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Insights into Lomaiviticin Biosynthesis. Isolation and Structure Elucidation of (−)-Homoseongomycin

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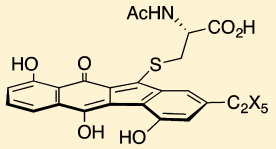
Supporting Information

ABSTRACT: The dimeric diazofluorenes known as the lomaiviticins are produced by the marine bacterium *Salinispora pacifica* DPJ-0019. Investigation of the fermentation broth of DPJ-0019 has yielded the first monomeric benzo[*b*]fluorene isolated from this species, (−)-homoseongomycin (**13**). (−)-Homoseongomycin (**13**) is related to the known natural product seongomycin (**10**), which is co-produced with the monomeric diazofluorenes known as the kinamycins. We describe the synthesis of the isotopically labeled derivative homoseongomycin-d_5 (**14**), via the intermediacy of the diazofluorene "prelomaiviticin-d_5" (**12**). Our studies establish that (−)-homoseongomycin (**13**) may be derived from prelomaiviticin (**11**) and suggest that **13** and **10** are shunt or detoxification metabolites in lomaiviticin and kinamycin biosynthesis, respectively.



X = H, (−)-homoseongomycin (**13**)
X = D, (−)-homoseongomycin-d_5 (**14**)

Bacteria of the genus *Salinispora*, first discovered by Fenical and co-workers in 2003,[1] have proven to be a rich reservoir of structurally diverse bioactive natural products including the salinosporamides,[2] the lymphostins,[3] and the lomaiviticins.[4] (−)-Lomaiviticins A and B (**1**, **2**, respectively) are cytotoxic, dimeric, C_2-symmetric bacterial metabolites that were first isolated from *Salinispora pacifica* 37L366 in 2001 (Figure 1A).[4a] The carbon skeleton of (−)-lomaiviticins A and B (**1**, **2**) comprises two diazotetrahydrobenzo[*b*]fluorene (diazofluorene) functional groups linked by a single carbon−carbon bond.[5] We recently reported the isolation of (−)-lomaiviticins C−E (**3**−**5**, respectively),[4b] which contain a hydroxyfulvene in place of one of the diazo groups, from *Salinispora pacifica* DPJ-0019.[6]

Two studies toward elucidating the biosynthesis of the lomaiviticins have been reported. The lomaiviticin biosynthetic gene cluster was recently characterized in *Salinispora tropica* CNB-440.[7] In addition, the biosynthesis of the aminosugar residue of the lomaiviticins has been studied in *E. coli*.[8] By comparison, the biosynthesis of the related monomeric diazofluorenes known as the kinamycins (see **6**−**8**)[9] has been extensively studied.[5a] In the latter pathway, the metabolite prekinamycin (**9**, Figure 1B)[10] was suggested to serve as a precursor to the kinamycins by a series of D-ring oxidations and acyl transfer steps (Figure 1B).[5a] The related metabolite seongomycin (**10**) is co-produced with the kinamycins.[11] No evidence for incorporation of seongomycin (**10**) in kinamycin biosynthesis was presented. Given the structural homology between the lomaiviticins and the kinamycins, it seems plausible that the lomaiviticins are synthesized by dimerization of two monomeric intermediates, and a putative dimerase enzyme has been identified in *S. tropica* CNB-440.[7] At present, the timing of dimerization remains undefined, and no monomeric precursors have been identified in the fermentation broth of the producing organisms.

Herein, we report the isolation and structure determination of (−)-homoseongomycin (**13**), which constitutes the first monomeric metabolite related to the lomaiviticins that has been isolated from DPJ-0019. We also describe an efficient synthesis of an isotopically labeled derivative of "prelomaiviticin" (prelomaiviticin-d_5, **12**) and demonstrate that this intermediate can be efficiently converted to (−)-homoseongomycin-d_5 (**14**). This latter result suggests that both seongomycin (**10**) and (−)-homoseongomycin (**13**) are derived from the corresponding diazofluorenes **9** and **11** by reduction and addition of *N*-acetyl-L-cysteine. This work provides the isotopically labeled derivative prelomaiviticin-d_5 (**12**) for feeding studies.

