LETTERS

Total Synthesis of Nannocystin A

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Supporting Information

ABSTRACT: Nannocystin A is a 21-membered cyclodepsipeptide showing remarkable anticancer properties. Described is the total synthesis of nannocystin A, which features an asymmetric vinylogous Mukaiyama aldol reaction for efficient assembly of the penultimate open-chain precursor and a pivotal intramolecular Heck cross-coupling for the final macrocyclization.



S econdary metabolites from microorganisms constitute a large reservoir of valuable chemotherapeutic agents such as antibiotics and anticancer drugs.¹ Recently, a class of cyclic depsipeptides named nannocystins were isolated independently by two research teams through cultivation of the myxobacterial strains *Nannocyctis* sp. ST201196 and *Nannocyctis* sp. MB1016, respectively.^{2,3} Nannocystin A (1) features a 21-membered polyketide–tripeptide macrocycle bearing a novel α,β -epoxy amide substructure, along with nine chiral centers and two continuous *E*-configured alkenes (Figure 1). Brönstrup et al. showed that 1 displayed potent antiproliferative activities toward 14 cancer cell lines at low nanomolar levels, and it retained excellent inhibitory effect against the drug-resistant cell line MDA-A1 (IC₅₀ 12 nM) compared to the related cell line





MDA-MB231 $(IC_{50} 6.5 \text{ nM})$.² In contrast, the reference drug docetaxel suffered a considerable drop (nearly 2000-fold) in activity against MDA-A1 (IC_{50} 570 nM) with respect to MDA-MB231 (IC_{50} 0.3 nM). These results demonstrated the potential of **1** as a novel lead compound for the development of anticancer drugs.

Parallel to Brönstrup's research,² Hoepfner et al. found that 1 displayed differential inhibitive properties (IC₅₀ values ranging from 0.5 μ M to 5 nM) against 472 cancer cell lines.³ Different from some known actin-binding cyclodepsipeptides such as chondramide C,⁴ jasplakinolide,⁵ seragamide A,⁶ and miuraenamide,⁷ the primary target of 1 was identified to be the eukaryotic translation elongation factor (eEF1 α).

To date, only preliminary studies have been carried out on their mechanism of action and pharmaceutical applications. Moreover, the issue regarding whether the epoxide moiety in **1** is a critical component of the pharmacophore is still unknown.^{2,3} Hence, there is an urgent need to explore the chemical synthesis of nannocystin A, the leading species from the natural nannocystin congeners. More important, systematic structure variations will ensue to gain insight into key structural elements that account for its high antiproliferative properties and aid in developing more potent $eEF1\alpha$ -targeting analogues. As the first step toward this aim, we report herein the total synthesis of nannocystin A (**1**) in an efficient route.⁸

Retrosynthetically, the steric environment of the 6E,8E-diene from the southern polyketide segment is less congested than

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that of the northern tripeptide segment in 1 (Figure 2). Accordingly, the C7-C8 bond tethering the two E-alkenes is



our preferred site for macrocyclization. In the synthetic direction, a ring-closing intramolecular E-selective Heck crosscoupling was envisioned to fulfill the task.9,10 Further disconnection of the open-chain precursor 2 gave rise to five building blocks, including the vinyl iodide bearing epoxy acid 3, N-methyl-L-isoleucine 4, 3,5-dichloro-D-tyrosine 5, N-Boc-3hydroxy-D-valine 6, and homoallylic alcohol 7. The synthesis of 3 called for an asymmetric vinylogous Mukaiyama aldol reaction¹¹ to construct the carbon skeleton and a Sharpless asymmetric epoxidation¹² to install the chiral epoxide group. The other fragments 4-7 were easily accessible according to the literature.

The forward synthesis commenced with the preparation of 3 (Scheme 1). Following Kobayashi's protocol,¹¹ 8 was subjected to TiCl₄-mediated vinylogous Mukaiyama aldol condensation with (E)-3-iodo-2-methylacrylaldehyde (21). Efficient control of stereoselectivity (dr >10:1) was observed in the reaction, with the predominant isomer 9 isolated in 51% yield after silica gel flash chromatography.¹⁴ After methylation and reduction, Letter





the allylic alcohol 11 was converted to 12 via Sharpless asymmetric epoxidation with excellent stereoselectivity (dr >10:1). Formation of 3 was secured through stepwise oxidations.

