Supporting information

Discovery of the Fibrinolysis Inhibitor AZD6564, Acting Via Interference of a Protein-Protein Interaction

Leifeng Cheng[†#], Daniel Pettersen[†*], Bengt Ohlsson[†], Peter Schell[†#], Michael Karle[†#], Emma Evertsson[†#], Sara Pahlén[†], Maria Jonforsen[†], Alleyn T. Plowright[†], Jonas Boström[†], Tomas Fex[†#], Anders Thelin[‡#], Constanze Hilgendorf[§], Yafeng Xue[□], Göran Wahlund[‡], Walter Lindberg[§], Lars-Olof Larsson[§], David Gustafsson[‡]

† Medicinal Chemistry, CVMD iMED

‡ Bioscience, CVMD iMED

§ DMPK, CVMD iMED

□ Discovery Sciences

AstraZeneca Mölndal, S-413 83 Mölndal, Sweden;

General synthetic details

All solvent and reagents used were reagent grade. Purity and characterization of compounds were established by a combination of liquid chromatography–mass spectroscopy (LCMS), and NMR analytical techniques. High resolution LCMS was detected on a Waters LCTp ToF MS using electrospray ionization (ESI-MS). The MS inlet consisted of a Waters Acquity UPLC system, and the separation was performed on a Waters C18 XBridge at 45-50 °C. The separation was obtained with a 2-95% ACN gradient over 3 min at pH 10 (40 mM NH_3 and 5 mM H_2CO_3). A measure of related impurities was assessed at 210 nM. ^{1}H NMR were recorded on a Bruker Avance DPX400 (400 MHz), AV500 (500 MHz) or AV600 (600 MHz) and were determined in $CHCl_3$-*d*, DMSO-d_6 and MeOH-d_4 with trimethylsilane (TMS) (0.00 ppm) or solvent peaks as the internal reference. Chemical shifts are reported in ppm relative to solvent signal at 7.26 ppm ($CDCl_3$), 2.50 ppm (DMSO) and 3.30 ppm (MeOH)**.**

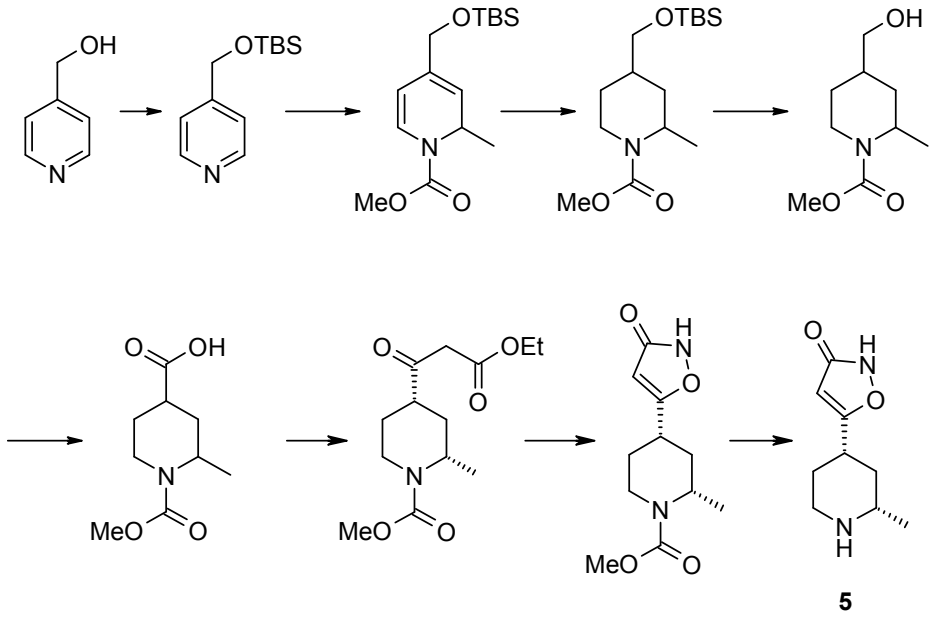
Synthesis of compounds 1-6

Compound 1
5-(1-Methylpiperidin-4-yl)isoxazol-3(2H)-one
A solution of 5-(piperidin-4-yl)isoxazol-3(2H)-one hydrobromide, **2** (19.3 mg, 0.077 mmol), formaldehyde (0.3 ml, 36%) and formic acid (0.15 ml) was heated in the microwave oven at 100°C for 60 min. LC/MS analysis showed formation of product. Purification using preparative reversed phase chromatography (pH=3, small column 0-15% ACN over 15 min, collect all at 232 nm). Fractions containing product were concentrated and then freeze dried to give 11 mg of **1**. HRMS Calculated for $[C_9H_{15}N_2O_2]$+: 183.1134; found: 183.1144.

Compound 2
2-Methyl-5-(piperidin-4-yl)isoxazol-3(2H)-one
A solution of tert-butyl 4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate (1 g, 3.3 mmol, purchased from Pharmacorel) in methanol (0.5 ml) was added to a solution of sodium hydroxide (143 mg, 3.6 mmol) in methanol/water (2.8 ml, 0.17 ml) at -30°C. After 10 min N-Me-hydroxylamine-HCl (0.56 g, 6.7 mmol) and sodium hydroxide (0.27 g, 6.7 mmol) in methanol (3 ml) and water (3 m) were added. Stirring was continued at -30°C during 2.5 h. The reaction solution was poured into conc. HCl (1.45 ml) at 80°C. The reaction was then heated at 80°C during 2h. The reaction solution was concentrated to give a yellow semi solid material (1.6g). The mixture was purified using preparative basic chromatography (pH=11, acetonitrile-buffer) which gave 94 mg product, yield 13%. ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.62 – 1.77 (m, 2H), 2 – 2.12 (m, 2H), 2.8 – 2.92 (m, 3H), 3.19 – 3.28 (m, 2H), 3.50 (s, 3H), 5.67 (s, 1H); HRMS Calculated for $[C_9H_{15}N_2O_2]$+: 183.1133; found: 183.1138.

Compounds 3,**4**
4-Methyl-5-(piperidin-4-yl)isoxazol-3(2H)-one
J. Med. Chem. **2002**, *45*, 2454-2468

Compound 5
Cis-5-[2-Methyl-4-piperidyl]isoxazol-3-one



4-((Tert-butyldimethylsilyloxy)methyl)pyridine
To a solution of pyridin-4-ylmethanol (25.7 g, 024 mmol) and imidazole (19.8 g, 0.29 mmol) in dry DMF (300 ml) and dry DCM (33 ml) under nitrogen atmosphere was added TBDMSCl (42.6 g, 0.29 mmol). The solution was allowed to stir for 18 h under which time a precipitate formed. The reaction mixture was then concentrated by removal of volatiles (about 100 ml) followed by addition of water (500 ml). The resulting mixture was then extracted with 1:1 heptane:ethylacetate (200 ml x3). The combined organic phases was washed with brine (x2), dried (magnesium sulfate), filtered and evaporated to yield an oil, 51.70 g (98%). ^{1}H NMR (600 MHz, $CDCl_3$) δ -0.01 (s, 6H), 0.82 (s, 9H), 4.63 (s, 2H), 7.13 (d, *J* = 5.90 Hz, 2H), 8.43 (d, *J* = 5.88 Hz, 2H).

Methyl 4-[[tert-butyl(dimethyl)silyl]oxymethyl]-2-methyl-2H-pyridine-1-carboxylate
To a suspension of 4-(tert-butyldimethylsilyloxy)methyl)pyridine (8.0 g, 35.8 mmol) in THF (50 mL) cooled to –30 °C was added methylmagnesium chloride (3 M in THF) (13.1 mL, 39.4 mmol, 1 M in THF) over 10 min. Methyl carbonochloridate (3.61 mL, 46.7 mmol) was added drop wise over 10 min. The reaction mixture was allowed to reach 0 °C over 2h. The organic solvents were evaporated, the reaction mixture was diluted with ethyl acetate, then washed with 1N HCl and brine. The organic layer was dried over magnesium sulfate and evaporated. The residue was purified by column chromatography (Biotage heptane:ethyl acetate using a gradient 0-15% ethyl acetate; snap 360 column) which gave the title compound as a slight yellow oil (2.48 g, 23%): (NMR complex); m/z (M^+) 298.

Methyl 4-((tert-butyldimethylsilyloxy)methyl)-2-methylpiperidine-1-carboxylate
To a solution of methyl 4-((tert-butyldimethylsilyloxy)methyl)-2-methylpyridine-1(2H)-carboxylate (2.48 g, 8.3 mmol) in ethyl acetate (50 mL) was added platinum(IV) oxide (0.07 g, 0.31 mmol). The suspension was hydrogenated at 6 bar H_2 atmosphere for 20 h. The mixture was filtered through Celite and the solvents were evaporated to give an oil (2.47 g, 98%): (NMR complex); m/z (M^+) 302.

Methyl 4-(hydroxymethyl)-2-methylpiperidine-1-carboxylate
To a suspension of methyl 4-((tert-butyldimethylsilyloxy)methyl)-2-methylpiperidine-1-carboxylate (2.47 g, 8.2 mmol) in tetrahydrofuran (15 mL) was added tetrabutylammonium fluoride (10.65 mL, 10.65 mmol, 1 M in THF) and the reaction mixture was stirred at room temperature for 90 min. The solvents were evaporated, the residue dissolved in ethyl acetate and washed with sat. $NaHCO_3$ (x 1), then brine (x 2). The organic layer was dried over

magnesium sulfate and evaporated. The residue was purified by column chromatography (Biotage 40%-90% gradient ethylacetate in heptane, 340 snap column), which resulted in the title compound (1.03 g, 67%): (NMR complex).

1-(methoxycarbonyl)-2-methylpiperidine-4-carboxylic acid

To a solution of methyl 4-(hydroxymethyl)-2-methylpiperidine-1-carboxylate (1.03 g, 5.50 mmol) in carbon tetrachloride (12 mL) was added sodium periodate (3.53 g, 16.5 mmol) and water (20 mL). Then acetonitrile (12 mL) was added to this mixture, followed by ruthenium(III) chloride (0.025 g, 0.12 mmol). The resulting biphasic mixture was stirred vigorously at room temperature for 2h. After 20 minutes a precipitate formed. The reaction mixture was diluted with water and DCM, the upper aqueous layer was extracted with DCM (x 3). The combined organic layers were dried over magnesium sulfate and evaporated to give an oil (0.90g, 81%): m/z (M^-) 200. The product was taken to the next step without further treatments.

Methyl 4-(3-ethoxy-3-oxo-propanoyl)-2-methyl-piperidine-1-carboxylate

A suspension of $MgCl_2$ (0.426g, 4.47mmol) and ethyl potassium malonate (1.142g, 6.71mmol) in dry THF (15ml) was stirred under N_2 at 50°C for 4h. In another flask was added carbonyldiimidazole (0.870g, 5.37mmol) portionwise to a solution of 1-(methoxycarbonyl)-2-methylpiperidine-4-carboxylic acid (0.90g, 4.47mmol) in dry THF (20° ml) at 5°C put under N_2. This solution was allowed to stir for 1h at 5°C . Then the malonate suspension was added dropwise via a flex needle to the carbonyldiimidazole suspension and the resulting mixture was stirred for 16h. LCMS analysis showed the desired product as the major compound in the reaction mixture. Concentration of the reaction mixture and the residue was taken up in EtOAc and H_2O. The aqueous phase was extracted once with EtOAc and the combined organic phases were washed with H_2O, Na_2CO_3(aq)and then dried (Na_2SO_4) and evaporated to give 1.29g. Purification using biotage (100 g column, grad 20-500% EtOAc/heptane 7 CV, collect at 220 nm, TLC-KMnO4 ok). The two isomers were isolated as trans (0.311g) and cis (0.496g). Yield of cis racemate 41%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.13 (d, *J* = 6.64 Hz, 3H), 1.27 (t, *J* = 7.15, 7.15 Hz, 3H), 1.65 – 1.73 (m, 1H), 1.79 – 1.93 (m, 2H), 1.99 – 2.05 (m, 1H), 2.68 – 2.76 (m, 1H), 3.05 – 3.13 (m, 1H), 3.50 (s, 2H), 3.68 (s, 3H), 3.83 – 3.9 (m, 1H), 4.12 (q, *J* = 6.69, 6.69, 6.69 Hz, 1H), 4.19 (q, *J* = 7.13, 7.14, 7.14 Hz, 2H).

Cis-methyl-2-methyl-4-(3-oxoisoxazol-5-yl)piperidine-1-carboxylate

A solution of cis methyl 4-(3-ethoxy-3-oxopropanoyl)-2-methylpiperidine-1-carboxylate (0.496 g, 1.83 mmol) in MeOH (1.35 mL) was added to a solution of NaOH (0.077 g) in MeOH/H_2O (1.5 ml/ 0.1 mL) at -30°C. After 10 min was added hydroxylamine hydrochloride (0.254 g, 3.66 mmol) and sodium hydroxide (0.069 ml, 3.66 mmol) in MeOH (1.8 mL) and H_2O (1.8 mL) added. Stirring was continued at -30°C for 30 min. The reaction solution was poured into 6M HCl (2.7 ml) at 80°C and heated at 80°C for 30 min. Concentration of the organic solvent and extraction with DCM (x2). The organic layer was dried using a phase separation and evaporation to give 0.345 g waxy oil. Purification using preparative LC (2 injections pH=3, small column 20-40% ACN over 30 min, collect 232 nm peak level 60) gave 230 mg methyl cis-2-methyl-4-(3-oxoisoxazol-5-yl)piperidine-1-carboxylate, yield 52%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.08 (d, *J* = 6.77 Hz, 3H), 1.81 – 1.92 (m, 2H), 1.98 – 2.11 (m, 2H), 2.94 – 3.03 (m, 1H), 3.13 – 3.24 (m, 1H), 3.69 (s, 3H), 3.85 – 3.95 (m, 1H), 4.19 (q, *J* = 6.55, 6.56, 6.56 Hz, 1H), 5.70 (s, 1H).

Cis-5-[2-Methyl-4-piperidyl]isoxazol-3-one

Cis-methyl-2-methyl-4-(3-oxoisoxazol-5-yl)piperidine-1-carboxylate was stirred in HBr (33% in AcOH) for 16h. Evaporation of solvents gave brown oil that was purified using prepLC (pH=11, small column, inject sample dissolved in water/MeOH, 0-15% ACN over 15 min. collect 232 nm) gave 46 mg, yield 60%. ^{1}H NMR (600 MHz, MeOH-d_4) δ 1.29 (d, *J* = 6.35 Hz, 3H), 1.57 (q, *J* = 12.40, 12.41, 12.41 Hz, 1H), 1.7 – 1.82 (m, 1H), 2.05 – 2.22 (m, 2H), 3.02 – 3.14 (m, 2H), 3.20 (s, 1H), 3.36 – 3.45 (m, 1H), 5.74 (s, 1H).

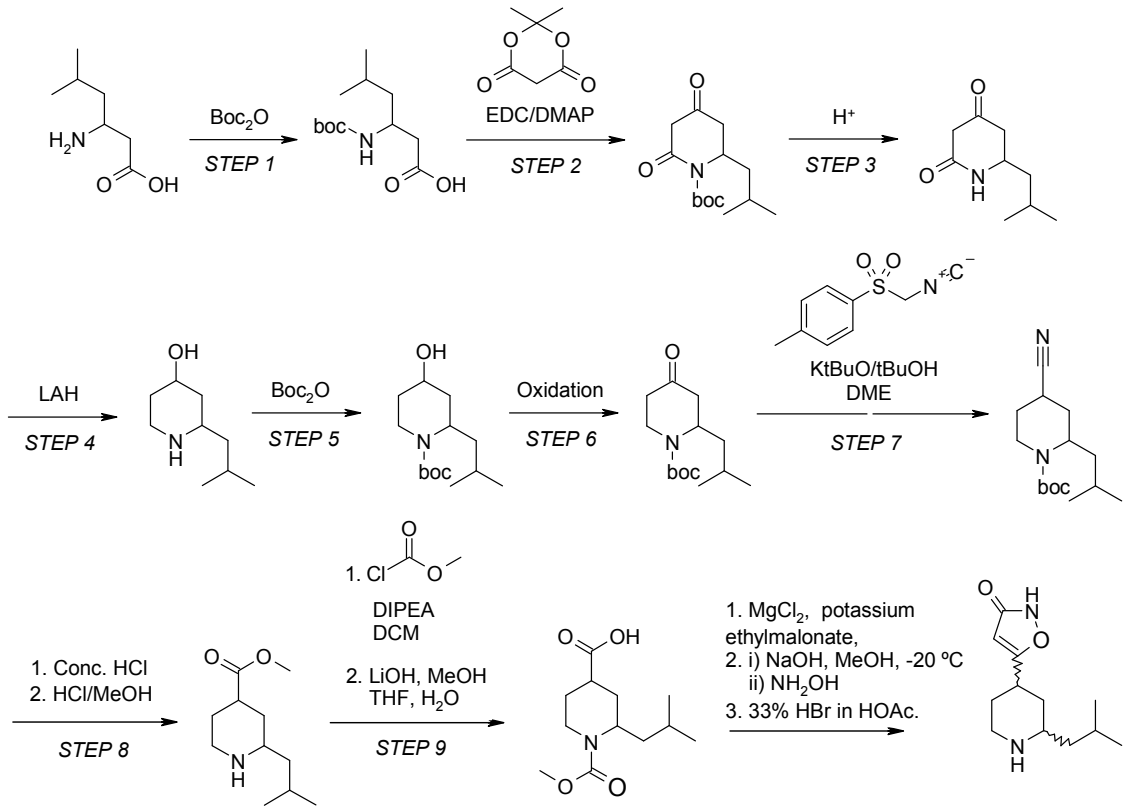
Compound 6
3-(piperidin-4-yl)isoxazol-5(2H)-one hydrochloride

Tert-butyl 4-(5-oxo-2,5-dihydroisoxazol-3-yl)piperidine-1-carboxylate
To a solution of tert-butyl 4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate (1420 mg, 4.74 mmol) in EtOH (40 ml) was added hydroxylamine-HCl (989 mg, 14.23 mmol) followed by DIPEA (1839 mg, 14.23 mmol). The reaction solution was stirred at rt under nitrogen for 16 h. The reaction solution was concentrated and purified using prepLC (pH=3, large column, 5-75% ACN over 20 min, collect 254 nm) to yield tert-butyl 4-(5-oxo-2,5-dihydroisoxazol-3-yl)piperidine-1-carboxylate (717 mg, 56 %) as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.51 – 1.61 (m, 2H), 1.87 (d, *J* = 12.70 Hz, 2H), 2.58 – 2.69 (m, 1H), 2.81 (br s, 2H), 3.36 (s, 2H), 4.16 (br s, 2H).

