Supplementary Information for

The hydrophobically-tagged MDM2-p53 interaction inhibitor Nutlin-3a-HT is more potent against tumor cells than Nutlin-3a

Florian Nietzold[†], Stefan Rubner[†], and Thorsten Berg[*]

Table of contents

Figure S1

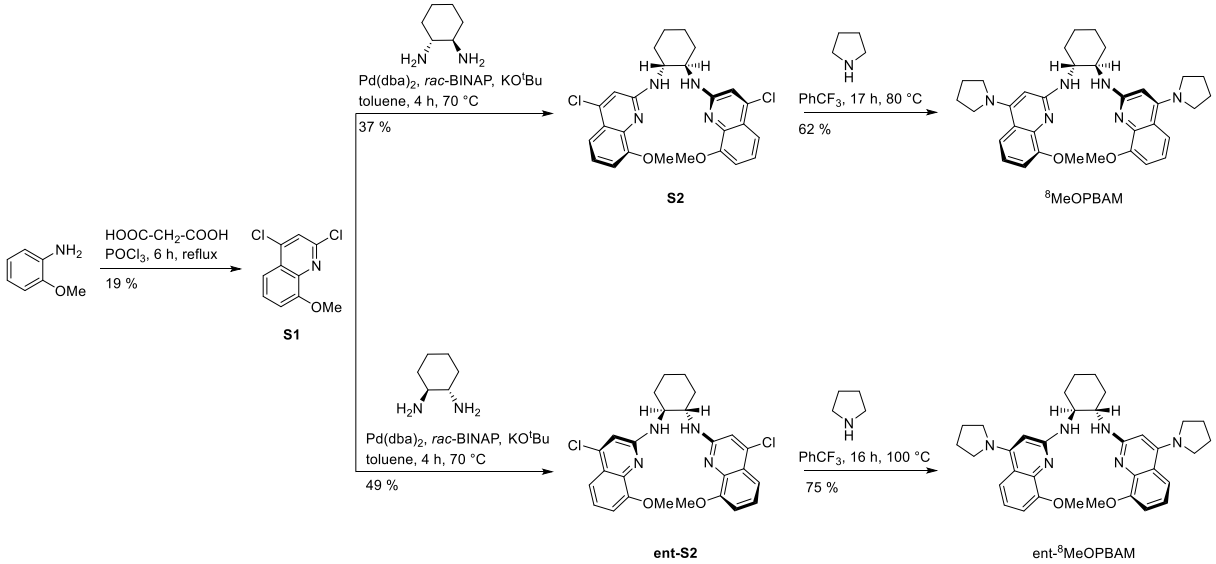


Figure S1: A) Preparation of the 4-pyrrolidine bis(amidine) catalyst ^{8}MeOPBAM following the protocol by Johnston *et al*. (upper section)[1] and of ent-^{8}MeOPBAM (lower section) as described in this manuscript.

Figure S2

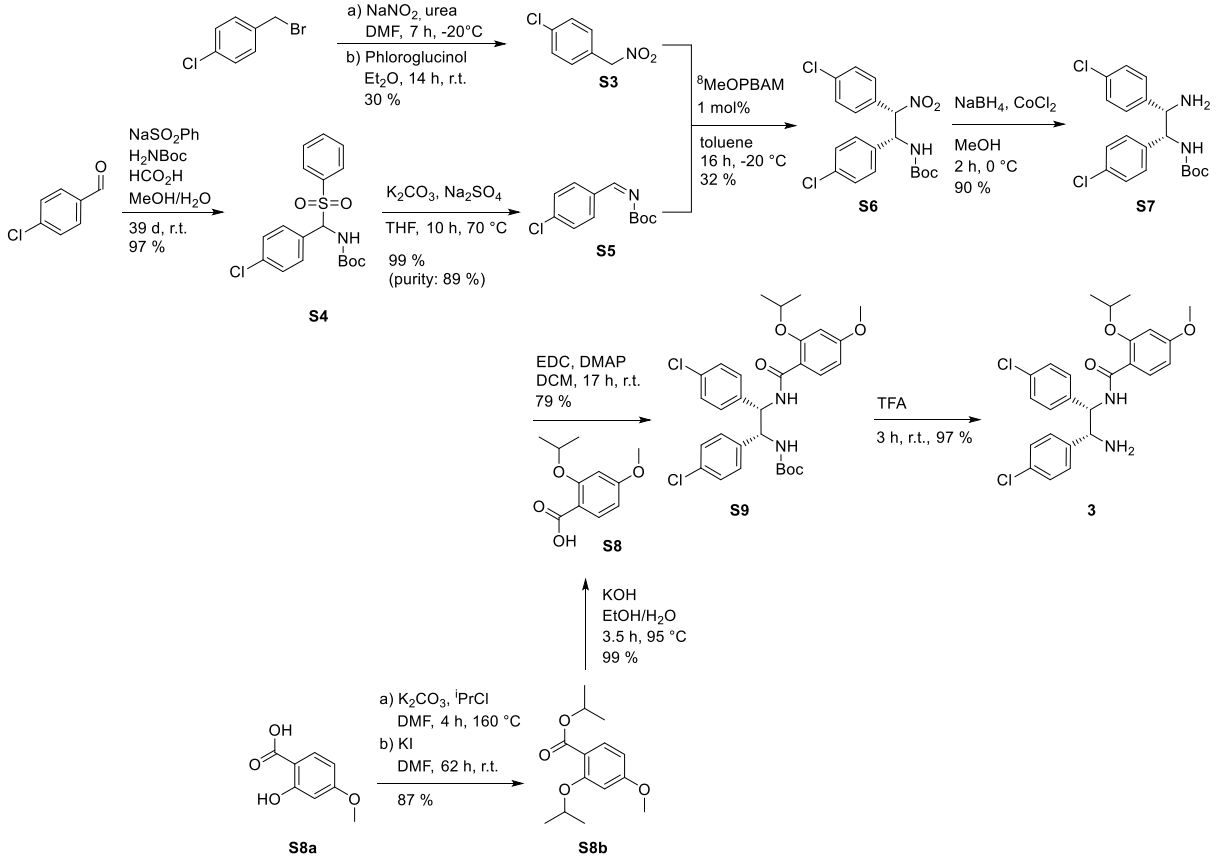


Figure S2: Synthesis of imidazoline precursor **3** following the protocol by Johnston *et al*.[1]

Figure S3

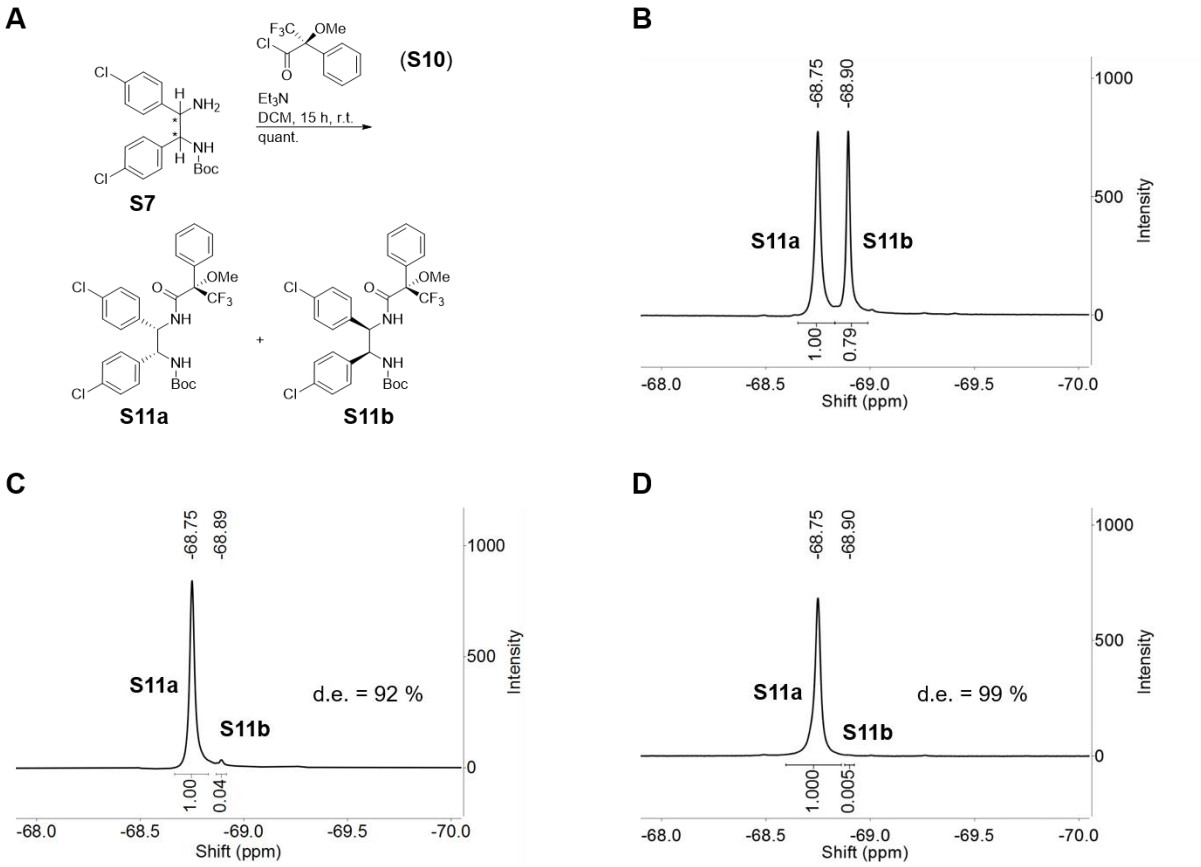


Figure S3. A) Synthesis of Mosher amides **S11a/b**. B) ^{19}F-NMR of Mosher amides **S11a/b** generated by mixing **S6** and **ent-S6** in a 1:1 ratio, reduction of the mixture to **S7**, and further reaction of **S7** with **S10**. C) ^{19}F-NMR of Mosher amides generated from **S7** obtained with ^{8}MeOPBAM without crystallization of **S6** (d.e. = 92 %). D) ^{19}F-NMR of Mosher amides generated from **S7** obtained with ^{8}MeOPBAM after crystallization of **S6** (d.e. = 99 %).

