ORGANIC LETTERS

2007
Vol. 9, No. 1
65−67

Total Synthesis and Biological Evaluation of Verticipyrone and Analogues

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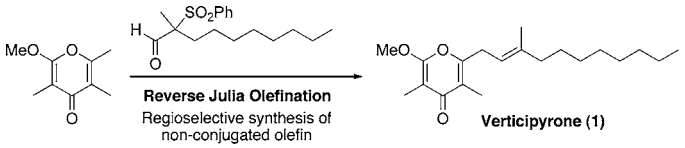
Received October 24, 2006

ABSTRACT

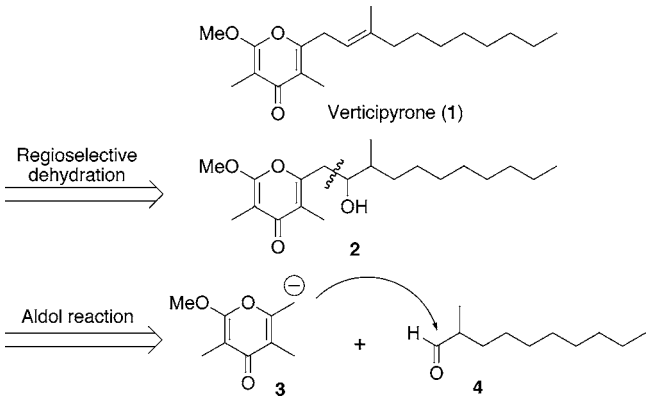


Total synthesis of verticipyrone, a novel NADH-fumarate reductase inhibitor, has been accomplished by a convergent approach using novel "Reverse Julia olefination" method. During total synthetic studies, we also prepared and evaluated several synthetic verticipyrone analogues, some of which exhibited more potent antiparasitic activity than the natural verticipyrone.

In the course of our screening for NADH-fumarate reductase (NFRD)[1] inhibitors, verticipyrone (**1**) was isolated from the fermentation broth of a fungal strain, *Verticillium* sp. FKI-1083.[2] Verticipyrone (**1**) inhibited NFRD of *Ascaris suum* with an IC_{50} value of 4.1 nM and showed selective inhibition against its target enzyme, complex I, of helminth mitochondria. Furthermore, verticipyrone (**1**) showed anthelmintic activity against *Caenorhabditis elegans* and *Artemia salina*. It therefore may become a good candidate as a new antiparasitic agent. These useful biological activities of **1** attracted our attention and recently prompted us to undertake a total synthesis study.[3] In this paper, we wish to report the total synthesis and biological evaluation of verticipyrone and its analogues.

We envisioned developing a convergent synthesis of **1** that would provide verticipyrone analogues with varying combination of pyrones and side chains. Our synthetic plan is shown in Scheme 1. Verticipyrone (**1**) would be obtained via aldol reaction between γ-pyrone **3** and aldehyde **4** followed by regioselective dehydration of the resulting alcohol **2**.

Scheme 1. Retrosynthetic Analysis of Verticipyrone (**1**)



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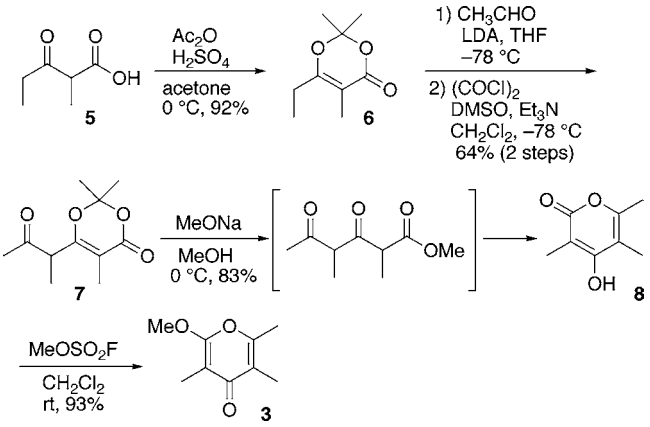
(1) For recent reviews, see: (a) Komuniecki, R.; Tielens, A. G. M. In *Molecular Medical Parasitology*; Marr, J. J., Nilsen, T. W., Komuniecki, R. W., Eds.; Academic Press: London, 2003; Chapter 14, p 339. (b) Kita, K.; Nihei, C.; Tomitsuka, E. *Curr. Med. Chem.* **2003**, *10*, 2535. (c) Shiomi, K.; Ōmura, S. *Proc. Jpn. Acad., Ser. B* **2004**, *80*, 245.

