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Solution-phase total synthesis of the hydrophilic natural product argifin using 3,4,5-tris(octadecyloxy)benzyl tag

Tomoyasu Hirose [a,b], Takako Kasai [b], Takafumi Akimoto [c], Ayako Endo [b], Akihiro Sugawara [a,b], Kazuo Nagasawa [c], Kazuro Shiomi [a,b], Satoshi Ōmura [a,b,*], Toshiaki Sunazuka [a,b,*]

[a] Kitasato Institute for Life Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan
[b] Graduate School of Infection Control Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan
[c] Tokyo University of Agriculture and Technology, Department of Biotechnology and Life Science, Faculty of Technology, 2-24-16 Nakamachi, Koganei, Tokyo 184-8588, Japan

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ABSTRACT

A solution-phase total synthesis of argifin using 3,4,5-tris(octadecyloxy)benzyl tag as a hydrophobic protective group of carboxylic acid was developed to produce 44% overall yield for 16 linear steps. Argifin, a novel class of natural product chitinase inhibitor, is a highly water-soluble cyclic pentapeptide, so hitherto, only solid-phase synthesis techniques have been used to conveniently prepare the compound and its derivatives. 3,4,5-Tris(octadecyloxy)benzyl alcohol (HO-TAGa) and its esters are highly crystalline materials and highly capable of dissolving in less-polar solvents such as dichloromethane, benzene, THF, etc., but insoluble in polar solvents such as methanol and DMSO. The combination of HO-TAGa and Fmoc-based peptide synthesis, together with simple purification by recrystallization from MeOH solution, furnished an efficient and practical route of argifin production in the liquid-phase.

1. Introduction

Chitinases cleave chitin, present in a wide range of organisms, including bacteria, fungi, insects, viruses, higher plants and animals,[1–5] into oligomers of *N*-acetyl-D-glucosamine. Chitinase inhibitors thus have significant potential, especially as possible fungicides and pesticides,[6,7] because chitin and chitin synthases have so far not been found in mammals. However, acidic mammalian chitinase (AMCase) and chitotriosidase, two of a few human chitinases, have been documented.[2,8,9] Although several chitinase enzymes are utilized by mammals to hydrolyze chitin encountered by inhalation and ingestion, the endogenous substrate and physiological functions for mammalian chitinases are currently unknown. Inhibition of AMCase results in decreased airway inflammation and airway hyper-responsiveness in a mouse model, the activity being part of the mechanism of T-helper type 2 (Th2) cytokine-driven inflammatory response in asthma.[10] Recently, Cole and his co-workers carried out high-throughout screening against AMCase to target for human asthma, and identified small molecule inhibitors.[11] Subsequently, the chitinase of the filarial nematode *Onchocerca volvulus*, which causes the neglected tropical disease onchocerciasis, was discovered, chitin metabolism being implicated in the larval development of *O. volvulus*.[12] Actually, closantel,[13] a veterinary anthelmintic with known proton ionophore activities, was identified as a potent and specific inhibitor of filarial chitinases by Janda and his co-workers.[14] Thus, many inhibitors of chitinases have been reported from other research groups. Among them, allosamidin,[15] styloguanidines,[16] CI-4,[17] and psammaplin A[18] have been identified as naturally occurring materials. Our research group has also reported a novel class of naturally occurring chitinase inhibitors, named argadin (**1**)[19] and argifin (**2**),[20] during screening of 11,900 extracts from soil microorganisms (Fig. 1). Because the original sources do not produce **1** and **2** in sufficient quantities, nor provide a supply of their analogues for biological tests, total synthesis of both compounds has been established by our group[21] and Eggleston's group,[22] independently. These natural products consist of novel cyclic pentapeptide skeletons, and possess some hydrophilic amino acid moieties. Therefore, **1** and **2** have very hydrophilic features, and are soluble in H_2O and DMSO, but not soluble in less-polar solvents such as CH_2Cl_2, ethers, etc. These features create significant difficulties in traditional solution-phase

* Corresponding authors. Tel./fax: +81 3 5791 6340 (T.S.); tel.: +81 3 5791 6101; fax: +81 3 3444 8360 (S.O.); e-mail addresses: omuras@insti.kitasato-u.ac.jp (S. Ōmura), sunazuka@lisci.kitasato-u.ac.jp (T. Sunazuka).

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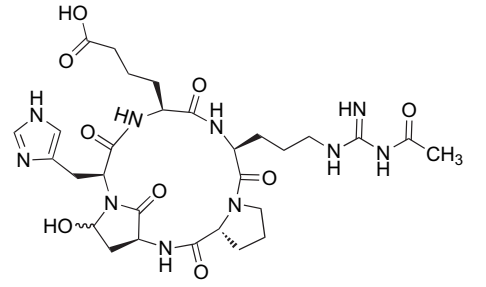
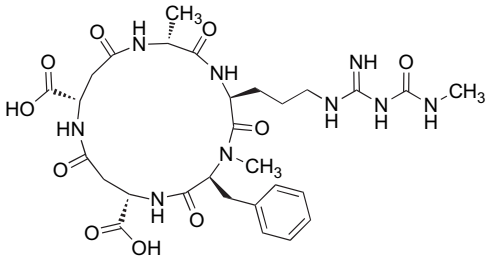


Argadin (**1**)

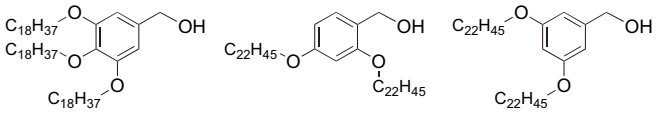


Argifin (**2**)

Fig. 1. Structures of argadin (**1**) and argifin (**2**).

synthesis, and contributed to development of the solid-phase synthesis needed to supply these natural products. In the case of synthesis of **2**, we and Eggleston's group have independently applied solid-phase synthesis approaches to conveniently supply **2** and its derivatives, including acyclic peptide fragments, and evaluated inhibitory activities against chitnases.[21b,22c] Since the solid-phase total synthesis of **2** has been completed, we started to investigate structure/activity relationships of **2** using computer-aided rational molecular design,[23] NMR spectroscopy, and computational analysis of interaction between ligands and chitinases,[24] and an in situ click chemistry approach[25] to generate highly potent inhibitors against bacterial and mammalian chitinases. Although the solid-phase approaches offer many advantages with regard to compound isolation and ease of handling, especially high polar and hydrophilic materials, the insoluble nature of resins-supported compounds makes it difficult to monitor the reactions in heterogeneous conditions, the characterization of the each synthetic intermediate, and the reagent accessibilities to the reactive site on the solid support. Moreover, to supply **2** as a standard material for chitinase inhibitors, we often encountered a complication in the purification process of **2** after cleaving from resin at the final step in the solid-phase total synthesis due to contamination of by-products, which were generated on the solid-supported substrates through each reaction sequence. To overcome these problems, a fluorous system involving fluorous-tagging an organic substrate in a solution-phase has been proposed.[26] To date, fluorous systems have relied on a simple solid–liquid extraction procedure over fluorous reverse-phase silica gel. This allows purification of the products from the reaction mixture using simple chromatography, as well as the characterization of products by common methods using NMR and mass spectrum. However, it is expensive, involving relatively high costs of reagents, such as fluorous tag and fluorous reverse-phase silica gel.

In 2001, Tamiaki and his co-workers first reported a novel protecting group, 3,4,5-tris(octadecyloxy)benzyl alcohol (**3**; HO-TAGa) (Fig. 2), as a hydrophobic tag, for application in peptide synthesis to construct peptide libraries.[27] Soon after, Chiba and his co-workers developed a liquid-phase system, and in addition, a cycloalkane-based thermomorphic system with **3**.[28] They also reported



3 (HO-**TAGa**) **4** (HO-**TAGb**) **5** (HO-**TAGc**)

Fig. 2. Structures of the substituted benzyl alcohols (**3**–**5**).

preparation methods and applications for two different hydrophobic tags, 2,4-didocosyloxybenzyl alcohol (**4**; HO-TAGb) and 3,5-didocosyloxybenzyl alcohol (**5**; HO-TAGc) (Fig. 2).[28e] The substituted benzyl alcohols are much effective for protecting group in a conventional peptide synthesis using the Fmoc strategy for tags **3**, **4**, and **5**, and the Boc strategy for tag **5**. The tagged-peptides can be soluble and subjected to chemical transformation in less-polar solvents, and easily purified by recrystallization from polar solvents. So far, peptide syntheses using the substituted benzyl tags have been demonstrated for construction of linear small peptide libraries[27] and preparation of an antagonistic peptide of TNF-α.[28e] However, there are no reported applications of the substituted benzyl tags for the total synthesis of the natural product. Here, we describe a first use of 3,4,6-tris(octadecyloxy)benzyl tag in the total synthesis of the hydrophilic cyclic peptide natural product argifin in the solution-phase.

