Natural Products Synthesis

Determination by Asymmetric Total Synthesis of the Absolute Configuration of Lucilactaene, a Cell-Cycle Inhibitor in p53-Transfected Cancer Cells**

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The tumor-suppressor gene p53 is involved in important cellular events, such as cell-cycle control and apoptosis.^[1] The p53 gene is lost or mutated in many types of human tumors. Small molecules that induce cell-cycle arrest or apoptosis in a p53-independent manner or allow mutant p53 to alter a conformationally active form of p53 may be good candidates for treating various types of cancers.^[2] Recently we isolated lucilactaene (1), which arrests cell-cycle progression in the G1 phase at the nonpermissive temperature of 37 °C in HI299/ tsp53 cells, from *Fusarium* sp. RK97-94.^[3] Lucilactaene (1) is a synthetically challenging molecule because of its rare hexahydro-3a-hydroxy-5-oxo-2*H*-furo[3,2-b]pyrrol-6-yl ring system and its substituted and conjugated *E*,*E*,*E*,*E*,*E* pentaene moiety, which is unstable to acid, base, and light.

Along with lucilactaene (1), we isolated a known neuronal-cell-protecting compound, NG-391 (2),^[4] which possesses the same pentaene portion but a different y-lactam moiety, and which is probably biosynthesized from the same intermediate as 1.^[3] These natural products 1 and 2 are close structural relatives of fusarins A and C^[5] nonmutagenic metabolites of Fusarium moniliforme. Though the biosynthetic pathways that lead to 1 and 2 remain unclear, the following is a plausible pathway based on the proposed biosynthetic route to fusarin C:[5] The fully elaborated polyketide reacts with homoserine aldehyde by an intramolecular Knoevenagel reaction to form the unmodified 1,5dihydropyrrol-2-one 3, a possible common key intermediate of 1 and 2, after cleavage of the thioester (NADPH reduction) and condensation (Scheme 1). In the case of NG-391 (2), the remaining steps are epoxidation and oxidation to form the hemiaminal, either by ether hydroxylation α to the nitrogen atom or oxidation to an imine and addition of water (the

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reactions might occur in the reverse order). In the case of lucilactaene (1), an intramolecular Michael reaction and oxidation to form the hemiaminal are the remaining reactions (again the order of reactions might be reversed). Another possibility for the biosynthesis of 1 is via 2 through intramolecular epoxide-ring opening by the primary hydroxy group, followed by reduction.

A similar side chain is also found in epolactaene, a neuritogenic compound isolated from the fungal strain *Penicillium* sp. BM-1689-P.^[6] Clarification of the structure–activity relationships of lucilactaene (1), NG-391 (2), epolactaene, and their derivatives is highly desirable for elucidating their mechanism of action. We completed the first total syntheses of $2^{[7]}$ and epolactaene,^[8] and developed a biologically more potent molecule, epolactaene tertiary butyl ester (ETB).^[9] Recently, we revealed that both epolactaene and ETB bind to human Hsp60 and inhibit Hsp60 chaperon activity in vitro and in cultured cells,^[9] whereas Kobayashi and co-workers reported that epolactaene is an inhibitor of mammalian topoisomerases α and β in vitro.^[10]

The absolute configuration of NG-391 (2) was determined by us by asymmetric total synthesis.^[7] The optical rotation of lucilactaene (1) is zero in two different solvents (methanol and chloroform), which indicates the possibility that 1 is racemic. That 1 should be racemic appears strange when one considers its structural resemblance with 2. Because of the interesting biological properties of 1, its lability, and its rare structure, and because of the puzzle concerning its absolute configuration, we have investigated its asymmetric total synthesis by a biomimetic route.

On the basis of the proposed biosynthetic pathway, we planned to synthesize **1** from the key intermediate **4**, which

corresponds to the key biosynthetic intermediate **3**. We had already synthesized **2** from **4**.^[7] The remaining steps from **4** to lucilactaene (**1**) would be hydrolysis of the nitrile group, an intramolecular Michael reaction with the hydroxy group $(\mathbf{R}' = \mathbf{H})$ as the nucleophile, and functional-group transformations (Scheme 2). However, all attempts, including changing of the order in which the reactions were carried out, were unsuccessful.



Scheme 2. Proposed synthetic approach to lucilactaene from 4.

We therefore considered an approach to 1 from NG-391(2)^[7] (Scheme 3). The formation of methyl ether 6, followed by reductive removal of the epoxide to give an alkene 5, an



Scheme 3. Retrosynthetic analysis of lucilactaene.

intramolecular Michael reaction, and deprotection would afford lucilactaene (1). For this approach to be successful the reactions would have to proceed under mild conditions to avoid decomposition of the labile pentaene moiety. Methyl ether formation and the Michael reaction must proceed with



Scheme 1. Proposed biosynthesis of lucilactaene (1) and NG-391 (2). ACP = acyl carrier protein, NADPH = nicotinamide adenine dinucleotide phosphate, PCP = peptidyl carrier protein.

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high diastereoselectivity for 1 to be generated with high enantiomeric excess.