10.1021/ol0626140 CCC: $37.00
Published on Web 12/05/2006

On the basis of this synthetic plan, we initially prepared γ-pyrone **3** as follows (Scheme 2). Known β-ketoacid **5**[4] was

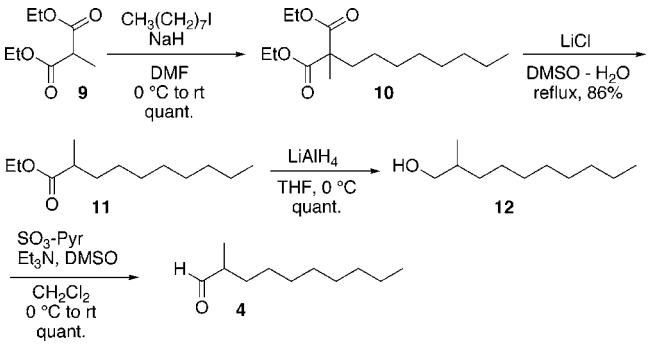
Scheme 2. Synthesis of γ-Pyrone (**3**)



treated with mixture of acetone and Ac_2O in the presence of catalytic amount of concd H_2SO_4[5] to give the dioxinone **6** in 92% yield. Dioxinone **6** was converted into ketone **7** in 64% yield for two steps by aldol reaction with acetaldehyde followed by oxidation[6] of the resulting alcohol under Swern conditions.[7] Ketone **7** was then treated with sodium methoxide in methanol to give the γ-hydroxy-α-pyrone **8** in 83% yield via deprotection of the acetonide group and successive cyclization of the resulting diketoester. Regioselective *O*-methylation of **8** with methylfluorosulfonate[8] provided α-methoxy-γ-pyrone **3** in 93% yield.

The side chain aldehyde **4** was prepared as illustrated in Scheme 3. Alkylation of diethyl methylmalonate **9** with NaH

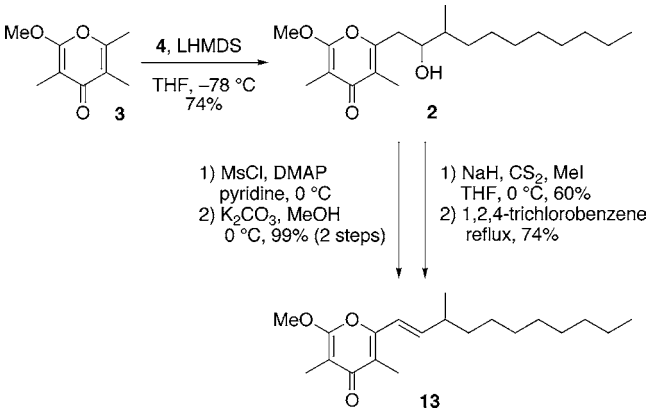
Scheme 3. Synthesis of Aldehyde (**4**)



and iodooctane gave **10** quantitatively. Decarboxylation[9] of **10** with LiCl in aqueous DMSO solution afforded **11** in 86% yield. Reduction of the ethyl ester **11** by $LiAlH_4$ and oxidation[10] of resulting alcohol **12** gave the desired aldehyde **4** quantitatively.

Aldol reaction between γ-pyrone **3** and aldehyde **4** with LHMDS gave aldol adduct **2** in 74% yield (Scheme 4). The

Scheme 4. Aldol Reaction of γ-Pyrone (**3**) and Aldehyde (**4**)



desired nonconjugated olefin of **1** from aldol adduct **2** was unsuccessful, though elimination of mesylate under basic conditions and thermal decomposition of xanthate were attempted. Further isomerization[11] of **13** into **1** was also unsuccessful.

Although aldol adduct **2** could not be converted to the desired non-conjugated olefin, the aldol reaction between γ-pyrone and aldehyde was useful for SAR studies on verticipyrone libraries. This observation prompted us to design an appropriate aldehyde to selectively generate a nonconjugated olefin after aldol reaction. Therefore, α-phenylsulfonyl aldehyde **16** was designed as it would allow reductive elimination of β-hydroxysulfone via a novel "Reverse Julia olefination" process after aldol reaction.

α-Phenylsulfonyl ester **14** was alkylated sequentially to give the α-dialkyl ester **15** in 82% overall yield (Scheme 5). Reduction of the ethyl ester by DIBAL afforded α-phenylsulfonyl aldehyde **16** in 99% yield. Coupling the γ-pyrone **3** and aldehyde **16** using LDA gave the Julia hydroxysulfone **17** in 74% yield. Finally, acetylation and reductive elimination afforded nonconjugated olefin **1** (59%) and its *Z*-isomer **18** (33%). Synthetic verticipyrone (**1**) was identical to the natural product in all respects (^{1}H and ^{13}C NMR, IR, FAB-MS, and inhibitory activity against NFRD).

