Université de Montréal

Synthesis of Heterocyclic Compounds of Medicinal Relevance

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Mémoire présenté à la Faculté des Études Supérieures

En vue de l' obtention du grade de

Maître ès Sciences (M.Sc.)

En Chimie

December 2003

Université de Montréal

Faculté des Études Surpérieures

Ce Mémoire intitulé :

Synthesis of Heterocyclic Compounds of Medicinal Relevance

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Dr. Stephen Hanessian	directeur de recherche

Mémoire accepté le :

Abstract

My research was involved in the synthesis of heterocyclic compounds of medicinal relevance. Firstly, we designed and synthesized a novel 2-pyridone precursor related to ABT-719, a well-known antibacterial compound. An advanced intermediated was reached, but difficulties in the last steps precluded the synthesis of the intended bicyclic azaquinoline.

The second project focused on the synthesis of a small library of 28 compounds as Rho kinase inhibitors. The core structure was an amino piperidine, which was diversified as sulfonamides and amides. Modest activity was found with one of the compounds.

The third project was involved in the synthesis of a series of monocyclic acylguanidines as Na^+/H^+ exchanger inhibitors. Biological testing identified four potent inhibitors.

Keywords: heterocycle, 2-pyridone, DNA gyrase, piperidine, Rhokinase inhibitor, acylguanidine, Na^+/H^+ exchanger (NHE-1) inhibitor.

Résumé

Ma recherche décrit la synthèse de composés hétérocycliques d'importance biologique. En premier lieu, nous avons fait le design et la synthèse d'un nouvel analogue de type 2-pyridone basé sur la structure d'un composé antibactérien bien connu, le ABT-719. Un composé intermédiaire avancé a été atteint, mais des difficultés lors des dernières étapes ont mené à l'abandon de l'azaquinoline bicyclique désirée.

Le deuxième projet décrit la synthèse d'une petite librairie de 28 produits consistant en deux séries de dérivés sulfonamides et amides de pipéridines comme étant inhibiteurs de la Rho-kinase. Une activité modeste a été trouvée avec un des analogues.

Le troisième projet consiste en la synthèse d'acylguanidines monocycliques comme inhibiteurs potentiels de canaux Na^+/H^+ (Na^+/H^+ échangeur, NHE-1). Basé sur les structures connues d'inhibiteurs NHE, nous avons synthétisé une petite librairie de dérivés acylguanidines. Les analyses biologiques ont identifié quatre inhibiteurs potentiels.

Mots clefs: hétérocycle, 2-pyridone, ADN gyrase, pipéridine, Rhokinase inhibiteurs, acylguanidine, Na^+/H^+ échangeur (NHE-1) inhibiteur.

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Abbreviation

$[\alpha]_D$	Specific rotation
Ac	Acetyl
Boc	*tert*-Butoxycarbonyl
Bp	Boiling point
Bu	Butyl
t-Bu	*tert*-Butyl
δ	Chemical shift in ppm
c	Concentration in milligrams per milliliter
Calcd.	Calculated
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DIPEA	N, N-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	N, N-Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPA	Diphenylphosphoryl azide
EDC	1-(3-Dimethyllaminopropyl)-3-ethylcarbodiimide hydrochloride
EtOAc	Ethyl acetate
Et	Ethyl
eq	Equivalent
ether	Diethyl ether
h	Hours (s)
Hex	Hexane
HOBt	1-Hydroxybenzotriazole
HRMS	High resolution mass spectrum
Hz	Hertz
IC_{50}	Concentraction of inhibition at 50%
IR	Infrared spectroscopy
J	Coupling constant

LDA	Lithium diisopropylamide
Me	Methyl
mg	Milligram
min	Minute
mL	Milliliter
mmol	Millimole
Mp	Melting point
MS	Mass spectrum
NMR	Nuclear magnetic resonance
Ph	Phenyl
PMB	*p*-Methoxylbenzyl
ppm	Parts per million
psi	Pounds per square inch
rt	Room temperature
Satd	Saturated
SAR	Structure activity relationship
TBAI	Tetrabutylammonium iodide
Tf	Trifluoromethanesulfonyl
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilane
Ts	4-Toluenesulfonyl
μL	Microliter
Wt	Weight

Acknowledgment

I would like to take this opportunity to express my sincere gratitude to Professor Stephen Hanessian, for his guidance and inspiration as well as giving me this training opportunity to improve my chemistry.

I wish to thank the support of my husband and my lovely son as well as my friends and colleagues for their friendship and help, especially Dr. Vinh Pham. My thanks also go to madams Carol Major, Elaine Fournelle and Lyne Laurin.

Finally, I would sincerely like to thank Professor Stephen Hanessian for revising the manuscript and Dr. Eric Therrien for preparing the Résumé.

CHAPTER 1

Synthesis of a novel of 2-pyridone analogue

Like the quinolones, the 2-pyridones are DNA gyrase inhibitors. The mechanism of inhibiting bacteria by 2-pyridones is very similar to that of the quinolones.[5]

1.2 The inhibition mechanism of quinolones

Quinolone-type drugs have a unique capacity to trap the intermediate (DNA gate) by stabilizing the enzyme-DNA complex as illustrated in Figure 1.2. More importantly, such a process leads to the formation of a cleavable complex.

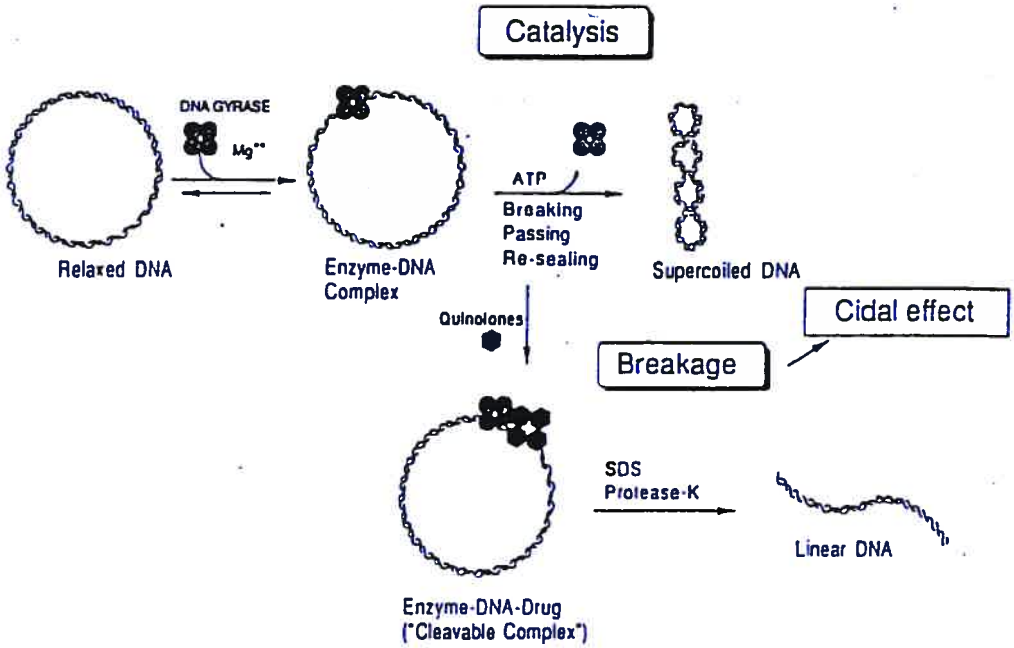


Figure 1.2 Schematic presentation of the catalytic function of DNA gyrase and the bactericidal effect of quinolone antibacterials (Reproduced from Shen, L.L. *Adv in Pharmacology*, 1994, *29A*, 285).

1.3 Mode of action of quinolones

A model for the inhibition of DNA gyrase is shown in Figure 1.3. Quinolone molecules are shown as solid and hatched rectangles that represent the drug self-association, and binding to a gyrase-induced DNA site during the intermediate gate-opening step of DNA supercoiling process via hydrogen bonds to the unpaired bases indicated by doted lines. Gyrase A subunits

1.1 DNA gyrase inhibitors

DNA gyrase[1] is a bacterial motor protein in a class known as topoisomerases, which is responsible for controlling the topological properties of DNA (e.g. amount of supercoiling or catenation). Most topoisomerases can relax supercoiled DNA, which is an energetically favourable process. DNA gyrase is unique amongst this class enzyme, because it can introduce supercoils as well remove them. Quinolones were new generarion broad-spectrum antibacterial agents[2] developed in 1980s'. The mechanism of action of quinolones is inhibition of DNA gyrase.[3]

Four-generations of quinolone drugs have been developed.[4] First-generation drugs (e.g., nalidixic acid) achieve minimal serum levels. Second-generation quinolones (e.g., ciprofloxacin) have increased Gram-negative and systemic activity. Third-generation drugs (e.g., levofloxacin) have expanded activity against Gram-positive bacteria and atypical pathogens. Fourth-generation quinolone drugs (currently only trovafloxacin) add significant activity against anaerobes. We chose ciprofloxacin as a representative of quinolone and compared its structure with 2-pyridone. Figure 1.1 shows that changing the position and number of nitrogen in ciprofloxacin can generate two structures related to 2-pyridones.

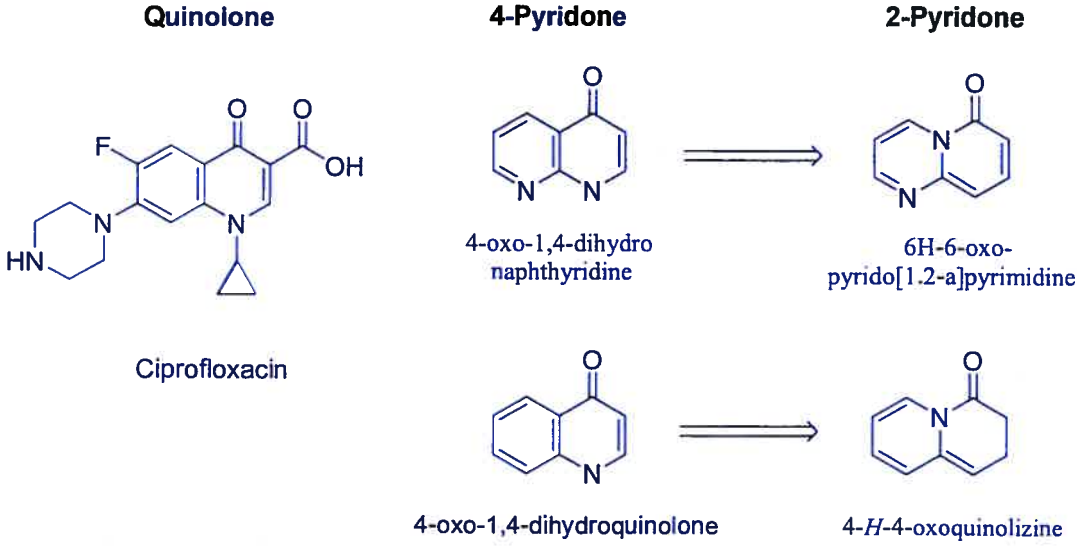


Figure 1.1 Structures of core units of 4-quinolone and 2-pyridone

form covalent bonds between Tyr-122 and the 5' end of the DNA chain, and the subsequent opening of the DNA chains along the 4-bp staggered cuts results in a locally denatured DNA bubble, which is an ideal site for the drug to bind. When a relaxed DNA is used, ATP is required for the induction of the drug-binding site. Dashed curves mimic the shape of the DNA gyrase, a tetramer of two A and two B subunits as revealed by the electron microscope image of the *M. luteus* enzyme. Figure 1.4 is a 3-dimensional presentation of the model.

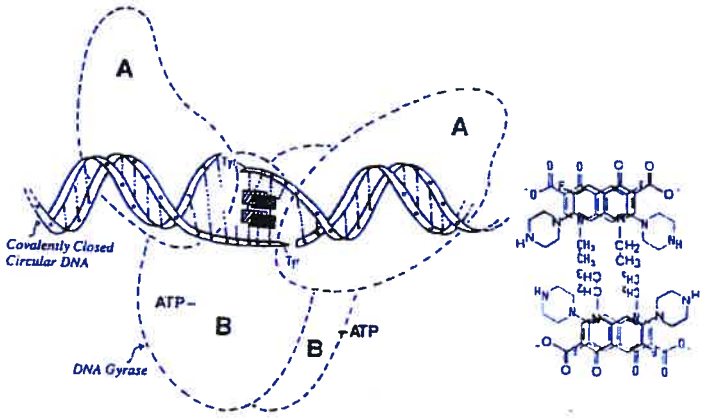


Figure 1.3 Head to tail association allows H-bonding to DNA (reproduced from Shen, L.L. *Adv in Pharmacology*, **1994**, *29A*, 285)

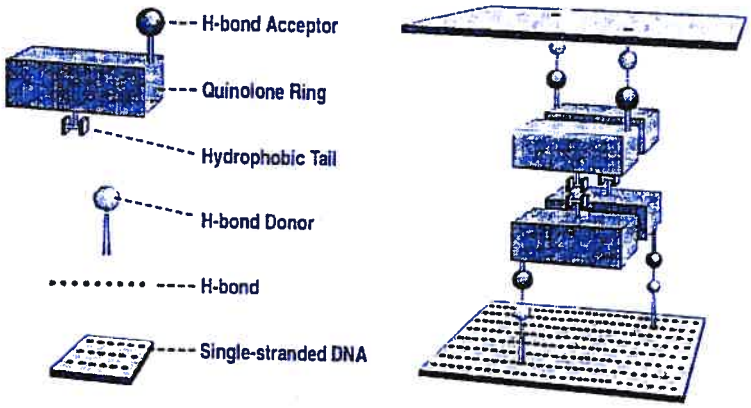
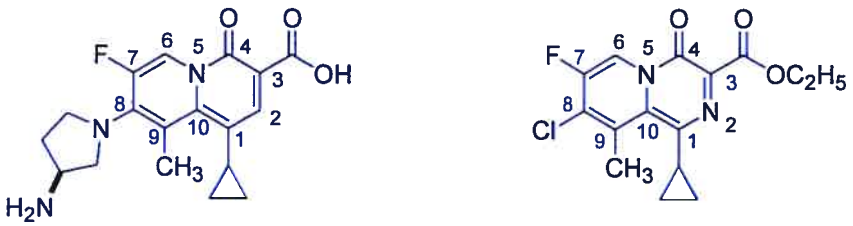


Figure 1.4 Inhibition 3D model for quinolone antibiotics (reproduced from Shen, L.L. *Adv in Pharmacology*, **1994**, *29A*, 285)

1.4 2-Pyridone: A new quinolone analogue

In an effort to discover novel antibacterials related to the known fluoroquinolones[6] such as ciprofloxacin,[7] scientists at Abbott Laboratories explored the chemistry of new series. This involved transposition of the nitrogen of 4-quinolones to the bridgehead position at C_5 quinolone membering) yielding two novel heterocyclic nuclei related to a 2-pyridone, 6H-6-oxo-pyrido[1,2-a]pyrimidine and 4-H-4-oxoquinolizine (see Figure 1.1), which had not previously been evaluated as antibacterial agents and were found to be potent inhibitors of DNA gyrase. In addition, the so-called 2-pyridones also possess favorable physiochemical and pharmacokinetic properties.[8, 9]

ABT-719 is a potential antibiotic compound,[10] which was synthesized by scientists at Abbott Laboratories. The chemical structure of ABT-719 is similar to fluoroquinolone, the difference being transfer of the N atom to position 5. The target compound, 8-chloro-1-cyclopropyl-7-fluoro-9-methyl-4-oxo-1, 4-dihydro-pyrido [1.2-a] pyrazine-3-carboxylic acid ethyl ester is a novel class of 2-pyridone core compared to ABT-719 (Figure 1.5), The main difference is in the replacement of C-5. This leads to a 5-aza-isoquinolone-type structure.



ABT-719 **1.1 target compound**

Figure 1.5 Structures of ABT-719 and a target compound

1.5 Synthesis of 8-Chloro-1-cyclopropyl-7-fluoro-9-methyl-4-oxo-4H-pyrido[1,2a]-pyrazine-3-carboxylic acid ethyl ester

We envisaged the disconnections shown in Figure 1.6 for the synthesis of the bicyclic core. A series of aromatic substitutious would lead to the C-2 branched acid, which would be subjected to a Curtius rearrangement.

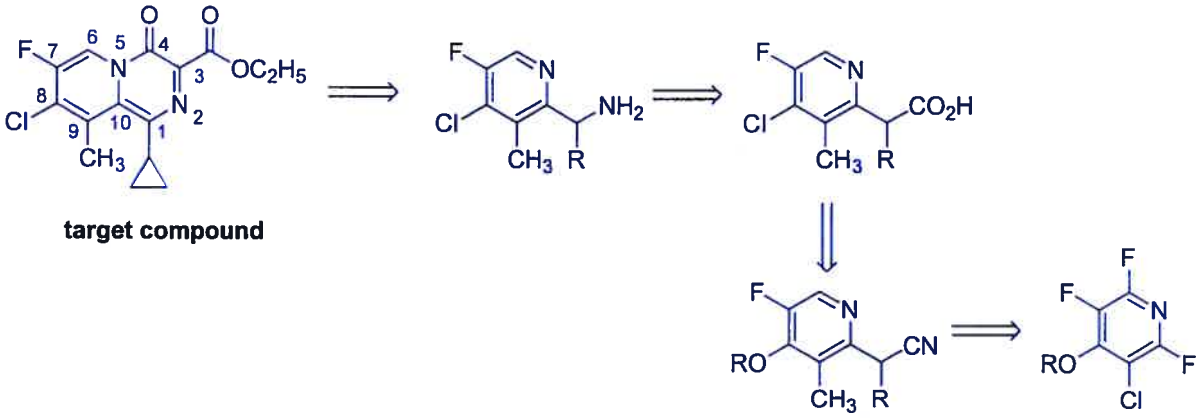
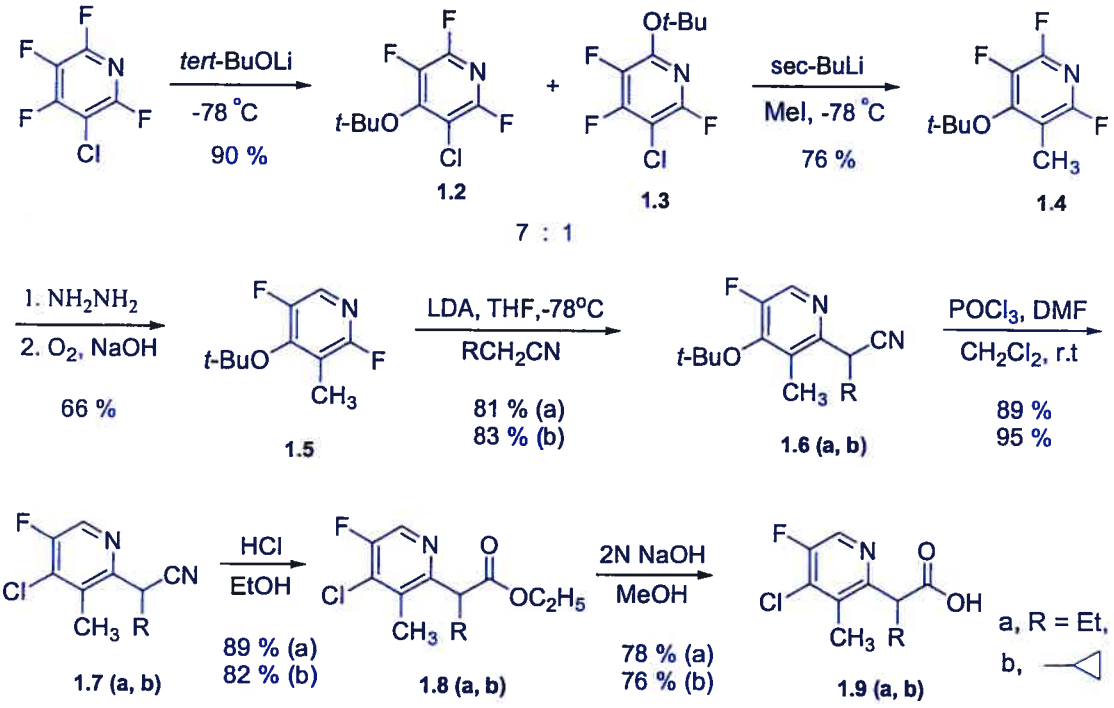


Figure 1.6 Retrosynthesis of the target compound

2,4,5,6-Tetrafluoro-3-chloro-pyridine was treated with lithium *tert*-butoxide to give two-regioisomeric products **1.2** and **1.3**[11] (Scheme 1.1). The next step involved metallation of aryl chloride by *sec*-butyl lithium followed by alkylation with methyl iodide to afford compound **1.4**. The fluorine in the 6 position was substituted with hydrazine and the product was oxidized to yield compound **1.5**. The carbanion formed by treatment of a substituted acetonitrile with LDA was then used to introduce carbon branching at position 2 to give **1.6**. Treatment of **1.6** with $POCl_3$ effected the cleavage of the OtBu group and the replacement by chlorine to give **1.7**. Hydrolysis of the nitrile group with ethanolic HCl afforded the ester[12] **1.8**, which was subsequently converted to the carboxylic acid **1.9**.[13]

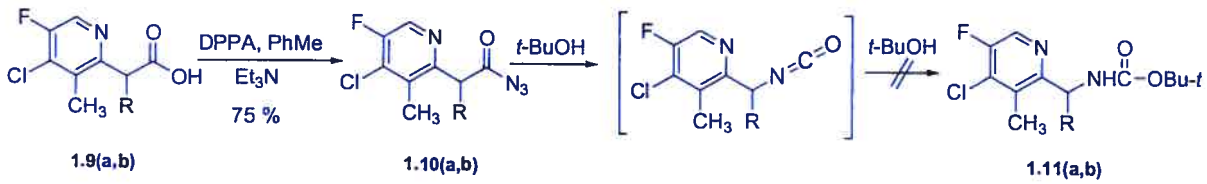
Scheme 1.1 Synthesis of the 2-pyridone analogue



The Curtius rearrangement [11, 14] was tried in the presence of DPPA and TEA. However, heating the compound **1.10** in *tert*-butyl alcohol didn't lead to compound **1.11**. Using the smaller methanol didn't help to produce the carbamate (Scheme 1.2).

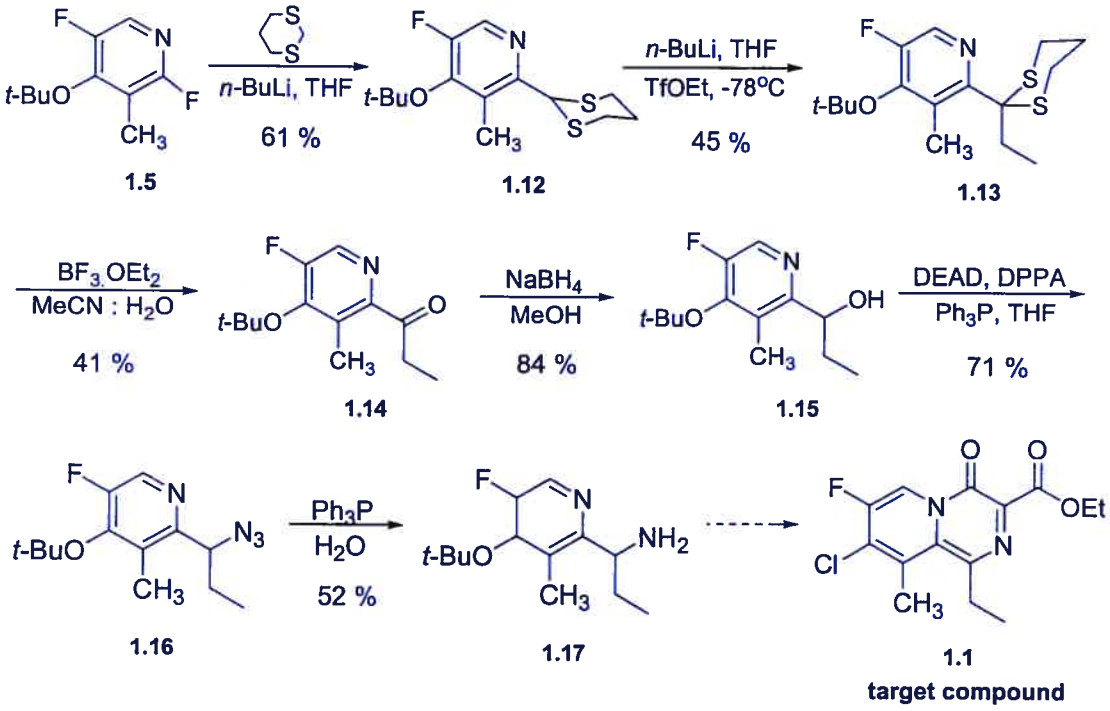
Unfortunately the standard conditions for a Curtius rearrangement (DPPA, Et_3N) and heating in *t*-BuOH failed to give the desired rearranged N-Boc derivative **1.11**, Even though can obeserve the formation of the isocyanate by IR spectraoscopy.

Scheme 1.2 Attempted Curtius rearrangement



We therefore considered an alterative strategy as shown in Scheme 1.3. Compound **1.5** was reacted with 1,3-dithiane in the presence of *n*-BuLi[15] to give 4-*tert*-butoxy-2-(2-ethyl-[1,3]dithian-2-yl)-5-fluoro-3-methyl-pyridine **1.12**, which was alkylated by ethyl triflate after deprotonation by *n*-BuLi to give compound **1.13**.[16] Removal of the 1.3-dithiane with BF_3 OEt_2 in MeCN and H_2O[17] gave **1.14**. Reduction of the ketone with $NaBH_4$ gave alcohol **1.15**,[18] which was transformed to the azide **1.16** under Mitsunobu condition.[19] Triphenylphosphine was employed to reduce[20] the azide to give amine **1.17**.

Scheme 1.3 Alternative methods

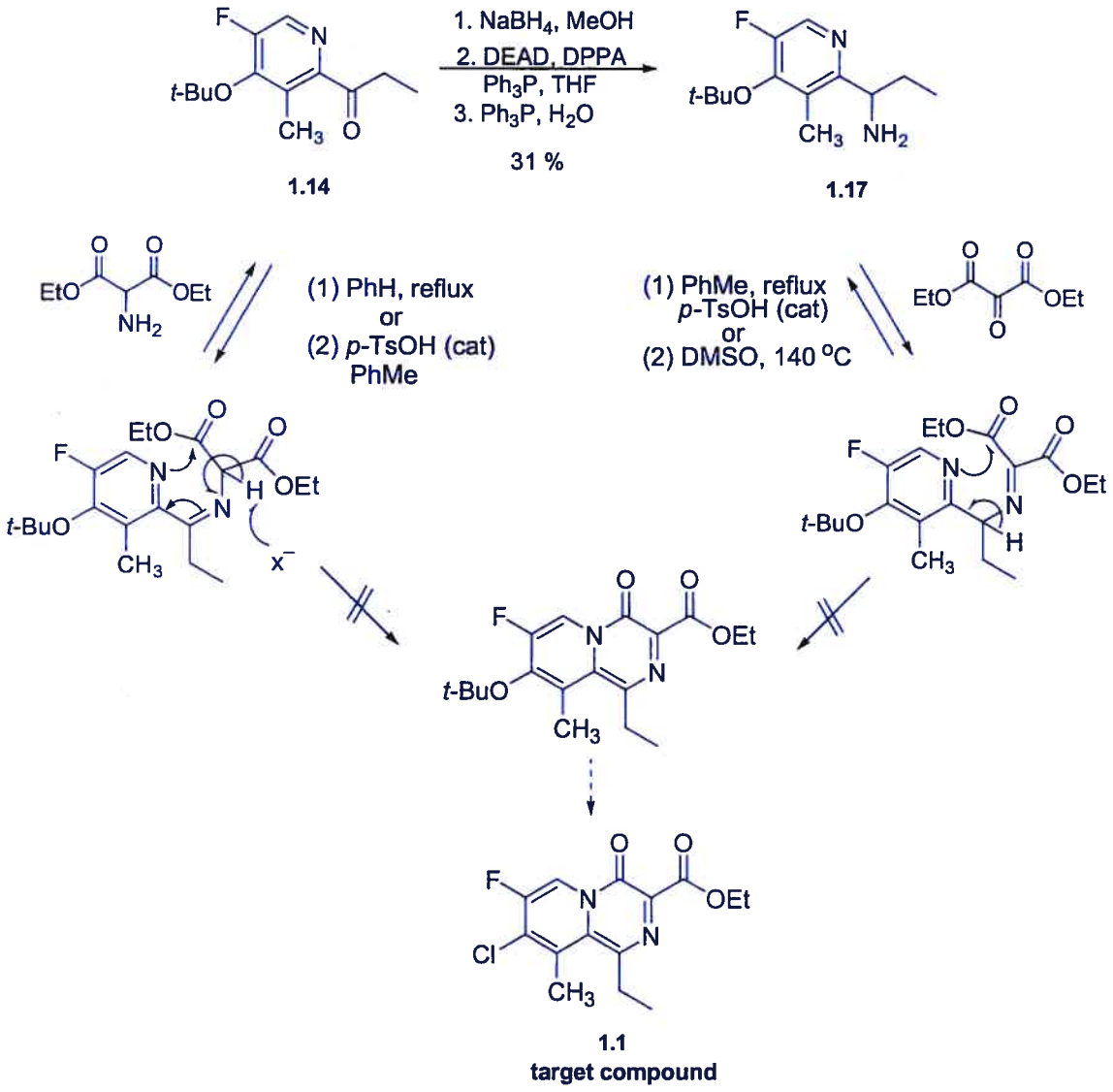


We had effectively introduced the amino propyl group at C-2 of the pyridine nucleus by this method of branching. The remaining was to construct the pyrazine unit en route to intended target.

From compounds **1.14** and **1.17**, we tried to prepare the six-membered ring using diethyl malonate derivatives.[21a, b] Unfortunately, we were not successful in effecting the desired condensations.

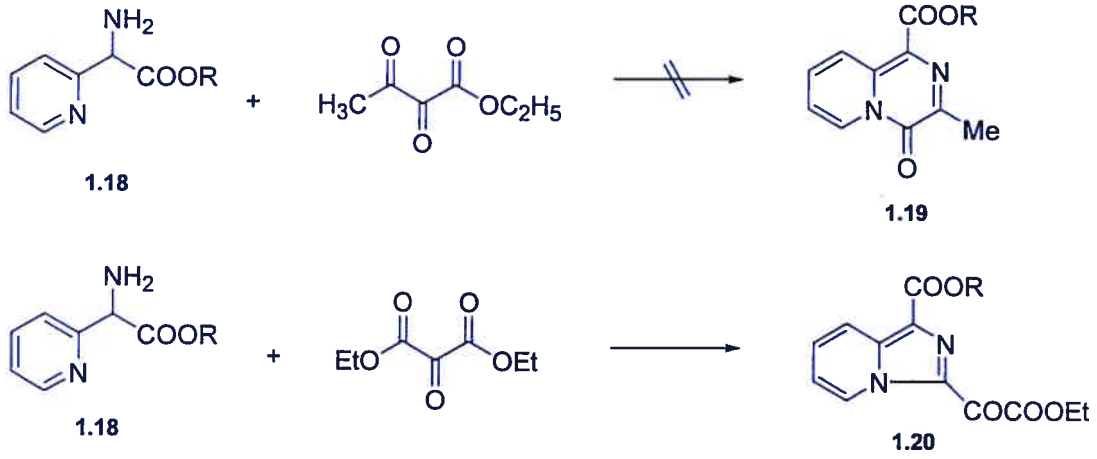
Scheme 1.4 shows in one series of reactions where the ketone **1.14** was treated with diethyl 2-aminomalonate under acid catalysis. We envisaged formation of an imine followed by cyclization. In a second attempt, we reacted the aminopropyl pyridone with diethyl 2-oxo-malonate under acid catalysis, expecting to get the same imine. In both cases starting materials **1.14** and **1.17** were recovered with and without the loss of the *tert*-Bu group. The literature has hardly any precedence for the formation of the intended ring structure as shown in Scheme 1.5.[22, 23]

Scheme 1.4 Attempts toward target compound 1.1



Thus, starting from compound **1.18**, the reaction was conducted under varying conditions such as boiling glacial acetic acid or in polyphosphoric acid at 80 °C. In no case compound **1.19** was formed. Also with diethyl oxalate only **1.20** was formed although the bicycle **1.19** is theoretically possible. (Scheme 1.5)

Scheme 1.5 Examples of cyclization



1.6 Conclusion

Although we were successful in preparing suitably functionalized pyridines, the construction of the desired 6-azaisoquinoline nucleus could not be achieved. It is possible that the pyridine nitrogen is too weakly basic to affect cyclization as shown in Scheme 1.4.

1.7 General experimental notes

Melting points (mp) were measured on a Fisher-Johns apparatus, and they are uncorrected. Unless otherwise specified, all non-aqueous reactions were carried out under a nitrogen atmosphere, using oven-dried glassware, and all reaction solvents were removed by rotary evaporator. All solvents in dry reactions were distilled over calcium hydride. Unless other stated, the reagents were purchased from Aldrich Chemical Co.

Analytical thin layer chromatography (TLC) was performed using EM Reagent 0.25 mm silica gel 60-F plates. Visualization of the developed chromatogram was performed by UV

absorbance. Nuclear magnetic resonance of proton spectra (^{1}H NMR) and carbon-13 (^{13}C NMR) were recorded on a Bruker AMX-300, or Bruker AMX-400 spectrometer in a deuterated solvent as indicated using the signal from the residual non-deuterated solvent, $CHCl_3$ (H, δ = 7.27 ppm; C, δ = 77.23 ppm) as internal reference. Chemical shifts (δ) and coupling constants (J) are expressed in ppm (part per million) and Hz (Hertz), respectively. The abbreviations used for the description of the peaks are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; dd, doublet of doublet; doublet of triplets. DEPT-135 experiments were performed routinely, methyl (CH_3) and methine (CH) give positive signal (+), methylene (CH_2) gives a negative signal (-), and tertracarbon give no signal (0). All chemical shifts are measured from the centre of the resolved peaks, the unresolved multiple and broad peaks are normally indicated as a range.

