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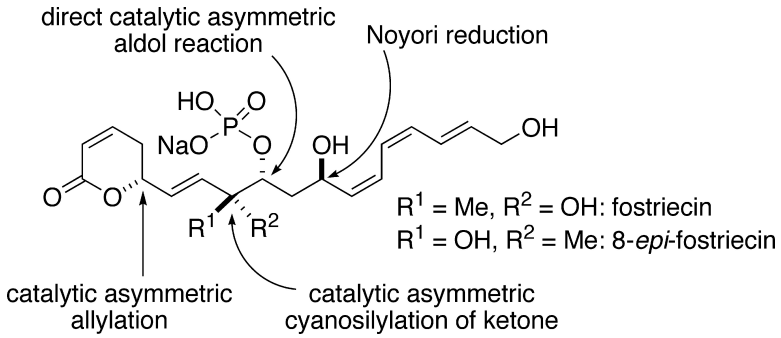

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Catalyst-Controlled Asymmetric Synthesis of Fostriecin and 8-*epi*-Fostriecin

Keisuke Maki,[†] Rie Motoki,[†] Kunihiko Fujii,[†] Motomu Kanai,*[,†] Takayasu Kobayashi,[‡] Shinri Tamura,[‡] and Masakatsu Shibasaki*[,†]

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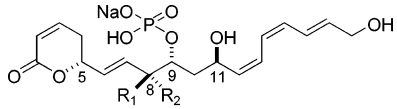
Abstract: Catalytic asymmetric synthesis of the natural antibiotic fostriecin (CI-920) and its analogue 8-*epi*-fostriecin and evaluation of their biological activity are described. We used four catalytic asymmetric reactions to construct all of the chiral centers of fostriecin and 8-*epi*-fostriecin; cyanosilylation of a ketone, Yamamoto allylation, direct aldol reaction, and Noyori reduction, two of which were developed by our group. Catalytic enantioselective cyanosilylation of ketone **13** produced the chiral tetrasubstituted carbon at C-8. Both enantiomers of the product cyanohydrin were obtained with high enantioselectivity by switching the center metal of the catalyst from titanium to gadolinium. Yamamoto allylation constructed the C-5 chiral carbon in the α,β-unsaturated lactone moiety. A direct catalytic asymmetric aldol reaction of an alkynyl ketone using LLB catalyst constructed the chirality at C-9 with the introduction of a synthetically versatile alkyne moiety, which was later converted to *cis*-vinyl iodide, the substrate for the subsequent Stille coupling for the triene synthesis. Noyori reduction produced the secondary alcohol at C-11 from the acetylene ketone **6** with excellent selectivity. Importantly, all the stereocenters were constructed under catalyst control in this synthesis. This strategy should be useful for rapid synthesis of stereoisomers of fostriecin.

Introduction

Fostriecin (**1**, CI-920) is a structurally unique antibiotic isolated from *Streptomyces pulveraceus* by a research group at Warner Lambert-Parke Davis in 1983 (Figure 1).[1] It displays cytotoxic activity against a broad range of cancerous cell lines such as leukemia, lung cancer, breast cancer, and ovarian cancer in vitro, and also antitumor activity against leukemia in vivo.[2] Initially, fostriecin was considered to be a topoisomerase II poison; however, there is no relation between this mechanism and its cancer suppression activity.[3] It is proposed that the anticancer activity is derived from the perturbation of the mitotic entry checkpoint through potent inhibition of protein phosphatases (PP). Specifically, fostriecin has the most selective serine/threonine phosphatase 2A (PP2A) inhibition known to date (10^4 times greater selectivity for PP2A versus PP1).[4] Due to this unique property, fostriecin is expected to be a novel and potent lead compound for antitumor drugs.[5] Considering the importance of protein phosphorylation and dephosphorylation reactions in living organisms, it might also be a valuable biological tool. Unfortunately, the clinical trial of fostriecin at the National Institute of Cancer was halted in phase I due to its instability and unpredictable purity.[5] Therefore, stable analogues of fostriecin are in high demand.



R_1 = Me, R_2 = OH ; fostriecin (**1**)
R_1 = OH, R_2 = Me ; 8-*epi*-fostriecin (**2**)

Figure 1. Structures of fostriecin (**1**) and 8-*epi*-fostriecin (**2**).

Only the two-dimensional structure of fostriecin was known[6] until Boger's pioneering synthetic and degradation studies in 1997 to determine the relative and absolute configuration of fostriecin.[7] Boger and colleagues subsequently achieved the first total synthesis in 2001.[8] Since then, several total syntheses have

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[‡] Tohoku University.

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(7) Boger, D. L.; Hikota, M.; Lewis, B. M. *J. Org. Chem.* **1997**, *62*, 1748.

