# Atroposelective Total Synthesis of Cihunamide B

Longhui Yu, Hugh Nakamura*

**Abstract:** A short, scalable synthesis towards cihunamide B (**1**) is reported. The feature of this report is the decagram-scale $S_NAr$ reaction of *L*-tryptophan derivatives followed by atroposelective Larock macrocyclization. This strategy allowed the scalable construction of a Trp-Trp cross-linkage with unprecedented atropisomerism. It has also enabled the scalable synthesis of cihunamide B (**1**), which is expected to be a potential antibacterial agent.

Structures with atropisomerism are widely found in artificial catalysts, natural products, and pharmaceuticals, and in recent years, the development of selective synthetic methods for atropisomers has been reported.[1] Cihunamide B (**1**) is a cyclic peptide recently isolated from deep-sea marine sediments near Jeju Island, Korea, by Kim and Oh *et al*.[2] Structurally, it has an unprecedented structure with an atropisomeric Trp-Trp cross-linkage within a highly strained fused macrocyclic peptide consisting of four natural amino acid units (Figure 1). The atropisomeric configurations of the cihunamides were determined by electronic circular dichroism (ECD) spectra and DFT calculations.[2] In terms of biological activity, it shows antibacterial activity (MIC = 8-16 μg/mL) against Gram-positive pathogens (*Enterococcus faecalis* and *Staphylococcus aureus*), making it a potential antibiotic seed compound for drug discovery. However, since only 1.2 mg of cihunamide B (**1**) has been isolated from nature, the details of its mechanism involved in bioactivity study are still unclear.[2] As reported by the WHO in the last decade, antibiotic overuse has led to a serious spread of multidrug-resistant and super-multidrug-resistant bacteria worldwide, making further development of antibiotics essential.[3] The biosynthetic pathway is thought to be the oxidative formation of a C-N bond between two tryptophans catalyzed by CYP450.[2] The synthesis of the structurally and bioactively intriguing cihunamide B (**1**) has not yet been reported so far. On the basis of this background, an atroposelective and scalable synthesis of cihunamide B (**1**) has been developed in this report.

The synthetic approach towards cihunamide B (**1**) is shown in Figure 1. The development of a rapid and scalable synthesis of cihunamide B (**1**) is of paramount importance in this study. Thus, a robust synthesis strategy was employed that could withstand scalable synthesis. In particular, we planned to use inexpensive and simple building blocks, *L*-tryptophan derivative **2**, 1-bromo-3-fluoro-2-nitrobenzene **3**, and *L*-isoleucine derivative **4**, *L*-aspartic acid derivative **5** and alkyne fragment **6** as starting materials in a reliable reaction that proceeds under mild conditions. The unprecedented unique atrop-C-N bond was planned to be synthesized by introducing an aromatic ring through the $S_NAr$ reaction of **3** with the tryptophan derivative **2**, followed by an intramolecular Larock indole synthesis of the alkyne moiety of **6**.