(−)-Homoseongomycin (**13**) was isolated by UV−vis-guided fractionation (310 nm) of the fermentation broth of DPJ-0019. Detailed fermentation conditions have been described.[4b] Briefly, the bacteria were grown in SPYESS media in the presence of HP-20 beads for 9 d at 28 °C. The beads were isolated by filtration and collected, and the collected beads were washed with MeOH. Concentration of the MeOH extracts and purification by reversed-phase chromatography afforded a dark green solid mass (500 mg/L), which was further purified by reversed-phase HPLC (CH_3CN−H_2O gradient containing 0.1% TFA).[12] (−)-Homoseongomycin (**13**) was obtained as a dark purple solid, in yields of 13−20 mg/L over several fermentations. Purified (−)-homoseongomycin (**13**) is soluble in DMF and DMSO but has limited solubility in MeOH, CH_2Cl_2, and THF.

HRMS analysis of **13** indicated a molecular formula of $C_{24}H_{21}NO_7S$. The structure of **13** was elucidated by 1D- and 2D-NMR analysis and IR spectroscopy. NMR data are presented in Table 1. C−H correlations were established by HMQC and HMBC experiments. An exchangeable proton at δ

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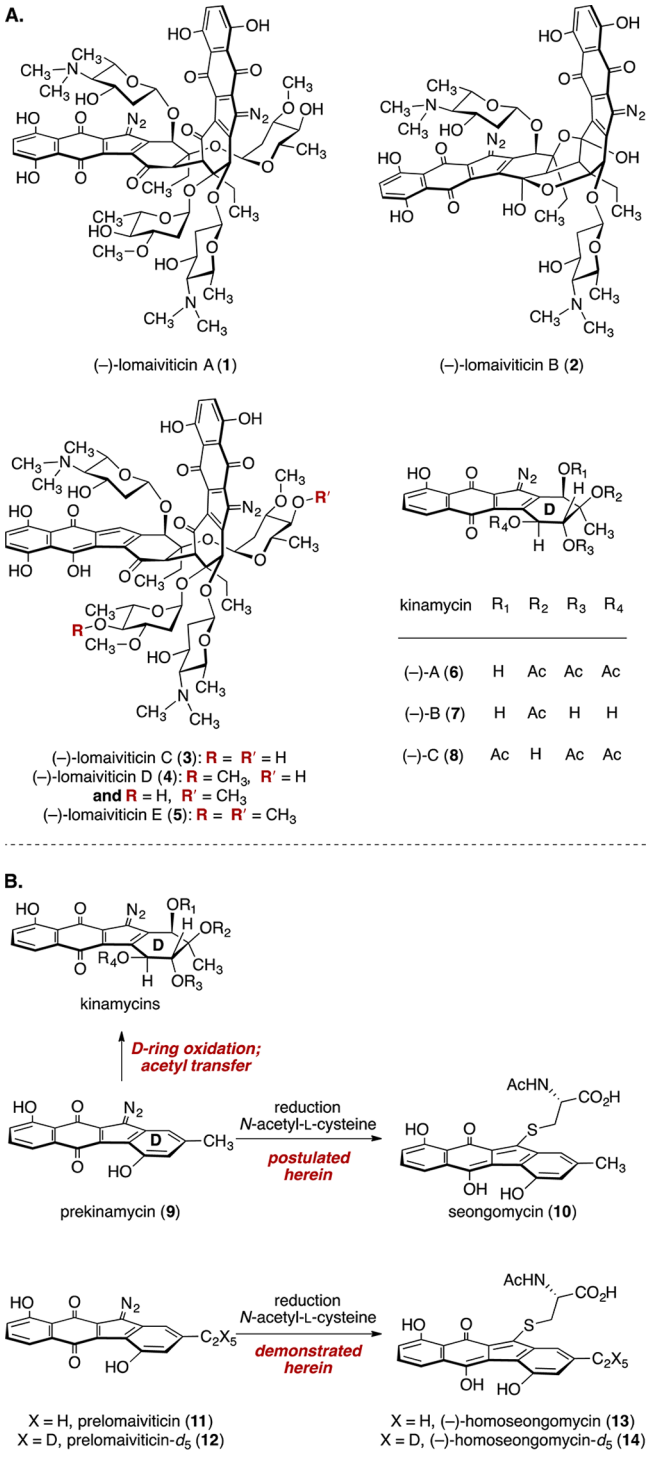


