SCIENCE CHINA
Chemistry

• ARTICLES •

June 2012 Vol.55 No.6: 1101–1107
doi: 10.1007/s11426-011-4478-5

Improved synthesis of rupintrivir

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Received August 31, 2011; accepted October 12, 2011; published online January 5, 2012

An improved synthesis of rupintrivir (AG7088) was accomplished using three amino acids (L-glutamic acid, D-4-fluorophenylalanine, and L-valine) as the building blocks. The key fragment ketomethylene dipeptide isostere was constructed with the valine derivative and phenylpropionic acid derivative, followed by coupling with a lactam derivative and an isoxazole acid chloride to provide AG7088 totally in eight steps.

rupintrivir, AG7088, synthesis

1 Introduction

Over 100 deaths were reported in the outbreaks of hand, foot and mouth disease (HFMD) over the past two years in China, and thousands of people were admitted to hospitals with moderate to severe symptoms of this disease. HFMD, commonly caused by coxsackievirus A16 (CVA16) or enterovirus 71 (EV71), can proceed to serious complications such as meningitis, which can be fatal to small infants and children [1]. Unfortunately, there is no specific treatment for HFMD; therefore, there is an urgent need to discover effective drugs against this viral infection. AG7088 was developed originally to combat human rhinovirus (HRV), which is the major cause of the common cold. In Phase-II clinical trials, AG7088 demonstrated moderate antiviral and clinical efficacy. Because of a lack of efficacy in natural infection studies, further development was stopped. However, AG7088 has received attention again in recent years, because it is now considered a potent inhibitor of EV71 [2–5]. The compound has been shown to inhibit the viral chymotrypsin-like protease ($3C^{pro}$), which is required for proteolytic processing of the large polyprotein translated from the viral RNA genome and is thus essential for the propagation of the virus [6]. As the safety of AG7088 has been confirmed, the development of AG7088 will be a shortcut for HFMD therapy. In addition, AG7088 derivative have also been shown to have antiviral activity against severe acute respiratory syndrome (SARS) [7–9]. Although AG7088 possesses good antiviral activity and has received much attention, there is limited information available in the literature concerning methods for its synthesis. Therefore, an efficient synthesis method for AG7088 is important and will benefit the development of antiviral drugs of this type. In this paper, we provide details of investigation and improvement of a new synthetic route for the preparation of AG7088.

As shown in the retrosynthetic analysis outlined in Figure 1, AG7088 was prepared from three fragments: the lactam derivative (A), the ketomethylene dipeptide isostere (B), and an isoxazole acid chloride (C). The original synthetic route, reported by Dragovich *et al.*, was coupling the ketoacid (B) with the lactam derivative (A) and the isoxazole acid chloride (C). Using this approach, AG7088 was syn-

