平成 26 年度　博士学位論文

糖代謝関連疾患治療を目指した低分子化合物の創製研究

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目次

略語

本論文中で用いられる略語表記法一覧 (アルファベット順)

Ac	acetyl
aq.	aqueous
Ar	aryl
t-Bu	*tert*-butyl
Bn	benzyl
Boc	*tert*-butoxycarbonyl
conc.	concentrated
DAG	diacylglycerol
DHAP	dihydroxyacetone phosphate
DHN	1,4-dihydroxynonene
DIBAL-H	diisobutylaluminium hydride
DMAP	4-(*N, N*-dimethyamino)pyridine
DMF	*N, N*-dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethylsulfoxide
EC_{50}	half maximal effective concentration
ELISA	enzyme-linked immune sorbent assay
ent	enantiomer
Et	ethyl
FT-IR	fourier transform infrared spectroscopy
GSH	glutathione
HbA1c	hemoglobin A1c
IC_{50}	half maximal inhibitory concentration
iNOS	inducible nitric oxide synthase
Me	methyl
HRMS	high resolution mass spectrometry
mp	melting point
MS	mass spectrometry
NF-κB	nuclear factor κB
NMO	*N*-methylmorpholine *N*-oxide

NMR	nuclear magnetic resonance
NO	nitric oxide
NOE	nuclear Overhauser effect
PCC	pyridinium chlorochromate
PKC	protein kinase C
Ph	phenyl
PLC	phospholipase C
Py	pyridine
quant.	quantitative
ROS	radical oxygen species
rt	room temperature
sat.	saturated
TBAF	tetrabutylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TMP	2,2,6,6-tetramethylpiperidine
TNF-α	tumor necrosis factor α
Ts	*p*-toluenesulfonyl

序論

　生体のエネルギー源かつ構成成分として機能する糖は重要な生体内分子の一つである。この糖の中でもグルコースは脳の活動を維持するために必須の分子として知られている。一方, マンノースやガラクトースをはじめとする単糖から構成される糖鎖はタンパク質や脂質と複合体を形成し, 細胞間の認識や相互作用, 組織の保護作用など様々な役割を担っている。このように糖は広範な生理作用を有するため, 糖の正常な代謝機能が失われることは様々な疾患の発症につながる。

　糖代謝が関連する疾患の代表例としては糖尿病が挙げられる。糖尿病の中でもその約 90%を占める 2 型糖尿病は, インスリン抵抗性やインスリンの分泌低下などの特徴を示す疾患である。サイレントキラーとも呼ばれる糖尿病は, それ自体は特に目立った自覚症状を示さないが, 長期にわたる高血糖状態の持続は3大合併症に代表される重篤な糖尿病性合併症につながることから, 適切に血糖コントロールを行うことが糖尿病治療においては重要である。しかしながら, 適切に血糖コントロールをしていても個人差などにより糖尿病性合併症を発症してしまうケースがあり, 優れた糖尿病性合併症治療薬が少ない現在において, 効果的な薬剤の開発は重要な研究課題である。一方, 糖尿病治療薬においては様々な作用機序に基づく治療薬が存在し, 近年は dipeptidyl peptidase-IV (DPP-IV) 阻害薬や Glucagon-like peptide-1 (GLP-1) 受容体作動薬といったインクレチン関連薬の台頭が著しい。これらの薬剤は新規性に加え, グルコース濃度依存的にインスリン分泌を促すことから, 低血糖を起こしにくい薬剤としても注目されている。しかしながら, 患者数が激増している糖尿病の治療薬開発においては, 患者の利便性向上や治療選択肢を広げるために現在でも精力的に研究が展開されている。

　また, 上述した糖鎖の生理的な役割は完全には明らかにされていないが, ウィルス, 細菌による感染症やがんの発生, 転移などの疾患発症に関与するといわれている。この糖鎖の形成, 修飾に深く関与しているグリコシダーゼをうまくコントロールする化合物は, 基礎研究などのツールとしてだけでなく, 医薬品開発など幅広い応用研究を発展させる可能性がある。

　以上の背景のもと, 著者は糖代謝関連疾患治療を目指し, 医薬品候補化合物

として期待できる低分子化合物の創製あるいはそれら分子の創出を可能とする手法を確立すること目的として研究を実施した。第一章では糖尿病性合併症 (神経障害) 治療における標的酵素である aldo-keto reductase family 1, member B1 (AKR1B1) 選択的阻害剤の開発，第二章では糖尿病の治療標的である GLP-1 に焦点を当て，そして第三章では様々な病態に密接に関連するグリコシダーゼを標的とした研究を展開した。