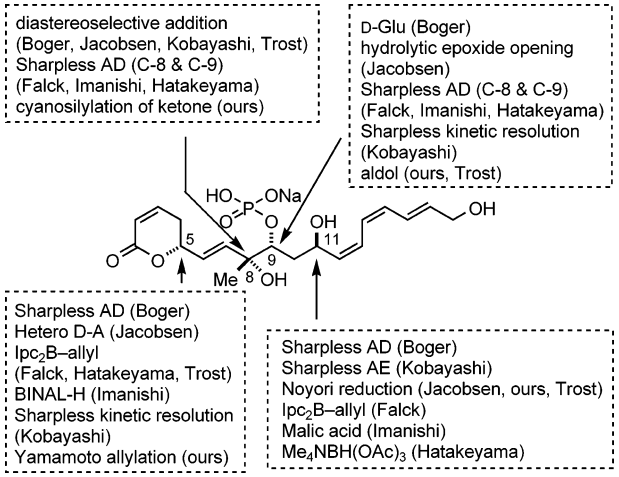
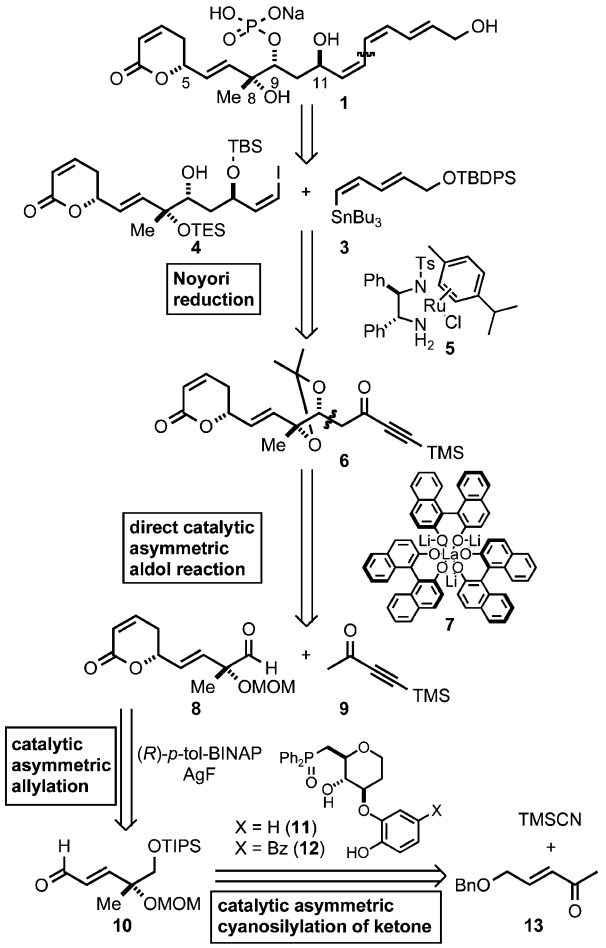


Figure 2. Summary of the stereocontrol strategies in the total syntheses of fostriecin.

been reported,[9,10] including one from our group.[11] Stereoselective construction of fostriecin's four chiral carbons is the key for success of the total synthesis. The strategies utilized in the reported total syntheses are summarized in Figure 2. Although various methods are available for construction of the chiral trisubstituted carbons at C-5, 9, and 11, there are few methods available for the chiral tertiary alcohol at C-8. Our synthesis is unique in that all the stereogenic centers were constructed using external asymmetric catalysts. Specifically, the tertiary alcohol at C-8 was constructed through catalytic enantioselective cyanosilylation of a ketone developed in our group.[12] An advantage of our synthesis over that of other groups is its applicability to the synthesis of stereoisomers of fostriecin. For example, it is possible to synthesize 8-*epi*-fostriecin **2** just by switching the enantioselectivity of the cyanosilylation. It is not very straightforward to synthesize **2** using the synthetic methods of the other groups. Investigation of the stereochemistry and biological activity relationship is the starting point for understanding the protein−drug interactions. In this sense, we are especially interested in the effect of the configuration of the C-8 tertiary alcohol of fostriecin and, more generally, in how the configuration of the chiral tetrasubstituted carbon is distinguished in nature. In this paper, we report catalytic asymmetric synthesis of fostriecin and its epimer 8-*epi*-fostriecin and the effect of the configuration of the tertiary alcohol (C-8) on their biological activity.

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



Results and Discussion

Synthetic Plan. One of our focal points in this project was to assess and demonstrate the power of catalytic asymmetric reactions in the practical synthesis of a biologically active compound with a considerably complex structure. Thus, we planned to construct all of the stereogenic centers using four catalytic asymmetric reactions. This strategy will be advantageous for rapid synthesis of stereoisomer analogues of fostriecin, if each reaction proceeds with catalyst control.

Our retrosynthesis is shown in Scheme 1. Because of its instability, the triene moiety should be introduced at a late stage through Stille coupling of vinyl tin **3** and vinyl iodide **4**.[13] The stereogenic center at C-11 of **4** should be produced through Noyori transfer hydrogenation[14] of alkynyl ketone **6**. The secondary alcohol at C-9 can be constructed through a direct catalytic asymmetric aldol reaction between aldehyde **8** and alkynyl methyl ketone **9** promoted by a heterobimetallic LLB complex **7**.[15] α,β-Unsaturated lactone can be constructed through Yamamoto allylation[16] of aldehyde **10**, acryloylation, and ring closing metathesis. Finally, we planned to apply catalytic

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(13) Jacobsen, Imanishi, Hatakeyama, and Falck utilized Stille or Suzuki cross-coupling for the triene construction. Vinyl iodide **4** is the common intermediate of Jacobsen, Imanishi, Hatakeyama, and Kobayashi.
(14) Matsumura, K.; Hashiguchi, S.; Ikariya, T.; Noyori, R. *J. Am. Chem. Soc*. **1997**, *119*, 8738.
(15) (a) Yoshikawa, N.; Yamada, Y. M. A.; Das, J.; Sasai, H.; Shibasaki, M. *J. Am. Chem. Soc*. **1999**, *121*, 4168. (b) Yamada, Y. M. A.; Yoshikawa, N.; Sasai, H.; Shibasaki, M. *Angew. Chem., Int. Ed. Engl*. **1997**, *36*, 1871.
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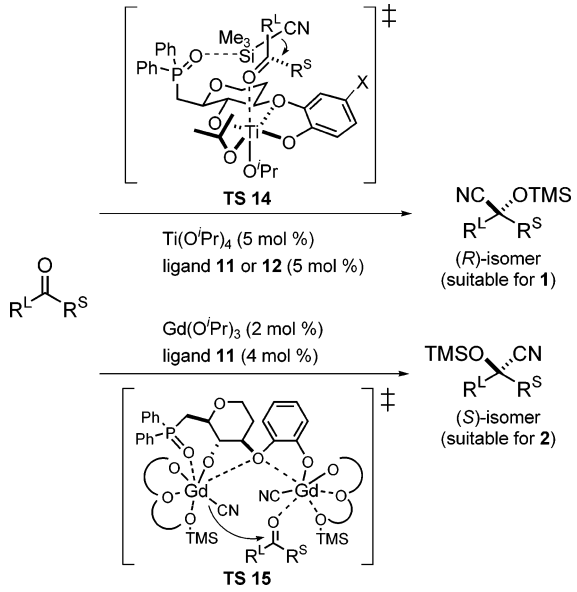
Scheme 2. Catalytic Enantioselective Cyanosilylation of Ketones

