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Process Development of (2-Nitrophenylcarbamoyl)-(*S*)-prolyl-(*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide (SDZ NKT343)

Mahavir Prashad,* Kapa Prasad, Oljan Repic, and Thomas J. Blacklock

Process Research and Development, Chemical and Analytical Development, Novartis Institute for Biomedical Research, 59 Route 10, East Hanover, New Jersey 07936, U.S.A.

Walter Prikoszovich

Process Research and Development, Chemical and Analytical Development, Novartis Pharma AG, CH 4002, Basel, Switzerland

Abstract:

(2-Nitrophenylcarbamoyl)-(*S*)-prolyl-(*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide (1; SDZ NKT343) is a human NK-1 tachykinin receptor antagonist. The development of a robust process for a multikilogram scale, chromatography-free preparation of this compound is described. The new four-step synthesis was based on a convergent approach, which utilized a peptide coupling of 1-[(2-nitrophenylamino)carbonyl]-L-proline (11) with free base of (*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide hydrochloride (4) as the key step in the presence of 1,3-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole as coupling agents. A scale-up of the well-known mixed anhydride coupling method, using isobutyl chloroformate, to produce 4 was found to be problematic due to coupling of the amine at the undesired carbonyl group of the mixed anhydride. This problem was overcome. The drug substance, initially an amorphous powder, was obtained with the desired purity without any chromatography. A process for crystallization of 1 was also developed.

Introduction

SDZ NKT343 (**1**) is a potent and selective antagonist for the human NK-1 tachykinin receptor discovered by Walpole et al. at Novartis Pharmaceuticals Corporation.[1,2] It has demonstrated potent oral analgesic activity in guinea pig models of chronic inflammatory and neuropathic pain.[1,2] The discovery synthesis of **1** is depicted in Scheme 1. Their original synthesis utilized commercially available BOC-L-3-(2-naphthyl)alanine (**2**) as the starting material. Coupling of **2** with *N*-benzylmethylamine in the presence of isobutyl chloroformate and 4-methylmorpholine in dichloromethane yielded amide **3**, which was treated with HCl gas in dioxane to obtain **4** as the hydrochloride salt. Reaction of **4** with BOC-L-proline in the presence of isobutyl chloroformate and 4-methylmorpholine in dichloromethane yielded the dipeptide **5**, which was treated with HCl gas in dioxane to obtain **6** as the hydrochloride salt. Treatment of **6** with 2-nitrophenyl isocyanate in dichloromethane, followed by a silica gel chromatography and precipitation with ethyl acetate and hexane produced **1** as an amorphous powder.

Results and Discussion

The synthesis of **1**, as depicted in Scheme 1, was simple, straightforward, and utilized standard peptide chemistry. Because of these reasons we decided to retain the same synthetic route to prepare the initial 1 kg of **1**, but to replace undesirable solvents such as dichloromethane and dioxane with ethyl acetate. Because **1** was an amorphous solid, a silica gel chromatography was necessary. The first scale-up of this synthesis, however, did not give **1** with the desired 98% purity. An HPLC analysis of **1** indicated that it contained three by-products, which were isolated by HPLC, and their structures were assigned on the basis of spectroscopic data as methyl-migrated analogue **7**, desmethyl analogue **8**, and isobutyl carbamate **9** (Figure 1). Their structures were further confirmed by comparison of spectral data with those of authentic samples. Interestingly, compound **7** was a single diastereomer. This fact was confirmed by synthesis of both isomers starting from (*S*)-α-methylbenzylamine and (*R*)-α-methylbenzylamine. Compound **7** was identical in all respects to the sample prepared from (*S*)-α-methylbenzylamine, thus confiming the (*S*)-configuration at the newly created stereogenic center in **7**. The mechanism of its formation during the conversion of **4** to **5** is speculative, but it is probably a base-catalyzed intramolecular diastereofacial-selective methyl migration (Scheme 2). The selectivity arises because one face in the intermediate **I** is blocked to the base by the bulky naphthyl group. Although it is less likely because of the apparent high diastereoselectivity, the mechanism could also be an intermolecular demethylation and methylation via chloromethane. The desmethyl impurity **8** emerged because the raw material, *N*-benzylmethylamine, was contaminated with benzylamine and not because of intermolecular methyl transfer, as demonstrated later. The isobutyl carbamate impurity **9** was formed by acid-catalyzed loss of *tert*-butyl carbamate in **5** and subsequent reaction with isobutyl chloroformate during step **4** to **5**.

Having identified the structures of these three impurities and the possible pathways of their formation, we made the

(1) Walpole, C.; Ko, S. Y.; Brown, M.; Beattie, D.; Campbell, E.; Dickenson, F.; Swan, S.; Hughes, G. A.; Lemaire, M.; Lerpiniere, J.; Patel, S.; Urban, L. *J. Med. Chem.* **1998**, *41*, 3159−3173.

(2) Ko, S. Y.; Walpole, C. WO 96/18643, June 20, 1996.

