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A Challenging Synthesis of the Highly Functionalized Echinocandin ASP9726: A Successor of Micafungin

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Supporting Information

ABSTRACT: Here, we describe a practical, scalable, and challenging synthesis of the highly functionalized novel echinocandin ASP9726 (**1**) starting from the natural product FR901379 (**3**), which is a starting material of micafungin (**2**). The synthesis includes transformations that address significant synthetic challenges due to the need to control the chemoselectivity of the reactions during modification of the highly functionalized peptide core. In the present study, we discovered an efficient, high-yielding route to ASP9726 (**1**) that is suitable for large-scale production. Namely, dehydration of carboxamide (**14**) to nitrile (**15**) was accomplished by use of EDC·HCl with pyridine. Further, the transformation of nitrile (**15**) to primary amine (**17**) was conducted via hydrogenation with Sponge Nickel catalyst without decomposition, followed by one-pot debenzylation with Pd/C. Reductive amination between primary amine (**17**) with dihydroxyacetone (DHA) was accomplished using 2-picoline/borane complex as a reducing agent in MeOH, yielding 66.6 kg of peptide core unit (**18**). After the $C_{15}H_{31}$ chain cleavage by bioconversion, reductive amination between the core peptide unit (**4**) and side chain (**10**) was achieved in high yield by making use of *tert*-butyl amine/borane complex as a reducing agent. Consequently, highly pure ASP9726 (**1**) was obtained in a practical manner without using silica gel or ODS column chromatography purification in any step. Overall yield was drastically increased from 0.71% to 13.8% compared to that of the prior synthetic method.

1. INTRODUCTION

ASP9726 (**1**; Figure 1) is a novel echinocandin with potent *Aspergillus* hyphal growth inhibition and significantly improved minimum inhibitory concentrations against *Candida parapsilosis* and echinocandin-resistant *Candida* compared to caspofungin. Further, this compound **1** is expected to be a successor of micafungin (**2**; Figure 1), which was launched by Astellas Pharma Inc. in 2002 and received approval from the U.S. Food and Drug Administration in 2005. ASP9726 (**1**) has shown potent efficacy in treating systemic candidiasis and aspergillosis with no concerns of side effects.[1] For the preparation of **1**, some chemical modifications of the peptide core are required to accomplish the synthesis. These transformations present significant synthetic challenges due to the need to control the chemoselectivity of the reactions during modification of the highly functionalized peptide core. Chemical reactions with the natural product FR901379 (**3**)[1f,2] which is a starting material of micafungin[1] and its derivatives also present numerous processing issues as a result of physical characteristics that are inherent to this class of lipopeptides (e.g., poor solubility in organic solvents, micellar and soap-like behavior, hygroscopicity in the solid state, and instability to both strong acid and strong base). Under these difficult circumstances, in the medicinal chemistry synthetic method, final product **1** was prepared in a lab-scale synthesis starting from the natural product **3** (Scheme 1). However, we encountered several drawbacks to this medicinal chemistry method, with issues summarized below.

1.1. Issues of the Medicinal Chemistry Method.

- Low yield in steps a, b, f, l, and n; further, overall yield was only 0.71%.
- Inefficient protection and deprotection steps were required.
- ODS column chromatography was necessary in all steps that should be avoided in large-scale synthesis because it requires large amounts of solvent. Actually it is impossible to prepare 100 kg-scale of **1** in this manner.
- Use of 1,4-dioxane, which represents an environmental hazard.
- Use of $NaBH_3CN$ as a reducing agent in steps k and n, which is toxic and has a sluggish rate of reaction.
- Final product **1** was unstable, presenting difficulties during operations including aging of the slurry, filtration, and drying.

Therefore, a practical and efficient new synthetic method for the large-scale synthesis of compound **1** was required.

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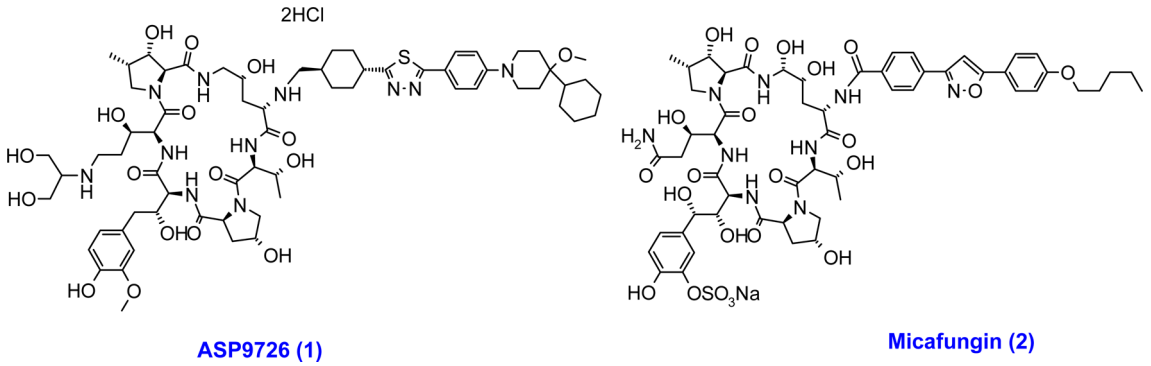
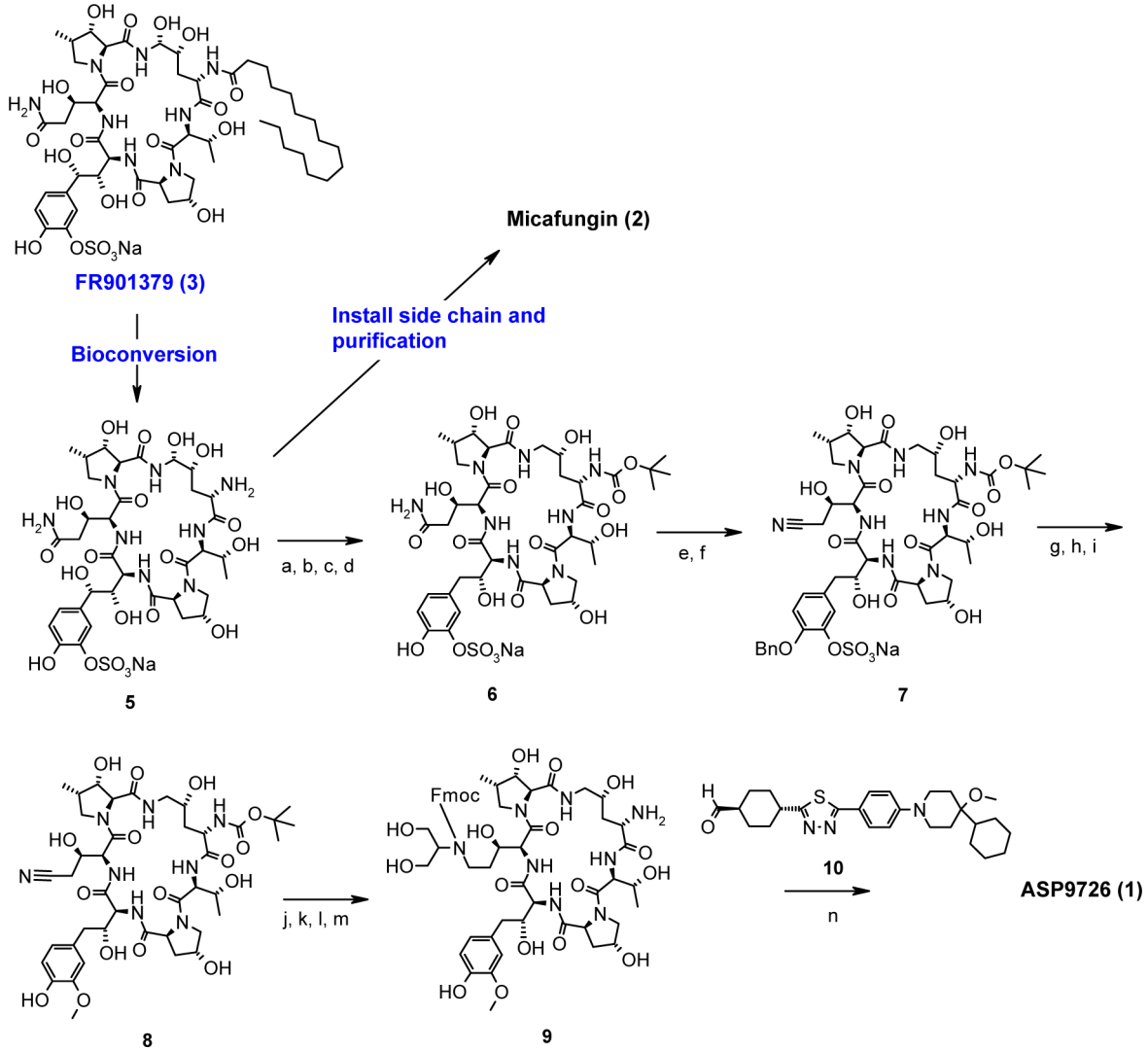


Figure 1. Structure of ASP9726 (**1**) and micafungin (**2**).

Scheme 1. Medicinal synthetic method for ASP9726 (1)[a]



[a]Reagents and conditions: (a) benzyl chloroformate, THF, pH 6.86 standard buffer solution, ODS column chromatography, 68%; (b) Et_3SiH, TFA, CH_2Cl_2, ODS column chromatography, 45%; (c) H_2, Pd/C, H_2O, ODS column chromatography, 73%; (d) $(Boc)_2O$, NaOH, H_2O, 1,4-dioxane, ODS column chromatography, 88%; (e) BnBr, LiOH-H_2O, DMF, ODS column chromatography, 85%; (f) MsCl, $NaHCO_3$, *i*-Pr_2NEt, zeolite, DMF, ODS column chromatography, 41%; (g) 10% HCl−MeOH, MeOH, ODS column chromatography; 86%; (h) MeI, LiOH·H_2O, DMF, ODS column chromatography, 76%; (i) H_2, Pd/C, MeOH, ODS column chromatography, quant.; (j) $NaBH_4$, $CoCl_2$-$6H_2O$, MeOH, H_2O, ODS column chromatography, 85%; (k) dihydroxyacetone (DHA), $NaBH_3CN$, AcOH, MeOH, ODS column chromatography, 73%; (l) Fmoc-Cl, *i*-Pr_2NEt, DMF, ODS column chromatography, 46%; (m) TFA, Et_3SiH, CH_2Cl_2, ODS column chromatography, 83%; (n) **10**, $NaBH_3CN$, AcOH, MeOH, DMF, $CHCl_3$, then piperidine, ODS column chromatography; 67%. Overall yield: 0.71%.