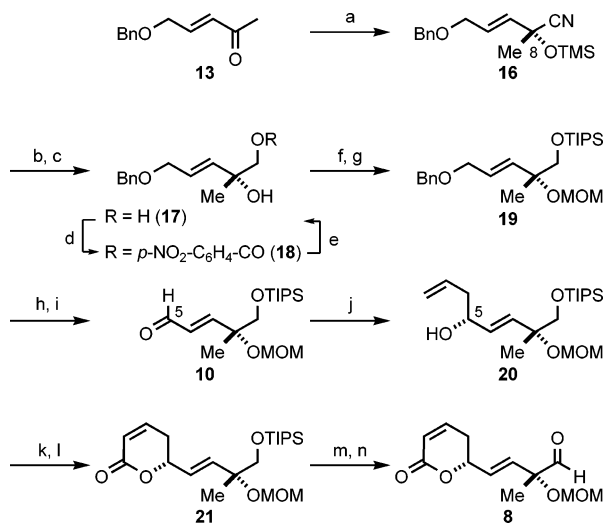


enantioselective cyanosilylation of ketone **13** developed in our group[12] to control the C-8 stereocenter.

Synthesis of Fostriecin (1). Our synthesis of fostriecin began with the catalytic asymmetric cyanosilylation of ketone **13**. A titanium complex derived from ligand **11** or **12** and Ti(O^{i}Pr)$_4$ in a 1:1 ratio generally gives (*R*)-ketone cyanohydrins with excellent enantioselectivity, while a gadolinium complex derived from **11** and Gd(O^{i}Pr)$_3$ in a 2:1 ratio generally gives (*S*)-ketone cyanohydrins (Scheme 2).[12] In natural fostriecin synthesis, the former reaction was used. Both the reactivity and selectivity of this reaction heavily depended on the protective group of the allylic alcohol of the substrate. Examination of several substrates[17] indicated that the benzyl group was the protective group of choice. Thus, using 5 mol % of the catalyst derived from ligand **11**, product **16** was obtained in 94% yield with 71% ee (−20 °C, 36 h). Enantioselectivity was improved using ligand **12** containing a benzoyl group at the catechol,[12b] and the product was obtained in 86% yield and 87% ee (−25 °C, 48 h). The higher enantioselectivity obtained when using **12** compared to **11** might be partly due to the enhanced Lewis acidity of titanium by the electron-withdrawing group at the catechol, which would more efficiently stabilize the hypothetical dual activation transition state **14**. This reaction was performed on a 50 g scale without a significant change in efficiency (93%, 85% ee). The chiral ligand **12** was recovered in 95% yield after silica gel column chromatography, which could be reused at least several times without any loss of catalyst activity.

The cyanohydrin **16** was transformed to the primary alcohol **17** via ethanolysis and $NaBH_4$ reduction (Scheme 3). Enantiomerically pure **17** was obtained after converting to the *p*-nitrobenzoyl ester **18**, recrystallization, and hydrolysis (78% yield in three operations). Subsequent protection of the primary and tertiary alcohols with TIPS and MOM groups, respectively, followed by debenzylation led to the allyl alcohol, which was oxidized by MnO_2 to give the allylation precursor **10**.[18] To

Scheme 3 [a]



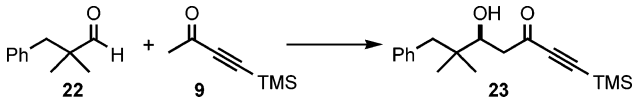
[a] Reagents and conditions: (a) Ti(O^{i}Pr)$_4$ (5 mol %), ligand **12** (5 mol %), TMSCN, THF, −25 °C, 93%, 85% ee; (b) 6 N HCl/EtOH, 60 °C, 83%; (c) $NaBH_4$, MeOH, 88%; (d) *p*-NO$_2$−C$_6$H$_4$COCl, py, CH_2Cl_2, 100%; recryst. from CH_2Cl_2/hexane, 78% (>99% ee); (e) K_2CO_3, MeOH−CH_2Cl_2, 100%; (f) TIPSCl, imidazole, DMF, 96%; (g) MOMCl, ^{i}Pr$_2$NEt, CH_2Cl_2, 100%; (h) Na, NH_3, −78 °C, 93%; (i) MnO_2, CH_2Cl_2, 93%; (j) AgF (20 mol %), (*R*)-*p*-tol-BINAP (20 mol %), allyltrimethoxysilane, MeOH, −20 °C, 80% (d.r. = 28:1); (k) acryloyl chloride, Et_3N, CH_2Cl_2, 76%; (l) first generation Grubbs catalyst (15 mol %), CH_2Cl_2, 94%; (m) 3HF−Et_3N, THF, 90%; (n) DMP, CH_2Cl_2, 92%.

construct the chiral lactone part, we investigated several catalytic asymmetric allylations.[19] Yamamoto's AgF-catalyzed reaction gave the best results in the current system. Diastereoselectivity was as high as 28:1 in favor of the desired isomer **20**.[20] α,β-Unsaturated lactone **21** was constructed from **20** through treatment with acryloyl chloride and ring closing olefin metathesis using the first generation Grubbs' catalyst.[21] Deprotection of the primary alcohol and oxidation with Dess−Martin periodinane (DMP) produced aldehyde **8**, the substrate for the catalytic asymmetric aldol reaction.

