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Concept and synthetic approach for a kilogram scale synthesis of octa-D-arginine amide nonahydrochloride salt

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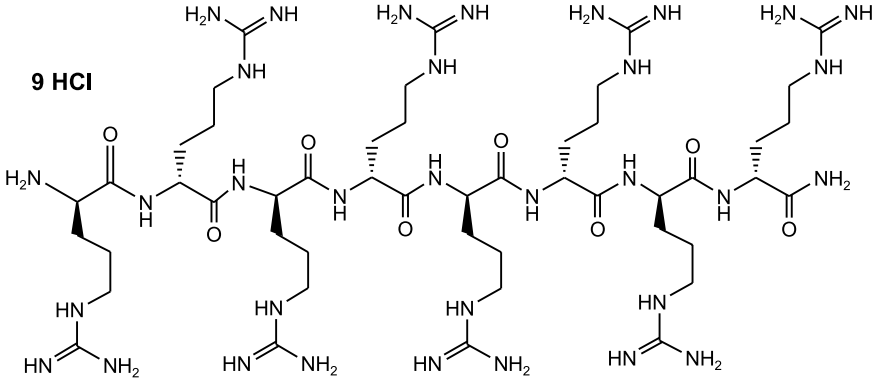
Abstract—Oligomers of arginine, such as octa-D-arginine amide, are excellent transporters for active drugs through cell membranes and tissue. The synthesis of octa-D-arginine amide, as the nonahydrochloride salt, was approached via a solution phase synthetic route involving the preparation of an octa-D-ornithine intermediate, which was then converted into the desired octa-D-arginine compound through a guanidinylation step. The multi-step synthesis was carried out at pilot scale, resulting in the preparation of 700 g of the target molecule. No chromatographic purification was needed at any step of the process.

1. Introduction

Timing and development of scalable processes are critical to the successful production of a peptide-based drug.[1] Although solid phase synthesis is a common approach used to prepare oligopeptides, this method can have limitations, such as the high cost of excess reagents and protected amino acids, the high volume of solvents used, potential limited scalability, and the usual requirement for a chromatographic purification once the peptide is cleaved from the resin. A solution phase synthesis appeared to be a more attractive approach for the preparation of our target molecule, octa-D-arginine amide nonahydrochloride salt[2–11] (**6**) (Scheme 1) in terms of robustness, scalability, and the potential to minimize labor-intensive purification. The limited availability and cost of protected D-arginine derivatives led us to explore the development of a synthesis in which D-ornithine is substituted for the D-arginine residues. A per-guanidinylation step would then



Scheme 1. Octa-D-arginine amide hydrochloride.

Keywords: Amino acids and derivatives; Arginine; Guanidinylation; Peptide transport; Peptide process development; Minimal isolation peptide synthesis.
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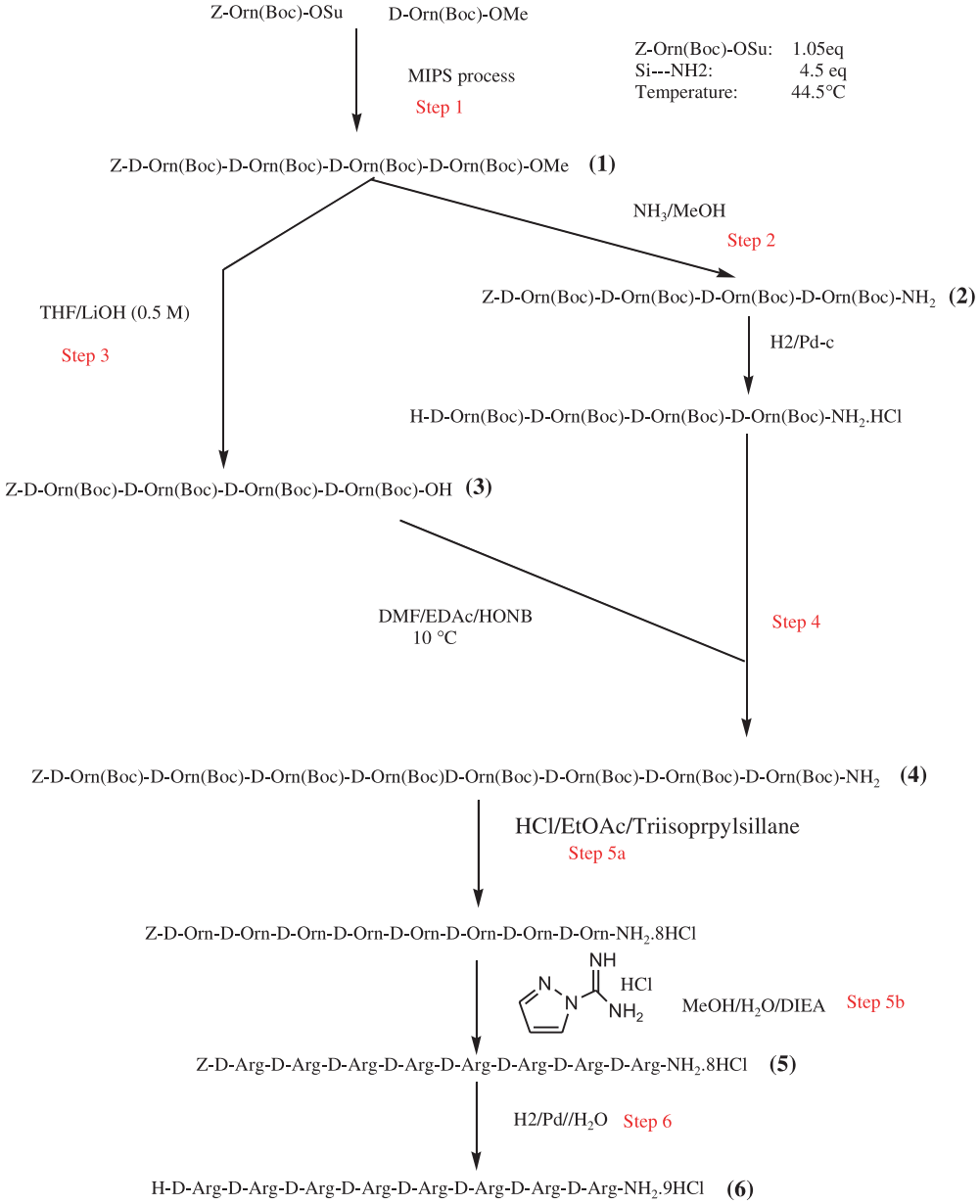
be used for conversion of the 8 ornithine residues to the corresponding arginine residues.[12–18]

