MedChemComm

Electronic Supplementary Information

Peptidomimetic Nitriles as Selective Inhibitors for the Malarial Cysteine Protease Falcipain-2

Veronika Ehmke,[a] Falco Kilchmann,[a] Cornelia Heindl,[b] Kunqiang Cui,[c] Jin Huang,[c] Tanja Schirmeister[b] and François Diederich*[a]

[a] *Laboratory of Organic Chemistry, ETH Zürich, Wolfgang-Pauli-Strasse 10, 8093 Zürich, Switzerland. Fax: (+41)44-632-1109; E-mail: diederich@org.chem.ethz.ch*

[b] *Institute of Pharmacy and Food Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany.*

[c] *School of Pharmacy, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China.*

Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Table of Contents

Figure 1ESI SI-1

Figure 2ESI SI-2

Biological Activities SI-3

Synthesis SI-5

Materials and Methods SI-5

Reaction Schemes 1ESI-4ESI for Ligands **6**–**9** SI-8

Synthetic Protocols for Ligands **1a**–**i**, **6**–**9** SI-10

Literature SI-54

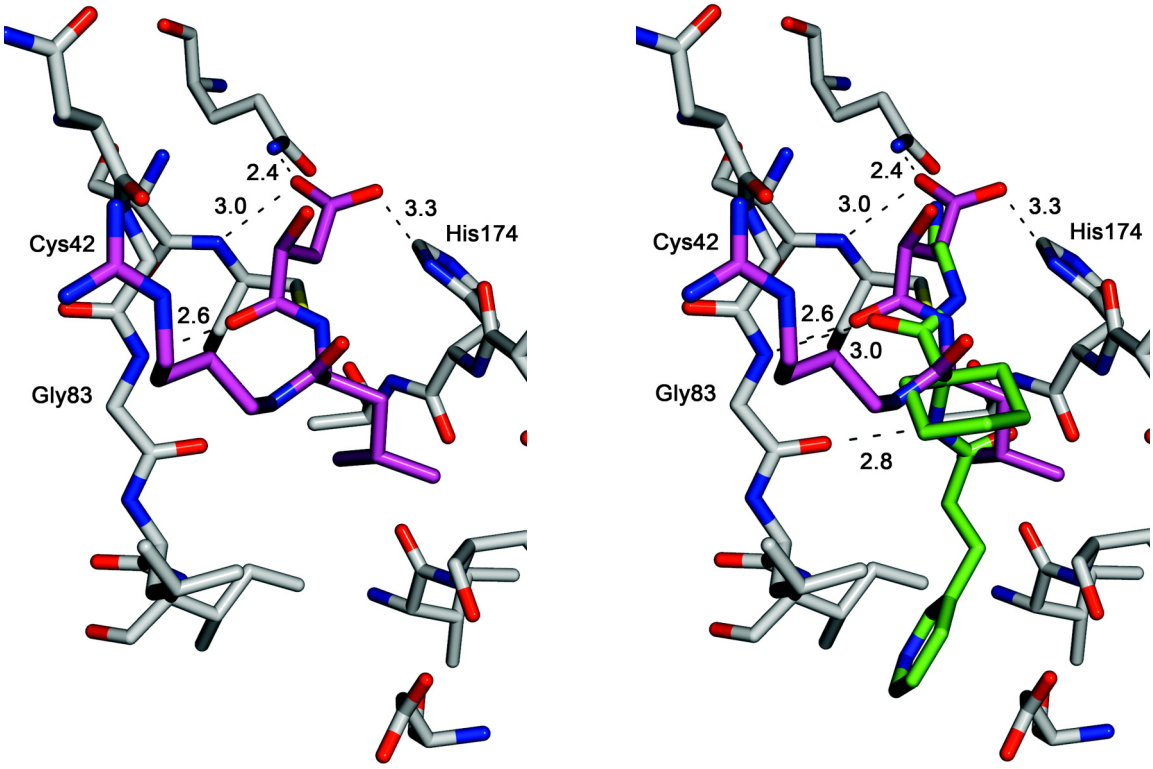


Fig. 1ESI Left: X-ray crystal structure of falcipain-2 (PDB code: 3BPF)[1] with bound protease inhibitor E64. Right: Superimposition of E64 with the proposed binding mode of compound **1a** showing the similar hydrogen bond pattern and positioning in the active site. Color code: $C_{falcipain-2}$ grey, C_{E64} magenta, C_{ligand} green, O red, N blue and S yellow. Hydrogen bond distances between heavy atoms are shown as dotted lines and given in Å.

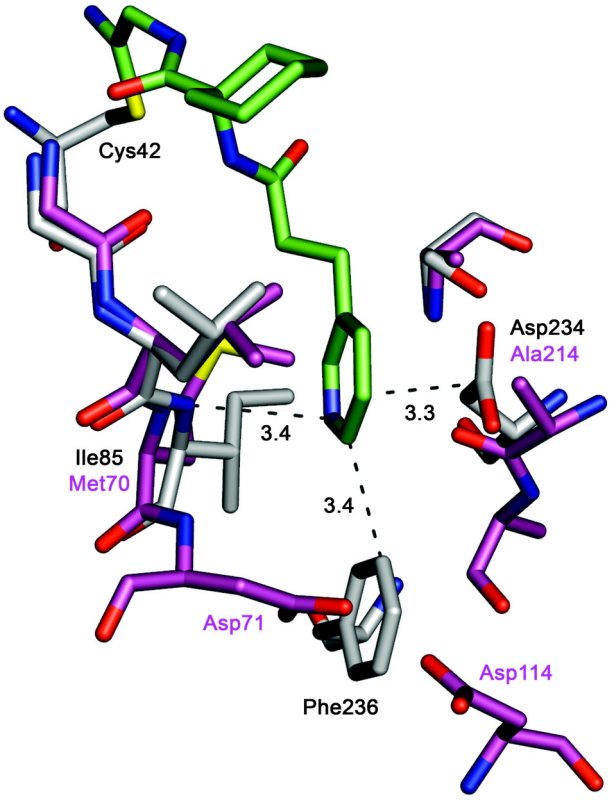


Fig. 2ESI Superimposition of important amino acids lining the S2 pocket of falcipain-2 (PDB code: 3BPF, grey)[1] and cathepsin L (PDB code: 2XU1, magenta)[2] with covalently bound inhibitor **1a** docked into the active site of falcipain-2. The pocket of falcipain-2 is less extended revealing attractive van der Waals contacts which are lost in the case of cathepsin L. Color code: $C_{falcipain-2}$ grey, C_{catL} magenta, C_{ligand} green, O red, N blue and S yellow. Intermolecular distances between heavy atoms are shown as dotted lines and given in Å.

Biological Activities

Enzyme assays: The falcipain-2 assay was performed as described previously.[3, 4] An initial screen was performed to identify compounds with an inhibition higher than 35% at an inhibitor concentration of 20 µM. For active compounds, continuous assays with progress curve methods[5] were carried out to determine the corresponding inhibition constants. Conditions for cathepsin L and B assays were taken from reference 6. For the assay with α-chymotrypsin, the following conditions were used: substrate: Suc-Leu-Tyr-AMC (*Bachem*) 75 µM; buffer: 50 mM TRIS·HCl, pH 8.0, 100 mM NaCl and 5 mM EDTA. For the determination of the dissociation constants K_i, inhibitors were used at seven different inhibitor concentrations spanning from weak inhibition to nearly total inhibition of the enzyme. Fluorescence increase resulting from hydrolysis of the substrate was measured over a period of 10 min. The residual enzyme activities v_i for various inhibitor concentrations were fitted against the inhibitor concentrations using the Dixon equation $v_0/v_i = 1 + ([I]/K_i^{app})$[7] where v_0 is the enzyme activity in the absence and v_i the enzyme activity in the presence of inhibitor (resulting from the slopes of the respective progress curves), yielding the apparent dissociation

constants K_i^{app}. The true K_i values were calculated by correction to zero substrate concentration using the Cheng-Prusoff equation $K_i = K_i^{app}/(1 + [S]/K_m])$.[8]

Kinetic constants are average values of at least two independent assays, each performed in duplicate. *GraFit*® software version 5.0.13 (*Erithacus Software Ltd.*, UK, 2006) was used to calculate kinetic constants.

Synthesis

Materials and Methods

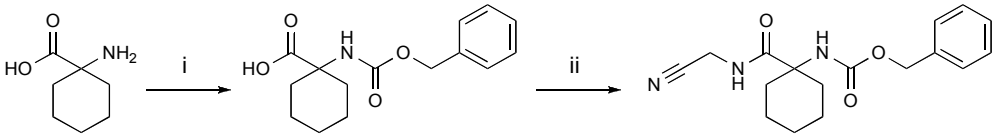
Solvents and reagents were purchased from *Acros*, *Aldrich* and *Fluka* at reagent-grade and used without further purification. All reactions were carried out in oven-dried glassware and under argon atmosphere unless otherwise stated. Solvents for extraction or flash column chromatography were of technical quality and distilled before use. Dry solvents (CH_2Cl_2, DMF, MeCN and MeOH) for reactions were purified by a solvent drying system from *LC Technology Solutions Inc. SP-105* under nitrogen atmosphere (H_2O content < 10 ppm as determined by *Karl-Fischer* titration). Other solvents were purchased in *p.a.* quality. All products were dried under high vacuum (10^{-2} Torr) before analytical characterisation. Thin-layer chromatography was carried out on glass plates coated with SiO_2-60 UV_{254} from *Merck*. Visualisation was achieved by UV light at 245 nm or staining with a solution of ninhydrin (1.5 g) in *n*-butanol (100 mL) and glacial acetic acid (3 mL). Flash column chromatography (FC) was perfomed using SiO_2-60 (230-400 mesh, particle size 0.040-0.063 mm) from *Fluka* with a head pressure of 0.1-0.4 bar. The eluent compositions used are reported

individually in parentheses. Liquid chromatography/mass spectrometry (LC/MS) for reaction control was performed on an *Ultimate 3000* series LC instrument combined with a *MSQ Plus* mass spectrometer from *Dionex*, using *Zorbax Eclipse Plus C18* columns (30 x 3 mm; 3.5 µm pore size) from *Agilent*. Melting points (m.p.) were determined on a *Büchi B-540* capillary melting point apparatus and are uncorrected. IR Spectra were recorded on a *Perkin-Elmer FT-IR 1600* spectrometer (*ATR-unit, Attenuated Total Reflection*). The spectra were measured between 4000–600 cm^{-1}. Absorption bands are reported in wavenumbers (cm^{-1}). NMR spectra (^{1}H, ^{13}C) were measured on a *Varian Gemini-300*, *Mercury-300*, *Bruker ARX-300*, *AV-400* or *DRX-400* spectrometer at 298 K using the solvent peak as an internal reference. Coupling constants (*J*) are given in Hz. The resonance multiplicity is described as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Broad signals are described as br. (broad). High-resolution electrospray ionization mass spectroscopy (HR-MS-ESI) spectra were measured on a *Bruker maXis ESI-Q-TOF* spectrometer. The relevant signals are reported in *m/z* units. Elemental analyses were performed by the Mikrolabor at the Laboratorium für Organische Chemie, ETH Zürich. Nomenclature follows the suggestions of the

computer program *ACD/Name 9* (*Advanced Chemistry Development Inc.*).

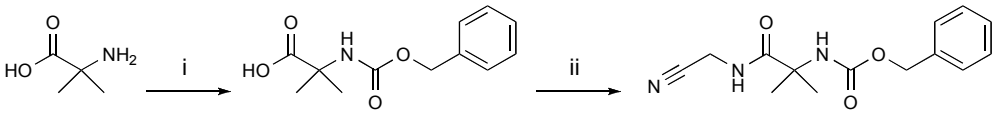
For some compounds, two rotamers could be observed in the ^{1}H and ^{13}C NMR spectra as their interconversion was sufficiently slow on the NMR time scale. These cases are mentioned with the individual compounds.

Reaction Schemes 1ESI–4ESI for Ligands 6–9



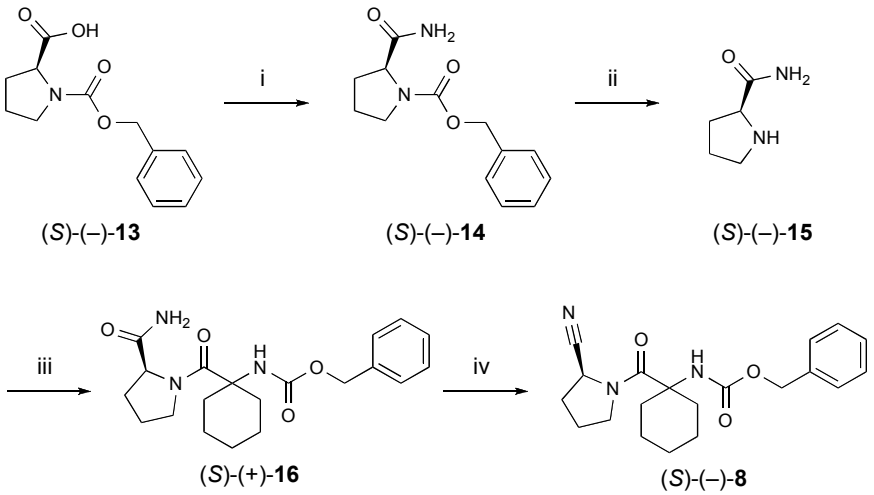
2 **10** **6**

Scheme 1ESI (i) Et_3N, *N*-(benzyloxycarbonyloxy)-succinimide, H_2O/MeOH 2:1, 25 °C, 72 h, 75%; (ii) aminoacetonitrile bisulfate, TBTU, HOBt, $^{i}Pr_2NEt$, DMF, 0–25 °C, 18 h, 52%; (TBTU = 2-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, HOBt = 1-hydroxy-1*H*-benzotriazole).