Before performing the catalytic asymmetric aldol reaction with an alkynyl ketone in the actual system, its feasibility was tested using a model aldehyde **22**, because this type of reaction had not been explored.[22] Neither the lithium enolate nor zinc enolate of **9** provided the target molecule **23** in reasonable yield (Table 1, entries 1 and 2). On the other hand, Lewis acid−Brönsted base two-center asymmetric catalyst[23] LLB (10 mol %) promoted the reaction in good yield with high enantioselectivity (entry 3). Less satisfactory results were obtained when the more basic LLB/KOH system[15a] was utilized (entry 4), probably because the highly basic catalyst facilitated the retroaldol reaction. Application of the best reaction conditions to the actual system (**8** + **9**) afforded the desired aldol adduct with 3.6:1 diastereomeric ratio in 65% yield.[24] All attempts to

(17) TBS, TBDPS, MOM, Bz, Ac, and PMB groups were tested. See Supporting Information (SI) for details.
(18) In the previous communication, debenzylation and oxidation were conducted with LiDBB and TPAP, respectively. The current reaction conditions produced higher chemical yield and reproducibility.
(19) Keck allylation (Keck, G. E.; Krishnamurthy, D.; Grier, M. C. *J. Org. Chem.* **1993**, *58*, 6543) gave unsatisfactory results, perhaps due to the coordination of the MOM-oxy group to the titanium of the catalyst. See SI for details.
(20) The C-5 configuration was determined after conversion to **4** by comparing the ^{1}H and ^{13}C NMR with Imanishi's NMR chart. The stereoselectivity was consistent with Yamamoto's transition state model. AgF-catalyzed reaction in the absence of the chiral ligand gave a 1:1 diastereomixture.
(21) Grubbs, R. H.; Chang, S. *Tetrahedron* **1998**, *54*, 4413.
(22) Trost recently reported a catalytic enantioselective aldol reaction using an alkynyl ketone as a donor: Trost, B. M.; Fetes, A.; Shireman, B. T.; *J. Am. Chem. Soc*. **2004**, *126*, 2660.
(23) For a recent review on two-center asymmetric catalysis, see: Shibasaki, M.; Kanai, M.; Funabashi, K. *Chem. Commun*. **2002**, 1985.

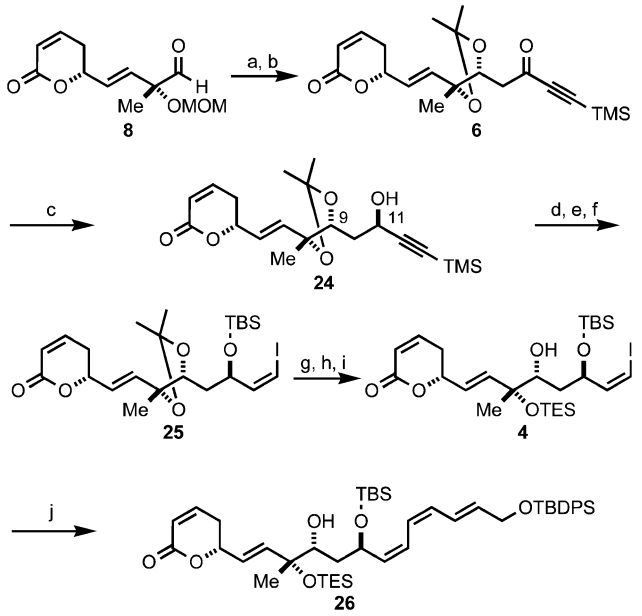
Table 1. Direct Catalytic Enantioselective Aldol Reaction of Alkynyl Ketone in a Model System



entry	promoter	additive	temp. (°C)	time (h)	yield (%)	ee (%)
1[a]	LDA	none	−78	1	28	
2[a,b]	LDA	$ZnCl_2$	0	12	0	
3[c]	(*R*)-LLB	none	−20	22	76	87
4[c,d]	(*R*)-LLB	KOH, H_2O	−40	64	63	60

[a] 2 equiv of **9** and 1 equiv of LDA were used. [b] 1 equiv of $ZnCl_2$ to **9** was used as an additive. [c] 6 equiv of **9** and 10 mol % of (*R*)-LLB were used. [d] 9 mol % of KOH and 10 mol % of H_2O were used as additives.

Scheme 4 [a]



[a] Reagents and conditions: (a) (*S*)-LLB (10 mol %), **9**, THF, −20 °C, 65% (d.r. = 3.6:1); (b) 2,2-dimethoxypropane, PPTS, acetone, 80%; (c) Noyori's catalyst **5** (10 mol %)−KOH (10 mol %), ^{i}PrOH, 49%; (other isomers: 18%); (d) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C, 73%; (e) NIS, $AgNO_3$, acetone, 88%; (f) NBSH, Et_3N, THF−^{i}PrOH, 40% (6% recovery); (g) 1 M HCl aq. MeOH, 47%; (h) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C; TESOTf, −78 °C to −10 °C, 52%; (i) 1 M HCl aq.−THF−CH_3CN (1:3:6), −10 °C, 52%; (j) $PdCl_2(CH_3CN)_2$, **3**, DMF, 85%.

improve the moderate selectivity, including catalyst tuning by modifying the BINOL structure, were unsuccessful.[25]

With the desired aldol in hand, construction of the remaining stereogenic center at C-11 was performed using Noyori hydrogenation. Thus, after acetonide formation to generate **6**, Noyori's transfer hydrogenation effectively produced the secondary alcohol **24** in a highly stereoselective manner (d.r. = >15:1, Scheme 4). Diastereomerically pure **24** was obtained by preparative TLC purification.[26] At this stage, all the chiral centers of fostriecin were introduced. **24** was transformed to *cis*-vinyl iodide **25** via three steps: TBS protection of the secondary alcohol at C-11, iododesilylation of the TMS acetylene,[27] and diimide reduction of the iodoalkyne using *p*-nitrobenzenesulfonylhydrazide (NBSH).[28] By modifying Imanishi's procedure, properly protected vinyl iodide **4** was afforded in three steps. Stille coupling between vinyl tin **3**[29] and **4** under ligand-free conditions[30] proceeded uneventfully to form **26**, the common intermediate of the fostriecin synthesis developed by Jacobsen, Imanishi, and Hatakeyama. Thus, the formal total synthesis of fostriecin was accomplished.