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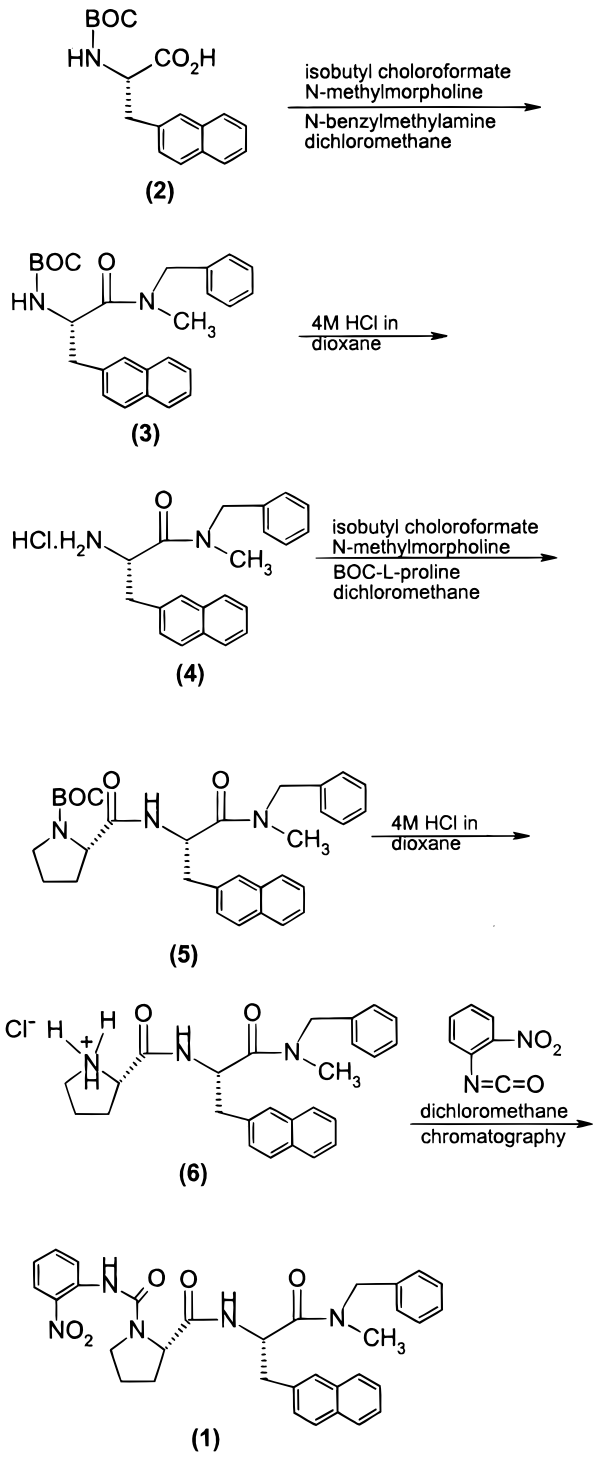
Scheme 1



following modifications to minimize/eliminate their formation. Because we were not able to find a source of pure *N*-benzylmethylamine, we treated the commercial material with 3% of ethyl trifluoroacetate[3] at 0 °C before use, which reacted preferentially with benzylamine, thus blocking its further participation in the coupling reaction. Coupling of **2** with *N*-benzylmethylamine, pretreated with ethyl trifluoroacetate, in the presence of isobutyl chloroformate and 4-methylmorpholine in ethyl acetate, yielded **3**, which was

(3) Takahashi, S.; Ohashi, T.; Watanabe, K. EP 0239063, March 24, 1987.

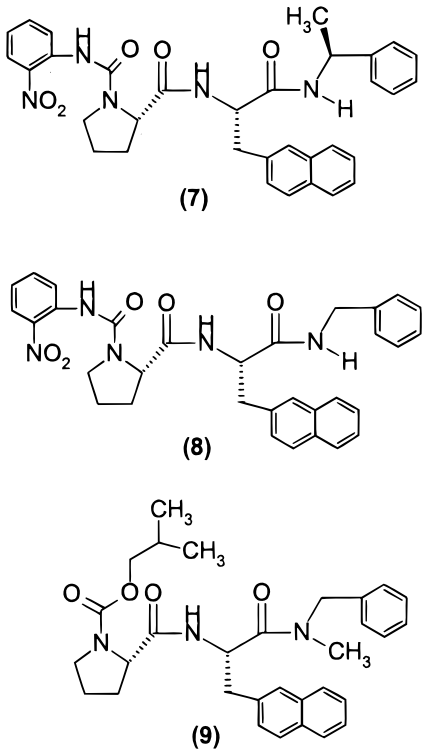
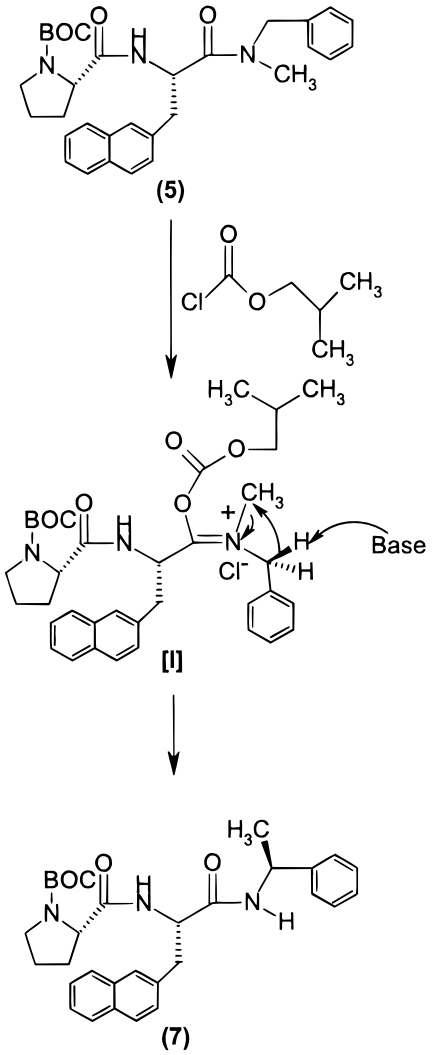