The synthetic process, as described in Scheme 2, resulted in the preparation of 0.7 kg of the desired compound, isolated as a hydrochloride salt (**6**). The main features of the process were the synthesis of a key crystalline tetrapeptide ester intermediate (**1**) with only one isolation, followed by the preparation of two crystalline tetrapeptide intermediates (**2**) and (**3**) from the common tetrapeptide ester (**1**). Coupling of the N-terminal deprotected (**2**) with C-terminal deprotected (**3**) gave the fully protected octa-D-ornithine intermediate (**4**), which upon global removal of 8 amine-protecting groups followed by global guanidinylation of the free amines in one step gave the desired D-arginine residues. No chromatographic purification was used at any step of the process. At the final step a simple activated carbon treatment of the desired octa-D-arginine amide hydrochloride salt (**6**) in water was sufficient for purification.

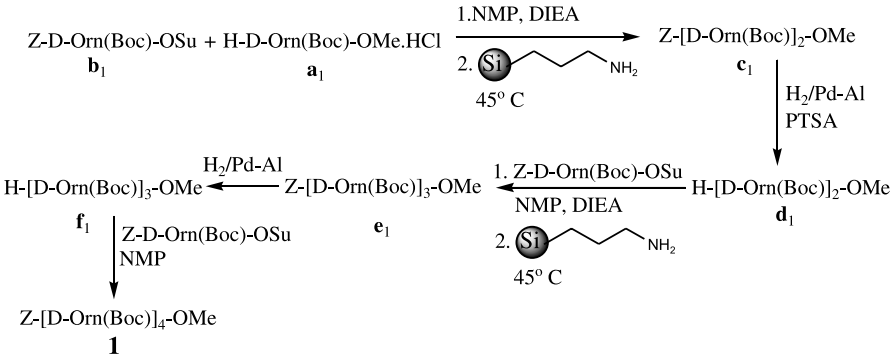
2. Results and discussion

In step 1, *Z*-[D-Orn(Boc)]$_4$-OMe (**1**) (Scheme 3), was prepared following a continuous process (MIPS, minimal isolation peptide synthesis) to minimize the isolation steps of the intermediate dipeptide and tripeptide.

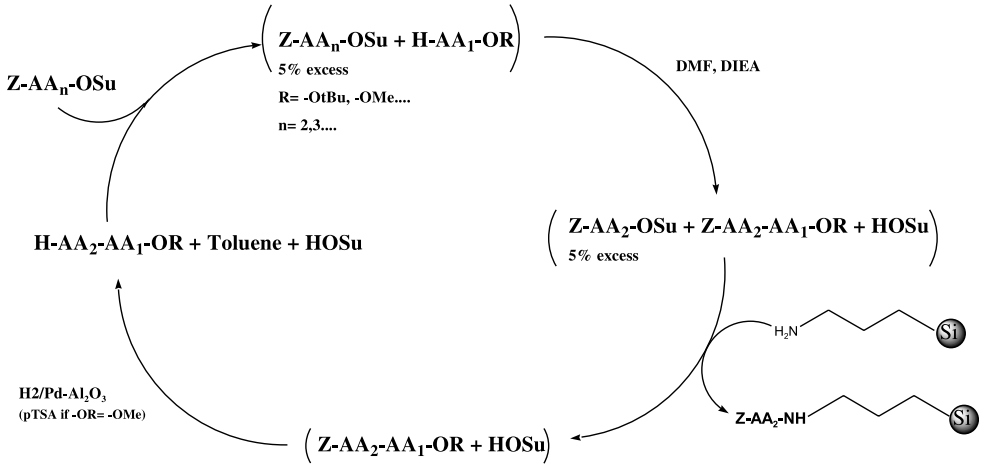
The general procedure for conducting the MIPS process is described in Scheme 4 below. Excess *N*-benzyloxycarbonyl-protected amino acid *N*-hydroxysuccinimide ester[19] (*Z*-AA-OSu) is used to drive the acylation to completion. Residual *Z*-AA-OSu is captured/scavenged by the addition of amine-derivatized insoluble resin or silicagel,[20,21] which



Scheme 2. Octa-D-arginine amide—overall process.



Scheme 3. M.I.P.S process step 1. *Z*-[D-Orn(Boc)$_4$-OMe.



Scheme 4. MIPS process cycle.

is then removed from the reaction mixture by filtration. Subsequent *N*-deprotection of the soluble peptide by hydrogenolysis completes one MIPS cycle. The process can be repeated ($n-1$) times for a peptide of (n) residues in length (Scheme 4).

In the present case, the hydrochloride salt of H-D-Orn(Boc)-OMe (**a$_1$**) was coupled with a 5% molar excess of the preactivated *N*-hydroxysuccinimide ester *Z*-D-Orn(Boc)-OSu (**b$_1$**), in *N*-methylpyrrolidinone in the presence of DIEA, to form *Z*-[D-Orn(Boc)]$_2$-OMe (**c$_1$**). Once the coupling was complete, the excess of *Z*-D-Orn(Boc)-OSu was scavenged with an aminosilica gel, which was then removed by filtration. The remaining solution was then hydrogenated in the presence of *p*-toluenesulfonic acid, giving H-[D-Orn(Boc)]$_2$-OMe·*p*TSA (**d$_1$**). The same process described above was repeated to obtain the tripeptide intermediate, *Z*-[D-Orn(Boc)]$_3$-OMe (**e$_1$**). The solution was carried through an additional hydrogenolysis/coupling reaction. No scavenging reaction was necessary for removal of the excess active ester at this stage. The NMP solution of the fully-protected tetrapeptide (**1**) was diluted with isopropyl acetate and the resulting mixture was washed with aqueous solutions, followed by distillation of the organic layer. The fully-protected tetrapeptide was then crystallized from methanol/isopropylacetate/heptane.