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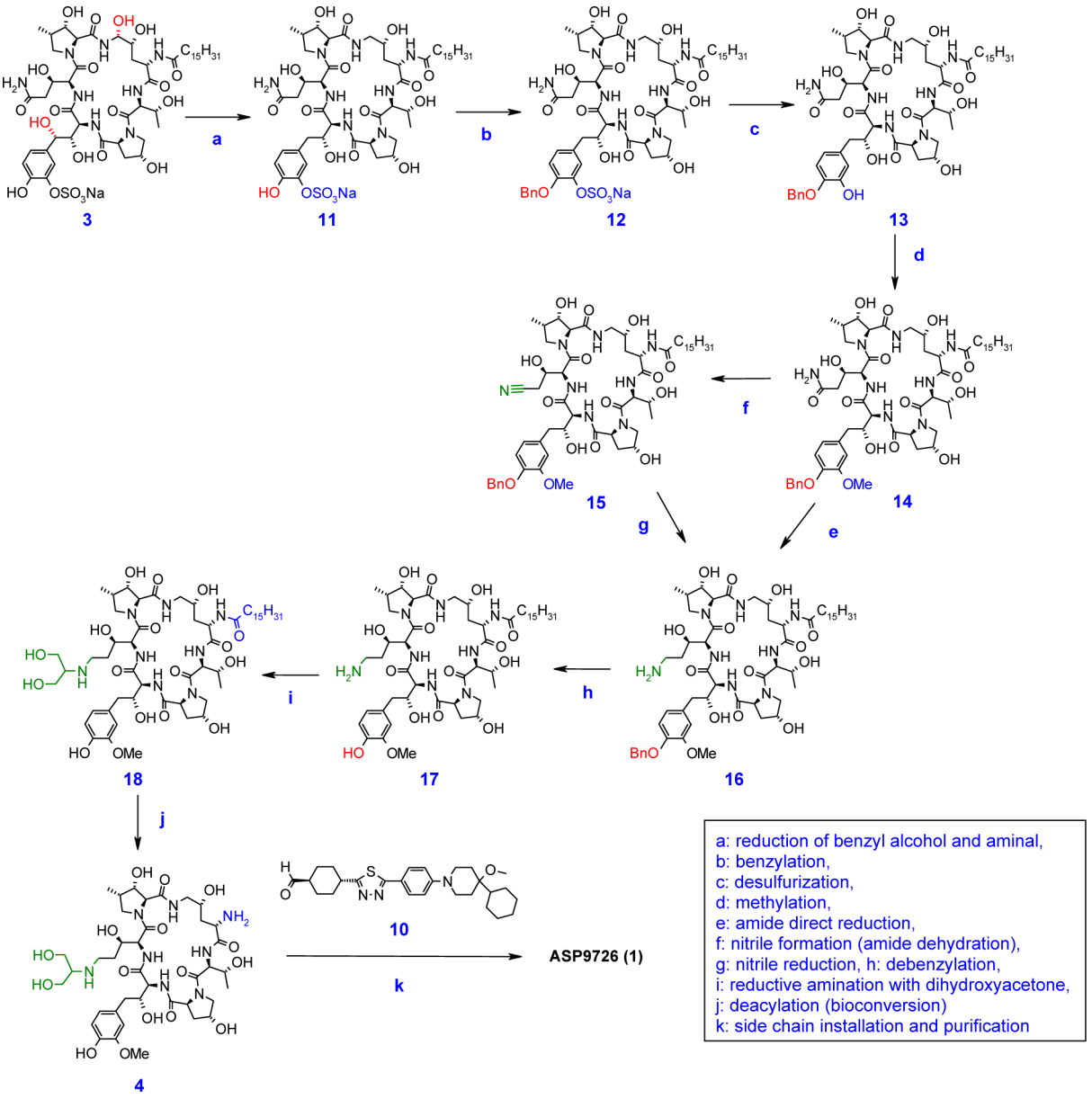
Scheme 2. New synthetic approach of 1



2. RESULTS AND DISCUSSION

2.1. New Synthetic Strategy and Brief Summary. To resolve many of the significant issues with the medicinal chemistry method, we considered a new synthetic strategy for the preparation of **1** (Scheme 2).

The synthesis of ASP9726 (**1**) from FR901379 (**3**) requires deacylation of the $C_{15}H_{31}$ chain by bioconversion and the installation of a side chain (**10**). Further, we considered that this $C_{15}H_{31}$ side chain might potentially be useful as a protection group of the primary amine because this strategy does not need inefficient protection and deprotection steps. In this strategy, we discovered an efficient, high-yielding route to ASP9726 (**1**) that is suitable for large-scale production. The synthesis includes dehydration of carboxamide (**14**) to nitrile (**15**) that was accomplished using EDC·HCl with pyridine without decomposition. Further, the transformation of nitrile (**15**) to primary amine (**17**) was conducted via hydrogenation with Sponge Nickel catalyst without degradation, followed by one-pot debenzylation with Pd/C. Reductive amination between primary amine (**17**) with dihydroxyacetone (DHA) was accomplished using 2-picoline/borane complex as a reducing agent in MeOH, yielding 66.6 kg of peptide core unit (**18**). After the $C_{15}H_{31}$ chain cleavage by bioconversion, reductive amination between the core peptide unit (**4**) and side chain (**10**) which was prepared in nine steps[3] was accomplished in high yield with *tert*-butyl amine/borane complex as a reducing agent. Consequently, highly pure ASP9726 (**1**) was obtained in a practical manner without using silica gel or ODS column chromatography purification in any step. Overall yield was drastically increased from 0.71% to 13.8% compared to that of the prior synthesis.

2.1.1. Selective Reduction of Benzylalcohol and Aminal with Silane Reducing Agent. The selective silane reduction of aminal moiety and benzyl alcohol moiety was examined. As reducing agents, $NaBH_4$ or silane reagents[4] such as Ph_3SiH, Et_3SiH, TBDMSiH were tested with AcOH or TFA. We found that the combination of TBDMSiH and TFA gave the best reaction profile, and the desired **11** was obtained in a high yield

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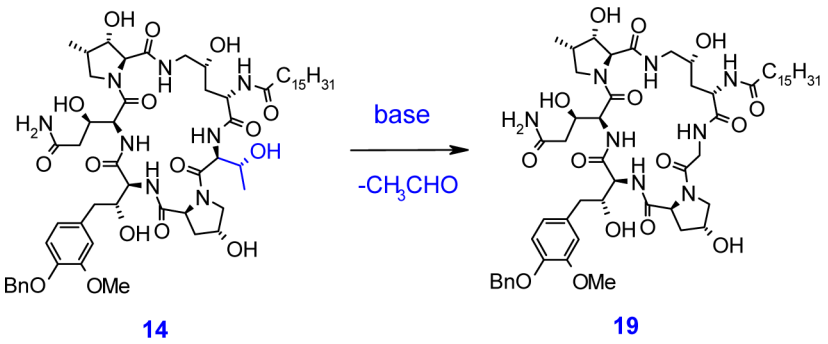

Figure 2. Structure of retro-aldol impurity **19**.

(>95%). The highly reproducible and robust method of selective reduction of benzylalcohol and aminal with a silane reducing agent was feasible for large-scale synthesis, producing 160 kg of **crude 11**. After purification with DIAION HP20SS,[5] **11** was used in the next benzylation step.

2.1.2. Benzylation, Desulfurization and Methylation. Following telescoping benzylation with benzyl bromide and LiOH, desulfurization under acidic conditions and further methylation with methyl iodide and LiOH were accomplished with good yield (86.8% in three reactions). LiOH gave the best reaction profile for benzylation and methylation in comparison with any other bases such as KOH or NaOH because of its improved solubility in DMF. However, a large amount of LiOH produced impurities derived from retro-aldol threonine moiety (Figure 2), as identified by LC−MS. Therefore, determining the appropriate amount of LiOH was a critical factor for this reaction, and 1.2 equiv of LiOH was ultimately selected as the optimum amount for the preparation of **14** with minimization of the formation of **19**.

2.1.3. Direct Reduction of Amide to Primary Amine. The direct reduction of amide **14** to primary amine **16** requires chemoselective reduction of the primary amide in the presence of other amides and hydroxyl groups. The borane complex, ionic aluminum, and boron hydride are the most common reducing agents for carboxamide; however, other amides are generally reduced more readily than primary amides with these reducing agents. Despite the potential drawbacks, we investigated the direct reduction of an amide to a primary amine (Table 1). As predicted, these reaction conditions did not produce a good reaction profile. Many unknown impurities appeared, such as over-reduced impurities during the reaction, even though the reaction was conducted at low temperature. The combination of BSTFA and BH_3·THF complex did not work well in this reaction.[6] Given these findings, we determined that direct reduction would not be suitable for this peptide core, and a stepwise reaction might be more appropriate for large-scale synthesis of the highly functionalized core unit.

Table 1. Direct reduction of amide to primary amine[a]

entry	conditions	results[b]
1	Red-Al/THF−toluene	yield: 3%, complex mixture
2	DIBAL-H/THF	yield: 5%, complex mixture
3	$LiAlH_4$/THF	yield: 1%, complex mixture
4	BH_3·THF complex/THF	yield: 1%, complex mixture
5	BH_3·$S(CH_3)_2$ complex/THF	yield: 2%, complex mixture
6	$CoCl_2$/$NaBH_4$/THF	yield: 16%, complex mixture
7	BSTFA then BH_3·THF/THF[6]	yield: 14%, complex mixture

[a]Reaction was carried out at −30 to 25 °C. [b]HPLC method B (see Experimental Section).

*2.1.4. Stepwise Approach to Primary Amine (**16**).* Therefore, a more effective two-step procedure was developed through dehydration to the nitrile followed by a catalytic hydrogenation. In the medicinal chemistry method, dehydration was conducted by using MsCl, $NaHCO_3$, and *i*-Pr_2NEt, resulting in 39% yield (Table 2, entry 1). This condition was problematic for large-scale synthesis, since **14** and product **15** decomposed during the reaction, resulting in a low yield. To solve this critical issue, study of the dehydration conditions was conducted (Table 2).

Table 2. Dehydration of Amide 14 to Nitrile 15

entry	reagents	conditions	results[a] **14**/**15**/ others
1	MsCl (× 1.1 mol), $NaHCO_3$ (× 1.1 mol), *i*-Pr_2NEt (× 1.1 mol), zeolite/DMF	medicinal method	5/56/39
2	cyanuric chloride (× 2.5 mol)/DMF[6]	−30 °C to rt	2/67/31
3	cyanuric chloride (× 2.5 mol)/DMF[6]	−10 °C	6/64/30
4	cyanuric chloride (× 2.5 mol)/DMF, water (250 mol %)[5]	−30 °C to rt	no reaction
5	cyanuric chloride (× 2.5 mol)/NMP[6]	−30 °C to rt	no reaction
6	Vilsmeier reagent (× 2.5 mol)/DMF	−30 °C to rt	2/65/33
7	Vilsmeier reagent (× 2.5 mol)/NMP	−30 °C to rt	5/64/31
8	dichlorotriazine (× 2.5 mol)/DMF	−30 °C to rt	70/25/5
9	Vilsmeier reagent (× 2.2 mol), pyridine (× 4.4 mol)/DMF	−30 °C to rt	78/10/12
10	Vilsmeier reagent (× 2.2 mol), $NaHCO_3$ (× 4.4 mol)/DMF	−30 °C to rt	75/11/14
11	2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT, × 2.5 mol)/DMF	−30 °C to rt	no reaction
12	1-chloro-*N*,*N*,2-trimethyl-1-propenylamine (Ghosez reagent, × 2.5 mol)/DMF	−30 °C to rt	no reaction
13	$(COCl)_2$(× 3.0 mol), pyridine (× 6.0 mol)/DMF	−30 °C to rt	11/58/31

[a]HPLC method B (see Experimental Section).