Figure 1. (A) Structures of lomaiviticins A–E (**1**–**5**) and kinamycins A–C (**6**–**8**). (B) Structures of prekinamycin (**9**), seongomycin (**10**), prelomaiviticin (**11**), (–)-homoseongomycin (**13**), and isotopically labeled derivatives.

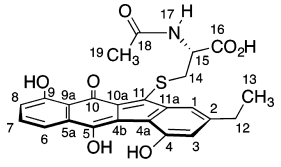
13.54 [confirmed by solvent exchange (CD_3OD) and HMQC experiments] and a three-proton spin system in the range of δ 7.52–6.95 (COSY) were diagnostic of a juglone residue. A quartet at δ 2.58, integrating for 2 H and coupled to a triplet at δ 1.19 (3H, J = 7.5 Hz), suggested the presence of an arylethyl substructure. HMBC correlations between a signal at δ 6.63 (H-3) and carbon resonances at δ 28.4 (C-12), 149.9 (C-4), and 114.4 (C-1), as well as a singlet at δ 7.09 (H-1) correlated

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data for (–)-Homoseongomycin (13)[a]

position	δ_C, type (125 MHz)	δ_H, mult. (J in Hz, 500 MHz)	HMBC (H→C)
1	114.4, CH	7.09, s	12, 11, 4a, 4, 3
2	143.9, C		
3	114.3, CH	6.63, s	12, 4a, 4, 1
4	149.9, C		
4a	121.2, C		
4b	114.8, C		
5	152.5, C		
5a	134.7, C		
6	116.1, CH	7.36, br d (7.5)	9a, 8, 7, 5
7	135.7, CH	7.52, dd (8.0, 8.0)	9, 6, 5a
8	119.7, CH	6.95, br d (8.0)	9, 9a, 6
9	162.6, C		
9-OH		13.54, s	9, 8, 6
9a	116.1, C		
10	184.3, C		
10a	128.3, C		
11	141.3, C		
11a	140.6, C		
12	28.4, CH_2	2.58, q (8.0)	13, 3, 2, 1
13	15.6, CH_3	1.19, t (7.5)	12, 2
14	34.9, CH_2	3.80, dd (13.5, 4.5)	16, 15, 11
		3.45, dd (11.0, 7.5)	
15	52.4, CH	4.37, dt (8.5, 4.5)	18, 16, 14
16	171.8, C		
17		8.27, d (8.0)	19, 18, 15
18	169.3, C		
19	22.2, CH_3	1.73, s	18

[a]NMR spectra were recorded in DMSO-d_6 at 24 °C. Carbons 4b, 10, 10a, and 11a were assigned by analogy to seongomycin (**10**).

to carbons at δ 114.3 (C-3), 28.4 (C-12), and 121.2 (C-4a), established this as a 3-ethyl-5,6-disubstituted phenol. ^{1}H and ^{13}C NMR data designated an *N*-acetyl cysteine subunit, which was connected to the core off the molecule at C-11 (δ 141.3) by HMBC. Collectively, these data suggested a structure similar to seongomycin (**10**). The remaining carbon–carbon connectivity was assigned by HMBC experiments and by analogy to seongomycin (**10**). The absolute configuration of the cysteine side chain was not rigorously established and was assumed to be the same as that of seongomycin (**10**).