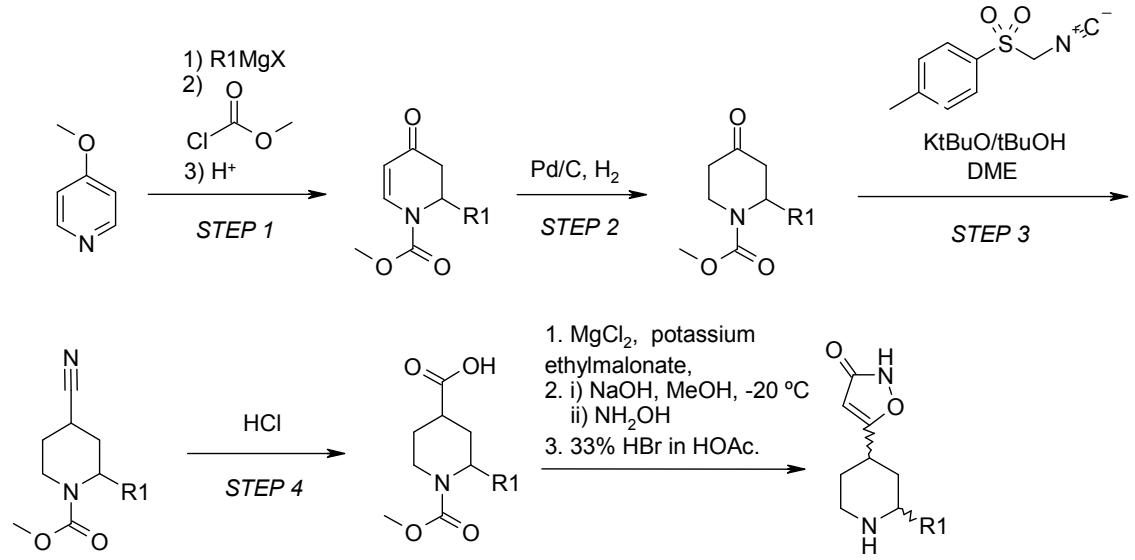
3-(piperidin-4-yl)isoxazol-5(2H)-one hydrochloride
To a solution of tert-butyl 4-(5-oxo-2,5-dihydroisoxazol-3-yl)piperidine-1-carboxylate (717 mg, 2.67 mmol) in EtOH (10 ml) under nitrogen was added HCl in dioxane (15.75 g, 15 ml).The solution was stirred for 1.5 h and then evaporated to give a slight yellow solid. Purified using prepLC(pH=11, large column, 0-8% ACN over 8 min, collect 251 nm) to yield an almost white solid as free amine. The solid was dissolved in water and HCl (4M in dioxane, 0.7 ml) was added and the solution was again freeze dried to give the title compound (333 mg, 61 %) as a HCl-salt. ^{1}H NMR (600 MHz, MeOH-d_4) δ 1.79 – 1.91 (m, 2H), 2.05 – 2.15 (m, 2H), 2.74 – 2.83 (m, 1H), 3.03 – 3.14 (m, 2H), 3.31 (s, 2H), 3.35 – 3.45 (m, 2H), HRMS Calculated for $[C_8H_{13}N_2O_2]$+: 169.0977; found: 169.0986.

Synthesis of compounds **14**-**25**

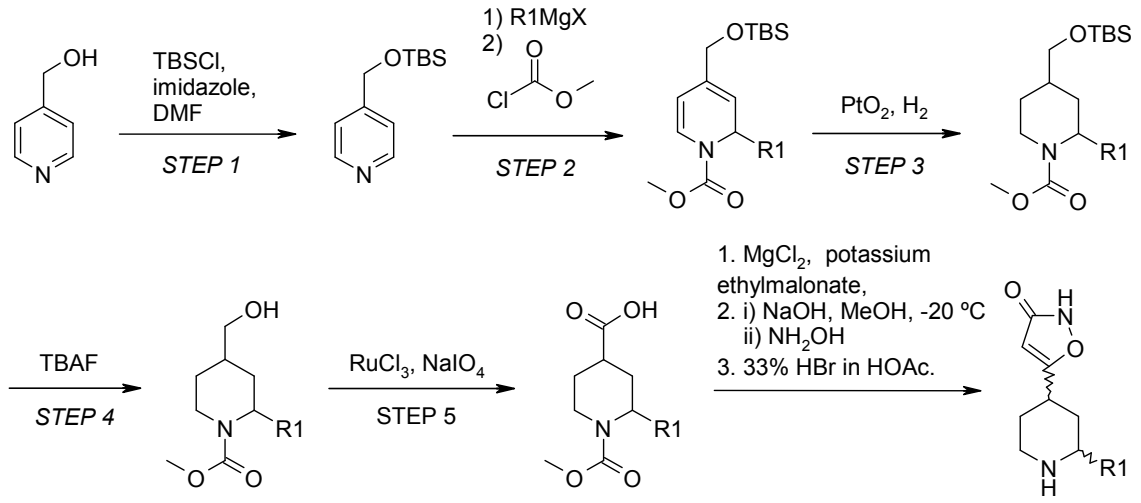
Method A



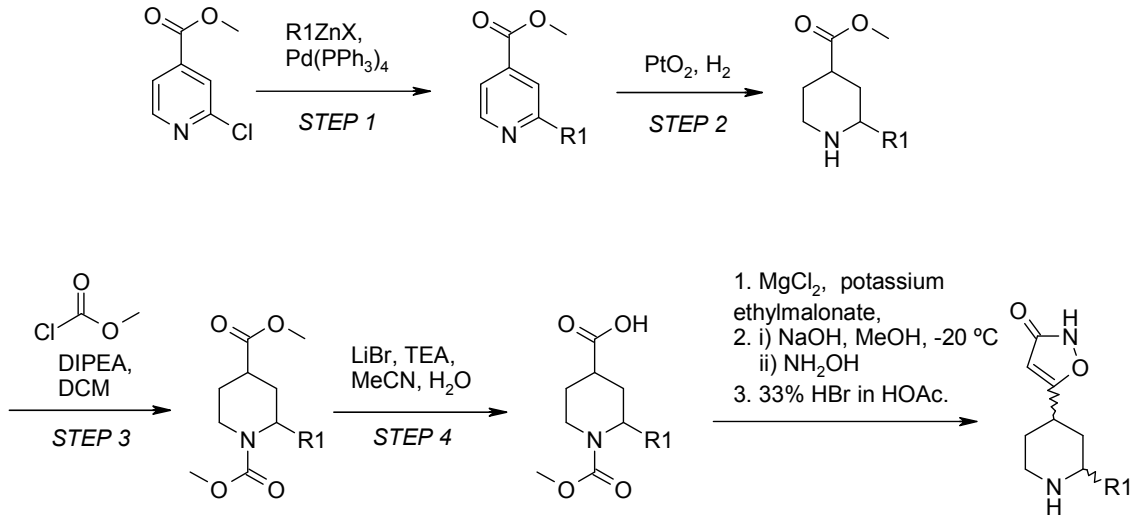
Method B



Method C



Method D



Compound 14 (Method B)
5-((2S,4R)-2-Benzylpiperidin-4-yl)isoxazol-3(2H)-one

Step 1: Methyl 2-benzyl-4-oxo-3,4-dihydropyridine-1(2H)-carboxylate
4-Methoxypyridine (1 g, 9.2 mmol) was dissolved in THF (20 mL). The solution was put under nitrogen atmosphere and cooled to -25 ºC. Then was added dropwise benzylmagnesium chloride (2M in THF) and a suspension was formed. After stirring at -30 ºC for 10 min was added methyl chloroformate (0.92 ml, 11.9 mmol) over 1 min. Stirring was continued at -30 ºC for 40 min and then HCl (10%, 20 ml) was added. The mixture was stirred for 10 min and then concentrated. The aqueous phase was extracted with ethyl acetate (x2) and the organic phase was dried over magnesium sulfate and evaporated to give 1.4 g oil. The reaction product was taken to the next step without further handling.

Step 2: Methyl 2-benzyl-4-oxopiperidine-1-carboxylate
Crude methyl 2-benzyl-4-oxo-3,4-dihydropyridine-1(2H)-carboxylate (crude 1.4 g from step 1) was dissolved in ethyl acetate and hydrogenated for 20 h at 4 bar over Pd/C (5%). The suspension was filtrated through a silica plug and then evaporated to give an oil. Purification

using automated column chromatography (Biotage) (100 g column, grad 0-60% EtOAc/heptane over 10 CV) gave methyl 2-benzyl-4-oxopiperidine-1-carboxylate (0.75 g, 32% over two reaction steps). ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.38 (m, 2H), 2.50 (m, 2H), 2.72 (m, 1H), 2.83 (m, 1H), 3.38 (m, 1H), 3.66 (s, 3H), 4.30 (m, 1H), 4.79 (m, 1 H), 7.15 – 7.30 (m, 5H); MS m/z 2.48 $(M+H)^+$.

Step 3: Methyl-2-benzyl-4-cyanopiperidine-1-carboxylate
To a solution of methyl 2-benzyl-4-oxopiperidine-1-carboxylate (0.72 g, 2.93 mmol) in DME (15 ml), put under nitrogen atmosphere, were added, at the same time, a solution of 1-(isocyanomethylsulfonyl)-4-methylbenzene (0.86g, 4.29 mmol) in DME (10 ml) and potassium tert-butoxide (8.8 ml, 1 M in tBuOH) over 20 min, keeping the temperature below 0 C. The solution was then allowed to stir at -20 °C for 2 h and then allowed to varm to RT over night. To the orange reaction mixture was added H_2O (15 ml) and the solution was stirred for 20 min and then extracted with EtOAc (3x15 ml). The organic phases were combined and dried over sodium sulfate and evaporated to give 1.0 g as a brown residue. Purification using automated column chromatography (Biotage) (100 g column, grad 20-60% EtOAc/heptane) yielded methyl-2-benzyl-4-cyanopiperidine-1-carboxylate (0.56 g, 75%) as a mixture of cis and trans isomers: ^{1}H NMR (600 MHz, $CDCl_3$) Trans isomer: δ 1.75 (m, 2H), 1.99 (m, 1H), 2.28 (m, 1H), 2.78 (m, 1H), 2.91 (m, 2H), 3.02 (m, 1H), 3.60 (s, 3H), 4.19 (m, 1H), 4.60 (m, 1H), 7.18-7.38 (m, 5H). Cis isomer: δ 1.75 (m, 2H), 1.99 (m, 2H), 2.98 (m, 2H), 3.3 (m, 2H), 3.62 (s, 3H), 4.19 (m, 1H), 4.45 (m, 1H), 7.22-7.38 (m, 5H).

Step 4: 2-Benzyl-1-(methoxycarbonyl)piperidine-4-carboxylic acid
To methyl-2-benzyl-4-cyanopiperidine-1-carboxylate (0.56 g, 2.1 mmol) was added conc. HCl (5 mL). The mixture was heated at 90 C for 60 min and then allowed to reach room temperature and diluted with water (10 ml). The aqueous phase was then extracted with EtOAc (3x10 ml) and the combined organic phases was dried over magnesium sulfate and evaporated to give 2-benzyl-1-(methoxycarbonyl)piperidine-4-carboxylic acid (420 mg, 72%) as an oil. m/z 276 (M-H). The product was taken to the next step without further handling.

Step 5: Trans-methyl 2-benzyl-4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate and cis-methyl 2-benzyl-4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate
A suspension of magnesium chloride (3.11 g, 32.66 mmol) and ethyl potassium malonate (8.34 g, 48.99 mmol) in dry THF (70 mL) was stirred under nitrogen atmosphere at 50°C for 4 h (flask 1). In another flask was added carbonyldiimidazole (6.35 g, 39.19 mmol) portionwise to a suspension of 2-benzyl-1-(methoxycarbonyl)piperidine-4-carboxylic acid (9.057 g, 32.66 mmol) in dry THF (70 mL) at 5°C under nitrogen atmosphere. This reaction mixture was stirred for 1 h at 5°C (flask 2). The contents of flask 2 was then added dropwise to flask 1 and the resulting mixture was stirred for 24 h. The reaction mixture was concentrated and the residue was partitioned between EtOAc and H_2O. The aqueous phase was extracted once with EtOAc and the combined organic phases were washed with H_2O, satd Na_2CO_3 and then dried over sodium sulfate and evaporated to give 11.02 g oil. Purification using automated column chromatography (Biotage) (2 runs - 340 g column, grad 10-60% EtOAc/heptane) yielded trans-methyl 2-benzyl-4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate (6.35 g): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.23 - 1.32 (m, 3H), 1.41 – 1.66 (m, 3H), 1.70 – 2.11 (m, 2H), 2.53 – 3.10 (m, 4H), 3.33 – 3.77 (m, 4H), 4.00 – 4.35 (m, 3H), 4.42 – 4.99 (m, 1H), 7.08 – 7.37 (m, 5H); MS m/z 348 $(M+H)^+$ and cis-methyl 2-benzyl-4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate (2.15 g): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.25 (m, 3H), 1.61 – 1.96 (m, 4H), 2.64 – 2.79 (m, 2H), 2.80 – 3.10 (m, 2H), 3.44 (s, 2H), 3.63 (s, 3H), 3.84 – 4.00 (m, 1H), 4.03 – 4.25 (m, 3H), 7.15 – 7.42 (m, 5H); MS m/z 348 $(M+H)^+$.

Step 6: Cis-methyl 2-benzyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate
A solution of cis-methyl 2-benzyl-4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate (2,15 g, 6.18 mmol) in MeOH (0.75 mL) was added to a solution of NaOH (0.26 g, 6.55 mmol) in MeOH/H_2O (5 mL/0.3 mL) at –30°C. After 10 minutes was added a solution of hydroxylamine

hydrochloride (0.86 g, 12.4 mmol) and NaOH (0.49 g, 12.4 mmol) in MeOH (6.1 mL) and H_2O (6.1 mL). Stirring was continued at -30°C for 30 minutes. The reaction mixture was then poured into concentrated HCl (7 mL) held at 80°C. The solution was stirred for 30 minutes. The organic solvent was evaporated and the aqueous phase extracted with ether (x3). The combined organic phases were dried over sodium sulfate, and evaporated to yield a solid, 2.2 g. The compound was purified by preparative HPLC on a Kromasil C8 column (10 µm 250x20 ID mm) using a gradient of 20-70% acetonitrile in H_2O/MeCN/FA 95/5/0.2 buffer, over 18 minutes with a flow of 19 mL/minutes. The title compound was isolated (1.1 g, 57%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.79 – 2.16 (m, 4H), 2.63 (dd, *J* = 8.26, 13.36 Hz, 1H), 2.82 (dd, *J* = 5.86, 13.37 Hz, 1H), 2.89 – 3.02 (m, 1H), 3.07 – 3.21 (m, 1H), 3.61 (s, 3H), 3.93 – 4.04 (m, 1H), 4.2 – 4.33 (m, 1H), 5.71 (s, 1H), 7.03 – 7.34 (m, 5H); MS m/z 317 $(M+H)^+$.

Step 7: (2S,4R)-Methyl 2-benzyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate
Racemic cis-methyl 2-benzyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (1.1 g) was subjected to chiral separation using Chiralcel OD, mobile phase heptane/EtOH/FA 90/10/0.1 at 40°C which resulted in (2S,4R)-methyl 2-benzyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (0.47 g). $[\alpha]^{20}_D$ -23.8 (ACN, c = 1).

Step 8: 5-((2S,4R)-2-Benzylpiperidin-4-yl)isoxazol-3(2H)-one
Following the same procedure as described in 17, Step 8 using (2S,4R)-methyl 2-benzyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate yielded the title compound (0.23 g, 26%); HRMS Calculated for $[C_{15}H_{19}N_2O_2]$+: 259.1447; found: 259.1433. ^{1}H NMR identical to title compound 17.

Compound 15 (Method B)
5-((2S,4S)-2-Benzylpiperidin-4-yl)isoxazol-3(2H)-one

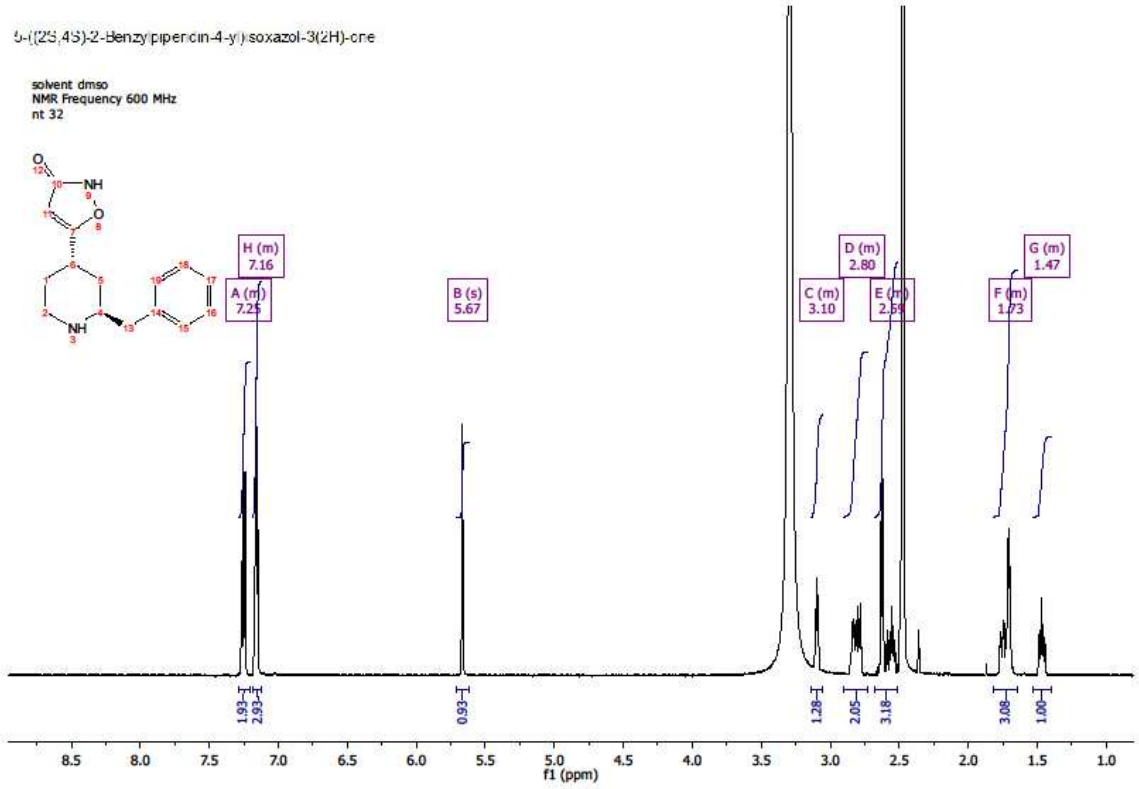
See **14** for step 1-5.