The methodology system of cyanuric chloride in DMF that was used for the synthesis of caspofungin acetate[6] gave a good reaction profile. However, in this case, unacceptable levels of unknown impurities were observed in the HPLC analysis (entries 2 and 3). In contrast, unfortunately, the addition of 250 mol % of water (*in situ* hydroxy dichlorotriazine, **20**, Figure 3)[6]

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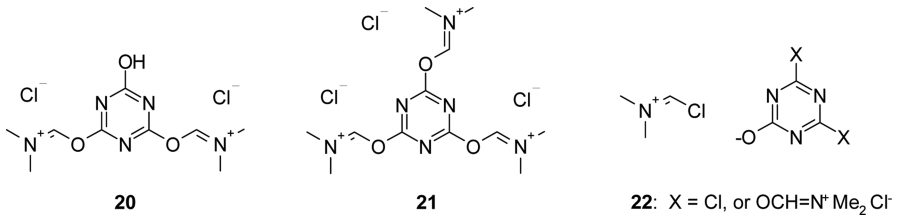

Figure 3. Proposed structures of chemically active species.

gave no reaction in this case (entry 4). Further, while reaction was attempted with cyanuric chloride in NMP, the reaction ultimately did not occur (entry 5). These results suggested that chemically active species were generated between cyanuric chloride and DMF. In contrast, Vilsmeier reagent in DMF or NMP gave good conversion, but a number of unknown impurities were observed in HPLC analysis (entries 6 and 7). To improve the ratio of the formation of impurities, bases such as pyridine or sodium bicarbonate were added to the reaction mixture with Vilsmeier reagent. However, these additions resulted in low reactivity for this reaction (entries 9 and 10). To confirm the reactivity of this carboxamide **14**, dichlorotriazine, CDMT, and Ghosez reagent were evaluated in DMF solvent (entries 8, 11, and 12). The use of dichlorotriazine gave medium activity, which should have been higher than that of hydroxy dichlorotriazine **20** (entry 4), whereas CDMT and Ghosez reagent gave no reaction at all. Therefore, we determined that the direct reaction of carboxamide with cyanuric chloride was infeasible, and that the active source would likely be either or both compound **21** and **22** (Figure 3). Using a similar approach, *in situ* generation of Vilsmeier reagent was tested, such as via oxalyl chloride and pyridine in DMF (entry 13). However, this approach did not improve the reaction profile, with nearly identical results in comparison with the cyanuric chloride and Vilsmeier method. In conclusion, these methodologies did not give a satisfactorily clean reaction, required low temperature, and carried a risk of degradation in large-scale synthesis.

To improve the reaction profile for the formation of the nitrile moiety, various kinds of dehydration agents were tested for this reaction (Table 3). A mild coupling agent EDC·HCl gave the best reaction profile, with a 72% yield (HPLC area) of desired **15** and minimized formation of impurities (entry 1).

Table 3. Screening of dehydration agents[a]

entry	reagents	conditions	results[b] **14**/**15**/others
1	EDC·HCl (× 10 mol)	rt to 50 °C, 27 h	2/72/26
2	CIP (× 10 mol)	rt to 50 °C, 27 h	56/27/17[c]
3	TFFH (× 10 mol)	rt to 50 °C, 24 h	31/10/59[c]
4	DEPBT (× 10 mol)	rt to 50 °C, 24 h	73/2/25[c]
5	HATU (× 10 mol)	rt to 50 °C, 24 h	no reaction
6	PyBop[6] (× 10 mol)	rt to 50 °C, 24 h	no reaction
7	DPPA (× 10 mol)	rt to 50 °C, 24 h	no reaction
8	CMPI (× 10 mol)	rt to 50 °C, 24 h	no reaction
9	T3P[7] (× 10 mol)	rt to 50 °C, 24 h	no reaction
10	^{i}BuOC(=O)Cl (× 10 mol)	rt to 50 °C, 24 h	no reaction
11	CDMT (× 10 mol)	rt to 50 °C, 24 h	no reaction

[a]Pyridine (× 28 mol), NMP (× 7 vol/wt). [b]HPLC area % ratio, HPLC method C (see Experimental Section). [c]Reaction stopped even though there remained further reaction time.

Other coupling agents such as CIP (2-chloro-1,3-dimethylimidazolidium hexafluorophosphate), TFFH (tetramethylfluoroformamidinium hexafluorophosphate), and DEPBT (3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one) were less reactive than EDC·HCl (entries 2−4). Other conditions such as HATU (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate), PyBop (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate),[7] DPPA (diphenylphosphoryl azide), CPMI (2-chloro-1-methylpyridinium iodide), T_3P (propylphosphonic anhydride),[8] ^{i}BuOC(=O)Cl, and CDMT did not give the desired nitrile **15** (entries 5−11).

To improve the yield of **15** with EDC·HCl, a base screening test was attempted (Table 4). One of the weak and nonbulky

Table 4. Optimization of dehydration with EDC·HCl[a]

entry	base	results[b] **14**/**15** HPLC ratio
1	pyridine	61/39
2	2,6-lutidine	75/25
3	2,4,6-collidine	82/18
4	DMAP	97/3
5	Et_3N	97/3
6	DIPEA	98/2
7	NMM	93/7
8	DBU	complex mixture
9	$NaHCO_3$	99/1

[a]Reaction temp: rt to 50 °C, reaction time: 4 h, base: × 28 mol, solvent: NMP. [b]HPLC area % ratio, HPLC method C (see Experimental Section).

bases, pyridine, gave the best conversion among other bulky bases such as 2,6-lutidine or 2,4,6-collidine (entries 2 and 3). Other bases of different bulkiness did not work for this reaction (entries 4−9).

To increase the yield of **15**, we also attempted to use other solvents such as THF and MeCN with EDC·HCl and pyridine. However, no solvent improved the yield of **15**. From the point of view of large-scale synthesis, the EDC·HCl method was considered to be better than the Vilsmeier approach. After the isolation of **crude 15**, purification was conducted using SEPABEADS SP207SS.[5]

2.1.5. Catalytic Hydrogenation of Nitrile to Amine. In the next step, the condition of catalytic hydrogenation was investigated. First, catalyst screening was attempted (Table 5). Results showed that $CoCl_2/NaBH_4$ in MeOH gave unacceptable levels of impurities on HPLC analysis. In addition, desired product was gradually decomposed in the reaction mixture; therefore, an ODS column was necessary for purification of **15** (entry 1). Further, Pt/C gave the undesired hydrolysis of nitrile to carboxamide without reducing nitrile (entry 2). The use of Rh on Alumina[9] gave undesirable results with the formation of unacceptable levels of impurities, and the

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Table 5. Hydrogenation of nitrile to amine[a]

entry	catalysts	solvents	results[b]
1	$CoCl_2/NaBH_4$[1f,5]	MeOH/water = 5/1	85% yield, ODS column purification was necessary
2	Pt/C	EtOH/NH_3 aq = 10/1	hydrolysis of nitrile occurred, 48% yield.
3	5% Rh on alumina[8]	EtOH/NH_3 aq = 10/1	**15**/**16**=22/48, yield 45%
4	Pd/C (K-type)	EtOH/NH_3 aq = 10/1	main product: **23**
5	Pd/C (P-type)	EtOH/NH_3 aq = 10/1	main product: **23**
6	Pd/C (NX-type)	EtOH/NH_3 aq = 10/1	main product: **23**
7	Pd/C (STD-type)	EtOH/NH_3 aq = 10/1	main product: **23**
8	Sponge Cobalt OFT-55	EtOH/NH_3 aq = 10/1	**15**/**16** = 82/18
9	Sponge Cobalt OFT-MS	EtOH/NH_3 aq = 10/1	**15**/**16** = 77/23
10	Sponge Cobalt ODHT-60	EtOH/NH_3 aq = 10/1	**15**/**16** = 85/15
11	Sponge Nickel NDT-90	EtOH/NH_3 aq = 10/1	**15**/**16** = 34/66
12	Sponge Nickel NDHT-90	EtOH/NH_3 aq = 10/1	**15**/**16** = 4/96
13	Sponge Nickel DHT-90M	EtOH/NH_3 aq = 10/1	**15**/**16** = 37/63

[a]Hydrogen pressure: 43−58 psi, 40 °C, 8−9 h. [b]HPLC area% ratio, HPLC method D (see Experimental Section).

yield of the desired compound was poor (less than 50%; entry 3). Screening of various kinds of Pd/C catalysts[10] did not give the desired reaction, as the main product was debenzylated undesired compound **23** without the nitrile moiety (entries 4−7, Figure 4). To overcome these issues, Sponge Cobalt catalysts

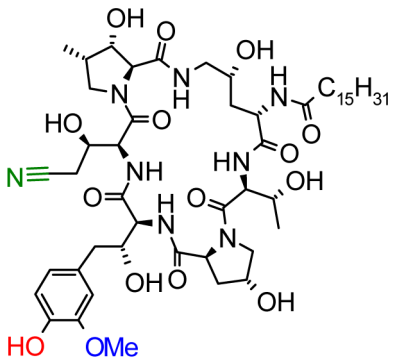

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Figure 4. Structure of debenzylated impurity **23**.

and Sponge Nickel catalysts were evaluated (entries 8−13).[11] Surprisingly, one of the nickel catalysts, NDHT-90, allowed the reaction to progress markedly cleanly, and the yield was up to 90% (entry 12).

2.1.6. Optimization of the Reaction Condition. To improve the yield of amine **16**, optimization of reaction conditions, such as reaction temperature, hydrogen pressure (30 to 100 psi), and the concentration of aqueous NH_3 in EtOH were investigated. Reaction pressure with hydrogen was not found to be an important factor; in contrast, reaction temperature and the concentration of aqueous NH_3 were found to be critical factors. An almost 1:1 ratio of EtOH and aqueous NH_3 and a 50 °C reaction temperature gave the best reaction profile, with the reaction completing within 5 h. After reaction completion, amine compound **16** was isolated as an amorphous solid. Unfortunately, critical problems were encountered during filtration because of the amorphous-like character of amine **16**, which resulted in a slow filtration speed and deliquescent powder. Even after attempts to isolate amine **16** as a hydrochloric acid salt, the condition of the filtrate was not improved. To resolve these issues, we attempted one-pot reduction of nitrile **15** to amine **16** following deprotection of the benzyl group to afford **17**. After the completion of the nitrile reduction with Ni catalyst, Pd/C catalyst was added to the reaction mixture and the mixture was aged under 30 psi hydrogen pressure for 1 h. After completion of the debenzylation, the desired debenzylated amine **17** was isolated as an HCl salt. The condition of the filtrate was better than that of **16**, it gave the faster rate during the filtration. This highly practical and reproducible method was successfully demonstrated in large-scale synthesis and yielded 63.0 kg of intermediate **17**·**HCl**.

2.1.7. Reductive Amination with Dihydroxyacetone. In the medicinal chemistry method, reductive amination of the primary amine with dihydroxyacetone was conducted with $NaBH_3CN$ as a reducing agent. However, the use of $NaBH_3CN$ should be avoided due to safety concerns. Further, ODS column chromatography purification also should be avoided because the point of view of large-scale operation. To resolve these issues, the medicinal group investigated improved conditions using $NaBH_4$ in DMF. Unfortunately, this methodology encountered fatal issues in scale-up synthesis. HPLC chromatograms of this reaction are shown in Figure 5.

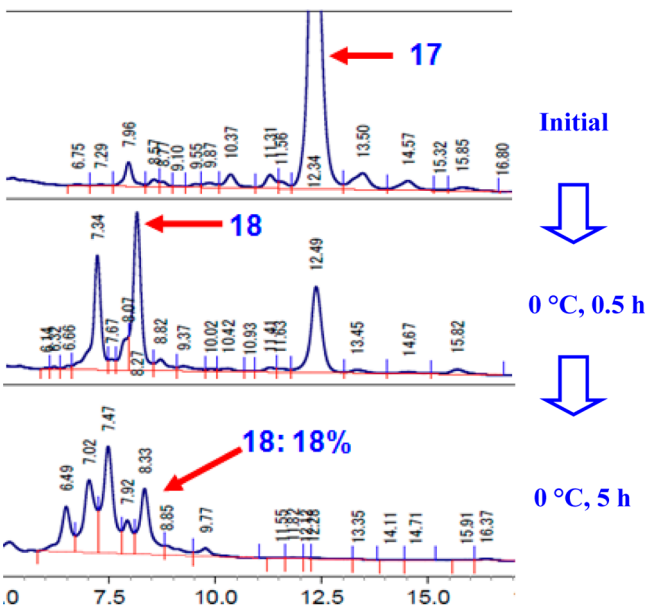

Figure 5. HPLC chromatograms of reductive amination with $NaBH(OAc)_3$ in DMF (HPLC method E, see Experimental Section).

After 30 min at 0 °C, desired compound **18** appeared, and the ratio of starting material **17** was decreased in HPLC analysis. However, impurities were also detected in HPLC analysis, and after 5 h, the target material **18** had almost entirely decomposed, with a number of impurities observed. From these

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results, we concluded that hydride reduction of imine was not suitable for this type of poly peptide compound.

