Total Synthesis

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Concise Total Synthesis of Nannocystin A

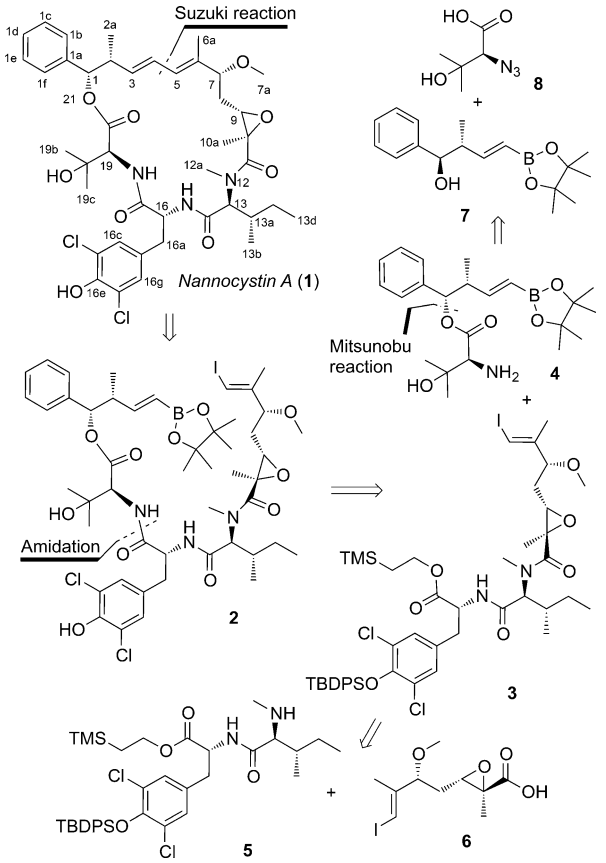
Linping Liao[+], Jingjing Zhou[+], Zhengshuang Xu, and Tao Ye**

Abstract: *Nannocystin A, a structurally unique 21-membered macrocyclic depsipeptide with low nanomolar inhibitory activity against elongation factor 1A, was synthesized according to a strategy involving the vinylogous Mukaiyama aldol reaction, Sharpless epoxidation, olefin metathesis, the Mitsunobu reaction, and a palladium-catalyzed intramolecular Suzuki coupling of a highly complex cyclization substrate. The overall synthesis is efficient and paves the way for preparation of analogues for drug development efforts.*

Natural products have long been a source of useful biologically active compounds for the development of new drugs.[1] The chemical synthesis of natural products presents opportunities to modify the structure, with the ultimate aim of improving the activity or physicochemical/biological properties of the lead molecule.[2] Synthesis is crucial in the establishment of structure–activity relationships (SAR), since the ability to establish a practical synthetic route suitable for analogue generation is a prerequisite of such studies.[3] We have been interested in macrocyclic natural products and view their syntheses as playing a key role in structural confirmation, structural modification, and subsequent activity control.[4] Herein, we report a highly efficient total synthesis of nannocystin A, a structurally unique macrocyclic depsipeptide that shows significant antitumor activity.

In 2015, the laboratories of Brönstrup[5] and Hoepfner[6] independently disclosed the isolation of nannocystin A (**1**) from the myxobacterium *Nannocystis* sp. Nannocystin A possesses a 21-membered macrocycle that contains both peptide and polyketide domains. These comprise highly modified amino acids, such as 3-hydroxy-valine, 3,5-dichloro-tyrosine, and *N*-methyl-isoleucine, and an oxygenated and methylated polyketide part with a conjugated diene and an epoxyamide moiety. In addition, a total of nine stereogenic centers are present in the carbon backbone of nannocystin A. Its structure was determined through a combination of spectral analysis, chemical degradation, and derivatization studies. The relative and absolute configurations of the nine stereocenters was established by extensive NMR spectroscopic analysis and chemical degradation,[5] and confirmed by single-crystal X-ray analysis.[5,6] Nannocystin A displays powerful growth-inhibition activity against a wide variety of human cancer cell lines at subnanomolar concentrations.[5,6] This antiproliferative activity appears to be mediated by the selective inhibition of elongation factor 1A (eEF1A).[6] Since this mechanism of action[7] is shared by plitidepsin,[8] which is originally from the marine tunicate *Aplidium albicans* and is currently in advanced clinical trials for multiple myeloma and T-cell lymphoma, it is clear that nannocystin A represents a promising lead compound for the development of new anticancer agents.

