Supplementary Material

The Design and Enzyme-Bound Crystal Structure of Indoline Based Peptidomimetic Inhibitors of Hepatitis C Virus NS3 Protease

Jesus M Ontoria, Stefania Di Marco, Immacolata Conte, M. Emilia Di Francesco, Cristina Gardelli, Uwe Koch, Victor G. Matassa, Marco Poma, Christian Steinkühler, Cinzia Volpari, and Steven Harper*

Chemistry: General Methods

Dess-Martin periodinane was prepared using the method of Ireland.[1] THF was dried over sodium-benzophenone ketyl prior to use. All other reagents and solvents were obtained from commercial suppliers and were used without further purification. With the exception of routine deprotection steps, all reactions were performed in oven-dried (110 °C) glassware under an atmosphere of nitrogen. Organic extracts were dried over anhydrous sodium sulfate (Merck). Flash chromatography filtrations were performed using 230-400 mesh silica gel 60 (Merck) as the stationary phase. Proton nmr spectra were recorded on Bruker AM series spectrometers and unless otherwise stated were recorded at 300 K and 400 MHz. Chemical shifts are reported in parts per million downfield from tetramethyl silane, and are measured using the residual resonance from the deuterated solvent as reference. The spectra of ketoacids **7a-j** are complicated in some instances by partial hydration of the ketone group. Where appropriate, the signals from both the keto and hydrate forms are reported. Assignments of protons with reference to their attached heavy atom is made using the following notation: $H_{\text{indoline2}}$ (etc.) for protons at the indoline C_2-C_7 positions; $H_{\text{thiophene2}}$ (etc.) for protons on thiophene rings; Leu-H_α (etc) for protons on leucine amino acid fragments; F_2Abu- H_α for protons on 4,4-difluoroaminobutyric amino acid fragments. Mass spectra were recorded on a Perkin Elmer API-100 using electrospray ionization, and only molecular ions are reported. High resolution mass spectrometry was performed using a Q-TOF hybrid system (QSTAR-XL, Applied Biosystems, Framingham MA) using an offline nano-ESI source (Protona). Preparative scale reversed-phase high performance liquid chromatography (RP-HPLC) was performed on a Waters DeltaPrep system incorporating a 486 absorbance detection unit operating at 220 nM. In all cases linear gradients of binary mixtures of MeCN (containing 0.1 % trifluoroacetic acid) (solvent A) and water (containing 0.1 % trifluoroacetic acid) (solvent B) were used as the mobile phase. The conditions used were: Method 1: 10 % solvent A (2 min) to 90 % solvent A over 10 min then isocratic; Stationary phase: Waters Symmetry 100mm x 20 mm, 5µm; Flow rate: 20 mL/min; Method

* To whom correspondence should be addressed. Phone +39 06 91093444. Fax +39 06 91093225. E-mail: Steven_Harper@Merck.com.

2: 10 % solvent A (5 min) to 37 % solvent A over 17 min then to 90 % solvent A over 15 min then isocratic. Method 3: 10 % solvent A (5 min) to 70 % solvent A over 25 min then isocratic; Stationary phase: Machery-Nagel Nucleosil 100-7 C_{18} 250 mm x 21 mm; Flow rate: 20 mL/min Stationary phase: Merck HiBar; Flow rate: 15 mL/min. Analytical scale RP-HPLC was performed using the mobile phases described above and the following gradients: Method 1: 10% solvent A (1 min) to 90 % solvent A over 7 min then isocratic; Stationary phase: Waters Symmetry C_{18} (150 mm x 3.9 mm, 5 μm); Flow rate: 1 mL/min. Method 2: 30% solvent A (1 min) to 90 % solvent A over 9 min then isocratic. Stationary phase: Waters Xterra C_{18} (100 x 4.6 mm, 5 μm); Flow rate: 1 mL/min. In all cases single isomers of final products **7** were assessed >95% pure under these conditions.

General route to 2-alkyl-2-indoline carboxylic esters (3). 1-*tert*-Butyl 2-methyl 2-cyclohex-2-en-1-ylindoline-1,2-dicarboxylate (3b)

A solution of KHMDS (7.21 mL, 3.61 mmol, 0.5 M in toluene) in THF (6 mL) was cooled to –78 °C and treated dropwise with a solution of **2a** (0.50 g, 1.80 mmol) in THF (5 mL). The mixture was warmed to -30 °C for 0.5 h then cooled again to -78 °C. A solution of 3-bromocyclohexene (1.45 g, 9.01 mmol) in THF (3 mL) was added over 0.5 h by syringe pump and the mixture was warmed to room temperature over 5 h. The reaction was diluted with EtOAc and aqueous NH_4Cl (1 N) and the organic layer was separated. The aqueous layer was extracted twice with EtOAc and the combined organic phases were washed with brine and dried. After removal of the organic solvent the residual oil was filtered through a short path of silica gel (petroleum ether/EtOAc, 97:3) to afford the title compound (456 mg, 70 %) as a pale oil. ^{1}H NMR (DMSO-d_6; 330 K, 1:1* mixture of rotomers about the carbamate C-N bond) δ 7.82-7.59 (app br s, 1H, $H_{\text{indoline7}}$), 7.20-7.11 (m, 2H, $H_{\text{indoline5,4}}$), 6.94 and 6.92* (t, J = 7.3 Hz and J* = 7.1 Hz, 1H, $H_{\text{indoline6}}$), 5.87-5.80 and 5.73* (m and dd, J* = 10.4, 2.8 Hz, 1H, C_6H_9-H_2), 5.87-5.80 and 5.35* (m and d*, J* = 10.4 Hz, 1H, C_6H_9-H_3), 3.66 and 3.64* (s, 3H, OCH_3), 3.45-3.40 and 3.34-3.28* (br m, 1H, C_6H_9-H_1), 3.25 and 3.22* (d, J = 17.0 Hz and J* = 17.4 Hz, 1H, $H_{\text{indoline3}}$), 3.15 and 3.12* (d, J = 17.0 Hz and J* = 17.4 Hz, 1H, $H_{\text{indoline3}}$), 2.38-2.29 and 1.96*-1.85* (m, 3H, C_6H_9-$H_{4,}H_{6eq}$), 1.82-1.60 (m, 1H, C_6H_9-H_{5eq}), 1.52-1.42 (m, 11H, C_6H_9-$H_{5ax,}H_{6ax,}$ $C(CH_3)_3$); MS (ES^+) m/z 358 $(M + H)^+$.

Using the required alkyl halide (see table 1 in manuscript) and a synthetic procedure analogous to that described above for **3b**, compounds **3c-d** were prepared from **2a** and compound **3e** was prepared from **2b**.

1-*tert*-butyl 2-methyl 2-(cyclobutylmethyl)indoline-1,2-dicarboxylate (3c)

^{1}H NMR (500 MHz; DMSO-d_6; 330 K) δ 7.75-7.50 (br s, 1H, $H_{\text{indoline7}}$), 7.15 (t, J = 7.6 Hz, 1H, $H_{\text{indoline5}}$), 7.11 (d, J = 7.6 Hz, 1H, $H_{\text{indoline4}}$), 6.90 (t, J = 7.6 Hz, 1H, $H_{\text{indoline6}}$), 3.73 (s, 3H, OCH_3), 3.36 (d, J = 15.7 Hz, 1H, $H_{\text{indoline3}}$), 3.13 (d, J = 15.7 Hz, 1H, $H_{\text{indoline3}}$), 2.47-2.39 (m, 1H, CH_2C$_4$H$_7$), 2.20-2.05 (m, 1H, C$_4H_7$), 1.95-1.85 (m, 1H, CH_2C$_4$H$_7$), 1.82-1.71 (m, 1H, C$_4H_7$), 1.70-1.50 (m, 5H, C$_4H_7$), 1.44 (s, 9H, C(CH_3)$_3$).

