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Concise Synthesis of Pochonin A, an HSP90 Inhibitor

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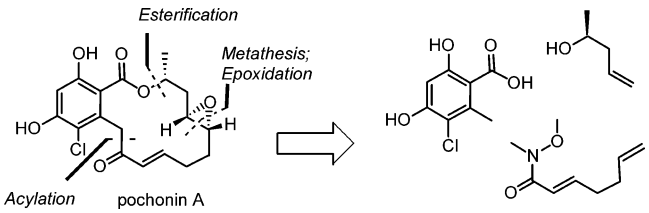
ABSTRACT



An expedient synthesis of (–)-pochonin A is reported (seven steps). This natural product is closely related to radicicol and was shown to be a 90 nM inhibitor of HSP90.

Pochonin A (Figure 1) is part of a new family of resorcyclic macrolides isolated by Hellwig and co-workers from the fermentation broths of *Pochonia chlamydosporia*.[1] These compounds were identified from a high throughput screen for inhibition of the herpes simplex virus (HSV) helicase primase, an ATPase-dependent enzyme complex. Our interest in these compounds stems from the discovery that radicicol[2] (Figure 1) is a competitive antagonist of ATP for HSP90[3] (an ATPase-dependent chaperone) as there is no obvious structural or topological relationship between the resorcyclides and ATP. Interestingly, the pochonins were also shown to be devoid of the estrogenic activity found with the structurally related zearalenone.[4] The different biological activity found with the structurally similar resorcyclides may be in part rationalized by the conformational diversity that is achievable within this class of compounds as we have shown for pochonin C versus radicicol.[5] Conformational analysis of radicicol and related analogues led us to speculate that pochonin D might also be an HSP90 inhibitor, a fact that was subsequently verified experimentally.[6] The high potential and widespread interest in HSP90 inhibition[7,8] recently led us to examine pochonin A.

A direct conversion of pochonin D into pochonin A via epoxidation (DMDO, *m*CPBA) was poorly stereoselective

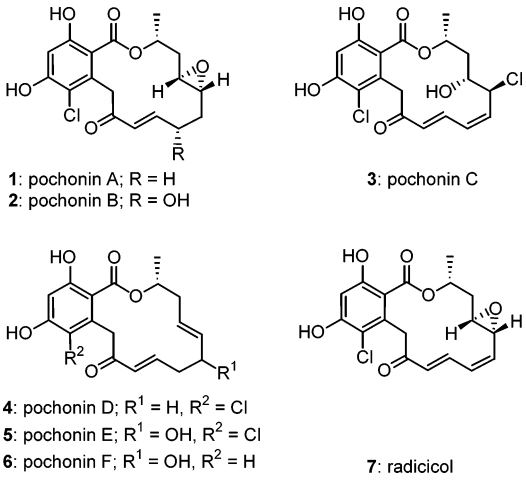


Figure 1. Structures of the pochonin family members.

(1) Hellwig, V.; Mayer-Bartschmid, A.; Mueller, H.; Greif, G.; Kleymann, G.; Zitzmann, W.; Tichy, H.-V.; Stadler, M. *J. Nat. Prod.* **2003**, *66*, 829.
(2) Delmotte, P.; Delmotte-Plaquee, J. *Nature* **1953**, *171*, 344.
(3) Roe, S. M.; Prodromou, C.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *J. Med. Chem.* **1999**, *42*, 260.
(4) Anke, H. In *Fungal Biotechnology;* Anke, T., Ed.; Chapman & Hall: Weinheim, Germany, 1997; p 186.
(5) Barluenga, S.; Moulin, E.; Lopez, P.; Winssinger, N. *Chem. Eur. J.* **2005**, *11*, 4935.

