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Chemical and Biological Studies of Nakiterpiosin and Nakiterpiosinone

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Abstract: Nakiterpiosin and nakiterpiosinone are two related C-nor-D-homosteroids isolated from the sponge *Terpios hoshinota* that show promise as anticancer agents. We have previously described the asymmetric synthesis and revision of the relative configuration of nakiterpiosin. We now provide detailed information on the stereochemical analysis that supports our structure revision and the synthesis of the originally proposed and revised nakiterpiosin. In addition, we herein describe a refined approach for the synthesis of nakiterpiosin, the first synthesis of nakiterpiosinone, and preliminary mechanistic studies of nakiterpiosin's action in mammalian cells. Cells treated with nakiterpiosin exhibit compromised formation of the primary cilium, an organelle that functions as an assembly point for components of the Hedgehog signal transduction pathway. We provide evidence that the biological effects exhibited by nakiterpiosin are mechanistically distinct from those of well-established antimitotic agents such as taxol. Nakiterpiosin may be useful as an anticancer agent in those tumors resistant to existing antimitotic agents and those dependent on Hedgehog pathway responses for growth.

Introduction

Terpios is a genus of thin, encrusting sponges that harbor large amounts of symbiotic bacteria.[1] These sponges are known to aggressively compete with corals for space by epizoism. During 1981−1985, large patches (up to 1000 m in length) of the cyanobacteriosponge *Terpios hoshinota* were observed in Okinawa. They killed large populations of corals in that region and were known as "black disease". Uemura and co-workers[2] hypothesized that *T. hoshinota* could secrete toxic compounds and kill the covered corals. In a search for these toxins, they isolated 0.4 mg of nakiterpiosin and 0.1 mg of nakiterpiosinone from 30 kg of the sponges. Both compounds inhibited the growth of P388 mouse leukemia cells with a mean inhibitory concentration (IC_{50}) of 10 ng/mL. The structures of these compounds were originally assigned as **3** and **4** on the basis of NMR experiments (Figure 1). They bear a unique molecular skeleton and several unusual functional groups. To date, they are the only C-nor-D-homosteroids isolated from a marine source. We recently proposed a revision of their relative configurations to those shown in **1** and **2** by analyzing spectroscopic data for their fragments. We further synthesized

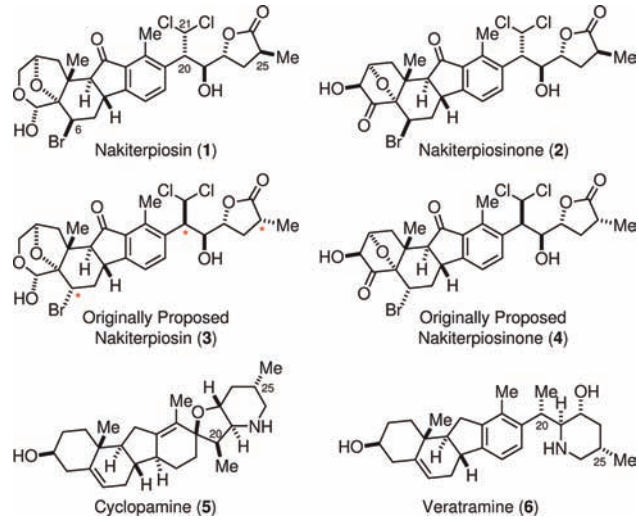

Figure 1. Structures of C-nor-D-homosteroids **1**−**6**.

1 and **3** and confirmed that the structure of nakiterpiosin should be **1**.[3] We present herein the details of our synthetic studies and spectroscopic analysis of nakiterpiosin (**1**) and 6,20,25-*epi*-nakiterpiosin (**3**). In addition, we report in this article an improved synthesis of **1**, the first synthesis of nakiterpiosinone (**2**), and the results of our preliminary biological studies of **1**.

C-nor-D-homosteroids are skeletally rearranged steroids in which the C ring is contracted and the D ring expanded by one carbon. The first known, and arguably best known, members

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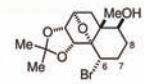
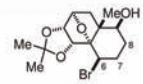
Table 1. Probing the C-6 Configurations of Nakiterpiosin and Nakiterpiosinone

	Nakiterpiosin	Nakiterpiosinone	 (6*S*)-**7**	 (6*R*)-**8**	 (6*S*)-**9**	 (6*R*)-**10**
J_{H6-H7a}	2.7 Hz	2.3 Hz	12.2 Hz	3.2 Hz	11.6 Hz	3.2 Hz
J_{H6-H7b}	1.4 Hz	1.4 Hz	5.6 Hz	2.8 Hz	5.5 Hz	2.8 Hz

of this family of natural products are cyclopamine (**5**) and veratramine (**6**). The discovery of these veratrum alkaloids is a rather interesting story.[4] Ranchers in the Rocky Mountain region had long been puzzled by a mysterious birth defect in their sheep. They found that 1−20% of the lambs were born as "chattos" (or "monkey faces") when the mother sheep grazed in the national forests in central Idaho during the summer. The Poisonous Plant Research Laboratory of the U.S. Department of Agriculture was contacted in 1954 to investigate this "malformed lamb disease". After 11 years of work, they found that ewes that grazed on corn lily (*Veratrum californicum*) on the *14th* day of gestation would give birth to cyclopic lambs, while the ewes were left unaffected. They further found that **5** was responsible for the one-eye face malformation and that **6** led to leg deformity.[4a] However, it was not until 30 years later that the molecular target of **5** was identified to be Smoothened (Smo).[5] Suppression of Hedgehog (Hh) signaling by inhibition of Smo with small molecules has since been pursued as a new strategy for cancer treatment.[6] The semisynthesis of **5** and **6** was first accomplished by Masamune and Johnson in 1967[7a−e] and recently by Giannis.[7g] A formal synthesis was also reported by Kutney in 1975.[7f]

Results and Discussion

Revision of the Relative Stereochemistry. The unique molecular structures and strong P388 growth inhibition activity of nakiterpiosin **1** and nakiterpiosinone **2** prompted us to initiate a research program to explore their laboratory synthesis[8] and biological functions. At the onset of this project, we noticed that the C-20 and C-25 configurations of the originally proposed structures for nakiterpiosin and nakiterpiosinone (i.e., **3** and **4**, respectively)[2] were opposite to those of cyclopamine **5** and veratramine **6** (Figure 1) and other "normal" steroids. In addition, the C-6 and C-20 configurations of **3** and **4** could not be easily rationalized on the basis of biogenesis analysis.[10] We further considered that the reported nuclear Overhauser effect data for the natural products[2] provided little support for the proposed stereochemistry. Most importantly, the ^{1}H NMR spectra of our synthetic intermediates leading to **3** displayed significantly different H-6 and H-21 splitting patterns than the natural products. We therefore decided to first study the relative stereochemistry of nakiterpiosin and nakiterpiosinone using model systems.

In order to probe the C-6 stereochemistry of the natural products, we synthesized a series of compounds (**7**−**10**)[11] that bear the A,B,C ring systems of nakiterpiosin and nakiterpiosinone. We found that the J_{H6-H7} values of **7** and **9**, which bear the same C-6 configuration as nakiterpiosin and nakiterpiosinone, were very different from those of the natural products (Table 1).[12] On the other hand, we found that the corresponding C-6 epimers (**8** and **10**) and the natural products exhibited similar H-6 splitting patterns. We therefore concluded that the C-6 stereocenters in nakiterpiosin and nakiterpiosinone have the *R* configuration.

In regard to the stereochemistry of the side chain of nakiterpiosin and nakiterpiosinone, we first focused on the C-20/C-22/C-23 relative configuration. We synthesized all four possible diastereomers (**11**−**14**)[11] and compared their NMR spectra with those of the natural products. We found that the

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(9) For a review of structure revisions of natural products through synthetic studies, see: Nicolaou, K. C.; Snyder, S. A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1012–1044.

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(11) For details, see the Supporting Information.

(12) The chemical shifts of H-6 in **7**−**10** and the residual H_2O are too close in CD_3OD for the accurate measurement of the coupling constants. We therefore recorded their ^{1}H NMR spectra in $CDCl_3$. We reasoned that the rigid polycyclic ring systems of **7**−**10** would adopt conformations similar to those of the natural products even in different solvents. Therefore, the coupling constants should not be significantly influenced by the NMR solvent.

