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ORIGINAL ARTICLE

Solid-phase total synthesis of the chitinase inhibitor Argadin using a supported acetal resin

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A versatile solid-phase total synthesis was applied to the rapid preparation of Argadin, a natural product isolated and characterized as a cyclopentapeptide by our group, which possesses superior inhibitory activity against family-18 chitinases. The synthetic strategy includes peptide synthesis by using an Fmoc (9-fluorenylmethoxycarbonyl) protective group, macrolactamization, acetylguanylation and formation of hemiaminal accompanied by total deprotection, including cleavage from resin.

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INTRODUCTION

Chitinases hydrolyze chitin, a linear homopolymer of *N*-acetyl-D-glucosamine, that is present in a wide range of organisms, including bacteria, fungi, insects, viruses, higher plants and animals, and have a variety of roles in the biological world.[1–3] Chitinases are currently classified into two different families of glycosyl hydrolases, namely family-18 and family-19, on the basis of amino acid sequence similarities.[4] Family-18 contains chitinases from various organisms, whereas family-19 chitinases are only found in plants and *Streptomyces* species. As chitin is a major component of fungal cell walls, the exoskeletons of crustaceans and insects, as well as the microfilarial sheaths of parasitic nematodes, family-18 chitinases have important physiological roles in these organisms.[1–3] Family-18 chitinase inhibitors have thus become a subject of interest owing to their potential as anti-fungal agents,[5] pesticides for controlling pests[6] and promise as insecticides to control disease vectors, such as malaria.[7] They also offer significant potential for the treatment of asthma and other diseases in humans.[8]

During the screening for chitinase inhibitors, two new cyclic pentapeptides, Argadin (**1**)[9] and Argifin (**2**),[10–12] were isolated from the cultured broths of *Clonostachys* sp. FO-7314 and *Gliocladium* sp. FTD-0668, respectively, by our research group, and found to be potent chitinase inhibitors of blowfly (*Lucilia cuprina*)[10] and *Serratia marcescens* chitinases (*Sm*Chi),[13–16] both of which are family-18 chitinases (Figure 1). Developments of practical and efficient total syntheses of **1** and **2** are important objectives, as the original sources do not produce these cyclic peptides with sufficient quantities. In fact, the total syntheses of **1** and **2**, involving hybrid approaches of solid- and liquid-phase reaction sequences, have been reported by Dixon *et al.*[17,18] They developed an improved synthetic route for **2** and analogs on the basis of an all-solid-phase approach.[19] More recently, we have independently reported the total synthesis of **2** accomplished by solid-phase synthetic protocols for the entire reaction sequence, except for the final cleavage step from resin. We have also evaluated acyclic peptide analogs of **2**[20] and have investigated the application of *in situ* click chemistry with *Sm*Chi to swiftly generate more potent chitinase inhibitors using the simplified structure of natural **2**.[21]

In this study, we describe our efficient solid-phase total synthesis of **1** using glycerol polystyrene resin along with only one-time HPLC separation at the final step.

RESULTS AND DISCUSSION

Our retrosynthetic analysis for Argadin (**1**) is outlined in Scheme 1. The cyclic peptide structure of **1** allowed us to adopt a solid-phase synthetic strategy on the basis of an application of an 9-fluorenylmethoxycarbonyl (Fmoc) protective group for the amine of amino acid fragments with allyl protection on the carboxylic acid of the L-aspartic-β-semialdehyde[22] unit. This strategy enables cyclization of the linear precursor that is still attached to a solid support by the side chain of an L-aspartic-β-semialdehyde residue. Our synthetic strategy of **1** offers many advantages, including: (i) the anchorage of the first residue (C terminal) to resin through the side-chain aldehyde function; (ii) the selected glycerol polystyrene resin acts as a protective group of aldehyde for preventing the formation of sensitive cyclic

