1,4-Oxazine #-secretase (BACE1) inhibitors: from hit generation to orally bioavailable brain penetrant leads.

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1,4-Oxazine β-secretase 1 (BACE1) inhibitors: from hit generation to orally bioavailable brain penetrant leads.

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BACE1 inhibitor, Morpholine, 1,4-Oxazine, Amidine, Alzheimer’s disease
ABSTRACT: 1,4-Oxazines are presented, which show good in vitro inhibition in enzymatic and cellular BACE1 assays. We describe lead optimization focused on reducing the amidine pKₐ whilst optimizing interactions in the BACE1 active site. Our strategy permitted modulation of properties such as permeation and especially P-glycoprotein efflux. This led to compounds which were orally bioavailable, centrally active and which demonstrated robust lowering of brain and CSF Aβ levels respectively in mouse and dog models. The amyloid lowering potential of these molecules makes them valuable leads in the search for new BACE inhibitors for the treatment of Alzheimer’s Disease.

INTRODUCTION

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder and the major cause of dementia in the elderly. According to the World Health Organization (WHO) dementia affects 47.5 million people worldwide, a number that is projected to triple by 2050 with the aging population.¹² AD is characterized by progressive deposition of amyloid and misfolded Tau, followed by neurodegeneration and loss of function, leading ultimately to death.³ Of the many attempts to target the underlying pathogenesis, amyloid lowering approaches have made the most progress so far, albeit without delivering a therapy to date.⁴ Amyloid oligomer and plaque formation is thought to occur when the balance between non-amyloidogenic (α-secretase mediated) and the amyloidogenic processing of amyloid precursor protein (APP) is shifted (familial AD) or clearance of amyloid is impaired (sporadic AD).⁵⁶ In the amyloidogenic pathway, β-secretase 1 (BACE1) cleaves APP producing a 99 amino acid length soluble peptide fragment called C99, which is the rate-limiting step in Aβ formation. This peptide is further processed by γ-secretase to 36-43 amino acid length Aβ species, of which the longer isoforms,
especially Aβ42, are the most fibrillogenic and neurotoxic. Consequently both β-secretase and γ-secretase are being pursued as targets to modulate Aβ production. Since its discovery in 1999, BACE1 has been a highly challenging target for drug discovery, and only after years of research medicinal chemists have managed progressing small molecule BACE1 inhibitors in clinical trials. A breakthrough in the development of non-peptidomimetic BACE1 inhibitors was the identification of amidine- and guanidine-containing small molecules. Compared to previous peptidomimetic and amino-alcohol derived inhibitors, these molecules form a salt bridge and hydrogen bond interactions with Asp32 and Asp228 in the catalytic site of BACE1 in an optimal way (see schematic in Chart 1). The use of a quaternary center alpha to the amidine or guanidine function permits substituents to enter adjacent binding pockets such as S2’, S1 and S3. The S3 pocket can be efficiently targeted via amide-tethered biaryl systems (Chart 1). In these amidine prototypes, the central aromatic ring (A) is a direct substituent on the quaternary center. The A-ring and amide nitrogen occupy the S1 pocket, whereas the distal aromatic ring (B) extends into the S3 pocket. The B-ring is generally a 2-pyridyl or 2-pyrazinyl ring, allowing for a quasi-coplanar orientation with the A-ring. The amide NH establishes an interaction with the backbone carbonyl oxygen of Gly230. The fourth substituent on the quaternary center (methyl as shown in the examples in Chart 1) is directed towards the S2’ pocket. As such, the field has progressed towards potent inhibitors with low molecular weight and good ligand efficiency.

A challenge in the development of amidine and guanidine-based BACE1 inhibitors has been to modulate the intrinsic high basicity of the amidine function. In our previously reported benzoguanidine series, we found this to be a key factor for obtaining compounds with favorable pharmacokinetic (PK) properties, as high basicity (pKₐ ~ 10-11) has been associated
with unfavorable tissue distribution and P-glycoprotein (P-gp) mediated efflux.\textsuperscript{14} Amidine-based warheads with pK\textsubscript{a} below 9 have been reported by a number of companies\textsuperscript{15} in the form of acyl- and sulfonylguanidines,\textsuperscript{16} isoureas\textsuperscript{17} and isothioureas\textsuperscript{18} for which representative examples 1 and 2 are shown in Chart 1.\textsuperscript{19,20} We have previously reported on the \textit{de novo} design and synthesis of piperazinones 3 and 4 as BACE1 inhibitors with moderate enzymatic and cellular activity.\textsuperscript{21} Interestingly, the amide function embedded in the ‘warhead’ of 3 and 4 helped to decrease the basicity of the amidine leading to compounds with \textit{in vivo} activity. However, the blood brain barrier crossing with this piperazinone series was found to be sub-optimal, and concomitantly subcutaneous administration of high doses was needed to achieve significant \textit{in vivo} reduction of Aβ peptides in mice.

![Pharmacophore of BACE1 inhibitors targeting the S3 pocket (X= CH, N)](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>BACE1 IC\textsubscript{50}</th>
<th>hAβ42 cell IC\textsubscript{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (LY-2886721, Eli Lilly)</td>
<td>15.5 nM</td>
<td>6.6 nM</td>
</tr>
<tr>
<td>2 (MK-8931, Merck)</td>
<td>3.2 nM</td>
<td>1.3 nM</td>
</tr>
<tr>
<td>3 (Janssen)</td>
<td>324 nM</td>
<td>36 nM</td>
</tr>
<tr>
<td>4 (Janssen)</td>
<td>23 nM</td>
<td>28 nM</td>
</tr>
<tr>
<td>5, \textit{This paper}</td>
<td>\textit{See diagram}</td>
<td>\textit{See diagram}</td>
</tr>
</tbody>
</table>
Chart 1. Representative overview of reported amidine containing BACE1 inhibitors targeting the S3 pocket. Internally generated enzymatic and cellular data are shown.

Herein we report the design and synthesis of novel 1,4-oxazines (5) as alternative warheads to the previously described piperazinones 3 and 4. Lead optimization strategies to modify the pKₐ of the amidine function resulted compounds (2R,3R)-7a and (2R,3R)-7d with a robust oral effect in lowering of Aβ peptides in mouse and dog models.

CHEMISTRY

In order to access 1,4-oxazines 3R-6a-c, acetophenones 13a,b were converted to the corresponding amino nitriles rac-14a,b via a Strecker reaction (Scheme 1). Acidic hydrolysis of the cyano group and subsequent esterification of the resulting carboxylic acids rac-15a,b led to the aminoesters rac-16a,b. Reduction of rac-16a,b with lithium aluminium hydride (LAH) provided the racemic amino alcohols rac-17a,b. Separation of enantiomers via chiral supercritical fluid chromatography (SFC) followed by determination of the absolute stereochemistry by vibrational circular dichroism (VCD) provided the desired enantiomers 2R-17a,b.22 The lactam rings 5R-18a,b were constructed next via a one-pot two step procedure involving amide formation at -15 °C in the presence of DIPEA, followed by Williamson etherification with t-BuOK. Subsequently, the corresponding amidine derivatives 5R-20a,b were obtained by sequential thionation of 5R-18a,b with P₂S₅, followed by aminolysis of the resulting thioamides 5R-19a,b with aqueous ammonia. Amination reaction on the bromoarene under Buchwald-type conditions using benzophenone imine as the nitrogen source led to 5R-21a,b. Finally, the target compounds 3R-6a-c were obtained via HATU or DMTMM mediated coupling of 5R-21a,b with the corresponding carboxylic acids.
Scheme 1. Synthesis route towards 1,4-oxazines 3R-6a-c.\(^a\)

\[ \text{Reagents and conditions: (a) TMSCN, NH}_4\text{Cl, 7 N NH}_3/\text{MeOH, rt, 4 d; (b) 6 N HCl, reflux, 16 h; (c) H}_2\text{SO}_4, \text{MeOH, reflux, 16 h; (d) LiAlH}_4, \text{THF, 0 °C, 1 h; (e) Chiral SFC separation; (f) i. chloroacetyl chloride, DIPEA, THF, -78 °C, 30 min, ii. }^{t}\text{-BuOK, 0 °C, 90 min; (g) }^{t}\text{-BuONa, toluene, 80 °C, 7 h, ii. 1 N HCl, rt, 16 h (j) 5-chloropyridine-2-carboxylic acid, HATU, }^{N,N}\text{-dimethylaniline, DCM, rt, 5 h; (k) 5-methoxypyrazine-2-carboxylic acid, DMTMM, MeOH, rt, 3 h.}\]

The synthesis routes for target molecules (2S,3R)-7a, (2R,3R)-7a, (2S,3R)-8, (2R,3R)-8, (2R,3R)-9 and (2S,3R)-9 bearing electron withdrawing groups (EWG) at the C-2 position start from the common intermediate 2R-15b (Scheme 2), which was obtained after chiral SFC separation of the racemate 15b and VCD characterization. Thus, 2R-15b was cyclized to the morpholinedione 3R-22 upon reaction with chloroacetyl chloride in a two-step procedure: first the amide bond was formed by treatment with NaOH in 1,4-dioxane, followed by aqueous work-up and subsequent lactonization by addition of NaHCO\(_3\) in DMF. Compound 3R-22 proved to be
a valuable precursor to access all envisioned modifications (I-III, Chart 2). Reduction of the lactone carbonyl with DIBAL-H to the corresponding hemi-acetal, followed by fluorination with DAST provided 5R-23 as an inseparable 60:40 mixture of 6R and 6S diastereomer. Addition of Ruppert-Prakesh reagent (TMS-CF3) to 3R-22 provided hemi-ketal 5R-24 as an unassigned 3:1 mixture of 6S/6R diastereomers. Interestingly 5R-24 also reacted smoothly with DAST providing the 6-fluoro-6-trifluoromethyl-1,4-oxazine derivatives (5R,6R)-25 and (5R,6S)-25 as inseparable mixture in a 25:75 diastereomeric ratio. Hemi-ketal 5R-24 was also chlorinated by reaction with SOCl2 leading to 5R-26. Treatment of 5R-26 with metallic zinc in acetic acid at 100 °C provided the CF3 derivative 5R-27, as a 80:20 mixture of 6R and 6S diastereomer respectively.

Scheme 2. Synthesis of 1,4-oxazine-3-one precursors for BACE1 targeting amidines."
Reagents and conditions: (a) i. Chloroacetyl chloride, 1 M NaOH, 1,4-dioxane, 2 h, ii. DMF, NaHCO₃, 80 °C, 3 h; (b) DIBAL-H, THF, -78 °C, 2 h; (c) DAST, DCM, 0 °C, 40 min (5R-23) to 2 h (5R-25); (d) TMSCF₃, TBAT, THF, 0 °C to rt, 20 min; (e) SOCl₂, DCM, 0 °C, 30 min, then add pyridine, 0 °C, 30 min; (f) Zn, AcOH, 80 °C, 3 h.

As shown in Scheme 3, 6R/6S diastereomeric mixtures 5R-23, 5R-25 and 5R-27 were converted to the target amidines (5R,6S)-31, (5R,6R)-31, (5R,6S)-32, (5R,6R)-32, (5R,6R)-33 and (5R,6S)-33 following a similar reaction sequence to that previously described for 3R-6a-c in Scheme 1.

The only modification in the synthesis route was the conversion of the bromoarene to the aniline, for which we found a copper-catalyzed reaction with sodium azide to provide superior yields to the Buchwald-Hartwig protocol. For occupying the S3 pocket the 5-methoxypyrazin-2-yl group was consistently used to allow for a comparison of properties between the different warheads in final compounds (2S,3R)-7a, (2R,3R)-7a, (2S,3R)-8, (2R,3R)-8, (2R,3R)-9 and (2S,3R)-9.

Scheme 3. Preparation of 1,4-oxazine-based amidines C-2 substituted with different EWG."
Reagents and conditions: (a) P$_2$S$_5$, THF, 70 °C, 2-4 h; (b) 33 % NH$_3$ (aq), 60-80 °C, 24-48 h; (c) NaN$_3$, CuI, DMEDA, Na$_2$CO$_3$, DMSO, 110 °C, 1-4 h; (d) 5-methoxypyrazine-2-carboxylic acid, DMTMM, MeOH, 0 °C, 2-6 h.

A novel stereoselective route using Ellmann’s sulfonamide was developed for the synthesis of compound (2R,3R)-10 (Scheme 4). Condensation of S-Ellmann’s sulfonamide with phenylglyoxylic ester (37) in the presence of Ti(O$i$Pr)$_4$ yielded the S-sulfoximine S-38. Conveniently, complete transesterification of the methyl ester with isopropanol occurred during the condensation step generating the isopropyl ester, which would be less reactive to the addition of cyclopropyl magnesium bromide in the subsequent reaction step. Similar to the protocol described in ref. 22, treatment of S-38 with cyclopropylmagnesium bromide led exclusively to the imine addition product 2R-39 with R-configuration at the quaternary center. Ester hydrolysis followed by the cleavage of the sulfoxamide S-N bond led to amino acid 3R-40, which was next cyclized to the lactone 3R-41 following a similar route to the one previously described for 3R-22. Reduction of 3R-41 with DIBAL-H followed by reaction with DAST provided 5R-42 as a 3:2 mixture of 6R:6S diastereomers. Next, thionation with P$_2$S$_5$ to 5R-43 and subsequent aminolysis, yielded the expected (5R,6R)-44 after separation of the minor (5R,6S)-diastereoisomer by column chromatography. Finally, copper-catalyzed coupling of (5R,6R)-44 with sodium azide to give aniline (5R,6R)-45, followed by amide formation with 5-methoxypyrazine-2-carboxylate yielded compound (2R,3R)-10.
Scheme 4. Enantioselective synthesis of C-3 cyclopropyl substituted 1,4-oxazine 9.a

Reagents and conditions: (a) S-tBuSONH2, Ti(OiPr)4, heptane, 80 °C, 24 h; (b) cPrMgBr, DCM, -78 °C, 30 min; (c) 1 M NaOH, MeOH, reflux, 4 h; (d) 4 N HCl in 1,4-dioxane, rt, 1 h; (e) i. chloroacetyl chloride, 1 M NaOH, 1,4-dioxane, 1 h, ii. NaHCO3, DMF, 80 °C, 2 h; (f) DIBAL-H, THF, -78 °C to rt, 2 h; (g) DAST, DCM, 0 °C, 40 min; (h) P2S5, THF, 70 °C, 4 h; (i) 33 % NH3 (aq), 60 °C, 21 h; (j) NaN3, CuI, DMEDA, Na2CO3, DMSO, 110 °C, 1 h; (k) 5-methoxypyrazine-2-carboxylic acid, DMTMM, MeOH, 0 °C, 6 h.

Racemic compounds (2R*,3R*)-10 and (2R*,3R*)-11 were prepared as shown in Scheme 5.

First, ketones 46a,b were converted to the α-aminonitriles rac-47a,b via a Strecker reaction.

Direct acidic hydrolysis of the nitrile group in rac-47a,b to the carboxylic acids rac-49a,b was not successful in refluxing HCl (aq) due to rapid decarboxylation under the reaction conditions.

This could be circumvented by the hydrolysis of the nitriles in rac-47a,b to the corresponding amides rac-48a,b with HBr in acetic acid, followed by basic hydrolysis with NaOH to rac-49a,b.

The standard lactonization procedure involving initial N-acylation of rac-49a,b with chloroacetyl...
chloride led also to decarboxylation. Fortunately, $O$-alkylation of rac-49a,b with tert-butyl chloroacetate to rac-50a,b followed by selective TFA-mediated hydrolysis of the tert-butyl ester yielded the amino acid derivatives rac-51a,b. Intramolecular cyclization of rac-51a,b with Mukaiyama reagent provided 1,4-oxazine-2,5-diones rac-52a,b. A similar reaction sequence to those previously described was used for the transformation of rac-52a,b into the target molecules rac-(2$R^\ast$,3$R^\ast$)-11 and rac-(2$R^\ast$,3$R^\ast$)-12. However, since the amidine function in rac-(5$R^\ast$,6$R^\ast$)-56a,b was found to be more reactive towards a peptidic coupling than the aminopyridine fragment using DMTMM, the former needed to be selectively protected with a Boc group giving rac-(2$R^\ast$,3$R^\ast$)-57a,b prior to coupling reaction with the 5-methoxypyrazine-2-carboxylic acid. Subsequent cleavage of the Boc protecting group in rac-(5$R^\ast$,6$R^\ast$)-58a,b provided rac-(2$R^\ast$,3$R^\ast$)-11 and rac-(2$R^\ast$,3$R^\ast$)-12, respectively.
Scheme 5. Synthesis of 1,4-oxazines rac-(2R*,3R*)-11 and rac-(2R*,3R*)-12 containing a pyridyl as A-ring.$^a$

Reagents and conditions: (a) TMSCN, NH$_4$Cl, NH$_3$/MeOH, 12 °C, 4 d; (b) 33 % HBr/AcOH, reflux, 12 h; (c) 1 M NaOH, 65 °C, 16 h; (d) ClCH$_2$CO$_2$Bu, Cs$_2$CO$_3$, DMF, rt, 3 h; (e) TFA, rt, 15 min; (f) 2-chloro-1-methylpyridinium iodide, DIPEA, DCM, reflux, 4 h; (g) DIBAL-H, THF, -78 °C to rt, 90 min; (h) DAST, DCM, 0 °C to rt; (i) P$_2$S$_5$, THF, 70 °C, 3 h; (j) 33 % NH$_3$ (aq), 80 °C, 2 h; (k) NaN$_3$, CuI, DMEDA, Na$_2$CO$_3$, DMSO, 110 °C, 24 h; (l) Boc$_2$O, DCM/ACN 1:1, rt, 22 h; (m) 5-methoxypyrazine-2-carboxylic acid, HATU, DIPEA, DMF, 50 °C, 3 h; (n) TFA, rt, 15 min.
RESULTS AND DISCUSSION

Our early benzoguanidine series provided us with an understanding of the optimal binding arrangement of the amidine motifs at the catalytic aspartates.\(^{13}\) This series suffered from high basicity, examples having measured pK\(_a\)'s in range of 11 and 12, which we associated with issues of poor permeation, brain penetration and P-gp efflux. We therefore emphasized the need to find similar amidine and guanidine binding motifs but with reduced basicity, assessed using calculated pK\(_a\) values. To implicitly capture elements of protein flexibility proposals were docked into multiple BACE1 crystal structures. At this preliminary stage we were using an entirely in silico approach as previously described.\(^{21}\) A similar rational design concept but with different method of execution has been reported by others.\(^{24}\) Alongside the previously reported piperazinones 3 and 4,\(^{21}\) the morpholine series 5 was prioritized highly by our approach. The docking of sample 1,4-oxazine molecules showed them to score particularly well, in fact, they were the best of all ideas profiled and docked optimally into the protein structure PDB 2VA7.\(^{12}\) With regards to the basicity, the 1,4-oxazine was predicted to be in an ideal range.\(^{25}\) For instance, whilst our previous benzoguanidines had a calculated basic pK\(_a\) of 11.1, an oxazine example such as 5-methyl-5-phenyl-5,6-dihydro-2H-1,4-oxazin-3-amine had a calculated basic pK\(_a\) of 6.4 (Scheme 6).\(^{25}\) This apparently placed the 1,4-oxazines in a good range of basicity, being protonated in the acidic endosome environment in which BACE1 is active and therefore binding at the catalytic aspartates. Such a pK\(_a\) would be expected to provide a balanced degree of protonation at higher physiological pH, beneficial for CNS drugs.\(^{26}\) However, comparison with the experimental pK\(_a\) reveals inaccuracy in the calculated value. Deeper analysis of this was performed once more examples were synthesised, and as the later discussion reveals, we may have been fortunate to have prioritised the oxazines via this in silico approach.
The morpholine derivatives 3R-6a-c were synthesized as early prototypes to set the baseline for potency and physicochemical properties of this class (Table 1). During the course of our research these molecules also got reported in an extensive warhead exploration published by Hoffmann-La Roche and Siena Biotech.19,27 Based on previous SAR derived from the exploration of the piperazinone series 4, 5-chloropyridine-2-carboxamide and 5-methoxy-2-pyrazinecarboxamide were chosen as B-rings to target the S3 pocket in the morpholine probe compounds 3R-6a-c. In addition, A-ring variations without (3R-6a) and with (3R-6b) a fluorine atom in para-position to the aniline on the A ring (see pharmacophore, Chart 1) were synthesized to assess its influence on pKₐ and potency. Compounds 3R-6a-c had satisfactory inhibitory activity in the BACE1 enzymatic assay, with IC₅₀'s of 44, 22 and 44 nM respectively. This potency nicely translated into an inhibition of Aβ42 production of 9.1 nM (3R-6a), 4.1 nM (3R-6b) and 5.4 nM (3R-6c) in a human neuroblastoma cell line. In vivo reduction in Aβ peptides is assessed in mouse, hence determination of the cellular activity of 3R-6a-c in a mouse neuroblastoma cell line was also done and found to correlate well with the values obtained with the human cell line.
Table 1. *In vitro* profile of morpholines 3R-6a-c.\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>3R-6a</th>
<th>3R-6b</th>
<th>3R-6c</th>
</tr>
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<tbody>
<tr>
<td>BACE1 IC(_{50}) (nM)</td>
<td>44</td>
<td>22</td>
<td>44</td>
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<tr>
<td>hAβ42 cell IC(_{50}) (nM)</td>
<td>9.1</td>
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<td>mAβ42 cell IC(_{50}) (nM)</td>
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<td>4.3</td>
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<tr>
<td>hLM (% met. @ 15 min)</td>
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<td>0</td>
<td>1</td>
</tr>
<tr>
<td>mLM (% met. @ 15 min)</td>
<td>6</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>f(_u) (brain, r, %)</td>
<td>2.9</td>
<td>3.1</td>
<td>10.1</td>
</tr>
<tr>
<td>f(_u) (plasma, h, %)</td>
<td>43</td>
<td>34</td>
<td>56</td>
</tr>
<tr>
<td>f(_u) (plasma, m, %)</td>
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<td>21</td>
<td>32</td>
</tr>
<tr>
<td>P(_{app}) A&gt;B (nm/s)</td>
<td>n. d.</td>
<td>38</td>
<td>37</td>
</tr>
<tr>
<td>P(_{app}) A&gt;B +elacridar (nm/s)</td>
<td>n. d.</td>
<td>231</td>
<td>154</td>
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<tr>
<td>P(_{app}) ratio</td>
<td>n. d.</td>
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<tr>
<td>pK(_a)</td>
<td>9.6</td>
<td>9.2</td>
<td>9.0</td>
</tr>
</tbody>
</table>

\(^a\)See SI for assay details.

