehyde **6** by two repetitive VMARs with the *N,O*-ketene acetal **7** from the known aldehyde **8**. Based on the total synthesis of **1a′**, **1b′** could be directly converted by chemoselective hydrolysis of the acetyl group without cleavage of the macrolactone skeleton.

We initially synthesized the α,β-unsaturated aldehyde **6** from the commercially-available (*S*)-2-methyl-1-butanol (**9**) through the first VMAR as shown in Scheme 2. The acid **10** was prepared from the alcohol **9** according to the procedure reported by Rezanka *et al.*,[7] then the coupling of **10** with a chiral oxazolidinone **11** was performed using a mixed
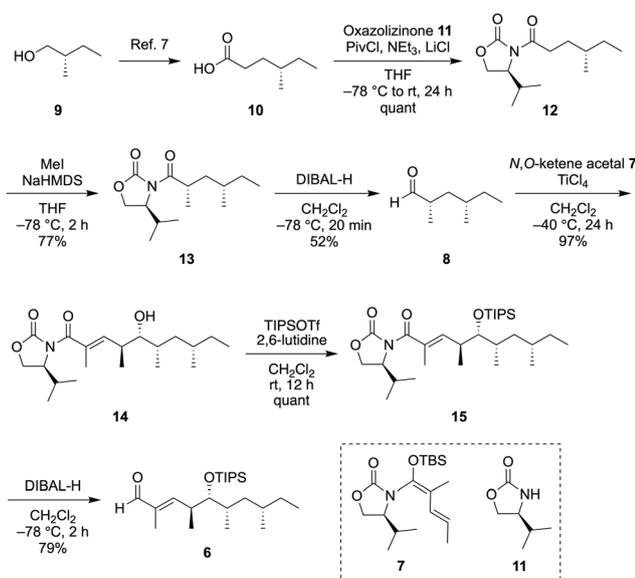
Scheme 2 Synthesis of α,β-unsaturated aldehyde **6** through the 1st VMAR.

anhydride method (PivCl/Et₃N/LiCl) to afford the acylated compound **12** in 80% yield. After diastereoselective alkylation of **12** leading to **13**, partial reduction of **13** using DIBAL-H provided the desired aldehyde **8**. Next, the first VMAR with the aldehyde **8** was attempted using TiCl₄ at −40 °C, and the desired **14** was successfully obtained in quantitative yield as a single diastereomer.[8] The resulting hydroxy group in **14** was protected by a TIPS group to provide the silyl ether **15**, which was subjected to partial reduction using DIBAL-H at −78 °C to afford the desired α,β-unsaturated aldehyde **6** in 79% yield.

The second VMAR with the α,β-unsaturated aldehyde **6** was investigated, and the details of the investigation are shown in Table 1. We initially attempted the same reaction conditions as used for the 1st VMAR with the saturated aldehyde **8**, however, the desired product **16**[8] was obtained in 14% yield along with the recovery of the aldehyde **6** (entry 1). On the other hand, the *N,O*-ketene acetal **7** was completely consumed under the stated conditions, indicating that the decomposition of **7** could be faster than the desired coupling due to the low reactivity of the α,β-unsaturated aldehyde **6**. Therefore, the reaction using an excess amount of **7** was next attempted, resulting in a slight improvement of the yield of the desired product **16** up to 25% (entry 2). In addition, an increase in the reaction temperature did not significantly improve the yield and selectivity (entry 3). To promote the first VMAR using the low reactive **6**, we next investigated the reaction in the presence of an excess amount of TiCl₄ and the *N,O*-ketene acetal **7**. As expected, VMAR of the α,β-unsaturated aldehyde **6** proceeded, but the stereoselectivity of VMAR was completely inverted to provide undesired **17** in 58% yield (entry 4). Hosokawa *et al.* previously reported that the VMAR of saturated aldehydes promoted by an excess Lewis acid selectively provided the *syn*-aldol product,[9] therefore, an equivalent of the Lewis acid should be strictly controlled to achieve the *anti*-selective VMAR with an α,β-unsaturated aldehyde. Based on the above results and further investigations,[10] we attempted the reaction using 0.5 eq. of TiCl₄ at −40 °C, resulting in an improvement of the yield of the desired **16** up to 36% (brsm 67%) along with the recovery of substrates **6** and **7** (entry 5).

