塩基性調整による中枢移行性獲得の応用研究

~ BACE1 阻害活性を有する

1,3-オキサジン誘導体の創製 ~

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略語表

Aβ	amyloid β peptide
Ac	acetyl
ACD	Advanced Chemistry Development
AcOH	acetic acid
AD	Alzheimer disease
AIBN	azobis(isobutyronitrile)
APP	amyloid precursor protein
ax	axial
BACE1	β-site APP-cleaving enzyme 1
Boc_2O	di-*tert*-butyl dicarbonate
B/P	brain-to-plasma ratio
CHO cell	chinese hamster ovary cells
CNS	central nervous system
CSF	cerebrospinal fluid
CYP	cytochrome P450
DAST	*N,N*-diethylaminosulfur trifluoride
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DIBAL	diisobutylaluminium hydride
DIEA	*N,N*-diisopropylethylamine
DLB	dementia with Lewy bodies
DMA	*N,N*-dimethylacetamide
DMAP	4-Dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMP	Dess-Martin periodinane
EC_{50}	median effect concentration
EDC-HCl	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
ER	efflux ratio
Et_3N	triethylamine

Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
eq	equatorial
FDA	Food and Drug Administration
FTLD	frontotemporal lobar degeneration
GPCR	G protein-coupled receptor
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate
hERG	human Ether-a-go-go-Related Gene
HOBt	1-hydroxy-1*H*-benzotriazole
HPBCD	2-hydroxypropyl-β-cyclodextrin
HTS	high throughput screening
HTRF	homogeneous time resolved fluorescence
IC_{50}	half maximal inhibitory concentration
IPE	diisopropyl ether
iv	intravenous injection
KHMDS	potassium hexamethyldisilazide
LC/MS	liquid chromatography – mass spectrometry
LDA	lithium diisopropylamide
LHMDS	lithium bis(trimethylsilyl)amide
LLC-PK1	Lilly Laboratories cell-porcine kidney 1
MC	methylcellulose
MDCK	madin-darby canine kidney
MDR1	multiple drug resistance 1
Me	methyl
MeCN	acetonitrile
MeOH	methanol
Ms	mesyl
Ms_2O	methanesulfonic anhydride

NMO	*N*-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
PDB	protein data bank
PG	propylene glycol
P-gp	P-glycoprotein
po	*per os*
PPTS	pyridinium *p*-toluenesulfonate
rt	room temperature
sAPP β	soluble amyloid precursor protein β
SAR	structure-activity relationships
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TMS	trimethylsilyl
TMSCl	trimethylsilyl chloride
VaD	vascular dementia

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緒論

中枢神経系薬の創薬研究

創薬研究において、標的とする組織への移行性を考慮することは、効率的に薬効を発現する化合物探索に重要な事柄である。経口投与の場合、生体にとって異物である薬物が、目的臓器に到達するまでには、様々なバリアが存在する。例えば、消化管の吸収過程における吸収障壁であったり、肝臓の初回通過効果などが挙げられる。特に中枢神経系に到達するためには、血液-脳関門を透過しなければならない。血液-脳関門は脳の毛細血管内に存在し、脳毛細血管内皮細胞とその一部を接着するペリサイト、それら周囲を取り巻くアストロサイトから構成される。抹消組織の毛細血管は有窓構造であり、薬物は内皮細胞間の間隙を通過し移行することが可能であるが、脳の毛細管内皮細胞間は強固なタイトジャンクション（密着結合）で構成されるため、血液中の遊離型薬物は細胞間隙を自由に通過することはできない[1)]。遊離型薬物が脳細胞間液中に侵入するためには、内皮細胞を経細胞的に通過する必要がある。このため中枢神経系の薬物は、脂溶性や大きさが制限される。また脳毛細血管内皮細胞には、様々なトランスポーターが発現しており、選択性と方向性をもった物質輸送をしている。必要な栄養素を血液中から脳内に取り込む輸送系と、不要な代謝物や異物を積極的に排出する輸送系があり、薬物に対しては多くの場合、この排出系のトランスポーターの作用を回避する必要がある[2)]。つまり中枢系に到達し、効果的に作用をもたらすためには、経細胞的に通過しやすいことを前提として、排出輸送系の影響を受けにくい、あるいは最小限に抑える化合物デザインが要求される。

また、創薬研究においては安全性の獲得が重要である。中枢神経系の神経伝達物質の一つにモノアミンがあるが、これらが関与するアミン作動性の GPCR やチャネル、トランスポーターを標的とする創薬研究では、薬物候補品の多くは塩基性化合物である。塩基性官能基を有する化合物は hERG チャネル阻害活性を示しやすいと言われており[3)]、中枢神経系薬剤の探索研究において hERG 阻害活性の低減が課題になることが多い。hERG チャネルは、細胞内外の各種イオン濃度の勾配によって生じる膜電位に応答して活性化されるカリウムチャネルであり、心臓の拍動における活動電位の迅速な終結（再分極）に大きく寄与している。hERG チャネルの阻害は QTc 延長、それに続く心室性不整脈の発現につながると言われていて、実際に 1990 年代に hERG 阻害によって引き起こされる致死性不整脈が原因で、複数の承認薬が市場から撤退した[4)]。近年では医薬品の承認申請に hERG チャネルへの影響を検証することが要求されている[5)]。不幸にも最適化している化合物に hERG 阻害活性

が認められた場合、その阻害活性の低減は解決しなければならない課題となる。

筆者もアルツハイマー病治療薬になりうるβセクレターゼ 1（BACE1）阻害剤の探索研究を通して、塩基性のリード化合物の最適化に取り組み、これら中枢移行性と hERG 阻害活性の課題に直面した。そこで、著者は中枢移行性を持ち *in vivo* で有効な薬理作用を示し、hERG 阻害活性を回避した安全な化合物の探索研究に着手した。

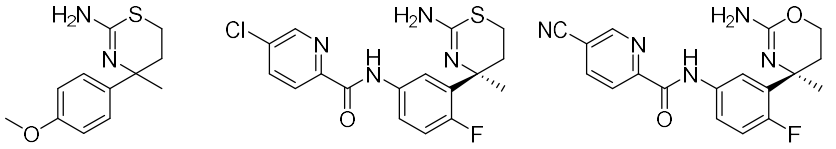
βセクレターゼ 1（BACE1）

2019 年には世界的に 5000 万人以上が認知症を罹患していて、2050 年には認知症患者の数は 1 億 5000 万人に達すると予想されている [6]。認知症には、アルツハイマー型認知症（AD），血管性認知症（VaD），レビー小体型認知症（DLB），前頭側頭葉変性症（FTLD）の 4 つの病型がある。病型別の割合では、アルツハイマー型認知症が最も多く、約 60~80%を占めると言われる [7]。AD に罹患すると、記憶だけでなく、思考や行動、言語、知覚、判断といった高次脳機能が低下し、生活機能や遂行機能に障害が生じる。AD 発症前は問題なくできていたことが徐々にできなくなり、日常生活や社会生活を自立して送ることが難しくなり、場合によっては介護が必要になる。実際に AD の個人の医療費と長期介護費は、個人的、公的資産に大きな影響を及ぼし、米国では認知症患者のケアにかかる総費用は、2020 年の 3050 憶ドルから、2050 年には 1.1 兆ドルを超えると推定されている [8]。 ドネペジルやメマンチンなどの AD 治療薬は用いられているが、一時的に病気の症状を和らげるだけで病気の進行を止めるものではなく、本邦では、予防や根治のための治療法は見出されていない [9]。患者だけでなく、家族や周囲の人の生活に影響を及ぼす AD の効果的な疾患修飾薬を開発することは社会的な課題である。

AD の組織病理学的特徴は、神経原線維変化、凝集、過剰リン酸化されたタウたんぱく質、および 38~43 のアミノ酸からなるアミロイドβペプチド（Aβ）で構成されるアミロイド斑が知られている。家族性アルツハイマー病の遺伝的解析から、Aβの産生、蓄積の異常がアルツハイマー病の発症に関与しているアミロイド仮説が広く支持されている。そのため、Aβの産生、蓄積を抑える戦略は、AD 疾患修飾に重要と考えられている [10]。2021 年 6 月に Aβ抗体であるアデュカヌマブがアメリカで FDA の迅速承認を受けた。その認知機能の改善効果はまだ確定的ではないが、アルツハイマー病患者の Aβの減少は確認されていて、Aβの産生を抑える薬剤はアルツハイマー病の疾患修飾薬になりうると注目を集めている。Aβは、アミロイド前駆体タンパク質（APP）からβセクレターゼ 1（BACE1）とγセクレ

ターゼの働きによって生成する[11]。APP が、BACE 1 と呼ばれるタイプ I 型の膜貫通性のアスパラギン酸プロテアーゼによって切断され、C99 と呼ばれる膜貫通領域を持った切断産物と可溶性 APPβ（sAPPβ）が生じる。その後γセクレターゼが C99 を膜内で切断し、38~43 アミノ酸長のアイソフォームを含む Aβを生じる。γセクレターゼも Aβ産生に関わる創薬ターゲットと考えられているが、γセクレターゼは細胞分化に重要な役割を果たすNotch シグナルの一端を担っているため、その阻害は多くの恒常性機能に影響を及ぼす。Notch シグナルに影響することなく、C99 の切断のみを止めることは難しく、γセクレターゼ阻害剤の探索は困難であるとされている[12]。これらを背景に BACE1 阻害剤は、安全なアルツハイマー病疾患修飾薬になりうると世界中で注目されており、1999 年に BACE1 が同定されて以来、経口投与可能な、脳移行性がある BACE1 阻害剤の探索が行われてきた[13]。

BACE1 阻害剤を見出すため、我々は HTS を実施し、チアジン誘導体 **0-1** を見出した（**Figure 0-1**）。このチアジン **0-1** を起点に Hit to lead SAR を実施し、良好な細胞活性を示すチアジン **0-2** を見出した。しかしながら、チアジン誘導体 **0-4**（LY-2886721）や **0-5**（atabecestat）はヒトにおいて反応性代謝物の生成が原因で肝障害を引き起こすことが報告されている[13c]（**Figure 0-2**）。チアジンの肝障害リスクを避けるために硫黄原子を酸素原子に変えたオキサジン **0-3** を合成し評価した。その結果、オキサジン **0-3** は良好な細胞活性を示したものの、*in vivo* で薬効を示さなかった。その原因を探るために、中枢移行性および代表的な排出系トランスポーターである P-糖たんぱく質（P-gp）の影響を評価を実施した。その結果、オキサジン **0-3** は高い P-gp 基質性を示したことから（P-gp ER = 36）、そのことが原因で中枢移行性が低く（BrKp = 0.15）、*in vivo* 薬効を示さなかったと考察した。また、hERG 阻害活性評価を実施した結果、高い hERG 阻害活性を示した（hERG（5 μM）= 78% inhibition）。そこで筆者らは、オキサジン **0-3** を起点に P-gp 基質性と hERG 阻害活性を低減し、*in vivo* で有意な Aβ減少を示す BACE1 阻害剤の探索研究に着手した。



0-1

BACE1 IC_{50}: 73 μM
Cell Aβ IC_{50}: 2.6 μM

0-2

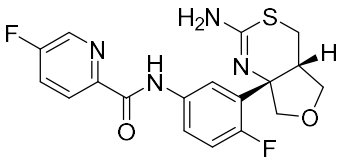
BACE1 IC_{50}: 6.7 nM
Cell Aβ IC_{50}: 0.84 nM

0-3

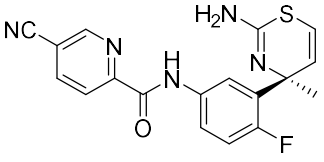
BACE1 IC_{50}: 118 nM
Cell Aβ IC_{50}: 3.7 nM
P-gp ER[a] : 36
BrKp (mice)[b] : 0.15
hERG(5 μM)[c] : 78 %

[a]Efflux ratio measured in MDCK cells transfected with human MDR1 at absorption systems. [b]Total brain -to-plasma ratio determined by *iv* dose at 0.5 h. [c]% inhibition at 5 μM measured in CHO cells.

Figure 0-1. HTS Hit 0-1 and Initial Leads 0-2 and 0-3



0-4 (LY-2886721)



0-5 (atabecestat)

Figure 0-2. Thiazine compounds

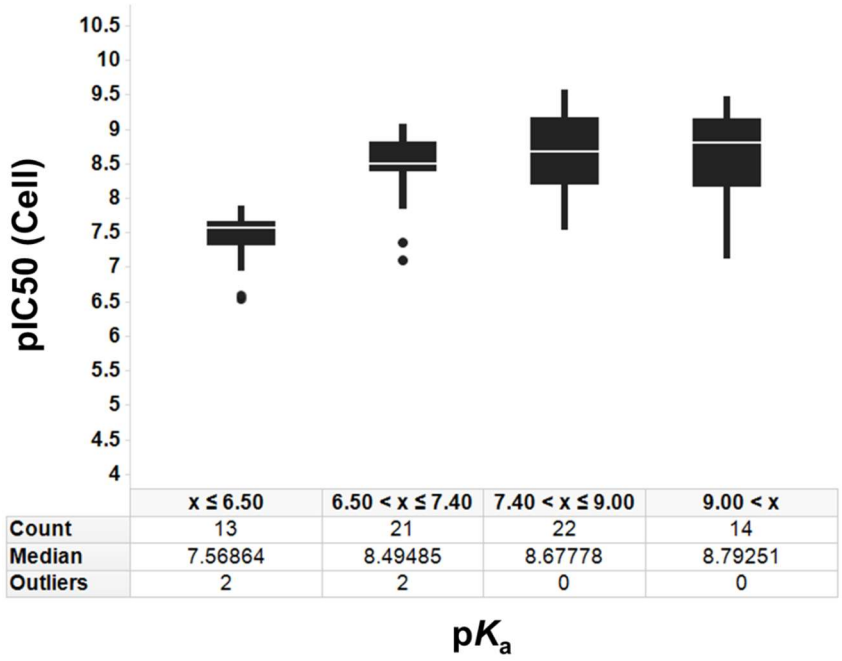
本論

第 1 章　オレフィン型オキサジン誘導体の創薬研究

第 1 節　中枢移行性改善を指向した化合物デザイン

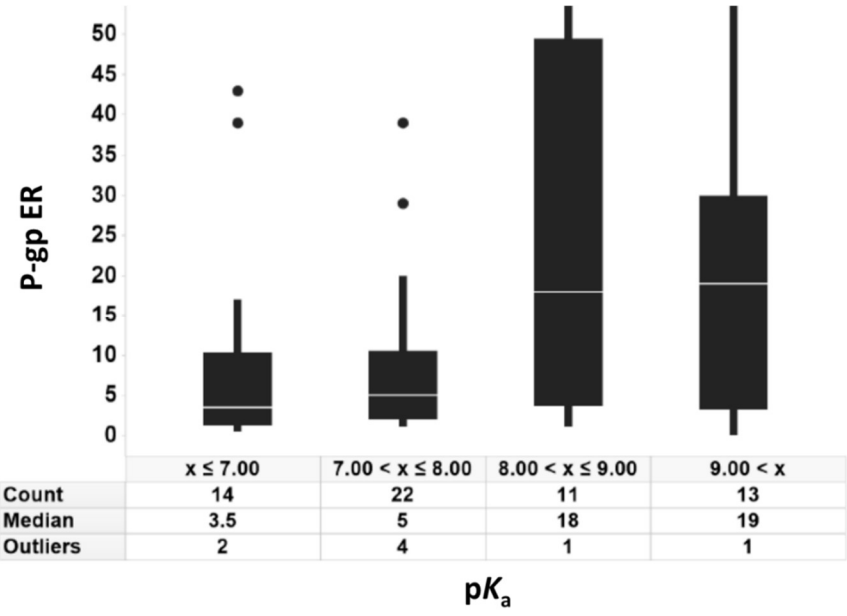
第 1 項　pK_a 至適範囲の考察

承認されている CNS 薬の多くは P-gp 基質ではないことから、P-gp 基質性の低減は必要である [14]。P-gp は 12 回膜貫通型トランスポーターであり、脂溶性が高い、塩基性および中性化合物が基質になりやすいと報告されていて、塩基性の中枢神経系薬物において、pK_a を下げることが、P-gp 基質性を低減する方法として知られている [14]。また、膜透過性の改善や水素結合供与体および受容体の低減、極性表面積、分子量、ratotable bond の数の低減も P-gp 回避に有効とされている [2]。一方で hERG 阻害活性においては、塩基性と脂溶性が高い化合物は阻害活性を示しやすいことが報告されていて、hERG 阻害を低減させる有効な方法の一つは塩基性を下げることである [3]。高脂溶性化合物は膜透過性には有利に作用するため、P-gp 基質性と hERG 阻害活性の 2 つの課題を解決する共通した方法として、塩基性の低減を試みた。塩基性を調整するに際し、適切な塩基性の範囲の設定を計画した。アミジン部位はファーマコフォアの一つであり、その塩基性の程度は活性値に影響すると考え、活性値と塩基性の相関から下限の設定に着手した。化合物の BACE1 阻害活性を評価するために細胞アッセイを採用しており、さらにこの細胞アッセイは生理学的な pH 7.4 で測定している。細胞アッセイで高活性を示す化合物の *in vivo* での効果も再現できていることから、細胞アッセイで考察することが重要と考えた。アミジン部位の pK_a と細胞活性の相関を確認した結果（**Figure 1-1**）、pK_a が 6.5 以下では活性が消失している化合物が多く見られたことから、pK_a の下限値を 6.5 に設定した。P-gp 基質性においても同様に pK_a との相関を調査した結果（**Figure 1-2**）、pK_a が 8 を超える化合物は高い P-gp 基質性を示したため、目標とする pK_a を 6.5 から 8.0 に設定した。



	x ≤ 6.50	6.50 < x ≤ 7.40	7.40 < x ≤ 9.00	9.00 < x
Count	13	21	22	14
Median	7.56864	8.49485	8.67778	8.79251
Outliers	2	2	0	0

Figure 1-1. Distribution of cellular IC_{50} values (shown as -log(cellular IC_{50})) binned by pK_a. All the compounds used in the box plot, submitted before the start of this research, had biochemical IC_{50} values of <100 nM with experimental pK_a values.

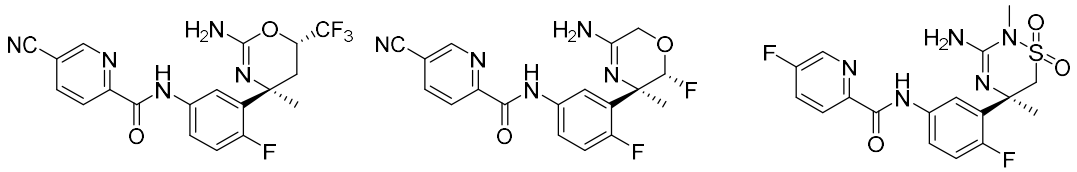


	x ≤ 7.00	7.00 < x ≤ 8.00	8.00 < x ≤ 9.00	9.00 < x
Count	14	22	11	13
Median	3.5	5	18	19
Outliers	2	4	1	1

Figure 1-2. P-gp ER ratio binned by pK_a. All the compounds used in the box plot submitted before the start of this research.

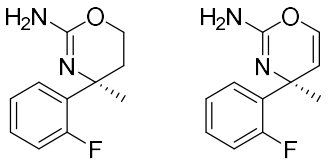
第２項　オレフィン型オキサジンの着想

ジヒドロオキサジン **0-3** の pK_a を測定した結果、pK_a = 9.8 を示した。これまでに BACE1 阻害剤の探索研究において、アミン部位の塩基性を下げるために、フッ素やスルホンなどの電気求引基を用いた例が報告されている [15]（**Chart 1-1**）。中枢移行性を有利にするために、分子量の増加を避けることが望ましく、オキサジン環内に二重結合を導入したオキサジン **B** をデザインした。二重結合の効果により窒素原子上の孤立電子対が非局在化することで、塩基性が低減できると考えた。ACD ソフトウェアで pK_a を予測することが可能であり [16]、アミノオキサジン **A** のアミン部分の pK_a を予測した結果、pK_a = 10.1 を示した。これは実測値の 9.8 に近く、類縁化合物も同様に良好な予測を示したことから、ACD ソフトウェアを用いてオキサジン **B** の pK_a を予測した。その結果、7.0 と予測され、第 1 項で設定した至適 pK_a の範囲内であったことから、オキサジン **B** 誘導体の合成に着手した。



1-1 (Roche)　　**1-2** (Janssen)　　**1-3** (Merck: verubecestat)

Chart 1-1. BACE1 Inhibitors **1-1, 1-2**, and **1-3** Identified Utilizing a pK_a-Lowering Strategy



	A	**B**
ACD pK_a[a]:	10.1	7.0
pK_a measured:	9.8	this work

Figure 1-3. Design of novel oxazine **B** from dihydrooxazine **A.**

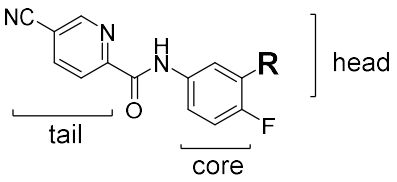
[a]The pK_a values were calculated with ACD/Percepta as the imine tautomer.

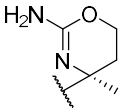
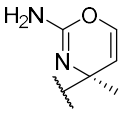
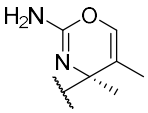
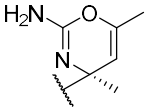
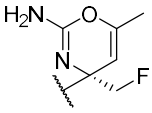
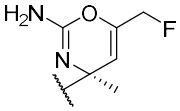
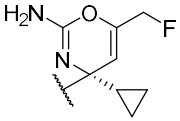
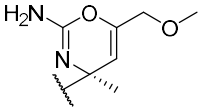
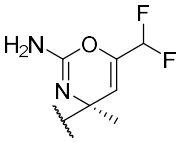
第 2 節　化合物 **1-8** の創製

第 1 項　オレフィン型オキサジンの最適化研究

オキサジン **1-4** を合成したところ、実際の pK_a は 7.7 であった (**Table 1-1**)。細胞活性は、7.9 nM であり、若干の活性低下が見られた。hERG 阻害活性に改善は見られなかったものの（hERG inhib at 5 μM = 85 %)、P-gp 基質性は狙い通りに下げることできた（P-gp ER = 7.5)。活性向上を含めたプロファイルの最適化を目指し、オキサジン環上への置換基の導入を試みた。5 位にメチル基を導入したオキサジン **1-5** は、活性が低下したが、6 位に導入したオキサジン **1-6** は活性向上を示した。pK_a はわずかに増加したが（pK_a = 7.9)、P-gp 基質性が悪化した（ER = 33)。P-gp 基質性の改善を目指し、さらなる塩基性の低下を狙って **1-6** を起点にフッ素原子の導入を試みた。4 位のメチル基にフッ素原子を導入したオキサジン **1-7** は、P-gp 基質性と hERG 阻害活性が低下したものの、活性が大きく低下した（cellular IC_{50} = 17 nM)。**1-7** の pK_a は 6.3 であり、設定した至適 pK_a を逸脱していていることから、活性が低下した原因は、低すぎる塩基性であると考えられる。6 位のメチル基にフッ素原子を導入したオキサジン **1-8** は、活性を維持し（cellular IC_{50} = 3.6 nM)、pK_a が低下したことから（pK_a = 6.9)、P-gp 基質性、hERG 活性が改善した（P-gp ER = 12、hERG inhib at 5 μM = 69 %)。4 位にシクロプロピル基を導入した **1-9** は、活性が低下し（cellular IC_{50} = 70 nM)、4 位へのかさ高い置換基の導入は許容されなかった。6 位にメトキシメチル基を導入した **1-10** は塩基性が低下したものの（pK_a = 7.4)、フッ素原子ほどの効果はなく P-gp 基質性は改善しなかった（ER = 47)。脂溶性の低下（LogD = 2.0）およびアクセプター原子が増えたことが原因であると考えている。6 位をジフルオロメチル基に変換した **1-11** は活性が低下しており（cellular IC_{50} = 110 nM)、化合物 **1-4** と同様に低すぎる塩基性が原因と考察している（pK_a = 6.4)。

Table 1-1. Exploration of Head-Part

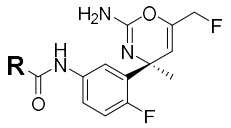


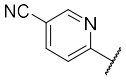
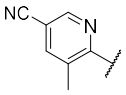
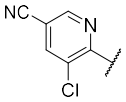
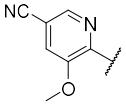
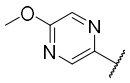
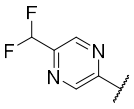
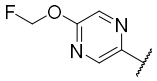
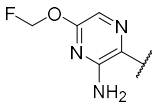
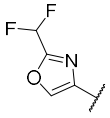
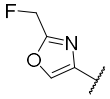
compd	R	IC_{50} (nM)[a] BACE1[b]	IC_{50} (nM)[a] Cellular Aβ[c]	P-gp ER[d]	hERG inhib (%) at 5 μM[e]	Log D (7.4)[f]	pK_a[g]
0-3		118	3.7	36	78	0.024	9.8
1-4		218	7.9	7.5	85	2.0	7.7
1-5		408	11	44	77	1.5	8.0
1-6		65.6	4.4	33	76	2.2	7.9
1-7		103	17	9.0	43	2.5	6.3
1-8		59.0	3.6	12	69	2.3	6.9
1-9		329	70	12	59	2.9	6.8
1-10		73.7	15	47	52	2.0	7.4
1-11		63.9	110	8.7	56	2.9	6.4

[a]Values represent the mean values of two experiments. [b]Biochemical HTRF-based assay. [c]IC_{50} determined by measuring the levels of secreted total Aβ in human APP-transfected human neuroblastoma (SH-SY5Y) cells via an HTRF-based assay. [d]Efflux ratio measured in MDCK cells transfected with human MDR1 at Absorption Systems. [e]% inhibition at 5 μM measured in CHO cells transfected with hERG channels using an automated patch clamp system. [f]LogD determined in 1-octanol/phosphate buffer at pH 7.4. [g]pK_a determined by capillary electrophoresis.

1-8 のプロファイルを最適化するために、tail 部分の変換を実施した（**Table 1-2**）。**1-8** の血漿中の安定性には改善の余地が残されていた。カルボキシエステラーゼの加水分解によるものと考え、ピリジン環のオルト位への置換基導入を試みた。オルト位に置換基を導入した **1-12**, **1-13** および **1-14** は狙い通りに半減期は増大したものの、P-gp 基質性が高くなった。**1-8** においてはピリジンの窒素原子が、アミド NH の水素原子と分子内水素結合を形成することで、ドナー性プロトンの影響をマスクしていたと考えられる。一方でオルト位に置換基を導入した **1-12** は、オルト位のメチル基とアミドのカルボニル間の立体障害からねじれが生じ、**1-8** で見られたような分子内水素結合が弱くなることで、ドナー性プロトンのマスク効果が減弱し、P-gp 基質性が高くなったと考察している。**1-13** や **1-14** の P-gp 基質性の悪化も同様に、オルト位の置換基とカルボニル基との間に生じる静電的な反発が原因と思われる。一連の化合物の hERG 阻害活性の低下も、アミドのドナー性プロトンの影響と考えている。分子内水素結合を形成しうることを考慮し、ピリジン環のピラジンへの変換を実施した。**1-15** は hERG 阻害活性および P-gp 基質性の改善が見られたが、低い血漿中安定性を示した。メトキシ基を持つ **1-15** はラット代謝安定性も低いことから、フルオロメトキシ基やジフルオロメチル基に変換した結果、ミクロソームの代謝安定性は改善したが、血漿中安定性の改善には至らなかった。オルト位にアミノ基を導入した **1-18** は、血漿中安定性が改善し、ドナーを増やしたにもかかわらず、P-gp 基質性は、**1-17** と同程度であった。このことは、アミドのカルボニル基と分子内水素結合を形成しているためと考えている。さらにオキサゾールへと変換した **1-19** および **1-20** は、P-gp 基質であった。オキサゾールの窒素原子とアミドの水素間の距離がピリジンやピラジンと比較して長くなるため、ドナー性プロトンのマスク効果が小さくなり、P-gp 基質性が高くなったと思われる。**1-18** は、**1-8** と比較して hERG 阻害活性と P-gp 基質性に関して良好なプロファイルを示したものの、ヒトミクロソーム安定性は 67%と中程度であった。結局のところ、Cellular IC_{50}、ヒトミクロソーム安定性を含め化合物 **1-8** が良好なバランスを示したため、**1-8** を先の評価に進めた。

Table 1-2. Optimization of Tail-Part



compd	R	IC_{50} (nM)[a] BACE1[b]	Cellular Aβ[c]	P-gp ER[d]	hERG 5 μM (%)[e]	RLM HLM (%)[f]	rat PK[g] CL (ml/min/kg)[h]	Plasma $t_{1/2}$ (h)[i]	*B/P*[j]	Log D (7.4)[k]
1-8		59.0	3.6	12	69	55 88	16.8	20.4	0.90	2.3
1-12		66.6	8.4	18	50	34 63	21.8	55.3	0.62	2.5
1-13		94.9	12	35	30	49 57	30.6	56.7	0.41	2.5
1-14		238	56	44	22	60 85	36.6	69.4	0.089	1.9
1-15		241	22	2.7	30	13 80	64.9	14.3	2.9	2.7
1-16		313	72	8.5	33	44 84	48.1	9.20	2.7	2.5
1-17		139	31	5.7	40	52 86	34.4	10.5	2.4	2.6
1-18		34.8	6.6	4.9	20	35 67	45.3	22.3	2.6	3.1
1-19		94.1	6.5	25	35	57 84	28.4	9.30	0.30	2.6
1-20		48.4	5.8	16	18	39 76	55.0	26.7	0.38	2.2

[a]Values represent the mean values of two experiments. [b]Biochemical HTRF-based assay. [c]IC_{50} determined by measuring the levels of secreted total Aβ in human APP-transfected human neuroblastoma (SH-SY5Y) cells via a HTRF-based assay. [d]Efflux ratio measured in MDCK cells transfected with human MDR1 at Absorption Systems. [e]% inhibition at 5 μM measured in CHO cells transfected with hERG channels using an automated patch clamp system. [f]Rat liver microsomal (RLM) and human liver microsomal (HLM) stability. % remaining after 30 min incubation. [g]Sprague-Dawley rats. [h]Plasma clearance dosed iv at 0.5 mg/kg as a solutution in DMA (n = 2). [i]in vitro plasma half life determined at 0,4 and 24 h. [j]Brain-to-plasma ratio determined by iv dose at 0.5 h. [k]LogD determined in 1-octanol/phosphate buffer at pH 7.4.

第 2 項　化合物 **1-8** の *in vivo* 評価

化合物 **0-3** および **1-8** の中枢移行性を確認するためラットカセットドージング評価を実施した（**Table 1-3**）。その結果、オキサジン **0-3** の Brain / Plasma ratio は 0.29 であったが、**1-8** のそれは 0.9 であり、中枢移行性が改善した。さらに、P-gp ノックアウトマウスを用いて、P-gp の影響を検証した（**Table 1-4**）。P-gp ノックアウトマウスの B/P ratio は両化合物ともに、4 を超えていて良好な中枢移行性を示したが、野生型マウスにおける化合物 **0-3** の B/P ratio は 0.15 であった。KO/WT ratio は 32 であり、P-gp の影響で中枢移行性が妨げられていることが示された。化合物 **1-8** の KO/WT は **0-3** に比べて低いことから、化合物 **1-8** の脳移行性の改善には P-gp の影響が大きいことが示唆される。ただし化合物 **1-8** の KO/WT は 3.6 であり、efflux ratio も 12 であることから、完全に P-gp 基質性を回避できたとは言い難く、さらなる中枢神経系の薬剤として改善の余地を残している。

Table 1-3. Phamacokinetic properties of compounds **0-3** and **1-8** in Sprague-Dawley rat using a cassette method.

			rat, iv, 0.5 mg/kg, $n = 2$[a]				rat, po, 1 mg/kg, $n = 2$[b]		
compd	RLM (%)[c]	$f_{u,s}$ / $f_{u,b}$[d]	CL (ml/min/kg)[e]	Vd_{ss} (L/kg)[f]	*B/P*[g]	$K_{p,uu}$[h]	AUC (ng·h/ml)[i]	C_{max} (ng/ml)[j]	*F* (%)[k]
0-3	104	0.41 / 0.080	37.2	6.1	0.29	0.057	328	32	73
1-8	55	0.16 / 0.066	16.8	3.4	0.90	0.37	617	77	52

[a]Dosed as a suspension of test compounds in 0.5% methylcellulose (MC, 400 cP). [b]Dosed as a solution of test compounds in DMA/PG = 1/1. [c]% remaining in rat liver microsomes after 30 min incubation. [d]$f_{u,s}$ = Fraction unbound in rat serum. $f_{u,b}$ = Fraction unbound in rat brain. [e]Total clearance. [f]Volume of distribution at steady state. [g]Total brain-to-plasma ratio measured at 0.5 h. [h]Unbound brain-to-plasma ratio measured at 0.5 h. Plasma area under the concentration-time curve. [j]Maximal plasma concentration. [k]Oral bioavailability.

Table 1-4. Pharmacokinetic Profiles of **0-3** and **1-8** in Wild-type and mdr1a(-/-) Mice.

		po, 2 or 10 mg/kg, $n = 3$[a]					
compd	P-gp ER[b]	dose (mg)	mouse[c]	C_b (ng/g)[d]	C_p (ng/ml)[e]	*B/P*[f]	KO/WT[g]
0-3	36	2	WT	14.5	102	0.15	32
			KO	546	118	4.7	
1-8	12	2	WT	92.6	76.1	1.2	3.6
			KO	318	71.5	4.4	

[a]Dosed as a suspension of test compounds in 0.5% MC. [b]Efflux ratio measured in MDCK cells transfected with human MDR1 at Absorption Systems. [c]KO = mdr1a (-/-) knockout mouse; WT = wild-type (C57BL/6J) mouse (vehicle = 0.5% MC). [d]Brain concentration at 2 h time point. [e]Plasma concentration at 2 h time point. [f]Brain-to-plasma ratio (C_b/C_p). [g]KO/WT = (B/P in KO)/(B/P in WT).

1-8 のラットおよびイヌの薬物動態の評価を実施した（**Table 1-5**）。1, 3, 10 mg/kg を経口投与したところ血漿中薬物濃度に用量依存性が見られ、中程度のクリアランス、バイオアベイラビリティを示した。中枢移行性を精査するためにラットに 3 mg/kg を経口投与し、脳内および CSF 中の薬物濃度を測定した（**Table 1-6**）。投与 3 時間後の C_b/ C_p は 0.83、非結合薬物濃度比 $C_{b,u}$ / $C_{p,u}$ は 0.34 であることから P-gp 基質であることが伺える。C_{CSF}/$C_{p,u}$ は 0.61 であり、CSF 中の薬物濃度は高い値を示した。P-gp は血液脳関門においては、基質を血液側に排泄するが、血液脳脊髄液関門においては基質を CSF 中に取り込むことが知られている [14c]。このため、P-gp 基質である **1-8** の C_{CSF}/$C_{p,u}$ は高くなったと考えられる。

Table 1-5. Pharmacokinetic properties of **1-8** at multiple doses in rat

	rat, po, n = 3[a]		
dose	C_{max} (ng/ml)[b]	AUC (ng·h/ml)[c]	F (%)[d]
1	70.0 ± 9.0	358 ± 20	33
3	274 ± 22	2040 ± 570	62
10	965 ± 233	4990 ± 1000	46

	rat, iv, n = 3[e]			
dose	CL (ml/min/kg)[f]	Vd_{ss} (L/kg)[g]	$t_{1/2}$[h]	AUC (ng·h/ml)[i]
2	15.4 ± 2.1	2.93 ± 0.33	2.45 ± 0.53	2190 ± 300

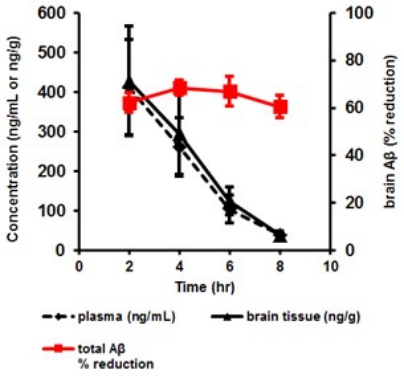
[a]Dosed as a suspension of **1-8** in 0.5% MC. [b]Maximal plasma concentration. [c]Plasma area under the curve (*po*). [d]Oral bioavailability. [e]Dosed as a solution of **1-8** in 0.5% MC. [f]Total clearance. [g]Volume of distribution at steady state. [h]Half-life (*iv*). [i]Plasma area under the curve (*iv*).

Table 1-6. CNS availability data for **1-8** in rat

	time after administration / po, 3 mg/kg, n = 3[a]			
	1 h	3 h	5 h	7 h
C_p (ng/ml)[b]	70.0 ± 9.0	51.0 ± 5.8	19.5 ± 4.7	19.6 ± 4.0
C_b (ng/g)[c]	48.9 ± 1.6	42.2 ± 8.4	16.1 ± 5.8	15.5 ± 4.0
$C_{b,u}$ (ng/g)[d]	3.2 (8.4 nM)	2.8 (7.2 nM)	1.1 (2.8 nM)	1.0 (2.7 nM)
C_{CSF} (ng/ml)[e]	7.5 ± 1.2 (20 nM)	4.9 ± 0.4 (13 nM)	2.4 ± 1.4 (6.3 nM)	1.6 ± 0.4 (4.2 nM)
C_b/C_p[f]	0.70	0.83	0.83	0.79
$C_{b,u}$/$C_{p,u}$[g]	0.29	0.34	0.34	0.32
C_{CSF}/$C_{p,u}$[h]	0.67	0.61	0.78	0.51

[a]Dosed as a suspension of **1-8** in 0.5% MC. [b]Plasma concentration. [c]Brain concentration. [d]Unbound brain concentration ($C_{b,u}$ = C_b × $f_{u,b}$). [e]Cerebrospinal fluid (CSF) concentration. [f]Total brain-to-plasma ratio (B/P or Kp). [g]Unbound brain-to-plasma ratio ($K_{p,uu}$). $C_{b,u}$ = C_b × $f_{u,b}$ (unbound brain fraction in rat). $C_{p,u}$ = C_p × $f_{u,s}$ (unbound plasma fraction in rat). [h]CSF-to- unbound plasma ratio ($K_{p,uu}$ (CSF)).

ICR マウス[17]に **1-8** を 10mg/kg 経口投与し、脳内の Aβ量を測定した（**Figure 1-4**）。その結果、4 時間で最大の Aβ減少 69 %を示し、8 時間後も持続的な脳内 Aβ減少を示した。8 時間後の脳内非結合薬物濃度は 6.9 nM であり、IC_{50}（3.6 nM）よりも 1.9 倍高く、マウスの持続的な Aβ減少を説明している。



Time (h)	C_p (ng/mL)[a]	C_b (ng/mL)[b]	$C_{b,u}$ (ng/mL)[c]	B/P[d]	Aβ reduction (%)
2	413 ± 121	428 ± 138	28.2	1.03	62 ± 4.0
4	262 ± 74	295 ± 103	19.5	1.13	69 ± 3.2
6	104 ± 35	125 ± 35	8.25	1.22	67 ± 6.3
8	38.8 ± 9.6	39.2 ± 11	2.58	1.02	61 ± 4.8

[a]Total plasma concentration. [b]Total brain concentration. [c]Unbound brain concentration ($C_{b,u} = C_b \times f_{u,b}$) [d]Total brain-to-plasma ratio.

Figure 1-4. Total Aβ reduction in mouse after an oral dose of **1-8** at 10 mg/kg (n = 4). Compound **1-8** was dosed as a solution of 20% HPBCD.

イヌの Aβ産生に関わる病理学的変化はヒトと類似していることが報告されている[18]。イヌはマウスとヒトの橋渡し研究において有用な種であり、Aβを 50%減少させることができる血漿中薬物濃度（EC_{50}）を決定するために、**1-8** を 0.31, 1.25, 5 mg/kg で経口投与し、投与後 4, 8, 25 時間後の Aβ量を測定した。用量依存的な Aβの減少が見られ、1.25 mg/kg においては、投与後 8 時間後に 70%の減少率を示した。EC_{50} の値を算出した結果、62 ng/ml（18 nM）であり、$C_{CSF}/C_{p,u}$ は 0.56 であるので（**Table 1-7**）、脳内の Free 体薬物濃度は 10 nM と推定され、細胞活性の 3 倍であり（IC_{50} = 3.6 nM）、Aβ減少が説明できた。

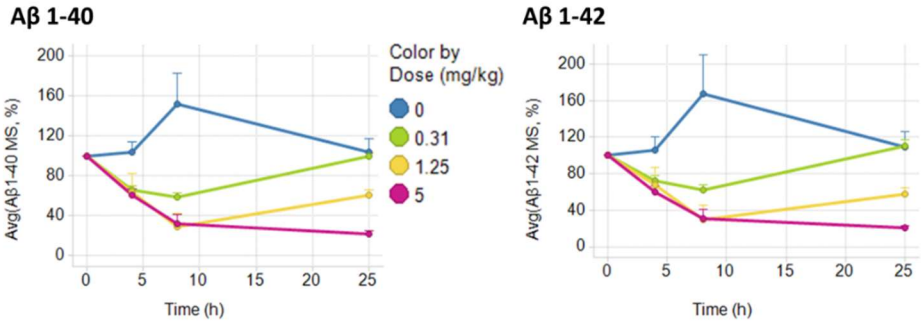


Figure 1-5. CSF Aβ1－40 and Aβ1－42 reduction in beagle dog after oral doses of **1-8** at 0.31, 1.25, and 5 mg/kg (n = 4). Compound **1-8** was dosed as a solution of 20% HPBCD.

Table 1-7. CNS availability data for **1-8** in dog

po (day7), 0.75 mg/kg, $n = 3$[a]							
10 h, ng/ml				24 h, ng/ml			
C_p	$C_{p,u}$[b]	C_{CSF}[c]	$C_{CSF}/C_{p,u}$[d]	C_p	$C_{p,u}$	C_{CSF}	$C_{CSF}/C_{p,u}$
107	11.8	6.8	0.58	27.7	3.05	1.7	0.56

[a]After 7 days of once daily dosing (po, 0.5% MC), cisternal CSF samples were collected from anesthetized dogs at 10 and 24 h time points. [b]Unbound plasma concentration ($C_{p,u} = C_p \times f_{u,s}$ (unbound plasma fraction in dog)). [c]CSF concentration. [d]CSF-to-unbound plasma ratio ($K_{p,uu}$ (CSF)).

麻酔下のモルモットに **1-8** を 3, 10, 30 mg/kg の用量を 30 分間隔で、10 分間漸増的に静脈内投与し、心毒性評価を実施した。3, 10 および 30 mg/kg の静脈内投与終了時の非結合薬物濃度は、0.907 μM, 4.31 μM, 13.4 μM であった。30 mg/kg において **1-8** は媒体対照群と比較して、QTc 間隔の持続時間を 13.9 %増加させた。このことから、モルモットの心血管安全性の無毒性用量（NOAEL）は 4.3 μM であり、イヌ EC_{50} と比較して安全性マージンは 239 倍であった。

イヌにおいても心毒性評価を実施した。イヌの PK と上の NOAEL から 15, 30, 100 mg/kg で経口投与した。30 mg/kg では、動脈血圧、心拍数、PR 間隔、QTc 延長は認められなかったが、100 mg/kg で QTc 間隔の有意な増加 (16.1 %) が観察された。30 および 100 mg/kg の非結合最大濃度は、1.40 μM,、および 3.21 μM であり、**1-8** の NOAEL は、1.4 μM であった。これは、hERG IC_{50} 値の 2.0 μM に近く、イヌ EC_{50} の 78 倍の安全性マージンを確認した。このことから、**1-8** は心毒性における十分な安全性を有していると考えている。

Table 1-8. Cardiovascular safety study for **1-8** in guinea pig and dog

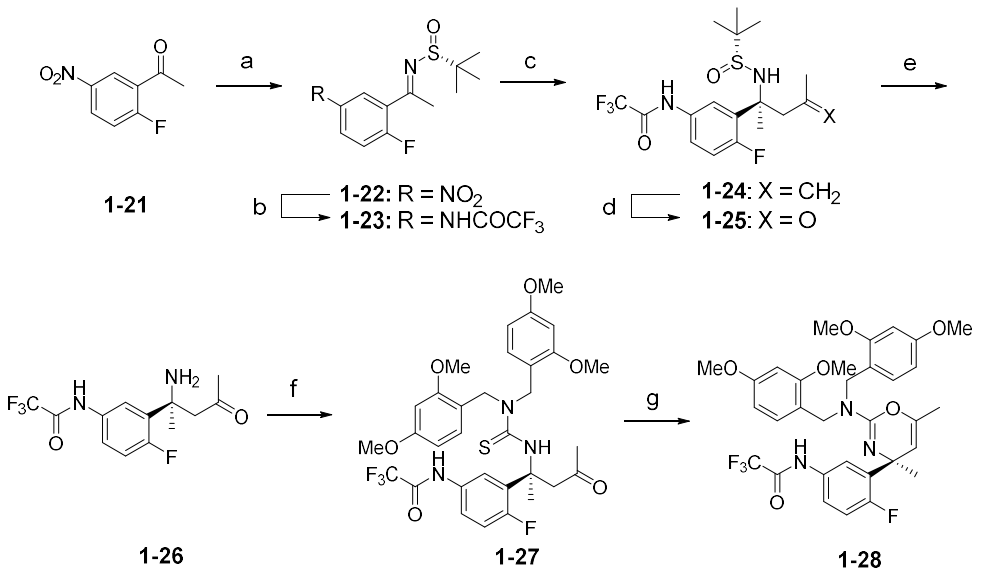
	$f_{u,s}$	Dose (mg)	C_p (μg/mL)[a]	$C_{p,u}$ (μM)[b]	QTc prolongation(%)
guinea pig	0.42	3	0.83	0.91	3.9
		10	3.93	4.31	6.0
		30	12.2	13.4	13.9
dog	0.11	30	4.9	1.4	4.9
		100	11.2	3.21	16.1

[a]Total plasma concentration. [b]Unbound plasma concentration ($C_{p,u} = C_p \times f_{u,s}$ (unbound plasma fraction)).

第３節　オレフィン型オキサジン誘導体の合成

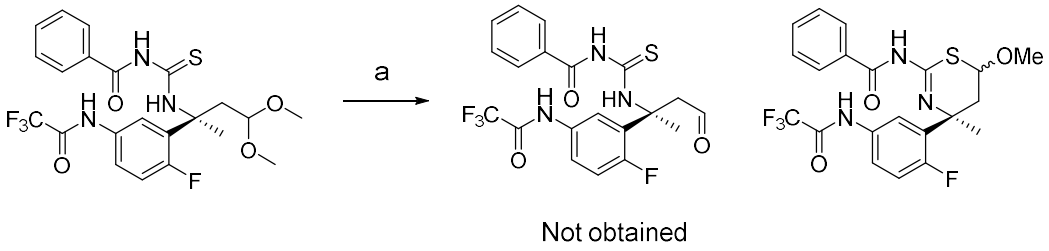
第１項　オレフィン型オキサジンの新規環化反応の開発

はじめに 6-メチルオキサジン誘導体 **1-28** は、**Scheme 1-1** に示された経路で合成した。ケトン **1-21** に対し $Ti(OEt)_4$ を用いて、キラルな(*R*)-2-メチルプロパン-2-スルフィンアミド[19]と縮合させることで、キラルなケチミン **1-22** を得た。ニトロ基の還元を行い、生じたアミンをトリフルオロアセチル基で保護し、アミド **1-23** に導いた。**1-23** に対し、イソブテニルマグネシウムブロミドを立体選択的に作用させ、単一のジアステレオマーとして **1-24** を得た。ハリースオゾン分解反応にて **1-25** に導き、酸性条件に付すことでスルフィニル基を除去しアミン **1-26** を得た。チオホスゲンを用いて、イソチオシアナートとした後に、ビス-ジメトキシベンジルアミンを作用させることで、チオウレア **1-27** を得た。ヨウ化メチルを用いて、メチルスルフィドとした後に、DIEA を加え加熱することで、目的とするオキサジン **1-28** を得た[20]。しかしながら、本反応は 2 工程で 19%と低収率であった。さらに、6-*H*オキサジン合成の前駆体アルデヒド **1-30** を得るために、**1-29** を塩酸で処理したところ、チオウレアとの環化反応が進行し **1-30** を得ることができなかった（**Scheme 1-2**）。



Scheme 1-1. Synthesis of Oxazine **1-28**[a]

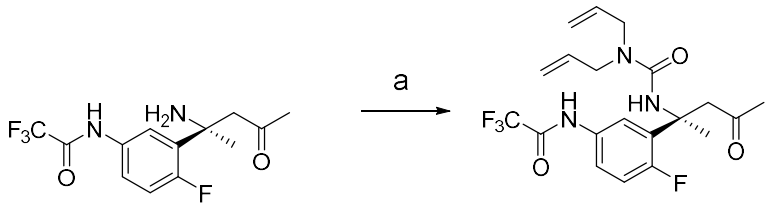
[a]Reagents and conditions: (a) (*R*)-2-methylpropane-2-sulfinamide, $Ti(OEt)_4$, THF, 65 °C, 65%; (b) (i) Fe, NH_4Cl, toluene−H_2O, 80 °C, (ii) TFAA, Et_3N, THF, −25 °C, 78%; (c) 2-methylallylmagnesium chloride, THF, −78 °C, 72%; (d) O_3, DCM, −78 °C then Et_3N, 100%; (e) 4 M HCl in dioxane, rt, 84%; (f)(i) thiophosgene, K_2CO_3, toluene, H_2O, 0 °C, 100%,(ii) bis(2,4-dimethoxybenzyl)amine, THF, rt, 97%; (g) (i) MeI, DIEA, MeCH, rt, 93%, (ii) DIEA, MeCN, reflux, 19%.



1-29 **1-30** **1-31**

Scheme 1-2. Synthetic approach for aldehyde **1-30**[a]
[a]Reagents and conditions: (a) 2 M HClaq, THF, rt.

チオウレアを中間体に 6-*H* オキサジンを合成することは困難であると判断し、ウレアを中間体としたオキサジンの環化反応の開発を試みた。酸性条件での脱水反応の検討を想定し[21]、**Scheme 1-3** の方法で、ウレア **1-32** の合成を行った。アミン **1-26** にトリホスゲンを用いて、イソシアナートを得た後に、ジアリルアミンを作用させ **1-32** を得た。

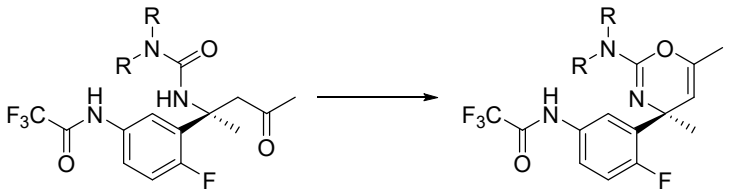


1-26 **1-32**

Scheme 1-3. Synthesis of Urea **1-32**[a]
[a]Reagents and conditions: (a) (i) K_2CO_3, triphosgene, EtOAc, H_2O, 0 °C; (ii) diallylamine, THF, rt, 2 steps 62%.

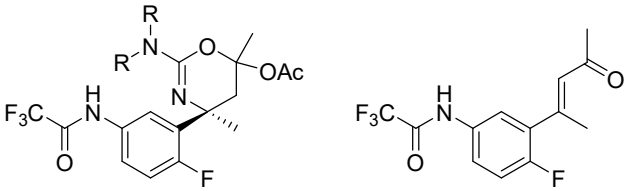
1-32 に対して、脱水反応に用いられる条件を検討した結果（**Table 1-8**)、酢酸中、P_2O_5 を用いた際に望む環化体 **1-33** が得られた（**Entry 2**)。副生成物として 7:3 のジアステレオマー混合物 **1-34** が得られ、これは溶媒に酢酸を用いたことが原因と考えた。溶媒をアセトニトリルに変更した結果、**1-34** の生成が抑えられ、**1-33** の収率の改善が見られた（**Entry 3**)。P_2O_5 の当量を減らして実施したが、目的物の生成は確認できなかった（**Entry 4**)。脱水試薬として Burgess 試薬（methyl *N*-(triethylammoniumsulfonyl)carbamate ）[22]を用いた結果、高収率で目的物を得ることができ、室温で実施しても問題なく進行した（**Entry 5, 6**)。

Table 1-8. Optimization of the reaction conditions using various dehydration reagents.



1-32 R = allyl **1-33** R = allyl

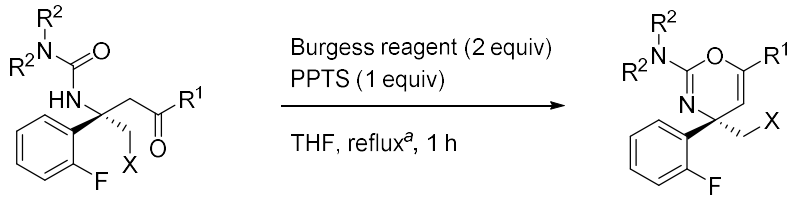
Entry	Reagent (equiv)	Solvent	Temp	Yield **1-30** (%)
1	-	AcOH	120	ND[a]
2	P_2O_5 (15)	AcOH	120	26[b]
3	P_2O_5 (15)	MeCN	70	54
4	P_2O_5 (2)	MeCN	70	ND[c]
5	Burgess reagent (2)	THF	80	83
6	Burgess reagent (2)	THF	rt	88



1-34 **1-35**

[a]**1-33** was not detected by LC/MS. [b]**1-34** (diastereo mixture 7:3) was obtained in 24% yield. [c]The urea group was eliminated to give **1-35**.

この条件をアルデヒド **1-36** に適応した結果、6-*H*オキサジンを 13%の収率で得ることができた。反応機構を考慮すると酸性条件で加速すると考え、PPTS を加えた結果、収率が 27%に改善した。他の中間体 **1-37** および **1-38** においても問題なく環化反応は進行し、望むオキサジン **1-40** および **1-41** が得られた（**Scheme 1-4**）。反応機構は **Scheme 1-5** を想定している。これは、酢酸中で **1-34** が得られたことと、PPTS を添加することで収率が改善したことがこの機構を支持していると考察している。



1-36: R^1 = H, R^2 = 2,4-dimethoxybenzyl, X = H

1-37: R^1 = CH_2OAc, R^2 = 2,4-dimethoxybenzyl, X = H

1-38: R^1 = Me, R^2 = 2,4-dimethoxybenzyl, X = F

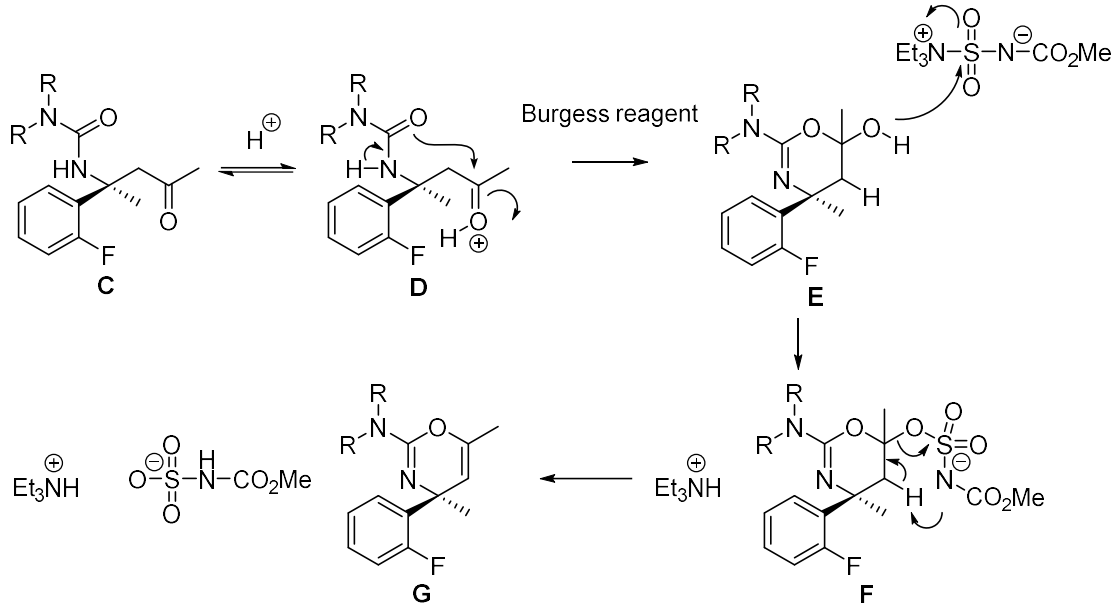
1-39: R^1 = H, R^2 = 2,4-dimethoxybenzyl, X = H (27% yield)

1-40: R^1 = CH_2OAc, R^2 = 2,4-dimethoxybenzyl, X = H (41% yield)

1-41: R^1 = Me, R^2 = 2,4-dimethoxybenzyl, X = F (62% yield)

Scheme 1-4. Synthesis of amino-1,3-oxzines **1-39, 1-40, 1-41** using Burgess reagent.

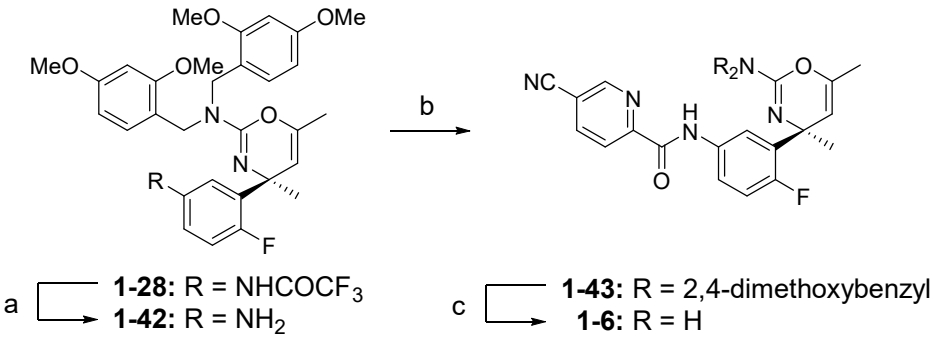
[a]The cyclization reaction of **1-41** proceeded at room temperature.



Scheme 1-5. Plausible mechanism for the cyclization of urea **C** using Burgess reagent.

第２項　オレフィン型オキサジン誘導体の合成

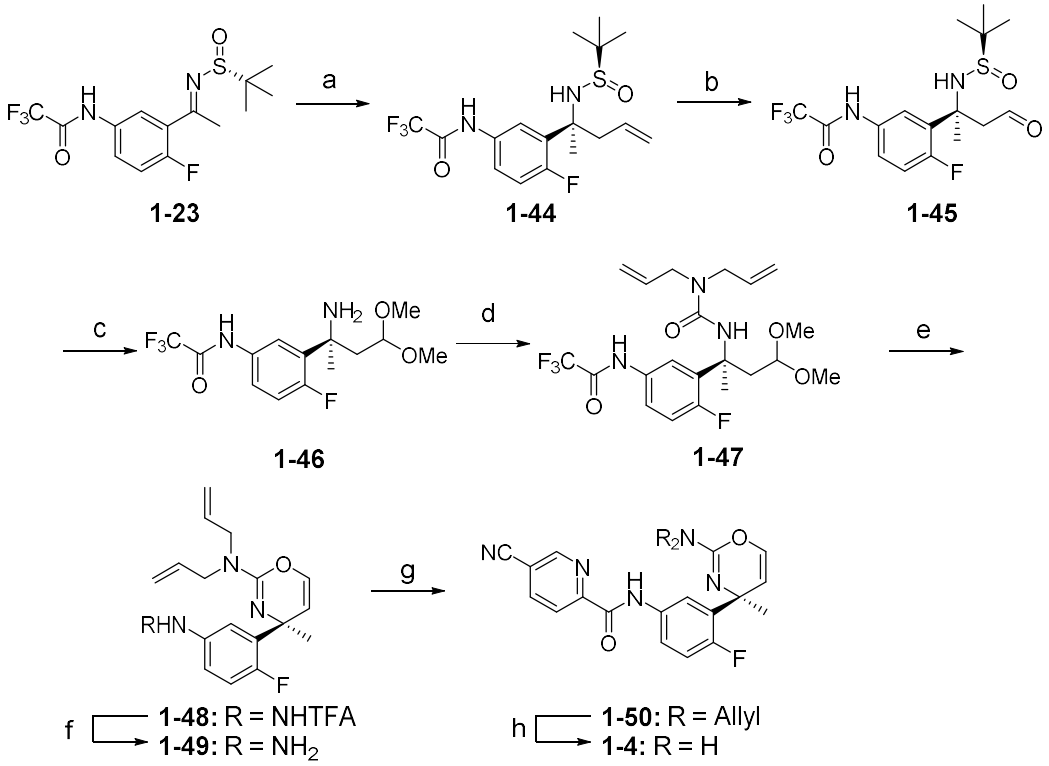
オキサジン **1-6** は前項の中間体より、**Scheme 1-6** に示した経路で合成した。すなわち、トリフルオロアセチル基を炭酸カリウムを用いて除去しアミン **1-42** に導いた後に、HATU を用いてアミド **1-43** に導いた。最後にアニソールと TFA を用いてジメトキシベンジル基を除去し、オキサジン **1-6** を得た。なお、**1-6** の絶対配置は単結晶 X 線構造解析で決定した（実験の部参照）。



Scheme 1-6. Synthesis of Oxazine **1-6**[a]

[a]Reagents and conditions: (a) K_2CO_3, THF－MeOH－H_2O, 40 ℃, 56%; (b) HATU, DIEA, 5-cyanopicolinic acid, DMF, rt, 92%; (h) anisole, TFA, 80 ℃, 48%.

