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Process for the Preparation of an Amorphous, Peptide-like Diabetes Drug: Approach to a Chromatography-Free Process

Yasuhiro Sawai,* Taihei Yamane,† Motoki Ikeuchi, Shinji Kawaguchi, Masatoshi Yamada, and Mitsuhisa Yamano

Chemical Development Laboratories, CMC Center, Takeda Pharmaceutical Company Limited, 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532-8686, Japan

Abstract:

A manufacturing process suitable for large-scale production of the peptide-like amorphous compound *N*-((2*R*)-1-{(3*R*)-6-chloro-3-[(dimethylamino)methyl]-3,4-dihydroquinolin-1(2*H*)-yl}-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)-1-[(1-methyl-1*H*-indol-2-yl)carbonyl]piperidine-4-carboxamide (1) as a drug for treating diabetes has been developed. The first kilogram quantities of 1 were prepared *via* a single-chromatography process that employed recyclable cation-exchange resin chromatography for an amorphous intermediate (2*R*)-2-amino-1-[(3*R*)-6-chloro-3-[(dimethylamino)methyl]-3,4-dihydroquinolin-1(2*H*)-yl]-3-(1*H*-indol-3-yl)-propan-1-one (*syn*-3a). We have also developed a chromatography-free process that involves a combination purification of extraction of *syn*-3a with crystallization of *syn*-3a·0.25H_3PO_4·0.5H_2O. The latter process afforded amorphous compound 1 with >98% purity by HPLC area analysis, the same quality as that provided by the former process.

Introduction

Peptide-based drug molecules are prevalent in drug discovery studies that target receptors or enzymes.[1−3] In most cases, a lead peptide is converted into a low-molecular weight peptide mimetic compound with peptide-like structure by reducing the number of peptide bonds and introducing general organic molecules other than natural amino acids.[4,5] The peptide-like compounds can acquire more favorable pharmaceutical properties than those of the original molecules, such as high stability against proteolytic degradation, good absorption in the gastrointestinal tract after oral ingestion, and slow excretion through liver and kidneys.[6] However, they often exhibit unfavorable physical properties; for example, they are frequently obtained as amorphous solids with poor solid properties.

When peptide like compounds and their salts are not available in crystalline or cocrystalline form, there is no choice but to develop them as amorphous active pharmaceutical ingredients (APIs).[7] In this case, the purification method of amorphous APIs has become a pivotal issue for their process development because conventional purification such as that using silica gel chromatography is undesirable for large-scale production.[8] Alternative purification methods suitable for large-scale manufacturing may include extraction-based purification and certain types of chromatography with a recyclable stationary phase, such as ion-exchange resin, chelate resin, and synthetic absorbent. In most cases, a chromatography-free process is considered the most desirable, although it is difficult to develop.[9]

In the course of a drug discovery study for diabetes mellitus, *N*-((2*R*)-1-{(3*R*)-6-chloro-3-[(dimethylamino)methyl]-3,4-dihydroquinolin-1(2*H*)-yl}-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)-1-[(1-methyl-1*H*-indol-2-yl)carbonyl]piperidine-4-carboxamide (**1**), which has a tetrapeptide-like structure with three peptide bonds, was discovered by our discovery team.[10] API **1** was obtained only as a stable amorphous solid that has relatively high glass-transition temperature (103−105 °C). The discovery synthesis of **1** started from the preparation of chiral tetrahydroquioline fragment (*S*)-**2** *via* an enzymatic esterification process (Scheme 1).[10] The resultant (*S*)-**2** was converted to *syn*-**3a** *via* peptide coupling with Fmoc-D-tryptophan followed by Fmoc-deprotection. The subsequent peptide coupling of *syn*-**3a** with carboxylic acid **4** provided **1** in gram quantities. Amorphous API **1** was purified by silica-gel chromatography and alumina chromatography, which are to be avoided in large-scale production if possible.

In order to support preclinical pharmacological and toxicological evaluations and potential clinical applications, it was necessary to develop a manufacturing process for multikilogram quantities of **1**. The most critical issue in process development was the purification of amorphous API **1**. In addition to this challenge, the purification of the penultimate intermediate, (2*R*)-

* Author to whom correspondence may be sent. E-mail: Sawai_Yasuhiro@takeda.co.jp.

† Current address: Production Control Department, Pharmaceutical Production Division, Takeda Pharmaceutical Company Limited, 1-1, Doshomachi 4-chome, Chuo-ku, Osaka 540-8645, Japan.

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(5) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699–1720.

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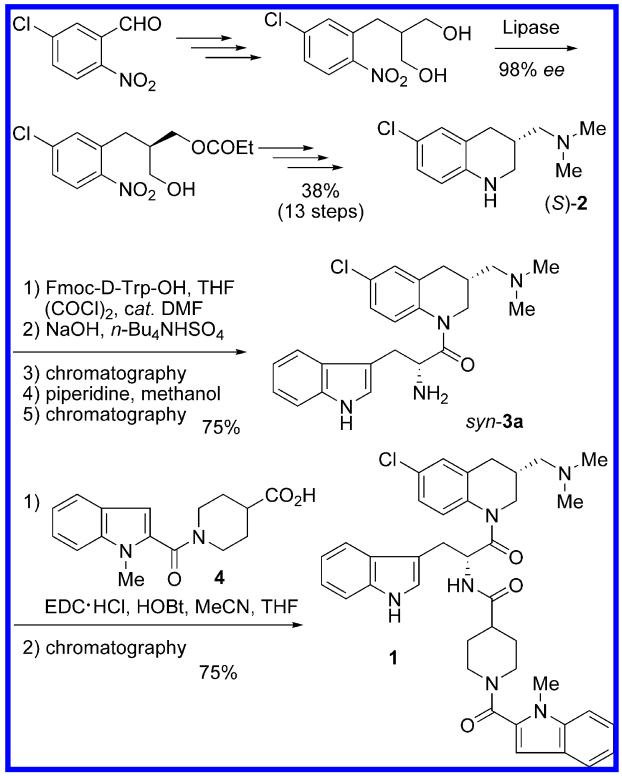
Scheme 1. **Discovery synthesis of 1**

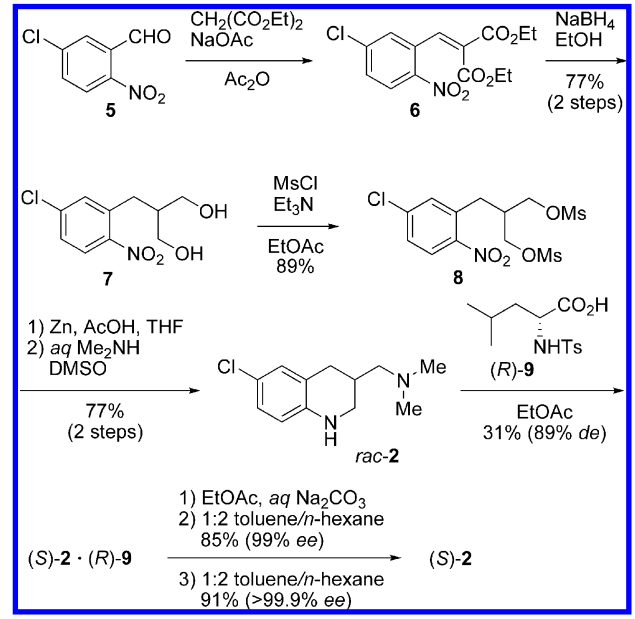


2-amino-1-[(3*R*)-6-chloro-3-[(dimethylamino)methyl]-3,4-dihydroquinolin-1(2*H*)-yl]-3-(1*H*-indol-3-yl)-propan-1-one (*syn*-**3a**), was a major issue, because it was also obtained as an amorphous solid. If high-quality *syn*-**3a** was not prepared, a significant amount of impurities derived from *syn*-**3a** would remain in **1**. These impurities would have a profound impact on the development of **1**.