2-Methyl 1-*tert*-butyl 2-{[5-chlorothien-2-yl]methyl}indoline-1,2-dicarboxylate (3d)

^{1}H NMR (DMSO-d_6; 335 K) δ 7.59 (app br s, 1H, $H_{\text{indoline7}}$), 7.10 (t, J = 7.5 Hz, 1H, $H_{\text{indoline5}}$), 7.03 (d, J = 7.5 Hz, 1H, $H_{\text{indoline4}}$), 6.87 (t, J = 7.5 Hz, 1H, $H_{\text{indoline6}}$), 6.80-6.75 (m, 2H, C$_4H_2$ClS), 3.82-3.69 (m, 4H, OCH_3, $H_{\text{indoline3}}$), 3.45 (d, J = 15.2 Hz, 1H, $H_{\text{indoline3}}$), 3.35 (d, J = 17.2 Hz, 1H, CH_2C$_4$H$_2$ClS), 3.30 (d, J = 17.2 Hz, 1H, CH_2C$_4$H$_2$ClS), 1.51 (s, 9H, C(CH_3)$_3$).

2-Benzyl 1-*tert*-butyl 2-{[2-(*tert*-butoxycarbonyl)thien-3-yl]methyl}indoline-1,2-dicarboxylate (3e)

^{1}H NMR (DMSO-d_6; 330 K) δ 7.47 (app br s, 1H, $H_{\text{indoline7}}$), 7.45 (d, J = 4.7 Hz, 1H, $H_{\text{thiophene5}}$), 7.30 (s, 5H, C$_6H_5$), 6.99 (t, J = 7.4 Hz, 1H, $H_{\text{indoline6}}$), 6.86 (d, J = 7.4 Hz, 1H, $H_{\text{indoline4}}$), 6.74 (t, J = 7.4 Hz, 1H, $H_{\text{indoline5}}$), 6.67 (d, J = 4.7 Hz, 1H, $H_{\text{thiophene4}}$), 5.21 (d, J = 12.4 Hz, 1H, OCH_2Ph), 5.17 (d, J = 12.4 Hz, 1H, OCH_2Ph), 4.11 (d, J = 14.2 Hz, 1H, $H_{\text{indoline3}}$), 3.66 (d, J = 14.2 Hz, 1H, $H_{\text{indoline3}}$), 3.25 (app s, 2H, CH_2C$_4$H$_2$S-CO$_2$^{t}Bu), 1.47 (s, 9H, C(CH_3)$_3$), 1.44 (s, 9H, (CH_3)$_3$); MS (ES$^+$) m/z 550 (M + H)$^+$.

1-*tert*-Butyl-2-methyl-6-(2-*tert*-butoxy-2-oxoethoxy)indoline-1,2-dicarboxylate (3i)

To a suspension of methyl 6-benzyloxyindole-2-carboxylate (1.60 g, 5.71 mmol) in acetonitrile (40 mL), di-*tert*-butyldicarbonate (1.37 g, 6.28 mmol) and DMAP (137 mg, 1.12 mmol) were added. The mixture was stirred for 12 h then concentrated to afford a residue that was purified by filtration through silica gel (petroleum ether: EtOAc, 9:1) to afford 1-

tert-butyl-2-methyl-6-benzyloxyindoline-1,2-dicarboxylate (1.78 g, 82 %) as a solid. A portion of this material (1.75 g, 4.59 mmol) was dissolved in MeOH (120 mL), treated with Pd/C (175 mg, 10 % w/w) and stirred under hydrogen (60 psi) for 18 h. The suspension was purged with nitrogen then filtered through celite and concentrated to afford **4** (1.29 g, 96 %) as a solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.45 (br s, 1H, $H_{indoline7}$), 6.92 (d, J = 7.6 Hz, 1H, $H_{indoline4}$), 6.45 (d, J = 7.6 Hz, 1H, $H_{indoline5}$), 4.85 (m, 1H, $H_{indoline2}$), 3.74 (s, 3H, OCH_3), 3.42 (dd, J = 16.6 Hz, 11.9 Hz, 1H, $H_{indoline3}$), 3.05 (dd, J = 16.6 Hz, 4.8 Hz, 1H, $H_{indoline3}$), 1.48 (s, 9H, C(CH_3)$_3$).

A portion of **4** (0.47 g, 1.59 mmol) in DMF (10 mL) was treated with Cs_2CO_3 (0.77 g, 2.39 mmol) and *tert*-butylbromoacetate (0.28 mL, 1.91 mmol). The mixture was stirred for 5 h then diluted with CH_2Cl_2 and water. The organic layer was separated, washed with brine and dried. After removal of the solvent the residue was filtered through silica gel (petroleum ether/EtOAc, 9:1) to give the title compound (0.60 g, 93 %) as a foam. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.55 (br s, 1H, $H_{indoline7}$), 6.98 (d, J = 7.6 Hz, 1H, $H_{indoline4}$), 6.57 (d, J = 7.6 Hz, 1H, $H_{indoline5}$), 5.00-4.80 (m, 1H, $H_{indoline2}$), 4.50 (s, 2H, OCH_2), 3.75 (s, 3H, OCH_3), 3.44 (dd, J = 16.6 Hz, 11.9 Hz, 1H, $H_{indoline3}$), 3.05 (dd, J = 16.6 Hz, 4.8 Hz, 1H, $H_{indoline3}$), 1.48 (s, 18H, 2 x C(CH_3)$_3$).

General route to keto-acids 7. 3-({*N*-[(2,3-dihydro-1*H*-indol-2-yl)carbonyl]-L-leucyl}amino)-5,5-difluoro-2-oxopentanoic acid trifluoroacetate (7a-c)

A solution of N-*tert*-butyloxycarbonyl-indoline-2-carboxylate (100 mg, 0.38 mmol) in CH_2Cl_2 (10 mL) was treated with DIEA (0.20 mL, 1.14 mmol) and H-(L)-Leu-(D,L)-F_2AbuCH(OH)CO_2Me[2] (152 mg, 0.46 mmol). HATU (289 mg, 0.76 mmol) was added and the mixture was stirred for 36 h. After dilution with EtOAc and saturated aqueous $NaHCO_3$ the organic layer was separated, washed with aqueous HCl (1 N) and brine then dried. After removal of the solvent the residual oil was filtered through a short path of silica gel (petroleum ether/EtOAc, 2:1) to afford **6a** (110 mg, 53 %) as a foam.

A portion of the material from above (70 mg, 0.13 mmol) was dissolved in CH_2Cl_2 (60 mL) then treated with Dess-Martin periodinane (165 mg, 0.39 mmol) and *tert*-BuOH (28.8 mg, 0.39 mmol). After 2.5 h the oxidation was judged complete by electro-spray mass spectrometry, and the mixture was diluted with EtOAc and washed with a 1:1 mixture of saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ (4 x) and then brine. The dried organic phase was concentrated to afford a solid which was dissolved in a 65/35/5 mixture of TFA/CH_2Cl_2/H_2O (10 mL) then stirred at room temperature for 0.5 h. The solvent was removed and the residue was taken up in MeOH (5 mL) and aqueous NaOH (3.3 mL, 1