Further profiling of 3R-6a-c revealed that they had good metabolic stability in human (hLM) and mouse (mLM) liver microsomes, low binding to human and mouse plasma proteins and moderate non-specific binding to rat brain homogenate (f\(_u\) in plasma or brain). The experimental pK\(_a\) values were determined for 3R-6a-c and were found to be higher than predicted (3R-6a 9.6; 3R-6b 9.2; 3R-6c 9.0). The effect of a fluorine substituent in the A-ring was modest as seen for compound 3R-6b which showed only 0.4 Log unit (2.5 fold) reduction in pK\(_a\) when compared with the non-fluorine substituted analogue 3R-6a. In line with previous observations that high pK\(_a\) is associated with increased P-gp efflux,\(^{20}\) high ratios of apparent permeability (P\(_{app}\)) in presence or absence of the P-gp inhibitor elacridar were measured for 3R-6b and 3R-6c: 6.0 and
4.2 respectively. Subcutaneous (s. c.) administration of a 30 mg/kg dose of 3R-6b to mouse
(Table 2, A) showed modest brain levels (315 ng/g) at 2 h compared to plasma concentration
(2397 ng/mL), resulting in a low brain/plasma ratio ($K_p$) of 0.13. These low concentrations in the
brain translated into a marginal in vivo reduction of Aβ peptides in the brain of wild type (WT)
CF-1 mice (Table 2, B) 2 h after dosing. When the same experiment was repeated using P-gp
deficient CF-1 mice a more favorable brain/plasma ratio ($K_p$) of 1.1 was observed, which
resulted into a much more pronounced reduction of Aβ peptides in brain compared to the one
observed in WT animals. This experiment confirmed the detrimental role of P-gp efflux in the
brain penetration of 3R-6b.

Table 2. Brain levels (A) and brain Aβ reduction (B) of 3R-6b in WT and P-gp deficient CF-1
mice.$^a$

<table>
<thead>
<tr>
<th></th>
<th>3R-6b levels at 2 h</th>
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<tr>
<td></td>
<td>Plasm ng/mL</td>
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<tr>
<td><strong>WP CF1</strong></td>
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<tr>
<td><strong>P-gp def. CF1</strong></td>
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$^a$
30 mg/kg, s. c. (n=8); 3R-6b was formulated with 20 % SBEβCD at pH 3.5.

Whilst lower than our old benzoguanidine series the measured pKₐ values were higher than expected from the initial calculations mentioned above. This prompted a deeper inspection of the accuracy of the calculated pKₐ’s on these unusual amidine containing ring systems. A selection of 42 compounds containing a cyclic amidine substructure from our compound collection was subjected to experimental pKₐ measurement (data in SI). The selection set encompassed multiple heterocyclic chemotypes and covering approximately a 10 log unit range in calculated pKₐ. Molecules with multiple ionizable centers were in general avoided. The pKₐ’s were calculated with a variety of methods, and the correlation with experiment is shown in SI. In contrast to previous studies comparing performance across global datasets, ADMET predictor performed better than ACD for these amidine ring systems (R² = 0.88 compared to R² = 0.75 for best ACD case). This was especially true in the relevant pKₐ range of 6 to 10. These results prompted us to reassess our 1,4-oxazine series with calculations on specific prototypes (Scheme 0).
6) confirming improved performance when using AP.\textsuperscript{29} This reiterates the need to calibrate the performance of pK\textsubscript{a} calculations for novel chemical systems.

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<th>pKa Experiment</th>
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<th>pKa calculated ACD\textsuperscript{b}</th>
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<td></td>
<td>8.1</td>
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<tr>
<td></td>
<td>8.0</td>
<td>4.6</td>
<td>9.9</td>
<td>6.7</td>
</tr>
</tbody>
</table>

**Legend**

- a Tautomer
- b Tautomer

**Scheme 6.** Comparison of the calculated pK\textsubscript{a} to experiment for prototype 1,4-oxazines using ACD v2014 and AP.

The crystal structure of 3R-6a with BACE1 was solved at 2.6 Å resolution (PDB code 5CLM, Figure 1). The result confirmed a binding mode similar to that seen for other bisarylamide substituted amidine BACE1 inhibitors.\textsuperscript{17} Namely, the amidine group makes the key and strong network of interactions with the catalytic aspartate dyad (Asp32 and Asp228) involving several hydrogen bonds and a charge-charge interaction between the protonated amidine and anionic acid groups. The A-ring (phenyl) occupies the S1 pocket, and the B-ring (5-chloro-2-pyridyl) enters the S3 pocket. The S1 pocket is formed by amino acids including Phe108 whereas the S3 is bordered by the presence of Thr232 and amino acids from the 10s loop such as Gly11, Gln12...
and Gly13 (10s loop, blue in Figure 1). Para substituents on the B-ring approach the salt bridge formed by Arg307 and Glu339. Between the A- and B-ring, the amide N-H forms an H-bond to the backbone carbonyl of Gly230. This bisarylamide has become a widely used group in substituted amidine BACE1 inhibitors, likely because of the optimal shape complementarity with the S1 and S3 pockets.\textsuperscript{15}

Based on the finding that P-gp efflux was influenced by the basicity of the amidine containing warhead, several options for chemical modification of the 1,4-oxazine ring by the introduction of electron withdrawing substituents were possible to reduce its high pK\textsubscript{a}. Close inspection of the 1,4-oxazine cycle reveals that the two possible sp\textsuperscript{3} carbon substitution points have different environments. The carbon which is between the oxygen and the amidine (C-6) is more exposed towards solvent. On the other hand, the carbon between the oxygen and the quaternary carbon, (C-2), would direct its substituents towards the active site flap (green in Figure 1). The flap is a well-known feature of the BACE1 binding site, it is flexible and more open at lower pH, correlating with higher enzyme activity.\textsuperscript{30} It is known to adopt different conformations dependent on the bound inhibitor. Molecules are reported which interact with amino acids such as Tyr71,\textsuperscript{13} (Figure 1) and the adjacent Thr72 for example.\textsuperscript{30} Therefore, given that the amidine warhead was satisfying the interactions at the catalytic aspartates, we set out to target alternative contacts with the flap. Specifically, C-2 position provided an ideal vector to interact with the aromatic ring of Tyr71, whilst steric factors would limit the size of the proposed substituents.
**Figure 1.** Crystal structure of 3R-6a in BACE1 (1-454). Amino acids as labelled. Active site flap highlighted in green and 10s loop in blue.

An overview of the different modifications explored at C-2 position of the 1,4-oxazine warhead is shown in Chart 2. A fluorine (I), CF₃ (II) and a combination of both groups (III) were selected as optimal C-2 substituents to further modulate pKₐ of 3R-6c whilst interacting with the active site flap.

**Chart 2.** Scope of exploration at C-2 position to modulate pKₐ.
BACE1 enzymatic and cellular activity, experimental pKₐ values, Pₐₚp ratios and Log D values for 3R-6c and fluorinated 1,4-oxazines (2S,3R)-7a, (2R,3R)-7a, (2S,3R)-8, (2R,3R)-8, (2R,3R)-9 and (2S,3R)-9 are provided in Table 3. Interestingly, the 2R-fluoro substituent in (2R,3R)-7a was found to be highly effective in reducing the basicity of the amidine: a pKₐ of 7.8 was measured, which is a decrease by 1.2 log units when compared with the C-2 non-substituted oxazine 3R-6c. This was aligned with our expectations based on the AP calculated values for this substitution (Scheme 6). The reduction in pKₐ was not detrimental for the primary enzymatic and cellular activity, with the discrepancy between both assays decreased when compared to 3R-6c. This is a general trend observed for our amidine-based BACE1 inhibitors: the lower the pKₐ, the smaller the discrepancy becomes between enzymatic and cellular activity. A possible explanation is that highly basic inhibitors accumulate in the acidic endosomes where BACE1 is most active. As such they display apparently higher activity in cellular than biochemical assays, whereas lower pKₐ results in cellular activity in closer accordance with the one in the biochemical assay due to a more uniform distribution of the compound in cellular compartments. Furthermore, compound (2R,3R)-7a showed good permeability in a LLC-MDR cell line (Pₐₚp 201 nm/s with elacridar) and a low Pₐₚp ratio of 1.1 providing additional support to the hypothesis that lowering the amidine pKₐ would have beneficial effects in Pₐₚp ratios. Interestingly, while in the same pKₐ range, the diastereomeric (2S,3R)-7a had a much reduced BACE1 IC₅₀ of 102 nM, which translated in an equally reduced cellular activity of 93 nM. We hypothesized that this potency difference could be attributed to the anomeric effect, which favors the 2-fluoro substituent in the axial position. QM calculations indeed confirmed this effect stabilizes the bioactive conformation for (2R,3R)-7a (see SI). However in the case of (2S,3R)-7a the conformation with the 2-fluorine in axial position places the phenyl in the unfavorable equatorial position for
binding to BACE1. \(^{32}\) An additional CF\(_3\) group at C-2 position such as 2\(S\)-C(F)CF\(_3\) disubstitution in (2\(S\),3\(R\))-8 reduces pK\(_a\) by another log unit ((2\(S\),3\(R\))-8, pK\(_a\) = 6.8), while maintaining a potency of 20 nM in the BACE1 enzymatic assay. Unfortunately, the 2\(S\)-C(F)CF\(_3\) disubstitution had a detrimental effect on the cellular activity and compound (2\(S\),3\(R\))-8 was \(~ 6\)-fold less potent than 3\(R\)-6c. Interestingly, the diastereoisomeric 2\(R\)-C(F)CF\(_3\) 1,4-oxazine (2\(R\),3\(R\))-8 displayed poor inhibitory activity in both enzymatic and cellular assays. This drop in potency could be rationalized after a close inspection of the crystal structure (Figure 1) of 3\(R\)-6c, which suggests that the CF\(_3\) group in (2\(R\),3\(R\))-8 would be directed into the face of Tyr71, producing an unfavorable interaction between the negative electrostatic properties of both the CF\(_3\) and the \(\pi\) cloud of Tyr71. A similar but less pronounced trend in enzymatic activity was also observed for the 2-C(H)CF\(_3\) diastereoisomeric pair (2\(R\),3\(R\))-9 (IC\(_{50}\) = 29 nM) and (2\(S\),3\(R\))-9 (IC\(_{50}\) = 110 nM), although in this case it did not translate in a difference in cellular activity: (2\(R\),3\(R\))-9 IC\(_{50}\) = 13 nM vs (2\(S\),3\(R\))-9 IC\(_{50}\) = 14 nM. Compound (2\(R\),3\(R\))-9, with a pK\(_a\) of 7.8, illustrated again the ability of the electron withdrawing ability of the CF\(_3\) group to reduce the amidine pK\(_a\) and its beneficial contribution to a lower P\(_{\text{app}}\) ratio of 1.2. Similarly, while the permeability for (2\(S\),3\(R\))-8 was not determined, close analogues with different B-rings showed low P\(_{\text{app}}\) ratios in the 1.2 (5-chloropyridine-2-carboxamide) to 1.6 (4-cyanopyridyl-2-carboxamide) range.
Table 3. Comparison of BACE1 enzymatic and cellular activities, pKₐ’s and P_app ratios for different C-2 substituted 1,4-oxazine warheads.ᵃᵇ

![Chemical structure](image)

<table>
<thead>
<tr>
<th></th>
<th>3R-6c</th>
<th>(2S,3R)-7a</th>
<th>(2R,3R)-7a</th>
<th>(2S,3R)-8</th>
<th>(2R,3R)-9</th>
<th>(2S,3R)-9</th>
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</thead>
<tbody>
<tr>
<td>BACE1 IC₅₀ (nM)</td>
<td>44</td>
<td>102</td>
<td>12</td>
<td>20</td>
<td>316</td>
<td>29</td>
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<tr>
<td>hAβ42 cell IC₅₀ (nM)</td>
<td>5.4</td>
<td>93</td>
<td>9.1</td>
<td>34</td>
<td>324</td>
<td>13</td>
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<tr>
<td>P_app A&gt;B +elacridar (nm/s)</td>
<td>154</td>
<td>n. d.</td>
<td>201</td>
<td>n. d.</td>
<td>n. d.</td>
<td>160</td>
</tr>
<tr>
<td>P_app ratio</td>
<td>4.2</td>
<td>n. d.</td>
<td>1.1</td>
<td>n. d.</td>
<td>n. d.</td>
<td>1.2</td>
</tr>
<tr>
<td>LogD (pH 7.4)</td>
<td>n. d.</td>
<td>n. d.</td>
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<td>3.43</td>
<td>n. d.</td>
<td>2.52</td>
</tr>
<tr>
<td>pKᵦ</td>
<td>9.0</td>
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<td>7.8</td>
<td>6.8</td>
<td>6.9</td>
<td>7.8</td>
</tr>
</tbody>
</table>

ᵃSee SI for assay details.ᵇn. d.: not determined

LogD was also measured for the most promising leads (2R,3R)-7a, (2S,3R)-8 and (2R,3R)-9, with (2R,3R)-7a showing the lowest value (LogD = 2.04). From the data obtained compound (2R,3R)-7a showed the most balanced profile with good enzymatic and cellular activity, low pKᵦ and LogD values, and good permeability and P_app ratio. Since sugars with anomeric fluorines are known to be hydrolytically unstable,³³ the chemical stability of (2R,3R)-7a was assessed by
LCMS-based experiments under a variety of conditions ranging from acidic to basic media and different temperatures after seven days (see SI). In these experiments, (2R,3R)-7a was found to be sufficiently stable at room temperature up to one week in buffer at pH 7.4 (92% remaining) and DMSO (100% remaining) to warrant further exploration.

Analysis of the crystal structure of 3R-6a suggested that space is available around the methyl substituent at the quaternary center in the S2’ pocket, hence a limited synthetic effort was devoted to better fill this pocket. Compound (2R,3R)-10 bearing a C-2 fluoro substituent and a cyclopropyl group at position C-3 was selected as a potential target. BACE1 enzymatic and cellular activity, metabolic stability data and pKₐ values are given for compounds (2R,3R)-10 and (2R,3R)-7a in Table 4. An approximately 5-fold decrease in enzymatic activity was measured for (2R,3R)-10 (IC₅₀ = 66 nM) compared to (2R,3R)-7a (IC₅₀ = 12 nM), however, a much more significant 66-fold drop in cellular activity was also observed (2R,3R)-10: IC₅₀ = 603 nM vs (2R,3R)-7a: IC₅₀ = 9.1 nM). Compared to (2R,3R)-7a, (2R,3R)-10 showed increased metabolic stability in hLM, but a slightly higher turnover in mLM. Finally, no difference in pKₐ between (2R,3R)-7a and (2R,3R)-10 was measured. We hence decided to maintain the methyl group at the quaternary center for further optimization of (2R,3R)-7a.
Table 4. Modification on the quaternary methyl group. 

![Chemical structure](image)

<table>
<thead>
<tr>
<th></th>
<th>(2R,3R)-7a</th>
<th>(2R,3R)-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>BACE1 IC₅₀ nM</td>
<td>12</td>
<td>66</td>
</tr>
<tr>
<td>hAB42 cell IC₅₀ nM</td>
<td>9.1</td>
<td>603</td>
</tr>
<tr>
<td>pKₐ</td>
<td>7.8</td>
<td>7.8</td>
</tr>
</tbody>
</table>

*See Experimental Section for assay details.

To generate additional SAR on potency and pKₐ variation of the A-ring was studied next. Analogues rac-(2R*,3R*)-11 and rac-(2R*,3R*)-12 in which the A-phenyl ring is replaced by a 2- or 4-pyridyl ring were synthesized. BACE1 enzymatic and cellular activity, metabolic stability data and pKₐ values are given for compounds (2R,3R)-7a, rac-(2R*,3R*)-11, rac-(2R*,3R*)-12 in Table 5. Interestingly the reduced basicity of the amidines was maintained when replacing the phenyl A-ring in (2R,3R)-7a by a 2-pyridyl as in rac-(2R*,3R*)-11 (pKₐ = 7.8) and the regioisomeric 4-pyridyl analogue rac-(2R*,3R*)-12 (pKₐ = 7.6). In addition, the pKₐ value of the pyridyl nitrogen in rac-(2R*,3R*)-11 and rac-(2R*,3R*)-12 was measured to be 2.6 and 2.8 respectively and hence no protonation of this ring is expected to occur under physiological conditions. Unfortunately, while the pKₐ of rac-(2R*,3R*)-11 and rac-(2R*,3R*)-12 was in the desired range, the presence of the pyridyl ring was found to be detrimental for BACE1 activity,
with the 2-pyridyl isomer \textit{rac-}(2R*,3R*)-\textbf{11} significantly less potent than (2R,3R)-\textbf{7a} and \textit{rac-}(2R*,3R*)-\textbf{12} only active in the \textmu M range.

\textbf{Table 5.} Overview of A-ring modifications.$^a$

<table>
<thead>
<tr>
<th></th>
<th>(2R,3R)-\textbf{7a}</th>
<th>\textit{rac-}(2R*,3R*)-\textbf{11}</th>
<th>\textit{rac-}(2R*,3R*)-\textbf{12}</th>
</tr>
</thead>
<tbody>
<tr>
<td>BACE1 IC$_{50}$ nM</td>
<td>12</td>
<td>74</td>
<td>3467</td>
</tr>
<tr>
<td>h\textalpha\beta42 cell IC$_{50}$ nM</td>
<td>9.1</td>
<td>257</td>
<td>1445</td>
</tr>
<tr>
<td>pK$_a$</td>
<td>7.8</td>
<td>7.8; 2.6</td>
<td>7.6; 2.8</td>
</tr>
</tbody>
</table>

$^a$See Experimental Section for assay details.

