Organic LETTERS

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Total Syntheses of Nannocystins A and A0, Two Elongation Factor 1 Inhibitors

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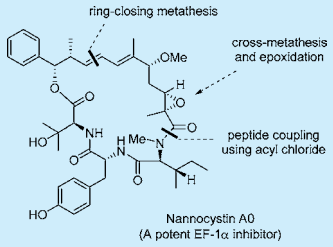
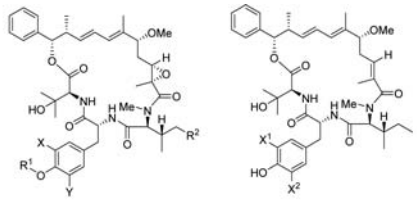
Supporting Information

ABSTRACT: Asymmetric total syntheses of nannocystins A and A0 were achieved in a convergent route starting from simple materials. Nannocystin family natural products bear potent anticancer activity as elongation factor 1 inhibitors. In this synthesis, the challenging tertiary amide bond was constructed by peptide coupling between an acyl chloride and a secondary amine. A late-stage ring-closing metathesis reaction successfully rendered the macrocycle. This efficient synthetic strategy should be applicable to other nannocystins and analogues and therefore should benefit future structure−activity relationship studies.



Natural products serve as a main source of therapeutics because of their unique structures and structural diversity.[1] About half of the current small-molecule drugs on the market are natural products or their synthetic analogues. When new natural products with unprecedented structural features are isolated, scientists often find new mechanisms of action for these natural products in biological systems, which could lead to new drug development, as exemplified by research on calicheamicin and rapamycin.[2] In June 2015, in searching for new bioactive natural products, Hoffmann et al.[3] reported a family of cyclodepsipeptides called nannocystins (Figure 1) with potent antiproliferative activities against a number of cancer cell lines at nanomolar concentrations. For example, the IC_{50}'s of nannocystin A (**1**) against HCT116, PC3, and HL60 are 1.2, 1.0, and 12 nM, respectively. Almost simultaneously, Krastel et al.[4] also discovered the nannocystins and conducted a more comprehensive investigation. They found that **1** showed antiproliferative properties against 472 cancer cell lines in the nanomolar concentration range. Moreover, a series of experimental and computational studies by Krastel et al. strongly suggested that the target protein of nannocystins is elongation factor 1-α (EF-1α). Elongation factors secure accuracy in the translation process and are important in protein synthesis.[5] Cancer cells tend to overexpress elongation factors to expedite protein production.[6] Therefore, compounds that target elongation factors, such as nannocystins, may serve as lead candidates for anticancer therapy. Attracted by their potent biological activity and urged by subsequent structure−activity relationship studies, we embarked on the total synthesis of nannocystins. Here we report the first asymmetric total syntheses of nannocystins A (**1**) and A0 (**3**).[7]



nannocystins	X	Y	R^1	R^2
nannocystin A, **1**	Cl	Cl	H	Me
nannocystin A1, **2**	H	Cl	H	Me
nannocystin A0, **3**	H	H	H	Me
nannocystin B, **4**	Cl	Cl	H	H
nannocystin B1, **5**	H	Cl	H	H
nannocystin A2, **6**	Cl	Br	H	Me
nannocystin A3, **7**	Br	H	H	Me
nannocystin Ax, **8**		X_1 = Cl,	X_2 = Cl	
nannocystin Ay, **9**		X_1 = Br,	X_2 = H	
nannocystin D1, **10**	Cl	Cl	Me	Me
nannocystin D2, **11**	Cl	Cl	$-(CH_2)_2NH_2$	Me

Figure 1. Natural nannocystins (**1**−**9**) and their synthetic analogues (**10** and **11**).

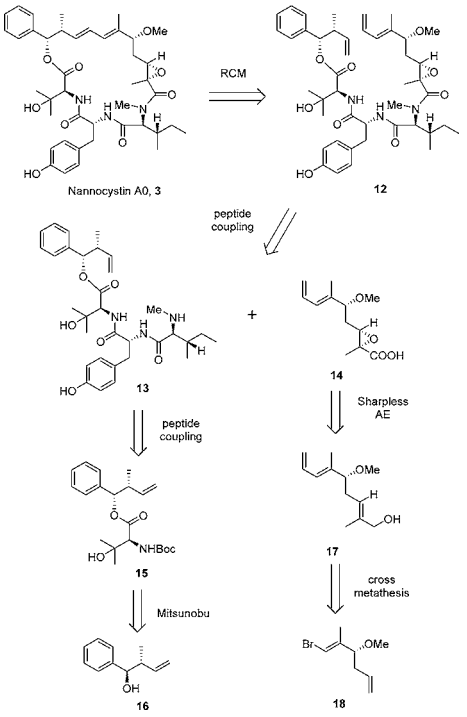
We sought a convergent synthesis of nannocystins. Our retrosynthetic analysis is shown in Scheme 1. We planned to take advantage of the ring-closing metathesis (RCM) reaction of **12** to assemble the macrocycle. The RCM strategy for macrocycle synthesis is a robust method, as documented in the literature.[8] Disconnection of the tertiary amide bond of **12** would lead to peptide fragment **13** and polyketide motif **14** with approximately equal complexity. Compound **13** can be derived from ester **15** through a peptide coupling reaction, and ester **15** should be easily prepared from homoallylic alcohol **16**. On the other hand, carboxylic acid **14** could be made via an asymmetric epoxidation of alcohol **17** followed by oxidation. Allylic alcohol **17** could be made via a regioselective cross-metathesis reaction of the simple compound **18**, the asymmetric allylation product of (*E*)-3-bromomethacrolein.

