Phenyl bioisosteres in medicinal chemistry: Discovery of novel γ-secretase modulators as a potential treatment for Alzheimer's disease

H. Ratni, K. Baumann, P. Bellotti, X. A. Cook, L. G. Green, T. Luebbers, M. Reutlinger, A. F. Stepan, W. Vifian*

[1]F. Hoffmann-La Roche Ltd., pRED, Pharma Research & Early Development, Roche Innovation Center Basel, Grenzacherstrasse 124, 4070 Basel, Switzerland

Supplementary informations

In vitro cellular Aβ secretion assay:

Human neuroglioma H4 cells stably overexpressing human APP695 isoform with the Swedish double mutation (K595N/M596L) were plated at 30,000 cells/well/100 µl in 96-well plates in IMDM media containing 10% FCS, 0.2 mg/l Hygromycin B and incubated at 37°C, 5% CO2. 3-4 hr post plating, test compounds were diluted in culture media and 50 µl is added to the wells as 1.5-fold concentrate to achieve the final concentration. Compound incubation is performed for 24 hr. Final doses typically range from 4 µM down to 0.0013 µM in half-log steps resulting in a eight point dose response curve. Appropriate controls using vehicle only and reference compound were applied to this assay. The final concentration of Me2SO was 0.4%.

After incubation at 37°C, 5% CO2, the supernatant was subjected to quantification of secreted Aβ peptides by the means of an AlphaLisa assay kits (Perkin Elmer). 20 µl of the cell culture supernatant was transferred to an assay plate. Then 10 µl of a mixture of the AlphaLisa coupled capture antibody and the biotinylated detection antibody was added and incubated for 3 hours at room temperature while softly shaking the assay plate. After a further addition of 20 µl of the Donor beads the assay plate was incubated for 30 min at room temperature and constant shaking without exposure to direct light. The assay plate was then read on a Paradigm AlphaLisa Reader using the build-in program with excitation at 680 nm and emission at 570 nm.

The measured signals were then used to calculate IC50 values for inhibition of Aβ peptides secretion by nonlinear regression fit analysis using XLfit 5.3 software (IDBS).

Lipophilicity (log D) determination by high-throughput shake-flask

The applied methods called CAMDIS© (**CA**rrier **M**ediated **DI**stribution **S**ystem) for the determination of distribution coefficients are derived from the conventional 'shake flask' method. CAMDIS© is carried out in 96-well microtiterplates in combination with the novel DIFI©-tubes constructed by Roche, which provide a hydrophobic layer for the octanol phase. The experiment starts with the accurate coating of the hydrophobic layer (0.45 mm PVDF membranes), which is fixed on the bottom of each DIFI©-tube: Each membrane is impregnated with exactly 1.0 mL 1-octanol by a robotic system (Microfluidic Dispenser BioRAPTR, Bechman Coulter). To expand the measurement range down to logD= -0.5, the procedure is carried at two different octanol/water ratios. One with a overplus of octanol for hydrophilic compounds (logD<1) and one with a low volume of octanol for the lipophilic compounds (logD>1). Therefore, some DIFI©-tubes are filled with 15 µl 1-octanol. The coated membranes are then connected to a 96-well plate which has been prefilled with exactly 150 mL of the selected aqueous buffer solution (25 mM Phosphate, pH 7.4). The buffer solution already contains the compound of interest with a starting concentration of 100 mM. The resulting sandwich construct guarantees that the membrane is completely dipped in the buffered sample solution. The plate is then sealed and shaken for 24 hours at room temperature (23°C). During this time the substance is distributed between the layer, the octanol and the buffer solution. After distribution equilibrium is reached the DIFI©-tubes are easily disassembled from the top of the 96-well plate, so that the remaining sample concentration in the aqueous phase can be analyzed by LC/MS. In order to know the exact sample concentration before incubation with 1-octanol, a part of the sample solution is connected to DIFI©-tubes without impregnation. The distribution coefficient is then calculated from the difference in concentration in the aqueous phase with and without impregnation and the ratio of the two phases. The preparation of the sample solutions is carried out by a TECAN robotic system (RSP 100, 8 channels).

Solubility Determination (Lysa Assay).

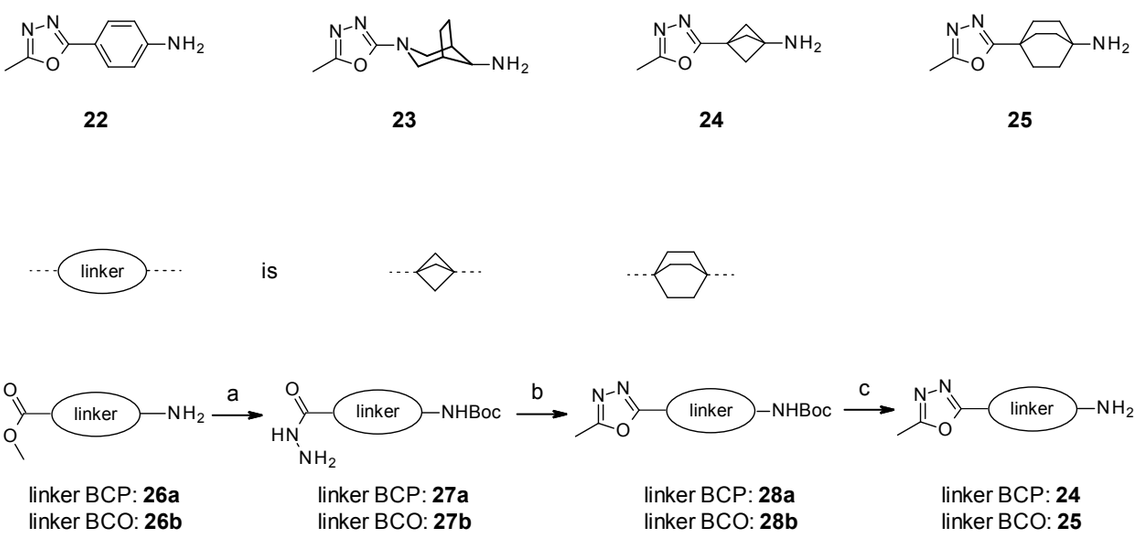
Samples were prepared in duplicate from 10 mM DMSO stock solutions. After evaporation (1h) of DMSO with a centrifugal vacuum evaporator (Genevac Technologies), the

compounds were dissolved in 0.05 M phosphate buffer (pH 6.5), stirred for 1 h, and shaken for 2 h. After one night, the solutions were filtered using a microtiter filter plate (Millipore MSDV N65), and the filtrate and its 1/10 dilution were then analyzed by direct UV measurement or by HPLC-UV. In addition, a four point calibration curve was prepared from the 10 mM stock solutions and used for the solubility determination of the compounds. Starting from 10 mM stock solution, the measurement range for MW 500 was 0−666 µg/mL.

Compound Synthesis and Characterization. Chemistry.

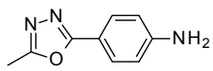
Reactions were carried out under argon atmosphere. Unless otherwise mentioned, all reagents and chemicals were obtained from commercial suppliers and used without further purification. All reactions were followed by TLC (TLC plates F254, Merck) or LCMS (liquid chromatography-mass spectrometry) analysis. The purity of final compounds as measured by HPLC was at least above 95%. Flash column chromatography was carried out either using cartridges packed with silica gel (Isolute Columns, Telos Flash Columns) or on glass columns on silica gel 60 (32-60 mesh, 60Å). LC-MS high resolution spectra were recorded with a Agilent LC-system consisting of Agilent 1290 high pressure system, an Agilent 1290 multisampler and a Agilent 6545 QTOF. The separation was achieved on a Zorbax Eclipse Plus C18 1,7 µm 2.1*50mm column at 55°C; A=0.02% formic acid in Water; B= acetonitrile with 0.01% formic acid at flow 0.8 mL/min. gradient: 0 min 5%B, 0.3 min 5%B, 4.5 min 99 %B 5 min 99%B

1 Preparation of the amino-linker-oxadiazole derivatives 22-25.



Scheme 1.

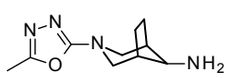
1.a. Preparation of 4-(5-methyl-1,3,4-oxadiazol-2-yl)aniline **22**



22

This derivative was purchase from a commercial source

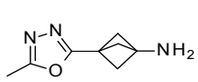
1.b. Preparation of 3-(5-methyl-1,3,4-oxadiazol-2-yl)-3-azabicyclo[3.2.1]octan-8-amine **23**



23

This derivative was prepared according to our reported synthesis (*J. Med. Chem.* **2020,** *63* (15), 8534-8553.

1.c. Preparation of 3-(5-methyl-1,3,4-oxadiazol-2-yl)bicyclo[1.1.1]pentan-1-amine **24**



24

According to scheme 1.

Step a: i. To a stirred solution of methyl 3-aminobicyclo[1.1.1]pentane-1-carboxylate hydrochloride **26a** (1.05 g, 5.91 mmol) in THF (30 mL) was added boc-anhydride (1.42 g, 1.51 mL, 6.5 mmol) and iPr_2Net (5.1 mL, 29.6 mmol). The reaction was stirred over night at RT, concentrated under vacuo, and redissolved in EtOAc (50 mL) and washed successively with an aqueous solution of saturated $NaHCO_3$ (25 mL), 3% citric acid (25 mL) and brine (25 mL). The organic phase was dried over Na_2SO_4, concentrated udner vacuo and a column chromatography (EtOAC / Heptane) yielded (1.20 g, 84% yield) of tert-butyl N-[3-(5-methyl-1,3,4-oxadiazol-2-yl)-1-bicyclo[1.1.1]pentanyl]carbamate as a white solid.

^{1}H NMR: (300 MHz, $CDCl_3$) δ 4.9 (br s, 1H), 3.63-3.75 (s, 3H), 2.20-2.36 (s, 6H), 1.38-1.51 (s, 9H). **LCMS** (ES) found: 186.1 $(M-C_4H_8)^+$

ii. To a stirred solution of tert-butyl N-[3-(5-methyl-1,3,4-oxadiazol-2-yl)-1-bicyclo[1.1.1]pentanyl]carbamate (0.77 g, 3.2 mmol) in MeOH (5 mL) was added hydrazine hydrate, 80% in water, (2.5 mL, 41 mmol). The mixture was heated at 80°C for 15 minutes, cooled down to RT and concentrated under vacuo to afford (0.77 g, quantitative yield) tert-butyl N-[3-(hydrazinecarbonyl)-1-bicyclo[1.1.1]pentanyl]carbamate **27a** as a white solid.

