Supplementary Material for the Manuscript - Synthesis of new DPP-4 inhibitors based on a novel tricyclic scaffold

Carolin Schwehm, Jin Li, Hongmei Song, Xiao Hu, Barrie Kellam, Michael Stocks

Contents

General Methods All reactions with air- and humidity- sensitive reagents were carried out under an atmosphere of nitrogen. The flasks were flushed with nitrogen. The liquids were added using plastic syringes. All starting materials, reagents and solvents were purchased from commercial suppliers e.g. Acros, Sigma Aldrich, Fluorochem, KeyOrganics and Merck. All solvents were either analytical reagent or HPLC grade and were supplied by Fisher Scientific. Dry solvents were used for all reactions and were purchased from Sigma Aldrich and VWR. The petrolether had a boiling point of 40 to 60°C. $CDCl_3$ was supplied by Cambridge Isotope Laboratories, Inc, $MeOD_4$ was supplied by Sigma Aldrich. All reactions were monitored for completion using TLC. Therefore commercially available pre-coated silica gel 60 F_{254} aluminium plates from Merck were used. Visualization of the spots was carried using the UV-lamp (λ=254 nm) and stained with $KMnO_4$ or iodine and subsequently heated. Flash column chromatography was carried out using silica gel, technical grade, pore size 60Å, 230-400 mesh particle size, 40-60 μm particle size purchased from Sigma Aldrich. The ^{13}C- and ^{1}H-NMR spectra were recorded using a *Bruker AV (II) 500* spectrometer with a magnetic field strength of 9.4T. This is corresponding to a resonance frequency of 400 MHz for protons and around 100 MHz for the ^{13}C nucleus. All ^{13}C-NMR are ^{1}H-broadband-decoupled. The chemical shifts δ are given in [ppm] and all coupling constants *J* are given in [Hz]. The spectra are referenced to the signal of the deuterated solvent: $CDCl_3$ (δ_H= 7.26, δ_C= 77.16 ppm) or $MeOD_4$ (δ_H= 3.31, δ_C= 49.00 ppm). The following abbreviations were used to describe signal shapes and multiplicity: s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublet), ddd (doublet of doublet of a doublet), dddd (doublet of a doublet of a doublet of a doublet), t (triplet), dt (doublet of a triplet), q (quartet), dq (doublet of a quartet) and m (multiplet). Furthermore 2D-NMR experiments: COSY and HSQC were used for the assignment of the signals and the processing of the NMR data was carried out using the NMR software TopSpin 3.0. All high-resolution mass spectra (HRMS)- time to flight electrospray were recorded on a Waters 2795 spectrometer by electrospray ionization (TOF ES) and the LC-MS spectra were performed on a Shimadzu UFLCXR system coupled to an Applied Biosystems API2000, and visualized at 220 nm (channel 2) and 254 nm (channel 1). LC-MS was carried out using a Phenomenex Gemini-NX C18 110A, column (50 mm x 2 mm x 3μm) at a flow rate 0.5 ml/min over a 5 min period. RP-HPLC was performed using a Waters 2767 sample manager, Waters 2525 binary gradient module, and visualised at 220 nm with a Waters 2487 dual wavelength absorbance detector. Spectra were analysed using MassLynx. Preparative HPLC was performed using a Phenomenex Gemini-NX 5u C18 110A, AXIA packed column (160 mm x 21.2 mm) at a flow rate of 20 mL/min,

typically starting with 90% water/ 10% acetonitrile and progressing to 100% acetonitrile over 40 min. The water phase contained 0.01% ammonia. Preparative HPLC gradient method reported doesn't include requilibration time.

Docking studies

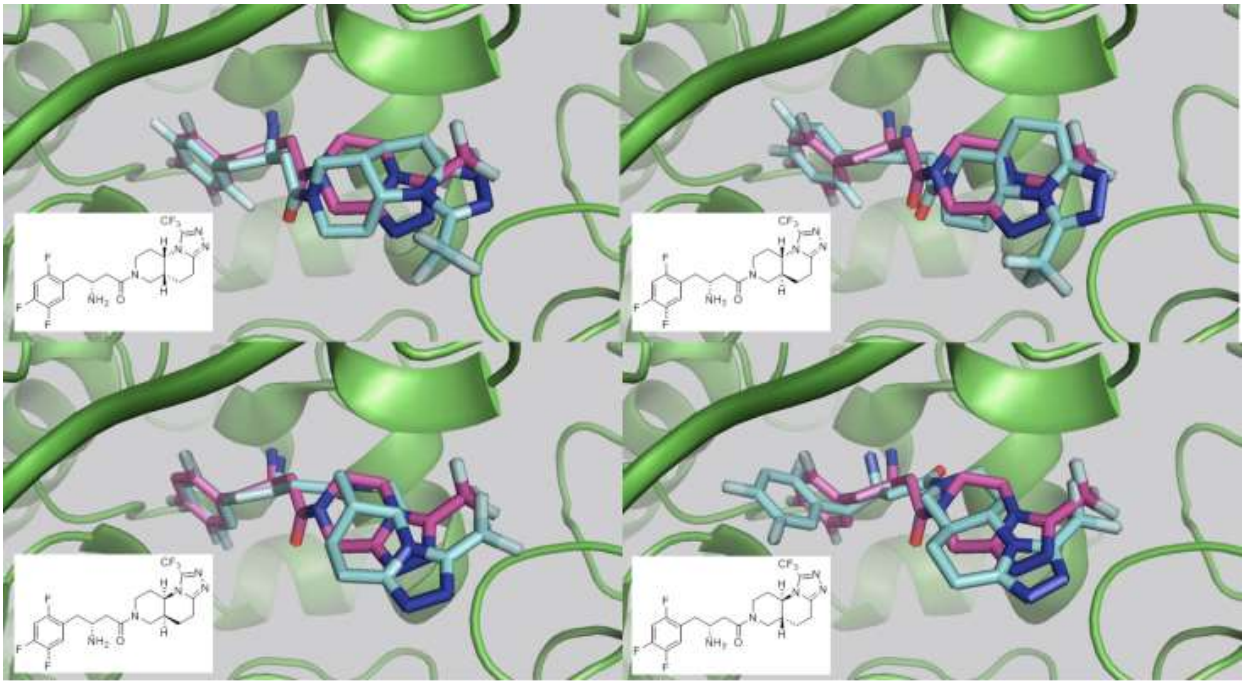
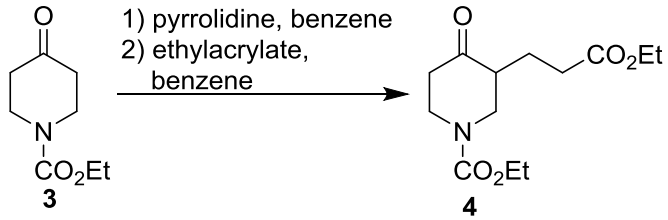


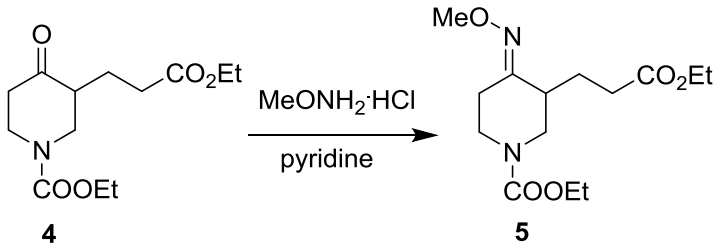
Figure s1 Compounds **2a** and **2b** as two 2 possible diastereomers (blue) docked into the DPP-4 active site (pdb code 1X70) shown overlaid with sitagliptin (magenta). The image was generated using PyMol.

Experimental Procedures



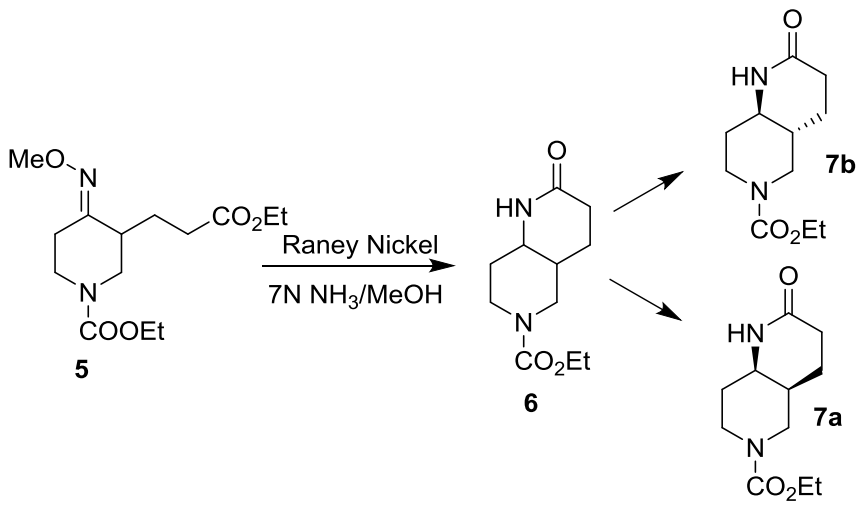
Ethyl 3-(3-ethoxy-3-oxopropyl)-4-oxopiperidine-1-carboxylate (4) To a solution of Ethyl-oxo-piperidone (**3**) (20.0 g, 17.6 mL, 116 mmol, 1.0 eq.) in benzene (240 mL) was added pyrrolidine (11.2 g, 13 mL, 158 mmol, 1.35 eq.) and refluxed over night with a Dean-Stark trap. After one night the red mixture was distilled to remove benzene, water and pyrrolidine. The excess of benzene and pyrrolidine was reduced under reduced pressure. The dark red residue was again dissolved in benzene (240 mL) and ethyl acrylate (23.2 g, 25.2 mL, 232 mmol, 2.0 eq.) and refluxed

for 20h. Afterwards water (88 mL) was added to the black red solution and the mixture was again refluxed for 2h. The phases were separated and the organic phase was dried over $MgSO_4$, filtered and the residue was evaporated under reduced pressure. The crude product was purified by flash chromatography (ethyl acetate/petrol ether: 1:4) and ethyl 3-(3-ethoxy-3-oxopropyl)-4-oxopiperidine-1-carboxylate was synthesized as a slightly yellow oil (22 g, 69%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 4.19 (q, *J*= 7.1, 14.1 Hz, 2H), 4.12 (q, *J*= 7.0, 14.0 Hz, 2H), 4.15-4.11 (m, 2H), 3.38 (ddd, *J*= 5.5, 9.0, 14.3 Hz, 1H), 3.04 (bs, 1H), 2.51-2.32 (m, 5H), 2.06 (m, 1H), 1.64-1.55 (m, 1H), 1.29 (t, *J*= 7.1 Hz, 3H), 1.25 (t, *J*= 7.2 Hz, 3H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 208.7, 173.1, 155.4, 62.1, 60.6, 49.3, 48.4, 43.9, 31.7, 22.4, 14.8, 14.3, LCMS *m/z* 272.2 $[M+H]^+$, 2.49 min.



