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Total synthesis of epicoccamides A and D via olefin cross-metathesis

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ABSTRACT

Epicoccamides A and D were synthesized through a route that utilizes fragment coupling via olefin cross-metathesis as a key step. The right-hand segment of the epicoccamides was synthesized by a tandem O-acylation–migration reaction, and the left-hand segments were stereoselectively synthesized through a modified version of Crich's β-selective mannosylation. The previously assigned absolute configuration of the epicoccamide D was confirmed, and that of epicoccamide A was assigned as (5*S*,2′*S*) based on the NMR and CD spectra. This Letter provides the first example of the total synthesis of epicoccamide A.

Naturally occurring tetramic acids constitute an important class of bioactive natural products and have attracted increasing interest among natural product chemists and synthetic organic chemists.[1] In most cases, tetramic acids are found in their 3-acylated forms, in which various substituents or stereogenic centers are present in the acyl group.[2] Epicoccamides are naturally occurring 3-acyltetramic acids bearing β-mannosylated fatty acid chains. Epicoccamide A (**1**), originally termed simply epicoccamide, was isolated from the jellyfish-derived fungus *Epicoccum purpurascens* (also known as *Epicoccum nigrum*) by Wright and co-workers in 2003,[3] whereas epicoccamides B–D (**2**–**4**) were isolated by Hertweck et al. in 2007 from an *Epicoccum* sp. growing within the fruiting body of the tree fungus *Pholiota squarrosa* (Fig. 1).[4] Recently, epicoccamide A was also isolated from an endophytic fungus *Epicoccum* sp. CAFTBO associated with *Theobroma cacao*.[5] The structurally related, bioactive, glycosylated tetramic acids ancorinosides (**5**)[6] and virgineones (**6**)[7] have been isolated from the marine sponges *Ancorina* sp. and *Lachnum virgineum*, respectively. Interestingly, epicoccamides B–D showed weak to moderate antiproliferative effects on mouse fibroblast (L-929) and human leukemia cell lines (K-562) and cytotoxicity against HeLa cells. No significant biological activity has yet been reported for epicoccamide A. Based on our interest in the structure–activity relationships of epicoccamides, we attempted to synthesize these compounds.

In the initial stages of our project, the absolute configuration of the epicoccamides had yet to be determined. Hertweck et al. suggested that epicoccamide D comprised a 2:3 mixture of isomers with mixed stereochemistry at C-5 based on the HPLC analysis of its degradation products, although the possibility of isomerization during derivatization could not be fully excluded.[4] Recently, Loscher and Schobert reported the first total synthesis of epicoccamide D.[8] These researchers concluded that the absolute configuration of the natural epicoccamide D is 5*S*,2′*S* based on comparisons of its ^{13}C NMR spectra and specific rotation values. These findings led us to investigate the stereochemical assignments of epicoccamides A and D.

From a structural point of view, the highly polar tetramic acid and sugar portions of the compounds are located in the terminal sites of the molecules and are linked together by simple long chains. Because it is well known that 3-acyltetramic acids are biosynthesized through a Dieckmann-type condensation of the corresponding β-ketoacid with the amino acid moiety,[9] epicoccamides and their related compounds should be biologically constructed from the following three major elements of primary metabolites: amino acids, fatty acids, and sugars. It is therefore reasonable to employ these three elements as the starting materials in the total synthesis of such natural products. We recently reported both the total synthesis of virgineone aglycone and the stereochemical assignment of virgineone.[10] Our synthetic methodology featured fragment coupling via olefin cross-metathesis. This methodology enabled us to synthesize various 3-acyltetramic acid derivatives simply by changing the starting amino acids or fatty acids. In the present study, we focused on the total synthesis of epicoccamides through the application of our established methodology. This Letter describes the results of our synthetic studies of a model compound and epicoccamides A and D and the NMR analysis results, which confirmed Schobert's stereochemical assignment of