Table 1 Investigation of 2nd VMAR with α,β-unsaturated aldehyde **6**

Entry	Conditions				Yield (%)	
	6 (eq.)	7 (eq.)	TiCl₄ (eq.)	Temp.(°C)	16	17
1	3.0	1.0	1.5	−40	14	0
2	1.0	0.5	1.0	−40	25	3
3	1.0	1.5	1.0	−20	26	3
4	1.0	5.0	4.0	−20	0	58[a]
5	1.0	1.5	0.5	−40	36[b]	0

[a] brsm 82%. [b] brsm 67%.
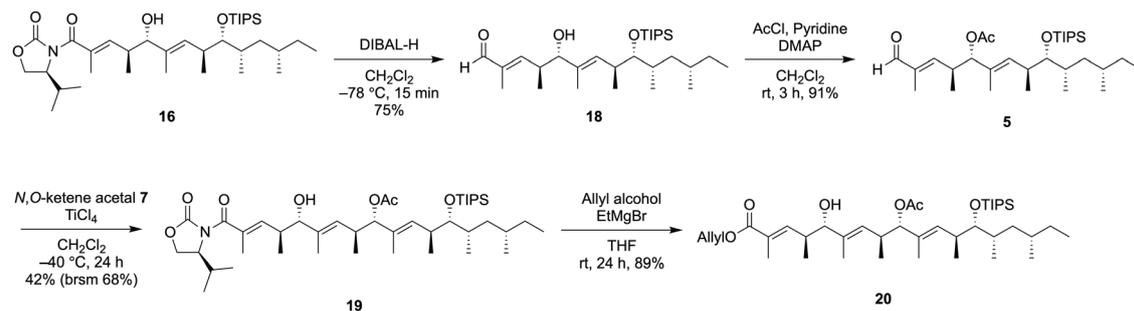
Scheme 3 Synthesis of allyl ester **20**.

We next investigated the preparation of the allyl ester **20** through the third VMAR (Scheme 3).

Reductive removal of the chiral auxiliary in **16** using DIBAL-H provided the aldehyde **18**, and the hydroxy group in the resulting **18** was protected by an acetyl group to provide **5** in 90% yield. The third VMAR with the aldehyde **5** was carried out in the same manner as the second VMAR, leading to the desired **19**[8] in 42% yield along with the recovery of aldehyde **5** (brsm 68%). Finally, the removal of the chiral auxiliary in **19** using AllyloxyMgBr provided the allyl ester **20** without losing the acetyl group.

For the construction of a macrolactone skeleton, the cyclization precursor **2** was prepared as shown in Scheme 4. Removal of the allyl group in **20** using Pd(PPh₃)₄/morpholine led to the acid **4**, which was amidated with a phenylalanine derivative **3** to afford the amide **21** in quantitative yield. The secondary hydroxy group in **21** was protected with an Alloc group to provide the amide **22**, and the removal of the MEM and TIPS groups in **22** was investigated under acidic conditions. However, simultaneous removal of both protecting groups failed using various acidic conditions due to the decomposition of **22**, therefore, stepwise deprotection was next investigated. The MEM group was readily removed using 2 M HCl/dioxane at

room temperature, and the remaining TIPS ether was successfully cleaved by treatment with 30% aq. HF/acetonitrile to provide the desired cyclization precursor **2** in 74% yield.
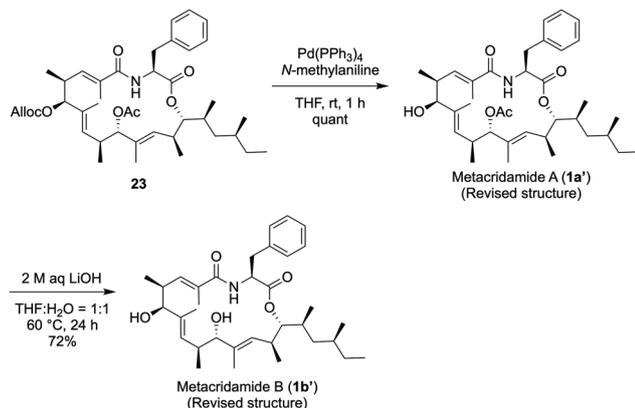
Macrolactonization of the resulting cyclization precursor **2** was next investigated, and the results are summarized in Table 2.

