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A Practical Synthesis of the Pseudotripeptide RC-1291

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Abstract:

The rapid process development of a scaleable synthesis of the pseudotripeptide RC-1291 for preclinical and clinical evaluation is described. By employing a nontraditional *N*-to-*C* coupling strategy, the peptide chain of RC-1291 was assembled in high yield, with minimal racemization and in an economical manner by introducing the most expensive component last. A one-pot deprotection/crystallization procedure was developed for the isolation of RC-1291 free base, which afforded the target compound in excellent yield and with a purity of >99.5% without chromatographic purification.

Introduction

RC-1291 (**1**) is a ghrelin agonist, binding to and stimulating the growth hormone secretagogue receptor and is currently under clinical evaluation as its HCl salt for cancer cachexia.[1] This complex metabolic syndrome manifests itself in progressive weight loss, anorexia, and persistent erosion of body cell mass in response to a malignant growth.[2]

To supply the necessary amounts for a rapid preclinical and clinical evaluation of RC-1291, a synthesis was required which could not only provide the initial quantities needed but also serve as framework for future process optimization to allow for larger-scale manufacturing. Another requirement for a new synthesis was that it should afford the target molecule in a purity of >98% without chromatography.

Results and Discussion

The discovery route for RC-1291 is shown in Scheme 1.

While this route was capable of supplying small amounts of the desired product, it suffered from several disadvantages:

- The peptide chain was built up in a traditional fashion from the *C*-terminus to the *N*-terminus, so that the expensive building block **3**[3] had to be introduced early in the synthesis.
- The peptide couplings used the potentially explosive reagent 1-hydroxy-7-azabenzotriazole (HOAt)[4] as an activator and were performed in dimethylacetamide, which led to volume-inefficient workups.
- Both Boc deprotections were performed by introducing HCl gas directly to a solution of the substrate in EtOAc and suffered from byproduct formation.
- After removal of the Boc protecting group, RC-1291 was converted to the free base and then allowed to react with fumaric acid to give an (amorphous) fumarate salt. All attempts to upgrade the purity of this material by precipitation from a variety of solvents were unsuccessful.

It was therefore decided to investigate the alternative strategy shown in Scheme 2 which would introduce expensive hydrazide **3** late in the synthesis. Replacement of HOAt in dimethylacetamide (DMA) for the coupling reactions, HCl gas for removal of the Boc protecting groups, and the amorphous fumaric acid salt of RC-1291 were the other key goals of the process optimization.

It was envisioned to build the peptide chain starting from the *N*-terminal 4-aminoisobutyric acid (Aib) residue and sequentially attach the D-tryptophan (D-Trp) residue and then amino acid derivative **3**. The major advantage of this route was considered to be that expensive hydrazide **3** was introduced late in the synthesis. However, possible racemization of the tryptophan residue was a significant concern because extensive racemization of the amino acid residue next to an Aib group during carbodiimide activation has been described in the literature.[5] Base-catalyzed racemization of the D-tryptophan moiety also was a concern in the hydrolysis of ester **9** to acid **8**. Therefore, the initial goal was to prove that this route could produce RC-1291 without racemization.

Step 1: Coupling of BocAibOH (6) with D-Tryptophan. Initial studies briefly examined whether **6** and free D-tryptophan (**10**) could be coupled without a protecting group on the carboxylic acid of the D-tryptophan residue. Thionyl chloride, ethyl chloroformate, isobutyl chloroformate, and pivaloyl chloride were examined as coupling agents, but all afforded complex product mixtures from which the desired product **8** was obtained in 30−35% yield after purification by multiple precipitations from MTBE/heptane mixtures. Better results were obtained when the carboxylic acid group of D-tryptophan was protected as the corresponding methyl ester; the coupling of **6** with methyl ester **11** was achieved in 89% yield using isobutyl chloroformate, but the

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(1) Ankersen, M. U.S. Patent 6,576,648 B2, 2003.

(2) Kern, K. A.; Norton, J. A. *J. Parenteral Enteral Nutr.* **1988**, *12*, 286.

(3) For the synthesis of the Boc-protected precursor of hydrazide **3**, see: Garcia-Rubio, S.; Wilson, C. D.; Renner, D. A.; Rosser, J. O.; Patra, D.; Reid, J. G.; Pines, S. H. *Org. Process Res. Dev.* **2004**, *8*, 360.

(4) For a comparison of the effectiveness and safety of several catalysts (including HOAt) for promoting imidazolide couplings, see: Dunn, P. J.; Hoffmann, W.; Kang, Y.; Mitchell, J. C.; Snowden, M. J. *Org. Process Res. Dev.* **2005**, *9*, 956.

(5) Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243.

