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Letter

Total Synthesis and Stereochemical Assignment of Penicitide A

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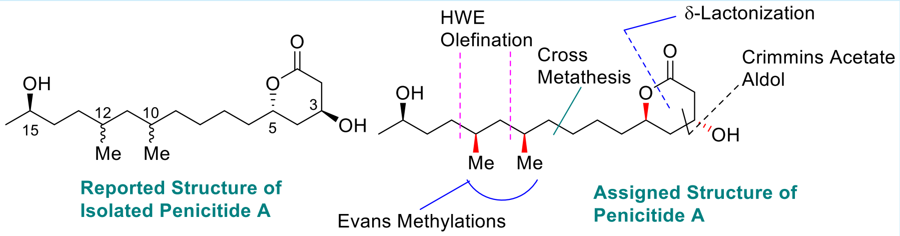

ABSTRACT: Stereoselective total synthesis of marine secondary metabolite penicitide A has been accomplished for the first time following a convergent approach. The salient feature of this study includes Horner−Wadsworth−Emmons (HWE) olefination, Evans methylations, Crimmins acetate aldol reaction, and cross olefin metathesis. Our synthetic study established the stereochemistry of unassigned C-10 and C-12 centers and also disclosed the absolute configurations of C-3 and C-5 stereocenters.

Marine fungi are known to be rich sources of architecturally attractive and biologically active secondary metabolites.[1] Chemical synthesis of these metabolites is the subject of great interest to the scientific community for their unambiguous structural assignment and for access to an ample supply of the natural products and their analogues for biological evaluations.[2] During the bioassay-guided isolation of endophytic fungi, *Penicillium chrysogenum* QEN-24S, obtained from an unidentified marine red algal species of the genus *Laurencia*, Wang and co-workers, in 2011, discovered the polyketide natural products penicitides A (**1**) and B (**2**) (Figure 1).[3] Penicitide A showed moderate cytotoxicity against pathogen *A. brassicae* and the human hepatocellular liver carcinoma (HepG2) cell line while penicitide B lacked such activities. The structures of these molecules were partially assigned by the isolation group using spectroscopic methods. Penicitide A consists of a linear structure terminated with a δ-lactone moiety. There are five streocenters in the molecule among which three are hydroxylated and two are methylated. On the other hand, penicitide B possesses a structure similar to that of penicitide A with a small difference at the C-12 center, which is a tetrasubstituted center bearing an additional hydroxy group.[3] The stereochemistry of both C-10 and C-12 centers remains undetermined in both these molecules to date. The interesting architectural feature of penicitides A and B along with our continual interest[4] in natural products synthesis prompted us to envisage their chemical synthesis with the goal to develop efficient synthetic routes and, most importantly, to address the uncertainties in their structural assignments. We initially embarked upon cytotoxic penicitide A. In this manuscript we disclose a convergent and flexible synthetic route for penicitide A along with its full stereochemical assignment for the first time.

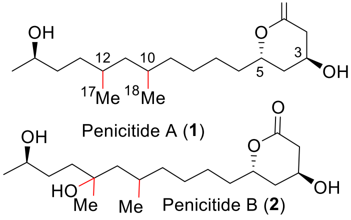

Figure 1. Reported structures of isolated penicitides A (**1**) and B (**2**).

The retrosynthetic analysis of penicitide A is outlined in Scheme 1. The stereochemistry of the C-10 and C-12 centers of penicitide A remained undisclosed during its isolation. In 2012, Breit et al.[5] predicted their relative configurations as *syn* from comparison with known 1,3-deoxypropionate units using NMR spectroscopic methods. However, there was no further confirmation of their absolute configurations. We planned to adopt a different strategy where possible stereo tetrads **1(a−d)** would be prepared to compare their spectroscopic data with those reported for the isolated penicitide A. This could give useful NMR spectroscopy data for all the possible stereoisomers thereby allowing NMR assignment of the isolated natural product. Thus, we planned to construct compounds **1(a−d)** from compounds **3(a−d)** and δ-lactone **4** using cross olefin metathesis as one of key strategic steps. Compounds