　詳細について以下に論述する。

各論

## 第一章　選択的 AKR1B1 阻害剤開発を目指したチオアミド誘導体の創製

第一節　AKR1B1 及び関連疾患について

　近年のライフスタイルの変化に伴い，糖尿病患者が激増しており，2014年現在の世界の有病者数は 3 億 8670 万人，20-79 歳の成人の有病率は 8.3%にまで達し，年々増加の一途を辿っている。中でもアジアをはじめとする新興国での増加傾向は顕著であり，有用な薬剤の開発は急務である。

　糖尿病の真の恐ろしさは高血糖持続に伴う糖尿病性合併症の発症にある。そのため，糖尿病患者においては適切に血糖コントロールを行うと同時に合併症の発症を予防することが重要である。この血糖コントロールについては近年，インクレチン関連薬と呼ばれる薬剤の台頭が目覚ましく，詳しくは第二章で触れる。本章ではもう一方の糖尿病性合併症について以下記述する。

　糖尿病性合併症の中でも網膜症，腎症，神経障害の 3 つの細小血管障害は 古くより 3 大合併症として認識されている。網膜症は，高血糖状態が 5-10 年続くことにより網膜など眼底に存在する細小血管が閉塞する，あるいは破裂することで，末期には失明につながる可能性がある疾患である [1)-5)]。腎症は高血糖状態により腎機能障害が引き起こされることでタンパク尿などを発症する疾患であり，進行すると透析治療が必要になることがある [6)]。神経障害も同様に高い血糖状態が続くことによって神経が障害をきたす疾患であり，自律神経が障害されると起立性低血圧症や排尿障害などが，末梢神経が障害されると四肢末端の痛みや痺れなどがそれぞれ引き起こされる [7)]。以上のように糖尿病性合併症発症の第一要因は，長期にわたる高血糖状態であり血糖をコントロールすることが合併症の発症，進展を妨げることに有効である [8)-9)]。しかしながら，糖尿病は自覚症状が少なく，ある程度病気が進行した後で病気が発覚するケースや適切な血糖コントロールを行っているにもかかわらず，糖尿病性合併症を引き起こすケースもあり，優れた糖尿病性合併症治療薬の開発が望まれている。

糖尿病性合併症の発生機序は諸説挙げられている。その中でも AKR1B1 (以下に示す別名も使用されている: EC 1. 1. 1. 21, ALR2, AR, aldose reductase) は糖尿病性合併症の発症に深く関与する酵素として特に注目されている。以下に詳細を記述する。

