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Efficient Synthesis of (*S*)-2-(Cyclopentyloxycarbonyl)-amino-8-nonenoic Acid: Key Building Block for BILN 2061, an HCV NS3 Protease Inhibitor

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Abstract:

A new procedure for the practical synthesis of (*S*)-2-(cyclopentyloxycarbonyl)amino-8-nonenoic acid, a key building block for BILN 2061, an HCV NS3 protease inhibitor, has been developed. The key step features a kinetic resolution of racemic 2-acetylamino-8-nonenoic acid with acylase I. In addition, the undesired (*R*)-2-acetylamino-8-nonenoic acid was recycled after racemization. The procedure was implemented for the production of (*S*)-2-(cyclopentyloxycarbonyl)amino-8-nonenoic acid on pilot-plant scale.

Introduction

BILN 2061, a new HCV NS3 protease inhibitor, was recently designed on the basis of a rational approach involving the use of traditional medicinal chemistry, parallel synthesis, and structural data for the optimization of binding and downstream biopharmaceutical properties.[1] It has shown good oral bioavailability and antiviral effect in humans infected with HCV genotype 1.[2] The selection of BILN 2061 as a candidate for further clinical studies necessitated a practical synthesis suitable for pilot-plant scale.

The structure of BILN 2061 features a 15-membered macrocyclic tripeptide. In our retrosynthetic approach (Scheme 1), the 15-membered macrocyclic tripeptide can be constructed by Ru-catalyzed ring-closing metathesis (RCM) of an acyclic tripeptide precursor **1**,[3] followed by etherification with **2**. In turn, the acyclic tripeptide precursor **1** can be synthesized by fragment condensation of three unnatural α-amino acids derivatives, **3**, **4**, and **5**.

(*S*)-2-(*tert*-Butyloxycarbonyl)amino-8-nonenoic acid had been prepared on small scale by asymmetric hydrogenation of (*Z*)-2-acetamido-2,8-nonadienoate using (*S*,*S*)-Et-DUPHOSRh(COD)OTf,[4] followed by the amide-to-carbamate conversion[5] and saponification.[3] In this paper, we describe an alternative synthesis of building block (*S*)-2-(cyclopentyloxycarbonyl)amino-8-nonenoic acid (**3**), by kinetic enzymatic resolution of the corresponding racemate with acylase I. This procedure could be reproduced in the pilot plant, after simple operational adjustments, on a scale up to 100 kg.

Results and Discussion

Because α-amino acids constitute one of the most important classes of natural products exhibiting important biological functions, a wide variety of synthetic methods are available for their preparation.[6] We focused on the application of inexpensive acylase I (EC 3.5.1.14) to the L-selective cleavage of *N*-acetyl amino acids, according to the extensive studies by Whitesides.[7] Acylase I enzymes isolated from porcine kidney (PKA) and the fungus *Aspergillus* sp. (AA) are both commercially available, inexpensive, stable in aqueous solution, and possess high specific activity. Considering the fact that acetylamino acids are good substrates of acylase I and are readily prepared, we chose 2-acetamido carboxylic acid (±)-**9** as our target (Scheme 2) for the enzymatic resolution. This would allow us to introduce the cyclopentyloxy group required in BILN 2061 by a simple acylation of the free amino group of **10** obtained by enantioselective hydrolysis of (±)-**9**.

7-Chloro-1-heptene (**7**) was prepared using a known literature procedure.[8] Thus, cross-coupling of 1-1-bromo-4-chlorobutane (**6**) with allylmagnesium bromide in the presence of dilithium tetrachlorocuprate in THF gave **7** in good yield. Although THF solutions of dilithium tetrachlorocuprate are commercially available, we prefer its in situ preparation by simply mixing 2 equiv of anhydrous lithium chloride with anhydrous copper (II) chloride in THF. This THF solution of Li_2CuCl_4 prepared in situ was as effective as the commercially available one. Alkylation of diethyl acetami-