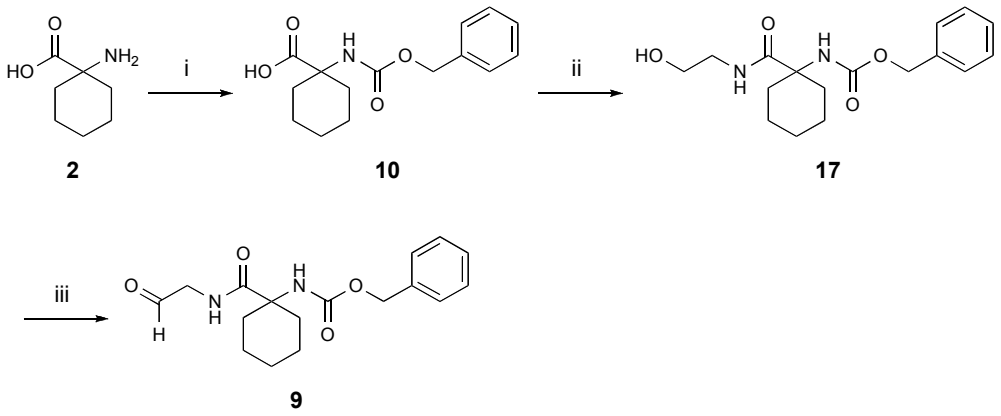


11 **12** **7**

Scheme 2ESI (i) Et_3N, *N*-(benzyloxycarbonyloxy)-succinimide, H_2O/MeOH 2:1, 25 °C, 72 h, 95%; (ii) aminoacetonitrile bisulfate, TBTU, HOBt, $^{i}Pr_2NEt$, DMF, 0–25 °C, 18 h, 45%.



Scheme 3ESI (i) Boc_2O, NH_4HCO_3, pyridine, MeCN, 25 °C, 18 h, 93%; (ii) H_2, Pd/C, MeOH, 25 °C, 3.5 h, 97%; (iii) **11**, TBTU, HOBt, $^{i}Pr_2NEt$, DMF, 0–25 °C, 18 h, 85%; (iv) cyanuric chloride, DMF, 0 °C, 7.5 h, 93%.



Scheme 4ESI (i) Et_3N, *N*-(benzyloxycarbonyl-oxy)succinimide, H_2O/MeOH 2:1, 25 °C, 72 h, 75%; (ii) 2-aminoethanol, TBTU, HOBt, $^{i}Pr_2NEt$, DMF, 0–25 °C, 15 h, quant.; (iii) Dess-Martin periodinane, CH_2Cl_2, 25 °C, 4 h, 65%.

Synthetic Protocols for Ligands 1a–i and 6–9

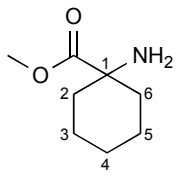
General procedure (GP-A) for the amide coupling with TBTU: A solution of the carboxylic acid (1 eq.) in DMF at 0 °C was treated successively with (1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU, 2 eq.), 1-hydroxy-1*H*-benzotriazole (HOBt, 2 eq.) and $^{i}Pr_2NEt$ (2–3 eq.). The mixture was stirred at this temperature for 20 min. After addition of the amine (1 eq.), the mixture was allowed to warm to 25 °C and stirred for 15–20 h. The solvent was removed *in vacuo*. The residue was taken up in H_2O and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$ or $NaSO_4$, filtered and evaporated. The residue was purified according to experimental details mentioned individually.

General procedure (GP-B) for the saponification of methyl esters: A solution of the methyl ester (1 eq.) in THF/MeOH/H_2O 2:2:1 was treated with $LiOH \cdot H_2O$ (3 eq.). The mixture was stirred at 40 °C for 4–6 h and then the solvent was evaporated. The residue was partitioned between H_2O and EtOAc. The aqueous layer was separated, the pH adjusted to pH 2 using 1 M HCl and extracted with EtOAc. The combined organic layers were washed with H_2O,

dried over $MgSO_4$ or $NaSO_4$, filtered and evaporated. The presence of the carboxylic acid was confirmed by LC/MS analysis. The isolated carboxylic acids were directly used for the next transformation without further purification or analyses.

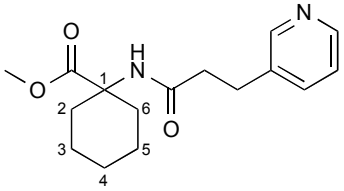
General procedure (GP-C) for the Cbz-protection of amines: A solution of the amine (1 eq.) in H_2O/MeOH 2:1 was treated dropwise with Et_3N (1.1 eq) at 25 °C. A solution of *N*-(benzyloxycarbonyloxy)succinimide (1 eq.) in MeCN was added and the mixture stirred at this temperature for 24–72 h. The solvent was evaporated *in vacuo*. The residue was dissolved in H_2O and the pH adjusted to pH 8 using a saturated aqueous $NaHCO_3$ solution. The aqueous solution was washed with Et_2O, acidified with 1 M HCl to pH 3 and extracted with EtOAc. The combined organic layers were washed with H_2O, dried over $MgSO_4$ or $NaSO_4$, filtered and evaporated to give the pure product.

Methyl 1-Aminocyclohexanecarboxylate (3)[9]



Thionyl chloride (15.3 mL, 0.21 mol) was added to a solution of 1-aminocyclohexanecarboxylic acid (**2**) (10.0 g, 0.07 mol) in MeOH (250 mL) at 0 °C over 30 min. The mixture was allowed to warm to 25 °C and stirred for 60 h at this temperature. MeOH was removed *in vacuo* and the remaining yellow solid dissolved in H_2O (150 mL). The pH was adjusted to pH 9 with a saturated aqueous Na_2CO_3 solution (~50 mL). The mixture was extracted with CH_2Cl_2 (2 x 200 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give **3** (10.31 g, 94%) as a pale yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.24–1.48 (m, 8 H; H–C(3, 4, 5), NH_2), 1.50–1.60 (m, 2 H; H_{ax}–C(2, 6)), 1.75–1.90 (m, 2 H; H_{eq}–C(2, 6)), 3.61 ppm (s, 3 H; Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.79 (2 C, C(3, 5)), 25.35 (C(4)), 35.33 (2 C, C(2, 6)), 51.84 (Me), 57.22 (C(1)), 177.73 ppm (*C*OOMe); HR-ESI-MS: *m/z* (%): 158.1176 (100, $[M + H]^+$, calcd for $C_8H_{16}NO_2^+$: 158.1176).

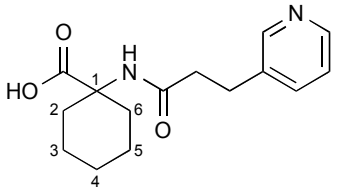
Methyl 1-[3-(Pyridin-3-yl)propanecarboxamido]cyclohexane-carboxylate (4a)



General procedure GP-A starting from 3-(pyridin-3-yl)-propionic acid (1.00 g, 6.62 mmol), TBTU (4.25 g, 13.23 mmol), HOBt (1.79 g, 13.23 mmol), $^{i}Pr_2NEt$ (2.28 mL, 13.23 mmol) and **3** (1.04 g, 6.62 mmol) in DMF (35 mL). Purification by FC (SiO_2; CH_2Cl_2/MeOH 40:1) gave **4a** (1.71 g, 89%) as a pale yellow oil. R_f = 0.44 (SiO_2; CH_2Cl_2/MeOH 10:1); ^1H NMR (300 MHz, $CDCl_3$): δ = 1.18–1.37 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.47–1.57 (m, 3 H; H_{eq}–C(3, 4, 5)), 1.73–1.83 (m, 2 H; H_{ax}–C(2, 6)), 1.93–1.97 (m, 2 H; H_{eq}–C(2, 6)), 2.61 (t, J = 7.4 Hz, 2 H; $COCH_2$), 3.02 (t, J = 7.4 Hz, 2 H; $COCH_2CH_2$), 3.63 (s, 3 H; OMe), 6.52 (br. s, 1 H; NH), 7.31–7.43 (m, 1 H; arom. H), 7.68–7.86 (m, 1 H; arom. H), 8.40–8.51 ppm (m, 2 H; 2 arom H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.38 (2 C, 2 CH_2), 25.06 (CH_2), 28.39 ($COCH_2$), 36.79 (2 C, 2 CH_2), 36.79 ($COCH_2CH_2$), 52.24 (OMe), 59.04 (C(1)), 138.27 (arom. CH), 139.61 (arom. CH), 142.42 (arom. C), 144.65 (arom. CH), 147.01 (arom. CH), 170.95 (CO), 174.52 ppm (CO); IR (ATR): $\tilde{\nu}$ = 3290 (w), 2939 (m), 2858 (w), 1733 (s), 1649 (m), 1530 (m), 1446 (m), 1383 (m), 1276 (m), 1234 (s), 1162

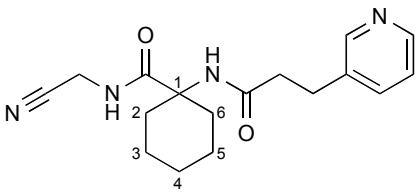
(m), 1069 (s), 984 (m), 902 (w), 804 (w), 781 (m), 739 (s), 704 (m), 650 cm^{-1} (w); HR-ESI-MS: *m/z* (%): 291.1708 (100, $[M + H]^+$, calcd for $C_{16}H_{23}N_2O_3^+$: 291.1703).

1-[3-(Pyridin-3-yl)propanamido)cyclohexanecarboxylic Acid (5a)



General procedure GP-B starting from **4a** (1.54 g, 5.30 mmol) and $LiOH \cdot H_2O$ (668 mg, 15.91 mmol) in THF/MeOH/H_2O 2:2:1 (10 mL). Carboxylic acid **5a** (904 mg, 61%) was obtained as a white solid and used for the next step without further characterisation.

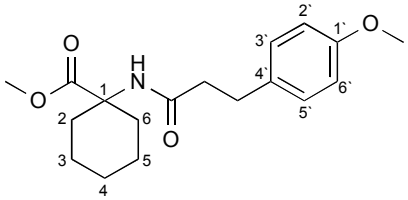
***N*-(Cyanomethyl)-1-(3-pyridin-3-yl)propaneamido)cyclo-hexanecarboxamide (1a)**



General procedure GP-A starting from **5a** (300 mg, 1.09 mmol), TBTU (698 mg, 2.17 mmol), HOBt (294 mg, 2.17 mmol), $^{i}Pr_2NEt$ (562 µL, 3.26 mmol) and aminoaceto-nitrile bisulfate (184 mg, 1.20 mmol) in DMF (10 mL). Purification by FC (SiO_2; CH_2Cl_2/MeOH 10:1) gave **1a**

(120 mg, 35%) as a pale yellow oil. R_f = 0.30 (SiO_2; CH_2Cl_2/MeOH 10:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.22–1.32 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.53–1.56 (m, 3 H; H_{eq}–C(3, 4, 5)), 1.74–1.80 (m, 2 H; H_{ax}–C(2, 6)), 2.01–2.05 (m, 2 H; H_{eq}–C(2, 6)), 2.58 (t, J = 7.5 Hz, 2 H; $COCH_2$), 2.94 (t, J = 7.4 Hz, 2 H; $COCH_2CH_2$), 4.07 (d, J = 5.7 Hz, 2 H; CH_2CN), 6.64 (br. s, 1 H; NH), 7.23 (dd, J = 7.7, 4.8 Hz, 1 H; arom. CH), 7.55 (dt, J = 7.8, 1.8 Hz, 1 H; arom. CH), 7.85 (t, J = 5.7 Hz, 1 H; $NHCH_2$), 8.34–8.42 ppm (m, 2 H; 2 arom. CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.26 (CH_2), 25.01 (CH_2), 27.76 (CH_2CN), 28.51 ($COCH_2CH_2$), 31.91 (CH_2), 37.61 ($COCH_2$), 60.18 (C(1)), 116.56 (CN), 123.64 (arom. CH), 136.24 (arom. C), 136.39 (arom. CH), 147.64 (arom. CH), 149.57 (arom. CH), 172.70 (CO), 175.08 ppm (CO); IR (ATR): $\tilde{\nu}$ = 3327 (w), 3033 (w), 2934 (m), 2858 (w), 1652 (s), 1510 (S9, 1450 (m), 1423 (m), 1347 (w), 1290 (m), 1252 (m), 11659 (m), 1150 (w), 1029 (w), 999 (w), 960 (w), 909 (w), 804 (m), 712 (s), 630 cm^{-1} (w); HR-ESI-MS: m/z (%): 315.1810 (100, $[M + H]^+$, calcd for $C_{17}H_{23}N_4O_2^+$: 315.1816).