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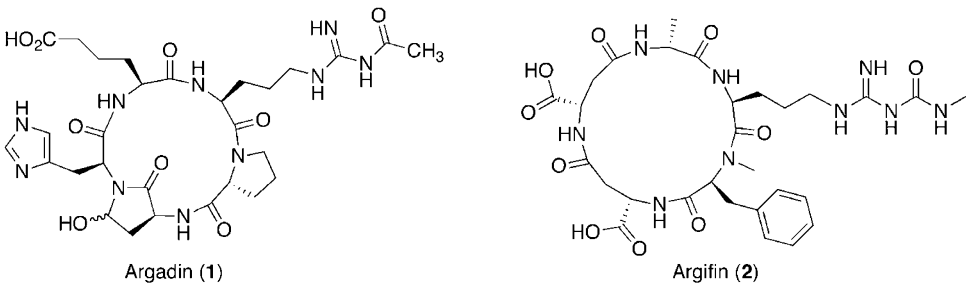
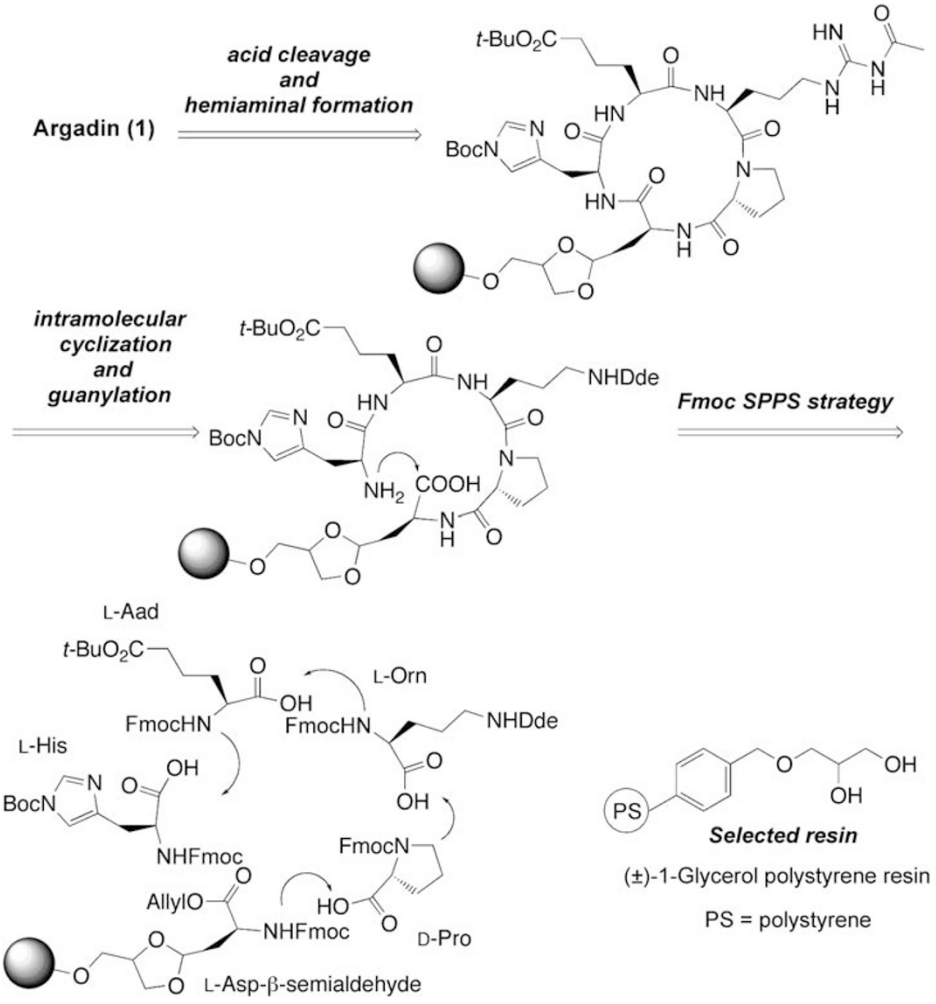


	IC_{50} (μM)		
	*Sm*ChiA	*Sm*ChiB	*Sm*ChiC$_1$
Argadin (**1**)	0.016 ± 0.0040	0.033 ± 0.0028	>30
Argifin (**2**)	0.025 ± 0.0024	6.4 ± 1.0	>30

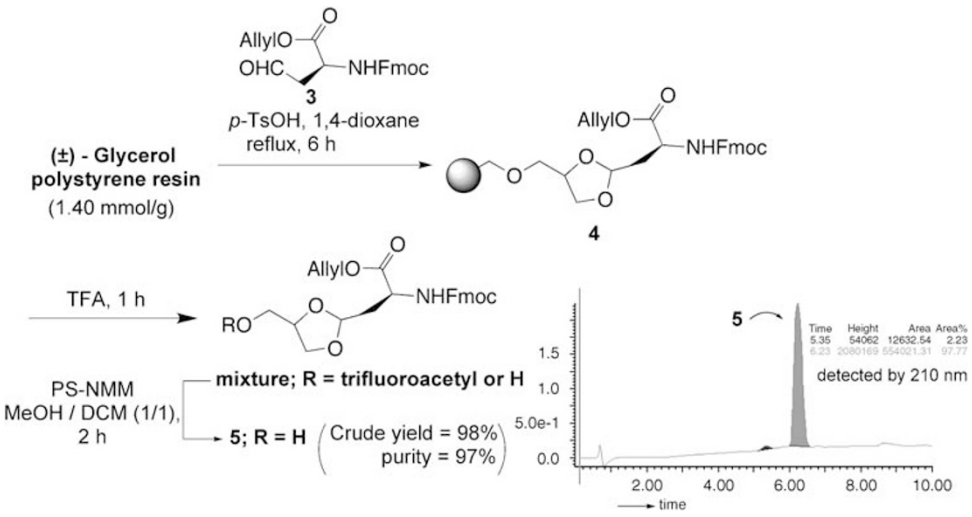
Figure 1 Argadin (**1**) and Argifin (**2**) and the IC_{50} values against *Serratia marcescens* chitinases (*Sm*Chi) A, B and C_1.