To proceed to step 2 (Scheme 2), half of step 1, *Z*-[D-Orn(Boc)]$_4$-OMe (**1**) was converted to the corresponding C-terminal amide, *Z*-[D-Orn(Boc)]$_4$-NH$_2$ (**2**), via treatment with a solution of anhydrous ammonia in methanol. The ammonolysis was performed at a temperature of no more than 10 °C in order to minimize racemization and impurity formation, which were observed at room temperature. Following distillation of the ammonia, the material was isolated by crystallization from methanol/isopropyl acetate/heptane.

Saponification of the second half of step 1 material led to *Z*-[D-Orn(Boc)]$_4$-OH (**3**) (step 3; Scheme 2). The saponification was carried out with lithium hydroxide in a mixture of tetrahydrofuran and water. The temperature was maintained at no more than 10 °C to control racemization of the C-terminal residue, which was observed at room temperature. The reaction was then quenched by adjusting to pH 4 with aqueous HCl solution. The product was extracted into isopropyl acetate. After a series of aqueous washes, the organic layer was distilled and *Z*-[D-Orn(Boc)]$_4$-OH (**3**) was crystallized from methanol/isopropylacetate/heptane.

These conditions of saponification were chosen in order to avoid pre-mature removal of the benzyloxycarbonyl protecting group under basic aqueous conditions. A summary of the different conditions tried during lab studies is reported in Table 1.

Table 1. Conditions examined for removal of methyl ester

	Saponification	Temperature (°C)	*Z*-[D-Orn(Boc)]$_4$-OH HPLC peak area (%)
NMP/NaOH (1 N) (1.3 equiv)	Not complete	0	35
MeOH/NaOH (1 N) (1.3 equiv)	Not complete	0	95
NMP/LiOH (1 N) (1.3 equiv)	Complete (slow overnight)	0	35
MeOH/LiOH (1 N) (1.3 equiv)	Complete (slow overnight)	0	95
THF/LiOH (1 N) (1.3 equiv)	Complete (4 h)	0	99.7
THF/LiOH (0.5 N) (1.5 equiv)	Complete (4 h)	10	99.9

Catalytic hydrogenation of *Z*-[D-Orn(Boc)]$_4$-NH_2 (**2**) was carried out in dimethylformamide in the presence of 5% palladium on alumina (35 psi, 25 °C). Complete removal of the benzyloxycarbonyl group went smoothly to afford a dimethylformamide solution of the tetrapeptide H-[D-Orn(Boc)]$_4$-NH_2.

The free amine solution was coupled with *Z*-[D-Orn(Boc)]$_4$-OH (**3**) in the presence of *N*-ethyl-*N*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDAc·HCl) and *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB)[22,23] to obtain the fully protected octapeptide, *Z*-[D-Orn(Boc)]$_8$-NH_2 (**4**), which precipitated from the reaction mixture by addition of water. The crude, protected octapeptide was isolated by filtration. The wet cake was redissolved in a mixture of acetone and water and the desired product was precipitated by additional water. This re-precipitation procedure resulted in an increase in purity from 89.5 to 92.5%.

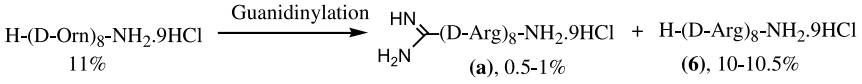
HONB was chosen as a coupling auxiliary because of the low levels of racemization (0.3%) that were observed in lab studies on this segment condensation. Other coupling conditions that were attempted for the condensation of *Z*-[D-Orn(Boc)]$_4$-OH with H-[D-Orn(Boc)]$_4$-NH_2 are summarized in Table 2.

The deprotection of *Z*-[D-Orn(Boc)]$_8$-NH_2 (**4**) was accomplished with HCl in ethyl acetate in the presence of triisopropylsilane as a scavenger at ambient temperature. This is a solid-to-solid reaction. After completion of the reaction, the product was filtered and dried under a nitrogen atmosphere.

Guanidinylation of *Z*-D-(Orn)$_8$-NH_2·8HCl was achieved with 1-pyrazole-1-carboxamidine hydrochloride salt[5–17,18] and diisopropylethylamine in methanol–water. Upon reaction completion, isopropyl alcohol was added to precipitate the product, *Z*-(D-Arg)$_8$-NH_2·8HCl (**5**). While most of the diisopropylethylamine hydrochloride salt (DIEA·HCl), formed during the guanidinylation was removed, some of this salt (3.5 wt%) remained trapped with the solids. *Z*-(D-Arg)$_8$-NH_2 (**5**) was precipitated from a methanol solution by addition of isopropanol to give product with trace (0.01 wt%) DIEA·HCl.

The intermediate at step 4, *Z*-[D-Orn(Boc)]$_8$-NH_2 (**4**), was obtained with a purity of >92 pa%, as judged by HPLC. Several impurities formed during step 5, that is the synthesis of the protected octa-arginine amide *Z*-(D-Arg)$_8$-NH_2 (**5**). The impurities are primarily attributed to the global-Boc removal step. A summary of these impurities, which were then carried to the guanidinylation step and indentified by LC/MS, is reported below:

1. Partial removal of *Z* protecting group during the Boc removal step. During the guanidinylation reaction step, about 10% of final product, H-(D-Arg)$_8$-NH_2 (**6**), was observed, as well an over-guanidinylated (**a**) side product (0.5–1%) (Scheme 5).