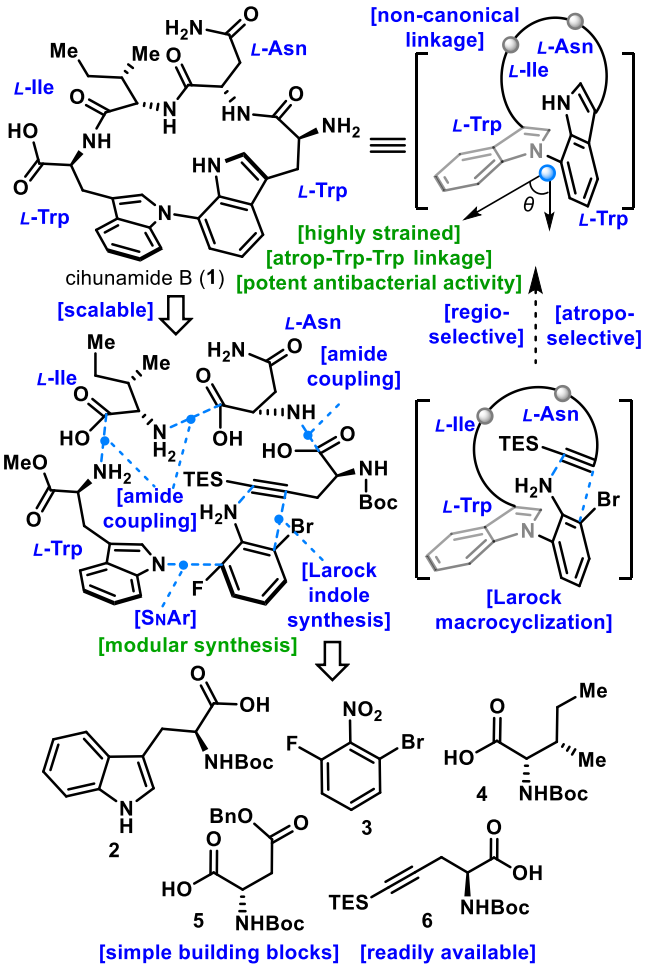


**Figure 1.** A synthetic approach to cihunamide B (**1**).

For the efficient and scalable synthesis of cihunamide B (**1**), this intramolecular Larock indole synthesis needs to proceed in an atroposelective manner. The atroposelectivity of the C-N bond was expected to be controlled by substrate control to achieve the desired atroposelectivity.

The synthesis of cihunamide B (**1**) begins with the $S_NAr$ reaction of the inexpensive 1-bromo-3-fluoro-2-nitrobenzene **3** and *N*-Boc-*L*-tryptophan **2**.[4] **2** and the commercially available 1-bromo-3-fluoro-2-nitrobenzene **3** were linked by the $S_NAr$ reaction by using NaOH in DMSO under room temperature to synthesize **7**, a fragment essential for the intramolecular Larock indole synthesis. It is worth noting that the $S_NAr$ reaction was carried out on a 40-gram scale and **7** was quantitatively obtained from **2** in a single step without any side reactions such as racemization. It was found that the use of free carboxylic acid is important to prevent racemization. The resulting **7** was then treated with HCl/MeOH to deprotect the Boc group and promote the methyl esterification simultaneously, followed by amide coupling with **4** using HATU to afford **8** in 88% yield. The resulting **8** was converted to **9** in 89% by deprotection of the Boc group by HCl and subsequent amide coupling with **5** by HATU. It is worth noting that the C-N bond between the tryptophan and the aromatic ring

