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Syntheses and Biological Evaluation of Irciniastatin A and the C1−C2 Alkyne Analogue

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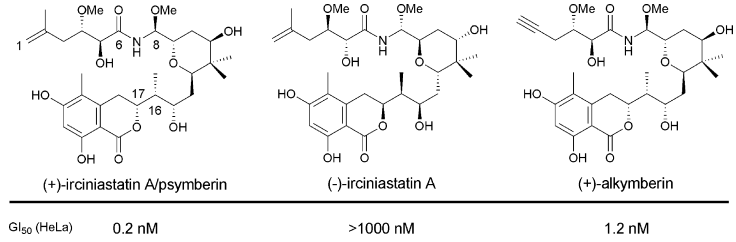
ABSTRACT



Syntheses of both natural (+)- and unnatural (−)-irciniastatin A (aka psymberin) as well as a C1−C2 alkyne analogue of (+)-irciniastatin A have been achieved. The key features of the syntheses include a highly regioselective epoxide-opening reaction and a late-stage assembly of C1−C6, C8−C16, and C17−C25 fragments. (+)-Alkymberin retained a high level of cytotoxicity, whereas (−)-irciniastatin A showed almost no activity. These results suggest that (+)-alkymberin could be a useful enantio-differential probe for mode-of-action study.

In 2004, (+)-irciniastatin A (**1**)[1] and psymberin,[2] new pederin-type natural products, were isolated by the Pettit group from marine sponge *Ircinia ramosa* and by the Crews group from marine sponge *Psammocinia* sp. (Figure 1). In addition to (+)-irciniastatin A (**1**), the Pettit group also isolated the C11 ketone analogue, named (−)-irciniastatin B. (+)-Irciniastatin A (**1**) has been shown to exhibit extremely potent and selective cytotoxicity against certain human cancer cell lines.[1,2]

The promising therapeutic potential coupled with the limited availability of these natural products has attracted significant attention from the synthetic community. In 2005, the first total synthesis of (+)-psymberin was achieved by the De Brabander group, who demonstrated that (+)-irciniastatin A and (+)-psymberin are identical, as repre-

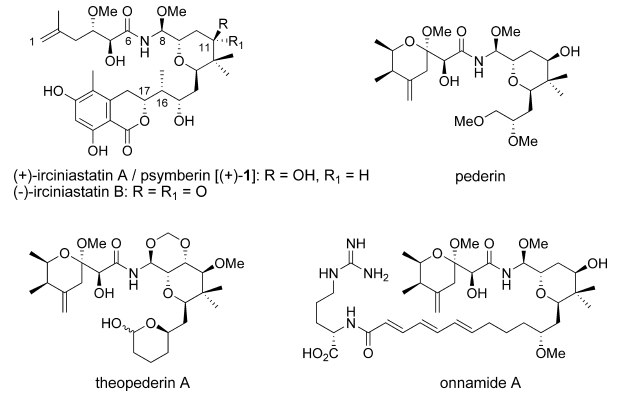


Figure 1. Pederin-type natural products.

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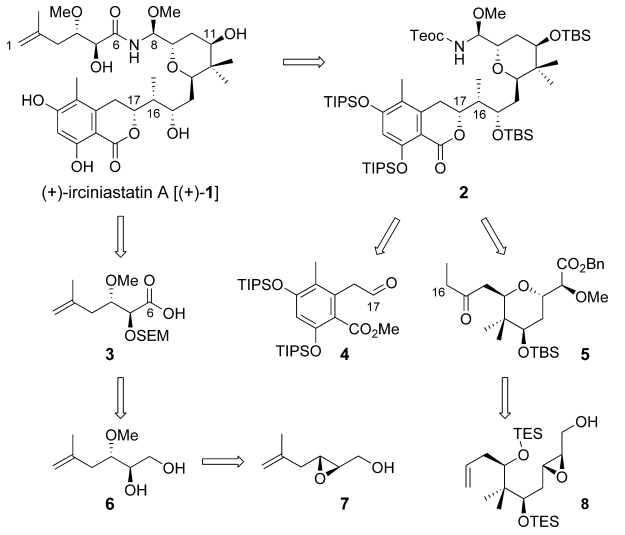
sented by (+)-**1**.[3] To date, several total[4−6] and formal[7] syntheses as well as SAR studies[8,9] have been reported. Among them, the Schering-Plough group has reported that the substituents at C4 and C5 are important for the cytotoxicity, and that the C1−C2 double bond is not essential for activity.[9] Also, preliminary biological studies using natural and synthetic samples have suggested that (+)-irciniastatin A (**1**) might have a different mode-of-action from that of other pederin family members (Figure 1).[2,8]

Intrigued by these results, we, several years ago, started a synthetic program that would enable acquisition of possible isomers and analogues. As a part of this program, we herein describe the syntheses and biological evaluation of both enantiomers of irciniastatin A (**1**), and (+)-"alkymberin", a C1−C2 alkyne analogue of natural (+)-**1**.

