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SECTION 1: General Methods

NMR spectra were recorded on a Bruker AMX400 spectrometer and are referenced to internal tetramethylsilane (CDCl_3) or the residual proton signal of DMSO assigned a chemical shift of 2.49 ppm. Peak multiplicities are denoted by the following abbreviations: app, apparent; br, broad; s, singlet; d, doublet; t, triplet; q^4 , quartet; q^5 , quintet; m, multiplet (asterisk indicates overlapping signal). Respective reference values of 77.00 and 39.50 ppm were used for the ^{13}C solvent signals of chloroform and DMSO. ^{19}F NMR spectra were recorded on a Bruker AC200 instrument using external trifluoromethylbenzene in chloroform, assigned a chemical shift of +63.00 ppm, as the standard. IR spectra were recorded on a Mattson Research Series spectrophotometer. FAB mass spectra were recorded on either Autospec VG or Kratos MS50 instruments (at the Department of Chemistry, Université de Montréal). Elemental analyses were performed on a Carlo Erba EA1106 CHN analyzer. Optical rotation measurements were carried out in a Perkin-Elmer 241 polarimeter. UV spectra were obtained using a Perkin-Elmer Lambda 7 UV/VIS instrument. Analytical HPLC employed either of the following systems: A) Vydac C18 10 mm analytical column (24 x 4.6 mm), mobile phase: acetonitrile / 0.06 % trifluoroacetic acid (TFA) in water / 0.06 % TFA; B) Symmetry shield C8 10 mm analytical column (15 x 3.9 mm), mobile phase: acetonitrile in 20 mM Na_2HPO_4 , pH 9.0. Flash chromatography was performed on Merck silica gel 60 (0.040-0.063 mm) using nitrogen pressure. Analytical thin-layer chromatography (TLC) was carried out on precoated (0.25 mm) Merck silica gel F-254 plates. Anhydrous grade (Aldrich) dichloromethane and *N,N*-dimethylformamide were employed and tetrahydrofuran was distilled from lithium aluminium hydride immediately prior to use.

SECTION 2: Synthesis of peptidyl HCMV protease inhibitors (3) and (5). Detailed procedures are provided for the peptidyl methyl ketone **3** and its corresponding alcohols **5**. Physical data for intermediates and final products, as well as [^{13}C]-**3**, are given. Refer to Scheme S1.

Weinreb amide of *N*-Boc-L-alanine (A)

Carbonyl diimidazole (23.0 g, 0.142 mol) was added to stirred solution of *N*-Boc-alanine (20.0 g, 0.106 mol) cooled to 0°C and the reaction stirred under a nitrogen atmosphere. After 0.5 h, triethylamine (19.8 mL, 0.142 mol) and *N,O*-dimethylhydroxylamine hydrochloride (13.85 g, 0.142 mol) were added and the stirring at 0°C continued for 0.5 h after which time the reaction was allowed to warm to ambient temperature. After 14 h, the solution was poured into ethyl ether (800 mL) and the organic phase washed with hydrochloric acid (1 N, 3 x 250 mL), saturated sodium bicarbonate (250 mL) and brine (250 mL). The ether phase was dried (MgSO_4), filtered and evaporated *in vacuo* to yield amide **A** as colourless crystals (23.1 g, 94%) which were used without further purification.

^1H NMR (400 MHz, CDCl_3 , TMS): δ 1.29 (d, 3H, $J = 7.0$, Ala- α -Me), 1.42 (s, 9H, t-Bu), 3.19 (s, 3H, NMe), 3.75 (s, 3H, OMe), 4.65 (br. m, 1H, Ala- α -H), 5.23 (br d, 1H, $J = 7.3$, NH);

MS (FAB-thioglycerol) m/e 233 (MH^+ , 92 %), 177 ($[\text{MH}^+ - \text{C}_4\text{H}_8]$, 100), 133 ($[\text{MH}^+ - \text{Boc}]$, 86), 91 (26), 57 (70).

***N*-Boc-L-alanyl methyl ketone (B)**

Methylolithium (1 M in ethyl ether, 60.0 mL, 60.0 mmol) was added slowly to a stirred solution of Weinreb amide **A** (5.07 g, 21.8 mmol) in anhydrous tetrahydrofuran (230 mL) at -78°C under a nitrogen atmosphere and the resulting solution stirred for 1.5 h. The reaction was then quenched with saturated ammonium chloride (100 mL) and extracted with ethyl acetate (100 + 400 mL). The combined organic phases were washed with water (500 mL) and brine (500 mL), and then

dried (MgSO_4), filtered and evaporated *in vacuo* to yield **B** as a pale yellow oil which partially solidified upon standing (4.45 g, quantitative) and which was used without further purification.

