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Total Synthesis of Nannocystin Ax

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Total synthesis of nannocystin Ax, a natural depsipeptide isolated from myxobacteria recently, has been accomplished concisely. By following a convergent strategy, the target molecule was assembled from three fragments. Each fragment can be synthesized expeditiously from readily achievable compounds. The key elements in this total synthesis features Kobayashi's remote asymmetric induction with vinylketene silyl N, O-acetal, Roush's asymmetric crotylboration of aldehyde, Mitsunobu's esterification and macrocyclization via Stille cross-coupling.

Natural products have been playing crucial role in R & D of new drugs as a pivotal source with diversified structures and intriguing bioactivities.[1] Chemical synthesis of natural products and their analogs offers opportunities for study on structure-activity relationships (SAR) of novel compounds and their mechanism of action in biological systems, promoting the possibility of identifying pharmaceutically new chemical entities (NCE). In 2015, the Brönstrup group[2] and the Hoepfner group[3] independently reported isolation and characterization of a family of depsipeptides named nannocystins (Fig. 1) with potent activity against cancer cell lines at nanomolar concentrations. Structurally, 21-membered macrocycles of these natural products consist of a polyketide motif and a tripeptide motif, involving derivative of an unnatural D-tyrosine, with 7-9 stereogenic centers. Furthermore, genetic and proteomic studies indicated that the primary target of nannocystins is the eukaryotic translation elongation factor 1α (EF-1α), and the epoxide moiety is not an essential feature of the pharmacophore. EF-1α is the identified binding protein of didemnin B,[4] a cyclic depsipeptide with failure in phase II clinical trial, so nannocystins would serve as alternative candidates of new anticancer therapeutics. Due to long-lasting enthusiasm toward depsipeptides from synthetic community,[5] nannocystins have become hot targets with the intriguing bioactivities as soon as their isolation. Actually, total syntheses of nannocystins A and A0 have been achieved by the Wang group,[6a] and total synthesis of nannocystin A has also been accomplished by the Ye group[6b] and the Chen group.[6c] Recently, we completed total synthesis of Nannocystin Ax and herein present our related synthetic efforts.

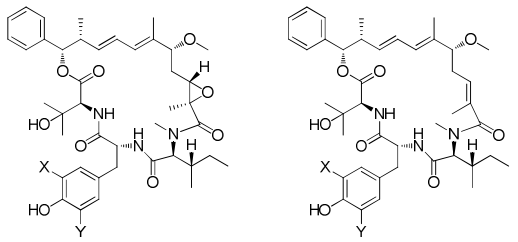

Fig. 1 Representative Structures of Nannocystin A, Ax, A3 and Ay

To fulfil an expeditious total synthesis, we preferred a convergent synthetic strategy (Scheme 1). As intramolecular cross-coupling plays conspicuous role in construction of macrocycles,[7] we planned to employ Stille coupling on compound **2** to realize macrocyclization of nannocystin Ax. The cross coupling precursor **2** can be assembled from the amine **3** and the acid **4** through amidation. Compound **3** could be constructed via Mitsunobu esterification from the amino acid **5** and the alcohol **6**. Formation of **4** could be accessed from the dipeptide **7** and compound **8**. These two fragments could be achievable from several known compounds, i.e. **9**-**12**.

Our total synthesis was initiated by preparation of the dipeptide **7** from the known compound **9**, which is accessible from D-tyrosine (Scheme 2).[8] Protecting the phenol in **9** with chloromethyl methyl ether in the presence of diisopropylethylamine afforded **13** in 79% yield. Amidation between **13** and the known compound **10**[9] afforded compound **14** in 85% yield, Boc group of which was selectively deprotected in the presence of MOM ether with trimethylsilyl triflate and 2, 6-lutidine, to deliver the dipeptide

