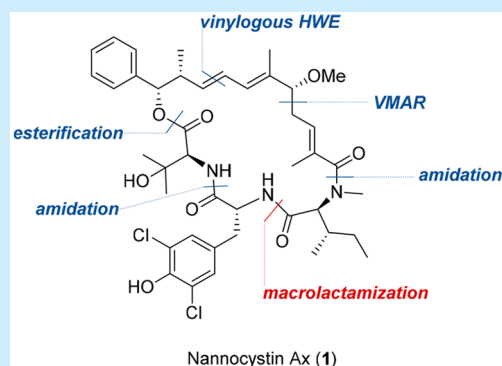


Total Synthesis of Nannocystin Ax

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Supporting Information

ABSTRACT: The total synthesis of nannocystin Ax in an overall yield of 11% with 14 steps as the longest linear sequence is reported. Nannocystin Ax is a cytotoxic 21-membered depsipeptide and was isolated from the myxobacterial genus *Nannocystis* sp. The synthesis uses a vinylogous Horner–Wadsworth–Emmons reaction (HWE) and a vinylogous Mukaiyama aldol reaction (VMAR) as the key steps for the construction of the polyketide fragment. The macrocycle was closed via a macrolactamization reaction using COMU.



Natural products are a major source of drugs used in the treatment of various diseases. They have been particularly successful as antibiotics and anticancer agents.¹ An important source of those secondary metabolites are myxobacteria, from which a variety of structurally different compounds with diverse biological activities and unique modes of action have been isolated.^{2,3} Recently the groups of Hoepfner (Novartis)⁴ and Brönstrup (Sanofi)⁵ isolated a new family of 21-membered macrocyclic depsipeptides from the myxobacterial genus *Nannocystis* sp. All members of the nannocystin family consist of a polyketide and a tripeptide hemisphere. Differences are present in the peptidic parts as well as in the presence of the α,β -epoxyamide moiety, which is present in nannocystin A (2), but not in nannocystin Ax (1) (Figure 1). Studies of the biological activity showed that all of the isolated compounds exhibit antiproliferative activity against various cancer cell lines in the nanomolar range ($IC_{50} = 2.6 \text{ nM}^4$ and 0.6 nM^5 for 2; $IC_{50} = 5.4 \text{ nM}$ for 1; HCT-116) and that the epoxide does not seem to be part of the pharmacophore. As a primary target for the nannocystins, the eukaryotic translation elongation factor 1α (EF-1 α) was proposed. Overexpression of EF-1 α is observed in various cancer cells,⁶ but its role in tumor biology still remains unclear. While several studies claim anti-apoptotic properties and a negative influence for chemotherapy,⁷ other studies propose pro-apoptotic properties.^{8,9} However, a correlation between overexpression of EF-1 α and a negative prognosis for gastric cancer has been shown by Yang.¹⁰ These findings make the nannocystins interesting candidates for the development of new drugs. Consequently, it is not surprising that several groups have contributed to the field of nannocystins. Four total syntheses of nannocystin A (2) have been achieved by the groups of Wang,¹¹ Ye,¹² Chen¹³ and He,^{16c} and one of nannocystin Ax (1) has been reported by the

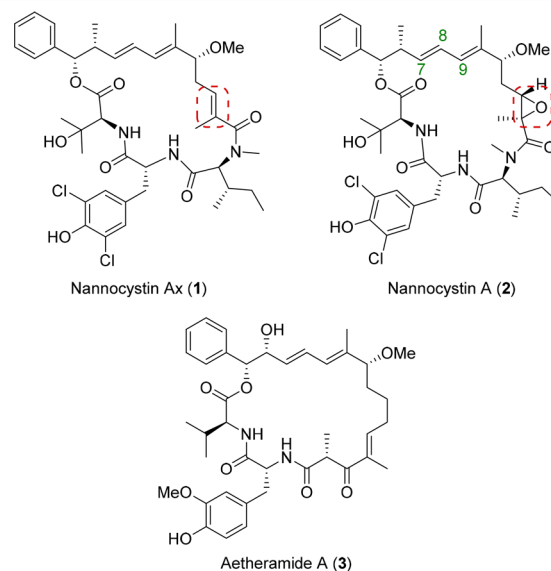


Figure 1. Structures of nannocystin A (2), Ax (1) and aetheramide A (3).