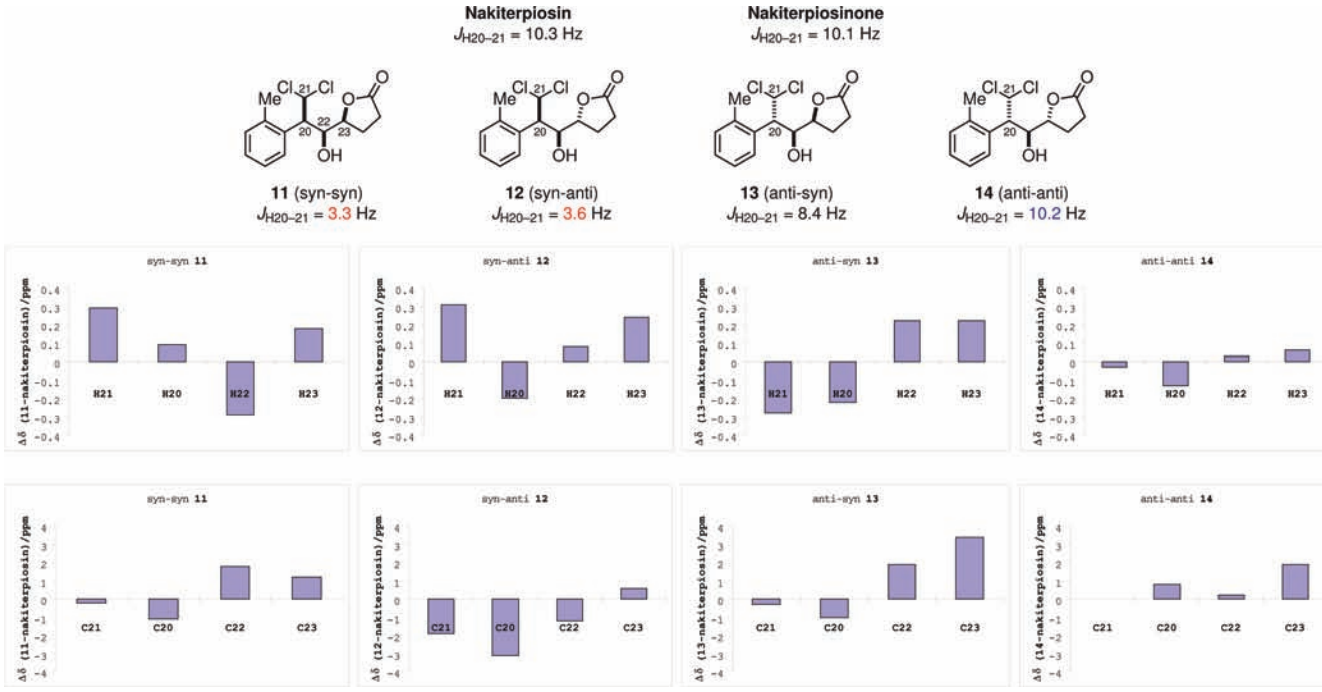

Figure 2. Probing the C-20/C-22/C-23 configurations of nakiterpiosin and nakiterpiosinone.

Table 2. Studies of the C-25 Configurations of Nakiterpiosin and Nakiterpiosinone

	Nakiterpiosin	Nakiterpiosinone	**15** (anti-anti-cis)	**16** (anti-anti-trans)
$J_{H20-H21}$	10.3 Hz	10.1 Hz	10.2 Hz	10.1 Hz
$J_{H23-H24a}$	8.2 Hz	8.2 Hz	10.0 Hz	8.0 Hz
$J_{H23-H24b}$	3.7 Hz	3.7 Hz	5.8 Hz	3.9 Hz
$J_{H24a-H25}$	8.4 Hz	8.2 Hz	12.1 Hz	8.3 Hz
$J_{H24b-H25}$	N/A	9.2 Hz	8.8 Hz	9.4 Hz

$^3J_{H-H}$ coupling constants and ^1H and ^{13}C chemical shifts of **11** (syn−syn), **12** (syn−anti) and **13** (anti−syn) were considerably different from those of the natural products (Figure 2). In particular, the $J_{H20-H21}$ values for **11** (syn−syn) and **12** (syn−anti) indicated a gauche instead of an anti H-20/H-21 conformation. Only **14** (anti−anti) exhibited ^1H and ^{13}C NMR spectra similar to those of the natural products.[13] We therefore revised the C-20 stereochemistry of nakiterpiosin and nakiterpiosinone to be *S*.

After revising the C-20 stereochemistry, we turned our attention to the C-25 configuration of nakiterpiosin and nakiterpiosinone and synthesized the two model substrates **15** and **16**.[11] We found that the coupling constants and ^1H and ^{13}C NMR chemical shifts of (25*S*)-**16** (anti−anti−trans) but not (25*R*)-**15** (anti−anti−cis) matched well with those of the natural products (Table 2 and Figure 3). In particular, the $J_{H23-H24a}$, $J_{H23-H24b}$, and $J_{H23-H25}$ values for **15** and the natural products were significantly different. We therefore determined that the C-25 stereocenters in nakiterpiosin and nakiterpiosinone have the *S* configuration.

The revised structures of nakiterpiosin **1** and nakiterpiosinone **2** have the same configurations at C-20 and C-25 as **5** and **6**. The C-6 and C-20 configurations are also consistent with the stereochemical outcomes of enzymatic halogenation of the steroid (Figure 4).[9] The C-21 chlorine atoms of nakiterpiosin are likely introduced by nonheme iron halogenase through radical chlorination. It was expected that this reaction would lead to retention of the C-20 configuration. On the other hand, the C-6 bromine atom is likely introduced by vanadium-dependent bromoperoxidase through bromoetherification. It is therefore expected that the C-5,6 bromohydrin would exist in an anti stereochemical relationship.

On the basis of these results, we proposed the revision of the relative stereochemistry of nakiterpiosin and nakiterpiosinone to that shown for **1** and **2**, respectively. Indeed, we found that the ^1H and ^{13}C NMR spectra of our synthetic sample of **1** agree with those of the natural product. In contrast, those of synthetic

(13) The ^{13}C NMR spectrum of nakiterpiosin was misreferenced by ca. −2 ppm in the original reports. We adjusted the reported data by +2.0 ppm in this article.

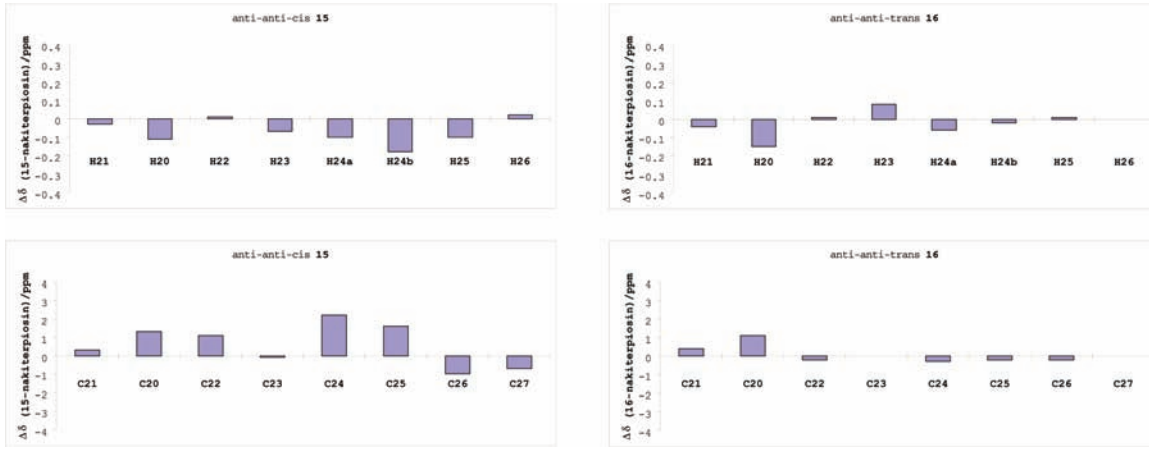

Figure 3. Probing the C-25 configurations of nakiterpiosin and nakiterpiosinone.

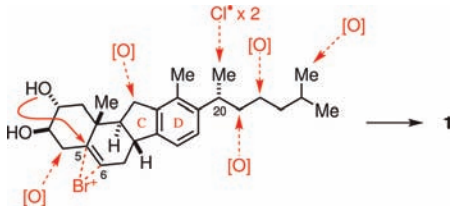

Figure 4. Biogenetic analysis of nakiterpiosin.