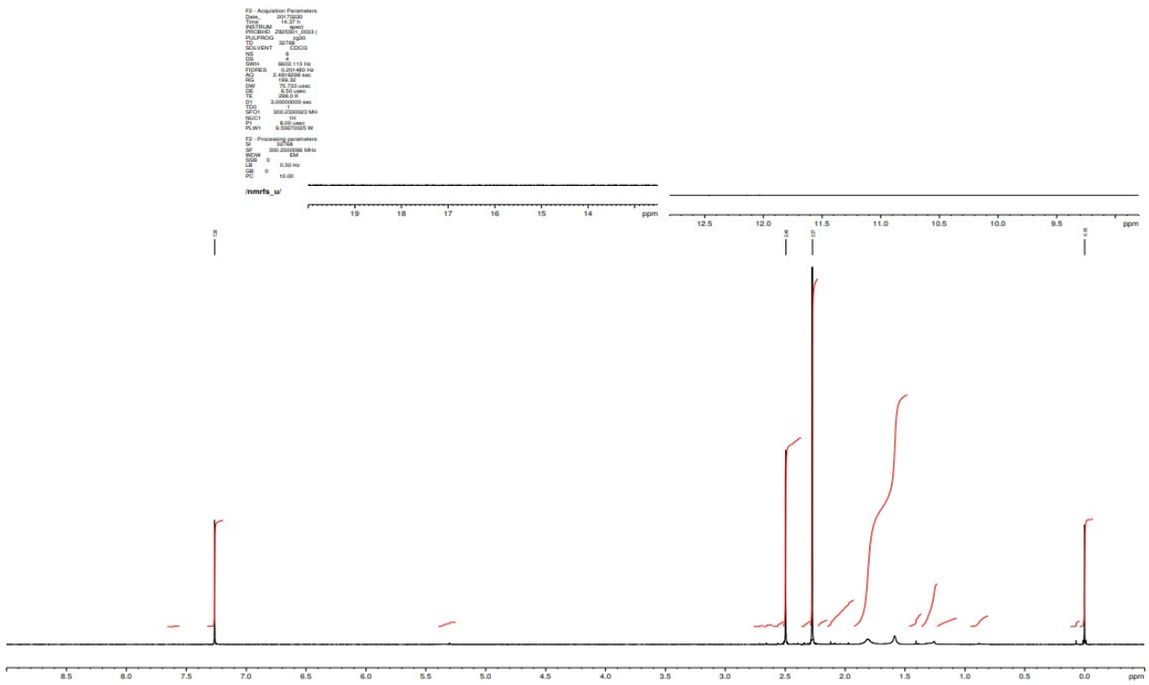
^{1}H NMR: (300 MHz, $CDCl_3$) δ 6.8 (br s, 1H), 5.0 (br s, 1H), 3.8 (br s, 2H), 2.3 (s, 6H), 1.4-1.5 (m, 9H). **LCMS** (ES) found: 242.2 $(M + H)^+$

Step b: tert-Butyl N-[3-(hydrazinecarbonyl)-1-bicyclo[1.1.1]pentanyl]carbamate **27a** (0.77 g, 3.2 mmol) was suspended in EtOAc (14 mL) before acetic acid (0.22 mL, 3.83 mmol), Et_3N (1.8 mL, 12.8 mmol) and propylphosphonic anhydride solution (50 wt. % in EtOAc; 4.7 mL, 8.0 mmol) were added providing a pale yellow solution. The reaction was heated in a microwave at 100°C fpr 15 minutes, and then at 140°C for 30 minutes and then cooled down to RT. The reaction was concentrated under high vacuum, and the residue dissolved in EtOAc, washed with brine. The organic phase was separated and dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (30-80% EtOAc in heptane) to yield (0.74 g, 87% yield) tert-butyl N-[3-(5-methyl-1,3,4-oxadiazol-2-yl)-1-bicyclo[1.1.1]pentanyl]carbamate **28a** as a white solid.

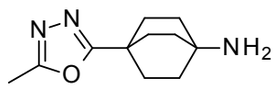
^{1}H NMR: (300 MHz, $CDCl_3$) δ 5.0 (br s, 1H), 2.5 (s, 6H), 3.8 (br s, 2H), 2.4 (s, 3H), 1.4-1.5 (m, 9H). **LCMS** (ES) found: 266.2 $(M + H)^+$

Step c: To a CH_2Cl_2 (19 mL) solution of tert-butyl N-[3-(5-methyl-1,3,4-oxadiazol-2-yl)-1-bicyclo[1.1.1]pentanyl]carbamate **28a** (0.74 g, 2.8 mmol) was added TFA (4.3 mL, 55 mmol). After stirring at RT for 45 minutes, the reaction was concentrated under vacuo, redissolved in CH_2Cl_2 and washed with an aqueous saturated solution of $NaHCO_3$, and then brine. The organic phase was dried over Na_2SO_4, and concentrated under high vacuum to yield (0.37 g, 78% yield) 3-(5-methyl-1,3,4-oxadiazol-2-yl)bicyclo[1.1.1]pentan-1-amine **24** as a white solid.

^{1}H NMR: (300 MHz, $CDCl_3$) δ 2.5 (s, 3H), 2.3 (s, 6H). **LCMS** (ES) found: 166.1 $(M + H)^+$



1.d. Preparation of 4-(5-methyl-1,3,4-oxadiazol-2-yl)bicyclo[2.2.2]octan-1-amine **25**



25

According to scheme 1.

Step a: i. To a stirred solution of methyl 4-aminobicyclo[2.2.2]octane-1-carboxylate **26b** (1.50 g, 8.19 mmol) in THF (40 mL) was added boc-anhydride (1.97 g, 2.09 mL, 9.0 mmol) and iPr_2Net (4.3 mL, 24.6 mmol). The reaction was stirred over night at RT, concentrated under vacuo, and redissolved in EtOAc (50 mL) and washed successively with an aqueous solution of saturated $NaHCO_3$ (25 mL), 3% citric acid (25 mL) and brine (25 mL). The organic phase was dried over Na_2SO_4, concentrated under vacuo to yield (2.29 g, 99% yield) of methyl 4-(tert-butoxycarbonylamino)bicyclo[2.2.2]octane-1-carboxylate as a white solid.

^{1}H NMR: (300 MHz, $CDCl_3$) δ 3.6 (s, 3H), 1.8-1.9 (m, 12H), 1.4 (s, 9H). **LCMS** (ES) found: 228.2 $(M + H)^+$

ii. To a stirred solution of methyl 4-(tert-butoxycarbonylamino)bicyclo[2.2.2]octane-1-carboxylate (2.29 g, 8.1 mmol) in MeOH (13.5 mL) was added hydrazine hydrate, 80% in water, (6.4 mL, 105 mmol). The mixture was heated at 80°C for 17 hours, cooled down to RT and concentrated under vacuo to afford (2.1 g, 85% yield) tert-butyl N-[4-(hydrazinecarbonyl)-1-bicyclo[2.2.2]octanyl]carbamate **27b** as a white solid.

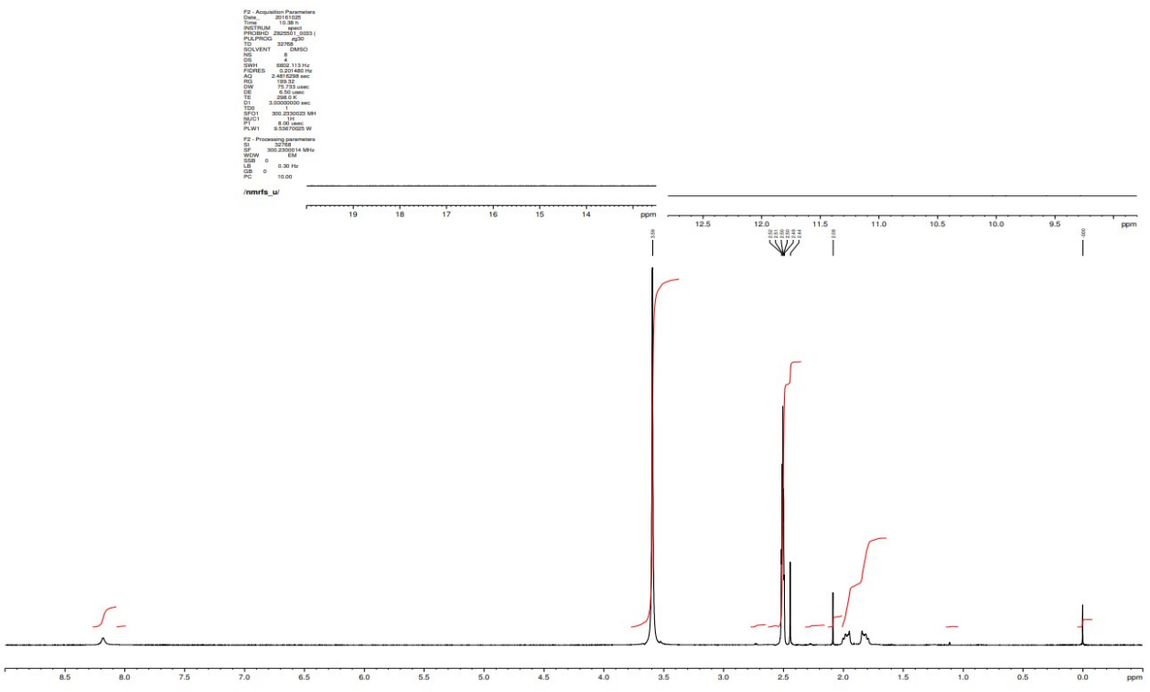
^{1}H NMR: (300 MHz, $CDCl_3$) δ 8.7 (br s, 1H), 6.4 (br s, 1H), 4.1 (br s, 2H), 1.6-1.7 (br s, 12H), 1.3 (s, 9H). **LCMS** (ES) found: 284.2 $(M + H)^+$

Step b: tert-Butyl N-[4-(hydrazinecarbonyl)-1-bicyclo[2.2.2]octanyl]carbamate **27b** (2.1 g, 6.8 mmol) was suspended in EtOAc (25 mL) before acetic acid (0.47 mL, 8.2 mmol), Et_3N (3.8 mL, 27.3 mmol) and propylphosphonic anhydride solution (50 wt. % in EtOAc; 10.2 mL, 17.1 mmol) were added providing a pale yellow solution. The reaction was heated in a microwave at 150°C for 30 minutes, and then cooled down to RT. The reaction was concentrated under high vacuum, and the residue dissolved in EtOAc, washed with brine. The organic phase was separated and dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (0-50% EtOAc in heptane) to yield (1.49 g, 70% yield) tert-butyl N-[4-(5-methyl-1,3,4-oxadiazol-2-yl)-1-bicyclo[2.2.2]octanyl]carbamate **28b** as a white solid.

