Discovery of BACE-1 inhibitors using an integrated computational and experimental approach

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Doctor of Philosophy

The University of Leeds
School of Chemistry

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The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

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Abstract

There are numerous complementary approaches to facilitate the identification of novel inhibitors for biological targets, including high throughput screening and fragment-based drug discovery. Computational tools are often employed to predict binding pose and affinity of the new inhibitors. In this thesis an integrated computational and experimental approach to identify novel inhibitors is described. The approach involves the design of a virtual library of likely synthetically accessible lead-like molecules, followed by virtual high throughput screening (vHTS) against target protein. To exemplify the approach, BACE-1 was selected as an example target protein. BACE-1 is responsible for the formation of amyloid plaque in brains affected by Alzheimer’s disease and therefore is a potential target for the treatment of the disease.

A virtual library of lead-like molecules was generated based on diversity-oriented synthesis methods established in our laboratory. The library underwent virtual high throughput screening (vHTS) against BACE-1 by using eHiTS and two families of putative inhibitors were identified with high predicted ligand efficiency (cLE). The in silico approach employed to identify novel putative BACE-1 inhibitors is schematically represented as follows.

A focused library based on the selected putative inhibitors was designed and synthesised, and biological activity was assessed via a fluorimetric assay. Structure-activity relationship (SAR) studies were conducted to rationalise the activity of the inhibitors and to confirm the validity of the integrated approach in identifying new inhibitors for biological targets. A novel series of BACE-1 inhibitors was identified and is herein described.
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Abbreviations and nomenclature

°C  Celsius degrees
2D  two dimensional
3D  three dimensional
Å  Ångström
A (Ala)  alanine
Aβ  amyloid β
Ac  acetyl
App  apparent
APP  amyloidal precursor protein
Ar  aromatic
BACE  β-site amyloidal precursor protein cleaving enzyme
Bn  benzyl
Boc  tert-butyloxy carbonyl
b.p.  boiling point
br  broad
Bu  butyl
ca.  circa; about
CTF-99  carboxy-terminal fragment of 99 amino acids
δ  chemical shift
d  doublet
D (Asp)  aspartate
DBU  1,8-diazabicyclo[5.4.0]undec-7-ene
de novo  anew
DEAD  diethyl azodicarboxylate
dd  double doublet
ddd  double double of doublet
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ddd</td>
<td>double double double of doublet</td>
</tr>
<tr>
<td>ddt</td>
<td>double doublet of triplet</td>
</tr>
<tr>
<td>DIBAL</td>
<td>diisobutylaluminium hydride</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-diisopropylethylamine</td>
</tr>
<tr>
<td>fDIPES</td>
<td>diisopropyl(3,3,4,4,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecy)silyl</td>
</tr>
<tr>
<td>DMB</td>
<td>3,4 dimethoxy benzyl</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DOS</td>
<td>diversity-oriented synthesis</td>
</tr>
<tr>
<td>DPPA</td>
<td>diphenylphosphoryl azide</td>
</tr>
<tr>
<td>dq</td>
<td>double quadruplet</td>
</tr>
<tr>
<td>dt</td>
<td>double triplet</td>
</tr>
<tr>
<td>dtd</td>
<td>double triplet of doublet</td>
</tr>
<tr>
<td>E</td>
<td>entgenen</td>
</tr>
<tr>
<td>E (Glu)</td>
<td>glutamate</td>
</tr>
<tr>
<td>e.g.</td>
<td>exampli gratia; for example</td>
</tr>
<tr>
<td>Et</td>
<td>ethyl</td>
</tr>
<tr>
<td>et al.</td>
<td>et alii; and others</td>
</tr>
<tr>
<td>eHiTS</td>
<td>electronic high-throughput screening</td>
</tr>
<tr>
<td>EI</td>
<td>electron impact</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>eq.</td>
<td>equivalent</td>
</tr>
<tr>
<td>ES</td>
<td>electron spray</td>
</tr>
<tr>
<td>F (Phe)</td>
<td>phenylalanine</td>
</tr>
<tr>
<td>FBDD</td>
<td>fragment-based drug design</td>
</tr>
<tr>
<td>FGI</td>
<td>functional group interconversion</td>
</tr>
<tr>
<td>F.U.</td>
<td>fluorescence unit</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>g</td>
<td>grams</td>
</tr>
<tr>
<td>G (Gly)</td>
<td>glycine</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>H(His)</td>
<td>histidine</td>
</tr>
<tr>
<td>hept</td>
<td>heptuplet</td>
</tr>
<tr>
<td>(v)HTS</td>
<td>(virtual) high-throughput screen</td>
</tr>
<tr>
<td>HE</td>
<td>hydroxyethylene</td>
</tr>
<tr>
<td>HEA</td>
<td>hydroxyethylamine</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>high-resolution mass spectrometry</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>I (Ile)</td>
<td>isoleucine</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>50% inhibitory concentration</td>
</tr>
<tr>
<td>in silico</td>
<td>performed by computer simulation</td>
</tr>
<tr>
<td>in situ</td>
<td>in place</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>'Pr</td>
<td>isopropyl</td>
</tr>
<tr>
<td>K (Lys)</td>
<td>lysine</td>
</tr>
<tr>
<td>Kᵢ</td>
<td>constant of inhibition</td>
</tr>
<tr>
<td>J</td>
<td>spin-spin coupling constant</td>
</tr>
<tr>
<td>L (Leu)</td>
<td>leucine</td>
</tr>
<tr>
<td>LC/MS</td>
<td>liquid chromatography–mass spectrometry</td>
</tr>
<tr>
<td>(c)LE</td>
<td>(computational) ligand efficiency</td>
</tr>
<tr>
<td>log</td>
<td>logarithm in base 10</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>M (Met)</td>
<td>methionine</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre</td>
</tr>
<tr>
<td>mM</td>
<td>milli mole</td>
</tr>
<tr>
<td>MOM</td>
<td>methoxymethyl</td>
</tr>
<tr>
<td>m.p.</td>
<td>melting point</td>
</tr>
<tr>
<td>μL</td>
<td>microlitre</td>
</tr>
<tr>
<td>Ms</td>
<td>mesyl</td>
</tr>
<tr>
<td>M.W.</td>
<td>molecular weight</td>
</tr>
<tr>
<td>m/z</td>
<td>mass to charge ratio</td>
</tr>
<tr>
<td>n</td>
<td>number</td>
</tr>
<tr>
<td>N (Asn)</td>
<td>asparagine</td>
</tr>
<tr>
<td>N/A</td>
<td>not available</td>
</tr>
<tr>
<td>n-Bu</td>
<td>normal butyl</td>
</tr>
<tr>
<td>nHA</td>
<td>number of heavy atoms</td>
</tr>
<tr>
<td>NIS</td>
<td>N-iodosuccinimide</td>
</tr>
<tr>
<td>nM</td>
<td>nanomolar</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>nOe</td>
<td>nuclear Overhauser effect</td>
</tr>
<tr>
<td>Ns</td>
<td>o-nitrophenylsulfonyl</td>
</tr>
<tr>
<td>Ns’</td>
<td>p-nitrophenylsulfonyl</td>
</tr>
<tr>
<td>P</td>
<td>partition coefficient</td>
</tr>
<tr>
<td>pdb</td>
<td>protein data bank</td>
</tr>
<tr>
<td>Petrol</td>
<td>petroleum spirit (b.p. 40-60 °C)</td>
</tr>
<tr>
<td>PG</td>
<td>protecting group</td>
</tr>
<tr>
<td>pH</td>
<td>potential of hydrogen</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
</tbody>
</table>
Pr  propyl
Py  pyridine
q   quadruplet
Q (Gln)  glutamine
R (Arg)  arginine
$R_F$  solvent front
r.t.  room temperature
$\sigma$  standard deviation
s   singlet
S (Ser)  serine
SAR(s)  structure-activity relationship(s)
SBDD  structure-based drug design
t   triplet
T (Thr)  threonine
td  triple doublet
tdd  triple double doublet
tt   triple triplet
$i' Bu$  tert-butyl
tert  tertiary
Tf  triflate; trifluoromethanesulfonate
TFA  trifluoroacetic acid
THF  tetrahydrofuran
TLC  thin layer chromatography
TMS  trimethylsilyl or trimethylsilane
TR-FRET  time-resolved fluorescence resonance energy transfer
V (Val)  valine
v/v  volume solute/volume solution
<table>
<thead>
<tr>
<th>Term</th>
<th>Translation</th>
</tr>
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<tbody>
<tr>
<td>via</td>
<td>by way of</td>
</tr>
<tr>
<td>v</td>
<td>wavelenght</td>
</tr>
<tr>
<td>W (Trp)</td>
<td>tryptohaan</td>
</tr>
<tr>
<td>w/v</td>
<td>weight/volume</td>
</tr>
<tr>
<td>Y (Tyr)</td>
<td>tyrosine</td>
</tr>
<tr>
<td>Z</td>
<td>zusammen</td>
</tr>
</tbody>
</table>
Chapter 1. Introduction

This introductory Chapter is composed of two parts. The first part gives an overview of the drug discovery process (Section 1.1) and of methods to identify and generate lead molecules for biological targets (Section 1.2). The second part introduces the protein BACE-1, the biological target chosen for this project (Section 1.3) and gives an overview of known BACE-1 inhibitors (Section 1.4). Finally an outline of the project is provided (Section 1.5).

1.1 Drug discovery process: from leads to drug candidates

The discovery of drugs for biological targets is a long process, which starts by identifying initial molecules, called hits or leads, having affinity for a specific biological target. Lead molecules can be identified by a variety of screening methods (Section 1.2); once a lead is identified, studies of its properties are performed in order to understand the role that different functional groups have in binding the target. Structure-activity relationship (SAR) studies facilitate lead optimisation and ultimately a drug candidate is nominated.

The physico-chemical properties of optimal oral drugs can be described by the Lipinski “rule of five”. This rule provides guidance of the ideal molecular weight (M.W. \( \leq 500 \)) and lipophilicity (\( \log P \leq 5 \)), and of the number of H-bonding atoms (number of H-bond donors \( \leq 5 \), number of H-bond acceptors \( \leq 10 \))\(^1\) that a drug should have to allow for oral bioavailability, absorption and permeation. Since drug candidates are developed from leads, physico-chemical properties of leads have been defined as well. Good leads generally have molecular weight between \( 200 \leq \text{M.W.} \leq 350 \), lipophilicity value in the range of \( -1 \leq \log P \leq +3 \),\(^2\) number of H-bonding donors \( \leq 4 \), number of H-bond acceptors \( \leq 8 \).\(^3\)

\(^1\)\( P \) is the partition coefficient which expresses the solubility of a unionised compound in two immiscible solvents, \( n \)-octanol and water. \( P = \frac{[\text{solute}]_{\text{octanol}}}{[\text{solute}]_{\text{water}}} \). \( \log P \) is used as a measurement of lipophilicity.
A clearer visual representation of lead development into drug candidates is provided by Nadin et al.\textsuperscript{2} (Figure 1). The chemical space of bioactive small molecules can be represented by lipophilicity and molecular weight. Typically, the drug discovery process results in a progression towards higher molecular weight and lipophilicity values. Drug candidates space is represented below $\text{M.W.} \leq 400$, logP $\leq 4$, since it is often observed that molecules with these physico-chemical properties have more chance of becoming successful drugs.\textsuperscript{4} In Nadin's representation it is also shown that fragments, defined as small molecules having a molecular weight between $100 \leq \text{M.W.} \leq 300$ and low lipophilicity, $-1.5 \leq \log P \leq 1.5$, are a good starting point to develop leads.

\textbf{Figure 1. Chemical space of drug candidates, leads, and fragments.} Representation of the chemical space explored during drug discovery process. clogP is a predicted measurement of logP estimated from a computed algorithm which sum logP values of small parts of a molecule.\textsuperscript{5} Adapted from Nadin et al.\textsuperscript{2}

A summary of physico-chemical properties of drug-like, lead-like and fragment-like molecules is given in Table 1. It is worth mentioning that lead-likeness criteria were defined in a broader way ($\text{M.W.} \leq 460$ and logP $\leq 4$) before Nadin's definition in 2012. Nadin's chemical space definition can be considered an up-to-date guideline to develop and design optimal leads (Section 1.2.4).
Table 1. Guidelines of physico-chemical properties of drug-like, lead-like and fragment-like molecules

<table>
<thead>
<tr>
<th>Drug-like molecules$^1$</th>
<th>Lead-like molecules$^2$</th>
<th>Fragment-like molecules$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.W. ≤ 500</td>
<td>200 ≤ M.W. ≤ 350</td>
<td>100 ≤ M.W. ≤ 300</td>
</tr>
<tr>
<td>logP ≤ 5</td>
<td>−1 ≤ logP ≤ +3</td>
<td>−1.5 ≤ logP ≤ +1.5</td>
</tr>
<tr>
<td>n H-bond donors ≤ 5</td>
<td>n H-bond donors ≤ 4$^3$</td>
<td>n H-bond donors ≤ 3$^7$</td>
</tr>
<tr>
<td>n H-bond acceptors ≤ 10</td>
<td>n H-bond acceptors ≤ 8$^3$</td>
<td>n H-bond acceptors ≤ 3$^7$</td>
</tr>
<tr>
<td>n aromatic rings ≤ 5$^8$</td>
<td>n aromatic rings ≤ 3$^8$</td>
<td>$^-$</td>
</tr>
<tr>
<td>$^-$</td>
<td>16 ≤ nHA$^b$ ≤ 26</td>
<td>$^-$</td>
</tr>
</tbody>
</table>

$^a$Not defined $^b$nHA: number of heavy atoms, corresponding to number of non-hydrogen atoms. nHA is related to the molecular weight, therefore is not always defined.

1.2 Approaches to identify lead compounds

1.2.1 High throughput screening

Approaches to identify leads can be categorised as: high throughput screening (HTS), fragment-based design and virtual high throughput screening (vHTS). HTS refers to screening of large compound libraries (up to 100,000 per day$^9$) against a biological target by means of established and validated biochemical (e.g. ELISA$^{10}$), biophysical (e.g. TR-FRET$^{11}$) or biological assays (e.g. cell based$^{12}$) which can measure compound binding affinity. HTS has been widely used by pharmaceutical companies since the mid-1990s.$^9$ The screening is generally automated, molecules are assayed in a μM concentration or lower, and up to 1536 compounds are screened in a run. It is useful to apply when there is no knowledge about the structure of a biological target, as any identified hits can give indication about relevant structure features. When information about the structure of likely active molecules is available, HTS is also applied to focused library subsets. This approach was successful for a range of biological targets, e.g. protein kinases, transferases, proteases and nuclear hormone receptors.$^{13}$
1.2.2 Fragment-based design

Fragment-based approaches were developed with the idea of identifying key fragment-sized molecules, able to bind discrete sites of a biological target, and to link them in order to build a lead molecule. These approaches have the potential to combine active fragments in many possible ways, building a variety of potentially active leads. Physico-chemical properties of fragments, have been defined according to the “rule of three”: M.W. \( \leq 300 \), logP \( \leq 3 \), number of H-bond donors and acceptors \( \leq 3 \) (Table 1). The “rule of three” is used as a good guideline to prepare libraries of fragments, which can be also chosen for their commercial availability and solubility at high concentrations (mM range). Methods of screening fragments include: directed binding assays (e.g. ELISA), NMR, mass-spectrometry and X-ray. Once active fragments are identified, they can be linked with chemical spacers known from previous inhibitors; alternatively a technique of “in situ assembly” can be employed. This technique consists of adding reactive fragments in the presence of the biological target and analysing the higher affinity products obtained.

The process of growing fragments into leads is often assisted by computational docking programs, which contain virtual library of linkers. The so-called de novo generation software use the coordinates of a known binding pose of a fragment in a target binding site and connect this fragment to virtual linkers and other fragments directly in the target binding site. The resulting virtual leads are docked and ranked according to predicted binding affinity values or ligand efficiency, which expresses the affinity per heavy atom (see Section 1.2.3.1). Examples of de novo generation software are shown in Table 2. Due to the potential of combining fragments in different ways, fragment-based approaches enable efficient coverage of chemical space.
Virtual screening is used in drug discovery to predict binding affinity of virtual or known compounds against a specific biological target or to design variations of known active molecules. Virtual screening can help to identify putative leads; therefore the software predictions of lead binding affinity need to be proven by established assays. Virtual screening methods can be differentiated into structure-based and ligand-based methods.

Structure-based virtual screening requires knowledge of the binding site of a biological target to predict binding poses of ligands and to estimate their binding affinity through scoring functions. The prediction of binding poses can be performed according to different docking programs; using stochastic or systematic methods. Stochastic methods perform random simulations of ligand binding poses until
convergence criteria are met. In the Monte Carlo method, for example, a ligand is randomly placed in the binding site and its degree of thermal motion is gradually decreased, until a stable state with minimum energy is reached. Systematic methods decompose ligands into fragments and dock them independently in a protein binding site; then the final binding pose is built on the basis of those docking results. The reconstruction of a ligand structure from fragments is performed through incremental construction or conformational search algorithm (for more details, see Table 3).

The estimation of binding affinity for the predicted ligand binding poses is made using scoring functions. Scoring functions are based on mathematical models and can be categorised as force-field, empirical and knowledge based. Force-field scoring functions assess the free energy of binding by deriving the enthalpy of binding at gas phase as sum of van der Waals and electrostatic intramolecular interactions. Intramolecular strain energy (angle, torsional, steric strain), entropic and desolvation energies are also included. Empirical scoring functions estimate the binding affinity from a series of energy terms: van der Waals, ionic, hydrogen bond, hydrophobic interactions and surface complementarity. The knowledge-based scoring function uses databases of known inhibitor-protein complexes to compare distance between ligand and protein atom types and to derive a favourable interaction. The ligand binding energy is derived from a function of distribution of atom pair interactions. The mathematical models, on which scoring functions are based, do not always consider entropic or flexibility factors in the estimation of binding affinity. Scoring functions are therefore limited and implementations of their mathematical models are still challenging.

Ligand-based virtual screening aims to enrich active compounds. Libraries of (virtual or real) ligands are compared with an active compound of reference according to structural and pharmacophoric similarity. The similarity search algorithm can perform a structure comparison by superimposing each ligand with the compound of reference (small molecule alignment search), or by considering electrostatic and conformational properties (pharmacophore similarity search). As an outcome, ligands are ranked according to similarity to the active compounds.
Table 3. Summary of virtual screening method.23

<table>
<thead>
<tr>
<th>Stochastic method</th>
<th>Characteristic</th>
<th>Examples of software</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monte Carlo</td>
<td>Cycles of simulations of ligand poses in the protein binding site are applied.</td>
<td>Autodock, ICM</td>
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<tr>
<td></td>
<td>The initial thermal motion of a ligand is gradually decreased until reaching a</td>
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<tr>
<td></td>
<td>minimum energy.</td>
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<tr>
<td>Genetic algorithm</td>
<td>Initial ligand states are defined according to conformation and orientations,</td>
<td>DOCK, GOLD</td>
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<tr>
<td></td>
<td>and then they are modified by random gene-fashion crossover and mutation.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The resulting new ligands are selected according to their fit.26</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Systematic method</th>
<th>Characteristic</th>
<th>Examples of software</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incremental construction algorithm</td>
<td>Ligands are broken into fragments by cutting at rotating bond positions.</td>
<td>DOCK 4.0, FlexX,</td>
</tr>
<tr>
<td></td>
<td>Set of rigid fragments, called anchors, are docked against the binding site.</td>
<td>Hammerhead</td>
</tr>
<tr>
<td></td>
<td>Flexible fragments are matched to anchors and docked considering different torsion angles.</td>
<td></td>
</tr>
<tr>
<td>Conformational search</td>
<td>Ligands are broken into fragments and a set of rigid fragments are docked.</td>
<td>eHiTS, FLOG</td>
</tr>
<tr>
<td></td>
<td>Only fragment poses which correspond to the original ligand structure are kept and flexible chains are added to these specific rigid fragment poses.</td>
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1.2.3.1 Criteria for ligand selection: ligand efficiency

Ligand efficiency (LE)27 is widely employed in the literature to evaluate the binding quality of ligands of different sizes to a target protein. LE is defined as the free energy of binding, \( \Delta G^0 \), of a ligand divided by its number of heavy atom, nHA (Equation 1). LE helps to identify ligands which bind to a target protein efficiently. Ligands with a large number of heavy atoms can show a high binding affinity, but they are less efficient than ligands showing the same binding affinity and having a smaller number of heavy atoms (Figure 2).28
Equation 1. Ligand efficiency definition.

\[ LE = \frac{\Delta G^o}{nHA} \]

**Figure 2. Representation of the ligand efficiency concept.** Cartoon of a protein binding site occupied by two ligands with the same binding affinity but of different sizes. A) The ligand is filling all the cavities of the protein binding site by interacting with five different groups, two of them with low-quality binding (orange and brown colour elements). B) The ligand is filling all the cavities of the protein binding site by interacting with four groups, each giving high-quality binding. Adapted from Kitchen et al.28

It is often reported in the literature\textsuperscript{29, 30} that when values of $K_i$ and $IC_{50}$ of ligands are available, they can be used in their logarithmic forms ($\log$) to calculate LE, in place of $\Delta G^o$, according to Equation 2.\textsuperscript{31} The difference between $\Delta G^o$ and $\log(K_i)$ is, in fact, a factor of 1.37, as shown in Equation 3.\textsuperscript{32} Therefore the LE' values calculated with $\log(K_i)$ would only differ from the LE values calculated with $\Delta G^o$ by the same factor (Equation 4).

\[ LE' = \frac{\log(K_i)}{nHA} \approx \frac{\log(IC_{50})}{nHA} \]

**Equation 2. Ligand efficiency calculated with $\log(K_i)$ and $\log(IC_{50})$.\textsuperscript{31}**

\textsuperscript{b} In Equation 2 the difference between $K_i$ and $IC_{50}$ is taken in account\textsuperscript{29}
\[ \Delta G^\circ = -2.30 \times RT \times \log(K_i) = -1.37 \times \log(K_i) \approx -1.37 \times \log(\text{IC}_{50}) \]

**Equation 3. Correlation between \( \Delta G^\circ \) and \( \log(K_i) \) or \( \log(\text{IC}_{50}) \).**

\[ \text{LE} = 1.37 \times \text{LE}' \]

**Equation 4. Difference between LE and LE'.**

The definitions of LE and LE', as expressed in the **Equation 1** and 2, can also be applied to assess the quality of binding of the virtual ligands. Docking software predict the binding affinity of virtual ligands giving a score which expresses \( \log(K_i) \); therefore this score can be used to calculate computed/predicted ligand efficiency, cLE or cLE', according to **Equation 5**. In this report LE' and cLE' will be used to compare quality of binding of known BACE-1 inhibitors (Section 1.4) or predicted inhibitors (Section 2.1.3)

\[ \text{cLE} = -1.37 \times \frac{\text{score}}{\text{nHA}}; \quad \text{cLE}' = -\frac{\text{score}}{\text{nHA}} \]

**Equation 5. Computed ligand efficiency, cLE and cLE'.**

### 1.2.4 Diversity-oriented synthesis

Combinatorial libraries of compounds of biological interest and large compound collections of pharmaceutical companies tend to lack diversity in shape and in structure, being mainly populated by flat compounds (2-D shape) which differ by their appendages, rather than their skeleton. An analysis of ten commercially available libraries, performed by Baell in 2012, showed that vendor libraries of ca. 400,000 compounds contained only 6,000 lead-like molecules, which lack diversity. Since the biological activity of compounds depends on their structural features, compound libraries with a high degree of structural diversity increase the probability of interaction with different biological targets. Therefore a demand for developing more structurally diverse libraries of lead-like molecules has risen in drug discovery.

It has been demonstrated that a convenient strategy to create a library of skeletally diverse compounds is to start from a range of different chemotypes, rather than
extending or varying the structure of a single chemotype. A successful synthetic approach towards diversity enriched libraries is that of diversity-oriented synthesis (DOS). The principle of DOS is to generate multiple scaffolds by performing parallel modification of starting materials to access a series of structurally diverse building blocks/chemotypes. These are able to undergo further diversification. A schematic representation of the DOS approach is given Figure 3.

**Figure 3. Diversity-oriented synthesis vs combinatorial library synthesis approach.**
A) A traditional approach in combinatorial library synthesis is based on variation of one chemotype, to obtain structure variations able to address a specific biological target. B) Diversity-oriented synthesis approach is based on a series of parallel modifications of an initial starting material to achieve a range of chemotypes leading to structurally diverse scaffolds. Adapted from Galloway et al. 33

A DOS strategy which uses a “reagent-based” approach was developed by Pizzarini et al. 36 (Scheme 1). The strategy employed one poly-functionalised starting material, the aminopropargylic alcohol 1, which was exposed to a series of different reagents to give three different chemotypes, compounds 2, 3 and 4, respectively via enyne metathesis, derivatisation with acetyl anhydride and intramolecular nucleophilic aromatic substitution. The resulting chemotypes contained different pairs of functional groups which were reacted intramolecularly under different conditions to give diverse cyclic scaffolds. For example, compound 3 was modified via enyne metathesis and the Pauson-Khand reaction, 37 giving scaffolds 5 and 6; while compound 4 underwent two
different cyclisation via Pauson-Khand reaction and enyne metathesis to lead to scaffolds 7 and 8.

Scheme 1. Example of reagent-based DOS, applied to the poly-functionalised aminopropargylic alcohol 1. Synthetic pathway developed by Pizzirani et al.\textsuperscript{36} Conditions: a) Hoveyda-Grubbs second-generation catalyst, ethylene, toluene, r.t.; b) acetic anhydride, Et\textsubscript{3}N, DMAP, CH\textsubscript{2}Cl\textsubscript{2}, 0 °C; c) NaH, THF, -10 °C; d) Hoveyda-Grubbs second-generation catalyst, ethylene, toluene, 45 °C; e) and f) [Co(CO)\textsubscript{8}], triethylamine N-oxide, THF, r.t.; g) Hoveyda-Grubbs first-generation catalyst, CH\textsubscript{2}Cl\textsubscript{2}, r.t., then Pb(OAc)\textsubscript{4}.

In our research group, DOS methodologies have been developed extensively to prepare lead-like molecules following a “substrate-based approach”.\textsuperscript{33} This approach uses a range of starting materials to couple under the same conditions, to form key
building blocks. Those building blocks contain functional groups, able to pair together and provide skeletally diverse molecules. This methodology was employed to prepare a variety of heterocyclic scaffolds based on piperazine, 1,4-diazepanes and 1,5 diazocanes, and a series of alkaloid-like scaffolds. In the case of heterocyclic scaffolds, a series of commercially available aminoalcohols was protected and then reacted with a range of cyclic sulfamidates to give cyclisation precursors. These precursors underwent to cyclisation to lead to a variety of heterocyclic scaffolds (Scheme 2).

Scheme 2. Example of substrate-based DOS to prepare lead-like heterocycles. Coupling of a range of N-nosyl amino alcohols with cyclosulfamidates led to the preparation of cyclisation precursors, able to cyclise to give diverse heterocyclic scaffolds. Ns: o-nitrophenylsulfonyl, Ns': p-nitrophenylsulfonyl. Adapted from James et al.

For the preparation of alkaloid-like molecules, a range of unsaturated building blocks was synthesised by attachment of propagating and terminating appendages to initial starting materials. The resulting cyclisation precursors underwent metathesis cascade reactions to yield skeletally diverse scaffolds. The initial building blocks were attached to a fluorous-tagged linker which facilitated the purification of the final scaffolds via fluorous solid-phase extraction (Scheme 3).
13

1.2.5 Combining diversity oriented synthesis and virtual screening

In this thesis a new approach to design novel bioactive lead molecules is described. To demonstrate the validity of the approach, it was applied to the BACE-1 protein. The approach combined in silico generation of a library of skeletally diverse lead-like
scaffolds, with virtual screening of this library against BACE-1. The generation of skeletally diverse lead-like scaffolds was obtained by designing a synthetic protocol based on DOS methodology. The aims in developing such an approach was, on one hand, to explore the chemical space of lead-like molecules by means of computer-aided design of DOS protocols; and on the other hand, to identify novel active leads, able to inhibit BACE-1, a target of pharmaceutical interest. Moreover, it was hoped to contribute in implementing lead discovery approaches, by combining the prediction of activity of lead-like scaffolds with information of their synthetic accessibility. An overview of the project is described in Section 1.5. In the following Section 1.3 the protein BACE-1 is introduced.

1.3 BACE-1

1.3.1 Involvement in Alzheimer’s disease

BACE-1 is a transmembrane human aspartic protease, known as ‘β-site amyloid precursor protein cleaving enzyme’ (BACE), which is expressed in high levels in the brain, mostly by neurons, and also in the peripheral tissues and in the pancreas14,15 in low level. BACE-1 enzymatic activity is displayed only in the brain,15 where it is involved in the early stage of formations of β-amyloid plaques, insoluble accumulations of neurotoxic amino acidic fragments recognised as the major symptoms of Alzheimer’s disease. Alzheimer’s disease is a neurodegenerative disease which affects memory, cognitive functions and behaviour. The causes of Alzheimer’s disease are still unknown, but the molecular mechanism associated with the disease is believed to start with the “amyloid cascade”.40 The amyloid cascade is a process which leads to the formation of neurotoxic peptides of 40/42 amino acids, called β-amyloid peptides Aβ40 and Aβ42, from the amyloid precursor protein (APP). APP is a substrate for different proteases, but its cleavage by BACE-1 starts an amyloidogenic pathway.

APP is cleaved by BACE-1 at the M-D bond contained in the sequence KMDAE, producing two fragments: an Aβ N-terminus soluble fragment, called APPsβ, and a C-terminus fragment, called CTF99.40 The CTF99 fragment is then heterogeneously cleaved by γ-secretase, releasing neurotoxic peptides Aβ40 and Aβ42. In the metabolism
of APP another alternative and ‘non-amyloidogenic’ pathway is initiated by the zinc metalloproteinase α-secretase; this pathway prevails in most cell types (Figure 4).\textsuperscript{41}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{amyloidogenic_nonamyloidogenic_pathway}
\caption{Amyloidogenic and non-amyloidogenic pathway of APP substrate. The APP can undergo two alternative and competing metabolic pathways. The major and non-amyloidogenic pathway, on the left, is started by α-secretase and precludes the formation of Alzheimer’s Aβ peptide. The amyloidogenic pathway, on the right, is started by β secretase. Adapted from Cole \textit{et al.}\textsuperscript{40}}
\end{figure}

Aβ\textsubscript{40} and Aβ\textsubscript{42} are insoluble peptides which aggregate to form β-amyloid plaques; they are formed in the brain cells, but they can be transported outside spreading their damaging effect.\textsuperscript{42} According to the amyloid cascade hypothesis, the formation of β-amyloid plaques has two main consequences: the activation of an inflammatory response and the hyperphosphorylation of Tau protein. The inflammatory response relies principally on the activation of brain glial cells, which degenerate and produce oxygen free radicals causing damage and neurons death.\textsuperscript{43} The hyperphosphorylation of Tau protein induces pairing of helical filaments of the protein which accumulate, resulting in the so called toxic “neurofibrillary tangles”\textsuperscript{44}.

It has been demonstrated that BACE-1 inhibition reduces Aβ peptide levels in mouse brain and up-regulates the α-secretase non amyloidogenic pathway.\textsuperscript{45} While BACE-1 total inhibition caused development of cognitive dysfunctions in mice,\textsuperscript{46} since
BACE-1 is necessary for specific hippocampal memory processes, its partial inactivation may not affect normal learning and memory processes. Therefore BACE-1 has been considered a good therapeutic target for the disease.

1.3.2 Structure and catalytic mechanism

Due to its pharmaceutical interest, BACE-1 has been targeted and studied widely in the last three decades. There are more than 250 crystal structures of BACE-1 in complex with inhibitors, and BACE-1 structural features and catalytic mechanism have been disclosed. The main characteristics of BACE-1 are: a flexible region, located in front of the substrate binding cleft, and the presence of two conserved water molecules in its catalytic site. These characteristics are in common with other aspartic proteases, with which BACE-1 shows from 24 to 52% of sequence identity (e.g. 29% with cathepsin D, 52% with BACE-2, 24% with renin, 27% with pepsin).

The BACE-1 flexible region, called “flap”, is a β hairpin structure composed of 14 residues (from Lys65 to Glu79) and a sequence of 7 amino acids (from Ala101 to Lys107) connected by antiparallel H-bond interactions and forming three strands which cover the binding cleft. The role of this flexible region in other aspartyl proteases is unclear; while X-ray structures of numerous of BACE-1 complexes revealed that this flexible region induces a conformational change in the protein, from an open and closed position, allowing the insertion of APP substrate in the catalytic site. The substrate-free BACE-1 structure (apo-structure) adopts an open conformation in which the flap is distant from the binding cleft and APP substrate can access into the active site. A closed conformation has been observed when BACE-1 is in complex with inhibitors and the flap residues move up to ca. 4.5 Å closer to the binding cleft (Figure 5).
Figure 5. Features and conformations of the flexible region of BACE-1, called “flap”. A) Apo-structure of BACE-1 (pdb code 1W50) with the flexible region, flap, highlighted in yellow. The flap is covering the binding cleft. B) Example of protein adopting open flap conformation (pdb code: 1W50). C) Example of closed flap conformation: BACE-1 is in complex with an inhibitor (pdb code 2XJT) and the flap surface is overlapping with the top surface of the protein, which limits the binding cleft. Visualised with Discovery Studio 3.0.
In the flap region Tyr71 is involved in key H-bond interactions which determine the protein conformational change. In the open conformation, Tyr71 forms a H-bond (CO backbone) with Gly74 (NH backbone) of ca. 2.8 Å of length. In the closed conformation, Tyr71 can be oriented towards Trp76 or towards Lys107 and can form new H-bonds with the O atom of the OH group of Tyr71 side chain (Figure 6).

Figure 6. Example of H-bond interactions formed in the flap regions of BACE-1. 14 amino acids (from Lys65 to Glu79) and 7 amino acid sequences (from Ala101 to Lys107) connected by antiparallel H-bonds, which constitutes the flap region. A) H-bonds formed in a flap open conformation; Tyr71 and Gly74 (yellow colour) form a characteristic H-bond between Tyr71 (CO backbone) and Gly74 (NH backbone) (pdb code 1W50). B) H-bonds formed in a flap closed conformation (pdb code 2XJT). A new H-bond is formed between Tyr71 (OH) and Lys107 (CO backbone). Visualised with Discovery Studio 3.0.
As with other aspartyl proteases, BACE-1 contains two proximal aspartyl residues, Asp228 and Asp32, and two conserved water molecules in the catalytic site. One water molecule (Wat1) is located between Asp32 and Asp228 and seems to assist the catalytic process of the natural substrate. The second molecule (Wat2) is part of a conserved network of hydrogen-bonds which involves Ser35 (Figure 7). The proteolysis mechanism of BACE-1 is an acid-base catalysed mechanism mediated by Wat1 and by the Asp228/Asp32 pair (see Figure 8).

Figure 7. Water molecules located in the catalytic site of BACE-1. The two conserved water molecules, Wat1 and Wat2, form a network of H-bond (green) with the proteolytic Asp228 and Asp32, and with Ser35.

Figure 8. Catalytic mechanism of BACE-1. The conserved water molecule Wat1, located between the two catalytic aspartates, takes part in the proteolytic mechanism of APP substrate. Asp32 is represented in its mono-protonated form, which is likely to be assumed at pH 4.5, at which BACE-1 is active.
As shown in Figure 8, one catalytic aspartate, Asp32, seems to assume a monoprotonated state where the inner oxygen contains the proton.\textsuperscript{55} It has been reported that BACE-1 optimal activity is at pH 4.5.\textsuperscript{52} The binding of the inhibitor resulted to be pH dependent: at pH 5.0 the inhibitor binding seems to occur simultaneously with protein conformational switching, whilst at pH 4.5 proteolysis of the inhibitor occurs.

### 1.3.3 Specificity for the APP substrate

The specificity of BACE-1 for APP has been explained from studies of mutations of APP. The cleavage point of APP is the M596-D597 bond of the EVKMDAEF sequence. According to the standard nomenclature of proteases, these residues are labelled P4-P3-P2-P1*P1'-P2'-P3'-P4' and the cleavage point is indicated by * (Figure 9).

![Figure 9. Amino acids sequence of BACE-1 APP substrate, flanking the cleavage point. The cleavage point between M and D is in highlighted in red.](image)

The sequence EVNL*DAEF of the Swedish mutant is very efficiently hydrolysed by BACE-1; while a mutation of M596 with V at P1 position reduces the cleavage.\textsuperscript{57} Sauder \textit{et al.}\textsuperscript{58} explained this difference of substrate preference by modelling interactions formed by P1*P1' residues of APP substrates with BACE-1 residues located in the catalytic site. The P1' residue of APP can form a salt bridge with Arg235 of BACE-1; while hydrophobic contacts can be established by the P1 residue with Leu30 and Ile118 of BACE-1, located in hydrophobic pocket of the
protein, and with Tyr71 located in the flap region. These interactions could show BACE-1 specificity for a negative charge at P1’ and for a hydrophobic residue at P1. They also reveal a difference with most aspartyl proteases, which have a preference for hydrophobic residues at P1’ (Figure 10).

Figure 10. BACE-1 region of interactions with P1 and P1’ residue of APP substrate. A hydrophobic region containing Leu30 and Ile118 interacts with P1 lipophilic residue of APP, M, together with the lipophilic Tyr71, located in the flap. A positively charged region, containing Arg235, shows preference for interaction with negatively charged P1’ residue of APP, D. Visualised with Discovery Studio 3.0.

The Swedish mutant substrate, containing Leu at P1, can maintain hydrophobic contacts with Leu30, Ile118 and Tyr71 of BACE-1. When the P1 residue of APP is mutated to V, one of the methyl groups of V can be oriented towards the catalytic Asp32 of BACE-1 and interferes with the catalytic activity; at the same time hydrophobic interactions with Leu30, Ile118 and Tyr71 would be suppressed. This could explain why BACE-1 hydrolyses the Swedish mutant substrate more efficiently.
The BACE-1 specificity for APP is also determined by other interactions with APP residues P2, P3, P2' and P3'. These residues seem to occupy specific pockets of BACE-1, which are named according to the contacts made with APP residues (Figure 11). The study of pocket interactions was important in the development of BACE-1 inhibitors.

**Figure 11. BACE-1 pockets.** BACE-1 pockets are separated by the flap region (in yellow) and are numbered according to the interactions made with residues P1, P2, P3 and P1', P2', P3' of APP substrates. Visualised with Discovery Studio 3.0.

### 1.4 Overview of BACE-1 inhibitors

BACE-1 inhibitors can be separated into two major categories: peptidomimetic inhibitors and non-peptidomimetic inhibitors. The first attempts to design BACE-1 inhibitors were based on structural modifications of the APP substrate which led to a series of peptides containing a transition state isostere modification in the substrate backbone. Further research was directed to the discovery of a series of potent and lower molecular weight peptidomimetic inhibitors (M.W. ~700), but the need to improve the inhibitor brain penetration forced the investigation towards a series of small non-peptidomimetic inhibitors (M.W. <500). The design of these inhibitors has proven to be extremely difficult due to the large size of the active site of BACE-1 (> 10000Å). 59, 60
1.4.1 The first peptidomimetic inhibitor: compound OM99-2

The first peptidomimetic inhibitor, OM99-2, reported by Tang and co-workers\textsuperscript{57} was based on an octapeptide sequence of EVNL*AAEF in which the L-A bond was substituted by a hydroxyethylene transition-state isostere (Figure 12). The design of the inhibitor was based on the substitution of the P1’ residue of the Swedish mutant EVNL*DAEF. The crystal structure of BACE-1 in complex with OM99-2 shows that the inhibitor forms a network of H-bonds in the active site, which involves the catalytic Asp32 and Asp228. OM99-2 is a nanomolar inhibitor of BACE-1 ($K_i$=1.6 nM).\textsuperscript{61}

![Figure 12. Structure of the OM99-2 inhibitor. 2D structure and representation of H-bonds formed between the OM99-2, first BACE-1 inhibitor,\textsuperscript{57} and BACE-1 residues in the catalytic site. The inhibitor structure is based on a reduced form of the Swedish mutant peptide, having only eight amino acids. A hydroxyethylene isostere group is contained at the centre of the sequence (highlighted in red) in replacement of the peptidic bond L-D, in position P1 and P1’. The amino acids are represented according to the likely charge state assumed at pH 4.5, at which BACE-1 is active.](image)

Improvement of the OM99-2 structure led to the peptide analogue ELDL*AVEF. This inhibitor, OM00-3, forms three new H-bonds with NH and CO groups of the backbone of residue E and with the NH of the backbone of residue F in P3’ and P4’ position. Its $K_i$ value is 0.3 nM (Figure. 13).\textsuperscript{50} In the BACE-1 complex formed with OM99-2 and with OM00-3 inhibitor, the water molecule Wat1, located between the two catalytic aspartates, is displaced by the inhibitor in the binding site.\textsuperscript{62}
Figure. 13. Structure of the OM00-3 inhibitor. 2D structure and representation of H-bonds formed between the OM00-3 inhibitor and BACE-1 residues in the catalytic site. The OM00-3 inhibitor contains an hydroxyethylene bond between P1-P1’ residue (in evidence in red). The E and F residues of the inhibitor are involved in new H-bonds with BACE-1.

1.4.2 Inhibitors incorporating a transition state isostere as a central core

Peptide analogues containing a transition state isostere were proven to be effective inhibitors of the BACE-1 enzyme. A key structural feature of these compounds is the hydrogen bonds formed between the secondary hydroxyl group with the Asp32 and Asp228 residues of BACE-1 in the catalytic site (as shown in Figures 12 and 13). A series of BACE-1 inhibitors containing this hydroxyl group has been reported; the structures are based on statine, tert-hydroxy motif, hydroxyethylene (HE), aminoethylene and hydroxyethylamine (HEA); units that are non-cleavable isosteres of the transition state (Figure 14).
Figure 14. Inhibitors incorporating a transition state isostere. A) Representation of the central core of transition state isostere-type inhibitors. The hydroxyl group or the primary amine is incorporated in replacement of a peptide bond. B) HE isostere inhibitor designed by Wångsell et al.\textsuperscript{65} C) Aminoethylene isostere inhibitor designed by Yang et al.\textsuperscript{67} D) HEA isostere inhibitor designed by Stachel et al.\textsuperscript{68} Red circles highlight the isostere central core of the inhibitors.

The inhibitors presented in Figure 14 show a higher lipophilic character in comparison with the peptidomimetic inhibitors OM99-2 and OM00-3. The aromatic groups were introduced in the structure to fill BACE-1 hydrophobic pockets. In the case of the inhibitor 13, an interaction in the S1 pocket of BACE-1 was established by increasing the size of a lipophilic group at P1; while the S2 pocket was occupied by the sulfonyl groups, and the S2' was filled with a benzylic group (Figure 15).\textsuperscript{65}
Figure 15. Example of interactions of the HE inhibitor 13 in BACE-1 pockets. 3D representation of the conformation assumed by BACE-1 in complex with the HE inhibitor 13. S1, S2 and S3 pockets of BACE-1 are occupied by the aryl group at P1, the sulfonyl group at S2 and the benzylic group at P3. Another benzyl group at P2' occupies S2' pocket of BACE-1. Adapted from Wångsell et al.66 (pdb code 3IXK). Visualised with Discovery Studio 3.0.

1.4.3 Reducing inhibitor peptidic character, towards macrocyclic inhibitors

Most of the peptide-like BACE-1 inhibitors showed a limited brain penetration due to their large molecular weight and their affinity for P-glycoprotein.69 Some structural adjustments were made to reduce molecular weight and peptide character, such as removing the P3-P4 amide segment and modifying the P2-P3 positions. Machauer et al.70 modified the structure of the inhibitor OM99-2 by: replacing Asn chain at P2 with a methyl group, methylating the N atom at P2-P3 bond, and ring closing the P1-P3 extremities. A macrocyclic inhibitor was derived, compound 16, which displayed a sub-micromolar activity, IC$_{50}$= 0.15 µM (Scheme 4).
Scheme 4. Example of a macrocyclic inhibitor derived from OM99-2 inhibitor structure. Gradual modifications of the inhibitor OM99-2, which led to the macrocyclic inhibitor 16. The length of the amino acid chain of OM99-2 was reduced by eliminating P3-P4 and P3'-P4' fragments and by methylation of N atom at P2-P3 bond. Cyclisation of lateral chain in P1-P3 gave the macrocycle 16. The isostere central core (highlighted in red) was kept in the macrocycle structure. Adapted from Machauer et al. 

