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名古屋市立大学学位論文

シクロヘキサン含有スピロ環を有する
新規GPR119アゴニストの探索合成研究

令和2年度（2020年6月）

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[1]. 本論文は、2020 年 6 月に名古屋市立大学大学院薬学研究科において審査されたものである。

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[2]. 本論文は、学術情報雑誌に収載された次の報文を基礎とするものである。

1. K. Harada, J. Mizukami, S. Kadowaki, I. Matsuda, T. Watanabe, Y. Oe, Y. Kodama, K. Aoki, K. Suwa, S. Fukuda, S. Yata, T. Inaba

Design and synthesis of novel and potent GPR119 agonists with a spirocyclic structure

Bioorg. Med. Chem. Lett. **2018**, 28, 1228.

2. K. Harada, J. Mizukami, T. Watanabe, G. Mori, M. Ubukata, K. Suwa, S. Fukuda, T. Negoro, M. Sato, T. Inaba

Lead generation and optimization of novel GPR119 agonists with a spirocyclic cyclohexane structure

Bioorg. Med. Chem. Lett. **2019**, 29, 373.

3. K. Harada, J. Mizukami, T. Watanabe, G. Mori, M. Ubukata, K. Suwa, S. Fukuda, T. Negoro, M. Sato, T. Inaba

Optimization of oxadiazole derivatives with a spirocyclic cyclohexane structure as novel GPR119 agonists

Bioorg. Med. Chem. Lett. **2019**, 29, 2100.

[3]. 本論文の基礎となる研究は、日本たばこ産業株式会社において稲葉隆之博士および佐藤元秀博士の指導の下に行われた。

略語表

Ac	Acetyl	アセチル
AUC	Area under the curve	曲線下面積
AZADO	2-Azaadamantane <i>N</i> -oxyl	2-アザアダマンタン- <i>N</i> -オキシル
BA	Bioavailability	生物学的利用能
BINAP	2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl	2,2'-ビス(ジフェニルホスフィノ)-1,1'-ビナフチル
Bn	Benzyl	ベンジル
Boc	<i>tert</i> -Butoxycarbonyl	<i>tert</i> -ブトキシカルボニル
Bu	Butyl	ブチル
cAMP	Cyclic adenosine monophosphate	環状アデノシンーリン酸
CDI	1,1'-carbonyldiimidazole	1,1'-カルボニルジイミダゾール
CL	Clearance	クリアランス
CSA	10-Camphorsulfonic acid	10-カンファースルホン酸
CYP	Cytochrome P450	シトクロム P450
DavePhos	2-Dicyclohexylphosphino-2'-(<i>N,N</i> -dimethylamino)biphenyl	2-ジシクロヘキシルホスフィノ-2'-(<i>N,N</i> -ジメチルアミノ)ビフェニル
DHP	3,4-Dihydro-2 <i>H</i> -pyran	3,4-ジヒドロ-2 <i>H</i> -ピラン
DMAD	<i>N,N,N',N'</i> -tetramethylazodicarboxamide	<i>N,N,N',N'</i> -テトラメチルアゾジカルボキサミド
DMF	Dimethylformamide	ジメチルホルムアミド
DMSO	Dimethyl sulfoxide	ジメチルスルホキシド
DPPA	Diphenylphosphoryl azide	ジフェニルリン酸アジド
EC ₅₀	Half-maximal effective concentration	50%効果濃度
ESI	Electrospray ionization	エレクトロスプレーイオン化
Et	Ethyl	エチル
FeSSIF	Fed state simulated intestinal fluid	摂食時における人工腸液
Fsp ³	Fraction of sp ³	化合物中の全炭素に占める sp ³ 炭素の割合
<i>gem</i> -	Geminal	ジェミナル
HEK293	Human embryonic kidney cells 293	ヒト胎児腎細胞 293
HOBt	1-Hydroxybenzotriazole	1-ヒドロキシベンゾトリアゾール
HRMS	High resolution mass spectrometer	高分解能質量分析
HTRF	Homogeneous time-resolved	均一系時間分解蛍光

	fluorescence	
IA	Inherent activity	固有活性
IC ₅₀	Half-maximal inhibitory concentration	50%阻害濃度
iv	Intravenous	静脈内の
JP1	Japanese Pharmacopoeia 1st fluid for a dissolution test adjusted to pH 1.2	pH 1.2 の日本薬局方溶出試験第 1 液
LDA	Lithium diisopropylamide	リチウムジイソプロピルアミド
LiHMDS	Lithium bis(trimethylsilyl)amide	リチウム (ビストリメチルシリル) アミド
LLE	Ligand-lipophilicity efficiency	リガンド脂溶性効率
LogP	Partition coefficient	分配係数
Me	Methyl	メチル
mp	Melting point	融点
MRT	Mean residence time	平均滞留時間
Ms	Microsomes	ミクロソーム
NBS	<i>N</i> -Bromosuccinimide	<i>N</i> -ブロモスクシンイミド
NMP	<i>N</i> -Methylpyrrolidone	<i>N</i> -メチルピロリドン
NMR	Nuclear magnetic resonance spectroscopy	核磁気共鳴スペクトル
Ph	Phenyl	フェニル
PK	Pharmacokinetics	薬物動態
po	Per os	経口投与
PPTS	Pyridinium <i>p</i> -toluenesulfonate	パラトルエンスルホン酸ピリジニウム
Pr	Propyl	プロピル
<i>rac</i>	Racemic	ラセミの
TBAF	Tetrabutylammonium fluoride	テトラブチルアンモニウムフルオリド
TBDMS	<i>tert</i> -butyldimethylsilyl	<i>tert</i> -ブチルジメチルシリル
THF	Tetrahydrofuran	テトラヒドロフラン
THP	Tetrahydropyran	テトラヒドロピラン
Ts	<i>p</i> -Toluenesulfonyl	パラトルエンスルホニル
Vdss	Volume of distribution at steady state	定常状態における分布容積
WSC	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride	1-エチル-3-(3-ジメチルアミノプロピル)カルボジイミド塩酸塩

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第1章 緒言

第1節 糖尿病

糖尿病は、血糖低下作用を有する唯一のホルモンであるインスリンの分泌低下・不全または作用不足のため高血糖状態が持続する疾患である。国際糖尿病連合（International Diabetes Federation: IDF）が発行した「糖尿病アトラス第9版2019」によると、世界の糖尿病患者数は4億6300万人に達し、2045年には約7億人にまで増加すると予測されている¹。なお、日本、中国、東南アジア諸国、オーストラリアを含む西太平洋地域は、世界最多の糖尿病患者（約1億6000万人）を有する地域であり、その理由として、遺伝要因（インスリン分泌量が少ない人種）と環境要因（経済発展による食の欧米化）が考えられる²。日本においては、厚生労働省が実施した「2016年国民健康・栄養調査」によると、糖尿病患者が約1000万人いるとされ、糖尿病の可能性を否定できない者（糖尿病予備群）の約1000万人を含めると、全人口の約6人に1人が糖尿病患者またはその予備群と推定される³。

糖尿病の病態は、その初期では無自覚・無症状で進行することが多いが、慢性的な高血糖状態により糖尿病合併症として細小血管合併症（網膜症、腎症、神経障害）や大血管合併症（虚血性心疾患、脳血管障害、閉塞性動脈硬化症）を発症及び進展する^{4,5}。糖尿病性網膜症では、網膜に変化をきたし、視力低下を認め、最悪の場合失明に至る。糖尿病性腎症では、腎臓の糸球体に傷害を受け、腎機能障害や腎不全を発症し、腎不全の場合には人工透析が必要となる。糖尿病性神経障害では、神経細胞の代謝障害や毛細血管の循環障害による壊疽を発症し、四肢の切断に至る場合がある。

糖尿病の病型は、1型糖尿病と2型糖尿病に大別される⁵。1型糖尿病は、主に自己免疫の作用により膵臓のインスリン産生細胞である膵ランゲルハンス島内β細胞が破壊され、インスリンの分泌が不全となり発症する。1型糖尿病患者数は糖尿病全体の約1%であり、日本での年間発症率は10万人につき1～2人とされる。なお、発症時期は幼少時が多い。一方、2型糖尿病は、様々な遺伝因子や環境因子（過食、ストレス、肥満、加齢など）によってインスリンの分泌能の低下やインスリン抵抗性が惹起され、インスリンの作用が不足し発症する。2型糖尿病患者数は糖尿病全体の9割以上を占め、40歳以上での発症が多いとされるが、近年若年層の発症が増加している。

糖尿病の診断には、血液検査と75 g経口ブドウ糖負荷試験（oral glucose tolerance test: OGTT）が用いられる⁵。早朝空腹時血糖値が126 mg/dL以上または75 g OGTTにおける2時間血糖値が200 mg/dL以上に加えて、慢性的な高血糖状態を表す指標として用いられるヘモグロビンA1c（HbA1c）が6.5%以上の場合、糖尿病と診断される。

糖尿病の治療では、適切な血糖値コントロールが基本方針となる⁵。HbA1c 6.0%未満を血糖正常化として目指す目標、7.0%未満を合併症予防のための目標としてそれぞれ設定されている。糖尿病の治療方法として、食事療法、運動療法、薬物療法が挙げられる。食事療

法では、性、年齢、体重、肥満度、身体活動量、血糖値、合併症の有無などを考慮し、エネルギー摂取量が決定される（Figure 1）⁵。

エネルギー摂取量	=	目標体重	×	身体活動レベルと 病態による エネルギー係数
		65歳未満：[身長（m）] ² ×22 65～74歳：[身長（m）] ² ×22～25 65～74歳：[身長（m）] ² ×22～25*		軽い労作：25～30 kcal/kg 普通の労作：30～35 kcal/kg 重い労作：35～ kcal/kg

*：75歳以上の後期高齢者では現体重に基づき、フレイル、（基本的）ADL（日常生活動作）低下、併発症、体組成、身長の変化、摂食状況や代謝状態の評価を踏まえ、適宜判断する。

Figure 1. Calculation of recommended energy intake.

運動療法では、できれば毎日、少なくとも週3～5回、強度が中等度の有酸素運動を20～60分間を含め、計150分以上の運動が推奨される⁵。食事療法、運動療法を2～3カ月継続し、目標の血糖値コントロールを達成できない場合、薬物療法の開始が検討される。

第2節 糖尿病治療薬（血糖低下薬）

薬物療法に用いられる主な糖尿病治療薬（血糖低下薬）として、①インスリン、②インスリン分泌促進薬（スルホニルウレア（sulfonylurea：SU）薬、速攻型インスリン分泌促進薬（グリニド薬））、③インスリン抵抗性改善薬（ビグアナイド薬、チアゾリジン（thiazolidines：TZD）薬）、④α-グルコシダーゼ阻害薬、⑤ナトリウム/グルコース共輸送体2（sodium-glucose cotransporter 2：SGLT2）阻害薬、⑥インクレチン関連薬（グルカゴン様ペプチド-1（glucagon-like peptide 1：GLP-1）アゴニスト、ジペプチジルペプチダーゼ-4（dipeptidyl peptidase-4：DPP-4）阻害薬）が知られている⁵。

①インスリン

インスリンは、膵臓に存在するランゲルハンス島のβ細胞から分泌されるペプチドホルモンの一種であり、血糖を下げる唯一のホルモンである。インスリン療法では、インスリンを体外から皮下注射により補充し、インスリン分泌を司る膵臓の負担を減らし、その機能回復を図る。副作用として、低血糖と体重増加が挙げられる。

②インスリン分泌促進薬

・SU薬

膵臓β細胞のSU受容体に結合し、膵臓からのインスリン分泌を促進する作用を有する。副作用として、低血糖が挙げられる。

・速攻型インスリン分泌促進薬（グリニド薬）

SU薬と同様、膵臓β細胞のSU受容体に結合し、膵臓からのインスリン分泌を促進する作用を有し、SU薬よりも素早くその薬効を示す特徴がある。副作用として、SU薬と同様、低血糖が挙げられる。

③インスリン抵抗性改善薬

・ ビグアナイド薬

主に肝臓からの糖の放出を抑える作用を示す他、インスリン抵抗性改善により筋肉や脂肪組織における糖の取り込みを促進させる作用、腸管（小腸）からの糖吸収を抑える作用を有し、これらの複数の作用によって血糖値を是正する効果を示す。副作用として、乳酸アシドーシスが挙げられる。

・ TZD薬

インスリン抵抗性を改善し、組織（筋肉、脂肪）での糖取り込み、糖利用の改善や肝臓での糖放出を抑えることで血糖値を改善する作用を有する。副作用として、体重増加、浮腫が挙げられる。

④α-グルコシダーゼ阻害薬

α-グルコシダーゼは、ショ糖（砂糖）等二糖類をブドウ糖（グルコース）に変換する酵素である。α-グルコシダーゼ阻害薬は、食後の急激な血糖値の上昇を抑制する。副作用として、低血糖、消化器症状（下痢・放屁）が挙げられる。

⑤SGLT2阻害薬

SGLTは、細胞内外のナトリウムイオンの濃度差を駆動力として、糖を細胞内に取り込む能動輸送を担うトランスポーターである。SGLTには、SGLT1とSGLT2の2種類のサブタイプが知られ、腎臓の近位尿細管に存在するSGLT2は、腎臓の糸球体でろ過されたグルコースを再吸収する機能を有する。SGLT2阻害薬は、近位尿細管においてグルコースの再吸収を阻害して尿糖排泄量を増加させることで、インスリン非依存的血糖低下作用を有する。副作用として、脱水、尿路・性器感染症が知られている。

⑥インクレチン関連薬

・ DPP-4阻害薬

DPP-4は、体内に食物が入った後にグルコース濃度依存的なインスリン分泌を促す作用を示すGLP-1、GIPなどインクレチン（後述）を分解し、不活性化する酵素である。DPP-4阻害薬は、DPP-4によるインクレチンの分解を阻害し、インクレチンの作用に則したグルコース濃度依存的なインスリン分泌を促すことで、血糖低下作用を示す。単独投与では低血糖を起こす頻度は低いとされるが、SU薬との併用投与時には低血糖に注意が必要である。

・ GLP-1アゴニスト（受容体作動薬）

GLP-1アゴニストは、DPP-4による分解を受け難い（DPP-4抵抗性）ヒトGLP-1アナログであり、長時間にわたりGLP-1の作用が継続することで血糖低下作用を示す。副作用として、DPP-4阻害薬と同様、SU薬との併用投与での低血糖が知られている。これまでに上市されているGLP-1アゴニストでは皮下注射が必要であったが、2019年に世界初の経口GLP-1アゴニストであるセマグルチドが米国および日本で承認申請された。

以上、主な糖尿病治療薬について簡潔に紹介した。その中で、単剤での低血糖と体重増加リスクが低い糖尿病治療薬であるインクレチン関連薬⁶について、次節で詳しく述べる。

第3節 インクレチン関連薬

インクレチン（incretin）とは、体内に食物など栄養素が入った後にグルコース濃度依存的なインスリン分泌を促す消化管ホルモンの総称である⁷。インクレチンは、グルコースを経口投与した際に経静脈投与に比べてより多くのインスリン分泌が促進される現象（インクレチン効果）が観られたことを契機に発見された。インクレチンとして、グルコース依存性インスリン分泌刺激ポリペプチド（glucose-dependent insulintropic polypeptide : GIP）とGLP-1が知られる。GIPは小腸上部のK細胞において、GLP-1は小腸下部および大腸のL細胞において、それぞれ分泌される。消化管で分泌されたGIPとGLP-1は、膵臓に発現するそれぞれの受容体に作用し、膵β細胞でのインスリン分泌を促進すると共に、膵α細胞でのグルカゴン（肝臓での糖新生を促進するホルモン）分泌を抑制することで血糖を低下させる作用を有する。また、インクレチンの膵保護作用として、膵β細胞の分化誘導、アポトーシス抑制、膵β細胞重量の増加効果が知られている。また、GLP-1受容体は膵臓のみならず脳、心臓、消化管、肝臓にも発現し、GLP-1の膵外作用として食欲抑制作用、胃排出遅延作用等が知られている。ただし、これらインクレチンは血中でDPP-4により速やかに分解され、血中半減期はGLP-1では2分、GIPでは5分と短い。

DPP-4阻害薬は、GLP-1などインクレチンを分解するDPP-4を阻害することで、活性型GLP-1濃度を維持し、血糖低下作用を示すと考えられる（Figure 2）。また、活性型GLP-1のアミノ酸配列の一部が変換されDPP-4抵抗性が付与されたGLP-1アゴニストは、長時間作用型の活性型GLP-1として薬理作用を示すと考えられる。



Figure 2. Incretin-based drugs.

第4節 GPR119 アゴニスト

DPP-4やGLP-1受容体以外に、GLP-1などインクレチンに関連するターゲットとして、Gタンパク質共役型受容体119（GPR119）が知られている。GPR119は、創薬ターゲットとして知られているアンジオテンシン受容体、ドーパミン受容体、エンドセリン受容体等を含むクラスA（ロドプシン様）Gタンパク質共役型受容体の一つである（Figure 3）⁸。

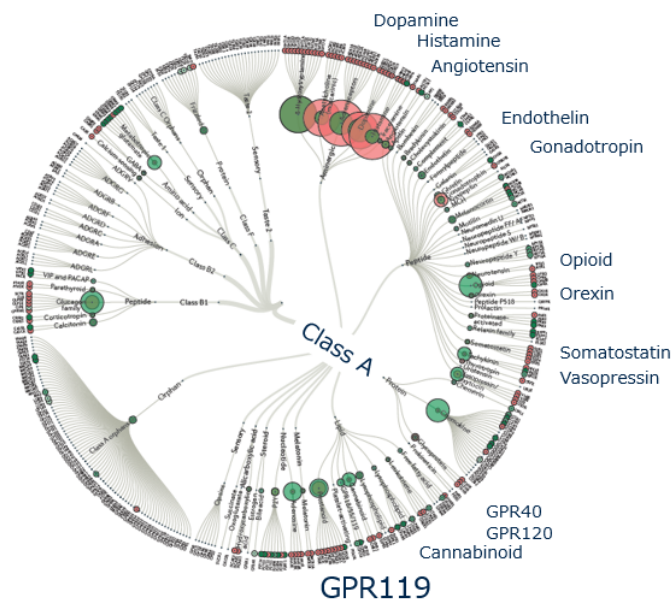


Figure 3. GPCR Tree.

GPR119は、ヒトおよび齧歯類の消化管L細胞および膵β細胞に高発現しており、ラットでは脳での発現が報告されている（Figure 4）^{9,10}。

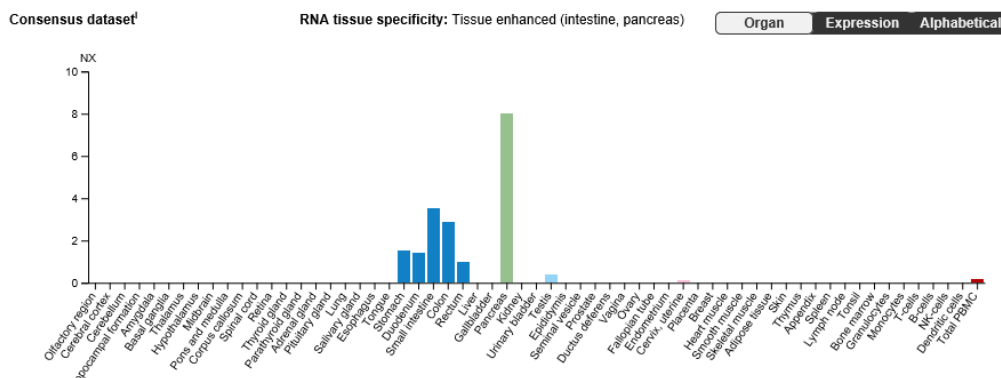


Figure 4. Expression overview of GPR119.

内因性GPR119アゴニストとして、オレイン酸エタノールアミド (oleoylethanolamide: OEA),

オレイン酸リゾホスファチジルコリン (oleoyl-lysophosphatidylcholine : oleoyl-LPC) などが知られている (Figure 5) ¹¹。

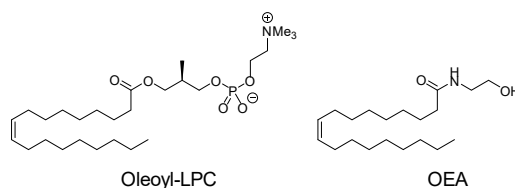


Figure 5. Endogenous GPR119 agonists.

GPR119アゴニストは、Gαsと共役するGPR119に結合して細胞内のアデニル酸シクラーゼを活性化し、サイクリックAMP (cAMP) の上昇を介して腸管でのGLP-1等インクレチンおよび膵β細胞での糖濃度依存的インスリン分泌促進作用を示すことが報告されている (Figure 6) ¹¹。

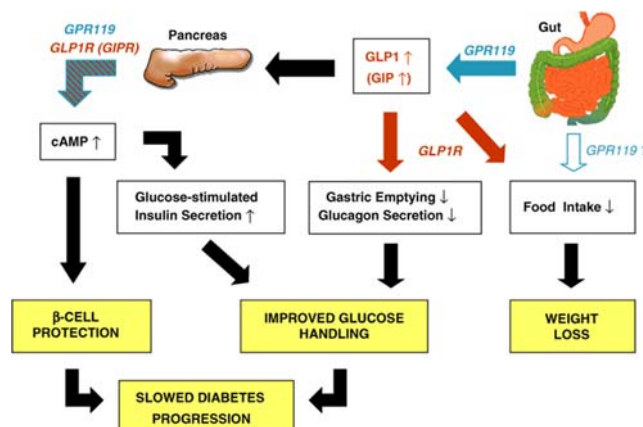


Figure 6. Proposed mechanisms of GPR119 agonist action.

以上のことから、GPR119アゴニストは、DPP-4阻害薬やGLP-1アゴニストと同様、低血糖と体重増加リスクが低い新規メカニズムの糖尿病治療薬として期待されるⁱ。なお、臨床試験に移行したGPR119アゴニストが複数報告されている (Figure 7) ⁶。

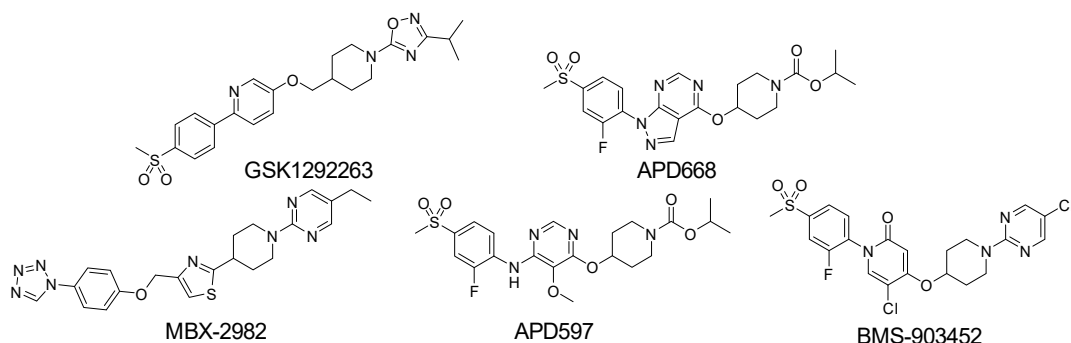


Figure 7. Clinical GPR119 agonists with disclosed structures.

ⁱ GPR119 アゴニストの抗肥満薬・抗高脂血症薬としての可能性を示唆する報告がある ^{9,11}。

第5節 研究方針と論文の概要

GPR119アゴニストは、低血糖と体重増加リスクが低い新規メカニズムの糖尿病治療薬として注目され、数多くの製薬企業により研究開発が為されてきた。実際、GPR119アゴニストとして様々なケモタイプの化合物が報告され、複数の化合物は臨床試験に移行していた。こうした背景の下、筆者は強力な薬効と高い安全性を有する新規GPR119アゴニストの創出に着手した。

本論文では、筆者が日本たばこ産業株式会社において取り組んだ探索合成研究について論じる。第2章では、既知GPR119アゴニストに多くみられる*N*-置換ピペリジン環の代替構造としてシクロヘキサン含有スピロ環を有する、新規GPR119アゴニストの創出について論じる。第3章では、シクロヘキサン含有スピロ環を有し、脂溶性が低減した新規GPR119アゴニストの創出について論じる。第4章では、*in vivo*ポテンシー向上を目指したシクロヘキサン含有スピロ環を有するオキサジアゾール誘導体の最適化について論じる。

第2章 シクロヘキサン含有スピロ環を有する新規 GPR119 アゴニストの創出

第1節 背景と戦略

本研究開始当時、既知 GPR119 アゴニストの多くは、Figure 8 に示すように、右側にカルバマートまたはヘテロアリール基 (R) で置換されたピペリジン環、左側にメチルスルホニル基またはヘテロアリール基 (X) で置換されたフェニル基と、これら2つの部分を連結するリンカーで構成されていた。なかでも、右側の *N*-置換ピペリジン環は、多くの GPR119 アゴニストの共通構造であった⁶。

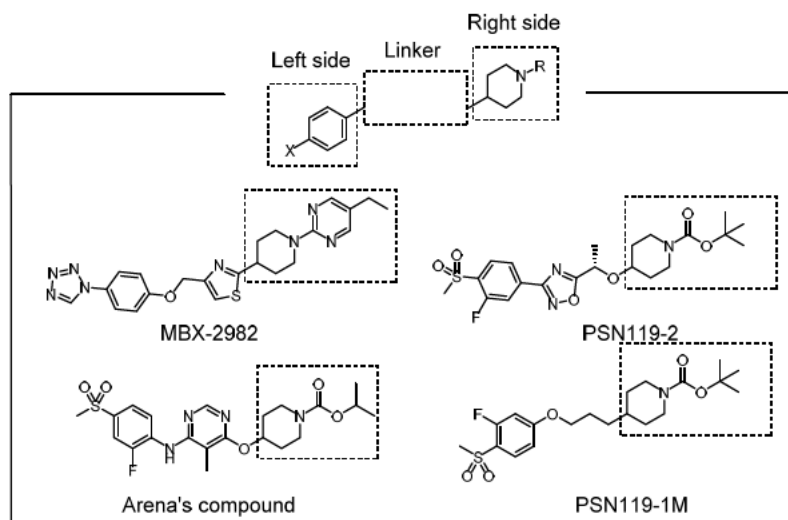


Figure 8. Typical GPR119 agonists.

本章では、新規GPR119アゴニストを創出するため、既知GPR119アゴニストに共通する右側の*N*-置換ピペリジン環に代わる構造を探索することにした。その際、フレキシブルなリンカーを有するPSN119-1M¹²に着目し、誘導体を合成することにした。

PSN119-1M誘導体には薬物代謝酵素であるシトクロムP450 (CYP) 阻害活性が認められたことを受け、筆者は右側の*N*-置換ピペリジン環に代わる構造として、ターゲットタンパク質に対する基質特異性が高くCYP等のオフターゲットに作用するリスクが低いと期待される、三次元性（指標として、化合物中の全炭素に占める sp^3 炭素の割合 (F_{sp^3}) が知られる^{13, 14)}が高いスピロ環構造¹⁵をデザインすることにした。また、右側にスピロ環構造を有するGPR119アゴニストの報告例はこれまでなかったことから¹⁶、スピロ環構造の利用は安全性と新規性の両面で有利になると考えた。なお、*N*-置換ピペリジン環が占有する空間との相同性を考慮し、シクロヘキサン環上4位にスピロ炭素を有するシクロヘキサン含有スピロ環をデザインすることにした (Figure 9)¹⁷。

PSN119-1M 誘導体の *N*-置換ピペリジン環をシクロヘキサン含有スピロ環に置換した各種誘導体を合成することにした。さらに、最適化した化合物については、CYP 阻害活性評価

に加えて、ラットを用いた耐糖能試験 (ipGTT) により GPR119 アゴニストの薬理作用として想定される糖濃度依存的なインスリン分泌を伴う血糖上昇抑制作用の有無を確認することにした。

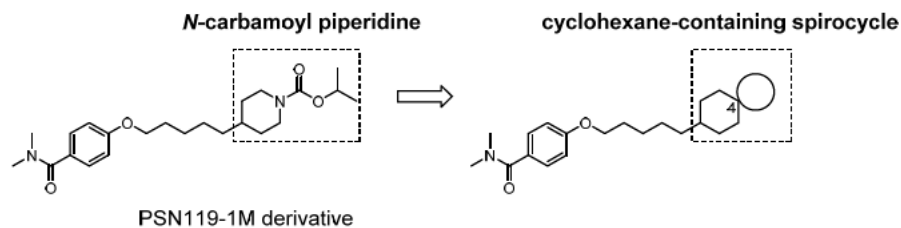


Figure 9. Cyclohexane-containing spirocycle as an alternative of *N*-carbamoyl piperidine.

第2節 合成

第1節で論じた戦略に基づき、最終的に以下の化合物を合成した (Figure 10)。なお、PSN119-1M の左側ベンゼン環にはフッ素原子が置換しているが、合成を単純化する目的から、筆者はフッ素原子の導入を行わないことにした。

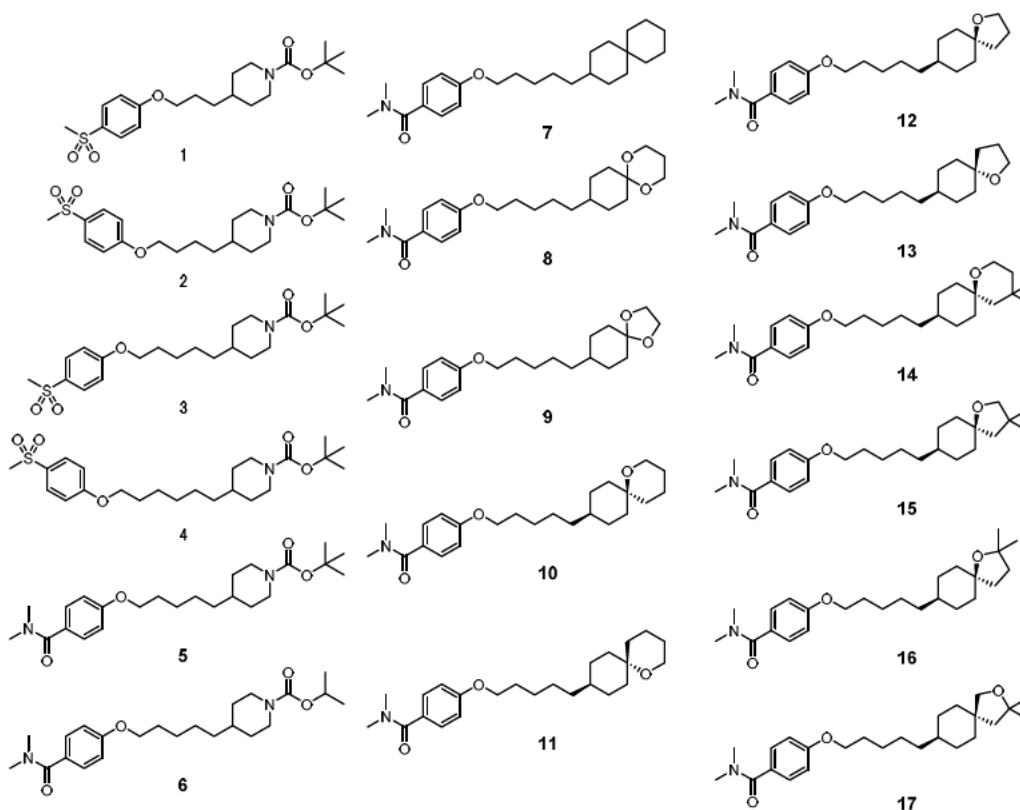
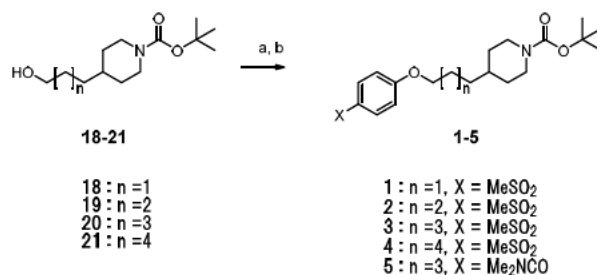


Figure 10. Structures of synthesized compounds in Chapter 2.

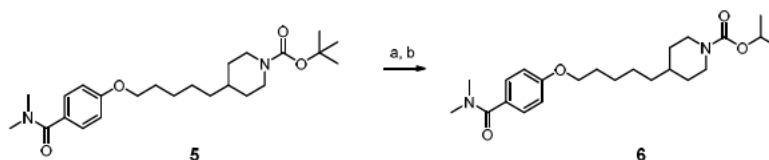
PSN119-1M 周辺化合物 **1–5** については、Scheme 1 に示す方法により合成した。市販の *tert*-ブトキシカルボニル (Boc) ピペリジンアルコール **18–21** をメシル化し、続く対応するフェ

ノールとの反応により **1-5** を得た。



Scheme 1. Reagents and conditions: (a) MeSO₂Cl, NEt₃, CHCl₃, 0 °C to rt; (b) 4-(methylsulfonyl)phenol (for **1-4**) or 4-hydroxy-*N,N*-dimethylbenzamide (for **5**), Cs₂CO₃, DMF, 80 °C, 54–90% (2 steps).

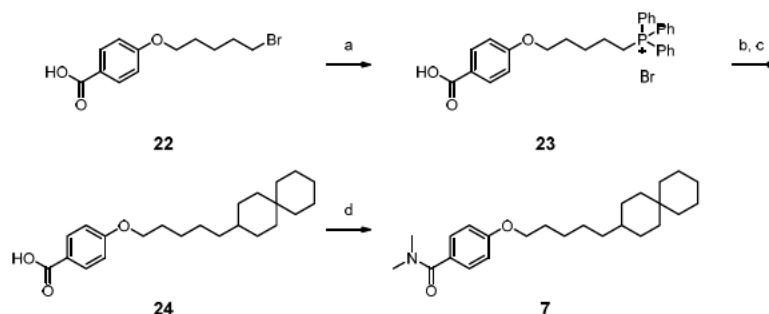
化合物 **6** の合成については, Scheme 2 に示すように **5** の脱 Boc 化, 続くクロロギ酸イソプロピルとの反応により行った。



Scheme 2. Reagents and conditions: (a) 4M HCl-dioxane, rt; (b) isopropyl chloroformate, NEt₃, CHCl₃, 0 °C to rt, 94% (2 steps).

各種シクロヘキサン含有スピロ環化合物 **7-17** の合成について, 以下に示した。

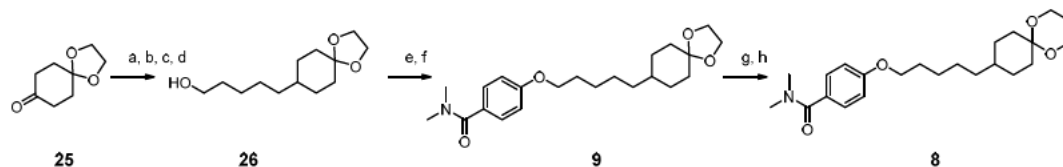
化合物 **7** については, Scheme 3 に示す方法により合成した。ブロミド **22** より誘導したホスホニウム塩 **23** とスピロ[5.5]ウンデカン-3-オンとの Wittig 反応, 続くオレフィン部の水素添加により得られたカルボン酸 **24** を経て, **7** へと誘導した。



Scheme 3. Reagents and conditions: (a) triphenylphosphine, toluene, 130 °C; (b) spiro[5.5]undecan-3-one, KO-*t*-Bu, THF, 0 °C to rt; (c) H₂ (15 psi), Pd-C, MeOH, rt, 14% (3 steps); (d) dimethylamine hydrochloride, NEt₃, WSC, HOBT·H₂O, DMF, 0 °C to rt, 82%.

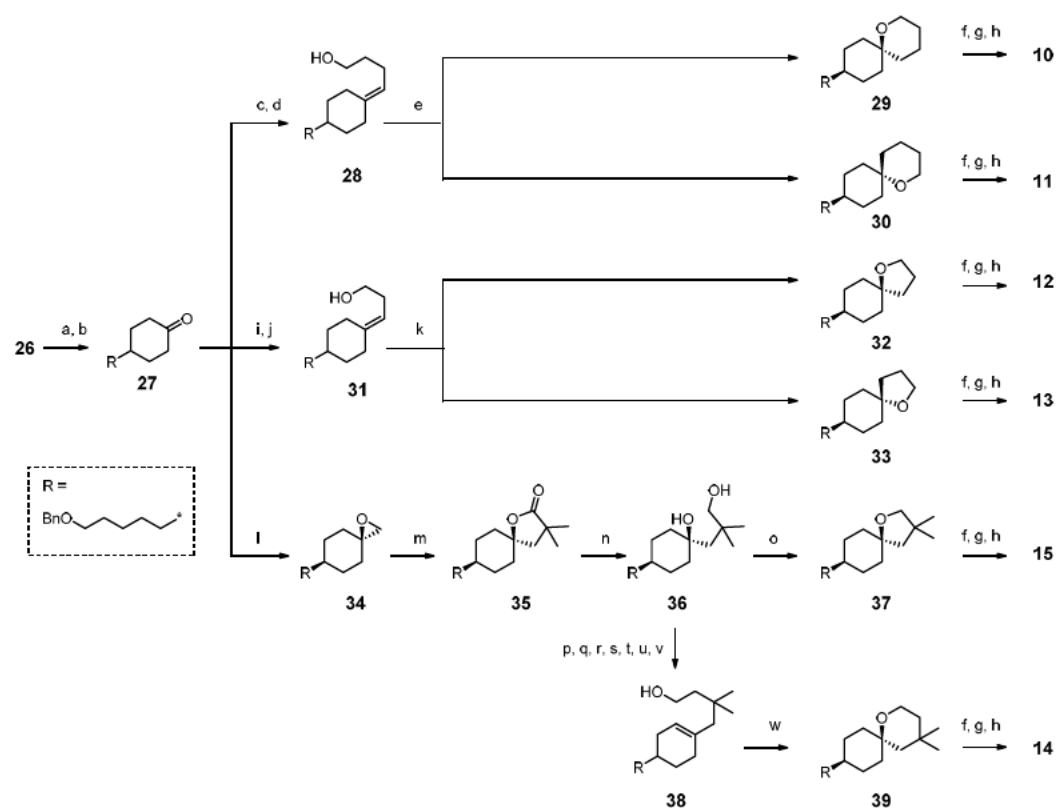
化合物 **8** と **9** の合成を Scheme 4 に示した。スピロ[4.5]デカノン **25** を出発原料とし, Wittig

反応等を経てアルコール **26** を合成した。**26** のメシル化、続く 4-ヒドロキシ-*N,N*-ジメチルベンズアミドとの反応により **9** を得た。**9** の脱ケタール化、続く 1,3-プロパンジオールとの反応により、スピロ[5.5]ケタール **8** へと誘導した。



Scheme 4. Reagents and conditions: (a) (4-carboxybutyl)triphenylphosphonium bromide, KO-*t*-Bu, THF, 0 °C to rt; (b) WSC, MeOH, rt, 50% (2 steps); (c) H₂ (15 psi), Pd-C, MeOH, rt, 62%; (d) LiAlH₄, THF, 0 °C, 98%; (e) MeSO₂Cl, NEt₃, CHCl₃, 0 °C to rt; (f) 4-hydroxy-*N,N*-dimethylbenzamide, Cs₂CO₃, DMF, 80 °C, 90% (2 steps); (g) AcOH, H₂O, 100 °C; (h) 1,3-propanediol, PPTS, toluene, 140 °C, 86% (2 steps).

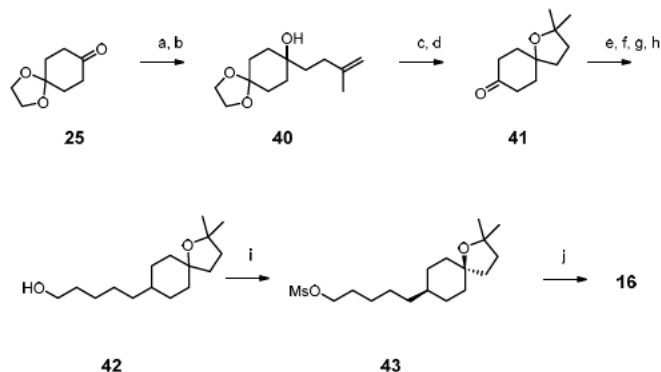
化合物 **10–15** については、Scheme 5 に示す方法で合成した。まず、アルコール **26** を出発原料とし、O-ベンジル化と脱ケタール化によりシクロヘキサノン **27** を得た。次に、化合物 **10–13** の合成のため、**27** に対して Wittig 反応を利用して 4 炭素および 3 炭素ユニットを導入し、エン-アルコール **28** と **31** をそれぞれ合成した。酸 (BF₃·OEt₂ や Amberlyst-15) を用いた **28**, **31** のカチオン環化反応、続くシリカゲルカラムクロマトグラフィーを用いたジステレオマー分離により、**28** より **29**, **30** を、**31** より **32**, **33** をそれぞれ得た。また、化合物 **15** の合成のため、**27** とスルホキシニウムイリドとの反応により、高立体選択的 (93 : 7 d.r.) にシス体のエポキシド **34** を調整した¹⁸。**34** とイソ酪酸メチルのリチウムエノラートとの反応によりスピロラクトン **35** に導いた後、ヒドリド還元により得られたジオール **36** のトシル化と続く環化により、スピロエーテル **37** を得ることができた。また、化合物 **14** の合成のため、**36** より 7 工程を経てエン-アルコール **38** に変換した後、酸を用いたカチオン環化反応、続くカラムクロマトグラフィーを用いたジアステレオマー分離によりシス体のスピロエーテル **39** を得た。ベンジルエーテル **29**, **30**, **32**, **33**, **37**, **39** の脱ベンジル化、続くメシル化、対応するフェノールとの反応により、目的物 **10–15** へと誘導した。



Scheme 5. Reagents and conditions: (a) BnBr, NaH, DMF, 50 °C; (b) 2N HCl, acetone, rt, 70% (2 steps); (c) 3-(ethoxycarbonyl)propyl triphenylphosphonium bromide, KO-*t*-Bu, THF, 0 °C to rt; (d) LiAlH₄, THF, 0 °C, 86% (2 steps); (e) BF₃·OEt₂, CHCl₃, rt, then separation by flash chromatography on silica gel, 22% for **29**, 26% for **30**; (f) H₂ (15 psi), Pd-C, MeOH, rt; (g) MeSO₂Cl, NEt₃, CHCl₃, 0 °C to rt; (h) 4-hydroxy-*N,N*-dimethylbenzamide, Cs₂CO₃, DMF, 80 °C, 68-99% (3 steps); (i) {3-[(*tert*-butyldimethylsilyl)oxy]propyl}triphenylphosphonium bromide, which was readily prepared from (3-bromopropoxy)-*tert*-butyldimethylsilane, KO-*t*-Bu, THF, 0 °C to rt; (j) 1M TBAF in THF, THF, rt; (k) Amberlyst-15, toluene, 80 °C, then separation by flash chromatography on silica gel, 26% (3 steps) for **32**, 23% (3 steps) for **33**; (l) trimethylsulfoxonium iodide, KO-*t*-Bu, DMSO, 50 °C, 99%; (m) methyl isobutyrate, LDA, THF, rt; (n) LiAlH₄, THF, 0 °C, 70% (2 steps); (o) *p*-toluenesulfonyl chloride, pyridine, 90 °C, 88%; (p) Ac₂O, pyridine, *N,N*-dimethylaminopyridine, CHCl₃, rt; (q) thionyl chloride, pyridine, 0 °C; (r) 4N aq. NaOH, THF, MeOH, rt; (s) Dess-Martin reagent, CHCl₃, 0 °C to rt; (t) methoxymethyltriphenylphosphonium chloride, KO-*t*-Bu, THF, rt, 60% (5 steps); (u) PPTS, THF, rt; (v) NaBH₄, MeOH, rt, 74% (2 steps); (w) Amberlyst-15, toluene, 60 °C, 42%.