Our preliminary study showed that the coupling reaction of *syn*-**3a** with **4** proceeded relatively cleanly to produce **1** and that *syn*-**3a** was obtained with a considerable amount of byproduct, which included a large amount of the residue derived from the Fmoc protecting group. On the basis of these results, we expected that the chromatography at the final step could be omitted if the final coupling reaction was carried out with the highest possible purity *syn*-**3a** under appropriate conditions. In contrast, it seemed difficult to prepare high-quality *syn*-**3a** without chromatographic purification. From the viewpoint of large-scale manufacturing, even if there were no other alternative purification methods except chromatography for the amorphous compounds, it is desirable that the recyclable resin chromatography of the most efficient separation mode be incorporated into the process at the most effective step.

Hence, we initially approached a single-chromatography process by formulating a purification strategy for the entire process as follows: (1) *syn*-**3a** was purified at as high a level as possible by recyclable resin chromatography, (2) the generation of impurities at the final step was minimized by optimizing the reaction conditions, and (3) API **1** was purified by extraction without chromatography. As a further study, we approached a chromatography-free process by replacing the chromatography with extraction-based purification of *syn*-**3a**. The former (first generation) process successfully produced the kilogram quantities of **1** necessary for preclinical evaluations. The latter (second generation) process could provide **1** of the same quality as that of the one provided by the former process without any chromatography. Herein, we describe the process development of peptide-like amorphous API **1** with particular focus on the purification of amorphous intermediate *syn*-**3a**.[11]

Scheme 2. **Synthesis of (*S*)-2**



Results and Discussion

Synthesis of (*S*)-2. The discovery synthesis *via* the enzymatic esterification process required 13 steps for the preparation of (*S*)-**2** (Scheme 1). In order to develop a shorter process, we attempted optical resolution *via* diastereomeric salt formation of *rac*-**2**, which can be prepared in five steps (Scheme 2).

Knoevenagel condensation of commercially available aldehyde **5** with diethyl malonate in the presence of sodium acetate in acetic anhydride quantitatively provided α,β-unsaturated diester **6**.[12,13] After typical extraction workup, crude oil **6** was used without a further purification in the next step. The reduction of both double bond and esters with sodium tetrahydroborate afforded saturated diol **7** in 77% yield.[14] After bismethanesulfonylation of diol, the nitro group of **8** was reduced using zinc in acetic acid. This reaction proceeded chemoselectively and was accompanied with *in situ* intramolecular cyclization. The resultant cyclic intermediate reacted with dimethylamine to give *rac*-**2** in 77% yield.

Then, various types of chiral acids were evaluated for their potential utility as resolving agents for *rac*-**2**. The screening revealed that *N*-Ts-leucine **9** was the most effective for resolving *rac*-**2**. Interestingly, the configuration of preferentially crystallized salt depended on the solvent used. For example, (*R*)-**9**

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(13) Lewandowska, E.; Kinastowski, S.; Wnuk, S. F. *Can. J. Chem.* **2002**, *80*, 192–199.
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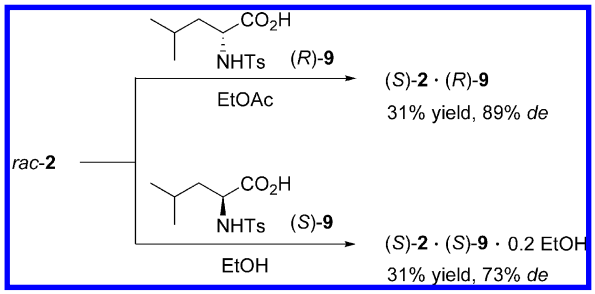
Scheme 3. **Resolution of *rac*-2 *via* diastereomeric salt formation with (*R*)-9 or (*S*)-9**



gave the salt with (*S*)-**2** from ethyl acetate and the salt with (*R*)-**2** from ethanol. Proton NMR study demonstrated that using (*R*)-**9** in ethyl acetate gave (*S*)-**2**·(*R*)-**9** as a solvent-free crystal, while using (*S*)-**9** in ethanol gave (*S*)-**2**·(*S*)-**9** as a solvate of ethanol (Scheme 3).[15] Under the optimum conditions, (*S*)-**2**·(*R*)-**9** or (*S*)-**4**·(*S*)-**9**·0.2EtOH was obtained in 31% yield with 89% de or in 31% yield with 73% de, respectively. The resolution efficiency with (*R*)-**9** was higher than that with (*S*)-**9**. The subsequent salt splitting of (*S*)-**2**·(*R*)-**9** followed by the crystallization of free base from 1:2 toluene/*n*-hexane improved the optical purity to give (*S*)-**2** in 85% yield with 99% ee.[16] It should be noted that the synthetic process of **1** did not involve any crystallization purification after the introduction of chiral parts, and thus the undesired epimer was difficult to remove.[17] The preparation of high optical purity (*S*)-**2** was one of the key-points for the successful preparation of high-quality **1**. Therefore, we carried out additional recrystallization of (*S*)-**2** that achieved further improvement of the optical purity to >99.9% ee.

This process employed potentially hazardous nitro compounds; therefore, we conducted a safety evaluation before the first scale-up campaign. Differential scanning calorimetry (DSC) analysis of four nitro compounds revealed that they did not produce significant safety issues arising from their thermochemical behavior.[18] Therefore, we carried out pilot-plant campaigns, and multikilogram quantities of (*S*)-**2** were successfully obtained through the seven-step process.[19]

Scheme 4. **Synthesis of *syn*-3a**

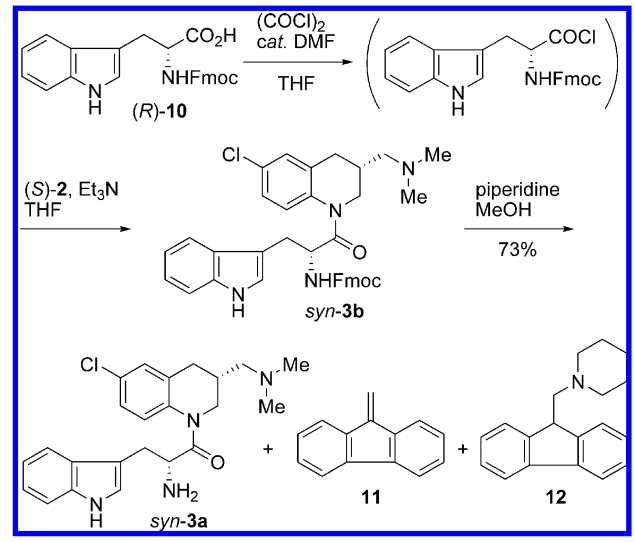


Table 1. **Optimization of the coupling reaction of (*S*)-2 with (*R*)-10[a]**

entry	(*R*)-**10** (equiv)	base	equiv	*syn*-**3b** yield (%)
1	3.0	NaOH[b]	1.1	87
2	3.0	*i*-Pr_2NEt	1.1	57
3	3.0	*i*-Pr_2NEt	1.6	86
4	1.5	*i*-Pr_2NEt	1.4	87
5	1.5	Et_3N	1.4	90

[a] 1.2 equiv of $(COCl)_2$ was used. [b] 0.1 equiv of *n*-Bu_4NHSO_4 was added.