Step 6: Trans-methyl 2-benzyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate
A solution of trans-methyl 2-benzyl-4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate (6.35 g, 18.3 mmol) in MeOH (2 mL) was added to a solution of NaOH (0.77 g, 19.4 mmol) in MeOH/H_2O (15 mL/0.9 mL) at –30°C. After 10 minutes was added a solution of hydroxylamine hydrochloride (2.54 g, 36.6 mmol) and NaOH (1.46 g, 36.6 mmol) in MeOH (18 mL) and H_2O (18 mL). Stirring was continued at -30°C for 30 minutes. The reaction mixture was then poured into concentrated HCl (20 mL) held at 80°C. The solution was stirred for 30 minutes. The organic solvent was evaporated and the aqueous phase extracted with DEE (x3). The organic phase was dried over soium sulfate and evaporated to give a solid, 3.68 g, which was purified by preparative HPLC on a Kromasil C8 column (10 µm 250x20 ID mm) using a gradient of 20-70% acetonitrile in H_2O/MeCN/FA 95/5/0.2 buffer, over 18 minutes with a flow of 19 mL/minutes. Trans-methyl-2-benzyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (2.54 g, 54.6 %) was isolated as a solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.47 – 1.73 (m, 2H), 1.81 – 2.18 (m, 2H), 2.73 – 3.02 (m, 2H), 3.01 – 3.21 (m, 2H), 3.37 – 3.84 (m, 3H), 4.04 – 4.41 (m, 1H), 4.43 – 4.82 (m, 1H), 5.55 – 5.84 (m, 1H), 6.99 – 7.41 (m, 5H); MS m/z 317 $(M+H)^+$.

Step 7: (2S,4S)-Methyl 2-benzyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate
Racemic trans-methyl 2-benzyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (2.54 g, 8.04 mmol) was subjected to chiral separation using Chiralcel IA, mobile phase heptane/EtOH/FA 80/20/0.4/0.1, which resulted in (2S,4S)-methyl 2-benzyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (0.99 g).

Step 8: 5-((2S,4S)-2-Benzylpiperidin-4-yl)isoxazol-3(2H)-one

To (2S,4S)-methyl 2-benzyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (0.99 g) was added HBr (33 % in HOAc, 20 mL) and the solution was stirred for 8 h. The volatiles were concentrated and the residues purified by preparative HPLC on a XBridge C18 column (10 µm 250x50 ID mm) using a gradient of 0-40 % acetonitrile in H_2O/MeCN/NH_3 95/5/0.2 buffer over 15 minutes with a flow of 100 mL/minutes. The title compound was isolated (0.62 g, 30%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.4 – 1.53 (m, 1H), 1.65 – 1.81 (m, 3H), 2.51 – 2.68 (m, 3H), 2.73 – 2.9 (m, 2H), 3.06 – 3.13 (m, 1H), 5.67 (s, 1H), 7.13 – 7.19 (m, 3H), 7.21 – 7.29 (m, 2H); $[\alpha]^{20}_D$ +47.0 (MeOH/H_2O 1:1, c = 1); HRMS Calculated for $[C_{15}H_{19}N_2O_2]$+: 259.1447; Found: 259.1449.



Compound 16 (Method B)
5-((2R,4R)-2-Benzylpiperidin-4-yl)isoxazol-3(2H)-one

See **14** for step 1-5. See **15** for step 6.

Step 7: <u>(2R,4R)-Methyl 2-benzyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate</u>
The title compound was obtained in the preparation of 15, Step 7 (0.93 g).

Step 8: <u>5-((2R,4R)-2-Benzylpiperidin-4-yl)isoxazol-3(2H)-one</u>
Following the same procedure as described in 15, Step 8 using ((2R,4R)-methyl 2-benzyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate the title compound (0.55 g, 27%) was obtained. $[\alpha]^{20}_D$ -43.0 (MeOH/H_2O 1:1, c = 1); HRMS Calculated for $[C_{15}H_{19}N_2O_2]$+: 259.1447; found: 259.1449. ^{1}H NMR identical to title compound 15.

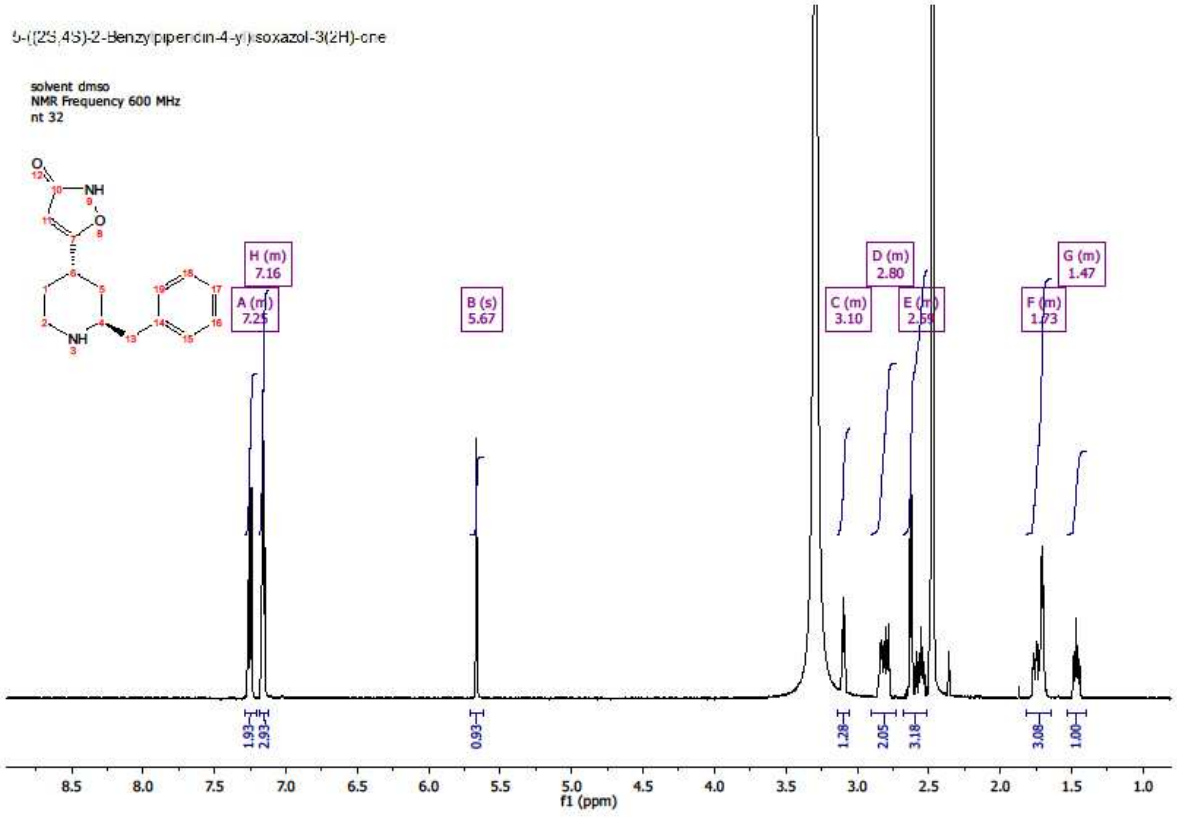
Compound 17 (Method B)

5-((2R,4S)-2-Benzylpiperidin-4-yl)isoxazol-3(2H)-one

See **14** for step 1-6.

Step 7: (2R,4S)-Methyl 2-benzyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate
The title compound was obtained in the preparation of 14, Step 7 (0.47 g).

Step 8: 5-((2R,4S)-2-Benzylpiperidin-4-yl)isoxazol-3(2H)-one
To (2R,4S)-methyl 2-benzyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (0.47 g) was added HBr (33% in HOAc, 10 mL) and the solution was stirred for 16 h. The volatiles were concentrated and the residue purified by preparative HPLC on a XBridge C18 column (10 µm 250x50 ID mm) using a gradient of 0-40% acetonitrile in H_2O/MeCN/NH_3 95/5/0.2 buffer over 15 minutes with a flow of 100 mL/minutes. The title compound was isolated (0.26 g, 29 %): ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.07 (q, *J* = 12.33, 12.33, 12.36 Hz, 1H), 1.35 (qd, *J* = 4.01, 12.40, 12.41, 12.41 Hz, 1H), 1.67 – 1.82 (m, 2H), 2.42 – 2.76 (m, 5H), 2.92 – 3.01 (m, 1H), 5.66 (s, 1H), 7.14 – 7.2 (m, 3H), 7.26 (t, *J* = 7.51, 7.51 Hz, 2H); ^{13}C NMR (126 MHz, MeOH-d_4) δ 176.74, 175.16, 137.25, 130.44, 129.95, 128.28, 95.06, 58.49, 45.65, 41.51, 34.88, 34.57, 28.53; $[\alpha]^{20}_D$ +67.8 (MeOH/H_2O 1:1, c = 1); HRMS Calculated for $[C_{15}H_{19}N_2O_2]$+: 259.1447; found: 259.1442.



Compound 18 (Method A)
5-((2S,4S)-2-Isobutylpiperidin-4-yl)isoxazol-3(2H)-one

Step 1: 3-(*tert*-Butoxycarbonylamino)-5-methylhexanoic acid
To a solution of DL-β-homo leucine (45 g, 0.31 mol) in 1N NaOH (1 L) at 0°C, a solution of $(Boc)_2O$ (87.8 g, 0.403 mol) in 1,4-dioxane (500 mL) was added dropwise. The reaction

mixture was stirred at room temperature overnight, cooled to 0°C and neutralized with 1 N HCl (ca. 1000 mL). The solid was filtered off and dried under vacuum to yield 3-(*tert*-butoxycarbonylamino)-5-methylhexanoic acid (48.0 g, 63 %) as a solid.

Step 2: *tert*-Butyl 2-isobutyl-4,6-dioxopiperidine-1-carboxylate
To a stirred solution of 3-(*tert*-butoxycarbonylamino)-5-methylhexanoic acid (44.0 g, 0.179 mol) in DCM (800 mL) at 0°C, EDC hydrochloride (51.56 g, 0.269 mol), DMAP (32.8 g, 0.269 mol) and Meldrum's acid (25.8 g, 0.179 mol) were added. The reaction mixture was stirred at room temperature for 3 h, washed with 1 N $KHSO_4$ (500 mL), dried over sodium sulfate and concentrated. The residue was dissolved in dry ethyl acetate (1.5 L) and heated under reflux overnight. The reaction mixture was washed with 1 N $KHSO_4$ (500 mL), brine (500 mL), dried over sodium sulfate and concentrated. The residue was purified by column chromatography using petroleum ether and EtOAc (65:35) as eluent to the title compound (35 g, 72 %).

Step 3: 6-Isobutyl-piperidine-2,4-dione
To a solution of *tert*-butyl 2-isobutyl-4,6-dioxopiperidine-1-carboxylate (30 g, 0.114 mol) in dry 1,4 dioxane (300 mL), HCl (3 M in 1,4 dioxane, 100 mL) was added and stirred at room temperature for 3 h. The reaction mixture was concentrated and purified by crystallization using diethyl ether to yield 6-isobutyl-piperidine-2,4-dione (14 g, 74 %) as a white solid.

Step 4: 2-Isobutyl-piperidin-4-ol
To a stirred ice cooled suspension of LAH (16.1 g, 0.414 mol) in THF (100 mL), a solution of 6-isobutyl-piperidine-2,4-dione (14.0 g, 0.083 mol) in THF (100 mL) was added dropwise under N_2. The reaction mixture was warmed to room temperature and stirred under N_2 for 48 h. The reaction mixture was cooled to 0°C and quenched with water (16.1 mL), 1 N NaOH (16.1 mL) and water (16.1 mL). The reaction mixture was filtered and the solid was washed with hot THF (100 mL). The filtrate was concentrated to yield 2-isobutyl-piperidin-4-ol as a solid (9 g, crude).

Step 5*:* *tert*-Butyl-4-hydroxy-2-isobutylpiperidine-1-carboxylate
2-Isobutyl-piperidin-4-ol (9.0 g, 0.057 mol) was taken up in a 50:50 solution of THF and satd $NaHCO_3$ (100 mL) and the mixture was cooled to 0°C. A solution of $(Boc)_2O$ (13.7 g, 0.063 mol) in THF (50 mL) was added dropwise. The resulting solution was stirred at room temperature overnight. The reaction mixture was concentrated and extracted with EtOAc (100 mL). The organic phase was washed with water (100 mL), brine (100 mL), dried over sodium sulfate and concentrated. The concentrated product was purified by column chromatography using 20% of EtOAc in petroleum ether to yield a mixture of two compounds. The mixture was further purified by preparative HPLC to yield *tert*-butyl-4-hydroxy-2-isobutylpiperidine-1-carboxylate as a pale yellow liquid (4.5 g, 39.5 %).

Step 6: 2-Isobutyl-4-oxo-piperidine-1-carboxylic acid *tert*-butyl ester
To a stirred solution of *tert*-butyl-4-hydroxy-2-isobutylpiperidine-1-carboxylate (4.6 g, 0.018 mol) in DCM (50 mL) at 0°C, 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one (9.1 g, 0.02146 mol) was added portionwise and the resulting solution was warmed to room temperature and stirred under N_2 overnight. The reaction mixture was quenched with satd $NaHCO_3$ solution (50 mL) and was filtered through a Celite® pad. The filtrate was extracted several times with DCM (50 mL), the combined organic phases were washed with water (50 mL), brine (50 mL), dried over sodium sulfate and concentrated. The concentrated product was purified by column chromatography using 10 % EtOAc in petroleum ether to yield the product with HPLC purity 90%. The impure product was further purified by preparative HPLC to yield 2-isobutyl-4-oxo-piperidine-1-carboxylic acid *tert*-butyl ester as a liquid (2.1 g, 46 %).

Step 7: *tert*-Butyl 4-cyano-2-isobutylpiperidine-1-carboxylate
To a solution of 2-Isobutyl-4-oxo-piperidine-1-carboxylic acid *tert*-butyl ester (4.55 g, 17.8 mmol) in DME (100 ml), put under nitrogen atmospere, were added at the same time, a

solution of 1-(isocyanomethylsulfonyl)-4-methylbenzene (5.22 g, 26.7 mmol) in DME (100 ml) and potassium tert-butoxide (53.5 ml, 1 M in tBuOH) over 30 min, keeping the temperature below 0 C. The solution was then allowed to stir at -20 C for 2 h and then allowed to varm to RT over night. To the orange reaction mixture was added H_2O (200 ml) and the solution was stirred for 20 min and then extracted with DEE x 3 and EtOAc x 3. The organic phases were combined and dried over magnesium sulfate and evaporated to give 6.43 g as a brown semisolid. The prodcut was taken to the next step without further purification. MS m/z 267 $(M+H)^+$.

Step 8: 2-Isobutyl-piperidine-4-carboxylic acid methyl ester
Crude *tert*-butyl-4-cyano-2-isobutylpiperidine-1-carboxylate (6.43 g, ca 24.2 mmol) was stirred in conc. HCl (35.7 g) in an open microwave vial for 10 min. Then it was heated in the micro at 140 C for 30 min. The solvent was evaporated and the residue redissolved in HCl in MeOH (25 ml, 1.25 M). The mixture was heated in the micro at 130 C for 20 min. Concentration gave a black residue that was uptaken in water and neutralised with solid $NaHCO_3$. The water phase was then extracted with DEE x 3, EtOAc x 3 and DCM x 4 to yield 2.75 g black oil. MS m/z 200 $(M+H)^+$. The prodcut was taken to the next step without further purification.

Step 9: 2-Isobutyl-1-(methoxycarbonyl)piperidine-4-carboxylic acid
To a solution of crude 2-isobutyl-piperidine-4-carboxylic acid methyl ester (2.60 g, 13.0 mmol) and DIPEA (4.55 ml, 26.1 mmol) in DCM (130 ml), under nitrogen atmosphere, was added methyl chloroformate (1.31 mL, 17.0 mmol) dropwise during 5 min. Stirring was continued for 1h. The reaction mixture was washed with $NaHCO_3$ (sat), dried through a phase separator and evaporated to give a darkbrown oil. Purification on biotage (2 injections) (100g column, EtOAc-Hept grad 0-50 10 CV) yielded 2.36 g as a yellow oil. The residue was dissolved in THF (40 ml) followed by addition of LiOH (0.26 g, 11.0 mmol), MeOH (30 ml) and H_2O (30 ml). The reaction mixture was stirred at rt under nitrogen atmosphere for 16 h. The solvents were evaporated and the resiude uptaken in water. The pH was adjusted to <2 by addition of HCl (10%). The aqueous phase was then extracted with DCM (x5) and the organic extracts were pooled, dried (phase sep) and evaporated to give crude 2-isobutyl-1-(methoxycarbonyl)piperidine-4-carboxylic acid. MS m/z 242 $(M-H)^-$. The product was taken to the next step without further purification.