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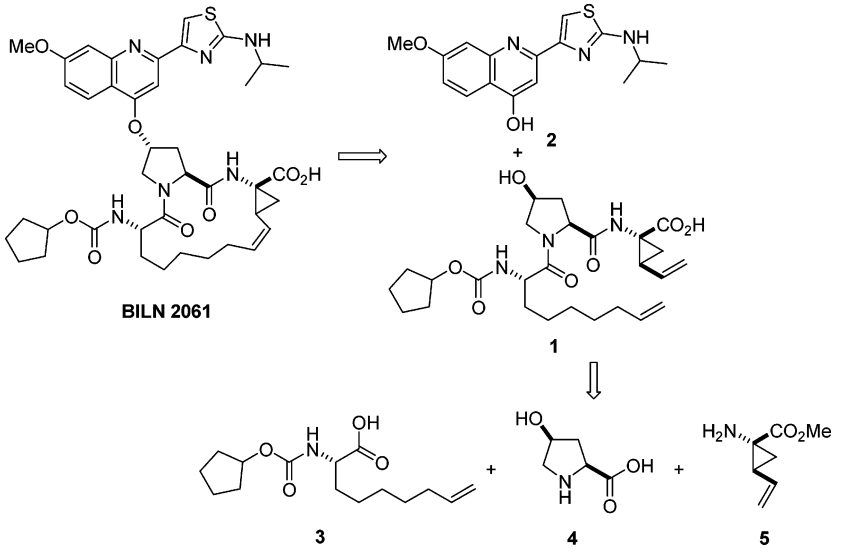
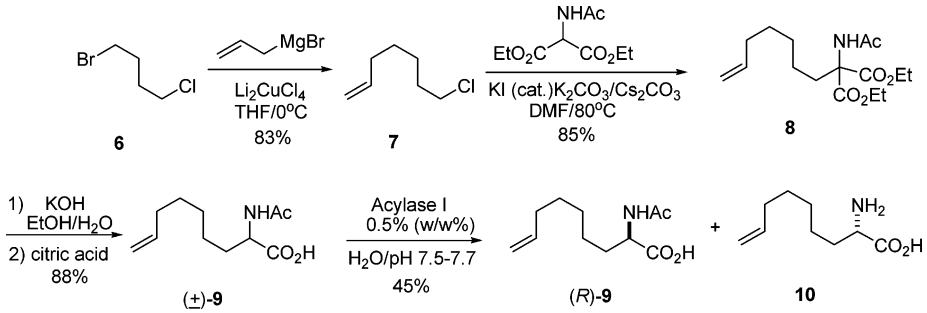
Scheme 1. **Retrosynthetic analysis**



Scheme 2. **Synthesis of (*S*)-2-amino-8-nonenoic acid**



domalonate with the crude **7** was accomplished in 85% yield by heating the mixture at 80 °C in DMF for 16 h with a 4:1 mixture of potassium carbonate and cesium carbonate as base and a catalytic amount of potassium iodide.[9] With only potassium carbonate as base, the alkylation was sluggish, taking 92 h to reach completion. It is worth mentioning that the initial attempt to prepare **8** using 7-bromo-1-heptene as alkylating agent proceeded much more readily than that using **7**. However, preparation of 7-bromo-1-heptene by the same cross coupling of 1,4-dibromobutane with allylmagnesium bromide gave only a 40% of the monobromide, due to the formation of 1,9-decadiene, the product of double allylation. Intermediate **8** can be also used without purification/isolation. Saponification of the crude **8** was effected by treatment with potassium hydroxide in aqueous ethanol at 60 °C. Upon concentration, the aqueous reaction mixture was neutralized with citric acid to yield (±)-**9** in 88% yield after recrystallization from isopropanol/water.

This racemic material was then subjected to kinetic resolution with 0.5% (w/w) acylase I in water at pH 7.5−8.0 in the presence of small amounts of Co(II), an enzyme cofactor. When the reaction mixture was heated to 37 °C for 30 min, we noticed the precipitation of the amino acid **10** and little variation of the solution pH.[10] After 7 h at 37 °C, the solid product was collected simply by filtration to provide **10** in ≥45% yield with >99% ee as determined by HPLC on chiral support; the reaction was halted when an assay on the solution indicated 95% ee for enriched (*R*)-**9**. There was no need for a periodic addition of sodium hydroxide solution to maintain the pH at 7.5−8.0, as normally required for this type of enzymatic resolution, since the pH of the reaction mixture showed little change.