Figure S4

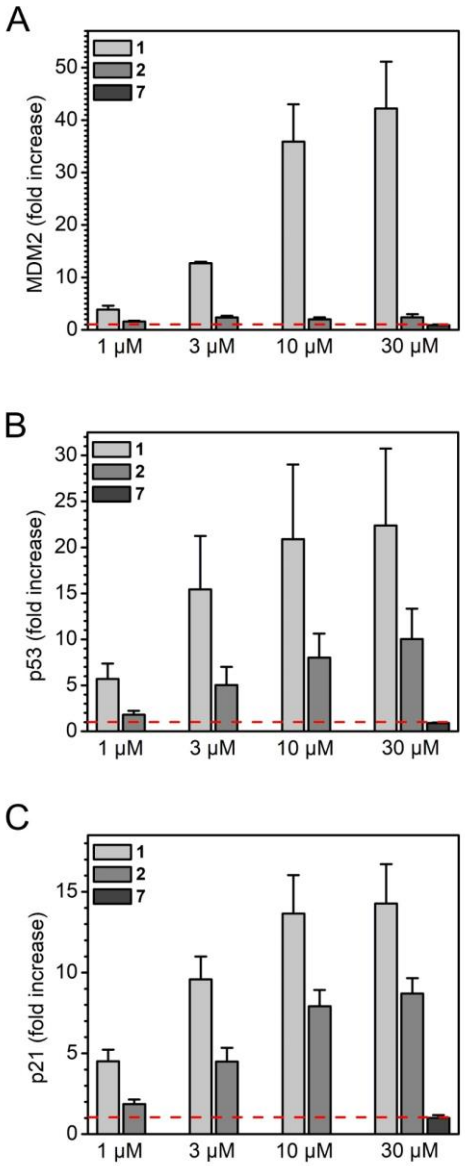


Figure S4. Quantification of the protein levels of A) MDM2, B) p53, and C) p21 from Western Blots shown in Figure 4. Mean values and standard deviations from three independent experiments are shown. The protein levels in the presence of DMSO are highlighted by the red dotted lines ("1-fold increase").

Figure S5

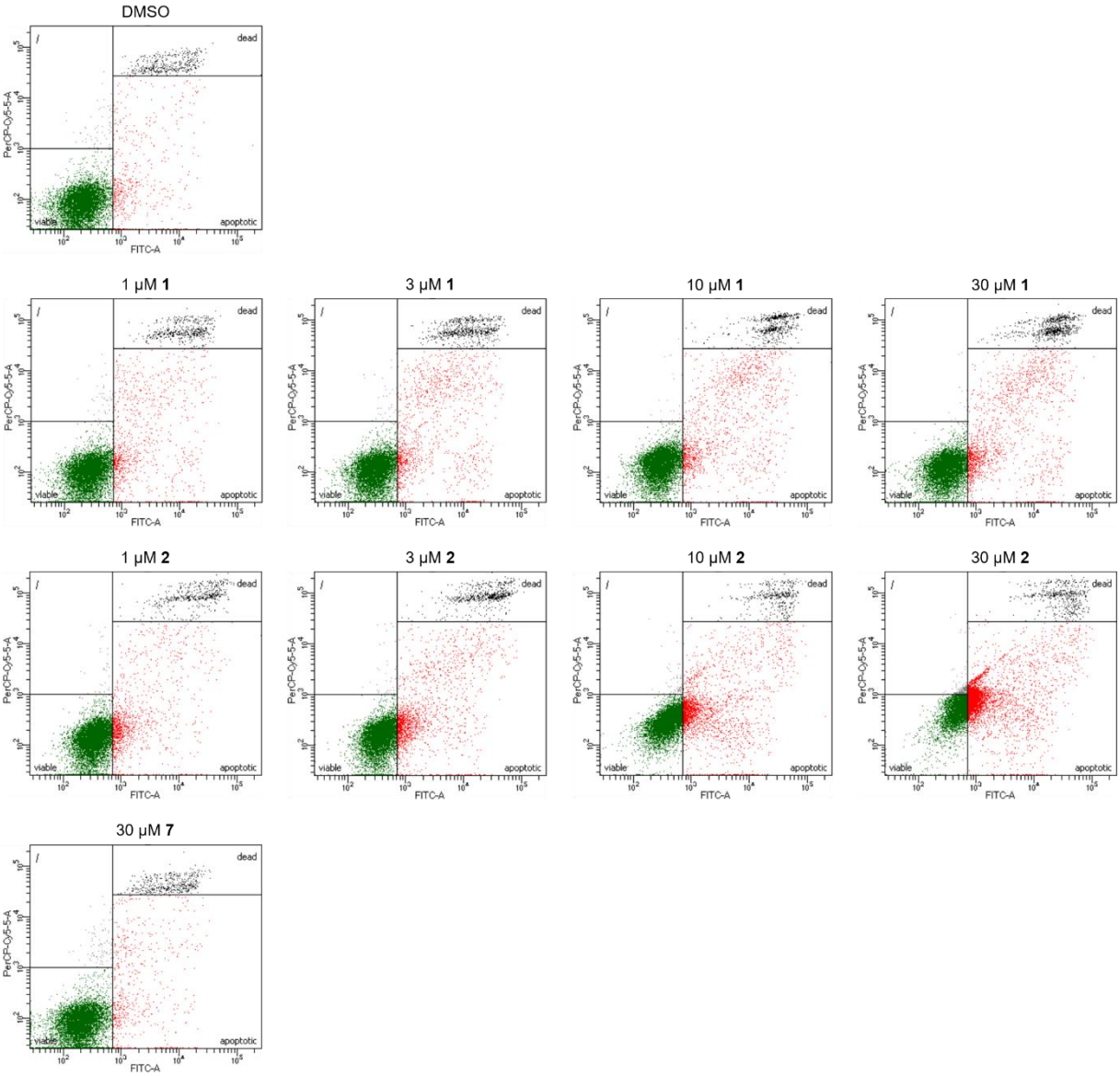


Figure S5. Representative results of flow cytometry analysis of HCT-116 cells treated with the indicated concentrations of Nutlin-3a (**1**), Nutlin-3a-HT (**2**), and control compound **7** for 24 h. Apoptotic cells, characterized by FITC Annexin V staining and the absence of propidium iodide staining, are depicted in the lower right-hand quadrant of the flow cytometry plot.

Figure S6

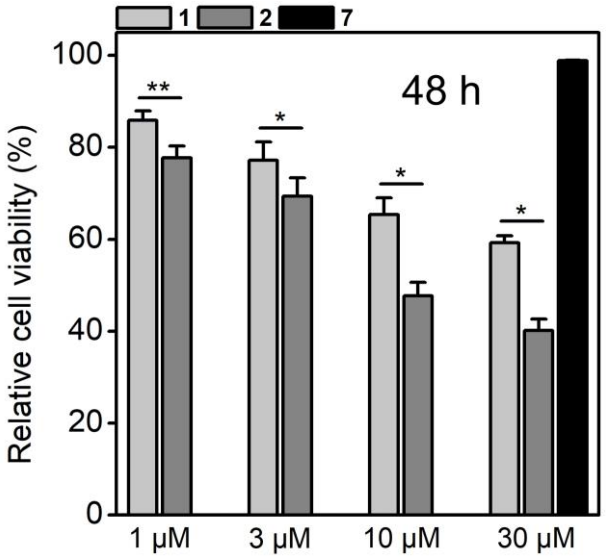


Figure S6. Effect of test compounds **1**, **2**, and **7** on the viability of HCT-116 cells after 48 h of exposure. Cell viability in the presence of DMSO control was defined as 100 %. Mean values and standard deviations are shown. *p<0.05, **p<0.01 (t-test, two-tailed, paired).

General information on synthetic methods

All chemicals were purchased from abcr, Acros Organics, Alfa Aesar, Sigma Aldrich, TCR and VWR international. Dry solvents for water sensitive reactions were either bought and used without further drying or prepared in our laboratory. DCM was dried over calcium hydride, THF over sodium, and ethanol over sodium and diethyl phthalate. All water or air sensitive reactions were carried out in oven-dried glassware under an argon atmosphere. All water used was deionized.

NMR spectroscopy

^{1}H-, ^{13}C- and ^{19}F-spectra were recorded on Varian Mercury plus 400 MHz, Varian Mercury plus 300 MHz, Bruker Avance III HD 400 MHz, Bruker DRX-400 400 MHz or Bruker Fourier-300 300 MHz spectrometers. The spectra were referenced to the signal of the undeuterated solvent (^{1}H-NMR: $CDCl_3$ 7.26 ppm, MeOH-d_4 3.31 ppm, DMSO-d_6 2.50; ^{13}C-NMR: $CDCl_3$ 77.16 ppm) or, in case of ^{19}F-NMR spectra, to CCl_3F as external standard.

Mass spectrometry

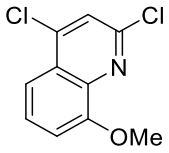
High resolution electro spray ionization (HR-ESI) mass spectra were recorded on ESI-TOF micrOTOF Bruker Daltonics coupled to ultimate HPLC LC packings and ESI-qTOF Impact II Bruker Daltonics coupled to Dionex Ultimate 3000 UHPLC ThermoFisher mass spectrometers. Exact molecular masses were calculated using ChemDraw Professional 16.0 Version software.

UV/VIS and IR spectroscopy

UV/VIS spectra were recorded on a JASCO V-630 spectrometer. Infrared spectra were recorded on a JASCO FT-IR-4100 type A spectrometer. Samples were measured in a KBr-pellet or as a film.

Chemical synthesis and spectroscopic characterization

2,4-Dichloro-8-methoxyquinoline (**S1**)[1]



A 2-necked flask equipped with reflux condenser and gas-washing bottle was charged with malonic acid and cooled with an ice bath (20.0 g, 192 mmol, 1.0 eq.). $POCl_3$ (77 mL) was added and the mixture was stirred before 2-methoxyaniline (23.6 g, 192 mmol, 1.0 eq.) was added dropwise over a period of 10 minutes. The suspension was refluxed for 6 h, cooled to room temperature while stirring and poured over crushed ice. 6 M aqueous NaOH solution (350 mL) was added to the ice-cooled mixture until pH > 8 was reached. After stirring of the suspension for 60 h, the solid was filtered and dried. Soxhlet extraction of the crude solid with 500 mL hexane for 24 h and evaporation of the extract solvent provided **S1** as a pale brown solid.

Yield: 8.38 g (19 %). Lit.[1]: 9%.

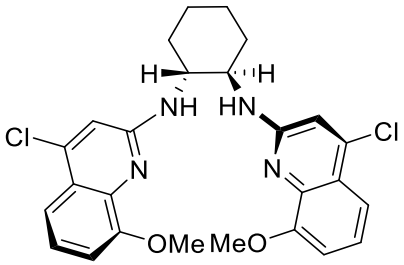
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.77 (dd, *J* = 8.5, 1.1 Hz, 1H), 7.58 (dd, *J* = 8.2, 8.2 Hz, 1H), 7.55 (s, 1H), 7.15 (dd, *J* = 7.9, 1.1 Hz, 1H), 4.08 (s, 3H) ppm.

^{13}C-NMR (101 MHz, $CDCl_3$): δ = 155.0, 149.1, 144.5, 140.1, 128.3, 126.5, 122.9, 115.8, 110.0, 56.5 ppm.

