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# Total Synthesis of Thaxtomin A and Its Stereoisomers and Findings of Their Biological Activities

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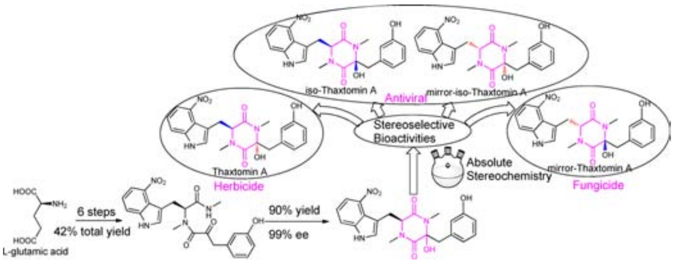
## ABSTRACT



**The first and facile total synthesis of thaxtomin A and its three stereoisomers has been achieved. The synthetic approach involves intramolecular nucleophilic cyclization of an amide toward a ketoamide group to produce a *C*-hydroxydiketopiperazine scaffold. The most amazing discovery was that each of the four stereoisomers of TA exhibits different phytotoxic, fungicidal, and antiviral activities.**

The growing need for new pesticides with new molecular target sites and the desire for "greener" pest management chemicals is increasingly the focus of interest in natural products.[1] Thaxtomins are a unique family of phytotoxins generated as microbial metabolites possessing a unique 4-nitroindole moiety.[2] Thaxtomin A (TA), the most predominant and active member of such a family, was initially isolated in 1989.[3] TA attracted extensive attention due to its established role as a virulence factor in the common scab potato disease and its new herbicidal mode of action.[4,5] Recent research indicated that TA is a green and safe herbicide to rice[6] and also may be used as a selective agent to elevate the overall levels of common scab resistance in the potato breeding populations.[7] The most distinguish

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structural feature of TA is the *C*-hydroxydiketopiperazine scaffold. In fact, such a scaffold exists in several classes of natural products (Figure 1).[8] Thus, a facile synthetic approach to produce the *C*-hydroxydiketopiperazine scaffold is very desirable for total synthesis and further structural modification of these natural products. Furthermore, the chemical synthesis may shed light on how the stereochemical properties of chiral pesticide TA have played a role in its biological activities, which has never been answered previously.

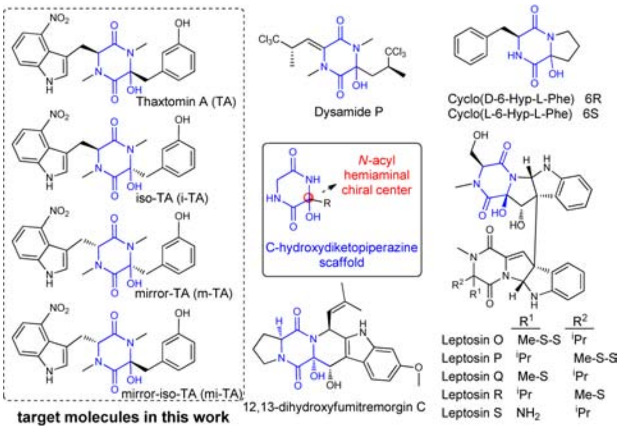
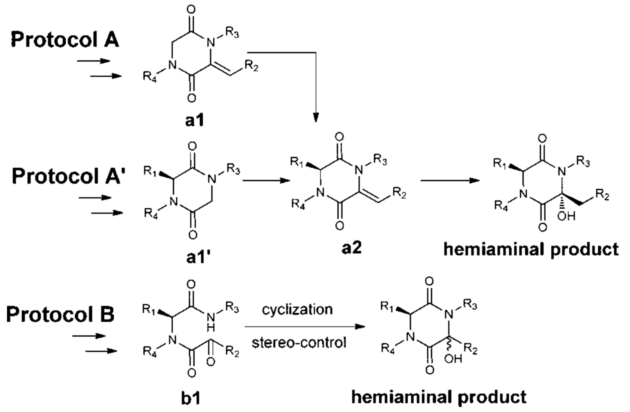