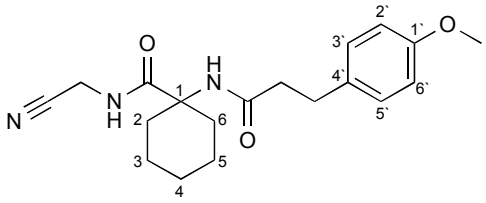
Methyl 1-[3-(4-Methoxyphenyl)propanamido]cyclohexane-carboxylate (4b)



General procedure GP-A starting from 3-(4-methoxy-phenyl)propionic acid (1.26 g, 7.00 mmol), TBTU (4.50 g, 14.00 mmol), HOBt (1.89 g, 14.00 mmol), $^{i}Pr_2NEt$ (2.43 mL, 14.00 mmol) and **3** (1.21 g, 7.70 mmol) in DMF (37 mL). Purification by FC (SiO_2; CH_2Cl_2/MeOH 25:1) gave **4b** (2.12 g, 95%) as a yellow oil. R_f = 0.69 (SiO_2; CH_2Cl_2/MeOH 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.22–1.27 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.52–1.59 (m, 3 H; H_{eq}–C(3, 4, 5)), 1.75–1.83 (m, 2 H; H_{ax}–C(2, 6)), 1.92–1.96 (m, 2 H; H_{eq}–C(2, 6)), 2.48 (t, J = 7.5 Hz, 2 H; $COCH_2$), 2.90 (t, J = 7.5 Hz, 2 H; $COCH_2CH_2$), 3.68 (s, 3 H; CO_2Me), 3.78 (s, 3 H; OMe), 5.58 (br. s, 1 H; NH), 6.81–6.85 (m, 2 H; H–C(2′, 6′)), 7.12–7.15 ppm (m, 2 H; H–C(3′, 5′)); ^{13}C NMR (100 MHz, $CDCl_3$; assignments based on an HSQC spectrum): δ = 21.30 (2 C, C(3, 5)), 25.12 (C(4)), 30.53 (CH_2Ph), 32.31 (2 C, C(2, 6)), 37.91 ($COCH_2$), 52.06 (CO_2Me), 55.20 (PhO*Me*), 58.60 (C(1)), 113.82 (2 C, C(2′, 6′)), 129.28 (2 C, C(3′ ,5′)), 132.87 (C(4′)), 157.98 (C(1′)), 171.90 (CO_2Me), 174.70 ppm (CONH); IR (ATR): $\tilde{\nu}$ = 3298 (br. w), 2938 (m), 2858 (w), 2250 (w), 1736 (m),

1645 (s), 1612 (m), 1584 (w), 1511 (s), 1452 (m), 1359 (w), 1291 (m), 1277 (m), 1240 (s), 1177 (m), 1163 (m), 1137 (m), 1107 (s), 1071 (m), 1035 (m), 986 (w), 907 (m), 883 (w), 853 (w), 823 (m), 784 (w), 729 (s), 645 cm^{-1} (m); HR-ESI-MS: *m/z* (%): 342.1666 (100, $[M + Na]^+$, calcd for $C_{18}H_{25}NNaO_4^+$: 342.1681).

_N_-(Cyanomethyl)-1-[3-(4-methoxyphenyl)propanamido]cyclo-hexanecarboxamide (1b)

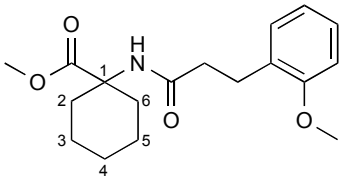


A solution of **4b** (495 mg, 1.55 mmol) in THF/MeOH/H_2O 2:2:1 (7 mL) was treated with $LiOH \cdot H_2O$ (195 mg, 4.66 mmol) and stirred at 25 °C for 48 h. The solvent was removed *in vacuo*. The residue was dissolved in H_2O (10 mL) and the pH adjusted to pH 2 using 2 M HCl. The solution was extracted with EtOAc (3 x 30 mL) and the solvent evaporated *in vacuo* to give the crude acid **5b** as a white solid. The residue was dissolved in DMF (9 mL) and treated with TBTU (905 mg, 2.82 mmol), HOBt (381 mg, 2.82 mmol) and $^{i}Pr_2NEt$ (0.49 mL, 2.8 mmol) at 0 °C. The resulting mixture was stirred at 0 °C for 20 min. Aminoacetonitrile bisulfate (239 mg, 1.55 mmol) was added and the solution allowed to warm to 25 °C. After stirring

for 17 h at this temperature, DMF was evaporated *in vacuo*. H_2O (50 mL) was added and the suspension extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with H_2O (5 x 50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. FC (SiO_2; CH_2Cl_2/MeOH 25:1) gave **1b** (159 mg, 33%) as a white solid. R_f = 0.23 (SiO_2; CH_2Cl_2/MeOH 25:1); m.p. 114–115 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.18–1.28 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.51–1.58 (m, 3 H; H_{eq}–C(3, 4, 5)), 1.79–1.85 (m, 2 H; H_{ax}–C(2, 6)), 1.98–2.02 (m, 2 H; H_{eq}–C(2, 6)), 2.56 (t, J = 7.3 Hz, 2 H; $COCH_2$), 2.92 (t, J = 7.3 Hz, 2 H; $COCH_2CH_2$), 3.80 (s, 3 H; OMe), 4.05 (d, J = 5.8 Hz, 2 H; CH_2CN), 5.45 (br. s, 1 H; C(1)NH), 6.85–6.87 (m, 2 H; H–C(2′, 6′)), 7.13–7.15 (m, 2 H, H–C(3′, 5′)), 7.65 ppm (br. t, J = 5.5 Hz, 1 H; CONH); ^{13}C NMR (100 MHz, $CDCl_3$; assignments based on an HSQC spectrum): δ = 21.16 (2 C, C(3, 5)), 24.97 (C(4)), 27.62 (CH_2CN), 30.86 (CH_2Ph), 32.95 (2 C, C(2, 6)), 38.75 ($COCH_2$), 55.33 (PhO*Me*), 60.36 (C(1)), 114.14 (2 C, C(2′, 6′)), 116.20 (CN), 129.32 (2 C, C(3′, 5′)), 132.05 (C(4′)), 158.35 (C(1′)), 173.47 (C(1)NH*C*O), 174.52 ppm (CONH); IR (ATR): $\tilde{\nu}$ = 3326 (m), 3002 (w), 2934 (w), 2854 (w), 1661 (s), 1640 (s), 1611 (m), 1532 (s), 1510 (s), 1462 (m), 1450 (m), 1407 (w), 1370 (w), 1348 (w), 1295 (m), 1241 (s), 1212 (w), 1177 (m), 1147 (m), 1111 (m), 1034 (s), 1001 (m), 946 (w), 931 (w), 911 (w), 896 (w),

852 (w), 838 (m), 808 (s), 786 (w), 745 (w), 705 (m), 653 (m), 631 cm^{-1} (m); HR-ESI-MS: *m/z* (%): 344.1955 (100, $[M + H]^+$, calcd for $C_{19}H_{26}N_3O_3^+$: 344.1929); elemental analysis calcd (%) for $C_{19}H_{25}N_3O_3$ (343.42): C 66.45, H 7.34, N 12.24, O 13.98; found: C 66.54, H 7.31, N 12.25, O 13.98.

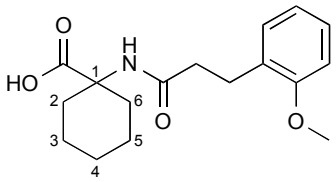
Methyl 1-[3-(2-Methoxyphenyl)propanamido)cyclohexane-carboxylate (4c)



General procedure GP-A starting from 3-(2-methoxyphenyl)-propionic acid (500 mg, 2.78 mmol), TBTU (1.78 g, 5.55 mmol), HOBt (750 mg, 5.55 mmol), $^{i}Pr_2NEt$ (956 µL, 5.55 mmol) and **3** (480 mg, 3.05 mmol) in DMF (10 mL). Purification by FC (SiO_2; CH_2Cl_2/MeOH 100:1) gave **4c** (796 mg, 90%) as a white solid. R_f = 0.51 (SiO_2; CH_2Cl_2/MeOH 10:1); m.p. 122–123 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.23–1.32 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.52–1.61 (m, 3 H; H_{eq}–C(3, 4, 5)), 1.75–1.84 (m, 2 H; H_{ax}–C(2, 6)), 1.92–1.98 (m, 2 H; H_{eq}–C(2, 6)), 2.51 (t, *J* = 7.6 Hz, 2 H; $COCH_2$), 2.95 (t, *J* = 7.6 Hz, 2 H; $COCH_2CH_2$), 3.68 (s, 3 H; OMe), 3.84 (s, 3 H; OMe), 5.56 (br. s, 1 H; NH), 6.84–6.89 (m, 2 H; 2 arom. CH), 7.15–7.22 ppm (m, 2 H; 2 arom. CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.39 (CH_2), 25.14 (CH_2),

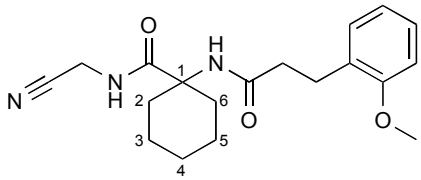
26.40 ($COCH_2$), 32.38 (CH_2), 36.44 ($COCH_2CH_2$), 52.24 (OMe), 55.27 (OMe), 58.57 (C(1)), 110.30 (arom. CH), 120.58 (arom. CH), 127.59 (arom. CH), 128.96 (arom. C), 130.17 (arom. CH), 157.37 (arom. C), 172.06 (CO), 174.63 ppm (CO); IR (ATR): $\tilde{\nu}$ = 3299 (w), 3060 (w), 2948 (m), 2893 (w), 1736 (s), 1640 (s), 1601 (w), 1538 (s), 1493 (m), 1465 (m), 1437 (m), 1354 (w), 1316 (w), 1298 (w), 1289 (w), 1263 (m), 1238 (s), 1219 (s), 1201 (s), 1134 (s), 1116 (m), 1029 (m), 1006 (m), 978 (m), 905 (m), 855 (w), 844 (w), 829 (w), 815 (w), 756 (s), 749 (s), 735 (m), 710 (w), 636 cm^{-1} (m); HR-ESI-MS: *m/z* (%): 320.1856 (100, $[M + H]^+$, calcd for $C_{18}H_{26}NO_4^+$: 320.1856); elemental analysis calcd (%) for $C_{18}H_{25}NO_4$ (319.40): C 67.69, H 7.89, N 4.39; found: C 67.53, H 7.87, N 4.54.

1-[3-(2-Methoxyphenyl)propanamido)cyclohexanecarboxylic Acid (5c)



General procedure GP-B starting from **4c** (740 mg, 2.32 mmol) and $LiOH \cdot H_2O$ (292 mg, 6.95 mmol) in THF/MeOH/H_2O 2:2:1 (10 mL). Carboxylic acid **5c** (640 mg, 90%) was obtained as a white solid and used for the next step without further characterisation.

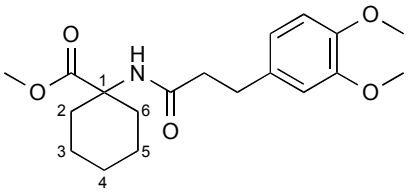
_N_-(Cyanomethyl)-1-[3-(2-methoxyphenyl)propanamido]cyclohexanecarboxamide (1c)



General procedure GP-A starting from **5c** (300 mg, 0.98 mmol), TBTU (631 mg, 1.96 mmol), HOBt (265 mg, 1.96 mmol), $^{i}Pr_2NEt$ (508 µL, 2.95 mmol) and aminoacetonitrile bisulfate (167 mg, 1.08 mmol) in DMF (10 mL). Purification by FC (SiO_2; CH_2Cl_2/MeOH 100:1→50:1) gave **1c** (271 mg, 80%) as a white solid. R_f = 0.61 (SiO_2; CH_2Cl_2/MeOH 10:1); m.p. 108–109 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.17–1.31 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.50–1.52 (m, 3 H; H_{eq}–C(3, 4, 5)), 1.69–1.77 (m, 2 H; H_{ax}–C(2, 6)), 1.96–2.00 (m, 2 H; H_{eq}–C(2, 6)), 2.55 (t, J = 7.6 Hz, 2 H; $COCH_2$), 2.91 (t, J = 7.5 Hz, 2 H; $COCH_2CH_2$), 3.78 (s, 3 H; OMe), 3.95 (d, J = 5.7, 2 H, CH_2CN), 6.22 (br. s, 1 H; NH), 6.81–6.87 (m, 2 H; 2 arom. CH), 7.09–7.20 (m, 2 H; 2 arom. CH), 7.53 ppm (t, J = 5.7 Hz, 1 H; $NHCH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.19 (CH_2), 25.06 (CH_2), 26.31 (CH_2), 27.71 ($COCH_2CH_2$), 31.87 (CH_2), 36.44 ($COCH_2$), 55.34 (OMe), 59.83 (C(1)), 110.55 (arom. CH), 116.54 (CN), 120.59 (arom. CH), 127.82 (arom. CH), 128.56 (arom. C), 129.92 (arom. CH), 157.36 (arom. C), 173.54 (CO), 175.27 ppm (CO); IR (ATR): $\tilde{\nu}$ = 3320 (m), 3000 (w), 2944 (w), 1663 (s), 1649 (s), 1600 (w), 1530 (s), 1492 (s), 1462

(m), 1438 (m), 1407 (w), 1377 (m), 1355 (w), 1288 (m), 1255 (m), 1174 (m), 1155 (m), 1111 (m), 1052 (m), 1030 (m), 1000 (w), 961 (w), 932 (w), 896 (w), 854 (w), 749 (s), 726 (w), 666 cm^{-1} (m); HR-ESI-MS: *m/z* (%): 344.1971 (100, $[M + H]^+$, calcd for $C_{19}H_{26}N_3O_4^+$: 344.1969).