(–)-homoseongomycin (**13**)

Chemical synthesis of (–)-homoseongomycin-d_5 (**14**) confirmed these assignments (Scheme 1).[13] Beginning with cyclohex-2-en-1-one (**15**), copper-catalyzed 1,4-addition of ethyl-d_5-magnesium iodide followed by oxidation of the resulting ketone (not shown) formed 5-(ethyl-d_5)-cyclohex-2-en-1-one (**16**, 56%, two steps). Copper-catalyzed 1,4-addition of trimethylsilylmethylmagnesium chloride, trapping of the resulting enolate with chlorotrimethylsilane, and palladium-mediated oxidation provided the β-(trimethylsilylmethyl)-α,β-unsaturated ketone **17** (66%, two steps). Fluoride-mediated

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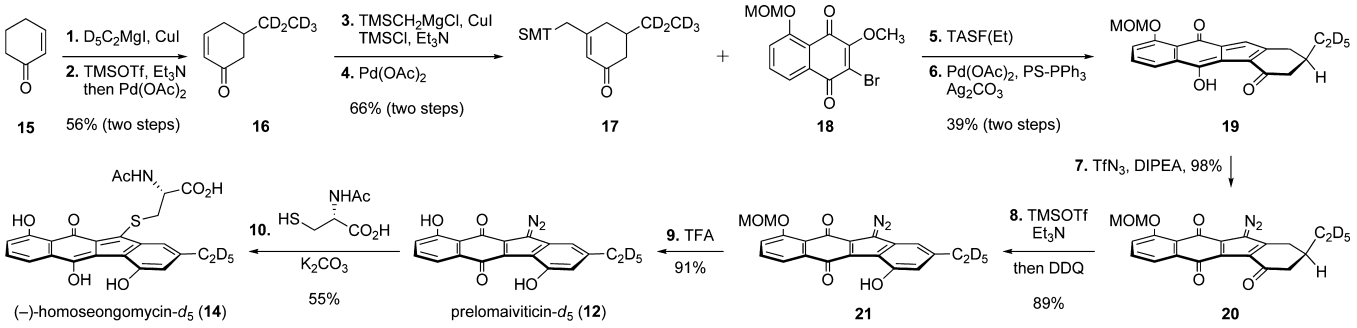
Scheme 1. Synthesis of Prelomaiviticin-d_5 (12) and Homoseongomycin-d_5 (14)[a]



[a]Conditions: (1) D_5C_2MgI, CuI, Et_2O, −40 °C, 92%. (2) Et_3N, TMSOTf, CH_2Cl_2, 0 °C; then $Pd(OAc)_2$, CH_3CN, 24 °C, 61%. (3) $TMSCH_2MgCl$, CuI, HMPA, Et_3N, TMSCl, THF, −30 → −60 → −78 °C. (4) $Pd(OAc)_2$, CH_3CN, 24 °C, 66%, two steps. (5) TASF(Et), CH_2Cl_2, −78 °C, 72%. (6) $Pd(OAc)_2$, polymer-supported PPh_3, Ag_2CO_3, toluene, 80 °C, 54%. (7) TfN_3, DIPEA, CH_3CN, 24 °C, 98%. (8) TMSOTf, Et_3N, CH_2Cl_2, 0 °C; then DDQ, CH_2Cl_2t-BuOH pH 7 buffer, 24 °C, 89%. (9) TFA, CH_2Cl_2, 24 °C, 91%. (10) *N*-Ac-L-cysteine, K_2CO_3, DMF, 24 °C, 55%.

coupling of the β-(trimethylsilylmethyl)-α,β-unsaturated ketone **17** with *O*-(methoxymethyl)-2-bromo-3-methoxyjuglone (**18**)[13] generated a γ-quinonylated enone (not shown) that was cyclized by heating with palladium acetate in the presence of polymer-supported triphenylphosphine (PS-PPh_3), to form the hydroxyfulvene **19** (39%, two steps). Diazo transfer to the hydroxyfulvene **19** (trifluoromethanesulfonyl azide) provided the diazofluorene **20** (98%). Enoxysilane generation (trimethylsilyl trifluoromethanesulfonate, triethylamine) followed by oxidation (1,2-dichloro-5,6-dicyanobenzoquinone, DDQ) formed the phenol **21** (89%).