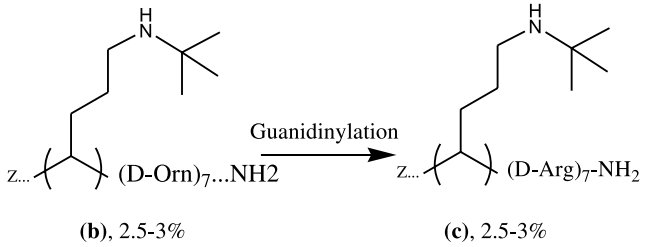
Scheme 5. Over guanidinylated impurity.

2. During the removal of the Boc protecting groups, a mixture of mono-*t*-butylated impurities was observed (**b**). These impurities were then carried to the next step, resulting in the formation of a partially guanidinylated impurity (**c**) at the same level as (**b**). An investigation to improve the Boc deprotection reaction conditions resulted in a mixture of HCl/EtOAc/triisopropylsilane showing the most promise. Under these conditions, the level of *t*-butylated impurities was decreased from 8 to 2.5% (Scheme 6).

Table 2. Racemization study during segment condensation

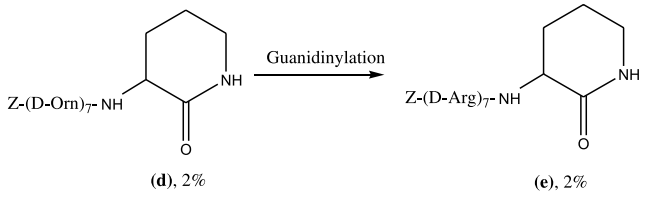
	Temperature (°C)	Racemization level of *Z*-DDDLDDDD-NH_2 [D=D-Orn(Boc)- and L=L-Orn(Boc)] (%)
DMF/EDAc in water/HOBt·H_2O	−10	1.1
DMF/EDAc/HOBt	−10	0.7
DMF/EDAc/HOBt	0	0.9
DMF/EDAc/HOBt	Room temperature	3.1
DMF/EDAc in water/HONB	10	0.3
DMF/EDAc/HONB	10	0.3

The level of racemization was measured by HPLC.



Scheme 6. *tert*-Butylation side reaction.

3. During the deprotection, lactam formation occurred at the C-terminal ornithine residue (**d**), which led to the formation of a partially guanidinylated impurity (**e**) (Scheme 7).



Scheme 7. Lactam formation.

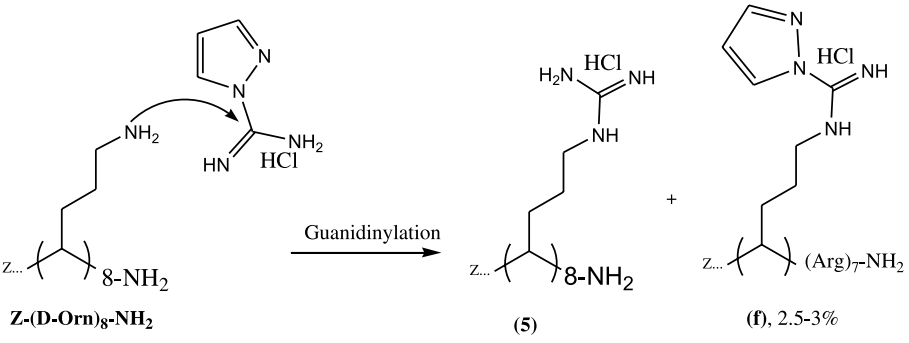
4. Impurity (**f**) formed during the guanidinylation. Formation of this impurity is attributed to ammonia serving as the leaving group in place of pyrazole. This side reaction was minimized when the guanidinylation reaction was carried out in methanol/water as the reaction solvent rather than a *N*-methylpyrrolidinone/water mixture (Scheme 8).

Hydrogenolysis of Z-(D-Arg)$_8$-NH$_2$ was carried out in H_2O with 10 wt% of 5% Pd/Al_2O_3 in the presence of 1 equiv HCl. The crude product solution was subjected to two carbon treatments to effect purification. Among the eight types of carbon evaluated, L3S provided adequate purification with best recovery. Laboratory experiments showed that two carbon treatments (1.8×loading for each) gave product at 94 pa% and an overall recovery of 65%. The pyrazole·HCl by-product from the guanidinylation step was also effectively removed. A summary of these experiments is reported in Tables 3 and 4.

The solution was then concentrated to a volume of 8 L by reverse osmosis and the final product was isolated by lyophilization.

3. Conclusion

A solution phase synthesis of H-(D-Arg)$_8$-NH$_2$·9HCl (**6**) was developed and demonstrated at the kilogram scale. The minimum isolation process for the preparation of tetrapeptide (**1**) was an efficient approach to this key intermediate. Conversion of the fully protected octa-D-ornithine intermediate (**4**) to the protected octa-D-arginine intermediate (**5**) avoided the need to develop what would be a more costly and complicated process if protected D-arginine was the starting material. Given the need and opportunity, the process as described herein could be used for the preparation of larger quantities of octa-D-arginine amide hydrochloride.



Scheme 8. Guanidinylation side reaction.

Table 3. Carbon treatment of H-(D-Arg)$_8$-NH$_2$·9HCl[a]

	H-(D-Arg)$_8$-NH$_2$·9HCl HPLC peak area (%)	H-(D-Arg)$_8$-NH$_2$·9HCl recovery (%)
L3S	89.4	91
5SC/G	88.5	79
ENO-PC	85.7	70
CPL	85.9	71
5% Pd/C	86.2	90
10% Pd/C	86.4	91
Darco G60	89.5	85

[a] Crude H-(D-Arg)$_8$-NH$_2$·9HCl at 77.2 peak area % was used for the study.