Finally, a limited SAR exploration of the B-ring in (2R,3R)-\textbf{7a} using different pyridine-2-yl carboxylic acids was performed following a similar synthetic route. A set of representative compounds (2R,3R)-\textbf{7a-d} along with BACE1 primary activity, data from broader profiling in ADME-tox assays and physicochemical properties, are given in Table 6. Although in general excellent enzymatic and cellular activity data were obtained for all compounds having a pyridyl B-ring ((2R,3R)-\textbf{7b-d}) subtle differences were observed regarding their \textit{in vitro} ADME properties. For instance, while (2R,3R)-\textbf{7b} and (2R,3R)-\textbf{7d} retained excellent metabolic stability...
across tested species, the trisubstituted pyridine (2R,3R)-7c was notably unstable in mLm. All compounds showed a low potential to inhibit CYP450 isoforms 1A2, 3A4, 2C9 and 2C19 compared to their IC50’s. The strongest inhibition of the 2D6 isoform was observed for compound (2R,3R)-7a with an IC50 of 600 nM, still leading to a 50-fold margin when compared with the enzymatic BACE1 activity (IC50 = 12 nM). In addition, all compounds displayed high free fractions in plasma across species (5.0-60 % free) and a reasonable free fraction in rat brain tissue (1.0-4.4 % free). Cardiovascular safety of the compounds was first assessed using a hERG channel patch-clamp assay indicating that the B-ring has a strong effect on hERG inhibition. For instance, while the 5-methoxypyrazine (2R,3R)-7a showed a relatively weak inhibition of the hERG channel at 3 µM (33%), the pyridyl derivative (2R,3R)-7b showed a much stronger hERG inhibition (78%) at the same concentration. This strong interaction could be mitigated by installing a methyl group on the pyridine as in (2R,3R)-7c (52 % inhibition). Finally the 4-cyanopyridyl derivative (2R,3R)-7d showed 56 % hERG inhibition at 3 µM. As could be expected for this 2R-F substituted warhead, Papp ratios for analogues (2R,3R)-7b-d were generally acceptable, with (2R,3R)-7d being a notable exception with an Papp ratio of 3.6.
Table 6. Overview of B-ring modifications.\textsuperscript{a, b}

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|}
\hline
 & (52,63)-7a & (2R,3R)-7b & (2R,3R)-7c & (2R,3R)-7d \\
\hline
\hline
BACE1 IC\textsubscript{50} nM & 12 & 7.4 & 6.9 & 7.6 \\
\hline
hAβ42 cell IC\textsubscript{50} nM & 9.1 & 5.2 & 3.3 & 8.1 \\
\hline
hLM (% met. @ 15 min) & 31 & 0 & 16 & 0 \\
\hline
mLM (% met. @ 15 min) & 3 & 17 & 98 & 2 \\
\hline
dLM (% met. @ 15 min) & 70 & 7 & 21 & 0 \\
\hline
CYP450 inh. <10 µM & 2D6 (0.6) & none & none & 2D6 (4.3) \\
\hline
f\textsubscript{u} (brain, r, %) & 4.4 & 1.9 & 1.0 & 3.9 \\
\hline
f\textsubscript{u} (plasma, h, %) & 60 & 18 & 11 & 41 \\
\hline
f\textsubscript{u} (plasma, m, %) & 28 & 14 & 5.0 & 30 \\
\hline
f\textsubscript{u} (plasma, d, %) & 34 & n. d. & n. d. & 42 \\
\hline
hERG PX (% inh. @ 3 µM) & 33 & 78 & 52 & 56 \\
\hline
P\textsubscript{app} A>B (nm/s) & 176 & 85 & 93 & 51 \\
\hline
P\textsubscript{app} A>B + elacridar (nm/s) & 201 & 167 & 142 & 183 \\
\hline
P\textsubscript{app} ratio & 1.1 & 2.0 & 1.5 & 3.6 \\
\hline
pK\textsubscript{a} & 7.8 & 7.9 & 7.4 & 7.9 \\
\hline
\end{tabular}
\caption*{\textsuperscript{a}See Experimental Section for assay details. \textsuperscript{b}n. d.: not determined}
\end{table}

From the exploration of the B-ring, compounds (2R,3R)-7a and (2R,3R)-7d showed the best balance between potency, hERG liability, and \textit{in vitro} P-gp efflux liability, and therefore were progressed to \textit{in vivo} PK/PD evaluation. The results obtained are summarized in Table 7. In these experiments, compounds were dosed orally (p. o.) to male Swiss mice at 10 mg/kg. Plasma
levels are shown for three time points. For each time point, one hemisphere of the brain was used to conduct bioanalysis, and the other hemisphere for AlphaLisa quantification of Aβ. In line with the measured $P_{app}$ ratios of 1.1 and 3.6, a higher brain uptake relative to plasma was observed for compound $(2R,3R)$-$7a$ ($K_p = 0.65$) compared to $(2R,3R)$-$7d$ ($K_p = 0.33$). Gratifyingly, a robust Aβ42 reduction of 62 % for $(2R,3R)$-$7a$ and 57 % for $(2R,3R)$-$7d$ was observed in brain homogenate at 4 h after administration, corresponding to brain levels of 195 ng/g for $(2R,3R)$-$7a$ and 98 ng/g for $(2R,3R)$-$7d$. This substantial reduction in Aβ42 is in line with their high in vitro potancy (resp. 12 and 7.6 nM) and good free fraction in brain. Moreover, this significant reduction of Aβ42 in brain is maintained up to 7-8 h.

**Table 7.** Mouse pharmacokinetic profile of $(2R,3R)$-$7a$ and $(2R,3R)$-$7d$ with associated Aβ levels in brain.$^a$
(2R,3R)-7d levels

\[ K_p = 0.33^b \]

Plasma ng/mL  Brain ng/g

(2R,3R)-7d Aβ (% versus control)

\[ 2 \text{ h} \quad 4 \text{ h} \quad 7 \text{ h} \]

Abeta42  Abeta Total

\[ 2 \text{ h} \quad 4 \text{ h} \quad 7 \text{ h} \]

\[ a \]

10 mg/kg p. o. Male Swiss SPF mouse fasted (n=6); (2R,3R)-7a formulated with 20 % HPβCD at pH 3.9; (2R,3R)-7d formulated with 20 % SBEβCD at pH 3.5. \[ b \] Calculated with AUC0-last, see SI.

Since (2R,3R)-7a suffers from a high metabolic turnover in dog liver microsomes (dLM, 70 % metabolized after 15 min), the more metabolically stable (2R,3R)-7d was selected for subsequent dog efficacy studies. Dog in vivo plasma concentrations and measured Aβ levels in cerebrospinal fluid (CSF) after administration of increasing doses from 0.08 to 10 mg/kg are given in Table 8. From this study it can be seen that there is a linear relation between dose and plasma levels with an exceptionally long half-life of about 25 h, which underscores the chemical stability of these fluoromorpholines under physiological conditions. In agreement with this, a dose dependent reduction of Aβ42 was observed in CSF, with the 2.5 mg/kg dose showing over 90 % reduction of CSF Aβ42 up to 50 h post dosing.
Table 8. Dog *in vivo* plasma levels and CSF Aβ42 levels of (2R,3R)-7d.*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BACE1 IC₅₀ nM</td>
<td>7.6</td>
</tr>
<tr>
<td>hAβ42 cell IC₅₀ nM</td>
<td>8.1</td>
</tr>
<tr>
<td>dLM (% met. after 15 min)</td>
<td>0</td>
</tr>
<tr>
<td>Cₘₐₓ (2.5 mg/kg, ng/mL)</td>
<td>571 ± 131</td>
</tr>
<tr>
<td>T½ (2.5 mg/kg, h)</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>Tₘₐₓ (2.5 mg/kg, h)</td>
<td>2.5 ± 1.0</td>
</tr>
<tr>
<td>CSF Aβ42 dog EC₅₀ (ng/mL)</td>
<td>~ 20</td>
</tr>
</tbody>
</table>

*Male (n=2/dose group) and female (n=2/dose group) Beagle dog fasted; (2R,3R)-7d formulated with 20 % HPβCD at pH 3.5.*

CONCLUSIONS
In summary, a novel class of 1,4-oxazine-based BACE1 inhibitors has been identified through rational design. The medicinal chemistry optimization effort through careful structural warhead modification to fine tune the amidine pK_a along with BACE1 primary activity, has resulted in the identification of 2-fluoro-1,4-oxazines (I, Chart 2). Lead optimization efforts identified (2R,3R)-7a and (2R,3R)-7d as potent orally bio-available BACE1 inhibitors displaying a robust reduction of Aβ peptides in mice. This amyloid reduction was also confirmed for (2R,3R)-7d in dog, where this compound was able to dose dependently decrease Aβ levels in CSF up to 50 h post dosing. Challenges remain to navigate the narrow physical chemical property space, especially balancing brain penetration with other critical parameters like metabolic stability and cardiovascular safety (hERG inhibition). Further optimization studies using fluoromorpholines and the other fluorinated warheads described above are under way and will be disclosed in due time.

EXPERIMENTAL SECTION

**Enzymatic BACE1 assay.** Primary BACE1 enzymatic activity was assessed by a FRET assay using an amyloid precursor protein (APP) derived 13 amino acids peptide that contains the ‘Swedish’ Lys-Met/Asn-Leu mutation of the APP beta-secretase cleavage site as a substrate (Bachem cat No. M-2465) and soluble BACE1(1-454) (Aurigene, Custom made). This substrate contains two fluorophores, (7-methoxycoumarin-4-yl) acetic acid (Mca) is a fluorescent donor with excitation wavelength at 320 nm and emission at 405 nm and 2,4-dinitrophenyl (Dnp) is a proprietary quencher acceptor. The increase in fluorescence is linearly related to the rate of proteolysis. In a 384-well format, BACE1 is incubated with the substrate and the inhibitor. The amount of proteolysis is directly measured by fluorescence measurement in the Fluoroskan.
microplate fluorometer (Thermo scientific). For the low control no enzyme was added to the reaction mixture.

**Cellular Aβ assay.** Cellular activity was assessed using a SKNBE2 (human) or Neuro-2a (mouse) neuroblastoma cell line expressing the wild type Amyloid precursor protein (hAPP695). The compounds are diluted and added to these cells, incubated for 18 hours and then measurements of Aβ42 and Aβtotal are taken. Aβ42 and Aβtotal are measured by a sandwich αlisa assay using biotinylated antibody (AbN/25) attached to streptavidin-coated beads and antibody (cAb42/26) conjugated acceptor beads. In the presence of Aβ42, the beads come into close proximity. The excitation of the donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the acceptor beads, resulting in light emission.

**Metabolic stability assay.** To test for metabolic stability, compounds (1 μM) were incubated at 37 °C with mouse, rat and human liver microsomes at a protein concentration of 1 mg protein/mL microsomal protein, 1 mM NADPH, 1 mM MgCl₂, and 0.1 M phosphate buffer, pH 7.4. DMSO Stock solutions (5 mM) of each compound were diluted with acetonitrile:water (1:1) to provide a working stock solution at 0.1 mM. The total incubation volume was 0.5 mL with a final total solvent content of 0.01% (v/v) DMSO and 0.5% (v/v) acetonitrile. The reaction was initiated by the addition of 100 μL pre-warmed NADPH solution. The incubation mixture was sampled at 15 min, and the sample quenched with 200 μL acetonitrile, centrifuged and analyzed using a specific HPLC-MS/MS technique. The percentage metabolized was taken as the disappearance of test compound at 15 min.

**CYP450 inhibition assay.** The potential to reversibly inhibit the major human P450 isoforms (CYPs 1A2, 2C9, 2C19, 2D6, and 3A4 was determined using recombinantly expressed human
CYPs. Specific probe substrates were used for each CYP isoform which were known to be selectively metabolized to defined fluorescent metabolites. Each test compound was incubated with individual CYPs over a concentration range up to 10 µM. At the end of the incubation, the level of fluorescence was measured on a plate reader. The level of fluorescence in the presence and absence of test compound was used to determine the IC$_{50}$ against each CYP isoform.

**Plasma protein binding assay.** The binding to mouse, dog and human plasma proteins was determined by Rapid Equilibrium Dialysis (RED Device, Thermo Fisher Scientific, Geel, Belgium). The RED device consists of a 48 well plate containing disposable inserts bisected by a semi-permeable membrane creating two chambers. A 300 µL aliquot of plasma containing test compound at 5 µM was placed one side and 500 µL of phosphate buffered saline (PBS) the other. The plate was sealed and incubated at approximately 37 °C for 4.5 h. After 4.5 h samples were removed and both the plasma and buffer compartment and analyzed for test compound using a specific HPLC-MS/MS method to estimate free and bound concentrations.

**Non-specific binding to brain tissue assay.** The *in vitro* non-specific binding of compounds to rat brain homogenate was determined using the RED Device (see above). Each test compound was diluted with rat brain homogenate, prepared following a 1:10 dilution with PBS, to achieve a final concentration of 5 µM. The plate was incubated at approximately 37 °C for 5 h. After 5 h samples were removed from both the brain homogenate and buffer compartment and analyzed for test compound using a specific HPLC-MS/MS method to estimate free and bound concentrations.

**In vitro permeability/P-gp efflux assay.** The *in vitro* permeability and potential to be transported by P-glycoprotein (P-gp) was determined using an LLC cell line transfected with human MDR1 (P-glycoprotein). Each test compound (5 µM) was added to either the apical (A)
side of a confluent monolayer of LLC-MDR1 cells and permeability towards the basolateral (B) direction measured by monitoring the appearance of the test compound on the opposite side of the membrane using a specific LC-MS/MS method. Permeability was assessed in with and without elacridar (GF 120918, CAS 143851-98-3), a well-known P-gp inhibitor. The A>B+elacridar/A>B ratio (P_app ratio) was calculated and used to determine if the test compound was subject to efflux by P-gp.

**Mouse in vivo PK and Aβ quantification.** Male CD1 Swiss Specific Pathogen Free (SPF) mice (Charles River company, Germany) were dosed p. o. or s. c. with the formulated (20% HPβCD) compound. After the indicated time of treatment, the animals were sacrificed and Aβ levels were analyzed. Blood was collected by decapitation and exsanguinations in EDTA-treated collection tubes. Blood was centrifuged at 1900 g for 10 min at 4 °C and the plasma recovered and flash frozen for later analysis. The brain was removed from the cranium and hindbrain. The cerebellum was removed, and the left and right hemisphere were separated. The left hemisphere was stored at -18 °C for quantitative analysis of test compound levels. The right hemisphere was rinsed with phosphate buffered saline (PBS) buffer and immediately frozen on dry ice and stored at -80 °C until homogenization for biochemical assays. The other hemisphere is homogenized and centrifuged and processed for the quantification of Aβ total and Aβ42 via ELISA as described previously. Briefly for the quantification of Aβ total Aβ42 the antibody pair JRF/rAb/2 and 4G8 or JRF/cAb42/26 and JRF/rAb/2 antibody was used for capturing and detection respectively.

**Dog in vivo PK and Aβ quantification.** Female beagle dogs were dosed p. o. with the formulated (20% HPβCD) compound or vehicle. At the indicated time points CSF was sampled in conscious dogs from the lateral ventricle. Quantification of Aβ42 in dog CSF was performed
using MesoScale Discovery (MSD)’s electrochemiluminescence detection technology as described previously.\textsuperscript{34}

\textbf{pK}_a \textbf{assay.} Dissociation constants were determined at 25 °C by potentiometric titration of a solution of the compound of interest using a Sirius GlpKa apparatus, and values were calculated using the Henderson-Hasselbach equation. For poorly soluble compounds, titrations were performed with MeOH as co-solvent. In this case pK\textsubscript{a} was calculated via Yasuda-Shedlovsky extrapolation.

\textbf{LogD assay.} The LogD of compounds was determined chromatographically at Sirius Analytical Ltd.\textsuperscript{36}

\textbf{Analytical methods.} All final compounds were characterized by \textsuperscript{1}H NMR and LC/MS. \textsuperscript{1}H Nuclear Magnetic Resonance spectra were recorded on Bruker spectrometers: 360 MHz, DPX-400 MHz and AV-500 MHz. Purity of all final compounds was \textgeq95\% by NMR. For the \textsuperscript{1}H spectra, all chemical shifts are reported in part per million (\(\delta\)) units, and are relative to the residual signal at 7.26 and 2.50 ppm for CDCl\textsubscript{3} and DMSO, respectively. All the LC/MS analyses were performed using an Agilent G1956A LC/MS quadrupole coupled to an Agilent 1100 series liquid chromatography (LC) system consisting of a binary pump with degasser, autosampler, thermostated column compartment and diode array detector. The mass spectrometer (MS) was operated with an atmospheric pressure electro-spray ionization (API-ES) source in positive ion mode. The capillary voltage was set to 3000 V, the fragmentor voltage to 70 V and the quadrupole temperature was maintained at 100°C. The drying gas flow and temperature values were 12.0 L/min and 350 °C, respectively. Nitrogen was used as the nebuliser gas, at a pressure of 35 psig. Data acquisition was performed with Agilent Chemstation software.

Analyses were carried out on a YMC pack ODS-AQ C18 column (50 mm long x 4.6 mm I.D.; 3
μm particle size) at 35 °C, with a flow rate of 2.6 mL/min. A gradient elution was performed from 95% (Water + 0.1% Formic acid)/5% Acetonitrile to 5% (Water + 0.1% Formic acid)/95% Acetonitrile in 4.8 min; the resulting composition was held for 1.0 min; from 5% (Water + 0.1% formic acid)/95% Acetonitrile to 95% (Water + 0.1% formic acid)/5% Acetonitrile in 0.2 min. The standard injection volume was 2 μL. Acquisition ranges were set to 190-400 nm for the UV-PDA detector and 100-1400 m/z for the MS detector. Optical rotations measurements were carried out on a 341 Perkin Elmer polarimeter in the indicated solvents.

**Synthetic protocols.**

*N-*{3-[(3R)-5-Amino-3-methyl-3,6-dihydro-2H-1,4-oxazin-3-yl]phenyl}-5-chloropyridine-2-carboxamide (3R-6a). 5-Chloro-2-pyridinecarboxylic acid (0.27 g, 1.72 mmol) was added to a stirred solution of intermediate 5R-21a (0.235 g, 1.145 mmol) in DCM (10 mL) at rt. Then, N,N-dimethylaniline (0.218 mL, 1.72 mmol) was added and after stirring for 5 min at rt HATU (0.500 g, 1.32 mmol) was added. The mixture was stirred at rt for 5 h. The mixture was diluted with water and sat. aq. aqueous Na₂CO₃ and extracted with DCM. The organic layer was separated, dried (Na₂SO₄), filtered and the solvents evaporated *in vacuo*. The crude product was purified by flash column chromatography (silica; 7 N solution of ammonia in MeOH/DCM 0/100 to 4/96). The desired fractions were collected and concentrated *in vacuo*. The resulting product was triturated with diisopropyl ether, filtered and dried. The product was purified again by flash column chromatography (silica; 7 N solution of ammonia in MeOH/EtOAc 0/100 to 4/96). The desired fractions were collected and concentrated *in vacuo* to yield 3R-6a (0.16 g, 41% yield). ¹H NMR (500 MHz, CDCl₃) δ 9.85 (br s, 1H), 8.56 (d, J=2.0 Hz, 1H), 8.25 (d, J=8.4 Hz, 1H), 7.88 (dd, J=2.3, 8.4 Hz, 1H), 7.77 (br d, J=8.1 Hz, 1H), 7.71-7.74 (m, 1H), 7.37 (t, J=7.9 Hz, 1H), 7.20 (d, J=7.8 Hz, 1H), 4.25 (br s, 2H), 4.16 (d, J=15.5 Hz, 1H), 4.09 (d, J=15.5 Hz, 1H), 3.74
(d, J=11.4 Hz, 1H), 3.62 (d, J=11.4 Hz, 1H), 1.56 (s, 3H); LC-MS m/z 345 [M+H]^+; [α]_{D}^{20} = -88.8 (c = 0.68 in DMF).

*N-[3-[(3R)-5-Amino-3-methyl-3,6-dihydro-2H-1,4-oxazin-3-yl]-4-fluorophenyl]-5-chloropyridine-2-carboxamide (3R-6b).* 5-Chloro-2-pyridinecarboxylic acid (155 mg, 0.99 mmol) was added to a mixture of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl morpholinium chloride (297 mg, 1.08 mmol) in MeOH (4 mL). The mixture was stirred for 5 min at rt, after which it was cooled to 0 ºC and a solution of aniline 5R-21b in MeOH (4 mL) was added. Then the mixture was stirred at rt for 3 h. The reaction was quenched with half-saturated aq Na₂CO₃ solution and extracted with DCM. The organic layer was separated, dried (Na₂SO₄), filtered and concentrated in vacuo. The crude product was purified by flash column chromatography (silica; 7 N solution of ammonia in MeOH/DCM 0/100 to 3/97). The desired fractions were collected and the solvents evaporated in vacuo and subsequently triturated with heptane, sonicated, filtered and dried in vacuo at 50 ºC to yield 3R-6b as a white solid (253 mg, 78% yield). ¹H NMR (400 MHz, CDCl₃) δ 9.82 (br s, 1H), 8.54 (d, J=2.3 Hz, 1H), 8.23 (d, J=8.3 Hz, 1H), 7.93 (td, J=3.6, 8.3 Hz, 1H), 7.87 (dd, J=2.3, 8.5 Hz, 1H), 7.67 (dd, J=2.8, 6.9 Hz, 1H), 7.05 (dd, J=8.9, 11.4 Hz, 1H), 4.14 (d, J=15.3 Hz, 1H), 4.06 (d, J=15.3 Hz, 1H), 3.92 (dd, J=0.9, 11.4 Hz, 1H), 3.82 (d, J=11.4 Hz, 1H), 1.58 (s, 3H) (2H exchanged); ¹³C NMR (101 MHz, DMSO-d₆) δ 161.45, 156.16, 156.36 (d, J=242.0 Hz, 1C), 148.49, 147.02, 137.80, 134.18, 134.11 (d, J=14.6 Hz, 1C), 133.80 (br d, J=2.3 Hz, 1C), 123.80, 122.63 (d, J=4.6 Hz, 1C), 120.67 (d, J=8.5 Hz, 1C), 115.70 (d, J=25.4 Hz, 1C), 70.74 (d, J=5.8 Hz, 1C), 62.32, 54.70 (d, J=4.2 Hz, 1C), 26.30 (d, J=4.2 Hz, 1C); LC-MS m/z 363 [M+H]^+; [α]_{D}^{20} = +31.9 (c = 0.69 in DMF); m. p. = 146.7 ºC.