HRMS (ESI): *m/z* calcd for $C_{10}H_7Cl_2NO+H^+$: 227.9977 $[M+H]^+$; found: 227.9970.

R_f: 0.75 (2.5 % MeOH in DCM).

(1*R*,2*R*)-*N1*,*N2*-bis(4-chloro-8-methoxyquinolin-2-yl)cyclohexane-1,2-diamine (**S2**)[1]



A solution of (1*R*,2*R*)-cyclohexane-1,2-diamine (0.890 g, 7.79 mmol, 1.0 eq.), quinoline **S1** (3.56 g, 15.6 mmol, 2.0 eq.), $Pd(dba)_2$ (0.090 g, 0.16 mmol, 0.02 eq.), *rac*-BINAP (0.097 g, 0.16 mmol, 0.02 eq.) and potassium *tert*-butoxide (2.62 g, 23.4 mmol, 3.0 eq.) in 40 mL dry

toluene was stirred for 4 h at 70 °C. The reaction mixture was cooled to room temperature, diluted with DCM, filtrated and washed with DCM and toluene. The solvent was removed under reduced pressure and the raw product was purified by column chromatography (25 – 50 % ethyl acetate in hexane) to provide **S2** as a yellow solid.

Yield: 1.03 g (37 %). Lit.[1]: 62%.

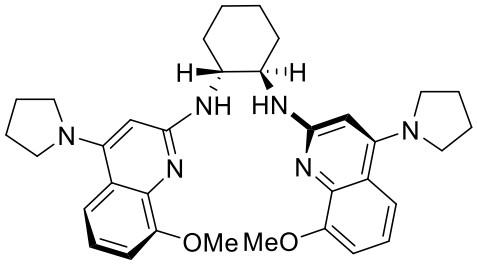
^{1}H-NMR (400 MHz, DMSO-d_6): δ = 7.72 (s, br, 2H), 7.42 (dd, *J* = 8.1, 1.5 Hz, 2H), 7.21 – 7.10 (m, 4H), 6.85 (s, 2H), 3.94 (s, 6H), 3.92 (s, 2H), 2.34 – 2.23 (m, 2H), 1.81 – 1.71 (m, 2H), 1.43 – 1.33 (m, 4H) ppm.

^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 156.0, 153.4, 142.3, 140.3, 122.4, 122.1, 116.4, 112.8, 110.1, 56.8, 56.5, 32.7, 24.9 ppm.

HRMS (ESI): *m/z* calcd for $C_{26}H_{26}Cl_2N_4O_2$+H^+: 497.1506 [*M*+H]$^+$; found: 497.1515.

R_f: 0.49 (50 % ethyl acetate in hexane).

(1*R*,2*R*)-*N1*,*N2*-bis(8-methoxy-4-(pyrrolidin-1-yl)quinolin-2-yl)cyclohexane-1,2-diamine (^{8}MeOPBAM)[1]



A solution of **S2** (1.00 g, 2.01 mmol, 1.0 eq.) and pyrrolidine (0.66 mL, 8.04 mmol, 4.0 eq.) in 20 mL dry trifluorotoluene was stirred for 17 h at 80 °C. The solvent was removed and the residue was purified by column chromatography (5 % MeOH in DCM) to provide ^{8}MeOPBAM as a colorless solid.

Yield: 0.704 g (62 %). Lit.[1]: 48%.

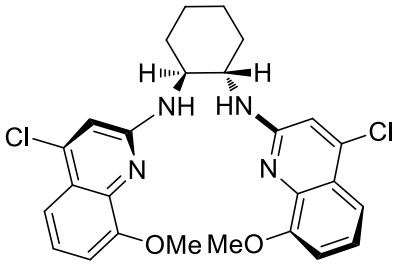
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.49 (dd, *J* = 8.3, 1.4 Hz, 2H), 6.97 – 6.84 (m, 4H), 5.81 (s, br, 1H), 5.52 (s, 2H), 4.04 (s, 2H), 4.01 (s, 6H), 3.39 – 3.28 (m, 4H), 3.25 – 3.15 (m, 4H), 2.32 (d, *J* = 9.7 Hz, 2H), 1.90 – 1.82 (m, 8H), 1.82 – 1.75 (m, 2H), 1.53 – 1.40 (m, 4H) ppm.

^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 157.7, 153.7, 141.6, 119.4, 118.5, 117.3, 108.4, 93.0, 56.3, 56.2, 51.8, 33.1, 25.6, 24.9 ppm.

HRMS (ESI): *m/z* calcd for $C_{34}H_{42}N_6O_2$+H^+: 567.3442 [*M*+H]$^+$; found: 567.3448.

R_f: 0.32 (5 % MeOH in DCM).

(1*S*,2*S*)-*N1*,*N2*-bis(4-chloro-8-methoxyquinolin-2-yl)cyclohexane-1,2-diamine (**ent-S2**)



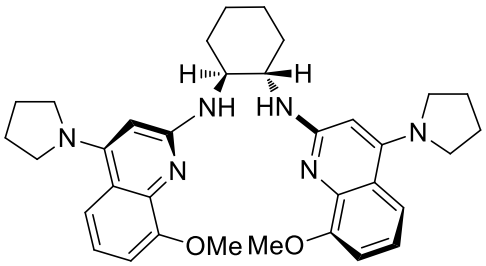
A solution of (1*S*,2*S*)-cyclohexane-1,2-diamine (200 mg, 1.75 mmol, 1.0 eq.), quinoline **S1** (800 mg, 3.50 mmol, 2.0 eq.), $Pd(dba)_2$ (20 mg, 35.0 µmol, 0.02 eq.), *rac*-BINAP (22 mg, 35.0 µmol, 0.02 eq.) and potassium *tert*-butoxide (589 mg, 5.25 mmol, 3.0 eq.) in 18 mL dry toluene was stirred for 4 h at 70 °C. The reaction mixture was cooled to room temperature, diluted with DCM, filtrated and washed with DCM and toluene. The solvent was removed under reduced pressure and the raw product was purified by column chromatography (25 % ethyl acetate in hexane) to provide **ent-S2** as a yellow solid.

Yield: 426 mg (49 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.54 (dd, *J* = 8.3, 1.3 Hz, 2H), 7.17 (t, *J* = 8.0 Hz, 2H), 7.02 (dd, *J* = 7.7, 1.3 Hz, 2H), 6.60 (s, 2H), 6.24 (s, br, 2H), 4.06 (s, 8H), 2.46 – 2.38 (m, 2H), 1.87 – 1.78 (m, 2H), 1.53 – 1.36 (m, 4H) ppm.

R_f: 0.49 (50 % ethyl acetate in hexane).

(1*S*,2*S*)-*N1*,*N2*-bis(8-methoxy-4-(pyrrolidin-1-yl)quinolin-2-yl)cyclohexane-1,2-diamine (ent-^{8}MeOPBAM)



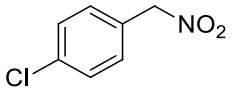
A solution of bismethoxyquinolinecyclohexane-1,2-diamine **ent-S2** (50 mg, 0.10 mmol, 1.0 eq.) and pyrrolidine (33 µL, 0.40 mmol, 4.0 eq.) in 0.3 mL dry trifluorotoluene was stirred for 16 h at 100 °C. The solvent was removed and the residue was purified by column chromatography (5 % MeOH in DCM) to provide ent-^{8}MeOPBAM as a colorless solid.

Yield: 42 mg (75 %).

^{1}H-NMR (400 MHz, MeOH-d_4): δ = 7.54 (d, *J* = 7.8 Hz, 2H), 7.05 – 6.95 (m, 4H), 5.48 (s, 1H), 5.31 (s, 2H), 3.98 (s, 6H), 3.95 (s, 2H), 3.29 – 3.18 (m, 4H), 3.10 – 2.94 (m, 4H), 2.20 – 2.09 (m, 2H), 1.90 – 1.76 (m, 10H), 1.53 – 1.45 (m, 4H) ppm.

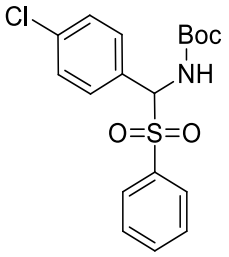
R_f: 0.32 (5 % MeOH in DCM).

1-Chloro-4-(nitromethyl)benzene (**S3**) [1-2]



Note of caution: As previously reported by Johnston *et al*.,[1] this compound is a lachrymator. Therefore, the reaction and the following work-up should take place in a well-ventilated fume hood. A solution of sodium nitrite (10.1 g, 146 mmol, 1.5 eq.) and urea (11.7 g, 195 mmol, 2.0 eq.) in 130 mL dry DMF was cooled to -50 °C by a cryostat. 1-(bromomethyl)-4-chlorobenzene (20.0 g, 97.3 mmol, 1.0 eq.) was added and 17 mL of dry DMF were used to rinse the remaining solid to the reaction solution. The mixture was warmed to -20 °C and stirred for 7 h before being poured to 500 mL ice water in a separating funnel. The mixture was extracted four times with a total of 350 mL Et_2O. Phloroglucinol (9.82 g, 77.9 mmol, 0.8 eq.) was added to the combined organic extracts and the suspension was stirred for 14 h. 400 mL water was added to the mixture and the aqueous phase was separated without shaking to avoid emulsion. The organic phase was washed five times with water and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure to obtain 12.4 g of a red oil. This oil was purified by column chromatography (1 – 3 % ethyl acetate in hexane) to isolate **S3** as a yellow solid.
Yield: 5.05 g, 30 % (contains 6% 4-chlorobenzaldehyde). Lit.[1]: 42%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.44 – 7.38 (m, 4H), 5.41 (s, 2H) ppm.
^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 136.5, 131.5, 129.5, 128.2, 79.3 ppm.
R_f: 0.37 (10 % ethyl acetate in hexane).

Tert-butyl ((4-chlorophenyl)(phenylsulfonyl)methyl)carbamate (**S4**) [1]



4-chlorobenzaldehyde (45.0 g, 320 mmol, 1.0 eq.), sodium benzoate (70.1 g, 427 mmol, 1.3 eq.) and *tert*-butyl carbamate (25.0 g, 214 mmol, 0.67 eq.) were suspended in a mixture of 202 mL methanol and 404 mL H_2O. Formic acid (16.1 mL, 427 mmol, 1.3 eq.) was added and the mixture was stirred for 9 days at room temperature before the residue was filtered, rinsed with Et_2O and dried on the filter. The colorless solid was transferred to a beaker, suspended and washed in Et_2O (ca. 250 mL), filtered and dried. This procedure was repeated four times. The product was dried at high vacuum to obtain 57.1 g of a colorless solid. All filtrates were combined and Et_2O was evaporated. The reaction mixture was stirred for 30 additional days before being filtered and rinsed with Et_2O. The process of solid transfer, washing with Et_2O,

filtering and drying as described previously was applied five times to provide another 21.7 g of substance of similar purity.