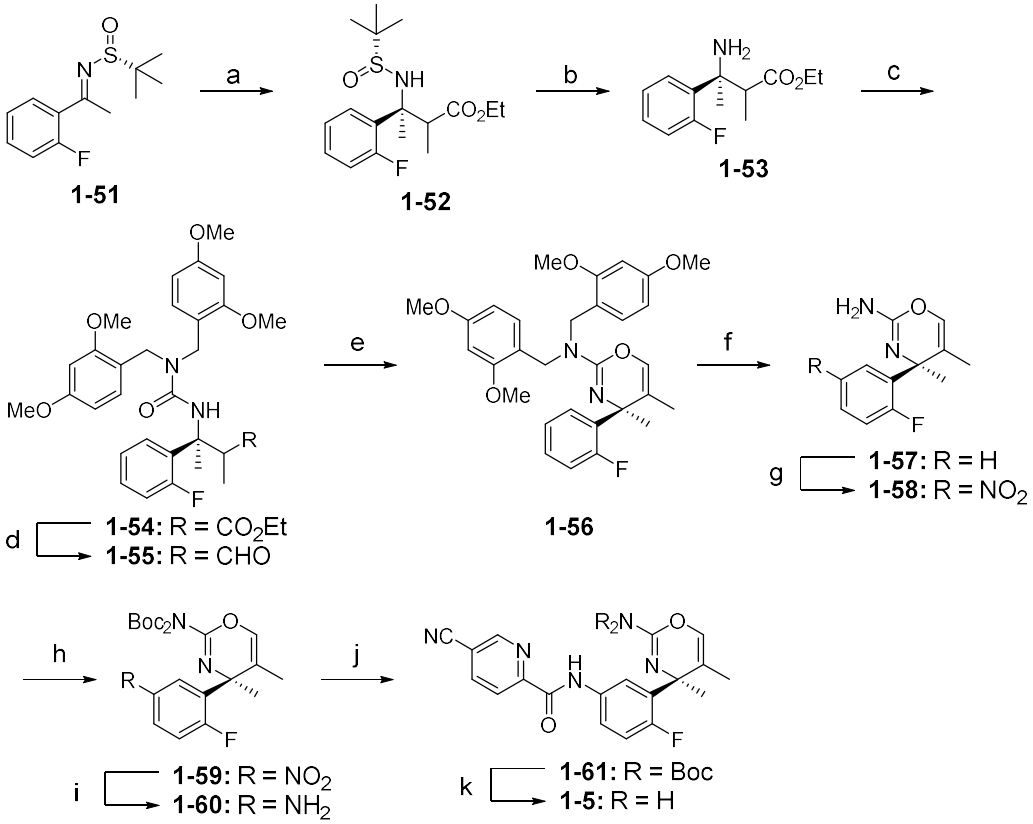
オキサジン **1-4** は **Scheme1-7**　に記載の方法で合成した。キラルなケチミン **1-23** に対し、アリルメチルマグネシウムブロミドを作用させ、**1-44** を得た。オゾン分解により、アルデヒド **1-45** に導いた後に、酸性条件にて、スルフィニル基の除去とアルデヒドのアセタール保護を同時に行い **1-46** とした。アルデヒドの脱保護を酸性条件で行うことを考慮し、ジアリルアミンとのウレア化を行い、**1-47** を得た。酸性条件にてアルデヒドに変換した後に、Burgess 試薬を用いてオキサジン **1-48** を合成した。トリアセチル基の脱保護後、生じたアミン **1-49** と縮合反応を行い、さらにアリル基を $Pd(PPh_3)_4$ を用いて除去し、オキサジン **1-4** を得た。絶対配置は **1-11** と同じ中間体を用いたため、**1-11** の絶対配置を決定することで確認した(実験の部参照)。



Scheme 1-7. Synthesis of Oxazine **1-4**[a]

[a]Reagents and conditions: (a) allylmagnesium bromide, THF, −78 °C, 40%; (b) O_3, DCM, −78 °C then Et_3N, 88%; (c) 2 M HCl in MeOH, rt, 56%; (d) triphosgene, Et_3N, diallylamine, H_2O − EtOAc, 0 °C, 82%; (e) (i) aq H_2SO_4, acetone, (ii) Burgess reagent, PPTS, THF, reflux, 24%; (f) K_2CO_3, THF − MeOH − H_2O, 40 °C, 87%; (g) EDC, HOBt, DMAP, 5-cyanopicolinic acid, DMF, rt, 92%; (h) 1,3-dimethylbarbituric acid, $Pd(PPh_3)_4$, DCM, reflux, 58%.

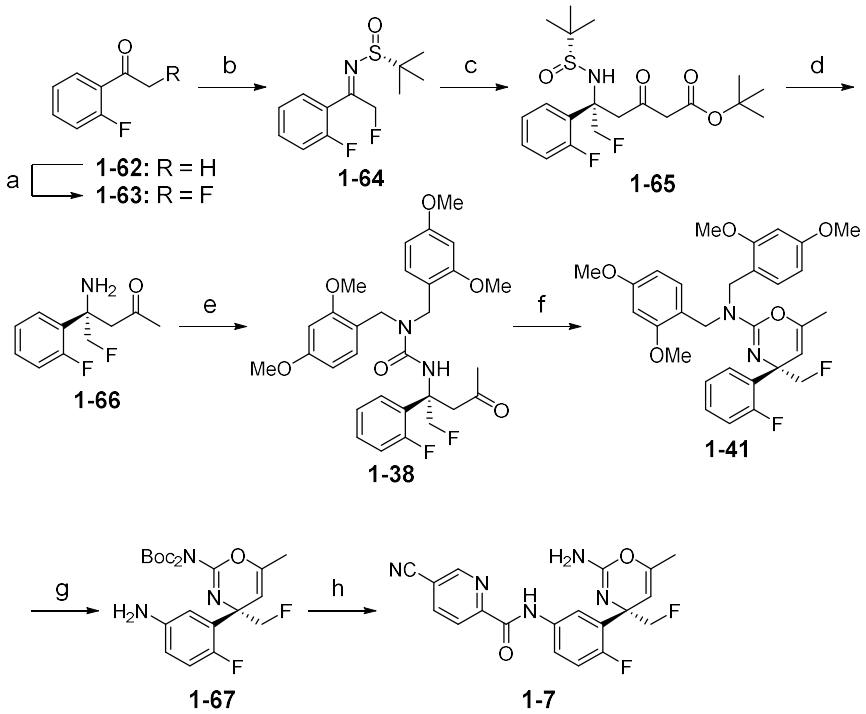
5-メチルオキサジン **1-5** は **Scheme 1-8** に従って合成した。キラルなスルフィニルイミン **1-51** に対し、調製したチタンエステルエノラートを作用させ、**1-52** をジアステレオマー混合物として得た。混合物のまま、酸性条件にてスルフィニル基を除去し、ウレア **1-54** に導いた。DIBAL を用いてエステルをアルデヒドに還元し、Burgess 試薬を用いてオキサジン **1-56** を得た。2 つのジメトキシベンジル基を除去、次いでニトロ化を行い、アミジン部位を Boc 基で保護し **1-59** を得た。ニトロ基を鉄と塩化アンモニウムを用いて還元し、ピコリン酸と縮合、Boc 基の除去を行い目的物 **1-5** を得た。絶対配置は **2-4** と同じ中間体を用いたため、**2-4** の絶対配置を決定することで確認した（実験の部参照）。



Scheme 1-8. Synthesis of Oxazine **1-5**[a]

[a]Reagents and conditions: (a) ethyl propionate, LDA, $TiCl(O\textit{i}\text{-}Pr)_3$, THF, −78 °C, 91%; (b) 4 M HCl in dioxane, MeOH, rt, 100%; (c) 4-nitrophenyl chloroformate, $NaHCO_3$, bis(2,4-dimethoxybenzyl)amine, EtOAc−H_2O, rt, 100%; (d) DIBAL, DCM, −78 °C, 60%; (e) Burgess reagent, PPTS, THF, reflux, 53%; (f) anisole, TFA, 80 °C, 92%; (g) HNO_3, H_2SO_4−TFA, −20 °C, 100%; (h) Boc_2O, DMAP, DCM, rt. 91%; (i) Fe, NH_4Cl, EtOH−THF−H_2O, 60 °C, 77%; (j) HATU, DIEA, 5-cyanopicolinic acid, DMF, rt, 100%; (k) HCO_2H, rt, 76%.

4-フルオロメチルオキサジン **1-7** は **Scheme 1-9** に記載の方法で合成した。ケトン **1-62** を TMS エノラートに変換し、セレクトフルオルを用いて **1-63** を合成した。スルフィニルイミンに導き、チタンエノラートを立体選択的に作用させ、キラルな **1-65** を得た。4 mol/L の塩化水素-1,4-ジオキサン溶液を用いてスルフィニル基の除去と *tert*-ブチルエステルの開裂を行い、6 mol/L の塩酸による脱炭酸反応によってアミン **1-66** に導いた。これ以降は **Scheme 1-8** と同様の方法で、**1-7** を合成した。**1-7** の絶対配置は単結晶 X 線構造解析で決定した（実験の部参照)。

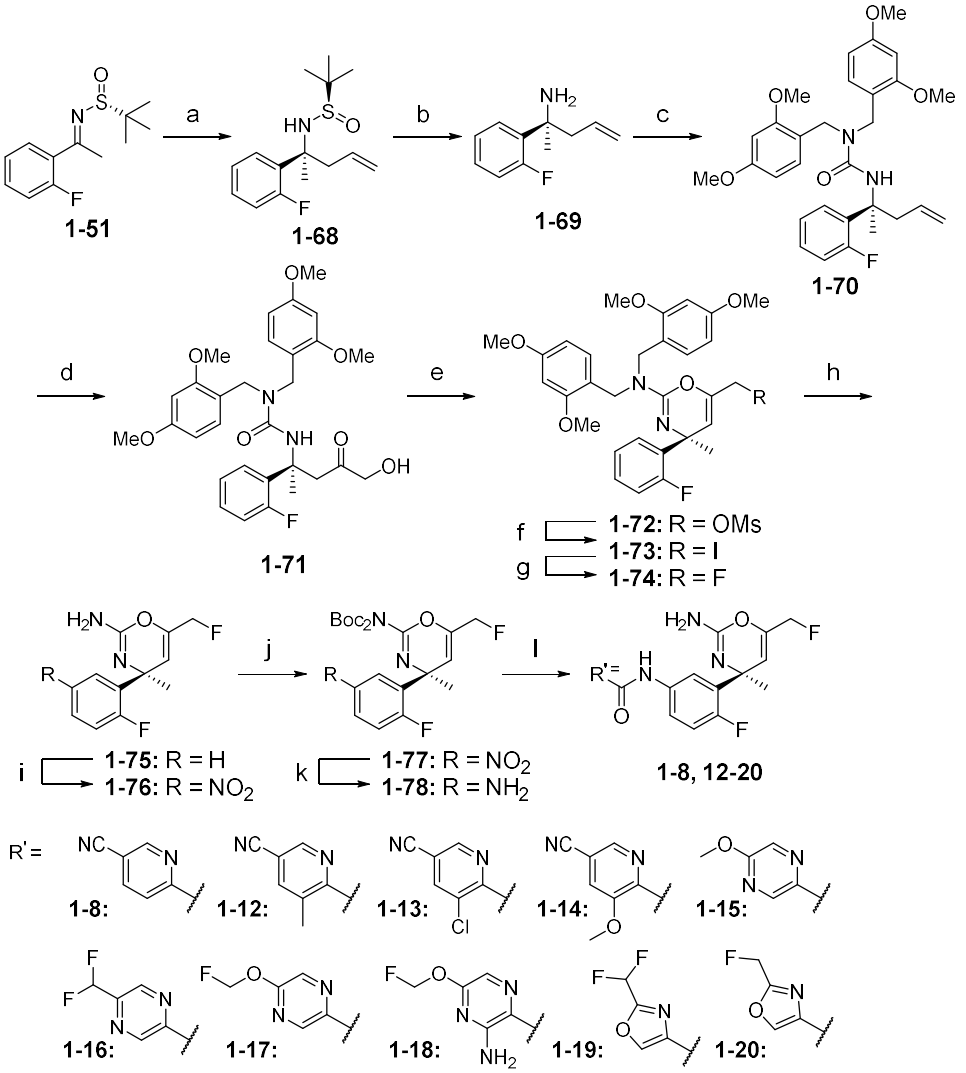


Scheme 1-9. Synthesis of Oxazine **1-7**[a]

[a]Reagents and conditions: (a) (i) LHMDS, TMSCl, THF, −78 ℃, (ii) Selectfluor, MeCN, 4 ℃ to rt, 89%; (b) 2-methylpropane-2-sulfinamide, $Ti(OEt)_4$, DCM, 40 ℃, 71%; (c) *tert*-butyl acetoacetate, LDA, $TiCl(O\textit{i}\text{-}Pr)_3$, THF, −78 ℃, 81%; (d) (i) 4 M HCl in dioxane, 60 ℃, (ii) 6 M HCl in H_2O, 85 ℃, 100%; (e) 4-nitrophenyl chloroformate, $NaHCO_3$, bis(2,4-dimethoxybenzyl)amine, EtOAc−H_2O, rt, 81%; (f) Burgess reagent, PPTS, THF, reflux, 82%; (g) (i) anisole, TFA, 80 ℃, (ii) HNO_3, H_2SO_4−TFA, −20 ℃, (iii) Boc_2O, DMAP, DCM, rt, (iv) Fe, NH_4Cl, EtOH−THF−H_2O, 60 ℃, 35% (4 steps); (h) (i) HATU, DIEA, 5-cyanopicolinic acid, DMF, rt, (ii) HCO_2H, rt, 72% (2 steps).

6-フルオロメチルオキサジン **1-8** の合成を **Scheme 1-10** に示した。スルフィニルイミン **1-51** にアリルマグネシウムブロミドを作用させ、キラルな **1-68** を合成した。酸性条件にてスルフィニル基を除去し、ウレア化を行い **1-70** を得た。酢酸中、過マンガン酸カリウムを

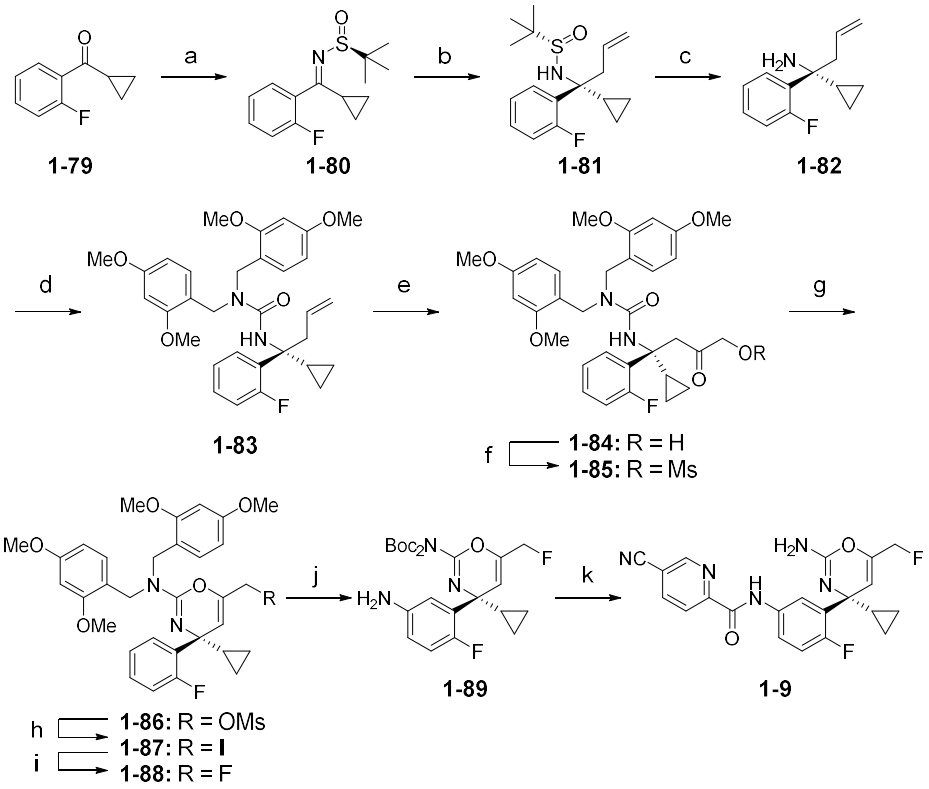
用いてαヒドロキシケトン **1-71** に導いた[23]。ヒドロキシ基をメシラートに変換し、環化反応を行い、**1-72** を得た。オキサジン環化後にヒドロキシ基を DAST 等を用いてフッ素化することも可能であったが低収率であり、メシラート **1-72** をヨウ素化し、次いでフッ素化することで収率が改善した。これ以降は **Scheme1-8** と同様の方法で、**1-8** を合成した。また、種々のカルボン酸[24]と HATU を用いた縮合反応で **1-12**－**20** を合成した。絶対配置は **1-11** と同じ中間体を用いたため、**1-11** の絶対配置を決定することで確認した（実験の部参照）。



Scheme 1-10. Synthesis of Oxazine **1-8, 12**－**20**[a]

[a]Reagents and conditions: (a) allylmagnesium bromide, Et_2O, －60 to －20 ℃, 72%; (b) 4 M HCl in EtOAc, MeOH, 0 ℃, quant; (c) 4-nitrophenyl chloroformate, K_2CO_3, bis(2,4-dimethoxybenzyl)amine, EtOAc－H_2O, 0 ℃ to rt, 82%; (d) $KMnO_4$, AcOH, acetone－H_2O, rt, 68%; (e) (i) Ms_2O, DCM, 0 ℃, (ii) Burgess reagent, THF, rt, 40%; (f) NaI, acetone, rt, 95%; (g) AgF, MeCN, rt, 98%; (h) anisole, TFA, 80 ℃, quant; (i) HNO_3, H_2SO_4－TFA, －20 ℃, 94%; (j) Boc_2O, DMAP, THF, 0 ℃, 90%; (k) Fe, NH_4Cl, EtOH－THF－H_2O, 60 ℃, 85%; (l) (i) HATU, RCO_2H, DIEA, DCM, rt, (ii) HCO_2H, rt, 61－95%.

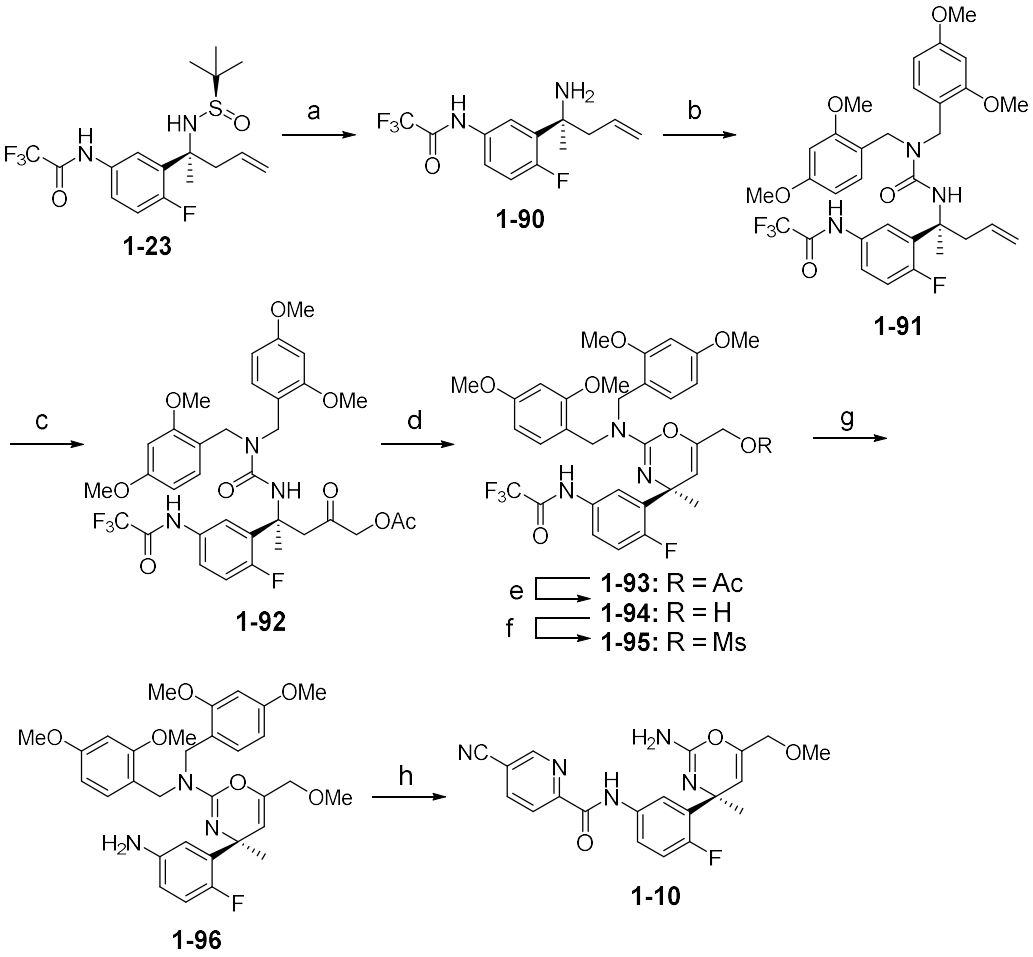
1-9 の合成は **Scheme 1-11** に記載の方法で合成した。オキサジンの 4 位がメチル基やフルオロメチル基の場合は、(*R*)-2-メチルプロパンスルフィンアミドを用いて、*4S*-オキサジン誘導体を得たが、4 位がシクロプロピル基の場合は、*4R*-オキサジンが得られた。このため(*S*)-2-メチルプロパンスルフィンアミドを用いてスルフィニルイミン **1-80** を合成した。アリルマグネシウムブロミドを付加し **1-81** とした後に、酸性条件にてスルフィンアミドを除去し、ウレア **1-83** に導いた。これ以降は **Scheme 1-10** と同様の方法で合成し、**1-9** の絶対配置は単結晶 X 線構造解析で決定した（実験の部参照)。



Scheme 1-11. Synthesis of Oxazine **1-9**[a]

[a]Reagents and conditions: (a) (*S*)-2-methylpropane-2-sulfinamide, $Ti(OEt)_4$, toluene, 80 °C, 63%; (b) allylmagnesium bromide, THF, −78 °C, 45%; (c) 2 M HCl in MeOH, rt, 100%; (d) 4-nitrophenyl chloroformate, $NaHCO_3$, bis(2,4-dimethoxybenzyl)amine, EtOAc−H_2O, rt, 100%; (e) $KMnO_4$, NaOAc, acetone−H_2O−AcOH, rt, 68%; (f) Ms_2O, Et_3N, DCM, 0 °C, 100%; (g) Burgess reagent, PPTS, THF, reflux, 60%; (h) NaI, acetone, rt, 76%; (i) AgF, MeCN, rt, 95%; (j) (i) anisole, TFA, 80 °C, (ii) HNO_3, H_2SO_4−TFA, −20 °C, (iii) Boc_2O, DMAP, DCM, rt, (iv) Fe, NH_4Cl, EtOH−THF−H_2O, 60 °C, 75% (4 steps); (h) (i) HATU, DIEA, 5-cyanopicolinic acid, DMF, rt, (ii) HCO_2H, rt, 81% (2 steps).

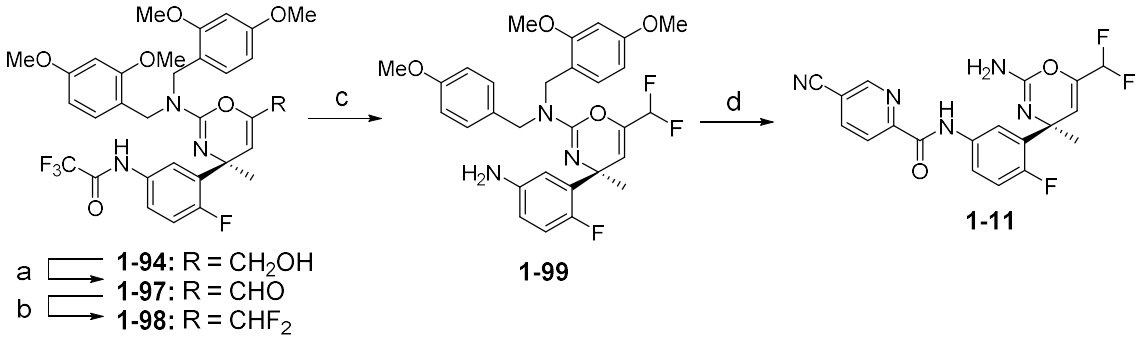
1-10 の合成は **Scheme 1-12** に記載した方法で合成した。キラルなスルフィンアミド **1-23** を酸性条件に付すことでアミン **1-90** に導き、ウレア化を行い **1-91** を得た。四酸化オスミウムを用いてジオールを得た後に、1 級アルコールのみをアセチル化、次いで 2 級アルコールを酸化し、**1-92** を合成した。Buregess 試薬を用いてオキサジン **1-93** にした後に、アセチル基を加水分解し、生じたアルコールをメシレートに変換した。NaOMe を用いてメトキシ基の付加とトリフルオロアセチル基の除去を行い **1-96** を得た。ピコリン酸と縮合し、最後にジメトキシベンジル基の除去を行い、**1-10** を合成した。絶対配置は **1-11** と同じ中間体を用いたため、**1-11** の絶対配置を決定することで確認した（実験の部参照)。



Scheme 1-12. Synthesis of Oxazine **1-10**[a]

[a]Reagents and conditions: (a) 2 M HCl in dioxane, MeOH, rt, 100%; (b) 4-nitrophenyl chloroformate, $NaHCO_3$, bis(2,4-dimethoxybenzyl)amine, EtOAc－H_2O, rt, 100%; (c) (i) $K_2OsO_4{\cdot}2H_2O$, NMO, acetone－H_2O, rt, (ii) Ac_2O, DMAP, pyridine－DCM, 0 ℃, (iii) DMP, DCM, rt, 99%; (d) Burgess reagent, PPTS, THF, reflux, 53%; (e) NaOMe, MeOH, rt, 88%; (f) Ms_2O, Et_3N, DCM, 0 ℃, 91%; (g) (i) NaOMe, MeOH, 40 ℃, (ii) K_2CO_3, MeOH－THF－H_2O, 50 ℃, 84%; (h) (i) HATU, DIEA, 5-cyanopicolinic acid, DMF, rt, (ii) anisole, TFA, 80 ℃, 63%.

Scheme 1-13 に 6-ジフルオロメチルオキサジン 1-11 の合成を記載した。1-94 のアルコールを酸化しアルデヒドに変換した後に、DAST を用いてジフルオロメチル基へと変換した。これ以降は Scheme 1-12 と同様の方法で 1-11 を得た。1-11 の絶対配置は単結晶 X 線構造解析によって確認した（実験の部参照）。



Scheme 1-13. Synthesis of Oxazine **1-11**[a]

[a]Reagents and conditions: (a) DMP, DCM, rt, 98%; (b) DAST, DCM, 0 °C, 30%; (c) K_2CO_3, THF－MeOH－H_2O, 40 °C, 95%; (d) (i) HATU, DIEA, 5-cyanopicolinic acid, DMF, rt, (ii) anisole, TFA, 80 °C, 53%.

第２章　ジヒドロ型オキサジン誘導体の創製研究

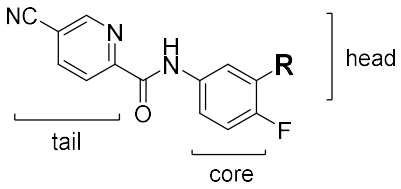
第１節　化合物 2-17 の創製

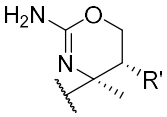
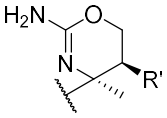
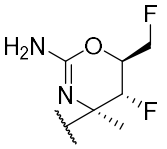
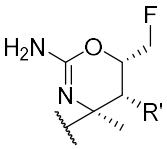
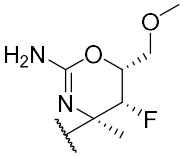
第１項　ジヒドロ型オキサジン誘導体の最適化研究

第１章で、pK_a を低減することが、P-gp 基質性と hERG 阻害活性の回避につながり、BACE1 阻害剤に対する pK_a の至適範囲が 6.5~8 であること、さらにオキサジン環内に２重結合を導入することで、pK_a を低減し、*in vivo* 薬効を示す化合物を同定したことを説明した。pK_a を低減する方法として、電子求引基の導入も知られていて[15]、筆者は電子求引基の導入により *in vivo* で薬効を示す化合物を見出すことも検討した。

ロッシュはオキサジンの 5 位にフッ素原子を導入し、有意な Aβ 減少を示すオキサジン **2-1** を報告した[13a]。P-gp 基質性は低下したものの、hERG 阻害活性は改善しておらず、前述した至適 pK_a を考慮しても改善の余地があるため、**2-1** を起点に探索研究を行った（**Table 2-1**）。まず **2-1** の 5 位の置換基変換を実施し、**2-1** を起点化合物とすることの妥当性を検証した。フッ素原子をメチル基に変換した結果、塩基性は **0-3** に対して低下せずに活性の向上も見られなかった。**2-1** の立体異性体である **2-3** を合成した結果、大幅な活性の低下が見られた。塩基性と活性の点から **2-1** が起点化合物に適していると判断し、さらなる塩基性低下を指向して 6 位にフルオロアルキル基を導入した **2-5** をデザインした。**2-5** は狙い通りに塩基性が低下し、P-gp 基質性および hERG 阻害活性が改善したものの、活性の低下が見られた。**2-5** の立体異性体である **2-6** を評価した結果、活性が向上した。電子求引基であるメトキシ基を導入した **2-7** や **2-8** を評価したが、フッ素原子ほどの塩基性を下げる効果はなく、P-gp や hERG 活性に改善は見られなかった。活性、P-gp 基質性、hERG 阻害活性において良好なプロファイルを示した **2-6** の更なる最適化研究に着手した。

Table 2-1. Exploration of the Head-Part



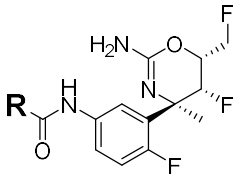
compd	R	R'	BACE1 IC_{50} (nM)[a]	P-gp ER[b]	hERG inhib at 3 μM[c]	pK_a[d]
0-3		H	118	20	66	9.8
2-1		F	47.6	4.0	68	8.1
2-2		Me	83.6	35	63	10.4
2-3		F	1170	1.2	85	7.6
2-4		Me	71.5	13	74	9.8
2-5			120	2.2	50	7.1
2-6		F	11.9	3.0	55	7.3
2-7		OMe	27.6	6.3	62	7.9
2-8			25.8	9.3	78	7.6

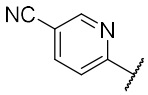
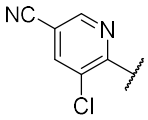
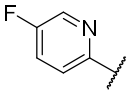
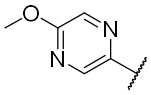
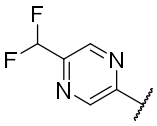
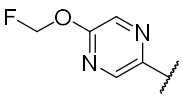
[a]Values represent the mean values of at least two determinations. Biochemical HTRF-based assay. An IC_{50} value for a reference compound (cas# 797035-11-1) was 22.6 nM. [b]Efflux ratio measured in LLC-PK1 cells transfected with human MDR1. [c]% inhibition at 3 μM measured in CHO cells transfected with hERG channels using an automated patch clamp system. [d]pK_a determined by capillary electrophoresis.

高活性で P-gp 基質性が改善した化合物を見出したものの、hERG 阻害活性に関しては改善の余地があり、ピコリンアミド部の最適化を行った（**Table 2-2**）。オルト位に塩素原子を導入した **2-9** の hERG 阻害活性は低下したものの、P-gp 基質性は向上した。シアノ基をフッ素原子に変換した **2-10** は、活性の低下が見られた。ピリジンをピラジンに変換した **2-11** は活性は維持し、P-gp 基質性、hERG 阻害活性の改善が見られた。一方で、ヒト代謝安定性の低下が見られた。ヒト代謝安定性が低い原因はメトキシ基と考え、ジフルオロメチル基、

フルオロメトキシ基に変換したところ、代謝安定性の改善が見られた。化合物 **2-13** は活性、P-gp 基質性、hERG 阻害活性において良好なプロファイルを示した。一方で、CYP2D6 阻害活性の増加が見られた。

Table 2-2. Optimization of the Tail-Part

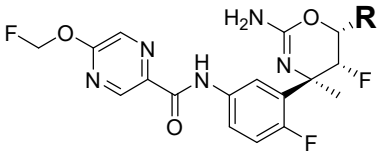


compd	R	IC_{50} (nM)[a] BACE1[b]	Cellular Aβ[c]	P-gp ER[d]	hERG 3 μM (%)[e]	HLM RLM (%)[f]	CYP2D6 IC_{50} (μM)[g]
2-6		11.9	0.58	3.0	55	110 97	10
2-9		12.9	1.7	16	43	77 98	>20
2-10		25.8	3.4	1.9	39	95 95	12
2-11		17.9	1.6	1.9	22	9.4 107	<1.0
2-12		27.4	3.7	0.90	35	98 124	<1.0
2-13		11.7	1.5	1.4	36	93 107	<1.0

[a]Values represent the mean values of at least two determinations. [b]Biochemical HTRF-based assay. An IC_{50} value for a reference compound (cas# 797035-11-1) was 22.6 nM. [c]IC_{50} determined by measuring the levels of secreted Aβ40 in human APP-transfected human neuroblastoma (SH-SY5Y) cells via a HTRF-based assay. An IC_{50} value for a reference compound (cas# 797035-11-1) was 25.9 nM. [d]Efflux ratio measured in LLC-PK1 cells transfected with human MDR1. [e]% inhibition at 3 μM measured in CHO cells transfected with hERG channels using an automated patch clamp system. [f]% remaining in human (HLM) and rat (RLM) liver microsomes after 30 min. [g]CYP2D6 inhibition measured with 5 μM dextromethorphan in human microsomes.

CYP2D6 の阻害活性を低減する方法として、塩基性、あるいは脂溶性を下げることが有効であると報告されている[25]。6 位の最適化においては検討の余地が残されていたため、**2-13** を起点に最適化を行った（**Table 2-3**）。まず塩基性を下げるため、6 位にジフルオロエチル基を導入した結果、化合物 **2-14** の塩基性が低下し、CYP2D6 の阻害活性が低下した。LogD は増加しているため、この改善は塩基性を下げたことに関係していると考察している。CYP2D6 の阻害活性の低下は、立体的なかさ高さが原因とも考えられたので、フルオロシクロプロピルに変換した。**2-15** の塩基性は **2-13** と同程度であったが、CYP2D6 阻害活性は 3.5 μMを示した。立体的な効果が CYP2D6 の阻害活性に影響したと考察し、モノフルオロエチル基に変換し、2 つのジアステレオマーを評価した結果、良好なプロファイルを満たす **2-17** を見出した。オキサジン環 6 位に相当する位置に CYP2D6 のポケットに対し立体的な制限が存在することを、Brodney らがチアジン誘導体の X 線複合体構造解析から報告していて[26]、**2-17** の CYP2D6 への親和性の低下は、6 位に相当する位置に置換基を導入したことで生じた CYP2D6 との立体障害が原因であると考察している。

Table 2-3. Optimization of 1,3-Dihydro-Oxazines at the 6-Position



compd	R	IC_{50} (nM)[a] BACE1[b]	Cellular Aβ[c]	P-gp ER[d]	hERG 3 μM (%)[e]	HLM RLM (%)[f]	CYP2D6 IC_{50} (μM)[g]	pK_a[h] LogD[i]
2-13		11.7	1.5	1.4	36	93 107	<1.0	7.4 1.8
2-14		8.74	1.9	1.4	25	105 101	5.1	6.5 3.2
2-15		20.5	3.0	0.79	26	97 100	3.5	7.2 2.8
2-16		16.0	2.1	2.2	16	103 103	<1.0	7.1 2.4
2-17		11.8	1.1	1.8	32	98 105	3.4	7.2 2.3

[a]Values represent the mean values of at least two determinations. [b]Biochemical HTRF-based assay. An IC_{50} value for a reference compound (cas# 797035-11-1) was 22.6 nM. [c]IC_{50} determined by measuring the levels of secreted Aβ40 in human APP-transfected human neuroblastoma (SH-SY5Y) cells via a HTRF-based assay. An IC_{50} value for a reference compound (cas# 797035-11-1) was 25.9 nM. [d]Efflux ratio measured in LLC-PK1 cells transfected with human MDR1. [e]% inhibition at 3 μM measured in CHO cells transfected with hERG channels using an automated patch clamp system. [f]% remaining in rat (RLM) and human (HLM) liver microsomes after 30 min. [g]CYP2D6 inhibition measured with 5 μM dextromethorphan in human microsomes. [h]pK_a determined by capillary electrophoresis. [i]Log D determined in 1-octanol/phosphate buffer at pH 7.4.

第２項　ジヒドロ型オキサジン誘導体の *in vivo* 評価

得られた化合物の薬物動態評価を実施した（**Table 2-4**）。化合物 **0-3** は P-gp 基質であり、中枢移行性は 0.29 であったが、塩基性を低減し、P-gp 基質性を低下させた **2-6** および **2-17** の中枢移行性は 0.62 および 2.2 であり改善が見られた。

Table 2-4. Pharmacokinetic Properties of Key Compounds for Sprague-Dawley Rat

	RLM	Serum	P-gp	rat, iv, 0.5 mg/kg, *n* = 2			rat, po, 1 mg/kg, *n* = 2		
compd	(%)[a]	f_u[b]	ER[c]	CL (ml/min/kg)[d]	Vd_{ss} (L/kg)[e]	*B*/*P*[f]	AUC (ng·h/ml)[g]	C_{max} (ng/ml)[h]	*F* (%)[i]
0-3	104	0.41	20	37	6.1	0.29	328	32	73
2-6	97	0.27	3.0	5.3	4.5	0.62	2390	101	73
2-13	107	0.33	1.4	8.3	3.5	1.6	885	75	44
2-14	101	ND	1.4	9.3	5.4	1.9	660	58	35
2-17	105	ND	1.8	11	6.9	2.2	630	50	43

[a]% remaining in rat liver microsomes after 30 min incubation. [b]Fraction unbound in rat serum. ND = not determined; the f_u values for **2-14** and **2-17** were not determined due to these instability in rat plasma. [c]Efflux ratio measured in LLC-PK1 cells transfected with human MDR1. [d]Total clearance. [e]Volume of distribution at steady state. [f]Total brain-to-plasma ratio. [g]Plasma area under the curve. [h]Maximal plasma concentration. [i]Oral bioavailability.

2-14 および **2-17** の Aβ減少率を ICR マウスを用いて評価した（**Figure 2-1**）。ICR マウスに 3 mg/kg の用量で **2-14** を経口投与し、2,4 および 6 時間後の血漿中、脳内薬物濃度と Aβ量を測定した。**2-14** の投与後 2 時間後の脳内薬物濃度（C_b）は 1027 ng/mL で Aβ量を 66 %減少させた。その際の非結合薬物濃度（$C_{b,u}$）は 19.5 ng/mL（43 nM）で IC_{50} 値（1.9 nM）を上回っていた。3 mg/mL の経口投与で十分な薬効を確認したため、1 mg/mL の低用量で検証した。投与後 2 時間後の $C_{b,u}$ は 12 nM で、Aβ量の 52 %の減少を示した。次に **2-17** の Aβ減少率を評価した。3 mg/kg で、6 時間にわたって Aβの持続的な低下を示した。1 mg/kg の最大の Aβ減少率は 44 %であり、投与後 6 時間の脳内非結合薬物濃度（$C_{b,u}$）は 3.05 ng / mL、6.9 nM と推定され、細胞活性を十分に満たしていた。両化合物ともに $C_{b,u}$ / $C_{p,u}$ は 1 に近く、P-gp の影響は少ないとみられる。さらに Aβ減少率は化合物 **2-14** が高くこれは脳内非結合薬物濃度が高いことに起因していると考察している。

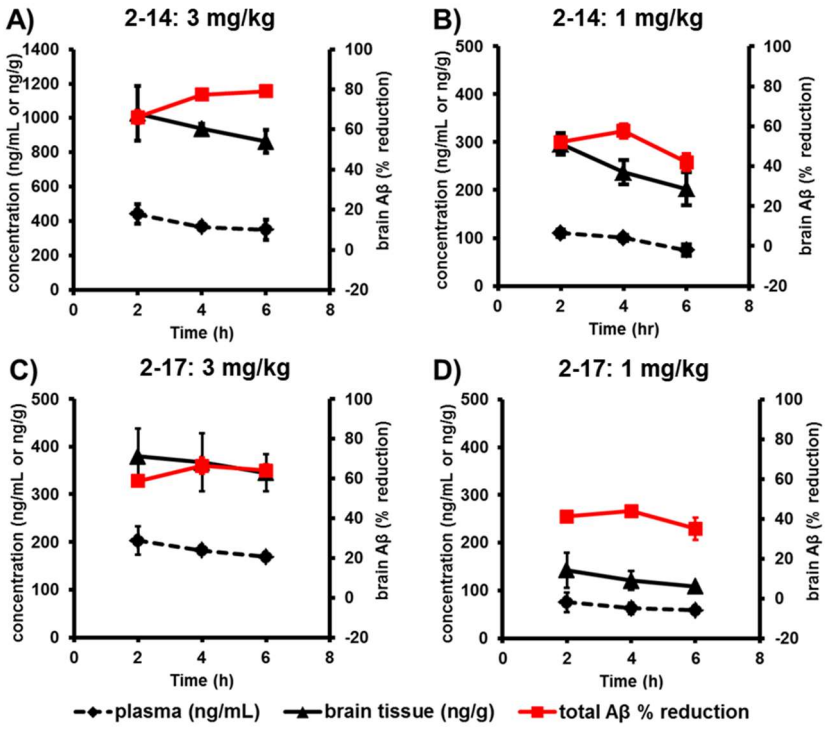


Figure 2-1. Total Aβ reduction of **2-14** and **2-17** in the brain of male ICR mice (n = 4) after oral dosing of **2-14** and **2-17** as a solution of 20% HPBCD. A) **2-14** was dosed at 3 mg/kg. B) **2-14** at 1 mg/kg. C) **2-17** at 3 mg/kg. D) **2-17** at 1 mg/kg.

Table 2-5. Total Aβ Reduction, Plasma and Brain Concentrations, and Brain Penetration of **2-14** and **2-17** in Male ICR Mice[a]

compd	$f_{u,s}$ / $f_{u,b}$[b]	Dose (mg)	Time (h)	C_p (ng/mL)[c]	C_b (ng/mL)[d]	$C_{b,u}$ (ng/mL)[e]	B/P[f]	$C_{b,u}/C_{p,u}$[g]	Aβ reduction (%)
2-14	0.037 / 0.019	3	2	441 ± 58	1027 ± 158	19.5	2.3	1.2	66 ± 3.1
			4	366 ± 17	939 ± 28	17.8	2.6	1.3	77 ± 1.4
			6	350 ± 58	864 ± 66	16.4	3.5	1.8	79 ± 1.1
		1	2	110 ± 8.0	296 ± 22	5.62	2.7	1.4	52 ± 2.4
			4	100 ± 6.0	237 ± 25	4.50	2.4	1.2	58 ± 3.5
			6	74.6 ± 12	202 ± 35	3.84	2.7	1.4	42 ± 4.2
2-17	0.065 / 0.028	3	2	203 ± 30	380 ± 58	10.6	1.9	0.81	59 ± 1.2
			4	182 ± 5.9	367 ± 61	10.3	2.0	0.87	66 ± 4.0
			6	169 ± 7.4	345 ± 39	9.66	2.0	0.88	64 ± 3.1
		1	2	75.2 ± 20	143 ± 37	4.00	1.9	0.82	41 ± 1.9
			4	62.3 ± 12	121 ± 20	3.39	1.9	0.84	44 ± 1.8
			6	58.3 ± 5.7	109 ± 5.7	3.05	1.9	0.81	35 ± 5.6

[a]Dosed as a solution of test compounds in 20% HPBCD. [b]$f_{u,s}$ = Fraction unbound in mouse serum. $f_{u,b}$ = Fraction unbound in rat brain (the rat $f_{u,b}$ values were used for the calculations). [c]Total plasma concentration. [d]Total brain concentration. [e]Unbound brain concentration ($C_{b,u} = C_b \times f_{u,b}$) [f]Total brain-to-plasma ratio. [g]Unbound brain-to-plasma ratio ($K_{p,uu}$). $C_{p,u} = C_p \times f_{u,s}$ (unbound plasma fraction in mouse).

2-17 のイヌ [18a]の非タンパク結合率を算出し（$f_{u,s}$ = 0.038）、**2-17** の Aβ減少率を評価した。0.16、0.31、0.63 mg/kg を経口投与し、4、8、25、49 時間後の脳脊髄液（CSF）中の薬物濃度と Aβ量を測定した。0.63 mg/kg 投与時において、25 時間後に最大 75%の減少を示し、その際の血漿中薬物濃度（C_p）、CSF 中薬物濃度（C_{CSF}）はそれぞれ 86 ng/mL、3.3 ng/mL であった。0.16 および 0.31 mg/kg を投与した場合でも、投与後 8 時間後に 45 %および 64 %の Aβ減少を示し、25 時間後に 43 %および 59 %の減少を維持した。0.16 mg/kg の 25 時間後の CSF 中の薬物濃度は 0.77 ng/mL（1. 7 nM）であり、細胞活性（cellular IC_{50} = 1.1 nM ）とほぼ同等であった。$C_{CSF}/C_{p,u}$ = 0.92 であり、マウスと同様トランスポーターの影響を受けていないことが示唆された。Aβを 50 %減少させる薬物濃度 EC_{50} は 32 ng/mL（73 nM）であり、$EC_{50,u}$ は 2.8 nM であった。以上の結果は、細胞活性（IC_{50} = 1.1 nM）に近く、Aβ減少を説明している。

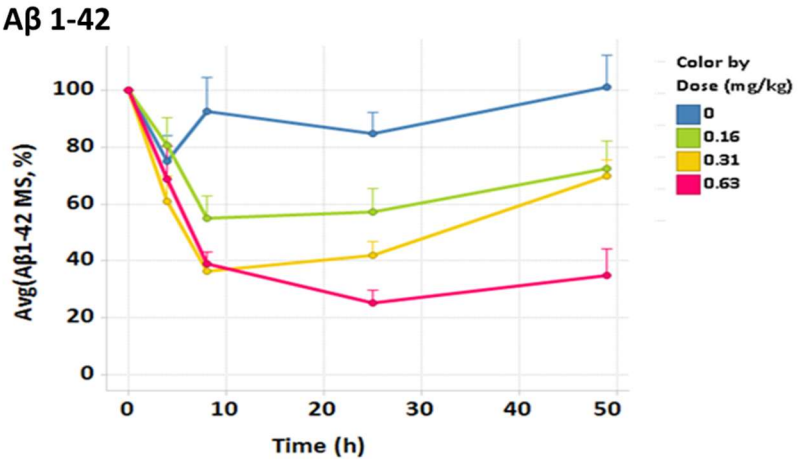


Figure 2-2. CSF Aβ1－42 reduction in beagle dog after oral dosing of **2-17** at 0.16, 0.31, and 0.63 mg/kg (n = 4) as a solution of 20% HPBCD.

第 3 項　BACE1 阻害活性とリガンド配座解析の考察

化合物 **2-14** と BACE1 複合体の 2.2Å 分解能の構造解析に成功した（**Figure 2-3**）。アミジンがアスパラギン酸（Asp）32 および 228 と相互作用し、フルオロフェニル部は、擬アキシアルに配向し、S1 ポケットを満たしていた。ピラジン環は S3 ポケットを満たし、アミドの NH がグリシン 230 と相互作用していることが確認できた。これまで報告されてきたアミジン誘導体と同様の結合モードを示すことが確認できた [15]。一方で、オキサジン環上の 6 位のジフルオロエチル基や、5 位のフッ素原子はタンパクとの相互作用は見られなかった。オキサジン **0-3** と比較して、**2-6** の BACE1 活性が向上した要因をコンフォメーション解析から解明を試みた。

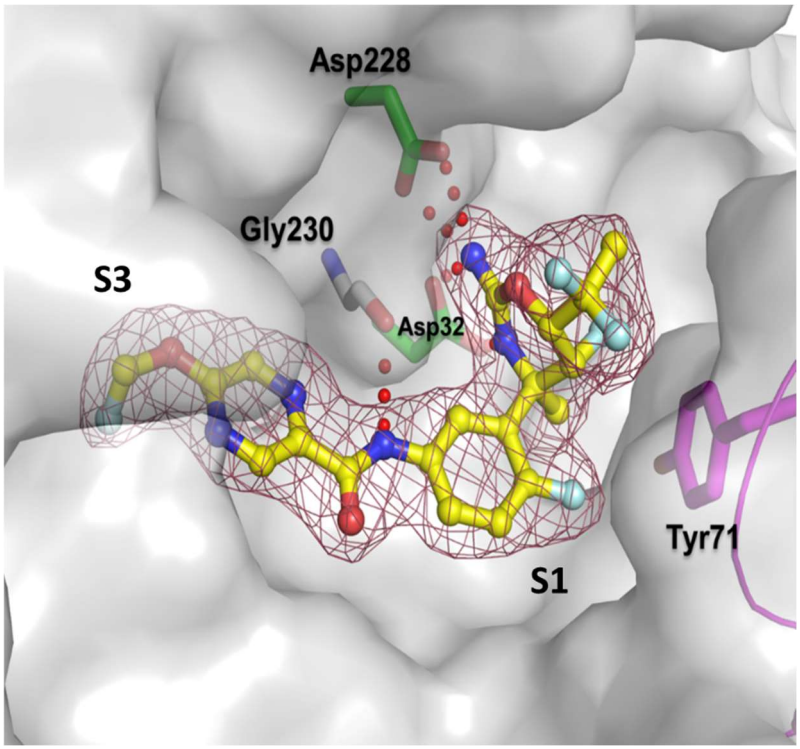


Figure 2-3. Cocrystal structure of compound **2-14** bound to human BACE1 (PDB: 5YGX). Purple mesh shows |Fo| - |Fc| map contoured at 2.0 σ in which ligand coordinates are omitted in calculation. Compound **2-14** is shown in ball-and-stick style with carbon atoms colored yellow. Key residues of the catalytic residues Asp32 and Asp228 (green), Gly230 (gray), and Tyr71 (purple) are shown in stick style. All nitrogen, oxygen, and fluorine atoms are colored blue, red, and sky blue, respectively. Dashed lines represent hydrogen bond interactions between **2-14** and BACE1.

2-6 における 5 位と 6 位の置換基導入で活性が向上した原因を解明するためにコンフォメーション解析を実施した。オキサジン環上の置換基の効果を検証するため、同一の tail 部を持つ **0-3, 2-1, 2-3, 2-5** および **2-6** の最安定コンフォメーションを計算した。**0-3** の最安定コンフォメーションを計算した結果、フルオロフェニル基が擬アキシアルに配向した配座（疑アキシアル：pseudoaxial confomation）が最安定であり、複合体構造の **2-14** のコンフォメーションに近似していた。次に **2-1** および **2-3** の最安定コンフォメーションを計算したところ、**2-1** は、**0-3** と同様の配座が最安定であったが、**2-3** はフルオロフェニル基が擬エクアトリアルに配向した配座（擬エクアトリアル：pseudoequatorial confomation）が最安定コンフォメーションであった。**2-3** は活性が大きく低下したことから、擬アキシアルと擬エクアトリアルの変形エネルギーが活性に関与していると考え、5 化合物の変形エネルギー（ΔE ax-eq（kcal/mol））を算出し、活性との相関を確認した（**Figure 2-4**）。その結果、擬アキシアル構造が最安定コンフォメーションであり、変形エネルギーが大きい **2-6** が最も活性が強く、活性と変形エネルギーに相関がみられた。複合体構造からは、オキサジン環上の 5 位と 6 位の置換基と、タンパクとの間に相互作用は見られなかったが、**2-6** は 5 位と 6 位の置換基が、活性コンフォメーションである擬アキシアル構造を安定化したため、活性が向上したと考察している。置換基のコンフォメーションに与える効果は以下のように考察している（**Figure 2-5**）。**2-1** においては擬アキシアルコンフォメーションを取った際に、5 位のフッ素原子の反結合性軌道と 6 位の C-H 結合との間に $\sigma_{C\text{-}H}$- $\sigma^*_{C\text{-}F}$ 相互作用が働くことで安定化される [27]。一方で **2-3** の場合、擬アキシアル構造を取った場合、5 位のフッ素原子はベンゼン環上のフッ素原子と静電的な反発が生じるため不利になり、擬エクアトリアル構造を取った場合には、フッ素原子の反結合性軌道と重なる 6 位の C-H 結合が存在するため安定化する。**2-5** は擬アキシアル構造において 6 位のフルオロメチル基が 4 位のフェニル基と 1,3-ジアキシアル相互作用により不利になることで擬エクアトリアルが優位になり、**2-6** においては、擬エクアトリアル構造の際に、6 位のフルオロメチル基と 4 位のメチル基との間に 1,3-ジアキシアル相互作用が働くために、擬アキシアル構造が優位となり活性が向上したと説明される。同様に有意な *in vivo* 薬効を示した **2-17** の変形エネルギーを計算した [28]。ΔE ax-eq は-5.48 kcal/mol であり、**2-6** と同程度のエネルギー差で擬アキシアル構造が優位となり、高い活性を示したと考えられる。

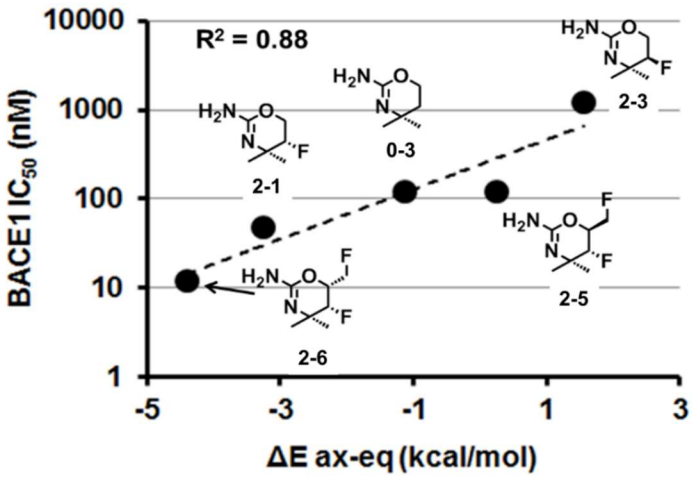


Figure 2-4. Plots of BACE1 IC_{50} vs ΔE for compounds **0-3**, **2-1**, **2-3**, **2-5** and **2-6**.

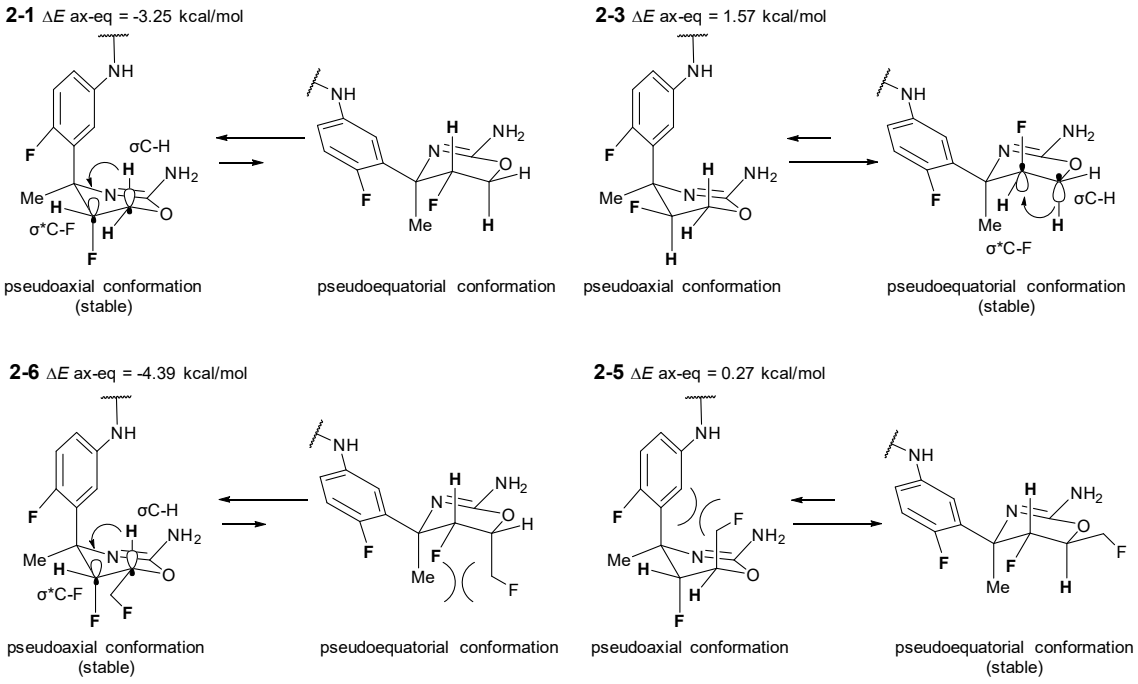
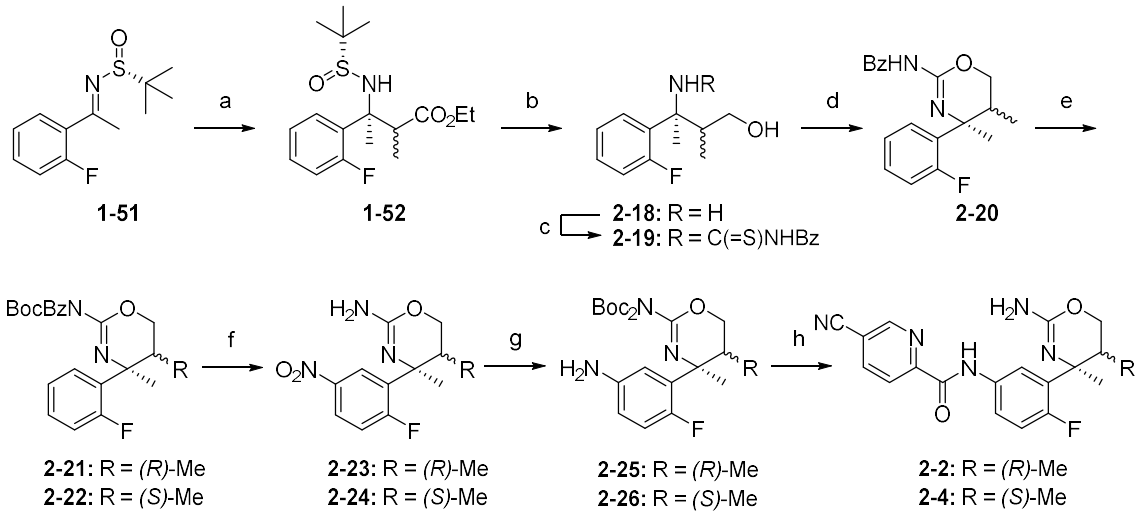


Figure 2-5. Schematic diagrams of dihydro-oxazines.

第２節　ジヒドロ型オキサジン誘導体の合成

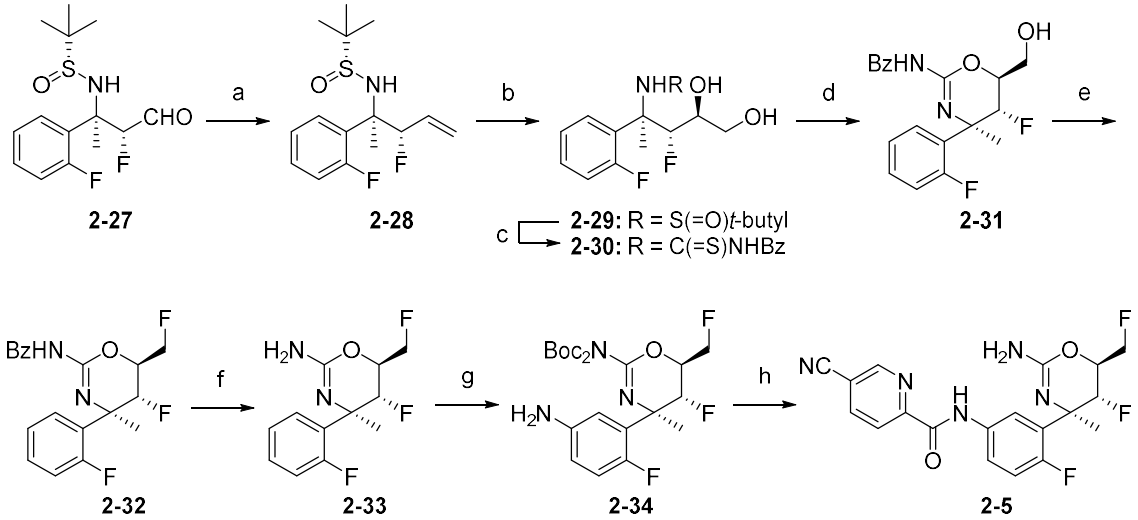
Scheme 2-1 に 5-メチルオキサジン **2-2** および **2-4** の合成を記載した。チタンエノラートを調製し、**1-51** に作用させ **1-52** を合成した。3:1 の混合物で得られ、分離することができなかったので、混合物のまま次の反応に進めた。エステルを還元し、アルコールに導いた後に、酸性条件にてアミノアルコール **2-18** を得た。ベンゾイルイソチオシアネートを用いてチオウレアとした後に、EDC-HCl を用いてオキサジン **2-20** を合成した。アミジン部を Boc 基でさらに保護した **2-21** および **2-22** はカラムクロマトグラフィーで分離可能であった。それぞれのジアステレオマーに対し、炭酸カリウムを用いてベンゾイル基の除去を行い、酸性条件にて脱 Boc 化を行いアミジン **2-23** および **2-24** を得た。これ以降は **Scheme 1-10** と同様の方法で合成した。なお立体化学に関しては、**2-4** の誘導体の単結晶 X 線構造解析にて立体配置を決定した（実験の部参照）。



Scheme 2-1. Synthesis of Oxazine **2-2, 2-4**[a]

[a]Reagents and conditions: (a) *n*-BuLi, diisopropylamine, methylpropionate, $ClTi(O\textit{i}\text{-}Pr)_3$, THF, −78 °C, 47%, *dr* = 3:1; (b) (i) $NaBH_4$, MeOH, THF, 0 °C to rt, (ii) 4 M HCl in EtOAc, MeOH, rt; (c) benzoyl isothiocyanate, DCM, rt, 89% over 3 steps, *dr* = 5:1; (d) EDC-HCl, MeCN, rt, 95%, *dr* = 5:1; (e) Boc_2O, DMAP, DCM, rt, 15% and 78% for **2-21** and **2-22**; (f) (i) K_2CO_3, THF, MeOH, H_2O, rt, (ii) TFA, rt; then HNO_3, H_2SO_4, −20 °C; (g) (i) Boc_2O, DMAP, DCM, rt, 58−76% over 3 steps, (ii) Fe, NH_4Cl, EtOH, THF, H_2O, 60 °C, 81−83%; (h) (i) 5-cyanopicolinic acid hydrate, HATU, DIEA, DCM, rt, (ii) formic acid, rt, 68−73% over 2 steps.