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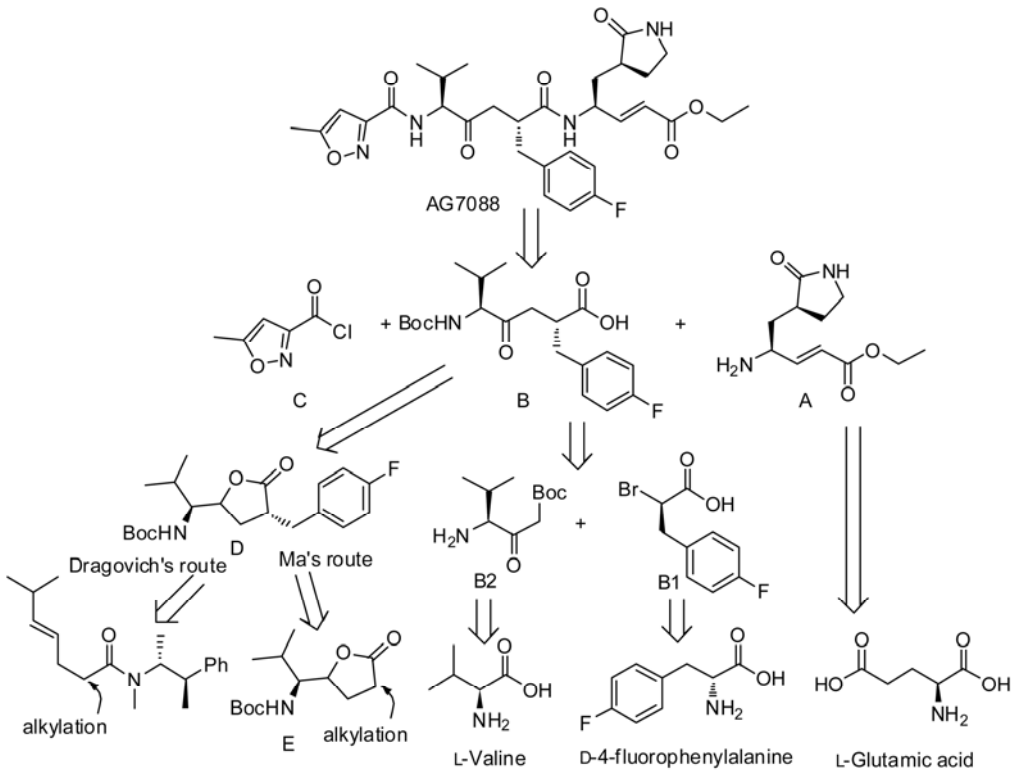
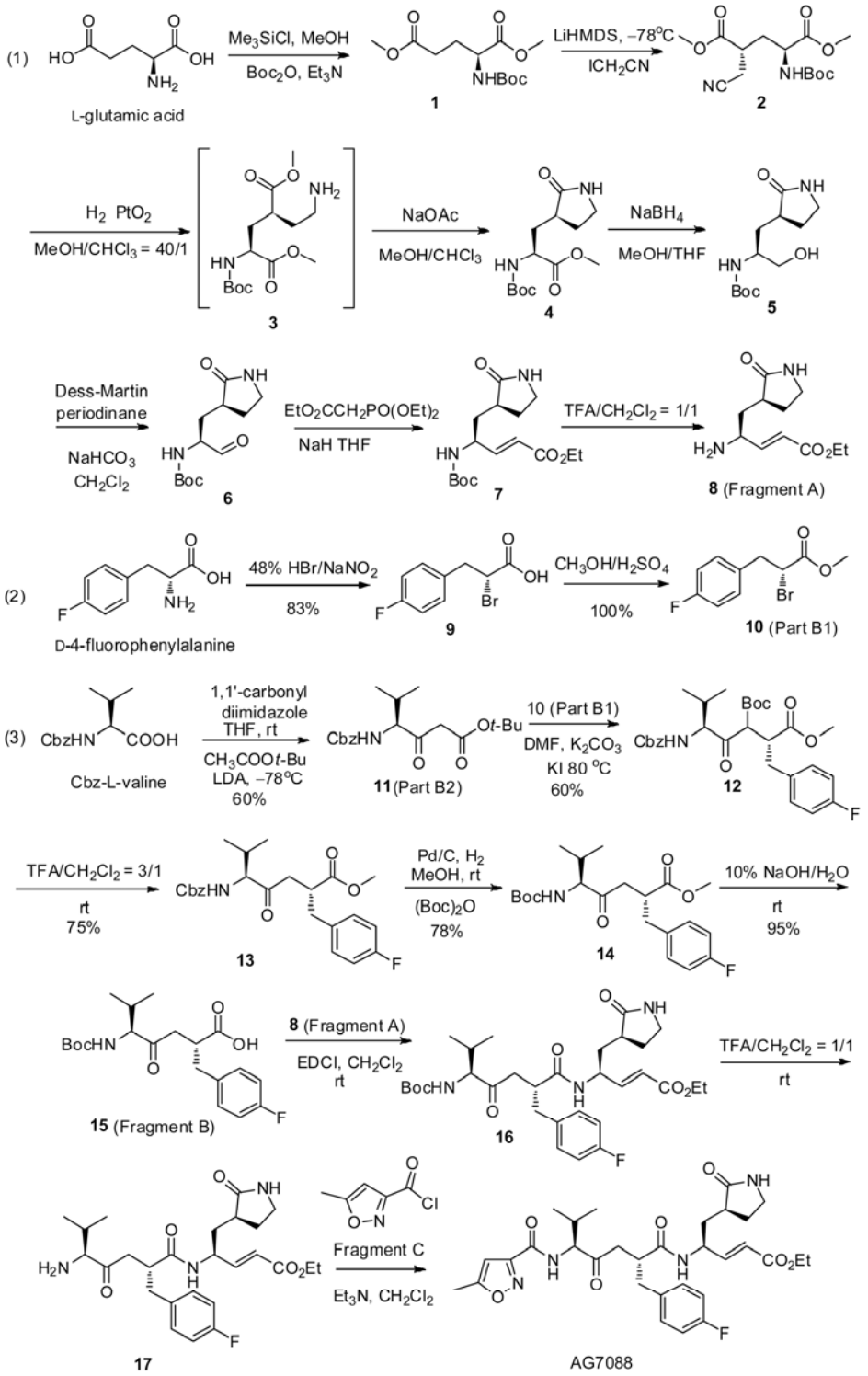