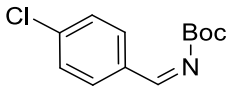
Yield: 78.8 g (97 **%**, contains 3 % 4-chlorobenzaldehyde). Lit.[1]: 89%.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.91 (d, *J* = 7.6 Hz, 2H), 7.66 (dd, *J* = 7.4, 7.4 Hz, 1H), 7.56 (dd, *J* = 7.7, 7.7 Hz, 2H), 7.43 – 7.36 (m, 4H), 5.89 (d, *J* = 10.8 Hz, 1H), 5.65 (d, *J* = 10.8 Hz, 1H), 1.26 (s, 9H) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 153.5, 136.8, 136.3, 134.3, 130.3, 129.6, 129.3, 129.2, 128.6, 81.6, 73.4, 28.1 ppm.

R_f: 0.27 (20 % ethyl acetate in hexane).

(*E*)-*tert*-butyl 4-chlorobenzylidenecarbamate (**S5**) [1]



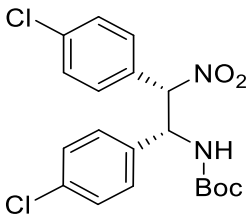
Sulfone **S4** (20.6 g, 54.0 mmol, 1.0 eq.), potassium carbonate (52.3 g, 378 mmol, 7.0 eq.) and sodium sulfate (61.4 g, 432 mmol, 8.0 eq.) were suspended in 450 mL dry THF and the mixture was refluxed for 10 h. The suspension was cooled to room temperature, filtered and washed with Et_2O. The filtrate was concentrated under reduced pressure to obtain **S5** as a colorless solid.

Yield: 12.8 g (99 %, contains 11 % of 4-chlorobenzaldehyde). Lit.[1]: 95%.

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.84 (s, 1H), 7.94 (d, *J* = 8.3 Hz, 2H), 7.62 (d, *J* = 8.5 Hz, 2H), 1.51 (s, 9H) ppm.

R_f: 0.59 (20 % ethyl acetate in hexane).

Tert-butyl ((1*R*,2*S*)-1,2-bis(4-chlorophenyl)-2-nitroethyl)carbamate (**S6**) [1]



A solution of **S3** (2.63 g, 15.3 mmol, 1.1 eq.) and ^{8}MeOPBAM (0.083 g, 0.15 mmol, 0.01 eq.) in 63 mL dry toluene was cooled to -20 °C by a cryostat. A solution of imine **S5** (3.50 g, 14.6 mmol, 1.0 eq) in 7 mL dry toluene in a separate flask was added in one-fifth aliquots every 30 min to the stirring reaction mixture. After complete addition, the solution was stirred for 16 h at -20 °C. The suspension was filtered and hexane was used to rinse residual solid from the flask. The solid was washed with cold toluene and hexane before being dried on the filter. The solid (2.13 g) was recrystallized from 18 mL toluene to provide **S6** as a colorless solid.

Yield: 1.90 g (32 %).

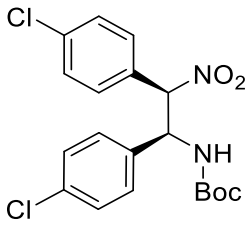
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.50 (d, *J* = 8.4 Hz, 2H), 7.40 (d, *J* = 8.5 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.28 (d, *J* = 8.5 Hz, 2H), 5.75 (d, *J* = 9.9 Hz, 1H), 5.59 (dd, *J* = 10.1, 10.1 Hz, 1H), 4.82 (s, 1H), 1.28 (s, 9H) ppm.

^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 154.2, 136.6, 135.6, 134.9, 130.1, 129.7, 129.3, 129.1, 128.6, 93.2, 80.8, 56.3, 28.0 ppm.

HRMS (ESI): *m/z* calcd for $C_{19}H_{20}Cl_2N_2O_4$+Na^+: 433.0698 [*M*+Na]$^+$; found: 433.0709.

R_f: 0.63 (25 % ethyl acetate in hexane).

Tert-butyl ((1S,2*R*)-1,2-bis(4-chlorophenyl)-2-nitroethyl)carbamate (**ent-S6**)



A solution of **S3** (50 mg, 0.29 mmol, 1.1 eq.) and ent-^{8}MeOPBAM (1.6 mg, 2.77 µmol, 0.01 eq.) in 1.3 mL dry toluene was cooled to -20 °C by a cryostat. A solution of imine **S5** (66 mg, 0.28 mmol, 1.0 eq) in 0.1 mL dry toluene in a separate flask was added in one-fifth aliquots every 30 min to the stirring reaction mixture. After complete addition, the solution was stirred for 18 h at -20 °C. The suspension was filtered and washed with cold toluene and hexane before being dried on the filter. The solid was recrystallized from toluene to provide **ent-S6b** as a colorless solid.

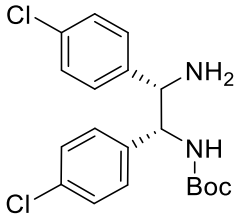
Yield: 46 mg, (38 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.50 (d, *J* = 8.4 Hz, 2H), 7.40 (d, *J* = 8.5 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.28 (d, *J* = 8.7 Hz, 2H), 5.76 (d, *J* = 9.9 Hz, 1H), 5.58 (dd, *J* = 9.7, 9.6 Hz, 1H), 4.75 (d, *J* = 9.1 Hz, 1H), 1.28 (s, 9H) ppm.

R_f: 0.63 (25 % ethyl acetate in hexane).

R_f: 0.19 (20 % ethyl acetate in hexane).

Tert-butyl ((1*R*,2*S*)-2-amino-1,2-bis(4-chlorophenyl)ethyl)carbamate (**S7**) [1]



A solution of **S6** (1.11 g, 2.70 mmol, 1.0 eq.) and $CoCl_2$ (0.348 g, 2.70 mmol, 1.0 eq) in 37 mL dry methanol was stirred and cooled with an ice bath. Sodium borohydride (0.511 g, 13.5 mmol, 5.0 eq.) was added in portions over a period of 45 min. The solution was stirred for 2 h at 0 °C before being quenched with water, 1 M aqueous HCl until pH 5 and 3 M aqueous ammonia

until pH 9 was reached. The suspension was filtrated and the residue was washed with water. The flask was changed before the residue was dissolved with DCM. The remaining water was removed in a separating funnel and the organic phase was dried over Na_2SO_4. After filtration the solvent was removed under reduced pressure obtaining **S7** as a colorless solid.

Yield: 0.93 g (90 %). Lit.[1]: 94%.

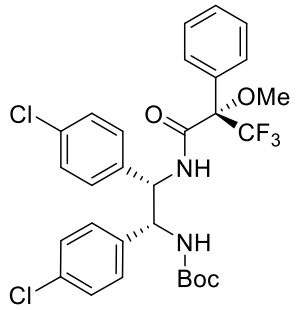
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.26 – 7.18 (m, 4H), 7.01 (d, *J* = 8.1 Hz, 2H), 6.93 (d, *J* = 8.0 Hz, 2H), 5.42 (s, 1H), 4.77 (s, 1H), 4.23 (s, 1H), 1.35 (s, 9H) ppm.

^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 155.2, 140.5, 133.5, 128.9, 128.6, 128.4, 80.0, 59.4, 28.4 ppm.

HRMS (ESI): *m/z* calcd for $C_{19}H_{22}Cl_2N_2O_2$+H^+: 381.1131 [*M*+H]$^+$; found: 381.1132.

R_f: 0.33 (50 % ethyl acetate in hexane).

Tert-butyl ((1*R*,2*S*)-1,2-bis(4-chlorophenyl)-2-((*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamido)-ethyl)carbamate (**S11a**)



Carbamate **S7** (7.0 mg, 0.018 mmol, 1.0 eq.) and Et_3N (5.1 µL, 0.037 mmol, 2.0 eq.) were dissolved in 0.5 mL DCM. The solution was stirred and cooled to 0 °C before (*R*)-(-)-MOSHER'S acid chloride **S10** (5.2 µL, 0.028 mmol, 1.5 eq.) was added. The mixture was stirred at room temperature for 15 h before being quenched by sodium bicarbonate. The solution was extracted three times with DCM and the organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to obtain **S11a** as a colorless solid.

Yield: 11 mg (quant).

d.e.: 99 % by ^{19}F-NMR.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.54 – 7.48 (m, 1H), 7.44 – 7.39 (m, 2H), 7.39 – 7.30 (m, 5H), 7.30 – 7.26 (m, 2H), 7.24 – 7.16 (m, 2H), 6.93 (d, *J* = 8.1 Hz, 2H), 6.78 (d, *J* = 8.1 Hz, 2H), 5.33 – 5.25 (m, 1H), 5.12 (s, br, 1H), 4.99 – 4.90 (m, 1H), 3.55 (s, 1H), 3.43 (s, 3H), 1.37 (s, 9H) ppm.

^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 166.2, 155.7, 136.2, 135.3, 134.2, 134.1, 132.5, 129.7, 129.0, 128.9, 128.6, 128.5, 127.6, 125.2, 122.3, 80.9, 58.0, 55.4, 28.3 ppm.

^{19}F-NMR (377 MHz, $CDCl_3$): δ = -68.75 ppm.

HRMS (ESI): *m/z* calcd for $C_{29}H_{29}Cl_2F_3N_2O_4$+$Na^+$: 619.1349 [*M*+Na]$^+$; found: 619.1356.

UV/VIS ($CHCl_3$): λ_{max} = 362, 312, 269, 262, 239 nm.

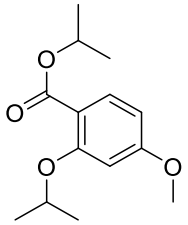
IR (KBr): $\tilde{\nu}$ = 3383, 2980, 2930, 2849, 1681, 1523, 1494, 1455, 1392, 1368, 1305, 1290, 1250, 1168, 1092, 1015, 718, 565 cm^{-1}.

R_f: 0.75 (50 % ethyl acetate in hexane).

Mp: 224 - 226 °C.

$[\alpha]_D^{23}$: -29 ° (0.95 mg/100 mL, $CHCl_3$).

Isopropyl 2-isopropoxy-4-methoxybenzoate (**S8b**) [1]



A solution of 2-hydroxy-4-methoxybenzoic acid **S8a** (9.70 g, 57.7 mmol, 1.0 eq.) and potassium carbonate (31.9 g, 231 mmol, 4.0 eq.) in 250 mL dry DMF was stirred for 15 min. Isopropyl chloride (26.3 mL, 288 mmol, 5.0 eq.) was added and the solution was refluxed for 4 h. The mixture was cooled to room temperature and potassium iodide (0.96 g, 5.77 mmol, 0.1 eq.) was added. The solution was stirred at room temperature for 62 h before a second batch of potassium iodide (541 mg, 3.26 mmol, 0.1 eq.) was added. The mixture was refluxed for 3.5 h and TLC indicated full conversion of starting material. The solution was quenched by slow addition of 120 mL 3 M aqueous HCl and extracted seven times with a total of 500 mL Et_2O. The organic phase was washed with 2 M aqueous sodium carbonate solution and water, dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure to provide **S8b** as a pale yellow solid.