*N-[3-[(3R)-5-Amino-3-methyl-3,6-dihydro-2H-1,4-oxazin-3-yl]-4-fluorophenyl]-5-methoxypyrazine-2-carboxamide (3R-6c).* Starting from 5R-19b (100 mg, 0.448 mmol) and
following the same procedure as for 3R-6b the corresponding oxazinamine 3R-6c was obtained (112 mg, 70% yield). $^1$H NMR (500 MHz, CDCl$_3$) δ 9.49 (br s, 1H), 9.00 (d, $J=1.2$ Hz, 1H), 8.13 (d, $J=1.2$ Hz, 1H), 7.91 (ddd, $J=2.9$, 4.0, 8.7 Hz, 1H), 7.64 (dd, $J=2.6$, 6.9 Hz, 1H), 7.05 (dd, $J=8.7$, 11.6 Hz, 1H), 4.21 (br s, 2H), 4.13 (d, $J=15.5$ Hz, 1H), 4.06 (s, 3H), 4.05 (d, $J=15.5$ Hz, 1H), 3.92 (dd, $J=1.2$, 11.5 Hz, 1H), 3.81 (d, $J=11.5$ Hz, 1H), 1.58 (s, 3H); 13C NMR (101 MHz, DMSO-d$_6$) δ 161.61, 161.28, 156.18, 156.30 (d, $J=242.0$ Hz, 1C), 142.10, 141.39, 137.95, 133.93 (d, $J=2.7$ Hz, 1C), 133.55, 122.69, 120.72 (d, $J=8.1$ Hz, 1C), 115.66 (d, $J=25.0$ Hz, 1C), 70.76 (d, $J=6.9$ Hz, 1C), 62.32, 54.70 (d, $J=3.9$ Hz, 1C), 54.26, 26.29 (d, $J=4.2$ Hz, 1C); LC-MS m/z 360 [M+H$^+$]; $[\alpha]_{20}^D = +32.2$ (c = 0.61 in DMF); m. p. = 178.8 ºC.

N-[3-[(2R,3R)-5-Amino-2-fluoro-3-methyl-3,6-dihydro-2H-1,4-oxazin-3-yl]-4-fluorophenyl]-5-methoxypyrazine-2-carboxamide ((2R,3R)-7a). Starting from (5R,6R)-34 (2.42 g, 6.1 mmol) and following the same procedure as for 3R-6b the corresponding (2R,3R)-7a was obtained as a white solid (1.5 g, 65% yield). $^1$H NMR (360 MHz, CDCl$_3$) δ 9.46 (s, 1H), 8.98 (d, $J=1.5$ Hz, 1H), 8.11 (d, $J=1.5$ Hz, 1H), 7.83 (ddd, $J=2.7$, 4.1, 8.9 Hz, 1H), 7.52 (dd, $J=2.7$, 6.8 Hz, 1H), 7.07 (dd, $J=8.8$, 11.3 Hz, 1H), 6.04 (d, $J=52.3$ Hz, 1H), 4.59 (br s, 2H), 4.26 (d, $J=15.7$ Hz, 1H), 4.06 (s, 3H), 4.03 (d, $J=15.7$ Hz, 1H), 1.65 (t, $J=2.0$ Hz, 3H); 13C NMR (101 MHz, DMSO-d$_6$) δ 161.60, 161.38, 156.16, 155.67 (d, $J=241.4$ Hz, 1C), 141.46, 137.91, 134.37 (d, $J=2.2$ Hz, 1C), 133.50, 131.61 (dd, $J=5.5$, 13.6 Hz, 1C), 121.89 (br d, $J=2.9$ Hz, 1C), 121.55 (d, $J=8.8$ Hz, 1C), 115.97 (d, $J=25.7$ Hz, 1C), 105.00 (dd, $J=7.3$, 225.9 Hz, 1C), 57.83 (dd, $J=4.8$, 26.8 Hz, 1C), 56.37 (d, $J=2.9$ Hz, 1C), 54.24, 25.11 (br d, $J=2.9$ Hz, 1C); LC-MS m/z 378 [M+H$^+$]; $[\alpha]_{20}^D = +117.3$ (c = 0.69 in DMF); m. p. = 212.4 ºC.

N-[3-[(2S,3R)-5-Amino-2-fluoro-3-methyl-morpholin-3-yl]-4-fluorophenyl]-5-methoxypyrazine-2-carboxamide ((2S,3R)-7a). Starting from (5R,6S)-34 (0.040 g, 0.166 mmol) and
following the same procedure as for 3R-6b the corresponding (2S,3R)-7a was obtained (0.019 g, 30%). $^1$H NMR (400 MHz, CDCl$_3$) δ 9.52 (br s, 1H), 9.01 (d, $J$=1.3 Hz, 1H), 8.13 (d, $J$=1.3 Hz, 1H), 8.03 (ddd, $J$=2.9, 4.2, 8.8 Hz, 1H), 7.86 (dd, $J$=2.7, 6.7 Hz, 1H), 7.07 (dd, $J$=8.8, 11.4 Hz, 1H), 6.09 (dd, $J$=1.5, 51.5 Hz, 1H), 4.29 (d, $J$=15.6 Hz, 1H), 4.16 (d, $J$=15.6 Hz, 1H), 4.06 (s, 3H), 1.55 (s, 3H) (2H exchanged); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 162.25, 160.97, 156.59 (d, $J$=242.8 Hz, 1C), 153.97, 141.89, 137.35, 133.78 (d, $J$=2.2 Hz, 1C), 133.35, 131.25 (d, $J$=13.9 Hz, 1C), 120.65 (d, $J$=5.1 Hz, 1C), 120.06 (d, $J$=8.8 Hz, 1C), 116.28 (d, $J$=25.7 Hz, 1C), 105.12 (dd, $J$=9.2, 226.3 Hz, 1C), 57.89 (dd, $J$=4.4, 25.7 Hz, 1C), 56.38, 26.35 (t, $J$=4.0 Hz, 1C); LC-MS m/z 378 [M+H]$^+$; m. p. = 211.8 ºC.

$N$-{3-[(2R,3R)-5-Amino-2-fluoro-3-methyl-3,6-dihydro-2H-1,4-oxazin-3-yl]-4-fluorophenyl}-5-chloropyridine-2-carboxamide ((2R,3R)-7b).

Starting from (5R,6R)-34 (500 mg, 2.07 mmol) and following the same procedure as for 3R-6b the corresponding amide (2R,3R)-7b was obtained as a white solid (600 mg, 76%). $^1$H NMR (360 MHz, DMSO-$d_6$) δ 10.74 (s, 1H), 8.78 (d, $J$=2.2 Hz, 1H), 8.19 (dd, $J$=2.2, 8.8 Hz, 1H), 8.14 (d, $J$=8.8 Hz, 1H), 7.76-7.84 (m, 1H), 7.74 (dd, $J$=2.6, 7.3 Hz, 1H), 7.18 (dd, $J$=8.8, 11.7 Hz, 1H), 6.07 (br s, 2H), 5.87 (d, $J$=53.8 Hz, 1H), 4.04 (d, $J$=16.1 Hz, 1H), 3.94 (d, $J$=16.1 Hz, 1H), 1.49 (s, 3H); $^{13}$C NMR (91 MHz, DMSO-$d_6$) δ 161.61, 156.21, 155.75 (d, $J$=241.5 Hz, 1C), 148.51, 147.04, 137.83, 134.30 (d, $J$=2.1 Hz, 1C), 134.23, 131.67 (dd, $J$=5.5, 13.8 Hz, 1C), 123.93, 121.98, 121.60 (d, $J$=8.3 Hz, 1C), 116.05 (d, $J$=25.6 Hz, 1C), 105.01 (dd, $J$=6.9, 226.3 Hz, 1C), 57.85 (dd, $J$=4.5, 26.6 Hz, 1C), 56.39 (d, $J$=3.5 Hz, 1C), 25.15 (br d, $J$=5.5 Hz, 1C); LC-MS m/z 381 [M+H]$^+$; m. p. = 220.4 ºC.

$N$-{3-[(2R,3R)-5-Amino-2-fluoro-3-methyl-3,6-dihydro-2H-1,4-oxazin-3-yl]-4-fluorophenyl}-5-chloro-3-methylpyridine-2-carboxamide hydrochloride ((2R,3R)-7c).
Starting from (5R,6R)-34 (150 mg, 0.38 mmol) and following the same procedure as for 3R-6b the corresponding amide (2R,3R)-7c was obtained. The purified compound (2R,3R)-7c was dissolved in isopropanol/DIPE and a few drops of a 6 N HCl solution in isopropanol were added. The resulting precipitate was collected after evaporation of the solvents yielding (2R,3R)-7c as a white solid (77 mg, 47%, HCl salt). 1H NMR (360 MHz, DMSO-d6) δ 11.18 (s, 1H), 10.80 (s, 1H), 9.68 (s, 1H), 8.98 (s, 1H), 8.60 (d, J=2.6 Hz, 1H), 8.05-8.07 (m, 1H), 8.01 (ddd, J=2.6, 4.2, 9.0 Hz, 1H), 7.75 (dd, J=2.6, 7.3 Hz, 1H), 7.33 (dd, J=9.0, 11.9 Hz, 1H), 6.15 (d, J=50.5 Hz, 1H), 4.76 (d, J=17.9 Hz, 1H), 4.67 (d, J=17.9 Hz, 1H), 2.58 (s, 3H), 1.73 (s, 3H); 13C NMR (101 MHz, DMSO-d6) δ 163.98, 162.16, 154.88 (d, J=243.1 Hz, 1C), 147.75, 144.36, 139.66, 135.82, 135.45 (d, J=2.3 Hz, 1C), 132.70, 126.52 (dd, J=4.2, 12.3 Hz, 1C), 122.23 (d, J=8.5 Hz, 1C), 119.63, 117.12 (d, J=24.7 Hz, 1C), 104.28 (dd, J=5.8, 232.7 Hz, 1C), 58.19 (dd, J=4.2, 25.4 Hz, 1C), 56.99 (d, J=4.2 Hz, 1C), 21.71 (dd, J=2.5, 4.8 Hz, 1C), 18.88; LC-MS m/z 395 [M+H]+; m. p. = 129.4 ºC.

N-{3-[2R,3R)-5-Amino-2-fluoro-3-methyl-3,6-dihydro-2H-1,4-oxazin-3-yl]-4-fluorophenyl}-5-cyanopyridine-2-carboxamide ((2R,3R)-7d). Starting from (5R,6R)-34 (834 mg, 3.46 mmol) and following the same procedure as for 3R-6b the corresponding amide (2R,3R)-7d was obtained as a white solid (615 mg, 48%). 1H NMR (500 MHz, DMSO-d6) δ 10.86 (s, 1H), 9.19 (d, J=2.0 Hz, 1H), 8.57 (dd, J=2.0, 8.1 Hz, 1H), 8.27 (d, J=8.1 Hz, 1H), 7.78-7.83 (m, 1H), 7.76 (dd, J=2.6, 7.2 Hz, 1H), 7.18 (dd, J=8.7, 11.8 Hz, 1H), 6.05 (br s, 2H), 5.87 (d, J=53.7 Hz, 1H), 4.04 (d, J=15.9 Hz, 1H), 3.94 (d, J=15.9 Hz, 1H), 1.49 (s, 3H); 13C NMR (101 MHz, DMSO-d6) δ 161.17, 156.21, 155.87 (d, J=242.1 Hz, 1C), 152.54, 151.42, 142.10, 134.08 (d, J=2.2 Hz, 1C), 131.70 (dd, J=5.9, 13.2 Hz, 1C), 122.34, 122.08 (br d, J=2.2 Hz, 1C), 121.74 (d, J=8.8 Hz, 1C), 116.55, 116.06 (d, J=25.7 Hz, 1C), 111.48, 104.98 (dd, J=7.3, 225.9
Hz, 1C), 57.84 (dd, \(J=4.8, 26.8\) Hz, 1C), 56.38 (d, \(J=3.7\) Hz, 1C), 25.09 (br d, \(J=3.7\) Hz, 1C);
LC-MS m/z 372 [M+H]+; [\(\alpha\)]\textsubscript{D}^20 = +125.0 (c = 0.51 in DMF); m. p. = 223.4 °C.

\[N\{-3\-[(2S,3R)-5-Amino-2-fluoro-3-methyl-2-(trifluoromethyl)-3,6-dihydro-2\textsubscript{H}-1,4-oxazin-\textsubscript{3-yl}]\-4-fluorophenyl\}-5-methoxypyrazine-2-carboxamide ((2S,3R)-8).\]

Starting from (5R,6S)-35 (0.20 g, 0.65 mmol) and following the same procedure as for 3R-6b the corresponding (2S,3R)-8 was obtained (0.13 g, 45% yield). "H NMR (360 MHz, CDCl\textsubscript{3}) \(\delta\) 9.49 (br s, 1H), 9.02 (d, \(J=1.3\) Hz, 1H), 8.16 (d, \(J=1.3\) Hz, 1H), 7.84 (dd, \(J=2.9, 7.3\) Hz, 1H), 7.64 (td, \(J=3.3, 8.8\) Hz, 1H), 7.02 (dd, \(J=8.8, 12.4\) Hz, 1H), 4.44-4.56 (m, 2H), 4.34 (br s, 2H), 4.07 (s, 3H), 1.87 (br s, 3H); 13C NMR (101 MHz, DMSO-d\textsubscript{6}) \(\delta\) 161.62, 161.56, 157.33 (d, \(J=245.8\) Hz, 1C), 154.03, 141.57, 137.96, 134.14 (d, \(J=2.2\) Hz, 1C), 133.46, 127.24 (dd, \(J=4.8, 10.6\) Hz, 1C), 122.75 (d, \(J=1.5\) Hz, 1C), 121.92 (d, \(J=9.5\) Hz, 1C), 120.65 (dq, \(J=35.2, 288.6\) Hz, 1C), 116.16 (d, \(J=26.4\) Hz, 1C), 106.52 (qd, \(J=32.3, 240.6\) Hz, 1C), 59.02, 58.41 (br d, \(J=30.1\) Hz, 1C), 54.26, 25.17;

LC-MS m/z 444 [M-H]-; [\(\alpha\)]\textsubscript{D}^20 = +75.6 (c = 0.17 in MeOH); m. p. = 80.0 °C.

\[N\{-3\-[(2R,3R)-5-Amino-2-fluoro-3-methyl-2-(trifluoromethyl)-3,6-dihydro-2\textsubscript{H}-1,4-oxazin-\textsubscript{3-yl}]\-4-fluorophenyl\}-5-methoxypyrazine-2-carboxamide ((2R,3R)-8).\]

Starting from (5R,6R)-35 (0.2 g, 0.65 mmol) and following the same procedure as for 3R-6b the corresponding (2R,3R)-8 was obtained (200 mg, 69% yield). "H NMR (360 MHz, CDCl\textsubscript{3}) \(\delta\) 9.49 (br s, 1H), 9.02 (d, \(J=1.5\) Hz, 1H), 8.15 (d, \(J=1.5\) Hz, 1H), 7.83-7.89 (m, 1H), 7.67 (td, \(J=2.4, 6.9\) Hz, 1H), 7.04 (dd, \(J=8.8, 12.1\) Hz, 1H), 4.53 (d, \(J=15.7\) Hz, 1H), 4.46 (d, \(J=15.7\) Hz, 1H), 4.38 (br s, 2H), 4.07 (s, 3H), 1.83 (s, 3H); 13C NMR (101 MHz, DMSO-d\textsubscript{6}) \(\delta\) 161.60, 161.38, 157.26 (d, \(J=245.8\) Hz, 1C), 153.70, 141.43, 137.97, 133.56 (d, \(J=2.2\) Hz, 1C), 133.47, 128.28 (d, \(J=11.0\) Hz, 1C), 125.27, 121.76 (d, \(J=8.8\) Hz, 1C), 121.00 (dq, \(J=35.2, 289.8\) Hz, 1C), 115.55 (d, \(J=27.1\) Hz, 1C), 106.34 (qd, \(J=33.8, 204.7\) Hz, 1C), 59.07 (d, \(J=2.9\) Hz, 1C), 58.42 (dd, \(J=2.9, 25.7\) Hz, 1C),
54.24, 24.13 (br d, J=6.6 Hz, 1C); LC-MS m/z 444 [M-H]; [α]_{D}^{20} = -128.6 (c = 0.22 in MeOH); m. p. = 198.4 °C.

**N-3-{(2R,3R)-5-Amino-3-methyl-2-(trifluoromethyl)-3,6-dihydro-2H-1,4-oxazin-3-yl}-4-fluorophenyl}-5-methoxypyrazine-2-carboxamide ((2R,3R)-9).** Starting from (5R,6R)-36 (0.20 g, 0.69 mmol) and following the same procedure as for 3R-6b the corresponding (2R,3R)-9 was obtained (0.19 g, 65% yield). ¹H NMR (360 MHz, CDCl₃) δ 9.55 (br s, 1H), 9.02 (d, J=1.5 Hz, 1H), 8.15 (d, J=1.5 Hz, 1H), 8.02 (dd, J=2.9, 4.4, 8.8 Hz, 1H), 7.89 (dd, J=2.7, 6.8 Hz, 1H), 7.05 (dd, J=8.8, 11.7 Hz, 1H), 4.65 (q, J=8.2 Hz, 1H), 4.32 (br s, 2H), 4.23 (s, 2H), 4.07 (s, 3H), 1.68 (s, 3H); ¹³C NMR (91 MHz, DMSO-d₆) δ 161.66, 161.35, 156.95 (d, J=243.0 Hz, 1C), 155.06, 141.43, 137.97, 133.97 (d, J=13.1 Hz, 1C), 122.70 (d, J=3.5 Hz, 1C), 121.22 (d, J=9.0 Hz, 1C), 124.33 (q, J=286.5 Hz, 1C), 115.39 (d, J=24.9 Hz, 1C), 73.25 (q, J=26.3 Hz, 1C), 59.45, 54.78 (d, J=3.5 Hz, 1C), 54.30, 28.57 (d, J=2.8 Hz, 1C); LC-MS m/z 428 [M+H]; [α]_{D}^{20} = -42.4 (c = 0.18 in MeOH); m. p. = 252.5 °C.

**N-3-{(2S,3R)-5-Amino-3-methyl-2-(trifluoromethyl)-3,6-dihydro-2H-1,4-oxazin-3-yl}-4-fluorophenyl}-5-methoxypyrazine-2-carboxamide ((2S,3R)-9).** Starting from (5R,6S)-36 (0.10 g, 0.34 mmol) and following the same procedure as for 3R-6b the corresponding (2S,3R)-9 was obtained (0.034 g, 23% yield). ¹H NMR (400 MHz, DMSO-d₆) δ 10.44 (br s, 1H), 8.88 (d, J=1.3 Hz, 1H), 8.38 (d, J=1.3 Hz, 1H), 7.94 (dd, J=2.6, 7.5 Hz, 1H), 7.84-7.91 (m, 1H), 7.11 (dd, J=8.8, 12.1 Hz, 1H), 5.76 (br s, 2H), 4.41 (q, J=7.6 Hz, 1H), 4.26 (d, J=15.5 Hz, 1H), 4.20 (d, J=15.5 Hz, 1H), 4.01 (s, 3H), 1.56 (s, 3H); ¹³C NMR (101 MHz, DMSO-d₆) δ 161.63, 161.44, 156.66 (d, J=243.6 Hz, 1C), 154.73, 141.50, 137.91, 134.26 (d, J=2.2 Hz, 1C), 133.47, 132.04 (d, J=10.3 Hz, 1C), 121.29 (d, J=3.7 Hz, 1C), 120.82 (d, J=8.8 Hz, 1C), 124.16 (q, J=283.9 Hz, 1C),
starting from \((5R,6R)-45\) (0.20 g, 0.75 mmol) and following the same procedure as for \(3R-6b\) the corresponding \((2R,3R)-10\) was obtained (0.15 g, 50% yield). \(^1\)H NMR (360 MHz, CDCl\(_3\)) \(\delta\) 9.46 (s, 1H), 8.99 (d, \(J=1.5\) Hz, 1H), 8.13 (d, \(J=1.46\) Hz, 1H), 7.82 (dd, \(J=2.93, 4.03, 8.78\) Hz, 1H), 7.39 (dd, \(J=2.74, 6.77\) Hz, 1H), 7.08 (dd, \(J=8.78, 11.71\) Hz, 1H), 6.22 (d, \(J=52.33\) Hz, 1H), 4.44 (br s, 2H), 4.22 (d, \(J=15.37\) Hz, 1H), 4.06 (s, 3H), 3.99 (d, \(J=15.37\) Hz, 1H), 1.65 (dt, \(J=3.29, 8.42\) Hz, 1H), 0.50-0.60 (m, 1H), 0.47 (d, \(J=15.00\) Hz, 1H), 0.38 (qd, \(J=4.88, 9.51\) Hz, 1H), 0.17-0.26 (m, 1H); \(^{13}\)C NMR (101 MHz, DMSO-\(d_6\)) \(\delta\) 161.57 (br d, \(J=5.1\) Hz, 1C), 161.35, 157.37, 155.70 (d, \(J=240.6\) Hz, 1C), 141.45, 137.93, 134.24 (d, \(J=2.2\) Hz, 1C), 133.50, 131.79 (br d, \(J=12.5\) Hz, 1C), 121.92 (d, \(J=3.7\) Hz, 1C), 121.36 (d, \(J=8.8\) Hz, 1C), 115.90 (d, \(J=25.7\) Hz, 1C), 105.37 (dd, \(J=8.4, 226.3\) Hz, 1C), 58.75 (dd, \(J=5.1, 27.9\) Hz, 1C), 56.95 (d, \(J=2.9\) Hz, 1C), 54.24, 16.02 (d, \(J=6.6\) Hz, 1C), 2.20, -1.60; LC-MS m/z 404 [M+H]^+. 