^1H NMR (400 MHz, CDCl_3 , TMS): δ 1.34 (d, 3H, $J = 7.3$, Ala- α -Me), 1.44 (s, 9H, *t*-Bu), 2.20 (s, 3H, C(O)Me), 4.30 (m, 1H, Ala- α -H), 5.24 (br s, 1H, NH)

(spectrum agrees with literature values for racemic product - Mansour, T. S. & Evans, C. A., (1989) *Synth. Commun.* 19 667.)

MS (FAB-thioglycerol) m/e 188 (MH^+ , 37 %), 132 ($[\text{MH}^+ - \text{C}_4\text{H}_8]$, 100), 91 (41), 88 ($[\text{MH}^+ - \text{Boc}]$, 61), 57 (66).

***N*-Boc-L-alanyl ethyl alcohol (C)**

Sodium borohydride (907 mg, 24.0 mmol) was added to a stirred solution of ketone **B** (4.09 g, 21.8 mmol) in tetrahydrofuran (42 mL) containing methanol (10 mL) at 0°C and the reaction stirred for 2 h. The solution was then diluted with ethyl ether (170 mL) and quenched by the slow addition of aqueous citric acid (10% w/v, 85 mL). The aqueous phase was extracted with ether (3 x 85 mL) and the combined organic phases washed with saturated sodium bicarbonate (170 mL) and brine (170 mL), dried (MgSO_4), filtered and evaporated *in vacuo* to yield the alcohols **C** as a white solid (4.15 g, 100%) which was used without further purification.

^1H NMR (400 MHz, CDCl_3 , TMS): **major isomer**: δ 1.09 (d, 3H, $J = 7.0$ Hz, Ala- α -Me), 1.14 (d, 3H, $J = 6.4$, CH(OH)Me), 1.45 (s, 9H, *t*-Bu), 3.60 (br. m, 1H, Ala- α -H), 3.85 (dq⁴, $J = 3.2, 6.4$, CH(OH)), 4.62* (br s, 1H, NH); **minor isomer**: δ 1.16 (d, 3H, $J = 7.0$ Hz, Ala- α -Me), 1.19 (d, 3H, $J = 6.4$, CH(OH)Me), 1.45 (s, 9H, *t*-Bu), 3.56 (br. m, 1H, Ala- α -H), 3.69 (dq⁴, $J = 4.7, 6.4$, CH(OH)), 4.62* (br s, 1H, NH);

MS (FAB-thioglycerol) m/e 190 (MH^+ , 52 %), 134 ($[\text{MH}^+ - \text{C}_4\text{H}_8]$, 100), 90 ($[\text{MH}^+ - \text{Boc}]$, 87), 57 (48).

***N*-Boc-L-alanyl ethyl alcohol benzyl ether (D)**

The mixture of alcohols **C** (995 mg, 5.26 mmol) was added to a stirred suspension of sodium hydride (211 mg, 60% oil disp., 5.28 mmol) in dry *N,N*-dimethylformamide (14 mL) cooled to 0°C followed by tetra-*n*-butylammonium iodide (97 mg, 0.26 mmol). After 0.5 h, benzyl bromide (0.75 mL, 6.31 mmol) was added and the reaction allowed to warm to ambient temperature under a nitrogen atmosphere. After 1 day, the mixture was diluted with ethyl ether / ethyl acetate (1:1 v/v, 100 mL) and washed with water (50 mL), saturated sodium bicarbonate (50 mL) and brine (50 mL). The organic phase was then dried (MgSO₄), filtered and evaporated *in vacuo* yielding an orange oil which was purified by chromatography over silica gel (6:1 to 4:1 hexane/ethyl acetate) to yield the benzyl ethers **D** as a colourless oil (644 mg, 44%):

¹H NMR (400 MHz, CDCl₃, TMS): **major isomer**: δ 1.12 (d, 3H, *J* = 7.0 Hz, Ala-α-Me), 1.16 (d, 3H, *J* = 6.4, CH(OR)Me), 1.43 (s, 9H, *t*-Bu), 3.56-3.64* (br. m, 1H, CH(OR)), 3.66-3.77 (br. m, 1H, Ala-α-H), 4.44 & 4.62 (ABq⁴, 2H, *J* = 11.8, CH₂Ph), 4.62* (br s, 1H, NH), 7.23-7.37* (m, 5H, Ph); **minor isomer**: δ 1.17 (d, 3H, *J* = 6.7 Hz, Ala-α-Me), 1.18 (d, 3H, *J* = 6.4, CH(OH)Me), 1.44 (s, 9H, *t*-Bu), 3.50 (dq⁴, 1H, *J* = 2.7, 6.4, CHOR), 3.56-3.64* (br. m, 1H, Ala-α-H), 4.43 & 4.62 (ABq⁴, 2H, *J* = 11.8, CH₂Ph), 4.62* (br s, 1H, NH), 7.23-7.37* (m, 5H, Ph);

MS (FAB-thioglycerol) *m/e* 280 (MH⁺, 37 %), 224 ([MH⁺- C₄H₈], 82), 180 ([MH⁺- Boc], 46), 91 ([C₇H₇⁺], 100), 57 (27).