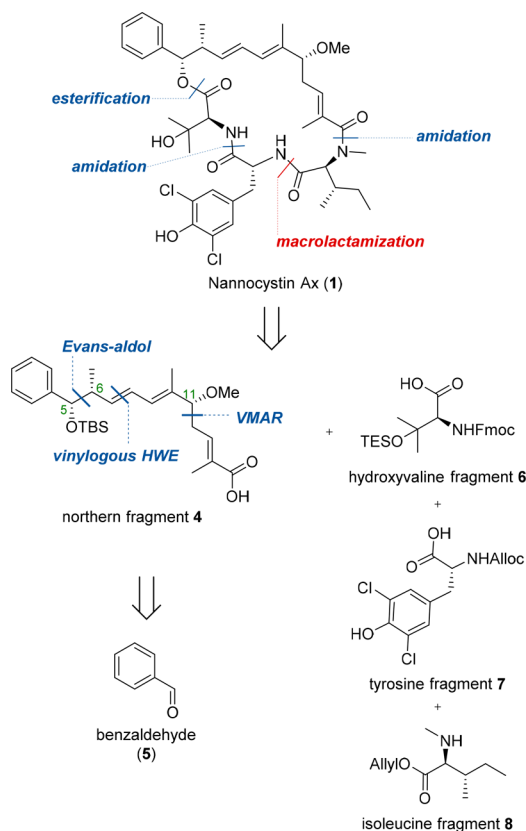
group of Liu.¹⁴ Also, the structurally related depsipeptides aetheramides A (3) (Figure 1) and B were recently synthesized in our laboratories¹⁵ and by the groups of Guo, He¹⁶ and Prasad.¹⁷ In the context of our program to establish syntheses for complex polyketidal natural products,¹⁸ we aimed at synthesizing nannocystin Ax.

Received: July 11, 2017

Published: August 15, 2017

Our retrosynthetic approach (Scheme 1) for the synthesis of nannocystin Ax (**1**) is based on the division of **1** into a

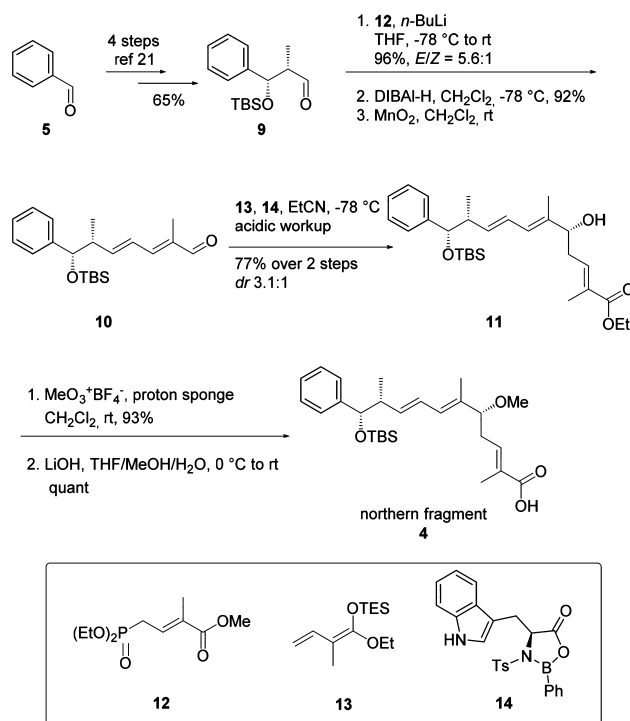
Scheme 1. Retrosynthetic Analysis



polyketide and a tripeptide segment. We envisioned closing the macrocycle via a macrolactamization between the tyrosine and isoleucine parts. This represents a new approach for closure of the macrocycle compared with the other published total syntheses of nannocystins. In those syntheses, a cross-coupling between C8 and C9^{12–14} or a metathesis between C7 and C8¹¹ was performed. The linear precursor could be generated from northern fragment **4** and the three peptide fragments **6**, **7**, and **8**¹⁹ via sequential amidation or esterification reactions. The key steps for the synthesis of **4** were a vinyllogous Horner–Wodsworth–Emmons (HWE) reaction¹⁵ and an asymmetric vinyllogous Mukaiyama aldol reaction (VMAR)²⁰ to install the C11 stereocenter.