　正常な血糖状態において，生体内に存在する大部分の glucose は hexokinase により代謝される。しかしながら，糖尿病などの疾患により高血糖状態が続くと hexokinase による代謝機能が飽和し，25-30%の glucose が代替経路によって代謝されるようになる [10]。この代替経路は polyol 経路と呼ばれ，glucose が AKR1B1 により sorbitol に変換され，さらに sorbitol dehydrogenase (EC 1. 1. 1. 14, SD) により fructose に代謝される経路を指す。Polyol 経路が活性化されることによる sorbitol , fructose の産生，補酵素 NADPH の過剰消費が，浸透圧ストレス，酸化ストレス，糖化ストレス，炎症性ストレス，生体内ストレスなどの生体内ストレスを引き起こし，最終的には各種合併症の発症につながっていく (**Figure 1**)。
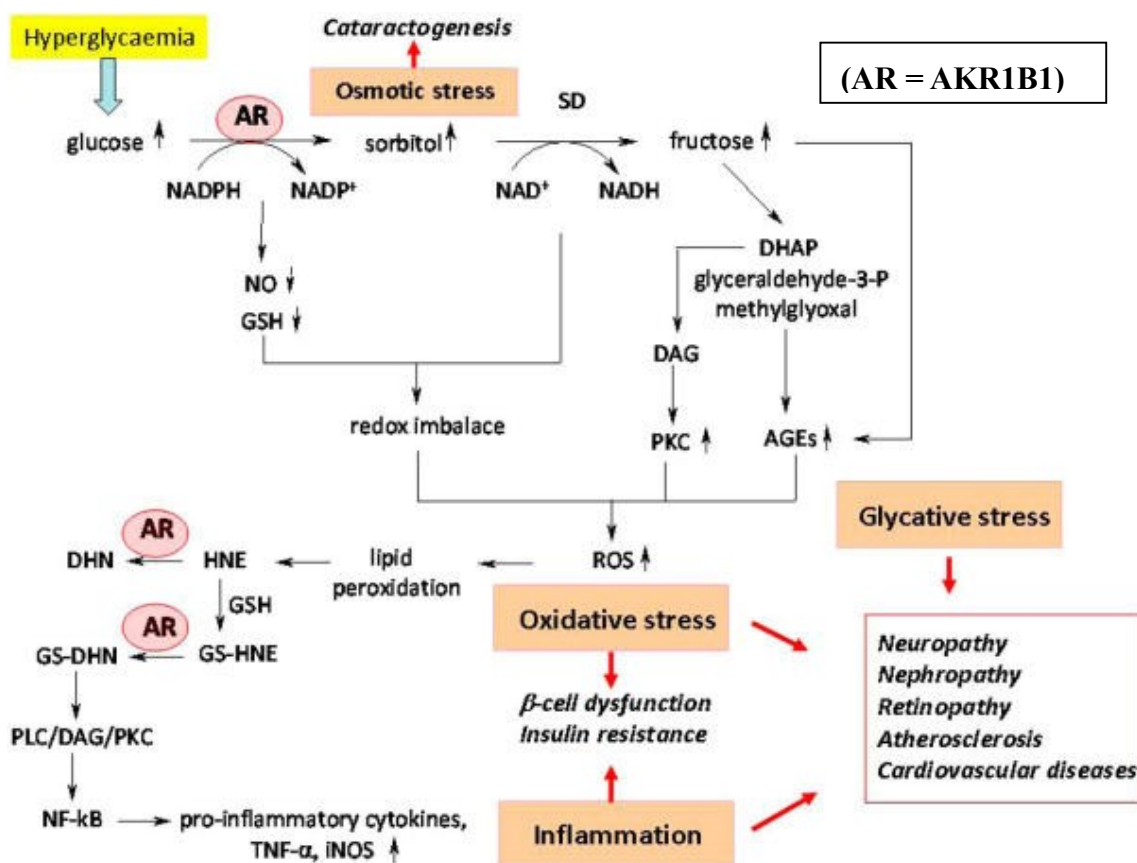
Figure 1. Role of AR (AKR1B1) in the development of diabetic complications. (*J. Med. Chem.* Article ASAP, **DOI:** 10.1021/jm500907avより抜粋)

従って，polyol 経路の第一段階の代謝過程を担う AKR1B1 を阻害することは，その下流に位置する生体内分子の生成を抑制し，ひいては糖尿病性合併症治療につながると考えられる [11)-14)]。以下に，既存の AKR1B1 阻害薬 (ARIs：AKR1B1 inhibitors) の構造を示す(**Figure 2**) [15)]。
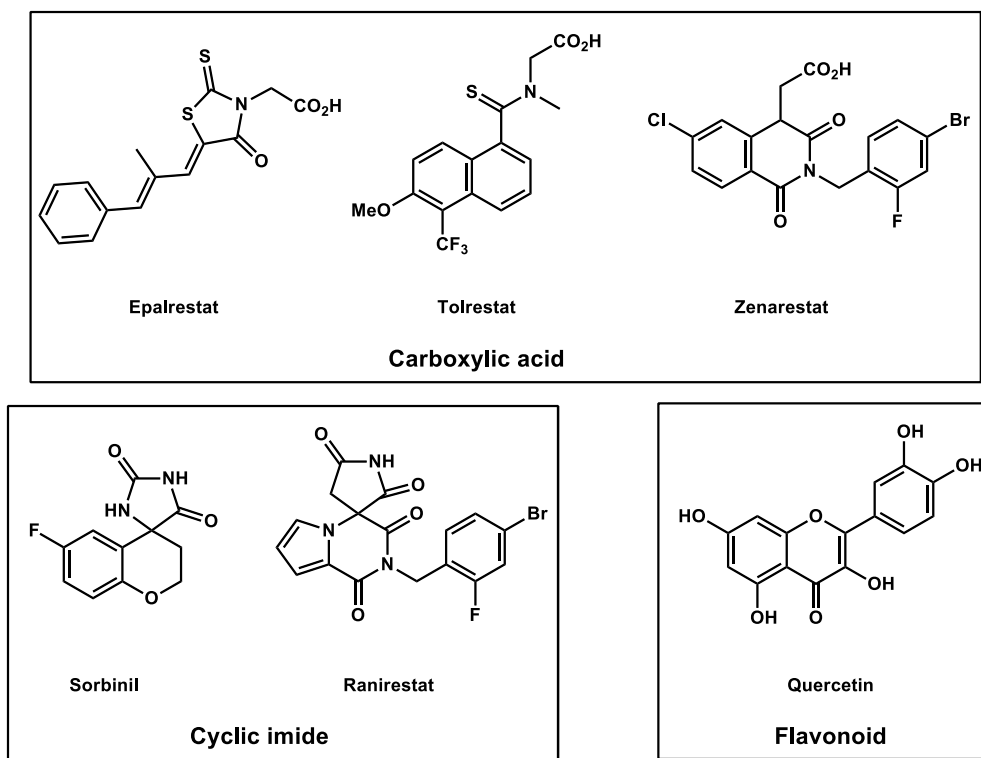
Figure 2. Structures of ARIs.