4 **5**

Ethyl 3-(3-ethoxy-3-oxopropyl)-4-(methoxyimino) piperidine-1-carboxylate (5) To a solution of ethyl 3-(1-((ethylperoxy)-l2-methyl)-4-oxopiperidin-3-yl)propanoate (**4**) (3.0 g, 11.0 mmol, 1.0 eq.) in pyridine (21 mL) was added *O*-methylhydroxylamine hydrochloride (1.11 g, 13.3 mmol, 1.2 eq.) and the reaction was stirred at room temperature under a nitrogen atmosphere overnight. The reaction mixture was evaporated and diluted with diethyl ether (150 mL) and water (150 mL). The organic phase was washed with hydrochloric acid (1M, 60 mL) and brine (150 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure to yield ethyl 3-(3-ethoxy-3-oxopropyl)-4-(methoxyimino)piperidine-1-carboxylate (**5**) as a yellow/orange oil (2.80 g, 84%, 1:1.5 diastereomeric mixture). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 4.16-4.08 (m, 4H), 3.85 (s, 2H), 3.78 (s, 1H,), 3.61 (m, 1H), 3.39 (m, 1H), 3.23 (m, 1H), 2.92-2.67 (m, 2H), 2.43-2.21 (m, 4H), 1.95 (m, 1H), 1.82-1.72 (m, 1H), 1.26, 1.25 (2t, *J*= 7.47 Hz, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 173.2, 173.0, 157.1, 155.7, 61.7, 61.5, 61.3, 60.5, 48.4, 44.3, 42.7, 40.6, 31.9, 24.9, 24.8, 14.7, 14.3, HRMS *m/z*: $C_{14}H_{24}N_2O_5$ calculated 301.1758 $[M+H]^+$, found 301.1699.

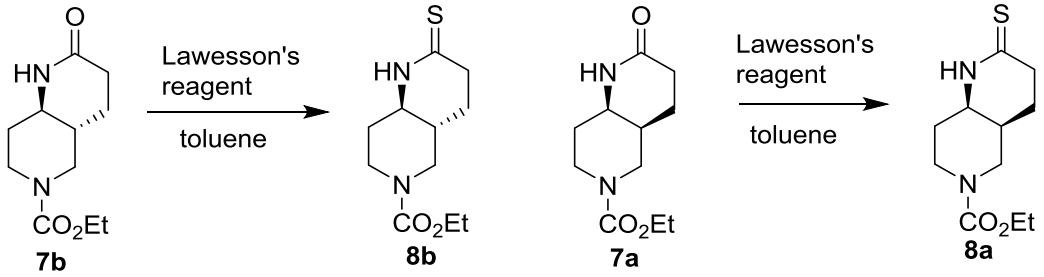


5 **6**

Ethyl 2-oxooctahydro-1,6-naphthyridine-6(2H)-carboxylate (6) To a stirred solution of ethyl 3-(3-ethoxy-3-oxopropyl)-4-(methoxyimino)piperidine-1-carboxylate (**5**) (200 mg, 0.66 mmol,

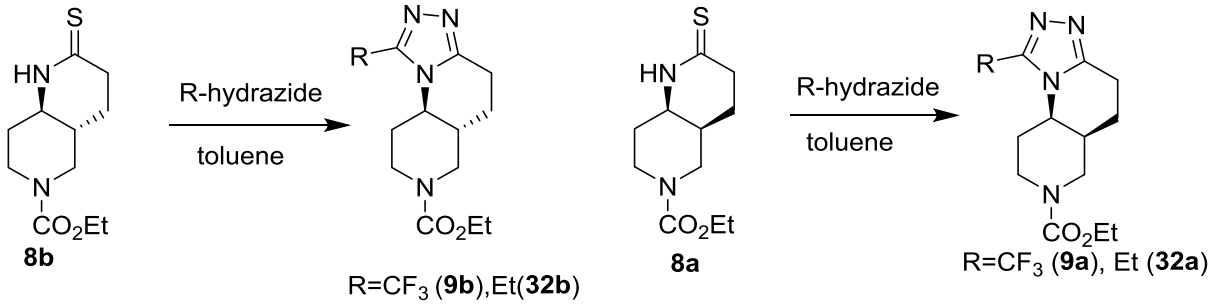
1.0 eq.) in methanolic NH_3 (7N, 5 mL) was added Raney nickel (50 mg, 50% slurry in water) and the resulting mixture was stirred under an hydrogen atmosphere overnight. The reaction mixture was filtered through Celite and the filter cake was washed with methanol (3 x 10 mL). The solvent was evaporated under reduced pressure and the crude product was purified by flash chromatography (ethyl acetate / petrol ether / methanol 9:1:2) to yield the 2 isomers of ethyl 2-oxooctahydro-1,6-naphthyridine-6(2*H*)-carboxylate (**6**) : isomer 1: 40 mg, 26%; isomer 2: 90 mg, 60%, combined yield: 130 mg, 86%, both as colorless oils). 1. isomer (**7b**) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 6.20 (bs, 1H), 4.24, (bm, 2H), 4.12 (q, *J*= 7.6, 14.8 Hz, 2H), 3.06 (dddd, *J*= 4.0, 9.8, 12.3 Hz, 1H), 2.81-2.74 (m, 1H), 2.52-2.34 (m, 3H), 1.83-1.76 (m, 2H), 1.55-1.41 (m, 3H), 1.24 (t, *J*= 7.4 Hz, 3H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 172.2, 155.4, 61.7, 56.4, 47.3, 42.3, 38.9, 32.2, 30.9, 24.5, 14.7.HMRS *m/z*: $C_{14}H_{19}N_2O_3$ calculated 227.1390 $[M+H]^+$, found 227.1265.

2 isomer (**7a**) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.33 (bs, 1H), 4.11-4.04 (m, 2H), 3.57-3.51 (m, 3H), 3.40-3.36 (m, 1H), 3.26-3.20 (m, 1H), 3.15 (t, *J*= 6.9 Hz, 2H), 2.03-1.98 (m, 1H), 1.86-1.62 (m, 4H), 1.20 (t, *J*= 7.2 Hz, 3H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 172.6, 155.6, 61.7, 50.6, 44.7, 40.5, 32.7, 30.5, 28.9, 22.0, 14.9, HRMS *m/z*: $C_{14}H_{19}N_2O_3$ calculated 227.1390 $[M+H]^+$, found 227.1229.



Ethyl 2-thioxooctahydro-1,6-naphthyridine-6(2H)-carboxylate (8) To a solution of ethyl 2-oxooctahydro-1,6-naphthyridine-6(2H)-carboxylate (**7a/7b**) (80 mg, 0.35 mmol, 1.0 eq.) in toluene (1 mL) was added Lawesson's reagent (71 mg, 0.17 mmol, 0.5 eq.) and the mixture was refluxed for 20 min. The reaction mixture was evaporated under reduced pressure and purified by flash chromatography (ethyl acetate/ petrol ether: 10:1) to yield ethyl 2-thioxooctahydro-1,6-naphthyridine-6(2H)-carboxylate (**8a/8b**) as a colorless waxy oil. 1.isomer (**8b**): (90%) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 8.93 (bs, 1H), 4.24 (bs, 2H), 4.12 (q, *J*= 7.2, 14.1 Hz, 2H), 3.14-3.03 (m, 2H), 2.92-2.74 (m, 2H), 2.46 (bs, 1H), 1.95 (dq, *J*= 2.4, 5.5, 12.3 Hz, 1H), 1.77-1.72 (m, 1H), 1.58-1.36 (m, 3H), 1.23 (t, *J*= 6.9 Hz, 3H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 202.9, 155.4, 61.8, 59.0, 47.5, 42.4, 39.1, 37.9, 31.1, 24.3, 14.7; LCMS *m/z* 243.2 $[M+H]^+$, 2.16 min.

2.isomer (**8a**): (94%) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 8.34 (bs, 1H), 4.12 (dq, *J*= 2.3, 7.2, 14.4 Hz, 2H,), 3.64-3.52 (m, 3H), 3.46-3.42 (m, 1H), 3.33-3.27 (m, 1H), 2.93 (qt, *J*= 7.1, 19.6, 40.4 Hz, 2H), 2.10-2.06 (m, 1H), 1.88-1.72 (m, 4H), 1.25 (t, *J*= 7.0 Hz, 3H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 202.1, 155.7, 61.7, 53.1, 44.9, 40.6, 37.7, 31.7, 29.3, 21.4, 14.9, HRMS m/z: $C_{11}H_{19}H_2O_2S$ calculated 243.1162 [M+H]+, found 243.1003.



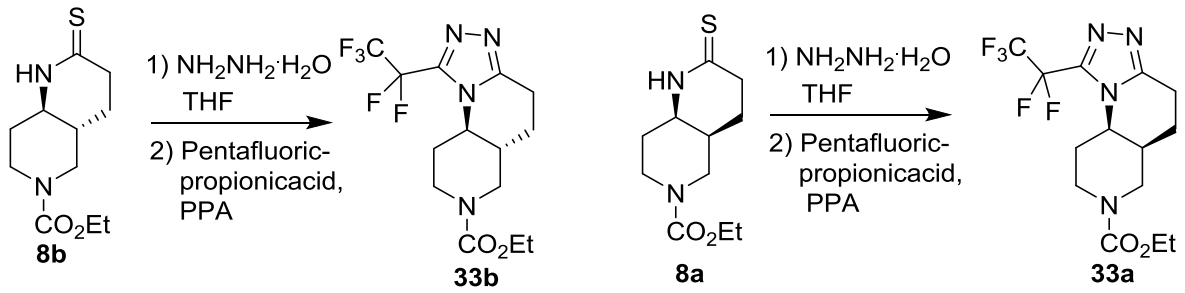
General procedure 1,2,4 triazole formation To a solution of ethyl 2-thioxooctahydro-1,6-naphthyridine-6(2H)-carboxylate (**8a/8b**) (2.43 mmol, 1 eq.) in toluene (20 mL) was added the corresponding hydrazide (4.86 mmol, 2 eq.) and the mixture was refluxed at 110 °C for two nights. The solvent was evaporated and the crude product was purified by flash chromatography (dichloromethane/ methanol 10:1) to yield triazole.