For the synthesis of northern fragment **4** (Scheme 2), benzaldehyde (**5**) was converted into literature-known aldehyde **9**²¹ in four steps by utilizing an Evans aldol reaction to install the stereocenters at C5 and C6. Afterward, a vinyllogous HWE olefination¹⁵ with phosphonate **12**²² provided the desired acrylic ester in very good yield with a good *E/Z* ratio of 5.6:1 in favor of the desired C7/C8 *E* isomer. After a reduction–oxidation sequence, aldehyde **10** was submitted to the vinyllogous Mukaiyama aldol reaction with TES-ketene acetal **13** using a protocol developed in our group that employs a chiral oxazaborolidinone Lewis acid generated in situ from *N*-Ts-L-tryptophan and dichlorophenylborane.²⁰ Coordination between the aldehyde and the chiral Lewis acid leads to attack from the less hindered *re* face of the aldehyde, giving preferentially the *R*-configured hydroxy group at C11. Alcohol **11** was obtained in good yield but with moderate

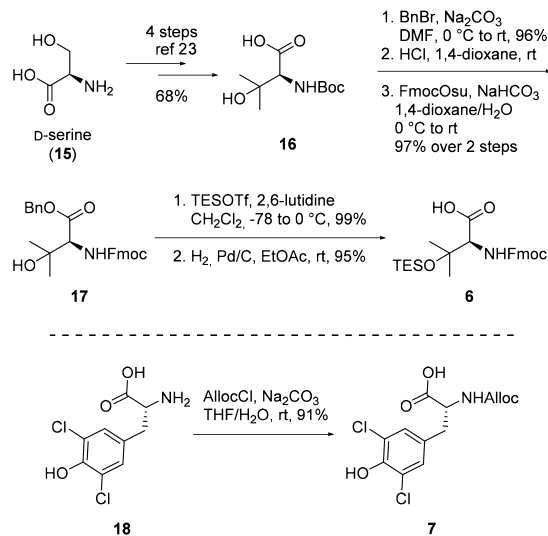
Scheme 2. Synthesis of the Northern Fragment 4



diastereoselectivity for the desired product (3.1:1). Unfortunately, the undesired diastereomer could not be fully separated at this stage, but it was removed during further steps. Finally, the hydroxy function of **11** was converted to the corresponding methyl ether and the ethyl ester was hydrolyzed, providing the northern fragment **4**. The acid was directly used in the next step, as it was not stable upon storage.

The synthesis of β -hydroxyvaline fragment **6** (Scheme 3) started from known *N*-Boc-L- β -hydroxyvaline (**16**), which is commercially available but could also be synthesized starting from D-serine (**15**) using a literature-known protocol.²³ Afterward, the carboxylic acid was protected as a benzyl ester, the amine protecting group was changed from Boc to Fmoc, and the tertiary alcohol was protected as the TES ether. The

