Supporting Information

BIM-46174 Fragments as Potential Ligands of G Proteins

Jim Küppers,[a] Tobias Benkel,[b] Suvi Annala,[b] Gregor Schnakenburg,[c] Evi Kostenis[b] and Michael Gütschow*,[a]

[a]*Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, 53121 Bonn, Germany.*
[b]*Molecular, Cellular and Pharmacobiology Section, Institute for Pharmaceutical Biology, University of Bonn, Nussallee 6, 53115 Bonn, Germany.*
[c]*Institute of Inorganic Chemistry, University of Bonn, Gerhard-Domagk-Str. 1, 53121 Bonn, Germany.*

Table of Contents

Effect of different fragment concentrations on CCh- and PLCβ3 ΔXY-induced IP1 production

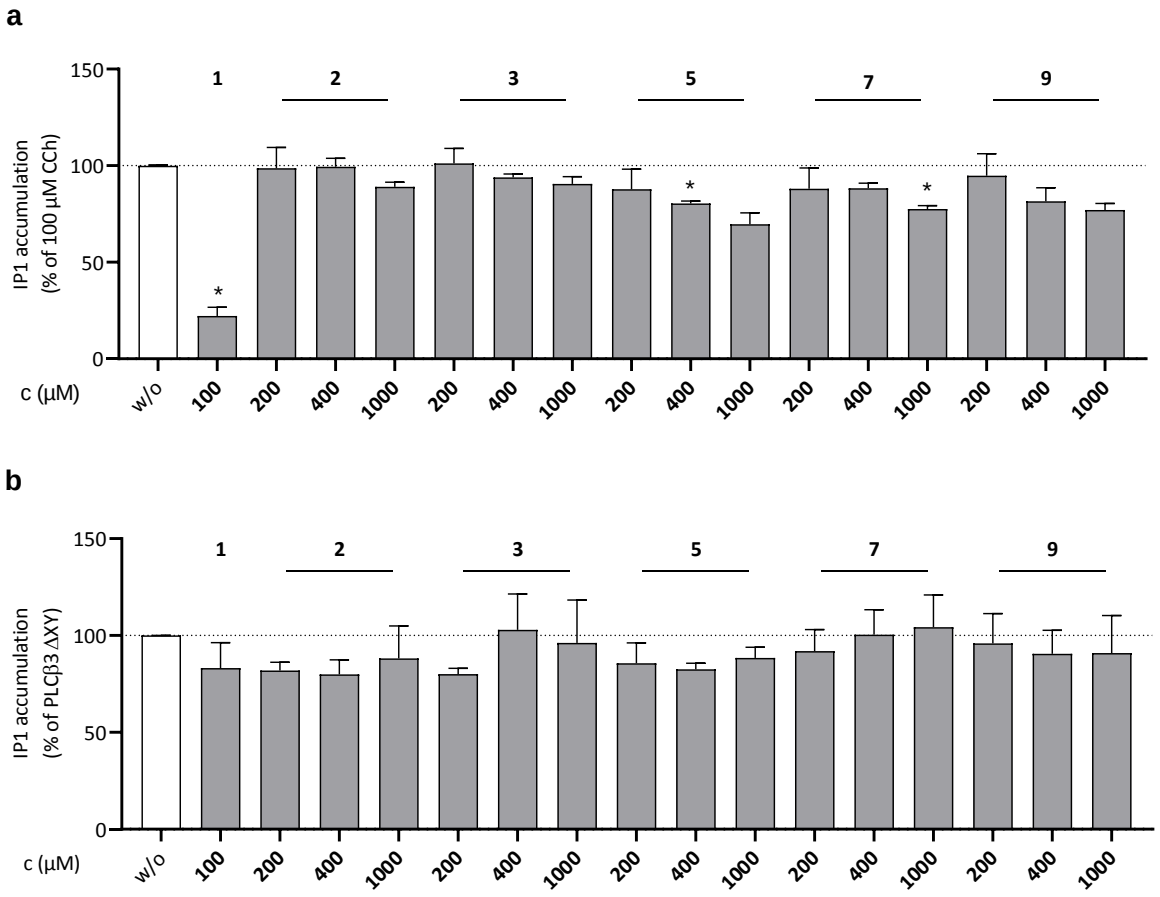


Figure S1. Effect of different fragment concentrations on CCh- and PLCβ3 ΔXY-induced IP1 production. (**a**) CCh induced IP1 production in cells pretreated with high concentrations of the respective fragments and (**b**) IP1 measurements of HEK293 wt cells transiently transfected with the constitutively active PLCβ1 ΔXY variant and incubated with the respective fragments for 2 h. Data are means ± s.e.m. of three independent experiments. * $P < 0.05$ compared to w/o using Dunnett´s multiple comparisons analysis after one-way ANOVA.

Protocols for biological assays

Cell culture and transfections. HEK293 wild-type (wt) cells were obtained from the American Type Culture Collection (ATCC). HEK293 wt cells were cultured in DMEM (Invitrogen) containing 10% foetal bovine serum (PAN biotech, Germany), 100 U mL^{-1} penicillin and 100 mg mL^{-1} streptomycin (Invitrogen) in a humidified CO_2 incubator at 37 °C and 5% CO_2. For PLCβ3 experiments, HEK293 wt cells were transiently transfected with either pcDNA3.1(+) or a constitutively active PLCβ3 mutant (PLCβ3 ΔXY)[1,2] cDNA using linear polyethylenimine (PEI, 25K, Polysciences, Inc.) in suspension. Briefly, 3 µL PEI and 1 µg cDNA (1:1 pcDNA: PLCβ3 ΔXY) were suspended in 100 µL OptiMEM (Invitrogen). After adding the PEI solution to the cDNA, the mixture was incubated for 15 min at room temperature. Meanwhile, the appropriate amount of cell suspension was prepared (1×10^6 cells in 3 mL DMEM). After the incubation, the transfection mixture was added to the cell suspension and gently mixed. The resulting solution was pipetted into a 6-well plate and incubated at 37 °C, 5% CO_2. Cells were analysed 48 h after transfection.

Inositol monophosphate (IP1) accumulation assay. The amount of intracellular IP1 was measured in a 384-well format using the HTRF-IP1 kit (Cisbio) as per manufacturer's instructions. Cell suspension was dispensed with a density of 60,000 cells/well into 384-well plates (7 µL/well). After 15 min incubation at 37 °C, 3.5 µL stimulation buffer containing BIM-46174 (**1**) or a BIM-fragment (**2**-**9**) was added and incubated for 2 h at 37 °C. Hereafter, 3.5 µL stimulation buffer containing 100 µM carbachol (CCh) was added. For experiments using PLCβ3 ΔXY, 3.5 µL stimulation buffer was lacking CCh. After another incubation at 37 °C for 35 min, IP1-*d*2 conjugate (3 µL) followed by terbium cryptate-labelled anti-IP1 antibody (3 µL) was added. Time-resolved fluorescence at 620 and 665 nm was measured with the Mithras LB 940 multimode reader after incubation at room temperature for 60 min, and the ratios of the signals were calculated as described previously.[3]

Colorimetric cell viability determination. Viability of HEK cells was assessed using a fluorimetric detection of resorufin (CellTiter-BlueTM cell viability assay, Promega). Specifically, cells were seeded (80 µL) at a density of 25,000 cells per well into black 96-well poly-D-lysine-coated plates with clear bottom and cultivated overnight. On the following day, 20 µL of BIM-46174 (**1**) or a BIM-fragment (**2**-**9**) were added to the cells and incubated for

22 h, followed by the addition of 20 μL Cell-Titer-BlueTM reagent per well. After shaking for 10 s, the plates were further incubated for 2 h. Fluorescence (excitation 560 nm, emission 590 nm) was measured using a FlexStation 3 Benchtop Multimode Plate Reader. Data were expressed as percentage of cell viability relative to buffer treatment.

Data and statistical analysis. Statistical and graphical analyses were performed using GraphPad Prism 8 software (GraphPad Software Inc., San Diego, CA). Data were analysed by one-way ANOVA with Dunnett´s post hoc analysis. Significance levels are given as: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. All data points throughout the manuscript represent the means ± s.e.m.

X-ray crystal structure of compound 4

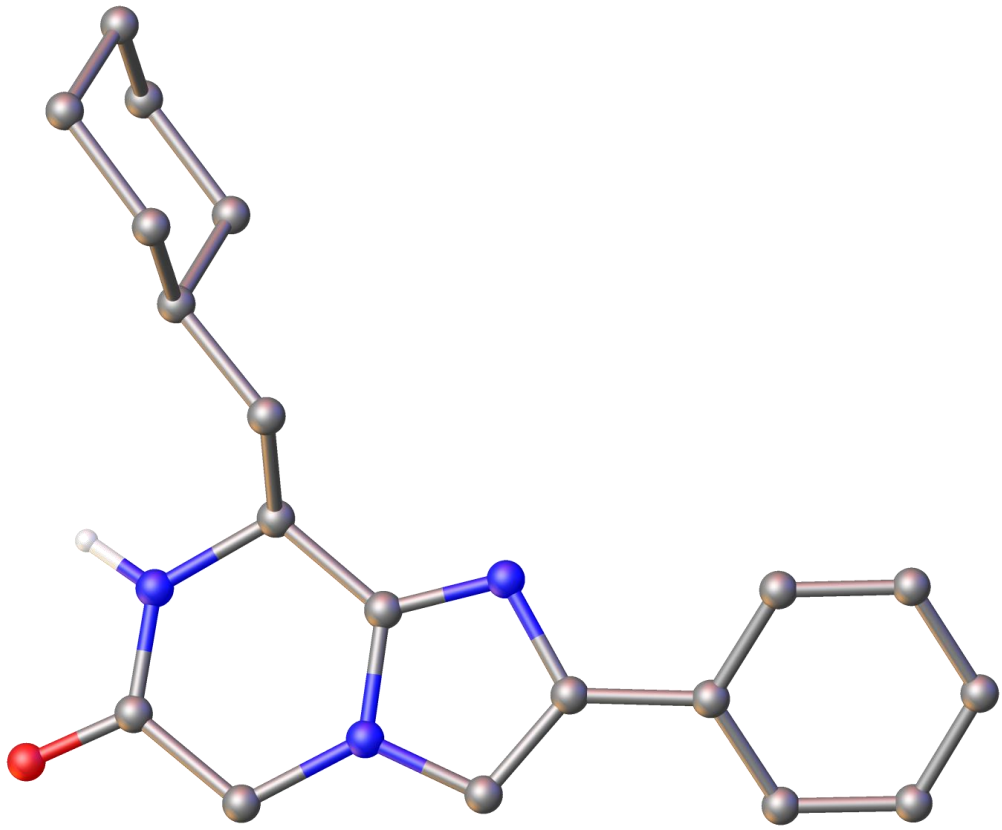


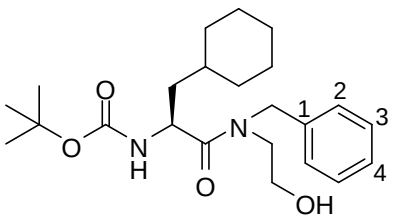
Figure S2. X-ray crystal structure of compound **4**. In the crystal form, compound **4** appears as a dimer due to interaction of the lactam NH of one molecule with the oxygen of the other. For specified hydrogen bonds (with esds except fixed and riding H), bond lengths and non-bonded distances for the N-H…O' and N'-H'…O system, respectively, are as follows, 0.88 Å and 0.88 Å (NH), 2.00 Å and 2.06 Å (H…O), 2.868(2) Å and 2.924(2) Å (N…O), respectively. The N-H…O' and N'-H'…O angels are 169.2 ° and 167.4 °, respectively. The X-ray crystallographic data collection of **4** was performed on a Bruker D8 Venture diffractometer (Photon I detector) at 150(2) K. The diffractometer was equipped with a low-temperature device (Oxford Cryostream 800, Oxford Cryosystems) and used mirror optic monochromated Cu Kα radiation (λ = 1.54178 Å). Intensities were measured by fine-slicing ϕ- and ω-scans and corrected for background, polarization, and Lorentz effects. Semi-empirical absorption corrections were applied for all data sets by using Bruker's SADABS program. The structure was solved by direct methods and refined anisotropically by the least-squares procedure implemented in the ShelX program system.[4-5] The hydrogen atoms were included isotropically using the riding model on the bound carbon atoms. CCDC 1915249 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/getstructures.

General chemical methods

Thin-layer chromatography was carried out on Merck (Darmstadt, Germany) aluminum sheets, silica gel 60 F254. Detection was performed with UV light at 254 nm. Preparative column chromatography was performed on Merck silica gel (0.063-0.200 mm, 60 Å). R_f values are related to thin-layer chromatography with the eluent of the corresponding column chromatography as mobile phase. When a gradient was used, the R_f value was determined with the final eluent as mobile phase. Melting points were determined on a Büchi (Essen, Germany) 510 oil bath apparatus. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker Avance DRX 500 and ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra on a Bruker Avance III 600 NMR spectrometer. Chemical shifts δ are given in ppm referring to the signal center using the solvent peaks for reference: DMSO-d_6 2.49/39.7 ppm. LC-MS analyses were carried out on an API2000 (Applied Biosystems, Darmstadt, Germany) mass spectrometer coupled to an Agilent (Santa Clara, CA, USA) 1100 LC system using a Phenomenex Luna C18 column (Phenomenex, Aschaffenburg, Germany; 50 × 2.0 mm, particle size 3 µm). Purity of the compounds was determined using the diode array detector (DAD) of the LC-MS instrument between 200 and 400 nm. HRMS were recorded on a microTOF-Q (Bruker, Köln, Germany) mass spectrometer connected to a Dionex (Thermo Scientific, Braunschweig, Germany) Ultimate 3000 LC via an ESI interface using a Nucleodur C18 Gravity column (50 × 2.0 mm I.D., 3 µm, Macherey-Nagel, Düren, Germany).

Procedures for the synthesis and analytical data of all compounds

(*S*)-*tert*-Butyl 1-(benzyl(2-hydroxyethyl)amino)-3-cyclohexyl-1-oxopropan-2-ylcarbamate (**11**).