　ARIsはその構造的特徴からepalrestat, tolrestatなどに代表されるカルボン酸グループ, sorbinil, ranirestatなどの環状イミドグループ, quercetinに代表されるフラボノイドグループの主に3つのグループに分類されている。このように様々な医薬品候補化合物が古くから開発されているにもかかわらず，多くの開発品目は，臨床試験において副作用，毒性が発現してしまうケースや期待通りに薬効が得られないケースなどの問題からドロップアウトしている。例えば，カルボン酸グループに属するzenarestatは肝障害や腎障害を引き起こし，環状イミドグループに属するsorbinilは皮疹などの過敏性反応が副作用として強く発現することが報告されている[15)]。現在本邦においてAKR1B1阻害に基づきepalrestat (糖尿病性神経障害に適応) 一品目のみが臨床利用されているものの，本薬剤が有色である

ことに伴い，長期服用により汗，涙，尿が着色するなどの理由から服薬コンプライアンスが守られないといった臨床利用上の問題点が指摘されている。

　一方，生体内における酸化ストレスの亢進は様々な炎症性疾患につながることが知られているが，近年，この炎症性疾患において AKR1B1 が過剰発現していることが明らかになり，マウスやラットにおいてではあるが，実際に既存のARIs が炎症性疾患を改善することが確認されている [16)-20)]。この事実は，ARIs が糖尿病性合併症のみならず，がん，敗血症，アテローム性動脈硬化症，関節リウマチなどの炎症性疾患治療にも応用可能であることを示唆している。従って，上述した副作用の発症を抑えることは医薬品開発の進展に貢献することにつながる。

　この副作用発症の原因のひとつとして AKR1B1 の同属酵素である AKR1A1 (EC 1. 1. 1. 2, ALR1, aldehyde reductase)[21)] やさらに近年，類縁酵素であるAKR1B10 が同定され，これらの酵素に対する阻害が考えられている。そのため，AKR1A1 ならびに，AKR1B10 に対する阻害作用を示さず，AKR1B1 への高い酵素阻害活性を獲得することがより安全性の高い薬剤につながると考えられる。

　このAKR1A1は最終糖化産物 advanced glycation end products (AGEs) の前駆体である2-oxoaldehydes, 3-deoxyglucosone, methylglyoxalなどの毒性を示すアルデヒドの解毒に関与している。他方，AKR1B10は，AKR1A1 と同様にHNE, glyceraldehydeといったアルデヒドの解毒に関与している酵素である。また，本酵素はAKR1B1とアミノ酸配列が71%一致し[22)]，さらに高次構造も極めて類似しており，その相同性の高さから選択性を獲得することは容易ではなく，AKR1B1阻害剤は同時にAKR1B10阻害剤としても機能してしまうことが多い。実際，本研究開始当時，AKR1B1に選択的な阻害剤についての報告例は皆無であった。