From the many disconnections one can envision for assembly of the macrocycle of nannocystin A, we chose a strategy based on intramolecular Suzuki coupling (Scheme 1). This approach requires the preparation of



Scheme 1. Retrosynthetic analysis of nannocystin A.

a precursor (**2**) carrying C5–C6 vinyl iodide and C3–C4 vinyl boronate functionalities for the cyclization process under the catalytic influence of palladium(0). Further analysis of the precursor **2** suggested that it could be assembled from two subunits (**3**, **4**) through amide coupling. The vinyl iodide

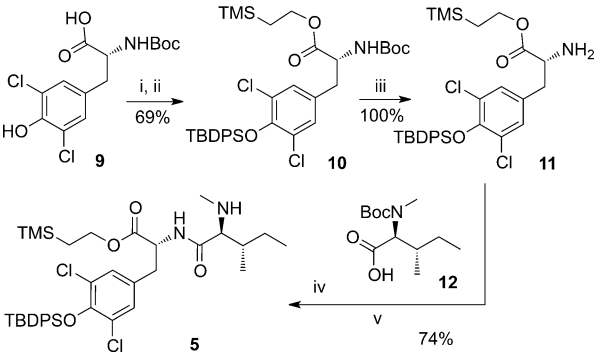
[*] L. Liao,[+] Dr. J. Zhou,[+] Dr. Z. Xu, Prof. Dr. T. Ye
Laboratory of Chemical Genomics, Engineering Laboratory for Chiral Drug Synthesis, School of Chemical Biology and Biotechnology
Peking University Shenzhen Graduate School
Xili, Nanshan District, Shenzhen, 518055 (China)
E-mail: xuzs@pkusz.edu.cn
yet@pkusz.edu.cn

[+] These authors contributed equally to this work.

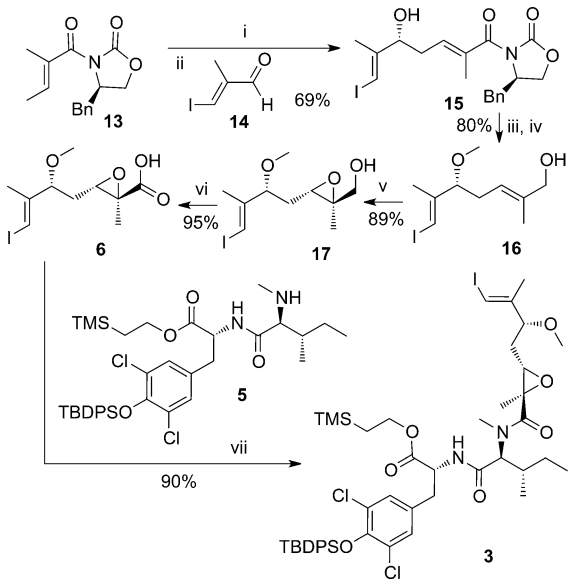
Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under http://dx.doi.org/10.1002/anie.201606679.

containing fragment **3** was planned to result from the assembly of dipeptide **5** and the epoxy acid **6**, while a Mitsunobu esterification between alcohol **7** and acid **8** was envisioned to deliver vinyl boronate **4**.

The starting point for the implementation of this strategy was dipeptide **5** (Scheme 2), which was obtained in five steps



Scheme 2. Synthesis of dipeptide **5**. Conditions: i)TTTBDPSCl, imid., CH_2Cl_2; ii) TMSE-OH, DMAP, EDCI, CH_2Cl_2; iii) TFA, CH_2Cl_2; iv) **12**, PyAOP, DIPEA, CH_2Cl_2; v) TFA, CH_2Cl_2. TBDPSCl = *tert*-butyl-(chloro)diphenylsilane, imid. = imidazole, DMAP = *N*,*N*-4-dimethylaminopyridine, TFA = trifluoroacetic acid, PyAOP = (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, DIPEA = diisopropylethylamine.