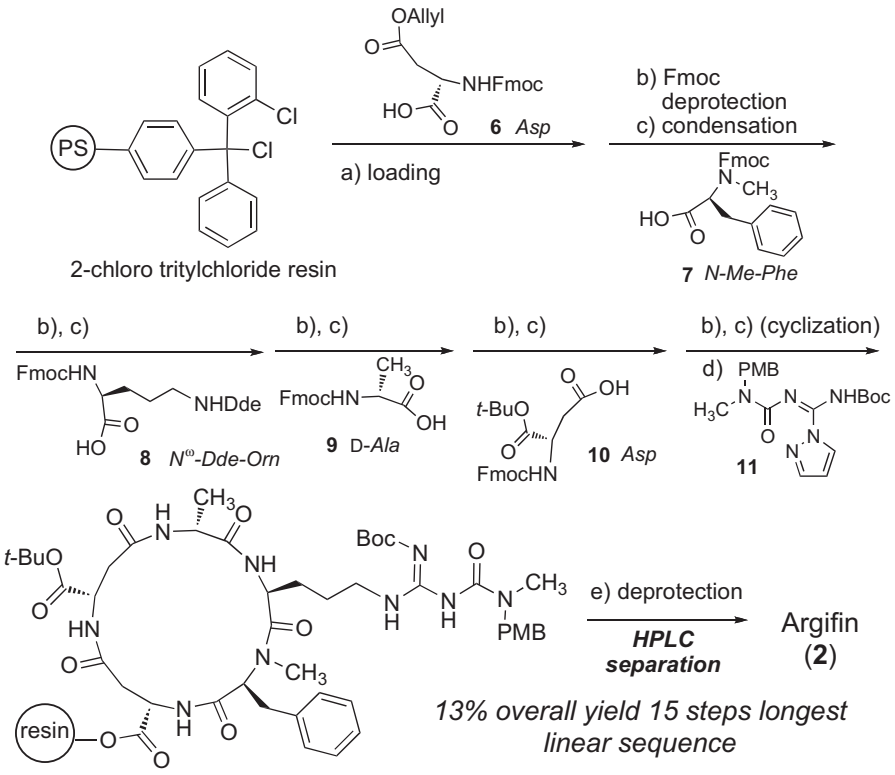
2. Results and discussion

In our first-generation synthesis of argifin (**2**) (Scheme 1),[21b] we utilized 2-chlorotrityl chloride resin to adopt a solid-phase synthetic strategy on the basis of Fmoc strategy attributed to the highly hydrophilic properties of Asp x2 and Arg x1 (=Orn as a synthetic fragment) amino acid moieties of **2**. The linear peptide was composed of blocks Fmoc-Asp(OAllyl)OH (**6**), Fmoc-*N*-Me-Phe-OH (**7**), Fmoc-Orn(Dde)-OH (**8**) [Dde; 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl],[29] Fmoc-D-Ala-OH (**9**), and Fmoc-Asp-O*t*-Bu (**10**) on the resin. Subsequently, the cyclic peptide was formed and the $N^{(\omega)}$-methylcarbamoylguanidino group was introduced with 1*H*-pyrazole-1-[*N*-(*tert*-butoxycarbonyl)-*N*′-(*N*-*p*-methoxybenzylcarbamoyl-*N*-methyl)]-carboxamidine (**11**) to the primary amine of Orn after Dde deprotection. Finally, deprotection of all protecting groups, including 2-chlorotrityl resin, was performed under acidic condition to afford **2**. This synthetic strategy necessitated 15 steps as longest linear reaction sequence from the step of loading to resin of **6** in overall 13% yield. However, the HPLC purification process of **2** from a mixture of products after final deprotection (Fig. 3) proved to be problematic, requiring very careful time-consuming work and reduction in overall yield. This problem should be originated in the inefficiencies of the each reaction on solid-phase synthesis was therefore not as efficient as would be desired.

In order to improve the efficiency of preparation of **2**, we developed a second-generation synthesis based upon a liquid-phase approach by a modification of the 2-chlorotrityl resin in the first-generation synthesis to a hydrophobic tag **3**. The work-up procedure of each reaction with tag **3** is shown in Fig. 4. All of the tagged synthetic compounds can be purified by simple recrystallization with polar solvents, such as MeOH, and silica gel flash chromatography, if necessary.

2.1. Synthesis of linear pentapeptide intermediate

At first, 3,4,5-tris(octadecyloxy)benzyl alcohol (**3**; HO-TAGa) was prepared from commercially available methyl gallate (**12**), using modified Tamiaki's protocol (Scheme 2).[27] The trialkylated product (**13**) could be easily purified by crystallization, which was performed by the addition of EtOH (more appropriate than



Scheme 1. Our first-generation total synthesis of argifin (**2**).

MeOH in this process) to CH_2Cl_2 solution of crude products, including di- and monoalkylated **12**, to yield colorless solid **13** in 76% yield as a pure form. Reduction with $LiAlH_4$ led to **3** (HO-TAGa) in excellent yield after recrystallization from MeOH solution.

According to first-generation strategy of **2**, our liquid-phase approach began with the condensation of the carboxylic acid at the α-position in Fmoc-Asp(OAllyl)-OH (**6**) and alcohol of **3**, using DCC and DMAP in CH_2Cl_2 to afford tagged amino acid [Fmoc-Asp(OAllyl)-*O*-TAGa] quantitatively after recrystallization from

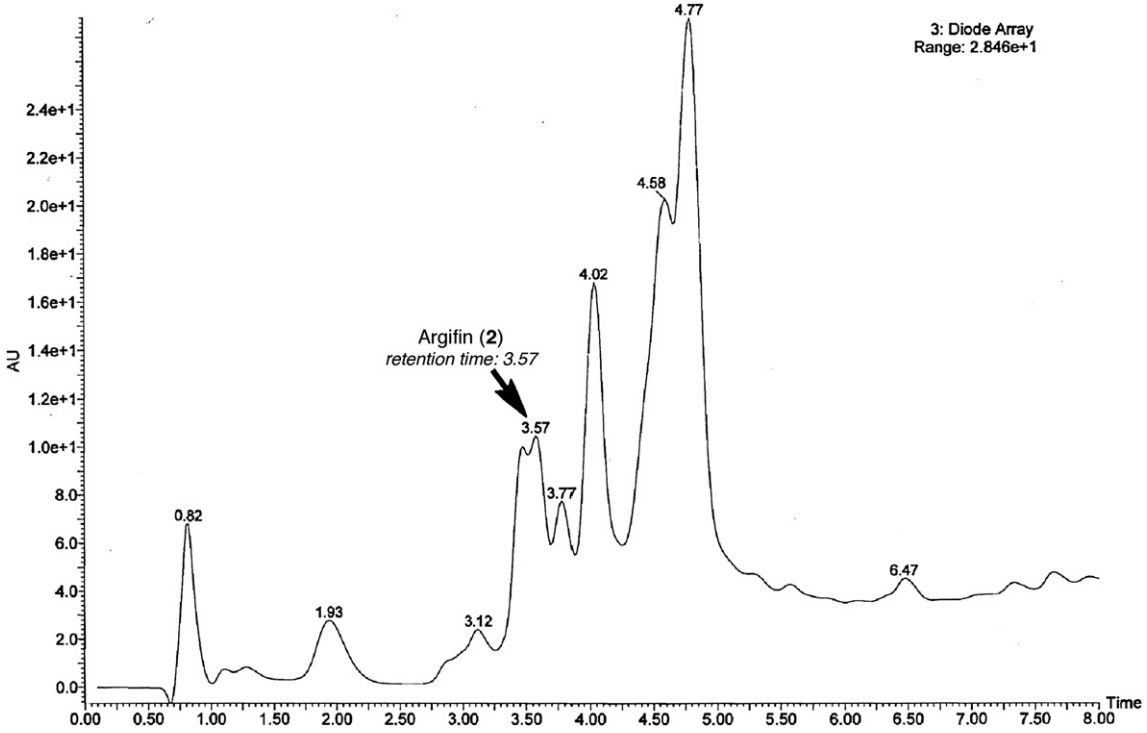


Fig. 3. HPLC chart of deprotected crude compounds at the final step of our solid-phase total synthesis of **2**.[21b] HPLC condition: column Senshu Pak PEGASIL ODS 2ø×50 mm; gradient 10% MeCN (0.05% TFA)/H_2O (0.1% TFA) to 100% MeCN (0.05% TFA) over 8.0 min; flow rate 0.3 mL/min, detect 200–400 nm; temperature 20 °C.

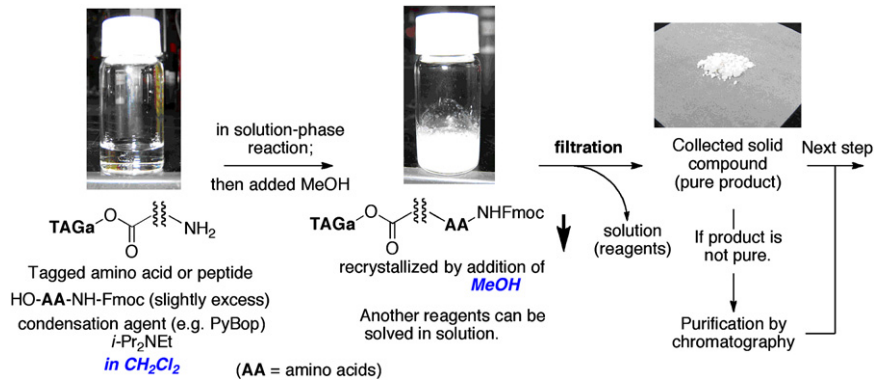
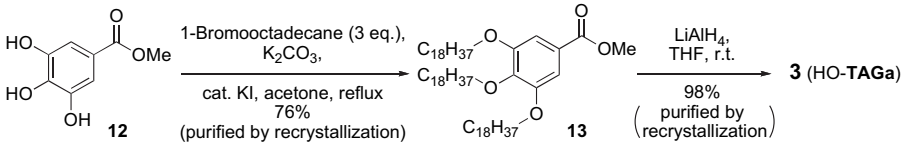
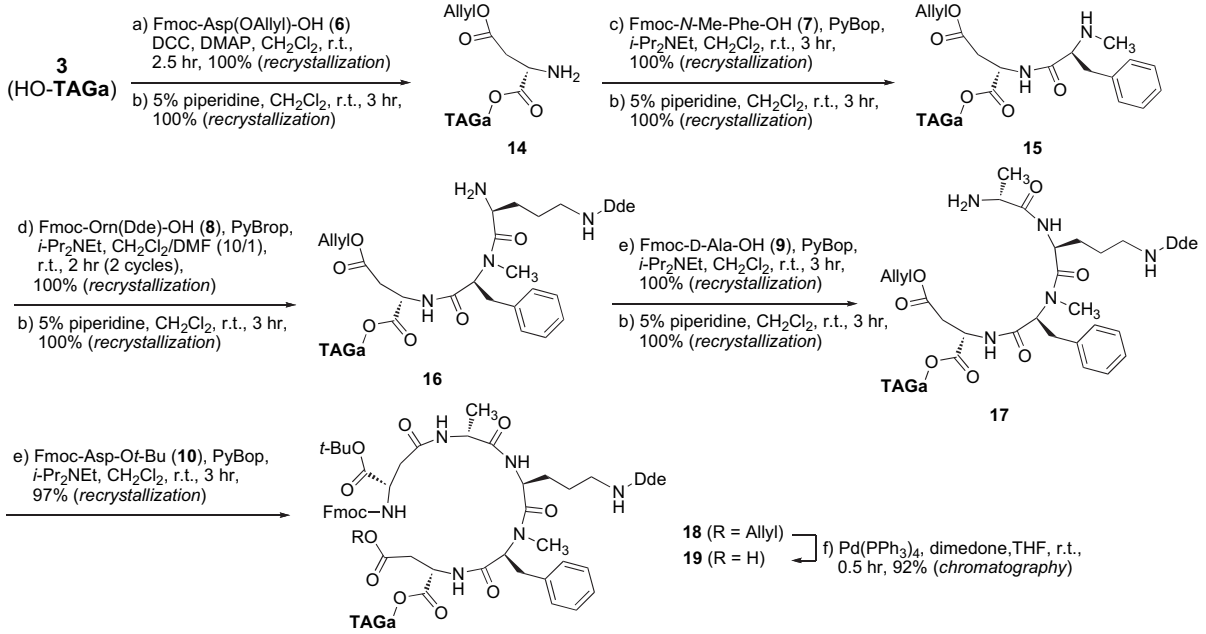


Fig. 4. General procedure of the liquid-phase strategy of **2** with simple separation by recrystallization or silica gel flash chromatography of each product on tag **3**.



Scheme 2. Preparation of **3** (HO-TAGa).