HRMS (FAB-thioglycerol) *m/e* calcd. C₁₆H₂₆O₃N (MH⁺) 280.1913, observed 280.1922.

***N*-Boc-L-(*N,N*-dimethylasparargyl)-L-alanyl ethyl alcohol benzyl ether (E)**

The carbamates **D** (562 mg, 2.01 mmol) were dissolved in a solution of hydrogen chloride in anhydrous dioxane (4N, 8.0 mL) and the reaction stirred at ambient temperature for 1.5 h, after which time the solvent was evaporated *in vacuo*. The resulting syrup was dissolved in benzene (10 mL) and evaporated to give a white solid which was dried under high vacuum for 1h. The amine hydrochloride thus obtained was dissolved in anhydrous dichloromethane (10 mL), *N*-Boc-

N,N-dimethylasparagine added, and the solution cooled to 0°C. HOBt (543 mg, 4.02 mmol) was then added followed by diisopropylethylamine (0.70 mL, 4.0 mmol) and the reaction stirred for 10 min. EDAC was then added and the pH of the solution raised to pH 8 with additional amine base followed by removal of the ice bath. After 2.5 h, the solution was extracted with ethyl acetate (2 x 50 mL) and washed with aqueous citric acid (10% w/w, 50 mL), aqueous sodium carbonate (10% w/w, 50 mL) and brine (50 mL). The combined organic phases were then dried (Na₂SO₄), filtered and evaporated *in vacuo* to afford a pale brown foam which was purified by chromatography over silica gel (3.5:1 ethyl acetate / hexane to ethyl acetate, v/v) to yield the dipeptides **E** as a colourless syrup (743 mg, 88 %):

58:42 mixture of diastereomers (purity of >96 %) by HPLC (conditions A):

¹H NMR (400 MHz, CDCl₃, TMS): **major isomer:** δ 1.14 (d, 3H, *J* = 7.3 Hz, Ala-α-Me), 1.15 (d, 3H, *J* = 6.4, CH(OBn)Me), 1.40 (s, 9H, *t*-Bu), 2.46-2.54* (m, 1H, Asn(Me₂)-β-H_A), 2.89 & 2.95 (two s, 2 x 3H, NMe₂), 3.12-3.22* (m, 1H, Asn(Me₂)-β-H_B), 3.56 (dq⁴, 1H, *J* = 6.4, 3.8, CH(OBn), 3.99-4.08* (m, 1H, Ala α-H), 4.46 & 4.55 (ABq⁴, 2H, *J* = 11.8, CH₂Ph), 4.47-4.54* (m, 1H, Asn(Me₂)-α-H), 6.13-6.22* (br. m., 1H, BocNH), 7.13 (br. d, 1H, *J* = 7.1, AlaNH), 7.23-7.37* (m, 5H, Ph); **minor isomer:** δ 1.12 (d, 3H, *J* = 6.4, CH(OBn)Me), 1.18 (d, 3H, *J* = 6.7, Ala-α-Me), 1.40 (s, 9H, *t*-Bu), 2.46-2.54* (m, 1H, Asn(Me₂)-β-H_A), 2.89 & 2.96 (two s, 2 x 3H, NMe₂), 3.12-3.22* (m, 1H, Asn(Me₂)-β-H_B), 3.51 (dq⁴, 1H, *J* = 6.4, 2.9, CH(OBn), 3.99-4.08* (m, 1H, Ala α-H), 4.44 & 4.60 (ABq⁴, 2H, *J* = 12.1, CH₂Ph), 4.47-4.54* (m, 1H, Asn(Me₂)-α-H), 6.13-6.22* (br. m., 1H, Boc-NH), 6.95 (br. d, 1H, *J* = 7.3, Ala-NH), 7.23-7.37* (m, 5H, Ph).

MS (FAB-thioglycerol) *m/e* 422 (MH⁺, 100 %), 366 ([MH⁺- C₄H₈], 21), 322 ([MH⁺- Boc], 70), 277 (12), 187 (48), 132 (41), 115 (91).