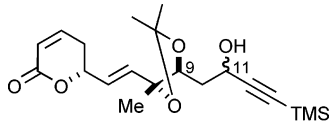
Synthesis of 8-*epi*-Fostriecin. Despite intensive studies, the structure−activity relationship of fostriecin, its binding feature to PP, and the molecular mechanism of PP inhibition are not yet clarified.[2] Specifically, the structure−activity relationship of **1** is limited to information obtained by either molecular modeling calculations or compounds that could be derivatized from **1** and its natural analogues. Three important pieces of information confirmed so far are as follows: (1) the α,β-unsaturated lactone is a fundamental motif for the potent activity and PP2A selectivity of **1**; (2) the phosphate ester at C-9 is essential; (3) the terminal allylic alcohol is not necessary.[31] To obtain further insight into the binding mode of **1** to PP, we were interested in the effect of the configuration of chiral carbons on fostriecin's biological activity, especially the effect of chiral tertiary alcohol at C-8. The methyl group at C-8 is proposed to be a common pharmacophore in naturally occurring PP1 and PP2A inhibitors, and it mimics the methyl group of phosphothreonine, a PP substrate.[32] Currently, the role of the tertiary alcohol is not well understood, but it might mimic the hydroxy group of the substrate threonine or displace the enzyme's metal-bound water nucleophile in the active site.[2a] Because our synthetic strategy is advantageous for preparing other isomers of fostriecin, we extended our strategy to the synthesis of 8-*epi*-fostriecin (**2**). A main interest in this synthetic endeavor is whether catalyst control dominates over substrate control in this linear system.

The first reaction was (*S*)-selective cyanosilylation of **13** using a gadolinium complex (2 mol %) derived from ligand **11** (Scheme 5). Product **27**, which is the enantiomer of the previous **16**, was obtained in 95% yield with 86% ee. This reaction was performed on a 120 g scale. **27** was effectively converted to the allylation precursor **28** following the procedure for the fostriecin synthesis. Yamamoto allylation of this substrate produced the desired isomer **29** with excellent selectivity (d.r. = 16:1). Thus, stereochemistry at C-8 did not significantly affect stereoselectivity in this allylation, and the catalyst chirality

(24) The diastereomeric ratio was determined based on the ^{1}H NMR analysis of the crude mixture of the aldol reaction. The diastereomers were not separable at this stage. The relative configuration of C-8 and C-9 was determined by NOE measurement on acetonide **6**.

(25) For a beneficial effect of BINOL substitution on a catalytic asymmetric nitroaldol reaction, see: Shibasaki, M.; Sasai, H.; Arai, T. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1236.

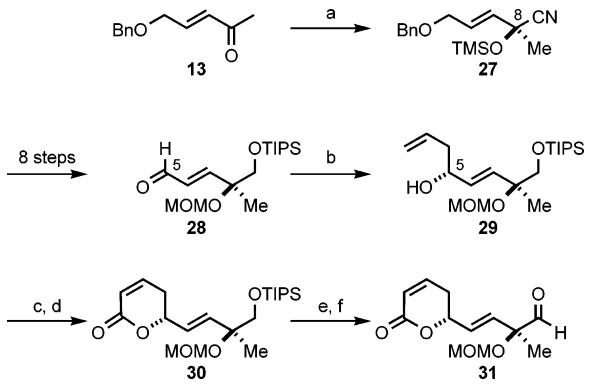
(26) Other diastereomers (9*S*,11*S* and 9*S*,11*R*) were obtained in 14% and 4% isolated yield. The relative configuration of C-9 and C-11 was determined by NOE measurement. See ref 11 for details.



11-β-OH: (9*S*,11*S*)
11-α-OH: (9*S*,11*R*)

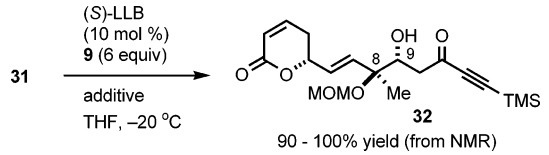
(27) Nishikawa, T.; Shibuya, S.; Hosokawa, S.; Isobe, M. *Synlett* **1994**, 485.
(28) Myers, A. G.; Zheng, B.; Morassaghi, M. *J. Org. Chem.* **1997**, *62*, 7507.
(29) Mapp, A. K.; Heathcook, C. H. *J. Org. Chem.* **1999**, *64*, 23.
(30) Stille, J. K.; Groh, B. L. *J Am. Chem. Soc.* **1987**, *109*, 813.
(31) (a) Buck, S. B.; Hardouin, C.; Ichikawa, S.; Soenen, D. R.; Gauss, G.-M.; Hwang, I.; Swingle, M. R.; Bonness, K. M.; Honkanen, R. E.; Boger, D. L. *J. Am. Chem. Soc.* **2003**, *125*, 15694. (b) Leopold, W. R.; Shillis, J. L.; Mertus, A. E.; Nelson, J. M.; Roberts, B. J.; Jackson, R. C. *Cancer Res.* **1984**, *44*, 1928. (c) Fry, D. W.; Besserer, J. A.; Boritzki, T. J. *Cancer Res.* **1984**, *44*, 3366. (d) Mamber, S. W.; Okasinski, W. G.; Pinter, C. D.; Tunac, J. B. *J. Antibiot.* **1986**, *39*, 1467.
(32) Gauss, C.-M.; Sheppeck, J. E., II; Narin, A. C.; Chamberlin, R. *Bioorg. Med. Chem.* **1997**, *5*, 1751.