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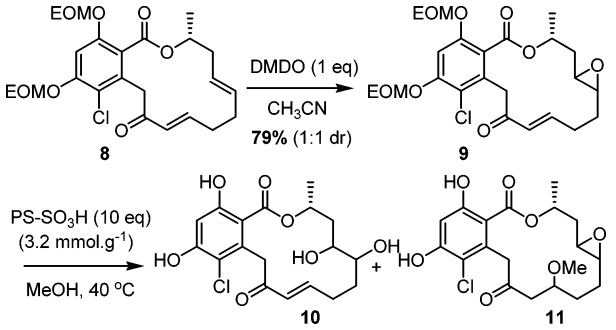
and did not appear to be improvable due to the lack of solubility of pochonin D at low temperature. Epoxidation of the previously reported EOM[9]-protected pochonin D (**8**, Scheme 1) afforded a 1:1 ratio of diastereoisomers **9**.

Scheme 1. From Bis-EOM Pochonin D to Pochonin A



Deprotection of the EOM group using sulfonic acid resin (Novabiochem, 70−90 mesh, MP) in methanol led to epoxide opening (**10**), as well as conjugate addition of methanol (**11**). A screen of other protic acids such as acetic acid or hexafluoro-2-propanol and Lewis acids such as $Sc(OTf)_3$ did not yield a suitable solution. We therefore turned our attention toward a synthesis that would rely on silyl-protected phenols (Figure 2). Thus, persilylation of benzoic acid **14**

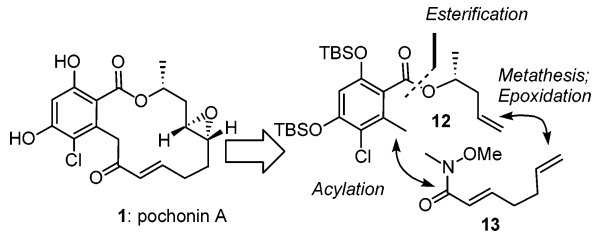
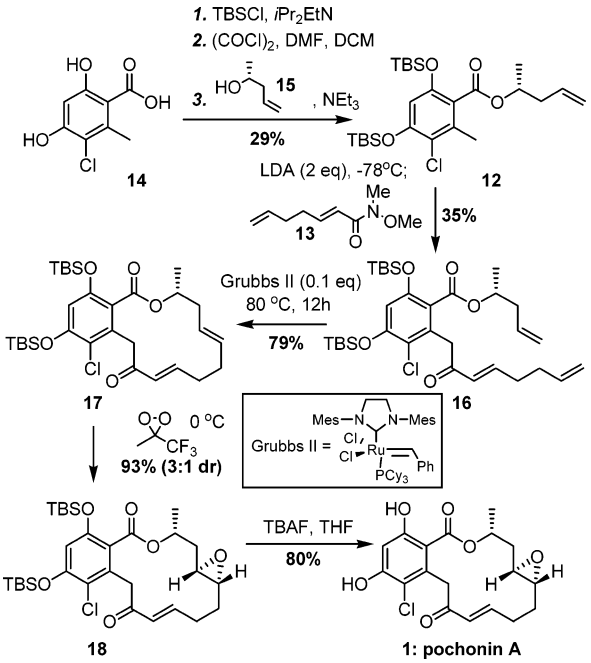


Figure 2. General retrosynthetic analysis.

(Scheme 2), followed by "acid-free" conversion of the silyl ester to the acyl chloride[10] yielded key intermediate **12** upon

Scheme 2. Synthesis of Pochonin A



esterification with alcohol **15**. The low yield obtained was attributed to the lability of the *ortho* TBS group, although it should be noted that these three steps were carried out without purification of the intermediates and as such this sequence was quite practical. Deprotonation of the benzylic methylene followed by reaction with Weinreb amide **13** afforded metathesis precursor **16** in modest yield. Ring-closing metathesis using second generation Grubbs catalyst[11] under thermodynamic conditions[12] afforded macrocyle **17** in good yield and excellent *cis*/*trans* ratio (<5% *cis*).