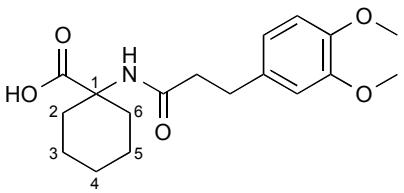
Methyl 1-[3-(3,4-Dimethoxyphenyl)propanamido]cyclohexane-carboxylate (4d)



General procedure GP-A starting from 3-(3,4-dimethoxy-phenyl)propionic acid (1.00 g, 4.76 mmol), TBTU (3.05 g, 9.51 mmol), HOBt (1.29 g, 9.51 mmol), $^{i}Pr_2NEt$ (1.64 mL, 9.51 mmol) and **3** (823 mg, 5.23 mmol) in DMF (40 mL). Purification by FC (SiO_2; CH_2Cl_2/MeOH 80:1) gave **4d** (1.46 g, 89%) as a yellow oil. R_f = 0.64 (SiO_2; CH_2Cl_2/MeOH 10:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.16–1.28 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.49–1.56 (m, 3 H; H_{eq}–C(3, 4, 5)), 1.70–1.80 (m, 2 H; H_{ax}–C(2, 6)), 1.89–2.03 (m, 2 H; H_{eq}–C(2, 6)), 2.46 (t, *J* = 7.5 Hz, 2 H; $COCH_2$), 2.86 (t, *J* = 7.6 Hz, 2 H; $COCH_2CH_2$), 3.63 (s, 3 H; CO_2Me), 3.80–3.81 (m, 6 H; 2 OMe), 5.72 (br. s, 1 H; NH), 6.68–6.76 ppm (m, 3 H; 3 arom. H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.31 (CH_2), 25.06 (CH_2), 31.00 ($COCH_2$), 32.32 (CH_2), 38.19

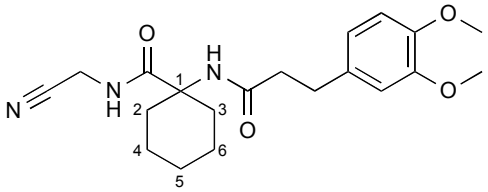
($COCH_2CH_2$), 52.20 (CO_2Me), 55.78 (OMe), 55.93 (OMe), 58.65 (C(1)), 111.35 (arom. CH), 111.83 (arom. CH), 120.18 (arom. CH), 133.49 (arom. C), 147.44 (arom. C), 148.88 (arom. C), 171.64 (CO), 174.58 ppm (CO); IR (ATR): $\tilde{\nu}$ = 3380 (w), 2936 (m), 2860 (w), 1735 (m), 1719 (m), 1651 (m), 1607 (w), 1591 (w), 1514 (s), 1452 (m), 1358 (w), 1292 (m), 1233 (s), 1157 (s), 1137 (s), 1070 (m), 1027 (s), 985 (m), 955 (w), 935 (w), 902 (w), 855 (w), 806 (w), 783 (w), 759 (m), 737 (m), 704 cm^{-1} (m); HR-ESI-MS: *m/z* (%): 350.1958 (100, $[M + H]^+$, calcd for $C_{19}H_{28}NO_5^+$: 350.1962).

1-[3-(3,4-Dimethoxyphenyl)propanamido]cyclohexane-carboxylic Acid (5d)



General procedure GP-B starting from **4d** (1.07 g, 3.06 mmol) and $LiOH \cdot H_2O$ (385 mg, 9.19 mmol) in THF/MeOH/H_2O 2:2:1 (10 mL). Carboxylic acid **5d** (890 mg, 86%) was obtained as a white solid and used for the next step without further characterisation.

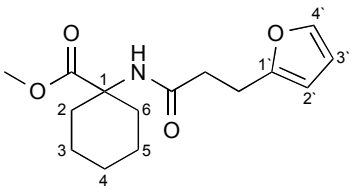
_N_-(Cyanomethyl)-1-[3-(3,4-dimethoxyphenyl)propanamido]-cyclohexanecarboxamide (1d)



General procedure GP-A starting from **5d** (500 mg, 1.49 mmol), TBTU (957 mg, 2.98 mmol), HOBt (403 mg, 2.98 mmol), $^{i}Pr_2NEt$ (771 µL, 4.47 mmol) and aminoacetonitrile bisulfate (253 mg, 1.64 mmol) in DMF (10 mL). Purification by FC (SiO_2; CH_2Cl_2/MeOH 50:1) gave **1d** (302 mg, 54%) as a colourless foam. R_f = 0.50 (SiO_2; CH_2Cl_2/MeOH 10:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.19–1.24 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.51–1.56 (m, 3 H; H_{eq}–C(3, 4, 5)), 1.76–1.82 (m, 2 H; H_{ax}–C(2, 6)), 1.99–2.02 (m, 2 H; H_{eq}–C(2, 6)), 2.57 (t, J = 7.4 Hz, 2 H; $COCH_2$), 2.91 (t, J = 7.4 Hz, 2 H; $COCH_2CH_2$), 3.83 (s, 3 H; OMe), 3.85 (s, 3 H; OMe), 4.05 (d, J = 5.7 Hz, 2 H; CH_2CN), 5.75 (br. s, 1 H; NH), 6.75–6.83 (m, 3 H; 3 arom. H), 7.67 ppm (t, J = 5.1 Hz, 1 H; $NHCH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.14 (CH_2), 24.97 (CH_2), 27.66 (CH_2), 31.04 ($COCH_2CH_2$), 31.91 (CH_2), 38.53 ($COCH_2$), 55.97 (OMe), 55.99 (OMe), 60.20 (C(1)), 111.50 (arom. CH), 111.98 (arom. CH), 116.40 (CN), 120.16 (arom. CH), 132.84 (arom. C), 147.71 (arom. C), 149.04 (arom. C), 173.38 (CO), 174.82 ppm (CO); IR (ATR): $\tilde{\nu}$ = 3315 (w), 2934 (w), 1662 (s), 1512 (s),

1449 (m), 1256 (s), 1245 (s), 1138 (s), 1024 (s), 896 (w), 845 (w), 807 (m), 763 cm^{-1} (m); HR-ESI-MS: *m/z* (%): 374.2084 (100, $[M + H]^+$, calcd for $C_{20}H_{28}N_3O_4^+$: 374.2074).

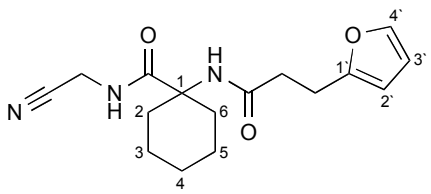
Methyl 1-[3-(Furan-2-yl)propanamido]cyclohexanecarboxylate (4e)



General procedure GP-A starting from 3-(2-furyl)propionic acid (981 mg, 7.00 mmol), TBTU (4.50 g, 14.00 mmol), HOBt (1.89 g, 14.00 mmol), $^{i}Pr_2NEt$ (2.43 mL, 14.00 mmol) and **3** (1.21 g, 7.70 mmol) in DMF (37 mL). Purification by FC (SiO_2; cyclohexane/EtOAc 3:2) gave **4e** (1.82 g, 94%) as a pale yellow solid. R_f = 0.15 (SiO_2; heptane/EtOAc 2:1); m.p. 84–87 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26–1.38 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.57–1.64 (m, 3 H; H_{eq}–C(3, 4, 5)), 1.78–1.85 (m, 2 H; H_{ax}–C(2, 6)), 1.97–2.00 (m, 2 H; H_{eq}–C(2, 6)), 2.56 (t, *J* = 7.4 Hz, 2 H; $COCH_2$), 2.98 (t, *J* = 7.3 Hz, 2 H, $COCH_2CH_2$), 3.69 (s, 3 H; OMe), 5.65 (br. s, 1 H; NH), 6.05 (dd, *J* = 3.2, 0.9 Hz, 1 H; H–C(2′)), 6.29 (dd, *J* = 3.2, 1.9 Hz, 1 H; H–C(3′)), 7.31 ppm (dd, *J* = 1.9, 0.8 Hz, 1 H; H–C(4′)); ^{13}C NMR (100 MHz, $CDCl_3$; assignments based on an HSQC spectrum): δ = 21.37 (2 C, C(3, 5)), 23.90 ($COCH_2CH_2$), 25.10 (C(4)), 32.34 (2 C, C(2,

6)), 34.75 (CO*C*$H_2$$CH_2$), 52.25 (OMe), 58.73 (C(1)), 105.63 (C(2′)), 110.30 (C(3′)), 141.13 (C(4′)), 154.30 (C(1′)), 171.12 (OCONH), 174.51 ppm (O*C*–C(1)); IR (ATR): $\tilde{\nu}$ = 3355 (m), 3109 (w), 3034 (w), 2952 (w), 2936 (w), 2907 (w), 2858 (w), 1717 (s), 1664 (s), 1599 (w), 1527 (m), 1466 (w), 1454 (m), 1436 (m), 1385 (w), 1372 (w), 1359 (w), 1325 (w), 1298 (s), 1289 (m), 1267 (w), 1249 (m), 1238 (s), 1210 (m), 1166 (m), 1148 (s), 1140 (m), 1073 (m), 1013 (m), 1029 (w), 1013 (m), 996 (w), 974 (w), 948 (w), 882 (w), 856 (w), 803 (m), 785 (w), 754 (s), 743 (m), 719 (w), 699 (m), 629 (m), 614 (m), 603 cm^{-1} (m); HR-ESI-MS: *m/z* (%): 280.1657 (99, $[M + H]^+$, calcd for $C_{15}H_{22}NO_4^+$: 280.1504), 235.9973 (100, $[M + H - CO_2]^+$, calcd for $C_{14}H_{22}NO_2^+$: 236.1606); elemental analysis calcd (%) for $C_{15}H_{21}NO_4$ (279.33): C 64.50, H 7.58, N 5.01; found: 64.53, H 7.56, N 5.05.

_N_-(Cyanomethyl)-1-(3-(furan-2-yl)propanamido)cyclohexane-carboxamide (1e)

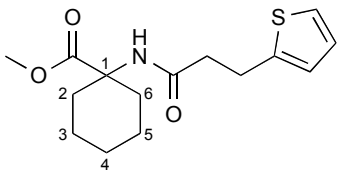


A solution of **4e** (800 mg, 2.86 mmol) and $LiOH·H_2O$ (361 mg, 8.58 mmol) in THF/MeOH/H_2O 2:2:1 (15 mL) was stirred at 25 °C for 3 d. The solvent was removed *in vacuo*. The

resulting mixture was dissolved H_2O (10 mL) and the pH adjusted to pH 2 using 2 M HCl. The mixture was extracted with EtOAc (3 x 30 mL) and the solvent evaporated *in vacuo* to give the crude acid **5e** as a white solid. The residue was dissolved in DMF (18 mL) and treated with TBTU (1.84 g, 5.74 mmol), HOBt (775 mg, 5.74 mmol) and $^{i}Pr_2NEt$ (0.99 mL, 5.7 mmol) at 0 °C. The resulting mixture was stirred at 0 °C for 20 min. Aminoacetonitrile bisulfate (486 mg, 3.16 mmol) was added and the solution allowed to warm to 25 °C. After stirring for 17 h at 25 °C, DMF was evaporated *in vacuo*. H_2O (50 mL) was added and the suspension extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with H_2O (5 x 50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. FC (SiO_2; CH_2Cl_2/MeOH 25:1) gave **1e** (336 mg, 39%) as a white solid. R_f = 0.13 (SiO_2; CH_2Cl_2/MeOH 25:1); m.p. 123–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.27–1.35 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.57–1.64 (m, 3 H; H_{eq}–C(3, 4, 5)), 1.83–1.90 (m, 2 H; H_{ax}–C(2, 6)), 2.02–2.06 (m, 2 H; H_{eq}–C(2, 6)), 2.61 (t, J = 7.0 Hz, 2 H; $COCH_2$), 3.02 (t, J = 6.9 Hz, 2 H, $COCH_2CH_2$), 4.10 (d, J = 5.8 Hz, 2 H; CH_2CN), 5.49 (br. s, 1 H; C(1)–NH), 6.10 (dd, J = 3.2, 0.8 Hz, 1 H; H–C(2′)), 6.34 (dd, J = 3.2, 1.9 Hz, 1 H; H–C(3′)), 7.36 (dd, J = 1.9, 0.8 Hz, 1 H; H–C(4′)) 7.68 ppm (t, J = 5.4 Hz, 1 H, CH_2NH); ^{13}C NMR (100 MHz, $CDCl_3$; assignments based on

an HSQC spectrum): δ = 21.20 (2 C, C(3, 5)), 24.10 ($COCH_2CH_2$), 24.97 (C(4)), 27.60 (CH_2CN), 31.94 (2 C, C(2, 6)), 35.51 ($COCH_2CH_2$), 60.40 (C(1)), 106.12 (C(2′)), 110.53 (C(3′)), 116.19 (CN), 141.52 (C(4′)), 153.77 (C(1′)), 173.04 (OCONH), 174.45 ppm (C(1)*C*O); IR (ATR): $\tilde{\nu}$ = 3300 (m), 3122 (w), 3036 (w), 2947 (m), 2847 (w), 1659 (s), 1645 (m), 1598 (w), 1526 (s), 1448 (m), 1463 (w), 1423 (m), 1372 (w), 1342 (w), 1296 (m), 1269 (w), 1253 (m), 1204 (w), 1169 (m), 1140 (m), 1116 (w), 1076 (w), 1047 (w), 1016 (m), 1002 (m), 984 (w), 930 (w), 906 (m), 896 (w), 853 (w), 798 (m), 778 (w), 742 (w), 720 (s), 707 (m), 665 (w), 641 (m), 620 cm^{-1} (s); HR-ESI-MS: *m/z* (%): 326.1472 (100, $[M + Na]^+$, calcd for $C_{16}H_{21}N_3NaO_3^+$: 326.1481); elemental analysis calcd (%) for $C_{16}H_{21}N_3O_3$ (303.36): C 63.35, H 6.98, N 13.85, O 15.82; found: C 63.44, H 6.88, N 13.88, O 15.92.