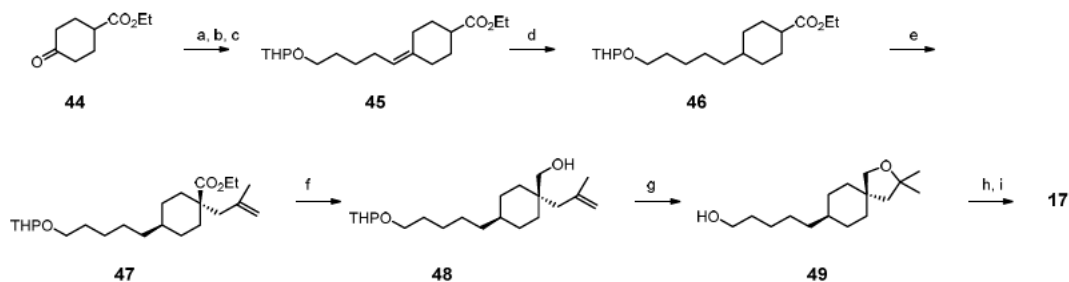
化合物 **16** の合成について, Scheme 6 に示した。**25** のエポキシ化, 続くメタアリルマグネシウムクロリドとの反応により, アルコール **40** を得た。**40** のカチオン環化反応, 脱ケタール化によりスピロケトン **41** へと導いた。Scheme 4 で示した方法と同様に, 鎖状リンカーに

相当する 5 炭素ユニットを有するアルコール **42** へと導いた後、メシル化し、ジアステレオマー分離により所望のメシラート **43** を得た。**43** と 4-ヒドロキシ-*N,N*-ジメチルベンズアミドとの反応により **16** を合成した。



Scheme 6. Reagents and conditions: (a) trimethylsulfoxonium iodide, KO-*t*-Bu, DMSO, 50 °C; (b) 0.25 M methallylmagnesium chloride in THF, Et₂O, 0 °C, 62% (2 steps); (c) *p*-TsOH·H₂O, CHCl₃, 0 °C to rt; (d) AcOH, H₂O, rt, 82% (2 steps); (e) (4-carboxybutyl)triphenylphosphonium bromide, KO-*t*-Bu, THF, 0 °C to rt; (f) WSC, MeOH, rt, 68% (2 steps); (g) H₂ (15 psi), Pd-C, MeOH, rt; (h) LiAlH₄, THF, 0 °C, 95% (2 steps); (i) MeSO₂Cl, NEt₃, CHCl₃, 0 °C to rt, then separation by flash chromatography on silica gel, 55%; (j) 4-hydroxy-*N,N*-dimethylbenzamide, Cs₂CO₃, DMF, 80 °C, 78%.

化合物 **17** の合成を Scheme 7 に示した。シクロヘキサノン **44** を出発原料とし、Scheme 4 や Scheme 6 に示した方法で、鎖状リンカーに相当する 5 炭素ユニットを有するテトラヒドロピラニル (THP) エーテル **46** に変換した。**46** をリチウムエノラートとして 3-クロロ-2-メチルプロペンと反応させることにより、高立体選択的にエステル **47** を得た (92 : 8 d.r.)¹⁹。**47** のヒドリド還元により得られたエン-アルコール **48** のトシル酸を用いたカチオン環化反応によりスピロエーテル **49** を合成し、メシラートを経て **17** へと誘導した。



Scheme 7. Reagents and conditions: (a) (4-carboxybutyl)triphenylphosphonium bromide, KO-*t*-Bu, DMF, 0 °C to rt; (b) CDI, NaBH₄, THF, H₂O, 0 °C to rt; (c) DHP, *p*-TsOH·H₂O, toluene, rt, 84% (3 steps); (d) H₂ (60 psi), Pd-C, EtOAc, rt, 99%; (e) 3-chloro-2-methylpropene, LDA, THF, -20 °C to rt, 85%; (f) LiAlH₄, THF, 40 °C, 100%; (g) *p*-TsOH·H₂O, MeOH, 70 °C, 46%; (h) MeSO₂Cl, NEt₃, EtOAc, 0 °C to rt; (i) 4-hydroxy-*N,N*-dimethylbenzamide, Cs₂CO₃, DMF, 80 °C, 38% (2 steps).

化合物 **15** および **17** の立体化学は、類縁体の単結晶 X 線構造解析により決定できた。また、化合物 **10–14**, **16** の立体化学に関しては ^1H NMR より推定した。

第 3 節 化合物の評価と考察

右側の *N*-置換ピペリジン構造に代わる新規構造のテンプレートを探索するために合成した、PSN119-1M 周辺化合物のアゴニスト活性を評価した。その結果を Table 1 に示した。

GPR119 アゴニスト活性としては、ヒト GPR119 安定発現 HEK293 細胞を用いて、HTRF 法にて測定した cAMP 産生量を指標に EC_{50} 値と固有活性 (inherent activity : IA) を算出した。IA については GPR119 の内因性リガンドであるオレオイルエタノール (OEA) の最大活性を 100% として、 EC_{50} 値については OEA の最大活性の 50% に達したときの化合物濃度として算出した。

Table 1. GPR119 agonistic activity of piperidine derivatives **1–6**.

Compound	X	n	R	GPR119 EC_{50} (nM)	IA ^a (%)
1	MeSO ₂ -	3	<i>t</i> -Bu	353	106
2	MeSO ₂ -	4	<i>t</i> -Bu	209	100
3	MeSO ₂ -	5	<i>t</i> -Bu	14	93
4	MeSO ₂ -	6	<i>t</i> -Bu	78	72
5	Me ₂ NCO-	5	<i>t</i> -Bu	5	97
6	Me ₂ NCO-	5	<i>i</i> -Pr	10	102

^a Percent of maximum response of oleoylethanolamide (OEA).

PSN119-1M と同じ鎖長 (3 炭素ユニット) のリンカーを有する化合物 **1** のアゴニスト活性 (EC_{50}) は 353 nM であった。鎖状リンカーの鎖長 (3–6 炭素ユニット) について、アゴニス

ト活性に対する効果を調べた結果、5炭素ユニットのリンカーを有する化合物**3**は、異なる鎖長の化合物**1**, **2**, **4**と比べて最も強いアゴニスト活性 ($EC_{50} = 14 \text{ nM}$) を示すことがわかった。また、6炭素ユニットのリンカーを有する化合物**4**については、その固有活性は72%に留まり、パーシャルアゴニスト様であった。以上のことから、リンカーの全長は、フルアゴニストとして強いアゴニスト活性を示す上で重要であることが示唆された。

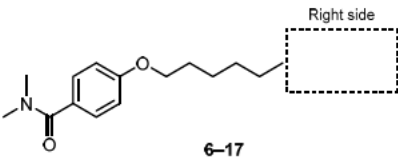
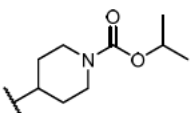
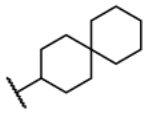
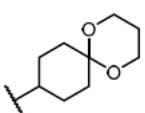
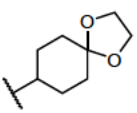
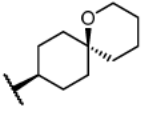
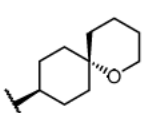
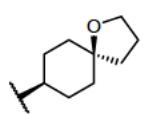
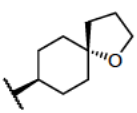
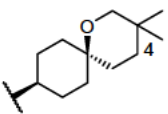
最も強いアゴニスト活性を示した**3**の溶解性について評価したところ、摂食時における人工腸液 (FeSSIF) に対する溶解性が低い ($15.9 \mu\text{M}$ in FeSSIF) ことが明らかとなった。そこで、左側ベンゼン環上4位置換基の効果を調べたところ、メチルスルホニル基の代わりに、*N,N*-ジメチルカルバモイル基²⁰を有する化合物**5**は、**3**と比べて強いアゴニスト活性およびFeSSIFに対する高い溶解性を示した ($EC_{50} = 5 \text{ nM}$, $74.2 \mu\text{M}$ in FeSSIF)。次に、脂溶性低減による溶解性の向上と酸に対する安定性を考慮して合成した、**5**の右側ピペリジン環上Boc基の代わりにイソプロポキシカルボニル基を有する化合物**6**は強いアゴニスト活性および**5**より良好な溶解性 ($EC_{50} = 10 \text{ nM}$, $221.8 \mu\text{M}$ in FeSSIF) を示すことがわかった。

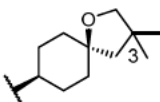
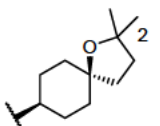
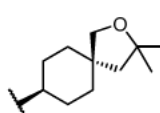
以上の結果から、右側の*N*-置換ピペリジン環に代わる新規構造のテンプレートとして、**5**や**6**が有する5炭素ユニットのリンカーと、4位に*N,N*-ジメチルカルバモイル基で置換された左側ベンゼン環を選抜した。

このように、**6**は強いアゴニスト活性と良好な溶解性を示したが、後述するようにCYP 2C8, 2C9, 2C19に対して阻害活性を示すことが明らかとなった (いずれも $IC_{50} < 10 \mu\text{M}$, Table 3)。一般に、三次元性の高い化合物は、ターゲットタンパク質への特異性が高く、CYPなどオフターゲットに作用するリスクが低いと考えられている。

そこで、筆者は化合物 **6** の右側の *N*-カルバモイルピペリジン環を、シクロヘキサンおよび飽和ヘテロ環から構成される各種スピロ環に置換した各種誘導体 **7-17** のアゴニスト活性を評価した (Table 2)。

Table 2. GPR119 agonistic activity of cyclohexane-containing spirocyclic derivatives **7–17**.

<div style="text-align: center;">  <p>Right side</p> <p>6–17</p> </div>			
Compound	Right side	GPR119 EC ₅₀ (nM)	IA ^a (%)
6		10	102
7		27	91
8		29	96
9		88	92
10		36	96
11		21	96
12		95	84
13		132	89
14		7	110

15		4	99
16		511	64
17		4	104

^a Percent of maximum response of oleoylethanolamide (OEA).

全て炭素で構成されたスピロ[5.5]環を有する化合物 **7**, また環状ケタール **8**, **9** がアゴニスト活性を示したことから, 第 1 節で述べたデザインの妥当性が確認された。また, 酸に対する安定性を考慮し, 環状ケタール **8**, **9** の 2 つの酸素原子のうち 1 つを炭素原子としたスピロエーテル **10–13** の評価結果から, スピロ[5.5]エーテル **10**, **11** は **8** と, スピロ[4.5]エーテル **12**, **13** は **9** と, それぞれ同等のアゴニスト活性を示すことがわかった。以上の結果から, 環状ケタールに含まれる 2 つの酸素原子は, アゴニスト活性に大きな影響を与えないことが示唆された。

一方, **10–13** のアゴニスト活性は **6** と比べて数倍弱かった。ここで, 互いの構造を比較し, **10–13** には **6** の右側カルバマート基上イソプロピル基に相当する脂溶性基が不足していることに注目した。右側カルバマート基上脂溶性基の嵩高さは, 強力なアゴニスト活性を示す上で重要であることが既に報告されていた²¹。そこで, **10** のテトラヒドロピラン環や **12** のテトラヒドロフラン環上に, かさ高い脂溶性基として *gem*-ジメチル基の導入を検討した²²。

テトラヒドロピラン環 4 位に *gem*-ジメチル基を有する化合物 **14** のアゴニスト活性は, **10** と比べて 5 倍程度強く, ピペリジン誘導体 **6** と同等であった。また, テトラヒドロフラン環 2 位に *gem*-ジメチル基を有する化合物 **16** では, **12** と比べてアゴニスト活性が弱かった。一方, 3 位に *gem*-ジメチル基を有する化合物 **15** および酸素原子の位置が異なる **17** では, それぞれ **12** および **6** より強力なアゴニスト活性 ($EC_{50} = 5 \text{ nM}$) を示した。本結果より, 脂溶性基 (*gem*-ジメチル基) は, 強力なアゴニスト活性を発現する上で重要であり, その位置がスピロ環により適切に規定されたことが示唆された。

以上, **6** の *N*-カルバモイルピペリジン環の代替構造として, シクロヘキサン含有スピロ環を有し, 強力なアゴニスト活性を示す化合物 **15**, **17** を見出すことに成功した。なお, **15** ($\text{CLog P} = 5.26$) より脂溶性が低く, 溶解性が良好な **17** ($\text{CLog P} = 5.10$, $441.5 \text{ } \mu\text{M}$ in FeSSIF) について, 更なる評価を実施した。

まず, **17** の CYP 阻害活性について評価した (Table 3)。その結果, CYP 2C8, 2C9, 2C19

に対して阻害活性を示した **6** ($F_{sp^3} = 0.65$) より三次元性が高い **17** ($F_{sp^3} = 0.72$) では、阻害活性は認められなかった (いずれも $IC_{50} > 10 \mu M$)。

Table 3. Inhibitory activity of **6** and **17** against seven CYP isoforms.

Compound	IC ₅₀ (μM) (% inhibition of 10μM test compound)						
	1A2	2C8	2D6	2C9	2C19	3A4(M)	3A4(T)
6	>10 (8%)	8.8 (53%)	>10 (11%)	5.0 (69%)	6.7 (69%)	>10 (8%)	>10 (8%)
17	>10 (7%)	>10 (30%)	>10 (18%)	>10 (38%)	>10 (30%)	>10 (0%)	>10 (17%)

次に、GPR119 アゴニストとしてのコンセプト確認を目的に、Sprague-Dawley (SD) ラットを用いた腹腔内グルコース負荷による耐糖能試験 (ipGTT) を実施した (n = 5) (Figure 11)。**17** を経口投与した 30 分後にグルコースを腹腔内に投与した。グルコース投与後、血漿中グルコース濃度を 2 時間、インスリン濃度を 1 時間まで測定した。その結果、グルコース負荷前である 0 分の時点では、**17** 投与群の血糖値およびインスリン値は、媒体 (Vehicle) 群のそれらと差異はなかった。また、グルコース負荷後、**17** の 0.3 mg/kg, 1 mg/kg 投与群の血糖値は、Vehicle 群と比べてそれぞれ 14%, 33%低下し、インスリン値は上昇した。以上、**17** の糖濃度依存的なインスリン分泌を伴う血糖上昇抑制作用が確認されたⁱⁱ。

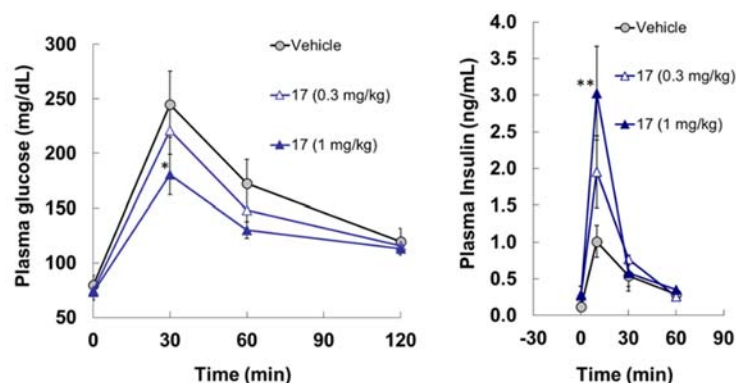


Figure 11. Results of an intraperitoneal glucose tolerance test model of compound **17** (dosed at 0.3 and 1 mg/kg, respectively) in Sprague-Dawley rats. Each data represents mean \pm S.D. (n = 5). *: $p < 0.05$ vs. Vehicle, **: $p < 0.01$ vs. Vehicle (Dunnett's test).

ⁱⁱ **17** の代謝物 *gem*-ジメチル基の一つのメチル基がヒドロキシルメチル基およびカルボキシル基に置換した化合物が代謝物として同定された。

第4節 小括

新規 GPR119 アゴニストの創出を目指し、既知 GPR119 アゴニストの多くに共通する右側の *N*-置換ピペリジン環に代わる構造として、シクロヘキサン含有スピロ環を見出した。右側にシクロヘキサン含有スピロ環を有する誘導体を合成した結果、代表化合物 **17** の創出に成功した。**17** は、ピペリジン誘導体 **6** より強力なアゴニスト活性 ($EC_{50} = 4 \text{ nM}$) を示した。また、**6** より三次元性の高い **17** は、**6** で認められた CYP 阻害活性を示さなかった。さらに、ラットを用いた耐糖能試験において、想定したコンセプトに則した **17** の糖濃度依存的なインスリン分泌を伴う血糖上昇抑制作用が確認された。

第3章 シクロヘキサン含有スピロ環を有する新規リード化合物の創出と最適化

第1節 背景と戦略

第2章で糖濃度依存的なインスリン分泌を伴う血糖上昇抑制作用を示した化合物 **17** について、糖尿病治療薬として更なる開発を進めることにした。しかしながら、ラットを用いた探索的毒性試験において、**17** は眼毒性を示すことが明らかとなった。一般に、化合物の脂溶性は毒性発現原因の一つと考えられ、**17** が示した眼毒性は、その高い脂溶性 (CLogP = 5.1) に起因すると考察した²³。

本章では、**17** より低脂溶性の新規 GPR119 アゴニストを探索することにした。既知 GPR119 アゴニストのうち **17** より低脂溶性が期待できるリンカーと左側パートを有する MBX-2982, PSN119-2, Arena's compound (第2章中 Figure 8) と **17** の右側パートであるシクロヘキサン含有スピロ環を組み合わせた化合物 **50-52** をデザインし、合成することにした (Figure 12)。合成した3化合物のうち、リガンド脂溶性効率 (ligand-lipophilicity efficiency : LLE (pEC₅₀ - CLogP)²⁴) を指標に、アゴニスト活性と脂溶性のバランスが良い (LLE が高い) リード化合物を選抜することを考えた。

続く最適化では、アゴニスト活性と脂溶性のバランスが良好 (LLE が高い) で高い溶解性や代謝安定性を示す化合物取得を目指し、構造変換を行うことにした。また、最適化した化合物については、ラット PK プロファイルおよび血糖上昇抑制作用の有無を確認することにした。

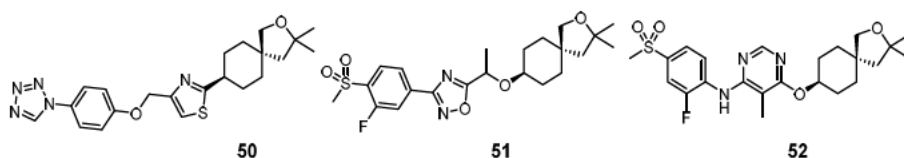


Figure 12. Lead candidates with a cyclohexane-containing spirocycle.

第2節 合成

第1節で論じた戦略に基づき，最終的に以下の化合物を合成した（Figure 13）。

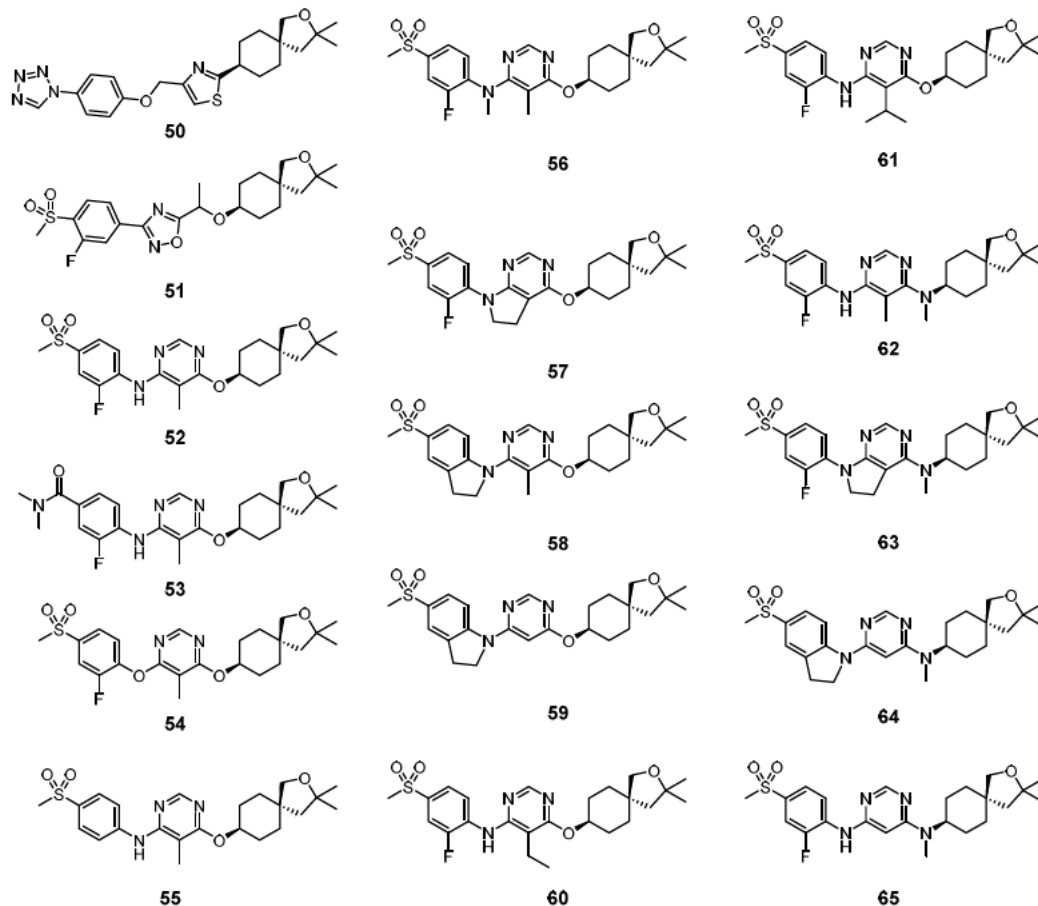
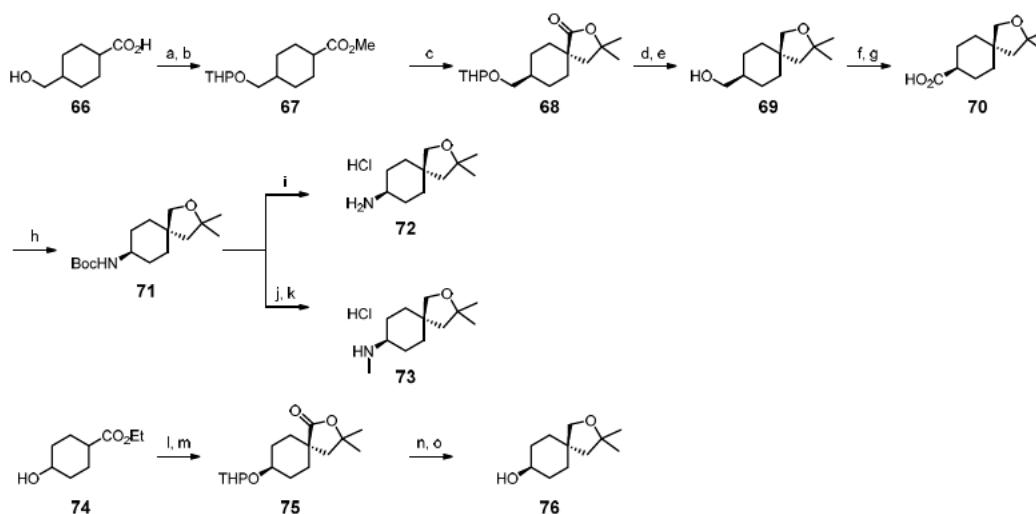


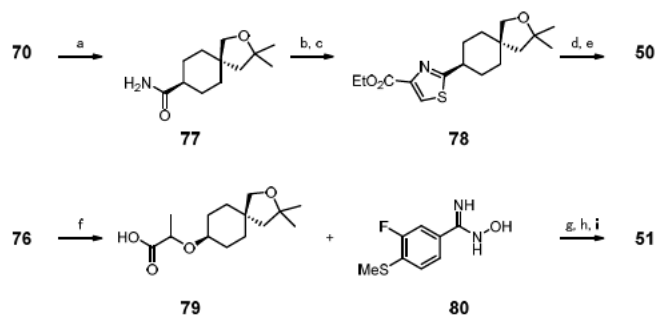
Figure 13. Structures of synthesized compounds in Chapter 3.

シクロヘキサン含有スピロ環を有する中間体 **70**, **72**, **73**, **76** について，Scheme 8 に示す方法で合成した。カルボン酸 **66** の誘導体であるエステル **67** より発生させたりチウムエノラートとイソブチレンオキシドとの反応により，スピロラクトン **68** が高立体選択的（98 : 2 d.r.）に得られた。**68** のヒドリド還元，続く酸を用いたカチオン環化反応により得られたスピロエーテル **69** を経て，段階的な酸化反応によりスピロカルボン酸 **70** を調整した。**70** の Curtius 転位反応により Boc 体 **71** とした後，**71** の脱 Boc 化により第一級アミン **72**，Boc 基のヒドリド還元により *N*-メチルアミン **73** をそれぞれ塩酸塩として単離した。スピロアルコール **76** については，エステル **74** を出発原料とし，**69** と同様の方法で合成した。



Scheme 8. Reagents and conditions: (a) MeI, K₂CO₃, DMF, rt; (b) DHP, (-)-CSA, toluene, rt; (c) isobutylene oxide, LiHMDS, THF, rt, 62% (3 steps); (d) LiAlH₄, THF, 0 °C to rt, 79%; (e) *p*-TsOH·H₂O, MeOH, 85 °C, 87%; (f) Dess-Martin reagent, CHCl₃, 0 °C; (g) NaClO₂, NaH₂PO₄, 2-methyl-2-butene, *t*-BuOH, H₂O, rt, 54% (2 steps); (h) DPPA, NEt₃, KO-*t*-Bu, dioxane, 90 °C, 97%; (i) HCl, AcOEt, rt, 66%; (j) LiAlH₄, THF, reflux; (k) HCl, AcOEt, rt, 47% (2 steps); (l) DHP, (-)-CSA, toluene, rt; (m) isobutylene oxide, LiHMDS, THF, rt, 69% (2 steps); (n) LiAlH₄, THF, 0 °C to rt; (o) *p*-TsOH·H₂O, MeOH, rt, 81% (2 steps).

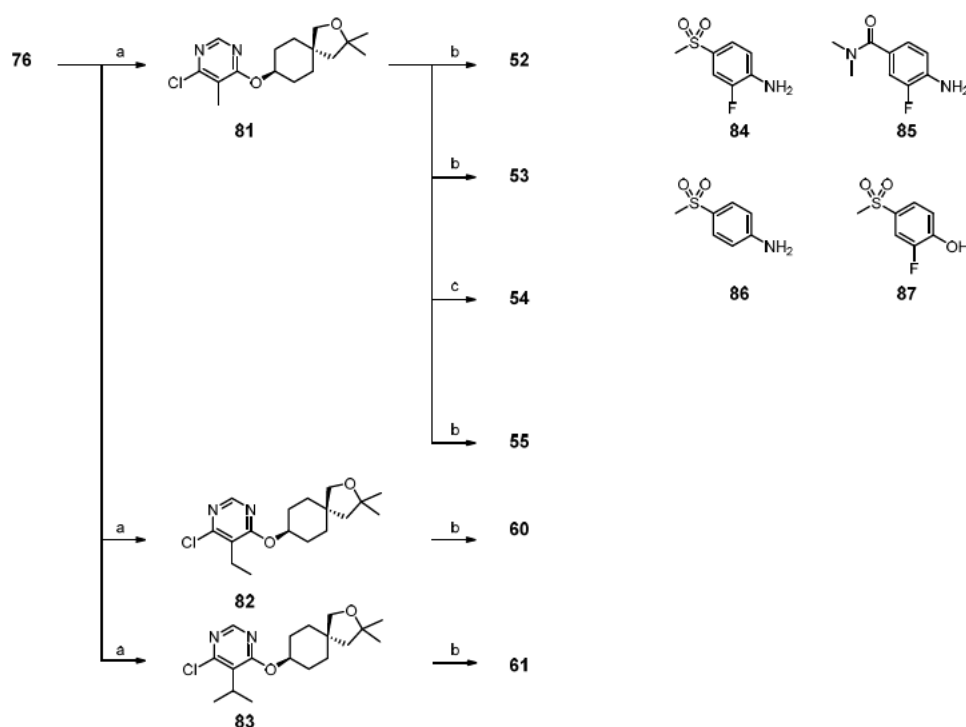
チアゾール誘導体 **50** およびオキサジアゾール誘導体 **51** については、**70** および **76** よりそれぞれ合成した (Scheme 9)。スピロカルボン酸 **70** 由来のカルボキサミド **77** のチオアミド化, 続くプロモピルビン酸エチルとの反応により, チアゾール **78** へと導いた。続いて, エステルのヒドリド還元により生成した第一級アルコールと 4-(1*H*-テトラゾール-1-イル)フェノールとの光延反応により **50** が得られた。一方, **51** を合成するために, まずスピロアルコール **76** と 2-プロモプロパン酸との反応により, カルボン酸 **79** を合成した。続いて, **79** と *N*-ヒドロキシルアミジン **80** との縮合によりオキサジアゾール環を構築し, 続くメチルチオ基の酸化により **51** へと誘導した。



Scheme 9. Reagents and conditions: (a) NH₄Cl, NEt₃, EDC, HOBt, CH₃CN, rt; (b) Lawesson's reagent, THF, reflux; (c) ethyl bromopyruvate, EtOH, rt, 31% (3 steps); (d) LiAlH₄, THF, 0 °C to rt;

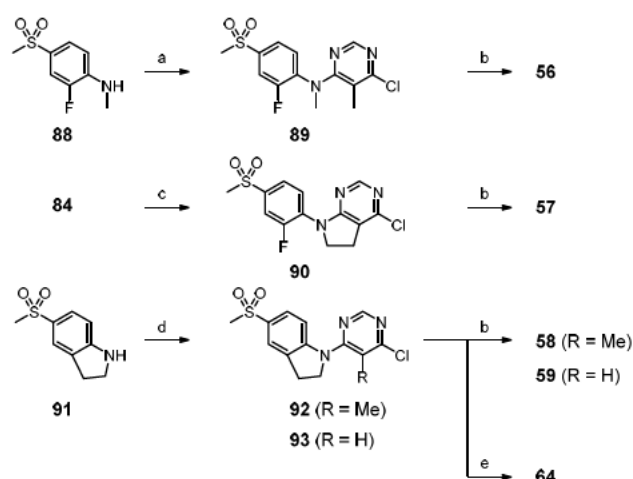
(e) 4-(1*H*-tetrazol-1-yl)phenol, PPh₃, DMAD, toluene, rt, 76% (2 steps); (f) 2-bromopropanoic acid, NaH, dioxane, rt; (g) EDC, HOBT, *i*-Pr₂NEt, DMF, rt; (h) toluene, reflux; (i) *m*-CPBA, CHCl₃, 0 °C, 34% (4 steps).

ピリミジン誘導体 **52–55**, **60**, **61** の合成については, スピロアルコール **76** と対応する 4,6-ジクロロピリミジンとの反応, 続く対応するアニリン **84–86** またはフェノール **87** との反応により行った (Scheme 10)。



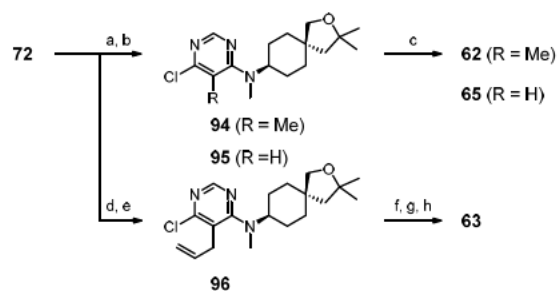
Scheme 10. Reagents and conditions: (a) the corresponding 4,6-dichloropyrimidine, NaH, THF, rt, 83–93%; (b) the corresponding aniline **84–86**, Pd(OAc)₂, 1,1'-bis(di-*t*-butylphosphino)ferrocene, NaO-*t*-Bu, dioxane, 100 °C, 12–57%; (c) **87**, K₂CO₃, TBAI, DMSO, 130 °C, 67%.

次に, ピリミジン誘導体 **56–59**, **64** の合成を, 対応するアニリン **84**, **88**, **91** を出発原料として行った (Scheme 11)。対応するクロロピリミジン誘導体との反応で得た中間体 **89**, **90**, **92**, **93** とスピロアルコール **76** との反応により化合物 **56–59** へと誘導した。また, 中間体 **92** とスピロアミン塩酸塩 **73** との反応により化合物 **64** を合成した。



Scheme 11. Reagents and conditions: (a) 4,6-dichloro-5-methylpyrimidine, Pd(OAc)₂, NaO-*t*-Bu, 1,1'-bis(di-*t*-butylphosphino)ferrocene, dioxane, 120 °C, 16%; (b) **76**, NaH, THF, reflux, 22–69%; (c) 2-(4,6-dichloropyrimidin-5-yl)acetaldehyde, NaBH(OAc)₃, THF, rt, 85%; (d) the corresponding 4,6-dichlorolpyrimidine, *n*-PrOH, reflux, 39%; (e) **73**, Pd(OAc)₂, DavePhos, K₂CO₃, dioxane, 105 °C, 5%.

ピリミジン誘導体 **62**, **63**, **65** の合成については、スピロアミン塩酸塩 **72** を出発原料として行った (Scheme 12)。スピロアミン塩酸塩 **72** と対応する 4,6-ジクロロピリミジンとの反応, 続く *N*-メチル化により中間体 **94–96** を得た。**94**, **95** とアニリン **84** とのカップリング反応により, **62**, **65** へと誘導した。また **96** を, 四酸化オスミウムを用いた酸化的開裂, 続くアニリン **84** との還元的アミノ化と酸性条件下での加熱により **63** へと誘導した。



Scheme 12. Reagents and conditions: (a) the corresponding 4,6-dichlorolpyrimidine, K₂CO₃, DMSO, rt; (b) MeI, NaH, DMF, rt, 25–70% (2 steps); (c) **84**, PdCl₂(dppf), (±)-BINAP, NaO-*t*-Bu, DMF, 80 °C, 22–38%; (d) 4,6-dichloro-5-allylpyrimidine, *i*-Pr₂NEt, K₂CO₃, DMF, 80 °C; (e) MeI, NaH, DMF, 60 °C, 26% (2 steps); (f) NaIO₄, K₂OsO₄·2H₂O, acetone, H₂O, rt, 10%; (g) **84**, NaBH(OAc)₃, TFA, CHCl₃, rt; (h) conc. HCl, *n*-PrOH, 100 °C, 66% (2 steps).

第3節 結果と考察

リード化合物の候補として合成した, MBX-2982, PSN119-2, Arena's compound (第2章中 Figure 8) と化合物 **17** の右側パートであるシクロヘキサン含有スピロ環を組み合わせた化合物 **50–52** の評価結果を Table 4 に示した。

Table 4. Lead candidates with a cyclohexane-containing spirocycle.

Compound	Left side-Linker	<div style="text-align: center;"> <p>17, 50-52</p> </div>			
		GPR119 EC ₅₀ (nM)	IA ^a (%)	CLogP ^b	LLE ^c
17		4	104	5.1	3.3
50		85	79	3.7	3.3
51		89	109	3.4	3.6
52		13	91	4.0	4.0

^a Percent of maximum response of oleoylethanolamide (OEA).

^b The ClogP value was calculated using a software from ChemAxon.

^c LLE = pEC₅₀ - CLogP.

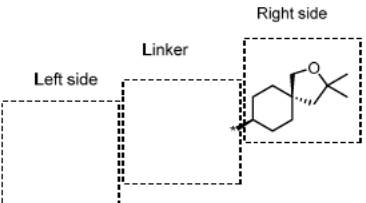
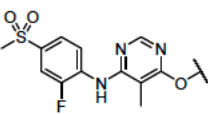
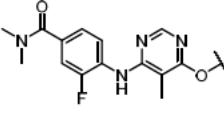
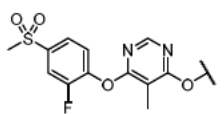
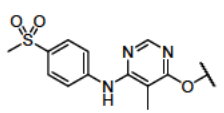
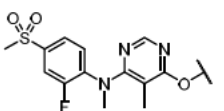
化合物 **50–52** はいずれもアゴニスト活性を示したことから, **17** のシクロヘキサン含有スピロ環は, 種々の既知 GPR119 アゴニストの *N*-置換ピペリジンと代替可能なことが示唆された。脂溶性 (CLogP) が **17** より低い上記3化合物のうち, LLE が最も高いピリミジン誘導体 **52** を, アゴニスト活性と脂溶性との良好なバランスを示すリード化合物として選抜した。

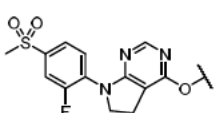
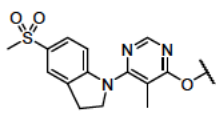
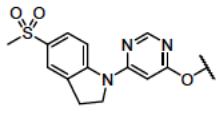
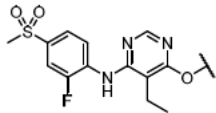
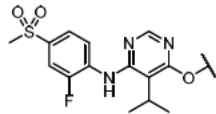
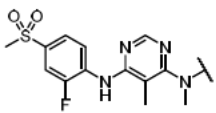
しかし, **52** の場合, pH 1.2 の日本薬局方溶出試験第1液 (JP1) および FeSSIF に対する溶解性が低いことが明らかとなった。一般に, 溶解性の低い化合物は低経口吸収性が懸念

される。

次に、ピリミジン誘導体の溶解性の改善をめざして合成した化合物 **52–62** の評価結果を Table 5 に示した。

Table 5. SAR and physicochemical properties of pyrimidine derivatives.

<div style="text-align: center;">  </div>							
52-62							
Compound	Left side -Linker	GPR119		CLogP ^b	LLE ^c	JP1 ^d (μM)	FeSSIF (μM)
		EC ₅₀ (nM)	IA ^a (%)				
52		13	91	4.0	4.0	5.3	15.8
53		33	101	4.5	3.7	128.5	65.2
54		59	62	4.1	3.2	<0.5	33.4
55		139	92	3.9	3.0	4.4	8.6
56		>1,000	17	4.2	-	13.0	42.5

57		5	90	3.7	4.6	18.9	15.2
58		>1,000	38	4.1	-	2.8	25.5
59		15	87	3.6	4.2	7.0	19.8
60		42	82	4.4	3.0	4.6	67.5
61		98	61	4.7	2.3	0.7	4.3
62		45	128	4.3	3.1	>475	49.8

^a Percent of maximum response of oleylethanolamide (OEA).

^b The CLogP value was calculated using a software from ChemAxon.

^c LLE = pEC₅₀ - CLogP.

^d Japanese Pharmacopoeia 1st fluid for a dissolution test adjusted to pH 1.2.

まず、左側ベンゼン環上置換基について、溶解性やアゴニスト活性に対する効果を調べた。第2章では、メチルスルホニル基を有する化合物 **3** と比較し、ジメチルカルバモイル基を有する化合物 **5** が、良好な溶解性を示したことを述べた。今回、化合物 **52** のメチルスルホニル基をジメチルカルバモイル基に代えた化合物 **53** の場合、JP1 および FeSSIF に対して良好な溶解性を示したが、**52** と比べて3倍程度アゴニスト活性が弱かった。フレキシビ

リティの高いリンカーを有する **5** と異なり、ピリミジンリンカーを有する **52** では、左側ベンゼン環上置換基が適切な位置に配置されなかったためと考察した。

次に、溶解性に影響する因子の一つとして、化合物の平面性に着目した。化合物の平面性は結晶パッキングに影響すると考えられることから、化合物の平面性を崩すと結晶パッキングが弱まり化合物の溶解性が向上することが期待される²⁵。**52** では、ピリミジン環および左側ベンゼン環が同一平面上にあるコンホメーションが低溶解性の原因と推察した。

そこで、コンホメーションに影響すると考えられるピリミジン環と左側ベンゼン環の連結原子や環上置換基について、溶解性およびアゴニスト活性に対する効果を調べた。ピリミジン環と左側ベンゼン環を連結する窒素原子 (NH) の代わりに酸素原子を有する化合物 **54** や、左側ベンゼン環上にフルオロ基を有さない化合物 **55** では、溶解性の変化は小さく、アゴニスト活性は **52** と比べて減弱した。また、*N*-メチル体 **56** では、**52** と比べて溶解性が若干改善されたものの、アゴニスト活性は著しく弱かった ($EC_{50} > 1000$ nM)。以上の結果から、ベンゼン環とピリミジン環が同一平面上にあるコンホメーション、すなわち共平面性が高活性を発現する上で重要なことが示唆された。特に、**56** では、窒素原子上メチル基とピリミジン環上 5 位メチル基との立体反発により、共平面性が崩れ、アゴニスト活性が大きく減弱したものと推察した。

この立体反発を解消するために合成した環化体 **57** では、強いアゴニスト活性 ($EC_{50} = 5$ nM) が認められた²⁶。また、**52** の窒素原子上水素原子とベンゼン環上フルオロ基との間で形成すると考えられた分子内水素結合ⁱⁱⁱに発想を得て合成したインドリン誘導体 **58** では、アゴニスト活性が著しく弱かったのに対して、ピリミジン環上 5 位メチル基を有さない化合物 **59** には、**52** と同程度のアゴニスト活性が確認された²⁷。

52 のピリミジン環上 5 位メチル基の代わりに、より嵩高い置換基 (エチル基, イソプロピル基) を有する化合物の場合、ベンゼン環とピリミジン環の共平面性を維持しつつ結晶パッキングは弱まることが期待される。しかし、エチル基 (**60**) への変換では、FeSSIF に対する溶解性は向上したが、アゴニスト活性は 3 倍程度減弱した。また、イソプロピル基 (**61**) への変換では、脂溶性の増大のためか溶解性の改善は認められず、アゴニスト活性はさらに減弱した。

次に、溶解性向上の別のアプローチとして、塩基性官能基の導入を検討した。その結果、**52** のピリミジン環とシクロヘキサン環を連結する酸素原子の代わりに、メチル化された窒素原子を有する化合物 **62** は、酸性の JP1 に対して良好な溶解性を示すことが明らかとなった。ただし、**62** のアゴニスト活性は **52** と比べて 3 倍程度弱かった。

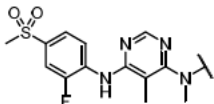
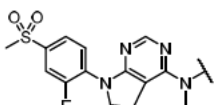
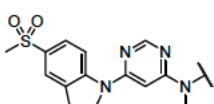
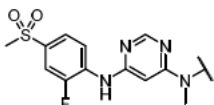
ここで、比較的高い溶解性と中程度のアゴニスト活性を示した **53** と **62** について、ヒトおよびラット肝ミクロソーム (Ms) を用いて代謝安定性を評価した。その結果、**53** では肝 Ms 中インキュベーション後 1 時間での残存率が 77% (ヒト) および 44% (ラット) であっ

ⁱⁱⁱ **52** および **55** が非極性溶媒に難溶であったため、分子内水素結合の形成について NMR により確認できなかった。

たのに対して、**62** では91%（ヒト）および93%（ラット）であった。

より良好な溶解性と代謝安定性を示した **62** を基に、活性の向上を目指して合成した化合物 **63–65** の評価結果を Table 6 に示した。

Table 6. Fine tuning of pyrimidine derivatives.