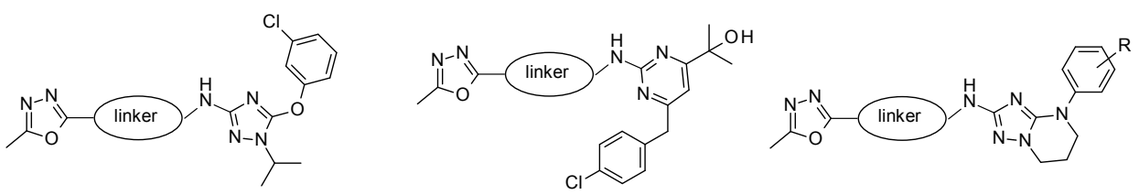
^{1}H NMR: (300 MHz, $CDCl_3$) δ 4.4 (br s, 1H), 2.5 (s, 3H), 1.9-2.1 (m, 12H), 1.4 (s, 9H). **LCMS** (ES) found: 308.2 $(M + H)^+$

Step c: To an acetone (17 mL) solution of tert-butyl N-[4-(5-methyl-1,3,4-oxadiazol-2-yl)-1-bicyclo[2.2.2]octanyl]carbamate **28b** (1.46 g, 4.7 mmol) was added an aqueous solution of HCl (30%, 3.5 mL, 42.7 mmol). After stirring at RT for 4 hours, the resulting white precipitate was collected, suspended in CH_2Cl_2 and washed with an aqueous saturated solution of $NaHCO_3$, and then brine. The organic phase was dried over Na_2SO_4, and concentrated under high vacuum to yield (1.12 g, 97% yield) 4-(5-methyl-1,3,4-oxadiazol-2-yl)bicyclo[2.2.2]octan-1-amine **25** as a white solid.

^{1}H NMR: (300 MHz, DMSO) δ 8.1 (br s, 2H), 2.4 (s, 3H), 1.7-2.0 (m, 12H). **LCMS** (ES) found: 208.2 $(M + H)^+$



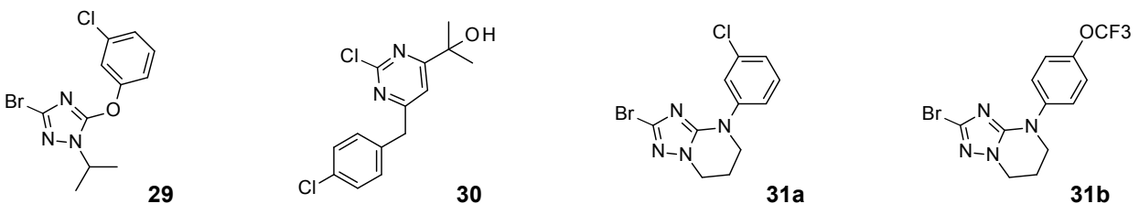
2. Preparation of final derivatives 5-20



5-8 **9-12** **13-20**

Figure 1.

2.1 Preparation of **29-31**



29 **30** **31a** **31b**

The 3-bromo-5-(3-chlorophenoxy)-1-isopropyl-1,2,4-triazole **29** was prepared according to our procedure reported in WO2018087018. The 2-[2-chloro-6-[(4-chlorophenyl)methyl]pyrimidin-4-yl]propan-2-ol **30** was prepared according to our procedure reported in WO2012/116965 and US20110201605. Finally, the compounds 2-bromo-4-(3-chlorophenyl)-6,7-dihydro-5H-[1,2,4]triazolo[1,5-a]pyrimidine **31a** and 2-bromo-4-[4-(trifluoromethoxy)phenyl]-6,7-dihydro-5H-[1,2,4]triazolo[1,5-a]pyrimidine **31b** were made according to our procedures reported in WO2018060300.

2.2 Preparation of compounds **5-20**

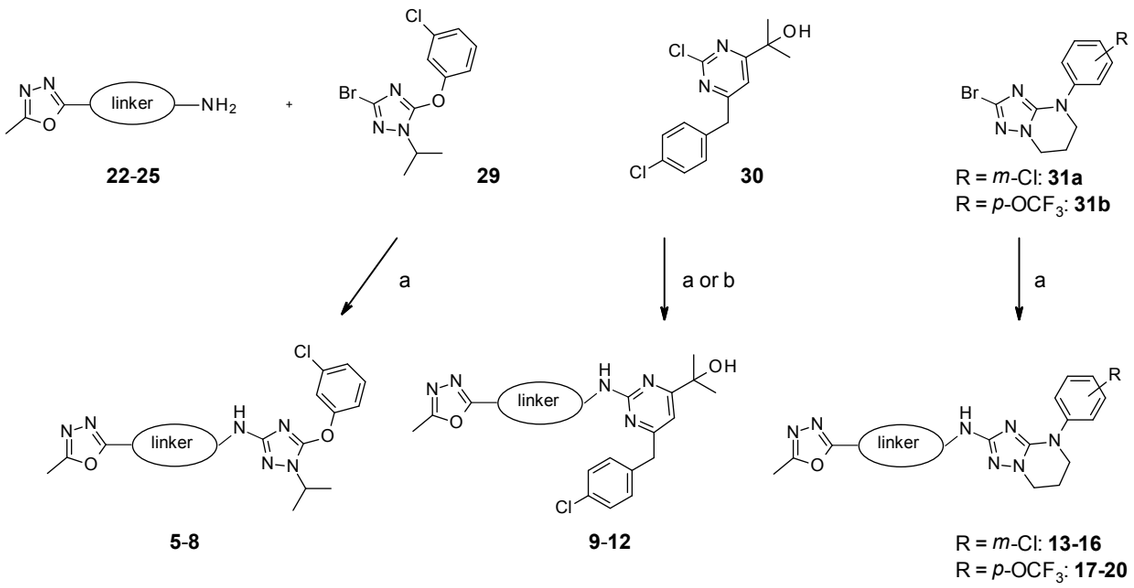
Those derivatives **5-20** were prepared according to the scheme 2 below using either a buchwald coupling or a nucleophilic aromatic substitution between the amino-linker derivatives **22-25** and the bromo-triazoles and chloro-pyrimidine compounds **29-31**.

a) General conditions for the buchwald conditions

To a solution of an halid-derivatives **29-31** (1 eq) in MeTHF was added 1 equivalent of an amino-linker intermediate **22-25**. The reaction mixture was degased and $Pd_2(dba)_3$ (0.05 eq.), *t*Bu-Xphos (0.1 eq) and NaO*t*Bu (6.0 eq.) were added. The reaction mixture was heated at 80-100°C until completion of the reaction (usually between 0.5 and 8 hours) and concentrated under vacuo. A purification was done either by column chromatography or reverse phase preparative HPLC to afford the desired product.

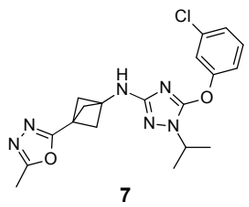
b) General condition for the SNAr

To a solution of the halid-derivative **30** (1 eq) in NMP was added 1 equivalent of an amino-linker intermediate **23-25** and *i*Pr_2NEt (6.0 eq). The reaction mixture was heated in a microwave at 150-175°C until completion of the reaction (usually between 0.5 and 1.5 hour) and concentrated under vacuo. A purification was done either by column chromatography or reverse phase preparative HPLC to afford the desired product.



Scheme 2.

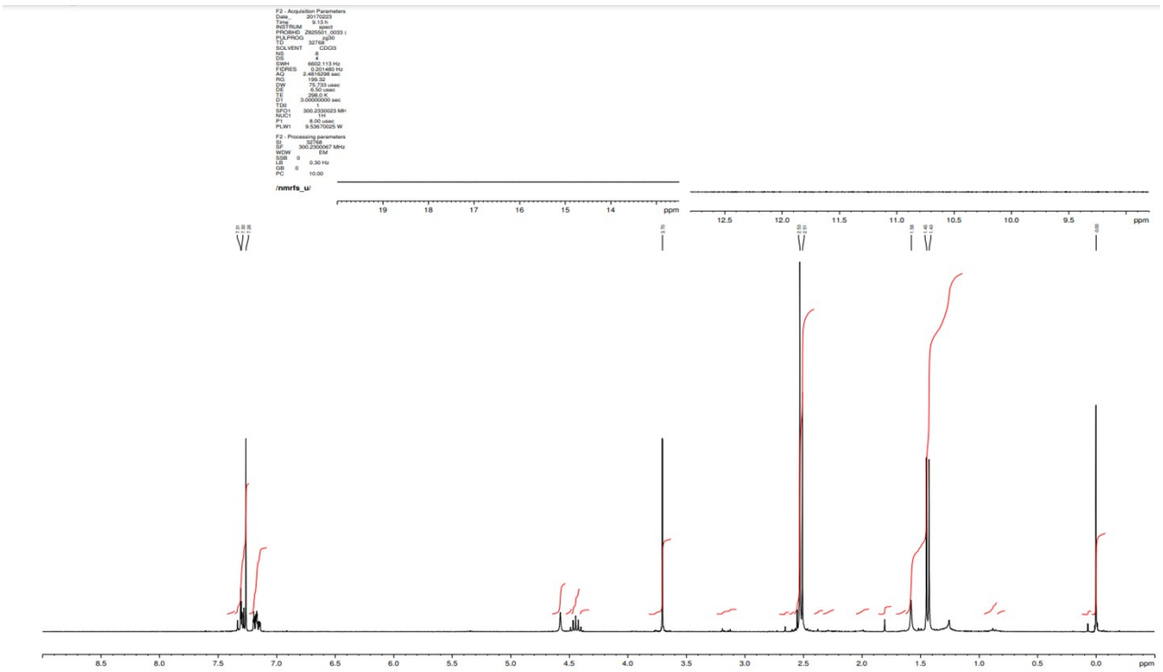
Preparation of **7**



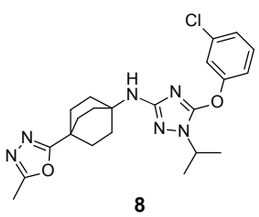
7

Using a Buchwald type coupling between 3-(5-methyl-1,3,4-oxadiazol-2-yl)bicyclo[1.1.1]pentan-1-amine **24** and 3-bromo-5-(3-chlorophenoxy)-1-isopropyl-1,2,4-triazole **29**, was obtained (39 mg, 32% yield) 5-(3-chlorophenoxy)-1-isopropyl-N-[3-(5-methyl-1,3,4-oxadiazol-2-yl)-1-bicyclo[1.1.1]pentanyl]-1,2,4-triazol-3-amine **7** as an oil.

