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

Tsubasa Watanabe,[†] Takamichi Imaizumi,[†] Takumi Chinen,[‡] Yoko Nagumo,[‡] Masatoshi Shibuya,[†] Takeo Usui,[‡] Naoki Kanoh,[†] and Yoshiharu Iwabuchi*[,†]

Department of Organic Chemistry, Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba, Sendai 980-8578, Japan, and Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai, Tsukuba 305-8572, Japan

iwabuchi@mail.pharm.tohoku.ac.jp

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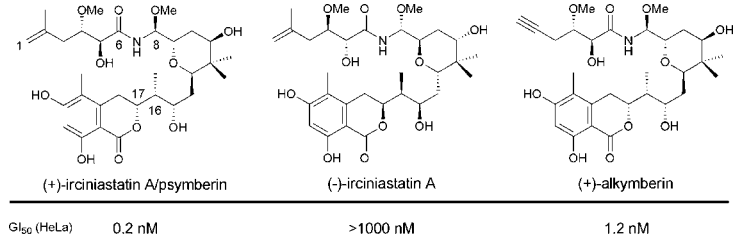
ABSTRACT



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-

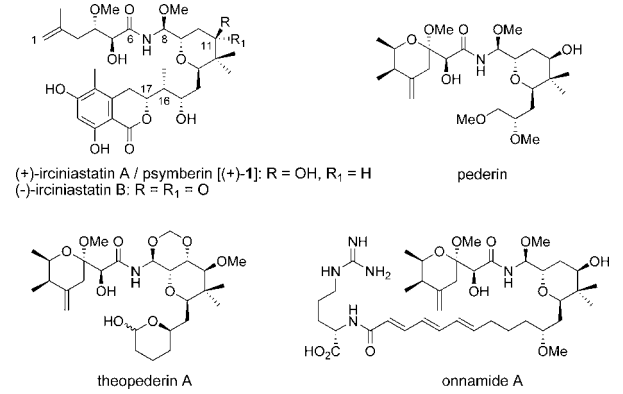


Figure 1. Pederin-type natural products.

[†] Tohoku University.
[‡] University of Tsukuba.

(1) Pettit, G. R.; Xu, J.-P.; Chapuis, J.-C.; Pettit, R. K.; Tackett, L. P.; Doubek, D. L.; Hooper, J. N. A.; Schmidt, J. M. *J. Med. Chem.* **2004**, *47*, 1149.
(2) Cichewicz, R. H.; Valeriote, F. A.; Crews, P. *Org. Lett.* **2004**, *6*, 1951.

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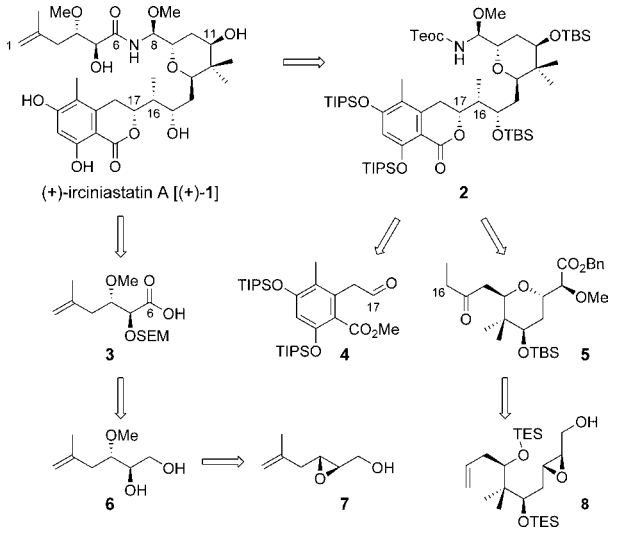
sented by (+)-**1**.[3] To date, several total[4−6] and formal[7] syntheses as well as SAR studies[8,9] have been reported. Among them, the Schering-Plough group has reported that the substituents at C4 and C5 are important for the cytotoxicity, and that the C1−C2 double bond is not essential for activity.[9] 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**

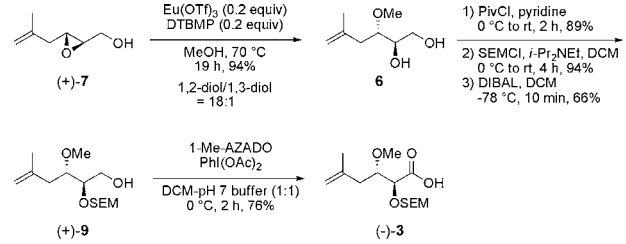
Scheme 1. Retrosynthetic Analysis of (+)-Irciniastatin A (**1**)