Acid-catalyzed deprotection of the methoxymethyl protecting group provided prelomaiviticin-d_5 (**12**, 91%). Treatment of prelomaiviticin-d_5 (**12**) with *N*-acetyl-L-cysteine and potassium carbonate generated (−)-homoseongomycin-d_5 (**14**, 55%). NMR spectroscopic data for (−)-homoseongomycin-d_5 (**14**) closely matched those of (−)-homoseongomycin (**13**), and equimolar mixtures of the natural and synthetic material exhibited similar chromatographic and spectroscopic properties (HPLC, ^1H, ^{13}C NMR analysis). The mild conversion of prelomaiviticin-d_5 (**12**) to (−)-homoseongomycin-d_5 (**14**) observed here suggests that prekinamycin (**9**) and prelomaiviticin (**11**) are converted to the seongomycins **10** and **13** by thiol addition intracellularly or in the fermentation media.

In summary, we have described the isolation and structure elucidation of (−)-homoseongomycin (**13**), a monomeric metabolite related to the lomaiviticins. We have provided evidence that the seongomycins are readily formed from diazofluorenes, suggesting they are shunt or detoxification metabolites in the biosynthesis of the kinamycins and lomaiviticins. The synthesis of prelomaiviticin-d_5 (**12**) described herein enables feeding studies to probe lomaiviticin biosynthesis.

(−)-Homoseongomycin (13): amorphous, purple solid; $[\alpha]_D^{20}$ −525 (*c* 0.001, CH_3OH); UV (0.1% TFA in CH_3CN–H_2O, diode array detector) λ_{max} (log ε) 225, 229, 234, 282 nm; IR (ATR-FTIR) ν_{max} 3333 (br), 1621 (s), 1585 (s), 1451 (m) cm^{-1}; ^1H and ^{13}C NMR data (500 and 125 MHz, DMSO-*d*) see Table 1; ^1H NMR (400 MHz, DMF-d_7) δ 13.66 (1H, s, C-9-OH), 8.34 (1H, d, *J* = 8.0 Hz, H-17), 7.56 (1H, dd, *J* = 8.0, 8.0 Hz, H-7), 7.42 (1H, br s, H-6), 7.24 (1H, br s, H-1), 6.97 (1H, br s, H-8), 6.81 (1H, br s, H-3), 4.69 (1H, ddd, *J* = 8.0, 4.8, 4.8 Hz, H-15), 3.98 (1H, br s, H-14), 3.72 (1H, br s, H-14), 2.64 (2H, br s, H-12), 1.83 (3H, s, H-19), 1.24 (3H, t, *J* = 7.6 Hz, H-13); ^1H NMR (500 MHz, CD_3OD) δ 7.42 (1H, dd, *J* = 8.0, 8.0 Hz, H-7), 7.35 (1H, d, *J* = 7.5 Hz, H-6), 7.15 (1H, s, H-1), 6.87 (1H, d, *J* = 8.0 Hz, H-8), 6.64 (1H, s, H-3), 4.57 (1H, dd, *J* = 8.5, 4.0 Hz, H-15), 4.01 (1H, dd, *J* = 13.5, 4.5 Hz, H-14), 3.55 (1H, dd, *J* = 14.0, 9.0 Hz, H-14), 2.65–2.58 (1H, m, H-12), 1.77 (3H, s, H-19), 1.26 (3H, t, *J* = 7.5 Hz, H-13); ^{13}C NMR (125 MHz, DMF-d_7) δ 185.7 (C), 172.9 (C), 170.6 (C), 164.4 (C), 152.1 (C), 151.1 (C), 145.8 (C), 144.3 (C), 143.3 (C), 136.7 (CH), 135.7 (C), 130.1 (C), 122.2 (C), 120.7 (CH), 117.4 (CH), 117.2 (CH), 116.6 (C), 116.2 (CH), 115.7 (C), 53.9 (CH), 36.6 (CH_2), 29.8 (CH_2), 22.8 (CH_3), 16.3 (CH_3); HRMSESI *m*/*z* 468.1132 $[M + H]^+$ (calcd for $C_{24}H_{22}NO_7S$, 468.1117).

■ ASSOCIATED CONTENT

Supporting Information

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

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Notes

The authors declare no competing financial interest.

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