N) then stirred for 15 min. The mixture was diluted with H_2O and the pH was adjusted to 3 by addition of aqueous HCl (1 N). The mixture was extracted with EtOAc and the dried organic phase was concentrated to afford a residue that was purified by RP-HPLC (method 3) to give three fractions containing the title compound. Fraction 1 (**7a**, 6.7 mg, 9.5 %, retention time 19.0-19.6 min, 1:1* mixture of diastereoisomers by ^{1}H nmr. Both the keto and hydrate forms (7:1) are observed. Only the keto form is assigned) ^{1}H NMR (DMSO-d_6) δ 8.78 and 8.71* (d, J = 7.0 Hz and J* = 6.8 Hz, 1H, F_2Abu-N*H*), 7.90 and 7.89* (d, J = 8.2 Hz and J* = 8.4 Hz, 1H Leu-N*H*), 7.02 (d, J = 7.0 Hz, 1H, $H_{\text{indoline4}}$), 6.96 (t, J = 7.4 Hz, 1H, $H_{\text{indoline6}}$), 6.72-6.57 (m, 2H, $H_{\text{indoline7,5}}$), 6.08 and 6.05* (tm, J_{HF} = 55.4 Hz and J^*_{HF} = 56.4 Hz, 1H, F_2Abu-H_γ), 4.89 and 4.79* (m, 1H, F_2Abu-H_α), 4.39-4.22 (m, 2H, Leu-H_α, $H_{\text{indoline2}}$), 3.30 (m, 1H, $H_{\text{indoline3}}$), 2.96-2.83 (m, 1H, $H_{\text{indoline3}}$), 2.44-2.03 (m, 2H, F_2Abu-H_β), 1.68-1.37 (m, 3H, Leu-H_β, Leu-H_δ), 0.93-0.78 (m, 6H, Leu-H_δ); MS (ES^+) m/z 426 $(M + H)^+$; Analytical RP-HPLC retention time 5.93 min and 6.03* min (method 1); Fraction 2 (**7b**, 3.0 mg, 4 %, retention time 18.0-18.8 min, single diastereoisomer (keto form only) observed by ^{1}H nmr.) ^{1}H NMR (DMSO-d_6) δ 8.70 (d, J = 7.0 Hz, 1H, F_2Abu-N*H*), 7.83 (d, J = 8.2 Hz, 1H, Leu-N*H*), 6.99 (d, J = 7.5 Hz, 1H, $H_{\text{indoline4}}$), 6.94 (t, J = 7.5 Hz, 1H, $H_{\text{indoline6}}$), 6.60-6.57 (m, 2H, $H_{\text{indoline7,5}}$), 6.05 (tm, J_{HF} = 57.5 Hz, 1H, F_2Abu-H_γ), 4.90-4.82 (m, 1H, F_2Abu-H_α), 4.38-4.26 (m, 1H, Leu-H_α), 4.25 (dd, J = 10.2 Hz, 8.7 Hz, 1H, $H_{\text{indoline2}}$), 3.25 (dd, J = 16.1 Hz, 10.2 Hz, 1H, $H_{\text{indoline3}}$), 2.88 (dd, J = 16.1 Hz, 8.7 Hz, 1H, $H_{\text{indoline3}}$), 2.39-2.30 (m, 1H, F_2Abu-H_β), 2.22-2.08 (m, 1H, F_2Abu-H_β), 1.68-1.58 (m, 1H, Leu-H_γ), 1.54-1.41 (m, 2H, Leu-H_β), 0.89 (d, J = 6.6 Hz, 3H, Leu-H_δ), 0.85 (d, J = 6.6 Hz, 3H, Leu-H_δ); MS (ES^+) m/z 426 $(M + H)^+$; Analytical RP-HPLC retention time 6.21 min (method 1); Fraction 3 (**7c**, 3.7 mg, 5 %, retention time 20.6-21.0 min, single diastereoisomer by ^{1}H nmr. Both the keto and hydrate forms (8:1) are observed. Only the keto form is assigned) ^{1}H NMR (DMSO-d_6) δ 8.82 (d, J = 6.9 Hz, 1H, F_2Abu-N*H*), 7.87 (d, J = 8.3 Hz, 1H, Leu-N*H*), 7.01 (d, J = 7.2 Hz, 1H, $H_{\text{indoline4}}$), 6.95 (t, J = 7.2 Hz, 1H, $H_{\text{indoline6}}$), 6.69-6.56 (m, 2H, $H_{\text{indoline7,5}}$), 6.08 (tm, J_{HF} = 56.3 Hz, 1H, F_2Abu-H_γ), 4.82-4.75 (m, 1H, F_2Abu-H_α), 4.41-4.33 (m, 1H, Leu-H_α), 4.26 (dd, J = 10.2 Hz, 8.2 Hz, 1H, $H_{\text{indoline2}}$), 3.29 (dd, J = 16.2 Hz, 10.2 Hz, 1H, $H_{\text{indoline3}}$), 2.91 (dd, J = 16.2 Hz, 8.2 Hz, 1H, $H_{\text{indoline3}}$), 2.45-2.08 (m, 2H, F_2Abu-H_β), 1.59-1.36 (m, 3H, Leu-H_β, Leu-H_γ), 0.85 (d, J = 6.3 Hz, 3H, Leu-H_δ), 0.84 (d, J = 6.3 Hz, 3H, Leu-H_δ); MS (ES^+) m/z 426 $(M + H)^+$; Analytical RP-HPLC retention time 6.93 min (method 1).

3-({*N*-[(2-cyclohexyl-2,3-dihydro-1*H*-indol-2-yl)carbonyl]-L-leucyl}amino)-5,5-difluoro-2-oxopentanoic acid trifluoroacetate (7d, 7e)

A solution of **3b** (427 mg, 1.19 mmol) in a 1:1 mixture of H_2O / THF (6 mL) was treated with aqueous NaOH (3 mL, 2 N). The solution was stirred at 90 °C for 72 h then cooled to

0 °C, diluted with H_2O and EtOAc, and acidified to pH 2 by dropwise addition of aqueous HCl (1 N). The organic layer was separated, and the aqueous layer was extracted EtOAc. The combined organic layers were washed with brine and dried. Removal of the solvent afforded 389 mg (95 %) of **5b** as a solid. MS (ES^+) m/z 344 $(M + H)^+$.

A portion the material from above (100 mg, 0.29 mmol) was taken up in CH_2Cl_2 (5 mL) and treated with DIEA (0.103 mL, 0.58 mmol) and H-(L)-Leu-OBn (75.1 mg, 0.29 mmol). HATU (110.7 mg, 0.29 mmol) was added and the mixture was stirred for 12 h then diluted with EtOAc and saturated aqueous $NaHCO_3$. The organic layer was separated, washed with brine and dried. Removal of the organic solvent afforded a residue that was filtered through a short path of silica gel (toluene/EtOAc, 98:2) to give a solid; MS (ES^+) m/z 547 $(M + H)^+$. This material was taken up in MeOH (10 mL) and treated with Pd/C (10 mg, 10 % w / w). The solution was stirred under hydrogen (30 psi) for 1 h then purged with nitrogen and filtered. The filtrate was concentrated to afford N-{[1-(*tert*-butoxycarbonyl)-2-cyclohexyl-2,3-dihydro-1*H*-indol-2-yl]carbonyl}-L-leucine 57 mg (43 %) as a white solid; ^{1}H NMR (DMSO-d_6) δ (1:1* mixture of diastereoisomers) 7.85 and 7.79* (app br s, 1H, $H_{\text{indoline7}}$), 7.60 and 7.55* (d, J = 8.1 and 8.1* Hz, 1H, Leu-N*H*), 7.17 (d, J = 7.5 Hz, 1H, $H_{\text{indoline4}}$), 7.13 (t, J = 7.5 Hz, 1H, $H_{\text{indoline5}}$), 6.94 (t, J = 7.5 Hz, 1H, $H_{\text{indoline6}}$), 4.28 (m, 1H, Leu-H_α), 3.56 and 3.52* (d, J = 16.4 and J* = 16.4 Hz, 1H, $H_{\text{indoline3}}$), 3.15 (m, 1H, $H_{\text{indoline3}}$ partly obscured by residual water signal), 2.81-2.72 and 2.70-2.66* (m, 1H, C_6H_{11}-H_1), 1.95-1.59 (m, 7H, Leu-H_γ, Leu-H_β, C_6H_{11}-H_{2eq}, C_6H_{11}-H_{2eq}), 1.54 and 1.53* (s, 9H, $C(CH_3)_3$), 1.43-1.33 (m, 1H, C_6H_{11}-H_{4eq}), 1.30-0.90 (m, 5H, C_6H_{11}-H_{2ax}, C_6H_{11}-H_{3ax}, C_6H_{11}-H_{4ax}), 0.88 and 0.85* (d, J = 6.5 and J* = 6.0 Hz, 3H, Leu-H_δ), 0.87 and 0.77* (d, J = 6.5 and J* = 6.0 Hz, 3H, Leu-H_δ); MS (ES^+) m/z 458 $(M + H)^+$.