Figure 1.

further processed to **1**. In this way the amounts of **8** in **1** were minimized to <0.05%. Because **1** still contained methyl-migrated impurity **7**, it clearly suggested that desmethyl analogue **8** was not formed due to an intermolecular methyl migration. Apart from solving the interference of benzylamine, the scale-up of the coupling of **2** with *N*-benzylmethylamine was problematic because of the side reaction, shown in Scheme 3, leading to the formation of isobutyl carbamate of *N*-benzylmethylamine (**10**) from the mixed anhydride of the starting material **2** (Table 1). This problem was overcome by adding additional amounts of isobutyl chloroformate, 4-methylmorpholine, and *N*-*N*-benzylmethylamine to the reaction mixture. The formation of **10** was of no consequence because it was stable under acidic conditions, which were used in the next step to cleave the BOC-protecting group in **3** with HCl gas, and was easily removed during the crystallization of **4**.

Optimization of the reaction conditions for the coupling of HCl salt **4** with BOC-L-proline to give **5** reduced the amounts of **7** in **1** from 2 to 0.1%. The changes included (a) the conversion of the HCl salt **4** to the free base with NaOH, thus lowering chloride concentration in the mixture, which could cause the methyl migration, and (b) using less isobutyl chloroformate, which can react with **5** and increase the acidity of the benzylic protons. The conversion of the HCl salt **4** to the free base also made the process simpler in that a solution of the free base was conveniently added to the reaction, compared to the addition of solid HCl salt **4**. The free base generation step also removed residual L-3-(2-naphthyl)alanine, present in **4**, into the aqueous layer as the water-soluble sodium salt. Simultaneously, two unacceptable solvents in this synthesis, dichloromethane and dioxane, were replaced with ethyl acetate. In addition, using an excess of

Scheme 2



4-methylmorpholine in the coupling step of the free base of **4** with BOC-L-proline further minimized the formation of impurity **9**. Finally, coupling of **6**, obtained by deblocking the BOC group in **5** with HCl gas, with 2-nitrophenyl isocyanate was carried out in isopropyl acetate. It yielded pure **1** after silica gel chromatography. Precipitation of **1** with ethyl acetate and hexane gave the amorphous drug substance from which the residual solvents could not be removed to the desired levels even upon drying in vacuo for an extended period of time. To circumvent this problem, an alternate method for the precipitation of **1**, which involved the addition of a solution of **1** in ethanol to water, was developed. This modification yielded **1** that met all specifications, including solvent residue. This modified linear synthesis was satisfactorily scaled-up in the pilot plant on a multi-kilogram scale to yield **1** of the desired purity.

Our next task was to eliminate the silica gel chromatography, which had been necessary to purify the drug substance and to remove impurities present in the commercial 2-nitrophenyl isocyanate used in the last step. Since **1** had not yet crystallized, we focused our attention on eliminating the silica gel chromatography by designing a new route. We reasoned that a convergent approach (Scheme 4), utilizing a peptide coupling of two pure penultimate intermediates:

Scheme 3

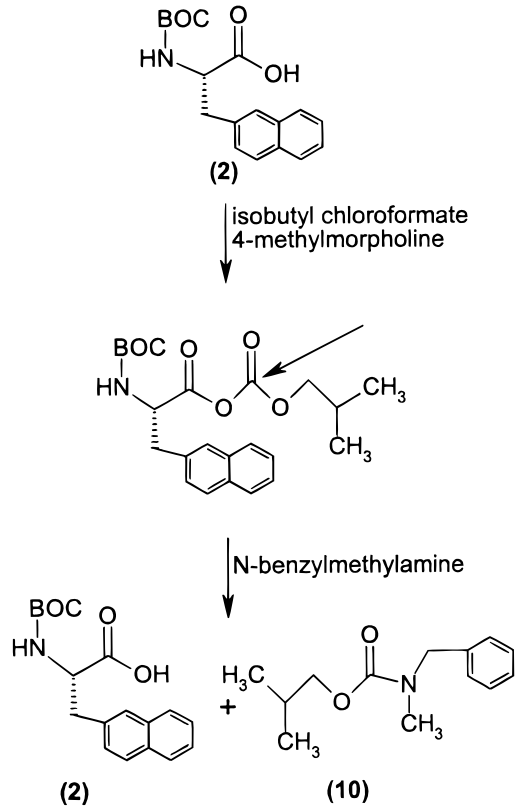
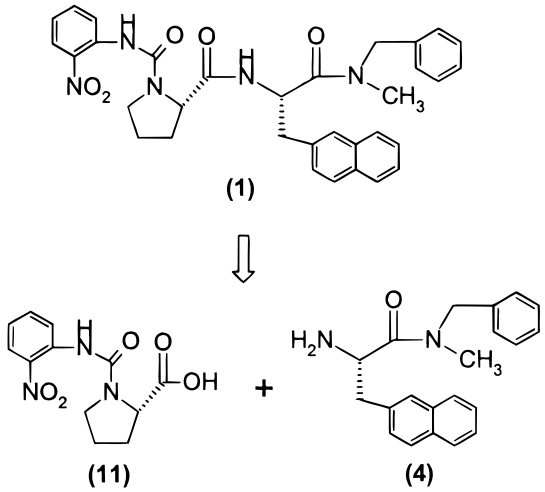