***N*-Boc-L-(α -*tert*-butylglycyl)-L-(*N,N'*-dimethylasparagyl)-L-alanyl ethyl alcohol benzyl ether (F)**

The coupling of **E** (690 mg) and *N*-Boc-*t*-butylglycine was carried out as described above except no cooling of the reaction was carried out. Chromatography of the crude syrup, obtained after the standard workup, over silica gel (4:1 ethyl acetate / hexane to ethyl acetate, v/v) afforded tripeptide **F** as a colourless foam (753 mg, 86%):

61:39 mixture of diastereomers (purity of >98 %) by HPLC (conditions A):

^1H NMR (400 MHz, CDCl_3 , TMS): **major isomer:** δ 0.98 (s, 9H, TBG-*t*-Bu), 1.128 (d, 3H, $J = 7.0$ Hz, Ala- α -Me), 1.134 (d, 3H, $J = 6.4$, CH(OBn)Me), 1.44 (s, 9H, Boc-*t*-Bu), 2.38-2.48* (m, 1H, Asn(Me₂)- β -H_A), 2.89 & 2.94 (two s, 2 x 3H, NMe₂), 3.08 (dd, 1H, $J = 2.9, 16.5$, Asn(Me₂) β -H_B), 3.54 (dq⁴, 1H, $J = 6.4, 4.1$, CH(OBn)), 3.83* (br. d, 1H, $J = 8.3$, TBG- α -H), 4.02-4.10* (m, 1H, Ala α -H), 4.47 & 4.53 (ABq⁴, 2H, $J = 11.8$, CH₂Ph), 4.75-4.83* (m, 1H, Asn(Me₂)- α -H), 5.19-5.30* (br. m., 1H, Boc-NH), 7.23-7.37 (m, 6H, Ph & Asn(Me₂)-NH), 7.56 (br. d, 1H, $J = 7.3$, Ala-NH); **major isomer:** δ 0.96 (s, 9H, TBG-*t*-Bu), 1.10 (d, 3H, $J = 6$ Hz, CH(OBn)Me), 1.16 (d, 3H, $J = 6.4$, Ala- α -Me), 1.44 (s, 9H, Boc-*t*-Bu), 2.38-2.48* (m, 1H, Asn(Me₂)- β -H_A), 2.90 & 2.96 (two s, 2 x 3H, NMe₂), 3.16 (dd, 1H, $J = 2.9, 16.5$, Asn(Me₂) β -H_B), 3.50 (dq⁴, 1H, $J = 6, 3.5$, CH(OBn)), 3.83* (br. d, 1H, $J = 8.3$, TBG- α -H), 4.02-4.10* (m, 1H, Ala α -H), 4.44 & 4.58 (ABq⁴, 2H, $J = 12.1$, CH₂Ph), 4.75-4.83* (m, 1H, Asn(Me₂)- α -H), 5.19-5.30* (br. m., 1H, BocNH), 7.11 (br. d, $J = 7.9$, Asn(Me₂)-NH), 7.23-7.37 (m, 6H, Ph), 7.52 (br. d, 1H, $J = 7$, Ala-NH).

MS (FAB-thioglycerol) m/e 535 (MH⁺, 77 %), 479 ([MH⁺- C₄H₈], 16), 300 (25), 115 (100).

***N*-*tert*-Butylacetyl-L-(α -*tert*-butylglycyl)-L-(*N,N'*-dimethylasparagyl)-L-alanyl ethyl alcohol benzyl ether (G)**

The coupling of **F** (382 mg) and *tert*-butylacetic acid was carried out as described above for the preparation of **F**. Tetrapeptide **G** was obtained as a colourless foam after the standard workup and was used without further purification (357 mg, 94%):

61:39 mixture of diastereomers (purity of >97 %) by HPLC (conditions A):