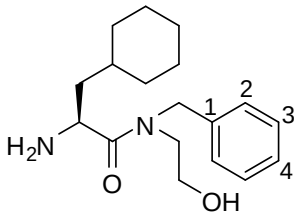


(*S*)-2-(*tert*-Butoxycarbonylamino)-3-cyclohexylpropanoic acid (**10**, 21.7 g, 80.0 mmol) and HATU (45.6 g, 120 mmol) were dissolved in anhydrous CH_2Cl_2 (290 mL) under nitrogen atmosphere and stirred at room temperature for 0.5 h. Subsequently, 2-(benzylamino)ethanol (11.5 g, 76.0 mmol) and triethylamine (24.3 g, 240 mmol) were added and stirring was

continued overnight. The solvent was then evaporated, the remaining oil diluted with H_2O (180 mL) and extracted with EtOAc (3 × 280 mL). The combined organic layer was washed with brine (280 mL), dried over Na_2SO_4, filtered and evaporated. The residue was purified by preparative column chromatography using a gradient of petroleum ether/EtOAc (3:2) to (1:1) (R_f = 0.5) as eluent to give **11** as a colourless oil (24.6 g, 80%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 0.75 – 0.97 (m, 2H, Cy-H), 0.99 – 1.28 (m, 4H, Cy-H), 1.33 and 1.38 (2 × s, 9H, C(CH_3)$_3$), 1.42 – 1.71 (m, 6H, CH_2-Cy, Cy-H), 1.75 – 1.81 (m, 1H, Cy-H), 3.18 (ddd, ^2J = 16.0 Hz, ^3J = 10.9 Hz, ^3J = 6.2 Hz, 1H, N-C*H*H-CH_2), 3.31 – 3.49 (m, 1H, N-CH*H*-CH_2), 3.55 (ddt, ^2J = 13.0 Hz, ^3J = 5.9 Hz, ^3J = 3.0 Hz, 2H, CH_2-OH), 4.39 – 4.72 (m, 3H, NH-C*H*, N-CH_2-C_6H_5), 4.82 (t, ^3J = 5.1 Hz, 1H, OH), 6.90 – 6.97 (m, 1H, NH), 7.14 – 7.31 (m, 4H, 2-H, 3-H), 7.35 (app t, ^3J = 7.5 Hz, 1H, 4-H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.8 (Cy-C), 26.1 (Cy-C), 26.2 (Cy-C), 28.3 (C(*C*H_3)$_3$), 31.8 (Cy-C), 33.6 (Cy-C), 33.7 (Cy-C), 39.0 (CH_2-Cy), 47.9, 48.7 (N-*C*H_2-CH_2, N-*C*H_2-C_6H_5), 48.9 (NH-CH), 59.3 (CH_2-OH), 78.1 (*C*(CH_3)$_3$, 126.9 (C-4), 127.3 (C-2), 128.4 (C-3), 138.2 (C-1), 155.8 (O-CO-NH), 173.4 (CO-N). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 200–400 nm), 97% purity, *m/z* = 405.3 ([M+H]$^+$).

(*S*)-2-Amino-*N*-benzyl-3-cyclohexyl-*N*-(2-hydroxyethyl)propanamide (**12**).

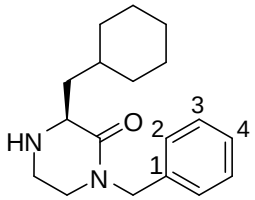


Compound **11** (24.3 g, 60.0 mmol) was dissolved in anhydrous CH_2Cl_2 (144 mL) and TFA (36 mL) was added. The mixture was stirred at room temperature for 2 h. After evaporating the solvents, the residue was redissolved in CH_2Cl_2 (140 mL) and again evaporated to dryness. This step was repeated 5 times. Finally, the residue was dissolved in CH_2Cl_2 (560 mL) and washed with sat. aq. $NaHCO_3$ solution (840 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by column chromatography on silica gel using EtOAc / 7 N ammonia in MeOH (9:1) (R_f = 0.5) as eluent to yield **12** as a clear, yellowish oil (7.12 g, 39%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 0.75 – 0.84 (m, 1H, Cy-H), 0.85 – 0.96 (m, 1H, Cy-H), 1.07 – 1.18 (m, 3H, Cy-H), 1.21 (ddd, ^2J = 13.5 Hz, ^3J = 8.7 Hz, ^3J = 4.9 Hz, 1H, C*H*H-Cy), 1.35 (ddd, ^2J = 13.1 Hz, ^3J = 8.1 Hz, ^3J = 4.9 Hz, 1H, CH*H*-Cy), 1.40 – 1.50 (m, 1H, Cy-H),

1.52 – 1.69 (m, 5H, Cy-H), 3.44 – 3.56 (m, 4H, N-CH_2-CH_2), 3.76 (dd, ^3J = 8.8 Hz, ^3J = 4.9 Hz, 1H, C*H*-CH_2-Cy), 4.33 (d, ^2J = 15.1 Hz, 1H, N-C*H*H-C_6H_5), 4.56 – 4.70 (m, 1H, OH), 4.75 (d, ^2J = 15.1 Hz, 1H, N-CH*H*-C_6H_5), 7.16 – 7.26 (m, 4H, 2-H, 3-H), 7.28 – 7.32 (m, 2H, NH_2), 7.33 – 7.38 (m, 1H, 4-H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.9 (Cy-C), 26.1 (Cy-C), 26.3 (Cy-C), 32.5 (Cy-C), 33.8 (Cy-C), 33.9 (Cy-C), 43.2 (CH_2-Cy), 47.8, 48.0, 48.6 (N-*C*H_2-CH_2, N-*C*H_2-C_6H_5, H_2N-CH), 59.1 (CH_2-OH), 126.5 (C-4), 127.5 (C-2), 128.5 (C-3), 138.5 (C-1), 176.5 (C=O). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 200–400 nm), 98% purity, *m/z* = 304.9 ($[M+H]^+$).

(*S*)-1-Benzyl-3-(cyclohexylmethyl)piperazin-2-one (**13**).

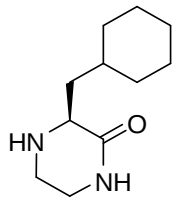


To a solution of compound **12** (6.09 g, 20.0 mmol) and triphenylphosphine (9.97 g, 38.0 mmol) in anhydrous THF (184 mL) was added diisopropyl azodicarboxylate (7.48 g, 37.0 mmol). After stirring the mixture at room temperature for 5 h, the solvent was evaporated. The crude residue was purified by preparative column chromatography using EtOAc (100%) (R_f = 0.5) as eluent. The product-containing fractions were combined and evaporated to dryness. The remaining residue was dissolved in anhydrous EtOAc (40 mL) under argon atmosphere and treated with 1 M HCl in EtOAc (8 mL). The precipitate was filtered off, washed with anhydrous EtOAc (120 mL) and dried *in vacuo*. The white solid was dissolved in sat. aq. $NaHCO_3$ solution (100 mL) and extracted with EtOAc (3 × 120 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated to give **13** as a colourless oil (2.00 g, 35%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 0.75 – 0.83 (m, 1H, Cy-H), 0.86 – 0.95 (m, 1H, Cy-H), 1.07 – 1.24 (m, 3H, Cy-H), 1.40 (ddd, ^2J = 13.9 Hz, ^3J = 9.7 Hz, ^3J = 4.5 Hz, 1H, C*H*H-Cy), 1.43 – 1.50 (m, 1H, Cy-H), 1.57 – 1.66 (m, 4H, Cy-H), 1.68 (ddd, ^2J = 13.5 Hz, ^3J = 9.4 Hz, ^3J = 3.7 Hz, 1H, CH*H*-Cy), 1.72 – 1.77 (m, 1H, Cy-H), 2.72 – 2.78 (m, 1H, NH-C*H*H), 2.93 (dt, ^2J = 12.9 Hz, ^3J = 4.3 Hz, 1H, NH-CH*H*), 3.08 (dt, ^2J = 11.6 Hz, ^3J = 4.1 Hz, 1H, CH_2-C*H*H-N), 3.16 – 3.22 (m, 1H, CH_2-CH*H*-N), 3.28 (dd, ^3J = 9.7 Hz, ^3J = 3.7 Hz, 1H, C*H*-CH_2-Cy), 4.43 (d, ^2J = 14.8 Hz, 1H, N-C*H*H-C_6H_5), 4.51 (d, ^2J = 14.8 Hz, 1H, N-CH*H*-C_6H_5), 7.18 – 7.22 (m, 2H, 2-H), 7.22 – 7.26 (m, 1H, 4-H), 7.29 – 7.34 (m, 2H, 3-H). One proton signal

(NH) is not recognizable. ^{13}C NMR (150 MHz, DMSO-d_6) δ 25.9 (Cy-C), 26.2 (Cy-C), 26.4 (Cy-C), 31.9 (Cy-C), 33.5 (Cy-C), 34.1 (Cy-C), 41.0 (NH-CH_2), 47.8, 49.4 (CO-N-(CH_2)$_2$), 56.3 (*C*H-CH_2-Cy), 127.2 (C-4), 127.7 (C-2), 128.6 (C-3), 137.7 (C-1), 170.9 (C=O). One carbon signal (CH_2-Cy) is obscured by the solvent peak. LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 200–400 nm), 97% purity, *m/z* = 286.8 ([M+H]$^+$).

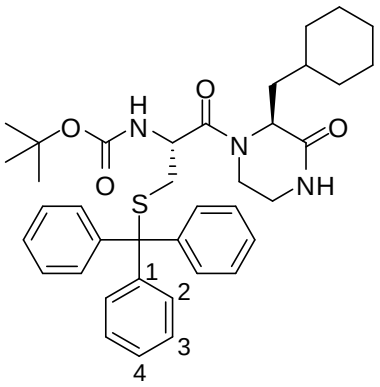
(*S*)-3-(Cyclohexylmethyl)piperazin-2-one (**14**).



Na (1.00 g, 43.5 mmol) was added to liquid NH_3 (60 mL) at -78 °C until a blue colour persisted. A solution of compound **13** (1.71 g, 6.00 mmol) in anhydrous THF (10 mL) was added and stirred for 20 min. Subsequently, H_2O (40 mL) and sat. aq. NH_4Cl solution (2 mL) were added dropwise at -78 °C. The mixture was extracted with EtOAc (5 × 80 mL) and the combined organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography using a gradient of EtOAc/MeOH (9:1) to (4:1) (R_f = 0.2) to obtain **14** as an orange solid (0.62 g, 54%); mp 84–87 °C.

^{1}H NMR (600 MHz, DMSO-d_6) δ 0.72 – 0.80 (m, 1H, Cy-H), 0.84 – 0.92 (m, 1H, Cy-H), 1.05 – 1.25 (m, 3H, Cy-H), 1.32 (ddd, ^2J = 14.1 Hz, ^3J = 9.9 Hz, ^2J = 4.5 Hz, 1H, C*H*H-Cy), 1.40 – 1.48 (m, 1H, Cy-H), 1.57 – 1.64 (m, 6H, Cy-H, CH*H*-Cy), 1.69 – 1.76 (m, 1H, Cy-H), 2.65 – 2.72 (m, 1H, CH-NH-C*H*H), 2.88 (dt, ^2J = 12.8 Hz, ^3J = 4.2 Hz, 1H, CH-NH-CH*H*), 3.03 – 3.08 (m, 1H, C*H*H-NH-CO), 3.10 – 3.15 (m, 2H, CH*H*-NH-CO, C*H*-CH_2-Cy), 7.44 (s, 1H, NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 25.8 (Cy-C), 26.1 (Cy-C), 26.4 (Cy-C), 31.8 (Cy-C), 33.3 (Cy-C), 34.0 (Cy-C), 39.1, 40.9 (CH_2-Cy, *C*H_2-NH-CO), 42.4 (CH-NH-*C*H_2), 55.8 (*C*H-CH_2-Cy), 171.9 (C=O). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 200–400 nm), *m/z* = 197.0 ([M+H]$^+$).

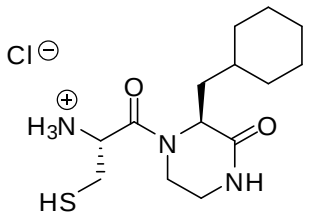
tert-Butyl (*R*)-1-((*S*)-2-(cyclohexylmethyl)-3-oxopiperazin-1-yl)-1-oxo-3-(tritylthio)propan-2-ylcarbamate (**16**).



(*R*)-2-(*tert*-Butoxycarbonylamino)-3-(tritylthio)propanoic acid (4.17 g, 9.00 mmol) and *N*,*N'*-dicyclohexylcarbodiimide (1.86 g, 9.00 mmol) were stirred in anhydrous CH_2Cl_2 (55 mL) under argon atmosphere for 0.5 h. Subsequently, (*S*)-3-(cyclohexylmethyl)piperazin-2-one (**14**, 0.59 g, 3.00 mmol) in CH_2Cl_2 (35 mL) and *N*,*N*-diisopropylethylamine (0.39 g, 3.00 mmol) were added and the reaction mixture was stirred at room temperature under argon atmosphere for 48 h. After removing the solvent *in vacuo*, the residue was treated with a mixture of petroleum ether/EtOAc (7:3) (200 mL). The white urea was filtered off, the filtrate was evaporated to dryness and the crude oily residue was purified by preparative column chromatography on silica gel using a gradient of petroleum ether/EtOAc (1:1) to EtOAc (100%) (R_f = 0.6) to obtain **16** as a yellow oil (1.42 g, 74%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 0.67 – 0.75 (m, 1H, Cy-H), 0.78 – 0.89 (m, 1H, Cy-H), 1.00 – 1.08 (m, 2H, Cy-H), 0.97 – 1.18 (m, 1H, Cy-H), 1.34 (s, 9H, C(CH_3)$_3$), 1.44 – 1.73 (m, 7H, Cy-H, CH_2-Cy), 1.80 – 1.88 (m, 1H, Cy-H), 2.35 – 2.47 (m, 2H, S-CH_2), [2.80 – 2.90 (m, 1H), 2.97 – 3.12 (m, 1H), 3.22 – 3.28 (m, 1H) and 3.54 – 3.62 (m, 1H, N-CH_2-CH_2)], 4.07 (app q, ^3J = 7.7 Hz, 1H, NH-C*H*), 4.63 – 4.70 (m, 1H, C*H*-CH_2-Cy), 7.19 – 7.37 (m, 16H, 2-H, 3-H, 4-H, O-CO-NH), 7.78 (s, 1H, CH-CO-N*H*). ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.6 (Cy-C), 25.8 (Cy-C), 26.2 (Cy-C), 28.3 (C(*C*H_3)$_3$), 32.2 (Cy-C), 33.0 (Cy-C), 33.5 (Cy-C), 38.1, 38.5, 41.0 (CH_2-Cy, CH_2-NH, S-CH_2), 50.0, 52.5 (NH-CH, N-CH_2), 59.9 (*C*H-CH_2-Cy), 66.5 (*C*(C_6H_5)$_3$), 78.4 (*C*(CH_3)$_3$), 127.0 (C-4), 128.2 (C-2), 129.2 (C-3), 144.4 (C-1), 155.0 (O-CO-NH), 169.2, 169.4 (CH-*C*O-NH, CO-N). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), *m/z* = 642.0 ([M+H]$^+$).