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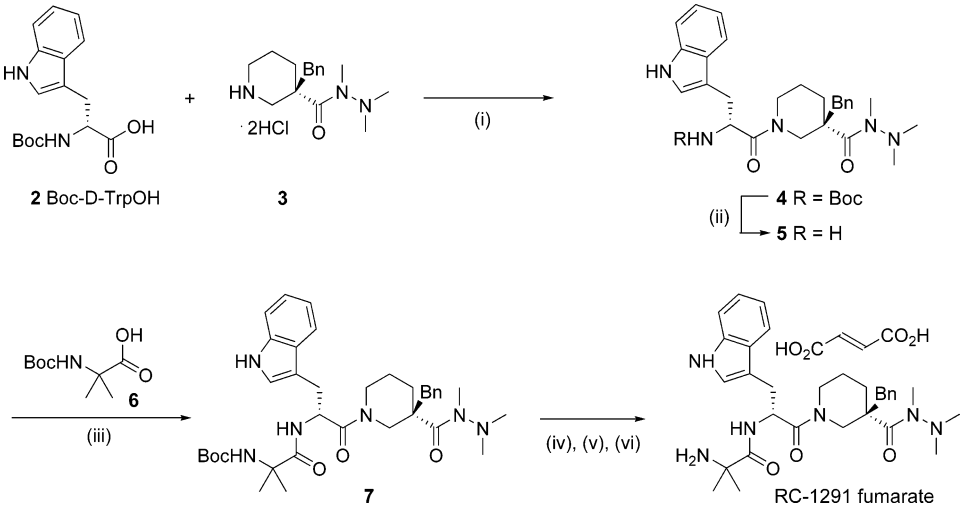
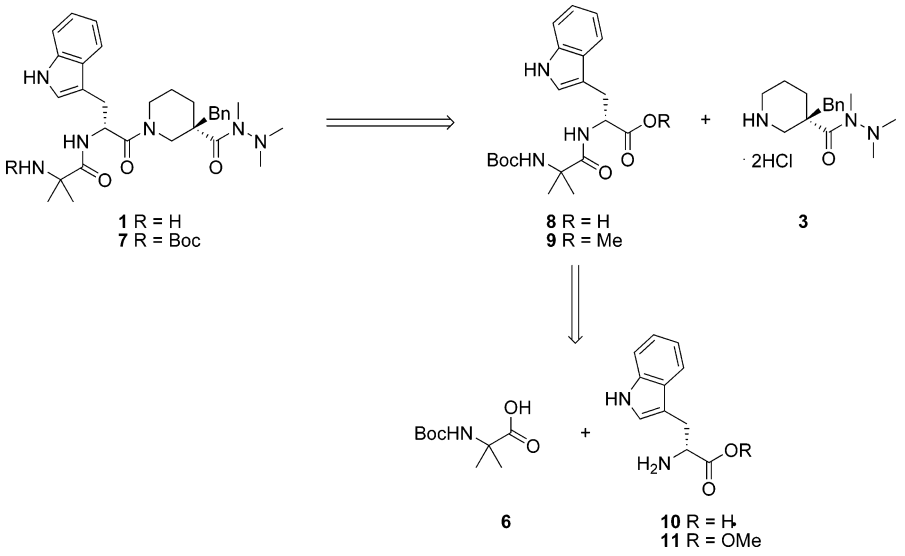
Scheme 1. **Discovery route for RC-1291**[a]



[a] Reaction conditions: (i) HOAt, 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (EDCI), *N*-methylmorpholine, dimethylacetamide, 95%; (ii) HCl, EtOAc, 58%; (iii) HOAt, EDCI, *N*-methylmorpholine, dimethylacetamide, 90%; (iv) HCl, EtOAc; (v) Na_2CO_3, CH_2Cl_2; (vi) fumaric acid, EtOAc, 2-propanol, 66% from **7**.

Scheme 2. **Proposed alternative synthesis of RC-1291**



product was contaminated with isobutoxycarbonyl-D-TrpOMe that was not readily removed.

During a screen of alternative standard coupling conditions it was found that 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) gave an almost quantitative yield of dipeptide **9**. The additional cost of using EDCI/HOBt was outweighed by the simple aqueous workup that these water soluble reagents would provide and the need to prove or disprove the *N*-to-*C* coupling approach so that clinical trials could start as quickly as possible. The first-generation procedure involved treating **6** with EDCI and HOBt in CH_2Cl_2 for 45 min before adding a solution of the activated ester into a slurry of **11** in dichloromethane. The reaction reproducibly gave 90−100% yield on a 1- to 10-g scale, but a new (unidentified) byproduct formed when the procedure was performed on a 750-g scale.[6] While the impurity was removed during the subsequent crystallization, its formation reduced the yield of desired product **9** to 53%. The main difference in the two preparations was that in the large-scale run, EDCI and HOBt were added sequentially, while on the small-scale runs they were premixed. Later investigations (Table 1) showed that premixing of EDCI and HOBt was crucial for consistently achieving a high yield of the desired product.