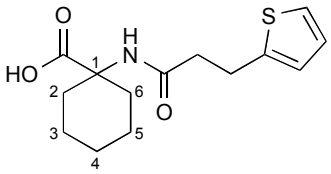
Methyl 1-[3-(Thiophen-2-yl)propanamido)cyclohexane-carboxylate (4f)



General procedure GP-A starting from 3-(2-thienyl)-propionic acid (500 mg, 3.20 mmol), TBTU (2.06 g, 6.40 mmol), HOBt (865 mg, 6.40 mmol), $^iPr_2NEt$ (1.10 mL,

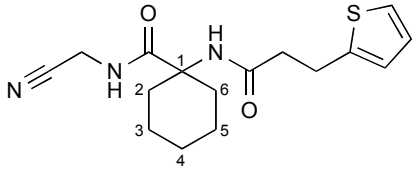
6.40 mmol) and **3** (553 mg, 3.52 mmol) in DMF (10 mL). Purification by FC (SiO_2; CH_2Cl_2/MeOH 100:1) gave **4f** (890 mg, 94%) as a pale yellow solid. R_f = 0.54 (SiO_2; CH_2Cl_2/MeOH 10:1); m.p. 74–75 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.23–1.31 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.54–1.62 (m, 3 H; H_{eq}–C(3, 4, 5)), 1.75–1.85 (m, 2 H; H_{ax}–C(2, 6)), 1.95–1.99 (m, 2 H; H_{eq}–C(2, 6)), 2.57 (t, J = 7.4 Hz, 2 H; $COCH_2$), 3.17 (t, J = 7.3 Hz, 2 H; $COCH_2CH_2$), 3.68 (s, 3 H; OMe), 5.61 (br. s, 1 H; NH), 6.82–6.84 (m, 1 H; arom. CH), 6.89–6.92 (m, 1 H; arom. CH), 7.11–7.13 ppm (m, 1 H; arom. CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.36 (CH_2), 25.08 (CH_2), 25.64 ($COCH_2$), 32.34 (CH_2), 38.30 ($COCH_2CH_2$), 52.27 (OMe), 58.76 (C(1)), 123.51 (arom. CH), 124.92 (arom. CH), 126.88 (arom. CH), 143.38 (arom. C), 170.90 (CO), 174.49 ppm (CO); IR (ATR): $\tilde{\nu}$ = 3259 (w), 3065 (w), 2942 (m), 2861 (w), 1734 (s), 1632 (s), 1544 (s), 1463 (w), 1440 (m), 1359 (m), 1273 (s), 1238 (s), 1202 (m), 1162 (m), 1070 (s), 1041 (w), 985 (m), 965 (w), 937 (w), 901 (w), 853 (m), 827 (m), 787 (w), 738 (w), 699 cm^{-1} (s); HR-ESI-MS: *m/z* (%): 296.1314 (100, $[M + H]^+$, calcd for $C_{15}H_{22}NO_2S^+$: 296.1315); elemental analysis calcd (%) for $C_{15}H_{21}NO_3S$ (296.40): C 60.99, H 7.17, N 4.74; found: C 60.91, H 7.03, N 4.87.

1-[3-(Thiophen-2-yl)propanamido)cyclohexanecarboxylic Acid (5f)



General procedure GP-B starting from **4f** (838 mg, 2.84 mmol) and LiOH·H_2O (357 mg, 8.51 mmol) in THF/MeOH/H_2O 2:2:1 (10 mL). Carboxylic acid **5f** (747 mg, 94%) was obtained as a white solid and used for the next step without further characterisation.

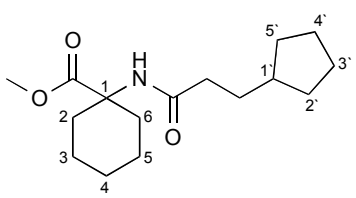
***N*-(Cyanomethyl)-1-[3-(thiophen-2-yl)propanamido]cyclo-hexanecarboxamide (1f)**



General procedure GP-A starting from **5f** (300 mg, 1.07 mmol), TBTU (685 mg, 2.13 mmol), HOBt (288 mg, 2.13 mmol), $^{i}Pr_2NEt$ (551 µL, 3.20 mmol) and aminoaceto-nitrile bisulfate (181 mg, 1.17 mmol) in DMF (10 mL). Purification by FC (SiO_2; CH_2Cl_2/MeOH 100:1→50:1) gave **1f** (295 mg, 87%) as a white solid. R_f = 0.44 (SiO_2; CH_2Cl_2/MeOH 10:1); m.p. 111–112 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.17–1.31 (m, 3 H; H_{ax}-C(3, 4, 5)), 1.53–1.64 (m, 3 H; H_{eq}-C(3, 4, 5)), 1.78–1.88 (m, 2 H; H_{ax}-C(2, 6)), 1.99–2.04 (m, 2 H; H_{eq}-C(2, 6)), 2.63 (t, J = 7.0 Hz, 2 H;

$COCH_2$), 3.21 (t, *J* = 7.0 Hz, 2 H; $COCH_2CH_2$), 4.06 (d, *J* = 5.8 Hz, 2 H; CH_2CN), 5.48 (br. s, 1 H; NH), 6.86 (dd, *J* = 3.4, 1.1 Hz, 1 H; arom. CH), 6.95 (dd, *J* = 5.1, 3.4 Hz, 1 H; arom. CH), 7.18 (dd, *J* = 5.1, 1.2 Hz, 1 H; arom. CH), 7.60 ppm (t, *J* = 5.4 Hz, 1 H; $NHCH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.17 (CH_2), 24.95 (CH_2), 25.77 ($COCH_2CH_2$), 27.64 (CH_2CN), 31.95 (CH_2), 38.89 ($COCH_2$), 60.48 (C(1)), 116.16 (CN), 123.99 (arom. CH), 125.30 (arom. CH), 127.09 (arom. CH), 142.77 (arom. C), 172.76 (CO), 174.37 ppm (CO); IR (ATR): $\tilde{\nu}$ = 3346 (w), 3301 (m), 1666 (s), 1646 (s), 1534 (s), 1509 (m), 1442 (w), 1411 (m), 1368 (w), 1284 (m), 1263 (w), 1219 (m), 1197 (m), 1110 (w), 1001 (w), 963 (w), 909 (w), 853 (w), 699 (s), 677 cm^{-1} (m); HR-ESI-MS: *m/z* (%): 320.1433 (100, $[M + H]^+$, calcd for $C_{16}H_{22}N_3O_2S^+$: 320.1427); elemental analysis calcd (%) for $C_{16}H_{21}N_3O_2S$ (319.43): C 60.16, H 6.63, N 13.15; found: C 60.14, H 6.52, N 13.14.

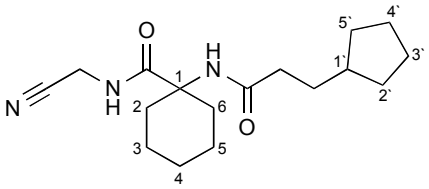
Methyl 1-(3-Cyclopentylpropanamido)cyclohexanecarboxylate (4g)



General procedure GP-A starting from 3-cyclopentyl-propionic acid (999 µL, 7.00 mmol), TBTU (4.50 g,

14.00 mmol), HOBt (1.89 g, 14.00 mmol), $^{i}Pr_2NEt$ (2.43 mL, 14.00 mmol) and **3** (1.21 g, 7.70 mmol) in DMF (35 mL). Purification by FC (SiO_2; cyclohexane/EtOAc 3:2) gave **4g** (0.77 g, 77%) as a white solid. R_f = 0.24 (SiO_2; heptane/EtOAc 2:1); m.p. 84–85 °C; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.07–1.15 (m, 2 H; CH_2), 1.26–1.88 (m, 17 H), 2.02 (d, J = 13.9 Hz, 2 H; H_{eq}–C(2, 6)), 2.20–2.24 (m, 2 H; $COCH_2$), 3.70 (s, 3 H; OMe), 5.58 ppm (br. s, 1 H; NH); ^{13}C NMR (100 MHz, $CDCl_3$; assignments based on an HSQC spectrum): δ = 21.53 (2 C, C(3, 5)), 25.15 (2 C, C(3′, 4′)), 25.18 (C(4)), 31.76 ($COCH_2CH_2$), 32.42 (br., 2 C, C(2, 6)), 32.44 (br., 2 C, C(2′, 5′)), 35.88 ($COCH_2$), 39.66 (C(1′)), 52.23 (OMe), 58.58 (C(1)), 172.79 (OCONH), 174.63 ppm (CO_2Me); IR (ATR): $\tilde{\nu}$ = 3252 (w), 3064 (w), 2945 (m), 2923 (m), 2858 (m), 1740 (s), 1732 (s), 1636 (s), 1549 (s), 1451 (m), 1430 (m), 1374 (w), 1359 (w), 1335 (w), 1288 (m), 1275 (m), 1237 (s), 1203 (m), 1182 (m), 1166 (m), 1071 (s), 1043 (w), 1008 (w), 985 (m), 933 (w), 900 (w), 852 (w), 805 (w), 784 (w), 736 (w), 718 (m), 700 (m), 615 cm^{-1} (w); HR-ESI-MS: m/z (%): 282.2208 (100, $[M + H]^+$, calcd for $C_{16}H_{28}NO_3^+$: 282.2024); elemental analysis calcd (%) for $C_{16}H_{27}NO_3$ (281.39): C 68.29, H 9.67, N 4.98, O 17.06; found: C 68.23, H 9.64, N 5.02, O 17.00.

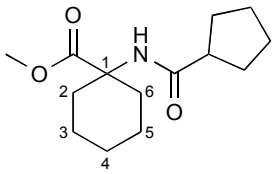
_N_-(Cyanomethyl)-1-(3-cyclopentylpropanamido)cyclohexane-carboxamide (1g)



A solution of **4g** (800 mg, 2.85 mmol) and $LiOH \cdot H_2O$ (358 mg, 8.55 mmol) in THF/MeOH/H_2O 2:2:1 (15 mL) was stirred at 25 °C for 3 d. The solvent was removed *in vacuo*. The residue was dissolved in H_2O (10 mL) and the pH adjusted to pH 2 using 2 M HCl. The solution was extracted with EtOAc (3 x 30 mL) and the solvent evaporated *in vacuo* to give the crude acid **5g** as a white solid. The residue was dissolved in DMF (18 mL) and treated with TBTU (1.83 g, 5.70 mmol), HOBt (770 mg, 5.70 mmol) and $^{i}Pr_2NEt$ (0.99 mL, 5.7 mmol) at 0 °C. The resulting solution was stirred at 0 °C for 20 min. Aminoacetonitrile bisulfate (483 mg, 3.14 mmol) was added and the mixture allowed to warm to 25 °C. After stirring for 24 h at this temperature, the solvent was evaporated *in vacuo*. H_2O (50 mL) was added and the suspension extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with H_2O (5 x 50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. FC (SiO_2; CH_2Cl_2/MeOH 25:1) gave **1g** (417 mg, 48%) as a white solid. R_f = 0.29 (SiO_2; CH_2Cl_2/MeOH 9:1); m.p. 142–144 °C; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.09–1.14 (m, 2 H; CH_2), 1.31–1.94

(m, 17 H), 2.12 (d, *J* = 14.0 Hz, 2 H; H_{eq}–C(2, 6)), 2.26–2.30 (m, 2 H; $COCH_2$), 4.13 (d, *J* = 5.8 Hz, 2 H; CH_2CN), 5.40 (br. s, 1 H; HN–C(1)), 8.21 ppm (t, *J* = 5.2 Hz, 1 H; CH_2NH); ^{13}C NMR (100 MHz, $CDCl_3$; assignments based on an HSQC spectrum): δ = 21.40 (2 C, C(3, 5)), 25.06 (2 C, C(3′, 4′)), 25.13 (C(4)), 27.61 (CH_2CN), 31.89 ($COCH_2CH_2$), 32.08 (br., 2 C, C(2, 6)), 32.45 (2 C, C(2′, 5′)), 36.63 ($COCH_2$), 39.67 (C(1′)), 60.38 (C(1)), 116.17 (CN), 174.64 (OCONH), 175.04 ppm (OC–C(1)); IR (ATR): $\tilde{\nu}$ = 3302 (m), 2944 (m), 2858 (m), 1665 (s), 1644 (s), 1646 (s), 1511 (s), 1451 (m), 1408 (m), 1353 (w), 1286 (m), 1263 (m), 1254 (m), 1199 (w), 1172 (m), 1156 (w), 1112 (m), 1044 (w), 1000 (w), 963 (w), 934 (w), 911 (w), 897 (m), 854 (w), 838 (w), 809 (w), 787 (w), 671 (m), 619 cm^{-1} (w); HR-ESI-MS: *m/z* (%): 328.1995 (100, $[M + Na]^+$, calcd for $C_{17}H_{27}N_3NaO_2^+$: 328.2001); elemental analysis calcd (%) for $C_{17}H_{27}N_3O_2$ (305.42): C 66.85, H 8.91, N 13.76, O 10.48; found: C 67.00, H 8.79, N 13.81, O 10.63.