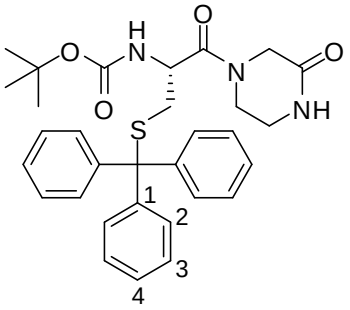
(*R*)-1-((*S*)-2-(Cyclohexylmethyl)-3-oxopiperazin-1-yl)-3-mercapto-1-oxopropan-2-aminium chloride (**2**).



Compound **17** (1.28 g, 2.00 mmol) was dissolved in anhydrous CH_2Cl_2 (15 mL) under argon atmosphere and a mixture of triisopropylsilane (3.17 g, 20.0 mmol) and TFA (15 mL) was added. After stirring at room temperature for 18 h, the solvent was evaporated to dryness without heating. The oily residue was treated with *n*-hexane (78 mL). After decanting the organic phase, the remaining oil was dissolved in anhydrous EtOAc (44 mL) containing 1,4-dithiothreitol (0.62 g, 4.00 mmol) and stirred for 2 h under argon atmosphere. Subsequently, a solution of 1 M HCl in EtOAc (5 mL) was added to the mixture, stirred for a further hour and the formed precipitate was filtered off, washed with anhydrous EtOAc (88 mL) and dried *in vacuo*. The crude solid was purified by column chromatography using chloroform / 7 N ammonia in MeOH (10:1) (R_f = 0.7). After evaporating the combined, product-containing fractions, the oily residue was redissolved in a mixture of 1,4-dithiothreitol (0.62 g, 4.00 mmol) in anhydrous EtOAc (54 mL) and anhydrous MeOH (10 mL), stirred again for 2 h under argon atmosphere and treated with 1 M HCl in EtOAc (5 mL) while stirring was prolonged for a further hour. To achieve complete precipitation of the salt, *n*-hexane (30 mL) was added to the mixture. The precipitate was filtered off, washed with anhydrous EtOAc (88 mL) and dried *in vacuo* to give a white solid (0.12 g, 18%); mp 174–176 °C.

^{1}H NMR (600 MHz, DMSO-d_6) δ 0.76 – 0.84 (m, 1H, Cy-H), 0.84 – 0.92 (m, 1H, Cy-H), 1.07 – 1.25 (m, 3H, Cy-H), 1.35 – 1.42 (m, 1H, Cy-H), 1.55 – 1.64 (m, 6H, Cy-H, CH_2-Cy), 1.67 – 1.74 (m, 1H, Cy-H), 2.75 – 2.94 (m, 2H, HS-CH_2), 3.02 (t, ^3J = 8.6 Hz, 1H, SH), [3.15 – 3.25 (m, 2H), 3.48 (ddd, ^2J = 14.7 Hz, ^3J = 11.0 Hz, ^3J = 4.2 Hz, 1H) and 3.90 – 4.03 (m, 1H, N-CH_2-CH_2)], 4.49 – 4.56 (m, 1H, $^+H_3$N-C*H*), 4.61 – 4.69 (m, 1H, C*H*-CH_2-Cy), 7.94 – 8.02 (m, 1H, NH), 8.37 – 8.57 (m, 3H, NH_3^+). ^{13}C NMR (150 MHz, DMSO-d_6) δ 24.9 (HS-CH_2), 25.6 (Cy-C), 25.8 (Cy-C), 26.3 (Cy-C), 32.7 (Cy-C), 33.1 (Cy-C), 33.2 (Cy-C), 38.8, 39.0, 40.6 (CH_2-Cy, N-CH_2, CH_2-NH), 51.4 ($^+H_3$N-CH), 53.2 (*C*H-CH_2-Cy), 165.8, 169.0 (CO-NH, CO-N). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 200–400 nm), *m/z* = 299.9 ($[M+H]^+$). HRMS, calcd. for $C_{14}H_{25}N_3O_2S$: $[M+H]^+$ *m/z* 300.1740, found: 300.1739.

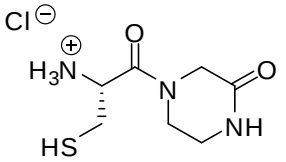
(*R*)-*tert*-Butyl 1-oxo-1-(3-oxopiperazin-1-yl)-3-(tritylthio)propan-2-ylcarbamate (**17**).



(*R*)-2-(*tert*-Butoxycarbonylamino)-3-(tritylthio)propanoic acid (13.9 g, 30.0 mmol) and *N*,*N'*-dicyclohexylcarbodiimide (6.19 g, 30.0 mmol) were stirred in anhydrous CH_2Cl_2 (180 mL) under argon atmosphere for 0.5 h. Subsequently, piperazin-2-one (**15**, 1.00 g, 10.0 mmol) in CH_2Cl_2 (120 mL) and *N*,*N*-diisopropylethylamine (1.29 g, 10.0 mmol) were added and the reaction mixture was stirred at room temperature and argon atmosphere for 48 h. After removing the solvent *in vacuo*, the residue was treated with a mixture of petroleum ether/EtOAc (7:3) (700 mL). The white urea was filtered off, the filtrate was evaporated to dryness and the crude oily residue was purified by preparative column chromatography on silica gel using EtOAc/MeOH (9:1) (R_f = 0.6) as eluent to obtain **17** as a colourless oil (5.07 g, 93%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 1.33 (s, 9H, C(CH_3)$_3$), 2.98 – 3.12 (m, 2H, S-CH_2), [3.20 – 3.26 (m, 1H), 3.37 – 3.43 (m, 1H), 3.48 – 3.57 (m, 1H, N-CH_2-CH_2)], 3.70 – 3.97 (m, 2H, CH_2-CO), 4.13 (app q, ^3J = 7.7 Hz, 1H, NH-C*H*), 7.21 – 7.36 (m, 16H, 2-H, 3-H, 4-H, O-CO-NH), 7.98 – 8.06 (m, 1H, CH_2-CO-N*H*). The signal for one N-CH_2-CH_2 hydrogen is obscured by the water peak. ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.2 (C(*C*H_3)$_3$), 33.4 (CH_2-NH), 41.7 (S-CH_2), 46.0 (N-*C*H_2-CH_2), 48.3 (NH-*C*H), 50.1 (*C*H_2-CO), 66.4 (*C*(C_6H_5)$_3$), 78.6 (*C*(CH_3)$_3$), 126.9 (C-4), 128.2 (C-2), 129.3 (C-3), 144.5 (C-1), 154.9 (O-CO-NH), 166.2 (CH_2-*C*O-NH), 168.6 (CO-N). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 97% purity, *m/z* = 563.5 ([M+NH_4]$^+$).

(*R*)-3-Mercapto-1-oxo-1-(3-oxopiperazin-1-yl)propan-2-aminium chloride (**3**).

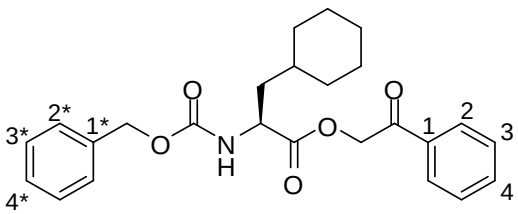


Compound **17** (4.91 g, 9.00 mmol) was dissolved in anhydrous CH_2Cl_2 (68 mL) under argon atmosphere and a mixture of triisopropylsilane (14.3 g, 90.0 mmol) and TFA (68 mL) was

added. After stirring at room temperature for 18 h, the solvent was evaporated to dryness without heating. The oily residue was treated with *n*-hexane (340 mL). After decanting the organic phase, the remaining oil was dissolved in anhydrous EtOAc (130 mL) containing 1,4-dithiothreitol (2.78 g, 18.0 mmol) and stirred for 2 h under argon atmosphere. Subsequently, a solution of 1 M HCl in EtOAc (18 mL) was added to the mixture, stirred for a further hour and the formed precipitate was filtered off, washed with anhydrous EtOAc (260 mL) and dried *in vacuo*. The crude solid was purified by column chromatography using chloroform / 7 N ammonia in MeOH (5:1) (R_f = 0.1). After evaporating the combined, product-containing fractions, the oily residue was redissolved in a mixture of 1,4-dithiothreitol (2.78 g, 18.0 mmol) in anhydrous EtOAc (110 mL) and anhydrous MeOH (55 mL), stirred again for 2 h under argon atmosphere and treated with 1 M HCl in EtOAc (18 mL) while stirring was prolonged for a further hour. To achieve complete precipitation of the salt, *n*-hexane (125 mL) was added to the mixture. The precipitate was filtered off, washed with anhydrous EtOAc (260 mL) and dried *in vacuo* to give a white solid (0.99 g, 46%); mp 95–98 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.75 – 2.95 (m, 2H, HS-CH_2), 3.01 and 3.11 (each t, ^3J = 8.8 and 8.9 Hz, 1H, SH), [3.15 – 3.21 (m, 1H), 3.21 – 3.33 (m, 1H), 3.71 (ddd, ^2J = 13.7 Hz, ^3J = 7.1 Hz, ^3J = 4.3 Hz, 1H) and 3.75 – 3.81 (m, 1H, N-CH_2-CH_2)], 3.87 – 4.08 (m, 1H, C*H*H-CO), 4.13 – 4.25 (m, 1H, CH*H*-CO), 4.45 – 4.57 (m, 1H, $^+H_3$N-C*H*), 8.11 – 8.20 (m, 1H, NH), 8.37 – 8.52 (m, 3H, NH_3^+). ^{13}C NMR (125 MHz, DMSO-d_6) δ 24.5, 24.8 (HS-CH_2), 42.3 (CH_2-NH), 46.1 (N-*C*H_2-CH_2), 48.5 (N-*C*H_2-CO), 51.3, 51.4 ($^+H_3$N-CH), 165.4, 165.8, 166.0, 166.1 (CO-NH, CO-N). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 200–400 nm), *m/z* = 204.0 ($[M+H]^+$). HRMS, calcd. for $C_7H_{13}N_3O_2S$: $[M+H]^+$ *m/z* 204.0801, found: 204.0798.

(*S*)-2-Oxo-2-phenylethyl 2-(benzyloxycarbonylamino)-3-cyclohexylpropanoate (**20**).

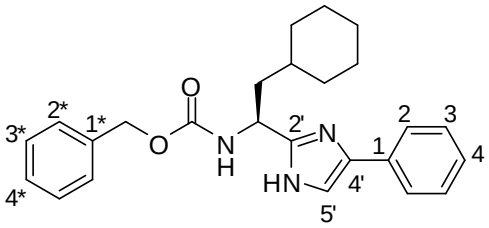


As described,[6] (*S*)-2-(benzyloxycarbonylamino)-3-cyclohexylpropanoic acid (**18**, 6.11 g, 20.0 mmol) was dissolved in anhydrous DMF (50 mL) and K_2CO_3 (3.32 g, 24.0 mmol) was added. After 10 min of stirring at room temperature, 2-bromoacetophenone (4.38 g, 22.0 mmol) was added and the reaction mixture was stirred at room temperature for a further 4 h. The mixture

was then diluted with H_2O (120 mL) and extracted with EtOAc (240 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chromatography on silica gel using a gradient of petroleum ether/EtOAc (20:1) to (10:1) (R_f = 0.1) to yield colourless crystals (6.94 g, 82%); mp 87–89 °C.

^{1}H NMR (600 MHz, DMSO-d_6) δ 0.80 – 0.89 (m, 1H, Cy-H), 0.90 – 0.99 (m, 1H, Cy-H), 1.09 – 1.24 (m, 2H, Cy-H), 1.37 – 1.46 (m, 1H, Cy-H), 1.57 – 1.77 (m, 8H, CH_2-Cy, Cy-H), 4.25 (ddd, ^3J = 10.4 Hz, ^3J = 8.0 Hz, ^3J = 4.6 Hz, 1H, C*H*-CH_2-Cy), 5.03 (d, ^2J = 12.8 Hz, 1H, C*H*H-O-CO-NH), 5.06 (d, ^2J = 12.4 Hz, 1H, CH*H*-O-CO-NH), 5.43 (d, ^2J = 16.9 Hz, 1H, C*H*H-CO), 5.57 (d, ^2J = 16.9 Hz, 1H, CH*H*-CO), 7.28 – 7.37 (m, 5H, 2*-H, 3*-H, 4*-H), 7.53 – 7.57 (m, 2H, 3-H), 7.66 – 7.70 (m, 1H, 4-H), 7.78 (d, ^3J = 8.1 Hz, 1H, NH), 7.93 – 7.97 (m, 2H, 2-H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 25.7 (Cy-C), 26.0 (Cy-C), 26.2 (Cy-C), 31.6 (Cy-C), 33.3 (Cy-C), 33.6 (Cy-C), 38.3 (CH_2-Cy), 51.6 (*C*H-CH_2-Cy), 65.6 (*C*H_2-O-CO-NH), 66.9 (*C*H_2-CO), 127.8, 128.0 (C-2*, C-3), 128.0 (C-4*), 128.5 (C-3*), 129.1 (C-2), 134.0 (C-4), 134.1 (C-1), 137.2 (C-1*), 156.3 (O-CO-NH), 172.7 (CH-*C*O-O), 192.7 (CH_2-*C*O). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 93% purity, *m/z* = 424.3 ($[M+H]^+$), 441.2 ($[M+NH_4]^+$).

(*S*)-Benzyl 2-cyclohexyl-1-(4-phenyl-1*H*-imidazol-2-yl)ethylcarbamate (**22**).