Table 1. **Coupling of 2 with *N*-benzylmethylamine**

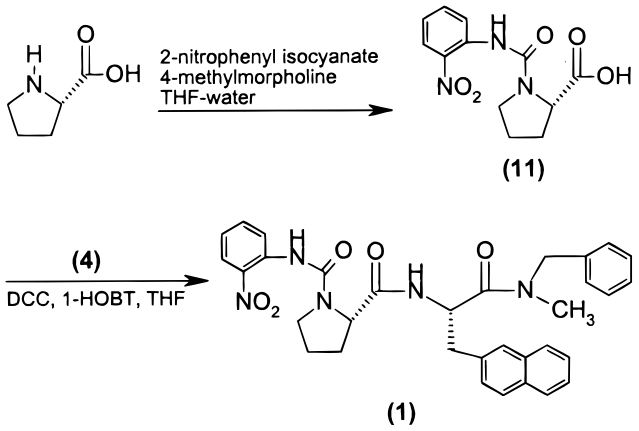
entry	scale of **2**	% of **2** after first additon of reagents	% of **2** after addition of more reagents
1	15.7 g	<3	
2	157 g	10	
3	394 g	22	<4
4	6.1 kg	25	<4

Scheme 4



1-[(2-nitrophenylamino)carbonyl]-L-proline (**11**) and **4**, should overcome the impurity problem. While a synthesis of pure **4** was already in place, **11** should be easily accessible in pure form from L-proline and 2-nitrophenyl isocyanate,

Scheme 5



probably with an acid−base work-up or a recrystallization to remove impurities coming from commercial 2-nitrophenyl isocyanate. As hoped, reaction of L-proline with 2-nitrophenyl isocyanate in a mixture of THF and water in the presence of 4-methylmorpholine followed by an extractive work-up yielded pure **11** in 80% yield (Scheme 5). Coupling of **11** with the free base of **4** in the presence of isobutyl chloroformate and 4-methylmorpholine in THF yielded **1**, but it was contaminated with two by-products, **12** and **13** (Figure 2), which were isolated by a silica gel chromatography and characterized on the basis of spectroscopic data. Compound **12** formed by an intramolecular cyclization in the mixed anhydride intermediate **II**, and **13** formed by a reaction of the primary amino group in **4** at the undesired carbonyl group in **II**. Whereas treatment of a solution of crude **1** in toluene with aqueous NaOH removed **12** via the ring opening of **12** to **11**, which was removed as a water soluble sodium salt, **13** could not be removed without chromatography.

Coupling of **11** with the free base of **4** was then studied with several coupling agents as listed in Table 2. Coupling with (2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HBTU)[4] and 2,2′-dithiobis(benzothiazole)[5] yielded **1** which required chromatographic purifications. Cyanuric chloride[6] gave pure **1**; however, it utilized pyridine as a base which was not so desirable for a scale-up due to unpleasant odor and toxicity. Coupling with 2-chloro-4,6-dimethoxy-1,3,5-triazine[7,8] was unsatisfactory as it yielded a 1:1 mixture of **1** and the by-product **12**. *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, which gives water-soluble urea as the by-product, afforded pure **1** in 90% yield without a chromatography, unfortunately, it was too expensive an agent (>$2000/kg) for a large scale preparation. Next we studied the coupling with the cheap and readily available 1,3-dicyclohexylcarbodiimide,[9,10] a widely used reagent in peptide chemistry, although a well-recognized problem with this reagent is the removal of the resulting 1,3-dicyclohexylurea by-product to the desired

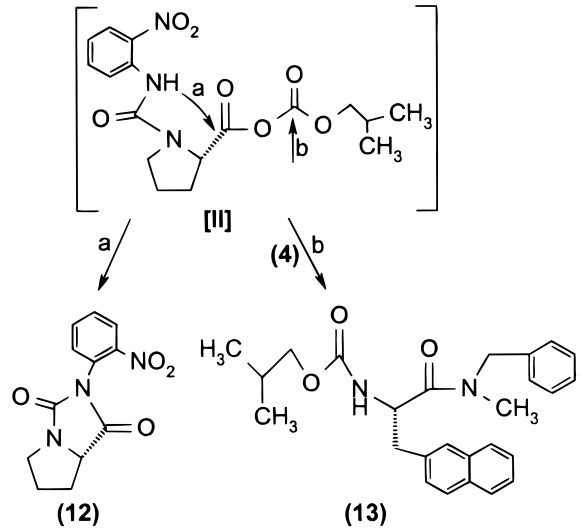


Figure 2.