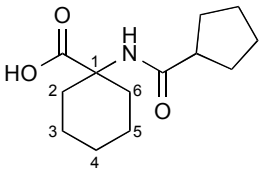
Methyl 1-(Cyclopentanecarboxamido)cyclohexanecarboxylate (4h)



General procedure GP-A starting from cyclopentanecarboxylic acid (0.95 mL, 8.76 mmol), TBTU (5.63 g, 17.52 mmol), HOBt (2.37 g, 17.52 mmol), $^{i}Pr_2NEt$ (3.02 mL, 17.52 mmol) and **3** (1.52 g, 9.64 mmol) in DMF (35 mL). Purification by FC (SiO_2; CH_2Cl_2/MeOH 40:1) gave **4h** (1.71 g, 89%) as a pale yellow solid. R_f = 0.57 (SiO_2; CH_2Cl_2/MeOH 10:1); m.p. 83–84 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.26–1.87 (m, 16 H), 2.00–2.05 (m, 2 H; H_{eq}–C(2, 6)), 2.52–2.62 (m, 1 H; CH), 3.69 (s, 3 H; OMe), 5.55 ppm (br. s, 1 H; NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.53 (CH_2), 25.20 (CH_2), 25.94 (CH_2), 30.21 (CH_2), 32.43 (CH_2), 45.51 (CH), 52.21 (OMe), 58.55 (C(1)), 174.70 (CO), 175.64 ppm (CO); IR (ATR): $\tilde{\nu}$ = 3343 (w), 2933 (m), 2865 (m), 1730 (s), 1637 (s), 1528 (s), 1466 (w), 1445 (m), 1384 (m), 1312 (m), 1277 (w), 1222 (s), 1202 (s), 1156 (m), 1138 (s), 1112 (m), 1011 (m), 993 (m), 906 (m), 867 (w), 830 (w), 791 (w), 752 cm^{-1} (m); HR-ESI-MS: *m/z* (%): 254.1749 (100, $[M + H]^+$, calcd for $C_{14}H_{24}NO_3^+$: 254.1751); elemental analysis calcd (%) for $C_{14}H_{23}NO_3$

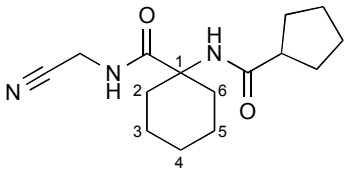
(253.34): C 66.37, H 9.15, N 5.53; found: C 66.36, H 9.08, N 5.81.

1-(Cyclopentanecarboxamido)cyclohexanecarboxylic Acid (5h)



General procedure GP-B starting from **4h** (1.98 g, 7.82 mmol) and $LiOH \cdot H_2O$ (984 mg, 23.45 mmol) in THF/MeOH/H_2O 2:2:1 (10 mL). Carboxylic acid **5h** (1.75 g, 94%) was obtained as a white solid and used for the next step without further characterisation.

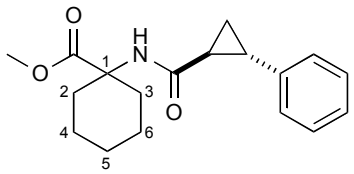
***N*-(Cyanomethyl)-1-(cyclopentanecarboxamido)cyclohexane-carboxamide (1h)**



General procedure GP-A starting from **5h** (300 mg, 1.25 mmol), TBTU (805 mg, 2.51 mmol), HOBt (339 mg, 2.51 mmol), $^{i}Pr_2NEt$ (648 µL, 3.76 mmol) and aminoacetonitrile bisulfate (213 mg, 1.25 mmol) in DMF (10 mL). Purification by FC (SiO_2; CH_2Cl_2/MeOH 50:1) gave **1h** (259 mg, 74%) as a white solid. R_f = 0.53 (SiO_2; CH_2Cl_2/MeOH 10:1); m.p. 176–177 °C; ^{1}H NMR (400 MHz,

$CDCl_3$): δ = 1.25–1.43 (m, 3 H), 1.54–1.82 (m, 9 H), 1.85–1.94 (m, 4 H), 2.11–2.14 (m, 2 H; H_{eq}–C(2, 6)), 2.56–2.64 (m, 1 H; CH), 4.12 (d, J = 5.8 Hz, 2 H; CH_2CN), 5.37 (br. s, 1 H; NH), 8.27 ppm (br. s, 1 H; N*H*CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.37 (CH_2), 25.07 (CH_2), 25.92 (CH_2), 27.59 (CH_2), 30.57 (CH_2), 32.05 (CH_2), 46.39 (CH), 60.35 (C(1)), 116.15 (CN), 174.71 (CO), 178.12 ppm (CO); IR (ATR): $\tilde{\nu}$ = 3299 (m), 2945 (m), 2860 (m), 1664 (s), 1640 (s), 1536 (s), 1450 (m), 1407 (m), 1287 (m), 1235 (s), 1204 (w), 1169 (w), 1112 (m), 1046 (w), 998 (m), 912 (m), 842 (w), 783 (w), 711 cm^{-1} (w); HR-ESI-MS: *m/z* (%): 278.1852 (100, $[M + H]^+$, calcd for $C_{15}H_{24}N_3O_2^+$: 278.1863); elemental analysis calcd (%) for $C_{15}H_{23}N_3O_2$ (277.37): C 64.96, H 8.36, N 15.15; found: C 64.93, H 8.29, N 15.16.

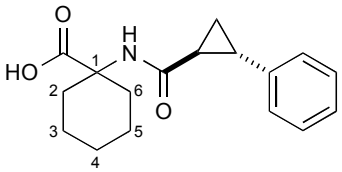
Methyl 1-(*trans*-2-Phenylcyclopropanecarboxamido)cyclo-hexanecarboxylate ((±)-4i)



General procedure GP-A starting from trans-2-phenylcyclopropane-carboxylic acid (1.00 g, 6.17 mmol), TBTU (3.96 g, 12.33 mmol), HOBt (1.67 g, 12.33 mmol), ^{i}Pr_2NEt (2.13 mL, 12.33 mmol) and **3** (1.07 g, 6.78 mmol) in

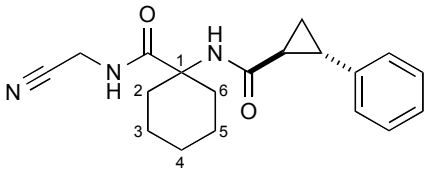
DMF (35 mL). Purification by FC (SiO_2; CH_2Cl_2/MeOH 80:1) gave (±)-**4i** (1.72 g, 84%) as a pale yellow foam. R_f = 0.71 (SiO_2; CH_2Cl_2/MeOH 10:1); ^1H NMR (300 MHz, $CDCl_3$): δ = 1.19–1.26 (m, 1 H; CH), 1.28–1.52 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.56–1.71 (m, 5 H; H_{eq}–C(3, 4, 5), 2 CH), 1.81–1.90 (m, 2 H; H_{ax}–C(2, 6)), 2.01–2.05 (m, 2 H; H_{eq}–C(2, 6)), 2.44 (ddd, J = 9.1, 6.3, 4.2 Hz, 1 H; C*H*Ph), 3.71 (s, 3 H; OMe), 5.87 (br. s, 1 H; NH), 7.06–7.30 ppm (m, 5 H; 5 arom. H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.00 (CH_2), 21.49 (2 C, 2 CH_2), 24.96 (*C*HPh), 25.17 (CH_2), 26.60 (*C*HCO), 32.49 (CH_2), 32.64 (CH_2), 52.27 (OMe), 59.01 (C(1)), 126.00 (arom. CH), 126.26 (arom. CH), 128.45 (arom. CH), 140.90 (arom. C), 171.28 (CO), 174.56 ppm (CO); IR (ATR): $\tilde{\nu}$ = 3381 (m), 3004 (w), 2935 (m), 2962 (w), 1718 (s), 1661 (s), 1606 (w), 1585 (w), 1525 (s), 1500 (m), 1454 (m), 1434 (m), 1409 (w), 1350 (w), 1294 (m), 1279 (m), 1247 (s), 1233 (s), 1198 (m), 1161 (m), 1094 (w), 1083 (m), 1071 (s), 1046 (m), 985 (m), 954 (w), 936 (m), 921 (w), 902 (w), 838 (w), 782 (w), 759 (s), 705 (s), 686 cm^{-1} (m); HR-ESI-MS: *m/z* (%): 302.1747 (100, $[M + H]^+$, calcd for $C_{18}H_{24}NO_3$: 302.1751).

1-(*trans*-2-Phenylcyclopropanecarboxamide)cyclohexane-carboxylic Acid ((±)-5i)



General procedure GP-B starting from (±)-**4i** (1.54 g, 5.12 mmol) and $LiOH \cdot H_2O$ (645 mg, 15.36 mmol) in THF/MeOH/H_2O 2:2:1 (10 mL). Carboxylic acid (±)-**5i** (1.36 mg, 93%) was obtained as a white solid and used for the next step without further characterisation.

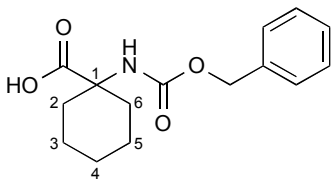
***N*-(Cyanomethyl)-1-(*trans*-2-phenylcyclopropanecarboxamido)-cyclohexanecarboxamide ((±)-1i)**



General procedure GP-A starting from (±)-**5i** (300 mg, 1.04 mmol), TBTU (670 mg, 2.09 mmol), HOBt (282 mg, 2.09 mmol), $^{i}Pr_2NEt$ (540 µL, 3.13 mmol) and aminoacetonitrile bisulfate (180 mg, 1.15 mmol) in DMF (10 mL). Purification by FC (SiO_2; CH_2Cl_2/MeOH 50:1) gave (±)-**1i** (302 mg, 54%) as a white solid. R_f = 0.50 (SiO_2; CH_2Cl_2/MeOH 10:1); m.p. 201–203 °C; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.25–1.46 (m, 4 H), 1.56–1.70 (m, 5 H), 1.89–1.96 (m, 2 H; H_{ax}–C(2, 6)), 2.08–2.12 (m, 2 H; H_{eq}–C(2,

6)), 2.47–2.52 (m, 1 H; CHPh), 4.13 (d, J = 5.6 Hz, 2 H; CH_2CN), 5.60 (br. s, 1 H; NH), 7.09–7.31 (m, 5 H; 5 arom. H), 8.13 ppm (t, J = 4.8 Hz, 1 H; N*H*CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.33 (CH_2), 21.33 (2 C, 2 CH_2), 25.03 (*C*HPh), 25.64 (CH_2), 27.02 (*C*HCO), 27.60 (2 C, 2 CH_2), 32.21 (CH_2), 60.71 (C(1)), 116.16 (CN), 126.04 (2 C, 2 arom. CH), 126.61 (arom. CH), 128.59 (2 C, 2 arom. CH), 140.05 (arom. C), 173.42 (CO), 174.45 ppm (CO); IR (ATR): $\tilde{\nu}$ = 3299 (m), 2939 (m), 2855 (w), 1665 (s), 1636 (s), 1541 (s), 1513 (s), 1454 (m), 1434 (m), 1402 (m), 1347 (m), 1284 (m), 1255 (m), 1236 (s), 1201 (m), 1113 (m), 1077 (w), 1046 (w), 1025 (m), 999 (w), 954 (m), 934 (m), 913 (m), 900 (w), 853 (w), 801 (w), 754 (m), 707 (m), 691 (s), 650 cm^{-1} (s); HR-ESI-MS: *m/z* (%): 326.1865 (100, $[M + H]^+$, calcd for $C_{19}H_{24}N_3O_2^+$: 326.1863); elemental analysis calcd (%) for $C_{19}H_{23}N_3O_2$ (325.41): C 70.13, H 7.12, N 12.91; found: C 69.98, H 7.05, N 13.01.

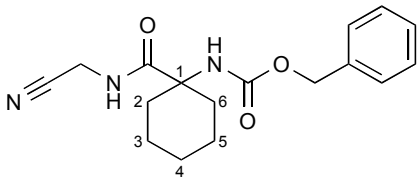
1-{[(Benzyloxy)carbonyl]amino}cyclohexanecarboxylic Acid (10)[10]



General procedure GP-C starting from 1-aminocyclohexane-carboxylic acid (**2**) (5.17 g, 36.1 mmol), Et_3N (5.50 mL, 40.0 mmol) in H_2O/MeCN 2:1 (45 mL) and *N*-(benzyloxy-

carbonyloxy)succinimide (9.00 g, 36.1 mmol) in MeCN (12 mL). **10** (7.38 g, 74%) was obtained as a white solid. m.p. 152–153 °C, Lit.[10] 152–154 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 1.26–1.51 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.63–1.69 (m, 3 H; H_{eq}–C(3, 4, 5)), 1.83–1.92 (m, 2 H; H_{ax}–C(2, 6)), 2.05–2.09 (m, 2 H; H_{eq}–C(2, 6)), 5.01 (br. s, 1 H; NH), 5.12 (s, 2 H; CH_2O), 7.37 ppm (s, 5 H; 5 arom. H); ^{13}C NMR (100 MHz, CD_3OD): δ = 21.04 (2 C, C(3, 5)), 25.09 (C(4)), 32.09 (2 C, C(2, 6)), 58.80 (C(1)), 65.73 (OCH_2), 127.26 (2 arom. CH), 127.46 (arom. CH), 128.00 (2 arom. CH), 137.00 (arom. C), 156.42 (OCONH), 177.06 ppm (CO_2H); HR-ESI-MS: *m/z* (%): 300.1205 (30, $[M + Na]^+$, calcd for $C_{15}H_{19}NNaO_4^+$: 300.1212), 278.1380 (100, $[M + H]^+$, calcd for $C_{15}H_{20}NO_4^+$: 278.1348), 234.1482 (83, $[M + H - CO_2]^+$, calcd for $C_{14}H_{20}NO_2^+$: 234.1449).