Synthesis of *syn*-3a. The next task was to improve the coupling reaction of (*S*)-**2** with *N*-Fmoc-D-tryptophan (*R*)-**10** (Scheme 4). Improvement of the reaction can reduce the load on the purification of amorphous compound *syn*-**3a**. This reaction required strong activation for the carboxyl function of D-tryptophan, such as that provided by acyl chloride, because the ring nitrogen of (*S*)-**2** has a low nucleophilicity arising from the 6-chloro substituent on the tetrahydroquinoline ring.[20] The Fmoc protective group was selected for the α-nitrogen protection of D-tryptophan because of its stability under acidic conditions, which was derived from acyl chloride and its removability under mild basic conditions. Although *syn*-**3b** was obtained in 87% yield under the discovery synthesis conditions (Table 1, entry 1), poor reproducibility was observed. The use of strongly basic sodium hydroxide was suspected to promote the hydrolysis of acyl chloride intermediate thus leading to the inconsistent results. Therefore, we attempted to use less basic

(15) Powder X-ray diffraction of each of (*S*)-**2**·(*R*)-**9** and (*R*)-**2**·(*R*)-**9**, which were prepared from enantiopure (*S*)-**2** and (*R*)-**2** in ethanol or ethyl acetate, demonstrated that (*S*)-**2**·(*R*)-**9** consistently showed the same pattern, while (*R*)-**2**·(*R*)-**9** showed two types of patterns, depending on the solvent used. For the resolution of both enantiomers *via* diastereomeric salt formation using a single configuration of resolving agent, see: (a) Hirose, T.; Begum, M.; Islam, M. S.; Taniguchi, K.; Yasutake, M. *Tetrahedron: Asymmmetry* **2008**, *19*, 1641–1646. (b) Taniguchi, K.; Aruga, M.; Yasutake, M.; Hirose, T. *Org. Biomol. Chem.* **2008**, *6*, 458–463. (c) Taniguchi, K.; Sakurai, R.; Sakai, K.; Yasutake, M.; Hirose, T. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 1084–1090. (d) Sakai, K.; Sakurai, R.; Nohira, H.; Tanaka, R.; Hirayama, N. *Tetrahedron: Asymmmetry* **2004**, *15*, 3495–3500. (e) Sakai, K.; Sakurai, R.; Hirayama, N. *Tetrahedron: Asymmmetry* **2004**, *15*, 1073–1076.

(16) The enantiomeric excess of **2** was determined by HPLC. HPLC conditions: column, Chiralpak AD-RH (4.6 mm × 150 mm); mobile phase, 0.05 M *aq* KH_2PO_4 containing 0.1% of Et_3N (pH 7.5)/MeCN (50:50); flow rate, 0.5 mL/min; column temperature, 25 °C; detection, UV 254 nm.The recrystallization of (*S*)-**2**·(*R*)-**9** of 88% de in ethyl acetate gave the product in 82% yield with 99% de.

(17) Use of (*S*)-**2** of low optical purity led to the formation of *syn*-**3a** in low diastereomeric ratio, because *anti*-**3b** was generated much faster than *syn*-**3b** and the undesired *anti*-isomer was difficult to remove from the desired *syn*-isomer.

(18) DSC analysis shows that these nitro compounds have sufficiently high exothermic onset temperatures (**5**, 218 °C; **6**, 328 °C; **7**, 302 °C; **8**, 252 °C), although they have large gross heating values (**5**, 1513 J/g; **6**, 363 J/g; **7**, 755 J/g; **8**, 1091 J/g). Comprehensive safety evaluations should be conducted before further attempts to scale up.

(19) It should be noted that reduction reaction using sodium borohydride in alcohol solvent can cause safety concerns including latent and abrupt release of heat. For the control of heat generation on large-scale usage of sodium borohydride in alcohol, see: (a) Flanagan, R. C.; Xie, S.; Millar, A. *Org. Process Res. Dev.* **2008**, *12*, 1307–1312. (b) Belecki, K.; Berliner, M.; Bibart, R. T.; Meltz, C.; Ng, K.; Phillips, J.; Ripin, D. H. B.; Vetelino, M. *Org. Process Res. Dev.* **2007**, *11*, 754–761.

(20) The reactions of (*S*)-**2** with *N*-protected D-tryptophans (*N*-Fmoc-D-Trp-OH, *N*-Boc-D-Trp-OH or *N*-Cbz-D-Trp-OH) using condensation reagent 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC·HCl) did not give the desired amides. For the intramolecular *N*-acylation of tetrahydroquinoline using *N*,*N*′-dicyclohexylcarbodiimide (DCC), see: Somayaji, V.; Brown, R. S. *J. Org. Chem.* **1986**, *51*, 2676–2686.

tertiary amine instead of sodium hydroxide. Using 1.1 equiv of diisopropylethylamine (DIEA) with 1.2 equiv of oxalyl chloride, however, caused the yield of *syn*-**3b** to be significantly decreased (entry 2). After extensive investigation into the amount of reagents, we found that using approximately 1.5 equiv of DIEA with 1.2 equiv of oxalyl chloride brought the yield back to the former level (entry 3). The acyl chloride formation and the subsequent coupling reaction could be conducted in one-pot. Although a large excess of (*R*)-**10** was necessary for completion of the coupling reaction under the discovery synthesis conditions (entry 1), the amount of (*R*)-**10** was successfully reduced to 1.5 equiv without a yield decrease (entry 4), and even in the case using triethylamine *syn*-**3b** was obtained in 90% yield (entry 5). Applying the optimum conditions, an efficient and robust process was accomplished.

After typical extraction workup, *syn*-**3b** was used without a further purification in the subsequent Fmoc-deprotection reaction (Scheme 4). Although the Fmoc protective group worked effectively during the coupling reaction,[21] dibenzofulvene **11** and its piperidine adduct **12** appeared during deprotection and were difficult to remove. Success in reducing of the amount of (*R*)-**10** at the coupling reaction step was also advantageous for the purification of amorphous compound *syn*-**3a**, because the amounts of **11** and **12** were consequently reduced. However, *syn*-**3a** still contained more than a stoichiometric amount of these byproduct, almost a half molar amount of *N*-H-D-tryptophan derived from the excess (*R*)-**10**, and a wide variety of low-level impurities.

Cation-Exchange Resin Chromatography of *syn*-3a. According to the purification strategy, we approached recyclable resin chromatography for the removal of these impurities. The screening of the separation mode of the chromatography using cation-exchange resin, chelate resin, and synthetic adsorbent revealed that cation-exchange resin chromatography (CEC) was effective for the purification of *syn*-**3a**. In particular, the weakly acidic cation-exchange resin DIAION WK100, which consists of a methacrylate resin matrix, was the most efficient stationary phase. Figure 1 illustrates the concept behind the CEC procedure for *syn*-**3a**: (1) a crude mixture was charged onto the column, which had been filled with DIAION WK100 preconditioned in its H-form,[22] (2) impurities were eluted while *syn*-**3a** remained adsorbed to the H-form resin, and (3) *syn*-**3a** was exchanged with sodium cations and eluted from Na-form resin.