Table 1. **Effect of premixing of the coupling reagents**

entry	HOBt/EDCI premixed	product **9** (area %)	residual **11** (area %)
1	no	64	36
2	yes	95	5

Subsequent studies on the coupling of **8** and **3** (Step 3, vide infra) identified 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine (DHOBt)[7] as an alternative to HOBt in this

(6) Because the impurity was easily removed during the subsequent recrystallization and because of the time constraints of having to deliver material for the preclinical and clinical evaluation of RC-1291, characterization of this impurity was not performed.

(7) (a) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788. (b) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 2024. (c) Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1995**, *60*, 3561.

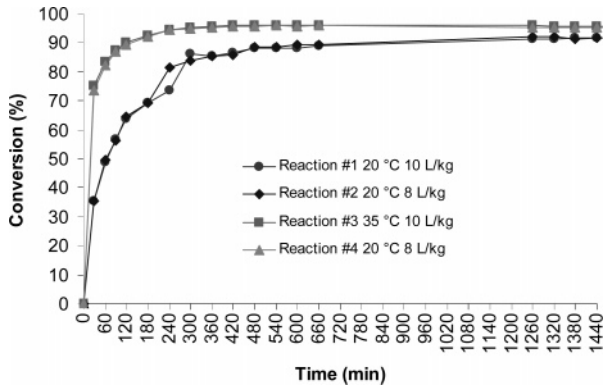


Figure 1. **Conversion as a function of temperature and concentration.**

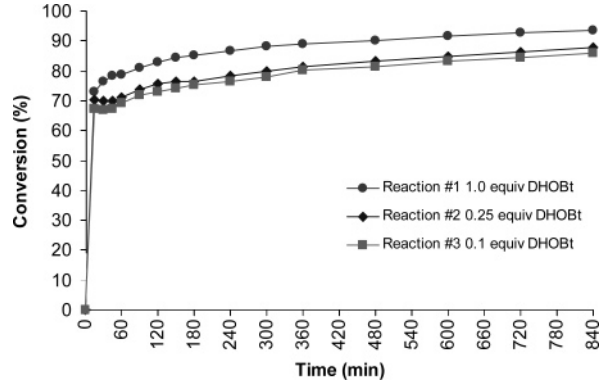


Figure 2. **Effect of the DHOBt concentration.**

peptide coupling. It was found that adding a mixture of DHOBt, EDCI, and triethylamine in dichloromethane to a mixture of **6** and **11**·HCl and triethylamine in dichloromethane afforded a near quantitative yield of dipeptide **9**. The advantage of this process was that it avoided the need to monitor the formation of the (unstable) active ester in the first-generation process. Further optimization of this coupling was performed using a Mettler Toledo MultiMax automated workstation equipped with a robotic sampler to prepare solutions for HPLC analysis. The data collected from these experiments showed that the rate of reaction and the overall conversion improved as the temperature was increased to 35 °C (Figure 1) and that DHOBt was acting as a catalyst, so that the loading could be reduced from 100 to 25 mol % (Figure 2).

An RC1 study showed that there was little heat accumulation at 35 °C (Figure 3) and that the adiabatic temperature rise was only 26 K.

Thus, the coupling of **6** and **10** was performed at gentle reflux (38 °C) when scaled up to 7.4 mol scale. Workup simply consisted of a water wash to remove the bulk of the water-soluble coupling agents and their byproducts, followed by a swap of the solvent from dichloromethane to MTBE. The MTBE solution was used directly in the next step after it was rinsed with dilute acid and base to remove any unreacted starting materials.

Step 2: Hydrolysis of Ester 9 to give Acid 8. Base-catalyzed racemization of the D-Trp residue was a concern in the hydrolysis of BocAib-D-TrpOMe (**9**) to BocAib-D-TrpOH (**8**), but chiral HPLC analysis of the initial studies showed that no racemization occurred when the reaction was performed at ambient temperature in methanol containing 1 equiv of 1 N NaOH. Complete reaction took 24 h under these conditions, and extraction of the product from the homogeneous mixture required an excessive amount of solvent; as a result, an improved biphasic procedure was developed and performed on kilogram scale. In this procedure, an aqueous solution of 5% NaOH was stirred with the solution of **9** in MTBE produced from the coupling of **6** and **11**. Chiral HPLC analysis showed that no racemization occurred in the biphasic procedure, even when a 25% aqueous solution of NaOH was used. After complete reaction, any nonpolar impurities and residual starting material remained in the organic layer, while the product was dissolved in the aqueous layer. After phase separation, the aqueous layer was acidified and the product back-extracted into fresh MTBE. A key discovery was that dipeptide **8** could be crystallized from dichloromethane since this removed trace impurities formed in the first two steps of the sequence and ensured that RC-1291 could be obtained in consistently high purity. In the kilo-lab the MTBE was removed on a rotary evaporator to afford a foam. A portion of dichloromethane was then added and evaporated to chase out the remaining MTBE. Six volumes of dichloromethane were then added to the foam while maintaining 35−40 °C. Dipeptide **8** crystallized on cooling to room temperature. Two batches of **8** were prepared using the biphasic process (7.4 mol scale each), and both afforded the desired product in 85−86% yield from **6** with a chemical and chiral purity of >99%.