^1H NMR (400 MHz, CDCl_3 , TMS): **major isomer:** δ 0.99 (s, 9H, DATBG-*t*-Bu), 1.043 (s, 9H, TBG-*t*-Bu), 1.129 (d, 3H, $J = 7.3$ Hz, Ala- α -Me), 1.130 (d, 3H, $J = 6.4$, CH(OBn)Me), 2.10 & 2.13 (ABq 4 , 2H, $J = 13.0$, DATBG- α -H) 2.38-2.46* (m, 1H, Asn(Me $_2$)- β -H $_A$), 2.89 & 2.91 (two s, 2 x 3H, NMe $_2$), 3.03 (dd, 1H, $J = 2.9, 16.5$, Asn(Me $_2$) β -H $_B$), 3.54 (dq 4 , 1H, $J = 6.4, 3.8$, CH(OBn), 4.00-4.12* (m, 1H, Ala α -H), 4.22 (d, 1H, $J = 8.6$, TBG- α -H), 4.47 & 4.53 (ABq 4 , 2H, $J = 11.8$, CH $_2$ Ph), 4.76-4.85* (m, 1H, Asn(Me $_2$)- α -H), 6.03* (d, 1H, $J = 8.6$, TBG-NH), 7.23-7.37 (m, 6H, Ph & Asn(Me $_2$)-NH), 7.47 (br. d, 1H, $J = 7.3$, Ala-NH); **minor isomer:** δ 0.97 (s, 9H, DATBG-*t*-Bu), 1.038 (s, 9H, TBG-*t*-Bu), 1.11 (d, 3H, $J = 6.2$, CH(OBn)Me), 1.16 (d, 3H, $J = 7.0$ Hz, Ala- α -Me), 2.08 & 2.12 (ABq 4 , 2H, $J = 13.0$, DATBG- α -H) 2.38-2.46* (m, 1H, Asn(Me $_2$)- β -H $_A$), 2.90 & 2.94 (two s, 2 x 3H, NMe $_2$), 3.12 (dd, 1H, $J = 3.0, 16.4$, Asn(Me $_2$) β -H $_B$), 3.50 (dq 4 , 1H, $J = 6.2, 3.2$, CH(OBn), 4.00-4.12* (m, 1H, Ala α -H), 4.21 (d, 1H, $J = 8.9$, TBG- α -H), 4.43 & 4.59 (ABq 4 , 2H, $J = 11.7$, CH $_2$ Ph), 4.76-4.85* (m, 1H, Asn(Me $_2$)- α -H), 6.03* (d, 1H, $J = 8.6$, TBG-NH), 7.09 (d, 1H, $J = 8.6$, Asn(Me $_2$)-NH), 7.23-7.37 (m, 6H, Ph), 7.45 (br. d, 1H, $J = 8$, Ala-NH).

MS (FAB-thioglycerol) m/e 533 (MH $^+$, 71 %), 425 ([MH $^+$ -BnOH], 10), 354 (14), 322 (30), 115 (100).

***N*-tert-Butylacetyl-L-(α -*tert*-butylglycyl)-L-(*N,N'*-dimethylasparagyl)-L-alanyl ethyl alcohol (5)**

A solution benzyl ethers **G** (327 mg, 0.614 mmol) in methanol (20 mL) containing 10% palladium-carbon (43 mg) was vigorously stirred under a balloon of hydrogen at ambient temperature. After 2.5 h, the reaction was filtered (Millex) and the filtrate evaporated *in vacuo* to afford the alcohols **5** as a colourless foam (272 mg, quant.) which were used without further purification:

Approximately 65:35 mixture of diastereomers by NMR. Purity of >98 % by HPLC (conditions A)

^1H NMR (400 MHz, CDCl_3 , TMS): **major isomer:** δ 1.035 (s, 9H, DATBG-*t*-Bu), 1.05 (s, 9H, TBG-*t*-Bu), 1.09 & 1.11 (two d, 2 x 3H, $J = 6.7$ Hz, Ala- α -Me & CH(OBn)Me), 2.17* (br. s, 2H, DATBG- α -H) 2.49* (dd, 1H, $J = 7.0, 16.2$, Asn(Me₂)- β -H_A), 2.92 & 3.01 (two s, 2 x 3H, NMe₂), 3.15 (dd, 1H, $J = 3.5, 15.9$, Asn(Me₂)- β -H_B), 3.37 (br. s, 1H, OH), 3.62-3.85* (m, 1H, CHOBN), 3.87-3.95* (m, 1H, Ala α -H), 4.11 (d, 1H, $J = 7.3$, TBG- α -H), 4.72-4.78* (m, 1H, Asn(Me₂)- α -H), 6.04* (d, 1H, $J = 7.0$, TBG-NH), 7.12 (d, 1H, $J = 7.6$, Asn(Me₂)-NH), 7.57 (d, 1H, $J = 7.6$, Ala-NH); **minor isomer:** δ 1.039 (s, 9H, DATBG-*t*-Bu), 1.05 (s, 9H, TBG-*t*-Bu), 1.15 & 1.17 (two d, 2 x 3H, $J = 6.7$ Hz, Ala- α -Me & CH(OBn)Me), 2.17* (br. s, 2H, DATBG- α -H) 2.49* (dd, 1H, $J = 7.0, 16.2$, Asn(Me₂)- β -H_A), 2.92 & 3.01 (two s, 2 x 3H, NMe₂), 3.17 (dd, 1H, $J = 3.5, 15.9$, Asn(Me₂)- β -H_B), 3.62-3.85* (m, 2H, CHOBN & OH), 3.87-3.95* (m, 1H, Ala α -H), 4.10 (d, 1H, $J = 6.7$, TBG- α -H), 4.72-4.78* (m, 1H, Asn(Me₂)- α -H), 6.04* (d, 1H, $J = 7.0$, TBG-NH), 7.01 (d, 1H, $J = 7.8$, Asn(Me₂)-NH), 7.60 (d, 1H, $J = 8.0$, Ala-NH);

MS (FAB-thioglycerol): m/e 443 (MH⁺, 100 %), 425 ([MH⁺ - H₂O], 6), 398 ([MH⁺ - C₂H₅O], 20), 354 (9), 232 (40), 115 (81).