^{1}H NMR: (300 MHz, $CDCl_3$) δ ppm: 7.28-7.35 (m, 2H), 7.14-7.21 (m, 2H), 4.58 (s, 1H), 4.45 (spt, 1H, *d*=6.6 Hz), 2.53 (s, 6H), 2.51 (s, 3H), 1.46 (d, *J*=6.6 Hz, 6H) . **LCMS** (ES) found: 401.2 / 403.2 (^{35}Cl/^{37}Cl: 3:1) $(M + H)^+$



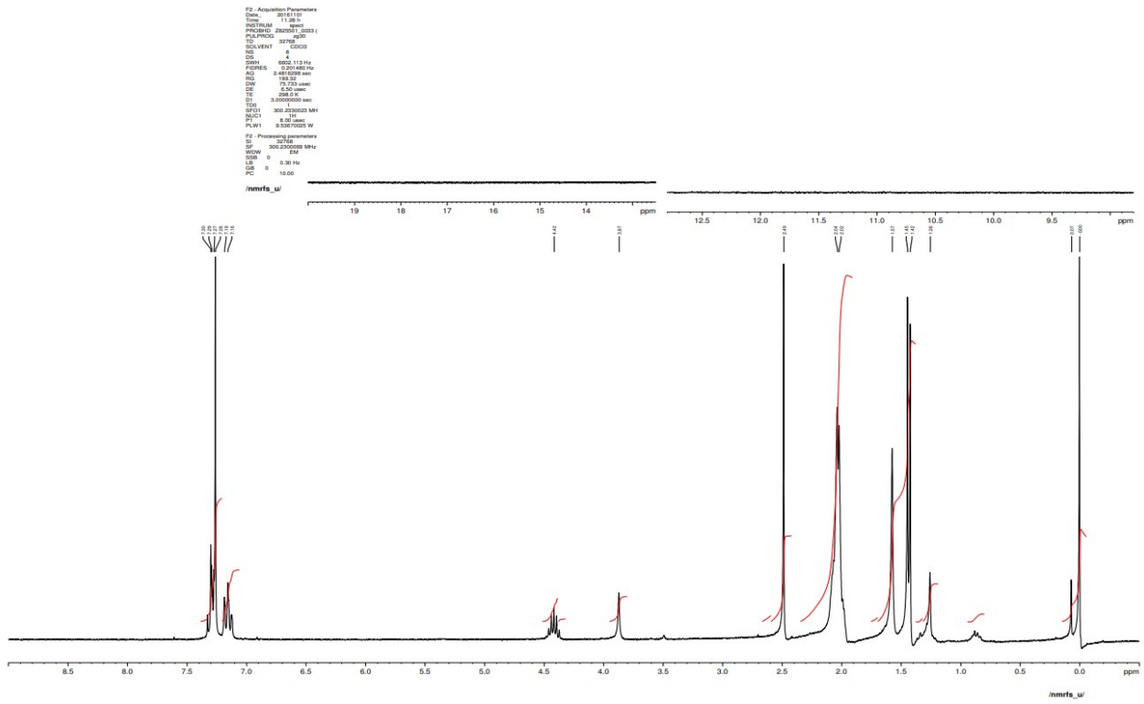
Preparation of **8**



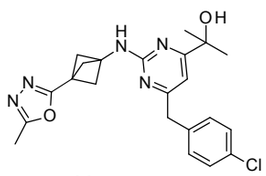
8

Using a Buchwald type coupling between 4-(5-methyl-1,3,4-oxadiazol-2-yl)bicyclo[2.2.2]octan-1-amine **25** and 3-bromo-5-(3-chlorophenoxy)-1-isopropyl-1,2,4-triazole **29**, was obtained (26 mg, 15% yield) 5-(3-chlorophenoxy)-1-isopropyl-N-[4-(5-methyl-1,3,4-oxadiazol-2-yl)-1-bicyclo[2.2.2]octanyl]-1,2,4-triazol-3-amine **8** as a white solid.

^{1}H NMR: (600 MHz, $CDCl_3$) δ ppm: 7.27-7.33 (m, 2H), 7.16-7.20 (m, 1H), 7.12-7.16 (m, 1H), 4.42 (m, 1H), 2.48-2.79 (s, 3H), 1.94-2.13 (m, 12H), 1.44 (d, *J*=6.6 Hz, 6H). **LCMS** (ES) found: 443.3 / 445.3 ($^{35}Cl/^{37}Cl$: 3:1) $(M + H)^+$