Macrocyclic inhibitors demonstrated a low molecular weight, potential for high cell permeability and good proteolytic stability. Structural differences of these inhibitors relied on the lateral chain linked to the ring (which contains the isostere unit); on the number of atoms contained in the ring (generally between 15 and 17 membered) and on the rigidity of the ring. Examples are provided in Figure 16.
**Figure 16. Other macrocyclic inhibitors.** A) Inhibitor designed by Hanessian et al.\textsuperscript{72}, containing a HE unit in the later chain (highlighted in red). B) Inhibitor designed by Lecher et al.\textsuperscript{73}, containing a HEA unit in the later chain (highlighted in red) and a longer and more rigid ring structure.

Further structural development brought to eliminate of the isostere core, as shown in the macrolactone inhibitor 19. Compound 19 contains a primary amine which forms key H-bond interactions with the catalytic Asp32 and Asp228.\textsuperscript{74} The macrolactone 19 occupies S1, S2 and S3 pockets of BACE-1 (Figure 17).

**Figure 17. Macrolactone inhibitor 19.** A) 2D structure and representation of the key H-bonds (green dotted line) formed by the inhibitor 19 with Asp32 and Asp228 of BACE-1. The inhibitor does not contain an isostere core; the primary amine is responsible for forming key H-bond interactions. B) 3D representation of the conformation assumed by BACE-1 in complex with the inhibitor 19: S1, S2 and S3 pockets are filled by aromatic groups (pdb code 2QZK). Adapted by Moore et al.\textsuperscript{74} Visualised with Discovery Studio 3.0.
1.4.4 Discovery of non-peptidomimetic inhibitors

Since 2006, many BACE-1 non-peptidomimetic inhibitors were discovered by fragment-based screening and optimised by computer modelling and SAR studies. These inhibitors have different structures but they all contain a heterocyclic unit and a central primary amine, which forms key H-bond interactions with the Asp32 and Asp228 in the BACE-1 catalytic site. These inhibitors can be divided into: pyridinyl aminohydantoin,75-77 aminopyridine,78, 79 acylguanidine,80, 81 dihydroquinazoline,82 aminimidazole,83 and spiropyrrolidine84 inhibitors. X-ray structures of BACE-1 in complex with these inhibitors revealed that BACE-1 adopts an open or semi-closed flap conformation. The water molecule located between the two catalytic aspartates, Wat1, is displaced by the inhibitors. Examples of interactions formed by these heterocyclic inhibitors in the BACE-1 catalytic site are reported in Figures 18-21. Value of activity against BACE-1 and respective LE' are shown; values of activity against BACE-2, a homologue of BACE-1 with 52% of sequence similarity, are also shown when available.

Figure 18. Pyridinyl aminohydantoin inhibitor 20. A) 2D structures and representation of key H-bond (green dotted line) formed by the inhibitor 20 with Asp32 and Asp228 of BACE-1. B) 3D representation of the conformation assumed by BACE-1 in complex with the inhibitor 20: S1, S3 pockets are filled by aromatic groups. The flap is open (pdb code 3IN4), adapted from Malamas et al.75 Visualised with Discovery Studio 3.0.
Figure 19. Aminopyridine inhibitor 21. A) 2D structures and representation of key H-bond (green dotted line) and π interactions (orange dotted line) formed by inhibitor 21 with BACE-1 residues in the catalytic site. B) 3D representation of the conformation assumed by BACE-1 in complex with the inhibitor 21: S1, S3 pockets are filled by aromatic groups. The flap is semi-closed (pdb code 2OHU), adapted from Congreve et al.\textsuperscript{78} Visualised with Discovery Studio 3.0.

Figure 20. Acylguanidine inhibitor 22 and dihydroquinazoline inhibitor 23. 2D structures and representation of key H-bond interactions (green dotted line) formed by inhibitors 22 and 23 in with BACE-1 residues in the catalytic site. A) Acylguanidine inhibitor 22 designed by Cole et al.\textsuperscript{81} B) Dihydroquinazoline inhibitor 23 designed by Baxter et al.\textsuperscript{82}
1.4.5 An interesting spiropiperidine iminohydantoin inhibitor

Among the series of non-peptidomimetic BACE-1 inhibitors identified by fragment-based drug design, there is the interesting spiropiperidine iminohydantoin inhibitor 26.\(^8\) The X-ray structure of the inhibitor 26 in complex with BACE-1 showed a particular feature of H-bond interactions. The water molecule located between the two catalytic aspartates, Wat1, is not displaced but forms a H-bond network with the N of the piperidine group of the inhibitor and with the BACE-1 catalytic aspartates. Another water molecule forms H-bonds with the secondary amine group of the inhibitor and with Gly34 and Asp228. This type of inhibition through water molecules is a new mode of inhibition (Figure 22). The inhibitor 26 also forms H-bonds with Phe108 and Thr72, located in the flap region, locking the protein in an open conformation.
Figure 22. Spiropiperidine iminohydantoin inhibitor 26. 2D (A) and 3D (B) representation of the binding pose of the inhibitor 26 in the BACE-1 catalytic site. H-bond interactions are represented as green dotted line, water molecules are highlighted in red. The characteristic of the spiropiperidine inhibitor 26 is to inhibit BACE-1 through a network of H-bonds with two bridge water molecules, one of which is the conserved water molecule Wat1. Adapted from Barrow et al.\textsuperscript{85} (pdb code 3FKT). Visualised with Discovery Studio 3.0.

Inspired by this kind of water bridge inhibition mode, Brodney et al.\textsuperscript{86} designed a series of a spirocyclic sulfamide inhibitors. Among those, the inhibitor 27 assumes a binding pose similar to the previously shown inhibitor 26. Wat1 is involved in a H-bond with the N of the piperidine group of the inhibitor and forms a network of H-bonds with the catalytic aspartates of BACE-1. Another water molecule forms a H-bond with Asp228. The X-ray structure shows also another H-bond formed by the O-alkyl group of the aromatic substituent of the inhibitor with a third water molecule (Figure 23).
Figure 23. Spirocyclic sulfamide inhibitor 27. 2D (A) and 3D (B) representation of the binding pose of the inhibitor 27 in the BACE-1 catalytic site. H-bond interactions are represented as green dotted line, water molecules are highlighted in red. The inhibitor has a binding pose similar to inhibitor 26 (Figure 22). The conserved water molecule Wat1 is involved in a bridge network of H-bonds. Two other water molecules are present in the catalytic site region. The sulfamide ring is involved in a H-bond with the NH (backbone) of Gln73 in the flap region. Adapted from Brodney et al.86 (pdb code 4FM7). Visualised with Discovery Studio 3.0.

1.4.6 Inhibitor designed through computational methods

Virtual screening was also successful in discovering new structures for BACE-1 inhibitors. In 2010 Xu et al.87, 88 identified two new BACE-1 inhibitors by screening in silico libraries of commercially available compounds with lead-like properties (Section 1.1). One inhibitor was based on a pyrazole-derived central core and the other on a benzothiazole central core (Figure 24).
Figure 24. Inhibitors identified via virtual screening by Xu et al. 87, 88 A) Inhibitor 28 contains a pyrazole-derived central core. B) Inhibitor 29 contains a benzothiazole central core.

In 2013 Mok et al. 89 employed a de novo generation software, SPROUT, to design a series of biphenylacetamide BACE-1 inhibitors. Among those, the inhibitor 30 showed an IC₅₀ of 33 μM (Figure 25). In 2014 Viklund et al. 90 generated novel cores for BACE-1 inhibitors based on de novo design of lead molecules and computational prediction of their affinity, permeability and synthetic feasibility. They derived and synthesized seven active cores, containing variation elements from known cores (Figure 26).

Figure 25. Inhibitors designed through de novo generation software. Inhibitor design by Mok et al. 89 using SPROUT.
Figure 26. Cores for BACE-1 inhibitors identified by de novo design. Seven cores designed by Viklund et al.\textsuperscript{90}

1.4.7 Challenges of BACE-1 inhibitor design, failure in clinical trials

Despite the wide range of BACE-1 inhibitors designed, only few of them entered into clinical trials. To enter a phase I clinical trial BACE-1 inhibitors require good cellular potency (nM range) and selectivity for BACE-1 vs BACE-2 and vs cathepsin D (100-fold). Inhibitors designed by Eli Lily, AstraZeneca and Roche from 2009 onwards failed phase I studies due to the poor blood-brain barrier permeability. Currently only two inhibitors are in phase II and phase III trials, one is E\textsuperscript{2609} of Eisai (phase II) and the other is MK-8931 of Merck (phase III).

Due to the failure of clinical studies, a new approach based on antibody therapies was proposed in 2011 to target BACE-1. A human antibody, able to target BACE-1 and to induce conformational changes on residues 157-170 of the protein, was developed by Atwal et al.\textsuperscript{91} Residues 157-170 of BACE-1 normally adopt $\alpha$-helical structure when the protein binds APP substrate. In the antibody-BACE-1 complex these residues assume a random loop structure, which seems to prevent APP substrate to reach the catalytic site of the protein. The anti BACE-1 antibody therapy showed in mice high selectivity and effective reduction of $\beta$-amyloidal peptides.

1.5 Project outline

The project described herein was directed to develop a novel computational and experimental approach to design lead-like bioactive molecules by generating a virtual library of likely synthetic accessible lead-like compounds based on DOS strategy. To
investigate the approach, BACE-1 protein was chosen as biological target, and the virtual library of lead-like compounds was screened *in silico* against BACE-1.

A virtual library of likely synthetic accessible lead-like compounds was generated by following DOS methodologies developed in the Nelson group. A protocol of synthesis was designed on the basis of known chemical reactions and commercially available reactants, and was built using Pipeline Pilot software. The virtual library was screened *in silico* against BACE-1 using eHits software. Putative BACE-1 inhibitors were identified from the virtual screening according to criteria of ligand efficiency (cLE’) and were selected for synthesis. Two synthetic routes were investigated to prepare a focused library of putative inhibitors. The biological activity of the focused library was assessed *via* a fluorimetric assay.

This report is composed as follows. Chapter 2 describes computational tools (Pipeline Pilot and eHiTS) and strategy envisaged to design lead-like putative BACE-1 inhibitors. Chapter 3 describes two synthetic routes towards a focused library of putative BACE-1 inhibitors. Chapter 4 presents results of the biological activity of putative BACE-1 inhibitors; describes further SAR studies and provides a discussion of the obtained results.
Chapter 2. Structure-based design of putative bioactive molecules for BACE-1

The following Chapter describes a novel computational approach to identify new putative bioactive molecules for BACE-1. The approach involved the design of a virtual library of likely synthetically accessible lead-like compounds based on diversity-oriented synthesis (DOS) and the virtual screening of this library against BACE-1. An overview of the approach, including a description of the computational tools employed for the enumeration of a virtual library (Pipeline Pilot) and for virtual screening (eHiTS), is presented in Section 2.1. Demonstration of the validity of the virtual screening method is given in Section 2.2. A detailed description of the performed computational process is shown in Section 2.3 and analysis of the identified putative inhibitors is presented in Section 2.4.

2.1 Overview of the computational approach

The proposed computational approach combined the in silico enumeration of likely synthetically accessible compounds using DOS and virtual high-throughput screening (vHTS). The approach was directed towards the identification of novel small cyclic BACE-1 inhibitors, able to interact with the catalytic aspartates, Asp32 and Asp228, and/or with two water molecules located in the catalytic site of BACE-1 (similarly to the inhibitor 26, Section 1.4.5).

Two libraries of cyclic molecules were generated using Pipeline Pilot. The first library, library A, was optimised after analysing the results of an initial vHTS round against BACE-1. The optimisation of library A led to the generation of the more focused library B. From each library, lead-like molecules were selected according to specific structural and lipophilicity parameters: number of heavy atoms, $16 \leq nHA \leq 23$ for library A and $16 \leq nHA \leq 27$ for library B; number of aromatic rings $\leq 2$; value of the atomic-based prediction partition coefficient, $92 \leq \text{AlogP}^c \leq 3.5$. The number of saturated rings $> 0$ was also considered as a parameter for the selection of molecules in

\*AlogP is an algorithm estimation of the partition coefficient, $P_{octanol/water}$, based on the contribution of different atoms according to their physicochemical properties.
order to verify that only cyclic molecules were generated from the enumeration of the two virtual libraries.

The selected lead-like molecules underwent vHTS using two BACE-1 structures: one containing two water molecules in the catalytic site and the other one without water molecules. After the vHTS of library B, putative inhibitors were chosen for synthesis. The overall computational approach is shown in Figure 27.

![Computational Approach Diagram](image)

**Figure 27. Summary of the computational approach which led to the identification of novel putative BACE-1 inhibitors.** 1) The approach starts with the enumeration of a virtual library of likely synthetically accessible molecules, library A. The generated library was filtered according to lead-like parameters and the resulting molecules were screened *in silico* against the target protein BACE-1. Promising ligands were identified and analysed. 2) The initial library A was optimised and the more focused library B was obtained. Library B underwent the same computational process to identify putative inhibitors of BACE-1.

### 2.1.1 Pipeline Pilot

Pipeline Pilot\textsuperscript{93, 94} is a scientific informatics platform which can be used to generate virtual compound libraries. Pipeline Pilot allows the creation of configurable protocols to manage, order and analyse scientific data, and to enumerate combinatorial processes. Configurable protocols can be built by assembling specific components which store,
The chemistry components in Pipeline Pilot are able to: perform virtual reactions, modify chemical structures, enumerate libraries, calculate different molecular properties, search molecules according to substructures and similarities, and cluster and filter compounds. An example of a configurable protocol designed in Pipeline Pilot to enumerate a virtual library of structurally diverse compounds is illustrated in Figure 28.

**Figure 28. Configurable protocol designed in Pipeline Pilot to enumerate a virtual library of structurally diverse compounds.** The protocol was built with a series of components. Firstly starting materials and reactants are read in the form of a ChemDraw file (components “Reader” 1 and 2, on the left), then a series of virtual reactions are performed through chemistry components. The data are then processed (removing duplicates and generating a 3D conformation per molecule) and stored in the form of SD file. The resulting molecules are then visualised as HTML and Excel File (components on the right).
2.1.2 eHiTS

eHiTS\textsuperscript{23} (electronics \textbf{H}igh \textbf{T}hroughput \textbf{S}creening) is one of the many docking programs (Section 1.2.3) employed in the virtual screening of molecules. eHiTS contains a systematic structure search algorithm to predict the binding pose of a ligand in a protein target site,\textsuperscript{23} and an empirical scoring function to estimate binding affinity. The structure search algorithm in eHiTS identifies rigid fragments and flexible chains contained in the ligand structure, and docks rigid fragments separately in any available location of the protein binding site. The docked poses of the fragments which are in proximity to each other are considered “pose sets” and are combined together through a graph matching algorithm. The ligand flexible chains are connected to the “pose sets”, without changing their original conformation, and the overall structure of the ligand is then reconstructed. The reconstructed ligand structure is then optimised by considering low energy rotamers or by resolving steric clashes. The predicted ligand pose is scored according to an empirical scoring function.

The scoring function detects interactions between surface points of a ligand’s rigid fragments and of a protein binding site to determine the initial score. Surface points are classified according to chemical properties and are divided into 23 different types: metals, H-bond donors, weak H-bond donors, lone pairs, π-electrons, halogens, positively charged hydrogens, \textit{etc.} An estimation of the binding affinity of a ligand is derived from a statistic sum of the ligand surface point types and it is expressed as a negative value on a logarithmic scale.\textsuperscript{23} The estimated ligand binding affinity can be correlated with the ligand inhibition constant for the target protein, $K_i$, as follows: an eHiTS score of \(-7.0\) would correspond to an estimated $10^{-7}$ M inhibitor.

Protonation states of the ligand and the protein binding site are important factors in predicting the ligand binding pose and its affinity. eHiTS scores all possible protonation states of the ligand and the protein in one run and selects the best protonation state for each single interaction. eHiTS can screen thousands of molecules in one run, enabling virtual high throughput screening.
2.1.3 Ligand selection

As shown in Figure 1 (Section 2.1), promising ligands were selected from virtual screening of library A and putative inhibitors were selected from virtual screening of library B. The criteria of selection of promising ligands and putative inhibitors were based on values of cLE’ (Section 1.2.3.1) and molecular weight: cLE’ ≥ 0.27 or 0.28 and 200 ≤ M.W. ≤ 460. The cLE’ was calculated from the eHiTS score according to Equation 6. The limiting value of cLE’ was chosen according to the distribution of data, in order to focus on the best 1-2% of cLE’.

\[ \text{cLE’} = -\frac{\text{eHiTS score}}{\text{nHA}} \]

**Equation 6.** Computed ligand efficiency calculated from eHiTS scores.

The range of molecular weight and lipophilicity chosen to select promising ligands and putative BACE-1 inhibitors (200 ≤ M.W. ≤ 460 and 0 ≤ AlogP ≤ 3.5) respects the lead-likeness criteria suggested by Monge et al.\(^6\) (M.W. ≤ 460 and logP ≤ 4). According to the chemical space definition of Nadin et al.\(^2\) (Section 1.1), instead, this range includes mostly lead-like compounds and a small part of drug like-compounds (Figure 29). The selection of promising ligands and putative inhibitors selection was performed by using a specific protocol designed in Pipeline Pilot (Figure 30).

**Figure 29.** Chemical space explored in the identification of putative bioactive molecules. The blue area shows the chemical space explored in the proposed approach to identify bioactive molecules. According to the representation of chemical space of Nadin et al.,\(^2\) this area includes mostly lead-like and part of drug-like molecules.

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**41**
Figure 30. Protocol designed in Pipeline Pilot to select virtual ligands identified by vHTS against BACE-1. The results files of any vHTS rounds, containing eHiTS scores, are used to calculate cLE'. The results are filtered according to cLE' and M.W. values, and are visualised in a scatter plot of AlogP vs cLE'. 
2.2 Testing the applicability of eHiTS to BACE-1

The docking method employed by eHiTS was validated before being applied to the virtual screening of libraries A and B. To verify the applicability of eHiTS to BACE-1 and to confirm the validity of eHiTS in predicting ligand binding poses, a docking test was performed. The test consisted of reproducing the binding pose of a known inhibitor in the catalytic site of BACE-1 using eHiTS. The known co-crystal structure of the spiropiperidine iminohydantoin inhibitor 26 bound to BACE-1, referred as 3FKT hereafter, was chosen for the docking test (Figure 31). The compound 26, a 3 μM inhibitor, inhibits BACE-1 via a hydrogen bonding network with the conserved water molecule Wat1, located between the catalytic aspartates, and another water molecule contained in the protein catalytic site (Section 1.4.5).

![Figure 31. 2D representation of the inhibitor 26 in complex with BACE-1.](image)

BACE-1: IC$_{50}$ = 3.0 μM, LE' = 0.18

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*3FKT corresponds to the PDB access number of the complex formed between the inhibitor 26 and BACE-1.
To reproduce the mode of interaction of the inhibitor 26 with BACE-1, a compatible structure had to be derived from 3FKT. The coordinates corresponding to the inhibitor 26, as well as the coordinates corresponding to all water molecules, except for the two in proximity to the catalytic aspartates, were removed. The resulting structure is shown in Figure 32.

![Figure 32. Derived BACE-1 structure for docking test.](image)

A) Original X-ray co-crystal structure of the 3FKT (1.90 Å). B) Derived BACE-1 structure used for docking test. The coordinates of the ligand and the water molecules, except for the ones in proximity to the catalytic aspartates, were removed. Structures visualised with Discovery Studio 3.0.

25 different three-dimensional conformations of the inhibitor 26 were generated and docked against BACE-1. The resulting binding poses were analysed and sorted according to their eHiTS score. The top five binding poses predicted by eHiTS are reported in Table 4.

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*The three-dimensional conformations of the inhibitor 26 were generated using Corina*
Table 4. Top scoring poses of inhibitor 26 predicted by eHiTS software.

<table>
<thead>
<tr>
<th>Entry</th>
<th>eHiTS predicted pose</th>
<th>eHiTS score</th>
<th>Predicted (grey) vs experimental (blue) pose&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td><img src="image1.png" alt="Predicted Pose 1" /></td>
<td>–7.05</td>
<td><img src="image2.png" alt="Experimental Pose 1" /></td>
</tr>
<tr>
<td>2</td>
<td><img src="image3.png" alt="Predicted Pose 2" /></td>
<td>–7.03</td>
<td><img src="image4.png" alt="Experimental Pose 2" /></td>
</tr>
<tr>
<td>3</td>
<td><img src="image5.png" alt="Predicted Pose 3" /></td>
<td>–6.89</td>
<td><img src="image6.png" alt="Experimental Pose 3" /></td>
</tr>
<tr>
<td>4</td>
<td><img src="image7.png" alt="Predicted Pose 4" /></td>
<td>–6.88</td>
<td><img src="image8.png" alt="Experimental Pose 4" /></td>
</tr>
<tr>
<td>5</td>
<td><img src="image9.png" alt="Predicted Pose 5" /></td>
<td>–6.82</td>
<td><img src="image10.png" alt="Experimental Pose 5" /></td>
</tr>
</tbody>
</table>

<sup>a</sup>Binding poses visualised with Discovery Studio 3.0
The predicted binding pose ranked as second, according to its eHiTS score, was in good agreement with the pose of the inhibitor 26 (Table 4, entry 2). The root mean square deviation (RMSD) of heavy atom position between this predicted binding pose and the co-crystal structure pose of the inhibitor 26 was of 1.36 Å; in line with the accepted limited value of Å ≤ 2.0 for reliable prediction. This predicted binding pose forms one H-bond between the piperidine-N atom of the inhibitor 26 and Wat1 in the catalytic site of BACE-1 (Figure 33).

![Figure 33. Superimposed binding poses of the spiropiperidine iminohydantoin inhibitor 26 in the catalytic site of BACE-1. Predicted (grey) and experimental (blue) binding poses of the inhibitor 26 in complex with BACE-1. The green dotted lines show the network of H-bond interactions for the experimental binding pose, red circles indicate the water molecules. The inhibitor binding pose predicted by eHiTS forms a H-bond with Wat1 (dark green dotted line), while the inhibitor 26 in complex with BACE-1 forms other three main H-bonds: one with another water molecule in proximity of Asp228, one with Phe108 and another one with NH (backbone) of Gln73. Visualised with Discovery Studio 3.0.](image)

\[\text{PDB access number 3FKT}\]
The H-bond between the secondary amino group of the inhibitor and the water molecule in proximity of Asp228, observed in 3FKT (Figure 31), was not detected. Other two H-bonds with BACE-1 residues, Gln73 and Phe108, located in the flap region (Section 1.3.2) were also missed. Nevertheless, the orientation of the inhibitor 26 in the catalytic site of BACE-1 was correctly reproduced and the reliability of eHiTS was therefore demonstrated.

2.3 Design of a virtual library of likely synthetically accessible lead-like compounds

A virtual library of likely synthetically accessible lead-like molecules was enumerated using Pipeline Pilot. In each case, it was envisaged that a scaffold might be assembled from an initial coupling of two main building blocks and the resulting intermediate could be varied by a sequence of established reactions. The virtual synthesis of each scaffold was performed in parallel from a range of different building blocks and a library of structurally diverse scaffolds was generated according to the DOS principle (Section 1.2.4).

In order to ensure synthetic accessibility as far as possible, a toolbox of previously established reactions and a pool of commercially available reagents were applied to the protocol. The protocol started with a series of virtual coupling reactions between nucleophile and electrophile building blocks (e.g. nucleophilic opening of cyclic sulfamidates, Ir-catalysed asymmetric amination of an allylic carbonate), and then proceeded with virtual derivatisation reactions on the resulting intermediates with isocyanates or aryl bromide reagents. The resulting compounds underwent one or two virtual cyclisation reactions (e.g. metathesis, Pd-catalysed aminoarylation) followed by virtual removal of protecting groups. A series of virtual skeletally diverse molecules was obtained and further modified by virtual derivatisation reactions. The list of virtual reactions and building blocks employed to enumerate the library, library A, is reported in Appendices 1.1 and 1.3.

The resulting compounds were filtered according to lead-like parameters (Section 2.1) and a library of ca. 85,000 likely synthetically accessible compounds, library A,
was obtained. Examples of cyclic compounds obtained from the enumeration of library A are represented in Figure 34.

Figure 34. Protocol of virtual synthesis employed to enumerate virtual library A. Examples of virtual products obtained at each stage are shown.
2.3.1 Identification of promising ligands

Libray A was docked against BACE-1 in order to identify putative ligands. A first vHTS was conducted using a randomly-selected sample of 10% of the molecules,\(^a\) in order to identify representative classes of molecules contained in the library. The resulting eHiTS scores were used to calculate the cLE'. The most promising putative ligands were chosen on the basis of cLE' value, cLE' ≥ 0.27.\(^b\) The structures of those ligands were analysed and clustered into eight main substructures (Figure 35).

![Figure 35. Eight main substructures of the most promising virtual ligands of library A.](image)

All the compounds in library A that contained one of the above substructures were selected. The resulting 29,084 molecules were docked against BACE-1. The 131 most promising compounds, with cLE' ≥ 0.28,\(^b\) were analysed: 68% of the compounds contained the substructure e, 35% the substructure c and 7% the substructures d and h. Examples of those compounds and their distribution according to lipophilicity (AlogP) and cLE', values are reported in Figure 36.

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\(^a\)The three-dimensional conformations of the representative 10% sample of library A were generated using Corina\(^a\)5 (one three-dimensional conformation for each molecule).

\(^b\)The limiting value of cLE' was chosen according to the distribution of data, in order to focus on the best 1% of cLE'.
Figure 36. Promising putative BACE-1 ligands with cLE' ≥ 0.28 contained in library A. Each symbol represents a single ligand described by AlogP and cLE'. Representative ligands are coloured according to their substructure defined in Figure 35. 131 ligands were identified following vHTS of 29,084 molecules. The substructures c and e contained mainly 2 or 3 saturated or aromatic rings, while the substructure d and h contained 1 or 2 saturated or aromatic rings. Graph reproduced with Dotmatics Vortex.

2.3.2 Optimisation of library A and identification of two families of putative BACE-1 inhibitors

The protocol used for the generation of library A was modified in order to create a more focused virtual library of putative BACE-1 inhibitors. A further series of virtual derivatisation reactions, performed with a variety of secondary amines, was added at the end of the protocol and a broader variety of virtual derivatisation reagents (aryl bromide and isocyanates) was used. The list of virtual derivatisation reactions and of the reagents employed is provided in Appendices 1.2 and 1.3.
Five million molecules were generated and filtered according to the established lead-like parameters (Section 2.1) and a virtual library of ca. 55,000 molecules was derived; library B. The overall protocol for the generation of the virtual library B is illustrated in Figure 37. It was observed that molecules generated from library B showed more substituted structures than members of library A (Figure 38).
Figure 37. Protocol of virtual synthesis employed to enumerate virtual library B. Examples of virtual products obtained at each stage are shown. The protocol of virtual synthesis of library A was implemented with additional virtual derivatisation reactions (below the dotted line) to give more substituted structures (16 ≤ nHA ≤ 27).
Figure 38. Comparison of virtual molecules obtained by in silico enumeration of libraries A and B. The molecules contained in library B showed a more substituted structures than in library A, and number of heavy atoms was $16 \leq n_{HA} \leq 27$.

*Library B* was docked against BACE-1$^a$ and the eHiTS scores were used to calculate cLE'. A larger proportion of molecules had cLE' $\geq 0.28$, compared to *library A* (ca. 900 vs 131); confirming that more promising virtual ligands were present in the focused *library B*. Two main families of putative inhibitors were identified amongst all the virtual ligands with cLE' $\geq 0.28$. The amino tetrahydropyridine family, containing the substructure e (Figure 35), represented 37% of all the ligands; the imidazolidinone family, containing the substructure e (Figure 35), represented 63% of all the ligands. Their distribution according to AlogP and cLE' values are reported in Figure 39.

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$^a$Three-dimensional conformations were generated using Corina$^{95}$(one three-dimensional conformation for each molecule).
Figure 39. Putative BACE-1 inhibitors, cLE’ ≥ 0.28 identified from library B. Each symbol represents a single ligand described by AlogP and cLE’. Representative ligands are coloured according to their substructure defined in Figure 35. 900 ligands were identified following vHTS of ca. 55,000 molecules. The amino tetrahydropyridine family of putative inhibitors (substructure e) contained mainly 1 or 2 saturated or aromatic rings, while the imidazolidinone family of putative inhibitors (substructure c) contained mainly 2 to 4 saturated or aromatic rings. Graph reproduced with Dotmatics Vortex.

2.4 Analysis of the predicted binding poses of the putative inhibitors

The predicted binding poses of the two identified families of putative inhibitors for BACE-1 were analysed. For both families the key predicted interactions with the catalytic site of BACE-1 were H-bond interactions formed through the primary amine contained in the putative inhibitor structures. The predicted H-bond interactions were directed towards the water molecules and/or the catalytic aspartates, Asp32 and Asp228. The primary amine is predicted to be protonated at the pH at which BACE-1 is active.
(pH 4.5). Other predicted H-bond interactions were formed with Lys107 and Phe108 contained in the flap region of BACE-1 or with Arg235. Examples of the predicted binding poses of the two main families of putative inhibitors are shown in Figure 40.

When docking was conducted without including the water molecules in the catalytic site of BACE-1, the H-bond interactions between the putative inhibitor's protonated primary amine, $\text{NH}_3^+$, and the catalytic aspartate residues, Asp32 and Asp228, were still predicted (illustrations given in the Appendix 2.1). The binding pose prediction was therefore consistent, orienting the putative inhibitors in the catalytic site of BACE-1 in the same direction, either in presence or in absence of the water molecules.
Figure 40. Predicted binding pose of representative members of the identified families of BACE-1 putative inhibitors. A) and C) 2D structure of the imidazolidinone putative inhibitor 31 (A) and of the amino tetrahydropyridine putative inhibitor 32 (C) in their protonated state. B) and D) 3D image of the predicted binding pose of the inhibitors 31 (B) and 32 (D). The predicted H-bond interactions are shown with green dotted lines, π-cation interactions are shown in orange line, and red circles indicate water molecules. The water molecules, Asp228 and Asp32 form H-bonds with the protonated primary amine, NH₃⁺, of the putative inhibitor 31 (B) and 32 (D). Arg235 forms a π-cation interaction with one pyridine ring of the putative inhibitor 31 (B). Lys107 and Phe108, both located in the flap region form H-bond with the other protonated primary amine, NH₃⁺, of the putative inhibitor 32 (D). Visualised with Discovery studio 3.0.
2.4.1 Validation of the predicted binding poses with additional docking software

A representative set (eight molecules) of the two identified families of putative BACE-1 inhibitors was studied using the Maestro suite (Schrödinger).\textsuperscript{101} The lengths of the predicted H-bonds formed in the catalytic site of BACE-1 were measured. The predicted length range was of 2.4-2.9 Å for H-bonds formed with Asp32 and Asp228, and 2.2-2.5 Å for H-bonds formed with the water molecules. These lengths were in agreement with the range of 2.5-3.2 Å reported in the literature for H-bonds formed in the catalytic site of BACE-1.\textsuperscript{77, 86} Representative examples are provided in Appendix 2.2.

The predicted binding poses of the representative putative inhibitors were analysed using Macromodel, a specific component within Maestro, in order to assess their conformational energy. Macromodel was used to minimise the conformational energy of raw eHiTS binding poses inside the catalytic site of BACE-1. Macromodel applied a force field method to calculate the putative energy of a ligand, using an iterative function of distances and angles between atoms and keeping the coordinates of the protein unchanged. Representative examples of energy-minimised binding poses of the putative inhibitors are represented in Figure 41. The root-mean square deviation (RMSD) of heavy atom positions between raw and minimised conformations was in the range of 1.8 ≤ Å ≤ 1.3, in line with the accepted limited value of Å ≤ 2.0 for reliable prediction.\textsuperscript{96}
Figure 41. Evolution of raw binding poses upon conformational energy minimisation for selected putative inhibitors. A) and C) 2D structure representation of the imidazolidinone and the amino tetrahydropyridine putative inhibitors 33 and 34. B) and D) Superimposed structures of the energy-minimised (grey) and raw (green) binding poses for the putative inhibitors 33 and 34.

The binding poses obtained after the minimisation of their conformational energy were compared with the raw eHiTS binding poses using SPROUT. SPROUT is a fragment-based ligand design program that identifies protein binding regions, favourable protein hydrogen bonding and hydrophobic sites.\textsuperscript{102, 103} In SPROUT, the ALIGATOR module (\texttt{Algorithms for LIGAnd Testing and Ordering of Results}) contains a scoring function which can help investigating the ligand binding poses generated by other \textit{in silico} docking software (e.g. eHiTS). The scoring function of SPROUT has a logarithmic scale and hydrophobic interactions represent the main score parameter. The scores have a negative value and represent the predicted log(K_i) of the ligand for the protein.\textsuperscript{a} The binding poses of the eight representative putative inhibitors, predicted by eHiTS and Maestro, were scored using SPROUT.

\textsuperscript{a}A score of $\sim$7.0 would correspond to an estimated $10^{-7}$ M inhibitor.
The resulting SPROUT scores for selected putative inhibitors are illustrated in Table 5 and in Table 6. As a general outcome, the SPROUT scores of binding poses with minimised conformational energy were similar to the raw ones (Table 5 and 6, column 3). Only one exception was observed (Table 5, entry 1). This similarity in the SPROUT scores for raw and energy minimised binding poses showed that the binding poses predicted by eHiTS were reliable.

It was noticed that the SPROUT scores were higher by 3.5 logarithm units than the eHiTS scores (Table 5 and 6 column 3 and 4); thus predicting $10^{-4}$ M inhibitors. These differences could be attributed to the different scoring functions employed by the two docking programs: H-bonding and $\pi$-cation interactions are weighted more in eHiTS, while lipophilic interactions and cavity filling are weighted more in SPROUT. Despite the scores differences, the interactions predicted by eHiTS were confirmed by SPROUT. A summary of the predicted non-covalent interactions formed by representative ligands of the two promising families in the catalytic site of BACE-1 is given in Table 5 and 6.
Table 5. eHiTS and SPROUT scores and predicted non-covalent interactions of selected imidazolidinone putative inhibitors in BACE-1.

<table>
<thead>
<tr>
<th>Entry</th>
<th>2D representation of predicted non-covalent interactions</th>
<th>Score</th>
<th>Main H-bond interactions</th>
<th>Other interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SPROUT&lt;sup&gt;a&lt;/sup&gt; (SPROUT)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>eHiTS</td>
<td>eLE&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>−3.99</td>
<td>−7.59</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(−5.06)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>−3.90</td>
<td>−7.78</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(−4.02)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Raw eHiTS binding poses scored by SPROUT; <sup>b</sup>energy-minimised binding poses (Macromodel/Maestro) scored by SPROUT; <sup>c</sup>calculated with eHits score
Table 6. eHiTS and SPROUT scores and predicted interactions of selected amino tetrahydropyridine putative inhibitors in BACE-1.

<table>
<thead>
<tr>
<th>Entry</th>
<th>2D representation of predicted non-covalent interactions</th>
<th>Score</th>
<th>Main H-bond interactions</th>
<th>Others interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SPROUT&lt;sup&gt;a&lt;/sup&gt; (SPROUT)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>eHiTS</td>
<td>eLE&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>–4.58</td>
<td>–7.08</td>
<td>0.31</td>
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<td></td>
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<td>–3.37</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>(–3.30)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Raw eHiTS binding poses scored by SPROUT; <sup>b</sup> energy-minimised binding poses (Macromodel/Maestro) scored by SPROUT; <sup>c</sup>calculated with eHits score
2.4.2 Design of a focused library of putative inhibitors

The two families of putative inhibitors identified by vHTS showed similar interactions with the catalytic site of BACE-1. The selection of one family over the other for synthesis was determined by their respective chemical structural features. An evaluation of their structural novelty, potential for diversification, and chemical stability were made.

To assess the novelty of the putative BACE-1 inhibitors, structures were compared with the ones of known BACE-1 inhibitors. Elements of novelty were found in both putative inhibitor scaffolds. The imidazolidinone scaffold (Table 7 entry 1) differed from the spiropiperidine imihydantoin inhibitor 26 and from the 2-aminoimidazole-4-one inhibitors in the position of the amino group and the number of substituent groups around the cyclic urea ring. The amino tetrahydropyridine scaffold entry (Table 7, entry 2) differed from known aminopyridine inhibitors for the position in the N atom, and the number of double bonds in the ring; and from a tetrahydropyridine inhibitor for the position and type of substituent groups on the ring. As a consequence, both putative inhibitor scaffolds were considered novel chemotypes for BACE-1.

Table 7. Comparison of structure features of putative BACE-1 inhibitors with known BACE-1 inhibitors.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Putative BACE-1 inhibitors(^a)</th>
<th>Known BACE-1 inhibitors(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="Image1" alt="Imidazolidinone scaffold" /></td>
<td><img src="Image2" alt="Spiropiperidine imihydantoin inhibitor 26" /></td>
</tr>
<tr>
<td>2</td>
<td><img src="Image4" alt="Amino tetrahydropyridine scaffold" /></td>
<td><img src="Image5" alt="Aminopyridine inhibitors" /></td>
</tr>
</tbody>
</table>

\(^a\)The central core of the molecules is shown; \(^b\)Only one example in the literature.
In terms of potential for diversification, the imidazolidinone scaffold represented the most diverse family of putative BACE-1 inhibitors. In this scaffold the central core can be diversified at three different positions, allowing for a broad investigation of binding effect of substituent groups. In the case of the amino tetrahydropyridine scaffolds, only two positions of diversification can be considered (Figure 42).

![Figure 42. Positions of diversification in the structures of the two families of putative inhibitors.](image)

Regarding chemical stability, it was noticed that the amino tetrahydropyridine scaffold can be subjected to tautomerisation into the $\alpha,\beta$ unsaturated ketone, giving a reactive Michael acceptor. From those considerations, the more potentially diverse and stable family of imidazolidinone compounds was chosen for synthesis. A focused library of these compounds was designed (Figure 43). Binding pose predictions, hydrophilicity, and variation of geometry and length of the substituent groups were considered in the library design.

![Figure 43. Focused library of putative BACE-1 inhibitors.](image)

2.5 Summary

The design of a library of putative BACE-1 inhibitors was presented in this Chapter. The design approach consisted of enumerating a virtual library of lead-like
compounds and screening those compounds \textit{in silico} against BACE-1. The virtual library enumeration was based on diversity oriented synthesis, to generate skeletally diverse molecules from an initial pool of building blocks. The protocol employed for the virtual library enumeration included established chemical reactions and commercially available reactants to ensure synthetic accessibility as far as possible.

The virtual screening of the library was performed using eHiTS. The eHiTS screening method was validated and the predicted binding poses of the putative BACE-1 inhibitors were also analysed using Maestro Macromodel and SPROUT. The non-covalent interactions predicted by eHiTS were confirmed. Two families of putative BACE-1 inhibitors were identified, both containing elements of novelty in comparison to known BACE-1 inhibitors. One family was chosen over the other for synthesis, on the basis of potential points of diversification and chemical stability. A focused library of imidazolidinone putative inhibitors was then designed; the synthesis of these compounds is discussed in Chapter 3.
Chapter 3. Synthesis of a library of imidazolidinones

The following Chapter describes the synthetic routes to a library of BACE-1 putative inhibitors based on the imidazolidinone structure. Two different potential synthetic routes are presented, both including a cyclisation reaction on a series of N-allyl urea substrates to lead to the imidazolidinone scaffold (Section 3.1). The first synthetic route is based upon a Pd-catalysed aminoarylation and is described in Section 3.2; the second synthetic route is based upon an iodine-mediated cyclisation and is described in Section 3.3.

3.1 Identification of two possible synthetic routes

A variety of imidazolidinones was identified as putative inhibitors for the BACE-1 by virtual high throughput screening. A generic representation of the putative inhibitors is given by structure 35 (Scheme 5). Two possible synthetic routes were envisaged for the imidazolidinone 35. The synthetic route A was suggested by the protocol of virtual reactions employed for the enumeration of virtual libraries A and B (Section 2.3); while the synthetic route B was envisaged from literature precedence. The two routes lead to two different starting materials: the allylic amines 38 and 41.
Scheme 5. Retrosynthetic analysis of target imidazolidinones. The groups \( R^1 \), \( R^2 \) and Ar were chosen according to the virtual library hits (see Figure 43, Section 2.4.2).

Following route A, the target imidazolidinone 35 might be obtained by functional group interconversions (FGIs) of cyclic precursor 36, itself be prepared by Pd-catalysed aminoarylation of the \( N \)-allyl urea 37. The \( N \)-allyl urea 37 might be synthetised from the allylic amine 38. According to route B, two FGIs of imidazolidinone 35 might lead to the precursor 39. Compound 39 might be formed via iodine-mediated cyclisation of the \( N \)-allyl urea 40, derived from the allylic amine 41. The investigation of the viability of these two routes is described in Sections 3.2 and 3.3.

3.2 Synthetic route to the target imidazolidinones based upon a Pd-catalysed aminoarylation

The synthesis of a first imidazolidinone was planned on the basis of literature examples of a Pd-catalysed aminoarylation. Wolfe et al. reported the use of the phenylurea 42 as substrate for the aminoarylation (Scheme 6).\(^{107}\) Here the formation of the new C-N bond provided the cyclic urea system and a 2-naphthalenyl was concomitantly introduced.
Scheme 6. Example of a Pd-catalysed aminoylation

Wolfe et al. also applied the Pd-catalysed aminoarylation to a series of N-allyl phenylureas containing an alkyl group at the allylic position (Scheme 7, A). Following these examples, the reaction scope was extended and adapted to our synthetic purpose. A variety of N-allyl phenylureas containing different functional group substitutions at the allylic position was chosen for synthesis, keeping the target imidazolidinone 44 in mind (Scheme 7, B).

Scheme 7. Other substrates for Pd-catalysed aminoarylations. A) Substituted N-allyl ureas employed in the literature. B) Designed N-allylureas to investigate Pd-catalysed aminoarylation in order to achieve the final amine 44.

3.2.1 Synthesis of substrates for the Pd-catalysed aminoarylation

A first substrate for the Pd-catalysed aminoarylation, compound 45, was synthesised starting from the allylic carbonate building block 46 (Scheme 8). The allylic
carbonate 46 was prepared in four steps from 2-aminoethanol following a literature procedure (Scheme 9).

![Scheme 8. Allylcarbonate 46, precursor of compound 45, substrate for Pd-catalysed aminoarylation.](image)

2-Aminoethanol was treated with di-tert-butyl dicarbonate under solvent free conditions to form the carbamate 47. The carbamate 47 then underwent an one pot Parikh–Doering oxidation\(^{109}\)/Wittig reaction\(^{110}\) by treatment with sulfur trioxide pyridine, triethylamine and dimethylsulfoxide (DMSO) in CH\(_2\)Cl\(_2\), followed by addition of the triphenylphosphorane Ph\(_3\)PCHCO\(_2\)Et. The resulting unsaturated ester 48 was obtained with >98:<2 E:Z selectivity. The reduction of the ester 48 to the corresponding alcohol 49 was achieved by treatment with diisobutylaluminium hydride (DIBAL) and borontrifluoride diethyletherate in CH\(_2\)Cl\(_2\) at \(-78\) °C. Finally, treatment of the alcohol 49 with methylchloroformate and pyridine in CH\(_2\)Cl\(_2\) gave the allylic carbonate 46.