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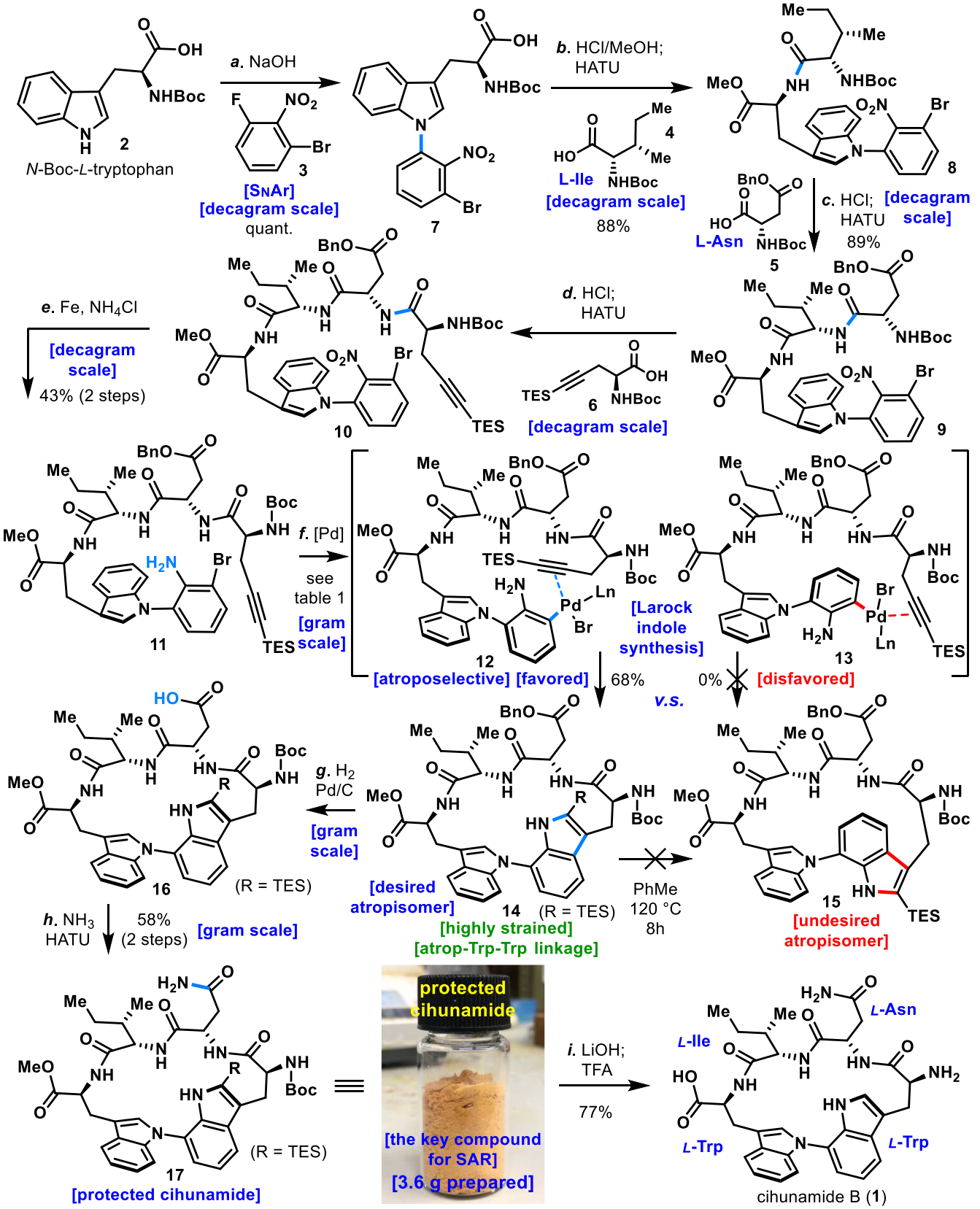


**Scheme 1.** Atroposelective total synthesis of cihunamide B (**1**)[a]

[a]Reagents and conditions: ***(a)*** **3** (1.42 eq.), NaOH, DMSO, quant. ***(b)*** $SOCl_2$, MeOH**, 4** (1.5 eq.), HATU (1.5 eq.), DIPEA (5.0), DMF, 88%; ***(c)*** $SOCl_2$, MeOH**, 5** (1.5 eq.), HATU (1.5 eq.), DIPEA (5.0), DMF, 89%; ***(d)*** 4M HCl, DCM**, 6** (1.06 eq.), HATU (1.2 eq.), DIPEA (4.0), DMF; ***(e)*** Fe, $NH_4Cl$, 50 °C, 43% (2 steps); ***(f)*** $Pd(OAc)_2$ (1.0 eq.), $tBu_3P{\cdot}HBF_4$ (2.0 eq.), DIPEA (10.0 eq.), toluene, 68%; ***(g)*** Pd/C, $H_2$; ***(h)*** $NH_4Cl$ (5.0 eq.), HATU (1.5 eq.) DIPEA (12.0 eq.), DMF; ***(i)*** LiOH (4.1 eq.), THF/$H_2O$/MeOH, then TFA, DCM, 77%; DMSO = Dimethyl sulfoxide; DMF = *N*,*N*-dimethylformamide; DIPEA = diisopropylethylamine.

