A Simple Synthesis of 4-Substituted 2-(3-Hydroxy-2-oxo-1-phenethylpropylcarbamoyl)pyrrolidine-1-carboxylic Acid Benzyl Esters as Novel Cysteine Protease Inhibitors

Seikwan Oh[a], Jae-Chul Jung[b], and Mitchell A. Avery[c]

[a] Department of Neuroscience and Medical Research Institute, School of Medicine, Ewha Womans University, Seoul 158-710, South Korea
[b] Ewha Global Challenge, BK21 and Medical Research Institute, School of Medicine, Ewha Womans University, Seoul 158-710, South Korea
[c] Department of Medicinal Chemistry, School of Pharmacy, National Center for Natural Products Research & Department of Chemistry, University of Mississippi, University, MS 38677-1848, USA

Reprint requests to Dr. Jae-Chul Jung. Fax: +82-02-2650-5791. E-mail: jcjung@rexgenebio.co.kr or jcjung10@yahoo.co.kr

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A convenient synthesis of 4-substituted 2-(3-hydroxy-2-oxo-1-phenethylpropylcarbamoyl)pyrrolidine-1-carboxylic acid benzyl esters **17** and **18** as new cysteine protease inhibitors is described. The synthetic key strategies involve the diazocarbonyl insertion reaction of *N*-Boc-L-homophenylalanine (**1**) by diazomethane, acetylation of the bromoketone **2** with sodium acetate, and condensation of acids **12**, **14** with (3*S*)-3-amino-2-oxo-5-phenyl-pentyl acetate monohydrochloride (**4**) in good yield.

Key words: Pyrrolidine-1-carboxylic Acid Benzyl Esters, Diazocarbonyl Insertion Reaction, Acetylation, Coupling Reaction, Cysteine Protease Inhibitors

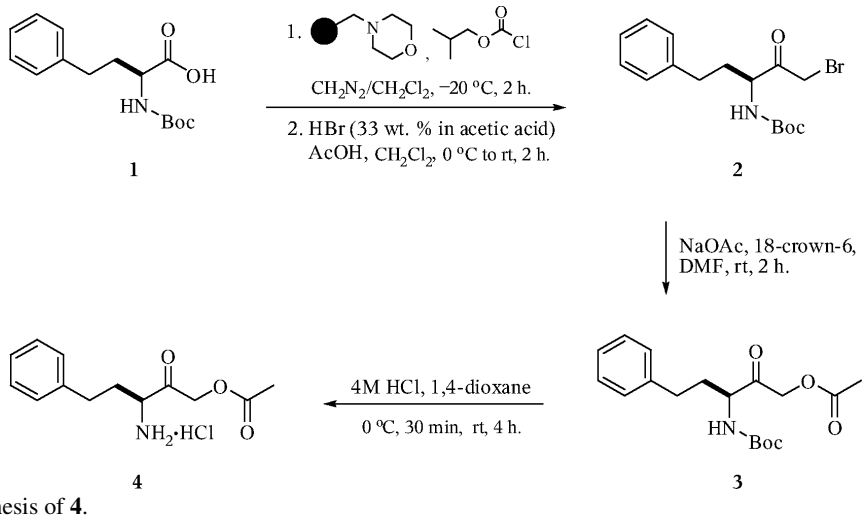
Introduction

Cysteine proteases have become a significant class of drugs for the chemotherapy [1], many of which possess widespread pharmacological properties and are related with various therapeutic fields such as oncology [2], osteoporosis [3], arthritis [4], bacterial [5], virus [6], cardiovascular [7], and malaria [8]. Cysteine proteases of malaria parasites offer potential new chemotherapeutic targets. Also, cysteine protease inhibitors blocked hemoglobin hydrolysis of parasites and their inhibitory effects against parasites generally correlated with the inhibition of falcipain-2 [9]. Some compounds also cured mice infected with otherwise lethal malaria infections. Current research priorities are to better characterize the biological roles and biochemical features of the falcipains. In addition, efforts to identify optimal falcipain inhibitors as antimalarials are underway. Recently, the Micale group [10] reported novel peptidomimetic cysteine protease inhibitors having significant inhibitory activity against falcipains 2A and 2B. The Mauger group [11] developed the synthesis of reversible and irreversible cysteine protease inhibitors using polymer supported methods. The McKerrow group [12] demonstrated that the irreversible inhibitors of cruzain can cure parasitic infections *in vitro*. Finally, Ellman *et al*. [13] described the crystal structure of ketone-based inhibitors of the cysteine protease cruzain.