MeOH (Scheme 3). The tagged amino acid was subsequently subjected to four deprotection-coupling cycles to construct the linear pentapeptide (**18**) by standard Fmoc peptide synthesis, using PyBop. In case of the condensation of Fmoc-Orn(Dde)-OH (**8**) with dipeptide (**15**), the use of PyBrop as the appropriate coupling reagent in CH_2Cl_2/DMF (10/1) by twofold operation afforded the tripeptide product (**16**) with complete consumption of **15**. The tag synthesis protocol enabled reduction of the amounts of all reagents, such as amino acid building blocks, condensation reagents, and others, compared to the first-generation solid-phase approach. In the solid-phase approach, each amino acid building block generally required 3.0 equiv in appropriate solvent and removal of the Fmoc protecting group was accomplished in 20% piperidine/CH_2Cl_2 solution, consuming all starting materials in the process. In solution-phase, however, the amounts of each building block could be reduced to 1.1–1.5 equiv and the Fmoc deprotection process was fulfilled in 5% piperidine/CH_2Cl_2 solution. Of course, other reagents could also be greatly reduced for all steps compared with our first-generation approach. Each reaction step of coupling and deprotection from the process of tag-attachment of **6** could be monitored by thin-layer chromatography, and each product could be purified by recrystallization in quantitatively yields from MeOH, and directly identified by NMR and mass spectrogram. After the synthesis of the linear pentapeptide (**18**) was accomplished without chromatographic purification, deprotection on the C-terminal was achieved by subjecting **18** to $Pd(PPh_3)_4$ treatment in the presence of dimedone in THF to eliminate the allyl group. At this stage, the product was further purified by silica gel flash chromatography to yield pure carboxylic acid (**19**) in 92% yield (unexpected adsorption of Pd reagents to tagged-peptide in MeOH was observed).



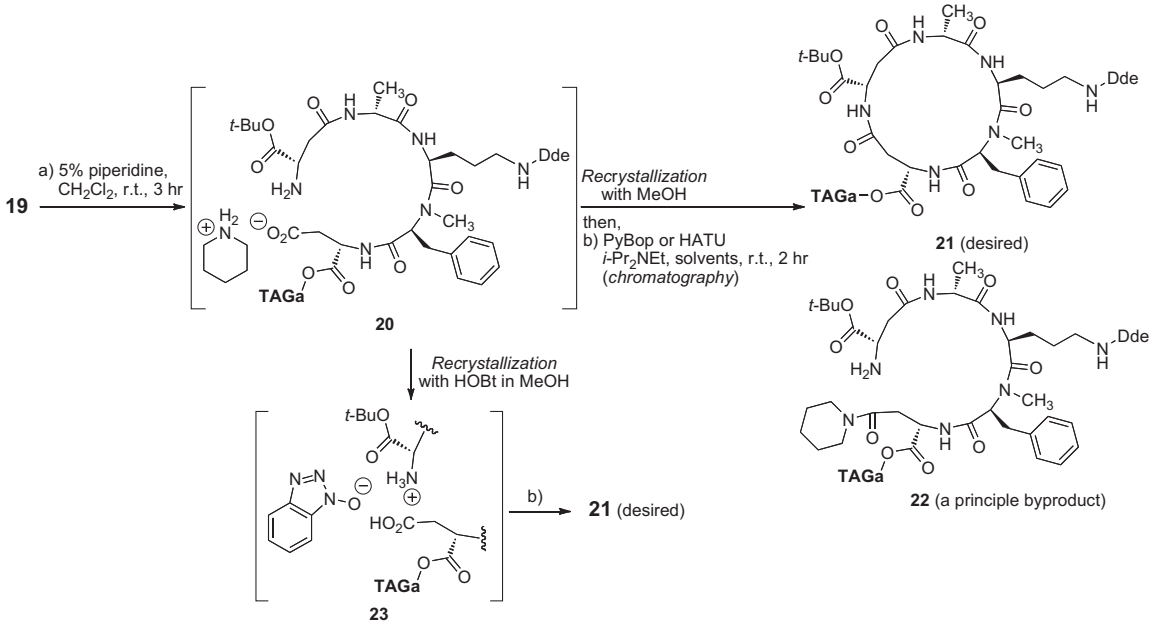
Scheme 3. Liquid-phase synthesis of linear pentapeptide **19**.

2.2. Macrolactamization and completion of total synthesis of argifin

Having the linear pentapeptide, macrolactamization was carried out after deprotection of Fmoc at the N-terminal of the peptide (**19**) (Table 1). As a first attempt, **19** was subjected to standard protocol to remove Fmoc with piperidine, followed by the recrystallization from MeOH to yield the amine. In this process, changing the polarity substrate on tag led to defective solid final product, which was then forcibly subjected to macrolactamization with HATU (entry 1) or PyBop (entry 2) in the presence of *i*-Pr_2NEt, furnishing the desired cyclic peptide **21** in 60% yield as a best after silica gel flash chromatography separation, but usually in low yields. This problem obviously attributed to formation of piperidinium (**20**) after Fmoc deprotection, which formed defective solid product and resulted in many by-products, including **22** as a principal (12% yield, when product **21** was obtained in 60% yield). To overcome this, we used a MeOH solution of HOBt as a recrystallization solvent after Fmoc deprotection of **19**. HOBt is a good crystalline material and has appropriate acidity, with pK_a 4.0 value in H_2O,[30] stronger than the acidity of acetic acid (pK_a 4.76) and much weaker than TFA (pK_a 0.23).[31] This means that HOBt can cleave the ionic bond between piperidine and the carboxylic acid of linear peptide, but not the ester bond of tag, and create a primary amine of the linear peptide to form an ammonium salt (**23**), which may be a convenient solid for recrystallization from MeOH. Because of that HOBt is a typical additive for a condensation reaction and a part of PyBop, HOBt of salt will not prevent macrolactam formation. Actually, the addition of HOBt was very effective in forming solid product from MeOH solution and improving the yield of macrolactamization process. After optimization of conditions, a combination of recrystallization from 100 mM HOBt in MeOH, followed by cyclization with PyBop in CH_2Cl_2 produced 85% yield of desired **20** with high reproducibility from the linear peptide **19** after silica gel flash chromatography separation (entry 6).

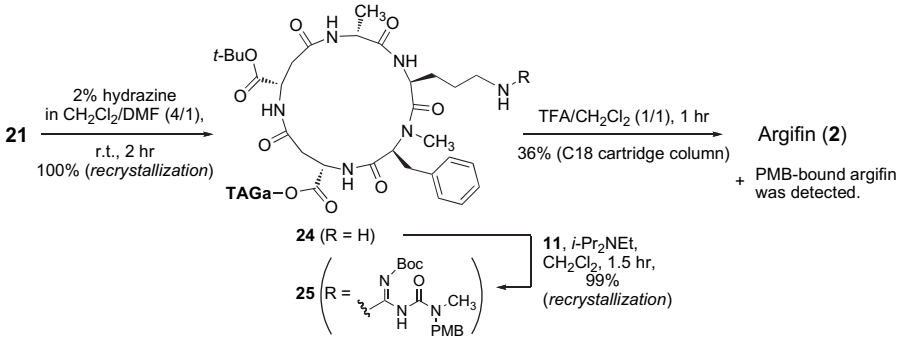
The cyclic pentapeptide (**21**) was synthesized by practical and convenient methods. The final stages of the total synthesis involved introduction of the $N^{(\omega)}$-methylcarbamoylguanidino group at the Orn moiety. According to our first-generation approach, the Dde protecting group was removed by treatment with 2% hydrazine solution in CH_2Cl_2/DMF (4/1), followed by recrystallization purification from MeOH to afford the primary amine (**24**) quantitatively (Scheme 4). Then, $N^{(\omega)}$-methylcarbamoylguanidino group was effectively introduced to **24** using pyrazole reagent (**11**)[21b] in the presence of *i*-Pr_2NEt to afford fully protected argifin (**25**) in 99% yield. Finally, deprotection of all protecting groups including TAGa with TFA/CH_2Cl_2 (1/1) afforded argifin (**2**) in 36% yield after simple C18-cartridge column chromatography (Scheme 4). A remarkable improvement over the solid-phase approach is achieved because soluble mixture in H_2O for the final deprotection process contained only two principal products, argifin (**2**) and PMB-bound argifin (the PMB group might migrate to another position under deprotection, but this has yet to be confirmed), which were easily separated by short cartridge column (Fig. 5). Therefore, no labor-intensive and

Table 1
Results of macrolactamization of **19**



Entry	Step a; deprotection[a]	Step b; macrolactamization[b]		
	Solvents for recrystallization	Reagents (4.5 equiv)	Solvents	Yields of **21**[c] (%)
1	MeOH only	HATU	CH_2Cl_2/DMF (10/1)	26–60[d,e]
2	MeOH only	PyBop	CH_2Cl_2/DMF (10/1)	16–30[e]
3	10 mM HOBt in MeOH	HATU	CH_2Cl_2/DMF (10/1)	84
4	10 mM HOBt in MeOH	PyBop	CH_2Cl_2/DMF (10/1)	67
5	100 mM HOBt in MeOH	PyBop	CH_2Cl_2/DMF (10/1)	77
6	100 mM HOBt in MeOH	PyBop	CH_2Cl_2	85

[a] All reactions were carried out in 5% piperidine/CH_2Cl_2 at room temperature for 3 h.
[b] All reactions were carried out with *i*-Pr_2NEt (18 equiv) at room temperature for 2 h, and products were purified by flash column chromatography on SiO_2.
[c] Isolated yields for two steps.
[d] A principal byproduct (**22**) was isolated in 12% yield, when desired product (**21**) was produced in 60% yield.
[e] Low reproducibility and many unidentified by-products were observed.



Scheme 4. The completion of liquid-phase total synthesis of argifin (**2**).