Scheme 5 [a]



[a] Reagents and conditions: (a) $Gd(O^iPr)_3$ (2 mol %), ligand **11** (4 mol %), TMSCN, EtCN, −65 °C, 95%, 86% ee; (b) AgF (5 mol %), (*R*)-*p*-tol-BINAP (5 mol %), allyltrimethoxysilane, MeOH, −20 °C, 88% (d.r. = 16:1); (c) acryloyl chloride, Et_3N, CH_2Cl_2, 0 °C, 87%; (d) second generation Grubbs catalyst (2 mol %), CH_2Cl_2, reflux, 93%; (e) 3HF−Et_3N, THF, 50 °C, 82%; (f) DMP, H_2O (0.5 equiv), CH_2Cl_2, 94%.

Table 2. Additive Effect in Direct Catalytic Asymmetric Aldol Reaction of **31**



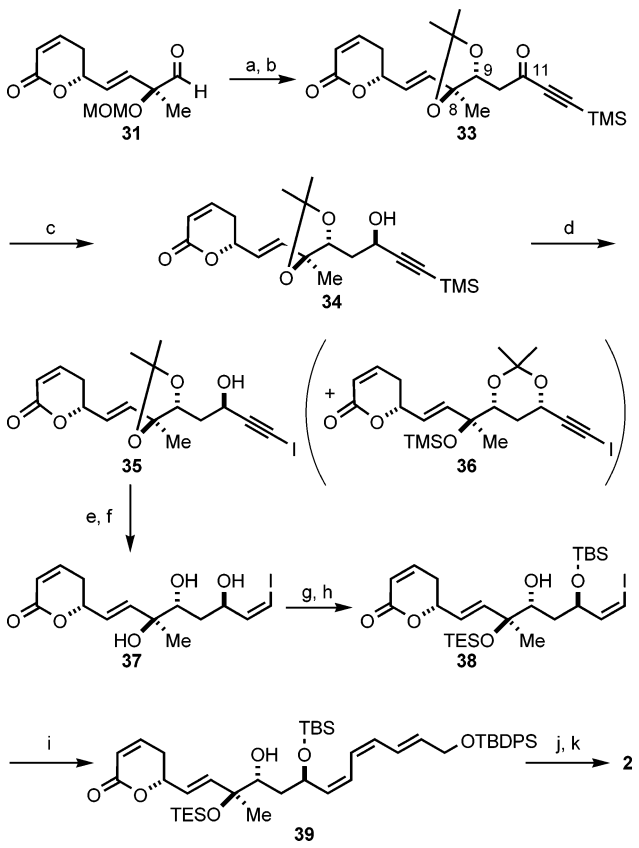
entry	additive (mol %)	time (h)	d.r. (*anti*/*syn*)
1	none	25	2:1
2	LiOTf(10)	24	3:1
3	LiOTf(20)	4	4:1
4	LiOTf(30)	10	2.5:1
5	NaOTf(10)	15	1.5:1
6	KOTf(10)	24	2:1
7	$LiClO_4$(10)	34	2:1
8	$LiPF_6$(10)	34	2:1

determined the stereochemical outcome. This reaction was followed by ester formation with acryloyl chloride and ring closing metathesis catalyzed by the second generation Grubbs' ruthenium complex[33] to form α,β-unsaturated lactone **30**. The TIPS group was then removed, and the resultant primary alcohol was oxidized to the aldol precursor **31** by DMP.[34] This oxidation, however, was tricky, and the yield of **31** was variable. Further investigations clarified that a small amount of water was essential for reproducible conversion. Thus, aldehyde **31** was obtained in 94% yield in the presence of 0.5 equiv of H_2O. A similar beneficial effect of water in DMP oxidation was reported by Schreiber et al.[35]

The (*S*)-LLB-catalyzed asymmetric aldol reaction of aldehyde **31** and alkynyl ketone **9** was then performed under the optimized conditions in natural fostriecin synthesis. Although the reaction proceeded under catalyst control in favor of the desired isomer **32**, the diastereoselectivity was only 2:1 (Table 2, entry 1).[36] In contrast to the case of natural fostriecin synthesis, the stereoselectivity of the catalyst did not match the 1,2-chirality transfer raised by the C-8 chiral tertiary alcohol in this case. To improve the selectivity, we intensively investigated the effect of additives (Table 2). Among the additive salts screened, 20 mol % LiOTf gave the best results;[37] the diastereomeric ratio was improved to 4:1 (entry 3). Other alkali metal triflates and other lithium counterions other than triflate gave almost the same diastereoselectivity as that in the absence of additives.

(33) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett*. **1999**, *1*, 953.
(34) Other oxidants such as PDC, TPAP, and IBX did not promote the reaction. Swern oxidation gave the product in 67% yield.