Next, union of N-Boc-3-hydroxy-D-valine (6) and homoallylic alcohol 7 was pursued. Unfortunately, condensation of 6 and 7 under standard esterification conditions (EDC/DMAP, DIC/DMAP)¹⁵ failed to give the desired product, presumably due to steric hindrance. Hence, we turned our attention to the Mitsunobu reaction,¹⁶ which required the use of *anti*-homoallylic alcohol 13^{17} as the coupling partner (Scheme 2).





To our delight, Mitsunobu reaction between 13 and 6 proceeded smoothly to give 14 in 70% yield. The Boc group of 14 was then removed with TFA to liberate the amine moiety ready to be coupled to the tyrosine carboxyl group.

With the key building blocks 3 and 15 in hand, the next stage was the preparation of the Heck precursor 2 via amide coupling reactions. Incorporation of 3 into the tripeptide derivative turned out to be a nontrivial task. The major challenge was the development of a suitable peptide coupling sequence that is compatible with the potentially reactive epoxy group. By testing different coupling strategies, we eventually found a satisfactory approach to 2 as shown in Scheme 3.

Starting from TBS-protected tyrosine derivative 16, which was prepared from commercially available D-tyrosine in three ⁸ coupling of **16** and *N*-methyl-*N*-Fmoc-L-isoleucine steps, (17) followed by Fmoc deprotection produced the dipeptide 19. A second amide coupling was accomplished in 52% yield under standard condensation conditions using HATU and DIPEA. Treatment of the resulting 20 with lithium hydroxide led to the hydrolysis of both the methyl ester and the TBS

Scheme 3. Completion of the Total Synthesis of 1



group, which was accompanied by a third amide condensation with **15** to afford the penultimate open-chain intermediate **2**. Finally, intramolecular Heck macrocyclization proceeded smoothly to give the product **1** in 58% yield. It was noteworthy that the labile epoxy group was well tolerated in the Heck macrocyclization conditions. The spectra (¹H NMR, ¹³C NMR, HRMS) of our synthetic **1** are in good agreement with the literature data,³ thus completing the total synthesis of nannocystin A.

In summary, total synthesis of nannocystin A 1 was achieved from readily available starting materials in 4.1% overall yield and 10 steps for the longest linear sequence. On the basis of the established route, synthesis and biological evaluation of structural analogues of 1 are currently in progress and will be reported in due course.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.or-glett.6b02729.

Detailed experimental procedures, spectroscopic data, and ¹H and ¹³C NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) (a) Demain, A. L. Appl. Microbiol. Biotechnol. 1999, 52, 455-463.
 (b) Verpoorte, R. Drug Discovery Today 1998, 3, 232-238.

(c) Newman, D. J.; Cragg, G. M. J. Nat. Prod. 2012, 75, 311–335.

(2) Hoffmann, H.; Kogler, H.; Heyse, W.; Matter, H.; Caspers, M.; Schummer, D.; Klemke-Jahn, C.; Bauer, A.; Penarier, G.; Debussche, L.; Brönstrup, M. Angew. Chem., Int. Ed. **2015**, 54, 10145–10148.

(3) Krastel, P.; Roggo, S.; Schirle, M.; Ross, N. T.; Perruccio, F.; Aspesi, P., Jr.; Aust, T.; Buntin, K.; Estoppey, D.; Liechty, B.; Mapa, F.; Memmert, K.; Miller, H.; Pan, X.; Riedl, R.; Thibaut, C.; Thomas, J.; Wagner, T.; Weber, E.; Xie, X.; Schmitt, E. K.; Hoepfner, D. *Angew. Chem., Int. Ed.* **2015**, *54*, 10149–10154.

(4) (a) Waldmann, H.; Hu, T. S.; Renner, S.; Menninger, S.; Tannert, R.; Oda, T.; Arndt, H. D. Angew. Chem., Int. Ed. 2008, 47, 6473–6477.
(b) Eggert, U.; Diestel, R.; Sasse, F.; Jansen, R.; Kunze, B.; Kalesse, M. Angew. Chem., Int. Ed. 2008, 47, 6478–6482.