2.1.8. Reductive Amination with Catalytic Hydrogenation. Since hydride reduction of imine was not feasible, we investigated catalytic hydrogenation conditions (Table 6).

Table 6. Reductive amination with catalytic hydrogenation[a]

entry	catalyst (× 1 wt)	**17**/**18** (HPLC area % ratio)[b]
1	Sponge Nickel NDT-90	86/14
2	Sponge Nickel NDHT-90	94/6
3	Sponge Nickel DHT-90M	93/7
4	Sponge Cobalt OFT-55	64/36
5	Sponge Cobalt OFT-MS	83/17
6	Sponge Cobalt ODHT-60	85/15
7	Pd/C PE-type	64/36
8	Pd/C P-type	70/30
9	Pd/C K-type	35/65
10	Pd/C STD-type	68/32
11	Pd/C NX-type	11/89
12	Pt/C	10/90

[a]DHA (5 equiv), hydrogen pressure: 30 psi, 40 °C, 8−9 h; solvent: NMP/MeOH = 1/1, 30 °C, 7 h. [b]HPLC method E (see Experimental Section).

Hydrogenation with Pt/C was selected for the reductive amination with dihydroacetone (entry 12) but failed to provide reproducible results, even when the reaction was conducted under the same conditions with the same reactor. Actually, in an 80-kg scale synthesis, reductive amination stopped at almost half conversion. In addition, further addition of Pt/C catalyst and DHA gave decompositions of both starting material and target compound without proceeding to the desired reaction. It was a critical issue for scale-up synthesis. At that time, the desired compound was recovered by temporary treatment; however, the yield was quite low. According to the consumption of hydrogen gas during the reaction, there was a possibility that DHA could be reduced to the triol impurity without reducing the imine intermediate. To resolve this critical issue, we focused on the character of the dihydroxyacetone and its dimer.[12] Although, the reaction was conducted with commercially available DHA monomer or dimer of DHA under the same conditions to prevent the hydrogenation of DHA, no differences were noted between results of these reactions. Subsequently, we attempted the reaction with a mild reducing agent in the presence of a protic solvent such as MeOH, which favors imine formation (Table 7).

As we expected, excess amount of $NaBH(OAc)_3$ was required in MeOH solvent; however, the desired product was obtained without decomposition. On the contrary, an alkyl amine/borane complex such as 5-ethyl-2-methylpyridine/borane,[13] triethylamine/borane, or *t*-$BuNH_2$/borane did not give a good reaction profile (entries 4−6). Fortunately, the borane complex with a relatively weak base such as the pyridine/borane complex[14] or the 2-picoline/borane complex[15] in MeOH gave excellent results; the desired compound was obtained without decomposition in good yield, more than 90% as an HCl salt. From the point of view of safety operation, 2-picoline/borane complex was selected as a reducing agent, and the highly efficient and reproducible procedure was demonstrated in a large-scale synthesis yielding 66.6 kg of **18**·**HCl**.

2.1.9. Installation of Side Chain. Deacylation of $C_{15}H_{31}$ moiety was accomplished using acylase *Streptomyces* sp. no. 6907,[16] in 45% yield. We then assessed the utility of the side-chain unit installation method. First, SN_2 substitution reaction of the primary amine with side chains such as mesylate **24** and triflate compound **25** were investigated (Scheme 3). However, only a trace amount of desired product **1** was detected in HPLC analysis (HPLC method G, see Experimental Section).

Table 7. Screening of reducing agents for reductive amination[a]

entry	reducing agent	**17**/**18**/ others	comments[b]
1	$NaBH(OAc)_3$	ND/80/20	excess amount of reagent was needed for full conversion
2	pyridine borane	ND/90/10	risk for the safety operation
3	2-picoline borane	ND/92/8	best condition
4	5-ethyl-2-methylpyridine borane[14]	10/67/23	not completed, and impurities were observed
5	triethylamine borane	23/50/27	not completed, and impurities were observed
6	*t*-$BuNH_2$ borane	33/41/26	not completed, and impurities were observed

[a]MeOH solvent; reaction temp: −5 to 25 °C. [b]HPLC method E (see Experimental Section).

Given our findings, we concluded that reductive amination with side-chain aldehyde **10**[3] would be better for this peptide core **4**, as the reaction was able to be conducted under milder conditions than the SN_2 reaction. The medicinal chemistry method used $NaBH_3CN$ as a reducing agent, which should be avoided in large-scale synthesis from the point of view of the safety operation (Table 8, entry 1). To accomplish the complicated reductive amination between highly water-soluble peptide core **3** and hydrophobic side chain **10**, we screened several reducing agents (Table 8).

As described in Table 8, $NaBH_3CN$ produced poor results due to competitive reduction of the side-chain aldehyde to alcohol (entry 1). Further, $NaBH(OAc)_3$ also failed to reduce the imine intermediate, and competitive reduction of side-chain aldehyde to alcohol was also observed (entry 3). In contrast, one of the alkyl amines, borane *t*-$BuNH_2$·BH_3 complex, resulted in a clean reaction profile, and imine was reduced at around 25 °C (entry 4). To optimize this reaction, we compared AcOH to other acids such as TFA or formic acid. TFA gave the best result among the examined acids. Further, base screening was also conducted to improve the yield of desired **1** and minimize the formation of dialkylated impurities. A number of bases were assessed, including TEA, *N*,*N*-diisopropylethylamine (DIPEA), pyridine, *N*-methyl morpholine (NMM), and sodium bicarbonate. The weak base, *N*-methyl morpholine, gave the best results, with relatively little formation of impurities. Using the above acid and base, yield of **1** was improved from 61% to more than 90% due to the minimization of the formation of dimeric impurities. Since peptide core **4** was isolated as a free base instead of dihydrochloric acid salt in the second-generation synthesis, we evaluated the optimal amount of acid, finding that the use of 1.65 equiv of HCl was required to minimize the formation of dimeric impurities.

2.1.10. Purification Method. In the next stage, we investigated purification methods that did not use ODS column chromatography. Many kinds of synthetic adsorbents that could be used repeatedly after regeneration, such as SEPABEADS,[5] SP207SS,[5] DIAION HP20SS,[5] and DIAION HP20,[16] were tested, with DIAION HP20SS found to be the most efficient

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Scheme 3. Installation of side chain

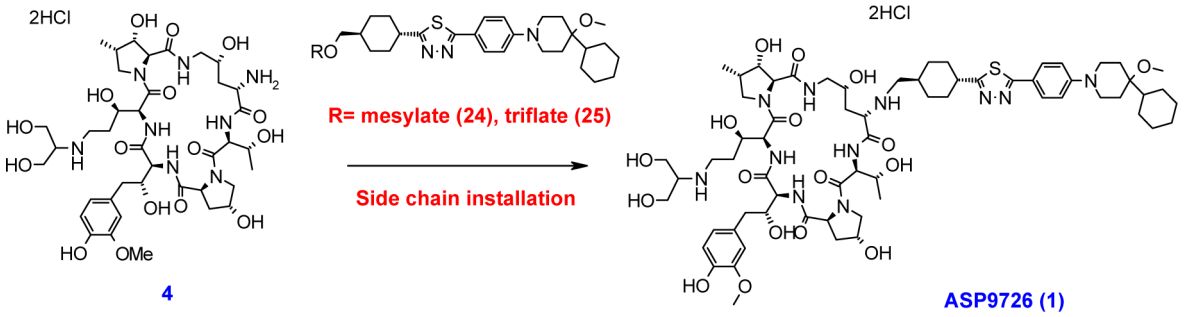

Table 8. Reductive amination for side-chain installation[a]

entry	reducing agents	HPLC yield of **1** (%)[b]
1	$NaBH_3CN$ × 2.5 mol, AcOH × 3.2 mol, Et_3N × 2.0 mol	61%; competitive reduction of side-chain aldehyde observed
2	2-picoline/borane × 2.5 mol, AcOH × 3.2 mol, Et_3N × 2.0 mol	82%
3	$NaBH(OAc)_3$ × 2.5 mol, AcOH × 3.2 mol, Et_3N × 2.0 mol	1%; imine was not reduced and competitive reduction, aldehyde to alcohol of side-chain aldehyde observed
4	*t*-$BuNH_2{\cdot}BH_3$ × 2.5 mol, AcOH × 3.2 mol, Et_3N × 2.0 mol	92%

[a]Reaction was carried out at −5 to 50 °C, solvent: DMF/$CHCl_3$/MeOH, [b]HPLC yield was determined in comparison with standard sample (HPLC method E, see Experimental Section).

resin synthetic adsorbent. In addition, we found that the most efficient method of regenerating the DIAION HP20SS[5] was: wash with (1) acetone, (2) water, (3) 1:1 acetone:1 M aqueous NaOH, (4) water, (5) 0.1 M aqueous H_2SO_4, (6) water, (7) acetone, (8) 0.1 M aqueous NaOH, (9) water, and finally (10) 0.5 M aqueous $NaHCO_3$.

2.1.11. Improvement of Stability of Final Product. We encountered a significant issue concerning the stability of the final product **1**, which gradually decomposed during stirring slurry, drying, and storage. In the early stage of development, **1** was isolated as a HCl salt. However, the element analysis of the final product indicated that these samples had 2.5−2.7 equiv of HCl even if 2 equiv of HCl was added against the result of assay with HPLC analysis. We suspected that the additional HCl might be the cause of the instability of **1**. To resolve this critical issue, the adequate pH range to prepare 2.0 equiv of HCl salt was investigated. The relationship between pH and HCl concentration of **1** is shown in Figure 6. To obtain the desired 2.0 equiv of HCl salt, the pH should be prepared between 5.0 to 6.0, with a target point of 5.5.

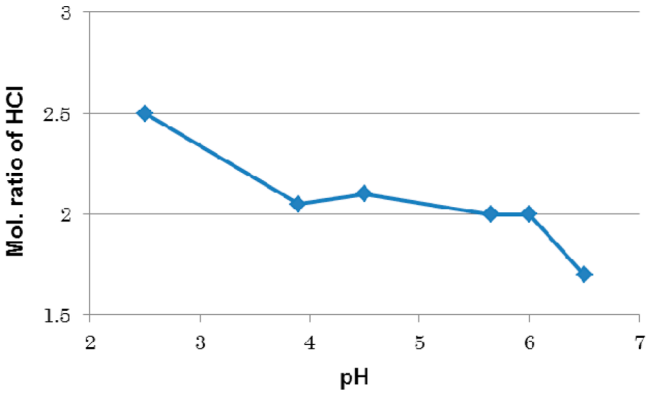

Figure 6. Relationship between pH and amount of HCl in ASP9726 (**1**).

We also conducted a stability test at 40 °C/75% RH for the various kinds of samples that have a different molar ration of HCl (Figure 7). Stability of **1** was dramatically improved with a decrease in additional amount of HCl.

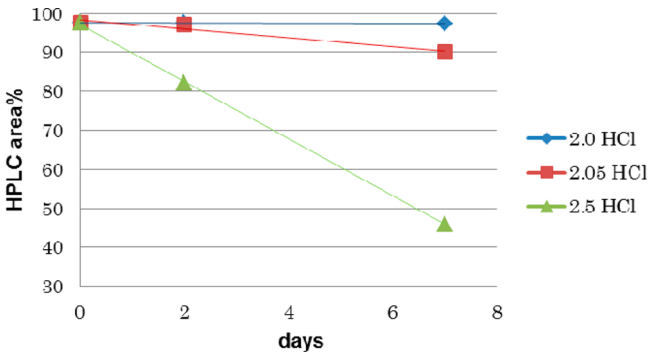

Figure 7. Results of the stability test at 40 °C/75% RH.