Ethyl 1-(trifluoromethyl)-4,5,5a,8,9,9a-hexahydro-[1,2,4]triazolo[4,3-a][1,6]naphthyridine-7(6H)-carboxylate: 1.isomer (**9b**): (40 %) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 4.35 (bs, 2H), 4.12 (q, *J*= 7.2, 14.0 Hz, 2H), 3.82 (dt, *J*= 3.3, 11.7 Hz, 1H), 3.30-3.24 (ddd, *J*= 1.49, 5.06, 17.15 Hz, 1H), 2.97-2.87 (m, 2H), 2.70 (bs, 1H), 2.55 (d, *J*= 11.91 Hz, 1H), 1.97 (ddt, *J*= 1.42, 6.31, 13.19 Hz, 1H), 1.87-1.78 (qq, *J*= 2.34, 12.31 Hz, 1H), 1.70 (dq, *J*= 5.05, 12.42 Hz, 1H), 1.56 (dq, *J*= 5.05, 12.84 Hz, 1H), 1.24 (t, *J*= 7.11 Hz, 3H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 155.2, 154.9, 117.9, 114.9, 62.0, 61.0, 47.6, 42.5, 41.0, 30.8, 23.5, 22.9, 14.7, HRMS *m/z*: $C_{13}H_{18}F_3N_4O_2$ calculated 319.1376 $[M+H]^+$, found 319.1276.

2.isomer (**9a**): (80 %) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 4.45 (dt, *J*= 4.3, 11.5 Hz, 1H), 4.32 (bs, 2H), 4.12 (m, 2H), 3.27 (dd, *J*= 7.4, 17.7 Hz, 1H), 3.10 (bs, 1H), 2.96 (ddd, *J*= 7.6, 12.2, 18.9 Hz, 1H), 2.84 (bs, 1H), 2.22 (m, 1H), 2.15 (dd, *J*= 5.4, 12.6 Hz, 2H), 1.97 (m, 1H), 1.79 (dq, *J*= 5.4, 12.6, 24.2 Hz, 1H), 1.25 (t, *J*= 6.9 Hz, 3H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 155.6, 153.0, 119.9 (q), 61.8, 54.3, 47.0, 42.2, 33.5, 28.9, 20.8, 18.7, 14.5, HRMS *m/z*: $C_{13}H_{18}F_3N_4O_2$ calculated 319.1376 $[M+H]^+$, found 319.1045.

Ethyl 1-ethyl-4,5,5a,8,9,9a-hexahydro-[1,2,4]triazolo[4,3-a][1,6]naphthyridine-7(6H)-carboxylate 1.isomer (**32b**): (84 %) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 4.36 (bs, 2H), 4.16 (q, *J*= 7.5, 14.5 Hz, 2H), 3.65 (td, *J*= 3.3, 10.9 Hz, 1H), 3.21 (ddd, *J*=1.6, 5.0, 16.7 Hz, 1H), 2.96-2.70 (m, 5H), 2.61 (m, 1H), 1.94 (dd, *J*= 5.7, 13.1 Hz,1H), 1.83-1.76 (m, 1H), 1.73 (dq, *J*=4.6, 12.4 Hz, 1H), 1.57 (dq, *J*=5.3, 12.9 Hz, 1H), 1.39 (t, *J*= 7.6 Hz, 3H), 1.27 (t, *J*= 7.3 Hz, 3H) ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 171.9, 155.0, 154.4, 151.7, 61.6, 59.1, 47.5, 42.2, 40.8, 30.3, 26.9, 24.1, 22.5, 21.1, 14.4, 11.3 HRMS *m/z*: $C_{14}H_{22}N_4O_2$ calculated 279.1816 $[M+H]^+$, found 279.1455.

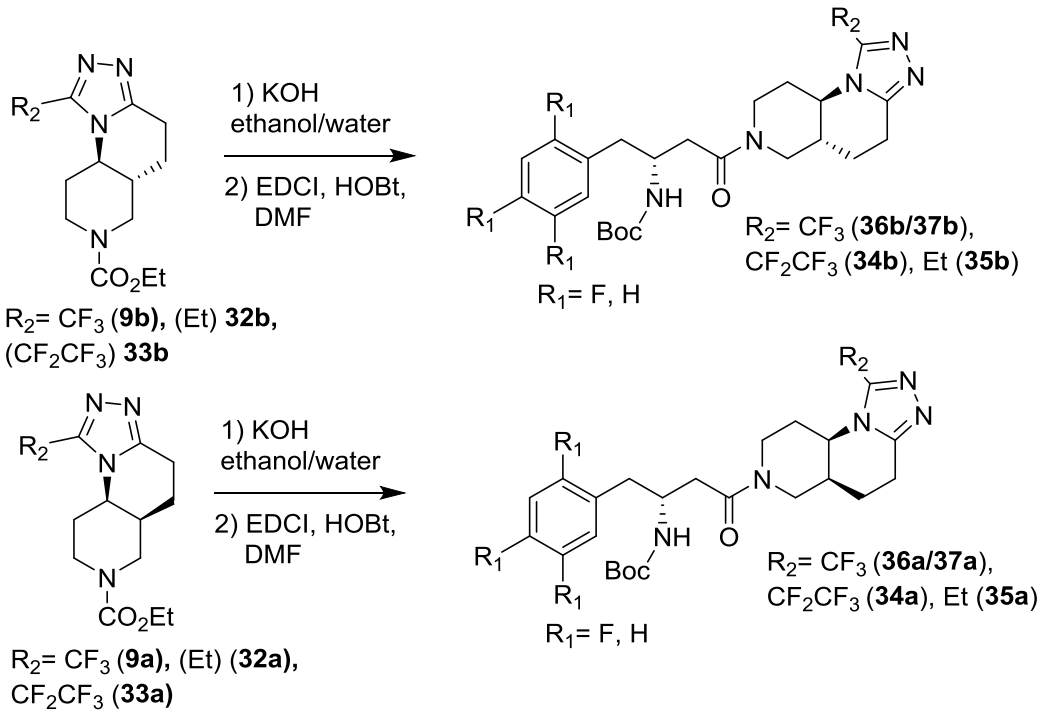
2.isomer (**32a**): (60%) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 4.29 (bs, 2H), 4.16-4.10 (m, 3H), 3.18 (dd, *J*= 6.5, 17.8 Hz, bs, 2H), 2.90 (m, 2H), 2.73 (dq, *J*=8.0, 15.4, 26.8 Hz, 2H), 2.16 (bd, *J*=12.5 Hz, 1H), 1.97 (dq, *J*= 6.25, 15.1, 25.9 Hz, 1H), 1.80 (bd, *J*= 11.6 Hz, 2H), 1.76 (dq, *J*= 5.3, 13.4 Hz, 1H), 1.43 (t, *J*= 7.1 Hz, 3H), 1.26 (t, *J*= 8.0, 3H) ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 171.1, 155.7, 153.7, 150.0, 61.8, 52.2, 47.5, 42.4, 34.1, 28.7, 21.3, 19.7, 18.4, 14.7, 11.5 HRMS *m/z*: $C_{14}H_{22}N_4O_2$ calculated 279.1816 $[M+H]^+$, found 279.2473.



Ethyl 1-(perfluoroethyl)-4,5,5a,8,9,9a-hexahydro-[1,2,4]triazolo[4,3-a][1,6]naphthyridine-7(6H)-carboxylate (33) To a solution of ethyl 2-thioxooctahydro-1,6-naphthyridine-6(2H)-carboxylate (**8a/8b**) (380 mg, 1.57 mmol, 1 eq.) in THF (16 mL) was added hydrazine hydrate (0.64 mL, 50-60%) and the mixture was heated to 60°C for one hour. The reaction was cooled to rt and the solvent was removed under an nitrogen atmosphere. To the greenish oil pentafluoro propionic acid (0.46 mL, 0.71 g, 4.37 mmol, 2.8 eq.) was added followed by polyphosphoric acid and the mixture was refluxed for 2 days at 150°C. The reaction mixture was neutralized with NH_4OH and the aqueous phase was extracted with ethyl acetate (3 x 40 mL). The combined organic phases were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography (dichloromethane/ methanol 10:1) to yield ethyl 1-(perfluoroethyl)-4,5,5a,8,9,9a-hexahydro-[1,2,4]triazolo[4,3-a][1,6]naphthyridine-7(6H)-carboxylate (**33a/33b**) as a yellowish oil.

1.isomer (**33b**): (8 %), ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 4.39 (bs, 2H), 4.15 (q, *J*= 7.60, 14.03 Hz, 2H), 3.86 (td, *J*=2.9, 11.6 Hz, 1H), 3.32 (dd, *J*= 5.2, 16.9 Hz, 1H), 3.01-2.90(m, 2H), 2.75 (bs, 1H), 2.66 (d, *J*=14.0 Hz, 1H), 2.03-1.98 (m, 1H), 1.90- 1.81 (m, 1H), 1.74 (ddd, *J*= 4.8, 12.7, 23.8 Hz, 1H), 1.59, (ddd, *J*= 4.8, 13.8, 26.1 Hz, 1H), 1.27 (t, *J*=7.1 Hz, 3H), HRMS *m/z* $C_{14}H_{17}F_5N_4O_2$ calculated 369.1344 $[M+H]^+$, found 369.1207.

2.isomer (**33a**): (34 %) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 4.45 (dt, *J*= 5.14, 12.17 Hz, 1H), 4.20 (bs, 2H), 4.07-3.99 (m, 2H), 3.16 (dd, *J*= 6.1, 18.1 Hz, 1H), 3.05 (bs, 1H), 2.87-2.79 (m, 2H), 2.19 (bd, *J*= 12.6 Hz, 1H), 2.05-1.93 (m, 2H), 1.89-1.83 (m, 1H), 1.72 (ddd, *J*= 4.4, 11.9, 23.9, 37.9 Hz, 1H), 1.15 (t, *J*=7.07 Hz, 3H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 155.5, 153.1, 141.4, 61.7, 54.7, 54.6, 50.1, 46.9, 42.2, 33.5, 29.1, 20.1, 18.5, 14.4 LC/MS *m/z*: 369.0 $[M+H]^+$.