Yield: 12.7 g (87 %). Lit.[1]: 93%.

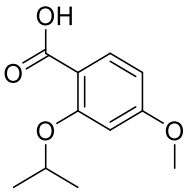
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.83 – 7.71 (m, 1H), 6.48 (d, *J* = 2.3 Hz, 1H), 6.47 (d, *J* = 1.8 Hz, 1H), 5.21 (hept, *J* = 6.3 Hz, 1H), 4.55 (hept, *J* = 6.1 Hz, 1H), 3.81 (s, 3H), 1.37 (d, *J* = 6.0 Hz, 6H), 1.33 (d, *J* = 6.3 Hz, 6H) ppm.

^{13}C-APT-NMR (75 MHz, $CDCl_3$): δ = 166.0, 163.7, 159.5, 133.6, 115.1, 105.0, 102.0, 71.7, 67.7, 55.5, 22.2 ppm.

HRMS (ESI): *m/z* calcd for $C_{14}H_{20}O_4$+Na^+: 275.1254 [*M*+Na]$^+$; found: 275.1244.

R_f: 0.43 (10 % ethyl acetate in hexane).

2-Isopropoxy-4-methoxybenzoic acid (**S8**) [1]



A solution of isopropyl ester **S8b** (12.0 g, 47.6 mmol, 1.0 eq.) and KOH (8.01 g, 143 mmol, 3.0 eq.) in 132 mL ethanol and 26 mL water was stirred for 3.5 h at 95 °C. The reaction mixture was cooled to room temperature, ethanol was removed under reduced pressure and the solution was diluted with water. 3 M aqueous HCl was added until pH 1 was reached and the mixture was extracted three times with Et_2O. The combined extracts were dried over Na_2SO_4, filtered and the solvent was evaporated to obtain **S8** as an off-white solid.

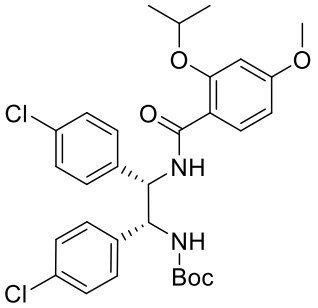
Yield: 9.89 g (99 %). Lit.[1]: 94%.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 10.96 (s, br, 1H), 8.14 (d, *J* = 8.8 Hz, 1H), 6.63 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.52 (d, *J* = 2.3 Hz, 1H), 4.81 (hept, *J* = 6.1 Hz, 1H), 3.86 (s, 3H), 1.49 (d, *J* = 6 Hz, 6H) ppm.

^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 165.5, 165.0, 157.9, 135.7, 111.6, 107.0, 101.0, 74.1, 55.9, 22.2 ppm.

HRMS (ESI): *m/z* calcd for $C_{11}H_{14}O_4$+Na^+: 233.0784 [*M*+Na]$^+$; found: 233.0794.

Tert-butyl((1*R*,2*S*)-1,2-bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxybenzamido)ethyl) carbamate (**S9**)[1]



A solution of carbamate **S7** (909 mg, 2.38 mmol, 1.0 eq.) and benzoic acid **S8** (501 mg, 2.38 mmol, 1.0 eq.) in 21 mL dry DCM was cooled to 0 °C. EDC (594 mg, 3.10 mmol, 1.3 eq.) and DMAP (29 mg, 0.24 mmol, 0.1 eq.) were added and the solution was stirred for 17 h at room temperature. The mixture was diluted with DCM, filtrated and washed by a sequence of 1:1 DCM/hexane, 4:1 H_2O/hexane, hexane, H_2O, hexane. The solid was dried at high vacuum to provide 383 mg of a colorless solid. The organic solvents of the filtrate were removed under reduced pressure and the precipitate was filtrated. The solid was washed by the same procedure again and dried to produce an additional 700 mg of **S9** as a colorless solid.

Yield: 1.083 g (79 %). Lit.[1]: 83%.

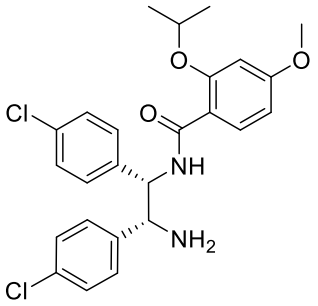
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.38 (s, 1H), 8.19 (d, *J* = 8.8 Hz, 1H), 7.28 – 7.24 (m, 2H), 7.23 – 7.19 (m, 2H), 7.01 (d, *J* = 8.0 Hz, 2H), 6.96 – 6.91 (m, 2H), 6.59 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.46 (d, *J* = 2.3 Hz, 1H), 5.88 (s, br, 1H), 5.77 (d, *J* = 8.3 Hz, 1H), 5.11 – 5.04 (m, 1H), 4.68 (hept, *J* = 6.1 Hz, 1H), 3.84 (s, 3H), 1.38 (s, 9H), 1.33 – 1.19 (m, 6H) ppm.

^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 165.8, 163.8, 157.4, 155.2, 137.1, 137.0, 134.6, 133.8, 133.6, 128.9, 128.7, 128.5, 114.4, 105.4, 100.4, 80.1, 71.7, 59.8, 56.8, 55.7, 28.5, 22.2, 21.8 ppm.

HRMS (ESI): *m/z* calcd for $C_{30}H_{34}Cl_2N_2O_5$+Na^+: 595.1737 [*M*+Na]$^+$; found: 595.1736.

R_f: 0.50 (ethyl acetate/hexane 1:1).

N-((1*S*,2*R*)-2-amino-1,2-bis(4-chlorophenyl)ethyl)-2-isopropoxy-4-methoxybenzamide (**3**) [1]



Carbamate **S9** (744 mg, 1.30 mmol, 1.0 eq.) was dissolved in 13 mL DCM and TFA (1.50 mL, 19.5 mmol, 15 eq.) was added to the solution. The reaction mixture was stirred for 3 h at room temperature, cooled with an ice bath and quenched with saturated aqueous $NaHCO_3$ solution. Water, DCM and 3 M NaOH were added and the suspension was stirred for 15 min. The organic phase was separated and the aqueous phase was extracted twice with DCM. The combined organic extracts were dried over Na_2SO_4, filtrated and concentrated under reduced pressure to obtain **3** as a colorless solid.

Yield: 596 mg (97 %). Lit.[1]: 92%.

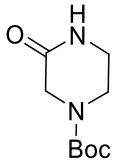
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.91 (d, *J* = 8.0 Hz, 1H), 8.16 (d, *J* = 8.8 Hz, 1H), 7.29 (d, *J* = 5.5 Hz, 2H), 7.25 – 7.22 (m, 2H), 7.09 – 7.05 (m, 2H), 7.05 – 7.01 (m, 2H), 6.59 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.52 (d, *J* = 2.3 Hz, 1H), 5.46 (dd, *J* = 8.1, 4.9 Hz, 1H), 4.79 (hept, *J* = 6.1 Hz, 1H), 4.44 (d, *J* = 4.9 Hz, 1H), 3.87 (s, 3H), 1.48 (d, *J* = 5.0 Hz, 3H), 1.47 (d, *J* = 5.0 Hz, 3H) ppm.

^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 164.8, 163.5, 157.4, 141.0, 137.0, 134.3, 133.4, 133.3, 129.3, 128.5, 128.4, 128.3, 115.2, 105.3, 100.5, 71.7, 59.2, 58.8, 55.7, 22.4, 22.2 ppm.

HRMS (ESI): *m/z* calcd for $C_{25}H_{27}Cl_2N_2O_3^+$: 473.1393 [*M*+H]$^+$; found: 473.1405.

R_f: 0.50 (5 % MeOH and 1 % $EtMe_2N$ in DCM).

Tert-butyl 3-oxopiperazine-1-carboxylate (**4a**)[3]



A solution of 2-oxopiperazine (2.90 g, 29.0 mmol, 1.0 eq.) and Boc_2O (9.49 g, 43.5 mmol, 1.5 eq.) in 100 mL dry DCM was stirred for 18 h at room temperature. The solvent was removed under reduced pressure and the residue was purified by column chromatography (0 – 5 % MeOH in DCM) to provide **4a** as a colorless solid.

Yield: 5.45 g (94 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.94 (s, br, 1H), 4.07 (s, 2H), 3.61 (t, *J* = 5.4 Hz, 2H), 3.37 (td, *J* = 5.5, 2.7 Hz, 2H), 1.46 (s, 9H) ppm.

^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 168.3, 154.0, 81.0, 41.3, 28.5 ppm.

HRMS (ESI): *m/z* calcd for $C_9H_{16}N_2O_3$+Na^+: 223.1053 [*M*+Na]$^+$; found: 223.1060.

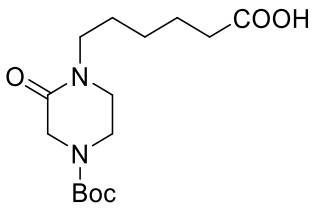
UV/VIS (DCM): λ_{max} = 276, 228 nm.

IR (KBr): $\tilde{\nu}$ = 3445, 3201, 2980, 1694, 1665, 1401, 1366, 1339, 1283, 1244, 1174, 1131, 1002, 817, 773 cm^{-1}.

R_f: 0.41 (5 % MeOH in DCM).

Mp: 160 °C.

6-(4-(*Tert*-butoxycarbonyl)-2-oxopiperazin-1-yl)hexanoic acid (**4b**)



A round bottom flask was charged with NaH (60 % dispersion in mineral oil, 0.599 g, 15.0 mmol, 3.0 eq.) which was washed three times with hexane. 20 mL dry DMF was added and the suspension was cooled with an ice bath. *N*-Boc-protected 2-oxopiperazine **4a** (1.00 g, 4.99 mmol, 1.0 eq.) and 6-bromohexanoic acid (1.17 g, 5.99 mmol, 1.2 eq.) were slowly added and the mixture was stirred for 15 h at room temperature. The suspension was quenched with H_2O, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (1 % AcOH in ethyl acetate) to provide **4b** as a colorless oil.

Yield: 1.41 g (75 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 4.07 (s, 2H), 3.63 (t, *J* = 5.4 Hz, 2H), 3.40 (t, *J* = 7.5 Hz, 2H), 3.33 (t, *J* = 5.4 Hz, 2H), 2.36 (t, *J* = 7.4 Hz, 2H), 1.67 (quin, *J* = 7.5 Hz, 2H), 1.58 (quin, *J* = 7.8 Hz, 2H), 1.47 (s, 9H), 1.41 – 1.33 (m, 2H) ppm.