Step 10: Cis-methyl 2-isobutyl-4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate
The compound was prepared as described in 14, Step 5 from crude 2-isobutyl-1-(methoxycarbonyl)piperidine-4-carboxylic acid (2.28 g, 9.39 mmol), magnesium chloride (0.95 g, 10.0 mmol), ethyl potassium malonate (2.54 g, 14.9 mmol) and carbonyldiimidazole (1.98 g, 12.2 mmol) which resulted in the title compound (0.59 g, 20%).

Step 11: Cis-methyl 2-isobutyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate
The compound was prepared as described in 15, Step 6 starting from cis-methyl 2-isobutyl-4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate (0.59 g, 1.9 mmol) which resulted in the title compound (0.29 g, 51%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.85 (2 d, 6H), 1.15 (m, 1H), 1.29 – 1.40 (m, 1H), 1.50 (m, 1H), 1.82 – 1.93 (m, 2H), 2.04 (m, 2H), 2.91 – 3.05 (m, 1H), 3.06 – 3.20 (m, 1H), 3.69 (s, 3H), 3.93 (m, 1H), 4.17 (m, 1H), 5.70 (s, 1H). MS m/z 283 $(M+H)^+$.

Step 12: Cis-5-(2-isobutylpiperidin-4-yl)isoxazol-3(2H)-one
Racemic cis-methyl 2-isobutyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (0.29 g, 1.03 mmol) was stirred in HBr (12 mL, 33% in HOAc) for 24 h. The volatiles were concentrated and the residue purified by preparative HPLC on a XBridge C18 column (10 µm 250x50 ID mm) using a gradient of 0-25% acetonitrile in H_2O/MeCN/NH_3 95/5/0.2 buffer over 10 minutes with a flow of 100 mL/min. The title compound (160 mg) was isolated. ^{1}H NMR (600 MHz, d_2o) δ 0.79 (2 d, 6H), 1.34 – 1.51 (m, 3H), 1.54 – 1.72 (m, 2H), 2.13 (m, 1H), 2.25 (m, 1H), 2.99 (m, 2H), 3.21 (m, 1H), 3.38 (m, 1H), 5.61 (s, 1H).

Step 13: 5-((2S,4S)-2-Isobutylpiperidin-4-yl)isoxazol-3(2H)-one
A racemic mixture of cis-5-(2-isobutylpiperidin-4-yl)isoxazol-3(2H)-one was subjected to chiral separation using Chiralpak IC, mobile phase heptane/EtOH/FA/TEA 60/40/0.4/0.2 which resulted in the title compound (85 mg, 37%). $[\alpha]^{20}_{D}$ +19.5 (H_2O, c = 1); HRMS Calculated for $[C_{12}H_{21}N_2O_2]$+: 225.1603; found: 225.1593. ^{1}H NMR identical to the racemix mixture, step 12.

Compound 19 (Method C)
5-((2R,4S)-2-Neopentylpiperidin-4-yl)isoxazol-3(2H)-one

Step 1: 4-((*tert*-Butyldimethylsilyloxy)methyl)pyridine
To a solution of pyridin-4-ylmethanol (25.7 g, 0.24 mol) and imidazole (19.8 g, 0.29 mol) in dry DMF (300 mL) and dry DCM (33 mL) under nitrogen atmosphere was added TBDMSCl (42.6 g, 0.29 mol). The solution was stirred for 18 h under which time a precipitate formed. The reaction mixture was concentrated by removal of volatiles (about 100 mL) followed by addition of water (500 mL). The resulting mixture was extracted with 1:1 heptane:EtOAc (200 mL x3). The combined organic phases were washed with brine (x2), dried (magnesium sulfate), filtered and evaporated to yield 4-((*tert*-butyldimethylsilyloxy)-methyl)pyridine (51.70 g, 98%) as an oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ -0.01 (s, 6H), 0.82 (s, 9H), 4.63 (s, 2H), 7.14 (m, 2H), 8.43 (m, 2H).

Step 2: Methyl 4-((*tert*-butyldimethylsilyloxy)methyl)-2-neopentylpyridine-1(2H)-carboxylate
To a suspension of 4-(*tert*-butyldimethylsilyloxy)methyl)pyridine (8.23 g, 36.84 mmol) in THF (60 mL) cooled to –30°C was added neopentylmagnesium chloride (40.5 mL, 40.5 mmol, 1 M in THF) over 10 minutes. Methyl carbonochloridate (3.77 mL, 47.9 mmol) was added dropwise over 10 minutes. The reaction mixture was allowed to reach 0°C over 2 h. The organic solvents were evaporated, the reaction mixture was diluted with ethyl acetate, then washed with 1 N HCl and brine. The organic layer was dried over magnesium sulfate and evaporated to give the crude residue. The residue was purified by automated flash column chromatography on a Biotage® KP-SIL 340g column. A gradient of 15:1 to 10:1 heptane:EtOAc was used as mobile phase to give the title compound (9.3 g, 71 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ -0.00 (s, 3H), 0.09 (s, 3H), 0.87 (s, 18H), 1.09 – 1.81 (m, 2H), 1.94 – 2.44 (m, 1H), 3.70 (s, 3H), 4.22 – 4.90 (m, 2H), 5.12 – 5.61 (m, 1H), 5.69 – 5.97 (m, 1H), 6.25 – 6.79 (m, 1H). MS m/z 354 $(M+H)^+$.

Step 3: Methyl 4-((*tert*-butyldimethylsilyloxy)methyl)-2-neopentylpiperidine-1-carboxylate
To a solution of methyl 4-((*tert*-butyldimethylsilyloxy)methyl)-2-neopentylpyridine-1(2H)-carboxylate (9.3 g, 26.3 mmol) in ethyl acetate (150 mL) was added platinum(IV) oxide (0.60 g, 2.63 mmol). The suspension was hydrogenated at 6 bar H_2 atmosphere for 15 h. The mixture was filtered through Celite and the solvents were evaporated to give the product (9.3 g, 99 %) as an oil. MS m/z 358 $(M+H)^+$.

Step 4: Methyl 4-(hydroxymethyl)-2-neopentylpiperidine-1-carboxylate
To a suspension of methyl 4-((*tert*-butyldimethylsilyloxy)methyl)-2-neopentylpiperidine-1-carboxylate (9.40 g, 26.30 mmol) in tetrahydrofuran (100 mL) was added tetrabutylammonium fluoride (34.2 mL, 34.2 mmol, 1 M in THF) and the reaction mixture was stirred at room temperature for 90 minutes. The solvents were evaporated, the residue dissolved in DCM and washed with satd $NaHCO_3$. The organic layer was dried over magnesium sulfate and evaporated. The residue was purified by automated flash column chromatography on a Biotage® KP-SIL 340g column. A gradient from 30% to 100% EtOAc in heptane was used as eluent, which resulted in the title compound.

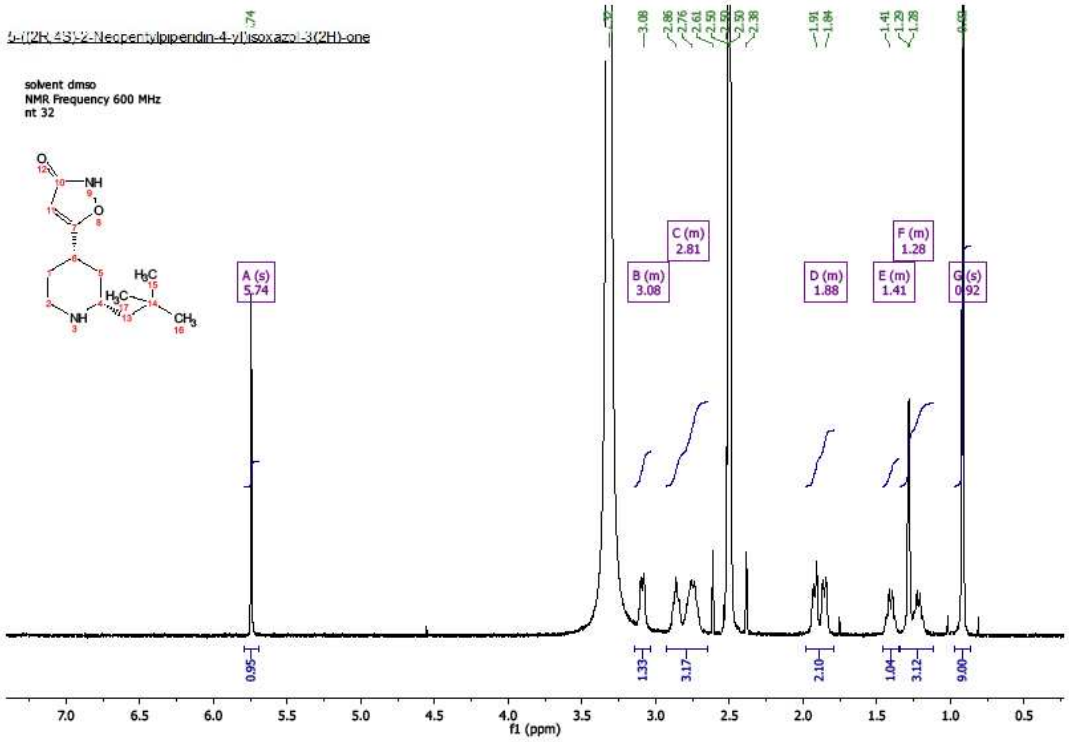
Step 5: 1-(Methoxycarbonyl)-2-neopentylpiperidine-4-carboxylic acid
To a solution of methyl 4-(hydroxymethyl)-2-neopentylpiperidine-1-carboxylate (5.7 g, 23.42 mmol) in carbon tetrachloride (47 mL) was added sodium periodate (15.0 g, 70.3 mmol) and water (70.5 mL). Acetonitrile (47 mL) was added to this mixture, followed by ruthenium(III) chloride (0.11 g, 0.52 mmol). The resulting biphasic mixture was stirred vigorously at room temperature for 2 h. The reaction mixture was diluted with water and DCM, the aqueous layer was extracted with DCM (x 3). The combined organic layers were dried over magnesium sulfate and evaporated to give the product as an oil (5.9 g, 98 %) MS m/z 256 $(M-H)^-$.

Step 6: Cis-methyl 4-(3-ethoxy-3-oxopropanoyl)-2-neopentylpiperidine-1-carboxylate
A suspension of magnesium chloride (2.381 g, 25.01 mmol) and ethyl potassium malonate (5.57 g, 32.70 mmol) in dry THF (60 mL) was stirred under nitrogen atmosphere at 50°C for 4 h (flask 1). In another flask was added carbonyldiimidazole (3.74 g, 23.08 mmol) in one portion to a suspension of 1-(methoxycarbonyl)-2-neopentylpiperidine-4-carboxylic acid (4.95 g, 19.24 mmol) in dry THF (60 mL) at rt under nitrogen atmosphere. This reaction mixture was stirred for 4 h at rt (flask 2). The contents of flask 2 was then added dropwise to flask 1 and the resulting mixture was stirred for 16 h. 1N HCl was added. The resulting solution was vigorously stirred for 10 min. The aqueous layer was extracted three times with ethyl acetate. The combined organic phases were washed with 1 N HCl, satd $NaHCO_3$ and brine and then dried over magnesium sulfate and evaporated. Purification using automated column chromatography (Biotage) (340 g column, grad 20-70% EtOAc/heptane) yielded Cis-methyl 4-(3-ethoxy-3-oxopropanoyl)-2-neopentylpiperidine-1-carboxylate (2.40 g, 38 %).

Step 7: Cis-methyl 2-neopentyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate
The compound was prepared as described in 15, Step 6 starting from cis-methyl 4-(3-ethoxy-3-oxopropanoyl)-2-neopentylpiperidine-1-carboxylate (2.68 g, 8.19 mmol) which resulted in cis-methyl 2-neopentyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (1.60 g, 66 %) : 1H NMR (400 MHz, $CDCl_3$) δ 0.89 (s, 9H), 1.18 (dd, *J* = 5.60, 14.31 Hz, 1H), 1.45 (dd, *J* = 7.01, 14.29 Hz, 1H), 1.80 - 1.92 (m, 2H), 1.97 - 2.17 (m, 2H), 2.94 - 3.02 (m, 1H), 3.11 - 3.23 (m, 1H), 3.71 (s, 3H), 3.88 - 3.99 (m, 1H), 4.22 - 4.32 (m, 1H), 5.72 (s, 1H); m/z (MH^+) 297.

Step 8: (2R,4S)-Methyl 2-neopentyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate
Following the procedure described in 15, Step 7, racemic cis-methyl 2-neopentyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (1.60 g, 5.4 mmol) was subjected to chiral separation using Chiralcel IC mobile phase heptane/IPA/FA 60/40/0.1 which resulted in (2R,4S)-methyl 2-neopentyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (0.8 g, 2.7 mmol).

Step 9: 5-((2R,4S)-2-Neopentylpiperidin-4-yl)isoxazol-3(2H)-one
Starting from (2R,4S)-methyl 2-neopentyl-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (0.8 g, 2.7 mmol) and following the procedure described in 15, Step 8 the title compound was obtained (0.44 g, 69 %): ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.92 (s, 9H), 1.11 – 1.34 (m, 3H), 1.35 – 1.46 (m, 1H), 1.79 – 1.98 (m, 2H), 2.65 – 2.93 (m, 3H), 3.03 – 3.14 (m, 1H), 5.74 (s, 1H); ^{13}C NMR (101 MHz, CH_4-d_4) δ 177.39, 174.72, 95.42, 54.83, 49.32, 45.50, 37.13, 34.75, 31.19, 30.07, 28.06; $[\alpha]^{20}_D$ +43.8 (MeOH/H_2O 1:1, c = 1); HRMS calculated for $[C_{13}H_{23}N_2O_2]$+: 239.1759; found: 239.1753.



Compound 20 (Method C)
5-((2S,4S)-2-(3,3-Dimethylbutyl)piperidin-4-yl)isoxazol-3(2H)-one

Step 1: 4-((*tert*-Butyldimethylsilyloxy)methyl)pyridine
To a solution of pyridin-4-ylmethanol (25.7 g, 0.24 mol) and imidazole (19.8 g, 0.29 mol) in dry DMF (300 mL) and dry DCM (33 mL) under nitrogen atmosphere was added TBDMSCl (42.6 g, 0.29 mol). The solution was stirred for 18 h under which time a precipitate formed. The reaction mixture was concentrated by removal of volatiles (about 100 mL) followed by addition of water (500 mL). The resulting mixture was extracted with 1:1 heptane:EtOAc (200 mL x3). The combined organic phases were washed with brine (x2), dried (magnesium sulfate), filtered and evaporated to yield 4-((*tert*-butyldimethylsilyloxy)-methyl)pyridine (51.70 g, 98%) as an oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ -0.01 (s, 6H), 0.82 (s, 9H), 4.63 (s, 2H), 7.14 (m, 2H), 8.43 (m, 2H).

Step 2: Methyl 4-((*tert*-butyldimethylsilyloxy)methyl)-2-(3,3-dimethylbutyl)pyridine-1(2H)-carboxylate
4-((*tert*-Butyldimethylsilyloxy)methyl)pyridine (5.58 g, 25 mmol) was dissolved in dry THF (50 mL) under nitrogen and the mixture cooled to -15°C. (3,3-Dimethylbutyl)magnesium chloride (0.5 M in THF) (50 mL, 25 mmol) was added dropwise during 20 min to yield a yellow solution which was stirred at -15°C for 30 min. Then, methyl carbonochloridate (2.5 mL, 32 mmol) was added during 1 min. The reaction was continued at -15°C for 30 min and then the mixture was cooled to -60°C. After 2 h the temperature had reached room temperature. Water (20 mL) was added and the solvent evaporated. The aqueous phase was extracted with DCM (x2) and the combined organic phase passed through a phase separator. The solvent was evaporated to yield a yellow oil. The residue was purified via Biotage (0 => 10 % EtOAc in heptane) to yield methyl 4-((*tert*-butyldimethylsilyloxy)methyl)-2-(3,3-dimethylbutyl)pyridine-1(2H)-carboxylate (3.67 g, 44 %) as a colourless oil. MS m/z 368 $(M+H)^+$

Step 3: Methyl 4-((*tert*-butyldimethylsilyloxy)methyl)-2-(3,3-dimethylbutyl)piperidine-1-carboxylate

To a solution of methyl 4-((*tert*-butyldimethylsilyloxy)methyl)-2-(3,3-dimethylbutyl)-pyridine-1(2H)-carboxylate (3.63 g, 9.87 mmol) in ethyl acetate (60 mL) was platinum (IV) oxide (224 mg, 1 mmol) added. Hydrogenated at 6 bar in a Büchi hydrogenator for 3.5 h. The catalyst was filtered off and the filtrate evaporated to yield methyl 4-((*tert*-butyl-dimethylsilyloxy)methyl)-2-(3,3-dimethylbutyl)piperidine-1-carboxylate (3.62 g, 99 %) as a colourless oil. MS m/z 372 $(M+H)^+$

Step 4: Methyl 2-(3,3-dimethylbutyl)-4-(hydroxymethyl)piperidine-1-carboxylate

Methyl 4-((*tert*-butyldimethylsilyloxy)methyl)-2-(3,3-dimethylbutyl)piperidine-1-carboxylate (3.606 g, 9.7 mmol) was dissolved in THF (50 mL) and TBAF (1M in THF) (13 mL, 13 mmol) added. Stirred at room temperature for 3.5 h. The solvent was evaporated. Redissolved in DCM and washed with satd $NaHCO_3$. The organic phase was passed through a phase separator and evaporated to yield an oil. The residue was purified via Biotage (eluent 30-70% EtOAc in heptane) to yield methyl 2-(3,3-dimethylbutyl)-4-(hydroxymethyl)piperidine-1-carboxylate (2.45 g, 98 %) as a colourless oil. MS m/z 258 $(M+H)^+$

Step 5: 2-(3,3-Dimethylbutyl)-1-(methoxycarbonyl)piperidine-4-carboxylic acid

Methyl 2-(3,3-dimethylbutyl)-4-(hydroxymethyl)piperidine-1-carboxylate (2.44 g, 9.49 mmol) was dissolved in CCl_4 (20 mL) and acetonitrile (20 mL). Sodium periodiate (6.09 g, 28.5 mmol) was added followed by water (30 mL) and ruthenium (III) chloride (43 mg, 0.21 mmol). The resulting suspension was stirred at room temperature for 3 h 50 min. The reaction mixture was diluted with DCM (100 mL) and water (100 mL). The aqueous layer was extracted with DCM (x3) and the combined organic phase passed through a phase separator and evaporated to yield crude 2-(3,3-dimethylbutyl)-1-(methoxycarbonyl)piperidine-4-carboxylic acid (2.42 g, 94 %) as a black solid. MS m/z 272 $(M+H)^+$

Step 6: Cis-methyl 2-(3,3-dimethylbutyl)-4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate

A suspension of magnesium chloride (0.852 g, 8.94 mmol) and ethyl potassium malonate (2.283 g, 13.42 mmol) in dry THF (40 mL) was stirred under nitrogen atmosphere at 50°C for 3.5 h (flask 1). In another flask was added carbonyldiimidazole (1.740 g, 10.73 mmol) portionwise to a solution of 2-(3,3-dimethylbutyl)-1-(methoxycarbonyl)piperidine-4-carboxylic acid (2.427 g, 8.94 mmol) in dry THF (40 mL) at 0°C under nitrogen atmosphere. This solution was stirred for 1.5 h (flask 2). The contents of flask 1 was added over 10 min to flask 2 and the resulting mixture was stirred at room temperature for 20 h. The reaction mixture was concentrated and the residue was taken up in EtOAc and H2O. The aqueous phase was extracted once with EtOAc and the combined organic phase was washed with H_2O, satd $NaHCO_3$ and then dried over Na_2SO_4, filtered and evaporated to give a black oil. Separation using Biotage (340g column, grad 20-30% EtOAc in heptane 7 CV) yielded cis-methyl 2-(3,3-dimethylbutyl)-4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate (1.74 g, 72 %) as a yellow oil. Cis-isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (s, 9H), 1.03 – 1.95 (m, 10H), 2.01 – 2.10 (m, 1H), 2.65 – 2.75 (m, 1H), 2.98 – 3.08 (m, 1H), 3.51 (s, 2H), 3.69 (s, 3H), 3.83 – 4.02 (m, 2H), 4.20 (q, *J* = 7.14, 7.14, 7.14 Hz, 2H). MS m/z 342 $(M+H)^+$.