Compound	Left side -Linker	62-65			LLE ^c	hMs ^d (%)	rMs ^d (%)	JP1 ^e /FeSSIF (μM)
		GPR119 EC ₅₀ (nM)	IA ^a (%)	CLogP ^b				
62		45	128	4.3	3.1	91	93	>475 / 49.8
63		39	118	4.0	3.4	99	94	>475 / 75.3
64		16	108	3.9	3.9	89	80	418.2 / 24.3
65		19	120	3.7	4.0	103	96	473.3 / 31.1

^a Percent of maximum response of oleoylethanolamide (OEA).

^b The ClogP value was calculated using a software from ChemAxon.

^c LLE = pEC₅₀ - CLogP.

^d Percent of compound (5 μM) remaining after 1 hour incubation with human or rat liver microsomes (0.2 μg/mL).

^e Japanese Pharmacopoeia 1st fluid for a dissolution test adjusted to pH 1.2.

比較的強いアゴニスト活性を示したエーテル **57** および **59** (Table 5) に対応する *N*-メチル体 **63** および **64** は、良好な溶解性と代謝安定性を示し、特に、インドリン誘導体 **64** は **62** と比較して 3 倍強いアゴニスト活性を示した。**64** は **59** と同程度のアゴニスト活性を示したのに対して、**63** のアゴニスト活性は **57** と比べて 8 倍程度弱かった。この結果から、ピリミジン 5 位の置換基と *N*-メチル基との立体反発によりコンホメーションが変化し、アゴニスト活性が低下したものと推察した。予想通り、**62** に対するピリミジン 5 位無置換体 **65** は、**62** と同様良好な溶解性および代謝安定性を示すとともに、**62** と比べて 2 倍程度強いアゴニスト活性を示した。また、**65** の脂溶性 (CLogP=3.7) は、第 2 章で見出した化合物 **17** (CLogP=5.1) と比べて低く、高い LLE 値 (4.0) を示した。

以上、化合物 **17** より低脂溶性の新規 GPR119 アゴニストとして、ピリミジン誘導体 **65** を見出すことに成功した。

Table 7. Pharmacokinetic profiles of **65** in Sprague-Dawley rats (n = 2).

iv (1 mg/kg)				po (3 mg/kg)	次に、SD ラットを用いて、化合物 65 の PK プロファイルを確認した (Table 7)。その結果、 65 は長時間作用型の薬効が期待できる持続性と、高い経口吸収性を示すことがわかった。
t1/2 β (h)	CL (L/h/kg)	Vdss (L/kg)	MRT (h)	BA (%)	
3.7	0.8	4.0	5.1	99	

65 について、SD ラットを用いた腹腔内グルコース負荷による耐糖能試験 (ipGTT) を実施した (n = 5)。**65** (10 mg/kg) を経口投与した 16 時間後にグルコースを腹腔内投与し、グルコース投与 0 分, 30 分, 60 分後にそれぞれ血漿中グルコース濃度を測定した。その結果、グルコース負荷直後のグルコース濃度は、媒体群と **65** 投与群の間ではほぼ差異がなかった。また、**65** 投与群のグルコース投与 30 分, 60 分後のグルコース濃度 (mean \pm S.D.) は、媒体群と比べてそれぞれ 14% ($p < 0.01$ (Student's t-test)), 11%低かった。以上、化合物 **65** の持続的な血糖上昇抑制作用が確認された。

第 4 節 小括

化合物 **17** より低脂溶性の新規 GPR119 アゴニストを創出するため、**17** より低脂溶性が期待できるリンカーと左側パートを有する MBX-2982, PSN119-2, Arena's compound (第 2 章中 Figure 8) と **17** の右側パートであるシクロヘキサン含有スピロ環を組み合わせた化合

物 **50–52** を合成した。合成した 3 化合物にアゴニスト活性が認められたことから、**17** のシクロヘキサン含有スピロ環は種々の既知 GPR119 アゴニストの *N*-置換ピペリジン環に代替可能であることが示唆された。LLE 値を指標に、アゴニスト活性と脂溶性の良好なバランスを示したピリミジン誘導体 **52** をリード化合物として選抜した。アゴニスト活性と脂溶性を維持しつつ溶解性の向上を目指して最適化を行った結果、**17** より低脂溶性の代表化合物 **65** を見出した。**65** は、高い経口吸収性と持続的な PK プロファイルに基づいた長時間作用型の薬効（血糖上昇抑制作用）を示すことが確認された。

第4章 *in vivo* ポテンシー向上を目指したシクロヘキサン含有スピロ環を有するオキサジアゾール誘導体の最適化

第1節 背景と戦略

本章では、化合物**65**よりさらに低用量で長時間作用型の薬効が期待される新規GPR119アゴニストを探索することにした。一般に、血漿中の化合物は、血漿タンパク質と結合した“結合型”と、血漿タンパク質から遊離した“非結合型”の平衡状態にあり、“非結合型”がターゲットタンパク質に結合して薬効を発現すると考えられる。そこで、*in vivo*ポテンシーの観点では、血漿タンパク結合性が低い化合物が有利であると考えた。一方、化合物の非結合型は、代謝や排泄を受けるため、持続性の観点では血漿タンパク結合性と共に代謝安定性が重要と考えた。

そこで、第3章で見出した化合物**50–52**のうち、ヒトおよびラット血漿タンパク結合率が最も低かったオキサジアゾール誘導体**51**を新たなリード化合物として選抜することにした (Figure 14)。

続く最適化では、低い血漿タンパク結合性且つ強いアゴニスト活性に加えて、高い代謝安定性を併せ示す化合物の取得を目指すことにした。なお、血漿タンパク結合性は脂溶性とおおよそ正の相関関係にあるため、構造変換の際、脂溶性の増大に留意することにした。また、最適化した化合物については、ラットPKプロファイルおよび血糖上昇抑制作用の有無を確認することにした。

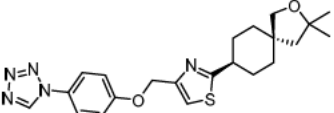
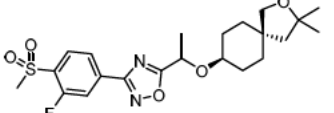
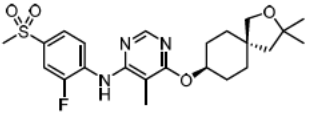
			
	50	51	52
GPR119 EC ₅₀	85 nM	89 nM	13 nM
CLogP	3.7	3.4	4.0
LLE	3.3	3.6	4.0
plasma protein binding			
human / rat	98.6% / 98.5%	96.7% / 97.7%	98.3% / 98.1%

Figure 14. Plasma protein binding of lead candidates with a cyclohexane-containing spirocycle.

第2節 合成

第1節で論じた戦略に基づき，以下の化合物を合成した（Figure 15）。

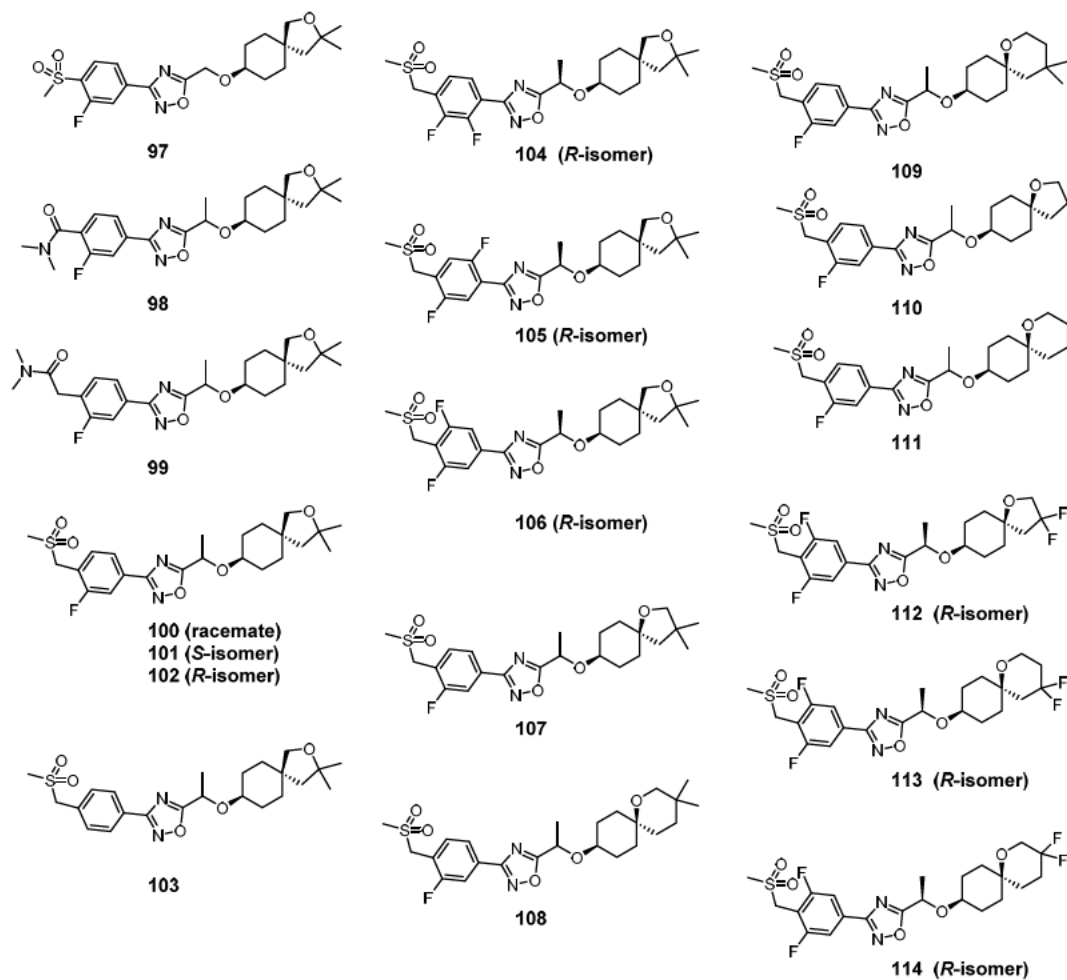
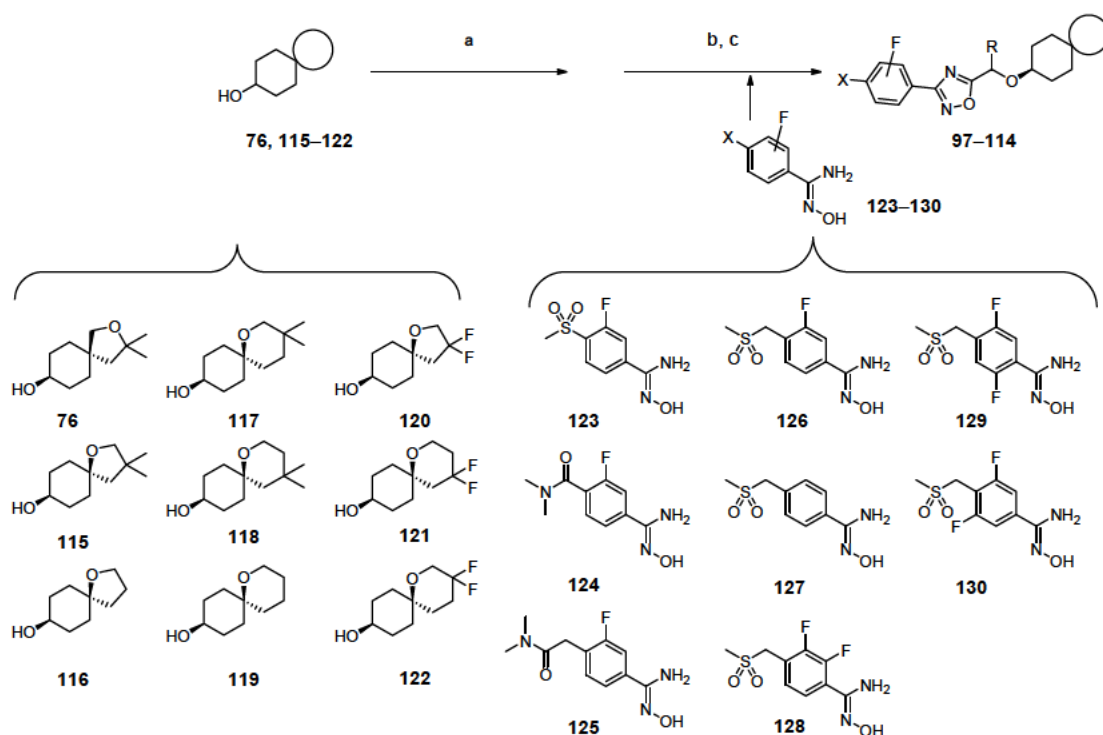


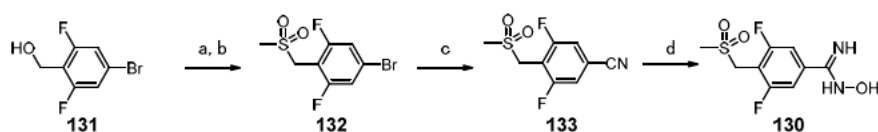
Figure 15. Structures of synthesized compounds in Chapter 4.

化合物 97–114 の一般的な合成を Scheme 13 に示す。水素化ナトリウム存在下，対応するスピロアルコールをプロモ酢酸または 2-プロモプロピオン酸と反応させ，対応する *N*-ヒドロキシルアミジンとの縮合を経て，目的物 97–114 へと誘導した。



Scheme 13. Reagents and conditions: (a) bromoacetic acid or 2-bromopropanoic acid, NaH, dioxane, rt to 90 °C, 30–91%; (b) the corresponding *N*-hydroxyl amidine **123–130**, EDC, HOBT, *i*-Pr₂NEt, DMF, rt; (c) NMP, 120 °C, 12–69% (2 steps).

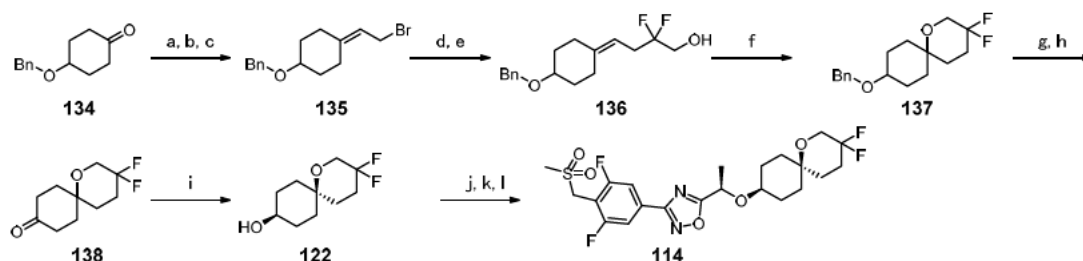
1 例として、化合物 **114** の全合成を Scheme 14, 15 に示した。対応する *N*-ヒドロキシルアミジン **130** については、Scheme 14 に示す方法で合成した。すなわち、ベンジルアルコール **131** のブロモ化、続くメチルスルフィン酸ナトリウムとの反応により、スルホン **132** を得た。パラジウム触媒存在下、**132** とフェロシアン化カリウムとの反応²⁸により得られたニトリル **133** を経て、**130** へと誘導した。



Scheme 14. Reagents and conditions: (a) MeSO₂Cl, NEt₃, EtOAc, 0 °C; (b) LiBr, MeSO₂Na, DMF, rt to 80 °C, 92% (2 steps); (c) K₄[Fe(CN)₆]·3H₂O, Pd(OAc)₂, Na₂CO₃, DMA, 130 °C, 73%; (d) NH₂OH·HCl, K₂CO₃, EtOH, H₂O, 90 °C, 75%.

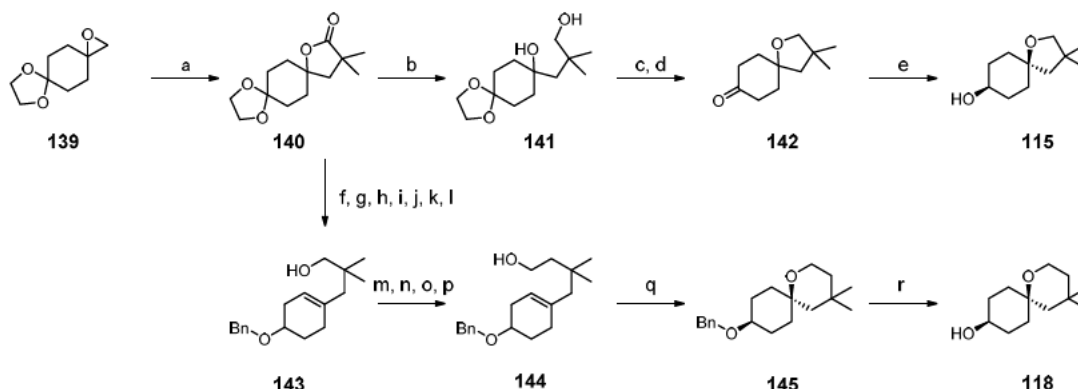
対応するスピロアルコール **122** および化合物 **114** の合成については、Scheme 15 に示した。シクロヘキサノン **134** より 3 工程で誘導したアリルブロミド **135** に対し、亜鉛末とシアン化銅存在下でのプロモジフルオロ酢酸エチルとの Reformatsky 反応、続くエステルのヒドリド還元により、エン-アルコール **136** とした。トシル酸を用いた **136** のカチオン環化反応

によりスピロエーテルを構築した。生成物 **137** の脱ベンジル化，続く第二級アルコールの酸化により得られたスピロケトン **138** のヒドリド還元を行い，シス体のアルコール **122** を高立体選択的 (98:2 d.r.) に得た²⁹。**122** と *S* 体の 2-ブロモプロピオン酸との反応，続く **130** との縮合反応により **114** へと導いた。



Scheme 15. Reagents and conditions: (a) triethyl phosphonoacetate, EtONa, EtOH, THF, 0 °C to rt, 92%; (b) diisobutylaluminum hydride, toluene, -78 °C, 93%; (c) NBS, PPh₃, THF, DMF, 0 °C to rt, 25%; (d) ethyl bromodifluoroacetate, Zn, CuCN, THF, rt, 17%; (e) LiAlH₄, THF, 0 °C, 92%; (f) *p*-TsOH·H₂O, toluene, 100 °C, 80%; (g) H₂ (15 psi), 20% Pd(OH)₂-C, MeOH, rt, 85%; (h) 1-Me-AZADO, NaClO, KBr, tetrabutylammonium bromide, NaHCO₃, CHCl₃, H₂O, 0 °C, 93%; (i) LiAlH₄, THF, 0 °C, 90%; (j) (*S*)-(-)-2-bromopropanonic acid, NaH, dioxane, rt, 67%; (k) **130**, WSC, HOBt, *i*-Pr₂NEt, DMF, rt; (l) NMP, 120 °C, 27%.

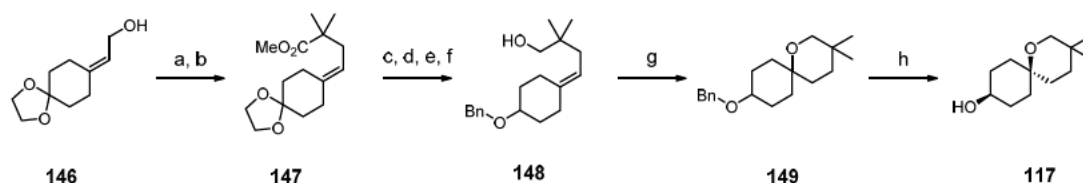
スピロ[4.5]アルコール **115** とスピロ [5.5] アルコール **118** については，エポキシド **139** から得られたスピロラクトン **140** を経由して合成した (Scheme 16)。すなわち，スピロ[4.5]アルコール **115** の場合，スピロラクトン **140** より誘導したジオール **141** にピリジン中メタンサルホニルクロリドを作用させてテトラヒドロフラン環を形成させた後，酢酸中加熱することで得られたシクロヘキサノン **142** を還元して合成した。また，スピロ[5.5]アルコール **118** の場合，**140** から 7 工程を経て得られたアルコール **143** を増炭しエン - アルコール **144** をまず合成した。続いて，**144** のカチオン環化反応，続くジアステレオマー分離により，シス体のスピロエーテル **145** を得た。**145** の脱ベンジル化により **118** へと導いた。



Scheme 16. Reagents and conditions: (a) methyl isobutyrate, LDA, THF, -20 °C to rt, 97%; (b)

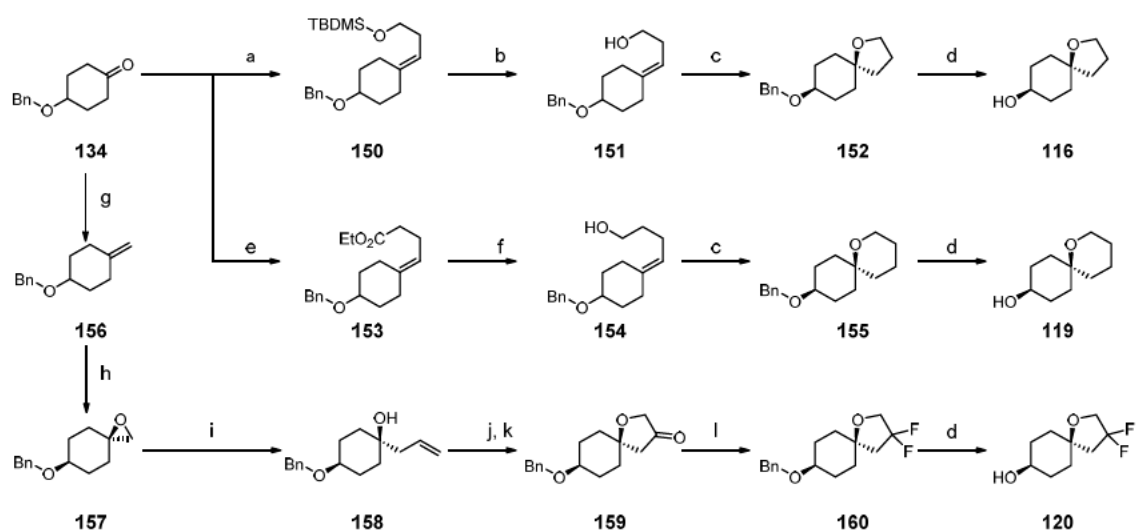
LiAlH₄, THF, 0 °C to rt, 97%; (c) MeSO₂Cl, pyridine, 0 °C to rt, (d) AcOH, H₂O, 100 °C, 90% (2 steps); (e) NaBH₄, MeOH, rt, then separation by column chromatography on silica gel, 36%; (f) AcOH, H₂O, 100 °C, 90%; (g) NaBH₄, MeOH, rt; (h) BnBr, NaH, THF, 0 °C to rt, 56% (2 steps); (i) LiAlH₄, THF, 0 °C; (j) Ac₂O, pyridine, *N,N*-dimethylaminopyridine, CHCl₃, 0 °C; (k) SOCl₂, pyridine, rt; (l) 4N aq. NaOH, THF, MeOH, rt, 89% (4 steps); (m) Dess-Martin reagent, CHCl₃, 0 °C; (n) methoxymethyltriphenylphosphonium chloride, KO-*t*-Bu, THF, 0 °C to rt; (o) PPTS, THF, H₂O, rt; (p) NaBH₄, MeOH, rt, 17% (4 steps); (q) Amberlyst-15, toluene, 90 °C, then separation by column chromatography on silica gel, 32%; (r) H₂ (15 psi), Pd(OH)₂-C, MeOH, rt, 99%.

スピロ[5.5]アルコール **117** の合成について、Scheme 17 に示した。アリルアルコール **146** のクロロ化、続くイソ酪酸メチル由来のリチウムエノラートとの反応により、エステル **147** を得た。**147** から 4 工程を経て得られたエン-アルコール **148** のカチオン環化反応により、スピロエーテル **149** がジアステレオマー混合物として得られた。**149** を脱ベンジル化し、ジアステレオマーをシリカゲルクロマトグラフィーで分離して、シス体の **117** を得た。



Scheme 17. Reagents and conditions: (a) triphosgene, pyridine, hexane, 0 °C; (b) methyl isobutyrate, LDA, THF, -10 °C to rt, 65% (2 steps); (c) AcOH, H₂O, 100 °C; (d) NaBH₄, MeOH, rt; (e) BnBr, NaH, THF, 0 °C to rt; (f) LiAlH₄, THF, 0 °C, 27% (4 steps); (g) BF₃·OEt₂, CHCl₃, rt; (h) H₂ (15 psi), Pd-C, MeOH, rt, then separation by column chromatography on silica gel, 26% (2 steps).

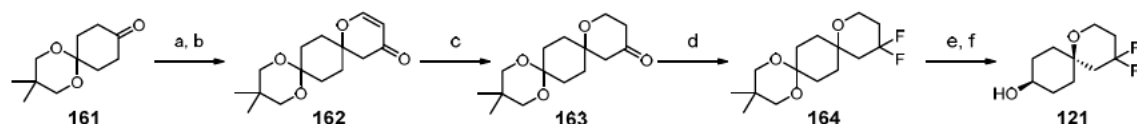
スピロ[4.5]アルコール **116** とスピロ [5.5]アルコール **119**、およびジフルオロ化されたスピロ[4.5]アルコール **120** については、シクロヘキサノン **134** を中間体として用いて合成した (Scheme 18)。 **116** および **119** の場合、対応するリンイリドを用いて 3 炭素または 4 炭素ユニットを導入することでシリルエーテル **150**、エステル **153** をそれぞれ合成した。**150**、**153** から脱シリル化または還元によりエン-アルコール **151**、**154** へそれぞれ誘導した。続いて、Amberlyst-15 存在下トルエン中加熱し、ジアステレオマーを分離して、所望のスピロエーテル **152**、**155** を得た。**120** の場合、ケトン **134** 由来のオレフィン **156** に対して、メタクロロ過安息香酸を用いたエポキシ化、続くジアステレオマー分離を行い、シス体のエポキシド **157** をまず得た。続いて、**157** とビニルマグネシウムクロリドとの反応により、オレフィン **158** に導いた。**158** の酸化的環化³⁰、生成した第二級アルコールの酸化により、スピロケトン **159** とした。**159** の脱酸素素的フッ素化反応³¹により、ジフルオロ体 **160** を得た。ベンジルエーテル **152**、**155**、**160** の脱ベンジル化により、アルコール **116**、**119**、**120** へとそれぞれ誘導した。



Scheme 18. Reagents and conditions: (a)

3-[(*tert*-butyldimethylsilyl)oxypropyl]triphenylphosphonium bromide, which was readily prepared from (3-bromopropoxy)-*tert*-butyldimethylsilane, NaH, DMF, 0 °C, 72%; (b) 1 M TBAF in THF, THF, rt, 88%; (c) Amberlyst-15, toluene, 90 °C, then separation by column chromatography on silica gel, 40–52%; (d) H₂ (15 psi), Pd(OH)₂-C, MeOH, rt, 97–99%; (e) 3-(ethoxycarbonyl) propyltriphenylphosphonium bromide, KO-*t*-Bu, THF, 0 °C to rt; (f) LiAlH₄, THF, 0 °C, 70% (2 steps); (g) methyltriphenylphosphonium bromide, KO-*t*-Bu, THF, 0 °C; (h) *m*-CPBA, NaHCO₃, CHCl₃, 0 °C, then separation by column chromatography on silica gel, 16% (2 steps); (i) 1 M vinylmagnesium bromide in THF, THF, 0 °C to rt, 79%; (j) NaIO₄, *t*-BuOH, H₂O, 50 °C; (k) Dess-Martin reagent, CHCl₃, 0 °C, 37% (2 steps); (l) bis(2-methoxyethyl)aminosulfur trifluoride, CH₂Cl₂, rt, 90%.

ジフルオロ化されたスピロ[4.5]アルコール **121** の合成を Scheme 19 に示した。はじめに、シクロヘキサノン **161** と Rewal ジエンとのヘテロ Diels-Alder 反応³²により、スピロエノン **162** を合成した。**162** のオレフィン部分の水素添加により得られたケトン **163** の脱酸素的フッ素化反応により、環状ケタール **164** を得た。酢酸を用いた脱ケタール化により得られたケトンのヒドリド還元、続くカラムクロマトグラフィーを用いたジアステレオマー分離により、シス体の **121** を得ることができた。

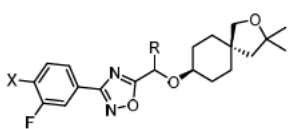


Scheme 19. Reagents and conditions: (a) *trans*-3-(*tert*-butyldimethylsilyloxy)-*N,N*-dimethyl-1,3-butadiene-1-amine, 2-BuOH, rt; (b) AcCl, Et₂O, -78 °C, 63% (2 steps); (c) H₂ (15 psi), Pd-C, MeOH, rt, 69%; (d) bis(2-methoxyethyl)aminosulfur trifluoride, CHCl₃, rt; (e) AcOH, H₂O, 100 °C, 49% (2 steps); (f) NaBH₄, MeOH, 0 °C, then separation by flash chromatography on silica gel, 85%.

第3節 結果と考察

第1節の方針に基づき選抜した **51** のアゴニスト活性は、これまでに見出した **17** ($EC_{50} = 4\text{ nM}$) や **65** ($EC_{50} = 19\text{ nM}$) と比べて弱かったため、まずアゴニスト活性の向上を目指して、左側ベンゼン環上置換基の変換を行った。評価結果を Table 8 に示した。

Table 8. SAR of oxadiazole derivatives with a spirocyclic cyclohexane structure.



51, 97-102

Compound	X	R	GPR119 EC_{50} (nM)	IA ^a (%)	CLogP ^b
51	MeSO ₂ -	Me (<i>rac</i>)	89	109	3.4
97	MeSO ₂ -	H	261	103	2.9
98	Me ₂ NCO-	Me (<i>rac</i>)	2,457	86	3.8
99	Me ₂ NCOCH ₂ -	Me (<i>rac</i>)	69	131	3.9
100	MeSO ₂ CH ₂ -	Me (<i>rac</i>)	44	130	3.3
101	MeSO ₂ CH ₂ -	Me (<i>S</i>)	430	106	3.3
102	MeSO ₂ CH ₂ -	Me (<i>R</i>)	27	106	3.3

^a Percent of maximum response of oleoylethanolamide (OEA).

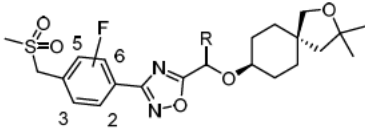
^b The ClogP value was calculated using software from ChemAxon.

化合物 **51** のリンカー部脱メチル体 ($R = H$) **97** では、アゴニスト活性は **51** と比べて約3倍弱かった。また、化合物 **51** の左側ベンゼン環上メチルスルホニル基を、化合物 **17** と同様ジメチルカルバモイル基に変換したところ、アゴニスト活性は大きく減弱した (化合物 **98**)。一方、これまでの検討により強力なアゴニスト活性を発現する上で重要と考えられた、

化合物の全長を調節するため、ジメチルカルバモイル基とベンゼン環の間にメチレン基を導入した化合物 **99** は、**98** より強いアゴニスト活性を示した。同様に、メチルスルホニル基とベンゼン環の間にメチレン基を有する化合物 **100** でも、期待通り **51** より強いアゴニスト活性を示した。この結果から、ベンゼン環上置換基のうち、水素結合アクセプター（カルバモイル基のカルボニル酸素原子やスルホニル基のスルホニル酸素原子）の位置が、強いアゴニスト活性を示すうえで重要なことが示唆された。化合物 **100** はラセミ体であるが、光学活性体をそれぞれ評価したところ、*R* 体 **101** が *S* 体 **102** より約 15 倍強いアゴニスト活性を示すことがわかった ($EC_{50} = 27$ nM)。

次に、複数の既報 GPR119 アゴニストでは、ベンゼン環上フルオロ基によるアゴニスト活性向上効果が報告されていたこともあり⁶、次に **100** を基に左側ベンゼン環上フルオロ基のアゴニスト活性に対する効果を調べた (Table 9)。

Table 9. Effects of F atom(s) on GPR119 agonistic activity.



100, 102-106

Compound	Position of F	R	GPR119 EC_{50} (nM)	IA ^a (%)	CLogP ^b
100	3-F	Me (<i>rac</i>)	44	130	3.3
103	none	Me (<i>rac</i>)	426	120	3.1
102	3-F	Me (<i>R</i>)	27	106	3.3
104	2-F, 3-F	Me (<i>R</i>)	301	77	3.4
105	3-F, 6-F	Me (<i>R</i>)	64	118	3.4
106	3-F, 5-F	Me (<i>R</i>)	5	109	3.4

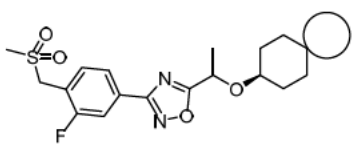
^a Percent of maximum response of oleoylethanolamide (OEA).

^b The ClogP value was calculated using software from ChemAxon.

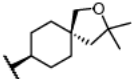
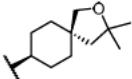
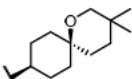
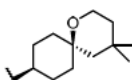
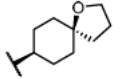
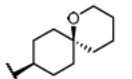
フルオロ基を有しない **103** では、**100** と比べて 10 倍程度アゴニスト活性が弱かったことから、3 位フルオロ基はアゴニスト活性発現に重要なことが示唆された。そこで、3 位を含む 2 つのフルオロ基を有する光学活性な化合物群を評価した。3 位の他に 2 位または 6 位にフルオロ基を有する化合物 **104**, **105** のアゴニスト活性は **102** と比べて弱かった。一方、3 位および 5 位にフルオロ基を有する化合物 **106** は、強いアゴニスト活性 ($EC_{50} = 5 \text{ nM}$) を示した。なお、**106** の脂溶性 ($CLogP = 3.4$) およびヒト血漿タンパク結合率 (ヒト血漿タンパク結合率 (plasma protein binding in human : hPPB) = 96.5%, ラット血漿タンパク結合率 (plasma protein binding in rat : rPPB) = 94.0%) は、リード化合物 **51** と同程度であった。

次に、**106** の代謝安定性を評価した結果、ヒト肝 Ms に対する代謝安定性はラット肝 Ms と比べて低いことがわかった (インキュベーション後 1 時間での残存率が 65% (ヒト), 83% (ラット))。第 2 章で見出した **17** のシクロヘキサン含有スピロ環上 *gem*-ジメチル基が代謝部位であったこともあり、**100** を基にシクロヘキサン含有スピロ環部の代謝安定性に対する効果を調べた (Table 10)。

Table 10. Effect of cyclohexane-containing spirocycle structures.



100, 107-111

Compound	Right side	GPR119 EC ₅₀ (nM)	IA ^a (%)	CLogP ^b	hMs ^c (%)	rMs ^c (%)
100		44	130	3.3	62	86
107		31	115	3.4	15	55
108		25	109	3.8	0	56
109		29	113	3.7	0	53
110		720	114	2.6	49	54
111		86	112	3.0	39	64

^a Percent of maximum response of oleoylethanolamide (OEA).

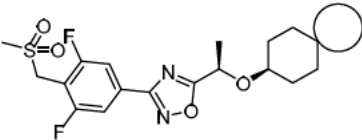
^b The CLogP value was calculated using software from ChemAxon.

^c Percent of compound (5 μ M) remaining after 1 hour incubation with human or rat liver microsomes (0.2 mg/mL).

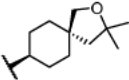
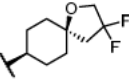
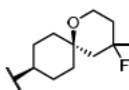
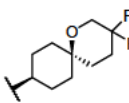
まず、シクロヘキサン含有スピロ環の酸素原子の位置，*gem*-ジメチル基の位置および環員数の代謝安定性に対する効果を調べるため，化合物 **107–109** を評価した。化合物 **100** と同程度のアゴニスト活性を示した **107–109** の代謝安定性は，**100** と比べて低く，ヒトではより顕著であった。一方，*gem*-ジメチル基を有さない化合物 **110** や **111** では，対応する *gem*-ジメチル体 **107–109** と比べてアゴニスト活性は弱かったが，ヒトでの代謝安定性の改善が認められた。これらの結果から，**17** と同様オキサジアゾール誘導体においても，シクロヘキサン含有スピロ環上 *gem*-ジメチル基は代謝部位であることが示唆された。

次に、ヒトでの代謝安定性の向上を目指した最適化を目的に、**106** を基にシクロヘキサン含有スピロ環上 *gem*-ジメチル基を変換した化合物の評価結果を Table 11 に示した。

Table 11. Final optimization of oxadiazole derivatives.



106, 112-114

Compound	Right side part	GPR119	IA ^a	CLogP ^b	hMs ^c	rMs ^c
		EC ₅₀ (nM)	(%)		(%)	(%)
106		5	109	3.4	65	83
112		7	116	3.1	83	85
113		27	106	3.3	97	91
114		6	112	3.3	98	98

^a Percent of maximum response of oleoylethanolamide (OEA).

^b The CLogP value was calculated using software from ChemAxon.

^c Percent of compound (5 μ M) remaining after 1hour incubation with human or rat liver microsomes (0.2 mg/mL).

gem-ジメチル基を *gem*-ジフルオロ基に置換することで、代謝安定性の向上に加えて、脂溶性の低下に伴う血漿タンパク結合性の低下を期待した³³。*gem*-ジフルオロ体 **112** は、同じくスピロ [4.5] 環を有する化合物 **106** と比べて良好なヒトにおける代謝安定性 (83%) を示し、同程度のアゴニスト活性 (EC₅₀ = 7 nM) を示した。**112** では、**106** よりも脂溶性 (CLogP = 3.1) が低く、タンパク結合率は低かった (hPPB = 93.4%, rPPB = 91.0%)。次に、スピロ [5.5] 環を有する化合物 **113**, **114** を評価したところ、**112** と比べてヒトおよびラットにおいて良好な代謝安定性を示した。特に **114** は、**106** と同程度の強力なアゴニスト活性 (EC₅₀ = 6 nM) と、低い血漿タンパク結合性 (hPPB = 92.5%, rPPB = 91.4%) を示した。

以上、第3章で見出したピリミジン誘導体 **65** より強力なアゴニスト活性と低い血漿タンパク結合性、良好な代謝安定性を示すオキサジアゾール誘導体 **114** を見出すことに成功し

た。

Table 12. Pharmacokinetic profiles of **114** in Sprague-Dawley rats (n = 2).

iv (1 mg/kg)				po (3 mg/kg)
t1/2 β (h)	CL (L/h/kg)	Vdss (L/kg)	MRT (h)	BA (%)
5.1	0.5	3.5	6.4	43

次に、**114**のPKプロファイルの評価した (Table 12)。その結果、**114**は**65**と同様、長時間作用型の薬効が期待できるプロファイルを示した。

65 より低用量での長時間作用型の薬効が期待された **114** について、Sprague-Dawley ラットを用いた腹腔内グルコース負荷による耐糖能試験 (ipGTT) を実施した (n = 6) (Figure 16)。化合物 **114** を経口投与した 16 時間後にグルコースを腹腔内投与した。グルコース投与後、血漿中グルコース濃度を 2 時間、インスリン濃度を 1 時間測定した。その結果、化合物 **114** は、糖濃度依存的なインスリン分泌を伴う強力な血糖上昇抑制作用を示した。化合物 **114** の 1 mg/kg, 3 mg/kg 投与時、グルコースの AUC は Vehicle 群と比べて、それぞれ 10%, 12% 低下した。

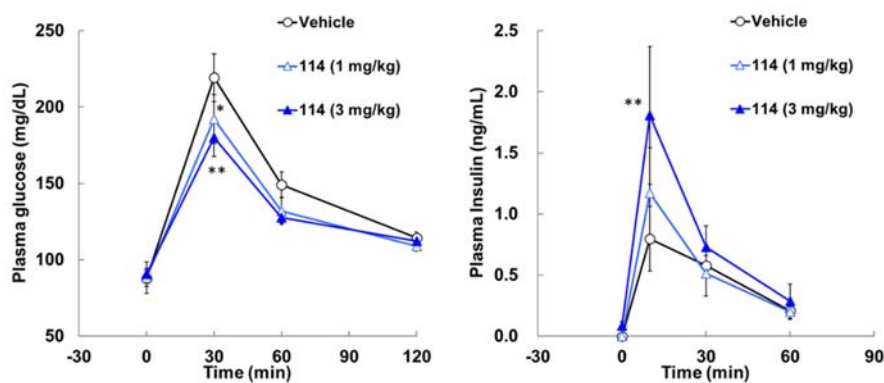


Figure 16. Effect of **114** on plasma glucose and insulin levels in ipGTT in rats. Each data represents mean \pm S.D. (n = 6). *: $p < 0.05$ vs. Vehicle, **: $p < 0.01$ vs. Vehicle (Dunnett's test).

