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Total Synthesis of NW-G01, a Cyclic Hexapeptide Antibiotic, and 34-epi-NW-G01

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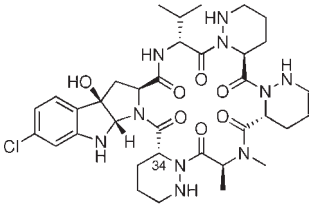
ABSTRACT



NW-G01

NW-G01, a cyclic hexapeptide antibiotic, and 34-epi-NW-G01 were synthesized by the highly stereoselective convergent approach for the first time, thereby unambiguously determining the absolute structure of NW-G01.

The severe resistance of many organisms against existing antibiotics is intensifying in the medical fields. This situation requires the advent of new classes of antibiotics. In the course of a screening program for such antibiotics, Wu and co-workers isolated NW-G01 from the fermentation broth of *Streptomyces alboflavus* 313 in 2009.[1] This compound exhibits potent antibacterial activity against gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) but is ineffective against gram-negative bacteria. The comparison of the MIC value of NW-G01 (7.81 μg mL^{-1}) against MRSA with that of ampicillin ($>$100 μg mL^{-1}), a representative β-lactam antibiotic, suggests that NW-G01 is expected to provide an excellent scaffold for the development of new antibiotics.[1a] Based on the detailed NMR and X-ray analyses as well as Marfey's amino acid analysis, the absolute structure of NW-G01 was initially assigned to structure **1** consisting of D-valine, *N*-methyl-L-alanine, (*R*)-piperazic acid, two molecules of (*S*)-piperazic acid, and a characteristic chlorinated pyrroloindoline moiety.[1b] However, it was later revised as structure **2**, a C34-epimer of **1** (Figure 1).[2] The 18-membered cyclic hexapeptide structure is related to the monomeric structures of himastatin[3,4] and chloptosin,[5,6] antitumor dimeric cyclohexapeptides. In connection with a project directed toward the synthesis of chloptosin,[7] we became interested in the synthesis of NW-G01 due to its intriguing biological and structural features. We herein describe the first total synthesis of NW-G01 and its C34-epimer, thereby unambiguously determining the absolute structure of NW-G01 as depicted in **2**.

(1) (a) Guo, Z.; Shen, L.; Ji, Z.; Zhang, J.; Huang, L.; Wu, W. *J. Antibiot*. **2009**, *62*, 201. (b) Guo, Z.; Ji, Z.; Zhang, J.; Deng, J.; Shen, L.; Liu, W.; Wu, W. *J. Antibiot*. **2010**, *63*, 231.

(2) Guo, Z.; Ji, Z.; Zhang, J.; Deng, J.; Shen, L.; Liu, W.; Wu, W. *J. Antibiot*. **2010**, *63*, 733.

(3) Leet, J. E.; Schroeder, D. R.; Krishnan, B. S.; Maston, J. A.; Doyle, T. W.; Lam, K. S.; Hill, S. E.; Lee, M. S.; Whitney, J. L.; Krishnan, B. S. *J. Antibiot*. **1996**, *49*, 299 and references therein.

(4) Total synthesis: (a) Kamenecka, T. M.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 2993. (b) Kamenecka, T. M.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 2995. (c) Kamenecka, T. M.; Danishefsky, S. J. *Chem.—Eur. J.* **2001**, *7*, 41.

(5) Umezawa, K.; Ikeda, Y.; Uchihata, Y.; Naganawa, H.; Kondo, S. *J. Org. Chem.* **2000**, *65*, 459.

(6) Total synthesis:(a) Yu, S.-M.; Hong, W.-X.; Wu, Y.; Zhong, C.-L.; Yao, Z.-J. *Org. Lett*. **2010**, *12*, 1124. (b) Oelke, A. J.; France, D. J.; Hofmann, T.; Wuitschik, G.; Ley, S. V. *Angew. Chem., Int. Ed.* **2010**, *49*, 6139. (c) Oelke, A. J.; France, D. J.; Hofmann, T.; Wuitschik, G.; Ley, S. V. *Chem.—Eur. J.* **2011**, *17*, 4183.

(7) Shibahara, S. Thesis, Nagasaki University, 2011. Our synthetic study toward chloptosin was presented at the 36th Symposium on Progress in Organic Reactions and Synthesis: Applications in the Life Sciences (Nagoya, Japan, November 2, 2010, abstract paper pp 182−183).

