

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

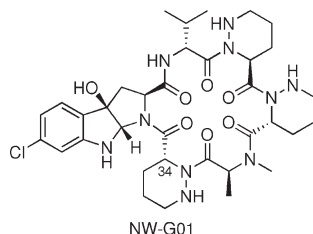
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ABSTRACT



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 $\mu\text{g mL}^{-1}$) against MRSA with that of ampicillin ($> 100 \mu\text{g mL}^{-1}$), 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**.

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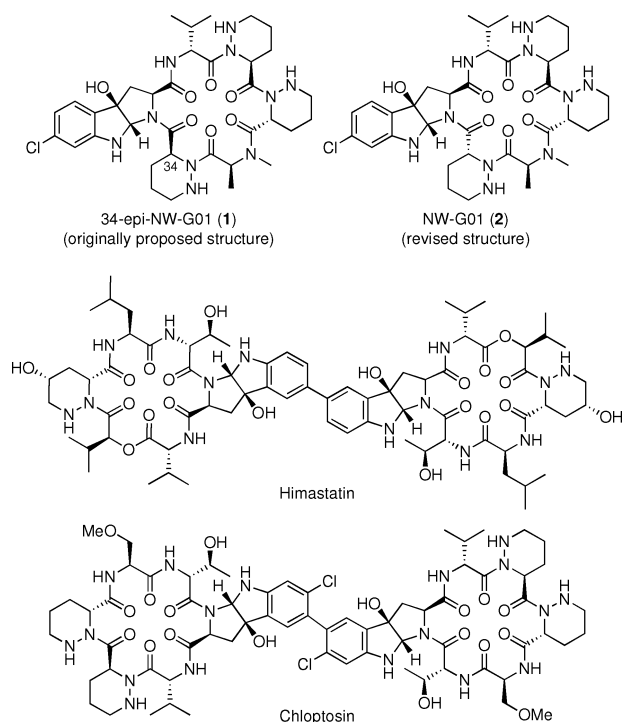
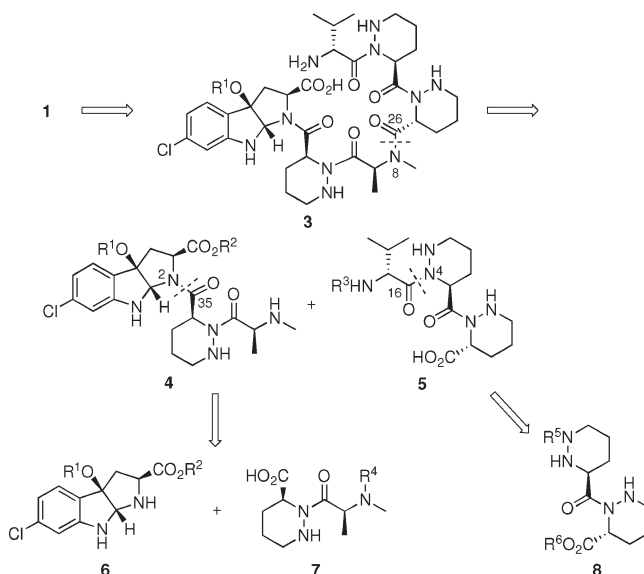


Figure 1. Related cyclohexapeptides with a pyrroloindoline.

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 studies⁷ 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 piperazid 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 synthesis⁹ (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 $\text{Mg}(\text{ClO}_4)_2$ 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** ($\text{R}^1 = \text{H}$) 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 piperazid acid synthesis¹³ (Scheme 3). Oxazolidinone **17**, prepared from 5-bromovaleric acid,¹³ was subjected to hydrazination/cyclization conditions to furnish piperazid 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 benzylloxycarbonylation in 65% overall yield. The enantiomeric

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

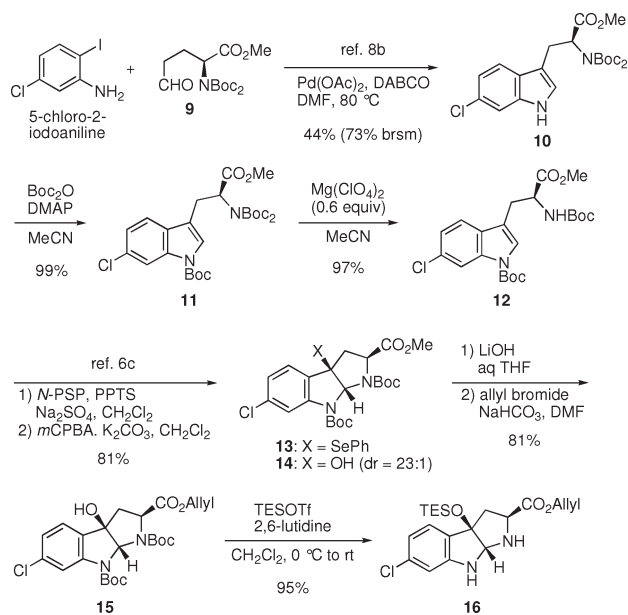
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Scheme 2. Synthesis of Pyrroloindoline 16

