Synthesis and Bioactivity of a Macrocidin B Stereoisomer

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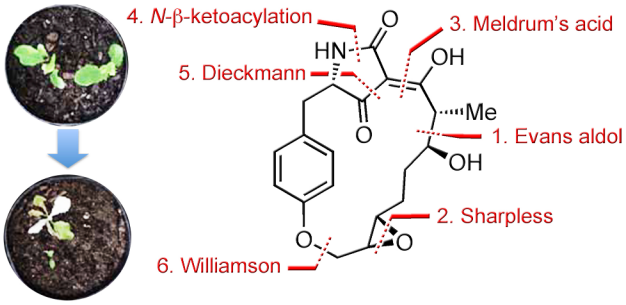


Supporting Information Placeholder

ABSTRACT: A stereoisomer of macrocidin B, a presumed metabolite of the fungus *Phoma macrostoma*, was synthesized in 18 steps and 2.7% yield from protected L-tyrosine which was *N*-β-ketoacylated with a fully functionalized octanoyl Meldrum's acid. Dieckmann condensation gave a 3-acyltetramic acid which was macrocyclized via Williamson etherification between the phenol and *epi*-bromohydrin termini. This macrocidin B stereoisomer showed a weaker herbicidal effect than macrocidin A, and no similar inhibitory effect on biofilms of *Staphylococcus aureus*.

The macrocidins A (**1**) and B (**2**) are fungal metabolites, isolated in minute quantities in 2003 by a Dow AgroSciences group from field isolates of the fungus *Phoma macrostoma* Montagne dwelling on Canada thistles.[1] Their unique structure is distinguished by an 18-membered macrolactam, comprising a 3-acyltetramic acid and a *para*-cyclophane (Fig. 1). The absolute configuration of macrocidin A was established through a single crystal X-ray structure analysis by Graupner *et al.*[1] and confirmed via two total syntheses by Pfaltz, Suzuki *et al.* in 2010[2] and our group in 2016.[3] The structure of macrocidin B remained uncertain, mainly for want of a sufficient quantity of isolate. Graupner's group assumed *S*-configurations for the stereogenic centers at C-atoms 5, 6' and 7' as in macrocidin A (**1**), leaving the configurations at C-2' and C-3' unassigned. On biosynthetic grounds, we thought the 2'*R*,3'*S*-diastereoisomer **2a**, out of the four conceivable ones, to be a likely candidate for a synthesis of natural macrocidin B, as it retains the 2'*R* configuration of macrocidin A.

The macrocidins induce growth inhibition and chlorosis, i.e. bleaching and withering, of various broadleaf weeds. They work by interfering with the electron transfer and the light harvesting complex in photosystem II and the phytoene synthase and desaturase in the biosynthesis of chlorophyll and carotenoids.[4] Due to this unique mode of herbicidal action the macrocidins are promising new crop protection leads. Quite a few, mostly non-stereoselective, syntheses of simplified derivatives and purported further *Phoma* metabolites were reported over the last decade.[5]