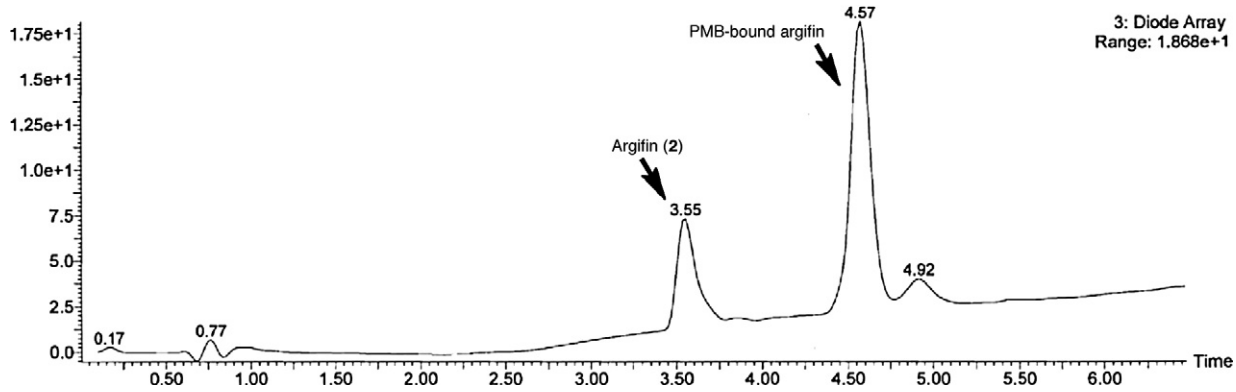
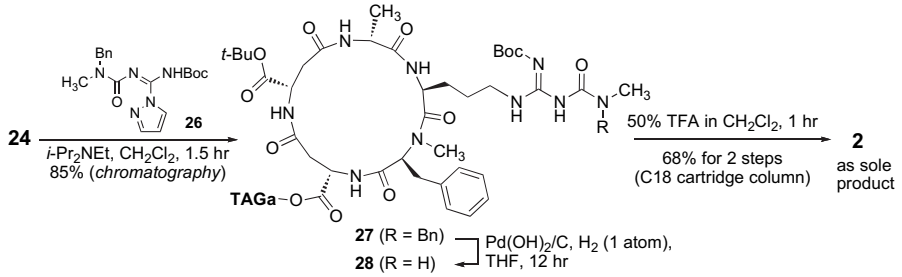


Fig. 5. HPLC chart for the soluble crude products into H_2O of final deprotection in Scheme 4. HPLC condition: same as described in Fig. 3.

time-consuming HPLC purification at the final isolation was required, directly contrasting with our first-generation solid-phase approach (Fig. 3). PMB protective group of **25** or PMB-bound argifin tolerated under the conditions of hydrogenolysis with Pd/C or $Pd(OH)_2/C$ and oxidative cleavage with DDQ/H_2O. Therefore, the stepwise deprotection of PMB and TAGa from **25** could not be achieved.

We also attempted to use 1*H*-pyrazole-1-[*N*-(*tert*-butoxycarbonyl)-*N*′-(*N*-benzylcarbamoyl-*N*-methyl)]carboxamidine (**26**) instead of **11** for introduction of the N^{ω}-methylcarbamoylguanidino group to **24** (Scheme 5). PMB-bound argifin was always accompanied with desired **2** from the final deprotection of **25** and, from our observations, this PMB group could not be removed after releasing the hydrophobic tag from core substrate under acidic conditions. Therefore, deprotection of the *N*-substitution of the guanidyl function must be conducted before the final step to overcome this problem. A novel *N*-(*tert*-butoxycarbonyl)-*N*′-(*N*-benzylcarbamoyl-*N*-methyl)-guanidino reagent (**26**) was synthesized according to our previous protocol for the preparation of **11** (Scheme 6), then subjected to guanidine formation of **24** to furnish **27** in 85% yield after silica gel flash chromatography. Subsequently, the benzyl protecting group was selectively deprotected with $Pd(OH)_2/C$ under H_2 atmosphere in THF to afford the crude debenzyl product (**28**), after removal of insoluble reagent by filtration. The crude product was then subjected to final process under the same condition as in Scheme 4, yielding solely desired product **2**, which was further purified by simple C18-cartridge column chromatography to afford **2** in 68% in pure form. This moderate yield from **27** to **2** may be due to a part of the tagged cyclic peptide being adsorbed to $Pd(OH)_2/C$ under the deprotection of the benzyl group and eliminated by the work-up process. Irrespectively, it could be satisfactorily improved by utilization of the benzyl protecting group for overall yield from **24** to **2**. The synthesized **2** was identical in all respects with authentic **2**.[21b]

Our first-generation approach produced 13% overall yield for 15 linear steps, with one time HPLC separation. However, the newly



Scheme 5. Alternative synthesis of argifin (**2**) from **24**.

accomplished liquid-phase approach is significantly more efficient and practical, proceeding in 44% overall yield for 16 linear steps without HPLC purification process, and with 10 times recrystallization and 4 times flash chromatography, including the purification of argifin.

3. Conclusion

In the case of multi-step synthesis of the very hydrophilic target compound, a hydrophobic tag 3,4,5-tris(octadecyloxy)benzyl (TAGa) was effective in improving the traditional organic synthesis processes. The second-generation total synthesis of argifin has been achieved via an efficient and practical strategy using HO-TAGa in liquid-phase, proving to be a major improvement on the existing solid-phase approach. So far, the application of HO-TAGa and its related compounds has not been reported in the field of the synthesis of natural products. This novel synthetic route not only renders the method of large amount production of a valuable natural product argifin, it also represents the first use of HO-TAGa for complex natural product synthesis.

4. Experimental section

4.1. General

Fmoc-Asp(OAllyl)OH (**6**), Fmoc-*N*-Me-Phe-OH (**7**), Fmoc-D-Ala-OH (**9**), and Fmoc-Asp-O*t*-Bu (**10**), ByBop, PyBrop, and HATU were purchased from Watanabe Chemical Industries, Ltd. (Hiroshima, Japan). Dehydrated tetrahydrofuran (THF), dehydrated CH_2Cl_2, dehydrated DMF, dehydrated acetone, MeOH, EtOH, and trifluoroacetic acid (TFA) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Methyl gallate (**12**) was purchased from Tokyo Chemical Industry. Co., Ltd. (Tokyo, Japan). C18-cartridge column (Sep-Pak® Plus C18) was purchased from Waters Co. (MA, U.S.A.). Pre-coated silica gel plates with a fluorescent indicator (Merck 60 F_{254}) were used for analytical and preparative thin-layer chromatography. Flash column chromatography was carried out with Kanto Chemical silica gel (Kanto Chemical Co., Inc., Silica gel 60N, spherical neutral, 0.040–0.050 mm, Cat.-No. 37563-84). ^{1}H NMR spectra were recorded at 270 MHz or 400 MHz or 500 MHz and ^{13}C NMR spectra were recorded at 67.5 MHz or 100 MHz or 125 MHz on JEOL JNM-EX270 (270 MHz) or Varian XL-400 (400 MHz) or JEOL ECA500 (500 MHz). Chemical shifts are expressed in parts per million downfield from internal solvent peaks $CHCl_3$ (7.26 ppm, ^{1}H NMR), pyridine (8.71 (br), 7.55 (br), 7.19 (br) ppm, ^{1}H NMR), DMF (8.01 (br), 2.91(5), 2.74 (5) ppm, ^{1}H NMR), $CDCl_3$ (77.0 ppm, ^{13}C NMR), pyridine-d_5 (123.5, 135.5, 149.2 ppm, ^{13}C NMR), DMF-d_7 (30.1, 35.2, 167.7 ppm, ^{1}H NMR), D_2O (the end of both fields; 0, 200 ppm, ^{13}C NMR) and *J* values are given in hertz. The coupling patterns are expressed by s (singlet), d (doublet), t (triplet), m (multiplet). All infrared spectra were measured on a Horiba FT-210 spectrometer. High-resolution mass spectra were measured on a JEOL JMS-700V HA mass spectrometer. HPLC analysis was conducted on a Waters 2795 Separation Module with Alliance® HT (Column; Senshu Pak PEGASIL ODS 2ø×50 mm: condition of HPLC; gradient 10% MeCN (0.05% TFA)/H_2O (0.1% TFA) to 100% MeCN (0.05% TFA) over 8 min, flow rate 0.3 mL/min, detect 210–400 nm, temperature 20 °C). Melting points were measured on OptiMelt (Stanford Research Systems) apparatus.

4.2. Synthetic procedures

*4.2.1. Methyl 3,4,5-tris(octadecyloxy)benzoate (**13**).* K_2CO_3 (54.0 g, 0.391 mol), 1-bromooctadecane (43.5 g, 0.130 mol), and potassium iodide (195 mg, 1.17 mmol) were added to a solution of methyl gallate (**12**) (8.0 g, 0.0434 mol) in dehydrated acetone (310 mL) at room temperature, and the reaction mixture was then warmed to 70 °C. After stirring for 48 h, the reaction mixture was cooled to room temperature and concentrated. The crude residue was then dissolved into CH_2Cl_2 (2.3 L) and washed with H_2O (2.3 L). The organic layer was concentrated and redissolved into CH_2Cl_2 (400 mL), which was then recrystallized by the addition of EtOH (800 mL) to furnish pure **13** as a colorless powder (30.7 g, 76%). All physical data for **13** matched with the data in Tamiaki's paper.[27]

*4.2.2. 3,4,5-Tris(octadecyloxy)benzyl alcohol (**3**) (HO-TAGa).* To a solution of $LiAlH_4$ (2.5 g, 0.0652 mol) in dehydrated THF (500 mL) was added a solution of **13** (30.7 g, 0.0326 mol) in dehydrated THF (590 mL) at room temperature, and the reaction mixture was stirred for 1.5 h. The reaction mixture was then cooled to 0 °C and quenched carefully by the addition of H_2O (2.5 mL) and 15% NaOH aq solution (7.4 mL), the resultant mixture was then warmed to room temperature. After stirring for 1 h, H_2O (7.4 mL) was added to the mixture followed by anhydrous $MgSO_4$ (10 g), the resultant mixture being filtered and concentrated. The crude product was dissolved into CH_2Cl_2 (300 mL) and recrystallized by the addition of MeOH (2 L) to furnish pure **3** as a colorless powder (29.2 g, 98%). All physical data for **3** matched with the data in Tamiaki's paper.[27]

4.2.3. Fmoc-Asp(OAllyl)-O-TAGa. To a solution of **3** (HO-TAGa) (3.0 g, 3.28 mmol) in CH_2Cl_2 (66 mL) was added Fmoc-Asp(OAllyl)-OH (**6**) (1.69 g, 4.26 mmol), 4-dimethylaminopyridine (80 mg, 0.66 mmol), and *N,N′*-dicyclohexylcarbodiimide (880 mg, 4.26 mmol) at 0 °C. After stirring for 15 min at 0 °C, the reaction mixture was warmed to room temperature and stirred for further 1 h. The reaction mixture was then cooled to 0 °C, and ice-cool MeOH (330 mL) was added. The resulting heterogeneous solution was stirred for further 10 min at 0 °C, and the colorless precipitate was filtered and washed with additional MeOH (66 mL) to afford Fmoc-Asp(OAllyl)-*O*-TAGa (4.23 g, 100%) as a colorless powder. R_f=0.72 (silica gel, hexane/EtOAc=3/1); IR ν max (cm^{-1}) (KBr) 3312 (w), 2920 (s), 1738 (m); $[\alpha]_D^{21}$ +4.87 (*c* 1.0, $CHCl_3$); mp 87–89 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 7.76 (d, *J*=7.3 Hz, 2H), 7.58 (d, *J*=7.6 Hz, 2H), 7.40 (t, *J*=7.0 Hz, 2H), 7.29 (t, *J*=7.6 Hz, 2H), 6.51 (s, 2H), 5.93–5.78 (complex m, 2H), 5.29 (dd, *J*=17.8, 1.4 Hz, 1H), 5.23 (dd, *J*=10.5, 1.1 Hz, 1H), 5.10 (s, 2H), 4.70 (m, 1H), 4.55 (d, *J*=5.9 Hz, 2H), 4.33 (complex m, 3H), 3.92 (m, 6H), 3.10 (dd, *J*=16.2, 4.6 Hz, 1H), 2.90 (dd, *J*=18.6, 4.6 Hz, 1H), 1.75 (m, 6H), 1.35 (m, 90H), 0.88 (t, *J*=6.5 Hz, 9H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ (ppm) 170.5, 170.4, 155.9, 153.2, 143.8, 143.6, 141.2, 138.3, 131.5, 130.0, 127.7, 127.0, 125.0, 119.9, 118.7, 106.9, 77.6, 77.0, 76.5, 73.3, 69.1, 67.9, 67.2, 65.6, 50.5, 47.0, 36.6, 31.9, 30.3, 29.7, 29.6, 29.4, 29.4, 26.1, 14.1 (many signals, especially alkyl chains, overlapped.); HRMS (FAB, NBA matrix) *m/z*: 1291.0240 [$(M+H)^+$ calcd for $C_{83}H_{136}O_9N$: 1291.0215].

