First Stereoselective Synthesis of Penicillenol A1 via Novel O- to C-Acyl Rearrangement of O-Acyltetramic Acid

First stereoselective synthesis of penicillenol A_1 via novel *O*- to *C*-acyl rearrangement of *O*-acyltetramic acid

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The first stereoselective synthesis of penicillenol A_1 has been accomplished in 9 steps from L-threonine. The 3-acyltetramic acid core of penicillenol A_1 was constructed by successful *O*- to *C*-acyl rearrangement.

Key words: stereoselective synthesis, penicillenol A_1, *O*- to *C*-acyl rearrangement, 4-*O*-acyltetramic acid, 3-acyltetramic acid

Penicillenols A_1 and A_2 are tetramic acid derivatives recently isolated from the culture broth of a fungus *Penicillium* sp., an endophytic fungus associated with *Aegiceras corniculatum* (Figure 1).[1] Penicillenol A_1 (**1**) has been identified as a potent cytotoxic agent (HL-60, IC_{50} = 0.76 μM), displaying much higher activity than penicillenol A_2 (**2**). Although their planar structures and the absolute stereochemistry of the chiral center at C5 were elucidated by the analysis of NMR and CD spectra, absolute configurations of the C6 and C9 stereocenters remained unspecified. Here we report an efficient synthesis of penicillenol A_1 in an effort to establish the absolute configurations.

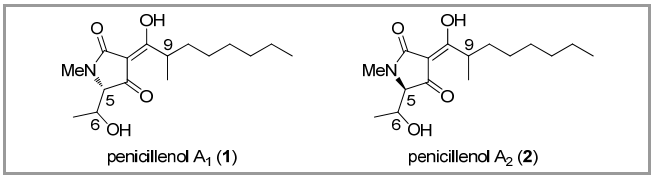


Figure 1 Structures of penicillenols A_1 and A_2.

Our synthetic plan for penicillenol A_1 is outlined in Figure 2. We expected that natural penicillenol A_1 would have (6*R*)-configuration, considering that the tetramic acid moiety is biosynthetically derived from L-threonine.[1,2] Accordingly, we decided to synthesize two possible diastereomers of (6*R*)-penicillenol A_1 by employing acylation of tetramic acid **3** with chiral carboxylic acid **4**.

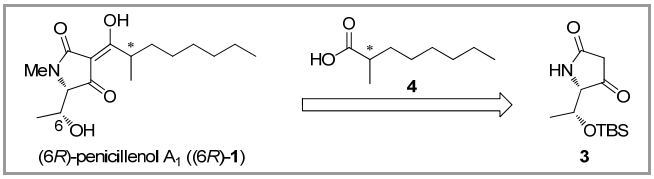
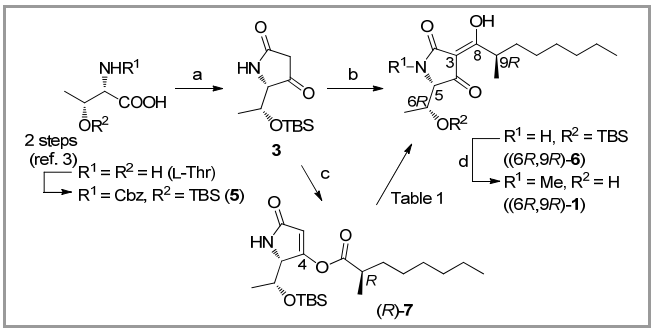


Figure 2 Retrosynthetic analysis of (6*R*)-penicillenol A_1

The synthesis commenced with the preparation of the TBS-protected tetramic acid **3** (Scheme 1). Condensation of L-threonine derivative **5**[3] with Meldrum's acid in the presence of *N*,*N*'-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP), followed by the cyclization with concomitant loss of carbon dioxide and acetone provided *N*-Cbz derivative of **3**.[4] The Cbz group was removed under hydrogenation conditions to furnish desired **3** in 38% yield (three steps) from **5**.