Step 7: Cis-methyl 2-(3,3-dimethylbutyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate

A solution of cis-methyl 2-(3,3-dimethylbutyl)-4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate (1.78 g, 5.22 mmol) in MeOH (3.9 mL) was added to a solution of NaOH (288 mg, 7.2 mmol) in MeOH/H2O (4.4 mL / 0.3 mL) at -30°C. After 10 min was added hydroxylamine hydrochloride (726 mg, 10.45 mmol) and NaOH (418 mg, 10.45 mmol) in MeOH (5.2 mL) and H2O (5.2 mL). Stirring was continued at -30°C for 30 min. The reaction solution was poured into 6 M HCl (7.7 mL) at 80°C and heated at 80°C for 1 h. Concentration of the organic solvent and extraction with DCM (x2), drying using a phase separator and

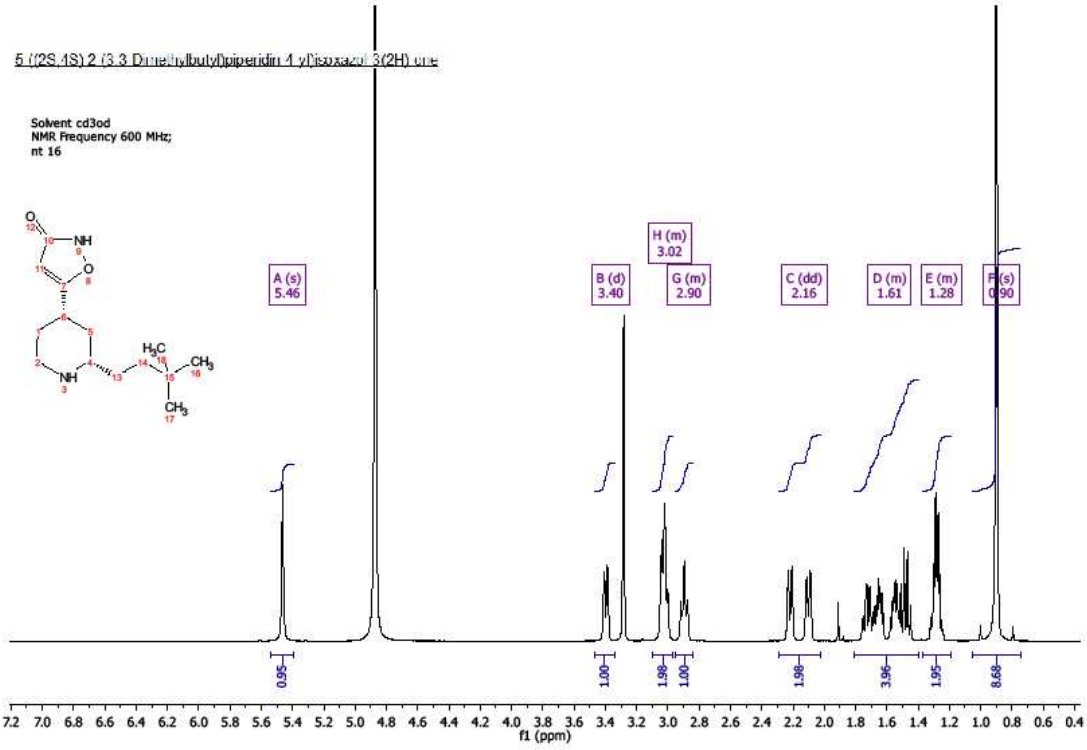
evaporation gave an orange oil. The compound was purified by preparative HPLC on a Kromasil C8 column (10 µm 250x50 ID mm) using a gradient of 30-60% Acetonitrile in H2O/MeCN/FA 95/5/0.2 buffer over 30 minutes with a flow of 100 mL/min. Cis-methyl 2-(3,3-dimethylbutyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (717 mg, 44 %) was isolated. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (s, 9H), 1.01 – 1.19 (m, 2H), 1.23 – 1.48 (m, 2H), 1.83 – 1.96 (m, 2H), 1.98 – 2.11 (m, 2H), 2.94 – 3.04 (m, 1H), 3.09 – 3.19 (m, 1H), 3.70 (s, 3H), 3.91 – 4.06 (m, 2H), 5.71 (d, *J* = 0.91 Hz, 1H). MS m/z 311 $(M+H)^+$

Step 8: (2S,4S)-Methyl 2-(3,3-dimethylbutyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate

Cis-methyl 2-(3,3-dimethylbutyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (717 mg, 2.31 mmol) was subjected to chiral preparative HPLC (Column: Chiralpak IC (250x20), 5 µm particle size, mobile phase: Heptane/IPA 80/20, flow rate 18 mL/min) to yield (2S,4S)-methyl 2-(3,3-dimethylbutyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)-piperidine-1-carboxylate (341 mg, 48 %), Chiral purity 99.9 %ee, Optical rotation $[\alpha]_D^{20} = +36.5$ (acetonitrile, c=1)

Step 9: 5-((2S,4S)-2-(3,3-Dimethylbutyl)piperidin-4-yl)isoxazol-3(2H)-one

(2S,4S)-Methyl 2-(3,3-dimethylbutyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (341 mg, 1.1 mmol) was dissolved in hydrogen bromide (33 % in acetic acid, 20 mL) and the mixture was stirred at room temperature for 24 h. The solvent was evaporated and the compound was purified by preparative HPLC on a XBridge C18 clumn (10 µm 250x50 ID mm) using a gradient of 5-30% Acetonitrile in H2O/MeCN/NH3 95/5/0.2 buffer over 15 minutes with a flow of 100 mL/min. 5-((2S,4S)-2-(3,3-Dimethylbutyl)piperidin-4-yl)-isoxazol-3(2H)-one (235 mg, 85 %) was isolated. ^{1}H NMR (600 MHz, MeOH-d_4) δ 0.90 (s, 9H), 1.19 – 1.37 (m, 2H), 1.4 – 1.81 (m, 4H), 2.16 (dd, *J* = 13.85, 70.70 Hz, 2H), 2.84 – 2.95 (m, 1H), 2.97 – 3.1 (m, 2H), 3.40 (d, *J* = 11.52 Hz, 1H), 5.46 (s, 1H); HRMS Calculated for $[C_{14}H_{24}N_2O_2+H]^+$: 253.1916. Found: 253.1898.



Compound 21 (Method D)
5-((2S,4S)-2-(Cyclohexylmethyl)piperidin-4-yl)isoxazol-3(2H)-one

Step 1: Methyl 2-benzylisonicotinate
Methyl 2-chloroisonicotinate (4.29 g, 25 mmol) and $Pd(PPh_3)_4$ (1.156 g, 1.00 mmol) were dissolved in THF (60 mL) to give a yellow solution. Then benzylzinc(II) bromide (0.5 M in THF) (75 mL, 37.50 mmol) was added. The resulting brown mixture was warmed to 60°C in an oil-bath for 18 h. The reaction mixture was quenched by addition of methanol, then diluted with ethyl acetate and washed with satd NH_4Cl and water. The combined organic layers were dried over magnesium sulfate and evaporated. The residue was purified via Biotage, Thompsson 160 g Silica, eluent isocratic heptanes/ethyl acetate 9:1 over 1 CV, then linear gradient 9:1-75:25 over 6 CV. Product containing fractions were evaporated to give methyl 2-benzylisonicotinate as an orange liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.91 (s, 3H), 4.22 (s, 2H), 7.16 – 7.53 (m, 5H), 7.64 – 7.74 (m, 2H), 8.70 (d, *J* = 5.07 Hz, 1H).

Step 2: Methyl 2-(cyclohexylmethyl)piperidine-4-carboxylate
Methyl 2-benzylisonicotinate (1.29 g, 5.67 mmol) and PtO_2 (0.13 g, 0.57 mmol) were added to acetic acid (50 mL). The reaction mixture was hydrogenated in a Büchi hydrogentor at 8 bar at room temperature for 3 days. Methanol (100 mL) was added and the catalyst filtered off. The solvent was evaporated. The crude product was partitioned between Na_2CO_3 (aq) and ethyl acetate. The organic phase was isolated, dried over Na_2SO_4, filtered through Celite® and the solvent was evaporated. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.75 – 0.95 (m, 2H), 1.04 – 1.73 (m, 13H), 1.81 – 1.92 (m, 2H), 2.36 (tt, *J* = 3.72, 3.72, 12.33, 12.33 Hz, 1H), 2.51 – 2.57 (m, 1H), 2.61 (td, *J* = 2.59, 12.32, 12.36 Hz, 1H), 3.04 – 3.18 (m, 1H), 3.64 (s, 3H).

Step 3: Dimethyl 2-(cyclohexylmethyl)piperidine-1,4-dicarboxylate
Methyl 2-(cyclohexylmethyl)piperidine-4-carboxylate (1.79 g, 7.48 mmol), methyl carbonochloridate (1.06 g, 11.2 mmol) and DIPEA (1.93 g, 15.0 mmol) were added to dichloromethane (60 mL) at room temperature and stirred for 2 h. The reaction mixture was diluted with diethylether and washed with water. The organic phase was dried over magnesium sulfate, filtered through Celite® and the solvent was evaporated. Crude product 2.2 g. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.74 – 0.99 (m, 2H), 1.02 – 2.06 (m, 15H), 2.5 – 2.62 (m, 1H), 3 – 3.14 (m, 1H), 3.61 – 3.74 (m, 6H), 3.82 – 3.95 (m, 1H), 4.14 – 4.31 (m, 1H).

Step 4: 2-(Cyclohexylmethyl)-1-(methoxycarbonyl)piperidine-4-carboxylic acid
Dimethyl 2-(cyclohexylmethyl)piperidine-1,4-dicarboxylate (2.20 g, 7.40 mmol) was dissolved in THF (25 mL) and water (25 mL). LiOH (0.266 g, 11.1 mmol) was added and the resulting mixture was stirred at room temperature overnight and heated under reflux for 30 min. The reaction mixture was partitioned between 1 M $KHSO_4$ and diethyl ether. The organic phase was dried (Na_2SO_4), filtered through Celite® and the solvent was evaporated. The crude product was dissolved again in THF (25 mL) and water (25 mL). LiOH (0.23 g) was added to the reaction flask. The reaction mixture was stirred overnight. The reaction was worked up as above to give the product. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.74 – 1 (m, 2H), 1.04 – 2.03 (m, 15H), 2.55 – 2.65 (m, 1H), 3 – 3.15 (m, 1H), 3.67 (s, 3H), 3.88 (d, *J* = 10.34 Hz, 1H), 4.16 – 4.29 (m, 1H).

Step 5: Cis-Methyl 2-(cyclohexylmethyl)-4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate
Ethyl potassium malonate (1.75 g, 10.3 mmol) and $MgCl_2$ (0.665 g, 6.88 mmol) were added to dry THF (50 mL). The reaction flask was stirred vigorously 4 h at 50°C (flask 1). 2-(Cyclohexylmethyl)-1-(methoxycarbonyl)piperidine-4-carboxylic acid (1.95 g, 6.88 mmol) and carbonyldiimidazole (1.34 g, 8.26 mmol) were added to dry THF (50 mL) at 5°C (flask 2). The contents of flask 2 was added to flask 1 at room temperature. The reaction mixture was evaporated to remove most of the THF. The crude was partitioned between water and diethyl ether. The organic phase was isolated, dried with magnesium sulfate, filtered through Celite®

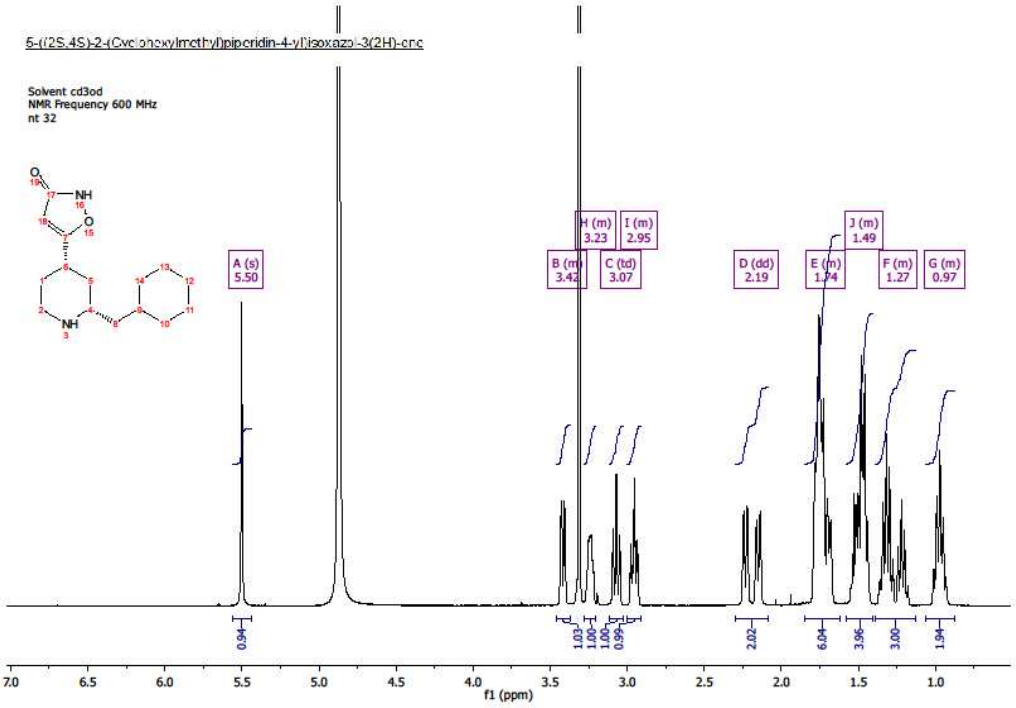
and the solvent was evaporated. The residue was purified by automated column chromatography using the Biotage equipment. Gradient eluation using ethylacetate-heptane, started 15-85 and ended 40-60 which gave cis-methyl 2-(cyclohexylmethyl)-4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate (1.28 g, 53 %). Cis-isomer ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.72 – 1.96 (m, 19H), 2.02 (dt, *J* = 6.19, 6.19, 14.07 Hz, 1H), 2.61 – 2.71 (m, 1H), 2.97 – 3.05 (m, 1H), 3.48 (s, 2H), 3.66 (s, 3H), 3.85 (dd, *J* = 4.08, 13.97 Hz, 1H), 4.06 – 4.24 (m, 3H).

Step 6: (2S,4S)-Methyl 2-(cyclohexylmethyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)-piperidine-1-carboxylate

A solution of cis-methyl 2-(cyclohexylmethyl)-4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate (1.28 g, 3.62 mmol) in MeOH (3 mL) was added dropwise to a solution of NaOH (0.159 g) in MeOH/H_2O (3 mL/0.2 mL) at -30°C. After stirring for 10 minutes a solution of hydroxylamine hydrochloride (0.50 g, 7.24 mmol) and NaOH (0.29 g, 7.24 mmol) in methanol/water (5 mL/5 mL) was added at -30°C. Stirring was continued for 30 minutes at -30°C. The solution was added dropwise to HCl (6M) at 80°C. Stirred 30 minutes at 80°C. The reaction mixture was partitioned between water and ethyl acetate. The organic phase was isolated, dried with Na_2SO_4, filtered through Celite® and the solvent was evaporated. Acidic reversed phase chromatography, gradient 35% to 75% acetonitrile gave cis-methyl 2-(cyclohexylmethyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (0.62 g, 53.1 %). Chiral separation using Chiralpac IC, mobile phase heptane/isopropyl alcohol 80/20 at the temperature 40°C gave (2S,4S)-methyl 2-(cyclohexylmethyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (0.27 g), e.e 98.5%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.67 – 1.67 (m, 13H), 1.77 – 1.86 (m, 2H), 1.94 – 2.05 (m, 2H), 2.86 – 2.98 (m, 1H), 3.03 – 3.15 (m, 1H), 3.65 (s, 3H), 3.82 – 3.93 (m, 1H), 4.08 – 4.21 (m, 1H), 5.65 (d, *J* = 0.87 Hz, 1H); $[\alpha]_D^{20} = +29.9$ (MeCN, c = 1).