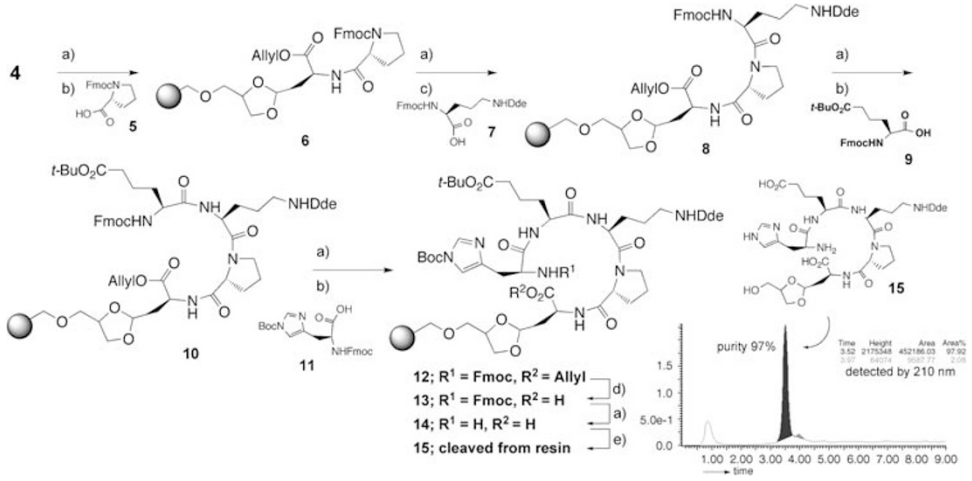
Scheme 1 Synthetic plan of **1**. Boc, *t*-butoxycarbonyl; Dde, 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl; Fmoc, 9-fluorenylmethoxycarbonyl; SPPS, solid-phase peptide synthesis.

hemiaminal; (iii) on resin, cyclization includes a stepwise selective deprotection of the C and N terminals, followed by intramolecular condensation; (iv) single-step operation to convert to the N^{ω}-acetylarginine residue obtained from ornithine by acetylguanylation; and (v) the whole reaction sequence can be carried out on resin, except for the cleavage from resin and hemiaminal formation at the final step.

The synthesis of **1** commenced with the loading of the solid support of the *N*-Fmoc-*O*-allyl-protected aspartic acid β-semialdehyde residue (**3**),[22] anchored by a side chain. The aldehyde in **3** was loaded onto (±)-glycerol polystyrene resin (1.40 mmol g^{-1}) in the presence of catalytic TsOH under reflux in 1,4-dioxane to yield **4** with a loading of > 1.33 mmol g^{-1} (Scheme 2). To confirm the loading yield, cleavage



Scheme 2 Loading of **3** onto resin and liquid chromatography (LC)-UV analysis of cleaved compound **5**. Ts, *p*-toluenesulfonyl; TFA, trifluoroacetic acid; PS-NMM, polystyrene-*N*-methylmorpholine.

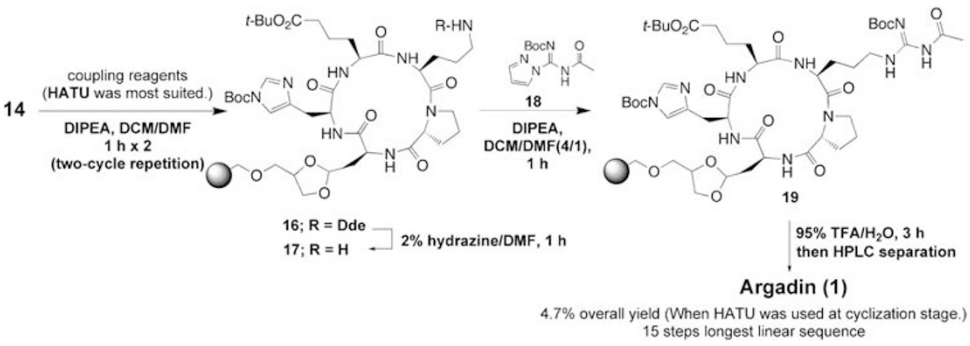


Scheme 3 Solid-phase synthetic scheme of linear pentapeptide **14** and liquid chromatography (LC)-UV analysis of cleaved compound **15**; (a) 20% piperidine/DMF, 1 h; (b) PyBOP, (DIPEA), dichloromethane (DCM)/DMF (4/1), 2 h; (c) HBTU, DIPEA, DCM/DMF (4/1), 2 h; (d) $Pd(PPh_3)_4$, dimedone, THF, 1 h; (e) (1) TFA, 1 h, (2) polystyrene-*N*-methylmorpholine (PS-NMM), MeOH/DCM (1/1), 2 h. PyBOP, (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; DIPEA, diisopropylethylamine; HBTU, *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate.

from the resin **4** with 100% of TFA for 1 h, followed by treatment with morpholinomethyl polystyrene resin (polystyrene-*N*-methylmorpholine (PS-NMM)) in $MeOH/CH_2Cl_2$ (1/1) for 2 h, resulted in the desired glycerol bearing **3** (**5**) in 98% crude yield with 97% purity (calculated using the liquid chromatography (LC)-UV analysis).