Scheme 1 *Reagents and conditions*: (a) (i) Meldrum's acid, DCC, DMAP, CH_2Cl_2, 0 ºC; (ii) EtOAc, reflux; (iii) H_2, Pd/C, EtOAc, 38% (three steps); (b) (*R*)-**4**, DCC, DMAP, CH_2Cl_2, then Et_3N, trace; (c) (*R*)-**4**, *i*-BuOCOCl, *N*-methylmorpholine, Et_2O, 0 ºC, then, 3, Et_3N, rt, 95%; (d) (i) NaHMDS, MeI, THF, -40 ºC, 78%; (ii) 2% HCl/MeOH, 0 ºC, 78%.

With tetramic acid **3** in hand, we turned our attention to the preparation of the C3-acylated target product obtained from the reaction with (*R*)-2-methyloctanoic acid ((*R*)-**4**)[5] according to the reported reaction conditions.[6] Although initial conversion from **3** into 4-*O*-acyltetramic acid ((*R*)-**7**) was achieved by the reaction with (*R*)-**4** in the presence of DCC and DMAP, *O*- to *C*-acyl rearrangement with Et_3N proceeded slowly to give only a trace amount of desired (6*R*,9*R*)-**6**. This low reactivity would be attributed to the steric hindrance around a carboxyl group.[7] Therefore, we next attempted a stepwise approach that uses 4-*O*-acyltetramic acid (*R*)-**7** prepared in 95% yield from the *O*-acylation of **3** with the mixed anhydride of (*R*)-**4** and isobutyl formate.

The results of *O*- to *C*-acyl rearrangement with (*R*)-**7** are summarized in Table 1. When the 4-*O*-acyl intermediate (*R*)-**7** was treated with DMAP and Et_3N, only a trace amount of the 3-acyltetramic acid (6*R*,9*R*)-**6** was obtained (entry 1). No significant change in conversion was observed at a higher temperature (entry 2), whereas a dramatic improvement was observed in the reaction with calcium chloride to afford desired (6*R*,9*R*)-**6** in 65%

isolated yield after separation of a trace amount of C5-isomerized product by silica gel column chromatography (entry 3). It is worth noting that the acyl rearrangement failed to give any desired compound in the absence of DMAP (entry 4), and, on the one hand, the reaction worked well without Et_3N to give (6*R*,9*R*)-**6** in enhanced 75% yield after chromatographic purification (entry 5). These results suggest that DMAP played a significant role in activation of the acyl moiety, which would be in close contact with the liberated tetramic acid via chelation of Ca^{2+} ions, promoting the rearrangement to give the desired product in good yields, although the exact mechanistic details of this process are still under investigation.[8]

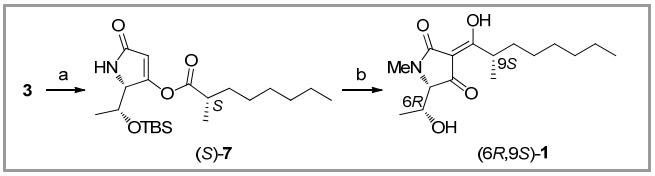
Table 1 Acyl rearrangement of (*R*)-**7** to (6*R*,9*R*)-**6**.

Entry	DMAP (equiv)	Et_3N (equiv)	$CaCl_2$ (equiv)	Solvent	Temp. (°C)	Time (h)	Yield (%)[a]
1	0.3	1.2	-	CH_2Cl_2	rt	96	trace
2	0.3	1.2	-	$ClCH_2CH_2Cl$	50	24	trace
3	0.3	1.2	1.5	CH_2Cl_2	rt	6	65
4	-	1.2	1.5	CH_2Cl_2	rt	12	0
5	1.5	-	1.5	CH_2Cl_2	rt	4	75

[a] Isolated yield of (6*R*,9*R*)-**6**

Having achieved the preparation of the 3-acyltetramic acid structure, we focused on the final steps of the synthesis. After considering the reaction conditions, *N*-methylation of amide was achieved by treating deprotonated (6*R*,9*R*)-**6** with excess amount of iodomethane at -40 ºC. In the course of this reaction, no detectable epimerization of the C5 stereocenter was observed. Finally, removal of TBS protecting group was conducted under acidic conditions to complete the first stereoselective synthesis of (6*R*,9*R*)-penicillenol A_1 ((6*R*,9*R*)-**1**) in 16% overall yield from **5**.[9]