Figure 1 Retrosynthetic analysis of AG7088 (rupintrivir).

thesized with a total yield of about 0.6% in 13 steps [10, 11]. In this route, the lactam derivative (A) was obtained using natural glutamic acid within 13 steps [10], and the key building block ketoacid (B) was obtained through a lactone (D) using diastereoselective alkylation of (−)-pseudoephedrine-derived amide with *p*-fluorobenzyl bromide and subsequent lactonization was promoted by *N*-bromosuccinimide (NBS) as the key steps [11]. Then Tian *et al.* improved the synthesis of the lactam derivative (A) and simplified the reaction step to 8 steps [12]. Ma *et al*. improved the synthesis of the ketoacid (B) via the chiral alkylation of the lactone (E), avoiding use of the chiral catalyst as the key step. Based on Tian's work, they obtained AG7088 using L-valine as the starting material in about 7% yield in 16 steps [13, 14]. Meanwhile, Chng *et al*. reported a synthetic method of AG7088 using the isoxazole acid (C) as the starting material; however, the use of toxic reagents and the harsh reaction condition of this method might limit its synthetic usefulness [15]. Shie *et al*. reported an alternative route to synthesize the ketoacid (B) and obtained AG7088 using Cbz-L-valine as the starting material in 15.3% yield and 12 steps [16]. However, the harsh reaction conditions and inconvenient chromatographic fractionation were disadvantageous. Based on Shie's work, we improved the synthesis of the ketoacid (B) from two parts: the 2-substituted propionic acid B1 and the valine derivative B2. In this improved route, the synthesis of part B1 was accomplished using commercially available 4-fluorophenylalanine as the material in one step and the product was easily purified. An S_N2 reaction of part B1 and part B2 provided the ketoacid (B) without using chiral ligands or low temperature, and was much more convenient than chiral synthesis. Then we linked the ketoacid (B) with the lactam derivative (A) and the isoxazole acid chloride (C) to provide the final product AG7088 in 3 steps, which was much shorter than the one reported Shie *et al*. Finally, we obtained AG7088 in about 12% yield in 8 steps using Cbz-L-valine as the starting material.

2 Results and discussion

After a thorough retrosynthetic analysis, we designed a synthetic route for AG7088 (rupintrivir) preparation (Scheme 1). In this route, we started with the dianionic alkylation of N-Boc glutamic acid dimethyl ester **1** with iodoacetonitrile. As expected, this alkylation occurred in a highly stereoselective manner, giving **2** as the exclusive product (72% yield). In the following step, the cyano group in **2** was subjected to hydrogenation to generate the intermediate **3**. Then, the *in situ* cyclization of intermediate **3** afforded the lactam **4** in 40% yield (from **1**). The ester group in **4** was then reduced to the corresponding alcohol. The oxidization of the alcohol product **5** by Dess-Martin periodinane, followed by the Wittig reaction with an appropriate reagent, gave rise to the conjugated ester **7** in 65%



Scheme 1 Synthesis of AG7088.

yield (from **4**). The designed lactam derivative **8** (Fragment A) was generated by removal of the protecting group of **7**.

Compound **10** (Part B1) was prepared from D-4-fluorophenylalanine via bromination and methyl esterification. Meanwhile, the valine-derived malonate **11** was obtained via Claisen condensation. The S_N2 reaction with bromoacetate **10** and the valine-derived malonate **11** was followed by removal of the tert-butyloxycarbonyl (Boc) group to provide the ketoester **13**. The direct conversion of the benzyloxycarbonyl (Cbz) group to the Boc group in **14** was accomplished by catalytic hydrogenation of **13** in the presence of di-*tert*-butyl carbonate, which was followed by hy-

drolysis to give the key ketoacid **15** (Fragment B). In the final step, AG7088 was prepared by coupling the ketoacid **15** with the lactam derivative **8** (at the right side initially) and then the isoxazole acid chloride (Fragment C) on the left side at the end.