オキサジン **2-5** は **Scheme 2-2** に記載した方法で合成した。文献既知のアルデヒド **2-27** に対し、Wittig 反応を用いてアルケン **2-28** を合成した。ジアステレオ選択的にジヒドロキシル化を行いジオールを得た後に、スルフィンアミドを 2 工程でチオウレア **2-30** に変換した。EDC-HCl を用いてオキサジン **2-31** に導き、DAST を用いてヒドロキシ基をフッ素原子に変換した。相対配置は **2-31** の NOE にて決定した。これ以降は **Scheme 2-1** と同様の方法で **2-5** へと導いた。

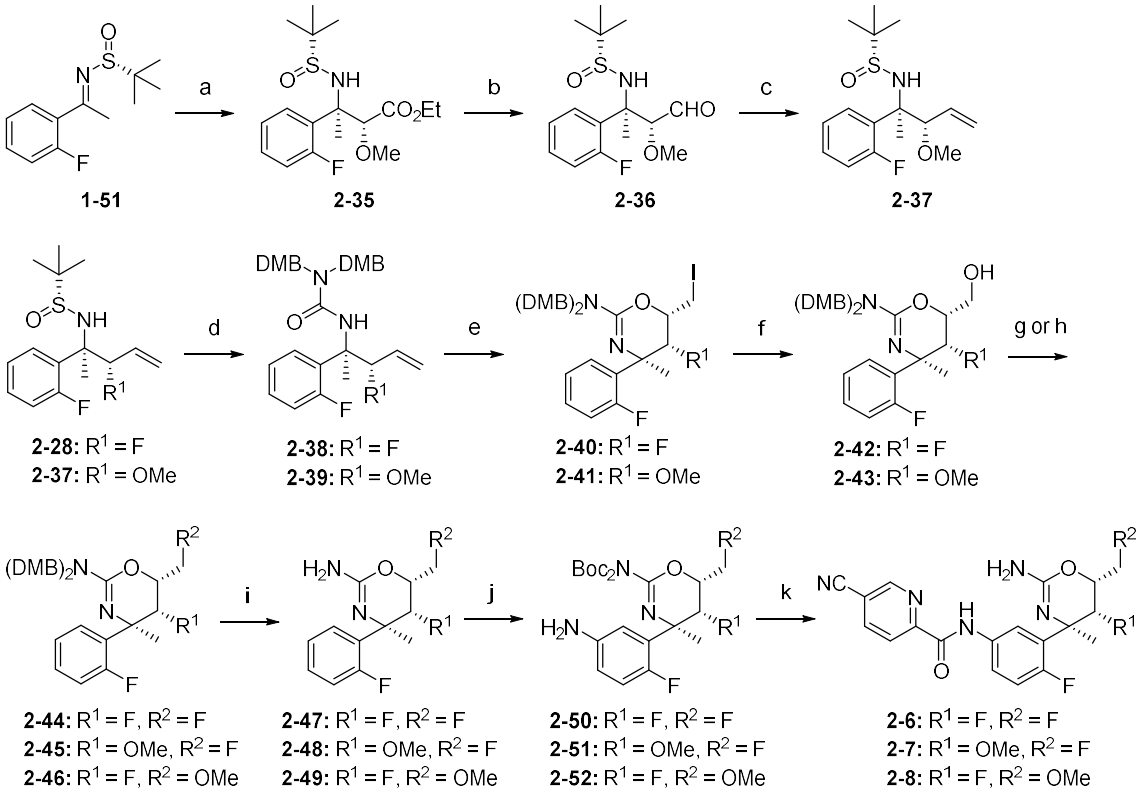


Scheme 2-2. Synthesis of Oxazine **2-5**[a]

[a]Reagents and conditions: (a) methyl triphenylphosphonium bromide, *tert*-BuOK, toluene, rt, 45%; (b) $K_2OsO_4{\cdot}2H_2O$, NMO, acetone, H_2O, rt, 70%; (c) (i) 4 M HCl in 1,4-dioxane, MeOH, rt, (ii) benzoyl isothiocyanate, DCM, rt, 88% over 2 steps; (d) EDC-HCl, MeCN, rt, quant.; (e) DAST, DCM, −78 ℃ to 0 ℃, 14%; (f) (i) Boc_2O, DMAP, THF, rt, (ii) K_2CO_3, MeOH, rt, (iii) TFA, DCM, rt, quant. over 3 steps; (g) (i) HNO_3, H_2SO_4, TFA, −20 ℃, (ii) Boc_2O, DMAP, THF, rt, 69% over 2 steps, (iii) Fe, NH_4Cl, EtOH, THF, H2O, 60 ℃, 83%; (h) (i) 5-cyanopicolinic acid hydrate, HATU, DIEA, DCM, rt, 97%, (ii) formic acid, rt, 75%.

Scheme 2-3 にオキサジン **2-6**, **2-7** および **2-8** の合成を記載した。5-メトキシオキサジン **2-7** を得るために、リチウムエノラートをキラルなスルフィニルイミン **1-51** に付加させ、**2-35** に導いた。**2-35** が主成績体であったが、ジアステレオマー混合物であり、分離できなかったので混合物のまま次工程へ進めた。エステルを還元し、生じたアルデヒド **2-36** に対し Wittig 反応を用いてアルケン **2-37** へ導いた。スルフィニル基を除去し、ウレア **2-39** に導いたところ、混入したジアステレオマーとの分離が可能であり、単一のジアステレオマーとして **2-39** を得た。**2-39** に対してヨウ素を作用させることで、立体選択的にオキサジン環化が進行し **2-41** を得た。ヨウ素原子はトリフルオロ酢酸銀を用いてヒドロキシ基に変換し、

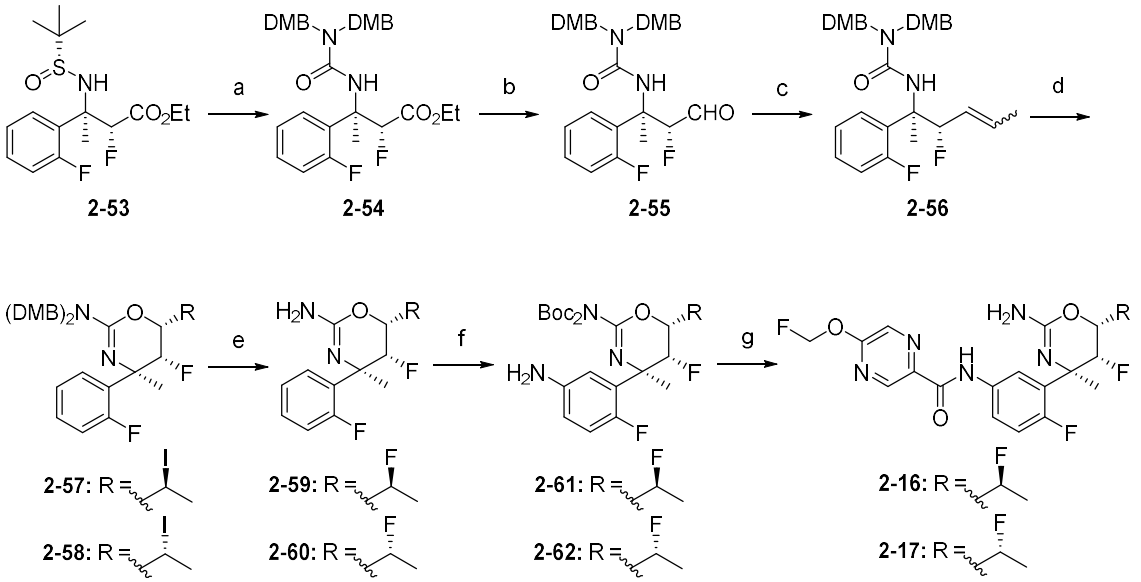
DAST を用いてフッ素化を行い **2-45** を合成した。ジメトキシベンジル基の除去を行い、**2-48** へと導いた後に、ニトロ化以降の反応は **Scheme 2-2** と同様の方法で合成した。**2-6** は **2-28** から **2-7** と同様の方法で合成した。**2-8** の中間体である **2-46** は **2-43** のヒドロキシ基をヨウ化メチルを用いてメトキシ基に変換し、合成した。それ以降は **2-6** と同様の方法で合成した。**2-6** および **2-7** の立体化学はそれぞれの誘導体の単結晶 X 線構造解析によって決定した（実験の部参照)。



Scheme 2-3. Synthesis of Oxazine **2-6**, **2-7**, **2-8**[a]

[a]Reagents and conditions: (a) *n*-BuLi, diisopropylamine, 2-methoxyacetate, THF, −78 °C, 89%; (b) DIBAL, DCM, −78 °C, 72%; (c) methyltriphenylphosphonium bromide, *tert*-BuOK, toluene, rt, 47%; (d) (i) 4 M HCl in 1,4-dioxane, MeOH, rt, (ii) 4-nitrophenyl carbonochloridate, $NaHCO_3$, bis(2,4-dimethoxybenzyl)amine, EtOAc, H_2O, 0 °C to rt, 88% over 2 steps; (e) iodine, MeCN, rt, 60%; (f) silver trifluoroacetate, $MeNO_2$, H_2O, 80 °C, 65%; (g) DAST, DCM, −78 °C to rt, 48 and 76% for **2-44** and **2-45**; (h) NaH, MeI, THF, rt, 86% of **2-46**; (i) TFA, 80 °C, 38−97%; (j) (i) HNO_3, H_2SO_4, TFA, −20 °C, (ii) Boc_2O, DMAP, THF, rt, 51−100% over 2 steps, (iii) Fe, NH_4Cl, EtOH, THF, H_2O, 60 °C, or H_2, Pd/C, MeOH, rt; (k) (i) 5-cyanopicolinic acid hydrate, HATU, DIEA, DCM or DMF, rt, (ii) formic acid, rt, 50−91% over 3 steps.

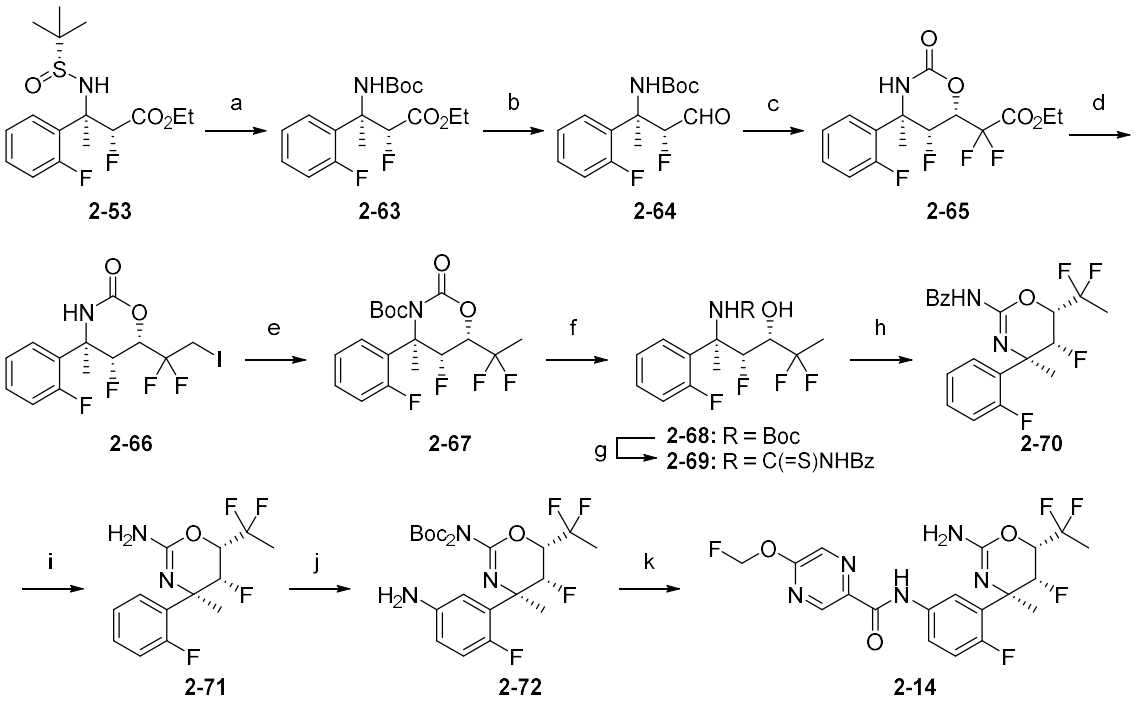
オキサジン **2-16** および **2-17** は **Scheme 2-4** の方法で合成した。**2-53**[15a)]に対し、2 工程でウレア **2-54** に導いた後に、DIBAL を用いた還元反応、続く Wittig 反応により **2-56** を 2 つの幾何異性体の混合物として合成した。混合物を氷冷下、ヨウ素と反応させることで環化反応を行った。この際、*Z*体は完全に消費され、カラムクロマトグラフィーで **2-57** と未反応の **2-56** の *E*体は分離可能であった。回収した **2-56** は室温で環化反応を行うことで、**2-58** に導いた。それ以降は **Scheme 2-3** のオキサジン **2-6** と同様の方法で合成した。**2-16** の絶対配置は **2-59** の誘導体の単結晶 X 線構造解析で決定した（実験の部参照）。



Scheme 2-4. Synthesis of Oxazine **2-16, 17**[a]

[a]Reagents and conditions: (a) (i) 4 M HCl in 1,4-dioxane, MeOH, rt; (ii) 4-nitrophenyl carbonochloridate, $NaHCO_3$, bis(2,4-dimethoxybenzyl)amine, EtOAc, H_2O, 0 ℃, quant. over 2 steps; (b) DIBAL, DCM, −78 ℃, 83%; (c) ethyltriphenylphosphonium bromide, KHMDS, THF, 0 ℃ to 50 ℃, 94%, *E*:*Z* = 2:3; (d) iodine, MeCN, 0 ℃, 58% of **2-57** and 22% of **2-58**; (e) (i) KO_2, 18-crown-6, DMSO, rt; (ii) nonafluorobutanesulfonyl fluoride, DBU, toluene, rt, or DAST, DCM, −78 ℃ to rt; (iii) TFA, anisole, 80 ℃, 10−16% over 3 steps; (f) (i) HNO_3, H_2SO_4, TFA, −20 ℃, 72−82%; (ii) Boc_2O, DMAP, DCM, rt, 63−91%; (iii) H_2, Pd/C, THF, MeOH, rt, 86−94%; (g) (i) 5-(fluoromethoxy)pyrazine-2-carboxylic acid, HATU, DIEA, DMF, rt; (ii) formic acid, rt, 77−88% over 2 steps.

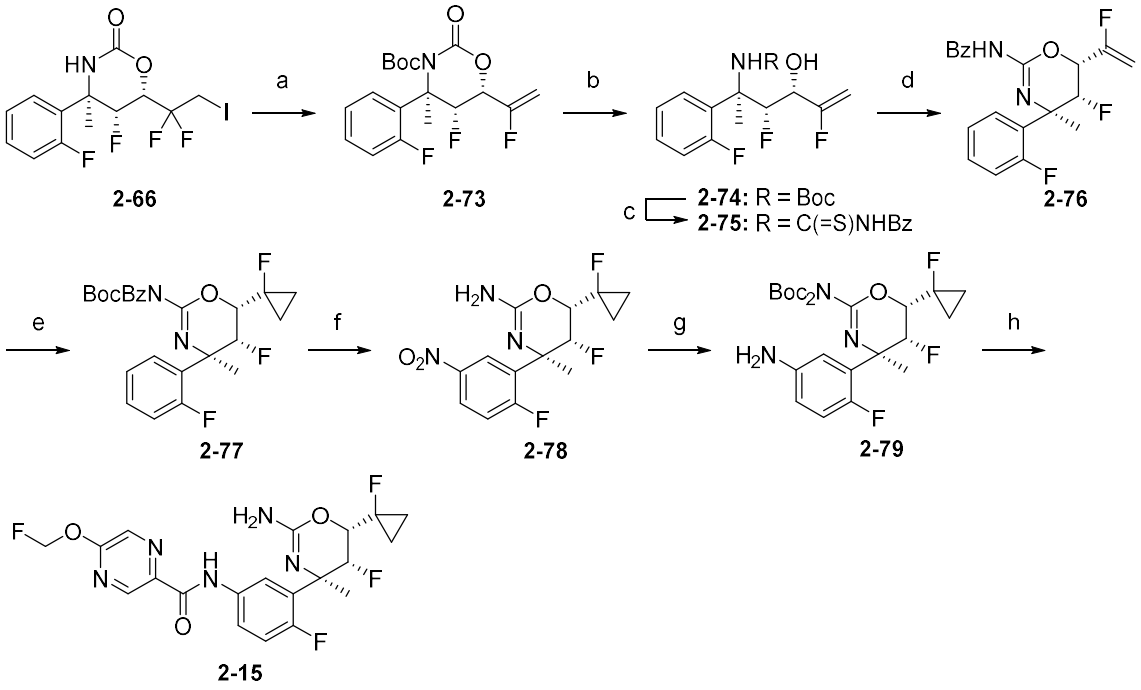
オキサジン **2-14** は **Scheme 2-5** に記載した方法で合成した。スルフィンアミド **2-53** を 2 工程で **2-63** に変換した後に、エステルを DIBAL で処理してアルデヒド **2-64** を合成した。2-ブロモ 2,2-ジフルオロアセテートとの Reformatsky 反応を行ったところ、生じたアルコールが Boc 基と反応し、カルバメート **2-65** が得られた。$LiBH_4$ を用いてアルコールに還元した後に、ヨウ素化を行い **2-66** に導いた。ラジカル還元反応にてヨウ素原子の除去を行い、Boc 基で保護を行い、環状カルバメートの加水分解により **2-68** を合成した。Boc 基を除去しチオウレア **2-69** に変換した後に、**2-5** と同様の方法でオキサジン **2-14** を合成した。**2-14** の立体化学は BACE1 との複合体構造解析によって決定した。



Scheme 2-5. Synthesis of Oxazine **2-14**[a]

[a]Reagents and conditions: (a) (i) 4 M HCl in 1,4-dioxane, MeOH, rt, (ii) Boc_2O, MeOH, 40 °C; (b) DIBAL, DCM, −78 °C; (c) Zn, 2-bromo-2,2-difluoroacetate, THF, reflux, 16% over 4 steps; (d) (i) $LiBH_4$, THF, rt, 60%, (ii) iodine, PPh_3, imidazole, THF, 80 °C, 93%; (e) (i) Boc_2O, DMAP, DCM, rt, quant., (ii) AIBN, *n*-Bu_3SnH, toluene, 80 °C, 71%; (f) $Ba(OH)_2{\cdot}8H_2O$, EtOH, H_2O, rt, 94%; (g) (i) 4 M HCl in 1,4-dioxane, MeOH, 50 °C, (ii) benzoyl isothiocyanate, DCM, rt, 85% over 2 steps; (h)EDC-HCl, MeCN, 50 °C, 97%; (i) K_2CO_3, MeOH, 80 °C, 92%, (j) (i) HNO_3, H_2SO_4, TFA, −20 °C, quant., (ii) Boc_2O, DMAP, DCM, rt, 91%, (iii) H_2, Pd/C, THF, MeOH, rt, 85%; (k) (i) 5-(fluoromethoxy)pyrazine-2-carboxylic acid, HATU, DIEA, DMF, rt, (ii) formic acid, rt, 91% over 2 steps.

オキサジン **2-15** は **Scheme 2-6** に記載の方法で合成した。**2-66** に対し、亜鉛を用いて、ヨウ素とフッ素原子の還元的脱離反応によってエキソオレフィンに変換し、Boc 化を行い **2-73** を得た。環状カルバメートを加水分解し、Boc 基をチオウレアに変換した後に、EDC-HCl を用いてオキサジン **2-76** を合成した。Boc 基で保護を行い、ジアゾメタンと反応させシクロプロパン誘導体 **2-77** に導いた。Bz 基を除去し、ニトロ化を行い **2-78** を得た。これ以降は **2-5** と同様の方法で **2-15** を合成した。



Scheme 2-6. Synthesis of Oxazine **2-15**[a]

[a]Reagents and conditions: (a) (i) Zn, DMA, H_2O, 80 °C, (ii) Boc_2O, DMAP, DCM, rt, 91% over 2 steps; (b) K_2CO_3, MeOH, rt; (c) (i) TFA, DCM, rt, 85% over 2 steps, (ii) benzoyl isothiocyanate, DCM, rt, 89%; (d) EDC-HCl, MeCN, 50 °C; (e) (i) Boc_2O, DMAP, DCM, rt, 95% over 2 steps, (ii) 1-methyl-1-nitrosourea, 30% NaOH, $Pd(OAc)_2$, Et_2O, H_2O, −20 °C; (f) (i) K_2CO_3, MeOH, rt, 35% over 2 steps, (ii) TFA, rt, then HNO_3, H_2SO_4, −20 °C to 0 °C, 35% over 2 steps; (g) (i) Boc_2O, DMAP, DCM, rt, 63%, (ii) H_2, Pd/C, THF, MeOH, rt, 83%; (h) (i) 5-(fluoromethoxy)pyrazine-2-carboxylic acid, HATU, DIEA, DMF, rt, (ii) formic acid, rt, 70% over 2 steps.

結語

中枢神経系の創薬研究において、適切な中枢移行性を有し安全な化合物を探索することが重要である。筆者はアルツハイマー病治療薬を指向し、BACE1 阻害活性を有するリード化合物を見出していたが、低い中枢移行性を示し、hERG 阻害活性も有していた。低い中枢移行性の原因は P-gp 基質であることが原因と特定し、塩基性を下げる構造変換を行うことで、P-gp 基質性を低減させ *in vivo* で有意な薬効を示す化合物を同定した。本研究成果は、塩基性が原因で P-gp 基質となっている化合物の最適化デザイン、中枢移行性の獲得に有用な知見を与えるものである。

本研究により得られた知見を下記に記す。

1. 複数の BACE1 阻害剤から活性と P-gp 基質性の回避において、アミジンの最適な pK_a の範囲を 6.5~8.0 に設定した。P-gpKO マウスを使った評価から、オキサジン **0-3** が低い中枢移行性を示す原因は、P-gp 基質であることを解明した。オキサジン **0-3** の pK_a は、9.8 であり、分子量を増加させずに、塩基性を低下させるために 2 重結合をオキサジン環内に導入した化合物 **1-3** をデザインした。評価したところ狙い通りに塩基性が低下し、P-gp 基質性が低減した。至適 pK_a に従い、最適化した結果、*in vivo* で有効性を示し、安全性を示す **1-8** を見出した。また、オレフィン型オキサジンの合成において新規なオキサジン環化反応を見出した。

2. 電子求引基を導入しアミジンの塩基性を調整することで、P-gp 基質性および hERG 阻害活性を低減させ、高い *in vitro* 活性を示す **2-13** を見出した。しかしながら、**2-13** は CYP2D6 阻害活性を有していた。立体的な効果を考慮しオキサジン環の 6 位を変換することで、CYP2D6 阻害が低減した **2-17** を見出した。**2-17** は *in vivo* において有意な Aβ 減少率を示した。高活性化合物の探索においては、BACE1 複合体構造解析から、フルオロフェニル基が擬アキシアルに配向する配座を最安定構造とする化合物は高活性を示しやすく、活性コンフォメーション（擬アキシアル）を安定化させることが BACE1 阻害活性の向上につながることを解明した。

なお、本研究成果は下記の論文として投稿し、受理、掲載された。上記論文中の文章、図は下記の論文より適宜引用した。

1. Fuchino, K.; Mitsuoka, Y.; Masui, M.; Kurose, N.; Yoshida, S.; Komano, K.; Yamamoto, T.; Ogawa, M.; Unemura, C.; Hosono, M.; Ito. H.; Sakaguchi, G.; Ando, S.; Ohnishi, S.; Kido, Y.; Fukushima, T.; Miyajima, H.; Hiroyama, S.; Koyabu, K.; Dhuyvetter, D.; Borghys, H.; Gijsen, H. J.M.; Yamano, Y.; Iso, Y.; Kusakabe, K.-I. Rational Design of Novel 1,3-Oxazine Based β-Secretase (BACE1) Inhibitors: Incorporation of a Double Bond To Reduce P-gp Efflux Leading to Robust Aβ Reduction in the Brain, *J. Med. Chem*. **2018**, *61*, 5122–5137.
2. Fuchino, K.; Nakahara, K.; Komano, K.; Asada, N.; Tadano, G.; Hasegawa, T.; Yamamoto, T.; Sako, Y.; Ogawa, M.; Unemura, C.; Hosono, M.; Ito, H.; Sakaguchi, G.; Ando, S.; Ohnishi, S.; Kido, Y.; Fukushima, T.; Dhuyvetter, D.; Borghys, H.; Gijsen, H. J. M.; Yamano, Y.; Iso, Y.; Kusakabe, K.-I. Discovery of Potent and Centrally Active 6-Substituted 5-Fluoro-1,3-dihydro-oxazine β-Secretase (BACE1) Inhibitors via Active Conformation Stabilization. *J. Med. Chem*. **2018**, *61*, 5525–5546.
3. Fuchino, K.; Masui, M.; Yoshida, S.; Kusakabe, K.-I. Facile Construction of an Amino-1,3-Oxazine Scaffold using Burgess Reagent Under Mild Conditions. *Tetrahedron Lett.* **2021***, 64,* 152684.

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実験の部

General Chemistry. All commercial reagents and solvents were used as received unless otherwise noted. Thin layer chromatography (TLC) analysis was performed using Merck silica gel 60 F_{254} thin layer plates (250 μm in thickness). Flash column chromatography was carried out on an automated purification system using Yamazen or Fuji Silysia prepacked silica gel columns. ^{1}H NMR spectra were recorded on a Bruker Advance 400 MHz. Spectral data are reported as follows: chemical shift (as ppm referenced to tetramethylsilane), multiplicity (s = singlet, d = doublet, dd = double doublets, dt = double triplet, t = triplet, q = quartet, m = multiplet, br = broad peak), coupling constant, and integration value. Analytical LC/MS was performed on a Shimadzu Shim-pack XR-ODS (C_{18}, 2.2 μm, 3.0 × 50 mm, a linear gradient from 10% to 100% B over 3 min and then 100% B for 1 min (A = H_2O + 0.1% formic acid, B = MeCN + 0.1% formic acid), flow rate 1.6 mL/min) using a Shimadzu UFLC system equipped with a LCMS-2020 mass spectrometer, LC-20AD binary gradient module, SPD-M20A photodiode array detector (detection at 254 nm), and SIL-20AC sample manager. The purity of all compounds used in the bioassays was determined by this method to be >95%. High resolution mass spectra were recorded on a Thermo Fisher Scientific LTQ Orbitrap using electrospray positive ionization.

第 1 章の合成

(*R*,*E*)-*N*-[1-(2-Fluoro-5-nitrophenyl)ethylidene)]-2-methylpropane-2-sulfinamide (1-22). To a solution of **1-21** (50.0 g, 273 mmol) and (*R*)-2-methylpropane-2-sulfinamide (36.4 g, 300 mmol) in THF (350 mL) was added $Ti(OEt)_4$ (106 g, 464 mmol) at room temperature, and the reaction mixture was stirred at 65 °C for 7 h. The mixture was allowed to cool to room temperature. To a solution of citric acid (166 g) in H_2O/toluene (600 mL/660 mL) was added the reaction mixture at 10 °C. The mixture was stirred for 2 h and filtered through Celite. The aqueous layer was separated and extracted with toluene. The combined organic layers were evaporated, the resulting residue was treated with IPE (210 mL), and the mixture was stirred at 0 °C for 2 h. The resulting solid was collected on a Kiriyama funnel and washed with cooled IPE (100 mL) to afford **1-22** (50.6 g, 65% yield) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 9H), 2.81 (s, 3H), 7.29 (t, *J* = 9.6 Hz, 1H), 8.30–8.34 (m, 1H), 8.55–8.57 (m, 1H). MS-ESI (*m/z*): 287 $[M + H]^+$.

(*R*,*E*)-*N*-[3-[1-[(*tert*-Butylsulfinyl)imino]ethyl]-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-23). A suspension of **1-22** (48.0 g, 168 mmol), Fe (32.8 g, 587 mmol), and NH_4Cl (40.4 g, 754 mmol) in toluene/H_2O (480 mL/192 mL) was stirred at 80 °C for 6 h. The mixture was cooled to room temperature and diluted with EtOAc (150 mL) and H_2O (200 mL). The insoluble materials were filtered off, and the aqueous layer was separated and extracted with EtOAc. The combined organic layers were evaporated. The residue was dissolved in THF (156 mL) and Et_3N (58.1 mL, 419 mmol), and the mixture was cooled to –40 °C. To this mixture was added dropwise TFAA (29.6 mL, 210 mmol) in THF (10 mL) at this temperature over 10 min, and the mixture was stirred at –25 °C for 30 min. The reaction was quenched with saturated aq $NaHCO_3$ solution (50 mL) at –25 °C, and the mixture was allowed to warm to room temperature and diluted with EtOAc (150 mL) and H_2O (200 mL). The aqueous layer was separated and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and evaporated. The residue was treated with IPE, and the mixture was stirred at room temperature. The resulting solid was collected on a Kiriyama funnel to afford **1-23** (46.0 g, 78% yield) as an orange solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.24 (s, 9H), 2.70 (s, 3H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.84–7.88 (m, 1H), 8.03–8.06 (m, 1H), 11.44 (br s, 1H). MS-ESI (*m/z*): 353 $[M + H]^+$.

***N*-[3-[(*S*)-2-[[(*R*)-*tert*-Butylsulfinyl]amino]-4-methylpent-4-en-2-yl]-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-24).** To a solution of **1-23** (3.0 g, 8.51 mmol) in THF (30 mL) was added 2-methylallylmagnesium chloride (0.5 M in THF, 85 mL) at −78 °C for 2 h. The reaction was quenched with saturated aq NH_4Cl solution. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 20−40% EtOAc) to give **1-24** (2.5 g, 72% yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (s, 9H), 1.38 (s, 3H), 1.86 (s, 3H), 2.79 (d, *J* = 13.3 Hz, 1H), 2.92 (d, *J* = 13.4 Hz, 1H), 4.21 (s, 1H), 4.81 (s, 1H), 4.92 (d, *J* = 1.5 Hz, 1H), 7.05 (dd, *J* = 11.7, 8.7 Hz, 1H), 7.45−7.51 (m, 1H), 7.74 (dd, *J* = 6.8, 2.7 Hz, 1H), 8.37 (br s, 1H). MS-ESI (*m/z*): 409 $[M + H]^+$.

***N*-[3-[(*S*)-2-[[(*R*)-*tert*-Butylsulfinyl]amino]-4-oxopentan-2-yl]-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-25).** A solution of compound **1-24** (2.5 g, 6.1 mmol) in DCM (40 mL) was cooled to –78 °C, and ozone gas was bubbled into the solution. When the color of the reaction solution

changed to blue, the bubbling was stopped. Et_3N (2.5 mL) was added to the soution, which was then stirred for an addtional 2 h at –78 °C. The mixture was warmed to room temperature and diluted with EtOAc and H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated to give **1-25** (2.5 g, 100% yield) as a pink amorohous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (s, 9H), 1.73 (s, 3H), 2.10 (s, 3H), 3.35 (dd, *J* = 18.6, 2.5 Hz, 1H), 3.66 (d, *J* = 18.6 Hz, 1H), 5.35 (s, 1H), 6.99 (dd, *J* = 11.9, 8.7 Hz, 1H), 7.30−7.35 (m, 1H), 7.98 (dd, *J* = 7.2, 2.7 Hz, 1H). MS-ESI (*m/z*): 411 $[M + H]^+$.

(*S*)-*N*-[3-(2-Amino-4-oxopentan-2-yl)-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-26). To a solution of **1-25** (1.45 g, 3.53 mmol) in MeOH (10 mL) was added HCl (4 M in dioxane; 1.2 mL, 4.95 mmol) at room temperature. After being stirred for 1 h, the mixture was diluted with saturated aq $NaHCO_3$ solution and EtOAc. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to afford **1-26** (912 mg, 84% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 3H), 2.06 (s, 3H), 2.18 (br s, 2H), 2.86 (d, *J* = 17.8 Hz, 1H), 3.44 (d, *J* = 17.8 Hz, 1H), 7.01 (dd, *J* = 11.7, 8.7 Hz, 1H), 7.60−7.70 (m, 2H), 8.17 (br s, 1H). MS-ESI (*m/z*): 307 $[M + H]^+$.

(*S*)-*N*-[3-[2-[3,3-Bis(2,4-dimethoxybenzyl)thioureido]-4-oxopentan-2-yl]-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-27). To a solution of **1-26** (152 mg, 0.496 mmol) in toluene (5 mL)-H_2O (2.5 mL) was added potassium carbonate (137 mg), then the mixture was cooled in an ice bath. To the mixture was added a solution of thiophosgene (1.40 g) in toluene (1 mL) and stirred for 1 h at 0°C. H_2O was added to the reaction solution and the mixture was extracted with EtOAc. The organic layer was washed with H_2O and brine, successively. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure to give thioisocyanate compound (182 mg, quant.) as a brown solid. To a solution of thioisocyanate compound in THF (1.5 mL) was added bis(2,4-dimethoxybenzyl)amine (189 mg, 0.596 mmol) then the mixture was stirred for 1 h at room temperature. The solvent was evaporated under reduced pressure. H_2O and 2 M HCl were added to the residue and was extracted with EtOAc. The organic layer was washed with H_2O, sat. $NaHCO_3$, H_2O and brine, successively and dried over anhydrous $MgSO_4$. The solvent was evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 30−50% EtOAc) to give purified by column chromatography to give **1-27** (322 mg, 97% yield) as a yellow oil. ^{1}H-

NMR ($CDCl_3$) δ: 1.81 (s, 3H), 2.15 (s, 3H), 3.11 (d, *J* = 16.8 Hz, 1H), 3.79 (s, 6H), 3.80 (s, 6H), 4.46 (d, *J* = 16.8 Hz, 1H), 4.77 (br, 2H), 5.03 (br, 2H), 6.45−6.52 (m, 4H), 6.86 (br, 1H), 7.00 (dd, *J* = 11.3, 8.7 Hz, 1H), 7.11−7.20 (m, 3H), 7.52−7.57 (m, 1H), 7.93 (br, 1H).

(*S*)-*N*-[3-[2-[Bis(2,4-dimethoxybenzyl)amino]-4,6-dimethyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-28). To a solution of **1-27** (4.61 g, 6.93 mmol) in MeCN (20 mL) were added DIEA (2.42 mL, 13.9 mmol) and methyl iodide (1.97 g, 13.9 mmol) then the mixture was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure. H_2O was added to the residue and the mixture was extracted with EtOAc. The organic layer was successively washed with 1 mol/L HCl, H_2O, sat. $NaHCO_3$, H_2O and brine, and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure to give carbamimidothioate (4.36 g, 93% yield) as a yellow gum. To a solution of carbamimidothioate (4.36 g, 6.41 mmol) in MeCN (20 mL) was added DIEA (3.36 mL, 19.2 mmol) then the mixture was heated under reflux for 48 h. H_2O was added to the reaction solution and the mixture was extracted with EtOAc. The organic layer was successively washed with H_2O and brine, and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure and the obtained residue was purified by column chromatography to give **1-28** (820 mg, 20% yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (s, 3H), 1.76 (s, 3H), 3.76 (s, 6H), 3.79 (s, 6H), 4.29 (d, *J* = 16.2 Hz, 2H) , 4.81 (d, *J* = 16.2 Hz, 2H), 5.30 (d, *J* = 2.5 Hz, 1H) , 6.43−6.47 (m, 4H), 6.98 (dd, *J* = 11.1, 8.7 Hz, 1H), 7.14−7.18 (m, 3H), 7.40−7.44 (m, 1H), 7.80−7.86 (m, 1H). MS-ESI (*m/z*): 632 $[M + H]^+$.

(*S*)-*N*-[3-[2-(3,3-Diallylureido)-4-oxopentan-2-yl]-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-32). To a mixture of compound **1-26** (477 mg, 1.58 mmol) and K_2CO_3 (1076 mg, 7.79 mmol) in EtOAc (4.77 mL) and H_2O (1.9 mL) was added triphosgene (462 mg, 1.56 mmol) at 0 °C. After stirring for 2 h at same temperature, the reaction mixture was poured into H_2O. The aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was dissolved in THF (8 mL). To the mixture was added diallylamine (0.23 mL, 1.87 mmol), and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was poured into H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient:

20-50% EtOAc) to give **1-32** (412 mg, 62% yield) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.81 (s, 3H), 2.12 (s, 3H), 2.94 (d, *J* = 16.1 Hz, 1H), 3.39 (d, *J* = 15.9 Hz, 1H), 3.89 (t, *J* = 4.4 Hz, 4H), 5.18−5.26 (m, 4H), 5.78−5.88 (m, 2H), 6.11 (s, 1H), 6.95 (dd, *J* = 11.7, 8.8 Hz, 1H), 7.48−7.55 (m, 2H), 8.67 (s, 1H). MS-ESI (*m/z*) = 430 $[M + H]^+$.

Experimental procedure for Entry 2 in Table 1-8. A mixture of compound **1-32** (98 mg, 0.23 mmol) and P_2O_5 (486 mg, 3.42 mmol) in AcOH (2.0 mL) was stirred at 120 °C for 2 h. The reaction mixture was poured into ice-cooled aqueous K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 20-50% EtOAc) to give **1-33** (24 mg, 26%) as a colorless amorphous and **1-34** (26 mg, 24%) as a colorless amorphous. **1-33**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ:1.53 (s, 3H), 1.78 (s, 3H), 3.87 (dd, *J* = 16.1, 5.3 Hz, 2H), 4.11−4.18 (m, 2H), 5.14−5.23 (m, 4H), 5.27 (d, *J* = 1.9 Hz, 1H), 5.85−5.96 (m, 2H), 7.01 (dd, *J* = 10.9, 8.8 Hz, 1H), 7.60−7.67 (m, 2H), 7.78 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 18.2, 32.3 (d, *J* = 3.6 Hz), 40.0, 54.8 (d, *J* = 3.6 Hz), 104.9 (d, *J* = 5.1 Hz), 116.0, 116.6 (d, *J* = 25.0 Hz), 117.2, 120.0 (d, *J* = 8.0 Hz), 120.7 (d, *J* = 5.1 Hz), 130.7, 134.8, 138.0 (d, *J* = 14.6 Hz), 144.8, 148.3, 154.5 (d, *J* = 36.4 Hz), 157.3 (d, *J* = 249.9 Hz). MS-ESI (*m/z*) = 412 $[M + H]^+$. **1-34**: ^{1}H NMR (400 MHz, $CDCl_3$) (diastereo mixture 7:3) δ:1.20 (s, 2.1H), 1.48 (s, 2.1H), 1.55 (s, 0.9H), 1.67 (s, 0.9H), 1.71 (s, 2.1H), 1.78 (d, *J* = 11.6 Hz, 0.3H), 1.93 (d, *J* = 11.6 Hz, 0.3H), 2.08 (s, 0.9H), 3.02−3.08 (m, 0.3H), 3.51 (d, *J* = 14.8 Hz, 0.7H), 3.78−3.90 (m, 2H), 4.09−4.20 (m, 2H), 5.13−5.22 (m, 4H), 5.84−5.96 (m, 2H), 6.99−7.05 (m, 1H), 7.54−7.70 (m, 2H), 7.79 (br, 1H). MS-ESI (*m/z*) = 472 $[M + H]^+$.

Experimental procedure for Entry 3 in Table 1-8. A mixture of compound **1-32** (35 mg, 0.08 mmol) and P_2O_5 (174 mg, 1.22 mmol) in MeCN (1.0 mL) was stirred at 80 °C for 1 h. The reaction mixture was poured into ice-cooled aqueous K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 20-50% EtOAc) to give **1-33** (18 mg, 54%) as a colorless amorphous.

Experimental procedure for Entry 5 in Table 1-8. To a solution of **1-32** (50 mg, 0.12 mmol) in THF (1 mL) was added Burgess reagent (56 mg, 0.23 mmol). The mixture was stirred at reflux for 1 h. The mixture was diluted with EtOAc and sat. $NaHCO_3$. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 20-50% EtOAc) to give **1-33** (40 mg, 83%) as a colorless amorphous.

(*S*)-*N*,*N*-Bis(2,4-dimethoxybenzyl)-4-(2-fluorophenyl)-4-methyl-4*H*-1,3-oxazin-2-amine (1-39). To a solution of **1-36** (260 mg, 0.49 mmol) and PPTS (125 mg, 0.49 mmol) in THF (3 mL) was added Burgess reagent (236 mg, 0.99 mmol). The mixture was stirred at reflux for 1 h. The mixture was diluted with EtOAc and sat. $NaHCO_3$. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 20-50% EtOAc) to give **1-39** (68 mg, 27%) as a colorless oil.^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.60 (s, 3H), 3.74 (s, 6H), 3.81 (s, 6H), 4.42 (d, *J* = 16.2 Hz, 2H), 4.63 (d, *J* = 16.1 Hz, 2H), 5.57 (dd, *J* = 5.9, 3.1 Hz, 1H), 6.35 (d, *J* = 6.1 Hz, 1H), 6.42−6.48 (m, 4H), 6.92−7.00 (m, 2H), 7.11−7.19 (m, 3H), 7.55 (t, *J* = 7.6 Hz, 1H). MS-ESI (*m/z*) = 507 $[M + H]^+$.

(*S*)-[2-[Bis(2,4-dimethoxybenzyl)amino]-4-(2-fluorophenyl)-4-methyl-4*H*-1,3-oxazin-6-yl]methyl acetate (1-40). Compound **1-40** was prepared in a manner similar to that for **1-39** in 41% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.60 (s, 3H), 1.90 (s, 3H), 3.75 (s, 6H), 3.81 (s, 6H), 4.40−4.46 (m, 4H), 4.63 (d, *J* = 16.1 Hz, 2H), 5.67 (d, *J* = 3.3 Hz, 1H), 6.42−6.46 (m, 4H), 6.93−7.00 (m, 2H), 7.12−7.18 (m, 3H), 7.52 (td, *J* = 8.1, 1.8 Hz, 1H). MS-ESI (*m/z*) = 579 $[M + H]^+$.

(*S*)-*N*,*N*-Bis(2,4-dimethoxybenzyl)-4-(fluoromethyl)-4-(2-fluorophenyl)-6-methyl-4*H*-1,3-oxazin-2-amine (1-41). Compound **1-41** was prepared at room temperature in a manner similar to that for **1-39** in 62% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.80 (s, 3H), 3.75 (s, 6H), 3.81 (s, 6H), 4.13−4.27 (m, 1H), 4.49−4.69 (m, 5H), 5.27 (d, *J* = 2.0 Hz, 1H), 6.42−6.48 (m, 4H), 6.94−7.00 (m, 2H), 7.16−7.22 (m, 3H), 7.61 (td, *J* = 7.9, 1.4 Hz, 1H). MS-ESI (*m/z*) = 539 $[M + H]^+$.

(*S*)-4-(5-Amino-2-fluorophenyl)-*N*,*N*-bis(2,4-dimethoxybenzyl)-4,6-dimethyl-4*H*-1,3-oxazin-2-amine (1-42). To a solution of **1-28** (7.03 g, 11.1 mmol) in THF (35.0 mL), MeOH (35.0 mL), and

H_2O (35.0 mL) was added K_2CO_3 (3.08 g, 22.3 mmol). The mixture was stirred at 40 °C for 18 h. After being cooled to room temperature, the mixture was diluted with EtOAc and H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated to give **1-42** (3.31 g, 56% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 3H), 1.73 (s, 3H), 3.19 (br s, 2H), 3.76 (s, 6H), 3.80 (s, 6H), 4.31 (d, *J* = 16.3 Hz, 2H) , 4.81 (d, *J* = 16.3 Hz, 2H), 5.27 (d, *J* = 2.2 Hz, 1H), 6.34−6.39 (m, 1H), 6.44−6.50 (m, 4H), 6.60 (dd, *J* = 6.7, 3.0 Hz, 1H), 6.71 (dd, *J* = 11.6, 8.6 Hz, 1H), 7.19 (d, *J* = 7.9 Hz, 2H). MS-ESI (*m/z*): 536 $[M + H]^+$.

(*S*)-*N*-[3-[2-[Bis(2,4-dimethoxybenzyl)amino]-4,6-dimethyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (1-43). To a solution of **1-42** (86.3 mg, 0.161 mmol), 5-cyanopocolinic acid monohydrate (34.8 mg, 0.209 mmol) was added EDC-HCl (40.2 mg, 0.21 mmol). The mixture was stirred at room temperature for 20 h. The mixture was diluted with EtOAc and saturated aq $NaHCO_3$ solution. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0–50% EtOAc) to give **1-43** (99 mg, 92% yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58 (s, 3H), 1.76 (s, 3H), 3.71 (s, 6H), 3.77 (s, 6H), 4.36 (d, *J* = 16.1 Hz, 2H) , 4.78 (d, *J* = 16.1 Hz, 2H), 5.33 (d, *J* = 3.4 Hz, 1H), 6.42−6.46 (m, 4H), 7.02 (dd, *J* = 11.2, 8.7 Hz, 1H), 7.19−7.25 (m, 2H), 7.41 (dd, *J* = 6.7, 2.9 Hz, 1H), 8.05−8.11 (m, 1H), 8.18 (dd, *J* = 8.2, 2.0 Hz, 1H), 8.39 (d, *J* = 8.2 Hz, 1H), 8.90 (d, *J* = 2.0 Hz, 1H), 9.50 (br s, 1H). MS-ESI (*m/z*): 666 $[M + H]^+$.

(*S*)-*N*-[3-(2-Amino-4,6-dimethyl-4*H*-1,3-oxazin-4-yl)-4-fluorophenyl]-5-cyanopicolinamide (1-6). A mixture of **1-43** (99 mg, 0.15 mmol) and anisole (112 mg, 1.04 mmol) in TFA (10 mL) was stirred at 80 °C for 15 h. The reaction mixture was warmed to room temperature and evaporated. The residue was diluted with EtOAc and 10% aq Na_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; $CHCl_3$/MeOH, gradient: 0–3% MeOH) to give **1-6** (25.9 mg, 48% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61 (s, 3H), 1.80 (s, 3H), 4.38 (br s, 2H) , 5.27 (dd, *J* = 2.8, 1.3 Hz, 1H), 7.03 (dd, *J* = 11.4, 8.9 Hz, 1H), 7.67 (dd, *J* = 6.9, 2.9 Hz, 1H), 7.86−7.92 (m, 1H), 8.17 (dd, *J* = 8.1, 2.0 Hz, 1H), 8.39 (d, *J* = 8.1 Hz,

1H), 8.82 (d, *J* =2.0 Hz, 1H), 9.81 (br s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 18.3, 32.0, 54.7, 100.6, 104.6, 112.3, 116.0, 116.8 (d, *J* = 24.8 Hz), 119.3 (d, *J* = 4.8 Hz), 122.3 (d, *J* = 8.0 Hz), 132.9, 136.8 (d, *J* = 13.9 Hz), 141.2, 144.4 (d, *J* =17.5 Hz), 148.7, 150.7, 152.3, 154.4 (d, *J* = 243.5 Hz), 159.9. HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{19}H_{17}FN_5O_2$, 366.1366; found, 366.1365.

***N*-[3-[(*S*)-2-[[(*R*)-*tert*-Butylsulfinyl]amino]pent-4-en-2-yl]-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-44)**. To a solution of **1-23** (10 g, 28.4 mmol) in THF (100 mL) was added allyl magnesium bromide (1 M in THF, 100 mL) at −78 °C for 1 h. The reaction mixture was quenched with saturated aq NH_4Cl solution. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0−30% EtOAc) to give **1-44** (4.5 g, 40% yield) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.21 (s, 9H), 1.68 (s, 3H), 2.79 (dd, *J* = 13.4, 7.3 Hz, 1H), 2.92 (dd, *J* = 13.4, 6.8 Hz, 1H), 4.16 (s, 1H), 5.10 (d, *J* = 9.6 Hz, 1H), 5.13 (d, *J* = 17.2 Hz, 1H), 5.51–5.65 (m, 1H), 6.95 (dd, *J* = 10.6, 10.1 Hz, 1H), 7.49–7.54 (m, 1H), 7.61–7.64 (m, 1H), 9.96 (s, 1H). MS-ESI (*m/z*): 395 $[M + H]^+$.

***N*-[3-[(*S*)-2-[[(*R*)-*tert*-Butylsulfinyl]amino]-4-oxobutan-2-yl]-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-45).** A solution of compound **1-44** (4.5 g, 11.4 mmol) in DCM (90 mL) was cooled to –78 °C, and ozone gas was bubbled into the solution. When the color of the reaction solution changed to blue, the bubbling was stopped. Et_3N (4.7 mL) was added to the soution, which was then stirred for an addtional 1 h at –78 °C. The mixture was warmed to room temperature and diluted with EtOAc and H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated to give **1-45** (4.0 g, 88% yield) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.11 (s, 9H), 1.79 (s, 3H), 3.18 (s, 2H), 5.49 (s, 1H), 7.20 (dd, *J* = 12.2, 8.8 Hz, 1H), 7.69 (ddd, *J* = 8.8, 4.0, 2.7 Hz, 1H), 7.84 (dd, *J* = 7.2, 2.7 Hz, 1H), 9.56 (s, 1H), 11.3 (s, 1H). MS-ESI (*m/z*): 397 $[M + H]^+$.

(*S*)-*N*-[3-(2-Amino-4,4-dimethoxybutan-2-yl)-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-46). To a solution of **1-45** (24.7 g, 62.3 mmol) in MeOH (250 mL) was added a solution of HCl (2 M in MeOH, 156 mL, 312 mmol). The mixture was stirred at room temperature for 22 h. The reaction mixture was evaporated, and then the residue was diluted with EtOAc. The mixture was poured into

saturated aq $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 10–50% EtOAc) to give **1-46** (11.7 g, 56% yield) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.79 (s, 3H), 2.04 (dd, *J* = 14.4, 7.2 Hz, 1H), 2.34–2.41 (m, 1H), 3.20 (s, 3H), 3.21 (s, 3H), 4.08–4.16 (m, 1H), 7.06 (dd, *J* = 11.4, 8.7 Hz, 1H), 7.59–7.64 (m, 1H), 7.69 (dd, *J* = 7.2, 3.0 Hz, 1H), 8.04 (br s, 1H). MS-ESI (*m/z*): 443 $[M + H]^+$.

(*S*)-*N*-[3-[2-(3,3-Diallylureido)-4,4-dimethoxybutan-2-yl]-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-47). To a solution of **1-46** (6.62 g, 19.6 mmol) and K_2CO_3 (13.5 g, 98.0 mmol) in EtOAc (40 mL) and H_2O (26 mL) was added a solution of triphosgene (5.81 g, 19.6 mmol) in EtOAc (25 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h, and then a solution of diallylamine (5.70 g, 58.7 mmol) in EtOAc (13 mL) was added. The resulting mixture was stirred at 0 °C for 2 h and diluted with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 10–50% EtOAc) to give **1-47** (7.39 g, 82% yield) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.92–1.96 (m, 4H), 2.34 (dd, *J* = 14.7, 5.4 Hz, 1H), 3.33 (s, 3H), 3.34 (s, 3H), 3.86 (dd, *J* = 16.8, 4.8 Hz, 2H), 3.97 (dd, *J* = 16.8, 4.8 Hz, 2H), 4.34 (t, *J* = 5.4 Hz, 1H), 5.26 (m, 4H), 5.80–5.90 (m, 2H), 6.51 (s, 1H), 6.90 (dd, *J* = 11.7, 8.7 Hz, 1H), 7.38–7.42 (m, 1H), 7.57 (dd, *J* = 7.5, 2.7 Hz, 1H), 9.14 (s, 1H). MS-ESI (*m/z*): 462 $[M + H]^+$.

(*S*)-*N*-[3-[2-(Diallylamino)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-48). To a solution of **1-47** (7.39 g, 16.0 mmol) in acetone (100 mL) was added a solution of H_2SO_4 (1 M in H_2O; 48 mL). The mixture was stirred at room temperature for 2 h. The mixture was diluted with EtOAc and H_2O. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated to give a residue (6.36 g) as a yellow oil. The residue was dissolved in THF (50 mL). Burgess regent (7.30 g, 30.6 mmol) and pyridinium *p*-toluenesulfonate (7.70 g, 30.6 mmol) were added to the solution, and the reaction mixture was refluxed for 7 h. After cooling to room temperature, the mixture was diluted with EtOAc and saturated aq $NaHCO_3$ solution. The aqueous layer was separated

and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0–20% EtOAc) to give **1-48** (1.45 g, 24% yield) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.60 (s, 3H), 3.85 (dd, *J* = 16.2, 5.7 Hz, 2H), 4.12–4.19 (m, 2H), 5.20–5.27 (m, 4H), 5.57 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.83–5.95 (m, 2H), 6.38 (d, *J* = 6.0 Hz, 1H), 6.97–7.04 (m, 1H), 7.62–7.66 (m, 2H), 7.76 (br s, 1H). MS-ESI (*m/z*): 398 $[M + H]^+$.

(*S*)-*N*,*N*-Diallyl-4-(5-amino-2-fluorophenyl)-4-methyl-4*H*-1,3-oxazin-2-amine (1-49). To a solution of **1-48** (624 mg, 1.57 mmol) in THF (2.0 mL), MeOH (2.5 mL), and H_2O (2.5 mL) was added K_2CO_3 (651 mg, 4.71 mmol). The mixture was stirred at 60 °C for 7 h. After being cooled to room temperature, the mixture was diluted with EtOAc and H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated to give **1-49** (412 mg, 87% yield) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 3H), 3.49 (br s, 2H), 3.86 (dd, *J* = 15.7, 5.6 Hz, 2H), 4.05 (dd, *J* = 15.7, 5.6 Hz, 2H), 5.13–5.21 (m, 4H), 5.55 (dd, *J* = 6.1, 3.0 Hz, 1H), 5.81–5.92 (m, 2H), 6.34 (d, *J* = 6.1 Hz, 1H), 6.47–6.52 (m, 1H), 6.77 (dd, *J* = 11.7, 8.6 Hz, 1H), 7.03–7.06 (m, 1H). MS-ESI (*m/z*): 302 $[M + H]^+$.

(*S*)-5-Cyano-*N*-[3-[2-(diallylamino)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]picolinamide (1-50). To a solution of **1-49** (50.9 mg, 0.169 mmol), 5-cyanopocolinic acid monohydrate (33.7 mg, 0.203 mmol), 1-hydroxybenzotriazole hydrate (31.0 mg, 0.203 mmol), and DMAP (2.06 mg, 0.017 mmol) in DMF (1 mL) was added EDC-HCl (38.9 mg, 0.203 mmol). The mixture was stirred at room temperature for 2 h. The mixture was diluted with EtOAc and saturated aq $NaHCO_3$ solution. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0–30% EtOAc) to give **1-50** (66.9 mg, 92% yield) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.60 (s, 3H), 3.85 (dd, *J* = 16.2, 5.6 Hz, 2H), 4.15 (dd, *J* = 16.2, 5.6 Hz, 2H), 5.20–5.26 (m, 4H), 5.58 (dd, *J* = 6.1, 3.0 Hz, 1H), 5.88–5.99 (m, 2H), 6.38 (d, *J* = 6.1 Hz, 1H), 7.03 (dd, *J* = 11.2, 8.6 Hz, 1H), 7.74–7.79 (m, 1H), 7.92–7.96 (m, 1H), 8.20 (dd, *J* = 8.1, 2.0 Hz, 1H), 8.43 (dd, *J* = 8.1, 1.0 Hz, 1H), 8.90 (d, *J* = 1.0 Hz, 1H), 9.79 (br s, 1H). MS-ESI (*m/z*): 432 $[M + H]^+$.

(*S*)-*N*-[3-(2-Amino-4-methyl-4*H*-1,3-oxazin-4-yl)-4-fluorophenyl]-5-cyanopicolinamide (1-4). To a solution of **1-50** (66.9 mg, 0.155 mmol) and 1,3-dimethylbarbituric acid (145 mg, 0.930 mmol) in DCM (1.0 mL) was added $Pd(PPh_3)_4$ (17.9 mg, 0.016 mmol). The mixture was refluxed for 2 h. The mixture was diluted with EtOAc and saturated aq $NaHCO_3$ solution. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (amino silica gel; hexane/EtOAc, gradient: 20–50% EtOAc) to give **1-4** (32 mg, 58% yield) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.56 (s, 3H), 4.11 (br s, 2H), 5.56 (dd, *J* = 6.1, 2.5 Hz, 1H), 6.37 (d, *J* = 6.1 Hz, 1H), 7.06 (dd, *J* = 11.2, 8.6 Hz, 1H), 7.67 (dd, *J* = 4.1, 3.0 Hz, 1H), 7.91–7.95 (m, 1H), 8.20 (dd, *J* = 8.1, 2.0 Hz, 1H), 8.43 (d, *J* = 8.1 Hz, 1H), 8.89 (dd, *J* = 6.1, 2.0 Hz, 1H), 9.85 (s, 1H). MS-ESI (*m/z*): 352 $[M + H]^+$.

Ethyl (3*S*)-3-[[(*R*)-*tert*-butylsulfinyl]amino]-3-(2-fluorophenyl)-2-methylbutanoate (1-52). To a solution of diisopropylamine (1.18 mL, 8.29 mmol) in THF (10 mL) was added dropwise *n*-BuLi (2.69 M in hexane; 3.08 mL, 8.29 mmol) at −78 °C. After being stirred for 15 min at 0 °C, to the mixture was added dropwise ethyl propionate (0.95 mL, 8.29 mmol) followed by chlorotriisopropoxy titanium (2.67 mL, 11.2 mmol) in THF (3 mL) at −78 °C. After being stirred for 30 min, **1-51** (1.0 g, 4.14 mmol) in THF (7 mL) was added dropwise to the mixture at −78 °C. The reaction mixture was stirred for 30 min at −78°C. The reaction was quenched with saturated aq NH_4Cl solution. The resulting mixture was filtered through Celite, and the filtrate was extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0−30% EtOAc) to give **1-52** (1.3 g, 91% yield) as a colorless oil. This material was obtained as a mixture of diastreomers.^{1}H NMR (400 MHz, $CDCl_3$) (diastereo mixture 3:1) δ 1.06−1.16 (m, 6H), 1.26 (s, 2.25H), 1.28 (s, 6.75H), 1.83 (s, 0.75H), 1.95 (s, 2.25H), 3.14 (q, *J* = 7.2 Hz, 0.25H), 3.42 (q, *J* = 7.2 Hz, 0.75H), 3.99−4.02 (m, 2H), 4.90 (s, 0.75H), 5.48 (s, 0.25H), 6.98−7.15 (m, 2H), 7.26−7.28 (m, 1H), 7.39−7.41 (m, 0.75H), 7.44-7.49 (m, 0.25H). MS-ESI (*m/z*): 344 $[M + H]^+$.

Ethyl (2*S*,3*S*)-3-amino-3-(2-fluorophenyl)-2-methylbutanoate (1-53). To a solution of **1-52** (1.30 g, 3.79 mmol) in MeOH (13 mL) was added HCl (4 M in dioxane; 1.3 mL, 5.20 mmol) at room temperature. After being stirred for 2 h, the mixture was diluted with saturated aq $NaHCO_3$ solution

and EtOAc. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to afford **1-53** (957 mg, quant) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) (diastereo mixture 3:1) δ 0.88 (t, *J* = 7.2 Hz, 0.75H), 0.98 (d, *J* = 7.2 Hz, 2.25H), 1.18−1.24 (m, 3H), 1.51 (s, 0.75H), 1.58 (s, 2.25H), 1.82 (br s, 2H), 3.20−3.30 (m, 1H), 3.78−3.84 (m, 0.5H), 4.08−4.12 (m, 1.5H), 6.99−7.03 (m, 1H), 7.10−7.13 (m, 1H), 7.23−7.26 (m, 1H), 7.53−7.57 (m, 1H). MS-ESI (*m/z*): 240 $[M + H]^+$.

Ethyl (2*S*,3*S*)-3-[3,3-Bis(2,4-dimethoxybenzyl)ureido]-3-(2-fluorophenyl)-2-methylbutanoate (1-54). To a solution of **1-53** (957 mg, 3.79 mmol) and $NaHCO_3$ (1.11 g, 13.3 mmol) in EtOAc (10 mL) and H_2O (5.0 mL) was added 4-nitrophenyl chloroformate (764 mg, 3.79 mmol) at 0 °C. After being stirred for 30 min, bis(2,4-dimethoxybenzyl)amine (1.20 g, 3.79 mmol) was added. The resulting mixture was stirred at 0 °C for 2 h. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 20−50% EtOAc) to give **1-54** (2.31 g, 100% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) (diastereo mixture 3:1) δ 0.78 (d, *J* = 7.2 Hz, 2.25H), 0.84 (t, *J* = 7.2 Hz, 0.75H), 0.93 (d, *J* = 7.2 Hz, 0.75H), 1.12 (t, *J* = 7.2 Hz, 2.25H), 1.94 (s, 0.75H), 1.96 (s, 2.25H), 2.98 (q, *J* = 7.2 Hz, 0.75H), 3.06 (q, *J* = 7.2 Hz, 0.25H), 3.77 (s, 6H), 3.81 (s, 6H), 3.92−4.02 (m, 2H), 4.34–4.60 (m, 4H), 5.96 (s, 0.75H), 6.33 (s, 0.25H), 6.44−6.47 (m, 4H), 6.96−6.99 (m, 1H), 7.03−7.05 (m, 1H), 7.15−7.22 (m, 3H), 7.26−7.28 (m, 1H). MS-ESI (*m/z*): 583 $[M + H]^+$.