第4節 小括

シクロヘキサン含有スピロ環を有するリード候補化合物 **50-52** のうち、血漿タンパク結合性が低いオキサジアゾール誘導体 **51** を新規リード化合物として選抜した。**51** の構造変換

の結果, 強力なアゴニスト活性と低い血漿タンパク結合性を示す代表化合物 **114** を見出した。持続的な PK プロファイルを示した **114** は, 化合物投与 16 時間後のラット ipGTT において, 第 3 章で見出したピリミジン誘導体 **65** と比べて低い投与量 (1 または 3 mg/kg) で血糖上昇抑制作用を示した。なお, **114** の投与量は, 臨床試験に移行した MBX-2982 の投与量と比べて少なかった。さらに, 第 2 章で見出した **17** とは異なり, **114** にはラットを用いた探索的毒性試験において重篤な毒性は認められなかった。

第5章 総括

新規GPR119アゴニストの探索合成研究を行い、多くの既知GPR119アゴニストにみられるN-置換ピペリジン環に代わる新規構造を探索し、シクロヘキサン含有スピロ環構造を見出した (Figure 17)。対応するN-置換ピペリジン誘導体**6**とは異なり、三次元性の高いシクロヘキサン含有スピロ環誘導体**17**について、CYP阻害活性は認められなかった。次に、**17**より低脂溶性の新規GPR119アゴニストを探索した。シクロヘキサン含有スピロ環構造を有する複数のリード化合物の最適化を実施し、ラットにおいて長時間作用型の薬効を示すピリミジン誘導体**65**およびオキサジアゾール誘導体**114**を見出した。特に、**114**はラットで低用量 (1 mg/kg) から強力かつ持続的な薬効を示すとともに探索的毒性試験において重篤な毒性が認められなかった。

臨床移行したGPR119アゴニストが複数報告されているが、未だ上市された薬剤はない。本研究で見出した化合物の非臨床および臨床の研究用ツールとしての活用を期待したい。

三次元性の高いスピロ環構造を活用したCYP阻害活性の低減、フルオロ基を利用した代謝安定性の改善、化合物の血漿タンパク結合性に着目した*in vivo*ポテンシーの向上等、本研究中で示した各課題解決の方策は創薬化学全般に応用可能と考える。

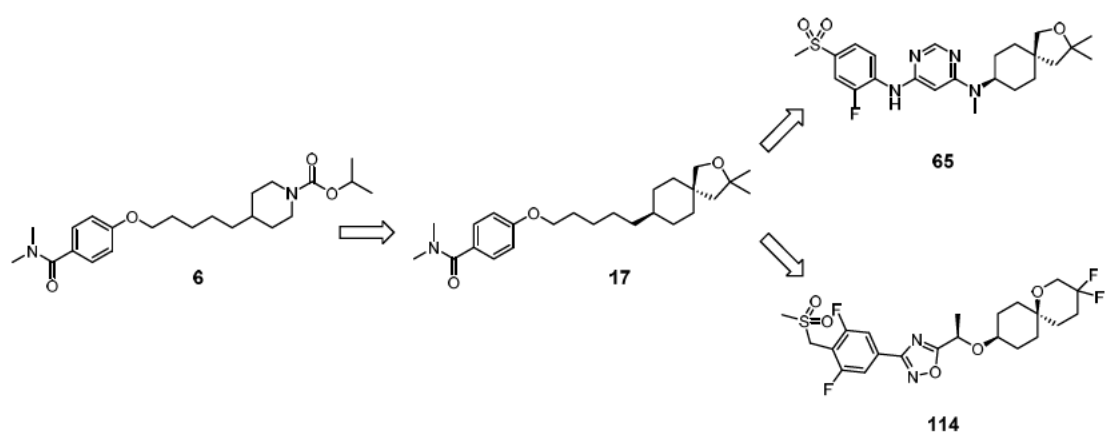


Figure 17. Summary of this work.

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Experimental section

General

Solvents and reagents were obtained from commercial suppliers and used as received. Flash column chromatography was performed using Merck 230–400 mesh silica gel 60. Melting points were determined using a Büchi 535 melting point apparatus or a Stanford Research Systems MPA100 OptiMelt melting point apparatus. ^1H NMR and ^{13}C NMR spectra were recorded on JEOL RESONANCE Inc. JNM-AL400, JEOL ALPHA300W, Varian MERCURY plus-AS400, Bruker BioSpin K.K. AV400, AMX-300, AVANCE III 400, or Agilent Technologies Inc. 400-MR spectrometer in the indicated solvent. Chemical shifts (δ) are reported in parts per million relative to internal standard tetramethylsilane. Optical rotation was measured using a Rudolph Autopol V automatic polarimeter at a wavelength of 589 nm. IR spectra were recorded on a Perkin-Elmer Inc. Spectrum One FT-IR spectrometer. Combustion analyses were performed with an elemental vario EL III, and all values were within $\pm 0.4\%$ of the calculated values. High-resolution mass spectra (HRMS) analyses were performed on a Thermo Fisher Scientific LTQ Orbitrap Velos mass spectrometer, which is equipped with Agilent 1290 Infinity LC. Low-resolution mass spectra (MS) analyses were performed on either a Finnigan TSQ-700 mass spectrometer in FAB ionization mode or an Agilent 1100 series LC/MSD mass spectrometer in ESI ionization mode.

Experiments concerning Chapter 2

***tert*-Butyl 4-{3-[4-(methylsulfonyl)phenoxy]propyl}piperidine-1-carboxylate (1)**

To a solution of *tert*-butyl 4-(3-hydroxypropyl)piperidine-1-carboxylate **18** (200 mg, 0.82 mmol) in CHCl₃ (2 ml) were added triethylamine (0.137 ml, 0.99 mmol) and methanesulfonyl chloride (0.07 ml, 0.90 mmol) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was washed with H₂O (1 ml). The organic layer was dried over Na₂SO₄, filtered, and concentrated *in vacuo* to give crude *tert*-butyl 4-{3-[(methylsulfonyl)oxy]propyl}piperidine-1-carboxylate (270 mg) as a colorless oil. The crude material was used without purification in the next step.

A suspension of crude *tert*-butyl 4-{3-[(methylsulfonyl)oxy]propyl}piperidine-1-carboxylate (270 mg), 4-(methylsulfonyl)phenol (141 mg, 0.82 mmol), and cesium carbonate (323 mg, 0.99 mmol) in DMF (3 ml) was stirred at 80 °C for 3 h. After cooling to room temperature, the reaction mixture was quenched with H₂O (1 ml). The precipitate was filtered off and recrystallized from *n*-hexane/EtOAc to give compound **1** (293 mg, 90%) as white crystals; mp 129–130 °C. IR (neat) 1692, 1593, 1398, 1364, 1314, 1294, 1275, 1260, 1234, 1167, 1138, 1094, 1003, 972, 964, 837, 772, 554, 490 cm⁻¹. ¹H NMR (CDCl₃) δ: 7.91–7.81 (m, 2H), 7.05–6.95 (m, 2H), 4.20–4.06 (m, 2H), 4.06–3.97 (m, 2H), 3.02 (s, 3H), 2.75–2.62 (m, 2H), 1.89–1.77 (m, 2H), 1.74–1.64 (m, 2H), 1.46 (s, 9H), 1.46–1.38 (m, 3H), 1.19–1.06 (m, 2H). ¹³C NMR (DMSO-*d*₆) δ: 162.3, 153.7, 132.3, 129.1, 114.8, 78.3, 68.2, 43.8, 43.4, 34.8, 32.1, 31.6, 28.0, 25.5. Anal. Calcd for C₂₀H₃₁NO₅S: C, 60.43; H, 7.86; N, 3.52. Found: C, 60.44; H, 7.92; N, 3.53.

***tert*-Butyl 4-{4-[4-(methylsulfonyl)phenoxy]butyl}piperidine-1-carboxylate (2)**

Compound **2** was prepared from *tert*-butyl 4-(4-hydroxybutyl)piperidine-1-carboxylate **19** and 4-(methylsulfonyl)phenol in a manner similar to that described for compound **1**. White crystals (65%); mp 97–99 °C. IR (neat) 1690, 1593, 1420, 1364, 1312, 1294, 1275, 1261, 1244, 1227, 1167, 1138, 1092, 1034, 1001, 968, 941, 833, 768, 548, 534, 488 cm⁻¹. ¹H NMR (CDCl₃) δ: 7.91–7.81 (m, 2H), 7.05–6.96 (m, 2H), 4.14–4.05 (m, 2H), 4.05–3.98 (m, 2H), 3.02 (s, 3H), 2.74–2.58 (m, 2H), 1.86–1.75 (m, 2H), 1.71–1.61 (m, 2H), 1.54–1.36 (m, 12H), 1.35–1.27 (m, 2H), 1.16–1.00 (m, 2H). ¹³C NMR (DMSO-*d*₆) δ: 162.3, 153.5, 132.3, 129.1, 114.7, 78.2, 67.9, 44.0, 35.4, 35.0, 31.6, 28.5, 28.0, 22.3. Anal. Calcd for C₂₁H₃₃NO₅S: C, 61.29; H, 8.08; N, 3.40. Found: C, 61.35; H, 8.09; N, 3.36.

***tert*-Butyl 4-{5-[4-(methylsulfonyl)phenoxy]pentyl}piperidine-1-carboxylate (3)**

Compound **3** was prepared from *tert*-butyl 4-(4-hydroxypentyl)piperidine-1-carboxylate **20** and 4-(methylsulfonyl)phenol in a manner similar to that described for compound **1**. White crystals (54%); mp 122–123 °C. IR (neat) 1690, 1593, 1400, 1364, 1312, 1294, 1275, 1256, 1244, 1165, 1138, 1092, 1003, 970, 964, 835, 770, 550, 538, 509, 490 cm⁻¹. ¹H NMR (CDCl₃) δ: 7.98–7.72 (m, 2H), 7.09–6.92 (m, 2H), 4.13–3.99 (m, 4H), 3.03 (s, 3H), 2.73–2.61 (m, 2H), 1.86–1.76 (m, 2H), 1.69–1.60 (m, 2H), 1.51–1.32 (m, 14H), 1.31–1.22 (m, 2H), 1.14–1.01 (m, 2H). ¹³C NMR (DMSO-*d*₆) δ: 162.8,

154.0, 132.6, 129.3, 115.1, 78.5, 68.2, 44.2, 36.1, 35.3, 32.0, 28.6, 28.2, 25.9, 25.7. Anal. Calcd for $C_{22}H_{35}NO_5S$: C, 62.09; H, 8.29; N, 3.29. Found: C, 62.03; H, 8.24; N, 3.15.

***tert*-Butyl 4-{6-[4-(methylsulfonyl)phenoxy]hexyl}piperidine-1-carboxylate (4)**

Compound **4** was prepared from *tert*-butyl 4-(4-hydroxyhexyl)piperidine-1-carboxylate **21** and 4-(methylsulfonyl)phenol in a manner similar to that described for compound **1**. White crystals (78%); mp 83–87 °C. 1H NMR (DMSO- d_6) δ : 7.84–7.79 (m, 2H), 7.16–7.10 (m, 2H), 4.09–4.01 (m, 2H), 3.96–3.81 (m, 2H), 3.14 (s, 3H), 2.74–2.56 (m, 2H), 1.77–1.67 (m, 2H), 1.64–1.56 (m, 2H), 1.44–1.23 (m, 16H), 1.23–1.14 (m, 2H), 0.98–0.84 (m, 2H). Anal. Calcd for $C_{23}H_{37}NO_5S$: C, 62.84; H, 8.48; N, 3.19. Found: C, 62.64; H, 8.43; N, 3.15.

***tert*-Butyl 4-{5-[4-(dimethylcarbamoyl)phenoxy]pentyl}piperidine-1-carboxylate (5)**

Compound **5** was prepared from *tert*-butyl 4-(4-hydroxypentyl)piperidine-1-carboxylate and 4-hydroxy-*N,N*-dimethylbenzamide in a manner similar to that described for compound **1**. White crystals (86%); mp 77–78 °C. IR (neat) 1690, 1622, 1605, 1491, 1450, 1393, 1364, 1275, 1240, 1217, 1179, 1167, 1146, 1121, 1099, 1084, 1059, 1047, 1036, 1020, 1009, 961, 866, 841, 820, 779, 762, 725, 621, 484 cm^{-1} . 1H NMR (DMSO- d_6) δ : 7.41–7.26 (m, 2H), 7.06–6.81 (m, 2H), 4.80–4.68 (m, 1H), 4.02–3.86 (m, 4H), 2.93 (s, 6H), 2.79–2.61 (m, 2H), 1.76–1.67 (m, 2H), 1.67–1.57 (m, 2H), 1.46–1.27 (m, 5H), 1.26–1.19 (m, 2H), 1.18–1.14 (m, 6H), 1.02–0.88 (m, 2H). ^{13}C NMR (DMSO- d_6) δ : 180.1, 169.6, 164.0, 139.2, 138.4, 124.1, 88.5, 77.7, 53.7, 46.1, 45.3, 42.0, 38.7, 38.3, 35.9, 35.8. Anal. Calcd for $C_{24}H_{38}N_2O_4$: C, 68.87; H, 9.15; N, 6.69. Found: C, 68.78; H, 9.05; N, 6.56.

Isopropyl 4-{5-[4-(dimethylcarbamoyl)phenoxy]pentyl}piperidine-1-carboxylate (6)

To a solution of compound **5** (88 mg, 0.22 mmol) and 1,4-dioxane (1 ml) was added 4N HCl 1,4-dioxane solution (1.00 ml, 4.00 mmol) at room temperature. After stirring at room temperature, the reaction mixture was concentrated *in vacuo*. The resulting precipitate was suspended in $CHCl_3$ (2 ml). To the suspension were added triethylamine (0.10 ml, 0.75 mmol) and isopropyl chloroformate (0.037 ml, 0.33 mmol) at 0 °C. After stirring at room temperature, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **6** (80 mg, 94%) as white crystals; mp 67–68 °C. IR (neat) 1686, 1624, 1387, 1236, 1215, 1107, 1082, 843, 621 cm^{-1} . 1H NMR (DMSO- d_6) δ : 7.41–7.26 (m, 2H), 7.06–6.81 (m, 2H), 4.80–4.68 (m, 1H), 4.02–3.86 (m, 4H), 2.93 (s, 6H), 2.79–2.61 (m, 2H), 1.76–1.67 (m, 2H), 1.67–1.57 (m, 2H), 1.46–1.27 (m, 5H), 1.26–1.19 (m, 2H), 1.18–1.14 (m, 6H), 1.02–0.88 (m, 2H). ^{13}C NMR (DMSO- d_6) δ : 169.8, 159.4, 154.1, 128.9, 128.2, 113.8, 67.4, 43.4, 35.7, 35.0, 31.6, 28.5, 25.6, 21.9. Anal. Calcd for $C_{23}H_{36}N_2O_4$: C, 68.29; H, 8.97; N, 6.92. Found: C, 68.26; H, 8.95; N, 6.99.

4-{[5-(Spiro[5.5]undecan-3-yl)pentyl]oxy}benzoic acid (24)

A suspension of 4-[(5-bromopentyl)oxy]benzoic acid **22** (5.74 g, 20.0 mmol) and

triphenylphosphine (5.51 g, 21.0 mmol) in toluene (28 ml) was stirred at 130 °C for 9 h. After cooling to room temperature, the resulting precipitate was filtered off and washed with toluene to give crude [5-(4-carboxyphenoxy)pentyl]triphenylphosphonium bromide **23** (4.93 g) as a white solid. The crude material was used without purification in the next step.

To a suspension of crude [5-(4-carboxyphenoxy)pentyl]triphenylphosphonium bromide **23** (833 mg) and potassium *tert*-butoxide (171 mg, 1.52 mmol) in THF (5.0 ml) was added dropwise a solution of spiro[5.5]undecan-3-one (210 mg, 1.26 mmol) in THF (2.1 ml) at 0 °C. After stirring to room temperature for 2 h, the reaction mixture was quenched with 2N HCl aqueous solution (4 ml, 8.00 mmol). The resulting mixture was extracted with EtOAc. The combined organic layer was washed with H₂O and brine. The organic layer was dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude 4-{[5-(spiro[5.5]undecan-3-ylidene)pentyl]oxy}benzoic acid (450 mg) as a white solid. The crude material was used without purification in the next step.

To a solution of crude 4-{[5-(spiro[5.5]undecan-3-ylidene)pentyl]oxy}benzoic acid (40 mg) in MeOH (1.0 ml) was added 10% Pd-carbon (50% wet). The reaction mixture was hydrogenated (60 psi) at room temperature overnight. The reaction mixture was filtered through a celite pad and concentrated *in vacuo* to give compound **24** (34.1 mg, 14% for 3 steps) as a white solid. ¹H NMR (DMSO-*d*₆) δ: 7.92–7.82 (m, 2H), 7.03–6.94 (m, 2H), 4.03 (t, *J* = 6.2 Hz, 2H), 1.79–1.67 (m, 2H), 1.62–1.53 (m, 2H), 1.50–1.27 (m, 14H), 1.20–1.16 (m, 5H), 1.08–0.91 (m, 3H).

***N,N*-Dimethyl-4-{[5-(spiro[5.5]undecan-3-yl)pentyl]oxy}benzamide (7)**

A suspension of compound **24** (34 mg, 0.095 mmol), 1-hydroxybenzotriazole monohydrate (22 mg, 0.143 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (27 mg, 0.143 mmol), dimethylamine hydrochloride (16 mg, 0.190 mmol) and triethylamine (0.027 ml, 0.190 mmol) in DMF (1 ml) was stirred at room temperature overnight. The reaction mixture was quenched with saturated NaHCO₃ aqueous solution (1 ml) and H₂O (4 ml). The resulting precipitate was washed with H₂O to give compound **7** (30 mg, 82%) as white crystals; mp 80–83 °C. ¹H NMR (CDCl₃) δ: 7.40–7.37 (m, 2H), 6.89–6.87 (m, 2H), 3.97–3.93 (m, 4H), 3.87 (t, 2H, *J* = 5.6 Hz), 3.07 (br s, 6H), 2.25–2.22 (m, 2H), 1.82–1.68 (m, 4H), 1.63–1.56 (m, 3H), 1.53–1.07 (m, 10H). HRMS (ESI) Calcd for C₂₅H₄₀NO₂ (M+H)⁺ *m/z* 386.3054, Found *m/z* 386.3044.

5-(1,4-Dioxaspiro[4.5]decan-8-yl)pentan-1-ol (26)

To a suspension of (4-carboxybutyl)triphenylphosphonium bromide (2.83 g, 6.40 mmol) and potassium *tert*-butoxide (1.50 g, 13.44 mmol) in THF (10 ml) was added dropwise a solution of 1,4-dioxaspiro[4.5]decan-8-one **25** (1.00 g, 6.40 mmol) in THF (5 ml) at 0 °C. After stirring at room temperature overnight, the reaction mixture was concentrated *in vacuo*. To the resulting precipitate, 1N HCl aqueous solution (10 ml, 10 mmol) was added. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude 5-(1,4-dioxaspiro[4.5]decan-8-ylidene)pentanoic acid (1.65 g) as a pale yellow

oil. The crude material was used without purification in the next step.

To a solution of crude 5-(1,4-dioxaspiro[4.5]decan-8-ylidene)pentanoic acid (1.65 g) in MeOH (10 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.59 g, 8.32 mmol) at room temperature. After stirring at room temperature, the reaction mixture was concentrated *in vacuo*. The residue was diluted with EtOAc (15 ml). The organic layer was washed with saturated NaHCO₃ aqueous solution and H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give methyl 5-(1,4-dioxaspiro[4.5]decan-8-ylidene)pentanoate (820 mg, 50 % for 2 steps) as a colorless oil. ¹H NMR (CDCl₃) δ: 5.16–5.08 (m, 1H), 3.97 (s, 4H), 3.67 (s, 3H), 2.35–2.18 (m, 6H), 2.10–2.00 (m, 2H), 1.72–1.61 (m, 6H).

To a solution of methyl 5-(1,4-dioxaspiro[4.5]decan-8-ylidene)pentanoate (820 mg, 3.22 mmol) in MeOH (8 ml) was added 10% Pd-carbon (50% wet). The reaction mixture was hydrogenated (60 psi) at room temperature overnight. The reaction mixture was filtered through a celite pad and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give methyl 5-(1,4-dioxaspiro[4.5]decan-8-yl)pentanoate (512 mg, 62%) as a colorless oil. ¹H NMR (CDCl₃) δ: 3.94 (s, 4H), 3.66 (s, 3H), 2.30 (t, *J* = 7.6 Hz, 2H), 1.77–1.66 (m, 4H), 1.65–1.45 (m, 4H), 1.38–1.12 (m, 7H).

To a suspension of LiAlH₄ (90 mg, 2.39 mmol) in THF (1 ml) was added dropwise a solution of methyl 5-(1,4-dioxaspiro[4.5]decan-8-yl)pentanoate (512 mg, 1.99 mmol) in THF (1 ml) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was quenched carefully with H₂O (0.09 ml), 4N NaOH aqueous solution (0.09 ml), and H₂O (0.27 ml) at 0 °C. The reaction mixture was stirred at room temperature for 1h, filtered through a celite pad and concentrated *in vacuo* to give compound **26** (446 mg, 98%) as a colorless oil. ¹H NMR (CDCl₃) δ: 3.95 (s, 4H), 3.70–3.57 (m, 2H), 1.83–1.66 (m, 4H), 1.66–1.45 (m, 4H), 1.42–1.12 (m, 9H).

4-{[5-(1,4-Dioxaspiro[4.5]decan-8-yl)pentyl]oxy}-*N,N*-dimethylbenzamide (**9**)

To a solution of compound **26** (446 mg, 1.95 mmol) in CHCl₃ (5 ml) were added triethylamine (0.33 ml, 2.34 mmol) and methanesulfonyl chloride (0.17 ml, 2.15 mmol) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was diluted with EtOAc (5 ml). The organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude 5-(1,4-dioxaspiro[4.5]decan-8-yl)pentyl methanesulfonate (580 mg) as a colorless oil. The crude material was used without purification in the next step.

A suspension of crude 5-(1,4-dioxaspiro[4.5]decan-8-yl)pentyl methanesulfonate (487 mg), 4-hydroxy-*N,N*-dimethylbenzamide (289 mg, 1.75 mmol), and cesium carbonate (673 mg, 2.07 mmol) in DMF (2 ml) was stirred at 80 °C for 3 h. After cooling to room temperature, the reaction mixture was quenched with H₂O (5 ml). The aqueous layer was extracted with EtOAc. The organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified

by column chromatography on silica gel (CHCl₃/acetone) to give compound **9** (538 mg, 90 % for 2 steps) as white crystals; mp 80–81 °C. ¹H NMR (DMSO-*d*₆) δ: 7.37–7.33 (m, 2H), 6.96–6.92 (m, 2H), 3.99 (t, 2H, *J* = 6.9 Hz), 3.82 (s, 4H), 2.94 (s, 6H), 1.75–1.60 (m, 6H), 1.45–1.07 (m, 11H). HRMS (ESI) Calcd for C₂₂H₃₄NO₄ (M+H)⁺ *m/z* 376.2482, Found *m/z* 376.2478.

4-{[5-(1,5-Dioxaspiro[5.5]undecan-9-yl)pentyl]oxy}-*N,N*-dimethylbenzamide (8)

A solution of compound **9** (538 mg, 1.43 mmol) in AcOH (2 ml) and H₂O (1 ml) was stirred at 100 °C overnight. After cooling to room temperature, the reaction mixture was diluted with H₂O (5 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with saturated NaHCO₃ aqueous solution and H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude *N,N*-dimethyl-4-{[5-(4-oxocyclohexyl)pentyl]oxy}benzamide (452 mg) as a white solid. The crude material was used without purification in the next step.

A suspension of crude *N,N*-dimethyl-4-{[5-(4-oxocyclohexyl)pentyl]oxy}benzamide (70 mg), 1,3-propanediol (0.02 ml, 0.275 mmol) and pyridinium *p*-toluenesulfonate (3 mg, 0.011 mmol) in toluene (2 ml) was stirred at 140 °C overnight. After cooling to room temperature, the reaction mixture was diluted with EtOAc (5 ml). The organic layer was washed with saturated NaHCO₃ aqueous solution and H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **8** (74 mg, 86% for 2 steps) as white crystals; mp 72–75 °C. IR (neat) 2932, 2857, 1622, 1609, 1574, 1489, 1474, 1449, 1404, 1385, 1379, 1366, 1309, 1254, 1246, 1213, 1175, 1153, 1144, 1105, 1074, 1049, 1036, 1026, 999, 968, 943, 928, 914, 893, 862, 845, 816, 764, 723, 692, 642, 623, 554, 515, 507, 490, 463, 447, 430 cm⁻¹. ¹H NMR (CDCl₃) δ: 7.40–7.37 (m, 2H), 6.89–6.87 (m, 2H), 3.97–3.93 (m, 4H), 3.87 (t, 2H, *J* = 5.6 Hz), 3.07 (br s, 6H), 2.25–2.22 (m, 2H), 1.82–1.68 (m, 4H), 1.63–1.56 (m, 3H), 1.53–1.07 (m, 10H). ¹³C NMR (DMSO-*d*₆) δ: 169.8, 159.3, 128.9, 128.1, 113.8, 97.1, 67.4, 58.3, 58.1, 36.1, 35.8, 31.9, 28.5, 28.2, 26.2, 25.7, 25.2. Anal. Calcd for C₂₃H₃₅NO₄: C, 70.92; H, 9.06; N, 3.60. Found: C, 70.69; H, 9.12; N, 3.60.

4-[5-(Benzyloxy)pentyl]cyclohexan-1-one (27)

A suspension of 5-(1,4-dioxaspiro[4.5]decan-8-yl)pentan-1-ol **26** (57.9 g, 253 mmol), benzyl bromide (45.1 ml, 380 mmol) and NaH (60% oil dispersion, 13.2 g, 329 mmol) in DMF (500 ml) was heated at 50 °C for 3 h. After cooling to room temperature, the reaction mixture was carefully poured into ice-water. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude 8-[5-(benzyloxy)pentyl]-1,4-dioxaspiro[4.5]decane (65.2 g) as a colorless oil. The crude material was used without purification in the next step.

A solution of crude 8-[5-(benzyloxy)pentyl]-1,4-dioxaspiro[4.5]decane (65.2 g) and 2N HCl aqueous solution (100 ml, 200 mmol) in acetone (500 ml) was stirred at room temperature overnight. The reaction mixture was quenched with 4N NaOH aqueous solution (50 ml, 200 mmol). The

aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **27** (48.9 g, 70% for 2 steps) as a colorless oil. ¹H NMR (CDCl₃) δ: 7.39–7.24 (m, 5H), 4.51 (s, 2H), 3.51–3.44 (m, 2H), 2.42–2.26 (m, 4H), 2.09–1.99 (m, 2H), 1.76–1.59 (m, 3H), 1.46–1.27 (m, 8H).

4-{4-[5-(Benzyloxy)pentyl]cyclohexylidene}butan-1-ol (**28**)

To a suspension of 3-(ethoxycarbonyl)propyl triphenylphosphonium bromide (10.0 g, 21.9 mmol) in THF (100 ml) was added potassium *tert*-butoxide (2.46 g, 21.9 mmol) at 0 °C under an argon atmosphere. After stirring at 0 °C for 30 min, a solution of compound **27** (3.00 g, 10.9 mmol) in THF (20 ml) was added to the reaction mixture at 0 °C. After stirring at room temperature for 1.5 h, the reaction mixture was quenched with saturated NH₄Cl aqueous solution (20 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude ethyl 4-{4-[5-(benzyloxy)pentyl]cyclohexylidene}butanoate (3.56 g) as a colorless oil. The crude material was used without purification in the next step.

To a suspension of LiAlH₄ (360 mg, 9.48 mmol) in THF (5 ml) was added dropwise a solution of crude ethyl 4-{4-[5-(benzyloxy)pentyl]cyclohexylidene}butanoate (3.53 g) in THF (15 ml) at 0 °C under an argon atmosphere. After stirring at 0 °C for 3 h, the reaction mixture was quenched carefully with H₂O (0.36 ml), 4N NaOH aqueous solution (0.36 ml), and H₂O (1.08 ml) at 0 °C. The reaction mixture was stirred at room temperature for 1h, filtered through a celite pad and concentrated *in vacuo* to give compound **28** (3.12 g, 86% for 2 steps) as a colorless oil. ¹H NMR (CDCl₃) δ: 7.38–7.25 (m, 5H), 5.13–5.05 (m, 1H), 3.68–3.59 (m, 2H), 3.50–3.42 (m, 2H), 2.20–1.94 (m, 4H), 1.89–1.83 (m, 2H), 1.83–1.67 (m, 2H), 1.66–1.58 (m, 3H), 1.42–1.13 (m, 8H), 1.01–0.81 (m, 2H).

9-[5-(Benzyloxy)pentyl]-1-oxaspiro[5.5]undecane (**29**, **30**)

To a solution of compound **28** (3.12 g, 9.44 mmol) in CHCl₃ (30 ml) was added dropwise boron trifluoride diethyl ether complex (1.41 g, 9.91 mmol) at 0 °C. After stirring at room temperature overnight, the reaction mixture was quenched with saturated NaHCO₃ aqueous solution (10 ml) at 0 °C. The aqueous layer was extracted with CHCl₃. The combined organic layer was washed with saturated NaHCO₃ aqueous solution and H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **29** (*cis*-isomer, less polar, 707 mg, 22%) and compound **30** (*trans*-isomer, more polar, 834 mg, 26%) as colorless oils.

compound **29**: ¹H NMR (CDCl₃) δ: 7.38–7.25 (m, 5H), 4.50 (s, 2H), 3.63–3.56 (m, 2H), 3.50–3.42 (m, 2H), 2.03–1.93 (m, 2H), 1.66–1.57 (m, 4H), 1.54–1.41 (m, 4H), 1.41–1.02 (m, 13H).

compound **30**: ¹H NMR (CDCl₃) δ: 7.40–7.23 (m, 5H), 4.51 (s, 2H), 3.74–3.65 (m, 2H), 3.51–3.42

(m, 2H), 1.94–1.84 (m, 2H), 1.72–1.47 (m, 10H), 1.40–1.14 (m, 9H), 1.04–0.91 (m, 2H).

3-{4-[5-(Benzyloxy)pentyl]cyclohexylidene}propan-1-ol (31)

To a suspension of {3-[(*tert*-butyldimethylsilyloxy)propyl]triphenylphosphonium bromide (3.54 g, 6.34 mmol), which was readily prepared from (3-bromopropoxy)-*tert*-butyldimethylsilane, in THF (50 ml) was added potassium *tert*-butoxide (771 mg, 6.87 mmol) at 0 °C under an argon atmosphere. After stirring at 0 °C for 30 min, a solution of compound **27** (1.45 g, 5.28 mmol) in THF (10 ml) was added to the reaction mixture at 0 °C. After stirring at room temperature overnight, the reaction mixture was quenched with saturated NH₄Cl aqueous solution (20 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude (3-{4-[5-(benzyloxy)pentyl]cyclohexylidene}propoxy)-*tert*-butyldimethylsilane (1.47 g) as a colorless oil. The crude material was used without purification in the next step.

To a solution of crude (3-{4-[5-(benzyloxy)pentyl]cyclohexylidene}propoxy)-*tert*-butyldimethylsilane (1.47 g) in THF (10 ml) was added 1M tetrabutylammonium fluoride solution in THF (4.70 ml, 4.70 mmol) at room temperature. After stirring at room temperature overnight, the reaction mixture was concentrated *in vacuo*. The residue was diluted with EtOAc (20 ml). The organic layer was washed with H₂O, saturated NaHCO₃ aqueous solution, and brine. The organic layer was dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude compound **31** (982 mg) as a pale yellow oil. The crude material was used without purification in the next step.

8-[5-(Benzyloxy)pentyl]-1-oxaspiro[4.5]decane (32, 33)

A mixture of crude compound **31** (400 mg) and Amberlyst 15 (100 mg) in toluene (5 ml) was heated at 80 °C for 2 h. The reaction mixture was filtered through a celite pad and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **32** (*cis*-isomer, less polar, 180 mg, 26% for 3 steps) and compound **33** (*trans*-isomer, more polar, 158 mg, 23% for 3 steps) as colorless oils.

compound **32**: ¹H NMR (CDCl₃) δ: 7.37–7.23 (m, 5H), 4.50 (s, 2H), 3.84–3.77 (m, 2H), 3.49–3.43 (m, 2H), 1.92–1.83 (m, 2H), 1.74–1.48 (m, 8H), 1.40–1.11 (m, 10H).

compound **33**: ¹H NMR (CDCl₃) δ: 7.38–7.25 (m, 5H), 4.50 (s, 2H), 3.85–3.79 (m, 2H), 3.50–3.43 (m, 2H), 1.94–1.84 (m, 2H), 1.80–1.57 (m, 8H), 1.51–1.40 (m, 2H), 1.40–1.14 (m, 2H), 0.99–0.85 (m, 2H).

***cis*-6-[5-(Benzyloxy)pentyl]-1-oxaspiro[2.5]octane (34)**

To a suspension of trimethylsulfoxonium iodide (6.28 g, 28.5 mmol) in DMSO (60 ml) was added potassium *tert*-butoxide (2.95 g, 26.3 mmol) at room temperature under an argon atmosphere. After stirring at 50 °C for 30 min, a solution of compound **27** (6.02 g, 21.9 mmol) in DMSO (40 ml) was added to the reaction mixture at 50 °C. After stirring at room temperature for 1.5 h, the reaction

mixture was poured into ice-water (500 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo* to give compound **34** (6.35 g, 99%) as a colorless oil. ¹H NMR (CDCl₃) δ: 7.38–7.24 (m, 5H), 4.51 (s, 2H), 3.51–3.43 (m, 2H), 2.64 (s, 2H), 1.91–1.77 (m, 2H), 1.77–1.68 (m, 2H), 1.68–1.58 (m, 2H), 1.43–1.20 (m, 11H).

***cis*-8-[5-(Benzyloxy)pentyl]-3,3-dimethyl-1-oxaspiro[4.5]decan-2-one (35)**

To a solution of methyl isobutyrate (3.45 g, 33.8 mmol) in THF (15 ml) was added dropwise 2 M lithium diisopropyl amide solution in THF (17.7 ml, 35.4 mmol) at -20 °C under an argon atmosphere. After stirring at -20 °C, to the reaction mixture was added dropwise a solution of compound **34** (4.87 g, 16.9 mmol) in THF (50 ml) at 0 °C. The reaction mixture was allowed to warm up to room temperature. After stirring at room temperature overnight, the reaction mixture was quenched with saturated NH₄Cl aqueous solution (20 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude compound **35** (5.09 g) as a colorless oil. The crude material was used without purification in the next step.

***cis*-4-[5-(Benzyloxy)pentyl]-1-(3-hydroxy-2,2-dimethylpropyl)cyclohexan-1-ol (36)**

To a suspension of LiAlH₄ (581 mg, 15.3 mmol) in THF (40 ml) was added dropwise a solution of crude compound **35** (5.09 g) in THF (15 ml) at 0 °C. After stirring at 0 °C for 3 h, the reaction mixture was quenched carefully with H₂O (0.58 ml), 4N NaOH aqueous solution (0.58 ml), and H₂O (1.74 ml) at 0 °C. The reaction mixture was stirred at room temperature for 1h, filtered through a celite pad and concentrated *in vacuo* to give compound **36** (5.45 g, 70% for 2 steps) as a white solid. ¹H NMR (CDCl₃) δ: 7.37–7.26 (m, 5H), 4.51 (s, 2H), 3.67 (br s, 1H), 3.51–3.40 (m, 4H), 2.38 (br s, 1H), 1.89–1.78 (m, 2H), 1.68–1.54 (m, 6H), 1.42–1.05 (m, 12H), 0.98 (s, 6H).

***cis*-8-[5-(Benzyloxy)pentyl]-3,3-dimethyl-1-oxaspiro[4.5]decane (37)**

To a solution of compound **36** (4.35 g, 12.0 mmol) in pyridine (45 ml) was added *p*-toluenesulfonyl chloride (2.29 g, 12.0 mmol) at 0 °C. After stirring at 90 °C, the reaction mixture was concentrated *in vacuo*. The residue was diluted with 1N HCl aqueous solution (30 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed saturated NaHCO₃ aqueous solution and H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **37** (3.54 g, 88%) as a colorless oil. ¹H NMR (CDCl₃) δ: 7.36–7.25 (m, 5H), 4.50 (s, 2H), 3.50–3.42 (m, 4H), 1.85–1.76 (m, 2H), 1.66–1.46 (m, 6H), 1.38–1.12 (m, 11H), 1.07 (s, 6H).

4-{4-[5-(Benzyloxy)pentyl]cyclohex-1-en-1-yl}-3,3-dimethylbutan-1-ol (38).

To a solution of compound **36** (1.50 g, 4.14 mmol), pyridine (0.502 ml, 6.20 mmol) and *N,N*-dimethylaminopyridine (50.5 mg, 0.414 mmol) in CHCl₃ (15 ml) was added acetic anhydride (0.469 ml, 4.96 mmol) at room temperature. After stirring at room temperature for 3 h, the reaction

mixture was concentrated *in vacuo*. The residue was diluted with EtOAc (15 ml). The organic layer was washed with H₂O, 1N HCl aqueous solution and brine, dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude 3-{4-[5-(benzyloxy)pentyl]-1-hydroxycyclohexyl}-2,2-dimethylpropyl acetate (1.94 g) as a colorless oil. The crude material was used without purification in the next step.

To a solution of crude 3-{4-[5-(benzyloxy)pentyl]-1-hydroxycyclohexyl}-2,2-dimethylpropyl acetate (1.94 g) in pyridine (10 ml) was added dropwise thionyl chloride (0.60 ml, 8.27 mmol) at 0 °C. After stirring at 0 °C for 2 h, the reaction mixture was concentrated *in vacuo*. The residue was diluted with EtOAc (10 ml). The organic layer was washed with 1N HCl aqueous solution and brine, dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude 3-{4-[5-(benzyloxy)pentyl]cyclohex-1-en-1-yl}-2,2-dimethylpropyl acetate (1.65 g) as an orange oil. The crude material was used without purification in the next step.

To a solution of crude 3-{4-[5-(benzyloxy)pentyl]cyclohex-1-en-1-yl}-2,2-dimethylpropyl acetate (1.65 g) in THF (5 ml) and MeOH (5 ml) was added 4N NaOH aqueous solution (2.07 ml, 8.28 mmol) at room temperature. After stirring at room temperature overnight, to the reaction mixture was added 2N HCl aqueous solution (4.14 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude 3-{4-[5-(benzyloxy)pentyl]cyclohex-1-en-1-yl}-2,2-dimethylpropan-1-ol (1.53 g) as a pale yellow oil. The crude material was used without purification in the next step.

To a solution of crude 3-{4-[5-(benzyloxy)pentyl]cyclohex-1-en-1-yl}-2,2-dimethylpropan-1-ol (1.53 g) in CHCl₃ (15 ml) was added Dess-Martin reagent (2.11 g, 4.96 mmol) at 0 °C. After stirring at room temperature for 3 h, the reaction mixture was quenched with saturated Na₂SO₃ aqueous solution (10 ml). The reaction mixture was concentrated *in vacuo*. The residue was diluted with EtOAc (15 ml). The organic layer was washed with saturated NaHCO₃ aqueous solution and brine, dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude 3-{4-[5-(benzyloxy)pentyl]cyclohex-1-en-1-yl}-2,2-dimethylpropanal (1.60 g) as a pale yellow oil. The crude material was used without purification in the next step.

To a suspension of methoxymethyltriphenylphosphonium chloride (2.84 g, 8.27 mmol) in THF (30 ml) was added potassium *tert*-butoxide (928 mg, 8.27 mmol) at 0 °C under an argon atmosphere. After stirring at 0 °C for 30 min, a solution of crude 3-{4-[5-(benzyloxy)pentyl]cyclohex-1-en-1-yl}-2,2-dimethylpropanal (1.60 g) in THF (10 ml) was added dropwise to the reaction mixture at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was quenched with saturated NH₄Cl aqueous solution (20 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give

[(5-[4-(4-methoxy-2,2-dimethylbut-3-en-1-yl)cyclohex-3-en-1-yl]pentyl)oxy)methyl]benzene (915 mg, 60% for 5 steps) as a colorless oil as a mixture of *E*, *Z*-isomers. ¹H NMR (CDCl₃) δ: 7.40–7.24 (m, 5H), 6.18–6.11 (m, 0.5H), 5.70–5.62 (m, 0.5H), 5.38–5.29 (m, 2H), 4.84–4.77 (m, 0.5H), 4.51 (s, 2H), 4.23–4.19 (m, 0.5H), 3.55–3.43 (m, 5H), 2.16–1.95 (m, 6H), 1.73–1.57 (m, 4H), 1.49–1.12 (m, 7H), 1.11–1.05 (m, 3H), 1.01–0.96 (m, 3H).

To a solution of [(5-[4-(4-methoxy-2,2-dimethylbut-3-en-1-yl)cyclohex-3-en-1-yl]pentyl)oxy)methyl]benzene (855 mg, 2.31 mmol) in THF (50 ml) was added pyridinium *p*-toluenesulfonate (5.8 mg, 0.023 mmol) at room temperature. After stirring at room temperature overnight, the reaction mixture was quenched with saturated NaHCO₃ aqueous solution and concentrated *in vacuo*. The residue was diluted with EtOAc. The organic layer was washed with H₂O and brine, dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude 4-{4-[5-(benzyloxy)pentyl]cyclohex-1-en-1-yl}-3,3-dimethylbutanal (326 mg) as a colorless oil. The crude material was used without purification in the next step.

To a suspension of NaBH₄ (96 mg, 2.53 mmol) in MeOH (5 ml) was added dropwise a solution of crude 4-{4-[5-(benzyloxy)pentyl]cyclohex-1-en-1-yl}-3,3-dimethylbutanal (451 mg) in THF (5 ml) at 0 °C. After stirring at room temperature for 30 min, the reaction mixture was quenched with acetone (5 ml) concentrated *in vacuo*. The residue was diluted with EtOAc. The organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **38** (334 mg, 74% for 2 steps) as a colorless oil. ¹H NMR (CDCl₃) δ: 7.39–7.20 (m, 5H), 5.39–5.31 (m, 1H), 4.51 (s, 2H), 3.78–3.63 (m, 2H), 3.53–3.42 (m, 2H), 2.20–1.90 (m, 4H), 1.88 (s, 2H), 1.75–1.03 (m, 19H), 0.89 (s, 6H).

***cis*-9-[5-(Benzyloxy)pentyl]-4,4-dimethyl-1-oxaspiro[5.5]undecane (39)**

A mixture of compound **38** (334 mg, 0.931 mmol) and Amberlyst 15 (33 mg) in toluene (3 ml) was heated at 60 °C overnight. The reaction mixture was filtered through a celite pad and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **39** (less polar, 139 mg, 42%) and *trans*-isomer of compound **39** (more polar, 120 mg, 36%) as colorless oils.

compound **39**: ¹H NMR (CDCl₃) δ: 7.40–7.21 (m, 5H), 4.56–4.43 (m, 2H), 3.71–3.58 (m, 2H), 3.54–3.40 (m, 2H), 2.01–1.90 (m, 2H), 1.70–1.55 (m, 2H), 1.50–1.40 (m, 2H), 1.39–1.08 (m, 1H), 0.99 (s, 6H).

4-{[5-(*cis*-1-Oxaspiro[5.5]undecan-9-yl)pentyl]oxy}-*N,N*-dimethylbenzamide (10)

To a solution of compound **29** of 9-[5-(benzyloxy)pentyl]-1-oxaspiro[5.5]undecane (705 mg, 2.13 mmol) in MeOH (10 ml) was added 5% Pd-carbon (50% wet). The reaction mixture was hydrogenated (60 psi) at room temperature overnight. The reaction mixture was filtered through a

celite pad and concentrated *in vacuo* to give crude 5-(1-oxaspiro[5.5]undecan-9-yl)pentan-1-ol (666 mg) as a colorless oil. The crude material was used without purification in the next step.