levels without chromatography. However, in our case, coupling of **11** and **4** in the presence of this agent gave satisfactory results. Thus, combining acid **11** with the free base of **4** in the presence of 1,3-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole in THF at 0 °C followed by warming to room temperature gave a crude mixture, which was filtered to remove precipitated 1,3-dicyclohexylurea. Addition of sodium bicarbonate, removal of THF, and extraction with toluene yielded the crude product that contained unreacted **11**, cyclic by-product **12**, and 1,3-dicyclohexylurea. Treatment of the toluene layer with NaOH converted **12** to the sodium salt of the starting acid **11**, which was removed in the aqueous layer. At the same time more of 1,3-dicyclohexylurea precipitated which was removed by a filtration of the organic layer. Removal of toluene followed by precipitation with ethanol and water yielded **1** of the desired purity in 86% yield. Analysis of **1** indicated that it contained <0.2% of 1,3-dicyclohexylurea. Thus, the silica gel chromatography was successfully eliminated by changing the synthetic strategy. Surprisingly, the drug substance, precipitated with ethanol and water, did not filter well upon scale-up. This filtration problem was overcome by enhancing the filtration rate by using a new "salt trick" which involved the addition of a small amount of saturated NaCl solution to the suspension of **1** (after precipitation with ethanol and water). This modification led to an excellent filtration of the drug substance.

The enantiopurity of **1** was ascertained by a chiral HPLC method, which indicated that none of the other three possible diastereomers (**14**−**16**, Figure 3) were present in **1** in detectable amounts. These results confirmed that there was no racemization during the coupling with 1,3-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole.

Our initial attempts to obtain **1** as a crystalline material failed. However, this compound crystallized from a formulated mixture after a long period of time. The seeds, so obtained, were then used to develop a recrystallization procedure for this drug substance. Crystallization of this material proved frustrating as it would initially oil out from

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(6) Venkataraman, K.; Wagle, D. R. *Tetrahedron Lett.* **1979**, *32*, 3037−3040.
(7) Kaminski, Z. J. *Synthesis* **1987**, 917−920
(8) Kaminski, Z. J. *Tetrahedron Lett.* **1985**, *26*, 2901−2904.
(9) Williams, A.; Ibrahim, I. T. *Chem. Rev.* **1981**, *81*, 589−636.
(10) Mikolajczyk, M.; Kielbasinski, P. *Tetrahedron* **1981**, *37*, 233−284.

Table 2. **Coupling of 4 and 11 with different agents**

entry	coupling agent	conditions	results and conclusions
1	IBCF	(a) **11** (41.4 mmmol), **4** (40 mmol), IBCF (41.2 mmol), 4-methylmorpholine (41.4 mmol), THF, −15 °C to rt; (b) toluene/aqueous NaOH	yield 90%; product contained 1.5% of **13**, which could not be removed without chromatography
2	HBTU	(a) **11** (52.5 mmol), **4** (50 mmol), 1-HOBT (50 mmol), HBTU (51.5 mmol), 4-methylmorpholine (155 mmol), THF, 0 °C to rt; (b) toluene/aqueous NaOH	yield 89%; contained 1.5% of free base of **4**, which could not be removed without chromatography
3	DTBBT	(a) **11** (4.8 mmol), **4** (4.0 mmol), DTBBT (5.3 mmol), triethylamine (4.8 mmol), triethyl phosphite (5.2 mmol), THF, 3 °C to rt; (b) toluene/aqueous NaOH	yield 90%; impurities from DTBBT could not be removed without chromatography
4	CC	(a) CC (13.5 mmol), pyridine (40.5 mmol), THF, 0 °C; then **11** (10 mmol), **4** (9.58 mmol), pyridine (19 mmol), 0 °C; (b) toluene/aqueous NaOH	yield 82%; use of pyridine was not desirable for scale-up
5	CDMT	(a) CDMT (5.8 mmol), **11** (5.92 mmol), **4** (5.64 mmol), 4-methylmorpholine (5.93 mmol), THF, −5 to 0 °C rt; (b) toluene/aqueous NaOH	1:1 mixture of **1** and by-product **12**
6	WSC·HCl	(a) **11** (10.5 mmol), **4** (10 mmol), 1-HOBT (10 mmol), WSC·HCl (11 mmol), THF, 0 °C to rt; (b) toluene/aqueous NaOH	90% yield; desired purity but coupling agent was too expensive ($2250/kg)
7	DCCI	(a) **11** (78.73 mmol), **4** (75 mmol), 1-HOBT (75 mmol), DCCI (75 mmol), THF, 0 °C to rt; (b) toluene/aqueous NaOH	yield 86%; desired purity

[a] ICBF: isobutyl chloroformate; HBTU: (2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate; DTBBT: 2,2′-dithiobis(benzothiazole); CC: cyanuric chloride; CDMT: 2-chloro-4,6-dimethoxy-1,3,5-triazine; WSC·HCl: *N*-(3-dimethyl aminopropyl)-*N*′-ethylcarbodiimide hydrochloride; DCCI: 1,3-dicyclohexylcarbodiimide; 1-HOBT: 1-hydroxybenzotriazole.