3 Conclusion

In summary, we have improved the synthetic route for AG7088 production using an easy and practical method. This route has many merits compared to previous methods, such as the non-usage of chiral catalysts, fewer reaction steps, low-cost starting materials, and convenient chromatographic fractionation.

4 Experimental

*(2S,4R)-Dimethyl 2-(tert-butoxycarbonylamino)-4-(cyanomethyl)pentanedioate **2***

To a solution of *N*-Boc-L-glutamic acid dimethyl ester (**1**, 6.0 g, 21.8 mmol) in THF (60 mL) was added dropwise a solution of lithium bis(trimethylsilyl)amide in THF (47 mL, 1 M) at −78 °C under an argon atmosphere. The resulting dark mixture was stirred at −78 °C for 1 h. At the same time, iodoacetonitrile (3.72 g, 2.33 mol) was added dropwise to the dianion solution over a period of 1 h while maintaining the temperature below −70 °C. The reaction mixture was stirred at −78 °C for additional 2 h and the disappearance of the starting material (**1**) was confirmed by TLC analysis. The reaction was quenched with methanol (3 mL) and a pre-cooled acetic acid in THF solution (2.7 mL HOAc/20 mL THF) was added. After stirring for 30 min, the cooling bath was removed. The reaction mixture was allowed to warm up to room temperature and then poured into a brine solution (40 mL). The layers were separated, and the organic layer was concentrated to afford dark brown oil. The crude residue was purified by flash column chromatography (petroleum ether/ethyl acetate = 4/1) to give product **2** as colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.23 (1H, d, J = 9.0 Hz), 4.43–4.36 (1H, m), 3.77(1H, s), 3.76 (1H, s), 2.89–2.69 (3H, m), 2.20–2.14 (2H, m), 1.45 (9H, s). ESI-MS (*m*/*z*): 315 $(M + H)^+$.

*(S)-Methyl 2-(tert-butoxycarbonylamino)-3-((S)-2-oxopyrrolidin-3-yl)propanoate **4***

In a hydrogenation flask was placed compound **2** (5.0 g, 15.9 mmol), 5 mL of chloroform and 60 mL of methanol before the addition of PtO_2. The resulting mixture was pressurized to hydrogen and mechanically stirred at room temperature for 12 h. Then the mixture was filtered over Celite. NaOAc (8.46 g, 31.8 mmol) was added to the filtrate before the resulting mixture was stirred at 60 °C for 12 h. The reaction was quenched with water (30 mL). The suspension was extracted with ethyl acetate. The organic layers were combined, dried ($MgSO_4$), and filtered. The light brown filtrate was concentrated and purified by silica gel column chromatography (petroleum ether/ethyl acetate = 4/1) to give the product **4** as colorless oil. ^{1}H NMR ($CDCl_3$) δ 6.02 (1H, br), 5.49 (1H, d, J = 7.8 Hz), 4.27–4.33 (1H, m), 3.72 (3H, s), 3.31–3.36 (2H, m), 2.40–2.49 (2H, m), 2.06–2.16 (1H, m), 1.77–1.89 (2H, m), 1.41 (9H, s). ESI-MS (*m*/*z*): 287 $(M + H)^+$.

*tert-Butyl (S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-ylcarbamate **6***

The complex **4** (1.0 g, 3.49 mmol) was dissolved in methanol (40 mL), then $NaBH_4$ (0.53 g, 14 mmol) was added under ambient conditions. The reaction mixture was stirred at room temperature for 2 h. Then the reaction was quenched with water (30 mL). The suspension was extracted with ethyl acetate. The organic layers were combined, dried, and filtered. The filtrate was evaporated to dryness and then dissolved in dichloromethane, then Dess-Martin periodinane (1.48 g, 3.49 mmol) and $NaHCO_3$ (0.37 g, 3.49 mmol) were added. The resulting mixture was stirred at room temperature for 1 h. The mixture was concentrated and purified by column chromatography on silica gel (dichloromethane/methanol = 100/1) to give the product **6** as a white solid. ^{1}H NMR ($CDCl_3$) δ 9.73 (1H, s), 6.02 (1H, br), 5.48 (1H, d, J = 7.8 Hz), 4.36–4.25 (1H, m), 3.38–3.32 (2H, m), 2.50–2.44 (2H, m), 2.11–2.03 (1H, m), 1.88–1.76 (2H, m), 1.43 (9H, s). ESI-MS (*m*/*z*): 279 $(M + Na)^+$.