To a solution of crude 5-(1-oxaspiro[5.5]undecan-9-yl)pentan-1-ol (120 mg, 0.50 mmol) in CHCl_3 (2 ml) were added triethylamine (0.105 ml, 0.75 mmol) and methanesulfonyl chloride (0.046 ml, 0.60 mmol) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was diluted with EtOAc (5 ml). The organic layer was washed with saturated NaHCO_3 aqueous solution, H_2O and brine. The organic layer was dried over Na_2SO_4 , filtered, and concentrated *in vacuo* to give crude 5-(1-oxaspiro[5.5]undecan-9-yl)pentyl methanesulfonate (158 mg) as a colorless oil. The crude material was used without purification in the next step.

A suspension of crude 5-(1-oxaspiro[5.5]undecan-9-yl)pentyl methanesulfonate (158 mg), 4-hydroxy-*N,N*-dimethylbenzamide (83 mg, 0.55 mmol) and cesium carbonate (326 mg, 1.00 mmol) in DMF (2 ml) was stirred at 80 °C for 3 h. After cooling to room temperature, the reaction mixture was quenched with H_2O (1 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with 1N NaOH aqueous solution and H_2O , dried over MgSO_4 , filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **10** (132 mg, 68%) as white crystals; mp 95–96 °C. IR (neat) 2920, 1618, 1603, 1491, 1460, 1389, 1248, 1231, 1217, 1172, 1082, 1049, 1042, 1003, 991, 843, 820, 814, 764, 625, 486 cm^{-1} . ^1H NMR (CDCl_3) δ : 7.52–7.36 (m, 2H), 6.99–6.86 (m, 2H), 4.13–3.95 (m, 2H), 3.60 (t, 2H, $J = 5.4$ Hz), 3.05 (s, 6H), 2.04–1.06 (m, 23H). ^{13}C NMR ($\text{DMSO}-d_6$) δ : 170.1, 159.7, 129.2, 128.4, 114.0, 70.5, 67.7, 59.7, 37.1, 36.9, 36.8, 33.7, 28.8, 27.5, 26.3, 26.0, 18.8. Anal. Calcd for $\text{C}_{24}\text{H}_{37}\text{NO}_3$: C, 74.38; H, 9.62; N, 3.61. Found: C, 74.36; H, 9.66; N, 3.54.

4-{{5-(*trans*-1-Oxaspiro[5.5]undecan-9-yl)pentyl}oxy}-*N,N*-dimethylbenzamide (11)

Compound **11** was prepared from compound **30** in a manner similar to that described for compound **10**. White crystals (76%); mp 111–112 °C. IR (neat) 2921, 1605, 1389, 1240, 1219, 1179, 1084, 1032, 853, 764, 625 cm^{-1} . ^1H NMR (CDCl_3) δ : 7.40–7.37 (m, 2H), 6.89–6.87 (m, 2H), 3.97 (t, 2H, $J = 6.6$ Hz), 3.69 (t, 2H, $J = 5.2$ Hz), 3.05 (s, 6H), 1.91–1.20 (m, 21H), 1.02–0.97 (m, 2H). ^{13}C NMR ($\text{DMSO}-d_6$) δ : 170.1, 159.6, 129.2, 128.4, 114.1, 72.1, 67.7, 60.1, 36.4, 35.4, 34.2, 31.7, 28.8, 28.4, 26.6, 26.2, 25.9, 18.8. Anal. Calcd for $\text{C}_{24}\text{H}_{37}\text{NO}_3$: C, 74.38; H, 9.62; N, 3.61. Found: C, 74.23; H, 9.69; N, 3.67.

4-{{5-(*cis*-1-Oxaspiro[4.5]decan-8-yl)pentyl}oxy}-*N,N*-dimethylbenzamide (12)

Compound **12** was prepared from compound **32** in a manner similar to that described for compound **10**. White crystals (78%); mp 87–88 °C. IR (neat) 2936, 2918, 1624, 1607, 1491, 1468, 1458, 1441, 1404, 1389, 1294, 1238, 1221, 1175, 1084, 1069, 1040, 1011, 972, 847, 824, 762, 729, 625, 563, 482 cm^{-1} . ^1H NMR (CDCl_3) δ : 7.39–7.37 (m, 2H), 6.89–6.87 (m, 2H), 3.97 (t, 2H, $J = 6.6$ Hz), 3.80 (t, 2H, $J = 6.7$ Hz), 3.05 (s, 6H), 1.92–1.20 (m, 21H). ^{13}C NMR ($\text{DMSO}-d_6$) δ : 170.1, 159.6, 129.2, 128.4, 114.0, 80.5, 67.7, 66.0, 37.7, 36.6, 36.3, 35.9, 29.2, 28.8, 26.3, 26.0, 25.1. Anal. Calcd for

C₂₃H₃₅NO₃: C, 73.96; H, 9.44; N, 3.75. Found: C, 73.94; H, 9.45; N, 3.75.

4-[[5-(*trans*-1-Oxaspiro[4.5]decan-8-yl)pentyl]oxy]-*N,N*-dimethylbenzamide (13)

Compound **13** was prepared from compound **33** in a manner similar to that described for compound **10**. White crystals (86%); mp 93–94 °C. IR (neat) 2924, 2851, 1605, 1572, 1499, 1443, 1389, 1244, 1180, 1084, 1042, 920, 851, 764, 625 cm⁻¹. ¹H NMR (CDCl₃) δ: 7.40–7.37 (m, 2H), 6.89–6.87 (m, 2H), 3.97 (t, 2H, *J* = 6.5 Hz), 3.81 (t, 2H, *J* = 6.7 Hz), 3.05 (s, 6H), 1.94–1.19 (m, 19H), 0.97–0.91 (m, 2H). ¹³C NMR (DMSO-*d*₆) δ: 170.1, 159.6, 129.1, 128.4, 114.1, 82.6, 67.6, 65.7, 36.9, 36.3, 36.0, 33.9, 30.7, 28.8, 26.5, 25.9, 25.7. Anal. Calcd for C₂₃H₃₅NO₃: C, 73.96; H, 9.44; N, 3.75. Found: C, 74.02; H, 9.49; N, 3.65.

4-[[5-(*cis*-4,4-Dimethyl-1-oxaspiro[5.5]undecan-9-yl)pentyl]oxy]-*N,N*-dimethylbenzamide (14)

Compound **14** was prepared from compound **39** in a manner similar to that described for compound **10**. White crystals (90%); mp 97–100 °C. IR (neat) 2920, 2847, 1618, 1605, 1491, 1476, 1462, 1437, 1408, 1389, 1368, 1298, 1248, 1219, 1171, 1132, 1076, 1065, 1047, 1036, 1026, 1011, 980, 920, 847, 820, 762, 719, 623, 559, 486 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 7.36–7.32 (m, 2H), 6.95–6.91 (m, 2H), 3.97 (t, 2H, *J* = 6.5 Hz), 3.54–3.49 (m, 2H), 2.93 (s, 6H), 1.90–1.82 (m, 2H), 1.74–1.65 (m, 2H), 1.43–1.21 (m, 8H), 1.18–1.07 (m, 9H), 0.94 (s, 6H). ¹³C NMR (DMSO-*d*₆) δ: 170.4, 159.9, 129.5, 128.7, 114.4, 71.2, 68.0, 57.3, 50.1, 38.8, 37.1, 36.9, 36.1, 31.5, 29.1, 28.7, 28.3, 26.6, 26.3. Anal. Calcd for C₂₆H₄₁NO₃: C, 75.14; H, 9.94; N, 3.37. Found: C, 75.06; H, 10.01; N, 3.25

4-[[5-(*cis*-3,3-Dimethyl-1-oxaspiro[4.5]decan-8-yl)pentyl]oxy]-*N,N*-dimethylbenzamide (15).

Compound **15** was prepared from compound **37** in a manner similar to that described for compound **10**. White crystals (77%); mp 84 °C. IR (neat) 2914, 2849, 1605, 1574, 1493, 1474, 1450, 1406, 1391, 1366, 1300, 1348, 1219, 1177, 1080, 1051, 1038, 1022, 1003, 949, 905, 845, 822, 764, 750, 723, 623, 554, 486 cm⁻¹. ¹H NMR (CDCl₃) δ: 7.41–7.37 (m, 2H), 6.91–6.86 (m, 2H), 3.97 (t, 2H, *J* = 6.7 Hz), 3.48 (s, 2H), 3.06 (br s, 6H), 1.85–1.73 (m, 4H), 1.59–1.13 (m, 13H), 1.51 (s, 2H), 1.08 (s, 6H). ¹³C NMR (DMSO-*d*₆) δ: 170.1, 159.6, 129.1, 128.4, 114.0, 81.4, 77.6, 67.7, 53.1, 36.9, 36.6, 35.9, 29.2, 28.8, 27.4, 26.3, 26.0. Anal. Calcd for C₂₅H₃₉NO₃: C, 74.77; H, 9.79; N, 3.49. Found: C, 74.73; H, 9.88; N, 3.55.

8-(3-Methylbut-3-en-1-yl)-1,4-dioxaspiro[4.5]decan-8-ol (40)

To a suspension of trimethylsulfoxonium iodide (5.72 g, 26.0 mmol) in DMSO (20 ml) was added potassium *tert*-butoxide (2.69 g, 24.0 mmol) at room temperature under an argon atmosphere. After stirring at 50 °C for 30 min, a solution of compound **25** (3.12 g, 20.0 mmol) in DMSO (10 ml) was added to the reaction mixture at 50 °C. After stirring at room temperature for 1.5 h, the reaction mixture was poured into ice-water (50 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude 1,7,10-trioxadispiro[2.2.4.2]dodecane (3.42 g) as a colorless oil. The crude material was used without purification in the next step.

To a solution of crude 1,7,10-trioxadispiro[2.2.4.2]dodecane (3.42 g) in diethyl ether (35 ml) was added dropwise 0.25 M methallylmagnesium chloride solution in THF (48.2 ml, 24.1 mmol) at 0 °C under an argon atmosphere. After stirring at room temperature overnight, the reaction mixture was quenched with saturated NH₄Cl aqueous solution (20 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **40** (2.78 g, 62%) as a colorless oil. ¹H NMR (CDCl₃) δ: 4.76–4.69 (m, 2H), 4.02–3.90 (m, 4H), 2.17–2.08 (m, 2H), 1.98–1.85 (m, 2H), 1.78–1.73 (m, 3H), 1.72–1.54 (m, 8H).

2,2-Dimethyl-1-oxaspiro[4.5]decan-8-one (41)

To a solution of compound **40** (2.78 g, 12.3 mmol) in CHCl₃ (25 ml) was added *p*-toluenesulfonic acid monohydrate (117 mg, 0.615 mmol) at 0 °C. After stirring at room temperature overnight, the reaction mixture was quenched with saturated NaHCO₃ aqueous solution (20 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give crude 10,10-dimethyl-1,4,9-trioxadispiro[4.2.4⁸.2⁵]tetradecane (2.47 g) as a colorless oil. The crude material was used without purification in the next step.

A solution of crude 10,10-dimethyl-1,4,9-trioxadispiro[4.2.4⁸.2⁵]tetradecane (2.47 g) in AcOH (20 ml) and H₂O (5.5 ml) was stirred at room temperature for 3 h. The reaction mixture was diluted with H₂O (50 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with saturated NaHCO₃ aqueous solution and H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **41** (1.72 g, 82%) as a colorless oil. ¹H NMR (CDCl₃) δ: 2.78–2.64 (m, 2H), 2.32–2.20 (m, 2H), 2.03–1.76 (m, 8H), 1.30 (s, 6H).

5-(2,2-Dimethyl-1-oxaspiro[4.5]decan-8-yl)pentan-1-ol (42)

To a suspension of (4-carboxybutyl)triphenylphosphonium bromide (8.39 g, 18.9 mmol) and potassium *tert*-butoxide (3.55 g, 31.6 mmol) in THF (25 ml) was added dropwise a solution of compound **41** (1.72 g, 9.46 mmol) in THF (5 ml) at 0 °C. After stirring at room temperature overnight, the reaction mixture was concentrated *in vacuo*. To the resulting precipitate, 1N HCl aqueous solution (40 ml, 40 mmol) was added. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude 5-(2,2-dimethyl-1-oxaspiro[4.5]decan-8-ylidene)pentanoic acid (9.67 g) as a pale yellow oil. The crude material was used without purification in the next step.

To a solution of crude 5-(2,2-dimethyl-1-oxaspiro[4.5]decan-8-ylidene)pentanoic acid (9.67 g) in MeOH (30 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.91 g, 10 mmol) at room temperature. After stirring at room temperature, the reaction mixture was

concentrated *in vacuo*. The residue was diluted with EtOAc. The organic layer was washed with saturated NaHCO₃ aqueous solution and H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give methyl 5-(2,2-dimethyl-1-oxaspiro[4.5]decan-8-ylidene)pentanoate (2.07 g, 68 % for 2 steps) as a colorless oil. ¹H NMR (CDCl₃) δ: 5.11–5.03 (m, 1H), 3.67 (s, 3H), 2.39–2.22 (m, 4H), 2.07–1.96 (m, 4H), 1.91–1.77 (m, 4H), 1.72–1.48 (m, 6H), 1.24 (s, 6H).

To a solution of methyl 5-(2,2-dimethyl-1-oxaspiro[4.5]decan-8-ylidene)pentanoate (2.07 g, 7.38 mmol) in MeOH (20 ml) was added 10% Pd-carbon (50% wet). The reaction mixture was hydrogenated (60 psi) at room temperature overnight. The reaction mixture was filtered through a celite pad and concentrated *in vacuo* to give crude methyl 5-(2,2-dimethyl-1-oxaspiro[4.5]decan-8-yl)pentanoate (2.14 g) as a colorless oil. The crude material was used without purification in the next step.

To a suspension of LiAlH₄ (287 mg, 7.56 mmol) in THF (10 ml) was added dropwise a solution of crude methyl 5-(2,2-dimethyl-1-oxaspiro[4.5]decan-8-yl)pentanoate (2.14 g) in THF (5 ml) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was quenched carefully with H₂O (0.28 ml), 4N NaOH aqueous solution (0.28 ml), and H₂O (0.86 ml) at 0 °C. The reaction mixture was stirred at room temperature for 1h, filtered through a celite pad and concentrated *in vacuo* to give compound **42** (1.79 g, 95% for 2 steps) as a colorless oil. ¹H NMR (CDCl₃) δ: 3.69–3.59 (m, 2H), 1.90–1.45 (m, 10H), 1.41–1.11 (m, 17H).

***cis*-5-(2,2-Dimethyl-1-oxaspiro[4.5]decan-8-yl)pentyl methanesulfonate (43)**

To a solution of compound **43** (489 mg, 1.92 mmol) in CHCl₃ (5 ml) were added triethylamine (0.401 ml, 2.88 mmol) and methanesulfonyl chloride (0.197 ml, 2.50 mmol) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was washed with H₂O (5 ml). The organic layer was dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **43** (less polar, 355 mg, 55%) and *trans*-isomer of compound **43** (252 mg, 38%) as a colorless oil.

compound **43**: ¹H NMR (CDCl₃) δ: 4.24–4.18 (m, 2H), 3.00 (s, 3H), 1.78–1.71 (m, 6H), 1.69–1.62 (m, 2H), 1.54–1.48 (m, 2H), 1.42–1.17 (m, 17H).

4-[[5-(*cis*-2,2-Dimethyl-1-oxaspiro[4.5]decan-8-yl)pentyl]oxy]-*N,N*-dimethylbenzamide (16)

A suspension of compound **43** (351 mg, 1.06 mmol), 4-hydroxy-*N,N*-dimethylbenzamide (209 mg, 1.27 mmol), and cesium carbonate (518 mg, 1.59 mmol) in DMF (5 ml) was stirred at 80 °C for 3 h. After cooling to room temperature, the reaction mixture was quenched with H₂O (10 ml). The aqueous layer was extracted with EtOAc. The organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc) to give compound **16** (333 mg, 78 %) as white crystals; mp 74–75 °C. IR (neat) 2930, 2913, 2849, 1605, 1572, 1495, 1476, 1454, 1439, 1406, 1391, 1373, 1360, 1306, 1252, 1221,

1206, 1175, 1148, 1113, 1084, 1045, 1007, 989, 887, 845, 822, 766, 721, 691, 619, 490 cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$) δ : 7.37–7.31 (m, 2H), 6.96–6.91 (m, 2H), 3.98 (t, 2H, $J = 6.5$ Hz), 2.93 (br s, 6H), 1.77–1.65 (m, 6H), 1.59–1.51 (m, 2H), 1.51–1.43 (m, 2H), 1.43–1.10 (m, 11H), 1.14 (s, 6H). ^{13}C NMR ($\text{DMSO}-d_6$) δ : 170.1, 159.6, 129.2, 128.4, 114.0, 81.2, 80.1, 67.7, 38.0, 37.9, 37.7, 36.5, 36.4, 30.1, 29.0, 28.8, 26.4, 26.0. Anal. Calcd for $\text{C}_{25}\text{H}_{39}\text{NO}_3$: C, 74.77; H, 9.79; N, 3.49. Found: C, 74.74; H, 9.81; N, 3.55.

Ethyl 4-{5-[(tetrahydro-2H-pyran-2-yl)oxy]pentyl}cyclohexane-1-carboxylate (45)

To a suspension of ethyl 4-oxocyclohexanecarboxylate **44** (25.0 g, 147 mmol) and (4-carboxybutyl) triphenylphosphonium bromide (68.4 g, 154 mmol) in DMF (50 ml) was added dropwise a solution of potassium *tert*-butoxide (35.3 g, 315 mmol) in DMF (170 ml) at 0 $^\circ\text{C}$. After stirring at room temperature overnight, H_2O (170 ml) was poured into the reaction mixture. The aqueous layer was extracted with toluene (2×200 ml). To the aqueous layer was added dropwise a 6N HCl aqueous solution (52.5 ml, 315 mmol) at 0 $^\circ\text{C}$. The resulting mixture was extracted with toluene. The combined organic layers were dried over MgSO_4 , filtered, and concentrated *in vacuo* to give crude 5-[4-(ethoxycarbonyl)cyclohexylidene]pentanoic acid (39.2 g) as a pale yellow oil. The crude material was used without purification in the next step.

To a solution of crude 5-[4-(ethoxycarbonyl)cyclohexylidene]pentanoic acid (39.2 g) in THF (150 ml) at room temperature was added 1,1'-carbonyldiimidazole (26.2 g, 162 mmol). After stirring at room temperature for 2 h, to a suspension of NaBH_4 (6.67 g, 176 mmol) in H_2O (150 ml) was added dropwise the reaction mixture at 0 $^\circ\text{C}$. After stirring at room temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was diluted with toluene (150 ml). The organic layer was washed with H_2O , saturated NaHCO_3 aqueous solution, and brine. The organic layer was dried over MgSO_4 , filtered, and concentrated *in vacuo* to give crude ethyl 4-(5-hydroxypentylidene)cyclohexane-1-carboxylate (33.1 g) as a pale yellow oil. The crude material was used without purification in the next step.

To a solution of crude ethyl 4-(5-hydroxypentylidene)cyclohexane-1-carboxylate (33.1 g) in toluene (100 ml) were added 3,4-dihydro-2H-pyran (13.5 ml, 159 mmol) and *p*-toluenesulfonic acid monohydrate (1.30 g, 6.83 mmol) at room temperature. After stirring at room temperature for 1 h, the reaction mixture was washed with saturated NaHCO_3 aqueous solution, dried over MgSO_4 , filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **45** (40.3 g, 84% for 3 steps) as a pale yellow oil. ^1H NMR ($\text{DMSO}-d_6$) δ : 5.14–5.03 (m, 1H), 4.55–4.45 (m, 1H), 4.09–3.98 (m, 2H), 3.77–3.66 (m, 1H), 3.66–3.52 (m, 1H), 3.46–3.36 (m, 1H), 3.36–3.25 (m, 1H), 2.53–2.39 (m, 1H), 2.19–2.08 (m, 1H), 2.08–1.24 (m, 19H), 1.21–1.08 (m, 3H).

Ethyl 4-{5-[(tetrahydro-2H-pyran-2-yl)oxy]pentyl}cyclohexane-1-carboxylate (46)

To a solution of compound **45** (40.3 g, 124 mmol) in EtOAc (100 ml) was added 5% Pd-carbon

(50% wet). The reaction mixture was hydrogenated (60 psi) at room temperature for 5 h. The reaction mixture was filtered through a celite pad and concentrated *in vacuo* to give compound **46** (40.5 g, 99%) as a colorless oil. ¹H NMR (DMSO-*d*₆) δ: 4.56–4.45 (m, 1H), 4.13–3.93 (m, 2H), 3.79–3.65 (m, 1H), 3.65–3.49 (m, 1H), 3.47–3.36 (m, 1H), 3.36–3.24 (m, 1H), 2.25–2.11 (m, 1H), 1.94–1.65 (m, 4H), 1.65–1.35 (m, 8H), 1.35–1.08 (m, 12H), 0.97–0.78 (m, 2H).

Ethyl

***cis*-1-(2-methylallyl)-4-{5-[(tetrahydro-2H-pyran-2-yl)oxy]pentyl}cyclohexane-1-carboxylate (47)**

To a 2 M lithium diisopropyl amide solution in THF (48.0 ml, 96.0 mmol) was added dropwise a solution of compound **46** (30.0 g, 91.9 mmol) in THF (24 ml) at -20 °C under an argon atmosphere. After stirring at -20 °C for 30 min, to the reaction mixture was added 3-chloro-2-methylpropene (12.6 ml, 112 mmol) at -20 °C. The reaction mixture was allowed to warm up to room temperature. After stirring at room temperature for 2.5 h, the reaction mixture was quenched with H₂O (40 ml). The aqueous layer was extracted with toluene. The combined organic layers were dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to afford compound **47** (29.8 g, 85%) as a pale yellow oil. ¹H NMR (DMSO-*d*₆) δ: 4.79–4.74 (m, 1H), 4.62–4.58 (m, 1H), 4.54–4.49 (m, 1H), 4.09–4.01 (m, 2H), 3.75–3.66 (m, 1H), 3.62–3.54 (m, 1H), 3.45–3.36 (m, 1H), 3.33–3.25 (m, 1H), 2.29 (br s, 1H), 2.15 (br s, 1H), 2.11–2.01 (m, 1H), 1.75–1.64 (m, 1H), 1.63–1.54 (m, 5H), 1.54–1.35 (m, 7H), 1.32–1.20 (m, 5H), 1.20–1.04 (m, 8H), 0.95–0.78 (m, 2H).

***(trans*-1-(2-Methylallyl)-4-{5-[(tetrahydro-2H-pyran-2-yl)oxy]pentyl}cyclohexyl)methanol (48)**

To a suspension of LiAlH₄ (3.00 g, 79.1 mmol) in THF (70 ml) was added dropwise a solution of compound **47** (28.0 g, 73.6 mmol) in THF (30 ml) at 40 °C. After stirring at 40 °C for 3 h, the reaction mixture was quenched carefully with H₂O (3 ml), 4N NaOH aqueous solution (3 ml), and H₂O (9 ml) at 0 °C. The reaction mixture was stirred at room temperature for 1h, filtered through a celite pad and concentrated *in vacuo* to give compound **48** (24.9 g, 100%) as a colorless oil. ¹H NMR (DMSO-*d*₆) δ: 4.85–4.77 (m, 1H), 4.67–4.59 (m, 1H), 4.56–4.49 (m, 1H), 4.32–4.25 (m, 1H), 3.76–3.66 (m, 1H), 3.64–3.54 (m, 1H), 3.46–3.35 (m, 1H), 3.30–3.21 (m, 3H), 1.99 (br s, 1H), 1.90 (br s, 1H), 1.78–1.64 (m, 4H), 1.64–1.34 (m, 11H), 1.33–1.21 (m, 4H), 1.21–0.89 (m, 7H).

5-(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)pentan-1-ol (49)

A solution of compound **48** (24.9 g, 73.6 mmol) and *p*-toluenesulfonic acid monohydrate (625 mg, 3.29 mmol) in MeOH (75 ml) was stirred at 70 °C overnight. After cooling to room temperature, the reaction mixture was quenched with 4N NaOH aqueous solution (0.9 ml). The reaction mixture was extracted with toluene. The organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to afford compound **49** (8.62 g, 46%) as a colorless oil. ¹H NMR (DMSO-*d*₆) δ:

4.33–4.26 (m, 1H), 3.50 (s, 2H), 3.39–3.32 (m, 2H), 1.67–1.60 (m, 2H), 1.57–1.54 (m, 2H), 1.49 (s, 2H), 1.43–1.33 (m, 3H), 1.33–1.18 (m, 6H), 1.18–1.06 (m, 8H), 0.95–0.78 (m, 2H).

4-[5-(*cis*-3,3-Dimethyl-2-oxa-spiro[4.5] dec-8-yl)-pentyloxy]-*N,N*-dimethyl-benzamide (17)

To a solution of compound **49** (4.19 g, 16.5 mmol) in EtOAc (22 ml) were added triethylamine (2.60 ml, 18.7 mmol) and methanesulfonyl chloride (1.40 ml, 18.1 mmol) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was diluted with EtOAc (20 ml). The reaction mixture was washed with H₂O and brine. The organic layer was dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The precipitate was washed with *n*-hexane/EtOAc to give crude 5-(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)pentyl methanesulfonate (3.17 g) as a white solid. The crude material was used without purification in the next step.

A suspension of crude 5-(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)pentyl methanesulfonate (100 mg), 4-hydroxy-*N,N*-dimethylbenzamide (55 mg, 0.33 mmol), and cesium carbonate (117 mg, 0.36 mmol) in DMF (0.3 ml) was stirred at 80 °C for 3 h. After cooling to room temperature, the reaction mixture was quenched with H₂O (1 ml). The precipitate was filtered off and recrystallized from *n*-hexane/EtOAc to give compound **17** (78 mg, 38% for 2 steps) as white crystals. mp 108–109 °C. IR (neat) 2911, 2847, 1616, 1603, 1574, 1491, 1474, 1458, 1445, 1406, 1391, 1364, 1298, 1240, 1229, 1211, 1171, 1150, 1082, 1040, 1024, 1009, 837, 818, 762, 725, 619, 552, 486 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 7.37–7.33 (m, 2H), 6.96–6.92 (m, 2H), 3.98 (t, 2H, *J* = 6.4 Hz), 3.52 (s, 2H), 2.94 (s, 6H), 1.74–1.54 (m, 6H), 1.50 (s, 2H), 1.42–1.24 (m, 6H), 1.20–1.12 (m, 9H), 0.93–0.82 (m, 2H). ¹³C NMR (DMSO-*d*₆) δ: 169.8, 159.3, 128.9, 128.1, 113.8, 78.9, 73.0, 67.4, 53.4, 44.1, 36.2, 36.1, 36.0, 30.1, 28.9, 28.5, 26.0, 25.7. Anal. Calcd for C₂₅H₃₉NO₃: C, 74.77; H, 9.79; N, 3.49. Found: C, 74.76; H, 9.99; N, 3.56.

Experiments concerning Chapter 3

Methyl 4-[[tetrahydro-2*H*-pyran-2-yl]oxy]methyl}cyclohexane-1-carboxylate (**67**)

To a solution of 4-(hydroxymethyl)cyclohexane-1-carboxylic acid **66** (25.5 g, 161 mmol) in DMF (130 ml) were added K₂CO₃ (26.7 g, 193 mmol) and iodomethane (12.0 ml, 193 mmol) at room temperature. After stirring at room temperature for 3 h, H₂O (500 ml) was poured into the reaction mixture. The aqueous layer was extracted with EtOAc. The organic layer was washed with saturated NaHCO₃ aqueous solution, H₂O and brine, dried over Na₂SO₄ and concentrated *in vacuo* to give crude methyl 4-(hydroxymethyl)cyclohexane-1-carboxylate (35.0 g) as a pale yellow oil. The crude material was used without purification in the next step.

To a solution of crude methyl 4-(hydroxymethyl)cyclohexane-1-carboxylate (35.0 g) in toluene (150 ml) were added 3,4-dihydro-2*H*-pyran (16.2 ml, 177 mmol) and (-)-10-camphorsulfonic acid (374 mg, 1.61 mmol) at room temperature. After stirring at room temperature for 3 h, H₂O (50 ml) was poured into the reaction mixture. The aqueous layer was extracted with toluene. The organic layer was washed with saturated NaHCO₃ aqueous solution, H₂O and brine, dried over Na₂SO₄ and concentrated *in vacuo* to give crude compound **67** (43.1 g) as a colorless oil. The crude material was used without purification in the next step.

cis-3,3-Dimethyl-8-[[tetrahydro-2*H*-pyran-2-yl]oxy]methyl}-2-oxaspiro[4.5]decan-1-one (**68**)

To a 1 M lithium bis(trimethylsilyl)amide in THF solution (290 ml, 290 mmol) was added dropwise a solution of crude compound **67** (43.1 g) and isobutylene oxide (125 ml, 1.41 mol) in THF (50 ml) at 0 °C under an argon atmosphere. After stirring at room temperature for 1 h, saturated NH₄Cl aqueous solution (500 ml) was poured into the reaction mixture. The aqueous layer was extracted with EtOAc. The organic layer was washed with saturated NaHCO₃ aqueous solution, H₂O and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) and recrystallized from *n*-hexane (120 ml) to give compound **68** (29.9 g, 62% for 3 steps) as a white solid. ¹H NMR (CDCl₃) δ: 4.55–4.57 (m, 1H), 3.88–3.83 (m, 1H), 3.71–3.67 (m, 1H), 3.53–3.47 (m, 1H), 3.32–3.28 (m, 1H), 2.03–1.99 (m, 2H), 1.99 (s, 2H), 1.86–1.77 (m, 1H), 1.77–1.66 (m, 4H), 1.66–1.47 (m, 6H), 1.43 (s, 6H), 1.43–1.38 (m, 2H).

cis-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl-methanol (**69**)

To a suspension of LiAlH₄ (3.83 g, 101 mmol) in THF (40 ml) was added dropwise a solution of compound **68** (29.9 g, 101 mmol) in THF (100 ml) at 0 °C under an argon atmosphere. After stirring at 0 °C for 15 min, the reaction mixture was quenched with H₂O (3.8 ml), 4*N* NaOH aqueous solution (3.8 ml), H₂O (11.4 ml), and diluted with diethyl ether (150 ml). The reaction mixture was stirred at room temperature for 30 min, filtered through a celite pad and concentrated *in vacuo*. The precipitate was washed with *n*-hexane to give 1-(1-(hydroxymethyl)-4-[[tetrahydro-2*H*-pyran-2-yl]oxy]methyl}cyclohexyl)-2-methylpropan-2-ol

(24.1 g, 79%) as a white solid. ^1H NMR (CDCl_3) δ : 4.55–4.57 (m, 1H), 3.88–3.82 (m, 1H), 3.67 (s, 2H), 3.57–3.53 (m, 1H), 3.52–3.46 (m, 1H), 3.22–3.18 (m, 1H), 1.91–1.88 (m, 2H), 1.85–1.78 (m, 1H), 1.74–1.67 (m, 1H), 1.62–1.51 (m, 7H), 1.48 (s, 2H), 1.33 (s, 6H), 1.20–1.08 (m, 4H).

To a solution of compound **69** (24.1 g, 80.2 mmol) in MeOH (250 ml) was added *p*-toluenesulfonic acid monohydrate (763 mg, 4.01 mmol) at room temperature. After stirring at 85 °C for 4 h, H_2O (100 ml) was poured into the reaction mixture. The aqueous layer was extracted with EtOAc. The organic layer was dried over MgSO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **69** (13.8 g, 87%) as a colorless oil. ^1H NMR (CDCl_3) δ : 3.65 (s, 2H), 3.46–3.44 (m, 2H), 1.82–1.76 (m, 2H), 1.72–1.66 (m, 2H), 1.58 (s, 2H), 1.50–1.41 (m, 2H), 1.41–1.31 (m, 2H), 1.26 (s, 6H), 1.03–0.93 (m, 2H).

***cis*-3,3-Dimethyl-2-oxaspiro[4.5]decane-8-carboxylic acid (70)**

To a solution of compound **69** (5.00 g, 25.2 mmol) in CHCl_3 (50 ml) was added Dess-Martin reagent (11.7 g, 27.7 mmol) at 0 °C. After stirring at 0 °C for 1 h, saturated $\text{Na}_2\text{S}_2\text{O}_3$ aqueous solution (50 ml) was poured into the reaction mixture. After stirring at room temperature for 15 min, the precipitate was filtered. The aqueous layer was extracted with CHCl_3 . The organic layer was washed with saturated NaHCO_3 aqueous solution and brine, dried over MgSO_4 and concentrated *in vacuo* to give crude 3,3-dimethyl-2-oxaspiro[4.5]decane-8-carbaldehyde (4.67 g) as a pale yellow oil. The crude material was used without purification in the next step.

To a solution of crude 3,3-dimethyl-2-oxaspiro[4.5]decane-8-carbaldehyde (4.67 g) in *tert*-butanol (45 ml) and H_2O (15 ml) were added 2-methyl-2-butene (10.1 ml, 95.2 mmol), NaH_2PO_4 (2.85 g, 23.8 mmol) and NaClO_2 (2.69 g, 23.8 mmol) at room temperature. After stirring at room temperature for 1 h, saturated $\text{Na}_2\text{S}_2\text{O}_3$ aqueous solution (50 ml) and 1N NaOH aqueous solution (50 ml) were poured into the reaction mixture at 0 °C. The aqueous layer was washed with diethyl ether. The aqueous layer was neutralized with 2N HCl aqueous solution and extracted with EtOAc. The organic layer was dried over MgSO_4 and concentrated *in vacuo* to give compound **70** (2.91 g, 54% for 2 steps) as a white solid. ^1H NMR ($\text{DMSO}-d_6$) δ : 12.01 (br s, 1H), 3.52 (s, 2H), 2.19–2.12 (m, 1H), 1.75–1.70 (m, 2H), 1.66–1.63 (m, 2H), 1.52 (s, 2H), 1.40–1.29 (m, 4H), 1.16 (s, 6H).

***tert*-Butyl (*cis*-3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)carbamate (71)**

To a solution of compound **70** (1.80 g, 8.49 mmol) in dioxane (18 ml) were added diphenylphosphoryl azide (2.20 ml, 10.2 mmol) and triethylamine (1.42 ml, 10.2 mmol) at room temperature. After stirring at room temperature for 1.5 h, the mixture was heated at 90 °C and stirred for 3 h. After cooling at room temperature, potassium *tert*-butoxide (2.89 g, 25.5 mmol) was added to the reaction mixture. After stirring at room temperature for 2 h, the reaction mixture was quenched with H_2O (5 ml) at 0 °C. The aqueous layer was extracted with EtOAc. The organic layer was

washed with H₂O and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **71** (2.32 g, 97%) as a white solid. ¹H NMR (CDCl₃) δ: 4.39 (br s, 1H), 3.64 (s, 2H), 3.41 (br s, 1H), 1.90–1.83 (m, 2H), 1.76–1.70 (m, 2H), 1.62–1.55 (m, 4H), 1.43 (s, 9H), 1.25 (s, 6H), 1.21–1.10 (m, 2H).

***cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-amine hydrochloride (72)**

To a solution of compound **71** (2.30 g, 8.13 mmol) in EtOAc (8 ml) was added 4N HCl in EtOAc (4.10 ml, 16.3 mmol) at room temperature. After stirring at room temperature overnight, the reaction mixture was cooled at 0 °C. The resulting precipitate was washed with EtOAc to give compound **72** (1.17 g, 66%) as a white solid. ¹H NMR (DMSO-*d*₆) δ: 7.85 (br s, 3H), 3.53 (s, 2H), 2.98–2.91 (m, 1H), 1.82–1.77 (m, 2H), 1.72–1.68 (m, 2H), 1.52 (s, 2H), 1.41–1.28 (m, 4H), 1.17 (s, 6H).

***cis*-N,3,3-Trimethyl-2-oxaspiro[4.5]decan-8-amine hydrochloride (73).**

A suspension of LiAlH₄ (57 mg, 1.52 mmol) and compound **72** (216 mg, 0.76 mmol) in THF (2 ml) was stirred under reflux for 1 h. After cooling to 0 °C, the reaction mixture was quenched with H₂O (0.057 ml), 4N NaOH aqueous solution (0.057 ml), H₂O (0.171 ml), and diluted with THF (5 ml). The reaction mixture was stirred at room temperature for 30 min, filtered through a celite pad and concentrated *in vacuo* to give crude *N*,3,3-trimethyl-2-oxaspiro[4.5]decan-8-amine (122 mg) as a pale yellow oil. The crude material was used without purification in the next step.

To a solution of crude *N*,3,3-trimethyl-2-oxaspiro[4.5]decan-8-amine (122 mg) in EtOAc (1.5 ml) was added 4N HCl in EtOAc (1.24 ml, 0.310 mmol) at room temperature. After stirring at room temperature for 1 h, the resulting precipitate was washed with EtOAc to give compound **73** (104 mg, 47% for 2 steps) as a white solid. ¹H NMR (DMSO-*d*₆) δ: 8.65 (br s, 2H), 3.54 (s, 2H), 2.95–2.84 (m, 1H), 2.55–2.44 (m, 3H), 1.96–1.83 (m, 2H), 1.79–1.69 (m, 2H), 1.52 (s, 2H), 1.41–1.27 (m, 4H), 1.17 (s, 6H).

***cis*-3,3-Dimethyl-8-[(tetrahydro-2*H*-pyran-2-yl)oxy]-2-oxaspiro[4.5]decan-1-one (75)**

To a solution of ethyl 4-hydroxycyclohexane-1-carboxylate **74** (50.0 g, 290 mmol) in toluene (250 ml) were added 3,4-dihydro-2*H*-pyran (29.1 ml, 319 mmol) and (-)-10-camphorsulfonic acid (337 mg, 1.45 mmol) at room temperature. After stirring at room temperature for 6 h, H₂O (50 ml) was poured into the reaction mixture. The aqueous layer was extracted with toluene. The organic layer was washed with saturated NaHCO₃ aqueous solution, H₂O and brine, dried over Na₂SO₄ and concentrated *in vacuo* to give crude ethyl 4-[(tetrahydro-2*H*-pyran-2-yl)oxy]cyclohexane-1-carboxylate (89.0 g) as a colorless oil. The crude material was used without purification in the next step.

To a 1 M lithium bis(trimethylsilyl)amide in THF solution (523 ml, 523 mmol) was added dropwise a solution of crude ethyl 4-[(tetrahydro-2*H*-pyran-2-yl)oxy]cyclohexane-1-carboxylate (89.0 g) and isobutylene oxide (222 ml, 2.49 mol) in THF (200 ml) at 0 °C under an argon atmosphere. After stirring at room temperature for 2 h, saturated NH₄Cl aqueous solution (500 ml) was poured into the

reaction mixture. The aqueous layer was extracted with EtOAc. The organic layer was washed with saturated NaHCO₃ aqueous solution, H₂O and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was recrystallized from diisopropyl ether (150 ml) to give compound **75** (56.5 g, 69% for 2 steps) as a white solid. ¹H NMR (CDCl₃) δ: 4.8–4.6 (m, 1H), 3.9–3.8 (m, 2H), 3.6–3.4 (m, 1H), 2.3–1.8 (m, 5H), 2.0 (s, 2H), 1.7–1.6 (m, 1H), 1.6–1.3 (m, 14H).

***cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-ol (76)**

To a suspension of LiAlH₄ (7.59 g, 200 mmol) in THF (75 ml) was added dropwise a solution of compound **75** (56.5 g, 200 mmol) in THF (900 ml) at 0 °C under an argon atmosphere. After stirring at 0 °C for 15 min, the reaction mixture was quenched with H₂O (7.6 ml), 4N NaOH aqueous solution (7.6 ml), H₂O (22.8 ml), and diluted with diethyl ether. The reaction mixture was stirred at room temperature for 30 min, filtered through a celite pad and concentrated *in vacuo*. The precipitate was washed with diisopropyl ether/*n*-hexane to give crude 1-{1-(hydroxymethyl)-4-[(tetrahydro-2*H*-pyran-2-yl)oxy]cyclohexyl}-2-methylpropan-2-ol (50.9 g) as a white solid. The crude material was used without purification in the next step.

To a solution of crude 1-{1-(hydroxymethyl)-4-[(tetrahydro-2*H*-pyran-2-yl)oxy]cyclohexyl}-2-methylpropan-2-ol (50.9 g) in MeOH (500 ml) was added *p*-toluenesulfonic acid monohydrate (1.69 g, 8.89 mmol) at room temperature. After stirring at 85 °C for 3 h, H₂O (100 ml) was poured into the reaction mixture. The aqueous layer was extracted with EtOAc. The organic layer was dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **76** (30.0 g, 81% for 2 steps) as a colorless oil. ¹H NMR (CDCl₃) δ: 3.7–3.6 (m, 3H), 1.9–1.7 (m, 4H), 1.6 (s, 2H), 1.5–1.3 (m, 4H), 1.3 (s, 6H).

Ethyl 2-(*cis*-3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)thiazole-4-carboxylate (78)

To a solution of compound **70** (400 mg, 1.88 mmol) in acetonitrile (5 ml) were added NH₄Cl (504 mg, 9.42 mmol), triethylamine (1.31 ml, 9.42 mmol), 1-hydroxybenzotriazole monohydrate (346 mg, 2.26 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (433 mg, 2.66 mmol) at room temperature. After stirring at room temperature, the reaction mixture was concentrated *in vacuo*. The residue was diluted with H₂O. The aqueous layer was extracted with CHCl₃. The organic layer was dried over MgSO₄ and concentrated *in vacuo* to give crude 3,3-dimethyl-2-oxaspiro[4.5]decan-8-carboxamide **77** (384 mg) as a white solid. The crude material was used without purification in the next step.

A suspension of crude 3,3-dimethyl-2-oxaspiro[4.5]decan-8-carboxamide **77** (384 mg) and Lawesson's reagent (512 mg, 1.26 mmol) in THF (5 ml) was stirred under reflux for 2h. After cooling to 0 °C, the reaction mixture was quenched with saturated NaHCO₃ aqueous solution. The aqueous layer was extracted with EtOAc. The organic layer was washed with H₂O, dried over MgSO₄ and concentrated *in vacuo*. The residue was diluted with *n*-hexane and EtOAc, filtered

through a celite pad and concentrated *in vacuo* to give crude 3,3-dimethyl-2-oxaspiro[4.5]decane-8-carbothioamide (168 mg) as a white solid. The crude material was used without purification in the next step.

A solution of crude 3,3-dimethyl-2-oxaspiro[4.5]decane-8-carbothioamide (168 mg) and ethyl bromopyruvate (173 mg, 0.80 mmol) in EtOH (2 ml) was stirred under reflux for 2 h. After cooling to room temperature, the reaction mixture was quenched with saturated NaHCO₃ aqueous solution (2 ml). The aqueous layer was extracted with EtOAc. The organic layer was washed with H₂O, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **78** (175 mg, 31% for 3 steps). ¹H NMR (CDCl₃) δ: 8.0 (s, 1H), 4.4–4.3 (m, 2H), 3.7 (s, 2H), 3.1–3.0 (m, 1H), 2.1–2.1 (m, 2H), 1.9–1.8 (m, 2H), 1.6 (s, 2H), 1.6–1.5 (m, 4H), 1.4–1.3 (m, 3H), 1.3 (s, 6H).