***N*-*tert*-Butylacetyl-L-(α -*tert*-butylglycyl)-L-(*N,N'*-dimethylasparagyl)-L-alanyl methyl ketone (3)**

Alcohols **5** (200 mg, 0.452 mmol) were dissolved in anhydrous dichloromethane (10 mL) containing *N,N*-dimethylformamide (0.5 mL) and Dess-Martin periodinane (383 mg, 0.904 mmol) added. After stirring at ambient temperature for 2.5 h under a nitrogen atmosphere, the reaction was diluted with dichloromethane (10 mL) and a mixture of 10% sodium thiosulfate / saturated sodium bicarbonate (1:1 v/v, 50 mL) was added and the phases vigorously stirred. After 0.5 h, the clear phases were extracted with ethyl acetate (50 + 30 mL) and washed with saturated sodium bicarbonate (4 x 50 mL) and brine (50 mL). The combined organic phases were then dried (Na₂SO₄), filtered and evaporated *in vacuo* to a colourless foam. The product was dissolved in boiling ethyl acetate and the solution allowed to cool resulting in the precipitation of a

white solid. The material was filtered, washed with cold solvent and dried under vacuum, affording methyl ketone **3** (112 mg, 56%):

Purity of >99 % by HPLC (conditions A)

^1H NMR (400 MHz, CDCl_3 , TMS): δ 1.04 (s, 18H, 2 x *t*-Bu), 1.34 (d, 3H, $J = 7.3$ Hz, Ala- α -Me), 2.11 & 2.14 (ABq⁴, 2H, $J = 13.0$, DATBG- α -H) 2.17 (s, 3H, C(O)Me), 2.50 (dd, 1H, $J = 7.0$, 16.5, Asn(Me₂)- β -H_A), 2.94 & 3.00 (two s, 2 x 3H, NMe₂), 3.13 (dd, 1H, $J = 3.2$, 16.5, Asn(Me₂)- β -H_B) 4.20 (d, 1H, $J = 8.6$, TBG- α -H), 4.46 (app. q⁵, 1H, $J_{\text{app}} = 7.3$, Ala α -H), 4.84 (app dt, 1H, $J_{\text{app}} = 3.2$, 7.3, Asn(Me₂)- α -H), 6.03 (d, 1H, $J = 8.3$, TBG-NH), 7.43 (d, 1H, $J = 7.6$, Asn(Me₂)-NH), 7.68 (d, 1H, $J = 6.7$, Ala-NH).

MS (FAB-thioglycerol) *m/e* 441 (MH⁺, 100 %), 354 ([MH⁺ - AlaMe], 11), 230 ([Asn(Me₂)AlaMe + H⁺], 21), 212 (19), 184 (11), 115 (74);

^{13}C NMR (100.6 MHz, CDCl_3): 16.72, 26.29, 26.85, 29.86, 31.00, 34.30, 34.77, 35.53, 37.34, 49.38, 50.76, 54.99, 61.36, 170.45, 170.54, 171.04, 171.89, 206.59;

HRMS (FAB-thioglycerol): *m/e* calcd. C₂₂H₄₁O₅N₄ (MH⁺) 441.3077, observed 441.3112;

$[\alpha]_{\text{D}}^{22} = +47.3^\circ$; (*c* 0.516, CHCl_3);

UV: $\lambda_{\text{max}} = 282$ nm ($\epsilon = 46$, MeOH);

IR (KBr): 1713 cm^{-1} .

^{13}C Carbonyl-labelled Methyl Ketone ([^{13}C]-3)

The ^{13}C carbonyl labelled methyl ketone was prepared in a manner identical to that described for its unlabelled counterpart employing *N-t*-Boc-L-alanine-1- ^{13}C (Cambridge Isotopes) as the starting material. The final product was purified by preparative HPLC (Partisil 10 OCD-3 C₁₈ column, mobile phase of conditions A).