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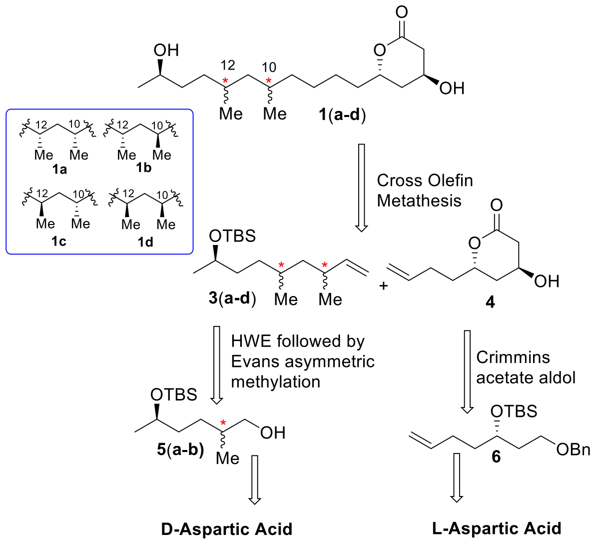
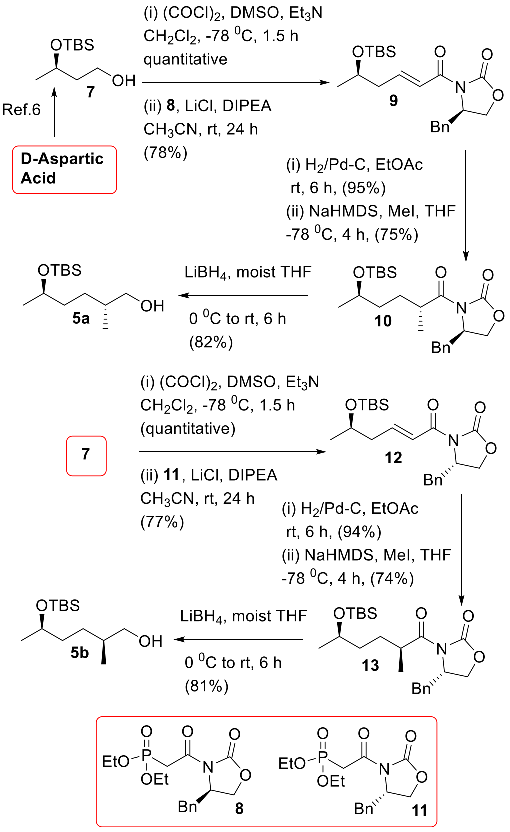
Scheme 1. Retrosynthetic Analysis of Penicitide A



3(a–d) could be synthesized from D-aspartic acid via the intermediates 5(a–b) following Horner–Wadsworth–Emmons (HWE) olefination and Evans methylation as the pivotal steps. The *δ*-lactone 4 could be prepared from compound 6 using Crimmins acetate aldol as one of the key steps which subsequently could be synthesized from L-aspartic acid.

The synthesis of compounds **5a** and **5b** was commenced with the known alcohol **7**[6] prepared from D-aspartic acid following the literature procedure (Scheme 2). Alcohol **7** was oxidized to the corresponding aldehyde using Swern conditions and concomitantly subjected to HWE olefination[4a,7] using the known phosphonate[4a,8] **8** in the presence of LiCl/DIPEA to prepare compound **9** with preference for *E*-selectivity. Compound **9** was then hydrogenated and subsequently subjected to Evans methylation[4a,9] in the presence of NaHMDS/MeI to obtain compound **10** as the exclusive isomer which further reacted with $LiBH_4$ to access alcohol **5a** in very good overall yield. Following exactly the same chemistry of compound **5a**, alcohol **7** was converted to compound **5b** via intermediates **12** and **13** where phosphonate **11** was used in the HWE olefination step.