Retrosynthetically, (+)-irciniastatin A (**1**) was divided into the C1−C6 acyclic side chain **3** and protected hemiaminal **2**, which in turn disconnected in a retro-aldol fashion into C17−C25 aldehyde fragment **4** and C8−C16 tetrahydropyran fragment **5** (Scheme 1). It should be noted that intermediates **3**

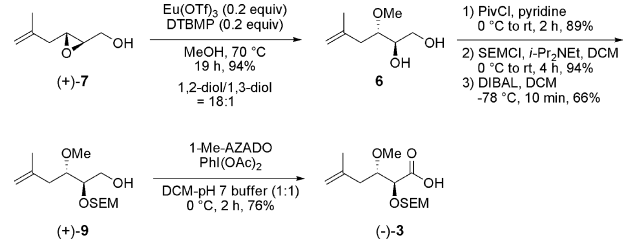
Scheme 1. Retrosynthetic Analysis of (+)-Irciniastatin A (**1**)



and **5** could be derived from epoxy alcohols **7** and **8**, respectively. To synthesize not only fragments **3** and **5** but also their isomers for SAR study, we planned to utilize a Sharpless asymmetric epoxidation (SAE) chemistry[10] as a key reaction. For example, enantiomers and diastereomers of **6** could be synthesized by the regioselective ring-opening of those of epoxy alcohol **7**, which could be easily obtained using an SAE strategy.

The synthesis of **3** commenced with regioselective ring-opening of known epoxy alcohol (+)-**7**[11] with MeOH (Scheme 2). Initially, we tried Sharpless condition using Ti(O*i*Pr)$_4$ as

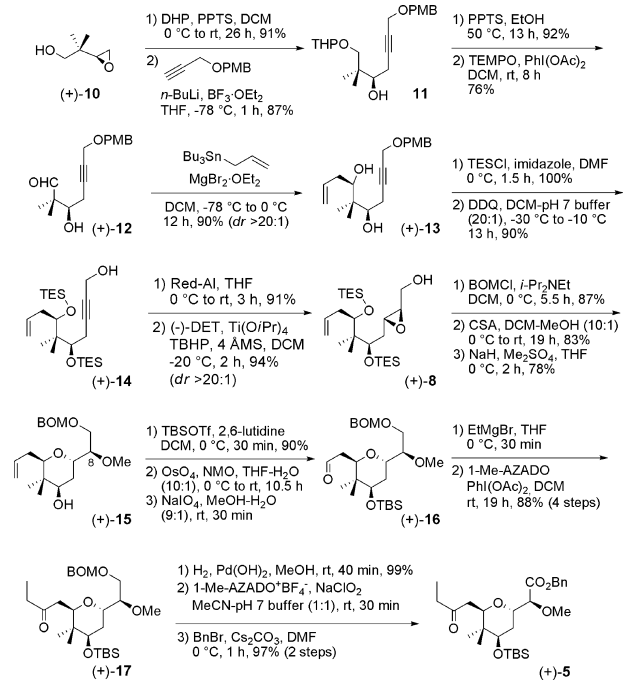
Scheme 2. Synthesis of the Acyclic Side Chain (−)-**3**



Lewis acid,[12] but the yield and selectivity were unsatisfactory (54%, 1,2-diol:1,3-diol = 3:1). To improve this situation, we screened various Lewis acids (BF_3•OEt_2, $MgBr_2$ etc.) and finally found that Eu(OTf)$_3$ gave the desired 1,2-diol **6**, which is inseparable from the corresponding 1,3-diol, in high yield and selectivity (>20:1). The loading of Eu(OTf)$_3$ could be reduced to a catalytic amount when it was used with 0.2 equiv of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP), giving comparable selectivity (18:1).[13] The obtained 1,2-diol **6** was converted via the usual three steps to the primary alcohol (+)-**9**, which was oxidized using 1-Me-AZADO[14] to furnish carboxylic acid (−)-**3**.

The synthesis of **5** began with a known epoxy alcohol (+)-**10**,[15] which was derived from commercially available (−)-pantolactone (Scheme 3). After protection of the primary

Scheme 3. Synthesis of C8−C16 Fragment (+)-**5**



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hydroxyl group in (+)-**10**, the epoxide was opened regioselectively with lithium acetylide and $BF_3{\cdot}OEt_2$[16] to give alcohol **11**. Deprotection and subsequent TEMPO oxidation afforded aldehyde (+)-**12**.