The synthesis of acid **14** started from the known compound (*E*)-3-bromomethacrolein (**19**) (Scheme 2).[9] Enantioselective allylation of **19** using Antilla's method provided alcohol **20** in

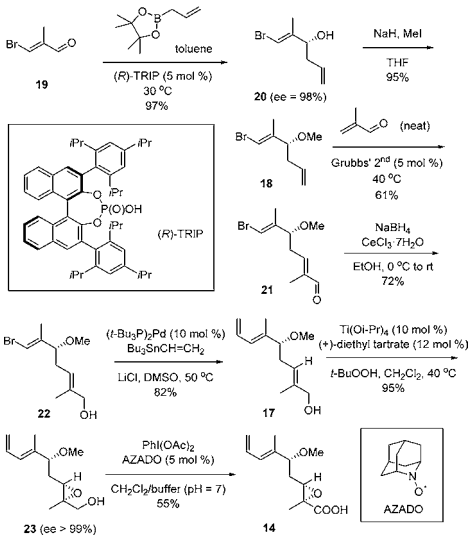
Received: August 6, 2016
Published: September 6, 2016

DOI: 10.1021/acs.orglett.6b02352
Org. Lett. 2016, 18, 4702−4705

Scheme 1. Retrosynthetic Analysis



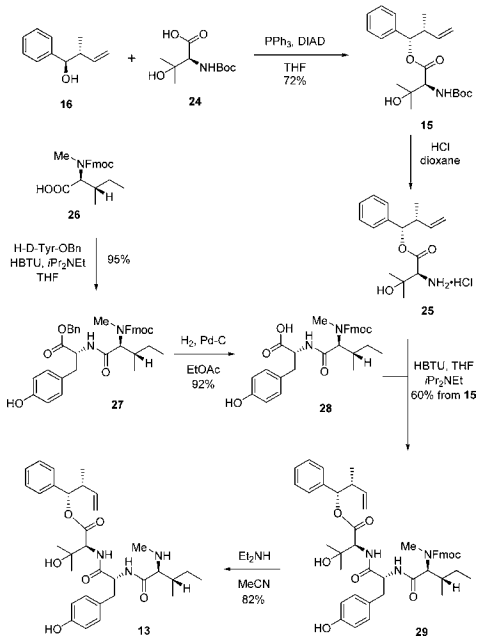
Scheme 2. Synthesis of Acid 14



97% yield with 98% ee.[10] The optical purity of **20** was determined from its UV-active derivative **17** (vide infra). The free hydroxyl group in **20** was methylated by treatment with sodium hydride and iodomethane in 95% yield. Methyl ether **18** underwent cross-metathesis with the monosubstituted olefin regio- and stereoselectively using Grubbs' second-generation catalyst and neat methacrolein as the solvent,[11] furnishing enal **21** in 61% yield. A prolonged reaction time (36 h) and excess methacrolein was necessary to obtain a good yield of **21** in this step. Luche reduction of enal **21** in ethanol smoothly rendered allylic alcohol **22** in 72% yield.[12] The next goal was to introduce the diene functionality via cross-coupling reactions. We found the more active palladium catalyst $Pd(Pt\text{-}Bu_3)_2$ to be superior to $Pd(PPh_3)_4$ in the Stille coupling reaction with tri-*n*-butylvinylstannane, furnishing diene **17** in 82% yield.[13] Sharpless asymmetric epoxidation of allylic alcohol **17** gave the desired epoxide **23** in 95% yield with amplified optical purity (>99% ee).[14] Finally, direct oxidation of alcohol **23** to acid **14** using iodosobenzene diacetate and a catalytic amount of 2-azaadamantane-*N*-oxyl (AZADO) successfully furnished acid **14** in 55% yield.[15]

The assembly of peptide motif **13** started with the synthesis of **15** via Mitsunobu reaction between the known compounds **16** and **24** (Scheme 3).[16,17] Deprotection of *tert*-butyl