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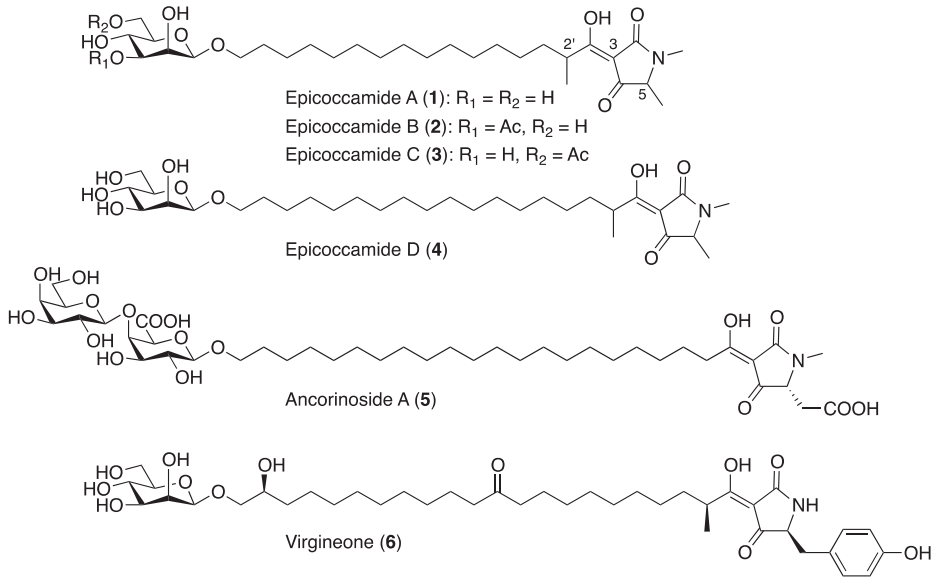
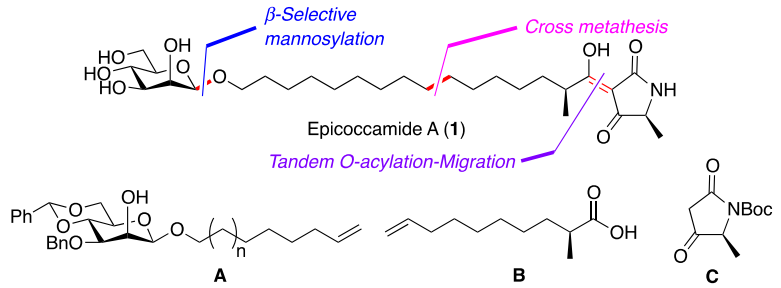


Figure 1. Structures of epicoccamides and related naturally occurring 3-acyltetramic acids.

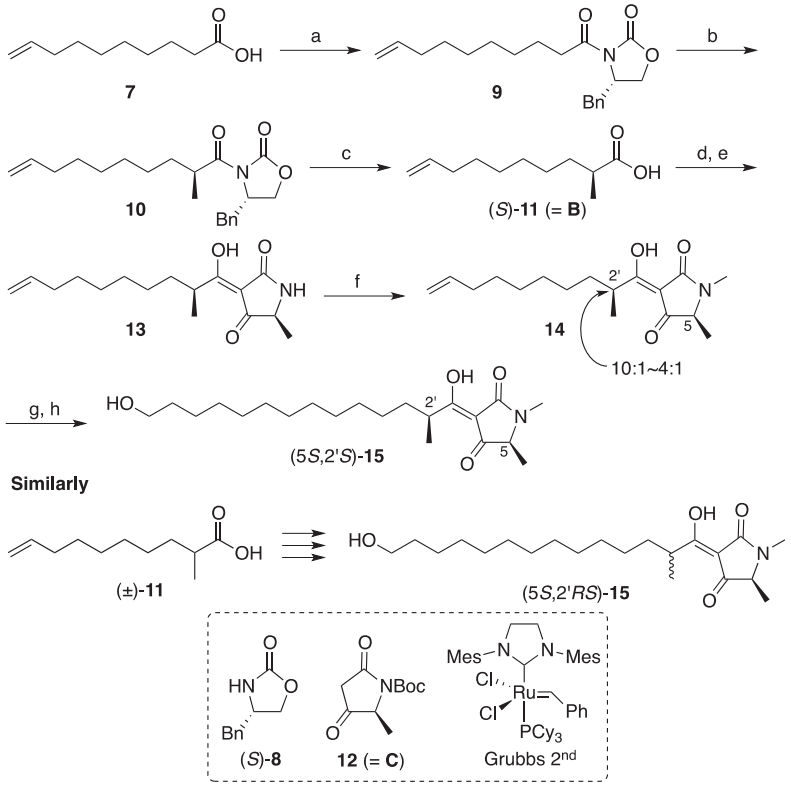
epicoccamide D and allowed the assignment of the absolute configuration of the natural epicoccamide A.