Low resolution mass spectra (MS) and high resolution mass spectra (HRMS) were respectively determined on a VG Micro Mass 1212 and a kratos MS-50 TCTA mass spectrometer by using methods of desorption chemical ionization (Cl) or fast atom bombardment (FAB).

Infrared (IR) spectra were recorded on Perkin-Elmer FTIR Paragon 1000 spectrophotometer in a chloroform solution with a sodium chloride cell, or mixture film with KBr. Only characteristic peaks are reported.

Optical rotations ([α]) were measured at room temperature using a Perkin-Elmer polarimeter, modele 241 apparatus with a sodium lamp (wavelength of 589 nm) at ambient temperature using a 10 cm-lenghtcell containing 1 mL of a solution prepared at the indicated concentration (c, g /100 mL).

Chromatography

Flash chromatography was done by general procedure using Kieselgel (Merck 9385, 230-400 mesh) silica gel. Thin Layer Chromatography (TLC) was performed using commercial available glass plates coated with Silica Gel 60 F254 with 0.25 mm thickness (Merck, Kieselgel $60F_{254}$).

TLC visualization

UV 254 lamp was used to observe the UV visible compounds and to evaluate the advancement of the reaction. Chemical visualization was done using one of the following solutions:

(a) Molybdate/Ceric sulfate solution.

Ammonium molybdate (VI) tetrahydrate: $(NH_4)_6Mo_7O_{24}.4H_2O$	50g
Ammonium cerium (IV) sulfate dihydrate: $(NH_4)_4Ce(SO_4).2H_2O$	20g
Concentratedd sulphuric acid: H_2SO_4	200 mL
Distilled water: H_2O	1200 mL

(b) Ninhydrin solution.

Ninhydrin dihydrate	2 g
Butanol	600 mL
Acetic acid	18 mL

(c) $KMnO_4$ solution.

A 10% aqueous solution of $KMnO_4$ was used with olefin compounds.

Reagents

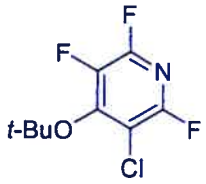
Commercially available were purchased from Aldrich, Sigma or Lancaster and used without further purification. All commercially unavailable reagents were prepared.

Solvents

EtOAc, Hexane, and dichloromethane (DCM) were distilled prior to chromatography and general use.

Toluene, THF, DCM and ether were dried using the dry alumina column. Triethylamine, diisopropylamine benzene and methanol were distilled over calcium hydride.

All reaction were carried out under argon. The entire flasks were flame-dried under vacuum. All needles and syringes were dried under vacuum before to use. Yields refer to chromatographically pure products.



4-*tert*-Butoxy-3-chloro-2, 5, 6-trifluoropyridine (1.2)

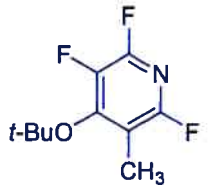
A solution of *tert*-BuOLi (7.5 g, 93.7 mmol) in THF (75ml) was cooled to –78 °C in a dry-ice/acetone bath. A solution of 2,4,5,6-tetrafluoro-3-chloropyridine (22.5 g, 84.9 mmol) (Aldrich, 70% pure) mixed with 2,4,5,6-tetrafluoro-3-chloropyridine 30% in THF (45 mL) was added dropwise. The reaction mixture was stirred for 2 h at –78 °C, then at ambient temperature overnight. The reddish brown mixture was concentrated at ~30 °C, hexane (100mL) and celite (~5 g) were added and the mixture was stirred for 30 min. The solid was removed by filtration the solvent was removed under reduced pressure to yield a colorless liquid (18.4 g, 90%) as a mixture of **1.2** (4-butoxy, desired) and 1.3 (6-butoxy, undesired) in ratios of 7:1. The two compounds could be separated by flash column chromatography (ethyl acetate:hexane 3:97) to get pure compound **1.2** (15.1g, 71%)

^{1}H NMR (400 MHz, $CDCl_3$) δ: **1.2** 1.52 (s, 9H); **1.3**: 1.61 (s, 9H);

^{13}C NMR (400 MHz, $CDCl_3$) δ: 163.9, 159.1, 150.7, 132.1, 104.3, 73.1, 28.4.

^{19}F NMR (400 MHz, $CDCl_3$) δ (ppm): **1.2**: -73.75 (dd, J = 14.2, 23.2 Hz, 1F), -89.71 (dd, J = 14.2, 21.9 Hz, 1F), -152.42 (t, J = 22 Hz, 1F); **1.3**: -74.95 (dd, J = 9.0, 24.5 Hz, 1F), -121.69 (dd, J = 9.0, 18.1 Hz, 1F), -162.47 (dd, J = 18.1, 24.5 Hz, 1F).

MS (M^+): 239.03



4-*tert*-Butoxy-3-methyl-2, 5, 6-trifluoropyridine (1.4)

A 250mL three-necked flask equipped with a mechanical stirrer, a graduated addition funnel and a digital thermometer was charged with compound **1.2** (6.06 g, 0.0252 mol) and THF (23 mL). The internal temperature of the mixture was cooled to –70 °C using a dry ice / acetone bath. A solution of *sec*-BuLi (24 ml, 1.3 M in cyclohexane, 0.0313 mol) was added via syringe to this above stirred solution over a period of 1.0 h. The speed of addition was adjusted as to maintain an internal temperature between –61 to –70 °C. After the addition was completed, stirring was continued for an additional 1 h in a dry ice / acetone bath. MeI (2.38 ml, 0.0383 mol) was added over ~15 min, the lithium salt dissolved and the internal temperature rose quickly to –39 °C. The mixture was stirred for 1 h at ambient temperature. The reaction was quenched with saturated aqueous NH_4Cl (7 mL) and extracted with 100 mL of ether. The extract was washed with water (1 x 25 mL), brine (2 x 15 mL), dried over $MgSO_4$, and concentrated to give the crude product (7.15 g). This material was distilled under reduced pressure to give **1.4** (4.21 g, 76%) as a pale yellow liquid, bp. 75-81 °C (7.5 mmHg). It was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.12 (s, 3H), 1.47 (s, 9H).

^{13}C NMR (400 MHz, $CDCl_3$) δ 164.3, 161.0, 149.8, 130.6, 105.1, 71.1, 28.5. 11.5.

^{19}F NMR) (400 MHz, $CDCl_3$) δ -75.91 (dd, J = 15.0, 22.1 Hz, 1F), -93.17 (dd, J = 15.0, 22.1 Hz, 1F), -156.54 (m, 1F).

HRMS: $C_{10}H_{12}F_3NO$ (M^+); Calcd.: 219.0897; found: 219.0881.

4-*tert*-Butoxy-2, 5-difluoro-3-methylpyridine (1.5)

A solution of **1.4** (4.2 g, 19.2 mmol) and hydrazine monohydrate (>98%, 2.33 ml, 0.048 mol) in methanol (7.5 mL) was refluxed for 9 h. The methanol was removed and the residue was dissolved in methylene chloride (10 mL) and washed with water (2 x 5 mL). Solvent was distilled under reduced pressure, leading an orange oil. It was redissolved in methanol (21.3 mL). To this was added aqueous sodium hydroxide (20%, 11.3 mL), and air was passed through the solution for 6 days with vigorous stirring. The methanol was removed under vacuum at 30-35 °C, the residue was dissolved in ether (38 mL), washed with water (1 x 15 mL), 10% HCl (1 x 10 mL), saturated brine (1 x 20 mL), and dried over $MgSO_4$. The solvent was removed and the residue was purified by flash chromatography (ethyl acetate:hexane 5:95) to afford 2.56 g of **1.5** (66.5%) as a colorless liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (br, H), 2.18 (d, J = 1.5 Hz, 3H), 1.43 (d, J = 1.5 Hz, 9H).

^{13}C NMR (400 MHz, $CDCl_3$) δ 162.5, 158.2, 143.7, 135.4, 106.6, 75.1, 28.5, 11.3.

^{19}F NMR (400 MHz, $CDCl_3$) δ -73.37 (d, J = 24.5, 1F), -142.17 (d, J = 24.5 Hz, 1F)

MS: 201.10.

HRMS: $C_{10}H_{13}F_2NO$ (M^+); Calcd.: 201.1072; found: 201.1088.

2-(4-*tert*-Butoxy-5-fluoro-3-methyl-2-pyridyl)-2-cyclopropylacetonitrile (1.6 a)

LDA was formed by adding n-BuLi (2.5 M in hexanes, 15 mL, 37.5 mmol), dropwise to a stirred solution of diisopropylamine (5.15 mL, 73.5 mmol) in THF (15 mL) at –78 °C. The reaction was allowed to stir at 0 °C for 15 min and then cooled to –78 °C with a dry-ice/acetone bath. Cyclopropylacetonitrile (3.0 g, 37.0 mmol) in anhydrous THF (7.5 mL) was added over a period of 15 min to the above solution of LDA, keeping the internal

temperature between –51 and –67 °C. The mixture was stirred for an additional 35 min at the same temperature. To the above solution, **1.5** (3.0 g, 14.9 mmol) in THF (7.5 mL) was added over 20 min maintaining an internal temperature of –65 to –71 °C. The cooling bath was removed and stirring was continued for 30 min. When the temperature reached –30 °C, an exothermic reaction was observed and the temperature rose quickly to 17 °C. The reaction was quenched with saturated aqueous NH_4Cl (10 mL) and was extracted with ether (50 mL). The extract was washed with saturated brine, dried over $MgSO_4$ and concentrated. The excess cyclopropylacetonitrile was removed at 40-45 °C at 0.2 mmHg. The residue was purified by flash chromatography (ethyl acetate:hexane 5:95) to give **1.6a** (3.25 g, 83%) as a colorless liquid, which solidified on standing.

Mp. 52-54 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 3.75 (d, J = 7.16 Hz, 1H), 2.28 (s, 3H), 1.48 (m, 1H), 1.42 (s, 9H), 0.73 (m, 1H), 0.63 (m, 1H), 0.50 (m, 2H).

^{13}C NMR (400 MHz, $CDCl_3$) δ 157.1, 154.7, 153.4, 150.7, 149.9, 137.2, 85.3, 38.9, 29.6

HRMS: $C_{15}H_{20}N_2OF$ (M+1); Calcd.: 263.1560; found: 263.1565.

2-(4-*tert*-Butoxy-5-fluoro-3-methyl-2-pyridyl)-butyronitrile (1.6b)

The procedure was same as **1.6a** to give **1.6b** as a colorless solid (0.8g, 81%)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.12 (s, 1H), 3.91 (t, J = 7.1, 1H), 2.13 (s, 3H), 1.85 (m, 2H), 1.30 (s, 9H), 0.82 (t, J = 7.1 Hz, 3H).

^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 154.9, 152.4, 150.9, 150.9, 149.6, 136.2, 84.6, 38.3, 29.3, 26.6, 12.7, 12.0.

HRMS: $C_{14}H_{20}FN_2OF$ (M+1); Calcd.: 251.1538; found: 251.1527.

2-(4-Chloro-5-fluoro-3-methyl-2-pyridyl)-2-cyclopropylacetonitrile (1.7a)

To a solution of **1.6a** (2.25 g, 8.65 mmol) and DMF (3.4 mL, 43.9 mmol) in anhydrous methylene chloride (19 mL), $POCl_3$ (3.17 mL, 34.0 mmol) was added slowly with an ambient temperature bath cooling since there was a delayed exothermic reaction. The solution was stirred overnight before being poured into crushed ice. (Caution: make sure $POCl_3$ is consumed before doing the extraction!). The mixture was extracted with methylene chloride (2 x 30 mL). The combined extracts were washed with water (1x 15 mL), saturated aqueous $NaHCO_3$ (1x 15 mL), water (2x 10 mL), dried over $MgSO_4$, and concentrated. The product was purified by flash chromatography (ethyl acetate:hexane 1:4) to yield **1.7a** as a pale yellow solid (1.83 g, 95%).

Mp. 43 – 44 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 3.80 (d, J = 8.0 Hz, 1H), 2.49 (s, 3H), 1.50 (m, 1H), 0.77 (m, 1H), 0.66 (m, 1H), 0.58 (m, 1H), 0.48 (m, 1H).

^{13}C NMR (400 MHz, $CDCl_3$) δ: 161.1, 158.2, 136.6, 133.1, 128.8, 117.7, 34.1, 29.3, 18.7, 13.7, 11.2.

HRMS: $C_{11}H_{10}ClFN_2$ (M^+); Calcd.: 224.0484; found: 224.0489.

2-(4-Chloro-5-fluoro-3-methyl-2-pyridyl)-butyronitrile (1.7b)

The procedure was same as **1.6b** to give **1.7b** (0.72 g, 89 %)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.39 (s, 1H). 4.06 (t, J = 7 Hz, 2H), 2.45 (s, 3H), 2.1 (m, 1H), 1.1 (t, J = 6.5 Hz, 3H).

^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 156.7, 154.1, 150.3, 135.9, 131.8, 119.8, 39.1, 26.7, 15.6, 12.8.

HRMS: $C_{10}H_{10}ClFN_2$ (M^+); Calcd.: 213.0542; found: 213.0529.

Ethyl 2-(4-chloro-5-fluoro-3-methyl-2-pyridyl)-2-cyclopropylacetate (1.8a)

A solution of **1.7a** (1.36 g, 6.0 mmol) in ethanol (0.9 mL) was added to a solution of ethanol (10 mL) saturated with HCl gas (~4 g) at 0 °C, which was prepared by the dropwise addition of concentrate H_2SO_4 onto $CaCl_2$. The reaction was stirred for 3 h at 0 °C. To this solution was added H_2O (0.9 mL). The reaction was heated at 80 °C for 2 h. The mixture was poured over ice to give a total volume of 40 mL. This solution was neutralized with 50% NaOH to pH 8 while maintaining a temperature less than 0 °C. The solid was filtered, dissolved in CH_2Cl_2, and the residual water layer removed. The organic layer was dried over $MgSO_4$ and evaporated and purified by flash chromatography (ethyl acetate:hexane 2:8) to provide **1.8a** as a pure tan solid (1.34 g, 82%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.36 (s, 1H), 3.23 (d, J = 9 Hz, 1H), 0.12 2.40 (s, 3H), 1.67 (m, 1H), 1.20 (t, J = 7 Hz, 3H), 0. 0.76 (m, 1H), 0.53 (m, 1H), 0.38 (m, 1H), 0.12 (m, 1H).

^{13}CNMR (400 MHz, $CDCl_3$) δ: 172.0, 156.1, 154.1, 153.5, 136.2, 132.7, 66.4, 45.9, 24.3, 18.9 15.8, 12.9.

HRMS: $C_{13}H_{15}ClFNO_2$ (M^+); Calcd.: 271.0871; found: 271.0904.

2-(4-Chloro-5-fluoro-3-methyl-2-pyridyl)-butyric acid ethyl ester (1.8b)

The procedure was same as **1.8a** to give **1.8b** (0.72 g, 89%)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.35 (s, 1H). 4.10 (q, J = 6.3 Hz, 2H), 3.82 (d, J = 8.7 Hz, 1H), 2.44 (s, 3H), 2.09 (m, 1H), 1.95 (m, 1H), 1.21 (t, J = 6.5 Hz, 3H), 1.85 (t, J = 6.1 Hz)

^{13}C NMR (400 MHz, $CDCl_3$) δ: 172.62, 155.9, 154.4, 153.4, 135.4, 132.6. 61.3, 52.1, 24.8, 15.8, 14.5, 12.5.

HRMS: $C_{12}H_{15}ClFNO_2$ (M^+); Calcd.: 259.0834; found: 259.0849.

2-(4-Chloro-5-fluoro-3-methyl-2-pyridyl)-2-cyclopropylacetic acid (1.9a)

A solution of **1.8a** (1.34 g, 5.8 mmol) in 10% NaOH (10 mL) was heated to 90 °C for 2 h. After cooling the residue was removed by filtration. The solution was adjusted to pH 5 with 18% HCl at 0 °C and a white solid precipitated. The solid was collected by filtration and dried under vacuum to give (0.98 g, 76%) of pure compound **1.9a**.

^{1}H NMR (400 MHz, D_2O) δ: 8.12 (s, 1H), 2.94 (d, J = 9.9 Hz, 1H), 2.21 (s, 3H), 1.32(m, 1H), 0.59 (m, 1H), 0.36 (m, 1H), 0.34 (m, 1H), 0.06 (m, 1H).

^{13}C-NMR (400 MHz, D_2O) δ: 172.5, 156.1, 154.3, 153.5, 135.4, 133.6, 23.7, 18.3, 15.0, 11.7.

HRMS: $C_{11}H_{11}ClFNO_2$ (M^+); Calcd.: 243.0594; found: 243.0613.

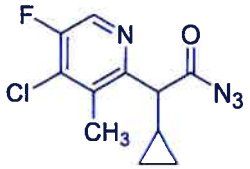
2-(4-Chloro-5-fluoro-3-methyl-2-pyridyl)-butyric acid (1.9b)

The procedure was same as **1.9a** to give **1.9b** (0.68 g, 78%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 4.06 (q, J = 7 Hz, 2H), 2.57 (s, 3H), 2.1 (m, 1H), 1.95 (m, 1H), 0.90 (t, J = 6.2 Hz, 3H).

^{13}C-NMR (400 MHz, $CDCl_3$) δ 175.9, 156.2, 153.9, 153.7, 134.5, 134.3, 50.9, 26.6, 15.7, 12.2.

HRMS: $C_{10}H_{11}ClFNO_2$ (M^+); Calcd.: 231.0462; found: 231.0465.



(4-Chloro-5-fluoro-3-methyl-2-pyridyl)-cyclopropyl-acetyl azide (1.10a)

To a solution of acid **1.9a** (243 mg, 1.0 mmol) in toluene (10 mL) at 0 °C was added triethylamine (396 µL, 3.0 mmol) follow by diphenylphosphoryl azide (430 µL, 2.0 mmol). The ice bath was removed and after 1.5 h of stirring at the room temperature, the reaction was diluted with ether and H_2O solution 45 mL (5:1). The layers were separated, and the aqueous layer was extracted with ether (2 x 15 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (10 mL) and saturated aqueous NaCl (10 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give crude product, which was purified by a very short column (ethyl acetate:hexane 5:95) to give **1.10a**. (174 mg, 65%)

IR: υ = 2139.1, 1725.9 cm^{-1}

^{1}H NMR (400 MHz, D_2O) δ: 8.4 (s, 1H), 3.2 (d, 2H), 2.4 (s, 3H), 1.7 (m, 2H), 1.24 (2 H), 0.8 (m, 1H), 0.27 (m, 1H).

^{13}C-NMR (400 MHz, D_2O) δ: 172.5, 156.1, 154.3, 153.5, 135.4, 133.6, 15.0, 12.9, 5.2, 3.8.

4-*tert*-Butoxy-2-[1,3] dithian-2-yl-5-fluoro-3-methyl-pyridine (1.12)

A solution of 1,3-dithiane (0.78 g, 6.5 mmol) in THF (degassed with argon, 6.5 mL) was cooled to –45°C and n-butyl lithium (hexane, 2.62 mL, 6.5 mmol) was added dropwise over 15 min. the reaction mixture was stirred for 2 h at –40 °C and then for 2 h at 0 °C. The solution was cooled to –40 °C and the pyridine **1.5** (0.41 g, 2.2 mmol) was added dropwise, then stirred for 2 h at –40 °C. The reaction mixture was quenched by the addition of aqueous NH_4Cl and extracted with CH_2Cl_2 (50 mL x 3). The combined organic layers were dried over Na_2SO_4, concentrated under reduced pressure, and then purified by flash column chromatography (ethyl acetate:hexane 15:85) to give 2-dithianyl pyridine **1.12** as a white solid (0.43 g, 61%).

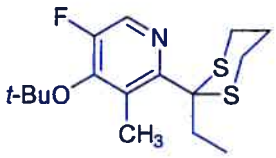
Mp. 125 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ: δ 8.27 (s, 1H), 5.32 (s, 1H), 3.02 (m, 4H), 2.36 (s, 3H), 2.15 (m, 1H), 2.01 (s, 1H), 1.39 (d, J = 1.1 Hz, 9H).

^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): δ 155.2, 153.35 149.5, 136.5, 129.4, 84.5, 51.7, 32.1, 29.47, 25.9, 12.8 .

MS (M^+): 301.1, 245.0, 212.1, 160.0, 147.1, 106.0.

HRMS: $C_{14}H_{20}FNOS_2$ (M^+); Calcd.: 301.097036; found : 301.097521.



4-*tert*-Butoxy-2-(2-ethyl-[1,3]dithian-2-yl)-5-fluoro-3-methyl-pyridine (1.13)

A solution of 2-dithianyl pyridine **1.12** (301 mg, 1 mmol) in THF (degassed with argon, 2.5 mL) was cooled to -78 °C, and n-butyl lithium (hexane, 550 µL, 1.1 mmol) was added

dropwise over 15 min. The resultant solution was stirred at -78 °C for 30 min and then TfOEt (155 μL, 1.2 mmol) was added. The mixture was stirred in –40 °C for 1h, the cold bath was removed, the temperature was raised to 0 °C for 1h, when a dark red color was appeared. Quenched the reaction by the addition of saturated NH_4Cl solution, extracted with CH_2Cl_2 (3 x 15 mL), washed with $NaHCO_3$ and H_2O, dried over Na_2SO_4. Concentration in vacuo and purification by flash chromatography (ethyl acetate:hexane 10:90) afforded **1.13** (148 mg, 45%) as a yellowish white solid.

Mp. 114 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.39 (s, 1H). 4.06 (q, J = 7 Hz, 2H), 3.23 (d, J = 9 Hz, 1H), 2.43 (s, 3H), 2.1 (m, 1H), 1.05 (t, J = 7 Hz, 3H)

^{13}C NMR (400 MHz, $CDCl_3$) δ: 156.5, 153.9, 149.4, 137.2, 129.9, 85.2, 51.3, 41.2, 33.4, 29.7, 26.1, 13.0, 9.8.

MS (M^+): 329.1, 296.1, 273.1, 240.1, 216.1, 160.0, 1487.1, 106.0.

HRMS: $C_{16}H_{24}FNOS_2$ (M^+); Calcd.: 329.1283; found: 329.1299.

1-(4-*tert*-Butoxy-5-fluoro-3-methyl-2-pyridyl)-propan-1-one (1.14)

Red mercuric oxide (432 mg, 2.0 mmol), boron trifluoride diethyl etherate (252 μL, 2.0 mmol) and 15% aqueous tetrahydrofuran (10 mL/g of dithiane) were stirred vigorously in a three-neck flask equipped with a dropping funnel and a nitrogen inlet tube. Compound **1.13** (330 mg, 1.0 mmol) was dissolved in the minimum of THF and was added *via* the dropping funnel in the course of 10-15 min under nitrogen. Stirring was maintained for 10-20 min after addition was complete. The red mercuric oxide gradually dissolved and a white precipitate appeared. Ethyl ether (5 mL) was then added, the precipitated salts were filtered, and the ether was washed to pH 10 with saturated Na_2CO_3, and to neutrality with satd. NaCl, after drying over Na_2SO_4, the ether was evaporated under vacuum and purified by flash chromatography (ethyl acetate: hexane 7:93) to yield compound **1.14** (98 mg, 41%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.28 (s, 1H), 3.12 (dd, J = 6.4 Hz, 2H), 2.4, 3H), 1.40 (d, J = 1.4 Hz, 9H), 1.14 (t, J = 6.3 Hz, 3H).

^{13}C-NMR (400 MHz, $CDCl_3$) δ: 204.5, 155.8, 154.2, 150.9, 135.6, 133.6, 84.9, 33.77, 29.5, 13.8, 8.5.

HRMS: $C_{13}H_{18}FNO_2$ (M^+); Calcd.: 239.1349; found: 239.1357.

1-(4-*tert*-Butoxy-5-fluoro-3-methyl-2-pyridyl)-propan-1-ol (1.15)

Sodium borohydride (113.5 mg, 3.0 mmol) was added portionwise over 30 min to a cooled (ice bath) stirring suspension of ketone 1.14 (120 mg, 0.5 mmol) in anhydrous MeOH (3 mL). Complete dissolution was obtained at the end of the addition. The ice bath was removed, and stirring was continued for 8 h. Monitoring by TLC (20% acetone-hexane on silica gel) confirmed that the reaction had gone to completion. The reaction mixture was concentrated to a residue that was diluted with H_2O and extracted with CH_2Cl_2 (3 x 15 mL). The organic extract were combined, dried over anhydrous Na_2SO_4, and concentrated to colourless oil. The crude product was purified by flash chromatography (ethyl acetate: hexane 1:4) to afford the alcohol **1.15** (109 mg, 91%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.22 (s, 1H), 4.71 (s, 1H), 4.45 (m, 1H), 2.18 (s, 3H), 1.72 (m, 1H), 1.53 (m, 1H), 1.41 (d, J = 1.3 Hz, 9H). 0.95 (t, J = 6.2 Hz, 3H)

^{13}C-NMR (400 MHz, $CDCl_3$) δ: 157.7, 154.9, 149.7, 134.8 127.5, 84.4, 71.4, 30.9, 29.5, 12.1, 10.1.

MS (M^+): 241.1, 212.1, 185.1, 156.0, 147.1

HRMS: $C_{13}H_{20}FNO_2$ (M^+); Calcd.: 241.1478; found: 241.1472

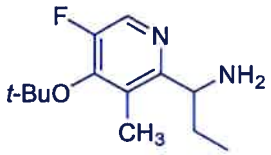
2-(1-Azido-propyl)-4-*tert*-butoxy-5-fluoro-3-methyl-pyridine (1.16)

To a stirred solution of **1.15** (24 mg, 0.1 mmol), triphenylphosphine (52.4 mg, 0.2 mmol) and diisopropyl azodicarboxylate (42 µL, 0.2 mmol) in dry THF, a solution of diphenylphosphoryl azide (44 µL, 0.2 mmol) was added over a period of 15 minutes and stirring continued for about 24 h. after which when the solvent was removed from the reaction mixture on a rotary evaporator under reduced pressure. The thick oily liquid was purified by flash chromatography (ethyl acetate:hexane 1:9) to afford **1.16** as a colourless liquid (37.5 mg, 71%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.32 (s, 1H), 4.38 (t, J = 7.1 Hz, 1H), 2.28 (s, 3H), 2.04 (m, 2H), 1.43 (s, 9H). 0.97 (t, J = 6.9 Hz, 3H)

^{13}C-NMR (400 MHz, $CDCl_3$) δ: 157.2, 152.3, 149.7, 131.9, 128.4, 83.1, 71.3, 30.2, 29.1, 12.4, 11.4

HRMS: $C_{13}H_{19}FN_4O$ (M^+); Calcd.: 266.1548; found: 266.1521



1-(4-tert-Butoxy-5-fluoro-3-methyl-2-pyridyl)-propylamine (1.17)

A mixture of azide **1.16** (113.15 mg, 0.5 mmol), triphenylphosphine (262 mg, 1.0 mmol), and water (18 µL, 1.0 mmol) was stirred in THF (15 mL) for 24h. The mixture was concentrated and the residual oil was purified by flash chromatography ($CHCl_2$:MeOH:NH_4OH 85:14:1) to give pyridine amine **1.17**.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.25 (s, 1H), 4.12 (m, 1H), 2.25 (s, 3H), 1.73 (m, 2H), 1.42 (d, J = 1.0 Hz, 9H). 0.89.

^{13}C-NMR (400 MHz, $CDCl_3$) δ: 156.1, 154.7, 148.4, 131.3, 127.7, 83.2, 70.1, 30.9, 29.4, 11.7, 10.0.

HRMS: $C_{13}H_{21}FN_2O$ (M^+); Calcd.: 240.1627; found: 240.1639.

Attempted cyclization

A. From ketone **1.14**

Commercial diethyl aminomalonate hydrochloride was converted to its free amine by stirring in ethanol with excess potassium carbonate for about 1 h. The solids were then filtered and the ethanol removed in vacuo. Diethyl aminomalonate was subsequently distilled at reduced pressure (10 Torr) using a Kugelrohr apparatus. This material was stored in a refrigerator, and it maintained its integrity for several days as determined by 1H NMR.

The aminomalonate (263 mg, 1.5 mmol) was dissolved in toluene (7.5 mL), the ketone **1.14** (583 mg, 1.5 mmol) was added to the mixture, the reaction flask was fitted with a Dean-Stark apparatus and heated to reflux. After 13 h, the mixture was cooled, and the toluene was removed in vacuo. After column chromatography, starting material was recovered.

B. From amine **1.17**

To a solution of compound **1.17** (264 mg, 1.1 mmol) in benzene (30mL) were added diethyl 2-oxomalonate (161 μL, 1.0 mmol) and *p*-toluenesulfonic acid (9.5 mg, 0.05 mmol) under an argon atmosphere. The reaction mixture was heated at reflux for 20 h with azeotropic removal. The solvent was evaporated, and the residue was purified with Kugelrohr distillation to give 4-hydroxyl starting material derivative of **1.17** (loss of *t*-Bu).

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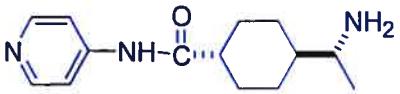
CHAPTER 2

Synthesis of Rho kinase inhibitors

2.1 The mechanism of action of Y-27632 — an inhibitor of Ca^{2+}-sensitizing enzyme

Abnormal smooth muscle contractility may be a major cause of disease states such as hypertension, and a smooth-muscle relaxant that modulates this process would be useful therapeutically. Smooth-muscle contraction is regulated by the cytosolic Ca^{2+} concentration and by the Ca^{2+} sensitivity of myofilaments. The former activates myosin light-chain kinase and the latter is achieved partly by inhibition of myosin phosphatase. Calcium sensitization of smooth muscle is mediated by a Rho-associated protein kinase in hypertension.[1]



Y-27632

Figure 2.1 Structure of Y-27632

Narumiya and colleagues[1] have identified a drug (Y-27632) that inhibits the activity of a Ca^{2+}-sensitizing enzyme (Rho-associated kinase) leading to a reduction of high blood pressure in experimental animals (Figure 2.1) Activation of receptors coupled to certain guanine-nucleotide-binding proteins G releases intracellular Ca^{2+} that binds to calmodulin (Cam), and this complex activates myosin light-chain kinase (MLCK). By phosphorylating the regulatory light chain of myosin (MLC_{50}) in smooth muscle, MLCK causes vascular smooth muscle to contract and the lumen of blood vessels to narrow. Many of the same receptors also activate RhoA and, with the help of guanine-nucleotide exchange factors (GEFs), dissociate cytosolic RhoA-GDP from guanine-nucleotide dissociation inhibitor (GDI), which allows the exchange of GTP for GDP on RhoA. The active RhoA-GTP activates Rho-associated kinase, which phosphorylates and so inhibits-myosin phosphatase. Myosin phosphatase dephosphorylates smooth-muscle myosin, causing the smooth muscle to relax and blood vessels to dilate. Y-27632 inhibits Rho-associated kinase, thereby blocking the inhibition of smooth muscle myosin phosphatase[2] and Ca^{2+} sensitization.[3] Although Ca^{2+} is the main activator of smooth-muscle contraction (through MLCK), the level of force can

be modulated independently of it.[4] Figure 2.2 shows the mechanism of inhibition.[5] We wished to test the activity of a small library of substituted piperidines as Rho-kinase inhibitors (Figure 2.3).

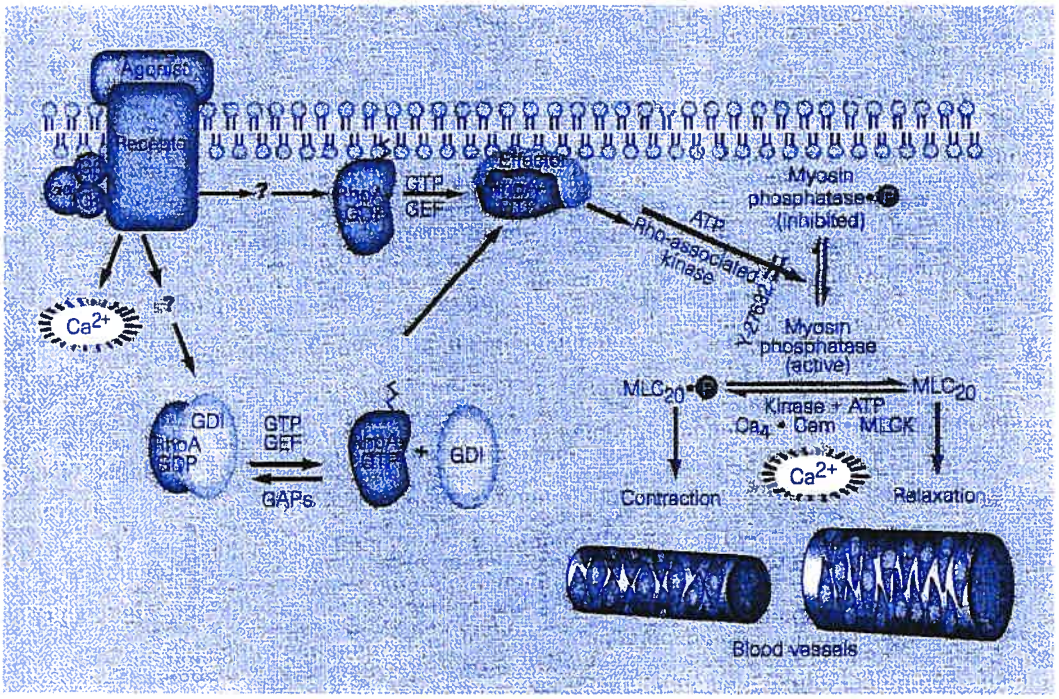
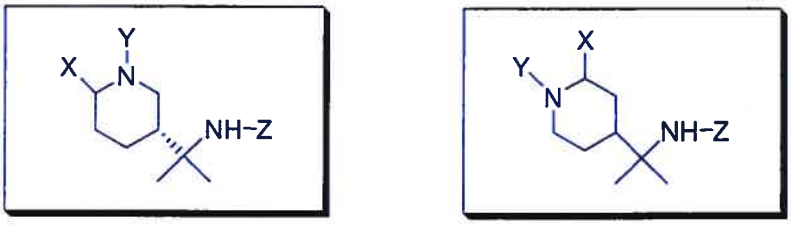


Figure 2.2 The mechanism of action of Y-27632 27632 – an inhibition of the activity of Ca^{2+}-sensitizing enzyme (Reproduced from Somlyo, A. P. *Nature*, **1997**, *389*, 908).