General Method for amide coupling

To a solution of the substituted triazole (**9a/9b, 32a/32b, 33a/33b**) (0.41 mmol, 1 eq.) in ethanol (0.44 mL) and water (2.50 mL) was added KOH (1.80 mmol, 4.4 eq.) and the mixture was refluxed at 110 °C for one night. The reaction mixture was extracted with DCM (10 mL). The organic phase was dried over MgSO4, filtered and the solvent was evaporated and the crude product was used as it is. To a solution of amine (0.33 mmol, 1 eq.) in DMF (1 mL) was added (R)-3-((tert-butoxycarbonyl)amino)-4-(2,4,5-trifluorophenyl)butanoic acid (**11**)/ (R)-3-((tert-butoxycarbonyl)amino)-4-phenylbutanoic acid (**10**) (0.34 mmol, 1.05 eq), EDCI (0.38 mmol, 1.2 eq) and HOBt (0.38 mmol, 1.2 eq.) and the mixture was stirred at rt overnight. The reaction mixture was diluted with ethyl acetate (20 mL) and saturated aqueous $NaHCO_3$ (20 mL). The water phase was extracted with ethyl acetate (3 x 20 mL). The combined organic phases were washed with saturated aqueous NaCl (20 mL), dried over Na_2SO_4, filtered and the crude product was purified by flash chromatography (dichloromethane/ methanol: 10:1) to yield amide (**34a/34b, 35a/35b, 36a/36b, 37a/37b**).

tert-Butyl ((2R)-4-oxo-4-(1-(trifluoromethyl)-4,5,5a,8,9,9a-hexahydro-[1,2,4]triazolo[4,3-a][1,6]naphthyridin-7(6H)-yl)-1-(2,4,5-trifluorophenyl)butan-2-yl)carbamate1.isomer (**36b**): Purity (100%) by HPLC (t_R= 11.17 min), (91 %) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.06 (m, 1H), 6.88 (m, 1H), 5.33 (m, 1H), 4.87 (m, 1H), 4.01 (m, 1H), 3.91 (dt, *J*= 4.1, 11.3 Hz, 2H), 3.33 (m, 1H), 3.22 (m, 1H), 2.99-2.93. (m, 2H), 2.74-2.46 (m, 5H), 2.07-1.63 (m, 4H), 1.35 (s, 9H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 169.2, 157.4, 155.4, 154.9, 154.6, 150.0 (3), 147.9 (2), 147.6 (2), 145.5 (2), 143.3, 142.9, 122.0, 121.7, 120.3, 119.3, 119.1, 105.6 (2), 105.3 (2), 79.6, 60.8 (2), 49.3, 48.4, 45.2, 44.2 (2), 41.5 (2), 40.8 (2), 40.2, 36.5, 33.1, 31.3, 30.5, 28.3, 23.6, 22.7 (2), HRMS *m/z*: $C_{25}H_{30}N_5F_6O_3$ calculated 562.2247 $[M+H]^+$, found 562.2310, calculated 506.1621 $[M-t\text{Bu}]^+$, found 506.1642, calculated 462.1723 $[M\text{-Boc}]^+$, found 462.1769.

2.isomer (**36a**): Purity (100 %) by HPLC (t_R=11.20 min), (77 %), ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.03 (m, 1H), 6.85 (m, 1H), 5.42 (dt, *J*= 8.3, 40.3 Hz, 1H), 4.84-4.75 (m, 1H), 4.51 (m, 1H), 4.11-4.01 (m, 1H), 3.97-3.86 (m, 1H), 3.40 (m, 1H), 3.28-3.11 (m, 1H), 2.97-2.84 (m, 4H), 2.72-2.47 (m, 2H), 2.31 (bs, 1H), 2.14 (m, 1H), 2.02-1.72 (m, 3H), 1.34-1.31 (2m, 9H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 169.2, 155.4, 152.9, 152.5, 142.8, 119.3, 119.1, 117.7, 105.7, 105.5, 105.3, 79.7, 54.2, 48.8 (3), 48.4, 44.8, 44.2, 40.1 (2), 36.7, 36.1, 33.8 (2), 33.7 (2), 33.5, 33.0, 31.1, 29.7, 28.4, 21.1,20.9, 19.1, 18.9, HRMS *m/z*: $C_{25}H_{30}N_5F_6O_3$ calculated 562.2247 $[M+H]^+$ found 562.2108, calculated 506.1621 $[M-t\text{Bu}]^+$, found 506.1627, calculated 462.1723 $[M\text{-Boc}]^+$, found 462.1449.

tert-Butyl ((2R)-4-oxo-1-phenyl-4-(1-(trifluoromethyl)-4,5,5a,8,9,9a-hexahydro-[1,2,4]triazolo[4,3-a][1,6]naphthyridin-7(6H)-yl)butan-2-yl)carbamate

1.isomer (**37b**): Purity (100%) by HPLC (t_R= 10.53 min), (75%) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.25-7.14 (m, 5H), 5.44-5.20 (m, 1H), 4.80 (t, *J*= 16.8 Hz, 1H), 4.12-4.05 (m, 1H), 3.93-3.76 (m, 2H), 3.28-3.06 (m, 2H), 3.00-2.75 (m, 5H), 2.61-2.40 (m, 3H), 2.03-1.50 (m, 3H), 1.35, 1.34 (2s, 9H) ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 169.6, 155.5, 154.9, 138.2 (4), 129.4, 129.2, 128.5, 128.4, 126.6, 126.4, 79.4, 60.7 (4), 49.3 (2), 45.2 (2), 44.2 (2), 41.3, 40.8, 40.5, 40.3, 40.1, 28.3, 23.4, 23.3, 22.7, 22.6 HRMS *m/z*: $C_{25}H_{32}N_5F_3O_3$ calculated *5*08.2530 $[M+H]^+$, found 508.2567, calculated 452.1965 $[M-t\text{Bu}]^+$, found 452.1946, calculated 408.2006 $[M\text{-}Boc]^+$, found 408.2015.

2.isomer (**37a**): Purity (100 %) by HPLC (t_R=10.50 min), (80%) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.26-7.16 (m, 5H), 5.49 (m, 0.5 H), 5.4 (m, 0.5 H), 4.83-4.73 (m, 1H), 4.51-4.44 (m, 1H), 4.15-4.05 (m, 1H), 3.89-3.67 (m, 1H), 3.32-2.78 (m, 7H), 2.64-2.40 (m, 2H), 2.40-2.13 (m, 4H), 1.54-1.34 (m, 9H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 170.4, 155.5, 138.2, 129.4 (3), 128.6 (3), 126.6 (4), 54.1, 48.7 (2),44.7 (2), 44.1 (2), 40.1, 39.9, 33.8, 33.6 (2), 28.4, 20.9 (2),18.8 (2) HRMS *m/z*: $C_{25}H_{32}N_5F_3O_3$ calculated *5*08.2530 $[M+H]^+$, found 508.2540, calculated 452.1904 $[M-t\text{Bu}]^+$, found 452.1946.

tert-Butyl ((2R)-4-oxo-4-(1-(perfluoroethyl)-4,5,5a,8,9,9a-hexahydro-[1,2,4]triazolo[4,3-a][1,6]naphthyridin-7(6H)-yl)-1-(2,4,5-trifluorophenyl)butan-2-yl)carbamate

1 isomer (**34b**):, HPLC (t_R =19.43 min) (90 %)^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.06 (m, 1H), 6.88 (m, 1H), 5.38 (m, 1H), 4.87 (m, 1H), 4.10 (m, 1H), 4.01 (m, 1H), 3.35-3.25 (m, 2H), 3.03-2.87 (m, 5H), 2.75-2.45 (m, 3H), 2.08-1.94 (m, 1H), 1.85-1.60 (m, 3H), 1.40-1.32 (m, 9H), LC/MS 612.3 $[M+H]^+$

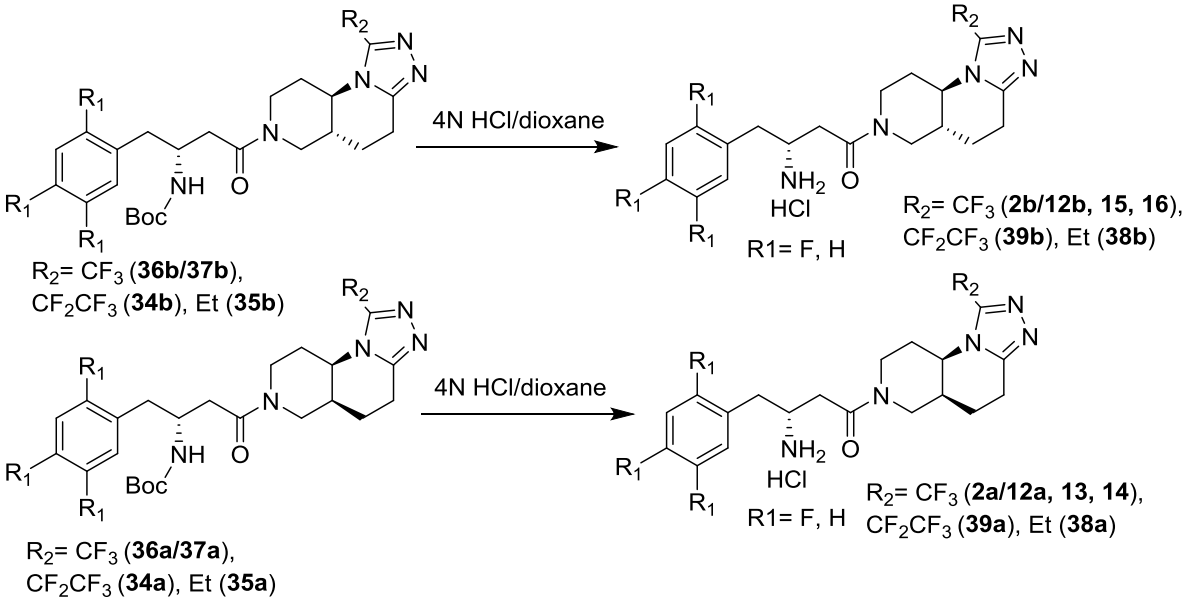
2.isomer (**34a**): HPLC (t_R =18.85 min) (98 %) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.05 (m, 1H), 6.85 (m, 1H), 5.65-5.20 (m, 1H), 4.86-4.73 (m, 0.5H), 4.59-4.48 (m, 0.5H), 4.44-4.24 (m, 0.5H), 4.17- 4.01 (m, 1.5H), 3.96-3.84 (m, 1H), 3.41 (m, 2H), 3.09-2.75 (m, 6H), 2.72-2.43 (m, 2H), 2.36-2.16 (m, 1H), 2.11-1.77 (m, 2H), 1.38-1.30 (m, 9H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 169.8, 169.6, 155.4, 155.0, 153.2, 152.8, 150.1, 147.6 (3), 141.6, 121.9, 119. 5(3), 105.6 (4), 79.6, 54.6, 48.7 (2), 44.8, 44.2 (2), 40.1 (2), 36.8, 33.7 (3), 29.8, 29.0, 28.3, 20.9 (2), 18.6, HRMS *m/z* $C_{26}H_{28}F_8N_5O_3$ calculated 612.2215 $[M+H]^+$, found 612.2228, calculated 556.1589 $[M-t\text{Bu}]^+$, found 556.1579, calculated 512.1691 $[M\text{-Boc}]^+$, found 512.1726.

tert-Butyl ((2R)-4-(1-ethyl-4,5,5a,8,9,9a-hexahydro-[1,2,4]triazolo[4,3-a][1,6]naphthyridin-7(6H)-yl)-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-yl)carbamate

1.isomer **(35b)**: (60 %) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 6.99 (m, 1H), 6.80 (m, 1H), 5.54 (m, 1H), 4.73 (2d, *J*= 14.0, 15.2 Hz, 1H), 4.03-3.83 (m, 3H), 3.69 (dt, *J*= 3.5, 11.2 Hz, 1H), 3.22- 3.06 (m, 2H),

2.94-2.37 (m, 8H), 1.92 (m, 1H), 1.75-1.40 (m, 3H), 1.26 (m, 12H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.1, 157.4, 155.4, 154.9, 154.6, 154.4, 151.8 (2), 150.0, 147.6 (2), 143. 2, 122.(2), 119.0 (3), 105.3 (4), 79.4, 59.2 (2), 49.4 92),48.2, 45.2, 44.0, 41.4, 40.8, 40.0, 33.0, 31.0, 30.9, 30.2 (2), 28.2, 24.3, 22. 6 (2), 21.2 (2), 11.4 HRMS *m/z*: $C_{26}H_{34}N_5F_3O_3$ calculated 522.2687 $[M+H]^+$, found 522.4608.