As a part of our medicinal chemistry program dealing with the development of new antimalarial derivatives, we required (3*S*)-3-amino-2-oxo-5-phenyl-pentyl acetate monohydrochloride (**4**) as an important fragment in order to generate novel cysteine protease inhibitors. We would like to report herein an efficient synthesis of 4-substituted 2-(3-hydroxy-2-oxo-1-phenethylpropylcarbamoyl)-pyrrolidine-1-carboxylic acid benzyl esters **17** and **18**, starting from *N*-Boc-L-homophenylalanine (**1**).

Results and Discussion

The synthesis of (3*S*)-3-amino-2-oxo-5-phenyl-pentyl acetate monohydrochloride (**4**) as a key intermediate is outlined in Scheme 1. We have used commercially available *N*-Boc-L-homophenylalanine (**1**) which was treated with freshly prepared diazomethane [14] in the presence of *N*-methylmorpholine-polystyrene and isobutyl chloroformate in quantitative yield. The diazo compound was readily subjected to



Scheme 1. Synthesis of **4**.

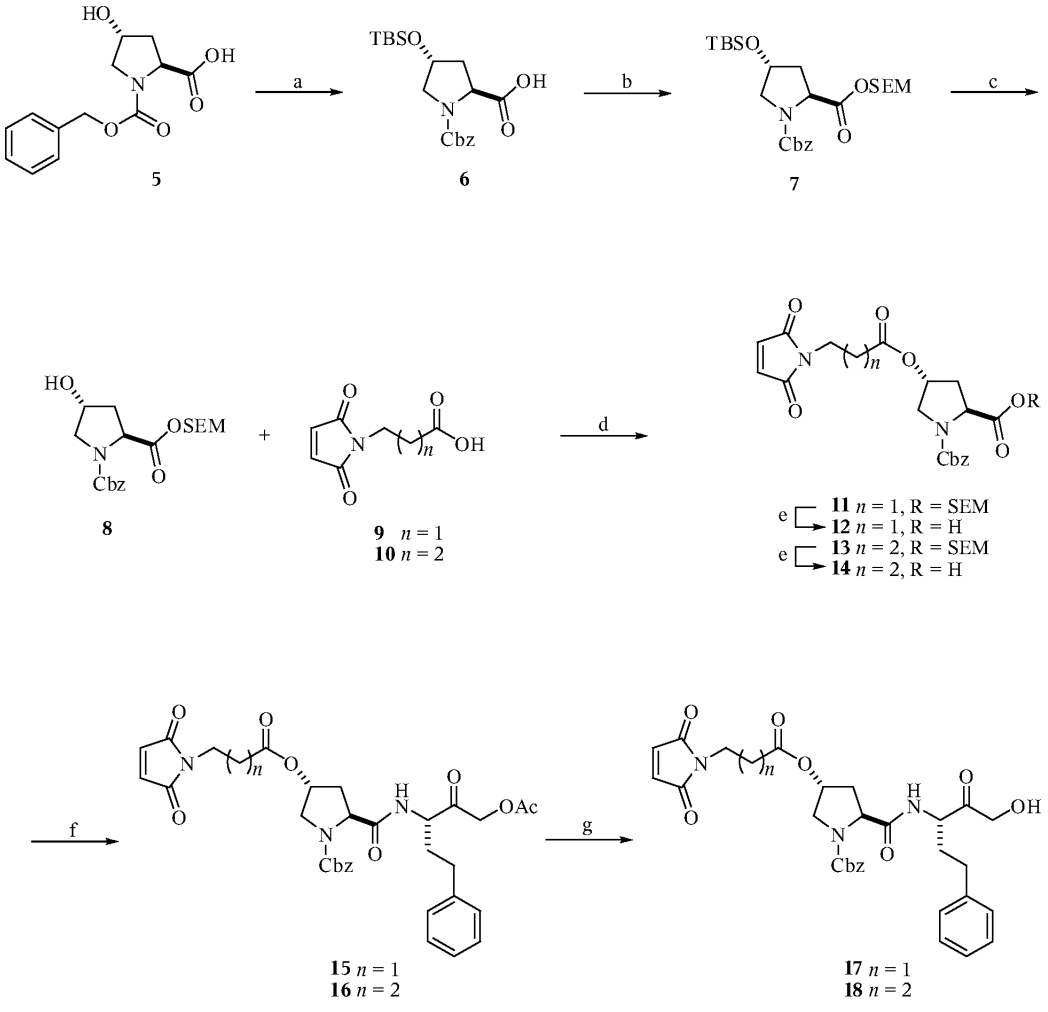
a substitution reaction using hydrogen bromide solution (33 wt.-% in acetic acid) in dichloromethane to obtain bromoketone **2** in 90 % yield [11]. Subsequent acetoxylation of **2** was accomplished by treatment with NaOAc and 18-crown-6 as a phase transfer catalyst in dry DMF and gave **3** in convenient handling and with high yield [15]. Removal of the *tert*-butyloxycarbonyl (Boc) protecting group from acetate **3** was achieved with 4 M HCl in 1,4-dioxane to generate **4** in good yield.