4.2.4. General procedure for Fmoc deprotection. Fmoc protected amino acid or peptide was dissolved into 5% piperidine/CH_2Cl_2 (0.036 M for substrate) at room temperature, and the solution was stirred for 3 h. The reaction mixture was subsequently cooled to 0 °C and ice-cool MeOH (generally five times excess of CH_2Cl_2) was added. The resulting heterogeneous solution was stirred for a further 10 min at 0 °C, and the colorless precipitate was filtered and washed with additional MeOH to afford the corresponding pure amine.

*4.2.5. Asp(OAllyl)-O-TAGa (**14**).* Following the procedure described for general procedure for Fmoc deprotection, Fmoc-Asp(OAllyl)-*O*-TAGa (4.23 g, 3.28 mmol) was converted to **14** (3.51 g, 100%) as a colorless powder. R_f=0.67 (silica gel, $CHCl_3$/MeOH=20/1); IR (KBr) ν (cm^{-1}) 3386 (w), 2917 (s), 1735 (w); $[\alpha]_D^{21}$–3.48 (*c* 1.0, $CHCl_3$); mp 55–56 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 6.52 (s, 2H), 5.87 (m,

1H), 5.30 (dd, J=17.3, 1.6 Hz, 1H), 5.22 (dd, J=10.5, 1.4 Hz, 1H), 5.06 (s, 2H), 4.56 (d, J=5.7 Hz, 2H), 3.94 (m, 6H), 3.86 (dd, J=7.0, 4.9 Hz, 1H), 2.80 (dd, J=17.8, 4.6 Hz, 1H), 2.75 (dd, J=16.5, 7.0 Hz, 1H), 1.76 (m, 6H), 1.36 (m, 90H), 0.88 (t, J=6.2 Hz, 9H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ (ppm) 174.0, 171.0, 153.2, 138.3, 131.8, 130.3, 118.4, 107.0, 77.5, 77.0, 76.5, 73.4, 69.1, 67.4, 65.4, 38.9, 31.9, 30.3, 29.7, 29.6, 29.4, 29.3, 26.1, 22.6, 22.6, 14.1, 14.0 (many signals, especially alkyl chains, overlapped.); HRMS (FAB, NBA matrix) m/z: 1068.9565 [$(M+H)^+$; calcd for $C_{68}H_{126}O_7N$: 1068.9534].

4.2.6. Fmoc-N-Me-Phe-Asp(OAllyl)-O-TAGa. At room temperature, to a solution of **14** (3.51 g, 3.28 mmol) in CH_2Cl_2 (66 mL) was added Fmoc-*N*-Me-Phe-OH (**7**) (1.45 g, 3.61 mmol), *i*-Pr_2NEt (1.42 mL, 8.20 mmol), and PyBop (2.05 g, 3.93 mmol), and the mixture was stirred for 3 h. Then, the reaction mixture was then recrystallized by the procedure described in Section 4.2.3 to afford Fmoc-*N*-Me-Phe-Asp(OAllyl)-*O*-TAGa (4.76 g, 100%) as a colorless powder. R_f=0.48 (silica gel, hexane/EtOAc=3/1); IR (KBr) ν (cm^{-1}) 3399 (w), 2917 (s), 1683 (m); $[\alpha]_D^{21}$ −17.8 (c 1.0, $CHCl_3$); mp 49–51 °C; ^{1}H NMR (400 MHz, pyridine-d_5, 90 °C) δ (ppm) 7.84 (d, J=6.1 Hz, 2H), 7.65–7.29 (complex m, 11H), 6.92 (s, 2H), 5.95 (m, 1H), 5.36 (s, 2H), 5.27 (m, 2H), 4.65 (d, J=5.1 Hz, 2H), 4.49–4.12 (complex m, 5H), [4.29 (t, J=4.3 Hz), 4.17 (t, J=4.1 Hz), total 6H, due to rotamer], 3.63 (d, J=3.8 Hz, 1H), 3.60 (d, J=3.8 Hz, 1H), 3.31 (dd, J=11.1, 4.6 Hz, 1H), 3.19 (dd, J=15.7, 4.6 Hz, 1H), 3.12 (s, 3H), [2.08 (m), 1.93 (m), total 6H, due to rotamer], 1.42 (m, 90H), 0.97 (t, J=5.1 Hz, 9H); ^{13}C NMR (100 MHz, pyridine-d_5, 90 °C) δ (ppm) 171.4, 171.2, 171.0, 170.6, 170.5, 154.1, 144.8, 141.9, 139.8, 138.6, 132.8, 131.5, 129.9, 129.6, 129.3, 128.9, 128.2, 127.6, 127.6, 127.0, 126.9, 125.6, 125.5, 121.7, 120.5, 120.3, 118.2, 108.4, 73.8, 70.0, 68.1, 67.9, 67.8, 65.6, 61.0, 50.5, 49.7, 47.9, 39.7, 37.1, 36.8, 35.3, 32.2, 31.2, 31.1, 30.1, 30.1, 30.0, 30.0, 29.8, 29.6, 26.7, 26.7, 22.9, 14.2 (many signals, especially alkyl chains, overlapped.); HRMS (FAB, NBA) m/z: 1474.0873 [$(M+Na)^+$, calcd for $C_{93}H_{146}O_{10}N_2Na$: 1474.0875].

*4.2.7. N-Me-Phe-Asp(OAllyl)-O-TAGa (**15**).* Following the procedure described for general procedure for Fmoc deprotection, Fmoc-*N*-Me-Phe-Asp(OAllyl)-*O*-TAGa (3.00 g, 2.07 mmol) was converted to **15** (2.55 g, 100%) as a colorless powder. R_f=0.21 (silica gel, hexane/EtOAc=3/1); IR (KBr) ν (cm^{-1}) 3336 (w), 2916 (s), 1730 (w), 1469 (w), 987.4(w); $[\alpha]_D^{22}$ −10.5 (c 1.0, $CHCl_3$); mp 64–66 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 8.08 (d, J=8.9 Hz, 1H), 7.25 (m, 4H), 6.50 (s, 2H), 5.85 (m, 1H), 5.29 (dd, J=17.6, 1.4 Hz, 1H), 5.22 (dd, J=10.5, 1.6 Hz, 1H), 5.06 (s, 2H), 4.93 (m, 1H), 4.52 (d, J=5.4 Hz, 2H), 3.94 (m, 6H), 3.18 (complex m, 2H), 3.07 (dd, J=16.7, 4.6 Hz, 1H), 2.79 (dd, J=16.7, 4.6 Hz, 1H), 2.64 (dd, J=13.5, 9.7 Hz, 1H), 2.23 (s, 3H), 1.75 (m, 6H), 1.36 (m, 90H), 0.88 (t, J=6.2 Hz, 9H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ (ppm) 173.5, 170.5, 170.2, 153.1, 138.2, 137.3, 131.6, 130.0, 129.0, 128.6, 126.8, 118.5, 106.9, 77.5, 77.0, 76.5, 73.3, 69.0, 67.8, 65.9, 65.5, 47.9, 39.2, 36.4, 35.2, 31.9, 31.9, 30.3, 29.7, 29.6, 29.6, 29.4, 29.3, 26.1, 22.6, 14.1, 14.1 (many signals, especially alkyl chains, overlapped.); HRMS (FAB, NBA matrix) m/z: 1230.0372 [$(M+H)^+$, calcd for $C_{78}H_{137}O_8N_2$: 1230.0375].

4.2.8. Fmoc-Orn(Dde)-N-Me-Phe-Asp(OAllyl)-O-TAGa. At room temperature, to a solution of **15** (2.55 g, 2.07 mmol) in CH_2Cl_2/DMF (10/1) (42 mL) was added Fmoc-Orn(Dde)-OH (**8**) (1.61 g, 3.11 mmol), *i*-Pr_2NEt (1.08 mL, 6.21 mmol), and PyBrop (1.45 g, 3.11 mmol), and the solution was stirred for 3 h. The reaction mixture was then cooled to 0 °C and ice-cool MeOH (210 mL) was added. The resulting heterogeneous solution was stirred for a further 10 min at 0 °C, and the colorless precipitate was filtered and washed with additional MeOH to afford the mixture of **15** and Fmoc-Orn(Dde)-*N*-Me-Phe-Asp(OAllyl)-*O*-TAGa, which was again subjected to condensation reaction under the same conditions. After replicating the condensation reaction twice, the reaction mixture was recrystallized again by the procedure described above to afford pure Fmoc-Orn(Dde)-*N*-Me-Phe-Asp(OAllyl)-*O*-TAGa (3.59 g, 100%) as a colorless powder. R_f=0.38 (siliga gel, hexane/EtOAc/AcOH=1/1/0.1); IR (KBr) ν (cm^{-1}) 3288 (w), 2917 (s), 1575 (m), 1118 (m); $[\alpha]_D^{23}$−18.1 (c 1.0, $CHCl_3$); mp 57–58 °C; ^{1}H NMR (400 MHz, DMF, 80 °C) δ (ppm) 7.98 (d, J=5.1 Hz, 2H), 7.93 (d, J=5.1 Hz, 2H), 7.52 (d, J=5.1 Hz, 2H), 7.44 (d, J=5.1 Hz, 2H), 7.36–7.28 (complex m, 8H), 6.82 (s, 2H), 6.01 (m, 1H), 5.41 (dt, J=11.6, 1.1 Hz, 1H), 5.30 (dd, J=7.2, 0.7 Hz, 1H), 5.21 (s, 2H), 5.26–4.97 (complex m, 3H), 4.66 (d, J=3.3 Hz, 2H), [4.14 (t, J=4.2 Hz), 4.06 (t, J=4.5 Hz), total 6H, due to rotamer], 3.50–2.76 (complex m, 9H), 2.63 (m, 3H), 2.41 (s, 4H), 1.86 (m, 6H), 1.54–1.26 (complex m, 94H), 1.10 (s, 6H), 1.09 (t, J=4.9 Hz, 9H); ^{13}C NMR (125 MHz, DMF, 80 °C); 171.8, 154.7, 145.0, 141.5, 139.8, 134.1, 133.0, 132.6, 131.7, 130.3, 129.9, 128.6, 128.5, 127.7, 125.0, 122.6, 121.3, 118.6, 109.6, 108.8, 108.8, 80.5, 74.4, 70.8, 70.7, 68.3, 67.7, 66.5, 66.1, 64.7, 56.0, 54.2, 53.2, 52.4, 51.0, 44.4, 44.1, 43.9, 43.0, 40.9, 38.3, 37.6, 34.1, 33.5, 33.0, 31.7, 31.6, 31.4, 31.2, 31.0, 30.9, 30.8, 30.6, 30.6, 30.4, 30.4, 29.1, 27.5, 27.4, 27.1, 26.6, 23.7, 18.2, 14.8 (many signals were overlapped, and some appropriate signals were disappeared due to the equilibrium of rotamer.); HRMS (FAB, NBA matrix) m/z: 1752.2551 [$(M+Na)^+$, calcd for $C_{108}H_{168}O_{13}N_4Na$: 1752.2506].