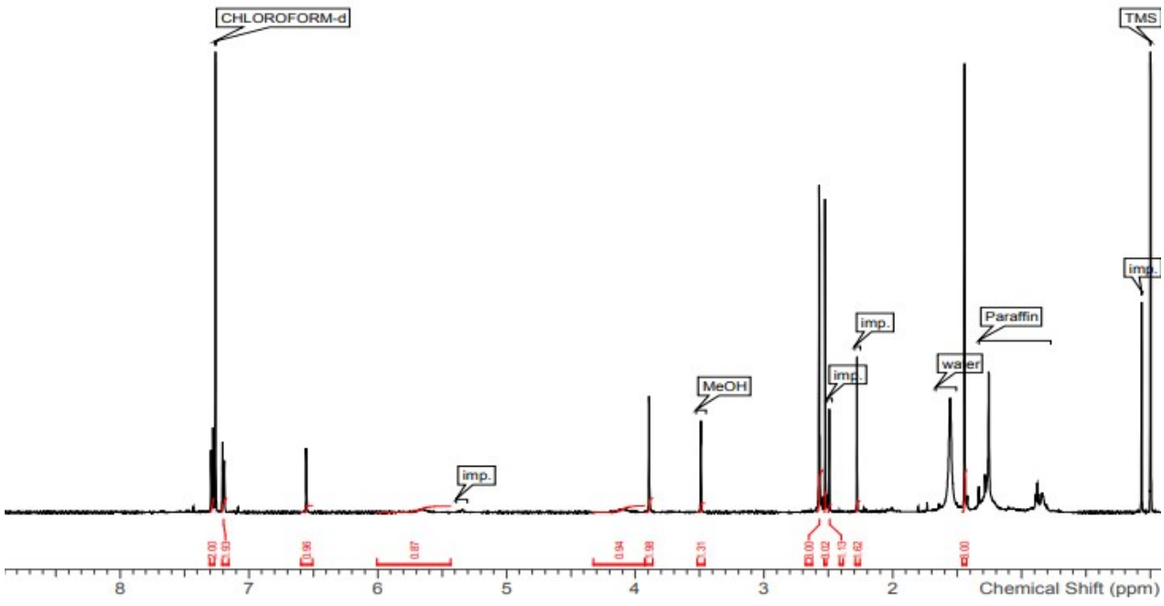
Preparation of **11**

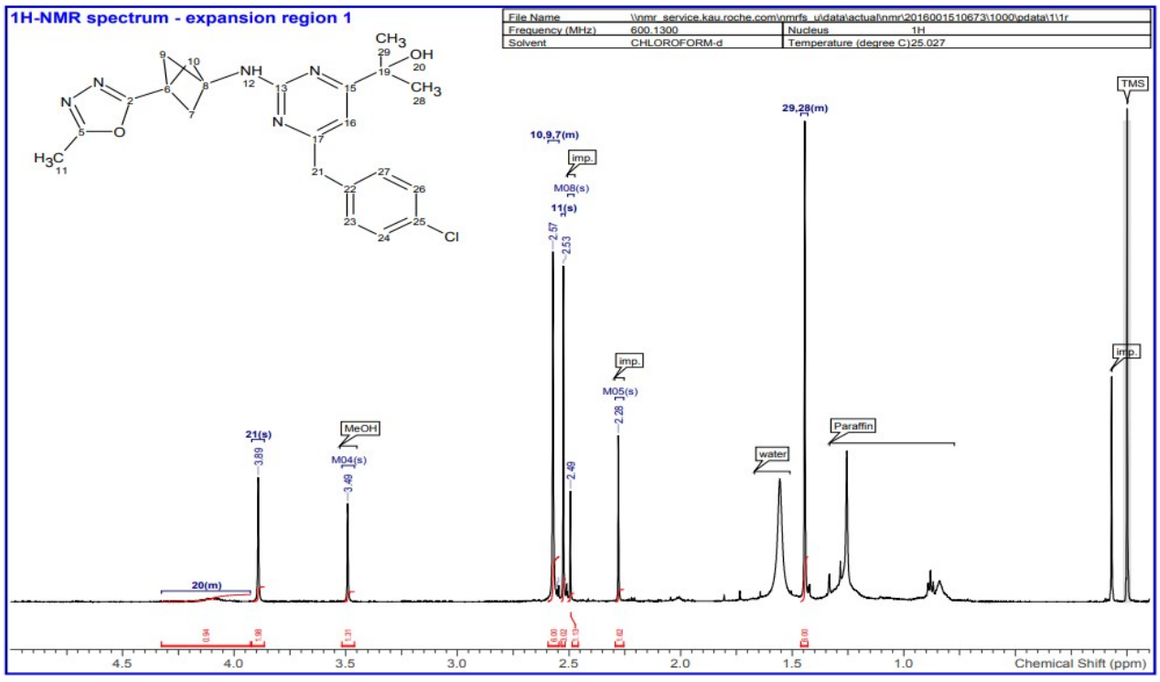


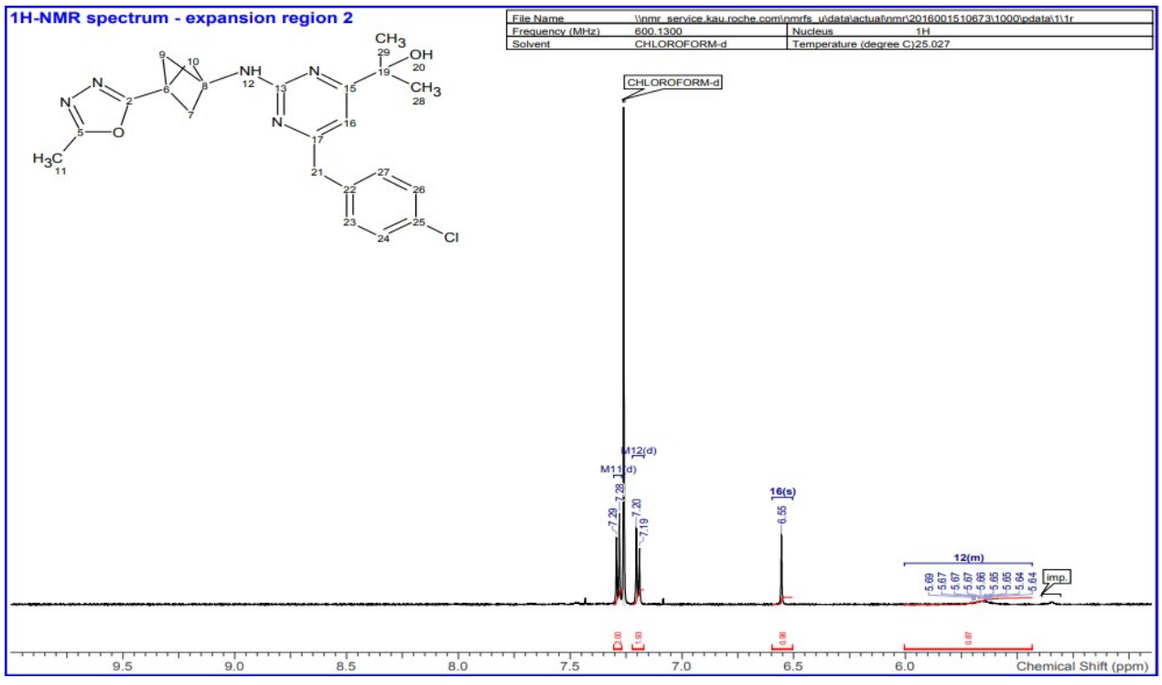
11

Using a SNAr coupling between 3-(5-methyl-1,3,4-oxadiazol-2-yl)bicyclo[1.1.1]pentan-1-amine **24** and 2-[2-chloro-6-[(4-chlorophenyl)methyl]pyrimidin-4-yl]propan-2-ol **30**, was obtained (10 mg, 16% yield) 2-[6-[(4-chlorophenyl)methyl]-2-[[3-(5-methyl-1,3,4-oxadiazol-2-yl)-1-bicyclo[1.1.1] pentanyl]amino]pyrimidin-4-yl]propan-2-ol **11** as a white solid.

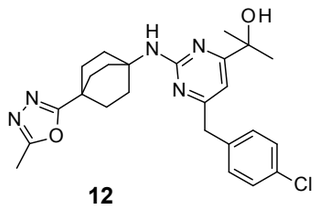
^{1}H NMR: (300 MHz, $CDCl_3$) δ ppm: 7.80-8.02 (m, 1H), 7.34-7.38 (m, 2H), 7.30 (br d, *J* = 8 Hz, 2H), 6.83 (s, 1H), 5.13 (s, 1H), 3.80-3.94 (m, 2H), 2.47 (s, 3H), 2.39-2.45 (m, 6H), 1.35 (s, 6H). **LCMS** (ES) found: 426.2 / 428.2 ($^{35}Cl/^{37}Cl$: 3:1) $(M + H)^+$







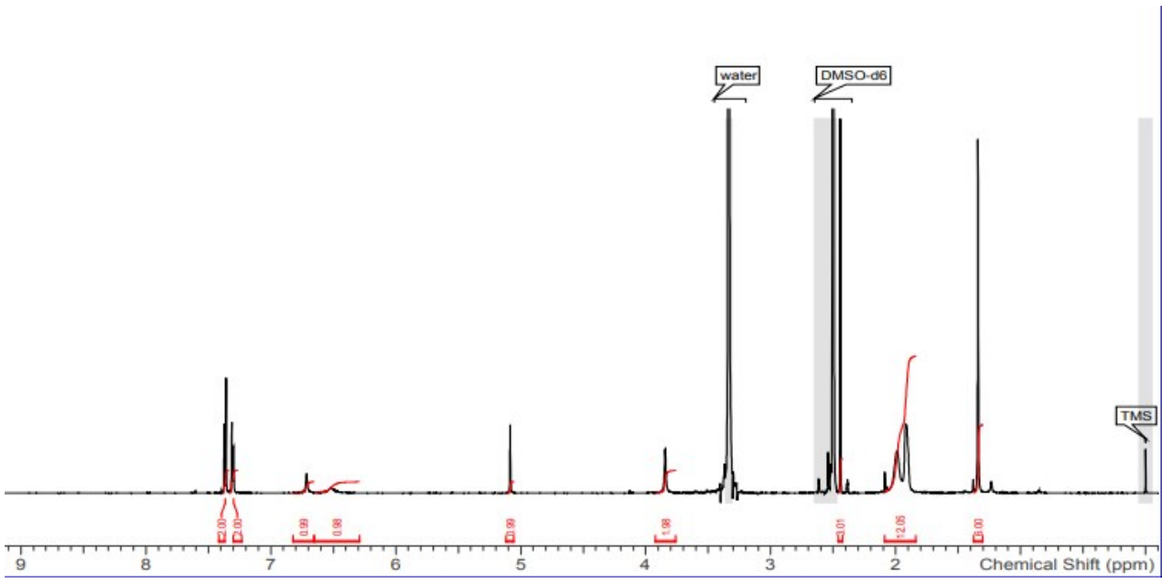
Preparation of **12**



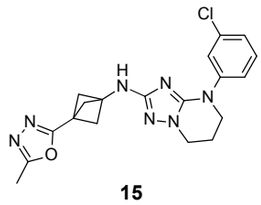
12

Using a SNAr coupling between 4-(5-methyl-1,3,4-oxadiazol-2-yl)bicyclo[2.2.2]octan-1-amine **25** and 2-[2-chloro-6-[(4-chlorophenyl)methyl]pyrimidin-4-yl]propan-2-ol **30**, was obtained (28 mg, 14% yield) 2-[6-[(4-chlorophenyl)methyl]-2-[[4-(5-methyl-1,3,4-oxadiazol-2-yl)-1-bicyclo[2.2.2] octanyl]amino]pyrimidin-4-yl]propan-2-ol **12** as a white solid.

^{1}H NMR: (600 MHz, DMSO) δ ppm: 7.34-7.39 (m, 2H), 7.26-7.33 (m, 2H), 6.71 (br s, 1H), 6.51 (br s, 1H), 5.08 (s, 1H), 3.84 (br s, 2H), 2.44 (s, 3H), 1.84-2.09 (m, 12H). **LCMS** (ES) found: 468.4 / 470.4 (^{35}Cl/^{37}Cl: 3:1) $(M + H)^+$



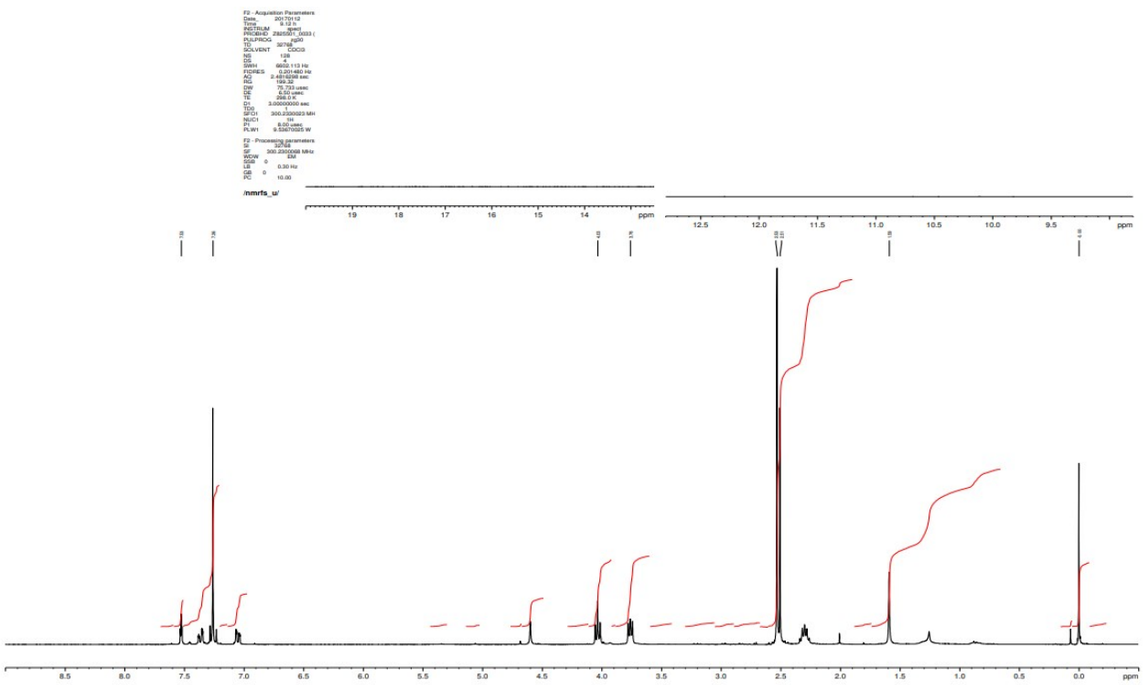
Preparation of **15**



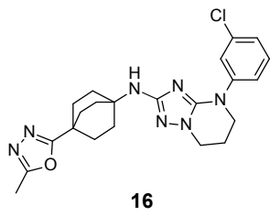
15

Using a Buchwald type coupling between 3-(5-methyl-1,3,4-oxadiazol-2-yl)bicyclo[1.1.1]pentan-1-amine **24** and 2-bromo-4-(3-chlorophenyl)-6,7-dihydro-5H-[1,2,4]triazolo[1,5-a]pyrimidine **31a**, was obtained (7 mg, 6% yield) 4-(3-chlorophenyl)-N-[3-(5-methyl-1,3,4-oxadiazol-2-yl)-1-bicyclo[1.1.1]pentanyl]-6,7-dihydro-5H-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine **15** as an oil.