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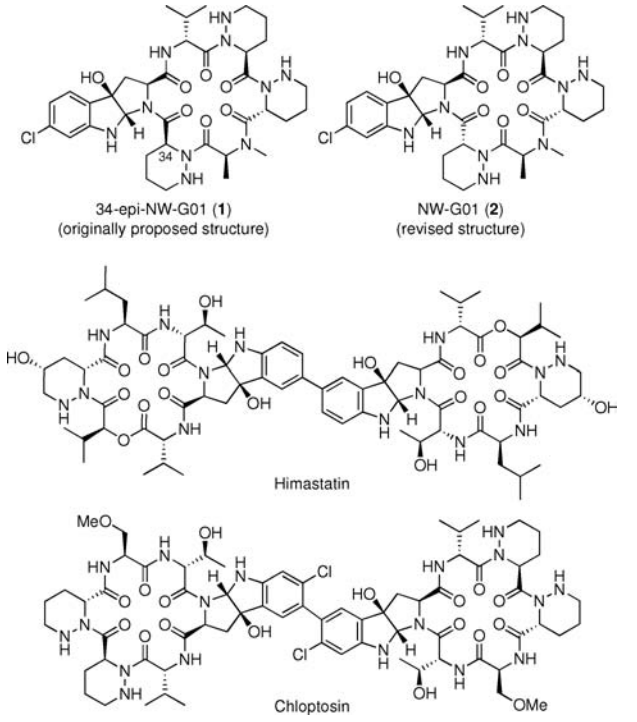
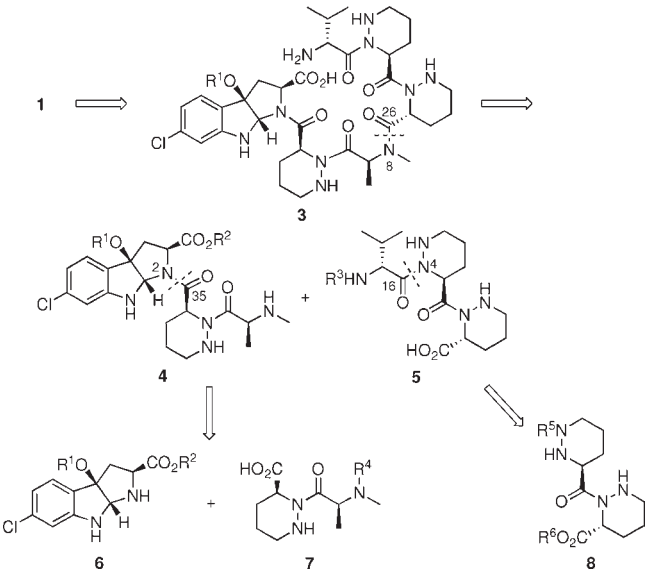


Figure 1. Related cyclohexapeptides with a pyrroloindoline.

Since the absolute structure of NW-G01 had been proposed to be **1**[1b] when we started this project, we initially addressed the synthesis of **1**. Our synthetic plan is illustrated in Scheme 1. In the synthesis of chloptosin, Yao and co-workers demonstrated[6a] that it was very difficult to install pyrroloindoline **6** in either penta-, tetra-, or tripeptide residue by amidation at the N2 position due to its low nucleophilicity. In addition, in the course of our synthetic studies[7] on this family of cyclohexapeptides, we often observed that piperazid acid derivatives are very reluctant coupling partners with bulky peptides. Taking into account the poor reactivities of **6** and piperazic acid, we retrosynthetically sought to address the formation of the macrocycle from **3**. Disconnection at N8−C26 affords two key intermediates **4** and **5**, which can be further disconnected at N2−C35 and N4−C16 respectively giving three important building blocks **6**, **7**, and **8**.

