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Synthesis and Structure Revision of Nakiterpiosin

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Nakiterpiosin (**1**) is a marine sponge metabolite that exhibits potent cytotoxicity against the P388 murine leukemia cell line (GI_{50} 10 ng/mL) (Figure 1).[1] It was the first C-nor-D-homosteroid isolated from a marine source. Its unique chemical structure and strong P388 growth inhibition property prompted us to initiate a research program to explore its laboratory synthesis[2] and biological function. We report herein the synthesis and structure revision[3] of nakiterpiosin.

The C-nor-D-homosteroids are skeletally rearranged steroids with their C-ring contracted and D-ring expanded by one carbon. The veratrum alkaloid cyclopamine (**3**) and veratramine (**4**) are arguably the best known members.[4,5] The teratogenic alkaloid **3** inhibits the Hedgehog (Hh) signaling by binding to Smoothened (Smo),[6,7] and the chronotropic alkaloid **4** induces serotonin (5-HT) syndrome.[8] While **1** possesses potent cytotoxicity against P388, its molecular target is not known. Furthermore, the complete biological profile of **1** could not be obtained because of the scarcity of the material. From 30 kg of sponge *T. hoshinota*, only 0.4 mg of nakiterpiosin was obtained. Its chemical structure was assigned as **2** in the original reports.[1]

We were puzzled by the inconsistency between the C-20 stereochemistry reported for **2** and **3**/**4** and therefore set out to probe the relative stereochemistry of nakiterpiosin. Our model studies indicated potential misassignment of the C-6, C-20, and C-25 stereogenic centers.[9] We next considered the biogenesis of the halogen atoms[10] of nakiterpiosin to rationalize the C-6 and C-20 stereochemistry. We envisioned that the C-21 chlorine atoms of nakiterpiosin might be introduced by radical chlorination and the C-6 bromine atom by bromoetherification (as shown in **5**) to result in retention of the C-20 configuration and the anti C-5,6 bromohydrin stereochemistry. Taken together, these considerations led us to propose **1** to be the correct structure of nakiterpiosin. Indeed, we found that the ^1H and ^{13}C NMR spectra of our synthetic sample of **1** agreed with those of the natural product.[11] In contrast, those of synthetic **2**[9,11] and the natural product are significantly different. We thus revised the relative stereochemistry of nakiterpiosin to be that indicated in **1**, which shares the same configuration at the C-20 and C-25 positions with **3** and **4**.

Our synthetic strategy is outlined in Figure 2. We dissected **1** into fragments **6** and **7** and constructed the central cyclopentanone ring with a carbonylative cross-coupling reaction[12] and a photo-Nazarov cyclization reaction.[13] The electrophilic coupling component **6** was synthesized by an intramolecular Diels−Alder reaction,[14] and the nucleophilic coupling component **7** by vinylogous Mukaiyama aldol reaction.[15]

To synthesize **6**, we first converted acid **8**[16] to the corresponding Weinreb amide and then set the C-6 stereocenter by Noyori reduction[17] (Scheme 1). The in-water hydrogenation protocol[18] provided significant enhancement of the reaction rate, allowed low catalyst loading and suppressed the formation of the undesired lactone. Subsequently, isopropenyl Grignard addition to **9** followed by Me_2AlCl-promoted intramolecular Diels−Alder reaction gave the exo cycloaddition product. The C-6 hydroxyl group controlled the stereoselectivity[19] of this Diels−Alder reaction to afford single diastereomer. The C-6 hydroxyl group was then activated with an

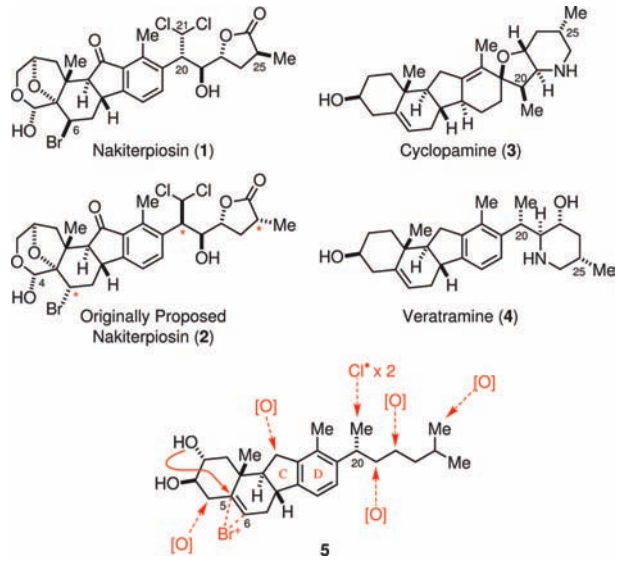


Figure 1. The C-nor-D-homosteroids.