**Table 1**: Optimization of the Larock macrocyclization of **11**.

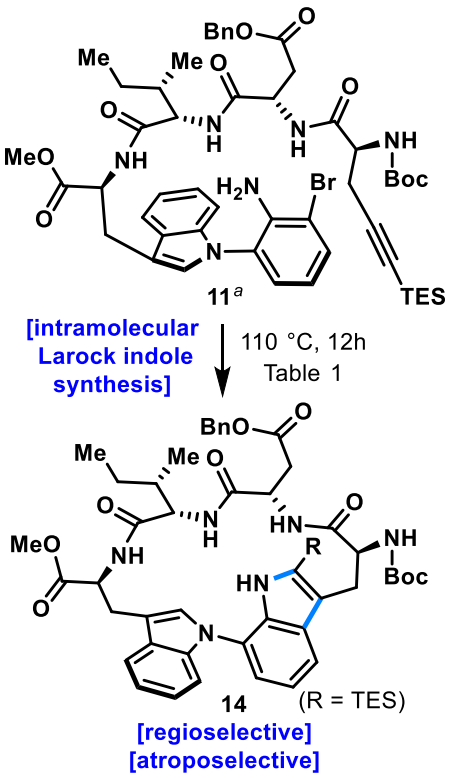


Table 1

| entry | [Pd] 0.4 eq. | [Ligand] 0.8 eq. | [base] 10 eq. | [solvent][b] 1 ml | yield[c] |
|---|---|---|---|---|---|
| 1 | $Pd(OAc)_2$ | $PPh_3$ | DIPEA | PhMe | trace |
| 2 | $Pd(OAc)_2$ | $PPh_2Me$ | DIPEA | PhMe | 18% |
| 3 | $Pd(OAc)_2$ | $P(Cy)_3$ | DIPEA | PhMe | 20% |
| 4 | $Pd(OAc)_2$ | dppe (0.4) | DIPEA | PhMe | 10% |
| 5 | $Pd(OAc)_2$ | dppf (0.4) | DIPEA | PhMe | 7% |
| 6 | $Pd(t\mathrm{Bu_3P})_2$ | — | DIPEA | PhMe | 38% |
| 7 | $Pd(OAc)_2$ | $t\mathrm{Bu_3P{\cdot}HBF_4}$ | DIPEA | PhMe | 51% |
| 8[d] | $Pd(OAc)_2$ | $t\mathrm{Bu_3P{\cdot}HBF_4}$ | DIPEA | PhMe | 68% |
| 9 | $Pd(OAc)_2$ | $t\mathrm{Bu_3P{\cdot}HBF_4}$ | $Et_3N$ | PhMe | 26% |
| 10 | $Pd(OAc)_2$ | $t\mathrm{Bu_3P{\cdot}HBF_4}$ | DBU | PhMe | decomp. |
| 11 | $Pd(OAc)_2$ | $t\mathrm{Bu_3P{\cdot}HBF_4}$ | $Na_2CO_3$ | PhMe | trace |
| 12 | $Pd(OAc)_2$ | $t\mathrm{Bu_3P{\cdot}HBF_4}$ | $K_2CO_3$ | PhMe | trace |
| 13 | $Pd(OAc)_2$ | $t\mathrm{Bu_3P{\cdot}HBF_4}$ | DIPEA | MeCN | trace |
| 14 | $Pd(OAc)_2$ | $t\mathrm{Bu_3P{\cdot}HBF_4}$ | DIPEA | PhMe/DMF = 1/1 | 14% |
| 15 | $PdCl_2$ | $t\mathrm{Bu_3P{\cdot}HBF_4}$ | DIPEA | PhMe | 35% |

[a] All reactions were performed on 25 mg scale. [b] Dry solvents were used. [c] Isolated yields. [d] The reaction was performed on 2.12 g scale. 1 eq. $Pd(OAc)_2$, 2 eq. $t\mathrm{Bu_3P{\cdot}HBF_4}$ and 80 ml of PhMe were used for this scale.

in **9** was stable and no decomposition or other side reactions occurred from **7** over several steps, including strongly acidic conditions.

Next, we attempted to attach the alkyne fragment **6** required for intramolecular Larock indole synthesis.[5] In Larock indole synthesis, the TMS group is usually used as an alkyne fragment.[5] However, the TMS group was sometimes removed during the reaction in Larock indole synthesis, which leads to a decrease in regioselectivity. Therefore, we prepared an alkyne fragment **6**, which has a TES group that is robust and bulkier than the TMS group. To begin with the Larock macrocyclization, **9** was easily deprotected by HCl, and the resulting amine was coupled with the prepared **6**[7a] by HATU to synthesize **10**. The synthesis of Trp-Trp cross-links with atropisomerism has not been reported so far. Therefore, the development of scalable macrocyclization is an important issue in this study. The cyclization precursor **11**, necessary for the investigation of the intramolecular Larock indole synthesis, was obtained from **10** in 43% yield (2 steps) by selective reduction of the nitro group with Fe dust and $NH_4Cl$.