Table 4. Carbon purification using L3S[a]

	HPLC peak area (%)	Pyrazole peak area (%)	Recovery (%)
1st Carbon treatment	90.73	1.63	81.6
2nd Carbon treatment	93.62	0.15	80.5

[a] Crude H-(D-Arg)$_8$-NH$_2$·9HCl at 79.9% peak area % and pyrazole at 6.1% peak area % was used for the study.

4. Experimental

4.1. General

All starting materials were obtained from commercial suppliers and used as received. Ultrapure aminosilica gel was obtained from SiliCycle, Inc, Que., Canada. Carbon treatment was performed with carbon L3S from CECA S.A (France). Triisopropylsilane, 99%, was obtained from Johnson Mathey. All reactions were performed under an atmosphere of nitrogen. Analytical thin layer chromatography was performed on MERCK 5×10 cm SG-60F 250 μ precoated silicagel plates. Visualization was accomplished with iodine vapor. RP-HPLC analysis were performed on Agilant 100 Series HPLC, using analytical columns Chromolith RP-18e, 100×4.6 mm; for compounds **1**, **2**, **3** and Water Symmetry300 C18, 250×4.6 mm, 5 μm; for compounds **4**, **5**, **6**; with UV detection (λ=205 nm). The products were eluted utilizing a solvent gradient [*A*= water:acetonitrile:perchloric acid (850:150:1); *B*=water:-acetonitrile:perchloric acid (150:850:1); for compounds **1**, **2**, **3** and *A*=0.1% perchloric acid in water; *B*=0.1% perchloric acid in water/acetonitrile 5:95%; for compounds **4**, **5**, **6**]. The reverse osmosis was performed on a Millipore unit with a Nanomax-50 membrane (1.8″ cartridge). The lyophilization was performed with an FTS tray freeze dryer. NMR spectra were measured on a Varian UI500 magnetic resonance spectrometer (^{1}H NMR spectra at 500 MHz, ^{13}C NMR spectra at 125 MHz). Mass spectra were collected with an Agilent Series 1100 LC/MS using electrospray (ES-MS). The column was Technikrom Kromasil KR100-5C18-250A, 250×4.6 nm, (λ=205 nm); elution with *A*= 0.1% trifluoroacetic acid in water; *B*=0.1% trifluoroacetic acid in acetonitrile. For compounds **1**, **2**, **3**, and **4**, the mass reported corresponds to the structure with the loss of a Boc protecting group (100), which is common with ES-MS.

4.1.1. Preparation of *Z*-[D-Orn(Boc)]$_4$-OMe (1). *Coupling stage for Z*-[D-Orn(Boc)]$_2$-OMe (**c$_1$**). To a reactor were charged successively 2.0 kg of H-D-Orn(Boc)-OMe·HCl (**a$_1$**) and 3.4 kg of *Z*-D-Orn(Boc)-OSu (**b$_1$**) (5% molar excess). *N*-Methylpyrrolidinone (27.0 kg) was charged to the same reactor to dissolve the solids under agitation at an internal temperature of 20±5 °C. Diisopropylethylamine (1.0 kg) was then charged to the reactor and the mixture was stirred for 2–3 h. An in process sample was taken for TLC and HPLC analysis. Analytical results indicated that no starting material, H-D-Orn(Boc)-OMe·HCl (**a$_1$**), was detected and the reaction mixture was used directly in the next step.

Scavenging stage for Z-[D-Orn(Boc)]$_2$-OMe (**c$_1$**). To a reactor was charged 1.2 kg of Si-amine (amine-derivatized insoluble silicagel), then the coupling reaction mixture from above was charged to the same reactor. *N*-Methylpyrrolidinone (5.0 kg) was used as a rinse. The mixture was stirred at 45±5 °C. An in process sample was pulled after 12 h for HPLC analysis. The disappearance of *Z*-D-Orn(Boc)-OSu was monitored by HPLC. Analytical results indicated that no starting material, *Z*-D-Orn(Boc)-OSu, was detected. The temperature was adjusted to 20±5 °C and the reaction mixture was filtered through a filter pot to a hydrogenolysis vessel, which was previously charged with 1.5 kg of *p*-toluenesulfonic acid and 0.25 kg of 5% palladium on alumina. Two rinses with *N*-methylpyrrolidinone (4.5 kg) were used, then the mixture carried to the next step.

*Hydrogenolysis stage, H-[D-Orn(Boc)]$_2$-OMe·pTSA (**d$_1$**).* The solution was hydrogenated at approximately 40 psi while maintaining the temperature at 20±5 °C. Every 2 h the reactor was subjected to 2 vent/purge cycles to remove CO_2 generated from the *Z* group hydrogenolysis. After 6 h, an in process sample was pulled to monitor the reaction by HPLC. Analytical result showed the reaction was complete (*Z*-[D-Orn(Boc)]$_2$-OMe (**c$_1$**)=0.0%).

Coupling stage for Z-[D-Orn(Boc)]$_3$-OMe (**e$_1$**). The hydrogenated solution was filtered through a filter pot from the hydrogenolysis vessel to a reactor, which was previously charged with 3.3 kg of *Z*-D-Orn(Boc)-OSu. *N*-Methylpyrrolidinone (4.5 kg) was used as a rinse of the hydrogenolysis vessel and the filter pot. The solution was mixed for 15 min to dissolve the solids, then diisopropylethylamine (1.1 kg) was charged to the reactor and the mixture was stirred at an internal temperature of 20±5 °C. An in process sample was taken after 4 h for HPLC analysis. Analytical results indicated that no starting material, H-[D-Orn(Boc)]$_2$-OMe (**d$_1$**), was detected.