To generate the 4-substituted 2-(3-hydroxy-2-oxo-1-phenethylpropylcarbamoyl)pyrrolidine-1-carboxylic acid benzyl esters **17** and **18**, (2*S*,4*R*)-4-(hydroxy)-1-(benzyloxycarbonyl)pyrrolidine-2-(2-trimethylsilylethoxymethoxy) ester (**8**) was prepared from (2*S*, 4*R*)-4-(hydroxy)-1-(benzyloxycarbonyl)pyrrolidine-2-carboxylic acid (**5**) [16]. Acid **5** was protected with *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) and 2,6-lutidine to yield the fully protected compound, followed by hydrolysis with AcOH in THF/H_2O (8 : 2, v/v) to give (2*S*, 4*R*)-4-(*tert*-butyldimethylsilyloxy)-1-(benzyloxycarbonyl)pyrrolidine-2-carboxylic acid (**6**) in 99 % yield over two steps, which was subsequently treated with 2-(trimethylsilyl)ethoxymethyl chloride (SEM-Cl) in the presence of diisopropylethylamine (DIPEA) to give the SEM ester, which was treated with tetrabutylammonium fluoride (TBAF) to afford the secondary alcohol **8** in 92 % yield over two steps. Compound **8** was then coupled with acids **9** [17] or **10** [18] in the presence of bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) in dichloromethane to generate the SEM esters **11** and **13** in 95 % and 92 % yield, respectively [19], which were readily hydrolyzed by magnesium bromide, nitromethane, and 1-butanethiol in ether to give acids **12** and **14** in 72 % and 66 % yield, respectively. Acids **12** and **14** were then coupled with **4** using ethyl(dimethylaminopropyl)carbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBt) to give amides **15** and **16** in 85 % and 81 % yield, respectively [20]. On the other hand, (3*S*)-3-amino-2-oxo-5-phenyl-pentyl acetate monohydrochloride (**4**) was also coupled to acids **12** and **14** in the presence of 2-(7-aza-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and DIPEA in dichloromethane to give amides **15** and **16** in 80 % and 78 % yield, respectively, which were subsequently treated with potassium carbonate in MeOH/CH_2Cl_2/H_2O (8 : 1 : 1, v/v) to afford esters **17** and **18** in 81 % and 80 % yield, respectively (Scheme 2).

The *in vitro* antimalarial activity of 4-substituted 2-(3-hydroxy-2-oxo-1-phenethylpropylcarbamoyl)-pyrrolidine-1-carboxylic acid benzyl esters **17** and **18** was evaluated in their inhibition of the plasmodium falciparium cystein protease falcipain. These compounds exhibited good efficacy (IC_{50}: 86.2 μM for **17**, 106.5 μM for **18**) comparable to that of artemisinin in their *in vitro* antimalarial activity [21].

Conclusion

In conclusion, an efficient preparation of the 4-substituted 2-(3-hydroxy-2-oxo-1-phenethylpropylcarbamoyl)pyrrolidine-1-carboxylic acid benzyl esters



Scheme 2. (a) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 1 h; then AcOH, THF/H_2O (8 : 2, v/v), 0 °C, 1 h (99 %); (b) SEM-Cl, DIPEA, CH_2Cl_2, 0 °C to r. t., 1 h (95 %); (c) TBAF, THF, 0 °C, 1 h (97 %); (d) BOP-Cl, TEA, CH_2Cl_2, r. t., 24 h (95 % for **11**, 92 % for **13**); (e) $MgBr_2$, $MeNO_2$, *n*-BuSH, CH_2Cl_2, r. t., 1 h (72 % for **12**, 66 % for **14**); (f) **4**, HOBt, EDCI, TEA, CH_2Cl_2, r. t., 16 h (85 % for **15**, 81 % for **16**); or HATU, DIPEA, CH_2Cl_2, r. t., 16 h (80 % for **15**, 78 % for **16**); (g) $K_2CO_3 \cdot 3/2H_2O$, MeOH/CH_2Cl_2/H_2O (8 : 1 : 1, v/v), −10 °C to 10 °C, 30 min (81 % for **17**, 80 % for **18**).

17 and **18** has been described. The key fragments were **4**, prepared from *N*-Boc-L-homophenylalanine, and **8**. We found that compounds **17** and **18** exhibit high efficacy, comparable to artemisinin, in their *in vitro* antimalarial activity. We expect that the simple syntheses of the new carboxamides **17** and **18** and their key fragments are useful for the modification of cysteine protease inhibitors.