　以上の背景をもとに，著者はAKR1B1に選択的な低分子化合物の創製を目的として研究に着手した。

第二節　AKR1B1 選択的阻害剤の合成とその活性評価

　著者らのグループでは，薬用植物である*Evodia rutaecarpa* (呉茱萸) に含まれるrhetsinine (**Figure 3**) がAKR1B1に対し阻害作用を示し，sorbitolの蓄積を抑制することを既に明らかにしている[23]。そこで，阻害作用の増強と先に述べたAKR1A1やAKR1B10との酵素阻害選択性の向上を目指し，rhetsinineの構造をモチーフとした誘導体の合成に着手した。
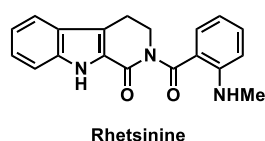
Rhetsinine

Figure 3. Structure of Rhetsinine.

　前節で既存のARIsは構造的特徴によって3種類に大別されていることは既に述べたが，その中でカルボン酸タイプに共通して含まれる酢酸ユニットを導入することで活性の向上を期待した。そこで，化合物**4**の合成を行った (**Scheme 1**)。文献既知の三環系ラクトン**1a**[24] に対しneat条件下*p*-fluorobenzylamineを用いラクタム**2a**としたのち，ブロモ酢酸メチルを用いて酢酸ユニットを導入し，最後に加水分解により化合物**4**を合成した。
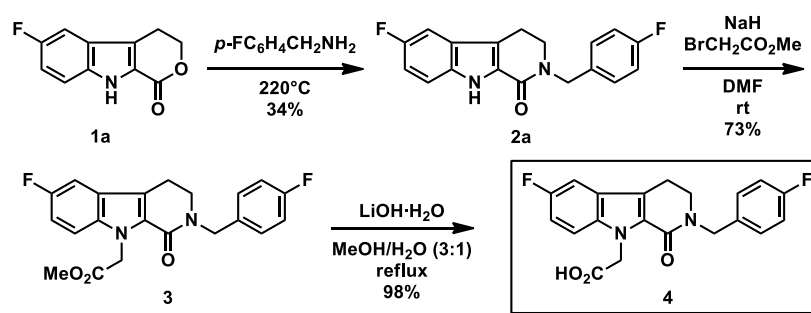
Scheme 1. Synthesis of **4**.