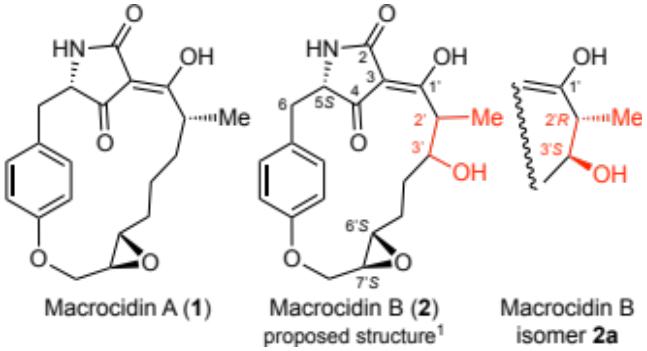
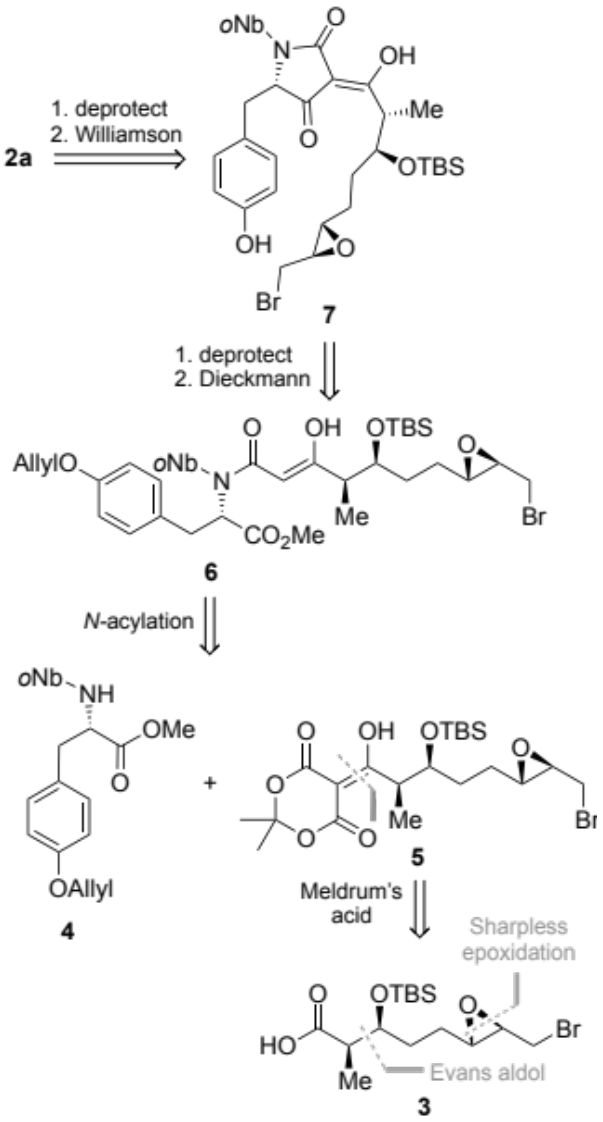


Figure 1. Structures of macrocidins A (**1**), B (**2**) and isomer **2a**

Our retrosynthetic approach to macrocidin B isomer (5*S*,2'*R*,3'*S*,6'*S*,7'*S*)-**2a** is outlined in scheme 1. It differs from our synthesis of macrocidin A,[3] where we 3-acylated a tyrosine-derived tetramic acid under Yoshii–Yoda[6,7] conditions (DMAP, NEt_3, $CaCl_2$) with a carboxylic acid identical to **3** but lacking the β-OTBS group. This reaction proceeded via a 4-*O*-acyltetramate intermediate. In an analogous reaction with carboxylic acid **3**, the corresponding 4-*O*-acyltetramate formed yet did not rearrange to the desired 3-acyltetramic acid under the same or any other conditions. So, drawing on our experience with the synthesis of F-14329,[8] a 3-acyltetramic acid carrying two stereogenic centers at C-2' and C-4', we intended to first *N*-acylate protected L-tyrosine ester **4** with a β-ketoacyl derivative **5** of carboxylic acid **3**. This can be obtained from