The resin-bound amino acid **4** was subsequently subjected to four deprotection-coupling cycles to construct the linear pentapeptide by standard Fmoc solid-phase peptide synthesis using PyBOP ((benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate) activation for peptide formation, except for the condensation between 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl (Dde)-protected Fmoc-Orn-OH (**7**)[23,24] and D-Pro, which was accomplished using HBTU (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate) activation. Each amino acid coupling step was monitored by the LC-UV analysis for the cleaved material using the acid deprotection processes. After the synthesis of the linear pentapeptide (**12**) was accomplished, deprotection on both the C and N terminals was achieved by subjecting the resin-bound **12** to $Pd(PPh_3)_4$ treatment in the presence of dimedone in DMF under Ar atmosphere to eliminate the allyl function. Subsequently, the Fmoc group was removed by piperidine to afford the precursor of the cyclic peptide compound (**14**), which was then converted to the crude product (**15**) with 97% purity, as determined by the LC-UV analysis (as shown in Scheme 3).

Macrolactamization of **14** under the PyBOP activation condition for 1 h of reaction time proceeded with low efficiency to yield the cyclic peptide **16**, which was converted to final Argadin (**1**) with very low quantity (<1% isolated yield after HPLC separation) by submitting a 3-reaction sequence, deprotection of Dde by 2% NH_2NH_2 in DMF, acetylguanylation using 1-*H*-pyrazole-1-[*N*-(*tert*-butoxycarbonyl)]-*N′*-acetylcarboxamidine (**18**),[25] followed by the formation of hemiaminal accompanied by total deprotection, including resin cleavage, to roughly elucidate the conversion ratio from **14** to **15** (Scheme 4). Although the two-cycle repetition reaction for lactamiza-



Scheme 4 Completion of solid-phase total synthesis of **1**. HATU=*O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate; DIPEA, diisopropylethylamine.

tion with **14** (PyBOP, 1 h×2) was attempted to improve the conversion yield, the production of **1** was still low (~1% yield). To obtain a sufficient quantity of **1**, various condensation reagents, such as HBTU, *O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU) and PyBrop, were tested. Optimally, when **14** was subjected to the macrolactamization using HATU in the presence of diisopropylethylamine (DIPEA) in CH_2Cl_2/DMF (4/1) for 1 h over a two-cycle repetition, Argadin (**1**) was isolated in 4.7% of the overall yield, after undergoing the three-step reaction sequence and final separation by HPLC (Scheme 4). All spectral data (^{1}H-, ^{13}C-NMR in DMSO-d_6 with 1.7% TFA and HR-MS) and activities of synthetic **1** were found to be identical with those of natural **1**. However, the optical rotations of synthetic ($[\alpha]_D$=−17.0 in H_2O; *c* 0.04) and natural **1** ($[\alpha]_D$=+52.1 in H_2O; *c* 0.1)[9] did not match, partly because of the optical rotational value of **1** being strongly influenced by the counter anion of acids, and the fact that it was difficult to create identical conditions under which to measure the optical rotation. HPLC profiles of both synthetic and natural **1** showed a 3:1 to 7:1 mixture of diastereoisomers at the hemiaminal position. The ratio of diastereomers was also influenced by acidic additives (TFA, AcOH or HCO_2H), which are usually added into a mobile phase of HPLC, and the stock solution of Argadin samples to stabilize Argadin itself. The diastereomeric ratio of hemiaminal may affect the optical rotational value of Argadin. Therefore, we examined the inhibitory activity of synthetic **1** against chitinases, and its activity was almost the same as that of natural **1**.

In conclusion, we completed the solid-phase total synthesis of Argadin (**1**) using a supported acetal resin. This synthesis was concise and required 15 steps in the longest linear sequence from (±)-glycerol polystyrene resin, with HPLC separation after cleavage from resin to yield **1** in 4.7% of the overall yield. The process allows us to speedily generate a variety of analogs in structure–activity relationship studies. Ongoing studies, aimed at the synthesis and biological evaluation of peptide analogs, designed using computer-aided rational molecular calculations[26] using information obtained from X-ray cocrystallography of Argadin-chitinase, are currently under way in our laboratories.