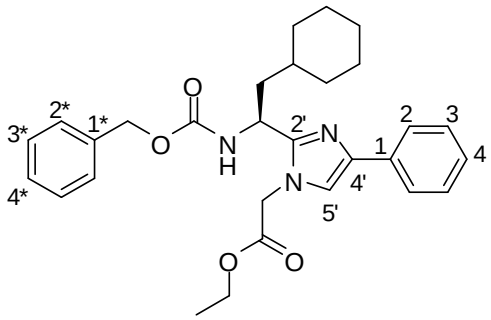


As described,[6] compound **20** (5.08 g, 12.0 mmol) was dissolved in anhydrous toluene (60 mL) and ammonium acetate (13.9 g, 180 mmol) was added to the solution. The reaction mixture was heated to reflux for 3 h with a drying tube attached to the condenser. The solvent was evaporated and the residue was suspended in EtOAc (240 mL). The organic layer was washed with H_2O (2 × 120 mL) and brine (120 mL), and then dried over Na_2SO_4, filtered, and evaporated to dryness. The crude residue was purified by preparative column chromatography using a gradient of petroleum ether/EtOAc (10:1) to (1:1) (R_f = 0.7) to give **22** as yellow solid (4.45 g, 92%); mp 92–94 °C (lit.[7] mp 102–103 °C).

^{1}H NMR (600 MHz, DMSO-d_6) δ 0.82 – 0.89 (m, 1H, Cy-H), 0.89 – 0.98 (m, 1H, Cy-H), 1.06 – 1.21 (m, 2H, Cy-H), 1.22 – 1.32 (m, 1H, Cy-H), 1.54 – 1.77 (m, 8H, CH_2-Cy, Cy-H), 4.73 – 4.80 (m, 1H, C*H*-CH_2-Cy), 5.00 (d, ^2J = 12.8 Hz, 1H, C*H*H-O), 5.08 (d, ^2J = 12.7 Hz,

1H, CH*H*-O), 7.13 – 7.48 (m, 9H, 2*-H, 3*-H, 4*-H, 3-H, 4-H, 5'-H), 7.59 (d, ^{3}J = 8.7 Hz, 1H, O-CO-NH), 7.71 – 7.74 (m, 2H, 2-H), 11.85 (s, 1H, imidazole-NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 25.8 (Cy-C), 26.0 (Cy-C), 26.2 (Cy-C), 32.1 (Cy-C), 33.2 (Cy-C), 33.8 (Cy-C), 41.6 (CH_2-Cy), 47.1 (*C*H-CH_2-Cy), 65.4 (CH_2-O), 112.4 (C-5'), 124.3 (C-2), 125.9 (C-4), 127.7 (C-2*), 127.9 (C-4*), 128.4, 128.5 (C-3, C-3*), 135.2 (C-1), 137.38 (C-1*), 139.6 (C-4'), 149.6 (C-2'), 156.0 (C=O). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 100% purity, *m/z* = 404.3 ($[M+H]^+$).

(*S*)-Ethyl 2-(2-(1-(benzyloxycarbonylamino)-2-cyclohexylethyl)-4-phenyl-1*H*-imidazol-1-yl)acetate (**24**).

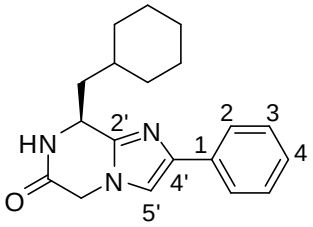


Compound **22** (2.02 g, 5.00 mmol) was dissolved in anhydrous DMF (8.5 mL) and Cs_2CO_3 (4.07 g, 12.5 mmol) was added to the solution. After stirring for 0.5 h at room temperature, ethyl 2-bromoacetate (1.00 g, 6.00 mmol) dissolved in anhydrous DMF (5.6 mL) was added dropwise over a period of 0.5 h. The mixture was stirred for a further 2.5 h at room temperature, then quenched with iced H_2O (40 mL) and extracted with EtOAc (3 × 40 mL). The organic layer was washed with brine (40 mL) and H_2O (2 × 40 mL), and dried over Na_2SO_4, filtered and evaporated to dryness. The crude residue was purified by preparative column chromatography using petroleum ether/EtOAc (10:1) (R_f = 0.1) as eluent to yield **24** as yellowish oil (2.03 g, 83%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 0.76 – 0.88 (m, 1H, Cy-H), 0.89 – 0.98 (m, 1H, Cy-H), 1.06 – 1.16 (m, 3H, Cy-H), 1.21 (t, ^{3}J = 7.0 Hz, 3H, CH_3), 1.26 – 1.36 (m, 1H, Cy-H), 1.58 – 1.66 (m, 4H, Cy-H), 1.68 – 1.75 (m, 2H, C*H*H-Cy, Cy-H), 1.87 – 1.95 (m, 1H, CH*H*-Cy), 4.10 – 4.16 (m, 2H, H_3C-CH_2), 4.66 – 4.73 (m, 1H, C*H*-CH_2-Cy), 4.90 – 5.10 (m, 4H, CH_2-N, CH_2-O-CO-NH), 7.15 – 7.20 (m, 1H, 4-H), 7.26 – 7.37 (m, 7H, 2*-H, 3*-H, 4*-H, 3-H), 7.51 (s, 1H, 5'-H), 7.68 – 7.72 (m, 2H, 2-H), 7.77 (d, ^{3}J = 8.7 Hz, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.1 (CH_3), 25.8 (Cy-C), 26.0 (Cy-C), 26.2 (Cy-C), 32.0 (Cy-C), 33.4 (Cy-C), 33.6 (Cy-C), 40.5 (CH_2-Cy), 44.1, 46.9 (*C*H-CH_2-Cy, CH_2-N), 61.3 (H_3C-*C*H_2), 65.5 (*C*H_2-O-

CO-NH), 117.9 (C-5'), 124.2 (C-2), 126.3 (C-4), 127.6 (C-2*), 127.8 (C-4*), 128.4, 128.6 (C-3, C-3*), 134.5 (C-1), 137.3 (C-1*), 138.5 (C-4'), 149.3 (C-2'), 156.1 (O-CO-NH), 168.4 (O-*C*O-CH_2). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 97% purity, *m/z* = 490.3 ($[M+H]^+$).

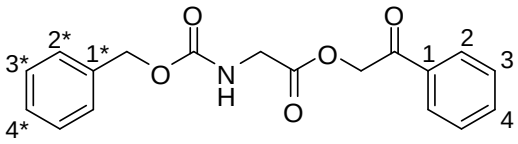
(*S*)-8-(Cyclohexylmethyl)-2-phenyl-7,8-dihydroimidazo[1,2-*a*]pyrazin-6(5*H*)-one (**4**).



Compound **24** (1.96 g, 4.00 mmol) was dissolved in anhydrous MeOH (32 mL) containing Pd/C (10% Pd) and was hydrogenated under atmospheric pressure at room temperature for 24 h. The catalyst was filtered off through celite and washed twice with MeOH (27 mL). The removal of the solvent *in vacuo* yielded a yellow solid (1.00 g, 81%) which exhibited the required purity without further purification; mp 195–198 °C (lit.[7] mp 206–207 °C). The product was recrystallized from EtOAc to obtain crystals for X-ray analysis.

^{1}H NMR (500 MHz, DMSO-d_6) δ 0.83 – 0.95 (m, 2H, Cy-H), 1.08 – 1.27 (m, 3H, Cy-H), 1.56 – 1.72 (m, 7H, CH_2-Cy, Cy-H), 1.71 – 1.79 (m, 1H, Cy-H), 4.59 – 4.65 (m, 2H, C*H*H-N, C*H*-CH_2-Cy), 4.70 (dd, ^2J = 17.7 Hz, ^4J = 1.3 Hz, 1H, CH*H*-N), 7.16 – 7.21 (m, 1H, 4-H), 7.31 – 7.36 (m, 2H, 3-H), 7.51 (s, 1H, 5'-H), 7.69 – 7.74 (m, 2H, 2-H), 8.59 (d, ^3J = 2.9 Hz, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.7 (Cy-C), 25.9 (Cy-C), 26.2 (Cy-C), 32.6 (Cy-C), 32.7 (Cy-C), 33.1 (Cy-C), 44.3 (CH_2-Cy), 47.0, 48.0 (CH_2-N, *C*H-CH_2-Cy), 114.2 (C-5'), 124.4 (C-2), 126.5 (C-4), 128.6 (C-3), 134.4 (C-1), 140.7 (C-4'), 143.1 (C-2'), 165.0 (C=O). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 98% purity, *m/z* = 310.1 ($[M+H]^+$). HRMS, calcd. for $C_{19}H_{23}N_3O$: $[M+H]^+$ *m/z* 310.1914, found: 310.1928.

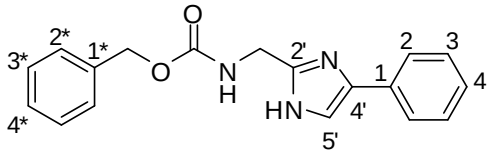
2-Oxo-2-phenylethyl 2-(benzyloxycarbonylamino)acetate (**21**).



2-(Benzyloxycarbonylamino)acetic acid (**19**, 6.28 g, 30.0 mmol) was dissolved in anhydrous DMF (76 mL) and K_2CO_3 (4.98 g, 36.0 mmol) was added. After 10 min of stirring at room temperature, 2-bromoacetophenone (6.56 g, 33.0 mmol) was added and the reaction mixture was stirred at room temperature for a further 4 h. The mixture was then diluted with H_2O (160 mL) and extracted with EtOAc (320 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness. The crude residue was purified by recrystallization from EtOAc/Et_2O to yield white needles (5.99 g, 61%); mp 102–104 °C (lit.[8] mp 103 °C).

^{1}H NMR (500 MHz, DMSO-d_6) δ 3.95 (d, ^3J = 6.2 Hz, 2H, NH-CH_2), 5.05 (s, 2H, CH_2-O-CO-NH), 5.54 (s, 2H, O-CH_2-CO), 7.28 – 7.38 (m, 5H, 2*-H, 3*-H, 4*-H), 7.53 – 7.58 (m, 2H, 3-H), 7.67 – 7.71 (m, 1H, 4-H), 7.75 (t, ^3J = 6.2 Hz, 1H, NH), 7.94 – 7.98 (m, 2H, 2-H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 42.1 (NH-CH_2), 65.7 (*C*H_2-O-CO-NH), 66.9 (O-*C*H_2-CO), 127.8, 127.9 (C-3, C-2*), 128.0 (C-4*), 128.5 (C-3*), 129.0 (C-2), 134.0 (C-4), 134.1 (C-1), 137.1 (C-1*), 156.6 (O-CO-NH), 169.9 (CH_2-*C*O-O), 192.6 (O-CH_2-*C*O). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 97% purity, *m/z* = 328.1 ([M+H]$^+$), 345.2 ([M+NH_4]$^+$).

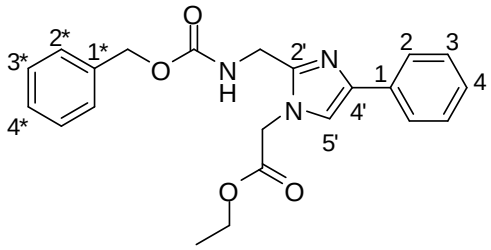
Benzyl (4-phenyl-1*H*-imidazol-2-yl)methylcarbamate (**23**).



Compound **21** (5.90 g, 18.0 mmol) was dissolved in anhydrous toluene (84 mL) and ammonium acetate (20.8 g, 270 mmol) was added to the solution. The reaction mixture was heated to reflux for 3 h with a drying tube attached to the condenser. The solvent was evaporated and the residue was suspended in EtOAc (280 mL). The organic layer was washed with H_2O (2 × 140 mL) and brine (140 mL), and then dried over Na_2SO_4, filtered, and evaporated to dryness. The crude residue was purified by preparative column chromatography using a gradient of petroleum ether/EtOAc (4:1) to (3:7) (R_f = 0.3) yielding **23** as a yellow solid (1.83 g, 33%); mp 174–176 °C (lit.[9] mp 171– 174 °C).

^{1}H NMR (500 MHz, DMSO-d_6) δ 4.27 (d, ^{3}J = 5.9 Hz, 2H, NH-CH_2), 5.06 (s, 2H, CH_2-O), 7.13 – 7.18 (m, 1H, 4-H), 7.28 – 7.39 (m, 7H, 2*-H, 3*-H, 4*-H, 3-H), 7.48 (s, 1H, 5'-H), 7.70 – 7.75 (m, 3H, 2-H, O-CO-NH), 11.91 (s, 1H, imidazole-NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 38.5 (NH-CH_2), 65.6 (CH_2-O), 112.8 (C-5'), 124.3 (C-2), 126.0 (C-4), 127.9 (C-2*), 127.9 (C-4*), 128.5 (C-3, C-3*), 135.1 (C-1), 137.2 (C-1*), 139.8 (C-4'), 145.8 (C-2'), 156.5 (C=O). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 97% purity, *m/z* = 308.0 ([M+H]$^{+}$).

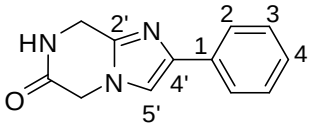
Ethyl 2-(2-((benzyloxycarbonylamino)methyl)-4-phenyl-1*H*-imidazol-1-yl)acetate (**25**).