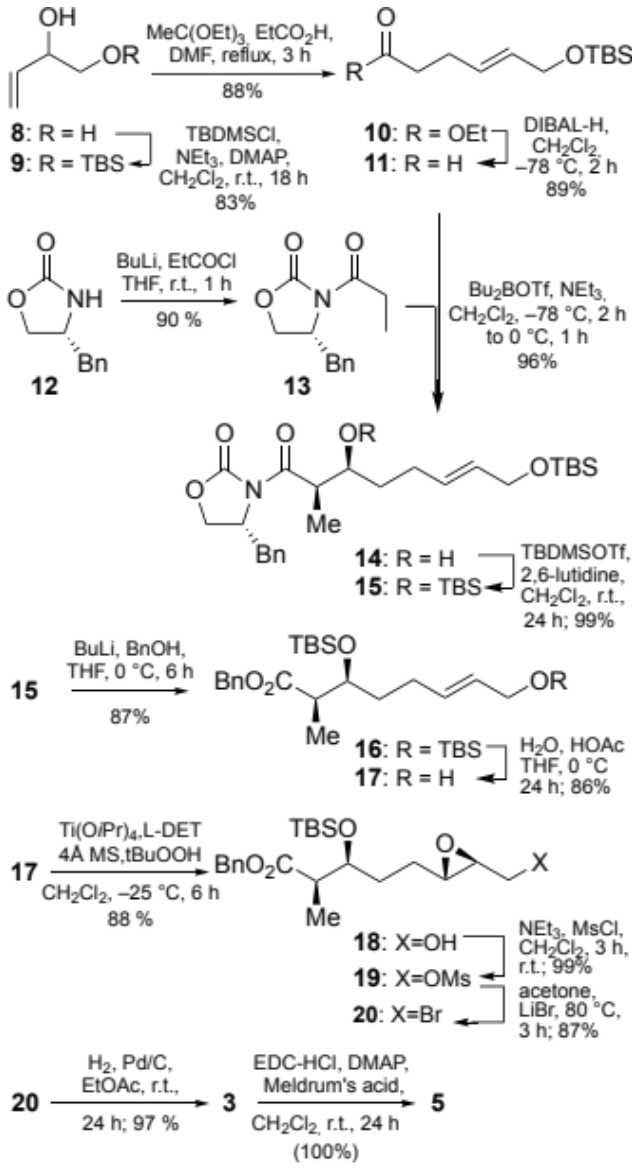
condensation with Meldrum's acid. A Dieckmann cyclization of the resulting *N*-β-ketoamide **6**, followed by deprotection of the phenolic OH-group, was to afford **7**, the immediate precursor for a ring-closing Williamson etherification. Final N,O-deprotection should give the target compound **2a**.

Scheme 1. Retrosynthesis of Macrocidin B Isomer 2a



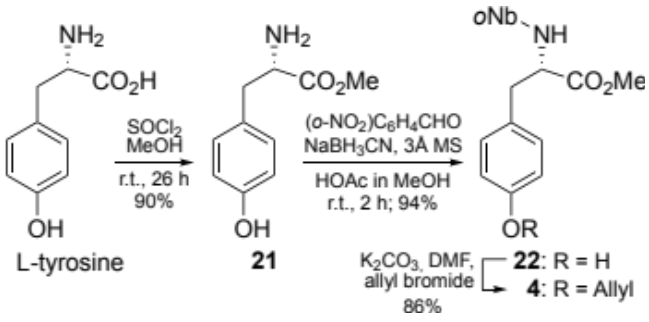
Carboxylic acid **3** was prepared starting from but-1-en-3,4-diol (**8**). Its primary hydroxy group was TBS-protected affording allylic alcohol **9**. This was reacted with triethyl orthoacetate to give ethyl hex-4-enoate **10** via an *E*-selective Johnson-Claisen rearrangement (Scheme 2). DIBAL-H reduction of **10** gave aldehyde **11**. This was submitted to a *syn*-selective aldol reaction with Evans imide **13**, prepared by propionylation of oxazolidinone **12**. The β-hydroxy group of the resulting aldol **14** was TBS-protected. This gave bis(silyl ether) **15**. Its amide bond was cleaved by treatment with LiOBn. This rendered the corresponding benzyl ester **16**. Cleavage of the primary TBS group liberated allyl alcohol **17** which was submitted to a Sharpless epoxidation furnishing alcohol **18**. This was mesylated to give **19** which, upon treatment with LiBr in acetone, afforded ω-bromoester **20**. Hydrogenolysis of the latter selectively led to carboxylic acid **3** in 34% yield related to diol **8** (11 steps) without affecting the bromide. Acid **3** was activated with EDC-HCl and then reacted with Meldrum's acid to give acylant agent **5**.