EXPERIMENTAL SECTION

General procedure

Fmoc-D-Pro-OH (**5**), Fmoc-Ada(O*t*-Bu)-OH (**9**) and Fmoc-(Boc)His-OH (**11**) were purchased from Watanabe Chemical Industries (Hiroshima, Japan). (±)-Glycerol polystyrene resin (1.4 mmol g^{-1}) was purchased from NovaBiochem (Tokyo, Japan). Dry pyridine, THF, DMF, MeOH, 1,4-dioxane and CH_2Cl_2 were purchased from Kanto Chemical (Tokyo, Japan). ^{1}H-NMR spectra were recorded at 400 MHz, and ^{13}C-NMR spectra were recorded at 100 MHz on Varian XL-400 (Varian; 400 MHz). The chemical shifts are expressed in p.p.m. downfield from internal solvent peaks and DMSO (2.49 p.p.m., ^{1}H-NMR), DMSO-d_6 (39.7 p.p.m., ^{13}C-NMR) and *J*-values are expressed in hertz. The coupling patterns are expressed by s (singlet), d (doublet), dd (double doublet), ddd (double double doublet), m (multiplet) or br (broad). High-resolution mass spectra were measured on a JEOL JMS-DX300 spectrometer (JEOL, Tokyo, Japan). HPLC analysis was conducted on a Waters 2795 Separation Module (Nihon Waters KK, Tokyo, Japan) with Alliance HT (column; Senshu Pak-PEGASIL ODS 2 ϕ×50 mm (Senshu Scientific Co. Ltd., Tokyo, Japan): condition of HPLC; gradient 10% MeCN (0.05% TFA)/H_2O (0.1% TFA) to 100% MeCN (0.05% TFA) over 8 min, flow 0.3 ml min^{-1}, detect 210 nm, temp 20 °C). HPLC purification of Argadin (**1**) was performed on a Hitach ELITE LaChrom (column; Senshu Pak PEGASIL ODS 20 ϕ×250 mm with a flow rate of 8 ml min^{-1}. The mobile phase was 0.05% TFA in 8% MeCN/H_2O). The solid-phase total synthesis of Argadin was carried out in a MicroKan microreactor initially filled with 30 mg of (±)-glycerol polystyrene resin-bound amino acid residue (**4**).

Loading of *N*-(9-Fluorenyl)methoxycarbonyl-(*O*Allyl)-L-Homoserinealdehyde (3) onto glycerol polystyrene resin; Fmoc-L-Hse-*O,O*-hydroxymethylethylideneacetal-*O*-PS (4)

(±)-Glycerol polystyrene resin (1.54 g, 2.0 mmol) was swelled in 1,4-dioxane (20 ml) for 30 min at room temperature. Fmoc-(*O*Allyl)-L-homoserinealdehyde (**3**)[22] (1.4 g, 4.0 mmol) and $TsOH \cdot H_2O$ (0.75 g, 0.4 mmol) was added to the solution of resin, and the reaction mixture was heated to 90 °C using a Dean—Stark trap. After heating for 8 h, the reaction was cooled to room temperature, filtered to collect the resin product, which was washed with pyridine (10 ml×2), Py/H_2O (1/1) (10 ml×2), H_2O (10 ml×2), THF (10 ml×2), DMF (10 ml×4), dichloromethane (DCM) (10 ml×4) and dried *in vacuo* to afford the desired resin-bound product **4**.

General procedure for deprotection of the Fmoc group

The MicroKan microreactor with 30 mg resin was placed into a 20 ml screw vial and swollen in DCM (1.5 ml) for 1 h and thereafter filtered. It was sequentially treated with a solution of 20% piperidine in DMF (1.5 ml). The mixture was vigorously agitated at room temperature. After being agitated for 1 h, the mixture was filtered, washed with DMF (5 ml×4), DCM (5 ml×4) and dried *in vacuo* to afford the corresponding resin-bound amine products.

General procedure for peptide coupling

The MicroKan microreactor with 30 mg resin was placed into a 20 ml screw vial and swollen in DMF (1.5 ml) for 1 h and thereafter filtered. It was treated with a cocktail of each amino acid (3.0 equiv.) [Fmoc-D-Pro-OH (**5**), Fmoc-Ada(O*t*-Bu)-OH (**9**) and Fmoc-(Boc)His-OH (**11**)], PyBop (3.0 equiv.), *N,N*-diisopropylethylamine (6.0 equiv.) in DCM/DMF (4/1, 1.5 ml), except for the case of coupling between dipeptide **6** and Fmoc-Orn(Dde)-OH (**7**) [**7** (3.0 equiv.), HBTU (3.0 equiv.) and *N,N*-diisopropylethylamine (6.0 equiv.) in DCM/DMF (4/1, 1.5 ml) for 1 h]. The mixture was vigorously agitated at room temperature. After being agitated for 2 h, the mixture was filtered, washed with

DMF (5 ml×4), DCM (5 ml×4) and dried *in vacuo* to afford the corresponding peptides.

Procedure for deprotection of the allyl group of 12

The MicroKan microreactor with **12** was placed into a 20 ml screw vial and swollen in THF (1.5 ml) for 1 h under Ar atmosphere, and the remaining THF was thereafter drained by a syringe. To the MicroKan microreactor with **12** was sequentially added a well-mixed solution of $Pd(PPh_3)_4$ (1.5 equiv.) and dimedone (10 equiv.) in THF (1.5 ml) through a syringe under Ar atmosphere. The mixture was vigorously agitated at room temperature under the Ar atmosphere. After being agitated for 1 h, the mixture was filtered, washed with THF (5 ml×4), DMF (5 ml×4), DCM (5 ml×4) and dried *in vacuo* to afford **13**.