Scheme 1 shows our retrosynthetic analysis of the targeted epicoccamides. In Schobert's synthesis of epicoccamide D, the researchers employed a stereo-inversion at C-2 of the β-glucoside, asymmetric hydrogenation, and late-stage Lacy-Dieckmann condensation to construct the β-mannoside, the C-2′ stereogenic center, and the tetramic acid portions of the compound, respectively.[8] In contrast, we employed β-selective mannosylation for the synthesis of segment **A**, Evans asymmetric alkylation for the synthesis of segment **B**, and tandem O-acylation–migration for the construction of the 3-acyltetramic acid portion. Based on this convergent approach, various epicoccamide stereoisomers and derivatives could be synthesized by employing the appropriate segments.

Scheme 2 shows our synthesis of model compound **15**, which does not include the mannose moiety. Because Wright et al. reported the NMR data of the aglycone of epicoccamide A, which was prepared by the hydrolysis of the natural product, we assumed that a comparison of the NMR spectra of **15** with the reported spectra would enable us to assign the absolute configuration of the natural product. First, the optically active carboxylic acid segment **B** was prepared from commercially available **7** via the Evans asymmetric alkylation of **9**.[11] The diastereomeric purity of **10** was estimated to be better than 98:2 by ^{1}H NMR analysis. Removal of the chiral auxiliary gave (*S*)-**11** (=**B**). Tandem O-acylation–migration between **11** and the known compound **12**,[12] followed by the deprotection of the Boc group, afforded **13** without epimerization of the two stereogenic centers. In the next N-methylation step, decomposition of the desired product was observed when more than 2.5 equiv of a base, such as NaHMDS, were employed. The decomposition was suppressed to some extent when less than 2.2 equiv of NaHMDS were employed, although this reaction was unreproducible. The optimized conditions were the same as those reported by Yoda and co-workers for their total synthesis of penicillenols.[13] Although Yoda did not observe any detectable epimerization of their N-methylation substrate, our substrate (**13**) was prone to epimerization at C-2′ under basic conditions, leading to the isolation of **14** as an inseparable diastereomeric mixture. The bulky side chain at C-5 may play a role in hindering deprotonation at C-2′ in Yoda's synthesis. Unfortunately, we could not determine the precise diastereomeric ratio of the product by ^{1}H NMR analysis, although ^{13}C NMR allowed a rough estimation of a 10:1∼4:1 ratio. Next, the partially epimerized **14** was coupled with the readily available 5-hexen-1-ol by olefin cross-metathesis using the Grubbs second-generation catalyst,[14] and the resulting double bond was hydrogenated over catalytic Pd/C to give the model compound (5*S*,2′*S*)-**15**. Similarly, (5*S*,2′*RS*)-**15** was prepared from (±)-**11**. Comparing the ^{1}H and ^{13}C NMR spectra of the synthetic (2′*S*)- and (2′*RS*)-**15**, we concluded that the natural epicoccamide D is not a diastereoisomeric mixture and that Hertweck's suggestion is incorrect. The ^{1}H NMR spectra of (2′*RS*)-**15** recorded in CD_3OD or $CDCl_3$ showed two pairs of doublets corresponding to the methyl group at C-2′, whereas only the doublet of the major isomer was observed in the ^{1}H NMR spectrum of (2′*S*)-**15**. However, it was difficult to determine the relative configurations of the natural epicoccamides by ^{1}H NMR due to the relatively small differences between the



Scheme 1. Retrosynthetic analysis of epicoccamides.