2.isomer (**35a**): (31 %) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.05-6.97 (m, 1H), 6.87- 6.79 (m, 1H), 5.60-5.43 (ddd, *J*= 9.9, 13.4, 37.4 Hz, 1H), 4.76 (d, *J*= 14.2 Hz, 0.5H), 4.70 (d, *J*= 14.2 Hz, 0.5 H), 4.20 (m, 1H), 4.07 (m, 1H), 3.89 (m, 1H), 3.37 (m, 0.5 H), 3.16 (m, 1.5H), 2.95-2.73 (m, 3H), 2.70-2.49 (m, 5H), 2.20 (m, 1H), 1.97-1.59 (m, 4H), 1.36 (t, *J*= 7.9 Hz, 3H), 1.30, 1.28 (2s, 9H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 169.6, 169.5, 157.4 (2), 155.4, 155.0, 154.9, 153.6(2), 150.0, 149.7, 149.4, 119.1, 119.1, 105.3 (4), 51.8 (2), 48.9, 45.0, 44.0, 39.9, 34.1 (2), 33.9 (2), 32.8, 29.1, 28.3, 21.0, 20.9, 19.4, 18.2, 11.4 (2) HRMS *m/z*: $C_{26}H_{34}N_5F_3O_3$ calculated 522.2687 $[M+H]^+$, found 522.2107, calculated 466.2061 $[M-t\text{Bu}]^+$, found 466.1626.



General method for deprotection To amide (**34a/34b, 35a/35b, 36a/36b, 37a/37b**) (0.08 mmol, 1 eq.) was added 4N HCl in dioxane (0.5 mL) and stirred at rt for 30 min. The HCl solution was evaporated under N_2 gas to yield HCl salt of amine (**2a/2b, 12a/12b, 13,14,15,16, 38a/38b, 39a/39b**) as oils.

(3R)-3-Amino-1-(1-(trifluoromethyl)-4,5,5a,8,9,9a-hexahydro-[1,2,4]triazolo[4,3-a][1,6]naphthyridin-7(6H)-yl)-4-(2,4,5-trifluorophenyl)butan-1-one

Compounds were separated using chiral HPLC using Diacel Chiralpak IC, 5 µm, 20x250 mm at 15 ml/min at 265 nm over 30 min. The solvents were iso-hexane containing 0.2% diethylamine and ethanol in a 30:70 ratio to give two separated compounds per isomer.

1.isomer (**2b,15, 16**): A: Purity (100 %) by HPLC (t_R= 7.87 min), ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.10-7.03 (m, 1H), 6.93-6.87 (m, 1H), 4.91-4.82 (m, 1H), 4.04 (2 d, *J*= 13.7, 14.8 Hz, 1H), 3.89 (dt, *J*= 3.8, 11.1 Hz, 1H), 3.56 (m, 1H), 3.36-3.29 (m, 1H), 3.23 (dt, *J*= 2.6, 13.9 Hz, 1H), 3.00-2.91 (m, 2H), 2.81-2.68 (m, 3H), 2.63-2.33 (m, 3H), 2.07-1.93 (m, 1H), 1.82-1.58 (m, 4H)

B: Purity (100 %) by HPLC (t_R= 15.13 min), ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.11-7.03 (m, 1H), 6.95-6.88 (m, 1H), 4.93-4.83 (m, 1H), 4.07 (d, *J*= 14.0 Hz, 1H), 3.96-3.87 (m, 1H), 3.58-3.52 (m, 1H), 3.38-3.30 (m, 1H), 3.21 (dt, *J*= 2.5, 13.3 Hz, 1H), 3.05-2.91 (m, 2H), 2.82-2.74 (m, 1H), 2.70-2.62 (m, 2H), 2.52- 2.35 (m, 3H), 2.08-1.97 (m, 1H), 1.83-1.58 (m, 4H)

^{13}C-NMR (100 MHz, $MeOD_4$) δ/ppm 169.8 (2), 169.7, 163.4, 159.2, 157.9, 156.8, 152.1 (2), 149.6, 149.3, 146.9, 144.5, 144.1, 120.7, 120.5 (2), 117.8, 107.2, 107.0, 106.9, 106.7, 73.5, 72.4, 63.1, 63.0, 62.1, 45.9, 44.7, 43.7, 41.2 (2), 40.6, 35.1 (2), 34.9 (2), 32.3, 31.3, 30.7, 22.7 (2), 22.6 LC/MS (A) m/z: 462.2 $[M+H]^+$ 1.95 min, (B) m/z: 462.2 $[M+H]^+$, 1.97 min,

2.isomer **(2a, 13, 14**): A: Purity (100 %) by HPLC (t_R= 5.78 min), ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.10-7.03 (m, 1H), 6.95-6.88 (m, 1H), 4.87 (dd, *J*= 1.8, 14.8 Hz, 1H), 4.53 (dt, *J*= 4.8, 11.3 Hz, 1H), 3.94 (t, *J*= 15.0 Hz, 1H), 3.58-3.51 (m, 1H), 3.43-3.13 (m, 2H), 3.04-2.87 (m, 1H), 2.80-2.74 (m, 2H), 2.67-2.63 (m, 1H), 2.47-2.39 (m, 1H), 2.29-2.22 (m, 2H), 2.09-2.01 (m, 2H), 1.89-1.84 (m, 2H), 1.74 (m, 2H)

B: Purity (99 %) by HPLC (t_R= 18.86 min), ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.10-7.01 (m, 1H), 6.95-6.87 (m, 1H), 4.86 (dt, *J*= 1.5, 13.4 Hz, d, *J*= 14.0 Hz, 1H), 4.52 (dt, *J*= 4.7, 14.9 Hz, 1H), 3.95 (t, *J*= 14.3 Hz, 1H), 3.59 (m, 1H), 3.49-3.10 (m, 2H), 3.04-2.93 (m, 1H), 2.87 (dd, *J*= 2.8, 13.7 Hz, 1H), 2.78 (dd, *J*= 5.7, 13.1 Hz, 1H), 2.67-2.59 (m, 1H), 2.44-2.28 (m, 3H), 2.20-1.95 (m, 3H), 1.88-1.68 (m, 3H)

^{13}C-NMR (100 MHz, $MeOD_4$) δ/ppm 170.4 (2), 170.0 (2), 159.2, 156.5, 156.3, 149.7, 149.3, 147.0, 146.9, 144.3, 143.9, 120.7, 120.5, 120.1, 117.4, 107.2 (2), 106.9, 106.7, 73.5, 72.4, 62.1, 57.1, 45.4 (2), 44.4, 43.7, 34.8, 34.0, 33.8, 32.4, 32.3, 28.9, 21.0, 18.3, 18.2, HRMS *m/z*: $C_{20}H_{22}N_5F_6O$ calculated 462.1723 $[M+H]^+$, found 462.1732. (B) m/z: 462.2 $[M+H]^+$ 1.95 min,

(3R)-3-Amino-4-phenyl-1-(1-(trifluoromethyl)-4,5,5a,8,9,9a-hexahydro-[1,2,4]triazolo[4,3-a][1,6]naphthyridin-7(6H)-yl)butan-1-one

1.isomer (**12b**): Purity (100 %) by HPLC (t_R= 7.80 min), ^{1}H-NMR (400 MHz, $MeOD_4$) δ/ppm 7.40-7.29 (m, 5H), 4.80-4.64 (m, 1H), 3.97-3.79 (m, 2H), 3.52-3.42 (m, 1H), 3.26-2.34 (m, 12H), 2.14-1.82 (m, 3H) ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 170.5 (4), 136.9 (3), 130.4 (4), 130.2 (2), 128.6, 121.1, 118.9, 55.4, 51.5 (4), 45.8, 44.6 (2), 41.1, 40.9, 39.6, 39.4 (3), 34.7, 34.6 (3), 34.2, 30.2, 30.0, 29.5, 24.2, 21.6 (4), 19.7, 19.5 (2), 19.3 HRMS *m/z*: $C_{20}H_{24}N_5F_3O$ calculated 408.2006 $[M+H]^+$, found 408.1962.

2.isomer (**12a**): Purity (100 %) by HPLC (t_R= 7.85 min), ^{1}H-NMR (400 MHz, $MeOD_4$) δ/ppm 7.39-7.26 (m, 5H), 4.80-4.70 (m, 1H), 4.19 (t, *J*= 10.3 Hz, 1H), 4.05 (dd, *J*=13.6, 26.7 Hz, 1H), 3.93-3.83 (m, 1H), 3.25-3.19 (m, 4H), 3.09-2.53 (m, 6H), 2.07-1.65 (m, 3H), ^{13}C-NMR (100 MHz, $MeOD_4$) δ/ppm 170.1, 136.8, 130.4, 130.1, 128.6, 61.8, 59.9, 51.5, 46.2, 44.7, 41.9, 41.7, 41.4, 41.3, 39.5, 34.8, 34.6, 23.9, 23.7, 23.3 LC/MS *m/z*: 408.3 $[M+H]^+$

(3R)-3-amino-1-(1-(perfluoroethyl)-4,5,5a,8,9,9a-hexahydro-[1,2,4]triazolo[4,3-a][1,6]naphthyridin-7(6H)-yl)-4-(2,4,5-trifluorophenyl)butan-1-one

1.isomer (**39b**): ^{1}H-NMR (400 MHz, $MeOD_4$) δ/ppm 7.39-7.32 (m, 1H), 7.28-7.21 (m, 1H), 4.78-4.70 (m, 1H), 4.22 (m, 1H), 4.04 (m, 1H), 3.87-3.81 (m, 1H), 3.71 (m, 1H), 3.21-3.18 (m, 1H), 3.13-2.93 (m, 6H), 2.90-2.78 (m, 2H), 2.73-2.60 (m, 2H), 2.06-1.94 (m, 1H), 1.92-1.83 (m, 1H), 1.80-1.66 (m, 1H), ^{13}C-NMR (100 MHz, $MeOD_4$) δ/ppm 169.9, 169.7, 157.3 (2), 120.6 (2), 120.4, 107.3, 107.1,

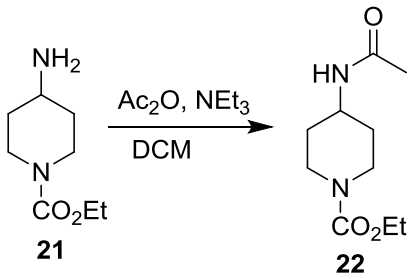
107.0, 106.9, 62.0, 46.3, 44.6, 42.3, 41.7 (2), 41.3, 35.2, 32.7, 23.9, 23.8, 23.4, LC/MS m/z 512.2 $[M+H]^+$, 2.09 min.