Experimental Section

Reactions requiring anhydrous conditions were performed with the usual precautions for rigorous exclusion of air and moisture. Tetrahydrofuran was distilled from sodium benzophenone ketyl prior to use. Thin layer chromatography (TLC) was performed on precoated silica gel G and GP uniplates from Analtech and visualized with 254 nm UV light. Flash chromatography was carried out on silica gel 60 [Scientific Adsorbents Incorporated (SAI), particle size 32 – 63 μm, pore size 60 Å]. ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX 500 at 500 and 125 MHz, respectively. The chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane, and *J* values are in Hz. Infrared (IR) spectra were obtained on an ATI Mattson FT/IR spectrometer. Mass spectra were recorded with a Waters Micromass ZQ LC-Mass system, and high-reso-

lution mass spectra (HRMS) were measured with a Bruker BioApex FTMS system by direct injection using an electrospray interface (ESI). When necessary, chemicals were purified according to the reported procedures [22].

*(3S)-3-(tert-Butoxycarbonyl)amino-2-oxo-5-phenyl-pentyl acetate (**3**)*

To a stirred solution of **2** (1.3 g, 3.6 mmol) in dry DMF (5 mL) were added NaOAc (0.6 g, 7.2 mmol) and 18-crown-6 (0.04 g, 0.14 mmol), and the mixture was stirred at r. t. for 2 h. The reaction mixture was filtered, diluted with dichloromethane (20 mL) and washed with saturated aqueous NH_4Cl solution (8 mL) and brine (8 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 15 % ethyl acetate in *n*-hexane) to give **3** (1.0 g, 82 %) as a white solid. R_f = 0.3 (12 % ethyl acetate/*n*-hexane). – M. p. 84 – 87 °C. – $[\alpha]_D^{23} = +5.1$ (c = 1.0, $CHCl_3$). – IR (neat, NaCl): ν = 3356 (NH), 2978, 2933, 1738 (CO), 1710 (CO), 1498, 1455, 1234, 1052, 865 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 7.35 – 7.16 (m, 5H, Ar-H), 5.12 (br s, 1H, NH), 4.83 (d, J = 16.0, 1H, CH_2), 4.77 (d, J = 16.0, 1H, CH_2), 4.45 – 4.37 (m, 1H, CH), 2.71 (t, J = 7.5, 2H, CH_2), 2.15 (s, 3H, CH_3), 2.23 – 2.19 (m, 1H, CH_2), 1.91 – 1.82 (m, 1H, CH_2), 1.48 (s, 9H, Me_3). – ^{13}C NMR ($CDCl_3$, 125.76 MHz): δ = 202.7 (CO), 169.9 (COO), 155.2 (CONH), 140.4, 128.5, 128.4, 126.2, 80.4 (C), 66.7 (CH_2), 56.4 (CH), 33.4 (CH_2), 31.7 (CH_2), 28.6 (Me_3), 20.8 (CH_3). – HRMS: m/z = 358.1620 (calcd. 358.1630 for $C_{18}H_{25}NO_5Na$, $[M+Na]^+$).

*(3S)-3-Amino-2-oxo-5-phenyl-pentyl acetate monohydrochloride (**4**)*

To a stirred solution of acetate **3** (0.38 g, 1.1 mmol) in dry 1,4-dioxane (5 mL) was added dropwise hydrochloric acid (2.3 mL, 8.8 mmol, 4 M in 1,4-dioxane sol) at 0 °C, and the mixture was stirred at r. t. for 4 h. The reaction mixture was evaporated *in vacuo*, and the residue was treated with ether (10 mL). The solid was filtered and dried under reduced pressure to give **4** (0.27 g, 85 %) as a beige solid. R_f = 0.3 (12 % ethyl acetate/*n*-hexane). – M. p. 130 – 131 °C. – IR (neat, NaCl): ν = 3300 – 2500 (NH_2), 1740 (CO), 1684 (CO), 1454, 1228, 1033, 702 cm^{-1}. – ^1H NMR ([D_6]-DMSO, 500.14 MHz): δ = 8.77 (br s, 3H, NH_2), 7.35 – 7.18 (m, 5H, Ar-H), 5.12 (d, J = 22.0, 1H, CH_2), 4.92 (d, J = 22.0, 1H, CH_2), 4.30 (t, J = 5.0, 1H, CH), 2.80 – 2.62 (m, 2H, CH_2), 2.28 – 2.17 (m, 1H, CH_2), 2.11 (s, 3H, CH_3), 2.12 – 2.01 (m, 1H, CH_2). – ^{13}C NMR ([D_6]-DMSO, 125.76 MHz): δ = 200.4 (CO), 169.9 (COO), 140.8, 128.8, 128.7, 126.6, 66.9 (CH_2), 55.9 (CH), 32.1 (CH_2), 30.8 (CH_2), 21.0 (CH_3). – HRMS: m/z = 272.1065 (calcd. 272.1053 for $C_{13}H_{19}ClNO_3$, $[M+H]^+$).