Further, for the preparation of preclinical samples, the final product was isolated using large amounts of acetone (vol/wt) and EtOH aq (vol/wt). However, this method required long filtration times (15 min/2 g-scale), and the wet cake was difficult to transfer to a dryer because of the muddy character. To resolve these issues, we evaluated methods of precipitation. EtOAc was added to a solution of ASP9726 in EtOH−water, which resulted in drastically improved filtration speed (from 15 to 0.5 min in 2-g scale synthesis). SEM images of these obtained solids are shown in Figure 8. Agglutinated powder was observed on the right SEM picture that should be suitable for the filtration.

3. CONCLUSION

A challenging synthesis of the highly functionalized echinocandin ASP9726 was accomplished. A novel echinocandin, ASP9726 (**1**), was prepared in an efficient and scalable method from fermentation product **3**, in an overall yield that was

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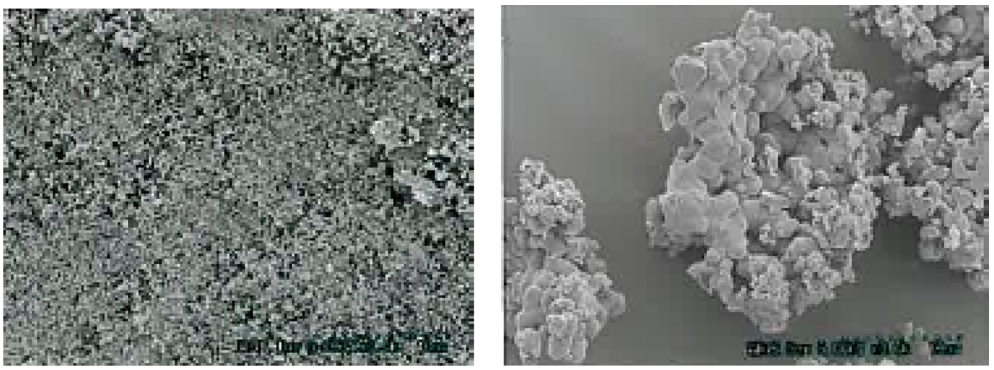

Figure 8. SEM images of ASP9726. (Left) A solution of ASP9726/EtOH−water was added to acetone (3000×). (Right) EtOAc was added to a solution of ASP9726 in EtOH−water (3000×).

dramatically improved in comparison with the medicinal chemistry method (13.8% vs 0.71%). This synthesis features complex and unstable substrates reacting with appropriate reagents and conditions that can provide chemoselective reactions. The formation of primary amine was achieved by the dehydration of nitrile with EDC·HCl in pyridine followed by hydrogenation with Sponge Ni (NDHT-90). The reductive amination of primary amine with dihydroxyacetone was accomplished by using 2-picoline/borane in MeOH. Further, reductive amination of core peptide **4** with side chain **10** was achieved using of *t*-BuNH_2/borane complex. In this study, practical purification and precipitation methods were developed, and pure final product **1** was obtained.

4. EXPERIMENTAL SECTION

General Information. Starting materials, reagents, and solvents were obtained from commercial suppliers and used without further purification. ^{1}H NMR spectra were recorded on a Varian Unity Inova 600 (600 MHz) spectrometer. Chemical shifts are reported in ppm from the solvent resonance DMSO-d_6 (2.50 ppm) or the tetramethylsilane (0.0 ppm) resonance as the internal standard [DMSO-d_6]. Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, and brs = broad singlet) and coupling constants (Hz). ^{13}C NMR spectra were recorded on a Unity Inova 600 (150 MHz) spectrometer with complete proton decoupling. Chemical shifts are reported in ppm from the solvent resonance as the internal standard [tetramethylsilane (0.0 ppm) in DMSO-d_6]. Structures are assigned by ^{1} H, ^{13}C, DEPT, COSY, TOCSY, HSQC, and HMBC spectra. HR-mass spectra were measured using LC/MS-IT-TOF (Shimadzu) or LTQ Orbitrap Discovery (Thermo Fisher Scientific). HPLC was performed using a Hitachi D-2500 or D-7500 system and Agilent 1100. HPLC methods are described below; see Table 9 for the gradient program.

HPLC Method A. Column: YMC-Pack ODS-AM, 5 μm, 4.6 mm I.D. × 150 mm (YMC), elution with 20 mM phosphate buffer (KH_2PO_4: 1.36 g + Na_2HPO_4·12H_2O 3.58g + Sodium 1-decanesulfonate: 1.22 g/water 1000 mL, adjusted pH 6.86 with aq H_3PO_4)/CH_3CN (1/1), over 30 min, 1.0 mL/min, at 35 °C, with UV detection at 210 nm; **3**: 5.1 min; mono reduced intermediate: 6.6 min, **11**, 7.6 min.

HPLC Method B. Column: YMC-Pack ODS-AM, 5 μm, 4.6 mm × 150 mm I.D. (YMC), elution with 20 mM phosphate buffer (KH_2PO_4: 1.36 g + Na_2HPO_4·12H_2O 3.58g + Sodium 1-decanesulfonate: 1.22 g/water 1000 mL, adjusted pH 6.86 with aq H_3PO_4)/CH_3CN (4/6), over 30 min, 1.0 mL/min, at 35 °C, with UV detection at 210 nm; DMF: 1.8 min, **11**: 3.4 min; **12**, 4.2 min, **13**: 9.9 min, **14**: 11.9 min.

HPLC Method C. Column: YMC-Pack ODS-AM, 5 μm, 4.6 mm × 150 mm I.D. (YMC), elution with 10 mM K_2HPO_4 buffer (adjusted 6.86 with aq H_3PO_4)/CH_3CN (2/8), over 30 min, 1.0 mL/min, at 35 °C, with UV detection at 210 nm; DMF: 1.8 min, **14**: 3.5 min; **15**: 5.1 min.

HPLC Method D. Column: YMC-Pack ODS-AM, 5 μm, 4.6 mm × 150 mm I.D. (YMC), elution with phosphate buffer (KH_2PO_4: 1.36 g + Na_2HPO_4·12H_2O 3.58 g + sodium 1-decane sulfonate: 1.22 g/water 1000 mL, adjusted pH 6.86 with aq H_3PO_4)/CH_3CN (4/6), over 40 min, 1.0 mL/min, at 35 °C, with UV detection at 210 nm; DMF: 1.8 min, **15**: 18.0 min; **16**: 6.4 min, **17**: 3.5 min.

HPLC Method E. Column: Unison UK-C18, 3 μm, 4.6 mm I.D. × 250 mm (Imtakt), elution with 10 mM K_2HPO_4 buffer (adjusted pH 7.5 with aq H_3PO_4)/CH_3CN (1/1), over 20 min, 1.0 mL/min, at 35 °C, with UV detection at 210 nm; **17**: 11.0 min, **18**: 8.0 min.

HPLC Method F. Column: Unison UK-C18, 3 μm, 4.6 mm I.D. × 150 mm (Imtakt), elution with 20 mmol/L aq $NH_4H_2PO_4$ buffer (pH 4.0 with H_3PO_4)/CH_3CN (11/9), over 25 min, 1.0 mL/min, at 15 °C, with UV detection at 210 nm; **18**: 13.0 min.

HPLC Method G. Column: YMC-Pack ProC18, AS-302, 5 μm, 4.6 mm I.D. × 150 mm (YMC), elution with phosphate buffer (pH 7.5)/CH_3CN (55/45), over 40 min, 1.0 mL/min, at 40 °C, with UV detection at 210 nm; **4**: 1.4 min, **1**: 11.8 min, imine intermediate: 13.5 min.

HPLC Method H. Column: Unison UK-Phenyl, AS-302, 3 μm, 4.6 mm I.D. × 250 mm (Imtakt), elution A: 200 mM $NaClO_4$ aq (adjusted pH 2.0 with $HClO_4$)/CH_3CN (7/3), elution B: 200 mM $NaClO_4$ aq (adjusted pH 2.0 with $HClO_4$)/CH_3CN (2/8), at 50 °C, with UV detection at 210 nm, **1**: 34 min. Gradient program; see Table 9.

Table 9. Gradient program of HPLC Method H

	0 min	40 min	70 min	80 min
elution A	100%	100%	0%	0%
elution B	0%	0%	100%	100%

Sodium 5-[(2*R*)-2-{(2*R*,6*S*,9*S*,11*R*,14*aS*,15*S*,16*S*,20*S*,23*S*,25*aS*)-20-[(1*R*)-3-amino-1-hydroxy-3-oxopropyl]-9-hexadecanamido-2,11,15-trihydroxy-6-[(1*R*)-1-hydroxyethyl]-16-methyl-5,8,14,19,22,25-hexaoxotetracosahydro-1*H*-dipyrrolo[2,1-

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c:2′,1′-*l*][1,4,7,10,13,16]hexaazacyclohenicosin-23-yl}-2-hydroxyethyl]-2-hydroxyphenyl sulfate (**11**).

To a solution of *tert*-butyldimethylsilane (29.8 kg, 256 mol) and trifluoroacetic acid (381.4 kg, 3345 mol) was added **3** (51.2 kg, 42.8 mol) at 0−10 °C, and the mixture was stirred at 9 °C for 8 h; HPLC analysis subsequently indicated that <1% **3** remained (HPLC method A). The reaction mixture was then added to a solution of water (512 L), anhydrous sodium acetate (51.2 kg, 624 mol), and NaCl (102.4 kg) at 5−20 °C, and, during addition, the pH was adjusted to between 6 and 8 with an aqueous solution of sodium hydroxide prepared in another vessel. After the addition, the pH was adjusted to 6.4, and the resulting slurry was stored at 18 °C for 12 h without agitation. The batch was then stirred, filter aid (Radiolite, 102.4 kg) was added, and the mixture was filtered and washed with an aqueous solution of NaCl (NaCl 20.5 kg/water 102 L). The resulting filtrate was added to water (1024 L), and the batch was agitated at 21 °C for 5 h, filtered, and washed with water (512 L). To the resulting filtrate was added NaCl (384 kg), and the mixture was agitated at 22 °C for 14 h, filtered, and washed with an aqueous solution of NaCl (NaCl 30.7 kg/water 154 L). Loss to filtrate was less than <1%. The wet cake was dried in vacuo at 40 °C to afford the desired **crude 11** (52.5 kg, 105.4% yield). This procedure was repeated three times, and 160.3 kg of **crude 11** was prepared in total (108% yield). 160.3 kg of **crude 11** was purified using DIAION HP20SS[5] (eluent: water/MeOH = 6/4) in seven batches. Regeneration method of the DIAION HP20SS[5] was as follows: wash with (1) DMF, (2) MeOH, (3) water/MeOH = 1/1. Desired fractions were combined and concentrated in vacuo to 640 L. To the residue was added DMF (640 L), and the solution was concentrated in vacuo to 640 L. The resulting solution was used in the next step without isolation. The analytically pure sample of **11** was purified with ODS column chromatography.

HR-MS (HPLC/MS ESI-TOF) calcd for $C_{51}H_{82}N_8O_{19}S$ 1141.5344 $[M - H]^-$, found 1141.5332 $[M - H]^-$.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.71 (1H, s), 8.08 (2H, d, J = 7.8 Hz), 7.71 (1H, brs), 7.55 (1H, brs), 7.34 (1H, d, J = 8.4 Hz), 7.19 (1H, s), 6.97 (1H, s), 6.76−6.80 (2H, m), 6.71 (1H, d, J = 8.4 Hz), 5.36 (1H, brs), 5.14−5.19 (2H, m), 5.10 (1H, d, J = 6.6 Hz), 4.86−4.97 (1H, brs), 4.83−4.85 (1H, m), 4.81 (1H, d, J = 5.4 Hz), 4.75 (1H, d, J = 6.0 Hz), 4.14−4.47 (8H, m), 3.98−4.02 (1H, m), 3.92−3.96 (1H, m), 3.77−3.83 (2H, m), 3.71 (1H, d, J = 10.8 Hz), 3.38−3.43 (1H, m), 3.17−3.22 (1H, m), 2.92 (1H, d, J = 13.2 Hz), 2.38−2.47 (3H, m), 2.30−2.36 (1H, m), 2.21−2.27 (1H, m), 2.14−2.20 (1H, m), 2.02−2.13 (2H, m), 1.84−1.93 (2H, m), 1.63−1.72 (1H, m), 1.40−1.47 (2H, m), 1.17−1.32 (24H, m), 1.03 (3H, d, J = 6.0 Hz), 0.96 (3H, d, J = 6.6 Hz), 0.86 (3H, t, J = 6.9 Hz).