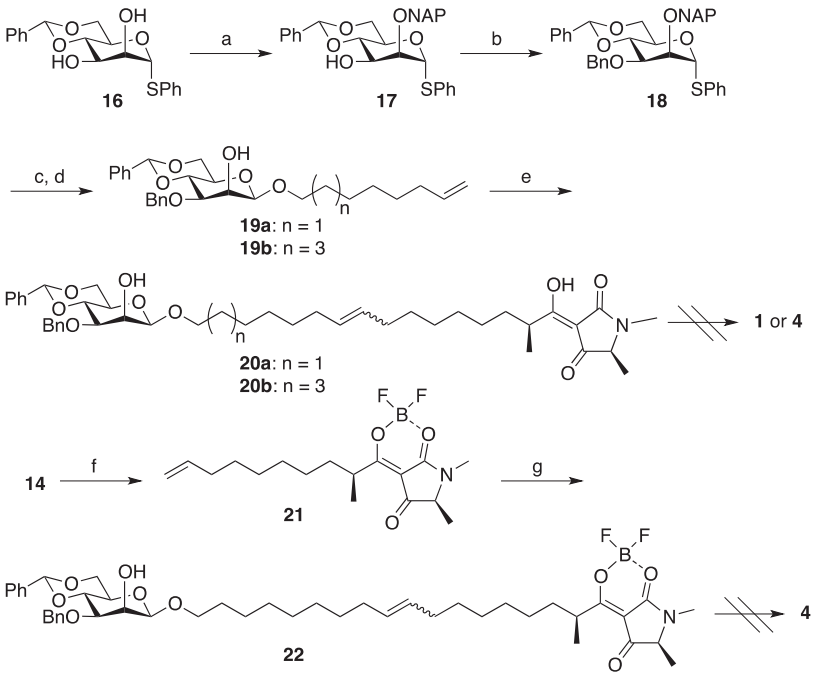
Scheme 2. Synthesis of model compounds. Reagents, conditions, and yields: (a) (i) PivCl, LiCl, Et_3N, THF, −20 °C; (ii) *n*BuLi, (*S*)-**8**, THF, −78 °C then mixed anhydride, 94%; (b) NaHMDS, MeI, THF, −78 °C, 96%, ds >98:2; (c) LiOH, H_2O_2, THF, H_2O, 74%; (d) DCC, **12**, DMAP, CH_2Cl_2; (e) TFA, CH_2Cl_2, 77% in two steps; (f) NaHMDS, MeI, THF, −78 °C to −40 °C, 83%; (g) Grubbs second-generation catalyst, 5-hexene-1-ol, CH_2Cl_2, reflux; (h) H_2, Pd/C, MeOH, 28% in two steps.

diastereomers. In contrast, the ^{13}C NMR spectra clearly showed significant differences between the two diastereomers when the spectra were recorded in $CDCl_3$. Fortunately, Hertweck reported the ^{13}C NMR spectra of epicoccamides B–D recorded in $CDCl_3$.[4] Because the spectrum of the synthetic (5*S*,2′*S*)-**15** was in good agreement with the reported data, we assigned the absolute configurations of epicoccamides B–D as 5*S*,2′*S*, in agreement with Schobert's work. Because Wright and co-workers only reported NMR data recorded in CD_3OD, it was impossible to assign the absolute configuration of epicoccamide A at this stage. We assumed, however, that it should be the same as the configurations of epicoccamides B–D because these compounds are derived from a common biogenetic origin.

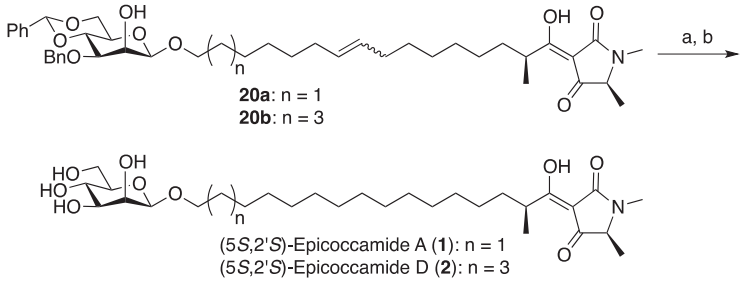
We then synthesized epicoccamides A and D with the 5*S*,2′*S* configuration, as shown in Schemes 3 and 4. First, the glycosyl donor **18** was prepared from the known compound **16**[15] by protection of the 2- and 3-hydroxy groups as NAP (2-naphtylmethyl) and benzyl ethers. The β-mannosides were prepared according to a slightly modified Crich's condition[16] followed by deprotection of the NAP group with DDQ to give **19a** and **19b** as mixtures of inseparable anomeric isomers (~10:1). By switching the chain lengths of the glycosyl acceptors, the segments for the synthesis of epicoccamides A and D were easily prepared. The couplings of **19a** or **19b** with the 3-acyltetramic acid segment **14** were successfully carried out by olefin cross-metathesis using Grubbs second-generation catalyst, yielding **20a** and **20b**. Unfortunately, the final remaining tasks, which comprised the deprotection of the benzylidene acetal and benzyl groups, were troublesome. Under catalytic hydrogenation conditions, with or without TFA, the reactions gave complex mixtures. Prior to deprotection of the benzylidene acetal and benzyl groups of **20**, decomposition of the starting material and product was indicated by a TLC analysis of the reaction mixture. Although decomposition was noted in the hydrogenation step in the synthesis of **15**, it could be avoided by stopping the reaction after a relatively short time. Because it was unlikely that the sugar moiety of **20** decomposed under such conditions, the decomposition likely occurred at the 3-acyltetramic acid moiety. To overcome these issues, we attempted the protection of the 3-acyltetramic acid moiety as a cyclic borate according to the methods reported by Loscher and Schobert.[8] As the model compound, **14** was treated with BF_3 etherate to afford **21**, and the cross-metathesis of **14** and **21** gave the coupled product **22** in moderate yield. Unfortunately, the hydrogenation and concomitant removal of the protecting groups were unsuccessful and gave only a complex mixture under the reported conditions (H_2, Pd/C, MeOH).