4-[[4-(1*H*-Tetrazol-1-yl)phenoxy]methyl]-2-(*cis*-3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)thiazole (50)

To a suspension of LiAlH₄ (20 mg, 0.54 mmol) in THF (2 ml) was added dropwise a solution of compound **78** (173 mg, 0.54 mmol) in THF (2 ml) at 0 °C under an argon atmosphere. After stirring at 0 °C for 30 min, the reaction mixture was quenched with H₂O (0.02 ml), 4N NaOH aqueous solution (0.02 ml), H₂O (0.06 ml), and diluted with EtOAc (5 ml). The reaction mixture was stirred at room temperature for 30 min, filtered through a celite pad and concentrated *in vacuo* to give crude (2-(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)thiazol-4-yl)methanol (142 mg) as a pale yellow solid. The crude material was used without purification in the next step.

To a solution of crude (2-(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)thiazol-4-yl)methanol (70 mg), 4-(1*H*-tetrazol-1-yl)phenol (48 mg, 0.30 mmol) and triphenylphosphine (130 mg, 0.50 mmol) in toluene (1 ml) was added *N,N,N,N*-tetramethylazodicarboxamide (86 mg, 0.50 mmol) at room temperature. After stirring at room temperature for 2 h, the reaction mixture was quenched with H₂O. The aqueous layer was extracted with EtOAc. The organic layer was washed with H₂O, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **50** (81 mg, 76% for 2 steps) as white crystals; mp 137–139 °C. IR (neat) 1520, 1456, 1204, 1277, 1260, 1233, 1202, 1169, 1092, 1036, 1016, 995, 870, 862, 833, 820, 746, 731, 710, 527 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 9.98 (s, 1H), 7.84–7.80 (m, 2H), 7.63 (s, 1H), 7.31–7.27 (m, 2H), 5.22 (s, 2H), 3.60 (s, 2H), 3.01–2.91 (m, 1H), 2.03–1.93 (m, 2H), 1.81–1.71 (m, 2H), 1.57 (s, 2H), 1.54–1.45 (m, 4H), 1.19 (s, 6H). ¹³C NMR (DMSO-*d*₆) δ: 175.6, 158.8, 150.4, 142.1, 127.0, 122.8, 79.0, 73.0, 65.7, 53.1, 43.6, 40.6, 35.3, 30.4, 28.9. Anal. Calcd for C₂₂H₂₇N₅O₂S: C, 62.09; H, 6.40; N, 16.46. Found: C, 62.17; H, 6.38; N, 16.11.

5-{*rac*-1-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]ethyl}-3-[3-fluoro-4-(methylsulfonyl)phenyl]-1,2,4-oxadiazole (51)

To a suspension of NaH (60% oil dispersion, 104 mg, 4.34 mmol) in dioxane (1 ml) was added

dropwise a solution of compound **76** (200 mg, 1.09 mmol) in dioxane (1 ml) at room temperature under a nitrogen atmosphere. After stirring at room temperature for 1 h, a solution of 2-*rac*-bromopropionic acid (167 mg, 1.09 mmol) in dioxane (1 ml) was added dropwise at room temperature. After stirring at room temperature overnight, the reaction mixture was quenched with H₂O (1 ml). The aqueous layer was washed with toluene. The aqueous layer was neutralized with 2N HCl aqueous solution and extracted with EtOAc. The organic layer was dried over Na₂SO₄ and concentrated *in vacuo* to give crude 2-[(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]propanoic acid **79** (174 mg) as a pale yellow oil. The crude material was used without purification in the next step.

To a solution of crude 2-[(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]propanoic acid **79** (174 mg) in DMF (1.5 ml) were added 1-hydroxybenzotriazole monohydrate (156 mg, 1.02 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (195 mg, 1.02 mmol), *i*-Pr₂NEt (0.36 ml, 2.04 mmol) and 3-fluoro-*N*-hydroxy-4-methylsulfanyl-benzamidine **80** (136 mg, 0.68 mmol) at room temperature under a nitrogen atmosphere. After stirring at room temperature for 2 h, EtOAc (3 ml) and 5% KHSO₄ aqueous solution (1 ml) were poured into the reaction mixture. The organic layer was washed with saturated NaHCO₃ aqueous solution and brine, dried over Na₂SO₄ and concentrated *in vacuo*. The resulting residue was dissolved with toluene (2.5 ml). After stirring under reflux for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give crude 5-{1-[(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]ethyl}-3-[3-fluoro-4-(methylthio)phenyl]-1,2,4-oxadiazole (165 mg) as a colorless oil. The crude material was used without purification in the next step.

To a solution of crude 5-{1-[(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]ethyl}-3-[3-fluoro-4-(methylthio)phenyl]-1,2,4-oxadiazole (165 mg) in CHCl₃ (2.0 ml) was added portionwise *m*-chloroperoxybenzoic acid (75 wt%, 149 mg, 0.862 mmol) at 0 °C. After stirring at 0 °C for 2 h, the reaction mixture was quenched with 10% Na₂S₂O₃ aqueous solution. The aqueous layer was extracted with CHCl₃. The combined organic layer was washed saturated NaHCO₃ aqueous solution and H₂O, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **51** (168 mg, 34% for 4 steps) as white crystals; mp 86–87 °C. IR (neat) 1433, 1315, 1292, 1152, 1134, 1103, 1067, 1055, 1040, 947, 926, 887, 878, 849, 772, 739, 590, 569, 525, 503, 480, 459, 444, 426 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 8.12–8.04 (m, 3H), 5.10 (q, 1H, *J* = 6.5 Hz), 3.55–3.49 (m, 1H), 3.52 (s, 2H), 3.41 (s, 3H), 1.86–1.79 (m, 1H), 1.72–1.59 (m, 3H), 1.55 (d, 3H, *J* = 6.5 Hz), 1.52 (s, 2H), 1.40–1.28 (m, 4H), 1.16 (s, 3H), 1.15 (s, 3H). ¹³C NMR (DMSO-*d*₆) δ: 181.3, 165.7, 160.1, 157.5, 133.2, 130.6, 130.5, 123.8, 115.6, 79.4, 75.6, 74.2, 66.6, 43.6, 43.5, 32.9, 29.3, 28.9, 28.5, 19.8. Anal. Calcd for C₂₂H₂₉FN₂O₅S: C, 58.39; H, 6.46; N, 6.19. Found: C, 58.40; H, 6.48; N, 6.13.

4-Chloro-6-[(*cis*-3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]-5-methylpyrimidine (**81**)

To a suspension of NaH (60% oil dispersion, 187 mg, 4.67 mmol) in THF (5 ml) was added dropwise a solution of compound **76** (718 mg, 3.79 mmol) in THF (3.0 ml) at room temperature under a nitrogen atmosphere. After stirring at room temperature for 30 min, to the reaction mixture was added 4,6-dichloro-5-methylpyrimidine (677 mg, 4.67 mmol) at room temperature. After stirring at room temperature overnight, the reaction mixture was quenched with H₂O (3 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **81** (1.09 g, 93%) as a white solid. ¹H NMR (CDCl₃) δ: 8.37 (s, 1H), 5.29–5.05 (m, 1H), 3.69 (s, 2H), 2.21 (s, 3H), 1.98–1.87 (m, 2H), 1.86–1.75 (m, 2H), 1.71–1.46 (m, 6H), 1.28 (s, 6H).

4-Chloro-6-[(*cis*-3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]-5-ethylpyrimidine (**82**)

Compound **82** was prepared from compound **76** and 4,6-dichloro-5-ethylpyrimidine in a manner similar to that described for compound **81**. A white solid (83%).

4-Chloro-6-[(*cis*-3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]-5-isopropylpyrimidine (**83**)

Compound **83** was prepared from compound **76** and 4,6-dichloro-5-isopropylpyrimidine in a manner similar to that described for compound **81**. A white solid (89%).

6-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]-*N*-[2-fluoro-4-(methylsulfonyl)phenyl]-5-methylpyrimidin-4-amine (**52**)

To a solution of compound **81** (200 mg, 0.64 mmol), 2-fluoro-4-(methylsulfonyl)aniline **84** (182 mg, 0.96 mmol), sodium *tert*-butoxide (147 mg, 1.54 mmol) and 1,1'-bis(di-*tert*-butylphosphino)ferrocene (30.4 mg, 0.06 mmol) in dioxane (4 ml) was added Pd(OAc)₂ (7.2 mg, 0.03 mmol) at room temperature under an argon atmosphere. After stirring at 100 °C for 3 h, the reaction mixture was quenched with H₂O. The aqueous layer was extracted with CHCl₃. The organic layer was washed with H₂O and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **52** (77 mg, 26%) as white crystals; mp 198 °C. IR (neat) 1618, 1605, 1566, 1514, 1483, 1472, 1456, 1429, 1418, 1377, 1366, 1337, 1327, 1294, 1285, 1246, 1192, 1140, 1115, 1101, 1076, 1053, 1013, 968, 895, 870, 827, 781, 770, 762, 691, 600, 550, 534, 509, 494, 488, 446 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 8.56 (s, 1H), 8.17 (s, 1H), 7.82–7.76 (m, 2H), 7.72–7.69 (m, 1H), 5.08–5.04 (m, 1H), 3.57 (s, 2H), 3.24 (s, 3H), 2.06 (s, 3H), 1.88–1.80 (m, 2H), 1.73–1.66 (m, 2H), 1.58 (s, 2H), 1.57–1.51 (m, 2H), 1.48–1.42 (m, 2H), 1.18 (s, 6H). ¹³C NMR (DMSO-*d*₆) δ: 166.1, 159.0, 155.5, 153.9, 153.0, 135.9, 135.8, 133.3, 133.2, 125.3, 123.2, 114.7, 114.5, 99.1, 79.5, 74.7, 72.2, 43.6, 43.4, 32.6, 28.9, 28.3, 8.3. Anal. Calcd for C₂₃H₃₀FN₃O₄S: C, 59.59; H, 6.52; N, 9.06. Found: C, 59.57; H, 6.54; N, 9.11.

4-({6-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]-5-methylpyrimidin-4-yl}amino)-3-fluor

***o*-*N,N*-dimethylbenzamide (53)**

Compound **53** was prepared from compound **81** and 4-amino-3-fluoro-*N,N*-dimethylbenzamide **85** in a manner similar to that described for compound **52**. White crystals (57%); mp 206 °C. IR (neat) 1630, 1616, 1574, 1489, 1445, 1429, 1414, 1395, 1366, 1290, 1273, 1153, 1130, 1109, 1080, 1049, 1028, 862, 837, 783, 621, 586 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 8.31 (s, 1H), 8.12 (s, 1H), 7.56–7.52 (m, 1H), 7.31–7.28 (m, 1H), 7.23–7.20 (m, 1H), 5.08–5.04 (m, 1H), 3.58 (s, 2H), 2.97 (s, 6H), 2.05 (s, 3H), 1.89–1.81 (m, 2H), 1.75–1.67 (m, 2H), 1.61–1.51 (m, 4H), 1.50–1.42 (m, 2H), 1.19 (s, 6H). ¹³C NMR (DMSO-*d*₆) δ: 168.6, 165.9, 159.8, 156.5, 154.1, 154.0, 133.3, 133.2, 129.0, 128.9, 123.1, 114.8, 114.6, 97.7, 79.7, 79.4, 79.0, 78.7, 74.9, 72.1, 50.7, 43.8, 32.8, 29.0, 28.6, 8.3. Anal. Calcd for C₂₅H₃₃FN₄O₃: C, 65.77; H, 7.29; N, 12.27. Found: C, 65.62; H, 7.34; N, 12.24.

4-[(3,3-*cis*-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]-6-[2-fluoro-4-(methylsulfonyl)phenoxy]-5-methylpyrimidine (54)

A suspension of 4-chloro-6-[(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]-5-methylpyrimidine **81** (100 mg, 0.32 mmol), 2-fluoro-4-(methylsulfonyl)phenol **86** (61 mg, 0.32 mmol), K₂CO₃ (53 mg, 0.38 mmol) and tetrabutylammonium iodide (77 mg, 0.21 mmol) in DMSO (0.5 ml) was heated at 130 °C under an argon atmosphere overnight. After cooling to room temperature, the reaction mixture was diluted with EtOAc (5 ml). The organic layer was washed with H₂O and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **54** (106 mg, 67%) as white crystals; mp 160 °C. IR (neat) 1591, 1572, 1499, 1449, 1402, 1366, 1315, 1283, 1271, 1240, 1196, 1146, 1113, 1070, 1053, 1020, 968, 930, 905, 885, 874, 843, 783, 773, 758, 677, 600, 536, 517, 492, 457, 446 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 8.27 (s, 1H), 8.00–7.97 (m, 1H), 7.85–7.82 (m, 1H), 7.68–7.64 (m, 1H), 5.17–5.11 (m, 1H), 3.59 (s, 2H), 3.32 (s, 3H), 2.14 (s, 3H), 1.92–1.85 (m, 2H), 1.76–1.69 (m, 2H), 1.66–1.57 (m, 4H), 1.52–1.44 (m, 2H), 1.19 (s, 6H). ¹³C NMR (DMSO-*d*₆) δ: 168.1, 166.0, 154.7, 154.1, 152.3, 144.2, 144.1, 138.9, 125.1, 124.3, 124.2, 116.0, 115.8, 101.3, 79.5, 74.7, 73.6, 43.6, 43.2, 32.5, 28.9, 28.2, 7.3. Anal. Calcd for C₂₃H₂₉FN₂O₅S: C, 59.47; H, 6.29; N, 6.03. Found: C, 59.50; H, 6.29; N, 6.08.

6-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]-5-methyl-*N*-[4-(methylsulfonyl)phenyl]pyrimidin-4-amine (55)

Compound **55** was prepared from compounds **81** and 4-(methylsulfonyl)aniline **86** in a manner similar to that described for compound **52**. White crystals (12%); IR (neat) 1614, 1564, 1506, 1449, 1435, 1416, 1337, 1323, 1296, 1275, 1254, 1231, 1146, 1105, 1092, 1045, 1022, 1013, 959, 930, 881, 841, 789, 770, 700, 619, 532, 498, 486, 451 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 8.75 (s, 1H), 8.28 (s, 1H), 7.91–7.89 (m, 2H), 7.81–7.79 (m, 2H), 5.10–5.05 (m, 1H), 3.57 (s, 2H), 3.14 (s, 3H), 2.08 (s, 3H), 1.88–1.81 (m, 2H), 1.73–1.66 (m, 2H), 1.60–1.51 (m, 4H), 1.49–1.41 (m, 2H), 1.18 (s, 6H). HRMS (ESI) Calcd for C₂₃H₃₂N₃O₄S (M+H)⁺ *m/z* 446.2108, Found *m/z* 446.2103.

6-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]-5-ethyl-*N*-[2-fluoro-4-(methylsulfonyl)phenyl]pyrimidin-4-amine (60)

Compound **60** was prepared from compound **82** and 2-fluoro-4-(methylsulfonyl)aniline **84** in a manner similar to that described for compound **52**. White crystals (22 %); mp 176–177 °C. IR (neat) 1616, 1603, 1560, 1514, 1439, 1420, 1368, 1339, 1310, 1290, 1273, 1256, 1233, 1192, 1144, 1107, 1067, 1047, 1024, 959, 914, 901, 876, 831, 814, 800, 775, 758, 687, 598, 532, 505, 490, 449 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 8.63 (s, 1H), 8.15 (s, 1H), 7.78–7.69 (m, 3H), 5.12–5.06 (m, 1H), 3.57 (s, 2H), 3.26 (s, 3H), 2.62 (q, 2H, *J* = 7.5 Hz), 1.87–1.81 (m, 2H), 1.73–1.67 (m, 2H), 1.63–1.60 (m, 2H), 1.60 (s, 2H), 1.50–1.44 (m, 2H), 1.18 (s, 6H), 1.09 (t, 3H, *J* = 7.5 Hz). ¹³C NMR (DMSO-*d*₆) δ: 165.9, 158.3, 155.9, 154.2, 153.4, 136.2, 133.2, 126.0, 123.1, 114.6, 104.7, 79.6, 75.0, 72.0, 43.6, 43.4, 32.5, 28.9, 28.3, 15.5, 12.3. Anal. Calcd for C₂₄H₃₂FN₃O₄S: C, 60.36; H, 6.75; N, 8.80. Found: C, 60.40; H, 6.82; N, 8.75.

6-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]-*N*-[2-fluoro-4-(methylsulfonyl)phenyl]-5-isopropylpyrimidin-4-amine (61)

Compound **61** was prepared from compound **83** and 2-fluoro-4-(methylsulfonyl)aniline **84** in a manner similar to that described for compound **52**. White crystals (28 %); mp 187–188 °C. IR (neat) 1560, 1504, 1483, 1437, 1404, 1364, 1298, 1275, 1256, 1213, 1138, 1125, 1080, 1067, 1043, 966, 937, 910 885, 868, 843, 810, 770, 692, 658, 598, 546, 523, 498, 459, 407 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 8.72 (s, 1H), 8.12 (s, 1H), 7.76–7.73 (m, 1H), 7.71–7.63 (m, 2H), 5.17–5.13 (m, 1H), 3.56 (s, 2H), 3.27 (q, 1H, *J* = 7.0 Hz), 3.25 (s, 3H), 1.88–1.81 (m, 2H), 1.74–1.61 (m, 4H), 1.61 (s, 2H), 1.52–1.46 (m, 2H), 1.29 (d, 6H, *J* = 7.0 Hz), 1.20 (s, 6H). ¹³C NMR (DMSO-*d*₆) δ: 166.5, 158.1, 153.9, 153.3, 135.9, 133.8, 125.5, 123.1, 114.6, 108.5, 79.7, 75.3, 71.9, 43.6, 43.4, 32.4, 28.9, 28.1, 23.8, 19.9. Anal. Calcd for C₂₅H₃₄FN₃O₄S: C, 61.08; H, 6.97; N, 8.55. Found: C, 61.17; H, 7.05; N, 8.51.

6-Chloro-*N*-[2-fluoro-4-(methylsulfonyl)phenyl]-*N*,5-dimethylpyrimidin-4-amine (89)

To a solution of 4,6-dichloro-5-methylpyrimidine (173 mg, 1.06 mmol), 2-fluoro-*N*-methyl-4-(methylsulfonyl)aniline **88** (108 mg, 0.53 mmol), sodium *tert*-butoxide (127 mg, 1.33 mmol) and 1,1'-bis(di-*tert*-butylphosphino)ferrocene (25.1 mg, 0.05 mmol) in dioxane (2 ml) was added Pd(OAc)₂ (5.9 mg, 0.03 mmol) at room temperature under an argon atmosphere. After stirring at 120 °C for 4 h, the reaction mixture was quenched with H₂O (0.5 ml). The aqueous layer was extracted with CHCl₃. The organic layer was washed with H₂O and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **89** (54 mg, 16%) as a pale yellow solid. ¹H NMR (CDCl₃) δ: 8.59–8.49 (m, 1H), 7.78–7.67 (m, 2H), 7.22–7.10 (m, 1H), 3.51 (s, 3H), 3.09 (s, 3H), 2.25 (s, 3H).

6-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]-*N*-[2-fluoro-4-(methylsulfonyl)phenyl]-*N*,5-dimethylpyrimidin-4-amine (56)

To a suspension of NaH (60% oil dispersion, 8.5 mg, 0.21 mmol) in THF (1 ml) was added

dropwise a solution of compound **76** (32.4 mg, 0.18 mmol) in THF (0.5 ml) at room temperature under a nitrogen atmosphere. After stirring for 30 min, compound **89** (54.3 mg, 0.16 mmol) was added to the reaction mixture. After stirring at 50 °C for 3 h, the reaction mixture was quenched with H₂O (3 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **56** (17 mg, 22%) as a white solid. ¹H NMR (CDCl₃) δ: 8.42 (s, 1H), 7.67–7.64 (m, 2H), 7.13–7.09 (m, 1H), 5.17–5.13 (m, 1H), 3.69 (s, 2H), 3.44 (s, 3H), 3.07 (s, 3H), 1.98–1.90 (m, 2H), 1.83–1.76 (m, 2H), 1.70–1.58 (m, 5H), 1.65 (s, 2H), 1.60–1.49 (m, 2H), 1.28 (s, 6H). Anal. Calcd for C₂₄H₃₂FN₃O₄S: C, 60.36; H, 6.75; N, 8.80. Found: C, 60.03; H, 6.75; N, 8.63.

4-Chloro-7-[2-fluoro-4-(methylsulfonyl)phenyl]-6,7-dihydro-5H-pyrrolo[2,3-*d*]pyrimidine (**90**)

To a solution of 2-fluoro-4-(methylsulfonyl)aniline **84** (4.02 g, 21.3 mmol) in CHCl₃ (5 ml) and TFA (21 ml) was added portionwise NaBH(OAc)₃ (5.82 g, 27.5 mmol) at 0 °C under an argon atmosphere. After stirring at 0 °C, to the solution added dropwise a solution of 2-(4,6-dichloropyrimidin-5-yl)acetaldehyde (3.50 g, 18.3 mmol) at 0 °C. After stirring at room temperature overnight, the reaction mixture was concentrated *in vacuo*. The residue was diluted with H₂O (10 ml) and saturated NaHCO₃ aqueous solution. The aqueous layer was extracted with CHCl₃. The combined organic layer was dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CHCl₃/EtOAc) to give compound **90** (4.93 g, 82%) as a white solid. ¹H NMR (CDCl₃) δ: 8.37 (s, 1H), 8.04–7.97 (m, 1H), 7.81–7.73 (m, 2H), 4.31–4.21 (m, 2H), 3.33–3.24 (m, 2H), 3.07 (s, 3H).

4-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]-7-[2-fluoro-4-(methylsulfonyl)phenyl]-6,7-dihydro-5H-pyrrolo[2,3-*d*]pyrimidine (**57**)

Compound **57** was prepared from compounds **76** and **90** in a manner similar to that described for compound **56**. White crystals (64%); mp 189–190 °C. IR (neat) 1587, 1570, 1506, 1485, 1458, 1435, 1408, 1310, 1256, 1240, 1215, 1150, 1126, 1082, 1049, 1002, 959, 916, 881, 866, 827, 775, 756, 633, 598, 542, 517, 486, 467, 455, 417 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 8.23 (s, 1H), 8.00–7.96 (m, 1H), 7.87–7.83 (m, 1H), 7.77–7.74 (m, 1H), 5.13–5.09 (m, 1H), 4.17–4.13 (m, 2H), 3.59 (s, 2H), 3.27 (s, 3H), 3.07–3.02 (m, 2H), 1.92–1.85 (m, 2H), 1.75–1.69 (m, 2H), 1.58 (s, 2H), 1.57–1.43 (m, 4H), 1.19 (s, 6H). ¹³C NMR (DMSO-*d*₆) δ: 166.1, 163.6, 156.6, 155.5, 152.9, 136.9, 133.1, 124.8, 123.3, 115.6, 102.3, 79.4, 74.3, 72.5, 50.3, 43.5, 43.3, 32.9, 28.9, 28.6, 22.1. Anal. Calcd for C₂₄H₃₀FN₃O₄S: C, 60.61; H, 6.36; N, 8.84. Found: C, 60.34; H, 6.41; N, 8.78.

1-(6-Chloro-5-methylpyrimidin-4-yl)-5-(methylsulfonyl)indoline (**92**)

A suspension of 4,6-dichloro-5-methylpyrimidine (600 mg, 3.68 mmol) and 5-(methylsulfonyl)indoline **91** (726 mg, 3.68 mmol) in isopropanol (6 ml) was heated at 100 °C for 8 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was diluted

with CHCl₃ (10 ml). The organic layer was washed with saturated NaHCO₃ aqueous solution, and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CHCl₃/EtOAc) to give compound **92** (460 mg, 39%) as a white solid. ¹H NMR (CDCl₃) δ: 8.58 (s, 1H), 7.80–7.68 (m, 2H), 6.89–6.81 (m, 1H), 4.31–4.21 (m, 2H), 3.30–3.21 (m, 2H), 3.04 (s, 3H), 2.28 (s, 3H).

1-(6-Chloropyrimidin-4-yl)-5-(methylsulfonyl)indoline (93)

Compound **93** was prepared from 4,6-dichloropyrimidine and 5-(methylsulfonyl)indoline **91** in a manner similar to that described for compound **92**. A white solid (80%). ¹H NMR (DMSO-*d*₆) δ: 8.70–8.67 (m, 1H), 8.61–8.54 (m, 1H), 7.82–7.73 (m, 2H), 7.10 (s, 1H), 4.22–4.10 (m, 2H), 3.37–3.24 (m, 2H), 3.15 (s, 3H).

1-{6-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]-5-methylpyrimidin-4-yl}-5-(methylsulfonyl)indoline (58)

Compound **58** was prepared from compounds **76** and **92** in a manner similar to that described for compound **56**. White crystals (69%); mp 196 °C. IR (neat) 1560, 1489, 1474, 1381, 1364, 1298, 1287, 1256, 1179, 1159, 1138, 1113, 1070, 1049, 1020, 1005, 962, 953, 893, 866, 820, 789, 766, 569, 534, 519, 511, 488, 459 cm⁻¹. ¹H NMR (CDCl₃) δ: 8.43 (s, 1H), 7.70 (s, 1H), 7.69–7.66 (m, 1H), 6.67–6.65 (m, 1H), 5.23–5.17 (m, 1H), 4.26–4.20 (m, 2H), 3.73 (s, 2H), 3.23–3.19 (m, 2H), 3.02 (s, 3H), 2.03 (s, 3H), 2.00–1.93 (m, 2H), 1.86–1.81 (m, 2H), 1.72–1.63 (m, 4H), 1.59–1.52 (m, 2H), 1.29 (s, 6H). ¹³C NMR (DMSO-*d*₆) δ: 168.1, 159.7, 154.5, 150.2, 132.5, 131.5, 127.0, 123.6, 111.1, 106.3, 79.6, 79.3, 78.9, 78.6, 74.8, 73.0, 52.4, 50.6, 44.2, 43.7, 32.7, 29.0, 28.3, 27.5, 11.4. Anal. Calcd for C₂₅H₃₃N₃O₄S: C, 60.61; H, 6.36; N, 8.84. Found: C, 63.36; H, 7.09; N, 8.75.

1-{6-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]pyrimidin-4-yl}-5-(methylsulfonyl)indoline (59)

Compound **59** was prepared from compounds **76** and **93** in a manner similar to that described for compound **56**. White crystals (47%); mp 230–232 °C. IR (neat) 1576, 1535, 1483, 1474, 1449, 1439, 1418, 1366, 1302, 1288, 1273, 1227, 1211, 1172, 1132, 1111, 1078, 1070, 1049, 1042, 999, 982, 955, 891, 868, 843, 831, 820, 770, 746, 677, 571, 532, 503, 496, 459 cm⁻¹. ¹H NMR (CDCl₃) δ: 8.57 (d, 1H, *J* = 8.9 Hz), 8.50 (s, 1H), 7.79–7.77 (m, 1H), 7.71 (br s, 1H), 5.95 (s, 1H), 5.19–5.13 (m, 1H), 4.11–4.03 (m, 2H), 3.71 (s, 2H), 3.32–3.28 (m, 2H), 3.04 (s, 3H), 1.99–1.92 (m, 2H), 1.86–1.79 (m, 2H), 1.66–1.60 (m, 4H), 1.58–1.50 (m, 2H), 1.28 (s, 6H). ¹³C NMR (DMSO-*d*₆) δ: 169.1, 160.3, 157.0, 147.8, 133.3, 132.8, 127.1, 123.4, 114.7, 89.7, 79.4, 79.2, 78.9, 78.5, 74.3, 72.6, 48.7, 44.0, 43.5, 32.8, 28.9, 28.4, 26.3. Anal. Calcd for C₂₄H₃₁N₃O₄S: C, 63.00; H, 6.83; N, 9.18. Found: C, 62.69; H, 6.79; N, 9.13.

***N*-(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)-*N*-methyl-6-[5-(methylsulfonyl)indolin-1-yl]pyrimidin-4-amine (64)**

A suspension of compound **93** (200 mg, 0.65 mmol), compound **72** (149 mg, 0.68 mmol) and

K₂CO₃ (196 mg, 1.42 mmol) in DMSO (2 ml) was heated at 130 °C for 4 h. After cooling to room temperature, the reaction mixture was diluted with H₂O (5 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O and brine, dried over MgSO₄ and concentrated *in vacuo* to give crude *N*-(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)-6-[5-(methylsulfonyl)indolin-1-yl]pyrimidin-4-amine (218 mg) as a colorless solid. The crude material was used without purification in the next step.

To a solution of crude *N*-(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)-6-[5-(methylsulfonyl)indolin-1-yl]pyrimidin-4-amine (123 mg) was added NaH (60% oil dispersion, 13 mg, 0.32 mmol) in DMF (3 ml) at room temperature. After stirring at room temperature for 20 min, iodomethane (25.0 µl, 0.40 mmol) was added to the reaction mixture. After stirring at room temperature overnight, the reaction mixture was quenched with H₂O (2 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CHCl₃/acetone) to give compound **64** (82 mg, 48% for 2 steps) as white crystals; mp 227 °C. IR (neat) 1587, 1574, 1504, 1479, 1420, 1391, 1364, 1300, 1283, 1256, 1223, 1175, 1136, 1113, 1072, 1047, 1026, 974, 881, 868, 839, 795, 766, 746, 573, 557, 529, 505, 492, 459, 442, 420 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 8.54–8.51 (m, 1H), 8.29 (s, 1H), 7.69–7.66 (m, 2H), 5.78 (s, 1H), 4.53 (br s, 1H), 4.13–4.09 (m, 2H), 3.66 (s, 2H), 3.27–3.24 (m, 2H), 3.13 (s, 3H), 2.87 (s, 3H), 1.79–1.74 (m, 2H), 1.58–1.46 (m, 8H), 1.18 (s, 6H). ¹³C NMR (DMSO-*d*₆) δ: 162.0, 159.3, 156.4, 148.5, 132.9, 131.9, 127.1, 123.2, 114.5, 84.3, 78.9, 72.6, 53.2, 48.7, 44.0, 43.5, 35.2, 28.9, 26.6, 26.2. Anal. Calcd for C₂₅H₃₄N₄O₃S: C, 63.80; H, 7.28; N, 11.90. Found: C, 63.48; H, 7.29; N, 11.84.

6-Chloro-*N*-(*cis*-3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)-*N*,5-dimethylpyrimidin-4-amine (94)

Compound **94** was prepared from compound **72** and 4,6-dichloro-5-methylpyrimidine in a manner similar to that described for compound **95** as below. A white solid (25% for 2 steps).

6-Chloro-*N*-(*cis*-3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)-*N*-methylpyrimidin-4-amine (95)

To a solution of compound **72** (158 mg, 0.73 mmol) and 4,6-dichloropyrimidine (119 mg, 0.80 mmol) in DMSO (0.8 ml) was added K₂CO₃ (222 mg, 1.60 mmol) at room temperature. After stirring at room temperature for 4 h, H₂O was poured into the reaction mixture. The aqueous layer was extracted with EtOAc. The organic layer was washed with H₂O and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give 6-chloro-*N*-(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)pyrimidin-4-amine (170 mg, 79%) as a white solid.

To a solution of 6-chloro-*N*-(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)pyrimidin-4-amine (158 mg, 0.729 mmol) in DMF (2 ml) was added NaH (60% oil dispersion, 34.0 mg, 0.862 mmol) at room temperature. After stirring at room temperature for 20 min, iodomethane (8.00 µl, 0.862 mmol) was

added to the reaction mixture. After stirring at room temperature for 2 h, H₂O (0.5 ml) was poured into the reaction mixture. The resulting precipitate was washed with H₂O to give compound **95** (159 mg, 89%) as a white solid.

***N*⁴-(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)-*N*⁶-[2-fluoro-4-(methylsulfonyl)phenyl]-*N*⁴,5-dimethylpyrimidine-4,6-diamine (**62**)**

Compound **62** was prepared from 2-fluoro-4-(methylsulfonyl)aniline **84** and compound **94** in a manner similar to that described for compound **65** as below. White crystals (38%); mp 166 °C. IR (neat) 1622, 1603, 1572, 1557, 1514, 1477, 1468, 1458, 1449, 1414, 1393, 1358, 1341, 1308, 1236, 1188, 1242, 1113, 1080, 1072, 1036, 976, 962, 872, 864, 841, 764, 598, 561, 552, 538, 527, 509, 494 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 8.34 (s, 1H), 8.12 (s, 1H), 7.87–7.83 (m, 1H), 7.76–7.73 (m, 1H), 7.70–7.67 (m, 1H), 3.64 (s, 2H), 3.62–3.54 (m, 1H), 3.24 (s, 3H), 2.77 (s, 3H), 2.11 (s, 3H), 1.77–1.74 (m, 2H), 1.63–1.57 (m, 4H), 1.53 (s, 2H), 1.48–1.40 (m, 2H), 1.18 (s, 6H). ¹³C NMR (DMSO-*d*₆) δ: 166.0, 159.0, 153.3, 152.2, 134.7, 134.0, 124.0, 123.2, 114.4, 100.4, 78.9, 72.6, 58.0, 53.1, 43.5, 35.4, 32.2, 28.9, 26.7, 13.6. Anal. Calcd for C₂₄H₃₃FN₄O₃S: C, 60.48; H, 6.98; N, 11.76. Found: C, 60.31; H, 7.06; N, 11.54.

***N*⁴-(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)-*N*⁶-[2-fluoro-4-(methylsulfonyl)phenyl]-*N*⁴-methylpyrimidine-4,6-diamine (**65**)**

To a solution of compound **95** (207 mg, 0.70 mmol) in DMF (4 ml) were added 2-fluoro-4-(methylsulfonyl)aniline **84** (132 mg, 0.70 mmol), (±)-BINAP (35.0 mg, 0.06 mmol), PdCl₂(dppf) (28.0 mg, 3.50 μmol), and sodium *tert*-butoxide (74.0 mg, 0.77 mmol) at room temperature under an argon atmosphere. After stirring at 80 °C for 3 h, H₂O was poured into the reaction mixture. The aqueous layer was extracted with EtOAc. The organic layer was washed with H₂O and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **65** (71 mg, 22%) as white crystals; mp 222–226 °C. IR (neat) 1626, 1609, 1574, 1508, 1491, 1476, 1443, 1414, 1339, 1310, 1281, 1240, 1175, 1140, 1113, 1072, 1049, 1022, 976, 897, 866, 837, 804, 762, 569, 538, 525, 494, 457, 426 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 9.13 (s, 1H), 8.67–8.62 (m, 1H), 8.24 (s, 1H), 7.75–7.72 (m, 1H), 7.67–7.65 (m, 1H), 6.27 (s, 1H), 4.27 (br s, 1H), 3.66 (s, 2H), 3.20 (s, 3H), 2.82 (s, 3H), 1.80–1.77 (m, 2H), 1.57–1.44 (m, 8H), 1.19 (s, 6H). ¹³C NMR (DMSO-*d*₆) δ: 161.8, 159.8, 156.7, 152.2, 149.8, 133.7, 132.3, 123.6, 120.9, 113.9, 86.3, 78.9, 72.6, 53.2, 43.6, 35.3, 28.9, 26.6. Anal. Calcd for C₂₃H₃₁FN₄O₃S: C, 59.72; H, 6.76; N, 12.11. Found: C, 59.84; H, 6.80; N, 12.14.

5-Allyl-6-chloro-*N*-(*cis*-3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)-*N*-methylpyrimidin-4-amine (96**)**

A suspension of 4,6-dichloro-5-allylpyrimidine (255 mg, 1.35 mmol), compound **72** (400 mg, 1.35 mmol), K₂CO₃ (373 mg, 2.69 mmol) and *N,N*-diisopropylethylamine (0.471 ml, 2.70 mmol) in DMF (5 ml) was heated at 80 °C for 4 h. After cooling to room temperature, the reaction mixture was

diluted with H₂O (10 ml). The aqueous layer was extracted EtOAc. The combined organic layer was washed with H₂O and brine, dried over MgSO₄ and concentrated *in vacuo* to give crude 5-allyl-6-chloro-*N*-(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)pyrimidin-4-amine (432 mg) as a pale yellow solid. The crude material was used without purification in the next step.

To a solution of crude 5-allyl-6-chloro-*N*-(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)pyrimidin-4-amine (232 mg) was added NaH (60% oil dispersion, 41 mg, 1.04 mmol) in DMF (4 ml) at room temperature. After stirring at room temperature for 20 min, iodomethane (96.4 μ l, 1.04 mmol) was added to the reaction mixture. After stirring at room temperature overnight, the reaction mixture was quenched with H₂O (2 ml). The aqueous layer was extracted EtOAc. The combined organic layer was washed with H₂O and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CHCl₃/EtOAc) to give compound **96** (68 mg, 26% for 2 steps) as pale yellow solid. ¹H NMR (CDCl₃) δ : 8.32 (s, 1H), 6.10–5.97 (m, 1H), 5.25–5.17 (m, 1H), 5.10–4.99 (m, 1H), 3.98–3.87 (m, 1H), 3.73–3.68 (m, 2H), 3.44–3.37 (m, 2H), 2.88 (s, 3H), 1.89–1.80 (m, 2H), 1.72–1.39 (m, 6H), 1.58 (s, 2H), 1.26 (s, 6H).

***N*-(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)-7-[2-fluoro-4-(methylsulfonyl)phenyl]-*N*-methyl-6,7-dihydro-5H-pyrrolo[2,3-*d*]pyrimidin-4-amine (63)**

To a solution of compound **96** (68 mg, 0.19 mmol) and K₂OsO₄·2H₂O (2.5 mg, 6.79 μ mol) in acetone (1 ml) and H₂O (1 ml) added sodium periodate (85 mg, 0.40 mmol) at room temperature under an argon atmosphere. After stirring at room temperature for 3 h, the reaction mixture was filtered through a celite pad. The aqueous layer was extracted CHCl₃. The combined organic layer was washed with H₂O and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was filtered through silica gel to give crude 2-{4-chloro-6-[(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)(methyl)amino]pyrimidin-5-yl}acetaldehyde (10 mg) as a blown oil. The crude material was used without purification in the next step.

To a solution of 2-fluoro-4-(methylsulfonyl)aniline **84** (5.9 mg, 0.03 mmol) in CHCl₃ (1.5 ml) and TFA (3 ml) was added portionwise NaBH(OAc)₃ (8.6 mg, 0.04 mmol) at 0 °C under an argon atmosphere. After stirring at 0 °C, to the solution was added dropwise a solution of crude 2-{4-chloro-6-[(3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)(methyl)amino]pyrimidin-5-yl}acetaldehyde (10 mg) at 0 °C. After stirring at room temperature overnight, the reaction mixture was concentrated *in vacuo*. The residue was diluted with H₂O (2 ml) and saturated NaHCO₃ aqueous solution. The aqueous layer was extracted with CHCl₃. The combined organic layer was dried over MgSO₄ and concentrated *in vacuo*. The residue was diluted with isopropanol (2 ml) and concentrated HCl aqueous solution (1 ml). The resulting solution was heated at 100 °C for 3 h. After cooling to room temperature, the reaction mixture was quenched with saturated NaHCO₃ aqueous solution (3 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was

washed with H₂O and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CHCl₃/acetone) to give compound **63** (6 mg, 10% for 3 steps) as a colorless solid. ¹H NMR (CDCl₃) δ: 8.18 (s, 1H), 7.99–7.95 (m, 1H), 7.72–7.67 (m, 2H), 4.39–4.32 (m, 1H), 4.09–4.04 (m, 2H), 3.73 (s, 2H), 3.41–3.37 (m, 2H), 3.04 (s, 3H), 3.02 (s, 3H), 1.91–1.87 (m, 2H), 1.70–1.54 (m, 8H), 1.27 (s, 6H). MS (ESI) m/z 489.2 (M+H)⁺.

Experiments concerning Chapter 4

5-Bromo-1,3-difluoro-2-[(methylsulfonyl)methyl]benzene (**132**)

To a solution of 4-bromo-2,6-difluorobenzyl alcohol **131** (5.00 g, 22.4 mmol) in EtOAc (40 ml) were added triethylamine (3.75 ml, 26.9 mmol) and methanesulfonyl chloride (1.91 ml, 24.6 mmol) at 0 °C. After stirring at 0 °C for 30 min, H₂O (20 ml) and 1N HCl aqueous solution (5 ml) were poured into the reaction mixture. The aqueous layer was extracted with EtOAc. The organic layer was washed with saturated NaHCO₃ aqueous solution, H₂O and brine, dried over MgSO₄ and concentrated *in vacuo* to give crude 4-bromo-2,6-difluorobenzyl methanesulfonate (6.69 g) as a colorless oil. The crude material was used without purification in the next step.

To a solution of crude 4-bromo-2,6-difluorobenzyl methanesulfonate (6.69 g) in DMF (25 ml) was added lithium bromide (2.14 g, 24.6 mmol) at room temperature. After stirring at room temperature for 1 h, sodium methanesulfinate (2.79 g, 24.6 mmol) was added at room temperature. After stirring at 80 °C for 1 h and cooling to room temperature, H₂O (50 ml) was poured into the reaction mixture. The resulting precipitate was washed with H₂O to give compound **132** (5.85 g, 92% for 2 steps) as a white solid. ¹H NMR (CDCl₃) δ: 7.25–7.19 (m, 2H), 4.34 (s, 2H), 2.92 (s, 3H).

3,5-Difluoro-4-[(methylsulfonyl)methyl]benzonitrile (**133**)

To a solution of compound **132** (5.00 g, 17.5 mmol) in DMA (30 ml) were added potassium hexacyanoferrate(II) trihydrate (3.70 g, 8.77 mmol), Na₂CO₃ (1.86 g, 17.5 mmol) and Pd(OAc)₂ (98.0 mg, 0.438 mmol) at room temperature under an argon atmosphere. After stirring at 130 °C for 3 h and cooling to room temperature, the reaction mixture was diluted with EtOAc (15 ml), filtered through a celite pad and concentrated *in vacuo*. The resulting precipitate was washed with H₂O to give compound **133** (2.96 g, 73%) as a beige solid. ¹H NMR (DMSO-*d*₆) δ: 8.00–7.83 (m, 2H), 4.70 (s, 2H), 3.15 (s, 3H).

3,5-Difluoro-*N'*-hydroxy-4-[(methylsulfonyl)methyl]benzimidamide (**130**)

To a suspension of compound **133** (4.76 g, 20.6 mmol) in EtOH (29 ml) and H₂O (9.5 ml) were added K₂CO₃ (1.71 g, 12.4 mmol) and NH₂OH·HCl (1.72 g, 24.7 mmol) at room temperature. The reaction mixture was heated at 80 °C and stirred for 3 h. After cooling to room temperature, H₂O (48 ml) was poured into the reaction mixture. The resulting precipitate was washed with H₂O to give compound **130** (4.08 g, 75%) as a beige solid. ¹H NMR (DMSO-*d*₆) δ: 10.02 (br s, 1H), 7.51–7.42 (m, 2H), 6.03 (br s, 2H), 4.59 (s, 2H), 3.08 (s, 3H).