^{1}H NMR: (300 MHz, $CDCl_3$) δ ppm: 7.53 (t, *J* = 2.0 Hz, 1H),7.32-7.40 (m, 1H), 7.20-7.30 (m. 1H), 7.0-7.1 (m, 1H), 4.60 (br s, 1H), 3.99-4.07 (m, 2H), 3.72-3.80 (m, 2H), 2.51 (s, 3H), 2.50 (s, 6H), 2.30 (dt, J = 11.6, 6.0 Hz, 2H). **LCMS** (ES) found: 398.2 / 400.2 (^{35}Cl/^{37}Cl: 3:1) $(M + H)^+$



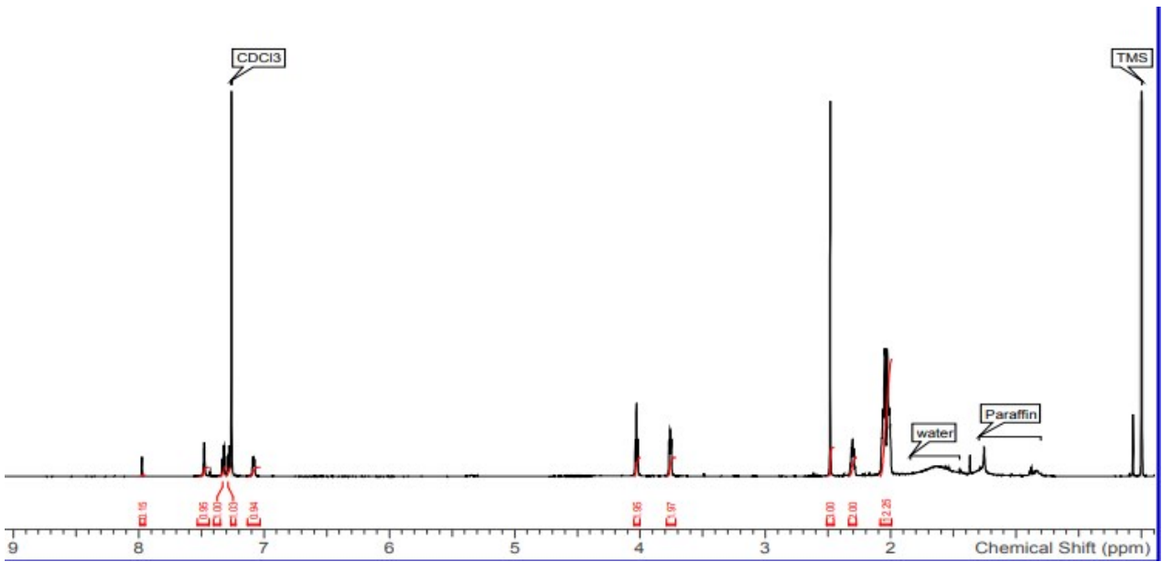
Preparation of **16**



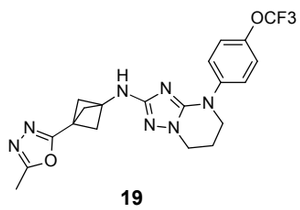
16

Using a Buchwald type coupling between 4-(5-methyl-1,3,4-oxadiazol-2-yl)bicyclo[2.2.2]octan-1-amine **25** and 2-bromo-4-(3-chlorophenyl)-6,7-dihydro-5H-[1,2,4]triazolo[1,5-a]pyrimidine **31a**, was obtained (29 mg, 46% yield) 4-(3-chlorophenyl)-N-[4-(5-methyl-1,3,4-oxadiazol-2-yl)-1-bicyclo[2.2.2]octanyl]-6,7-dihydro-5H-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine **16** as a white solid.

^{1}H NMR: (600 MHz, $CDCl_3$) δ ppm: 7.97 (s, 1H), 7.44-7.53 (m, 1H), 7.31-7.36 (m, 1H), 7.28-7.30 (m, 1H), 7.03-7.13 (m, 1H), 4.03 (t, J = 6.0 Hz, 2H), 3.72-3.79 (m, 2H), 2.48 (s, 3H), 2.30-2.40 (m, 2H), 1.99-2.08 (m, 12H). **LCMS** (ES) found: 440.2 / 442.2 ($^{35}Cl/^{37}Cl$: 3:1) $(M + H)^+$



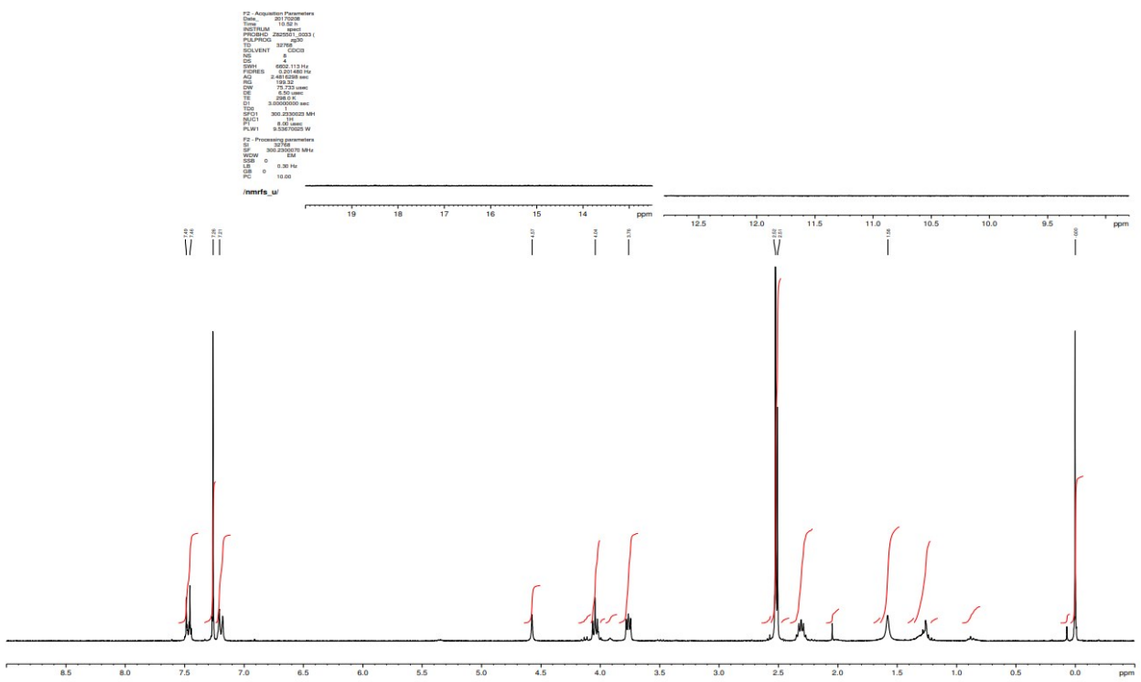
Preparation of **19**



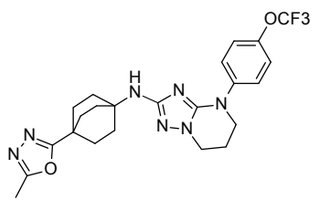
19

Using a Buchwald type coupling between 3-(5-methyl-1,3,4-oxadiazol-2-yl)bicyclo[1.1.1]pentan-1-amine **24** and 2-bromo-4-[4-(trifluoromethoxy)phenyl]-6,7-dihydro-5H-[1,2,4]triazolo[1,5-a]pyrimidine **31b**, was obtained (15 mg, 10% yield) N-[3-(5-methyl-1,3,4-oxadiazol-2-yl)-1-bicyclo[1.1.1]pentanyl]-4-[4-(trifluoromethoxy)phenyl]-6,7-dihydro-5H-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine **19** as a white solid.

^{1}H NMR: (300 MHz, $CDCl_3$) δ ppm: 7.43-7.51 (m, 2H),7.10-7.19 (m, 2H), 4.57 (s, 1H), 4.04 (t, *J* = 6 Hz, 2H), 3.75 (t, *J* = 6 Hz, 2H), 2.52 (s, 3H), 2.50 (s, 6H), 2.31 (q, *J* = 6 Hz, 2H). **LCMS** (ES) found: 448.2 $(M + H)^+$