Apparently, after L-selective cleavage of the acetamide bond, the resulting acetic acid partially neutralizes the amino acid sodium salt formed to the free amino acid **10**, which precipitates out of solution, driving the equilibrium. As a result, the pH of the solution is self-buffered. The virtually full precipitation of the desired **10** from the reaction mixture allowed an exceedingly simple isolation, and the self-adjusting pH of the reaction mixture obviated the need to periodically add base to the reaction mixture. These two features make the process practical, robust, and easy to perform on large scale.

As typical of other α-amino acids, the undesired (*R*)-**9** could be recycled through racemization. To illustrate this, the (*R*)-enriched **9** sodium salt was fully racemized by heating in water containing Ac_2O.[11] After two cycles of racemization, an overall 71% yield of **10** could be obtained.

We next turned to the preparation of the fragment **3** with optically pure **10**. Treatment of **10** with commercially

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(10) In a few cases, the precipitation of (*S*)-**15** was delayed, leading to a drop in pH. It rose once precipitation began.

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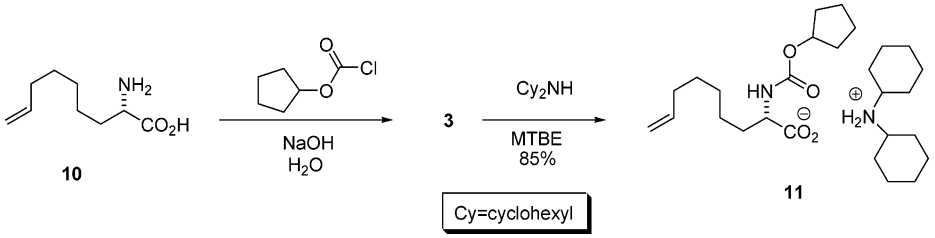
Scheme 3. **Completion of the synthesis**



available cyclopentyl chloroformate under Schotten−Baumann conditions gave **3** in quantitative yield. The crude, oily material may be used directly for the formation of the tripeptide precursor **1**. However, for more convenient handling and long-term storage of this material, the fragment **3** was isolated as the highly crystalline dicyclohexylamine salt **11** in 85% yield by a simple treatment of the crude **3** with dicyclohexylamine in methyl *tert*-butyl ether (MTBE, Scheme 3).

Conclusion

In summary, we have developed a practical procedure for preparation of (*S*)-2-(cyclopentyloxycarbonyl)amino-8-nonenoic acid (**3**) with >99% ee in 28% overall yield over six steps. A recycling process was established for the kinetic resolution to permit more complete conversion of the material to the desired L-amino acid. This can boost the overall yield to ≥40%. The above synthesis of building block **11** was scaled, with minor operational adjustments, to >100 kg, as part of the manufacturing of BILN 2061 for clinical development.[13]

Experimental Section

General. Acylase I was purchased from Sigma and stored at 0 °C as instructed. All other reagents and solvents were purchased from commercial sources and used without further drying and purification. NMR spectra of all compounds were recorded on a Bruker-Biospin DPX 400 NMR spectrometer. Enantiomeric excess determinations were carried out on a Chiralpak AD-RH column (0.46 cm × 15 cm) using 0.02% phosphoric acid in water and acetonitrile as eluent (65:35), using a flow rate of 0.7 mL/min at a column temperature of 35 °C and a detection wavelength of 205 nm. The analyte concentration was typically 1−2 mg/mL in water/acetonitrile (1:1). NMR assays were carried out by adding a weighed amount of ethylene carbonate to a determined volume of the solution to be analyzed (in $CDCl_3$), and quantitating the analyte by NMR integration vs the known concentration of internal standard.

1-Chlorohept-6-ene (7). To a solution of LiCl (69.0 g, 1.63 mol) in THF (7.0 L) was added and $CuCl_2$ (109 g, 0.821 mol), and the mixture was stirred at 20 °C for 1 h under N_2. The solution was then cooled to 0 °C, and 1-bromo-4-chlorobutane (**6**, 700 g, 4.08 mol) was added. A solution of 1 M allylmagnesium bromide (5.50 L, 5.50 mol) in THF was added over a period of 30 min at 0 °C. The mixture was stirred at this temperature for 2 h and then quenched with 10% H_2SO_4 (5.0 L) followed by addition of MTBE (9.0 L). The organic layer was washed with water (2 × 6.0 L) and 3% NaCl (6.0 L) and was distilled (20 °C, 100 mmHg) to a low volume. Assay by ^{1}H NMR indicated 450 g of **7** in the residue (83%). This crude material, which contained 50% w/w THF admixed with **7**, was taken to the next step without further purification. The physical data (NMR, IR) obtained on this product were identical to those reported in the literature.[12]