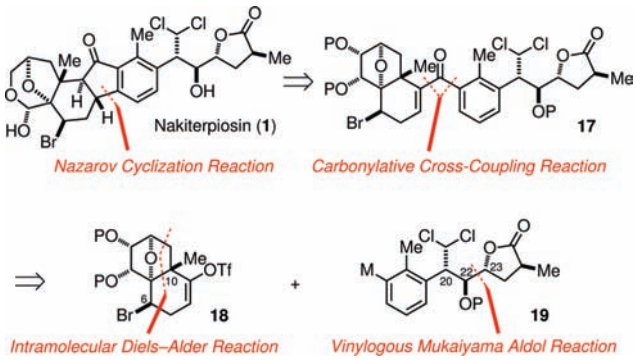

Figure 5. Retrosynthetic analysis for nakiterpiosin.

3 and the natural product are significantly different. We thus revised the relative stereochemistry of nakiterpiosin to be that indicated in **1**, which has the same configuration at C-20 and C-25 as cyclopamine **5** and veratramine **6**.

Synthetic Plans. After determining the correct stereochemistry of nakiterpiosin and nakiterpiosinone, we sought to develop a general strategy to target these two steroid derivatives. Our first-generation approach[3] is outlined in Figure 5. We opted to construct the central cyclopentanone ring at a late stage for convergence reasons. We envisioned that a carbonylative cross-coupling reaction[14−20] together with a Nazarov cyclization reaction[21−23] could be used to effectively assemble this cyclopentanone ring. We recognized that the Nazarov cyclization of vinyl aryl ketone **17** would involve a disruption of the aromaticity and would therefore have an activation energy significantly higher than that of the divinyl systems.[22] However, we believed that a suitable set of reaction conditions could be found to realize this highly efficient plan. We also expected that **17** could be used as a versatile intermediate for synthesizing not only **1** but also **2**.

It should be noted that the carbonylative cross-coupling of **18** and **19** is also considerably challenging because of their sensitive functionalities and congested reaction sites. Compared with the recent advancement in transition-metal-catalyzed cross-coupling,[24,25] catalytic carbonylative cross-coupling is relatively underdeveloped.[15] Suppression of the direct coupling reactions when more nucleophilic coupling components are used remains challenging. In general, the Stille and Suzuki carbonylative coupling reactions are more successful because of the lower rate of transmetalation. We later found that the nearly

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neutral conditions of the Stille carbonylative reaction are crucial to the successful implementation of this strategy for the coupling of highly acid- and base-sensitive **18** and **19**.

For the synthesis of the coupling fragments, we envisioned that the electrophilic coupling component **17** could be synthesized by an intramolecular Diels−Alder reaction[26,27] and the nucleophilic coupling component **18** by a vinylogous Mukaiyama aldol reaction.[28,29] It should be noted that intramolecular Diels−Alder reactions of furan derivatives are normally exo-selective and much less kinetically and thermodynamically favored because of the aromaticity of furan and the ring strain of the product.[26b] The exo selectivity should lead to the desired relative stereochemistry between the oxo bridge and angular methyl group. We planned to use the C-6 stereogenic center to control the diastereoselectivity of this reaction. The use of a substituent group on the tether α to the diene to control the diastereoselectivity was first studied by Roush, Taber, and Boeckman.[27] Since the C-6 bromine atom resides at the axial position, we anticipated that introduction of the bromine atom into the cycloaddition product with an inversion of configuration would set up the correct C-6 configuration.

In terms of the vinylogous Mukaiyama aldol reaction, we planned to use the C-20 stereocenter to control the installation of the C-22 and C-23 stereocenters. We were able to use this versatile approach to obtain a diverse array of C-20/C-22/C-23 diastereomers (**11**−**16**: syn−syn, syn−anti, anti−syn, anti−anti) for the previously described model studies.[11] The absolute stereochemistries of **18** and **19** were established by the Noyori reduction[30] and Sharpless epoxidation[31] reactions.

First-Generation Synthesis of Nakiterpiosin. We previously reported our first synthetic approach to **1** (Scheme 1).[3] This sequence commenced with the Friedel−Crafts acylation of furan (**20**) with succinic anhydride (**21**)[32] and the formation of Weinreb amide **23** from acid **22**. Subsequently, a Noyori reduction[30] was used to set the C-6 stereochemistry. While a significant amount of dehydrated lactone byproduct was formed under the conventional NEt_3/HCOOH azeotropic conditions,[30a,b] we found that the in-water protocol developed by Xiao[30c−e] provided significant enhancement of the reaction rate and allowed low catalyst loading. Alcohol **24** was obtained in 91% ee without the formation of the undesired lactone. The dieneno-

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Scheme 1. Synthesis of the Electrophilic Coupling Component **30**

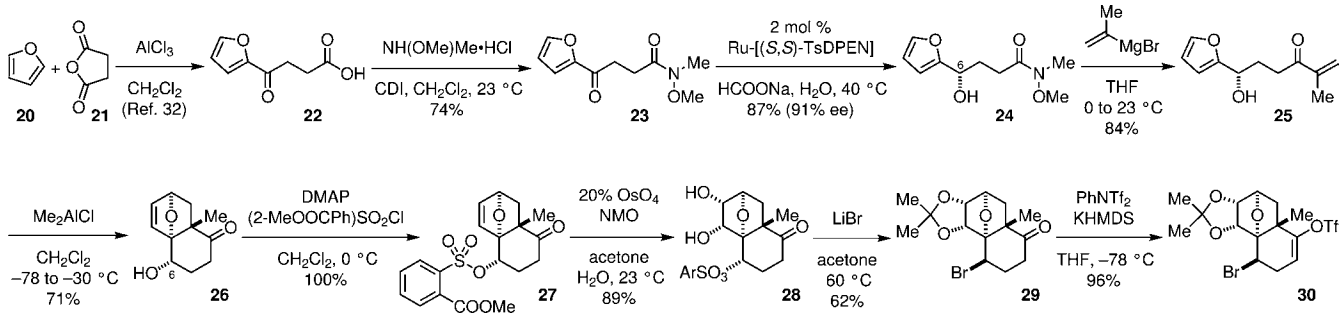

Table 3. Influence of the Lewis Acid on the Intramolecular Diels–Alder Reaction of **25**

entry	Lewis acid	equiv	**25**/**26**	note
1	Me_2AlCl	2.5	0:100	
2	Me_2AlCl	1.0	30:70	
3	Me_2AlCl	0.1	100:0	
4	$MeAlCl_2$	1.0	15:85	with decomposition
5	$MeAlCl_2$	0.1	100:0	

phile was then introduced into **24** by addition of isopropenyl Grignard reagent, giving enone **25** and setting the stage for the Diels–Alder cyclization.

It has been shown that the conversion in the intramolecular furan Diels–Alder reaction is highly dependent on the relative basicity of the reactant and product and the stoichiometry of the Lewis acids.[26b] We found that for the reaction of **25**, 2.5 equiv of Me_2AlCl provided the best yield of **26** (Table 3), indicating that the reaction was driven by the complexation of Me_2AlCl to **26**. The reaction proceeded with good stereochemical control and gave only the desired exo product with an equatorial hydroxyl group. When the C-6 hydroxyl group of **25** was protected as a triethylsilyl (TES) ether, the reaction proceeded with the opposite enantiomeric preference and provided the exo product with an axial TES ether group.[11]

Since the cycloaddition product **26** was not stable and underwent a retro-Diels–Alder reaction upon gentle heating or exposure to other Lewis acids, we dihydroxylated the olefin before the introduction of the bromine atom to prevent the retro-Diels–Alder reaction. The steric congestion around the C-6 position necessitated the use of an electron-deficient arylsulfonate group (**27**) for 6-OH activation. Thus, treatment of **28** with lithium bromide in warm acetone allowed the introduction of the bromine atom with inversion of configuration and concomitant acetonide protection to give **29**. The use of an electron-deficient sulfonate was crucial to the success of this S_N2 reaction. 4-Toluenesulfonate (Ts) was unreactive and 4-nitrophenylsulfonate (Ns) less reactive under these reaction conditions. The absolute and relative configurations of **29** were confirmed by X-ray analysis.[11] Finally, enol triflate installation completed the synthesis of the electrophilic coupling component **30**.

The synthesis of the nucleophilic coupling component **47** (Scheme 2) started with reduction of acid **31** to aldehyde **33** followed by Horner–Wadsworth–Emmons reaction and reduction of the resulting ester to afford **35**. Asymmetric installation of the C-20 stereocenter was achieved by Sharpless epoxidation with 92% ee.[31] The resulting alcohol was protected as a *tert*-butyldimethylsilyl (TBS) ether and then subjected to the pinacol-type rearrangement using Yamamoto's conditions.[33] We previously reported that aldehyde **38** was obtained with significant erosion of enantiomeric purity (71% ee).[3] We have since determined that the loss of ee occurred during workup of the reaction and purification of **38** by column chromatography. Quenching the reaction with 1 N HCl aqueous solution instead of sodium fluoride afforded **38** with 90% ee. Aldehyde **38** should be used directly without purification. We also found that while low conversions were observed when catalytic amounts of Yamatomo's aluminum salt were used for the reaction of **37**, only 5 mol % Cr(TPP)(OTf)[33e] was needed to drive this reaction to completion. However, a signification amount of ketone was also obtained (aldehyde/ketone = 3:1).