Scavenging stage for Z-[D-Orn(Boc)]$_3$-OMe (**e$_1$**). To a reactor was charged 1.4 kg of Si-amine, then the coupling reaction mixture was charged to the same reactor. *N*-Methylpyrrolidinone (5.0 kg) was used as a rinse. The mixture was stirred at 45±5 °C. An in process sample was pulled after 36 h for HPLC analysis. The disappearance of *Z*-D-Orn(Boc)-OSu was monitored by HPLC. Analytical results indicated that 0.67% of *Z*-D-Orn(Boc)-OSu was detected and the reaction was called complete. The temperature was adjusted to 20±5 °C and the reaction mixture was filtered through a filter pot to a hydrogenation vessel, which was previously charged with 0.3 kg of 5% palladium on alumina. Two rinses with *N*-methylpyrolidinone (4.5 kg) were used and the mixture was carried to the next step.

*Hydrogenolysis stage, H-[D-Orn(Boc)]$_3$-OMe (**f$_1$**).* The solution was hydrogenated at approximately 40 psi while maintaining the temperature at 20±5 °C. After 2 h the reactor was subjected to 2 vent/purge cycles to remove CO_2 generated from the *Z* group hydrogenolysis. After 3 h, an in process sample was pulled to monitor the reaction by HPLC. The analytical result showed the reaction was complete (*Z*-[D-Orn(Boc)]$_3$-OMe (**e$_1$**)=0.0%).

Coupling stage for Z-[D-Orn(Boc)]$_4$-OMe (**1**). The hydrogenated solution was filtered through a filter pot from the

hydrogenolysis vessel to a reactor, which was previously charged with 3.24 kg of *Z*-D-Orn(Boc)-OSu. *N*-Methylpyrrolidinone (4.5 kg) was used as a rinse of the hydrogenolysis vessel and the filter pot. The mixture was stirred at an internal temperature of 20±5 °C. An in process sample was taken after 3 h for HPLC analysis. Analytical results indicated that no starting material, H-[D-Orn(Boc)]$_3$-OMe (**f$_1$**), was detected.

The reaction mixture was diluted with 116 kg of isopropyl acetate and the resulting solution was washed with purified water (89 kg). The organic layer was then distilled under vacuum at a jacket temperature of 45 °C to a volume of approximately 40 L. To the reactor was charged 11 kg of methanol and the mixture was stirred at about 40 °C until all solids dissolved. The temperature was then adjusted to about 20 °C and 124 kg isopropyl acetate was charged to the reactor. Crystalline solids formed and after 6 h heptane (18 kg) was charged to the reactor to complete the crystallization. Solids were filtered and rinsed with isopropyl acetate (30 kg). The wet cake was dried under vacuum at 50 °C for 8 h (sample tested for loss on drying showed 0% LOD) to give 5.26 kg (72%) of **1**, with a purity of 95.4% by HPLC. MS 924 $[M+H-(Boc)]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35 (s, 36H), 1.39 (m, 8H), 1.45–1.69 (m, 8H), 2.89 (m, 8H), 3.60 (s, 3H), 3.97 (td, J=8.54, 4.73 Hz, 1H), 4.21–4.25 (m, 3H), 4.96–5.01 (m, J=12.50 Hz, 1H), 5.00–5.04 (m, J=12.70 Hz, 1H), 6.72–6.76 (m, 4H), 7.31–7.36 (m, 5H), 7.39 (m, 1H, N), 7.85–7.88 (m, 2H), 8.20 (d, J=7.17 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.2, 29.3–29.6, 39.3–39.5, 51.7, 51.6, 51.9–52.0, 54.4, 65.4, 77.4, 127.6–128.3, 137.0, 155.5–155.6, 155.9, 171.6, 171.2, 171.7, 173.3. HRMS (EI) *m/z* 1023.5953 (calcd for $C_{49}H_{83}N_8O_{15}$ 1023.5972).

4.1.2. Preparation of *Z*-[D-Orn(Boc)]$_4$-NH$_2$ (2). To a reactor were charged 2.5 kg of *Z*-[D-Orn(Boc)]$_4$-OMe (**1**) and 22 kg of methanol. The mixture was stirred at 45 °C until solids were dissolved. The temperature was then adjusted to 5 °C and 7.5 kg of anhydrous ammonia was charged to the reactor while maintaining the temperature at no more than 15 °C. At the end of the addition, the temperature was adjusted to 10 °C and the solution was mixed. An in process sample was pulled after 43 h for HPLC analysis. Analytical results indicated that 0.9% starting material, *Z*-[D-Orn(Boc)]$_4$-OMe (**1**), remained and the reaction was called complete (in-process limit for *Z*-[D-Orn(Boc)]$_4$-OMe (**1**) was set at 1.0%).

The temperature was adjusted to 20 °C and the contents of the reactor were distilled under vacuum at this temperature for about 40 min. The distillation was then carried out at 45 °C under vacuum until the mixture reached 8 L. To the reactor was charged 5 kg of methanol and the mixture was stirred at a 45 °C until solids dissolved. The temperature was then adjusted to 20 °C and 54 kg of isopropyl acetate was charged to the reactor. The mixture was stirred for 12 h to crystallize *Z*-[D-Orn(Boc)]$_4$-NH$_2$ (**2**). Heptane (14 kg) was charged to the reactor to complete the crystallization. After 3 h, the solids were filtered and rinsed with a mixture of methanol (1.5 kg)/isopropyl acetate (17 kg)/heptane (4.3 kg). The wetcake was dried under vacuuum at 50 °C for 8 h (LOD 0%) to give 1.92 kg (76%) of product, with a purity of 97% by HPLC. MS 910 $[M+H-(Boc)]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35 (s, 36H), 1.36 (m, 8H), 1.46–1.61 (m, 8H), 2.89 (m, 8H), 3.98 (td, J=8.54, 4.73 Hz, 1H), 4.21–4.25 (m, 3H), 4.97–5.01 (m, J=12.50 Hz, 1H), 5.01–5.05 (m, J=12.50 Hz, 1H), 6.73–6.76 (m, 4H), 7.01–725 (s, 2H), 7.31–7.36 (m, 5H), 7.39 (m, 1H), 7.73 (d, J=7.63 Hz, 1H), 7.91 (d, J=7.78 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.9–26.1, 28.5, 29.3–29.6, 39.5, 51.9, 52.2–52.3, 54.4, 65.4, 77.4, 127.6–128.3, 136.9, 155.5, 155.9, 171.1, 171.5, 171.8, 173.3. HRMS (EI) *m/z* 1008.5967 (calcd for $C_{48}H_{82}N_9O_{14}$ 1008.5976).