Scheme 2. Synthesis of Compounds 5a and 5b

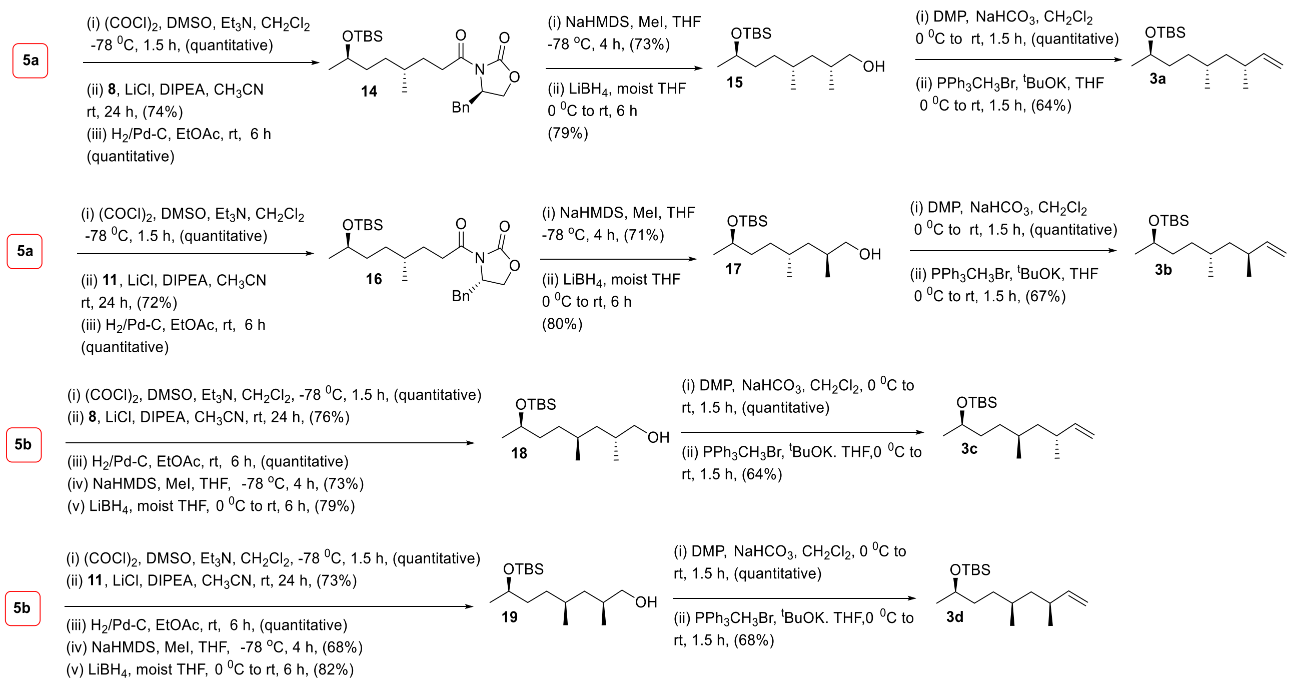


The synthesis of coupling partners **3(a–d)** for one-half of penicitide A is depicted in Scheme 3. Alcohol **5a** was oxidized to the corresponding aldehyde by Swern conditions which was treated with phosphonate **8** following HWE olefination in the presence of LiCl/DIPEA and subsequently hydrogenated to obtain compound **14**. Next, compound **14** was subjected to Evans methylation in the presence of NaHMDS/MeI to access the corresponding methylated product as a single isomer. The methylated compound was then reduced to compound **15** using $LiBH_4$ and further oxidized using DMP followed by one carbon Wittig olefination to produce compound **3a** in good overall yield. Similarly, compound **3b** was synthesized from alcohol **5a** in the presence of phosphonate **11** via intermediates **16** and **17**. Compounds **3c** and **3d** were also prepared from alcohol **5b** through intermediates **18** and **19**, respectively, following a route similar to those for compounds **3a** and **3b**.

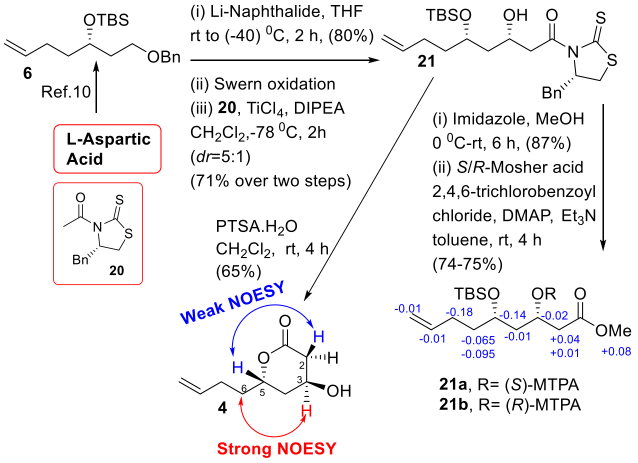
The synthesis of coupling partner **4** is described in Scheme 4. The known compound **6**,[10] prepared from L-aspartic acid following literature procedure, was subjected to debenzylation in the presence of Li/naphthalene[4a,11] to afford the corresponding alcohol in 80% yield. Alcohol was then oxidized to the corresponding aldehyde under Swern conditions and subsequently subjected to Crimmins acetate aldol reaction[4e,12] with the known thiazolidione **20**[4e,12c] in the presence of $TiCl_4$/DIPEA to obtain compound **21** as the major product (*dr* = 5:1) which was separated from its minor counterpart by column chromatography. The presence of a weak cross peak between H5 and one of the protons on C2 and a strong cross peak between H3 and protons on C6 in NOESY correlation supported the structure of compound **4**. To confirm further the absolute stereochemistry of the newly generated hydroxy center, compound **21** was converted to (*S*)- and (*R*)-MTPA esters **21a** and **21b**, respectively, following the reported procedure.[4e,13] All of the protons of the pairs of Mosher esters were assigned by ^1H NMR. The positive $\Delta\delta$ values $[\Delta\delta = \delta S - \delta R]$ (Scheme 4) recorded for H-2 protons from esters **21a** and **21b** clearly established the desired (*S*)-configuration of the originated hydroxy center. Next, compound **21** was treated with PTSA to afford compound **4**.