Step 7: 5-((2S,4S)-2-(Cyclohexylmethyl)piperidin-4-yl)isoxazol-3(2H)-one

HBr (33 % in acetic acid) (5 mL) was added to (2S,4S)-methyl 2-(cyclohexylmethyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (0.27g, 0.85 mmol). The reaction was stirred vigorously overnight. The solvent was evaporated. Purification using PrepLC (pH=11, small column, sample dissolved in acetonitrile/water (40/60), gradient 15-55, 20 minutes) gave the title compound (75 mg, 33 %). ^{1}H NMR (600 MHz, MeOH-d_4) δ 0.88 – 1.06 (m, 2H), 1.13 – 1.39 (m, 3H), 1.4 – 1.58 (m, 4H), 1.62 – 1.85 (m, 6H), 2.19 (dd, *J* = 14.06, 49.79 Hz, 2H), 2.91 – 3 (m, 1H), 3.07 (td, *J* = 2.95, 13.12, 13.14 Hz, 1H), 3.2 – 3.28 (m, 1H), 3.37 – 3.46 (m, 1H), 5.50 (s, 1H); HRMS calculated for $[C_{15}H_{25}N_2O_2]$+: 265.1916; found: 265.1939.



Compound 22 (Method B)
5-((2S,4S)-2-Phenethylpiperidin-4-yl)isoxazol-3(2H)-one

Step 1: Methyl 4-oxo-2-phenethyl-3,4-dihydropyridine-1(2H)-carboxylate
4-Methoxypyridine (9.30 mL, 91.64 mmol) was dissolved in THF (150 mL) under nitrogen atmosphere and cooled to -15°C. Phenethylmagnesium chloride (93 mL, 93.47 mmol, 1 M in THF) was added dropwise and a suspension was formed. After stirring at –20°C for 30 minutes methyl chloroformate (9.23 mL, 119.13 mmol) was added over 1 minute. Stirring was continued at -10°C for 1 h and then HCl (10%) was added. The mixture was stirred for 20 minutes and then concentrated. The aqueous phase was extracted with ether (x2) and the organic phase was dried (magnesium sulfate) and evaporated to yield methyl 4-oxo-2-phenethyl-3,4-dihydropyridine-1(2H)-carboxylate (21.7 g, 82 %) as an oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.98 (m, 2H), 2.54 (m, 2H), 2.69 (m, 1H), 2.81 (m, 1H), 3.83 (s, 3H), 4.62 (m, 1H), 5.33 (m, 1H), 7.12 – 7.38 (m, 5H), 7.72 (m, 1H); MS m/z 260 $(M+H)^+$.

Step 2: Methyl 4-oxo-2-phenethylpiperidine-1-carboxylate
Methyl 4-oxo-2-phenethyl-3,4-dihydropyridine-1(2H)-carboxylate (21.7 g, ca 75 mmol) was hydrogenated over Pd/C (5%) in EtOAc at 5 bar for 20 h. The mixture was filtered through a silica plug and then evaporated to give the product as an oil (19.8 g). ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.69 (m, 1H), 1.80 (m, 1H), 2.26 (m, 2H), 2.41 (m, 1H), 2.47 – 2.70 (m, 3H), 3.16 (m, 1H), 3.69 (m, 3H), 4.15-4,48 (br m, 2H), 7.14-7.30 (m, 5H). MS 262 m/z $(M+H)^+$.

Step 3: Methyl 4-cyano-2-phenethylpiperidine-1-carboxylate
To a solution of methyl 4-oxo-2-phenethylpiperidine-1-carboxylate (19.8 g, 75.7 mmol) in DME (250 mL) under nitrogen atmosphere was simultaneously added toluene-4-sulfonylmethyl isocyanide (16.6 g, 85.0 mmol, in 250 mL DME) and potassium *tert*-butoxide (197 mL, 1 M in *tert*-butanol) over 1 h so that the temperature was kept below -10°C. The mixture was then stirred at -10°C for 2 h and allowed to reach ambient temperature over 16 h. To the orange reaction mixture was added H_2O (400 mL), it was stirred for 20 minutes and then extracted with ether (x 3) and EtOAc (x 3). The organic phases were combined, dried over Na_2SO_4 and evaporated to give 24.8 g of residue. Flash chromatography using

EtOAc/heptane (30-80% gradient EtOAc) gave the product (13.2 g, 64%) as a mixture of diastereomers (major cis isomer). MS m/z 273 $(M+H)^+$. Cis isomer: ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.76 (m, 1H), 1.88 (m, 1H), 1.92 – 2.07 (m, 2H), 2.07 – 2.15 (m, 1H), 2.24 – 2.38 (m, 1H), 2.55 – 2.72 (m, 2H), 2.96 (m, 1H), 3.16 – 3.29 (m, 1H), 3.69 (s, 3H), 4.10 (m, 1H), 4.35 (m, 1H), 7.15 – 7.35 (m, 5H).

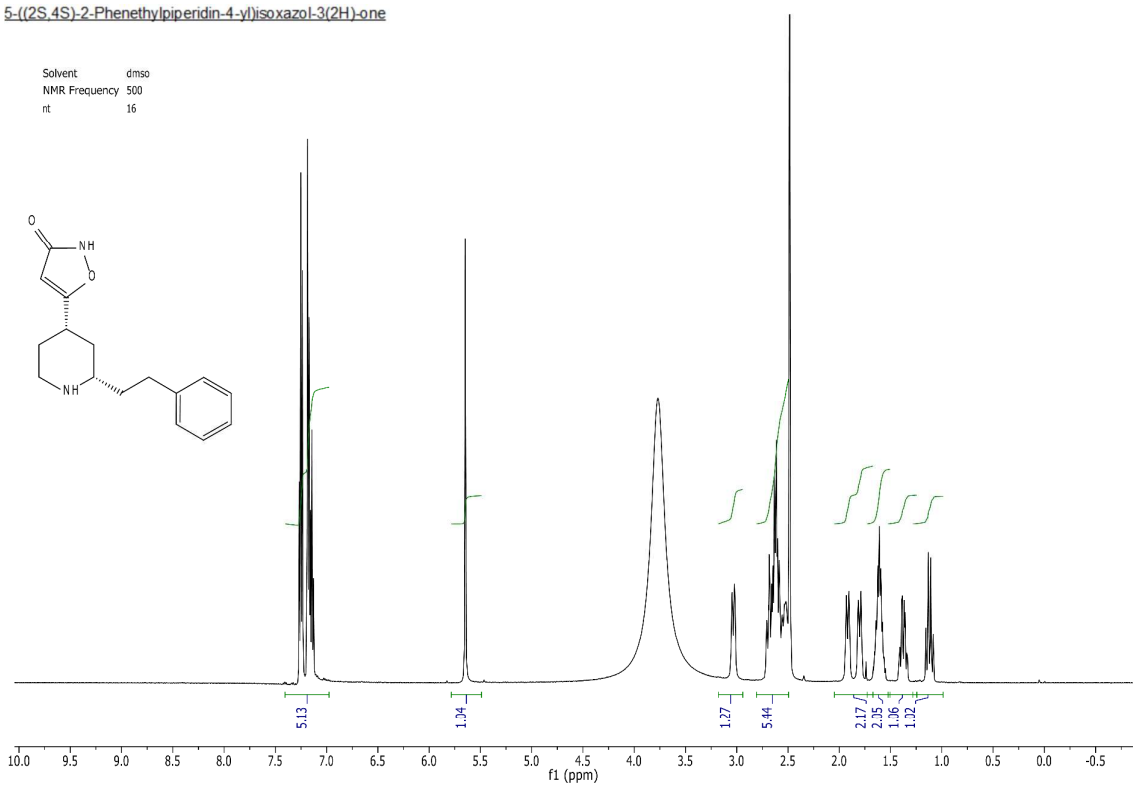
Step 4: 1-(Methoxycarbonyl)-2-phenethylpiperidine-4-carboxylic acid
To methyl 4-cyano-2-phenethylpiperidine-1-carboxylate (13.2 g, 48.5 mmol) in a microwave reaction vial was added 6 M HCl. The mixture was heated to 100°C for 30 min in a single node microwave reactor. The aqueous phase was extracted with EtOAc and the resulting organic phase was washed once with 10% HCl, dried over magnesium sulfate and evaporated to give crude product 3.47 g. MS m/z 290 $(M-H)^-$.

Step 5: Cis-methyl 4-(3-ethoxy-3-oxopropanoyl)-2-phenethylpiperidine-1-carboxylate
The compound was prepared as described in 14, Step 5 starting from crude 1-(methoxycarbonyl)-2-phenethylpiperidine-4-carboxylic acid (5.7 g, 19.6 mmol), magnesium chloride (1.86 g, 19.6 mmol), ethyl potassium malonate (4.99 g, 29.3 mmol) and carbonyldiimidazole (3.81 g, 23.5 mmol) which resulted in cis-methyl 4-(3-ethoxy-3-oxopropanoyl)-2-phenethylpiperidine-1-carboxylate (0.29 g, 4 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.25 (t, *J = 7.1 Hz,* 3H), 1.63 – 1.82 (m, 3H), 1.91 (m, 3H), 2.00 – 2.12 (m, 1H), 2.53 – 2.66 (m, 2H), 2.71 (m, 1H), 3.09 (m, 1H), 3.49 (s, 2H), 3.69 (s, 3H), 3.89 (m, 1H), 4.08 (m, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 7.15 – 7.31 (m, 5H). MS m/z 362 $(M+H)^+$.

Step 6: Cis-methyl 4-(3-oxo-2,3-dihydroisoxazol-5-yl)-2-phenethylpiperidine-1-carboxylate
The compound was prepared as described in 15, Step 6 starting from cis-methyl 4-(3-ethoxy-3-oxopropanoyl)-2-phenethylpiperizdine-1-carboxylate (0.29 g, 0.82 mmol) resulting in the title compound (0.07 g, 26%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.65 (m, 1H), 1.79 – 2.14 (m, 5H), 2.60 (m, 2H), 3.00 (m, 1H), 3.20 (m, 1H), 3.70 (s, 3H), 3.92 (m, 1H), 4.10 (m, 1H), 5.71 (s, 1H), 7.10 – 7.29 (m, 5H); MS m/z 331 $(M+H)^+$.

Step 7: (2S,4S)-Methyl 4-(3-oxo-2,3-dihydroisoxazol-5-yl)-2-phenethylpiperidine-1-carboxylate
Following the procedure described in 15, Step 7, racemic cis-methyl 4-(3-oxo-2,3-dihydroisoxazol-5-yl)-2-phenethylpiperidine-1-carboxylate (0.07 g, 0.22 mmol) was subjected to chiral separation using Chiralcel IC, mobile phase heptane/IPA 80/20 at 40°C which resulted in (2S,4S)-methyl 4-(3-oxo-2,3-dihydroisoxazol-5-yl)-2-phenethylpiperidine-1-carboxylate (0.032 g, 0.1 mmol).

Step 8: 5-((2S,4S)-2-Phenethylpiperidin-4-yl)isoxazol-3(2H)-one
Starting from (2S,4S)-methyl 4-(3-oxo-2,3-dihydroisoxazol-5-yl)-2-phenethylpiperidine-1-carboxylate (0.032 g) and following the procedure described in 15, Step 8 could the title compound be obtained (12.6 mg, 21%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.15 (m, *J* = 12.4 Hz, 1H), 1.38 (m, *J* = 12,4; 4.5 Hz, 1H), 1.60 (m, 2H), 1.79 (br d, *J* = 12.8 Hz, 1H), 1.95 (br d, *J* = 12.8 Hz, 1H), 2.45 – 2.75 (m, 5H), 3.05 (m, 1H), 5.77 (s, 1H), 7.15 – 7.30 (m, 5H); ^{13}C NMR (126 MHz, CH_4-d_4) δ 177.01, 174.74, 141.85, 129.46, 129.17, 127.14, 95.17, 56.87, 45.25, 36.99, 34.63, 34.25, 32.13, 28.30; $[\alpha]^{20}_D$ +54.1 (MeOH/H_2O 1:1, c = 1); HRMS calculated for $[C_{16}H_{21}N_2O_2]$+: 273.1603; found: 273.1601.



Compound 23 (Method D)
5-((2R,4S)-2-(2-Fluorobenzyl)piperidin-4-yl)isoxazol-3(2H)-one

Step 1: Methyl 2-(2-fluorobenzyl)isonicotinate
Methyl 2-chloroisonicotinate (5.2 g, 30.31 mmol) and $Pd(PPh_3)_4$ (0.700 g, 0.61 mmol) were dissolved under nitrogen in THF (100 mL) and (2-fluorobenzyl)zinc(II) chloride (0.5 M in THF) (90 mL, 45.00 mmol) was added and the brown solution was stirred at 60°C for 18 h. The reaction was quenched by addition of methanol (50.0 mL). The solution was diluted with EtOAc, washed with NH_4Cl (aq) and dried over Na_2SO_4, then evaporated. The compound was purified in 2 runs via Biotage, SNAP 340g KP-SIL, eluent isocratic heptane/ethyl acetate 8:2 for 2 CV, then linear gradient heptane/ethyl acetate 8:2 to 5:5 over 5 CV to give methyl 2-(2-fluorobenzyl)isonicotinate (5.42 g, 73%) as a yellow oil. ^1H NMR (400 MHz, $CDCl_3$) 3.92 (s, 3H), 4.25 (s, 2H), 7.00 – 7.13 (m, 2H), 7.18 – 7.30 (m, 2H), 7.64 – 7.74 (m, 2H), 8.69 (d, 1H). MS m/z 246 $(M+H)^+$

Step 2: Methyl 2-(2-fluorobenzyl)piperidine-4-carboxylate
Methyl 2-(2-fluorobenzyl)isonicotinate (5.42 g, 22.08 mmol) was dissolved in acetic acid (50 mL) and platinum(IV) oxide (0.251 g, 1.10 mmol) added. The resulting mixture was hydrogenated in a Büchi hydrogenator for 4 h at room temperature and 5 bar. The catalyst was filtered off and washed with MeOH and the eluate evaporated. DCM and 10 % K_2CO_3 were added and the phases separated. The water phase was extracted with DCM and the combined organic phase washed with water, passed through a phase separator and evaporated to yield methyl 2-(2-fluorobenzyl)piperidine-4-carboxylate (4.49 g, 81 %) MS m/z 252 $(M+H)^+$

Step 3: Dimethyl 2-(2-fluorobenzyl)piperidine-1,4-dicarboxylate

To a solution of methyl 2-(2-fluorobenzyl)piperidine-4-carboxylate (4.49 g, 17.85 mmol) and DIPEA (9.35 mL, 53.55 mmol) in DCM (100 mL) was added methyl chloroformate (1.798 mL, 23.21 mmol) in DCM (50 mL). The reaction mixture was stirred for 1.5 h. The organic phase was washed with satd $NaHCO_3$. The phases were separated and the organic phase dried using a phase separator to yield crude dimethyl 2-(2-fluorobenzyl)piperidine-1,4-dicarboxylate (6.16 g, 112 %). MS m/z 310 $(M+H)^+$

Step 4: 2-(2-Fluorobenzyl)-1-(methoxycarbonyl)piperidine-4-carboxylic acid

Dimethyl 2-(2-fluorobenzyl)piperidine-1,4-dicarboxylate (6.16 g, crude) was dissolved in acetonitrile (45 mL) and water (0.9 mL). Lithium bromide (13.84 g, 159.39 mmol) and triethylamine (11.05 mL, 79.69 mmol) were added and the mixture was heated at reflux overnight. Water (90 mL) and MTBE were added. The organic phase was extracted with water (x2). To the pooled aqueous layer was added MTBE and the solution was acidified to pH 1 with 2 M HCl and then extracted with MTBE (x2). The combined organic layer was dried over Na_2SO_4 and evaporated to give 2-(2-fluorobenzyl)-1-(methoxycarbonyl)piperidine-4-carboxylic acid (4.48 g, 76 %) as a slightly yellow semisolid. MS m/z 296 $(M+H)^+$

