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Data availability

The data supporting this article have been included as part of the Supplementary Information. Crystallographic data for *Z*-**8** and the derivative of **14** have been deposited at the CCDC as CCCDC2343575 and CCDC2343576, respectively, and can be obtained from https://www.ccdc.cam.ac.uk.

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The total synthesis of 1,4a-di-*epi*-*ent*-pancratistatin, a novel stereoisomer of the anti-tumor *Amaryllidaceae* alkaloid pancratistatin, was achieved in 14 steps starting from D-mannitol. The construction of the pancratistatin skeleton involved conjugate addition of organocuprate to a nitrosoolefin, which was generated in situ from inososе oxime. This was followed by stereoselective reduction of the oxime to an amine and site-selective formylation. Biological evaluations revealed that the newly synthesized compounds exhibit cytotoxicity toward cancer cells and significant ferroptosis inhibitory activity. These compounds constitute a promising small-molecule library for the development of potent bioactive agents.

(+)-Pancratistatin (**1**), an *Amaryllidaceae* alkaloid characterized by a highly oxygenated hexahydrophenanthridinone core with six contiguous chiral centers on the cyclohexane ring, was first isolated by Pettit and co-workers from the bulbs of the Hawaiian plant *Pancratium littorale* (Figure 1).[1] Pancratistatin has been shown to inhibit the growth of cancer cells both in vitro and in vivo[2] and to possess antiviral properties.[3] These bioactivities, coupled with its challenging structural features, have garnered considerable interest from biologists and synthetic chemists alike. Numerous total syntheses of pancratistatin have been reported,[4] including the first racemic synthesis by the Danishefsky group[4a] and the first asymmetric synthesis by Hudlicky.[4b] More recently, Liu achieved a gram-scale total synthesis featuring a stereoselective Michael addition to a nitro olefin, followed by a Henry reaction.[4r]

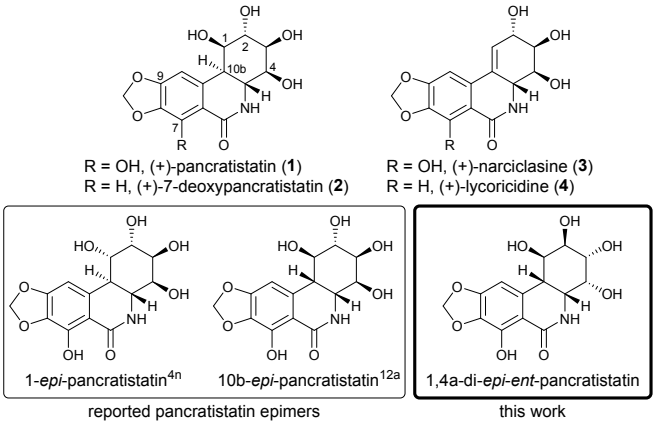

Fig. 1 Structures of Pancratistatin and Related Alkaloids.

Related alkaloids, such as (+)-7-deoxypancratistatin (**2**),[1] (+)-narciclasine (**3**),[5] and (+)-lycoricidine (**4**),[6] have also demonstrated a broad spectrum of bioactivities, sustaining the interest in these compounds. To explore the structure–activity relationship, numerous unnatural analogs have been synthesized.[7] Among these, various modifications to pancratistatin's structure have been assessed. An aromatic-ring modified analog, (±)-7,9-dideoxypancratistatin, synthesized by Alons, showed that the removal of the C-9 oxygen functionality resulted in a significant decrease in bioactivity compared to compound **2**.[8] Lactone analogs created by Chapleur displayed negligible biological activity, underscoring the importance of the lactam ring.[9] Modifications to the cyclohexane ring have been extensively studied, with a multitude of analogs, including multideoxy[10] and C1-functionalized variants,[2h,11] being reported. Notably, C1-aza derivatives produced by Marion[11a] and C-1 benzoyl esters synthesized by Pettit[11b] and Hudlicky[2h] exhibited markedly enhanced bioactivity compared to the parent compound **1**, attributed to improved solubility. However, for cyclohexane-ring epimers, only 1- and 10b-epimers have been synthesized (Figure 1).[4n,12a] Including 7-deoxy derivatives, additional five

[a] *Graduate School of Pharmaceutical Sciences, Tokushima University, Shomachi, Tokushima 770-8505, Japan.*
[b] *Research Cluster on "Key Material Development", Tokushima University, Shomachi, Tokushima 770-8505, Japan.*
[c] *Faculty of Pharmacy, Yasuda Women's University, Asaminami-ku, Hiroshima 731-0153, Japan.*
[d] *School of Pharmacy, Hyogo Medical University, Chuo-ku, Kobe, Hyogo 650-8530, Japan*