Scheme 2. Syntheses of Carboxylic Acid 3 and Acylant 5



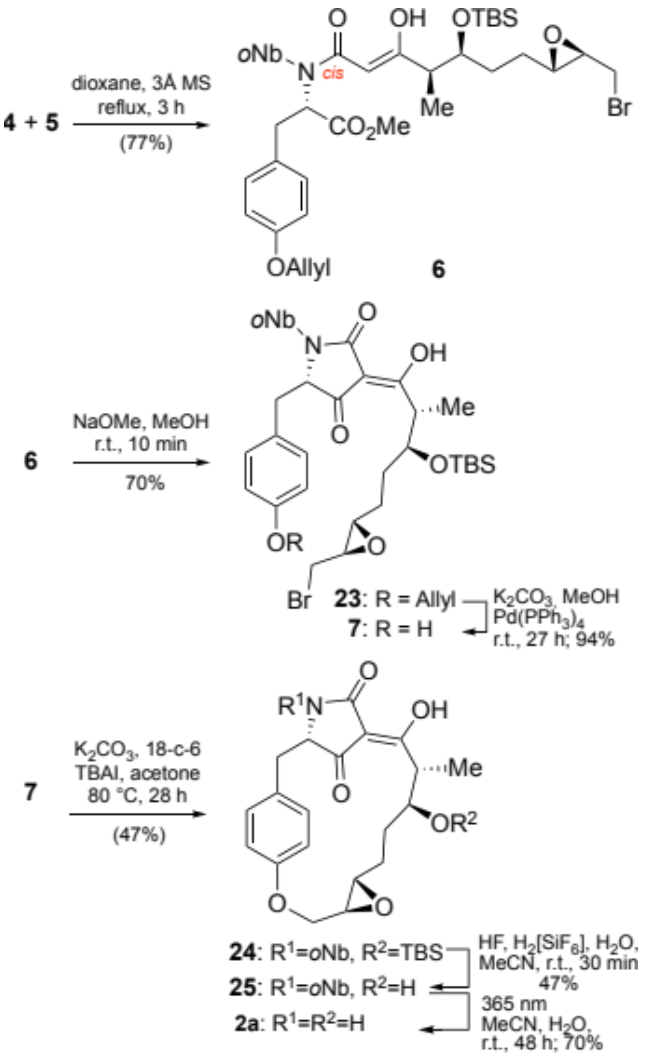
β-Ketoester **5** was then used to *N*-acylate methyl *O*-allyltyrosinate **4** carrying a photocleavable *ortho*-nitrobenzyl (*o*Nb)[9] residue on the nitrogen atom. The latter was prepared from L-tyrosine in three steps and 73% yield as shown in scheme 3. Treatment of L-tyrosine with a mixture of methanol and $SOCl_2$ afforded methyl ester **21**. Its reaction with *o*-nitrobenzaldehyde and $NaBH_3CN$ gave derivative **22** which was *O*-allylated under basic conditions affording building block **4**.

Scheme 3. Synthesis of Bisprotected Amino Ester 4



Refluxing a mixture of compounds **4** and **5** in dioxane over molecular sieve afforded *N*-β-ketoamide **6** in 77% crude yield (Scheme 4). The bulky *o*Nb group of **6** locks the amide bond in the *cis* conformation, which facilitates the subsequent Dieckmann cyclization between adjacent enolate and carboxyl carbon atoms.[2,8] Treating compound **6** with NaOMe in methanol for 10 min gave the Dieckmann condensation product, 3-acyltetramic acid **23**, in 70% yield. It was de-allylated in 94% yield to leave the ω-bromophenol **7** as immediate precursor of the intended macrocyclizing Williamson etherification. Under conditions similar to those we had already applied for the Williamson etherification leading to macrocidin A,[3] we obtained the *N*,*O*-bisprotected macrocidin B derivative **24** in 47% crude yield. Crucial for this macrocyclization to proceed in good yield is a strongly nucleophilic phenolate anion in the vicinity of an iodide. The latter was generated in situ by TBAI. We assume that the crown ether sequesters part of the potassium near the phenolate, increasing its nucleophilicity. The remainder of the potassium forms a *Z*-configured chelate complex with the 3-acyltetramic acid moiety, forcing the acyl sidechain to point toward the phenolate. Selective removal of the TBS group from **24** afforded the mono-protected macrocidin B derivative **25** in 47% yield. Photocleavage of the *o*Nb group under a UV lamp (365 nm) finally gave the target macrocidin B isomer **2a** in 70% yield and in 2.7% overall yield (18 steps).