2.isomer (**39a**): ^{1}H-NMR (400 MHz, $MeOD_4$) δ/ppm 7.40-7.31 (m, 1H), 7.27-7.19 (m, 1H), 4.77-4.66 (m, 2H), 4.01 (m, 2H), 3.90-3.81 (m, 2H), 3.56-3.47 (m, 1H), 3.27-3.02 (m, 5H), 2.95-2.65 (m, 2H), 2.50-2.41 (m, 1H), 2.22-1.86 (m, 4H) ^{13}C-NMR (100 MHz, $MeOD_4$) δ/ppm 170.4 (2), 170.1, 165.0, 159.2 (2), 156.8, 156.6, 152.1, 151.9, 149.6, 149.5, 149.3, 147.0 (2), 147.0, 146.9, 142.9, 120.7, 120.5, 107.2, 107.1 (2), 106.7, 92.2, 73.5,72.4, 63.5, 62.1, 60.6, 57.7, 45.4, 44.5, 43.8, 40.1, 37.4, 34.8 (2), 34.0, 33.9, 32.4, 32.3, 29.8, 29.1, 21.0, 18.1 (2), 17.9, LC/MS *m/z* 512.2 $[M+H]^+$, 2.08 min.

(3R)-3-Amino-1-(1-ethyl-4,5,5a,8,9,9a-hexahydro-[1,2,4]triazolo[4,3-a][1,6]naphthyridin-7(6H)-yl)-4-phenylbutan-1-one hydrochloride

1.isomer (**38b**): Purity (100 %) by HPLC (t_R= 7.30 min), ^{1}H-NMR (400 MHz, $MeOD_4$) δ/ppm 7.31-7.23 (m, 1H), 7.18-7.10 (m, 1H), 4.71 (m, 1H), 4.28 (m, 1H), 4.11-4.00 (m, 2H), 3.87 (m, 1H), 3.38-2.62 (m, 12H), 2.01-1.59 (m, 4H), 1.35 (m, 3H) ^{13}C-NMR (100 MHz, $MeOD_4$) δ/ppm 170.2, 170.0, 156.8, 120.6, 120.5, 107.2, 107.1 (2), 106.8, 54.4, 45.6 (2), 44.5, 41.0, 40.9, 34.8, 34.7 (2), 34.2, 34.0, 32.4, 32.3, 32.2, 28.8 (2), 28.1, 20.8 (2), 18.9, 18.8 (2), 18.6, 10.2 (2) HRMS *m/z*: $C_{21}H_{26}N_5F_3O$ calculated 422.2166 $[M+H]^+$, found 422.1171.

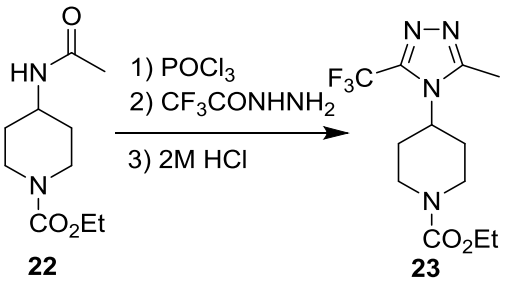
2.isomer (**38a**): Purity (100 %) by HPLC (t_R= 7.22 min), ^{1}H-NMR (400 MHz, $MeOD_4$) δ/ppm 7.43-7.34 (m, 1H), 7.28-7.19 (m, 1H), 4.81-4.65 (m, 2H), 4.10 (m, 0.5H), 3.91 (m, 0.5H), 3.89-3.79 (m, 1H), 3.29-3.19 (m, 3H), 3.14-2.45 (m, 8H), 2.37 (m, 1H), 2.17-1.81 (m, 5H), 1.45 (t, *J*= 7.3 Hz, 3H) ^{13}C-NMR (100 MHz, $MeOD_4$) δ/ppm 169.8, 169.7, 169.6, 120.6, 120.4, 107.3, 107.1, 107.0, 106.8, 61.0 (2), 46.2, 44.7, 41.6 (2), 41.2 (2), 34.9 (3), 34.7, 32.4, 31.3 (2), 30.7, 24.1, 23.8 (2), 22.7, 21.7, 11.1 (2), HRMS *m/z*: $C_{21}H_{26}N_5F_3O$ calculated 422.2166 $[M+H]^+$, found 422.1016.



Ethyl 4-acetamidopiperidine-1-carboxylate (22) To ethyl 4-aminopiperidine-1-carboxylate (**21**) (500 mg, 0.5 mL, 2.9 mmol, 1 eq.) in dichloromethane (12 mL) was added acetic acid anhydride (325 mg, 0.3 mL, 3.2 mmol, 1.1 eq.) and triethylamine (878 mg, 1.2 mL, 8.7 mmol, 3 eq.) and the reaction mixture was stirred overnight. The mixture was diluted with dichloromethane and the organic phases were washed with water. The organic phases were dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure to yield ethyl 4-acetamidopiperidine-1-carboxylate (**22**) (520 mg, 84%) as a white solide.

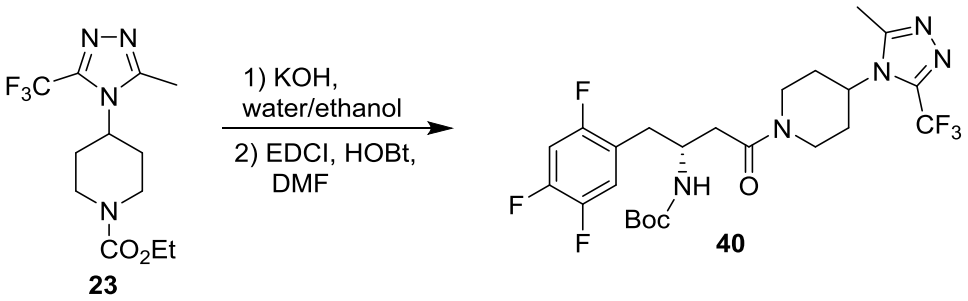
^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 5.49 (bs, 1H), 4.12 (q, *J*= 7.5, 14.6 Hz, 4H), 3.93 (m, 1H), 2.89 (t, *J*= 12.6 Hz, 2H), 1.97 (s, 3H), 1.91 (d, J=14.2 Hz, 2H), 1.34-1.27 (m, 2H), 1.25 (t, *J*= 7.1 Hz, 3H),

^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 169.5, 155.6, 61.5, 46.7, 42.9, 32.1, 23.6, 14.8.



Ethyl 4-(3-methyl-5-(trifluoromethyl)-4H-1,2,4-triazol-4-yl)piperidine-1-carboxylate (23) $POCl_3$ (0.39 g, 0.24 mL, 2.6 mmol, 3 eq.) was added to ethyl 4-acetamidopiperidine-1-carboxylate (**22**) (180 mg, 0.84 mmol, 1 eq.) in a mixture of chloroform (0.55 mL) and pyridine (0.41 mL) at 0°C. The solvent was evaporated under nitrogen using toluene and leave standing overnight. To the brownish slurry in chloroform (0.5 mL) trifluoroacetic acid hydrazide (164 mg, 1.28 mmol, 1.5 eq.) was added and the mixture was refluxed for 5h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (20 mL). The aqueous phase was extracted with dichloromethane (3 x 20 mL) and the combined organic phases were dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. To the residue was added 2M HCl (1.2 mL) and the mixture was refluxed for 4h. The reaction mixture was evaporated under nitrogen and diluted with saturated aqueous $NaHCO_3$ (20 mL) and dichloromethane (20 mL). The aqueous phase was extracted with dichloromethane (2 x 20 mL), dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The crude product was purified by flash chromatography (dichloromethane/ methanol 10:1) to yield ethyl 4-(3-methyl-5-(trifluoromethyl)-4H-1,2,4-triazol-4-yl)piperidine-1-carboxylate (**23**) (30 mg, 15%).

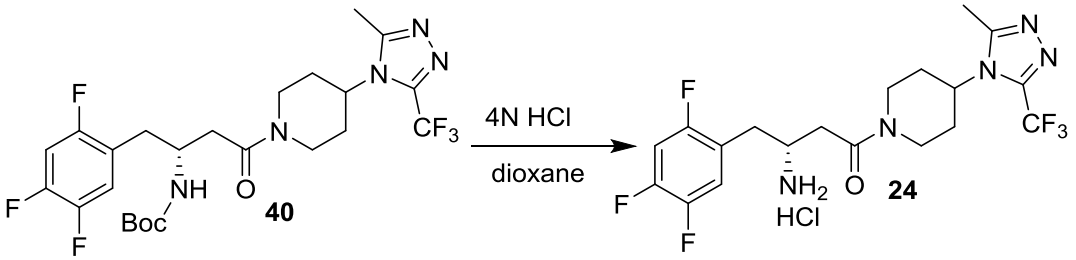
^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 4.26-4.19 (m, 3H), 4.02 (q, *J*= 6.7, 14.4 Hz, 2H), 2.81-2.68 (bt, *J*= 11.7 Hz, 2H), 2.47 (s, 3H), 1.95 (dq, *J*=5.0, 12.7, 24.7 Hz, 2H), 1.84 (m, 2H), 1.15 (t, *J*=7.2 Hz, 3H)
^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 154.9, 153.7, 119.7 (q), 61.6, 55.7, 49.9, 43.1, 30.4, 14.4, 13.2
LCMS *m/z*: 307.1 $[M+H]^+$, 2.41 min.



tert-Butyl (R)-(4-(4-(3-methyl-5-(trifluoromethyl)-4H-1,2,4-triazol-4-yl)piperidin-1-yl)-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-yl)carbamate (40) To ethyl 4-(3-methyl-5-(trifluoromethyl)-4H-1,2,4-triazol-4-yl)piperidine-1-carboxylate (**23**) (30 mg, 0.098 mmol, 1 eq.) in ethanol (84 µL) and water (0.48 mL) was added KOH (36 mg, 0.64 mmol, 6.5 eq.) and the mixture was refluxed for overnight. The reaction mixture was diluted with water (5 mL) and the aqueous phase was extracted with dichloromethane (6 x 10 mL). The organic phases were dried over Na_2SO_4, filtered and the solvents were evaporated. The crude amine (12 mg, 0.051 mmol, 1 eq.) in DMF (1 mL) was added (R)-3-((tert-butoxycarbonyl)amino)-4-(2,4,5-trifluorophenyl)butanoic acid (**11**) (19 mg, 0.057 mmol, 1.1eq.) followed by EDCI (12.5 mg, 0.08 mmol, 1.4 eq.) and HOBt (8.7 mg, 0.057 mmol, 1

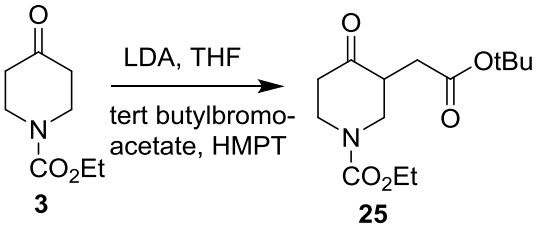
eq.) and stirred for overnight at rt. The crude mixture was evaporated under reduced pressure and the crude product was purified by flash chromatography (dichloromethane/ methanol 10:1) and *tert*-butyl (R)-(4-(4-(3-methyl-5-(trifluoromethyl)-4H-1,2,4-triazol-4-yl)piperidin-1-yl)-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-yl)carbamate (**40**) (25 mg, 93%) was yielded.