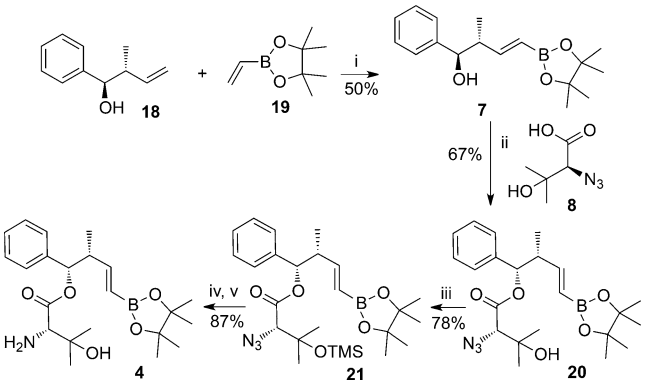


Scheme 3. Synthesis of fragment **3**. Conditions: i) NaHMDS, TBSCl, THF, −78 °C; ii) **14**, $TiCl_4$, CH_2Cl_2, −35 °C; iii) Proton sponge, $Me_3O^+BF_4^-$; iv) $LiBH_4$, THF/MeOH, 0 °C, 3 h; v) $Ti(OPr^i)_4$, L-(+)-DET, TBHP, 4ÅMS, DCM; vi) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2; $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, Bu^tOH/H_2O, 0 °C; vii) BEP, DIPEA, $MeCN/CH_2Cl_2$. HMDS = 1,1,1,3,3,3-hexamethyldisilazane, TBS = *tert*-butyldiphenylsilyl, THF = tetrahydrofuran, TBHP = *tert*-butyl hydroperoxide, BEP = 2-bromo-1-ethyl pyridinium tetrafluoroborate.

from the commercially available Boc-3,5-dichloro-L-tyrosine (**9**). The phenolic hydroxy group of **9** was protected as the *tert*-butyldiphenylsilyl ether and the carboxy group was protected as a fluoride-labile 2-trimethylsilylethyl (TMSE) ester to give **10** in 69 % yield. Trifluoroacetic acid promoted removal of the Boc group of **10** followed by coupling of the resulting amine (**11**) with *N*-Boc-*N*-methyl-L-isoleucine (**12**)[9] afforded dipeptide **5** in 74 % yield.

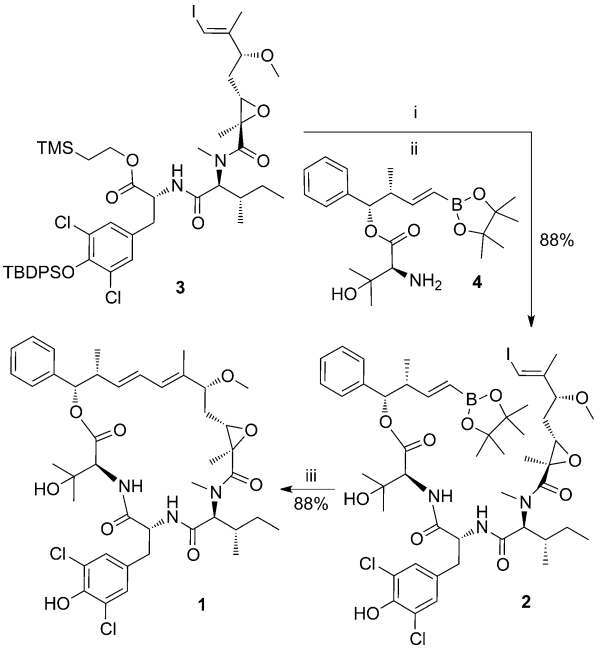
With fragment **5** in hand, we next turned our attention to the synthesis of key intermediate **3** (Scheme 3). Treatment of (4*R*)-3-[(2*E*)-2-methyl-1-oxo-1-buten-1-yl]-4-(phenylmethyl)-2-oxazolidinone (**13**)[10] with NaHMDS and TBSCl afforded the corresponding vinylketene silyl N,O-acetal, and subsequent $TiCl_4$-mediated vinylogous Mukaiyama aldol reaction[11] with aldehyde **14**[10] gave the aldol adduct **15** in 69 % yield. The resultant secondary alcohol was methylated by using Meerwein's salt (Me_3OBF_4) and a proton sponge[12] in dichloromethane. The chiral auxiliary was reductively cleaved by the use of $LiBH_4$ to provide the desired allylic alcohol **16** along with a minor amount of 1,4-reduction product. Sharpless epoxidation of **16** with (+)-diethyl tartrate (DET) gave epoxy alcohol **17** in 89 % yield as a single isomer, as demonstrated by ^{1}H-NMR and ^{13}C-NMR analysis. Sequential Dess–Martin[13] and Pinnick[14] oxidations of the primary hydroxy group in **17** afforded carboxylic acid **6**, which was used directly without further purification. Condensation of acid **6** with dipeptide **5** was attempted by employing various coupling reagents. Only when 2-bromo-1-ethyl pyridinium tetrafluoroborate (BEP)[15] was employed as a coupling agent, the reaction afforded amide **3** in 90 % yield. To our surprise, the condensation resulted generally in sluggish reactions and low yields when other coupling reagents such as *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI), 1-hydroxy-7-azabenzotriazole (HOAt),[16a,c] HATU,[16b,c] or PyAOP[17] were used.