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

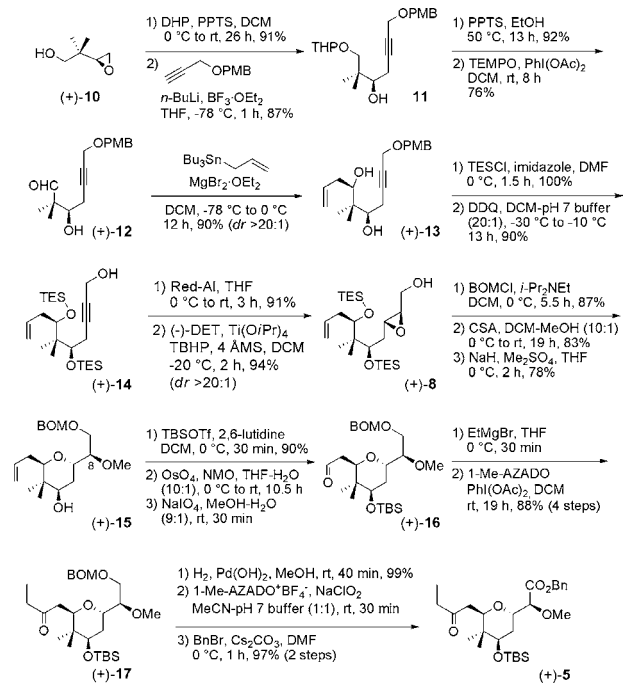
Scheme 2. Synthesis of the Acyclic Side Chain (−)-**3**



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**



(3) Jiang, X.; Garcia-Fortanet, J.; De Brabander, J. K. *J. Am. Chem. Soc.* **2005**, *127*, 11254.
(4) Huang, X.; Shao, N.; Palani, A.; Aslanian, R.; Buevich, A. *Org. Lett.* **2007**, *9*, 2597.
(5) Smith, A. B., III; Jurica, J. A.; Walsh, S. P. *Org. Lett.* **2008**, *10*, 5625.
(6) Crimmins, M. T.; Stevens, J. M.; Schaaf, G. M. *Org. Lett.* **2009**, *11*, 3990.
(7) Shangguan, N.; Kiren, S.; Williams, L. J. *Org. Lett.* **2007**, *9*, 1093.
(8) Jiang, X.; Williams, N.; De Brabander, J. K. *Org. Lett.* **2007**, *9*, 227.
(9) Huang, X.; Shao, N.; Huryk, R.; Palani, A.; Aslanian, R.; Seidel-Dugan, C. *Org. Lett.* **2009**, *11*, 867.
(10) Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.

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]

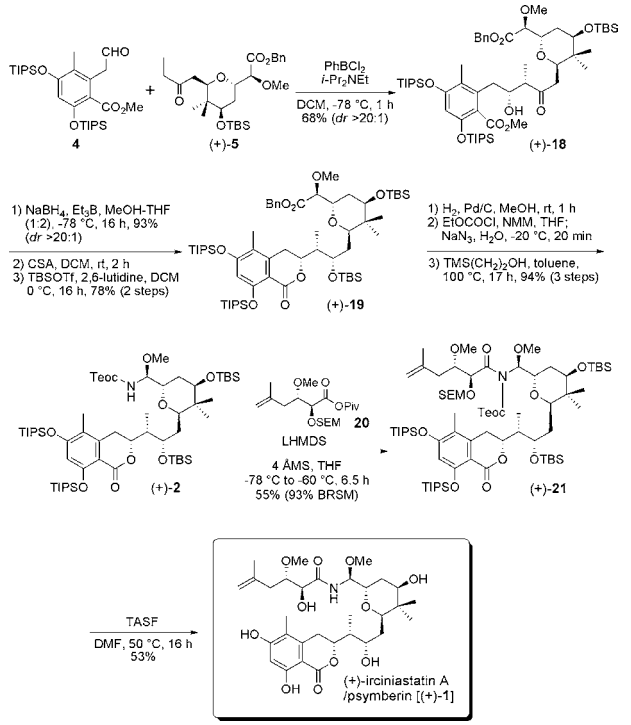
Coupling of (+)-**2** with the acyclic side chain fragment proved to be a difficult task. Initially, we examined the coupling reaction of (+)-**2** with several derivatives of the carboxylic acid (−)-**3**, which never yielded the desired product. After intensive effort, we realized that Teoc-protected 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.