The synthesis of another diastereomer (6*R*,9*S*)-**1** was also performed in the same manner as for (6*R*,9*R*)-**1** (Scheme 2). Thus, 4-*O*-acyltetramic acid (*S*)-**7** was synthesized by the acylation of **3** with (*S*)-**4** and converted to (6*R*,9*S*)-**1** in 36% yield (three steps).[10]



Scheme 2 *Reagents and conditions*: (a) (*S*)-**4**, *i*-BuOCOCl, *N*-methylmorpholine, Et_2O, 0 ºC, then, **3**, Et_3N, rt, 95%; (b) (i) DMAP, $CaCl_2$, CH_2Cl_2, 60%; (ii) NaHMDS, MeI, THF, -40 ºC, 82%; (iii) 2% HCl/MeOH, 0 ºC, 74%.

As a matter of fact, ^1H NMR spectra of both synthetic penicillenols A_1 closely resembled those reported in the literature,[1] and detailed analysis of ^{13}C NMR spectra of them indicated that ^{13}C NMR spectra of (6*R*,9*S*)-**1** closely matched that of the natural product relative to that of (6*R*,9*R*)-**1**.[11,12] However, the absolute value of optical rotation of (6*R*,9*S*)-**1** ($[\alpha]_D^{29}$ -68.5 (*c* 0.618, MeOH)) was unexpectedly found to be inconsistent with that reported in the literature ($[\alpha]_D^{29}$ -864.5 (*c* 0.155, MeOH)). From these results, it can be deduced that the natural sample may be contaminated with some impurities.[13]

In conclusion, we have achieved the first stereoselective syntheses of (6*R*,9*R*)- and (6*R*,9*S*)-penicillenol A_1 in 9 steps starting from L-threonine. As a consequence of the spectroscopic investigations, natural penicillenol A_1 has been found to have a (5*S*,6*R*,9*S*)-absolute configuration. Our synthetic approach involves novel acyl rearrangement mediated by calcium chloride. The syntheses of (6*S*)-derivatives of penicillenols A_1 and further investigations on the role of calcium chloride are now in progress.

Acknowledgment

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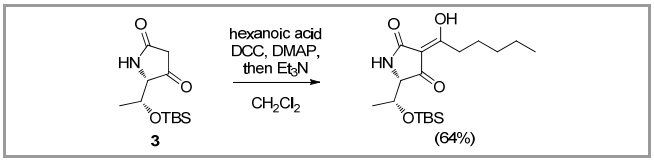
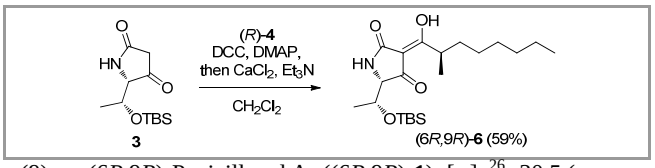
(7) Under the same conditions, one-pot acylation between **3** and hexanoic acid gave 3-acyltetramic acid in 64% yield.



(8) The addition of $CaCl_2$ also improved the yield in one-pot procedure. For example, the reaction of **3** with (*R*)-**4** in the presence of $CaCl_2$ afforded (6*R*,9*R*)-**6** in 59% yield, whereas a trace amount of (6*R*,9*R*)-**6** was obtained in the absence of $CaCl_2$ (see scheme 2).