Epoxidation of the unconjugated olefin was optimal when carried out with methyl(trifluoromethyl)-dioxirane generated in situ.[13] It afforded protected pochonin A in excellent yield as an inseparable 3:1 diastereomeric mixture. It is interesting to note that epoxidation with *m*CPBA or DMDO only proceeded at room temperature and did not give any diastereomeric excess. Attempts to further improve the stereoselectivity of the epoxidation using epoxone[14] were not productive. Deprotection of the products obtained from the epoxidation afforded a separable diastereomeric mixture and confirmed that the major product was indeed the desired pochonin A.[15] Although the overall synthetic sequence was short and could be carried out in only a few days, the poor yield in the acylation reactions led us to consider alternative protecting groups. Eventually, we decided to use SEM ethers

(6) Moulin, E.; Zoete, V.; Barluenga, S.; Karplus, M.; Winssinger, N. *J. Am. Chem. Soc.* **2005**, *127*, 6999.

(7) Neckers, L.; Neckers, K., Heat-shock protein 90 inhibitors as novel cancer chemotherapeutics−an update. *Exp. Opin. Emerg. Drugs* **2005**, *10*, 137. Workman, P. *Curr. Cancer Drug Targets* **2003**, *3*, 297.

(8) For recent examples, see: Shen, G.; Blagg, B. S. *Org. Lett.* **2005**, *7*, 2157. Yu, X. M.; Shen, G.; Neckers, L.; Blake, H.; Holzbeierlein, J.; Cronk, B.; Blagg, B. S. *J. Am. Chem. Soc.* **2005**, *127* (37), 12778. Cheung, K.-M.; Matthews, T. P.; James, K.; Rowlands, M. G.; Boxall, K. J.; Sharp, S. Y.; Maloney, A.; Roe, S. M.; Prodromou, C.; Pearl, L. H.; Aherne, G. W.; McDonald, E.; Workman, P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3338. Dymock, B. W. et al *J. Med. Chem.* **2005**, *48*, 4212. Llauger, L.; He, H.; Kim, J.; Aguirre, J.; Rosen, N.; Peters, U.; Davies, P.; Chiosis, G., *J. Med. Chem.* **2005**, *48*, 2892. Kreusch, A.; Han, S.; Brinker, A.; Zhou, V.; Choi, H.-S.; He, Y.; Lesley, S. A.; Caldwell, J.; Gu, X.-J., *Bioorg. Med. Chem. Lett* **2005**, *15*, 1475. Vilenchik, M.; Solit, D.; Basso, A.; Huezo, H.; Lucas, B.; He, H.; Rosen, N.; Spampinato, C.; Modrich, P.; Chiosis, G. *Chem. Biol.* **2004**, *11* (6), 787.

(9) EOM (ethoxymethyl) because of the easier availability for EOM-Cl compared to MOM-Cl.

(10) Wissner, A.; Grudzinskas, C. V. *J. Org. Chem.* **1978**, *43*, 3972.

(11) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953. Chatterjee, A. K.; Morgan, J. P.; Scholl, M.; Grubbs, R. H. *J. Am. Chem. Soc.* **2000**, *122*, 3783.

(12) Lee, C. W.; Grubbs, R. H. *Org. Lett.* **2000**, *2*, 2145.