The synthesis of pyrroloindoline fragment **6** commenced with the preparation of **10** from 5-chloro-2-iodoaniline and aldehyde **9**[8] by Zhu's tryptophan synthesis[9] (Scheme 2). Compound **10** was then converted to *N*,*N*′-di-Boc derivative **12** by a protection−deprotection sequence. However, the second deprotection step turned out to be problematic under conventional acidic conditions.[10] After considerable exploration, we eventually found that, upon treatment of

Scheme 1. Retrosynthetic Analysis of **1**



11 with 0.6 equiv of $Mg(ClO_4)_2$ in acetonitrile at room temperature,[11] selective removal of one of the *N*-Boc groups was cleanly achieved to provide **12** in almost quantitative yield. According to the procedure reported by Ley and co-workers,[6c,12] **12** was then converted to **14** diastereoselectively (dr = 23:1) via **13** by selenocyclization followed by oxidative deselenation. For the subsequent assembly of the cyclic hexapeptide, the methyl ester of **14** was switched to an allyl ester group by successive hydrolysis and allylation to give **15**. In our preliminary examination, we found that a free tertiary alcohol in pyrroloindoline **6** (R^1 = H) hampered the coupling with carboxylic acid **7**. Thus, the hydroxy group of **15** was masked as its TES ether by treatment with TESOTf which also promoted the concomitant removal of two Boc groups to afford pyrroloindoline **16** in excellent yield.

Fragment **7** was prepared as dipeptide **23** starting with Hale's piperazic acid synthesis[13] (Scheme 3). Oxazolidinone **17**, prepared from 5-bromovaleric acid,[13] was subjected to hydrazination/cyclization conditions to furnish piperazic acid derivative **18** in 63% yield. Hydrolysis of **18** followed by allyl esterification of the resulting carboxylic acid afforded allyl ester **19**, which was then converted to (*S*)-**20** via removal of the Boc groups and selective benzyloxycarbonylation in 65% overall yield. The enantiomeric

(8) Padrón, J. M.; Kokotos, G.; Martín, T.; Markidis, T.; Gibbons, W. A.; Martín, V. S. *Tetrahedron: Asymmetry* **1998**, *9*, 3381.

(9) (a) Jia, Y.; Zhu, J. *Synlett* **2005**, 2469. (b) Jia, Y.; Zhu, J. *J. Org. Chem.* **2006**, *71*, 7826.

(10) Ley and co-workers[6c] pointed out the difficulty of this transformation on scale-up.

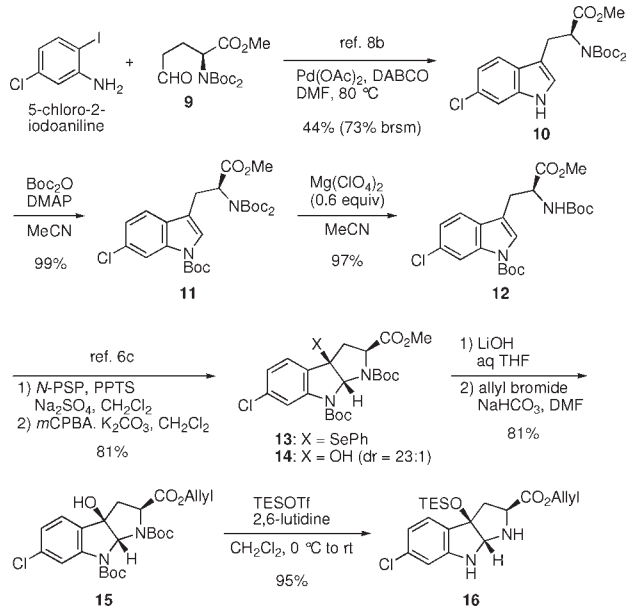
(11) Hernández, J. N.; Ramírez, M. A.; Martín, V. S. *J. Org. Chem.* **2003**, *68*, 743.

(12) (a) Marsden, S. P.; Depew, K. M.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1994**, *116*, 11143. (b) Ley, S. V.; Cleator, E.; Hewitt, P. R. *Org. Biomol. Chem.* **2003**, *1*, 3492. (c) Hewitt, P. R.; Cleator, E.; Ley, S. V. *Org. Biomol. Chem.* **2004**, *2*, 2415. (d) Crich, D.; Huang, X. *J. Org. Chem.* **1999**, *64*, 7218. (e) Crich, D.; Banerjee, A. *Acc. Chem. Res.* **2007**, *40*, 151.