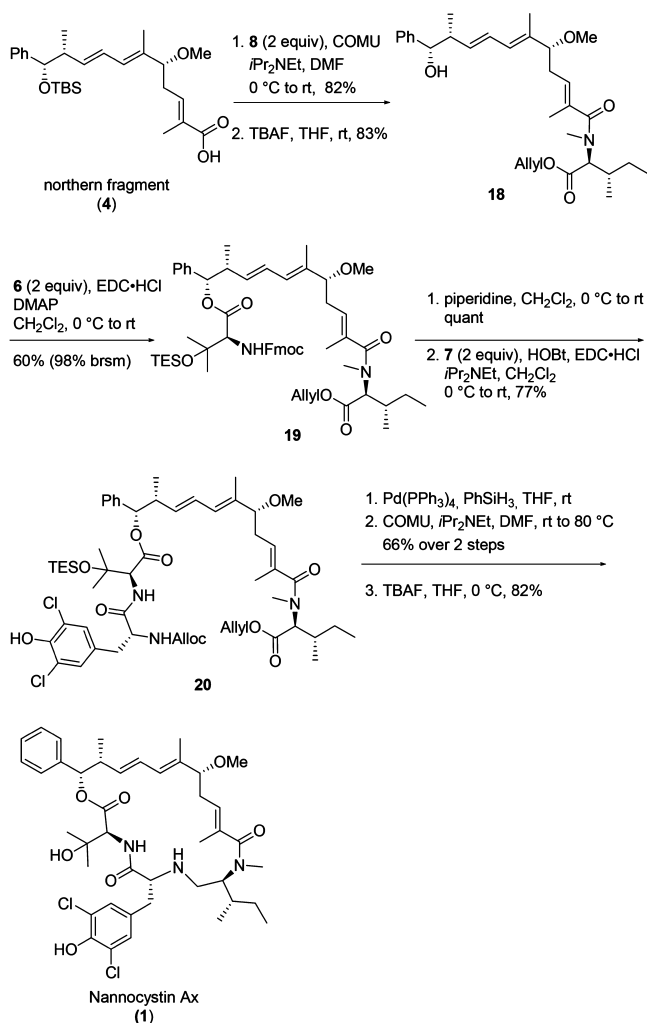
Scheme 3. Synthesis of Peptide Fragments 6 and 7



benzyl group was then cleaved via hydrogenation. Tyrosine fragment 7 (Scheme 3) was synthesized by Alloc protection of known 3,5-dichloro-D-tyrosine (18).²⁴ Isoleucine fragment 8 can be obtained from L-isoleucine using the literature procedure.¹⁹

Amidation between northern fragment 4 and isoleucine fragment 8 (Scheme 4) proved to be challenging because of the

Scheme 4. Endgame of the Total Synthesis



presence of the *N*-methylated amine.²⁵ For this coupling, several sets of conditions were investigated (e.g., PyBrop, PyClop, Oxyma, EDC·HCl, and DMAP), and it was found that (1-cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU)²⁶ gave the best results (82%) for these substrates. We also tried to apply these conditions to di- and tripeptide fragments but were not able to isolate the desired products. After removal of the TBS protecting group with TBAF, esterification of the obtained alcohol 18 with hydroxyvaline fragment 6 was achieved. For this it was necessary to protect the tertiary alcohol of 6, preventing it from elimination. Regrettably, only a 60% yield of the desired ester 19 was isolated. Fortunately, most of alcohol 18 could be recovered from the reaction, whereas this was not possible for hydroxyvaline fragment 6. Removal of the Fmoc protecting group enabled amidation with tyrosine fragment 6 using HOBT and EDC·HCl. On a small scale (20 μmol), a good

yield of 77% was obtained, whereas we observed a drop in yield during scale-up (~0.1 mmol) as well as the formation of diastereomers (60%, dr ~ 2:1). After simultaneous removal of the Allyl and Alloc groups, we were able to perform the macrolactamization using COMU, yielding the macrocycle in 66% yield. Deprotection of the tertiary TES ether with TBAF led to nannocystin Ax (1) in good yield.

In conclusion, the total synthesis of nannocystin Ax (1) with a longest linear sequence of 14 steps and an overall yield of 11% starting from building block 9 has been accomplished. In contrast to the other published total syntheses of nannocystin A (2)^{11–13} and 1,¹⁴ our synthesis closed the macrocycle via an amidation reaction instead of cross-coupling or metathesis. The stepwise introduction of the three peptide fragments in our approach could allow the incorporation of modified fragments in order to provide derivatives that could be used for further biological investigations.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b02112.

Experimental procedures and spectral data for compounds described herein (PDF)

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Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge the spectroscopy department of the Institute of Organic Chemistry for their support. Furthermore, we thank Dr. Phillip Krastel (Novartis Institutes for BioMedical Research) for providing the NMR spectrum of natural nannocystin Ax.

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