A solution of the above product (52 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) was treated with HOBt (17.4 mg, 0.11 mmol), DIEA (0.02 mL, 0.11 mmol) and (+/-)-H-difluoroAbu-$CH(OH)CO_2Me$ (24.9 mg, 0.11 mmol). EDC (21.7 mg, 0.11 mmol) was added and the mixture was stirred for 12 h at room temperature. The solution was diluted with EtOAc and aqueous HCl (5 mL, 1 N). The organic layer was separated, washed with brine and dried. Removal of the solvent and filtration through silica gel (petroleum ether/EtOAc, 2:1) afforded (46 mg, 67 %) of **6b** as a foam. This material was dissolved in CH_2Cl_2 (2 mL) containing *tert*-BuOH (16.4 mg, 0.22 mmol) and then treated with Dess-Martin periodinane (93.8 mg, 0.22 mmol). The reaction was monitored by electro-spray mass

spectrometry and was judged complete after 6 h. The mixture was diluted with EtOAc and washed successively with a 1:1 mixture of saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ (4 x), water and brine. The dried organic extracts were concentrated to afford a solid that was dissolved in a 65/35/5 mixture of TFA/CH_2Cl_2/H_2O (2 mL). After stirring for 1 h the solution was concentrated to dryness, and the residue was taken up in a mixture of MeOH (5 mL) and aqueous NaOH (1 mL, 1 N). After 0.5 h the solution was diluted with EtOAc and H_2O and cooled to 0 °C. The pH was adjusted to 2 by addition of aqueous HCl (1 N) and the organic layer was separated, washed with brine and dried. The solvents were removed, and the residue was purified by RP-HPLC (method 1) to afford two fractions containing the title compound. Fraction 1 (**7e**, 2.7 mg, 6 %, retention time 9.6-9.7 min, 0.05:0.05:0.2:1 mixture of diastereoisomers by ^{1}H nmr. Both the keto and hydrate* forms (1:1*) of the major diastereoisomer are observed and are assigned) ^{1}H NMR (DMSO-d_6) δ 8.81 and 7.85* (d, J = 7.2 Hz and J* = 8.3 Hz, 1H, F_2Abu-N*H*), 7.70-7.63 (m, 1H, Leu-N*H*), 7.00-6.86 (m, 2H, $H_{\text{indoline4,6}}$), 6.58 (d, J = 7.2 Hz, 1H, $H_{\text{indoline7}}$), 6.52 (t, J = 7.2 Hz, 1H, $H_{\text{indoline5}}$), 6.20-5.86 and 6.10*-5.75* (tm, J = 55.7 Hz and J* = 57.6 Hz, 1H, F_2Abu-H_γ), 4.75-4.70 and 4.10-4.01* (m, 1H, F_2Abu-H_α), 4.25-419 (m, 1H, Leu-H_α), 3.10-3.01 (m, 2H, $H_{\text{indoline3}}$), 2.55-2.45 (m, obscured by residual solvent signal, 1H, C_6H_{11}-H_1), 2.39-2.20 and 2.10-1.89* (m, 2H, F_2Abu-H_γ), 1.81 (m, 1H, Leu-H_γ), 1.70-1.31 (m, 8H, Leu-H_β, C_6H_{11}-H_{2eq}, C_6H_{11}-H_{3eq}, C_6H_{11}-H_{4eq}), 1.21-0.97 (m, 5H, C_6H_{11}-H_{2ax}, C_6H_{11}-H_{3ax}, C_6H_{11}-H_{4ax}), 0.93-0.74 (m, 6H, Leu-H_δ); MS (ES^+) m/z 508 $(M + H)^+$; Analytical RP-HPLC: retention time (major isomer) 8.38 min (method 1), 7.35 min (method 2); HRMS calculated for $C_{26}H_{34}F_2N_3O_5$ $(M - H)^-$ 506.2466; found 506.2479. Fraction 2 (**7d**, 7.2 mg, 16 %, retention time 9.7-10.5 min, 1:1*:1**:0.2 mixture of diastereoisomers by ^{1}H nmr. Both the keto and hydrate forms (95:5) are observed. Only the keto form of the major three isomers is assigned). The keto form of the major three isomers is assigned. ^{1}H NMR (DMSO-d_6) δ 8.86 and 8.70* and 8.69** (d, J = 7.2 Hz and J* = 6.9 Hz and J** = 6.9 Hz, 1H, F_2Abu-N*H*), 7.74-7.65 (m, 1H, Leu-N*H*), 6.95-6.85 (m, 2H, $H_{\text{indoline4,6}}$), 6.60-6.50 (m, 2H, $H_{\text{indoline5,7}}$), 6.25-5.70 (m, 1H, F_2Abu-H_γ), 4.96 and 4.88* and 4.75** (m, 1H, F_2Abu-H_α), 4.38-4.28 (m, 1H, Leu-H_γ), 3.11-2.95 (m, 2H, $H_{\text{indoline3}}$), 2.55-2.45 (m, obscured by residual solvent signal, 1H, C_6H_{11}-H_1), 2.41-2.27 (m, 1H, F_2Abu-H_β), 2.26-2.05 (m, 1H, Leu-H_β), 1.78 (m, 1H, Leu-H_γ), 1.70-1.30 (m, 7H, Leu-H_β, C_6H_{11}-H_{2eq}, C_6H_{11}-H_{3eq}, C_6H_{11}-H_{4eq}), 1.25-1.00 (m, 5H, C_6H_{11}-H_{2ax}, C_6H_{11}-H_{3ax}, C_6H_{11}-H_{4ax}), 0.92-0.72 (m, 6H, Leu-H_δ); MS (ES^+) m/z 508 $(M + H)^+$; Analytical RP-

HPLC: retention time 8.30 min, 8.43* min, 8.79** min (method 1), 7.25 min, 7.50* min, 7.60** min (method 2); HRMS calculated for $C_{26}H_{34}F_2N_3O_5$ $(M - H)^-$ 506.2466; found 506.2464.