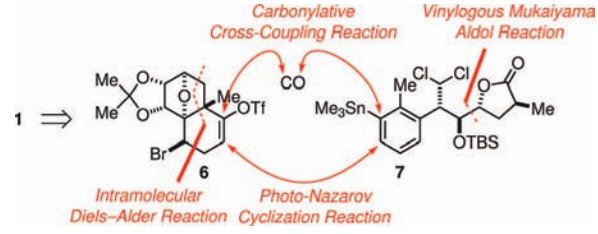
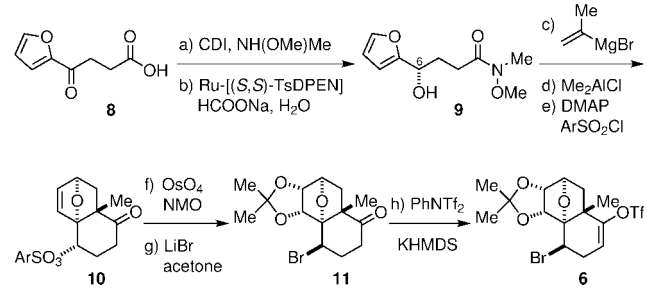


Figure 2. Our synthetic approach to **1**.

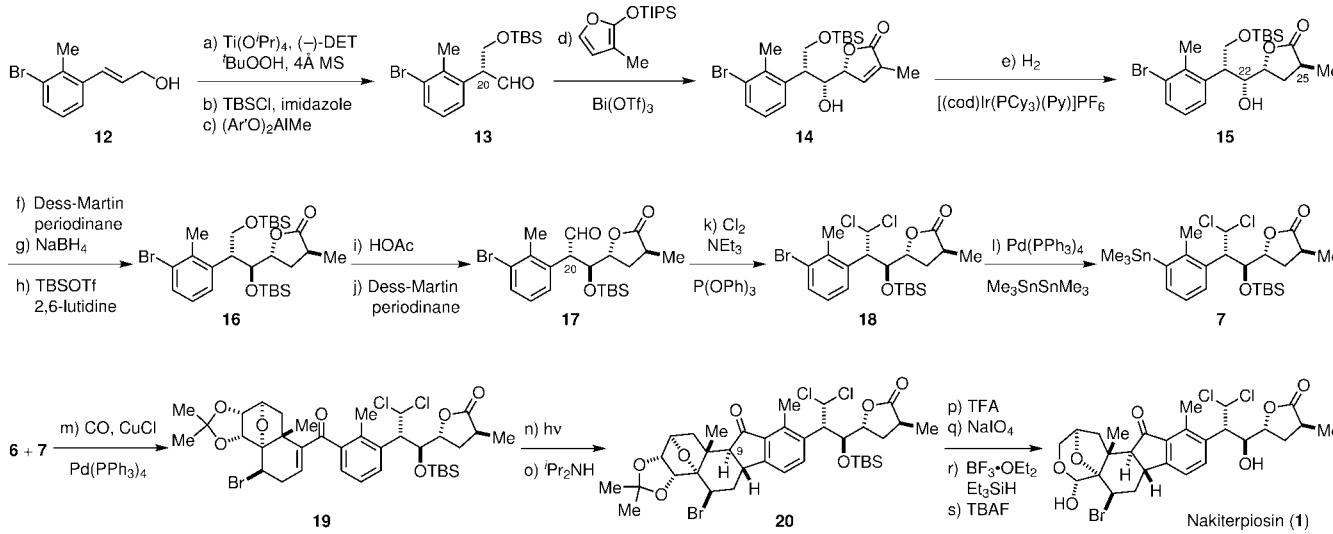
Scheme 1. The Synthesis of **6**[a]