Jones and co-workers reported that a 3-acyltetramic acid derivative is relatively unstable under hydrogenation conditions (H_2, Pd/C, EtOH).[17] However, in our synthesis of virgineone aglycone, decomposition of the product was not observed (H_2, $Pd(OH)_2$/C, TFA, *t*-BuOH).[10] The instability of the 3-acyltetramic acid derivatives under hydrogenation conditions may depend on the steric hindrance of the substituents at C-5. In an effort to avoid decomposition, we studied the effects of a number of hydrogenation conditions on the tetramic acid segments **14** and **21** (Table 1). Although we could not find the ideal, decomposition-free conditions for the hydrogenation of **14**, we found that Pearlman's catalyst in ethyl acetate was effective for the hydrogenation of **14** with only slight decomposition of the product **23** (entry 4). The hydrogenation of **21** resulted in decomposition under hydrogenation conditions (entry 5). Although the cyclic borate ester moiety of **21** was readily hydrolyzed in MeOH, the terminal olefin was isomerized to give the internal olefin mixtures **24** (entry 6).

Employing the established procedure, we sought to finalize the total synthesis of epicoccamides (Scheme 4). The benzylidene acetal groups of the cross-coupling products **20a** and **20b** were hydrolyzed with TFA, and this step was followed by the

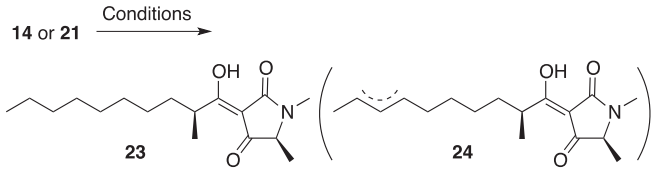


Scheme 3. β-Selective mannosylation and olefin cross-metathesis. Reagents, conditions, and yields: (a) NaOH aq., NAPBr, Bu_4NHSO_4, CH_2Cl_2, reflux, 57%; (b) NaH, BnBr, DMF, 0 °C to rt, 95%; (c) 1-benzenesulfinyl piperidine (BSP), 2,4,6-tri-*t*-butylpyrimidine (TTBP), Tf_2O, MS3A, 1-hexene, 7-octen-1-ol or 9-decen-1-ol, CH_2Cl_2, −78 °C to rt; (d) DDQ, CH_2Cl_2, 0 °C to rt; 86% in two steps for **19a** or 51% in two steps for **19b**; (e) **14**, Grubbs second-generation catalyst, toluene, reflux; (f) BF_3OEt_2, CH_2Cl_2, reflux, 75%; (g) **19b**, Grubbs second-generation catalyst, toluene, reflux, 47%.



Scheme 4. Completion of the total synthesis of epicoccamides A and D. Reagents, conditions, and yields: (a) TFA, CH_2Cl_2, H_2O; (b) $Pd(OH)_2$/C, H_2, EtOAc, 20% in three steps for **1** or 16% in three steps for **4**.