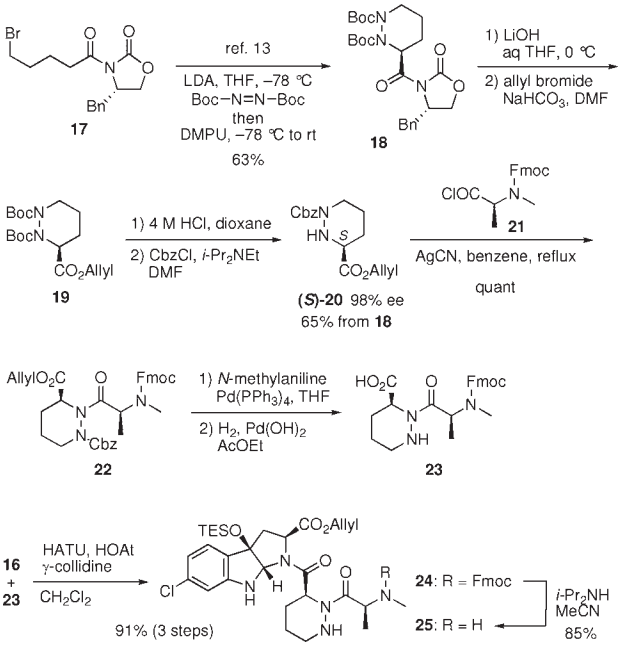
(13) Hale, K. J.; Cai, J.; Delisser, V.; Manaviazar, S.; Peak, S. A.; Bhatia, G. S.; Collins, T. C.; Jogiya, N. *Tetrahedron* **1996**, *52*, 1047.

(14) (a) Hale, K. J.; Cai, J.; Williams, G. *Synlett* **1998**, 149. (b) Hale, K. J.; Lazarides, L. *Org. Lett.* **1998**, *4*, 1903.

Scheme 2. Synthesis of Pyrroloindoline **16**



Scheme 3. Synthesis of Key Intermediate **25**

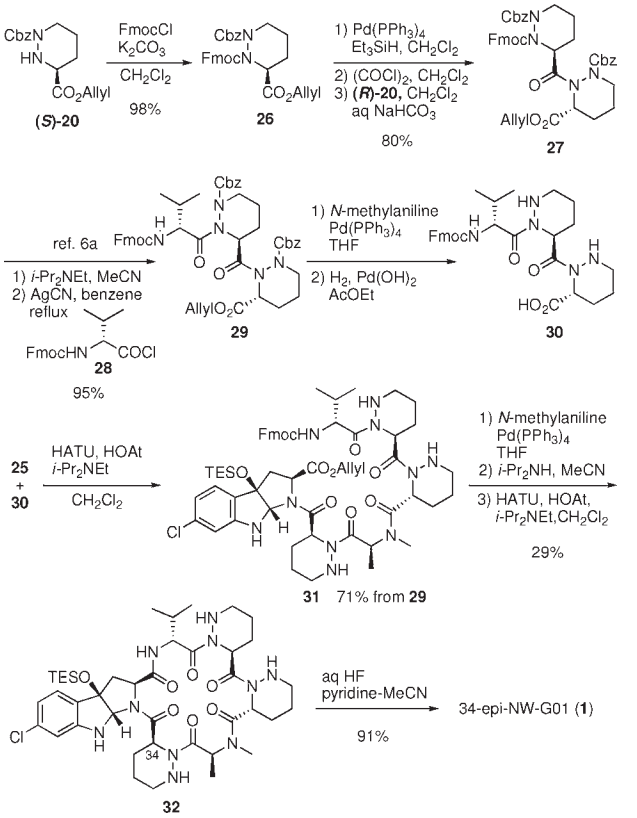


purity of (*S*)-**20** was determined to be 98% ee by chiral HPLC analysis. This compound was then reacted with acid chloride **21**, derived from commercially available *N*-methyl-Fmoc-L-alanine, in the presence of AgCN[14] to give **22** quantitatively. Upon successive removal of the allyl and Cbz groups, **22** furnished practically pure dipeptide **23**, which was used for the next reaction without purification.

The union of two fragments **16** and **23** was cleanly achieved under the conditions using HATU, HOAt, and γ-collidine to provide tripeptide **24** in 91% overall yield from **22**. Exposure of **24** to diisopropylamine afforded **25** corresponding to the envisaged key intermediate **4**. It is important to note that the unprotected free amine of **23** did not impede the condensation at all. In addition, the Cbz group of **22** should be removed prior to the coupling with **16**, because the chlorine atom in the pyrroloindoline moiety did not survive under hydrogenolytic deprotection conditions.