The synthesis of compounds **1(a–d)** is shown in Scheme 5. Alkenes **3a** and **4** were subjected to cross olefin metathesis.[4f,14] Different catalysts (G-II, HG-I) were screened at this stage in different reaction conditions (Table 1). G-II in CH_2Cl_2 (entry 2) functioned best compared to others regarding reaction time and yields. The metathesized product **22** was then hydrogenated and reacted with $PTSA{\cdot}H_2O$ to access compound **1a** in 60% yield (based on recovered starting material). A similar strategy was adopted to prepare compounds **1(b–d)** from alkenes **3(b–d)** using alkene **4**. NMR data of all the synthesized compounds were recorded and compared (see Tables S1 and S2 in the Supporting Information). The major mismatch in the ^1H NMR of compounds **1b** and **1c** was

Scheme 3. Synthesis of Coupling Partners 3(a−d)



Scheme 4. Synthesis of Coupling Partner 4



observed for the H-11 proton. The chemical shift of the H-11 proton, for both compounds, appeared at δ 1.06, 1.23 ppm whereas these protons in the isolated penicitide A were observed at δ 0.93, 1.22 ppm. Some discrepancies in the coupling constants of 17H and 18H for both the compounds were also observed (for compound **1b**, $^{3}J_{17H\text{-}12H}$ = 6.7 Hz and $^{3}J_{18H\text{-}10H}$ = 6.9 Hz, for compound **1c**, $^{3}J_{17H\text{-}12H}$ = $^{3}J_{18H\text{-}10H}$ = 5.2 Hz; reported $^{3}J_{17H\text{-}12H}$ = $^{3}J_{18H\text{-}10H}$ = 6.4 Hz). Moreover, anomalies were observed in the ^{13}C NMR for compound **1b** at C-8, C-13, C-14, C-17, C-18 (observed at δ 26.81, 33.90, 36.99, 19.69, 19.63 ppm, respectively; reported at δ 26.39, 32.67, 36.55, 20.28, 20.26 ppm, respectively) and for compound **1c** at C-8, C-9, C-14, C-17, C-18 (observed at δ 26.84, 37.73, 36.83, 19.59, 19.59 ppm, respectively; reported at δ 26.39, 36.55, 36.55, 20.28, 20.26 ppm, respectively). The optical rotation of compounds **1b** {observed $[\alpha]^{20}_{D} = -3.9$ (*c* 0.31, MeOH)} and **1c** {observed $[\alpha]^{20}_{D} = +5.0$ (*c* 0.4, MeOH)} differed significantly from the data of isolated penicitide A {reported $[\alpha]^{20}_{D} = +42.9$ (*c* 0.14, MeOH)}. This clearly discarded the possibility of a *trans*- relationship between C-10 and C-12 methyls in the isolated molecule which reconfirmed further the observation of Breit et al.[5] On the

Scheme 5. Completion of Total Synthesis of Compounds 1(a−d)