With the key intermidiate **11** in hand, the construction of a unique Trp-Trp linkage by Larock macrocyclization was investigated (Table 1). Initially, a variety of ligands were investigated using 0.4 eq. $Pd(OAc)_2$, toluene as solvent, and 10 eq. DIPEA as base. Interestingly, it was found that the use of 0.8 eq. $PPh_3$ as a monodentate ligand under heating at 110 °C for 12 h yielded the highly strained cyclic peptide **14** with atrop-Trp-Trp bond, albeit in trace amounts. (Table 1, entry 1). Next, Larock macrocyclization was attempted using 0.8 eq. $PPh_2Me$, and the desired cyclized compound **14** was successfully obtained as a single compound in 18% (entry 2). It is worth noting that the Larock macrocyclization of **11** proceeds with perfect atroposelectivity and regioselectivity. Furthermore, when $PCy_3$, which is more electron-rich than $PPh_3$, was used as a ligand, a slight yield improvement was observed and the Larock macrocyclization proceeded with a 20% isolated yield (entry 3). Bidentate ligands such as dppe and dppf were not effective for this macrocyclization (entries 4 and 5). On the other hand, Larock macrocyclization of indole skeleton into cyclic peptides and synthesis of complex alkaloids was reported by Reisman, Boger, Baran, and Sarlah group in their pioneering work.[6,7] Based on these background studies, we performed this Larock macrocyclization using 0.4 eq. $Pd(t\mathrm{Bu_3P})_2$. It was found that the yield was improved and the desired cyclized product **14** was obtained in an isolated yield of 38% (entry 6). This result indicates that bulky and electron-rich ligands are effective for this reaction. Further investigation of the reaction to improve the yield revealed that the use of 0.4 eq. $Pd(OAc)_2$ and 0.8 eq. $t\mathrm{Bu_3P{\cdot}HBF_4}$ was crucial for this reaction, and the atrop-Trp-Trp linkage was successfully constructed in 51% isolated yield (entry 7). The reaction was also found to proceed well at the gram scale, and compound **14** was well obtained at a larger scale (68%, entry 8). The reaction was then attempted using a different base. However, the yield of $Et_3N$ dropped to 26%, indicating that a bulky base is essential for this Larock macrocyclization (entry 9). Inorganic bases such as $Na_2CO_3$ and $K_2CO_3$ showed little progress in this macrocyclization due to solubility problems (entries 11 and 12). On the other hand, the effect of the solvent on the reaction was investigated, and the reaction hardly progressed with MeCN, and the isolated yield was 14% with PhMe/DMF = 1/1 (entries 13 and 14). In the case of $PdCl_2$, the macrocyclization proceeded, but the isolated yield was 35% (entry 15). From these results, it is concluded that the most effective condition for the Larock macrocyclization is the use of $Pd(OAc)_2$ and $t\mathrm{Bu_3P{\cdot}HBF_4}$ at 110 °C in PhMe in the presence of DIPEA (entries 7 and 8).

The synthesis of the highly strained atrop-Trp-Trp linkage by macrocyclization of **11** is noteworthy in that the cyclization **14** is obtained with perfect atroposelectivity. The atroposelectivity of this reaction is addressed (Scheme 1). First, in the Larock

macrocyclization of the cyclization precursor **11**, Pd undergoes an oxidative addition to Ar-Br bond. Then, the **12** formed in the reaction system is subjected to a regioselective migratory insertion onto the neighboring triple bond, avoiding the TES group, to obtain **14** with the desired atrop-Trp-Trp linkage. On the other hand, **15** with undesired atrop-Trp-Trp linkage is not generated at all via **13**. This is probably due to the energetic disadvantage of the process via **13** in the transition state of the reaction. To confirm the stability of the atrop-C-N bond of **14**, **14** was heated in PhMe at 110 ºC for 8 h and 1H NMR experiment was performed. However, **15** was not observed and only **14** was recovered. The atrop-C-N bond of **14** was found to be thermodynamically stable.[2] To complete the total synthesis of cihunamide B (**1**), only the conversion of benzyl ester **14** to the primary amide and the removal of the protecting groups remain. **14** obtained by Larock macrocyclization was derivatized to carboxylic acid **16** by hydrogenation in the presence of Pd/C. Subsequently, the amidation of **16** with $NH_3$ was successfully carried out by HATU to afford 3.62 g of protected cihunamide **17**. Finally, the protected cihunamide **17** was subjected to hydrolysis of the Me-ester, and the total synthesis of cihunamide B (**1**) was completed by deprotection of the Boc and TES groups with TFA.