Purity of >99 % by HPLC (conditions A and B);

^1H NMR (400 MHz, DMSO- d_6): δ 0.91 & 0.95 (two s, 2 x 9H, 2 x *t*-Bu), 1.14 (dd, 3H, $^3J_{\text{H-H}} = 7.0$ Hz, $^3J_{\text{H-C}} = 4.1$, Ala- α -Me), 2.02 & 2.19 (ABq⁴, 2H, $J = 12.7$, DATBG- α -H) 2.05 (d, 3H, $^2J_{\text{H-C}} = 6.0$, $^{13}\text{C}(\text{O})\text{Me}$), 2.66 (A of ABX, 1H, $J = 6.4$, 16.2, Asn(Me₂)- β -H_A), 2.73 (B of ABX, 1H, $J = 6.4$, 16.2, Asn(Me₂)- β -H_B), 2.79 & 2.95 (two s, 2 x 3H, NMe₂), 4.08-4.19 (m, 2H, TBG- α -H & Ala α -H), 4.59 (app dd, 1H, $J_{\text{app}} = 6.7$, 13.7, Asn(Me₂)- α -H), 7.60 (d, 1H, $J = 8.3$, TBG-NH), 8.01 (d, 1H, $J = 6.7$, Ala-NH), 8.16 (d, 1H, $J = 7.3$, Asn(Me₂)-NH);

MS (FAB-thioglycerol) *m/e* 442 (MH⁺, 82 %), 354 ([MH⁺ - AlaMe], 17), 231 ([^{13}C -Asn(Me₂)AlaMe + H⁺], 29), 184 (19), 115 (100);

^{13}C NMR (100.6 MHz, DMSO- d_6): 15.60, 25.66 (d, $^1J_{\text{C-C}} = 40.5$ Hz, $^{13}\text{C}(\text{O})\text{Me}$), 26.62, 29.61, 30.51, 33.72, 34.01, 34.73, 36.58, 48.22, 49.52, 54.30 (d, $^1J_{\text{C-C}} = 42.1$ Hz, Ala- α -C), 60.17, 169.26, 170.13, 171.50, 171.19, 207.81;

HRMS (FAB-thioglycerol): *m/e* calcd. C₂₁H₄₁O₅N₄¹³C (MH⁺) 442.3110, observed 442.3097.

SECTION 3: Physical properties of peptidyl HCMV protease inhibitors. The physical properties of the peptidyl HCMV protease inhibitors: trifluoromethyl ketones (**1**) and (**6**), pentafluoroethyl ketones (**2**) and [^{13}C]-(**2**), and α -ketoamide (**4**) are provided.

Trifluoromethyl ketone (**1**)

Purity of >99 % by HPLC (conditions A);

^1H NMR (400 MHz, DMSO- d_6): δ 8.20 and 8.10 (2 x d, $J = 7.3$, 7.0 Hz, 1H), 7.62 (d, $J = 8.9$, 1H), 7.46-7.40 (m, 1H), 6.95-6.75 (m, 1H), 4.56 (q⁵, $J = 6.7$, 1H), 4.19 and 4.16 (2 x d, $J = 8.9$, 8.9, 1H), 4.12-4.05 (m, 1H), 2.95 and 2.94 (2 x s, 3H), 2.79 (s, 3H), 2.75-2.60 (m, 1H), 2.20-2.15 (m, 1H), 2.06-2.00 (m, 1H), 1.07-1.04 (m, 3H), 0.95 (s, 9H) 0.90 (s, 9H);

MS (FAB-thioglycerol) 495 (MH⁺), 513 (MH⁺+H₂O);

IR (KBr): 1635 cm⁻¹;

HRMS (FAB-thioglycerol): *m/e* calcd. C₂₂H₃₈F₃N₄O₅ (MH⁺) 495.2794, observed 495.2777.

Pentafluoroethyl ketone (2)

Purity of >98 % by HPLC (conditions A);

$^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): 3:1 mixture of diastereomers, δ 8.20 and 8.14 (2 x d, $J = 7.5, 7.5$ Hz, 1H), 7.66 (d, $J = 9.0, 1\text{H}$), 7.51 (d, $J = 8.8, 1\text{H}$), 6.91 (m, 2H), 4.58 (m, 1H), 4.16 (m, 2H), 2.95 and 2.80 (2 x s, 6H), 2.70 (m, 2H), 2.11 (m, 2H), 1.08 (d, $J = 6.6, 3\text{H}$), 0.95 (s, 9H), 0.91 (s, 9H);

IR (KBr): 1685, 1618 cm^{-1} ;

MS (FAB-thioglycerol) m/e 545 (MH^+), 563 ($\text{MH}^+ + \text{H}_2\text{O}$);

HRMS (FAB-thioglycerol): calcd. for $\text{C}_{23}\text{H}_{38}\text{O}_5\text{N}_4\text{F}_5$ (MH^+) 545.2762, found: 545.2775.