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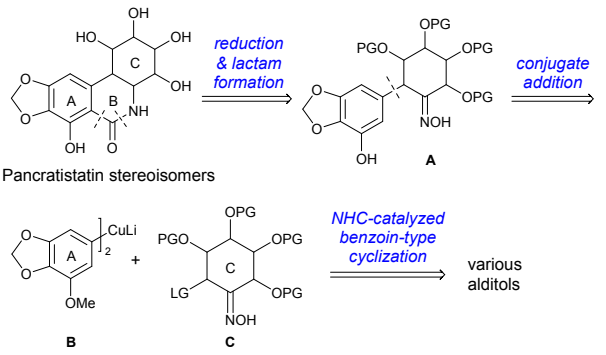
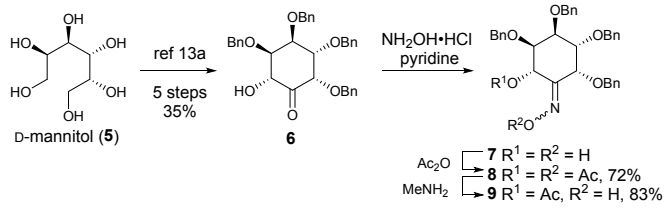
diastereomers—1,10b-di-*epi*-, 2-*epi*-, 2,4-di-*epi*-, 10b-*epi*-, and *ent*-isomers—have been reported,[12b–f] yet the variation remains limited, with only anticancer activity being evaluated.

We have previously reported the synthesis of inositol derivatives utilizing N-heterocyclic carbene (NHC)-catalyzed intramolecular benzoin cyclization. This methodology affords straightforward access to a variety of inositols and their derivatives, including *allo*-, *chiro*-, *epi*-, *muco*-, *myo*-, *neo*-, and *scyllo*-inositols.[13] Aiming to contribute to structure–activity relationship studies and to develop a novel bioactive-compound-like molecular library, we devised a concise and stereodivergent synthetic strategy for pancratistatin stereoisomers. The retrosynthetic analysis, illustrated in Scheme 1, posits that pancratistatin stereoisomers could be derived from oxime **A** through reduction followed by lactam formation.[4q,r,14,15] Oxime **A** would be accessible via conjugate addition of organocuprate **B** to a nitrosoolefin, generated in situ from oxime **C** which contains a chlorine atom as a leaving group at the α-position (LG = Cl).[16] We anticipated that various stereoisomers of oxime **C** could be selectively synthesized from commercially available alditols through NHC-catalyzed benzoin cyclization.[13]



Scheme 1 Retrosynthetic Analysis of Pancratistatin Stereoisomers.

Our synthetic approach began with chiral inosose **6**, readily prepared on a large scale from D-mannitol (**5**) using our previously reported method.[13a] Although initial attempts at α-chlorination of **6** or its corresponding oxime **7** were unsuccessful, we successfully converted **6** into α-acetoxy oxime **9** (*E*/*Z* 1:1) bearing an acetoxy group as an alternative leaving group through oxime formation followed by diacetylation and subsequent monodeacetylation,[17] as depicted in Scheme 2.

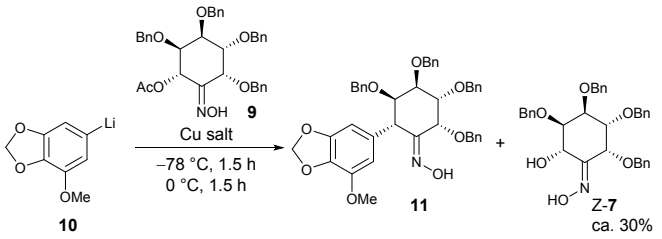


Scheme 2 Synthesis of C-Ring Fragment **9**.

With the C-ring fragment **9** secured, we proceeded to the arylation reaction with the A-ring unit **10** (Table 1). Following established protocols,[16] the reaction was initially performed in THF using CuCN as the copper(I) salt. To our delight, acetate functioned as a good leaving group in this reaction instead of chloride, yielding the arylated product **11** with a 29% yield and excellent diastereoselectivity (dr >50:1, entry 1). The influence of ligands was then investigated. The addition of tetramethylethylenediamine (TMEDA) as a ligand resulted in an increased yield of 37% (entry 2), while employing dimethoxyethane (DME) further enhanced the yield to 45% (entry 3). Attempts with other solvents such as diethyl ether and toluene, as well as copper salts like CuTC and CuOAc, did not lead to yield improvements (entries 4–7).