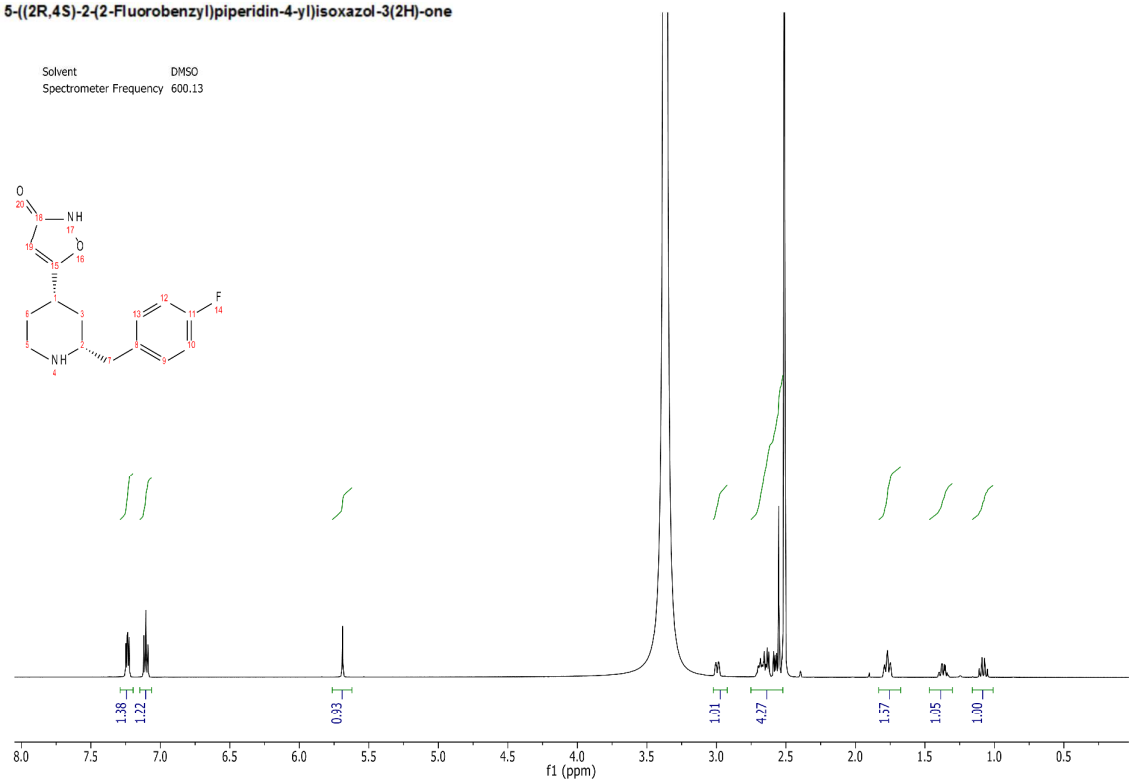
Step 5: Cis-methyl 4-(3-ethoxy-3-oxopropanoyl)-2-(2-fluorobenzyl)piperidine-1-carboxylate
A suspension of magnesium chloride (2.60 g, 27.33 mmol) and ethyl potassium malonate (4.65 g, 27.33 mmol) in dry THF (80 mL) was stirred under nitrogen atmosphere at 50°C for 2.5 h (flask 1). In another flask di(1H-imidazol-1-yl)methanone (3.69 g, 22.77 mmol) was added portionwise to a solution of 2-(2-fluorobenzyl)-1-(methoxycarbonyl)piperidine-4-carboxylic acid (4.483 g, 15.18 mmol) in dry THF (20 mL) at 0°C under nitrogen atmosphere. The ice bath was removed and the solution was stirred for 2 h at room temperature (flask 2). The contents of flask 1 was added slowly to flask 2 and the resulting mixture was stirred for 19 h under nitrogen. The reaction mixture was concentrated and the residue was taken up in EtOAc and H_2O. The aqueous phase was extracted once with EtOAc and the combined organic phases were washed with H_2O, 10 % Na_2CO_3 and then dried over Na_2SO_4. The compound was purified further via Biotage, 2 runs, SNAP 340g KP-SIL, first 8:2 for 2 CV then linear gradient heptanes/ethyl acetate 8:2 to 3:7 over 7CV. Cis-methyl 4-(3-ethoxy-3-oxopropanoyl)-2-(2-fluorobenzyl)piperidine-1-carboxylate (3.19 g, 58 %) was isolated. ^1H NMR (400 MHz, $CDCl_3$) δ 1.20-1.30 (t, *J* = 7.4 Hz, 3H), 1.63 – 1.99 (m, 4H), 2.63-2.76 (m, 2H), 2.82 (dd, *J* = 6.1; 14.3.4 Hz, 1H), 2.95 (m, 1H), 3.46 (s, 2H), 3.53 (s, 3H), 3.85-3.99 (m, 1H), 4.17 (q, *J* = 7.4 Hz, 2H), 4.22 (m, 1H), 6.93 – 7.08 (m, 2H), 7.09 – 7.22 (m, 2H). MS m/z 366 $(M+H)^+$.
z
Step 6: Cis-methyl 2-(2-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate
Cis-methyl 4-(3-ethoxy-3-oxopropanoyl)-2-(2-fluorobenzyl)piperidine-1-carboxylate (1.95 g, 5.34 mmol) was dissolved in MeOH (20 mL) and cooled to -40°C. Sodium hydroxide (0.213 g, 5.34 mmol) dissolved in water (2.000 mL) was added dropwise and the colourless solution continued to stir at -40°C for 30 min. Hydroxylamine (50 % by weight in water, 0.327 mL, 5.34 mmol) was added dropwise. The resulting solution was stirred at -40°C for 3 h. The mixture was then rapidly poured into a prewarmed (80°C) solution of 6 M hydrogen chloride (25 mL, 150.00 mmol) and the mixture continued to stir at 80°C for 20 min. The solvent was evaporated and MTBE/water added. The phases were separated and the organic phase dried over Na_2SO_4 and evaporated to yield a slightly yellow foam. The compound was purified by preparative HPLC on a Kromasil C8 column (10 µm 250x50 ID mm) using two injections with a gradient of 15-75% Acetonitrile in H2O/MeCN/AcOH 95/5/0.2 buffer over 30 minutes with a flow of 100 mL/min. Cis-methyl 2-(2-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (1.09 g, 61%) was isolated. ^1H NMR (400 MHz, $CDCl_3$) δ 1.80 – 1.98 (m, 3H), 2.07 (m, 1H), 2.60 (dd, *J* = 7.6; 13.1, 1H), 2.76 (dd, *J* = 7.6; 13.1 Hz 1H), 2.91-3.02 (m, 1H), 3.12-3.24 (m, 1H), 3.52 (s, 3H), 3.95-4.05 (m, 1H), 4.29 – 4.40 (m, 1H), 5.72 (s, 1H), 6.94 – 7.12 (m, 3H), 7.12 – 7.20 (m, 1H). MS m/z 335 $(M+H)^+$

Step 7: (2R,4S)-Methyl 2-(2-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate

Cis-methyl 2-(2-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (1.09 g, 3.26 mmol) was subjected to chiral preparative HPLC (Column: Chiralpak AS (250x20), 5 mm particle size, mobile phase: Heptane/EtOH 80/20, flow rate 18 mL/min) to yield (2R,4S)-methyl 2-(2-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (492 mg, 45 %), Chiral purity 98.2 %ee. Optical rotation $[\alpha]_D^{20} = -3$ (acetonitrile, c=1)

Step 8: 5-((2R,4S)-2-(2-Fluorobenzyl)piperidin-4-yl)isoxazol-3(2H)-one

(2R,4S)-Methyl 2-(2-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (492 mg, 1.47 mmol) was stirred in hydrogen bromide (33 % in AcOH) overnight (19 h). Evaporation of solvents and purification by preparative HPLC (Instrument: FractionLynx I, Mobilphase: gradient 5-95% MeCN in 0.2% NH_3, pH 10, Column: Xbridge Prep C18 5µm OBD 19*150 mm) yielded 5-((2R,4S)-2-(2-fluorobenzyl)piperidin-4-yl)isoxazol-3(2H)-one (504 mg, 124%). The sample contained DMSO and acetic acid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04 (m, *J* = 11.7 Hz, 1H), 1.32 (m, *J* = 3.4; 11.7 Hz, 1H), 1.70 (m, 2.08-2.22 (m, 2H), 2.89 – 3.18 (m, 4H), 3.38-3.51 (m, 2H), 5.66 (s, 1H), 7.11 – 7.23 (m, 2H), 7.28 – 7.41 (m, 2H). HRMS Calcd for $[C_{15}H_{17}FN_2O_2+H]+$: 277.1352. Found: 277.1344.



Compound 24 (Method D)
5-((2R,4S)-2-(3-Fluorobenzyl)piperidin-4-yl)isoxazol-3(2H)-one

Step 1: Methyl 2-(3-fluorobenzyl)isonicotinate

Methyl 2-chloroisonicotinate (5.6 g, 32.64 mmol) and $Pd(PPh_3)_4$ (0.754 g, 0.65 mmol) were dissolved in THF (100 mL) under nitrogen and (3-fluorobenzyl)zinc(II) chloride (0.5 M in THF) (100 mL, 50.00 mmol) was added. The brown solution was stirred at 60°C for 4 h. The

reaction was quenched by addition of methanol (50 mL), diluted with EtOAc and washed with NH_4Cl. The organic layer was dried over Na_2SO_4, filtered and evaporated to yield a yellow oil. The compound was purified in 2 runs via Biotage, SNAP 340g KP-SIL, eluent isocratic heptane/ethyl acetate 8:2 for 2 CV, then linear gradient heptane/ethyl acetate 8:2 to 5:5 over 5 CV to yield methyl 2-(3-fluorobenzyl)isonicotinate (6.766 g, 85%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.93 (s, 3H), 4.21 (s, 2H), 6.88 – 7.00 (m, 2H), 7.02 – 7.07 (m, 1H), 7.22 – 7.30 (m, 1H), 7.66 – 7.73 (m, 2H), 8.68 – 8.73 (m, 1H). MS m/z 246 $(M+H)^+$

Step 2: Methyl 2-(3-fluorobenzyl)piperidine-4-carboxylate
Methyl 2-(3-fluorobenzyl)isonicotinate (6.766 g, 27.59 mmol) was dissolved in acetic acid (70 mL) and platinum(IV) oxide (0.313 g, 1.38 mmol) added. The resulting mixture was hydrogenated in a Büchi hydrogenator at room temperature and 5 bar for 4.5 h. More platinum(IV) oxide (0.313 g, 1.38 mmol) was added and the hydrogenation continued at 5 bar for 2 h 40 min. The catalyst was filtered off, washed with MeOH and the eluate evaporated. DCM and 10 % K_2CO_3 were added and the phases separated. The water phase was extracted with DCM and the combined organic phase washed with brine, passed through a phase separator and evaporated to yield crude methyl 2-(3-fluorobenzyl)piperidine-4-carboxylate (7.6 g, 110%) as a brown oil. MS m/z 252 $(M+H)^+$

Step 3: Dimethyl 2-(3-fluorobenzyl)piperidine-1,4-dicarboxylate
Methyl 2-(3-fluorobenzyl)piperidine-4-carboxylate (7.6 g, 30.24 mmol) was dissolved in DCM (200 mL) and DIPEA (6.32 mL, 36.29 mmol), then methyl carbonochloridate (3.3 mL, 41.91 mmol) was added. The solution was stirred at room temperature for 2 h. The reaction mixture was washed with 0.1 M HCl and satd $NaHCO_3$. The organic phase was passed through a phase separator and evaporated to yield dimethyl 2-(3-fluorobenzyl)piperidine-1,4-dicarboxylate (9.30 g, 99%) as a brown oil. MS m/z 310 $(M+H)^+$

Step 4: 2-(3-Fluorobenzyl)-1-(methoxycarbonyl)piperidine-4-carboxylic acid
Dimethyl 2-(3-fluorobenzyl)piperidine-1,4-dicarboxylate (9.153 g, 29.59 mmol) was dissolved in acetonitrile (100 mL) and water (2 mL), then lithium bromide (20.56 g, 236.72 mmol) was added. Triethylamine (16.41 mL, 118.36 mmol) was added and the resulting brown suspension was heated at reflux for 2 h. Water (100 mL) and MTBE (300 mL) were added. The organic phase was extracted with water (x2). The pooled aqueous layer was acidified to pH 1 with 3.8 M HCl and then extracted with MTBE (x2). The combined organic layer was washed with water, dried over Na_2SO_4, filtered through a Celite containing filter and evaporated. 2-(3-Fluorobenzyl)-1-(methoxycarbonyl)piperidine-4-carboxylic acid (7.07 g, 81%) was isolated as a yellow solid. MS m/z 296 $(M+H)^+$

Step 5: Cis-methyl 4-(3-ethoxy-3-oxopropanoyl)-2-(3-fluorobenzyl)piperidine-1-carboxylate
2-(3-Fluorobenzyl)-1-(methoxycarbonyl)piperidine-4-carboxylic acid (7.112 g, 24.08 mmol) was dissolved in methyl THF (150 mL) and di(1H-imidazol-1-yl)methanone (5.86 g, 36.13 mmol) added. The suspension was stirred at room temperature under nitrogen for 3.5 h (flask 1). In a separate flask potassium 3-ethoxy-3-oxopropanoate (7.38 g, 43.35 mmol) was suspended in methyl THF (150 mL) and magnesium chloride (4.13 g, 43.35 mmol) added. The suspension was stirred at 50°C under nitrogen for 3 h (flask 2). The orange suspension in flask 1 was now added to the white suspension in flask 2. The resulting yellow suspension was stirred under nitrogen at room temperature for 3 days. The mixture was acidified to pH 1 with 3.8 M HCl and MTBE added. The phases were separated and the organic phase extracted with water, satd $NaHCO_3$ and water. Evaporated the solvents to yield an orange oil. 42 % of the oil was purified on Biotage (20% => 50 % EtOAc in heptane, 6 CV + 50 %, 4 CV; Biotage® KP-SIL 340g column) to yield cis-methyl 4-(3-ethoxy-3-oxopropanoyl)-2-(3-fluorobenzyl)piperidine-1-carboxylate (1.8 g, 49 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (t, *J* = 7.4 Hz, 3H), 1.56 – 1.93 (m, 4H), 2.62 – 2.73 (m, 2H), 2.83 – 3.03 (m, 2H), 3.41 (s, 2H), 3.59 (s, 3H), 3.83 – 3.93 (m, 1H), 4.07 – 4.20 (m, 3H), 6.82 – 6.93 (m, 3H), 7.15 – 7.22 (m, 1H). MS m/z 366 $(M+H)^+$.

Step 6: Cis-methyl 2-(3-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate

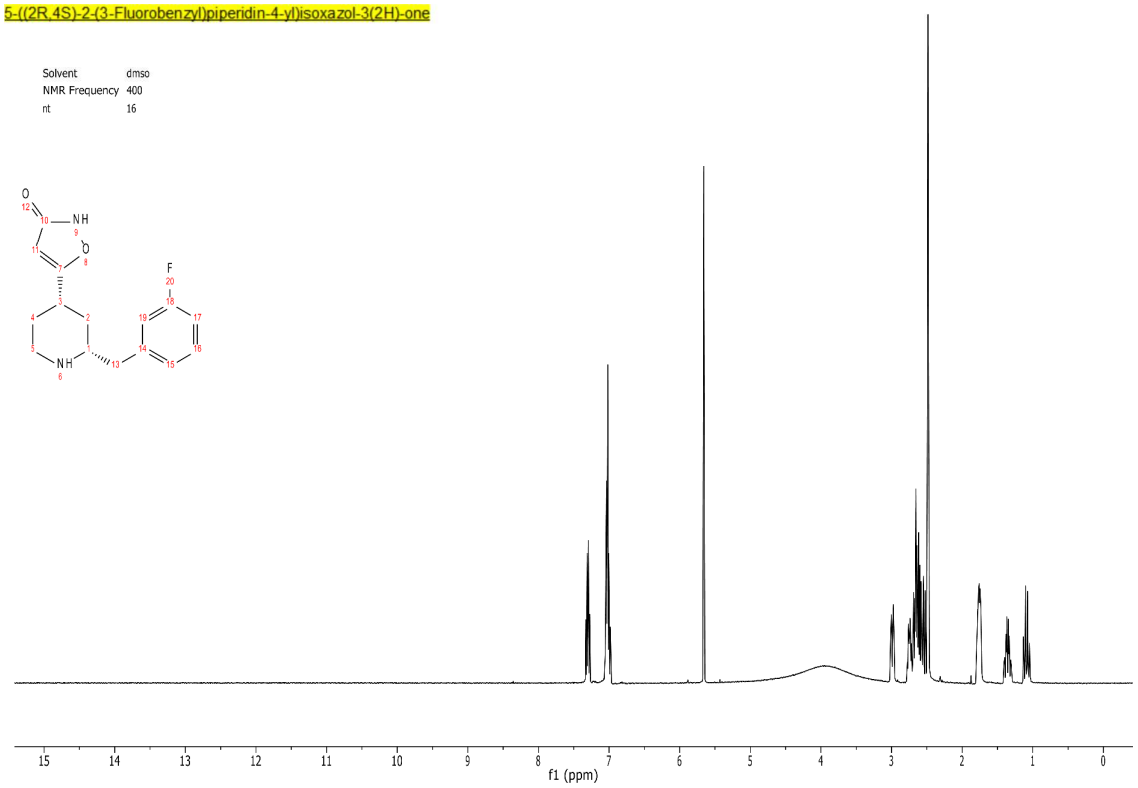
Cis-methyl 4-(3-ethoxy-3-oxopropanoyl)-2-(3-fluorobenzyl)piperidine-1-carboxylate (1.826 g, 5.00 mmol) was dissolved in MeOH (20 mL) and cooled to -40°C under nitrogen. Sodium hydroxide (0.200 g, 5.00 mmol) dissolved in water (2.000 mL) was added during 20 min and the yellow solution continued to stir at -40°C for 20 min. Hydroxylamine (50 % by weight in water, 0.306 mL, 5.00 mmol) was added during 8 min. The resulting solution was stirred at -40°C for 3.5 h. The mixture was then rapidly poured into a prewarmed (80°C) solution of 6 M hydrogen chloride (25 mL, 150.00 mmol) and the mixture continued to stir at 80°C for 20 min. The solvent was evaporated and DCM/water added. The phases were separated and the organic phase passed through a phase separator and evaporated to yield a brown semi-solid. The compound was purified by preparative HPLC in 2 injections on a XBridge C18 column (10 µm 250x50 ID mm) using a gradient of 0-25 % Acetonitrile in H2O/MeCN/NH3 95/5/0.2 buffer over 20 minutes with a flow of 100 mL/min. Cis-methyl 2-(3-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (1.141 g, 68%) was isolated as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.83 – 2.15 (m, 4H), 2.58 – 2.67 (dd, *J* = 8.3; 13.5 Hz, 1H), 2.77 – 2.86 (dd, *J* = 8.3; 13.5 Hz, 1H), 2.93 – 3.03 (m, 1H), 3.10 – 3.21 (m, 1H), 3.63 (s, 3H), 3.96 – 4.05 (m, 1H), 4.22 – 4.32 (m, 1H), 5.73 (s, 1H), 6.81 – 6.95 (m, 3H), 7.18 – 7.27 (m, 1H). MS m/z 335 $(M+H)^+$

Step 7: (2R,4S)-Methyl 2-(3-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate

Cis-methyl 2-(3-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (1.141 g, 3.42 mmol) was subjected to chiral preparative HPLC (Column: Chiralpak IC (250x20), 5 µm particle size, mobile phase: Heptane/THF 80/20, flow rate 18 mL/min) to yield (2R,4S)-methyl 2-(3-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (545 mg, 48 %), Chiral purity 99.7 %ee, Optical rotation $[\alpha]_D^{20} = +17.8$ (acetonitrile, c=1)