The allylic carbonate 46 was then employed in the synthesis of the allylic amine 50, precursor of the substrate for the Pd-catalysed aminoarylation. The conversion of the allylic carbonate 46 into the allylic amine 50 was achieved via an Ir-catalysed allylic amination (Table 8).\(^{98}\) The allylic carbonate 46 was treated with aniline in DMSO or THF as solvent in presence of 2 mol% of each of the precatalyst [Ir(dbcot)Cl]\(_2\),\(^{111,112}\)
phosphoramidite chiral ligands (S,S aS)-51 and (R,R aR)-52 and n-butyl amine. The active catalyst was formed in situ. The reaction was performed using a 1:1 mixture of the two chiral ligands (S,S aS)-51 and (R,R aR)-52 in order to obtain the product as a racemate. The reaction was attempted in DMSO and THF; similar yields were obtained in both solvents (Table 8) and, as a general trend, the yield improved with the scale of the reaction (entry 1 and 3 and entry 2, 4).

Table 8. Synthesis of the allylic amine 50.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Scale 46 (mmol)</th>
<th>Solvent</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.50</td>
<td>DMSO</td>
<td>49</td>
</tr>
<tr>
<td>2</td>
<td>0.50</td>
<td>THF</td>
<td>43</td>
</tr>
<tr>
<td>3</td>
<td>0.75</td>
<td>DMSO</td>
<td>61</td>
</tr>
<tr>
<td>4</td>
<td>1.2</td>
<td>THF</td>
<td>69</td>
</tr>
</tbody>
</table>

The dbcot ligand, the precatalyst [Ir(dbcot)Cl]$_2$ and the phosphoramidite ligands (S,S aS)-51 and (R,R aR)-52 were prepared according to literature procedures.
The allylic amine 50 was ultimately converted into the N-allyl urea 45. The formation of the N-allyl urea 45 required the treatment of 50 with an excess of ethylisocyanate in CH$_2$Cl$_2$, stirring at reflux for 2 days (Scheme 10). The urea 45 was prepared in 58% yield.

Scheme 10. Synthesis of the N-allyl urea 45.

To prepare other substituted N-allyl ureas, an alternative concise approach was subsequently adopted. The synthesis of the N-allyl urea 54 was accomplished in two steps consisting of a Petasis multicomponent reaction,$^{116}$ followed by an urea formation (Scheme 11). The allylic amine 53 was isolated as a racemate after combining glycoaldehyde dimer, vinyl boronic acid pinacol ester and aniline in H$_2$O–THF. A range of conditions was investigated (Table 9) and ultimately the best yield was obtained at 30 °C over 4 days. The conversion of the allylic amine 53 into the respective urea 54 was performed by treatment with an excess of ethylisocyanate in CH$_2$Cl$_2$ in 80% yield.

Scheme 11. Preparation of the substituted N-allyl urea 54.
Table 9. Reaction conditions employed in the preparation of the allylic amine 53.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Scale PhNH₂ (mmol)</th>
<th>Time (days)</th>
<th>Temperature ( °C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>50</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>3</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>4</td>
<td>30</td>
<td>45</td>
</tr>
</tbody>
</table>

Three additional substrates for the Pd-catalysed aminoarylation were prepared from the N-allyl urea 54. The N-allyl urea 54 was converted into the NsBoc-protected amine 55, the MOM-protected alcohol 56 and the azide 57 (Scheme 12).

Scheme 12. Conversion of the N-allyl urea 54 into the N-allyl ureas 55, 56 and 57.

The NsBoc protected amine 55 was prepared under Fukuyama–Mitsunobu conditions in 53% yield, by treating the N-allyl urea 54 with NsNHBOc, triphenylphosphine and DEAD. The protected alcohol 56 was obtained in a 41% yield stirring the alcohol 54 with methyl chloromethyl ether (MOMCl) and N,N-diisopropylethylamine (DIPEA) base in CH₂Cl₂. The formation of the azide 57 was achieved from compound 54 using 1,8-diazadicyclo[5.4.0]undec-7-ene (DBU) and diphenylphosphoryl azide (DPPA). Initially the reaction was conducted in DMF stirring from 1 to 3 days obtaining up to 34% yield (entry 1 and 2, Table 10). When toluene was employed as solvent, the yield improved to 63% (entry 3, Table 10).
Table 10. Study of the formation of the azide 57 from the alcohol 54.

<table>
<thead>
<tr>
<th>Entry</th>
<th>DBU, DPPA (eq.)</th>
<th>solvent</th>
<th>Time (days)</th>
<th>Yield (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.0</td>
<td>DMF</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>6.0</td>
<td>DMF</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
<td>toluene</td>
<td>3</td>
<td>63</td>
</tr>
</tbody>
</table>

3.2.2 Investigation of the Pd-catalysed aminoarylation

The Pd-catalysed aminoarylation reaction was initially reproduced using the known substrate 42 and then it was investigated using the prepared N-allyl urea substrates 45, 54, 55, 56 and 57. In each case, the relevant substrate was treated with 2-bromonaphthalene and NaO\(^{1}\)Bu in the presence of a 5 mol% of Pd\(_2\)(dba)\(_3\) complex and 10 mol% of phosphine ligand Xantphos in toluene, stirring the resulting mixture at reflux for 2-3 days (Table 11).
Table 11. Results of the Pd-catalysed aminoarylations performed on different N-allyl ureas.

![Diagram of the reaction](image)

<table>
<thead>
<tr>
<th>Entry&lt;sup&gt;b&lt;/sup&gt;</th>
<th>N-allyl urea&lt;sup&gt;a&lt;/sup&gt;</th>
<th>R</th>
<th>ArBr&lt;sup&gt;a&lt;/sup&gt; (eq.)</th>
<th>NaO'Bu (eq.)</th>
<th>product</th>
<th>yield (%)</th>
</tr>
</thead>
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<td>H</td>
<td>2.0</td>
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</tr>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>CH₃OH</td>
<td>2.0</td>
<td>1.8</td>
<td><img src="image" alt="Product 53" /></td>
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<tr>
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</tr>
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<td>4</td>
<td>55</td>
<td>CH₂NsNBoc</td>
<td>2.0</td>
<td>1.8</td>
<td><img src="image" alt="Product 59" /></td>
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<td></td>
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<td><img src="image" alt="Product 60" /></td>
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</tr>
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<td>6</td>
<td>57</td>
<td>CH₂N₃</td>
<td>3.0</td>
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<td></td>
<td></td>
<td><img src="image" alt="Product 61" /></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Ar = 2-naphthyl; <sup>b</sup>All the substrates were reacted for 3 days, only in the case of entry 2 the reaction lasted 2 days; <sup>c</sup>The starting material was recovered in 15% yield.
From the \( N \)-allyl ureas 42, 56 and 57, the expected imidazolidinones 43, 60 and 61 were obtained (entry 1, 5 and 6). In the case of the \( N \)-Boc substrate 45, the \( N \)-arylated imidazolidinone 58 was isolated in 4% yield (entry 2). In this reaction, an excess of base was employed since no product was recovered by using one equivalent of \( \text{NaO}^+\text{Bu} \). From the \( N \)-allyl urea 55, containing Nosyl-\( N \)-Boc amino group, a product of the competing Heck reaction,\(^{119} \) compound 59, was obtained in 13% yield (entry 4). The starting material and the amine precursor, compound 53, were recovered from the reaction of the \( N \)-allyl urea 54 (entry 3). A mechanistic rationalisation of these reactions is provided in Sections 3.2.2.2 and 3.2.2.3.

3.2.2.1 Determination of the stereochemistal outcome of the Pd-catalysed aminoarylation

The 500 MHz \(^1\text{H}\)-NMR spectrum of the imidazolidinone 43 was studied to help assign the relative configuration of the other imidazolidinone products. A nOe experiment of the imidazolidinone 43 was performed (Figure 44). A nOe enhancement (6%) of the proton 5-\( H_A \) was recorded when the proton 4-\( H \) was irradiated and \textit{vice versa}, while no nOe enhancement was recorded between proton 4-\( H \) and 5-\( H_B \). Therefore a \textit{syn} relationship was assigned between the proton 4-\( H \) and 5-\( H_A \).

![Figure 44. Results of the nOe experiment on the imidazolidinone 43.](image)

No nOe was observed between proton 4-\( H \) and 5-\( H_B \).

The relative configuration of the imidazolidinones 58, 60 and 61 was assigned on the basis of the magnitude of the coupling constant, \( J \), of the diagnostic proton signals in the urea ring system, protons 4-\( H \) and 5-\( H \). The protons 4-\( H \) and 5-\( H_A \) of the imidazolidinone 43 in \textit{syn} relationship showed a \( J \) value of 8.9 Hz, while the protons 4-\( H \) and 5-\( H_B \) in \textit{anti} relationship showed a \( J \) value of 6.0 Hz. Proton 4-\( H \) in the imidazolidinones 58, 60 and 61 all showed a \( J \) value between 3.0-4.0 Hz, therefore the
relative configuration assigned was trans. The magnitude of the observed coupling constants was in accordance with the Karplus relation. A summary of the J values of all the synthesised imidazolidinones is provided in the Appendix 3 (Table 24).

3.2.2.2 Rationalisation of the stereochemical outcome of the Pd-catalysed aminoarylation

The stereochemical outcome of the Pd-catalysed aminoarylation may be rationalised using the mechanism reported by Wolfe et al (Scheme 13). The Pd-phosphine complex, formed in situ, is proposed to undergo oxidative addition to 2-bromonaphthalene to start the catalytic cycle of the reaction. Once the urea substrate is deprotonated by NaO'Bu, an Ar-Pd-amido complex is formed, followed by syn insertion across the alkene. From the resulting cyclic urea species, the formation of the aryl-C bond occurs via a reductive elimination leading to the product and to the regeneration of the Pd(0) catalyst.

Scheme 13. Catalytic cycle of the Wolfe reaction. Adapted from Wolfe et al.¹

According to the mechanism, the stereo-determining step is the olefin syn addition across the alkene which leads to the intermediate. The substituent R at the allylic postion in the imidazolidinones 58, 60 and 61 determines the facial selectivity of the reaction. The N-Pd bond would attack the olefin from the opposite side to the R group,
through the favoured conformation 64, favouring the formation of the trans imidazolidinones (Scheme 14).

Scheme 14. Rationalisation of the stereochemical outcome of the Pd-aminoarylation.

3.2.2.3 Proposed mechanism for the formation of the N-arylated imidazolidinone

A mechanism for the formation of the N-arylated imidazolidinone 58, obtained from the N-allyl urea 45, is here proposed (Scheme 15). Under the high temperature of the reaction the Boc group was removed\textsuperscript{121,122} either before or after the product formation, therefore, in the Scheme 15 the R group can represent either the Boc group or a proton.
According to the mechanism proposed, after the formation of the Pd-amido complex 67 from the allylurea 66 and the Pd active species 65, the syn addition of the olefin would occur. The resulting cyclic intermediate 68 would undergo a hydrogen transfer and a concomitant Pd exchange, between the amino group and the carbon attached to the Pd, to give a new Pd-N complex 69. The species 69 would undergo a reductive elimination to provide the compound 58 and regenerate the catalyst.

3.2.3 Practicability of the route based upon the Pd-catalysed aminoarylation

The investigation of the Pd-catalysed aminoarylation on different N-allyl ureas showed that the protected alcohol 56 was tolerated under the reaction conditions, giving the imidazolidinone 60 in 35\% yield. A possible synthetic pathway towards the synthesis of the final imidazolidinone 44, starting from the precursor 60 was envisaged (Scheme 16).
Scheme 16. Envisaged synthetic steps towards the preparation of the target imidazolidinone 44.

The synthesis of the final imidazolidinone 44 would consist of three steps: deprotection of the alcohol group, subsequent displacement with azide and final reduction to the amino group. The envisaged synthetic pathway would have to be applied to each desired compound, making the synthetic route longer and not necessarily practical. Moreover, the Pd-catalysed aminoarylation is not known with heteroaromatic-containing ureas and the insertion of a heteroaryl group is limited to the 2-pyridyl group.\(^1\) Contextually the preparation of the heteroaromatic N-allyl urea substrates would need to be planned differently. In Section 3.2.2, the alcohol 53, precursor of the N-allyl urea 54, was prepared using the Petasis reaction from aniline, but the use of amines containing a heteroaromatic group is not known for the Petasis reaction.\(^{116,123}\) Due to these considerations, the synthetic route based upon the Pd-catalysed aminoarylation was not pursued. A potentially more viable synthetic route based upon an iodine-mediated cyclisation was investigated (Section 3.3).

3.3 Synthetic route to the target imidazolidinones based upon an iodine-mediated cyclisation reaction

An alternative synthesis of the target imidazolidinones was investigated according to the envisaged retrosynthetic route B (Section 3.1). In this synthesis, the key urea ring system would be prepared through an iodine-mediated cyclisation of the N-allyl urea substrate. The synthesis was first explored using benzylallylamine, expanding the scope of a literature procedure (Section 3.3.1),\(^{124}\) and then was extended to the specific N-allyl urea substrates (Section 3.3.3).
3.3.1 Exploration of synthetic route from benzylallylic amine

The synthesis of imidazolidinones via an iodine-mediated cyclisation of \( N \)-allylurea was firstly reported by Moody et al. in 2010.\textsuperscript{124} The reaction, performed on three different \( N \)-allylurea substrates, gave yields between 67 and 83\% (Scheme 17). In order to expand the scope of the iodine-medicated cyclisation for our synthetic purpose, the preparation of the imidazolidinone 73 was initially attempted (Scheme 18).

![Scheme 17. Literature example of iodine-mediated cyclisation of \( N \)-allylureas.](image1)

![Scheme 18. Study of the iodine-mediated cyclisation on benzylallylamine.](image2)

Benzyllallylamine 70 was prepared in 47\% yield by treating benzyl bromide with an excess of allylamine and potassium carbonate. The \( N \)-allyl urea 71 was then obtained in 95\% yield by treatment of the benzyllallylic amine 70 with phenylisocyanate in \( \text{CH}_2\text{Cl}_2 \). The imidazolidinone 73 was synthesised by treatment of the urea 71 with trimethylsilyl trifluoromethanesulfonate and triethylamine in \( \text{CH}_2\text{Cl}_2 \) and then with iodine in \( \text{THF} \). The cyclic urea 73 was isolated in 53\% yield. Presumably, the reaction proceeds via an \( O \)-silyl imidate species 72.
Subsequently, the synthetic steps for the transformation of the imidazolidinone 73 into the amine 75 were undertaken (Scheme 19). The iodide 73 was treated with sodium azide in DMF to give the azide 74 in a 37% yield. The azide 74 was used without further purification in the following reduction. The imidazolidinone 75 was obtained in 81% yield following a Staudinger reaction, using triphenylphosphine in THF–H₂O (11:1) with stirring at r.t. for 18 h.

![Scheme 19. Conversion of the imidazolidinone 73 into the amine 75.](image)

### 3.3.2 Synthesis of the target imidazolidinones

This synthetic route was then extended to a series of N-allyl urea substrates containing a substituent at the allylic position. The choice of the heteroaromatic and alkyl groups to incorporate in the N-allyl ureas was determined by the commercial availability of the reactants. As a consequence, the initial design of a focused library of imidazolidinones (Figure 43, Section 2.4.2) was readapted, taking in consideration computational predictions of binding affinity of the new substituent groups against BACE-1. The resulting diverse N-allyl ureas selected for the synthesis are illustrated in Figure 45. An N-allyl urea substrate without substitution at the allylic position was also included in the series.

![Figure 45. Structure of the designed N-allyurea substrates for the iodine-mediated cyclisation reaction.](image)
3.3.2.1 Synthesis of substrates for the iodine-mediated cyclisation

The use of the building blocks 76 and 77, containing respectively a benzyl and isoxazole group at the allylic position, was envisaged for the preparation of the designed substituted N-allyl ureas (Scheme 20). The substituted allylic amine 76 was prepared in two steps following a literature precedence.\textsuperscript{126} Then, the same reaction conditions were applied to prepare the amine 77 (Scheme 21).

Scheme 20. Allylic amines building block 76 and 77 for N-allyl ureas.

Benzophenone was condensed with allylamine in presence of titatium isopropoxide in toluene at 80 °C to give the resulting imine 78 in 98% yield. Deprotonation of the imine 78 by n-butyl lithium, followed by addition of the relevant electrophile (chloroisoxazole 79 or benzyl chloride), led to formation of the substituted imine intermediate which was hydrolysed to give the substituted allylic amine 76 and 77 in 50% and 49% yield respectively.
Reductive amination was employed to prepare a series of secondary amines from compounds 76 and 77 and from allylamine (Table 12). In the majority of cases, the relevant aldehyde was reacted with the relevant amine in MeOH at r.t. and the resulting imine was reduced with sodium borohydride. The products did not require purification. The secondary amines 80-83 were obtained in 98%, 45%, 65% and 43% yield respectively (entry 1-4). The secondary amine 84 (entry 5) was prepared by treating the allylic amine 76 with acetone and magnesium sulfate in EtOH at r.t. and the resulting imine was reduced with sodium borohydride to yield the amine 84 in 61% yield.

Table 12. Synthesis of the secondary amines 80-84, precursors of the N-allyl urea substrates for the iodine-mediated cyclisation reaction.

<table>
<thead>
<tr>
<th>Entry</th>
<th>aldehyde</th>
<th>product</th>
<th>R</th>
<th>R¹</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OCHO</td>
<td>80</td>
<td>O</td>
<td>O</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>Cl-CHO</td>
<td>81</td>
<td>Cl</td>
<td>Cl</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>Cl-CHO</td>
<td>82</td>
<td>Cl</td>
<td>Cl</td>
<td>65</td>
</tr>
<tr>
<td>4</td>
<td>Cl-CHO</td>
<td>83</td>
<td>H</td>
<td>Cl</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>O</td>
<td>84</td>
<td></td>
<td></td>
<td>61⁴</td>
</tr>
</tbody>
</table>

⁴Reaction conditions: 1) Acetone (4 eq.), MgSO₄, EtOH, r.t., 2 days; 2) NaBH₄ (3.0 eq.), 0 °C-r.t., 1 day

The secondary amines 80-84 were converted into a range of N-allyl ureas (85-92) by refluxing with the relevant isocyanate in CH₂Cl₂ for 1 or 2 days (Table 13). The N-allyl ureas 85, 88, 89, 90 and 91 (entry 1, 4, 5, 6, 7) were obtained in similar yield,
between 77-87%; lower yields, between 58-70%, were achieved with the \(N\)-allyl ureas 86, 87 and 92 (entry 2, 3 and 8).

**Table 13. Preparation of a variety of \(N\)-allyl ureas from the amine 85-92.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>(R)</th>
<th>(R^1)</th>
<th>(R^2)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>85</td>
<td><img src="image1" alt="Structure" /></td>
<td><img src="image2" alt="Structure" /></td>
<td><img src="image3" alt="Structure" /></td>
<td>77</td>
</tr>
<tr>
<td>2(^a)</td>
<td>86</td>
<td><img src="image4" alt="Structure" /></td>
<td><img src="image5" alt="Structure" /></td>
<td><img src="image6" alt="Structure" /></td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>87</td>
<td><img src="image7" alt="Structure" /></td>
<td><img src="image8" alt="Structure" /></td>
<td><img src="image9" alt="Structure" /></td>
<td>58</td>
</tr>
<tr>
<td>4</td>
<td>88</td>
<td><img src="image10" alt="Structure" /></td>
<td><img src="image11" alt="Structure" /></td>
<td><img src="image12" alt="Structure" /></td>
<td>82</td>
</tr>
<tr>
<td>5</td>
<td>89</td>
<td><img src="image13" alt="Structure" /></td>
<td><img src="image14" alt="Structure" /></td>
<td><img src="image15" alt="Structure" /></td>
<td>87</td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td><img src="image16" alt="Structure" /></td>
<td><img src="image17" alt="Structure" /></td>
<td><img src="image18" alt="Structure" /></td>
<td>80</td>
</tr>
<tr>
<td>7</td>
<td>91</td>
<td><img src="image19" alt="Structure" /></td>
<td><img src="image20" alt="Structure" /></td>
<td><img src="image21" alt="Structure" /></td>
<td>81</td>
</tr>
<tr>
<td>8(^a)</td>
<td>92</td>
<td><img src="image22" alt="Structure" /></td>
<td><img src="image23" alt="Structure" /></td>
<td><img src="image24" alt="Structure" /></td>
<td>68</td>
</tr>
</tbody>
</table>

\(^a\)Reaction time extended to 2 days

### 3.3.2.2 Synthesis of a focused library of imidazolidinones

The iodine-mediated cyclisation and the final steps of the synthesis of the target imidazolidinones were initially investigated using the \(N\)-allyl urea 85 and then were applied to the \(N\)-allyl ureas 86 and 87 (Scheme 22). The relevant \(N\)-allyl urea was first
treated with trimethylsilyl trifluoromethanesulfonate and triethylamine in CH$_2$Cl$_2$ at r.t. and then with iodine in THF. Due to concerns about the stability of these products, the crude products were used in the next step without further purification. The displacement of the iodide with sodium azide was achieved by stirring in DMF at r.t. overnight. The azide product was obtained with trans relative configuration. The final imidazolidinones were obtained by reducing the relative azides under Staudinger$^{125}$ conditions, by treating the relevant azide with trimethylphosphine in THF-H$_2$O (11:1).

Scheme 22. Final steps of synthesis of a focused library of imidazolidinones.

The azide compounds prepared from the N-allyl urea 85, 86 and 87 contained traces of triethylamine salt and in some cases the final imidazolidinones contained traces of trimethylphosphium oxide. Therefore the iodine-mediated cyclisation on the next N-allyl urea substrates, 88-92, was performed using DBU base instead of triethylamine, and triphenylphosphine was employed in the azide reduction instead of trimethylphosphine. The imidazolidinone 96, prepared from the N-allyl urea 86, gave the lactam 97 after purification (Figure 46). The results of the synthesis are summarised in Table 14.

Figure 46. Imidazolidinone 96 and lactam 97. The lactam 97 was recovered after purification by ion exchange column of compound 96.
Table 14. Summary of synthesis of a focused library of imidazolidinones.

<table>
<thead>
<tr>
<th>Entry</th>
<th>N-allyl urea</th>
<th>R</th>
<th>R¹</th>
<th>R²</th>
<th>Azide formation yield (%)</th>
<th>trans: cis</th>
<th>Final product yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
<td>93, 16</td>
<td>trans</td>
<td>94, 18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt; 98: &lt; 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>86</td>
<td></td>
<td></td>
<td></td>
<td>95, 20</td>
<td>trans</td>
<td>lactam 97</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt; 98: &lt; 2</td>
<td></td>
<td>8e</td>
</tr>
<tr>
<td>3</td>
<td>87</td>
<td></td>
<td></td>
<td></td>
<td>98, 20</td>
<td>trans</td>
<td>99, 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt; 98: &lt; 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4ᵇ</td>
<td>88</td>
<td>H</td>
<td></td>
<td></td>
<td>100, 18</td>
<td>trans</td>
<td>101, 7</td>
</tr>
<tr>
<td>5ᵇ</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
<td>102, 7</td>
<td>trans</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt; 98: &lt; 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6ᵇᶜ</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
<td>103, 18</td>
<td>trans</td>
<td>104, 27ᵇ</td>
</tr>
<tr>
<td>7ᵇᶜ</td>
<td>91</td>
<td></td>
<td></td>
<td></td>
<td>105</td>
<td>telescoped</td>
<td></td>
</tr>
<tr>
<td>8ᵇᶜ</td>
<td>92</td>
<td></td>
<td></td>
<td></td>
<td>107</td>
<td>trans</td>
<td>&lt;1 mgᵇʰⁱ obtained</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt; 98: &lt; 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ᵃYield of purified product over two steps;ᵇDBU was employed in place of Et₃N;ᶜPPh₃ was used in place of PMe₃;ᵈA trace of triethylamine salt was present;ᵉTrimethylphosphonium oxides was contained in 10%;ᶠThe imidazolidinone 99 was purified by HPLC;ᵍReaction not attempted;ʰThe products 104, 106 and 107 were purified by column chromatography;ᵢInsufficient material for full characterisation.
In general, the yields of the preparation of the azides and amines from the substituted N-allyl ureas were surprisingly lower compared to that obtained during the synthesis of the imidazolidinone 75 (Section 3.3.1). Presumably the poor yields obtained in the synthesis of the azide were affected by the outcome of the iodine-mediated cyclisation. The iodine-mediated cyclisations were monitored by LCMS and TLC and were not quenched until the N-allyl urea starting material was completely consumed, but the 500 MHz $^1$H-NMR analysis of the reaction mixture of the iodide or of the azide crude revealed the presence of the N-allyl urea precursors in 20-35%. This could be explained by the hydrolysis of the O-silyl imidate species, which could lead to the N-allyl urea precursors, preventing the formation of the iodide compounds.

The final amines were also obtained in low yields. The purification of the amines through analytical methods, HPLC and mass directed chromatography, gave yields between 8-18% (entry 1-4). Better yields, ~28%, were obtained when the amines were purified via column chromatography (entry 6-7). Despite the low yields, the synthetic route based upon the iodine-mediated cyclisation allowed the preparation of a focused library of imidazolidinones to test against the biological target, BACE-1.

### 3.3.2.3 Determination of the relative configuration of products

By analogy to the analysis reported in Section 3.2.2.1, the relative configuration of the imidazolidinones in Table 7 was assigned on the basis of $J$ values of the diagnostic proton 4-H/5-H. Firstly the protons of the imidazolidinone 75 were assigned by a nOe experiment. A nOe enhancement (1.6%) of the proton 5-H$_A$ was recorded when the proton 4-H was irradiated and vice versa, while no nOe enhancement was showed between proton 4-H and 5-H$_B$ (Figure 47). Therefore a syn relationship was assigned between the proton 4-H and 5-H$_A$.

![Figure 47. Results of the nOe experiment on the imidazolidinone 75. No nOe enhancement was observed between protons 4-H and 5-H$_B$.](image-url)
The imidazolidinone 75 had a $J$ value of 9.0 Hz between the syn-related protons 4-H/5-H$_A$, while the $J$ value between the anti-related protons 4-H/5-H$_B$ was 5.1 Hz.\cite{124} The protons 4-H/5-H of all the imidazolinone products had $J$ values in the range of 3.5-6.0 Hz, as a consequence, the relative configuration of those compounds was assigned as trans. A summary of the diagnostic $J$ values of all the synthesised imidazolidinones is provided in the Appendix 3 (Table 24).

3.3.2.4 Rationalisation of the stereochemical outcome of the urea ring formation

The stereochemical outcome of the synthesised imidazolidinones is controlled by the formation of the respective cyclic iodine precursors from the $N$-allyl urea substrates; therefore the mechanism of the iodine-mediated cyclisation can provide a rationalisation of the stereochemical outcome. The substituted $N$-allyl urea substrates were first treated with triethylamine and trimethylsilyl trifluoromethanesulfonate to presumably form the $O$-silyl imidate species 108 (Scheme 23).

![Scheme 23. Rationalisation of the stereochemical outcome of the iodine-mediated cyclisation.](image)

Ar$^1$ = 3,5-dimethyl-4-yi-isoxazole
Ar$^2$= furan-2-yi or 4-chlorophenyl
R= 3,5-dimethyl-4-yi-isoxazole or ethylacetyl

Treatment with iodine is believed to trigger iodonium ion formation, species 109. The formation of the iodonium ion species is considered reversible;\cite{127} therefore the stereochemistry of the reaction is likely determined by the intramolecular nucleophilic attack of the nitrogen of the urea system onto the iodonium ion. The attack would favour the formation of a five-membered ring and the new formed C-I bond would be
oriented to the opposite side in respect to the methyl-aryl substituent, avoiding clashing interactions. The resulting compound 110 would have *trans* geometry.

### 3.4 Summary

Two synthetic routes towards the imidazolidinone compounds, putative inhibitors of BACE-1, were described in this Chapter. The first one was based upon the Pd-catalysed aminoarylation on *N*-allyl ureas, containing different functional groups at the allylic position: NHBoc, NsBocN, OH, N₃, OMOM. The second one was based upon the iodine-mediated cyclisation of *N*-allyl ureas containing different aromatic group at the allylic position.

The investigation of the Pd-catalysed aminoarylation showed that the *N*-allyl urea 56, containing the MOM protected alcohol at the allylic position, was a suitable substrate for the reaction, giving the imidazolidinone 60 in 34% yield. Further studies to convert the imidazolidinone 60 into the target one, compound 44, containing a primary amino group, or to prepare a range of MOM substituted *N*-allyl ureas, containing different heteroaromatic and alkylic group at N atoms, were not carried on.

The route based upon the iodine-mediate cyclisation was applied to eight different *N*-allyl urea substrates and led to the synthesis of a small family of six target imidazolidinones and seven azide precursors. Surprisingly, the yields of the iodine-mediated cyclisation and of the final two steps of synthesis were low. The biological activity of those compounds will be discussed in Chapter 4.

An analysis of the relative configuration of the imidazolidinones, prepared through both synthetic routes, was also described in the Chapter. A summary of the diagnostic $J$ value of those compounds is reported in Appendix 3, Table 24. Mechanisms to rationalise the stereochemical outcomes of the Pd-catalysed aminoarylation and the iodine-mediated cyclisation were also discussed.
Chapter 4. Biological activity evaluation of BACE-1 imidazolidinone putative inhibitors

The following Chapter presents results of the biological activity of the synthesised imidazolidinone compounds against BACE-1. The biological activity evaluation was performed through an \textit{in vitro} assay based on fluorescence quenching (Section 4.1). Initially, measurements of biological activity at single concentration were performed; subsequently, dose responses were carried out for selected molecules (Section 4.2). After a discussion of the biological results, new structural analogues of active inhibitors were synthesised and tested against BACE-1 (Section 4.3). Studies of biological selectivity were also performed by assessing the biological activity of selected inhibitors against BACE-2, a homologue of BACE-1 (Section 4.4).

4.1 Assay for biological activity evaluation against BACE-1

The biological activity of the synthesised imidazolidinone compounds against BACE-1 was assessed using an \textit{in vitro} fluorescent competitive inhibition assay. The assay uses a Swedish mutant of BACE-1 natural substrate containing a fluorescent tag, FAM, and a quencher, TAMRA, at its two extremities (Figure 49). The distance between the fluorescent tag and quencher is such that, under UV light excitation, the fluorescence of the donor is quenched.

![Figure 48. Swedish mutant of BACE-1 natural substrate containing a fluorescent tag and a quencher at its extremities. The peptide sequence of the Swedish mutant at the centre of the figure is represented by amino acid symbols.](image-url)
When BACE-1 cleaves the substrate, the fluorescent tag is separated from the quencher and a fluorescence signal is emitted. In presence of a BACE-1 inhibitor, the substrate cleavage is partially or totally inhibited; as a consequence, the fluorescence signal emission is also reduced or suppressed. The difference between the signal observed in absence and in presence of a BACE-1 inhibitor is used to assess the inhibition. A schematic illustration of the fluorescence quenching assay is provided in Figure 49.

**Figure 49. Fluorescent quenching assay illustration.** A) BACE-1 cleaves the substrate, the fluorescent tag, FAM, is separated from the quencher, TAMRA, and it emits a fluorescence signals. B) An inhibitor competes with the substrate in interacting with BACE-1, therefore the substrate cleavage is partially inhibited and a reduced fluorescent emission is observed.

### 4.2 Biological activity measurements

The biological activity of imidazolidinone putative inhibitors was measured at the initial concentration of 100 μM in DMSO. The commercially-available inhibitor β-secretase IV (10 μM in DMSO) was used in the assay as a positive control; DMSO-only (5-10%) was used as a negative control. Details regarding the protocol of the fluorescent quenching assay are provided in Section 5.4. The designed imidazolidinone compounds 94, 97, 99, 101, 104 and 106, their azide precursors and an
iodo precursor, 111, were assayed. The lipophilic imidazolidinone 43, 60, 73, 74 and 75, which were not included in the putative inhibitor design but were prepared to establish the synthetic route of target compounds, were also assayed. The imidazolidinones showing \( \geq 25\% \) inhibition were also assayed for a dose response to determine their IC\(_{50}\). The ligand efficiency, LE' was calculated from the IC\(_{50}\) values (see Equation 2, Section 1.2.3.1, page 8). The results of these in vitro assays are summarised in Table 15 and Table 16. In cases in which was not possible to determine the full dose response, due to solubility or stability issues, the concentration of the putative inhibitor at 50\% of inhibition was reported. Examples of dose response curves are illustrated in Figure 50 further data are reported in Appendix 4.1.

\[ \text{Intermediate of the synthesis of the imidazolidinone 99.} \]
Table 15. Biological activity of amino and azido imidazolidinones against BACE-1.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Inhibition at 100 μM (%)</th>
<th>IC₅₀ (μM)</th>
<th>LE' a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Compound 1" /></td>
<td>33 ± 11</td>
<td>250 ± 4</td>
<td>0.12</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2" alt="Compound 2" /></td>
<td>39 ± 2</td>
<td>720 ± 20</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3" alt="Compound 3" /></td>
<td>17 ± 2</td>
<td>- b</td>
<td>- b</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4" alt="Compound 4" /></td>
<td>13 ± 4</td>
<td>- b</td>
<td>- b</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5" alt="Compound 5" /></td>
<td>9 ± 5</td>
<td>- b</td>
<td>- b</td>
</tr>
<tr>
<td>6</td>
<td><img src="image6" alt="Compound 6" /></td>
<td>12 ± 4</td>
<td>- b</td>
<td>- b</td>
</tr>
<tr>
<td>7</td>
<td><img src="image7" alt="Compound 7" /></td>
<td>6 ± 1</td>
<td>- b</td>
<td>- b</td>
</tr>
<tr>
<td>Entry</td>
<td>Compound</td>
<td>Inhibition at 100 μM (%)</td>
<td>IC$_{50}$ (μM)</td>
<td>LE’$^a$</td>
</tr>
<tr>
<td>-------</td>
<td>----------</td>
<td>--------------------------</td>
<td>----------------</td>
<td>--------</td>
</tr>
<tr>
<td>8</td>
<td><img src="image" alt="Compound 8" /></td>
<td>28 ± 5</td>
<td>750 ± 20$^c$</td>
<td>$^d$</td>
</tr>
<tr>
<td>9</td>
<td><img src="image" alt="Compound 9" /></td>
<td>30 ± 3</td>
<td>590 ± 20</td>
<td>0.10</td>
</tr>
<tr>
<td>10</td>
<td><img src="image" alt="Compound 10" /></td>
<td>10 ± 2</td>
<td>$^b$</td>
<td>$^b$</td>
</tr>
<tr>
<td>11</td>
<td><img src="image" alt="Compound 11" /></td>
<td>8 ± 5</td>
<td>$^b$</td>
<td>$^b$</td>
</tr>
<tr>
<td>12</td>
<td><img src="image" alt="Compound 12" /></td>
<td>no inhibition observed</td>
<td>$^b$</td>
<td>$^b$</td>
</tr>
<tr>
<td>13</td>
<td><img src="image" alt="Compound 13" /></td>
<td>7 ± 2</td>
<td>$^b$</td>
<td>$^b$</td>
</tr>
</tbody>
</table>

$^a$LE’ calculated as $-\log(\text{IC}_{50})/nHA$ (see Equation 2, Section 1.2.3.1). $^b$Not determined; $^c$Concentration at 50% inhibition, $^d$Not calculated, IC$_{50}$ value not available.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Inhibition at 100 μM (%)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
<th>LE&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Compound 1" /></td>
<td>34 ± 12</td>
<td>260 ± 20</td>
<td>0.17</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2.png" alt="Compound 2" /></td>
<td>28 ± 1</td>
<td>805 ± 13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Compound 3" /></td>
<td>15 ± 7</td>
<td>-&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4.png" alt="Compound 4" /></td>
<td>16 ± 1</td>
<td>-&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5.png" alt="Compound 5" /></td>
<td>27 ± 5</td>
<td>260 ± 5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>LE<sup>a</sup> calculated as –log(IC<sub>50</sub>)/nHA (see Equation 2, Section 1.2.3.1). <sup>b</sup>Not determined; <sup>c</sup>Concentration at 50% inhibition, <sup>d</sup>Not calculated, IC<sub>50</sub> value not available
Figure 50. Dose response curves of imidazolidinone 99 and 75 against BACE-1. Data fitted with a sigmoidal dose response model using Origin Pro 8.6.

IC$_{50}$ = 250 ± 4 μM

IC$_{50}$ = 260 ± 20 μM
4.2.1 Analysis of structure-activity relationship

The biological results obtained with the designed series of imidazolidinones, summarised in Table 15, showed that the imidazolidinone 99 (entry 1, IC$_{50}$ = 250 µM) is the most active compound of the series and contains the best combination of substituent groups around the cyclic urea ring. In this compound, the two dimethyl isoxazole groups seem to be important for the activity observed. Replacing the dimethyl isoxazole at the N-3 with a pyridyl ring (entry 3, compound 104) resulted in an inhibition value at single concentration below the 25% threshold (17%). Replacing the dimethyl isoxazole at C-5 with a phenyl group (entry 2, compound 106) decreases the activity of 3-fold (IC$_{50}$ = 720 µM), and removing the dimethyl isoxazole at C-5 (entry 7, compound 101) causes a drastic fall of activity (6% inhibition at 100 µM).

A comparison of the azides 98 (entry 8, IC$_{50}$ = 750 µM) with the amines 99 (entry 1) shows that the azide group could be a viable surrogate of the primary amine, maintaining a moderate activity. This was shown also by the azide 103 (entry 9, IC$_{50}$ = 590 µM) to compare with the amine 106 (entry 2, IC$_{50}$ = 720 µM). The presence of an iodide group (entry 4, compound 111) or of a more constrain cyclic amidic bond (entry 6, compound 97) reduces significantly the activity (ca. 13% inhibition at 100 µM).

The importance of the dimethyl isoxazole groups at the N-3 and at C-5 was observed also in the azide compounds (entry 8-13). The substitution of the dimethyl isoxazole at the N-3 (entry 10, compound 102) and the removal of the dimethyl isoxazole at the C-5 (entry 13, compound 100) bring the activity below the 25% threshold (ca. 10% inhibition at 100 µM) in comparison with compound 98 (entry 8). Regarding the p-chlorobenzyl substituent, it appears to be preferred in comparison to the furan ring or the isopropyl group, as shown by the difference of activity between compound 99 (entry 1) and compounds 94 (entry 5, ca. 10% inhibition at 100 µM); and between compound 102 (entry 10, ca. 10% inhibition at 100 µM) and compound 107 (entry 12, no inhibition observed).

Among the lipophilic series of imidazolidinones, Table 2, the compound 75 (entry 1) resulted surprisingly active (IC$_{50}$ = 260 µM), showing an activity similar to the designed imidazolidinone 99 in Table 1 (entry 1). The azide analogue (entry 2) of the
imidazolidinone 74 demonstrated a lower level of activity (concentration at 50% inhibition = 805 μM), and the iodide analogue 73 (entry 3) did not show any activity (15% inhibition at 100 μM); confirming the trend of the imidazolidinones 99, 98, 111 in Table 1 (entry 1, 4, 7). It was interesting to note that the compound 60 (entry 5), containing a MOM protected alcohol group instead of the primary amine and a bulkier aromatic lipophilic substituent at the C-5, was active (27% inhibition at 100 μM). Unfortunately due to solubility issues, it was not possible to obtain the full dose response curve. As expected, the monosubstituted analogue 43 (entry 4) did not display any activity (15% inhibition at 100 μM).

In terms of ligand efficiency, the active compounds 99, 106 and 103 (Table 15, entry 1, 2 and 9) had similar ligand efficiency values (LE' = 99: 0.12, 106 and 103: 0.10). The lipophilic compound 75 (Table 16, entry 1) was the most ligand efficient, LE' = 0.17; as generally occurred with lipophilic compounds. The ligand efficiency values were generally lower than the predicted ones (cLE' = 99: 0.23, 106: 0.21, 103: 0.20 and 75: 0.26) and the activity values as well (eHiTS prediction of 10⁻⁷ M inhibitors).

The structures of the most active compounds were analysed in order to rationalise the activity results. As mentioned, the biological results revealed that in the case of the imidazolidinone 99 the two dimethyl isoxazole substituents seemed to be fundamental for activity (Figure 51). This might suggest that the two dimethyl isoxazoles could be involved either in key interactions in BACE-1 catalytic site or that their geometry could be more efficient in filling BACE-1 pockets, in comparison to phenyl or pyridine groups.

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⁹ Lipophilic compounds generally show higher ligand efficiency but less specific interactions with a biological target.
In order to shed light on this outcome, an analysis of the predicted binding poses of the imidazolidinones 99 was performed. According to the binding pose predicted by eHiTS, the dimethyl isoxazole attached to N-3 of the urea central core could be involved in a H-bond with Wat1, positioned between the two catalytic aspartates; while the primary amine could act as an H-bond donor and form a H-bond with Glu124 located in the flexible region of the protein, called flap (Section 1.3.2). At the same time the carbonyl group of the urea could act as a H-bond acceptor and be involved in an H-bond with Thr231 (Figure 52, panel A). In terms of protein surface interactions, the dimethyl isoxazole group at C-5 is predicted to being accommodated in the S3 pocket of BACE-1 (Section 1.3.3); the p-chlorobenzyl group is predicted to occupy the S2 pocket, and the H-bond with Glu124 is predicted to lock the protein in a semi-closed conformation (Figure 52, panel B).

**Figure 51. Structure activity relationship relevant to the imidazolidinone 99.**

IC$_{50}$ = 250 ± 4 µM  
not active  
IC$_{50}$ = 720 ± 20 µM  
not active
Figure 52. Predicted binding pose of the imidazolidinone 99 in BACE-1. A) Representation of the predicted H-bonds (green dotted lines) formed with aminoacidic residues in the catalytic site. The primary amine is predicted to form a H-bond with Glu124, located in the flexible region of BACE-1, while the O atom of the isoxazole at N-3 is predicted to form an H-bond with Wat1. B) Representation of the predicted surface interaction. The imidazolidinone 99 is predicted to fill S3 and S2 pockets of BACE-1 respectively with the dimethyl isoxazole at C-5 and with the $p$-chlorobenzyl group. Images correspond to the 4S, 5S enantiomer of the imidazolidinone 99, visualised by Discovery Studio 3.0.
The predicted binding pose of the imidazolidinone 99 seems to help rationalising the role of the isoxazoles. Keeping in mind limitations of computational prediction, it can still be stated that a dimethyl isoxazole group could interact with the conserved water molecule in BACE-1 catalytic site, forming H-bonds with its N or O atoms. Furthermore, it is known that heteroaromatic and aromatic groups can often be accommodated in BACE-1 pockets (See examples in Section 1.4.4); therefore it is plausible that the dimethyl isoxazole group can assume a similar role, and that the two methyl groups can contribute to occupy the pockets more extensively in comparison to pyridine and phenyl group (Figure 53).

Figure 53. Comparison of surface interactions between the imidazolidinone 99 and imidazolidinone 105 in BACE-1 catalytic site. A) Representation of surface interaction of the imidazolidinone 99 containing the dimethyl isoxazole group. B) Representation of surface interaction of the imidazolidinone 99, containing the phenyl group. The more extended surface of the dimethylisoxazole ring (A) occupies S3 pocket and partially S1 pocket, while the phenyl group (B) occupies only a part of S3 pocket. Visualised with Discovery Studio 3.0.

Regarding the lipophilic monosubstituted imidazolidinone 75 (Table 16, entry 1), it was interesting to note that the main structure difference with the inactive analogue 101 (Table 15, entry 7) was the N-aryl substituent group (Figure 54). This difference might suggest that, in this case, the isoxazole group is not involved in critical binding interactions. Other type of interactions, such as lipophilic one, might determine the activity of the monosubstituted imidazolidinone 75, which might assume a different binding pose in BACE-1 catalytic site, in respect to the imidazolidinone 99.
Figure 54. Comparison of two monosubstituted imidazolidinone analogues. The active imidazolidinone 75 differs from the not active imidazolidinone 101 mainly for the substituent group at N-3. This seems to suggest that lipophilic interactions have an important role for the activity of the imidazolidinone 75.