Next, we turned our attention to the construction of the vinyl boronate **7** (Scheme 4). Olefin cross-metathesis of the



Scheme 4. Synthesis of **4**. i) Hoveyda–Grubbs II, toluene; ii) **8**, PPh_3, DEAD, THF; iii) TMSOTf, TEA, THF; iv) PMe_3, THF/H_2O; v) HCl, CH_2Cl_2. TEA = triethylamine.

known homoallylic alcohol **18**[18] with vinyl pinacol boronate **19**[19] under the influence of the Hoveyda–Grubbs II catalyst furnished vinyl boronate **7** in 50 % yield as a single geometrical isomer.[20] Mitsunobu esterification[21] using diethylazodicarboxylate (DEAD) and triphenylphosphine (Ph_3P) to condense alcohol **7** with acid **8** furnished ester **20** in 67 % yield



Scheme 5. i) TASF, THF; ii) DIC, HOBt, NMM, CH_2Cl_2/DMF; iii) Pd-$(PPh_3)_4$, Ag_2O, H_2O/THF. TASF = tris(dimethylamino)sulfur (trimethylsilyl)difluoride, DIC = diisopropylcarbodiimide, HOBt = 1-hydroxybenzotriazole, NMM = *N*-methylmorpholine, DMF = *N*,*N*-dimethylformamide.

with the expected hydroxy configuration inversion.[22] Staudinger reduction[23] of azide **20** proved to be problematic, presumably due to participation of the neighboring hydroxy group.[24] To our satisfaction, protection of the tertiary alcohol of **20** as its TMS ether (**21**), followed by a trimethylphosphine-mediated reduction and acidic clevage of the TMS group afforded key intermediate **4** in 68% yield over two steps.

With the key intermediates **3** and **4** in hand, their assembly to nannocystin A (**1**) was undertaken (Scheme 5). The β-trimethylsilylethyl ester was removed through treatment with TASF in THF to afford the corresponding acid,[25] which was condensed with vinyl boronate **4** to afford amide **2** in 88% yield. Treatment of a solution of **2** in wet THF with Ag_2O and catalytic $Pd(PPh_3)_4$ effected an intramolecular Suzuki reaction[26] that provided nannocystin A in 88% yield.[27]

The optical rotation measured for the synthetic sample ($[\alpha]_D^{25} = -29.0$ ($c = 1.0$, MeOH)) did not exactly match the value reported for natural nannocystin A ($[\alpha]_D = -38.0$ ($c = 0.19$, MeOH)).[5,28,29] The ^{1}H NMR (500 MHz, [D_6]DMSO) spectrum, and ^{13}C NMR (125 MHz, [D_6]DMSO) data for the synthetic sample exactly matched those[6] reported for naturally derived nannocystin A.[30] The original assignment of the relative and absolute configuration of nannocystin A is thus unambiguously corroborated by this total synthesis.