The reaction using 2-methyl-6-nitrobenzoic anhydride (MNBA)/DMAPO[11] was initially attempted, however, the desired macrolactone **23** was not obtained (entry 1). In addition, the macrolactonization of **2** using 2,4,6-trichlorobenzoyl chloride (TCBC)/Et₃N[12] gave the same result as already described (entry 2). Under both conditions, the oxazolone **24** was generated by intramolecular cyclization of the amide group to an activated ester, indicating that the nucleophilicity of a carbonyl moiety in the amide group should be reduced, and protonation of the corresponding carbonyl group would prevent the formation of the undesired oxazolone **24**. Recently, Zhao *et al.* reported the ynamide-mediated macrolactonization in the presence of 5 mol% TsOH·H₂O leading to the corresponding macrolactone without epimerization.[13] After the formation of a vinyl ester intermediate from **2** and *N*-methylynetoluenesulfonamide (MYTsA),[14] macrolactonization by activation using 15 mol% TsOH·H₂O successfully provided the desired macrolactone **23** in 65% yield without forming the undesired products such as the oxazolone **24** (entry 3).
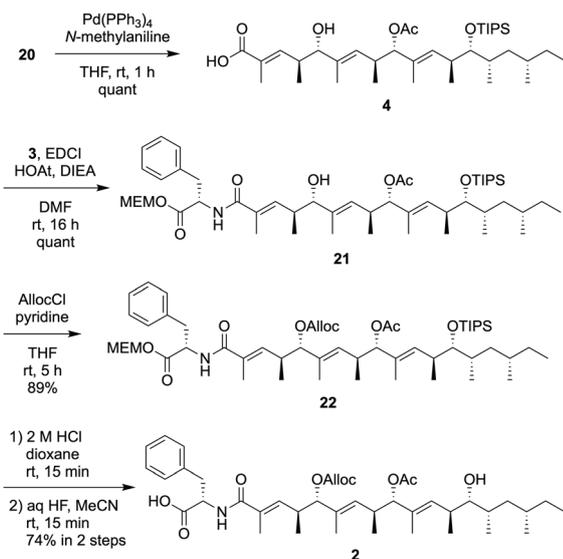
Scheme 4 Synthesis of the cyclization precursor **2**.

Table 2 Investigation of the macrolactonization of **2**

Entry	Conditions	Yield (%)	
		23	**24**
1	MNBA, DMAPO, toluene	0	42
2	TCBC, Et₃N, DMAP, toluene	0	56
3	1) (MYTsA), (CH₂)₂	65	Trace
	2) 15 mol% TsOH·H₂O, (CH₂)₂		

Scheme 5   Total synthesis of metacridamides A (**1a′**) and B (**1b′**)

Having the macrolactone **23** successfully in hand, the total synthesis of **1a′** and **1b′** was attempted as shown in Scheme 5. Removal of the Alloc group using Pd(PPh$_3$)$_4$/*N*-methylaniline smoothly proceeded to afford the desired **1a′** in quantitative yield.

In addition, direct conversion from **1a′** to **1b′** should be a challenging issue by hydrolysis without degradation of the macrolactone ring. Several investigations of the chemoselective removal of the acetyl moiety in **1a′** led us to find the best conditions (2 M aq LiOH in THF, 60 °C) to afford the desired **1b′** as the sole product. To our delight, the ^{1}H and ^{13}C NMR spectra of the synthetic **1a'** and **1b′** were identical to those of the natural products, resulting in the correction of the absolute configuration at the C-9 position to *S* as depicted in the ORTEP drawing of metacridamide A (CCDC 840273†). We evaluated the cytotoxicity of the synthetic metacridamides A and B against MCF-7 and HCT-116 cells. However, both compounds exhibited very weak cytotoxicity (>100 μM), while metacridamide A was reported to be cytotoxic at IC$_{50}$ = 11 μM against MCF-7 cells. These results indicated that impurities contained in the natural metacridamide A, for example compounds that showed signals at around 3.7 ppm observed in the reported ^{1}H NMR spectra, might induce the cytotoxic effect.

In summary, we have succeeded in the total synthesis and structure revision of metacridamides A and B. The plausible revised structures of the metacridamides were initially proposed by validation of the crystal structure of the natural products. The polyketide chain segment was successfully prepared using repetitive vinylogous Mukaiyama aldol reactions with an α,β-unsaturated aldehyde. Notably, strict control of an equivalent of TiCl$_4$ to the aldehyde was required to implement an excellent *anti*-selectivity, resulting in the successful elongation of the polyketide chain to provide the desired acid segment. Amidation with a phenylalanine derivative, followed by the stepwise removal of the MEM and TIPS groups afforded the cyclization precursor. Although the widely employed methods for macrolactonization (MNBA/DMAPO or TCBC/Et$_3$N/DMAP) were not suitable, the ynamide-mediated macrolactonization

smoothly proceeded in the presence of TsOH·H$_2$O leading to the macrolactone in an acceptable yield. The removal of the Alloc group using Pd(PPh$_3$)$_4$/*N*-methylaniline furnished the desired metacridamide A, which was subjected to a direct conversion by hydrolysis of the acetate moiety at the C-13 position to provide metacridamide B without decomposition of the macrolactone ring. The spectral data of both synthetic products were identical to those of the natural products, resulting in the revision of the absolute configuration of the C-9 position in metacridamides A and B to be *S*. The further biological studies including an evaluation of the cytotoxicity against other cancer cells will be reported in due course.

Conflicts of interest

There are no conflicts to declare.

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