Cyclization of 14

The MicroKan microreactor with **14** was placed into a 20 ml screw vial and swollen in DCM (1.5 ml) for 1 h and thereafter filtered. It was treated with a cocktail of HATU (2 equiv.) and DIPEA (4 equiv.) in DCM/DMF (4/1, 1.5 ml). The mixture was vigorously agitated at room temperature. After being agitated for 2 h, the mixture was filtered, washed with DMF (5 ml×4), DCM (5 ml×4) and dried *in vacuo*. The same procedure was repeated.

Procedure for deprotection of Dde in 16

The MicroKan microreactor with **16** was placed into a 20 ml screw vial and swollen in DMF (1.5 ml) for 1 h, and filtered. It was sequentially treated with a solution of 2% hydrazine monohydrate in DMF (1.5 ml). The mixture was vigorously agitated at room temperature. After being agitated for 1 h, the reaction mixture was filtered, washed with DMF (5 ml×4), DCM (5 ml×4) and dried *in vacuo* to afford **17**.

Procedure for acetylguanylation of 17

The MicroKan microreactor with **17** was placed into a 20 ml screw vial and swollen in DCM (1.5 ml) for 1 h and thereafter filtered. The MicroKan microreactor was sequentially treated with a 1.0 M solution of **18**[25] in DCM (126 µl) and *N,N*-diisopropylethylamine (43.9 µl, 252 µmol) in DCM/DMF (4/1, 1.5 ml). The mixture was vigorously agitated at room temperature. After being agitated for 2 h, the reaction mixture was filtered, washed with DMF (5 ml×4), DCM (5 ml×4) and dried *in vacuo* to afford **19**.

Cleavage from the resin and hemiaminal formation to yield 1

The MicroKan microreactor with **19** was placed into a 20 ml screw vial and swollen in DCM (1.5 ml) for 1 h and thereafter filtered. It was sequentially treated with a cocktail mixture of 95% TFA/H_2O (1.5 ml). The mixture was vigorously agitated at room temperature. After being agitated for 3 h, the reaction mixture was filtered and dried *in vacuo* to provide crude Argadin (**1**) (24 mg, 84.5%). The use of HPLC purification (8% MeCN/H_2O containing 0.05% TFA) furnished **1** (4.2 mg, 4.7%) as a colorless solid.

Synthetic Argadin

$[\alpha]_D$=17.0 (*c* 0.04, H_2O); ^{1}H-NMR (400 MHz, DMSO-d_6 with 1.7% TFA) δ: 14.19 (brs, 1H, His), 11.75 (s, 2H, η1 position of Arg), 9.14 (m, 2H, ε position of Arg), 9.00 (s, 1H, ε position of His), 8.68 (brs, 1H, η2 position of Arg), 8.61 (d, *J*=6.6 Hz, 1H, NH), 8.29 (d, *J*=8.0 Hz, 1H, NH), 7.46 (s, 1H, δ position of His), 7.19 (d, *J*=9.0 Hz, 1H, NH), 5.33 (d, *J*=6.2 Hz, 1H, γ position of Hse), 4.60 (m, 1H, α position of Pro), 4.58 (m, 1H, α position of His), 4.36 (m, 1H, α position of Hse), 4.11 (m, 1H, α position of Arg), 3.40 (m, 1H, δ position of Pro), 3.36 (m, 1H, β position of His), 3.31 (m, 1H, δ position of Pro), 3.30 (m, 1H, γ position of Pro), 3.27 (dd, *J*=9.0, 15.0 Hz, 1H, β position of His), 3.21 (m, 2H, δ position of Arg), 2.65 (m, 1H, β position of Hse), 2.20 (ddd, *J*=9.0, 17.0, 24.0 Hz, 1H, γ position of Aad), 2.12 (m, 3H, γ position of Aad, β position of Pro), 2.05 (s, 3H, acetyl), 1.74 (m, 2H, β position of Pro, γ position of Pro), 1.72 (m, 1H, β position of Arg), 1.66 (m, 2H, β position of Arg, β position of Hse), 1.58 (m, 1H, α position of Aad), 1.49 (m, 2H, γ position of Arg), 1.44 (m, 2H, β position of Aad); ^{13}C-NMR (100 MHz, DMSO-d_6 with 1.7% TFA) δ: 173.9 (ε position of Aad), 172.9 (-NHCO- of Hse), 173.2 (-COCH$_3$), 171.9 (-NHCO- of Aad), 171.4 (-NHCO- of Pro), 170.1 (-NHCO- of Arg), 169.9 (-NHCO- of His), 153.1 (ζ position of Arg), 134.5 (ε position of His), 129.4 (γ position of Aad), 117.0 (δ position of His), 78.4 (γ position of Hse), 59.4 (α position of Pro), 57.2 (α position of His), 53.2 (α position of Aad), 51.2 (α position of Arg), 48.9 (α position of Hse), 46.0 (δ position of Pro), 40.7 (δ position of Arg), 35.9 (β position of Hse), 33.1 (δ position of Aad), 31.8 (β position of Aad), 27.8 (β position of Pro), 27.2 (β position of Arg), 24.3 (γ position of Arg), 24.1 (-COCH$_3$), 23.6 (γ position of Pro), 22.5 (β position of His), 20.7 (γ position of Aad); HR-MS (PEG_ESI+_1000) calcd for $C_{29}H_{43}O_{10}N_9$: 675.3215 [M+H], found *m/z* : 675.3199 $[M+H]^+$.