3-[(*N*-{[2-(cyclobutylmethyl)-2,3-dihydro-1*H*-indol-2-yl]carbonyl}-L-leucyl)amino]-5,5-difluoro-2-oxopentanoic acid trifluoroacetate (7f, 7g)

A solution of **3c** (286 mg, 0.83 mmol) in a 1:1 mixture of THF/H_2O (6 mL) was treated with $LiOH.H_2O$ (310 mg, 4.14 mmol) and was heated to 80 °C for 16 h. The mixture was cooled and diluted with EtOAc and aqueous HCl (1 N). The organic layer was separated, washed with brine and dried. Removal of the solvent afforded the carboxylic acid **5c** (260 mg, 95 %). A portion of this material (240 mg, 0.72 mmol) was dissolved in CH_2Cl_2 (5 mL) and was treated with H-(L)-Leu-OBn (187 mg, 0.72 mmol) and HATU (317 mg, 0.83 mmol). DIEA (187 mg, 1.45 mmol) was added and the mixture was stirred for 16 h. After dilution with EtOAc and aqueous HCl (1 N) the organic phase was separated and washed with saturated aqueous $NaHCO_3$ and brine. The dried organic layer was concentrated, and the residue was purified by flash chromatography on SiO2 (EtOAc:petroleum ether, 5:95) to afford in the first fractions 171 mg (44 %) of a single diastereoisomer of N-{[1-(*tert*-butoxycarbonyl)-2-(cyclobutylmethyl)-2,3-dihydro-1*H*-indol-2-yl]carbonyl}-L-leucine (fraction A; MS (ES^+) m/z 535 $(M + H)^+$). The later fractions contained 110 mg (28 %) of a second diastereoisomer (fraction B; MS (ES^+) m/z 535 $(M + H)^+$).

A portion of the amide from fraction A (170 mg, 0.32 mmol) was dissolved in MeOH then treated with Pd/C (17 mg, 10 % w / w) and stirred under a positive pressure of hydrogen for 2 h. The mixture was filtered and the filtrate was concentrated to afford a solid. A portion of this material (130 mg, 0.29 mmol) was taken up with CH_2Cl_2 (5 mL) and treated with (D,L)-H-F_2Abu-CH(OH)CO_2Me (64.2 mg, 0.29 mmol) and HOBt (89.6 mg, 0.58 mmol). EDC (57.2 mg, 0.30 mmol) was added and the mixture was stirred for 16 h. EtOAc and aqueous HCl (1 N) were added and the organic layer was separated and washed with saturated aqueous $NaHCO_3$ and brine then dried. Removal of the solvent afforded **6c** (129 mg, 72 %) as a solid. MS (ES^+) m/z 610 $(M + H)^+$.

A portion of this material (120 mg, 0.20 mmol) was taken up in CH_2Cl_2 and treated with *tert*-BuOH (43.8 mg, 0.59 mmol) and Dess Martin periodinane (251 mg, 0.59 mmol). The reaction mixture was stirred for 25 min then judged complete by electro-spray mass

spectrometry. The mixture was diluted with EtOAc and washed successively with a 1:1 mixture of saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ (4 x), water and brine. The dried organic extracts were concentrated to afford a solid (120 mg) that was dissolved in a 65/35/5 mixture of TFA/CH_2Cl_2/H_2O (5 mL). After stirring for 1 h the solution was concentrated to dryness, and the residue was taken up in a mixture of MeOH (5 mL) and aqueous NaOH (1.5 mL, 1 N). After 15 min the solution was diluted with EtOAc and H_2O and cooled to 0 °C. The pH was adjusted to 2 by addition of aqueous HCl (1 N) and the organic layer was separated, washed with brine and dried. After removal of the solvent the residue was purified by RP-HPLC (method 1) to afford the title compound (**7f**, 18 mg, 15 %, retention time 8.7-9.4 min, 1:1* mixture of diastereoisomers (keto form only) by ^{1}H nmr) as a solid. ^{1}H NMR (DMSO-d_6) δ 8.82 and 8.70* (d, J = 6.7 Hz and J* = 7.0 Hz, 1H, F_2Abu-N*H*), 7.70 and 7.68* (d, J = 4.2 Hz and J* = 4.5 Hz, Leu-N*H*), 6.95 (d, J = 7.5 Hz, 1H, $H_{\text{indoline4}}$), 6.93 (t, J = 7.5 Hz, 1H, $H_{\text{indoline6}}$), 6.58 (d, J = 7.5 Hz, 1H, $H_{\text{indoline7}}$), 6.57 (t, J = 7.5 Hz, 1H, $H_{\text{indoline5}}$), 6.03 (tm, J = 56.3, 1H, F_2Abu-H_α), 4.87 and 4.74* (m, 1H, F_2Abu-H_α), 4.34-4.25 (m, 1H, Leu-H_α), 3.04 and 3.02* (d, J = 16.2 Hz and J* = 16.2 Hz, 1H, $H_{\text{indoline3}}$), 2.86 (d, J = 16.2 Hz, 1H, $H_{\text{indoline3}}$), 2.40-2.25 (m, 2H, CH_2C$_4$H$_7$, F_2Abu-H_β), 2.21-2.03 (m, 1H, F_2Abu-H_β), 1.94-1.83 (m, 2H, CH_2C$_4$H$_7$, C$_4H_7$), 1.81-1.55 (m, 5H, C$_4H_7$), 1.50-1.32 (m, 3H, Leu-H_β, Leu-H_γ), 0.90 and 0.89* (d, J = 6.5 Hz and J* = 6.5 Hz, 3H, Leu-H_δ), 0.86 (d, J = 6.5 Hz, 3H, Leu-H_δ); Analytical RP-HPLC: retention time 8.30 min and 8.51* min (method 1), 6.70 min and 6.98* min (method 2); MS (ES$^-$) m/z 492 (M - H)$^-$; HRMS calculated for $C_{25}H_{32}F_2N_3O_5$ (M - H)$^-$ 492.2310; found 492.2252.

Repetition of the above procedure with the carboxylic acid obtained in fraction B above afforded a residue that was purified by RP-HPLC (method 1) to afford the title compound (**7g**, 18 %, retention time 8.8-9.4 min, 1:1* mixture of diastereoisomers (keto form only) by ^{1}H nmr) as a solid. ^{1}H NMR (DMSO-d_6) δ 8.89 and 8.73* (d, J = 6.9 Hz and J* = 7.2 Hz, 1H), 7.72 and 7.70* (d, J = 3.4 Hz and J* = 3.4 Hz, 1H), 6.95 (d, J = 7.5 Hz, 1H), 6.93 (t, J = 7.5 Hz, 1H), 6.57 (d, J = 7.5 Hz, 1H), 6.56 (d, J = 7.5 Hz, 1H), 6.07 (tm, J_{HF} = 56.3 Hz, 1H), 4.94 and 4.76* (m, 1H), 4.41-4.23 (m, 1H), 3.10 (d, J = 16.1 Hz, 1H), 2.87 (d, J = 16.1 Hz, 1H), 2.44-2.25 (m, 2H), 2.34-1.96 (m, 1H), 1.97-1.84 (m, 2H), 1.82-1.53 (m, 7H), 1.46-1.32 (m 3H), 0.83-0.71 (m, 6H); MS (ES$^+$) m/z 494 (M + H)$^+$; Analytical RP-HPLC: retention time 8.61 min and 8.80* min (method 1), 6.58 min and 6.78* min (method 2); HRMS calculated for $C_{25}H_{32}F_2N_3O_5$ (M - H)$^-$ 492.2310; found 492.2279.

3-({*N*-[(2-{[5-chlorothien-2-yl]methyl}-2,3-dihydro-1*H*-indol-2-yl)carbonyl]-L-leucyl}amino)-5,5-difluoro-2-oxopentanoic acid trifluoroacetate (7h)

A solution of (**3d**) (490 mg, 1.20 mmol) in a 3:1:1 mixture of MeOH/THF/H_2O (12 mL) was treated with LiOH.H_2O (504 mg, 12.0 mmol). The mixture was heated under reflux for 6.5 h then cooled, acidified with aqueous HCl (1 N) and extracted with EtOAc. Concentration of the dried organics afforded the title compound 405 mg (88 %) of **5d** as a solid.