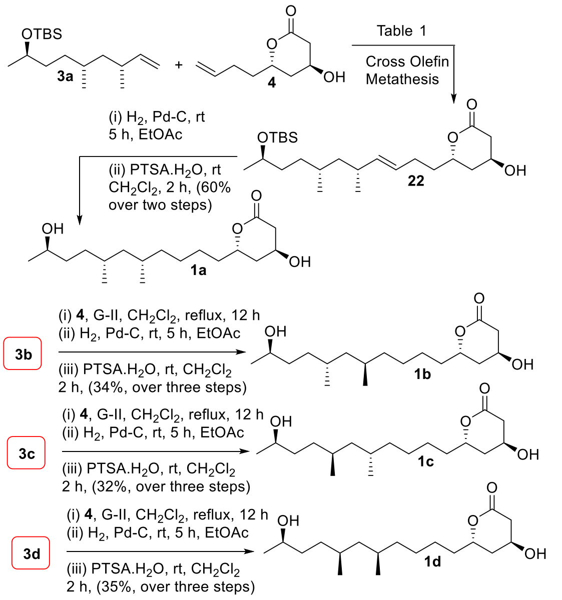


Table 1. Optimization of Cross Olefin Metathesis for Synthesis of Compound 22

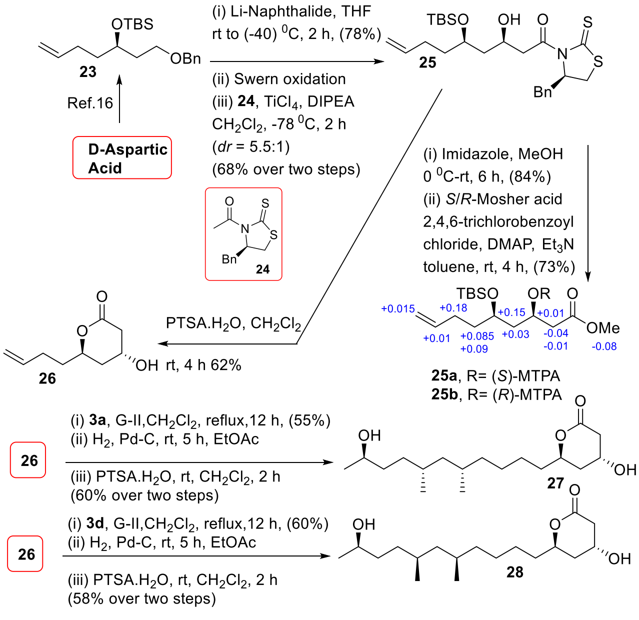
entry	catalyst	solvent	temp	time (h)	yield[a] (%)
1	G-II	toluene	reflux	24	25
2	G-II	CH_2Cl_2	reflux	12	60
3	HG-I	CH_2Cl_2	reflux	24	31
4	HG-I	toluene	reflux	24	20

[a]Yield was calculated based on recovered starting material.

other hand, the ^{1}H NMR data of both compounds **1a** and **1d** were in close agreement with the reported data except the coupling constants of 17H and 18H (for compound **1a**, $^3J_{17H\text{-}12H}$ = 5.7 Hz and $^3J_{18H\text{-}10H}$ = 5.8 Hz; for compound **1d**, $^3J_{17H\text{-}12H}$ = 3.9 Hz and $^3J_{18H\text{-}10H}$ = 4.1 Hz; reported $^3J_{17H\text{-}12H}$ = $^3J_{18H\text{-}10H}$ = 6.4 Hz). Moreover, major mismatches were also found in the ^{13}C NMR of compound **1a** at C-14, C-15 (observed at δ 36.76, 68.79 ppm, respectively; reported at δ 36.55, 68.56 ppm, respectively). The optical rotation of compound **1a** {observed $[\alpha]^{20}_{D}$ = −45.7 (*c* 0.35, MeOH)} differed completely from the reported value. The ^{13}C NMR of compound **1d** was in close agreement with the data of isolated penicitide A, but the optical rotation varied considerably {observed $[\alpha]^{20}_{D}$ = +21.0 (*c* 0.38, MeOH)}. This clearly confirmed that neither isomer **1a** nor **1d** could be the structure of the isolated penicitide A which compelled us to synthesize other possible diastereomers.