 ^{13}C Carbonyl-labelled Pentafluoroethyl ketone (^{13}C -2)

Purity of >98.5 % by HPLC (conditions A and B);

$^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ 8.20 (d, $J = 7.5$ Hz, 1H), 7.63 (d, $J = 9.0, 1\text{H}$), 7.51 (d, $J = 8.8, 1\text{H}$), 6.96 & 6.80 (two s, 2 x 0.8H, hydrate OH), 4.56 (m, 1H), 4.18 (m, 2H), 2.95 & 2.79 (2 x s, 6H), 2.68 (m, 2H), 2.18 & 2.02 (ABq, $J = 12.4, 2\text{H}$), 1.07 (dd, $^3J_{\text{H-C}} = 3.8, ^3J_{\text{H-H}} = 6.4, 3\text{H}$, Ala- α -Me), 0.95 (s, 9H), 0.91 (s, 9H);

$^{19}\text{F-NMR}$ (188.15 MHz, $\text{DMSO-}d_6$): 52.33 ppm (s, CF_3 of hydrate), 49.17 (s, CF_3 of ketone), 9.45 (t, $^2J_{\text{F-C}} = 24$ Hz, CF_2 of ketone), 8.48 (B of ABX, $^2J_{\text{F-F}} = 274$ Hz; $^2J_{\text{F-C}} = 24$ Hz, CF_2 of hydrate), 6.72 (A of ABX, $^2J_{\text{F-F}} = 274$ Hz, $^2J_{\text{F-C}} = 25$ Hz, CF_2 of hydrate);

MS (FAB-thioglycerol) m/e 546 ($[\text{MH}^+ + \text{H}_2\text{O}]$), 545 ($[\text{MH}^+]$);

HRMS (FAB-thioglycerol): calcd. for $\text{C}_{22}\text{H}_{38}\text{O}_5\text{N}_4\text{F}_5^{13}\text{C}$ (MH^+) 546.2796, found: 546.2812.

 α -Ketoamide (4)

Purity of 100 % by HPLC (conditions B);

$^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ 9.21 and 9.15 (2 x m, 1H), 8.14 and 8.09 (2 x d, $J = 7.3, 7.6$ Hz, 1H), 8.03 and 7.97 (2 x d, $J = 6.4, 5.7, 1\text{H}$), 7.60 (d, $J = 8.3, 1\text{H}$), 7.35 - 7.17 (m, 5H), 5.02-4.88 (m, 1H), 4.64-4.49 (m, 1H), 4.39-4.23 (m, 2H), 4.13 and 4.12 (2 x d, $J = 8.6, 8.6, 1\text{H}$), 2.92

and 2.91 (2 x s, 3H), 2.79 and 2.78 (2 x s, 3H), 2.74-2.54 (m, 2H), 2.19 (br d, $J = 12.4$, 1H), 2.03 and 2.02 (2 x d, $J = 12.4$, 12.7, 1H), 1.25 and 1.23 (2 x d, $J = 7.3$, 7.0, 3H), 0.94 and 0.91 (2 x s, 18H);

IR (KBr): 3316, 3075, 2958, 1641, 1529 cm^{-1} ;

MS (FAB-thioglycerol) m/e 560 (MH^+), 582 ($\text{M} + \text{Na}^+$);

HRMS (FAB-thioglycerol) m/e calcd. for $\text{C}_{29}\text{H}_{45}\text{N}_5\text{O}_6$ (MH^+) 560.3448, observed 560.3426.

Trifluoromethyl ketone (6):

white solid: mp 110-112 $^{\circ}\text{C}$;

$^1\text{H-NMR}$ (400 MHz, CDCl_3 , TMS): δ 3.60 (s, 1 H), 2.16-2.00 (m, 3 H), 1.76-1.62 (m, 12 H);

$^{13}\text{C-NMR}$ (100.6 MHz, CDCl_3): 174.19, 72.51, 38.68, 37.50, 36.51, 28.19;

MS (FAB-thioglycerol) m/e 236 (MH^+);

HRMS (FAB-thioglycerol) m/e calcd. for $\text{C}_{12}\text{H}_{18}\text{N}_3\text{O}_2$ (MH^+) 236.1399; observed 236.1389;

$[\alpha]_{\text{D}}^{22} = -36^{\circ}$ (c 1.60, MeOH);

Anal. calcd. for $\text{C}_{12}\text{H}_{17}\text{N}_3\text{O}_2$: C, 61.26; H, 7.28; N, 17.86. Found: C, 61.04; H, 7.43; N, 17.94.

Scheme S1 (Supplementary Material)