(9) (6*R*,9*R*)-Penicillenol A_1 ((6*R*,9*R*)-**1**): $[\alpha]_D^{26}$ -30.5 (*c* 0.276, MeOH); IR (NaCl) 3414, 2956, 2927, 2857, 1709, 1642, 1611, 1484, 1462, 1379, 1342, 1089 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 4.19 (dq, *J* = 4.6, 6.4 Hz, 1H, C*H*), 3.81 (d, *J* = 4.6 Hz, 1H, C*H*), 3.56 (m, 1H, C*H*), 2.98 (s, 3H, CH_3), 1.68 (m, 1H, CH_2), 1.49 (m, 1H, CH_2),1.33-1.21 (m, 8H, CH_2), 1.18 (d, *J* = 7.0 Hz, 3H, CH_3), 1.13 (d, *J* = 6.4 Hz, 3H, CH_3), 0.86 (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) 195.0 (*C*), 192.9 (*C*), 174.1 (*C*), 101.3 (*C*), 68.4 (*C*H), 66.6 (*C*H), 36.3 (*C*H), 33.9 (*C*H_2), 31.7 (*C*H_2), 29.1 (*C*H_2), 27.1 (*C*H_3), 27.0 (*C*H_2), 22.5 (*C*H_2), 17.5 (*C*H_3), 17.1 (*C*H_3), 14.0 (*C*H_3); Anal. Calcd for $C_{16}H_{27}NO_4$: C, 64.62; H, 9.15; N, 4.71. Found: C, 64.55; H, 9.08; N, 4.31.

(10) (6*R*,9*S*)-Penicillenol A_1 ((6*R*,9*S*)-**1**): $[\alpha]_D^{29}$ -68.5 (*c* 0.618, MeOH); IR (NaCl) 3425, 2956, 2928, 2857, 1708, 1642, 1610, 1486, 1460, 1378, 1342, 1089 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 4.19 (dq, *J* = 4.2, 6.3 Hz, 1H, C*H*), 3.80 (d, *J* = 4.2 Hz, 1H, C*H*), 3.56 (m, 1H, C*H*), 2.99 (s, 3H, CH_3), 1.68 (m, 1H, CH_2), 1.47 (m, 1H, CH_2),1.33-1.22 (m, 8H, CH_2), 1.18 (d, *J* = 6.9 Hz, 3H, CH_3), 1.14 (d, *J* = 6.6 Hz, 3H, CH_3), 0.87 (t, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) 194.7 (*C*), 192.8 (*C*), 174.1 (*C*), 100.9 (*C*), 68.5 (*C*H), 66.6 (*C*H), 36.4 (*C*H), 33.4 (*C*H_2), 31.5 (*C*H_2), 29.1 (*C*H_2), 27.1 (*C*H_3), 27.0 (*C*H_2), 22.4 (*C*H_2), 17.6 (*C*H_3), 17.0 (*C*H_3), 13.9 (*C*H_3); Anal. Calcd for $C_{16}H_{27}NO_4$: C, 64.62; H, 9.15; N, 4.71. Found: C, 64.86; H, 9.00; N, 4.31.

(11) Selected ^{13}C NMR data of natural and synthetic penicillenols A_1 around C9 center are shown.

	C3	C4	C8	C9	C10
natural **1**[a]	100.9	194.6	192.6	36.3	33.4
(6*R*,9*R*)-**1**[b]	101.2	195.0	192.9	36.3	33.9
$\Delta_{\delta 9R}$[c]	+0.3	+0.4	+0.3	0	+0.5
(6*R*,9*S*)-**1**[b]	100.9	194.7	192.8	36.4	33.4
$\Delta_{\delta 9S}$[c]	0	+0.1	+0.2	+0.1	0

[a] 150 MHz. [b] 75 MHz. [c] $\Delta_{\delta X} = \delta((6R,X)\text{-}\mathbf{1}) - \delta(\text{natural } \mathbf{1})$

(12) ^1H and ^{13}C NMR spectra of (5*R*)-isomer of (6*R*,9*R*)-**1**, which was prepared by isomerization of **3** (DBU, toluene, 50 ºC), was found to be quite similar to those of natural penicillenol A_2. This indicated that enantiomeric (5*S*,6*S*)-isomers should give NMR spectra similar to those of penicillenol A_2. Details of synthesis and characterization of (5*R*)-isomer of (6*R*,9*R*)-**1** will be presented in future work.

(13) Synthetic sample was obtained as a colorless oil after separation from orange-colored material by silica gel column chromatography. Due to the fact that the natural sample has been reported to be isolated as an yellow oil, we conclude that the previously reported value of optical rotation was obtained by measuring incompletely purified one.