Step 3: Coupling of BocAib-D-TrpOH (8) and Hydrazide 3. Preventing racemization of the D-tryptophan residue during the coupling of **8** with **3** proved to be the first of the two major challenges of developing the route shown in Scheme 2. Reverse-phase HPLC showed that a mixture of desired compound **7** having the (*R*,*R*) configuration and undesired **7a** with the (*S*,*R*) configuration was produced when **8** was treated with EDCI and HOBt at room temperature in dichloromethane followed by addition of hydrazide **3**.The data presented in Table 2 shows that the extent of racemization was dependent on the incubation time between addition of the EDCI/HOBt mixture and compound **3**.

The initial breakthrough came when it was found that racemization could be suppressed by treatment of a 1:1 mixture of **8** and **3** with 1.7 equiv of *n*-propylphosphonic acid cyclic anhydride (PPACA)[8] as the coupling agent in the presence of 7.5 equiv of triethylamine. The reaction was initially performed in EtOAc at −40 °C as described in the literature, but optimization studies showed that the reaction gave a higher yield in dichloromethane and that a reaction temperature of −10 °C was sufficient to suppress racemization. Less than <0.5% **7a** was produced under these conditions, and **7** could be obtained in >95% yield by mass with a purity of up to 96% after workup. Changing the dosing rate and the reaction temperature had only a minor influence on the yield or purity of **7**. This procedure was scaled-up to

(8) (a) Millbanks, C. In *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L. A., Ed.; Wiley: New York, 1995; p 4336. (b) Schwarz, M. *Synlett* **2000**, *9*, 1369.

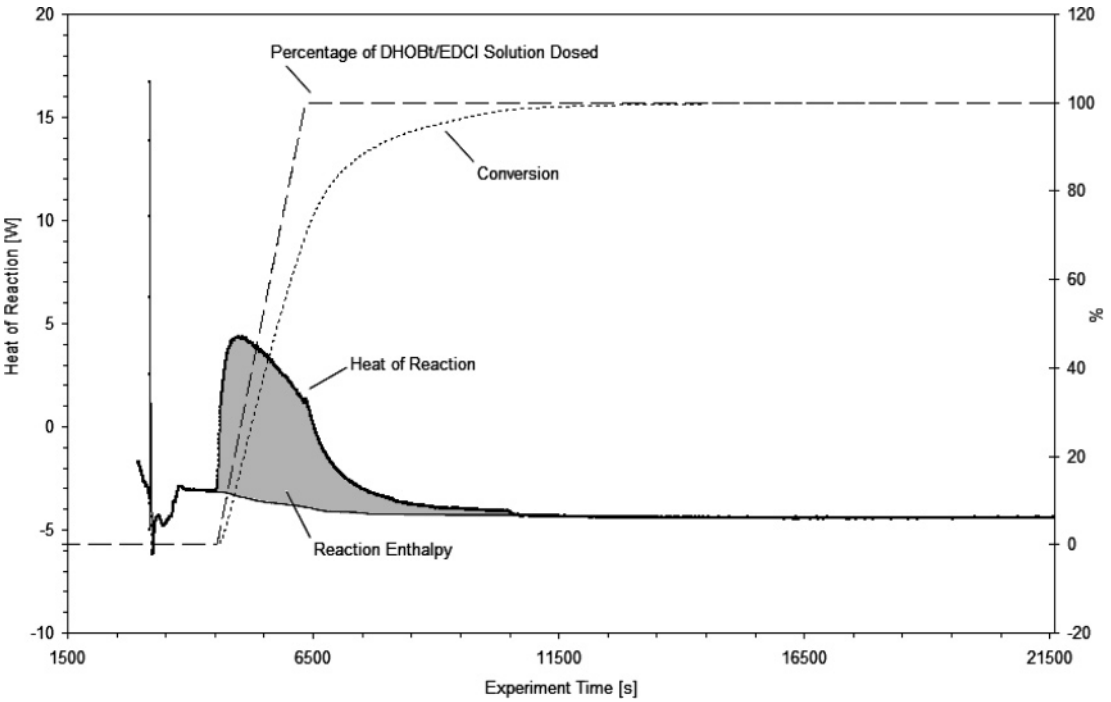
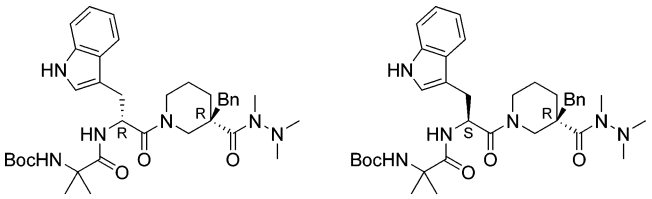


Figure 3. **Heat of reaction of the EDCI/DHOBt-mediated coupling of 8 with 3.**



7 (*R*, *R*) **7a (*S*, *R*)**

Figure 4. **Structure of pseudotripeptides 7 and 7a.**

Table 2. **Effect of the incubation time on the extent of racemization**

incubation time (min)	isomer **7** (%)	isomer **7a** (%)
0	87	13
1	82	18
5	79	21
20	64	36
120	55	45