The absolute stereochemistry of the C-15 hydroxy center of penicitide A was confirmed by Mosher's ester method whereas the relative configurations of both the C-3 and C-5 oxygenated centers were assigned by comparing their ^{13}C NMR data with two known analogues[15] bearing the 3-hydroxy δ-lactone moiety ($3\beta/5\alpha$ and $3\beta/5\beta$) attached with a heptyl side chain. It was observed that the ^{13}C NMR data of the C-3 and C-5 centers were in close agreement with the known $3\beta/5\alpha$ analogue which guided the isolation group to assign them as 3β and 5α.[3] As the absolute stereochemistry of these centers were not established, one should also consider their opposite relative configurations ($3\alpha/5\beta$). Thus, we planned to synthesize compounds **27** and **28** (Scheme 6) where both C-10 and C-12 methyls are *syn*- relative to each other. The synthesis began with the known compound **23**,[16] prepared from D-aspartic acid following a literature procedure, which was transmuted to compound **25** as the major isomer following the chemistry adopted for the synthesis of compound **21** where Crimmins acetate aldol in the presence of thaizolidine **24**[4e,12c] was used as one of the key steps. Compound **25** was separated from its minor aldol product (*dr* = 5.5:1). The absolute stereochemistry of newly generated hydroxy center of compound **25** was confirmed further by a modified Mosher ester method[4e] by converting it to (*S*)- and (*R*)-MTPA esters **25a** and **25b**. The negative $\Delta\delta$ values [$\Delta\delta = \delta S - \delta R$] (Scheme 6) obtained from their H-2 protons clearly supported the (*R*)-configuration of the center installed in the Crimmins aldol reaction. Compound **25** was then treated with PTSA to obtain compound **26**. It was further subjected to cross metathesis with olefin **3a** in the presence of G-II to afford the corresponding coupled product. The coupled product was then hydrogenated and subsequently desilylated to access compound **27** in good overall yield. Similarly compound **28** was also prepared from olefins **3d** and **26**. The ^{1}H NMR of compound **27** was in close agreement. But anomalies in the coupling constants of 17H and 18H were observed where *J* values differed from each other (observed $^3J_{17H\text{-}12H}$ = 6.1 Hz and $^3J_{18H\text{-}10H}$ = 6.4 Hz; reported $^3J_{17H\text{-}12H}$ = $^3J_{18H\text{-}10H}$ = 6.4 Hz) (see Table S1 in the Supporting Information). Moreover, the ^{13}C NMR data revealed a major mismatch at C-14, C-15 compared to the reported values (observed at δ 36.78, 68.80 ppm, respectively; reported at δ 36.55, 68.56 ppm, respectively) (see Table S2 in the Supporting Information). Interestingly, the optical rotation of compound **27** {observed $[\alpha]^{20}_{D}$ = +42.1 (*c* 0.38, MeOH)} was in excellent agreement with the reported value {reported $[\alpha]^{20}_{D}$ = +42.9 (*c* 0.14, MeOH)}. On the other hand, the ^{1}H NMR and the optical rotation of compound **28** {observed $[\alpha]^{20}_{D}$ = +38.4 (*c* 0.52, MeOH)} were in good accordance with the reported data. Notably, the anomalies found in the coupling constant of methyl groups at C-10 and C-12 of compound **27** were not observed in this case ($^3J_{17H\text{-}12H}$ = $^3J_{18H\text{-}10H}$ = 6.5 Hz; reported $^3J_{17H\text{-}12H}$ = $^3J_{18H\text{-}10H}$ = 6.4 Hz). The ^{13}C NMR of compound **28** was also in close agreement (see Figure S1 in the Supporting Information). Our study revealed that the spectroscopic data of compound **28** were closest to the reported data for penicitide A compared to all the other isomers synthesized.

Scheme 6. Completion of Total Synthesis of Compounds 27 and 28



In summary, we have achieved the first total synthesis of penicitide A in 15 linear steps from the known intermediate **7** with an overall yield of 4.3%. Out of five stereocenters, two have been installed from D-aspartic acid whereas the rest have been generated chemically. A total of six stereoisomers [**1(a–d)**, **27**, **28**] of proposed structure of penicitide A have been synthesized. Comparison of their spectroscopic data clearly confirmed compound **28** as the structure of the isolated penicitide A where the stereochemistry of C-10 and C-12 centers was assigned and relative configurations of the C-3 and C-5 centers were disclosed as 3α and 5β opposite to the proposed relative configurations. The absolute stereochemistry of the C-3 and C-5 centers were established further as 3(*R*) and 5(*R*).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.9b04585.

Experimental procedures; spectroscopic data; copies of NMR (^{1}H and ^{13}C); HRMS of representative compounds; 2D-NMR data of compounds **4**, **1a**, **1d**, **27**, and **28**; NMR comparison (Table S1, Table S2, and Figure S1) of compounds **1(a-d)**, **27**, and **28** (PDF)

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Notes

The authors declare no competing financial interest.

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