*(2S,4R)-4-(tert-Butyldimethylsilyloxy)-1-(benzyloxycarbonyl)pyrrolidine-2-carboxylic acid (**6**)*

To a stirred solution of **5** (0.53 g, 2.0 mmol) in anhydrous dichloromethane (10 mL) was added 2,6-lutidine (0.7 g, 6.6 mmol), followed by TBSOTf (1.6 g, 6.0 mmol) at 0 °C, and the mixture was stirred at the same temperature for 1 h, diluted with dichloromethane (5 mL) and washed with saturated aqueous NH_4Cl solution (10 mL). The organic phase was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was treated with AcOH (1.2 mL) in THF/H_2O (12 mL, 8 : 2, v/v) at 0 °C and the mixture was stirred at that temperature for 1 h. The resulting mixture was evaporated *in vacuo*, and the residue was treated with dichloromethane (10 mL). The organic solution was washed with saturated aqueous NH_4Cl solution (10 mL) and brine (10 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 5 % methanol in dichloromethane) to give **6** (0.75 g, 99 %) as a colorless oil. R_f = 0.6 (5 % MeOH/CH_2Cl_2). – $[\alpha]_D^{23} = -36.5$ (c = 0.8, $CHCl_3$). – IR (neat, NaCl): ν = 3417 (OH), 3034, 2955, 2867, 1713 (CO), 1423, 1359, 1255, 1120, 1022, 837 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): Mixture of two rotamers. δ = 7.41 – 7.27 (m, 5H, Ar-H), 7.14 (br s, 1H, CO_2H), 5.26 – 5.13 (m, 2H, CH_2), 4.57 – 4.42 (m, 2H, 2 × CH), 3.71 – 3.61 (m, 1H, CH_2), 3.57 – 3.42 (m, 1H, CH_2), 2.31 – 2.19 (m, 1H, CH_2), 2.18 – 2.07 (m, 1H, CH_2), 0.89 (s, 1/2 × 9H, $SiCMe_3$), 0.88 (s, 1/2 × 9H, $SiCMe_3$), 0.12 (s, 3H, Si-Me), 0.08 (s, 3H, Si-Me). – ^{13}C NMR ($CDCl_3$, 125.67 MHz): Mixture of two rotamers. δ = 177.4, 176.5, 175.9, 155.9, 154.4, 136.3, 136.1, 128.4, 128.3, 128.0, 127.8, 127.7, 127.5, 70.4, 69.9, 67.8, 67.4, 58.5, 57.9, 55.4, 55.0, 40.2, 38.7, 18.4, 18.3, −3.2, −4.3, −4.4, −4.5. – HRMS: m/z = 380.1888 (calcd. 380.1893 for $C_{19}H_{30}NO_5SiNa$, $[M+Na]^+$).

*(2S,4R)-4-(Hydroxy)-1-(benzyloxycarbonyl)pyrrolidine-2-(2-trimethylsilylethoxymethoxy) ester (**8**)*

To a stirred solution of **6** (0.38 g, 1.0 mmol) and DIPEA (0.14 g, 1.1 mmol) in anhydrous dichloromethane (10 mL) was added dropwise SEM-Cl (0.18 g, 1.1 mmol) at 0 °C, and the mixture was stirred at the same temperature for 1 h, diluted with dichloromethane (5 mL) and washed with saturated aqueous NH_4Cl solution (10 mL). The organic phase was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was treated with TBAF (2.0 g, 2.0 mmol) in anhydrous THF (10 mL) at 0 °C, and the mixture was stirred at that temperature for 1 h, warmed to r. t. and diluted with dichloromethane (10 mL). The solution was washed with saturated aqueous NH_4Cl solution (12 mL) and brine (12 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue

was purified by flash column chromatography (silica gel, *n*-hexane/ethyl acetate/methanol = 75 : 20 : 5, v/v) to give **8** (0.36 g, 92 %, two steps yield) as a colorless oil. – R_f = 0.4 (5 % MeOH/CH_2Cl_2). – $[\alpha]_D^{23}$ = −46.9 (*c* = 0.2, $CHCl_3$). – IR (neat, NaCl): ν = 3315 (OH), 2985, 1718 (CO), 1455, 1289, 1168, 1071, 836 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): Mixture of two rotamers. δ = 7.39 – 7.31 (m, 5H, Ar-H), 6.12 (s, 2H, CH_2), 5.05 (d, *J* = 7.2 Hz, 2H, CH_2), 4.18 (d, *J* = 6.5 Hz, 1H, CH), 3.60 (d, *J* = 7.0 Hz, 2H, CH_2), 3.45 – 3.18 (m, 4H), 2.21 – 1.89 (m, 2H), 0.88 – 0.79 (m, 2H, CH_2), 0.04 (s, 1/2 × 9H, $SiMe_3$), 0.03 (s, 1/2 × 9H, $SiMe_3$). – HRMS: *m/z* = 396.1833 (calcd. 396.1842 for $C_{19}H_{30}NO_6Si$, $[M+H]^+$).