2.2 Piperidine derivatives as potential inhibitors of Rho kinase

Based on the structure of Y-27632, we postulated that 1,3- and 1,4-substituted piperidine derivatives might have inhibitory activity against Rho Kinase. The intended derivatives and their provenance are shown in Figure 2.3.



X = H, Ph

Y = SO_2R_1; H

Z = H; SO_2R_1; COR_2

X = H

Y = H

Z = H; SO_2R_1; COR_2

Figure 2.3 The target compounds

The common starting material was cyclopentenone, which would undergo a proline catalyzed Michael addition (see 2.4) to give the corresponding adduct. Beckmann rearrangement would then lead to the corresponding lactams. Using this synthetic strategy we prepared five different piperidine cores as shown in Figure 2.4. The sites of diversification are shown in Figure 2.5.

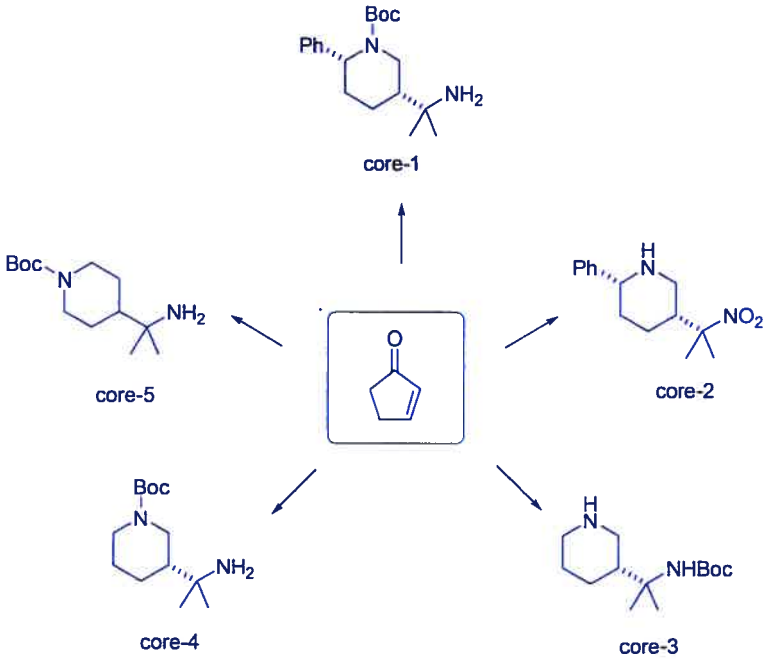


Figure 2.4 Synthesis of five piperidine cores

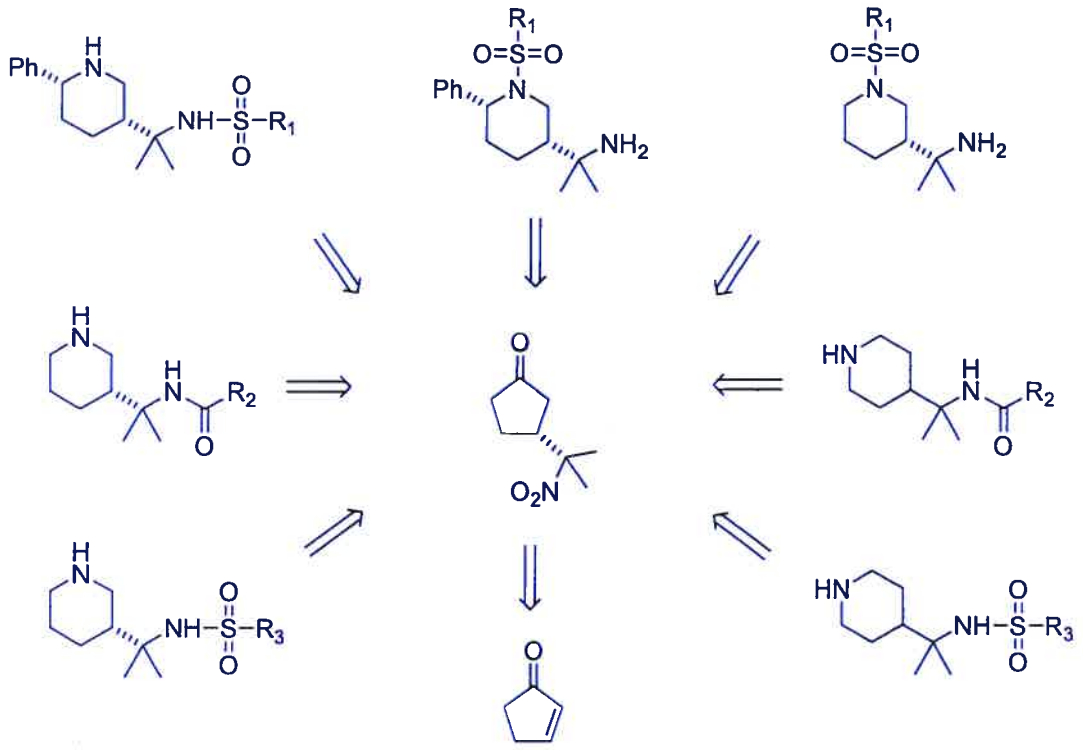


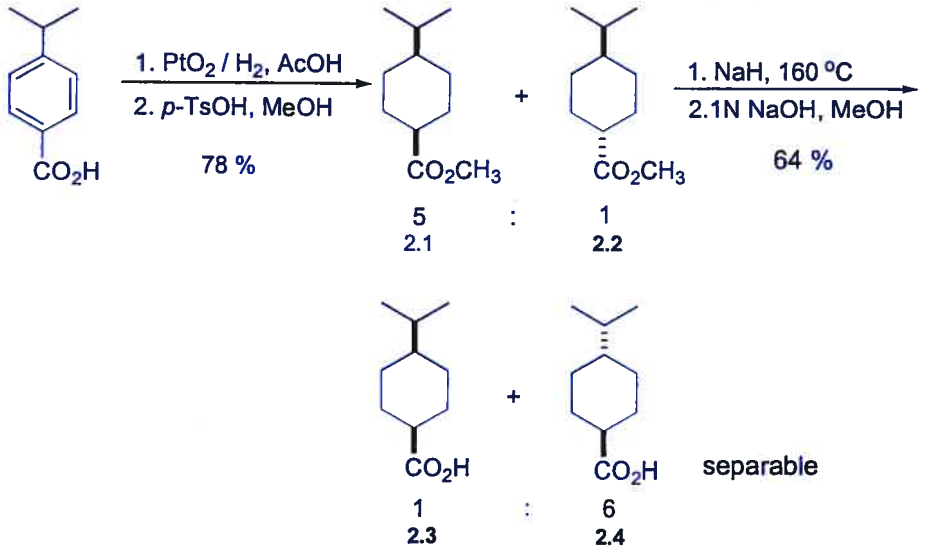
Figure 2.5 Retrosynthesis of substituted piperidine derivatives

2.3 Synthesis of intermediates

2.3.1 Synthesis of *trans* 4-isopropyl-cyclohexanecarboxylic acid 2.4

Isopropyl benzoic acid was hydrogenated with platinum oxide to give the *cis*-4-isopropyl-cyclohexanecarboxylic acid as the major product. Esterification, isomerization and hydrolysis gave the desired *trans* carboxylic acid **2.4**[6] as the major diasteremer

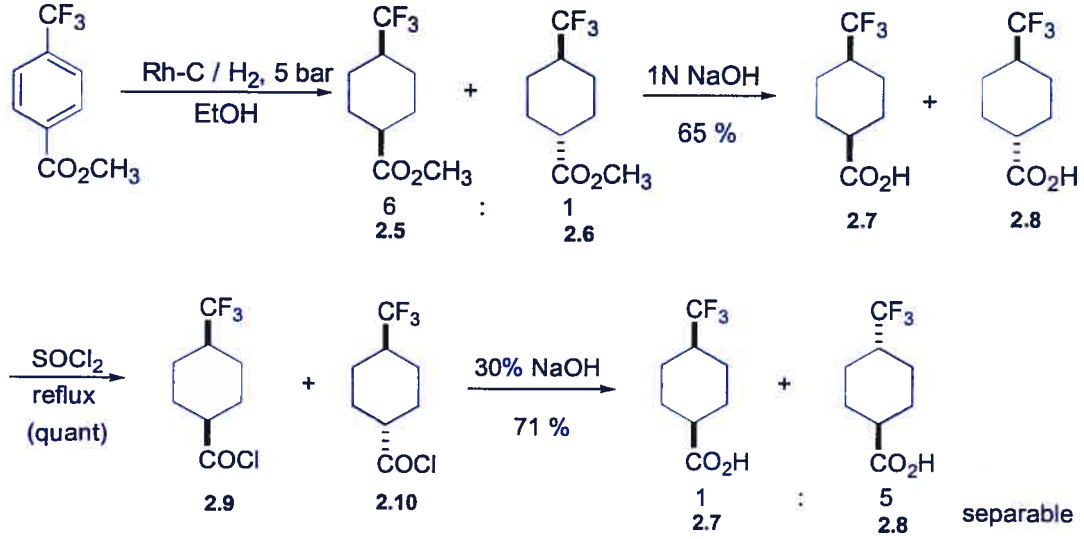
Scheme 2.1 Synthesis of *trans* 4-isopropyl-cyclohexanecarboxylic acid **2.4**



2.3.2 Synthesis of trans-4-(trifluoromethyl)cyclohexanecarboxylic acid 2.8

Methyl 4-trifluoromethylbenzoate was reduced and hydrolyzed to afford the *cis*-4-(trifluoromethyl)cyclohexanecarboxylic acid **2.7** as a major product following a proceduce in the patant literature.[7] Reaction with thionyl chloride and treatment with sodium hydroxide resulted in the *trans* acid **2.8** as a major product.

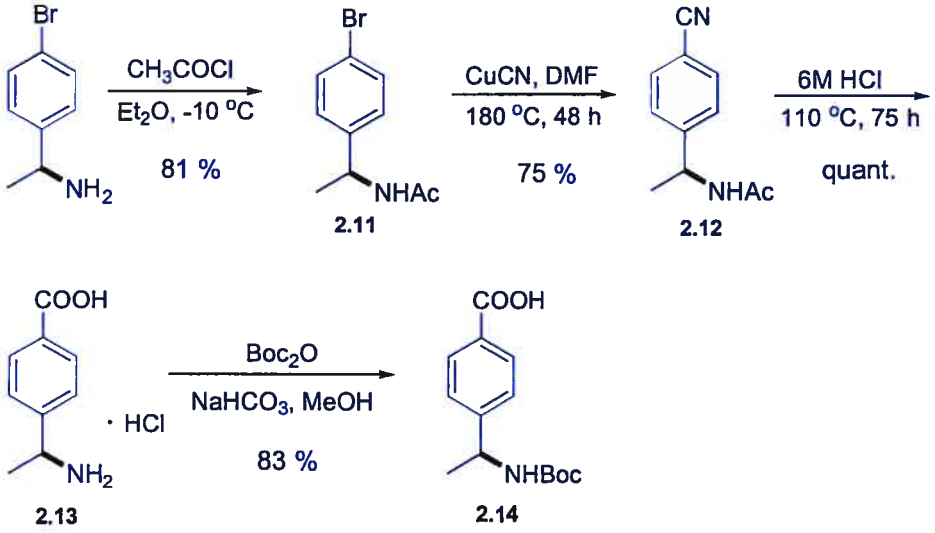
Scheme 2.2 Synthesis of *trans*-4-(trifluoromethyl)cyclohexanecarboxylic acid **2.8**



2.3.3 Synthesis of 4-(1-tert-butoxycarbonylaminoethyl)benzoic acid 2.14

Using optically pure 1*S*-(4-bromophenyl) ethylamine, three steps[8] were necessary to obtain optically pure compound **2.13**, which was protected[9] with Boc_2O to give compound **2.14.**

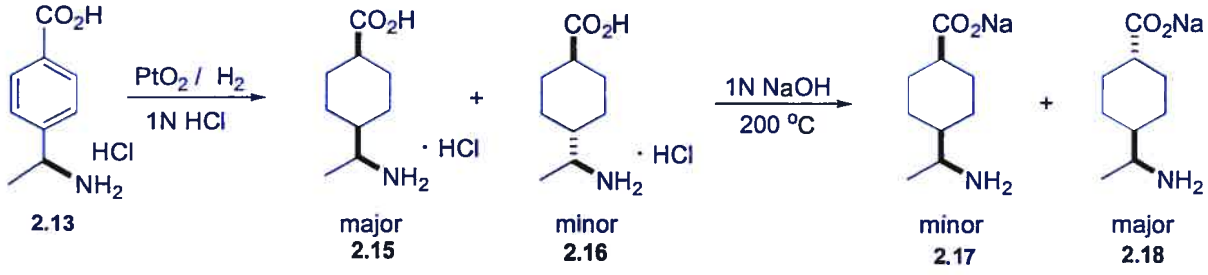
Scheme 2.3 Synthesis of 4-(1-*tert*-butoxycarbonylamino-ethyl)-benzoic acid **2.14**

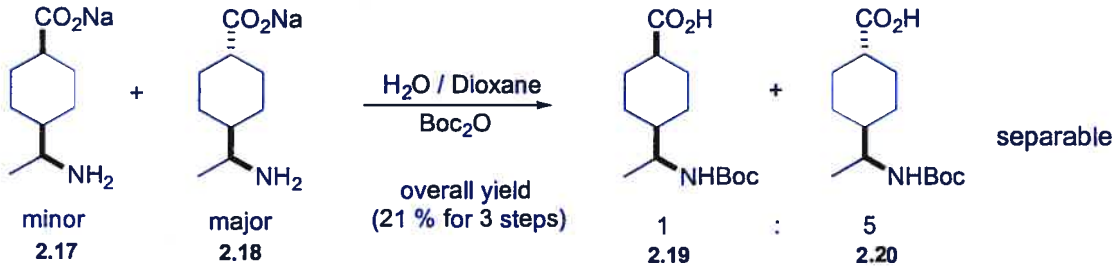


2.3.4 Synthesis of *trans* (*S*)-(1-*tert*-butoxycarbonylaminoethyl) cyclohexanecarboxylic acid 2.20

Compound **2.13** was reduced,[10] isomerized and protected to give optically pure **2.20**. (Scheme 2.4)

Scheme 2.4 Synthesis of *trans*-(*S*)-4-(1-*tert*-butoxycarbonylaminoethyl) cyclohexanecarboxylic acid **2.20**



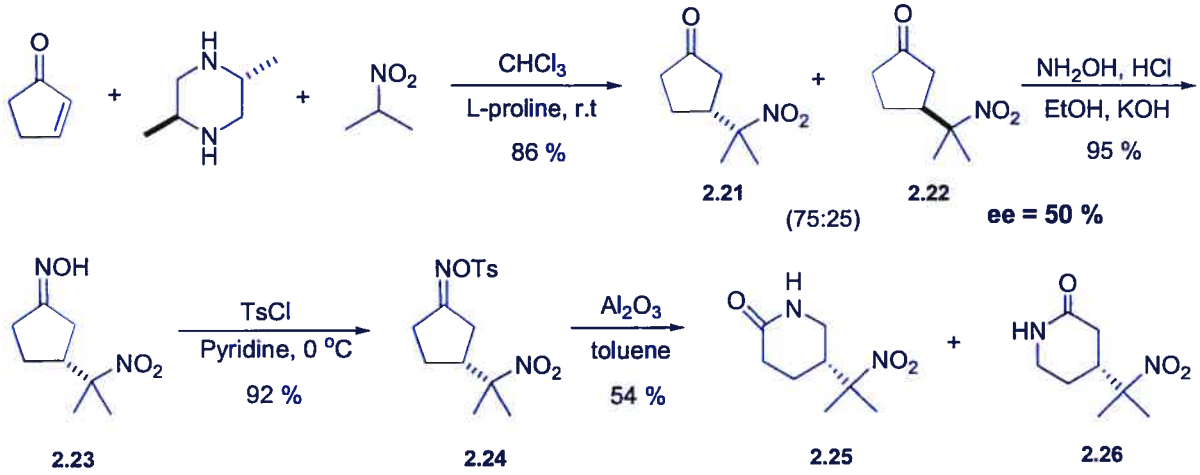


2.4 Synthesis of piperidine derivatives

2.4.1 Michael addition and Beckmann rearrangement.

2-Nitropropane was introduced by Michael addition to 2-cyclopentenone catalyzed by L-proline to give enantioenriched **2.21**,[11] which reacted with hydroxylamine to afford the oxime **2.23**.[12] Unfortunately the enantioselectivity of this reaction is mediocre compared to that using cyclohexenone. Nevertheless, we proceeded with the modestly enriched mixture towards the intended mini-library. Compound **2.23** was protected by *p*-TsCl,[12] and then subjected to a Beckmann rearrangement[13] in the presence of Al_2O_3 to give a regioisomeric mixture **2.25** and **2.26** in a proportion of 3:2 (Scheme 2.1). The stereochemistry indicated relates to the enriched isomer (75: 25 *R/S*).

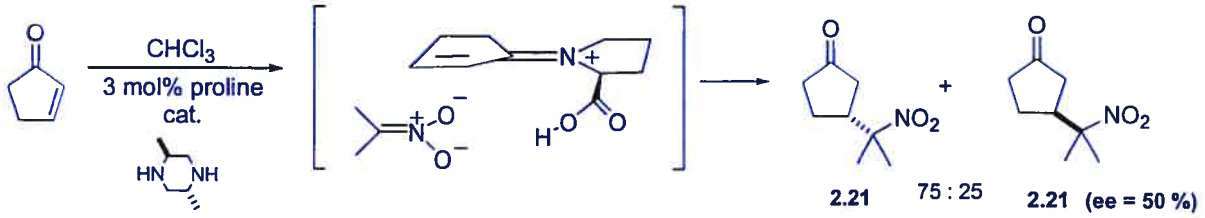
Scheme 2.5 Synthesis of 3- and 4- substituted δ-lactams



It is not possible to derive clear a mechanistic pathway,[11] but it is known that the proline-catalyzed addition in presence of 2,5-dimethylpiperazine shows a complex non-linear effect.

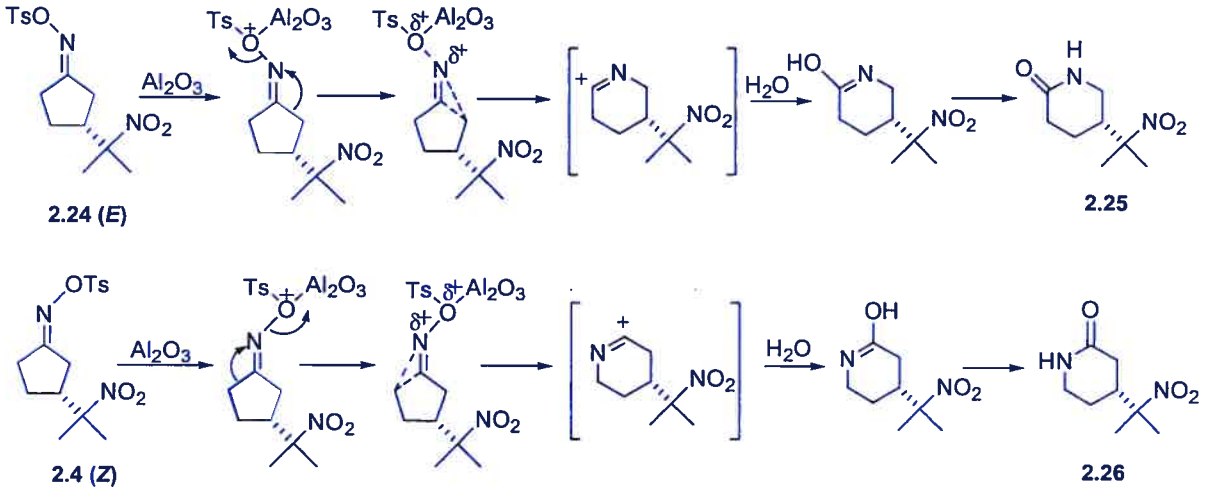
L-Proline first reacts with 2-cyclopentenone to give an iminium ion, which is attacked by nitropropane anion to give the *R*-product **2.21** as the major isomer. Although several secondary and tertiary bases were used as additives, only *trans*-2,5-dimethylpiperiazine gave a good ratio.

Scheme 2.6 Possible transition state model for the Michael addition



Beckmann rearrangement in the presence of dry alumina[13] afforded two constitutional isomeric 3-substituted and 4-substituted lactams because of the existing of *Z* and *E* two conformations in the oxime. A plausible mechanism is shown in Scheme 2.7. The mechanism involves conversion of the oxime hydroxyl group to a leaving group. Ionization and migration then occur as a concerted process, with the group, which is anti to the leaving group migrating. This results in formation of an iminium ion, which captures water. Eventually, hydrolysis leads to the lactams **2.25** and **2.26**.

Scheme 2.7 Mechanism of Beckmann rearrangement



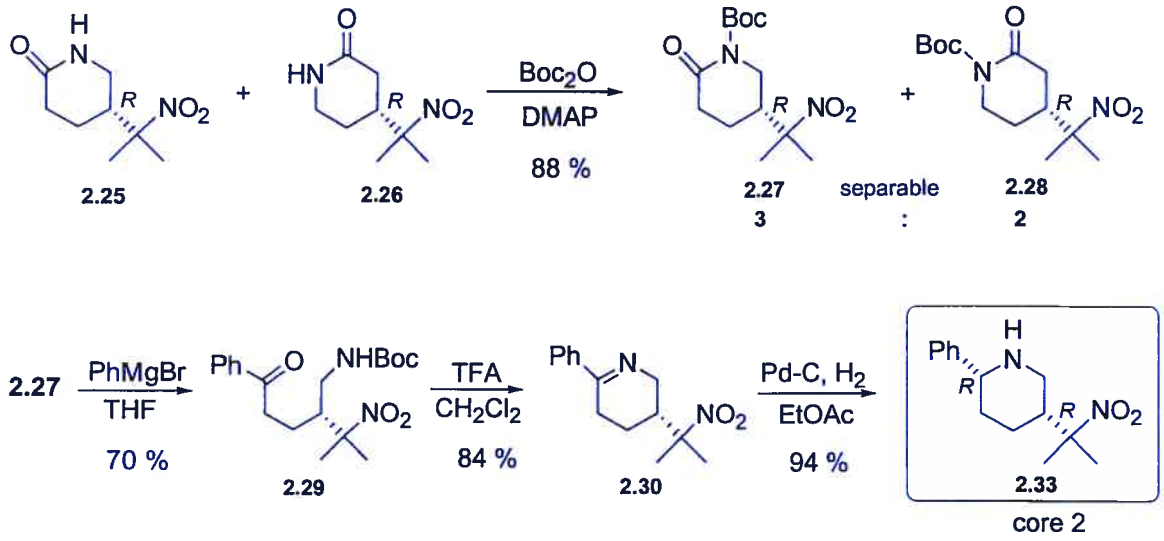
The structural arrangement was made from analysis of their ^{1}H NMR spectura. Thus the major isomer 2.25 shows a doublet of doublets for the C-6 methylene hydrogens next to the lactam NH. The minor isomer 2.26 showed a multiplet for the C-6 methylene hydrogens

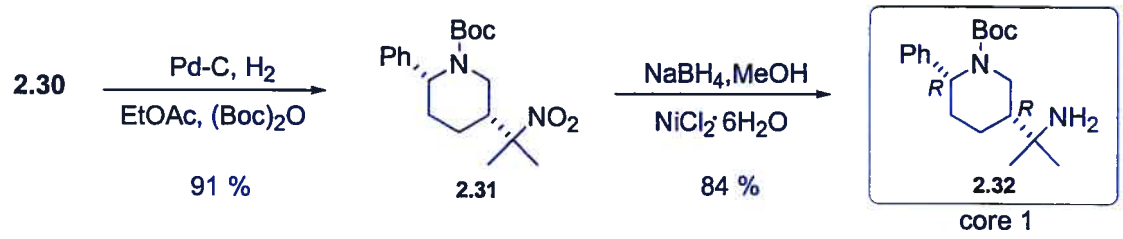
2.4.2. Synthesis of piperidine cores.

2.4.2.1 Synthesis of piperidine cores 2.32 and 2.33

The mixture of **2.25** and **2.26** was derivatized with Boc_2O to give separable *N*-protected lactams[14] **2.27** and **2.28**. The desired compound **2.27** was treated with a Grignard reagent[15] to open the ring to give compound **2.29**. Treatment with TFA[16] gave the imine **2.30**, followed by a 2-step reaction sequence protocol[17, 18] to give the amino *N*-Boc piperidine core compound **2.32**. Reduction[17] in the presence of Pd-C afforded the nitropiperidine core compound **2.33**. The relative stereochemistry of the 2-phenyl substituent was not determined, but it can be assumed to be *cis* by analyzing the coupling constants of benzylic proton by ^{1}H NMR,

Scheme 2.8 Synthesis of piperidine cores **2.32** and **2.33**

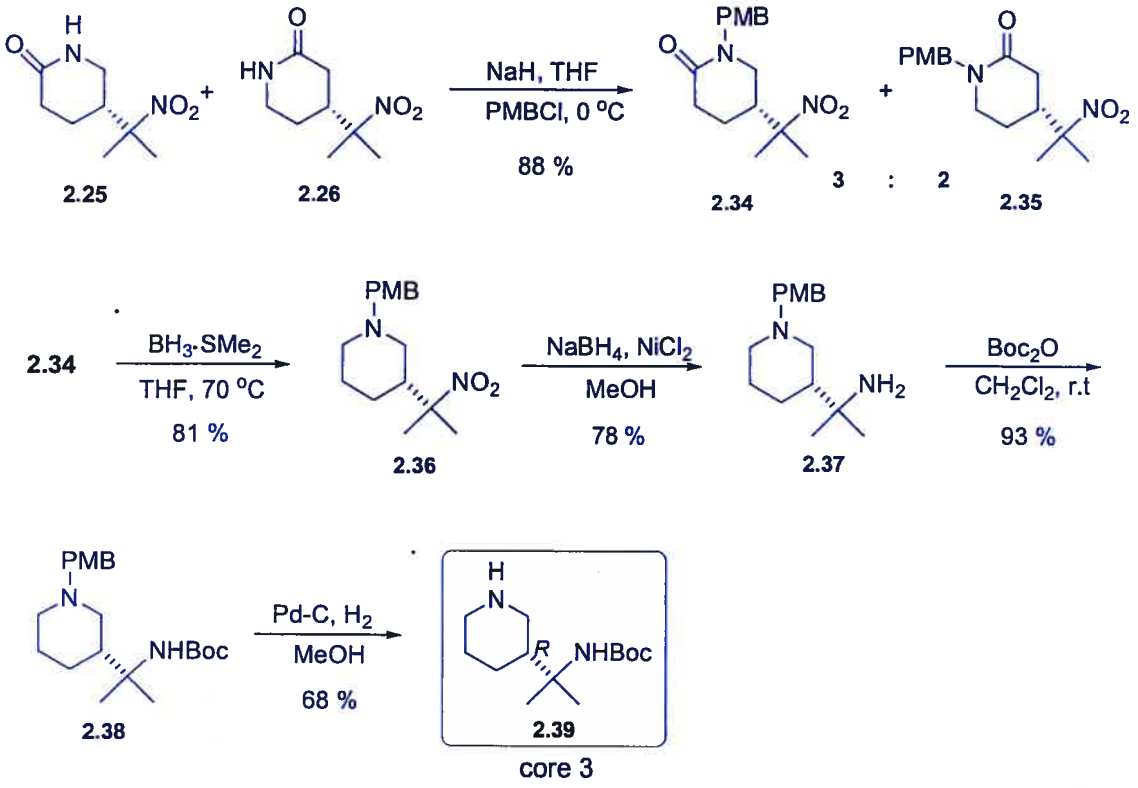




2.4.2.2 Synthesis of piperidine core 2.39

The mixture of lactams **2.25** and **2.26** was protected[19] with PMBCl to yield two separable compounds **2.34** and **2.35**. BH_3•SMe_2[20] followed by $NaBH_4$ and $NiCl_2$[21] reduced the major product to amine **2.37**. Protection of **2.37** with Boc_2O[22] and deprotection[23] of PMB lead to compound **2.39**. (Scheme 2.9)

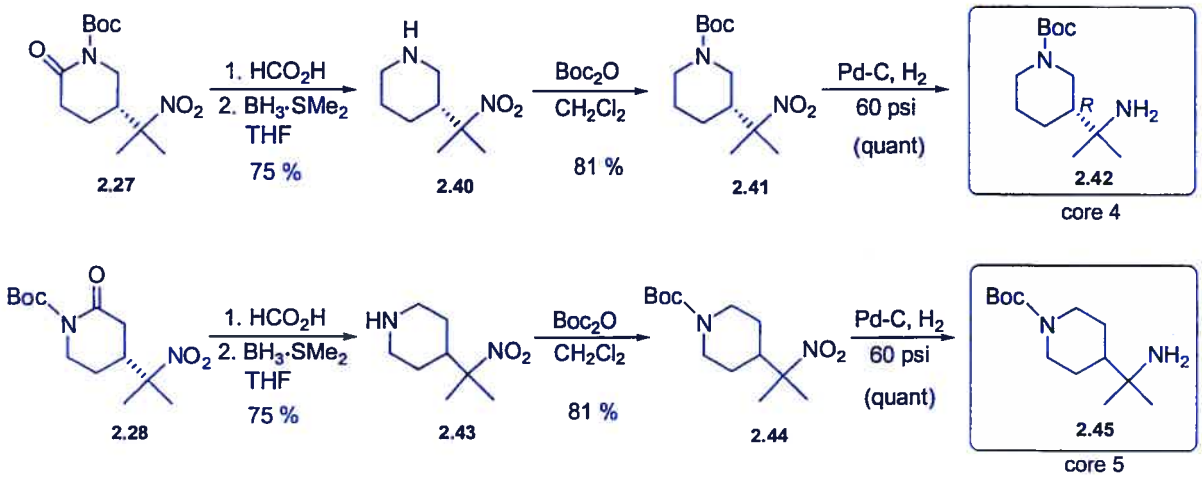
Scheme 2.9 Synthesis of piperidine core **2.39**



2.4.2.3 Synthesis of piperidine cores 2.42 and 2.45

Compounds **2.42** and **2.45** were prepared using a 4-step sequence from **2.27** and **2.28** as shown in Scheme 2.10. In this sequence the lactam was reduced with the borane dimethylsulfide complex,[20] the piperidine protected as the *N*-Boc derivative, and the nitro group reduced to the corresponding amine in the presence of Pd-C and hydrogen.[24]

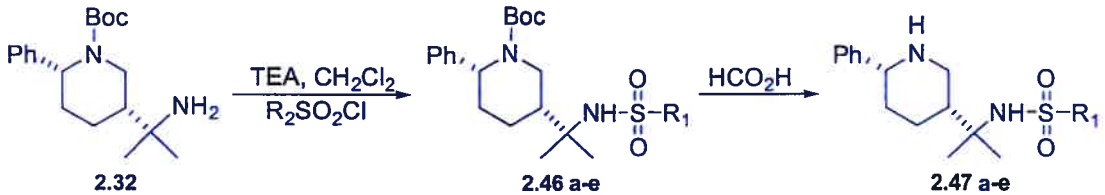
Scheme 2.10 Synthesis of piperidine cores **2.42** and **2.45**

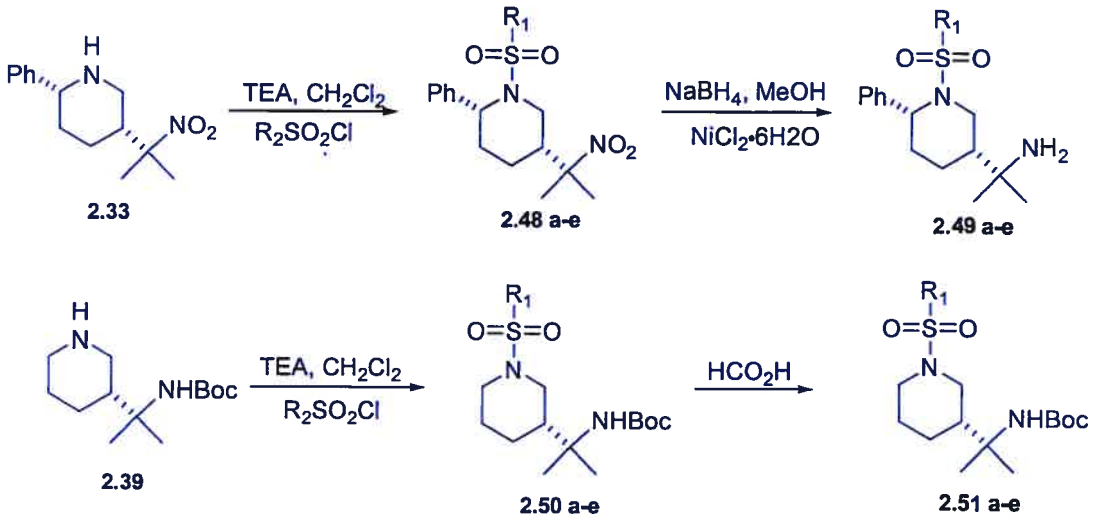


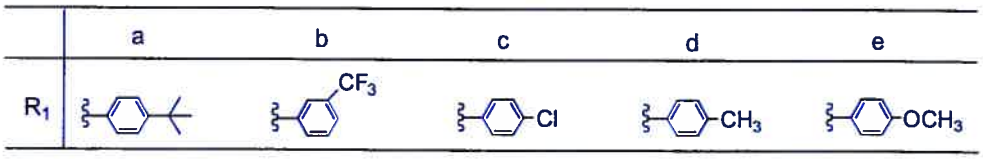
2.4.3 Synthesis of 3-substituted piperidine derivatives 2.47, 2.49, 2.51, 2.54, and 2.57

Piperidine cores **2.32**, **2.33**, and **2.39** reacted with substituted benzenesulfonyl chlorides to yield sulfonylamides[25] **2.46**, **2.48**, and **2.50**, which were deprotected[26] or reduced[21] to lead to the desired 3-substituted piperidine derivatives **2.47 a-e**, **2.49 a-e**, and **2.51 a-e** respectively (Scheme 2.11).