Natural Argadin

$[\alpha]_D$=+52.1 (*c* 0.1, H_2O);[9] ^{1}H-NMR (400 MHz, DMSO-d_6 with 1.7% TFA) δ: 14.19 (brs, 1H, His), 11.78 (s, 2H, η1 position of Arg), 9.16 (t, *J*=4.8 Hz, 2H, ε position of Arg), 8.97 (s, 1H, ε position of His), 8.68 (brs, 1H, η2 position of Arg), 8.58 (d, *J*=6.8 Hz, 1H, NH), 8.27 (d, *J*=7.9 Hz, 1H, NH), 7.43 (s, 1H, δ position of His), 7.16 (d, *J*=9.1 Hz, 1H, NH), 5.31 (d, *J*=6.2 Hz, 1H, γ position of Hse), 4.59 (m, 1H, α position of Pro), 4.58 (m, 1H, α position of His), 4.33 (ddd, *J*=4.3, 9.1, 11.6 Hz, 1H, α position of Hse), 4.10 (m, 1H, α position of Arg), 3.41 (ddd, *J*=11.4, 11.4, 11.4 Hz, 1H, δ position of Pro), 3.36 (dd, *J*=12.6, 19.9 Hz, 1H, β position of His), 3.31 (m, 1H, δ position of Pro), 3.28 (m, 1H, γ position of Pro), 3.26 (dd, *J*=9.6, 19.9 Hz, 1H, β position of His), 3.25 (m, 2H, δ position of Arg), 2.63 (ddd, *J*=6.2, 11.6, 19.4 Hz, 1H, β position of Hse), 2.21 (ddd, *J*=9.1, 16.9, 21.3 Hz, 1H, γ position of Aad), 2.17 (ddd, *J*=9.1, 16.9, 21.3 Hz, 2H, γ position of Aad), 2.11 (m, 1H, β position of Pro), 2.10 (s, 3H, acetyl), 1.75 (m, 2H, β position of Pro, γ position of Pro), 1.73 (m, 1H, β position of Arg), 1.66 (m, 2H, β position of Arg, β position of Hse), 1.57 (m, 1H, α position of Aad), 1.50 (m, 2H, γ position of Arg), 1.43 (m, 2H, β position of Aad).

^{13}C-NMR (100 MHz, DMSO-d_6 with 1.7% TFA) δ: 174.3 (ε position of Aad), 172.9 (-NHCO- of Hse), 172.7 (-COCH$_3$), 171.4 (-NHCO- of Aad), 171.1 (-NHCO- of Pro), 170.1 (-NHCO- of Arg), 169.9 (-NHCO- of His), 153.1 (ζ position of Arg), 134.5 (ε position of His), 129.4 (γ position of Aad), 117.0 (δ position of His), 78.7 (γ position of Hse), 59.8 (α position of Pro), 57.2 (α position of His), 53.2 (α position of Aad), 51.6 (α position of Arg), 49.0 (α position of Hse), 46.0 (δ position of Pro), 40.7 (δ position of Arg), 35.9 (β position of Hse), 33.1 (δ position of Aad), 31.8 (β position of Aad), 27.9 (β position of Pro), 27.4 (β position of Arg), 24.3 (γ position of Arg), 24.1 (-COCH$_3$), 23.6 (γ position of Pro), 22.5 (β position of His), 20.7 (γ position of Aad).

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1 Boot, R. G. *et al.* Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. *J. Biol. Chem.* **276,** 6770–6778 (2001).
2 Shahabuddin, M., Toyoshima, T., Aikawa, M. & Kaslow, D. C. Transmission-blocking activity of a chitinase inhibitor and activation of malarial parasite chitinase by mosquito protease. *Proc. Natl. Acad. Sci. USA* **90,** 4266–4270 (1993).
3 Shibata, Y., Foster, L. A., Bradfield, J. F. & Myrvik, Q. N. Oral administration of chitin down-regulates serum IgE levels and lung eosinophilia in the allergic mouse. *J. Immunol.* **164,** 1314–1321 (2000).
4 Matsuura, H *et al.* Nucleotide sequences of genes encoding allosamidin-sensitive and -insensitive chitinases produced by allosamidin-producing *Streptomyces*. *Biosci. Biotechnol. Biochem.* **67,** 2002–2005 (2003).
5 Hollis, T. *et al.* The X-ray structure of a chitinase from the pathogenic fungus *Coccidioides immitis*. *Protein Sci.* **9,** 544–551 (2000).
6 Sakuda, S., Isogai, A., Matsumoto, S. & Suzuki, A. Search for microbial insect growth regulators. II. Allosamidin, a novel insect chitinase inhibitor. *J. Antibiot.* **40,** 296–300 (1987).
7 Vinetz, J. M. *et al.* Chitinases of the avian malaria parasite *Plasmodium gallinaceum*, a class of enzymes necessary for parasite invasion of the mosquito midgut. *J. Biol. Chem.* **275,** 10331–10341 (2000).