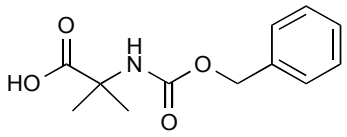
Benzyl (1-{[(Cyanomethyl)amino]carbonyl}cyclohexyl)carbamate (6)[11]



General procedure GP-A starting from **10** (0.879 g, 3.17 mmol), TBTU (2.04 g, 6.34 mmol), HOBt (0.857 g, 6.34 mmol), $^{i}Pr_2NEt$ (1.10 mL, 6.34 mmol) and aminoacetonitrile bisulfate (0.585 g, 3.80 mmol) in DMF (20 mL).

Purification by FC (SiO_2; cyclohexane/EtOAc 2:1) gave **6** (0.768 g, 77%) as a white solid. R_f = 0.44 (SiO_2; CH_2Cl_2/MeOH 16:1); m.p. 112–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.27–1.45 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.57–1.68 (m, 3 H; H_{eq}–C(3, 4, 5)), 1.87–1.93 (m, 2 H; H_{ax}–C(2, 6)), 2.01–2.06 (m, 2 H; H_{eq}–C(2, 6)), 4.14 (br. s, 2 H; CH_2CN), 4.96 (br. s, 1 H; OCONH), 5.12 (s, 2 H; CH_2O), 7.34–7.42 ppm (m, 5 H; 5 arom. H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.17 (2 C, C(3, 5)), 24.98 (C(4)), 27.64 (CH_2CN), 32.06 (br., 2 C, C(2, 6)), 59.58 (C(1)), 67.37 (OCH_2), 116.05 (CN), 128.22 (2 arom. CH), 128.51 (arom. CH), 128.70 (2 arom. CH), 135.79 (arom. C), 155.72 (OCONH), 174.61 ppm (CONH); HR-ESI-MS: *m/z* (%): 338.1466 (29, $[M + Na]^+$, calcd for $C_{17}H_{21}N_3NaO_3^+$: 338.1481), 316.1645 (100, $[M + H]^+$, calcd for $C_{17}H_{22}N_3O_3^+$: 316.1616).

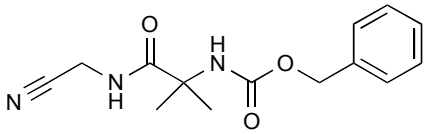
2-{[(Benzyloxy)carbonyl]amino}-2-methylpropanoic Acid (12)[12]



General procedure GP-C starting from 2-aminoisobutyric acid (**11**) (5.00 g, 48.49 mmol), Et_3N (7.40 mL, 53.34 mmol) in H_2O/MeCN 2:1 (40 mL) and *N*-(benzyloxycarbonyloxy)-succinimide (13.30 g, 53.34 mmol) in MeCN (15 mL). **12** (10.89 g, 95%) was obtained as a white solid.

R_f = 0.55 (SiO_2; *n*BuOH/AcOH/H_2O 4:1:1); m.p. 73–75 °C, Lit.[13] 74–75 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.58 (s, 6 H; 2 CH_3), 5.10 (s, 2 H; OCH_2), 5.40 (br. s, 1 H; NH), 7.30–7.38 ppm (s, 5 arom. H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 25.22 (2 CH_3), 56.35 ($C(CH_3)_2$), 67.05 (OCH_2), 128.22 (2 arom. CH), 128.33 (arom. CH), 128.68 (2 arom. CH), 136.27 (arom. C), 155.33 (CONH), 179.51 ppm (CO_2H); HR-ESI-MS: *m/z* (%): 260.0887 (100, $[M + Na]^+$, calcd for $C_{12}H_{15}NNaO_4^+$: 260.0893).

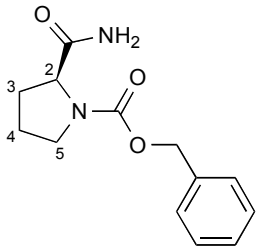
Benzyl [1-(Cyanomethylamino)-2-methyl-1-oxopropan-2-yl]-carbamate (7)



General procedure GP-A starting from **12** (474 mg, 2.00 mmol), TBTU (1.29 g, 4.00 mmol), HOBt (540 mg, 4.00 mmol), ^{i}Pr$_2$NEt (694 µL, 4.00 mmol) and aminoacetonitrile bisulfate (393 mg, 2.20 mmol) in DMF (10 mL). Purification by FC (SiO_2; heptane/EtOAc 1:1) gave **7** (246 mg, 45%) as a white solid. R_f = 0.41 (SiO_2; cyclohexane/EtOAc 1:1); m.p. 101–104 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.52 (s, 6 H; CMe_2), 4.10 (br. s, 2 H; CH_2CN), 5.12 (s, 2 H; OCH_2), 5.31 (br. s, 1 H; OCONH), 7.24 (br. s, 1 H; CONH), 7.34–7.40 ppm (m, 5 H; 5 arom. H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 25.48 (br., 2 C,

2 Me), 27.76 (CH_2CN), 57.09 (CMe_2), 67.26 (OCH_2), 116.15 (CN), 128.23 (2 arom. CH), 128.46 (arom. CH), 128.67 (2 arom. CH), 135.80 (arom. C), 155.51 (OCONH), 174.69 ppm (CONH); IR (ATR): $\tilde{\nu}$ = 3344 (w), 3283 (m), 3037 (w), 2985 (w), 2940 (w), 1684 (s), 1665 (s), 1534 (s), 1467 (w), 1454 (w), 1385 (w), 1369 (w), 1341 (w), 1279 (s), 1236 (m), 1186 (m), 1083 (m), 1029 (w), 1011 (w), 958 (m), 920 (w), 902 (w), 851 (w), 826 (w), 788 (w), 751 (m), 730 (m), 697 (m), 622 cm^{-1} (m); HR-ESI-MS: *m/z* (%): 298.1157 (100, $[M + Na]^+$, calcd for $C_{14}H_{17}N_3NaO_3^+$: 298.1168); elemental analysis calcd (%) for $C_{14}H_{17}N_3O_3$ (275.30): C 61.08, H 6.22, N 15.26, O 17.43; found: C 61.15, H 6.12, N 15.26, O 17.43.

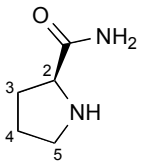
Benzyl (2*S*)-2-(Aminocarbonyl)1-pyrrolidinecarboxylate ((*S*)-(−)-14)[14]



A solution of (*S*)-1-(benzyloxycarbonyl)pyrrolidine-2-carboxylic acid ((*S*)-(−)-**13**) (10.00 g, 40.12 mmol), di-*tert*-butyl dicarbonate (11.38 g, 52.15 mmol), ammonium bicarbonate (3.81 g, 48.14 mmol) in MeCN (200 mL) was treated with pyridine (1.95 mL, 24.07 mmol) at 25 °C and

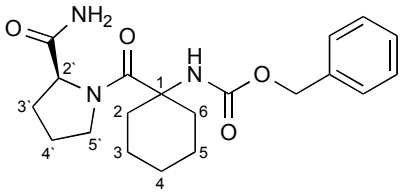
stirred at this temperature for 18 h. The solvent was removed *in vacuo*. The residue was partitioned between H_2O (150 mL) and EtOAc (250 mL). The organic layer was separated and the aqueous phase extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine (2 x 200 mL), dried over $MgSO_4$, filtered and the solvent evaporated. Compound (*S*)-(−)-**14** (9.30 g, 93%) was obtained as a white solid. R_f = 0.21 (SiO_2; EtOAc); m.p. 93–94 °C, Lit.[14] 91–93 °C; $[\alpha]_D^{20}$ = −104.8 (*c* = 0.2 in $CHCl_3$, Lit.[14] −100.6 (*c* = 0.5 in $CHCl_3$)); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.88–2.32 (m, 4 H; H–C(3), H–C(4)), 3.46–3.53 (m, 2 H; H–C(5)), 4.34 (s, 1 H; H–C(2)), 5.11–5.19 (m, 2 H; OCH_2), 5.69–6.02 (br. s, 2 H; NH_2), 7.34 ppm (s, 5 arom. H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.66 (C(4)), 28.41 (C(3)), 47.17 (C(5)), 60.33 (C(2)), 67.50 (OCH_2), 128.01 (2 arom. CH) 128.26 (arom. CH), 128.66 (2 arom. CH), 136.47 (arom. C), 156.19 (CON), 174.25 ppm ($CONH_2$); HR-ESI-MS: *m/z* (%): 249.1236 (100, $[M + H]^+$, calcd for $C_{13}H_{17}N_2O_3^+$: 249.1234).

(2*S*)-2-Pyrrolidinecarboxamide ((*S*)-(−)-15)[15]



A solution of (*S*)-(−)-**14** (2.69 g, 10.83 mmol) in MeOH (20 mL) was treated with palladium on charcoal (10% Pd) and hydrogen gas (1 bar) at 25 °C for 3.5 h. The suspension was filtered over celite and the solvent removed *in vacuo*. (*S*)-(−)-**15** (1.20 g, 97%) was obtained as a white solid. R_f = 0.26 (SiO_2; *n*BuOH/HOAc/H_2O 4:1:1); m.p. 101–102 °C, Lit.[15] 99–100 °C; $[\alpha]_D^{20}$ = −86.4 (*c* = 2.1 in EtOH, Lit. −99.5[15] (*c* = 2.0 in EtOH)); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.62–1.98 (m, 3 H; H–C(2), H–C(3)), 2.02 (s, 1 H ;NH), 2.08–2.21 (m, 1 H; H–C(2)), 2.87–3.05 (m, 2 H; H–C(5)), 3.72 (dd, *J* = 9.2, 5.5 Hz, 1 H; H–C(2)), 5.69 (br. s, 1 H; NH_2), 7.40 ppm (br. s, 1 H; NH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 26.25 (C(3)), 30.65 (C(2)), 47.33 (C(5)), 60.54 (C(2)), 178.61 ppm ($CONH_2$); HR-EI-MS: *m/z* (%): 114.0787 (20, $[M]^+$, calcd for $C_5H_{10}N_2O^+$: 114.0788), 70.0650 (100, $[M - CONH_2]^+$, calcd for $C_4H_8N^+$: 70.0651).

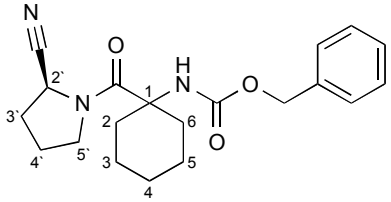
Benzyl (1-{[(2*S*)-2-(Aminocarbonyl)-pyrrolidinyl]-1-carbonyl}cyclohexyl)carbamate ((*S*)-(+)-**16)**



A solution of **10** (3.68 g, 13.28 mmol) in DMF/MeCN 1:1 (60 mL) was treated with TBTU (4.26 g, 13.28 mmol), HOBt (1.79 g, 13.28 mmol) and (*S*)-(−)-**15** (2.00 g, 13.28 mmol). The mixture was cooled to 0 °C and $^{i}Pr_2NH$ (6.53 mL, 46.48 mmol) was added dropwise. The mixture was allowed to warm to 25 °C, stirred at this temperature for 20 h and partitioned between EtOAc (25 mL) and brine (30 mL). The aqueous layer was extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with 0.1 M HCl (2 x 30 mL) and saturated aqueous $NaHCO_3$ solution (2 x 30 mL), dried over $MgSO_4$ and filtered. Evaporation and purification by FC (SiO_2; CH_2Cl_2/MeOH 15:1) gave (*S*)-(+)-**16** (4.24 g, 85%) as a white foam. R_f = 0.69 (SiO_2; CH_2Cl_2/MeOH 5:1); $[\alpha]_D^{20}$ = +51.8 (*c* = 0.5 in EtOH); ^{1}H NMR (400 MHz, $CDCl_3$; 1:1 mixture of rotamers, assignments based on a DQF-COSY spectrum): δ = 1.25–1.43 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.63–1.75 (m, 5 H; H_{eq}–C(3, 4, 5), H–C(4')), 1.75–1.94 (m, 2 H; H_{ax}–C(2, 6)), 2.07–2.13 (m, 4 H; H_{eq}–C(2, 6), H–C(3')), 3.09–3.15 (m, 1 H; H–C(5')), 3.57–3.63 (m, 1 H; H–C(5')), 4.55–4.59 (m, 1 H; H–C(2')), 4.98 (d, *J*

= 12.0 Hz, 1 H; OCH_2), 5.03–5.12 (br. s, 1 H; NH), 5.17 (br. s, 1 H; NH), 5.21 (br. d, *J* = 12 Hz, 1 H; OCH_2), 7.14 (br. s, 1 H; NH), 7.35 ppm (s, 5 arom. H); ^{13}C NMR (100 MHz, $CDCl_3$; assignments based on an HSQC spectrum): δ = 21.33 (2 C, C(3, 5)), 25.00 (C(4')), 25.85 (C(4)), 28.51 and 31.97 (2 C, C(2, 6)), 31.90 (C(3')), 48.07 (C(5')), 59.47 (C(1)), 62.57 (C(2')), 67.52 (OCH_2), 128.78 (2 arom. CH), 128.80 (arom. CH), 128.84 (2 arom. CH), 136.17 (arom. C), 155.22 (OCONH), 172.42 (CON), 174.85 ppm ($CONH_2$); IR (ATR): $\tilde{\nu}$ = 3415 (m), 3198 (w), 2947 (w), 1705 (m), 1665 (s), 1627 (s), 1601 (s), 1524 (m), 1449 (w), 1384 (s), 1323 (w), 1251 (s), 1092 (m), 979 (m), 745 (s), 697 (s), 633 cm^{-1} (m); HR-ESI-MS: *m/z* (%): 396.1891 (34, $[M + Na]^+$, calcd for $C_{20}H_{27}N_3NaO_4^+$: 396.1894), 374.2071 (100, $[M + H]^+$, calcd for $C_{20}H_{28}N_3O_4^+$: 374.2074); elemental analysis (%) calcd for $C_{20}H_{27}N_3O_4$ (373.45): C 6 4.32, H 7.29, N 11.25; found: C 64.03, H 7.47, N 11.09.