We then focused on the diastereoselective allylation of (+)-**12**. After extensive experimentation, we found that the reaction using allyltributylstannane and $MgBr_2$ proceeded in a highly diastereoselective manner to give diol (+)-**13**.[17] Protecting group manipulation and *trans*- reduction of the alkyne moiety provided the corresponding allyl alcohol, which was then subjected to SAE to give (+)-**8** in 94% yield and >20:1 diastereoselectivity. After protection of the primary hydroxyl group, treatment of the epoxide with CSA in $MeOH-CH_2Cl_2$ effected the deprotection of two TES groups and formation of the desired tetrahydropyran core.

Selective methylation of the C8-hydroxyl group was accomplished using Me_2SO_4 to give methyl ether (+)-**15** in 78% yield. Silylation of the remaining secondary alcohol in (+)-**15** followed by oxidative cleavage of the terminal olefin (OsO_4, NMO; $NaIO_4$) provided the corresponding aldehyde **16**. Treatment of crude **16** with ethylmagnesium bromide followed by oxidation of the resultant secondary alcohol using 1-Me-AZADO gave ketone (+)-**17** in 88% for 4 steps. Finally, cleavage of BOM ether, oxidation of the resultant alcohol using 1-Me-AZADO$^+BF_4^-$/$NaClO_2$,[18] and protection provided benzyl ester (+)-**5** in high yield.

Aldehyde **4** was prepared based on De Brabander's protocol.[3] The union of **4** with (+)-**5** was achieved by mixing the *Z*-boron enolate of (+)-**5** with aldehyde **4** at −78 °C to give the aldol product (+)-**18** in a highly diastereoselective manner (Scheme 4).[19] Reduction of (+)-**18** with $NaBH_4$ in the presence of Et_3B in MeOH provided the 1,3-*syn* diol (dr >20:1),[20] which was converted to lactone (+)-**19** in 78% yield in 2 steps. Hydrogenolysis of benzyl ester followed by a Curtius sequence using 2-(trimethylsilyl)ethanol as a nucleophile gave Teoc-protected hemiaminal (+)-**2** in high yield.[21,22]

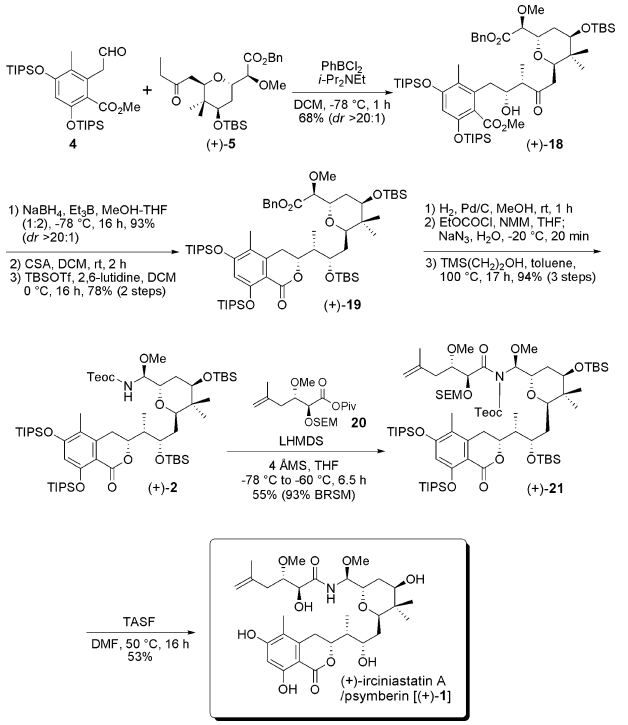
Coupling of (+)-**2** with the acyclic side chain fragment proved to be a difficult task. Initially, we examined the coupling reaction of (+)-**2** with several derivatives of the carboxylic acid (−)-**3**, which never yielded the desired product. After intensive effort, we realized that Teoc-protected hemiaminal (+)-**2** and pivaloate **20** were most suitable for this coupling reaction.[5] Finally, global deprotection using TASF provided (+)-irciniastatin A (**1**).

With a highly convergent and flexible route to access (+)-irciniastatin A (**1**) in hand, we then synthesized "alkymberin" (**29**), which bears an alkyne moiety at the C1−C2 position. As described, the C1−C2 olefin moiety has been reported to be unnecessary for cytotoxicity,[9] and the alkyne part is expected to be a useful handle for introducing several reporter tags using click chemistry.[23] Moreover, we decided to synthesize (−)-irciniastatin A (**1**) to examine whether irciniastatin A acts as a "ligand" or "chemical reagent" in cells. For example, the acyl aminal at C8 could be a good electrophilic reagent (i.e., acylimine) when the methoxy group at C8 was eliminated.