The initial biological evaluation of **1** was performed across a panel of two colon cancer cell lines (HCT116 and HT29), and cell proliferation was measured by MTS assay. The IC_{50} values for **1** were 4.4 nM (HCT116) and 12.2 nM (HT29). These data indicate that **1** inhibits the proliferation of HCT116 and HT29 cancer cells at comparable levels of potency, with IC_{50} values slightly higher than these reported for natural nannocystin A.[5] We also expanded the scope of our tests by screening the effect of **1** on liver cancer cells (SMMC7721 and Hep3B). In this case, the IC_{50} values for **1** were 1.2 nM (SMMC7721) and 0.5 nM (Hep3B).

In summary, the execution of a highly convergent strategy has led to the completion of the first total synthesis of nannocystin A. Overall, **1** was prepared in 10 steps for the longest linear sequence (32.5% overall yield) and 20 total steps from imide **13**[10] and aldehyde **14**.[10] This paves the way for the efficient preparation of analogues for drug development efforts. Extension of this strategy to the synthesis of various analogues and to establish a SAR profile for nannocystin A is currently underway.

Acknowledgements

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Keywords: anticancer agents · cyclodepsipeptides · nannocystin A · natural products · total synthesis

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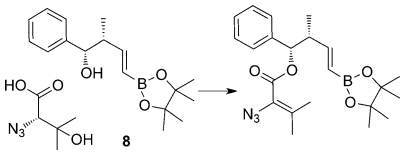
[22] Esterification of a diastereomeric alcohol (see below) with acid **8** under Yamaguchi conditions gave rise to the corresponding β-elimination product.



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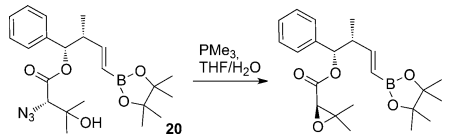
[24] Staudinger reduction of azide **20** gave rise to the corresponding epoxide as the major product.



[25] When a methyl ester, instead of the β-trimethylsilylethyl ester was employed as the protecting group for vinyl iodide **3**, the compound underwent extensively decomposition under either base hydrolysis or Me_3SnOH-induced cleavage conditions.

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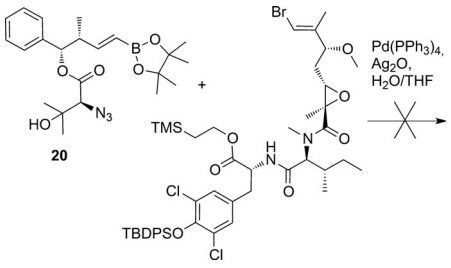
[27] Under identical conditions as for the synthesis of **1**, intermolecular Suzuki coupling between vinyl boronate **20** and vinyl bromide failed to deliver any of the desired products.



[28] The natural nannocystin A isolated by Professor Mark Brönstrup and co-workers (see Ref. [5]) was contaminated with various amounts of ammonium acetate. The exact amount of the ammonium acetate presented in the natural sample is unknown. (See the supporting information for details).

[29] The optical rotation for nanocystin A isolated by Dr. Dominic Hoepfner and co-workers (see Ref. [6]) was not determined/reported.

[30] For a detailed comparison of ^{1}H NMR spectrum of synthetic nannocystin A with that received from Drs. Dominic Hoepfner and Philipp Krastel, and a tabulated comparison of the ^{13}C NMR data of the synthetic nannocystin A with that reported by Dr. Dominic Hoepfner and co-workers (Ref. [6]), please see Sections 3.1 and 3.2 of the Supporting Information. It should be noted that the sample isolated by Professor Mark Brönstrup and co-workers (Ref. [5]) was contaminated with various amounts of ammonium acetate. Our NMR studies revealed that the ^{1}H NMR spectrum recorded for a sample containing synthetic nannocystin A and 3 equivalents of NH_4OAc, closely matched the spectrum reported by Professor Mark Brönstrup and co-workers. For details, please see Section 3.3 of the Supporting Information.

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