Moreover, a comparison between the monosubstituted imidazolidinones 75 and the more lipophilic bisubstituted imidazolidinone 60 (Table 16, entry 5), both containing a phenyl group, revealed that lipophilic aromatic groups at the “right side” of the urea core might play an important role for the activity (Figure 55). In order to investigate those lipophilic interactions and to understand the role of the OMOM group, the synthesis of a series of analogues of those molecules was designed and prepared (Section 4.3).

Figure 55. Comparison of the imidazolidinone 75 with the lipophilic compound 60.

4.3 Design of structural analogues of active compounds

A small library of analogues of lipophilic imidazolidinones was designed varying the structure of compounds 75 and 60. The analogues were designed in order to identify an alternative group to the primary amine and to evaluate the importance of the lipophilic interactions (Figure 56).
4.3.1 Synthesis of monosubstituted analogues

The alcohol \( 112 \) was prepared in two steps from the iodide \( 73 \) via displacement of iodide with acetate (Scheme 24). The iodide \( 73 \) was treated with silver acetate in acetic acid to give the intermediate \( 116 \) which was used without further purification. The intermediate \( 116 \) was converted into the alcohol \( 112 \) by treatment with potassium carbonate in MeOH. The alcohol \( 112 \) was obtained in 44% yield over two steps.

The synthesis of the imidazolidinone \( 113 \) was attempted using the established iodine-mediated cyclisation (Section 3.3.1). Commercially-available methyl allylamine was treated with phenylisocyanate in \( \text{CH}_2\text{Cl}_2 \) to give the urea \( 117 \), which was used without further purification. The urea \( 117 \) was first treated with trimethylsilyl trifluoromethanesulphonate and triethylamine in \( \text{CH}_2\text{Cl}_2 \) at room temperature, and then with iodine in THF. The resulting iodide intermediate \( 118 \) was mixed with sodium azide in DMF at room temperature overnight, giving the azide \( 119 \) in 14% yield over three steps. Treatment of the azide \( 119 \) with triphenylphospine and water in THF did not give the expected product (Scheme 25).
4.3.2 Synthesis of bisubstituted analogues

The imidazolidinone 114 was prepared from the precursor 60 (Section 3.2.2) in one step, via deprotection of the MOM group. The compound 60 was treated with few drops of hydrochloric acid in MeOH stirring at r.t., giving the alcohol 114 in 42% yield (Scheme 26).

The imidazolidinone 115 was prepared from the amine building block 120 (Scheme 27). The amine building block 120 was synthesised in 54% yield from the imine 78, using a similar methodology to the amines 76 and 77 (Scheme 21, Section 3.3.2.1), by treatment with n-butyl lithium and 2-(chloromethyl)naphthalene, followed by base hydrolysis. The amine 120 was then treated with acetylchloride and triethylamine in CH₂Cl₂, to give the acetamide 121. The acetamide 121 was reduced to give the ethyl allylamine 122 in 88% yield by treatment with lithium aluminium hydride in THF. The urea 123 was prepared from the allylamine 122 and phenyl isocyanate in CH₂Cl₂ in 47% yield and was employed in the iodine-mediated cyclisation to give imidazolidinone 124. The urea 123 was treated with trimethylsilyl trifluoromethanesulfonate and triethylamine in CH₂Cl₂ at r.t. and then with iodine in THF. The resulting iodine
intermediate was treated with sodium azide in DMF at r.t. overnight to give the azide 125, which was used without further purification. The azide 125 was treated with triphenylphosphine and water in THF to give the final imidazolidinone 115 in 25% yield over three steps.

Scheme 27. Synthesis of the imidazolidinone 115.

4.3.3 Biological evaluation of a second generation of analogues

The synthesised analogues of lipophilic imidazolidinones, compounds 112, 114 and 115, and the azide compound 119 were tested against BACE-1 through the established in vitro assay. The results are summarised in Table 17. In cases in which it was not possible to obtain the full dose response curve, due to solubility issues, the concentration at 50% of inhibition observed was reported.
Table 17. Biological activity of analogues of lipophilic imidazolidinones against BACE-1.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Inhibition at 100 μM (%)</th>
<th>IC\textsubscript{50} (μM)</th>
<th>LE\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="Compound 112" /></td>
<td>10 ± 1</td>
<td>_\textsuperscript{b}</td>
<td>_\textsuperscript{b}</td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="Compound 119" /></td>
<td>no inhibition observed</td>
<td>_\textsuperscript{b}</td>
<td>_\textsuperscript{b}</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="Compound 114" /></td>
<td>41 ± 12</td>
<td>1170 ± 83\textsuperscript{c}</td>
<td>_\textsuperscript{d}</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="Compound 115" /></td>
<td>25 ± 3</td>
<td>724 ± 12</td>
<td>0.12</td>
</tr>
</tbody>
</table>

\textsuperscript{a}LE' calculated as –log(IC\textsubscript{50})/nHA, (see Equation 2, Section 1.2.3.1); \textsuperscript{b}Not determined; \textsuperscript{c}Value of concentration at 50% inhibition; \textsuperscript{d}Not calculated, IC\textsubscript{50} value not available

The biological results showed that the alcohol group is not a suitable surrogate of the primary amine; since the monosubstituted analogues 112 (entry 1, 10% inhibition at 100 μM) and the bisubstituted analogues 114 (entry 3, concentration at 50% inhibition = 1170 μM) demonstrated lower activity than the respective amino compounds 75 (Table 16, entry 1, IC\textsubscript{50} = 260 μM) and 115 (Table 3, entry 4, IC\textsubscript{50} = 724 μM). The benzyl group at the N-1 of the urea central core seems to be critical for the activity of the monosubstituted series of imidazolidinones. Replacement of the benzyl group with a methyl group (compound 119, entry 2) suppressed any activity in comparison with the more lipophilic azide 74, (Table 16, entry 2, 28% inhibition at 100 μM). These results seem to indicate that, in this series of compounds, lipophilic groups at both side of the urea central core play a significant role in inhibiting BACE-1.
Regarding the series of bisubstituted imidazolidinones, the analogue 114, containing the alcohol group at C-4 (entry 3) displayed activity at single concentration above the threshold; but, once again, it was not possible to determine the full dose response due to solubility issues. A comparison of the concentrations at 50% inhibition revealed that the analogue 114 (entry 3) is ca. 5-fold less active than the analogue 60 containing the MOM group (Table 16, entry 5, concentration at 50% inhibition = 260 μM). The imidazolidinone 115, containing the amino group (entry 4), displayed modest activity: ca. 2.8-fold less than the monosubstituted compound 75 (Table 16, entry 1, IC₅₀ = 260 μM). This might suggest that the best position to incorporate a lipophilic group is at N-1, at the “right side” of the urea central core.

Illustrations of the predicted binding poses of the imidazolidinone 75 are provided in Figure 57. The imidazolidinone 75 is predicted to be involved in H-bond and π-cation interactions with Arg235, located at the top of BACE-1 catalytic site. The H-bond is formed through the carbonyl group, while the π-cation interactions are formed through both benzyl and phenyl group. These interactions seem to direct the primary amine towards the water molecules, allowing the formation of H-bonds (Figure 57, panel A). In terms of protein surface interactions, the phenyl group is predicted to occupy the S1’ pocket of BACE-1, while the benzyl group is predicted to be oriented towards the inner without occupying any pocket (Figure 57, panel B).
Figure 57. **Predicted binding pose of the imidazolidinone 75.** A) Representation of the predicted H-bonds (green dotted-lines) and π-cation interactions (orange dotted lines) in BACE-1 catalytic site. B) Representation of the predicted surface interactions: the benzylic group is not oriented towards the available pockets (right), the phenyl group is accommodated into S1’ pocket (left). Figures represented for the $R$ enantiomer of the imidazolidinone 75. Visualised with Discovery Studio 3.0.
The predicted binding pose of the imidazolidinone 75 might suggest a possible explanation about the importance of the aromatic groups for the activity against BACE-1. When both benzyl and phenyl group are attached to the two $N$ of the urea core, they could be oriented in the same direction in BACE-1 catalytic site and be involved in common binding interactions ($e.g.$ $\pi$-sigma, $\pi-\pi$ or $\pi$-cations interactions or pocket filling); which might act as an “anchor point” to direct the rest of the molecule towards the catalytic aspartates or Wat1, where the primary amine can form H-bonds. When the benzyl group is replaced with an alkyl group (in the case of the imidazolidinone 113, Table 17, entry 2) or when a bulkier aromatic group is incorporated in another position around the cyclic urea central core (in the case of the imidazolidinone 60, Table 16, entry 5), such as a common interaction cannot occur and the imidazolidinone scaffold might assume a less effective binding pose.

### 4.4 Assessing inhibitor selectivity for BACE-1

BACE-2 is an homolog of BACE-1, with 75% homology and 45% gene coding sequence identity.$^{128}$ BACE-2 is expressed in low level in brain and competes with BACE-1 for the same substrate, acting as an antagonist for BACE-1, similar to $\alpha$-secretase$^{129}$ (Section 1.3.1). The role of BACE-2 in Alzheimer’s disease is not fully understood yet, but seems to be marginal in comparison to BACE-1.$^{130}$ BACE-1 inhibitors are generally tested against BACE-2 in order to determine their selectivity for BACE-1. Therefore the two identified BACE-1 inhibitors, the imidazolidinone 99 and 75, were assayed in vitro against BACE-2 protein to determine their selectivity for either protein.

#### 4.4.1 Biological activity measurements

The described in vitro fluorescent competitive inhibition assay (Section 4.1) was employed to determine the activity of the imidazolidinone 99 and 75 against BACE-2. The biological activity was initially measured at 100 $\mu$M (5-10% of DMSO in buffer); then a dose response assay was performed. As for BACE-1, the commercially-available inhibitor $\beta$–secretase IV (100 $\mu$M in DMSO) was employed as a positive control DMSO-only (5-10%) was employed as a negative control. Details regarding the execution of the fluorescent quenching assay for BACE-2 are provided in Section 5.4.1.
The results of the \textit{in vitro} assays are summarised in Table 18; dose response curves are illustrated in Figure 58 and Figure 59.

\textbf{Table 18. Biological activity of imidazolidinones 99 and 75 against BACE-2.}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Inhibition at 100 ( \mu \text{M} ) (%)</th>
<th>IC(_{50}) (( \mu \text{M} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image.png" alt="Chemical Structure 99" /></td>
<td>10 ( \pm ) 3</td>
<td>(-^a)</td>
</tr>
<tr>
<td>2</td>
<td><img src="image.png" alt="Chemical Structure 75" /></td>
<td>8 ( \pm ) 4</td>
<td>647 ( \pm ) 20</td>
</tr>
</tbody>
</table>

\(^a\)Not determined, dose-response not observed.

\textbf{Figure 58. Comparison of activity of the imidazolidinone 99 against BACE-1 and BACE-2.} Data fitted with a sigmoidal dose response model using Origin Pro 8.6.
The imidazolidinone 99 and 75 displayed a similar value of inhibition against BACE-2 at single concentration, ca. 10%; but the dose response revealed different outcome. The imidazolidinone 75 showed an activity of ca. 647 μM, only 2.5-fold less active than against BACE-1, suggesting that the activity may stem from non-specific interactions. Regarding the imidazolidinone 99, although the IC₅₀ could not be determined with BACE-2, the compound showed at least 10-fold selective for BACE-1.

4.5 Summary

This Chapter described the evaluation of biological activity of a focused library of imidazolidinone putative inhibitors of BACE-1, and of a series of lipophilic analogues. The compounds were assayed at a single concentration (100 μM) and IC₅₀ values of the promising inhibitors were also determined. The best BACE-1 inhibitors, amongst the imidazolididones assayed, were compounds 75 and 99, showing an IC₅₀ value of ca. 250 μM.
The inhibitor 99, belonging to the designed focused library of compounds, showed the following key structure features: a hydrophilic dimethyl isoxazole ring at N-3 of the urea system, a p-chlorobenzyl group at N-1, another dimethyl isoxazole ring at the C-4 position and a primary amine at the C-5 position (Figure 60, panel A). The lipophilic imidazolidinone 99, which did not belong to the designed focused library of putative inhibitors, contained lipophilic substituents (benzyl and phenyl) at both the N atoms of the urea system and a primary amine at the C-4 position (Figure 60, panel B).

![Figure 60. Structures of the identified imidazolidinone BACE-1 inhibitors. A) Designed imidazolidinone 99, IC$_{50}$ = 250 μM, dimethyl isoxazole groups seem to be critical for activity. B) Lipophilic imidazolidinone 75, IC$_{50}$ = 260 μM, aromatic groups at the N atoms seem to be critical for activity.](image)

The designed imidazolidinone 99 demonstrated a good selectivity for BACE-1 vs the homolog BACE-2, being at least 10-fold less active against BACE-2. The lipophilic imidazolidinone 75, instead, was only 2.5-fold more active against BACE-1 than against BACE-2, suggesting that it might bind BACE-1 through non-specific lipophilic interactions. Given the good selectivity of the imidazolidinone 99, it can be considered a good novel lead molecule to be developed into a more potent BACE-1 inhibitor.
4.6 Conclusion and future directions

In this thesis a novel approach to design inhibitors was described and validated. The approach started with a computational design of a virtual library of skeletally diverse virtual lead-like compounds, which was screened \textit{in silico} against a target protein, BACE-1. From the virtual screening, identified putative inhibitors were selected for synthesis and tested \textit{in vitro} through a fluorimetric assay.

A virtual library of skeletally diverse lead-like compounds was generated on the basis of the diversity oriented synthesis approach (DOS) developed within the Nelson group. The approach used a virtual protocol of synthesis, in which an initial pool of virtual electrophile and nucleophile building blocks underwent a combinatorial virtual coupling, followed by a series of virtual chemical reactions: derivatisation, cyclisation and further derivatisation (Section 2.3, Figures 34 and 37). The virtual chemical reactions employed were previously established experimentally, and only commercially available reactants were included in the virtual library enumeration, in order to ensure synthetic accessibility as far as possible. A virtual library of ca. 55,000 cyclic lead-like molecules was generated. The virtual library was screened against BACE-1 using eHiTS software.

Two families of putative inhibitors of BACE-1 were identified after the vHTS with eHiTS: the amino tetrahydropyridine and the imidazolidinone family. Both families contained elements of novelty with respect to known BACE-1 inhibitors. The most potentially diverse family of imidazolidinone was selected for synthesis (Section 2.4.2). A focused library of this family was designed. The key features of the structure of imidazolidinones were a primary amine and a series of heteroaromatic or alkyl substituent positioned around the cyclic urea central core (Figure 61). According to the computational prediction of binding poses of this family, the primary amine would form key H-bond interactions with the catalytic Asp32 and Asp228 in BACE-1 catalytic site; while heteroaromatic group could fill pockets in BACE-1.
Two synthetic routes were envisaged for the synthesis of the imidazolidinone putative inhibitors of BACE-1, both based on a key cyclisation reaction of N-allyl ureas, to obtain the cyclic central core of the imidazolidinones. The first synthetic route was suggested by the virtual protocol of synthesis employed to enumerate a virtual library of lead-like compounds. The route was based upon the Pd-catalysed aminoarylation on N-allyl ureas containing different functional groups at the allylic position. The second synthetic route was based upon the iodine-mediated cyclisation of N-allyl ureas containing different aromatic groups at the allylic position (see Scheme 5, Section 3.1, page 66).

The investigation of the Pd-catalysed aminoarylation led to the identification of the imidazolidinone 60 (Figure 62), containing the MOM protected alcohol at the 4-CH₂ position, as a precursor of the final imidazolidinone, containing a primary amine at the same position. To apply this route to the designed imidazolidinones (Figure 61), further investigations regarding the synthesis of specific N-allyl urea substrates, containing heteroaromatic groups, were required. At the same time, chemical reactions to convert the MOM protected alcohol into a primary amine needed to be explored. As a consequence the route was abandoned.
Figure 62. Imidazolidinone 60, synthesised through the Pd-catalysed aminoarylation. The imidazolidinone 60 was identified as a possible precursor of a final imidazolidinone, containing a primary amine at 4-CH₂ position.

The second synthetic route, based upon the iodine-mediated cyclisation, was applied to eight different N-allyl urea substrates and led to the synthesis of a small library of six target imidazolidinones (Figure 63) and seven azide precursors. Surprisingly, the yields of the iodine-mediated cyclisation and of the final two steps of synthesis were low. The six target imidazolidinones, the azide precursors and a series of lipophilic imidazolidinones (synthesised during the exploration of the two synthetic routes) were assayed against BACE-1 through an established in vitro fluorimetric assay.

Figure 63. Focused library of six imidazolidinones, putative inhibitors of BACE-1.

Two compounds showed inhibitory capacity against BACE-1, with IC₅₀ value of ca. 250 μM (Figure 60, Section 4.5). The imidazolidinone 99, belonging to the designed library of putative inhibitors, showed a good selectivity (10-fold) for BACE-1 over its homologue BACE-2. The imidazolidinone 75, belonging to the series of lipophilic
compounds, was surprisingly active, but it did not show good selectivity for BACE-1 over BACE-2; being only 2.5-fold more active against BACE-1. These results suggested that the imidazolidinone 99 is likely to interact through promiscuous interactions, while the designed imidazolidinone 75 is likely to form specific interactions in BACE-1 catalytic site.

In conclusion, the biological results validated the computational approach employed to design a focused library of BACE-1 inhibitors. A novel imidazolidinone inhibitor, compound 99, exhibited a μM inhibitory capacity, 3-fold lower than the one predicted by the eHiTS, nevertheless it showed a good selectivity for BACE-1 over BACE-2. Therefore, it can be considered a good lead to be developed in a more potent BACE-1 inhibitor. Suggestions towards the optimisation of 99 are herein indicated.

A common strategy employed to develop initial leads of μM activity into potent BACE-1 inhibitor is to increase the length of alkyl substituents or adding lipophilic groups.49 These modifications generally aim to occupy BACE-1 pockets. Following this strategy, similar modifications can be suggested to further develop the imidazolidinone 99. Keeping the two dimethyl isoxazoles in the structure, critical for the activity, and the \( p \)-chloro benzyl group, which is preferred above a furan ring of an alkyl group (Section 4.2), the imidazolidinone 99 could be modified at the primary amine. The primary amine could be converted into an amide, by reaction with amino acids containing aromatic groups. Alternatively it could be modified by simple arylation (Figure 64). The aromatic group and the amino acid chains could occupy S2' or S1' BACE-1 pockets, which are predicted not to be occupied by the imidazolidinone 99 (Section 4.2.1, Figure 52).
Figure 64. BACE-1 μM inhibitor as lead molecule to develop into more potent inhibitors. A) Pyrrolidine compound discovered by Stachel et al.\textsuperscript{131} in 2012, and developed in a spiropyrrrolidine inhibitor of 0.03 uM. B) Imidazolidinone inhibitor 99, to be develop in a more potent inhibitors by addition of alkyl or aromatic groups.

The described computational approach had two main limitations: the difficulty of predicting both synthetic accessibility and inhibitory activity of the designed molecules. Regarding the synthetic accessibility, the protocol of synthesis designed in Pipeline Pilot was based on Pd-catalysed aminoarylation and was not successful. The investigation of the Pd-catalysed aminoarylation showed that functional groups such as azide, NHBoc, NsNBoc are not compatible with the reaction conditions. N-Allylurea substrates containing those functional groups at the allylic position did not give the expected product, or gave it in very low yield (Section 3.2.2, Table 11). Therefore a new synthetic route based on the iodine-mediated cyclisation was employed to prepare a focused library of imidazolidinones. Unfortunately, apart from few exceptions (compound 104, Section 3.3.2.2, Table 14, entry 6, and compound 115, Section 4.3.2, Scheme 27), the yield of the iodine–mediated cyclisation were poor. In order to improve the value of virtual libraries of structurally diverse compounds, designed \textit{in silico} according to our computational approach, it is necessary to refine them as the scope of the reactions included in the protocol of synthesis is defined. In our group, Richard Doveston has recently exemplified the synthesis of structurally diverse lead-like
scaffolds using a robust toolkit of reactions. This toolkit of reactions would be a good basis for the design a virtual library of compounds, having a greater of successful results in synthesis.

Regarding the prediction of inhibitory activity of novel molecules against biological target, the employed eHiTS software overestimated the potency of the imidazolidinone library. The predicted potency of compounds was of $10^{-7}$ M, while the measured potency was of ca. $10^{-4}$ M. eHiTS was reliable in reproducing the binding pose of the known inhibitor 26 (Section 1.4.4 and 2.2) and in predicting its activity (predicted activity of $10^{-7}$ M, real activity 3 μM) therefore it seemed to be a good choice to perform the vHTS of virtual libraries. As eHiTS requires a reference inhibitor to identify protein binding site and to orient new ligands in the protein, it is possible that scores of ligand binding poses, which are oriented similarly to the inhibitor of reference in the binding site, can be overestimated. It is worth mentioning that SPROUT prediction of potency for the imidazolidinone library was more accurate, $10^{-4}$ M. SPROUT scoring function differs from eHiTS scoring functions; in SPROUT pocket filling and lipophilic interactions weighted more than H-bond interactions. Therefore it is possible that given the characteristic of BACE-1 target, with six pockets available to be filled (Section 1.3.3), SPROUT could be more appropriate to predict a more reliable score.

The literature confirmed that prediction of activity of library of compounds against specific biological targets is still challenging. It has been reported that many docking software are able to reproduce correctly X-ray poses of an inhibitors in a biological target, but a reliable prediction of the free energy of binding is still very difficult.\textsuperscript{132,133} Factors such as protein flexibility and solvent are not always predictable. In this project, vHTS was performed in the presence and absence of two water molecules, located in the proximity of the catalytic Asp32 and Asp228 residues, and in both cases similar docking predictions were obtained. The similarity of outcomes, for displaceable water molecules, occurred often with most biological targets.\textsuperscript{134} As general advice, the use of more than one docking software can be advantageous in achieving a good prediction of potency, and verifying predicted ligand binding poses through X-ray and H\textsuperscript{1} NMR could be a good source of information to assess the quality of docking.
Despite the discussed limitations of the investigated approach, a novel lead molecule with $\mu$M potency against BACE-1, compound 99, has been successfully identified. The importance of the dimethyl isoxazole groups for the compound activity is an interesting factor, which has not yet been acknowledged in the literature. Moreover the approach opens a new pathway towards ligand identification, which differs from the currently used approach of fragment based-drug design or high throughput screening.
Chapter 5. Experimental

5.1 General experimental

All non-aqueous reactions were performed under an atmosphere of nitrogen unless otherwise stated. Water sensitive reactions were performed in oven dried glassware, cooled under nitrogen before use, or flame dried, and cooled under vacuum if stated. Solvents were removed under reduced pressure using a Büchi rotary evaporator and a Vacuubrand PC2001 Vario diaphragm pump, or a Genevac HT-4 evaporation system.

Tetrahydrofuran, dichloromethane, toluene, ethanol and acetonitrile were dried and purified by means of a PureSolv MD solvent purification system (Innovative Technology Inc.). Anhydrous N,N-dimethylformamide and 1,4-dioxane was obtained in Oxford sure/seal™ bottles from Sigma–Aldrich. All other solvents used were of chromatography or analytical grade. Ether refers to diethyl ether and petrol refers to petroleum spirit (b.p. 40-60 °C). Commercially available starting materials were obtained from Sigma–Aldrich, Fluka, Lancaster, Alfa Aesar or Acros Organics and used without further purification unless stated.

Thin layer chromatography was carried out on aluminium backed silica (Merck silica gel 60 F254) plates supplied by Merck. Visualisation of the plates was achieved using an ultraviolet lamp ($\lambda_{\text{max}} = 254$ nm), KMnO$_4$, anisaldehyde or ninhydrin. Flash column chromatography was carried out using silica (35-70 μm particles) with crude reaction mixtures loaded in dichloromethane (CH$_2$Cl$_2$) or the initial solvent system, or pre-absorbed onto silica. Where specified, flash column chromatography was carried using Isolera flash purification system of Biotage SNAP cartridge Kp-Sil instrument (Isolera four, version 1.3.3). SNAP cartridge columns of 25 g were used for 1.0-1.5 g of crude and SNAP cartridge column 50 g for 3-4 g of crude.

Mass-directed HPLC purifications were run on an Agilent 1260 Infinity Preparative HPLC system equipped with a Waters XBridge™ Prep C18 19 × 100 mm column, 5 μm particle size, on an acetonitrile or methanol/water gradient (5-95% acetonitrile or methanol over 8 minutes) and an Agilent 6120 Quadrupole system equipped with a quadrupole MS detector, using electrospray (ES) ionisation. Semipreparative HPLC
purifications were run on a Gynkotec system equipped with a Phenomenex®
Hyperclone ODS-C18 250 × 10 mm column, 5 µm particle size, on an acetonitrile/water
gradient (5-95% acetonitrile, 0.1% TFA, over 30 minutes).

All optical rotations were carried out at room temperature on a Perkin-Elmer AA-
1000 with a path length of 1 dm; concentrations are g/100 mL, the optical rotations are
given in 10⁻¹ deg cm² g⁻¹ and units are omitted for clarity. Infrared spectra were
recorded on a Perkin-Elmer One FT-IR spectrometer, the wavelengths (ν_max) are given
in cm⁻¹ and units are omitted for clarity.

Proton and carbon NMR data were combined on an Avance 500, Bruker DPX500
and DPX300 spectrometer. All shifts were recorded against and internal standard of
tetramethylsilane (TMS). Solvents (CDCl₃, DMSO-d₆ and MeOH-d₄) used for NMR
experiments were obtained from Sigma-Aldrich. Splitting patterns in this report have
been recorded in an abbreviated manner; app. (apparent), s (singlet), d (doublet), t
(triplet), q (quartet), m (multiplet). NMR data was recorded in the following format;
ppm (number of protons, splitting pattern, coupling constant (Hz), proton ID). Signal
assignments were made with the aid of COSY, DEPT 90 and 135, HMQC and HMBC.
NMR spectra were recorded at 300 K, unless stated.

Low resolution mass spectra data were recorded on a system comprising an Aligent
1200 series LC system comprising and a Bruker HCT Ultra ion trap mass spectrometer
using electrospray (ES) ionisation. The system used two solvent systems; MeCN/H₂O +
0.1% formic acid with a Phenomenex Luna C18 50 × 2 mm 5 micron column or
MeCN/H₂O with a Phenomenex Luna C18 50 × 2 mm 5 micron column.

Nominal and accurate mass spectrometry was routinely performed by Mrs Tanya
Marinko-Covell on a Waters-Micromass GCT spectrometer using electron impact (EI)
or on a Bruker Daltronics micrOTOF using ES ionisation. Accurate mass spectrometry
was also carried out using ES ionization with a Bruker Maxis Impact spectrometer. For
each compound, data of the most abundant isotope are reported.

In the experimental procedures for compounds, Session 5.3, novel compounds are
indicated in italics according to the handbook for postgraduate research students of the
University of Leeds. References of the preparation are indicated after the name of the
compounds.
5.2 General experimental procedure

A. General procedure for the preparation of the chiral phosphoramidites (S,S,R)-51 and (R,R,R)-52.\textsuperscript{113}

Triethylamine (4.0 mL, 28.0 mmol) was added slowly to a solution of phosphorus trichloride (0.6 mL, 7.0 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (15 mL) at 0 °C, then a solution of the relevant amine (bis[(R)-1-phenethyl]amine or bis[(S)-1-phenethyl]amine) (1.6 mL, 7.0 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (25 mL) was added by dropping funnel over 1 h at 0 °C. The reaction mixture was allowed to warm to r.t. and stirred for 18 h, then a solution of the relevant naphthal [(R)-BINOL or (S)-BINOL)] (2.0 g, 7.0 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (25 mL) was added followed by triethylamine (3.0 mL, 21.6 mmol) by dropping funnel over 1 h at 0 °C. The reaction mixture was allowed to warm to r.t., stirred for 5 h and quenched with cold water (30 mL) at 0 °C. The phases were separated, the aqueous phase was extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 × 25 mL), the combined organic phases were dried (MgSO\textsubscript{4}) and concentrated under reduced pressure to give a crude product.

B. General procedure for the preparation of the ureas 42, 45, 54, 71, 85-92 and 123.\textsuperscript{100}

The relevant isocyanate (2.0-10 eq.) was added slowly to a solution of the relevant amine (from 0.4 to 15.0 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (0.20 M) and the reaction mixture was heated under reflux and stirred. The reaction was monitored by TLC and LC/MS and, when judged complete, the mixture was concentrated under reduced pressure to give a crude product.

C. General procedure for the Pd-catalysed aminoarylation of the N-allyl ureas, to give the imidazolidinones 43, 58, 60 and 61.\textsuperscript{100}

Pd\textsubscript{2}(dba)\textsubscript{3} (5 mol%), Xantphos (10 mol%), NaO\textsubscript{t}Bu (1.8 eq.), 2-bromonaphthalen (1.5-3.0 eq.) were loaded in a flame-dried round bottom flask and the reaction vessel was purged with nitrogen and degassed (four times). The N-allylphenyl urea substrate (from 0.2 to 1.35 mmol) and toluene (4.0 mL/mmol of the urea substrate) were added and the resulting mixture was stirred at 110 °C for 2-3 days. The reaction was
monitored by TLC and LC/MS and, when judged complete, the reaction mixture was cooled to r.t. Then a saturated aqueous solution of ammonium chloride (5.0 mL/mmol of the urea substrate) was added and the mixture was extracted with EtOAc (3 × 4.0 mL/mmol of the urea substrate). The combined organic phases were dried (Na$_2$SO$_4$) and concentrated under reduced pressure to give a crude product.

D. General procedure for the preparation of substituted allylic amines 76, 77 and 120.

$n$-Butyl lithium (1.0 eq.) was added dropwise to a solution of the diphenyl methylene propenamine 78 (from 18.1 to 29.0 mmol) in THF (0.9 M) at −50 ºC and the resulting mixture was stirred at −50 ºC for 1 h. The relevant electrophile (1.0 eq.) was added portionwise as a solution of THF (6.0 M), the mixture was stirred at −50 ºC for 1 h and quenched with EtOH (0.7-1.2 mL). An aqueous solution of KHSO$_4$ (20% w/v, 1.2 mL/mmol of the propenamine) was added and the mixture was stirred overnight allowing the temperature to warm to r.t. Two phases were formed; the aqueous phase was separated, washed with methyl tert-butyl ether (25-35 mL) and CH$_2$Cl$_2$ (2 × 25 mL), basified at 0 ºC with an aqueous solution of NaOH (50% w/v) until pH 10 then extracted with CH$_2$Cl$_2$ (2 × 25 mL). The combined organic phases were washed with brine (2 × 25 mL), dried (Na$_2$SO$_4$) and concentrated under reduced pressure to give a crude product.

E. General procedure for the reductive amination of substituted allylic amines, to give compounds 80, 81 and 83.

The relevant aldehyde (1.2-2.4 eq.) was added to a solution of the relevant amine (from 1.2 to 8.0 mmol) in MeOH (0.20 M) and the resulting mixture was stirred at r.t. or heated under reflux for 6-24 h. NaBH$_4$ (3.0-5.0 eq.) was added at 0 ºC and the mixture was stirred at r.t. overnight. The solvent was removed under reduced pressure and the crude material was dissolved in EtOAc (~10-20 ml), washed with H$_2$O (2 x 10 mL) and brine (2 x 10 mL), dried (MgSO$_4$) and concentrated under reduced pressure to give a crude product.
F. General procedure for the preparation of the azides 74, 93, 95, 98, 100, 102, 103, 105 and 107\textsuperscript{124,137} from allyl ureas.

\[
\begin{array}{c}
\text{R}^2\text{N}=\text{N}\text{R}^1 \\
\text{R} \\
\rightarrow \\
\begin{array}{c}
\text{O} \\
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\text{N} \\
\text{R} \\
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\end{array}
\rightarrow
\begin{array}{c}
\text{R}^2\text{N}=\text{N}\text{R}^1 \\
\text{I} \\
\text{N}_3
\end{array}
\]

The iodo intermediates were prepared from the relevant allyl ureas according to the procedure of Moody \textit{et al.}\textsuperscript{124} Triethylamine or DBU (2.0 eq.) and trimethylsilyl trifluoromethansulfonate (2.0 eq.) were added to a solution of the relevant allyl urea (0.3-1.5 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (0.20 M) and the resulting mixture was stirred for 1 h at r.t., then concentrated under reduced pressure. The crude material was dissolved in THF (0.15 M), I\textsubscript{2} (2.0 eq.) was added and the resulting mixture was stirred at r.t. or heated under reflux overnight. Then the reaction mixture was poured into an aqueous solution of Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} (20\% w/v) and extracted with EtOAc (3 x 7-20 mL). The combined organic phases were dried (MgSO\textsubscript{4}) and concentrated under reduced pressure to give the relevant iodo intermediate as a crude product which was used without further purification.

The azide compounds were prepared from the relevant iodo intermediates according to the procedure of Knapp \textit{et al.}\textsuperscript{137} Sodium azide (4.0-6.0 eq.) was added to a solution of the relevant iodo intermediate in DMF (0.1 M) at 0 °C; the resulting mixture was stirred under reflux overnight, allowed to cool at r.t., poured in EtOAc (7-20 mL) and washed with H\textsubscript{2}O (3 x 5.0-15 mL) and brine (2 x 5-15 mL). The organic phase was dried (MgSO\textsubscript{4}) and concentrated under reduced pressure to give a crude product.

G. General procedure for the reduction of azides, to give the amines 75, 94, 97, 99, 101, 104, 106 and 115\textsuperscript{138}

Trimethylphosphine or triphenylphosphine (1.5 eq.) was added to a solution of the relevant azide in THF (0.1 M), H\textsubscript{2}O was added (1/10 of THF volume) and the resulting mixture was stirred under reflux for 2-3 days; then concentrated under reduced pressure to give a crude product.
5.3 Experimental procedures for compounds

*tert*-Butyl(2-hydroxyethyl)carbamate 47\(^{139}\)

The title compound was prepared according to the general method of Jia *et al*.\(^{139}\) Di-*tert*-butyl dicarbonate (14.5 g, 66.4 mmol) was added slowly at 0 °C to ethanolamine (4.0 mL, 66.4 mmol) and the resulting mixture was stirred at r.t. for 1 h. Water (40 mL) and EtOAc (40 mL) were added to the reaction mixture and the phases were separated. The aqueous phase was extracted with EtOAc (3 × 20 mL) and the combined organic phases were washed with brine (20 mL), dried (MgSO\(_4\)) and concentrated under reduced pressure to yield the title protected amine 47 as a colourless oil (10.4 g, 97%), which was used without further purification. \(R_F\): 0.6 (10:1 CH\(_2\)Cl\(_2\)–MeOH); \(\nu_{\text{max}}/\text{cm}^{-1}\) (film) 3358, 2978, 2934, 1693, 1530, 1367, 1280, 1172, 1070, 867; \(\delta_H\) (500 MHz; CDCl\(_3\)) 4.96 (1H, br s, NH), 3.70 (2H, q, \(J = 4.8\), 2-\(H_2\)), 3.29 (2H, q, \(J = 4.8\), 1-\(H_2\)), 2.47 (1H, br s, OH), 1.45 (9H, s, \('\text{Bu}\)); \(\delta_C\) (75 MHz, CDCl\(_3\)) 157.2 (CONH), 79.9 (C(\('\text{Bu}\))), 62.9 (C-2), 43.4 (C-1), 28.5 (\('\text{Bu}\)): \(m/z\) (ES) 184.1 (100%, [M+Na]+). Characterisation data were consistent with those reported in literature.\(^{139}\)

(E)-Ethyl 4-(*tert*-butoxycarbonylamino)but-2-enoate 48\(^{140}\)

The title compound was prepared according to the general method of Crisóstomo *et al*.\(^{140}\) Dimethyl sulfoxide (26.0 mL), triethylamine (26.5 mL) and (ethoxycarbonylmethylene) triphenylphosphorane (22.0 g, 63.2 mmol) were added to a solution of the carbamate 47 (5.1 g, 31.6 mmol) in CH\(_2\)Cl\(_2\) (210 mL). The reaction mixture was stirred until all reactants were solubilised, SO\(_3\)-Py complex (15.1 g, 91.8 mmol) was added at 0 °C and the reaction mixture was stirred at r.t. for 36 h. An aqueous solution of HCl (1.00 M) was added until pH ~ 2 and the phases were separated. The aqueous phase was extracted with CH\(_2\)Cl\(_2\) (3 × 30 mL) and the combined organic phases were washed with saturated solution of brine (3 × 50 mL), dried
(MgSO₄) and concentrated under reduced pressure. The crude product was purified by chromatographic column (80:20 petrol–EtOAc), to give the unsaturated ester 48 (1.10 g, 56%; E:Z >98:< 2) as pale yellow oil. Rₖ: 0.6 (60:40 petrol–EtOAc); νₘₐₓ/cm⁻¹ (film) 3361, 2980, 2933, 1662, 1662, 1523, 1367, 1174, 1042, 867; δₓ (500 MHz; CDCl₃) 6.88 (1H, dt, J 15.7 and 6.1, 3-H), 5.91 (1H, d, J 15.7, 2-H), 4.65 (1H, br. s, NH), 4.19 (2H, q, J 7.1, ethyl 1-H₂), 3.92 (2H, app. br s, 4-H₂), 1.45 (9H, s, t-Bu), 1.28 (3H, t, J 7.1, ethyl 2-H₃); δₓC (75 MHz; CDCl₃) 166.1 (C O₂Et), 155.5 (C ONH), 144.7 (C-3), 121.6 (C-2), 80.0 (C(1(Bu)), 60.5 (ethyl C-1), 41.5 (C-4), 24.5 (1(Bu), 14.3 (ethyl C-2); m/z (ES) 252.1 (100%, [M+Na]⁺). Characterisation data were consistent with those reported in literature.¹⁴⁰

(E)-tert-Butyl(4-hydroxybut-2-en-1-yl)carbamate 49¹¹¹

The title compound was prepared according to the general method of Moriwake et al.¹¹¹ The unsaturated ester 48 (3.1 g, 13.6 mmol) was dissolved in dry CH₂Cl₂ (31 mL) at −78 °C and trifluoride etherate (1.9 mL, 4.1 mmol) was added. The reaction mixture was stirred at −78 °C for 30 min, then diisobutylaluminium hydride in hexane (41 mL, 41.0 mmol, 1.0 M) was added slowly and the reaction mixture was stirred at −78 °C for 90 min. The reaction mixture was quenched with an aqueous saturated solution of NH₄Cl (30 mL) and filtered on Celite® eluting with EtOAc. The phases were separated, the aqueous phase was extracted with EtOAc (3 x 7.0 mL), the combined organic phases were washed with brine (3 x 7.0 mL), dried (MgSO₄) and concentrated under reduced pressure to give a crude product which was purified by column chromatography (50:50 petrol–EtOAc) to yield the alcohol 49 as a yellow oil (0.98 g, 38%). Rₖ: 0.5 (30:70 petrol–EtOAc); νₘₐₓ/cm⁻¹ (film) 3350, 2978, 2930, 1693, 1526, 1366, 1252, 1171, 970; δₓ (500 MHz; CDCl₃) 5.78 (1H, dt, J 15.7 and 5.0, 2-H), 5.72 (1H, dt, J 15.7 and 5.1, 3-H), 4.59 (1H, br s, NH), 4.13 (2H, d, J 5.1, 4-H₂), 3.75 (2H, app.br.s, 1-H₂), 1.44 (9H, s, t-Bu); δₓC (75 MHz; CDCl₃) 155.9 (CONH), 130.8 (C-2), 129.0 (C-3), 79.6.1 (C(1(Bu)), 63.1 (C-4), 42.0 (C-1), 28.7 (1(Bu); m/z (ES) 210.1 (100%, [M+Na]⁺). Characterisation data were consistent with those reported in literature.¹¹¹
(E)-tert-Butyl(4-methoxycarbonyl)oxy)but-2-en-1-yl)carbamate 46

The title compound was prepared according to the general method of Gramm et al. Methyl chloroformate (0.55 mL, 7.2 mmol) and pyridine (0.58 mL, 7.2 mmol) were added and to a solution of the alcohol 49 (1.0 g, 5.55 mmol) in CH₂Cl₂ (14 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C, allowed to warm up to r.t. and stirred overnight. An aqueous solution of NH₄Cl (1.0 M, 10 mL) was added, the phases were separated, the aqueous phase was extracted with CH₂Cl₂ (3 × 3.0 mL). The combined organic phases were dried (MgSO₄) and concentrated under reduced pressure to give a yellow oil that was purified by flash chromatography (99:1 CH₂Cl₂–MeOH) to give the carbamate 46 as a pale yellow oil (0.88 g, 65%). Rf: 0.6 (97:3 CH₂Cl₂–MeOH); νₓₓ/cm⁻¹ (film) 3351, 1750, 1696, 1236, 1168, 944, 792; δₓ (500 MHz; CDCl₃) 5.82 (1H, dt, J 15.3 and 4.8, 2-H), 5.72 (1H, dt, J 15.3 and 5.9, 3-H), 4.60 (2H, dd, J 5.9 and 0.7, 4-H₂), 4.56 (1H, br.s, NH), 3.78 (3H, s, OMe), 3.56 (2H, app.br.s, 1-H₂), 1.44 (9H, s, 'Bu); δₓ (75 MHz; CDCl₃) 156.1 (CO₂Me), 155.6 (CONH), 132.5 (C-2), 124.9 (C-3), 84.5 (C('Bu)), 67.7 (C-4), 54.9 (OCH₃), 41.5 (C-1), 28.5 (‘Bu); m/z (ES) 268.1 (100%, [M+Na]⁺). Characterisation data were consistent with those reported in literature.

tert-Butyl 2-(phenylamino)but-3-enylcarbamate 50

THF (2.5 mL) and n-butyl amine (4.8 μL, 50 μmol) were added to the [Ir(dbcot)Cl]₂ complex (20.4 mg, 25 μmol), phosphoramidites S,S aS-51 (13.1 mg, 25 μmol) and R,R aR-52 (13.1 mg, 25 μmol). The reaction mixture was stirred at 50 °C for 45 minutes, then the allyl carbonate 46 (0.30 g, 1.2 mmol) and aniline (167 μl, 1.8 mmol) were added and the resulting mixture was stirred at 55°C for 18 h. The solvent was removed under reduced pressure and the crude product was purified by flash
chromatography (80:20 petrol–EtOAc) to give the title allyl phenylamine 50 as a colourless amorphous solid (0.22 g, 69%). $R_F$: 0.7 (66:33 petrol–EtOAc); $\nu_{\text{max}}$/cm$^{-1}$ (film) 3388, 3321, 1667, 1603, 1517, 1498, 1165, 919; $\delta_H$ (500 MHz; CDCl$_3$) 7.15 (2H, dd, $J$ 8.5 and 7.3, phenyl 3- and 5-H), 6.70 (1H, app. t, $J$ 7.3, phenyl 4-H), 6.61 (2H, dd, $J$ 8.5 and 0.9, phenyl 2- and 6-H), 5.77 (1H, ddd, $J$ 17.2, 10.5 and 5.3, 3-H), 5.31 (1H, d, $J$ 17.2, 4-H$_A$), 5.22 (1H, d, $J$ 10.5, 4-H$_B$), 4.80 (1H, s, NHBOc), 4.18 (1H, s, NHPh), 3.95 (1H, app. dt, $J$ 7.7 and 5.3, 2-H), 3.40-3.22 (1H, m, 1-H$_A$), 3.29 (1H, ddd, $J$ 13.8, 7.7 and 6.1, 1-H$_B$), 1.45 (9H, s, tBu); $\delta_C$ (75 MHz; CDCl$_3$) 157.7 ($\text{C}=$ONH), 137.2 (phenyl C-1), 129.4 (phenyl C-3-and C-5), 117.7 (phenyl C-4), 117.1 (C-3), 117.0 (C-4), 113.5 (phenyl C-2 and C-6), 79.9 (C('Bu)), 57.3 (C-2), 45.1 (C-1), 28.5 ('Bu); m/z (ES) 263.17 (100%, [M+H]$^+$), HRMS Found: 263.1743 (C$_{15}$H$_{22}$N$_2$O$_2$, requires [M+H] 263.1754).