^{13}C NMR (150 MHz, DMSO-d_6) δ 172.7, 172.2, 171.6, 171.0, 170.4, 169.7, 169.5, 167.8, 147.4, 140.3, 129.7, 125.8, 124.1, 116.8, 73.9, 71.9, 69.0, 68.7, 68.6, 66.1, 65.8, 60.7, 56.6, 56.2, 55.7, 53.4, 51.0, 49.8, 42.2, 38.3, 37.5, 37.0, 34.9, 33.7, 32.0, 29.0, 28.9, 28.7, 28.6, 28.4, 25.2, 22.0, 18.9, 13.9, 10.8.

N-{(2*R*,6*S*,9*S*,11*R*,14*aS*,15*S*,16*S*,20*S*,23*S*,25*aS*)-20-[(1*R*)-3-Amino-1-hydroxy-3-oxopropyl]-23-{(1*R*)-2-[4-(benzyloxy)-3-hydroxyphenyl]-1-hydroxyethyl}-2,11,15-trihydroxy-6-[(1*R*)-1-hydroxyethyl]-16-methyl-5,8,14,19,22,25-hexaoxotetracosahydro-1*H*-dipyrrolo[2,1-*c*:2′,1′-*l*][1,4,7,10,13,16]-hexaazacyclohenicosin-9-yl}hexadecanamide (**13**).

Benzylation and Desulfurization. To a solution of **11** in DMF was added benzyl bromide (37.6 kg, 220 mol) and an aqueous solution of lithium hydroxide (LiOH 3.9 kg, 163 mol/water 160 L) at 19 to 23 °C. The reaction mixture was stirred at 23 °C for 1 h; HPLC analysis subsequently indicated that <1% **11** remained (HPLC method B). To the reaction mixture, 7 M NH_3 in MeOH (35 L) was added, and the mixture was aged at 24 °C for 4 h; HPLC analysis subsequently indicated that <1% benzyl bromide remained and formation of **12** had occurred (HPLC method B). The mixture was cooled to 7 °C, 2 M HCl/MeOH (594 L, 1370 mol) was added, and the mixture was warmed to 20 °C and aged for 17 h; HPLC analysis subsequently indicated that <1% **12** remained (HPLC method B). The batch was concentrated in vacuo to 640 L, and the residue was divided into three equal batches for isolation. To a solution of MeOH (27L)/water (2136 L), one of the divided batches was added at 25 °C, and the resulting slurry was agitated for 1 h, filtered, and washed with water (800 L). Loss to the filtrate was less than 0.1%. The wet cake was dried in vacuo at 40 °C to afford the desired **crude 13** (46.7 kg, 88% yield). This precipitation procedure was repeated three times, and 140.5 kg of **crude 13** was prepared in total. **Crude 13** was divided into two batches and washed with water as follows: **crude 13** (70.0 kg) was added to water (1400 L) at 25 °C and aged for 3 h, filtered, and washed with water (700 L). Loss to the filtrate was less than 0.1%. The wet cake was dried in vacuo at 40 °C to afford the desired **13** (66.9 kg, 95.6% yield). This precipitation procedure was repeated twice, and 132.5 kg of **13** was prepared in total (83.5% yield). The analytically pure sample of **13** was purified via ODS column chromatography.

Compound 12. HR-MS (HPLC/MS ESI-TOF) calcd for $C_{58}H_{88}N_8O_{19}S$ 1231.5814 $[M - H]^-$, found 1231.5810 $[M - H]^-$.

Compound 13. HR-MS (HPLC/MS ESI-TOF) calcd for $C_{58}H_{88}N_8O_{16}$ 1151.6246 $[M - H]^-$, found 1151.6221 $[M - H]^-$.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.89 (1H, s), 8.06−8.15 (2H, m), 7.69 (1H, brs), 7.51 (1H, brs), 7.46 (2H, d, J = 7.2 Hz), 7.28−7.40 (4H, m), 7.20 (1H, brs), 6.84 (1H, d, J = 8.4 Hz), 6.78 (1H, brs), 6.64−6.67 (1H, m), 6.48−6.51 (1H, m), 5.27 (1H, brs), 5.14−5.21 (2H, m), 5.06 (2H, s), 5.01−5.03 (1H, m), 4.94−5.00 (1H, brs), 4.83−4.90 (1H, m), 4.79−4.81 (1H, m), 4.74−4.77 (1H, m), 4.14−4.44 (8H, m), 3.98−4.03 (1H, m), 3.94−3.97 (1H, m), 3.78−3.85 (2H, m), 3.68−3.76 (1H, m), 3.38−3.44 (1H, brs), 3.17 (1H, t, J = 9.6 Hz), 2.92 (1H, d, J = 13.8 Hz), 2.37−2.47 (3H, m), 2.29−2.36 (1H, m), 2.21−2.28 (1H, m), 2.16−2.21 (1H, m), 2.01−2.15 (2H, m), 1.81−1.92 (2H, m), 1.63−1.72 (1H, m), 1.40−1.48 (2H, m), 1.15−1.32 (24H, m), 1.00−1.06 (3H, m), 0.95 (3H, d, J = 7.2 Hz), 0.85 (3H, t, J = 6.9 Hz).

^{13}C NMR (150 MHz, DMSO-d_6) δ 172.7, 172.2, 171.7, 171.0, 170.3, 169.8, 169.5, 167.7, 146.5, 144.8, 137.5, 131.3, 128.2, 127.5 (2 carbons), 119.8, 116.9, 114.0, 73.8, 72.0, 69.8, 69.0, 68.7, 68.6, 66.1, 65.8, 60.7, 56.6, 55.7, 53.5, 51.0, 49.9, 42.2, 38.3, 37.5, 36.9, 34.9, 33.7, 31.2, 29.0, 28.9, 28.7, 28.6, 28.4, 25.2, 22.0, 18.9, 13.9, 10.8.

N-{(2*R*,6*S*,9*S*,11*R*,14*aS*,15*S*,16*S*,20*S*,23*S*,25*aS*)-20-[(1*R*)-3-Amino-1-hydroxy-3-oxopropyl]-23-{(1*R*)-2-[4-(benzyloxy)-3-methoxyphenyl]-1-hydroxyethyl}-2,11,15-trihydroxy-6-[(1*R*)-1-hydroxyethyl]-16-methyl-5,8,14,19,22,25-hexaoxotetracosahydro-1*H*-dipyrrolo[2,1-*c*:2′,1′-*l*][1,4,7,10,13,16]-hexaazacyclohenicosin-9-yl}hexadecanamide (**14**).

Methylation. To a solution of **13** (132.5 kg, 115 mol) in DMF (663 L) was added methyl iodide (22.8 kg, 161 mol) at 25 °C. To this solution was added an aqueous solution of lithium hydroxide (LiOH: 3.7 kg, 154 mol/water 106 L) at 1 to

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15 °C and agitated at 24 °C for 6 h; HPLC analysis subsequently indicated that <3% **13** remained (HPLC method B). To the batch was added 7 M NH_3 in MeOH (25 L), and the solution was aged at 25 °C for 4 h. The mixture was divided into three equal batches for isolation. To a solution of NaCl (199 kg), conc. HCl (10.6 kg), and water (2077 L) was added one of the divided batches at 25 °C. The solution was then washed with MeOH (33 L) and agitated for 1 h, filtered, and washed with water (663 L). Loss to the filtrate was less than 0.1%. The wet cake was dried in vacuo at 50 °C to afford the desired **14** (47.1 kg, 105% yield). This precipitation procedure was repeated three times, and 139.1 kg of **14** was prepared in total (104% yield). The analytically pure sample of **14** was purified with ODS column chromatography.

HR-MS (HPLC/MS ESI-TOF) calcd for $C_{59}H_{90}N_8O_{16}$ 1165.6402 $[M - H]^-$, found 1165.6383 $[M - H]^-$.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.16 (1H, brs), 8.09 (1H, d, J = 7.2 Hz), 7.70 (1H, brs), 7.53 (1H, brs), 7.42−7.46 (2H, m), 7.37−7.41 (2H, m), 7.29−7.36 (2H, m), 7.21 (1H, brs), 6.92 (1H, d, J = 8.4 Hz), 6.79 (1H, brs), 6.75 (1H, brs), 6.67 (1H, d, J = 7.8 Hz), 5.25 (1H, brs), 5.19−5.20 (1H, m), 5.15−5.18 (1H, m), 5.03 (2H, s), 4.96−5.01 (2H, m), 4.84−4.89 (1H, m), 4.80 (1H, d, J = 5.4 Hz), 4.76−4.78 (1H, m), 4.38−4.44 (2H, m), 4.35 (1H, brs), 4.17−4.29 (5H, m), 4.00 (1H, t, J = 8.1 Hz), 3.95 (1H, brs), 3.79−3.86 (2H, m), 3.68−3.78 (4H, m), 3.38−3.46 (1H, m), 3.17 (1H, t, J = 9.6 Hz), 2.91 (1H, d, J = 13.2 Hz), 2.52−2.58 (1H, m), 2.45−2.49 (1H, m), 2.41−2.44 (1H, m), 2.29−2.36 (1H, m), 2.23−2.28 (1H, m), 2.17−2.22 (1H, m), 2.01−2.16 (2H, m), 1.84−1.97 (2H, m), 1.64−1.72 (1H, m), 1.40−1.48 (2H, m), 1.14−1.33 (24H, m),1.03 (3H, d, J = 6.0 Hz), 0.95 (3H, d, J = 6.6 Hz), 0.85 (3H, t, J = 6.9 Hz).

^{13}C NMR (150 MHz, DMSO-d_6) δ 172.8, 172.2, 171.7, 171.1, 170.4, 169.8, 169.5, 167.6, 148.6, 146.0, 137.2, 131.1, 128.2, 127.7, 127.6, 121.1, 113.5, 73.9, 72.3, 69.8, 69.0, 68.7, 66.1, 65.8, 60.8, 56.6, 55.7, 55.3, 53.5, 51.0, 49.9, 42.1, 38.3, 37.5, 36.9, 34.9, 33.6, 31.2, 29.0, 28.9, 28.8, 28.7, 28.6, 28.4, 25.2, 22.0, 18.9, 13.9, 10.8.

N-{(2*R*,6*S*,9*S*,11*R*,14*aS*,15*S*,16*S*,20*S*,23*S*,25*aS*)-23-{(1*R*)-2-[4-(Benzyloxy)-3-methoxyphenyl]-1-hydroxyethyl}-20-[(1*R*)-2-cyano-1-hydroxyethyl]-2,11,15-trihydroxy-6-[(1*R*)-1-hydroxyethyl]-16-methyl-5,8,14,19,22,25-hexaoxotetracosahydro-1*H*-dipyrrolo[2,1-*c*:2′,1′-*l*][1,4,7,10,13,16]hexaazacyclohenicosin-9-yl}hexadecanamide (**15**).