*4.2.9. Orn(Dde)-N-Me-Phe-Asp(OAllyl)-O-TAGa (**16**).* Following the procedure described for general procedure of Fmoc deprotection, Fmoc-Orn(Dde)-*N*-Me-Phe-Asp(OAllyl)-*O*-TAGa (3.59 g, 2.07 mmol) was converted to **16** (3.13 g, 100%) as a colorless powder. R_f=0.33 (silica gel, $CHCl_3$/MeOH=10/1); IR (KBr) ν (cm^{-1}) 3452 (w), 2918 (s), 1577 (w), 1119 (w); $[\alpha]_D^{23}$−25.9 (c 1.0, $CHCl_3$); mp 52–53 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 7.18 (m, 5H), 6.50 (s, 2H), 5.82 (m, 1H), 5.25 (m, 2H), 5.11 (d, J=11.9 Hz, 1H), 5.00 (d, J=11.6 Hz, 1H), 4.86 (m, 1H), 4.76 (m. 1H), 4.48 (d, J=5.9 Hz, 2H), 3.96 (m, 6H), 3.12–2.79 (complex, 7H), 2.82 (s, 3H), 2.48 (s, 3H), 2.36 (s, 3H), 2.34 (s, 1H), 1.76 (m, 6H), 1.57 (m, 2H), 1.24 (complex m, 92H), [1.04 (s), 1.01(s), total 6H, due to rotamer], 0.88 (t, J=6.6 Hz, 9H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ (ppm) 173.2, 172.0, 170.2, 170.1, 169.4, 153.1, 138.3, 137.6, 131.5, 130.0, 128.9, 128.7, 128.7, 128.4, 126.8, 118.8, 107.7, 106.9, 77.5, 77.2, 77.0, 76.5, 73.3, 69.1, 67.7, 65.6, 61.2, 51.2, 48.8, 42.9, 36.3, 33.3, 33.1, 31.8, 30.2, 30.0, 29.6, 29.6, 29.3, 29.3, 28.3, 28.2, 26.0, 25.5, 22.6, 17.8, 14.1, 14.0 (many signals, especially alkyl chains, overlapped); HRMS (FAB) m/z: 1530.1804 [$(M+Na)^+$, calcd for $C_{93}H_{158}O_{11}N_4Na$: 1531.1825].

4.2.10. Fmoc-D-Ala-Orn(Dde)-N-Me-Phe-Asp(OAllyl)-O-TAGa. At room temperature, to a solution of **16** (2.50 g, 1.66 mmol) in CH_2Cl_2 (33 mL) was added Fmoc-D-Ala-OH (**9**) (713 mg, 2.16 mmol), *i*-Pr_2NEt (0.87 mL, 4.98 mmol), and PyBop (1.30 g, 2.49 mmol), and the solution was stirred for 3 h. The reaction mixture was then recrystallized by the procedure described in Section 4.2.3 to afford Fmoc-D-Ala-Orn(Dde)-*N*-Me-Phe-Asp(OAllyl)-*O*-TAGa (2.99 g, 100%) as a colorless powder. R_f=0.51 (silica gel, $CHCl_3$/MeOH=10/1); IR (KBr) ν (cm^{-1}) 3286 (w), 2918 (s), 1576 (m), 1118 (m); $[\alpha]_D^{23}$−21.9 (c 1.0, $CHCl_3$); mp 58–59 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.99 (d. J=7.2 Hz, 1H), 7.76 (d, J=7.2 Hz, 1H), 7.73 (d, J=7.6 Hz, 1H), 7.58 (m, 2H), 7.41–7.11 (complex m, 10H), 6.48 (d, J=7.0 Hz, 2H), 5.78 (m, 1H), 5.37–4.73 (complex m, 6H), 4.49 (d, J=5.6 Hz, 1H), 4.46 (d, J=5.9 Hz, 1H), 4.40–4.18 (complex m, 3H), 3.91 (t, J=6.5 Hz, 6H), 3.36–2.86 (complex m. 6H), 2.83 (s, 3H), 2.48 (s, 3H), 2.33 (s, 4H), 1.74 (m, 6H), 1.64 (m, 2H), 1.25 (complex m, 95H), 1.01 (m, 6H), 0.88 (t, J=6.7 Hz, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 173.7, 173.4, 172.0, 171.9, 171.6, 170.7, 170.3, 170.21, 170.16, 169.3, 168.8, 153.2, 143.7, 141.3, 141.2, 138.4, 138.3, 137.7, 131.5, 131.4, 129.9, 129.0, 128.9, 128.0, 127.8, 127.7, 127.7, 127.1, 127.1, 127.0, 126.8, 125.1, 125.0, 120.0, 120.0, 118.9, 118.7, 107.9, 107.0, 73.5, 69.2, 68.1, 67.2, 65.8, 65.8, 62.1, 50.2, 49.4, 49.0, 49.4, 49.0, 48.5, 48.3, 42.8, 42.6, 31.9, 30.3, 30.0, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.1, 26.0, 22.3, 17.8, 14.1 (many signals, especially alkyl chains,

overlapped); HRMS (FAB, NBA matrix) *m/z*: 1823.2839 [(M+Na)$^+$, calcd for $C_{111}H_{173}O_{14}N_5Na$: 1823.2877].

*4.2.11. D-Ala-Orn(Dde)-N-Me-Phe-Asp(OAllyl)-O-TAGa (**17**).* Following the procedure described for general procedure of Fmoc deprotection, Fmoc-D-Ala-Orn(Dde)-*N*-Me-Phe-Asp(OAllyl)-*O*-TAGa (2.00 g, 1.10 mmol) was converted to **17** (1.74 g, 100%) as a colorless powder. R_f=0.37 (silica gel, $CHCl_3$/MeOH=15/1); IR (KBr) ν (cm^{-1}) 3271 (w), 2920 (s), 2848 (s), 1739 (w), 1641 (w), 1579 (w), 1506 (w), 1468 (w), 1441 (w), 1369 (w), 1336 (w), 1234 (w), 1207 (w), 1119 (w), 991 (w); $[\alpha]_D^{26}$ −26.3 (*c* 1.0, $CHCl_3$); mp 53–54 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 7.23 (m, 5H), [6.52 (s), 6.49 (s), total 2H, due to rotamer], 5.82 (m, 1H), 5.24 (m, 2H), [5.07 (s), 5.04(s), total 2H, due to rotamer], 4.49 (d, *J*=5.7 Hz, 2H), 5.12–4.76 (complex m, 3H), 3.94 (m, 6H), 3.47–2.87 (complex m, 7H), [2.88 (s), 2.84 (s), total 3H, due to rotamer], [2.51 (s), 2.49 (s), total 3H, due to rotamer], 2.36 (s, 4H), 1.73 (m, 6H), 1.60 (m, 2H), 1.35 (complex m, 95H), [1.03 (s), 1.01 (s), total 6H, due to rotamer], 0.87 (t, *J*=7.0 Hz, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 177.0, 175.3, 173.4, 173.2, 172.0, 170.2, 169.6, 168.8, 153.1, 138.2, 137.9, 136.6, 131.6, 131.4, 130.0, 129.8, 129.5, 128.8, 128.6, 128.4, 126.7, 126.6, 118.6, 107.8, 107.7, 106.8, 77.5, 77.2, 76.5, 73.3, 69.0, 67.7, 65.5, 62.0, 50.4, 50.1, 49.4, 48.9, 47.9, 47.8, 42.6, 35.9, 31.8, 30.2, 30.0, 29.9, 29.6, 29.5, 29.3, 29.2, 29.1, 26.0, 25.5, 22.6, 21.5, 21.1, 17.7, 14.0 (many signals, especially alkyl chains, overlapped); HRMS (ESI$^+$) *m/z*: 1579.2317 [(M+H)$^+$, calcd for $C_{96}H_{164}O_{12}N_5$: 1579.2377].

*4.2.12. Fmoc-Asp-Ot-Bu-D-Ala-Orn(Dde)-N-Me-Phe-Asp(OAllyl)-O-TAGa (**18**).* At room temperature, to a solution of **17** (1.74 g, 1.10 mmol) in CH_2Cl_2 (22 mL) was added Fmoc-Asp-O*t*-Bu (**10**) (587 mg, 1.43 mmol), *i*-Pr_2NEt (0.57 mL, 3.30 mmol), and PyBop (858 mg, 1.65 mmol), and the solution was stirred for 3 h. The reaction mixture was then recrystallized by the procedure described in Section 4.2.3 to afford **18** (2.11 g, 97%) as a colorless powder. R_f=0.44 (silica gel, $CHCl_3$/MeOH=15/1); IR (KBr) ν (cm^{-1}) 3286 (w), 2918 (s), 2850 (s), 1736 (m), 1680 (m), 1643 (m), 1577 (m), 1508 (m), 1468 (m), 1369 (m), 1336 (m), 1234 (m), 1159 (m), 1119 (m), 758 (m); $[\alpha]_D^{21}$ −14.6 (*c* 0.5, $CHCl_3$); mp 54–56 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.02 (d, *J*=7.2 Hz, 1H), 7.75 (d, *J*=7.5 Hz, 2H), 7.65 (d, *J*=7.1 Hz, 1H), 7.57 (d, *J*=7.4 Hz, 1H), 7.40–6.87 (complex m, 10H), 6.53 (s, 2H), 5.81 (m, 1H), 5.29–4.73 (complex m, 7H), 4.50 (d, *J*=5.7 Hz, 2H), 4.38–4.18 (complex m, 3H), 3.93 (m, 6H), 3.35–2.88 (complex m, 8H), 2.81 (s, 3H), 2.49 (s, 3H), 2.36 (s, 4H), 1.75 (m, 6H), 1.63 (m, 2H), 1.44 (m, 9H), 1.25 (complex m, 95H), 1.03 (m, 6H), 0.87 (t, *J*=6.9 Hz, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 173.5, 173.5, 173.4, 172.1, 171.8, 171.7, 170.6, 170.4, 170.4, 170.3, 170.2, 170.0, 169.3, 168.9, 156.2, 156.1, 153.2, 153.2, 143.9, 143.8, 141.2, 138.4, 137.8, 131.5, 131.4, 129.8, 129.6, 129.0, 128.9, 127.7, 127.0, 126.8, 125.2, 119.9, 118.9, 118.7, 107.9, 107.8, 107.0, 107.0, 82.3, 77.2, 73.4, 69.2, 68.2, 67.1, 65.9, 65.7, 62.1, 51.6, 50.8, 49.6, 49.0, 48.8, 48.6, 48.4, 47.1, 42.8, 42.6, 38.0, 37.8, 36.2, 34.1, 33.8, 34.1, 31.9, 30.3, 30.1, 30.0, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 28.2, 28.0, 27.9, 27.9, 26.1, 25.4, 24.8, 22.7, 17.9, 14.1 (many signals, especially alkyl chains, overlapped); HRMS (FAB, NBA matrix) *m/z*: 1994.3765 [(M+Na)$^+$, calcd for $C_{119}H_{186}O_{17}N_6Na$: 1994.3772].