Dibenzo[a,e]cyclooctatetraene (dbcot)$^{142}$

The title compound was prepared from $\alpha,\alpha'$-dibromo-\textit{o}-xylene according to the general method of Wuld et al.$^{142}$ Under an atmosphere of argon, lithium (granular dry, high in sodium) (0.79 g, 113.5 mmol) was added to THF (24 mL). $\alpha,\alpha'$-dibromo-\textit{o}-xylene (5.0 g, 18.4 mmol) was dissolved in THF (7.5 mL) by sonication and added portionwise to the lithium solution at 0 °C, to give a grey suspension that was allowed to warm to r.t. and then sonicated for 3 h. The solution was separated from the residual lithium via syringe and the solvent removed under reduced pressure to give a pale yellow oil that was dissolved in EtOAc. Florisil (17.7 g) was added, the solvent was removed under reduced pressure and the resulting mixture was purified eluting through a silica plug (95:5 cyclohexane–EtOAc) to give the 5, 6, 11, 12-tetrahydrodibenzo[a,e]cyclooctadiene 126, (1.54 g, 40%) as a colourless amorphous solid. $R_F$: 0.6 (80:20 cyclohexane–ether); $\nu_{\text{max}}$/cm$^{-1}$ (film) 3060, 3015, 2933, 2869, 2246, 1490, 1450, 1159, 1047; 909; 720; 690; $\delta_H$ (500 MHz; CDCl$_3$) 7.27-7.24 (8H, m, Ar
\((\text{C}H)_{7.01-6.97}, 3.06 (8\text{H}, s, 4 \times \text{CH}_2)\); \(\delta\text{C} (75 \text{ MHz}; \text{CDCl}_3) = 140.2 (\text{C}-13, \text{C}-14, \text{C}-15-\text{and C}-16), 129.8 (\text{C}-2, \text{C}-3, \text{C}-8 \text{ and C}-9), 126.2 (\text{C}-1, \text{C}-4, \text{C}-7 \text{ and C}-8), 35.4 (\text{C}-5, \text{C}-6, \text{C}-11-\text{ and C}-12); m/z (\text{EI}) 208.1 (100\%, \text{[M+H]}^+)\). Characterisation data were consistent with those reported in literature.\(^{142}\)

\(N\)-Bromosuccinimide (2.96 g, 14.9 mmol), recrystallised (water), was added to a solution of 126 (1.54 mg, 7.4 mmol) in \(\text{CCl}_4\) (52 mL) and the resulting mixture was stirred at 90 °C for 24 h under air. The reaction was allowed to reach r.t. and filtered; the solvent was removed under reduced pressure and the crude material was washed with water and sonicated. The water removed by azeotropical drying with toluene to give 5, 11-dibromo-5, 6, 11, 12-tetrahydrodibenzo[a,e]cycloctene 127, as an orange amorphous solid (2.4 g, 85\%). \(R_F = 0.5\) (80:20 cyclohexane–ether); \(\nu_{\text{max}}/\text{cm}^{-1}\) (film) 3063, 2928, 1715, 1489, 1450, 1146, 908; \(\delta\text{H} (500 \text{ MHz}; \text{CDCl}_3) 7.22-6.84 (8\text{H}, \text{m}, 8 \times \text{Ar CH}), 5.34 (2\text{H}, \text{dd, \(J\) 11.2 and 8.5, CHBr}), 4.30 (2\text{H}, \text{dd, \(J\) 14.2 and 11.3, \(\text{CH}_2\)H_B}), 3.66 (2\text{H}, \text{dd, \(J\) 14.3 and 8.5, \(\text{CH}_2\)H_B}); \(\delta\text{C} (75 \text{ MHz}; \text{CDCl}_3) = 138.4 (\text{C}-13 \text{ and C}-16), 136.4 (\text{C}-14 \text{ and C}-15), 130.9 (\text{C}-4 \text{ and C}-10), 130.8 (\text{C}-1 \text{ and C}-7), 129.1 (\text{C}-3 \text{ and C}-9), 127.9 (\text{C}-2 \text{ and C}-8), 52.9 (\text{C}-5 \text{ and C}-11), 43.7 (\text{C}-6 \text{ and C}-12); m/z (\text{EI}) 285.0 (30\%, \text{[M–Br]}^+), 79.9 (30\%, \text{[Br]}^+)\). Characterisation data were consistent with those reported in literature.\(^{142}\)

A solution of the dibromide 127 (2.4 g, 6.8 mmol) in THF (67 mL) was added at 0 °C to a suspension of \(^1\text{BuOK}\) (12.2 g, 109.3 mmol) in THF (150 mL). The reaction mixture was allowed to warm to r.t., stirred for 18 h and quenched with water (50 mL) at 0 °C. The phases were separated, the aqueous phase was extracted with chloroform (3 × 35 mL), the combined organic phases were dried (MgSO_4) and concentrated under reduced pressure to give a brown solid that was re-dissolved in CHCl_3. Florisil (7.0 g) was added, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography with Biotage instrument (gradient elution petrol–

\(^c\) Numbering according to the literature:

\(^d\) Numbering according to the literature:
EtOAc 100:0 → 95:5 in 10 column volumes) to give the dbcot as a colourless needle solid (0.34 g, 25%). Rf: 0.3 (petrol–Et₂O); m.p. 107-109 [lit. 109 °C];¹⁴³ ν_max/cm⁻¹ (film) 3047, 3008, 1950, 1922, 1476, 1446, 1208, 1093, 1041, 976; δ_H (500 MHz; CDCl₃) 7.17 (4H, dd, J 5.7 and 3.4, 1-, 4-, 7- and 10-H), 7.07 (4H, dd, J 5.6 and 3.4, 2-, 3-, 8- and 9-H), 6.77 (4H, s, 5-, 6-, 11- and 12-H); δ_C (75 MHz; CDCl₃) 137.2 (C-13, C-14, C-15 and C-16), 133.4 (C-5, C-6, C-11 and C-12), 129.3 (C-2, C-3, C-8 and C-9), 126.9 (C-1, C-4, C-7 and C-10); m/z (EI) 204.1 (100%, [M+H]^+). HRMS Found: 204.0939 (C₁₆H₁₂, M+H requires 204.0950. Characterisation data were consistent with those reported in literature.¹⁴²

**Chloro(dibenzo[a,e]cyclooctatraene)iridium(I)dimer, [Ir(dbcot)Cl]¹¹²**

The title compound was prepared according to the general method of Crabtree et al.¹¹² A solution of the dbcot (118 mg, 0.57 mmol) in CH₂Cl₂ (4.5 mL) was added by dropping funnel to solution of the [Ir(cod)Cl]₂ complex in CH₂Cl₂ (3.5 mL) and stirred for 20 minutes. The reaction mixture changed from red to yellow colour, the solvent was reduced to ca. 3.0 mL under flow of N₂ and cyclohexane (8.0 mL) was added slowly. The precipitated was filtered and dried under reduced pressure to give the Iridium complex [Ir(dbcot)Cl]₂ as a yellow amorphous solid (136 mg, 28%). ν_max/cm⁻¹ (film) 3066, 3020, 2978, 1488, 1462, 1384, 1218, 1003, 916, 829, 747; δ_H (500 MHz; CDCl₃); 6.99 (4H, dd, J 5.6 and 3.3, 1-, 4-, 7- and 10-H), 6.85 (4H, dd, J 5.7 and 3.2, 2-, 3-, 8- and 9-H), 5.32 (8H, s, 5-, 6-, 11- and 12-H); δ_C (75 MHz; CDCl₃) 145.8 (C-5, C-6, C-11 and C-12), 126.8 (C-2, C-3, C-8 and C-9), 126.2 (C-1, C-4, C-7 and C-10), 63.9 (C-13, C-14, C-15 and C-16); m/z (EI) 864.0 (100%, [M+H]^+). Characterisation data were consistent with those reported in literature.¹¹²

¹¹² Numbering according to the literature:

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¹⁴² Numbering according to the literature:
(S,S aS)-O,O’-[1,1’-binaphthyl-2,2’-diyl-N,N’-bis[1-phenethyl]phosphoramidite

The title compound 51 was prepared in 75% yield according to the general method of Mikhel et al.\textsuperscript{113} described in \textbf{general procedure A}. The crude product was purified by flash chromatography (95:5 petrol–EtOAc) to give a colourless needle solid. \(R_f\): 0.7 (80:20, petrol-EtOAc); m.p. 85-87 °C [lit. 88-89 °C\textsuperscript{145}]; \([\alpha]_D^{25}\) +16.5° (c. 1, CHCl\(_3\)) [lit. \([\alpha]_D^{22}\) +202 (c.0.79, CHCl\(_3\))\textsuperscript{146}]; \(\nu_{\max}/\text{cm}^{-1}\) (film) 3059, 2970, 1591, 1463, 1373, 1327, 1230, 1070, 946, 817; \(\delta_H\) (500 MHz; CDCl\(_3\)) 8.00 (1H, d, \(J\) 8.8 Ar CH), 7.92 (1H, dd, \(J\) 8.8 Ar CH), 7.81 (1H, d, \(J\) 8.4 and 1.2 Ar CH), 7.73 (1H, dd, \(J\) 8.7 Ar CH), 7.57 (1H, d, \(J\) 8.7 and 0.9 Ar CH), 7.44-7.32 (4H, m, Ar CH), 7.30-7.15 (13H, m, Ar CH), 4.42 (2H, dq, \(J\) 11.0 and 7.0 NCH), 1.68 (6H, d, \(J\) 7.1, CH\(_3\)); \(\delta_C\) (75 MHz; CDCl\(_3\)) 150.9, 150.8 (Ar C), 150.1, 143.5 (Ar CH), 133.3 (2 x Ar C), 133.2 (2 x Ar C), 131.8, 130.8, 130.7, 130.0, 128.7, 128.5 (Ar CH), 128.4 (2 x Ar CH), 128.1 (2 x Ar CH), 127.7, 127.5 (Ar CH), 127.1 (Ar CH), 126.5, 126.3, 125.2, 124.7, 122.9 (Ar CH), 122.8, 122.7 (Ar CH), 121.6, 121.5 (Ar C), 55.0, 54.9 (NCH), 23.5, 23.3 (CH\(_3\)); 2 X Ar C not observed; \(m/z\) (ES) 540.21 (100%, [M+H]\(^+\)). HRMS Found: 540.2087 (C\(_{36}\)H\(_{30}\)NO\(_3\)P M+H requires 540.2085). Characterisation data were consistent with those reported in literature.\textsuperscript{144}
The title compound 52 was prepared in 57% yield according to the general method of Mikhel et al. described in **general procedure A**. The crude product was purified by flash chromatography with Biotage instrument (gradient elution petrol–EtOAc 100:0 in 2 column volumes → 80:20 in 6 column volumes, dry loading of crude in 7.0 g of Florisil, loaded in 50 g SNAP cartridge column) to give a colourless needle solid. \( R_F: 0.7 \) (80:20, petrol-EtOAc); m.p. 84-86 °C [lit. 88-89 °C]; \([\alpha]_D^{25}: -13.1^\circ \) (c 1.0, CHCl3); \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 3058, 2969, 1590, 1463, 1327, 1230, 1117, 1070, 948; \( \delta_H \) (500 MHz; CDCl3) 8.00 (1H, d, \( J 8.8, \text{Ar CH} \)), 7.92 (1H, d, \( J 8.2, \text{Ar CH} \)), 7.81 (1H, dd, \( J 8.4 \) and 0.4, \( \text{Ar CH} \)), 7.73 (1H, d, \( J 8.8, \text{Ar CH} \)), 7.58-7.56 (1H, m, \( \text{Ar CH} \)), 7.44-7.31 (4H, m, \( \text{Ar CH} \)), 7.29-7.13 (13H, m, \( \text{Ar CH} \)), 4.42 (2H, dq, \( J 10.8 \) and 7.0, NCH), 1.69 (6H, d, \( J 7.1, \text{CH}_3 \)); \( \delta_C \) (75 MHz; CDCl3) 151.2, 151.1 (Ar C), 150.4, 143.7 (Ar CH), 133.6, 133.5 (Ar C), 133.4 (2 x Ar C), 132.0, 131.1, 131.0, 130.2, 128.9, 128.8, 128.7, 128.6 (Ar CH), 128.4 (2 x Ar CH), 127.9, 127.8, 127.3, 126.7, 126.5, 125.4, 125.0 (Ar CH), 124.9, 124.8 (Ar C), 123.2, 123.1, 123.0 (Ar CH), 121.9, 121.8 (Ar C), 55.2, 55.1 (NCH), 23.7, 23.6 (CH3); \( m/z \) (ES) 540.21 (100%, \([\text{M}+\text{H}]^+\)). HRMS Found: 540.2087 (C36H30NO2P M+H requires 540.2089). Characterisation data were consistent with those reported in literature.
** tert-Butyl 2-(3-ethyl-1-phenylureido)but-3-enylcarbamate 45**

The title compound 45 was prepared from allyl amine 50 (0.26 g, 1.0 mmol) and ethylisocyanate (0.6 mL, 5.0 mmol) in CH₂Cl₂ at 35 °C, according to general procedure B. The crude product was purified by flash chromatography (petrol–EtOAc 66:33) to give a pale amorphous solid (58% yield). R_F: 0.2 (66:33, petrol- EtOAc); ν_max/cm⁻¹ (film) 3344, 2975, 2930, 1710, 1650, 1514, 1274, 1173, 927, 709; δ_H (500 MHz; CDCl₃) 7.41 (2H, app. t, J 7.4, phenyl 2- and 6-H), 7.36 (1H, app.t, J 7.4 phenyl 4-H), 7.18 (2H, d, J 7.4 phenyl 3- and 5-H), 5.71 (1H, ddd, J 17.4, 10.2 and 7.5, 3-H), 5.17 (1H, dt, J 17.4 and 1.1, 4-Hₐ), 5.13 (d, J 10.2, 4-Hₐ), 5.00 (1H, app.tdd, J 7.5, 6.8 and 0.5, 2-H), 4.02 (1H, br. s, NHₐEt), 3.50-3.38 (1H, m, 1-Hₐ), 3.34-3.10 (3H, m, 1-Hₐ and ethyl 1-H₂), 1.43 (9H, s, t-Bu), 1.01 (3H, t, J 7.19, ethyl 2-H₃); δ_C (75 MHz; CDCl₃) 162.2 (NCONH), 157.9 (COOtBu), 137.7 (phenyl C-1), 135.6 (C-3), 130.9 (phenyl C-2 and C-6), 130.3 (phenyl C-3 and C-5), 128.8 (phenyl C-4), 118.6 (C-4), 80.2 (C'(Bu)₃), 58.8 (C-2), 43.5 (ethyl C-1), 35.9 (C-1), 28.8 ('Bu), 15.9 (ethyl C-2); m/z (ES) 356.19 (100%, MNa⁺); HRMS Found: 356.1945 (C₁₈H₂₇N₃O₃ M+Na requires 356.1951).

** 2-(Phenylamino)but-3-en-1-ol 53**

Vinylboronic acid pinacol ester (3.3 mL, 20.0 mmol) was added to a solution of glycoaldehyde dimer (1.20 g, 10.0 mmol) in water (100 mL) and the resulting mixture was stirred at 50 °C for 45 minutes, then allowed to warm to 30 °C. A solution of aniline (1.8 mL, 20.0 mmol) in THF (25 mL) was added and the resulting mixture was stirred for 4 days at 30 °C. An aqueous solution of HCl (1.0 M) was added until pH 1 and the mixture was extracted with CH₂Cl₂ (3 × 50 mL); then the combined aqueous phases were basified with an aqueous solution of NaOH (2.0 M) until pH 12 and extracted with
CH₂Cl₂ (3 × 100 mL). The combined organic phases were dried (Na₂SO₄) and concentrated under reduced pressure to give a crude product which was purified by flash chromatography (petrol–EtOAc 60:20) to give the title compound 53 as a yellow oil (1.5 g, 45%). Rf: 0.6 (20:80, petrol–EtOAc; νmax/cm⁻¹ (film): 3392, 2933, 1602, 1504, 1317, 1031, 751; δH (500 MHz; CDCl₃) 7.17 (2H, dd, J 8.5 and 7.3, phenyl 2- and 6-H), 6.74 (1H, dd, J 7.3 and 0.9, phenyl 4-H), 6.67 (2H, dd, J 8.5 and 0.9, phenyl 3- and 5-H), 5.81 (1H, ddd, J 17.3, 10.4 and 5.4, 4-H), 5.32 (1H, d, J 17.3, 3-H₂A), 5.25 (1H, d, J 10.4, 3-H₈), 4.04 (1H, app. q, J 6.0, 2-H), 3.95 (1H, br. s, NH), 3.8 (1H, dt, J 6.9, 5.5, 1-H₂A), 3.67-3.61 (1H, m, 1-H₂B), 1.80-1.77 (1H, m, OH); δC (125 MHz; CDCl₃) 147.3 (phenyl C-1), 136.3 (C-3), 129.2 (phenyl C-2 and C-6), 118.1 (phenyl C-4), 117.4 (C-4), 113.9 (phenyl C-3 and C-5), 64.9 (C-1), 57.6 (C-2); m/z (ES) 146.1 (100%, fragment [C₁₀H₁₁NH]⁺); HRMS Found: 164.1082 (C₁₀H₁₃NO requires M+H 164.107).

3-Ethyl-1-(1-hydroxybut-3-en-2-yl)-1-phenylurea 54²

Ethylisocyanate (1.0 mL, 12.8 mmol) was added at 0 °C to a solution allyl amine 53 (1.0 g, 6.4 mmol) in CH₂Cl₂ (27 mL) and the resulting mixture was stirred at 0 °C for 2 hours, then allowed to warm to r.t. and stirred overnight. Another equivalent of the ethylisocyanate was added to the reaction and the resulting mixture was stirred at r.t. for 2 days. The solvent was removed under reduced pressure to give a crude product which was purified by flash chromatography (petrol–EtOAc 60:40) to give the title compound 54 as a yellow oil (1.2 g, 80%). Rf: 0.4 (50:50, petrol-EtOAc); νmax/cm⁻¹ (film) 3440, 3368, 2972, 2932, 1643, 1595, 1515, 1282, 1065, 711; δH (500 MHz; CDCl₃) 7.45-7.43 (2H, m, phenyl 2- and 6-H), 7.40-7.36 (1H, m, phenyl 4-H), 7.26-7.23 (2H, m, phenyl 3- and 5-H), 5.75 (1H, ddd, J 17.4, 10.4 and 7.4, 3-H), 5.20 (1H, dt, J 17.4 and 1.3, 4-H₂A), 5.19-5.18 (1H, m, 4-H₈), 4.83 (1H, ddd, J 8.5, 7.4 and 4.1, 2-H), 4.07 (1H, br. s, NH), 3.84 (1H, ddd, J 11.5, 4.1 and 3.9, 1-H₂A), 3.74-3.67 (1H, m, 1-H₂B), 3.57 (1H, dd, J 8.8 and 3.3, OH), 3.19 (2H, dq, J 7.2 and 5.6, ethyl 1-H₂), 1.01 (3H, t, J 7.2, ethyl 2-H₃); δC (125 MHz; CDCl₃) 158.4 (CO), 139.8 (phenyl C-1), 134.2 (C-3), 130.1 (phenyl C-2)
and C-6), 129.9 (phenyl C-3 and C-5), 128.4 (phenyl C-4), 118.2 (C-4), 64.8 (C-1), 62.8 (C-2), 35.6 (ethyl C-1), 15.4 (ethyl C-2); m/z (ES) 257.0 (100%, [M+Na]+); HRMS Found: 257.1268 (C_{13}H_{18}N_{2}O requires M+Na 257.1260).

tert-Butyl 2-(3-ethyl-1-phenylureido)but-3-enyl(2-nitrophenylsulfonyl)carbamate

55

tert-Butyl 2-nitrophenylsulfonylcarbamate (0.31 g, 1.0 mmol) was added to a solution of the phenyl urea 54 (0.20 g, 0.85 mmol) in THF (8.5 mL) at 0 °C, followed by triphenylphosphine (0.33 g, 1.3 mmol) and by a dropwise addition of diethyl azodicarboxylate (0.2 mL, 1.27 mmol). The resulting mixture was stirred at 0 °C for 3 h then allowed to warm to r.t. and stirred overnight. The reaction mixture was diluted with EtOAc (40 mL), washed with an aqueous solution of HCl (1.0 M) (2 × 15 mL), with a saturated aqueous solution of NaHCO$_3$ (2 × 15 mL) and brine (2 × 15 mL). The combined organic phases were dried (Na$_2$SO$_4$) and concentrated under reduced pressure to give a crude product which was purified by flash chromatography (CH$_2$Cl$_2$–MeOH 98.5:1.5) to give the title compound 55 as a pale yellow oil (0.2 g, 53%). R$_F$: 0.55 (98:2, CH$_2$Cl$_2$ –MeOH); $\nu_{\text{max}}$/cm$^{-1}$ (film) 3508, 3004, 2145, 1704, 1660, 1422, 1367, 1230, 1093, 904; $\delta_{\text{H}}$ (500 MHz; CDCl$_3$) 8.34-8.31 (1H, m, Ns 3-H), 7.74-7.71 (3H, m, Ns 4-, 5- and 6-H), 7.45-7.40 (2H, m, phenyl 2- and 6-H), 7.36-7.34 (1H, m, phenyl 4-H), 7.33-7.31 (2H, m, phenyl 3- and 4-H), 5.99 (1H, dd, J 17.1, 10.1 and 9.0, 3-H), 5.28 (1H, ddd, J 17.1, 1.4 and 0.9, 4-H$_A$), 5.22 (1H, dd, J 10.1 and 1.6, 4-H$_B$), 4.88 (1H, app. m, 2-H), 4.33 (1H, dd, J 14.6 and 7.1, 1-H$_A$), 4.06 (1H, dd, J 14.6 and 7.4, 1-H$_B$), 4.02 (1H, t, J 5.2, NH), 3.25-3.16 (2H, app. m, ethyl 1-H$_2$), 1.37 (9H, s, t-Bu), 1.01 (3H, t, J 7.2, ethyl 2-H$_3$); $\delta_{\text{C}}$ (125 MHz; CDCl$_3$) 156.7 (CO urea), 150.4 (COO'Bu), 140.9 (Ns C-2), 140.1 (phenyl C-1), 134.8 (C-3), 133.9 (Ns C-3), 133.8 (Ns C-4 or C-5 or C-6), 133.2 (Ns C-1), 131.7 (Ns C-4 or C-5 or C-6), 130.1 (phenyl C-2 and C-6), 129.9 (phenyl C-3 and C-5), 128.1 (phenyl C-4), 124.3 (Ns C-4 or C-5 or C-6), 119.4 (C-4), 85 (C (t-Bu)), 61.9 (C-2), 50.2 (C-1), 35.4 (ethyl C-1), 27.8 (t-Bu), 15.4 (ethyl C-2); m/z
(ES) 541.14 (100% [M+Na]+); HRMS Found: 541.1739 (C_{24}H_{30}N_{4}O_{7}S requires M+Na 541.1727).

3-Ethyl-1-[1-(methoxymethoxy)but-3-en-2-yl]-1-phenylurea 56

\[ \text{N,N-Diisopropylethylamine (0.11 mL, 0.66 mmol) was added dropwise to a} \]
\[ \text{solution of the phenyl urea 54 (0.07 g, 0.33 mmol) in CH}_2\text{Cl}_2 (2 mL) at 0 \text{ °C, followed} \]
\[ \text{by portionwise addition of chloromethyl ethyl (50 μL, 0.66 mmol). The resulting} \]
\[ \text{mixture was stirred at 0 °C for 1.5 h, then allowed to warm to r.t. and stirred overnight.} \]
\[ \text{The reaction mixture was quenched with brine (2.0 mL) and extracted with CH}_2\text{Cl}_2 (2 × 3.0 mL),} \]
\[ \text{the combined organic phases were dried (Na}_2\text{SO}_4) \text{ and concentrated under} \]
\[ \text{reduced pressure to give a crude product which was purified by flash chromatography} \]
\[ \text{(petrol–EtOAc 50:50) to give the title compound 56 as a pale yellow oil (40 mg, 41%).} \]
\[ R_F: 0.5 \text{ (30:70, petrol–EtOAc); } \nu_{\text{max}}/\text{cm}^{-1} \text{ (film) 3400, 2987, 2212, 1654, 1648, 1508,} \]
\[ 1279, 1039, 919, 712; \delta_H (500 MHz; CDCl}_3) 7.42 (2H, app. t, J 7.4, phenyl 2- and 6-H),} \]
\[ 7.36 (1H, app. t, J 7.4, phenyl 4-H), 7.25 (2H, d, J 7.2, phenyl 3- and 5-H), 5.79 (1H,} \]
\[ \text{ddd, J 17.6, 10.5 and 7.3, 3-H), 5.26-5.16 (3H, m, 4-H}_A, 4\text{-H}_B, 2\text{-H}), 4.67 (1H, d, J 6.6,} \]
\[ \text{OCH}_3\text{H}_2\text{O}), 4.59 (1H, d, J 6.6, OCH}_3\text{H}_2\text{O}), 3.96 (1H, br. s, NH), 3.62 (1H, dd, J 10.3} \]
\[ \text{and 6.2, 1-H}_A), 3.58 (1H, dd, J 10.3 and 9.1, 1-H}_B), 3.35 (3H, s, OCH}_3), 3.17 (2H, dq, J} \]
\[ 14.5 \text{ and 7.0, ethyl 1-H}_2), 0.99 (3H, t, J 7.2, ethyl 2-H}_3); \delta_C (125 MHz; CDCl}_3) 157.0} \]
\[ \text{(CO), 144.9 (phenyl C-1), 135.3 (C-3), 130.8 (phenyl C-2 and C-6), 129.7 (phenyl C-3} \]
\[ \text{and C-5), 128.3 (phenyl C-4), 117.9 (C-4), 96.4 (OCH}_3\text{H}_2\text{O), 67.5 (C-1), 57.9 (OCH}_3),} \]
\[ 53.6 \text{ (C-2), 41.8 (ethyl C-1), 18.66 (ethyl C-2); } m/z \text{ (ES) 301.1 (100%, [M+Na]+); } \]
\[ \text{HRMS Found: 301.1532 (C}_{15}\text{H}_{22}\text{N}_2\text{O}_3 \text{ requires M+Na 301.1523).} \]
1-(1’-Azidobut-3’en-2’-yl)-3-ethyl-1-phenylurea 57

Diphenyl phosphoryl azide (0.7 mL, 3.2 mmol) was added to a solution of the phenyl urea 54 (0.25 g, 1.0 mmol) in toluene (27 mL) followed by DBU 0.5 mL, 3.2 mmol) and the resulting mixture was stirred at 90 °C for 3 days. The solvent was removed under reduced pressure to give a crude material which was partitioned in EtOAc (8.0 mL) and an aqueous solution of HCl (1.0 M, 8.0 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 x 15 mL), the combined organic phases were washed with water (2 x 15 mL), with a saturated aqueous solution of NaHCO$_3$ (2 x 15 mL) and brine (15 mL), dried (MgSO$_4$) and concentrated under reduced pressure. The crude product was purified by flash chromatography (petrol–EtOAc 60:40) to give the title compound 57 as an orange oil (0.17 g, 63%). $R_F$: 0.6 (40:60, petrol–EtOAc); $\nu_{\text{max}}$/cm$^{-1}$ (film) 3368, 2946, 2931, 2878, 1659, 1508, 1494, 1273, 923, 713; $\delta_H$ (500 MHz; CDCl$_3$) 7.46-7.42 (2H, m, phenyl 2- and 6-H), 7.40-7.36 (1H, m, phenyl 4-H), 7.26-7.23 (2H, m, phenyl 3- and 5-H), 5.88 (1H, ddd, $J$ 17.4, 10.4 and 7.6, 3-H), 5.23 (1H, app. dt , $J$ 17.4 and 1.1, 4-H$_A$), 5.20 (1H, app. dt , $J$ 10.4, and 1.1, 4-H$_B$), 4.91-4.86 (1H, app. m, 2-H), 4.01 (1H, br. s, NH), 3.65 (1H, dd, $J$ 12.4 and 8.8, 1-H$_A$), 3.4 (1H, dd, $J$ 12.4 and 6.0, 1-H$_B$), 3.19 (2H, qdd, $J$ 7.2, 5.6 and 1.7, ethyl 1-H$_2$), 1.01 (3H, t, $J$ 7.2, ethyl 2-H$_3$); $\delta_C$ (125 MHz; CDCl$_3$) 156.7 (CO), 139.5 (phenyl C-1), 134.9 (C-3), 130.3 (phenyl C-2 and C-6), 130.0 (phenyl C-3 and C-5), 128.4 (phenyl C-4), 118.6 (C-4), 59.8 (C-2), 52.6 (C-1), 35.5 (ethyl C-1), 15.5 (ethyl C-2); m/z (ES) 282.1 (100%, [M+Na]$^+$); HRMS Found: 282.1320 (C$_{13}$H$_{17}$N$_5$O requires M+Na 282.1325).
1-Allyl-3-ethyl-1-phenylurea 42

The title compound 42 was prepared according to the general method of Wolfe et al., described in general procedure B, from the N-allyl-aniline (2.0 g, 15.0 mmol) and ethylisocyanate (6.0 mL, 75.0 mmol) in CH₂Cl₂, stirring for 1 day. The crude product was purified by flash chromatography (petrol–EtOAc 75:25) to give a yellow oil (2.7 g, 88%). Rₚ: 0.2 (66:33, petrol–EtAc); νmax/cm⁻¹ (film) 3445, 3356, 2972, 2873, 1650, 1496, 1271, 1227, 921, 750; δH (500 MHz; CDCl₃) 7.43-7.38 (2H, m, phenyl 2- and 6-H), 7.31-7.28 (1H, m, phenyl 4-H), 7.24-7.21 (2H, m, phenyl 3- and 5-H), 5.90 (1H, ddt, J 17.7, 9.7 and 6.1, allyl 2-H), 5.08 (1H, app. dt, J 17.7 and 1.4 allyl 3-H), 5.06 (1H, app. dt, J 9.7 and 1.4 allyl 3-H), 4.28 (2H, dt, J 6.1 and 1.4, allyl 1-H), 4.26 (1H, br. s, NH), 3.21 (2H, dq, J 7.2 and 5.6, ethyl 1-H₂), 1.03 (3H, t, J 7.2, ethyl 2-H), 129.8 (phenyl C-2 and C-6), 128.4 (phenyl C-3 and C-5), 127.4 (phenyl C-4), 116.7 (allyl C-3), 52.1 (allyl C-1), 35.5 (ethyl C-1), 15.4 (ethyl C-2); m/z (ES) 227.2 (100%, [M+Na]⁺); HRMS Found: 227.1152 (C₁₂H₁₆N₂O, requires M+Na 227.1155). Characterisation data were consistent with those reported in literature.

3-Ethyl-4-(naphthalen-2-ylmethyl)-1-phenylimidazolidin-2-one 43

The title compound 43 was prepared according to the general method of Wolfe et al., described in general procedure C, from the allyl urea 42 (0.27 g, 1.35 mmol) and 2-bromonaphthalenyl (0.56 g, 2.7 mmol) stirring for 2 days. The crude product was purified by flash chromatography (petrol–EtOAc 80:20 → 70:30) to give a pale yellow
amorphous solid (0.21 g, 48%). \( R_F: 0.5 \) (60:30, petrol–EtOAc); \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 3380, 2955, 2926, 2854, 1702, 1597, 1261, 1124, 942, 762, 694; \( \delta_H \) (500 MHz; CDCl\(_3\)) 7.81-7.77 (3H, m, naphthalenyl 7-, 6- and 4-H), 7.63 (1H, s, naphthalenyl 1-H), 7.48-7.43 (4H, m, naphthalenyl 3- and 5-H, phenyl 2- and 6-H), 7.30 (1H, d, \( J 8.3 \), naphthalenyl 8-H), 7.26-7.21 (2H, m, phenyl 3- and 5-H), 6.95 (1H, t, \( J 7.3 \), phenyl 4-H), 4.08-4.02 (1H, m, \( 4-H \)), 3.68 (1H, dq, \( J 14.7 \) and 7.4, ethyl 1-\( H_A \)), 3.62 (1H, app. t, \( J 8.9 \), 5-\( H_A \)), 3.46 (1H, dd, \( J 9.1 \) and 6.0, 5-\( H_B \)), 3.35 (1H, dd, \( J 13.5 \) and 4.2, 4-\( CH_AH_B \)), 3.21 (1H, dq, \( J 14.0 \) and 7.0, ethyl 1-\( H_B \)), 2.76 (1H, dd, \( J 13.4 \) and 9.8, 4-\( CH_AH_B \)), 1.27-1.22 (3H, app. m, ethyl 2-\( H_3 \)); \( \delta_C \) (125 MHz; CDCl\(_3\)) 157.5 (\( C_O \)), 140.7 (phenyl C-1), 134.1 (naphthalenyl C-4a), 133.7 (naphthalenyl C-2), 132.3 (naphthalenyl C-8a), 128.9 (phenyl C-2 and C-6), 128.8 (naphthalenyl C-6- or C-7), 127.9 (naphthalenyl C-1), 127.8 (naphthalenyl C-6 or C-7), 127.6 (naphthalenyl C-4), 127.2 (naphthalenyl C-8), 126.5 (naphthalenyl C-3), 126 (naphthalenyl C-5), 122.3 (phenyl C-4), 117.4 (phenyl C-3 and C-5), 53 (C-4), 48 (C-5), 39.5 (4-\( CH_2 \)), 36.5 (ethyl C-1), 13.2 (ethyl C-2); \( m/z \) (ES) 683.4 (100%, [M\(_2^+\)Na\(^+\)]. Characterisation data were consistent with those reported in literature.\(^{100}\)

\((4S^*, 5S^*)\)-1-Ethyl-5-methyl-4-((naphthalen-2-ylamino)methyl)-3-phenyl imidazolin-2-one 58\(^{100}\)

The title compound 58 was prepared according to general procedure C\(^{100}\) from the allyl urea 45 (0.18 g, 0.53 mmol) and 2-bromonaphthalenyl (0.16 g, 0.80 mmol) stirring for 2 days in the presence of NaO'Bu (0.13 g, 1.3 mmol). The crude product was purified by flash chromatography (petrol–EtOAc 80:20 → 70:30) to give a yellow oil (2.0 mg, 4%). \( R_F: 0.25 \) (60:30, petrol–EtOAc); \( \nu_{\text{max}}/\text{cm}^{-1} \) (film); 3838, 3753, 2956, 2912, 2852, 1687, 1578, 1219, 1034, 939, 799; \( \delta_H \) (500 MHz; CDCl\(_3\)) 7.66 (1H, dd, \( J 8.1 \) and
0.6, naphthalenyl 5-H), 7.62 (1H, d, J 8.8, naphthalenyl 4-H), 7.57 (1H, dd, J 8.3 and 0.6, naphthalenyl 8-H), 7.50-7.48 (2H, dd, J 8.7 and 1.1, phenyl 2- and 6-H), 7.39-7.34 (3H, m, phenyl 3- and 5-H, naphthalenyl 7-H), 7.21 (1H, ddd, J 8.1, 6.8 and 1.2, naphthalenyl 6-H), 7.12 (1H, app. t, J 7.4, phenyl 4-H), 6.81 (1H, dd, J 8.7 and 2.4, naphthalenyl 3-H), 6.77 (1H, app. br. s, naphthalenyl 1-H), 6.71 (1H, ddd, J 8.1, 6.8 and 1.2, naphthalenyl 6-H), 6.71 (1H, app. br. s, naphthalenyl 1-H), 6.04 (1H, td, J 5.2 and 3.4, 4-H), 3.77-3.72 (1H, m, phenyl 3- and 5-H, naphthalenyl 7-H), 7.21 (1H, ddd, J 8.1, 6.8 and 1.2, naphthalenyl 6-H), 7.12 (1H, app. t, J 7.4, phenyl 4-H), 6.81 (1H, dd, J 8.7 and 2.4, naphthalenyl 3-H), 6.77 (1H, app. br. s, naphthalenyl 1-H), 6.04 (1H, td, J 5.2 and 3.4, 4-H), 3.77-3.72 (1H, m, 5-H), 3.58 (1H, dq, J 14.3 and 7.3, ethyl 1-H), 3.47 (1H, dd, J 13.2 and 5.1, 4-CH$_2$H$_3$), 3.43 (1H, dd, J 13.1 and 3.4, 4-CH$_2$H$_3$), 3.16 (1H, dq, J 14.2 and 7.2, ethyl 1-H), 1.35 (3H, d, J 6.2, methyl), 1.17 (3H, t, J 7.2, ethyl 2-H), NH not observed; δ$^1$C (125 MHz; CDCl$_3$) 157.8 (CO), 139.1 (phenyl C-1), 138.9 (naphthalenyl C-8a), 130.2 (naphthalene C-4), 129.4 (naphthalenyl C-2), 129.3 (phenyl C-2 and C-6), 127.9 (naphthalenyl C-4a), 127.8 (naphthalenyl C-5), 126.7 (naphthalenyl C-7), 126.1 (naphthalenyl C-8), 124.5 (phenyl C-4), 122.6 (naphthalenyl C-6), 121.9 (phenyl C-3 and C-5), 118.2 (naphthalenyl C-3), 105.3 (naphthalenyl C-1), 60.9 (C-4), 51.8 (C-5), 44.2 (4-CH$_2$), 36.2 (ethyl C-1), 19.5 (Me), 13.4 (ethyl C-2); m/z (ES) 360.1 (100%, [M+H]$^+$); HRMS Found: 382.1909 (C$_{23}$H$_{25}$N$_3$O, requires M+Na 382.1890).

(E)-N-(2-(3-ethyl-1-phenylureido)-4-(naphthalen-2-yl)but-3-enyl)-2-nitrobenzenesulfonamide 59$^{100}$

The title compound 59 was obtained from the allyl urea 55 (0.1 g, 0.19 mmol) and 2-bromonaphthalenyl (90 mg, 0.38 mmol) following general procedure C$^{100}$ and stirring for 2 days. The crude product was purified by flash chromatography (98.5:1.5 CH$_2$Cl$_2$–MeOH) followed by mass directed chromatography to give a yellow oil (13.6 mg, 13%). R$_F$: 0.25 (60:30, petrol–EtOAc); $\nu_{max}$/cm$^{-1}$ (film); 3063, 2930, 1649, 1541, 1513, 1362, 1276, 970, 731; δ$^1$H (500 MHz; CDCl$_3$) 8.30 (1H, dd J 7.7 and 1.5, Ns 3-H), 7.80 (1H, dd, J 7.8 and 1.5, Ns 5-H), 7.76 (1H, d, J 8.4, naphthalenyl 5-H or 8-H), 7.73 (1H, d, J 8.5, naphthalenyl 3-H or 4-H), 7.69 (1H, td, J 7.7 and 1.3, Ns 4-H), 7.64 (1H,
The title compound 60 was prepared from the allyl urea (55 mg, 0.2 mmol) 56 and 2-bromonaphthalenyl (91 mg, 2.2 mmol) according to general procedure C,\textsuperscript{100} stirring for 3 days. The crude product was purified by flash chromatography (petrol–EtOAc 80:20 → 70:30) to give a yellow oil (33 mg, 35%). R\textsubscript{F}: 0.25 (50:50, petrol–EtOAc); ν\textsubscript{max}/cm\textsuperscript{-1} (film); 3057, 2928, 2825, 1703, 1598, 1425, 1261, 1044, 918, 751; δ\textsubscript{H} (500 MHz; CDCl\textsubscript{3}) 7.82-7.79 (3H, m, naphthalenyl 7-, 6- and 4-H), 7.69 (1H, s, naphthalenyl 1-H), 7.51-7.44 (2H, m, naphthalenyl 3- or 5-H and 8-H), 7.39 (2H, d, J 8.5 phenyl 2- and 6-H), 7.35 (1H, d, J 7.5, naphthalenyl 3- or 5-H), 7.24 (2H, d, J 8.5 phenyl 3- and 5-H), 7.00 (1H, app. t, J 7.4, phenyl 4-H), 4.37 (1H, d, J 6.5, OCH\textsubscript{3}H\textsubscript{5}O), 4.35 (1H, d, J 6.5, OCH\textsubscript{3}H\textsubscript{5}O), 4.08 (1H, dt, J 6.3 and 3.1, 4-H), 4.03 (1H, ddd, J 8.3, 4.8 and 3.0, 5-H), 3.75 (1H, dq, J 14.0 and 7.5, ethyl 1-H\textsubscript{A}), 3.46 (1H, dd, J 10.4 and 6.3, 4-CH\textsubscript{3}H\textsubscript{5}B), 3.28-3.26 (2H, m, 4-CH\textsubscript{3}H\textsubscript{5}B and 5-CH\textsubscript{3}H\textsubscript{5}B), 3.12 (3H, s, OCH\textsubscript{3}), 3.13-3.09
(1H, m, ethyl 1-\(H_AH_B\)), 2.92 (1H, dd, \(J \) 13.7 and 8.3, 5-\(CH_AH_B\)), 1.22 (3H, t, \(J \) 7.2, ethyl 2-H3); \(\delta_c\) (125 MHz; CDCl\(_3\)) 156.6 (CO), 139 (phenyl C-1), 134 (naphthalenyl C-4a), 133.5 (naphthalenyl C-2), 132.3 (naphthalenyl C-8a), 128.8 (phenyl C-2 and C-6), 128.4 (naphthalenyl C-7- or C-6- or C-4), 128.0 (naphthalenyl C-1), 127.7 (naphthalenyl C-7- or C-6- or C-4), 127.5 (naphthalenyl C-7- or C-6- or C-4), 127.3 (naphthalenyl C-3 or C-5), 126.3 (naphthalenyl C-3 or C-5), 125.7 (naphthalenyl C-8), 123.1 (phenyl C-4), 120 (phenyl C-2 and C-6), 96.4 (OCH\(_A\)H\(_B\)O), 66.1 (4-\(CH_2\)), 57.9 (C-4), 55.8 (C-5), 55.2 (OCH\(_3\)), 39.2 (5-\(CH_2\)), 36.3 (ethyl C-1), 13.1 (ethyl C-2); \(m/z\) (ES) 427.1 (100%, [M+Na]+); HRMS Found: 427.2000 (C\(_{25}\)H\(_{28}\)N\(_2\)O\(_3\) requires M+Na 427.1992).