3-Fluoro-*N'*-hydroxy-4-(methylsulfonyl)benzimidamide (**123**)

Compound **123** was prepared from 3-fluoro-4-(methylsulfonyl)benzonitrile in a manner similar to that described for compound **130**. A beige solid (90%). ¹H NMR (DMSO-*d*₆) δ: 9.82 (br s, 1H), 8.15–8.01 (m, 3H), 5.92 (br s, 2H), 3.41 (s, 3H).

2-Fluoro-4-(*N'*-hydroxycarbamimidoyl)-*N,N*-dimethylbenzamide (**124**)

Compound **124** was prepared from 4-cyano-2-fluoro-*N,N*-dimethylbenzamide in a manner similar to that described for compound **130**. A white solid (85%). ¹H NMR (DMSO-*d*₆) δ: 9.83 (br s, 1H), 7.95–7.89 (m, 1H), 7.88–7.80 (m, 1H), 7.64–7.56 (m, 1H), 5.95 (br s, 2H), 3.03 (s, 3H), 2.86 (s, 3H).

2-(2-Fluoro-4-(*N'*-hydroxycarbamimidoyl)phenyl)-*N,N*-dimethylacetamide (125)

Compound **125** was prepared from 2-(4-cyano-2-fluorophenyl)-*N,N*-dimethylacetamide in a manner similar to that described for compound **130**. A white solid (80%). ¹H NMR (DMSO-*d*₆) δ: 9.84 (br s, 1H), 7.83–7.75 (m, 1H), 7.75–7.65 (m, 1H), 7.49–7.43 (m, 1H), 5.94 (br s, 2H), 3.82 (s, 2H), 3.08 (s, 3H), 2.87 (s, 3H).

3-Fluoro-*N'*-hydroxy-4-((methylsulfonyl)methyl)benzimidamide (126)

Compound **126** was prepared from 3-fluoro-4-((methylsulfonyl)methyl)benzonitrile in a manner similar to that described for compound **130**. A white solid (87%). ¹H NMR (DMSO-*d*₆) δ: 9.83 (br s, 1H), 7.61–7.40 (m, 3H), 5.91 (br s, 2H), 4.55 (s, 2H), 3.01 (s, 3H).

***N'*-Hydroxy-4-[(methylsulfonyl)methyl]benzimidamide (127)**

Compound **127** was prepared from 4-((methylsulfonyl)methyl)benzonitrile in a manner similar to that described for compound **130**. A white solid (87%). ¹H NMR (DMSO-*d*₆) δ: 9.64 (br s, 1H), 7.78–7.60 (m, 2H), 7.51–7.28 (m, 2H), 5.80 (br s, 2H), 4.47 (s, 2H), 2.89 (s, 3H).

2,3-Difluoro-*N'*-hydroxy-4-[(methylsulfonyl)methyl]benzimidamide (128)

Compound **128** was prepared from 2,3-difluoro-4-((methylsulfonyl)methyl)benzonitrile in a manner similar to that described for compound **130**. A white solid (76%). ¹H NMR (DMSO-*d*₆) δ: 9.83 (br s, 1H), 7.40–7.32 (m, 1H), 7.32–7.23 (m, 1H), 5.94 (br s, 2H), 4.64 (s, 2H), 3.05 (s, 3H).

2,5-Difluoro-*N'*-hydroxy-4-((methylsulfonyl)methyl)benzimidamide (129)

Compound **129** was prepared from 2,5-difluoro-4-((methylsulfonyl)methyl)benzonitrile in a manner similar to that described for compound **130**. A white solid (60%). ¹H NMR (DMSO-*d*₆) δ: 9.84 (br s, 1H), 7.46–7.29 (m, 2H), 5.89 (br s, 2H), 4.58 (s, 2H), 3.04 (s, 3H).

({4-(2-Bromoethylidene)cyclohexyl}oxy)methylbenzene (135)

To a solution of 20% EtONa in EtOH (7.50 g, 22.0 mmol) and THF (24 ml) was added dropwise triethyl phosphonoacetate (4.37 ml, 22.0 mmol) at 0 °C under a nitrogen atmosphere. After stirring at 0 °C for 15 min, a solution of 4-(benzyloxy)cyclohexanone **134** (3.00 g, 14.7 mmol) in THF (12 ml) was added dropwise to the reaction mixture at 0 °C. After stirring at room temperature for 30 min, EtOAc (30 ml) and 5% KHSO₄ aqueous solution (10 ml) were poured into the reaction mixture. The organic layer was washed with saturated NaHCO₃ aqueous solution and brine, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give ethyl 2-[4-(benzyloxy)cyclohexylidene]acetate (3.70 g, 92%) as a pale yellow oil.

To a solution of ethyl 2-[4-(benzyloxy)cyclohexylidene]acetate (3.80 g, 13.9 mmol) in toluene (30 ml) was added dropwise a 1.0 M diisobutylaluminum hydride in toluene solution (32.0 ml, 32.0

mmol) at -78 °C under a nitrogen atmosphere. After stirring at -78 °C for 1 h, the reaction mixture was quenched with MeOH (10 ml) and Rochelle salt aqueous solution (30 ml). After stirring at room temperature, the aqueous layer was extracted with EtOAc. The organic layer was washed with 5% KHSO₄ aqueous solution, saturated NaHCO₃ aqueous solution and brine, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give 2-[4-(benzyloxy)cyclohexylidene]ethan-1-ol (3.00 g, 93%) as a pale yellow oil.

To a solution of 2-[4-(benzyloxy)cyclohexylidene]ethan-1-ol (2.30 g, 9.90 mmol) in DMF (23 ml) were added triphenylphosphine (2.11 g, 11.9 mmol) and *N*-bromosuccinimide (3.12 g, 11.9 mmol) at 0 °C under a nitrogen atmosphere. After stirring at room temperature for 1 h, the reaction mixture was diluted with *n*-hexane (30 ml) and H₂O (30 ml). The organic layer was washed with H₂O and brine, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **135** (730 mg, 25%) as a pale yellow oil. ¹H NMR (CDCl₃) δ: 7.38–7.24 (m, 5H), 5.57–5.48 (m, 1H), 4.61–4.54 (m, 2H), 4.05–3.98 (m, 2H), 3.64–3.54 (m, 1H), 2.59–2.46 (m, 1H), 2.44–2.31 (m, 1H), 2.13–2.00 (m, 2H), 2.00–1.80 (m, 2H), 1.72–1.59 (m, 2H).

4-[4-(Benzyloxy)cyclohexylidene]-2,2-difluorobutan-1-ol (**136**)

To a suspension of activated zinc powder (226 mg, 3.46 mmol) in THF (5.1 ml) was added dropwise ethyl bromodifluoroacetate (854 mg, 4.21 mmol) at room temperature under a nitrogen atmosphere. After stirring at room temperature for 1 h, copper cyanide (310 mg, 3.46 mmol) was added at room temperature. After stirring at room temperature for 1 h, a solution of compound **135** (730 mg, 2.47 mmol) in THF (5.8 ml) was added dropwise at room temperature. After stirring at room temperature for 3 h, the reaction mixture was diluted with EtOAc (10 ml) and saturated NaHCO₃ aqueous solution (10 ml). The reaction mixture was filtered through a celite pad. The organic layer was washed with saturated NaHCO₃ aqueous solution and brine, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give ethyl 4-[4-(benzyloxy)cyclohexylidene]-2,2-difluorobutanoate (140 mg, 17%) as a pale yellow oil.

To a suspension of LiAlH₄ (158 mg, 4.16 mmol) in THF (9 ml) was added dropwise a solution of ethyl 4-[4-(benzyloxy)cyclohexylidene]-2,2-difluorobutanoate (1.88 g, 5.56 mmol) in THF (10 ml) at 0 °C under a nitrogen atmosphere. After stirring at 0 °C for 15 min, the reaction mixture was quenched with H₂O (0.158 ml), 4N NaOH aqueous solution (0.158 ml) and H₂O (0.474 ml), and diluted with EtOAc (20 ml). The reaction mixture was stirred at room temperature for 1 h, filtered through a celite pad and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **136** (1.51 g, 92%) as a pale yellow oil. ¹H NMR (DMSO-*d*₆) δ: 7.38–7.18 (m, 5H), 5.51–5.39 (m, 1H), 5.18–5.06 (m, 1H), 4.51 (s, 2H), 3.61–3.47 (m,

3H), 2.69–2.54 (m, 2H), 2.43–2.33 (m, 1H), 2.32–2.21 (m, 1H), 2.07–1.78 (m, 4H), 1.52–1.37 (m, 2H).

9-(Benzyloxy)-3,3-difluoro-1-oxaspiro[5.5]undecane (137)

To a solution of compound **136** (1.51 g, 5.10 mmol) in toluene (30 ml) was added *p*-toluenesulfonic acid monohydrate (291 mg, 1.53 mmol) at room temperature under a nitrogen atmosphere. After stirring at 100 °C for 3 h and cooling to room temperature, the reaction mixture was diluted with EtOAc (30 ml) and washed with saturated NaHCO₃ aqueous solution and brine, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **137** (1.20 g, 80%) as a pale yellow oil as a mixture of isomers.

cis-isomer: ¹H NMR (CDCl₃) δ: 7.38–7.26 (m, 5H), 4.51 (s, 2H), 3.72–3.59 (m, 3H), 2.11–1.97 (m, 2H), 1.82–1.64 (m, 10H).

trans-isomer: ¹H NMR (CDCl₃) δ: 7.37–7.26 (m, 5H), 4.55 (s, 2H), 3.72–3.62 (m, 2H), 3.43–3.31 (m, 1H), 2.10–1.96 (m, 4H), 1.90–1.78 (m, 2H), 1.72–1.59 (m, 4H), 1.31–1.20 (m, 2H).

3,3-Difluoro-1-oxaspiro[5.5]undecan-9-one (138)

To a solution of compound **137** (2.04 g, 6.88 mmol) in MeOH (8 ml) was added 20% Pd(OH)₂-C (50% wet). The reaction mixture was hydrogenated (15 psi) at room temperature for 4 h. The reaction mixture was filtered through a celite pad and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give 3,3-difluoro-1-oxaspiro[5.5]undecan-9-ol (1.20 g, 85%) as a colorless oil as a mixture of isomers.

To a solution of 3,3-difluoro-1-oxaspiro[5.5]undecan-9-ol (8.70 g, 42.2 mmol) in CHCl₃ (61 ml) were added 1-methyl-2-azaadamantane-*N*-oxyl (7.01 mg, 0.0422 mmol), saturated NaHCO₃ aqueous solution (61 ml), potassium bromide (502 mg, 4.22 mmol) and tetrabutylammonium bromide (680 mg, 2.11 mmol) at 0 °C. To the reaction mixture were added dropwise a mixture of NaClO aqueous solution (5% or more chlorite, 32.9 ml, 46.4 mmol) and saturated NaHCO₃ aqueous solution (29 ml) at 0 °C. After stirring at 0 °C, the reaction mixture was quenched with saturated Na₂S₂O₃ aqueous solution (35 ml). The organic layer was washed with saturated Na₂S₂O₃ aqueous solution and concentrated *in vacuo*. The resulting residue was dissolved with toluene (61 ml). The organic layer was washed with 1N HCl aqueous solution and H₂O, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **138** (8.00 g, 93%) as a white solid. ¹H NMR (CDCl₃) δ: 3.82–3.68 (m, 2H), 2.67–2.52 (m, 2H), 2.38–2.19 (m, 4H), 2.18–2.03 (m, 2H), 1.84–1.76 (m, 2H), 1.76–1.65 (m, 2H).

***cis*-3,3-Difluoro-1-oxaspiro[5.5]undecan-9-ol (122)**

To a suspension of LiAlH₄ (446 mg, 11.8 mmol) in THF (20 ml) was added dropwise a solution of compound **138** (8.00 g, 39.2 mmol) in THF (36 ml) at 0 °C under a nitrogen atmosphere. After stirring at 0 °C for 2 h, the reaction mixture was quenched with H₂O (0.446 ml), 4N NaOH aqueous solution (0.446 ml) and H₂O (1.34 ml), and diluted with EtOAc (80 ml). The reaction mixture was

stirred at room temperature for 1 h, filtered through a celite pad and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **122** (7.27 g, 90%) as a white solid. ¹H NMR (DMSO-*d*₆) δ: 4.48–4.42 (m, 1H), 3.67–3.55 (m, 2H), 3.46–3.34 (m, 1H), 2.10–1.95 (m, 2H), 1.93–1.83 (m, 2H), 1.60–1.48 (m, 4H), 1.42–1.29 (m, 2H), 1.29–1.16 (m, 2H).

5-(*R*)-{1-[(*cis*-3,3-Difluoro-1-oxaspiro[5.5]undecan-9-yl)oxy]ethyl}-3-{3,5-difluoro-4-[(methylsulfonyl)methyl]phenyl}-1,2,4-oxadiazole (114)

To a suspension of NaH (60% oil dispersion, 3.88 g, 96.8 mmol) in dioxane (20 ml) was added dropwise a solution of compound **122** (5.00 g, 24.2 mmol) in dioxane (15 ml) at room temperature under a nitrogen atmosphere. After stirring at room temperature for 1 h, a solution of 2-(*S*)-bromopropanoic acid (5.44 ml, 60.5 mmol) in dioxane (32.5 ml) was added dropwise at room temperature. After stirring at room temperature overnight, the reaction mixture was quenched with H₂O (30 ml). The aqueous layer was washed with toluene. The aqueous layer was neutralized with 2N HCl aqueous solution and extracted with EtOAc. The organic layer was dried over Na₂SO₄ and concentrated *in vacuo*. To a solution of the resulting residue in EtOH (46 ml) was added dropwise morpholine (17.3 ml) at room temperature. After stirring at 60 °C for 2 h and cooling to room temperature, the reaction mixture was quenched with H₂O (35 ml) and 6N HCl aqueous solution (16 ml). The aqueous layer was extracted with EtOAc. The organic layer was dried over Na₂SO₄ and concentrated *in vacuo* to give 2-(*R*)-[(*cis*-3,3-difluoro-1-oxaspiro[5.5]undecan-9-yl)oxy]propanoic acid (4.55 g, 67%) as a pale yellow oil. ¹H NMR (CDCl₃) δ: 4.21–4.01 (m, 1H), 3.70–3.62 (m, 2H), 3.48–3.39 (m, 1H), 2.12–1.96 (m, 4H), 1.86–1.75 (m, 2H), 1.74–1.59 (m, 3H), 1.48–1.41 (m, 3H), 1.32–1.20 (m, 3H).

To a solution of 2-(*R*)-[(*cis*-3,3-difluoro-1-oxaspiro[5.5]undecan-9-yl)oxy]propanoic acid (30 mg, 0.108 mmol) in DMF (0.6 ml) were added 1-hydroxybenzotriazole monohydrate (29 mg, 0.214 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (41 mg, 0.214 mmol), diisopropylethylamine (0.038 ml, 0.214 mmol) and compound **130** (43 mg, 0.163 mmol) at room temperature under a nitrogen atmosphere. After stirring at room temperature for 1 h, EtOAc (3 ml) and 5% KHSO₄ aqueous solution (1 ml) were poured into the reaction mixture. The organic layer was washed with saturated NaHCO₃ aqueous solution and brine, dried over Na₂SO₄ and concentrated *in vacuo*. The resulting residue was dissolved with NMP (1 ml). After stirring at 120 °C for 1 h and cooling to room temperature, the reaction mixture was diluted with EtOAc (3 ml). The organic layer was washed with H₂O and brine, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **114** (15 mg, 27% for 2 steps) as white crystals; mp 98–100 °C. IR (neat) 1433, 1327, 1132, 1107, 1084, 1063, 1049, 1030, 1018, 966, 935, 872, 854, 772, 503, 463, 413 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 7.80–7.73 (m, 2H), 5.15–5.08 (m, 1H), 4.69 (s, 2H), 3.68–3.58 (m, 2H), 3.55–3.46 (m, 1H), 3.13 (s,

3H), 2.09–1.78 (m, 5H), 1.67–1.51 (m, 6H), 1.51–1.36 (m, 2H), 1.33–1.20 (m, 2H). ¹³C NMR (DMSO-*d*₆) δ: 181.2, 165.7, 162.6, 160.1, 128.8, 122.0, 119.6, 117.1, 110.3, 108.9, 76.3, 70.6, 66.6, 62.2, 48.1, 40.6, 31.8, 30.1, 27.3, 27.0, 26.4, 19.8. Anal. Calcd for C₂₂H₂₆F₄N₂O₅S: C, 52.17; H, 5.17; N, 5.53. Found: C, 52.17; H, 5.16; N, 5.56. $[\alpha]_D^{20} = +47.786^\circ$ (*c* = 0.280, MeOH).

5-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]methyl}-3-[3-fluoro-4-(methylsulfonyl)phenyl]-1,2,4-oxadiazole (97)

Compound **97** was prepared from compound **76**, bromoacetic acid and compound **123** in a manner similar to that described for compound **114**. White crystals (56% for 2 steps); mp 118–119 °C. ¹H NMR (DMSO-*d*₆) δ: 8.16–8.00 (m, 3H), 4.92 (s, 2H), 3.60–3.47 (m, 3H), 3.41 (s, 3H), 1.89–1.73 (m, 2H), 1.72–1.59 (m, 2H), 1.53 (s, 2H), 1.48–1.24 (m, 4H), 1.17 (s, 6H). ¹³C NMR (DMSO-*d*₆) δ: 178.3, 165.8, 160.1, 157.5, 133.2, 130.7, 130.5, 123.8, 115.6, 79.4, 76.9, 74.3, 60.3, 43.6, 43.5, 32.8, 28.9, 28.4. Anal. Calcd for C₂₁H₂₇FN₂O₅S: C, 57.52; H, 6.21; N, 6.39. Found: C, 57.46; H, 6.25; N, 6.43.

4-(5-{*rac*-1-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]ethyl}-1,2,4-oxadiazol-3-yl)-2-fluoro-*N,N*-dimethylbenzamide (98)

Compound **98** was prepared from compound **76**, 2-*rac*-bromopropanoic acid and compound **124** in a manner similar to that described for compound **114**. A colorless oil (44% for 2 steps). ¹H NMR (DMSO-*d*₆) δ: 7.95–7.89 (m, 1H), 7.88–7.80 (m, 1H), 7.64–7.56 (m, 1H), 5.12–5.04 (m, 1H), 3.56–3.46 (m, 3H), 3.03 (s, 3H), 2.86 (s, 3H), 1.88–1.77 (m, 1H), 1.72–1.58 (m, 3H), 1.57–1.49 (m, 5H), 1.41–1.26 (m, 4H), 1.21–1.12 (m, 6H). HRMS (ESI) Calcd for C₂₄H₃₃FN₃O₄ (M+H)⁺ *m/z* 446.2446, Found *m/z* 446.2450.

2-[4-(5-{*rac*-1-[(*cis*-3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]ethyl}-1,2,4-oxadiazol-3-yl)-2-fluorophenyl]-*N,N*-dimethylacetamide (99)

Compound **99** was prepared from compound **76**, 2-*rac*-bromopropanoic acid and compound **125** in a manner similar to that described for compound **114**. A white solid (30% for 2 steps). ¹H NMR (DMSO-*d*₆) δ: 7.82–7.76 (m, 1H), 7.74–7.66 (m, 1H), 7.50–7.42 (m, 1H), 5.10–5.02 (m, 1H), 3.82 (s, 2H), 3.57–3.46 (m, 3H), 3.07 (s, 3H), 2.85 (s, 3H), 1.87–1.77 (m, 1H), 1.72–1.58 (m, 3H), 1.57–1.50 (m, 5H), 1.43–1.26 (m, 4H), 1.20–1.12 (m, 6H). MS (ESI) *m/z* 460.2 (M+H)⁺.

5-{*rac*-1-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]ethyl}-3-{3-fluoro-4-[(methylsulfonyl)methyl]phenyl}-1,2,4-oxadiazole (100)

Compound **100** was prepared from compound **76**, 2-*rac*-bromopropanoic acid and compound **126** in a manner similar to that described for compound **114**. White crystals (60% for 2 steps); mp 138–140 °C. IR (neat) 1439, 1312, 1265, 1171, 1132, 1111, 1084, 1049, 976, 878, 858, 847, 779, 741, 721, 515, 465, 451, 417 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 7.94–7.88 (m, 1H), 7.86–7.79 (m, 1H), 7.73–7.65 (m, 1H), 5.12–5.02 (m, 1H), 4.66 (s, 2H), 3.57–3.45 (m, 3H), 3.05 (s, 3H), 1.88–1.75 (m, 1H), 1.73–1.58 (m, 3H), 1.58–1.47 (m, 5H), 1.43–1.26 (m, 4H), 1.20–1.11 (m, 6H). ¹³C NMR

(DMSO-*d*₆) δ : 180.9, 166.3, 162.1, 159.6, 134.3, 128.3, 123.1, 119.7, 113.9, 79.3, 75.6, 74.3, 66.6, 52.9, 43.6, 32.9, 29.3, 28.9, 28.5, 19.8. HRMS (ESI) Calcd for C₂₃H₃₂FN₂O₅S (M+H)⁺ *m/z* 467.2010, Found *m/z* 467.2009.

5-{(S)-1-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]ethyl}-3-{3-fluoro-4-[(methylsulfonyl)methyl]phenyl}-1,2,4-oxadiazole (101)

Compound **101** was prepared from compound **76**, 2-(*R*)-bromopropanoic acid and compound **126** in a manner similar to that described for compound **114**. White crystals (45% for 2 steps); mp 112 °C. IR (neat) 1362, 1348, 1321, 1288, 1265, 1215, 1130, 1107, 1090, 1055, 1043, 974, 887, 876, 779, 772, 501, 471, 453 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ : 7.94–7.88 (m, 1H), 7.86–7.79 (m, 1H), 7.73–7.65 (m, 1H), 5.12–5.02 (m, 1H), 4.66 (s, 2H), 3.57–3.45 (m, 3H), 3.05 (s, 3H), 1.88–1.75 (m, 1H), 1.73–1.58 (m, 3H), 1.58–1.47 (m, 5H), 1.43–1.26 (m, 4H), 1.20–1.11 (m, 6H). ¹³C NMR (DMSO-*d*₆) δ : 180.9, 166.3, 162.1, 159.6, 134.3, 128.3, 123.1, 119.7, 113.9, 79.3, 75.5, 74.2, 66.6, 52.8, 51.0, 43.5, 32.8, 29.3, 28.9, 28.5, 19.9. Anal. Calcd for C₂₃H₃₁FN₂O₅S: C, 59.21; H, 6.70; N, 6.00. Found: C, 59.37; H, 6.75; N, 6.03. $[\alpha]_D^{20} = -37.209^\circ$ (*c* = 0.215, MeOH).

5-{(R)-1-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]ethyl}-3-{3-fluoro-4-[(methylsulfonyl)methyl]phenyl}-1,2,4-oxadiazole (102)

Compound **102** was prepared from compound **76**, 2-(*S*)-bromopropanoic acid and compound **126** in a manner similar to that described for compound **114**. White crystals (52% for 2 steps); mp 110 °C. ¹H NMR (DMSO-*d*₆) δ : 7.94–7.88 (m, 1H), 7.86–7.79 (m, 1H), 7.73–7.65 (m, 1H), 5.12–5.02 (m, 1H), 4.66 (s, 2H), 3.57–3.45 (m, 3H), 3.05 (s, 3H), 1.88–1.75 (m, 1H), 1.73–1.58 (m, 3H), 1.58–1.47 (m, 5H), 1.43–1.26 (m, 4H), 1.20–1.11 (m, 6H). ¹³C NMR (DMSO-*d*₆) δ : 180.9, 166.3, 162.1, 159.6, 134.3, 128.3, 123.1, 119.7, 113.9, 79.3, 75.5, 74.2, 66.6, 52.8, 51.0, 43.5, 32.8, 29.3, 28.9, 28.5, 19.9. Anal. Calcd for C₂₃H₃₁FN₂O₅S: C, 59.21; H, 6.70; N, 6.00. Found: C, 59.40; H, 6.62; N, 6.21.

5-{*rac*-1-[(*cis*-3,3-Dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]ethyl}-3-{4-[(methylsulfonyl)methyl]phenyl}-1,2,4-oxadiazole (103)

Compound **103** was prepared from compound **76**, 2-*rac*-bromopropanoic acid and compound **127** in a manner similar to that described for compound **114**. White crystals (60% for 2 steps); mp 141–142 °C. IR (neat) 1308, 1265, 1140, 1126, 1101, 1053, 883, 536, 455 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ : 8.07–8.02 (m, 2H), 7.65–7.57 (m, 2H), 5.11–5.01 (m, 1H), 4.60 (s, 2H), 3.56–3.44 (m, 3H), 2.94 (s, 3H), 1.87–1.78 (m, 1H), 1.71–1.58 (m, 3H), 1.56–1.50 (m, 5H), 1.40–1.27 (m, 4H), 1.18–1.12 (m, 6H). ¹³C NMR (DMSO-*d*₆) δ : 180.6, 167.1, 132.6, 131.7, 127.1, 125.9, 79.4, 75.5, 74.3, 66.6, 59.0, 43.6, 32.8, 29.3, 28.8, 28.5, 19.8. HRMS (ESI) Calcd for C₂₃H₃₃N₂O₅S (M+H)⁺ *m/z* 449.2105, Found *m/z* 449.2101.

3-{2,3-Difluoro-4-[(methylsulfonyl)methyl]phenyl}-5-{(R)-1-[(*cis*-3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]ethyl}-1,2,4-oxadiazole (104)

Compound **104** was prepared from compound **76**, 2-(*S*)-bromopropanoic acid and compound **128** in

a manner similar to that described for compound **114**. White crystals (35% for 2 steps); mp 103–105 °C. IR (neat) 1477, 1315, 1169, 1128, 1047, 883, 503, 455 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 7.93–7.85 (m, 1H), 7.54–7.47 (m, 1H), 5.14–5.07 (m, 1H), 4.73 (s, 2H), 3.56–3.45 (m, 3H), 3.07 (s, 3H), 1.87–1.78 (m, 1H), 1.72–1.59 (m, 3H), 1.57–1.50 (m, 5H), 1.41–1.28 (m, 4H), 1.19–1.14 (m, 6H). ¹³C NMR (DMSO-*d*₆) δ: 180.4, 163.4, 150.7, 149.3, 148.2, 146.7, 128.3, 124.7, 121.8, 116.2, 79.3, 75.6, 74.3, 66.6, 52.7, 51.0, 43.6, 32.8, 29.3, 28.9, 28.4, 19.8. Anal. Calcd for C₂₃H₃₀F₂N₂O₅S: C, 57.01; H, 6.24; N, 5.78. Found: C, 56.94; H, 6.31; N, 5.88.

3-{2,5-Difluoro-4-[(methylsulfonyl)methyl]phenyl}-5-[(*R*)-1-[(*cis*-3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]ethyl]-1,2,4-oxadiazole (105)

Compound **105** was prepared from compound **76**, 2-(*S*)-bromopropanoic acid and compound **129** in a manner similar to that described for compound **114**. White crystals (46% for 2 steps); mp 105–107 °C. IR (neat) 1489, 1312, 1290, 1263, 1165, 1142, 1126, 1053, 908, 893, 810, 777, 750, 519, 459 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 7.90–7.83 (m, 1H), 7.65–7.57 (m, 1H), 5.14–5.05 (m, 1H), 4.67 (s, 2H), 3.56–3.46 (m, 3H), 3.07 (s, 3H), 1.87–1.78 (m, 1H), 1.72–1.59 (m, 3H), 1.57–1.50 (m, 5H), 1.41–1.28 (m, 4H), 1.19–1.13 (m, 6H). ¹³C NMR (DMSO-*d*₆) δ: 180.3, 163.2, 158.0, 156.5, 155.5, 154.0, 121.7, 121.5, 121.2, 120.9, 116.8, 116.5, 79.3, 75.6, 74.3, 66.6, 52.7, 51.0, 43.5, 32.9, 29.2, 28.9, 28.4, 19.8. Anal. Calcd for C₂₃H₃₀F₂N₂O₅S: C, 57.01; H, 6.24; N, 5.78. Found: C, 57.01; H, 6.38; N, 5.97.

3-{3,5-Difluoro-4-[(methylsulfonyl)methyl]phenyl}-5-[(*R*)-1-[(*cis*-3,3-dimethyl-2-oxaspiro[4.5]decan-8-yl)oxy]ethyl]-1,2,4-oxadiazole (106)

Compound **106** was prepared from compound **76**, 2-(*S*)-bromopropanoic acid and compound **130** in a manner similar to that described for compound **114**. White crystals (53% for 2 steps); mp 94–96 °C. IR (neat) 1433, 1315, 1275, 1136, 1049, 1026, 964, 885, 868, 775, 745, 511, 467, 424 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 7.82–7.68 (m, 2H), 5.13–5.05 (m, 1H), 4.69 (s, 2H), 3.56–3.46 (m, 3H), 3.13 (s, 3H), 1.87–1.78 (m, 1H), 1.72–1.58 (m, 3H), 1.57–1.50 (m, 5H), 1.41–1.28 (m, 4H), 1.18–1.12 (m, 6H). ¹³C NMR (DMSO-*d*₆) δ: 181.2, 165.7, 162.6, 160.1, 128.8, 110.3, 109.0, 79.3, 75.6, 74.2, 66.6, 51.0, 48.1, 43.5, 40.7, 32.8, 29.2, 28.9, 28.4, 19.8. Anal. Calcd for C₂₃H₃₀F₂N₂O₅S: C, 57.01; H, 6.24; N, 5.78. Found: C, 57.16; H, 6.46; N, 5.80. [α]_D²⁰ = +36.250° (*c* = 0.240, MeOH).

5-{*rac*-1-[(*cis*-3,3-Dimethyl-1-oxaspiro[4.5]decan-8-yl)oxy]ethyl}-3-{3-fluoro-4-[(methylsulfonyl)methyl]phenyl}-1,2,4-oxadiazole (107)

Compound **107** was prepared from compound **115**, 2-*rac*-bromopropanoic acid and compound **126** in a manner similar to that described for compound **114**. White crystals (69% for 2 steps); mp 78–81 °C. ¹H NMR (DMSO-*d*₆) δ: 7.94–7.89 (m, 1H), 7.86–7.80 (m, 1H), 7.74–7.66 (m, 1H), 5.11–5.03 (m, 1H), 4.66 (s, 2H), 3.54–3.46 (m, 1H), 3.37 (s, 2H), 3.05 (s, 3H), 1.80–1.65 (m, 3H), 1.65–1.51 (m, 6H), 1.47 (s, 2H), 1.42–1.27 (m, 2H), 1.06–0.98 (m, 6H). ¹³C NMR (DMSO-*d*₆) δ: 180.9, 166.3, 162.1, 159.6, 134.3, 128.3, 123.1, 119.7, 114.0, 80.8, 77.3, 75.3, 66.5, 52.9, 51.1, 34.3, 28.6, 27.9,

27.0, 19.9. HRMS (ESI) Calcd for C₂₃H₃₂FN₂O₅S (M+H)⁺ m/z 467.2010, Found m/z 467.2007.

5-{*rac*-1-[(*cis*-3,3-Dimethyl-1-oxaspiro[5.5]undecan-9-yl)oxy]ethyl}-3-{3-fluoro-4-[(methylsulfonyl)methyl]phenyl}-1,2,4-oxadiazole (108)

Compound **108** was prepared from compound **117**, 2-*rac*-bromopropanoic acid and compound **126** in a manner similar to that described for compound **114**. White crystals (49% for 2 steps); mp 111–114 °C. ¹H NMR (DMSO-*d*₆) δ: 7.95–7.87 (m, 1H), 7.87–7.79 (m, 1H), 7.74–7.65 (m, 1H), 5.14–5.03 (m, 1H), 4.69–4.64 (m, 2H), 3.51–3.42 (m, 1H), 3.12 (s, 2H), 3.04 (s, 3H), 1.97–1.82 (m, 2H), 1.81–1.73 (m, 1H), 1.61–1.51 (m, 4H), 1.51–1.40 (m, 2H), 1.33 (s, 2H), 1.28–1.06 (m, 4H), 0.87–0.83 (m, 6H). HRMS (ESI) Calcd for C₂₄H₃₄FN₂O₅S (M+H)⁺ m/z 481.2168, Found m/z 481.2167.

5-{*rac*-1-[(*cis*-4,4-Dimethyl-1-oxaspiro[5.5]undecan-9-yl)oxy]ethyl}-3-{3-fluoro-4-[(methylsulfonyl)methyl]phenyl}-1,2,4-oxadiazole (109)

Compound **109** was prepared from compound **118**, 2-*rac*-bromopropanoic acid and compound **126** in a manner similar to that described for compound **114**. White crystals (54% for 2 steps); mp 107–110 °C. IR (neat) 2943, 1366, 1319, 1288, 1265, 1250, 1213, 1169, 1130, 1107, 1088, 1076, 1051, 1038, 976, 887, 876, 845, 783, 770, 716, 692, 596, 527, 496, 471, 451 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 7.94–7.89 (m, 1H), 7.87–7.80 (m, 1H), 7.73–7.66 (m, 1H), 5.11–5.04 (m, 1H), 4.67 (s, 2H), 3.58–3.51 (m, 2H), 3.49–3.39 (m, 1H), 3.05 (s, 3H), 1.92–1.72 (m, 3H), 1.60–1.38 (m, 6H), 1.29–1.12 (m, 6H), 0.99–0.89 (m, 6H). ¹³C NMR (DMSO-*d*₆) δ: 181.0, 166.3, 162.1, 159.6, 134.3, 128.3, 123.1, 119.7, 113.9, 76.4, 69.9, 66.5, 56.8, 52.9, 48.0, 38.1, 33.5, 30.7, 28.1, 27.4, 26.8, 19.9. Anal. Calcd for C₂₄H₃₃FN₂O₅S: C, 59.98; H, 6.92; N, 5.83. Found: C, 60.33; H, 7.13; N, 6.00.

5-{*rac*-1-[(*cis*-1-Oxaspiro[4.5]decan-8-yl)oxy]ethyl}-3-{3-fluoro-4-[(methylsulfonyl)methyl]phenyl}-1,2,4-oxadiazole (110)

Compound **110** was prepared from compound **116**, 2-*rac*-bromopropanoic acid and compound **126** in a manner similar to that described for compound **114**. A pale yellow oil (49% for 2 steps). ¹H NMR (DMSO-*d*₆) δ: 7.94–7.89 (m, 1H), 7.87–7.80 (m, 1H), 7.73–7.67 (m, 1H), 5.13–5.02 (m, 1H), 4.67 (s, 2H), 3.71–3.63 (m, 2H), 3.55–3.46 (m, 1H), 3.05 (s, 3H), 1.88–1.71 (m, 3H), 1.68–1.46 (m, 9H), 1.41–1.21 (m, 3H). HRMS (ESI) Calcd for C₂₁H₂₈FN₂O₅S (M+H)⁺ m/z 439.1698, Found m/z 439.1697.

5-{*rac*-1-[(1-Oxaspiro[5.5]undecan-9-yl)oxy]ethyl}-3-{3-fluoro-4-[(methylsulfonyl)methyl]phenyl}-1,2,4-oxadiazole (111)

Compound **111** was prepared from compound **116**, 2-*rac*-bromopropanoic acid and compound **126** in a manner similar to that described for compound **114**. White crystals (27% for 2 steps); mp 103–104 °C. IR (neat) 1306, 1260, 1132, 1105, 1078, 1049, 993, 897, 889, 878, 772, 471, 455 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 7.94–7.87 (m, 1H), 7.86–7.79 (m, 1H), 7.73–7.65 (m, 1H), 5.13–5.05 (m, 1H), 4.66 (s, 2H), 3.53–3.41 (m, 3H), 3.04 (s, 3H), 1.95–1.73 (m, 3H), 1.61–1.49 (m, 6H), 1.49–1.36 (m, 4H), 1.34–1.25 (m, 2H), 1.20–1.07 (m, 2H). ¹³C NMR (DMSO-*d*₆) δ: 180.9, 166.3, 162.1, 159.6,

134.3, 128.3, 123.1, 119.7, 114.0, 76.8, 69.6, 66.5, 59.6, 52.9, 35.2, 31.3, 26.9, 26.2, 25.7, 19.9, 18.7. Anal. Calcd for C₂₂H₂₉FN₂O₅S: C, 58.39; H, 6.46; N, 6.19. Found: C, 58.52; H, 6.58; N, 6.22.

5-*[(R)-1-[(*cis*-3,3-Difluoro-1-oxaspiro[4.5]decan-8-yl)oxy]ethyl]-3-{3,5-difluoro-4-[(methylsulfonyl)methyl]phenyl}-1,2,4-oxadiazole (112)*

Compound **112** was prepared from compound **120**, 2-(*S*)-bromopropanoic acid and compound **130** in a manner similar to that described for compound **114**. White crystals (43% for 2 steps); mp 130–133 °C. IR (neat) 1433, 1317, 1132, 1109, 1063, 1026, 880, 866, 505, 469 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 7.80–7.73 (m, 2H), 5.14–5.06 (m, 1H), 4.69 (s, 2H), 4.01–3.91 (m, 2H), 3.59–3.51 (m, 1H), 3.13 (s, 3H), 2.31–2.19 (m, 2H), 1.86–1.73 (m, 3H), 1.67–1.41 (m, 8H). ¹³C NMR (DMSO-*d*₆) δ: 181.2, 165.7, 162.6, 160.1, 133.0, 130.5, 128.8, 110.4, 108.9, 81.2, 74.7, 70.2, 66.7, 48.1, 44.9, 40.7, 32.5, 27.9, 27.1, 19.8. Anal. Calcd for C₂₁H₂₄F₄N₂O₅S: C, 51.22; H, 4.91; N, 5.69. Found: C, 51.20; H, 4.99; N, 5.75. [α]_D²⁰ = +38.364° (*c* = 0.220, MeOH).

5-*[(R)-1-[(*cis*-4,4-difluoro-1-oxaspiro[5.5]undecan-9-yl)oxy]ethyl]-3-{3,5-difluoro-4-[(methylsulfonyl)methyl]phenyl}-1,2,4-oxadiazole (113)*

Compound **113** was prepared from compound **121**, 2-(*S*)-bromopropanoic acid and compound **130** in a manner similar to that described for compound **114**. A pale yellow solid (12% for 2 steps). ¹H NMR (DMSO-*d*₆) δ: 7.81–7.72 (m, 2H), 5.15–5.04 (m, 1H), 4.69 (s, 2H), 3.73–3.65 (m, 2H), 3.53–3.43 (m, 1H), 3.13 (s, 3H), 2.00–1.75 (m, 7H), 1.65–1.57 (m, 1H), 1.56–1.51 (m, 3H), 1.51–1.39 (m, 2H), 1.38–1.26 (m, 2H). HRMS (ESI) Calcd for C₂₂H₂₆F₄N₂O₅S (M+H)⁺ *m/z* 507.1577, Found *m/z* 507.1571.

11,11-Dimethyl-1,4,9-trioxadispiro[4.2.4⁸.2⁵]tetradecan-10-one (140)

To a solution of methyl isobutyrate (5.78 g, 56.6 mmol) in THF (30 ml) was added dropwise 2 M lithium diisopropyl amide solution in THF (29.2 ml, 58.4 mmol) at -15 °C under an argon atmosphere. After stirring at -15 °C, a solution of 1,7,10-trioxadispiro[2.2.4⁶.2³]dodecane **139** (3.20 g, 18.9 mmol) in THF (30 ml) was added dropwise at -15 °C. The reaction mixture was allowed to warm up to room temperature. After stirring at room temperature overnight, the reaction mixture was quenched with saturated NH₄Cl aqueous solution (20 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **140** (4.40 g, 97%) as a pale yellow solid. ¹H NMR (CDCl₃) δ: 4.02–3.88 (m, 4H), 2.01 (s, 2H), 1.98–1.73 (m, 6H), 1.70–1.60 (m, 2H), 1.32 (s, 6H).

8-(3-Hydroxy-2,2-dimethylpropyl)-1,4-dioxaspiro[4.5]decan-8-ol (141)

To a suspension of LiAlH₄ (840 mg, 22.0 mmol) in THF (25 ml) was added dropwise a solution of compound **140** (4.40 g, 18.4 mmol) in THF (20 ml) at 0 °C under a nitrogen atmosphere. After stirring at room temperature for 2 h, the reaction mixture was quenched with H₂O (0.84 ml), 4N NaOH aqueous solution (0.84 ml) and H₂O (1.68 ml), and diluted with THF (50 ml). The reaction

mixture was stirred at room temperature for 1 h, filtered through a celite pad and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **141** (4.35 g, 97%) as a white solid. ¹H NMR (CDCl₃) δ: 3.99–3.85 (m, 4H), 3.44 (s, 2H), 3.24 (br s, 1H), 2.82 (br s, 1H), 1.89–1.64 (m, 6H), 1.64–1.55 (m, 4H), 0.97 (s, 6H).

3,3-Dimethyl-1-oxaspiro[4.5]decan-8-one (142)

To a solution of compound **141** (4.35 g, 17.8 mmol) in pyridine (35 ml) were added methanesulfonyl chloride (1.59 ml, 20.5 mmol) at 0 °C. After stirring at room temperature overnight, the reaction mixture was concentrated *in vacuo*. The residue was diluted with EtOAc (20 ml). The organic layer was washed with H₂O, 1N KHSO₄ aqueous solution, H₂O, saturated NaHCO₃ aqueous solution and H₂O. The organic layer was dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The residue (4.39 g) was diluted with AcOH (12 ml) and H₂O (3 ml). The reaction mixture was heated at 100 °C for 7 h. After cooling to room temperature, the reaction mixture was diluted with toluene (20 ml) and H₂O (10 ml). The organic layer was washed with H₂O, saturated NaHCO₃ aqueous solution and H₂O. The organic layer was dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **142** (3.62 g, 90% for 2 steps) as a colorless oil. ¹H NMR (CDCl₃) δ: 3.58 (s, 2H), 2.76–2.62 (m, 2H), 2.29–2.18 (m, 2H), 2.17–2.06 (m, 2H), 1.89–1.76 (m, 2H), 1.66 (s, 2H), 1.14 (s, 6H).

cis-3,3-Dimethyl-1-oxaspiro[4.5]decan-8-ol (115)

To a solution of compound **142** (1.13 g, 6.18 mmol) in MeOH (10 ml) was added dropwise a solution of NaBH₄ (280 mg, 7.42 mmol) in MeOH (10 ml) at room temperature. After stirring at room temperature for 30 min, the reaction mixture was concentrated *in vacuo*. The residue was diluted with H₂O (5 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CHCl₃/acetone) to give *trans*-isomer of compound **115** (less polar, 90 mg, 8%) and compound **115** (more polar, 407 mg, 36%) as white crystals.

trans-isomer of compound **115**: ¹H NMR (CDCl₃) δ: 3.85–3.74 (m, 1H), 3.49 (s, 2H), 1.99–1.87 (m, 2H), 1.80–1.69 (m, 2H), 1.63–1.48 (m, 2H), 1.59 (s, 2H), 1.46–1.30 (m, 2H).

compound **115**: ¹H NMR (CDCl₃) δ: 3.70–3.54 (m, 1H), 3.49 (s, 2H), 1.92–1.80 (m, 2H), 1.80–1.57 (m, 4H), 1.53 (s, 2H), 1.47–1.23 (m, 2H).