1,1-Bis(2,4-dimethoxybenzyl)-3-[(2*S*,3*S*)-2-(2-fluorophenyl)-3-methyl-4-oxobutan-2-yl]urea (1-55). To a solution of **1-54** (2.31g, 3.79 mmol) in DCM (16 mL) was added DIBAL (1.02 M in hexane; 13.9 mL, 14.2 mmol) at −78 °C. After being stirred for 1 h, the reaction was quenched with Rochelle's salt and stirred at room temperature. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 20−50% EtOAc) to give **1-55** (1.23 g, 60% yield) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) (diastereo mixture 3:1) δ 0.82 (d, *J* = 6.9 Hz, 2.25H), 0.98 (d, *J* = 6.9 Hz, 0.75H), 1.98 (s, 2.25H), 2.05 (s, 0.75H), 3.05 (q, *J* = 6.9 Hz, 0.25H), 3.23 (q, *J* = 6.9 Hz, 0.75H), 3.75 (s, 4.5H), 3.79 (s, 1.5H), 3.81 (s, 4.5H), 3.82 (s, 1.5H), 4.34−4.50 (m, 4H), 5.50 (s, 0.75H), 5.52 (s, 0.25H), 6.43−6.51

(m, 4H), 6.98−7.18 (m, 2H), 7.14−7.25(m, 4H), 9.47 (s, 0.25H), 9.68 (s, 0.75H). MS-ESI (*m/z*): 539 $[M + H]^+$.

(*S*)-*N*,*N*-Bis(2,4-dimethoxybenzyl)-4-(2-fluorophenyl)-4,5-dimethyl-4*H*-1,3-oxazin-2-amine (1-56). To a solution of **1-55** (1.23 g, 2.28 mmol) and PPTS (1.15 g, 4.57 mmol) in THF (10 mL) was added Burgess reagent (1.09 g, 4.57 mmol). The mixture was refluxed for 1.5 h. The mixture was cooled to room temperature and diluted with EtOAc and saturated aq $NaHCO_3$ solution. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 20−50% EtOAc) to give **1-56** (628 mg, 53% yield) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 3H), 1.72 (s, 3H), 3.70 (s, 6H), 3.80 (s, 6H), 4.40 (d, *J* = 16.2 Hz, 2H), 4.45 (d, *J* = 16.2 Hz, 2H), 6.20 (s, 1H), 6.37−6.46 (m, 4H), 6.98 (dd, *J* = 12.2, 8.2 Hz, 1H), 7.04 (m, 1H), 7.16 (d, *J* = 8.3 Hz, 2H), 7.16−7.19 (m, 1H), 7.36−7.40 (m, 1H). MS-ESI (*m/z*): 521 $[M + H]^+$.

(*S*)-4-(2-Fluorophenyl)-4,5-dimethyl-4*H*-1,3-oxazin-2-amine (1-57). A mixture of compound **1-56** (628 mg, 1.21 mmol) and anisole (922 μL, 2.31 mmol) in TFA (4.6 mL) was stirred at 80 °C for 20 h. The reaction mixture was cooled to room temperature and poured into aq K_2CO_3 solution and EtOAc at 0 °C. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (amino silica gel; hexane/EtOAc, gradient: 20−50% EtOAc) to give **1-57** (245 mg, 92% yield) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 3H), 1.72 (s, 3H), 3.97 (br s, 2H), 6.22 (s, 1H), 7.00 (dd, *J* = 12.3, 8.2 Hz, 1H), 7.10 (t, *J* = 7.6 Hz, 1H), 7.21−7.24 (m, 1H), 7.34 (t, *J* = 8.0 Hz, 1H). MS-ESI (*m/z*): 221 $[M + H]^+$.

(*S*)-4-(2-Fluoro-5-nitrophenyl)-4,5-dimethyl-4*H*-1,3-oxazin-2-amine (1-58). To a solution of **1-57** (235 mg, 1.07 mmol) in TFA (2 mL) was added sulfuric acid (0.5 mL) at −20 °C. After being stirred at this temperature for 10 min, to the mixture was added dropwise HNO_3 (72 μL, 1.60 mmol). The reaction mixture was stirred at −20 °C for 1 h and poured into aq K_2CO_3 solution and EtOAc. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column

chromatography (amino silica gel; hexane/EtOAc, gradient: 20−50% EtOAc) to give **1-58** (287 mg, 100% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 3H), 1.79 (s, 3H), 4.22 (br s, 2H), 6.28 (s, 1H), 7.18 (dd, *J* = 10.5, 9.0 Hz, 1H), 8.16−8.20 (m, 1H), 8.30 (dd, *J* = 6.9, 2.6 Hz, 1H). MS-ESI (*m/z*): 266 $[M + H]^+$.

***tert*-Butyl-*N*-[(tert-butoxy)carbonyl]-*N*-[(4*S*)-4-(2-fluoro-5-nitrophenyl)-4,5-dimethyl-4*H*-1,3-oxazin-2-yl]carbamate (1-59).** To a solution of **1-58** (277 mg, 1.03 mmol) and Boc_2O (0.53 mL, 2.30 mmol) in DCM (3 mL) was added DMAP (12.8 mg, 0.104 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0−30% EtOAc) to give **1-59** (442 mg, 91% yield) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (s, 3H), 1.51 (s, 18H), 1.86 (s, 3H), 6.36 (s, 1H), 7.18−7.22 (m, 1H), 8.20−8.23 (m, 1H), 8.37 (dd, *J* = 6.7, 2.5 Hz, 1H). MS-ESI (*m/z*): 466 $[M + H]^+$.

***tert*-Butyl-*N*-[(4*S*)-4-(5-amino-2-fluorophenyl)-4,5-dimethyl-4*H*-1,3-oxazin-2-yl]-*N*-[(*tert*-butoxy)carbonyl]carbamate (1-60).** A mixture of compound **1-59** (232 mg, 0.50 mmol), Fe (223 mg, 3.99 mmol), and NH_4Cl (320 mg, 5.98 mmol) in EtOH/THF/H_2O (3.0 mL/1.5 mL/1.5 mL) was stirred at 60 °C for 1 h. The reaction mixture was cooled to room temperature and filtered through Celite, which was washed with EtOAc and H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (amino silica gel; hexane/EtOAc, gradient: 20−50% EtOAc) to give **1-60** (166 mg, 77% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (s, 3H), 1.50 (s, 18H), 1.76 (s, 3H), 3.53 (s, 2H), 6.27 (s, 1H), 6.54−6.57 (m, 1H), 6.71−6.73 (m, 1H), 6.81 (dd, *J* = 11.2, 8.7 Hz, 1H). MS-ESI (*m/z*): 436 $[M + H]^+$.

***tert*-Butyl-*N*-[(tert-butoxy)carbonyl]-*N*-[(4*S*)-4-[5-(5-cyanopyridine-2-amido)-2-fluorophenyl]-4,5-dimethyl-4*H*-1,3-oxazin-2-yl]carbamate (1-61).** To a solution of **1-60** (135 mg, 0.310 mmol), 5-cyanopocolinic acid monohydrate (61.8 mg, 0.372 mmol), and DIEA (108 μL, 0.620 mmol) in DMF (2 mL) was added HATU (141 mg, 0.372 mmol). The reaction mixture was stirred at room temperature for 1 h and then diluted with EtOAc and saturated aq $NaHCO_3$ solution. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with H_2O and

brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0−30% EtOAc) to give **1-61** (175 mg, 100% yield) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 18H), 1.53 (s, 3H), 1.85 (s, 3H), 6.31 (s, 1H), 7.06−7.10 (m, 1H), 7.70−7.72 (m, 1H), 7.91−7.94 (m, 1H), 8.21 (d, *J* = 8.3 Hz, 1H), 8.43 (d, *J* = 8.3 Hz, 1H), 8.86 (s, 1H), 9.87 (s, 1H). MS-ESI (*m/z*): 566 $[M + H]^+$.

(*S*)-*N*-(3-(2-Amino-4,5-dimethyl-4*H*-1,3-oxazin-4-yl)-4-fluorophenyl)-5-cyanopicolinamide (1-5). A solution of compound **1-61** (175 mg, 0.310 mmol) in formic acid (2 mL) was stirred at room temperature for 1 h. The reaction mixture was poured into saturated aq $NaHCO_3$ solution, and the mixture was diluted with EtOAc. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (amino silica gel; hexane/EtOAc, gradient: 20−50% EtOAc) to give **1-5** (87 mg, 76% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 3H), 1.75 (s, 3H), 3.93 (br s, 2H), 6.24 (s, 1H), 7.06 (dd, *J* = 11.3, 9.0 Hz, 1H), 7.67−7.70 (m, 1H), 7.73−7.76 (m, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 8.44 (d, *J* = 8.0 Hz, 1H), 8.90 (s, 1H), 9.83 (s, 1H). MS-ESI (*m/z*): 366 $[M + H]^+$.

2-Fluoro-1-(2-fluorophenyl)ethan-1-one (1-63). To a solution of LHMDS (1.3 M in THF; 659 mL, 856 mmol) in THF (182 mL) was added dropwise compound **1-62** (91.0 g, 656 mmol) in THF (182 mL) at −78 °C over 30 min. After being stirred at this temperature for 10 min, TMSCl (109 mL, 856 mmol) was added dropwise over 30 min. The reaction mixture was maintained at −78 °C for 1 h and then allowed to warm to room temperature. Stirring was continued for 2 h at room temperature, and then the mixture was diluted with EtOAc (550 mL) and 20% aq NH_4Cl solution (910 mL). The aqueous layer was separated and extracted with EtOAc (270 mL). The combined organic layers were washed with brine (550 mL), dried over $MgSO_4$, filtered, and evaporated. The residue was dissolved in MeCN (910 mL). To this solution was added portionwise Selectfluor (257 g, 725 mmol) at 4 °C over 30 min. The reaction mixture was allowed to warm to room temperature and stirred for 10 h. The solvent was evaporated and then diluted with EtOAc (364 mL) and H_2O (364 mL). The aqueous layer was separated and extracted with EtOAc (364 mL). The combined organic layers were washed with H_2O (364 mL) and brine (364 mL), dried over $MgSO_4$, filtered, and evaporated. The residue was treated with hexane (200 mL), and the mixture was stirred at 0 °C for 1 h. The resulting solid was collected

and washed with cooled hexane (100 mL). The solid was dissolved in DCM (500 mL), and silica gel (206 g) was added to the mixture. The suspension was filtered off, and the resulting silica gel was washed with EtOAc−hexane (1:1, 200 mL × 3). The filtrate was evaporated, and the residue was treated with hexane (200 mL). The mixture was stirred at 0 °C for 1 h. The resulting solid was collected and washed with cooled hexane (100 mL) to afford **1-63** (91.0 g, 89% yield) as a tan solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45 (d, *J* = 48.0 Hz, 2H), 7.18 (t, *J* = 10.0 Hz, 1H), 7.31 (t, *J* = 7.6 Hz, 1H), 7.58−7.64 (m, 1H), 8.04 (t, *J* = 7.6 Hz, 1H). MS-ESI (*m/z*): 157 $[M + H]^+$.

(*R*,*Z*)-*N*-[2-Fluoro-1-(2-fluorophenyl)ethylidene]-2-methylpropane-2-sulfinamide (1-64). To a solution of **1-63** (74.6 g, 478 mmol) and (*R*)-2-methylpropane-2-sulfinamide (232 g, 1911 mmol) in DCM (373 mL) was added dropwise $Ti(OEt)_4$ (200 mL, 956 mmol) at room temperature, and the reaction mixture was stirred at 40 °C for 6 h and then allowed to cool to room temperature. The mixture was dissolved in MeCN (1.5 L), and then H_2O (60.3 mL) was added at room temperature. After being stirred for 10 min, $MgSO_4$ (74.6 g) was added. Stirring was continued for an additional 10 min at room temperature, and the insoluble materials were filtered off. The filtrate was evaporated, and the residue was diluted with EtOAc (750 mL) and H_2O (750 mL). The aqueous layer was separated and extracted with EtOAc (750 mL). The combined organic layers were evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0−20% EtOAc) to give **1-64** (88.2 g, 71% yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (s, 9H), 5.62−5.98 (m, 2H), 7.12 (d, *J* = 9.6 Hz, 1H), 7.21 (t, *J* = 7.6 Hz, 1H), 7.45 (dd, *J* = 13.6, 7.6 Hz, 1H), 7.46−7.56 (m, 1H). MS-ESI (*m/z*): 260 $[M + H]^+$.

***tert*-Butyl-(*S*)-5-[[(*R*)-*tert*-Butylsulfinyl]amino]-6-fluoro-5-(2-fluorophenyl)-3-oxohexanoate (1-65)**. Compound **1-65** was prepared in a manner similar to that for **1-52** using *tert*-butyl acetoacetate in 81% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (s, 9H), 1.46 (s, 9H), 3.29−3.66 (m, 4H), 4.77−5.21 (m, 2H), 5.21 (s, 1H), 7.00−7.07 (m, 1H), 7.15−7.20 (m, 1H), 7.28−7.36 (m, 1H), 7.48−7.54 (m, 1H). MS-ESI (*m/z*): 418 $[M + H]^+$.

(*S*)-4-Amino-5-fluoro-4-(2-fluorophenyl)pentan-2-one (1-66). A solution of compound **1-65** (7.2 g, 17.2 mmol) and HCl (4 M in dioxane; 70 mL) was stirred at 60 °C for 1 h. The reaction mixture was evaporated, and the residue was diluted with aq HCl solution (6 M in H_2O, 70 mL). The mixture was

stirred at 85 °C for 2 h, then allowed to cool to room temperature, and neutralized with aq NaOH solution. The mixture was extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 20−50% EtOAc) to give **1-66** (3.74 g, 100% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.05 (s, 3H), 2.29 (s, 2H), 3.17 (dd, *J* = 136.4, 17.2 Hz, 2H), 4.55 (ddd, *J* = 47.5, 41.5, 9.0 Hz, 2H), 7.01 (dd, *J* = 12.8, 8.1 Hz, 1H), 7.17 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.23−7.31 (m, 1H), 7.62−7.68 (m, 1H). MS-ESI (*m/z*): 214 $[M + H]^+$.

(*S*)-1,1-Bis(2,4-dimethoxybenzyl)-3-[1-fluoro-2-(2-fluorophenyl)-4-oxopentan-2-yl]urea (1-38). Compound **1-38** was prepared in a manner similar to that for **1-54** in 81% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04 (s, 3H), 3.17−3.66 (m, 2H), 3.74 (s, 6H), 3.80 (s, 6H), 4.41 (dd, *J* = 43.2, 16.2 Hz, 4H), 5.11 (ddd, *J* = 47.2, 12.8, 9.1 Hz, 2H), 6.31 (s, 1H), 6.44−6.47 (m, 4H), 6.97−7.15 (m, 4H), 7.20−7.33 (m, 2H). MS-ESI (*m/z*): 493 $[M + H]^+$.

***tert*-Butyl-*N*-[(4*S*)-4-(5-amino-2-fluorophenyl)-4-(fluoromethyl)-6-methyl-4*H*-1,3-oxazin-2-yl]-*N*-[(*tert*-butoxy)carbonyl]carbamate (1-67).** Compound **1-67** was prepared in a manner similar to that for **1-57** in 35% yield over 4 steps from **1-41**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (s, 18H), 1.88 (s, 3H), 3.54 (br s, 2H), 4.49 (ddd, *J* = 80.2, 47.7, 8.8 Hz, 2H), 5.16−5.17 (m, 1H), 6.52 (dt, *J* = 8.5, 3.3 Hz, 1H), 6.86 (dd, *J* = 11.4, 8.5 Hz, 1H), 6.92 (dd, *J* = 6.3, 3.3 Hz, 1H). MS-ESI (*m/z*): 454 $[M + H]^+$.

(*S*)-*N*-[3-[2-Amino-4-(fluoromethyl)-6-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (1-7). Compound **1-7** was prepared in a manner similar to that for **1-5** in 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.85 (s, 3H), 4.32 (br s, 2H), 4.34 (dd, *J* = 63.6, 13.2 Hz, 1H), 4.63 (dd, *J* = 63.6, 12.4 Hz, 1H), 5.22−5.23 (m, 1H), 7.04 (dd, *J* = 11.2, 8.8 Hz, 1H), 7.71−7.74 (m, 1H), 7.97−8.02 (m, 1H), 8.17−8.20 (m, 1H), 8.39−8.42 (m, 1H), 8.87−8.88 (m, 1H), 9.85 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 18.5, 58.9, 87.8, 99.0, 112.4, 115.9, 116.7 (d, *J* = 24.8 Hz), 120.5 (d, *J* = 8.8 Hz), 120.8 (d, *J* = 7.3 Hz), 122.3, 131.1(d, *J* = 10.9 Hz), 133.4, 141.3, 147.8, 150.3, 150.6, 152.2, 156.5 (d, *J* = 242.8 Hz), 160.0. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{15}F_2N_5O_2$, 384.1272; found, 384.1255. $[\alpha]_D^{20}$ + 37.6 (*c* 0.51, MeCN).

(*R*)-*N*-[(*S*)-2-(2-Fluorophenyl)pent-4-en-2-yl]-2-methylpropane-2-sulfinamide (**1-68).** Compound **1-68** was prepared in a manner similar to that for **1-44** in 72% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.22 (s, 9H), 1.81 (s, 3H), 2.82 (dd, *J* = 14.4, 7.5 Hz, 1H), 2.91 (dd, *J* = 14.4, 7.5 Hz, 1H), 3.99 (s, 1H), 5.07–5.11 (m, 1H), 5.12–5.19 (m, 1H), 5.46–5.56 (m, 1H), 7.00–7.07 (m, 1H), 7.09–7.14 (m, 1H), 7.26–7.31 (m, 1H), 7.35–7.41 (m, 1H). MS-ESI (*m/z*): 284 $[M + H]^+$.

(*S*)-2-(2-Fluorophenyl)pent-4-en-2-amine (1-69). Compound **1-69** was prepared in a manner similar to that for **1-53** in 100% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 3H), 2.48 (dd, *J* = 13.5, 7.8 Hz, 1H), 2.74 (dd, *J* = 13.5, 7.8 Hz, 1H), 4.99–5.09 (m, 2H), 5.47–5.61 (m, 1H), 6.98–7.05 (m, 1H), 7.06–7.12 (m, 1H), 7.17–7.22 (m, 1H), 7.39–7.45 (m, 1H). MS-ESI (*m/z*): 180 $[M + H]^+$.

(*S*)-1,1-Bis(2,4-dimethoxybenzyl)-3-[2-(2-fluorophenyl)pent-4-en-2-yl]urea (1-70). Compound **1-70** was prepared in a manner similar to that for **1-54** in 82% yield.^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.76 (s, 3H), 2.52 (dd, *J* = 13.2, 7.6 Hz, 1H), 2.89 (dd, *J* = 13.2, 7.6 Hz, 1H), 3.76 (s, 6H), 3.81 (s, 6H), 4.37-4.46 (m, 4H), 4.88–4.90 (m, 1H), 4.90–4.95 (m, 1H), 5.35 (s, 1H), 5.42–5.53 (m, 1H), 6.43–6.47 (m, 4H), 6.97 (ddd, *J* = 12.8, 8.4, 1.2 Hz, 1H), 7.05 (td, *J* = 7.6, 1.6 Hz, 1H), 7.14–7.20 (m, 3H), 7.27 (td, *J* = 8.4, 1.6 Hz, 1H). MS-ESI (*m/z*): 523 $[M + H]^+$.

(*S*)-1,1-Bis(2,4-dimethoxybenzyl)-3-[2-(2-fluorophenyl)-5-hydroxy-4-oxopentan-2-yl]urea (1-71). To a mixture of **1-70** (2.97 g, 5.68 mmol) in acetone (30 mL) and AcOH (2 M in H_2O; 9.0 mL) was added dropwise a solution of $KMnO_4$ (1.62 g, 10.2 mmol) and AcOH (1 M in H_2O; 15 mL) over 15 min on a water bath, and the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was quenched with 10% aq $NaHSO_3$ solution (10 mL) and then evaporated acetone. The resulting mixture was extracted with EtOAc, and the combined organic layers were washed with 10% aq Na_2CO_3 solution and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 50–80% EtOAc) to give **1-71** (2.13 g, 68% yield) as a while amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.77 (s, 3H), 3.07–3.11 (m, 1H), 3.76–3.81 (m, 1H), 3.77 (s, 6H), 3.81 (s, 6H), 3.90–3.93 (m, 1H), 4.07–4.09 (m, 1H), 4.36–4.48 (m, 4H), 5.87 (s, 1H), 6.43–6.49 (m, 4H), 6.97–7.06 (m, 2H), 7.13–7.21(m, 4H). MS-ESI (*m/z*): 555 $[M + H]^+$.

(*S*)-[2-[Bis(2,4-dimethoxybenzyl)amino]-4-(2-fluorophenyl)-4-methyl-4*H*-1,3-oxazin-6-yl]methyl methanesulfonate (1-72). To a solution of **1-71** (2.13 g, 3.84 mmol) and Et_3N (799 μL, 5.76 mmol) in DCM (20 mL) was added Ms_2O (736 mg, 4.22 mmol) at 0 °C, and the mixture was stirred at the same temperature for 30 min. The reaction mixture was diluted with saturated aq $NaHCO_3$ solution. The aqueous layer was separated and extracted with DCM. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated. The residue was diluted with THF (25 mL). To the mixture was added Burgess reagent (1.83 g, 7.68 mmol), and the reaction mixture was stirred at room temperature for 2 h. The mixture was diluted with H_2O, and the aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brain, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 30–50% EtOAc) to give **1-72** (944 mg, 40% yield) as a while amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.61 (s, 3H), 2.70 (s, 3H), 3.75 (s, 6H), 3.80 (s, 6H), 4.46 (d, *J* = 16.0 Hz, 2H), 4.55 (s, 2H), 4.66 (d, *J* = 16.0 Hz, 2H), 5.82 (d, *J* = 3.2 Hz, 1H), 6.43–6.46 (m, 4H), 6.93–7.00 (m, 2H), 7.13–7.18 (m, 3H), 7.46–7.51 (m, 1H). MS-ESI (*m/z*): 615 $[M + H]^+$.

(*S*)-*N*,*N*-Bis(2,4-dimethoxybenzyl)-4-(2-fluorophenyl)-6-(iodomethyl)-4-methyl-4*H*-1,3-oxazin-2-amine (1-73). To a solution of **1-72** (20.5 g, 33.4 mmol) in acetone (200 mL) was added NaI (10.0 g, 66.7 mmol) at room temperature. After stirring at the same temperature for 1 h, the mixture was filtered through Celite, and the filtrate was evaporated. The residue was diluted with EtOAc and H_2O, and the aqueous layer was separated and then extracted with EtOAc. The combined organic layers were washed with 10% aq $Na_2S_2O_3$ solution and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 20–40% EtOAc) to give **1-73** (20.5 g, 95% yield) as a pale yellow amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 3H), 3.72–3.73 (m, 2H), 3.75 (s, 6H), 3.81 (s, 6H), 4.48 (d, *J* = 16.0 Hz, 2H), 4.67 (d, *J* = 15.6 Hz, 2H), 5.72 (d, *J* = 3.2 Hz, 1H), 6.43–6.47 (m, 4H), 6.93–6.99 (m, 2H), 7.12–7.18 (m, 1H), 7.20 (d, *J* = 8.0 Hz, 2H), 7.51 (td, *J* = 8.4, 2.0 Hz, 1H). MS-ESI (*m/z*): 647 $[M + H]^+$.

(*S*)-*N*,*N*-Bis(2,4-dimethoxybenzyl)-6-(fluoromethyl)-4-(2-fluorophenyl)-4-methyl-4*H*-1,3-oxazin-2-amine (1-74). To a solution of **1-73** (20.5 g, 31.7 mmol) in MeCN (120 mL) was added AgF (10.1 g, 79.6 mmol) at room temperature. After being stirred at the same temperature for 16 h, the mixture was filtered through Celite, and the filtrate was evaporated. The residue was purified by

column chromatography (silica gel; hexane/EtOAc, gradient: 10–30% EtOAc) to give **1-74** (16.8 g, 98% yield) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.60 (s, 3H), 3.76 (s, 6H), 3.81 (s, 6H), 4.46 (d, *J* = 15.6 Hz, 2H), 4.65 (d, *J* = 16.0 Hz, 2H), 4.67 (d, *J* = 47.6 Hz, 2H), 5.76–5.78 (m, 1H), 6.44–6.46 (m, 4H), 6.93–6.99 (m, 2H), 7.12–7.19 (m, 3H), 7.48–7.52 (m, 1H). MS-ESI (*m/z*): 539 $[M + H]^+$.

(*S*)-6-(Fluoromethyl)-4-(2-fluorophenyl)-4-methyl-4*H*-1,3-oxazin-2-amine (1-75). Compound **1-75** was prepared in a manner similar to that for **1-57** in 100% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.65 (s, 3H), 4.20 (br s, 2H), 4.72 (d, *J* = 48.0 Hz, 2H), 5.70 (dd, *J* = 4.8, 2.4 Hz, 2H), 7.01 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.12 (td, *J* = 7.6, 1.2 Hz, 1H), 7.19–7.26 (m, 1H), 7.54 (td, *J* = 8.4, 2.0 Hz, 1H). MS-ESI (*m/z*): 239 $[M + H]^+$.

(*S*)-4-(2-Fluoro-5-nitrophenyl)-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-2-amine (1-76). Compound **1-76** was prepared in a manner similar to that for **1-58** in 94% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.65 (s, 3H), 4.30 (br s, 2H), 4.74 (d, *J* = 47.2 Hz, 2H), 5.69 (dd, *J* = 4.4, 2.8 Hz, 2H), 7.16 (dd, *J* = 10.4, 8.8 Hz, 1H), 8.12–8.16 (m, 1H), 8.53 (dd, *J* = 6.8, 2.8 Hz, 1H). MS-ESI (*m/z*): 284 $[M + H]^+$.

***tert*-Butyl-*N*-[(*tert*-butoxy)carbonyl]-*N*-[(4*S*)-4-(2-fluoro-5-nitrophenyl)-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-2-yl]carbamate (1-77).** Compound **1-77** was prepared in a manner similar to that for **1-59** in 90% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 18H), 4.76 (dd, *J* = 47.2, 3.6 Hz, 2H), 5.63 (dd, *J* = 4.0, 2.8 Hz, 1H), 7.22 (dd, *J* = 10.4, 9.2 Hz, 1H), 8.17–8.21 (m, 1H), 8.52 (dd, *J* = 6.8, 2.8 Hz, 1H). MS-ESI (*m/z*): 484 $[M + H]^+$.

***tert*-Butyl-*N*-[(4*S*)-4-(5-amino-2-fluorophenyl)-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-2-yl]-*N*-[(*tert*-butoxy)carbonyl]carbamate (1-78).** Compound **1-78** was prepared in a manner similar to that for **1-60** in 85% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 18H), 1.69 (s, 3H), 3.54 (s, 2H), 4.74 (dd, *J* = 47.2, 2.0 Hz, 2H), 5.66 (dd, *J* = 4.8, 1.6 Hz, 1H), 6.49–6.53 (m, 1H), 6.80–6.87 (m, 2H). MS-ESI (*m/z*): 454 $[M + H]^+$.

(*S*)-*N*-[3-[2-Amino-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (1-8). Compound **1-8** was prepared in a manner similar to that for **1-7** as a white

solid in 89% yield. ^{1}H-NMR (400MHz, $CDCl_3$) δ 1.66 (s, 3H), 4.26 (s, 2H), 4.74 (d, *J* = 47.7 Hz, 2H), 5.74 (dd, *J* = 5.1, 2.5 Hz, 1H), 7.06 (dd, *J* = 11.2, 9.1 Hz, 1H), 7.69 (dd, *J* = 6.8, 2.8 Hz, 1H), 7.89–7.93 (m, 1H), 8.20 (dd, *J* = 8.1, 2.0 Hz, 1H), 8.42 (d, *J* = 8.1 Hz, 1H), 8.89 (d, *J* = 2.0 Hz, 1H), 9.84 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 31.6, 54.5, 80.4 (d, *J* = 167.7 Hz), 110.7 (d, *J* = 8.0 Hz), 112.4, 115.9, 116.8 (d, *J* = 24.8 Hz), 119.3 (d, *J* = 5.1 Hz), 119.9 (d, *J* = 8.8 Hz), 122.4, 133.1, 135.6 (d, *J* = 13.9 Hz), 141.2, 142.9 (d, *J* =17.5 Hz), 148.2, 150.6, 152.3, 155.5 (d, *J* = 243.5 Hz), 156.5, 160.0. HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{19}H_{16}F_2N_5O_2$, 384.1267; found, 384.1261. $[\alpha]_D^{20}$ + 34.7 (*c* 0.51, MeCN).

(*S*)-*N*-[3-[2-Amino-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyano-3-methylpicolinamide (1-12). Compound **1-12** was prepared in a manner similar to that for **1-8** as a yellow solid in 77% yield. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.70 (s, 3H), 2.85 (s, 3H), 4.77 (d, *J* = 47.7 Hz, 2H), 5.80 (dd, *J* = 4.8, 2.6 Hz, 1H), 7.07 (dd, *J* = 11.3, 8.8 Hz, 1H), 7.71 (dd, *J* = 6.9, 2.7 Hz, 1H), 7.80–7.86 (m, 1H), 7.90–7.92 (m, 1H), 8.60–8.61 (m, 1H), 10.01 (br s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 20.6, 31.7, 54.4, 80.4 (d, *J* = 167.7 Hz), 110.8 (d, *J* = 8.0 Hz), 111.8, 115.9, 116.6 (d, *J* = 24.8 Hz), 119.3 (d, *J* = 4.4 Hz), 119.8 (d, *J* = 8.0 Hz), 133.4, 135.5 (d, *J* = 13.9 Hz), 136.6, 142.8 (d, *J* =17.5 Hz), 144.3, 147.6, 148.1, 149.4, 156.4 (d, *J* = 242.8 Hz), 161.8. HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{20}H_{17}F_2N_5O_2$, 398.1429; found, 398.1433.

(*S*)-*N*-[3-[2-Amino-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-3-chloro-5-cyanopicolinamide (1-13). Compound **1-13** was prepared in a manner similar to that for **1-8** as a yellow solid in 67% yield.^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.65 (s, 3H), 4.73 (d, *J* = 47.7 Hz, 2H), 5.73 (dd, *J* = 5.1, 2.5 Hz, 1H), 7.06 (dd, *J* = 11.4, 8.9 Hz, 1H), 7.59 (dd, *J* = 6.8, 2.8 Hz, 1H), 7.91–7.95 (m, 1H), 8.16 (d, *J* = 2.0 Hz, 1H), 8.76 (d, *J* = 2.0 Hz, 1H), 9.67 (s, 1H). MS-ESI (*m/z*): 418 $[M + H]^+$.

(*S*)-*N*-[3-[2-Amino-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyano-3-methoxypicolinamide (1-14). Compound **1-14** was prepared in a manner similar to that for **1-8** as a yellow solid in 83% yield. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.80 (s, 3H), 4.03 (s, 3H), 4.79 (d, *J* = 47.2 Hz, 2H), 5.79 (dd, *J* = 4.3, 2.3 Hz, 1H), 7.07 (dd, *J* = 11.4, 8.9 Hz, 1H), 7.61 (d, *J* = 6.9, 2.7 Hz, 1H), 7.59–7.64 (m, 2H), 8.46–8.47 (m, 1H), 9.69 (s, 1H). MS-ESI (*m/z*): 414 $[M + H]^+$.

(*S*)-*N*-[3-[2-Amino-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-methoxypyrazine-2-carboxamide (1-15). Compound **1-15** was prepared in a manner similar to that for **1-8** as a white solid in 70% yield. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.65 (s, 3H), 4.07 (s, 3H), 4.73 (d, *J* = 47.7 Hz, 2H), 5.73 (dd, *J* = 4.8, 2.3 Hz, 1H), 7.04 (dd, *J* = 11.4, 8.9 Hz, 1H), 7.65 (dd, *J* = 6.8, 2.8 Hz, 1H), 7.86–7.89 (m, 1H), 8.14 (s, 1H), 9.01 (s, 1H), 9.49 (s, 1H). MS-ESI (*m/z*): 390 $[M + H]^+$.

(*S*)-*N*-[3-[2-Amino-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-(difluoromethyl)pyrazine-2-carboxamide (1-16). Compound **1-16** was prepared in a manner similar to that for **1-8** as a yellow amorphous in 76% yield.^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.67 (s, 3H), 4.74 (d, *J* = 47.6 Hz, 2H), 5.75 (dd, *J* = 4.9, 2.6 Hz, 1H), 6.79 (t, *J* = 54.4 Hz, 1H), 7.07 (dd, *J* = 11.3, 8.8 Hz, 1H), 7.70 (dd, *J* = 6.9, 2.9 Hz, 1H), 7.86–7.92 (m, 1H), 8.91 (s, 1H), 9.51 (s, 1H), 9.62 (br s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 31.7, 54.4, 80.4 (d, *J* = 167.7 Hz), 110.6 (d, *J* = 8.7 Hz), 112.9 (t, *J* = 240.6 Hz), 116.8 (d, *J* = 24.8 Hz), 119.4 (d, *J* = 5.1 Hz), 120.0 (d, *J* = 8.7 Hz), 133.0, 135.6 (d, *J* = 13.8 Hz), 139.7, 143.0 (d, *J* =16.1 Hz), 143.7, 145.7, 147.9, 150.4, 156.6 (d, *J* = 243.5 Hz), 159.6. HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{18}H_{15}F_4N_5O_2$, 410.1240; found, 410.1252.

(*S*)-*N*-[3-[2-Amino-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-(fluoromethoxy)pyrazine-2-carboxamide (1-17). Compound **1-17** was prepared in a manner similar to that for **1-8** as a white solid in 59% yield. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.66 (s, 3H), 4.74 (d, *J* = 47.7 Hz, 2H), 5.74 (dd, *J* = 4.8, 2.5 Hz, 1H), 6.15 (d, *J* = 51.0 Hz, 2H), 7.05 (dd, *J* = 11.4, 8.8 Hz, 1H), 7.67 (dd, *J* = 6.9, 2.8 Hz, 1H), 7.85–7.91 (m, 1H), 8.26–8.27 (m, 1H), 9.06 (s, 1H), 9.48 (br s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 31.6, 54.5, 80.4 (d, *J* = 167.7 Hz), 95.8 (d, *J* = 222.4 Hz), 110.7 (d, *J* = 8.0 Hz), 116.7 (d, *J* = 24.8 Hz), 119.2 (d, *J* = 4.4 Hz), 119.9 (d, *J* = 8.8 Hz), 133.1, 133.4, 135.5 (d, *J* = 13.9 Hz), 139.8, 141.8, 143.0 (d, *J* =16.0 Hz), 147.9, 156.4 (d, *J* = 242.8 Hz), 159.3, 160.4. HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{18}H_{16}F_3N_5O_3$, 408.1283; found, 408.1283.

(*S*)-3-Amino-*N*-[3-[2-amino-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-(fluoromethoxy)pyrazine-2-carboxamide (1-18). Compound **1-18** was prepared in a manner similar to that for **1-8** as a colorless amorphous in 95% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.65 (s, 3H), 4.73 (d, *J* = 47.7 Hz, 2H), 5.74 (dd, *J* = 4.6, 2.4 Hz, 1H), 6.02 (d, *J* = 51.4 Hz, 2H), 7.01 (dd, *J* = 11.3, 8.8 Hz, 1H), 7.52 (s, 1H), 7.63–7.64 (m, 1H), 7.74–7.75 (m, 1H), 9.50 (s, 1H).

(*S*)-*N*-[3-[2-Amino-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-2-(difluoromethyl)oxazole-4-carboxamide (1-19). Compound **1-19** was prepared in a manner similar to that for **1-8** as a colorless amorphous in 64% yield. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.65 (s, 3H), 4.74 (d, *J* = 47.6 Hz, 2H), 5.73 (dd, *J* = 4.7, 2.5 Hz, 1H), 6.69 (t, *J* = 52.4 Hz, 1H), 7.04 (dd, *J* = 11.2, 8.8 Hz, 1H), 7.62 (dd, *J* = 6.8, 2.8 Hz, 1H), 7.76–7.82 (m, 1H), 8.38 (s, 1H), 8.61 (br s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 31.6, 54.4, 80.4 (d, *J* = 167.7 Hz), 106.1 (t, *J* = 239.1 Hz), 110.7 (d, *J* = 8.0 Hz), 116.7 (d, *J* = 24.8 Hz), 119.4 (d, *J* = 4.4 Hz), 120.1 (d, *J* = 8.0 Hz), 133.0, 135.7 (d, *J* = 13.9 Hz), 136.9, 143.0, 143.3 (d, *J* =16.0 Hz), 147.9, 154.4, 156.5 (d, *J* = 243.5 Hz), 157.1. HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{17}H_{14}F_4N_4O_3$, 399.1080; found, 399.1078.

(*S*)-*N*-[3-[2-Amino-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-2-(fluoromethyl)oxazole-4-carboxamide (1-20). Compound **1-20** was prepared in a manner similar to that for **1-8** as a colorless amorphous in 61% yield. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.73 (s, 3H), 4.76 (d, *J* = 47.4 Hz, 2H), 5.42 (d, *J* = 47.0 Hz, 2H), 5.76 (dd, *J* = 4.4, 2.4 Hz, 1H), 7.04 (dd, *J* = 11.3, 8.8 Hz, 1H), 7.64 (dd, *J* = 6.9, 2.7 Hz, 1H), 7.76–7.80 (m, 1H), 8.31–8.32 (m, 1H), 8.70 (br s, 1H). MS-ESI (*m/z*): 381 $[M + H]^+$.

(*S*,*E*)-*N*-[Cyclopropyl(2-fluorophenyl)methylene]-2-methylpropane-2-sulfinamide (1-80). A solution of **1-79** (13.8 g, 84 mmol), (*S*)-2-methylpropane-2-sulfinamide (15.3 g, 126 mmol), and $Ti(OEt)_4$ (38.4 g, 168 mmol) in toluene (70 mL) was stirred at 80 °C for 20 h. The reaction mixture was allowed to cool to room temperature and diluted with MeCN (280 mL) then H_2O (9.1 mL). The mixture was stirred for 30 min and filtered through Celite. The filtrate was evaporated, and the resulting residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 20−40% EtOAc) to give **1-80** (14 g, 63%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07−1.28 (m, 13H), 1.97 (br s, 1H), 7.10 (t, *J* = 6.9 Hz, 1H), 7.19 (t, *J* = 7.3 Hz, 1H), 7.25−7.31 (m, 1H), 7.40 (dd, *J* = 10.5, 5.7 Hz, 1H). MS-ESI (*m/z*): 268 $[M + H]^+$.

(*S*)-*N*-[(*R*)-1-Cyclopropyl-1-(2-fluorophenyl)but-3-en-1-yl]-2-methylpropane-2-sulfinamide (1-81). Compound **1-81** was prepared in a manner similar to that for **1-68** in 45% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.35−0.41 (m, 1H), 0.51−0.55 (m, 1H), 0.58−0.66 (m, 2H), 1.26 (s, 9H), 1.36 (m, 1H), 2.59 (dd, *J* = 13.7, 8.3 Hz, 1H), 3.08 (dd, *J* = 13.7, 6.0 Hz, 1H), 3.99 (s, 1H), 5.06 (d, *J* = 10.4 Hz, 1H),

5.14 (d, *J* = 16.8 Hz, 1H), 5.53 (m, 1H), 7.01 (dd, *J* = 12.7, 8.1 Hz, 1H), 7.15 (t, *J* = 7.6 Hz, 1H), 7.26 (m, 1H), 7.81 (t, *J* = 8.2 Hz, 1H). MS-ESI (*m/z*): 310 $[M + H]^+$.

(*R*)-1-Cyclopropyl-1-(2-fluorophenyl)but-3-en-1-amine (1-82). Compound **1-82** was prepared in a manner similar to that for **1-53** in 100% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.21−0.26 (m, 1H), 0.36−0.43 (m, 1H), 0.43−0.51 (m, 2H), 1.40−1.46 (m, 1H), 2.56 (dd, *J* = 13.8, 7.8 Hz, 1H), 2.87 (dd, *J* = 13.7, 7.2 Hz, 1H), 5.00 (d, *J* = 10.0 Hz, 1H), 5.07 (d, *J* = 17.1 Hz, 1H), 5.56−5.66 (m, 1H), 7.02 (dd, *J* = 12.8, 8.0 Hz, 1H), 7.10 (t, *J* = 7.5 Hz, 1H), 7.20−7.23 (m, 1H), 7.51 (t, *J* = 8.0 Hz, 1H). MS-ESI (*m/z*): 206 $[M + H]^+$.

(*R*)-3-[1-Cyclopropyl-1-(2-fluorophenyl)but-3-en-1-yl]-1,1-bis(2,4-dimethoxybenzyl)urea (1-83). Compound **1-83** was prepared in a manner similar to that for **1-54** in 100% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.15−0.25 (m, 2H), 0.38−0.46 (m, 2H), 1.46−1.55 (m, 1H), 2.65−2.71 (m, 1H), 3.11 (dd, *J* = 13.4, 6.7 Hz, 1H), 3.76 (s, 6H), 3.81 (s, 6H), 4.35−4.48 (m, 4H), 4.94 (d, *J* = 10.4 Hz, 1H), 4.98 (d, *J* = 17.6 Hz, 1H), 5.23 (s, 1H), 5.60−5.72 (m, 1H), 6.43−6.48 (m, 4H), 6.95 (dd, *J* = 12.7, 8.2 Hz, 1H), 7.03 (t, *J* = 7.6 Hz, 1H), 7.14−7.20 (m, 3H), 7.33 (t, *J* = 8.0 Hz, 1H). MS-ESI (*m/z*): 549 $[M + H]^+$.

(*R*)-3-[1-Cyclopropyl-1-(2-fluorophenyl)-4-hydroxy-3-oxobutyl]-1,1-bis(2,4-dimethoxybenzyl)urea (1-84). To a mixture of **1-83** (12.8 g, 23 mmol) and NaOAc (1.41 g, 17.3 mmol) in acetone/H_2O/AcOH (128 mL/109 mL/8.1 mL) was added $KMnO_4$ (6.65 g, 42 mmol), and the mixture was stirred at room temperature for 1 h. The reaction was quenched with $NaHSO_3$ (6.08 g, 57 mmol), and stirring was continued for 10 min. The insoluble materials were filtered off. The filtrate was diluted with EtOAc and saturated aq $NaHCO_3$ solution. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 20−50% EtOAc) to give **1-84** (9.20 g, 68%) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.12−0.00 (m, 2H), 0.28−0.34 (m, 1H), 0.41−0.45 (m, 1H), 1.86−1.93 (m, 1H), 3.18−3.24 (m, 1H), 3.30−3.35 (m, 1H), 3.79 (s, 6H), 3.81 (s, 6H), 4.05−4.22 (m, 3H), 4.38 (d, *J* = 16.2 Hz, 2H), 4.48 (d, *J* = 16.2 Hz, 2H), 5.66 (s, 1H), 6.45−6.50 (m, 4H), 6.92−7.05 (m, 3H), 7.11−7.15 (m, 2H), 7.16−7.22 (m, 1H). MS-ESI (*m/z*): 581 $[M + H]^+$.

(*R*)-4-[3,3-Bis(2,4-dimethoxybenzyl)ureido]-4-cyclopropyl-4-(2-fluorophenyl)-2-oxobutyl methanesulfonate (1-85). To a solution of **1-84** (5.50 g, 9.47 mmol) and Et_3N (2.63 mL, 18.9 mmol) in DCM (55 mL) was added Ms_2O (2.48 g, 14.2 mmol) at 0 °C. After being stirred at this temperature for 1 h, the reaction mixture was diluted with H_2O. The aqueous layer was separated and then extracted with DCM. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated to afford **1-85** (6.17 g, 99% yield) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ -0.15−-0.11 (m, 1H), -0.04−0.02 (m, 1H), 0.28−0.34 (m, 1H), 0.40−0.46 (m, 1H), 1.81−1.87 (m, 1H), 3.05 (s, 3H), 3.33 (dd, *J* = 15.2, 2.3 Hz, 1H), 3.80 (s, 6H), 3.82 (s, 6H), 4.00 (d, *J* = 15.2 Hz, 1H), 4.40 (d, *J* = 16.2 Hz, 2H), 4.48 (d, *J* = 15.2 Hz, 2H), 4.74 (d, *J* = 17.4 Hz, 1H), 4.86 (d, *J* = 17.4 Hz, 1H), 5.69 (s, 1H), 6.46−6.50 (m, 4H), 6.93−7.00 (m, 3H), 7.14−7.21 (m, 3H). MS-ESI (*m/z*): 659 $[M + H]^+$.

(*S*)-[2-[Bis(2,4-dimethoxybenzyl)amino]-4-cyclopropyl-4-(2-fluorophenyl)-4*H*-1,3-oxazin-6-yl]methyl methanesulfonate (1-86). Compound **1-86** was prepared in a manner similar to that for **1-56** in 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.16−0.21 (m, 1H), 0.28−0.34 (m, 2H), 0.52−0.61 (m, 1H), 1.48−1.55 (m, 1H), 2.70 (s, 3H), 3.77 (s, 6H), 3.81 (s, 6H), 4.40−4.44 (m, 2H), 4.53−4.63 (m, 4H), 5.84 (d, *J* = 3.0 Hz, 1H), 6.41−6.47 (m, 4H), 6.92−7.02 (m, 2H), 7.08−7.12 (m, 2H), 7.12−7.20 (m, 1H), 7.34 (t, *J* = 8.0 Hz, 1H). MS-ESI (*m/z*): 641 $[M + H]^+$.

(*S*)-4-Cyclopropyl-*N*,*N*-bis(2,4-dimethoxybenzyl)-4-(2-fluorophenyl)-6-(iodomethyl)-4*H*-1,3-oxazin-2-amine (1-87). To a solution of **1-86** (6.0 g, 9.4 mmol) in acetone (98 mL) was added NaI (2.8 g, 18.8 mmol) at room temperature. After being stirred for 20 h, the reaction mixture was filtered through Celite. The filtrate was evaporated and then diluted with EtOAc and saturated aq $NaHCO_3$ solution. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0−30% EtOAc) to give **1-87** (4.8 g, 76% yield) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.08−0.16 (m, 1H), 0.28−0.40 (m, 2H), 0.48−0.56 (m, 1H), 1.45−1.52 (m, 1H), 3.70−3.78 (m, 2H), 3.75 (s, 6H), 3.81 (s, 6H), 4.47 (d, *J* = 15.8 Hz, 2H), 4.60 (d, *J* = 15.8 Hz, 2H), 5.72 (d, *J* = 3.0 Hz, 1H), 6.42-6.47 (m, 4H), 6.92−6.99 (m, 2H), 7.12−7.17 (m, 3H), 7.39 (t, *J* = 8.0 Hz, 1H). MS-ESI (*m/z*): 673 $[M + H]^+$.

(*S*)-4-Cyclopropyl-*N*,*N*-bis(2,4-dimethoxybenzyl)-6-(fluoromethyl)-4-(2-fluorophenyl)-4*H*-1,3-oxazin-2-amine (1-88). To a solution of **1-87** (4.8 g, 7.11 mmol) in MeCN (48 mL) was added AgF (5.4 g, 42.6 mmol) at room temperature. After being stirred for 5 h, the reaction mixture was filtered through Celite. The filtrate was evaporated, and the residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0−30% EtOAc) to give **1-88** (3.83 g, 95%) as a white amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.10−0.18 (m, 1H), 0.28−0.37 (m, 2H), 0.50−0.56 (m, 1H), 1.48−1.54 (m, 1H), 3.75 (s, 6H), 3.81 (s, 6H), 4.42 (d, *J* = 15.9 Hz, 2H), 4.59 (d, *J* = 15.9 Hz, 2H), 4.68 (d, *J* = 47.6 Hz, 2H), 5.78 (t, *J* = 3.8 Hz, 1H), 6.42−6.46 (m, 4H), 6.91-7.00(m, 2H), 7.11−7.17 (m, 3H), 7.35 (t, *J* = 7.9 Hz, 1H). MS-ESI (*m/z*): 565 $[M + H]^+$.

***tert*-Butyl-*N*-[(4*S*)-4-(5-amino-2-fluorophenyl)-4-cyclopropyl-6-(fluoromethyl)-4*H*-1,3-oxazin-2-yl]-*N*-[(*tert*-butoxy)carbonyl]carbamate (1-89).** Compound **1-89** was prepared in a manner similar to that for **1-60** in 75% yield over 4 steps from **1-88**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.35−0.40 (m, 1H), 0.44−0.49 (m, 1H), 0.53−0.60 (m, 2H), 1.50 (s, 18H), 1.48−1.56 (m, 1H), 3.53 (br s, 2H), 4.75 (d, *J* = 47.6 Hz, 2H), 5.59−5.62 (m, 1H), 6.50−6.53 (m, 1H), 6.80−6.87 (m, 2H). MS-ESI (*m/z*): 465 $[M + H]^+$.

(*S*)-*N*-[3-[2-Amino-4-cyclopropyl-6-(fluoromethyl)-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (1-9). Compound **1-9** was prepared in a manner similar to that for **1-5** in 81% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.33−0.55 (m, 4H), 1.47−1.55 (m, 1H), 4.75 (d, *J* = 44.0 Hz, 2H), 5.61−5.65 (m, 1H), 7.07 (t, *J* = 9.6 Hz, 1H), 7.76−7.79 (m, 1H), 7.84−7.88 (m, 1H), 8.19 (d, *J* = 8.0 Hz, 1H), 8.42 (d, *J* = 8.0 Hz, 1H), 8.89 (s, 1H), 9.83 (s, 1H). MS-ESI (*m/z*): 410 $[M + H]^+$.

(*S*)-*N*-[3-(2-Aminopent-4-en-2-yl)-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-90). Compound **1-90** was prepared in a manner similar to that for **1-53** in 100% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 3H), 1.75−1.89 (br s, 2H), 2.47 (dd, *J* = 13.1, 8.1 Hz, 1H), 2.76 (dd, *J* = 13.1, 7.1 Hz, 1H), 5.03−5.11 (m, 2H), 5.46−5.58 (m, 1H), 7.01−7.08 (m, 1H), 7.52−7.60 (m, 2H), 8.25−8.36 (br s, 1H). MS-ESI (*m/z*): 291 $[M + H]^+$.

(*S*)-*N*-[3-[2-[3,3-Bis(2,4-dimethoxybenzyl)ureido]pent-4-en-2-yl]-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-91). Compound **1-91** was prepared in a manner similar to that for **1-54** in 100% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68 (s, 3H), 2.47 (dd, *J* = 13.4, 7.4 Hz, 1H), 2.87 (dd, *J* = 13.4,

7.4 Hz, 1H), 3.78 (s, 6H), 3.81 (s, 6H), 4.40 (s, 4H), 4.90−4.94 (m, 1H), 4.95−4.99 (m, 1H), 5.48 (s, 1H), 5.48−5.56 (m, 1H), 6.44−6.48 (m, 4H), 6.85 (dd, *J* = 11.8, 8.7 Hz, 1H), 7.16 (d, *J* = 8.1 Hz, 2H), 7.39 (dd, *J* = 7.3, 2.4 Hz, 1H), 7.52−7.56 (m, 1H), 8.76 (s, 1H). MS-ESI (*m/z*): 634 $[M + H]^+$.

(*S*)-4-[3,3-Bis(2,4-dimethoxybenzyl)ureido]-4-[2-fluoro-5-(2,2,2-trifluoroacetamido)phenyl]-2-oxopentyl acetate (1-92). A mixture of **1-91** (10.0 g, 15.9 mmol), $K_2OsO_4 \cdot 2H_2O$ (116 mg, 0.316 mmol), and NMO (4.44 g, 37.9 mmol) in acetone (50 mL) and H_2O (10 mL) was stirred at room temperature overnight. The reaction mixture was diluted with 10% aq Na_2CO_3 solution and EtOAc. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was dissolved in DCM (100 mL), and then pyridine (1.66 mL, 20.5 mmol) and DMAP (193 mg, 1.58 mmol) was added. To the mixture was added Ac_2O (1.57 mL, 16.57 mmol) at 0 °C. After being stirred at this temperature for 1 h, the reaction mixture was diluted with saturated aq $NaHCO_3$ solution and EtOAc. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was dissolved in DCM (160 mL), and to the solution was added Dess-Martin periodinane (7.37 g, 17.4 mmol) at room temperature, and the reaction mixture was stirred at this temperature for 1 h. The mixture was diluted with EtOAc and saturated aq $NaHCO_3$ solution. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 20−50% EtOAc) to give **1-92** (11.1 g, 99% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.75 (s, 3H), 2.14 (s, 3H), 3.21 (d, *J* = 15.9 Hz, 1H), 3.65 (d, *J* = 15.9 Hz, 1H), 3.78 (s, 6H), 3.80 (s, 6H), 4.38−4.44 (m, 2H), 4.45 (d, *J* = 17.0 Hz, 1H), 4.48−4.52 (m, 2H), 4.64 (d, *J* = 17.0 Hz, 1H), 5.92 (s, 1H), 6.43−6.48 (m, 4H), 6.97 (dd, *J* = 11.7, 8.7 Hz, 1H), 7.03 (dd, *J* = 7.2, 2.7 Hz, 1H), 7.13 (d, *J* = 9.1 Hz, 2H), 7.62−7.66 (m, 1H), 8.23 (s, 1H). MS-ESI (*m/z*): 708 $[M + H]^+$.

(*S*)-[2-[Bis(2,4-dimethoxybenzyl)amino]-4-[2-fluoro-5-(2,2,2-trifluoroacetamido)phenyl]-4-methyl-4*H*-1,3-oxazin-6-yl]methyl acetate (1-93). Compound **1-93** was prepared in a manner similar to that for **1-56** in 100% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58 (s, 3H), 1.92 (s, 3H), 3.76−3.79 (m. 12H), 4.31 (d, *J* = 15.2 Hz. 2H), 4.44 (d, *J* = 13.1 Hz, 1H), 4.53 (d, *J* = 13.1 Hz, 1H), 4.77 (d, *J* = 15.2

Hz, 2H), 5.68 (s, 1H), 6.44−6.46 (m, 4H), 6.97−7.02 (m, 1H), 7.14−7.18 (m, 3H), 7.48 (s, 1H), 7.82−7.84 (m, 1H). MS-ESI (*m/z*): 690 $[M + H]^+$.

(*S*)-*N*-[3-[2-[Bis(2,4-dimethoxybenzyl)amino]-6-(hydroxymethyl)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-94). To a solution of **1-93** (1.27 g, 1.84 mmol) in MeOH (13 mL) was added NaOMe (1.02 M in MeOH; 1.80 mL, 1.84 mmol). The mixture was stirred at room temperature for 1.5 h. The mixture was diluted with EtOAc and H_2O. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated to give **1-94** (1.05 g, 88% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58 (s, 3H), 3.77−3.79 (m, 13H), 3.99 (s, 2H), 4.33 (d, *J* = 16.2 Hz, 2H), 4.80 (d, *J* = 15.7 Hz, 2H), 5.60 (s, 1H), 6.44−6.47 (m, 4H), 6.98 (t, *J* = 9.6 Hz, 1H), 7.16−7.17 (m, 3H), 7.50 (s, 1H), 7.80−7.85 (m, 1H). MS-ESI (*m/z*): 648 $[M + H]^+$.

(*S*)-[2-[Bis(2,4-dimethoxybenzyl)amino]-4-[2-fluoro-5-(2,2,2-trifluoroacetamido)phenyl]-4-methyl-4*H*-1,3-oxazin-6-yl]methyl methanesulfonate (1-95). To a solution of **1-94** (200 mg, 0.309 mmol) and Et_3N (171 μL, 1.24 mmol) in DCM (3.0 mL) was added Ms_2O (134 mg, 722 mmol) at 0 °C, and the reaction mixture was allowed to warm to room temperature and stirred for 30 min. The mixture was diluted with saturated aq $NaHCO_3$ solution and DCM. The aqueous layer was separated and extracted with DCM. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 25−50% EtOAc) to give **1-95** (203 mg, 91% yield) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57 (s, 3H), 2.75 (s, 3H), 3.73 (s, 6H), 3.79 (s, 6H), 4.35 (d, *J* = 16.4 Hz, 2H), 4.57 (s, 2H), 4.48 (d, *J* = 16.0 Hz, 2H), 5.80−5.84 (m, 1H), 6.40−6.50 (m, 4H), 6.96−7.03 (m, 1H), 7.11−7.20 (m, 3H), 7.47−7.52 (m, 1H), 7.87−7.83 (m, 1H). MS-ESI (*m/z*): 726 $[M + H]^+$.

(*S*)-4-(5-Amino-2-fluorophenyl)-*N*,*N*-bis(2,4-dimethoxybenzyl)-6-(methoxymethyl)-4-methyl-4*H*-1,3-oxazin-2-amine (1-96). To a solution of **1-95** (263 mg, 0.363 mmol) in MeOH (2.6 mL) was added NaOMe (1.02 M in MeOH; 1.78 mL, 1.82 mmol) at room temperature, and the reaction mixture was stirred at 40 °C for 4.5 h. The mixture was allowed to cool to room temperature and diluted with THF (2.6 mL) and H_2O (2.6 mL). To this mixture was added K_2CO_3 (100 mg, 0.726 mmol), and the mixture was stirred at 50 °C for 6 h. After being cooled to room temperature, it was diluted with H_2O

and EtOAc. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 25−60% EtOAc) to give **1-96** (172 mg, 84% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 3H), 3.21 (br s, 2H), 3.26 (s, 3H), 3.75 (s, 6H), 3.78−3.82 (m, 2H), 3.80 (s, 6H), 4.33 (d, *J* = 21.6 Hz, 2H), 4.79 (d, *J* = 21.6 Hz, 2H), 5.59 (d, *J* = 4.4 Hz, 1H), 6.34 (dt, *J* = 10.8, 4.8 Hz, 1H), 6.42−6.45 (m, 3H), 6.46−6.48 (m, 1H), 6.58 (dd, *J* = 9.2, 4.0 Hz, 1H), 6.70 (dd, *J* = 15.2, 11.2 Hz, 1H), 7.19 (d, *J* = 10.4 Hz, 2H). MS-ESI (*m/z*): 566 $[M + H]^+$.

(*S*)-*N*-[3-[2-Amino-6-(methoxymethyl)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (1-10). Compound **1-10** was prepared in a manner similar to that for **1-6** in 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (s, 3H), 3.35 (s, 3H), 3.87 (s, 2H), 4.24 (s, 2H), 5.59 (d, *J* = 2.5 Hz,1H), 7.05 (dd, *J* = 11.4, 8.9 Hz, 1H), 7.67 (dd, *J* = 6.8, 2.8 Hz, 1H), 7.90−7.94 (m, 1H), 8.20 (dd, *J* = 8.1, 2.0 Hz, 1H), 8.42 (d, *J* = 8.1 Hz, 1H), 8.89 (d, *J* = 2.0 Hz,1H), 9.84 (s, 1H). MS-ESI (*m/z*): 396 $[M + H]^+$.

(*S*)-*N*-[3-[2-[Bis(2,4-dimethoxybenzyl)amino]-6-formyl-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-97). To a solution of **1-94** (296 mg, 0.46 mmol) in DCM (4.5 mL) was added Dess-Martin periodinane (252 mg, 0.59 mmol) at room temperature. After being stirred for 2 h, the reaction mixture was diluted with saturated aq $NaHCO_3$, 10% aq $Na_2S_2O_3$ solution, and EtOAc. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0−30% EtOAc) to give **1-97** (288 mg, 98% yield) as a white amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (s, 3H), 3.76 (s, 6H), 3.78 (s, 6H), 4.41 (d, *J* = 16.2 Hz, 2H), 4.82 (d, *J* = 16.2 Hz, 2H), 6.41−6.46 (m, 4H), 6.59 (s, 1H), 7.03 (t, *J* = 9.9 Hz, 1H), 7.15−7.20 (m, 3H), 7.45 (s, 1H), 7.88 (d, *J* = 6.6 Hz, 1H), 9.20 (s, 1H). MS-ESI (*m/z*): 646 $[M + H]^+$.

(*S*)-*N*-[3-[2-[Bis(2,4-dimethoxybenzyl)amino]-6-(difluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-2,2,2-trifluoroacetamide (1-98). To a solution of **1-97** (269 mg, 0.417 mmol) in DCM (4.0 mL) was added DAST (0.137 mL, 1.04 mmol) at 0 °C. The mixture was allowed to warm

to room temperature and stirred for 2.5 h. The reaction mixture was poured into ice-cooled aq $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0−20% EtOAc) to give **1-98** (82.0 mg, 30% yield) as a white amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (s, 3H), 3.76 (s, 6H), 3.79 (s, 6H), 4.35 (d, *J* = 16.7 Hz, 2H), 4.76 (d, *J* = 16.7 Hz, 2H), 5.79−6.06 (m, 2H), 6.43−6.46 (m, 4H), 7.01 (t, *J* = 10.4 Hz, 1H), 7.13−7.15 (m, 3H), 7.45 (s, 1H), 7.84 (s, 1H). MS-ESI (*m/z*): 668 $[M + H]^+$.

(*S*)-4-(5-Amino-2-fluorophenyl)-6-(difluoromethyl)-*N*,*N*-bis(2,4-dimethoxybenzyl)-4-methyl-4*H*-1,3-oxazin-2-amine (1-99). Compound **1-99** was prepared in a manner similar to that for **1-49** in 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (s, 3H), 3.22 (s, 2H), 3.76 (s, 6H), 3.80 (s, 6H), 4.34 (d, *J* = 16.0 Hz, 2H), 4.77 (d, *J* = 16.0 Hz, 2H), 5.89 (t, *J* = 54.0 Hz, 1H), 5.95−5.97 (m, 1H), 6.38 (dt, *J* = 8.4, 3.4 Hz, 1H), 6.44−6.47 (m, 4H), 6.52 (dd, *J* = 6.8, 3.0 Hz, 1H), 6.72 (dd, *J* = 11.4, 8.5 Hz, 1H), 7.15 (s, 1H), 7.18 (s, 1H). MS-ESI (*m/z*): 572 $[M + H]^+$.