*4.2.13. Fmoc-Asp-Ot-Bu-D-Ala-Orn(Dde)-N-Me-Phe-Asp-O-TAGa (**19**).* At room temperature, to a solution of **18** (1.00 g, 0.507 mmol) and dimethyl-1,3-cyclohexadione (dimedone) (142 mg, 1.01 mmol) in THF (10 mL) was added $Pd(PPh_3)_4$ (58.6 mg, 0.05 mmol) under N_2, and the solution was stirred for 30 min. The reaction mixture was then cooled to 0 °C, and ice-cool MeOH (50 mL) was added. The resulting heterogeneous solution was stirred for a further 10 min at 0 °C, and the light yellow precipitate was filtered and washed with additional MeOH. Further purification of crude product was carried out by flash chromatography ($CHCl_3$/MeOH=100/1 to 20/1) to afford **19** (906 mg, 92%) as a colorless powder. R_f=0.50 (silica gel, $CHCl_3$/MeOH=10/1); IR (KBr) ν (cm^{-1}) 3288 (w), 2924 (s), 2852 (s), 1728 (m), 1643 (m), 1574 (m), 1468 (m), 1452 (m), 1369 (m), 1335 (m), 1232 (m), 1157 (m), 1117 (m), 758 (w), 741 (w); $[\alpha]_D^{27}$ −28.5 (*c* 1.0, $CHCl_3$); mp 65–67 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.74 (d, *J*=3.0 Hz, 2H), 7.58 (d, *J*=4.4 Hz, 2H), 7.40–7.09 (complex m, 11H), 6.51 (s, 2H), 6.32 (br s, 1H), 5.24–4.78 (complex m, 5H), 4.45–4.15 (complex m, 3H), 3.92 (m, 6H), 3.41–2.70 (complex m, 11H), 2.52 (d, *J*=6.8 Hz, 3H), 2.36 (s, 4H), 1.76 (complex m, 8H), 1.43 (m, 9H), 1.25 (complex m, 95H), 1.01 (s, 6H), 0.88 (t, *J*=6.4 Hz, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 174.5, 173.6, 173.3, 172.2, 171.2, 170.9, 19.9, 169.6, 168.7, 156.1, 153.2, 143.8, 143.6, 141.2, 138.3, 129.9, 129.6, 129.1, 128.9, 128.6, 127.7, 127.1, 126.8, 126.6, 125.1, 120.0, 108.0, 127.9, 107.0, 106.8, 105.0, 82.4, 77.2, 73.4, 69.2, 68.0, 67.9, 67.3, 51.5, 49.4, 49.1, 48.4, 47.0, 43.3, 38.2, 33.4, 31.9, 30.3, 30.2, 30.1, 29.8, 29.8, 29.7, 29.7, 29.5, 29.4, 29.4, 28.9, 28.2, 28.2, 27.9, 27.8, 26.2, 22.7, 18.3, 17.9, 14.1 (many signals, especially alkyl chains, overlapped); HRMS (FAB, NBA matrix) *m/z*: 1954.3486 [(M+Na)$^+$, calcd for $C_{116}H_{182}O_{17}N_6Na$: 1954.3459].

*4.2.14. cyclo-(Asp-O-TAGa-N-Me-Phe-Orn(Dde)-D-Ala-Asp-Ot-Bu) (**21**).* At room temperature, **19** (200 mg, 0.103 mmol) was dissolved into 5% piperidine/CH_2Cl_2 (2.0 mL), and the solution was stirred for 3 h. Ice-cool 100 mM HOBt in MeOH (100 mL) was then added to the reaction mixture. The resulting colorless precipitate was filtered and washed with an additional 100 mM HOBt in MeOH to afford the corresponding ammonium salt (**23**) as a colorless solid, which was used for cyclization without further purification.

At room temperature, to a solution of crude **23** in CH_2Cl_2 (6.3 mL) were added *i*-Pr_2NEt (0.33 mL, 1.88 mmol) and PyBop (244 mg, 0.470 mmol), and the solution was stirred for 2 h. The reaction mixture was subsequently cooled to 0 °C, and ice-cool MeOH (32 mL) was added. The resulting heterogeneous solution was stirred for a further 10 min at 0 °C, and the light yellow precipitate was filtered and washed with additional MeOH. Further purification of crude product was carried out by flash chromatography ($CHCl_3$/MeOH=100/1 to 10/1) to afford **21** (148.8 mg, 85%) as a colorless solid. R_f=0.50 (silica gel, $CHCl_3$/MeOH=10/1); IR (KBr) ν (cm^{-1}) 3419 (w), 3288 (w), 2920 (s), 2850 (s), 1738 (w), 1639 (w), 1577 (w), 1506 (w), 1468 (w), 1456(w), 1441 (w), 1369 (w), 1334 (w), 1302 (w), 1286 (w), 1248 (w), 1234 (w), 1159 (w), 1119 (w), 721 (w), 704 (w), 633 (w); $[\alpha]_D^{22}$ −29.4 (*c* 1.0, $CHCl_3$); mp 73–75 °C; HRMS (FAB, NBA matrix) *m/z*: 1714.2711 [(M+Na)$^+$, calcd for $C_{101}H_{170}O_{14}N_6Na$: 1714.2673]; Compound **21** gave broad spectra for NMR in less-polar solvents due to the equilibrium of the rotamer, and each signal of **21** could not be assigned. Therefore, **21** was indirectly confirmed by NMR in 0.4% TFA/D_2O for the unprotected compound, which was prepared by deprotection of **24** under the condition of TFA/CH_2Cl_2 (1/1) (see Section 4.2.17).

*4.2.15. Asp-Ot-Bu-D-Ala-Orn(Dde)-N-Me-Phe-Asp(piperidyl)-O-TAGa (**22**).* R_f=0.58 ($CHCl_3$/MeOH=10/1); IR (KBr) ν (cm^{-1}) 3363 (w), 3296 (w), 2918 (s), 2850 (s), 1736 (w), 1678 (w), 1643 (w), 1577 (w), 1504 (w), 1468 (w), 1442 (w), 1369 (w), 1335 (w), 1302 (w), 1286 (w), 1252 (w), 1223 (w), 1157 (w), 1119 (w), 1014 (w); $[\alpha]_D^{25}$ −25.7 (*c* 1.0, $CHCl_3$); mp 53–55 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.23 (m, 5H), 6.50 (s, 2H), 5.16–4.48 (complex m, 5H), 3.97–3.56 (complex m, 12H), 3.42–2.75 (complex m, 7H), 2.64 (s, 3H), 2.60 (dd, *J*=9.6, 2.3 Hz, 1H), 2.48 (m, 3H), 2.36 (s, 4H), 1.82–1.12 (complex m, 118H), 1.03 (s, 6H), 0.87 (t, *J*=6.9 Hz, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 173.9, 173.4, 172.6, 172.0, 171.8, 171.6, 169.3, 153.4, 138.7, 137.1, 129.7, 129.6, 129.0, 127.2, 107.8, 107.5, 81.3, 77.4, 73.5, 69.3, 68.6, 61.6, 54.0, 49.4, 48.9, 48.1, 46.6, 43.2, 43.1, 35.4, 34.0, 32.0, 30.4, 30.1, 29.8, 29.8, 29.8, 29.7, 29.5, 29.45, 29.2, 28.4, 28.1, 26.4,

26.2, 26.2, 25.6, 25.1, 24.3, 22.8, 17.9, 16.1, 14.2 (many signals, especially alkyl chains, overlapped); HRMS (FAB, NBA matrix) *m*/*z*: 1799.3580 [(M+Na)$^+$, calcd for $C_{106}H_{181}O_{14}N_7Na$: 1799.3564].

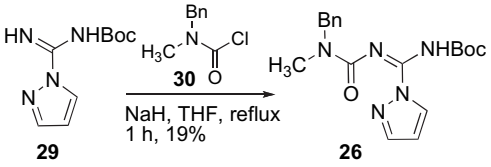
*4.2.16. cyclo-(Asp-O-TAGa-N-Me-Phe-Orn-D-Ala-Asp-Ot-Bu) (**24**).* At room temperature, **21** (57.3 mg, 0.034 mmol) was dissolved into 2% hydrazine CH_2Cl_2/DMF (4/1) solution, and the mixture was stirred for 2 h. The reaction mixture was then recrystallized by the procedure described in Section 4.2.3 to afford **24** (51.8 mg, 100%) as a colorless powder. R_f=0.29 (silica gel, $CHCl_3$/MeOH/30% NH_4OH=6/1/0.1); IR (KBr) ν (cm^{-1}) 3411 (w), 3288 (w), 2918 (s), 2850 (s), 1739 (w), 1645 (w), 1545 (w), 1467 (w), 1454 (w), 1371 (w), 1335 (w), 1238 (w), 1160 (w), 1119 (w); $[\alpha]_D^{26}$−26.2 (*c* 0.5, $CHCl_3$); mp 101–109 °C; HRMS (ESI$^+$) *m*/*z*: 1550.1806 [(M+Na)$^+$, calcd for $C_{91}H_{158}O_{12}N_6Na$: 1550.1835]. Compound **24** gave broad spectra for NMR in less-polar solvents due to the equilibrium of the rotamer, and each signal of **24** could not be assigned. Therefore, **24** was indirectly confirmed by NMR in 0.4% TFA/D_2O for the unprotected compound, which was prepared by deprotection of **24** under the condition of TFA/CH_2Cl_2 (1/1) (see Section 4.2.17).