(5) Tannert, R.; Milroy, L. G.; Ellinger, B.; Hu, T. S.; Arndt, H. D.; Waldmann, H. J. Am. Chem. Soc. 2010, 132, 3063–3077.

(6) Arndt, H. D.; Rizzo, S.; Nocker, C.; Wakchaure, V. N.; Milroy, L. G.; Bieker, V.; Calderon, A.; Tran, T. T.; Brand, S.; Dehmelt, L.; Waldmann, H. *Chem. - Eur. J.* **2015**, *21*, 5311–5316.

(7) Karmann, L.; Schultz, K.; Herrmann, J.; Muller, R.; Kazmaier, U. Angew. Chem., Int. Ed. 2015, 54, 4502–4507.

(8) During the preparation of our manuscript, two alternative total syntheses of nannocystin A were reported; see: (a) Huang, J.; Wang, Z. Org. Lett. **2016**, *18*, 4702–4705. (b) Liao, L.; Zhou, J.; Xu, Z.; Ye, T. Angew. Chem., Int. Ed. **2016**, *55*, 13263–13266.

(9) For leading reviews, see: (a) Beletskaya, I. P.; Cheprakov, A. V. *Chem. Rev.* **2000**, *100*, 3009–3066. (b) Dounay, A. B.; Overman, L. E. *Chem. Rev.* **2003**, *103*, 2945–2963.

(10) For recent examples of applying Heck macrocyclization in total synthesis, see: (a) Li, P.; Li, J.; Arikan, F.; Ahlbrecht, W.; Dieckmann, M.; Menche, D. J. Am. Chem. Soc. 2009, 131, 11678–11679. (b) Jägel, J.; Maier, M. E. Synthesis 2009, 2881–2892. (c) Li, P.; Li, J.; Arikan, F.; Ahlbrecht, W.; Dieckmann, M.; Menche, D. J. Org. Chem. 2010, 75, 2429–2444. (d) Dieckmann, M.; Rudolph, S.; Dreisigacker, S.; Menche, D. J. Org. Chem. 2012, 77, 10782–10788. (e) Symkenberg, G.; Kalesse, M. Angew. Chem., Int. Ed. 2014, 53, 1795–1798. (f) Reddy, K. M.; Yamini, V.; Singarapu, K. K.; Ghosh, S. Org. Lett. 2014, 16, 2658–2660. (g) Nguyen, M. H.; Imanishi, M.; Kurogi, T.; Smith, A. B. J. Am. Chem. Soc. 2016, 138, 3675–3678. (h) Das, S.; Paul, D.; Goswami, R. K. Org. Lett. 2016, 18, 1908–1911.

(11) Shirokawa, S.; Kamiyama, M.; Nakamura, T.; Okada, M.; Nakazaki, A.; Hosokawa, S.; Kobayashi, S. J. Am. Chem. Soc. **2004**, *126*, 13604–13605. For a recent review, see: Kalesse, M.; Cordes, M.; Symkenberg, G.; Lu, H.-H. Nat. Prod. Rep. **2014**, *31*, 563–594.

(12) Katsuki, T.; Sharpless, K. B. J. Am. Chem. Soc. 1980, 102, 5974–5976.

(13) See the Supporting Information for their preparation.

(14) The diastereoselectivity was estimated by examining the NMR spectroscopy of the crude product.

(15) Tsakos, M.; Schaffert, E. S.; Clement, L. L.; Villadsen, N. L.; Poulsen, T. B. *Nat. Prod. Rep.* **2015**, *32*, 605–632.

(16) Swamy, K. C. K.; Kumar, N. N. B.; Balaraman, E.; Kumar, K. *Chem. Rev.* **2009**, *109*, 2551–2651.

(17) For some representative synthesis of **13**, see: (a) Brown, H. C.; Bhat, K. S. *J. Am. Chem. Soc.* **1986**, 108, 5919–5923. (b) Corey, E. J.; Yu, C. M.; Kim, S. S. *J. Am. Chem. Soc.* **1989**, 111, 5495–5496. (c) Kim, H.; Ho, S.; Leighton, J. L. *J. Am. Chem. Soc.* **2011**, 133, 6517– 6520.

(18) See the Supporting Information for details.