^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 178.3, 165.9, 154.0, 81.0, 47.0, 46.4, 33.8, 28.5, 26.8, 26.3, 24.5 ppm.

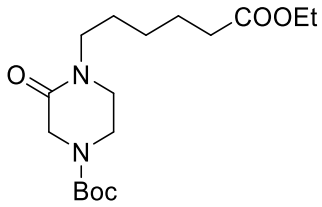
HRMS (ESI): *m/z* calcd for $C_{15}H_{26}N_2O_5$+Na^+: 337.1734 [*M*+Na]$^+$; found: 337.1732.

UV/VIS (DCM): λ_{max} = 276 nm.

IR (KBr): $\tilde{\nu}$ = 3440, 2974, 2932, 2861, 1729, 1699, 1627, 1505, 1421, 1367, 1343, 1234, 1205, 1163, 1126, 988, 939, 856, 768, 522 cm^{-1}.

R_f: 0.44 (1 % AcOH in ethyl acetate).

Tert-butyl 4-(6-ethoxy-6-oxohexyl)-3-oxopiperazine-1-carboxylate (**4c**)



Hexanoic acid **4b** (617 mg, 1.96 mmol, 1.0 eq.) was dissolved in 16 mL dry DCM and DCC (506 mg, 2.95 mmol, 1.5 eq.), DMAP (48 mg, 0.393 mmol, 0.2 eq.) and ethanol (0.344 mL, 5.89 mmol, 3.0 eq.) were added. The mixture was stirred for 21 h at room temperature and the solvent was removed under reduced pressure. The residue was suspended in DCM, filtered, concentrated under reduced pressure and purified by column chromatography (2 % MeOH in DCM) to obtain **4c** as a colorless oil.

Yield: 618 mg (92 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 4.12 (q, *J* = 7.1 Hz, 2H), 4.05 (s, 2H), 3.62 (t, *J* = 5.4 Hz, 2H), 3.39 (t, *J* = 7.5 Hz, 2H), 3.32 (t, *J* = 5.4 Hz, 2H), 2.29 (t, *J* = 7.4 Hz, 2H), 1.70 – 1.63 (m, 2H), 1.57 (t, *J* = 7.6 Hz, 2H), 1.46 (s, 9H), 1.37 – 1.31 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H) ppm.

^{13}C-APT-NMR (75 MHz, $CDCl_3$): δ = 173.7, 165.6, 154.0, 80.9, 60.4, 47.0, 46.4, 34.3, 33.9, 28.5, 26.9, 26.5, 25.0, 24.8, 14.4 ppm.

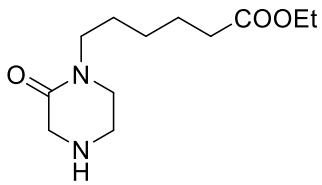
HRMS (ESI): *m/z* calcd for $C_{17}H_{30}N_2O_5$+Na^+: 365.2047 [*M*+Na]$^+$; found: 365.2041.

UV/VIS (DCM): λ_{max} = 264, 229 nm.

IR (Film): $\tilde{\nu}$ = 3324, 2977, 2927, 2850, 1733, 1699, 1654, 1626, 1575, 1418, 1366, 1241, 1170, 1126, 1031, 864, 768, 641, 595, 419 cm^{-1}.

R_f: 0.20 (2 % MeOH in DCM).

Ethyl 6-(2-oxopiperazin-1-yl)hexanoate (**4**)



A solution of *N*-Boc-protected 2-oxopiperazine **4c** (527 mg, 1.54 mmol, 1.0 eq.) in 12 mL DCM was cooled with an ice bath and TFA (1.78 mL, 23.1 mmol, 15 eq.) was added slowly. The mixture was stirred for 5 h at room temperature, treated with aqueous Na_2CO_3 solution and extracted with DCM. The combined organic layers were concentrated under reduced pressure and the residue was purified by column chromatography (9 % MeOH in DCM) to yield **4** as a colorless oil.

Yield: 306 mg (82 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 4.12 (q, *J* = 7.1 Hz, 2H), 3.51 (s, 2H), 3.37 (t, *J* = 7.5 Hz, 2H), 3.30 (t, *J* = 5.5 Hz, 2H), 3.07 (t, *J* = 5.5 Hz, 2H), 2.29 (t, *J* = 7.4 Hz, 2H), 1.95 (s, br, 1H), 1.65 (quin, *J* = 7.4 Hz, 2H), 1.57 (quin, *J* = 7.4 Hz, 2H), 1.34 (quin, *J* = 8.3 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 3H) ppm.

^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 173.7, 167.6, 60.4, 50.3, 47.9, 46.8, 43.3, 34.3, 26.8, 26.5, 24.8, 14.4 ppm.

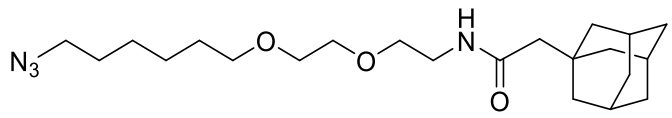
HRMS (ESI): *m/z* calcd for $C_{12}H_{22}N_2O_3$+H^+: 243.1703 [*M*+H]$^+$; found: 243.1712.

UV/VIS (DCM): λ_{max} = 249, 227 nm.

IR (Film): $\tilde{\nu}$ = 3470, 3296, 2936, 2863, 1732, 1634, 1499, 1459, 1433, 1374, 1345, 1180, 1159, 1032, 857, 753, 664 cm^{-1}.

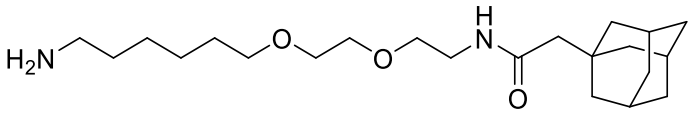
R_f: 0.28 (9 % MeOH in DCM).

2-((3*r*,5*r*,7*r*)-Adamantan-1-yl)-*N*-(2-(2-((6-azidohexyl)oxy)ethoxy)ethyl)acetamide (**6**)



Synthesis of **6** was carried out as described.[4]

2-((3*r*,5*r*,7*r*)-Adamantan-1-yl)-*N*-(2-(2-((6-aminohexyl)oxy)ethoxy)ethyl)acetamide (**5**)



To a solution of azide **6** (139 mg, 0.344 mmol, 1.0 eq.) and seven drops H_2O in degassed THF, Ph_3P (117 mg, 0.448 mmol, 1.3 eq.) was added. The reaction mixture was stirred for 2 h at room temperature and the solvent was removed under reduced pressure. The residue was purified by column chromatography (8 % MeOH and 1 % $EtMe_2N$ in DCM) to obtain **5** as a pale yellow oil.

Yield: 137 mg (quant.).

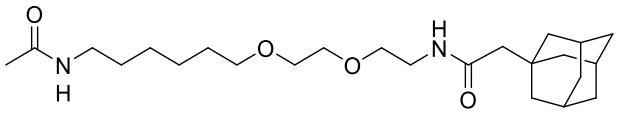
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.48 (s, br, 2H), 6.09 (s, br, 1H), 3.63 – 3.58 (m, 2H), 3.58 – 3.53 (m, 4H), 3.47 – 3.39 (m, 4H), 2.96 (t, *J* = 8.7 Hz, 2H), 1.96 (s,, br, 3H), 1.94 (s, 2H), 1.88 (s, 3H), 1.81 (q, *J* = 7.6 Hz, 2H), 1.73 – 1.55 (m, 13H), 1.44 – 1.35 (m, 2H) ppm.

^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 71.3, 70.3, 70.1, 51.8, 42.7, 39.9, 39.2, 36.9, 32.9, 29.3, 28.8, 27.5, 26.4, 25.6 ppm.

HRMS (ESI): *m/z* calcd for $C_{22}H_{40}N_2O_3$+H^+: 381.3112 [*M*+H]$^+$; found: 381.3112.

R_f: 0.29 (8 % MeOH and 1 % $EtMe_2N$ in DCM).

N-(2-(2-((6-acetamidohexyl)oxy)ethoxy)ethyl)-2-((3*r*,5*r*,7*r*)-adamantan-1-yl)acetamide (**7**)



A solution of amine **5** (24 mg, 0.063 mmol, 1.0 eq.), Et_3N (10.5 µL, 0.078 mmol, 1.2 eq.) and acetic anhydride (6.6 µL, 0.069 mmol, 1.1 eq.) in dry DCM was stirred for 2 h at room temperature. The mixture was washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$ solution and H_2O twice. The organic layer was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography (4 % MeOH in DCM) to yield **7** as a colorless solid.

Yield: 13 mg (49 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.97 (s, br, 1H), 5.63 (s, br, 1H), 3.67 – 3.61 (m, 2H), 3.61 – 3.55 (m, 4H), 3.51 – 3.43 (m, 4H), 3.29 – 3.21 (m, 2H), 1.99 (s, 3H), 1.98 (s, br, 3H), 1.95 (s, 2H), 1.76 – 1.57 (m, 14H), 1.52 (quin, *J* = 7.2 Hz, 2H), 1.43 – 1.32 (m, 4H) ppm.

^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 171.2, 170.2, 71.4, 70.4, 70.2, 70.1, 51.9, 42.8, 39.7, 39.2, 36.9, 32.9, 29.7, 29.6, 28.8, 26.8, 25.9, 23.5 ppm.

HRMS (ESI): *m/z* calcd for $C_{24}H_{42}N_2O_4+H^+$: 423.3217 $[M+H]^+$; found: 423.3210.

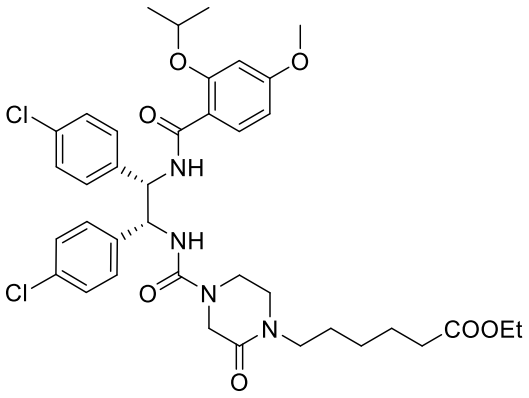
UV/VIS (DCM): λ_{max} = 228 nm.

IR (KBr): $\tilde{\nu}$ = 3444, 3272, 2928, 2902, 2848, 1639, 1559, 1450, 1372, 1293, 1139 cm^{-1}.

R_f: 0.28 (4 % MeOH in DCM).

Mp: 85 - 87 °C.