Dehydration. To a solution of **14** (69.3 kg, 59.4 mol) in NMP (485 L) was added pyridine (131.5 kg, 1662 mol) and EDC·HCl (136.6 kg, 713 mol). The mixture was warmed to 75 °C and aged for 6 h; HPLC analysis subsequently indicated that <5% **14** remained (HPLC method C). The batch was cooled to 40 °C, and EDC·HCl (34.1 kg, 178 mol) was added. The mixture was warmed to 75 °C and aged for 15 h; HPLC analysis subsequently indicated that <2% **14** remained (HPLC method C). The reaction mixture was then added to a mixture of EtOAc (1386 L) and water (693 L) at 27 °C. The resulting aqueous layer was extracted with EtOAc (693 L). These organic layers were combined and washed with an aqueous solution of NaCl (NaCl 139 kg/water 693 L) 5 times, and the resulting organic layer was concentrated in vacuo to 208 L. MeOH (347 L) was added to the residue, and the mixture was concentrated in vacuo to 208 L three times. This dehydration procedure was conducted in two batches. The combined resulting residue was purified with SEPABEADS SP207SS[5] in eight batches (elution: 82% aqueous MeOH then MeOH). The regeneration method of the SEPABEADS SP207SS[5] was performed as follows: wash with (1) DMF, (2) acetone, and (3) water/MeOH = 1/1. Desired fractions were combined, and the solution was divided into two batches, which were concentrated in vacuo to 277 L. MeOH (347 L) was then added to the residue, and the mixture was again concentrated in vacuo to 277 L. The residue was then added to a solution of NaCl (277.2 kg) in water (2772 L) at 20 °C, and the mixture was washed with MeOH (35 L) and agitated for 2 h, filtered, and washed with water (1040 L). Loss to the filtrate was less than 0.1%. The wet cake was dried in vacuo at 40 °C to afford the desired **15** (36.9 kg). This precipitation procedure was repeated twice, and 74.4 kg of **15** was prepared in total (54.5% yield). The analytically pure sample of **15** was purified with ODS column chromatography.

HR-MS (HPLC/MS ESI-TOF) calcd for $C_{59}H_{88}N_8O_{15}$ 1147.6296 $[M - H]^-$, found 1147.6290 $[M - H]^-$.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.23 (1H, d, J = 7.2 Hz), 7.94 (1H, d, J = 7.8 Hz), 7.68−7.75 (1H, m), 7.28−7.48 (7H, m), 6.93 (1H, d, J = 8.4 Hz), 6.72 (1H, s), 6.64−6.69 (1H, m), 5.85 (1H, d, J = 6.0 Hz), 5.18 (2H, d, J = 5.4 Hz), 5.00−5.08 (3H, m), 4.94−4.98 (1H, m), 4.83−4.88 (1H, m), 4.74−4.82 (2H, m), 4.35−4.45 (3H, m), 4.10−4.25 (5H, m), 4.00 (1H, t, J = 8.1 Hz), 3.93−3.97 (1H, m), 3.80−3.88 (2H, m), 3.73−3.78 (3H, m), 3.72 (1H, brs), 3.44−3.54 (1H, m), 3.20 (1H, t, J = 9.3 Hz), 2.89 (1H, d, J = 13.8 Hz), 2.78 (1H, d, J = 15.0 Hz), 2.48−2.65 (2H, m), 2.44−2.47 (1H, m), 2.30−2.38 (1H, m), 2.21−2.29 (1H, m), 2.01−2.12 (2H, m), 1.84−1.98 (2H, m), 1.62−1.72 (1H, m), 1.40−1.49 (2H, m), 1.14−1.34 (24H, m),1.04 (3H, d, J = 6.0 Hz), 0.95 (3H, d, J = 7.2 Hz), 0.85 (3H, t, J = 6.9 Hz).

^{13}C NMR (150 MHz, DMSO-d_6) δ 171.9 (2 carbons), 171.1, 170.3, 170.1, 169.3, 166.5, 148.6, 146.1, 137.2, 130.9, 128.3, 127.6, 121.0, 119.4, 113.4, 74.0, 72.5, 69.8, 69.1, 68.4, 68.0, 66.3, 65.7, 60.8, 56.3, 55.6, 55.2, 55.0, 53.6, 51.2, 49.7, 41.8, 37.4, 36.9, 35.0, 33.5, 31.2, 29.0, 28.9, 28.8, 28.6, 28.4, 25.3, 22.0, 21.6, 18.9, 13.9, 10.9.

N-{(2*R*,6*S*,9*S*,11*R*,14*aS*,15*S*,16*S*,20*S*,23*S*,25*aS*)-20-[(1*R*)-3-Amino-1-hydroxypropyl]-2,11,15-trihydroxy-6-[(1*R*)-1-hydroxyethyl]-23-[(1*R*)-1-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-ethyl]-16-methyl-5,8,14,19,22,25-hexaoxotetracosahydro-1*H*-dipyrrolo[2,1-*c*:2′,1′-*l*][1,4,7,10,13,16]hexaazacyclohenicosin-9-yl}hexadecanamide monohydrochloride (**17·HCl**).

Sponge Nickel NDHT-90[11] (36.5 kg wet) was washed with EtOH twice. Compound **15** (36.5 kg, 31.8 mol) in a solution of EtOH/28% NH_3 aq = 1/1 (365 L) was added to a 1500-L autoclave, and then prewashed sponge Nickel NDHT-90 was added. The autoclave was flushed with nitrogen gas three times and then with hydrogen gas three times. The hydrogenation was conducted under 33 psi pressure of hydrogen at 50 °C for 5 h; HPLC analysis subsequently indicated that <1% **15** remained and formation of **16** (HPLC method D). Then, 7.5% Pd/C[10] (3.7 kg, 50% wet) and EtOH (20 L) were added to the batch, and the reaction was conducted under 35 psi pressure of hydrogen at 35 °C for 1 h; HPLC analysis subsequently indicated that <1% **16** remained (HPLC method D). After the completion of the reaction, MeOH (146 L) was added to the mixture and filtered and then was washed with MeOH (220 L) and concentrated in vacuo to 292 L. EtOH (183 L) was added to the residue, and the mixture was concentrated in vacuo to 292 L. EtOH (183 L) was then added again to the residue, and the mixture was concentrated in vacuo to 292 L. EtOH (365 L) and 2 M HCl/EtOH were then added to the batch until pH reached 2.3. The batch was concentrated in vacuo to 146 L,

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poured into CH_3CN (1825 L) at 3 °C, and then washed with MeOH (36 L). The resulting slurry was aged at 3 °C for 1 h, filtered, and washed with CH_3CN (111 L). Loss to the filtrate was 2%. The wet cake was dried in vacuo at 50 °C to afford the desired **17·HCl** (30.8 kg). This hydrogenation procedure was repeated twice, and 63.0 kg of **17·HCl** was prepared in total (89.6% yield). The analytically pure sample of **17·HCl** was purified with ODS column chromatography.

Compound ***16***. HR-MS (HPLC/MS ESI-TOF) calcd for $C_{59}H_{92}N_8O_{15}$ 1151.6609 $[M - H]^-$, found 1151.6585 $[M - H]^-$

Compound ***17·HCl***. HR-MS (HPLC/MS ESI-TOF) calcd for $C_{52}H_{86}N_8O_{15}$ 1097.5907 $[M + Cl]^-$, found 1097.5884 $[M + Cl]^-$.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.71 (1H, s), 8.48 (1H, m), 8.33 (1H, d, J = 7.8 Hz), 7.76−7.81 (1H, m), 7.64−7.75 (3H, m), 7.62 (1H, brs), 7.36 (1H, d, J = 9.0 Hz), 6.62−6.69 (2H, m), 6.54−6.59 (1H, m), 5.33 (1H, brs), 5.14−5.24 (3H, m), 4.80−4.85 (1H, brs), 4.70−4.79 (3H, m), 4.46 (1H, brs), 4.42 (1H, brs), 4.32−4.38 (1H, m), 4.20−4.28 (2H, m), 4.16−4.19 (1H, m), 4.10−4.15 (1H, m), 4.02−4.08 (1H, m), 3.90−3.98 (3H, m), 3.82−3.88 (1H, m), 3.73 (3H, s), 3.69 (1H, d, J = 10.8 Hz), 3.42−3.51 (1H, m), 3.22 (1H, t, J = 9.3 Hz), 2.88−2.94 (1H, m), 2.78−2.87 (2H, m), 2.56−2.64 (1H, m), 2.50−2.54 (1H, m), 2.28−2.37 (1H, m), 2.18−2.24 (1H, m), 2.05−2.16 (2H, m), 1.83−1.98 (3H, m), 1.72−1.78 (1H, m), 1.65−1.71 (1H, m), 1.38−1.47 (2H, m), 1.13−1.34 (24H, m), 1.02 (3H, d, J = 6.0 Hz), 0.95 (3H, d, J = 7.2 Hz), 0.86 (3H, t, J = 6.9 Hz).

^{13}C NMR (150 MHz, DMSO-d_6) δ 172.8, 171.6, 171.2, 170.2, 170.1, 169.4, 167.2, 147.0, 144.6, 128.9, 121.3, 115.1, 113.4, 73.9, 73.0, 69.5, 69.1, 68.1, 65.5, 65.4, 61.2, 56.6, 55.7, 55.6, 55.2, 54.4, 51.3, 49.9, 42.0, 37.2, 36.9 (2 carbons), 34.9, 33.3, 31.2, 29.1, 29.0, 28.9, 28.7, 28.6, 28.3, 25.3, 22.0, 19.2, 13.9, 10.9.

N-{(2*R*,6*S*,9*S*,11*R*,14*aS*,15*S*,16*S*,20*S*,23*S*,25*aS*)-20-{(1*R*)-3-[(1,3-Dihydroxypropan-2-yl)amino]-1-hydroxypropyl}-2,11,15-trihydroxy-6-[(1*R*)-1-hydroxyethyl]-23-[(1*R*)-1-hydroxy-2-(4-hydroxy-3-methoxyphenyl)ethyl]-16-methyl-5,8,14,19,22,25-hexaoxotetracosahydro-1*H*-dipyrrolo[2,1-*c*:2′,1′-*l*][1,4,7,10,13,16]hexaazacyclohenicosin-9-yl}hexadecanamide monohydrochloride (**18·HCl**).

To a solution of **17·HCl** (30.7 kg, 27.9 mol) in MeOH (307 L) was added dihydroxyacetone (7.54 kg, 83.7 mol). The batch was cooled to 2 °C, and 2-picoline/borane complex (4.48 kg, 41.9 mol) was added. The mixture was then warmed to 23 °C and agitated for 5 h. Dihydroxyacetone (7.54 kg, 83.7 mol) was added at 23 °C, and the mixture was agitated for 12 h; HPLC analysis subsequently indicated that <1% **17** remained (HPLC method E). To the batch was added 2 M HCl/MeOH (49 L), and then the mixture was concentrated in vacuo to 92 L. MeOH (307 L) was added to the residue, and the mixture was concentrated in vacuo to 92 L, poured into CH_3CN (1535 L) at 3 °C, and washed with MeOH (30 L). The resulting slurry was agitated at 3 °C for 1 h, filtered, and washed with CH_3CN (93 L). Loss to the filtrate was 2%. The wet cake was dried in vacuo at 50 °C to afford the desired **18·HCl** (32.8 kg). This reductive amination procedure was repeated twice, and 66.6 kg of **18·HCl** was prepared in total (99.4% yield). The analytically pure sample of **18** was purified with ODS column.