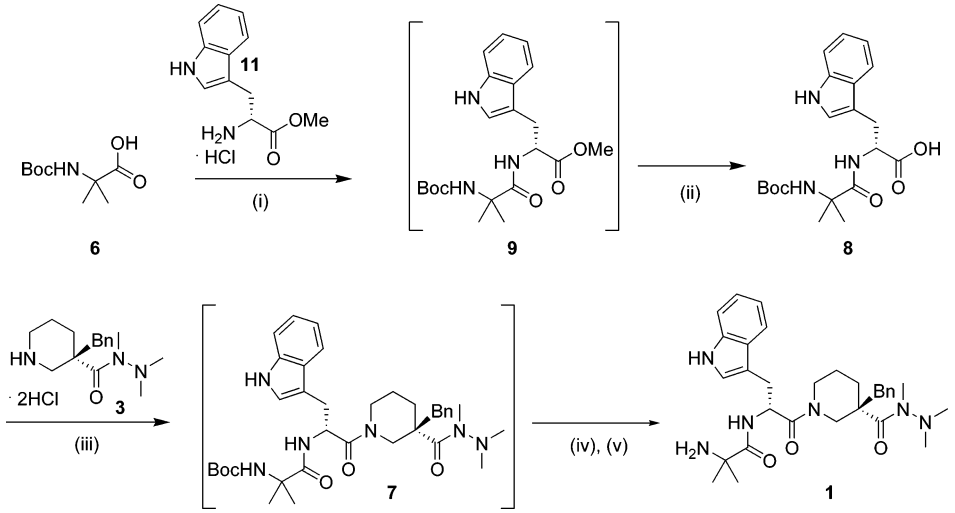
a 0.44 mol scale to make the initial batch of RC-1291 for preclinical studies.

Scaling the PPACA procedure to 0.44 mol indicated that the highly exothermic addition of PPACA would lead to an unacceptably long cycle time on further scale-up; thus, additional screening was performed to find coupling conditions that would suppress racemization in the coupling of **8** and **3**. During this screening it was found that replacing HOBt with DHOBt in an EDCI-activated coupling significantly reduced the amount of racemization. In this procedure, a mixture of DHOBt and EDCI was added to a mixture of dipeptide **8** and hydrazide **3** in dichloromethane at room temperature. The reaction conversion, as measured by HPLC, was 96% after 18 h. It was found that the nature of the base had a significant influence on the amount of racemization. No **7a** was detected by HPLC when triethylamine was used as base. The use of *N*-methylmorpholine (NMM) led to a faster reaction, but approximately 2% of **7a** was detected. A solvent screen showed that the reaction occurred with no racemization by using Et_3N as the base in tetrahydrofuran, dimethylformamide, dimethylacetamide, ethyl acetate, isopropyl acetate, acetonitrile, and acetone. However, dichloromethane was the only water-immiscible solvent in which triethylamine hydrochloride was soluble and did not separate from the reaction mixture as a sticky gum that adhered to the reactor walls. After complete reaction, residual starting materials and coupling agents were removed by acid/base extraction, and then the solution was treated with activated carbon to reduce the trace amounts of colored impurities. This procedure was scaled-up to a 1.2 mol scale and afforded compound **7** with a purity of 96.2%. This material was used for the deprotection without further purification.

Step 4: Boc Deprotection and Isolation of RC-1291. Conversion to RC-1291 was initially accomplished by adding HCl gas directly into a solution of **7** in EtOAc at room temperature. A number of phase transitions took place during the reaction, but ultimately the hydrochloride salt of **1** was directly isolated from the mixture by filtration. However, the purity of RC-1291 hydrochloride obtained in this fashion was below the target purity of 98%. The procedure was also volume inefficient, requiring >30 L/kg of solvent to afford a readily filtered precipitate.

Improving the purity of RC-1291 to >98% without chromatography proved to be the second major challenge of the project. A series of studies was performed to minimize byproduct formation in the preparation of **1**, but 96% was the highest purity obtained. The purity of RC-1291 was not improved by attempted partitioning of the reaction products between aqueous solutions of different pH and organic solvents, nor by treatment with inorganic clays and activated carbons in an attempt to preferentially absorb polar byprod-

Scheme 3. **New Route for the synthesis of pseudotripeptide RC-1291**[a]



[a] Reaction conditions: (i) DHOBt, EDCI, Et_3N, CH_2Cl_2; (ii) 5% NaOH, MTBE, 86% from **6**; (iii) DHOBt, EDCI, Et_3N, CH_2Cl_2 (iv) CH_3SO_3H, MeOH, 55 °C, (v) KOH, H_2O, cool to rt, 85% from **8**.

ucts. A wide range of acid salts of RC-1291 was prepared and screened in common organic solvents in an attempt to find a purification method via crystallization. These experiments showed that RC-1291 typically had a narrow supersaturation range that resulted in the formation of an oil or uncontrolled precipitation, which did not result in purification.