Scheme 2.11 Synthesis of 3-substituted piperidine derivatives **2.47, 2.49, and 2.51**

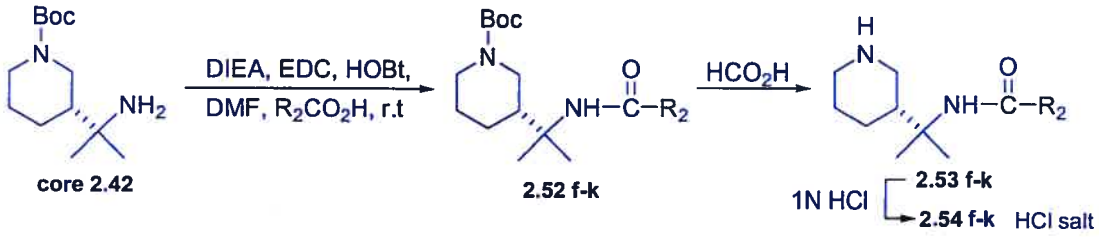


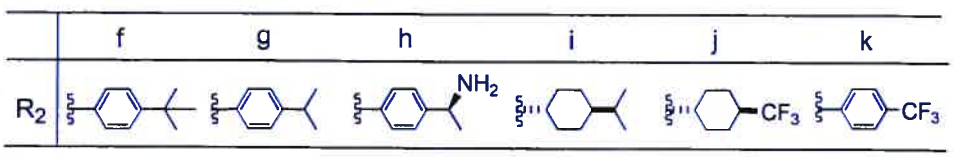




Core compound **2.42** was coupled[27] with substituted benzoic acids and saturated carboxylic acids to give compounds **2.52 f-k**, which were deprotected and treated with 1N HCl[28] to give hydrochloride salts **2.54 f-k**. (Scheme 2.12)

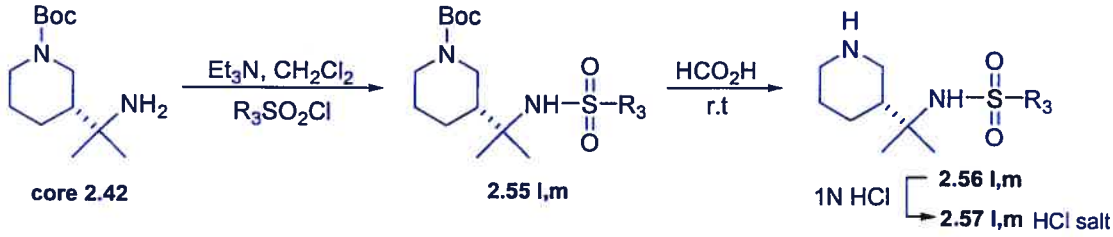
Scheme 2.12 Synthesis of 3-substituted piperidine derivatives **2.54**

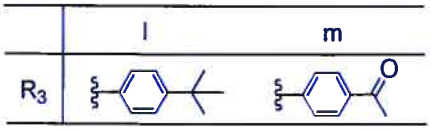




Core compounds **2.42** was reacted with sulfonyl chlorides to give compounds **2.55 l, m**, Deprotection and conversion to the hydrochloride salt gave **2.57 l, m** (Scheme 2.13).

Scheme 2.13 Synthesis of 3-substituted piperidine derivatives **2.57**

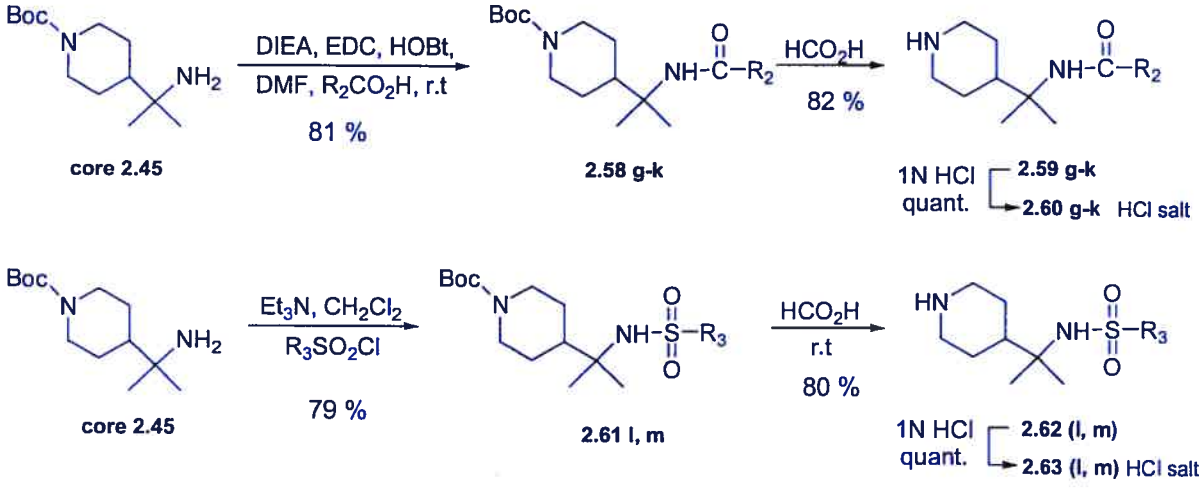




2.4.4 Synthesis of 4-substituted piperidine derivatives 2.60 and 2.63

Applying the same procedure that was used to prepare as **2.54** and **2.57** gave the 4-substituted compounds **2.60 g-k** and **2.63 l, m** as hydrochloride salts (Scheme 2.14).

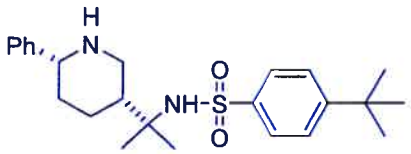
Scheme 2.14 Synthesis of 4-substituted piperidine derivatives **2.60** and **2.63**



R_3 is same as Scheme 2.13

2.5 Biological tests

The set of substituted piperidine derivatives was tested for inhibition of the Rho Kinase.[29, 30] Unfortunately only moderate inhibition was observed with 4-*tert*-butyl-*N*-[1-methyl-1-(6S-phenyl-piperidin-3R-yl)-ethyl]-benzenesulfonamide, **2.47a**.



30 % inhibition at 10 μM

Figure 2.6 Moderately active compound

2.6 Conclusion

We have prepared a small library of substituted piperidines as *N*-acyl and *N*-acylsulfonyl derivatives, starting with cyclopentenone using a recently developed Michael addition with nitroalkances. These compounds were obtained as enantiomerically partly enriched isomers (~50% ee corresponding to a 75:25 ratio of enantiomers in the 3-substituted piperidine series). Biological testing reveraled that only one analogue (**2.47a**) was moderately active as a Rho Kinase inhibitor.

2.7 Experimental notes (See Chapter1)

For some compounds the carbon resonances do not match the formulae due to signal overlap.

cis **2.1** *trans* **2.2**

4-Isopropylcyclohexanecarboxylic acid methyl ester (2.1) and (2.2)

Cuminic acid (1 g, 6.1 mmol) was hydrogenated in acetic acid (5 mL) in the presence of platinum oxide (50 mg) under 60 psi of hydrogen at room temperature. The reaction mixtures were continuously stirred for 2 h. The acetic acid was distilled off from the reaction mixture under reduced pressure, and 0.95 g of the mixture of *cis*- and *trans*-4-isopropylcyclohexane-carboxylic acid was obtained by distillation (113-116 °C, 1 mmHg). To a solution of this acid and methanol (15 mL), a catalytic amount of TsOH (10% wt) was added and the mixture was refluxed until starting material disappeared. Evaporation of methanol gave an oil which was purified by flash chromatography (ethyl acetate:hexane 5:95) to afford a mixture of **2.1** and **2.2** in a ratio of 3:1 (0.88 g, 79%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.60 (s, 3H), 2.58-2.14(m, 1H), 2.08-0.83 (m, 10H)
^{13}C-NMR (400 MHz, $CDCl_3$): δ 177.07(d), 51.84(d), 43.72(d), 43.50(d), 32.4(d), 29.40(d), 27.02(d), 20.29(d)

***trans*-4-(Isopropyl)cyclohexanecarboxylic acid (2.4)**

The mixture of esters **2.1** and **2.2** (0.85 g, 4.6 mmol) was isomerized in the presence of 60% sodium hydride (18.5 mg, 0.46 mmol) at 150 °C without solvent for 2 h. Water (3 mL) was carefully added to quench the reaction, extraction with CH_2Cl_2 (3x 20 mL), drying over anhydrous Na_2SO_4 gave 0.78 g of the *trans* methyl ester **2.2** and *cis* methyl ester **2.1** in a ratio of 6:1 after distillation (64 °C, 0.7 mmHg). The methyl ester was dissolved in 4.2 mL of methanol and hydrolyzed by 4.2 mL of 2 N NaOH for 10 min. The solution was acidified with 1N HCl to pH 2, and the powdery precipitate was filtered. The crude product **2.3** and **2.4** was recrystallized from 80% MeOH aqueous to afford the *trans* acid **2.4** (0.49g, 64 %) as a white solid.

^{1}H NMR (400 MHz, CD_3OD): 2.24 (tt, J = 12.24, J = 3.49 Hz, 1H), 2.04 (m, 2H), 1.81 (m, 2H), 1.40 (m, 3H), 1.04 (tt, J = 11.7, J = 4.1 Hz, 1H), 1.01 (m, 2H), 0.86 (d, J = 6.8 Hz, 6H).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 183.46, 43.76, 43.61, 33.14, 29.39, 29.18, 20.15.
HRMS: $C_{10}H_{18}O_2$ (M^+); Calcd.: 170.1307; found: 170.1302.

***trans*-4-(Trifluoromethyl)cyclohexanecarboxylic acid (2.8)**

A mixture of ethyl 4-trifluoromethylbenzoate (191 mg, 0.94 mmol), 2 mL of ethanol and 40 mg of rhodium/activated charcoal (5%) was hydrogenated for 5 h under a pressure of 5 bar and at a temperature of 60 °C. The mixture of ethyl ester **2.5** and **2.6** obtained after removal of the catalyst by filtration and removal of the solvent was suspended in 1 ml of water and treated with 140 mg of 30% NaOH solution, and the mixture was briefly heated to boiling and stirred at room temperature for 18 h. Acidification using hydrochloric acid gave the carboxylic acid (119 mg, 65% overall yield) as a *cis/trans* mixture.

A mixture of this acid (110 mg, 0.6 mmol) and 300 µL of thionyl chloride were boiled for 48 h. After the excess thionyl chloride was removed by distillation, 1 mL of water and 200 µL of 30% NaOH solution were added, and the reaction mixture was stirred at 60 °C for 2 h. Acidification and recrystallization from petroleum ether gave the pure *trans*-carboxylic acid **2.8** (78 mg, 71% overall yield) as a white solid.

Mp. 154-155 °C
^{1}H NMR (400 MHz, $CDCl_3$): δ 2.70 (tt, J = 13.4, J = 4.3 Hz, 1H), 2.09 (tt, J = 12.8, J = 4.1 Hz, 1H), 2.07 (m, 2H), 2.0 (m, 2H), 1.40 (m, 4H),
^{13}C-NMR (400 MHz, $CDCl_3$): δ 176.3, 42.5, 41.42, 3.59, 27.5, 27.3.
HRMS: $C_8H_{11}F_3O_2$ (M^+); Calcd.: 196.0711; found: 196.0718.

(*S*)-*N*-Ethanoyl-1-(4-bromophenyl)ethylamine (2.11)

A solution of (*S*)-1-(4-bromophenyl)ethylamine (0.5 g, 2.5 mol) and triethylamine (0.44 mL, 3.1 mmol) in anhydrous diethyl ether (100 mL) was cooled to 0 °C. Acetyl chloride (0.21 mL, 3.0 mmol) was added dropwise with vigorous stirring, the temperature being maintained at 0 °C. After allowing the mixture to warm to room temperature, water (100 mL) was added and the diethyl ether layer separated, washed with 0.1M HCl (200 mL) followed by water (2x100 mL) and finally dried over potassium carbonate. The off-white solid residue obtained after evaporation of the solvent was recrystallised from diethyl ether giving the title compound as colorless needle-like crystals (0.58 g, 81%)

Mp. 127 – 130 °C

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (d, J = 8.4 Hz, 2H), 7.18 (d, J = 8.4 Hz, 2H), 5.86 (s, 1H), 5.06 (m, 1H), 1.97 (s, 3H), 1.79 (s, 3H), 1.45 (d, J = 6.95 Hz, 3H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 170.9, 141.4, 131.6, 129.3, 121.1, 47.7, 23.6, 18.3

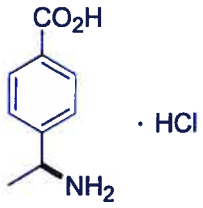
(*S*)-*N*-Ethanoyl-1- (4-cyanophenyl) ethylamine (2.12)

Copper (I) cyanide, CuCN (0.46 g, 2.6 mmol) was added to a solution of **2.11** (0.620 g, 4.9 mmol) in dry DMF (5 mL) and the suspension stirred vigorously at 180 °C for 48 h. A clear solution was obtained. The solvent was removed under reduced pressure and the residue taken into 6M HCl (20 mL). The resulting red-brown solution was extracted with

dichloromethane (5x 25 mL) and the organic extracts subsequently washed with water (100 mL) to give a colorless solution. The solvent was removed under reduced pressure to yield the compound **2.12** as a colorless solid (0.35g, 75%).

Mp. 187-189 °C

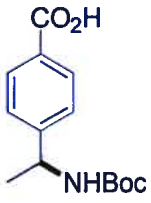
^{1}H NMR (400 MHz, CD_3OD): 7.67 (d, J = 8.18, 2H), 7.48 (d, J = 8.46 Hz, 2H), 5.0 (p, J = 1H), 4.89 (s, 1H), 1.97 (s, 3H), 1.43 (d, J = 7.09 Hz, 3H)
^{13}C NMR (CD_3OD): 171.5, 150.3, 132.5, 127.1, 118.8, 110.8, 46.5, 21.6, 21.2
IR (solid): ν =2227 (CN), 1637 cm^{-1} (C=O)



(*S*)-4-(1-Aminoethyl) benzoic acid hydrochloride (2.13)

Compound **2.12** (0.35 g, 1.8 mmol) was dissolved in 6M HCl (5 mL), and the solution heated at 108 °C for 75 h. The solvent was removed under reduced pressure to give **2.13** as a colorless solid (0.32 g), which was used in next step without purification.

IR (solid): ν =1703 cm^{-1} (C=O)
^{1}H NMR (400 MHz, CD_3OD): 8.13 (d, J = 8.23, 2H), 7.48 (d, J = 8.29 Hz, 2H), 4.60 (q, J = 3.64, 1H), 1.97 (s, 3H), 1.43 (d, J = 7.09 Hz, 3H)
^{13}C NMR (CD_3OD): 168.0, 143.5, 131.6, 130.6, 126.9, 51.0, 19.8

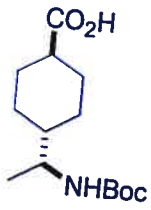


(*S*)-4-(1-*tert*-Butoxycarbonylamino-ethyl)-benzoic acid (2.14)

A mixture of **2.13** (0.3 g, 1.49 mmol), $(Boc)_2O$ (0.44 g, 2.0 mmol) and $NaHCO_3$ (0.38 g, 4.5 mmol) in MeOH (8 mL) was sonicated in a cleaning bath until the starting material was no longer detected. The solids were filtered and the solvent evaporated. The addition of 5 mL of ether caused the precipitation of small quantities of mineral solids. The process was repeated to give compound **2.14** as a white solid (0.31 g, 83%)

^{1}H NMR (400 MHz, CD_3OD): 7.98 (d, J = 8.26, 2H), 7.42 (d, J = 8.12 Hz, 2H), 4.70 (q, J = 3.44, 1H), 1.43 (s, 9H), 1.35 (d, J = 6.3 Hz, 3H), 1.24 (s, 1H)

^{13}C NMR (CD_3OD): 168.7, 156.6, 150.8, 129.9, 129.4, 125.9, 79.2, 65.4, 27.8. 21.9.



***trans*-(*S*)-4-(1-*tert*-Butoxycarbonylamino-ethyl)-cyclohexanecarboxylic acid (2.20)**

A solution of **2.13** (184 mg, 0.92 mmol) in 1N HCl (2 mL) was hydrogenated over PtO_2 (20 mg) at room temperature and atmospheric pressure. The catalyst was filtered off, and the solution was evaporated to dryness to give the crude products **2.15** and **2.16** (161 mg), which were heated in an autoclave at 200 °C for 10 h in 1N NaOH (5 mL). To the resulting solution was added activated carbon (30 mg), the suspension filtered and evaporated to give a mixture of sodium carboxylate **2.18** (108 mg) as the *trans* as a major product, which could not be separated from the minor *cis* isomer. The crude product was used in the next step.

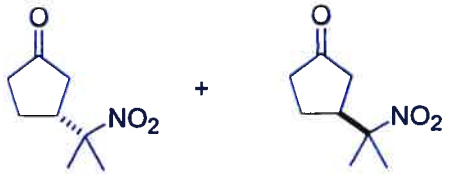
The crude product (96 mg) was dissolved in 1.5 mL water. To the solution added a mixture of $(Boc)_2O$ (327 mg, 1.5 mmol) and dioxane (1.8 mL), the reaction was stirred at room temperature until starting material was disappeared. 1N $KHSO_4$ aqueous was added to adjust the pH to 4 at 0 °C. Solvent was removed under reduced pressure to give **2.19** and **2.20**, which were separated by column chromatography (ethyl acetate:hexane 1:1) to give *trans* compound **2.20** (53 mg, 21%) as a white solid.

^{1}H NMR (400 MHz, CD_3OD): δ 3.42 (dd, J = 4.1, 13.1 Hz, IH), 2.55 (m, 1H), 2.09 (m, 2H), 1.42 (s, 9H), 1.26 (m, 3H), 1.04 (d, J = 6.72 Hz, 3H),

^{13}C NMR (400 MHz, CD_3OD): δ 178.9, 158.1, 79.7, 50.9, 43.6, 40.9, 28.8, 27.7, 27.6, 26.9, 29.9, 18.5

MS (M^+): 271.18, 144.10, 88.04

HRMS: $C_{14}H_{25}N_1O_4$ (M^+); Calcd.: 271.1784; found: 271.1792.



***R*-(+)-3-(2-Nitropropane-2-yl)cyclopentanone (2.21) and 3-epimer**

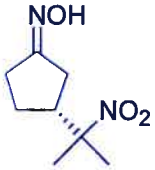
A mixture of 2-cyclopenten-1-one (0.5 mL, 5.2 mmol), 2-nitropropane (1 mL, 11.0 mmol), 2,5-dimethylpiperazine (0.6 mL, 5.3 mmol), a catalytic amount of L-proline (20.2 mg, 0.2 mmol), and 0.1 mL of water was stirred in reagent grade chloroform previously passed through a bed of Beckmann 1 grade basic alumina (40 mL) for 62 h at RT. The reaction mixture was diluted with CH_2Cl_2 and washed with aqueous HCl (3%). The organic phrase was dried with Na_2SO_4, filtered, concentrated and chromatographed on a silica gel column (ethyl acetate:hexane 1:5) to obtain a colorless oil (0.84 mg, 88%).

[α] $_D$: +21.3 (c 1.0, $CHCl_3$, R: S = 75:25; 50 % *ee*)

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.77 (m, 1H), 2.24 (m, 3H), 2.02 (m, 2H), 1.58 (m, 1H), 1.54 (d, J = 5.42 Hz, 3H).

^{13}C-NMR (400 MHz, $CDCl_3$) δ 216.1, 89.9, 46.0, 40.5, 38.9, 24.8, 23.8.

HRMS: $C_8H_{14}NO_3$ (M+1); Calcd.: 172.0974; found: 172.0977.



***R*-(+)-3-(2-Nitropropane-2-yl)cyclopentanoxime (2.23) and 3-epimer**

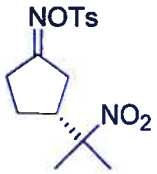
A solution of compound **2.21** (4.9 g, 28.9 mmol) in ethanol (75 mL) containing hydroxylamine hydrochloride (3.2 g, 44.5 mmol) and potassium hydroxide (3.1 g, 55 mmol in 5 mL water) was stirred at room temperature for 2.5 h. The precipate was filtered and washed with EtOAc. The solvent was removed under vacuum, the residue was partitioned between ethyl acetate and water. The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum to yield **2.23** (4.9 g, 92%, *E/Z* mixture) as colorless solid.

[α] $_D$: +23.3 (c 1.0, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.67 (m, 2H), 2.40 (m, 2H), 2.22 (m, 2H), 1.92 (m, 1H), 1.60 (d, J = 1.54 Hz, 6H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 163.9, 89.9, 32.1, 26.5, 25.7, 23.1, 22.8

HRMS: $C_8H_{15}N_2O_3$ (M+1); Calcd.: 187.1083; found: 187.1079



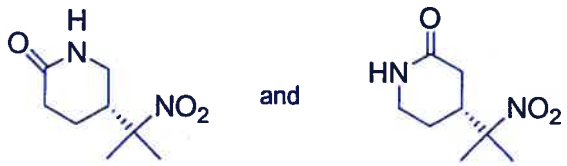
***R*-(+)-3-(2-Nitropropane-2-yl)cyclopentan-*O*-*p*-tosyloxime (2.24) and 3-epimer**

To a solution of **2.23** (3.55 g, 18.8 mmol) in pyridine (17.5 mL) at 0 °C was added *p*-toluenesulfonyl chloride (4.3 g, 22.5 mmol). The reaction mixture was stirred at 0-5 °C for 5 h, it was diluted with ethyl acetate (40 mL), washed with 1N cold HCl, saturated $NaHCO_3$ solution and brine, and dried over $MgSO_4$. The solvent was removed under vacuum to obtain an oil (6.1 g) which slowly solidified on standing. The compound **2.24** was used in the next step without further purification.

[α] $_D$: +22.4 (c 1.0, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 (d, J = 7.05, 2H), 7.35 (d, J = 7.76, 2H), 2.81-2.30 (m, 5H), 2.45 (s, 3H), 1.94 (m, 1H), 1.56 (m, 1H), 1.57 (d, J = 5.25 Hz, 6H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 173.6, 145.53, 132.9, 130.1, 129.2, 89.4, 47.6, 33.3, 31.6, 29.3, 26.8, 24.3, 22.1.



2.25 **2.26**

5*R*-(1-Methyl-1-nitro-ethyl)-piperidin-2-one (2.25) and 4*R*-(1-Methyl-1-nitro-ethyl)-piperidin-2-one (2.26) and their 3-epimers

Compound **2.24** (0.5 g, 21 mmol) was dissolved in 25 mL methanol, the mixture was absorbed on basic Beckmann grade I alumina. Toluene 50 mL was added and the solution was evaporated to dryness. The procedure was iterated several times until the starting material disappeared. The alumina was filtered and washed with methanol until all the product was resulted. Removed the solvent to give yellow solid, which was purified by silica gel column chromatography (MeOH:CH_2Cl_2 5:95) to afford a mixture of **2.25** and **2.26** as a white solid (201 mg, 45%),

The mixture was separated after protection with $(Boc)_2O$, treatment with formic acid gave pure compounds **2.25** and **2.26** respectively.

For (**2.25**) **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.10 (s, 1H) 3.26 (dd, J = 1.76, 12.9 Hz, 1H), 3.16 (dd, J = 1.81, 4.64 Hz, 1H), 2.51 (m, 2H), 2.37 (m, 1H), 1.98 (m, 1H), 1.61-1.57 (m, 1H), 1.60 (s, 3H), 1.59 (s, 3H), **^{13}C-NMR** (400 MHz, $CDCl_3$): δ 174.3, 89.9, 43.1, 42.2, 31.2, 23.8, 23.7, 22.8; **HRMS**: $C_{18}H_{15}N_2O_3$ (M+1); Calcd.: 187.1085; found: 187.1083

For (**2.26**) **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.9 (s, 1H), 3.37 (m, 1H), 3.25 (m, 1H), 2.44 (dd, J = 1.86, 13.2 Hz, 1H), 2.37 (dd, J = 1.75, 4.8 Hz, 1H), 2.25 (m, 1H), 1.78 (m, 1H), 1.62 (m, 1H), 1.56 (s, 3H), 1.54 (s, 3H), 1.52 (m, 1H); **^{13}C-NMR** (400 MHz, $CDCl_3$): δ 174.4, 89.9,

42.9, 41.8, 30.7, 23.8, 22.6, 22.3; **HRMS**: $C_{18}H_{15}N_2O_3$ (M+1); Calcd.: 187.1087; found: 187.1085.

2.27 **2.28**

5*R*-(1-Methyl-1-nitro-ethyl)-2-oxo-piperidine-1-carboxylic acid *tert*-butyl ester (2.27) and 4*R*-(1-Methyl-1-nitro-ethyl)-2-oxo-piperidine-1-carboxylic acid *tert*-butyl ester (2.28) and their epimer

To a solution of mixture of **2.25** and **2.26** (0.93 mg, 5 mmol) in anhydrous CH_2Cl_2 (25 mL) were added Et_3N (0.7 mL, 5.0 mmol), $(Boc)_2O$ (0.6 mg, 6.0 mmol), and DMAP (60 mg, 0.5 mmol) at room temperature. After stirring for 18 h at r.t, the solvent was evaporated and water (45 mL) was added. The resulting mixture was extracted with Et_2O (3x 30 mL), the combined organic layers were washed with 1 M $KHSO_4$, $NaHCO_3$, brine, and dried over Na_2SO_4. After filtration and evaporation of the solvent, the mixture was separated by silica gel column chromatography (ethyl acetate:hexane 1:4) to give *N*-Boc lactams **2.27** and **2.28** (0.69 g and 0.5 g respectively 88%) as white solids.

For (**2.27**) **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.77 (dd, J = 1.81, 13.27, 1H), 3.42 (dd, J = 1.79, 4.70 Hz, 1H), 2.6 (m, 2H), 2.40 (m, 1H), 1.85 (m, 1H), 1.61 (s, 3H), 1.59 (s, 3H), 1.57 (m, 1H), 1.47(s, 9H); **^{13}C-NMR** (400 MHz, $CDCl_3$): δ 171.0, 152.4, 90.4, 83.9, 45.5, 41.9, 33.9, 28.0, 23.5, 23.0, 21.5; **HRMS**: $C_{13}H_{23}N_2O_5$ (M+1); Calcd.: 287.1607; found: 287.1604.

For (**2.28**) **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.86 (m, 1H), 3.49 (m, 1H), 2.57 (dd, J = 14.82, 2.99, 2H), 2.30 (m, 1H), 1.87 (m, 1H), 1.55 (s, 6H), 1.50 (d, J = 3.31 Hz, 9H), 1.55-1.43 (m, 1H); **^{13}C-NMR** (400 MHz, $CDCl_3$): δ 169.5, 152.3, 90.5, 83.6, 45.0, 40.9, 36.3, 28.1, 24.6, 23.7, 22.2; **HRMS**: $C_{13}H_{23}N_2O_5$ (M+1); Calcd.: 287.1607; found: 287.1612.

[2*R*-(1-Methyl-1-nitro-ethyl)-5-oxo-5-phenyl-pentyl]-carbonic acid *tert*-butyl ester (2.29)

To a solution of compound **2.27** (464 mg, 1.62 mmol) in anhydrous THF (12 mL) under argon was added dropwise a 1.0 M PhMgBr (2.5 mL, 2.5 mmol) at -78 °C. The reaction mixture was slowly warmed to r.t and overnight, and then quenched with H_2O (5 mL). The organic phase was extracted with EtOAc (3 x 10 mL), dried over Na_2SO_4. The solution was evaporated and subjected to flash chromatography eluting with (ethyl acetate/hexane 1:9) to give **2.29** as a white solid (413 mg, 70%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, J = 8.02 Hz, 2H), 7.59-7.43 (m, 4H), 5.3 (m, 1H), 4.9 (s, 1H), 4.42 (m, 1H), 3.32-3.1 (m, 4H), 1.60 (s, 3H), 1.58 (s, 3H), 1.46 (m, 1H) 1.41 (s, 9H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 199.9, 156.1, 133.5, 128.9, 128.3, 125.6, 91.7, 79.8, 47.0, 40.9, 36.9, 28.5, 27.9, 24.0, 23.8

HRMS: $C_{19}H_{29}N_2O_5$ (M+1); Calcd.: 364.1281; found: 364.1299.

3*R*-(1-Methyl-1-nitro-ethyl)-6-phenyl-2, 3,4,5-tetrahydro-pyridine (2.30) and 3-epimers

Trifluoroacetic acid (1 mL) was added dropwise to the **2.29** (364 mg, 1.0 mmol) with stirring at 0 °C. The solution is stirred at room temperature overnight until the starting material disappeared, and then a 30% aqueous solution of sodium hydroxide was carefully added, with cooling at 0 °C, until pH 10-11 was reached. The organic base was extracted with CH_2Cl_2 (3x 10 mL), washed with brine, dried over Na_2SO_4, and evaporated. The crude imine

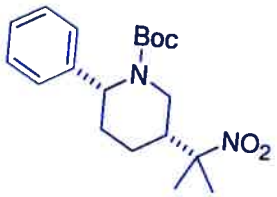
was purified by flash chromatography (ethyl acetate:hexane 1:4) to give **2.30** as a white solid (206 mg, 84%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (m, 2H), 7.38 (m, 3H), 4.0 (m, 1H), 3.48 (m, 1H), 2.89 (m, 1H), 2.63 (m, 1H), 2.37 (m, 1H), 1.81 (m, 1H), 1.63 (s, 3H), 1.60 (s, 3H), 1.58-1.47 (m, 1H).

^{13}C-NMR (400 MHz, $CDCl_3$): δ 165.5, 139.2, 130.0, 128.4, 126.1, 90.7, 50.6, 41.5, 28.1, 24.1, 22.1, 21.4.

MS (M^+): 246.1, 216.1, 200.1, 171.1, 148.1.

HRMS: $C_{14}H_{18}N_2O_2$ (M^+); Calcd.: 246.1368; found: 246.1377



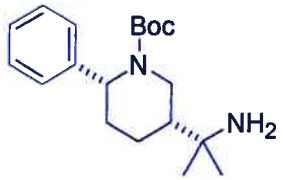
5*R*-(1-Methyl-1-nitro-ethyl)-2*R*-phenyl-piperidine-1-carboxylic acid *tert*-butyl ester (2.31)

To a solution of compound **2.30** (123 mg, 0.5 mmol) and EtOAc (5 mL) were added 10% Pd/C (0.20 g) and $(Boc)_2O$ (163 mg, 0.75 mmol), and hydrogenated at atmosphere overnight until the reaction was completed. The catalyst was filtered, and the solvent was removed under reduce pressure to give a crude product, which was purified by flash chromatography (ethyl acetate:hexane 1:4) to give **2.31** as a white solid (158 mg, 91%). The relative stereo chemistry was assumed to be *cis*.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7.14 (m, 5H), 4.78 (dd, J = 1.9, 10.58 Hz, 1H), 4.01 (dd, J = 1.87, 14.90 Hz, 1H), 3.41 (dd, J = 1.78, 5.0 Hz, 1H), 2.62 (m, 1H), 2.20 (m, 1H), 1.72 (m, 2H), 1.65 (s, 3H), 1.57 (s, 3H), 1.56-1.29 (m, 1H), 1.29 (s, 9H), 1.29 (m, 1H).

^{13}C-NMR (400 MHz, $CDCl_3$): δ 155.0, 143.6, 128.3, 126.6, 124.9, 91.6, 79.8, 57.1, 42.7, 38.7, 29.1, 28.2, 28.0, 23.2, 21.7, 21.1.

HRMS: $C_{19}H_{28}N_2O_4$ (M^+); Calcd.: 348.2049; found: 348.2041.