^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.08-6.99 (m, 1H), 6.88-6.81 (m, 1H), 5.52 (d, *J*= 9.0 Hz, 0.5H), 5.42 (d, *J*= 9.1 Hz, 0.5H), 4.86 (d, *J*= 14.0 Hz, 1H), 4.38-4.29 (m, 1H), 4.12-3.96 (m, 2H), 3.12 (dt, *J*= 3.1, 14.3 Hz, 1H), 2.90-2.82 (m, 2H), 2.69-2.49 (m, 6H), 2.15-1.92 (m, 4H), 1.31 (s, 9H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 169.2, 169.0, 155.4, 153.9 (2), 144.6, 144.2, 122.6, 122.0, 121.8, 119.2 (2), 117.2, 105.3 (4), 79.5, 55.7, 48.3, 45.0, 44.8, 41.0, 37.2, 33.3, 33.0, 31.2, 31.0, 20.4, 30.2, 28.3, 13.4 (2) LCMS m/z 550.3 $[M+H]^+$, 2.84 min.



(*R*)-3-Amino-1-(4-(3-methyl-5-(trifluoromethyl)-4H-1,2,4-triazol-4-yl)piperidin-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one hydrochloride (24) To *tert*-butyl (R)-(4-(4-(3-methyl-5-(trifluoromethyl)-4H-1,2,4-triazol-4-yl)piperidin-1-yl)-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-yl)carbamate (**40**) (25 mg, 0.04 mmol, 1 eq.) was added 4N HCl in dioxane (0.5 mL) and stirred at rt for 30 min. The HCl solution was evaporated under N_2 gas to yield HCl salt (**24**) as an oil.

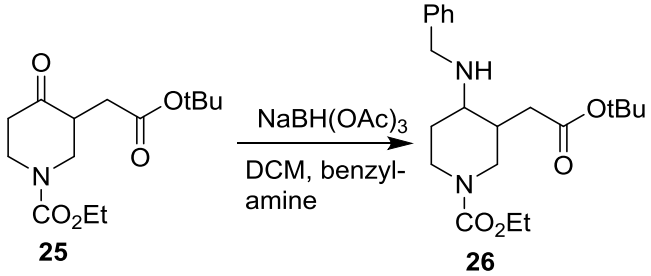
Purity (100 %) by HPLC (t_R= 8.37 min, 8.48 min), ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.41-7.34 (m, 1H), 7.27-7.20 (m, 1H), 4.78 (d, *J*=13.9 Hz, 1H), 4.57 (bs, 1H), 4.05 (d, *J*= 13.5 Hz, 1H), 3.86 (bs, 1H), 3.33-3.26 (m, 4H), 3.08 (m, 2H), 2.95 (d, *J*= 16.9 Hz, 1H), 2.84- 2.67 (m, 2H), 2.64, 2.62 (2s, 3H), 2.19-2.04 (m, 3H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 169.8, 169.7, 165.2, 163.4, 158.9 (2), 156.9 (3), 151,8, 151,7, 151.6, 149.8 (2), 149.7, 149.1 (2), 149.0 (2), 147.2 (2), 147.1 (2), 145.7, 145.3, 145.0, 144.7, 129.6, 121.7, 120.8 (2), 120.6, 119. 6, 117.4, 117.2, 115.3, 112.3, 107.1 106.9 (2), 106.7, 73.4, 72.3, 64.2, 62.0, 59.8, 50.3, 45.3, 41.7 (2), 37.7, 35.4,34.9 (3), 32.3 (3), 31.4, 31.3, 30.7 (2), 12.7 (2) LCMS m/z 450.2 $[M+H]^+$, 1.96 min.



Ethyl 3-(2-(tert-butoxy) 2-oxyethyl)-4-oxopiperidine-1-carboxylate (25) To a solution of diisopropylamine (5.6 g, 7.8 mL, 55.6 mmol, 1.9 eq.) in THF (175 mL) at 0°C was added *n*-BuLi (18.5 mL, 2.5M in hexane, 46.2 mmol, 1.6 eq.) and the mixture was stirred for 30 min. The mixture was cooled to -78°C and ethyl 4-oxopiperidine-1-carboxylate (**3**) (5.0 g, 4.4 mL, 29.2 mmol, 1 eq.) was added and the mixture was stirred for an additional 30 min at -78°C. A solution of *tert*-butylbromoacetate (9.2 g, 7.0 mL, 47.2 mmol, 1.62 eq.) in THF (17.5 mL) and HMPT (2.9 mL) was

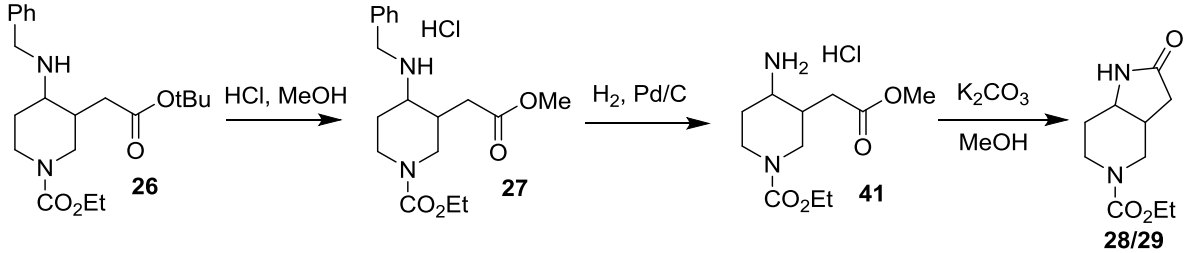
added and the yellow reaction mixture was warmed gradually to room temperature overnight. The mixture was quenched with saturated aqueous NH_4Cl (200 mL), the phases were separated and the aqueous phase was extracted with ethylacetate (3 x 200 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (petrol ether / ethyl acetate: 3:1) to yield ethyl 3-(2-(*tert*-butoxy)-2-oxoethyl)-4-oxopiperidine-1-carboxylate (**25**) in 50% yield.

^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 4.34 (bs, 2H), 4.13 (q, *J*= 7.0, 14.5 Hz, 2H), 3.21 (m, 1H), 2.94-2.80 (m, 2H), 2.64-2.50 (m, 2H), 2.39 (t, *J*= 3.9 Hz, 0.68 H), 2.35 (t, *J*= 4.3 Hz, 0.32 H), 2.25-2.18 (m, 1H), 1.45 (s, 9H), 1.28 (t, *J*= 7.1 Hz, 3H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 207.2, 170.6, 155.2, 80.9, 61.9, 47.9, 46.5, 43.6, 40.7, 32.7, 28.0, 14.6.



Ethyl 4-(benzylamino)-3-(2-(tert-butoxy)-2-oxoethyl)piperidine-1-carboxylate (26) To a solution of ethyl 3-(2-(*tert*-butoxy)-2-oxoethyl)-4-oxopiperidine-1-carboxylate (**25**) (1.43 g, 5.01 mmol, 1.0 eq.) in dichloroethane (22 mL) was added benzylamine (0.65 mL, 0.63 g, 5.90 mmol, 1.17 eq.) and sodium triacetoxyborohydride (1.80 g, 8.53 mmol, 1.70 eq.). The resulting mixture was stirred overnight under an atmosphere of dry nitrogen. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (150 mL) and the aqueous phase was extracted with ethyl acetate (3 x 150 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (petrol ether / ethyl acetate, 1:5) to yield ethyl 4-(benzylamino)-3-(2-(*tert*-butoxy)-2-oxoethyl)piperidine-1-carboxylate (**26**) as a clear colourless oil (1.77g, 94%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.34-7.31, 7.29-7.23 (m, 5H), 4.12 (q, *J* = 7.1, 14.3 Hz, 2H), 4.04-3.69 (m, 5H), 3.11-2.89 (m, 1H), 2.86-2.81 (m, 1H), 2.74-2.46 (m, 2H), 2.37-2.30 (m, 1H), 2.18-2.12 (m, 1H), 1.91-1.76 (m, 1H), 1.56-1.46 (m, 1H), 1.45 (9H, s), 1.24 (t, *J* = 6.8 Hz, 3H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 172.5, 155.8, 155.4, 140.6, 140.4, 128.4, 128.3 (2), 128.1 (2), 126.9 (2), 82.6, 80.4, 80.3, 62.2, 61.5, 61.2, 59.2, 50.9, 50.4, 45.9, 42.5, 42.4, 28.2, 14.7 (2), HRMS *m/z*: $C_{22}H_{33}N_2O_4$ calculated 377.2435 $[M+H]^+$, found 377.2422.

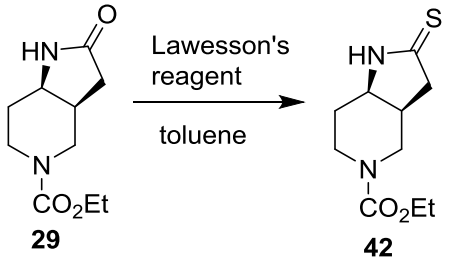


Ethyl 2-oxohexahydro-1H-pyrrolo[3,2-c]pyridine-5(6H)-carboxylate (28/29) To a solution of ethyl 4-(benzylamino)-3-(2-(tert-butoxy)-2-oxoethyl)piperidine-1-carboxylate (**26**) (1.70 g, 4.52 mmol, 1.0 eq.) in MeOH (50 mL) was added hydrochloric acid (0.6M, 4 mL) and the reaction

mixture was stirred for 4 days. A further aliquot of hydrochloric acid (conc., 1 mL) was added and the mixture was stirred for a further two days. The reaction mixture was evaporated under a reduced pressure and subsequently diluted in MeOH (30 mL) and dilute hydrochloric acid (0.5M, 2 mL). The reaction was stirred for 48 hours at room temperature. After evaporation the crude product was obtained as the hydrochloride salt and was used without further purification. To a solution of the hydrochloride salt (550 mg, 1.49 mmol, 1.0 eq.) in MeOH (15 mL) was added Pd/C (10% w/w, 100 mg) and the reaction mixture was stirred at room temperature overnight under an atmosphere of hydrogen. The reaction mixture was filtered through Celite and the filter cake was washed with MeOH (3 x 10 mL) and the filtrate was evaporated to ethyl 4-amino-3-(2-methoxy-2-oxoethyl)piperidine-1-carboxylate hydrochloride (**41**) which was used without further purification.
To a solution of crude product (1-(ethoxycarbonyl)-3-(2-methoxy-2-oxoethyl)piperidin-4-aminium chloride(**41**) in MeOH (6 mL) was added anhydrous K_2CO_3 (200 mg, 1.4 mmol) and the reaction mixture was stirred overnight at room temperature. The reaction mixture was quenched with water (30 mL) and the aqueous phase was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were dried over MgSO4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (ethyl acetate / petrol ether/ methanol: 10:1:1) to yield ethyl 2-oxohexahydro-1*H*-pyrrolo[3,2-*c*]pyridine-5(6*H*)-carboxylate (**28**/**29**) which were separated by flash column chromatography to afford two isomers: isomer 1 (168 mg, 16%) and isomer 2 (286 mg, 28%), combined: 454 mg, 45%)