The purification problem was solved only when it was found that the free base of RC-1291 (**1**) could be crystallized from methanol/water to afford >99.5% purity.[9] Amine APIs are typically not isolated as the free base because they are usually less bioavailable and more susceptible to aerobic oxidation compared to amine salts. Forced degradation studies showed that RC-1291 free base had excellent long-term stability, so it was decided to isolate and purify RC-1291 as the free base and then form the HCl salt.

Screening of different conditions for the conversion of **7** to **1** showed that methanesulfonic acid in EtOH or MeOH gave rapid deprotection with minimal byproduct formation. Methanol was preferred since this would allow the mixture to be telescoped into the crystallization procedure used to purify RC-1291. Optimization studies of the deprotection gave a readily monitored homogeneous reaction mixture with excellent throughput due to a high substrate concentration (5 L of MeOH per kilogram of **7**) and a short reaction time (<2 h) at 60 °C. Prolonged exposure of the solution of RC-1291 led to slow decomposition; thus, the reaction was quenched by adding a solution of aqueous KOH (5 L/kg) that both neutralized the methanesulfonic acid and established the correct solvent system for the crystallization. During the KOH quench the mixture was heated to reflux at approximately 70 °C, and then the mixture was allowed to cool to room temperature at 5 °C/h. This resulted in crystallization of RC-1291 free base that was readily isolated by filtration in >99.5% purity. The overall two-step yield from **8** was 85% based on weight when the process was evaluated on a 1.2 mol scale.

(9) Initial work for the crystallization of RC-1291 was performed by SSCI, Inc., West Lafayette, IN.

Conclusion

Scheme 3 summarizes the new route using a nontraditional peptide coupling sequence starting from the *N*-terminal residue that was developed for the clinical candidate RC-1291.

Compared to the initial conventional *C*-terminus to *N*-terminus approach, the *N*-to-*C* route has enabled the most expensive component **3** to be introduced in the final coupling without racemization. The undesirable coupling conditions of HOAt in DMA have been replaced with EDCI/DHOBt in dichloromethane which allows high throughput and simple workup procedures. The removal of the Boc group has been simplified by swapping HCl gas for methanesulfonic acid, and a telescoped procedure reproducibly affords RC-1291 as a readily filterable off-white powder consistently above 99.5% purity. The overall yield of **1** was increased from 32% to 73%, and RC-1291 is now available in quantities sufficient to allow its evaluation as a clinical candidate. Importantly, the convergent strategy outlined in Scheme 3 could be developed into a commercial manufacturing route to RC-1291 with relatively minor modifications to the reaction conditions to fit the plant and reduce the cost of goods.

Experimental Section

Reagents were purchased from commercial sources and used as received. Solvents for reactions and isolations were reagent grade and used without purification. Proton NMR spectra were obtained on a Bruker AMX500 or a Bruker AV500 spectrometer at 500 MHz using DMSO-d_6 as the solvent. Tetramethylsilane was used as an internal standard for the proton spectra. HRMS was performed by M-Scan Inc., West Chester, PA, using a PE Sciex Q-Star hybrid quadrupole/time-of-flight mass spectrometer. The mass spectrometer was internally calibrated using PEG 300 or PEG 600 + NaCl. HPLC analysis was performed on a Varian Prostar Series 210 liquid chromatograph with a Waters Symmetry C18 column (4.6 mm × 150 mm; 3.5 μm) using gradient elution with water (0.05% TFA) and acetonitrile (0.04% TFA).

Synthesis of (*S*)-2-(2-*tert*-Butoxycarbonylamino-2-methyl-propionylamino)-3-(1*H*-indol-3-yl)propionic Acid Methyl Ester (9). A 72-L flask equipped with a heating mantle, a digital thermocouple, a mechanical stirrer, a gas inlet, and a reflux condenser was charged with **6** (1.5 kg, 7.38 mol) and **11**·HCl (1.88 kg, 7.38 mol). Dichloromethane (15 L) and triethylamine (821 g, 8.12 mol) were added to the flask. The resulting white suspension was heated to 38 °C under nitrogen, and then a solution of DHOBt (301 g, 1.845 mol), EDCI·HCl (1.56 kg, 8.12 mol), and triethylamine (821 g, 8.12 mol) in dichloromethane (15 L) was added over a period of 30 min. Approximately 15 min after the end of the addition, the mixture became a homogeneous orange solution, which was stirred at 38 ± 2 °C for 3 h. (Conversion was monitored by HPLC.) The heating was discontinued, and ice-cold water (15 L) was added to the warm solution; the biphasic mixture was transferred to a 100-L separatory vessel. An additional portion of cold water (15 L) was added to the vessel, and the mixture was stirred rapidly for 15 min. The layers were separated, and the organic layer was concentrated in vacuo to give 4.05 kg of an orange oil, which was redissolved in MTBE (9 L). The solution was washed successively with a 10% aqueous solution of $NaHSO_4$ (9 L), then with a saturated aqueous solution of $NaHCO_3$ (9 L), and finally with a 1:1 mixture of water and saturated brine (4 L). The resulting orange MTBE solution was used in the next step without further purification.