*General procedure for the preparation of compounds **11** and **13** via coupling reaction of secondary alcohol **8** and acids **9** or **10***

To a solution of alcohol **8** (0.59 g, 1.5 mmol) in dichloromethane (10 mL) were added TEA (0.61 g, 6.0 mmol) and BOP-Cl (0.76 g, 3.0 mmol), followed by acids **9** or **10** (0.51 g for **9**; 0.55 g for **10**, 3.0 mmol) at 10 °C. The reaction mixture was stirred at r. t. for 24 h, diluted with dichloromethane (10 mL) and washed with saturated aqueous NH_4Cl solution (10 mL) and brine (10 mL). The organic phase was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, ethyl acetate/*n*-hexane/methanol = 15 : 80 : 5, v/v) to afford SEM esters **11** and **13** as viscous oils, respectively.

*(2S,4R)-4-[3-(2,5-Dioxo-2,5-dihydropyrrol-1-yl)propionyloxy]-1-(benzyloxycarbonyl)pyrrolidine-2-(2-trimethylsilylethoxymethoxy) ester (**11**)*

Yield: 95 %. R_f = 0.4 (33 % ethyl acetate/*n*-hexane). – $[\alpha]_D^{24}$ = −23.2 (*c* = 0.2, $CHCl_3$). – IR (neat, NaCl)): ν = 3031, 2978, 1722 (CO), 1701 (COO), 1412, 1165, 1080, 836 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 7.41 – 7.19 (m, 5H, Ar-H), 6.89 (s, 2H, HC=CH), 5.35 (d, *J* = 6.5 Hz, 2H, CH_2), 4.24 – 4.06 (m, 2H), 3.91 – 3.69 (m, 3H), 3.55 – 3.37 (m, 3H), 2.52 (d, *J* = 7.0 Hz, 2H, CH_2), 2.31 – 2.17 (m, 1H, CH_2), 2.15 – 2.06 (m, 1H, CH_2), 0.81 (t, *J* = 7.5 Hz, 2H, CH_2), 0.02 (s, 9H, CH_3). – HRMS: *m/z* = 547.2128 (calcd. 547.2112 for $C_{26}H_{35}N_2O_9Si$, $[M+H]^+$).

*(2S,4R)-4-[3-(2,5-Dioxo-2,5-dihydropyrrol-1-yl)butyryloxy]-1-(benzyloxycarbonyl)pyrrolidine-2-(2-trimethylsilylethoxymethoxy) ester (**13**)*

Yield: 92 %. R_f = 0.5 (*n*-hexane/ethyl acetate/methanol = 80 : 15 : 5; v/v). – $[\alpha]_D^{24}$ = −15.8 (*c* = 0.1, $CHCl_3$). – IR (neat, NaCl): ν = 3034, 2965, 1730 (CO), 1705 (COO), 1538, 1248, 1082, 835 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 7.40 – 7.16 (m, 5H, Ar-H), 6.85 (s, 2H, HC=CH), 5.35 (d, *J* = 7.0 Hz, 2H, CH_2), 4.31 – 4.10 (m, 2H), 3.88 – 3.64 (m, 2H), 3.43 – 3.25 (m, 6H), 2.45 – 2.06 (m, 6H, CH_2), 0.80 (t, *J* = 7.0 Hz, 2H, CH_2), 0.01 (s, 9H, CH_3). – HRMS: *m/z* = 561.2283 (calcd. 561.2268 for $C_{27}H_{37}N_2O_9Si$, $[M+H]^+$).

*General procedure for the preparation of compounds **15** and **16** via hydrolysis and coupling reaction of amides **11** and **13***

To a stirred solution of $MgBr_2$ (1.05 g, 5.70 mmol) and $MeNO_2$ (0.35 g, 5.70 mmol) in ether/dichloromethane (15 mL/35 mL) was added **11** or **13** (1.0 g for **11**; 1.05 g for **13**, 1.90 mmol) in ether/dichloromethane (15 mL/15 mL), and the mixture was stirred at r. t. for 1 h. The mixture was diluted with dichloromethane (40 mL) and quenched with water (30 mL) and then washed with saturated aqueous NH_4Cl solution (60 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give **12** (0.55 g, 72 %) or **14** (0.50 g, 66 %). Acids **12** or **14** were used *in situ* for the coupling reaction. To a stirred suspension of acids **12** or **14** (0.46 g for **12**; 0.47 g for **14**, 1.1 mmol) in dry dichloromethane (75 mL) were added HATU (2.5 g, 6.6 mmol), diisopropylamine (0.8 g, 6.0 mmol) and **4** (1.6 g, 5.8 mmol) at 5 °C. The mixture was stirred at r. t. for 16 h, diluted with dichloromethane (50 mL) and washed with saturated aqueous NH_4Cl solution (50 mL) and brine (60 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, ethyl acetate/*n*-hexane/methanol = 20 : 75 : 5, v/v) to give pure **15** or **16** as viscous oils.