[a] Reaction conditions: (a) CDI, NH(OMe)Me·HCl, CH_2Cl_2, 23 °C, 74%; (b) 2 mol % RuCl[(*S*,*S*)-TsDPEN](*p*-cymeme), HCOONa, H_2O, 40 °C, 87%, 91% ee; (c) $H_2C{=}C(Me)MgBr$, THF, 0 to 23 °C, 84%; (d) Me_2AlCl, CH_2Cl_2, −78 to −30 °C, 71%; (e) DMAP, $ArSO_2Cl$, CH_2Cl_2, 0 to 23 °C, 100%; Ar = 2-(MeOOC)Ph; (f) 20 mol % OsO_4, NMO, acetone, H_2O, 23 °C, 89%; (g) LiBr, acetone, 70 °C, 62%; (h) KHMDS, $PhNTf_2$, THF, −78 °C, 96%.

10.1021/ja808110d CCC: $40.75

Scheme 2. Synthesis of **7** and Completion of the Synthesis of **1**[a]



[a] Conditions: (a) 15 mol % $Ti(O^iPr)_4$, 18 mol % (−)-diethyl tartrate, ^{t}BuOOH, 4Å MS, CH_2Cl_2, −20 °C, 98%, 92% ee; (b) TBSCl, imidazole, DMF, 23 °C, 97%; (c) $(Ar'O)_2AlMe$, CH_2Cl_2, −78 °C, 79%, 71% ee; Ar′ = 4-Br-2,6-^{t}Bu$_2$-Ph; (d) 10 mol % $Bi(OTf)_3$, 3-Me-2-TIPSO-furan, CH_2Cl_2, −40 °C, 76% (56% conversion), dr 11:1, 60% ee; (e) H_2, 10 mol % $[(cod)Ir(PCy_3)(Py)]PF_6$, CH_2Cl_2, 23 °C, 97%, dr 6:1; (f) Dess−Martin periodinane, H_2O, CH_2Cl_2, 23 °C, 98%; (g) $NaBH_4$, THF, EtOH, −78 °C, 93%; (h) TBSOTf, 2,6-lutidine, CH_2Cl_2, 40 °C, 93%; (i) HOAc, THF, H_2O, 60 °C, 76%; (j) Dess-Martin periodinane, H_2O, CH_2Cl_2, 23 °C, 91%; (k) Cl_2, $P(OPh)_3$, NEt_3, CH_2Cl_2, −78 °C, 83%; (l) 45 mol % $Pd(PPh_3)_4$, $Me_3Sn-SnMe_3$, dioxane, 100 °C, 50%; (m) 1 atm CO, $Pd(PPh_3)_4$, CuCl, DMSO, 55 °C, 62%; (n) hν (350 nm), CH_3CN, 23 °C; (o) ^{i}Pr$_2$NH, MeOH, 50 °C, 60% for two steps; (p) TFA, CH_2Cl_2, H_2O, 23 °C; (q) $NaIO_4$, acetone, pH 7.4 buffer, 23 °C; (r) $BF_3\cdot OEt_2$, Et_3SiH, CH_2Cl_2, 0 to 23 °C, 44% for three steps; (s) TBAF, THF, 23 °C, 79%.

electron-deficient aryl sulfonate group for the introduction of the C-6 bromine atom. We found that **10** underwent retro-Diels−Alder reaction readily at elevated temperature, particularly in the presence of Lewis acids. This characteristic imposed significant challenges for the introduction of the C-6 bromine atom. We therefore dihydroxylated the olefin group to prevent the retro-Diels−Alder reaction even through this functionalization created serious steric congestion around the C-6 position. The C-6 bromine atom could then be introduced with the inversion of configuration and concomitant acetonide protection. The absolute and relative configurations of **11** were confirmed by X-ray analysis.[11] Finally, enol triflate installation completed the synthesis of **6**.