Compound **23** (0.61 g, 2.00 mmol) was dissolved in anhydrous DMF (3.5 mL) and Cs_2CO_3 (1.63 g, 5.00 mmol) was added to the solution. After stirring for 0.5 h at room temperature, ethyl 2-bromoacetate (0.40 g, 2.40 mmol) dissolved in anhydrous DMF (2.25 mL) was added dropwise over a period of 0.5 h. The mixture was stirred for a further 2.5 h at room temperature, then quenched with iced H_2O (15 mL) and extracted with EtOAc (3 × 15 mL). The organic layer was washed with brine (15 mL) and H_2O (2 × 15 mL), and dried over Na_2SO_4, filtered and evaporated to dryness. The crude residue was purified by preparative column chromatography using a gradient of petroleum ether/EtOAc (9:1) to (3:2) (R_f = 0.3) to yield **25** as yellow crystals (0.57 g, 72%); mp 100–103 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.21 (t, ^{3}J = 7.1 Hz, 3H, CH_3), 4.14 (q, ^{3}J = 7.1 Hz, 2H, H_3C-CH_2), 4.26 (d, ^{3}J = 6.0 Hz, 2H, NH-CH_2), 4.99 (s, 2H, CH_2-N), 5.03 (s, 2H, CH_2-O-CO-NH), 7.16 – 7.21 (m, 1H, 4-H), 7.28 – 7.37 (m, 7H, 2*-H, 3*-H, 4*-H, 3-H), 7.55 (s, 1H, 5'-H), 7.68 – 7.72 (m, 2H, 2-H), 7.78 (t, ^{3}J = 6.4 Hz, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.1 (CH_3), 36.8 (NH-CH_2), 47.1 (CH_2-N), 61.3 (H_3C-*C*H_2), 65.7 (*C*H_2-O-CO-NH), 118.3 (C-5'), 124.2 (C-2), 126.4 (C-4), 127.8 (C-2*), 127.9 (C-4*), 128.5, 128.6 (C-3, C-3*), 134.4 (C-1), 137.2 (C-1*), 138.7 (C-4'), 146.0 (C-2'), 156.4 (O-CO-NH), 168.4 (O-*C*O-CH_2). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 90% purity, *m/z* = 394.2 ([M+H]$^{+}$).

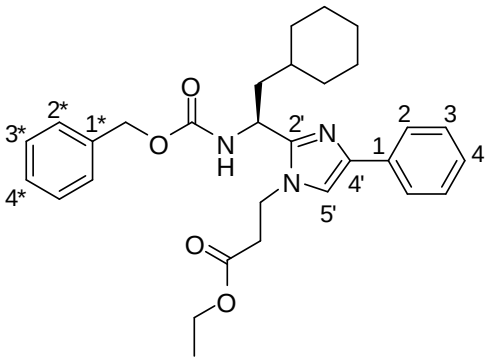
2-Phenyl-7,8-dihydroimidazo[1,2-*a*]pyrazin-6(5*H*)-one (**5**).



Compound **25** (0.39 g, 1.00 mmol) was dissolved in anhydrous MeOH (8 mL) containing Pd/C (10% Pd) and was hydrogenated under atmospheric pressure at room temperature for 24 h. The catalyst was filtered off through celite and washed twice with MeOH (6 mL). The removal of the solvent *in vacuo* yielded a brownish solid (0.09 g, 42%) which exhibited the required purity without further purification; mp >250 °C.

^{1}H NMR (600 MHz, DMSO-d_6) δ 4.44 – 4.47 (m, 2H, NH-CH_2), 4.62 – 4.64 (m, 2H, CH_2-N), 7.19 (tt, ^{3}J = 7.3 Hz, ^{4}J = 1.4 Hz, 1H, 4-H), 7.33 (t, ^{3}J = 7.7 Hz, 2H, 3-H), 7.54 (s, 1H, 5'-H), 7.70 – 7.75 (m, 2H, 2-H), 8.43 (app s, 1H, NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 39.5 (NH-CH_2), 47.1 (CH_2-N), 114.2 (C-5'), 124.4 (C-2), 126.5 (C-4), 128.7 (C-3), 134.4 (C-1), 139.3, 140.9 (C-2', C-4'), 165.0 (C=O). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 99% purity, *m/z* = 214.1 ([M+H]$^{+}$). HRMS, calcd. for $C_{12}H_{11}N_3O$: [M+H]$^{+}$ *m/z* 214.0975, found: 214.0989.

(*S*)-Ethyl 3-(2-(1-(benzyloxycarbonylamino)-2-cyclohexylethyl)-4-phenyl-1*H*-imidazol-1-yl)propanoate (**26**).

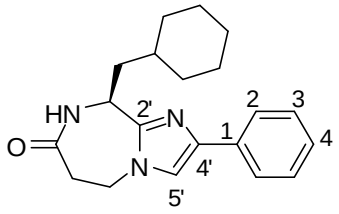


Compound **22** (2.02 g, 5.00 mmol) was dissolved in anhydrous DMF (8.5 mL) and Cs_2CO_3 (4.07 g, 12.5 mmol) was added to the solution. After stirring for 0.5 h at room temperature, ethyl 3-bromopropionate (1.09 g, 6.00 mmol) dissolved in anhydrous DMF (5.6 mL) was added dropwise over a period of 0.5 h. The mixture was stirred for a further 2.5 h at room temperature, then quenched with iced H_2O (40 mL) and extracted with EtOAc (3 × 40 mL). The organic layer was washed with brine (40 mL) and H_2O (2 × 40 mL), and dried over Na_2SO_4, filtered and evaporated to dryness. The crude residue was purified by preparative

column chromatography using petroleum ether/EtOAc (10:1) (R_f = 0.1) as eluent to yield **24** as a yellowish oil (2.22 g, 88%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 0.82 – 0.92 (m, 1H, Cy-H), 0.93 – 1.01 (m, 1H, Cy-H), 1.08 – 1.13 (m, 2H, Cy-H), 1.15 (t, ^3J = 7.2 Hz, 3H, CH_3), 1.29 – 1.35 (m, 1H, Cy-H), 1.55 – 1.76 (m, 6H, Cy-H, C*H*H-Cy), 1.78 – 1.83 (m, 1H, Cy-H), 1.89 (ddd, ^2J = 14.3 Hz, ^3J = 9.8 Hz, ^3J = 4.9 Hz, 1H, CH*H*-Cy), 2.75 – 2.87 (m, 2H, CO-CH_2), 4.00 – 4.09 (m, 2H, H_3C-CH_2), 4.13 (dt, ^2J = 13.9 Hz, ^3J = 6.6 Hz, 1H, C*H*H-N), 4.34 (dt, ^2J = 14.5 Hz, ^2J = 7.3 Hz, 1H, CH*H*-N), 4.85 – 4.91 (m, 1H, C*H*-CH_2-Cy), 4.98 (d, ^2J = 12.7 Hz, 1H, C*H*H-O-CO-NH), 5.07 (d, ^2J = 12.7 Hz, 1H, CH*H*-O-CO-NH), 7.13 – 7.18 (m, 1H, 4-H), 7.25 – 7.36 (m, 7H, 2*-H, 3*-H, 4*-H, 3-H), 7.55 (s, 1H, 5'-H), 7.68 (dd, ^3J = 7.8 Hz, ^4J = 1.4 Hz, 2H, 2-H), 7.77 (d, ^3J = 8.6 Hz, 1H, NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 14.2 (CH_3), 25.83 (Cy-C), 26.0 (Cy-C), 26.2 (Cy-C), 32.1 (Cy-C), 33.5 (Cy-C), 33.8 (Cy-C), 35.2 (CO-*C*H_2), 40.9 (CH_2-N), 44.3 (*C*H-CH_2-Cy), 60.4 (H_3C-*C*H_2), 65.4 (*C*H_2-O-CO-NH), 116.3 (C-5'), 124.2 (C-2), 126.2 (C-4), 127.6 (C-2*), 127.9 (C-4*), 128.4, 128.6 (C-3, C-3*), 134.6 (C-1), 137.4 (C-1*), 138.8 (C-4'), 148.8 (C-2'), 156.0 (O-CO-NH), 170.6 (O-*C*O-CH_2). One carbon signal (CH_2-Cy) is obscured by the solvent peak. LC-MS (ESI) (60% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 99% purity, *m/z* = 504.4 ($[M+H]^+$).

(*S*)-9-(Cyclohexylmethyl)-2-phenyl-8,9-dihydro-5*H*-imidazo[1,2-*a*][1,4]diazepin-7(6*H*)-one (**6**).

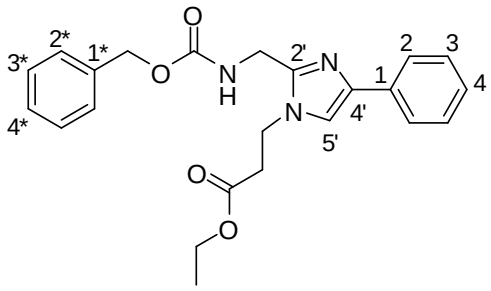


Compound **26** (2.01 g, 4.00 mmol) was dissolved in anhydrous MeOH (30 mL) containing Pd/C (10% Pd) and was hydrogenated under atmospheric pressure at room temperature for 24 h. The catalyst was filtered off through celite and washed twice with MeOH (23 mL). The solvent was evaporated to dryness, the residue redissolved in anhydrous EtOAc (40 mL) and toluenesulfonic acid (5%) was added. After heating the mixture to reflux for 16 h, the solvent was evaporated and the residue purified by column chromatography using EtOAc (100%) (R_f = 0.5) as eluent to yield white solid (0.71 g, 55%); mp 214–217 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 0.84 – 0.93 (m, 1H, Cy-H), 0.95 – 1.06 (m, 1H, Cy-H), 1.11 – 1.28 (m, 3H, Cy-H), 1.54 (dtt, ^2J = 11.8 Hz, ^3J = 8.2 Hz, ^3J = 4.1 Hz, 1H, Cy-H), 1.60

– 1.65 (m, 1H, Cy-H), 1.66 – 1.80 (m, 4H, C*H*H-Cy, Cy-H), 1.85 – 1.92 (m, 1H, Cy-H), 2.13 (ddd, ^{2}J = 13.9 Hz, ^{3}J = 9.1 Hz, ^{3}J = 4.6 Hz, 1H, CH*H*-Cy), 2.50 – 2.56 (m, 1H, CO-CH*H*), 3.31 – 3.39 (m, 1H, CO-C*H*H), 4.12 – 4.19 (m, 1H, C*H*H-N), 4.27 (dt, ^{2}J = 13.6 Hz, ^{3}J = 5.1 Hz, 1H, CH*H*-N), 4.75 (dt, ^{3}J = 9.8 Hz, ^{3}J = 5.0 Hz, 1H, C*H*-CH_2-Cy), 7.14 – 7.19 (m, 1H, 4-H), 7.30 – 7.35 (m, 2H, 3-H), 7.54 (s, 1H, 5'-H), 7.67 – 7.71 (m, 2H, 2-H), 7.84 (d, ^{3}J = 5.7 Hz, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.9 (Cy-C), 26.1 (Cy-C), 26.3 (Cy-C), 31.9 (Cy-C), 33.5 (Cy-C), 33.6 (Cy-C), 33.6 (CO-*C*H_2), 38.6 (CH_2-Cy), 43.4 (CH_2-N), 46.8 (*C*H-CH_2-Cy), 117.9 (C-5'), 124.3 (C-2), 126.3 (C-4), 128.6 (C-3), 134.5 (C-1), 137.9 (C-4'), 147.4 (C-2'), 172.9 (C=O). LC-MS (ESI) (60% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 99% purity, *m/z* = 324.1 ([M+H]$^+$). HRMS, calcd. for $C_{20}H_{25}N_3O$: [M+H]$^+$ *m/z* 324.2070, found: 324.2084.

Ethyl 3-(2-((benzyloxycarbonylamino)methyl)-4-phenyl-1*H*-imidazol-1-yl)propanoate (**27**).

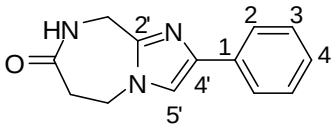


Compound **23** (0.61 g, 2.00 mmol) was dissolved in anhydrous DMF (3.5 mL) and Cs_2CO_3 (1.63 g, 5.00 mmol) was added to the solution. After stirring for 0.5 h at room temperature, ethyl 3-bromopropionate (0.43 g, 2.40 mmol) dissolved in anhydrous DMF (2.25 mL) was added dropwise over a period of 0.5 h. The mixture was stirred for a further 2.5 h at room temperature, then quenched with iced H_2O (15 mL) and extracted with EtOAc (3 × 15 mL). The organic layer was washed with brine (15 mL) and H_2O (2 × 15 mL), and dried over Na_2SO_4, filtered and evaporated to dryness. The crude residue was purified by preparative column chromatography using a gradient of petroleum ether/EtOAc (3:2) to (2:3) (R_f = 0.7) to yield **25** as yellow oil (0.38 g, 47%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.15 (t, ^{3}J = 7.1 Hz, 3H, CH_3), 2.86 (t, ^{3}J = 6.9 Hz, 2H, CO-CH_2), 4.07 (q, ^{3}J = 7.1 Hz, 2H, H_3C-C*H*$_2$), 4.25 (t, ^{3}J = 6.8 Hz, 2H, CH_2-N), 4.39 (d, ^{3}J = 5.8 Hz, 2H, NH-C*H*$_2$), 5.05 (s, 2H, C*H*$_2$-O-CO-NH), 7.19 – 7.24 (m, 1H, 4-H), 7.25 – 7.40 (m, 7H, 2*-H, 3*-H, 4*-H, 3-H), 7.67 (s, 1H, 5'-H), 7.68 – 7.71 (m, 2H, 2-H), 7.84 (t, ^{3}J = 5.6 Hz, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.1 (CH_3), 34.8 (CO-*C*H_2), 36.6 (NH-CH_2), 41.4 (CH_2-N), 60.4 (H_3C-*C*H_2), 65.7 (*C*H_2-O-CO-NH), 117.0 (C-5'), 124.4 (C-2), 126.7 (C-4),

127.8 (C-2*), 127.9 (C-4*), 128.4, 128.7 (C-3, C-3*), 133.3 (C-1), 137.1 (C-1*), 137.9 (C-4'), 145.3 (C-2'), 156.3 (O-CO-NH), 170.6 (O-*C*O-CH_2). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 96% purity, *m/z* = 408.1 ($[M+H]^+$).

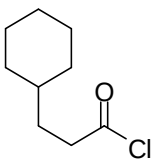
2-Phenyl-8,9-dihydro-5*H*-imidazo[1,2-*a*][1,4]diazepin-7(6*H*)-one (**7**).



Compound **27** (0.20 g, 0.50 mmol) was dissolved in anhydrous MeOH (6 mL) containing Pd/C (10% Pd) and was hydrogenated under atmospheric pressure at room temperature for 24 h. The catalyst was filtered off through celite and washed twice with MeOH (5 mL). The solvent was evaporated to dryness, the residue redissolved in anhydrous EtOAc (15 mL) and toluenesulfonic acid (5%) was added. After heating the mixture to reflux for 16 h, the solvent was evaporated and the residue was purified by column chromatography using EtOAc/MeOH (9:1) (R_f = 0.1) as eluent to give a white solid (352 mg, 31%); mp >250 °C.