The previously reported problem of the subsequent vinylogous Mukaiyama aldol reaction was also solved. The bismuth(III) triflate-catalyzed reaction between purified **38** (71% ee) and 3-methyl-2-(triisopropylsiloxy)furan yielded **39** with only 56% conversion, 11:1 dr, and 60% ee.[3] We recently found that 2 equiv of tin(II) triflate promoted this reaction with 83% conversion. Alcohol **39** was obtained from crude **38** as the only diastereomer in 80% isolated yield and 90% ee. For large-scale reactions, we found that boron trifluoride diethyl etherate was a more economical Lewis acid. Full conversion could be obtained and no loss of ee was observed. It provided **39** in comparable yields despite having a diastereoselectivity of only 4:1. Results from the survey of fourteen different Lewis acids can be found in the Supporting Information.

We previously used the Crabtree catalyst[29b,34] to direct the hydrogenation of **39** and set the desired C-25 configuration of **40** with moderate diastereoselectivity (6:1 dr). We later found that **40** could be obtained as the only diastereomer by conjugate reduction with L-Selectride. Overall, these new protocols are much more cost-effective and provide significantly improved yields and stereoselectivities of the desired products.

To invert the C-22 configuration, we oxidized **40** with Dess–Martin periodinane and reduced the resulting ketone with sodium borohydride. We then protected the C-22 alcohol as a TBS ether, selectively removed the C-21 TBS protecting group, and oxidized the primary alcohol. Introduction of the C-21 *gem*-dichloride was achieved by reacting aldehyde **45** with Cl_2/

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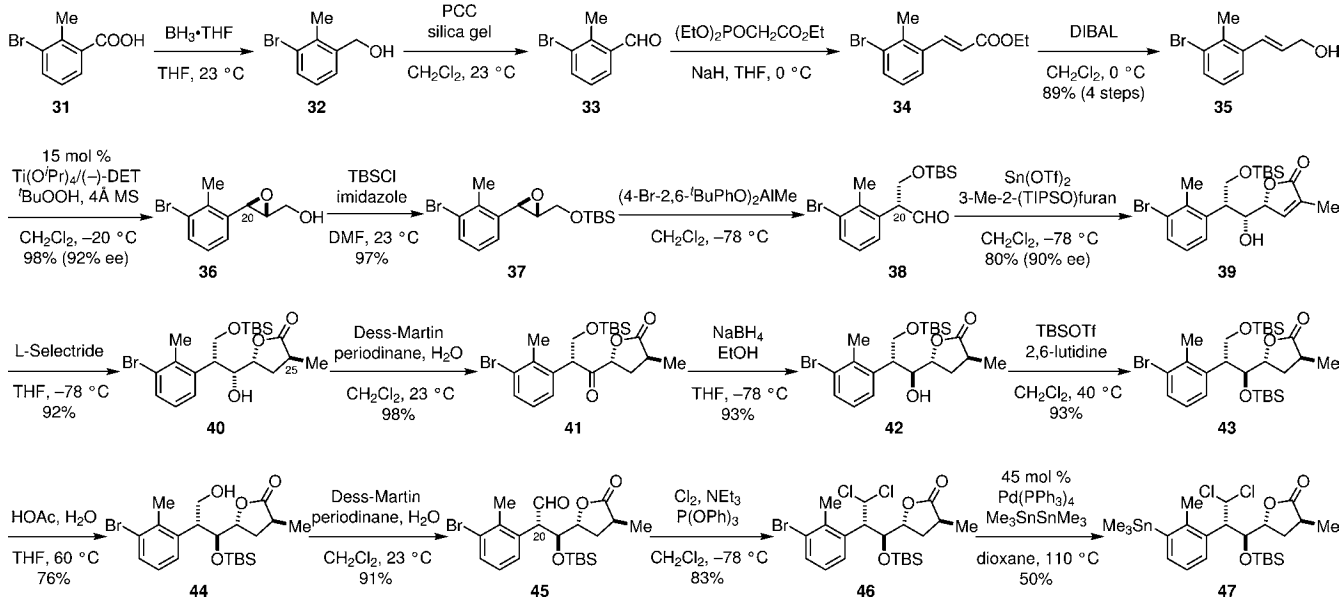
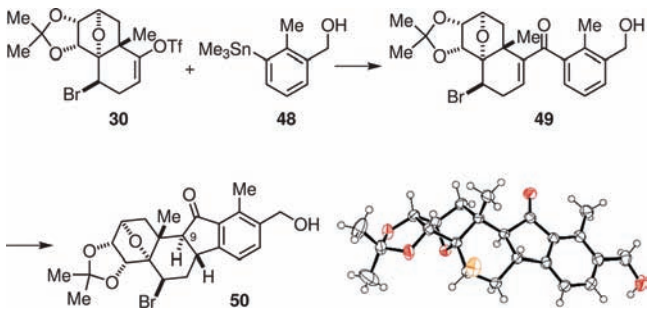
Scheme 2. Synthesis of the Nucleophilic Coupling Component **47**



Scheme 3. Model Studies of the Carbonylative Coupling/Nazarov Cyclization Strategy



$P(OPh)_3$.[35] The hydrazone method developed by Myers[36] was not compatible with the other functional groups of **45**. The relative and absolute stereochemistries of **46** were determined by single-crystal X-ray analysis after TBS deprotection.[11] The synthesis of the nucleophilic coupling component **47** was concluded by a palladium-catalyzed stannylation of **46**. Despite the high catalyst loading and moderate yield, this protocol continues to be the best method for the introduction of the trimethylstannane to **47**.

After the routes to both cross-coupling fragments had been established, our next goal was to realize the carbonylative coupling and Nazarov cyclization strategy to assemble the C ring of **1** and **2**. We used several model systems carrying a simplified side chain on the E ring to study these two key reactions (Scheme 3). We found that the carbonylative coupling of **30** and **48** could be achieved with a modified Stille's protocol to afford **49**.[16] After an extensive survey of the reaction conditions, we concluded that $Pd(PPh_3)_4$ is the best promoter, that copper(I) chloride additive[37] is crucial, and that dimethyl sulfoxide (DMSO) is the ideal solvent for this reaction. The use of catalytic amounts of $Pd(PPh_3)_4$ gave only small amounts of the desired product. No reaction occurred when $Pd(OAc)_2$ was used in combination with $AsPh_3$, $P(furyl)_3$, PCy_3, and $P(t\text{-}Bu)_3$ under otherwise identical conditions. The use of $Pd(OAc)_2/P(OMe)_3$ led mainly to decomposition. It is also worth noting that **49** was highly sensitive to both acids and bases. Addition of lithium chloride, prolonged heating, and employment of base-promoted carbonylative Suzuki coupling protocols led to the elimination of the bromine atom.

The sensitive nature and aromaticity of **49** made the implementation of the Nazarov cyclization strategy challenging. Normally, Lewis acid-promoted Nazarov cyclizations of aryl vinyl ketones require harsh reaction conditions or activated substrates.[22] Indeed, exposure of **49** to various Lewis acids resulted only in substrate decomposition. However, we found that irradiation of a solution of **49** in acetonitrile at 350 nm readily delivered the desired annulation product **50** in its enol form, which tautomerized to the ketone form as a 1:1 mixture of C-9 diastereomers upon addition of ammonium chloride. Treating this mixture with diisopropylamine in warm methanol gave the desired diastereomer **50**, the structure of which was confirmed by X-ray analysis.[11] The reaction can also be carried out in methanol to directly give the C-9 diastereomeric mixture of **50** with slightly lower yield. The photo-Nazarov cyclization reaction of aryl vinyl ketones was first reported by Smith and Agosta.[23c] Subsequent mechanistic studies by Leitich and Schaffner[23d,e] revealed the reaction mechanism to be a thermal electrocyclization induced by photolytic enone isomerization. The mildness of the reaction conditions and the selective activation of the enone functional group were key to the success of this reaction.