The most significant issue for the development of the CEC procedure was the separation of tertiary amine *syn*-**3a** and **12**. An extensive investigation into the concentration of sodium cations in mobile phase revealed that the sodium cations in 0.5% sodium chloride aqueous solution/methanol (20:80) selectively exchanged **12**, while the sodium cations in 5% sodium chloride aqueous solution/methanol (20:80) simultaneously exchanged both *syn*-**3a** and **12**. The CEC procedure has been developed

(21) For the peptide synthesis *via* amino acid chloride using Fmoc protective group, see: (a) Carpino, L. A.; Beyermann, M.; Wenschuh, H.; Bienert, M. *Acc. Chem. Res.* **1996**, *29*, 268–274. (b) Carpino, L. A. *Acc. Chem. Res.* **1987**, *20*, 401–407.

(22) The weakly acidic cation-exchange resin DIAION WK100 was preconditioned as follows: A column was filled with DIAION WK100 (100 L) using methanol and was successively eluted with the mobile phase as follows: water/methanol (200 L, 1:4), water/methanol (200 L, 1:1), water/methanol (200 L, 4:1), water (200 L), 1 M hydrochloric acid (300 L), water (300 L), and methanol (300 L).

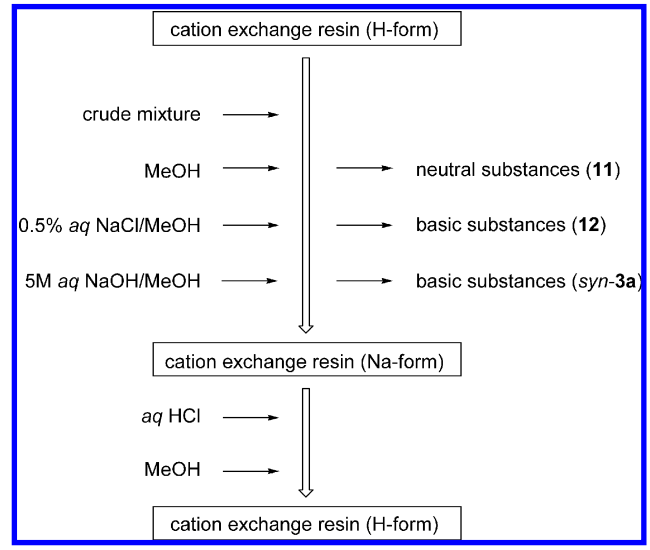


Figure 1. **CEC for *syn*-3a**

on the basis of this selective cation exchange. After eluting the neutral substances such as **11** and *N*-H-D-tryptophan by methanol, 5% sodium chloride aqueous solution/methanol (20:80) eluted **12** with low levels of other impurities. Then, 5 M sodium hydroxide aqueous solution/methanol (20:80) eluted *syn*-**3a**. This fraction gave amorphous solid *syn*-**3a** with 97% purity by HPLC area analysis and 73% yield based on (*S*)-**2**.

This process has been run on a kilogram scale, and the resin could be reused at least three times after reconditioning, in which the resin was converted from its Na-form to its H-form using hydrochloric acid and methanol. As discussed in more detail below, *syn*-**3a** was converted to **1** with >98% HPLC area. Therefore, we have developed a first-generation process of **1** suitable for large-scale production, which involved single chromatography throughout the entire process.

Extraction-Based Purification of *syn*-3a. Before the CEC was developed, all attempts to crystallize the salt of *syn*-**3a** with various types of acid were unsuccessful. However, when applying the high-quality amorphous solid *syn*-**3a**, which was purified by the CEC, to the salt crystallization study, the phosphate salt was successfully isolated as a stable crystal. The composition of the phosphate salt was determined by single-crystal X-ray analysis (Figure 2, top and side views of the phosphate salt). It was revealed that the crystal consists of four molecules of *syn*-**3a**, a molecule of phosphoric acid, and two molecules of water. One of the two molecules of water coordinates the phosphoric acid. The resulting four of the five hydrogen atoms interact with the nitrogen atoms of four molecules of *syn*-**3a**, and one of the five hydrogen atom interacts with an another water. Using 4 equiv of phosphoric acid in aqueous ethanol on the basis of the composition of the phosphate salt, crystalline *syn*-**3a**·$0.25H_3PO_4$·$0.5H_2O$ was obtained in the highest yield. The combination of the CEC with the subsequent phosphate salt crystallization enabled preparation of *syn*-**3a**·$0.25H_3PO_4$·$0.5H_2O$ with >99% HPLC area in 64% yield based on (*S*)-**2**.

Success in the salt crystallization allowed the preparation of highly purified *syn*-**3a** without chromatography. One of the possible methods was the use of extraction purification in combination with the phosphate salt crystallization. We inves-