Treatment of the above compound as described in the general procedure for **7a** afforded a residue that was purified by RP-HPLC (method 1) to afford the title compound (**7h**, 3.7 mg, 5 %, retention time 9.5-9.7 min, single diastereoisomer (keto-form only) by ^{1}H nmr) as a solid. ^{1}H NMR (DMSO-d_6) δ 8.45 (br s, 1H, F_2Abu-N*H*), 7.86 (d, *J* = 8.3 Hz, 1H, Leu-N*H*), 7.03-6.85 (m, 2H, $H_{\text{indoline4,6}}$), 6.84 (d, *J* = 3.7 Hz, 1H, $H_{\text{thiophene3}}$), 6.71 (d, *J* = 3.7 Hz, 1H, $H_{\text{thiophene4}}$), 6.61 (d, *J* = 7.4 Hz, 1H, $H_{\text{indoline7}}$), 6.54 (t, *J* = 7.4 Hz, 1H, $H_{\text{indoline5}}$), 6.01 (tm, J_{HF} = 56.1 Hz, 1H, F_2Abu-H_γ), 4.94-4,76 (m, 1H, F_2Abu-H_α), 4.44-4.21 (m, 1H, Leu-H_α), 3.27 (d, *J* = 15.3 Hz, 1H, CH_2C$_4$H$_2$ClS), 3.16 (d, *J* = 16.8 Hz, 1H, $H_{\text{indoline3}}$), 3.11 (d, *J* = 15.3 Hz, 1H, CH_2C$_4$H$_2$ClS), 2.99 (d, *J* = 16.8 Hz, 1H, $H_{\text{indoline3}}$), 2.49-2.23 (m, 1H, F_2Abu-H_β), 2.29-1.87 (m, 1H, F_2Abu-H_β), 1.65-1.25 (m, 3H, Leu-H_β, Leu-H_γ), 0.92-0.68 (m, 6H, Leu-H_δ); MS (ES^+) m/z 557 $(M + H)^+$; Analytical RP-HPLC: retention time 8.9 min (method 1), 7.78 min (method 2); HRMS calculated for $C_{25}H_{27}ClF_2N_3O_5S$ $(M-H)^-$ 554.1328; found 554.1304.

3-({*N*-[(2-{[2-(*tert*-butoxycarbonyl)thien-3-yl]methyl}-2,3-dihydro-1*H*-indol-2-yl)carbonyl]-L-leucyl}amino)-5,5-difluoro-2-oxopentanoic acid trifluoroacetate (7i-l)

A solution of **3e** (0.94 g, 1.71 mmol) in MeOH (50 mL) was treated with Pd/C (0.16 g, 30 % w/w) and stirred under an atmosphere of hydrogen for 18 h. The solution was purged with nitrogen, filtered and concentrated to give **5e** (0.78 g, 99 %) as an oil.

Treatment of the above compound as described in the general procedure for **7a** afforded a residue that was purified by RP-HPLC (method 1) to give four fractions containing the title compound: Fraction 1 (**7k**, 13.0 mg, 11 %, retention time 7.4-7.7 min, single diastereoisomer by ^{1}H nmr. Both the keto and hydrate* forms (2:1*) are observed and are assigned) ^{1}H NMR (DMSO-d_6; 330 K) δ 12.94 (br s, 1H, CO_2H), 8.72 and 7.73* (d, *J* = 6.8 Hz and *J** = 8.4 Hz, 1H, F_2Abu-N*H*), 7.73 (d, *J* = 8.4 Hz, 1H, Leu-N*H*), 7.58 (d, *J* = 5.1 Hz,

1H, $H_{thiophene5}$), 7.00 (d, J = 5.1 Hz, 1H, $H_{thiophene4}$), 6.91-6.84 (m, 2H, $H_{indoline4,6}$), 6.56 (d, J = 7.6 Hz, 1H, $H_{indoline7}$), 6.48 (t, J = 7.6 Hz, 1H, $H_{indoline5}$), 6.04 and 5.82* (tm, J_{HF} = 56.6 Hz and J_{HF}* = 55.9 Hz, 1H, F_2Abu-H_γ), 4.88-4.82 and 4.28-4.20* (m, 1H, F_2Abu-H_α), 4.37-4.30 (m, 1H, Leu-H_α), 3.74 (d, J = 13.4 Hz, 1H, $H_{indoline3}$), 3.24 (d, J = 13.4 Hz, 1H, $H_{indoline3}$), 3.09 (app s, 2H, CH_2C$_5$H$_3$O$_2$S), 2.35-2.10 (m, 2H, F_2Abu-H_β), 1.57-1.31 (m, 3H, Leu-H_β, Leu-H_γ), 0.92-0.77 (m, 6H, Leu-H_δ); MS (ES^+) m/z 566 $(M + H)^+$; Analytical RP-HPLC: retention time 7.05 min (method 1), 5.92 min (method 2); HRMS calculated for $C_{26}H_{28}F_2N_3O_7S$ $(M - H)^-$ 564.1616; found 564.1623; Fraction 2 (**7j**, 3.0 mg, 3 %, retention time 7.8-7.9 min, single diastereoisomer by ^{1}H nmr. Only the keto form is observed and assigned) ^{1}H NMR (300 MHz; DMSO-d_6; 300 MHz) δ 8.77 (d, J = 7.1 Hz, 1H, F_2Abu-NH), 7.73 (d, J = 8.5 Hz, 1H, Leu-NH), 7.59 (d, J = 5.1 Hz, 1H, $H_{thiophene5}$), 7.01 (d, J = 5.1 Hz, 1H, $H_{thiophene4}$), 6.93-6.80 (m, 2H, $H_{indoline4,6}$), 6.53 (d, J = 7.6 Hz, 1H, $H_{indoline7}$), 6.52 (d, J = 7.6 Hz, 1H, $H_{indoline5}$), 6.03 (tm, J_{HF} = 56.1 Hz, 1H, F_2Abu-H_γ), 4.82-4.72 (m, 1H, F_2Abu-H_α), 4.40-4.29 (m, 1H, Leu-H_α), 3.74 (d, J = 13.4 Hz, 1H, $H_{indoline3}$), 3.23 (d, J = 13.4 Hz, 1H, $H_{indoline3}$), 3.09 (app s, 2H, CH_2C$_5$H$_3$O$_2$S), 2.40-1.94 (m, 2H, F_2Abu-H_β), 1.56-1.32 (m, 3H, Leu-H_β, Leu-H_γ), 0.86 (app d, J = 6.0 Hz, Leu-H_δ); MS (ES^-) m/z 564 $(M - H)^-$; Analytical RP-HPLC: retention time 6.97 min (method 1), 4.92 min (method 2); HRMS calculated for $C_{26}H_{28}F_2N_3O_7S$ $(M - H)^-$ 564.1616; found 564.1582; Fraction 3 (**7i**, 3.0 mg, 3 %, retention time 7.9-8.1 min, single diastereoisomer by ^{1}H nmr. Only the keto form is observed and assigned) ^{1}H NMR (300 MHz; DMSO-d_6) δ 12.98 (br s, 1H, CO_2H), 8.76 (d, J = 7.0 Hz, 1H, F_2Abu-NH), 7.82 (d, J = 8.3 Hz, 1H, Leu-NH), 7.53 (d, J = 5.1 Hz, 1H, $H_{thiophene5}$), 7.04 (d, J = 5.1 Hz, 1H, $H_{thiophene4}$), 6.94-6.79 (m, 2H, $H_{indoline4,6}$), 6.51 (d, J = 7.8 Hz, 1H, $H_{indoline7}$), 6.43 (t, J = 7.8 Hz, 1H, $H_{indoline5}$), 6.10 (tm, J_{HF} = 56.6 Hz, 1H, F_2Abu-H_γ), 5.01-4.90 (m, 1H, F_2Abu-H_α), 4.41-4.27 (m, 1H, Leu-H_α), 3.72 (d, J = 13.6 Hz, 1H, $H_{indoline3}$), 3.29 (d, J = 13.6 Hz, 1H, $H_{indoline3}$), 3.09 (app s, 2H, CH_2C$_5$H$_3$O$_2$S), 2.44-2.02 (m, 2H, F_2Abu-H_β), 1.50-1.30 (m, 3H, Leu-H_β, Leu-H_γ), 0.81 (d, J = 6.3 Hz, 1H, Leu-H_δ), 0.79 (d, J = 6.3 Hz, 3H, Leu-H_δ); MS (ES^-) m/z 566 $(M - H)^-$; Analytical RP-HPLC: retention time 7.22 min (method 1), 5.44 min (method 2); HRMS calculated for $C_{26}H_{28}F_2N_3O_7S$ $(M - H)^-$ 564.1616; found 564.1598; Fraction 4 (**7l**, 14.6 mg, 13 %, retention time 8.1-8.4 min, single diastereoisomer by ^{1}H nmr. A mixture of the keto:hydrate* (3:1*) forms are observed and assigned) ^{1}H NMR (DMSO-d_6; 330 K) δ 12.98 (br s, 1H, CO_2H), 8.88 and 7.72* (d, J = 6.9 Hz and J* = 7.9 Hz, 1H, F_2Abu-NH), 7.81 (d, J = 8.6 Hz, 1H, Leu-NH), 7.53 (d, J = 5.2 Hz, 1H, $H_{thiophene5}$), 7.00 (d, J = 5.2 Hz, 1H, $H_{thiophene4}$), 6.91-6.82 (m,