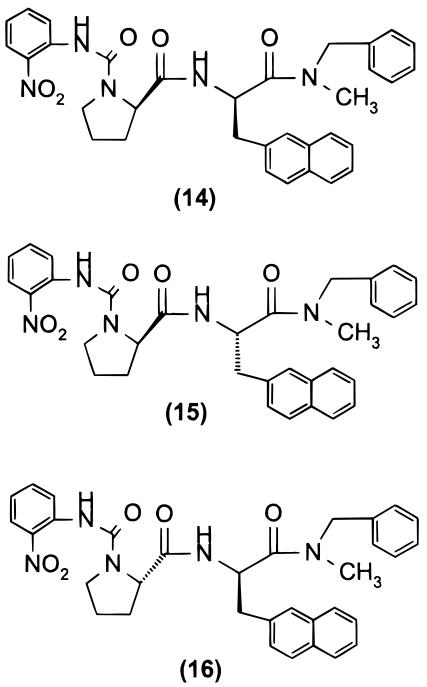

Figure 3.

various solvent systems and then turn into a crystalline material. Such behavior was not suitable for a scale-up. Finally, we found that **1** could be crystallized from ethanol without this problem, although the crystallization rate was slow. These crystallization conditions avoided the isolation of amorphous material.

This convergent synthesis was also successfully scaled-up on a multi-kilogram scale in the pilot plant, yielding the drug substance **1** of the desired purity without chromatography.

Finally, with crystallization conditions in hand we reinvestigated the coupling of free base of **4** using mixed anhydride method. Thus, coupling of free base of **4** with **11** in the presence of isobutyl chloroformate and *N*-benzyldimethylamine in toluene at −20 to −25 °C yielded the crude material which was recrystallized from ethanol to afford pure **1** in 70% yield.

Conclusions

In summary, a robust process for a large scale, chromatography-free preparation of **1** was developed. The key step in the four-step synthesis utilized a peptide coupling of **4** and **11** in the presence of 1,3-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole.

Experimental Section

All of the melting points are uncorrected. ^{1}H NMR spectra were recorded on a Bruker DPX 300 instrument. BOC-L-3-(2-naphthyl)alanine and BOC-L-proline were purchased from Synthetech Inc. Synthetic details on the modified linear synthesis of **1** are described in ref 2. The enantiopurity of **1** was determined on a Waters HPLC system (consisting of model 625 solvent delivery system, model 490 variable wavelength UV detector, and model 717 WISP autosampler) using a Phenomenex Chirex NGLY & DNP column (250 × 4.0 mm) and a mixture of hexane:1,2-dichloroethane:ethanol (50:15:1, v/v/v) as the mobile phase (isocratic at a flow rate of 0.5 mL/min and UV detector at 225 nm) at a column temperature at 50 °C. The retention times of **1**, **14**, **15**, and **16** were 43.0, 48.0, 56.0, and 61.0 min, respectively. The chemical purity of **1** was determined using a YMC-Pack ODS-AQ column (150 × 4.6 mm; 5 μm particle size, 120 Å pore size) and mobile phase A = 1.9 g ammonium acetate/0.9 L water/5.0 mL triethylamine/glacial acetic acid (pH =

4.0)/water to make up 1.0 L volume, B = acetonitrile/methanol (80:20 v/v mixture) at a flow rate of 1.0 mL/min and UV detector at 270 nm. The column temperature was 30 °C and gradient from 65% A/35% B to 25% A/75% B and then to 65% A/35% B was used. The run time was 50 min. The retention times of **11**, **12**, **8**, **7**, **1**, and **13** were 4.6, 8.1, 28.6, 31.1, 32.9, and 34.5 min, respectively. 1,3-Dicyclohexylurea contents in **1** were determined using a YMC-Pack ODS-AQ column (150 × 4.6 mm; 5 μm particle size, 120 Å pore size) and a mixture of methanol:water (75:25, v/v) as the mobile phase (isocratic at a flow rate of 1.0 mL/min and refractive index detector at a sensitivity of 256) with a column temperature at 35 °C. The retention time of 1,3-dicyclohexylurea was 4.5 min.

BOC-(S)-3-(2-Naphthyl)alanyl-*N*-benzyl-*N*-methylamide (3). To *N*-benzylmethylamine (187.4 g, 1.546 mol), cooled to 0−5 °C, was added ethyl trifluoroacetate (7.5 g, 52.79 mmol) dropwise over 15 min while maintaining the internal temperature at 0−5 °C. The reaction mixture was allowed to warm to 22 °C and stirred at 22 °C for 30 min. This mixture was held for further use in the next step.

A solution of BOC-L-3-(2-naphthyl)alanine (394 g, 1.249 mol) in ethyl acetate (5.6 L) was cooled to −15 °C and 4-methylmorpholine (174.4 g, 1.724 mol) was added over 5 min. After stirring the mixture for 10 min, a solution of isobutyl chloroformate (181.2 g, 1.327 mol) in ethyl acetate (125 mL) was added dropwise over 30 min while maintaining the internal temperature at −15 °C. After ethyl acetate was used to wash the addition funnel (50 mL) and added to the reaction mixture, the suspension was stirred at −15 °C for additional 30 min. A solution of the treated *N*-benzylmethylamine in ethyl acetate (125 mL) was added at a constant rate over 40 min while maintaining the internal temperature at −15 °C. After the mixture stirred at −15 °C for an additional 1 h, a solution of isobutyl chloroformate (36.2 g, 0.265 mol) in ethyl acetate (25 mL) was added over 10 min at −15 °C. Ethyl acetate (10 mL) was used to wash the addition funnel and added to the mixture. After the mixture stirred at −15 °C for additional 15 min, a solution of *N*-benzylmethylamine (37.4 g, 0.309 mol; pretreated with 1.5 g, 10.56 mmol of ethyl trifluoroacetate as above) in ethyl acetate (5 mL) was added at a constant rate over 10 min while maintaining the internal temperature at −15 °C. The reaction mixture was warmed to 22 °C over 1 h and stirred at 22 °C for an additional 1 h. After water was added (2.5 L) and the mixture was stirred for 5−10 min, the organic layer was separated and washed with 1 N HCl (1.9 L), water (1.8 L), 5% $NaHCO_3$ (1.5 L), water (1.5 L), and brine (1 L). The organic layer, containing BOC-(*S*)-3-(2-naphthyl)-alanyl-*N*-benzyl-*N*-methylamide (**3**, 522.4 g, yield 100%), was used in the next step.