HR-MS (HPLC/MS ESI-TOF) calcd for $C_{55}H_{92}N_8O_{17}$ 1171.6274 $[M + Cl]^-$, found 1171.6252 $[M + Cl]^-$.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.72 (1H, brs), 8.52 (1H, d, J = 6.6 Hz), 8.23−8.35 (2H, m), 8.20 (1H, brs), 7.86 (1H, d, J = 6.6 Hz), 7.55 (1H, brs), 7.34 (1H, d, J = 9.0 Hz), 6.62−6.70 (2H, m), 6.53−6.58 (1H, m), 5.41 (1H, brs), 5.05−5.35 (4H, m), 4.83 (1H, brs), 4.72−4.81 (2H, m), 4.48 (1H, brs), 4.42 (1H, br), 4.32−4.36 (1H, m), 4.23−4.24 (1H, m), 4.16−4.22 (2H, m), 4.08−4.14 (1H, m), 4.02−4.07 (1H, m), 3.97−3.99 (1H, m), 3.90−3.96 (2H, m), 3.84−3.89 (1H, m), 3.72−3.76 (3H, m), 3.61−3.71 (3H, m), 3.54−3.60 (2H, m), 3.48−3.52 (1H, m), 3.23−3.28 (1H, m), 3.18−3.24 (1H, m), 3.14 (1H, brs), 3.07 (1H, brs), 2.78−2.84 (1H, m), 2.58−2.64 (1H, m), 2.48−2.54 (1H, m), 2.30−2.40 (1H, m), 2.18−2.24 (1H, m), 2.06−2.15 (2H, m), 1.91−2.05 (3H, s), 1.68−1.80 (2H, m), 1.41−1.49 (2H, m), 1.13−1.30 (24H, m), 1.00 (3H, d, J = 5.4 Hz), 0.95 (3H, d, J = 7.2 Hz), 0.86 (3H, t, J = 6.9 Hz).

Two peaks for OH protons were not confirmed.

^{13}C NMR (150 MHz, DMSO-d_6) δ 173.1, 171.7, 171.1, 170.1 (2 carbons), 169.6, 167.3, 147.0, 144.6, 128.8, 121.3, 115.1, 113.5, 73.9, 73.2, 70.2, 69.1, 67.8, 65.4, 61.2, 59.1, 57.4, 57.1, 56.5, 55.5, 55.2, 54.5, 51.4, 49.9, 42.8, 41.6, 39.0, 37.2, 36.9, 35.0, 33.1, 31.2, 29.0, 28.9 (2 carbons), 28.7, 28.6, 28.3, 27.4, 25.4, 22.0, 19.1, 13.9, 11.0.

(2*R*,6*S*,9*S*,11*R*,14*aS*,15*S*,16*S*,20*S*,23*S*,25*aS*)-9-Amino-20-{(1*R*)-3-[(1,3-dihydroxypropan-2-yl)amino]-1-hydroxypropyl}-2,11,15-trihydroxy-6-[(1*R*)-1-hydroxyethyl]-23-[(1*R*)-1-hydroxy-2-(4-hydroxy-3-methoxyphenyl)ethyl]-16-methyloctadecahydro-19*H*-dipyrrolo[2,1-*c*:2′,1′-*l*][1,4,7,10,13,16]-hexaazacyclohenicosine-5,8,14,19,22,25(9*H*)-hexone (**4**).

To a solution of **18·HCl** (13.0 g, 11.1 mmol) in MeOH (1.0 L) was added water (2.0 L) and a solution of acylase *Streptomyces* sp. no. 6907 (0.46 L), and then the pH was adjusted to 8.0 with aq NaOH. The reaction mixture was then agitated at 37 °C for 6 h; HPLC analysis subsequently indicated that 0.1 mg/mL of **18** remained (HPLC method F). After the completion of the reaction, pH was adjusted from 8 to 3.5 with 78% aqueous solution of H_2SO_4, and the mixture was concentrated in vacuo, filtered through the filter aid, and washed with water. The pH of the resulting filtrate was adjusted to 2.5 with 78% aqueous solution of H_2SO_4, and then the mixture was concentrated in vacuo followed by polish filtration. MeOH (30 mL) was added to the resulting filtrate, and the pH was adjusted to 9.0 with aq NaOH. The resulting solution was purified with SP207SS[5] followed by SEPABEADS SP700.[5] The collected desired fractions were concentrated in vacuo to 32 mL. EtOAc (600 mL) was added to the residue, and the resulting slurry was agitated for 1 h, filtered, and washed with EtOAc (32 mL). The wet cake was dried in vacuo at 40 °C to afford the desired **4** (4.44 g, 44.6% yield).

HR-MS (HPLC/MS ESI-TOF) calcd for $C_{39}H_{62}N_8O_{16}$ 921.4176 $[M + Na]^+$, found 921.4188 $[M + Na]^+$.

^{1}H NMR (600 MHz, DMSO-d_6) δ 7.68−7.75 (1H, m), 7.64 (1H, brs), 7.52 (1H, brs), 7.42−7.48 (1H, m), 6.62−6.68 (2H, m), 6.52−6.58 (1H, m), 5.20−5.24 (1H, m), 5.16−5.19 (1H, m), 4.76−4.81 (1H, m), 4.73 (1H, brs), 4.36−4.48 (2H, m), 4.32 (1H, brs), 4.10−4.20 (3H, m), 4.00−4.06 (1H, m), 3.96−3.99 (1H, m), 3.80−3.89 (3H, m), 3.70−3.78 (4H, m), 3.48−3.56 (1H, m), 3.21−3.47 (7H, m), 3.10−3.20 (1H, m), 2.72−2.78 (1H, m), 2.58−2.67 (2H, m), 2.52−2.57 (1H, m), 2.45−2.48 (1H, m), 2.39−2.44 (1H, m), 2.32−2.38 (1H, m), 2.20−2.28 (1H, m), 1.85−1.98 (2H, m), 1.51−1.60 (1H, m), 1.40−1.50 (1H, m), 1.13−1.22 (1H, m), 1.09 (3H, d, J = 6.6 Hz), 0.95−0.99 (3H, m). Eight peaks for OH, NH and NH_2 protons were not confirmed.

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^{13}C NMR (150 MHz, DMSO-d_6) δ 174.6, 171.1, 170.4, 169.7, 169.3, 168.3, 147.0, 144.6, 128.8, 121.3, 115.1, 113.4, 73.4, 72.4, 69.8, 69.0, 68.5, 67.8, 65.9, 60.9 (2 carbons), 60.8, 60.7, 59.7, 56.7, 55.7, 55.4, 55.2, 53.6, 52.6, 50.9, 43.8, 43.3, 37.5, 37.1 (2 carbons), 32.8, 19.1, 10.8.

(2*R*,6*S*,9*S*,11*R*,14*aS*,15*S*,16*S*,20*S*,23*S*,25*aS*)-9-{[(*trans*-4-{5-[4-(4-cyclohexyl-4-methoxypiperidin-1-yl)phenyl]-1,3,4-thiadiazol-2-yl}cyclohexyl)methyl]amino}-20-{(1*R*)-3-[(1,3-dihydroxypropan-2-yl)amino]-1-hydroxypropyl}-2,11,15-trihydroxy-6-[(1*R*)-1-hydroxyethyl]-23-[(1*R*)-1-hydroxy-2-(4-hydroxy-3-methoxyphenyl)ethyl]-16-methyloctadecahydro-19*H*-dipyrrolo[2,1-*c*:2′,1′-*l*][1,4,7,10,13,16]hexaazacyclohenicosine-5,8,14,19,22,25(9*H*)-hexone dihydrochloride (**1**).

To a solution of **4** (3.5 g, 3.9 mmol) in DMF (21 mL) was added 2 M HCl/MeOH (3.22 mL, 6.44 mmol), MeOH (17.5 mL), and CH_2Cl_2 (17.5 mL). The batch was agitated for 0.5 h, and then **10** (2.18 g, 4.7 mmol) was added, followed by the addition of TFA (1.42 g, 12.5 mmol). To the resulting solution was added *t*-BuNH_2·BH_3 (0.51 g, 5.9 mmol) at −12 °C; the solution was warmed to 25 °C (warming rate: 10 °C/h) and agitated for 12 h; HPLC analysis subsequently indicated that <1% of **4** remained (HPLC method G). After quenching with 4 M HCl/EtOAc (14 mL, 56 mmol), CH_3CN (350 mL) was added to the mixture at 0 °C. The resulting slurry was then agitated at 0 °C for 1 h, filtered, and washed with CH_3CN (35 mL). The wet cake was dried in vacuo at 40 °C to afford the desired **crude 1** (5.71 g, 103% yield). The crude product (1.3 g, 0.91 mmol) was purified with HP20SS[5] elution in a solution of MeOH/water (7/3). pH of the collected desired fractions was adjusted from 2.57 to 6.10 with aq NaOH. Half of the volume of the solution was then purified with HP20SS[5] elution in MeOH/EtOH (1/1). The desired fraction was concentrated in vacuo to 8.8 mL, and the residue was added to EtOH (30 mL). The resulting mixture was concentrated in vacuo to 8.8 mL, and then EtOH (2.2 mL) and water (0.44 mL) was added. The pH was then adjusted to 5.68 with 2 M HCl/EtOH at 0 °C. EtOAc (55 mL) was added to the resulting solution at 0 °C, and the slurry was agitated for 1 h, filtered, and washed with EtOAc (5 mL). The wet cake was dried in vacuo at 40 °C to afford the desired **1** (0.443 g, 68.2% yield from **crude 1**; HPLC purity: 96.6%, HPLC method H).

HR-MS (ESI) calcd for $C_{66}H_{99}N_{11}O_{17}S$ 1350.70139 $[M + H]^+$, found 1350.70215 $[M + H]^+$.

^{1}H NMR (600 MHz, DMSO-d_6, 45 °C) δ 8.60 (1H, s), 8.48 (1H, brs), 8.39 (1H, brs), 8.16 (1H, brs), 7.72 (2H, d, J = 9.0 Hz), 7.62−7.71 (3H, m), 7.01 (2H, d, J = 9.6 Hz), 6.69−6.71 (1H, m), 6.64−6.68 (1H, m), 6.55−6.60 (1H, m), 5.44−5.50 (1H, m), 5.18−5.38 (6H, m), 4.74−4.82 (2H, m), 4.49 (1H, brs), 4.45 (1H, brs), 4.38−4.44 (1H, m), 4.18−4.25 (3H, m), 4.12−4.17 (2H, m), 4.04−4.11 (2H, m), 4.02 (1H, brs), 3.85−3.92 (1H, m), 3.72−3.78 (4H, m), 3.66−3.71 (4H, m), 3.60−3.65 (2H, m), 3.41 (1H, brs), 3.10−3.22 (5H, m), 3.02−3.10 (3H, m), 2.93−3.02 (2H, m), 2.85−2.90 (1H, brs), 2.80−2.84 (1H, m), 2.69−2.74 (1H, m), 2.64−2.68 (1H, m), 2.52−2.58 (1H, m), 2.35−2.44 (1H, m), 2.20−2.30 (2H, m), 2.10−2.18 (2H, m), 1.97−2.04 (1H, m), 1.92−1.96 (1H, m), 1.84−1.90 (1H, m), 1.70−1.82 (5H, m), 1.61−1.68 (7H, m), 1.50−1.60 (4H, m), 1.12−1.27 (7H, m), 1.04−1.10 (2H, m), 0.50−1.02 (5H, m). Two peaks for NH and HCl protons were not confirmed.

^{13}C NMR (150 MHz, DMSO-d_6, 45 °C) δ 172.7, 171.1, 170.0 (2 carbons), 169.3, 168.1, 167.2, 152.5, 147.2, 144.7, 129.1, 128.5 (2 carbons), 121.4, 118.5, 115.2, 114.2 (2 carbons), 113.7, 74.5, 73.2, 72.4, 68.9, 68.5, 68.2, 67.6, 67.4, 66.9, 64.6, 61.9, 60.2, 60.1, 57.5, 57.3, 57.2, 56.3, 55.9, 55.6, 55.5, 53.8, 52.2, 51.0, 47.1, 44.3, 43.0 (2 carbons), 42.2, 41.5, 38.5, 37.6, 37.2, 34.0, 33.5, 32.3 (2 carbons), 29.3 (2 carbons), 28.6 (2 carbons), 26.5 (2 carbons), 26.3 (2 carbons), 26.2, 19.6, 10.9.

■ ASSOCIATED CONTENT

Supporting Information

NMR data for the products. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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