Diethyl (Acetamido)(6-hepten-1-yl)propanedioate (8). Potassium iodide (305 g, 1.84 mol), potassium carbonate (635 g, 4.60 mol), and cesium carbonate (1.50 kg, 4.60 mol) were added to a mixture of crude **7** (prepared as above, pooled from three runs, 1.23 kg estimated by NMR assay, 9.20 mol) and diethyl acetamidomalonate (1.95 kg, 9.20 mol) in anhydrous DMF (8.0 L). The reaction mixture was stirred at 80 °C for 16 h, then was cooled to RT, and water (8.0 L) was added, followed by EtOAc (16 L). The organic layer was washed with water (2 × 8.0 L), and the organic solvent was distilled off. The residue, containing some residual ethyl acetate, was assayed by ^{1}H NMR, which showed 2.45 kg of **8** (85%). This crude material was used in the next step without further purification. A small aliquot was purified by flash column chromatography to give **8** as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10 (m, 2H), 1.25 (t, J = 7.2 Hz, 6 H), 1.33 (m, 4H), 2.02 (m, 2 H), 2.03 (s, 3H), 2.32 (dt, J = 2.4 and 6.0 Hz, 2H), 4.23 (q, J = 7.2 Hz, 4 H), 4.95 (dd, J = 1.6 and 15.6 Hz, 2H), 5.77 (m, 1H), 6.76 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 168.9, 168.2, 138.8, 114.4, 66.6, 62.4, 33.6, 32.0, 28.7, 28.6, 23.4, 23.0, 14.0; HRMS (APCI) calcd for $C_{16}H_{29}NO_5$ 314.1961, found 314.1976.

(±)-2-Acetamido-8-nonenoic acid (9). To a solution of diethyl (acetamido)(6-hepten-1-yl)propanedioate (**8**, 1.89 kg, 6.02 mol) in ethanol (10 L) and water (1.0 L) was added potassium hydroxide (1.01 kg, 18.1 mol) at RT. The solution was stirred at 60 °C for 16 h. The solution was partially evaporated (to ∼3 L), and diluted with water (6.0 L). The aqueous phase was washed with ethyl acetate (2 × 1.3 L), and the pH was adjusted to 4−5 by addition of citric acid (∼1.3 kg, 6.76 mol). The mixture was stirred at 100 °C for 6 h and then cooled to RT. The pH was further adjusted to 3.5 by adding 3 N HCl. The aqueous layer was extracted with ethyl acetate (2 × 3.0 L). Evaporation of the solvents was followed by recrystallization of the residue from isopropanol/water to give **9** (1.13 kg, 88%) as a white solid:

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(13) Yee, N. K.; Farina, V.; Houpis, I. N.; Haddad, N. Frutos, R. P.; Gallou, F.; Wang, X.-J.; Wei, X.; Simpson, R. D.; Feng, X.; Fuchs, V.; Xu, Y.; Tan, J.; Zhang, L.; Xu, J.; Smith-Keenan, L. L.; Vitous, J.; Ridges, M. D.; Spinelli, E. M.; Johnson, M.; Donsbach, K.; Nicola, T.; Brenner, M.; Winter, E.; Kreye, P.; Samstag, W. *J. Org. Chem.* **2006**, *71*, 7133−7145.

mp 129−131 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (m, 6H), 1.72 (m, 1 H), 1.88 (m, 1H), 2.03 (m, 2 H), 2.06 (s, 3H), 4.60 (dt, J = 5.6, 7.6 Hz, 1H), 4.97 (dd, J = 1.6, 15.6 Hz, 2H), 5.80 (m, 1H), 6.20 (d, J = 8 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 175.5, 171.5, 138.8, 114.4, 52.5, 33.6, 32.0, 28.7, 28.6, 25.0, 22.8; MS (CI): m/z 214 ($M^+ + 1$); Anal. Calcd for $C_{11}H_{19}NO_3$: C, 61.95; H, 8.98; N, 6.57. Found: C, 62.24; H, 9.17; N, 6.52.