To complete the synthesis of **1**, we carried out the carbonylative coupling of **30** and **47** under the optimized conditions to give **51**, which was effectively transformed to **53** upon photolysis and C-9 epimerization (Scheme 4). The previous

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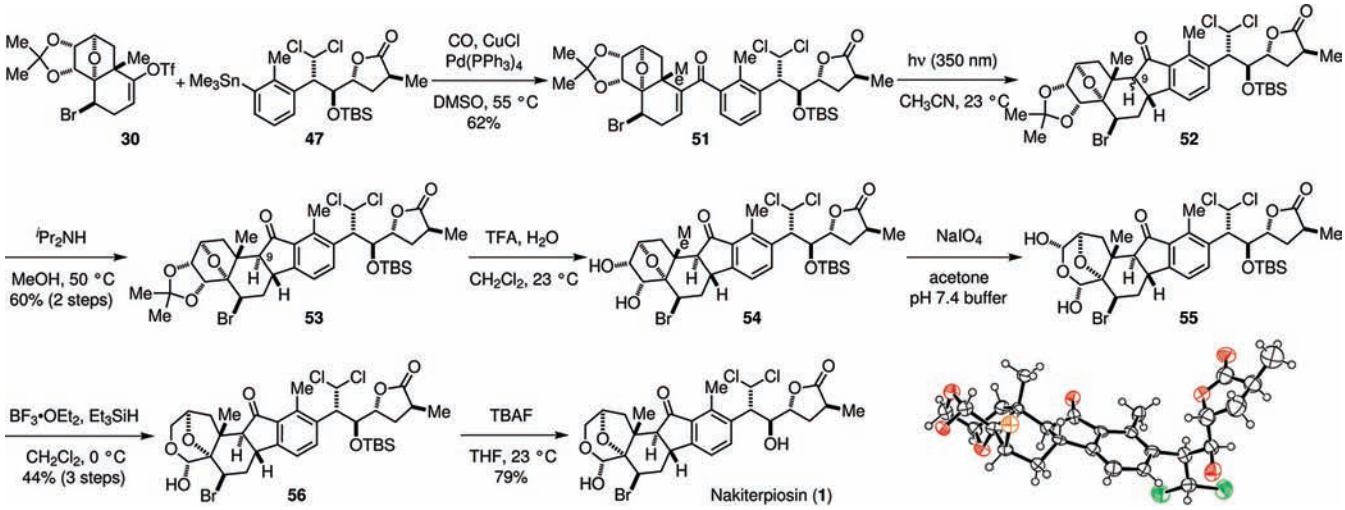
Scheme 4. Completion of the Synthesis of Nakiterpiosin (**1**)



stereochemical issues involving **51** were solved (99.5% ee and dr 10:1) because **47** could be prepared with high enantiomeric purity.

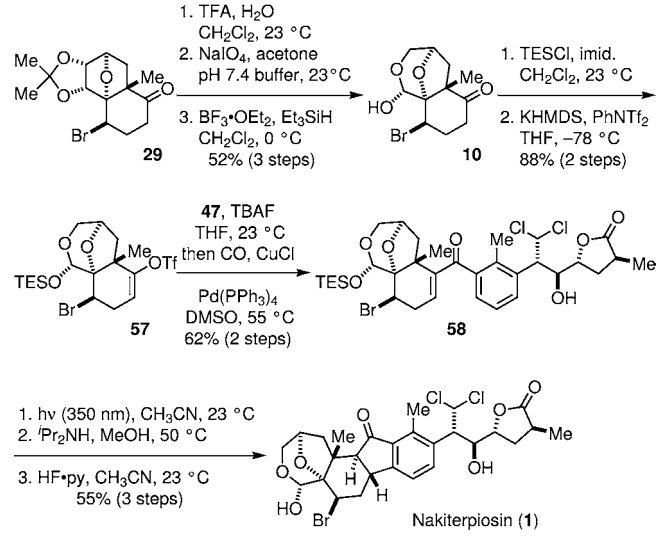
With the central C ring in place, the remaining task was to assemble the A ring of **1**. We first removed the acetonide protecting group of **53** and cleaved the diol of **54** to afford bishemiacetal **55**. Selective reduction of the less hindered hemiacetal of **55** followed by TBS deprotection[38] concluded the synthesis of **1**. A single crystal suitable for X-ray analysis[11] was recently obtained by evaporation of the solvent from a 30% ethyl acetate/hexanes solution of **1**, allowing unambiguous determination of its relative and absolute configurations.

To confirm our proposal for the structure revision of **1**, we compared the ^{1}H and ^{13}C NMR spectra of our synthetic **1** with those of the natural product.[2] We were pleased to find that the chemical shift differences for all peaks were within 0.03 and 0.2 ppm for the ^{1}H and ^{13}C signals, respectively, and that the splitting patterns for all of the ^{1}H signals were also consistent with those of the natural product. While the optical rotation of the natural product was not reported, the Mosher ester analysis of our synthetic **1** was consistent with the data reported for the natural product. The $\Delta\delta^{SR}$ of H-4 was found to be +0.03 ppm in the 4-Mosher ester of **1**, compared to +0.04 ppm for that of H-4 in the 4,22-di-Mosher ester of the natural product. We also determined that nakiterpiosin **1** is levorotatory in methanol with $[\alpha]_D^{20} = -33°$ (*c* 0.067, MeOH).

Second-Generation Synthesis of Nakiterpiosin. While the sequence shown in Scheme 4 successfully delivered **1**, it required an additional six steps after the convergent fragment coupling reaction. We anticipated that all of the functional groups of **1** could tolerate the carbonylative coupling and photo-Nazarov cyclization reactions. We therefore decided to perform the C-ring construction on a fully elaborated system to improve the overall efficiency of the synthesis of **1** (Scheme 5).

In ketone **29**, we introduced the A-ring functionality using the chemistry previously described, generating **10**. We then protected the hemiacetal and installed the triflate to give **57**. We also removed the TBS protecting group of **47** prior to the carbonylative coupling. Pleasingly, the fragment coupling and photo-Nazarov cyclization proceeded in good yields. Subsequent

Scheme 5. An Improved Synthesis of Nakiterpiosin (**1**)



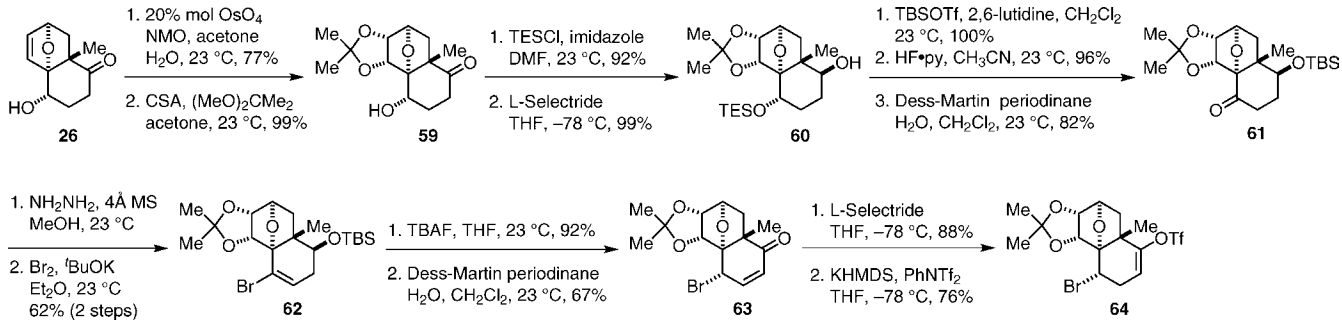
TES deprotection provided **1** with significantly improved efficiency. However, it was unfortunate that photolysis of des-TES-**58** resulted in significant amounts of decomposition.

Synthesis of 6,20,25-*epi*-Nakiterpiosin. To unambiguously determine the relative stereochemistry of nakiterpiosin, we also synthesized the originally proposed structure (6,20,25-*epi*-nakiterpiosin, **3**) and compared its NMR spectra with those of the natural product. Several approaches were explored in an attempt to introduce the C-6 bromine atom. We first sought to use the C-6 bromine atom as the stereochemistry-controlling element for the intramolecular Diels–Alder reaction and set its equatorial configuration. However, nucleophilic substitution of the C-6 hydroxyl group of **24** under various conditions failed to deliver the desired product. The use of a chelating leaving group[39] did not result in retention of configuration, and an S_N2 instead of S_Ni reaction occurred. Equally fruitless was the effort to replace the hydroxyl group of **26** or its derivatives with a bromine atom through radical chemistry. Eventually, we found that the desired C-6 configuration could be set through isomerization of vinyl bromide **62** (Scheme 6).