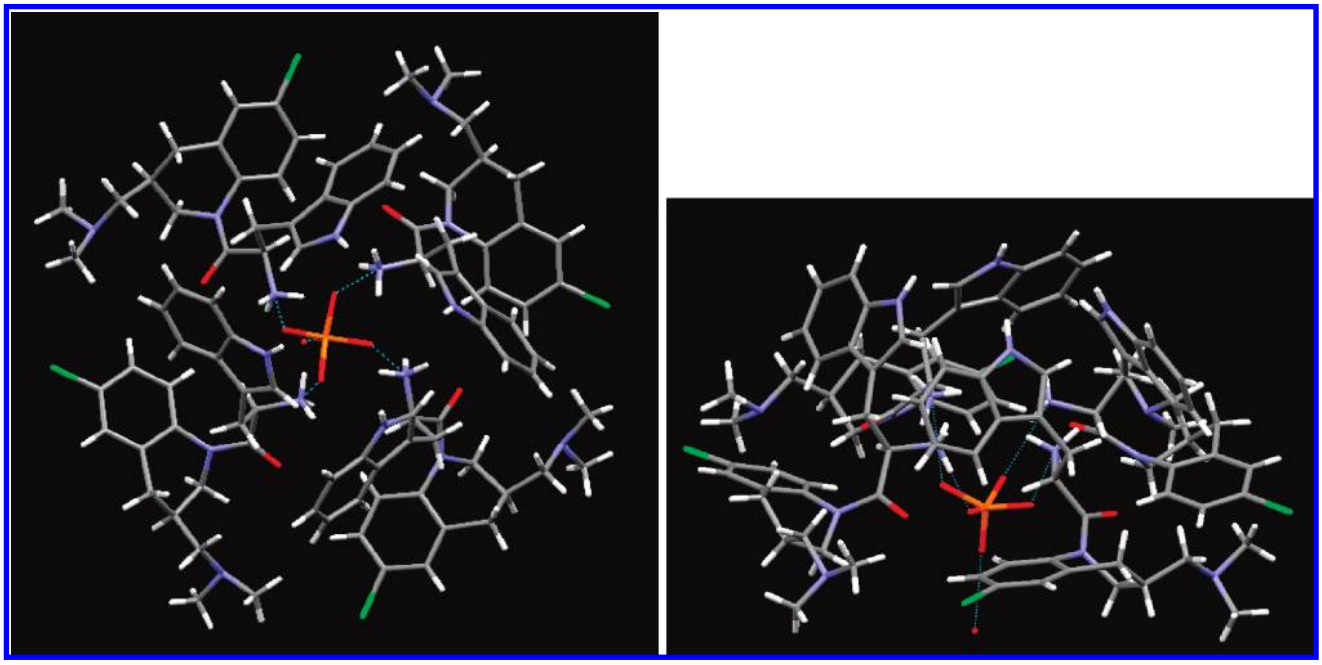


Figure 2. **Single-crystal X-ray analysis of *syn*-3a·0.25H_3PO_4·0.5H_2O.**

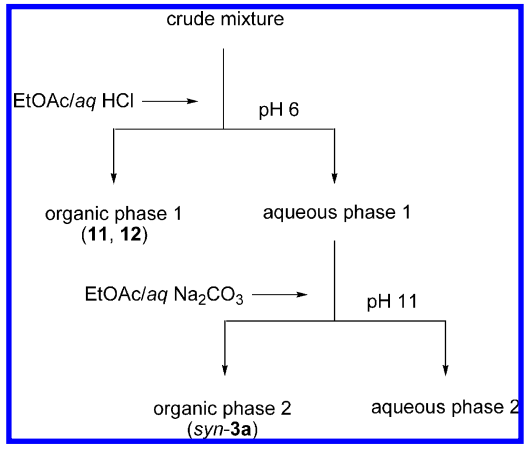
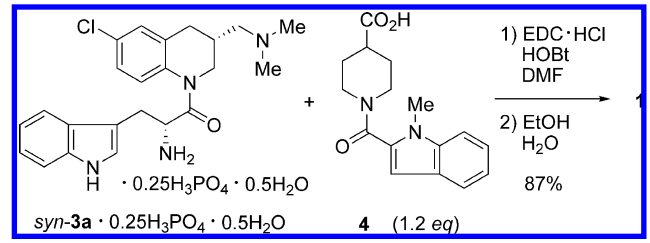


Figure 3. **Extraction of *syn*-3a.**

tigated the extraction conditions using a weakly acidic aqueous phase on the basis of the concept of CEC. Adjusting the aqueous phase to pH 6, *syn*-**3a** was effectively separated from major impurities **11** and **12** (Figure 3). Then, *syn*-**3a** was extracted with ethyl acetate after adjusting the aqueous phase to pH 11. *N*-H-D-Tryptophan was removed by a sequence of the extraction. Unfortunately, a wide variety of low-level impurities were carried downstream, and therefore, amorphous compound *syn*-**3a** was obtained with a purity of only 88% by HPLC area analysis. However, the subsequent phosphate salt crystallization from aqueous ethanol followed by an additional purification by reslurrying in 3:1 ethyl acetate/*n*-hexane successfully improved the purity to give *syn*-**3a** ·0.25H_3PO_4·0.5H_2O with >99% HPLC area in 68% yield based on (*S*)-**2**. Therefore, a second-generation chromatography-free process has been developed.

Synthesis of 1. The synthesis of **1** was completed with a peptide-coupling reaction of amine *syn*-**3a** with carboxylic acid **4** (Scheme 5), which was readily prepared with high purity from commercially available ethyl isonipecotinate with 1-methyindolyl-2-carboxylic acid. In order to reduce the burden on the

Scheme 5. **Synthesis of 1**



purification step, we minimized the impurities generated by optimizing the coupling reaction conditions. The coupling reactions using oxalyl chloride, pivaloyl chloride, ethyl chloroformate, or *N*, *N*′-carbonyldiimidazole (CDI) resulted in the formation of **1** with a considerable amount of byproduct. In contrast, with the use of condensation reagent 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC·HCl) with 1-hydroxybenzotriazole monohydrate (HOBt), the reaction was completed in a short time to give **1** with improved purity. The best result was obtained with 1.2 equiv each of **4**, EDC·HCl, and HOBt in DMF at ambient temperature. In particular, using 1.2 equiv of **4** was critical for the complete consumption of *syn*-**3a**, which was difficult to remove by extraction.

After the final peptide coupling reaction was carried out under the optimum conditions, extraction using aqueous potassium carbonate solution and ethyl acetate afforded amorphous powder **1** with >98% HPLC area from *syn*-**3a** or *syn*-**3a** ·0.25H_3PO_4·0.5H_2O without chromatographic purification. The residual **4** was removed by washing of organic layer with 5% aqueous potassium carbonate solution. After decolorization of the organic layer with activated carbon, solvent was switched to ethanol by vacuum concentration with additional ethanol. A simple and scalable precipitation was also developed: dropping 10 volumes of ethanol solution of **1** into 50 volumes of water at ambient temperature effectively provided amorphous powder. The wet powder of **1** was dried under reduced pressure until the water content is not more than 2% by Karl Fisher method.

Applying the optimum conditions, the pilot-plant campaigns successfully provided the kilogram quantities of amorphous API **1** in 87% yield with >98% HPLC area and >99.9% ee.[23] The alternative extraction-based purification of *syn*-**3a** allowed **1** to be prepared, without any chromatography, and of a quality matching that provided by the process involving CEC.

Conclusion

The process of manufacturing peptide-like API **1** suitable for large-scale production is required to support preclinical evaluations and potential clinical applications. The most critical issue to be addressed for the process development was the solid-state properties of **1** and the final intermediate *syn*-**3a**. These are both amorphous solids that are difficult to purify in scaled-up production. We initially considered a single-chromatography process that employed recyclable cation-exchange resin chromatography for *syn*-**3a**. The process successfully provided kilogram quantities of **1** with >98% HPLC area. As a further study, we attempted to replace the CEC with extraction-based purification. The chromatography-free process that involved the extraction purification of *syn*-**3a** and the successive crystallization of *syn*-**3a**·$0.25H_3PO_4$·$0.5H_2O$ provided **1** of the same quality as that provided by the former process.

Experimental Section

General. All materials were purchased from commercial suppliers and used without further purification. Melting points were recorded on a Büchi B-540 micromelting apparatus and were uncorrected. IR spectra were recorded on a Horiba FT-210 spectrophotometer. NMR spectra were run at 300 MHz (^{1}H) and 75 MHz (^{13}C) on a Bruker DPX-300 spectrometer. Chemical shifts are reported as δ values using tetramethylsilane as an internal standard, and coupling constants (J) are given in Hz. The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, and br = broad. Optical rotation values were recorded on a JASCO DIP-370 polarimeter under standard conditions. HPLC analyses were performed with Hitachi L-7000. Detection was effected with an ultraviolet absorption photometer (wavelength 254 nm). Purity was determined by HPLC and presented as an area percentage of the compound peak relative to the total area of all the peaks integrated. All compounds were judged to be of greater than 95% purity on the basis of ^{1}H NMR and HPLC analyses. The microanalyses and mass spectral analyses were carried out at Takeda Analytical Research Laboratories, Ltd.