Step 8: 5-((2R,4S)-2-(3-Fluorobenzyl)piperidin-4-yl)isoxazol-3(2H)-one

(2R,4S)-Methyl 2-(3-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (545 mg, 1.63 mmol) was dissolved in hydrogen bromide (33 % in acetic acid, 10 mL, 57.10 mmol) and the mixture stirred at room temperature for 23 h. The solvent was evaporated and the residue purified by preparative HPLC (Instrument:FractionLynx I, Mobilphase: gradient 5-95% MeCN in 0.2% NH_3, pH 10, Column: Xbridge Prep C18 5µm OBD 19*150 mm) to yield 5-((2R,4S)-2-(3-fluorobenzyl)piperidin-4-yl)isoxazol-3(2H)-one (286 mg, 63.5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.09 (m, *J* = 11.0 Hz, 1H), 1.36 (m, *J* = 3.9; 11.0 Hz 1H), 1.71 – 1.81 (m, 2H), 2.50 – 2.79 (m, 5H), 2.95 – 3.03 (m, 1H), 5.66 (s, 1H), 6.95 – 7.07 (m, 3H), 7.26 – 7.34 (m, 1H). HRMS Calculated for $[C_{15}H_{17}FN_2O_2+H]^+$: 277.1352. Found: 277.1343



Compound 25 (Method D)
5-((2R,4S)-2-(4-Fluorobenzyl)piperidin-4-yl)isoxazol-3(2H)-one

Step 1: Methyl 2-(4-fluorobenzyl)isonicotinate
Methyl 2-chloroisonicotinate (5.6 g, 32.64 mmol) and $Pd(PPh_3)_4$(0.754 g, 0.65 mmol) were dissolved under nitrogen in THF (100 mL) and (4-fluorobenzyl)zinc(II) chloride (0.5 M in THF) (100 mL, 50.00 mmol) was added. The brown solution was stirred at 60°C for 19 h. The reaction was quenched by addition of methanol (50.0 mL). The solution was diluted with EtOAc and washed with NH_4Cl (aq) and water. The organic layer was evaporated, dissolved in DCM and washed with NH4Cl (aq) and then dried by passage through a phase separator. The solvent was evaporated to yield a brown oil. The compound was purified in 2 runs via Biotage, SNAP 340g KP-SIL, eluent isocratic heptane/ethyl acetate 8:2 for 2 CV, then linear gradient heptane/ethyl acetate 8:2 to 5:5 over 5 CV to yield methyl 2-(4-fluorobenzyl)isonicotinate 7.08 g (88%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 (s, 3H), 4.18 (s, 2H), 6.94 – 7.02 (m, 2H), 7.19 – 7.27 (m, 2H), 7.65 – 7.69 (m, 2H), 8.68 – 8.71 (m, 1H). MS m/z 246 $(M+H)^+$

Step 2: Methyl 2-(4-fluorobenzyl)piperidine-4-carboxylate
Methyl 2-(4-fluorobenzyl)isonicotinate (5.06 g, 20.63 mmol) was dissolved in acetic acid (50 mL) and platinum(IV) oxide (0.234 g, 1.03 mmol) added. The resulting mixture was hydrogenated in a Büchi hydrogenator overnight at room temperature and 5 bar. The catalyst was filtered off and washed with MeOH and the eluate evaporated. DCM and 10 % K_2CO_3 were added and the phases separated. The aqueous phase was extracted with DCM and the combined organic phase washed with brine, passed through a phase separator and evaporated to yield methyl 2-(4-fluorobenzyl)piperidine-4-carboxylate (3.816 g, 73.6 %) as a yellow oil. MS m/z 252 $(M+H)^+$

Step 3: Dimethyl 2-(4-fluorobenzyl)piperidine-1,4-dicarboxylate

Methyl 2-(4-fluorobenzyl)piperidine-4-carboxylate (4.981 g, 19.82 mmol) was dissolved in DCM (150 mL) and DIPEA (4.14 mL, 23.79 mmol), then methyl carbonochloridate (1.873 mL, 23.79 mmol) was added. The solution was stirred at room temperature for 50 min. More methyl carbonochloridate (a few drops) were added and the reaction continued at room temperature for 1 h. The reaction mixture was washed with 0.1 M HCl and satd $NaHCO_3$. The organic phase was passed through a phase separator and evaporated to yield dimethyl 2-(4-fluorobenzyl)piperidine-1,4-dicarboxylate (5.82 g, 95 %) as a yellow oil. MS m/z 310 $(M+H)^+$

Step 4: 2-(4-Fluorobenzyl)-1-(methoxycarbonyl)piperidine-4-carboxylic acid
Dimethyl 2-(4-fluorobenzyl)piperidine-1,4-dicarboxylate (5.797 g, 18.74 mmol) was dissolved in acetonitrile (60 mL) and water (1.2 mL), then lithium bromide (13.02 g, 149.92 mmol) was added. Triethylamine (10.39 mL, 74.96 mmol) was added and the resulting yellow suspension was heated at reflux for 1.5 h. water (60 mL) and MTBE (120 mL) were added. The organic phase was extracted with water (x2). The pooled aqueous layer was acidified to pH 1 with 3.8 M HCl and then extracted with MTBE (x2). The combined organic layer was washed with water and evaporated. Traces of water were azeotropically removed by MeCN. 2-(4-Fluorobenzyl)-1-(methoxycarbonyl)piperidine-4-carboxylic acid (4.43 g, 80 %) was isolated as a beige solid. MS m/z 296 $(M+H)^+$

Step 5: Cis-methyl 4-(3-ethoxy-3-oxopropanoyl)-2-(4-fluorobenzyl)piperidine-1-carboxylate
2-(4-Fluorobenzyl)-1-(methoxycarbonyl)piperidine-4-carboxylic acid (4.428 g, 14.99 mmol) was dissolved in methyl THF (100 mL) and di(1H-imidazol-1-yl)methanone (3.65 g, 22.49 mmol) added. The suspension was stirred at room temperature overnight under nitrogen (flask 1). In a separate flask potassium 3-ethoxy-3-oxopropanoate (4.59 g, 26.99 mmol) was suspended in methyl THF (100 mL) and magnesium chloride (2.57 g, 26.99 mmol) added. The suspension was stirred at 50°C under nitrogen for 3 h (flask 2). The slightly yellow suspension in flask 1 was now added to the white suspension in flask 2. The resulting white suspension was stirred under nitrogen at room temperature overnight. The mixture was acidified to pH 1 with 3.8 M HCl and MTBE added. The phases were separated and the organic phase extracted with water, sat $NaHCO_3$ and water. Evaporated the solvents to yield a yellow oil. The diastereoisomers were separated on Biotage (0% => 70 % EtOAc in heptane, 7 CV; Biotage® KP-SIL 340g column). Mixed fractions were repurified on Biotage (30% => 65 % EtOAc in heptane, 10 CV; Biotage® KP-SIL 50g column). Cis-methyl 4-(3-ethoxy-3-oxopropanoyl)-2-(4-fluorobenzyl)piperidine-1-carboxylate (2.531 g, 57.3 %) was isolated. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.20 – 1.25 (t, *J* = 7.4 Hz, 3H), 1.61 – 1.68 (m, 1H), 1.77 – 1.91 (m, 3H), 2.64 – 2.73 (m, 2H), 2.82 – 2.87 (m, 1H), 2.94 – 3.00 (m, 1H), 3.43 (s, 2H), 3.60 (s, 3H), 3.90 (dd, 1H), 4.11 – 4.17 (m, 3H), 6.91 – 6.96 (m, 2H), 7.08 – 7.13 (m, 2H). MS m/z 366 $(M+H)^+$.

Step 6: Cis-methyl 2-(4-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate
Cis-methyl 4-(3-ethoxy-3-oxopropanoyl)-2-(4-fluorobenzyl)piperidine-1-carboxylate (2.53 g, 6.92 mmol) was dissolved in MeOH (20 mL) and cooled to -40°C under nitrogen. Sodium hydroxide (0.277 g, 6.92 mmol) dissolved in water (2.000 mL) was added during 10 min and the colourless solution continued to stir at -40°C for 20 min. Hydroxylamine (50 % by weight in water, 0.424 mL, 6.92 mmol) was added during 8 min. The resulting solution was stirred at -40°C for 2 h. The mixture was then rapidly poured into a prewarmed (80°C) solution of 6 M hydrogen chloride (30 mL, 180.00 mmol) and the mixture continued to stir at 80°C for 20 min. The solvent was evaporated and DCM/water added. The phases were separated and the organic phase passed through a phase separator and evaporated to yield a yellow solid. The compound was purified by preparative HPLC in 3 injections on a XBridge C18 column (10 µm 250x50 ID mm) using a gradient of 0-35 % Acetonitrile in H2O/MeCN/NH3 95/5/0.2 buffer over 20 minutes with a flow of 100 mL/min. Cis-methyl 2-(4-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (1.516 g, 65.5%) was isolated as a white solid.

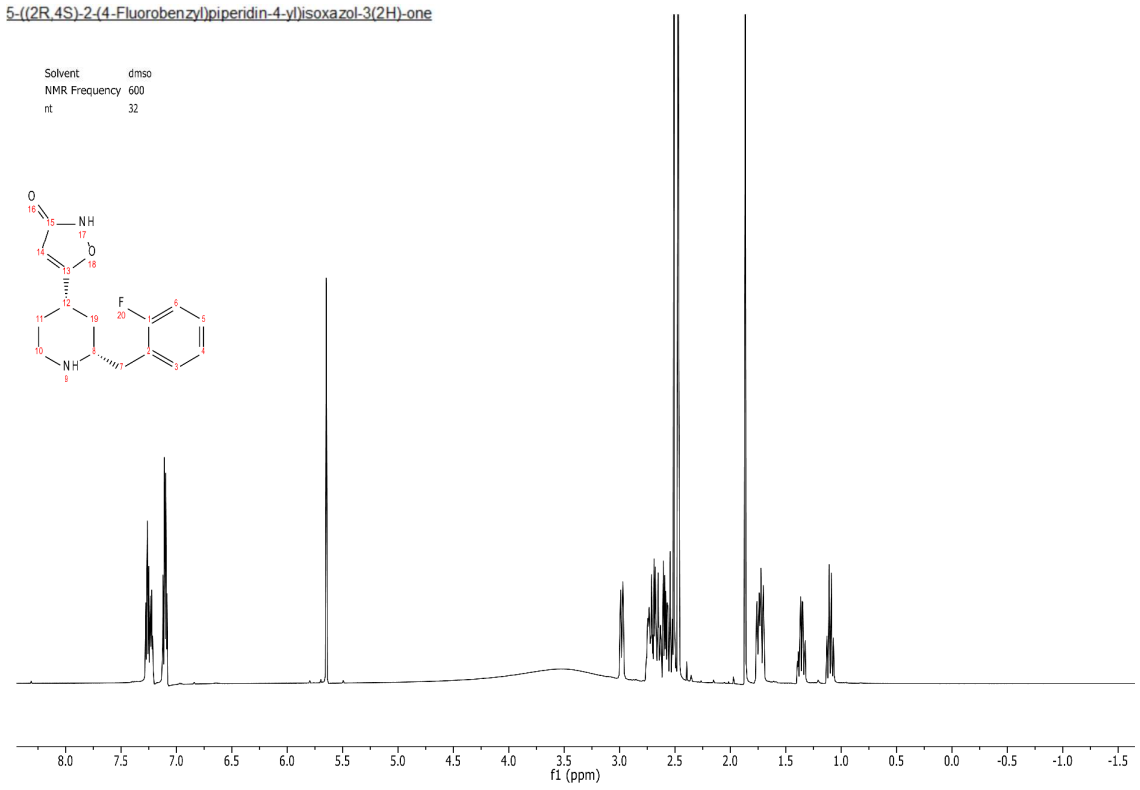
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.83 – 2.14 (m, 4H), 2.55 – 2.65 (m, 1H), 2.72 – 2.81 (m, 1H), 2.93 – 3.03 (m, 1H), 3.08 – 3.19 (m, 1H), 3.61 (s, 3H), 3.95 – 4.05 (m, 1H), 4.20 – 4.30 (m, 1H), 5.73 (s, 1H), 6.91 – 6.99 (m, 2H), 7.02 – 7.09 (m, 2H). MS m/z 335 $(M+H)^+$

Step 7: (2R,4S)-Methyl 2-(4-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate

Cis-methyl 2-(4-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (1.516 g, 4.53 mmol) was subjected to chiral preparative HPLC (Column: Chiralpak IC (250x20), 5 μm particle size, mobile phase: Heptane/(MTBE/MeOH 95/5) 50/50, flow rate 18 mL/min) to yield (2R,4S)-methyl 2-(4-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate 647 mg (43%), Chiral purity 99.9 %ee, Optical rotation $[\alpha]_D^{20} = +17.8$ (acetonitrile, c=1)

Step 8: 5-((2R,4S)-2-(4-Fluorobenzyl)piperidin-4-yl)isoxazol-3(2H)-one

(2R,4S)-Methyl 2-(4-fluorobenzyl)-4-(3-oxo-2,3-dihydroisoxazol-5-yl)piperidine-1-carboxylate (647 mg, 1.94 mmol) was dissolved in hydrogen bromide (33 % in acetic acid, 15 mL, 85.65 mmol) and the mixture stirred at room temperature overnight. The solvent was evaporated and the residue purified by preparative HPLC (Instrument: FractionLynx II, Mobilphase: gradient 5-95% MeCN in 0.2% NH_3, pH 10, Column: Xbridge Prep C18 5µm OBD 19*150 mm) to yield 5-((2R,4S)-2-(4-fluorobenzyl)piperidin-4-yl)isoxazol-3(2H)-one (360 mg, 67 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.08 (q, *J* = 12.1 Hz, 1H), 1.37 (dq, *J* = 4.5; 12.1 Hz, 1H), 1.73 – 1.81 (m, 2H), 2.44 – 2.73 (m, 5H), 2.96 – 3.03 (m, 1H), 5.70 (s, 1H), 7.07 – 7.14 (m, 2H), 7.21 – 7.27 (m, 2H). HRMS Calculated for $[C_{15}H_{17}FN_2O_2+H]^+$: 277.1352. Found: 277.1333



Assay details

Clotlysis

For details on potency testing in *in vitro* clotlysis (buffer and plasma) please refer to Boström, J.; Grant, A. J.; Fjellström, O.; Thelin, A.; Gustafsson, D. Potent Fibrinolysis Inhibitor discovered by shape and electrostatic complementary to the drug Tranexamic Acid. *J. Med. Chem.* **2013**, *56*, 3273-3280.

GABAa receptor binding assay

Rat brain membranes from male Sprague Dawley rats were prepared as described by *Jensen et. al.*Mol Pharmacol 2002, 61, 1377–1384.
On the day of assay membranes were thawed at room tempter and suspended to 1 µg/µL in 0.32 M sucrose, 10 mM Tris, 0.1 mM AEBSF, and 20 g/ml bacitracin, pH 7.4 using a Turrax blender for 3 x 5 sec. The assay were carried out in quadruplicate by incubating 60 µL brain membranes, 100 µL 58 pM [^{3}H-muscimol] and compound at various concentrations in a final volume of 200 µL. All constutuents dissolved in the buffer above. Non-specific binding were determined in the presence of bicuculline 90 µM. The mixture was then incubated on a microplate shaker (Denley Instruments, Billinghurst-West Sussex, UK) for 1 hr at room temperature, followed by rapid filtration through a glass fiber filter (Printed Filtermat B filters; PerkinElmer Wallac, Gaithersburg, MD) that had been presoaked in 0.3% polyethyleneimine, followed by a wash with TC buffer using a Tomtec cell harvester (Tomtec, Orange, CT). The filters were dried in a microwave at maximal effect for 1.5 min followed by incubation at 55°C for 45 min. MeltiLex B/HS scintillation sheets from PerkinElmer Wallac (Turku, Finland) were melted onto the filter, and radioactivity was determined in a Microbeta scintillation counter (PerkinElmer Wallac).

Assay details

Clotlysis

For details on potency testing in *in vitro* clotlysis (buffer and plasma) please refer to Boström, J.; Grant, A. J.; Fjellström, O.; Thelin, A.; Gustafsson, D. Potent Fibrinolysis Inhibitor discovered by shape and electrostatic complementary to the drug Tranexamic Acid. *J. Med. Chem.* **2013**, *56*, 3273-3280.

GABAa receptor binding assay

Rat brain membranes from male Sprague Dawley rats were prepared as described by *Jensen et. al.*Mol Pharmacol 2002, 61, 1377–1384.
On the day of assay membranes were thawed at room tempter and suspended to 1 µg/µL in 0.32 M sucrose, 10 mM Tris, 0.1 mM AEBSF, and 20 g/ml bacitracin, pH 7.4 using a Turrax blender for 3 x 5 sec. The assay were carried out in quadruplicate by incubating 60 µL brain membranes, 100 µL 58 pM [^{3}H-muscimol] and compound at various concentrations in a final volume of 200 µL. All constutuents dissolved in the buffer above. Non-specific binding were determined in the presence of bicuculline 90 µM. The mixture was then incubated on a microplate shaker (Denley Instruments, Billinghurst-West Sussex, UK) for 1 hr at room temperature, followed by rapid filtration through a glass fiber filter (Printed Filtermat B filters; PerkinElmer Wallac, Gaithersburg, MD) that had been presoaked in 0.3% polyethyleneimine, followed by a wash with TC buffer using a Tomtec cell harvester (Tomtec, Orange, CT). The filters were dried in a microwave at maximal effect for 1.5 min followed by incubation at 55°C for 45 min. MeltiLex B/HS scintillation sheets from PerkinElmer Wallac (Turku, Finland) were melted onto the filter, and radioactivity was determined in a Microbeta scintillation counter (PerkinElmer Wallac).

Relationship between pKa and permeabilitet of selected compounds.

Table shows relationship between measued Caco-2 Papp (1E-6 cm/s) vs measured delta pKa (difference in pKa for the piperidine functionality and the isoxazolone) for selected compounds.