The methyl ether was formed stereoselectively by the treatment of 2 with a catalytic amount of $TsOH \cdot H_2O$ in MeOH to afford β -methoxide 7 as a single isomer in which methanol had captured the acyliminium ion intermediate from the opposite face to that occupied by the epoxide (Scheme 4).^[11] The next planned transformation was the reductive removal of the epoxide. Although SmI₂ is known to convert α,β -epoxyketones into α,β -unsaturated ketones,^[12] in this case reductive demethoxylation is faster than epoxide removal. When epoxylactam 7 was treated with SmI₂, a 5-(2hydroxyethyl)-2-pyrrolidone derivative was formed. After some experimentation, it was found that the protecting group on the nitrogen atom of the amide affects the reactivity of the compound towards reductive demethoxylation. Thus, 7 was treated with Boc₂O in the presence of triethylamine and a catalytic amount of DMAP to give the bis-Boc-protected derivative 8 in 69% yield over two steps. The reductive removal of the epoxide with SmI₂ (2 equiv) now proceeded efficiently at low temperature without affecting the methoxy group to afford 9 in 75% yield. The two Boc protecting groups were then removed by treatment with CF₃CO₂H (TFA) in CH₂Cl₂ at room temperature, whereupon a spontaneous Michael reaction and the conversion of the methyl ether into a hydroxy group gave lucilactaene (1) in 28% yield, along with lucilactaene methyl ether (10) in 55% yield. Synthetic 1 exhibited identical spectroscopic properties to those of the natural product (¹H NMR, ¹³C NMR, IR).

The optical rotation of this synthetic lucilactaene (1) was zero, identical to that of the isolated natural product and markedly different to that of 10 ($[\alpha]_D + 36.6$ (c = 0.17, MeOH)). Lucilactaene methyl ether (10) can also be converted into 1 in 60% yield by treatment with TFA in CH₂Cl₂; again the optical rotation of the product is zero. The large difference in optical rotation between 1 and its methyl ether 10 strongly suggests that the lucilactaene (1) formed is racemic. If racemization occurs during the synthesis, it must be during the final treatment with acid. To better understand the facile racemization of **1**, the synthesis of optically pure **1** was investigated.

To avoid possible racemization, the final cleavage of the hemiaminal protecting group should be conducted under neutral conditions. To this end we developed a novel deprotection method. When NG-391 (2) was treated with PhSeCH₂CH₂OH^[13] in the presence of a catalytic amount of TsOH·H₂O in CH₂Cl₂ for 3 h at room temperature, phenylselenylethyl ether 11 was formed in 31% yield as a single isomer; 2 was recovered in 67% yield (Scheme 5). Although decomposition occurred upon longer treatment of 2 with acid, the repeated exposure of recovered 2 to acid led to its conversion into 11 in a high overall yield of 94%. Both the amide and the hydroxy groups were protected with Boc₂O to afford 12 in 73% yield. The sequence of steps involving reductive removal of the epoxide with SmI₂, removal of the Boc groups, and the Michael reaction proceeded as efficiently as for the methyl ether derivative 8 to afford the bicyclic compound 14 as a single isomer in 36% yield over two steps. The transformation of the 2-phenylselenylethoxy group into a hydroxy group could be carried out under mild reaction conditions in three novel steps: 1) The oxidation of the selenide to the selenoxide, which was isolated in good yield, proceeded smoothly at low temperature on treatment with dimethyldioxirane (DMD),^[14] without affecting the pentaene moiety. 2) The elimination of benzeneselenenic acid occurred at 60 °C in the presence of dabco to provide vinyl ether 16.^[15] 3) Final oxidative removal of the vinyl substituent was performed under neutral conditions by the use of DMD at low temperature $(-78 \,^{\circ}\text{C})$ to afford lucilactaene (1) in 56 % yield from 15 in optically pure form ($[\alpha]_D$ + 39.5 (c = 0.10, MeOH)).^[16]

It is clear from the asymmetric total synthesis that isolated natural lucilactaene (1) is racemic. The facile racemization raises another question: There is the possibility that natural 1 is optically active but that racemization proceeds during the purification process. To investigate this possibility, it was necessary to isolate 1 under nonracemizing conditions. We



Scheme 4. Synthesis of lucilactaene. Boc = tert-butoxycarbonyl, DMAP = 4-dimethylaminopyridine, Ts = p-toluenesulfonyl.

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Scheme 5. Synthesis of optically pure lucilactaene; dabco = 1,4-diazabicyclo[2.2.2]octane.

chose to use bicycle **17** as a model compound to establish such conditions.

Optically pure 17, prepared by the same method as that used for 1, was treated with various reagents, and after a certain period of time the optical purity of the recovered 17 was measured by HPLC analysis on a chiral phase; the results are summarized in Table 1. Under weakly acidic or basic conditions, for example, in the presence of PPTS in MeOH or NEt₃ in CH₂Cl₂, no racemization was observed. However, racemization occurred when 17 was treated with TFA/CH₂Cl₂ or K₂CO₃ in MeOH. It was also confirmed that no racemization occurred in the medium in which the fermentation was carried out. These results indicate that the purification of lucilactaene (1) should be performed under nearly neutral, mild reaction conditions. The racemization might occur via intermediates such as 18 or 19, which arise from a reversible retro-Michael reaction, followed by acyliminium ion formation or keto-amide formation, though the order of the reactions might be different (Scheme 6).