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(3) Recent synthetic approach for the other complex I inhibitors: (a) Ichimaru, N.; Abe, M.; Murai, M.; Senoh, M.; Nishioka, T.; Miyoshi, H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3555. (b) Schnermann, M. J.; Romero, F. A.; Hwang, I.; Nakamaru-Ogiso, E.; Yagi, T.; Boger, D. L. *J. Am. Chem. Soc.* **2006**, *128*, 11799.
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(10) Parikh, J. R.; Doering, W. v. E. *J. Am. Chem. Soc.* **1967**, *89*, 5505.
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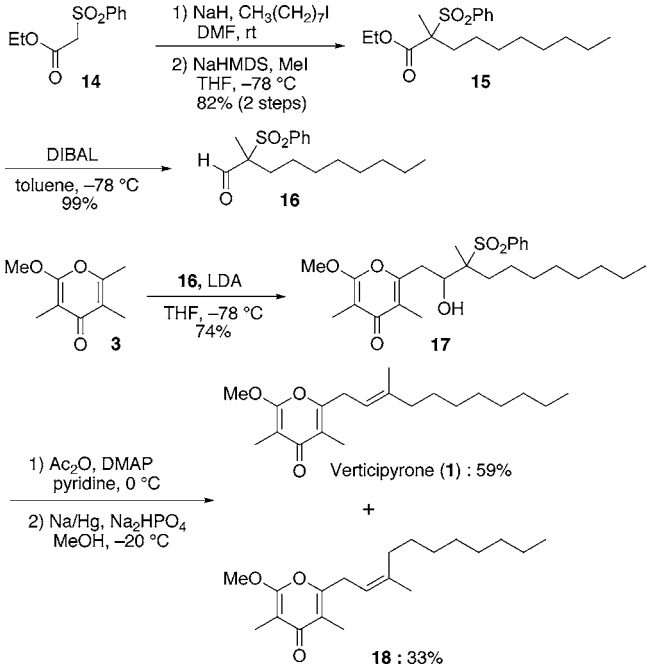
Scheme 5. Total Synthesis of Verticipyrone (**1**)



We then evaluated the NFRD inhibitory activities of several synthetic analogues[13,14] of **1** in order to establish a SAR profile (Figure 1). The assay method for the inhibitory activities (IC_{50}) against *A. suum* NFRD was the same as that reported previously.[15]

First, olefin isomers **13** and **18** and alkyl side chain analogue **19** showed similar NFRD inhibitory activities to **1**. This result suggested that the olefin in the side chain was not particularly important to activity. Fortunately, alcohol **20** showed 10-fold greater potency than **1**. Furthermore, alcohol **2** also showed potent activity. This suggested that the newly introduced hydroxyl group on the side chain might contribute to the observed activity. Surprisingly, analogue **21** showed no inhibitory activity. This finding suggested that the most important active center was the γ-pyrone moiety,

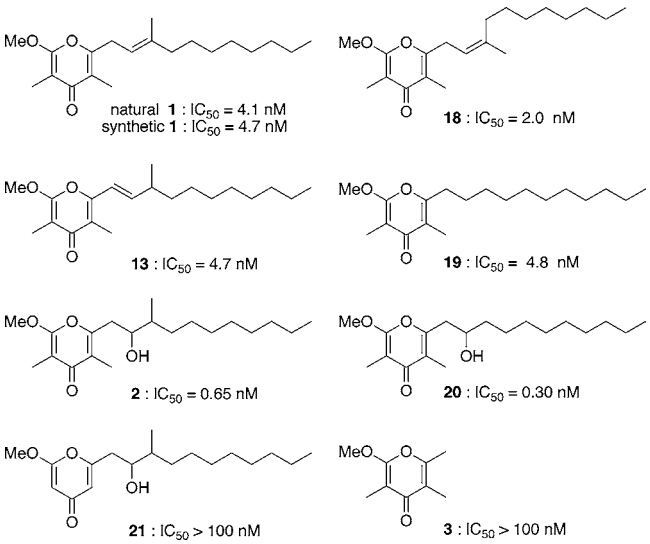


Figure 1. Inhibitory activity of synthetic **1** and analogues against NFRD.

and 3,5-dimethyl groups on a γ-pyrone moiety were an essential factor for inhibitory activity against NFRD. The γ-pyrone **3**, however, showed no activity. Thus, the long side chain was also important for the inhibitory activity against NFRD.

In conclusion, we have achieved a concise total synthesis of verticipyrone (**1**) using a reverse Julia olefination method. We have also synthesized some analogues and established a SAR profile, and found more potent active analogues, **2** and **20**, than the natural product. Additional investigation of the SAR and biological studies of verticipyrone are currently underway.

Acknowledgment. This work was supported by the Grant of 21st Century COE Progam, Ministry of Eduction, Culture, Sports, Science and Technology. We also thank Ms. Nakagawa, Ms. C. Sakabe, and Ms. N. Sato (School of Pharmaceutical Sciences, Kitasato University) for various instrumental analyses.

Supporting Information Available: Experimental procedures and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0626140

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(13) Syntheses of the verticipyrone anlogues are described in the Supporting Information.

(14) The analogues **2** and **21** were evaluated as a diastereomeric mixture. The diastereomeric ratio of both **2** and **21** was approximately 2:1, determined from ^{1}H NMR.

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