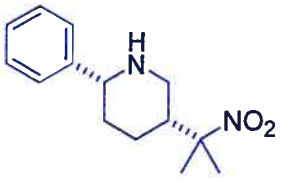
5*R*-(1-Amino-1-methyl-ethyl)-2*R*-phenyl-piperidine-1-carboxylic acid *tert*-butyl ester (2.32)

To a solution of $NiCl_2$•6H2O (124 mg, 0.52 mmol) in MeOH (2 mL) was added $NaBH_4$ (316 mg, 8.36 mmol) in small portions. After stirring for 0.5 h (sonication) compound **2.31** (348 mg, 1.0 mmol) was added. And the mixture was filtered through a short pad of Celite after 10 minutes. The Celite was washed with MeOH, and combined MeOH solution was concentrated. Addition of 1N NaOH to the residue, extraction with ether, and condensation of the ether layer gave the amine derivative **2.32** (262 mg, 84%), which was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7.16 (m, 5H), 4.78 (m, 1H), 4.01 (m, 1H), 3.43 (m, 1H), 2.63 (m, 1H), 2.14 (m, 1H), 1.75-1.56 (m, 2H), 1.66 (s, 3H), 1.58 (s, 3H), 1.43 (m, 1H), 1.29 (s, 9H), 1.29 (m, 1H),

^{13}C-NMR (400 MHz, $CDCl_3$): δ 155.7, 144.1, 129.1, 127.8, 125.4, 92.2, 80.5, 57.6, 43.3, 39.4, 29.7, 28.8, 24.1, 22.2, 21.6.

HRMS: $C_{19}H_{30}N_2O_2$ (M^+); Calcd.: 318.2307; found: 318.2305



5*R*-(1-Methyl-1-nitro-ethyl)-2*R*-phenyl-piperidine (2.33)

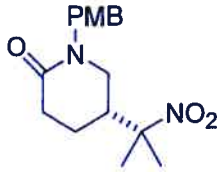
To a solution of compound **2.30** (123 mg, 0.5 mmol) and EtOAc (5 mL), 10% Pd/C (20 mg) was added and hydrogenated at atmosphere overnight until the reaction was completed. The

catalyst was filtered, and the solvent was removed under reduce pressure. The residue was purified by chromatography ($MeOH/CH_2Cl_2$ 5:95) to give **2.33** as a white solid (116 mg, 94 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34-7.25 (m, 5H), 3.56, (dd, J = 4.70, 11.2 Hz, 1H), 3.14 (dd, J = 4.3, 2.2 Hz, 1H), 2.28 (dd, J = 4.1, 1.9 Hz, 1H), 1.92 (m, 1H), 1.71 (m, 2H), 1.60 (s, 3H), 1.58 (s, 3H), 1.46 (m, 2H).

^{13}C-NMR (400 MHz, $CDCl_3$): δ 145.0, 128.9, 127.7, 126.9, 91.1, 62.2, 48.9, 45.7, 34.8, 27.0, 24.2, 26.8.

HRMS: $C_{14}H_{20}N_2O_4$ (M^+); Calcd.: 248.1527; found: 248.1523



1-(4-Methoxy-benzyl)-5*R*-(1-methyl-1-nitro-ethyl)-piperidin-2-one (2.34)

To a solution of mixture of **2.25** and **2.26** (330 mg, 1.8 mmol) in 3.5 mL of THF was added 45.0 mg (1.89 mmol) of NaH. The slurry was stirred for 15 min, and a solution of 168 μL (1.18 mmol) of neat p-methoxylbenzyl chloride was added, followed by 72 mg (0.016 mmol) of tetrabutylammonium iodide. The mixture was stirred for 21 h, and 1 mL of *tert*-BuOH was added. 5 mL of 5 % NH_4Cl was added slowly dropwise and the mixture was extracted with diethyl ether (3 x 15 mL). The organic extracts were combined, washed with 10 mL of brine, dried over Na_2SO_4. After filtration and evaporation of the solvent, the mixture was separated by silica gel column chromatography (ethyl acetate:hexane 3:7) to afford *p*-methoxybenzyl lactams **2.34** and **2.35** (0.28 g and 0.19 g respectively 87%) as a pale yellow solids.

For (**2.34**) **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.16 (d, J = 8.50 Hz, 2H), 6.85 (d, J = 8.3 Hz, 2H), 6.84 (d, J = 8.60 Hz, 2H), 4.53 (dd, J = 14.52, 1.8 Hz, 1H), 4.47 (dd, J = 4.9, 1.4 Hz, 1H), 3.78 (s, 3H), 3.06 (m, 1H), 2.58 (m, 1H), 2.45 (m, 2H), 1.80 (m, 1H), 1.55 (s, 3H), 1.50, (s, 1H), 1.19 (m, 1H); **^{13}C-NMR** (400 MHz, $CDCl_3$): δ 168.0, 159.5, 129.8, 129.3,

114.4, 90.6, 55.7, 49.8, 46.2, 42.0, 34.2, 25.0, 24.3, 22.0; **HRMS:** $C_{16}H_{22}N_2O_4$ (M+1), Calcd.: 307.1658; found: 307.1652;

For (**2.35**) **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.21 (dd J = 8.31 Hz, 2H), 6.92 (d, J = 8.28 Hz, 2H), 6.71 (d, J = 8.1 Hz, 2H), 4.51 (m, 1H), 4.40 (m, 1H), 3.78 (s, 3H), 3.2 (m, 2H), 2.54 (m, 1H), 2.21 (m, 1H), 1.70 (m, 1H), 1.51 (s, 3H), 1.48 (s, 1H), **^{13}C-NMR** (400 MHz, $CDCl_3$): δ 168.0, 159.5, 129.9, 129.2, 114.3, 90.6, 55.7, 49.8, 46.2, 41.9, 34.2, 25.0, 24.3, 21.9; **HRMS:** $C_{16}H_{22}N_2O_4$ (M+1), Calcd.: 307.1656; found: 307.1654

1-(4-Methoxy-benzyl)-3*R*-(1-methyl-1-nitro-ethyl)-piperidine (2.36)

To a solution of **2.34** (306 mg, 1.0 mmol) in 2 mL of THF was added 1.1 mL (0.11 mmol) of BH_3•SMe_2 (1 M in THF). After the solution was stirred at 70 °C for 2.5 h, the solvent was removed, 1N HCl was added and the solution was refluxed 30 min then cooled to 0 °C, CH_2C1_2 3mL and 0.5 mL of 1 N NaOH were added and the solution was stirred for 20 min, washed with water, brine, and dried (Na_2SO_4) and concentrated. Chromatography on silica gel (ethyl acetate:hexane 1:9) using 5-20% ethyl acetate/hexane gave **2.36** (207 mg, 71%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.14 (d, J = 8.34 Hz, 2H), 6.85 (d, J = 8.11 Hz, 2H), 3.78 (s, 3H), 3.49 (d, J = 13.06 Hz, 1H), 3.37 (d, J = 13.0 Hz, 1H), 2.81 (m, 2H), 2.28 (m, 1H), 1.78-1.54 (m, 5H), 1.52 (s, 3H), 1.51 (s, 1H), 1.04 (m, 1H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 159.1, 130.5, 130.5, 114.0, 91.2, 63.1, 55.6, 55.4, 53.6, 45.4, 26.1, 25.6, 24.0, 23.7

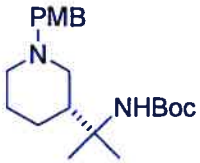
MS (M+1): 292.1, 246.1, 154.0, 136.0, 121.0, 107.0, 77.0

HRMS: $C_{19}H_{29}N_2O_5$ (M+1); Calcd.: 292.1787; found: 292.1781

1-[1-(4-Methoxy-benzyl)-piperidin-3*R*-yl]-1-methyl-ethylamine (2.37)

To a solution of $NiCl_2 \cdot 6H_2O$ (124 mg, 0.52 mmol) in MeOH (2 mL) was added $NaBH_4$ (316 mg, 8.36 mmol) in small portions. After stirring for 0.5 h (sonication) compound **2.36** (292 mg, 1.0 mmol) was added, and the mixture was filtered through a short pad of Celite after 10 minutes. The Celite was washed with MeOH, and combined MeOH solution was concentrated. Addition of 1N NaOH to the residue, extraction with ether (3 x 20 mL), and The organic extracts were combined, washed with brine, dried over Na_2SO_4. After evaporation of the solvent, the crude was purified by column chromatography (CH_2Cl_2:MeOH:NH_4OH 85:14:1) to gave the amine derivative **2.37** (214 mg, 82%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.18 (d, J = 8.28 Hz, 2H), 6.80 (d, J = 8.36 Hz, 2H), 3.74 (s, 3H), 3.47 (d, J = 12.95 Hz, 1H), 3.0 (m, 1H), 2.78 (m, 1H), 2.34 (d, J = 12.0 Hz, 1H), 1.72 (m, 4H), 147 (m, 2H), 1.0 (s, 6H), 1.0 (m, 2H).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 158.9, 131.0, 130.6, 113.9, 63.6, 56.1, 55.6, 54.0, 51.0, 47.8, 29.2, 28.7, 26.2, 25.9.
MS (M+1): 262.2, 245.2, 231.2, 205.1, 141.1, 84.1.
HRMS: $C_{16}H_{26}N_2O$ (M+1); Calcd.:262.2045; found: 262.2047.



{1-[1-(4-Methoxy-benzyl)-piperidin-3*R*-yl]-1-methyl-ethyl}-carbamic acid *tert*-butyl ester (2.38)

To a solution of the amine **2.37** (314 mg, 1.2 mmol) in CH_2Cl_2 (5 mL) was added Boc_2O (414 mg, 1.92 mmol) at room temperature, and the solution was stirred for 24 h. The solution

was concentrated, and the residue was purified by flash chromatography (ethyl acetate:hexane 3:7) to afford **2.38** (311 mg, 86%) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.22 (d, J = 8.49 Hz, 2H), 6.83 (d, J = 8.49 Hz, 2H), 3.78 (s, 3H), 3.42 (s, 2H), 2.81 (m, 1H), 2.65 (m, 1H), 1.92 (m, 1H), 1.69 (m, 1H), 1.57-0.93 (m, 6H), 1.41 (s, 9H), 1.24 (s, 6H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 158.4, 130.5, 129.9, 62.8, 55.2, 55.2, 54.3, 53.7, 43.7, 28.3, 25.5, 25.2, 24.9, 24.6.

MS (M+1): 363.2, 307.2, 204.1, 154.0, 121.0, 102.0.

HRMS: $C_{21}H_{35}N_2O_3$ (M+1); Calcd.: 363.2648; found: 363.2638.

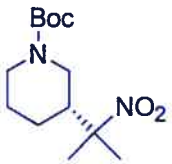
(1-Methyl-1-piperidin-3*R*-yl-ethyl)-carbamic acid *tert*-butyl ester 2.39

An ethyl acetate solution of **2.38** (362 mg, 1.0 mmol) was treated with $Pd(OH)_2/C$ (37 mg) and the mixture was stirred under a balloon containing hydrogen for 1.5 h. The reaction mixture was filtered, rinsed with MeOH, the filtrate was concentrated and the residue was chromatographed (MeOH: CH_2Cl_2 1:9) to afford **2.39** (196 mg, 80%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.58, 2.96 (m, 1H), 2.87 (m, 1H), 2.34 (m, 4H), 1.66 (m, 3H), 1.30 (S, 9H), 1.08 (S, 1H), 1.07 (S, 3H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 154.1, 78.2, 54.0, 47.7, 46.4, 44.7, 28.2, 26.9, 26.9, 24.5, 24.2.

HRMS: $C_{13}H_{26}N_2O_2$ (M^+); Calcd.: 242.2014; found: 242.2017



3*R*-(1-Methyl-1-nitro-ethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (2.41)

A solution of compound **2.27** (286 mg, 1 mmol) in formic acid (3 mL) was stirred for 1 h at rt. Evaporation of the solvent under vacuum gave a solid, which was used in the next step without purification. BH_3•SMe_2 (0.6 mL, 2M in THF) was added dropwise to a solution of above preparation in 10 mL dry THF at room temperature. The reaction mixture was refluxed overnight. After removal of solvent, the residue was treated with 15 mL saturated HCl-MeOH solution and refluxed for 30 min. The solvent was evaporated, 15 mL MeOH was added and subsequently removed under reduce pressure. The residue was further treated with 15 mL water and neutralized with K_2CO_3, the aqueous suspension was extracted with three portions of 15 mL dichloromethane. The combined extracts were dried over Na_2SO_4 and evaporated to give the crude product **2.40** (163 mg, 95%).

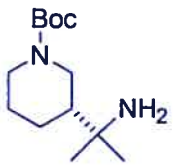
To a solution of the **2.40** (163 mg, 0.95 mmol) in CH_2Cl_2 (5 mL) was added Boc_2O (409 mg, 1.9 mmol) at room temperature, and the solution was stirred for 24 h. The solution was concentrated, and the mixture was purified by flash chromatography (ethyl acetate:hexane 3:7) to afford **2.41** (233 mg, 86%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.67 (m, 2H), 2.40 (m, 2H), 2.22 (m, 2H), 1.92 (m, 1H), 1.60 (d, J = 1.54 Hz, 6H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 155.0, 90.6, 80.2, 45.7, 45.0, 44.6, 28.7, 26.2, 25.5, 23.1, 21.7.

MS (M^+): 272.2, 217.1, 186.1, 168.1, 142.1, 130.1

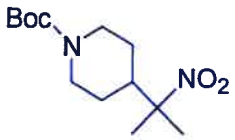
HRMS: $C_{13}H_{24}N_2O_4$ (M^+); Calcd.: 272.1736; found: 272.1737



3*R*-(1-Amino-1-methyl-ethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (2.42)

A solution of **2.41** (326 mg, 1.2 mmol) in MeOH (6 mL) was hydrogenated over 10% Pd/C (50 mg) at 60 psi at room temperature for 2 days until starting material was no longer detectable by TLC. The reaction mixture was filtered through Celite, the pad was washed with CH_2Cl_2 (5 mL), the solvent was removed and the residue was purified with column chromatography (CH_2Cl_2:MeOH:NH_4OH 90:9:1) to afford the desired product **2.42** as a colorless oil (264 mg, 91%).

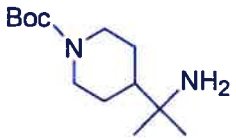
^{1}H NMR (400 MHz, $CDCl_3$): δ 4.22 (b, 1H), 4.05 (b, 1H), 2.49 (m, 4H), 1.44 (s, 9H), 1.92 (m, 1H) 1.68 (m, 1H), 1.39-1.14 (m, 3H)
^{13}C-NMR (400 MHz, $CDCl_3$): 155.2, 79.6, 52.0, 45.5, 44.0, 28.8, 28.2, 25.9, 24.1, 20.4.
MS (M^+): 242.2, 225.2, 184.1, 169.1, 72.1
HRMS: $C_{13}H_{36}N_2O_2$ (M^+); Calcd.: 242.1994; found: 242.1993.



4-(1-Methyl-1-nitro-ethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (2.44)

Using the same procedure as for **2.41** starting with **2.28** (286 mg, 1 mmol) afforded **2.44** (225 mg, 83 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.10 (m, 2H), 2.58 (m, 1H), 2.06 (m, 1H), 1.45 (s, 6H), 1.45-1.37 (m, 2H), 1.37 (s, 9H).
^{13}C-NMR (400 MHz, $CDCl_3$): 154.9, 94.7, 80.0, 45.65, 28.74, 27.1, 27.4, 26.8.
MS (M^+): 272.2, 217.1, 186.1, 168.1, 142.1, 130.1.
HRMS: $C_{13}H_{24}N_2O_4$ (M^+); Calcd.: 272.1734; found: 272.1739.



4-(1-Amino-1-methyl-ethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (2.45)

Using the same procedure as for **2.42** starting with **2.44** (286 mg, 1 mmol) afforded **2.45** (218 mg, 90%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.09 (b, 2H), 2.54 (m, 2H), 1.61 (m, 4H), 1.36 (s, 9H), 0.97 (s, 6H), 1.07 (m, 3H).

^{13}C-NMR (400 MHz, $CDCl_3$): 155.1, 79.6, 51.6, 47.9, 40.1, 28.8, 28.3, 27.0.

MS (M^+): 242.2, 197.1, 72.1.

HRMS: $C_{13}H_{26}N_2O_2$ (M^+); Calcd.: 242.1994; found: 242.1999.

General procedure for the preparation of sulfonamides (A)

To a dry THF (6 ml) solution of crude amine (1.0 mmol) was added Et_3N (0.4 mL, 3 mmol) and the mixture were cooled to 0 °C. Benzenesulfonyl chloride (1.2 mmol) was added and the solution was stirred at 0 °C for 10 min and then at room temperature for 24 h. After removal of THF, water was added and the product was extracted with CH_2Cl_2, washed with brine and dried over Na_2SO_4, Condensation and purification by flash column chromatography (10-50% ethyl acetate/hexane) afforded the product.

General procedure for the preparation of amides (B)

To a chilled solution of substituted benzoic acid (0.3 mmol), piperidine amine (48 mg, 0.2 mmol) and HOBt (46 mg, 0.3 mmol) in DMF (2mL) were added DIPEA (52 µL, 0.3 mmol) and EDC (58 mg, 0.3 mmol). After 1 h at 0 °C and 1 day at room temperature, the solution was evaporated. The residue was added CH_2Cl_2 and washed with $NaHCO_3$ and brine, dried over Na_2SO_4, and purified by flash column chromatography to give amide compounds.

Genereal procedure for reduction of nitro groups (C)

To a solution of $NiCl_2 \cdot 6H_2O$ (124 mg, 0.52 mmol) in MeOH (2 ml) was added $NaBH_4$ (316 mg, 8.36 mmol) in small portions. After stirring for 0.5 h (sonication) nitro compound (1.0 mmol) was added, and the mixture was filtered through a short pad of celite after 10 minutes. The Celite was washed with MeOH, and combined MeOH solution was concentrated. Addition of 1N NaOH to the residue, which were extraction with ether, washing with brine and drying over Na_2SO_4 gave a crude product, which was purified by flash column

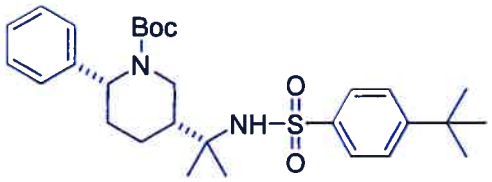
chromatography (CH_2Cl_2:MeOH:$NH_3 \cdot H_2O$ 90:9:1) to give the amine derivatives as white solids.

General procedure for *N*-Boc hydrolysis (D)

Excess formic acid was added to the *N*-Boc protected compound. Formic acid was distilled, the crude compound was purified with flash column chromatography (CH_2Cl_2:MeOH:NH_3·-H_2O 90:9:1) to give amine derivatives as give final compounds.

General procedure for the preparation of hydrochloride salts (E)

HCl (1 N, 3.0 mmol) was dropped into a stirred suspension of piperidine derivatives (1.0 mmol) in H_2O (5 mL). The filtered solution was frozen and then lyophilized to give the hydrochloride salt as white solids

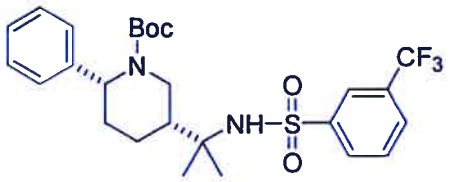


5*R*-[1-(4-*tert*-Butyl-benzenesulfonylamino)-1-methyl-ethyl]-2*R*-phenyl-piperidine-1-carboxylic acid *tert*-butyl ester (2.46a)

According to general procedure A, starting from **2.32** to give **2.46a** (23 mg, 81 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (d, J = 8.2 Hz, 2H), 7.48 (d, J = 8.1 Hz, 2H), 7.34-7.16 (m, 5H), 5.41 (s, 1H), 4.73 (m, 1H), 4.18 (m, 1H), 3.19 (dd, J = 6.8, 15.4 Hz, 1H), 2.14 (m, 1H), 1.90-1.78 (m, 2H), 1.521.58-1.39 (m, 2H), 1.39 (s, 9H), 1.38 (s, 9H), 1.20 (s, 3H), 1.19 (s, 3H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 156.3, 145.2, 136.8, 132.4, 128.7, 128.6, 127.1, 127.0, 125.8, 80.2, 58.5, 58.1, 41.2, 40.8, 30.9, 30.1, 28.9, 25.1, 24.6, 22.1

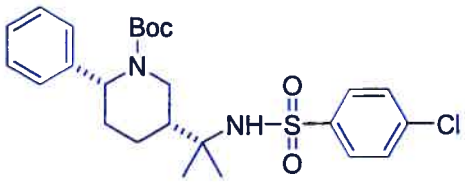


5*R*-[1-Methyl-1-(3-trifluoromethyl-benzenesulfonylamino)-ethyl]-2*R*-phenyl-piperidine-1-carboxylic acid *tert*-butyl ester (2.46b)

According to general procedure A, starting from **2.32** to give **2.46b** (23 mg, 81 %)

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (s, 1H), 8.05 (dd J = 8.1, 6.9 Hz, 1H), 7.88 (m, 1H), 7.62 (t, J = 87.9 Hz, 1H), 7.738-7.17 (m, 5H), 5.92 (s, 1H), 4.80 (m, 1H), 4.19 (m, 1H), 3.21 (m, 1h), 2.15 (m, 1H), 1.79 (m, 2H), 1.52 (m, 1H), 1.27 (m, 1H), 1.37 (s, 9H), 1.24 (s, 3H), 1.18 (s, 3H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 157.3, 143. 130.7, 130.1, 129.0, 129.0, 128.8, 127.2, 125.5, 124.7, 81.3, 59.4, 57.9, 46.6, 38.6, 30.0, 28.7, 28.0, 23.9, 21.4

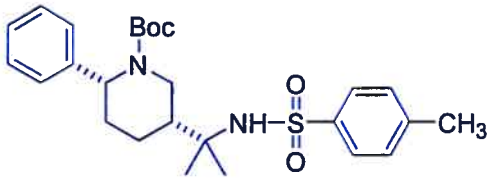


5*R*-[1-(4-Chloro-benzenesulfonylamino)-1-methyl-ethyl]-2*R*-phenyl-piperidine-1-carboxylic acid *tert*-butyl ester (2.46c)

According to general procedure A, starting from **2.32** to give **2.46c** (23 mg, 81 %)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (d, J = 8.27 Hz, 2H), 7.47 (d, J = 8.23 Hz, 2H), 7.31-7.17 (m, 5H), 5.65 (s, 1H), 4.78 (m, 12H), 4.15 (m, 1H), 3.12 (m, 1H), 2.19 (m, 1H), 1.81 (m, 21H0, 1.58-1.39 (m, 2H), 1.38 (s, 9H), 1.21 (s, 3H), 1.19 (s, 3H)

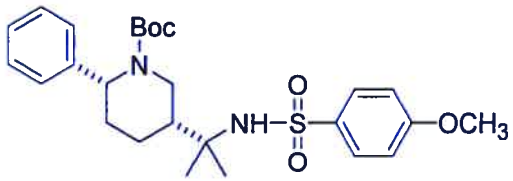
^{13}C-NMR (400 MHz, $CDCl_3$): δ 158.3, 147.2, 144.1, 132.9, 132.4, 128.7, 127.4, 127.0, 126,2, 81.6, 59.2, 58.4, 46.7, 38.5, 30.0, 28.9, 27.14, 23.5, 22.1



5*R*-[1-Methyl-1-(toluene-4-sulfonylamino)-ethyl]-2*R*-phenyl-piperidine-1-carboxylic acid *tert*-butyl ester (2.46d)

According to general procedure A, starting from 2.32 to give 2.46d (23 mg, 81%).

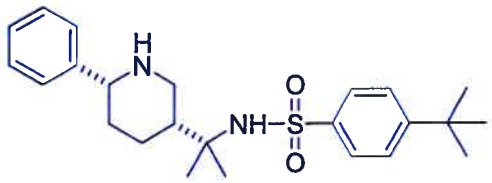
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (d, J = 7.1 Hz, 2H), 7.29-7.23 (m, 5H), 7.17 (d, J = 6.9 Hz), 5.36 (s, 1H), 4.78 (m, 1H), 4.16 (m, 1H), 3.48 (m, 2H), 3.21 (m, 1H), 2.42 (s, 3H), 2.16 (m, 1H), 1.84 (m, 2H), 1.52 (m, 2H), 1.35 (s, 9H), 1.23 (s, 3H), 1.16 (s, 3H), 1.16 (m, 1H).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 156.8, 145.8, 143.9, 132.1, 131.5, 128.8, 127.4, 127.2, 125.5, 81.1, 59.5, 57.9, 46.5, 38.7, 30.1, 28.7, 27.5, 23.7, 21.5.



5*R*-[1-(4-Methoxy-benzenesulfonylamino)-1-methyl-ethyl]-2*R*-phenyl-piperidine-1-carboxylic acid *tert*-butyl ester (2.46e)

According to general procedure A, starting from **2.32** to give **2.46e** (23 mg, 81%)

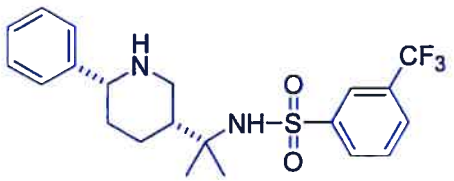
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, J = 8.1 Hz, 2H), 7.38-7.17 (m, 5H), 6.98 (d, J = 8.2 Hz, 2H, J = 8.23 Hz, 2H), 5.40 (s, 1H), 4.78 (m, 1H), 4.15 (m, 1H), 3.87 (s, 3H), 3.20 (m, 1H), 2.18 (m, 1H), 1.83 (m, 2H), 1.57-1.39 (m, 2H), 1.34 (s, 9H), 1.28 (m, 1H), 1.22 (s, 3H), 1.16 (s, 3H)
^{13}C-NMR (400 MHz, $CDCl_3$): δ 155.9, 145.0, 136.2, 131.6, 128.9, 128.8, 127.1, 127.0, 125.5, 80.1, 58.5, 57.6, 40.9, 39.4, 30.8, 28.6, 24.9, 24.1, 22.1



4-*tert*-Butyl-*N*-[1-methyl-1-(6*R*-phenyl-piperidin-3*R*-yl)-ethyl]-benzenesulfonamide (2.47a)

Acording to general procedure D, starting from **2.46a** to give **2.47a** (23 mg, 81%)

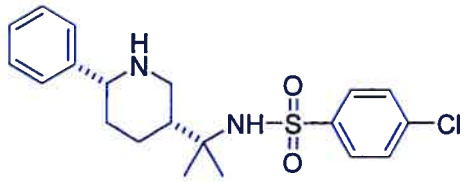
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, J = 8.2 Hz, 2H), 7.58 (d, J = 8.1 Hz, 2H), 7.37-7.21 (m, 5H), 4.9 (s, 1H), 3.50 (dd, J = 10.6, 1.8 Hz, 1H), 3.25 (m, 2H), 2.55 (m, 2H), 1.90 (m, 1H), 1.82 (m, 1H), 1.67 (m, 1H), 1.3 (s, 9H), 1.22 (s, 3H), 1.20 (s, 3H)
^{13}C-NMR (400 MHz, $CDCl_3$): δ 156.3, 145.2, 140.9, 128.8, 127.6, 127.2, 127.0, 126.3, 62.2, 59.1, 48.8, 47.3, 35.5, 35.1, 31.5, 26.6, 25.7, 25.6
HRMS: $C_{24}H_{34}N_2O_2S$ (M^+); Calcd.: 414.2341; found: 414.2338



***N*-[1-Methyl-1-(6*R*-phenyl-piperidin-3*R*-yl)-ethyl]-3-trifluoromethyl-benzenesulfonamide (2.47b)**

According to general procedure D, starting from **2.46b** to give **2.47b** (23 mg, 84%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (s, 1H), 8.1 (dd, J = 8.0, 3.1 Hz, 1H), 7.81 (d, J = 7.6 Hz, 1H), 7.65 (m, 1H), 7.37 (m, 5H), 4.7 (br, 1H), 3.52 (m, J = 11.4, 2.32 Hz, 1H), 3.27 (m, 1H), 2.57 (m, 1H), 1.94-1.72 (m, 3H), 1.48 (m, 1H), 1.29 (m, 1H), 1.23 (s, 3H), 1.21 (s, 3H)
^{13}C-NMR (400 MHz, $CDCl_3$): δ 156.3, 145.2, 140.9, 129.9, 129.1, 128.3, 128.0, 127.6, 114.1, 61.2, 55.9, 46.9, 45.9, 33.3, 28.7, 27.9, 23.0
HRMS: $C_{21}H_{25}F_3N_2O_2S$ (M^+); Calcd.: 426.1559; found: 426.1579



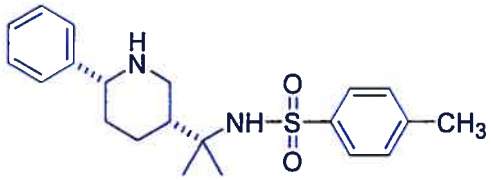
4-Chloro-*N*-[1-methyl-1-(6*R*-phenyl-piperidin-3*R*-yl)-ethyl]-benzenesulfonamide (2.47c)

According to general procedure D, starting from **2.46c** to give **2.47c** (27 mg, 87%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (d, J = 8.27 Hz, 2H), 7.47 (d, J = 8.23 Hz, 2H), 7.30 (m, 5H), 3.54 (m, 1H), 3.33 (m, 1H), 2.57 (m, 1H), 1.90 (m, 2H), 1.82 (m, 1H), 1.52 (m, 1H), 1.30 (m, 1H), 1.20 (s, 3H), 1.19 (s, 3H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 160.1, 142.5, 139.0, 129.6, 128.9, 128.8, 127.7, 127.1, 62.1, 59.5, 59.4, 48.5, 47.1, 26.4, 25.9, 25.1

HRMS: $C_{20}H_{25}ClN_2O_2S$ (M^+); Calcd.: 393.1325; found: 393.1331



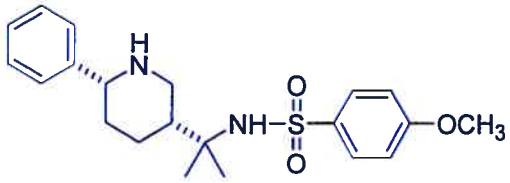
4-Methyl-*N*-[1-methyl-1-(6*R*-phenyl-piperidin-3*R*-yl)-ethyl]-benzenesulfonamide (2.47d)

According to general procedure D, starting from **2.46d** to give **2.47d** (19 mg, 87%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (d, J = 7.9 Hz, 2H), 7.40 (d, J = 8.1 Hz, 2H), 7.39-7.27 (m, 5H), 5.40 (br, 1H), 3.64 (dd, J = 11.4, 3.1 Hz, 1H), 3.49 (m, 1H), 2.67 (m, 1H), 2.42 (s, 3H), 1.92 (m, 3H), 1.65 (m, 1H), 1.35 (m, 1H), 1.18 (s, 9H), 1.17 (s, 9H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 156.7, 143.2, 141.1, 129.9, 129.0, 128.2, 127.3, 127.3, 61.8, 58.9, 46.1, 33.7, 26.1, 25.9, 24.5, 21.9

HRMS: $C_{21}H_{28}N_2O_2S$ (M^+); Calcd.: 372.1872; found: 372.1871



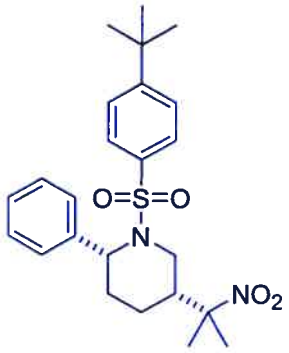
4-Methoxy-*N*-[1-methyl-1-(6*R*-phenyl-piperidin-3*R*-yl)-ethyl]-benzenesulfonamide (2.47e)

According to general procedure D, starting from **2.46e** to give **2.47e** (18 mg, 84%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (d, J = 8.9 Hz, 2H), 7.33-7.25 (m, 5H), 6.96 (d, J = 8.9 Hz, 2H), 4.55 (s, 1H), 3.87 (s, 3H), 3.52 (dd, J = 11.3, 2.3 Hz, 1H), 3.29 (m, 1H), 2.57 (m, 1H), 1.96-1.86 (m, 2H), 1.68 (m, 1H), 1.32 (m, 1H), 1.21 (s, 3H), 1.20 (s, 3H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 162.9, 135.6, 129.5, 128.9, 127.6, 127.0, 114.4, 62.3, 59.0 56.0, 48.7, 47.6, 35.1, 26.5, 25.7, 25.2

HRMS: $C_{21}H_{29}N_2O_3S$ (M^+); calcd.: 388.1821; found: 388.1819



1-(4-*tert*-Butyl-benzenesulfonyl)-5*R*-(1-methyl-1-nitro-ethyl)-2*R*-phenyl-piperidine (2.48a)

According to general procedure A, starting from **2.33** to give **2.48a** (22 mg, 83%)

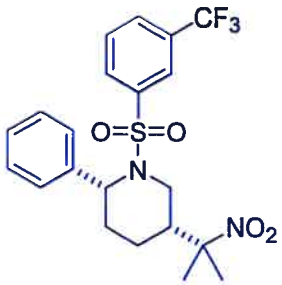
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26 (m, 4H), 7.09 (m, 5H), 4.25 (dd, J = 9.20, 5.56 Hz, 1H), 4.01 (dd, J = 12.06, 3.13 Hz, 1H), 3.02 (m, 1H), 2.58 (m, 2H), 1.91 (m, 2H), 1.70 (s, 3H), 1.63 (s, 3H), 1.31 (s, 9H), 1.30 (m, 1H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 163.9, 140.8, 131.1, 129.9, 128.3, 127.8, 127.5, 125.9, 61.4,

46.7, 44.1, 32.6. 31.5, 24.3, 23.6, 23.4

MS (M+1): 445.1, 398.2, 356.1, 307.1, 289.0, 247.1, 200.1, 173.1, 154.0, 136.0, 117.0

HRMS: $C_{24}H_{33}N_2O_4S$ (M+1); Calcd.: 445.2156; found: 445.2161

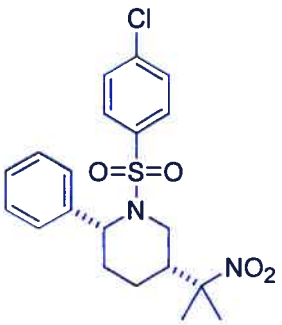


5*R*-(1-Methyl-1-nitro-ethyl)-2*R*-phenyl-1-(3-trifluoromethyl-benzenesulfonyl)piperidine (2.48b)

According to general procedure A, starting from **2.33** to give **2.48b** (23 mg, 81%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (d, J = 7.51 Hz, 2H), 7.53 (d, J = 7.80 Hz, 2H), 7.39 (m, 2H), 7.10 (m, 1H), 7.09 (m, 4H), 4.31 (dd, J = 9.71, 6.13 Hz, 1H), 4.08 (dd, J = 12.65, 4.71 Hz, 1H), 3.18 (dd, J = 12.78, 8.36 Hz 1H), 2.62 (m, 1H), 1.99 (m, 2H), 1.82 (m, 1H), 1.73 (s, 3H), 1.66 (s, 3h), 1.41 (m, 1H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 142.2, 138.7, 130.8, 129.6, 129.1, 128.8, 128.6, 128.4, 127.5, 124.5, 91.1, 62.3, 46.8, 44.4, 32.2, 24.2, 24.1, 23.5

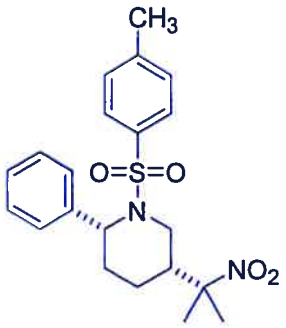


1-(4-Chloro-benzenesulfonyl)-5*R*-(1-methyl-1-nitro-ethyl)-2*R*-phenyl-piperidine (2.48c)

According to general procedure A, starting from **2.33** to give **2.48c** (25 mg, 81%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21 (m, 5H), 7.09 (m, 4H), 4.28 (dd, J = 9.94, 5.23 Hz, 1H), 3.99 (dd, J = 12.64, 4.79 Hz, 1H), 2.58 (m, 1H0, 1.94 (m, 1H), 1.72 (m, 1H), 1.70 (s, 3H), 1.64 (s, 3H), 1.39 (m, 1H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 161.9, 140.2, 131.0, 129.1, 128.9, 128.6, 128.4, 128.2, 91.3, 61.9, 45.5, 44.2, 32.5, 24.0, 23.8, 23.6,



5*R*-(1-Methyl-1-nitro-ethyl)-2*R*-phenyl-1-(toluene-4-sulfonyl)-piperidine (2.48d)

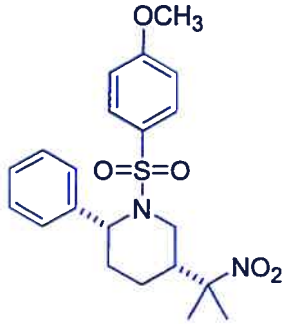
According to general procedure A, starting from **2.33** to give **2.48d** (24 mg, 81%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 (d, J = 8.84 Hz, 2H), 7.15 (m, 5H), 6.77 (d, J = 8.84 Hz, 2H), 4.18 (dd, J = 9.71, 6.13 Hz, 1H), 4.08 (dd, J = 9.04, 5.47 Hz, 1H), 3.95 (dd, J = 12.17, 4.29 Hz 1H), 3.83 (s, 3H), 2.88 (m, 1H), 2.52 (m, 1H), 1.90 (m, 2H), 1.68 (m, 1H), 1.67 (s, 3H), 1.61 (s, 3H), 1.39 (m, 1H).