As information about the racemization under a variety of conditions had been obtained, the production profile of lucilactaene (1) by *Fusarium* sp. RK97-94 was investigated further. All experiments were performed as rapidly as possible, with the temperature and pH value controlled carefully. The ethyl acetate extracts of the broth (supernatant) and the mycelia, which were obtained by centrifugation, were prepared under mild conditions at pH 7.0. The production profile of 1 in the broth is summarized in Table 2. The optical purity of 1 in the broth was very low (ca. 10% *ee*) throughout the fermentation. Moreover, the lucilactaene (1) in the mycelia was also nearly racemic (data not shown).

As shown in Scheme 1, lucilactaene (1) and NG-391 (2) may be biosynthesized from the same intermediate 3. Epoxidation and oxidation to form the hemiaminal produce 2; these two reactions proceed in this order, as 2 would otherwise be racemic. The absolute configuraTable 1: Racemization of the lucilactaene model 17.



1	none	23	24	100
2	AcOH/CH ₂ Cl ₂ (1:20)	23	3	100
3	PPTS in MeOH (0.005 м)	23	3	100
4	DMD in acetone (0.07м)	-78	0.5	100
5	Et_3N/CH_2Cl_2 (1:4)	23	3	100
6	TFA/CH ₂ Cl ₂ (1:100)	0	0.1	98
7	TFA/CH ₂ Cl ₂ (1:20)	0	0.25	57
8	TsOH·H ₂ O in CH ₂ Cl ₂ (0.013 м)	23	3	48
9	К ₂ CO ₃ in MeOH (0.15 м)	23	3	2
10	TFA/CH_2Cl_2 (1:4)	0	2.5	0
11	culture medium ^[b]	28	48	100

[a] Optical purity was determined by HPLC analysis on a chiral phase (chirapak AD-H). [b] Culture medium: 2% glucose, 1% soluble starch, 0.3% meat extract, 2.5% yeast extract, 0.05% NaCl, 0.005% K₂HPO₄, 0.05% CaCO₃, and 0.05% MgSO₄·H₂O adjusted to pH 7.2. PPTS = pyridinium *p*-toluenesulfonate.



Scheme 6. Racemization of 17 via 18 and/or 19.

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Table 2: Productior	profile of 1	by	Fusarium	sp.	RK97-94	in	the	broth.
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Entry	<i>t</i> [h]	pH ^[a]	PCV ^[b] [%]	Production of 1 [μg mL ⁻¹]	ee [%] ^[c]
1	24	7.4	10	0	n.d. ^[d]
2	48	7.2	30	0.014	10.0
3	72	8.3	45	0.084	10.3
4	96	8.5	50	0.71	6.9

[a] pH value of the fermentation broth. [b] Packed-cell volume (ν/ν). [c] Optical purity was determined by HPLC analysis on a chiral phase (chiralcel OD-RH). [d] Not determined.

tion of the epoxide can also be explained reasonably as arising from the selective epoxidation from the opposite face to that with the hydroxyethyl substituent. In the synthesis of **1**, a Michael reaction and oxidation to form the hemiaminal are the remaining steps. Should the Michael reaction be the first step, then we suspect that racemization must occur during the subsequent oxidation step, because the internal Michael addition is a spontaneous reaction, as demonstrated for the synthetic conversion of **13** into **14** without racemization. That is, there is an acidic moiety in the enzyme responsible for this oxidation which causes racemization If oxidation is the first step, a subsequent racemization process is conceivable (Scheme 7): Intermediate **3** may undergo tautomerism to a



Scheme 7. Possible racemization mechanism in the biosynthesis of lucilactaene.

2-hydroxypyrrole derivative, which could undergo epoxidation and isomerization. Racemization could occur via the achiral pyrrole tautomer. A subsequent Michael reaction would then afford racemic lucilactaene (1).

In summary, the labile natural product lucilactaene (1), which readily undergoes racemization, has been synthesized for the first time in optically pure form via a biomimetic pathway. The conditions under which racemization occurs were elucidated during this total synthesis. The careful isolation of lucilactaene (1) from both the broth and the mycelia under neutral, nonracemizing conditions demonstrated that the isolable natural product is in fact itself racemic. This total synthesis, which enabled verification of the absolute configuration of 1, has several noteworthy features: All the reactions from NG-391 (2) are mild enough not to

affect the labile E, E, E, E pentaene moiety; ether formation from 2 to 7 and 11 and the intramolecular Michael reaction from 9 to 10 or from 13 to 14 are both highly stereoselective; the reductive removal of the epoxide with SmI₂ without effecting demethoxylation, and the deprotection of a hemiaminal under neutral, oxidative conditions via vinyl ether 16 by using a newly developed phenylselenylethyl protecting group, are also useful transformations. Detailed biological studies on both enantiomers of lucilactaene are underway, the results of which will be reported in due course.

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