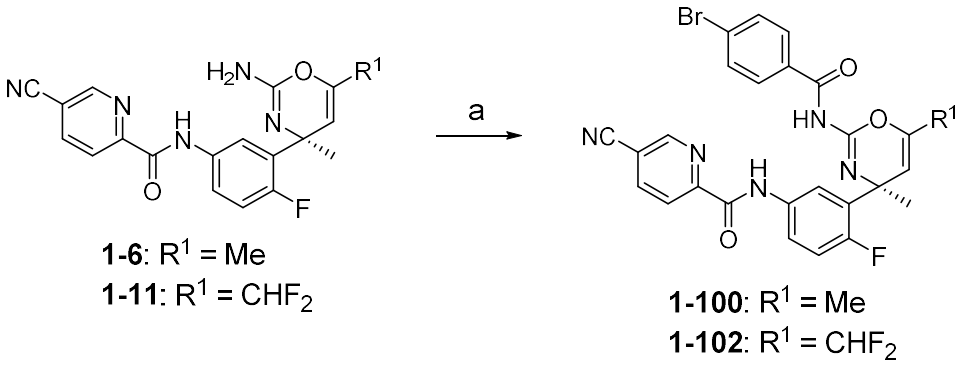
(*S*)-*N*-[3-[2-Amino-6-(difluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (1-11). Compound **1-11** was prepared in a manner similar to that for **1-6** in 53% yield (2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68 (s, 3H), 4.34 (s, 2H), 5.95−5.96 (m, 1H), 5.96 (t, *J* = 53.8 Hz, 1H), 7.08 (dd, *J* = 11.2, 8.6 Hz, 1H), 7.73 (dd, *J* = 6.8, 2.8 Hz, 1H), 7.88−7.92 (m, 1H), 8.20 (dd, *J* = 8.1, 2.0 Hz, 1H), 8.42 (d, *J* = 8.1 Hz, 1H), 8.88−8.89 (m, 1H), 9.85 (s, 1H). MS-ESI (*m/z*): 402 $[M + H]^+$.

Determination of the Stereochemistry for Compounds 1-6, 1-7, 1-9 and 1-11.

The stereochemistry of compounds **1-9** was confirmed by single X-ray structure analysis, while the other compounds **1-6**, **1-7**, and **1-11** required structural conversions to the corresponding amide derivatives, such as **1-100**, **1-101**, and **1-102**, to obtain high quality crystalline materials and acceptable X-ray diffraction patterns (Schemes S1 and S2). Data were collected on a Rigaku XtaLAB P200 system using thin-layer mirror monochromated Cu-K radiation (λ = 1.54187 Å) at 100 K. The ORTEP figures, detailed crystal data, and structure refinement are given in Figures S1−4 and Tables S1−S4. Coordinates, refinement details, and structure factors were deposited with the Cambridge

Crystallographic Data Centre (**1-9**: CCDC 1583140; **1-100**: CCDC 1583135; **1-101**: CCDC 1583100; **1-102**: CCDC 1583036)

Scheme S1. Synthesis of 4-Bromobenzamides 1-100 and 1-102[a]



1-6: R^1 = Me
1-11: R^1 = CHF_2

1-100: R^1 = Me
1-102: R^1 = CHF_2

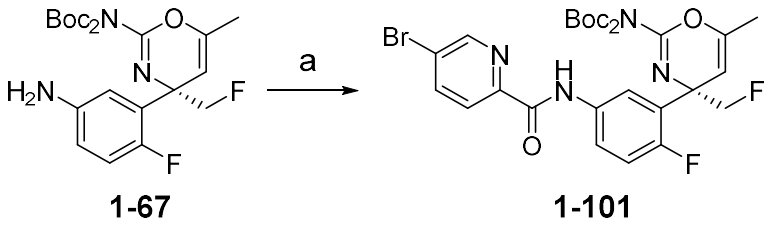
[a]Reagents and conditions: (a) 4-bromobenzoyl chloride, DCM, 0 °C, 55−67%.

(*S*)-*N*-[3-[2-(4-Bromobenzamido)-4,6-dimethyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (1-100). To a solution of **1-6** (15.0 mg, 0.041 mmol) and Et_3N (17.1 μL, 0.123 mmol) in DCM (0.3 mL) was 4-bromobenzoyl chloride (13.5 mg, 0.062 mmol) at 0 °C. After being stirred for 2 h, the reaction mixture was diluted with saturated aq $NaHCO_3$ and EtOAc. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0−30% EtOAc) and recrystallized from MeOH-H_2O to give **1-100** (15.0 mg, 67% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.89 (s, 3H), 2.05 (s, 3H), 5.34 (s, 1H), 7.13 (dd, *J* = 10.8, 8.6 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.69−7.72 (m, 1H), 7.76−7.80 (m, 1H), 8.11 (d, *J* = 8.4 Hz, 2H), 8.20 (dd, *J* = 8.0, 1.6 Hz, 1H), 8.39 (d, *J* = 8.0 Hz, 1H), 8.87 (d, *J* = 1.2 Hz, 1H), 9.85 (s, 1H), 11.75 (s, 1H). MS-ESI (*m/z*): 548 $[M + H]^+$.

(*S*)-*N*-[3-[2-(4-Bromobenzamido)-6-(difluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (1-102). Compound **1-102** was prepared in a manner similar to that for **1-100** in 55% yield and recrystallized from MeOH-H_2O. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (s, 3H), 6.03 (s, 1H), 6.19 (t, *J* = 54.0 Hz, 1H), 7.18 (dd, *J* = 10.8, 8.8 Hz, 1H), 7.57 (d, *J* = 8.4 Hz,

2H), 7.76−7.80 (m, 2H), 8.11 (d, J = 8.0 Hz, 2H), 8.21 (d, J = 8.4 Hz, 1H), 8.38 (d, J = 8.0 Hz, 1H), 8.88 (s, 1H), 9.87 (s, 1H), 11.69 (s, 1H). MS-ESI (*m/z*): 584 $[M + H]^+$.

Scheme S2. Synthesis of Boc-4-Bromobenzamide 1-101[a]



1-67 **1-101**

[a]Reagents and conditions: (a) HATU, DIEA, 5-bromopicolinic acid, DMF, rt, 54%.

***tert*-Butyl-*N*-[(4*S*)-4-[5-(5-bromopyridine-2-amido)-2-fluorophenyl]-4-(fluoromethyl)-6-methyl-4*H*-1,3-oxazin-2-yl]-*N*-[(*tert*-butoxy)carbonyl]carbamate (1-101).** Compound **1-101** was prepared in a manner similar to that for **1-61** in 54% yield and recrystallized from MeOH-H_2O. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (s, 3H), 1.90 (s, 3H), 4.42 (dd, J = 48.0, 8.8 Hz, 1H), 4.70 (dd, J = 47.2, 8.8 Hz, 1H), 5.21 (s, 1H), 7.07−7.12 (m, 1H), 7.52−7.55 (m, 1H), 8.04 (dd, J = 8.0, 1.6 Hz, 1H), 8.17 (d, J = 8.0 Hz, 1H), 8.22−8.26 (m, 1H), 8.60 (d, J = 1.6 Hz, 1H), 9.92 (s, 1H). MS-ESI (*m/z*): 637 $[M + H]^+$.

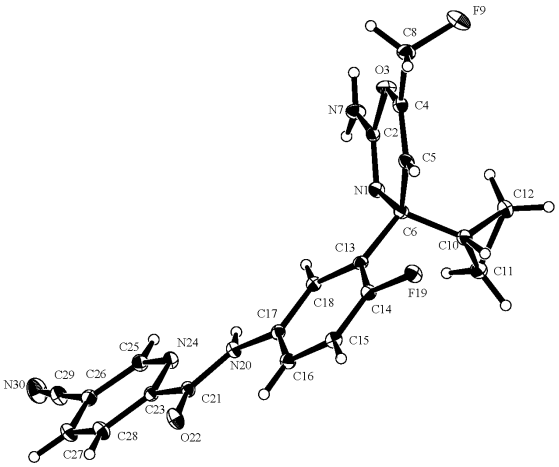


Figure S1. Single X-ray structure of compound **1-9**. An ORTEP figure with thermal ellipsoid set at the 30% probability level.

Table S1. Crystal Data and Structure Refinement for Compound 1-9

Empirical Formula	$C_{21}\ H_{17}\ F_2\ N_5\ O_2$
Formula Weight	409.39
Crystal Color, Habit	colorless, platelet
Crystal Dimensions	0.16 x 0.10 x 0.03 mm^3
Crystal System	Orthorhombic
Lattice Type	Primitive
Lattice Parameters	a = 25.574(5) Å
	b = 7.1978(11) Å
	c = 10.0658(19) Å
	$\alpha = \beta = \gamma = 90^o$.
	V = 1852.9(6) $Å^3$
Space Group	$P2_12_12$ (#18)
Z value	4
No. Observations (All reflections)	3350
No. Variables	272
Residuals: R1 (I>2.00s(I))	0.0271
Residuals: R (All reflections)	0.0274
Residuals: wR2 (All reflections)	0.0744
Goodness of Fit Indicator	1.085
Flack parameter (Parsons' quotients = 1346)	-0.03(5)
Max Shift/Error in Final Cycle	0.000
CCDC number	1583140

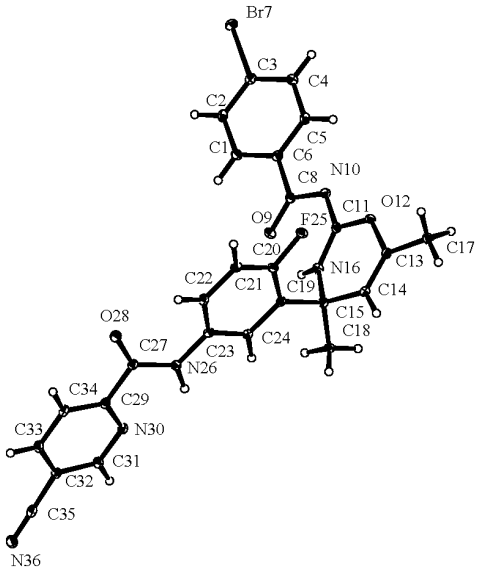


Figure S2. Single X-ray Structure of compound **1-100**. An ORTEP figure with thermal ellipsoid set at the 30% probability level.

Table S2. Crystal Data and Structure Refinement for Compound 1-100

Empirical Formula	$C_{26}\ H_{19}\ Br\ F\ N_5\ O_3$
Formula Weight	548.37
Crystal Color, Habit	colorless, prism
Crystal Dimensions	0.15 x 0.04 x 0.03 mm^3
Crystal System	Orthorhombic
Lattice Type	Primitive
Lattice Parameters	a = 7.528(2) Å
	b = 15.510(3) Å
	c = 20.104(4) Å
	$\alpha = \beta = \gamma = 90^o$.
	V = 2347.3(9) $Å^3$
Space Group	$P2_12_12_1$ (#19)
Z value	4
No. Observations (All reflections)	4249

No. Variables	327
Residuals: R1 (I>2.00s(I))	0.0210
Residuals: R (All reflections)	0.0213
Residuals: wR2 (All reflections)	0.0554
Goodness of Fit Indicator	1.017
Flack parameter (Parsons' quotients = 1760)	-0.019(6)
Max Shift/Error in Final Cycle	0.001
CCDC number	1583135

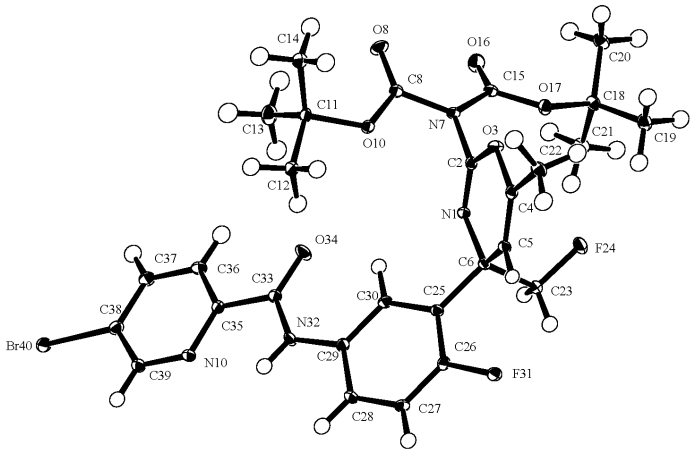


Figure S3. Single X-ray Structure of compound **1-101**. An ORTEP figure with thermal ellipsoid set at the 30% probability level.

Table S3. Crystal Data and Structure Refinement for Compound 1-101

Empirical Formula	$C_{28}H_{31}BrF_2N_4O_6$
Formula Weight	637.48
Crystal Color, Habit	colorless, prism
Crystal Dimensions	0.20 x 0.05 x 0.04 mm^3
Crystal System	Monoclinic
Lattice Type	Primitive
Lattice Parameters	a = 12.47800 Å
	b = 7.13560 Å

	c = 16.29640 Å
	α = γ = 90°. β = 96.53820°.
	V = 1441.56206 Å^3
Space Group	$P2_1$ (#4)
Z value	2
No. Observations (All reflections)	5115
No. Variables	377
Residuals: R1 (I>2.00s(I))	0.0267
Residuals: R (All reflections)	0.0270
Residuals: wR2 (All reflections)	0.0697
Goodness of Fit Indicator	1.054
Flack parameter (Parsons' quotients = 2222)	-0.017(7)
Max Shift/Error in Final Cycle	0.001
CCDC number	1583100

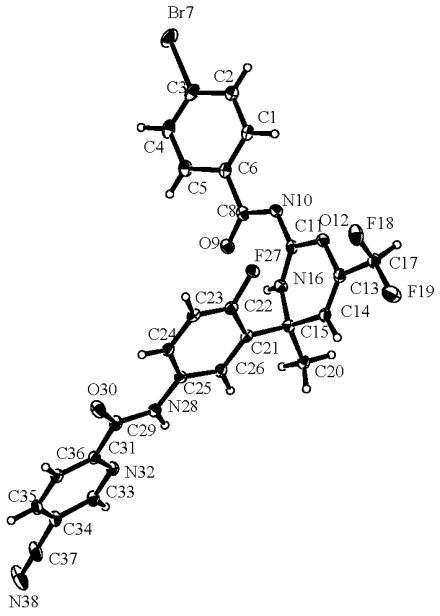


Figure S4. Single X-ray Structure of compound **1-102**. An ORTEP figure with thermal ellipsoid set at the 30% probability level.

Table S4. Crystal Data and Structure Refinement for Compound 1-102

Empirical Formula	$C_{26}\ H_{17}\ Br\ F_3\ N_5\ O_3$
Formula Weight	584.35
Crystal Color, Habit	colorless, platelet
Crystal Dimensions	0.19 x 0.03 x 0.02 mm^3
Crystal System	Monoclinic
Lattice Type	Primitive
Lattice Parameters	a = 11.008(6) Å
	b = 7.5623(18) Å
	c = 14.701(4) Å
	$\alpha = \gamma = 90^o$. $\beta = 103.334(11)^o$.
	V = 1190.8(8) $Å^3$
Space Group	$P2_1$ (#4)
Z value	2
No. Observations (All reflections)	4227
No. Variables	344
Residuals: R1 (I>2.00s(I))	0.0384
Residuals: R (All reflections)	0.0393
Residuals: wR2 (All reflections)	0.1035
Goodness of Fit Indicator	1.048
Flack parameter (Parsons' quotients = 1723)	-0.032(14)
Max Shift/Error in Final Cycle	0.001
CCDC number	1583036

(*3S*)-3-Amino-3-(2-fluorophenyl)-2-methylbutan-1-ol (2-18). A solution of **1-52** (2.00 g, 5.82 mmol) in THF/MeOH (1:1, 20 mL) was added $NaBH_4$ (220 mg, 5.82 mmol) at 0 °C. The mixture was stirred at the same temperature for 3 h, and additional $NaBH_4$ (1.32 g, 34.9 mmol) was added to the mixture. The resulting mixture was stirred at 0 °C for 4 h and quenched with aqueous 2 M HCl solution. The mixture was neutralized with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to give (*R*)-*N*-((*2S*)-2-(2-fluorophenyl)-4-hydroxy-3-methylbutan-2-yl)-2-methylpropane-2-sulfinamide. A mixture of this compound and HCl (4 M in EtOAc; 2.04 mL, 8.15 mmol) in MeOH (20 mL) was stirred at room temperature for 17 h. Additional HCl (4 M in EtOAc; 2.04 mL, 8.15 mmol) was added at room temperature and stirred at the same temperature for 1 h. The mixture was quenched with aqueous 2 M NaOH solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to give **2-18** (1.38 g, quant). This was used in the next step without further purification. MS-ESI (*m/z*): 198 $[M + H]^+$.

***N*-[[(*2S*)-2-(2-Fluorophenyl)-4-hydroxy-3-methylbutan-2-yl]carbamothioyl]benzamide (2-19).** A mixture of **2-18** (1.38 g) and benzoyl isothiocyanate (0.974 mL, 6.98 mmol) in DCM (10 mL) was stirred at room temperature for 30 min. Additional benzoyl isothiocyanate (0.487 mL, 3.49 mmol) was added at room temperature, and the mixture was stirred at the same temperature for 20 min. The mixture was evaporated, and the resulting residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 20−50% EtOAc) to give **2-19** (1.87 g, 89% over 2 steps) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$; diastereo mixture 5:1) δ 0.90 (d, *J* = 6.9 Hz, 0.5H) 1.24 (d, *J* = 6.9 Hz, 2.5H), 2.10−2.20 (m, 3H), 2.44−2.54 (m, 1H), 3.47−3.53 (m, 0.83H), 3.58−3.62 (m, 0.87H), 3.64−3.70 (m, 0.17H), 3.86−3.92 (m, 0.17H), , 6.98−7.04 (m, 1H), 7.10−7.16 (m, 1H), 7.23−7.29 (m, 1H), 7.30−7.36 (m, 1H), 7.45−7.54 (m, 1H), 7.57−7.63 (m, 1H),7.83−7.86 (m, 2H), 8.01 (brs, 1H), 11.6 (brs, 0.87H), 11.7 (brs, 0.13H). MS-ESI (*m/z*): 361 $[M + H]^+$.

***N*-[(*4S*)-4-(2-Fluorophenyl)-4,5-dimethyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]benzamide (2-20).** A mixture of **2-19** (1.87 g, 5.19 mmol) and EDC·HCl (1.79 g, 9.34 mmol) in MeCN (19 mL) was stirred at room temperature for 4.5 h. The mixture was diluted with H_2O, and the aqueous layer was separated

and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, 50% EtOAc) to give **2-20** (1.61 g, 95%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$; diastereo mixture 5:1) δ 0.87 (dd, *J* = 7.0, 2.0 Hz, 2.5H), 1.25−1.27 (m, 0.5H), 1.74 (s, 0.5H), 1.91 (d, *J* = 2.0 Hz, 2.5H), 2.53−2.58 (m, 0.87H), 2.68−2.72 (m, 0.13H), 4.01−4.15 (m, 1H), 4.42−4.46 (m, 1H), 7.07−7.13 (m, 1H), 7.13−7.23 (m, 1H), 7.31−7.46 (m, 3.87H), 7.47−7.52 (m, 1.13H), 8.23−8.27 (m, 2H), 11.5 (brs, 0.13H), 11.8 (brs, 0.87H). MS-ESI (*m/z*): 327 $[M + H]^+$.

***tert*-Butyl-benzoyl[(*4S*,*5R*)-4-(2-fluorophenyl)-4,5-dimethyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]carbamate (2-21) and *tert*-butyl benzoyl[(*4S*,*5S*)-4-(2-fluorophenyl)-4,5-dimethyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]carbamate (2-22).** A mixture of **2-20** (1.48 g, 4.53 mmol), Boc_2O (1.58 mL, 6.80 mmol), and DMAP (111 mg, 0.907 mmol) in DCM (15 mL) was stirred at room temperature for 1 h. The mixture was evaporated, and the residue was purified by flash column chromatography (silica gel; EtOAc/hexane, 50% EtOAc) to give **2-21** (300 mg, 15%) as a colorless amorphous and **2-22** (1.55 g, 78%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$; **2-21**) δ 1.17 (d, *J* = 7.2 Hz, 3H), 1.52 (d, *J* = 2.1 Hz, 3H), 1.58 (s, 9H), 2.37−2.44 (m, 1H), 3.96 (dd, *J* = 10.8, 3.0 Hz, 1H), 4.02 (dd, *J* = 10.8, 3.0 Hz, 1H), 7.00 (ddd, *J* = 12.5, 8.1, 1.2 Hz, 1H), 7.11 (td, *J* = 7.5, 1.2 Hz, 1H) 7.18−7.24 (m, 1H), 7.40−7.56 (m, 4H), 7.76 (dd, *J* = 8.5, 1.5 Hz, 2H). MS-ESI (*m/z*): 427 $[M + H]^+$. ^{1}H-NMR (400 MHz, $CDCl_3$; **2-22**) δ 0.73 (d, *J* = 7.2 Hz, 3H), 1.44 (s, 9H), 1.59 (d, *J* = 1.6 Hz, 3H), 2.44−2.50 (m, 1H), 4.06 (dd, *J* = 11.0, 2.9 Hz, 1H), 4.49 (dd, *J* = 11.0, 2.9 Hz, 1H), 6.94−7.01 (m, 2H), 7.15−7.20 (m, 1H) 7.18−7.24 (m, 1H), 7.37−7.54 (m, 3H), 7.78−7.80 (m, 2H). MS-ESI (*m/z*): 427 $[M + H]^+$.

(*4S*,*5R*)-4-(2-Fluoro-5-nitrophenyl)-4,5-dimethyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-23). A mixture of **2-21** (300 mg, 0.703 mmol) and K_2CO_3 (467 mg, 3.38 mmol) in THF/MeOH/H_2O (2:2:1, 5 mL) was stirred at room temperature for 1 h. The mixture was diluted with H_2O, and the aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0−100% EtOAc) to give *tert*-butyl ((*4S*,*5R*)-4-(2-fluorophenyl)-4,5-dimethyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl)carbamate (218 mg, 96%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.21 (d, *J* = 7.0 Hz, 3H), 1.51 (s, 9H), 1.68 (d, *J* = 1.1 Hz, 3H), 2.56−2.63 (m, 1H), 3.98 (d, *J* = 3.0 Hz, 2H), 7.07 (ddd, *J* = 12.3, 8.1, 1.0 Hz, 1H), 7.17 (td, *J* = 7.6,

1.2 Hz, 1H), 7.28−7.37 (m, 2H). A mixture of this compound (218 mg, 0.677 mmol) in TFA (3 mL) was stirred at room temperature for 2.5 h. The mixture was cooled to −20 °C and H_2SO_4 (0.75 mL) was added to the mixture, which was warmed to 0 °C and stirred at the same temperature for 5 min. After being cooled to −20 °C , HNO_3 (1.42 g/mL; 0.094 mL, 2.11 mmol) was added. The mixture was stirred at −20 °C for 40 min and quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to give **2-23** (376 mg). This was used in the next step without further purification. MS-ESI (*m/z*): 268 $[M + H]^+$.

(*4S*,*5S*)-4-(2-Fluoro-5-nitrophenyl)-4,5-dimethyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-24). A mixture of **2-22** (1.55 g, 3.63 mmol) and K_2CO_3 (1.21 g, 8.72 mmol) in THF/MeOH/H_2O (2:2:1, 25 mL) was stirred at room temperature for 18 h. The mixture was diluted with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to afford a residue. A mixture of this residue in TFA (3.4 mL) was stirred at room temperature for 3 h. The mixture was cooled to −20 °C, and H_2SO_4 (0.58 mL) was added to the mixture, which was warmed to 0 °C and stirred at the same temperature for 5 min. After being cooled to −20 °C, HNO_3 (1.42 g/mL; 0.488 mL, 10.9 mmol) was added. The mixture was stirred at −20 °C for 40 min and quenched with aqueous 2 M NaOH solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50−100% EtOAc) to give the crude product (mixture with **2-24** and the starting material). A mixture of this crude product in TFA (9 mL) and H_2SO_4 (5 mL) was stirred at 0 °C for 5 min. After being cooled to −20 °C , HNO_3 (1.42 g/mL; 0.244 mL, 5.45 mmol) was added. The mixture was stirred at −20 °C for 70 min and quenched with aqueous 2 M NaOH solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to give **2-24** (970 mg, 99% over 3 steps) as a yellow solid.^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.70 (d, *J* = 7.0 Hz, 3H), 1.60 (s, 3H), 2.46−2.49 (m, 1H), 3.94 (dd, *J* = 10.9, 2.4 Hz, 1H), 4.03 (brs, 1H), 4.47 (dd, *J* = 10.9, 2.4 Hz, 1H), 7.12 (dd, *J* = 10.2, 9.2 Hz, 2H), 8.11−8.14 (m, 1H), 8.77 (dd, *J* = 6.5, 2.8 Hz, 1H). MS-ESI (*m/z*): 268 $[M + H]^+$.

1,3-Bis(1,1-dimethylethyl) 2-[(4*S*,5*R*)-4-(5-amino-2-fluorophenyl)-5,6-dihydro-4,5-dimethyl-4*H*-1,3-oxazin-2-yl]imidodicarbonate (2-25). A mixture of **2-23** (376 mg, crude), Boc_2O (0.816 mL, 3.52 mmol), DMAP (214 mg, 1.76 mmol) in DCM (1.8 mL) was stirred at room temperature for 1 h. The mixture was quenched with saturated aqueous NH_4Cl solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, 25% EtOAc) to give di-*tert*-butyl ((*4S*,*5R*)-4-(2-fluoro-5-nitrophenyl)-4,5-dimethyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl)carbamate (251 mg, 76% over 3 steps) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.15 (d, *J* = 7.0 Hz, 3H), 1.54 (s, 18H), 1.95 (d, *J* = 2.3 Hz, 3H), 2.34−2.41 (m, 1H), 4.02−4.03 (m, 2H), 7.18 (dd, *J* = 11.0, 9.0 Hz, 1H), 8.15−8.19 (m, 1H), 8.48 (dd, *J* = 6.8, 3.0 Hz, 1H). MS-ESI (*m/z*): 468 $[M + H]^+$. A mixture of this compound (251 mg, 0.537 mmol), Fe (240 mg, 4.30 mmol), and NH_4Cl (345 mg, 6.44 mmol) in EtOH/THF/H_2O (2:1:1, 5 mL) was heated at 60 °C for 100 min. The mixture was allowed to cool to room temperature and diluted with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, 50% EtOAc) to give **2-25** (191 mg, 81%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.18 (d, *J* = 7.2 Hz, 3H), 1.52 (s, 3H), 1.53 (s, 18H), 2.42−2.46 (m, 1H), 3.50 (brs, 1H), 3.99 (ddd, *J* = 35.5, 10.7, 3.4 Hz, 1H), 6.48−6.52 (m, 1H), 6.78−6.85 (m, 2H). MS-ESI (*m/z*): 438 $[M + H]^+$.

1,3-Bis(1,1-dimethylethyl) 2-[(4*S*,5*S*)-4-(5-amino-2-fluorophenyl)-5,6-dihydro-4,5-dimethyl-4*H*-1,3-oxazin-2-yl]imidodicarbonate (2-26). A mixture of **2-24** (970 mg, 3.63 mmol), Boc_2O (2.11 mL, 9.07 mmol), DMAP (222 mg, 1.82 mmol) in DCM (20 mL) was stirred at room temperature for 1 h and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 20−25% EtOAc) to give di-*tert*-butyl ((*4S*,*5S*)-4-(2-fluoro-5-nitrophenyl)-4,5-dimethyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl)carbamate (980 mg, 58%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.78 (d, *J* = 7.0 Hz, 3H), 1.53 (s, 18H), 1.69 (d, *J* = 1.3 Hz, 3H), 2.56−2.58 (m, 1H), 4.15 (dd, J = 11.0, 1.5 Hz, 2H), 4.57 (dd, *J* = 11.0, 2.5 Hz, 2H), 7.18 (dd, *J* = 10.7, 8.9 Hz, 1H), 8.15−8.19 (m, 1H), 8.68 (dd, *J* = 6.8, 3.0 Hz, 1H). MS-ESI (*m/z*): 468 $[M + H]^+$. A mixture of this compound (980 mg, 2.10 mmol), Fe (937 mg, 16.8 mmol), and NH_4Cl (1.35 g, 25.2 mmol) in EtOH/THF/H_2O

(2:1:1, 20 mL) was heated at 60 °C for 100 min. The mixture was allowed to cool to room temperature and diluted with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, 50% EtOAc) to give **2-26** (763 mg, 83%) as a yellow amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.81 (d, *J* = 7.2 Hz, 3H), 1.52 (s, 18H), 1.64 (d, *J* = 0.8 Hz, 3H), 2.50−2.53 (m, 1H), 3.89 (brs, 1H), 4.09 (dd, *J* = 11.0, 2.1 Hz, 2H), 4.50 (dd, *J* = 11.0, 2.1 Hz, 2H), 6.49−6.52 (m, 1H), 6.81 (dd, *J* = 11.7, 8.5 Hz, 2H), 7.03 (dd, *J* = 6.7, 3.0 Hz, 2H). MS-ESI (*m/z*): 438 $[M + H]^+$.

General procedure for condensation with carboxylic acids and deprotection of the Boc group. A mixture of aniline derivatives (1.0 eq), 5-cyanopicolinic acid (1.05−1.20 eq), HATU (1.2 eq), and DIEA (2.0 eq) in DMF or DCM was stirred at room temperature. The mixture was quenched with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography to give Boc-protected amide. To a flask containing this compound was added an excess amount of formic acid (>100 eq) at room temperature. The mixture was stirred at room temperature and quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by flash column chromatography and/or recrystallization to obtain the target compound.

***N*-[3-[(*4S*,*5R*)-2-Amino-4,5-dimethyl-5,6-dihydro-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (2-2).** Compound **2-2** (39.7 mg, 68% over 2 steps) was prepared from **2-25** (70.0 mg, 0.160 mmol) in DCM for 1 h following the general procedure. White solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.14 (d, *J* = 7.0 Hz, 3H), 1.55 (d, *J* = 1.4 Hz, 3H), 2.44−2.48 (m, 1H), 3.85 (dd, *J* =10.8, 3.4 Hz, 1H), 3.90 (dd, *J* =10.8, 3.4 Hz, 1H), 7.07 (dd, *J* = 11.7, 8.8 Hz, 1H), 7.48 (dd, *J* = 6.9, 2.8 Hz, 1H), 7.95−7.98 (m, 1H), 8.20 (dd, *J* = 8.1, 1.9 Hz, 1H), 8.43 (d, *J* = 8.0 Hz, 1H), 8.90 (d, *J* = 1.4 Hz, 1H), 9.84 (brs, 1H). MS-ESI (*m/z*): 368 $[M + H]^+$.

***N*-[3-[(*4S*,*5S*)-2-Amino-4,5-dimethyl-5,6-dihydro-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (2-4).** Compound **2-4** (61.7 mg, 73% over 2 steps) was prepared from **2-26** (70.0

mg, 0.160 mmol) in DCM for 1 h following the general procedure. Yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.75 (d, *J* = 6.4 Hz, 3H), 1.64 (d, *J* = 1.1 Hz, 3H), 2.47−2.49 (m, 1H), 3.95 (dd, *J* = 10.8, 3.1 Hz, 1H), 4.46 (dd, *J* = 10.8, 3.1 Hz, 1H) 7.05 (dd, *J* = 11.5, 8.8 Hz, 1H), 7.76 (dd, *J* = 6.8, 2.8 Hz, 1H), 8.02−8.06 (m, 1H), 8.20 (dd, *J* = 8.2, 2.0 Hz, 1H), 8.43 (d, *J* = 8.0 Hz, 1H), 8.89 (d, *J* = 1.3 Hz, 1H), 9.89 (brs, 1H). MS-ESI (*m/z*): 368 $[M + H]^+$.

(*R*)-*N*-[(*2R*,*3S*)-3-Fluoro-2-(2-fluorophenyl)pent-4-en-2-yl]-2-methylpropane-2-sulfinamide (2-28). To a stirred suspension of methyltriphenylphosphonium bromide (10.1 g, 28.0 mmol) in toluene (90 mL) was added *tert*-BuOK (1.0 M in THF; 25.8 mL 25.8 mmol) at room temperature. The mixture was stirred at room temperature for 1 h, and a solution of **2-27** (3.40 g, 11.2 mmol) in toluene (60 mL) was added dropwise at 0 ˚C over 10 min. The mixture was stirred at room temperature for 30 min and quenched with saturated aqueous NH_4Cl solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 40−70% EtOAc) to give **2-28** (1.52 g, 45%) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.25 (s, 9H), 1.88 (s, 3H), 3.98 (brs, 1H), 5.30−5.59 (m, 3H), 5.78−5.91 (m, 1H), 7.07 (dd, *J* = 12.8, 8.3 Hz, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 7.31−7.39 (m, 1H), 7.45 (t, *J* = 7.6 Hz, 1H). MS-ESI (*m/z*): 302 $[M + H]^+$.

(*R*)-*N*-[(*2R*,*3R*,*4S*)-3-Fluoro-2-(2-fluorophenyl)-4,5-dihydroxypentan-2-yl]-2-methylpropane-2-sulfinamide (2-29). A mixture of **2-28** (1.08 g, 3.58 mmol), *N*-methylmorpholine *N*-oxide (1.05 g, 8.96 mmol), and potassium osmate dehydrate (264 mg, 0.717 mmol) in acetone/H_2O (3:1, 10 mL) was stirred at room temperature for 12 h. The mixture was quenched with aqueous $Na_2S_2O_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50−70% EtOAc) to give **2-29** (853 mg, 70%) as a amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.18 (s, 9H), 2.00 (s, 3H), 2.72 (brs, 1H), 3.62−3.66 (m, 1H), 3.79−3.83 (m, 1H), 4.10−4.14 (m, 1H), 4.84 (dd, *J* = 44.2, 8.7 Hz, 1H), 5.55 (d, *J* = 14.8 Hz, 1H), 7.06 (dd, *J* = 12.6, 8.2 Hz, 1H), 7.17 (dd, *J* = 16.9, 9.2 Hz, 1H), 7.32−7.33 (m, 1H), 7.41 (t, *J* = 8.2 Hz, 1H). MS-ESI (*m/z*): 336 $[M + H]^+$.

***N*-[[(*2R*,*3R*,*4S*)-3-Fluoro-2-(2-fluorophenyl)-4,5-dihydroxypentan-2-yl]carbamothioyl]benzamide (2-30).** To a solution of **2-29** (836 mg, 2.49 mmol) in MeOH (8 mL) was added HCl (4 M in 1,4-dioxane; 1.25 mL, 4.98 mmol) at 0 °C. The mixture was stirred at room temperature for 1 h. The mixture was quenched with aqueous Na_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to afford a residue. To a solution of this compound in DCM (6 mL) was added benzoyl isothiocyanate (0.34 mL, 2.54 mmol) at 0 °C. The mixture was stirred at room temperature for 3 h and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 40−70% EtOAc) to give **2-30** (867 mg, 88% over 2 steps) as a yellow amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.20 (s, 3H), 2.95 (d, *J* = 5.9 Hz, 1H), 3.77−3.89 (m, 1H), 4.08−4.14 (m, 1H), 4.10−4.14 (m, 1H), 5.08 (dd, *J* = 44.1, 8.3 Hz, 1H), 7.04 (dd, *J* = 12.4, 8.0 Hz, 1H), 7.17 (t, *J* = 7.5 Hz, 1H), 7.30 (t, *J* = 6.3 Hz, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.51 (t, *J* = 7.5 Hz, 1H), 7.62 (t, *J* = 7.3 Hz, 1H), 7.86 (t, *J* = 9.3 Hz, 2H), 8.82 (s, 1H), 11.8 (s, 1H).

***N*-[(*4R*,*5R*,*6S*)-5-Fluoro-4-(2-fluorophenyl)-6-(hydroxymethyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]benzamide (2-31).** A mixture of **2-30** (867 mg, 2.20 mmol) and EDC·HCl (843 mg, 4.40 mmol) in MeCN/DMF (10:1, 10 mL) was stirred at room temperature for 14 h. The mixture was diluted with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50−70% EtOAc) to give **2-31** (791 mg, quant.) as a yellow amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.88 (s, 3H), 3.64−3.70 (m, 2H), 4.69 (dt, *J* = 17.5, 5.1 Hz, 1H), 5.46 (dd, *J* = 47.5, 6.0 Hz, 1H), 7.14−7.24 (m, 2H), 7.36−7.46 (m, 3H), 7.49 (dd, *J* = 16.2, 9.0 Hz, 2H), 8.22 (dd, *J* = 16.2, 7.8 Hz, 2H), 11.8 (brs, 1H).

***N*-[(*4R*,*5R*,*6S*)-5-Fluoro-6-(fluoromethyl)-4-(2-fluorophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]benzamide (2-32).** To a solution of **2-31** (791 mg, 2.20 mmol) in DCM (25 mL) was added DAST (1.16 mL, 8.78 mmol) at −78 °C. The mixture was gradually warmed to 0 °C over 30 min and stirred at the temperature for 3 h. The mixture was quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 20−40% EtOAc) to give **2-32** (113

mg, 14%) as a white amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.88 (s, 3H), 4.14−4.50 (m, 2H), , 4.80−4.90 (m, 1H), 5.47 (dd, *J* = 46.8, 5.1 Hz, 1H), 7.00−7.24 (m, 2H), 7.41−7.53 (m, 5H), 8.24 (d, *J* = 7.5 Hz, 2H), 11.8 (brs, 1H).

(*4R*,*5R*,*6S*)-5-Fluoro-6-(fluoromethyl)-4-(2-fluorophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-33). A mixture of **2-32** (332 mg, 0.917 mmol), Boc_2O (0.32 mL, 1.38 mmol), and DMAP (11.2 mg, 0.092 mmol) in THF (6 mL) was stirred at room temperature for 45 min. The mixture was diluted with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to afford a residue. To a solution of this residue in MeOH (6 mL) was added K_2CO_3 (380 mg, 2.75 mmol) at 0 °C. The mixture was stirred at room temperature for 30 min and diluted with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 30−60% EtOAc) to give *tert*-butyl [(*4R*,*5R*,*6S*)-5-fluoro-6-(fluoromethyl)-4-(2-fluorophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]carbamate. To a solution of this compound in DCM (4 mL) was added TFA (1 mL) at room temperature. The mixture was stirred at room temperature for 1.5 h. The mixture was quenched with aqueous Na_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 80−100% EtOAc) to give **2-33** (237 mg, quant. over 3 steps) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.70 (s, 3H), 4.08−4.15 (m, 1H), 4.21−4.26 (m, 1H), 4.63 (ddt, *J* = 21.5, 13.1, 3.7 Hz, 1H), 5.11 (dd, *J* = 47.6, 5.4 Hz, 1H), 7.06 (ddd, *J* = 12.4, 8.1, 1.2 Hz, 1H), 7.17 (td, *J* = 7.7, 1.2 Hz, 1H), 7.26−7.33 (m, 1H), 7.50 (td, *J* = 8.1, 1.8 Hz, 1H). MS-ESI (*m/z*): 259 $[M + H]^+$.

1,3-Bis(1,1-dimethylethyl) 2-[(4*R*,5*R*,6*S*)-4-(5-amino-2-fluorophenyl)-5-fluoro-6-(fluoromethyl)-5,6-dihydro-4-methyl-4*H*-1,3-oxazin-2-yl]imidodicarbonate (2-34). To a solution of **2-33** (40.0 mg, 0.155 mmol) in TFA (0.32 mL) and H_2SO_4 (0.08 mL) was added HNO_3 (0.010 mL, 0.232 mmol) at −20 °C. The mixture was stirred at −20 °C for 45 min and quenched with aqueous Na_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to a residue. To a solution of this compound in

THF (1 mL) were added Boc_2O (0.079 mL, 0.341 mmol) and DMAP (1.9 mg, 0.015 mmol) at 0 °C. The mixture was stirred at the same temperature for 2 h and evaporated. The residue was diluted with EtOAc and H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 20−40% EtOAc) to give a di-*tert*-butyl [(*4R*,*5R*,*6S*)-5-fluoro-4-(2-fluoro-5-nitrophenyl)-6-(fluoromethyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]carbamate (53.7 mg, 69% over 2 steps) as a white amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.54 (s, 9H), 1.56 (s, 9H), 1.81 (s, 3H), 4.54−4.66 (m, 3H), 4.87 (dd, *J* = 48.3, 8.5 Hz, 1H), 7.22−7.26 (m, 1H), 8.24 (d, *J* = 8.7 Hz, 1H), 8.51 (d, *J* = 6.5 Hz, 1H). A mixture of this compound (53.7 mg, 0.107 mmol), Fe (47.7 mg, 0.853 mmol), and NH_4Cl (68.5 mg, 1.28 mmol) in EtOH/THF/H_2O (2:1:1, 1 mL) was heated at 60 °C for 1 h. The reaction mixture was allowed to cool to room temperature and diluted with EtOAc and H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 20−40% EtOAc) to give **2-34** (42.1 mg, 83%) as a yellow solid. MS-ESI (*m/z*): 474 $[M + H]^+$.

***N*-[3-[(*4R*,*5R*,*6S*)-2-Amino-5-fluoro-6-(fluoromethyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (2-5).** Compound **2-5** (26.0 mg, 73% over 2 steps) was prepared from **2-34** (42.1 mg, 0.089 mmol) following the general procedure. White solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.53 (s, 3H), 4.37 (d, *J* = 47.4 Hz, 2H), 4.70−4.78 (m, 1H), 4.94 (dd, *J* = 48.5, 6.6 Hz, 1H), 5.78 (brs, 2H), 7.17 (t, *J* = 10.2 Hz, 1H), 7.89 (s, 1H), 8.01 (d, *J* = 6.8 Hz, 1H), 8.29 (d, *J* = 8.0 Hz, 1H), 8.59 (d, *J* = 8.0 Hz, 1H), 9.21 (s, 1H), 10.9 (s, 1H). MS-ESI (*m/z*): 404 $[M + H]^+$.

Ethyl (*2R*,*3R*)-3-[[(*R*)-*tert*-butylsulfinyl]amino]-3-(2-fluorophenyl)-2-methoxybutanoate (2-35). To a solution of diisopropylamine (1.95 mL, 13.7 mmol) in THF (14 mL) was added *n*-BuLi (1.64 M in hexane; 8.09 mL, 13.3 mmol) dropwise at –78 °C. The mixture was stirred at the same temperature for 30 min, and then ethyl 2-methoxyacetate (1.46 mL, 12.4 mmol) was added dropwise to the mixture. After being stirred for 30 min at –78 °C, a solution of **1-51** (1.00 g, 4.14 mmol) in THF (6 mL) was added dropwise at −78 °C, and the mixture was stirred at the same temperature for 1 h. The reaction was quenched with saturated aqueous NH_4Cl solution, and the aqueous layer was separated and

extracted with EtOAc. The combined organic layers were washed with H_2O and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 20−40% EtOAc) to give **2-35** (1.32 g, 89%) as a yellow oil, which was a mixture with a minor diastereomer. ^{1}H-NMR (400 MHz, $CDCl_3$, diastereo mixture 4:1) δ 1.19–1.28 (m, 12H), 1.86 (s, 0.6H), 1.90 (s, 2.4H), 3.31 (s, 0.6H), 3.48 (s, 2.4H), 3.84−3.92 (m, 0.4H), 4.14–4.21 (m, 1.6H), 4.32−4.36 (m, 1H), 4.49 (s, 0.2H), 4.92 (s, 0.8H), 6.99–7.07 (m, 1H), 7.08−7.14 (m, 1H), 7.33–7.27 (m, 1H), 7.40−7.47 (m, 1H). MS-ESI (*m/z*): 360 $[M + H]^+$.

(*R*)-*N*-[(*2R*,3*R*)-2-(2-Fluorophenyl)-3-methoxy-4-oxobutan-2-yl]-2-methylpropane-2-sulfinamide (2-36). To a solution of **2-35** (1.32 g, 3.67 mmol) in DCM (13 mL) was added DIBAL (1.02 M in hexane; 11.9 mL, 12.1 mmol) dropwise at –78 °C. The mixture was stirred at the same temperature for 15 min and then quenched with saturated aqueous Rochelle's salt solution. The mixture was stirred vigorously at room temperature for 1.5 h, and the aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50−100% EtOAc) to give **2-36** (832 mg, 72%) as a colorless oil, which was a mixture with a minor diastereomer. ^{1}H-NMR (400 MHz, $CDCl_3$, diastereo mixture 4:1) δ 1.21 (s, 1.8H), 1.24 (s, 7.2H), 1.83 (s, 0.6H), 1.92 (s, 2.4H), 3.41 (s, 2.4H), 3.52 (s, 0.6H), 4.06 (s, 0.8H), 4.29 (s, 0.2H), 4.35 (s, 0.2H), 4.68 (s, 0.8H), 7.01–7.10 (m, 1H), 7.11–7.17 (m, 1H), 7.27−7.52 (m, 2H), 9.46 (s, 0.2H), 9.70 (s, 0.8H). MS-ESI (*m/z*): 316 $[M + H]^+$.

(*R*)-*N*-[(*2R*,*3S*)-2-(2-Fluorophenyl)-3-methoxypent-4-en-2-yl]-2-methylpropane-2-sulfinamide (2-37). To a solution of methyltriphenylphosphonium bromide (2.36 g, 6.59 mmol) in toluene (20 mL) was added *tert*-BuOK (1 M in THF; 6.07 mL, 6.07 mmol) at room temperature. The mixture was stirred at the same temperature for 30 min, and a solution of **2-36** (832 mg, 2.64 mmol) in toluene (13 mL) was added at 0 °C. The mixture was stirred at room temperature for 20 min and then quenched with saturated aqueous NH_4Cl solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0−70% EtOAc) to give **2-37** (390 mg, 47%) as a colorless oil, which was a mixture with a minor diastereomer. ^{1}H-NMR (400 MHz, $CDCl_3$, diastereo mixture 4:1) δ 1.20 (s, 1.8H), 1.21 (s, 7.2H), 1.76 (s, 0.6H), 1.83 (s, 2.4H), 3.22 (s,

2.4H), 3.34 (s, 0.6H), 4.01 (d, *J* = 7.5 Hz, 0.8H), 4.22 (d, *J* = 9.6 Hz, 0.2H), 4.30 (s, 0.8H), 4.37 (s, 0.2H), 5.05–5.37 (m, 2H), 5.45–5.71 (m, 1H), 6.96–7.05 (m, 1H), 7.07–7.14 (m, 1H), 7.24–7.32 (m, 1H), 7.38–7.46 (m, 1H). MS-ESI (*m/z*): 314 $[M + H]^+$.

1,1-Bis(2,4-dimethoxybenzyl)-3-[(*2R*,*3S*)-3-fluoro-2-(2-fluorophenyl)pent-4-en-2-yl]urea (2-38). A mixture of **2-28** (600 mg, 1.99 mmol) and HCl (4 M in 1,4-dioxane; 0.747 mL, 2.99 mmol) in MeOH (6 mL) was stirred at room temperature for 30 min. The mixture was quenched with aqueous $NaHCO_3$ solution, and the aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and evaporated to afford a residue. To a solution of this residue in EtOAc/H_2O (2:1, 6 mL) were added $NaHCO_3$ (586 mg, 6.97 mmol) and 4-nitrophenyl carbonochloridate (442 mg, 2.19 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, and then bis(2,4-dimethoxybenzyl)amine (696 mg, 2.19 mmol) was added to the mixture at 0 °C. After being stirred for 2 h at the same temperature, the mixture was diluted with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0−30% EtOAc) to give **2-38** (1.06 g, 98% over 2 steps) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.84 (s, 3H), 3.78 (s, 6H), 3.81 (s, 6H), 4.39 (s, 4H), 5.14 (d, *J* = 10.5 Hz, 1H), 5.27 (d, *J* = 17.3 Hz, 1H), 5.58-5.69 (m, 2H), 6.44 (s, 4H), 6.94−7.02 (m, 1H), 7.06−7.14 (m, 3H), 7.18−7.24 (m, 1H), 7.35−7.42 (m, 1H). MS-ESI (*m/z*): 541 $[M + H]^+$.

1,1-Bis(2,4-dimethoxybenzyl)-3-[(2*R*,3*S*)-2-(2-fluorophenyl)-3-methoxypent-4-en-2-yl]urea (2-39). A mixture of **2-37** (390 mg, 1.24 mmol) and HCl (4 M in 1,4-dioxane; 0.467 mL, 1.87 mmol) in MeOH (4 mL) was stirred at room temperature for 30 min. The mixture was quenched with aqueous $NaHCO_3$ solution, and the aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to afford a residue. To a solution of this residue in EtOAc/H_2O (2:1, 6 mL) were added $NaHCO_3$ (365 mg, 4.35 mmol) and 4-nitrophenyl carbonochloridate (275 mg, 1.37 mmol) at 0 °C. The mixture was stirred at 0 °C for 40 min, and then bis(2,4-dimethoxybenzyl)amine (434 mg, 1.37 mmol) was added at 0 °C. After being stirred for 2 h at the same temperature, the mixture was diluted with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with aqueous K_2CO_3 solution and evaporated. The residue was purified by flash column chromatography (silica gel;

EtOAc/hexane, gradient: 0−30% EtOAc) to give **2-39** (605 mg, 88% over 2 steps) as a white amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.86 (s, 3H), 3.16 (s, 3H), 3.77 (s, 6H), 3.81 (s, 6H), 3.93 (d, *J* = 7.3 Hz, 1H), 4.33−4.47 (m, 4H), 5.01−5.13 (m, 2H), 5.30−5.41 (m, 1H), 5.64 (s, 1H), 6.42−6.48 (m, 4H), 6.90−7.00 (m, 1H), 7.01−7.07 (m, 1H), 7.15−7.19 (m, 3H), 7.30−7.23 (m, 1H). MS-ESI (*m/z*): 553 $[M + H]^+$.

(*4R*,*5R*,*6S*)-*N*,*N*-Bis(2,4-dimethoxybenzyl)-5-fluoro-4-(2-fluorophenyl)-6-(iodomethyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-40). To a solution of iodine (995 mg, 3.92 mmol) in MeCN (70 mL) was added **2-38** (1.06 g, 1.96 mmol) in MeCN (30 mL) at 0 °C. The mixture was stirred at the same temperature for 3.5 h and then quenched with aqueous $NaHCO_3$ and $Na_2S_2O_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0−20% EtOAc) to give **2-40** (1.07 g, 82%) as a white amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.64 (s, 3H), 3.16−3.21 (m, 2H), 3.75 (s, 6H), 3.77−3.86 (m, 1H), 3.82 (s, 6H), 4.48 (d, *J* = 15.6 Hz, 2H), 4.61 (d, *J* = 15.6 Hz, 2H), 5.31 (d, *J* = 48.7 Hz, 1H), 6.42−6.49 (m, 4H), 6.97−7.07 (m, 2H), 7.25−7.17 (m, 3H), 7.38 (t, *J* = 7.8 Hz, 1H). MS-ESI (*m/z*): 667 $[M + H]^+$.

(*4R*,*5R*,*6S*)-*N*,*N*-Bis(2,4-dimethoxybenzyl)-4-(2-fluorophenyl)-6-(iodomethyl)-5-methoxy-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-41). To a solution of iodine (556 mg, 2.19 mmol) in MeCN (40 mL) was added **2-39** (605 mg, 1.10 mmol) in MeCN (20 mL) at 0 °C. The mixture was stirred at the same temperature for 1 h and then quenched with aqueous $NaHCO_3$ and $Na_2S_2O_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0−30% EtOAc) to give **2-41** (442 mg, 60%) as a white amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.63 (s, 3H), 3.15−3.26 (m, 2H),3.70−3.77 (m, 1H), 3.71 (s, 3H), 3.73 (s, 6H), 3.81 (s, 6H), 4.06 (s, 1H), 4.52 (s, 4H), 6.40−6.48 (m, 4H), 6.96−7.07 (m, 2H), 7.17−7.27 (m, 3H), 7.39 (t, *J* = 7.7 Hz, 1H). MS-ESI (*m/z*): 679 $[M + H]^+$.

[(*4R*,*5R*,*6R*)-2-[Bis(2,4-dimethoxybenzyl)amino]-5-fluoro-4-(2-fluorophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-6-yl]methanol (2-42). A mixture of **2-40** (1.07 g, 1.61 mmol) and silver trifluoroacetate (1.42 g, 6.42 mmol) in CH_3NO_2/H_2O (5:2, 15 mL) was heated at 80 °C for 7 h.

Additional silver trifluoroacetate (0.71 g, 3.21 mmol) was added, and stirring was continued at 80 °C for 17 h. The reaction mixture was allowed to cool to room temperature and quenched with aqueous $NaHCO_3$ solution. After the mixture was filtered through Celite, the aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0−50% EtOAc) to give **2-42** (506 mg, 57%) as a white amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.63 (s, 3H), 3.59−3.67 (m, 1H), 3.71−3.75 (m, 2H), 3.76 (s, 6H), 3.81 (s, 6H), 4.48 (d, *J* = 15.8 Hz, 2H), 4.63 (d, *J* = 15.8 Hz, 2H), 5.13 (d, *J* = 48.9 Hz, 1H), 6.43−6.50 (m, 4H), 6.94−7.06 (m, 2H), 7.17−7.25 (m, 3H), 7.39 (t, *J* = 8.1 Hz, 1H). MS-ESI (*m/z*): 557 $[M + H]^+$.

[(*4R*,5*R*,*6R*)-2-[Bis(2,4-dimethoxybenzyl)amino]-4-(2-fluorophenyl)-5-methoxy-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-6-yl]methanol (2-43). A mixture of **2-41** (442 mg, 0.651 mmol) and silver trifluoroacetate (576 mg, 2.61 mmol) in CH_3NO_2/H_2O (5:2; 7 mL) was heated at 80 °C for 7 h. Additional silver trifluoroacetate (288 mg, 1.30 mmol) was added, and stirring was continued at 80 °C for 17 h. The reaction mixture was allowed to cool to room temperature and quenched with aqueous $NaHCO_3$ solution. After the mixture was filtered through Celite, the aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0−50% EtOAc) to give **2-43** (241 mg, 65%) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.61 (s, 3H), 3.60−3.62 (m, 4H), 3.68−3.84 (m, 15H), 4.54 (s, 4H), 6.41−6.48 (m, 4H), 6.93−7.05 (m, 2H), 7.13−7.32 (m, 3H), 7.39 (t, *J* = 7.8 Hz, 1H). MS-ESI (*m/z*): 569 $[M + H]^+$.

(*4R*,*5R*,*6R*)-*N*,*N*-Bis(2,4-dimethoxybenzyl)-5-fluoro-6-(fluoromethyl)-4-(2-fluorophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-44). To a solution of **2-42** (506 mg, 0.909 mmol) in DCM (5 mL) was added DAST (0.400 mL, 2.73 mmol) at −78 °C. The mixture was stirred at room temperature for 2.5 h and then quenched with aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0−30% EtOAc) to give **2-44** (386 mg, 76%) as a white amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.64 (s, 3H), 3.76 (s, 6H), 3.82 (s, 6H), 3.90−4.07 (m, 1H), 4.36−4.68 (m, 6H), 5.18 (d, *J* =

48.7 Hz, 1H), 6.42−6.49 (m, 4H), 6.95−7.06 (m, 2H), 7.16−7.25 (m, 3H), 7.38 (t, *J* = 7.7 Hz, 1H). MS-ESI (*m/z*): 559 $[M + H]^+$.

(*4R*,*5R*,*6S*)-*N*,*N*-Bis(2,4-dimethoxybenzyl)-6-(fluoromethyl)-4-(2-fluorophenyl)-5-methoxy-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-45). To a solution of **2-43** (4.56 g, 8.02 mmol) in DCM (50 mL) was added DAST (3.18 mL, 24.1 mmol) at −78 °C. The mixture was stirred at room temperature for 28 h and then quenched with aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 20−40% EtOAc) to give **2-45** (2.84 g, 62%) as a yellow amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.62 (s, 3H), 3.62 (s, 3H), 3.74 (s, 6H), 3.81 (s, 6H), 3.86 (s, 1H), 3.89−4.01 (m, 1H), 4.37−4.60 (m, 6H), 6.40−6.48 (m, 4H), 6.94−7.05 (m, 2H), 7.16−7.28 (m, 3H), 7.37 (t, *J* = 7.8 Hz, 1H). MS-ESI (*m/z*): 571 $[M + H]^+$.

(*4R*,*5R*,*6R*)-*N*,*N*-Bis(2,4-dimethoxybenzyl)-5-fluoro-4-(2-fluorophenyl)-6-(methoxymethyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-46). To a solution of **2-42** (265 mg, 0.476 mmol) in THF (5 mL) were added NaH (60% dispersion in mineral oil; 57.1 mg, 1.43 mmol) and MeI (0.045 mL, 0.714 mL) at room temperature. The mixture was stirred at room temperature for 30 min and then quenched with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 10−40% EtOAc) to give **2-46** (234 mg, 86%) as a white amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.63 (s, 3H), 3.25 (s, 3H), 3.41−3.47 (m, 1H), 3.53−3.58 (m, 1H), 3.72−3.82 (m, 1H), 3.75 (s, 6H), 3.81 (s, 6H), 4.52 (dd, *J* = 78.7, 15.8 Hz, 4H), 5.15 (d, *J* = 48.5 Hz, 1H), 6.42−6.49 (m, 4H), 6.93−7.04 (m, 2H), 7.15−7.23 (m, 3H), 7.38 (t, *J* = 8.0 Hz, 1H). MS-ESI (*m/z*): 571 $[M + H]^+$.

(*4R*,*5R*,6R)-5-Fluoro-6-(fluoromethyl)-4-(2-fluorophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-47). A mixture of **2-44** (3.60 g, 6.44 mmol) and anisole (2.96 mL, 27.1 mmol) in TFA (15 mL) was heated at 80 °C for 24 h. The mixture was allowed to cool to room temperature and quenched with aqueous K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O, dried over Na_2SO_4, filtered, and evaporated. The

residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 30−100% EtOAc) to give **2-47** (1.62 g, 97%) as a white amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.78 (s, 3H), 4.20−4.29 (m, 1H), 4.64 (dd, *J* = 46.6, 5.0 Hz, 2H), 5.27 (d, *J* = 47.8 Hz, 1H), 7.10 (t, *J* = 10.2 Hz, 1H), 7.20−7.25 (m, 1H), 7.44−7.32 (m, 2H). MS-ESI (*m/z*): 259 $[M + H]^+$.

(*4R*,*5R*,*6S*)-6-(Fluoromethyl)-4-(2-fluorophenyl)-5-methoxy-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-48). A mixture of **2-45** (2.84 g, 4.98 mmol) and anisole (3.81 mL, 34.8 mmol) in TFA (23 mL) was heated at 80 °C for 6 h. The mixture was allowed to cool to room temperature and quenched with aqueous K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were diluted with aqueous HCl solution (2 M in H_2O) and back extracted with H_2O. The combined aqueous layers were basified with aqueous NaOH solution (2 M in H_2O) and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give **2-48** (1.01 g, 75%) as a white amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.66 (s, 3H), 3.65 (s, 3H), 3.90 (s, 1H), 3.95−4.03 (m, 1H), 4.46−4.50 (m, 1H), 4.58−4.62 (m, 1H), 6.99−7.07 (m, 1H), 7.14 (t, *J* = 7.9 Hz, 1H), 7.22−7.30 (m, 1H), 7.42 (t, *J* = 7.9 Hz, 1H). MS-ESI (*m/z*): 271 $[M + H]^+$.

(*4R*,*5R*,*6R*)-5-Fluoro-4-(2-fluorophenyl)-6-(methoxymethyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-49). A mixture of **2-46** (234 mg, 0.410 mmol) and anisole (0.314 mL, 2.87 mmol) in TFA (1.6 mL) was heated at 80 °C for 19 h. The mixture was allowed to cool to room temperature and quenched with aqueous K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 60−100% EtOAc) to give **2-49** (103 mg, 93%) as a white amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.65 (s, 3H), 3.35 (s, 3H), 3.53−3.65 (m, 2H), 3.90 (dt, *J* = 30.2, 6.3 Hz, 1H), 4.22 (brs, 2H), 5.13 (d, *J* = 48.0 Hz, 1H), 7.02 (dd, *J* = 12.0, 8.0 Hz, 1H), 7.13 (t, *J* = 7.5 Hz, 1H), 7.28−7.24 (m, 1H), 7.45 (t, *J* = 7.5 Hz, 1H). MS-ESI (*m/z*): 271 $[M + H]^+$.