4.1.3. Preparation of *Z*-[D-Orn(Boc)]$_4$-OH (3). To a reactor were charged 2.5 kg of *Z*-[D-Orn(Boc)]$_4$-OMe (**1**), tetrahydrofuran (22 kg), and purified water (6.7 kg). The contents of the reactor were mixed at room temperature until solids dissolved. The internal temperature was adjusted to 10 °C and 7.33 kg of 0.5 M lithium hydroxide solution was charged. The solution was mixed while maintaining the temperature at no more than 15 °C. An in process sample was pulled after 2 h for HPLC analysis. Analytical results indicated that 1.0% starting material, *Z*-[D-Orn(Boc)]$_4$-OMe (**1**), remained and the reaction was called complete. While maintaining the internal temperature at no more than 15 °C, 37.5 kg of 0.1 N HCl solution was charged to the reactor, resulting in a solution at pH<4. The reaction mixture was diluted with 30 kg of isopropyl acetate. The aqueous layer was separated and was back-extracted with 30 kg of isopropyl acetate. The organic layers were combined and washed with purified water (30 kg). The organic layer was then distilled under vacuum at 45 °C to a volume of approximately 15 L. To the reactor was charged 5 kg of methanol and the mixture was stirred at 45 °C until solids dissolved. The temperature was then adjusted to 20 °C and 52.5 kg of isopropyl acetate was charged to the reactor. The mixture was stirred for 12 h to effect crystallization of *Z*-[D-Orn(Boc)]$_4$-OH (**3**). Heptane (20.4 kg) was charged to the reactor to complete the crystallization. After 3 h the solids were filtered and rinsed with a mixture of methanol (1.2 kg)/isopropyl acetate (14 kg)/heptane (6 kg). The wet cake was dried under vacuum at 50 °C for 8 h to an LOD of 0% (limit 2%), to give 1.84 kg (77%) of product, with a purity of 97.2% by HPLC analysis. MS 909 $[M+H-(Boc)]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35 (s, 36H), 1.36 (m, 8H), 1.47–1.62 (m, 8H), 2.89 (m, 8H), 3.98 (m, 1H), 4.14 (m, 1H), 4.24–4.27 (m, 2H), 4.96–5.01 (m, J=12.50 Hz, 1H), 5.01–5.06 (m, J=12.50 Hz, 1H), 6.72–6.77 (m, 4H), 7.30–7.35 (m, 5H), 7.38 (m, 1H), 7.85–7.89 (m, 2H), 8.03 (m, 1H), 12.54 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.9–26.1, 28.3, 28.5–29.6, 39.5, 51.7, 51.9–52.1, 54.4, 65.4, 77.4, 127.7–128.3, 137.0, 155.6, 155.9, 171.3, 171.4, 171.8, 173.3. HRMS (EI) *m/z* 1009.5796 (calcd for $C_{48}H_{81}N_8O_{15}$ 1009.5816).

4.1.4. Preparation of *Z*-[D-Orn(Boc)]$_8$-NH$_2$ (4). To a reactor were charged 1.75 kg of *Z*-[D-Orn(Boc)]$_4$-NH$_2$ (**2**), 0.175 kg of palladium on alumina, and dimethylformamide (30 kg). The solution was then hydrogenated at 40 psi and 20 °C. An in-process sample was pulled after 3 h for HPLC analysis. Analytical results indicated that no starting material, *Z*-[D-Orn(Boc)]$_4$-NH$_2$ (**2**), was detected and the reaction was called complete. The catalyst was removed by

filtration and the filter cake was washed with dimethylformamide (15 kg).

To a second reactor were charged 1.67 kg of *Z*-[D-Orn(Boc)]$_4$-OH (**3**), 0.34 kg of HONb and the hydrogenated solution from above. The mixture was stirred until all solids dissolved, then the internal temperature was adjusted to 10 °C. To the same reactor was added a solution of EDAc·HCl (0.50 kg) in purified water (0.50 kg). The mixture was stirred at 10 °C. An in process sample was pulled after 6 h for HPLC analysis. Analytical results indicated that 0.33% starting material, *Z*-[D-Orn(Boc)]$_4$-OH (**3**), remained and the reaction was called complete. The internal temperature was adjusted to 20 °C. Under high agitation, 48 kg of purified water was charged to the mixture. The solution was stirred for 1 h. The precipitate was filtered and rinsed with a mixture of dimethylformamide (5 kg) and water (5 kg), followed by a rinse with 10 kg of water. The material was blown dry with nitrogen for 2 h, then the wet cake was recharged to the same reactor, followed by the addition of acetone (42 kg) and purified water (16 kg). The mixture was stirred until solids were dissolved, then 31 kg of purified water was slowly charged to the reactor. The mixture was stirred for 1 h. The precipitate was filtered and rinsed with a mixture of acetone (5 kg) and water (6 kg). The material was blown dry with nitrogen for 2 h, then the wet cake was dried under vacuum at 50 °C to an LOD of 0%. Upon completion of isolation and drying, 2.29 kg (71%) was obtained, with a purity of 92.5% (HPLC area %). MS/ES 1765 $[M+H-(Boc)]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35 (s, 72H), 1.36 (m, 16H), 1.62–1.46 (m, 16H), 2.89 (m, 16H), 3.98 (m, 1H), 4.14 (m, 1H), 4.21 (m, 6H), 4.97–5.01 (m, J=12.50 Hz, 1H), 5.01–5.05 (m, J=12.50 Hz, 1H), 6.71–6.76 (m, 8H), 7.01–7.25 (s, 2H), 7.31–7.35 (m, 5H), 7.40 (m, 1H), 7.72 (m, 1H), 7.89 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.9–26.1, 28.2, 29.4–29.6, 39.5, 52.0, 52.2, 52.4, 65.4, 77.4, 127.6–128.2, 136.9, 155.5, 156.0, 171.1, 171.4, 173.3. HRMS (EI) *m/z* for $[M+2H]^{2+}$ 933.0669 (calcd for $C_{88}H_{153}N_{17}O_{26}$ 933.0659).