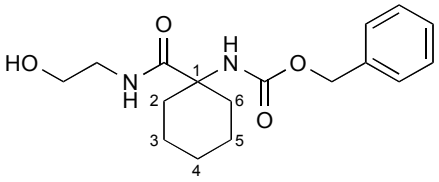
Benzyl (1-{[(2*S*)-2-Cyanopyrrolidinyl]-1-carbonyl}cyclo-hexyl)carbamate ((*S*)-(−)-8)



Cyanuric chloride (156 mg, 0.84 mmol) was added in one portion to an ice-cold solution of (*S*)-(+)-**16** (450 mg, 1.20 mmol) in DMF (10 mL). The mixture was stirred at 0 °C for 7.5 h, treated with ice-water (15 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with water (3 x 40 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give (*S*)-(−)-**8** (399 mg, 93%) as a white solid. R_f = 0.81 (SiO_2; CH_2Cl_2/MeOH 5:1); m.p. 170–171 °C; $[\alpha]_D^{20}$ = −64.0 (*c* = 0.3 in EtOH); ^{1}H NMR (400 MHz, $CDCl_3$; assignments based on an HSQC spectrum): δ = 1.24–1.45 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.55–1.65 (m, 5 H; H_{eq}–C(3, 4, 5), H–C(4')), 1.68–1.98 (m, 2 H; H_{ax}–C(2, 6)), 2.00–2.16 (m, 4 H; H_{eq}–C(2, 6), H–C(3')), 3.20–3.35 (br. s, 1 H; H_a–C(5')), 3.55–3.60 (m, 1 H; H_b–C(5')), 4.78 (br. s, 1 H; H–C(2')), 4.80–5.10 (br. m, 2 H; OCH_2, H–C(2')), 7.30–7.39 ppm (m, 5 arom. H); ^{13}C NMR (100 MHz, $CDCl_3$; assignments based on an HSQC spectrum): δ = 21.33 (2 C, C(3, 5)), 21.49 (C(4')), 25.01 (2 C, C(4)), 31.46 (C(3')), 32.47 (C(2, 6)), 47.33 (C(5')), 48.87 (C(2')), 59.36 (C(1)), 67.15 (OCH_2), 119.11

(CN), 128.59 (2 arom. CH), 128.70 (arom. CH), 128.88 (2 arom. CH), 172.25 (CO), 172.35 ppm (CO); IR (ATR): $\tilde{\nu}$ = 3348 (w), 2938 (w), 2861 (w), 1713 (s), 1619 (s), 1524 (s), 1455 (s), 1394 (s), 1281 (m), 1249 (s), 1198 (w), 1094 (m), 1078 (m), 1032 (w), 975 (m), 751 (m), 697 cm^{-1} (s); HR-ESI-MS: *m/z* (%): 373.2225 (100, $[M + NH_4]^+$, calcd for $C_{20}H_{29}N_4O_3^+$: 373.2234), 356.1966 (47, $[M + H]^+$, calcd for $C_{20}H_{26}N_3O_3^+$: 356.1969); elemental analysis (%) calcd for $C_{20}H_{25}N_3O_3$ (355.44): C 67.58, H 7.09, N 11.82; found: C 67.13, H 6.99, N 11.71.

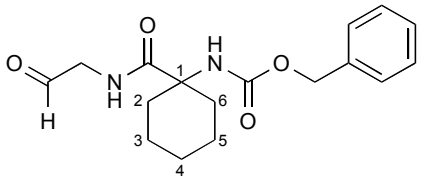
Benzyl (1-{[(2-Hydroxyethyl)amino]carbonyl}cyclohexyl)-carbamate (17)



General procedure GP-A starting from **10** (1.00 g, 3.61 mmol), TBTU (2.32 g, 7.22 mmol), HOBt (0.976 g, 7.22 mmol), $^{i}Pr_2NEt$ (1.25 mL, 7.22 mmol) and 2-amino-ethanol (262 µL, 4.33 mmol) in DMF (18 mL). Alcohol **17** (1.18 g, quant.) was obtained as a yellow oil without the need for further purification by FC. R_f = 0.20 (SiO_2; EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.19–1.42 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.55–1.57 (m, 3 H; H_{eq}–C(3, 4, 5)), 1.75–1.82 (m, 2 H; H_{ax}–C(2, 6)), 1.96–1.99 (m, 2 H; H_{eq}–C(2,

6)), 3.29 (br. s, 2 H; C*H*$_2$NH), 3.23 (br. s, 2 H; C*H*$_2$OH), 5.01 (s, 2 H; C*H*$_2$Ph), 5.78 (br. s, 1 H; OCONH), 7.00 (t, *J* = 5.8 Hz, 1 H; CONH), 7.26–7.29 ppm (m, 5 H; 5 arom. H); ^{13}C NMR (100 MHz, $CDCl_3$; assignments based on an HSQC spectrum): δ = 21.31 (2 C, C(3, 5)), 25.14 (C(4)), 32.06 (2 C, C(2, 6)), 42.32 (CH_2NH), 59.55 (C(1)), 61.50 (CH_2OH), 66.78 (OCH_2), 128.01 (2 arom. CH), 128.16 (arom. CH), 128.50 (2 arom. CH), 136.21 (arom. C), 155.49 (OCONH), 175.72 ppm (CONH); IR (ATR): $\tilde{\nu}$ = 3326 (m), 3061 (w), 2937 (m), 2855 (w), 2249 (w), 1716 (m), 1686 (s), 1650 (s), 1540 (s), 1514 (s), 1454 (m), 1447 (m), 1414 (m), 1374 (w), 1298 (m), 1279 (s), 1247 (s), 1216 (m), 1203 (s), 1174 (m), 1114 (m), 1088 (s), 1079 (s), 1041 (m), 974 (s), 935 (m), 915 (w), 850 (w), 819 (m), 781 (m), 750 (s), 732 (m), 700 (s), 620 cm^{-1} (s); HR-ESI-MS: *m/z* (%): 343.1627 (100, [*M* + Na]$^+$, calcd for $C_{17}H_{24}N_2NaO_4^+$: 343.1634).

Benzyl 1-[(2-Oxoethyl)carbamoyl]cyclohexylcarbamate (9)



A solution of **17** (390 mg, 1.22 mmol) in CH_2Cl_2 (60 mL) was treated with a 15-weight-% solution of Dess-Martin periodinane in CH_2Cl_2 (4.03 g, 1.46 mmol). The solution

was stirred at 25 °C for 4 h and then treated with a saturated aqueous $NaHCO_3$ solution (60 mL). The mixture was extracted with Et_2O (3 x 40 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. FC (SiO_2; heptane/EtOAc 3:5) gave **9** (251 mg, 65%) as a white foam. R_f = 0.35 (SiO_2; EtOAc); m.p. 106–110 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.27–1.46 (m, 3 H; H_{ax}–C(3, 4, 5)), 1.60–1.69 (m, 3 H; H_{eq}–C(3, 4, 5)), 2.06–2.10 (m, 2 H; H_{ax}–C(2, 6)), 1.96–1.99 (m, 2 H; H_{eq}–C(2, 6)), 4.16 (br. s, 2 H; OCHC*H*$_2$), 5.00 (s, 1 H; OCONH), 5.12 (s, 2 H; C*H*$_2$Ph), 7.21 (br. s, 1 H; CONH), 7.32 (br. s, 5 H; 5 arom. H), 9.63 ppm (s, 1 H; CHO); ^{13}C NMR (100 MHz, $CDCl_3$; assignments based on an HSQC spectrum): δ = 21.26 (2 C, C(3, 5)), 25.07 (C(4)), 32.21 (2 C, C(2, 6)), 50.36 (CH_2NH), 59.63 (C(1)), 67.09 (OCH_2), 128.19 (2 arom. CH), 128.35 (arom. CH), 128.62 (2 arom. CH), 136.04 (arom. C), 155.26 (OCONH), 174.88 (CONH), 196.79 ppm (CHO); IR (ATR): $\tilde{\nu}$ = 3326 (br. s), 3034 (w), 2933 (m), 2858 (m), 1703 (s), 1649 (s), 1514 (s), 1453 (s), 1403 (w), 1376 (w), 1338 (m), 1279 (s), 1243 (s), 1201 (m), 1172 (m), 1092 (s), 1029 (s), 973 (m), 909 (m), 857 (w), 805 (w), 779 (m), 731 (s), 695 (s), 645 (m), 605 cm^{-1} (m); HR-ESI-MS: *m/z* (%): 341.1476 (5, $[M + Na]^+$, calcd for $C_{17}H_{22}N_2NaO_4^+$: 341.1477), 268.9978 (100); elemental analysis calcd (%) for $C_{18}H_{22}N_3O_4$ (318.37):

C 69.70, H 7.70, N 12.83, O 9.77; found: C 69.76, H 7.62, N 12.57, O 9.82.

Literature

1 I. D. Kerr, J. H. Lee, K. C. Pandey, A. Harrison, M. Sajid, P. J. Rosenthal, L. S. Brinen, *J. Med. Chem.* **2009**, *52*, 852–857.

2 L. A. Hardegger, B. Kuhn, B. Spinnler, L. Anselm, R. Ecabert, M. Stihle, B. Gsell, R. Thoma, J. Dietz, J. Benz, J.-M. Plancher, G. Hartmann, D. W. Banner, W. Happ, F. Diederich, *Angew. Chem. Int. Ed.* **2011**, *50*, 314–318.

3 A. Breuning, B. Degel, F. Schulz, C. Büchold, M. Stempka, U. Machon, S. Heppner, C. Gelhaus, M. Leippe, M. Leyh, C. Kisker, J. Rath, A. Stich, J. Gut, P. J. Rosenthal, C. Schmuck, T. Schirmeister, *J. Med. Chem.* **2010**, *53*, 1951–1963.

4 F. Schulz, C. Gelhaus, B. Degel, R. Vicik, S. Heppner, A. Breuning, M. Leippe, J. Gut, P. J. Rosenthal, T. Schirmeister, *ChemMedChem* **2007**, *2*, 1214–1224.

5 W.-X. Tian, C.-L. Tsou, *Biochemistry* **1982**, *21*, 1028–1032.

6 R. Vicik, M. Busemann, C. Gelhaus, N. Stiefl, J. Schreiber, W. Schmitz, F. Schulz, M. Mladenovic, B. Engels, M. Leippe, K. Baumann, T. Schirmeister, *ChemMedChem* **2006**, *1*, 1126–1141.

7 M. Dixon, *Biochem. J.* **1972**, *129*, 197–202.

8 Y.-C. Cheng, W. H. Prusoff, *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

9 V. Wehner, H. Blum, M. Kurz, H. Stilz, *Synthesis* **2002**, *14*, 2023–2036.

10 M. Y. H. Lai, M. A. Brimble, D. J. Callis, P. W. R. Harris, M. S. Levi, F. Sieg, *Bioorg. Med. Chem.* **2005**, *13*, 533–548.

11 J. T. Palmer, C. Bryant, D. X. Wang, D. E. Davis, E. L. Setti, R. M. Rydzewski, S. Venkatraman, Z. Q. Tian, L. C. Burrill, R. V. Mendoca, E. Springman, J. McCarter, T. Chung, H. Cheung, J. W. Janc, M. McGrath, J. R. Somoza, P. Enriquez, Z. W. Yu, R. M. Strickley, L. Liu, M. C. Venuti, M. D. Percival, J. P. Falgueyret, P. Prasit, R. Oballa, D. Riendeau, R. N. Young, G. Wesolowski, S. B. Rodan, C. Johnson, D. B. Kimmel, G. Rodan, *J. Med. Chem.* **2005**, *48*, 7520–7534.

12 P. Rossi, F. Felluga, P. Tecilla, F. Formaggio, M. Crisma, C. Toniolo, P. Scrimin, *J. Am. Chem. Soc.* **1999**, *121*, 6948–6949.

13 P. Wipf, H. Heimgartner, *Helv. Chim. Acta* **1988**, *71*, 140–154.

14 V. Aureggi, V. Franckevicius, M. O. Kitching, S. Ley, D. A. Longbottom, A. J. Oelke, G. Sedelmeier, *Org. Synth.* **2008**, *85*, 72–87.

15 F. Herold, M. Dawidwoski, I. Wolska, A. Chodkowski, J. Kleps, J. Turlo, A. Zimniak, *Tetrahedron: Asymmetry* **2007**, *18*, 2019–2098.