*(1S,2S,4R)-2-(3-Acetoxy-2-oxo-1-phenethylpropylcarbamoyl)-4-[3-(2,5-dioxo-2,5-dihydropyrrol-1-yl)propionyloxy] pyrrolidine-1-carboxylic acid benzyl ester (**15**)*

Yield: 80 %. R_f = 0.3 (ethyl acetate/*n*-hexane/methanol = 20 : 75 : 5, v/v). – $[\alpha]_D^{24}$ = −10.2 (*c* = 0.1, $CHCl_3$). – IR (neat, NaCl): ν = 3379 (NH), 2935, 1736 (CO), 1708 (COO), 1527, 1414, 1231, 1125, 1068, 828 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 7.38 – 7.22 (m, 8H, Ar-H), 7.21 – 7.12 (m, 2H, Ar-H), 7.10 (br s, 1H, NH), 6.66 (s, 2H, HC=CH), 5.27 (s, 1H, CH), 5.17 (s, 2H, CH_2Ph), 4.77 (s, 1H, CH), 4.64 – 4.56 (m, 1H, CH_2), 4.49 – 4.01 (m, 1H, CH_2), 3.78 (t, *J* = 6.5 Hz, 2H, NCH_2), 3.73 – 3.64 (m, 2H, CH_2), 2.80 (s, 2H, CH_2), 2.68 – 2.56 (m, 4H), 2.49 – 2.37 (m, 1H), 2.28 – 2.16 (m, 2H), 2.11 (s, 3H, CH_3). – ^{13}C NMR ([D_6]-DMSO, 125.76 MHz): δ = 201.8 (CO), 171.5 (COO), 170.0, 169.8, 155.2, 141.3, 135.7, 134.1, 128.4, 128.2, 128.1, 127.9, 126.2, 73.5, 67.8, 66.7, 59.1, 55.5, 52.6, 38.9, 34.6, 33.8, 32.9, 32.7, 31.6, 20.7 (CH_3). – HRMS: *m/z* = 634.2418 (calcd. 634.2401 for $C_{33}H_{36}N_3O_{10}$, $[M+H]^+$).

*(1S,2S,4R)-2-(3-Acetoxy-2-oxo-1-phenethylpropylcarbamoyl)-4-[3-(2,5-dioxo-2,5-dihydropyrrol-1-yl)butyryloxy] pyrrolidine-1-carboxylic acid benzyl ester (**16**)*

Yield: 78 %. R_f = 0.3 (ethyl acetate/*n*-hexane/methanol = 20 : 75 : 5, v/v). – $[\alpha]_D^{24}$ = −31.4 (*c* = 0.14, $CHCl_3$). – IR

(neat, NaCl): ν = 3318 (NH), 3029, 2939, 1722 (CO), 1710 (COO), 1530, 1412, 1358, 1141, 1064, 830 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 7.37 – 7.21 (m, 8H, Ar-H), 7.20 – 7.13 (m, 2H, Ar-H), 7.01 (br s, 1H, NH), 6.67 (s, 2H, HC=CH), 5.28 (s, 1H, CH), 5.15 (s, 2H, CH_2Ph), 4.77 (s, 1H, CH), 4.64 – 4.55 (m, 1H, CH_2), 4.52 – 4.42 (m, 1H, CH_2), 3.71 (dd, J = 6.5, 7.0 Hz, 2H, NCH_2), 3.53 (t, J = 7.0 Hz, 2H, CH_2), 2.78 (s, 2H, CH_2), 2.71 – 2.39 (m, 4H), 2.36 – 2.16 (m, 3H), 2.10 (s, 3H, CH_3), 1.93 – 1.82 (m, 2H, CH_2). – ^{13}C NMR ([D_6]-DMSO, 125.76 MHz): δ = 201.8 (CO), 171.6 (COO), 170.5, 169.9, 161.1, 155.2, 140.1, 136.0, 134.0, 128.4, 128.1, 127.8, 127.8, 126.1, 73.1, 67.7, 66.7, 59.1, 55.5, 52.7, 38.9, 37.1, 34.8, 32.8, 32.7, 31.6, 24.0, 20.7 (CH_3). – HRMS: *m/z* = 648.2541 (calcd. 648.2557 for $C_{34}H_{38}N_3O_{10}$, $[M+H]^+$).