Ethyl 6-(4-(((1*R*,2*S*)-1,2-bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxybenzamido)ethyl) carbamoyl)-2-oxopiperazin-1-yl)hexanoate (**8**)



A solution of amine **5** (194 mg, 0.410 mmol, 1.0 eq.) and CDI (80 mg, 0.492 mmol, 1.2 eq.) in 15 mL dry DCM was stirred for 2 h at room temperature. Ethyl hexanoate **4** (157 mg, 0.647 mmol, 1.6 eq.) was added and the reaction mixture was stirred for 14 h at room temperature. The solution was washed two times with water and the organic phase was dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the residue was purified by column chromatography (2 % MeOH in DCM) to provide **8** as a colorless solid.

Yield: 261 mg (86 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.35 (d, *J* = 7.8 Hz, 1H), 8.28 (d, *J* = 8.9 Hz, 1H), 7.65 (d, *J* = 5.0 Hz, 1H), 7.31 – 7.26 (m, 2H), 7.20 – 7.13 (m, 2H), 6.98 – 6.92 (m, 2H), 6.91 – 6.85 (m, 2H), 6.62 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.45 (d, *J* = 2.3, 1H), 5.77 (dd, *J* = 7.9, 2.5 Hz, 1H), 5.11 (s, 1H), 4.65 (hept, *J* = 6.2 Hz, 1H), 4.17 – 4.06 (m, 4H), 3.85 (s, 3H), 3.79 – 3.67 (m, 1H), 3.64 – 3.52 (m, 1H), 3.41 (t, *J* = 7.4 Hz, 2H), 3.37 – 3.31 (m, 2H), 2.30 (t, *J* = 7.4 Hz, 2H), 1.72 – 1.51 (m, 4H), 1.41 – 1.29 (m, 2H), 1.5 (t, *J* = 7.2 Hz, 3H), 1.20 (d, *J* = 6.0 Hz, 3H), 1.14 (d, *J* = 6.0 Hz, 3H) ppm.

^{13}C-APT-NMR (75 MHz, $CDCl_3$): δ = 173.7, 167.4, 165.2, 164.2, 157.4, 156.1, 136.7, 136.6, 134.7, 134.1, 133.5, 129.6, 128.8, 128.6, 128.2, 113.7, 105.6, 100.5, 71.6, 62.0, 60.4, 58.0, 55.8, 47.9, 47.1, 46.5, 40.5, 34.3, 26.9, 26.5, 24.8, 22.1, 21.7, 14.4 ppm.

HRMS (ESI): *m/z* calcd for $C_{38}H_{46}Cl_2N_4O_7+H^+$: 741.2816 $[M+H]^+$; found: 741.2844.

UV/VIS (DCM): λ_{max} = 287, 258, 228 nm.

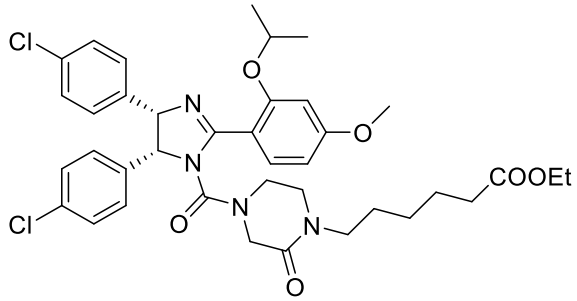
IR (Film): $\tilde{\nu}$ = 3018, 2925, 2850, 1645, 1604, 1531, 1493, 1215, 756, 667 cm^{-1}.

R_f: 0.30 (3 % MeOH in DCM).

Mp: 127 - 128 °C.

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{23}}$: +173 ° (0.53 mg/100 mL, $CHCl_3$).

Ethyl 6-(4-((4*S*,5*R*)-4,5-bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)-2-oxopiperazin-1-yl)hexanoate (**9**)



Trifluoromethanesulfonic anhydride (145 µL, 0.860 mmol, 2.5 eq.) was added to an ice-cooled solution of Ph_3PO (479 mg, 1.72 mmol, 5.0 eq.) in 5 mL dry DCM and the mixture was stirred for 20 min. Diamide **8** (255 mg, 0.344 mmol, 1.0 eq.) was dissolved in 5 mL dry DCM and added to the reaction mixture. The solution was stirred for 15 h at 45 °C and quenched with saturated aqueous $NaHCO_3$ solution. The mixture was extracted three times with DCM, dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography (20 % hexane in ethyl acetate – 100 % ethyl acetate) to yield **9** as a colorless solid.

Yield: 249 mg (quant.).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.58 (d, *J* = 8.5 Hz, 1H), 7.08 (d, *J* = 8.1 Hz, 2H), 7.03 (d, *J* = 8.2 Hz, 2H), 6.92 (d, *J* = 8.2 Hz, 2H), 6.86 (d, *J* = 8.1 Hz, 2H), 6.56 (dd, *J* = 8.5, 2.3 Hz,

1H), 6.46 (d, *J* = 2.3 Hz, 1H), 5.61 (d, *J* = 9.9 Hz, 1H), 5.51 (d, *J* = 9.9 Hz, 1H), 4.61 (hept, *J* = 6.1 Hz, 1H), 4.12 (q, *J* = 7.1, 2H), 3.85 (s, 3H), 3.76 (d, *J* = 17.8 Hz, 1H), 3.64 (d, *J* = 17.8 Hz, 1H), 3.48 – 3.39 (m, 1H), 3.32 – 3.20 (m, 2H), 3.20 – 3.10 (m, 1H), 2.93 (t, *J* = 5.4 Hz, 2H), 2.27 (t, *J* = 7.4 Hz, 2H), 1.61 (quin, *J* = 7.5 Hz, 2H), 1.39 (quin, *J* = 7.5 Hz, 2H), 1.38 (d, *J* = 6.0 Hz, 3H), 1.35 (d, *J* = 6.0 Hz, 3H), 1.29 – 1.21 (m, 5H) ppm.

^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 173.6, 164.1, 163.5, 161.0, 157.3, 154.2, 135.6, 134.6, 133.5, 133.2, 132.5, 129.3, 128.7, 128.3, 128.2, 104.8, 100.2, 71.3, 69.3, 60.4, 55.7, 49.9, 46.9, 45.5, 42.3, 34.2, 29.9, 26.7, 26.4, 24.7, 22.3, 22.2, 14.4 ppm.

HRMS (ESI): *m/z* calcd for $C_{38}H_{44}Cl_2N_4O_6$+H^+: 723.2711 [*M*+H]$^+$; found: 723.2727.

UV/VIS (DCM): λ_{max} = 327, 285, 229 nm.

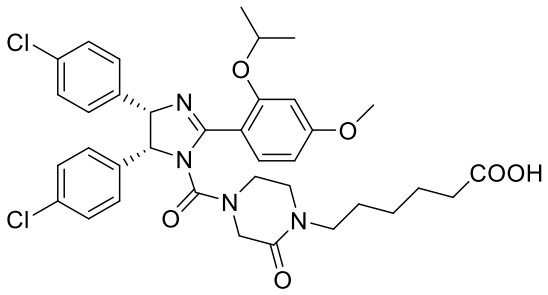
IR (Film): $\tilde{\nu}$ = 3438, 2978, 2917, 2848, 1731, 1652, 1605, 1493, 1411, 1385, 1278, 1231, 1204, 1167, 1143, 1109, 1091, 1035, 987, 879, 803, 757, 638, 586, 505, 449, 425, 403 cm^{-1}.

R_f: 0.31 (ethyl acetate).

Mp: 73 - 75 °C.

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{23}}$: -71 ° (0.68 mg/100 mL, $CHCl_3$).

6-(4-((4*S*,5*R*)-4,5-Bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)-2-oxopiperazin-1-yl)hexanoic acid (**10**)



Ethyl hexanoate **9** (249 mg, 0.344 mmol) was stirred in 0.5 M aqueous KOH in isopropanol for 2 h at room temperature. The organic solvent was removed under reduced pressure and the residue was diluted in DCM and H_2O. The solution was acidified with 1 M aqueous HCl to pH 6 and extracted several times with DCM. The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield **10** as a colorless solid.

Yield: 211 mg (88 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.57 (d, *J* = 8.5 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 2H), 7.00 (d, *J* = 8.5 Hz, 2H), 6.94 (d, *J* = 8.5 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 6.52 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.46 (d, *J* = 2.2 Hz, 1H), 5.54 (d, *J* = 9.8 Hz, 1H), 5.48 (d, *J* = 9.6 Hz, 1H), 4.60 (hept, *J* = 6.0 Hz, 1H), 3.79 (s, 3H), 3.73 (d, *J* = 17.7 Hz, 1H), 3.58 (d, *J* = 17.7 Hz, 1H), 3.43 – 3.31 (m, 1H), 3.20 – 3.04 (m, 3H), 2.82 (t, *J* = 6.3 Hz, 2H), 2.00 (t, *J* = 7.5 Hz, 2H), 1.45 (t, *J* = 7.4 Hz, 2H), 1.38 – 1.26 (m, 8H), 1.19 – 1.08 (m, 2H) ppm.

^{13}C-APT-NMR (75 MHz, $CDCl_3$): δ = 181.3, 164.7, 163.1, 160.3, 157.2, 154.9, 136.3, 135.5, 133.2, 133.0, 132.4, 129.5, 128.7, 128.2, 128.1, 113.7, 104.8, 100.2, 72.1, 71.0, 69.3, 55.7, 49.6, 46.6, 45.5, 42.6, 37.5, 26.5, 26.4, 25.7, 22.3, 22.2 ppm.

HRMS (ESI): *m/z* calcd for $C_{36}H_{40}Cl_2N_4O_6$+H^+: 695.2398 [*M*+H]$^+$; found: 695.2386.

UV/VIS (DCM): λ_{max} = 287, 252, 233 nm.

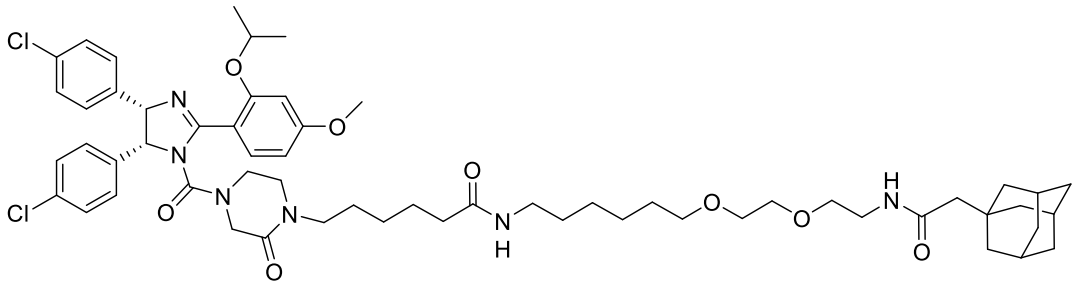
IR (Film): $\tilde{\nu}$ = 3417, 2979, 2928, 2849, 1660, 1605, 1575, 1493, 1410, 1345, 1203, 1091, 1015, 879, 802, 753, 594, 449, 402 cm^{-1}.