1,3-Bis(1,1-dimethylethyl)-2-[(4*R*,5*R*,6*R*)-4-(5-amino-2-fluorophenyl)-5-fluoro-6-(fluoromethyl)-5,6-dihydro-4-methyl-4*H*-1,3-oxazin-2-yl]imidodicarbonate (2-50). To a solution of **2-47** (1.62 g, 6.27 mmol) in TFA (13 mL) and H_2SO_4 (3.1 mL) was added HNO_3 (1.42 g/mL; 0.421 mL, 9.41 mmol) at −20 °C. The mixture was stirred at 0 °C for 15 min and quenched with aqueous

K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O, dried over Na_2SO_4, filtered, and evaporated to afford (4*R*,5*R*,6*R*)-5-fluoro-4-(2-fluoro-5-nitrophenyl)-6-(fluoromethyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.83 (s, 3H), 4.20−4.31 (m, 1H), 4.66 (dd, *J* = 46.2, 5.3 Hz, 2H), 5.27 (d, *J* = 46.8 Hz, 1H), 7.28−7.34 (m, 1H), 8.29 (d, *J* = 6.3 Hz, 1H), 8.37 (d, *J* = 6.3 Hz, 1H). To a solution of this compound in THF (20 mL) were added Boc_2O (4.36 mL, 18.8 mmol) and DMAP (306 mg, 2.51 mmol) at room temperature. The mixture was stirred at room temperature for 30 min and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0−30% EtOAc) to give di-*tert*-butyl [(*4R*,*5R*,*6R*)-5-fluoro-4-(2-fluoro-5-nitrophenyl)-6-(fluoromethyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]carbamate (3.15 g, quant. over 2 steps) as a white amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.56 (s, 18H), 1.74 (dd, *J* = 3.3, 1.8 Hz, 3H), 4.04−4.14 (m, 1H), 4.65 (dd, *J* = 46.2, 6.1 Hz, 2H), 5.22 (d, *J* = 47.3 Hz, 1H), 7.24−7.30 (m, 1H), 8.23−8.28 (m, 1H), 8.57 (dd, *J* = 6.7, 2.8 Hz, 1H). MS-ESI (*m/z*): 504 $[M + H]^+$. A mixture of this compound (1.50 g, 2.98 mmol) and Pd/C (10%; 154 mg) in MeOH (30 mL) was stirred at room temperature for 1.5 h under a hydrogen atmosphere. The mixture was filtered through Celite and evaporated to give **2-50** (1.41 g, quant.) as a white amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.53 (s, 18H), 1.70 (dd, *J* = 2.8, 1.5 Hz, 3H), 3.57 (s, 2H), 4.15−4.30 (m, 1H), 4.63 (dd, *J* = 46.3, 5.6 Hz, 2H), 5.19 (d, *J* = 47.7 Hz, 1H), 6.56 (dt, *J* = 8.5, 3.4 Hz, 1H), 6.90−6.85 (m, 2H). MS-ESI (*m/z*): 474 $[M + H]^+$.

1,3-Bis(1,1-dimethylethyl)-2-[(4*R*,5*R*,6*S*)-4-(5-amino-2-fluorophenyl)-6-(fluoromethyl)-5,6-dihydro-5-methoxy-4-methyl-4*H*-1,3-oxazin-2-yl]imidodicarbonate (2-51). To a solution of **2-48** (990 mg, 3.66 mmol) in TFA (8 mL) and H_2SO_4 (2 mL) was added HNO_3 (1.42 g/mL; 0.246 mL, 5.49 mmol) at −20 °C. The mixture was stirred at −20 °C to −10 °C for 1 h and quenched with aqueous K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated to afford a residue. To a solution of this residue in THF (10 mL) were added Boc_2O (2.46 mL, 11.4 mmol) and DMAP (232 mg, 1.90 mmol) at room temperature. The mixture was stirred at room temperature for 2.5 h and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 10−30% EtOAc) to give di-*tert*-butyl [(*4R*,*5R*,*6S*)-4-(2-fluoro-5-nitrophenyl)-6-

(fluoromethyl)-5-methoxy-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]carbamate (1.28 g, 66% over 2 steps) as a white amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.56 (s, 18H), 1.74 (dd, *J* = 3.3, 1.8 Hz, 3H), 3.71 (s, 3H), 4.04−4.14 (m, 1H), 4.65 (dd, *J* = 46.2, 6.1 Hz, 2H), 5.22 (d, *J* = 47.3 Hz, 1H), 7.24−7.30 (m, 1H), 8.23−8.28 (m, 1H), 8.57 (dd, *J* = 6.7, 2.8 Hz, 1H). MS-ESI (*m/z*): 516 $[M + H]^+$. A mixture of this compound (1.27 g, 2.46 mmol) and Pd/C (10%; 277 mg) in MeOH (10 mL) was stirred at room temperature for 2.5 h under a hydrogen atmosphere. The mixture was filtered through Celite and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 20−40% EtOAc) to give **2-51** (889 mg, 74%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.53 (s, 18H), 1.70 (dd, *J* = 2.8, 1.5 Hz, 3H), 3.57 (s, 3H), 4.15−4.30 (m, 1H), 4.63 (dd, *J* = 46.3, 5.6 Hz, 2H), 5.19 (d, *J* = 47.7 Hz, 1H), 6.56 (dt, *J* = 8.5, 3.4 Hz, 1H), 6.85−6.90 (m, 2H). MS-ESI (*m/z*): 486 $[M + H]^+$.

1,3-Bis(1,1-dimethylethyl)-2-[(4*R*,5*R*,6*R*)-4-(5-amino-2-fluorophenyl)-5-fluoro-5,6-dihydro-6-(methoxymethyl)-4-methyl-4*H*-1,3-oxazin-2-yl]imidodicarbonate (2-52). To a solution of **2-49** (103 mg, 0.381 mmol) in TFA (1 mL) and H_2SO_4 (0.25 mL) was added HNO_3 (1.42 g/mL; 0.026 mL, 0.572 mmol) at −20 °C. The mixture was stirred at 0 °C for 15 min and then quenched with aqueous K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O, dried over Na_2SO_4, filtered, and evaporated to afford a residue. To a solution of this compound in THF (2 mL) were added Boc_2O (0.265 mL, 1.14 mmol) and DMAP (18.6 mg, 0.152 mmol) at room temperature. The mixture was stirred at room temperature for 1 h and then evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0−30% EtOAc) to give di-*tert*-butyl [(*4R*,*5R*,*6R*)-5-fluoro-4-(2-fluoro-5-nitrophenyl)-6-(methoxymethyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]carbamate (171 mg, 87% over 2 steps) as a white amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.54 (s, 18H), 1.73 (s, 3H), 3.36 (s, 3H), 3.58−3.70 (m, 2H), 3.96 (dt, *J* = 28.5, 6.6 Hz, 1H), 5.19 (d, *J* = 47.0 Hz, 1H), 7.21−7.27 (m, 1H), 8.20−8.25 (m, 1H), 8.56 (dd, *J* = 6.4, 2.7 Hz, 1H). A mixture of this compound (171 mg, 0.332 mmol), Fe (148 mg, 2.65 mmol), and NH_4Cl (213 mg, 3.98 mmol) in EtOH/THF/H_2O (2:1:1, 4 mL) was heated at 60 °C for 2 h. After additional Fe (148 mg, 2.65 mmol) and NH_4Cl (213 mg, 3.98 mmol) were added, stirring was continued at 60 °C for 80 min. The reaction mixture was allowed to cool to room temperature and filtered through Celite. The aqueous layer was separated and extracted with EtOAc. The combined

organic layers were washed with H_2O, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0−40% EtOAc) to give **2-52** (108 mg, 67%) as a yellow amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.54 (s, 18H), 1.69 (s, 3H), 3.35 (s, 3H), 3.54 (s, 2H), 3.63 (d, *J* = 5.3 Hz, 2H), 4.03−4.07 (m, 1H), 5.16 (d, *J* = 47.5 Hz, 1H), 6.51−6.56 (m, 1H), 6.80−6.90 (m, 2H). MS-ESI (*m/z*): 486 $[M + H]^+$.

***N*-[3-[(*4R*,*5R*,*6R*)-2-Amino-5-fluoro-6-(fluoromethyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (2-6).** Compound **2-6** (2.75 g, 92% over 2 steps) was prepared from **2-50** (3.50 g, 7.39 mmol) following the general procedure. White solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.50 (s, 3H), 3.84−4.01 (m, 1H), 4.53 (dt, *J* = 48.7, 8.7 Hz, 1H), 4.74 (dd, *J* = 45.6, 10.0 Hz, 1H), 5.13 (d, *J* = 48.3 Hz, 1H), 5.87 (s, 2H), 7.22 (t, *J* = 10.2 Hz, 1H), 7.81−7.88 (m, 1H), 7.89−7.96 (m, 1H), 8.28 (d, *J* = 8.0 Hz, 1H), 8.59 (d, *J* = 8.0 Hz, 1H), 9.21 (s, 1H), 10.95 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 24.8, 57.8 (d, *J* = 16.0 Hz), 72.3 (d, *J* = 18.2 Hz), 81.6 (d, *J* = 164.0 Hz), 84.9 (d, *J* = 183.0 Hz), 112.6, 115.9, 117.3 (d, *J* = 24.4 Hz), 120.9, 121.4 (d, *J* = 4.4 Hz), 122.4, 132.4, 133.8, 141.3, 150.4, 150.7, 152.1, 155.9 (d, *J* = 242.8 Hz), 160.1. HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{19}H_{17}F_3N_5O_2$, 404.1329; found 404.1322.

***N*-[3-[(*4R*,*5R*,*6S*)-2-Amino-6-(fluoromethyl)-5-methoxy-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (2-7).** Compound **2-7** (103 mg, 82% over 2 steps) was prepared from **2-51** (150 mg, 0.309 mmol) following the general procedure. White solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.69 (s, 3H), 3.66 (s, 3H), 3.95 (s, 1H), 4.04−4.12 (m, 1H), 4.48−4.53 (m, 1H), 4.60−4.64 (m, 1H), 7.11 (dd, *J* = 11.4, 9.1 Hz, 1H), 7.45 (dd, *J* = 7.1, 2.8 Hz, 1H), 8.04−8.09 (m, 1H), 8.20 (dd, *J* = 8.1, 1.8 Hz, 1H), 8.42 (d, *J* = 8.1 Hz, 1H), 8.90 (d, *J* = 1.0 Hz, 1H), 9.87 (s, 1H). MS-ESI (*m/z*): 416 $[M + H]^+$.

***N*-[3-[(*4R*,*5R*,*6R*)-2-Amino-5-fluoro-6-(methoxymethyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (2-8).** Compound **2-8** (38.0 mg, 74% over 2 steps) was prepared from **2-52** (60.0 mg, 0.124 mmol) following the general procedure. Yellow powder. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.67 (s, 3H), 3.36 (s, 3H), 3.55−3.66 (m, 2H), 3.96 (dt, *J* = 29.8, 5.8 Hz, 1H), 5.16 (d, *J* = 48.0 Hz, 1H), 7.09 (dd, *J* = 11.1, 8.8 Hz, 1H), 7.50 (dd, *J* = 6.8, 2.8 Hz, 1H), 8.01−8.06

(m, 1H), 8.20 (dd, *J* = 8.1, 1.8 Hz, 1H), 8.42 (d, *J* = 8.1 Hz, 1H), 8.90 (s, 1H), 9.85 (s, 1H). MS-ESI (*m/z*): 416 $[M + H]^+$.

Ethyl (*2R*,*3R*)-3-[3,3-bis(2,4-dimethoxybenzyl)ureido]-2-fluoro-3-(2-fluorophenyl)butanoate (2-54). A mixture of **2-53** (15.0 g, 43.2 mmol) and HCl (4 M in 1,4-dioxane; 15.1 mL, 60.4 mmol) in MeOH (150 mL) was stirred at room temperature for 1 h. The mixture was quenched with aqueous $NaHCO_3$ solution, and the aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to afford a residue. To a solution of this residue in EtOAc/H_2O (2:1, 150 mL) were added $NaHCO_3$ (12.7 g, 151 mmol) and 4-nitrophenyl carbonochloridate (8.71 g, 43.2 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, and bis(2,4-dimethoxybenzyl)amine (13.7 g, 43.2 mmol) was then added at the same temperature. After being stirred for 1 h at 0 °C, the mixture was diluted with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with aqueous K_2CO_3 solution, H_2O, and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33−50% EtOAc) to give **2-54** (25.3 g, quant. over 2 steps) as a pale brown amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.97 (t, *J* = 7.2 Hz, 3H), 1.95 (s, 3H), 3.80 (s, 6H), 3.81 (s, 6H), 3.87 (dq, *J* = 10.6, 7.2 Hz, 1H), 4.01 (dq, *J* = 10.6, 7.2 Hz, 1H), 4.33 (d, *J* = 16.2 Hz, 2H), 4.42 (d, *J* = 16.2 Hz, 2H), 5.58 (d, *J* = 47.8 Hz, 1H), 6.07 (s, 1H), 6.42−6.49 (m, 4H), 6.98−7.03 (m, 1H), 7.06−7.10 (m, 1H), 7.18 (d, *J* = 8.8 Hz, 2H), 7.21−7.26 (m, 1H), 7.34−7.38 (m, 1H). MS-ESI (*m/z*): 587 $[M + H]^+$.

1,1-Bis(2,4-dimethoxybenzyl)-3-[(*2R*,*3R*)-3-fluoro-2-(2-fluorophenyl)-4-oxobutan-2-yl]urea (2-55). To a solution of **2-54** (25.3 g, 43.2 mmol) in DCM (125 mL) was added dropwise DIBAL (1.02 M in toluene; 127 mL, 130 mmol) at −78 °C. The mixture was stirred at −78 °C for 1 h and then quenched with EtOAc and aqueous Rochelle's salt solution. After being stirred for 2 h at room temperature, the aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33−50% EtOAc) to give **2-55** (19.5 g, 83%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.67 (s, 3H), 3.80 (s, 6H), 3.81 (s, 6H), 4.36 (d, *J* = 15.9 Hz, 2H), 4.43 (d, *J* = 15.9 Hz, 2H), 5.76 (d, *J* = 46.9 Hz, 1H), 5.77 (s, 1H),

6.43−6.51 (m, 4H), 7.00−7.20 (m, 4H), 7.22−7.35 (m, 2H), 9.51 (d, *J* = 10.0 Hz, 1H). MS-ESI (*m/z*): 543 $[M + H]^+$.

1,1-Bis(2,4-dimethoxybenzyl)-3-[(*2R*,*3S*,*EZ*)-3-fluoro-2-(2-fluorophenyl)hex-4-en-2-yl]urea (2-56). To a solution of ethyltriphenylphosphonium bromide (28.2 g, 76.0 mmol) and KHMDS (0.5 M in toluene; 143 mL, 71.3 mmol) in THF (129 mL) was added **2-55** (12.9 g, 23.8 mmol) in THF (80 mL) rapidly at 0 °C. The mixture was stirred at the same temperature for 30 min and then heated at 50 °C. After being stirred for 1 h at the same temperature, the reaction mixture was allowed to cool at 0 °C. The mixture quenched with H_2O at 0 °C, and the aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33−50% EtOAc) to give **2-56** (12.4 g, 94%, *E*:*Z* = 2:3) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$,) δ 1.52−1.57 (m, 3H), 1.82 (s, 1.2H), 1.87 (s, 1.8H), 3.78 (s, 6H), 3.81 (s, 6H), 4.31−4.47 (m, 4H), 5.27−5.35 (m, 1H), 5.45 (dd, *J* = 47.3, 6.8 Hz, 0.4H), 5.58−5.83 (m, 2.6H), 6.40−6.49 (m, 4H), 6.94−7.00 (m, 1H), 7.05−7.09 (m, 1H), 7.10−7.17 (m, 2H), 7.18−7.21 (m, 1H), 7.35−7.40 (m, 1H). MS-ESI (*m/z*): 555 $[M + H]^+$.

(*4R*,*5R*,*6S*)-*N*,*N*-Bis(2,4-dimethoxybenzyl)-5-fluoro-4-(2-fluorophenyl)-6-[(*S*)-1-iodoethyl]-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-57). To a solution of iodine (11.4 g, 44.7 mmol) in MeCN (500 mL) was added a solution of **2-56** (12.4 g, 22.4 mmol, *E*:*Z* = 2:3) in MeCN (125 mL) at 0 °C. After being stirred at the same temperature for 1.5 h, the mixture was quenched with aqueous $Na_2S_2O_3$ solution and then aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33−50% EtOAc) to give **2-57** (8.81 g, 58%) as a colorless amorphous and **2-56** (3.17 g, 26%, *E* isomer) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$, **2-57**) δ 1.63 (s, 3H), 1.84 (d, *J* = 7.0 Hz, 3H), 3.63 (dd, *J* = 28.6, 9.2 Hz, 1H), 3.75 (s, 6H), 3.82 (s, 6H), 4.08−4.16 (m, 1H), 4.49 (d, *J* = 15.9 Hz, 2H), 4.65 (d, *J* = 15.9 Hz, 2H), 5.37 (d, *J* = 48.8 Hz, 1H), 6.41−6.50 (m, 4H), 7.00 (dd, *J* = 11.9, 8.3 Hz, 1H), 7.06 (dd, *J* = 7.4, 7.4 Hz, 1H), 7.16−7.28 (m, 3H), 7.42 (dd, *J* = 8.3, 7.4 Hz, 1H). MS-ESI (*m/z*): 681 $[M + H]^+$. ^{1}H-NMR (400 MHz, $CDCl_3$, **2-56**, *E* isomer) δ 1.52−1.57 (m, 3H), 1.82 (s, 3H), 3.78 (s, 6H), 3.81 (s, 6H), 4.33−4.45 (m, 4H), 5.30 (ddd, *J* = 15.2, 14.0, 7.0 Hz, 1H), 5.45 (dd,

J = 47.3, 6.8 Hz, 1H), 5.60 (s, 1H), 5.70 (ddd, *J* = 15.2, 6.4, 3.6 Hz, 1H), 6.40−6.49 (m, 4H), 6.98 (dd, *J* = 12.5, 8.2 Hz, 1H), 7.05−7.09 (m, 1H), 7.13 (d, *J* = 8.5 Hz, 2H), 7.18−7.21 (m, 1H), 7.38 (dd, *J* = 8.2, 8.0 Hz, 1H). MS-ESI (*m/z*): 555 $[M + H]^+$.

(*4R*,*5R*,*6S*)-*N*,*N*-Bis(2,4-dimethoxybenzyl)-5-fluoro-4-(2-fluorophenyl)-6-[(*R*)-1-iodoethyl]-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-58). To a solution of iodine (1.05 g, 4.15 mmol) in MeCN (45 mL) was added a solution of **2-56** (1.15 g, 2.07 mmol, *E* isomer) in MeCN (15 mL) at 0 °C. After being stirred at room temperature for 20 h, the mixture was quenched with aqueous $NaHCO_3$ solution and then aqueous $Na_2S_2O_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33−50% EtOAc) to give **2-58** (1.20 g, 85%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.65 (s, 3H), 1.74 (d, *J* = 6.8 Hz, 3H), 3.61 (dd, *J* = 27.5, 10.0 Hz, 1H), 3.74 (s, 6H), 3.81 (s, 6H), 4.07−4.15 (m, 1H), 4.45 (d, *J* = 16.1 Hz, 2H), 4.61 (d, *J* = 16.1 Hz, 2H), 5.66 (d, *J* = 48.3 Hz, 1H), 6.41−6.49 (m, 4H), 7.00−7.08 (m, 2H), 7.19 (d, *J* = 8.2 Hz, 2H), 7.23−7.26 (m, 1H), 7.39−7.43 (m, 1H). MS-ESI (*m/z*): 681 $[M + H]^+$.

(*4R*,*5R*,*6R*)-5-Fluoro-6-[(*S*)-1-fluoroethyl]-4-(2-fluorophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-59). To a mixture of KO_2 (1.47 g, 20.6 mmol) and 18-crown-6 (5.45 g, 20.6 mmol) in DMSO (20 mL) was added **2-57** (3.51 g, 5.16 mmol) in DMSO (20 mL) at room temperature. The mixture was stirred at the same temperature for 25 min and poured into aqueous saturated $Na_2S_2O_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33−50% EtOAc) to give (*R*)-1-((*4R*,*5R*,*6R*)-2-(bis(2,4-dimethoxybenzyl)amino)-5-fluoro-4-(2-fluorophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-6-yl)ethan-1-ol (817 mg, 28%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.07 (d, *J* = 6.3 Hz, 3H), 1.66 (s, 3H), 3.35 (dd, *J* = 30.4, 7.8 Hz, 1H), 3.75 (s, 6H), 3.81 (s, 6H), 3.81−3.88 (m, 1H), 4.45 (d, *J* = 15.9 Hz, 2H), 4.61 (d, *J* = 15.9 Hz, 2H), 5.38 (d, *J* = 48.6 Hz, 1H), 6.41−6.50 (m, 4H), 6.98 (dd, *J* = 12.0, 8.2 Hz, 1H), 7.05 (dd, *J* = 7.7, 7.3 Hz, 1H), 7.17−7.28 (m, 3H), 7.42 (dd, *J* = 8.2, 7.7 Hz, 1H). MS-ESI (*m/z*): 571 $[M + H]^+$. A mixture of this compound (1.42 g, 2.49 mmol), nonafluorobutanesulfonyl fluoride (1.61 mL, 8.96 mmol), and DBU (1.34 mL, 8.96 mmol)

was stirred at room temperature for 12 h. The mixture was quenched with aqueous saturated NH_4Cl solution and 2 M HCl solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with 2 M NaOH solution, brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 20−100% EtOAc) to give (*4R,5R,6R*)-*N*,*N*-bis(2,4-dimethoxybenzyl)-5-fluoro-6-[(*S*)-1-fluoroethyl]-4-(2-fluorophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (1.00 g) as a colorless amorphous. MS-ESI (*m/z*): 573 $[M + H]^+$. A mixture of this compound (1.00 g) and anisole (1.34 mL, 12.2 mmol) in TFA (6.7 mL) was heated at 80 °C for 14 h. The mixture was allowed to cool to 0 °C and then quenched with aqueous K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50−100% EtOAc) to give **2-59** (252 mg, 37% over 2 steps) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.40 (dd, *J* = 24.1, 6.4 Hz, 3H), 1.77 (s, 3H), 3.89 (ddd, *J* = 30.2, 12.9, 7.8 Hz, 1H), 4.88 (ddq, *J* = 49.0, 7.8, 6.4 Hz, 1H), 5.25 (d, *J* = 47.2 Hz, 1H), 7.10 (dd, *J* = 12.3, 8.2 Hz, 1H), 7.26−7.29 (m, 1H), 7.32−7.45 (m, 2H). MS-ESI (*m/z*): 273 $[M + H]^+$.

(*4R*,*5R*,*6R*)-5-Fluoro-6-[(*R*)-1-fluoroethyl]-4-(2-fluorophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-60). To a mixture of KO_2 (435 mg, 6.11 mmol) and 18-crown-6 (1.62 g, 6.11 mmol) in DMSO (13 mL) was added **2-58** (1.04 g, 1.53 mmol) in DMSO (12 mL) at room temperature. The mixture was stirred at the same temperature for 30 min and poured into aqueous saturated $Na_2S_2O_3$ solution at 0 °C. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33−50% EtOAc) to give (*S*)-1-[(*4R*,*5R*,*6R*)-2-[bis(2,4-dimethoxybenzyl)amino]-5-fluoro-4-(2-fluorophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-6-yl]ethan-1-ol (291 mg, 33%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.07 (d, *J* = 6.3 Hz, 3H), 1.62 (s, 3H), 2.25 (brs, 1H), 3.34 (dd, *J* = 30.9, 8.0 Hz, 1H), 3.76 (s, 6H), 3.81 (s, 6H), 3.81−3.85 (m, 1H), 4.49 (d, *J* = 15.9 Hz, 2H), 4.67 (d, *J* = 15.9 Hz, 2H), 5.14 (d, *J* = 48.8 Hz, 1H), 6.42−6.51 (m, 4H), 6.97−7.05 (m, 2H), 7.17−7.29 (m, 3H), 7.35−7.39 (m, 1H). MS-ESI (*m/z*): 571 $[M + H]^+$. A solution of this compound (290 mg, 0.508 mmol) in DCM (6 mL) was added DAST (0.269 mL, 2.03 mmol) at −78 °C. After being stirred at room temperature, additional DAST

(0.130 mL, 1.02 mmol) was added. The mixture was stirred at room temperature for 23 h and then quenched with aqueous saturated $NaHCO_3$ solution at 0 °C. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33−50% EtOAc) to give (*4R,5R,6R*)-*N*,*N*-bis(2,4-dimethoxybenzyl)-5-fluoro-6-[(*R*)-1-fluoroethyl]-4-(2-fluorophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (153 mg, 53%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.17 (dd, *J* = 25.5, 6.1 Hz, 3H), 1.66 (s, 3H), 3.40−3.59 (m, 1H), 3.75 (s, 6H), 3.81 (s, 6H), 4.38−4.75 (m, 5H), 5.30 (d, *J* = 48.3 Hz, 1H), 6.40−6.50 (m, 4H), 6.93−7.08 (m, 2H), 7.15−7.29 (m, 3H), 7.39−7.43 (m, 1H). MS-ESI (*m/z*): 573 $[M + H]^+$. A mixture of this compound (237 mg, 0.413 mmol) and anisole (0.32 mL, 2.89 mmol) in TFA (1.6 mL) was heated at 80 °C for 17 h. The mixture was allowed to cool to 0 °C and then quenched with aqueous K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50−80% EtOAc) to give **2-60** (103 mg, 92%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.44 (dd, *J* = 25.3, 6.0 Hz, 3H), 1.85 (s, 3H), 3.79−3.89 (m, 1H), 4.79−4.94 (m, 1H), 5.44 (d, *J* = 46.4 Hz, 1H), 7.10−7.15 (m, 1H), 7.23−7.26 (m, 1H), 7.33−7.43 (m, 2H). MS-ESI (*m/z*): 273 $[M + H]^+$.

1,3-Bis(1,1-dimethylethyl)-2-[(4*R*,5*R*,6*R*)-4-(5-amino-2-fluorophenyl)-5-fluoro-6-[(1*S*)-1-fluoroethyl]-5,6-dihydro-4-methyl-4*H*-1,3-oxazin-2-yl]imidodicarbonate (2-61). To a solution of **2-59** (252 mg, 0.925 mmol) in H_2SO_4 (0.6 mL) and TFA (2.4 mL) was added HNO_3 (1.42 g/mL; 0.062 mL, 1.39 mmol) at −20 °C. The mixture was stirred at 0 °C for 30 min and quenched with aqueous K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50−100% EtOAc) to give (*4R*,*5R*,*6R*)-5-fluoro-4-(2-fluoro-5-nitrophenyl)-6-[(*S*)-1-fluoroethyl]-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (240 mg, 82%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.39 (dd, *J* = 24.2, 6.4 Hz, 3H), 1.65 (s, 3H), 3.67 (ddd, *J* = 30.9, 13.2, 7.4 Hz, 1H), 4.42 (brs, 2H), 4.86 (ddq, *J* = 48.8, 7.4, 6.4 Hz, 1H), 5.15 (d, *J* = 47.7 Hz, 1H), 7.21−7.26 (m, 1H), 8.20−8.24 (m, 1H), 8.44−8.47

(m, 1H). MS-ESI (*m/z*): 318 $[M + H]^+$. A solution of this compound (240 mg, 0.757 mmol), Boc_2O (0.439 mL, 1.89 mmol), and DMAP (18.5 mg, 0.151 mmol) in DCM (2.4 mL) was stirred at room temperature for 35 min and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 9−20% EtOAc) and then recrystallized from hexane/EtOAc to give di-*tert*-butyl [(*4R*,*5R*,*6R*)-5-fluoro-4-(2-fluoro-5-nitrophenyl)-6-[(*S*)-1-fluoroethyl]-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]carbamate (245 mg, 63%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.40 (dd, *J* = 24.1, 6.5 Hz, 3H), 1.52 (s, 18H), 1.72 (s, 3H), 3.80 (ddd, *J* = 29.9, 12.9, 7.2 Hz, 1H), 4.91 (ddq, *J* = 48.1, 7.2, 6.5 Hz, 1H), 5.19 (d, *J* = 47.2 Hz, 1H), 7.25−7.30 (m, 1H), 8.24−8.28 (m, 1H), 8.56−8.58 (m, 1H). MS-ESI (*m/z*): 518 $[M + H]^+$. A mixture of this compound (241 mg, 0.466 mmol) and Pd/C (10%; 24.8 mg) in THF/MeOH (2:1, 4.5 mL) was stirred at room temperature for 2 h under a hydrogen atmosphere. The mixture was filtered through Celite and then evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, 50% EtOAc) to give **2-61** (214 mg, 94%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.39 (dd, *J* = 24.1, 6.4 Hz, 3H), 1.52 (s, 18H), 1.68 (s, 3H), 3.58 (brs, 2H), 3.91 (ddd, *J* = 30.0, 13.4, 7.4 Hz, 1H), 4.88 (ddq, *J* = 48.8, 7.4, 6.4 Hz, 1H), 5.16 (d, *J* = 47.7 Hz, 1H), 6.55−6.57 (m, 1H), 6.82−6.91 (m, 2H). MS-ESI (*m/z*): 488 $[M + H]^+$.

1,3-Bis(1,1-dimethylethyl)-2-[(4*R*,5*R*,6*R*)-4-(5-amino-2-fluorophenyl)-5-fluoro-6-[(1*R*)-1-fluoroethyl]-5,6-dihydro-4-methyl-4*H*-1,3-oxazin-2-yl]imidodicarbonate (2-62). To a solution of **2-60** (103 mg, 0.378 mmol) in TFA (1.2 mL) and H_2SO_4 (0.3 mL) was added HNO_3 (1.42 g/mL; 0.025 mL, 0.567 mmol) at −20 °C. The mixture was stirred at 0 °C for 30 min and quenched with aqueous K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50−100% EtOAc) to give (*4R*,*5R*,*6R*)-5-fluoro-4-(2-fluoro-5-nitrophenyl)-6-((*R*)-1-fluoroethyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (86.3 mg, 72%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.40 (dd, *J* = 25.5, 6.1 Hz, 3H), 1.67 (s, 3H), 3.54−3.79 (m, 1H), 4.29 (brs, 2H), 4.72−4.91 (m, 1H), 5.30 (d, *J* = 47.2 Hz, 1H), 7.20−7.26 (m, 1H), 8.20−8.22 (m, 1H), 8.43−8.44 (m, 1H). MS-ESI (*m/z*): 318 $[M + H]^+$. A solution of this compound (86.3 mg, 0.272 mmol), Boc_2O (0.158 mL, 0.680 mmol), and DMAP (16.6 mg, 0.135 mmol) in DCM (2 mL) was stirred at room temperature for 50 min and then

evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, 25% EtOAc) to give di-*tert*-butyl [(*4R*,*5R*,*6R*)-5-fluoro-4-(2-fluoro-5-nitrophenyl)-6-[(*R*)-1-fluoroethyl]-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]carbamate (129 mg, 91%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.41 (dd, *J* = 25.5, 6.1 Hz, 3H), 1.53 (s, 18H), 1.74 (s, 3H), 3.65 (ddd, *J* = 27.4, 8.3, 4.8 Hz, 1H), 4.87 (ddq, *J* = 46.7, 8.3, 6.1 Hz, 1H), 5.35 (d, *J* = 46.9 Hz, 1H), 7.25−7.30 (m, 1H), 8.25−8.27 (m, 1H), 8.51−8.52 (m, 1H). MS-ESI (*m/z*): 518 $[M + H]^+$. A mixture of this compound (129 mg, 0.248 mmol) and Pd/C (10%, 26.4 mg) in THF/MeOH (2:1, 3 mL) was stirred at room temperature for 8 h under a hydrogen atmosphere. The mixture was filtered through Celite and then evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, 50% EtOAc) to give **2-62** (104 mg, 86%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.40 (dd, *J* = 25.4, 6.1 Hz, 3H), 1.53 (s, 18H), 1.70 (s, 3H), 3.56 (brs, 2H), 3.75−3.85 (m, 1H), 4.75−4.93 (m, 1H), 5.33 (d, *J* = 47.3 Hz, 1H), 6.55−6.57 (m, 1H), 6.83−6.89 (m, 2H). MS-ESI (*m/z*): 488 $[M + H]^+$.

***N*-[3-[(*4R*,*5R*,*6R*)-2-Amino-5-fluoro-6-[(*S*)-1-fluoroethyl]-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-(fluoromethoxy)pyrazine-2-carboxamide (2-16).** Compound **2-16** (55.6 mg, 88% over 2 steps) was prepared from **2-61** (70.0 mg 0.144 mmol) following the general procedure. White solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.38 (dd, *J* = 24.2, 6.4 Hz, 3H), 1.65 (s, 3H), 3.77 (ddd, *J* = 30.9, 13.4, 7.7 Hz, 1H), 4.33 (brs, 2H), 4.85 (ddq, *J* = 49.1, 7.7, 6.4 Hz, 1H), 5.18 (d, *J* = 47.9 Hz, 1H), 6.15 (d, *J* = 51.1 Hz, 2H), 7.11 (dd, *J* = 11.4, 8.9 Hz, 1H), 7.46 (dd, *J* = 6.8, 2.5 Hz, 1H), 8.04−8.07 (m, 1H), 8.30 (s, 1H), 9.08 (s, 1H), 9.52 (s, 1H). MS-ESI (*m/z*): 442 $[M + H]^+$.

***N*-[3-[(*4R*,*5R*,*6R*)-2-Amino-5-fluoro-6-[(*R*)-1-fluoroethyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-(fluoromethoxy)pyrazine-2-carboxamide (2-17).** Compound **2-17** (31.0 mg, 77% over 2 steps) was prepared from **2-62** (44.2 mg 0.091 mmol) following the general procedure. White solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.39 (dd, *J* = 25.3, 6.1 Hz, 3H), 1.68 (s, 3H), 3.61−3.72 (m, 1H), 4.73−4.88 (m, 1H), 5.35 (d, *J* = 47.6 Hz, 1H), 6.15 (dd, *J* = 51.0, 5.9 Hz, 2H), 7.08−7.13 (m, 1H), 7.51−7.52 (m, 1H), 7.97−7.99 (m, 1H), 8.29 (s, 1H), 9.09 (s, 1H), 9.52 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 17.3, 24.9, 57.8, 74.9, 84.3, 86.3, 95.8, 117.1, 117.4, 120.9, 132.7, 133.1, 134.0, 139.7, 141.8, 150.4, 155.8, 159.4, 160.4. MS-ESI (*m/z*): 442 $[M + H]^+$. HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{19}H_{20}F_4N_5O_3$, 442.1497; found 442.1490.

Ethyl-(*2R*,*3R*)-3-[(*tert*-butoxycarbonyl)amino]-2-fluoro-3-(2-fluorophenyl)butanoate (2-63). A mixture of **2-53** (16.1 g, 46.5 mmol) and HCl (4 M in 1,4-dioxane; 16.3 mL, 65.0 mmol) in MeOH (160 mL) was stirred at room temperature for 1.5 h. The mixture was quenched with aqueous $NaHCO_3$ solution, and then the aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. To a solution of this compound in MeOH (55 mL) was added Boc_2O (32.4 mL, 46.5 mmol) at room temperature. The mixture was heated at 60 °C for 4 h. The mixture was allowed to cool to room temperature and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33−50% EtOAc) to give **2-63** (21.4 g, 94%) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.03 (brs, 3H), 1.39 (brs, 9H), 1.96 (brs, 3H), 4.06 (brs, 2H), 5.34 (d, J = 47.2 Hz, 1H), 5.70 (brs, 1H), 7.01−7.06 (m, 1H), 7.09−7.13 (m, 1H), 7.25−7.35 (m, 2H). MS-ESI (*m/z*): 344 $[M + H]^+$.

***tert*-Butyl-[(*2R*,*3R*)-3-fluoro-2-(2-fluorophenyl)-4-oxobutan-2-yl]carbamate (2-64).** To a solution of **2-63** (11.4 g, 33.2 mmol) in DCM (110 mL) added DIBAL (1.02 M in toluene; 107 mL, 109 mmol) dropwise at −78 °C. After being stirred at −78 °C for 50 min, the mixture was quenched with EtOAc and aqueous Rochelle's salt solution and then stirred at room temperature for 1.5 h. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to give **2-64** (7.87 g, 79%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.39 (s, 9H), 1.74 (s, 3H), 5.07 (s, 1H), 5.53 (d, J = 46.8 Hz, 1H), 7.00−7.09 (m, 1H), 7.11−7.18 (m, 1H), 7.26−7.39 (m, 2H), 9.55 (d, J = 9.0 Hz, 1H). MS-ESI (*m/z*): 300 $[M + H]^+$

Ethyl-2,2-difluoro-2-[(*4R*,*5R*,*6S*)-5-fluoro-4-(2-fluorophenyl)-4-methyl-2-oxo-1,3-oxazinan-6-yl]acetate (2-65). A mixture of **2-64** (7.87 g, crude), zinc (4.84 g, 74.1 mmol), and 2-bromo-2,2-difluoroacetate (15.0 g, 74.1 mmol) in THF (150 mL) was heated at 70 °C for 1.5 h. The mixture was allowed to cool to 0 °C and then quenched with saturated aqueous NH_4Cl solution. The mixture was filtered through Celite. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33−50% EtOAc) to give **2-65** (1.40 g, 15%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.34 (t, J = 7.2 Hz, 3H), 1.78 (s, 3H), 4.34 (q, J = 7.2 Hz, 2H), 4.40 (ddd, J = 28.5, 13.7, 6.3 Hz, 1H), 5.49 (d, J = 47.4

Hz, 1H), 5.83 (s, 1H), 7.13−7.18 (m, 1H), 7.26−7.30 (m, 1H), 7.38−7.45 (m, 1H), 7.49−7.53 (m, 1H). MS-ESI (*m/z*): 350 $[M + H]^+$.

(*4R*,*5R*,*6S*)-6-(1,1-Difluoro-2-iodoethyl)-5-fluoro-4-(2-fluorophenyl)-4-methyl-1,3-oxazinan-2-one (2-66). A mixture of **2-65** (1.40 g, 4.01 mmol) and $LiBH_4$ (175 mg, 8.02 mmol) in THF (28 mL) was stirred at room temperature for 45 min. The mixture was quenched with saturated aqueous NH_4Cl solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50−100% EtOAc) to give (*4R*,*5R*,*6S*)-6-(1,1-difluoro-2-hydroxyethyl)-5-fluoro-4-(2-fluorophenyl)-4-methyl-1,3-oxazinan-2-one (740 mg, 60%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.79 (s, 3H), 1.97 (t, *J* = 7.1 Hz, 1H), 3.84−4.11 (m, 2H), 4.28 (ddd, *J* = 30.0, 13.2, 6.4 Hz, 1H), 5.47 (d, *J* = 46.9 Hz, 1H), 5.69 (s, 1H), 7.12−7.17 (m, 1H), 7.25−7.29 (m, 1H), 7.39−7.44 (m, 1H), 7.48−7.52 (m, 1H). A mixture of this compound (850 mg, 2.77 mmol), PPh_3 (2.90 g, 11.1 mmol), imidazole (753 mg, 11.1 mmol), and iodine (2.81 g, 11.1 mmol) in THF (17 mL) was heated at 80 °C for 16 h. The mixture was allowed to cool to 0 °C and then quenched with aqueous $Na_2S_2O_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50−100% EtOAc) to give **2-66** (1.07 g, 93%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.79 (s, 3H), 3.56−3.74 (m, 2H), 4.29 (dt, *J* = 29.5, 7.6 Hz, 1H), 5.41 (d, *J* = 47.2 Hz, 1H), 5.72 (s, 1H), 7.14−7.19 (m, 1H), 7.26−7.30 (m, 1H), 7.40−7.45 (m, 1H), 7.48−7.52 (m, 1H). MS-ESI (*m/z*): 417 $[M + H]^+$.

***tert*-Butyl-(*4R*,*5R*,*6S*)-6-(1,1-difluoroethyl)-5-fluoro-4-(2-fluorophenyl)-4-methyl-2-oxo-1,3-oxazinane-3-carboxylate (2-67).** A mixture of **2-66** (1.07 g, 2.57 mmol), Boc_2O (1.19 mL, 5.13 mmol), DMAP (157 mg, 1.28 mmol) in DCM (20 mL) was stirred at room temperature for 45 min. The mixture was evaporated, and the resulting residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33−50% EtOAc) to give *tert*-butyl (*4R*,*5R*,*6S*)-6-(1,1-difluoro-2-iodoethyl)-5-fluoro-4-(2-fluorophenyl)-4-methyl-2-oxo-1,3-oxazinane-3-carboxylate (1.33 g, quant.) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H), 1.97 (s, 3H), 3.56−3.74 (m, 2H), 4.31 (dt, *J* = 30.6, 7.6 Hz, 1H), 5.32 (d, *J* = 46.9 Hz, 1H), 7.12−7.17 (m, 1H), 7.25−7.29 (m, 1H),

7.39−7.44 (m, 1H), 7.46−7.50 (m, 1H). A mixture of this compound (1.33 g, 2.57 mmol), *n*-Bu_3SnH (1.64 mL, 6.16 mmol), and AIBN (63.2 mg, 0.385 mmol) in toluene (26 mL) was heated at 80 °C for 1 h. The mixture was allowed to cool to room temperature and evaporated. The residue was purified by flash column chromatography (amino silica gel; EtOAc/hexane, gradient: 0−80% EtOAc) to give **2-67** (717 mg, 71%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H), 1.75 (t, *J* = 19.8 Hz, 3H), 1.97 (s, 3H), 4.05 (ddd, *J* = 30.7, 10.4, 4.8 Hz, 1H), 5.27 (d, *J* = 46.9 Hz, 1H), 7.10−7.15 (m, 1H), 7.23−7.27 (m, 1H), 7.37−7.42 (m, 1H), 7.46−7.50 (m, 1H). MS-ESI (*m/z*): 392 $[M + H]^+$.

***tert*-Butyl-[(*2R*,*3R*,*4S*)-3,5,5-trifluoro-2-(2-fluorophenyl)-4-hydroxyhexan-2-yl]carbamate (2-68).** A mixture of **2-67** (717 mg, 1.83 mmol) and $Ba(OH)_2{\cdot}8H_2O$ (1.73 g, 5.50 mmol) in EtOH/H_2O (2:1, 18 mL) was stirred at room temperature for 1.5 h. The mixture was quenched with aqueous citric acid solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33−50% EtOAc) to give **2-68** (630 mg, 94%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.61 (t, *J* = 19.3 Hz, 3H), 2.02 (s, 3H), 2.67 (dd, *J* = 9.3, 3.8 Hz, 1H), 3.62 (brs, 1H), 5.14 (d, *J* = 44.5 Hz, 1H), 6.27 (brs, 1H), 7.02−7.08 (m, 1H), 7.15−7.18 (m, 1H), 7.26−7.32 (m, 1H), 7.37−7.41 (m, 1H).

***N*-[[(*2R*,*3R*,*4S*)-3,5,5-Trifluoro-2-(2-fluorophenyl)-4-hydroxyhexan-2-yl]carbamothioyl]benzamide (2-69).** A mixture of **2-68** (630 mg, 1.73 mmol) and HCl (4 M in 1,4-dioxane; 1.73 mL, 6.90 mmol) in MeOH (6 mL) was heated at 50 °C for 2.5 h. The mixture was cooled to room temperature and evaporated. The residue was quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. A mixture of the residue and benzoyl isothiocyanate (0.348 mL, 2.59 mmol) in DCM (3 mL) was stirred at room temperature for 3 h and then evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33−50% EtOAc) to give **2-69** (631 mg, 85% over 2 steps) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.69 (t, *J* = 19.2 Hz, 3H), 2.31 (s, 3H), 2.74 (dd, *J* = 9.9, 2.9 Hz, 1H), 3.83−3.92 (m, 1H), 5.41 (d, *J* = 44.4 Hz, 1H), 7.05−7.10 (m, 1H), 7.16−7.20 (m, 1H), 7.31−7.35 (m, 1H), 7.45−7.49 (m, 1H), 7.50−7.54 (m, 2H), 7.62−7.65 (m, 1H), 7.87 (d, *J* = 7.3 Hz, 2H), 8.91 (s, 1H), 11.80 (s, 1H). MS-ESI (*m/z*): 429 $[M + H]^+$.

***N*-[(*4R*,*5R*,*6S*)-6-(1,1-Difluoroethyl)-5-fluoro-4-(2-fluorophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]benzamide (2-70).** A mixture of **2-69** (631 mg, 1.47 mmol) and EDC·HCl (565 mg, 2.95 mmol) in MeCN (12 mL) was heated at 50 °C for 1.5 h. The mixture was allowed to cool to room temperature and then diluted with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33−50% EtOAc) to give **2-70** (564 mg, 97%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.83 (td, *J* = 19.7, 1.9 Hz, 3H), 1.89 (s, 3H), 4.11 (ddd, *J* = 29.1, 10.0, 5.0 Hz, 1H), 5.52 (d, *J* = 47.2 Hz, 1H), 7.14−7.19 (m, 1H), 7.22−7.26 (m, 1H), 7.38−7.48 (m, 4H), 7.51−7.55 (m, 1H), 8.27 (d, *J* = 8.0 Hz, 2H), 11.80 (s, 1H). MS-ESI (*m/z*): 395 $[M + H]^+$.

(*4R*,*5R*,*6S*)-6-(1,1-Difluoroethyl)-5-fluoro-4-(2-fluorophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-71). A mixture of **2-70** (554 mg, 1.41 mmol) and K_2CO_3 (1.17 g, 8.43 mmol) in MeOH (22 mL) was heated at 80 °C for 3 h. The mixture was allowed to cool to room temperature and then diluted with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50−80% EtOAc) to give **80** (375 mg, 92%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.66 (s, 3H), 1.71 (t, *J* = 19.7 Hz, 3H), 3.83 (ddd, *J* = 30.0, 10.8, 4.4 Hz, 1H), 4.29 (brs, 2H), 5.35 (d, *J* = 48.2 Hz, 1H), 7.03−7.08 (m, 1H), 7.15−7.19 (m, 1H), 7.26−7.30 (m, 1H), 7.42−7.46 (m, 1H). MS-ESI (*m/z*): 291 $[M + H]^+$.

1,3-Bis(1,1-dimethylethyl)-2-[(4*R*,5*R*,6*S*)-4-(5-amino-2-fluorophenyl)-6-(1,1-difluoroethyl)-5-fluoro-5,6-dihydro-4-methyl-4*H*-1,3-oxazin-2-yl]imidodicarbonate (2-72). To a solution of **2-71** (360 mg, 1.24 mmol) in H_2SO_4 (1 mL) and TFA (4 mL) was added HNO_3 (1.42 g/mL; 0.083 mL, 1.86 mmol) at −20 °C. The mixture was stirred at 0 °C for 15 min and quenched with aqueous K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to afford a residue. A mixture of the residue, Boc_2O (0.864 mL, 3.72 mmol), and DMAP (182 mg, 1.49 mmol) in DCM (7 mL) was stirred at room temperature for 1 h and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33% EtOAc) to give di-*tert*-butyl ((*4R*,*5R*,*6S*)-

6-(1,1-difluoroethyl)-5-fluoro-4-(2-fluoro-5-nitrophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl)carbamate (604 mg, 91% over 2 steps) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 18H), 1.69−1.79 (m, 3H), 1.73 (s, 3H), 3.88 (ddd, *J* = 28.9, 10.3, 4.3 Hz, 1H), 5.39 (d, *J* = 47.4 Hz, 1H), 7.26−7.31 (m, 1H), 8.26−8.28 (m, 1H), 8.50−8.52 (m, 1H). MS-ESI (*m/z*): 536 $[M + H]^+$. A mixture of this compound (604 mg, 1.13 mmol) and Pd/C (10%, 60.0 mg) in THF/MeOH (2:1, 12 mL) was stirred at room temperature for 3.5 h under a hydrogen atmosphere. The mixture was filtered through Celite and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50% EtOAc) and then recrystallized from hexane/EtOAc to give **2-72** (483 mg, 85%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 18H), 1.69 (s, 3H), 1.72 (t, *J* = 19.6 Hz, 3H), 3.56 (brs, 2H), 3.97−4.07 (m, 1H), 5.37 (d, *J* = 47.8 Hz, 1H), 6.56−6.58 (m, 1H), 6.80−6.82 (m, 1H), 6.87−7.00 (m, 1H). MS-ESI (*m/z*): 506 $[M + H]^+$.

***N*-[3-[(*4R*,*5R*,*6S*)-2-Amino-6-(1,1-difluoroethyl)-5-fluoro-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-(fluoromethoxy)pyrazine-2-carboxamide (2-14).** Compound **2-14** (57.6 mg, 91% over 2 steps) was prepared from **2-72** (70.0 mg, 0.138 mmol) following the general procedure. White solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.66 (s, 3H), 1.72 (t, *J* = 19.6 Hz, 3H), 3.89 (ddd, *J* = 29.7, 10.3, 4.5 Hz, 1H), 4.38 (brs, 2H), 5.38 (d, *J* = 48.1 Hz, 1H), 6.15 (d, *J* = 51.1 Hz, 2H), 7.09−7.14 (m, 1H), 7.50−7.51 (m, 1H), 7.98−8.04 (m, 1H), 8.29 (s, 1H), 9.08 (s, 1H), 9.51 (s, 1H). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 19.8, 23.8, 58.5, 74.8, 83.5, 95.8, 117.6, 120.4, 121.6, 131.0, 133.3, 134.4, 140.0, 141.9, 152.2, 155.4, 159.4, 160.6, 170.3. HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{19}H_{19}F_5N_5O_3$, 460.1403; found 460.1395.

***tert*-Butyl-(*4R*,*5R*,*6S*)-5-fluoro-4-(2-fluorophenyl)-6-(1-fluorovinyl)-4-methyl-2-oxo-1,3-oxazinane-3-carboxylate (2-73).** A mixture of **2-66** (538 mg, 1.29 mmol) and zinc (421 mg, 6.45 mmol) in DMA/H_2O (1:1, 5 mL) was heated at 80 °C for 2 h. The mixture was allowed to cool to 0 °C and quenched with aqueous $Na_2S_2O_3$ solution. The mixture was filtered through Celite, and the aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to afford (4*R*,5*R*,6*S*)-5-fluoro-4-(2-fluorophenyl)-6-(1-fluorovinyl)-4-methyl-1,3-oxazinan-2-one. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.80 (s, 3H), 4.45 (d, *J* = 28.2 Hz, 1H), 4.91 (ddd, *J* = 17.8, 3.9, 1.3 Hz, 1H), 5.00 (ddd, *J* = 14.8, 3.9, 1.3 Hz, 1H), 5.29 (d, *J* = 46.6 Hz, 1H), 6.02 (s, 1H), 7.12−7.17 (m, 1H), 7.25−7.29 (m, 1H), 7.38−7.43 (m,

1H), 7.51−7.55 (m, 1H). MS-ESI (*m/z*): 272 $[M + H]^+$. A mixture of this compound, Boc_2O (0.748 mL, 3.22 mmol), and DMAP (79.0 mg, 0.645 mmol) in DCM (3.5 mL) was stirred at room temperature for 1.5 h and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, 25% EtOAc) to give **2-73** (436 mg, 91% over 2 steps) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H), 1.97−1.98 (m, 3H), 4.46 (d, *J* = 29.3 Hz, 1H), 4.91 (ddd, *J* = 20.2, 4.0, 1.3 Hz, 1H), 4.99 (ddd, *J* = 12.3, 4.0, 1.3 Hz, 1H), 5.21 (d, *J* = 46.3 Hz, 1H), 7.10−7.15 (m, 1H), 7.25−7.27 (m, 1H), 7.36−7.42 (m, 1H), 7.58−7.52 (m, 1H). MS-ESI (*m/z*): 372 $[M + H]^+$.

***N*-[[(*2R*,*3R*,*4S*)-3,5-Difluoro-2-(2-fluorophenyl)-4-hydroxyhex-5-en-2-yl]carbamothioyl]benzamide (2-75).** A mixture of **2-73** (456 mg, 1.23 mmol) and K_2CO_3 (679 mg, 4.91 mmol) in MeOH (5 mL) was stirred at room temperature for 15 min. The mixture was quenched with saturated aqueous NH_4Cl solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to give **2-74** as a pale yellow amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H), 2.03 (s, 3H), 2.57 (brs, 1H), 4.07−4.13 (m, 1H), 4.63 (dd, *J* = 49.1, 3.1 Hz, 1H), 4.79 (dd, *J* = 17.7, 3.6 Hz, 1H), 5.03 (d, *J* = 43.9 Hz, 1H), 6.42 (brs, 1H), 7.03−7.08 (m, 1H), 7.14−7.19 (m, 1H), 7.26−7.32 (m, 1H), 7.39−7.43 (m, 1H). A mixture of **2-74** and TFA (1 mL) in DCM (5 mL) was stirred at room temperature for 1 h. The mixture was quenched with saturated aqueous $NaHCO_3$ and K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, 50% EtOAc) to give (*3S*,*4R*,*5R*)-5-amino-2,4-difluoro-5-(2-fluorophenyl)hex-1-en-3-ol (255 mg, 85% over 2 steps) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.77 (s, 3H), 3.89 (d, *J* = 31.0 Hz, 1H), 4.71 (ddd, *J* = 22.2, 2.9, 1.4 Hz, 1H), 4.79 (ddd, *J* = 10.9, 2.9, 1.4 Hz, 1H), 5.09 (dd, *J* = 44.9, 1.1 Hz, 1H), 7.07−7.13 (m, 1H), 7.21−7.24 (m, 1H), 7.32−7.38 (m, 1H), 7.56−7.61 (m, 1H). MS-ESI (*m/z*): 246 $[M + H]^+$. A mixture of this compound (255 mg, 1.04 mmol) and benzoyl isothiocyanate (0.209 mL, 1.56 mmol) in DCM (2.5 mL) was stirred at room temperature for 2.5 h and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 25−50% EtOAc) to give **2-75** (376 mg, 89%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 3H), 2.56 (d, *J* = 9.5 Hz, 1H), 4.36 (ddd, *J* = 25.2, 9.5, 5.0 Hz, 1H), 4.76 (dd, *J* = 48.9, 3.5 Hz, 1H), 4.85 (dd, *J* = 17.7, 3.5 Hz, 1H), 5.37 (d, *J* = 43.9 Hz, 1H), 7.07−7.10 (m,

1H), 7.17−7.20 (m, 1H), 7.31−7.35 (m, 1H), 7.46−7.56 (m, 3H), 7.62−7.65 (m, 1H), 7.87 (d, *J* = 7.4 Hz, 2H), 8.89 (s, 1H), 11.85 (s, 1H). MS-ESI (*m/z*): 409 $[M + H]^+$.

***N*-[(*4R*,*5R*,*6S*)-5-Fluoro-4-(2-fluorophenyl)-6-(1-fluorovinyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]benzamide (2-76).** A mixture of **2-75** (376 mg, 0.919 mmol) and EDC·HCl (352 mg, 1.84 mmol) in MeCN (7 mL) was heated at 50 °C for 1 h. The mixture was allowed to cool to room temperature and diluted with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to give **2-76** as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.91 (s, 3H), 4.52 (d, *J* = 27.7 Hz, 1H), 5.01 (dd, *J* = 9.0, 4.0 Hz, 1H), 5.09 (dd, *J* = 23.7, 4.0 Hz, 1H), 5.43 (d, *J* = 46.4 Hz, 1H), 7.11−7.26 (m, 2H), 7.39−7.47 (m, 4H), 7.51−7.55 (m, 1H), 8.25−8.28 (m, 2H), 11.81 (s, 1H). MS-ESI (*m/z*): 375 $[M + H]^+$.

***tert*-Butyl-benzoyl[(*4R*,*5R*,*6S*)-5-fluoro-6-(1-fluorocyclopropyl)-4-(2-fluorophenyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]carbamate (2-77).** A mixture of **2-76** and Boc_2O (0.427 mL, 1.84 mmol), and DMAP (22.5 mg, 0.184 mmol) in DCM (3.5 mL) was stirred at room temperature for 30 min and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, 20% EtOAc) to give *tert*-butyl benzoyl((*4R*,*5R*,*6S*)-5-fluoro-4-(2-fluorophenyl)-6-(1-fluorovinyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl)carbamate (417 mg, 96% over 2 steps) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9H), 1.55 (s, 3H), 4.40 (dd, *J* = 27.5, 2.8 Hz, 1H), 4.80 (dd, *J* = 49.4, 3.6 Hz, 1H), 4.95 (dd, *J* = 17.8, 3.6 Hz, 1H), 5.23 (d, *J* = 47.2 Hz, 1H), 7.03−7.08 (m, 1H), 7.15−7.19 (m, 1H), 7.27−7.33 (m, 1H), 7.43−7.58 (m, 4H), 7.78 (d, *J* = 7.3 Hz, 2H). MS-ESI (*m/z*): 475 $[M + H]^+$. To a solution of 30% aqueous NaOH solution (4 mL) and Et_2O (4 mL) was added 1-methyl-1-nitrosourea (905 mg, 4.39 mmol) at 0 °C to prepare a solution of diazomethane, and the mixture was stirred at the same temperature for 20 min. A suspension of the compound (417 mg, 0.878 mmol) and $Pd(OAc)_2$ (39.4 mg, 0.176 mmol) in Et_2O (4 mL) was added to the prepared diazomethane solution at −30 °C. The mixture was stirred at −20 °C for 3 h and quenched with H_2O and AcOH. Saturated aqueous $NaHCO_3$ solution was added to the mixture, which was then filtered through Celite. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, 13% EtOAc) to give **2-77** (259

mg). This was used in the next step without further purification. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.75−1.20 (m, 4H), 1.43 (s, 3H), 1.47 (s, 9H), 4.25 (dd, *J* = 28.5, 10.7 Hz, 1H), 5.27 (d, *J* = 47.6 Hz, 1H), 7.00−7.10 (m, 1H), 7.15−7.19 (m, 1H), 7.27−7.30 (m, 1H), 7.40−7.61 (m, 4H), 7.75−7.77 (m, 2H). MS-ESI (*m/z*): 489 $[M + H]^+$.

(*4R*,*5R*,*6S*)-5-Fluoro-4-(2-fluoro-5-nitrophenyl)-6-(1-fluorocyclopropyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (2-78). A mixture of **2-77** (259 mg) and K_2CO_3 (367 mg, 2.65 mmol) in MeOH (5 mL) was stirred at room temperature for 20 min. The mixture was diluted with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, 20% EtOAc) to give *tert*-butyl [(*4R*,*5R*,*6S*)-5-fluoro-4-(2-fluoro-5-nitrophenyl)-6-(1-fluorocyclopropyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]carbamate (117 mg, 35% over 2 steps) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.84−1.17 (m, 4H), 1.52 (s, 9H), 1.85 (s, 3H), 4.35 (dd, *J* = 28.1, 5.3 Hz, 1H), 5.48 (d, *J* = 46.9 Hz, 1H), 7.11−7.17 (m, 1H), 7.22−7.26 (m, 1H), 7.35−7.44 (m, 2H), 10.00 (brs, 1H). MS-ESI (*m/z*): 385 $[M + H]^+$. A mixture of this compound (117 mg, 0.305 mmol) in TFA (1 mL) was stirred at room temperature for 1 h. The mixture was cooled to −20 °C, and H_2SO_4 (0.25 mL) was added. After being stirred at 0 °C for 5 min, the mixture was cooled to −20 °C, and HNO_3 (1.42 g/mL; 0.041 mL, 0.916 mmol) was added. The mixture was stirred at 0 °C for 25 min and quenched with aqueous K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, 50% EtOAc) to give **2-78** (82.8 mg, 82%) as a yellow amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.78−0.85 (m, 1H), 0.91−1.07 (m, 2H), 1.08−1.19 (m, 1H), 1.66 (s, 3H), 4.05 (dd, *J* = 29.1, 9.9 Hz, 1H), 4.35 (brs, 2H), 5.33 (d, *J* = 47.6 Hz, 1H), 7.23 (dd, *J* = 10.7, 9.0 Hz, 1H), 8.21 (ddd, *J* = 9.0, 4.1, 2.9 Hz, 1H), 8.46 (dd, *J* = 6.7, 2.9 Hz, 1H). MS-ESI (*m/z*): 330 $[M + H]^+$.

1,3-Bis(1,1-dimethylethyl)2-[(4*R*,5*R*,6*S*)-4-(5-amino-2-fluorophenyl)-5-fluoro-6-(1-fluorocyclopropyl)-5,6-dihydro-4-methyl-4*H*-1,3-oxazin-2-yl]imidodicarbonate (2-79). A mixture of **2-78** (82.8 mg, 0.251 mmol), Boc_2O (0.175 mL, 0.754 mmol), and DMAP (30.7 mg, 0.251 mmol) in DCM (1 mL) was stirred at room temperature for 30 min and then evaporated. The residue

was purified by flash column chromatography (silica gel; EtOAc/hexane, 20% EtOAc) to give di-*tert*-butyl [(*4R*,*5R,6S*)-5-fluoro-4-(2-fluoro-5-nitrophenyl)-6-(1-fluorocyclopropyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]carbamate (83.8 mg, 63%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.77−0.84 (m, 1H), 0.92−1.09 (m, 2H), 1.11−1.22 (m, 1H), 1.53 (s, 18H), 1.74 (s, 3H), 4.23 (dd, *J* = 28.2, 8.2 Hz, 1H), 5.39 (d, *J* = 47.1 Hz, 1H), 7.25−7.30 (m, 1H), 8.24−8.28 (m, 1H), 8.56 (dd, *J* = 6.7, 2.9 Hz, 1H). MS-ESI (*m/z*): 530 $[M + H]^+$. A mixture of this compound (83.8 mg, 0.158 mmol) and Pd/C (10%; 8.4 mg) in THF/MeOH (1:2, 1.5 mL) was stirred at room temperature for 4.5 h under a hydrogen atmosphere. The mixture was filtered through Celite and then evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, 50% EtOAc) to give **2-79** (65.4 mg, 83%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.75−1.20 (m, 4H), 1.52 (s, 18H), 1.67−1.73 (m, 3H), 3.57 (brs, 2H), 4.31 (dd, *J* = 28.6, 10.0 Hz, 1H), 5.35 (d, *J* = 47.7 Hz, 1H), 6.54−6.58 (m, 1H), 6.83−6.90 (m, 2H). MS-ESI (*m/z*): 500 $[M + H]^+$.