**Figure 1.** Typical natural products containing *C*-hydroxydiketopiperazine.[8]

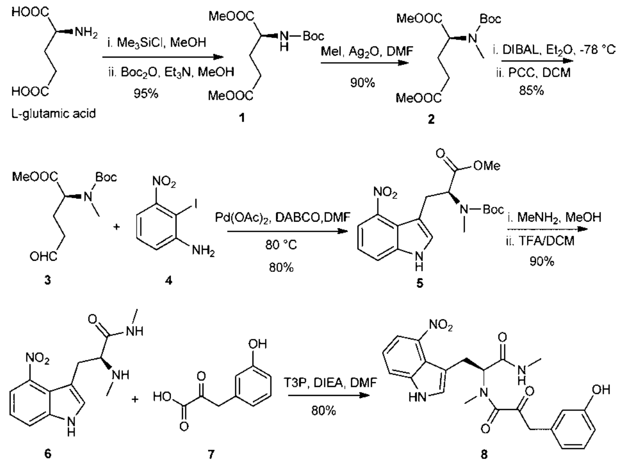
The total synthesis of TA has not been achieved. This might be attributed to the challenge of stereocontrolled formation of the *C*-hydroxydiketopiperazine.[9] When constructing a 2,5-diketopiperazine scaffold bearing an *N*-acyl hemiaminal chiral center which was observed in many nature products,[8] there were two established protocols as shown in Scheme 1. Protocol A or A′ features the dehydrated intermediate **a2**. However, when applying this protocol for the synthesis of TA, considering the incompatibility of the nitro group toward most of the reduction manipulations, and the high epimerization risk of the hemiaminal chiral center in basic or acidic media, it is difficult to find proper protecting groups, which are not only suitable for the preparation of **a2** from **a1** or **a1′** but also orthogonal in the final global deprotection. Such restrictions might well explain why only the synthesis of some structurally simplified TA derivatives rather than TA itself were accomplished to date.[9a,b] Protocol B provided hemiaminal product via intramolecular nucleophilic cyclization of an amide toward a ketoamide group. Despite of the scattering appearance of protocol B in literature,[10] it has never been well studied, especially on factors affecting the cyclization and stereochemistry.

**Scheme 1.** Protocols for Constructing *C*-Hydroxydiketopiperazine[9,10]



In this work, we report the first total synthesis of TA and its three stereoisomers through protocol B (Scheme 1) by investigating the cyclization reaction and its stereoselectivity in great detail. Moreover, the most amazing discovery was that each stereoisomer of TA exhibits different bioactivities, indicating the versatile role of *C*-hydroxydiketopiperazine scaffold for developing new biologically active chemicals.

**Scheme 2.** Synthesis of Compound **8**



Our synthesis started with the known dimethyl *N*-Boc-glutamate **1**[11] which was readily prepared from L-glutamic acid in two steps. Then, the N-methylation of **1** with MeI and Ag$_2$O in DMF produced **2** in a yield of 90%. The diester **2** was reduced by DIBAL-H, followed by the

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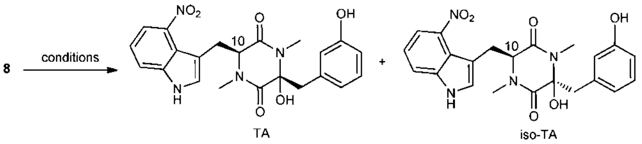
**Table 1.** Explorations on the Intramolecular Nucleophilic Cyclization



| conditions | results | conditions | conversion (%) | C-10 de[a] (%) |
|---|---|---|---|---|
| $CF_3COOH$, THF or MeOH | no reaction (–) | LiOH, MeOH | 100 (yield: 90%) | 67 |
| $CH_3COOH$, THF or MeOH | – | DABCO, MeOH | <5 | 90 |
| TsOH, THF or MeOH | – | DIEA, MeOH | <5 | 93 |
| LiCl, THF or MeOH | – | $Et_3N$, MeOH | <5 | 99 |
| $ZnCl_2$, THF or MeOH | – | $Et_3N$, LiCl, MeOH | <5 | 99 |
| $FeCl_3$, THF or MeOH | – | $Et_3N$, $ZnCl_2$, MeOH | <5 | 99 |
| $CuCl_2$, THF or MeOH | – | $Et_3N$, $FeCl_3$, MeOH | <5 | 99 |
| $AlCl_3$, THF or MeOH | – | $Et_3N$, $CuCl_2$, MeOH | <5 | 99 |
| $MgBr_2 \cdot Et_2O$, THF or MeOH | – | $Et_3N$, $AlCl_3$, MeOH | <5 | 99 |
| NaH, THF | – | $Et_3N$, $MgBr_2 \cdot Et_2O$, MeOH | 100 (yield: 90%) | 99 |