(S)-3-(2-Naphthyl)alanyl-*N*-benzyl-*N*-methylamide hydrochloride (4). To a solution of HCl gas (455.8 g, 12.5 mol) in ethyl acetate (2.2 L), cooled to an internal temperature at 10 °C, was added the above crude solution of BOC-(*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide (**3**, 522.4 g, 1.249 mol) in ethyl acetate over 30 min while maintaining the internal temperature below 20 °C. The mixture was stirred at 22 °C for 3 h and concentrated in vacuo until 5.0 L of the solvent was collected. The solids were collected by filtration, washed with ethyl acetate (1.2 L), and dried to afford pure (*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide hydrochloride (**4**, 406.2 g, 92% in two steps): mp 162−164 °C; ^{1}H NMR (300 MHz, CD_3OD) mixture of two rotamers δ 2.61 (s, 0.75 × 3H, major N−CH_3), 2.83 (s, 0.25 × 3H, minor N−CH_3), 3.26−3.42 (m, 2H), 4.22 (dd, 0.25 × 2H, J = 15 and 135 Hz, minor CH_2Nap), 4.5 (dd, 0.75 × 2H, J = 15 and 75 Hz, major CH_2Nap), 4.75−4.88 (m, 1H), 6.92−7.87 (m, 12H); MS (CI/isobutane) 319 (MH^+, free base); $[\alpha]^{23}_D$ +24.8° (c = 1, MeOH).

1-[(2-Nitrophenylamino)carbonyl]-L-proline (11). A solution of L-proline (115.13 g, 1.0 mol) in water (700 mL) was cooled to 8 °C (internal temperature) and THF (500 mL) was added over 10 min while maintaining the internal temperature at 10−15 °C. The solution was cooled to 8 °C (internal temperature) and 4-methylmorpholine (105.0 g, 1.04 mol) was added over 5 min while maintaining the internal temperature at 8 °C. A solution of 2-nitrophenyl isocyanate (164.12 g, 1.0 mol) in THF (150 mL) was added over 20 min while maintaining the internal temperature at 8 °C, and then the mixture was warmed to 22 °C over 1 h. After the mixture stirred at 22 °C for an additional 1 h, a solution of $NaHCO_3$ (100 g, 1.19 mol) in water (2.5 L) was added in 15 min, while the internal temperature was maintained at 22 °C. To the resulting mixture was added ethyl acetate (1.25 L), and the mixture stirred for 5 min. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 × 750 mL). The combined organic layers were discarded. The aqueous layer was acidified with concentrated HCl (650 mL), and the solids were collected by filtration. The solids were washed with water (2.0 L) and dried to afford pure 1-[(2-nitrophenylamino)carbonyl]-L-proline (**11**, 223.7 g, 80%): mp 171−173 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.4 (m, 4 H), 3.8 (m, 2 H), 4.73 (m, 1 H), 7.2 (m, 1 H), 7.72 (m, 1 H), 8.3 (m, 1 H), 8.78 (m, 1 H), 10.3 (s, 1 H), 11.0 (bs, 1 H); IR (KBr) 3433, 1738, 1683, 1587, 1503 cm^{-1}; MS (CI/isobutane) 280 (MH^+); $[\alpha]^{23}_D$ −48.9° (c = 1, MeOH).

(2-Nitrophenylcarbamoyl)-(S)-prolyl-(S)-3-(2-naphthyl)-alanyl-*N*-benzyl-*N*-methylamide (1). To a suspension of (*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide hydrochloride (**4**, 106.46 g, 0.3 mol) in isopropyl acetate (650 mL) was added 5% NaOH (450 mL) over 10 min while maintaining the internal temperature at 10−12 °C. The mixture was warmed to 22 °C over 30 min, and the organic layer was separated. The organic layer was washed with water (225 mL) and brine (100 mL) and then dried ($MgSO_4$). The organic layer was concentrated in vacuo to afford (*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide in free base form.