*(S,E)-Ethyl 4-(tert-butoxycarbonylamino)-5-((S)-2-oxopyrrolidin-3-yl)pent-2-enoate **7***

To a solution of triethyl phosphonoacetate (0.44 g, 1.95 mmol) in anhydrous THF, at −78 °C under an argon atmosphere, was added sodium hydride (78 mg, 1.95 mmol). The suspension was stirred for 15 min at the same temperature, and a solution of **6** (0.5 g, 1.95 mmol) in anhydrous THF was added dropwise. The reaction was gradually warmed to −50 °C and stirred for 2 h and upon completion was quenched by the addition of saturated aqueous NH_4Cl. The mixture was diluted with diethyl ether (100 mL) and the water layer was extracted with diethyl ether. The combined organic layer was dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (petroleum ether/ethyl acetate = 1/1) to afford **7** as white foam. $[\alpha]^{22}_D$ −6.7 (c 1.0 g/100 mL, $CHCl_3$) ^{1}HNMR ($CDCl_3$, 400 MHz) δ 6.86 (1H, dd, J = 15.6, 5.2 Hz), 6.47 (1H, br), 5.95 (1H, dd, J = 15.6, 1.6 Hz), 5.37 (1H, d, J = 8.4 Hz), 4.37–4.26 (1H, m), 4.19 (2H, q, J = 7.2 Hz), 3.37–3.32 (2H, m), 2.53–2.47 (2H, m), 2.05–1.98 (2H, m), 1.85–1.79 (1H, m), 1.62–1.56 (1H, m), 1.44 (9H, s), 1.28 (3H, t, J = 7.2 Hz); ESI-MS (*m*/*z*): 327 $(M + H)^+$.

(R)-2-Bromo-3-(4-fluorophenyl)propanoic acid ***9***

D-2-Amino-3-(4-fluorophenyl)propanoic acid (5.0 g, 27.3 mmol) was dispersed in 48%HBr (60 mL) and cooled to 0 °C with an ice bath. $NaNO_2$ (2.18 g, 32 mmol) was added by portion. The reaction was stirred for 1 h at 0 °C, then slowly warmed to room temperature, and stirred for another 10 h. The aqueous solution was extracted with 400 mL of EtOAc (4×100 mL), washed with 100 mL of brine and dried over Na_2SO_4. The solvent was evaporated and the crude material was purified on silica eluting with mixtures of hexane/EtOAc (2:1) to afford the product **9** (5.57 g, 83%) as colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.1 (1H, s), 7.18–7.15 (2H, m), 7.03–6.97 (2H, m), 4.34–4.31 (1H, m), 3.48–3.42 (1H, dd, *J* = 14.0 Hz, 8.4 Hz), 3.25-3.21 (1H, dd, *J* = 14.4 Hz, 7.2 Hz) ; ESI-MS (*m*/*z*): 245 $(M - H)^+$.

(R)-Methyl 2-bromo-3-(4-fluorophenyl)propanoate ***10***

Compound **9** (4.0 g) was dissolved in MeOH (30 mL), and 1 mL concentrated sulfuric acid was added. The reaction was stirred for 2 h at 70 °C. Then MeOH was evaporated, and 10 mL water was added, followed by extraction with 100 mL EtOAc, washing with 50 mL of saturated brine (2 × 25 mL) and drying over Na_2SO_4. The solvent was evaporated and the crude material was purified on silica eluting with mixtures of hexane/EtOAc (8:1) to afford the product **10** (3.93 g, 93%) as colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.20–7.16 (2H, m), 7.02–6.97 (2H, m), 4.38–4.34 (1H, m), 3.73 (3H, s), 3.46–3.40 (1H, dd, *J* = 14.0 Hz, 8.4 Hz), 3.24–3.19 (1H, dd, *J* = 14.4 Hz, 7.2 Hz). ESI-MS (*m*/*z*): 261 $(M + H)^+$.