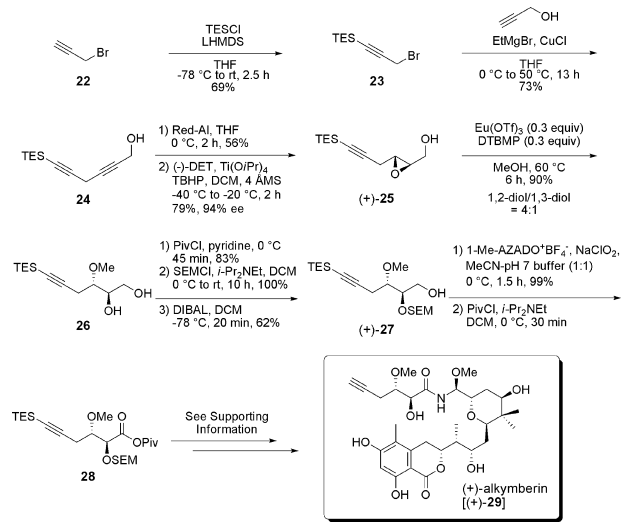
For the synthesis of (+)-alkymberin, we prepared alkyne side chain **28** based on the synthetic route previously established (Scheme 5). In the course of the synthesis, we found that the 1-Me-AZADO$^+BF_4^-$/$NaClO_2$ system was more effective for the oxidation of alcohol (+)-**27**. The resultant carboxylic acid was activated as mixed anhydride **28**, which was successfully coupled with (+)-**2** to furnish (+)-alkymberin [(+)-**29**].

To synthesize (−)-irciniastatin A (**1**), each enantiomer of the C1−C6 acyclic side chain [i.e., *ent*-**20**] and the C8−C16

Scheme 4. Total Synthesis of (+)-Irciniastatin A (**1**)

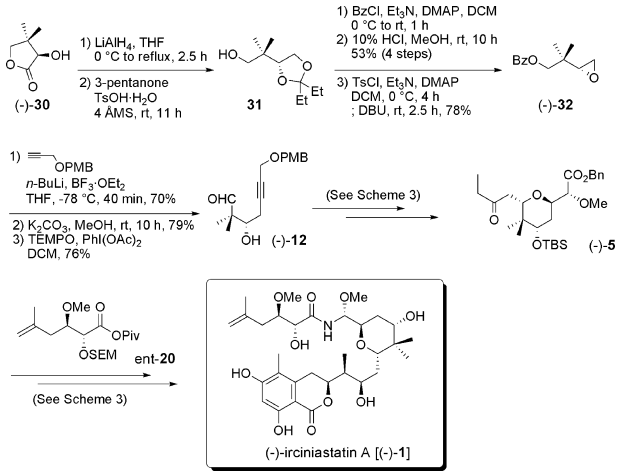


Scheme 5. Synthesis of (+)-Alkymberin [(+)-**29**]



tetrahydropyran fragment [(−)-**5**] is needed. The C1−C6 acyclic side chain *ent*-**20** was prepared using (+)-DET by SAE in the established route in Scheme 2. The C8−C16 tetrahydropyran fragment (−)-**5** was synthesized from aldehyde (−)-**12**, which was prepared in 8 steps from (−)-pantolactone (**30**) (Scheme 6).

Scheme 6. Synthesis of (−)-Irciniatstain A (**1**)



Synthetic (+)- and (−)-irciniastatin A (**1**) and (+)-alkymberin (**29**) were evaluated for their cytotoxicity against HeLa cells. As expected, (+)-alkymberin (**29**) retained a high level of cytotoxic activity [GI_{50} value of 1.2 nM for (+)-**1**, 0.2 nM for (+)-**29**]. In contrast, (−)-irciniastatin A (**1**) showed almost no cytotoxic activity (GI_{50} > 1000 nM). These results indicated that an enantio-differential recognition event occurs between (+)-irciniastatin A (**1**) and its cellular target; as such, (+)-alkymberin [(+)-**29**] is a good candidate for an enantio-differential probe[24] for mode-of-action study.[19]

In summary, we have accomplished syntheses of (+)- and (−)-irciniastatin A (**1**), as well as (+)-alkymberin (**29**), via a convergent synthetic route. Biological evaluation of these compounds suggested that (+)-alkymberin (**29**) can be a good enantio-differential probe for analyzing mode-of-action of (+)-irciniastatin A (**1**). Further studies on both SAR and the mode-of-action of irciniastatins are now in progress, and results will be reported in the near future.

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Supporting Information Available: Experimental procedures, characterization data, and copy of NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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