**rac-N-{2-[(2R*,3R*)-5-Amino-2-fluoro-3-methyl-3,6-dihydro-2H-1,4-oxazin-3-yl]pyridin-4-yl}-5-methoxy pyrazine-2-carboxamide (rac-(2R*,3R*)-11).** Di-tert-butyldicarbonate (0.85 g, 3.88 mmol) was added to 0.54 g (2.43 mmol) aniline rac-(5R*,6R*)-56a in 100 mL DCM/ACN (1:1), and the resulting mixture was stirred 22 h at rt. Then, all volatiles were evaporated at rt and the crude was purified by flash column chromatography (silica; 7 N solution of NH\(_3\) in MeOH/DCM 0/100 to 10/90). The product fractions were evaporated providing the Boc-amidine rac-(5R*,6R*)-57a as a yellow oil (0.29 mg, 37 % yield), and another fraction of starting material rac-(5R*,6R*)-56a was recovered (0.27 g, 49%).
HATU (0.18 g, 0.46 mg) was added to a solution of rac-(5R*,6R*)-57a (0.050 g, 0.154 mmol), 5-methoxypyrazine-2-carboxylic acid (0.071 g, 0.462 mmol) and DIPEA (0.16 mL, 0.925 mmol) in 2 mL dry DMF, and the resulting mixture was stirred 3 h at 50 °C. After cooling to rt, DCM and 2 N Na₂CO₃ were added to the mixture and it was stirred for 30 min at rt. The organic layer was separated and the aq layer was extracted with DCM. The combined organic layers were dried (Na₂SO₄), filtered and concentrated. The residue was purified by flash column chromatography (silica; 7 N solution of ammonia in MeOH/DCM 0/100 to 10/90). The desired fractions were collected and concentrated in vacuo yielding amide rac-(5R*,6R*)-58 as a transparent oil (33.1 mg, 47%).

Rac-(5R*,6R*)-58a (33.1 mg, 0.0719 mmol) was dissolved in TFA (3 mL) and stirred for 15 min. Then, the mixture was evaporated to dryness and sat. aq. NaHCO₃ and DCM were added. The biphasic mixture was stirred until gas evolution ceased, and then the organic layer was separated. The aq layer was extracted twice with DCM. The combined organic layers were dried (Na₂SO₄), filtered and the solvent was evaporated. The residue was purified by flash column chromatography (silica; 7 N solution of ammonia in MeOH/DCM 0/100 to 10/90). The desired fractions were collected and concentrated in vacuo yielding rac-(2R*,3R*)-11 as beige crystals, which were washed with diethyl ether and dried in vacuo at 50 °C (14.8 mg, 51%).

1H NMR (360 MHz, DMSO-d₆) δ 10.89 (br s, 1H), 8.92 (d, J=1.3 Hz, 1H), 8.44 (d, J=5.7 Hz, 1H), 8.43 (d, J=1.3 Hz, 1H), 7.96 (d, J=1.9 Hz, 1H), 7.75 (dd, J=1.9, 5.7 Hz, 1H), 6.02 (br s, 2H), 5.92 (d, J=54.5 Hz, 1H), 4.06 (d, J=16.1 Hz, 1H), 4.03 (s, 3H), 3.95 (d, J=16.1 Hz, 1H), 1.42 (d, J=0.7 Hz, 3H); 13C NMR (101 MHz, DMSO-d₆) δ 164.99, 162.49, 161.81, 156.02, 149.08, 145.79, 142.01, 137.49, 133.63, 112.39, 111.55 (d, J=1.9 Hz, 1C), 107.06 (d, J=225.4 Hz, 1C), 60.40 (d,
\( J = 25.4 \text{ Hz}, 1 \mathrm{C} \), \( 56.41 \) (d, \( J = 3.9 \text{ Hz}, 1 \mathrm{C} \), 54.36, 26.02; LC-MS m/z 361 [M+H]^+. \) m. p.:
decomposition around 230 °C.

\textit{rac-N-[4-[(2R*,3R*)-5-Amino-2-fluoro-3-methyl-3,6-dihydro-2H-1,4-oxazin-3-yl]pyridin-2-yl]-5-methoxypyrazine-2-carboxamide (rac-\textit{(2R*,3R*)-12}).} \textit{Di-}\textit{tert}-butyldicarbonate (0.234 g, 1.07 mmol) was added portionwise to a stirred solution of intermediate \textit{rac-}(5R*,6R*)-56b (0.200 g, 0.892 mmol), triethylamine (1.00 mL, 19.1 mmol) and 4-dimethylaminopyridine (0.006 g, 0.05 mmol) in THF (1 mL) at rt for 3 h. The mixture was quenched with sat. aq. NaHCO₃ solution. The aqueous layer was extracted with EtOAc. The organic layer was dried (MgSO₄), filtered and the solvents evaporated \textit{in vacuo} to yield the corresponding Boc-amidine \textit{rac-}(5R*,6R*)-57b (0.185 g, 64% yield) which was used as such in the then reaction step.

HATU (70.3 mg, 0.185 mmol) was added to a solution of \textit{rac-}(5R*,6R*)-57b (50.0 mg, 0.154 mmol), 5-methoxypyrazine-2-carboxylic acid (28.5 mg, 0.185 mmol) and DIPEA (79.7 mL, 0.462 mmol) in dry DMF (2 mL), and the resulting mixture was stirred overnight at rt. The solvent was evaporated and the residue was taken up in DCM and 2 N Na₂CO₃. The organic layer was separated and the aqueous layer was extracted with DCM. The combined organic layers were dried (Na₂SO₄), filtered and concentrated. The residue was purified by flash column chromatography (silica; 7 N solution of ammonia in MeOH/DCM 0/100 to 10/90). The desired fractions were collected and concentrated \textit{in vacuo} yielding providing \textit{rac-}(5R*,6R*)-58b as a transparent glass (50.5 mg, 71%).

\textit{Rac-}(5R*,6R*)-58b (50 mg) was dissolved in TFA (5 mL) and the resulting mixture was stirred for 15 min before it was evaporated to dryness. Sat. aq. NaHCO₃ and DCM were added, the biphasic mixture was stirred gas evolution ceased, and then the organic layer was separated. The aqueous layer was extracted twice with DCM. The combined organic layers were separated,
dried (MgSO₄) and the solvent was evaporated providing \( \textit{rac-}(2R^*,3R^*)-\text{12} \) as off-white crystals, which were triturated with diethyl ether, filtered and dried \( \textit{in vacuo} \) at 50 °C (34.2 mg, 87%). \(^1\)H NMR (360 MHz, DMSO-\( \text{d}_6 \)) \( \delta \) 10.06 (s, 1H), 8.95 (d, \( J=1.2 \) Hz, 1H), 8.45 (d, \( J=1.2 \) Hz, 1H), 8.32 (d, \( J=5.3 \) Hz, 1H), 8.28 (d, \( J=1.6 \) Hz, 1H), 7.24 (dd, \( J=1.6,5.3 \) Hz, 1H), 6.13 (br s, 2H), 5.82 (br d, \( J=52.7 \) Hz, 1H), 4.10 (d, \( J=15.4 \) Hz, 1H), 4.03 (s, 3H), 4.00 (d, \( J=15.4 \) Hz, 1H), 1.45 (d, \( J=0.7 \) Hz, 3H); \(^1\)C NMR (101 MHz, DMSO-\( \text{d}_6 \)) \( \delta \) 162.05, 161.06, 156.45 (br s, 2C), 150.88, 148.06, 141.55, 136.66, 134.04, 118.02, 111.18, 106.36 (d, \( J=227.3 \) Hz, 1C), 58.49 (d, \( J=25.8 \) Hz, 1C), 56.69 (d, \( J=3.9 \) Hz, 1C), 54.44, 25.69; LC-MS m/z 361 [M+H]⁺.

\( \text{rac-}2\text{-Amino-2-(3-bromophenyl)-propionitrile (rac-\text{14a}).} \) Trimethylsilylcyanide (20 g, 200 mmol) was added to a stirred solution of 3-bromoacetophenone \( \text{13a} \) (20 g, 100 mmol) and NH₄Cl (11 g, 200 mmol) in 7 N NH₃/Methanol (400 mL). The mixture was stirred at rt for 4 days. Then the solvent was evaporated \( \textit{in vacuo} \) and the residue was taken up in EtOAc (100 mL). The solid was filtered and the filtrate was evaporated \( \textit{in vacuo} \) to yield intermediate \( \text{rac-\text{14a}} \) (20 g, 86% yield) which was used in the then step without further purification. \(^1\)H NMR (360 MHz, DMSO-\( \text{d}_6 \)) \( \delta \) 7.78 (t, \( J=1.8 \) Hz, 1H), 7.60 (ddd, \( J=0.9,1.7,7.9 \) Hz, 1H), 7.56 (ddd, \( J=1.1,1.9,7.9 \) Hz, 1H), 7.39 (t, \( J=8.0 \) Hz, 1H), 3.15 (br s, 2H), 1.64 (s, 3H); LC-MS m/z 225 [M+H]⁺.

\( \text{rac-2-Amino-2-(5-bromo-2-fluorophenyl)propanenitrile (rac-\text{14b}).} \) Starting from 1-(5-bromo-2-fluorophenyl)ethanone (46.3 g, 213 mmol) and following the same procedure as for \( \text{rac-\text{14a}} \) the corresponding aminonitrile \( \text{rac-\text{14b}} \) was obtained (30 g, 60% yield). \(^1\)H NMR (360 MHz, CDCl₃) \( \delta \) 7.80 (dd, \( J=2.2,6.9 \) Hz, 1H), 7.48 (ddd, \( J=2.5,4.2,8.6 \) Hz, 1H), 7.03 (dd, \( J=8.6,10.8 \) Hz, 1H), 2.18 (br s, 2H), 1.86 (s, 3H); LC-MS m/z 243 [M+H]⁺.
**rac-2-Amino-2-(3-bromophenyl)-propionic acid (rac-15a).** Intermediate rac-14a (47 g, 209 mmol) was dissolved in acetic acid (250 mL) and HCl (37% in water, 240 mL) was added. Then the mixture was refluxed for 16 h, after which the reaction mixture was concentrated in vacuo. Water was added and the aqueous layer was washed with EtOAc. Then the aqueous layer was adjusted to pH 7 by slow addition of 25% aq. NaOH solution. The resulting solid was filtered, washed with water and diethyl ether, and dried under vacuum at 50 °C to yield intermediate rac-15a (36 g, 71% yield); LC-MS m/z 242 [M-H]-. NMR shifts were in accordance to those reported previously.37

**rac-2-Amino-2-(5-bromo-2-fluorophenyl)propanoic acid (rac-15b).** Starting from rac-14b (19.9 g, 81.9 mmol) and following the same procedure as for rac-15a the corresponding aminonitrile rac-15b was obtained (14.6 g, 68% yield). 1H NMR (360 MHz, DMSO-d6) δ 7.78 (br s, 1H), 7.64 (dd, J=2.6, 6.9 Hz, 1H), 7.55 (ddd, J=2.6, 4.2, 8.6 Hz, 1H), 7.18 (dd, J=8.6, 11.2 Hz, 1H), 1.64 (s, 3H) (2H exchanged); LC-MS m/z 260 [M+H]+.

**rac-Methyl 2-amino-2-(3-bromophenyl)propanoate (rac-16a).** Intermediate rac-15a (36 g, 147 mmol) was dissolved in MeOH (1 L). Then sulphuric acid (103 mL, 1.93 mol) was added and the reaction mixture was stirred at reflux temperature overnight. After cooling to rt, the solvent was evaporated in vacuo. The residue was dissolved in water, basified with aq. NaHCO₃ to pH 8 and extracted with EtOAc. The combined organic layers were dried (MgSO₄), filtered and evaporated to provide rac-16a (36 g, 89% yield). 1H NMR (500 MHz, CDCl₃) δ 7.68 (t, J=1.7 Hz, 1H), 7.37-7.44 (m, 2H), 7.21 (t, J=7.8 Hz, 1H), 3.72 (s, 3H), 1.95 (br s, 2H), 1.68 (s, 3H); LC-MS m/z 258 [M+H]+.

**rac-Methyl 2-amino-2-(5-bromo-2-fluorophenyl)propanoate (rac-16b).** Starting from rac-15b (14.6 g, 55.5 mmol) and following the same procedure as for rac-16a the corresponding
amino ester rac-16b was obtained (13.9 g, 87%). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) ppm 1.27 - 1.51 (m, 3 H), 2.51 (br s, 2 H), 3.55 - 3.71 (m, 3 H), 7.12 (dd, \(J = 10.9, 8.7\) Hz, 1 H), 7.49 (ddd, \(J = 8.7, 4.4, 2.5\) Hz, 1 H), 7.87 (dd, \(J = 7.0, 2.5\) Hz, 1 H); LC-MS m/z 276 [M+H]⁺.

\((2R)-2\)-Amino-\(2\)-(3-bromophenyl)-propan-1-ol (\(2R-17a\)). Ester rac-16a (7.5 g, 29.1 mmol) was dissolved THF (200 mL) and cooled to -15 °C. Then, LAH (1 M in THF; 22 mL, 22 mmol) was added dropwise while stirring. The mixture was left warming up slowly to 0 °C during 1 h. Then more THF (150 mL) was added and sat. aq. Na₂SO₄ was added dropwise until hydrogen evolution ceased. Then anhydrous Na₂SO₄ was added and the stirring was continued overnight at rt. The mixture was filtered over celite, rinsed with THF and the solvent evaporated in vacuo. The crude product was purified by flash column chromatography (silica; 7 N solution of ammonia in MeOH/DCM 0/100 to 3/97). The desired fractions were collected and concentrated in vacuo to yield rac-17a (5.70 g, 85% yield) as an oil. \(^1\)H NMR (500 MHz, CDCl₃) \(\delta\) 7.64 (t, \(J = 1.9\) Hz, 1H), 7.38-7.44 (m, 2H), 7.25 (t, \(J = 7.8\) Hz, 1H), 3.64 (d, \(J = 10.7\) Hz, 1H), 3.59 (d, \(J = 10.7\) Hz, 1H), 1.81 (br s, 3H), 1.46 (s, 3H); LC-MS m/z 230 [M+H]⁺.

Intermediate rac-17a (18.0 g) was separated into the corresponding enantiomers by preparative SFC on a Chiralpak® Diacel AD x 250 mm column using CO₂ and MeOH with 0.2% iPrNH₂ as mobile phase to yield amino alcohol \(2R-17a\) (7.21 g, 40% yield). \(^1\)H NMR (400 MHz, CDCl₃) \(\delta\) 7.63 (t, \(J = 1.8\) Hz, 1H), 7.38-7.43 (m, 2H), 7.25 (t, \(J = 7.9\) Hz, 1H), 3.64 (d, \(J = 10.7\) Hz, 1H), 3.58 (d, \(J = 10.7\) Hz, 1H), 1.78 (br s, 3H), 1.45 (s, 3H); LC-MS m/z 230 [M+H]⁺; \([\alpha]^{20}_D = -14.9\) (c = 0.29 in MeOH).

\((2R)-2\)-Amino-\(2\)-(5-bromo-2-fluorophenyl)propan-1-ol (\(2R-17b\)). Starting from rac-16b (14.2 g, 48.4 mmol) and following the same procedure as for rac-17a the corresponding aminonitrile rac-17b was obtained (12.0 g, 96% yield). \(^1\)H NMR (400 MHz, CDCl₃) \(\delta\) 7.63 (dd,
$J=2.5, 7.4 \text{ Hz, 1H}$, 7.36 (ddd, $J=2.5, 4.2, 8.5 \text{ Hz, 1H}$), 6.93 (dd, $J=8.5, 11.8 \text{ Hz, 1H}$), 3.82 (d, $J=10.6 \text{ Hz, 1H}$), 3.64 (dd, $J=1.2, 10.6 \text{ Hz, 1H}$), 1.89 (br s, 3H), 1.48 (d, $J=0.9 \text{ Hz, 3H}$); LC-MS m/z 248 [M+H$^+$].

rac-17b (16.6 g, 66.9 mmol) was purified by chiral SFC on (CHIRALPAK AD-H 5µm 250x20 mm). Mobile phase (0.3% isopropylamine, 80% CO$_2$, 20% MeOH), yielding 5.3 g 2R-17b (32% yield). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.62 (dd, $J=2.6, 7.2 \text{ Hz, 1H}$), 7.36 (ddd, $J=2.5, 4.3, 8.6 \text{ Hz, 1H}$), 6.93 (dd, $J=8.6, 11.8 \text{ Hz, 1H}$), 3.83 (d, $J=10.7 \text{ Hz, 1H}$), 3.65 (br d, $J=10.7 \text{ Hz, 1H}$), 2.61 (br s, 3H), 1.48 (s, 3H); LC-MS m/z 248 [M+H$^+$]; [$\alpha$]$^20_D$ = +2.0 (c = 0.61 in DMF).

(5R)-5-(3-Bromophenyl)-5-methylmorpholin-3-one (5R-18a). Chloroacetyl chloride (0.55 mL, 6.95 mmol) was added dropwise to a stirred solution of intermediate 2R-17a (1.6 g, 6.95 mmol) in THF (60 mL) and diisopropylethyl amine (1.44 mL, 8.34 mmol) at -78 ºC. The mixture was stirred for 30 min at -78 ºC. Then potassium tert-butoxide (1.95 g, 17.38 mmol) was added and the mixture was stirred at -15ºC and left warming up to 0 ºC during 90 min. The mixture was diluted with sat. aq. NH$_4$Cl and extracted with DCM. The organic layer was separated, dried (Na$_2$SO$_4$), filtered and the solvents evaporated in vacuo. The crude product was triturated with Et$_2$O, filtered and dried to yield intermediate 5R-18a (1.65 g, 88% yield) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$) δ 7.54 (t, $J=1.9 \text{ Hz, 1H}$), 7.46 (td, $J=1.3, 7.7 \text{ Hz, 1H}$), 7.31-7.35 (m, 1H), 7.28 (t, $J=7.5 \text{ Hz, 1H}$), 6.41 (br s, 1H), 4.19-4.28 (m, 2H), 3.82 (d, $J=11.8 \text{ Hz, 1H}$), 3.72 (d, $J=11.8 \text{ Hz, 1H}$), 1.67 (s, 3H); LC-MS m/z 311 [M+H$^+$]; [$\alpha$]$^20_D$ = −71.6 (c = 0.62 in DMF); m. p. = 135.2 ºC.

(5R)-5-(5-Bromo-2-fluorophenyl)-5-methylmorpholin-3-one (5R-18b). Starting from 2R-17b (5.1 g, 20.6 mmol) and following the same procedure as for 5R-18a the corresponding morpholinone 5R-18b was obtained (5.9 g, 89%). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.51 (dd, $J=2.6,
7.2 Hz, 1H), 7.43 (ddd, J = 2.6, 4.3, 8.7 Hz, 1H), 6.98 (dd, J = 8.7, 11.6 Hz, 1H), 6.60 (br s, 1H),
4.30 (d, J = 11.8 Hz, 1H), 4.15-4.26 (m, 2H), 3.72 (d, J = 11.8 Hz, 1H), 1.64 (s, 3H); LC-MS m/z
288 [M+H]+; [α]20 D = -53.4 (c = 0.67 in DMF); m. p. = 161.9 ºC.

(5R)-5-(3-Bromophenyl)-5-methylmorpholine-3-thione (5R-19a). THF (40 mL) was added
to a mixture of intermediate 5R-18a (1.14 g, 3.92 mmol) and phosphorus pentasulfide (0.704 g,
3.17 mmol) at rt. The mixture was stirred at 50 ºC for 50 min. Then the mixture was cooled to rt
and filtered over cotton and evaporated in vacuo. The crude product was purified by flash
column chromatography (silica; DCM). The desired fractions were collected and evaporated in
vacuo to yield the thioamide 5R-19a (1.05 g, 93% yield) as a yellow solid. 1H NMR (500 MHz,
CDCl3) δ 8.44 (br s, 1H), 7.45-7.52 (m, 2H), 7.26-7.33 (m, 2H), 4.56-4.65 (m, 2H), 3.86 (d,
J = 11.8 Hz, 1H), 3.77 (d, J = 7.0 Hz, 1H), 1.71 (s, 3H); LC-MS m/z 286 [M+H]+; [α]20 D = -190.0
(c = 0.6 in DMF).