Synthesis of 2-(5-Chloro-2-nitrobenzyl)propane-1,3-diol (7). To a solution of **5** (39.8 kg, 214.5 mol) and diethyl malonate (34.4 kg, 214.8 mol) in acetic anhydride (86.8 kg, 850.2 mol) was added sodium acetate (26.4 kg, 321.8 mol). The mixture was stirred at 90−95 °C for 5.5 h and then allowed to cool to room temperature. After adding water (119.4 kg), the mixture was stirred at room temperature for 1 h. Then water (119.4 kg) and toluene (206.6 kg) were added, and the layers were separated. The aqueous layer was extracted with toluene (206.6 kg). The combined organic layers were washed with water (3 × 477.6 kg), saturated *aq* $NaHCO_3$ (2 × 425.9 kg), and 5% *aq* NaCl (2 × 417.9 kg), and then concentrated *in vacuo*. Ethanol (158.0 kg) was added to the residue, and the resultant solution was concentrated *in vacuo* (twice). Then ethanol (126.6 kg) was added to the residue to give a clear solution. Under nitrogen atmosphere, to another reaction vessel containing ethanol (379.3 kg) was added sodium tetrahydroborate (24.4 kg, 645.0 mol) in small portions at −10−0 °C. The solution obtained above was added dropwise to the reaction vessel containing ethanol and sodium tetrahydroborate. The mixture was allowed to warm and was stirred at room temperature for 2.5 h. After cooling to 0 °C, 6 M hydrochloric acid (206.6 kg) was added dropwise. The mixture was allowed to warm to room temperature, and water (238.8 kg) was added. After stirring at room temperature for 1.5 h, the mixture was adjusted to pH 6 by adding 30% *aq* NaOH (48.1 kg) and then was concentrated to 320 L *in vacuo*. Water (238.8 kg) and *tert*-butylmethylether (235.6 kg) were added, and the layers were separated. The aqueous layer was extracted with *tert*-butylmethylether (235.6 kg). The combined organic layers were washed with water (2 × 398.0 kg) and then concentrated *in vacuo*. Toluene (68.9 kg) was added to the residue, and the resultant mixture was concentrated *in vacuo* (twice). After the addition of toluene (79.2 kg), the mixture was heated to 40 °C and stirred until a clear solution was obtained. Then the mixture was stirred at room temperature for 2 h and at 0−5 °C for 1.5 h. The resultant precipitate was collected by filtration, washed with cold toluene (34.6 kg), and dried *in vacuo* to give **7** (40.4 kg, 164.5 mol, 77% yield) as a white crystalline powder. Mp 59−60 °C; IR (ATR) ν 3282, 1519, 1333, 1028, 831 cm^{-1}; MS (ESI) m/z 246 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02−2.09 (m, 1H), 2.43 (t, J = 5.0 Hz, 2H), 3.00 (d, J = 7.3 Hz, 2H), 3.67−3.74 (m, 2H), 3.83−3.89 (m, 2H), 7.35 (dd, J = 2.3, 8.7 Hz, 1H), 7.43 (d, J = 2.3 Hz, 1H), 7.92 (d, J = 8.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.0, 42.9, 64.3, 126.6, 127.7, 132.9, 137.6, 139.3, 146.8; Anal. Calcd for $C_{10}H_{12}NO_4Cl$: C, 48.89; H, 4.92; N, 5.70; Cl, 14.43. Found: C, 48.92; H, 4.86; N, 5.76; Cl, 14.36.

Synthesis of 2-(5-Chloro-2-nitrobenzyl)propane-1,3-diyl Dimethanesulfonate (8). To a solution of **7** (40.3 kg, 164.0 mol) in ethyl acetate (181.8 kg) were successively added triethylamine (49.8 kg, 492.1 mol) and methanesulfonyl chloride (56.4 kg, 492.4 mol) at 0−10 °C. The mixture was stirred at 0−10 °C for 1 h and then allowed to warm to room temperature. After adding saturated *aq* $NaHCO_3$ (215.5 kg), the mixture was stirred at room temperature for 1 h and then extracted with ethyl acetate (181.8 kg). The organic layer was washed with saturated *aq* $NaHCO_3$ (215.5 kg), 10% *aq* NaCl (221.7 kg), 2 M hydrochloric acid (215.5 kg), 10% *aq* NaCl (221.7 kg), and 5% *aq* NaCl (6 × 211.8 kg) and then was concentrated *in vacuo*. Ethyl acetate (109.0 kg) was added to the residue, and the resultant mixture was allowed to cool to 0 °C and stirred for 1 h. Then diisopropylether (87.6 kg) was added dropwise, and the mixture was stirred at 0 °C for 2 h. The resultant precipitate was collected by filtration, washed with 1:1 ethyl acetate/diisopropylether (65.4 kg), and dried *in vacuo* to give **8** (58.8 kg, 146.3 mol, 89% yield) as a white crystalline powder. Mp 73−74 °C; IR (ATR) ν 1522, 1339, 1173, 945, 823, 522

(23) The enantiomeric excess of **1** was determined by HPLC. HPLC conditions: column, Chiralcel OD-RH (4.6 mm × 150 mm); mobile phase, 0.05 M *aq* $CH_3CO_2NH_4$/MeCN (40:60); flow rate, 1.0 mL/min; column temperature, 40 °C; detection, UV 250 nm.

cm^{-1}; MS (ESI) *m/z* 424 $(M + Na)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 2.56−2.64 (m, 1H), 3.04−3.08 (m, 8H), 4.23−4.36 (m, 4H), 7.42 (m, 1H), 8.03 (d, $J = 8.1$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.1, 37.5, 39.1, 67.4, 127.2, 128.7, 133.0, 135.1, 140.1, 147.4; Anal. Calcd for $C_{12}H_{16}NO_8S_2Cl$: C, 35.87; H, 4.01; N, 3.49; S, 15.96; Cl, 8.82. Found: C, 35.85; H, 3.79; N, 3.34; S, 16.01; Cl, 8.55.

Synthesis of 1-(6-Chloro-1,2,3,4-tetrahydroquinolin-3-yl)-*N*,*N*-dimethylmethanamine (*rac*-2). To a solution of **8** (58.7 kg, 146.1 mol) and acetic acid (307.7 kg) in THF (260.8 kg) was added zinc dust (95.6 kg, 1462.0 mol) at 0−5 °C. The mixture was stirred at 0 °C for 1 h and at 60 °C for 3 h. After cooling to room temperature, the precipitate was filtered off and washed with THF (260.8 kg). The combined filtrates were concentrated *in vacuo*. Ethyl acetate (264.6 kg) was added to the residue. The resultant solution was washed with water (3 × 293.4 kg), 5% *aq* $NaHCO_3$ (308.0 kg), and 5% *aq* NaCl (308.0 kg), and then concentrated *in vacuo*. Then DMSO (64.6 kg) was added to the residue, and the resultant solution was concentrated *in vacuo*. Then DMSO (64.6 kg) and 50% *aq* dimethylamine (252.3 kg, 2798.4 mol) were successively added dropwise, and the resultant mixture was stirred at 60 °C for 5 h. After the addition of water (293.4 kg), the mixture was stirred at room temperature for 1 h and at 0 °C for 5 h. The resultant precipitate was collected by filtration, washed with water (293.4 kg), and dried *in vacuo* to give *rac*-**2** (25.1 kg, 111.7 mol, 77% yield) as a white crystalline powder. Mp 94−95 °C; IR (KBr) ν 3246, 1604, 1495, 1304, 806 cm^{-1}; MS (ESI) *m/z* 225 $(M + H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 2.11−2.26 (m, 9H), 2.43 (dd, $J = 8.7$, 16.3 Hz, 1H), 2.81 (dd, $J = 4.4$, 16.1 Hz, 1H), 2.97 (t, $J = 9.7$ Hz, 1H), 3.38 (dd, $J = 1.4$, 11.2 Hz, 1H), 3.86 (br s, 1H), 6.39 (d, $J = 7.9$ Hz, 1H), 6.91 (d, $J = 7.8$ Hz, 1H), 6.92 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.2, 31.7, 45.4, 46.1, 63.1, 114.9, 121.3, 122.0, 126.6, 129.3, 143.2; Anal. Calcd for $C_{12}H_{17}N_2Cl$: C, 64.13; H, 7.62; N, 12.47; Cl 15.78. Found: C, 64.12; H, 7.60; N, 12.43; Cl, 15.67.