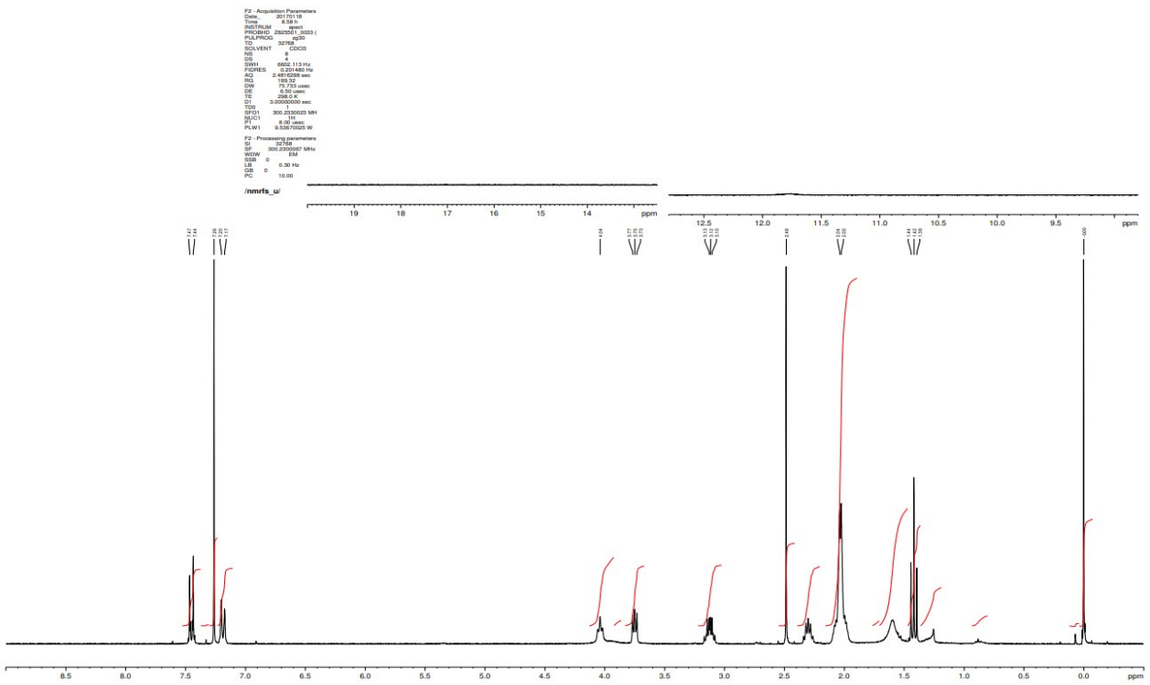


Preparation of **20**

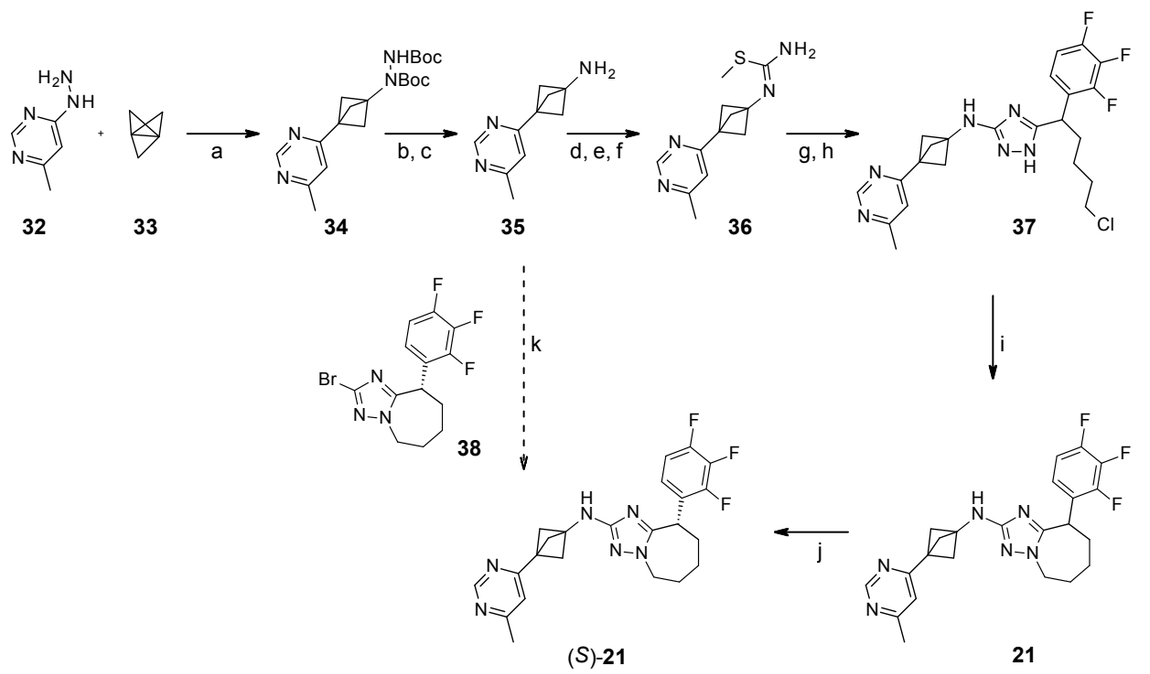


Using a Buchwald type coupling between 4-(5-methyl-1,3,4-oxadiazol-2-yl)bicyclo[2.2.2]octan-1-amine **25** and 2-bromo-4-[4-(trifluoromethoxy)phenyl]-6,7-dihydro-5H-[1,2,4]triazolo[1,5-a]pyrimidine **31b**, was obtained (36 mg, 36% yield) N-[4-(5-methyl-1,3,4-oxadiazol-2-yl)-1-bicyclo[2.2.2]octanyl]-4-[4-(trifluoromethoxy)phenyl]-6,7-dihydro-5H-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine **20** as a white solid.

^{1}H NMR: (600 MHz, $CDCl_3$) δ ppm: 7.44-7.47 (m, 2H), 7.17-7.21 (m, 2H), 4.01-4.06 (m, 2H), 3.71-3.78 (m, 2H), 2.48 (s, 3H), 2.30 (m, 2H), 1.93-2.10 (m, 12H). **LCMS** (ES) found: 490.3 $(M + H)^+$



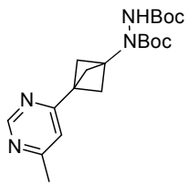
4.3 Preparation of compound (*S*)-21



Scheme 3 Reagents and conditions: (a) Fe(Pc), TBHP, Cs_2CO_3, di-*tert*-butyl azodicarboxylate, MeCN, -20°C to RT, 52%; b) 6M HCl in MeOH, RT; c) Raney Ni, H_2, MeOH, RT, quantitative; d) 1,1'-thiocarbonylbis(pyridin-2(1*H*)-one, DIPEA, DCM, RT; e) 7M NH_3 in MeOH, RT; f) MeI, EtOH, 75°C, 71%; g) 6-chloro-2-(2,3,4-trifluorophenyl)hexanoic acid, TEA, T3P®, DMF, RT; h) $N_2H_4 \cdot H_2O$, DMF, 60°C, 25%; i) LiCl, NaO^{t}Bu, DMF, 50°C, 74%; j) chiral HPLC separation; k) ^{t}BuXPhos, Cs_2CO_3, $Pd_2(dba)_3$, dioxane, 110°C, failed.

The compound (*S*)-**21** was prepared according to scheme 3:

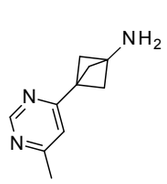
Step a: Preparation of di-*tert*-butyl 1- (3-(6- methylpyrimidin-4-yl)bicyclo[1.1.1]-pentan-1-yl)hydrazine-1,2-dicarboxylate **34**



In a two-necked round-bottom flask equipped with an argon inlet, [1.1.1]-propellane **33** in diethyl ether (35.4 ml, 0.1 M in Et_2O, 3.65 mmol) was diluted with dry MeCN (20 ml) under argon atmosphere and the mixture cooled to 20 °C. 4-hydrazinyl-6-methylpyrimidine hydrochloride **32** (1.17 g, 7.29 mmol) (preparation according to Bulletin des Societes Chimiques Belges, 1959, vol. 68, p. 30), di-*tert*-butyl azodicarboxylate (1.68 g, 7.29 mmol), Cs_2CO_3 (2.38 g, 7.29 mmol) and Fe(II) phathalocyanine (115 mg, 182 µmol) were added and the suspension was stirred for 5 minutes, then *tert*-butyl hydroperoxide 70% *w/w* in water (704 mg, 749 µl, 5.47 mmol) was added dropwise and the reaction was stirred for further 2 hours at -20° C and then allowed to reach room temperature over 12 hours. The reaction was quenched by addition of sat. aq. $Na_2S_2O_3$ (15 ml), then diluted with ethyl acetate (120 ml) and water (70 ml) and the layers were separated. The organic layer was washed with brine (50 ml), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash silica gel chromatography (eluent: *n*-heptane:EtOAc = 8:2 to 1:1) afforded di-*tert*-butyl 1- (3-(6- methylpyrimidin-4-yl)bicyclo[1.1.1]-pentan-1-yl)hydrazine-1,2-dicarboxylate **34** (1.42 g, 52%) as an off-white foam.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.02 (d, J = 1.2 Hz, 1H), 7.07 (s, 1H), 5.95-6.53 (m, 1H), 2.52 (s, 3H), 2.42 (s, 6H), 1.51 (s, 9H), 1.49 (s, 9H). **LCMS** (ES) found: 391.3 $(M+H)^+$

Steps b, c: Preparation of 3-(6-methylpyrimidin-4- yl)bicyclo[1.1.1]-pentan-1-amine **35**



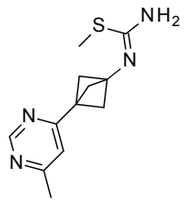
Step b: In a round-bottom flask, di-*tert*-butyl-1-(3-(6-methylpyrimidin-4-yl)bicyclo[1.1.1]pentan-1-yl)hydrazine-1,2-dicarboxylate (736 mg, 1.88 mmol) was dissolved in 6 M HCl in methanol (15 ml, 90 mmol) and the reaction was stirred at ambient temperature for 140 minutes, then the volatiles were removed *in vacuo* to afford 4-(3-hydrazineylbicyclo[1.1.1]pentan-1-yl)-6-methylpyrimidine hydrochloride (427 mg, quantitative yield) as a brown solid, which was used directly in the next step.

^{1}H NMR (300 MHz, CD_3OD) δ 9.29 (s, 1H), 7.88 (s, 1H), 2.76 (s, 3H), 2.44 (s, 6H). **LCMS** (ES) found: 191.2 $(M+H)^+$

Step c: In a round-bottom flask, 4-(3-hydrazineylbicyclo[1.1.1]pentan-1-yl)-6-methylpyrimidine hydrochloride (427 mg, 1.88 mmol) was dissolved in MeOH (10 ml) and a spatula of Raney Ni (50% water slurry) was added. An hydrogen balloon was attached to the main inlet and the reaction was stirred under an atmosphere of hydrogen for 22 hours. The reaction was filtered over of Dicalite® rinsing with MeOH and concentrated to afford 3-(6-methylpyrimidin-4-yl)bicyclo[1.1.1]-pentan-1-amine hydrochloride **35** (399 mg, quantitative yield) as a dark green solid, which was used without further purification.