(5R)-5-(5-Bromo-2-fluorophenyl)-5-methylmorpholine-3-thione (5R-19b). Starting from
5R-18b (5.3 g, 18.4 mmol) and following the same procedure as for 5R-19a the corresponding
thione 5R-19b was obtained (4.1 g, 72% yield). 1H NMR (400 MHz, CDCl3) δ 8.48 (br s, 1H),
7.46 (ddd, J = 2.5, 4.4, 8.5 Hz, 1H), 7.41 (dd, J = 2.4, 7.0 Hz, 1H), 7.01 (dd, J = 8.5, 11.6 Hz, 1H),
4.62 (d, J = 18.4 Hz, 1H), 4.54 (d, J = 18.4 Hz, 1H), 4.33 (d, J = 12.0 Hz, 1H), 3.74 (d, J = 12.0 Hz,
1H), 1.69 (d, J = 0.9 Hz, 3H); LC-MS m/z 304 [M+H]+; [α]20 D = -167.3 (c = 0.6 in DMF).

(5R)-5-(3-Bromophenyl)-5-methyl-5,6-dihydro-2H-1,4-oxazin-3-amine trifluoroacetate
salt (5R-20a). The thioamide 5R-19a (0.205 g, 0.716 mmol) and 32% aqueous ammonia solution
(12 mL) were stirred in a sealed tube at 60 ºC for 4 h. After cooling, the mixture was diluted with
water and extracted with DCM. The organic layer was separated, dried (Na2SO4), filtered and the
solvent evaporated in vacuo. DCM (15 mL) and TFA (0.25 mL) were added and the mixture was
concentrated in vacuo. To this residue, Et$_2$O and heptane were added and evaporated.

Diisopropyl ether was added, and the suspension was sonicated for 20 min and then stirred overnight at rt. The white precipitate was filtered and washed with diisopropyl ether and dried in vacuo to yield intermediate 5$R$-20a (0.19 g, 69% yield) as a white solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.57 (t, $J$=1.7 Hz, 1H), 7.36 (td, $J$=0.9, 7.9 Hz, 1H), 7.32 (br d, $J$=7.90 Hz, 1H), 7.20 (t, $J$=7.90 Hz, 1H), 4.13 (d, $J$=15.5 Hz, 1H), 4.05 (d, $J$=15.5 Hz, 1H), 3.68 (d, $J$=11.3 Hz, 1H), 3.56 (d, $J$=11.3 Hz, 1H), 3.32 (br s, 2H), 1.50 (s, 3H); LC-MS m/z 269 [M+H]$^+; $[\alpha]^{20}_D = -112.6$ (c = 0.66 in DMF).

(5$R$)-5-(5-Bromo-2-fluorophenyl)-5-methyl-5,6-dihydro-2$H$-1,4-oxazin-3-amine (5$R$-20b).

Starting from 5$R$-19b (4.1 g, 13.5 mmol) and following the same procedure as for 5$R$-20a the corresponding oxazinamn 5$R$-20b was obtained (4.1 g, 87% yield). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.70 (dd, $J$=2.6, 6.9 Hz, 1H), 7.32 (ddd, $J$=2.6, 4.1, 8.6 Hz, 1H), 6.90 (dd, $J$=8.5, 11.4 Hz, 1H), 4.21 (br s, 2H), 4.11 (d, $J$=15.6 Hz, 1H), 4.03 (d, $J$=15.6 Hz, 1H), 3.86 (dd, $J$=1.4, 11.3 Hz, 1H), 3.77 (d, $J$=11.5 Hz, 1H), 1.54 (d, $J$=0.9 Hz, 3H); LC-MS m/z 287 [M+H]$^+$.

(5$R$)-5-(3-Aminophenyl)-5-methyl-5,6-dihydro-2$H$-1,4-oxazin-3-amine (5$R$-21a). Toluene (1.5 mL) was added to a mixture of intermediate 5$R$-20a (0.05 g, 0.13 mmol), tris(dibenzylideneacetone)dipalladium(0) (0.012 g, 0.013 mmol), rac-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (0.024 g, 0.04 mmol) and sodium tert-butoxide (0.031 g, 0.326 mmol) in a sealed tube and under nitrogen at rt. The mixture was flushed with nitrogen for a few min and then benzophenone imine (0.028 mL, 0.17 mmol) was added and the mixture was stirred at 80°C for 7 h. After cooling, a mixture of 1 N HCl/THF (1/1.4 mL) was added and the mixture was stirred at rt overnight. The mixture was diluted with water and washed with EtOAc. The aqueous layer was basified with sat. aq. Na$_2$CO$_3$ and extracted with DCM/EtOH 9/1 (10
times). The combined organic layers were dried (Na₂SO₄), filtered and the solvents evaporated in vacuo. The crude product was purified by flash column chromatography (silica; 7 N solution of ammonia in MeOH/DCM 0/100 to 8/92). The desired fractions were collected and concentrated in vacuo to yield intermediate 5R-21a (0.012 g, 45% yield) as an oil. ¹H NMR (500 MHz, CDCl₃) δ 7.12 (t, J=7.9 Hz, 1H), 6.75-6.80 (m, 2H), 6.56 (br d, J=7.6 Hz, 1H), 4.13 (d, J=15.4 Hz, 1H), 4.06 (d, J=15.4 Hz, 1H), 3.68 (d, J=11.6 Hz, 1H), 3.60-3.75 (m, 2H), 3.57 (d, J=11.6 Hz, 1H), 3.09 (br s, 2H), 1.50 (s, 3H); LC-MS m/z 206 [M+H]⁺.

(5R)-5-(5-Amino-2-fluorophenyl)-5-methyl-5,6-dihydro-2H-1,4-oxazin-3-amine (5R-21b).

Toluene (1.5 mL) was added to a mixture of intermediate 5R-20b (3.2 g, 11.1 mmol), tris(dibenzylideneacetone)dipalladium(0) (1.02 g, 1.11 mmol), rac-2,2’-bis(diphenylphosphino)-1,1’-binaphthyl (2.08 g, 3.34 mmol) and sodium tert-butoxide (1.93 g, 20.1 mmol) in a sealed tube and under nitrogen at rt. The mixture was flushed with nitrogen for a few min and then benzophenone imine (3.74 mL, 22.3 mmol) was added and the mixture was stirred at 100 ºC for 2 h. After cooling the mixture was diluted with water and extracted with DCM. The organic layer was dried (Na₂SO₄), filtered and the solvents concentrated in vacuo. The crude product was purified by flash column chromatography (silica; 7 N solution of ammonia in MeOH/DCM 0/100 to 3/97). The desired fractions were collected and concentrated in vacuo to yield the intermediate benzophenone imine as a yellow foam (3.6 g, 83% yield). This imine (3.6 g, 9.29 mmol) was dissolved in HCl (0.6 M in 2-propanol, 66 mL) and the resulting mixture was stirred at rt for 30 min. Diethyl ether (400 mL) was added and the precipitated product (HCl salt of 5R-21b) was filtered and washed with ether. ¹H NMR (400 MHz, DMSO-d₆) δ ppm 1.61 (s, 3 H), 3.81 - 3.89 (m, 1 H), 4.08 (d, J=12.0 Hz, 1 H), 4.48 - 4.61 (m, 2 H), 6.63 - 6.88 (m, 3 H), 7.03 (br dd, J=12.1, 8.4 Hz, 2 H), 8.62 (s, 1 H), 9.26 (s, 1 H), 10.76 (s, 1 H). Since the HCl salt of 5R-21b...
was hygroscopic, the base was liberated prior to the next step. Hence 5R-21b was dissolved in MeOH and excess 7 N solution of ammonia in MeOH was added. Then all volatiles were evaporated in vacuo. The residue was purified by flash column chromatography (silica; 7 N solution of ammonia in MeOH 1/99 to 12/88). The desired fractions were collected and concentrated in vacuo to yield 5R-21b as an off white solid (1.6 g, 77%). LC-MS m/z 224 [M+H]+.

(3R)-3-(5-Bromo-2-fluorophenyl)-3-methylmorpholine-2,5-dione (3R-22). Enantiopure 5R-15a was obtained via preparative SFC of rac-15a on a Chiralpak® Diacel AD x 250 mm column using CO2 and MeOH with 0.2% iPrNH2 as mobile phase. To a cooled solution of intermediate 5R-15b (41.3 g, 145 mmol) in water (150 mL), a solution of chloroacetyl chloride (24 mL, 304.5 mmol) in 1,4-dioxane (75 mL) was added dropwise. Simultaneously, NaOH (5 M in water, 29 mL) was added to adjust the pH at 10-11. The reaction mixture was stirred at rt for 2 h. The organic layer was separated, and the aqueous layer extracted with Et2O. Then the aqueous layer was acidified with HCl (6 M in water) until pH 2. The precipitated white solid was collected by filtration, washed with water and dried. The obtained intermediate (42 g, 124 mmol) and NaHCO3 (20.8 g, 248 mmol) were dissolved in DMF (1000 mL), and the reaction mixture was stirred at 80 ºC for 3 h. The mixture was partially concentrated in vacuo, cooled to rt and then filtered over celite. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography (silica; MeOH/DCM 0/100 to 5/95). The desired fractions were collected and concentrated in vacuo to yield intermediate 3R-22 (36 g, 96% yield). 1H NMR (400 MHz, DMSO-d6) δ 9.12 (br s, 1H), 7.68 (ddd, J=2.5, 4.4, 8.7 Hz, 1H), 7.63 (dd, J=2.5, 6.9 Hz, 1H), 7.29 (dd, J=8.7, 11.2 Hz, 1H), 4.97 (d, J=16.4 Hz, 1H), 4.74 (d, J=16.4 Hz, 1H), 1.84 (s, 3H); LC-MS m/z 302 [M+H]+; [α]20 D = +31.6 (c = 0.53 in DMF); m. p. = 162.6 ºC.
(5R)-5-(5-Bromo-2-fluorophenyl)-6-fluoro-5-methylmorpholin-3-one (5R-23). A solution of intermediate 3R-22 (10 g, 21.5 mmol) in THF (105 mL) was cooled to -78 °C under N₂ atmosphere. Then, diisobutylaluminium hydride (1 M in toluene, 43 mL, 43 mmol) was slowly added. The reaction mixture was allowed to reach rt over 2 h. The reaction mixture was cooled down to 0 °C and quenched by the slow addition of aq. 1 N HCl solution. The mixture was then extracted with EtOAc, the organic layers were separated, dried (Na₂SO₄), filtered and the solvent evaporated in vacuo to yield the hydroxymorpholinone (6.6 g, 100% yield) as a 80:20 mixture of diastereoisomers which was used as such in the following reaction. The crude hydroxymorpholinone (6.3 g, 20.7 mmol) was dissolved in DCM (84 mL) and the reaction was cooled down to 0 °C. Then DAST (3 mL, 24.9 mmol) was added dropwise. After 20 min at 0 °C the reaction mixture was quenched with sat. aq. NaHCO₃ and extracted with DCM. The combined organic layers were dried (MgSO₄), filtered, and the solvent evaporated in vacuo. The crude product was suspended in diisopropyl ether, filtered and dried in vacuo at 60°C to yield intermediate 5R-23 (4.2 g, 66% yield) as an 80:20 mixture of 6R and 6S isomers. ¹H NMR (400 MHz, DMSO-d₆) δ 8.97 (s, 1H), 7.55-7.68 (m, 1.2H), 7.45 (dd, J=2.5, 7.3 Hz, 0.8H), 7.29 (dd, J=8.5, 11.8 Hz, 0.8H), 7.25 (dd, J=8.5, 12.00 Hz, 0.2H), 6.03 (d, J=50.9 Hz, 0.8H), 6.01 (d, J=50.7 Hz, 0.2H), 4.28 (d, J=16.3 Hz, 0.2H), 4.20 (d, J=16.4 Hz, 0.2H), 4.19 (d, J=16.6 Hz, 0.8H), 4.11 (d, J=16.6 Hz, 0.8H), 1.64 (s, 0.6H), 1.56 (s, 2.4H); LC-MS m/z 306 [M+H⁺].

(5R)-5-(5-Bromo-2-fluorophenyl)-6-hydroxy-5-methyl-6-(trifluoromethyl)morpholin-3-one (5R-24). To a solution of intermediate 3R-22 (11.6 g, 38.5 mmol) in THF (117 mL) was added tetrabutylammonium difluorotriphenylsilicate (2.08 g, 3.85 mmol). Then (trifluoromethyl)trimethylsilane (12.5 mL, 84.6 mmol) was added dropwise, and the reaction mixture was stirred at rt for 20 min. The mixture was quenched with aqueous NaCl and extracted...
with EtOAc. The combined organic layers were dried (MgSO₄), filtered and concentrated in vacuo to yield intermediate 5R-24 (14 g, 98% yield) as a 3:1 6R/6S mixture, which was used as such in the following step. ¹H NMR (500 MHz, DMSO-d₆) δ 8.51 (s, 1H), 8.39 (br s, 1H), 7.57-7.64 (m, 1H), 7.46 (br d, J=5.5 Hz, 1H), 7.18 (dd, J=8.8, 12.9 Hz, 1H), 4.31 (d, J=16.5 Hz, 1H), 4.17 (d, J=16.5 Hz, 1H), 1.72 (s, 3H); LC-MS m/z 372 [M+H]⁺; m. p. = 191.2 ºC.

(5R)-5-(5-Bromo-2-fluorophenyl)-6-fluoro-5-methyl-6-(trifluoromethyl)morpholin-3-one (5R-25). Intermediate 5R-24 (3.35 g, 9.00 mmol) was suspended in DCM (25 mL) and after cooling the reaction mixture at 0 ºC, DAST (1.32 mL, 10.80 mmol) was added dropwise. The reaction mixture was stirred at 0 ºC for 2 h and then quenched with sat. aq. NaHCO₃. The organic layer was separated and the aqueous layer was extracted with DCM. The combined organic layers were dried (MgSO₄), filtered and the solvent evaporated in vacuo. The crude product was purified by flash column chromatography (silica; MeOH/DCM 0/100 to 1/99). The desired fractions were collected and concentrated in vacuo to yield intermediate 5R-25 (3.15 g, 93% yield) as a 25:75 6R/6S mixture as an off white solid. ¹H NMR (500 MHz, CDCl₃) δ 7.61 (td, J=2.1, 7.1 Hz, 0.25H), 7.48-7.53 (m, 1H), 7.45 (dd, J=2.3, 7.2 Hz, 1H), 7.13 (br s, 1H), 7.00 (dd, J=8.7, 12.1 Hz, 0.25H), 6.99 (dd, J=8.7, 12.4 Hz, 0.75H), 4.52-4.59 (m, 1.25H), 4.48 (d, J=15.9 Hz, 0.75H), 2.00 (s, 0.75H), 1.96 (s, 2.25H); LC-MS m/z 374 [M+H]⁺.

(5R)-5-(5-Bromo-2-fluorophenyl)-6-chloro-5-methyl-6-(trifluoromethyl)morpholin-3-one (5R-26). Intermediate 5R-24 (14 g, 37.6 mmol) was dissolved in DCM (600 mL) and cooled to 0 ºC and then thionyl chloride (11.2 mL, 150 mmol) was added dropwise. The reaction mixture was stirred for 30 min at 0 ºC and then pyridine (18.2 mL, 225.7 mmol) was added. After 30 min the reaction was hydrolyzed with aq. 1 N HCl and then extracted with DCM. The organic layers were separated, dried (MgSO₄), filtered and evaporated in vacuo. The crude product was purified
by flash column chromatography (silica; 7 N solution of ammonia in MeOH/DCM 0/100 to 2/98). The desired fractions were collected and concentrated in vacuo to yield intermediate 5R-26 (6 g, 41% yield) as a mixture of diastereoisomers. $^1$H NMR (400 MHz, DMSO-d$_6$) δ 9.10 (br s, 1H), 7.69 (ddd, $J$=2.5, 3.9, 8.8 Hz, 1H), 7.48-7.62 (m, 1H), 7.27 (dd, $J$=8.8, 12.9 Hz, 1H), 4.76 (br d, $J$=16.2 Hz, 1H), 4.42 (dd, $J$=0.9, 16.8 Hz, 1H), 1.90 (s, 3H); LC-MS m/z 390 [M+H]$^+$. m. p. = 147.0 °C.

(5R)-5-(5-Bromo-2-fluorophenyl)-5-methyl-6-(trifluoromethyl)morpholin-3-one (5R-27).

To a solution of intermediate 5R-26 (3 g, 7.68 mmol) in acetic acid (136 mL), zinc (1.26 g, 19.2 mmol) was added. The reaction mixture was then stirred at 80 °C for 3 h, after that the reaction was filtered hot and concentrated in vacuo. The residue was dissolved in DCM and washed with ammonium hydroxide solution. The organic phase was separated, dried (MgSO$_4$) and the solvent concentrated in vacuo. The crude product purified by column chromatography (silica; 7 N solution of ammonia in MeOH/DCM 0/100 to 3/97). The desired fractions were collected and concentrated in vacuo to yield intermediate 5R-27 (2.7 g, 99% yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.42-7.52 (m, 2H), 7.16 (s, 1H), 6.99 (dd, $J$=9.1, 11.9 Hz, 1H), 4.48 (d, $J$=17.1 Hz, 1H), 4.34 (d, $J$=17.1 Hz, 1H), 1.92 (d, $J$=0.7 Hz, 3H); LC-MS m/z 356 [M+H]$^+$. 

(5R)-5-(5-Bromo-2-fluorophenyl)-6-fluoro-5-methylmorpholine-3-thione (5R-28).

THF (100 mL) was added to a mixture of intermediate 5R-23 (4.20 g, 13.7 mmol) and phosphorus pentasulfide (3.66 g, 16.5 mmol) at rt. The mixture was stirred at 70 °C for 3 h. Then the mixture was cooled to rt and filtered over cotton and evaporated in vacuo. The crude product was purified by flash column chromatography (silica; EtOAc/Heptane 0/1 to 1/0). The desired fractions were collected and evaporated in vacuo to yield the thioamide 5R-28 as a yellow solid (3 g, 68% yield, as a 62:38 6R/6S mixture).
(5R)-5-(5-Bromo-2-fluorophenyl)-6-fluoro-5-methyl-6-(trifluoromethyl)morpholine-3-thione (5R-29). Starting from 5R-25 (3.05 g, 8.15 mmol) and following the same procedure as for 5R-28 the corresponding thione 5R-29 was obtained (2.80 g, white foam, 88% yield) as a 25:75 6R/6S mixture. \(^1\)H NMR (500 MHz, DMSO-d\(_6\)) \(\delta\) 11.44 (br s, 0.25H), 11.30 (br s, 0.75H), 7.69-7.77 (m, 1H), 7.67 (br d, \(J=6.9\) Hz, 0.25H), 7.60 (dd, \(J=2.3, 7.2\) Hz, 0.75H), 7.30 (dd, \(J=8.8, 12.6\) Hz, 0.75H), 7.27 (dd, \(J=8.9, 12.7\) Hz, 0.25H), 5.07 (d, \(J=18.2\) Hz, 0.25H), 5.05 (d, \(J=17.9\) Hz, 0.75H), 4.78 (d, \(J=18.2\) Hz, 0.25H), 4.74 (d, \(J=18.2\) Hz, 0.75H), 1.92 (br s, 2.25H), 1.90 (br s, 0.75H); LC-MS m/z 390 [M+H]^+.

(5R)-5-(5-Bromo-2-fluoro-phenyl)-5-methyl-6-(trifluoromethyl)morpholine-3-thione (5R-30). Starting from 5R-27 (9.8 g, 27.5 mmol) and following the same procedure as for 5R-28 the corresponding thioamide 5R-30 was obtained (9.4 g, white foam, 92% yield) as a 80:20 6R/6S mixture.