4.1.5. Preparation of *Z*-(D-Arg)$_8$-NH$_2$·8HCl (5). To a reactor were charged 2.5 kg of *Z*-[D-Orn(Boc)]$_8$-NH$_2$ (**4**) and a mixture of ethyl acetate (60 kg)/anhydrous HCl (7.3 kg)/triisopropylsilane (10.6 kg). The slurry was stirred at 20 °C. An in process sample was pulled after 90 min for HPLC analysis. Analytical results indicated that no starting material, *Z*-[D-Orn(Boc)]$_8$-NH$_2$ (**4**), was detected. The solids were filtered and rinsed with ethyl acetate (30 kg), then the wet-cake was blown dry with nitrogen for 12 h to give *Z*-(D-Orn)$_8$-NH$_2$·8HCl (2.5 kg). The dried *Z*-(D-Orn)$_8$-NH$_2$·8HCl was charged to a reactor, followed by 3.4 kg of 1*H*-pyrazole-1-carboxamidine hydrochloride, water (2.7 kg), and methanol (16.2 kg). The mixture was stirred until solids dissolved. Diisopropylethylamine (4.2 kg) was charged and the mixture was stirred. An in process sample was pulled after 4 h for HPLC analysis. Analytical results indicated that no starting material, *Z*-(D-Orn)$_8$-NH$_2$·8HCl, was detected. The reaction mixture was slowly poured into 144 kg of isopropyl alcohol under high agitation to precipitate the product. The reactor was rinsed with 10 kg of methanol and the rinse was charged to the IPA slurry. After 20 min, the precipitate was filtered and rinsed with isopropyl alcohol (30 kg). The wet cake was dried under vacuum at 40 °C for 8 h to give 2.1 kg of product. The product was dissolved in methanol (16.5 kg). The solution was slowly poured into 63 kg of isopropyl alcohol under high mixing. The reactor was rinsed with methanol (1.6 kg) and the rinse was added to the IPA slurry, the precipitate was filtered and rinsed with isopropyl alcohol (8 kg). The wet cake was dried under vacuum at 55 °C for 12 h to give 1.44 kg (69%) product with a purity of 77% by HPLC. MS 701 $[M/2+H]^+$ (as free base), 467 $[M/3+H]^+$ (as free base). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.51 (m, 16H), 1.59–1.71 (m, 16H), 3.13 (m, 16H), 4.17 (m, 1H,), 4.27 (m, 7H), 4.97–5.01 (m, J=12.50 Hz, 1H), 5.01–5.06 (m, J=12.50 Hz, 1H), 7.14–7.51 (s, 2H), 7.31–7.36 (m, 5H) 7.03–7.47 (m, 24H), 7.50 (m, 1H), 7.84 (m, 8H), 8.22 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.0–25.1, 28.9–29.0, 40.3–40.4, 52.1, 52.1–52.5, 65.5, 127.7–128.4, 136.9, 157, 171.4–171.5, 171.8, 173.4. HRMS (EI) *m/z* for $[M+3H]^{3+}$ 467.6313 (calcd for $C_{56}H_{108}N_{33}O_{10}$ 467.6314).

4.1.6. Preparation of H-(D-Arg)$_8$-NH$_2$·9HCl (6). To a reactor were charged 2.0 kg of *Z*-(D-Arg)$_8$-NH$_2$·8HCl (**5**), 0.2 kg of 5% palladium on alumina, water (25 kg), and 0.115 kg of 12 N hydrochloric acid. The mixture was then hydrogenated at 30 psi and 20 °C. An in process sample was pulled after 4 h for HPLC analysis. Analytical results indicated that no starting material, *Z*-(D-Arg)$_8$-NH$_2$·8HCl (**5**), was detected. The catalyst was filtered and washed with water (25 kg). The hydrogenated solution was charged to a reactor followed by a rinse with water (3 kg). L3S activated carbon (2.4 kg) was charged and the mixture was stirred at ambient temperature. After 4 h the mixture was filtered and the carbon was washed with water (3×3 kg).

The combined filtrates were transferred to a reactor followed by a water rinse (3 kg). To the same reactor was charged 1.4 kg of L3S activated carbon and the mixture was stirred at ambient temperature. After 4 h the mixture was filtered and the carbon was washed with water (3×3 kg). The combined filtrates were concentrated by reverse osmosis from a volume of approximately 45–8 L. The concentrated solution was then frozen and lyophilized to give H-(D-Arg)$_8$-NH$_2$·9HCl (0.7 kg) with a purity of 94% by HPLC. MS 634 $[M/2+H]^+$ (as free base), 623 $[M/3+H]^+$ (as free base). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.51 (m, 16H), 1.60–1.71 (m, 16H), 3.13 (m, 16H), 4.17 (m, 1H), 4.27 (m, 7H), 7.04–7.49 (m, 24H), 7.50 (m, 2H), 7.14–7.52 (s, 2H), 7.84–7.93 (m, 8H), 8.02 (m, 1H), 8.23 (m, 4H), 8.33 (m, 1H), 8.76 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 24.2, 25.0, 28.8–29.0, 40.3–40.4, 52.1–52.5, 157.0, 169.8, 171.1, 171.2, 171.5, 173.4. HRMS (EI) *m/z* for $[M+3H]^{3+}$ 422.9527 (calcd for $C_{48}H_{102}N_{33}O_8$ 422.9524).

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