^{1}H NMR (300 MHz, CD_3OD) δ 8.92 (s, 1H), 7.37 (s, 1H), 2.52 (s, 3H), 2.41 (s, 6H). **LCMS** (ES) found: 176.1 $(M+H)^+$

Steps d, e, f: Preparation of 2-methyl-3-[3-(6-methylpyrimidin-4-yl)-1-bicyclo[1.1.1]pentanyl]iso-thiourea **36**



Step d: In a round-bottom flask, 3-(6-methylpyrimidin-4-yl)bicyclo[1.1.1]pentan-1-amine hydrochloride (399 mg, 1.88 mmol) and *i*Pr_2NEt (487 mg, 658 µl, 3.77 mmol) were dissolved in CH_2Cl_2 (10 ml), then 1,1'-thiocarbonylbis(pyridin-2(1*H*)-one) (657 mg, 2.83 mmol) was added in one portion and the reaction was stirred at room temperature for 90 minutes. The reaction mixture was directly absorbed onto silica gel and purified by flash silica gel chromatography chromatography (eluent: n-heptane:EtOAc = 7:3 to 1:1) to afford 4-(3-isothiocyanatobicyclo[1.1.1]pentan-1-yl)-6-methylpyrimidine (292 mg, 71%) as a light green oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.01 (d, *J* = 1.2 Hz, 1H), 7.04 (dd, *J* = 0.4, 0.8 Hz, 1H), 2.53 (s, 6H), 2.52 (s, 3H). **LCMS** (ES) found: 218.1 $(M+H)^+$

Step e: In a round-bottom flask, 4-(3-isothiocyanatobicyclo[1.1.1]pentan-1-yl)-6-methylpyrimidine (292 mg, 1.34 mmol) was dissolved in 7 M NH_3 in MeOH (3 ml, 21 mmol) and

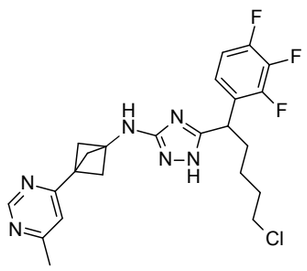
the reaction was stirred at room temperature for 35 minutes, then the reaction was concentrated *in vacuo* to afford 1-(3-(6-methylpyrimidin-4-yl)bicyclo[1.1.1]pentan-1-yl)thiourea (315 mg, quantitative yield) as an off-white solid, which was used without further purification.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.92 (d, J = 1.2 Hz, 1H), 7.31 (br s, 1H), 2.43 (s, 3H), 2.41 (s, 6H). **LCMS** (ES) found: 235.2 $(M+H)^+$

Step f: To a solution of 1-(3-(6-methylpyrimidin-4-yl)bicyclo[1.1.1]pentan-1-yl)thiourea (315 mg, 1.34 mmol) in EtOH (5 ml) was added iodomethane (92.5 µl, 1.48 mmol) and the reaction was heated to 75°C for 55 minutes, then the reaction was cooled to room temperature and concentrated *in vacuo* to afford methyl (3-(6-methylpyrimidin-4-yl)bicyclo[1.1.1]pentan-1-yl)carbamimidothioate hydroiodide **36** (497 mg, 98%) as an off-white foam, which was used without further purification.

^{1}H NMR (300 MHz, CD_3OD) δ 8.96 (d, J = 1.2 Hz, 1H), 7.40 (dd, J = 0.4, 0.8 Hz, 1H), 2.70 (s, 3H), 2.64 (s, 6H), 2.55 (s, 3H). **LCMS** (ES) found: 249.2 $(M+H)^+$

Steps g, h: Preparation of 5-[5-chloro-1-(2,3,4-trifluorophenyl)pentyl]-N-[3-(6-methylpyrimidin-4-yl)-1-bicyclo[1.1.1]pentanyl]-1H-1,2,4-triazol-3-amine **37**



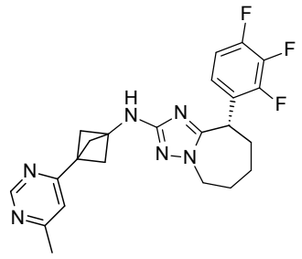
Step g: In a round-bottom flask, methyl (*E*)-*N*'-(3-(6-methylpyrimidin-4-yl)bicyclo[1.1.1]pentan-1-yl)carbamimidothioate hydroiodide (397 mg, 1.06 mmol), 6-chloro-2-(2,3,4-trifluorophenyl)hexanoic acid (preparation described in WO2018/83050 A1, 2018) (355 mg, 1.27 mmol) and Et_3N (515 µl, 3.69 mmol) were dissolved in dry DMF (3 ml) and T3P® (1.23 ml, 50% in DMF, 2.11 mmol) was added and the reaction was stirred at ambient temperature for 15 minutes, then the reaction was diluted with ethyl acetate (30 ml), washed three times with water (30 ml each time), dried over Na_2SO_4 and concentrated *in vacuo* to afford the crude amide (477 mg, 89%) which was directly without further purification.

Step h: The crude was then re-dissolved in DMF (3 ml), followed by the addition of hydrazine hydrate (78.3 µl, 1.58 mmol) and the reaction was stirred at 60°C for 22 hours. The reaction was

diluted with ethyl acetate (50 ml), washed three times with water (30 ml each time), then dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash silica gel chromatography (eluent: CH_2Cl_2:MeOH = 1:0 to 95:5) to afford 5-(5-chloro-1-(2,3,4-trifluorophenyl)pentyl)-*N*-(3-(6-methylpyrimidin-4-yl)bicyclo[1.1.1]pentan-1-yl)-4*H*-1,2,4-triazol-3-amine **37** (143 mg, 28%) as a light yellow foam.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.02 (d, *J* = 1.2 Hz, 1H), 7.11-7.23 (m, 1H), 7.09 (d, J = 0.8 Hz, 1H), 6.88-7.00 (m, 1H), 5.32 (s, 1H), 4.27 (t, J = 7.9 Hz, 1H), 3.50 (t, J = 6.7 Hz, 2H), 2.53 (s, 3H), 2.47 (s, 6H), 2.13-2.30 (m, 1H), 1.88-2.03 (m, 1H), 1.72-1.87 (m, 2H), 1.32-1.58 (m, 2H). **LCMS** (ES) found: 475.3 $(M+HCl)^+$

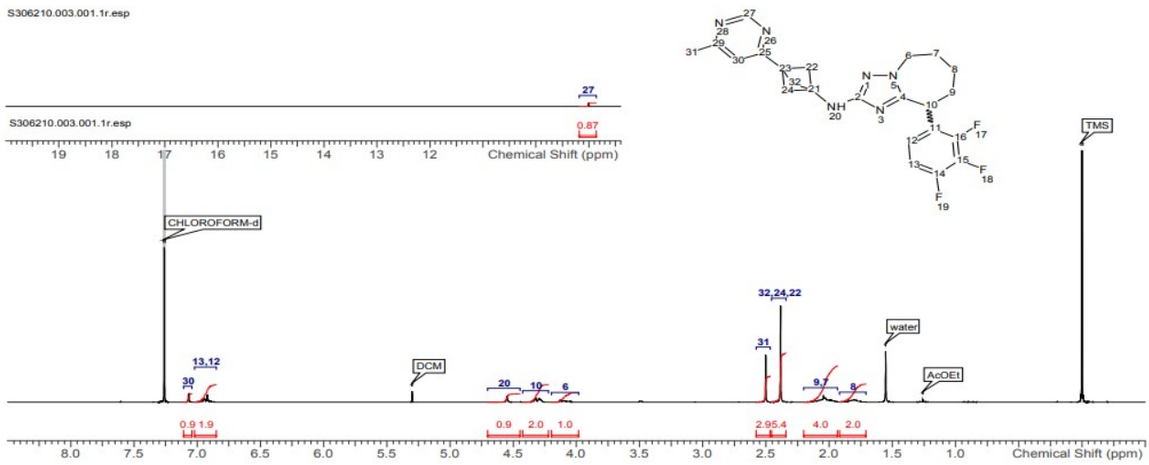
Steps i, j: Preparation of (*S*)-*N*-(3-(6-methylpyrimidin-4-yl)bicyclo[1.1.1]pentan-1-yl)-9-(2,3,4-trifluorophenyl)-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepin-3-amine **21**



Step i: To a solution of 5-(5-chloro-1-(2,3,4-trifluorophenyl)pentyl)-*N*-(3-(6-methylpyrimidin-4-yl)bicyclo[1.1.1]pentan-1-yl)-4*H*-1,2,4-triazol-3-amine (168 mg, 352 µmol) in dry DMF (3 ml) was added lithium chloride (14.9 mg, 352 µmol) and sodium *tert*-butoxide (34.5 mg, 352 µmol) and the reaction was stirred at 50°C for 70 minutes. After cooling to room temperature, the reaction was diluted with ethyl acetate (50 ml), washed three times with water (30 ml each time), the dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash silica gel chromatography (eluent: CH_2Cl_2:MeOH = 1:0 to 95:5) to afford *rac*-*N*-(3-(6-methylpyrimidin-4-yl)bicyclo[1.1.1]pentan-1-yl)-9-(2,3,4-trifluorophenyl)-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[1,5-*a*]azepin-2-amine **21** (155 mg, 74%) as light yellow solid.

Step j: Chiral preparative HPLC on a Reprosil Chiral NR afforded (*S*)-*N*-(3-(6-methylpyrimidin-4-yl)bicyclo[1.1.1]pentan-1-yl)-9-(2,3,4-trifluorophenyl)-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepin-3-amine (*S*)-**21** as a colourless solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.00 (s, 1H), 7.07 (s, 1H), 6.81-7.00 (m, 2H), 4.56 (s, 1H), 4.31 (br d, *J* = 9.1 Hz, 2H), 3.97-4.20 (m, 1H), 2.50 (s, 3H), 2.39 (s, 6H), 1.92-2.19 (m, 4H), 1.80 (br s, 2H). **LCMS** (ES) found: 441.3 $(M+H)^+$


S306210.003.001.1r.esp
S306210.003.001.1r.esp
Chemical Shift (ppm)
CHLOROFORM-d
DCM
water
AcOEt
TMS
Chemical Shift (ppm)