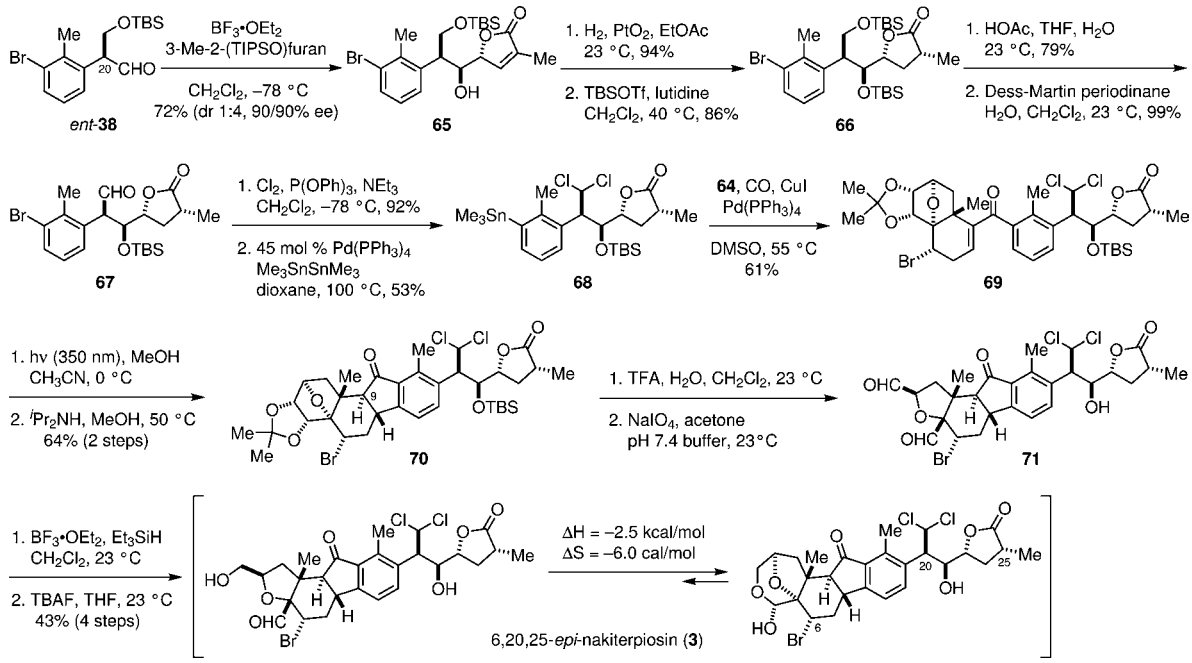
(38) For a convenient workup method, see: Kaburagi, Y.; Kishi, Y. *Org. Lett.* **2007**, *9*, 723–726.

(39) (a) Lepore, S. D.; Mondal, D. *Tetrahedron* **2007**, *63*, 5103–5122. (b) Braddock, D. C.; Pouwer, R. H.; Burton, J. W.; Broadwith, P. *J. Org. Chem.* **2009**, *74*, 6042–6049.

Scheme 6. Synthesis of **64** (6-*epi*-**30**)



Scheme 7. Synthesis of 6,20,25-*epi*-Nakiterpiosin (**3**)



Starting from **26**, the olefin was first dihydroxylated and the resulting diol protected as an acetonide. The C-6 hydroxyl group of **59** was then protected and the ketone group reduced to afford **60**. Subsequent TBS protection, TES deprotection, and alcohol oxidation provided **61**. The vinyl bromide was then introduced by the Barton−Myers method to give **62**.[36,40] Removal of the TBS protecting group and alcohol oxidation under Dess−Martin conditions induced olefin isomerization to yield enone **63**. Finally, conjugate reduction yielded a crystalline ketone, allowing unambiguous assignment of the C-6 configuration.[11] Conversion of the ketone to the enol triflate furnished electrophilic coupling component **64** (6-*epi*-**30**).

To set the syn−anti−cis configuration of the side chain, an anti-selective vinylogous aldol reaction was need. Despite the fact that efforts to reverse the syn selectivity failed, we found that a useful amount of the desired diastereomer could be obtained with any of the following Lewis acids: boron trifluoride diethyl etherate, zirconium(IV) chloride, ytterbium(III) triflate, or scandium(III) triflate.[11] Reaction of *ent*-**38** and 3-methyl-2-(triisopropylsiloxy)furan (Scheme 7) gave *syn-anti*-**65** as the minor diastereomer (syn−syn/syn−anti = 4:1). The C-25 configuration was then set by catalytic hydrogenation using Adams' catalyst without debromination. Protection of the alcohol as a TBS ether gave the desired *syn-anti-cis*-**66**.

With the protocols previously described for the synthesis of **47**, the *gem*-dichloromethyl and stannyl groups were then introduced to provide **68**. The relative stereochemistry of **68** was confirmed by X-ray analysis of a racemic sample after removal of the TBS protecting group.[11] Its carbonylative coupling with **64** and the subsequent photolysis reaction proceeded uneventfully to yield **70**. Interestingly, cleavage of the diol gave **71** as a bisaldehyde instead of a bisacetonide. Nevertheless, reduction of **71** with Et_3SiH in the presence of $BF_3 \cdot OEt_2$ induced the acetal formation. After TBS deprotection, 6,20,25-*epi*-nakiterpiosin **3** was obtained as an equilibrium mixture of the C-4 aldehyde and hemiacetal forms. Through variable-temperature NMR experiments, we determined the ΔH and ΔS values for this equilibrium to be −2.5 kcal/mol and −6.0 cal mol^{-1} K^{-1}, respectively.[11] By comparing the ^1H and ^{13}C NMR spectra of **1**, **3**, and the natural product,[2] we confirmed that the structure of nakiterpiosin should be **1** instead of **3** (Table 4 and Figures 6 and 7).

Synthesis of Nakiterpiosinone. After completion of the synthesis of **1**, we turned our attention to the related steroid nakiterpiosinone **2**. Initially, we planned to selectively oxidize the C-4 hydroxyl group of diol **28** or **54** to accomplish this goal. However, we encountered considerable difficulties in utilizing

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Table 4. Selected ^{1}H NMR Chemical Shifts and Coupling Constants of Nakiterpiosin

H no.	natural sample[a]	synthetic **1**[b]	synthetic **3**[c]	H no.	natural sample[a]	synthetic **1**[b]	synthetic **3**[c]
6	4.70 (dd) J = 2.7, 1.4 Hz	4.71 (dd) J = 3.4, 2.5 Hz	4.74 (dd) J = 12.3, 5.1 Hz	21	6.32 (d) J = 10.3 Hz	6.32 (d) J = 10.0 Hz	6.66 (d) J = 3.6 Hz
7a	2.28 (m)	2.29 (ddd) J = 13.7, 12.7, 3.4 Hz	2.22 (ddd) J = 12.3, 12.0, 12.0 Hz	22	4.39 (dd) J = 8.0, 3.8 Hz	4.41 (dd) J = 7.9, 3.2 Hz	4.52 (dd) J = 10.2, 2.3 Hz
7b	2.74 (ddd) J = 13.4, 2.7, 1.4 Hz	2.75 (ddd) J = 13.7, 2.5, 2.3 Hz	2.94 (ddd) J = 12.0, 5.1, 2.7 Hz	24a	1.71 (ddd) J = 12.8, 8.4, 8.2 Hz	1.72 (ddd) J = 13.2, 8.2, 8.1 Hz	2.00 (m)
8	3.58 (m)	3.55 (ddd) J = 12.7, 9.4, 2.3 Hz	3.03 (ddd) J = 12.0, 9.1, 2.7 Hz	24b	2.28 (m)	2.29 (ddd) J = 13.2, 4.8, 3.9 Hz	2.00 (m)

[a] Recorded at 800 MHz, as reported by Uemura et al. (ref 2). [b] Recorded at 600 MHz. [c] Recorded at 500 MHz.