^{1}H NMR (600 MHz, DMSO-d_6) δ 2.89 – 2.96 (m, 2H, CO-CH_2), 4.17 – 4.24 (m, 2H, CH_2-N), 4.38 (d, ^3J = 5.4 Hz, 2H, NH-CH_2), 7.17 (tt, ^3J = 7.3 Hz, ^4J = 1.3 Hz, 1H, 4-H), 7.32 (t, ^3J = 7.7 Hz, 2H, 3-H), 7.55 (s, 1H, 5'-H), 7.69 (dd, ^3J = 8.0 Hz, ^4J = 1.5 Hz, 2H, 2-H), 8.07 (t, ^3J = 5.4 Hz, 1H, NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 33.2 (CO-*C*H_2), 39.3 (NH-CH_2), 43.4 (*C*H_2-N), 117.7 (C-5'), 124.3 (C-2), 126.4 (C-4), 128.6 (C-3), 134.4 (C-1), 138.4 (C-4'), 144.1 (C-2'), 173.4 (C=O). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 99% purity, *m/z* = 228.1 ($[M+H]^+$). HRMS, calcd. for $C_{13}H_{13}N_3O$: $[M+H]^+$ *m/z* 228.1131, found: 228.1148.

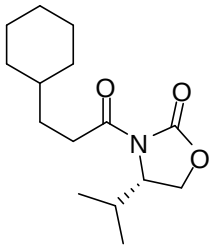
3-Cyclohexylpropanoyl chloride (**29**).



To a solution of 3-cyclohexylpropionic acid (**28**, 10.94 g, 70.0 mmol) in anhydrous CH_2Cl_2 (145 mL) was added dropwise thionyl chloride (10.2 mL) at 0 °C. The resulting mixture was

refluxed overnight and subsequently the excess solvent was evaporated. The resulting yellowish liquid was used without further purification and characterization (12.03 g, 98%).

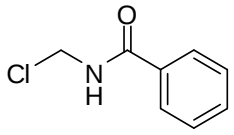
(*S*)-3-(3-Cyclohexylpropanoyl)-4-isopropyloxazolidin-2-one (**30**).



To a solution of (*S*)-4-isopropyloxazolidin-2-one (6.46 g, 50.0 mmol) in anhydrous THF (110 mL) was added dropwise the solution of *n*-butyllithium (20 mL, 50.0 mmol, 2.5 M in hexane) within 1 h at -78 °C. After additional stirring for 0.5 h at -78 °C, a solution of compound **29** (11.53 g, 66.0 mmol) in anhydrous THF (27 mL) was added dropwise within 15 min at a temperature below -70 °C and stirred for 0.5 h at the same temperature. Subsequently, the reaction mixture was warmed to room temperature within 15 min and sat. aq. NH_4Cl (45 mL) was added. The organic solvent was evaporated and the residue was quenched with H_2O (26 mL). The obtained mixture was extracted with CH_2Cl_2 (3 × 65 mL) and the combined organic layer was washed with 1 M solution of NaOH (65 mL), H_2O (65 mL), brine (65 mL), dried over Na_2SO_4, filtered and evaporated to dryness. The crude residue was purified by column chromatography using petroleum ether/EtOAc (8:1) (R_f = 0.1) to give a colourless oil (11.36 g, 85%).

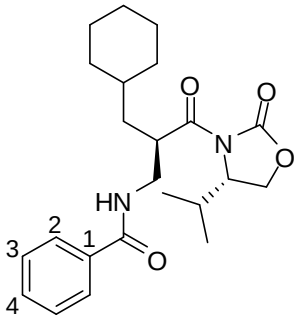
^{1}H NMR (600 MHz, DMSO-d_6) δ 0.77 (d, ^3J = 6.8 Hz, 3H) and 0.83 (d, ^3J = 7.0 Hz, 3H, CH(CH_3)$_2$), 0.84 – 0.90 (m, 2H, Cy-H), 1.06 – 1.19 (m, 3H, Cy-H), 1.19 – 1.25 (m, 1H, Cy-H), 1.38 – 1.48 (m, 2H, CH_2-Cy), 1.55 – 1.61 (m, 1H, Cy-H), 1.62 – 1.69 (m, 4H, Cy-H), 2.12 – 2.20 (m, 1H, C*H*(CH$_3$)$_2$), 2.73 (ddd, ^2J = 15.9 Hz, ^3J = 9.3 Hz, ^3J = 6.1 Hz, 1H, C*H*H-CO), 2.90 (ddd, ^2J = 15.8 Hz, ^3J = 9.3 Hz, ^3J = 6.1 Hz, 1H, CH*H*-CO), 4.25 (dd, ^2J = 8.9 Hz, ^3J = 3.0 Hz, 1H, C*H*H-O), 4.26 – 4.31 (m, 1H, CH*H*-O), 4.34 (ddd, ^3J = 8.0 Hz, ^3J = 3.9 Hz, ^3J = 3.0 Hz, 1H, N-CH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 14.7, 17.6 (CH(*C*H$_3$)$_2$), 25.9 (Cy-C), 25.9 (Cy-C), 26.2 (Cy-C), 28.4 (*C*H(CH$_3$)$_2$), 31.6 (Cy-C), 32.4 (Cy-C), 32.7, 32.7 (*C*H$_2$-CO, Cy-C), 36.8 (CH_2-Cy), 58.0 (N-CH), 63.5 (CH_2-O), 154.1 (N-CO-O), 172.8 (CH-*C*O-N). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 200–400 nm), 100% purity, *m/z* = 268.0 ([M+H]$^+$).

N-(Chloromethyl)benzamide (**32**).



N-(Hydroxymethyl)benzamide (**31**, 15.1 g, 100 mmol) was suspended in dry CH_2Cl_2 (150 mL). Thionyl chloride (35.69 g, 26.5 mL, 300 mmol) was added dropwise with stirring to the suspension. The resulting clear solution was poured into *n*-heptane (570 mL). The precipitate was immediately filtered off and washed with *n*-heptane to yield a white solid (13.1 g, 77%); mp 220–222 °C (lit.[10] mp 90–91 °C).

N-((*R*)-2-(Cyclohexylmethyl)-3-((*S*)-4-isopropyl-2-oxooxazolidin-3-yl)-3-oxopropyl)benzamide (**33**).

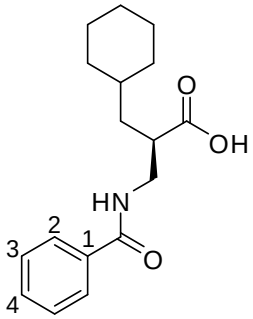


Compound **30** (10.7 g, 40.0 mmol) was dissolved in anhydrous CH_2Cl_2 (165 mL) and cooled to 0 °C. $TiCl_4$ (7.59 g, 4.39 mL, 40.0 mmol) was added slowly at 0 °C and the resulting yellow suspension was stirred at this temperature for 15 min. Next, triethylamine (4.05 g, 5.55 mL, 40.0 mmol) was added and the resulting dark red solution was stirred for 45 min at 0 °C. Subsequently, *N*-(chloromethyl)benzamide **32** (8.14 g, 48.0 mmol) was added and the mixture was stirred for 1 h at 0 °C. After addition of sat. aq. NH_4Cl solution (140 mL), the organic layer was washed with 1 M HCl (140 mL), and the HCl phase extracted with CH_2Cl_2 (175 mL). The combined organic layer was dried over Na_2SO_4, filtered and evaporated. The crude residue was purified using column chromatography petroleum ether/EtOAc (7:3) (R_f = 0.5) to yield a colourless oil (12.7 g, 79%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 0.63 (d, ^3J = 6.8 Hz, 3H) and 0.79 (d, ^3J = 7.1 Hz, 3H, CH(CH_3)$_2$), 0.80 – 0.90 (m, 2H, Cy-H), 1.07 – 1.21 (m, 3H, Cy-H), 1.23 – 1.34 (m, 2H, Cy-H, C*H*H-Cy), 1.52 – 1.59 (m, 2H, C*H*H-Cy, Cy-H), 1.60 – 1.75 (m, 4H, Cy-H), 2.11 – 2.20 (m, 1H, C*H*(CH$_3$)$_2$), 3.43 (ddd, ^2J = 13.4 Hz, ^3J = 8.1 Hz, ^3J = 6.0 Hz, 1H, NH-C*H*H), 3.47 – 3.54 (m, 1H, NH-CH*H*), 4.16 – 4.22 (m, 1H, CH-CO), 4.23 (dd, ^2J = 9.1 Hz, ^3J = 2.8 Hz, 1H,

C*H*H-O), 4.25 – 4.31 (m, 1H, CH*H*-O), 4.37 (dt, ^{3}J = 8.3 Hz, ^{3}J = 3.1 Hz, 1H, N-CH), 7.39 – 7.45 (m, 2H, 3-H), 7.46 – 7.51 (m, 1H, 4-H), 7.75 – 7.80 (m, 2H, 2-H), 8.46 (t, ^{3}J = 5.8 Hz, 1H, NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 14.5, 17.7 (CH(*C*H$_3$)$_2$), 25.8 (Cy-C), 25.9 (Cy-C), 26.2 (Cy-C), 28.0 (*C*H(CH$_3$)$_2$), 32.8 (Cy-C), 33.3 (Cy-C), 34.8 (Cy-C), 37.6 (CH$_2$-Cy), 40.2, 41.5 (NH-CH$_2$-CH), 58.3 (N-CH), 63.2 (CH$_2$-O), 127.3 (C-2), 128.3 (C-3), 131.2 (C-4), 134.5 (C-1), 153.7 (N-CO-O), 166.3 (CO-NH), 174.8 (CH-*C*O-N). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 100% purity, *m/z* = 401.0 ([M+H]$^{+}$).

(*R*)-3-Benzamido-2-(cyclohexylmethyl)propanoic acid (**34**).

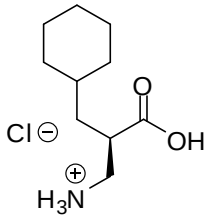


Compound **33** (12.0 g, 30 mmol) was dissolved in a mixture of THF / H_2O (4:1) (150 mL) and cooled to 0 °C. H_2O_2 (30% aq. soln., 120 mmol) and lithium hydroxide monohydrate (2.01 g, 48 mmol) were added and the mixture was stirred for 1 h at 0 °C. Subsequently, sat. aq. $NaHSO_3$ solution (24 mL) was added. Upon washing of the reaction mixture with CH_2Cl_2 (3 × 120 mL), the organic layer was concentrated to a volume of 60 mL and extracted with sat. aq. $NaHCO_3$ (120 mL). The combined aqueous layer was acidified to pH 1-2 with 6 M HCl and extracted with EtOAc (3 × 120 mL). The combined EtOAc phase was dried over Na_2SO_4, filtered and evaporated to dryness. The crude residue was purified by column chromatography using petroleum ether/EtOAc (1:1) + 1% AcOH (R_f = 0.6) as eluent to obtain white needles (4.51 g, 52%); mp 144–146 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 0.76 – 0.92 (m, 2H, Cy-H), 1.04 – 1.20 (m, 3H, Cy-H), 1.21 – 1.33 (m, 2H, Cy-H, CH*H*-Cy), 1.45 (ddd, ^{2}J = 12.4 Hz, ^{3}J = 9.2 Hz, ^{3}J = 4.8 Hz, 1H, C*H*H-Cy), 1.55 – 1.60 (m, 1H, Cy-H), 1.60 – 1.68 (m, 3H, Cy-H), 1.69 – 1.76 (m, 1H, Cy-H), 2.72 (dddd, ^{3}J = 9.6 Hz, ^{3}J = 7.8 Hz, ^{3}J = 6.2 Hz, ^{3}J = 4.6 Hz, 1H, CH-CO), 3.27 – 3.33 (m, 1H, NH-C*H*H), 3.39 (ddd, ^{2}J = 13.5 Hz, ^{3}J = 7.9 Hz, ^{3}J = 5.9 Hz, 1H, NH-CH*H*), 7.42 – 7.46 (m, 2H, 3-H), 7.48 – 7.52 (m, 1H, 4-H), 7.79 – 7.82 (m, 2H, 2-H), 8.51 (t, ^{3}J = 5.8 Hz, 1H, NH), 12.12 (s, 1H, CO-OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.8 (Cy-C), 25.9 (Cy-C),

26.2 (Cy-C), 32.4 (Cy-C), 33.3 (Cy-C), 35.3 (Cy-C), 37.5 (CH_2-Cy), 41.9, 42.7 (NH-CH_2-CH), 127.3 (C-2), 128.4 (C-3), 131.2 (C-4), 134.6 (C-1), 166.5 (CO-NH), 176.0 (CO-OH). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 200–400 nm), 100% purity, *m/z* = 290.2 ($[M+H]^+$).

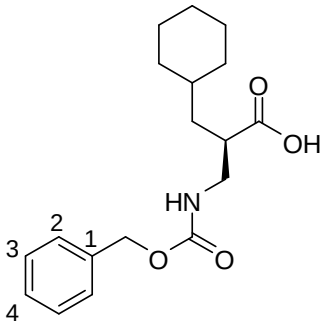
(*R*)-2-Carboxy-3-cyclohexylpropan-1-aminium chloride (**35**).



Compound **34** (4.34 g, 15.0 mmol) was dissolved in a mixture of conc HCl / AcOH / H_2O (20:10:10) (60 mL) and refluxed at 110 °C for 2 days. Subsequently, the reaction was diluted with H_2O (38 mL) and washed with Et_2O (3 × 225 mL). The aqueous layer was evaporated to give a white solid (2.69 g, 81%); mp 247–249 °C.