\((4S^*, 5S^*)\)-4-(Azidomethyl)-1-ethyl-5-(naphthalen-2-ylmethyl)-3-phenyl imidazolidin-2-one 61\(^{100}\)

The title compound 61 was prepared from the allyl urea 57 (70 mg, 0.27 mmol) and 2-bromonaphthalenyl (0.17 g, 0.80 mmol) according to general procedure C,\(^{100}\) stirring for 3 days. The crude product was purified by flash chromatography (petrol–EtOAc 80:20 \(\rightarrow\) 70:30) to give a yellow oil (6 mg, 5%). \(R_f\): 0.6 (50:50, petrol–EtOAc); \(\nu_{\text{max}}/\text{cm}^{-1}\) (film): 2925, 2105, 1704, 1501, 1423, 1260, 800, 752; \(\delta_H\) (500 MHz; CDCl\(_3\)) 7.85-7.81 (3H, m, naphthalenyl 7-, 6- and 4-H), 7.69 (1H, s, naphthalenyl 1-H), 7.52-7.46 (2H, m, naphthalenyl 3- and 8-H), 7.36-7.33 (3H, m, phenyl 2- and 6-H, naphthalenyl 5-H), 7.30-7.28 (2H, m, phenyl 3- and 5-H), 7.05 (1H, tt, \(J \) 7.5 and 1.0, phenyl 4-H), 4.08 (1H, app. dt, \(J \) 5.7 and 3.0, 5-H), 3.96 (1H, ddd, \(J \) 8.3, 4.2 and 3.0, 4-H), 3.78 (1H, dq, \(J \) 14.6 and 7.2, ethyl 1-H\(_A\)), 3.37-3.32 (2H, m, 4-\(CH_2\)H\(_B\) and 5-\(CH_2\)H\(_B\)), 3.18 (1H, dq, \(J \) 14.0 and 7.0, ethyl 1-H\(_B\)), 2.89-2.82 (2H, m, 4-\(CH_2\)H\(_B\) and 5-\(CH_2\)H\(_B\)), 1.27 (3H, t, \(J \) 7.2, ethyl 2-H3); \(\delta_c\) (125 MHz; CDCl\(_3\)) 159.4 (CO), 138.1 (phenyl C-1), 133.6 (naphthalenyl C-4a), 133.4 (naphthalenyl C-2), 132.5 (naphthalenyl C-8a), 129.1 (phenyl C-2 and C-6), 128.8 (naphthalenyl C-7 or C-6 or C-4), 128.1
(naphthalenyl C-1), 127.8 (naphthalenyl C-7 or C-6 or C-4), 127.6 (naphthalenyl C-7 or C-6 or C-4), 127.1 (naphthalenyl C-5), 126.5 (naphthalenyl C-3), 125.9 (naphthalenyl C-8), 123.9 (phenyl C-4), 120.7 (phenyl C-3 and C-5), 57.6 (C-5), 55.9 (C-4), 51.1 (5-CH₂), 39.2 (4-CH₂), 36.4 (ethyl C-1), 13.1 (ethyl C-2); m/z (ES) 408.1 (100%, [M+Na]+); HRMS Found: 408.1799 (C₂₃H₂₅N₅O requires M+Na 408.1795).

\[ \text{N-Benzylprop-2-en-1-amine}^{70} \]

The title compound 70 was prepared according to the method of Mukherjee et al.\textsuperscript{150} Benzylbromide (2.1 mL, 17.7 mmol) was added via syringe pump over 1 h to a suspension of K₂CO₃ (2.9 g, 21.0 mmol) in allyl amine (10.5 mL, 140.1 mmol) and the resulting mixture was stirred at r.t. for 2 days. The reaction mixture was filtered through Celite\textsuperscript{®} eluting with CH₂Cl₂; the filtrate was concentrated under reduced pressure and the crude product was purified by flash chromatography (petrol–EtOAc 50:50) to give a yellow oil (1.20 g, 47%). \( R_F \): 0.5 (10:90, petrol-EtOAc); \( \nu_{\max }/\text{cm}^{-1} \) (film) 3311, 3027, 2813, 1948, 1810, 1643, 1455, 1106, 918, 736; \( \delta_H \) (500 MHz; CDCl₃); 7.23 (1H, dd, J 8.7 and 1.4, phenyl 4-H), 5.87 (1H, ddt, J 17.1, 10.3 and 6.0, 2-H), 5.13 (1H, dd, J 17.1 and 1.4, 3-Hₐ), 5.04 (1H, dd, J 10.3 and 1.4, 3-Hₜ), 3.79 (1H, s, benzyl CH₂), 3.28 (2H, dt, J 6.0 and 1.4, 1-H₂), 1.39 (1H, br. s, NH); m/z (ES) 148.2 (100%, [M+H]+). Spectra corresponds to the one reported in the literature.\textsuperscript{150}
1-Allyl-1-benzyl-3-phenylurea 71\textsuperscript{100,151}

The title compound 71 was prepared according to general procedure B\textsuperscript{100} from phenylisocyanate (1.4 mL, 12.4 mmol) and the amine 70 (1.21 g, 8.2 mmol) in CH\textsubscript{2}Cl\textsubscript{2} stirring for 2 days. The crude product was purified by flash chromatography (petrol–EtOAc 70:30) to give a yellow oil (2.1 g, 95%). \(R_f\): 0.7 (50:50 petrol–EtOAc); \(\nu_{\text{max}}/\text{cm}^{-1}\) (film) 3031, 2984, 2922, 2278, 1645, 1441, 1231, 928, 754, 696; \(\delta_{\text{H}}\) (500 MHz; CDCl\textsubscript{3}) \(\delta\) 7.41-7.25 (m, 9H, Ar), 7.03 (1H, t, \(J\) 7.1, Ar), 6.45 (s, 1H, NH), 5.89 (1H, ddd, \(J\) 15.8, 11.9 and 5.4, allyl 2-H), 5.37-5.31 (2H, m, allyl 3-H\textsubscript{A} and 3-H\textsubscript{B}), 4.62 (2H, s, benzyl CH\textsubscript{2}), 3.98 (2H, d, \(J\) 5.4 Hz, allyl 1-H); \(\delta_{\text{C}}\) (125 MHz; CDCl\textsubscript{3}) 157.8 (C=O), 139.0, 137.6 (Ar C-1 and C-1'), 133.9 (C-2), 128.9, 128.8, 127.7, 127.5, 123.0, 119.7 (Ar CH), 117.5 (C-3), 50.7 (benzyl CH\textsubscript{2}), 50.1 (C-1); \(m/z\) (ES) 267.1 (100%, [M+H]\textsuperscript{+}); HRMS Found: 267.1485 (C\textsubscript{17}H\textsubscript{18}N\textsubscript{2}O requires M+H 267.1470). Characterisation data consistent with those reported in literature.\textsuperscript{151}

1-Benzyl-4-(iodomethyl)-3-phenylimidazolidin-2-one 73\textsuperscript{124}

Triethylamine (0.25 mL, 2.2 mmol) was added slowly to a solution of the benzyl allyl urea 71 (0.30 g, 1.1 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (5 mL) followed by trimethylsilyltrifluoromethansulfonate (0.40 mL, 2.0 mmol). The resulting mixture was stirred for 1 h at r.t. and concentrated under reduced pressure. The crude material was dissolved in THF (7.0 mL) and I\textsubscript{2} (0.57 g, 2.0 mmol) was added. The resulting mixture was stirred at r.t. overnight, then it was poured into an aqueous solution of Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} (20%, w/v, 40 mL) and extracted with EtOAc (3.0 \(\times\) 20 mL). The combined organic phases were dried (MgSO\textsubscript{4}) and concentrated under reduced pressure to give a crude
product which was purified by flash chromatography (petrol–EtOAc 60:40) to give the **title compound 73** as a pale yellow oil (0.24 g, 53%). $R_F$: 0.7 (60:40, petrol–EtOAc); $\nu_{\text{max}}$/cm$^{-1}$ (film) 2923, 2098, 1698, 1655, 1500, 1441, 1260, 1138, 1028, 755, 696, 525; $\delta_H$ (500 MHz; CDCl$_3$) 7.46 (2H, dd, $J$ 8.1 and 1.1, Ar CH), 7.39-7.30 (7H, m, Ar CH), 7.14 (1H, dd, $J$ 7.3 and 1.2, Ar CH), 4.56 (1H, d, $J$ 14.9, benzyl CH$_3$H$_B$), 4.41 (1H, d, $J$ 14.9, benzyl CH$_3$H$_B$), 4.35 (1H, app. tdd, $J$ 8.9, 4.7 and 2.5, 4-H), 3.54 (1H, app. t, $J$ 9.2, 5-H$_A$), 3.33 (1H, dd, $J$ 10.3 and 2.5, 4-CH$_3$H$_B$), 3.16 (1H, dd, $J$ 9.3 and 4.7, 5-H$_B$), 3.15 (1H, dd, $J$ 10.3 and 8.8, 4-CH$_2$); $\delta_C$ (125 MHz; CDCl$_3$) 157.4 (C O), 137.8, 136.5 (Ar C-1 and C-1'), 129.2, 128.7, 128.3 (Ar CH), 127.7, 124.3 (Ar C-4 and C-4'), 121.4 (Ar CH), 54.12 (C-4), 48.1 (C-5), 47.9 (benzyl CH$_2$), 8.0 (4-CH$_2$); $m/z$ (ES) 393.2 (100%, [M+H]$^+$); HRMS Found: 393.0456 (C$_{17}$H$_{17}$N$_2$O requires M+H 393.0458).

4-(Azidomethyl)-1-benzyl-3-phenylimidazolidin-2-one 74$^{137,151}$

Sodium azide (0.05 g, 0.8 mmol) was added portionwise at 0 °C to a solution of the imidazolidinone 73 (0.10 g, 0.25 mmol) in DMF (2.5 mL) and the resulting mixture was stirred at r.t overnight. EtOAc (5.0 mL) was added to the reaction mixture, the resulting mixture was washed with water (2 × 5.0 mL) and brine (2 × 50 mL). The combined organic phases were dried (MgSO$_4$) and concentrated under reduced pressure to give the title compound 74 as a white amorphous solid which was used without further purification (3 mg, 37%). $R_F$: 0.7 (40:60, petrol–EtOAc); $\nu_{\text{max}}$/cm$^{-1}$ (film) 2250, 2106, 1651, 1441, 1357, 1259, 1087, 907, 713, 695; $\delta_H$ (500 MHz; CDCl$_3$) 7.47 (2H, dd, $J$ 8.7 and 1.1, Ar CH), 7.40-7.34 (7H, m, Ar CH), 7.31 (1H, dd, $J$ 7.5 and 2.3, Ar CH), 4.51 (1H, dd, $J$ 15.0, benzyl CH$_3$H$_B$), 4.46 (1H, dd, $J$ 15.0, benzyl CH$_3$H$_B$), 4.38 (1H, dddd, $J$ 9.3, 6.4, 4.6 and 3.1, 4-H), 3.49 (1H, dd, $J$ 6.4 and 1.2, 4-CH$_3$H$_B$), 3.48 (1H, app. t, $J$ 9.2, 5-H$_A$), 3.38 (1H, dd, $J$ 12.7 and 3.1, 4-CH$_3$H$_B$), 3.24 (1H, dd, $J$ 9.2 and 4.6, 5-H$_B$); $\delta_C$ (125 MHz; CDCl$_3$) 157.5 (CO), 137.9, 136.6 (Ar C-1 and C-1'), 129.2, 128.7, 128.2 (Ar CH), 127.7, 124.3 (Ar C-4 and C-4'), 121.2 (Ar CH), 52.4 (4-CH$_2$), 51.4 (C-4), 48.0 (benzyl CH$_2$), 45.0 (C-5); $m/z$ (ES) 308.2 (100%, [M+H]$^+$);
HRMS Found: 330.1325 (C₁₇H₁₇N₅O requires M+H 330.35). Characterisation data were consistent with those reported in literature.¹⁵¹

4-(Aminomethyl)-1-benzyl-3-phenylimidazolidin-2-one 75¹³⁸

Triphenylphosphine (19 mg, 58 μmol) was added to a solution of the imidazolinone 74 (15 mg, 48 μmol) in THF-H₂O (550 μL of THF, 50 μl of H₂O) and the resulting mixture was stirred at 45 °C overnight. The solvent was removed to give a crude product which was purified by flash chromatography (petrol–EtOAc 50:50, then CH₂Cl₂–EtOH–NH₄OH 84.7:13.6:1.7) to give the title imidazolidinone 75 as a pale yellow oil (11 mg, 81%). Rₚ: 0.7 (70:30, CH₂Cl₂–MeOH); νmax/cm⁻¹ (film) 3384, 2920, 1697, 1598, 1500, 1439, 1261, 756; δH (500 MHz; CDCl₃) 7.5 (2H, dd, J 8.7 and 1.0, Ar CH), 7.36-7.28 (7H, m, Ar CH), 7.1 (1H, tt, J 7.8 and 1.0, Ar CH), 4.53 (1H, d, J 14.9, benzyl CHₐHₕ), 4.42 (1H, d, J 14.9, benzyl CHₐHₖ), 4.31-4.27 (1H, m, 4-H), 3.47 (1H, app. t, J 9.0, 5-Hₐ), 3.27 (1H, dd, J 9.0 and 5.1, 5-Hₖ), 2.93 (1H, dd, J 13.4 and 6.3, 4-CHₐHₕ), 2.83 (1H, app. d, J 13.4, 4-CHₐHₖ), 1.61 (2H, br. s, NH₂); δC (125 MHz; CDCl₃) 158.1 (CO), 138.6, 136.8 (Ar C-1 and C-1'), 129.0, 128.7, 128.3 (Ar CH), 127.6, 123.9 (Ar C-4 and C-4'), 121.1 (Ar CH), 54.7 (C-4), 48.4 (benzyl CH₂), 44.9 (C-5), 42.4 (4-CH₂); m/z (ES) 282.2 (100%, [M+H]+); HRMS Found: 282.1603 (C₁₇H₁₉N₃O requires M+H 282.1601).

N-(Diphenylmethylene)prop-2-en-1-amine 78¹²⁶

The title compound 78 was prepared according to the general method of Blacker et al.¹²⁶ Allyl amine (4.0 g, 77.0 mmol) was added to a solution of benzophenone (8.6 g,
47.0 mmol) in toluene (45 mL) at r.t., followed by titanium isopropoxide (9.4 mL, 32.0 mmol) and the resulting mixture was stirred at 80 °C overnight. After cooling down at r.t., water (2.0 mL) and toluene (35 mL) were added and the reaction mixture was stirred for 2 h at r.t., filtered through Celite®, washed with toluene (2 × 20 mL). The organic phases were concentrated under reduced pressure to give the title compound 78 as a yellow oil that was used without further purification (10.4 g, 98%).

\[ \text{R}_{	ext{F}}: 0.8 \ (20:80, \text{petrol–EtOAc}) ; \nu_{\text{max}}/\text{cm}^{-1} \ (\text{film}) \ 3059, 3024, 2912, 1957, 1661, 1464, 1314, 1287, 1179, 1074, 918, 780; \delta_{\text{H}} \ (500 \text{ MHz; CDCl}_3) \ 7.64 \ (2\text{H, dd, } J 8.4 \text{ and } 1.3, \text{ Ar CH}), \ 7.48 - 7.36 \ (4\text{H, m, Ar CH}), \ 7.32 - 7.31 \ (2\text{H, m, Ar CH}), \ 7.18 \ (2\text{H, dd, } J 7.7 \text{ and } 1.6, \text{ Ar CH}), \ 6.06 \ (2\text{H, ddt, } J 17.2, 10.5 \text{ and } 5.4, 2-\text{H}), \ 5.18 \ (1\text{H, dq, } J 17.2 \text{ and } 1.8, 3-\text{H}_A), \ 5.11 \ (1\text{H, dq, } J 10.5 \text{ and } 1.8, 3-\text{H}_B), \ 4.04 \ (2\text{H, dt, } J 5.4 \text{ and } 1.8, 1-\text{H}_2); \delta_{\text{C}} \ (75 \text{ MHz; CDCl}_3) \ 168.8 \ (\text{N=CPH}_2), \ 139.8, \ 136.7 \ (\text{Ar C}), \ 136.6 \ (\text{Ar CH}), \ 130.0 \ (\text{C-2}), \ 129, \ 128.5, \ 128.3, \ 128.1, \ 127.7 \ (\text{Ar CH}), \ 115.1 \ (\text{C-3}), \ 56.4 \ (\text{C-1}); \text{HRMS Found: } 222.1281 \ (C_{16}H_{15}N \text{ requires } M+H 222.1107). \]

Characterisation data were consistent with those reported in literature.

**1-phenylbut-3-en-2-amine 76**

The title compound 78 was prepared according to the general method of Blacker et al. The amine 76 (4.0 g, 18.1 mmol) and benzylchloride (2.1 mL, 18.1 mmol) were mixed in the presence of n-butyllithium (11.3 mL, 18.1 mmol) according to general procedure D. The crude product was purified by flash chromatography (petrol–EtOAc 80:20 → 70:30) to give a yellow oil (0.85 g, 50%).

\[ \text{R}_{	ext{F}}: 0.15 \ (99.5:0.5, \text{CH}_2\text{Cl}_2–\text{MeOH}); \nu_{\text{max}}/\text{cm}^{-1} \ (\text{film}) \ 3910, 3758, 2107, 1644, 1495, 1454, 1371, 1260, 1078, 994, 746; \delta_{\text{H}} \ (500 \text{ MHz; CDCl}_3) \ 7.3 \ (2\text{H, t, } J 7.3, \text{ Ar}), \ 7.26-7.19 \ (3\text{H, m, Ar}), \ 5.89 \ (1\text{H, ddd, } J 17.0, 10.3, 6.4, 3-\text{H}), \ 5.14 \ (1\text{H, d, } J 17.0, 4-\text{H}_A), \ 5.04 \ (1\text{H, d, } J 10.3, 4-\text{H}_B), \ 3.6 \ (1\text{H, ddd, } J 8.3, 6.4 \text{ and } 5.4, 2-\text{H}), \ 2.83 \ (1\text{H, dd, } J 13.3 \text{ and } 5.4, 1-\text{H}_A), \ 2.62 \ (1\text{H, dd, } J 13.3 \text{ and } 8.3, 1-\text{H}_B), \ 1.47 \ (2\text{H, br. s., NH}_2); \delta_{\text{C}} \ (75 \text{ MHz; CDCl}_3) \ 142.4 \ (\text{phenyl C-1}), \ 138.8 \ (\text{phenyl C-4}), \ 129.4 \ (\text{phenyl C-2 and C-6 or C-3 and C-5}), \ 128.4 \ (\text{phenyl C-2 and C-6 or C-3 and C-5}), \ 126.3 \ (\text{C-4}), \ 113.6 \ (\text{C-3}), \ 55.5 \ (\text{C-2}), \ 44.4 \ (\text{C-1}); \text{HRMS Found: } 148.1147 \]
(C_{10}H_{13}N, M+H requires 148.1121). Characterisation data were consistent with those reported in literature.\textsuperscript{126}

1-(3,5-dimethylisoxazol-4-yl)but-3-en-2-amine 77\textsuperscript{126}

The \textit{title compound 77} was prepared from the amine 78 (6.4 g, 29.0 mmol) and 4-chloromethyl-3,5-dimethylisoxazolyl (3.6 mL, 29.0 mmol) in the presence of \textit{n-Butyl lithium} (11.6 mL, 29.0 mmol) according to \textbf{general procedure D}.\textsuperscript{126} A yellow oil (2.4 g, 49\%) was obtained and used without further purification. \textit{R_F}: 0.1 (99.5:0.5, CH\textsubscript{2}Cl\textsubscript{2}–MeOH); \textit{\nu}_{\text{max}}/\text{cm}\textsuperscript{-1} (film) 3698, 2962, 1956, 1577, 1492, 1450, 1260, 1195, 1015, 923, 745; \delta\textsubscript{H} (500 MHz; CDCl\textsubscript{3}) 5.80 (1H, ddd, \textit{J} 17.2, 10.3 and 6.6, 3-H), 5.11 (1H, dt, \textit{J} 17.2 and 1.2, 4-H\textsubscript{A}), 5.05 (1H, dt, \textit{J} 10.3 and 1.2, 4-H\textsubscript{B}), 3.45 (1H, app. q, \textit{J} 6.6, 2-H), 2.43 (1H, app. d, \textit{J} 6.6, 1-H\textsubscript{A}), 2.42 (1H, app. d, \textit{J} 7.2, 1-H\textsubscript{B}), 2.33 (3H, s, isoxazolyl 5-\textit{CH}_3), 2.23 (3H, s, isoxazolyl 3-\textit{CH}_3), 1.49 (2H, br. s., NH\textsubscript{2}); \delta\textsubscript{C} (75 MHz; CDCl\textsubscript{3}) 166.0 (isoxazolyl C-5), 159.9 (isoxazolyl C-3), 141.8 (C-3), 114.5 (C-4), 110.5 (isoxazolyl C-4), 54.5 (C-2), 30.8 (C-1), 11.3 (isoxazolyl 5-\textit{CH}_3), 10.5 (isoxazolyl 3-\textit{CH}_3); HRMS Found 167.1190 (C\textsubscript{9}H\textsubscript{14}N\textsubscript{2}O requires M+H 167.1179).

1-(3,5-Dimethylisoxazol-4-yl)-N-(furanyl-2-ylmethyl)but-3-en-2-amine 80\textsuperscript{136}

The \textit{title compound 80} was prepared from the amine 77 (1.3 g, 7.9 mmol) and furanylaldehyde (0.8 mL, 9.5 mmol) according to \textbf{general procedure E}.\textsuperscript{136} stirring for 4 h at r.t., before adding NaBH\textsubscript{4} (2.0 eq.). A yellow oil (1.9 g, 98\%) was obtained and
used without further purification. $R_F$: 0.2 (99:1, CH$_2$Cl$_2$–MeOH); $\nu_{\text{max}}$/cm$^{-1}$ (film) 3307, 2978, 2931, 1651, 1636, 1455, 1424, 1195, 1148, 1011, 742; $\delta_H$ (500 MHz; CDCl$_3$) 7.33 (1H, dd, $J$ 1.8 and 0.8, furanyl 5-H), 6.29 (1H, dd, $J$ 17.1, 10.2 and 8.2, 3-H), 5.14 (1H, dddd, $J$ 17.1, 10.2 and 1.5, 4-$H_A$), 5.08 (1H, dddd, $J$ 17.1, 10.2 and 0.8, 4-$H_B$), 3.59 (1H, d, $J$ 13.6, benzyl $CH_AH_B$), 3.09 (1H, app. q, $J$ 7.2, 2-H), 2.48 (1H, ddd, $J$ 14.4 and 6.9, 1-$H_A$), 2.43 (1H, dd, $J$ 14.4 and 6.9, 1-$H_B$), 2.27 (3H, s, isoxazolyl 5-$CH_3$); m/z (ES) 247.1 (100%, [M+H]$^+$); HRMS Found: 247.1439 (C$_{14}$H$_{18}$N$_2$O$_2$, M+H requires 247.1441).

$N$-(4-Chlorobenzyl)-1-(3,5-dimethylisoxazol-4-yl)but-3-en-2-amine 81$^{136}$

![N-(4-Chlorobenzyl)-1-(3,5-dimethylisoxazol-4-yl)but-3-en-2-amine 81](image)

The title compound 81 was prepared from the amine 77 (1.3 g, 7.6 mmol) and $p$-chlorobenzaldehyde (2.6 mL, 18.2 mmol) according to general procedure E,$^{136}$ stirring at reflux overnight, before adding NaBH$_4$ (6.0 eq.). The crude product was dissolved in EtOAc (15 mL), extracted with a aqueous solution of HCl (2.0 M) (3.0 x 10 mL), then the combined water phases were basified with an aqueous solution of NaOH (50% w/v) until pH 10-11, and extracted with EtOAc (3.0 x 20 mL). The organic phases were dried (MgSO$_4$) and concentrated under reduced pressure to give a yellow oil (0.99 g, 45%). $R_F$: 0.6 (95:5, CH$_2$Cl$_2$–MeOH); $\nu_{\text{max}}$/cm$^{-1}$ (film) 3354, 2926, 1637, 1491, 1449, 1196, 1089, 1014, 924, 800; $\delta_H$ (500 MHz; CDCl$_3$) 7.27 (2H, d, $J$ 8.3, phenyl 3- and 5-H), 7.17 (2H, d, $J$ 8.3, phenyl 2- and 6-H), 5.61 (1H, ddd, $J$ 17.1, 10.1 and 8.2, 3-H), 5.15 (1H, d, $J$ 10.1, 4-$H_A$), 5.06 (1H, d, $J$ 17.1, 4-$H_B$), 3.79 (1H, d, $J$ 13.6, benzyl $CH_AH_B$), 3.59 (1H, d, $J$ 13.6, benzyl $CH_AH_B$), 3.09 (1H, app. q, $J$ 7.2, 2-H), 2.48 (1H, ddd, $J$ 14.4 and 6.9, 1-$H_A$), 2.43 (1H, dd, $J$ 14.4 and 6.9, 1-$H_B$), 2.27 (3H, s, isoxazolyl 5-
CH₃), 2.15 (3H, s, isoxazolyl 3-CH₃); δC (75 MHz; CDCl₃) 166.0 (isoxazolyl C-5), 159.9 (isoxazolyl C-3), 139.7 (phenyl C-1), 132.7 (C-3), 129.3 (phenyl C-2- and C-6), 128.5 (phenyl C-3- and C-5), 128.3 (phenyl C-4), 117.2 (C-4), 110.3 (isoxazolyl C-4), 60.8 (C-2), 50.4 (benzyl CH₂), 29.1 (C-1), 11.3 (isoxazolyl 5-CH₃), 10.4 (isoxazolyl 3-CH₃); m/z (ES) 291.3 (100%, [M+H]+); HRMS Found 291.1259 (C₁₆H₁₉ClN₂O, M+H requires 291.1272).

N-(4-Chlorobenzyl)-1-phenylbut-3-en-2-amine 82

\[
\begin{align*}
&\text{HN} \\
&\text{CH₂=CHCHCH₃} \\
&\text{Cl}
\end{align*}
\]

\(p\)-Chlorobenzaldehyde (0.7 g, 5.0 mmol) was added to a solution of the amine 76 (0.49 g, 3.3 mmol) in MeOH (0.25 M), followed by molecular sieves (4 Å) and the mixture was stirred at reflux overnight. The reaction was filtered through Celite®, concentrated under reduced pressure and resuspended in dry MeOH. NaBH₄ (2.0 eq.) was then added at 0 °C and the mixture was left stirring at r.t. overnight. The reaction mixture was quenched with water (5.0 mL), diluted with CH₂Cl₂ (20 mL) and the phases were separated. The aqueous phase was extracted with CH₂Cl₂ (3 x 10 mL) and the combined organic phases were dried (MgSO₄) and concentrated under reduced pressure to give a crude product, which was purified by flash chromatography (80:20 hexane–EtOAc) to give the title amine 82 as a colourless oil (0.6 g, 65%). Rᵢ: 0.4 (50:50, petrol–EtOAc); νmax/cm⁻¹ (film) 3346, 2922, 2849, 1491, 1455, 1090, 1014, 700; δH (500 MHz; CDCl₃) 7.34-7.26 (4H, m, Ar CH), 7.22 (2H, dd, J 8.0 and 2.0 Ar CH), 7.16 (2H, d, J 6.8, Ar CH), 7.08 (1H, d, J 8.2 Ar CH), 5.70 (1H, ddd, J 17.1, 10.3, 8.0, 3-H), 5.13 (1H, ddd, J 10.3, 1.6 and 0.6, 4-Hₐ), 5.09 (1H, ddd, J 17.1, 1.6 and 0.8, 4-Hₐ), 4.68 (1H, br. s., NH), 3.78 (1H, d, J 13.8 benzyl CH₃Hₐ), 3.57 (1H, d, J 13.8 benzyl CH₃Hₐ), 3.27 (1H, app. td, J 8.0 and 5.7, 2-H), 2.81 (1H, dd, J 13.5 and 5.7, 1-Hₐ), 2.75 (1H, dd, J 13.5 and 8.0, 1-Hₐ); δC (75 MHz; CDCl₃) 140.5 (Ar C), 138.4 (C-3), 132.5 (Ar C), 129.4, 129.3 (Ar CH), 128.7 (Ar C), 128.3, 126.5, 126.5 (Ar CH), 116.5 (C-4), 61.8 (C-2), 50.3 (benzyl CH₂), 42.5 (C-1); m/z (ES) 271.4 (100%, [M+H]+); HRMS Found 272.1194 (C₁₇H₁₈ClN, M+H requires 272.1200).
The title compound 83 was prepared from allylamine (0.4 mL, 5.2 mmol) and p-chlorobenzaldehyde (0.6 mL, 4.3 mmol) according to general procedure E,\textsuperscript{136} stirring at reflux for 4 h, before adding NaBH\textsubscript{4} (4 eq.). A yellow oil (0.33 g, 43\%) was obtained and used without further purification. R\textsubscript{F}: 0.4 (95:5, CH\textsubscript{2}Cl\textsubscript{2}–MeOH); ν\textsubscript{max}/cm\textsuperscript{-1} (film) 3368, 2927, 1643, 1491, 1090, 10115, 819; δ\textsubscript{H} (500 MHz; CDCl\textsubscript{3}) 7.28 (2H, d, J 8.4, phenyl 3- and 5-H), 7.25 (2H, d, J 8.4, phenyl 2- and 6-H), 5.91 (1H, ddd, J 7.17, 10.2 and 6.0, 2-H), 5.22 (1H, dd, J 17.1 and 1.5, 3-H\textsubscript{A}), 5.18 (1H, dt, J 10.2 and 1.1, 3-H\textsubscript{B}), 3.79 (2H, s, benzyl CH\textsubscript{2}), 3.25 (2H, dd, J 6.0 and 1.1, 1-H\textsubscript{3}), 1.55 (2H, br. s., NH\textsubscript{2}); δ\textsubscript{C} (75 MHz; CDCl\textsubscript{3}) 138.9 (C-3), 136.7 (phenyl C-1), 132.8 (phenyl C-4), 129.6 (phenyl C-3 and C-5), 128.6 (phenyl C-2 and C-6), 116.3 (C-4), 52.6 (benzyl CH\textsubscript{2}), 51.8 (C-2); m/z (ES) 181.9 (100\%, [M+H]\textsuperscript{+}); HRMS Found 182.0730 (C\textsubscript{9}H\textsubscript{14}N\textsubscript{2}O requires M+H 182.0731). Characterisation data were consistent with those reported in literature.\textsuperscript{153}

1-(3,5-Dimethylisoxazol-4-yl)-N-isopropylbut-3-en-2-amine 84\textsuperscript{154}

Acetone (0.17 mL, 2.4 mmol) was added to a solution of the amine 77 (0.2 g, 1.2 mmol) in EtOH (0.8 M, 1.5 mL), followed by MgSO\textsubscript{4} (15 mg) and the mixture was stirred at r.t. for 8 h; then NaBH\textsubscript{4} (0.1 g, 7.2 mmol) was added and the mixture was left stirring at r.t overnight. The reaction mixture was quenched with an aqueous solution of NaOH (1.0 M, 2.0 mL), EtOH was removed under reduced pressure and the remaining solution was extracted with EtOAc (3 x 10 mL). The combined organic phases were washed with brine (2 x 5.0 mL), dried (Na\textsubscript{2}SO\textsubscript{4}) and concentrated under reduced pressure to give the title amine 84 as a pale yellow oil (0.15 g, 61\%). R\textsubscript{F}: 0.2 (99.5:0.5,
CH$_2$Cl$_2$–MeOH); $\nu$$_{max}$/cm$^{-1}$ (film) 3365, 2962, 1660, 1638, 1454, 1426, 1195, 996, 921, 744; $\delta$$_H$ (500 MHz; CDCl$_3$) 5.56 (1H, ddd, J 17.1, 10.1 and 8.4, 3-H), 5.06 (1H, dd, J 10.1 and 1.1, 4-H$_A$), 4.99 (1H, dd, J 17.1 and 1.1, 4-H$_B$), 3.22 (1H, app. td, J 8.1, and 6.1, 2-H), 2.84 (1H, m, isopropyl CH), 2.50 (1H, dd, J 13.7 and 6.1, 1-H$_A$), 2.38 (1H, dd, J 13.7 and 7.8, 1-H$_B$), 2.33 (3H, s, isoxazolyl 5-C$_H$$_3$), 2.22 (3H, s, isoxazolyl 3-C$_H$$_3$), 1.06 (3H, d, J 6.1, isopropyl C$_H$$_3$), 0.97 (3H, d, J 6.2, isopropyl CH); $\delta$$_C$ (75 MHz; CDCl$_3$) 165.9 (isoxazolyl C-5), 159.9 (isoxazolyl C-3), 140.3 (C-3), 120.9 (C-4), 110.6 (isoxazolyl C-4), 59.1 (C-2), 45.7 (isopropyl C-1), 29.4 (C-1), 24.0 (isopropyl CH$_3$A), 22.0 (isopropyl CH$_3$B), 11.3 (isoxazolyl 5-C$_H$$_3$), 10.5 (isoxazolyl 3-C$_H$$_3$); m/z (ES) 209.1 (100%, [M+H]$^+$); HRMS Found: 209.1648 (C$_{12}$H$_{20}$N$_2$O, M+H requires 209.1651).

3-(3,5-Dimethylisoxazol-4-yl)-1-(1-(3,5-dimethylisoxazol-4-yl)but-3-en-2-yl)-1-(furanyl-2-ylmethyl)urea 85

The title compound 85 was prepared from the amine 80 (0.5 g, 2.1 mmol) and 4-isocyanato-3,5-dimethylisoxazolyl (0.7 mL, 6.45 mmol) in CH$_2$Cl$_2$ stirring at 35 °C for 1 day, according to general procedure B. The crude product was purified by flash chromatography (50:50, petrol–EtOAc) to give a yellow oil (0.60 g, 77%). R$_F$: 0.3 (60:40, petrol–EtOAc); $\nu$$_{max}$/cm$^{-1}$ (film) 3320, 2928, 1722, 1660, 1651, 1645, 1515, 1456, 1427, 1241, 1012, 917, 734 ; $\delta$$_H$ (500 MHz; CDCl$_3$) 7.41 (1H, dd, J 1.9 and 0.8, furanyl 5-H), 6.40 (1H, dd, J 3.2 and 1.9, furanyl 4-H), 6.27 (1H, dd, J 3.2 and 0.8, furanyl 3-H), 5.60 (1H, ddd, J 17.3, 10.5 and 6.1, 3-H), 5.92 (1H, br. s., NH), 5.26 (1H, ddd, J 17.3, 1.5 and 1.1, 4-H$_A$), 5.21 (1H, ddd, J 17.3, 1.5 and 1.1, 4-H$_B$), 4.73 (1H, app. td, J 7.7 and 6.1, 2-H), 4.37 (1H, d, J 17.0, CH$_A$H$_B$N), 4.33 (1H, d, J 17.0, CH$_A$H$_B$N), 2.78 (1H, d, J 14.6 and 7.8, 1-H$_A$), 2.70 (1H, d, J 14.6 and 7.5, 1-H$_B$), 2.30 (3H, s, isoxazolyl 5-C$_H$$_3$), 2.23 (3H, s, isoxazolyl 5'-C$_H$$_3$), 2.22 (3H, s, isoxazolyl 3-C$_H$$_3$), 2.08 (3H, s, isoxazolyl 3'-C$_H$$_3$); $\delta$$_C$ (75 MHz; CDCl$_3$) 166.3 (isoxazolyl C-5), 163.5
(isoxazolyl C-3), 160.1 (isoxazolyl C-5'), 158.26 (isoxazolyl C-3'), 156.0 (CO), 151.2 (furanyl C-2), 143.2 (furanyl C-5), 136.8 (C-3), 118.5 (C-4), 114.8 (isoxazolyl C-4'), 111.3 (furanyl C-4), 110.3 (isoxazolyl C-4), 108.9 (furanyl C-3), 59.1 (C-2), 42.8 (CH₂N), 25.7 (C-1), 11.6 (isoxazolyl 5-CH₃), 11.3 (isoxazolyl 5'-CH₃), 10.7 (isoxazolyl 3-CH₃), 9.8 (isoxazolyl 3'-CH₃); m/z (ES) 385.1 (100%, [M+H]⁺); HRMS Found: 407.1702 (C₂₀H₂₄N₄O₄, M+Na requires 407.1690).

**Ethyl 2-((3,5-dimethylisoxazol-4-yl)but-3-en-2-yl)-3-(furanyl-2-ylmethyl)ureido)acetate 86**

![Structure of compound 86](image)

The *title compound 86* was prepared from the amine 80 (0.5 g, 2.0 mmol) and ethyl 2-isocyanatoacetate (0.7 mL, 6.1 mmol) in CH₂Cl₂ stirring at 35 °C for 3 days, according to **general procedure B**. The crude product was purified by flash chromatography (99.5:0.5, CH₂Cl₂–MeOH) to give a yellow oil (0.52 g, 70%). Rₚ: 0.45 (95:5, CH₂Cl₂–MeOH); ν̇max/cm⁻¹ (film) 3383, 2982, 1748, 1644, 1526, 149, 1377, 1195, 1023, 933, 746; δH (500 MHz; CDCl₃) 7.37 (1H, d, J 1.8, furanyl 5-H), 6.33 (1H, dd, J 3.2 and 1.8, furanyl 4-H), 6.25 (1H, dd, J 3.2, furanyl 3-H), 5.92 (1H, ddd, J 17.3, 10.5 and 6.3, 3-H), 5.26 (1H, t, J 4.9 NH), 5.19 (1H, d, J 10.5, 4-H₁A), 5.13 (1H, d, J 17.3, 4-H₂B), 4.43 (1H, app. q, J 7.1, 2-H), 4.37 (1H, d, J 17.0, CH₄H₄B₂N), 4.25 (1H, d, J 17.0, CH₄H₄B₂N), 4.20 (2H, q, J 7.2, ethyl 1-H₂), 3.99 (2H, d, J 4.9, CH₂CO), 2.81 (1H, d, J 14.5 and 7.1, 1-H₁A), 2.70 (1H, d, J 14.5 and 8.1, 1-H₂B), 2.27 (3H, s, isoxazolyl 5-CH₃), 2.20 (3H, s, isoxazolyl 3-CH₃), 1.27 (3H, t, J 7.2 ethyl 2-H₂); δC (75 MHz; CDCl₃) 170.9 (CO₂Et), 166.0 (NCON), 159.7 (isoxazolyl C-5), 157.5 (isoxazolyl C-3), 151.0 (furanyl C-2), 142.6 (furanyl C-5), 136.0 (C-3), 117.9 (C-4), 110.7 (furanyl C-4), 110.4 (isoxazolyl C-4), 108.4 (furanyl C-3), 61.5 (ethyl C-1), 59.7 (C-2), 43.2 (CH₂NCONH), 43.0 (CH₂CO₂Et), 25.6 (C-1), 14.3 (ethyl C-2), 11.0 (isoxazolyl 5-CH₃), 10.4 (isoxazolyl 3-CH₃); m/z (ES) 398.1 (100%, [M+Na]⁺); HRMS Found: 376.1882 (C₁₉H₂₅N₃O₅, M+H requires 379.1867).
1-(4-Chlorobenzyl)-3-(3,5-dimethylisoxazol-4-yl)-1-(1-(3,5-dimethylisoxazol-4-yl)but-3-en-2-yl)urea 87

The title compound 87 was prepared from the amine 81 (0.3 g, 1.2 mmol) and 4-isocyanato-3,5-dimethylisoxazolyl (0.4 mL, 3.6 mmol) in CH₂Cl₂ stirring at 35 °C for 1 day, according to general procedure B. The crude product was dissolved in EtOAc (~10 mL), petrol was added until the precipitation was completed and the precipitate was removed by filtration. The filtrate was concentrated under reduced pressure to give a yellow oil (0.3 g, 58%). Rf: 0.4 (95:5, CH₂Cl₂–MeOH); νmax/cm⁻¹ (film) 3286, 2925, 1663, 1643, 1492, 1463, 1242, 1091, 932, 751; δH (500 MHz; CDCl₃) 7.35 (2H, d, J 8.4, phenyl 3- and 5- H), 7.20 (2H, d, J 8.4, phenyl 2- and 6- H), 5.93 (1H, ddd, J 17.1, 10.7 and 6.6, 3- H), 5.44 (1H, br. s., NH), 5.25-5.18 (2H, m, 4-Hₐ and 4-Hₐ'), 4.85 (1H, dt, J 8.0 and 6.8, 2-H), 4.51 (1H, d, J 13.6, benzyl CH₂Hₐ), 4.30 (1H, d, J 13.6, benzyl CHₐHₐ'), 2.75 (1H, dd, J 14.5 and 8.0, 1-Hₐ), 2.67 (1H, dd, J 14.5 and 7.3, 1-Hₐ'), 2.27 (3H, s, isoxazolyl 5-CH₃), 2.21 (3H, s, isoxazolyl 5'-CH₃), 2.06 (3H, s, isoxazolyl 3-CH₃), 1.90 (3H, s, isoxazolyl 3'-CH₃); δc (75 MHz; CDCl₃) 165.9 (isoxazolyl C-5), 163.4 (isoxazolyl C-5'), 159.7 (isoxazolyl C-3), 157.9 (isoxazolyl C-3'), 155.7 (CO), 135.8 (C-3), 135.3 (phenyl C-1), 134.0 (phenyl C-4), 129.3 (phenyl C-3 and C-6), 127.8 (phenyl C-2 and C-6), 119.0 (C-4), 114.1 (isoxazolyl C-4'), 110.3 (isoxazolyl C-4), 58.5 (C-2), 47.9 (benzyl CH₂), 25.6 (C-1), 11.2 (isoxazolyl 5-CH₃), 10.7 (isoxazolyl 3-CH₃), 10.3 (isoxazolyl 5'-CH₃), 9.4 (isoxazolyl 3'-CH₃); m/z (ES) 451.4 (100%, [M+Na]+); HRMS Found 451.1523 (C₂₂H₂₃ClN₄O₃, M+Na requires 451.1507).
The title compound 88 was prepared from the amine 83 (0.3 g, 1.8 mmol) and 4-isocyanato-3,5-dimethylisoxazolyl (0.6 mL, 5.3 mmol) in CH₂Cl₂ stirring at 35 °C for 2 days, according to general procedure B.¹⁰⁰ The crude product was dissolved in EtOAc (~10 mL), petrol was added until the precipitation was completed and the precipitate was removed by filtration. The filtrate was concentrated under reduced pressure to give a yellow oil (0.47 g, 82%). Rf: 0.7 (80:20, petrol–EtOAc); νmax/cm⁻¹ (film) 3295, 2926, 1723, 158, 1635, 1515, 1492, 1248, 1092, 1014, 795; δH (500 MHz; MeOD-d₄) 7.38 (2H, d, J 8.6, phenyl 3- and 5-H), 7.31 (2H, d, J 8.6, phenyl 2- and 6-H), 5.88 (1H, ddd, J 17.1, 10.3 and 5.1, 2-H), 5.25 (1H, d, J 10.3 and 1.4, 3-Hₐ), 5.23 (1H, dd, J 17.1, 3-Hₕ), 4.89 (2H, s, benzyl CH₂), 4.00 (1H, d, J 5.1, 1-H₂), 2.28 (3H, s, isoxazolyl 5-CH₃), 2.15 (3H, s, isoxazolyl 3-CH₃); δC (75 MHz; MeOD-d₄) 165.8 (isoxazolyl C-5), 160.4 (isoxazolyl C-3), 158.8 (CO), 138.1 (phenyl C-1) 134.3 (C-2), 134.2 (phenyl C-4), 134.2 (phenyl C-2 and C-6), 130.1 (phenyl C-3 and C-5), 117.1 (C-3), 116.4 (isoxazolyl C-4), 50.4 (benzyl CH₂), 10.6 (isoxazolyl 5-CH₃), 9.3 (isoxazolyl 3-CH₃); m/z (ES) 342.7 (100%, [M+Na]⁺) HRMS Found 320.1168 (C₁₆H₁₉ClN₃O₂ requires MH⁺ 320.1160).

The title compound 89 was prepared from the amine 81 (0.28 g, 1.0 mmol) and ethylisocyanate (0.23 mL, 3.0 mmol) in CH₂Cl₂ stirring at 35 °C for 1 day, according to general procedure B.¹⁰⁰ The crude product was dissolved in EtOAc (~15 mL), washed
with an aqueous saturated solution of NaHCO₃ (2 x 7.0 mL) and brine (2 x 7.0 ml). The organic phase was dried (MgSO₄) and concentrated under reduced pressure to give a yellow oil (0.3 g, 87%). Rf: 0.3 (94:6, CH₂Cl₂–MeOH); νmax/cm⁻¹ (film) 3357, 2972, 2930, 1633, 1530, 1450, 1293, 114, 929, 746; δH (500 MHz; CDCl₃) 7.30 (2H, d, J 8.3, phenyl 3- and 5- H), 7.12 (2H, d, J 8.3, phenyl 2- and 6-H), 5.88 (1H, ddd, J 17.2, 10.4 and 6.7, 3-H), 5.16 (1H, dd, J 17.2 and 1.0 4-Hₐ), 5.12 (1H, dd, J 10.4 and 1.0 4-Hₐ), 4.55 (1H, app. q, J 7.0, 2-H), 4.38 (1H, d, J 17.4, benzyl CHₐHₐ), 4.19 (1H, br. s., NH), 4.15 (1H, d, J 17.4, benzyl CHₐHₐ), 3.18 (2H, m, ethyl 1-Hₐ2), 2.79 (1H, dd, J 14.3 and 7.1, 1-Hₐ), 2.60 (1H, dd, J 14.3 and 8.1, 1-Hₐ), 2.26 (3H, s, isoxazolyl 5-CH₃), 2.19 (3H, s, isoxazolyl 3-CH₃), 1.00 (3H, t, J 7.2, ethyl 2-Hₐ2); δC (75 MHz; CDCl₃) 165.9 (isoxazolyl C-5), 159.7 (isoxazolyl C-3), 157.9 (CO), 136.2 (phenyl C-1), 136.1 (C-3), 133.4 (phenyl C-4), 129.1 (phenyl C-3 and C-5), 127.9 (phenyl C-2 and C-6), 118.2 (C-4), 110.2 (isoxazolyl C-4), 59.2 (C-2), 48.5 (benzyl CH₂), 35.6 (ethyl C-1), 25.7 (C-1), 15.4 (ethyl C-2), 11.1 (isoxazolyl 5-CH₃), 10.3 (isoxazolyl 3-CH₃); m/z (ES) 384.5 (100%, [M+Na]+); HRMS Found 362.1634 (C₁₉H₂₄ClN₃O₂, M+H requires 362.1629).