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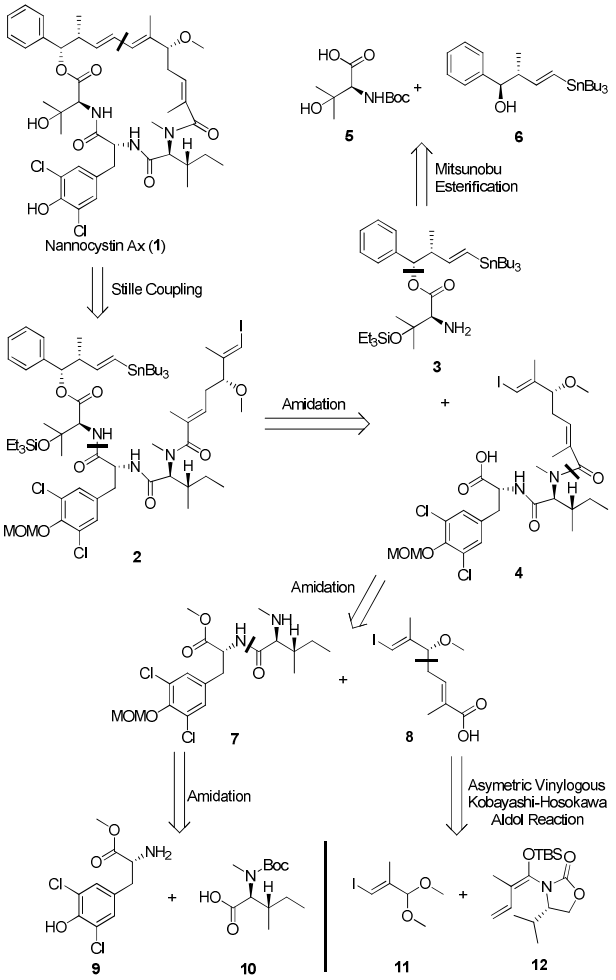
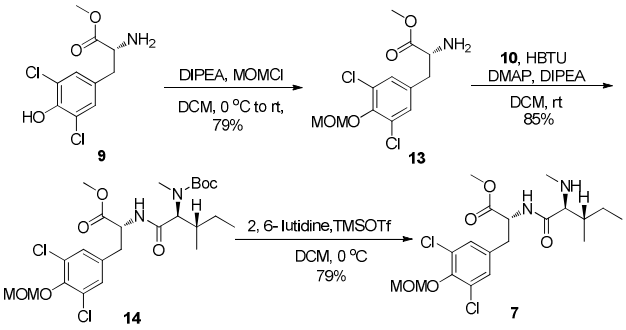


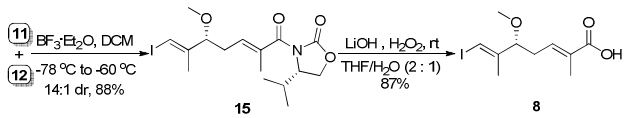
Scheme 1 Retrosynthetic Analysis

7 in 79% yield.[10]

Then we started to synthesize the acid **8** from the known compound **11** (Scheme 3).[11] The vinylogous Mukaiyama aldol reaction has been developed and widely applied in natural product synthesis.[12] By incorporating an Evans auxiliary into vinylketene silyl N, O-acetal, Kobayashi *et al* developed an asymmetric aldol reaction of aldehyde.[13a] This methodology was further extended to aldol reaction between E, E-



Scheme 2 Synthesis of the Dipeptide **7**

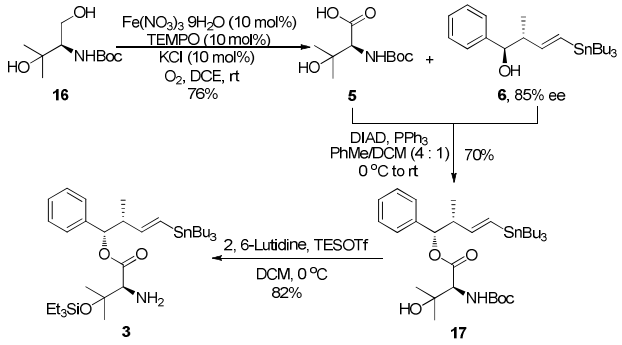


Scheme 3 Synthesis of Acid **8**

vinylketene silyl N, O-acetal and acetals by Hosokawa *et al*.[13b] However, asymmetric aldol reaction between an acetate-type vinylketene silyl N, O-acetal and acetal has never been tested. To our delight, aldol reaction of **11** with acetate-type vinylketene silyl N, O-acetal **12**,[13c] mediated by $BF_3{\cdot}Et_2O$, afforded the aldol adduct **15** with 14:1 dr in 88% yield. The freshly generated allylic chiral center in **15** was introduced through remote asymmetric induction of the Evans auxiliary in **12**.[13b] Hydrolysis of this chiral auxiliary was undertaken with lithium hydroxide and hydrogen peroxide to furnish the acid **8** in 87% yield with 89% ee.

Based on a recent green methodology developed by the Ma group,[14] Iron-catalyzed oxidation of a known compound **16** produced the oxidized N-Boc-L-valine **5**, a known aminoacid Scheme 4).[15] Then compound **6** was prepared with 85% ee according to Roush's enantioselective synthesis of E-δ-stannyl-anti-homoallylic alcohol via aldehyde crotylboration.[16] Since chirality at benzylic carbon in **6** was inconsistent with the desired one in target molecule, its absolute configuration was inversed by reacting **5** with **6** in Mitsunobu's condition to give compound **17** with proper stereochemistry in 70% yield with 10:1 dr. Removal of Boc protection with protonic acids resulted in destannylation of **17**. So 2, 6-lutidine and triethylsilyl trifluoromethanesulfonate was employed to achieve deprotection of tert-butyl carbamate,[10] while silyl protection of the tertiary alcohol was achieved at the same time to afford compound **3** with 14:1 dr after purification.