Another key intermediate **5** was prepared as **30** from (*S*)-**20** following the known procedure[6a] (Scheme 4). After attachment of an Fmoc group to (*S*)-**20**, palladium-catalyzed cleavage of the allyl group followed by chlorination yielded the acid chloride, which was condensed with (*R*)-**20**[15] under Schotten–Baumann conditions to give dipeptide **27** in 80% yield. After removal of the Fmoc group of **27**, the resulting amine was reacted with acid chloride **28**, derived from commercially available Fmoc-D-valine, in the presence of AgCN to afford tripeptide **29** in 95% yield. Palladium-catalyzed cleavage of the allyl ester group of **29** and subsequent hydrogenolytic removal of the Cbz groups furnished practically pure tripeptide **30**.

Scheme 4. Synthesis of 34-epi-NW-G01 (**1**)



With two key tripeptide fragments **25** and **30** in hand, we then addressed the synthesis of NW-G01. Coupling of **25**

(15) Prepared in 98% ee in the same manner as described for the preparation of (*S*)-**19**.

with **30** was achieved using HATU, HOAt, and Hünig's base to afford **31** in 71% yield from **29**. After the allyl and Fmoc groups of **31** were unveiled, the resulting amino acid was exposed to condensation conditions using HATU, HOAt, and Hünig's base to form cyclic hexapeptide **32** in moderate yield. Finally, desilylation of **32** produced the originally proposed NW-G01 (**1**). Unfortunately, however, the spectral data and specific rotation of the synthetic substance did not match with those of the natural product, although the X-ray crystallography[16] showed the structure to be identical to the originally proposed structure **1**. Right after the completion of our total synthesis of **1**, Wu and co-workers reported the revised structure **2** for NW-G01, which possesses the *R*-configuration at C34.

For the synthesis of NW-G01 (**2**), the required tripeptide **35** was prepared in 37% (59% brsm) overall yield starting from (*R*)-**20** via the attachment of *N*-methyl-L-alanine and pyrroloindole moieties (Scheme 5). Then, the coupling of two tripeptides **30** and **35** was achieved in the same manner as described in Scheme 4 to provide hexapeptide **36** in 85% yield. After successive removal of the allyl and Fmoc groups, the resulting amino acid was subjected to macrocyclization to afford cyclohexapeptide **37** in 25% overall yield. Finally, removal of the TES group led to the completion of the total synthesis of NW-G01 (**2**). The spectral data, melting point, and specific rotation exhibited good agreement with those of natural NW-G01.

In conclusion, we have accomplished the first total synthesis of NW-G01 (**2**) as well as 34-epi-NW-G01 (**1**) in 40 steps (20 steps in the longest linear sequence) in 5% overall yield from 5-bromovaleric acid.

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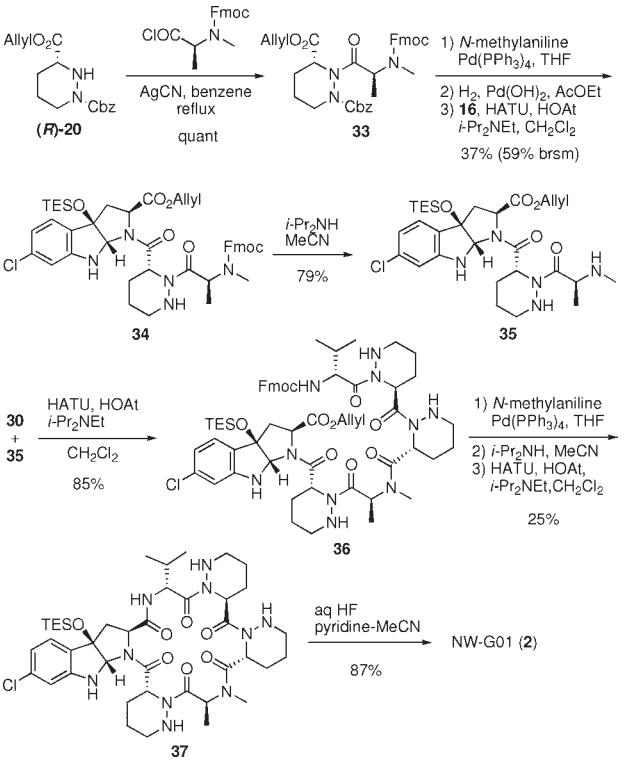
(16) The crystallographic data (CCDC 831625) can be obtained free of charge from the Cambridge Crystallographic Data centre via www.ccdc.cam.ac.uk/data_request/cif.

Scheme 5. Synthesis of NW-G01 (**2**)



Supporting Information Available. Experimental procedures and characterization data for all new compounds, and the X-ray crystallographic data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.