^{13}C-NMR (400 MHz, $CDCl_3$): δ 143.1, 140.3, 136.7, 129.5, 128.7, 128.4, 128.1, 127.9, 91.3, 61.6, 46.6, 43.8, 32.9, 23.7, 22.4, 21.6.

MS (M+1): 403.1, 356.1, 307.1, 289.0, 247.1, 200.1, 184.1, 173.1, 154.1, 136.0, 117.0.

HRMS: $C_{21}H_{27}N_2O_4S$ (M+1); Calcd.: 403.1696; found: 403.1692.



1-(4-Methoxy-benzenesulfonyl)-5*R*-(1-methyl-1-nitro-ethyl)-2*R*-phenyl-piperidine (2.48e)

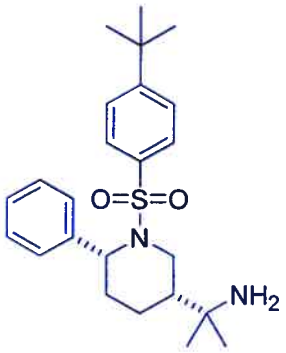
According to general procedure A, starting from **2.33** to give **2.48e** (27 mg, 79%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 (d, *J* = 8.93 Hz, 2H), 7.15 (m, 5H), 6.77 (d, *J* = 8.92 Hz, 2H), 4.18 (dd, *J* = 9.20, 5.56 Hz, 1H), 3.98 (dd, *J* = 12.06, 3.13 Hz, 1H), 3.84 (s, 3H), 2.88 (dd, J = 12.15, 9.34 Hz, 1H), 2.52 (m, 1H), 1.90 (m, 2H), 1.68 (m, 1H), 1.61 (s, 3H), 1.39 (m, 1H).

^{13}C-NMR (400 MHz, $CDCl_3$): δ 163.9, 140.8, 131.1, 129.9, 128.4, 128.2, 127.1, 91.0, 61.5, 56.0, 46.6, 44.0, 32.8, 24.3, 23.6, 23.5.

MS (M+1): 419.1, 372.1, 307.1, 289.0, 200.0, 171.0, 154.1, 136.0, 123.0.

HRMS: $C_{21}H_{27}N_2O_5S$ (M+1); Calcd.: 419.1641; found.: 419.1638.



(3*R*)-1-[1-(4-*tert*-Butyl-benzenesulfonyl)-6*R*-phenyl-piperidin-3-yl]-1-methyl-ethylamine (2.49a)

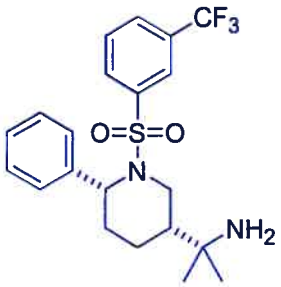
According to general procedure C, starting from **2.48a** to give **2.49a** (25 mg, 82%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37 (d, *J*= 8.46 Hz, 2H), 7.30 (d, *J* = 8.48 Hz, 2H), 7.17-7.11 (m, 5H), 4.26 (m, 1H), 4.05 (m, 1H), 2.30 (mk, 1H), 1.88 (m, 2H), 1.76 (m, 2H), 1.50 (br, 2H), 1.31 (s, 9H), 1.30 (m, 1H), 1.16 (s, 3H), 1.13 (s, 3H).

^{13}C-NMR (400 MHz, $CDCl_3$): δ 160.0, 141.6, 136.4, 128.2, 128.1, 127.6, 126.4, 125.9, 61.1, 46.7, 46.2, 35.4, 233.1, 31.5, 29.2, 28.4, 23.0.

MS (M+1): 415.2, 398.2, 307.1, 289.1, 226.1, 154.0, 136.0, 116.9.

HRMS: $C_{24}H_{34}N_2O_2S$ (M+1); Calcd.: 415.2419; found: 415.2419.



(3*R*-1-Methyl-1-[6*R*-phenyl-1-(3-trifluoromethyl-benzenesulfonyl)-piperidin-3-yl]ethyl-amine (2.49b)

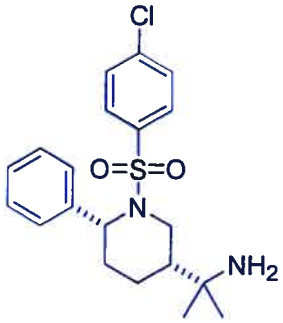
According to general procedure C, starting from **2.48b** to give **2.49b** (15 mg, 75%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (d, *J*= 7.51 Hz, 2H), 7.53 (d, *J*= 7.80 Hz, 2H), 7.39 (m, 2H), 7.10 (m, 1H), 7.09 (m, 4H), 4.31 (dd, *J*= 9.71, 6.13 Hz, 1H), 4.08 (dd, *J*= 12.65, 4.71 Hz, 1H), 3.18 (dd, *J*= 12.78, 8.36 Hz 1H), 2.62 (m, 1H), 1.99 (m, 2H), 1.82 (m, 1H), 1.73 (s, 3H), 1.66 (s, 3h), 1.41 (m, 1H).

^{13}C-NMR (400 MHz, $CDCl_3$): δ 161.3, 142.2, 138.7, 132.1, 129.58, 128.79, 128.57, 128.36, 91.14, 62.29, 46.76, 44.39, 32.25, 32.25, 24.17, 24.10, 23.52.

MS (M+1): 427.1, 238.0, 173.1, 136.0, 116.9.

HRMS: $C_{21}H_{26}F_3N_2O_2S$ (M+1); Calcd.: 427.1654; found: 427.1667.



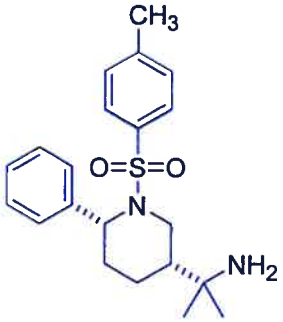
3*R*-1-[1-(4-Chloro-benzenesulfonyl)-6*R*-phenyl-piperidin-3-yl]-1-methyl-ethylamine (2.49c)

According to general procedure C, starting from **2.48c** to give **2.49c** (18 mg, 77%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47 (d, J = 7.86 Hz, 2H), 7.34 (d, J = 7.50 Hz, 2H), 7.20 (m, 5H), 4.27 (dd, J = 14.1, 7.09 Hz, 1H), 4.02 (dd, J = 14.0, 7.68 Hz, 1H), 2.96 (dd, J = 11.8, 9.31 Hz, 1H), 1.88 (m, 2H), 1.76 (m, 2H), 1.56 (br, 2H), 1.31 (m, 1H), 1.15 (s, 3H), 1.13 (s, 3H).

MS (M+1): 393.1, 307.1, 289.0, 154.0, 137.0, 119.9.

HRMS: $C_{20}H_{26}ClN_2O_2S$ (M+1); Calcd.: 393.1404; found: 393.1394.



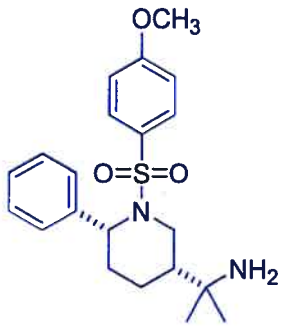
3*R*-1-Methyl-1-[6*R*-phenyl-1-(toluene-4-sulfonyl)-piperidin-3-yl]-ethylamine, 2.49d

According to general procedure C, starting from **2.48d** to give **2.49d** (18mg, 76%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 (d, J= 8.84 Hz, 2H), 7.15 (m, 5H), 6.77 (d, J = 8.84 Hz, 2H), 4.18 (dd, J = 9.71, 6.13 Hz, 1H), 4.08 (dd, J = 9.04, 5.47 Hz, 1H), 3.95 (dd, J = 12.17,

4.29 Hz 1H), 3.83 (s, 3H), 2.88 (m, 1H), 2.52 (m, 1H), 1.90 (m, 2H), 1.68 (m, 1H), 1.67 (s, 3H), 1.61 (s, 3H), 1.39 (m, 1H).

HRMS: $C_{21}H_{29}ClN_2O_2S$ (M+1); Calcd.: 373.5248; found: 373.52463.



3*R*-1-[1-(4-Methoxy-benzenesulfonyl)-6*R*-phenyl-piperidin-3-yl]-1-methyl-ethylamine, 2.49e

According to general procedure C, starting from **2.48e** to give **2.49e** (19 mg, 78%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 (d, J = 8.93 Hz, 2H), 7.15 (m, 5H), 6.77 (d, J = 8.92 Hz, 2H), 4.18 (dd, J = 9.20, 5.56 Hz, 1H), 3.98 (dd, J = 12.06, 3.13 Hz, 1H), 3.84 (s, 3H), 2.88 (dd, J = 12.15, 9.34 Hz, 1H), 2.52 (m, 1H), 1.90 (m, 2H), 1.68 (m, 1H), 1.61 (s, 3H), 1.39 (m, 1H).

^{13}C-NMR (400 MHz, $CDCl_3$): δ 162.8, 141.9, 130.8, 130.0, 128.3, 127.9, 127.6, 114.1, 61.1, 55.9, 52.0, 46.9, 46.0, 33.2, 29.1. 28.3, 23.1.

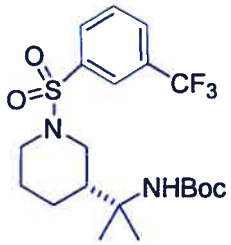
MS (M+1): 389.2, 307.1, 200.0, 154.0, 132.8, 116.9.

HRMS: $C_{21}H_{29}N_2O_3S$ (M+1); Calcd.: 389.1896; found: 389.1899.

3*R*-{1-[1-(4-*tert*-Butyl-benzenesulfonyl)-piperidin-3-yl]-1-methyl-ethyl}-carbamic acid *tert*-butyl ester (2.50a)

According to general procedure A, starting from **2.39** to give **2.50a** (25 mg, 82%).

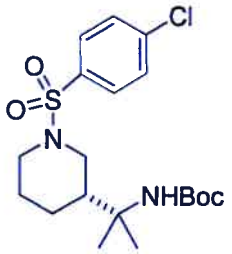
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (d, J = 8.41 Hz, 2H), 7.52 (d, J = 8.41 Hz, 2H), 4.01 (m, 1H), 3.79 (m, 1H), 2.4 (s, 2H), 2.13 (m, 1H), 2.03 (m, 1H), 1.88 (m, 1H), 1.77 (m, 1H), 1.61 (m, 2H), 1.13 (s, 3H), 1.11 (s, 3H), 0.98 (m, 1H).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 163.33, 130.14, 128.71, 114.52, 55.97, 54.45, 48.20, 46.85, 43.93, 28.83, 25.58, 25.50, 25.33.



3*R*-{1-Methyl-1-[1-(3-trifluoromethyl-benzenesulfonyl)-piperidin-3-yl]-ethyl}-carbamic acid tert-butyl ester (2.50b)

According to general procedure A, starting from **2.39** to give **2.50b** (25 mg, 82%).

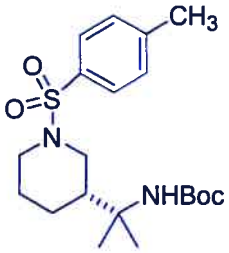
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (s, 1H), 8.0 (d, J = 7.89 Hz,1H), 7.86 (d, J = 7.89 Hz, 2H), 7.70 (d, J = 7.82, 7.87 Hz, 1H), 4.05 (m, 1H), 3.84 (m, 1H), 2.45 (s, 2H), 2.17 (m, 1H), 2.08 (m, 1H), 1.90 (m, 1H), 1.82 (m, 1H), 1.60 (m, 2H), 1.38 (s, 9H), 1.13 (s, 3H), 1.10 (s, 3H), 0.99 (m, 1H).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 139.0, 131.2, 130.3, 129.65, 124.9, 48.1, 79.8, 46.9, 46.7, 28.8, 28.1, 25.4, 25.2.



3*R*-{1-[1-(4-Chloro-benzenesulfonyl)-piperidin-3-yl]-1-methyl-ethyl}-carbamic acid *tert*-butyl ester (2.50c)

According to general procedure A, starting from **2.39** to give **2.50c** (25 mg, 82%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (d, J = 7.96 Hz,1H), 7.55 (d, J = 7.89 Hz, 2H), 4.02 (m, 1H), 3.78 (m, 1H), 3.78 (s, 2H), 3.78 (brs, 2H), 2.02 (m, 2H), 1.89 (m, 1H), 1.80 (m, 1H), 1.64 (m, 2H), 1.82 (m, 1H), 1.39 (s, 9H), 1.17 (s, 3H), 1.14 (s, 3H), 0.99 (m, 1H)
MS (M+1): 417.1, 361.1, 343.1, 300.1, 241.2, 154.0, 136.0, 120.0
HRMS: $C_{19}H_{29}ClN_2O_4S$ (M+1); Calcd.: 417.1615; found: 417.1598



3*R*-{1-Methyl-1-[1-(toluene-4-sulfonyl)-piperidin-3-yl]-ethyl}-carbamic acid *tert*-butyl ester (2.50d)

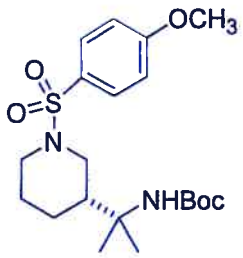
According to general procedure A, starting from **2.39** to give **2.50d** (25 mg, 82%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (d, J = 8.0 Hz, 2H), 7.21 (d, J = 7.84 Hz, 2H), 3.89 (m, 1H), 3.81 (m, 1H), 3.4-2.8 (s, 2H), 2.43 (s, 3H), 2.09 (dd, J = 12.0, 9.39, Hz, 1H), 1.99 (m, 1H), 1.87 (m, 1H), 1.78 (m, 1H), 1.59 (m, 2H), 1.37 (s, 9H), 1.12 (s, 3H), 1.11 (s, 3H), 0.96 (m, 1H).

^{13}C-NMR (400 MHz, $CDCl_3$): δ 143.8, 133.7, 130.1, 128.1, 51.5, 80.8, 48.7. 46.9, 46.6, 28.4, 27.9, 25.4, 25.2, 21.2.

MS (M+1): 397.2, 341.1, 307.1, 280.1, 241.2, 154.0, 136.0, 123.0.

HRMS: $C_{20}H_{32}N_2O_4S$ (M+1); Calcd.: 397.2161; found: 397.2160.



3*R*-{1-[1-(4-Methoxy-benzenesulfonyl)-piperidin-3-yl]-1-methyl-ethyl}-carbamic acid *tert*-butyl ester (2.50e)

According to general procedure A, starting from **2.39** to give **2.50e** (25 mg, 82%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (d, J = 8.87 Hz, 2H), 7.0 (d, J = 8.90 Hz, 2H), 4.04 (m, 1H), 3.87 (s, 3H), 3.76 (m, 1H), 2.03 (m, 2H), 1.84 (m, 1H), 1.78 (m, 1H), 1.62 (m, 1H), 1.38 (s, 9H), 1.25 (s, 3H), 1.23 (s, 1H), 0.98 (m, 1H).

^{13}C-NMR (400 MHz, $CDCl_3$): δ 163.28, 130.23, 128.21, 114.62, 56.0, 48.09, 46.88, 44.0, 25.26, 25.22.

MS (M+1): 413.1, 357.1, 339.1, 313.1, 296.1, 289.1, 241. 214.0, 185.1, 171.0, 154.1, 136..0, 124.1.

HRMS: $C_{20}H_{33}N_2O_5S$ (M+1); Calcd.: 413.2110; found: 413.2101.

3*R*-1-[1-(4-*tert*-Butyl-benzenesulfonyl)-piperidin-3-yl]-1-methyl-ethylamine (2.51a)

According to general procedure A, starting from **2.50a** to give **2.51a** (25 mg, 82%).

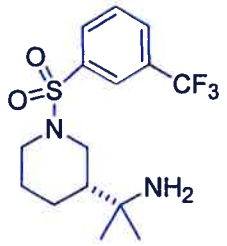
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (d, J = 8.41 Hz, 2H), 7.52 (d, J = 8.41 Hz, 2H), 4.01 (m, 1H), 3.79 (m, 1H), 2.4 (s, 2H), 2.13 (m, 1H), 2.03 (m, 1H), 1.88 (m, 1H), 1.77 (m, 1H), 1.61 (m, 2H), 1.35 (s, 9H), 1.13 (s, 3H), 1.11 (s, 3H), 0.98 (m, 1H).

^{13}C-NMR (400 MHz, $CDCl_3$): δ 163.3, 130.1, 128.7, 114.5, 56.0, 55.9, 54.5, 48.2, 46.9, 43.9, 28.8, 25.6, 25.5, 25.3.

163.33, 130.14, 128.71, 114.52, 86.02, 55.97, 54.45, 48.20, 46.85, 43.93, 28.83, 25.58, 25.50, 25.33.

MS (M+1): 339.2, 322.2, 307.1, 289.1, 154.0, 136.0, 124.0.

HRMS: $C_{18}H_{31}N_2O_2S$ (M+1); Calcd.: 339.2124; found: 339.2106.



3*R*-1-Methyl-1-[1-(3-trifluoromethyl-benzenesulfonyl)-piperidin-3-yl]-ethylamine, 2.51b

According to general procedure D, starting from **2.50b** to give **2.51b** (25 mg, 82%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (s, 1H), 8.0 (d, J = 7.89 Hz,1H), 7.86 (d, J = 7.89 Hz, 2H), 7.70 (d, J = 7.82, 7.87 Hz, 1H), 4.05 (m, 1H), 3.84 (m, 1H), 2.45 (s, 2H), 2.17 (m, 1H), 2.08 (m, 1H), 1.90 (m, 1H), 1.82 (m, 1H), 1.60 (m, 2H), 1.13 (s, 3H), 1.10 (s, 3H), 0.99 (m, 1H).

^{13}C-NMR (400 MHz, $CDCl_3$): δ 165.1, 139.0, 131.2, 130.3, 129.6, 124.9, 52.1, 48.1, 46.9, 46.7, 28.8, 28.1, 25.4, 25.2.

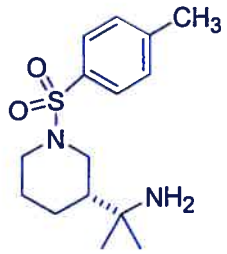
MS (M^+): 350.1, 338.2.

HRMS: $C_{15}H_{21}F_3N_2O_2S$ (M^+); Calcd: 350.1276; found: 350.1282.

3*R*-{1-[1-(4-Chloro-benzenesulfonyl)-piperidin-3-yl]-1-methyl-ethylamine (2.51c)

According to general procedure D, starting from **2.50c** to give **2.51c** (25 mg, 82%).

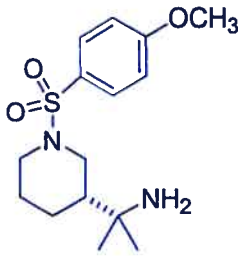
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (d, J = 7.96 Hz,1H), 7.55 (d, J = 7.89 Hz, 2H), 4.02 (m, 1H), 3.78 (m, 1H), 3.78 (s, 2H), 3.78 (brs, 2H), 2.02 (m, 2H), 1.89 (m, 1H), 1.80 (m, 1H), 1.64 (m, 2H), 1.82 (m, 1H), 1.17 (s, 3H), 1.14 (s, 3H), 0.99 (m, 1H).
MS (M+1): 317.1, 307.1, 289.1, 54.0, 136.0.
HRMS: $C_{14}H_{21}ClN_2O_2S$ (M+1); Calcd.: 317.1091; found: 317.1090.



3*R*-{1-Methyl-1-[1-(toluene-4-sulfonyl)-piperidin-3-yl]-ethylamine (2.51d)

According to general procedure D, starting from **2.50d** to give **2.51d** (25 mg, 82%).

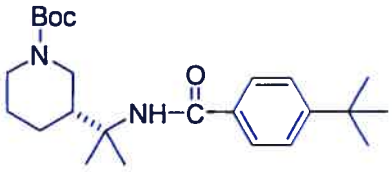
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (d, J = 8.10 Hz, 2H), 7.32 (d, J = 7.92 Hz, 2H), 3.98 (m, 1H), 3.78 (m, 1H), 3.4-2.8 (s, 2H), 2.43 (s, 3H), 2.09 (dd, J = 12.0, 9.39, Hz, 1H), 1.99 (m, 1H), 1.87 (m, 1H), 1.78 (m, 1H), 1.59 (m, 2H), 1.12 (s, 3H), 1.11 (s, 3H), 0.96 (m, 1H).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 143.8, 133.7, 130.1, 128.1, 51.5, 48.1, 46.9, 46.6, 28.4, 28.0, 25.4, 25.2, 21.9.
HRMS: $C_{15}H_{24}N_2O_2S$ (M^+); Calcd: 296.1559; found: 296.1569.



3*R*-1-[1-(4-Methoxy-benzenesulfonyl)-piperidin-3-yl]-1-methyl-ethylamine (2.51e)

According to general procedure D, starting from **2.50e** to give **2.51e** (25 mg, 82%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, J = 8.87 Hz, 2H), 7.35 (d, J = 8.90 Hz, 2H), 4.04 (m, 1H), 3.85 8 (m, 1H), 3.4-2.8 (s, 2H), 2.43 (s, 3H), 2.09 (dd, J = 12.0, 2.3, Hz, 1H), 1.99 (m, 1H), 1.87 (m, 1H), 1.78 (m, 1H), 1.59 (m, 2H), 1.12 (s, 3H), 1.11 (s, 3H), 0.96 (m, 1H).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 16.2, 130.2, 128.2, 114.6, 56.0, 48.1, 46.9, 45.7, 29.3, 28.7, 25.3, 25.2, 21.2.
MS (M+1): 313.1, 296.1, 214.0, 171.1, 132.8, 124.0.
HRMS: $C_{15}H_{24}N_2O_3S$ (M+1); Calcd.: 312.1508; found: 312.1504.

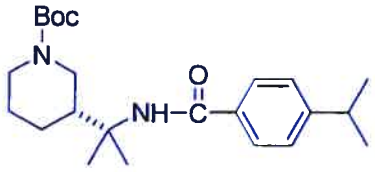


3*R*-[1-(4-*tert*-Butyl-benzoylamino)-1-methyl-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (2.52f)

According to general procedure B, starting from **2.42** to give **2.52f** (27 mg, 81%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (d, J = 8.15, 2H), 7.25 (d, J = 8.18, 2H), 5.88 (s, 1H), 4.25 (s, 2H), 4.05 (m, 1H), 2.94 (m, 1H), 2.53(m, 2H), 2.30 (m, 1H), 1.95 (m, 1H), 1.67 ((m, 1H), 1.45 (s, 6H), 1.40 (s, 9H), 1.24 (s, 9H), 1.20 (m, 1H).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 169.36, 155.86, 134.14, 128.2, 126.21, 59.02, 46.83, 43.12, 35.63, 31.57, 26.59, 26.38, 24.64, 24.64.

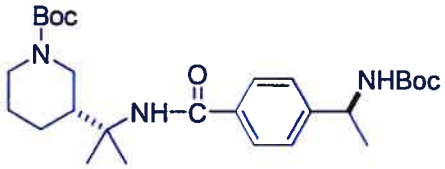
HRMS: $C_{24}H_{38}N_2O_3$ (M+1); Calcd.: 402.2882; found: 402.2887.



3*R*-[1-(4-Isopropyl-benzoylamino)-1-methyl-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (2.52g)

According to general procedure B, starting from **2.42** to give **2.52g** (25 mg, 82%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (d, J = 7.99 Hz, 2H), 7.30 (d, J = 8.26 Hz, 2H), 4.19 (m, 1H), 4.05 (s, 1H), 2.95 (m, 1H), 2.59-2.32 (m, 3H), 1.87 (m, 1H), 1.1.72 (m, 1H)
^{13}C-NMR (400 MHz, $CDCl_3$): δ
HRMS: $C_{23}H_{36}N_2O_3$ (M+1); Calcd.: 388.2726; found: 388.2729

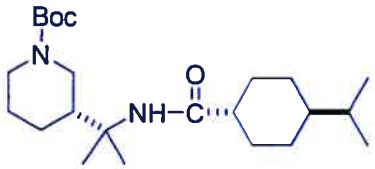


3*R*-{1-[4-(1*S*-*tert*-Butoxycarbonylamino-ethyl)-benzoylamino]-1-methyl-ethyl}-piperidine-1-carboxylic acid *tert*-butyl ester (2.52 h)

According to general procedure B, starting from **2.42** to give **2.52h** (25 mg, 81%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (d, J = 8.48, 2H), 7.31 (d, J = 8.12, 2H), 5.90 (s, 1H), 4.95 (m, 1H), 4.76 (m, 1H), 4.11, (m, 2H), 2.50 (m, 2H), 2.22 (m, 1H), 1.87 (m, 1H), 1.67 (m, 1H), 1.42 (s, 3H), 1.39 (m, 22H), 1.20 (m, 2H), 3H), 1.14 (s, 3H), 1.29 (m, 2H)
^{13}C-NMR (400 MHz, $CDCl_3$): δ 166.98, 155.42, 155.19, 148.12, 134.87, 127.45, 126.29, 79.97, 79.77m 66.25 (60.81), 56.21 (50.39), 44.36 (43.55), 28.8, 26.2, 25.85, 24.98, 24.75, 23.11, 15.67 (14.61)
MS (M+1): 490.1, 434.2, 388.2, 334.2, 307.1, 209.1, 192.1, 154.0, 136.0

HRMS: $C_{27}H_{44}N_3O_5$ (M+1); Calcd.: 490.3281; found: 490.3295



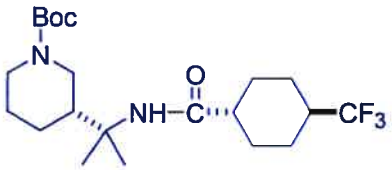
3*R*-{1-[(*trans*-4-Isopropyl-cyclohexanecarbonyl)-amino]-1-methyl-ethyl}-piperidine-1-carboxylic acid *tert*-butyl ester (2.52i)

According to general procedure B, starting from 2.42 to give 2.52i (21 mg, 82%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.23 (m, 1H), 4.10 (m, 2H), 2.55 (m, 1H), 2.43 (m, 1H), 2.19-2.1.89 (M, 7H), 1.80 (m, 1H), 1.68 (m, 1H), 1.54 (m, 3H), 1.43 (s, 9H), 1.35 (m, 2H), 1.31 (s, 3H), 1.28 (s, 3H), 1.16 (m, 1H),

^{13}C-NMR (400 MHz, $CDCl_3$): δ 175.97, 155.24, 79.74, 55.35, 46.96, 43.61, 43.45, 33.17, 30.41, 30.32, 29.39, 28.86, 26.04, 25.81, 25.01, 24.74, 20.14

HRMS: $C_{23}H_{42}N_2O_3$ (M+1); Calcd.: 394.3195; found: 394.3191



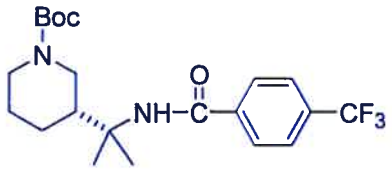
3*R*-{1-Methyl-1-[(*trans*-4-trifluoromethyl-cyclohexanecarbonyl)-amino]-ethyl}-piperidine-1-carboxylic acid *tert*-butyl ester (2.52j)

According to general procedure B, starting from **2.42** to give **2.52j** (23 mg, 81%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.23 (m, 1H), 4.10 (m, 2H), 2.55 (m, 1H), 2.43 (m, 1H), 2.19-2.1.89 (M, 7H), 1.80 (m, 1H), 1.68 (m, 1H), 1.54 (m, 3H), 1.43 (s, 9H), 1.35 (m, 2H), 1.31 (s, 3H), 1.28 (s, 3H), 1.16 (m, 1H),

^{13}C-NMR (400 MHz, $CDCl_3$): δ 174.71, 155.25, 79.81, 55.85, 55.64, 45.7343.55, 41.68, 41.42, 28.86, 28.52, 28.45, 26.05, 25.82, 25.03, 24.95, 24.61, 23.99

HRMS: $C_{21}H_{35}F_2N_2O_3$ (M+1); Calcd.: 420.5094; found: 420.5089



3*R*-[1-Methyl-1-(4-trifluoromethyl-benzoylamino)-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (2.52k)

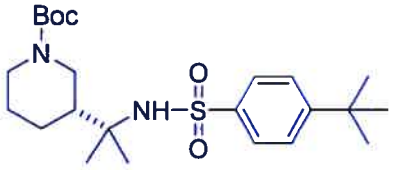
According to general procedure B, starting from **2.42** to give **2.52k** (24 mg, 84%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (d, J = 7.76 2H), 7.67 (d, J = 7.88, 2H), 6.07 (s, 1H), 4.20 (s, 1H), 4.07 (m, 1H), 2.55 (m, 2H), 2.22 (m, 1H), 1.87 (m, 1H), 1.72 (m, 1H), 1.46 (s, 3H), 1.43 (s, 3H), 1.41 (s, 9H), 1.23 (m, 2H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 165.97, 155.23, 139.44, 127.63, 126.02, 125.97, 125.92,79.9, 56.7, 43.62, 28.8, 26.19, 25.83, 24.98, 24.5

MS (M+1): 415.2, 359.1, 341.1, 31501, 231.1, 190.1, 168.1, 154.0, 136.0, 124.1

HRMS: $C_{21}H_{28}F_3N_2O_3$ (M+1); Calcd.: 415.2052; found: 415.2040



3*R*-[1-(4-*tert*-Butyl-benzenesulfonylamino)-1-methyl-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (2.55l)