(S)-tert-Butyl 4-(benzyloxycarbonyl)amino-5-methyl-3-oxohexanoate ***11***

Cbz-L-Valine (3.0 g, 11.9 mmol) was dissolved in 15 mL of dry THF. To this solution was added 1,1′-carbonyldiimidazole (2.13 g, 13.1 mmol) with stirring under nitrogen at room temperature. Diisopropylamine lithium (LDA, 2 M THF solution, 3.3 equiv) under nitrogen was diluted with 10 mL of THF and cooled to −78 °C. To the LDA solution was added *tert*-butyl acetate (4.87 g, 42 mmol). After 1 h the Boc-amino acid/imidazole solution was cooled to −78 °C and added to the enolate solution under nitrogen. The reaction was allowed to stir for 2 h at −78 °C, then quenched with 2 mL water and allowed to warm to room temperature. The aqueous solution was extracted with 200 mL of EtOAc (2 × 100 mL), and washed with 50 mL of brine, and dried over Na_2SO_4. The solvent was evaporated and the crude material was purified on silica eluting with mixtures of hexane/EtOAc (4:1) to afford the product **11** (2.08 g, 60%) as yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.35 (5H, s), 5.48–5.46 (1H, d, *J* = 8.4 Hz), 5.09 (2H, s), 4.46–4.42 (1H, dd, *J* = 8.8 Hz, 4.0 Hz), 3.43 (2H, s), 2.28–2.24 (1H, m), 1.44 (9H, s), 1.04–1.02 (3H, d, *J* = 6.8 Hz), 0.81–0.79 (3H, d, *J* = 6.8 Hz); ESI-MS (*m*/*z*): 348 $(M - H)^+$.

(3R)-1-tert-Butyl 4-methyl 2-((S)-2-(benzyloxycarbonylamino)-3-methylbutanoyl) -3-(4-fluorobenzyl)succinate ***12***

To a solution of **11** (2.16 g, 6.18 mmol) in DMF (10 mL) was added compound **10** (1.77 g, 6.80 mmol), K_2CO_3 (2.56 g, 18.54 mmol), potassium iodide (216 mg, 10%), and the reaction mixture was then stirred for 8 h at 80 °C until the disappearance of the starting material **10**. The reaction mixture was poured into 60 mL water. The aqueous solution was extracted with 100 mL of EtOAc (2 × 50 mL) and washed with 100 mL of saturated brine (2 × 50 mL) and dried over Na_2SO_4. The solvent was evaporated and the crude material was purified on silica eluting with mixtures of hexane/EtOAc (4:1) to afford the product **12** (1.96 g, 60%) as a white solid. $[\alpha]^{22}_D$ +6.6 (c 1.0 g/100 mL, $CHCl_3$) ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.39–7.33 (7H, m), 7.03–6.92 (2H, m), 5.50–5.48 (1H, d, *J* = 8.4 Hz), 5.14 (2H, s), 4.54–4.52 (1H, m), 4.14–4.10 (1H, m), 3.74 (3H, s), 3.68–3.66 (1H, m), 3.65–3.63 (1H, m), 3.16–3.08 (1H, m), 2.26–2.22 (1H, m), 1.42 (9H, s), 0.98–0.96 (3H, d, *J* = 6.8 Hz), 0.88–0.86 (3H, d, *J* = 6.8 Hz); ESI-MS (*m*/*z*): 552 $(M + Na)^+$.

(2R,5S)-Methyl 5-(benzyloxycarbonylamino)-2-(4-fluorobenzyl)-6-methyl-4-oxo heptanoate ***13***

To a mixture solution (TFA:CH_2Cl_2=3:1, 4 mL) was added compound **12** (430 mg, 0.81 mmol), and the reaction was stirred for 4 h at 30 °C. Then the solvent was evaporated, and 10 mL water was added, followed by extraction with 50 mL EtOAc, washing with 50 mL of saturated brine (2 × 25 mL) and drying over Na_2SO_4. The solvent was evaporated and the crude material was purified on silica, eluting with mixtures of hexane/EtOAc (2:1) to afford the product **13** (262 mg, 75%). $[\alpha]^{22}_D$ +13.6 (c 0.15 g/100 mL, $CHCl_3$) ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.38–7.32 (6H, m), 7.24–7.21 (1H, m), 7.03–6.96 (2H, m), 5.42–5.40 (1H, d, *J* =8.4Hz), 5.09 (2H, s), 4.32–4.25 (1H, m), 3.65 (3H, s), 3.63–3.61 (1H, m), 3.58–3.56 (1H, m), 2.48–2.40 (2H, m), 2.36–2.30 (1H, m), 2.26–2.23 (1H, m), 0.98–0.96 (3H, d, *J* = 6.8 Hz), 0.74–0.72 (3H, d, *J* = 6.8 Hz); ESI-MS (*m*/*z*): 452 $(M + Na)^+$.