(*S*)-2-Amino-8-nonenoic Acid (10). To a slurry of racemic **9** (670 g, 3.14 mol) in water (7.0 L) were added sodium hydroxide (125 g, 31.2 mol) and $CoCl_2 \cdot 6H_2O$ (500 mg, 2.10 mmol) at RT to form a solution. The pH was adjusted to 7.8 by addition of 0.1 N NaOH. The solution was heated to 38.5 °C under stirring, and acylase I (0.7 g) was added. The reaction mixture was stirred at 38.5 °C for 7 h. After adjusting the pH to 7.6 using acetic acid (if needed), the slurry was cooled to RT and filtered. The resulting filter cake was washed with water (1.0 L), and a 10:1 mixture of water/methanol (0.75 L). The solid was dried to give **10** (230 g, 45%) as an off-white solid: mp 200−201 °C dec. HPLC on chiral support indicated >99% ee. ^{1}H NMR (400 MHz, NaOD/D_2O) δ 1.28 (m, 4H), 1.36 (m, 2 H), 1.49 (m, 1 H), 1.55 (m, 1H), 2.02 (m, 2 H), 3.17 (t, J = 6 Hz, 1H), 4.97 (dd, J = 10, 18 Hz, 2H), 5.61 (m, 1H); ^{13}C NMR (NaOD/D_2O) δ 183.0, 139.4, 113.1, 55.2, 33.7, 32.0, 27.3, 27.0, 23.8. MS (CI): m/z 172 ($M^+ + 1$); Anal. Calcd for $C_9H_{17}NO_2$: C, 63.13; H, 10.01; N, 8.18. Found: C, 62.79; 10.11; N, 7.90.

Dicyclohexylamine Salt of (*S*)-2-(Cyclopentyloxycarbonyl)amino-8-nonenoic Acid (11). To a stirred slurry of **10** (228 g, 1.33 mol) in a 1:1 mixture of THF/water (4 L) was added sodium hydroxide (53 g, 1.33 mol) to form a solution. Sodium carbonate (142 g, 1.33 mol) was added, followed by addition of cyclopentyl chloroformate (230 g, 1.47 mol) at RT over a period of 35 min. The reaction mixture was stirred at RT for an additional 30 min and diluted with water (3 L). The aqueous layer was washed with ethyl acetate (1.5 L); 1 N HCl was added to the solution to bring the pH to 3. The mixture was then extracted with *tert*-butyl methyl ether (4 L). Dicyclohexylamine (275 g, 1.50 mol) was added to the extracts over a period of 30 min at 50 °C. The suspension was cooled to room temperature and stirred for an additional 4 h. The slurry was filtered, and the filter cake was washed with methyl *tert*-butyl methyl ether (0.5 L). The solid was dried to give dicyclohexylamine salt **11** (530 g, 85%) as a white solid: mp 90−93 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 (m, 2H), 1.25 (m, 14 H), 1.60 (m, 10 H), 1.69 (m, 8H), 2.02 (m, 6 H), 3.64 (t, J = 6 Hz, 1H), 4.96 (m, 3H), 5.76 (m, 1H), 6.22 (d, J = 6.8 Hz, 1 H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 174.3, 155.2, 138.7, 114.5, 75.6, 55.0, 51.6, 33.1, 32.4, 32.3, 29.2, 28.5, 28.3, 25.1, 24.8, 24.2, 23.2; MS (CI): m/z 284 ($M^+ + 1$); Anal. Calcd for $C_{27}H_{48}N_2O_4$: C, 69.79; H, 10.41; N, 6.03. Found: C, 69.52; H, 10.49; N, 5.80.

Racemization of (*R*)-2-Acetamido-8-nonenoic Acid [(*R*)-9]. To a solution of the sodium salt of (*R*)-2-acetamido-8-nonenoic acid [(*R*)-(**9**)] (850.0 g, 3.36 mol) in water (8 L) was added acetic anhydride (1.5 L, 16.20 mol) at 60 °C over a period of 6 h with stirring under nitrogen. The reaction mixture was stirred at 60 °C for an additional 16 h and cooled to RT. Concentrated HCl was added to bring the pH to about 3. The mixture was extracted with ethyl acetate (2 × 3 L). The combined extracts were washed with water (1 L) and concentrated to dryness. The NMR weight % assay and HPLC analysis on chiral support indicated 847 g of racemic **9** in the organic residue (99%). This crude material was directly taken to the next cycle of resolution without purification.

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