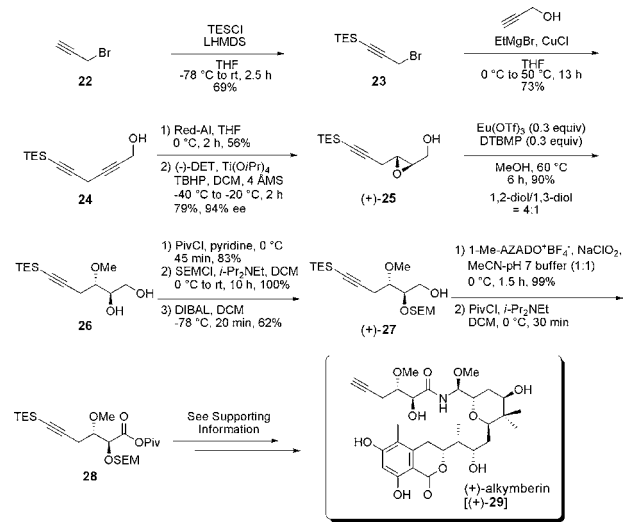
For the synthesis of (+)-alkymberin, we prepared alkyne side chain **28** based on the synthetic route previously established (Scheme 5). In the course of the synthesis, we found that the 1-Me-AZADO$^+BF_4^-$/$NaClO_2$ system was more effective for the oxidation of alcohol (+)-**27**. The resultant carboxylic 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**)

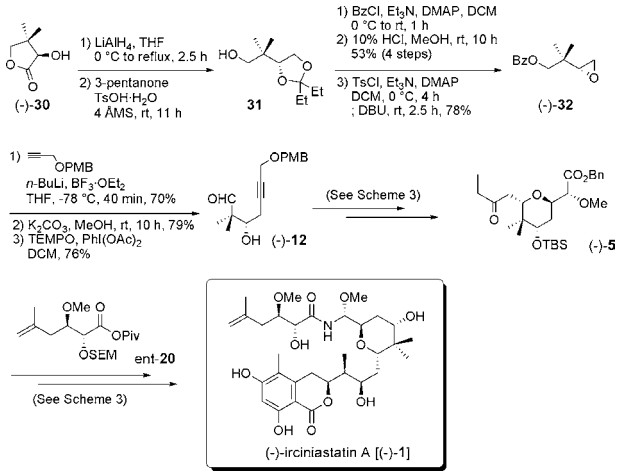


Scheme 5. Synthesis of (+)-Alkymberin [(+)-**29**]



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.

Acknowledgment. We thank Dr. Shinichi Nishimura (Kyoto University) for his helpful suggestions and comments.

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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(11) Alegret, C.; Santacana, F.; Riera, A. *J. Org. Chem.* **2007**, *72*, 7688.
(12) Caron, M.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 1557.
(13) These details will be reported elsewhere.
(14) Shibuya, M.; Tomizawa, M.; Suzuki, I.; Iwabuchi, Y. *J. Am. Chem. Soc.* **2006**, *128*, 8412.
(15) Lavallée, P.; Ruel, R.; Grenier, L.; Bissonnette, M. *Tetrahedron Lett.* **1986**, *27*, 679.
(16) Yamaguchi, M.; Hirao, I. *Tetrahedron Lett.* **1983**, *24*, 391.
(17) De Brabander, J. K.; Vandewalle, M. *Synthesis* **1994**, 855.
(18) Shibuya, M.; Sato, T.; Tomizawa, M.; Iwabuchi, Y. *Chem. Commun.* **2009**, 1739.
(19) Evans, D. A.; Calter, M. A. *Tetrahedron Lett.* **1993**, *34*, 6871.
(20) Chen, K.-M.; Hardtmann, G. E.; Prasad, K.; Repiè, O.; Shapiro, M. J. *Tetrahedron Lett.* **1987**, *28*, 155.
(21) Weinstock, J. *J. Org. Chem.* **1961**, *26*, 3511.
(22) Smith, A. B., III; Safonov, I. G.; Corbett, R. M. *J. Am. Chem. Soc.* **2002**, *124*, 11102.
(23) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
(24) Nakamura, Y.; Miyatake, R.; Ueda, M. *Angew. Chem., Int. Ed.* **2008**, *47*, 7289.