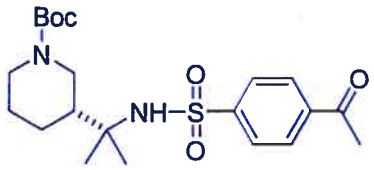
According to general procedure A, starting from **2.42** to give **2.55l** (23 mg, 79%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (d, J = 8.27, 2H), 7.46 (d, J = 8.49, 2H), 4.76 (s, 1H), 4.18 (s, 1H), 4.05 (m, 1H), 2.50 (m, 1H), 2.37, 1.87 (m, 2H), 1.64 ((m, 1H), 1.53 (m, 1H), 1.46 (s, 9H), 1.33 (s, 9H), 1.19 (s, 3H), 1.15 (s, 3H),

^{13}C-NMR (400 MHz, $CDCl_3$): δ 156.31, 155.22, 140.87, 127.07, 126.29, 79.84, 79.74, 59.76, 58.78, 46.59, 35.48, 31.48, 28.84, 27.02, 25.74, 25.06

MS (M+1): 439.2, 383.2, 365.2, 339.2, 307.1, 289.0, 254.1, 197.1, 170.1, 154.0, 136.0, 126.1

HRMS: $C_{23}H_{39}N_2O_4S$ (M+1); Calcd.: 439.2631; found: 439.2641



3*R*-[1-(4-Acetyl-benzenesulfonylamino)-1-methyl-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (2.55m)

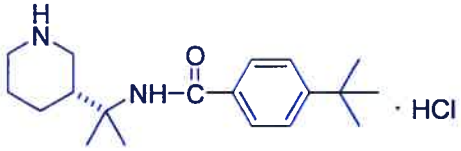
According to general procedure A, starting from **2.42** to give **2.55m** (11 mg, 81%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (d, J = 8.48, 2H), 7.97 (d, J = 8.52, 2H), 5.42 (s, 1H), 2.64 (s, 3H), 2.52 (m, 1H), 2.36 (m, 1H), 1.87 (m, 1H), 1.60 (m, 2H), 1.16 (s, 3H), 1.14 (s, 3H), 1.29 (m, 2H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 197.33, 155.19, 155.0 147.87, 139.96, 129.29, 127.52, 79.93, 79.84, 60.17, 59.29, 46.57, 28.82, 27.3, 26.95, 25.7, 25.17, 25.02

MS (M+1): 425.1, 369.1, 351.1, 325.1, 240.0, 170.1, 154.0, 126.1

HRMS: $C_{21}H_{33}N_2O_5S$ (M+1); Calcd.: 425.2110; found: 425.2106



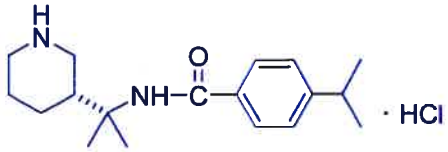
4-*tert*-Butyl-*N*-(1-methyl-1-piperidin-3*R*-yl-ethyl)-benzamide hydrochloride (2.54f)

According to general procedure D and E, starting from **2.52f** to give **2.54f** (11 mg, 84%)

^{1}H NMR (400 MHz, CD_3OD): δ 7.68 (d, J = 8.45, 2H), 7.48 (d, J = 8.50, 2H), 3.17 (m, 1H), 3.07 (m, 1H), 2.57 (m, 2H), 1.85 (m, 2H), 1.56 (m, 11.33 (m, 1H)

^{13}C-NMR (400 MHz, CD_3OD): δ 169.36, 154.96, 133.14, 127.14, 125.34, 56.02, 45.59, 42.65, 34.73, 30.56, 25.49, 25.38, 23.7, 23.59

HRMS: $C_{19}H_{30}N_2O$ (M+1); Calcd.: 302.2358; found: 302.2354



4-Isopropyl-*N*-(1-methyl-1-piperidin-3*R*-yl-ethyl)-benzamide hydrochloride (2.54g)

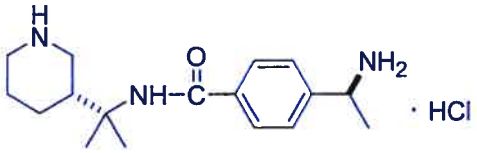
According to general procedure D and E, starting from **2.52g** to give **2.54g** (17 mg, 81%)

^{1}H NMR (400 MHz, CD_3OD): δ 7.87 (d, J = 7.83 Hz, 2H), 7.63 (d, J = 7.90 Hz, 2H), 6.21 (s, 1H), 3.49 (m, 1H), 3.31 (m, 1H), 2.89 (m, 3H), 1.88 (m, 1H), 1.43 (s, 3H), 1.38 (s, 3H), 1.24 (d, J = 6.35 Hz, 6H). 1.20 (m, 2H)

^{13}C-NMR (400 MHz, CD_3OD): δ 167.92, 153.23, 132.99, 127.44, 127.07, 66.29, 55.88, 45.93, 44.18, 41.51, 34.46, 25.25, 24.59, 24.45, 24.19, 23.09. 15.69

MS: (M+1) 289.2, 206.1, 125.1, 84.1

HRMS: $C_{18}H_{29}N_2O$ (M+1); Calcd.: 289.22154; found: 289.2156



4-(1*S*-Amino-ethyl)-*N*-(1-methyl-1-piperidin-3*R*-yl-ethyl)-benzamide hydrochloride (2.54h)

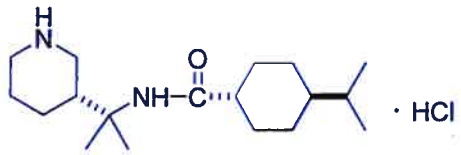
According to general procedure D and E, starting from **2.52h** to give **2.54h** (9 mg, 81%)

^{1}H NMR (400 MHz, CD_3OD): δ 7.72 (d, J = 8.32 Hz, 2H), 7.44 (d, J = 8.15, 2H), 4.09 (q, J = 6.60 Hz, 1H), 3.12 (m, 1H), 3.20 (m, 1H), 2.51 (m, 3H), 1.83 (m, 2H), 1.53 (m, 1H), 1.39 (m. 9H), 1.1.33 (m, 1H)

^{13}C-NMR (400 MHz, CD_3OD): δ 167.92, 153.23, 132.99, 127.44, 127.07, 66.29, 55.88, 45.93, 44.18, 41.51, 34.46, 25.25, 24.59, 24.45, 24.19, 23.09. 15.69

MS (M+1): 289.2, 206.1, 125.1, 84.1

HRMS: $C_{17}H_{27}N_3O$ (M+1); Calcd.: 289.2154; found: 289.2156



***trans*-4-Isopropyl-cyclohexanecarboxylic acid (1-methyl-1-piperidin-3*R*-yl-ethyl)-amide hydrochloride (2.54i)**

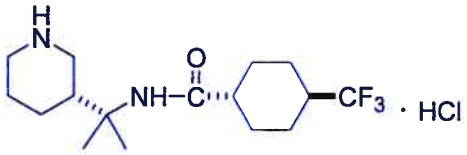
According to general procedure D and E, starting from **2.52i** to give **2.54i** (13 mg, 82%)

^{1}H NMR (400 MHz, CD_3OD): δ 3.0 (m, 2H), 2.42 (m, 2H), 2.25 (m, 1H), 2.1 (m, 1H), 1.76 (m, 6H), 1.45 (m, 4H), 1.25 (m, 7H), 1.12 (m, 3H), 0.88 (d, J = 6.78 Hz 6H)

^{13}C-NMR (400 MHz, CD_3OD): δ 177.69, 55.09, 46.0, 43.81, 43.37, 33.11, 29.97, 29.16, 26.39, 25.73, 23.74, 23.61, 19.15

MS (M^+): 294.3, 211.2, 125.1, 98.1

HRMS: $C_{18}H_{34}N_2O$ (M^+); Calcd.: 294.2671; found: 294.2678



***trans*-4-(Trifluoromethyl)cyclohexanecarboxylic acid (1-methyl-1-piperidin-3*R*-yl-ethyl)amide hydrochloride (2.54j)**

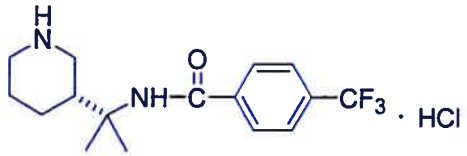
According to general procedure D and E, starting from **2.42j** to give **2.54j** (15 mg, 83%)

^{1}H NMR (400 MHz, CD_3OD): δ 3.31 (m, 1H), 3.04 (m, 2H), 2.47 (m, 2H), 2.27 (m,. 1H), 2.15 (m, 2H0, 1.98 (m, 2H), 1.84 (m, 4H), 1.49 (m, 3H), 1.38-1.30 (m, 6H), 1.26 (s, 6H)

^{13}C-NMR (400 MHz, CD_3OD): δ 166.59, 55.14, 45.79, 44.72, 43.02, 41.47, 41.21, 28.08, 25.92, 25.48, 24.35, 23.59

MS (M^+): 320.2, 318.2, 236.1, 125.1, 84.1

HRMS: $C_{16}H_{27}F_3N_2O$ (M^+): Calcd.: 320.2075; found: 320. 2083



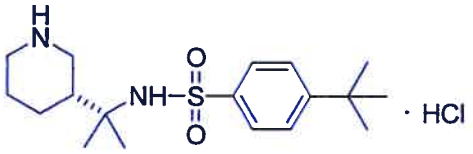
_N_-(1-Methyl-1-piperidin-3*R*-yl-ethyl)-4-trifluoromethyl-benzamide hydrochloride, (2.54k)

According to general procedure D and E, starting from **2.52k** to give **2.54k** (15 mg, 83%)

^{1}H NMR (400 MHz, CD_3OD): δ 7.87 (d, J = 8.12, 2H), 7.65 (d, J = 8.28 Hz, 2H), 3.12 (m, 1H), 3.01 (m, 1H), 2.48 (m, 3H), 1.83 (m, 2H), 1.53 (m, 1H), 1.45 (S, 3H), 1.40 (s, 3H). 1.32 (m, 1H),

^{13}C-NMR (400 MHz, CD_3OD): δ 168.45, 138.90, 128.04, 127.73, 125.85, 56.33, 45.95, 44.60, 44.118, 41.78, 25.18, 24.72, 24.48, 23.69, 23.09

HRMS: $C_{16}H_{21}F_3N_2O$ (M^+); Calcd.: 314.1612; found: 314.1607



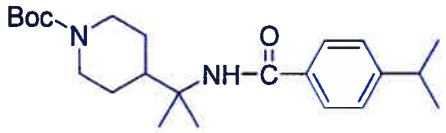
4-*tert*-Butyl-_N_-(1-methyl-1-piperidin-3*R*-yl-ethyl)-benzenesulfonamide hydrochloride (2.57l)

According to general procedure D and E, starting from **2.55l** to give **2.57l** (18 mg, 86%)

^{1}H NMR (400 MHz, CD_3OD): δ 3.14 (m, 2H), 2.47 (m, 2H), 2.26 (m, 1H), 2.14 (m, 1H), 1.99 (m, 2H), 1.82 (m, 4H), 1.49 (m, 3H), 31.38-1.26 (m, 9H)

^{13}C-NMR (400 MHz, CD_3OD): δ 166.59, 55.14, 45.79, 44.72, 43.02, 41.47, 28.08, 25.92, 25.48, 24.35, 23.59

HRMS: $C_{18}H_{31}N_2O_2S$ (M+1); Calcd.: 339.2104; found: 339.2101



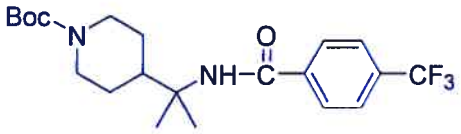
4-[1-(4-Isopropyl-benzoylamino)-1-methyl-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (2.58g)

According to general procedure B, starting from **2.45** to give **2.58g** (24 mg, 81%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63 (d, J = 8.26, 2H), 7.24 (d, J = 8.15, 2H), 5.87 (s, 1H), 4.13 (m, 1H), 2.91 (m, 1H), 2.64 (m, 2H), 2.43 (m, 2H), 1.65 (m, 2H),

^{13}C-NMR (400 MHz, $CDCl_3$): 167.15, 155.13, 152.89, 133.57, 127.19, 127.0, 79.79, 56.85, 44.54, 42.75, 34.44, 34.44, 28.86, 27.26, 24.86, 24.20

HRMS: $C_{23}H_{35}N_2O_3$ (M-1); Calcd.: 387.2648; found: 387.2637



4-[1-Methyl-1-(4-trifluoromethyl-benzoylamino)-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (2.58k)

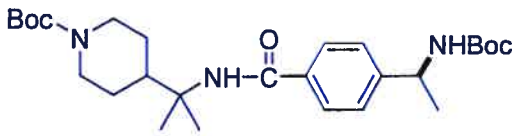
According to general procedure B, starting from **2.45** to give **2.58k** (26 mg, 80%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (d, J = 8.12, 2H), 7.65 (d, J = 8.20, 2H), 5.76 (s, 1H), 4.16 (m, 2H), 2.91 (m, 1H), 2.65 (m, 2H), 2.41 (m, 1H), 1.64 (m, 2H), 1.43 (s, 9H), 1.38 (s, 6H), 1.21 (m, 2H)

^{13}C-NMR (400 MHz, $CDCl_3$): 165.9, 155.1, 139.3, 127.6, 126.0, 125.9, 79.8, 57.4, 46.1, 45.7, 44.8, 42.8, 28.8, 27.3, 24.8

MS (M+1): 415.2, 407.3, 389.2,

HRMS: $C_{21}H_{28}N_2O_3F_3$ (M+1); Calcd.: 415.2209; found: 415.2199



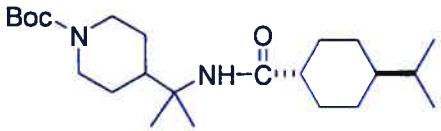
4-{1-[4-(1*S*-*tert*-Butoxycarbonylamino-ethyl)-benzoylamino]-1-methyl-ethyl}-piperidine-1-carboxylic acid *tert*-butyl ester (2.58h)

According to general procedure B, starting from **2.45** to give **2.58h** (21 mg, 81%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (d, J = 8.04 Hz, 2H), 7.34 (d, J = 7.73 Hz, 2H), 5.84 (s, 1H), 4.83 (m, 2H), 4.10 (m, 2H), 2.66 (m, 1H), 1.66 (m, 2H), 1.43 (s, 18H), 1.41 (d, J = 7.61 Hz, 3H), 1.36 (s, 6H), 1.21 (m, 2H)

^{13}C-NMR (400 MHz, $CDCl_3$): 167.0, 155.4, 155.1, 148.2, 134.8, 127.4, 126.3, 80.0 , 79.73, 66.27, 26.97, 50.41, 42.79, 28.87, 28.76, 27.27, 24.85, 23.12, 15.69

HRMS: $C_{27}H_{42}N_3O_5$ (M-1); Calcd.: 489.3124; found: 488.3125



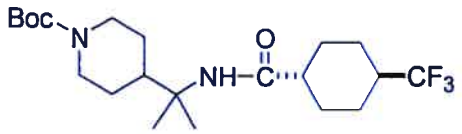
4-{1-[(*trans*-4-Isopropyl-cyclohexanecarbonyl)-amino]-1-methyl-ethyl}-piperidine-1-carboxylic acid *tert*-butyl ester (2.58i)

According to general procedure B, starting from **2.45** to give **2.58i** (25 mg, 82%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.23 (s, 1H), 4.07 (m, 2H), 2.59 (m, 2H), 2.24 (m, 1H), 1.79 (m, 5H), 1.54 (m, 2H), 1.40 (s, 9H), 1.32 (m, 3H), 1.20 (s, 6H), 1.16-0.84 (m, 5H), 0.80 (d, J = 6.78 Hz, 6H),

^{13}C-NMR (400 MHz, $CDCl_3$): 175.9, 155.1, 79.6, 56.0, 46.9, 44.9, 44.2, 43.6, 42.6, 33.1, 30.4, 29.4, 28.8, 27.2, 24.8, 20.1

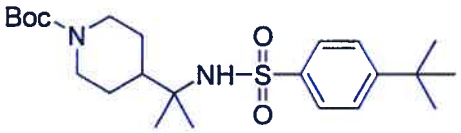
HRMS: $C_{23}H_{42}N_2O_3$ (M+1); Calcd.: 394.5912; found: 394.5917



4-{1-Methyl-1-[(*trans*-4-trifluoromethyl-cyclohexanecarbonyl)amino]-ethyl}-piperidine-1-carboxylic acid *tert*-butyl ester (2.58j)

According to general procedure B, starting from **2.45** to give **2.58j** (26 mg, 81%)

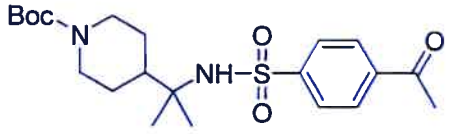
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.30 (s, 1H), 4.08 (m, 2H), 2.59 (m, 2H), 2.23 (m, 1H), 1.87 (m, 6H), 1.52 (m, 4H), 1.41 (s, 9H), 1.28 (m, 2H), 1.22 (s, 6H), 1.12 (m, 2H)m, 2H).
^{13}C-NMR (**400** MHz, $CDCl_3$): 174.7, 155.1, 79.71, 56.29, 45.58, 42.64, 28.46, 24.75,
HRMS: $C_{21}H_{35}F_3N_2O_3$ (M^+); Calcd.: 420.2612; found: 420.2619.



4-[1-(4-*tert*-Butyl-benzenesulfonylamino)-1-methyl-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (2.61l)

According to general procedure A, starting from **2.45** to give **2.61l** (25 mg, 79%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (d, J = 8.7, 2H), 7.48 (d, J = 8.66, 2H), 4.98 (s, 1H), 4.09 (s, 1H), 2.49 (m, 2H), 1.63 (m, 3H), 1.42 (s, 9H), 1.33 (s, 9H), 1.11 (s, 6H), 1.10 (m. 2H)
^{13}C-NMR (400 MHz, $CDCl_3$): 156.40, 155.01, 140.83, 127.08, 126.29, 79.78, 59.79, 46.60, 44.4, 43.6, 35.50, 31.53, 28.84, 27.01, 25.1.
MS (M+1): 307.1, 260.1, 121.0.
HRMS: $C_{23}H_{37}N_2O_4S$ (M-1); Calcd.: 437.2474; found: 437.2466.



4-[1-(4-Acetyl-benzenesulfonylamino)-1-methyl-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (2.61m)

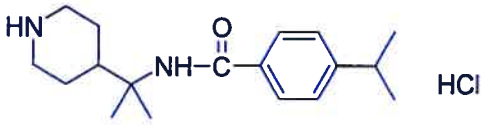
According to general procedure A, starting from **2.45** to give **2.61m** (11 mg, 79%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (d, J = 8.47 Hz, 2H), 7.97 (d, J = 8.50 Hz, 2H), 4.98 (s, 1H), 1.09 (m, 2H), 2.65 (s, 3H), 2.60 (m, 2H), 1.62 (m, 2H), 1.43 (s, 9H), 1.21 (m, 2H), 1.11 (s, 6H)

^{13}C-NMR (400 MHz, $CDCl_3$): 197.26, 155.03, 147.8, 140.0, 129.3, 127.6, 79.9, 60.2, 46.2, 44.4, 43.7, 28.8, 27.3, 27.0, 25.1.

MS (M+1): 425.21, 389.25, 369.15.

HRMS: $C_{21}H_{33}N_2O_5S$ (M+1); Calcd.: 425.2110; found: 425.2122.



4-Isopropyl-*N*-(1-methyl-1-piperidin-4-yl-ethyl)-benzamide hydrochloride (2.60g)

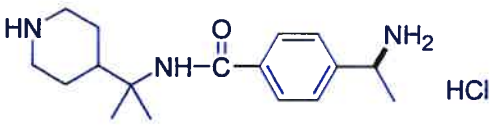
According to general procedure D and E, starting from **2.58g** to give **2.60g** (17 mg, 82%)

^{1}H NMR (400 MHz, CD_3OD): δ 7.65 (d, J = 8.28, 2H), 7.26 (d, J = 8.15, 2H), 5.84 (s, 1H), 3.13 (m, 2H), 2.94 (m, 1H), 2.63 (m, 2H), 2.32 (m, 1H), 2.03 (s, 1H), 1.71 (m, 1H), 1.36 (s, 6H), 1.31 (m, 2H), 1.25 (d, J = 6.92, 6H)

^{13}C-NMR (400 MHz, CD_3OD): 167.1, 152.8, 133.8, 127.2, 127.0, 57.0, 47.4, 44.3, 43.4, 34.5, 28.2, 24.8, 24.2

MS (M+1): 289.2, 154.1, 136.0, 124.1

HRMS: $C_{18}H_{29}N_2O$ (M+1); Calcd.: 289.2280; found: 289.2270



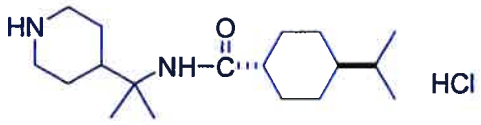
4-(1*S*-Amino-ethyl)-*N*-(1-methyl-1-piperidin-4-yl-ethyl)-benzamide hydrochloride (2.60h)

According to general procedure D and E, starting from **2.58h** to give **2.60h** (11 mg, 79%)

^{1}H NMR (400 MHz, CD_3OD): δ 7.71 (d, J = 8.24 Hz, 2H), 7.44 (d, J = 8.13 Hz, 2H), 4.08 (q, J = 6.69 Hz, 4H), 3.14 (m, 2H), 2.62 (m, 2H), 2.42 (m, 2H), 1.70 (m, 2H), 1.39 (s, 9H), 1.38 (d, J = 6.41, 3H)

^{13}C-NMR (400 MHz, CD_3OD): 169.1, 150.7, 134.7, 127.5, 125.9, 65.9, 56.9, 51.0, 46.3, 42.7, 27.1, 24.3, 23.3

HRMS: $C_{17}H_{28}N_3O$ (M^+); Calcd.: 289.2154; found: 289.2156



***trans*-4-(Isopropylcyclohexane)carboxylic acid (1-methyl-1-piperidin-4-yl-ethyl)amide hydrochloride (2.60i)**

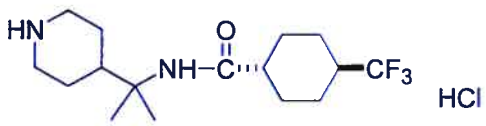
According to general procedure D and E, starting from **2.58i** to give **2.60i** (14 mg, 78%)

^{1}H NMR (400 MHz, CD_3OD): δ 3.16, (m, 2H), 2.62 (m, 2H), 2.27 (m, 1H), 2.10 (m, 1H), 1.79 (m, 4H), 1.67 (m, 9H), 1.43-1.29 (m, 5H), 1.25 (s, 6H), 1.04 (m, 3H), 0.88 (d, J = 6.79 Hz, 6H),

^{13}C-NMR (400 MHz, CD_3OD): 156.01, 141.81, 126.73, 125.97, 59.0, 46.28, 46.13, 34.96, 30.55, 27.17, 24.0

MS (M+1): 295.3, 289.1, 154.1, 137.0, 120.0

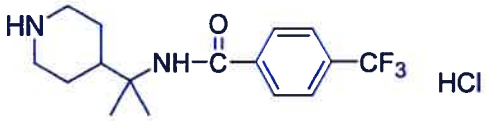
HRMS: $C_{18}H_{35}N_2O$ (M+1); Calcd.: 295.2749; found: 295.2748



***trans*-4-(Trifluoromethyl-cyclohexane)carboxylic acid (1-methyl-1-piperidin-4-yl-ethyl)-amide hydrochloride (2.60j)**

According to general procedure D and E, starting from **2.58j** to give **2.60j** (17 mg, 82%)

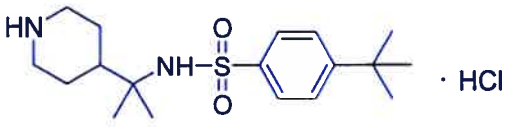
^{1}H NMR (400 MHz, CD_3OD): δ 3.06 (m, 2H), 2.52 (t, J = 11.0 Hz, 2H), 2.22 (m, 3H), 1.98 (m, 2H), 1.86 (m, 2H), 1.62 (m, 2H), 1.51 (m, 2H), 1.33 (m, 4H), 1.24 (s, 6H),
^{13}C-NMR (**400** MHz, CD_3OD): 176.25, 55.87, 46.48, 44.71, 42.75, 41.46, 28.10, 27.27, 24.35, 23.31.
HRMS: $C_{16}H_{27}N_2OF_3$ (M^+); Calcd.: 320.2075; found: 320.2084.



***N*-(1-Methyl-1-piperidin-4-yl-ethyl)-4-trifluoromethyl-benzamide hydrochloride (2.60k)**

According to general procedure D and E, starting from **2.58k** to give **2.60k** (15 mg, 81%)

^{1}H NMR (400 MHz, CD_3OD): δ 7.80 (d, J = 8.12, 2H), 7.65 (d, J = 8.20, 2H), 5.76 (s, 1H), 4.16 (m, 2H), 2.91 (m, 1H), 2.65 (m, 2H), 2.41 (m, 1H), 1.64 (m, 2H), 1.43 (s, 9H), 1.38 (s, 6H), 1.21 (m, 2H).
^{13}C-NMR (400 MHz, CD_3OD): 167.9, 140.0, 128.1, 125.4, 125.4, 57.1, 46.1, 42.3, 26.6, 24.8, 23.2.
HRMS: $C_{16}H_{21}F_3N_2O$ (M^+); Calcd.: 314.1606; found: 314.1609.



4-*tert*-Butyl-*N*-(1-methyl-1-piperidin-4-yl-ethyl)-benzenesulfonamide hydrochloride, (2.63l)

According to general procedure D and E, starting from **2.61l** to give **2.63l** (18 mg, 79%)

^{1}H NMR (400 MHz, CD_3OD): δ 7.81 (d, J = 8.67, 2H), 7.53 (d, J = 8.64, 2H), 3.12 (m, 2H), 2.39 (m, 2H), 1.63 (m, 3H), 1.36 (s, 9H), 1.19 (m, 9H), 1.11 (s, 6H).

MS (M+1): 339.2, 154.1, 126.1.

HRMS: $C_{18}H_{30}N_2O_2S$ (M+1); Calcd.: 339.2100; found: 339.2106.

2.8 References

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CHPTER 3

Synthesis of acylguanidines as Na^+/H^+ antiporter inhibitors

3.1 Na^+/H^+ antiporters

Most cardioprotective drugs act at different key regulatory cardiovascular cascades to elicit beneficial effects in ischemic heart disease. β-Adrenoreceptor antagonists, α-adrenoreceptor antagonists, ACE inhibitors, endothelin antagonists, adenosine-related agents, Ca^+ channel blockers, Na^+ channel openers, and Na^+/H^+ exchanger inhibitors are some of the known agents of clinical importance.[1,2]

Activation of sodium/hydrogen exchangers (NHE) may have an important role in ischemic cell death by means of intracellular overload of Na^+ and Ca^{2+}. Recent evidence has suggested that inhibitors of NHE have protective effects on myocardial ischemia both in vivo and in vitro.

The isoforms are integral plasma membrane proteins, which transport sodium ions in exchange for protons. Currently there are six known isoforms of NHE. NHE-1 is ubiquitous and plays a role in maintaining cellular pH, intracellular sodium ion concentration, and cell volume.[3] NHE-2 is present in all three major gastric epithelial cell types and is expressed in the small intestine, colon, and kidney.[4,5] NHE-3 is primarily found in renal epithelia, localized to the apical membrane,[6,7] where it has been implicated in the absorption of sodium. NHE-4 is found in the stomach and the collecting tubule of the renal inner medulla,[8] where it has been proposed to play a specialized role in volume regulation. NHE-5 is present in several nonepithelial tissues, including brain, spleen, and skeletal muscle, and its role are unknown.[9] NHE-6 is the first intracellular NHE. It has been identified on recycling endosomes but not in the inner membrane of mitochondria as primarily assessed.[10]

In myocardial ischemia and reperfusion, sodium hydrogen exchangers are activated by intracellular acidosis in cardiomyocytes leading to an increase in intracellular sodium and subsequent intracellular calcium overload. The calcium overload provokes severe arrhythmia.[11] In 1998, Merck (Darmstadt) started clinical trials with a benzyolguanidine (EMD 8531, Figure 3.1)[12] for the treatment in high-risk cardiac patients of acute myocardial infarction. Clearly, such "small molecule" lead compounds are of great interest, although efficacy in the treatment of ischemia, and selectivity still remain as unresolved issues.

Furthermore, even though the target has been associated with the Na^+/H^+ antiporter, and the subsequent physiological effects are damaging, the molecular basis of drug action for these compounds is not clear.

3.1.1 NHE-1 structure and cellular localization

Figure 3.1 represents the putative topological model[11] for the mammalian NHE-1, which consists of two principal domains: a 500–amino acid transmembrane domain and a 315–amino acid highly hydrophilic carboxyl-terminus cytoplasmic domain. The number of membrane-spanning units differs according to NHE isoform type, although NHE-1 contains 12 such spanning regions that are critical for the maintenance its function in terms of proton extrusion. The hydrophilic cytoplasmic region plays an important role in modulation of the exchanger, especially through phosphorylation-dependent reactions.[13] This region is NHE isoform specific, which likely accounts for differential regulation by diverse factors.

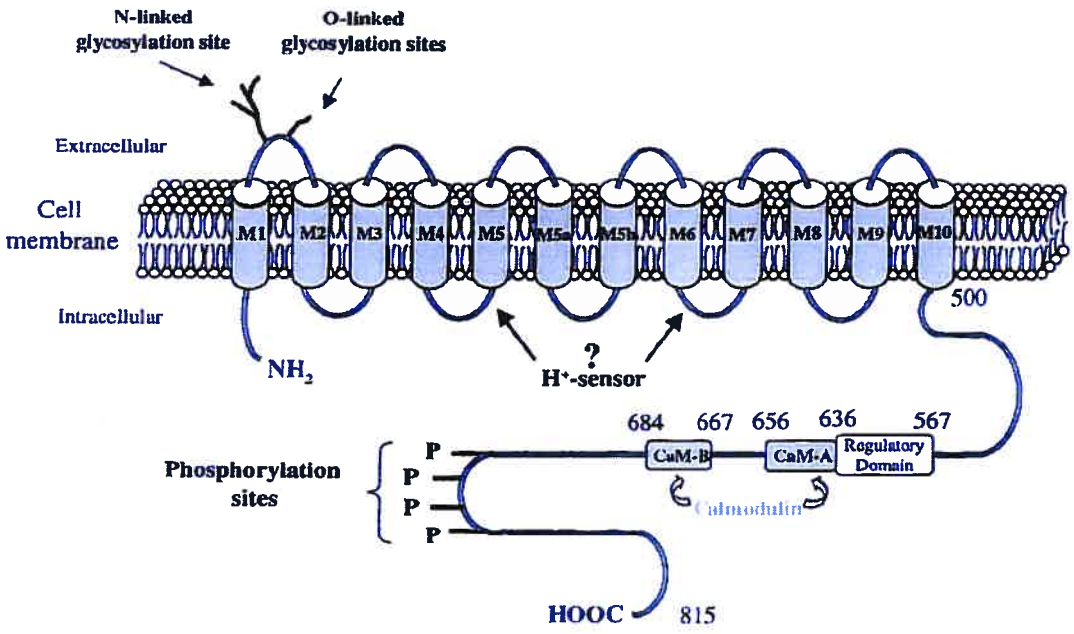


Figure 3.1 Structure and cellular localization. (Reproduced from Karmazyn, M.; Gan, X. T.; Humphreys, R. A.; Yoshida, H.; Kusumoto, K. J. *Circulation Research*. **1999**. *85*, 777-786.)

Putative topological model of 815–amino acid NHE-1 showing 12 transmembra-spanning segments and hydrophilic carboxyl terminus, with indications of proposed regulatory sites.

Localization of the putative H^+ sensor that accounts for the sensitivity of NHE to pH influx has not been confirmed but likely resides in the lipophilic terminus transmembrane region.

3.1.2 Mechanistic basis for NHE involvement in myocardial ischemic and reperfusion injury

Because NHE activation is associated with Na^+ influx ($[Na^+]_i$) the exchanger may also regulate $[Na^+]_I$ under some conditions. Indeed, activation of the NHE in the cardiac myocyte accounts for up to 50% of the basal membrane permeability to Na^+,[14] which may explain the mechanistic basis for the ability of amiloride an aromatic acylguanidine to decrease the cardiac effects of digitalis glycosides.[15]

Increasing $[Na^+]_i$ will also affect $[Ca^{2+}]_i$ levels in the cardiac cell that will affect cardiac function, especially under ischemia and reperfusion. As illustrated in Figure 3.2, the basis for NHE involvement in myocardial ischemic and reperfusion injury reflects a close interaction between ion-regulatory processes found in the cardiac cell, especially NHE, Na^+-Ca^{2+} exchange, and the Na^+-K^+ ATPase; indeed, inhibition of the latter during ischemia is an important prerequisite for NHE involvement in ischemic and reperfusion injury and forms the basis for a Na^+-dependent elevation in $[Ca^{2+}]_i$ levels resulting in cell injury. It is known that changes in pH_i and in cytosolic Ca^{2+} levels are closely related,[16] most likely because Na^+ entering via NHE activation is exchanged for Ca^{2+} via Na^+-Ca^{2+} exchange, leading to an increase in $[Ca^{2+}]_i$.