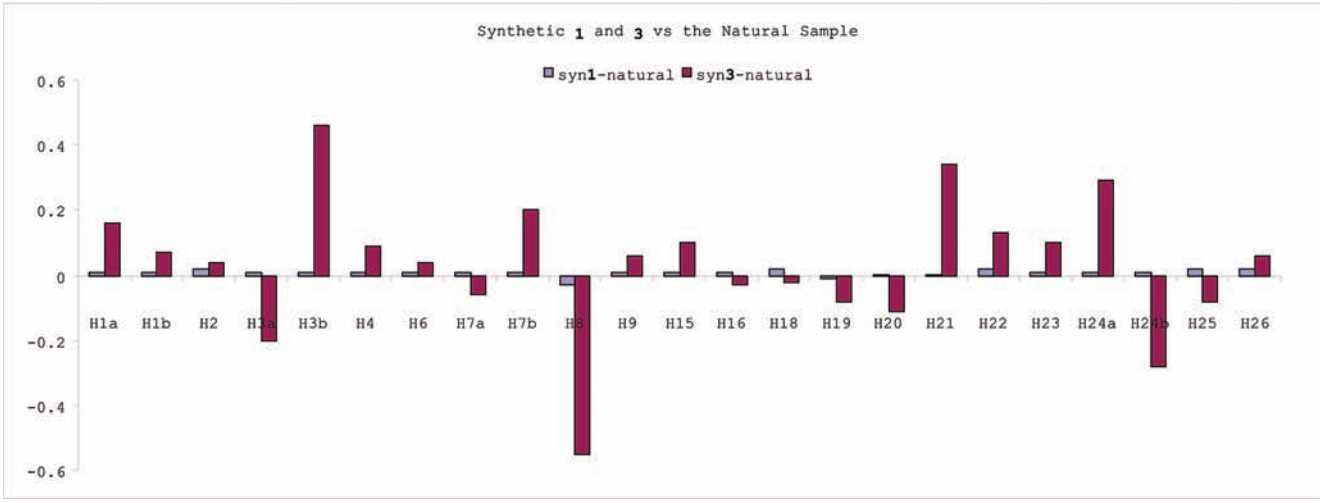


Figure 6. Differences in the chemical shifts in the ^{1}H NMR spectra of **1**, **3**, and the natural product.

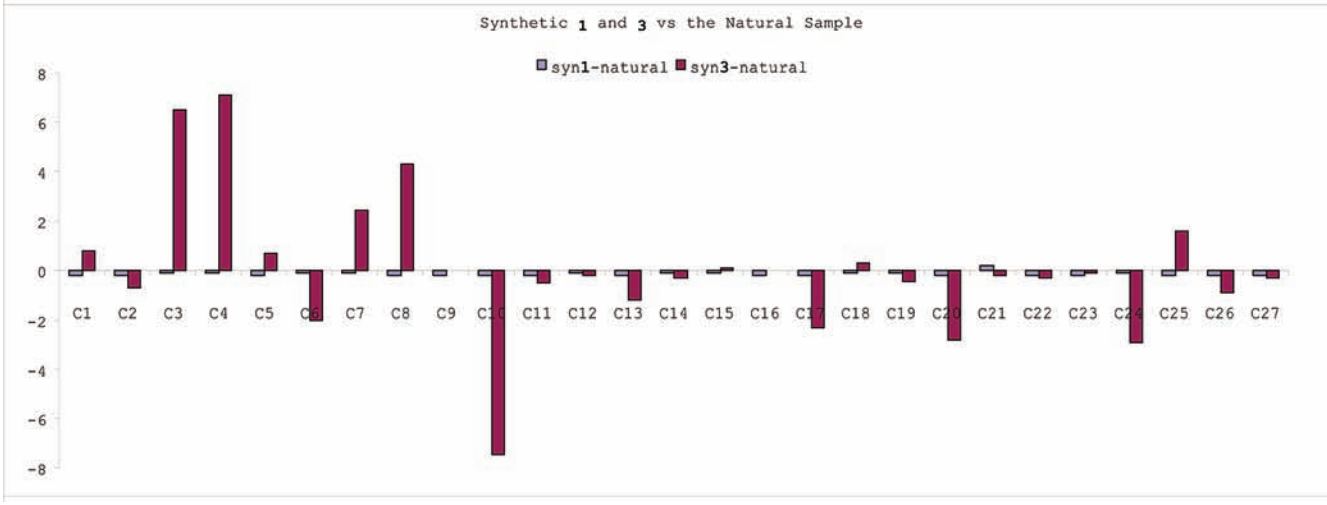
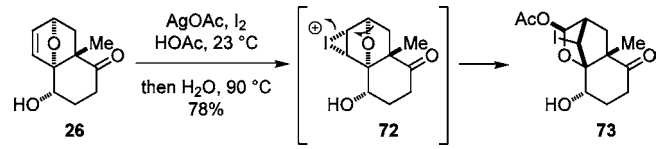


Figure 7. Differences in the chemical shifts in the ^{13}C NMR spectra of **1**, **3**, and the natural product.

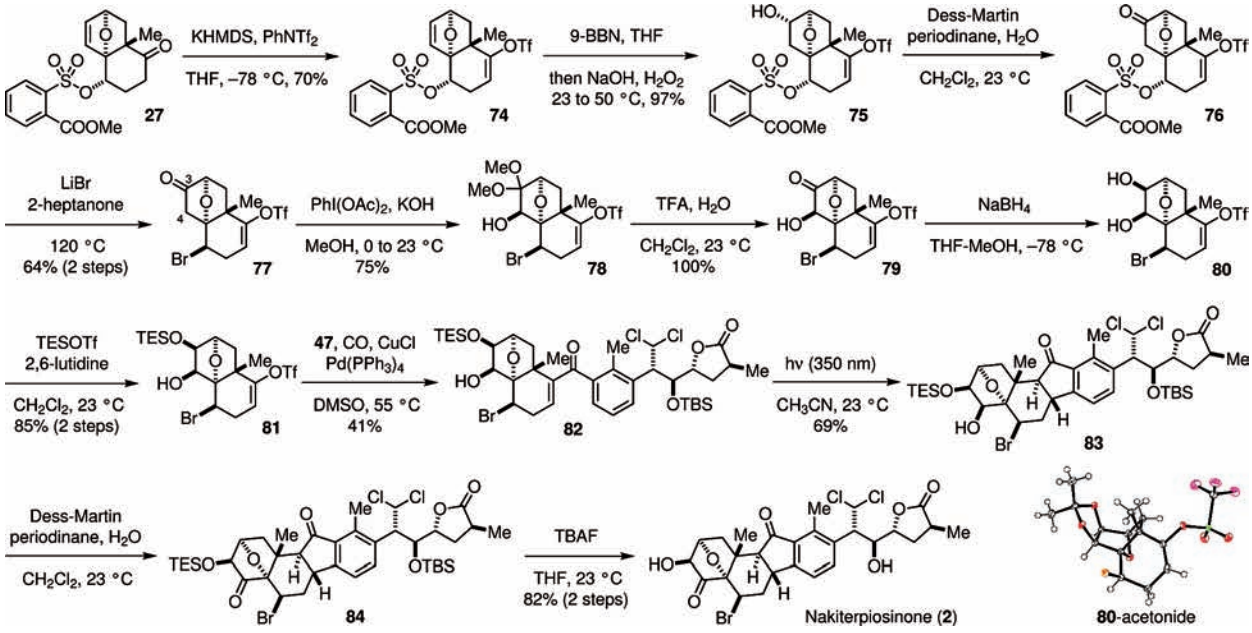
this strategy to synthesize **2.** First, despite the fact that we could selectively protect the C-3 hydroxyl group and oxidize the C-4 hydroxyl group of **28**, we were not able to perform C-3 epimerization without considerable decomposition. Next, our attempts to introduce a C-3 hydroxyl group to the more hindered β-face of **26** by Prévost−Woodward dihydroxylation[41] were complicated by the Wagner−Meerwein rearrangement of the [2.2.1] bicyclic system (Scheme 8).[42,43] Equally unsuccessful was the oxidation of diol **28** to the diketone because of diol cleavage or decomposition.[44] We therefore sought to install the C-3 stereocenter by an alternative approach.

Scheme 8. Rearrangement of the [2.2.1] Bicyclic System



Since the β-face of the A ring of **2** is significantly less hindered than its α-face, we decided to set the desired C-3 stereochemistry by reduction of the corresponding ketone (Scheme 9). Starting from **27**, the enol triflate was first installed,

Scheme 9. Synthesis of Nakiterpiosinone (**2**)



and the olefin of **74** was then hydroborylated with 9-bora[3.3.1]bicyclononane (9-BBN) to afford alcohol **75** as the only regioisomer after oxidative cleavage of the borane. Subsequently, the alcohol was oxidized to the ketone, and the bromine atom was introduced. The nucleophilic bromination reaction of **76** was much more difficult than that of **28**. Nevertheless, **77** could be obtained in good yield by reacting **76** with lithium bromide in 2-heptanone at 120 °C.