To a solution of 1-[(2-nitrophenylamino)carbonyl]-L-proline (**11**, 88.0 g, 0.315 mol) in THF (530 mL) was added a solution of above (*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide free base preparation in THF (200 mL) over 15 min while maintaining the internal temperature at 22 °C. THF (50 mL) was used to wash the addition funnel and added to the reaction mixture. 1-Hydroxybenzotriazole (40.54

g, 0.3 mol) was then added. The reaction mixture was stirred at 22 °C for 10 min to dissolve the solids and then cooled to 0 °C (internal temperature). A solution of 1,3-dicyclohexylcarbodiimide (62.53 g, 0.303 mol) in THF (100 mL) was added to the mixture over 15 min while maintaining the internal temperature at 0 °C. *(Note: 1,3-dicyclohexylcarbodiimide is a contact allergen).* The mixture was warmed to 22 °C over 30 min and stirred at 22 °C for 4 h. The reaction mixture was cooled to 0 °C and filtered. The filter cake was washed with cold (0 °C) THF (50 mL). To the filtrate was added 5% $NaHCO_3$ (750 mL) over 10 min, while the internal temperature was maintained at 22 °C (this treatment was necessary for safety reasons), followed by addition of toluene (750 mL). After the mixture stirred for 10 min, the organic layer was separated and concentrated in vacuo to remove 1.05 L of solvent. To the distillation residue solution in toluene was added 1 N NaOH solution (325 mL), and the mixture was stirred at 22 °C for 15 min. The mixture was filtered, and the filter cake was washed with toluene (2 × 10 mL). The organic layer was separated from the filtrate and stirred with 1 N NaOH (325 mL) at 22 °C for 1 h. The organic layer was separated and washed with water (3 × 300 mL) and brine (150 mL). The organic layer was filtered, and the filtrate was concentrated in vacuo until no further solvent distilled to give crude **1**.

Isolation of Amorphous 1. The crude **1** was dissolved in ethanol (400 mL, 190 proof) by heating at 50 °C to obtain a clear solution and concentrated in vacuo. The resulting residue was dissolved in ethanol (1.0 L, 190 proof) by heating at 45−50 °C to obtain a clear solution. The solution was cooled to 27−28 °C and added to water (4.5 L), precooled to 7−8 °C (internal temperature) over a period of 30 min while maintaining an internal temperature at 7−9 °C. The addition funnel was washed with ethanol (30 mL), and the washings were added to the suspension. The suspension was stirred at 7−9 °C for 30 min, and a saturated solution of NaCl (250 mL) was added. The solids were collected by filtration, washed with water (3 × 400 mL), dried at 43−45 °C (55−60 mmHg) to obtain pure **1** (150.0 g, 86%): mp 79−84 °C; ^{1}H NMR (300 MHz, $CDCl_3$) mixture of two rotamers d 1.73−2.02 (m, 3H), 2.2−2.35 (m, 1H), 2.8 (s, 0.66 × 3H, major N−CH_3), 2.97 (s, 0.34 × 3H, minor N−CH_3), 3.1−3.47 (m, 4H), 4.34−4.76 (m, 3H), 5.33−5.4 (m, 1H), 7.02−7.75 (m, 15H), 8.24 (dd, 1H, J = 8.7 Hz), 8.77 (dd, 1H, J = 8.7 Hz), 9.92 (s, 0.34 × 1H, minor NH), 10.03 (s, 0.66 × 1H, major NH); IR (KBr) 3346, 1680, 1646, 1610, 1586, 1500, 1453 cm^{-1}; MS (ESI) 580.2 (MH^+); $[\alpha]^{23}_D$ −60.2° (c =1, MeOH).

Isolation of Crystalline 1. The crude **1** was dissolved in ethanol (400 mL, 190 proof) by heating at 50 °C and concentrated in vacuo. This operation was repeated once more. The resulting residue was dissolved in ethanol (1.1 L, 190 proof) by heating at 45−50 °C to obtain a clear solution. The solution was cooled to 22 °C, and seeds of crystalline **1** (2.0 g) were added. The mixture was stirred at 22 °C for 40 h. The solids were collected by filtration, washed with cold (0−2 °C) ethanol (3 × 50 mL, 190 proof), and dried at 55−58 °C (60−65 mmHg) to obtain pure crystalline **1** (140.6 g, 81%): mp 116−120 °C.

Isobutyl Chloroformate Method for the Coupling of Free Base of 4 with 11. To a suspension of 1-[(2-nitrophenylamino)carbonyl]-L-proline (**11,** 3.1 g, 11.0 mmol) in toluene (10 mL) was added a solution of *N*-benzyldimethylamine (1.5 g, 11.0 mmol) in toluene (5 mL) at room temperature. The clear yellow solution was cooled to −20 to −25 °C, and a solution of isobutyl chloroformate (1.5 g, 11.0 mmol) in toluene (7 mL) was added over 10 min while the internal temperature was maintained at −20 to −25 °C. After the mixture stirred for an additional 30 min at −20 to −25 °C, a solution of (*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide free base (3.2 g, 10.0 mmol) in toluene (8 mL) was added over 10 min while the internal temperature was maintained at −20 to −25 °C. After stirring for additional 30 min at −20 to −25 °C, the reaction mixture was warmed to 0 °C. After a solution of sulfuric acid (1 mL) in water (15 mL) was added, the organic layer was separated and washed with water (10 mL), 5% NaOH (20 mL), and water (3 × 10 mL). The organic layer was concentrated in vacuo, and the residue was dissolved in 95% ethanol (27 mL). The resulting solution was stirred at 22 °C for 40 h. The solids were collected by filtration, washed with cold (0−2 °C) ethanol (2 × 3 mL), and dried to obtain pure crystalline **1** (4.1 g, 70%).

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