1-(4-Chlorobenzyl)-1-(1-(3,5-dimethylisoxazol-4-yl)but-3-en-2-yl)-3-(pyridin-3-yl)urea 90

![Chemical structure of 1-(4-Chlorobenzyl)-1-(1-(3,5-dimethylisoxazol-4-yl)but-3-en-2-yl)-3-(pyridin-3-yl)urea 90](image)

The title compound 90 was prepared from the amine 81 (0.4 g, 1.4 mmol) and 3-isocyanatopyridine (0.5 g, 4.5 mmol) in CH₂Cl₂ stirring at 35 °C for 1 day, according to general procedure B.¹⁰⁰ The crude product was purified by flash chromatography (99:1, CH₂Cl₂–MeOH) to give a yellow oil (0.5 g, 80%). Rf: 0.6 (95:5, CH₂Cl₂–MeOH); νmax/cm⁻¹ (film) 3588, 2923, 1731, 1655, 1533, 1483, 1421, 1227, 1089, 931, 801; δH (500 MHz; CDCl₃) 8.26 (1H, d, J 3.9, py 4-H), 8.16 (1H, s, py 2-H), 7.86-7.84 (1H, m, py 6-H), 7.30 (2H, d, J 8.3, phenyl 3- and 5- H), 7.29-7.26 (1H, m, py 5-H)ᵃ.

ᵃ Signal overlapping with δH 7.3
7.12 (2H, d, J 8.3, phenyl 2- and 6-H), 5.88 (1H, ddd, J 17.2, 10.4 and 6.7, 3-H), 5.16 (1H, dd, J 17.2 and 1.0, 4-H_A), 5.12 (1H, dd, J 10.4 and 1.0, 4-H_B), 4.55 (1H, app. q, J 7.0, 2-H), 4.38 (1H, d, J 17.4, benzyl CH_AH_B), 4.19 (1H, br. s., NH), 4.15 (1H, d, J 17.4, benzyl CH_AH_B), 2.79 (1H, dd, J 14.2 and 7.1, 1-H_A), 2.60 (1H, dd, J 14.2 and 8.1, 1-H_B), 2.26 (3H, s, isoxazolyl 5-CH_3), 2.19 (3H, s, isoxazolyl 3-CH_3); δ_C (75 MHz; CDCl_3) 166.2 (isoxazolyl C-5), 162.9 (isoxazolyl C-3), 159.6 (C_O), 155.14 (py C), 144.65, 141.65, (py CH), 138.6 (phenyl C-1), 135.9 (C-3), 135.2 (phenyl C-4), 129.5 (phenyl C-3 and C-5), 128.1 (phenyl C-2 and C-6), 127.3, 123.6 (py CH), 119.1 (C-4), 109.8 (isoxazolyl C-4), 59.6 (C-2), 49.3 (benzyl CH_2), 25.5 (C-1), 11.2 (isoxazolyl 5-CH_3), 10.3 (isoxazolyl 3-CH_3); m/z (ES) 411.2 (100%, [M+H]^+); HRMS Found 411.1614 (C_{22}H_{23}ClN_4O_2, M+H requires 411.1631).

1-(4-chlorobenzyl)-3-(3,5-dimethylisoxazol-4-yl)-1-(1-phenylbut-3-en-2-yl)urea 91

The title compound 91 was prepared from the amine 82 (0.35 g, 1.3 mmol) and 4-isocyanato-3,5-dimethylisoxazolyl (0.4 mL, 3.9 mmol) in CH_2Cl_2 stirring at 35 °C for 1 day, according to general procedure B. The crude product was purified by flash chromatography (70:30, petrol–EtOAc) to give a yellow oil (0.43 g, 81%). R_f: 0.4 (50:50, petrol–EtOAc); ν_{max}/cm^{-1} (film) 3311, 1367, 1494, 1465, 1245, 1089, 929, 748; δ_H (500 MHz; CDCl_3) 7.33-7.27 (5H, m, Ar), 7.23 (2H, dd, J 8.0 and 6.7, Ar), 7.13 (2H, d, J 8.5, Ar), 6.0 (1H, ddd, J 17.3, 10.5, 5.6, 3-H), 5.31 (1H, ddd, J 17.3 4-H_A), 5.25 (1H, ddd, J 10.5, 4-H_B), 5.00 (1H, app. dt, J 7.8, 5.5, 2-H), 4.51 (1H, d, J 17.1 benzyl CH_AH_B), 4.11 (1H, d, J 17.1 benzyl CH_AH_B), 3.08 (1H, dd, J 12.7 and 7.8, 1-H_A), 3.04 (1H, dd, J 12.7 and 5.5, 1-H_B), 2.08 (3H, s, isoxazolyl 5-CH_3), 1.93 (3H, s, isoxazolyl 3-CH_3); δ_C (75 MHz; CDCl_3) 163.2 (isoxazolyl 5-C), 157.9 (isoxazolyl C-3), 155.8 (C_O), 137.8 (C_Ar), 137.0 (C-3), 136.0, 133.7 (Ar C), 129.2, 129.1, 128.6, 128.5, 126.7 (Ar CH), 117.7 (C-4), 114.3 (isoxazolyl C-4), 60.4 (C-2), 48.3 (benzyl CH_2), 38.0 (C-1).
14.2 (isoxazolyl 5-CH$_3$), 10.8 (isoxazolyl 3-CH$_3$); HRMS Found: 410.1637 (C$_{23}$H$_{24}$ClN$_4$O$_2$, M+H requires: 410.1629).

1-(1-(3,5-dimethylisoxazol-4-yl)but-3-en-2-yl)-3-ethyl-1-isobutylurea 92$^{100}$

The title compound 92 was prepared from the amine 84 (80 mg, 0.4 mmol) and ethylisocyanate (0.1 mL, 1.2 mmol) in CH$_2$Cl$_2$ stirring at 35 °C for 2 days, according to general procedure B.$^{100}$ The crude product was purified by flash chromatography (97.5:2.5, CH$_2$Cl$_2$–MeOH) to give a yellow oil (80 mg, 68%). $R_p$: 0.4 (95:5, CH$_2$Cl$_2$–MeOH); $\nu_{\text{max}}$/cm$^{-1}$ (film) 3372, 2963, 1633, 1531, 1455, 1325, 1195, 1000, 919, 744; $\delta_{\text{H}}$ (500 MHz; CDCl$_3$) 6.07 (1H, ddd, $J$ 17.4, 10.5 and 5.4, 3-H), 5.14 (1H, dd, $J$ 10.5 and 1.2, 4-H$_A$), 5.08 (1H, dd, $J$ 17.4, 1.2, 4-H$_B$), 4.32 (1H, t, $J$ 4.7, NH), 4.07-4.01 (1H, m, isopropyl CH), 3.71-3.67 (1H, m, 2-H), 3.13 (2H, dd, $J$ 13.7 and 7.0, propyl 1-H$_2$), 2.90 (1H, dd, $J$ 14.6 and 8.3, 1-H$_A$), 2.76 (1H, dd, $J$ 14.6 and 7.0, 1-H$_B$), 2.23 (3H, s, isoxazolyl 5-CH$_3$), 2.16 (3H, s, isoxazolyl 3-CH$_3$), 1.47-1.43 (2H, m, $J$ 6.1, propyl 2-H$_2$), 1.03 (3H, d, $J$ 6.7, isopropyl CH$_3$A), 0.86 (3H, t, $J$ 7.4, propyl 3-H$_3$), 0.97 (3H, d, $J$ 6.27, isopropyl CH$_3$B); $\delta_{\text{C}}$ (75 MHz; CDCl$_3$) 166.3 (isoxazolyl C-5), 159.7 (isoxazolyl C-3), 157.3 (CO), 138.7 (C-3), 116.5 (C-4), 111.2 (isoxazolyl C-4), 55.9 (C-2), 47.2 (isopropyl C-1), 42.5 (propyl C-1), 25.3 (C-1), 23.4 (propyl C-2), 21.3 (isopropyl CH$_3$A), 21.1 (isopropyl CH$_3$B), 11.5 (isoxazolyl 5-CH$_3$), 11.0 (propyl C-3), 10.3 (isoxazolyl 3-CH$_3$); m/z (ES) 294.1 (100%, [M+H]$^+$); HRMS Found: 316.1987 (C$_{16}$H$_{27}$N$_3$O$_2$, M+Na requires 316.1995).
(4S*, 5S*)-4-(Azidomethyl)-3-(3,5-dimethylisoxazol-4-yl)-5-((3,5-dimethylisoxazol-4-yl) methyl)-1-(furan-2-ylmethyl)imidazolidin-2-one 93124,137

The title compound 93 was prepared from the allyl urea 85 (1.6 mmol) according to general procedure F. The crude product was purified by flash chromatography (98.5:1.5, CH2Cl2–MeOH) to give a yellow oil (108 mg, 16% over two steps). Rf: 0.6 (94:6, CH2Cl2–MeOH); νmax/cm−1 (film) 2932, 2109, 1711, 1441, 1254, 1011, 750; δH (500 MHz; CDCl3) 7.43 (1H, dd, J 1.9 and 0.9, furanyl 5-H), 6.39 (1H, dd, J 3.2 and 1.9, furanyl 4-H), 6.29 (1H, dd, J 3.2 and 0.9, furanyl 3-H), 4.91 (1H, d, J 15.8, NCHAHB), 4.05 (1H, d, J 15.8, NCHAHB), 3.55 (1H, ddd, J 7.7, 5.4 and 4.3, 5-H), 3.46 (1H, ddd, J 5.8, 4.3 and 3.6, 4-H), 3.13 (1H, dd, J 12.5 and 5.8, 4-CHAHB), 3.05 (1H, dd, J 12.5 and 3.6, 4-CHAHB), 2.82 (1H, dd, J 14.8 and 5.4, 5-CHAHB), 2.55 (1H, dd, J 14.8 and 7.7, 5-CHAHB), 2.34 (3H, s, isoxazolyl 5-CH3), 2.31 (3H, s, isoxazolyl 5-CH3), 2.15 (3H, s, isoxazolyl 3′-CH3); δC (75 MHz; CDCl3) 166.5 (isoxazolyl C-5), 166.3 (CO), 159.3 (isoxazolyl C-3), 158.1 (furanyl C-2), 157.0 (isoxazolyl C-5′), 149.7 (isoxazolyl C-3′), 142.8 (furanyl C-5), 113.2 (isoxazolyl C-4′), 110.7 (furanyl C-4), 109.2 (furanyl C-3), 108.4 (isoxazolyl C-4), 59.4 (C-5), 55.1 (C-4), 52.0 (5-CH2), 39.5 (CH2NCO), 26.5 (4-CH2), 11.3 (isoxazolyl 5-CH3), 11.1 (isoxazolyl 5′-CH3), 10.4 (isoxazolyl 3-CH3), 9.4 (isoxazolyl 3′-CH3); m/z (ES) 448.2 (100%, [M+Na]+); HRMS Found: 448.1720 (C20H23N7O4, M+Na requires 448.1704).
Ethyl 2-((4S*, 5S*)-5-(azidomethyl)-4-((3,5-dimethylisoxazol-4-yl)methyl)-3-(furan-2-yl methyl)-2-oxoimidazolidin-1-yl)acetate 95

The title compound 95 was prepared from the allyl urea 86 (1.4 mmol) according to general procedure F. The crude product was purified by flash chromatography (99.5:0.5, CH2Cl2–MeOH) to give a yellow oil (116 mg, 20% over two steps). Rf: 0.6 (94:6, CH2Cl2–MeOH); νmax/cm−1 (film) 2934, 2107, 1742, 1704, 1457, 1202, 1015, 754; δH (500 MHz; CDCl3, 325 K) 7.37 (1H, dd, J 1.8 and 0.8, furanyl 5-H), 6.34 (1H, dd, J 3.2 and 0.8, furanyl 4-H), 6.23 (1H, dd, J 3.2 and 0.8, furanyl 3-H), 4.85 (1H, d, J 15.8, 3-CH2A-HB), 4.43 (1H, d, J 18.1, CH3HB-CO2Et), 4.20 (2H, qd, J 7.1 and 3.3 ethyl 1-H2), 4.04 (1H, d, J 15.8, 3-CH2A-HB), 3.69 (1H, d, J 18.1, CH3HB-CO2Et), 3.37 (1H, app. td, J 5.3 and 4.1, 4-H), 3.29 (1H, ddd, J 9.1, 5.1 and 4.1, 5-H), 3.09 (1H, dd, J 12.6 and 5.2, 4-CH2A-HB), 3.04 (1H, dd, J 12.6 and 5.6, 4-CH3HB), 2.81 (1H, dd, J 14.4 and 5.1, 5-CH3HB), 2.54 (1H, dd, J 14.4 and 9.1, 5-CH3HB), 2.32 (3H, s, isoxazolyl 5-CH3), 2.17 (3H, s, isoxazolyl 3-CH3), 1.29 (3H, t, J 7.1, ethyl 2-H3); δC (75 MHz; CDCl3) 169.6 (CO2Et), 166.5 (isoxazolyl C-5), 159.4 (isoxazolyl C-3), 158.5 (NCON), 150.1 (furanyl C-2), 142.6 (furanyl C-5), 110.6 (furanyl C-4), 108.8 (furanyl C-3), 108.6 (isoxazolyl C-4), 61.4 (ethyl C-1), 57.6 (C-4), 55.5 (C-5), 51.4 (4-CH2), 43.5 (CH2CO2Et), 38.9 (3-CH2), 26.0 (5-CH2), 14.2 (ethyl C-2), 11.1 (isoxazolyl 5-CH3), 10.3 (isoxazolyl 3-CH3); m/z (ES) 439.4 (100%, [M+Na]+). HRMS Found: 439.2204 (C19H24N6O5, M+Na requires 439.2197).
(4S*, 5S*)-4-(Azidomethyl)-1-(4-chlorobenzyl)-3-(3,5-dimethylisoxazol-4-yl)-5-((3,5-dimethyl isoxazol-4-yl)methyl)imidazolidin-2-one 98\textsuperscript{124,137}

The title compound 98 was prepared from the allyl urea 87 (0.7 mmol) according to general procedure F.\textsuperscript{124,137} The crude product was purified by flash chromatography (99.5:0.5, CH\textsubscript{2}Cl\textsubscript{2}–MeOH) to give a yellow oil (65 mg, 20% over two steps). R\textsubscript{f}: 0.3 (95:5, CH\textsubscript{2}Cl\textsubscript{2}–MeOH); \nu\textsubscript{max}/\text{cm}\textsuperscript{-1} (film) 2926, 2109, 1688, 1492, 1440, 1424, 1218, 1200, 1062, 733; \delta\textsubscript{H} (500 MHz; CDCl\textsubscript{3}) 7.37 (2H, d, J 8.3, phenyl 3- and 5-H), 7.26 (2H, d, J 8.3, phenyl 2- and 6-H), 5.03 (1H, d, J 14.9, benzyl CH\textsubscript{A}H\textsubscript{B}), 4.12 (1H, d, J 14.9, benzyl CH\textsubscript{A}H\textsubscript{B}), 4.11 (1H, app. q, J 4.5, 5-H), 3.53 (1H, app. dt, J 8.6 and 5.0, 4-H), 3.32 (1H, dd, J 13.5 and 4.3, 5-CH\textsubscript{A}H\textsubscript{B}), 3.04 (1H, dd, J 13.5 and 4.1, 5-CH\textsubscript{A}H\textsubscript{B}), 2.72 (1H, dd, J 14.8 and 5.2, 4-CH\textsubscript{A}H\textsubscript{B}), 2.44 (1H, dd, J 14.8 and 8.6, 4-CH\textsubscript{A}H\textsubscript{B}), 2.25 (3H, s, isoxazolyl 5-CH\textsubscript{3}), 2.24 (3H, s, isoxazolyl 5′-CH\textsubscript{3}), 2.15 (3H, s, isoxazolyl 3-CH\textsubscript{3}), 2.09 (3H, s, isoxazolyl 3′-CH\textsubscript{3}); \delta\textsubscript{C} (75 MHz; CDCl\textsubscript{3}) 166.3 (isoxazolyl C-5), 158.9 (isoxazolyl C-3), 156.6 (isoxazolyl C-5′), 153.1 (CO), 134.3 (phenyl C-4), 134.1 (phenyl C-1), 129.6 (phenyl C-3 and C-5), 129.1 (phenyl C-2 and C-6), 107.8 (isoxazolyl C-4), 79.4 (C-5), 56.9 (C-4), 52.5 (5-CH\textsubscript{2}), 46.8 (benzyl CH\textsubscript{2}), 25.8 (4-CH\textsubscript{2}), 11.2 (isoxazolyl 5-CH\textsubscript{3}), 11.1 (isoxazolyl 5′-CH\textsubscript{3}), 10.3 (isoxazolyl 3-CH\textsubscript{3}), 9.9 (isoxazolyl 3′-CH\textsubscript{3}), isoxazolyl C-3′ and isoxazolyl C-4 not observed; m/z (ES) 470.4 (100%, [M+H]\textsuperscript{+}); HRMS Found: 470.1709 (C\textsubscript{22}H\textsubscript{24}ClN\textsubscript{7}O\textsubscript{3}, M+H requires 470.1702).
4-(Azidomethyl)-1-(4-chlorobenzyl)-3-(3,5-dimethylisoxazol-4-yl)imidazolidin-one \textsuperscript{100}\textsuperscript{124,137}

The title compound \textsuperscript{100} was prepared from the allyl urea \textsuperscript{88} (1.5 mmol) according to general procedure \textsuperscript{F}.\textsuperscript{124,137} The crude product was purified by flash chromatography (20:80, hexane–EtOAc) to give a yellow oil (97 mg, 18% over two steps). \textit{R}_F: 0.7 (96.5:3.5, CH\textsubscript{2}Cl\textsubscript{2}–MeOH); \textit{v}_{\text{max}}/cm\textsuperscript{-1} (film) 3404, 3226, 2107, 1682, 1443, 1254, 1045, 814; \textit{δ}_H (500 MHz; CDCl\textsubscript{3}) 7.35 (2H, d, \textit{J} 8.5, phenyl 3- and 5-\textit{H}), 7.31 (2H, d, \textit{J} 8.5, phenyl 2- and 6-\textit{H}), 4.65 (1H, app. ddt, \textit{J} 8.5, 6.3 and 4.4, 4-\textit{H}), 4.54 (2H, s, benzyl \textit{CH}_2), 3.58 (1H, dd, \textit{J} 13.3 and 4.3, 4-CH\textsubscript{A}H\textsubscript{B}), 3.50 (1H, app. t, \textit{J} 8.5, 5-H\textsubscript{A}), 3.42 (1H, dd, \textit{J} 13.3 and 4.5, 4-CH\textsubscript{A}H\textsubscript{B}), 3.27 (1H, dd, \textit{J} 8.6 and 6.3, 5-H\textsubscript{B}), 2.26 (3H, s, isoxazolyl 5-\textit{CH}\textsubscript{3}), 2.17 (3H, s, isoxazolyl 3-\textit{CH}\textsubscript{3}); \textit{δ}_C (75 MHz; CDCl\textsubscript{3}) 156.8 (isoxazolyl C-5), 156.4 (isoxazolyl C-3), 153.2 (CO), 134.6 (phenyl C-1), 133.8 (phenyl C-4), 129.5 (phenyl C-3 and C-5), 129.0 (phenyl C-2 and C-6), 122.9 (isoxazolyl C-4), 74.0 (C-4), 52.8 (benzyl CH\textsubscript{2}), 48.4 (C-5), 47.6 (4-CH\textsubscript{2}), 11.0 (isoxazolyl 3-CH\textsubscript{3}), 9.9 (isoxazolyl 5-CH\textsubscript{3}); \textit{m/z} (ES) 361.3 (100\%, [M+H]\textsuperscript{+}); HRMS Found: 361.1180 (C\textsubscript{16}H\textsubscript{17}ClN\textsubscript{6}O\textsubscript{2}, M+H requires 361.1179).
(4S*, 5S*)-4-(azidomethyl)-1-(4-chlorobenzyl)-5-((3,5-dimethylisoxazol-4-yl)methyl)-3-ethyl imidazolidin-2-one 102

The title compound 102 was prepared from the allyl urea 89 (0.9 mmol) according to general procedure F. The crude product was purified by flash chromatography (99.5:0.5, CH₂Cl₂–MeOH) to give a yellow oil (25 mg, 7% over two steps). Rᵗ: 0.8 (96:4, CH₂Cl₂–MeOH); νₑₑₑ/cm⁻¹ (film) 3456, 2930, 2109, 1695, 1087, 936; δₓ (500 MHz; CDCl₃) 7.30 (2H, dd, J 8.2, phenyl 3- and 5-H), 6.92 (2H, d, J 8.2, phenyl 2- and 6-H), 4.30 (1H, app. td, J 4.7 and 2.6, 5-H), 4.11 (1H, d, J 14.7, benzyl CH₄H₅), 4.04 (1H, d, J 14.7, benzyl CH₄H₅), 3.64 (1H, dd J 12.7 and 2.6, 5-CH₄H₅), 3.42 (1H, m, 4-H), 3.39 (1H, dd, J 12.7 and 6.1, 5-CH₄H₅), 3.18-3.12 (3H, m, 4-CH₄H₅ and ethyl 1-H₂), 2.75 (1H, dd, J 14.5 and 4.5, 4-CH₄H₅), 2.21 (3H, s, isoxazolyl 5-CH₃), 2.18 (3H, s, isoxazolyl 3-CH₃) 1.00 (3H, t, J 6.7, ethyl 2-H₃); δₓ (75 MHz; CDCl₃) 166.2 (isoxazolyl C-5), 159.6 (isoxazolyl C-3), 157.7 (CO), 134.7 (phenyl C-4), 134.1 (phenyl C-1), 129.4 (phenyl C-3 and C-5), 127.9 (phenyl C-2 and C-6), 110.5 (isoxazolyl C-4), 64.9 (C-5), 53.0 (C-4), 39.7 (benzyl CH₂), 35.6 (5-CH₂), 29.7 (ethyl C-1), 22.5 (4-CH₂), 15.3 (ethyl C-3), 10.9 (isoxazolyl 5-CH₃), 10.2 (isoxazolyl 3-CH₃); m/z (ES) 403.2 (100%, [M+H]+); HRMS Found: 403.1650 (C₁₉H₂₃ClN₂O₂, M+H requires 403.1649).
(4S*, 5S*)-4-(azidomethyl)-1-(4-chlorobenzyl)-5-((3,5-dimethylisoxazol-4-yl)methyl)-3-(pyridin-3-yl)imidazolidin-2-one 103

The title compound 103 was prepared from the allyl urea 90 (1.1 mmol) according to general procedure F. The crude product was purified by flash chromatography (99.2:0.8, CH$_2$Cl$_2$–MeOH) to give a yellow oil (89 mg, 18% over two steps). $R_F$: 0.2 (96:4, CH$_2$Cl$_2$–MeOH); $\nu_{\text{max}}$/cm$^{-1}$ (film) 2930, 2103, 1698, 1485, 1243, 1150, 1080, 801; $\delta_H$ (500 MHz; CDCl$_3$) 8.49 (1H, app. s, py 4-H), 8.41 (1H, s, py 2-H), 8.24 (1H, app. d, $J$ 3.9, py 6-H), 7.38 (2H, dd, $J$ 8.4, phenyl 3- and 5-H), 7.36-7.33 (1H, app. m, py 5-H), 7.28 (2H, d, $J$ 8.4, phenyl 2- and 6-H), 5.11 (1H, d, $J$ 15.3, benzyl CH$_A$H$_B$), 4.19 (1H, app. dd, $J$ 5.1 and 4.3, 5-H)$^a$, 4.15 (1H, d, $J$ 15.3, benzyl CH$_A$H$_B$), 3.99 (1H, app. dt, $J$ 9.0 and 5.1, 4-H), 3.35 (1H, dd, $J$ 13.4 and 4.1, 5-CH$_A$H$_B$), 3.01 (1H, dd, $J$ 13.4 and 4.2, 5-CH$_A$H$_B$), 2.76 (1H, dd, $J$ 14.7 and 5.3, 4-CH$_A$H$_B$), 2.44 (1H, dd, $J$ 14.7 and 9.0, 4-CH$_A$H$_B$), 2.25 (3H, s, isoxazolyl 5-CH$_3$), 2.07 (3H, s, isoxazolyl 3-CH$_3$); $\delta_C$ (75 MHz; CDCl$_3$) 166.3 (isoxazolyl C-5), 160.3 (isoxazolyl C-3), 159.0 (CO), 154.4, 145.7, 143.5 (py CH), 140.6 (py C-1) 134.2 (phenyl C-4), 130.2 (phenyl C-1), 129.7 (phenyl C-3 and C-5), 129.6 (phenyl C-2 and C-6), 107.9 (isoxazolyl C-4), 79.4 (C-5), 56.4 (C-4), 52.6 (5-CH$_2$), 46.8 (benzyl CH$_2$), 25.9 (4-CH$_2$), 11.2 (isoxazolyl 5-CH$_3$), 10.3 (isoxazolyl 3-CH$_3$); m/z (ES) 452.2 (100%, [M+H]$^+$); HRMS Found: 452.1601 (C$_{22}$H$_{22}$ClN$_7$O$_2$, M+H requires 452.1596).

$^a$ Overlapping with signal at $\delta_H$ 4.15
(4S*, 5S*)-4-(azidomethyl)-5-((3,5-dimethylisoxazol-4-yl)methyl)-3-isopropyl-1-propyl imidazolidin-2-one 107\textsuperscript{124,137}

The title compound 107 was prepared from the allyl urea 92 (0.3 mmol) according to general procedure F\textsuperscript{124,137}. The crude product was purified by flash chromatography (50:50, petrol–EtOAc) to give a yellow oil (13 mg, 13% over two steps). R\textsubscript{F}: 0.1 (99:1, CH\textsubscript{2}Cl\textsubscript{2}–MeOH); \textit{v}\textsubscript{max}/cm\textsuperscript{-1} (film) 2962, 2930, 2106, 1699, 1635, 1455, 1198, 1035, 931, \textit{\delta}\textsubscript{H} (500 MHz; CDCl\textsubscript{3}) 4.12 (1H, hept, J 6.9, isopropyl CH), 3.97 (1H, app. td, J 5.2 and 3.0, 5-H), 3.55 (1H, app. dt J 10.5 and 3.5, 4-H), 3.20-3.05 (2H, m, propyl 1-H\textsubscript{2}), 2.77 (1H, dd, J 14.6 and 3.9, 4-CH\textsubscript{A}H\textsubscript{B}), 2.77 (1H, dd, J 14.6 and 10.5, 4-CH\textsubscript{A}H\textsubscript{B}), 2.37 (3H, s, isoxazolyl 5-CH\textsubscript{3}), 2.26 (3H, s, isoxazolyl 3-CH\textsubscript{3}), 1.54-1.47 (2H, m, propyl 2-H\textsubscript{2}), 1.31 (1H, d, J 6.8, isopropyl CH\textsubscript{3A}), 1.26 (1H, d, J 6.9, isopropyl CH\textsubscript{3B}), 0.9 (3H, t, J 7.4, propyl-3H\textsubscript{3}); \textit{\delta}\textsubscript{C} (75 MHz; CDCl\textsubscript{3}) 170.3 (isoxazolyl C-5), 166.7 (isoxazolyl C-3), 159.2 (CO), 108.9 (isoxazolyl C-4), 77.8 (C-5), 55.7 (C-4), 53.1 (5-CH\textsubscript{2}), 48.3 (propyl C-1), 46.0 (isopropyl CH), 28.3 (4-CH\textsubscript{2}) 25.3 (propyl C-2), 22.0, 19.3 (isopropyl CH\textsubscript{3A} and CH\textsubscript{3B}) 15.6 (propyl C-3), 11.3 (isoxazolyl 5-CH\textsubscript{3}), 10.6 (isoxazolyl 3-CH\textsubscript{3}); m/z (ES) 335.0 (100%, [M+H]\textsuperscript{+}); HRMS Found: 335.2202 (C\textsubscript{16}H\textsubscript{28}N\textsubscript{6}O\textsubscript{2}, M+H requires 335.2190).
(4S*, 5S*)-4-(Aminomethyl)-3-(3,5-dimethylisoxazol-4-yl)-5-((3,5-dimethylisoxazol-4-yl) methyl)-1-(furan-2-ylmethyl)imidazolidin-2-one 94

The title compound 94 was prepared from the azide 93 (0.1 mmol) and trimethylphosphine (solution 1 M in THF, 0.16 mL, 0.16 mmol), according to general procedure G. The crude product was purified by HPLC (5:95, MeCN–H₂O in 45 minutes) to give a yellow oil (8.0 mg, 18%). Rf: 0.3 (95:5, CH₂Cl₂–MeOH); δH (500 MHz; CDCl₃) 7.43 (1H, d, J 1.9, furanyl 5-H), 6.33 (1H, dd, J 3.1 and 1.9, furanyl 4-H), 6.27 (1H, d, J 3.1 furanyl 3-H), 4.70 (1H, d, J 15.8, 3-CH₃H₅), 4.10 (1H, d, J 15.8, 3-CH₃H₅), 3.60 (1H, app. td, J 5.6 and 3.8, 5-H), 3.31 (1H, app. dt, J 7.6 and 3.6, 4-H), 2.73 (1H, app. d, J 5.8, 5-CH₃H₅), 2.72 (1H, app. d, J 5.4, 5-CH₃H₅), 2.49 (1H, dd, J 13.1 and 3.4, 4-CH₃H₅), 2.35 (1H, dd, J 13.1 and 7.6, 4-CH₃H₅), 2.29 (3H, s, isoxazolyl 5-CH₃), 2.11 (6H, s, isoxazolyl 5′-CH₃ and isoxazolyl 3-CH₃), 1.97 (3H, s, isoxazolyl 3′-CH₃), NH₂ not observed; δC (75 MHz; CDCl₃) 168.6 (isoxazolyl C-5), 168.2 (isoxazolyl C-5′), 161.5 (CO) 160.1 (isoxazolyl C-3), 159.8 (isoxazolyl C-3′), 151.5 (furanyl C-2), 144.2 (furanyl C-5), 111.7 (isoxazolyl C-4), 111.6 (furanyl C-4), 110.7 (isoxazolyl C-4′), 110.2 (furanyl C-3), 63.5 (C-5), 57.3 (C-4), 44.2 (5-CH₃), 40.3 (3-CH₂), 26.7 (4-CH₂), 11.3 (isoxazolyl 5-CH₃), 11.0 (isoxazolyl 5′-CH₃), 10.5 (isoxazolyl 3-CH₃), 9.7 (isoxazolyl 3′-CH₃); m/z (ES) 400.32 (100%, [M+H]+); HRMS Found: 400.1988 (C₂₀H₂₅N₅O₄ M+H requires 400.1979).

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a Insufficient quantity to perform the I.R. analysis
b Signal overlapping with the one at 2.73
The title compound 97 was prepared from the azide 95 (0.07 mmol) and trimethylphospine (solution 1M in THF, 0.1 mL, 0.1 mmol), according to general procedure G. The crude product was purified by HPLC (5:95, MeCN–H₂O in 45 minutes) to give a yellow oil (2.0 mg, 8%). \( R_f \): 0.2 (94:6, CH₂Cl₂–MeOH); \( \delta_H \) (500 MHz; CDCl₃) 7.39 (1H, dd, \( J \) 1.2 and 0.5, furanyl 5-H), 6.36 (1H, dd, \( J \) 2.8 and 1.2, furanyl 4-H), 6.27 (1H, dd, \( J \) 2.8 and 0.5, furanyl 3-H), 4.88 (1H, d, \( J \) 15.7, 2-CH₂A-HB), 4.49 (1H, d, \( J \) 18.1, 5-HA), 4.03 (1H, d, \( J \) 15.7 2-CH₂A-HB), 3.73 (1H, d, \( J \) 18.1, 5-HB), 3.36 (1H, app. dt \( J \) 10.6 and 3.7, 9-H), 3.12 (1H, app. dt, \( J \) 9.3, and 3.5, 1-H), 3.07 (1H, app. t, \( J \) 11.2, 8-HA), 2.90 (1H, dd, \( J \) 11.5 and 4.5, 8-HB), 2.85 (1H, dd, \( J \) 14.6, 5.3, 1-CH₂A-HB), 2.45 (1H, dd, \( J \) 14.6, 9.3, 1-CH₂A-HB), 2.33 (3H, s, isoxazolyl 5-CH₃), 2.17 (3H, s, isoxazolyl 3-CH₃), NH not observed; \( \delta_C \) (75 MHz; MeOD-\( d^4 \))\(^b\) 168.9 (CO₂NH), 166.9 (isoxazolyl C-5), 160.1 (isoxazolyl C-3), 159.1 (NCON), 150.9 (furanyl C-2), 144.3 (furanyl C-5), 111.6 (furanyl C-4), 110.3 (furanyl C-3), 109.0 (isoxazolyl C-4), 57.6 (ethyl C-1), 54.6 (C-9), 45.2 (1-CH₂), 44.8 (C-5), 39.7 (2-CH₂), 25.7 (C-8), 17.4 (isoxazolyl 5-CH₃), 16.9 (isoxazolyl 3-CH₃); m/z (ES) 367.1 (100%, [M+Na]+); HRMS Found: 367.1379 (C\(_{17}\)H\(_{20}\)N\(_4\)O\(_4\), M+Na requires 367.1377).

\(^a\) Quantity was not sufficient to perform the I.R. analysis
\(^b\) Quaternary carbons assigned through HMBC experiment
The title compound 98 was prepared from the azide 99 (0.11 mmol) and trimethylphospine (solution 1 M in THF, 0.17 mL, 0.17 mmol), according to general procedure G. The crude product was purified by mass directed chromatography (5:95, MeOH–H2O) to give a yellow oil (4.6 mg, 9%). RF: 0.2 (95:5, CH2Cl2–MeOH); δH (500 MHz; MeOD-d4) 7.55-7.46 (4H, m, phenyl 3-, 5-, 2- and 6-H), 5.01 (1H, d, J 15.5, benzyl CHAHB), 4.61 (app. dt, J 8.8 and 4.3, 5-H), 4.47 (1H, d, J 15.5, benzyl CHAHB), 3.79 (1H, app. dt J 6.4 and 4.9, 4-H), 3.02 (1H, dd, J 14.0 and 8.8, 5-CHAHB), 2.99 (1H, d, J 14.0 and 4.1, 5-CHAHB), 2.95 (1H, dd, J 15.1 and 4.9, 4-CHAHB), 2.50 (1H, dd, J 15.1 and 6.4, 4-CHAHB), 2.41 (3H, s, isoxazolyl 5-CH3), 2.26 (3H, s, isoxazolyl 5′-CH3), 2.23 (3H, s, isoxazolyl 3′-CH3), 2.15 (3H, s, isoxazolyl 3′-CH3), NH2 not observed; δC (75 MHz; MeOD-d4) 168.8 (isoxazolyl C-5), 161.1 (isoxazolyl C-3), 158.2 (isoxazolyl C-5′), 158.1 (CO), 154.4 (isoxazolyl C-3′), 135.3 (phenyl C-4), 131.0 (phenyl C-2 and C-6), 130.5 (phenyl C-1), 130.3 (phenyl C-3 and C-5), 114.2 (isoxazolyl C-4), 109.4 (isoxazolyl C-4′), 79.8 (C-5), 60.3 (C-4), 47.4 (benzyl CH2), 43.0 (5-CH2), 25.2 (4-CH2); 11.3 (isoxazolyl 5-CH3), 10.9 (isoxazolyl 5′-CH3), 10.4 (isoxazolyl 3-CH3), 9.9 (isoxazolyl 3′-CH3); m/z (ES) 444.3 (100%, [M+H]+); HRMS Found: 444.1800 (C22H26ClN3O3, M+H requires 444.1797).

* Insufficient quantity to perform the I.R. analysis
4-(Aminomethyl)-1-(4-chlorobenzyl)-3-(3,5-dimethylisoxazol-4-yl)imidazolidin-2-one 101

The title compound 101 was prepared from the azide 100 (0.27 mmol) and trimethylphospine (solution 1 M in THF, 0.41 mL, 0.41 mmol), according to general procedure G138. The crude product was purified by HPLC (5:95, MeCN–H2O in 45 minutes) to give a yellow oil (2.6 mg, 7%). Rf: 0.4 (95:5, CH2Cl2–MeOH); δH (500 MHz; DMSO-d6) 7.44 (2H, d, J 8.5, phenyl 3- and 5-H), 7.41 (2H, d, J 8.5, phenyl 2- and 6-H), 4.84 (1H, app. ddt, J 8.5, 7.7 and 4.0, 4-H), 4.52 (1H, d, J 15.3, benzyl CHA, B), 4.47 (1H, d, J 15.3, benzyl CHA, B), 3.59 (1H, app. t, J 8.5, 4-CH2A), 3.25 (1H, app d, J 8.5, 4-CH2A), 3.16 (1H, dd, J 13.8 and 4.0, 5-HA), 3.08 (1H, dd, J 13.8 and 7.7, 5-HA), 2.18 (3H, s, isoxazolyl 5-CH3), 2.03 (3H, s, isoxazolyl 3-CH3), NH2 not observed; δC (75 MHz; DMSO-d6) 156.2 (isoxazolyl C-5), 155.4 (isoxazolyl C-3), 153.0 (CO), 135.6 (phenyl C-4), 132.0 (phenyl C-1), 129.9 (phenyl C-2 and C-6), 128.5 (phenyl C-3 and C5), 95.9 (isoxazolyl C-4), 73.2 (C-4), 48.0 (4-CH2), 47.4 (benzyl CH2), 40.1 (C-5), 10.9 (isoxazolyl 5-CH3), 9.7 (isoxazolyl 3-CH3); m/z (ES) 335.1 (100%, [M+H]+); HRMS Found: 669.2457 (C16H19ClN4O2, M2+H requires 669.2465).

\(^a\) Insufficient quantity to perform the I.R. analysis

\(^b\) Signal masked by solvent
(4S*, 5S*)-4-(Aminomethyl)-1-(4-chlorobenzyl)-5-((3,5-dimethylisoxazol-4-yl)methyl)-3-(pyridin-3-yl)imidazolidin-2-one 104

The title compound 104 was prepared from the azide 103 (0.23 mmol) and triphenylphospine (90 mg, 0.34 mmol), according to general procedure G. The crude product was purified by flash chromatography (99:1, CH₂Cl₂–MeOH); to give a yellow oil (28 mg, 27%). Rf: 0.4 (92:8, CH₂Cl₂–MeOH); ν max/cm⁻¹ (film) 3407, 2922, 1695, 1488, 1432, 1086, 739; δH (500 MHz; CDCl₃) 8.61 (1H, d, J 2.5, py 2-H), 8.34 (1H, dd, J 4.5 and 1.1, py 4-H), 8.12 (1H, ddd, J 2.5 py 2-H), 8.06 (1H, d, J 8.4 and 4.5, py 5-H), 7.30 (1H, dd, J 8.4 and 4.5, py 5-H), 7.21 (2H, d, J 8.4, phenyl 2- and 6-H), 4.96 (1H, d, J 15.3, benzyl CH₂H₄), 4.02 (1H, d, J 15.3, benzyl CH₂H₄), 3.74 (1H, app. dt, J 6.3 and 3.2, 4-H), 3.59 (1H, ddd, J 8.5, 5.6 and 3.2, 5-H), 2.73 (1H, dd, J 14.7 and 5.6, 5-CH₂H₄), 2.69 (1H, dd, J 13.5 and 5.6, 4-CH₂H₄), 2.59 (1H, dd, J 13.3 and 3.0, 4-CH₂H₄), 2.44 (1H, dd, J 14.7 and 8.5, 5-CH₂H₄), 2.27 (3H, s, isoxazolyl 5-CH₃), 2.11 (3H, s, isoxazolyl 3-CH₃), NH₂ not observed; δC (75 MHz; CDCl₃) 166.2 (isoxazolyl C-5), 159.3 (isoxazolyl C-3), 156.6 (phenyl C-1), 144.6 (py C-1), 140.5, (py C-5), 135.5 (phenyl C-4), 134.0 (CO), 133.9 (py C-4) 129.6 (phenyl C-3 and C-5), 129.0 (phenyl C-2 and C-6), 127.5 (py C-2), 123.7 (py C-6), 108.3 (isoxazolyl C-4), 59.8 (C-4), 54.2 (C-5), 45.5 (benzyl CH₂), 41.8 (5-CH₂), 26.8 (4-CH₂), 11.2 (isoxazolyl 5-CH₃), 10.4 (isoxazolyl 3-CH₃); m/z (ES) 426.2 (100%, [M+H]⁺); HRMS Found: 426.1704 (C₂₂H₂₂ClN₅O₂, M+H requires 426.1691).
(4S*, 5S*)-4-(Aminomethyl)-5-benzyl-1-(4-chlorobenzyl)-3-(3,5-dimethylisoxazol-4-yl) imidazolidin-2-one 106$^{124,137,138}$

The azide 105 (0.08 mmol) was prepared from the allyl urea 91 (1.0 mmol) according to **general procedure F**$^{124,137}$ After elution through a silica plug (40:60, petrol–EtOAc) and evaporation under reduced pressure, the azide 105 was mixed with triphenylphosphine (0.03 g, 0.13 mmol) according to **general procedure G**$^{138}$ and the crude product was purified by flash chromatography (50:50 → 100:0, petrol–EtOAc); to give the **title imidazolidinone 106**, as a yellow oil (11 mg, 2.5% over three steps). $R_f$: 0.1 (10:90, petrol–EtOAc); $\nu_{\text{max}}$/cm$^{-1}$ (film) 2922, 1717, 1678, 1446, 1408, 1247, 1091, 1064, 725; $\delta_H$ (500 MHz; CDCl$_3$) 7.44 (3H, app. s, Ar CH), 7.39 - 7.36 (3H, m, Ar CH), 7.32 - 7.29 (1H, m, Ar CH), 7.28 (2H, dd, $J$ 6.8 and 1.3, Ar CH), 4.95 (1H, d, $J$ 15.7, benzyl $CH_AH_B$), 4.45 (1H, d, $J$ 15.7, benzyl CH$_A$H$_B$), 4.41 (1H, app. dt $J$ 6.9 and 4.7, 4-H), 3.99 (1H, app. dt, $J$ 6.7 and 4.7, 5-H), 3.07 (1H, dd, $J$ 13.8 and 4.5, 5-CH$_A$H$_B$), 2.99 (1H, dd, $J$ 13.8 and 6.7, 5-CH$_A$H$_B$), 2.54 (1H, dd, $J$ 13.7 and 6.9, 4-CH$_A$H$_B$), 2.47 (1H, dd, $J$ 13.7 and 4.6, 4-CH$_A$H$_B$), 2.15 (3H, s, isoxazolyl 5-CH$_3$), 2.04 (3H, s, isoxazolyl 3-CH$_3$), NH$_2$ not observed; $\delta_C$ (75 MHz; CDCl$_3$) 158.5 (isoxazolyl C-5), 158.3 (isoxazolyl C-3), 156.8 (CO), 137.2 (phenyl C-1), 137.0 (phenyl C-1'), 134.7 (phenyl C-4), 130.9, 130.8, 129.9 (Ar CH) 129.5 (phenyl C-4'), 128.3 (Ar CH), 125.4 (isoxazolyl C-4), 83.9 (C-4), 61.4 (C-5), 46.8 (benzyl CH$_2$), 45.5 (4-CH$_2$), 38.8 (5-CH$_2$), 10.8 (isoxazolyl 5-CH$_3$), 9.8 (isoxazolyl 3-CH$_3$); m/z (ES) 425.2 (100%, [M+H]$^+$); HRMS Found: 425.1744 (C$_{23}$H$_{25}$ClN$_4$O$_2$, M+H requires 425.1738).
To a solution of the allyl urea 87 (0.1 mmol) in CH$_2$Cl$_2$ (0.22 M), trimethyl-trifluoromethanesulfonate (0.1 mL, 0.56 mmol) was added, followed by DBU (90 µL, 0.6 mmol) and the resulting mixture was stirred at r.t. for 1h and half. The solvent was removed under reduced pressure, the crude material was dissolved in dry THF (0.16 M), iodine was added (2.0 eq.) and the mixture was left stirring at r.t overnight, then at reflux for 3 h. The reaction mixture was poured in an aqueous saturated solution of Na$_2$S$_2$SO$_4$ (2.0 mL) and extracted with EtOAc (3 x 1.0 mL); the combined organic phases were dried (MgSO$_4$) and concentrated under reduced pressure. The crude product was purified by mass directed chromatography (5:95, CH$_3$CN-H$_2$O) to give the imidazolidinone 111 as a yellow oil (6 mg, 12%).