Synthesis of (*S*)-2-(2-*tert*-Butoxycarbonylamino-2-methyl-propionylamino)-3-(1*H*-indol-3-yl)propionic Acid (8). The orange solution of **9** (approx 7.83 mol) in MTBE (9 L) was combined with a 5% aqueous solution of NaOH (9 L), and the resulting biphasic mixture was stirred vigorously overnight at ambient temperature. HPLC analysis of both layers showed that no more starting material remained and that the product was in the aqueous layer. The layers were separated, and the aqueous layer was transferred into a 100-L separatory vessel and fresh MTBE (9 L) added. The biphasic mixture was acidified with 2 M HCl to pH 2. After the mixture was stirred rapidly for 15 min, the layers were separated, and the aqueous layer was extracted with another portion (6 L) of MTBE. The combined organic layers were washed with saturated brine (4 L), dried over Na_2SO_4, and concentrated on a rotary evaporator to give an orange foam. The foam was diluted with dichloromethane (1.5 L) and the mixture concentrated again. The resulting orange foam was dissolved with stirring in warm (35−40 °C) dichloromethane (9 L) and then slowly allowed to cool to room temperature during which time a white precipitate formed. The solid was isolated by filtration, washed with dichloromethane (1.5 L), and dried in a vacuum oven to give **8** as a white powder (2.48 kg, 86% over two steps, >99% AUC by HPLC). HRMS (ESI) calcd for $C_{20}H_{27}N_3NaO_5$ $[M + Na]^+$ 412.1848, found 412.1868. ^{1}H NMR (DMSO-d_6) δ 12.67 (s, 1H), 10.83 (s, 1H), 7.51 (d, J = 7.5 Hz, 1H), 7.34 (m, 1H), 7.32 (d, J = 8 Hz, 1H), 7.09 (d, J = 2.5 Hz, 1H), 7.05 (t, J = 7.5 Hz, 1H), 6.97 (t, J = 7.5 Hz, 1H), 6.88 (bs, 1H), 4.49 (dd, J = 7.0, 13.5 Hz, 1H), 3.1−3.4 (m, 2H), 1.2−1.4 (m, 15 H). ^{13}C NMR (DMSO-d_6) δ 174.3, 173.2, 154.3, 136.1, 127.4, 123.7, 120.9, 118.34, 118.29, 111.3, 109.5, 78.2, 55.8, 52.8, 28.2, 27.3, 25.3, 24.9.

PPACA-Mediated Synthesis of (*R*,*R*)-{1-[2-[3-Benzyl-3-(*N*,*N*′,*N*′-trimethyl-hydrazinocarbonyl)piperidin-1-yl]-1-(1*H*-indol-3-ylmethyl)-2-oxo-ethylcarbamoyl]-1-methyl-ethyl}carbamic Acid *tert*-Butyl Ester (7). Dipeptide **8** (171 g, 0.439 mol) and hydrazide **3** (192 g, 0.551 mol) were charged into a jacketed 5-L three-necked round-bottom flask equipped with an N_2 inlet, a digital thermocouple, and a mechanical stirrer. The flask was flushed with nitrogen for 10 min; then dichloromethane (1.71 L) was added and the jacket temperature set to −10 °C. The first portion of Et_3N (153 mL) was added when the jacket temperature fell below 15 °C, and a second portion of Et_3N (307 mL) was added when the jacket temperature reached −10 °C. Meanwhile a 50 wt % solution of *n*-propylphosphonic acid cyclic anhydride in EtOAc (0.44 L, 0.746 mol) was mixed with CH_2Cl_2 (0.44 L) in a 1-L round-bottom flask. Immediately after the second portion of Et_3N was added, the *n*-propylphosphonic acid cyclic anhydride solution was added to the reaction mixture (subsurface) at a rate of approximately 3 mL/min. The temperature of the jacket was kept at −10 to −15 °C during the addition (total addition time was 4.5 h). One hour after completed addition, the reaction was quenched with water (50 mL), and after a further 15 min the jacket temperature was then set to 20 °C. An additional portion of water (1.7 L) was added once the reaction reached 20 °C. The organic phase was separated and the solvent removed to afford an amorphous solid. (*Note:* For the workup, the crude materials from two 0.439 mol scale reactions were combined.) The solid was dissolved in EtOAc (8 L) and washed twice with a solution of $NaHSO_4$ (170 g) in water (1.7 L). The organic phase was washed twice with a solution of NaOH (68 g) in water (1.7 L), then washed with water (3.4 L), and dried over Na_2SO_4; the solvent was removed on a rotary evaporator to afford **7** as a colorless amorphous foam (610 g, 91.3% AUC by HPLC).