3-[4-(Benzyloxy)cyclohex-1-en-1-yl]-2,2-dimethylpropan-1-ol (143)

A solution of compound **140** (3.82 g, 15.9 mmol) in AcOH (5 ml) and H₂O (3 ml) was heated at 100 °C overnight. After cooling to room temperature, the reaction mixture was diluted with H₂O (5 ml). The aqueous layer was extracted with CHCl₃. The combined organic layer was H₂O and saturated NaHCO₃ aqueous solution. The organic layer was dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give 3,3-dimethyl-1-oxaspiro[4.5]decane-2,8-dione (2.82 g, 90%) as a white

solid. ^1H NMR (CDCl_3) δ : 2.83–2.70 (m, 2H), 2.38–2.28 (m, 2H), 2.27–2.16 (m, 2H), 2.08 (s, 2H), 2.04–1.93 (m, 2H), 1.36 (s, 6H).

To a solution of 3,3-dimethyl-1-oxaspiro[4.5]decan-2,8-dione (2.82 g, 14.4 mmol) in MeOH (15 ml) was added NaBH_4 (543 mg, 14.4 mmol) at 0 °C. After stirring at 0 °C for 30 min, to the reaction mixture was added acetone (10 ml) and concentrated *in vacuo*. The residue was diluted with H_2O (5 ml). The aqueous layer was extracted with CHCl_3 . The combined organic layer was dried over MgSO_4 and concentrated *in vacuo* to give crude 8-hydroxy-3,3-dimethyl-1-oxaspiro[4.5]decan-2-one (3.36 g) as a white solid. The crude material was used without purification in the next step.

To a solution of crude 8-hydroxy-3,3-dimethyl-1-oxaspiro[4.5]decan-2-one (3.36 g) in THF (30 ml) was added NaH (60% oil dispersion, 1.15 g, 28.8 mmol) at 0 °C. After stirring at room temperature for 30 min, benzyl bromide (3.43 ml, 28.8 mmol) was added dropwise to the reaction mixture. After heating at 80 °C overnight, the reaction mixture was cooled to room temperature and quenched with H_2O (5 ml). The aqueous layer was extracted with CHCl_3 . The combined organic layer was dried over MgSO_4 , filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give 8-(benzyloxy)-3,3-dimethyl-1-oxaspiro[4.5]decan-2-one (2.33 g, 56% for 2 steps) as a pale yellow solid. ^1H NMR (CDCl_3) δ : 7.40–7.19 (m, 5H), 4.53 (s, 2H), 3.48–3.37 (m, 1H), 2.05–1.96 (m, 4H), 1.96–1.94 (s, 2H), 1.91–1.76 (m, 2H), 1.57–1.45 (m, 2H), 1.31 (s, 6H).

To a suspension of LiAlH_4 (308 mg, 8.11 mmol) in THF (10 ml) was added dropwise a solution of 8-(benzyloxy)-3,3-dimethyl-1-oxaspiro[4.5]decan-2-one (2.34 g, 8.11 mmol) in THF (10 ml) at 0 °C under a nitrogen atmosphere. After stirring at 0 °C for 2 h, the reaction mixture was quenched with H_2O (0.308 ml), 4N NaOH aqueous solution (0.308 ml) and H_2O (0.924 ml), and diluted with EtOAc (10 ml). The reaction mixture was stirred at room temperature for 1 h, filtered through a celite pad and concentrated *in vacuo* to give crude 4-(benzyloxy)-1-(3-hydroxy-2,2-dimethylpropyl)cyclohexan-1-ol (2.63 g) as a white solid. The crude material was used without purification in the next step.

To a solution of crude 4-(benzyloxy)-1-(3-hydroxy-2,2-dimethylpropyl)cyclohexan-1-ol (2.63 g), pyridine (1.21 ml, 12.2 mmol) and *N,N*-dimethylaminopyridine (99 mg, 0.811 mmol) was added acetic anhydride (0.920 ml, 9.74 mmol) at room temperature. After stirring at room temperature overnight, the reaction mixture was concentrated *in vacuo*. The residue was diluted with H_2O (10 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with 5% KHSO_4 aqueous solution and H_2O . The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give crude 3-[4-(benzyloxy)-1-hydroxycyclohexyl]-2,2-dimethylpropyl acetate (2.74 g) as a colorless oil. The crude material was used without purification in the next step.

To a solution of crude 3-[4-(benzyloxy)-1-hydroxycyclohexyl]-2,2-dimethylpropyl acetate (2.74 g)

in pyridine (10 ml) was added dropwise thionyl chloride (1.18 ml, 16.2 mmol) at 0 °C. After stirring at room temperature for 30 min, the reaction mixture was concentrated *in vacuo*. The residue was diluted with H₂O (5 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with 5% KHSO₄ aqueous solution and H₂O. The organic layer was dried over Na₂SO₄ and concentrated *in vacuo* to give crude 3-[4-(benzyloxy)cyclohex-1-en-1-yl]-2,2-dimethylpropyl acetate (2.60 g) as a pale yellow oil. The crude material was used without purification in the next step.

To a solution of crude 3-[4-(benzyloxy)cyclohex-1-en-1-yl]-2,2-dimethylpropyl acetate (2.60 g) in MeOH (20 ml) was added 4N NaOH aqueous solution (4.06 ml, 16.2 mmol) at room temperature. After stirring at room temperature for 2 h, the reaction mixture was quenched with 2N HCl aqueous solution (8.5 ml, 17.0 mmol) and concentrated *in vacuo*. The residue was diluted with H₂O (5 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **143** (1.95 g, 89% for 4 steps) as a pale yellow solid. ¹H NMR (CDCl₃) δ: 7.44–7.15 (m, 5H), 5.35–5.29 (m, 1H), 4.58–4.53 (m, 2H), 3.67–3.58 (m, 1H), 3.30 (s, 2H), 2.43–2.32 (m, 1H), 2.25–1.99 (m, 4H), 1.96–1.35 (m, 4H), 0.90 (s, 3H), 0.86 (s, 3H).

4-[4-(Benzyloxy)cyclohex-1-en-1-yl]-3,3-dimethylbutan-1-ol (**144**)

To a solution of compound **143** (1.95 g, 7.10 mmol) in CHCl₃ (20 ml) was added Dess-Martin reagent (4.52 g, 10.6 mmol) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was quenched with saturated Na₂SO₃ aqueous solution (10 ml). The organic layer was concentrated *in vacuo*. The residue was diluted with EtOAc (20 ml). The organic layer was washed with saturated NaHCO₃ aqueous solution and brine, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was filtered through a silica gel pad to give crude 3-[4-(benzyloxy)cyclohex-1-en-1-yl]-2,2-dimethylpropanal (1.69 g) as a colorless oil. The crude material was used without purification in the next step.

To a suspension of methoxymethyltriphenylphosphonium chloride (4.25 g, 12.4 mmol) in THF (40 ml) was added potassium *tert*-butoxide (1.39 g, 12.4 mmol) at 0 °C. After stirring at 0 °C for 30 min, a solution of crude 3-[4-(benzyloxy)cyclohex-1-en-1-yl]-2,2-dimethylpropanal (1.69 g) in THF (20 ml) was added dropwise to the reaction mixture at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was quenched with H₂O (5 ml). The reaction mixture was concentrated *in vacuo*. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na₂SO₄ and concentrated *in vacuo* to give crude ({[4-(4-methoxy-2,2-dimethylbut-3-en-1-yl)cyclohex-3-en-1-yl]oxy}methyl)benzene (1.65 g) as a pale yellow oil. The crude material was used without purification in the next step.

To a solution of crude

({[4-(4-methoxy-2,2-dimethylbut-3-en-1-yl)cyclohex-3-en-1-yl]oxy}methyl)benzene (1.65 g) in THF (30 ml) was added pyridinium *p*-toluenesulfonate (138 mg, 0.549 mmol) at room temperature. After stirring at room temperature overnight, the reaction mixture was quenched with H₂O (5 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na₂SO₄ and concentrated *in vacuo* to give crude 4-[4-(benzyloxy)cyclohex-1-en-1-yl]-3,3-dimethylbutanal (1.82 g) as a pale yellow oil. The crude material was used without purification in the next step.

To a solution of crude 4-[4-(benzyloxy)cyclohex-1-en-1-yl]-3,3-dimethylbutanal (1.82 g) in MeOH (10 ml) was added NaBH₄ (200 mg, 5.30 mmol) at 0 °C. After stirring at room temperature for 30 min, the reaction mixture was quenched with acetone (5 ml). The reaction mixture was concentrated *in vacuo*. The residue was diluted with H₂O (5 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **144** (360 mg, 17% for 4 steps) as a colorless oil. ¹H NMR (CDCl₃) δ: 7.39–7.17 (m, 5H), 5.31–5.22 (m, 1H), 4.57–4.52 (m, 2H), 3.77–3.65 (m, 2H), 3.66–3.55 (m, 1H), 2.44–2.33 (m, 1H), 2.18–2.03 (m, 3H), 1.98–1.81 (m, 3H), 1.75–1.60 (m, 1H), 1.57–1.47 (m, 3H), 0.89 (s, 6H).

***cis*-9-(Benzyloxy)-4,4-dimethyl-1-oxaspiro[5.5]undecane (145)**

A mixture of compound **144** (360 mg, 1.25 mmol) and Amberlyst-15 (50 mg) in toluene (5 ml) was heated at 90 °C overnight. The reaction mixture was filtered through a celite pad and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give *trans*-isomer of compound **145** (less polar, 142 mg, 39%) and compound **145** (more polar, 116 mg, 32%) as colorless oils.

trans-isomer of compound **145**: ¹H NMR (CDCl₃) δ: 7.38–7.19 (m, 5H), 4.50 (s, 2H), 3.70–3.61 (m, 2H), 3.58–3.49 (m, 1H), 1.85–1.55 (m, 8H), 1.52 (s, 2H), 1.37–1.29 (m, 2H), 0.99 (s, 6H).

compound **145**: ¹H NMR (CDCl₃) δ: 7.37–7.16 (m, 5H), 4.53 (s, 2H), 3.68–3.58 (m, 2H), 3.37–3.23 (m, 1H), 2.03–1.91 (m, 2H), 1.81–1.59 (m, 4H), 1.53 (s, 2H), 1.36–1.30 (m, 2H), 1.27–1.14 (m, 2H), 0.97 (s, 6H).

***cis*-4,4-Dimethyl-1-oxaspiro[5.5]undecan-9-ol (118)**

To a solution of compound **145** (190 mg, 0.659 mmol) in MeOH (2 ml) was added 20% Pd(OH)₂-C (50% wet). The reaction mixture was hydrogenated (15 psi) at room temperature for 4 h. The reaction mixture was filtered through a celite pad and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **118** (130 mg, 99%) as a colorless oil. ¹H NMR (CDCl₃) δ: 3.68–3.60 (m, 2H), 3.61–3.49 (m, 1H), 2.02–1.92 (m, 2H), 1.76–1.65 (m, 2H), 1.64–1.48 (m, 2H), 1.41–1.16 (m, 5H), 1.23 (s, 2H), 1.00–0.96 (m, 6H).

Methyl 2,2-dimethyl-4-(1,4-dioxaspiro[4.5]decan-8-ylidene)butanoate (147)

To a suspension of 2-(1,4-dioxaspiro[4.5]decan-8-ylidene)ethan-1-ol **146** (4.22 g, 22.9 mmol) and

triphosgene (3.40 g, 11.4 mmol) in *n*-hexane (50 ml) was added dropwise a solution of pyridine (3.47 ml, 45.8 mmol) in *n*-hexane (10 ml) at 0 °C. After stirring at 0 °C, the reaction mixture was quenched with H₂O (5 ml). The aqueous layer was extracted with *n*-hexane. The combined organic layer was washed with 5% KHSO₄ aqueous solution and brine, dried over Na₂SO₄ and concentrated *in vacuo* to give crude 8-(2-chloroethylidene)-1,4-dioxaspiro[4.5]decane (3.75 g) as a colorless oil. The crude material was used without purification in the next step.

To a solution of methyl isobutyrate (3.78 g, 37.0 mmol) in THF (20 ml) was added dropwise 2 M lithium diisopropyl amide solution in THF (18.5 ml, 37.0 mmol) at -78 °C under an argon atmosphere. After stirring at -78 °C for 30 min, a solution of crude 8-(2-chloroethylidene)-1,4-dioxaspiro[4.5]decane (3.75 g) in THF (20 ml) was added dropwise -78 °C. The reaction mixture was allowed to warm up to room temperature. After stirring at room temperature for 1h, the reaction mixture was quenched with saturated NH₄Cl aqueous solution (20 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H₂O, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **147** (4.02 g, 65% for 2 steps) as a pale yellow solid. ¹H NMR (CDCl₃) δ: 5.12–5.03 (m, 1H), 3.95 (s, 4H), 3.64 (s, 3H), 2.29–2.17 (m, 6H), 1.69–1.59 (m, 4H), 1.14 (s, 6H).

4-(4-(Benzyloxy)cyclohexylidene)-2,2-dimethylbutan-1-ol (**148**)

A solution of compound **148** (3.48 g, 12.9 mmol) in AcOH (14 ml) and H₂O (4 ml) was heated at 60 °C for 4 h. After cooling to room temperature, the reaction mixture was diluted with H₂O (5 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was H₂O and saturated NaHCO₃ aqueous solution. The organic layer was dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude methyl 2,2-dimethyl-4-(4-oxocyclohexylidene)butanoate (3.12 g) as a white solid. The crude material was used without purification in the next step.

To a solution of crude methyl 2,2-dimethyl-4-(4-oxocyclohexylidene)butanoate (3.12 g) in MeOH (26 ml) was added NaBH₄ (392 mg, 10.4 mmol) at 0 °C. After stirring at 0 °C for 30 min, the reaction mixture was quenched with acetone (5 ml) and concentrated *in vacuo*. The residue was diluted with H₂O and saturated NH₄Cl aqueous solution. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over MgSO₄, filtered, and concentrated *in vacuo* to give crude methyl 4-(4-hydroxycyclohexylidene)-2,2-dimethylbutanoate (2.40 g) as a pale yellow oil. The crude material was used without purification in the next step.

To a solution of crude methyl 4-(4-hydroxycyclohexylidene)-2,2-dimethylbutanoate (2.40 g) in THF (15 ml) was added NaH (60% oil dispersion, 552 mg, 13.8 mmol) at 0 °C. After stirring at room temperature for 30 min, benzyl bromide (1.63 ml, 13.8 mmol) was added dropwise to the reaction mixture. After stirring at room temperature overnight, the reaction mixture was quenched with saturated NH₄Cl aqueous solution (5 ml). The aqueous layer was extracted with EtOAc. The

combined organic layer was washed with brine, dried over MgSO_4 , filtered, and concentrated *in vacuo* to give crude methyl 4-[4-(benzyloxy)cyclohexylidene]-2,2-dimethylbutanoate (2.20 g) as a pale yellow oil. The crude material was used without purification in the next step.

To a suspension of LiAlH_4 (185 mg, 4.86 mmol) in THF (10 ml) was added dropwise a solution of crude methyl 4-[4-(benzyloxy)cyclohexylidene]-2,2-dimethylbutanoate (2.20 g) in THF (10 ml) at 0 °C under a nitrogen atmosphere. After stirring at 0 °C for 2 h, the reaction mixture was quenched with H_2O (0.185 ml), 4.0 M NaOH aqueous solution (0.185 ml) and H_2O (0.555 ml), and diluted with EtOAc (20 ml). The reaction mixture was stirred at room temperature for 1 h, filtered through a celite pad and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **148** (1.00 g, 27% for 4 steps) as a colorless oil. ^1H NMR (CDCl_3) δ : 7.42–7.16 (m, 5H), 5.22–5.12 (m, 1H), 4.55 (s, 2H), 3.60–3.50 (m, 1H), 3.35–3.27 (m, 2H), 2.56–2.44 (m, 1H), 2.35–2.25 (m, 1H), 2.07–1.84 (m, 5H), 1.66–1.39 (m, 2H), 1.35–1.21 (m, 2H), 0.86 (s, 6H).

9-(Benzyloxy)-3,3-dimethyl-1-oxaspiro[5.5]undecane (149)

To a solution of compound **148** (1.00 g, 3.47 mmol) in CHCl_3 (10 ml) was added dropwise boron trifluoride diethyl ether complex (542 mg, 3.82 mmol) at 0 °C. After stirring at room temperature overnight, the reaction mixture was quenched with saturated NaHCO_3 aqueous solution (10 ml) at 0 °C. The aqueous layer was extracted with CHCl_3 . The combined organic layer was washed with saturated NaHCO_3 aqueous solution and H_2O , dried over MgSO_4 , filtered, and concentrated *in vacuo*. The residue was filtered through a silica gel pad to give crude **149** (900 mg) as a colorless oil. The crude material was used without purification in the next step.

cis-3,3-Dimethyl-1-oxaspiro[5.5]undecan-9-ol (117)

To a solution of crude compound **149** (900 mg) in MeOH (9 ml) was added 20% $\text{Pd}(\text{OH})_2\text{-C}$ (50% wet). The reaction mixture was hydrogenated (15 psi) at room temperature overnight. The reaction mixture was filtered through a celite pad and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give *trans*-isomer of compound **117** (less polar, 250 mg, 36% for 2 steps) and compound **117** (more polar, 180 mg, 26% for 2 steps) as white crystals.

trans-isomer of compound **117**: ^1H NMR (CDCl_3) δ : 3.97–3.84 (m, 1H), 3.22 (s, 2H), 1.92–1.75 (m, 2H), 1.75–1.55 (m, 4H), 1.51–1.34 (m, 6H), 1.33–1.15 (m, 3H), 0.90 (s, 6H).

compound **117**: ^1H NMR (CDCl_3) δ : 3.64–3.52 (m, 1H), 3.22 (s, 2H), 2.05–1.95 (m, 2H), 1.77–1.65 (m, 2H), 1.63–1.46 (m, 3H), 1.45–1.08 (m, 8H), 0.90 (s, 6H).

{3-[4-(Benzyloxy)cyclohexylidene]propoxy}(tert-butyl)dimethylsilane (150)

To a suspension of {3-[(*tert*-butyldimethylsilyl)oxy]propyl}triphenylphosphonium bromide (11.4 g, 22.2 mmol), which was readily prepared from (3-bromopropoxy)-*tert*-butyldimethylsilane), in DMF (18 ml) was added NaH (60% oil dispersion, 888 mg, 22.2 mmol) at 0 °C under an argon atmosphere.

After stirring at 0 °C for 30 min, a solution of 4-(benzyloxy)cyclohexan-1-one **134** (2.27 g, 11.1 mmol) in THF (5 ml) was added to the reaction mixture at 0 °C. After stirring at room temperature for 2 h, the reaction mixture was quenched with saturated NH₄Cl aqueous solution (5 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane) to give compound **150** (2.87 g, 72%) as a colorless oil. ¹H NMR (CDCl₃) δ: 7.39–7.23 (m, 5H), 5.15–5.05 (m, 1H), 4.56 (s, 2H), 3.60–3.49 (m, 3H), 2.56–2.42 (m, 1H), 2.37–2.17 (m, 3H), 2.06–1.85 (m, 4H), 1.61–1.41 (m, 2H), 0.89 (s, 9H), 0.05 (s, 6H).

3-[4-(Benzyloxy)cyclohexylidene]propan-1-ol (**151**)

To a solution of compound **150** (2.87 g, 7.96 mmol) in THF (21 ml) was added 1 M tetrabutylammonium fluoride solution in THF (24 ml, 24.0 mmol) at room temperature. After stirring at room temperature overnight, the reaction mixture was concentrated *in vacuo*. The residue was diluted with EtOAc (30 ml). The organic layer was washed with H₂O, saturated NaHCO₃ aqueous solution and brine. The organic layer was dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **151** (1.73 g, 88%) as a pale yellow oil. ¹H NMR (CDCl₃) δ: 7.38–7.18 (m, 5H), 5.18–5.06 (m, 1H), 4.58 (s, 2H), 3.68–3.45 (m, 3H), 2.56–2.46 (m, 1H), 2.40–2.25 (m, 3H), 2.10–1.82 (m, 4H), 1.64–1.46 (m, 2H).

cis-8-(Benzyloxy)-1-oxaspiro[4.5]decane (**152**)

A mixture of compound **151** (676 mg, 2.75 mmol) and Amberlyst-15 (68 mg) in toluene (10 ml) was heated at 90 °C for 3 h. The reaction mixture was filtered through a celite pad and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **152** (less polar, 276 mg, 40%) and *trans*-isomer of compound **152** (more polar, 210 mg, 31%) as colorless oils.

compound **152**: ¹H NMR (CDCl₃) δ: 7.42–7.20 (m, 5H), 4.53 (s, 2H), 3.87–3.75 (m, 2H), 3.58–3.41 (m, 1H), 2.00–1.86 (m, 4H), 1.76–1.66 (m, 4H), 1.62–1.42 (m, 4H).

trans-isomer of compound **152**: ¹H NMR (CDCl₃) δ: 7.42–7.17 (m, 5H), 4.55 (s, 2H), 3.90–3.70 (m, 2H), 3.52–3.28 (m, 1H), 1.94–1.84 (m, 2H), 1.84–1.70 (m, 6H), 1.69–1.61 (m, 2H), 1.43–1.29 (m, 2H).

Ethyl 4-(4-(benzyloxy)cyclohexylidene)butanoate (**153**)

To a suspension of 3-(ethoxycarbonyl)propyltriphenylphosphonium bromide (13.4 g, 29.3 mmol) in THF (45 ml) was added potassium *tert*-butoxide (3.30 g, 29.4 mmol) at 0 °C. After stirring at 0 °C for 1 h, a solution of 4-(benzyloxy)cyclohexanone **134** (3.00 g, 14.7 mmol) in THF (15 ml) was added dropwise to the reaction mixture at 0 °C. After stirring at room temperature for 2 h, the reaction mixture was quenched with H₂O (5 ml). The reaction mixture was concentrated *in vacuo*. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine,

dried over Na₂SO₄ and concentrated *in vacuo*. The residue was filtered through a silica gel pad to give crude compound **153** (3.30 g) as a pale yellow oil. The crude material was used without purification in the next step.

4-[4-(Benzyloxy)cyclohexylidene]butan-1-ol (154)

To a suspension of LiAlH₄ (414 mg, 10.9 mmol) in THF (26 ml) was added dropwise a solution of crude compound **153** (3.30 g) in THF (26 ml) at 0 °C under a nitrogen atmosphere. After stirring at 0 °C for 30 min, the reaction mixture was quenched with H₂O (0.414 ml), 4N NaOH aqueous solution (0.414 ml) and H₂O (1.24 ml), and diluted with EtOAc (40 ml). The reaction mixture was stirred at room temperature for 1 h, filtered through a celite pad and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **154** (2.70 g, 70% for 2 steps) as a white solid. ¹H NMR (DMSO-*d*₆) δ: 7.37–7.08 (m, 5H), 5.14–5.03 (m, 1H), 4.50 (s, 2H), 4.37–4.25 (m, 1H), 3.60–3.46 (m, 1H), 3.41–3.33 (m, 2H), 2.43–2.33 (m, 1H), 2.25–2.14 (m, 1H), 2.04–1.73 (m, 6H), 1.48–1.33 (m, 4H).

cis-9-(Benzyloxy)-1-oxaspiro[5.5]undecane (155)

Compound **155** was prepared from compound **154** in a manner similar to that described for compound **152**. A colorless oil (40%). ¹H NMR (CDCl₃) δ: 7.39–7.20 (m, 5H), 4.56 (s, 2H), 3.66–3.56 (m, 2H), 3.42–3.26 (m, 1H), 2.07–1.96 (m, 2H), 1.84–1.74 (m, 2H), 1.73–1.56 (m, 4H), 1.56–1.46 (m, 2H), 1.44–1.35 (m, 2H), 1.22–1.08 (m, 2H).

{[(4-Methylenecyclohexyl)oxy]methyl}sbenzene (156)

To a suspension of methyltriphenylphosphonium bromide (3.57 g, 10.0 mmol) in THF (30 ml) was added potassium *tert*-butoxide (1.12 g, 10.0 mmol) at 0 °C. After stirring at 0 °C for 30 min, a solution of 4-(benzyloxy)cyclohexanone **134** (2.04 g, 10.0 mmol) in THF (20 ml) was added dropwise to the reaction mixture at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was quenched with H₂O (5 ml). The reaction mixture was concentrated *in vacuo*. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was filtered through a silica gel pad to give crude compound **156** (1.31 g) as a pale yellow oil. The crude material was used without purification in the next step.

cis-6-(Benzyloxy)-1-oxaspiro[2.5]octane (157)

To a solution of crude compound **156** (7.00g) in CHCl₃ (50 ml) was added portionwise *m*-chloroperoxybenzoic acid (75 wt%, 7.17 g, 41.5 mmol) at 0 °C. After stirring at 0 °C overnight, the reaction mixture was quenched with 10% Na₂S₂O₃ aqueous solution. The aqueous layer was extracted with CHCl₃. The combined organic layer was washed with saturated NaHCO₃ aqueous solution and H₂O, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give *trans*-isomer of compound **157** (less polar, 3.82 g, 35% for 2 steps) and compound **157** (more polar, 1.67 g, 16% for 2 steps) as colorless

oils.

trans-isomer of compound **157**: ^1H NMR (CDCl_3) δ : 7.37–7.21 (m, 5H), 4.54 (s, 2H), 3.67–3.57 (m, 1H), 2.63 (s, 2H), 2.01–1.91 (m, 2H), 1.91–1.69 (m, 4H), 1.49–1.36 (m, 2H).

compound **157**: ^1H NMR (CDCl_3) δ : 7.42–7.20 (m, 5H), 4.57 (s, 2H), 3.58–3.46 (m, 1H), 2.64 (s, 2H), 2.00–1.76 (m, 4H), 1.69–1.60 (m, 4H).

***cis*-1-Allyl-4-(benzyloxy)cyclohexan-1-ol (158)**

To a solution of compound **157** (1.67 g, 7.65 mmol) in THF (17 ml) was added dropwise 1 M vinylmagnesium bromide solution in THF (8.42 ml, 8.42 mmol) at 0 °C. After stirring at room temperature for 6 h, the reaction mixture was quenched with saturated NH_4Cl aqueous solution (5 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with H_2O , dried over MgSO_4 , filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **158** (1.49 g, 79%) as a pale yellow solid. ^1H NMR (CDCl_3) δ : 7.38–7.19 (m, 5H), 5.95–5.76 (m, 1H), 5.18–5.01 (m, 2H), 4.55 (s, 2H), 3.39–3.25 (m, 1H), 2.22–2.14 (m, 2H), 1.91–1.79 (m, 2H), 1.78–1.61 (m, 4H), 1.49–1.31 (m, 2H).

***cis*-8-(Benzyloxy)-1-oxaspiro[4.5]decan-3-one (159)**

To a solution of compound **158** (1.34 g, 5.11 mmol) and sodium periodate (1.31 g, 6.13 mmol) in *tert*-butanol (30 ml) and H_2O (10 ml) was added dropwise a solution of sodium hydrogen sulfite (1.28 g, 12.3 mmol) in H_2O (26 ml) at 50 °C. After stirring at 50 °C for 7 h, the reaction mixture was cooled to room temperature and quenched with 10% $\text{Na}_2\text{S}_2\text{O}_3$ aqueous solution (20 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give crude *cis*-8-(benzyloxy)-1-oxaspiro[4.5]decan-3-ol (520 mg) as a pale yellow oil. The crude material was used without purification in the next step.

To a solution of crude *cis*-8-(benzyloxy)-1-oxaspiro[4.5]decan-3-ol (520 mg) in CHCl_3 (8 ml) was added Dess-Martin reagent (1.34 g, 3.15 mmol) at 0 °C. After stirring at room temperature, the reaction mixture was quenched with saturated Na_2SO_3 aqueous solution (5 ml). The organic layer was concentrated *in vacuo*. The residue was diluted with EtOAc (20 ml). The organic layer was washed with H_2O and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **159** (486 mg, 37% for 2 steps) as a colorless oil. ^1H NMR ($\text{DMSO}-d_6$) δ : 7.39–7.22 (m, 5H), 4.50 (s, 2H), 3.95 (s, 2H), 3.48–3.37 (m, 1H), 2.39–2.35 (m, 2H), 1.89–1.77 (m, 2H), 1.77–1.60 (m, 4H), 1.57–1.43 (m, 2H).

***cis*-8-(Benzyloxy)-3,3-difluoro-1-oxaspiro[4.5]decane (160)**

To a solution of compound **159** (486 mg, 1.87 mmol) in CH_2Cl_2 (10 ml) was added dropwise bis(2-methoxyethyl)aminosulfur trifluoride (1.24 g, 5.61 mmol) at room temperature. After stirring at room temperature overnight, the reaction mixture was quenched with 4N NaOH aqueous solution (3 ml) at 0 °C. The aqueous layer was extracted with toluene. The combined organic layer was washed

with brine, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **160** (473 mg, 90%) as a colorless oil. ¹H NMR (DMSO-*d*₆) δ: 7.40–7.21 (m, 5H), 4.48 (s, 2H), 4.04–3.89 (m, 2H), 3.44–3.36 (m, 1H), 2.32–2.20 (m, 2H), 1.87–1.77 (m, 2H), 1.76–1.67 (m, 2H), 1.66–1.54 (m, 2H), 1.54–1.40 (m, 2H).

***cis*-1-Oxaspiro[4.5]decan-8-ol (116)**

To a solution of crude compound **152** (276 mg, 1.12 mmol) in MeOH (5 ml) was added 20% Pd(OH)₂-C (50% wet). The reaction mixture was hydrogenated (15 psi) at room temperature overnight. The reaction mixture was filtered through a celite pad and concentrated *in vacuo* to give compound **116** (173 mg, 99%) as a colorless oil.

¹H NMR (CDCl₃) δ: 3.89–3.76 (m, 3H), 1.99–1.85 (m, 4H), 1.79–1.64 (m, 4H), 1.58–1.34 (m, 5H).

***cis*-1-Oxaspiro[5.5]undecan-9-ol (119)**

Compound **119** was prepared from compound **155** in a manner similar to that described for compound **116**. A colorless oil (99%). ¹H NMR (DMSO-*d*₆) δ: 4.39–4.35 (m, 1H), 3.53–3.46 (m, 2H), 3.42–3.31 (m, 1H), 1.91–1.81 (m, 2H), 1.59–1.45 (m, 4H), 1.45–1.34 (m, 4H), 1.33–1.27 (m, 2H), 1.16–1.04 (m, 2H).

***cis*-3,3-Difluoro-1-oxaspiro[4.5]decan-8-ol (120)**

Compound **120** was prepared from compound **160** in a manner similar to that described for compound **116**. A white solid (99%). ¹H NMR (DMSO-*d*₆) δ: 4.52–4.43 (m, 1H), 4.01–3.90 (m, 2H), 3.51–3.40 (m, 1H), 2.28–2.17 (m, 2H), 1.85–1.72 (m, 2H), 1.62–1.38 (m, 6H).

3,3-Dimethyl-1,5,10-trioxadispiro[5.2.5⁹.2⁶]hexadec-11-en-13-one (162)

To a solution of 3,3-dimethyl-1,5-dioxaspiro[5.5]undecan-9-one **162** (16.3 g, 82.7 mmol) in 2-butanol (39 ml) was added dropwise *trans*-3-(*tert*-butyldimethylsilyloxy)-*N,N*-dimethyl-1,3-butadiene-1-amine (9.40 g, 41.3 mmol) at room temperature. After stirring at room temperature for 2 h, the reaction mixture was concentrated *in vacuo*. The residue was dissolved with Et₂O (78 ml). Acetyl chloride (3.53 ml, 49.6 mmol) was added dropwise to the solution at -78 °C. After stirring at -78 °C for 10 min, the reaction mixture was quenched with saturated NaHCO₃ aqueous solution (10 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **162** (6.93 g, 63% for 2 steps) as a white solid. ¹H NMR (CDCl₃) δ: 7.22 (d, *J* = 11.4 Hz, 1H), 5.36 (d, *J* = 19.3 Hz, 1H), 3.52 (s, 2H), 3.44 (s, 2H), 2.47 (s, 2H), 2.11–1.99 (m, 4H), 1.80–1.67 (m, 2H), 1.65–1.50 (m, 2H), 0.95 (s, 6H).

3,3-Dimethyl-1,5,10-trioxadispiro[5.2.5⁹.2⁶]hexadecan-13-one (163)

To a solution of compound **162** (3.50 g, 7.38 mmol) in MeOH (35 ml) was added 5% Pd-carbon (50% wet). The reaction mixture was hydrogenated (15 psi) at room temperature for 5 h. The reaction mixture was filtered through a celite pad and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give compound **163** (2.45 g,

69%) as a white solid. ^1H NMR (CDCl_3) δ : 7.22 (d, $J = 11.4$ Hz, 1H), 5.36 (d, $J = 19.3$ Hz, 1H), 3.52 (s, 2H), 3.44 (s, 2H), 2.47 (s, 2H), 2.11–1.99 (m, 4H), 1.80–1.67 (m, 2H), 1.65–1.50 (m, 2H), 0.95 (s, 6H). ^1H NMR (CDCl_3) δ : 3.99–3.91 (m, 2H), 3.50 (s, 2H), 3.47–3.41 (m, 2H), 2.46–2.38 (m, 2H), 2.32 (s, 2H), 2.06–1.90 (m, 2H), 1.85–1.64 (m, 4H), 1.59–1.44 (m, 2H), 0.94 (s, 6H).

13,13-Difluoro-3,3-dimethyl-1,5,10-trioxadispiro[5.2.5⁹.2⁶]hexadecane (164)

To a solution of compound **163** (2.45 g, 9.13 mmol) in CHCl_3 (25 ml) was added dropwise bis(2-methoxyethyl)aminosulfur trifluoride (6.73 ml, 36.5 mmol) at 0 °C. After stirring at room temperature for 3 h, the reaction mixture was quenched with MeOH (5 ml) and saturated NaHCO_3 aqueous solution (5 ml) at 0 °C. The aqueous layer was extracted with CHCl_3 . The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was filtered through a silica gel pad to give crude compound **164** (2.51 g) as a white solid. The crude material was used without purification in the next step.

cis-4,4-Difluoro-1-oxaspiro[5.5]undecan-9-ol (121)

A solution of crude compound **164** (2.51 g) in AcOH (5 ml) and H_2O (1 ml) was heated at 80 °C for overnight. After cooling to room temperature, the reaction mixture was diluted with H_2O (5 ml). The aqueous layer was extracted with toluene. The combined organic layer was washed with H_2O and saturated NaHCO_3 aqueous solution. The organic layer was dried over MgSO_4 , filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give 4,4-difluoro-1-oxaspiro[5.5]undecan-9-one (954 mg, 49% for 2 steps) as a colorless oil. ^1H NMR (CDCl_3) δ : 3.90–3.81 (m, 2H), 2.66–2.51 (m, 2H), 2.36–2.16 (m, 4H), 2.11–1.97 (m, 2H), 1.98–1.85 (m, 2H), 1.78–1.64 (m, 2H).

To a solution of 4,4-difluoro-1-oxaspiro[5.5]undecan-9-one (954 mg, 4.67 mmol) in MeOH (10 ml) was added NaBH_4 (124 mg, 3.28 mmol) at 0 °C. After stirring at 0 °C for 30 min, the reaction mixture was quenched with acetone (5 ml). The reaction mixture was concentrated *in vacuo*. The residue was diluted with H_2O (5 ml). The aqueous layer was extracted with EtOAc. The combined organic layer was dried over MgSO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give *trans*-isomer of compound **121** (less polar, 149 mg, 15%) and compound **121** (more polar, 820 mg, 85%) as white crystals.

trans-isomer of compound **121**: ^1H NMR (CDCl_3) δ : 3.99–3.91 (m, 1H), 3.83–3.73 (m, 2H), 2.02–1.73 (m, 8H), 1.71–1.62 (m, 2H), 1.61–1.42 (m, 3H).

compound **121**: ^1H NMR (CDCl_3) δ : 3.81–3.74 (m, 2H), 3.67–3.56 (m, 1H), 2.06–1.88 (m, 5H), 1.88–1.76 (m, 2H), 1.77–1.67 (m, 2H), 1.66–1.51 (m, 2H), 1.36–1.24 (m, 2H).

Experiments concerning biological activities and physicochemical properties

Measurements of GPR119 agonistic activity

GPR119 agonistic activity was evaluated in HEK293 cells overexpressing human GPR119 using a cAMP HiRange assay kit (Cisbio). The test compounds in stimulation buffer (Hanks' Balanced Salt Solution (Invitrogen), 20 mM HEPES, 0.1% bovine serum albumin, 0.1 mM 3-isobutyl-1-methylxanthine, and 0.1 mM 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone) were plated in 96-well half-area plates. The cells were plated onto the plates at 12500 cells/well in buffer mix (stimulation buffer with cAMP-d2, according to the manufacturer's protocol) then incubated for 30 min at room temperature. After that, anti-cAMP cryptate conjugate in lysis buffer was added into the wells and incubated for 3 h at room temperature in the dark. Cellular cAMP levels were measured using a micro plate reader. The EC₅₀ value of each compound was determined as the concentration of the test compound required to achieve 50% of the maximal oleoylethanolamide (OEA) stimulated response.

Measurements of solubility

The test compound solution (10 μ L) was placed in 96well plates, and DMSO was dried up with a centrifugal evaporator (model HT-4x, Genevac) for 2 h. JP1 or FeSSIF solvent (Each of 200 μ L) was added to each well. The plates were mixed at 2500 rpm at room temperature for 4 h. Incubation samples were filtered twice with 96well filtration plates (MSSLBPC50, Millipore). An aliquot of the second filtrates and acetonitrile were mixed, and injected into the LC/MS to quantify the amount of compounds in filtrates.

Measurements of inhibitory activities against six CYP isoforms

The test compounds (0.1, 1 or 10 μ M) with human liver microsomes (0.1 mg protein/mL) were preincubated for 20 min at 37 °C in a shaking water bath. Reactions were initiated by addition of NADPH-generating system (1.3 mM NADP⁺, 3.3 mM G-6-P, 0.4 IU/mL G6PDH, 3.3 mM MgCl₂) and a cocktail of probe substrates (See Table 13). After incubation for 20 min, the reactions were terminated by addition of acetonitrile containing SI labeled each makers as an internal standard. Samples were centrifuged at 3000 rpm for 20 min at room temperature. An aliquot of the supernatant was injected into LC/MS/MS. The inhibitory effects of the test compounds on the CYP activities were evaluated by measuring the specific metabolites of each CYP substrate.

Table 13. Cocktail of probe substrates

Enzyme	Probe substrate	Concentration of probe substrate	Marker activity of Enzyme
CYP3A4	Midazolam (M)	1 μ M	1-hydroxylation
	Testosterone (T)	10 μ M	6 β -hydroxylation
CYP2C9	Diclofenac	1 μ M	4-hydroxylation
CYP2D6	Bufuralol	1 μ M	1-hydroxylation
CYP1A2	Ethoxyresorufin	0.5 μ M	Resorufin
CYP2C8	Amodiaquine	0.05 μ M	Desethylamodiaquine
CYP2C19	(S)-mephenytoin	80 μ M	4-hydroxylation

Measurements of metabolic stability

The test compounds (5 μ M) were incubated with human or rat liver microsomes (0.2 mg protein/mL) at 37 °C for 0, 10 and 60 min. Reactions were terminated by adding 400 μ L of acetonitrile containing 0.1% formic acid. The samples were centrifuged (10000 rpm, 4 °C, 5 min), and aliquots (5 μ L) of the supernatant were injected into the UPLC/MS.

Protein binding assay

96-Well Equilibrium Dialysis Block, model HTD96a (HTDialysis, LLC) was used to determine the plasma protein binding. 150 μ L each of plasma side test solution containing 5 μ M compound and D-PBS were loaded in the plasma side and buffer side of the apparatus, respectively. After incubation at 37 °C for 5 hr, plasma side and buffer side samples were transferred into a deep well plate, and were mixed with twice the volume of acetonitrile, and were then centrifuged at (5000 rpm, 15 °C, 5 min). The concentrations of test compounds in the supernatant were determined by LC/MS/MS.

The percentage of the unbound fraction in plasma was calculated using the formula below.

Percentage of the unbound fraction (%)

= Concentration in buffer side solution after equilibrium dialysis (μ M) / Concentration in plasma side solution after equilibrium dialysis (μ M) \times 100

Protein binding in plasma was calculated using the formula below.

Protein binding (%) = 100 – percentage of unbound fraction (%)

Pharmacokinetics analysis in rats

Male CD (SD) rats (Charles River Laboratories (Japan)) were intravenously or orally administered a single dose of the test compounds at 1.0 mg/kg (DMSO, solution) or 3.0 mg/kg (0.5% methyl cellulose, suspension), respectively. After the administration, the plasma samples were collected over

a period of 25 h. Plasma samples were mixed with twice the volume of acetonitrile, and were then centrifuged at 4 °C and 11000×g for 5 min. The concentrations of test compounds in the supernatant were determined by LC/MS. The time-course of the plasma concentrations of the compounds were analyzed by non-compartmental analysis and the pharmacokinetic parameters were calculated.

Intraperitoneal glucose tolerance tests (ipGTT) in SD rats

The ipGTT was performed in the rats (7 weeks of age) after overnight fasting. Compounds were administered by oral gavage. After 30 minutes or 16 hours, glucose (1 g/kg of body weight) was administered intraperitoneally to rats and blood samples were collected from the tail vein prior to and at 10, 30 and 60 minutes after glucose loading. Plasma glucose levels were measured using an automatic analyzer (Hitachi7170S, Tokyo, Japan). Plasma insulin levels were measured with a rat-insulin ELISA kit (Morinaga Institute of Biological Science, Yokohama, Japan).

Statistical analysis

For the evaluation of the results, statistical analyses were performed for the ΔAUC_{0-2hr} of the plasma glucose levels and the ΔAUC_{0-1hr} of the plasma insulin levels as follows.

Differences between the Vehicle and Test article group was tested with an F-test, followed by Student's t test because the variances were equal. Differences were considered significant at $p < 0.05$ (two-sided).

For comparisons more than two groups, differences between the Vehicle and Test article groups were tested by the Bartlett's homogeneity of variance test, followed by Dunnett's multiple comparison test because the variances were equal. Differences were considered significant at $p < 0.05$ (two-sided).

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