(2R,5S)-Methyl 5-(tert-butoxycarbonylamino)-2-(4-fluorobenzyl)-6-methyl-4- oxoheptanoate ***14***

Pd/C (17 mg, 5%) was added to a solution of compound **13** (340 mg, 0.79 mmol) in MeOH (20 mL) at 25 °C. The mixture was stirred overnight and filtered. $(Boc)_2O$ (151 mg, 0.87 mmol) and Et_3N (0.13 mL) were added to the filtrate. The reaction was stirred for another 2 h. Then the solvent was evaporated, and 10 mL water was added, followed by extraction with 50 mL EtOAc, washing with 50 mL of saturated brine (2 × 25 mL) and drying over Na_2SO_4. The solvent was evaporated and the crude material was purified on silica eluting with mixtures of hexane/EtOAc (2:1) to afford the product **14** (244 mg, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.20–7.16 (2H, m), 7.02–6.97 (2H, m), 5.46–5.44 (1H, m), 4.33–4.27 (1H, m), 3.67 (3H, s), 3.61–3.59 (1H, m), 3.57–

3.54 (1H, m), 2.46–2.41 (2H, m), 2.35–2.30 (1H, m), 2.25–2.23 (1H, m), 1.40 (9H, s), 1.01–0.99 (3H, d, J = 6.8 Hz), 0.78–0.76 (3H, d, J = 6.8 Hz); ESI-MS (m/z): 418 $(M + H)^+$.

(2R,5S)-5-(tert-Butoxycarbonylamino)-2-(4-fluorobenzyl)-6-methyl-4-oxoheptanic acid ***15***

10% NaOH/H_2O (5 mL) was added to a solution of compound **14** (240 mg, 0.61 mmol) in ethanol (5 mL). The reaction was stirred for 1 h at room temperature. Then 2M HCl was added to the reaction solution until pH 1. Then the solution was extracted with 100 mL of EtOAc (2 × 100 mL) and washed with 50 mL of brine and dried over Na_2SO_4. The solvent was evaporated and the crude material was purified on silica, eluting with mixtures of hexane/EtOAc (2:1) to afford the product **15** (232 mg, 90%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.18–7.15 (2H, m), 7.03–6.98 (2H, m), 5.43–5.41 (1H, m), 4.29–4.24 (1H, m), 3.60–3.57 (1H, m), 3.55–3.53 (1H, m), 2.49–2.43 (2H, m), 2.33–2.20 (2H, m), 1.41 (9H, s), 0.98–0.96 (3H, d, J = 6.8 Hz), 0.76–0.74 (3H, d, J = 6.8 Hz); ESI-MS (m/z): 380 $(M - H)^+$.

(S,E)-Ethyl 4-((2R,5S)-5-(tert-butoxycarbonylamino)-2-(4-fluorobenzyl)-6-methyl-4-oxoheptanamido)-5-((S)-2-oxopyrrolidin-3-yl)pent-2-enoate ***16***