In conclusion, we have developed the first total synthesis of antibacterial cihunamide B (**1**). This work features (1) the formation of a C-N bond between *N*-Boc-*L*-tryptophan **2** and 1-bromo-3-fluoro-2-nitrobenzene **3** on a decagram scale utilizing inexpensive reagents through the $S_NAr$ reaction, and (2) the synthesis of a highly strained cyclic peptide by atroposelective Larock macrocyclization, resulting in the successful production of protected cihunamide **17** on a 3.6 g scale. Additionally, this synthetic pathway obviates the need for specialized apparatus or reagents, enabling the large-scale synthesis of protected cihunamide **17** using economical starting materials and readily accessible reagents. This development is anticipated to have far-reaching implications in biological research and related disciplines. Moreover, the utilization of protected cihunamide **17** allows for the possibility of conducting structure-activity relationship (SAR) studies.

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## Conflict of Interest

The authors declare no competing financial interest.

## Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

**Keywords:** total synthesis • macrocyclization • atropisomerism • atroposelective • Larock indole synthesis

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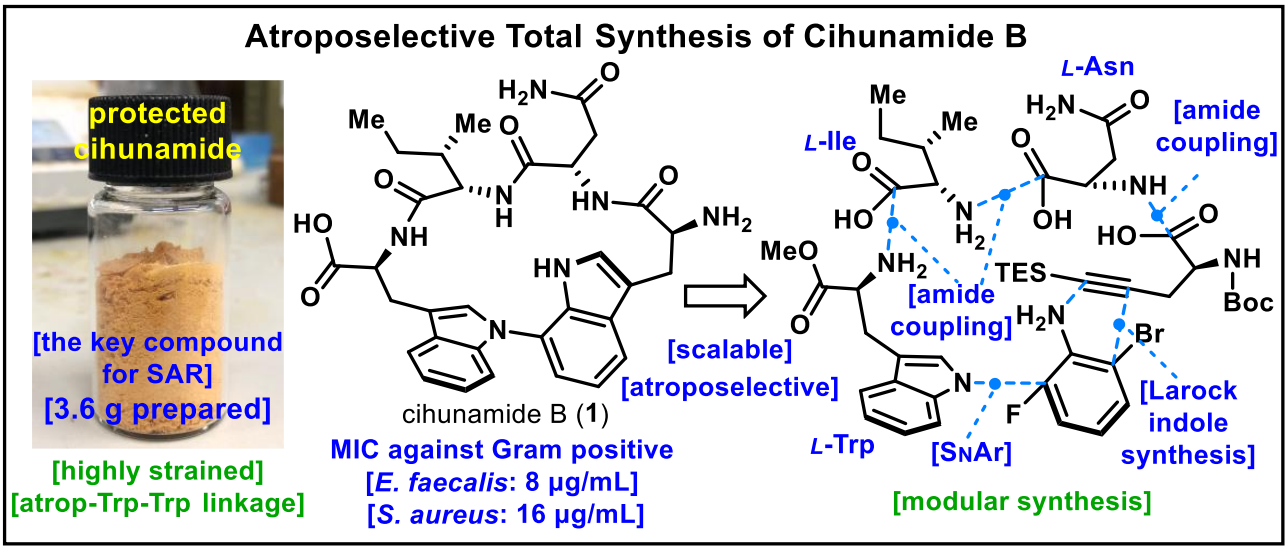


A short, scalable access to cihunamide B is reported herein. The highlights of this work include the C-N bond formation reaction through the $S_NAr$ reaction in decagram scale and the atroposelective Larock macrocyclization to construct the unprecedented highly strained atrop-Trp-Trp linkage.

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