R_f: 0.34 (5 % MeOH in DCM).

Mp: 133 - 134 °C.

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{23}}$: -84 ° (0.66 mg/100 mL, $CHCl_3$).

N-(6-(2-(2-(2-((3*r*,5*r*,7*r*)-adamantan-1-yl)acetamido)ethoxy)ethoxy)hexyl)-6-(4-((4*S*,5*R*)-4,5-bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)-2-oxopiperazin-1-yl)hexanamide (**2**)



11 (17 mg, 0.024 mmol, 1.0 eq.), HATU (11 mg, 0.029 mmol, 1.2 eq.) and DIPEA (14 µL, 0.083 mmol, 3.4 eq.) were dissolved in 0.5 mL dry DMF and the reaction mixture was stirred for 1 h at room temperature. A solution of amine **5** (13 mg, 0.025 mmol, 1.0 eq.) in 0.5 mL DMF was added and the mixture was stirred for 19 h at room temperature. The solvent was removed under reduced pressure and the residue was taken up in DCM and H_2O. The aqueous phase was extracted three times with DCM and the combined organic layers were dried over Na_2SO_4 and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography (3 % MeOH in DCM) to obtain **2** as a colorless oil.

Yield: 14 mg (54 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.58 (d, *J* = 8.5 Hz, 1H), 7.11 – 7.06 (m, 2H), 7.05 – 6.99 (m, 2H), 6.97 – 6.92 (m, 2H), 6.89 – 6.84 (m, 2H), 6.54 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.47 (d, *J* = 2.2 Hz, 1H), 5.90 (s, br, 1H), 5.57 (d, *J* = 9.8 Hz, 1H), 5.54 – 5.52 (m, 1H), 5.48 (d, *J* = 9.8 Hz, 1H), 4.61 (hept, *J* = 6.0 Hz, 1H), 3.84 (s, 3H), 3.78 – 3.65 (m, 2H), 3.62 – 3.58 (m, 2H), 3.58 – 3.53 (m, 4H), 3.47 – 3.36 (m, 5H), 3.32 – 3.07 (m, 5H), 2.92 (t, *J* = 5.4 Hz, 2H), 2.12 (t, *J* = 7.5 Hz, 2H), 1.96 (s, br, 3H), 1.92 (s, 2H), 1.73 – 1.66 (m, 4H), 1.66 – 1.55 (m, 12H), 1.53 – 1.40 (m, 4H), 1.40 – 1.30 (m, 10H), 1.28 – 1.21 (m, 2H) ppm.

^{13}C-APT-NMR (101 MHz, $CDCl_3$): δ = 172.9, 171.2, 164.4, 157.2, 135.1, 133.3, 133.0, 132.4, 129.4, 128.6, 128.2, 128.1, 104.7, 100.2, 71.4, 71.1, 70.4, 70.2, 70.1, 69.3, 55.7, 51.9, 49.9, 46.8, 45.5, 42.8, 42.3, 39.5, 39.2, 36.9, 36.5, 32.9, 29.7, 29.6, 28.8, 26.8, 26.6, 26.4, 25.9, 25.3, 22.2 ppm.

HRMS (ESI): *m/z* calcd for $C_{58}H_{78}Cl_2N_6O_8+H^+$: 1057.5331 $[M+H]^+$; found: 1057.5328.

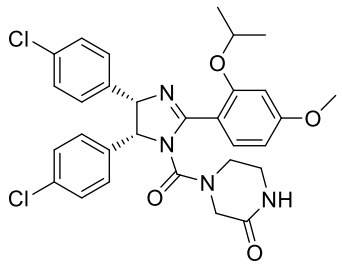
UV/VIS (DCM): λ_{max} = 286, 251, 230 nm.

IR (KBr): $\tilde{\nu}$ = 3434, 2927, 2848, 1645, 1510, 1493, 1464, 1411, 1247, 1204, 1111, 1091, 1034, 1015, 845, 731 cm^{-1}.

R_f: 0.15 (3 % MeOH in DCM).

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{23}}$: -63 ° (2.0 mg/100 mL, $CHCl_3$).

4-((4*S*,5*R*)-4,5-bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydro-1H-imidazole-1-carbonyl)piperazin-2-one (Nutlin-3a, **1**)



Nutlin-3a (**1**) was synthesized from **3** according to the procedure described by Johnston *et al*.[1]

Fluorescence polarization assays

The test compounds were analyzed in fluorescence polarization-based binding assays for their ability to inhibit binding of wild-type (wt) MDM2 (amino acids 1-119) to the 5-carboxyfluorescein (CF)-labeled peptide 5-CF-RFMDYWEGL-OH[5] derived from p53. The final concentration of buffer components used was 10 mM Tris/HCl pH 8.0, 50 mM NaCl, 1 mM EDTA, 0.1 % NP-40 substitute, 1 mM TCEP (as hydrochloride), and 2 % DMSO. Dilution series of the respective test compound in buffer were incubated with the protein (final concentration: 20 nM) for 60 min at room temperature, followed by addition of the fluorescent peptide (final concentration: 10 nM). 75 min after addition of the fluorescent peptide, fluorescence polarization (excitation: 485 nm, emission: 535 nm) was measured using a Tecan Infinite F500 384-well microplate reader. Data were analyzed using OriginPro 2017G software. Fluorescence polarization values were converted to % inhibition via the binding curve between fluorescent peptide and protein. Compound concentrations at which 50 % inhibition was observed are given as IC_{50} values. IC_{50} values were converted to K_i values using the published equation (K_d = 18.2 nM).[6]

Cell culture

HCT-116 cells were obtained from the DSMZ (Braunschweig, Germany), and were cultured in McCoy's 5A (Modified) Medium (Gibco Life Technologies), containing 10 % (v/v) fetal bovine serum and 1 % (v/v) penicillin/streptomycin (Gibco Life Technologies), at 37 °C, 5 % CO_2, and 95 % humidity.

Compound treatment and cell lysates

HCT-116 cells (3 x 10^5 cells per well) were seeded into 6-well plates (Corning). After adherence for 24 h, cells were treated with test compound at the indicated concentrations or DMSO (final DMSO concentration: 0.1 %) for 24 h. After compound incubation time, cells were washed twice with cold phosphate buffered saline (PBS) and lysed with lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 10 mM $Na_4P_2O_7$, 10 % glycerol, 1 % Triton X-100, 1 mM EDTA, with protease inhibitors 1 mM PMSF and 100 ng/ml aprotinin added freshly prior to use). Cell lysates were snap frozen in liquid nitrogen and stored at -80 °C. The protein concentration of the cell lysates was determined using a Bicinchoninic Acid (BCA) assay (Pierce™BCA Protein Assay Kit, Thermo Fisher Scientific) following the manufacturer‘s instructions.

Western Blot

Cell lysates (total protein amount of 40 µg) were separated on a 10 % polyacrylamide gel under denaturing conditions and transferred to a nitrocellulose membrane. Cellular levels of HDM2, p53, and p21 were assessed using rabbit monoclonal antibodies against the respective protein (1:1000, Cell Signaling) and reblotted with β-actin as loading control (1:1000, Cell Signaling). Membranes with bound primary antibody were incubated with secondary antibody swine anti-rabbit HRP (1:3000, Dako) and ECL was performed using Western Lightning Plus chemiluminescence reagent (Perkin-Elmer). Visualization was carried out using an ImageQuant digital imaging system (GE Healthcare) and quantitation was carried out using ImageJ software (NIH).[7] Experiments were carried out in triplicate.

Cell viability assay

HCT-116 cells (8 x 10^3 cells per well for 24 h incubation time; 4 x 10^3 cells per well for 48 h incubation time; 5 x 10^2 cells per well for 120 h incubation time) were seeded in 96-well plates (Corning). After adherence for 24 h, cells were treated with test compound at the indicated concentrations or DMSO (final DMSO concentration: 0.1 %) for 24 h, 48 h, or 120 h. Subsequently, 10 µl WST-1 solution (Roche, 1:10 dilution with cell culture medium) was added to each well, and incubated for 1 h. The absorbance at 450 nm was measured, using absorbance at 650 nm as a reference. Experiments were carried out in triplicate.

Apoptosis Assay

HCT-116 cells (7 x 10^4 cells per well) were seeded in 24-well plates (Corning). After adherence for 24 h, cells were treated with test compound at the indicated concentrations or DMSO (final DMSO concentration: 0.1 %) for 24 h. After compound incubation time, the supernatant from each well was collected, and cells were washed twice with warm PBS. Subsequently, cells were incubated and detached with Accutase (BD Bioscience) at 37 °C for 5 min. The supernatants were then returned to each well to neutralize the Accutase solution and the cell suspensions were centrifuged at 3,000 rpm, at 4 °C for 5 min. The cells were washed twice with cold PBS and centrifuged again. Subsequently, cells were stained using the FITC Annexin V Apoptosis Detection Kit I (BD Bioscience). Cells were resuspended in 1 x binding buffer and incubated with FITC annexin V and propidium iodide at 4 °C for 30 min. Apoptosis was measured using a LSR II flow cytometer (BD Bioscience) within 1 h. Experiments were carried out in quadruplicate.

Supplementary references

[1] T. A. Davis, A. E. Vilgelm, A. Richmond, J. N. Johnston, *J. Org. Chem.* **2013**, *78*, 10605-10616.

[2] N. Kornblum, H. O. Larson, R. K. Blackwood, D. D. Mooberry, E. P. Oliveto, G. E. Graham, *J. Am. Chem. Soc.* **1956**, *78*, 1497-1501.

[3] J. M. Kane, A. A. Carr, *Tetrahedron Lett.* **1980**, *21*, 3019-3020.

[4] S. Rubner, A. Scharow, S. Schubert, T. Berg, *Angew. Chem. Int. Ed.* **2018**, *57*, 17043-17047.

[5] D. J. Parks, L. V. Lafrance, R. R. Calvo, K. L. Milkiewicz, V. Gupta, J. Lattanze, K. Ramachandren, T. E. Carver, E. C. Petrella, M. D. Cummings, D. Maguire, B. L. Grasberger, T. Lu, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 765-770.

[6] Z. Nikolovska-Coleska, R. Wang, X. Fang, H. Pan, Y. Tomita, P. Li, P. P. Roller, K. Krajewski, N. G. Saito, J. A. Stuckey, S. Wang, *Anal. Biochem.* **2004**, *332*, 261-273.

[7] C. A. Schneider, W. S. Rasband, K. W. Eliceiri, *Nat. Methods* **2012**, *9*, 671-675.

NMR spectra

^{1}H and ^{13}C-APT-NMR of **2**