R$_F$: 0.7 (10:10:80, toluene–petrol–EtOAc); $\nu$$_{max}$/cm$^{-1}$ (film) 2926, 2853, 1686, 1632, 1424, 1261, 1088, 614; $\delta$H (500 MHz; CDCl$_3$) 7.37 (2H, d, $J$ 8.3, phenyl 3- and 5-H), 7.26 (2H, d, $J$ 8.3, phenyl 2- and 6-H), 5.02 (1H, d, $J$ 15.2, benzyl C$_2$H$_A$H$_B$), 4.09 (1H, d, $J$ 15.2, benzyl CH$_A$H$_B$), 4.04 (1H, ap. dt $J$ 6.5 and 4.5, 5-H), 3.54 (1H, ddd, $J$ 8.2, 5.4 and 4.4, 4-H), 3.02-2.99 (2H, m, 5-C$_2$H$_A$H$_B$ and 5-C$_4$H$_A$H$_B$), 2.71 (1H, dd, $J$ 8.2, 5.4 and 4.4, 4-H), 3.02-2.99 (2H, m, 5-C$_2$H$_A$H$_B$ and 5-C$_4$H$_A$H$_B$), 2.71 (1H, dd, $J$ 14.7 and 5.4, 4-CH$_A$H$_B$), 2.50 (1H, dd, $J$ 8.2, 5.4 and 4-CHA$_H_B$), 2.28 (3H, s, isoxazolyl 5-CH$_3$), 2.27 (3H, s, isoxazolyl 5'-CH$_3$), 2.17 (3H, s, isoxazolyl 3-CH$_3$), 2.12 (3H, s, isoxazolyl 3'-CH$_3$); $\delta$C (75 MHz; CDCl$_3$) 166.4 (isoxazolyl C-5), 159.1 (isoxazolyl C-3), 156.6 (isoxazolyl C-5'), 156.4 (CO), 152.4 (isoxazolyl C-3'), 134.3 (phenyl C-4), 129.6 (phenyl C-2 and C-6), 129.2 (phenyl C-3 and C-5), 120.3 (phenyl C-1), 108.1 (isoxazolyl C-4), 105.0 (isoxazolyl C-4'), 79.6 (C-5), 60.2 (C-4), 46.8 (benzyl CH$_2$), 25.8 (5-CH$_2$), 11.3 (isoxazolyl 5-CH$_3$), 11.3 (isoxazolyl 5'-CH$_3$), 10.4 (isoxazolyl 3-CH$_3$), 10.2 (isoxazolyl 3'-CH$_3$), 5.2 (4-CH$_2$); m/z (ES) 555.0 (100%, [M+H]$^+$); HRMS Found: 555.0664 (C$_{22}$H$_{24}$ClIN$_4$O$_3$, M+H requires 555.0670).
1-Benzyl-4-(hydroxymethyl)-3-phenylimidazolidin-2-one, 112\textsuperscript{155}

AgOAc (70 mg, 0.4 mmol) was added to a solution of the iodide 73 (70 mg, 0.17 mmol) in AcOH (1.7 mL) and the resulting mixture was stirred at 70 °C for three days. The mixture was allowed to reach r.t., then CH\textsubscript{2}Cl\textsubscript{2} (15 mL) and an aqueous saturated solution of NaHCO\textsubscript{3} (15 mL) were added portion wise. The phases were separated and the organic phase was washed with H\textsubscript{2}O (2 x 8.0 mL) and brine (2 x 8.0 mL), dried (MgSO\textsubscript{4}) and concentrated under reduced pressure to give a crude product 116 which was used without further purification.

K\textsubscript{2}CO\textsubscript{3} (30 mg, 0.22 mmol) was added to a solution of 116 (35 mg, 0.1 mmol) in MeOH (2.0 mL) and the resulting mixture was stirred at r.t. for 36 hours. The solvent was removed and the crude material was partitioned between CH\textsubscript{2}Cl\textsubscript{2} (5.0 mL) and H\textsubscript{2}O (5.0 mL); the phases were separated and the organic phase was washed with brine (2 x 2.0 mL), dried (MgSO\textsubscript{4}) and concentrated under reduced pressure. The crude product was purified by flash chromatography (50:50, hexane–EtOAc) to give the title imidazolidione 112 as a yellow oil (13.6 mg, 28% over two steps). R\textsubscript{F}: 0.35 (40:60, petrol–EtOAc); v\textsubscript{max}/cm\textsuperscript{-1} (film) 3369, 2961, 2927, 1681, 1446, 1260, 1088, 800; δ\textsubscript{H} (500 MHz; CDCl\textsubscript{3}) 7.50 (2H, d, J 8.3, Ar CH), 7.42-7.27 (7H, m, Ar CH), 7.11 (1H, t, J 7.5, Ar CH), 4.54 (1H, d, J 15.0, benzyl CH\textsubscript{A}H\textsubscript{B}), 4.42 (1H, d, J 15.0, benzyl CH\textsubscript{A}H\textsubscript{B}), 4.35 (1H, app. dtd, J 9.2, 5.1 and 3.0, 4-H), 3.77 (1H, dd, J 11.3 and 5.2, 4-CH\textsubscript{A}H\textsubscript{B}), 3.64 (1H, dd, J 11.3 and 3.0, 4-CH\textsubscript{A}H\textsubscript{B}), 3.47 (1H, app. t, J 9.2, 5-H\textsubscript{A}), 3.36 (1H, dd, J 8.9 and 5.1, 5-H\textsubscript{B}), OH not observed; δ\textsubscript{C} (75 MHz; CDCl\textsubscript{3}) 158.0 (CO), 129.1, 128.7, 128.2 (Ar 3 x CH), 127.6, 124.0 (Ar 2 x C), 121.1 (Ar CH), 61.3 (4-CH\textsubscript{2}), 54.3 (C-4), 48.0 (benzyl CH\textsubscript{2}), 44.4 (C-5); m/z (ES) 305.0 (100%, [M+Na]\textsuperscript{+}); HRMS Found: 305.1262 (C\textsubscript{17}H\textsubscript{18}N\textsubscript{2}O\textsubscript{2} M+Na requires 305.1260).
The urea 117 was prepared from methyl allyl amine (70 μL, 0.7 mmol) and phenylisocyanate (0.15 mL, 1.4 mmol) with stirring overnight according to general procedure B. The reaction mixture was filtered and concentrated under reduced pressure to give a crude product which was used without further purification for the preparation of the azide 119 according to general procedure F. The crude product was purified by flash chromatography (70:30, hexane–EtOAc) to give the title azide 119 as a white solid (22 mg, 14% over three steps). Rf: 0.2 (50:50, petrol–EtOAc); m.p. 62-65 °C; v_max/cm⁻¹ (film) 2933, 2868, 2094, 1687, 1494, 1427, 1377, 1248, 1160, 747; δ_H (500 MHz; CDCl₃) 7.44 (2H, m, phenyl 2- and 6-H), 7.36 (2H, m, phenyl 4-H), 7.12 (1H, tt, J 7.4 and 1.1, phenyl 3- and 5-H), 4.39 (1H, ddd, J 9.9, 6.6, 4.6 and 3.2, 4-H), 3.59 (1H, app. t, J 9.1, 5-H_A), 3.50 (1H, dd, J 12.6 and 6.6, 4-CH₄H_B), 3.44 (1H, dd, J 12.6 and 3.2, 4-CH₄H_B), 3.36 (1H, dd, J 9.1 and 4.6, 5-H_B), 2.9 (3H, s, CH₃); δ_C (75 MHz; CDCl₃) 157.9 (CO), 138.1 (phenyl C-1), 129.2 (phenyl C-2 and C-6), 124.2 (phenyl C-4), 121.1 (phenyl C-3 and C-5), 51.3 (C-4), 47.9 (4-CH₂), (C-5), 31.0 (CH₃); m/z (ES) 254.0 (100%, [M+Na]⁺); HRMS Found: 232.1202 (C₁₁H₁₃N₅O M+H requires 232.1193).
(4R*, 5S*)-1-Ethyl-4-(hydroxymethyl)-5-(naphthalen-2-ylmethyl)-3-phenyl imidazolidin-2-one, 114

A few drops of hydrochloric acid (12 M) were added to a solution of the imidazolidinone 60 (40 μmol) in MeOH (0.6 mL) and the resulting mixture was stirred at r.t. for 2 days. Water (1.0 mL) was added and the mixture was extracted with EtOAc; the organic phase was washed with brine (2 x 1.0 mL), dried (MgSO₄) and concentrated under reduced pressure to give a crude product which was purified by flash chromatography to give the title imidazolidinone 114 as an amorphous solid (6 mg, 42%). Rf: 0.25 (40:60, petrol–EtOAc); νmax/cm⁻¹ (film) 3385, 2931, 1677, 1501, 1431, 1261, 1060, 909, 750; δH (500 MHz; CDCl₃) 7.85-7.79 (3H, m, naphthalenyl 3-, 4- and 5- or 8-H), 7.65 (1H, s, naphthalenyl 1-H), 7.51-7.45 (2H, m, naphthalenyl 6- and 7-H), 7.39-7.34 (3H, m, naphthalenyl 5- or 8-H and phenyl 2- and 6-H), 7.28-7.28 (2H, m, phenyl 3- and 5-H), 7.03 (1H, app t, J 7.5, phenyl 4-H), 4.09 (1H, app dt, J 8.8 and 4.5, 5-H), 3.99 (1H, app dt, J 7.1 and 4.5, 4-H), 3.73 (1H, dq, J 14.7 and 7.3, ethyl 1-HA), 3.57 (1H, dd, J 11.7 and 4.7, 4-CH₂HB), 3.34 (1H, dd, J 13.7 and 4.7, 5-CH₃HB), 3.25-3.09 (2H, m, ethyl 1-HB and 4-CH₂HB), 2.88 (1H, dd, J 13.7 and 8.8, 5-CH₃HB), 1.24 (3H, t, J 7.1, ethyl 2-H); δC (75 MHz; CDCCl₃) 157.1 (CO), 138.8 (phenyl C-1), 133.7 (naphthalenyl C-2), 133.5, 132.4, (naphthalene C-1a and C-8a), 128.9 (phenyl C-3 and C-5), 128.6, 128.1, 127.7, 127.5, 127.2, 126.4, 125.8 (naphthalene 7 x CH), 123.6 (phenyl C-4), 120.5 (phenyl C-2 and C-6), 61.2 (4-CH₂), 59.6 (C-4), 55.2 (C-5), 39.5 (5-CH₂), 36.5 (ethyl C-1), 13.1 (ethyl C-2); m/z (ES) 383.2 (100%, [M+Na]⁺); HRMS Found: 383.1736 (C₂₃H₂₄N₂O₂ M+Na requires 383.1729).

 Signal masked by the solvent peak
1-(Naphthalen-2-yl)but-3-en-2-amine, 120

The title compound 120 was prepared from diphenyl methylene propenamine 78 (4.1 g, 18.4 mmol) and 2-chloromethylnaphthalenyl (3.2 mL, 18.4 mmol) in the presence of n-Butyl lithium (7.4 mL, 18.4 mmol) according to general procedure D.126 A yellow oil was obtained and used without further purification (0.3 g, 20%). $R_F$: 0.1 (90:10, petrol–EtOAc); $\nu_{\text{max}}$/cm$^{-1}$ (film) 3054, 2922, 1598, 1506, 1435, 1367, 994, 918, 814, 748; $\delta_H$ (500 MHz; CDCl$_3$) 7.83-7.78 (3H, m, naphthalenyl 3-, 4- and 5- or 8-H), 7.66 (1H, s, naphthalenyl 1-H), 7.48-7.43 (2H, m, naphthalenyl 6- and 7-H), 7.35 (1H, dd, $J$ 8.4 and 1.6, naphthalenyl 5- or 8-H), 5.94 (1H, ddd, $J$ 17.1, 10.3 and 6.26, 3-H), 5.17 (1H, dd, $J$ 17.1 and 1.4, 4-H$_A$), 5.06 (1H, dd, $J$ 10.3 and 1.4, 4-H$_B$), 3.74-3.69 (1H, m, 2-H), 3.00 (1H, dd, $J$ 13.3 and 5.6, 1-H$_A$), 2.79 (1H, dd, $J$ 13.3 and 8.3, 1-H$_B$), 1.5 (2H, br. s, NH$_2$); $\delta_C$ (75 MHz; CDCl$_3$) 142.4 (C-3), 136.2 (naphthalenyl C-4a), 133.4 (naphthalenyl C-2), 132.1 (naphthalenyl C-8a), 127.9, 127.8, 127.7, 127.6 127.5, 125.9, 125.3 (naphthalene 7 x CH), 113.6 (C-4), 55.3 (C-2), 44.5 (C-1); HRMS Found: 198.1277 (C$_{14}$H$_{15}$N M+H requires 198.1275).
Triethylamine (0.8 ml, 6.2 mmol) and acetyl chloride (0.5 mL, 7.3 mmol) were added at 0 °C to a solution of the amine 120 (0.70 g, 3.7 mmol) in CH₂Cl₂ and the resulting mixture was stirred at r.t. overnight. The mixture was filtered and concentrated under reduced pressure; the crude material was dissolved in the minimum amount of CH₂Cl₂, acetone was added until precipitation was completed and the precipitate was removed by filtration. The filtrate was concentrated under reduced pressure to give a crude product which was purified by flash chromatography (50:50, petrol–EtOAc) to yield the title acetamide 121 as yellow oil (0.48 g, 54%). Rf: 0.2 (40:60, petrol–EtOAc); νmax/cm⁻¹ (film) 3282, 3052, 1655, 1542, 1432, 1369, 1278, 990, 816; δH (500 MHz; CDCl₃) 7.83–7.77 (3H, m, naphthalenyl 3-, 4- and 5- or 8-H), 7.62 (1H, s, naphthalenyl 1-H), 7.49–7.42 (2H, m, naphthalenyl 6- and 7-H), 7.33 (1H, dd, J 8.3 and 1.0, naphthalenyl 5- or 8-H), 5.86 (1H, ddd, J 16.2, 10.2 and 5.4, 3-H), 5.38 (1H, d, J 6.8, NH), 5.11 (1H, d, J 16.2, 4-Hₐ), 5.10 (1H, d, J 10.2, 4-Hₐ), 4.89 (1H, app. dq, J 6.8 and 5.4, 2-H), 3.04 (1H, d, J 6.8, 1-Hₐ), 3.03 (1H, d, J 6.1, 1-Hₐ), 1.95 (3H, s, CH₃); δC (75 MHz; CDCl₃) 169.3 (CO), 137.5 (C-3), 134.7 (naphthalenyl C-4a), 133.4 (naphthalenyl C-2), 132.3 (naphthalenyl C-8a), 128.8, 128.0, 127.8, 127.6, 127.5, 126.1, 125.6 (naphthalene 7 x CH), 115.3 (C-4), 52.0 (C-2), 41.1 (C-1), 23.4 (CH₃); m/z (ES) 262.1 (100%, [M+Na]⁺); HRMS Found: 262.1205 (C₁₆H₁₇NO M+Na requires 262.1202).
LiAlH₄ (0.45 g, 12.0 mmol) was added at 0 °C to a solution of the amide 121 (0.48 g, 2.0 mmol) in THF, and the resulting mixture was allowed to warm to r.t. and stirred for 5 h, then at reflux overnight. An aqueous solution of NaOH (15% w/v) was added at 0 °C dropwise, then the mixture was filtered through Celite®, washed with THF and the filtrate concentrated under reduced pressure to give the title amine 122 as a yellow oil (0.4 g, 88%). Rf: 0.2 (92:8, CH₂Cl₂–MeOH); ν_max/cm⁻¹ (film) 3059, 2968, 1685, 1494, 1284, 1119, 747; δ_H (500 MHz; CDCl₃) 7.85-7.74 (3H, m, naphthalenyl 3-, 4-, and 5- or 8-H), 7.65 (1H, s, naphthalenyl 1-H), 7.49-7.41 (2H, m, naphthalenyl 6- and 7-H), 7.34 (1H, dd, J 8.4 and 1.5, naphthalenyl 5- or 8-H), 5.75 (1H, ddd, J 16.8, 10.5 and 8.1, 3-H), 5.09 (1H, d, J 16.8, 4-HA), 5.08 (1H, d, J 10.5, 4-HB), 3.44 (1H, app. q, J 7.4, 2-H), 3.02 (1H, dd, J 13.4 and 7.3, 1-HA), 2.97 (1H, dd, J 13.4 and 6.6, 1-HB), 2.73 (1H, dq, J 11.8 and 7.1, ethyl 1-HA), 2.52 (1H, dq, J 11.8 and 7.1, ethyl 1-HB), 1.06 (3H, t, J 7.1, ethyl 2-H₃), NH not observed; δ_C (75 MHz; CDCl₃) 140.0 (C-3), 135.8 (naphthalenyl C-4a), 133.5 (naphthalenyl C-2), 132.3 (naphthalenyl C-8a), 128.0, 127.9, 127.7, 127.6, 127.5, 126.0, 125.4, (naphthalene 7 x CH), 116.8 (C-4), 62.9 (C-2), 42.3 (C-1), 41.6 (ethyl C-1), 14.8 (ethyl C-2); m/z (ES) 226.1 (100%, [M+H]+). HRMS Found: 226.1598 (C₁₆H₁₉N M+H requires 262.1590).
The title compound 123 was prepared from the amine 122 (0.14 g, 0.6 mmol) and phenylisocyanate (0.15 mL, 1.3 mmol) in CH₂Cl₂ stirring at 35 °C for 3 hours, according to general procedure B. The crude product was purified by flash chromatography (70:30, petrol–EtOAc) to give a yellow oil (80 mg, 47%). Rᵢ: 0.7 (40:60, petrol–EtOAc); νₑₘₓ/cm⁻¹ (film) 2933, 2870, 1686, 1586, 1494, 1428, 1255, 1016, 696; δₜ (500 MHz; CDCl₃) 7.85-7.76 (3H, m, naphthalenyl 3-, 4- and 5- or 8-H), 7.68 (1H, s, naphthalenyl 1-H), 7.49-7.41 (2H, m, naphthalenyl 6- and 7-H), 7.39 (1H, dd, J 8.4 and 1.5, naphthalenyl 5- or 8-H), 7.23 (2H, d, J 7.2, phenyl 2- and 6-H), 7.21 (2H, d, J 7.2, phenyl 3- and 5-H), 7.00 (1H, dd, J 7.2 and 1.8, phenyl 4-H), 6.20 (1H, s, NH), 6.08 (1H, ddd, J 17.4, 10.5 and 5.2, 3-H), 5.31 (1H, d, J 17.4, 4-Hₐ), 5.29 (1H, d, J 10.5, 4-Hₐ), 4.79 (1H, app. q, J 6.8, 2-H), 3.35 (1H, dq, J 14.3 and 7.1, ethyl 1-Hₐ), 3.29 (1H, dd, J 13.6 and 7.0, 1-Hₐ), 3.19 (1H, dd, J 13.6 and 6.9, 1-Hₐ), 3.09 (1H, dq, J 14.3 and 7.1, ethyl 1-Hₐ), 1.13 (3H, t, J 7.1, ethyl 2-Hₐ); δₛ (75 MHz; CDCl₃) 155.0 (CₐO), 139.1 (phenyl C-1), 137.6 (C-3), 135.8 (naphthalenyl C-4a), 133.6 (naphthalenyl C-2), 132.3 (naphthalenyl C-8a), 128.8 (phenyl C-2 and C-6), 128.2, 127.8, 127.7, 127.6, 126.5, 126.1, 125.5 (naphthalene 7 x CH), 122.9 (phenyl C-4), 119.8 (phenyl C-3 and C-5), 117.0 (C-4), 60.5 (C-2), 40.5 (ethyl C-1), 38.5 (C-1), 15.0 (ethyl C-2); m/z (ES) 711.4 (100%, [2M+Na]+); HRMS Found: 367.1793 (C₂₃H₂₄N₂O M+Na requires 367.1780).
(4S*, 5S*)-4-(aminomethyl)-1-ethyl-5-(naphthalen-2-ylmethyl)-3-phenyl imidazolidin-2-one, compound 115\textsuperscript{124,137,138}

The azide 125 was prepared from the allyl urea 123 (1.0 mmol), according to general procedure F.\textsuperscript{124,137} After elution through a silica plug (40:60, petrol–EtOAc) and evaporation under reduced pressure, the azide 125 was mixed triphenylphosphine (30 mg, 0.13 mmol) and stirred for 3 days according to general procedure G.\textsuperscript{138} The crude product was purified by flash chromatography (50:50 \rightarrow 100:0, petrol–EtOAc) to give the title amine 115, as a yellow oil (22 mg, 25% over three steps). $R_f$: 0.1 (96:4, CH$_2$Cl$_2$–MeOH); $\nu_{\text{max}}$/cm$^{-1}$ (film) 3053, 2929, 1666, 1590, 1243, 1020, 754; $\delta_H$ (500 MHz; CDCl$_3$) 7.85-7.76 (3H, m, naphthalenyl 3-, 4-, and 5- or 8-H), 7.65 (1H, s, naphthalenyl 1-H), 7.52-7.44 (2H, m, naphthalenyl 6- and 7-H), 7.31 (1H, dd, $J$ 8.4 and 1.2, naphthalenyl 5- or 8-H), 7.22-7.19 (2H, m, phenyl 2- and 6-H), 7.03-7.01 (2H, m, phenyl 3- and 5-H), 6.94-6.91 (1H m, phenyl 4-H), 4.22 (1H, app. dt, $J$ 6.3 and 4.7, 4-H), 3.86 (1H, app. dt, $J$ 9.1 and 4.7, 5-H), 3.79 (1H, dq, $J$ 14.5 and 7.2, ethyl 1-H$_A$), 3.33 (1H, dd, $J$ 13.6 and 4.4, 5-CH$_A$H$_B$), 3.28 (1H, dq, $J$ 14.5 and 7.2, ethyl 1-H$_B$), 2.87 (1H, dd, $J$ 13.6 and 9.1, 5-CH$_A$H$_B$), 2.54 (1H, dd, $J$ 13.6 and 4.2, 4-CH$_A$H$_B$), 2.47 (1H, dd, $J$ 13.6 and 6.3, 4-CH$_A$H$_B$), 1.28 (3H, t, $J$ 7.1, ethyl 2-H$_3$); $\delta_C$ (75 MHz; CDCl$_3$) 151.9 (CO), 147.9 (phenyl C-1), 133.6 (naphthalenyl C-4a), 133.5 (naphthalenyl C-2), 132.4 (naphthalenyl C-8a), 128.7 (naphthalene CH), 128.5 (phenyl C-2 and C-6), 128.0, 127.7, 127.5, 127.1, 126.4, 125.9 (naphthalene 6 x CH), 123.5 (phenyl C-3 and C-5), 121.7 (phenyl C-4), 82.0 (C-5), 58.9 (C-4), 44.8 (5-CH$_2$), 39.0 (4-CH$_2$), 37.6 (ethyl C-1), 12.3 (ethyl C-2); m/z (ES) 360.2 (100%, [M+H]$^+$); HRMS Found: 360.2082 (C$_{23}$H$_{25}$N$_3$O M+H requires 360.2070).
5.4 General procedure for biological fluorimetric assay

Invidual compounds were assayed using a BACE fluorimetric assay performed in 96 well plates. The following materials were employed. Quenched fluorescent peptide substrate: Swedish mutant APP sequence (SEVNLDAEFK) tagged with FAM and TAMRA (FAM-SEVNLDAEFK-TAMRA). Enzymes: recombinant BACE-1 (R&D System) or recombinant BACE-2 (R&D System). Inhibitor: β-secretase Inhibitor IV (Merck Chemicals).

The assay protocol was adapted from Hussain et al. Recombinant BACE-1 (26.0 nM) or recombinant BACE-2 (3.0 nM) were diluted in enzyme buffer [100.0 mM sodium acetate, pH 4.5, 40.0 mM sodium chloride, 10% (v/v) glycerol, 0.2% (w/v) CHAPS]. The enzyme was incubated in the presence or absence of β-secretase Inhibitor IV (for positive control, at 0.01 mM for assaying BACE-1 and at 0.1 mM for assay BACE-2), DMSO (for negative control, 5% or 10%) or compounds (for assay compound activity) with shaking for 10 min at 25 °C. For detection of enzyme activity, the quenched fluorescent peptide substrate (5.0 μM) was diluted in substrate buffer [100.0 mM sodium acetate, pH 4.5, 0.06% Triton x-100]. Enzyme buffer and substrate buffer were used at 1:1 ratio in each well to a total volume of 100 μL. The fluorescence was measured using a Synergy HT Bio-Tek fluorimeter using KC4 software, with excitation and emission wavelengths set to 485 nm and 585 nm, respectively. Kinetic measurements over 20 minutes at 2 minutes intervals were performed, 2 duplicate readings were obtained and the results of each measurement were averaged during data processing. Each assay was performed at least three times. A representative 96-well plate layout is shown below (Figure 65).
The compounds were configured as follow for the assay: for single concentration tests, a solution of 100 μM of compound in enzyme buffer containing 5% or 10% DMSO (depending on compound solubility) was used, for dose response of selected compounds, an initial compound solution of 2000 μM or 1000 μM solution in DMSO (depending on compound solubility) was used and then serial diluted in DMSO in 5-9 steps, as low as 2.0 μM.

The data were processed as follows: the average value of fluorescence unit (FU) of wells A1 and A2 was deducted from the average value of fluorescent unit obtained from each measurement corresponding to either the individual compound, the negative or the positive control. The overall kinetic measurements for each well were plotted in a graph FU vs time. A linear fit was applied to the measurement points and slopes corresponding to individual compounds, negative or positive control were derived. The compound inhibition % was calculated as:

$$\frac{\text{compound slope} - \text{positive control slope}}{\text{negative control slope} - \text{positive control slope}} \times 100$$

The results were reported as mean ± SEM. Dose response data, expressed as percentage inhibition vs log[M], were fitted with a sigmoidal dose response model by using Origin Pro 8.6.

**Figure 65. 96 well plate layout of assessing BACE-1 and BACE-2 inhibition.**
5.4.1 Protocol to assess the optimal concentration of BACE-2 to use in a fluorimetric assay

In order to optimise the recombinant BACE-2 for the assay, four different concentrations were assayed. The assay was performed according to the protocol described in Section 1.4, with the following exceptions. Recombinant BACE-2 was used in 30.0 nM, 17.2 nM, 8.6 nM and 3.0 nM diluted in an enzyme buffer, and the samples were incubated in the absence or in the presence of β-secretase Inhibitor IV (at 0.01 mM for BACE-1 and at 0.1 mM for BACE-2). A layout of the 96 well plate prepared is shown below (Figure 66).

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>fluorescent quenched substrate only</td>
<td>recombinant BACE-1 (26.0 nM)</td>
<td>recombinant BACE-1 (26.0 nM) + inhibitor</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>recombinant BACE-2 (30.0 nM)</td>
<td>recombinant BACE-2 (17.2 nM)</td>
<td>recombinant BACE-2 (8.6 nM)</td>
<td>recombinant BACE-2 (3.0 nM)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>recombinant BACE-2 (30.0 nM) + inhibitor</td>
<td>recombinant BACE-2 (17.2 nM) + inhibitor</td>
<td>recombinant BACE-2 (8.6 nM) + inhibitor</td>
<td>recombinant BACE-2 (3.0 nM) + inhibitor</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Figure 66. 96 well plate prepared to assess the optimal concentration of BACE-2.
Appendix 1. Virtual library generation supplementary information

1.1 Virtual reactants employed in the virtual synthesis protocol to enumerate virtual library A (Section 2.3)

1.1.1 Nucleophile building blocks

Amine (trimethyl silyl and tert-Butoxycarbonyl are virtual protecting groups)
Sulfonamides (methylsulfonyl is a virtual protecting group)

\[
\begin{align*}
\text{MeO} &- \text{N} & \text{SO}_2 & \quad \text{Ph} &- \text{N} & \text{SO}_2 & \quad \text{MeO} &- \text{N} & \text{SO}_2 & \quad \text{Ph} &- \text{N} & \text{SO}_2 \\
\text{H} &- \text{N} & \text{SO}_2 & \quad \text{H} &- \text{N} & \text{SO}_2 & \quad \text{HO} &- \text{N} & \text{S} & \text{O}_2 & \quad \text{HO} &- \text{N} & \text{S} & \text{O}_2 \\
\end{align*}
\]

\( n = 1, 2 \)

Amino acids derivatives (trimethyl silyl and methylsulfonyl are virtual protecting groups)

\[
\begin{align*}
\text{HO} &- \text{C} & \text{N} & \text{SO}_2 & \quad \text{HO} &- \text{C} & \text{N} & \text{SO}_2 & \quad \text{HO} &- \text{C} & \text{N} & \text{SO}_2 & \quad \text{HO} &- \text{C} & \text{N} & \text{SO}_2 \\
\text{HO} &- \text{C} & \text{N} & \text{SO}_2 & \quad \text{HO} &- \text{C} & \text{N} & \text{SO}_2 & \quad \text{HO} &- \text{C} & \text{N} & \text{SO}_2 & \quad \text{HO} &- \text{C} & \text{N} & \text{SO}_2 \\
\text{HO} &- \text{C} & \text{N} & \text{SO}_2 & \quad \text{HO} &- \text{C} & \text{N} & \text{SO}_2 & \quad \text{HO} &- \text{C} & \text{N} & \text{SO}_2 & \quad \text{HO} &- \text{C} & \text{N} & \text{SO}_2 \\
\text{HO} &- \text{C} & \text{N} & \text{SO}_2 & \quad \text{HO} &- \text{C} & \text{N} & \text{SO}_2 & \quad \text{HO} &- \text{C} & \text{N} & \text{SO}_2 & \quad \text{HO} &- \text{C} & \text{N} & \text{SO}_2 \\
\end{align*}
\]

\( n = 1, 2 \)
1.1.2 Electrophile building blocks

Allyl carbonates (*tert*-Butoxycarbonyl is a virtual protecting group)

Cyclic sulfamidates (*tert*-Butoxycarbonyl is a virtual protecting group)
1.1.3 Derivatisation reagents

Acyl chlorides

Aryl bromides

Isocyanates and aryl aldehyde

Sulfonyl chlorides
1.2 Virtual reactants employed in the virtual synthesis protocol to enumerate virtual library B (Section 2.3.2)

Aryl bromides

Isocyanates
Secondary amines

Other derivatisation reagents employed were the acyl chlorides, aryl aldehyde and sulfonyl chlorides included in Appendix 1.1.3.

1.3 Virtual reactions employed in the virtual synthesis protocol for the enumeration of libraries A and B (Section 2.3 and 2.3.2)

Table 19. Virtual coupling reactions between initial nucleophiles and electrophiles

<table>
<thead>
<tr>
<th>Entry</th>
<th>Virtual reaction</th>
<th>Scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Iridium-catalysed asymmetric allylic amination with primary or secondary amines</td>
<td><img src="image1" alt="Scheme 1" /></td>
</tr>
<tr>
<td>2</td>
<td>Amide formation from primary or secondary amines</td>
<td><img src="image2" alt="Scheme 2" /></td>
</tr>
<tr>
<td>3</td>
<td>Nucleophilic opening of cyclic sulfamidates</td>
<td><img src="image3" alt="Scheme 3" /></td>
</tr>
</tbody>
</table>
Table 20. Virtual cyclisation reactions.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Virtual reaction</th>
<th>Scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Formation of cyclic urea</td>
<td><img src="image1.png" alt="Diagram" /></td>
</tr>
<tr>
<td></td>
<td>(5, 162, 163, 6, 162, 164, 7, 4 membered ring)</td>
<td><img src="image2.png" alt="Diagram" /></td>
</tr>
<tr>
<td></td>
<td>a)CDI  b)BuOK in THF, then HCl/H₂O</td>
<td><img src="image3.png" alt="Diagram" /></td>
</tr>
<tr>
<td>2</td>
<td>Formation of keto-piperidine</td>
<td><img src="image4.png" alt="Diagram" /></td>
</tr>
<tr>
<td></td>
<td>(6- and 7- membered ring)</td>
<td><img src="image5.png" alt="Diagram" /></td>
</tr>
<tr>
<td>3</td>
<td>Formation of cyclic secondary amine</td>
<td><img src="image6.png" alt="Diagram" /></td>
</tr>
<tr>
<td></td>
<td>(5-, 6- and 7- membered ring)</td>
<td><img src="image7.png" alt="Diagram" /></td>
</tr>
<tr>
<td></td>
<td>X= N, CH₂, O, C=O</td>
<td><img src="image8.png" alt="Diagram" /></td>
</tr>
<tr>
<td>4</td>
<td>Ring-closing metathesis</td>
<td><img src="image9.png" alt="Diagram" /></td>
</tr>
<tr>
<td></td>
<td>(5- and 6- membered ring)</td>
<td><img src="image10.png" alt="Diagram" /></td>
</tr>
<tr>
<td></td>
<td>X= N, CH₂, O, C=O</td>
<td><img src="image11.png" alt="Diagram" /></td>
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</tbody>
</table>
Table 21. Virtual Pd-catalysed cyclisation reactions.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Virtual reaction</th>
<th>Scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd-catalysed urea intramolecular amino arylation(^{100})</td>
<td><img src="image1" alt="Scheme" /></td>
</tr>
<tr>
<td></td>
<td>[C,H] (\text{N} \bigtriangledown \text{N}[C,H]) + ArBr (\rightarrow) [C,H] (\text{N} \bigtriangledown \text{N}[C,H])</td>
<td>cat. Pd(^{0})</td>
</tr>
<tr>
<td>2</td>
<td>Pd-catalysed intramolecular oxyarylation(^{167})</td>
<td><img src="image2" alt="Scheme" /></td>
</tr>
<tr>
<td></td>
<td>(\text{X} = \text{N, CH}_2, \text{O, C=O}) (\rightarrow) Ar</td>
<td>cat. Pd(^{0})</td>
</tr>
<tr>
<td>3</td>
<td>Pd-catalysed intramolecular aminoarylation(^{168}) from primary or secondary amines ((5-\text{and 6- membered ring}))</td>
<td><img src="image3" alt="Scheme" /></td>
</tr>
<tr>
<td></td>
<td>(\text{X} = \text{N, CH}_2, \text{O, C=O}) (\rightarrow) Ar</td>
<td>cat. Pd(^{0})</td>
</tr>
</tbody>
</table>
Table 22. Derivatisation reactions.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Virtual reaction</th>
<th>Scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acylation of primary or secondary amines\textsuperscript{151, 157}</td>
<td>![Acylation”的反应方程式]</td>
</tr>
<tr>
<td>2</td>
<td>Reductive amination of primary or secondary amines\textsuperscript{136}</td>
<td>![Reductive amination”的反应方程式]</td>
</tr>
<tr>
<td>3</td>
<td>Arylation of primary or secondary amines\textsuperscript{169, 170}</td>
<td>![Arylation”的反应方程式]</td>
</tr>
<tr>
<td>4</td>
<td>Urea formation from secondary amines and isocyanates\textsuperscript{100} or $N$-acyl chlorides\textsuperscript{171}</td>
<td>![Urea formation”的反应方程式]</td>
</tr>
<tr>
<td>5</td>
<td>Thiourea formation from secondary amines\textsuperscript{172}</td>
<td>![Thiourea formation”的反应方程式]</td>
</tr>
<tr>
<td>6</td>
<td>Sulfonamide formation from secondary amines\textsuperscript{173}</td>
<td>![Sulfonamide formation”的反应方程式]</td>
</tr>
</tbody>
</table>
Table 23. Protecting group removal reactions

<table>
<thead>
<tr>
<th>Entry</th>
<th>Virtual reaction</th>
<th>Scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Removal of Boc group\textsuperscript{174, 175}</td>
<td>\begin{align*} &amp; \text{[C,H]} \text{N} \text{O} \text{O} \ &amp; \text{[C,H]} \rightarrow \text{[C,H]} \text{N} \text{O} \text{O} \ &amp; \text{H} \end{align*}</td>
</tr>
<tr>
<td>2</td>
<td>Removal of Dmob group\textsuperscript{176}</td>
<td>\begin{align*} &amp; \text{O} \text{N} \text{R} \ &amp; \text{R} \rightarrow \text{H} \text{N} \text{R} \ &amp; \text{R} \end{align*}</td>
</tr>
<tr>
<td>3</td>
<td>Removal of sulfonamide\textsuperscript{177}</td>
<td>\begin{align*} &amp; \text{O} \text{N} \text{S=O} \ &amp; \text{R} \rightarrow \text{R} \text{N} \text{R} \ &amp; \text{H} \end{align*}</td>
</tr>
<tr>
<td>4</td>
<td>Removal of TMS group\textsuperscript{178, 179, 180}</td>
<td>\begin{align*} &amp; \text{R} \text{O} \text{Si} \ &amp; \rightarrow \text{R} \text{O} \text{H} \end{align*}</td>
</tr>
</tbody>
</table>
Appendix 2. Virtual screening supplementary information

2.1 Predicted binding pose of representative ligands in the BACE-1 catalytic site, free from water molecules (Section 2.4)

Predicted binding poses of representative putative inhibitors of the imidazolidinone and amino tetrahydropyridine families are illustrated herein (Figure 67). The predicted binding poses were obtained by docking library B against the BACE-1 structure which did not contain water molecules in the catalytic site. The ligands chosen in the following illustrations are the same as the one represented in Figures 40, Section 2.4. The binding poses show that H-bond interactions were predicted to be formed with the catalytic aspartate residues, Asp32 and Asp228, even without the intermediary involvement of water molecules. The primary amine of the ligands was predicted to be protonated in the catalytic site of BACE-1 (pH 4.5).
Figure 67. Predicted binding pose of representative members of the identified families of BACE-1 putative inhibitors. A) and C) 2D structure of the imidazolidinone putative inhibitor 31 (A) and of the amino tetrahydropiridine putative inhibitor 32 (C) in their protonated state. B) and D) 3D image of the predicted binding pose of the putative inhibitors 31 (B) and 32 (D) in the catalytic site of BACE-1. The predicted H-bond interactions are shown with green dotted lines. Asp32 and Asp228 form H-bonds with the protonated primary amine, NH₃⁺, of the putative inhibitor 31 (B). Asp32 and Asp228 form H-bonds with the protonated primary amine, NH₃⁺, of the putative inhibitor 32 (D), while Lys107, located in the flap region (Section 1.3.2), forms H-bond with the other protonated primary amine, NH₃⁺, of the putative inhibitor.
2.2 H-bond length evaluation (Section 2.4.1)

The length of the predicted H-bonds formed between the identified putative inhibitors and the BACE-1 catalytic site were measured and compared to the ones reported in X-ray crystal structures of BACE-1 complexes available in the literature. The length range was of 2.4-2.9 Å for H-bonds formed with Asp32 and Asp228, and 2.2-2.5 Å for the H-bonds formed with Wat1 and the other water molecules located in the catalytic site. These values were in agreement with the length range reported in the literature. Examples are illustrated in Figure 68 and Figure 69.

![Predicted binding poses BACE-1 putative inhibitors](image)

**Figure 68. Length of H-bonds formed amongst putative inhibitors and aminoacidic residues in the catalytic site of BACE-1.** Two-dimensional representation of the predicted H-bonds formed by the imidazolidinone putative inhibitors 31 (A) and the amino tetrahydropyridine putative inhibitors 32 (B). The H-bond lengths of the predicted H-bonds of the putative inhibitors is in the range of 2.4-2.8 Å.
Figure 69. Length of H-bonds formed amongst known inhibitors and aminoacidic residues in the catalytic site of BACE-1. Two-dimensional representation of the predicted H-bonds formed by the known inhibitors 23 \(^{28}\) (C) and 27 \(^{86}\) (D) (Section 1.4.4 and 1.4.5). The H-bond lengths in the known X-ray crystal structures are in the range of 2.5-3.1 Å; of 2.4-2.8 Å.
Appendix 3. Stereochemical assignment of the synthesised imidazolidinones (Section 3.2.2.1 and 3.3.2.3)

Table 24. Summary of the $J$ values of the signals of the diagnostic protons of the urea ring system in the synthesised imidazolidinones.

![Illustration of the imidazolidinone structure]

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<sup>a</sup> Where applicable, angles are marked in degrees.
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*aThe synthesised compounds were racemic, therefore the relative configuration is shown; bThe relative syn and anti relationship was confirmed by NOE experiment.
Appendix 4. Biological data supplementary information

4.1 Dose responses curves of active imidazolidinones (Section 4.2)

Figure 70. Dose response curves of the imidazolidinone 106 and 103. Compounds 106 and 103 were introduced in Section 4.2, Table 15, entry 2 and 9.
4.2 Assessing the optimal concentration of BACE-2 for biological fluorimetric assay

In order to assay selected compounds against BACE-2 (Section 4.4.1), a homolog of BACE-1, the optimal concentration of BACE-2 to use in the fluorimetric assay was evaluated. The fluorescence resulting from BACE-2 cleavage of the peptide substrate was compared to that emitted by BACE-1 (26.0 nM). The signal was recorded over 20 minutes, at intervals of 2 minutes; the results are shown below (Figure 72).
Figure 72. Fluorescence signal emitted by the fluorescent quenched peptide substrate, cleaved by BACE-2 enzyme. The most similar fluorescence emission trend, in comparison to BACE-1 at 26.0 nM (green line), was obtained using 3.0 nM of BACE-2 (pink line).

As shown in the graph, the relative fluorescence emission using ca. 10 times less BACE-2, 3.0 mM, (pink line) was similar to that seen with BACE-1, 26.0 nM, (green line). A higher quantity of BACE-2 (8.6, 17.2 and 30.0 nM) gave a much higher value of fluorescent emission. Inhibition of BACE-2 by β-secretase inhibitor IV was also assessed. β-secretase inhibitor IV was used at 0.01 nM to assay BACE-1 and at 0.1 mM to assay BACE-2*. The signal was recorder over 20 minutes, at intervals of 2 minutes; the results are shown below (Figure 73).

* Details of assay preparation are described in Section 5.4.1
Figure 73. Inhibition of BACE-2 by β-secretase inhibitor IV. Yellow and purple lines show inhibition of BACE-1 (yellow line) and BACE-2 (purple line) by β-secretase inhibitor IV, compared to non-inhibited BACE-1 (green line) and BACE-2 (pink line) activity.

The reduced fluorescence signals obtained when BACE-1 or BACE-2 cleaves the substrate, in the presence of the β-secretase inhibitor IV, showed a similar linear trend (yellow and purple line). For BACE-2 assays a concentration of 3.0 nM was used to assay selected compounds (Section 4.4 and 5.4).
Reference