Scheme 6 [a]



[a] Reagents and conditions: (a) (*S*)-LLB (10 mol %), LiOTf (20 mol %), **9**, THF, −20 °C; (b) 2,2-dimethoxypropane, 50 °C, 65% (for two steps, d.r. = 4:1); (c) Noyori's catalyst **5** (3.3 mol %), KOH (3 mol %), ^{i}PrOH, 81%; (d) NIS, $AgNO_3$ (0.6 equiv), EtOH (10 equiv), acetone, 0 °C, 87%; (e) NBSH, $NaHCO_3$, MeOH, 58% (31% recovery); (f) 1 M HCl aq. MeOH, 73%; (g) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C; TESOTf, −78 °C to −40 °C, 89%; (h) 1 M HCl aq.−THF−CH_3CN (1:3:6), −10 °C, 53% (20% recovery); (i) $PdCl_2(MeCN)_2$ (50 mol %), **3**, DMF, 40 °C, 85%; (j) $^iPr_2NP(Oallyl)_2$ (**40**), 1*H*-tetrazole, CH_2Cl_2, 0 °C; I_2, py−H_2O−THF (1:2:7), −20 °C (k) $Pd(PPh_3)_4$ (100 mol %), PPh_3, formamide, THF; 48% HF aq.−H_2O−MeCN (1:2:20), py, 50% (4 steps).

With the desired aldol **32** in hand, the next task was the construction of the C-11 stereocenter. Due to the instability of **32**, it was directly transformed to acetonide **33** without column chromatographic purification. As might be expected, Noyori reduction of **33** proceeded under catalyst-control irrespective of the configuration of C-8, giving product **34** with excellent selectivity (d.r. = >10:1, Scheme 6).[36] When the resulting propargyl alcohol **34** was subjected to the same iododesilylation conditions as the natural fostriecin synthesis (NIS and $AgNO_3$ in acetone), the desired iodoalkyne **35** was obtained in moderate yield (62%) with concomitant generation of an epimerized

(35) Meyer, S. D.; Schreiber, S. L. *J. Org. Chem*. **1994**, *59*, 7549.
(36) The relative configurations between C-8, C-9, and C-11 were determined by NOE and/or NOESY measurements on the **33** and **34** derivative and later by X-ray crystallographic analysis of **37**. See SI for details.
(37) It is proposed that the LLB−LiOTf oligomeric species is the actual asymmetric catalyst under these conditions. See: (a) Horiuchi, Y.; Gnanadesikan, V.; Ohshima, T.; Masu, H.; Katagiri, K.; Sei, Y.; Yamaguchi, K.; Shibasaki, M. *Chem.−Eur. J*. **2005**, *11*, 5195. (b) Gnanadesikan, V.; Horiuchi, Y.; Ohshima, T.; Shibasaki, M. *J. Am. Chem. Soc*. **2004**, *126*, 7782.

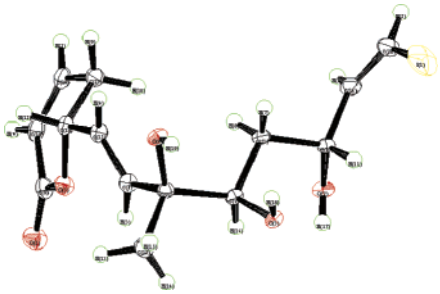


Figure 3. ORTEP drawing of **37**.

dioxane **36** (20%). Generation of Lewis acidic silyl species ($TMSNO_2$ or TMS-succinimide) during this reaction might cause the formation of **36**. Thus, Lewis acid mediated equilibration between the dioxolane and the dioxane, silicon trap of the tertiary alcohol to terminate this equilibrium, and epimerization at the propargylic position to the more stable isomer with the two substituents at the equatorial positions should produce **36**. To avoid the adverse effects of the Lewis acidic silyl species, we added EtOH as a scavenger. The yield of **35** then dramatically increased to 87%, with minimum generation of undesired **36** (2%).

cis-Reduction of the iodoalkyne **35** with NBSH, followed by deprotection in acidic media, gave triol **37** (Figure 3). X-ray crystallographic analysis of **37** unequivocally determined the stereochemistry of all the stereogenic centers. The two-step protection−deprotection sequence worked reasonably well as in the case of natural fostriecin synthesis, giving the coupling precursor **38**. The Stille coupling between **38** and **3** proceeded at 40 °C under ligandless conditions, and the triene was obtained in 85% yield. Careful temperature control was necessary for this Stille coupling reaction, because isomerization of the triene occurred gradually at 60 °C, while at room temperature the reaction was rather slow.

Finally, the phosphate moiety was added to the C-9 alcohol to complete the synthesis of **2**. We used allyl-protected phosphoramidite **40** for this phosphorylation, according to Hatakeyama's fostriecin synthesis. The application of Hatakeyama's conditions using TBHP as an oxidizing reagent of the initially formed phosphite, however, produced a complex mixture of products. TLC analysis of the reaction revealed that decomposition occurred at the oxidation step. Thus, changing the oxidizing reagent to I_2 and performing the reaction at −20 °C under careful TLC monitoring, the phosphorylation product was obtained in reasonable yield (80% based on TLC analysis). Deprotection of the phosphate and global desilylation were conducted under slightly modified conditions of the reported procedure.[9e] These reactions should also be quenched in 4 h, otherwise significant decomposition occurs. After purification through a short pad reversed-phase silica gel column chromatography,[38] the synthesis of 8-*epi*-fostriecin was achieved.

Biological Assay. Inhibitory activities of okadaic acid (control), fostriecin (**1**), and 8-*epi*-fostriecin (**2**) against PP1 and PP2A were evaluated. Four kinds of assays were conducted in which the enzymatic activity was determined by hydrolysis of phosphorylated histone (assay A),[39] phosphorylated casein (assay B), *p*-nitrophenyl phosphate (assay C),[40] or commercial phosphorylated peptide (assay D)[41] (Table 3). Although the IC_{50} values of okadaic acid were similar in the four assay systems (IC_{50} = 0.1−6 nM for PP2A and 0.1−2 μM for PP1), those of **1** and **2** were highly dependent on the phosphorylated substrates. **1** exhibited excellent PP2A selective inhibitory activity in assay A, as previously reported (2 nM for PP2A and >100 μM for PP1),[3f] but almost no selectivity or even slight PP1-selectivity in assays B−D. **2** was a weaker PP inhibitor than **1** in assays A−C. Specifically, **2** did not demonstrate inhibitory activity in assay C using *p*-nitrophenyl phosphate as the substrate, which is more precisely a substrate analogue of tyrosine phosphatase. On the other hand, **2** inhibited PP2A more selectively and with higher potency than **1** in assay D.