(5R, 6S)-5-(5-Bromo-2-fluorophenyl)-6-fluoro-5-methyl-2,6-dihydro-1,4-oxazin-3-amine ((5R,6S)-31) and (5R, 6R)-5-(5-bromo-2-fluorophenyl)-6-fluoro-5-methyl-2,6-dihydro-1,4-oxazin-3-amine ((5R,6R)-31). The crude thione 5R-28 (3.0 g, 9.3 mmol) was dissolved in 7 N solution of ammonia in MeOH (150 mL) and the reaction mixture was stirred in a sealed tube at 60 °C for 18 h. Next, the solvent was evaporated and the residue re-dissolved in 7 N solution of ammonia in MeOH (150 mL) and stirred in a sealed tube at 60 °C for another 18 h. Then the solvent was evaporated and the crude product purified by column chromatography (silica; 7 N solution of ammonia in MeOH/DCM 0/1 to 1/9). The desired fractions were collected and concentrated \textit{in vacuo} to yield the amidine (5R,6R)-31 (1.6 g, 56%), \(^1\)H NMR (360 MHz, CDCl\(_3\)) \(\delta\) 8.00 (dd, \(J=2.6, 6.9\) Hz, 1H), 7.35 (ddd, \(J=2.7, 4.3, 8.7\) Hz, 1H), 6.91 (dd, \(J=8.6, 11.5\) Hz, 1H), 6.04 (dd, \(J=1.8, 51.6\) Hz, 1H), 4.34 (br s, 2H), 4.26 (d, \(J=15.3\) Hz, 1H), 4.13 (d, \(J=15.3\) Hz, 1H),
1.51 (t, J=1.5 Hz, 3H); LC-MS m/z 305 [M+H]+; and (5R,6S)-31 (0.27 g, 9%); \(^{1}\)H NMR (360 MHz, CDCl\(_3\)) \(\delta\) 7.44 (dd, J=2.6, 6.9 Hz, 1H), 7.37 (ddd, J=2.7, 4.3, 8.7 Hz, 1H), 6.93 (dd, J=8.6, 11.5 Hz, 1H), 5.98 (d, J=51.9 Hz, 1H), 4.43 (br s, 2H), 4.25 (d, J=15.7 Hz, 1H), 3.99 (d, J=15.7 Hz, 1H), 1.61 (t, J=2.0 Hz, 3H); LC-MS m/z 305 [M+H]+.

(5R,6S)-5-(5-Bromo-2-fluorophenyl)-6-fluoro-5-methyl-6-(trifluoromethyl)-5,6-dihydro-2\(H\)-1,4-oxazin-3-amine ((5R,6S)-32) and (5R,6R)-5-(5-bromo-2-fluorophenyl)-6-fluoro-5-methyl-6-(trifluoromethyl)-5,6-dihydro-2\(H\)-1,4-oxazin-3-amine ((5R,6R)-32). Starting from 5R-27 (10 g, 25.6 mmol) and following the same procedure as for 5R-28 both diastereomers of 32 were separately isolated: (5R,6S)-32 (2.0 g, 21% yield), \(^{1}\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.44 (dd, J=2.5, 7.3 Hz, 1H), 7.40 (ddd, J=2.5, 4.0, 8.5 Hz, 1H), 6.89 (dd, J=8.7, 12.4 Hz, 1H), 4.25-4.55 (m, 4H), 1.79 (s, 3H); LC-MS m/z 305 [M+H]+, and (5R,6R)-32 (0.75 g, 8% yield), \(^{1}\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.58 (td, J=2.4, 7.0 Hz, 1H), 7.38 (ddd, J=2.6, 4.0, 8.7 Hz, 1H), 6.89 (dd, J=8.8, 12.0 Hz, 1H), 4.50 (d, J=15.4 Hz, 1H), 4.43 (d, J=15.3 Hz, 1H), 4.35 (br s, 2H), 1.74-1.81 (m, 3H); LC-MS m/z 373 [M+H]+.

(5R,6R)-5-(5-Bromo-2-fluorophenyl)-5-methyl-6-(trifluoromethyl)-2,6-dihydro-1,4-oxazin-3-amine ((5R,6R)-33) and (5R,6S)-5-(5-Bromo-2-fluorophenyl)-5-methyl-6-(trifluoromethyl)-2,6-dihydro-1,4-oxazin-3-amine ((5R,6S)-33). Starting from 5R-30 (6 g, 16.1 mmol) and following the same procedure as for (5R,6R)-31 the corresponding amidine diastereomers of 33 were separately isolated: (5R,6R)-33 (3.4 g, 59% yield), \(^{1}\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.02 (dd, J=2.6, 7.1 Hz, 1H), 7.36 (ddd, J=2.4, 4.2, 8.7 Hz, 1H), 6.89 (dd, J=8.7, 11.5 Hz, 1H), 4.59 (dq, J=1.1, 8.3 Hz, 1H), 4.32 (br s, 2H), 4.20 (d, J=0.8 Hz, 2H), 1.64 (d, J=1.2 Hz, 3H); LC-MS m/z 355 [M+H]+; \([\alpha]\)\(_D\) = -66.5 (c = 1.23 in DMF), and (5R,6S)-33 (0.75 g, 13% yield), which was isolated in impure form.
(5R,6R)-5-(5-Amino-2-fluorophenyl)-6-fluoro-5-methyl-5,6-dihydro-2H-1,4-oxazin-3-amine ((5R,6R)-34). Bromide (5R,6R)-31 (1.6 g, 5.24 mmol) was combined with NaN₃ (0.85 g, 13 mmol), CuI (1.25 g, 6.5 mmol) and Na₂CO₃ (1.1 g, 10.5 mmol) in DMSO (75 mL) and the reaction was degassed. After that, N,N’-dimethylethylenediamine (1 mL, 9.1 mmol) was added and the mixture was heated at 110 °C for 4 h. The reaction mixture was poured into DCM. Ammonium hydroxide (28% in water) was added and the organic layer was separated and washed three times with ammonium hydroxide. Then the organic layer was dried (MgSO₄), filtered and concentrated in vacuo. The crude product was purified by column chromatography (silica; 7 N solution of ammonia in MeOH in DCM 0/100 to 10/90). The desired fractions were collected and concentrated in vacuo to yield the corresponding aniline (5R,6R)-34 (0.3 g, 24% yield), ¹H NMR (400 MHz, CDCl₃) δ 6.83 (dd, J=8.5, 11.8 Hz, 1H), 6.62 (dd, J=2.9, 6.6 Hz, 1H), 6.53 (td, J=3.3, 8.5 Hz, 1H), 6.02 (d, J=52.5 Hz, 1H), 4.42 (br s, 2H), 4.22 (dd, J=0.7, 15.5 Hz, 1H), 3.97 (d, J=15.5 Hz, 1H), 3.56 (br s, 2H), 1.60 (t, J=2.0 Hz, 3H); LC-MS m/z 242 [M+H]⁺.

(5R,6S)-5-(5-Amino-2-fluorophenyl)-6-fluoro-5-methyl-5,6-dihydro-2H-1,4-oxazin-3-amine ((5R,6S)-34). Starting from (5R,6S)-31 (0.27 g) and following the same procedure as for (5R,6R)-34 the corresponding (5R,6S)-34 was obtained (0.040 g, 15% yield) ¹H NMR (360 MHz, CDCl₃) δ 7.22 (dd, J=3.1, 6.8 Hz, 1H), 6.81 (dd, J=8.4, 11.7 Hz, 1H), 6.45-6.54 (m, 1H), 6.05 (dd, J=1.5, 51.9 Hz, 1H), 4.70 (br s, 2H), 4.25 (d, J=15.5 Hz, 1H), 4.11 (d, J=15.5 Hz, 1H), 3.56 (br s, 2H), 1.48-1.53 (m, 3H).

(5R,6S)-5-(5-Amino-2-fluorophenyl)-6-fluoro-5-methyl-6-(trifluoromethyl)-5,6-dihydro-2H-1,4-oxazin-3-amine ((5R,6S)-35). Starting from (5R,6S)-32 (2.0 g, 5.36 mmol) and following the same procedure as for (5R,6R)-34 the corresponding (5R,6S)-35 was obtained (1.3
g, 78% yield). $^1$H NMR (360 MHz, CDCl$_3$) $\delta$ 6.80 (dd, $J$=8.6, 12.6 Hz, 1H), 6.64 (dd, $J$=2.2, 6.6 Hz, 1H), 6.59 (td, $J$=3.0, 8.2 Hz, 1H), 4.36-4.49 (m, 2H), 4.31 (br s, 2H), 3.51 (br s, 2H), 1.73-1.84 (m, 3H); [a]$^\text{20}_D$ = +95.8 ($c$ = 0.3 in MeOH).

(5$R$,6$R$)-5-(5-Amino-2-fluorophenyl)-6-fluoro-5-methyl-6-(trifluoromethyl)-5,6-dihydro-2H-1,4-oxazin-3-amine ((5$R$,6$R$)-35). Starting from (5$R$,6$R$)-32 (1.1 g, 2.95 mmol) and following the same procedure as for (5$R$,6$R$)-34 the corresponding (5$R$,6$R$)-35 was obtained (0.75 g, 82% yield). $^1$H NMR (360 MHz, CDCl$_3$) $\delta$ 6.73-6.85 (m, 2H), 6.58 (td, $J$=3.2, 8.6 Hz, 1H), 4.49 (d, $J$=15.7 Hz, 1H), 4.42 (d, $J$=15.7 Hz, 1H), 4.31 (br s, 2H), 3.51 (br s, 2H), 1.77 (s, 3H); LC-MS m/z 310 [M+H]$^+$.

(5$R$,6$R$)-5-(5-Amino-2-fluorophenyl)-5-methyl-6-(trifluoromethyl)-5,6-dihydro-2H-1,4-oxazin-3-amine ((5$R$,6$R$)-36). Starting from (5$R$,6$R$)-33 (3.4 g, 9.57 mmol) and following the same procedure as for (5$R$,6$R$)-34 the corresponding (5$R$,6$R$)-36 was obtained (2.5 g, 90% yield). $^1$H NMR (360 MHz, CDCl$_3$) $\delta$ 7.20 (dd, $J$=2.9, 6.6 Hz, 1H), 6.80 (dd, $J$=8.6, 11.9 Hz, 1H), 6.54 (td, $J$=3.6, 8.6 Hz, 1H), 4.61 (q, $J$=8.4 Hz, 1H), 4.27 (br s, 2H), 4.20 (s, 2H), 3.56 (br s, 2H), 1.64 (s, 3H); LC-MS m/z 292 [M+H]$^+$; [a]$^\text{20}_D$ = -94.9 ($c$ = 0.42 in MeOH).

(5$R$,6$S$)-5-(5-Amino-2-fluorophenyl)-5-methyl-6-(trifluoromethyl)-5,6-dihydro-2H-1,4-oxazin-3-amine ((5$R$,6$S$)-36). Starting from (5$R$,6$S$)-33 (0.3 g, 0.84 mmol) and following the same procedure as for (5$R$,6$R$)-34 the corresponding (5$R$,6$S$)-36 was obtained (0.10 g, 41% yield). LC-MS m/z 292 [M+H]$^+$.

Methyl 2-(5-bromo-2-fluorophenyl)-2-oxoacetate (37). Thionyl chloride (37 mL, 510 mmol) was added dropwise to a stirred solution of (2)-(5-bromo-2-fluoro-phenyl)-2-oxo-acetic acid (42 g, 170 mmol) in MeOH (456 mL) at 0 °C. The mixture was refluxed for 18 h. The solvents were evaporated in vacuo and the residue was partitioned between sat. aq. Na$_2$CO$_3$ and DCM. The
organic layer was separated, dried (MgSO₄), filtered and concentrated in vacuo to yield 37 (30 g, 68% yield) as a yellow oil.

**Isopropyl 2-(5-bromo-2-fluoro-phenyl)-2-[(S)-tert-butylsulfinyl]imino-acetate**

**(S-38).** Titanium(IV) isopropoxide (51.6 mL, 172 mmol) was added to a stirred mixture of 37 (30 g, 115 mmol) and (S)-2-methyl-2-propanesulfonamide (16.7 g, 138 mmol) in n-heptane (1000 mL). The mixture was stirred at 80 °C for 24 h. The mixture was partly concentrated in vacuo, then diluted with EtOAc. The mixture was cooled to rt and water was added. The resulting mixture was filtered through a pad of celite and rinsed with EtOAc and water. The organic layer was separated, dried (MgSO₄), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (silica; EtOAc/heptane 0/100 to 50/50). The desired fractions were collected and concentrated in vacuo to yield intermediate S-38 (40 g, 89% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.92 (dd, J=2.1, 6.2 Hz, 1H), 7.59 (ddd, J=2.5, 4.3, 8.7 Hz, 1H), 7.03 (dd, J=8.8, 10.6 Hz, 1H), 5.29 (spt, J=6.2 Hz, 1H), 1.39 (d, J=6.2 Hz, 3H), 1.38 (d, J=6.2 Hz, 3H), 1.36 (s, 9H); LC-MS m/z 392 [M+H]^+.

**Isopropyl (2R)-2-(5-bromo-2-fluorophenyl)-2-[[[(S)-tert-butylsulfinyl]amino]-2-cyclopropyl-acetate (2R-39).** A solution of cyclopropylmagnesium bromide (174 mL, 0.5 M, 87 mmol) was added dropwise over 45 min to a stirred solution of 24.4 g (62 mmol) iminoester S-38 in DCM (388 mL) at -78°C. The reaction mixture was stirred at -78°C for 30 min. Sat. aq. NH₄Cl was added and the reaction mixture was warmed to rt. The mixture was extracted with DCM and washed with water. The organic layer was separated and dried with MgSO₄, filtered and concentrated in vacuo to give intermediate 2R-39 (26.4 g 97% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.01 (dd, J=2.5, 6.5 Hz, 1H), 7.48 (ddd, J=2.3, 4.5, 8.7 Hz, 1H), 6.94 (dd, J=8.7, 10.3 Hz, 1H), 5.13 (spt, J=6.3 Hz, 1H), 4.97 (br s, 1H), 1.48 (tt, J=5.3, 8.2 Hz, 1H), 1.22 (d, J=6.2 Hz,
3H), 1.20 (s, 9H), 1.18 (d, J=6.0 Hz, 3H), 0.78-0.88 (m, 1H), 0.60-0.70 (m, 1H), 0.38-0.54 (m, 2H); LC-MS m/z 434 [M+H]+.

(2R)-2-Amino-2-(5-bromo-2-fluorophenyl)-2-cyclopropyl-acetic acid hydrochloride (2R-40). Intermediate 2R-39 (21 g, 48 mmol) was dissolved in MeOH (96 mL) and then 1 N NaOH (96 mL, 96 mmol) was added. The reaction mixture was refluxed for 4 h and then it was allowed to reach rt. The mixture was partitioned between water and EtOAc. The aqueous layer was separated, acidified with 1 N HCl and extracted with DCM. The combined organic extracts were dried over MgSO4, filtered and the solvent was evaporated in vacuo to yield the corresponding carboxylic acid as a white solid (15.5 g, 82% yield). This material was dissolved in dioxane (100 mL) and then HCl (4 N in dioxane, 29.6 mL, 118 mmol) was added dropwise. The resulting solution was stirred at rt for 1 h and the solvent was removed in vacuo. The residue was suspended in DIPE, filtered and dried in vacuo to give amino acid 2R-40 (HCl salt) as a white solid (10 g, 88% yield). 1H NMR (360 MHz, DMSO-d6) δ 14.33 (br s, 1H), 8.86 (br s, 3H), 7.98 (dd, J=2.4, 6.8 Hz, 1H), 7.74 (ddd, J=2.6, 4.4, 8.8 Hz, 1H), 7.35 (dd, J=9.0, 10.8 Hz, 1H), 1.70-1.82 (m, 1H), 0.56-0.92 (m, 4H); LC-MS m/z 286 [M-H]-; [a]$_{20}^{D}$ = -65.4 (c = 0.63 in MeOH).

(3R)-3-(5-Bromo-2-fluorophenyl)-3-cyclopropyl-morpholine-2,5-dione (3R-41). Amino acid 2R-40 (40 g, 123 mmol) was dissolved in THF (370 mL) and then 1 N NaOH (256 mL, 256 mmol) was added. The reaction mixture was cooled down to 0 °C and then a solution of 2-chloroacetyl chloride (24.5 mL, 308 mmol) in THF (50 mL) was added dropwise over 1 h at 15 °C while aq. NaOH was simultaneously added (to maintain the pH around 10-11). After the addition was finished, 12 N HCl was added carefully to the mixture until pH 2. The reaction mixture was concentrated in vacuo and the resulting precipitate was washed with DIPE and dried in vacuo. The resulting solid was dissolved in DMF (1 L) and then NaHCO$_3$ (19.8 g, 236 mmol)
was added. The reaction mixture was stirred at 80 °C for 6 h. The reaction mixture was partially
concentrated in a rotary evaporator and filtered through a pad of celite to remove the salts. The
solvent was removed in vacuo to give morpholine dione 3R-41 as a colourless oil (39 g, 99% yield). $^1$H NMR (360 MHz, DMSO-d$_6$) δ 8.74 (br s, 1H), 7.92 (dd, J=2.6, 6.9 Hz, 1H), 7.70 (ddd,
J=2.6, 4.8, 8.8 Hz, 1H), 7.32 (dd, J=8.8, 11.0 Hz, 1H), 5.12 (d, J=16.8 Hz, 1H), 4.84 (d, J=16.8
Hz, 1H), 1.62-1.72 (m, 1H), 0.84-0.97 (m, 1H), 0.58-0.73 (m, 2H), 0.41-0.52 (m, 1H); LC-MS
m/z 328 [M+H]$^+$; [α]$^20_D$ = +107.5 (c = 0.62 in DMF).

(5R)-5-(5-Bromo-2-fluorophenyl)-5-cyclopropyl-6-fluoromorpholin-3-one (5R-42).

Starting from 3R-41 (7.2 g, 21.94 mmol) and following the same procedure as for 5R-23 the
corresponding hemiacetal was obtained as 3:2 mixture of diastereoisomers, and further converted
to 5R-42 in 92% yield (6.0 g, 3:2 mixture of 6S/6R isomers). Hemiacetal: $^1$H NMR (600 MHz,
DMSO-d$_6$) δ 8.22 (s, 0.4H), 8.10 (s, 0.6H), 7.61 (dd, J=2.6, 7.0 Hz, 0.6H), 7.53-7.58 (m, 1H),
7.50 (ddd, J=2.5, 4.1, 8.7 Hz, 0.4H), 7.12-7.22 (m, 1H), 6.90 (d, J=4.7 Hz, 0.4H), 5.44 (dd,
J=1.2, 4.5 Hz, 0.4H), 5.38 (d, J=5.0 Hz, 0.6H), 4.09 (d, J=16.4 Hz, 0.4H), 4.07 (d, J=16.6 Hz,
0.6H), 3.98 (d, J=16.6 Hz, 0.6H), 3.96 (d, J=16.4 Hz, 0.4H), 1.52-1.65 (m, 1H), 0.43-0.59 (m,
1H), 0.14-0.43 (m, 3H) (1H exchanged); LC-MS m/z 330 [M+H]$^+$.

5R-42: $^1$H NMR (400 MHz, CDCl$_3$) δ 7.94 (br s, 0.4H), 7.51 (dd, J=2.5, 7.0 Hz, 0.4H), 7.39-7.48 (m, 1.6H), 7.20 (br s,
0.6H), 6.94-7.04 (m, 1H), 6.30 (dd, J=1.5, 51.0 Hz, 0.6H), 6.15 (d, J=49.9 Hz, 0.4H), 4.42 (d,
J=16.7 Hz, 0.4H), 4.33 (d, J=17.2 Hz, 0.6H), 4.28 (d, J=16.7 Hz, 0.4H), 4.14 (d, J=17.2 Hz,
0.6H), 1.58-1.68 (m, 1H), 0.22-0.74 (m, 4H); LC-MS m/z 332 [M+H]$^+$. This material was used
as a diasteromeric mixture in the following step.

(5R)-5-(5-Bromo-2-fluorophenyl)-5-cyclopropyl-6-fluoromorpholine-3-thione (5R-43).

Starting from 5R-42 (3.0 g, 9 mmol) and following the same procedure as for 5R-28 the
corresponding thioamide 5R-43 was obtained (2.8 g, 89% yield) as a mixture of
diastereoisomers, which was used as such for the next reaction step.

**(5R,6R)-5-(5-Bromo-2-fluorophenyl)-5-cyclopropyl-6-fluoro-2,6-dihydro-1,4-oxazin-3-amine (5R,6R)-44.** Starting from 5R-43 (2.8 g) and following the same procedure as for
(5R,6R)-31 the corresponding both diastereomers (5R,6R)-44 (0.98 g, 37% yield) and (5R,6S)-44 (1.16 g, 44% yield) were obtained. (5R,6R)-44: 1H NMR (360 MHz, CDCl3) δ 7.29-7.39 (m, 2H), 6.94 (dd, J = 8.8, 11.7 Hz, 1H), 6.14 (d, J = 52.0 Hz, 1H), 4.37 (br s, 2H), 4.20 (d, J = 15.4 Hz, 1H), 3.95 (d, J = 15.4 Hz, 1H), 1.59 (dq, J = 4.9, 8.6 Hz, 1H), 0.39-0.59 (m, 2H), 0.29-0.38 (m, 1H), 0.14-0.24 (m, 1H); LC-MS m/z 331 [M+H]+. (5R,6S)-44: 1H NMR (360 MHz, CDCl3) δ 7.94 (dd, J = 2.6, 6.95 Hz, 1H), 7.35 (ddd, J = 2.9, 4.2, 8.6 Hz, 1H), 6.93 (dd, J = 8.6, 11.5 Hz, 1H), 6.18 (dd, J = 2.2, 51.2 Hz, 1H), 4.29 (br s, 2H), 4.23 (d, J = 15.4 Hz, 1H), 4.09 (d, J = 15.4 Hz, 1H), 1.45 (dtt, J = 2.6, 5.4, 8.1 Hz, 1H), 0.37-0.50 (m, 1H), 0.13-0.33 (m, 3H); LC-MS m/z 331 [M+H]+.