With compounds **3**, **7** and **8** in hand, we embarked on assembling these fragments to finalize total synthesis of nannocystin Ax (Scheme 5). However, amidation between the dipeptide **7** and the acid **8** proved sluggish, probably due to steric repulsion between these two counterparts in transition state. Various condensing reagents, such as N-(3-



Scheme 4 Synthesis of the Amine **3**

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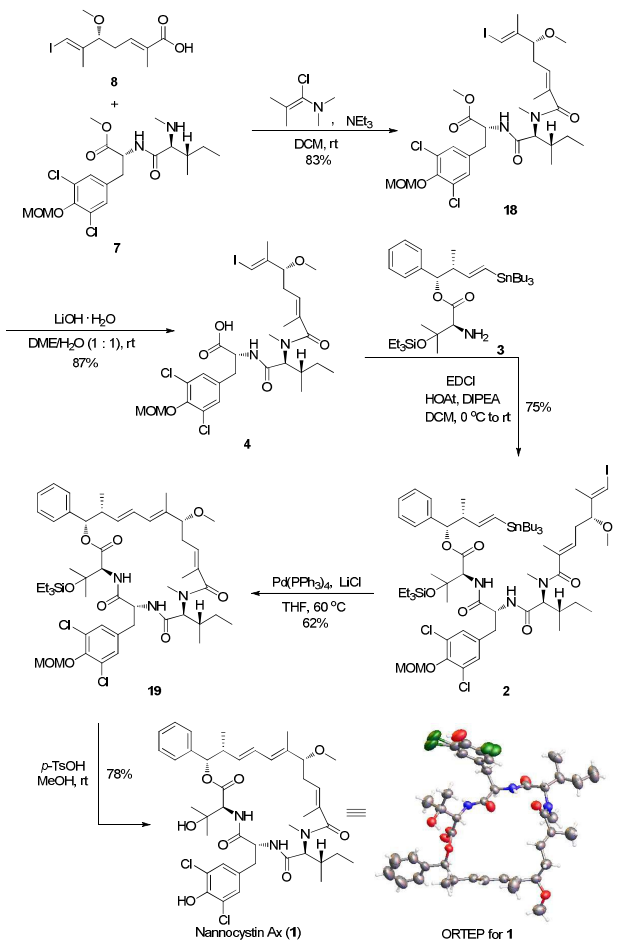

Scheme 5 Total Synthesis of nannocystin Ax

dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI),[17] 2, 4, 6-trichlorobenzoyl chloride,[18] HBTU,[19] HATU,[20] and HOAt,[21] were attempted, but all failed to yield the desired product. Although reactions upon treatment with oxalyl chloride,[22] pivaloyl chloride[23] behaved fertile, 1-chloro-N, N, 2-trimethylpropenylamine (Ghosez reagent)[24] successfully prompted condensation between **7** and **8** to furnish the amide **18** in 83% yield. Hydrolysis the methyl ester in **18** with lithium hydroxide afforded the acid **4** in 87% yield. The following condensation between **3** with **4** in the presence of EDCI, HOAt and DIPEA provided compound **2** in 75% yield. Then intramolecular Stille cross-coupling, mediated by tetrakis (triphenylphosphine) palladium and lithium chloride, afforded the macrocycle **19** in 62% yield.[25] After global deprotection of MOM ether and TES ether with *p*-toluenesulfonic acid, total synthesis of nannocystin Ax was accomplished in 78% yield.

In ^{1}H and ^{13}C NMR spectra of our synthetic nannocystin Ax dissolved in DMSO-d_6, a 5:1 mixture of conformers could be determined. Characterization data of the major isomer match those reported by the Hoepfner group very well.[3,26] One may argue that these minor signals in NMR spectra might be ascribed to impurities from side reaction in the final transformation from **19** to nannocystin Ax, so further experiments were executed to rule out this probability undoubtedly. First, ^{1}H NMR spectra of our synthetic sample in $(CD_3)_2CO$ and $CDCl_3$ indicated existence of two conformers in about 10:1 ratio. Second, variable temperature (VT) NMR experiments showed that the proton signals of both conformer merged together gradually from 25 to 70 ℃.[26] Third, a single crystal of nannocystin Ax was fortunately obtained out of methanol and its X-ray crystallography unequivocally evidenced its structure and purity; after dissolving this single crystal in DMSO-d_6, a spectrum consisting of a 5:1 mixture of conformers was obtained again. Accordingly, we completed total synthesis of nannocystin Ax and proved its existence as conformers in solution beyond question.

In summary, total synthesis of nannocystin Ax was completed from readily available starting materials. Establishment of this convergent synthetic strategy sets solid foundation for preparation of structural analogues of nannocystin Ax and study on structure-activity relationship.

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