Compound **15** (260 mg, 0.68 mmol) was dissolved in 10 mL of dry CH_2Cl_2. To this solution was added 1.1 equiv of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), and the mixture was stirred for 0.5 h at room temperature. Then compound **8** (195 mg, 0.68 mmol) and Et_3N (0.20 mL, 1.36 mmol) were added to the reaction. The reaction was stirred for another 6 h. The reaction mixture was poured into 10 mL water. The aqueous solution was extracted with 50 mL of CH_2Cl_2 (2 × 25 mL) and washed with 50 mL of saturated brine (2 × 25 mL) and dried over Na_2SO_4. The solvent was evaporated and the crude material was purified on silica, eluting with a mixture of CH_2Cl_2/MeOH (80:1) to afford the product **16** (252 mg, 63%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.36–7.34 (1H, d, J = 8.7 Hz), 7.08–7.06 (2H, m), 6.93–6.91 (2H, m), 6.61–6.58 (1H, dd, J = 11.7, 5.2 Hz), 6.06 (1H, m), 5.40–5.37 (1H, dd, J = 11.6, 1.4 Hz), 4.62–4.60 (1H, m), 4.51–4.38 (1H, m), 4.16–4.13 (2H, q, J = 6.8 Hz), 3.30–3.24 (2H, m), 3.11–3.04 (1H, m), 3.00–2.95 (1H, m), 2.88–2.81 (1H, m), 2.72–2.57 (3H, m), 2.19–2.02 (3H, m), 1.87–1.80 (1H, m), 1.78–1.62 (1H, m), 1.51–1.44 (1H, m), 1.42 (9H, s), 1.23–1.20 (3H, m), 0.90–0.88 (3H, d, J = 6.8 Hz), 0.79–0.77 (3H, d, J = 6.8 Hz); ESI-MS (m/z): 590 $(M + H)^+$.

(S,E)-Ethyl 4-((2R,5S)-2-(4-fluorobenzyl)-6-methyl-5-(5-methylisoxazole-3-carboxamido)-4-oxoheptanamido)-5-((S)-2-oxopyrrolidin-3-yl)pent-2-enoate AG7088

The compound **16** (70 mg, 0.12 mmol) was dissolved in 6 mL of dry CH_2Cl_2. To this solution was added 2 mL of TFA, and the mixture was stirred for 2 h at room temperature. Then the reaction mixture was evaporated in vacuum. The crude intermediate was resolved in 5 mL CH_2Cl_2, and 5-methylisoxazole-3-carbonyl chloride (20 mg, 0.14 mmol) and Et_3N (0.04 mL, 0.18 mmol) were added. The mixture was stirred for another 10 h at room temperature. The reaction mixture was diluted with 20 mL CH_2Cl_2, washed with 50 mL of saturated brine (2 × 25 mL), and dried over $NaSO_4$. The solvent was evaporated and the crude material was purified on silica eluting with mixtures of CH_2Cl_2/MeOH (40:1) to afford the product AG7088 (49 mg, 71%). $[\alpha]^{24}_D$ +31.7 (c 0.2 g/100 mL, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.35–7.33 (1H, d, J = 8.7 Hz), 7.10–7.06 (2H, m), 6.94–6.92 (2H, m), 6.60–6.57 (1H, dd, J = 11.7, 5.2 Hz), 6.47 (1H, s), 6.08 (1H, m), 5.43–5.40 (1H, dd, J = 11.6, 1.4 Hz), 4.65–4.63 (1H, dd, J = 8.7, 4.4 Hz), 4.48–4.39 (1H, m), 4.17–4.14 (2H, q, J = 6.8 Hz), 3.32–3.19 (2H, m), 3.08–3.00 (1H, m), 2.96–2.91 (1H, m), 2.89–2.80 (1H, m), 2.73–2.54 (3H, m), 2.51 (3H, s), 2.26–2.02 (3H, m), 1.87–1.74 (1H, m), 1.73–1.62 (1H, m), 1.49–1.41 (1H, m), 1.28–1.23 (3H, m), 0.94–0.92 (3H, d, J = 6.8 Hz), 0.78–0.76 (3H, d, J = 6.8 Hz). ^{13}CNMR ($CDCl_3$, 400 MHz): δ 206.7, 175.2, 173.1, 166.8, 163.4, 162.1, 160.0, 158.1, 148.2, 135.4, 131.6, 122.5, 115.7, 113.6, 102.1, 64.3, 61.5, 50.2, 44.7, 42.5, 41.2, 39.1, 35.3, 31.6, 29.4, 20.1, 18.2, 14.8, 12.6. ESI-MS (m/z): 599 $(M + H)^+$.

We gratefully acknowledge financial support from the National Natural Science Foundation of China (20872153, 21021063, 20720102040 and 81025017), the National Basic Research Program of China grant (2009CB918502), the Chinese Academy of Sciences (XDA01040305), and the SILVER project of the European Commission (contract HEALTH-F3-2010-260644). HR is supported by a Chinese Academy of Sciences Visiting Professorship for Senior International Scientists (2010T1S6).

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