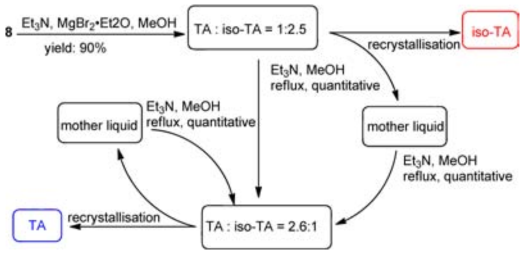
[a] C-10 de = (TA − m-TA)/(TA + mTA).

treatment with PCC to give aldehyde **3** in 85% yield. Direct coupling of aldehyde **3** with 3-nitro-2-iodoaniline **4**[12] under standard Pd-catalyzed indole synthesis condition provided the 4-nitrotryptophan derivative **5** in 80% yield. By using methylamine, the ester moiety was converted into the secondary amide, which was then deprotected by trifluoroacetic acid, the 4-nitrotryptophan derivatives **6** could be obtained in a 90% yield over these two steps. After extensive exploration, the fragment arylpyruvic acid **7**[13] was introduced to the specific secondary amide with the coupling agent T3P in 80% yield (Scheme 2).

With the product **8** in hand, the next task was to achieve the intramolecular cyclization, a well-recognized challenge to achieve. First, to avoid the epimerization of C-10, we have screened a variety of acids to catalyze such transformation, but such acid conditions did not catalyze the reaction. Second, although the C-hydroxydiketopiperazine was constructed under inorganic base condition, the epimerization of C-10 occurred, and the C-10 de values were all below 70%. Third, some organic bases were used to catalyze this reaction. Luckily, these did result in a significance increase in C-10 de values ($>$90%). Especially when treatment of compound **8** with $Et_3N$ in methanol, the C-10 de value was up to 99% with a ratio of TA/iso-TA (i-TA) of 2.6:1. However, the reaction conversion was only 5% under this condition. To increase the reaction conversion rate while maintaining high stereoselectivity, we screened a variety of Lewis acid into the $Et_3N$/MeOH reaction system. Ultimately, it was found that the treatment of compound **8** with $Et_3N/MgBr_2 \cdot Et_2O$ in Methanol under room temperature for 7 days could produce TA and its C-12 epimer i-TA in 90% yield with a ratio of TA/i-TA of 1:2.5 by $^1$H NMR analysis. The C-10 de value of TA is 99% (Table 1).

Although this represented a truly significant increase in synthetic efficiency for TA, the diastereoselectivity is not satisfactory. It was later found that the mixture of diastereoisomers from treatment with $E_3N$ in MeOH could reach an equilibrium state quantitatively and without C-10 de lost. The ratio of TA/i-TA was 2.6:1. After recrystallization from methanol, pure TA was obtained. Treatment of mother liquid with $E_3N$/MeOH converse the ratio of TA/i-TA to 2.6:1 again. Thus, this method may allow facile large-scale access to TA (Scheme 3).

**Scheme 3.** Preparation of TA and Iso-TA



The structure of TA and i-TA were confirmed by single-crystal X-ray analysis (Figure 2). The $^1$H NMR and $^{13}$C NMR spectra of synthetic TA were in good agreement with those of authentic samples.[3] Through the same synthetic route, mirror-TA (m-TA) and mirror-iso-TA (mi-TA) were synthesized using D-glutamic acid to replace L-glutamic acid as the starting material showed in Scheme 2.

Phytotoxic activity, fungicidal activity, and antiviral activity of these four TA stereoisomers were evaluated using reported procedures.[14] Generally, stereoisomers of chiral pesticides may differ with respect to biological

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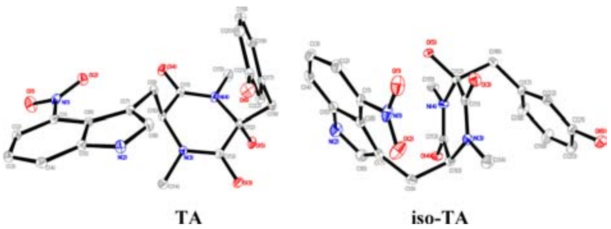