**(5R,6R)-5-(5-Amino-2-fluorophenyl)-5-cyclopropyl-6-fluoro-2,6-dihydro-1,4-oxazin-3-amine (5R,6R)-45.** Starting from (5R,6R)-44 (1.7 g, 5.1 mmol) and following the same
procedure as for (5R,6R)-34 the corresponding (5R,6R)-45 was obtained (0.81 g, 59% yield). 1H NMR (400 MHz, CDCl3) δ 6.83 (dd, J = 9.0, 11.8 Hz, 1H), 6.48-6.56 (m, 2H), 6.18 (d, J = 52.7 Hz, 1H), 4.17 (dd, J = 1.1, 15.4 Hz, 1H), 3.92 (d, J = 15.3 Hz, 1H), 3.53 (br s, 2H), 3.33 (m, 2H), 3.03 (br s, 2H), 1.60 (dq, J = 5.0, 8.4 Hz, 1H), 0.39-0.54 (m, 2H), 0.31-0.39 (m, 1H), 0.16-0.25 (m, 1H) (2H exchanged); LC-MS m/z 268 [M+H]+.

**rac-2-Amino-2-(4-bromopyridin-2-yl)propanenitrile (rac-47a).** Starting from 1-(4-bromo-2-pyridinyl)-ethanone 46a (18 g, 90 mmol) and following the same procedure as for 14a the
corresponding aminonitrile rac-47a was obtained as a white solid (11 g, 54% yield).
**rac-2-Amino-2-(2-chloropyridin-4-yl)propanenitrile (rac-47b).** Starting from 4-acetyl-2-chloropyridine 46b (18 g, 90 mmol) and following the same procedure as for 14a the corresponding aminonitrile rac-47b was obtained (11.4 g, 98% yield) as a yellow solid. LC-MS m/z 182 [M+H]^+.

**rac-2-(4-Bromopyridin-2-yl)alaninamide (rac-48a).** Nitrile rac-47a (23 g, 101.7 mmol) was dissolved in a solution of 48% HBr in acetic acid (200 mL) and the mixture was refluxed for 12 h. After cooling to rt, EtOAc (40 mL) was added and the precipitate was filtered off and washed with EtOAc (100 mL), then dried to give rac-48a as an off-white solid (25 g, 61% yield).

**rac-2-Amino-2-(2-chloro-4-pyridyl)propanamide (rac-48b).** Intermediate rac-47b (6 g, 33.04 mmol) was dissolved in HCl (1 M in AcOH, 165 mL) and HBr (33% in AcOH, 25 mL) and the mixture was stirred at 75 °C for 3 h. After cooling to rt, EtOAc (250 mL) was added and the precipitate was filtered off, washed with EtOAc (100 mL) and dried in vacuo to give rac-48b (9.7 g, 81% yield). LC-MS m/z 198 [M-H]^−.

**rac-2-(4-Bromopyridin-2-yl)alanine (rac-49a).** 1 N NaOH (412 mL, 412 mmol) was added to a solution of rac-48a (33.4 g, 82.4 mmol) in THF (1 L) at rt. The resulting mixture was stirred at 65 °C for 16 h. Then, the reaction mixture was half concentrated, then cooled with an ice bath and brought to pH 7 with 1 N HCl while stirring. A white precipitate formed, which was filtered, washed with diethyl ether, and dried in vacuo to provide rac-49a (13.2 g, 65% yield). ^1H NMR (360 MHz, DMSO-d6) δ 8.39 (d, J=5.5 Hz, 1H), 7.91 (d, J=1.5 Hz, 1H), 7.81 (br s, 3H), 7.60 (dd, J=1.8, 5.1 Hz, 1H), 1.64 (s, 3H). LC-MS m/z 245 [M+H]^+.

**rac-2-Amino-2-(2-chloropyridin-4-yl)propanoic acid (rac-49b).** Intermediate rac-48b (9.7 g, 26.84 mmol) was dissolved in NaOH (1 M in water, 134 mL) and the mixture was stirred at rt for 60 h. The reaction mixture was concentrated to half volume in vacuo and then cooled on an
ice bath. The pH of the solution was adjusted to pH = 7 by addition of HCl (1 N in water) and a white solid precipitated. The precipitate was filtered off, washed with Et₂O and dried in vacuo to give intermediate rac-49b (5.48 g, quant. yield) as a white solid. \(^1\)H NMR (360 MHz, DMSO-d₆) \(\delta 8.38 \ (d, \ J=5.1 \ Hz, \ 1H), \ 7.92 \ (br \ s, \ 3H), \ 7.59 \ (d, \ J=1.5 \ Hz, \ 1H), \ 7.50 \ (dd, \ J=1.5, \ 5.5 \ Hz, \ 1H), \ 1.63 \ (s, \ 3H); \) LC-MS m/z 199 [M-H].

**rac-\{[2-Amino-2-(4-bromopyridin-2-yl)propanoyl]oxy\}acetic acid trifluoroacetate salt (rac-51a).** Amino acid rac-49a (16.0 g, 65.3 mmol) and Cs₂CO₃ (63.8 g, 195.9 mmol) in dry DMF (500 mL) were stirred for 30 min. Then tert-butyl chloroacetate (9.33 mL, 65.3 mmol) was added, and the resulting mixture was stirred at rt for 3 h. Then, ice and DCM were added and the organic layer was separated. The aqueous layer was extracted with DCM. The combined organic layers were dried (MgSO₄), filtered and evaporated to dryness providing rac-50a as an orange oil (note: a high vacuum of 1-3 mbar is needed to remove a low volatile impurity). Ester rac-50a was dissolved in TFA (234 mL) and stirred at rt for 30 min. Then, the solvent was thoroughly evaporated providing a brown oil, which was treated with diethyl ether and stirred for about 10 min to get white solid which was filtered, washed with diethyl ether and dried in vacuo at 50 °C to give the TFA salt of rac-51a (8.95 g, 29 % yield over two steps). \(^1\)H NMR (360 MHz, DMSO-d₆) \(\delta 9.12 \ (br \ s, \ 4H), \ 8.56 \ (d, \ J=5.1 \ Hz, \ 1H), \ 8.10 \ (d, \ J=1.5 \ Hz, \ 1H), \ 7.84 \ (dd, \ J=1.6, \ 5.3 \ Hz, \ 1H), \ 4.65-4.87 \ (m, \ 2H), \ 1.95 \ (s, \ 3H). \) LC-MS m/z 303 [M+H].

**rac-\{[2-Amino-2-(2-chloropyridin-4-yl)propanoyl]oxy\}acetic acid trifluoroacetate salt (rac-51b).** Starting from rac-49b (5.29 g, 26.36 mmol) and following the same procedure as for rac-51a the corresponding acid rac-51b was obtained as a trifluoroacetate salt (6.12 g, 96%). \(^1\)H NMR (360 MHz, DMSO-d₆) \(\delta 9.50 \ (br \ s, \ 4H), \ 8.58 \ (d, \ J=5.4 \ Hz, \ 1H), \ 7.79 \ (d, \ J=1.5 \ Hz, \ 1H), \ 7.65 \ (dd, \ J=1.5, \ 5.4 \ Hz, \ 1H), \ 4.73-4.85 \ (m, \ 2H), \ 1.94 \ (s, \ 3H); \) LC-MS m/z 257 [M-H].
**rac-3-(4-Bromopyridin-2-yl)-3-methylmorpholine-2,5-dione (rac-52a).** Amino acid (TFA salt) rac-51a (12.3 g, 29.5 mmol) was suspended in DCM (764 mL) and 2-chloro-1-methylpyridinium iodide (8.3 g, 32.5 mmol) was added, followed by DIPEA (25.3 mL, 88.6 mmol). The reaction mixture was subsequently refluxed for 4 h. Then, the reaction mixture was concentrated *in vacuo* and purified by column chromatography (silica: Heptane/EtOAc 100/0 to 20/80). Evaporation of the product fractions provided 4.6 g rac-52a containing about 30% of impurity bearing a chloro instead of bromo on the pyridine 4-position (55% yield). ¹H NMR (360 MHz, DMSO-d₆) δ 9.18 (br s, 1H), 8.44 (d, J=5.1 Hz, 1H), 7.79 (d, J=1.5 Hz, 1H), 7.73 (dd, J=1.6, 5.3 Hz, 1H), 4.62 (s, 2H), 1.74 (s, 3H); LC-MS m/z 285 [M+H]+.

**rac-3-(2-Chloropyridin-4-yl)-3-methylmorpholine-2,5-dione (rac-52b).** Starting from rac-51b (5.29 g, 26.36 mmol) and following the same procedure as for rac-52a the corresponding acid rac-52b was obtained as white crystals (1.10 g, 19%). ¹H NMR (360 MHz, DMSO-d₆) δ 9.43 (br s, 1H), 8.51 (d, J=5.4 Hz, 1H), 7.41-7.43 (m, 1H), 7.39 (dd, J=1.6, 5.4 Hz, 1H), 4.69 (s, 2H), 1.71 (s, 3H); LC-MS m/z 241 [M+H]+; m. p. = 213.5 ºC.

**rac-(5R*,6R*)-5-(4-Bromo-2-pyridyl)-6-fluoro-5-methyl-morpholin-3-one (rac-53a).** Starting from rac-52a (4.3 g, 5.2 mmol) and following the same procedure as for 5R-23 the corresponding rac-(5R*,6R*)-53a was obtained as a single diastereomer (3.55 g, 81%). ¹H NMR (400 MHz, CDCl₃) δ 8.42 (dd, J=0.5, 5.3 Hz, 1H), 7.67 (dd, J=0.5, 1.8 Hz, 1H), 7.42 (dd, J=1.8, 5.0 Hz, 1H), 7.32 (br s, 1H), 6.06 (d, J=51.7 Hz, 1H), 4.38 (d, J=17.2 Hz, 1H), 4.19 (d, J=17.2 Hz, 1H), 1.68 (d, J=1.8 Hz, 3H); LC-MS m/z 289 [M+H]+; m. p. = 193.1 ºC.

**rac-(5R*,6R*)-5-(2-Chloro-4-pyridyl)-6-fluoro-5-methyl-morpholin-3-one**

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(rac-(5R*,6R*)-53b). Starting from rac-52b (1.42 g, 5.90 mmol) and following the same procedure as for 5R-23 the corresponding rac-(5R*,6R*)-53b was obtained as a transparent oil (985 mg, 69%, 65:35 mixture of diastereoisomers). $^1$H NMR (360 MHz, CDCl$_3$) δ 8.46 (d, $J$=5.5 Hz, 0.35H), 8.46 (d, $J$=5.5 Hz, 0.65H), 7.64 (br s, 0.65H), 7.60 (br s, 0.35H), 7.39-7.44 (m, 0.35H), 7.32-7.36 (m, 0.65H), 7.28-7.32 (m, 0.35H), 7.22 (dd, $J$=1.3, 5.3 Hz, 0.65H), 5.65 (d, $J$=50.1 Hz, 0.65H), 5.51 (d, $J$=49.0 Hz, 0.35H), 4.45 (d, $J$=16.7 Hz, 0.35H), 4.37 (d, $J$=16.8 Hz, 0.65H), 4.34 (d, $J$=16.7 Hz, 0.35H), 4.22 (d, $J$=16.8 Hz, 0.65H), 1.78 (d, $J$=1.5 Hz, 1.05H), 1.70 (d, $J$=1.5 Hz, 1.95H); LC-MS m/z 245 [M+H]$^+$. 

rac-(5R*,6R*)-5-(4-Bromopyridin-2-yl)-6-fluoro-5-methylmorpholine-3-thione (rac-54a). Starting from rac-(5R*,6R*)-53a (3.50 g, 12.11 mmol) and following the same procedure as for 5R-28 the corresponding rac-(5R*,6R*)-54a was obtained as white crystals (2.00 g, 54%). $^1$H NMR (360 MHz, CDCl$_3$) δ 8.43 (br d, $J$=5.1 Hz, 2H), 7.51 (d, $J$=1.8 Hz, 1H), 7.45 (dd, $J$=1.8, 5.1 Hz, 1H), 6.04 (dd, $J$=1.5, 51.6 Hz, 1H), 4.74 (d, $J$=18.7 Hz, 1H), 4.62 (d, $J$=18.7 Hz, 1H), 1.73 (d, $J$=1.8 Hz, 3H); LC-MS m/z 305 [M+H]$^+$. 

rac-(5R*,6R*)-5-(2-Chloropyridin-4-yl)-6-fluoro-5-methylmorpholine-3-thione (rac-54b). Starting from rac-(5R*,6R*)-53b (0.769 g, 1.53 mmol) and following the same procedure as for 5R-28 the corresponding rac-(5R*,6R*)-54b was obtained as a transparent oil (445 mg, 54%, 60:40 mixture of diastereoisomers). LC-MS m/z 259 [M-H]$^-$. 

rac-(5R*,6R*)-5-(4-Bromopyridin-2-yl)-6-fluoro-5-methyl-5,6-dihydro-2H-1,4-oxazin-3-amine (rac-(5R*,6R*)-55a). Starting from rac-(5R*,6R*)-54a (2.00 g, 6.55 mmol) and following the same procedure as for 5R-31 the corresponding rac-(5R*,6R*)-55a was obtained as white crystals (1.61 g, 85%). $^1$H NMR (360 MHz, DMSO-d$_6$) δ 8.45 (d, $J$=5.1 Hz, 1H), 7.53-
Starting from rac-(5R*,6R*)-54b (0.445 g, 1.53 mmol) and following the same procedure as for 5R-31 the corresponding amidine rac-(5R*,6R*)-55b was obtained as a white solid (300 mg, 72%). 1H NMR (400 MHz, DMSO-d6) δ 8.36 (d, J=5.3 Hz, 1H), 7.45 (d, J=1.5 Hz, 1H), 7.42 (dd, J=1.5, 5.3 Hz, 1H), 6.06 (br s, 2H), 5.88 (d, J=52.2 Hz, 1H), 4.06 (d, J=16.0 Hz, 1H), 3.97 (d, J=16.0 Hz, 1H), 1.40 (d, J=1.8 Hz, 3H); LC-MS m/z 245 [M+H]+; m. p. = 174.1 ºC. Note: the other diastereoisomer was discarded without NMR analysis.

rac-(5R*,6R*)-5-(4-Aminopyridin-2-yl)-6-fluoro-5-methyl-5,6-dihydro-2H-1,4-oxazin-3-amine (rac-(5R*,6R*)-56a). Starting from rac-(5R*,6R*)-55a (1.10 g, 3.82 mmol) and following the same procedure as for 5R-34 the corresponding rac-(5R*,6R*)-56a was obtained as white crystals (0.54 g, 64% yield). LC-MS m/z 225 [M+H]+.

rac-(5R*,6R*)-5-(2-Aminopyridin-4-yl)-6-fluoro-5-methyl-5,6-dihydro-2H-1,4-oxazin-3-amine (rac-(5R*,6R*)-56b). Starting from rac-(5R*,6R*)-55b (0.300 g, 1.23 mmol) and following the same procedure as for 5R-34 the corresponding aniline rac-(5R*,6R*)-56b was obtained as a transparent oil (200 mg, 72%). LC-MS m/z 225 [M+H]+.

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Author Contributions
The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

AUC$_{0\text{–}\text{last}}$, area under the curve until last time point sampled; Aβ, amyloid beta; BACE1, beta-site amyloid precursor protein cleaving enzyme 1; BINAP, 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl; C$_{\text{max}}$, maximum plasma concentration; CSF, cerebrospinal fluid; DAST, diethylaminosulfur trifluoride; dLM, dog liver microsomes; DMEDA, N,N'-dimethyleneediamine; DMTMM, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride; f$_{u}$, free (unbound) fraction; HATU, (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate); hLM, human liver microsomes; HPβCD, (2-Hydroxypropyl)-beta-cyclodextrin [128446-35-5]; K$_p$, brain-to-plasma ratio; n. d., not determined; mLMT, mouse liver microsomes; P$_{\text{app}}$, apparent permeability; p. o., per os (= oral); rLM, rat liver microsomes; SBEβCD, sulfobutylether β-cyclodextrin sodium salt [182410-00-0]; s. c., subcutaneous; T$_{1/2}$, half-life; TBAT, tetrabutylammonium difluorotriphenylsilmicate; T$_{\text{max}}$, time at which maximum plasma concentration is reached; WT, wild type.
ASSOCIATED CONTENT


REFERENCES


(25) Values calculated with ACD Labs software v10. pKa. Advanced chemistry development Inc. 110 Yonge Street, 14th Floor, Toronto, Ontario, Canada, M5C 1T4. 


(29) Two tautomeric forms were considered in the calculation with ACD. One with endocyclic double bond, and one with exocyclic double bond. Performance of ACD on the training set was worse using an endocyclic double bonded NH tautomer (see SI). However the endocyclic form is better for the final morpholine examples but still not as good as ADMET Predictor and introduces uncertainty of choosing the most appropriate tautomer.


(31) Please note the oxazine ring numbering reverses in the final compounds compared to the previous intermediates.

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TABLE OF CONTENTS GRAPHIC
(2R,3R)-7d
BACE1 IC$_{50}$ 7.6 nM
hAβ42 cell IC$_{50}$ 8.1 nM
Aβ dog EC$_{50}$ 20 ng/mL

pK$_a$ 9.6
non-brain penetrant

pK$_a$ 7.8
brain penetrant
Chart 1.
237x214mm (96 x 96 DPI)
Scheme 1.

300x198mm (96 x 96 DPI)
Scheme 2.
195x190mm (96 x 96 DPI)
Scheme 3.
276x184mm (96 x 96 DPI)
Scheme 4.
302x205mm (96 x 96 DPI)
Scheme 5.
187x202mm (96 x 96 DPI)
Table 1.

154x76mm (96 x 96 DPI)
Table 2. A
3R-6b levels at 2 h

\[ K_p = 0.13 \quad K_p = 1.1 \]

Plasma ng/mL
Brain ng/g

WT CF1
P-gp def. CF1

Table 2. A
110x83mm (150 x 150 DPI)
B

$3\text{R-}\text{6b A} \beta$ (\% versus control) at 2 h

![Graph showing $3\text{R-}\text{6b A} \beta$ at 2 h for different conditions.]

Table 2. B

136x83mm (150 x 150 DPI)
Scheme 6.
224x148mm (96 x 96 DPI)
Chart 2.
199x63mm (96 x 96 DPI)
Table 3. Top graphic
55x77mm (96 x 96 DPI)
Table 3. Graph in column 1
33x24mm (96 x 96 DPI)
Table 3. Graph in column 2
29x22mm (96 x 96 DPI)
Table 3. Graph in column 4
29x20mm (96 x 96 DPI)
Table 3. Graph in column 5
35x27mm (96 x 96 DPI)
Table 3. Graph in column 6
34x26mm (96 x 96 DPI)
Table 3. Graph in column 7
35x27mm (96 x 96 DPI)
Table 4. Top graph
54x75mm (96 x 96 DPI)
Table 4. Graph in column 2
52x28mm (96 x 96 DPI)
Table 4. Graph in column 3
48x28mm (96 x 96 DPI)
Table 5. Top graph

49x74mm (96 x 96 DPI)
Table 5. Graph in column 1
24x37mm (96 x 96 DPI)
Table 5. Graph in column 2
26x36mm (96 x 96 DPI)
Table 5. Graph in column 3
25x36mm (96 x 96 DPI)
Table 5. Graph in column 4
25x36mm (96 x 96 DPI)
Table 6. Top graph
48x72mm (96 x 96 DPI)
Table 6. Graph in column 1
30x34mm (96 x 96 DPI)
Table 5. Graph in column 2
40x30mm (96 x 96 DPI)
Table 5. Graph in column 3
35x31mm (96 x 96 DPI)
Table 5. Graph in column 4
35x31mm (96 x 96 DPI)
Table 6. Graph for column 5
38x30mm (96 x 96 DPI)
Table 7. A

(2R,3R)-7a levels

$K_p = 0.65^b$

Table 7. A
78x67mm (150 x 150 DPI)
Table 7. B

(2R,3R)-7a Aβ (% versus control)

![Graph showing Aβ levels at different times (2 h, 4 h, 8 h) for Abeta42 and Abeta Total.]

ACS Paragon Plus Environment
Table 7. (2R,3R)-7d levels

$K_p = 0.33^b$

<table>
<thead>
<tr>
<th>Time</th>
<th>Plasma ng/mL</th>
<th>Brain ng/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 h</td>
<td>600</td>
<td>200</td>
</tr>
<tr>
<td>4 h</td>
<td>400</td>
<td>100</td>
</tr>
<tr>
<td>7 h</td>
<td>300</td>
<td>70</td>
</tr>
</tbody>
</table>

Table 7. C
78x67mm (150 x 150 DPI)
(2R,3R)-7d
BACE1 IC<sub>50</sub> 7.6 nM
hAβ42 cell IC<sub>50</sub> 8.1 nM
Aβ dog EC<sub>50</sub> 20 ng/mL