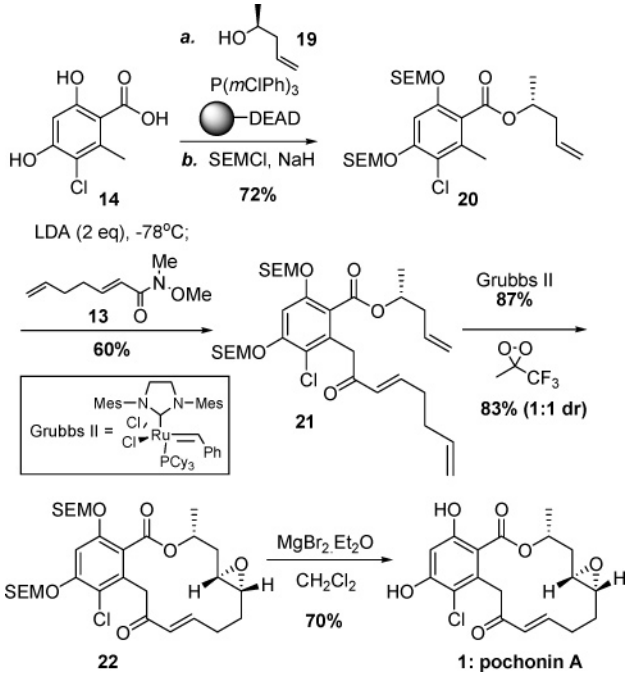
(13) Yang, D.; Wong, M.-K.; Yip, Y.-C. *J. Org. Chem.* **1995**, *60* (12), 3887.

(14) Tu, Y.; Wang, Z.-X.; Shi, Y. *J. Am. Chem. Soc.* **1996**, *118*, 9806. Wang, Z. X.; Tu, Y.; Frohn, M.; Shi, Y. *J. Org. Chem.* **1997**, *62*, 2328.

(15) ^{1}H NMR of pochinin A was kindly provided by Dr. Stadler (Bayer Health Care, Wuppertal, Germany).

on account of their stability toward basic conditions and their capacity for deprotection with TBAF (Scheme 3). Thus after

Scheme 3. Synthesis of Bis-SEM Protected Pochonin A



selective Mitsunobu reaction of benzoic acid **14** and alcohol **19** using polymer-bound DEAD and subsequent protection with SEM-Cl, ester **20** was isolated in 72% yield.[6] Deprotonation of **20** followed by addition of Weinreb amide **13** afforded the metathesis precursor **21** in 60% yield. Treatment of **21** with the second generation Grubbs catalyst under thermodynamic conditions afforded the corresponding macrocycle in 87% yield (<5% *cis* olefin), which was epoxidized under the same conditions as for the TBS-protected compound **17** [methyl(trifluoromethyl)-dioxirane], yielding compound **22** in 83% yield albeit in 1:1 diastereomeric ratio (inseparable). All attempts to deprotect the SEM groups using TBAF were unsuccessful, with the formation of coumarin being the major undesired reaction. To our great delight, we found that 8 equiv of $MgBr_2$ in dichloromethane at room temperature afforded the desired pochonin A and its diastereoisomer as a separable mixture. The yield was limited by reaction conversion, since longer times generated the corresponding bromohydrine by opening of the oxirane ring. It is important to note that in the case of EOM-protected product **9**, treatment with $MgBr_2$ was found to open the epoxide faster than to deprotect the EOM.

Pochonin A (**1**) and its diol analogue **10** were evaluated for HSP90 affinity in a competition assay with geldanamycin using a previously described assay.[16] Interestingly, pochonin A was a good ligand (90 nM), while analogue **10** was inactive. The inactivity of diol **10** may be rationalized by the different conformational profile of this compound relative to pochonin A or D as was calculated for radicicol and its diol-analogue.[6]

The synthesis described herein represents the first synthesis of pochonin A, thereby assigning a negative specific optical rotation to this product. It also led to the discovery that pochonin A is a good ligand for HSP90. The ability of these compounds to antagonize ATP and inhibit ATPase activity may be related to their inhibition of HCV helicase. The difference of selectivity in the epoxidation reaction depending on the phenol protecting groups may be ascribed to the conformational impact of the bulky silyl group on the *ortho* phenol.

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Supporting Information Available: Experimental procedures utilized and compound characterization including NMR of pochonin A. This material is available free of charge via the Internet at http://pubs.acs.org.

OL052263+

(16) Zhou, V.; Han, S.; Brinker, A.; Klock, H.; Caldwell, J.; Gu, X.-j. *Anal. Biochem.* **2004**, *331*, 349.