DHOBt-Promoted Synthesis of (*R*,*R*)-{1-[2-[3-Benzyl-3-(*N*,*N*′,*N*′-trimethyl-hydrazinocarbonyl)-piperidin-1-yl]-1-(1*H*-indol-3-ylmethyl)-2-oxo-ethylcarbamoyl]-1-methyl-ethyl}carbamic Acid *tert*-Butyl Ester (7). Dipeptide **8** (460.0 g, 1.181 mol) and hydrazide **3** (411.4 g, 1.181 mol) were mixed in methylene chloride (4.6 L) in a 22-L round-bottom flask equipped with a mechanical stirrer, a digital thermocouple, a gas (N_2) inlet, and a reflux condenser. Triethyl-
amine (251 g) was added, resulting in the formation of a homogeneous, dark-brown solution. A solution of DHOBt (212 g, 1.30 mol), EDCI hydrochloride (249.1 g, 1.299 mol), and triethylamine (131.5 g) in methylene chloride (4.6 L) was then added to the flask over a period of 5 min. The dark-orange solution was then stirred at 25 °C for 20 h at which point HPLC analysis of the reaction mixture showed a conversion from **8** to **7** of >95% (AUC). The solution was transferred into a separatory vessel and washed with water (9.6 L). The organic layer was concentrated on a rotary evaporator to afford an oily, orange residue, which was redissolved in ethyl acetate (9.5 L). The organic layer was washed sequentially with a 10% aqueous solution of $NaHSO_4$ (9.5 L), then a 1 M solution of NaOH (9.5 L), and finally with saturated brine (4 L). To the resulting solution were added anhydrous sodium sulfate (500 g) and activated carbon

(40 g), and the mixture was stirred for 15 min. The mixture was filtered through a pad of diatomaceous earth to afford a pale-yellow solution which was concentrated on a rotary evaporator, Methanol (3 L) was then added, and the solvent was evaporated again to yield crude **7** as an orange oil (850 g, 96.2% AUC by HPLC) that was used without further purification for the next step.

(*R*,*R*)-2-Amino-*N*-[2-[3-benzyl-3-(*N*,*N*′,*N*′-trimethyl-hydrazinocarbonyl)piperidin-1-yl]-1-(1*H*-indol-3-ylmethyl)-2-oxo-ethyl]-2-methyl-propionamide (1). Crude **7** (911 g; 1.28 mol theoretical)[10] was dissolved in methanol (4.12 L) in a 22-L round-bottom flask equipped with a mechanical stirrer, a temperature probe, a reflux condenser, a gas (N_2) inlet, and an addition funnel. The solution was heated to 55 °C; then methanesulfonic acid (269.5 g, 2.805 mol) was added over a period of 15 min. (Caution: gas evolution!) The solution was then heated to 60 °C for a period of 1 h, after which HPLC analysis showed that no **7** remained. The temperature of the reaction mixture was increased to reflux (68−72 °C) over a period of 35 min, while simultaneously adding a solution of KOH (85%, 210.4 g, 3.187 mol) in water (4.12 L). The clear, slightly yellow solution was then allowed to cool to 20 °C at a rate of 5 °C/h. The free base of RC-1291 (**1**) crystallized as a pale-yellow solid, which was isolated by filtration. The filter cake was washed with two portions of 50% aqueous methanol (500 mL each) and then dried under high vacuum at 20 ± 5 °C to afford **1** as an off-white, crystalline solid (595 g, 85% yield for two steps, >99.5% AUC by HPLC). HRMS (ESI) calcd for $C_{31}H_{43}N_6O_3$ $[M + H]^+$ 547.3397, found 547.3432. ^{1}H NMR (DMSO-d_6; 413 K) δ 10.30 (s, 1H), 7.85 (bs, 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.27 (d, J = 8.1 Hz, 1H), 7.1−7.2 (m, 3H), 6.95−7.0 (m, 5H), 5.07 (t, J = 6.3 Hz, 1H), 3.54 (d, J = 12.3 Hz, 1H), 3.36 (bs, 1H), 3.15−3.30 (m, 1H), 3.06 (dd, J = 7.2, 14.4 Hz, 1H), 2.96 (dd, J = 6.0, 14.3 Hz, 2H), 2.7−2.8 (m, 6H), 2.43 (m, 6H), 2.09 (bs, 1H), 1.73 (bs, 1H), 1.45−1.55 (m, 2H), 1.3−1.40 (m, 1H), 1.18 (s, 3H), 1.15 (s, 3H). ^{13}C NMR (DMSO-d_6; 413 K) δ 175.8, 173.4, 170.3, 137.0, 135.7, 129.0, 127.2, 127.1, 125.3, 122.9, 120.1, 117.6, 110.7, 109.4, 53.6, 49.0, 47.0, 42.7, 38.5, 30.7, 28.2, 28.0, 23.2, 21.1.

(10) Crude **7** from a 1.81 mol and a 0.1 mol scale coupling reaction was combined for the deprotection.

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