Notably, approximately 30% of compound **9** was consistently recovered as the Z-isomer of the deacetylated oxime (*Z*-**7**)[18] in these reactions, suggesting a significantly lower reactivity of *Z*-**9** compared to *E*-**9**. As anticipated, the reaction using *Z*-**9**, derived from the recovered *Z*-**7**, only yielded product **11** at 9% yield, with *Z*-**9** and deacetylated oxime *Z*-**7** being recovered in a combined yield of 90% (Table 1, entry 8). Fortunately, *Z*-**7** could be thermally isomerized to a 1:1 mixture of *E*/*Z* mixture of **7** (in toluene at 90 °C for 9 h) and reused. Thus, under optimal conditions (Table 1, entry 3), the yield of **11** based on the recovered starting material was 64%.

Table 1 Arylation of α-Acetoxy Oxime **9** with A-Ring Unit **10**[a]



Entry	solvent	Cu salt	ligand	yield of **11**[b]
1	THF	CuCN	none	29%
2	THF	CuCN	TMEDA	37%
3	THF	CuCN	DME	45% (64%)[c]
4	Et_2O	CuCN	DME	22%
5	toluene	CuCN	DME	24%
6	THF	CuTC	DME	28%
7	THF	CuOAc	DME	13%
8[d]	THF	CuCN	DME	9%

[a] Reactions were performed using **9** (0.40 mmol), Cu salt (1.20 mmol), and **10** (2.40 mmol) in the indicated solvent (0.04 M) for 1.5 h at −78 °C and then 1.5 h at 0 °C with or without a ligand (2.40 mmol). [b] Isolated yields. [c] The yield in parentheses is based on the recovered starting material. [d] *Z*-**9** was used instead of a 1:1 *E*/*Z* mixture.

The observed diastereoselectivity is explainable based on axial attack preference in addition reactions to cyclohexene derivatives (Scheme 3). To avoid steric repulsion between the approaching organocuprate and the pseudoaxial 4-benzyloxy group in an axial attack to half-chair conformer **A**, conjugate addition proceeded with conformation **B** to provide **11**.[16]

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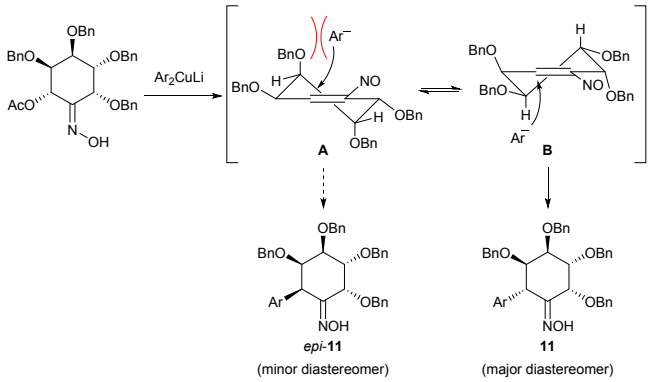
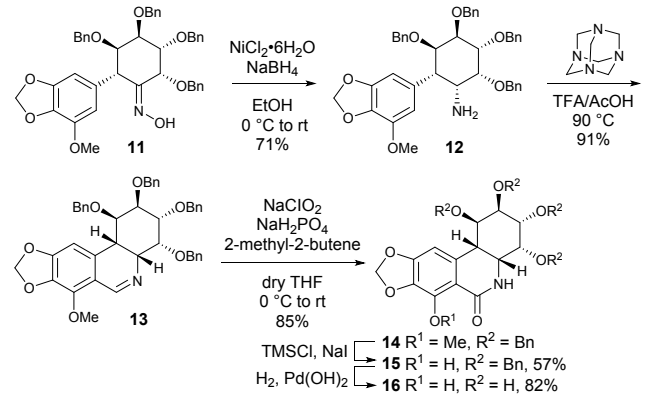


Scheme 3 Rationalization of the diastereoselectivity in the arylation.

The stereoselective reduction of the oxime C=N bond was achieved using $NaBH_4/NiCl_2{\cdot}6H_2O$ in ethanol, yielding *cis*-amine **12** as the sole diastereomer (Scheme 4). Other reduction conditions were tested, including $H_2/Pd(OH)_2$, SmI_2, Zn/AcOH, $BH_3{\cdot}THF$, *t*-$BuNH_2{\cdot}BH_3$,[13] $Me_4N(AcO)_3BH$,[13b,19] and MeI followed by $LiAlH_4$/NaOMe,[13a,20] but these attempts failed to produce the *trans*-isomer.