Table 3. IC_{50} Values (in μM) in Inhibition of PP2A and PP1[a]

assay	inhibitor	PP2A	PP1
A	okadaic acid	0.0001	0.1
	fostriecin (**1**)	0.002	>100
	8-*epi*-fostriecin (**2**)	4	>100
B	okadaic acid	0.006	1
	fostriecin (**1**)	5	4
	8-*epi*-fostriecin (**2**)	40	>1000
C	okadaic acid	0.004	0.2
	fostriecin (**1**)	6	9
	8-*epi*-fostriecin (**2**)	770	>4000
D	okadaic acid	0.001	2
	fostriecin (**1**)	40	4
	8-*epi*-fostriecin (**2**)	6	510

[a] Substrates of PPs are phosphorylated histone in assay A, phosphorylated casein in assay B, *p*-nitrophenyl phosphate in assay C, or commercial phosphorylated peptide or ProFluor Ser/Thr Phosphatase Assay Kit in assay D. Assays were performed at least three times.

Differences in the inhibitory activity and subtype selectivity between these three compounds are interesting from the viewpoint of protein−drug interactions and their inhibitory mechanisms. Although both okadaic acid and fostriecin are protein phosphatase inhibitors, the cellular responses caused by these compounds are different: okadaic acid has been reported to promote or suppress tumor formation depending on the duration of the drug application or cell type, while fostriecin is an antitumor agent. This contrasting outcome might be related to our observation that okadaic acid inhibited dephosphorylation of all four substrates examined with the IC_{50} values in the nanomolar range, while fostriecin's IC_{50} values varied over 4 orders of magnitude depending on the substrate. These results also suggest that fostriecin might selectively interfere with specific intracellular signaling pathways depending on the phosphorylated substrate proteins. Based on the results in assays B, C, and D, 8-*epi*-fostriecin (**2**) appears to have an even higher substrate specificity with a higher PP2A selectivity than natural fostriecin (**1**). The difference between **1** and **2** should reflect the difference in their three-dimensional structures,[42] and therefore interactions with the phosphatases. The stereochemistry at C-8 has a great influence on their biological activity, and this finding supports the proposed hypothesis that the methyl and hydroxyl groups at C-8 are important pharmacophores that

(38) 8-*epi*-Fostriecin **2** is not very stable. It decomposes partially on reversed-phase preparative TLC at 4 °C, and significantly on reversed-phase HPLC under buffered conditions at rt (eluent, MeCN/pH 6.8 phosphate buffer = 12:88).

(39) (a) Honkanen, R. E.; Zwillwe, J.; Moore, R. E.; Daily, S. L.; Khatra, B. S.; Dukelow, M.; Boynton, A. L. *J. Biol. Chem.* **1990**, *265*, 19401. (b) Huang, X.; Swingle, M. R.; Honkanen, R. E. *Methods Enzymol.* **2000**, *315*, 579.

(40) Zwiller, J.; Ogasawara, E. M.; Nakamoto, S. S.; Boynton, A. L. *Biochem. Biophys. Res. Commun.* **1988**, *155*, 767.

(41) The assay was performed using the commercially available ProFluor Ser/Thr Phosphatase Assay Kit.

mimic the substrate threonine. Considering its high selectivity, both in terms of the substrates and the protein phosphatases subtypes, 8-*epi*-fostriecin is a unique biological tool. Further evaluation of the anticancer activity of 8-*epi*-fostriecin is ongoing.

Conclusions

We accomplished a catalytic enantioselective synthesis of fostriecin and 8-*epi*-fostriecin. All of the stereogenic centers of these compounds were constructed by combining catalytic enantioselective cyanosilylation of a ketone, catalytic asymmetric allylation, a direct catalytic asymmetric aldol reaction using an alkynyl ketone, and Noyori reduction, two of which were developed in our laboratories. Importantly, these key reactions proceeded under catalyst control. This strategy should facilitate the synthesis of the other stereosiomers of fostriecin. Four kinds of biological assays to evaluate the phosphatase inhibitory activity were performed. The results clarified that the inhibitory activities of fostriecin and 8-*epi*-fostriecin were substrate-dependent, and the stereochemistry at C-8 greatly affected the biological activity. Furthermore, in one assay, 8-*epi*-fostriecin was a more selective and more potent PP2A inhibitor than natural fostriecin. Therefore, 8-*epi*-fostriecin might be useful as a unique biological tool.

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Supporting Information Available: Full experimental details including structure assignments and results of biological assays are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

JA0562043

(42) Preliminary conformation search by molecular mechanics calculations using CONFLEX5 [(a) Goto, H.; Osawa, E. *J. Am. Chem. Soc*. **1989**, *111*, 8950. (b) Goto, H.; Osawa, E. *J. Chem. Soc., Perkin Trans. 2* **1993**, 187. (c) Goto, H.; Osawa, E. *THEOCHEM* **1993**, *285*, 157. (d) Goto, H.; Kawashima, Y.; Kashimura, M.; Morimoto, S.; Osawa, E. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1647. (e) Osawa, E.; Goto, H.; Hata, T.; Deretey, E. *THEOCHEM* **1997**, *399*, 229.] indicated that natural fostriecin is conformationally more flexible than 8-*epi*-fostriecin. Among about 8000 conformers generated in the MMFF94S force field using this program, more than 90% of them were distributed in the 2 kcal/mol range from the most stable conformer in the case of 8-*epi*-fostriecin. In the case of natural fostriecin, however, ca. 75% of conformers existed in the same range.