We next found α-oxidation of ketone **77** to be particularly challenging. Oxidation by the Rubottom method, Davis reagent, or selenium(IV) oxide was unsuccessful.[45] However, reaction of **77** with $PhI(OAc)_2$ under basic conditions gave **78** in good yield.[46] While oxidation of the C-4 ketone regioisomer of **77** may deliver the desired protected hydroxyketone directly, we found that hydroborylation of **74** with the complementary regioselectivity did not proceed with good efficiency. Deprotection of the dimethyl acetal of **78** and subsequent reduction of the resulting ketone **79** gave cis diol **80** with the desired C-3 configuration. The structure of **80** was confirmed by X-ray structural analysis of its acetonide derivative. Selective protection of the C-3 hydroxyl group of **80** afforded the electrophilic coupling component **81**. Carbonylative coupling of **81** with **47** and the subsequent photo-Nazarov cyclization proceeded smoothly to give **83**. Interestingly, ketone **83** with the desired 9*S* configuration was obtained directly as the only diastereomer. Finally, oxidation of the hydroxyl group and removal of the TES and TBS protecting groups concluded the synthesis of **2**. The ^1H NMR spectrum of **2** agreed with that of the natural product,[2b,11] confirming the structure of nakiterpiosinone to be **2**.

Biological Studies of Nakiterpiosin. Uemura and co-workers[2] initially reported that nakiterpiosin and nakiterpiosinone inhibit the growth of P388 mouse leukemia cells with an IC_{50} of 10 ng/mL. However, the limited amount of material that could be isolated from the sponge hampered further efforts to investigate the anticancer potential of these compounds. Having resolved the resupply issue through total synthesis, we are now pursuing further mechanistic studies focused on the anti-cell-proliferation properties associated with **1**.

The steroid skeleton of **1** is related to that of cyclopamine **5**, a potent Hh pathway antagonist. **5** inhibits Hh signaling by influencing the movement of Smo,[5a−c] a seven-pass transmembrane protein, in and out of the primary cilium.[5d,e] The primary cilium is a microtubule-based assembly-point organelle for Hh pathway components.[47] Indeed, **1** suppressed Hh signaling at submicromolar concentrations in NIH3T3 mouse fibroblasts (Figure 8A). Surprisingly, as part of our efforts to evaluate the effects of **1** on the movement of Smo with respect to the primary

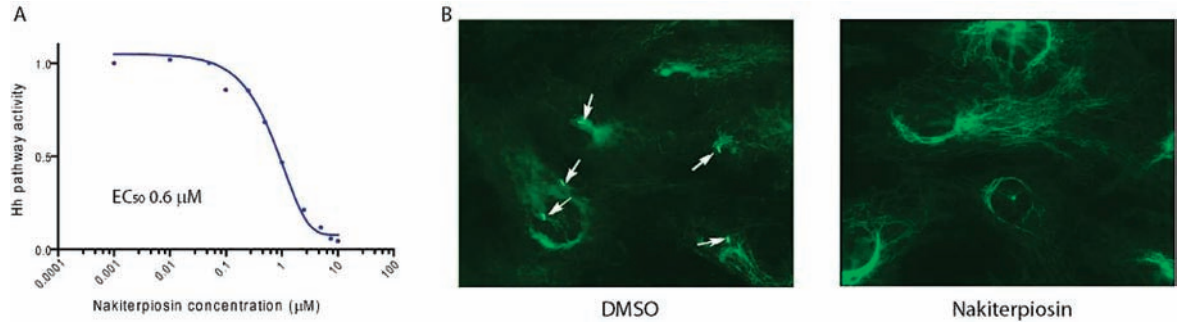


Figure 8. Effects of nakiterpiosin on Hh signaling and primary cilia. (A) Hh pathway response of Light2 cells (NIH3T3 cells with an Hh-specific firefly luciferase reporter) in the presence of nakiterpiosin. (B) NIH3T3 cells treated with nakiterpiosin, which show a compromised ability to form primary cilia as detected by antiacetylated tubulin antibody.

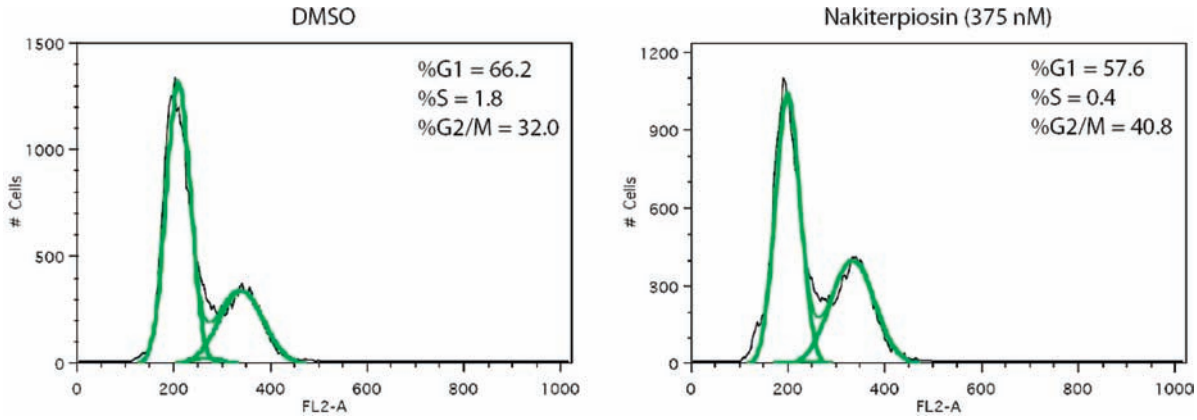


Figure 9. DNA profiles of nakiterpiosin-treated HeLa cells.

cilium, we observed that cell populations treated with nakiterpoisin show a loss of the primary cilium (Figure 8B).

Because the formation and retraction of the primary cilium is cell-cycle- and microtubule-dependent,[48] we suspected **1** to be an antimitotic agent. Indeed, flow cytometry experiments suggested that **1** induces cell-cycle arrest in the G2/M phase (Figure 9). We first determined the cell-growth IC_{50} of **1** in HeLa cells to be 375 nM. Subsequently, we incubated the HeLa cells with **1** at this concentration for 16 h and found the population of cells in the G2/M phase to be significantly increased relative to the DMSO control. Since the most common mechanism for G2/M arrest is the targeting of the microtubule assembly and it has been reported that JK184, a microtubule depolymerizing agent,[49] effectively regulates Hh signaling, we next examined the effect of **1** on microtubule dynamics. However, we found that in contrast to taxol and nocodazole, tubulin polymerization was not influenced in the presence of 5 μM **1** in an in vitro assay (Figure 10). Taken together, these results suggest that **1** may be useful in treatment of tumors resistant to existing antimitotic chemotherapeutic agents. Lastly, because of the frequency of mutations in Smo that render it resistant to Hh pathway antagonists currently under clinical development,[5c] considerable effort is being devoted to the identification of small molecules that act upstream or downstream of Smo.[50] The ability of **1** to block Hh pathway responses suggests that it may be particularly useful against Hh-dependent tumors.

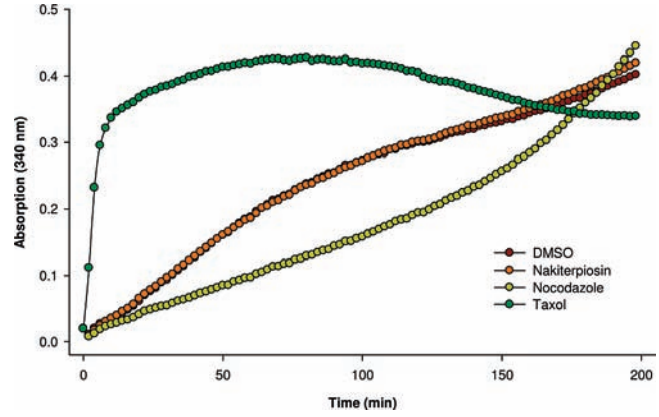


Figure 10. In vitro tubulin polymerization assays.

Summary

Through extensive spectroscopic analysis and convergent total synthesis, we have established the stereochemistry of nakiterpiosin (**1**) and nakiterpiosinone (**2**). Our synthetic route provides **1** in 21 steps with 5% overall yield along the longest linear sequence. Our preliminary biological studies show that **1** is antimitotic and has functions distinct from those of other well-established antimitotic agents such as taxol. Nakiterpiosin may be an alternative chemotherapeutic agent against tumors that are resistant to antitubulin agents and dependent on Hh signaling.

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Supporting Information Available: Complete refs 6e and 6g, ^{1}H and ^{13}C NMR spectra of **2**, calculation of the thermodynamic properties of **3**, details of the optimization of reaction parameters, experimental procedures, crystallographic data (CIF), and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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