Subsequently, our focus shifted to the construction of the lactam ring. Previous reports indicated that Bischler–Napieralski-type cyclization for the pancratistatine-like systems often encountered regioselectivity challenges.[4f,l,m,14] However, Duff formylation[4q,21] of compound **12** resulted in the regioselective formation of hexahydrophenanthridine **13** as the sole product. Subsequent oxidation[15] yielded compound **14** with a 77% overall yield from **12**. The regioselectivity and absolute configuration of these products were confirmed via X-ray crystallography. The final step involved the deprotection of **14** using TMSI, followed by hydrogenolysis using $H_2/Pd(OH)_2$ to obtain 1,4a-di-*epi*-*ent*-pancratistatin (**16**).



Scheme 4 Total Synthesis of 1,4a-Di-*epi*-*ent*-pancratistatin (**16**)

The biological activities of **16** and intermediates **12–15** were assessed. Initially, their cytotoxicity against human hepatoma Hep3B cells was evaluated at a concentration of 10 μM (Figure 2). Compounds **14–16** exhibited no cytotoxicity at this concentration, with cell viability comparable to untreated controls. In contrast, azacyclitol **12** and hydrophenanthridine **13** demonstrated strong and moderate cytotoxicity, respectively, resulting in approximately 95% and 60% cell mortality.

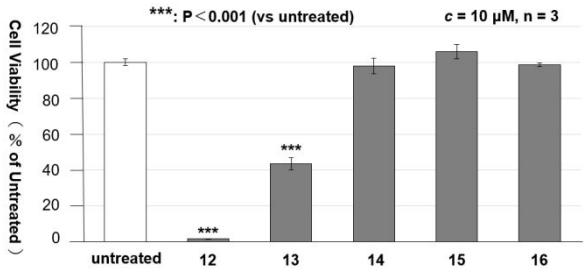


Fig. 2 Cytotoxicity of **12–16** toward Hepatoma Hep3B Cells at 10 μM.

Furthermore, the non-cytotoxic compounds **14–16** were subjected to ferroptosis inhibitory activity assays on Hep3B cells at 10 μM using erastin as the ferroptosis inducer.[22] Ferroptosis, a type of regulated cell death dependent on iron and lipotoxicity,[23] has been implicated in various neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and Huntington's disease,[24] and inhibitors of this pathway are considered potential therapeutic agents (Figure 3). Our findings revealed that while treatment with **16** had no impact on cell viability, compound **14** inhibited ferroptosis, improving cell viability to 70% in the presence of erastin. Remarkably, compound **15** exhibited potent ferroptosis inhibitory activity, comparable to ferrostatin-1 (Fer-1), a well- known ferroptosis inhibitor[22] used as the positive control, with approximately 90% of cells surviving erastin treatment.

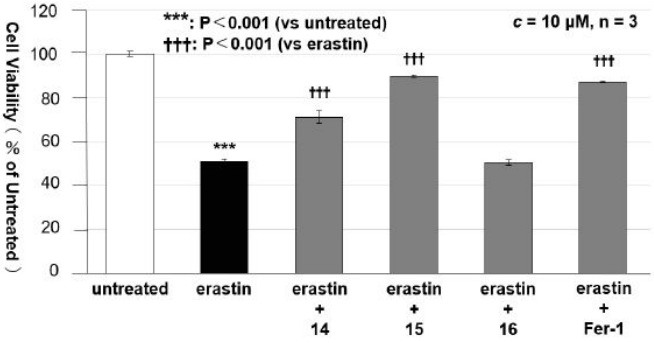


Fig. 3 Antiferroptosis Activity of **14–16** and Ferrostatin-1 (Fer-1) on Erastin-treated Hepatoma Hep3B Cells at 10 μM.

In summary, we performed efficient total synthesis of a pancratistatin stereoisomer via stereoselective inosose synthesis and a coupling reaction between organocuprate and an inosose-derived α-acetoxy oxime. We demonstrated for the first time that acetate can be utilized as a leaving group in this reaction instead of chloride. This approach exemplified our stereodivergent strategy toward pancratistatin isomers and led to the discovery of new compounds with either cytotoxic or antiferroptosis activity among the synthetic intermediates, which clearly indicates the importance of chemical synthesis of a novel stereoisomer of natural products even when its bioactivity is unknown. Therefore, pancratistatin stereoisomers and their intermediates hold promise as potential drug-seed candidates. Given the availability of various inosose stereoisomers from commercially accessible

alditols, our strategy offers a means to generate pancratistatin derivatives with diverse absolute configurations, thereby enriching the molecular library for biological testing and facilitating structure–activity relationship studies. Further applications of this synthetic approach to other natural products and non-natural derivatives are currently underway in our laboratory.

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Conflicts of interest

There are no conflicts to declare.

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