*General procedure for the preparation of compounds **17** and **18** via deacetylation of esters **15** and **16***

To a stirred solution of **15** or **16** (63 mg for **15**; 65 mg for **16**, 0.1 mmol) in a mixture of MeOH (4 mL), CH_2Cl_2 (0.5 mL) and H_2O (0.5 mL) were added K_2CO_3 3/2H_2O (33 mg, 0.2 mmol) in H_2O (0.5 mL) at −10 °C, and the mixture was stirred at −10 °C to 0 °C for 30 min, evaporated *in vacuo*, and the residue was treated with dichloromethane (12 mL) and then dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (silica gel, ethyl acetate/*n*-hexane/methanol = 23 : 75 : 7, v/v) to give pure **17** or **18** as white foams.

*(1S,2S,4R)-4-[3-(2,5-Dioxo-2,5-dihydropyrrol-1-yl)propionyloxy]-2-(3-hydroxy-2-oxo-1-phenethylpropylcarbamoyl) pyrrolidine-1-carboxylic acid benzyl ester (**17**)*

Yield: 81 %. R_f = 0.2 (ethyl acetate/*n*-hexane/methanol = 20 : 75 : 5, v/v). – $[\alpha]_D^{24}$ = −39.4 (*c* = 0.23, $CHCl_3$). – IR (neat, NaCl): ν = 3345 (OH), 2965, 1725 (CO), 1705 (COO), 1558, 1268, 1076, 838 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 7.41 – 7.16 (m, 11H, Ar-H, NH), 6.67 (s, 2H, HC=CH), 5.25 (s, 1H, CH), 5.16 (s, 2H, CH_2Ph), 4.73 (s, 1H, CH), 4.62 – 4.58 (m, 2H, CH_2), 4.50 – 3.98 (m, 4H, CH_2), 3.67 (t, J = 7.0 Hz, 2H, NCH_2), 3.71 – 3.59 (m, 2H, CH_2), 2.82 (s, 2H, CH_2), 2.69 – 2.61 (m, 2H), 2.50 – 2.35 (m, 1H), 2.24 – 2.11 (m, 2H). – ^{13}C NMR ([D_6]-DMSO, 125.76 MHz): δ = 201.6 (CO), 171.4 (COO), 170.5, 169.8, 155.3, 141.4, 135.8, 134.5, 128.4, 128.2, 128.1, 128.0, 127.8, 126.0, 73.8, 67.9, 67.1, 66.7, 59.3, 55.7, 52.4, 40.2, 34.8, 33.7, 33.1, 32.6, 31.5. – HRMS: *m/z* = 592.2282 (calcd. 592.2295 for $C_{31}H_{34}N_3O_9$, $[M+H]^+$).

*(1S,2S,4R)-4-[3-(2,5–Dioxo-2,5-dihydropyrrol-1-yl)butyryloxy]-2-(3-hydroxy-2-oxo-1-phenethylpropylcarbamoyl)pyrrolidine-1-carboxylic acid benzyl ester (**18**)*

Yield: 80 %. R_f = 0.2 (ethyl acetate/*n*-hexane/methanol = 20 : 75 : 5, v/v). – $[\alpha]_D^{24}$ = −23.6 (*c* = 0.18, $CHCl_3$). – IR (neat, NaCl): ν = 3351 (OH), 2943, 1722 (CO), 1698 (COO), 1565, 1248, 1168, 1083, 835 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 7.40 – 7.11 (m, 11H, Ar-H, NH), 6.68 (s, 2H, HC=CH), 5.26 (s, 1H, CH), 5.17 (s, 2H, CH_2Ph), 4.77 (s, 1H, CH), 4.62 – 4.56 (m, 2H, CH_2), 4.50 – 4.43 (m, 3H, CH_2), 3.71 (d, J = 7.0 Hz, 2H, NCH_2), 3.51 (t, J = 7.5 Hz, 2H, CH_2), 2.78 (s, 2H, CH_2), 2.70 – 2.36 (m, 2H), 2.34 – 2.15 (m, 3H), 1.91 – 1.83 (m, 2H, CH_2). – ^{13}C NMR ([D_6]-DMSO, 125.76 MHz): δ = 201.8 (CO), 171.4 (COO), 171.0, 169.8, 161.4, 155.4, 139.8, 136.1, 134.2, 128.6, 128.4, 128.1, 127.9, 126.5, 73.8, 67.9, 67.6, 66.4, 59.0, 55.2, 52.5, 38.9, 37.5, 33.9, 32.5, 32.9, 31.4, 23.9. – HRMS: *m/z* = 628.2289 (calcd. 628.2271 for $C_{32}H_{35}N_3O_9Na$, $[M+Na]^+$).

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