Scheme 4. Dieckmann and Williamson Cyclizations



As some chemical shifts in the ^{1}H and ^{13}C NMR spectra of Graupner's isolate[1] and our synthetic macrocidin B stereoisomer differ significantly (*cf.* Table S1 in the Supporting Information), both compounds are in all likelihood not identical. What is more, the NMR data of the isolate are under-reported and no further analytical data, such as specific optical rotation or ECD spectra, had been provided. Thus, the structure of the natural product remains uncertain. The next-likely candidate for a synthesis of macrocidin B would be the 2'*R*,3'*R*-diastereoisomer, retaining the configuration of macrocidin A at C-2'. However, unsuccessful attempts by us to detect, let alone isolate, a macrocidin B from fermentation broths of various *Phoma macrostoma* strains even cast some doubt on it being a natural metabolite in the first place.

Concentrated *Phoma macrostoma* cultures, formulated as broadcast granules, are used as bioherbicides in the US and Canada, mainly for turfgrass and landscape management. Their efficiency was attributed to their content of macrocidins. Due to the unavailablity of larger quantities of pure macrocidins A (**1**) and B (**2**) for extensive plant tests, it remained unclear which one of the two was the better herbicide. With gram quantities of pure synthetic macrocidin A (**1**) and macrocidin B isomer **2a** in hand, we now tested their herbicidal efficacy on green-house grown dandelion and thistles. For both species, macrocidins **1** and **2a** were applied as 100 mM solu-

tions in a mixture of isopropanol/water = 1:1 + 0.25% Tween 20 to four pots with two plants each. Their bleaching, withering, and necrotising effects were assessed after two and after five weeks, and a mortality factor, i.e. the percentage of eventually dead plants, was calculated (*cf*. Supporting Information for details and pictures of treated plants). Macrocidin A (**1**) exhibited the highest maximum herbicidal efficiency, causing 100% mortality of dandelions and 100% of thistles, five weeks after application (Figure 2). Macrocidin B isomer **2a** led to a mortality of 75% in dandelion and 38% in thistles under identical conditions. It should be noted that a macrocidin Z (**26**), featuring an *E*-alkene instead of the epoxide of macrocidin A, was recently isolated from *Phoma macrostoma* cultures and also synthesized.[10] It was shown by us to be less herbicidal than macrocidin A (**1**), yet with a selectivity for thistles over dandelion.[11] In contrast, as we now found, macrocidin B isomer **2a** is more active in dandelions, where it outperforms macrocidin Z, than in thistles.

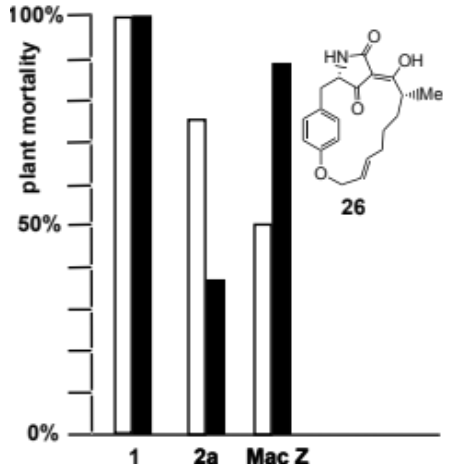


Figure 2. Percentage of final mortality of dandelions (white columns) and thistles (black columns) five weeks after treatment with 0.2 mL/plant of 100 mM solutions of synthetic macrocidins.

The synthetic macrocidin B isomer **2a** was also evaluated for other biological activities. Unlike macrocidins A and Z,[10] and other simplified synthetic macrocidin analogues,[11] our macrocidin B isomer had no inhibitory effect on the growth of *Staphylococcus aureus* biofilms, even when applied in concentrations as high as 250 µg/mL. More in line with other macrocidin derivatives, macrocidin B isomer **2a** exhibited no cytotoxicity against human cancer cells or mouse fibroblasts, and no antibiotic effect on a broad panel of microorganisms (*cf.* Supporting Information for details).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: xxx.

Experimental details of chemical syntheses; characterization of new compounds; NMR spectra and HPLC chromatograms; herbicidal effects on pot plants; assays for cytotoxicity, antibiofilm and antimicrobial effects.

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Notes

The authors declare no competing financial interest.

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