2H, $H_{indoline4,6}$), 6.52 (d, J = 7.6 Hz, $H_{indoline7}$)1H, 6.51 (t, J = 7.6 Hz, 1H, $H_{indoline5}$), 6.11 and 5.86* (tm, J_{HF} = 56.1 and J_{HF}* = 57.1, 1H, F_2Abu-H_γ), 4.83-4.77 and 4.35-4.25* (m, 1H, F_2Abu-H_α), 4.43-4.37 (m, 1H, Leu-H_α), 3.74 (d, J = 13.8 Hz, 1H, $H_{indoline3}$), 3.22 (d, J = 13.8 Hz, 1H, $H_{indoline3}$), 3.08 (app s, 2H, CH_2C$_5$H$_3$O$_2$S), 2.45-1.95 (m, 2H, F_2Abu-H_β), 1.50-1.31 (m, 3H, Leu-H_β, Leu-H_γ), 0.83-0.70 (m, 6H, Leu-H_δ); MS (ES$^+$) m/z 566 (M + H)$^+$; Analytical RP-HPLC: retention time 7.28 min (method 1), 5.59 min (method 2); HRMS calculated for $C_{26}H_{28}F_2N_3O_7S$ (M - H)$^-$ 564.1616; found 564.1623.

3-[(N-{[6-(carboxymethoxy)-2,3-dihydro-1*H*-indol-2-yl]carbonyl}-L-leucyl)amino]-5,5-difluoro-2-oxo-pentanoate trifluoroacetate (7m-p)

A solution of **3f** (0.25 g, 0.60 mmol) in a 3:1:1 mixture of MeOH:THF:H_2O (10 mL) was treated with LiOH.H_2O (0.10 g, 2.43 mmol) then stirred for 1.5 h. The mixture was acidified with aqueous HCl (1 N) and extracted with EtOAc. The dried organic phase was concentrated to afford a residue that was dissolved in CH_2Cl_2 (2 mL) and treated with *tert*-BuOH (40 mg, 0.57 mmol) and DMAP (30 mg, 0.26 mmol). The mixture was cooled to 0 °C and treated portionwise with EDC (110 mg, 0.57 mmol). The resulting solution was stirred at 4 °C for 16 h then diluted with CH_2Cl_2 and aqueous HCl (1 N). The organic layer was separated and washed with H_2O then dried. After removal of the solvent the residue was purified by flash chromatography on silica gel (CH_2Cl_2 / MeOH, 9:1) to afford 50 mg (21 %) of **5f** as an oily solid.

Treatment of the above compound as described in the general procedure for **7a** afforded a residue that was purified by RP-HPLC (method 2) to afford four fractions containing the title compound. Fraction 1 (**7m**, 3 mg, 1 %, retention time 22.5-23.5 min, single diastereoisomer by ^{1}H nmr. Both the keto and hydrate* forms (2:1*) are observed and are assigned) ^{1}H NMR (DMSO-d_6) δ 8.76 (s, 1H, F_2Abu-NH), 7.81 (d, J = 8.2 Hz, 1H, Leu-NH), 6.86 (d, J = 8.0 Hz, 1H, $H_{indoline4}$), 6.14 (s, 1H, $H_{indoline7}$), 6.11 (d, J = 8.0 Hz, 1H, $H_{indoline5}$), 6.08 and 6.07* (tm, J_{HF} = 55.9 and J_{HF}* = 55.9 Hz, 1H, F_2Abu-H_γ), 4.84-4.78 and 4.22-4.12* (m, 1H, F_2Abu-H_α), 4.53 (s, 2H, CH_2CO$_2$H), 4.35 (m, 1H, Leu-H_α), 4.25 (dd, J = 10.3 Hz, 8.2 Hz, 1H, $H_{indoline2}$), 3.20 (dd, J = 15.9 Hz, 10.3 Hz, 1H, $H_{indoline3}$), 2.82 (dd, J = 15.9 Hz, 8.2 Hz, 1H, $H_{indoline3}$), 2.61-1.97 (m, 2H, F_2Abu-H_β), 1.71-1.29 (m, 3H, Leu-H_β, Leu-H_γ), l.04 - 0.62 (m, 6H, Leu-H_δ); MS (ES$^+$) m/z 500 (M + H)$^+$; Fraction 2: (**7o**, 3 mg, 1 %, retention time 23.7-24.5 min, single diastereoisomer (keto form only) by ^{1}H nmr). ^{1}H NMR (DMSO-d_6) δ 8.60 (br s,