4.2.17. cyclo-(Asp-N-Me-Phe-Orn-D-Ala-Asp). At room temperature, **24** (24.8 mg, 0.016 mmol) was dissolved into TFA/CH_2Cl_2 (1/1) (1.6 mL), and the mixture was stirred for 1 h. The reaction mixture was then concentrated and dissolved into H_2O (1.6 mL). The resulting precipitate of HO-TAGa was filtered off and washed with additional H_2O (3.2 mL). The combined filtrates were concentrated to afford pure deprotected product *cyclo*-(Asp-*N*-Me-Phe-Orn-D-Ala-Asp) (9.4 mg, 100%) as a colorless solid. IR (KBr) ν (cm^{-1}) 3423 (s), 1685 (s), 1637 (m), 1433 (w), 1354 (w), 1209 (s), 1190 (m), 1138 (m), 845 (w), 800 (w), 725 (w); $[\alpha]_D^{24}$−6.4 (*c* 0.175, 1% TFA/H_2O); mp 234–238 °C (decomposed); ^{1}H NMR (500 MHz, 0.4% TFA/D_2O) δ (ppm) 7.35 (dd, *J*=15.5, 6.9 Hz, 3H), 7.30–7.20 (complex m, 2H), 5.09 (dd, *J*=11.5, 2.3 Hz, 1H), 4.79 (m, 1H), 4.55 (dd, *J*=12.0, 1.7 Hz, 1H), 4.35 (m, 1H), 4.15 (q, *J*=7.1 Hz, 1H), 3.13 (dd, *J*=14.1, 2.6 Hz, 1H), 3.07–2.80 (complex m, 3H), 2.85 (s, 3H), 2.77 (dd, *J*=14.0, 2.6 Hz, 1H), 2.64 (m, 2H), 2.50 (m, 1H), 1.44–0.99 (complex m, 4H), 1.30 (d, *J*=7.5 Hz, 3H); ^{13}C NMR (125 MHz, 0.4% TFA/D_2O) δ (ppm) 176.9, 176.5, 175.7, 175.5, 172.9, 172.9, 171.6, 139.1, 131.3, 130.8 (×2), 129.0 (×2), 64.0, 51.8, 51.3, 51.2, 50.2, 40.2, 39.2, 36.4, 34.8, 31.2, 27.7, 25.1, 18.3; HRMS (ESI$^+$) *m*/*z*: 599.2479 [(M+Na)$^+$, calcd for $C_{26}H_{36}O_9N_6Na$: 599.2441].

*4.2.18. cyclo-[Asp-O-TAGa-N-Me-Phe-Arg{N$^{\omega}$-tert-butoxycarbonyl-N$^{\omega'}$-(N-p-methoxybenzyl-N-methylcarbamoyl)}D-Ala-Asp-Ot-Bu] (**25**).* At room temperature, to a solution of **24** (21.4 mg, 0.014 mmol) in CH_2Cl_2 (0.28 mL) were added *i*-Pr_2NEt (15 μL, 0.084 mmol) and pyrazole reagent (**11**)[21b] (16.3 mg, 0.042 mmol), and the solution was stirred for 1.5 h. Then, the reaction mixture was recrystallized by the procedure described in Section 4.2.3 to afford **25** (25.5 mg, 99%) as a colorless solid. R_f=0.45 (silica gel, $CHCl_3$/MeOH=20/1); IR (KBr) ν (cm^{-1}) 3332 (w), 3286 (w), 2922 (s), 2852 (s), 1736 (w), 1716 (w), 1635 (w), 1592 (w), 1542 (w), 1512 (w), 1468 (w), 1456 (w), 1440 (w), 1415 (w), 1389 (w), 1369 (w), 1279 (w), 1246 (w), 1157 (w), 1117 (w), 1036 (w); $[\alpha]_D^{26}$−14.0 (*c* 0.5, $CHCl_3$); mp 65–67 °C; HRMS (FAB, NBA matrix) *m*/*z*: 1869.3378 [(M+Na)$^+$, calcd for $C_{107}H_{179}O_{16}N_9Na$: 1869.3368]. Compound **25** gave broad spectra for NMR in less-polar solvents due to the equilibrium of the rotamer, and each signal of **25** could not be assigned.

*4.2.19. Argifin (**2**) from **25**.* At room temperature, **25** (8.5 mg, 0.00492 mmol) was dissolved into TFA/CH_2Cl_2 (1/1) (0.49 mL), and the mixture was stirred for 1 h. The reaction mixture was then concentrated, and the residues dissolved into H_2O (2.0 mL). The resulting precipitate of HO-TAGa was filtered off and washed with additional H_2O (2.0 mL). The combined filtrates were concentrated to afford a mixture of argifin (**2**) and PMB-bound argifin, which were separated by C18-cartridge column chromatography (Sep-Pak® Plus C18) (5–10% MeCN/H_2O) to furnish pure argifin (**2**) (1.2 mg, 36%) as a colorless solid. All physical data for synthetic **2** produced via the novel liquid-phase approach matched with the data of authentic **2** reported previously.[21b] PMB-bound argifin was identified by high-resolution mass spectrum for a product peak at 4.57 min retention time of Fig. 5. HRMS-ESI *m*/*z*: 796.3619 [(M+H)$^+$; calcd for $C_{37}H_{50}O_{11}N_9$: 796.3630].

*4.2.20. Preparation of 1H-pyrazole-1-[N-(tert-butoxycarbonyl)-N′-(N-benzylcarbamoyl-N-methyl)]carboxamidine (**26**).* To a solution of **29**[21b] (1.10 g, 5.23 mmol) in THF (53 mL) was added 55% NaH, dispersed in paraffin liquid (460 mg, 10.5 mmol) at 0 °C. After stirring at 0 °C for 10 min, the reaction solution was allowed to warm up to room temperature, and then a solution of *N*-benzyl-*N*-methylamidoylchloride (**30**)[32] (2.9 g, 15.8 mmol) in THF (15.8 mL) was introduced to the reaction solution. The resulting mixture was stirred at reflux for 1 h and cooled to room temperature. After the mixture was diluted with EtOAc (110 mL), quenched with satd NH_4Cl aq solution (100 mL), and separated, the organic layer was washed with H_2O (100 mL×1), dried over Na_2SO_4, and concentrated to yield the crude product. Flash chromatography (hexane/EtOAc=3/1) afforded **26** (363 mg, 19%) as a colorless oil (Scheme 6). R_f=0.40 (silica gel, hexane/EtOAc=1/2); IR (KBr) ν (cm^{-1}): 2941 (w), 2359 (w), 1761 (s), 1641 (s), 1504 (s), 1242 (s), 1153 (s); ^{1}H NMR (270 MHz, CD_3OD) δ (ppm) 8.28 (br d, *J*=27.4 Hz, 1H), 7.73 (d, *J*=4.3 Hz, 1H), 7.30 (m, 4H), 6.50 (m, 1H), 4.58 (s, 2H), 2.88 (s, 1.7H, *N*–CH_3, rotamer), 2.84 (s, 1.3H, *N*–CH_3, rotamer), 1.50 (d, *J*=1.3 Hz, 9H); ^{13}C NMR (67.5 MHz, CD_3OD) δ (ppm) 162.6, [151.5, 151.4 –*N*H–*C*(=O)O–*t*-Bu, rotamer], 143.9, [142.2, 141.7 –(CH_3)*N*–*C*(=O)–*N*=, rotamer], 138.4, [130.1, 130.0 pyrazole, rotamer], [129.2, 129.1, 128.8, 128.5, 128.0, 127.9 Ph, rotamer], [110.4, 110.3 pyrazole, rotamer], [83.5, 84.4 –O*C*$(CH_3)_3$, rotamer], [54.5, 52.0 Ph–*C*H_2–*N*(Me)–, rotamer], [35.2, 33.3 –(O=C)*N*–CH_3, rotamer], [28.1, 28.0 –OC$(CH_3)_3$, rotamer]; HRMS (FAB, NBA matrix) *m*/*z*: 358.1876: [(M+H)$^+$, calcd for $C_{18}H_{24}O_3N_5$: 358.1879].



Scheme 6. Preparation of **26**.

*4.2.21. cyclo-[Asp-O-TAGa-N-Me-Phe-Arg{N$^{\omega}$-tert-butoxycarbonyl-N$^{\omega'}$-(N-benzyl-N-methylcarbamoyl)}D-Ala-Asp-Ot-Bu] (**27**).* Following the procedure described for the preparation of **25**, *cyclo*-(Asp-*O*-TAGa-*N*-Me-Phe-Orn-D-Ala-Asp-*O*t-Bu) (**24**) (18.6 mg, 0.012 mmol) was treated with *i*-Pr_2NEt (27.5 μL, 0.158 mmol) and pyrazole reagent (**26**) (26.1 mg, 0.073 mmol) in CH_2Cl_2 (0.24 mL) for 1.5 h to afford crude **27**, which was further purified by flash chromatography ($CHCl_3$/MeOH=100/1) to yield pure **27** (18.9 mg, 85%) as a colorless solid. R_f=0.42 (silica gel, $CHCl_3$/MeOH=20/1); IR (KBr) ν (cm^{-1}) 3332 (w), 3300 (w), 2924 (s), 2852 (s), 1739 (w), 1714 (w), 1633 (w), 1595 (w), 1537 (w), 1468 (w), 1454 (w), 1441 (w), 1415 (w), 1390 (w), 1369 (w), 1331 (w), 1250 (w), 1157 (w), 1119 (w), 1036 (w); $[\alpha]_D^{26}$−14.4 (*c* 1.0, $CHCl_3$); mp 58–62 °C; HRMS (FAB, NBA matrix) *m*/*z*: 1839.3285 [(M+Na)$^+$, calcd for $C_{106}H_{177}O_{15}N_9Na$: 1839.3262]; Compound **27** gave broad spectra for NMR in less-polar solvents due

to the equilibrium of the rotamer, and each signal of **27** could not be assigned.

*4.2.22. Argifin (**2**) from **27**.* To a solution of **27** (5.9 mg, 3.25 μmol) in THF (0.33 mL) was added 20% $Pd(OH)_2$ on carbon (20.7 mg, 0.0295 mmol) at room temperature. The heterogeneous solution was stirred for 4.5 h under H_2 atmosphere. The reaction mixture was then filtered to remove $Pd(OH)_2$/C and washed with THF (3 mL). The combined filtrates were concentrated, and the crude residue was suspended with ice-cool EtOH (5 mL), the resulting insoluble residue being collected by filtration and washed with additional ice-cool EtOH (5 mL) to afford crude product (**28**) as a black solid, which was used for further reaction without further purification.

At room temperature, crude **28** was dissolved into TFA/CH_2Cl_2 (1/1) (0.32 mL), and the mixture was stirred for 1 h. The reaction mixture was then concentrated, and residues dissolved in H_2O (1.6 mL). The resulting precipitate of HO-TAGa was filtered off and washed with additional H_2O (1.6 mL). The combined filtrates were concentrated to afford almost pure argifin (**2**), which was further purified by C18-cartridge column chromatography (Sep-Pak® Plus C18) (5–10% MeCN/H_2O) to furnish pure argifin (**2**) (1.5 mg, 68%) as a colorless solid. All physical data for synthetic **2** matched with the data of authentic **2** reported previously.[21b]

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