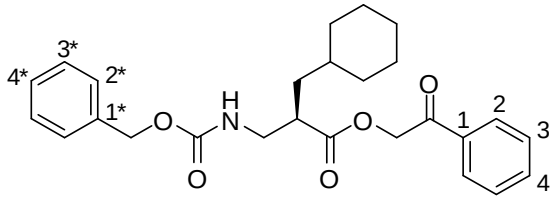
^{1}H NMR (600 MHz, DMSO-d_6) δ 0.80 – 0.88 (m, 2H, Cy-H), 1.05 – 1.21 (m, 3H, Cy-H), 1.22 – 1.30 (m, 1H, Cy-H), 1.36 (dt, ^2J = 13.7 Hz, ^3J = 6.9 Hz, 1H, C*H*H-Cy), 1.43 (dt, ^2J = 14.2 Hz, ^3J = 7.2 Hz, 1H, CH*H*-Cy), 1.56 – 1.61 (m, 1H, Cy-H), 1.61 – 1.67 (m, 3H, Cy-H), 1.69 – 1.74 (m, 1H, Cy-H), 2.68 – 2.74 (m, 1H, CH-CO), 2.78 (dd, ^2J = 12.8 Hz, ^3J = 5.0 Hz, 1H, $^+H_3$N-C*H*H), 2.94 (dd, ^2J = 12.8 Hz, ^3J = 8.3 Hz, 1H, $^+H_3$N-CH*H*), 8.12 (s, 3H, NH_3^+), 12.72 (s, 1H, CO-OH). ^{13}C NMR (150 MHz, DMSO) δ 25.8 (Cy-C), 25.8 (Cy-C), 26.2 (Cy-C), 32.5 (Cy-C), 32.8 (Cy-C), 34.7 (Cy-C), 37.1 (CH_2-Cy), 40.1, 40.3 ($^+H_3$N-CH_2-CH), 174.9 (CO-OH). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 200–400 nm), *m/z* = 186.2 ($[M+H]^+$).

(*R*)-3-(Benzyloxycarbonylamino)-2-(cyclohexylmethyl)propanoic acid (**36**).



Compound **35** (2.22 g, 10.0 mmol) and *N*-(benzyloxycarbonyloxy)succinimide (2.49 g, 10.0 mmol) were dissolved in THF (35 mL) and H_2O (10 mL), and triethylamine (4.05 g, 5.54 mL, 40.0 mmol) was added. The resulting clear solution was stirred at room temperature overnight. The mixture was diluted with EtOAc (125 mL), washed with 1 N HCl (3 × 60 mL), brine (60 mL), dried over Na_2SO_4, filtered and evaporated to dryness. The crude residue was purified by preparative column chromatography using petroleum ether/EtOAc (3:2) + 1% AcOH (R_f = 0.4) as eluent to give compound **36** as white solid (2.81 g, 88%); mp 86–88 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.73 – 0.81 (m, 1H, Cy-H), 0.82 – 0.88 (m, 1H, Cy-H), 1.05 – 1.26 (m, 5H, Cy-H), 1.33 – 1.39 (m, 1H, C*H*H-Cy), 1.53 – 1.67 (m, 4H, CH*H*-Cy, Cy-H), 1.67 – 1.71 (m, 1H, Cy-H), 2.50 – 2.55 (m, 1H, CH-CO), 3.02 (dt, ^2J = 12.9 Hz, ^3J = 6.0 Hz, 1H, NH-C*H*H), 3.14 (dt, ^2J = 13.7 Hz, ^3J = 6.9 Hz, 1H, NH-CH*H*), 5.00 (s, 2H, CH_2-O), 7.26 – 7.36 (m, 6H, 2-H, 3-H, 4-H, NH). One proton signal (CO-OH) is not recognizable. ^{13}C NMR (125 MHz, DMSO-d_6) δ 26.5 (Cy-C), 26.6 (Cy-C), 26.9 (Cy-C), 33.1 (Cy-C), 34.0 (Cy-C), 35.9 (Cy-C), 37.9 (CH_2-Cy), 43.7, 43.7 (NH-CH_2-CH), 66.0 (CH_2-O), 128.4 (C-2), 128.6 (C-4), 129.2 (C-3), 138.1 (C-1), 157.0 (O-CO-NH), 176.6 (CO-OH). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 200–300 nm), 98% purity, *m/z* = 320.1 ($[M+H]^+$), 337.0 ($[M+NH_4]^+$).

(*R*)-2-Oxo-2-phenylethyl 3-(benzyloxycarbonylamino)-2-(cyclohexylmethyl)propanoate (**38**).

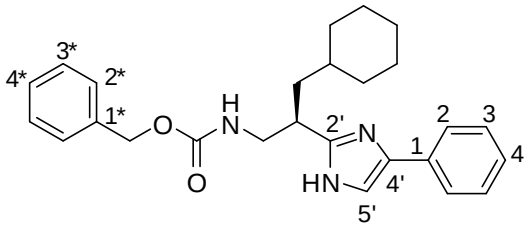


Compound **36** (4.79 g, 15.0 mmol) was dissolved in anhydrous DMF (38 mL) and K_2CO_3 (2.49 g, 18.0 mmol) was added. After 10 min of stirring at room temperature, 2-bromoacetophenone (3.28 g, 16.5 mmol) was added and the reaction mixture was stirred at

room temperature for a further 4 h. The mixture was then diluted with H_2O (100 mL) and extracted with EtOAc (200 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness. The crude residue was purified by column chromatography on silica gel using a gradient of petroleum ether/EtOAc (20:1) to (4:1) (R_f = 0.6) to yield colourless oil (4.92 g, 75%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 0.75 – 0.83 (m, 1H, Cy-H), 0.83 – 0.91 (m, 1H, Cy-H), 1.07 – 1.23 (m, 3H, Cy-H), 1.27 – 1.37 (m, 2H, Cy-H, C*H*H-Cy), 1.46 – 1.54 (m, 1H, CH*H*-Cy), 1.55 – 1.69 (m, 4H, Cy-H), 1.69 – 1.76 (m, 1H, Cy-H), 2.74 – 2.80 (m, 1H, C*H*-CH_2-Cy), 3.12 – 3.19 (m, 1H, NH-C*H*H), 3.23 – 3.29 (m, 1H, NH-CH*H*), 5.01 (d, ^2J = 12.8 Hz, 1H, C*H*H-O-CO-NH), 5.05 (d, ^2J = 12.9 Hz, 1H, CH*H*-O-CO-NH), 5.41 (d, ^2J = 16.7 Hz, 1H, C*H*H-CO), 5.47 (d, ^2J = 16.8 Hz, 1H, CH*H*-CO), 7.27 – 7.38 (m, 6H, 2*-H, 3*-H, 4*-H, NH), 7.53 – 7.57 (m, 2H, 3-H), 7.66 – 7.70 (m, 1H, 4-H), 7.92 – 7.96 (m, 2H, 2-H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.7 (Cy-C), 25.8 (Cy-C), 26.1 (Cy-C), 32.3 (Cy-C), 33.2 (Cy-C), 34.8 (Cy-C), 36.8 (CH_2-Cy), 42.8, 42.9 (NH-*C*H_2, *C*H-CH_2-Cy), 65.4 (*C*H_2-O-CO-NH), 66.5 (*C*H_2-CO), 127.7 (C-2*), 127.8 (C-4*), 127.9 (C-3), 128.4 (C-3*), 129.0 (C-2), 134.0 (C-4), 134.1 (C-1), 137.3 (C-1*), 156.3 (O-CO-NH), 173.6 (CH-*C*O-O), 193.1 (CH_2-*C*O). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 94% purity, *m/z* = 438.1 ($[M+H]^+$), 455.1 ($[M+NH_4]^+$).

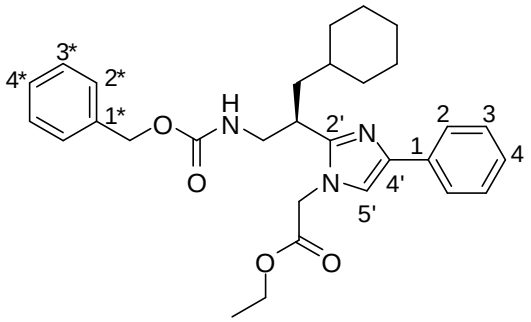
(*R*)-Benzyl 3-cyclohexyl-2-(4-phenyl-1*H*-imidazol-2-yl)propylcarbamate (**40**).



Compound **38** (4.38 g, 10.0 mmol) was dissolved in anhydrous toluene (46 mL) and ammonium acetate (11.6 g, 150 mmol) was added to the solution. The reaction mixture was heated to reflux for 3 h with a drying tube attached to the condenser. The solvent was evaporated and the residue was suspended in EtOAc (160 mL). The organic layer was washed with H_2O (2 × 80 mL) and brine (80 mL), and then dried over Na_2SO_4, filtered, and evaporated to dryness. The crude residue was purified by preparative column chromatography using a gradient of petroleum ether/EtOAc (4:1) to (1:1) (R_f = 0.7) to obtain **22** as orange, yellowish oil (1.80 g, 43%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 0.73 – 0.81 (m, 1H, Cy-H), 0.82 – 0.86 (m, 1H, Cy-H), 1.00 – 1.10 (m, 4H, Cy-H), 1.45 (ddd, ^2J = 13.6 Hz, ^3J = 8.6 Hz, ^3J = 4.8 Hz, 1H, C*H*H-Cy), 1.50 – 1.59 (m, 5H, Cy-H, CH*H*-Cy), 1.72 – 1.81 (m, 1H, Cy-H), 3.03 – 3.09 (m, 1H, C*H*-CH_2-Cy), 3.23 (t, ^3J = 6.5 Hz, 2H, NH-CH_2), 4.98 (d, ^2J = 12.7 Hz, 1H, C*H*H-O), 5.02 (d, ^2J = 12.7 Hz, 1H, CH*H*-O), 7.11 – 7.19 (m, 1H, 4-H), 7.24 – 7.39 (m, 8H, 2*-H, 3*-H, 4*-H, 3-H, O-CO-NH), 7.43 (s, 1H, 5'-H), 7.67 – 7.74 (m, 2H, 2-H), 11.83 (s, 1H, imidazole-NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 25.8 (Cy-C), 25.9 (Cy-C), 26.3 (Cy-C), 32.3 (Cy-C), 33.5 (Cy-C), 34.9 (Cy-C), 37.0 (CH_2-Cy), 39.4 (*C*H-CH_2-Cy), 45.1 (NH-CH_2), 65.3 (CH_2-O), 112.3 (C-5'), 124.4 (C-2), 125.9 (C-4), 127.7 (C-2*), 127.9 (C-4*), 128.5 (C-3, C-3*), 135.3 (C-1), 137.5 (C-1*), 139.6 (C-4'), 150.1 (C-2'), 156.4 (C=O). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 96% purity, *m/z* = 418.0 ($[M+H]^+$).

(*R*)-Ethyl 2-(2-(1-(benzyloxycarbonylamino)-3-cyclohexylpropan-2-yl)-4-phenyl-1*H*-imidazol-1-yl)acetate (**42**).

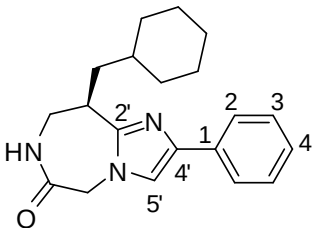


Compound **40** (1.67 g, 4.00 mmol) was dissolved in anhydrous DMF (6.6 mL) and Cs_2CO_3 (3.26 g, 10.0 mmol) was added to the solution. After stirring for 0.5 h at room temperature, ethyl 2-bromoacetate (0.80 g, 4.80 mmol) dissolved in anhydrous DMF (4.4 mL) was added dropwise over a period of 0.5 h. The mixture was stirred for a further 2.5 h at room temperature, then quenched with iced H_2O (30 mL) and extracted with EtOAc (3 × 30 mL). The organic layer was washed with brine (30 mL) and H_2O (2 × 30 mL), and dried over Na_2SO_4, filtered and evaporated to dryness. The crude residue was purified by preparative column chromatography using a gradient of petroleum ether/EtOAc (4:1) to (7:3) (R_f = 0.8) to yield **42** as colourless oil (1.31 g, 65%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 0.70 – 0.78 (m, 1H, Cy-H), 0.79 – 0.87 (m, 1H, Cy-H), 1.04 – 1.16 (m, 4H, Cy-H), 1.21 (t, ^3J = 7.1 Hz, 3H, CH_3), 1.45 (ddd, ^2J = 13.2 Hz, ^3J = 8.0 Hz, ^3J = 5.1 Hz, 1H, C*H*H-Cy), 1.52 – 1.60 (m, 5H, Cy-H), 1.73 (ddd, ^2J = 13.9 Hz, ^3J = 8.9

Hz, ^{3}J = 5.7 Hz, 1H, CH*H*-Cy), 2.96 – 3.03 (m, 1H, C*H*-CH_2-Cy), 3.06 (dt, ^{2}J = 12.9 Hz, ^{3}J = 6.3 Hz, 1H, NH-C*H*H), 3.18 (dt, ^{2}J = 12.6 Hz, ^{3}J = 6.1 Hz, 1H, NH-CH*H*), 4.11 – 4.18 (m, 2H, H_3C-CH_2), 4.85 (d, ^{2}J = 18.2 Hz, 1H, C*H*H-N), 4.93 (d, ^{2}J = 18.1 Hz, 1H, CH*H*-N), 4.99 (d, ^{2}J = 12.5 Hz, 1H, C*H*H-O), 5.02 (d, ^{2}J = 12.8 Hz, 1H, CH*H*-O), 7.17 (tt, ^{3}J = 7.5 Hz, ^{4}J = 1.3 Hz, 1H, 4-H), 7.27 – 7.36 (m, 8H, 2*-H, 3*-H, 4*-H, 3-H, NH), 7.47 (s, 1H, 5'-H), 7.70 (dd, ^{3}J = 8.1 Hz, ^{4}J = 1.4 Hz, 2H, 2-H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 14.2 (CH_3), 25.7 (Cy-C), 25.9 (Cy-C), 26.3 (Cy-C), 32.8 (Cy-C), 33.4 (Cy-C), 33.5 (Cy-C), 34.7 (CH_2-Cy), 39.1 (*C*H-CH_2-Cy), 45.3 (NH-CH_2), 46.5 (CH_2-N), 61.5 (H_3C-*C*H_2), 65.3 (CH_2-O), 117.3 (C-5'), 124.3 (C-2), 126.3 (C-4), 127.7 (C-2*), 127.9 (C-4*), 128.5, 128.6 (C-3, C-3*), 134.7 (C-1), 137.5 (C-1*), 138.8 (C-4'), 150.3 (C-2'), 156.4 (O-*C*O-NH), 168.5 (O-*C*O-CH_2). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 98% purity, *m/z* = 504.2 ($[M+H]^+$).