For the synthesis of **7**, we utilized the Sharpless asymmetric epoxidation reaction[20] of **12**[11] to set the C-20 stereochemistry (Scheme 2). The subsequent TBS protection and Yamamoto epoxide rearrangement reaction[21] gave aldehyde **13**. We note that aldehyde **13** was sensitive to both acidic and basic conditions and underwent elimination and racemization readily. The enantiomeric purity was eroded after the rearrangement reaction. We also found that $Bi(OTf)_3$ promoted the vinylogous Mukaiyama aldol reaction of **13** with good levels of diastereoselectivity.[22] However, further loss of enantiomeric purity could not be avoided. With the complete C-20−C-26 carbon framework of the side-chain in place, we then sought to set its anti−anti−trans configuration. The C-25 stereochemistry was set by a chelation-controlled hydrogenation of **14** using Crabtree's catalyst.[23,24] We then inverted the C-22 stereocenter of **15** and protected the hydroxyl group as TBS ether to give **16** with the desired anti−anti−trans configuration. Selective deprotection of the primary TBS ether followed by Dess-Martin oxidation yielded aldehyde **17**. The gem-dichloromethyl group was introduced by $Cl_2/P(OPh)_3$ to give **18**.[25] No epimerization of the C-20 stereocenter was observed. The X-ray analysis on des-TBS-**18** confirmed the absolute and relative configurations of **18**.[11] Finally, the palladium-catalyzed stannylation afforded the nucleophilic coupling component **7**.

The carbonylative coupling of **6** and **7** was achieved with a modified Stille's protocol[26] using $Pd(PPh_3)_4$/CuCl in DMSO under 1 atm CO. The CuCl additive and DMSO solvent provided dramatic rate enhancement[27] and were crucial to the success of this reaction. It is worth noting that **6** and **19** were highly sensitive to both acidic and basic reaction conditions. Addition of LiCl and prolonged heating led to the elimination of bromide. Enone **19** was obtained as a 4:1 mixture of inseparable diastereomers due to the diminished enantiomeric purity of **7**. Nonetheless, the minor diastereomer could be removed at a later stage (**20**).

With **19** in hand, we next explored the key Nazarov cyclization reaction to complete the construction of the C-nor-D-homosteroid skeleton of nakiterpiosin. Remarkably, irradiation of a solution of **19** in acetonitrile at 350 nm smoothly delivered the desired annulation product as a 1:1 mixture of C-9 diastereomers, which converged to **20** upon treating with diisopropylamine in methanol. The photo-Nazarov cyclization reaction of aryl vinyl ketones was first reported by Smith and Agosta.[28] Subsequent mechanistic studies by Leitich and Schaffner revealed the reaction mechanism to be thermal electrocyclization induced by photolytic enone isomerization.[29] The mildness of the reaction conditions and the selective activation of the enone functional group allowed the facile transformation of the densely functionalized **19** to **20**. It should be noted that the Lewis acid-promoted Nazarov cyclization of aryl vinyl ketones normally requires much harsher reaction conditions or activated substrates.[30] Indeed, exposure of the model systems of **19** with simplified side chains to various Lewis acids only resulted in substrate decomposition.

To complete the synthesis of **1**, we first removed the acetonide protecting group of **20** and cleaved the diol to afford the corresponding bis-hemiacetal. Selective reduction of the less hindered hemiacetal followed by TBS deprotection[31] furnished **1**. The spectroscopic data of **1** is fully consistent with that of the natural sample.[11] In contrast, synthetic **2**, which was obtained by a similar approach,[9,11] exhibits significantly different ^{1}H and ^{13}C NMR spectra. Notably, synthetic **2** existed as an equilibrium mixture of the C-4 hemiacetal and aldehyde forms.[11] We have also compared the chemical shifts of the Mosher esters of synthetic **1** with those

reported for the natural product and found that they were in agreement.[11,32]

In conclusion, the structure of nakiterpiosin is revised as **1**. The newly assigned structure is supported by total synthesis and biogenesis rationale. Our synthetic approach is highly convergent and allows for the synthesis of the analogues and derivatives of **1** for further biological studies. We are investigating the biological function and molecular target of **1**. These results will be reported in due course.

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Supporting Information Available: NMR spectroscopic data of synthetic **1** and **2**, CD spectrum of synthetic **1**, synthetic route for **2**, experimental procedures, crystallographic data, and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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