1 isomer (**28**) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 6.29 (bs, 1H), 4.34 (bm, 2H), 4.12 (q, *J* = 7.4, 13.3 Hz, 2H), 3.19 (dddd, *J* = 3.7, 10.2 Hz, 1H), 2.84-2.74 (m, 2H), 2.32 (dd, *J* =6.7, 15.7 Hz, 1H), 2.08 (dd, *J* = 12.9, 15.7 Hz, 1H), 2.01-1.87 (m, 2H), 1.54 (dddd, *J* = 4.4, 12.9, 24.5 Hz, 1H), 1.25 (t, *J* = 7.4 Hz, 3H);^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 178.2, 156.2, 61.5, 60.4, 59.5, 46.4, 43.8, 42.5, 34.9, 14.7, m.p. 104-105 °C, HRMS *m/z*: $C_{10}H_{17}N_2O_3$ calculated 213.1234 $[M+H]^+$, found 213.0807.

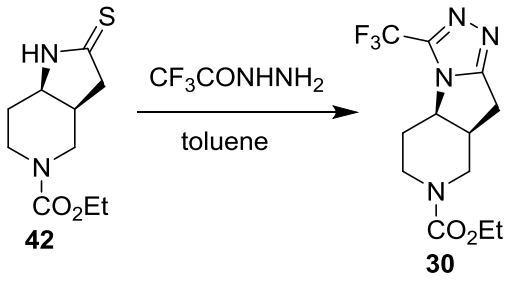
2. isomer (**29**) ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 7.22 (bs, 1H), 4.05 (q, *J* = 7.4, 14.4 Hz, 2H), 3.80 (q, *J* = 4.8, 11.5 Hz, 1H), 3.60 (dd, *J* = 5.1, 13.9 Hz, 1H), 3.46-3.41 (m, 1H), 3.24 (dddd, *J* = 3.6, 9.7, 13.4 Hz, 1H), 3.15 (dd, *J* = 7.1, 13.8 Hz, 1H), 2.52 (bs, 1H), 2.38 (dd, *J* =10.6, 17.9 Hz, 1H), 1.97 (dd, *J* = 4.7, 16.6 Hz, 1H), 1.87-1.78 (m, 1H), 1.70-1.62 (m, 1H), 1.18 (t, *J* = 7.0 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 178.4, 155.5, 61.5, 51.1, 43.6, 39.3, 34.9, 27.6, 21.2, 14.7, HRMS *m/z*: $C_{10}H_{16}KN_2O_3$ calculated 251.0793 $[M+K]^+$, found 251.0761.



Ethyl (3aS,7aR)-2-thioxooctahydro-5H-pyrrolo[3,2-c]pyridine-5-carboxylate (42)

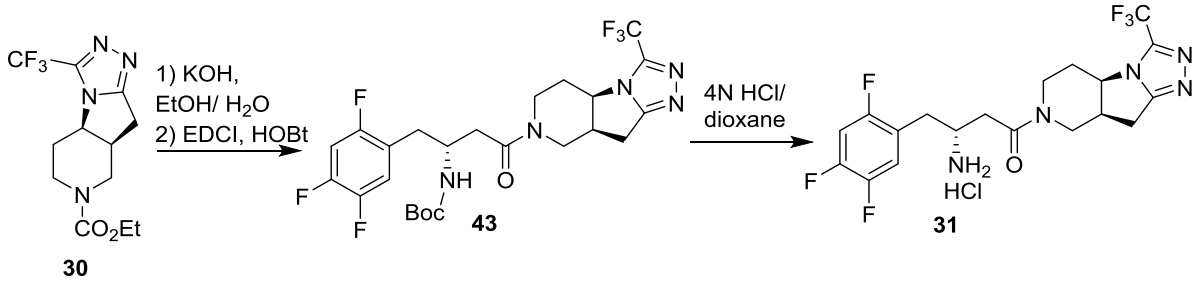
To a solution of ethyl 2-oxohexahydro-1*H*-pyrrolo[3,2-*c*]pyridine-5(6*H*)-carboxylate (**29**) (70 mg, 0.33 mmol, 1 eq.) in toluene (0.5 mL) was added Lawesson's reagent (77 mg, 0.19 mmol, 0.57 eq.) and refluxed at 110°C for 40 min. The mixture was evaporated under reduced pressure and the curde product was purified by flash chromatography (ethylacetate/petrolether: 10:1) to yield ethyl (3aS,7aR)-2-thioxooctahydro-5H-pyrrolo[3,2-c]pyridine-5-carboxylate (**42**) (50 mg, 66%) as a colorless oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 9.11 (bs, 1H), 4.10 (m, 3H), 3.71-3.68 (m, 2H), 3.23 (t, *J*=10.6 Hz, 1H), 3.11 (dd, *J*= 8.1, 13.9 Hz, 1H), 2.94 (dd, *J*= 6.9, 17.1 Hz, 1H), 2.66-2.58 (m, 2H), , 1.94-1.80 (m, 2H), 1.21 (t, *J*= 7.3 Hz, 3H) ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 204.9, 171.2, 155.7, 134.0, 61.7, 58.8,47.4, 42.8, 39.1, 26.3, 14.6.



Ethyl (4aR,8aS)-3-(trifluoromethyl)-4a,5,8a,9-tetrahydro-6H-[1,2,4]triazolo[4',3':1,5]pyrrolo[3,2-c]pyridine-7(8H)-carboxylate (30) To a solution of ethyl (3aS,7aR)-2-thioxooctahydro-5H-pyrrolo[3,2-c]pyridine-5-carboxylate (**42**) (54 mg, 0.26 mmol, 1 eq.) in toluene (2 mL) was added trifluoroacetic acid hydrazide (61 mg, 0.47 mmol, 2 eq.) and refluxed for 3 nights. The crude mixture was evaporated under reduced pressure and the crude product was purified by intensive flash chromatography (dichlormethane/methanol 10:1) to yield, ethyl (4aR,8aS)-3-(trifluoromethyl)-4a,5,8a,9-tetrahydro-6H-[1,2,4]triazolo[4',3':1,5]pyrrolo[3,2-c]pyridine-7(8H)-carboxylate (**30**) (70%) as a yellowish oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 4.60 (q, *J*= 7.0, 13.6 Hz, 1H), 4.17-4.11 (dq, *J*= 2.0, 7.1, 14.1 Hz, 2H), 3.91 (m, 1H), 3.80 (m, 1H), 3.45 (d, *J*= 13.4 Hz, 1H), 3.33 (m, 1H), 3.13 (dd, *J*= 8.2, 16.7 Hz, 2H), 2.82 (dd, *J*= 8.8 , 17.1 Hz, 1H), 2.30-2.22 (m, 1H), 1.84-1.73 (m, 1H), 1.24 (t, *J*= 6.8 Hz, 3H) ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 162.8, 157.9 (2), 141.2 (2), 117.4 (q), 62.0, 55.5, 42.3, 39.6, 27.7, 24.3, 14.6 HRMS *m/z*: $C_{12}H_{15}N_4O_2F_3$ calculated 305.1220 $[M+H]^+$, found 305.1140.



(R)-3-Amino-1-((4aR,8aS)-3-(trifluoromethyl)-4a,5,8a,9-tetrahydro-6H-[1,2,4]triazolo[4',3':1,5]pyrrolo[3,2-c]pyridin-7(8H)-yl)-4-(2,4,5-trifluorophenyl)butan-1-one hydrochloride (31) To a solution of carbamate (**30**) (54 mg, 0.17 mmol, 1eq.) in water/ethanol (1.25 mL:0.22 mL) was added potassium hydroxide (100 mg, 1.73 mmol, 10 eq) and refluxed at 110°C for one night. The mixture was diluted with water and dichloromethane and the aqueous phase was extracted with dichloromethane (6 x 10 mL). The combined organic phases were dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to yield amine (23 mg) as a colorless oil. The crude amine was diluted with DMF (1 mL) and HOBt (16 mg, 0.10 mmol) and EDCI (23 mg, 0.15 mmol) and (R)-3-((tert-

butoxycarbonyl)amino)-4-(2,4,5-trifluorophenyl)butanoic acid (**11**) (38 mg, 0.14 mmol). The mixture was stirred at rt overnight. The crude product was purified by flash chromatography to yield amide (**43**) (20 mg, 37%). LCMS *m/z*: 548.2 $[M+H]^+$, 2.09 min. To Boc-protected amide (**43**) (20 mg, 0.03mmol, 1 eq.) was added 4N HCl in dioxane (0.5 mL) and stirred at rt for 30 min. The HCl solution was evaporated under N_2 gas to yield HCl salt (**31**) as an oil.

Purity (100 %) by HPLC (t_R= 8.47 min), ^{1}H-NMR (400 MHz, $MeOD_4$) δ/ppm 7.40-7.30 (m, 1H), 7.27-7.17 (m, 1H), 4.29- 4.22 (m, 1H), 3.89-3.80 (m, 2H), 3.76-3.65 (m, 2H), 3.60-3.35 (m, 2H), 3.26-3.14 (m, 1H), 3.08-3.00 (m, 2H), 2.88-2.58 (m, 3H), 2.36 (m, 1H), 1.88 (m, 1H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm 173.4, 171.9, 171.1, 170.9, 170.5, 170.4, 165.1, 159.0, 158.9, 157.0, 156.9, 151.9, 149.9, 149.2 (2), 147.3 (2), 129.6, 128.3, 127.2, 120.8, 120.6, 120.5, 118.7, 11.5, 107.2 (3), 106.9, 106.8, 73.5, 72.4, 62.1, 57.2 (2), 56.9, 52.7, 45.2 (2), 43.7, 43.5 (2), 41.8 (2), 39.2, 36.4 (2), 35.2, 35.0, 34.9 (2), 32.3 (2), 28.6, 27.7 (2), 24.9, 24.8 (2), HRMS *m/z*: $C_{19}H_{19}N_5OF_6$ calculated 448.1567 $[M+H]^+$, found 448.1568.