8 Zhu, Z. *et al.* Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science* **304,** 1678–1682 (2004).
9 Arai, N. *et al.* Argadin, a new chitinase inhibitor, produced by *Clonostachys sp.* FO-7314. *Chem. Pharm. Bull.* **48,** 1442–1446 (2000).
10 Ōmura, S. *et al.* Argifin, a new chitinase inhibitor, produced by *Gliocladium sp.* FTD-0668. I. Taxonomy, fermentation, and biological activities. *J. Antibiot.* **53,** 603–608 (2000).
11 Arai, N., Shiomi, K., Iwai, Y. & Ōmura, S. Argifin, a new chitinase inhibitor, produced by *Gliocladium sp.* FTD-0668. II. Isolation, physico-chemical properties, and structure elucidation. *J. Antibiot.* **53,** 609–614 (2000).
12 Shiomi, K. *et al.* Structure of argifin, a new chitinase inhibitor produced by *Gliocladium sp. Tetrahedron Lett.* **41,** 2141–2143 (2000).
13 Suzuki, K. *et al.* Chitinases A, B, and C1 of *Serratia marcescens* 2170 produced by recombinant *Escherichia coli*: enzymatic properties and synergism on chitin degradation. *Biosci. Biotechnol. Biochem.* **66,** 1075–1083 (2002).
14 Horn, S. J. *et al.* Comparative studies of chitinases A, B and C from *Serratia marcescens. Biocatal. Biotransfor.* **24,** 39–53 (2006).
15 Rao, F. V. *et al.* Specificity and affinity of natural product cyclopentapeptide inhibitors against *A. fumigatus*, human, and bacterial chitinases. *Chem. Biol.* **12,** 65–76 (2005).
16 Houston, D. R. *et al.* High-resolution structures of a chitinase complexed with natural product cyclopentapeptide inhibitors: mimicry of carbohydrate substrate. *Proc. Natl. Acad. Sci. USA* **99,** 9127–9132 (2002).
17 Dixon, M. J., Andersen, O. A., van Aalten, D. M. F. & Eggleston, I. M. First synthesis of Argadin: a nanomolar inhibitor of family-18 chitinases. *Eur. J. Org. Chem.* 5002–5006 (2006).
18 Dixon, M. J., Andersen, O. A., van Aalten, D. M. F. & Eggleston, I. M. An efficient synthesis of argifin: a natural product chitinase inhibitor with chemotherapeutic potential. *Bioorg. Med. Chem. Lett.* **15,** 4717–4721 (2005).
19 Dixon, M. J. *et al.* Solid-phase synthesis of cyclic peptide chitinase inhibitors: SAR of the argifin scaffold. *Org. Biomol. Chem.* **7,** 259–268 (2009).
20 Sunazuka, T. *et al.* Argifin; efficient solid phase total synthesis and evaluation of analogues of acyclic peptides. *Bioorg. Med. Chem.* **17,** 2751–2758 (2009).
21 Hirose, T. *et al.* Chitinase inhibitors: extraction of the active framework from natural argifin and use of *in situ* click chemistry. *J. Antibiot.* **62,** 277–282 (2009).
22 Bayer, T., Riemer, C. & Kessler, H. A new strategy for the synthesis of cyclopeptides containing diaminoglutaric acid. *J. Peptide Sci.* **7,** 250–261 (2001).
23 Bycroft, B. W., Chan, W. C., Chhabra, S. R. & Hone, N. D. A novel lysine-protecting procedure for continuous flow solid phase synthesis of branched peptides. *J. Chem. Soc. Chem. Commun.* 778–779 (1993).
24 Chhabra, S. R. *et al.* An appraisal of new variants of Dde amine protecting group for solid phase peptide synthesis. *Tetrahedron Lett.* **39,** 1603–1606 (1998).
25 Hong, Y. *et al.* Preparation of amidino-urea serotonin receptor ligands. *PCT Int. Appl.* WO2002036554 A2 20020510 (2002).
26 Gouda, H. *et al.* Computer-aided rational molecular design of argifin-derivatives with more potent inhibitory activity against chitinase B from *Serratia marcescens. Bioorg. Med. Chem. Lett.* **19,** 2630–2633 (2009).