**Figure 2.** X-ray of TA and iso-TA.

activity, plant metabolism, environmental fate, and ecotoxicity.[15] It has been previously shown that the 10*S*,12*S* epimer of TA has reduced phytotoxic activity.[16] Our bioassay result showed that among four stereoisomers, only natural product TA with the 10*S*,12R configuration showed obvious growth inhibitory activity on dicotyledon weeds *B. campestris* and *A. retroflexus* (dichlobenil, a commercial herbicide, was used as a control, Table 2). This absolute stereoselectivity was also important in the antifungi activity. All four stereoisomers were tested in vitro against *Cercospora arachidicola* and *Physalospora piricola*. Only TA's enantiomer m-TA showed excellent activity, while the other three stereoisomers have weak or no inhibitory activity (Table 3). Furthermore, the fungicidal activities for m-TA and commercial fungicide chlorothalonil were compared. The $EC_{50}$ values of m-TA against *Cercospora arachidicola* and *Physalospora piricola* were 8.52 and 1.57 $\mu$g mL$^{-1}$, respectively, while the corresponding values for chlorothalonil were 8.64 and 7.33 $\mu$g mL$^{-1}$, respectively, indicating that m-TA has similar anti-*Cercospora arachidicola* activity as chlorothalonil and obviously better anti-*Physalospora piricola* activity than chlorothalonil. For our antiviral activity investigation, anti-tobacco mosaic virus (TMV) activities of these four TA stereoisomers were compared with a benchmark antiviral drug ribavirin. The assay results in Table 4 showed that i-TA displayed higher activity than ribavirin and mi-TA exhibited the same level of activity as ribavirin, while TA and m-TA did not exhibit inhibitory activity at all. These results showed that stereochemistry plays a determining role on their antiviral activities.

**Table 2.** Herbicidal Activity of Compounds (Percent Inhibition) (Rate = 1500g/ha)[a]

| | BC | | AR | | EC | | DS | |
|---|---|---|---|---|---|---|---|---|
| compd | pre | post | pre | post | pre | post | pre | post |
| TA | 25.0 | 80.0 | 30.0 | 70.0 | 40.0 | 30.0 | 45.0 | 10.0 |
| i-TA | 20.0 | 10.0 | 10.0 | 15.0 | 10.0 | 15.0 | 0 | 15.0 |
| m-TA | 10.0 | 10.0 | 10.0 | 5.0 | 10.0 | 0 | 0 | 10.0 |
| mi-TA | 0 | 5.0 | 10.0 | 15.0 | 0 | 0 | 0 | 10.0 |
| dichlobenil | 100 | 20.0 | 100 | 5.0 | 95.4 | 0 | 64.9 | 0 |

[a] BC = *B. campestris*, AR = *A. retroflexus*, EC = *E. crusgalli*, DS = *D. sanguinalis*; post = postemergence; pre = pre-emergence. All treatments were performed in triplicate.

**Table 3.** Fungicidal Activity of Compounds at a Dosage of 50 $\mu$g/mL (Percent Inhibition)[a]

| fungi | TA | i-TA | m-TA | mi-TA | CH |
|---|---|---|---|---|---|
| CA | 0 | 7.1 ± 0.6 | 78.6 ± 1.6 | 0 | 78.0 |
| PP | 43.9 ± 1.7 | 24.4 ± 0.6 | 96.0 ± 2.0 | 24.4 ± 1.3 | 89.0 |

[a] CA = *Cercospora arachidicola*, PP = *Physalospora piricola*, CH = *chlorothalonil*. Values are mean ± SD ($n = 3$).

**Table 4.** Antiviral Activity of Compounds against TMV at 500 $\mu$g/mL (Percent Inhibition)

| compd | inactivation efect | curative efect | protection efect |
|---|---|---|---|
| TA | 10.4 | 0 | 8.6 |
| i-TA | 42.3 | 38.9 | 44.2 |
| m-TA | 6.4 | 0 | 0 |
| mi-TA | 34.8 | 36.1 | 40.6 |
| ribavirin | 37.6 | 38.4 | 38.2 |

In conclusion, natural product TA and its three stereoisomers have been synthesized for the first time. A concerted catalytic condition using $Et_3N/MgBr_2 \cdot Et_2O$ has been developed to implement the construction of a *C*-hydroxydiketopiperazine scaffold by an intramolecular nucleophilic addition strategy. The convenient purification procedure of TA from diastereoisomeric mixtures and the conversion of i-TA into TA in situ make large scale production possible. The biological test results indicated that natural product TA has obvious growth inhibitory activity on dicotyledon weeds *B. campestris* and *A. retroflexus*, m-TA has simmilar antifungi activity as fungicide chlorothalonil, i-TA has higher anti-TMV activity than antiviral drug ribavirin, and mi-TA exhibited the same level of activity as ribavirin. The strict stereoselective properties of the four stereoisomers indicated that the *C*-hydroxydiketopiperazine scaffold represents a rich resource for developing new biologically active chemicals.

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**Supporting Information Available.** Experimental details, characterization data, $^1$H and $^{13}$C NMR spectra of new compounds, and X-ray data for TA and iso-TA (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.