1H, F_2Abu-N*H*), 7.83 (d, *J* = 8.0 Hz, 1H, Leu-N*H*), 6.86 (d, *J* = 7.8 Hz, 1H, $H_{indoline4}$), 6.14 (s, 1H, $H_{indoline7}$), 6.11 (d, *J* = 7.8 Hz, 1H, $H_{indoline5}$), 6.05 (tm, J_{HF} = 55.8 Hz, 1H, F_2Abu-H_γ), 4.90-4.82 (m, 1H, F_2Abu-H_α), 4.53 (s, 2H, CH_2CO_2H), 4.43-4.15 (m, 2H, Leu-H_α, $H_{indoline2}$), 3.19 (dd, *J* = 16.1 Hz, 10.7 Hz, 1H, $H_{indoline3}$), 2.80 (dd, *J* = 16.1 Hz, 8.3 Hz, 1H, $H_{indoline3}$), 2.61-1.87 (m, 2H, F_2Abu-H_β), 1.81-1.25 (m, 3H, Leu-H_β), 0.90 (d, *J* = 6.5 Hz, 3H, Leu-H_δ), 0.86 (d, *J* = 6.5 Hz, 3H, Leu-H_δ); MS (ES^+) m/z 500 $(M + H)^+$; Fraction 3 (**7p**, 4 mg, 1.5 %, retention time 25.0-26.0 min, single diastereoisomer (keto form only) by ^{1}H nmr). ^{1}H NMR (DMSO-d_6) δ 8.44 (br s, 1H, F_2Abu-N*H*), 7.82 (d, *J* = 8.1 Hz, 1H, Leu-N*H*), 6.86 (d, *J* = 7.9 Hz, 1H, $H_{indoline4}$), 6.14 (s, 1H, $H_{indoline7}$), 6.11 (d, *J* = 7.9 Hz, 1H, $H_{indoline5}$), 6.04 (tm, J_{HF} = 56.0 Hz, 1H, F_2Abu-H_γ), 4.88-4.80 (m, 1H, F_2Abu-H_α), 4.53 (s, 2H, CH_2CO_2H), 4.50-4.10 (m, 2H, Leu-H_α, $H_{indoline2}$), 3.20 (dd, *J* = 15.7 Hz, 10.9 Hz, 1H, $H_{indoline3}$), 2.82 (dd, *J* = 15.7 Hz, 8.3 Hz, 1H, $H_{indoline3}$), 2.63-1.83 (m, 2H, F_2Abu-H_β), 1.73-1.31 (m, 3H, Leu-H_β, Leu-H_γ), 0.98-0.72 (m, 6H, Leu-H_δ); MS (ES^+) m/z 500 $(M + H)^+$; Fraction 4 (**7n**, 4 mg, 1.5 %, retention time 27.6-28.6 min, single diastereoisomer by ^{1}H nmr. Both the keto and hydrate* forms (2:1*) are observed and are assigned). ^{1}H NMR (DMSO-d_6) δ 8.59 (br s, 1H, F_2Abu-N*H*), 7.83 (d, *J* = 8.2 Hz, 1H, Leu-N*H*), 6.86 (d, *J* = 8.0 Hz, 1H, $H_{indoline4}$), 6.14 (s, 1H, $H_{indoline7}$), 6.10 (d, *J* = 8.0 Hz, 1H, $H_{indoline5}$), 6.22 and 6.17* (tm, J_{HF} = 55.6 Hz and J_{HF}^* = 55.0 Hz, 1H, F_2Abu-H_γ), 4.82-4.77 and 4.22-4.12* (m, 1H, F_2Abu-H_α), 4.52 (s, 2H, CH_2CO_2H), 4.48-4.12 (m, 2H, Leu-H_α, $H_{indoline2}$), 3.19 (dd, *J* = 16.1 Hz, 10.5 Hz, 1H, $H_{indoline3}$), 2.81 (dd, *J* = 16.1 Hz, 8.3 Hz, 1H, $H_{indoline3}$), 2.55-1.95 (m, 2H, F_2Abu-H_β), 1.83-1.25 (m, 3H, Leu-H_β, Leu-H_γ), 1.02-0.7 (m, 6H, Leu-H_δ); MS (ES^+) m/z 500 $(M + H)^+$.

X-ray Structure Determination and Analysis.

The NS3 protein was expressed, purified and crystallized in complex with the NS4A cofactor peptide as previously described[3] The ternary complex with **7i** was prepared by addition of 5 mM inhibitor to the stabilized NS3J/4A crystals (in 4.5 M NaCl, 10 mM DTT, 0.1 M citrate buffer, pH 5.1), and equilibrating for two weeks before mounting. The X-ray diffraction data were collected, at 100 K, at the beam line ID14/H3 (ESRF, Grenoble), using 30% glycerol as cryoprotectant. Data were integrated and scaled with the HKL suite[4] and with the CCP4 suite[5]. Crystals belong to the space group $P6_1$ with two molecules in the asymmetric unit. A summary of the diffraction data is presented in Table 1. The starting model of the inhibited structure was obtained by rigid body of the refined native coordinates[3] within AmoRe.[6] The unique inhibitor was built into the initial, clearly interpretable $2F_o - F_c$ and $F_o - F_c$

density maps. Refinement, using a maximum likelihood target function, was performed with REFMAC[7]. All data were used (no σ cutoff) from 20 Å to the high resolution limit of the data set (Table 1). Modelling of solvent sites was executed with an automatic refinement program, ARP[8]. In the refinement, 5% of the data was set aside for use as a cross validation set[9]. Refinement was continued interspersed with manual model building with the program O.[10] The final statistics are given in Table 1 below. The coordinates of the structure have been deposited in the Protein Data Bank (Coordinate ID code: 1w3c; Structure factor code: r1w3csf).

Table 1 : Data collection and refinement statistics

Data set[1]	1. NS3/4A + inhibitor
a, b (Å)	92.411
c (Å)	80.007
Resolution (Å)[2]	2.3 (2.42-2.3)
R_{merge}(%)[3]	3.7 (19.5)
<I/σI>	15.7 (3.0)
Number of measurements	44,944
Number of unique observations	16,243
Completeness (%)	93.7 (95.0)
Refinement statistics	
Resolution (Å)	20-2.3
R-factor[4]	0.177
R_{free}[5]	0.240
R.m.s.d.[6] bond lengths (Å)	0.008
R.m.s.d. bond angles (Å)	0.030
Overall B-factor (Å^2)	51.15
φψ angle distribution[7]	
in core region	274 (92.6)
in additionally allowed reg	21 (7.1)
in generously allowed regi	1 (0.3)
in disallowed region	0 (0)
Number of atoms	
in structure	2,988

in NS3/4A	2,714
in inhibitor	78
solvent	196

[1] λ: data set 1, 0.91160 Å.

[2] Highest resolution of data set with highest resolution bin in parentheses.

[3] $R_{merge} = \sum_{hkl} \sum_{i=1}^{N} | < I^{hkl} > - I_i^{hkl} | \; / \sum_{hkl} \sum_{i=1}^{N} I_i^{hkl}$.

[4] R-factor = $\sum_h | F_o - F_c | \; / \sum_h | F_o |$.

[5] R_{free} is calculated from 5% of the data which were omitted during the course of the refinement.

[6] R.m.s.d. is the root mean square deviation from ideal geometry.

[7] As defined by PROCHECK[11]; the percentage distribution is given in parentheses.

Biochemical Assay Protocol

Compounds were assessed for activity against the HCV NS3 protease domain in the presence of NS4A cofactor peptide following the procedure we have described.[12] In this assay the NS3 enzyme used is a complex between the protease domain (residues 1027-1206 of NS3 followed by the solubilizing sequence ASKKKK) and a co-factor peptide (Pep4AK) that comprises residues 21-34 of NS4A and a solubilizing tag (KKKGSVVIVGRIILSGR-NH2). The synthetic substrates were based on the peptide sequences of the HCV polyprotein at the NS4A/NS4B (DEMEECASHLPYK) and NS5A/5B (EAGDDIVPCSMSYTWTGA) ring junctions and are denoted Pep4AB and Pep5AB respectively. Assays were performed in 60 µL hepes (50 mM), pH 7.5, 1 % (w / v) CHAPS, 15 % (v / v) glycerol, 10 mM DTT containing 80 µM Pep4AK. Buffer solutions were pre-incubated for 10 min with 10-200 nM protease and reactions were started by addition of 10 µM substrate. Six data points at differing substrate concentrations were measured in duplicate and were used to calculate kinetic parameters. Incubation times were chosen in order to obtain < 7 % substrate conversion and reactions were stopped by addition of 40 µL TFA (1 %). 90 µL samples were injected on a lichrospher C_{18} reversed phase cartridge column (4 mm x 125 mm x 5 um) connected to a Merck Hitachi chromatograph operating at 2.5 mL/min. A linear gradient of 10 % - 90 % MeCN over 15 min was used to separate substrate and cleavage product fragments. Peak monitoring was done by following tyrosine (λ_{ex} = 260 nm, λ_{em} = 305 nm) or tryptophan (λ_{ex} = 280 nm, λ_{em} = 350 nm) fluorescence. Cleavage products were quantitated by integration of the chromatograms with respect to appropriate standards. Kinetic parameters and IC_{50} values were

calculated form non-linear least squares fit of initial rates as a function of the substrate concentration assuming Michaelis-Menten kinetics; functionalities embedded in the Kaleidagraph and Sigmaplot software products were utilized for data fitting.

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