(*R*)-9-(Cyclohexylmethyl)-2-phenyl-8,9-dihydro-5*H*-imidazo[1,2-*d*][1,4]diazepin-6(7*H*)-one (**8**).

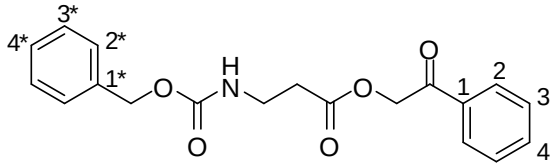


Compound **42** (1.01 g, 2.00 mmol) was dissolved in anhydrous MeOH (16 mL) containing Pd/C (10% Pd) and was hydrogenated under atmospheric pressure at room temperature for 24 h. The catalyst was filtered off through celite and washed twice with MeOH (12 mL). The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel using EtOAc (100%) (R_f = 0.5) as eluent to give a white solid (0.50 g, 77%); mp 104–107 °C.

^{1}H NMR (600 MHz, DMSO-d_6) δ 0.87 – 0.96 (m, 2H, Cy-H), 1.11 – 1.29 (m, 3H, Cy-H), 1.40 – 1.46 (m, 1H, C*H*H-Cy), 1.50 – 1.58 (m, 1H, Cy-H), 1.60 – 1.76 (m, 5H, Cy-H, CH*H*-Cy), 1.77 – 1.82 (m, 1H, Cy-H), 3.08 – 3.15 (m, 1H, C*H*-CH_2-Cy), 3.35 – 3.40 (m, 1H, NH-C*H*H), 3.60 (ddd, ^{2}J = 15.2 Hz, ^{3}J = 6.3 Hz, ^{3}J = 3.6 Hz, 1H, NH-CH*H*), 4.76 (d, ^{2}J = 14.6 Hz, 1H, C*H*H-N), 4.79 (d, ^{2}J = 14.8 Hz, 1H, CH*H*-N), 7.14 – 7.19 (m, 1H, 4-H), 7.30 – 7.35 (m, 2H, 3-H), 7.50 (s, 1H, 5'-H), 7.65 – 7.69 (m, 2H, 2-H), 8.15 (t, ^{3}J = 6.1 Hz, 1H, NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 25.9 (Cy-C), 26.1 (Cy-C), 26.4 (Cy-C), 32.1 (Cy-C), 33.7 (Cy-C), 33.8 (Cy-C), 36.4 (CH_2-Cy), 40.9 (NH-CH_2), 51.1 (CH_2-N), 117.6 (C-5'), 124.3 (C-2),

126.3 (C-4), 128.6 (C-3), 134.5 (C-1), 138.3 (C-4'), 149.1 (C-2'), 169.5 (C=O). One carbon signal (*C*H-CH_2-Cy) is obscured by the solvent peak. LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 98% purity, *m/z* = 324.0 ($[M+H]^+$). HRMS, calcd. for $C_{20}H_{25}N_3O$: $[M+H]^+$ *m/z* 324.2070, found: 324.2082.

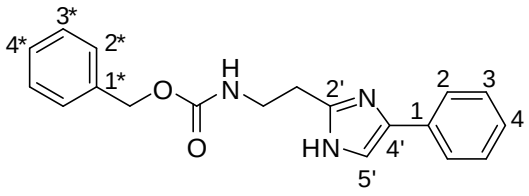
2-Oxo-2-phenylethyl 3-(benzyloxycarbonylamino)propanoate (**39**).



3-(Benzyloxycarbonylamino)propanoic acid (**37**, 6.70 g, 30.0 mmol) was dissolved in anhydrous DMF (76 mL) and K_2CO_3 (4.98 g, 36.0 mmol) was added. After 10 min of stirring at room temperature, 2-bromoacetophenone (6.56 g, 33.0 mmol) was added and the reaction mixture was stirred at room temperature for a further 4 h. The mixture was then diluted with H_2O (180 mL) and extracted with EtOAc (360 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness. The crude residue was purified by recrystallization from EtOAc/Et_2O to yield white needles (6.66 g, 65%); mp 74–76 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.63 (t, ^3J = 7.1 Hz, 2H, NH-CH_2-CH_2), 3.30 – 3.34 (m, 2H, NH-CH_2), 5.02 (s, 2H, CH_2-O-CO-NH), 5.47 (s, 2H, O-CH_2-CO), 7.27 – 7.38 (m, 6H, 2*-H, 3*-H, 4*-H, NH), 7.52 – 7.58 (m, 2H, 3-H), 7.66 – 7.71 (m, 1H, 4-H), 7.93 – 7.97 (m, 2H, 2-H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 34.0 (NH-CH_2-*C*H_2), 36.6 (NH-CH_2), 65.4 (*C*H_2-O-CO-NH), 66.6 (O-*C*H_2-CO), 127.8 (C-4*), 127.9 (C-3, C-2*), 128.5 (C-3*), 129.0 (C-2), 134.0 (C-4), 134.1 (C-1), 137.2 (C-1*), 156.2 (O-CO-NH), 170.7 (CH_2-*C*O-O), 192.8 (O-CH_2-*C*O). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 99% purity, *m/z* = 342.1 ($[M+H]^+$), 359.1 ($[M+NH_4]^+$).

Benzyl 2-(4-phenyl-1*H*-imidazol-2-yl)ethylcarbamate (**41**).

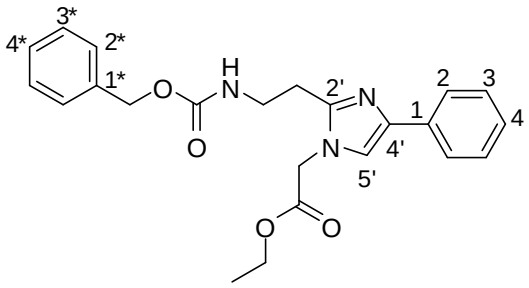


Compound **39** (6.14 g, 18.0 mmol) was dissolved in anhydrous toluene (84 mL) and ammonium acetate (20.8 g, 270 mmol) was added to the solution. The reaction mixture was

heated to reflux for 3 h with a drying tube attached to the condenser. The solvent was evaporated and the residue was suspended in EtOAc (280 mL). The organic layer was washed with H_2O (2 × 140 mL) and brine (140 mL), and then dried over Na_2SO_4, filtered, and evaporated to dryness. The crude residue was redissolved in a small volume of DMF and silica gel was added to this solution. The solvent was again evaporated. The crude product attached to silica gel was loaded to a silica gel containing column and purified using a gradient of petroleum ether/EtOAc (1:1) to (1:4) (R_f = 0.5) as eluent. Corresponding fractions were evaporated to give **41** as yellow solid (2.26 g, 39%); mp 141–143 °C.

^{1}H NMR (600 MHz, DMSO-d_6) δ 2.80 (t, ^3J = 7.5 Hz, 2H, NH-CH_2-CH_2), 3.34 – 3.39 (m, 2H, NH-CH_2), 5.02 (s, 2H, CH_2-O), 7.12 – 7.16 (m, 1H, 4-H), 7.26 – 7.41 (m, 8H, 2*-H, 3*-H, 4*-H, 3-H, O-CO-NH), 7.46 (s, 1H, 5'-H), 7.65 – 7.77 (m, 2H, 2-H), 11.87 (s, 1H, imidazole-NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ 28.8 (NH-CH_2-CH_2), 40.2 (NH-CH_2), 65.4 (CH_2-O), 112.3 (C-5'), 124.3 (C-2), 125.9 (C-4), 127.9 (C-2*), 127.9 (C-4*), 128.5 (C-3, C-3*), 135.2 (C-1), 137.3 (C-1*), 139.7 (C-4'), 146.0 (C-2'), 156.2 (C=O). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 98% purity, *m/z* = 322.0 ($[M+H]^+$).

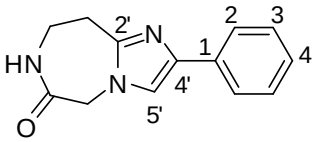
Ethyl 2-(2-(2-(benzyloxycarbonylamino)ethyl)-4-phenyl-1*H*-imidazol-1-yl)acetate (**43**).



Compound **41** (2.25 g, 7.00 mmol) was dissolved in anhydrous DMF (12 mL) and Cs_2CO_3 (5.70 g, 17.5 mmol) was added to the solution. After stirring for 0.5 h at room temperature, ethyl 2-bromoacetate (1.40 g, 8.40 mmol) dissolved in anhydrous DMF (8 mL) was added dropwise over a period of 0.5 h. The mixture was stirred for a further 2.5 h at room temperature, then quenched with iced H_2O (50 mL) and extracted with EtOAc (3 × 50 mL). The organic layer was washed with brine (50 mL) and H_2O (2 × 50 mL), and dried over Na_2SO_4, filtered and evaporated to dryness. The crude residue was purified by preparative column chromatography using a gradient of petroleum ether/EtOAc (9:1) to (3:2) (R_f = 0.1) to yield **43** as yellow crystals (2.25 g, 79%); mp 94–97 °C.

^{1}H NMR (600 MHz, DMSO-d_6) δ 1.22 (t, ^{3}J = 7.1 Hz, 3H, CH_3), 2.74 (t, ^{3}J = 7.5 Hz, 2H, NH-CH_2-CH_2), 3.35 – 3.44 (m, 2H, NH-CH_2), 4.17 (q, ^{3}J = 7.1 Hz, 2H, H_3C-CH_2), 4.91 (s, 2H, CH_2-N), 5.02 (s, 2H, CH_2-O-CO-NH), 7.17 (tt, ^{3}J = 7.3 Hz, ^{4}J = 1.2 Hz, 1H, 4-H), 7.28 – 7.36 (m, 8H, 2*-H, 3*-H, 4*-H, 3-H, NH), 7.50 (s, 1H, 5'-H), 7.68 – 7.71 (m, 2H, 2-H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 14.2 (CH_3), 26.6 (NH-CH_2-*C*H_2), 39.0 (NH-CH_2), 46.9 (CH_2-N), 61.4 (H_3C-*C*H_2), 65.4 (*C*H_2-O-CO-NH), 117.5 (C-5'), 124.2 (C-2), 126.3 (C-4), 127.9 (C-2*), 127.9 (C-4*), 128.5, 128.6 (C-3, C-3*), 134.6 (C-1), 137.3 (C-1*), 138.7 (C-4'), 146.6 (C-2'), 156.2 (O-CO-NH), 168.5 (O-*C*O-CH_2). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 90% purity, *m/z* = 408.1 ([M+H]$^{+}$).

2-Phenyl-8,9-dihydro-5*H*-imidazo[1,2-*d*][1,4]diazepin-6(7*H*)-one (**9**).



Compound **43** (1.63 g, 4.00 mmol) was dissolved in anhydrous MeOH (32 mL) containing Pd/C (10% Pd) and was hydrogenated under atmospheric pressure at room temperature for 24 h. The catalyst was filtered off through celite and washed twice with MeOH (24 mL). The removal of the solvent *in vacuo* yielded a brownish solid (0.91 g, 99%) which exhibited the required purity without further purification; mp 211–214 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 3.00 – 3.04 (m, 2H, NH-CH_2-CH_2), 3.53 – 3.61 (m, 2H, NH-CH_2), 4.79 (s, 2H, CH_2-N), 7.14 – 7.21 (m, 1H, 4-H), 7.32 (t, ^{3}J = 7.8 Hz, 2H, 3-H), 7.53 (s, 1H, 5'-H), 7.65 – 7.74 (m, 2H, 2-H), 8.16 (t, ^{3}J = 5.6 Hz, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 29.8 (NH-CH_2-*C*H_2), 37.1 (NH-CH_2), 50.9 (CH_2-N), 117.6 (C-5'), 124.2 (C-2), 126.3 (C-4), 128.6 (C-3), 134.4 (C-1), 138.6 (C-4'), 145.0 (C-2'), 169.3 (C=O). LC-MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH for 10 min, DAD 220–400 nm), 96% purity, *m/z* = 228.0 ([M+H]$^{+}$). HRMS, calcd. for $C_{13}H_{13}N_3O$: [M+H]$^{+}$ *m/z* 228.1131, found: 228.1149.

References

1. T. H. Charpentier, G. L. Waldo, M. O. Barrett, W. Huang, Q. Zhang, T. K. Harden and J. Sondek, *J. Biol. Chem.*, 2014, **289**, 29545–29557.
2. S. N. Hicks, M. R. Jezyk, S. Gershburg, J. P. Seifert, T. K. Harden and J. Sondek, *Mol. Cell*, 2008, **31**, 383–394.
3. J. Schmidt, N. J. Smith, E. Christiansen, I. G. Tikhonova, M. Grundmann, B. D. Hudson, R. J. Ward, C. Drewke, G. Milligan, E. Kostenis and T. Ulven, *J. Biol. Chem.*, 2011, **286**, 10628–10640.
4. G. M. Sheldrick, *Acta Cryst.*, 2015, **A71**, 3–8.
5. G. M. Sheldrick, *Acta Cryst.*, 2015, **C71**, 3–8.
6. J. Küppers, M. Hympánová, T. Keuler, A. J. Schneider, G. Schnakenburg and M. Gütschow, *Synthesis*, 2019, **51**, 1961–1968.
7. A. L. Schmitz, R. Schrage, E. Gaffal, T. H. Charpentier, J. Wiest, G. Hiltensperger, J. Morschel, S. Hennen, D. Häußler, V. Horn, D. Wenzel, M. Grundmann, K. M. Büllesbach, R. Schröder, H. H. Brewitz, J. Schmidt, J. Gomeza, C. Galés, B. K. Fleischmann, T. Tüting, D. Imhof, D. Tietze, M. Gütschow, U. Holzgrabe, J. Sondek, T. K. Harden, K. Mohr and E. Kostenis, *Chem. Biol.*, 2014, **21**, 890–902.
8. G. C. Stelakatos, A. Paganou and L. Zervas, *J. Chem. Soc. Perkin 1*, 1966, **13**, 1191–1199.
9. P. F. Carneiro, B. Gutmann, R. O. M. A. de Souza and C. O. Kappe, *ACS Sustainable Chem. Eng.*, 2015, **3**, 3445–3453.
10. J. J. Getz, R. J. Prankerd and K. B. Sloan, *J. Org. Chem.*, 1992, **57**, 1702–1706.