Synthesis of the Salt of 1-[(3*S*)-6-Chloro-1,2,3,4-tetrahydroquinolin-3-yl]-*N*,*N*-dimethylmethanamine with *N*-[(4-Methylphenyl)sulfonyl]-D-leucine ((*S*)-2·(*R*)-9). A suspension of *rac*-**2** (12.0 kg, 53.4 mol) and **(*R*)-9** (15.2 kg, 53.3 mol) in ethyl acetate (216.5 kg) was heated to 68 °C and stirred until a clear solution was obtained. Then the mixture was allowed to cool to room temperature and was stirred for 18 h. The resultant precipitate was collected by filtration, washed with ethyl acetate (21.6 kg), and dried *in vacuo* to give **(*S*)-2·(*R*)-9** (8.40 kg, 16.5 mol, 89.4% de, 31% yield) as a white crystalline powder. Mp 142−143 °C; IR (ATR) ν 2361, 1497, 1164, 569, 544 cm^{-1}; MS (ESI) *m/z* 286 $(M + H)^+$; $[\alpha]^{20}_D$ −0.6 (*c* 1.01, MeOH); ^1H NMR (300 MHz, DMSO-d_6) δ 0.68 (d, $J = 6.5$ Hz, 3H), 0.78 (d, $J = 6.6$ Hz, 3H), 1.35 (t, $J = 7.1$ Hz, 2H), 1.52−1.66 (m, 1H), 1.91−2.04 (m, 1H), 2.15−2.32 (m, 9H), 2.35 (s, 3H), 2.66−2.83 (m, 2H), 3.23 (d, $J = 9.4$ Hz, 1H), 3.55 (t, $J = 7.2$ Hz, 1H), 5.84 (br s, 1H), 6.41 (d, $J = 8.0$ Hz, 1H), 6.83 (d, $J = 9.0$ Hz, 1H), 6.85 (s, 1H), 7.33 (d, $J = 8.3$ Hz, 2H), 7.63 (d, $J = 8.2$ Hz, 2H), 7.88 (br s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.5, 21.8, 23.2, 24.4, 29.3, 31.5, 42.0, 44.6, 45.3, 55.0, 62.1, 114.8, 118.7, 121.3, 126.6, 127.1, 129.0, 129.8, 138.8, 142.8, 144.5, 174.1; Anal. Calcd for $C_{25}H_{36}N_3O_4SCl$: C, 58.87; H, 7.11; N, 8.24; S, 6.29; Cl, 6.95. Found: C, 58.72; H, 7.03; N, 8.13; S, 6.34, Cl, 6.72.

Synthesis of 1-[(3*S*)-6-Chloro-1,2,3,4-tetrahydroquinolin-3-yl]-*N*,*N*-dimethylmethanamine ((*S*)-2). A suspension of **(*S*)-2·(*R*)-9** (8.40 kg, 16.5 mol, 89.4% de) in 0.5 *N* sodium hydroxide aqueous solution (128.5 kg) was stirred at room temperature for 0.5 h, and then extracted with toluene (109.0 kg). The aqueous layer was extracted with toluene (36.3 kg). The combined organic layers were washed with water (2 × 42.0 kg) and concentrated *in vacuo*. Then 1:2 toluene/*n*-hexane (9.2 kg) was added to the residue. The resultant mixture was heated and stirred at 75 °C until clear solution was obtained. Then the solution was allowed to cool to room temperature and stirred for 20 h. The resultant precipitate was collected by filtration, washed with 1:2 toluene/*n*-hexane (2.3 kg), and dried *in vacuo* to give crude-**(*S*)-2** (3.14 kg, 14.0 mol, 98.6% ee, 85% yield) as a white crystalline powder. A suspension of crude-**(*S*)-2** (3.10 kg, 13.8 mol, 98.6% ee) in 1:2 toluene/*n*-hexane (6.7 kg) was heated and stirred at 80 °C until clear solution was obtained. Then the mixture was allowed to cool to room temperature and stirred for 3 h. The resultant precipitate was collected by filtration, washed with 1:2 toluene/*n*-hexane (2.4 kg), and dried *in vacuo* to give **(*S*)-2** (2.81 kg, 12.5 mol, > 99.9% ee, 77% yield (based on **(*S*)-2·(*R*)-9** used)) as a white crystalline powder. Mp 115−116 °C; IR (KBr) ν 3271, 1604, 1493, 1306, 814 cm^{-1}; MS (ESI) *m/z* 225 $(M + H)^+$; $[\alpha]^{20}_D$ +47.0 (*c* 0.96, MeOH); ^1H NMR (300 MHz, $CDCl_3$) δ 2.11−2.27 (m, 9H), 2.42 (dd, $J = 8.8$, 16.2 Hz, 1H), 2.81 (dd, $J = 3.2$, 15.4 Hz, 1H), 2.98 (t, $J = 9.5$ Hz, 1H), 3.38 (dd, $J = 1.6$, 11.2 Hz, 1H), 3.86 (br s, 1H), 6.39 (d, $J = 8.7$ Hz, 1H), 6.91 (d, $J = 9.0$ Hz, 1H), 6.92 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.2, 31.7, 45.4, 46.1, 63.1, 114.9, 121.3, 122.0, 126.6, 129.3, 143.2; Anal. Calcd for $C_{12}H_{17}N_2Cl$: C, 64.13; H, 7.62; N, 12.47; Cl, 15.78. Found: C, 64.16; H, 7.82; N, 12.43; Cl, 15.57.

Synthesis of (2*R*)-2-amino-1-[(3*R*)-6-chloro-3-[(dimethylamino)methyl]-3,4-dihydroquinolin-1(2*H*)-yl]-3-(1*H*-indol-3-yl)-propan-1-one (*syn*-3a) (First-Generation Process). To a solution of Fmoc-D-Trp-OH·THF (4.68 kg, 9.38 mol) and DMF (0.28 kg) in THF (25.0 L) was added dropwise oxalyl chloride (1.43 kg, 11.3 mol) at 0−10 °C. The mixture was stirred at 0−10 °C for 1 h. Then a solution of **(*S*)-2** (1.40 kg, 6.23 mol) and triethylamine (1.37 kg, 13.5 mol) in THF (12.5 L) was added dropwise, and the mixture was stirred at 0−10 °C for 3 h. After adding 5% *aq* $NaHCO_3$ (18.8 L), the mixture was allowed to warm to room temperature and was extracted with ethyl acetate (18.8 L). The organic layer was washed with water (18.8 L) and concentrated *in vacuo*. Methanol (75.0 L) and piperidine (3.77 kg, 44.3 mol) were successively added to the residue. After stirring at room temperature for 16.5 h, the precipitate was filtered off and washed with methanol (7.5 L). The combined filtrates were concentrated *in vacuo*, and then methanol (25.0 L) was added to the residue. After stirring at room temperature for 1 h, the precipitate was filtered off and washed with methanol (6.3 L). The combined filtrates were charged onto the column that was filled with DIAION WK100, which had been conditioned according to the method described in a footnote.[22] The crude mixture was successively eluted by

methanol (1125 L), 0.5% *aq* NaCl/methanol (1125 L, 20:80), and 5 M *aq* NaOH/methanol (1125 L, 20:80). The combined fractions containing *syn*-**3a** were adjusted to pH 7 by adding 2 M hydrochloric acid and then were concentrated *in vacuo*. Ethyl acetate (188 L) and sodium carbonate (18.8 kg) were added to the residue, and the layers were separated. The organic layer was washed with water (2 × 63.0 L) and then concentrated *in vacuo* to give amorphous solids containing *syn*-**3a** (1.88 kg, 4.57 mol, 73% yield (based on **(*S*)-2** used)).