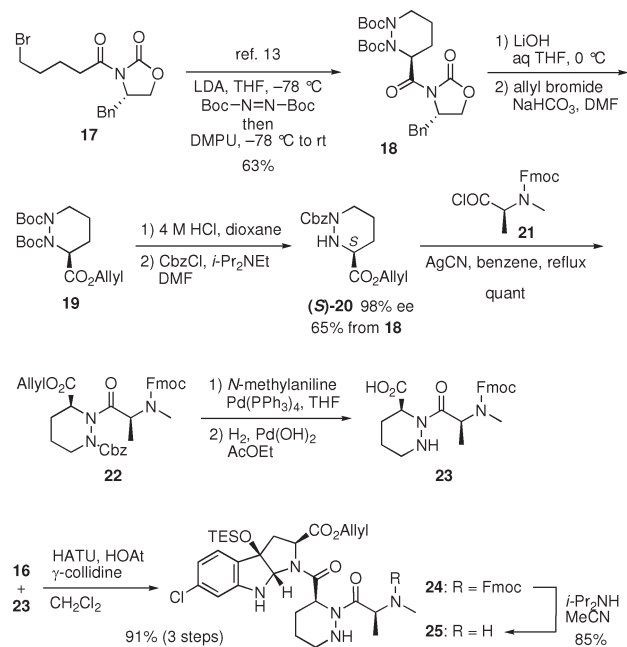


purity of (*S*)-**20** was determined to be 98% ee by chiral HPLC analysis. This compound was then reacted with acid chloride **21**, derived from commercially available *N*-methyl-Fmoc-L-alanine, in the presence of AgCN¹⁴ to give **22** quantitatively. Upon successive removal of the allyl and Cbz groups, **22** furnished practically pure dipeptide **23**, which was used for the next reaction without purification.

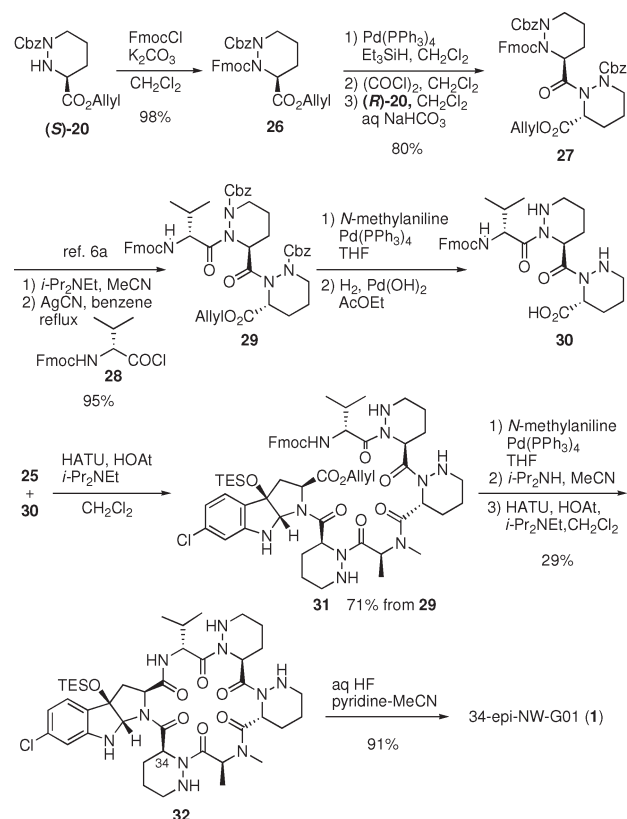
The union of two fragments **16** and **23** was cleanly achieved under the conditions using HATU, HOAt, and γ -collidine to provide tripeptide **24** in 91% overall yield from **22**. Exposure of **24** to diisopropylamine afforded **25** corresponding to the envisaged key intermediate **4**. It is 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 3. Synthesis of Key Intermediate 25



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



(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 crystallography¹⁶ 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.

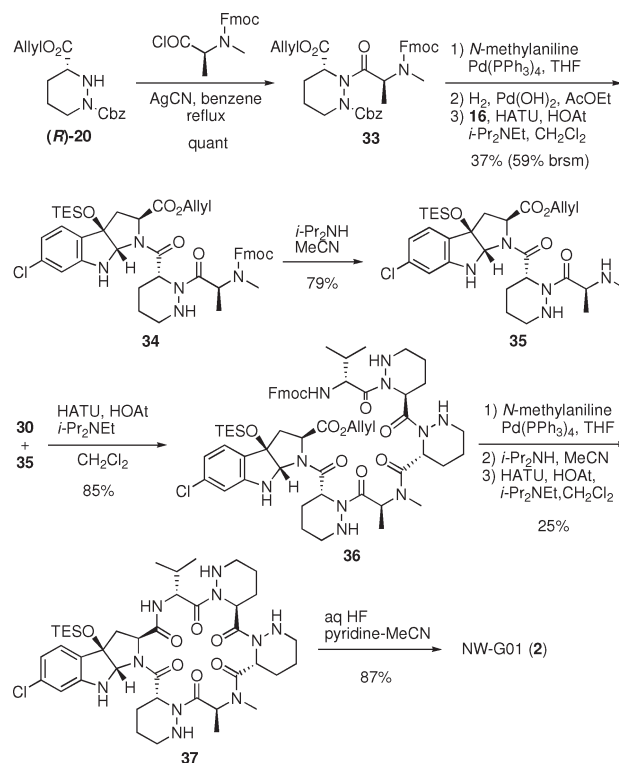
For the synthesis of NW-G01 (**2**), the required tripeptide **35** was prepared in 37% (59% brsm) overall yield starting from (*R*)-**20** via the attachment of *N*-methyl-L-alanine and pyrroloindole moieties (Scheme 5). Then, the coupling of two tripeptides **30** and **35** was achieved in the same 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**)



University) for providing us with natural NW-G01 and Associate Professor Hidehiro Uekusa (Department of Chemistry and Material Sciences Tokyo Institute of Technology) for X-ray crystallographic analysis of compound **1**.

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>.