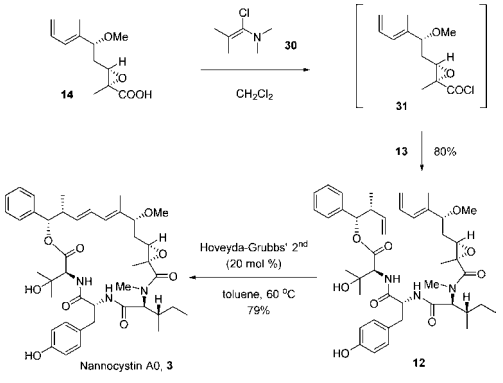
Scheme 3. Synthesis of Peptide Motif 13



carbamate **15** furnished ammonium salt **25**. *N*-Fmoc-protected *N*-methyl-L-isoleucine (**26**) was successfully coupled with D-tyrosine benzyl ester, affording dipeptide **27** in 95% yield. After hydrogenolysis of the benzyl group, acid **28** was connected with **25** using HBTU, giving tripeptide **29** in 60% yield. The free secondary amine **13** was produced by treatment of **29** with diethylamine in dry acetonitrile.

With fragments **13** and **14** in hand, we turned our attention to the coupling of the two termini of **13** and **14** to finish the total synthesis (Scheme 4). The carbonyl group in acid **14** is quite hindered because of the tetrasubstituted α-carbon. At the same time, isoleucine is a hindered amino acid in peptide

Scheme 4. Total Synthesis of Nannocystin A0

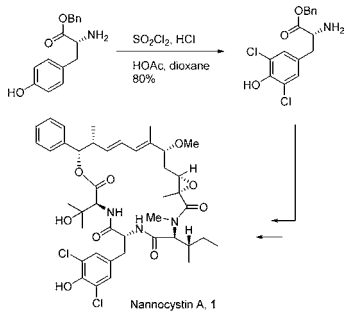


DOI: 10.1021/acs.orglett.6b02352
Org. Lett. 2016, 18, 4702−4705

coupling, especially when the nitrogen atom is alkylated.[18] Common peptide coupling reagents such as HATU, HBTU, EDCI, etc., failed to generate the coupled product **12**. Therefore, we focused on stronger acyl donors generated from **14**. Happily, we were able to make the corresponding acyl chloride **31** using Ghosez reagent **30**.[19] Crude **31** in a dichloromethane solution was added dropwise to **13** in dichloromethane at −20 °C, and the desired product **12** was obtained in 80% yield.[20] It should be noted that compounds **29**, **13**, and **14** had to be purified by preparative TLC to remove small amounts of impurities. In addition, compounds **14** and **31** are not very stable, probably because of the acid-sensitive diene moiety, and had to be used immediately after preparation. Finally, the target natural product nannocystin A0 (**3**) was synthesized successfully via ring-closing metathesis of **12** using the second-generation Hoveyda−Grubbs catalyst in 79% yield as a 4.6:1 ratio of isomers (**3** is the major isomer).[21] The functional group compatibility of this metathesis macrocyclization step is quite remarkable. Pure **3** was obtained after separation by reversed-phase HPLC. The NMR spectra of synthetic **3** were identical to those of natural nannocystin A0 reported by Krastel et al.[4]

The synthetic sequence for nannocystin A0 could be generalized to the synthesis of other nannocystins. Starting from 3,5-dichloro-D-tyrosine benzyl ester,[22] we prepared nannocystin A (**1**) in the same manner (Scheme 5; also see the Supporting Information). The NMR spectra of synthetic **1** also matched those of natural nannocystin A reported by Krastel et al.[4]

Scheme 5. Highlights of the Total Synthesis of Nannocystin A (1)



In summary, convergent total syntheses of nannocystins A and A0 were achieved in nine steps (longest linear sequence) from a simple starting material, (*E*)-3-bromomethacrolein. Noteworthy steps include the peptide coupling between a reactive acyl chloride and a hindered *N*-methyl-L-isoleucine moiety and the ring-closing metathesis macrocyclization. Our synthetic route will benefit future structure−activity relationship studies of nannocystins.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02352.

Detailed experimental procedures and characterizations of new compounds (PDF)
NMR data (ZIP)

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Notes

The authors declare the following competing financial interest(s): A patent application was filed by University at Albany, State University of New York.

■ ACKNOWLEDGMENTS

We are grateful to University at Albany, State University of New York, for financial support. We thank Prof. Alexander Shekhtman (SUNY-Albany), Dr. Xiaochuan Cai (Memorial Sloan-Kettering Cancer Center), and Dr. Rong Long (Columbia University) for NMR assistance. We also thank Dr. Philipp Krastel (Novartis Institutes for BioMedical Research) for providing the NMR spectra of natural nannocystins A and A0. Dr. Ying Wu is acknowledged for helping prepare this article. This work is dedicated to Professor Samuel J. Danishefsky (Columbia University and Memorial Sloan-Kettering Cancer Center) on the occasion of his 80th birthday.

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DOI: 10.1021/acs.orglett.6b02352
Org. Lett. 2016, 18, 4702−4705