Synthesis of *syn*-3a·0.25H_3PO_4·0.5H_2O (Second-Generation Process). A methanol solution containing *syn*-**3a** (2.18 kg, 5.30 mol) was prepared from (*S*)-**2** (1.40 kg, 6.23 mol) by the same procedure as the first-generation process without the CEC and the successive separation in 85% yield. A part of the methanol solution containing *syn*-**3a** (37.1 g, 90.3 mmol) was concentrated *in vacuo*. Ethyl acetate (200 mL) and water (100 mL) were added to the residue. The aqueous layer was adjusted to pH 6 by adding 2 M hydrochloric acid (200 mL), and then the layers were separated. The organic layer was extracted with water (50 mL). After adding ethyl acetate (200 mL) and Na_2CO_3 (50 g) to the combined aqueous layer, the layers were separated. The organic layer was washed with 5% *aq* NaCl (100 mL) and concentrated *in vacuo* to give crude-*syn*-**3a** (42.7 g). Phosphoric acid (0.60 mL, 0.85 M) was added to a solution of the crude-*syn*-**3a** (1.00 g) in ethanol (2.0 mL) and water (2.0 mL). After stirring at room temperature for 0.5 h, water (4.0 mL) was added dropwise. The mixture was allowed to cool to 0 °C and stirred for 1 h. The resultant precipitate was collected by filtration, washed with 10% *aq* ethanol (1.0 mL), and dried *in vacuo* to give crude-*syn*-**3a**·0.25H_3PO_4·0.5H_2O (858 mg). After the crude-*syn*-**3a**·0.25H_3PO_4·0.5H_2O (500 mg) was suspended in ethyl acetate/*n*-hexane (1.5 mL/0.5 mL) at room temperature for 0.5 h, the precipitate was collected by filtration, washed with *n*-hexane (1.0 mL), and dried *in vacuo* to give *syn*-**3a**·0.25H_3PO_4·0.5H_2O (430 mg, 0.978 mmol, 68% yield (based on **(*S*)-2** used)) as a white crystalline powder. Mp 159−166 °C; IR(KBr) ν 1658, 1485, 1090, 1043, 744 cm^{-1}; MS (FAB) *m/z* 411 $(M + H)^+$; $[\alpha]^{20}_D$ +202.1 (*c* 1.00, MeOH.); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.69−1.94 (m, 3H), 2.05 (s, 7H), 2.20−3.50 (m, 5H), 4.08−4.22 (m, 1H), 6.76−6.90 (m, 1H), 6.91−7.18 (m, 5H), 7.29 (d J = 8,1 Hz, 1H), 10.8 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 30.2, 32.5, 33.0, 45.5, 46.9, 52.5, 63.2, 110.1, 111.4, 118.2, 118.3, 120.9, 123.6, 125.7, 127.2, 127.6, 128.8, 136.2, 137.7, 174.9; Anal. Calcd for $C_{23}H_{27}N_4OCl$·0.25H_3PO_4·0.5H_2O: C, 62.16; H, 6.52; N, 12.61; Cl, 7.98; P, 1.74. Found: C, 62.16; H, 6.40; N, 12.47; Cl, 7.93; P, 1.77. Crystal data for *syn*-**3a**·0.25H_3PO_4·0.5H_2O: $C_{23}H_{27}ClN_4O$, 0.25H_3PO_4, 0.5H_2O, tetragonal, *I*4 (#79), a = 22.700 (3) Å, c = 8.762(3) Å, V = 4514.8(17) Å^3, Z = 8, D_{calc} = 1.308 g/cm^3, R = 0.056, R_w = 0.180. The Flack parameter,[24] 0.01(5), indicates the correct absolute configuration. Further details of the X-ray structure data are available on request from the Cambridge Crystallographic Data Centre (deposition number CCDC 761513).

***N*-((2*R*)-1-{(3*R*)-6-chloro-3-[(dimethylamino)methyl]-3,4-dihydroquinolin-1(2*H*)-yl}-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)-1-[(1-methyl-1*H*-indol-2-yl)carbonyl]piperidine-4-carboxamide (1).** A mixture of *syn*-**3a**·0.25H_3PO_4·0.5H_2O (1.40 kg, 3.15 mol), **4** (1.08 kg, 3.77 mol), EDC·HCl (0.73 kg), and HOBt (0.58 kg) in DMF (14.0 L) was stirred at room temperature for 2 h. Then the mixture was added to ethyl acetate (18.0 L) at 10 °C. After adding DMF (2.0 L) and ethyl acetate (10.0 L), the mixture was washed with 5% *aq* K_2CO_3 (2 × 14.0 L) and 10% *aq* NaCl (14.0 L) below 20 °C. Then activated carbon (0.14 kg) was added to the organic layer. After stirring at room temperature for 10 min, the activated carbon was filtered off and washed with ethyl acetate (4.2 L). The combined filtrates were concentrated *in vacuo*. Ethanol (4.2 L) was added to the residue, and the resultant solution was concentrated *in vacuo*. Then ethanol (12.0 L) was added to the residue, and the resultant solution was added dropwise to water (70.0 L) and rinsed with ethanol (2.0 L). After stirring at room temperature for 1 h, the resultant precipitate was collected by filtration, washed with water (7.0 L), and dried *in vacuo* to give **1** (1.89 kg, 2.75 mol, 87% yield, MW. 688.26, as 0.5 hydrate) as a white amorphous powder. Mp 135−145 °C; IR (ATR) ν 1630, 1442, 1270, 1214, 739 cm^{-1}; MS (ESI) *m/z* 679 $(M + H)^+$; $[\alpha]^{20}_D$ −151.4 (*c* 1.00, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.42−1.59 (m, 2H), 1.61−1.91 (m, 3H), 1.94−2.11 (m, 8H), 2.38−2.68 (m, 5H), 2.75−3.20 (m, 4H), 3.40−3.55 (m, 1H), 3.74 (s, 3H), 3.80−4.70 (m, 2H), 5.16 (br s, 1H), 6.62 (s, 1H), 6.79 (br s, 1H), 6.90−7.35 (m, 7H), 7.43−7.66 (m, 3H), 8.36 (br s, 1H), 10.77 (br s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.1, 30.2, 30.6, 41.2, 45.3, 47.1, 50.8, 63.5, 101.8, 109.1, 110.2, 111.2, 117.6, 118.0, 119.8, 120.7, 121.0, 122.6, 123.7, 125.7, 126.0, 126.7, 127.3, 132.4, 135.9, 137.1, 137.4, 161.8, 172.0, 173.8; Anal. Calcd for $C_{39}H_{43}N_6O_3Cl$·0.5H_2O: C, 68.06; H, 6.44; N, 12.21. Found: C, 68.36; H, 6.28; N, 12.21.

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