Figure 3.2 illustrates the interrelationships between ion-regulatory transporters[11] as a mechanism for NHE involvement in cardiac injury in the ischemic and reperfused myocardium. Activation of the exchanger occurs as a consequence of various intracellular and extracellular factors but most importantly as a result of intracellular acidosis. The increased influx of Na^+ cannot be removed efficiently because of inhibition of Na^+-K^+ ATPase.[14] As a result, $[Na^+]_i$ levels will increase, producing elevations in $[Ca^{2+}]_i$ levels via Na^+-Ca^{2+} exchange (NCE).[17] Most of the mammalian NHE's have been cloned.[3]

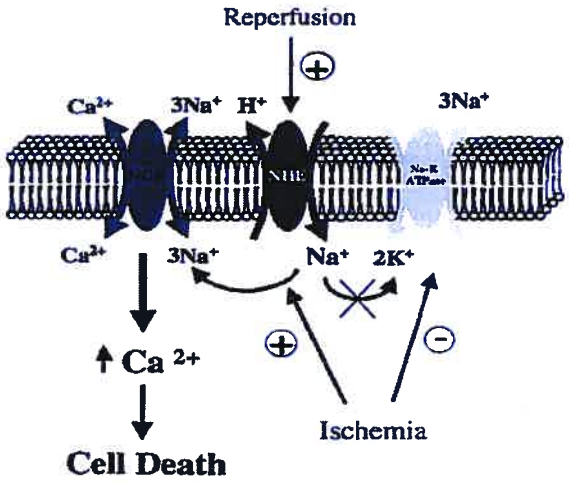


Figure 3.2 NHE involvement in myocardial ischemic and reperfusion injury (according to Karmazyn, M; Gan, X. T.; Humphreys, R. A.; Yoshida, H.; Kusumoto, K. J. *Circulation Research*. **1999**. *85*, 777-786)

3.1.3 Na^+/H^+ antiporter inhibitors under clinical development

The inhibition of NHE-1 demonstrates cardioprotective and antiarrhythmic effect in myocardial ischemia and reperfusion. In the last decade there has been a tremendous burst of activity in the pharmaceutical sector to design potent or clinically effective NHE-1 inhibitors. Several compounds are now in the clinical trials as early as 1998 but none have been marketed yet (Figure 3.3).

Amiloride was the first of a family of NHE inhibitors. Many NHE inhibitors have been developed recently, with marked selectivity for the NHE-1 isoform. These novel NHE inhibitors include cariporide (HOE-642), eniporide (EMD-96785), and zoniporide (CP-597, 396). Cariporide (HOE-642) was the first selective NHE-1 inhibitor discovered, and it is currently in phase III clinical trials as a potential treatment for myocardial infarction (MI) and ischemic damage. Another NHE-1 inhibitor eniporide is also reported to be in phrase II clinical trials.[18]

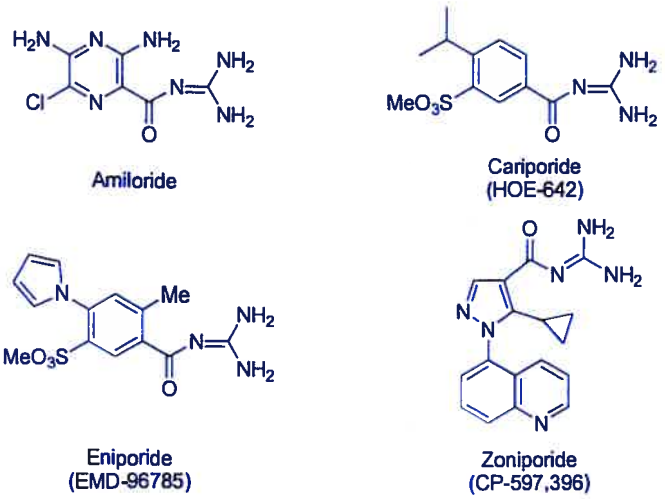


Figure 3.3 Na^+/H^+ antiporter inhibitors

3.1.4 Functional requirements of acylguanidines

It is clear that all the inhibitors have an acylguanidine unit that appears to be a prerequisite for biological activity. The methyl sulfonate, although present as an aryl substituent or as a cyclic variant (Figure 3.4),[19] appears to be optional. The majority of the core structures to which the aryl guanidine is attached are aromatic or heteroaromatic in nature. Although there are several NHE-1 subtypes, only one appears to be associated with cardioprotective activity.

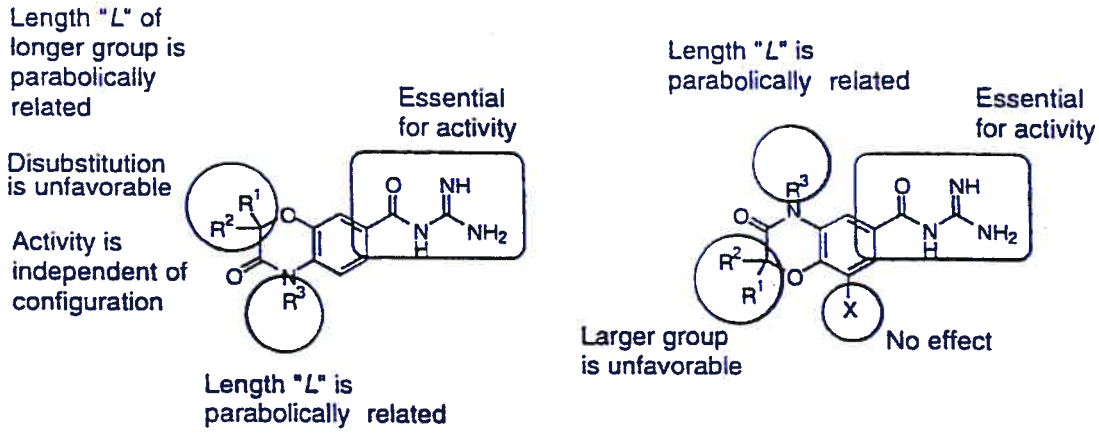
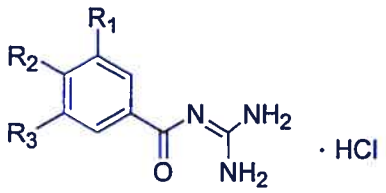


Figure 3.4 Functional and structure requirements for activity (Reproducted to Yamamoto, T.; Hori, M.; Watanabe, I.; Tsutsui, H.; Harada, K.; Ikeda, S.; Ohtaka, H. *Chem Pharm. Bull.* 1998, *46*, 1716)

3.2 Monocyclic acylguanidines

The opportunities for a chemical approach to drug design in search for an effective and selective NHE inhibitor are open for exploration. We chose substituted benzoic acids as templates to synthesize a series of monocyclic acylguandines related to the simple acylguanidines (Figure. 3.5)

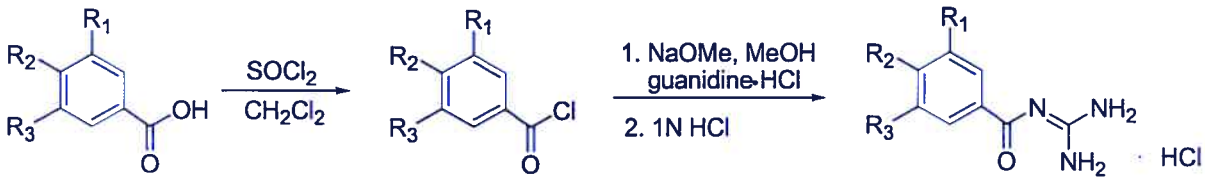


R_1, R_2, R_3 = X, alkyl, and H

Figure 3.5 The structure of monoacylguanidines

The first series consisted of a small library of thirteen compounds with variations in the aromatic subsistents R_1, R_2, and R_3 from **3.1 to 3.13** (Scheme 3.1). Following an established protocol, the appropriate benzoyl chloride prepared form the corresponding acid[20] was reacted with freshly prepared guanidine free base,[20, 21] and the product acidified.[22] The acylguanidines were isolated as hydrochloride salts.

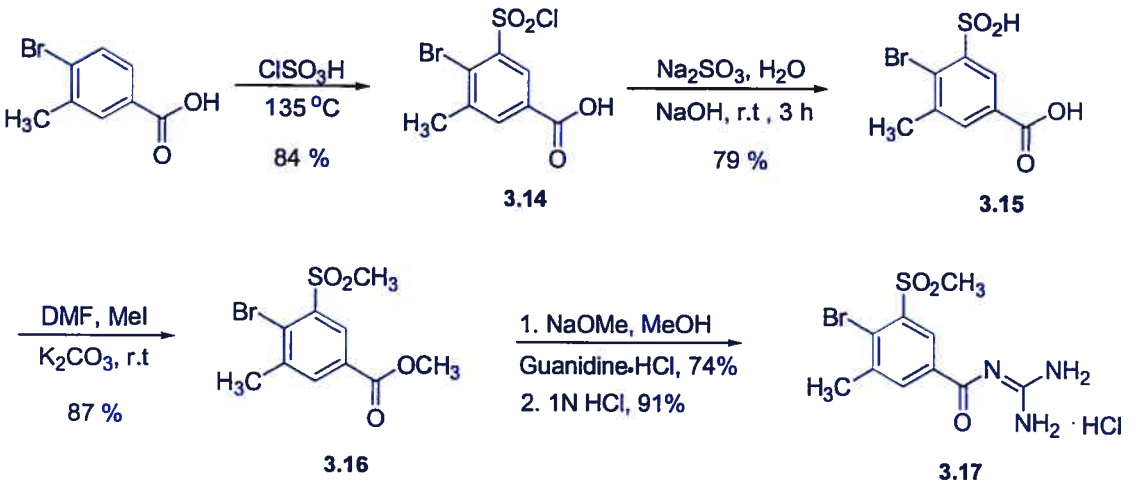
Scheme 3.1 The synthesis of substituted *N*-acylguanidines **3.1-3.13**



3.1-3.13

The synthesis of *N*-(4-bromo-3-methanesulfonyl-5-methyl-benzoyl)-guanidine **3.14** is shown in Scheme 3.2.

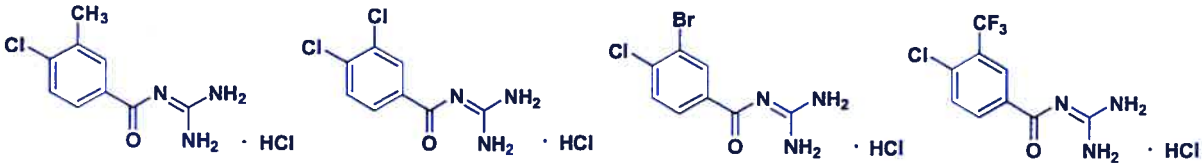
Scheme 3.2 *N*-(4-Bromo-3-methanesulfonyl-5-methyl-benzoyl)-guanidine **3.17**



Treatment of 4-bromo-3-methylbenzoic acid with chlorosulfonic acid[23] gave the chlorosulfonyl chloride **3.14**, which was transformed to the corresponding acid **3.15**. Methylation with MeI and K_2CO_3 afforded the bis-methyl ester **3.16**, which was treated with guanidine to give the intended acylguanidine **3.17** isolated as the hydrochloride salt.

3.3 Biological results

A set of acylguanidines was tested for inhibition of NHE-1 rat ventricular myocytes[24] courtesy of Professor Morris Karmazyn (University of Western Ontario). The activities expressed in IC_{50} are shown for the most active compound.



IC_{50} = 140 nM — **3.8**

IC_{50} = 718 nM — **3.9**

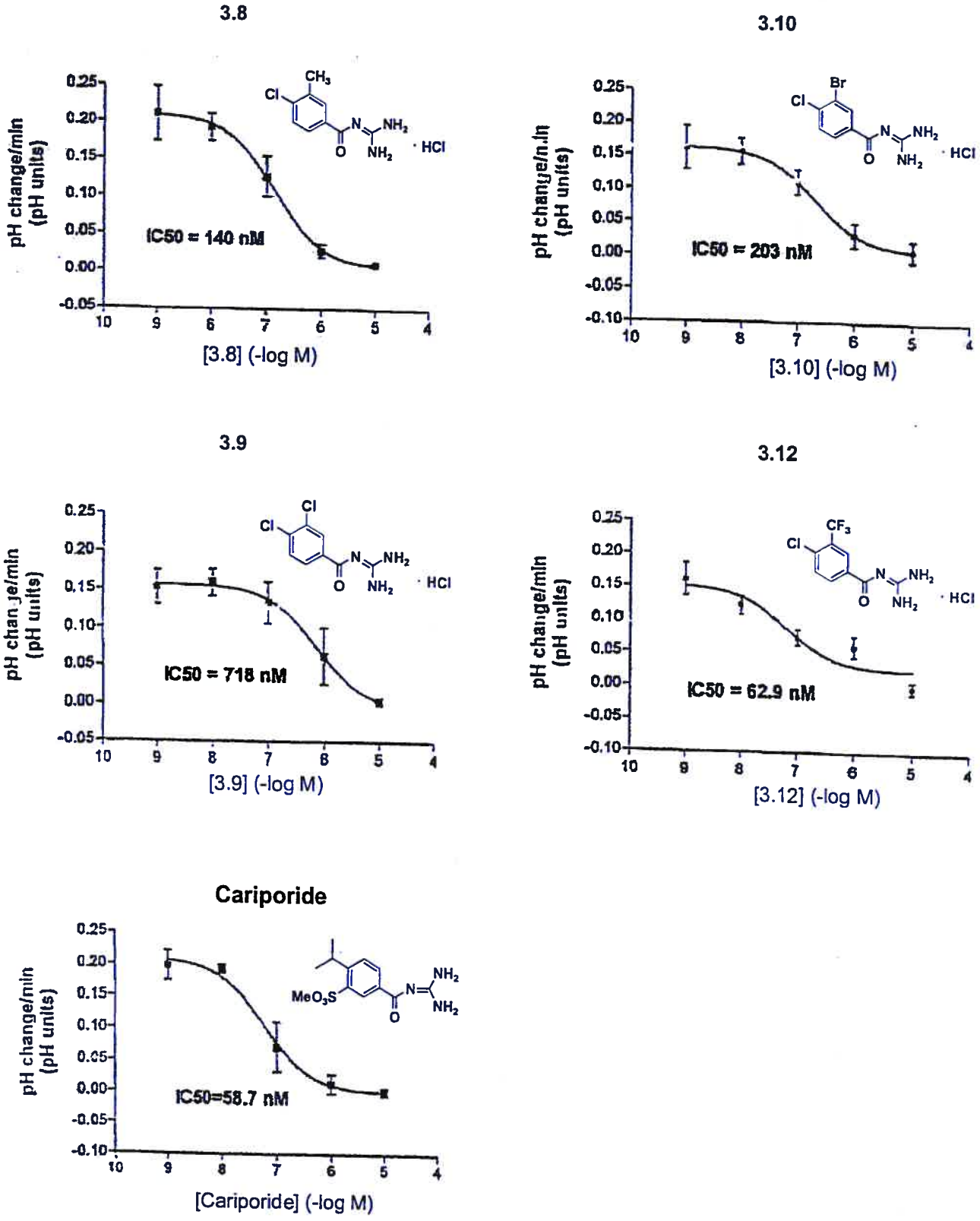
IC_{50} = 203 nM — **3.10**

IC_{50} = 63 nM — **3.12**

Figure 3.6 IC_{50} values for inhibition.

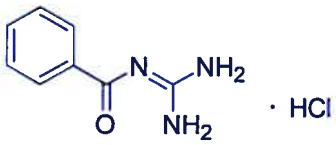
Typical curves showing reduction of intracellular pH shown for Cariporide (the standard) and the most potent analogues **3.8**, **3.9**, **3.10**, and **3.12** are show in Figure 3.7 (Figure 3.7).

Figure 3.7 Change in pH with concentration.



3.4 Experimental notes: (See Chapter1)

For some compounds the carbons resonances do not match the formulae due to signal overlap.



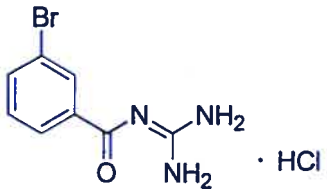
_N_-Benzoyl-guanidine hydrochloride (3.1)

Benzoic acid (122 mg, 1.0 mmol) was treated with $SOCl_2$ (1.3 mL, 18 mmol) at 120 °C for 2 h. Excess $SOCl_2$ was removed with the aid of a water pump, and the resulting acid chloride was used without further purification. Guanidine hydrochloride (382 mg, 4.0 mmol) was added to a methanol solution of NaOMe (4.0 mmol, 6 mL), the mixture was refluxed for 30 min. and filtered. The MeOH was removed in vacuo and the residue taken up in 1,2-dimethoxyethane (15 mL). The acid chloride in 1,2-dimethoxyethane (10 mL) then added to the guanidine solution (15 mL). After the mixture was stirred for 1 h at room temperature, the inorganic precipitate was removed and the filtrate was evaporated. The residue was purified by silica gel chromatography ($MeOH:CH_2Cl_2:NH_3{\cdot}H_2O$ 9:90:1) to give benzoyl-guanidine as a white solid (122 mg, 71%). HCl (1 N, 3.0 mmol) was dropped into a stirred suspension of benzoylacylguanidine (0.75 mmol) in H_2O (5 mL), The filtered solution was frozen and then lyophilized to give the hydrochloride salt **3.1** (141 mg, 95%).

Mp. 168 °C

^{1}H NMR (400 MHz, $CDOD_3$): δ 8.7.98 (d, J = 8.35 Hz, 2H), 7.19 (m, 3H); **^{13}C-NMR** (400 MHz, $CDOD_3$): δ 178.1, 164.3, 139.0, 132.3, 129.0, 126.2.

MS (M^+) 163.1; **HRMS**: $C_8H_{10}N_3O$ (M^+); Calcd.: 163.0746; found: 163.0749.

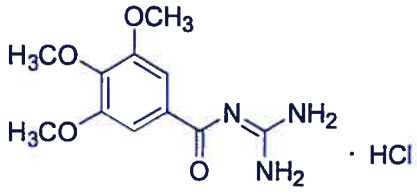


***N*-(3-Bromo-benzoyl)-guanidine hydrochloride (3.2)**

Same procedure as **3.1** afforded **3.3** (37 mg, 71%)

Mp. 139-142 °C

^{1}H NMR (400 MHz, $CDOD_3$): δ 8.22 (s, 1H), 7.99 (d, J = 8.65 Hz, 1H), 7.58 (d, J = 8.82 Hz, 1H), 7.30 (dd, J = 8.82, 8.65 Hz, 1H); **^{13}C-NMR** (400 MHz, $CDOD_3$): δ 177.0, 163.0, 141.2, 138.4, 133.81, 131.86, 129.75, 127.45; **HRMS:** $C_8H_8BrN_3O$ (M^+); Calcd.: 240.9851; found: 240.9855

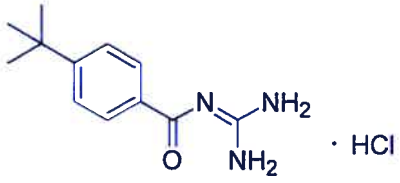


***N*- (3,4,5-Trimethoxy-benzoyl)-guanidine hydrochloride (3.3)**

Same procedure as **3.1** afforded **3.3** (61 mg, 74%).

Mp: 142-145 °C

^{1}H NMR (400 MHz, $CDOD_3$): δ 7.42 (s, 2H), 3.81 (s, 3H), 3.90 (S, 6H); **^{13}C-NMR** (400 MHz, $CDOD_3$): δ 173.5, 152.2, 139.1, 134.8, 134.5, 127.3, 106.7, 61.1; **MS** (M^+): 253.1, 226.1; **HRMS:** $C_{11}H_{15}N_3O_4$ (M^+); Calcd.: 253.1063; found: 253.1071



***N*-(4-*tert*-Butyl-benzoyl)-guanidine Hydrochloride (3.4)**

Same procedure as **3.1** afforded **3.4** (59 mg, 74%).

Mp. 165-167 °C

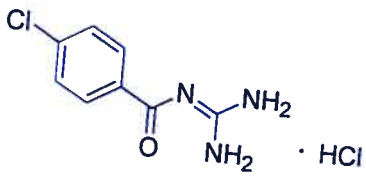
^{1}H NMR (400 MHz, $CDOD_3$): δ 7.92 (d, J = 8.65 Hz, 2H), 7.40 (d, J = 8.70 Hz, 2H), 1.32 (s, 9H); **^{13}C-NMR** (400 MHz, $CDOD_3$): δ 169.1, 153.2, 129.42, 128.68, 125.55, 124.83, 48.9, 24.8; **MS** (M^+): 219.1, 218.1, 204.1, 161.1; **HRMS:** $C_{12}H_{17}N_3O$ (M^+); Calcd.: 219.1372; found: 219.1378

***N*-(4-Bromo-3-methyl-benzoyl)-guanidine hydrochloride (3.5)**

Same procedure as **3.1** afforded **3.5** (34 mg, 71%).

Mp. 221-224 °C.

^{1}H NMR (400 MHz, $CDOD_3$): δ 7.93 (d, J = 8.45 Hz, 1H), 7.71 (dd, J = 8.52 Hz, 1H), 7.54 (d, J = 8.3 Hz, 1H), 2.41 (s, 3H); **^{13}C-NMR** (400 MHz, $CDOD_3$): δ 176.45, 163.04, 137.64, 137.58, 132.06, 131.11, 128.25, 127.83, 22.04;
MS (M^+): 255.0, 230.0, 192.2, 136.1; **HRMS:** $C_9H_{10}N_3Obr$ (M^+); Calcd.: 255.0007; found: 255.0003

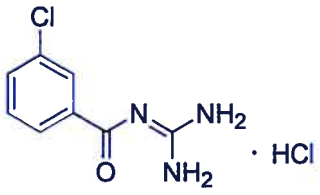


***N*-(4-Chloro-benzoyl)-guanidine hydrochloride (3.6)**

Same procedure as **3.1** afforded **3.6** (39 mg, 76%).

Mp. 239-241 °C.

^{1}H NMR (400 MHz, $CDOD_3$): δ 8.08 (d, J = 8.70 Hz, 2H), 7.35 (d, J = 8.68 Hz, 1H); **^{13}C-NMR** (400 MHz, $CDOD_3$): δ 178.2, 157.3, 131.11, 130.46, 128.87, 128.02, **MS** (M^+): 197.0, 139.0, 127.1, 113.0, 95.0, 77.0; **HRMS:** $C_8H_8ClN_3O$ (M^+); Calcd.: 197.0362; found: 197.0356

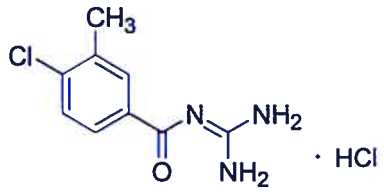


***N*-(3-Chloro-benzoyl)-guanidine hydrochloride (3.7)**

Same procedure as **3.1** afforded **3.7** (43 mg, 72%).

Mp. 212-213 °C.

^{1}H NMR (400 MHz, $CDOD_3$): δ 8.08 (m, 1H), 7.97 (d, J = 7.7 Hz, 1H), 7.46 (m, 1H), 7.34 (m, 1H); **^{13}C-NMR** (400 MHz, $CDOD_3$): δ 176.80, 163.98, 141.07, 164.02, 130.92, 129.50, 128.91, 127.09; **HRMS:** $C_8H_8ClN_3O$ (M^+): Calcd.: 197.0356; found: 197.0349

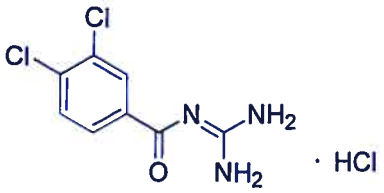


***N*-(4-Chloro-3-methyl-benzoyl)-guanidine hydrochloride (3.8)**

Same procedure as **3.1** afforded **3.8** (58 mg, 75%).

Mp. 201-202 °C

^{1}H NMR (400 MHz, $CDOD_3$): δ 7.98 (d, J = 1.0 Hz, 1H), 7.83 (d, J = 8.29, 1.6 Hz, 1H), 7.35 (d, J = 8.31 Hz, 1H), 2.39 (s, 3H); **^{13}C-NMR** (400 MHz, $CDOD_3$): δ 177.30, 163.74, 137.57, 135.55, 131.43, 128.56, 127.80, 124.3, 19.14; **HRMS:** $C_9H_{11}ClN_3O$ (M^+); Calcd.: 212.0510; found.: 211.0512

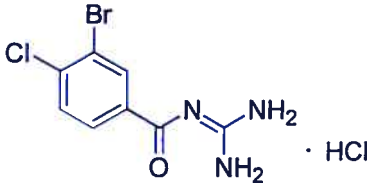


***N*-(3,4-Dichloro-benzoyl)-guanidine hydrochloride (3.9)**

Same procedure as **3.1** afforded **3.9** (54 mg, 72%).

Mp. 208 °C

^{1}H NMR (400 MHz, $CDOD_3$): δ 8.21 (d, J = 1.9 Hz, 1H), 7.96 (dd, J = 8.37, 2.0 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H); **^{13}C-NMR** (400 MHz, $CDOD_3$): δ 175.56, 163.97, 139.49, 134.87, 131.90, 130.89, 130.07, 128.39; **MS** (M+1): 232.0, 219.2, 202.1; **HRMS:** $C_8H_8Cl_2N_3O$ (M+1); Calcd.: 232.0044; found: 232.0051.



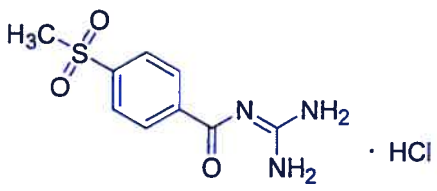
***N*-(3-Bromo-4-chloro-benzoyl)-guanidine hydrochloride (3.10)**

Same procedure as **3.1** afforded **3.10** (41 mg, 72%).

Mp. 215-216 °C

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.38 (s, 1H), 8.0 (d, J = 8.1 Hz, 1H), 7.53 (d, J = 8.2 Hz, 1H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 175.43, 163.75, 139.47, 136.87, 134.26, 129.92, 129.04, 121.59; **MS** (M+1): 275.9, 218.9, 154.0, 136.0; **HRMS:** $C_8H_8BrClN_3O$ (M+1); Calcd.: 275.9539; found: 275.9543.



***N*-(4-Methanesulfonyl-benzoyl)-guanidine hydrochloride (3.11)**

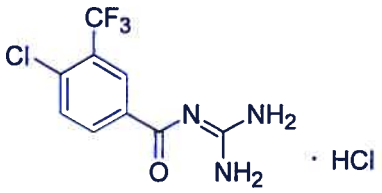
Same procedure as **3.1** afforded **3.11** (28 mg, 74%).

Mp. 205 °C

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (d, J = 8.6 Hz, 1H), 7.98 (d, J = 8.5 Hz, 1H), 3.15 (s, 3H)

^{13}C-NMR (400 MHz, $CDCl_3$): δ 181.2, 162.4, 144.3, 141.9, 130.2, 127.1, 41.5.

MS (M^+): 241.1, 218.9, 199.0, 180.9, 147.1, 88.1; **HRMS:** $C_9H_{12}N_3O_3S$ (M^+); Calcd.: 242.05227; found: 242.0523

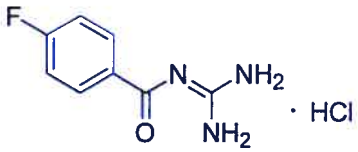


***N*-(4-Chloro-3-trifluoromethyl-benzoyl)-guanidine hydrochloride (3.12)**

Same procedure as **3.1** afforded **3.12** (48 mg, 76%).

Mp. 207 °C

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.48 (d J = 1.72 Hz, 1H), 8.24 (dd, J = 8.32, 1.70 Hz, 1H), 7.59 (d, J = 8.34 Hz, 1H); **^{13}C-NMR** (400 MHz, $CDCl_3$): δ 175.23, 164.03, 138.33, 134.49, 133.54, 131.30, 128.1793, 128.10, 48.90; **MS** (M+1): 266.1; **HRMS:** $C_9H_8ClF_3N_3O$ (M+1); Calcd.: 266.0234; found: 266.0238.



***N*-(4-Fluoro-benzoyl)-guanidine hydrochloride (3.13)**

Same procedure as **3.1** afforded **3.13** (34 mg, 71%).

Mp. 216-217 °C

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (d, J = 8.21 Hz, 2H), 7.10 (d, J = 8.34 Hz, 2H); **^{13}C-NMR** (400 MHz, $CDCl_3$): δ 177.3, 166.4, 135.3, 131.4, 114.7, 114.5.

MS: (M+1) 182.1, 154.0, 136.0, 123.0; **HRMS:** $C_8H_9CN_3OF$ (M+1), Calcd.: 182.0730; found: 182.0733.

4-Bromo-3-chlorosulfonyl-5-methyl-benzoic acid (3.14)

4-Bromo-3-methyl-benzoic acid (0.5g, 2.32 mmol) was added portionwise to chlorosulfonic acid (0.5 mL, 7.5 mol) in a cooling ice both at such a rate that the internal temperature remained at 20 °C. The resultant mixture was heated at 140 °C bath temperature for 3 h. After cooling, the mixture was added dropwise to stirred ice water (3.5 mL), and stirring was continued for an additional 30 min at 10 °C. The precipitate was collected by filtration and washed with ice water (1 mL) to give crude 4-chloro-5-(chlorosulfonyl)-3-methylbenzoic acid **3.14** (0.43 g) which was used directly in the next reaction.

4-Bromo-3-methyl-5- carboxybenzenesulfinic acid (3.15)

The crude compound **3.14** was added in portions to a solution of Na_2SO_3 (176 mg, 5.2 mmol) in H_2O (0.8 mL) at 15-20 °C. The pH was adjusted to 10 by addition of 32% aqueous NaOH.

Stirring was continued for an additional 3 h, then the mixture was left to stand overnight at room temperature. Acidification to pH 1 using 25% aqueous HCl at 0 °C afforded a precipitate, which was filtered, washed with ice water (0.5 mL) to give crude 4-bromo-3-methyl-5-carboxybenzenesulfinic acid. The crude was used in the next step without further purification.

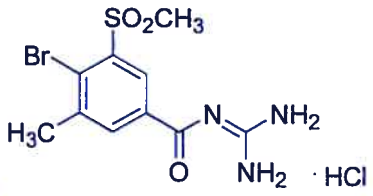
Mp. 137 °C

4-Bromo-3-methanesulfonyl-5-methyl-benzoic acid methyl ester (3.16)

MeI (656 μL, 10.5 mmol) was added to a suspension of **3.16** (0.43 g, mmol) in DMF (6 mL), and K_2CO_3 (1.59 g, 11.5 mmol) over a period of 1 h with stirring, and stirring was continued overnight at room temperature. DMF was removed under reduced pressure, and the residue was treated with water (10 mL), filtered off, and washed with water (2 mL). The resulting solid was air-dried and recrystallized from EtOAc (4 mL) to give **3.16** (0.34 g, 55% overall).

Mp. 146-147 °C

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (s, 1H), 8.10 (s, 1H), 3.85 (s, 3H), 3.25 (s, 3H), 2.58 (s, 3H); **^{13}C-NMR** (400 MHz, $CDCl_3$): δ 165.5, 141.2, 136.4, 130.1, 130.0, 128.3, 124.3, 53.1, 42.8, 24.3



***N*-(4-Bromo-3-methanesulfonyl-5-methyl-benzoyl)-guanidine hydrochloride (3.18)**

Free guanidine base was prepared by adding guanidine hydrochloride (382 mg, 4.0 mmol) to a sodium methoxide (NaOMe) solution, which was prepared from sodium (92 mg, 4.0 mmol) and MeOH (6 mL). The mixture was refluxed for 30 min. and filtered. To the filtrate was added 4-bromo-3-methanesulfonyl-5-methyl-benzoic acid methyl ester **3.16** (307 mg, 1.0 mmol), and the mixture was stirred for 2.5 h at 50 °C. After the mixture was cooled to room temperature, water (2.50 mL) was added, and the solution was stirred for 30 min and an additional 30 min with ice cooling while crystallization took place. The product was collected and recrystallized from MeOH, yielding **3.17** as white crystals (47 mg, 57%)

HCl (1 N, 0.1 mL) was added into a stirred suspension of **3.17** (25 mg, 1.15 mmol) in H_2O (3 mL), The filtered solution was frozen and then lyophilized to give the title compound as a solid (28 mg, 95%).

Mp: 260 °C.
^{1}H NMR (400 MHz, $CDOD_3$): δ 8.71 (s, 1H), 8.29 (s, 1H), 2.50 (s, 3H), 3.30 (s, 3H), 2.58)s, 3H); **^{13}C-NMR** (400 MHz, $CDOD_3$): δ 177.3, 163.7, 137.6, 137.3, 131.4, 128.6, 127.8, 123.5, 47.1, 19.1; **HRMS:** $C_{10}H_{12}BrN_3OS$ (M+1), Calcd.: 333.9782; found: 333.9779.

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Résumé

Ma recherche décrit la synthèse de composés hétérocycliques d'importance biologique. En premier lieu, nous avons fait le design et la synthèse d'un nouvel analogue de type 2-pyridone basé sur la structure d'un composé antibactérien bien connu, le ABT-719. Un composé intermédiaire avancé a été atteint, mais des difficultés lors des dernières étapes ont mené à l'abandon de l'azaquinoline bicyclique désirée.

Le deuxième projet décrit la synthèse d'une petite librairie de 28 produits consistant en deux séries de dérivés sulfonamides et amides de pipéridines comme étant inhibiteurs de la Rho-kinase. Une activité modeste a été trouvée avec un des analogues.

Le troisième projet consiste en la synthèse d'acylguanidines monocycliques comme inhibiteurs potentiels de canaux Na^+/H^+ (Na^+/H^+ échangeur, NHE-1). Basé sur les structures connues d'inhibiteurs NHE, nous avons synthétisé une petite librairie de dérivés acylguanidines. Les analyses biologiques ont identifié quatre inhibiteurs potentiels.

Mots clefs: hétérocycle, 2-pyridone, ADN gyrase, pipéridine, Rhokinase inhibiteurs, acylguanidine, Na^+/H^+ échangeur (NHE-1) inhibiteur.