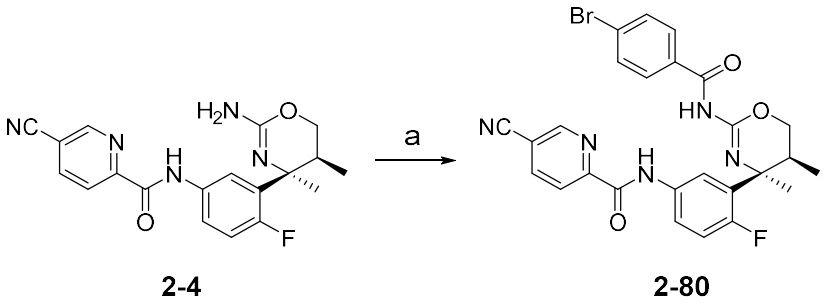
***N*-[3-[(*4R*,*5R*,*6S*)-2-Amino-5-fluoro-6-(1-fluorocyclopropyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-(fluoromethoxy)pyrazine-2-carboxamide (2-15).** Compound **2-15** (25.9 mg, 70% over 2 steps) was prepared from **2-79** (41.0 mg, 0.082 mmol) following the general procedure. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.76−1.20 (m, 4H), 1.67 (s, 3H), 4.11 (dd, *J* = 29.2, 11.8 Hz, 1H), 5.35 (d, *J* = 47.9 Hz, 1H), 6.15 (dd, *J* = 51.2, 4.3 Hz, 2H), 7.11 (dd, *J* = 11.3, 8.9 Hz, 1H), 7.50 (dd, *J* = 6.8, 2.6 Hz, 1H), 8.00−8.04 (m, 1H), 8.29 (s, 1H), 9.08 (s, 1H), 9.52 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 7.2 (d, *J* = 11.7 Hz), 8.7 (d, *J* = 10.2 Hz). 24.7 (d, *J* = 4.4 Hz), 58.3 (d, *J* = 21.9 Hz), 72.7 (d, *J* = 28.4 Hz), 78.6, 85.4 (d, *J* = 188.9 Hz), 95.8 (d, *J* = 222.4 Hz), 117.3 (d, *J* = 25.5 Hz), 120.8 (d, *J* = 8.7 Hz), 121.0 (d, *J* = 3.0 Hz), 132.8 (d, *J* = 5.9 Hz), 133.2, 134.0, 139.6, 141.8, 150.5, 155.7 (d, *J* = 242.8 Hz), 159.4, 160.5. HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{20}H_{19}F_4N_5O_3$, 454.1502; found, 454.1494.

Single X-ray Structure and NMR Analysis

The stereochemistry of compounds **2-4**, **2-6**, **2-7**, **2-16** was verified by single X-ray structure analysis of the corresponding derivatives **2-80**, **2-81**, **2-82**, and **2-83** to obtain high quality crystalline materials and acceptable X-ray diffraction patterns (**Schemes S3, S4**, and **S5**). Data collections were performed on a Rigaku XtaLAB P200 system at 100 K. The ORTEP figures, detailed crystal data, and structure refinement are given in Figures **S5−8** and Tables **S5−S8**. Coordinates, refinement details, and structure

factors were deposited with the Cambridge Crystallographic Data Centre (**2-80**: CCDC 1587386; **2-81**: CCDC 1587387; **2-82**: CCDC 1587393; **2-83**: CCDC 1587394). The relative stereochemistry of **2-5** was confirmed by 2D-NMR analysis based on the intermediate **2-31** (**Figure S9**).

Scheme S3. Synthesis of 4-Bromobenzamide 2-80

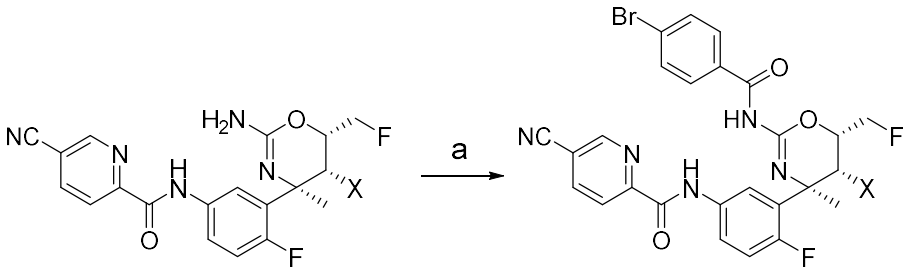


2-4 **2-80**

[a]Reagents and conditions: (a) 4-bromobenzoyl chloride, DCM, 0 °C, 74%.

***N*-[3-[(4*S*,5*S*)-2-(4-Bromobenzamido)-4,5-dimethyl-5,6-dihydro-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (2-80).** To a solution of **2-4** (13.7 mg, 0.037 mmol) and Et_3N (15.5 μL, 0.112 mmol) in DCM (0.3 mL) was added 4-bromobenzoyl chloride (12.3 mg, 0.056 mmol) at 0 °C. After being stirred for 2 h, the reaction mixture was diluted with saturated aq $NaHCO_3$ and EtOAc. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0−30% EtOAc) to give **2-80** (16 mg, 74% yield) and recrystallized from MeOH-H_2O as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (dd, *J* = 6.8, 1.6 Hz, 3H), 1.95 (s, 3H), 2.50-2.56 (m, 1H), 4.08 (dd, *J* = 11.6, 8.0 Hz, 1H), 4.40 (dd, *J* = 11.6, 3.6 Hz, 1H), 7.17 (dd, *J* = 11.2, 8.8 Hz, 1H), 7.47 (dd, *J* = 6.8, 2.4 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 2H), 8.03−8.07 (m, 1H), 8.13 (d, *J* = 8.4 Hz, 2H), 8.20 (dd, *J* = 8.0, 1.6 Hz, 1H), 8.40 (d, *J* = 8.4 Hz, 1H), 8.88 (s, 1H), 9.90 (s, 1H), 11.75 (s, 1H). MS-ESI (*m/z*): 550 $[M + H]^+$.

Scheme S4. Synthesis of 4-bromobenzamide 2-81 and 2-82

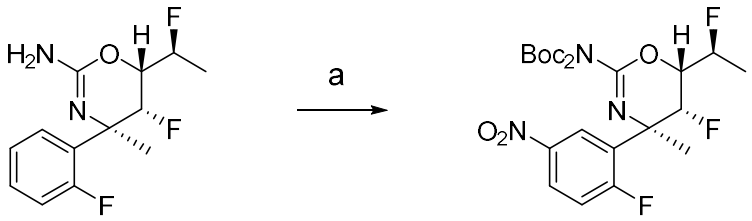


[a]Reagents and conditions: (a) 4-bromobenzoyl chloride, DCM, 0 °C, 68−74%.

***N*-[3-[(4*R*,5*R*,6*R*)-2-(4-bromobenzamido)-5-fluoro-6-(fluoromethyl)-4-methyl-5,6-dihydro-4H-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (2-81).** Compound **2-81** was prepared in a manner similar to that for **2-80** in 68% yield and recrystallized from MeOH-H_2O. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93 (s, 3H), 4.30−4.43 (m, 1H), 4.73 (dd, *J* = 45.6, 6.8 Hz, 2H), 5.38 (d, *J* = 46.8 Hz, 1H), 7.23 (dd, *J* = 11.6, 9.2 Hz, 1H), 7.46 (dd, *J* = 6.8, 2.4 Hz, 1H), 7.60 (d, *J* = 6.8 Hz, 2H), 8.15 (d, *J* = 6.8 Hz, 2H), 8.17−8.19 (m, 1H), 8.19−8.20 (m, 1H), 8.38 (dd, *J* = 8.0, 0.8 Hz, 1H), 8.84−8.85 (m, 1H), 9.90 (s, 1H), 11.76 (s, 1H). MS-ESI (*m/z*): 586 $[M + H]^+$.

***N*-[3-[(4*R*,5*R*,6*S*)-2-(4-bromobenzamido)-6-(fluoromethyl)-5-methoxy-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopicolinamide (2-82).** Compound **2-82** was prepared in a manner similar to that for **2-81** in 74% yield and recrystallized from MeOH-H_2O. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.90 (s, 3H), 3.73 (s, 3H), 4.11 (s, 1H), 4.31 (q, *J* = 6.8 Hz, 1H), 4.58−4.63 (m, 1H), 4.69−4.74 (m, 1H), 7.21 (dd, *J* = 11.2, 8.8 Hz, 1H), 7.35 (dd, *J* = 6.8, 2.4 Hz, 1H), 7.58 (d, *J* = 8.4 Hz, 2H), 8.13 (d, *J* = 8.4 Hz, 2H), 8.17−8.23 (m, 2H), 8.38 (d, *J* = 8.0 Hz, 1H), 8.84 (s, 1H), 9.89 (s, 1H), 11.58 (s, 1H). MS-ESI (*m/z*): 598 $[M + H]^+$.

Scheme S5. Synthesis of Oxazine 2-83



2-60 **2-83**

[a]Reagents and conditions: (a) (i) HNO_3, H_2SO_4, TFA, −20 °C, 82%, (ii) Boc_2O, DMAP, DCM, rt, 63%.

Di-*tert*-butyl-[(*4R*,*5R*,*6R*)-5-fluoro-4-(2-fluoro-5-nitrophenyl)-6-[(*S*)-1-fluoroethyl]-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-yl]carbamate (2-83). To a solution of **2-60** (252 mg, 0.925 mmol) in H_2SO_4 (0.6 mL) and TFA (2.4 mL) was added HNO_3 (1.42 g/mL; 0.062 mL, 1.39 mmol) at −20 °C. The mixture was stirred at 0 °C for 30 min and quenched with aqueous K_2CO_3 solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50−100% EtOAc) to give (*4R*,*5R*,*6R*)-5-fluoro-4-(2-fluoro-5-nitrophenyl)-6-((*S*)-1-fluoroethyl)-4-methyl-5,6-dihydro-4*H*-1,3-oxazin-2-amine (240 mg, 82%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.39 (dd, *J* = 24.2, 6.4 Hz, 3H), 1.65 (s, 3H), 3.67 (ddd, *J* = 30.9, 13.2, 7.4 Hz, 1H), 4.42 (brs, 2H), 4.86 (ddq, *J* = 48.8, 7.4, 6.4 Hz, 1H), 5.15 (d, *J* = 47.7 Hz, 1H), 7.21−7.26 (m, 1H), 8.20−8.24 (m, 1H), 8.44−8.47 (m, 1H). MS-ESI (*m/z*): 318 $[M + H]^+$. A solution of this compound (240 mg, 0.757 mmol), Boc_2O (0.439 mL, 1.89 mmol), and DMAP (18.5 mg, 0.151 mmol) in DCM (2.4 mL) was stirred at room temperature for 35 min and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 9−20% EtOAc) and then recrystallized from hexane/EtOAc to give **2-83** (245 mg, 63%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.40 (dd, *J* = 24.1, 6.5 Hz, 3H), 1.52 (s, 18H), 1.72 (s, 3H), 3.80 (ddd, *J* = 29.9, 12.9, 7.2 Hz, 1H), 4.91 (ddq, *J* = 48.1, 7.2, 6.5 Hz, 1H), 5.19 (d, *J* = 47.2 Hz, 1H), 7.25−7.30 (m, 1H), 8.24−8.28 (m, 1H), 8.56−8.58 (m, 1H). MS-ESI (*m/z*): 518 $[M + H]^+$.

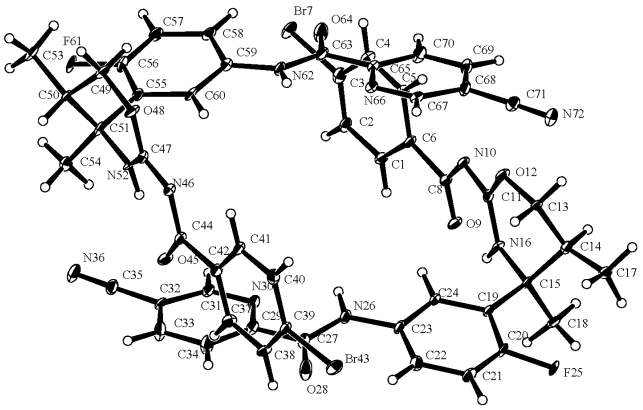


Figure S5. Single X-ray structure of compound **2-80**. An ORTEP figure with thermal ellipsoid set at the 30% probability level.

Table S5. Crystal Data and Structure Refinement for Compound 2-80

Empirical Formula	$C_{26}H_{21}BrFN_5O_3$
Formula Weight	550.39
Crystal Color, Habit	colorless, platelet
Crystal Dimensions	0.25 x 0.02 x 0.02 mm^3
Crystal System	Triclinic
Lattice Type	Primitive
Lattice Parameters	a = 6.6661(10) Å
	b = 13.163(3) Å
	c = 14.366(3) Å
	α = 81.30(3) ^{o}.
	β = 80.85(2) ^{o}.
	γ = 73.63(3) ^{o}.
	V = 1186.5(5) $Å^3$
Space Group	P1 (#1)
Z value	2
No. Observations (All reflections)	7774
No. Variables	653
Residuals: R1 (I>2.00s(I))	0.0646

Residuals: R (All reflections)	0.0655
Residuals: wR2 (All reflections)	0.1548
Goodness of Fit Indicator	1.062
Flack parameter (Parsons' quotients = 3034)	0.01(3)
Max Shift/Error in Final Cycle	0.000
CCDC number	1587386

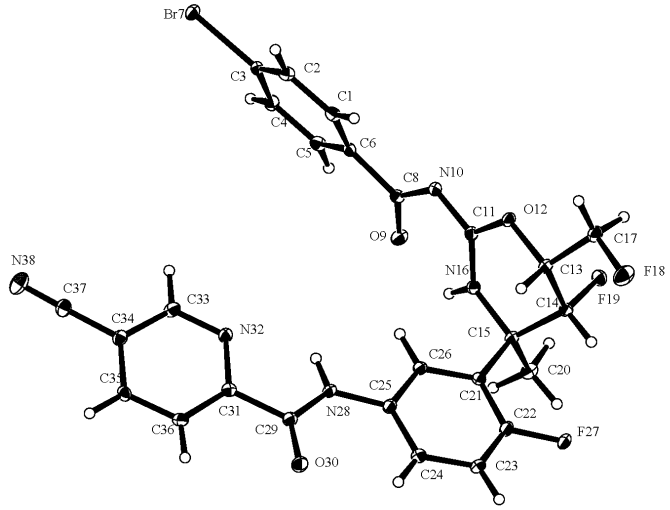


Figure S6. Single X-ray structure of compound **2-81**. An ORTEP figure with thermal ellipsoid set at the 30% probability level.

Table S6. Crystal Data and Structure Refinement for Compound 2-81

Empirical Formula	$C_{26}H_{19}BrF_3N_5O_3$
Formula Weight	586.37
Crystal Color, Habit	colorless, platelet
Crystal Dimensions	0.09 x 0.07 x 0.03 mm^3
Crystal System	Orthorhombic
Lattice Type	Primitive
Lattice Parameters	a = 8.1952(11) Å
	b = 9.5996(11) Å

	c = 30.634(3) Å
	V = 2410.0(5) Å^3
	$\alpha = \beta = \gamma = 90^o$.
Space Group	$P2_12_12_1$ (#19)
Z value	4
No. Observations (All reflections)	4306
No. Variables	344
Residuals: R1 (I>2.00s(I))	0.0360
Residuals: R (All reflections)	0.0374
Residuals: wR2 (All reflections)	0.0882
Goodness of Fit Indicator	1.042
Flack parameter (Parsons' quotients = 1639)	-0.039(15)
Max Shift/Error in Final Cycle	0.002
CCDC number	1587387

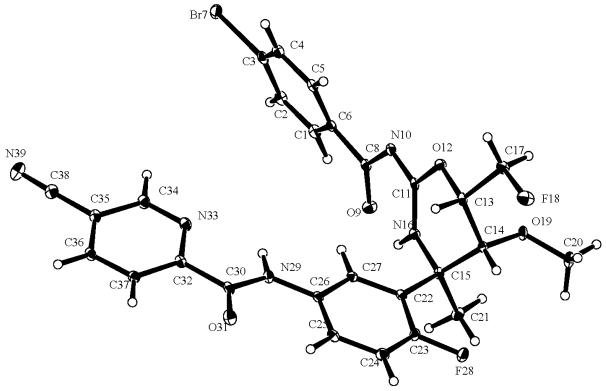


Figure S7. Single X-ray structure of compound **2-82**. An ORTEP figure with thermal ellipsoid set at the 30% probability level.

Table S7. Crystal Data and Structure Refinement for Compound 2-82

Empirical Formula	$C_{27}H_{22}BrF_2N_5O_4$
Formula Weight	598.40
Crystal Color, Habit	colorless, prism
Crystal Dimensions	0.20 x 0.06 x 0.04 mm^3
Crystal System	Orthorhombic
Lattice Type	Primitive
Lattice Parameters	a = 8.0391(11) Å
	b = 9.8135(13) Å
	c = 32.294(4) Å
	$\alpha = \beta = \gamma = 90^o$.
	V = 2547.7(6) $Å^3$
Space Group	$P2_12_12_1$ (#19)
Z value	4
No. Observations (All reflections)	4583
No. Variables	354
Reflection/Parameter Ratio	12.95
Residuals: R1 (I>2.00s(I))	0.0327
Residuals: R (All reflections)	0.0328
Residuals: wR2 (All reflections)	0.0931
Goodness of Fit Indicator	1.057
Flack parameter (Parsons' quotients = 1900)	-0.020(10)
Max Shift/Error in Final Cycle	0.001
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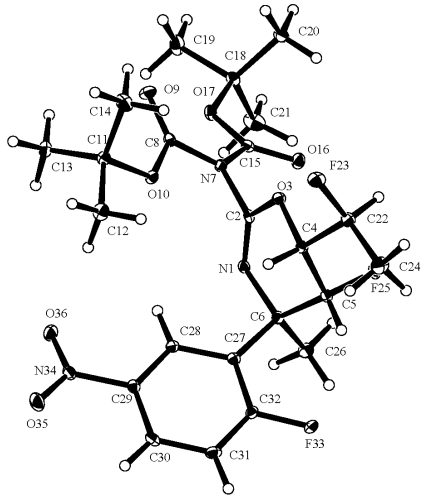

Figure S8. Single X-ray structure of compound **2-83**. An ORTEP figure with thermal ellipsoid set at the 30% probability level.

Table S8. Crystal Data and Structure Refinement for Compound 2-83

Empirical Formula	$C_{23}H_{30}F_3N_3O_7$
Formula Weight	517.50
Crystal Color, Habit	colorless, prism
Crystal Dimensions	0.11 x 0.09 x 0.08 mm^3
Crystal System	Orthorhombic
Lattice Type	Primitive
Lattice Parameters	a = 9.5866(14) Å
	b = 12.775(2) Å
	c = 20.441(3) Å
	$\alpha = \beta = \gamma = 90^o$.
	V = 2503.4(6) $Å^3$
Space Group	$P2_12_12_1$ (#19)
Z value	4
No. Observations (All reflections)	4519
No. Variables	333
Reflection/Parameter Ratio	13.57

Residuals: R1 (I>2.00s(I))	0.0319
Residuals: R (All reflections)	0.0322
Residuals: wR2 (All reflections)	0.0864
Goodness of Fit Indicator	1.069
Flack parameter (Parsons' quotients = 1884)	-0.04(5)
Max Shift/Error in Final Cycle	0.000
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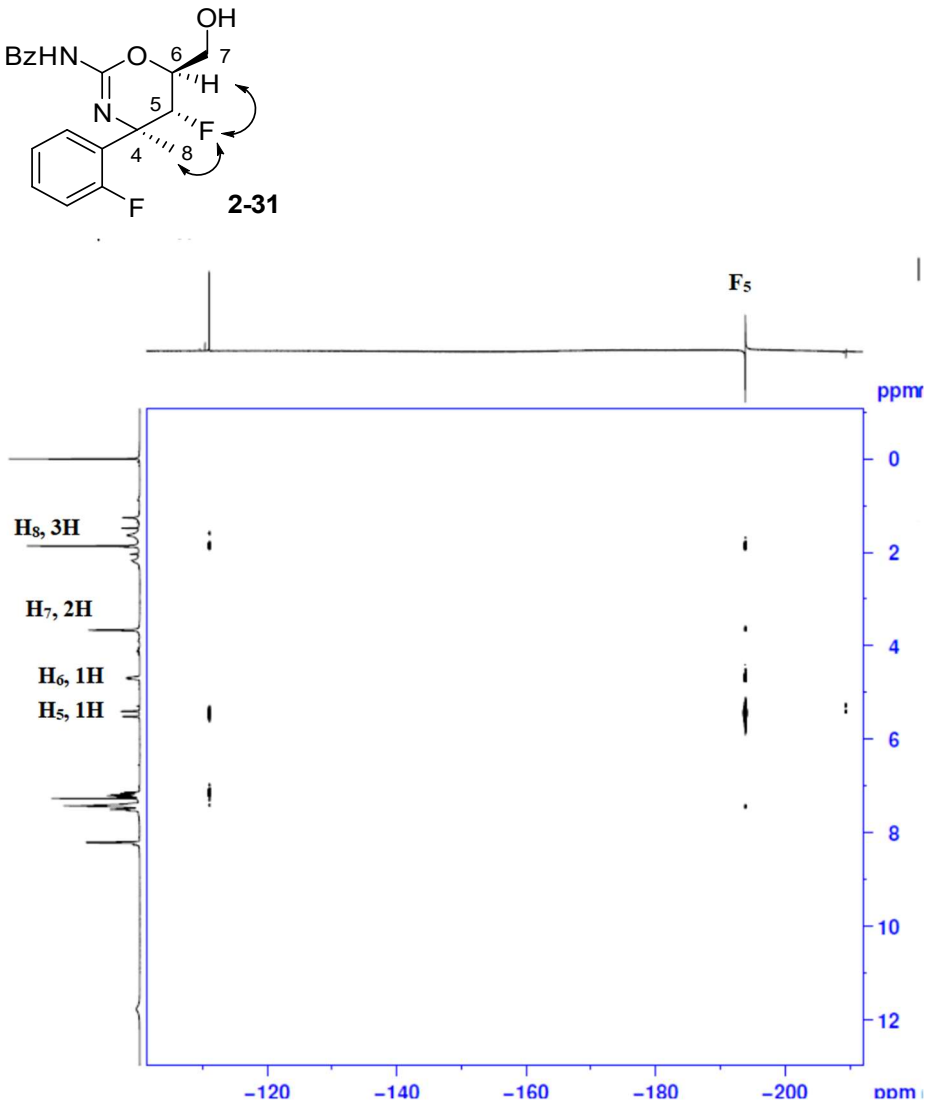
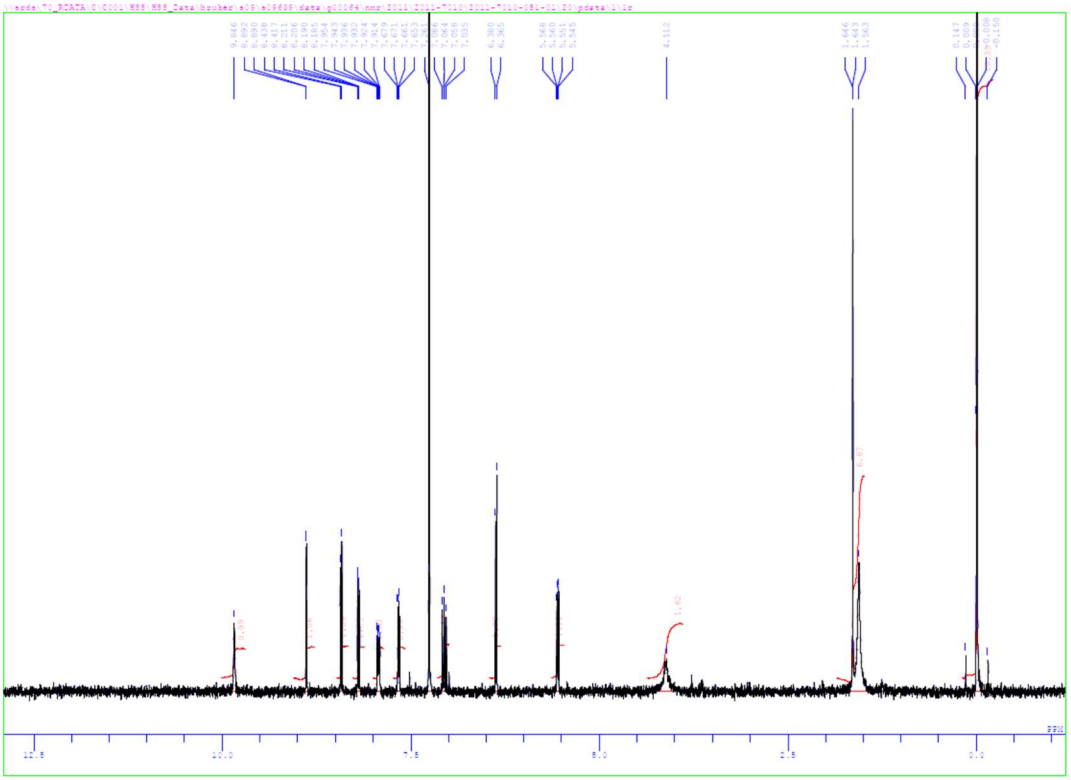


Figure S9. 2D-NMR analysis for compound **2-31**.

1-4



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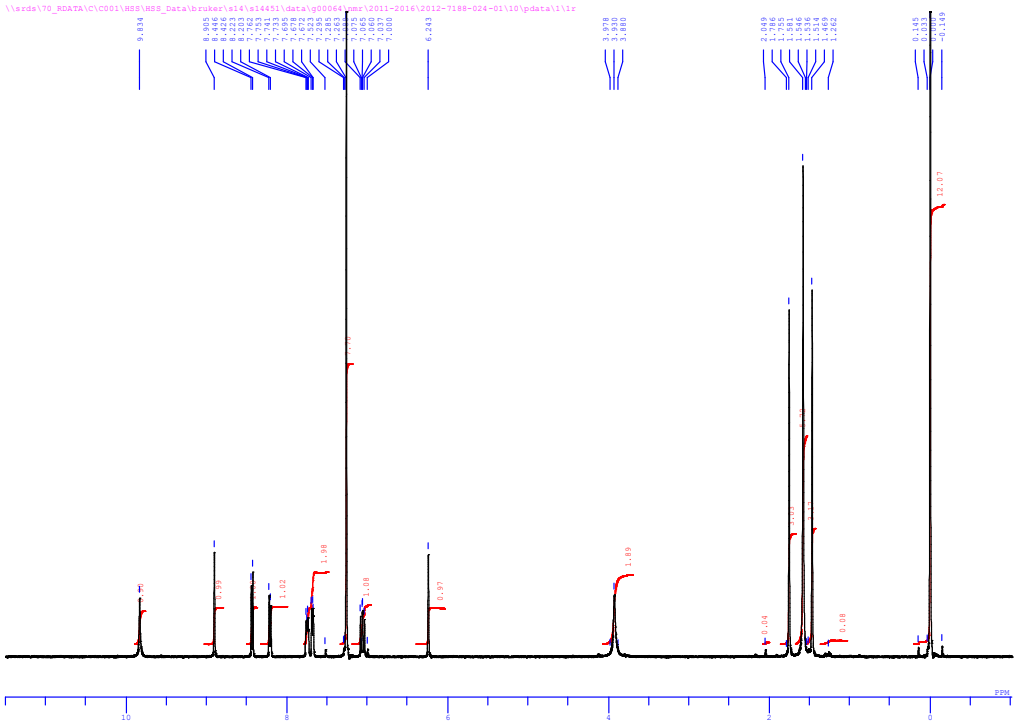

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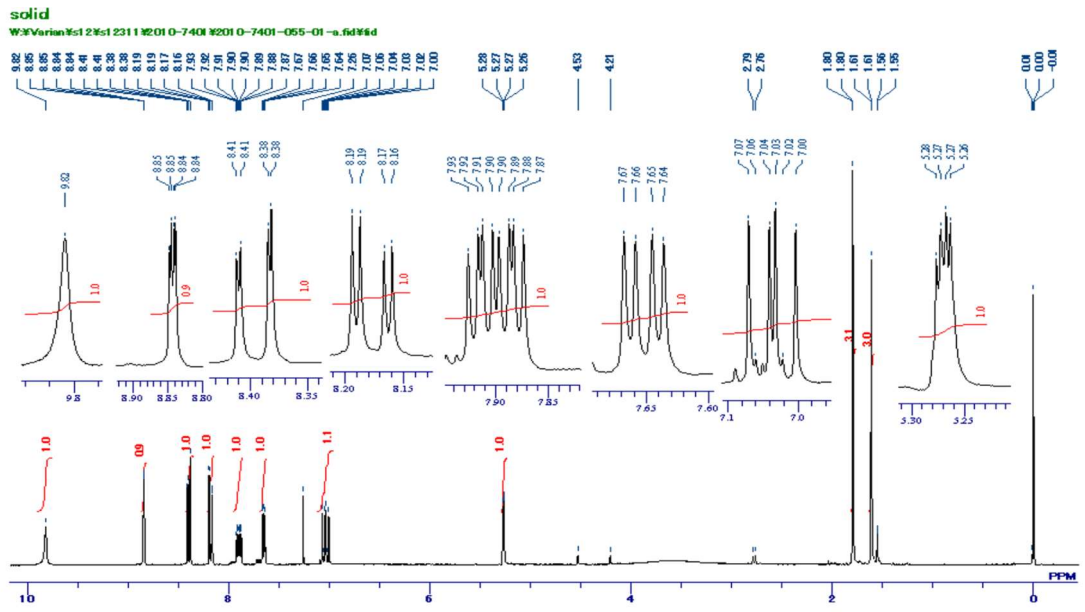

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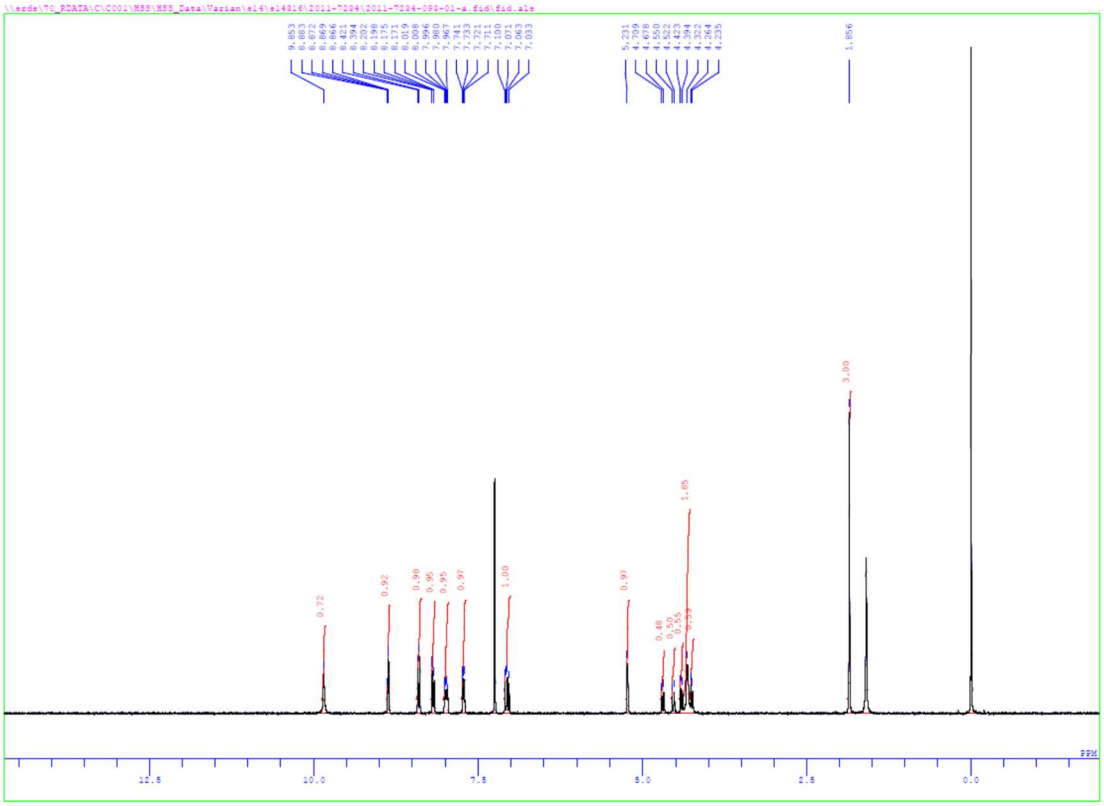

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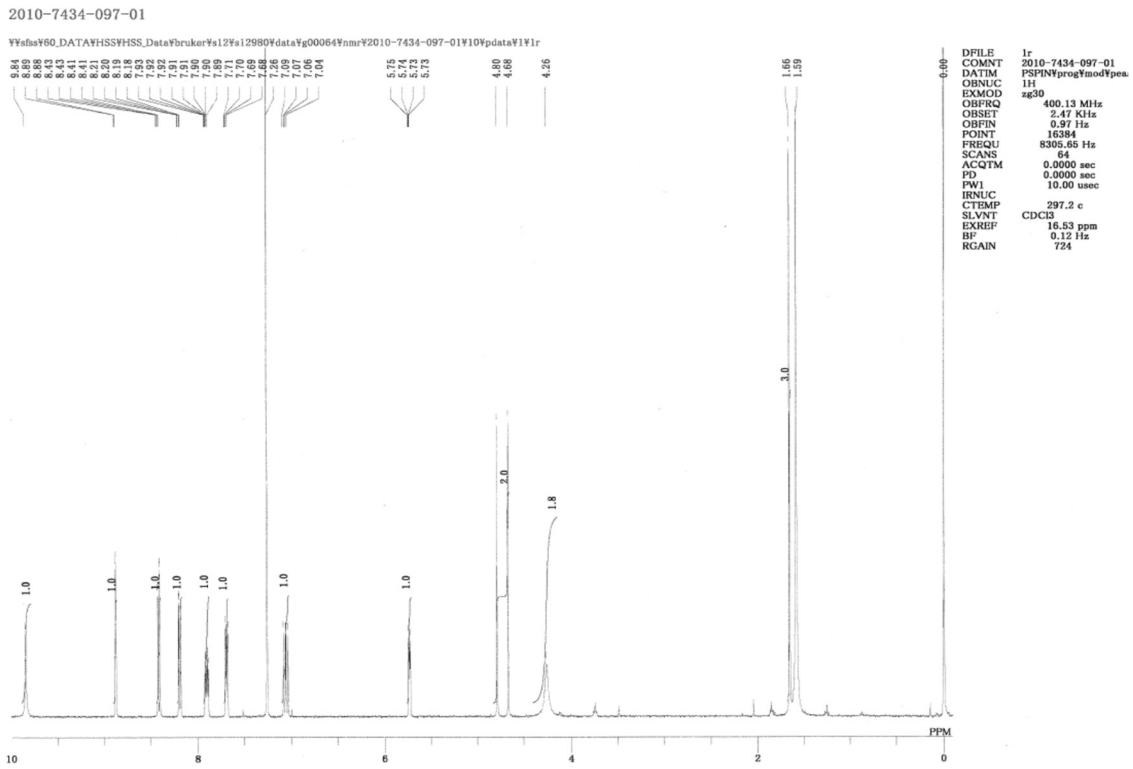
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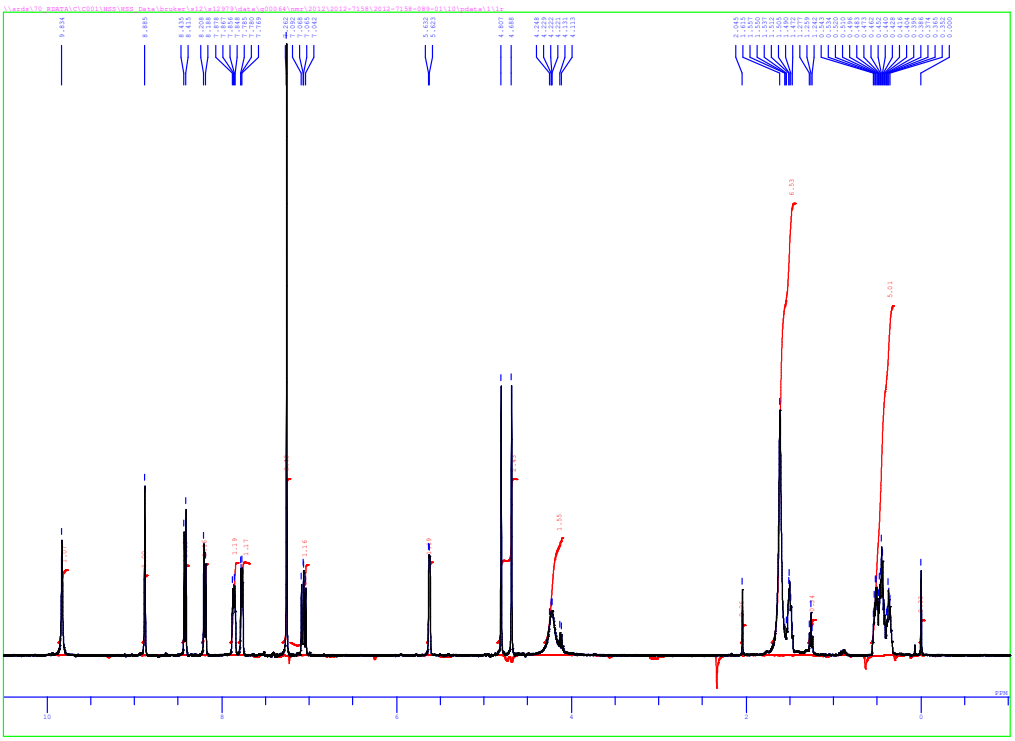

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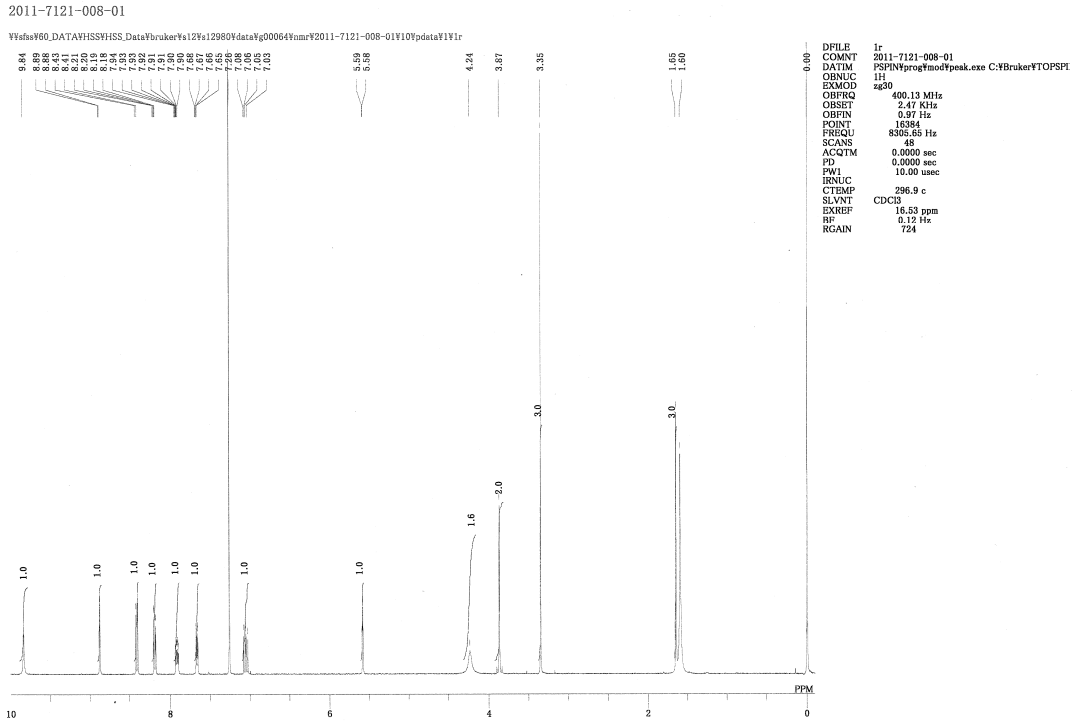
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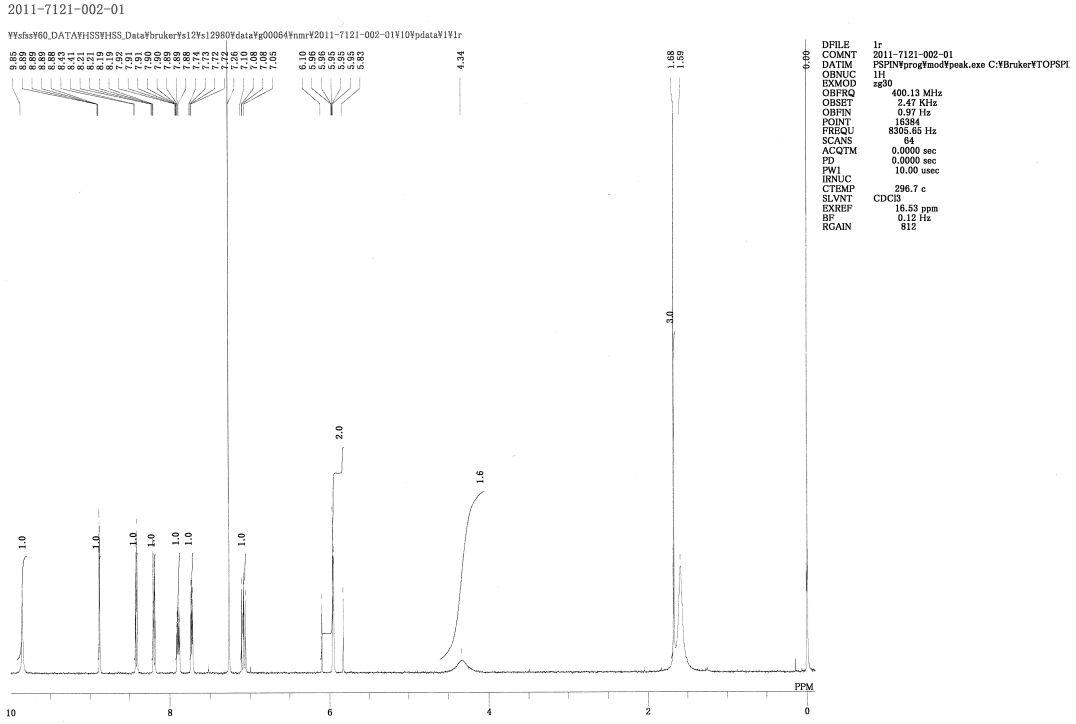
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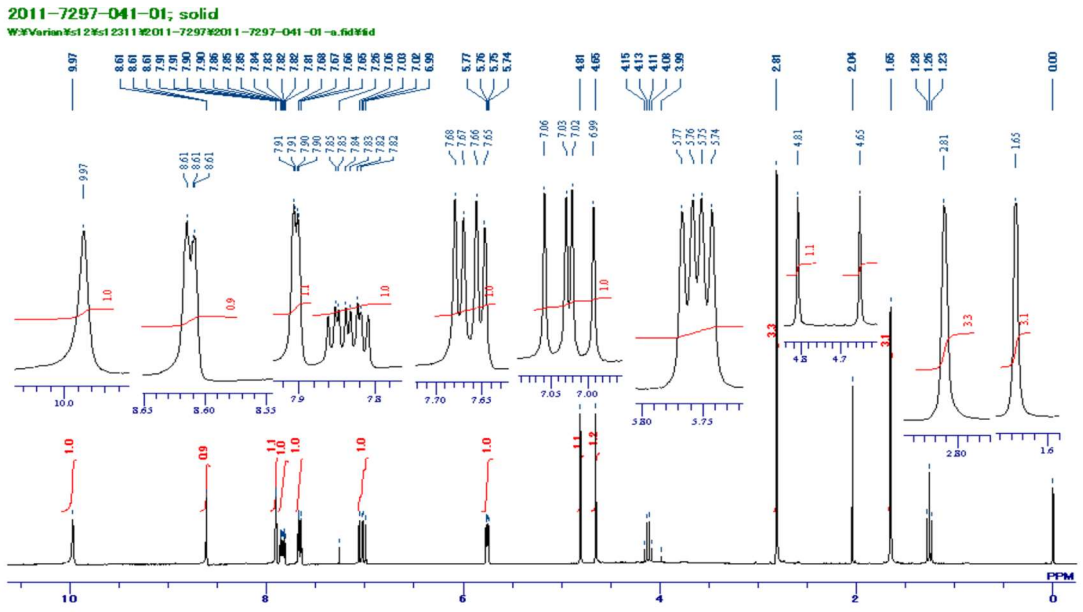

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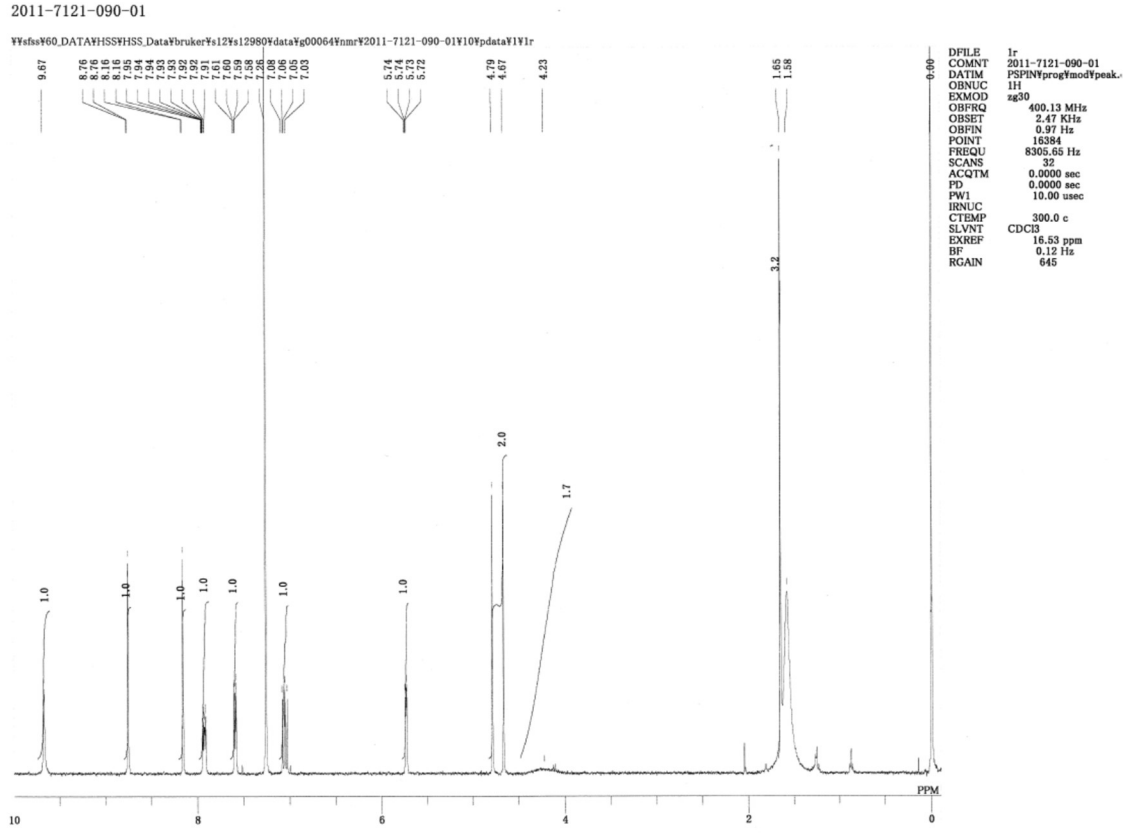

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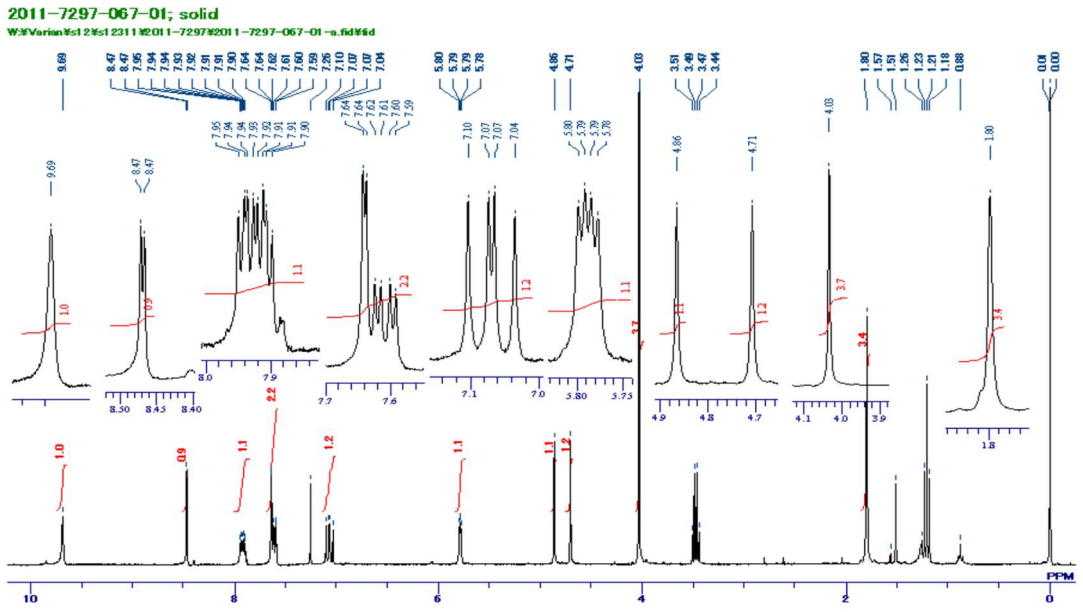

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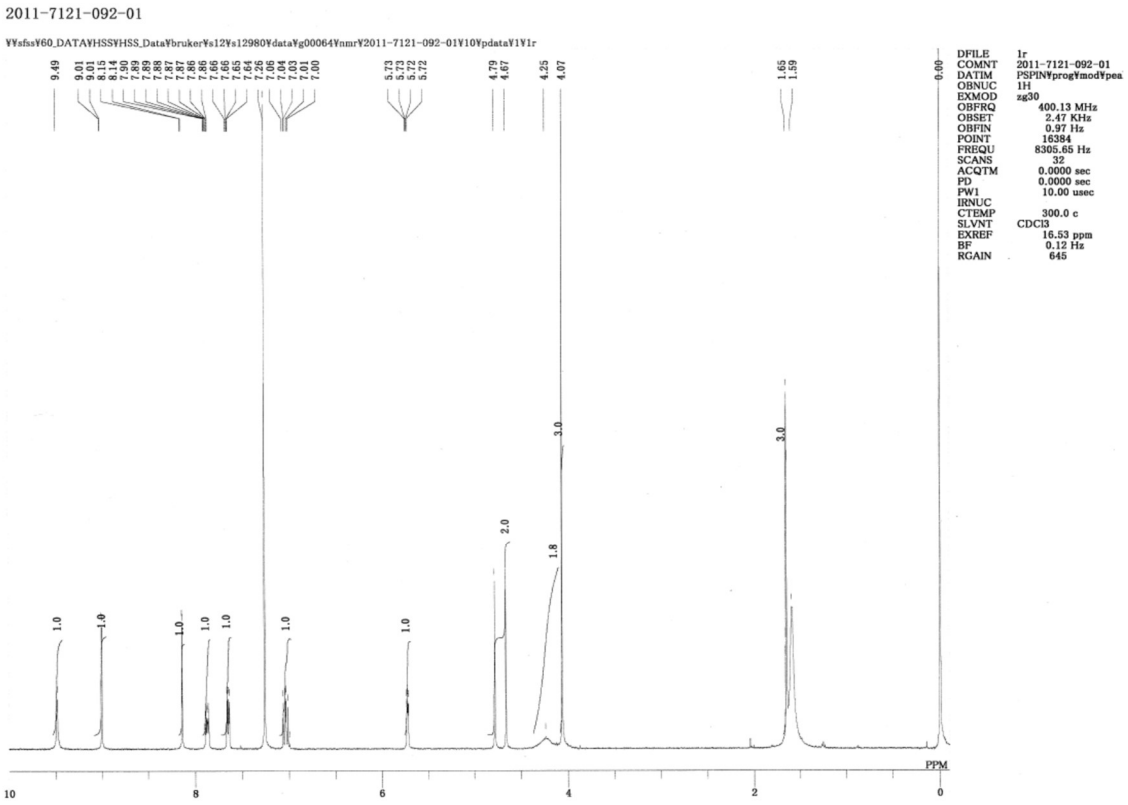

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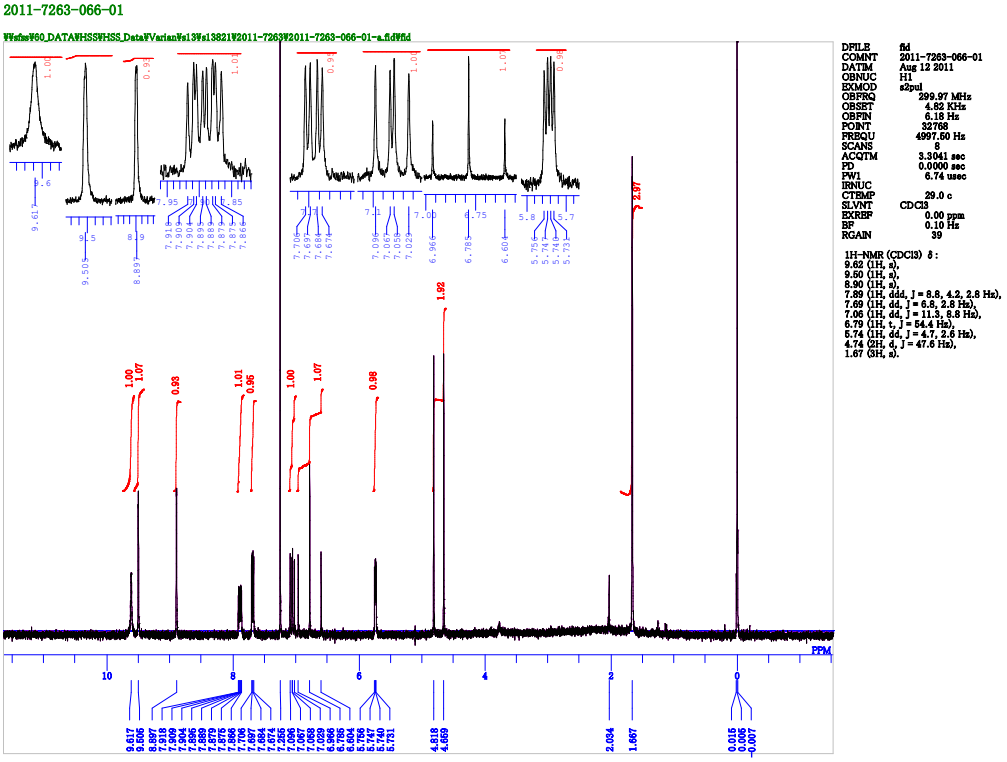
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7.69 (1H, dd, J = 6.8, 2.8 Hz),
7.06 (1H, dd, J = 11.3, 8.8 Hz),
6.79 (1H, t, J = 54.4 Hz),
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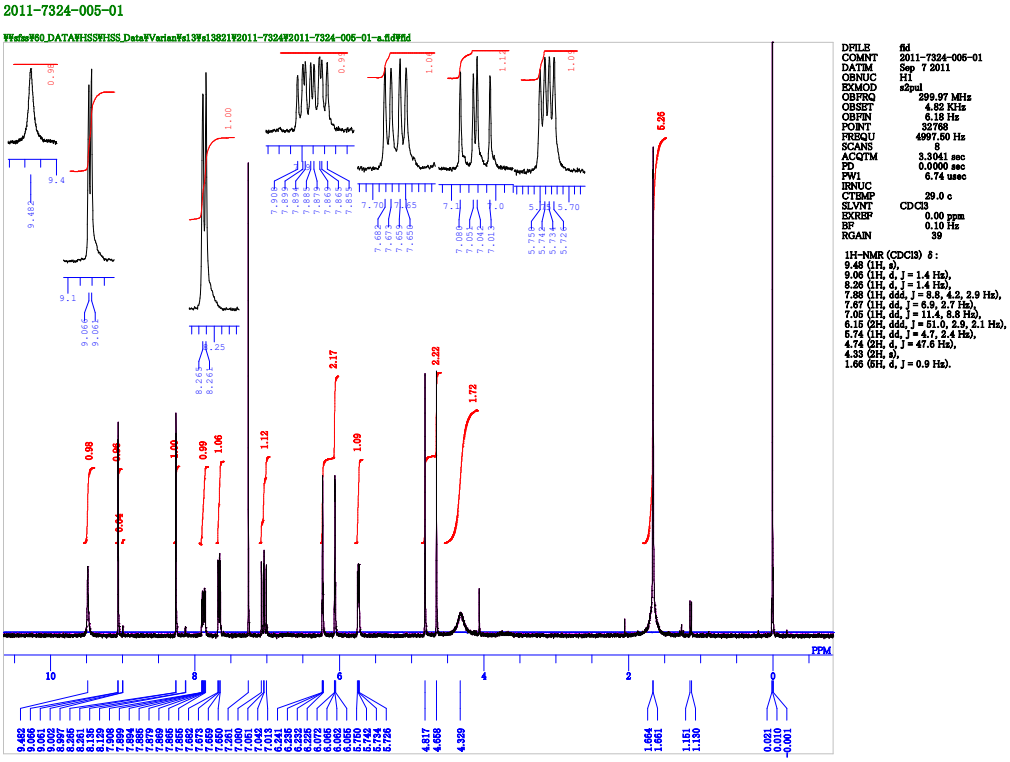
1.67 (3H, s).
PPM

1-17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7.88 (1H, ddd, J = 8.8, 4.2, 2.9 Hz),
7.67 (1H, dd, J = 6.9, 2.7 Hz),
7.05 (1H, dd, J = 11.4, 8.8 Hz),
6.15 (2H, ddd, J = 51.0, 2.9, 2.1 Hz),
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PPM

1-18

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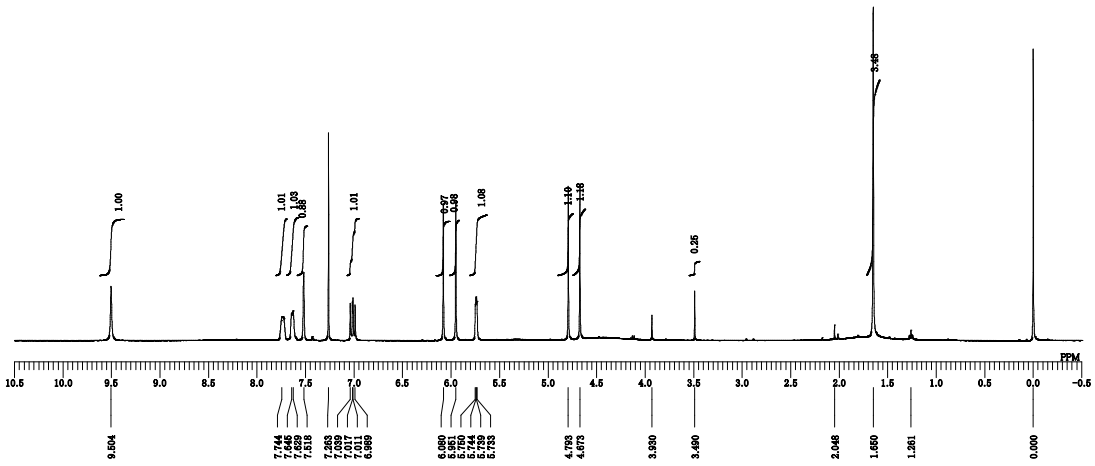

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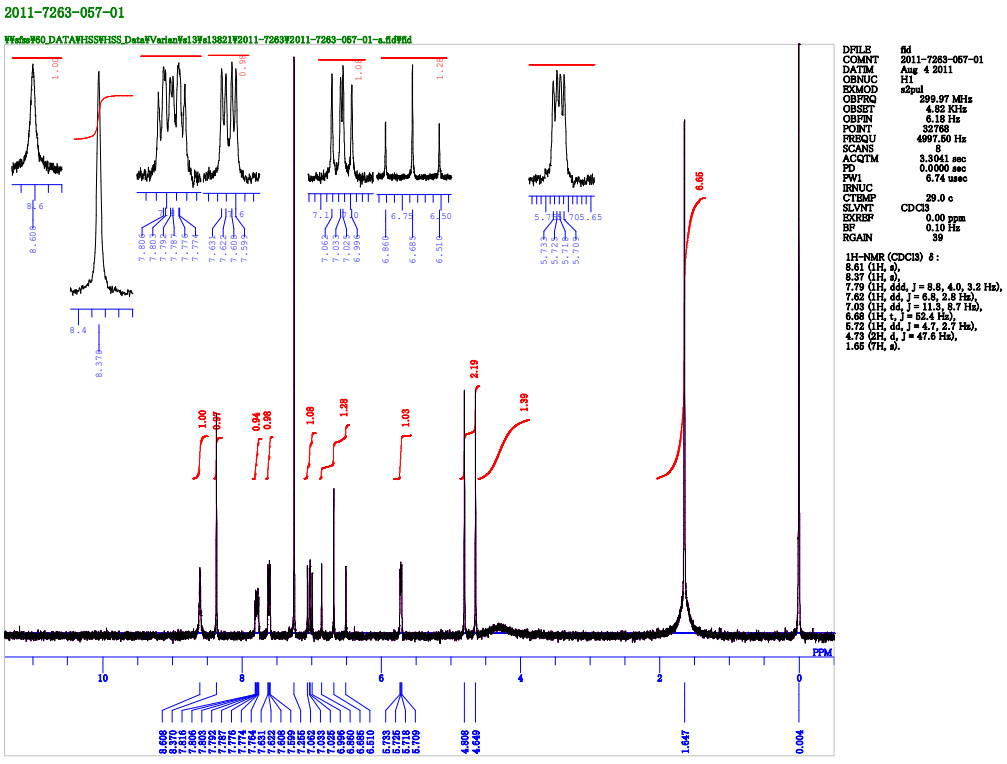