Table 1
Investigation of the hydrogenation reaction with model compounds



Entry	Substrate	Conditions	Result (yield%)
1	**14**	H_2, Pd/C, MeOH	Decomposition
2	**14**	H_2, Pd/C, EtOAc	Decomposition
3	**14**	Ar, Pd/C, EtOAc	No reaction
4	**14**	H_2, $Pd(OH)_2$/C, EtOAc	**23** (quant.)
5	**21**	H_2, Pd/C, MeOH	Decomposition
6	**21**	Ar, MeOH	**24** (quant.)

concomitant hydrogenolysis of a benzyl group and hydrogenation of a double bond with Pearlman's catalyst in ethyl acetate to give epicoccamides A and D in 15–20% isolated yields through the three transformations from **19a** and **19b**. Again, a prolonged reaction time resulted in decomposition of the products under the reaction conditions.

We then compared the ^1H and ^{13}C NMR spectra of the synthetic compounds with the reported spectra of epicoccamides A and D. As described above, the absolute configuration of the natural epicoccamide D was determined by Loscher and Schobert as 5*S*,2′*S* based on the specific rotation values and partial ^{13}C NMR data recorded in $CDCl_3$.[8] We confirmed this assignment with the aid of the ^{13}C NMR spectra of **15** and the synthetic epicoccamide D and found that the ^1H and ^{13}C NMR spectra of the synthetic **4** recorded in $CDCl_3$ were identical with those of the natural product. Loscher and Schobert reported the NMR spectra of their synthetic compound recorded in CD_3OD. However, it was very difficult to verify the consistency of our NMR spectra with their spectra because the use of CD_3OD as the solvent in our study led to slight variations in the data across a number of trials. In fact, clear differences were immediately apparent between our ^1H NMR spectrum and the reported spectrum: we observed a Me–N singlet signal at 2.95 ppm, whereas the reported spectrum showed small doublet signals at approximately 2.8 ppm. This inconsistency was noted by Loscher and Schobert.[18] Wright and co-workers reported the

NMR spectroscopic data of natural epicoccamide A recorded in CD_3OD. The ^1H spectrum of our synthetic epicoccamide A showed relatively a good agreement with the reported data and with a spectrum kindly provided by Professor König.[3] However, slight differences between the synthetic and natural epicoccamide A were observed in their ^{13}C NMR spectra. Broad signals may be expected for the tetramic acid portion of the compound due to the complex tautomerism observed in the NMR spectrum of 3-acyltetramic acid derivatives.[19] The differences observed in this study may be caused by a complex range of variables, such as pH, concentration, and temperature of the samples. We also measured the ^1H and ^{13}C NMR spectra of epicoccamide A in DMSO-d_6 and found that these spectra were relatively suitable for stereochemical assignment because they did not exhibit substantial pH or concentration dependency. Thus, we propose DMSO-d_6 as a good choice for the measurement of the NMR spectra of 3-acyltetramic acid derivatives. Our synthetic epicoccamide D exhibited relatively similar $[\alpha]_D$ values [$[\alpha]_D^{30} = -54.2$ (c 0.20, MeOH)] as the reported natural [$[\alpha]_D^{25} = -40.4$ (c 0.20, MeOH)][4] and synthetic epicoccamide D [$[\alpha]_D^{24} = -39$ (c 0.20, MeOH)].[8] Although our synthetic epicoccamide A exhibited considerably smaller $[\alpha]_D$ values [$[\alpha]_D^{25} = -40.6$ (c 0.10, EtOH)] than the natural epicoccamide A [$[\alpha]_D^{20} = -10.3$ (c 0.10, EtOH)], the CD spectrum of the synthetic epicoccamide A and those of the natural epicoccamide A matched. Thus, we concluded that the absolute configuration of the natural epicoccamide A is 5*S*,2′*S*. The clear difference of the $[\alpha]_D$ values between the synthetic and natural epicoccamide A might be due to the influence of pH of the samples.

In summary, we achieved the total synthesis of epicoccamides A and D using olefin cross-metathesis as the key segment-coupling reaction. This Letter constitutes the first example of the total synthesis of epicoccamide A. Although the stereochemical purities of the synthesized compounds were not optimal, our established methodology could be applied to the synthesis of related 3-acyltetramic acid natural products, such as virgineones and ancorinosides. Although the stereoisomers generated by N-methylation and β-selective mannosylation reactions may be eliminated through HPLC or related chromatographic techniques, the development of more efficient and highly stereoselective reactions for these transformations, as well as the synthesis of other epicoccamide derivatives, are currently underway in our laboratory. The assignment of the absolute configuration of epicoccamide D by Loscher and Schobert was confirmed by the analyses of **15** and epicoccamide D. Because the CD spectrum of the synthetic epicoccamide A and those of the natural epicoccamide A matched, we concluded that the absolute configuration of the natural epicoccamide A is 5*S*,2′*S*, identical to that of epicoccamide D. Structure–activity relationship studies of the epicoccamide derivatives are currently underway.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures and ^1H and ^{13}C NMR and CD spectra of the synthetic compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.06.040.