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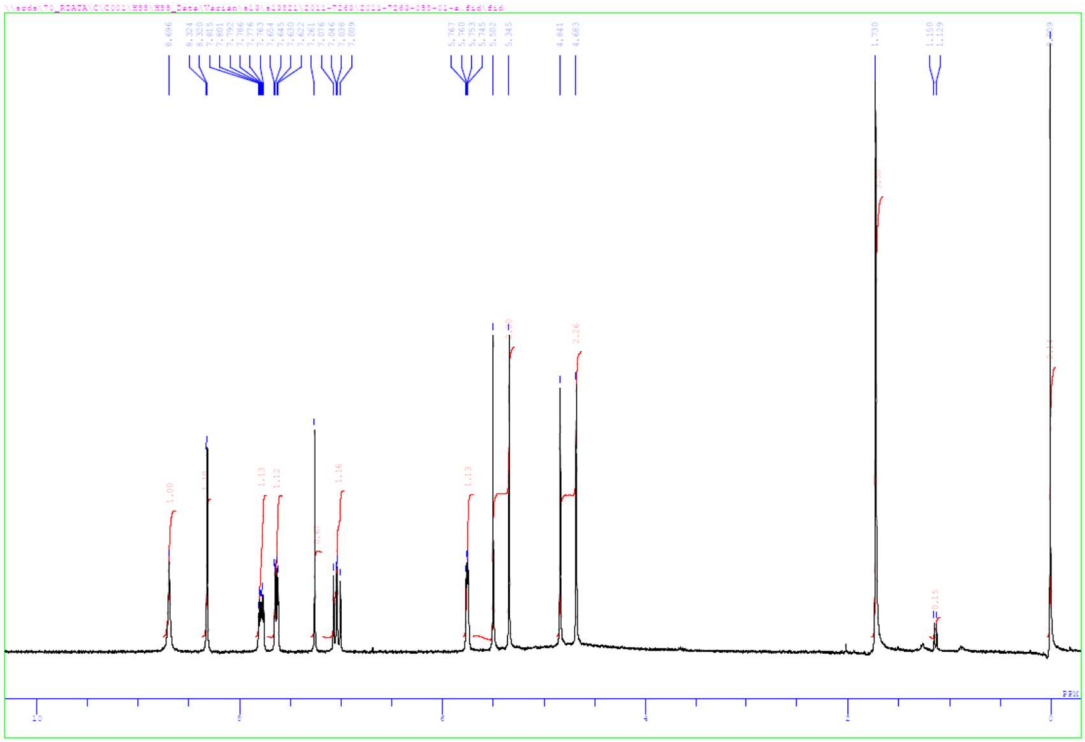

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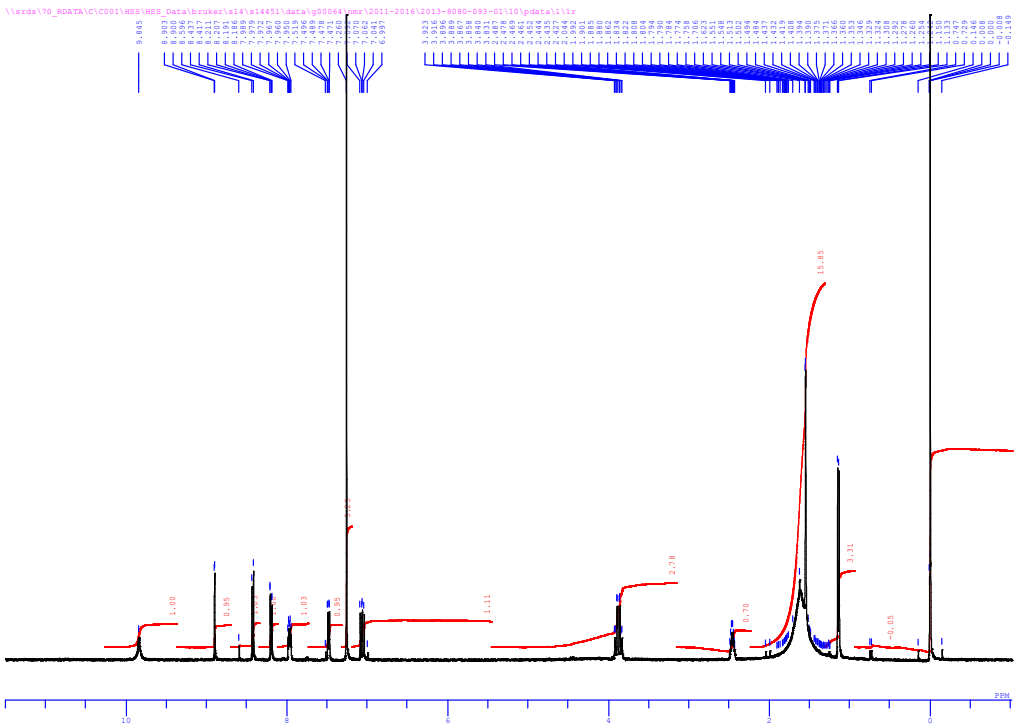

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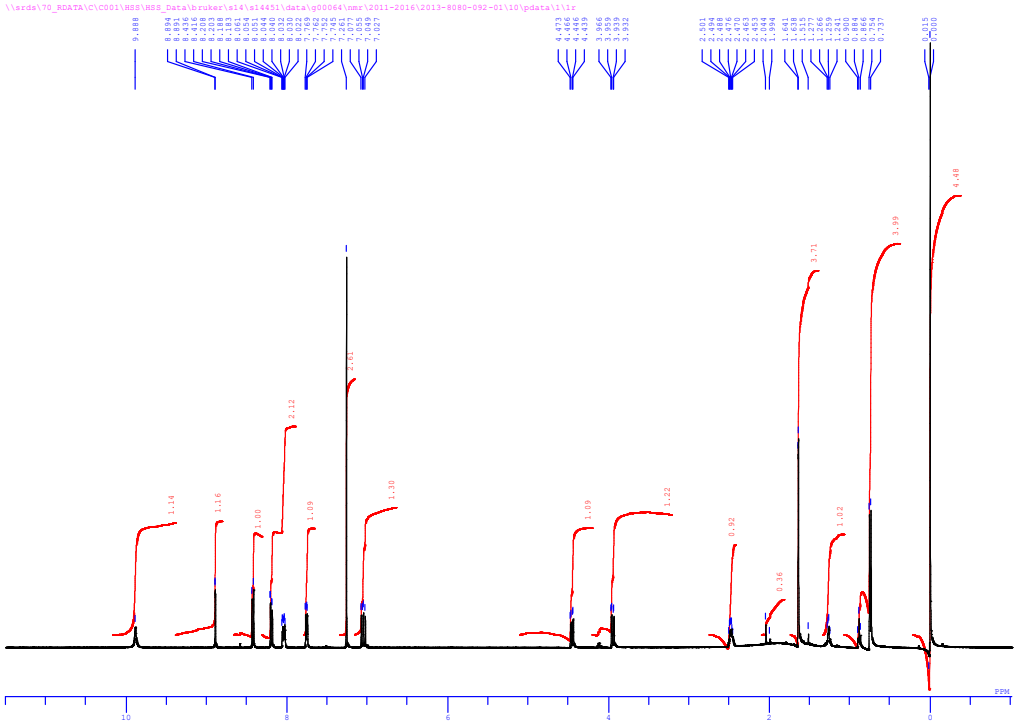

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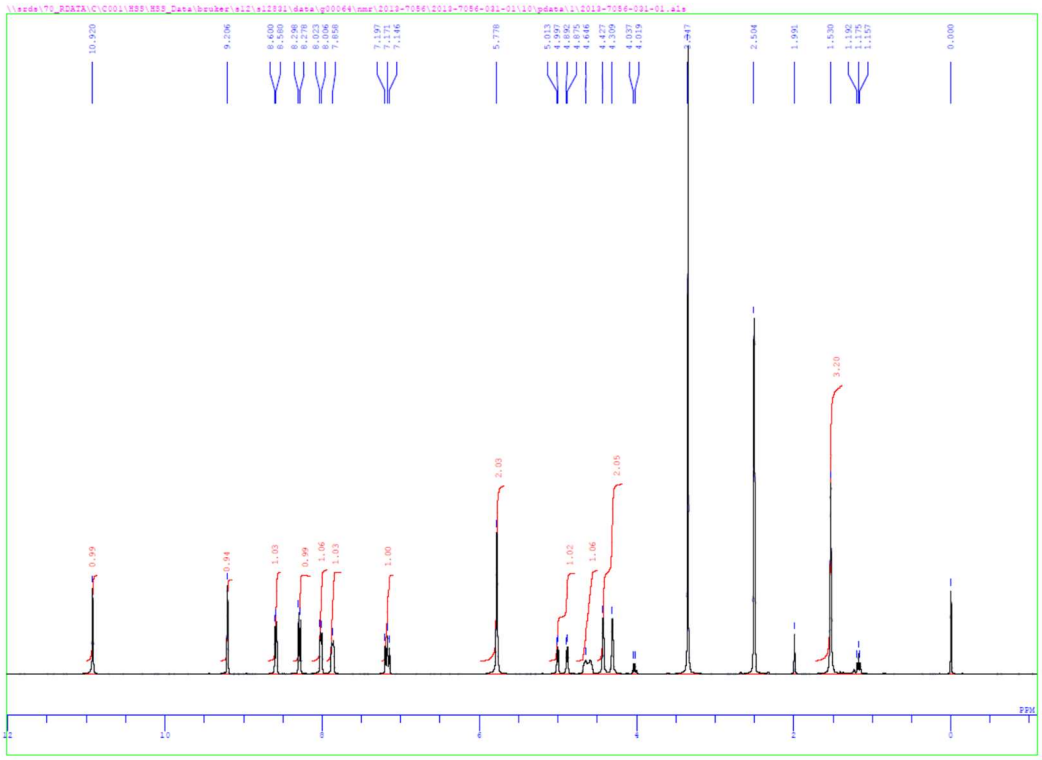

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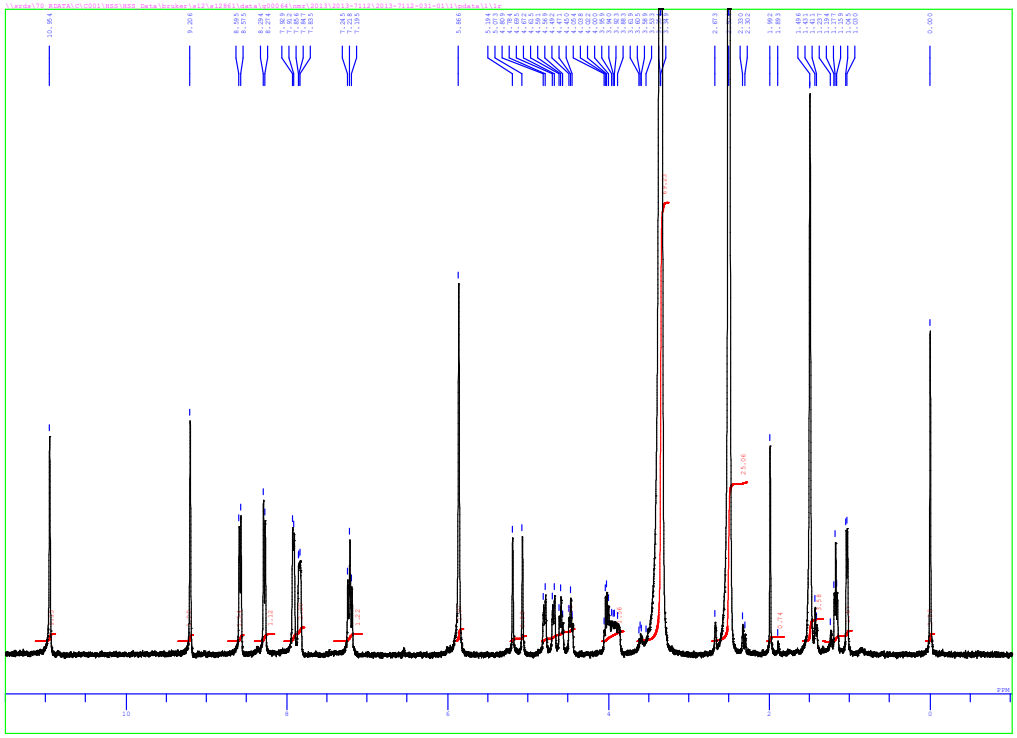

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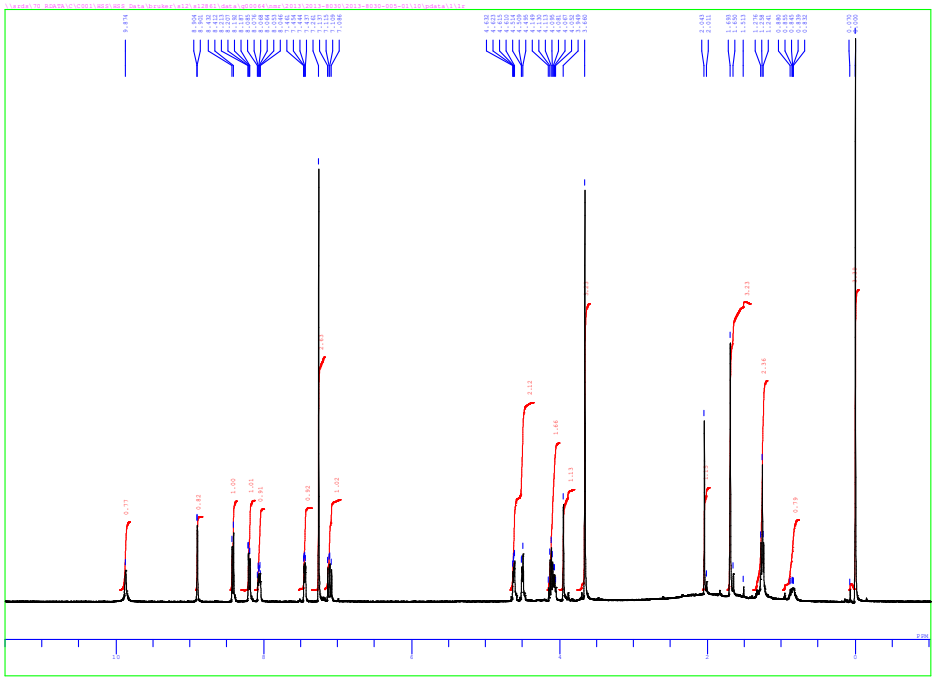

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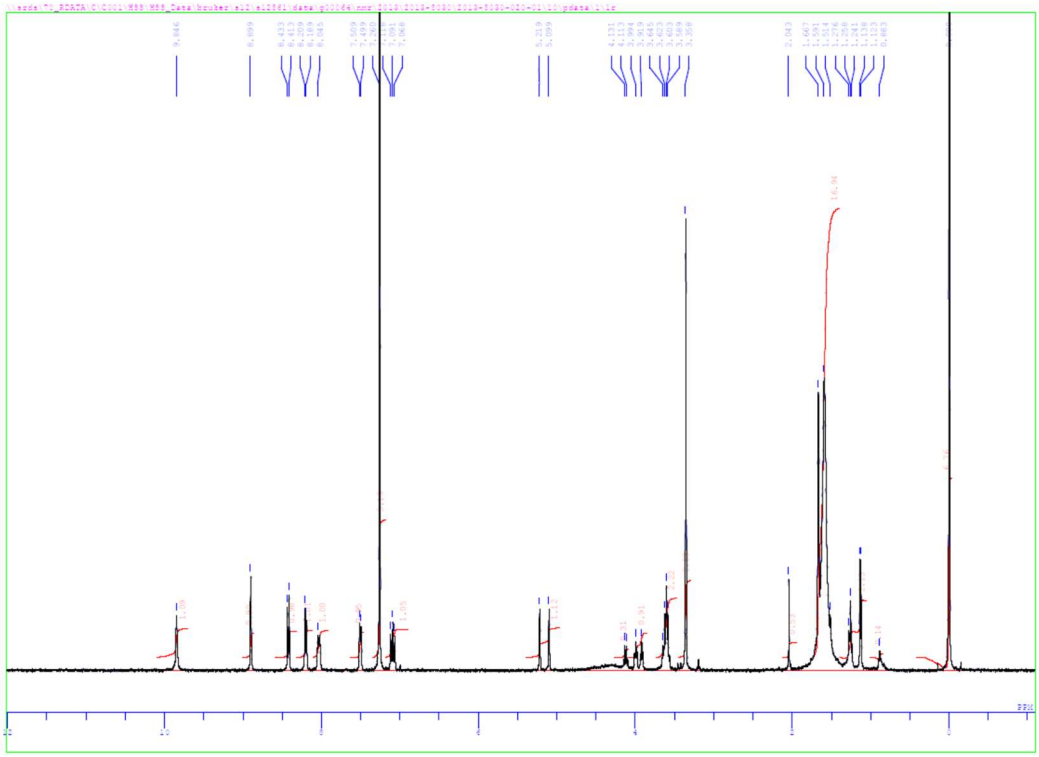

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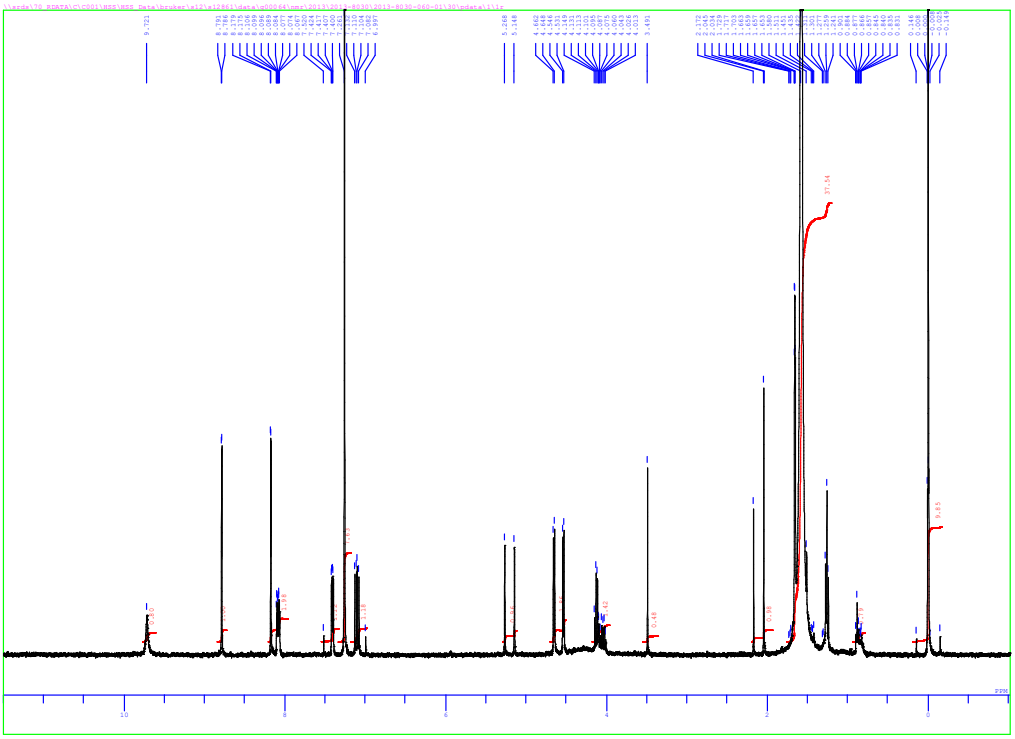

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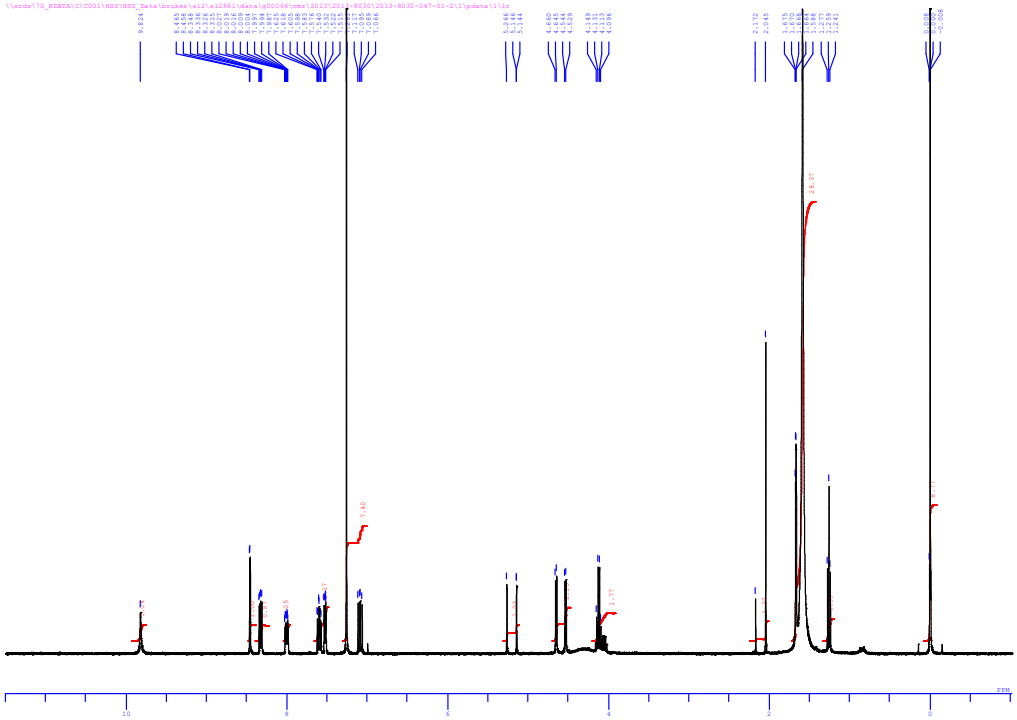

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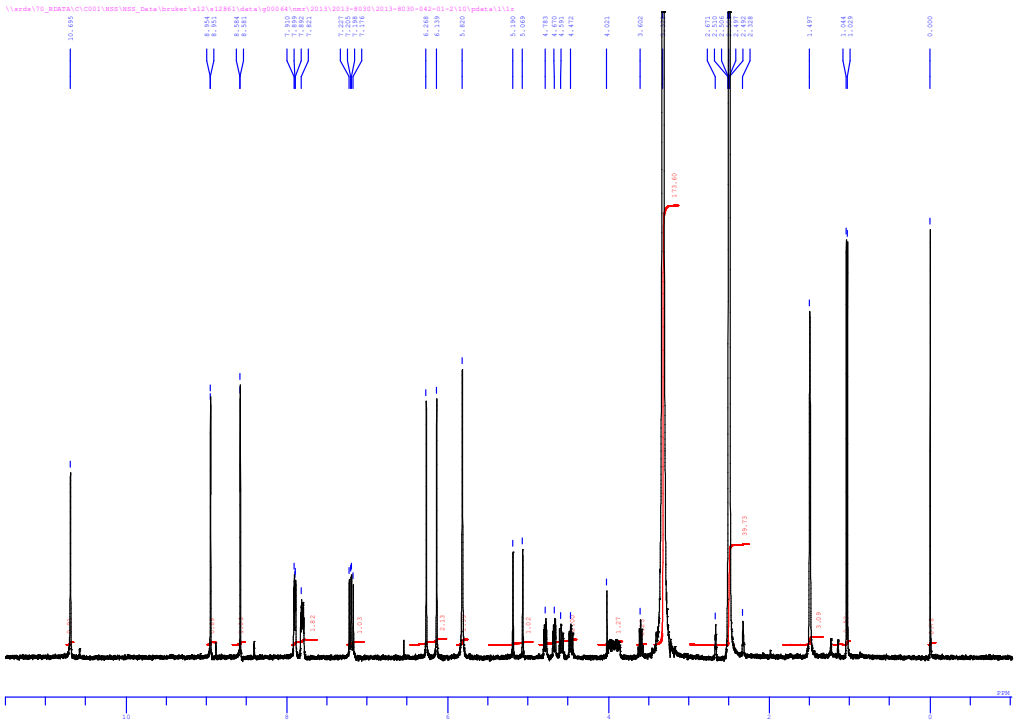

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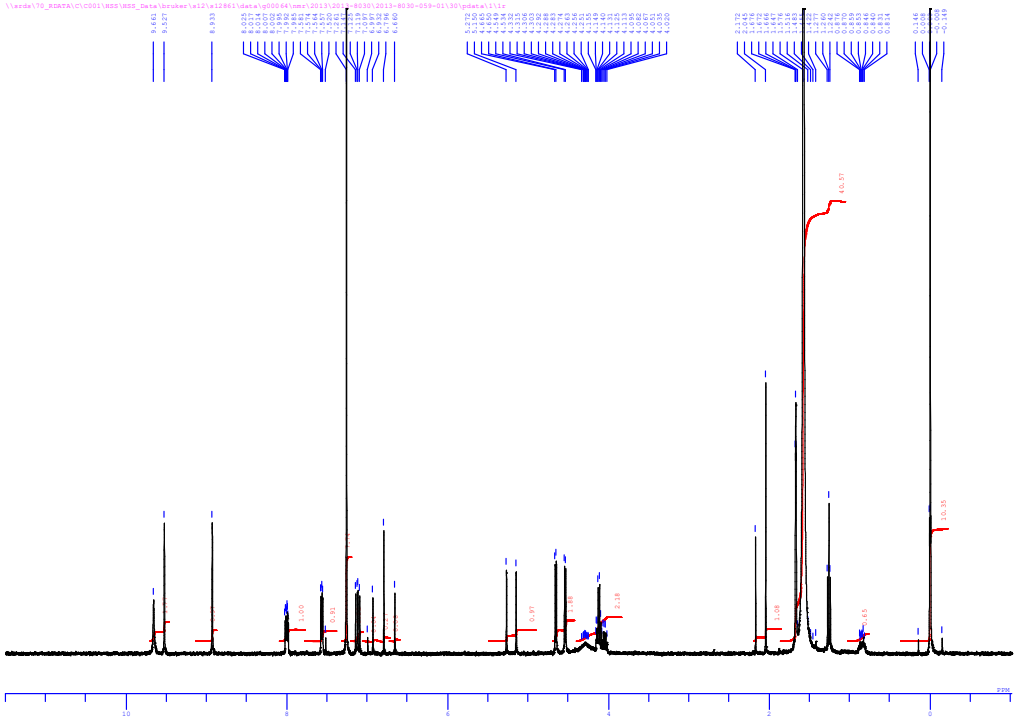

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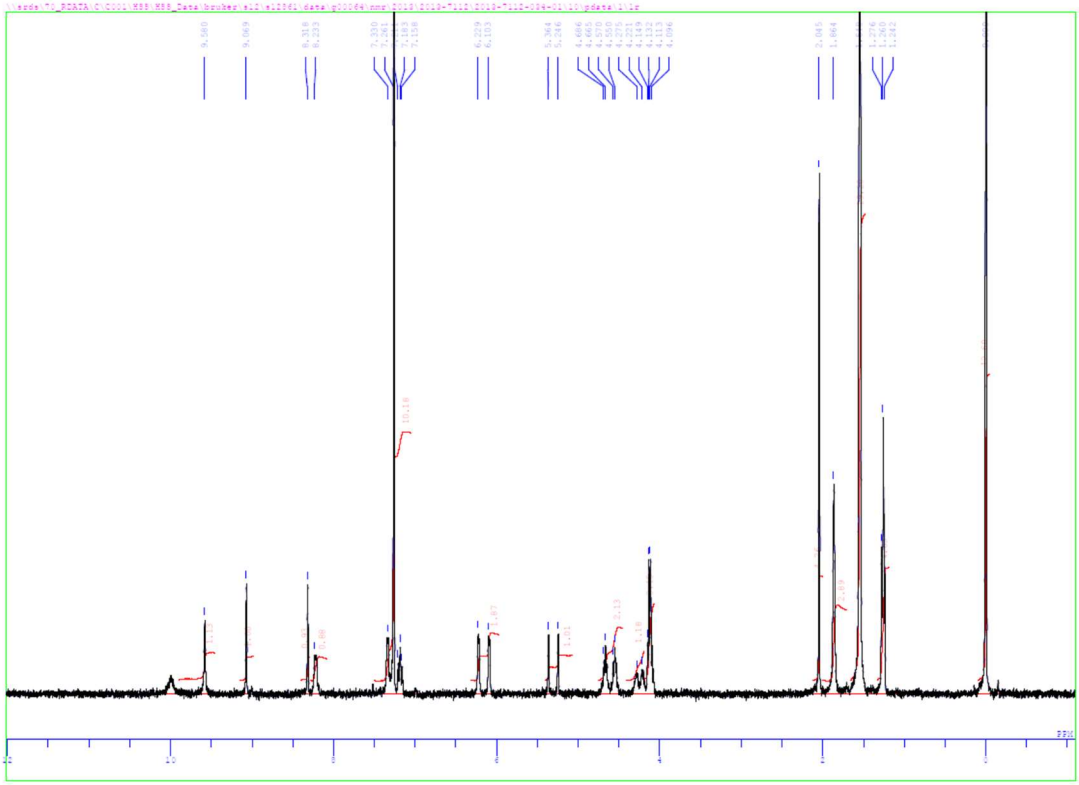

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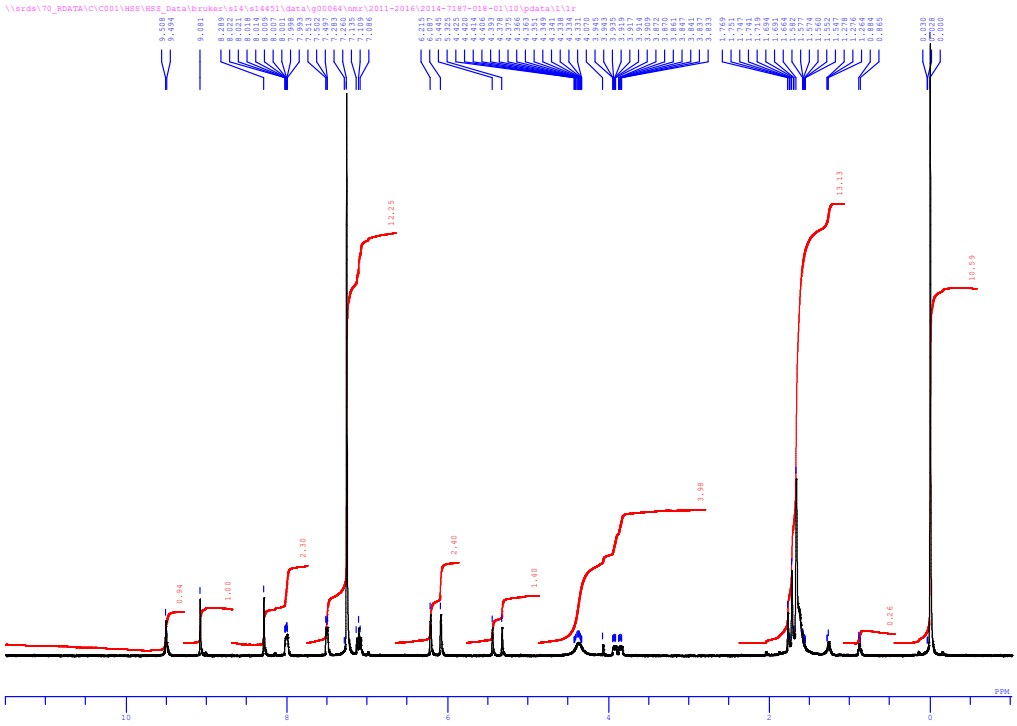

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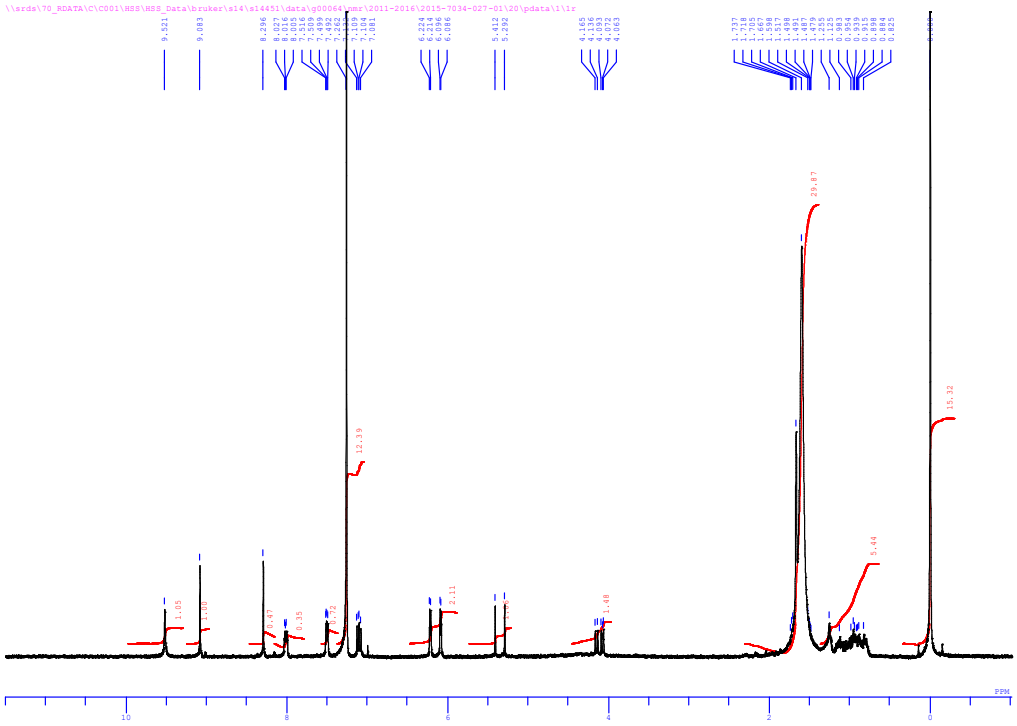

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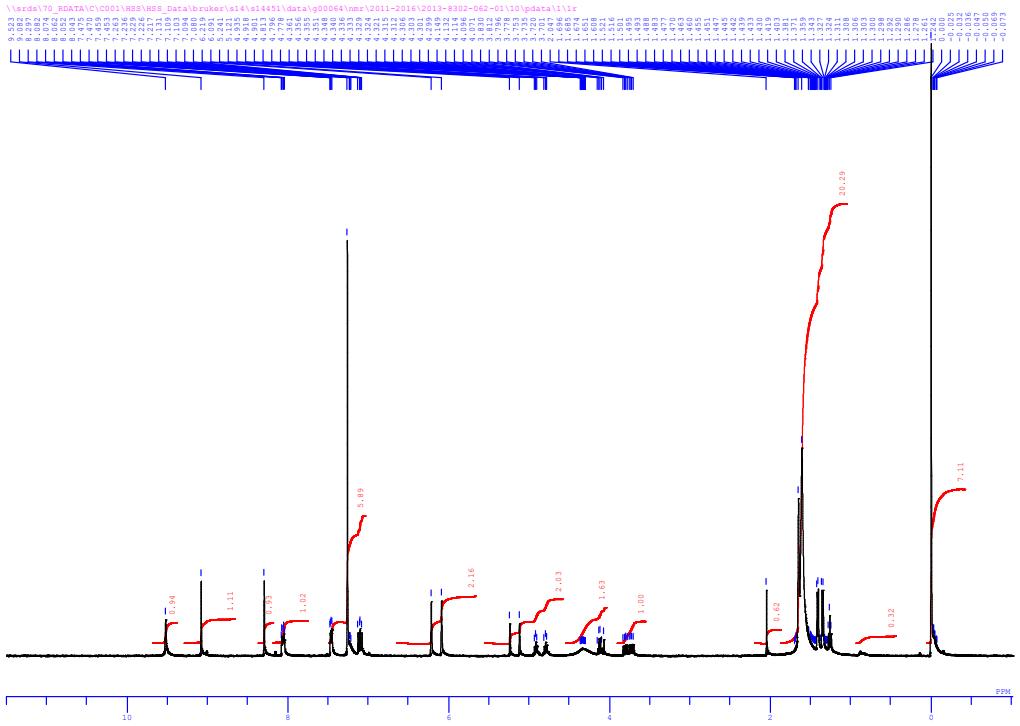

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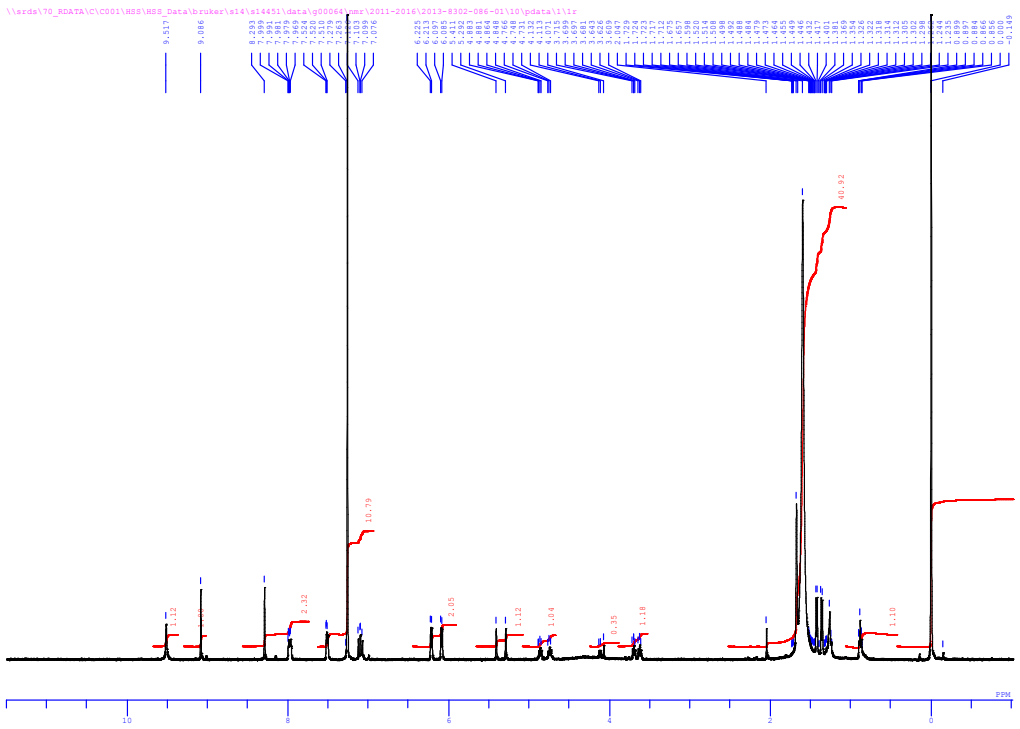

化合物の生物学的、物理学的評価

Biochemical BACE1 Assay

BACE1 activity was determined by a homogeneous time resolved fluorescence (HTRF) assay using a Swedish Lys-Met/ Asn-Leu mutation of the amyloid precursor protein (APP) β-secretase cleavage site, according to a previously published procedure[29]. The reference compound (cas# 797035-11-1)[30] showed an IC_{50} value of 22.6 ± 2.2 nM.

Cellular Aβ Assay

Neuroblastoma SH-SY5Y cells (SH/APPwt, ATCC CRL-2266) with human wild-type β-APP excessively expressed therein were prepared at 4×10^5 cells/mL, and 50 μL portions thereof were inoculated into each well of a 384-well culture plate (Corning) containing 0.5 μL of the test compound (DMSO solution). DMSO was present at 1% final concentration in the assay, and the amount of the cell culture was 50 μL. The plates were incubated for 24 h from the cell seeding, 5 μL of the culture supernatant were collected from each fraction followed by measurement of secreted Aβ1−40 using a homogeneous time resolved fluorescence (HTRF). Briefly, 5 μL of an HTRF measurement reagent (Amyloid β1−40 peptide; Cisbio Bioassays) and 5 μl of the culture supernatant were placed in a 384-well plate (a black plate: Corning) and mixed with each other, and the plate was kept overnight at 4 °C. The fluorescence intensity (620 nm and 665 nm) was measured using EnVision (Perkin Elmer Life Sciences). The Aβ1−40 amount was determined from the each fluorescence intensity ratio. The reference compound (cas# 797035-11-1) showed an IC_{50} value of 25.9 ± 2.8 nM.

P-gp MDCK Assay

The efflux ratios were determined for the wild-type MDCK cells transfected with human MDR1 gene at Absorption Systems[31]. Digoxin exhibited efflux ratios of 204 in MDCK cell transfected with MDR1 and 26.1 in the wild-type MDCK cells, and the net flux was 7.8. The P-gp ERs shown in the tables were the net flux.

P-gp LLC-PK1 Assay

MDR1 expressing LLC-PK1 cells (Becton Dickinson) and its parent cells were routinely cultured in Medium A (Medium 199 (Invitrogen) supplemented with 10% FBS (Invitrogen), gentamycin (0.05 mg/mL, Invitrogen) and hygromycin B (100 μg/mL, Invitrogen)) at 37 °C under 5% CO_2/95% O_2 gasses. For the transport experiments, these cells were seeded on a Transwell insert (96-well, pore size: 0.4 μm, Costar) at a density of 1.4×10^4 cells/insert with addition of Medium B (Medium 199 supplemented with 10% FBS and gentamycin at 0.05 mg/mL) to the feeder tray. These cells were incubated in a CO_2 incubator (5% CO_2/95% O_2 gases, 37 °C) with replacement of apical and basolateral culture medium every 48−72 h after seeding. These cells were used between 4 and 6 days after seeding. The medium in the culture insert seeded with MDR1 expressing cells or parent cells were removed by aspiration and rinsed with HBSS. The apical side (140 μL) or basolateral side (175 μL) was replaced with transport buffer containing a reference substrate, Digoxin, and the test compound and then a portion (50 μL) of transport buffer on the donor side was collected to estimate the initial concentration of the reference substrate and the test compounds. After incubation for a designated time at 37 °C, portions (50 μL) of transport buffer on the donor and receiver side were collected. The assay was performed in duplicate or triplicate. The reference substrate and the test compounds in the aliquot were quantified by LC/MS/MS.

The amounts that permeated across monolayers of MDR1-expressing cells and parent cells were determined, and permeation coefficients (P_{app}) were calculated using Excel using the following equitation:

$$P_{app}\ (\mathrm{cm/s}) = \frac{\text{Permeated amount (pmol)} \,/\, \text{area of cell membrane (cm}^2\text{)}}{\text{initial concentration (nM)} \,/\, \text{incubation time (s)}}$$

where the permeated amount was calculated from the permeation concentration (nM, concentration of the receiver side) of the substance after incubation for the designated time (s) multiplied by volume (mL), and 0.1433 (cm^2) was used as the area of the cell membrane.

The efflux ratio was calculated using the following equation:

$$\text{Efflux ratio} = \frac{P_{app}\ \text{(Basolateral to Apical)}}{P_{app}\ \text{(Apical to Basolateral)}}$$

The net flux was calculated using the following equation:

$$\text{Net flux} = \frac{\text{Efflux Ratio in MDR1 expressing cells}}{\text{Efflux Ratio in parent cells}}$$

Digoxin exhibited efflux ratios of 34.4 in LLC-PK1 cells transfected with MDR1 and 2.2 in LLC-PK1 cells, and the net flux was 15.6. The P-gp ER shown in the manuscript was the net flux.

Microsomal Stability Assay

The test compound was incubated at a defined substrate concentration of 0.1 μM in liver microsomes for 30 min at 37 °C. The liver microsomes used in this manuscript were as follows: Rat: Crj;CD(SD) male, 8weeks (prepared from Crj;CD(SD) rat in-house); Dog: Toyo or Marshall beagle, male/female (prepared in-house); Monkey: Cynomolgus monkey, male/female (prepared in-house); Human: pooled (purchased from XenoTech, LLC). The method was described in a previously published article.

CYP Inhibition Assay

The IC_{50} values of the test compounds for CYP1A2, 2C9, 2C19, 2D6, and 3A4 were determined using a substrate cocktail method in human liver microsomes. The substrate concentrations were 0.5 μM ethoxyresorufin for CYP1A2, 100 μM tolbutamide for CYP2C9, 50 μM S-mephenytoin for CYP2C19, 5 μM dextromethorphan for CYP2D6, and 1 μM terfenadine for CYP3A4. The range of the test compound concentration was 0 to 20 μM. The test compound, substrates, human liver microsomes (0.2 mg protein/mL), and 1 mM NADPH were mixed and incubated for 15 min at 37 °C. After centrifugation, the metabolites in the supernatant were measured using LC/MS/MS or a fluorescence plate reader. The remaining activity (%) was calculated at each concentration of the test compound compared to the solvent control (0.2% DMSO), and IC values were calculated by reverse presumption by a logistic model using a concentration and an inhibition rate.

Brain Tissue Binding Assay

The binding to rat brain homogenate was determined using the RED device as described previously.[15b)]

Plasma Protein Binding Assay in Serum

Equilibrium dialysis membrane was soaked in purified water and PBS. This membrane was placed into a dialysis cell. Formulations for intravenous administration were used for protein binding study. 4 µL of iv formulation was added to 996 µL of rat sera to obtain the serum sample at 2 µg/mL. Next, 450 µL of serum sample was placed into one side of the cell, and 450 µL of PBS was placed into the other side of the cell (n = 2). The cells were incubated at 37 °C for 24 h. After the incubation, 30 µL of serum sample and 270 µL of PBS sample were transferred into each tube. Next, 270 µL of blank PBS was added to the serum sample, and 30 µL of blank sera was added to PBS sample. The samples were mixed well and stored in a freezer until analysis. The supernatants obtained by protein precipitation of samples were analyzed by LC/MS/MS. The unbound fraction in serum ($f_{u,s}$) was determined with peak area of serum sample (A_{serum}) and PBS sample (A_{PBS}) as follows:

$$f_{u,s} = \frac{A_{PBS} \times \frac{10}{9}}{A_{serum} \times 10}$$

Plasma Stability Assay

The formulation for intravenous administration was diluted with DMSO to prepare 20 µg/mL solutions, and 10 µL of diluted solution was added to 990 µL of plasma and mixed to obtain the plasma sample at 200 ng/mL. The plasma samples were incubated for 4 and 24 h at 37 °C. The supernatants obtained by protein precipitation of plasma samples were analyzed by LC/MS/MS. The analytical method was calibrated using a standard curve constructed with blank plasma and known quantities of analytes. Similarly prepared quality control samples were included to monitor the accuracy and precision of the methodology. The remaining % at 24 h was calculated by comparing the concentration at 0 and 24 h. The $t_{1/2}$ in plasma was calculated assuming of first order degradation.

hERG Inhibition Assay

The in vitro potential for hERG potassium channel current inhibition was assessed in HEK293 or CHO cells expressing hERG channels using an automatic patch clamp system (QPatch system, Sophion Bioscience A/S). After a cell was retained at a membrane potential of −80 mV by whole cell patch

clamp method using the automated patch clamp system, hERG current was induced by the following protocol: leak current detect pulse stimulation at −50 mV for 0.1 s, depolarization pulse stimulation at +20 mV for 2 s. Tail peak current was measured by repolarization pulse stimulation at −50 mV for 2 s. Patch pipettes were filled with a solution consisting of 120 mM KCl, 5.4 mM $CaCl_2$, 1.8 mM $MgCl_2$, 31.3 mM/10 mM KOH/EGTA, 10 mmol/L HEPES, and 4 mM Na_2-ATP. After the generated current was stabilized, the vehicle application solution was applied to the cell under room temperature for 10 min, followed by the test substance solution. From the recorded hERG current, the absolute value of the tail peak current was measured based on the current value at the resting membrane potential using an analysis software (Falster Patch, Sophion Bioscience A/S). The percentage inhibition of the test compound against the pre-application values in tail peak currents was calculated from the mean values of tail peak currents for 3 pulses before (pre application) and after (post application) application using the described above software.

Animal Care and Use

All animal studies were performed with the approval of the Shionogi Animal Care and Use Committee.

PK Study in Wild-Type and *mdr1a* Knockout Mouse

The *in vivo* potential of P-gp substrate was assessed using *mdr1a* (-/-) B6 mouse, originally established by Shionogi & Co., Ltd., and the wild-type of C57BL/6J. Three animals were dosed at 2 or 10 mg/kg for each time point (0.5% MC suspension), and blood and brain samples were removed at selected time points (2 h) after dosing. Blood (0.3−0.7 mL) was collected via trunk blood collection with syringe containing anticoagulants (EDTA and heparin). Blood and brain samples were immediately placed on melting ice. Blood samples were centrifuged (1780*g* for 10 min) to obtain plasma. The plasma samples were transferred to a clean tube and stored in a −70 °C freezer until analysis. The brain samples were homogenized at a 1:3 ratio of tissue weight to mL of distilled water and transferred to a clean tube and stored in a −70 °C freezer until analysis. Plasma and brain samples were prepared using protein precipitation and analyzed by LC/MS/MS.

Rat PK Study

Male Sprague−Dawley rats (8 weeks old) were purchased from Charles River Laboratories. For the preliminary studies shown in Tables 1-2, 1-3 and 2-4 pharmacokinetic parameters were estimated from the plasma concentration measured by LC/MS/MS after a single oral or intravenous administration of multiple compounds suspended in 0.5% 400 cP MC or dissolved in *N*,*N*-dimethylacetamide (DMA)/propylene glycol (PG) (1/1, v/v) at 0.5 or 1 mg/kg to non-fasted rats (n = 2, cassette dosing), respectively. In the brain distribution study, blood samples were collected 30 min after intravenous dosing through the abdominal aorta with the rat under isoflurane anesthesia, and centrifuged for 10 min at 3500 rpm at 4 °C to obtain plasma. The plasma samples were transferred to separate tubes. The brain was removed and homogenized at a 1 to 3 ratio of tissue weight to mL of distilled water. Next, 200 µL of plasma sample and 200 µL of brain homogenate sample were transferred into separate tubes, and 200 µL of blank brain homogenate was added to the plasma sample while 200 µL of blank plasma was added to the brain homogenate sample. The samples were mixed well and stored in a freezer until analysis. The samples were analyzed by LC/MS/MS. The *B*/*P* ratios (K_p) were determined from the peak area of the plasma sample (A_{plasma}) and the brain homogenate sample (A_{brain}):

$$B/P\text{ratio }(K_p) = \frac{A_{brain} \times 4}{A_{plasma}}$$

For the PK study shown in Table 1-5 and 1-6, pharmacokinetic parameters were estimated from the plasma concentration measured after a single oral or intravenous administration of a discrete compound suspended in 0.5% 400 cP MC or dissolved in *N*,*N*-dimethylacetamide (DMA)/propylene glycol (PG) (1/1, v/v) at 3 or 2 mg/kg to non-fasted rats (n = 3), respectively. In the brain distribution study, blood samples were collected 1, 3, 5 and 7 hours after oral dosing through abdominal aorta under isoflurane anesthesia, centrifuged for 10 min at 3500 rpm at 4 °C to obtain plasma. The brain was removed and homogenized at a 1 to 3 ratio of tissue weight to mL of distilled water. CSF samples were also collected via the cisterna magna in isoflurane-anesthetized rats orally administered. The concentrations in plasma, brain homogenate and CSF were measured by LC/MS/MS. The detailed

method was described previously[32].

Mouse PK/PD Study

The test compound was dissolved in 20% hydroxylpropyl-β-cyclodextrin (the final concentration was adjusted to 2 mg/mL) and was orally administered to male Crl:CD1 (ICR) mouse (6 to 8 weeks old) at given doses. In the vehicle control group, only 20% hydroxylpropyl-β-cyclodextrin was administered, and an administration test was performed at 3 to 6 animals per group. A brain was isolated 1 to 6 h after administration, a cerebral hemisphere was isolated and weighed. The hemisphere was rapidly frozen in liquid nitrogen, and stored at −80 °C until extraction. The frozen cerebral hemisphere was transferred to a homogenizer tube containing ceramic beads in a 8-fold volume of the weight of an extraction buffer (containing 0.4% DEA (diethylamine), 50 mmol/L NaCl, Complete protease inhibitor (Roche)) and incubated on an ice for 20 min. Thereafter, the homogenization was done using MP BIO FastPrep-24 with Lysing matrix D 1.4 mm ceramic beads (20 s at 6 m/s). The tubes were spun down for 1 min, and the supernatant was transferred to a centrifugation tube and centrifuged at 221,000*g*, 4 °C for 50 min. After centrifugation, the supernatant was transferred to Nunc Maxisorp plate (Thermo Fisher Scientific) coating with antibody against *N*-terminal of β-amyloid for measuring total β-amyloid, and the plate was incubated overnight at 4 °C. The plate was washed with TBS-T (Tris buffered saline containing 0.05% Triton X-100), and HRP-conjugated 4G8 dissolved in PBS (pH 7.4) containing 0.1% casein was added in the plate and incubated at 4 °C for 1 h. After it had been washed with TBS-T, SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Scientific) was added to the plate. The chemi-luminescence counting was measured by ARVO MX 1420 Multilabel Counter (Perkin Elmer) as soon as possible. The lowering effect was calculated as a ratio compared to the brain total β-amyloid level of the vehicle control group of each test.

Dog PK/PD Study

The effect of the compounds on the beta-amyloid profile in cerebrospinal fluid (CSF) of Beagle dogs was tested in combination with pharmacokinetic (PK) follow up, according to the procedures

described previously. Upon oral administration of compound, the levels of Aβ1−38, Aβ1−39, Aβ1−40, and Aβ1−42 in CSF were measured at various time-points with immunoassay (Mesoscale electro chemiluminescence technology). The lowering effect was described as an EC_{50} value, which is defined as the plasma level of a tested compound (ng/mL) required for 50% lowering of Aβ in CSF. The EC_{50} value was determined after testing of the compound for dose response, using the statistical methods described previously.[18a)]

Guinea Pig Cardiovascular Safety Study

Animal species: Guinea pig (Slc:Hartley, 4−6 weeks old, male, $n = 4$)

Dosage: 3, 10, and 30 mg/kg.

Formulation: Composition of vehicle; dimethylacetamide (DMA):polyethylene glycol 400 (PEG400):distilled water = 1:7:2 (in principle). The test compounds were dissolved with DMA and then added PEG400 and distilled water. Finally, 1.5, 5, and 15 mg/mL solutions were prepared.

Dosing route and schedule: Intravenous infusion for 10 min (2 mL/kg). 0 to 10 min: 3 mg/kg, 30 to 40 min: 10 mg/kg, 60 to 70 min: 30 mg/kg. Vehicle was administered by the same schedule as above.

Group composition: Vehicle group and the test compound group (4 guinea pigs per group).

Evaluation items: Mean blood pressure [mmHg], Heart rate (derived from blood pressure waveform [beats/min]), QTc (ms), and Toxicokinetics (TK).

Experimental procedure: Guinea pigs were anesthetized with urethane (1.4 g/kg, ip), and polyethylene tubes were inserted into the carotid artery (for measuring blood pressure and sampling blood) and the jugular vein (for infusion test compounds). Electrodes were attached subcutaneously (Lead 2). Blood pressure, heart rate, and electrocardiogram (ECG) were measured using PowerLab system (ADInstruments).

Toxicokinetics (TK): Approximately 0.3 mL of blood (approximately 150 μL as plasma) were drawn from carotid artery with a syringe containing heparin sodium and cooled with ice immediately at each evaluation point. Plasma samples were obtained by centrifugation (4 °C, 10000 rpm, 9300*g*, 2 min). The procedure for separation of plasma was conducted on ice or at 4 °C. The obtained plasma (TK

samples) was stored in a deep freezer (set temperature: −80 °C).

Analysis methods: Mean blood pressure and heart rate were averaged a 30-second period at each evaluation time point. ECG parameters (QT interval [ms] and QTc were derived as the average waveform of a 10-second consecutive beats in the evaluation time points. QTc [Fridericia's formula; QTc = QT/(RR)1/3)] was calculated using the PowerLab (Registered trademark) system. The incidence of arrhythmia was visually evaluated for all ECG recordings (from 0.5 h before dosing to end of experiment) for all four animals.

Evaluation time points: Before (pre dosing), and 10, 25, 40, 55, 70, and 85 min after the first dosing.

Data analysis of QTc: Percentage changes (%) in QTc from the pre-dose value were calculated (the pre-dose value was regarded as 100%). Relative QTc was compared with vehicle value at the same evaluation point.

Dog Cardiovascular Safety Study

Animal: Beagle dogs (Marshall, 6 months-2 years old, male, $n = 4$)

Dosage: 30 and 100 mg/kg

Formulation: 0.5 w/v% methylcellulose solution, suspension (5 mL/kg)

Evaluation items: Blood pressure (systolic, diastolic, and mean [mmHg]), heart rate (derived from blood pressure waveform [beats/min]), ECG parameters (PR interval [ms], QRS duration [ms], RR interval [ms], QT interval [ms], and QTc [Fridericia's formula, ms]), and Toxicokinetics.

Experimental procedure: Four healthy animals previously implanted with a telemetry transmitter were used. A blood pressure-ECG transmitter (TL11M2-D70-PCT, Data Sciences International) was implanted in the dorsal subcutaneous layer, and the pressure sensor catheter of the transmitter was inserted into the femoral artery and placed at the abdominal aorta under pentobarbital anesthesia (30 mg/kg, *iv*). The ECG electrodes were placed on the right thoracic subcutaneous tissue and on the left abdominal subcutaneous tissue. The cardiovascular parameters were acquired continuously using a telemetry system from approximately 1 h before until 24 h after dosing. Each parameter was analyzed using an NOTOCORD hem (ver. 4.2.0,297, Notocord Systems S.A.).

Toxicokinetics (TK): Approximately 0.5 mL of blood (approximately 200 μL as plasma) were drawn from the cephalic vein with a syringe containing heparin sodium and cooled with ice immediately at each evaluation point. Plasma samples were obtained by centrifugation (4 °C, 3000 rpm, 1619*g*, 10 min). The procedure for separation of plasma was conducted on ice or at 4 °C. The obtained plasma samples (TK samples) were stored in a deep freezer (set temperature: −80 °C).

Analysis method: Blood pressure and heart rate were averaged for a 30 s period. ECG parameters) was derived as the average of a 10 consecutive beats at the evaluation points. QTc [Fridericia's formula; QTc = QT/(RR)1/3)] was calculated using Excel (Microsoft). The RR interval was used only for calculating QTc and was not evaluated. The incidence of arrhythmia was visually evaluated for all ECG recordings (from 0.5 h before dosing to end of experiment) for all animals.

Evaluation time points: Before (pre dosing), and 1, 2, 4, 6, 8, and 24 h after dosing.

Data analysis of QTc: Percentage changes (%) in QTc from the pre-dose value were calculated (the pre-dose value for % will be regarded as 100). Relative QTc was compared with vehicle value at the same evaluation point.

LogD Assay

To a glass vial were added 150 μL of octanol, 850 μL of phosphate buffer (pH 7.4), and 10 μL of test compound solution in DMSO (10 mM). The vials were sealed, and shaken for 1 h at room temperature. They were then centrifuged at 2000 rpm for 3 min. A volume of 20 μL of each octanol phase was transferred into a 96-well plate, and 180 μL of methanol was added to each octanol solution. A volume of 200 μL of each phosphate buffer phase was transferred into a 96-well plate. Quantification was performed by HPLC with an absolute calibration method. The LogD value was calculated using the following equation:

$$\mathrm{LogD} = \log \frac{[\text{concentration of the test compound in octanol phase}]}{[\text{concentration of the test compound in aqueous phase}]}$$

pK_a Assay

The pK_a values for the test compounds were determined using the capillary electrophoresis method

(CE)[33]. The experiments were performed on P/ACE MDQ (Beckman Coulter) equipped with untreated fused-silica (Beckman Coulter) capillaries of 50 μm ID, 30.2 cm total length, 20.0 cm effective length, and 20 μm aperture. Other CE conditions: current = 10 kV, electric field strength = 33,113 V/m, pressure = 0.7 psi, capillary temperature = 25 °C, electro osmotic flow marker = DMSO, PDA detection = 215 and 238 nm. Injection pressure = 0.5 psi and 0.5 s. To prepare a sample for injection, 4 μL of the test compound solution in DMSO (10 mM) was added to 6 μL of DMSO and 90 μL of water in a glass vial. L-Tryptophan was used as a control and exhibited pK_a values of 2.4 (acid) and 9.4 (base) with an error range of ±0.25.

BACE1 X-ray Crystallography

The X-ray structure for **2-14** was solved according to a method described previously[29].

Table S9. X-ray Data Collection and Refinement Statistics for Compound 2-14

Data collection	
Space group	$P6_122$
Cell dimensions (Å, °) [a]	$a = b = 101.55$, $c = 170.81$, $\gamma = 120$
Resolution (Å)[a]	50.78 - 2.2 (2.35 - 2.20)
R_{sym} [a, b]	0.111 (0.491)
<I / σI>[a]	12.9 (3.4)
Completeness (%) [a]	98.9 (95.5)
Total reflections [a]	180574 (2266)
Unique reflections [a]	26786 (3683)
Redundancy [a]	6.7 (6.2)
Refinement	
Resolution (Å) [a]	20 - 2.2 (2.257 – 2.200)
R_{work} [a, c] / R_{free} [a, d]	0.178 (0.210) / 0.207 (0.236)
No. atoms	
Protein	2947

Water	277
Ligand	32
Average B-factors	
Protein (Å^2)	34.0
Water (Å^2)	40.4
Ligand (Å^2)	26.8
Ramachandran Plot [e]	
Favoured (%)	97.6
Allowed (%)	2.1
Outlier (%)	0.3
R.m.s deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.403
PDB ID	5YGX

[a] Values in parentheses are for the highest resolution shell.

[b] $R_{sym} = \sum| I - <I>|/\sum I$, where I is the intensity of observation I and $<I>$ is the mean intensity of the reflection.

[c] $R_{work} = \sum||F_o| - |F_c||/\sum|F_c|$, where F_o and F_c are the observed and calculated structure factor amplitudes, respectively.

[d] R_{free} was calculated using a randomly selected 5% of the data set that was omitted through all stages of refinement.

[e] Ramachandran plot was obtained for all residues other than Gly and Pro.

Conformational Energy Calculations

Conformational analyses were performed using the MacroModel in the Schrödinger 2015 update 4 modeling package[34]. Each compound was subjected to 2500 steps of mixed torsional/low-mode sampling using OPLS3 force field to obtain the low energy conformations, followed by quantum mechanics (QM) energy optimizations on these low energy conformations by using Jaguar in the Schrödinger package. QM geometry optimizations were carried out using the B3LYP hybrid density

functional and the 6-31G** basis set. Single point energy calculations of the pseudoaxial and the pseudoequatorial conformations for each compound were performed using B3LYP/6-31G** with the Poisson-Boltzmann model to take the water solvation effect into account. The relative energies were calculated using these energies.

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28) **Figure 2-4** および **2-5** 内の **0-3**、**2-1**、**2-3**、**2-5**、**2-6** の変形エネルギー（ΔEax-eq(kcal/mol)）

は Schrödinger 2015 update 4 modeling package を用いて算出した（実験の部参照）。**2-17** は Schrödinger 2019 update 4 modeling package を用いて算出した。Schrödinger 2019 update 4 modeling package を用いて算出した **2-5** および **2-6** の変形エネルギーは 0.83 kcal/mol および-6.19 kcal/mol であった。

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