References and notes

1. (a) Ghisalberti, E. L. In *Studies in Natural Products Chemistry*; Atta-ur Rahman, Ed.; ; Elsevier: Amsterdam, 2003; Vol. 28, pp 109–163; (b) Schobert, R.; Schlenk, A. *Bioorg. Med. Chem.* **2008**, *16*, 4203–4221.
2. Royles, B. J. L. *Chem. Rev.* **1995**, *95*, 1981–2001.
3. Wright, A. D.; Osterhage, C.; König, G. M. *Org. Biomol. Chem.* **2003**, *1*, 507–510.
4. Wangun, H. V. K.; Dahse, H.-M.; Hertweck, C. *J. Nat. Prod.* **2007**, *70*, 1800–1803.
5. Talontsi, F. M.; Dittrich, B.; Schüffler, A.; Sun, H.; Laatsch, H. *Eur. J. Org. Chem.* **2013**, 3174–3180.
6. (a) Ohta, S.; Ohta, E.; Ikegami, S. *J. Org. Chem.* **1997**, *62*, 6452–6453; (b) Fujita, M.; Nakao, Y.; Matsunaga, S.; Seiki, M.; Itoh, Y.; van Soest, R. W. M.; Fusetani, N. *Tetrahedron* **2001**, *57*, 1229–1234; (c) Ohta, E.; Ohta, S.; Ikegami, S. *Tetrahedron* **2001**, *57*, 4699–4703.
7. (a) Ondeyka, J.; Harris, G.; Zink, D.; Basilio, A.; Vicente, F.; Bills, G.; Platas, G.; Collado, J.; González, A.; de la Cruz, M.; Martin, J.; Kahn, J. N.; Galuska, S.; Giacobbe, R.; Abruzzo, G.; Hickey, E.; Liberator, P.; Jiang, B.; Xu, D.; Roemer, T.; Singh, S. B. *J. Nat. Prod.* **2009**, *72*, 136–141; (b) Figueroa, M.; Raja, H.; Falkinham, J. O., III; Adcock, A. F.; Kroll, D. J.; Wani, M. C.; Pearce, C. J.; Oberlies, N. H. *J. Nat. Prod.* **2013**, *76*, 1007–1015.
8. Loscher, S.; Schobert, R. *Chem. Eur. J.* **2013**, *19*, 10619–10624.
9. (a) Du, L.; Lou, L. *Nat. Prod. Rep.* **2010**, *27*, 255–278; (b) Fisch, K. M. *RSC Adv.* **2013**, *3*, 18228–18247.
10. Yajima, A.; Ida, C.; Taniguchi, K.; Murata, S.; Katsuta, R.; Nukada, T. *Tetrahedron Lett.* **2013**, *54*, 2497–2501.
11. Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737–1739.
12. Tanaka, A.; Usuki, T. *Tetrahedron Lett.* **2011**, *52*, 5036–5038.
13. (a) Yoda, H.; Sengoku, T.; Wierzejska, J.; Takahashi, M. *Synlett* **2010**, 2944–2946; (b) Sengoku, T.; Nagae, Y.; Ujihara, Y.; Takahashi, M.; Yoda, H. *J. Org. Chem.* **2012**, *77*, 4391–4401.
14. Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18–29.
15. Boltje, T. J.; Li, C.; Boons, G.-J. *Org. Lett.* **2010**, *12*, 4636–4639.
16. Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015–9020.
17. Jones, R. C. F.; Bhalay, G.; Carter, P. A.; Duller, K. A. M.; Dunn, S. H. *J. Chem. Soc., Perkin Trans. 1* **1999**, 765–776.
18. Private communication.
19. (a) Gromak, V. V.; Avakyan, V. G.; Lakhvich, O. F. *J. Appl. Spectrosc.* **2000**, *67*, 205–215; (b) Jeong, Y.-C.; Moloney, M. G. *J. Org. Chem.* **2011**, *76*, 1342–1354.