　得られた化合物**4**の酵素阻害活性について評価を行った (**Table 1**)。AKR1B1に対する阻害活性 (IC$_{50}$) は48.6 μMを示し，epalrestatと比較するとその活性はかな
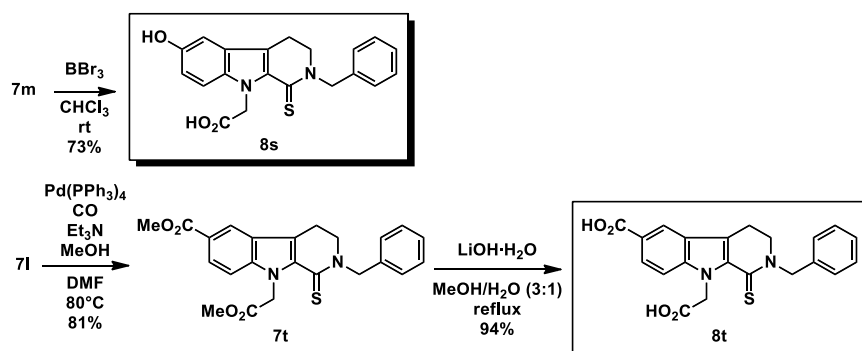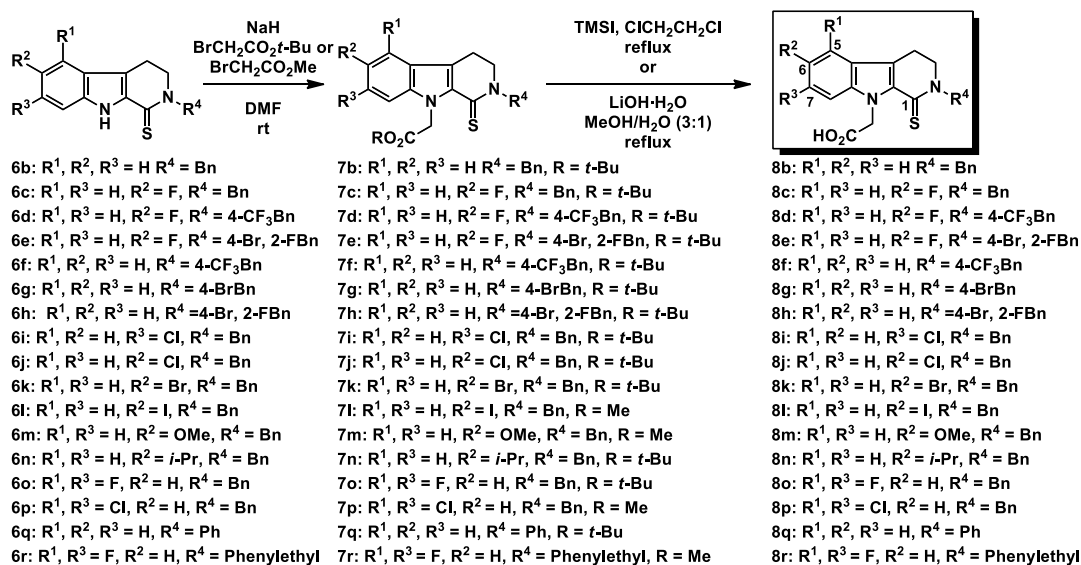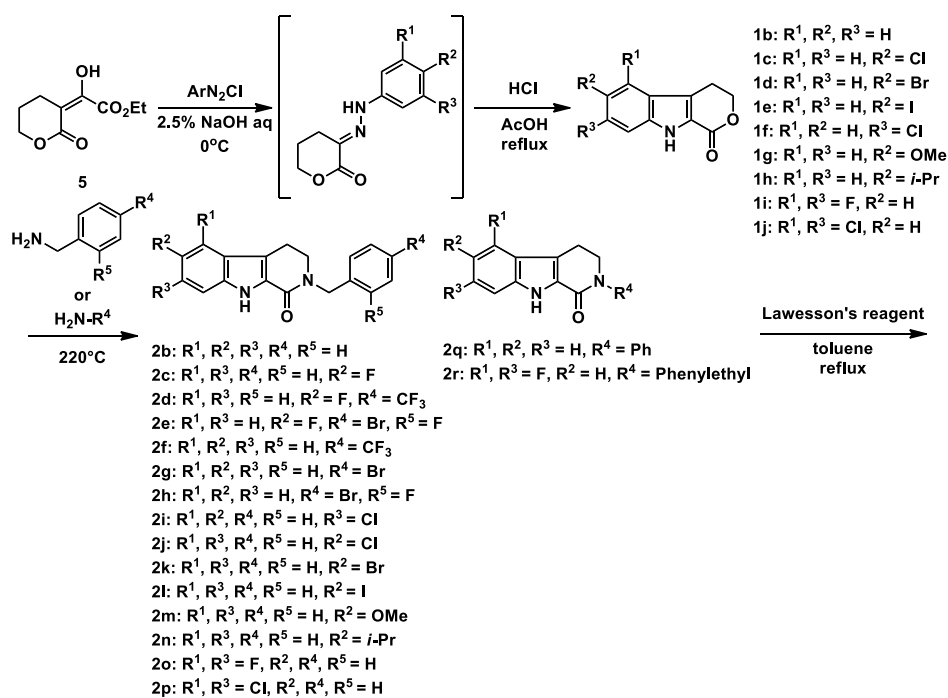
り劣る結果となった。そこで，活性の増強を目的として更なる分子デザインを行った。

Table 1. Inhibitory potency and selectivity of tricyclic carboxylic acids for AKR1A1, AKR1Bl and AKR1B10.

Compound	IC_{50} (μM)			selectivity index	
	AKR1A1	AKR1B1	AKR1B10	(AKR1A1/AKR1B1)	(AKR1B10/AKR1B1)
4	ND	48.6	ND	-	-
Epalrestat	2.6	0.10	0.33	26	3.3

ND: Not determined.

　既存のARIsの構造を再度見直すとepalrestatやtolrestatがチオアミド構造を有していたため，**4**のラクタム構造をチオアミド構造へと変換すれば高い阻害活性が得られるのではないかと期待し，チオアミド**8**の合成と活性評価を検討した。**Scheme 1**と同様，各種ラクタム**2b-2r**とし，Lawesson's reagentを用いてチオアミド**6b-6r**を得た。最後に酢酸ユニットを導入し**8b-8r**を合成した。化合物**8s**に関してはルイス酸であるBBr$_3$を用いて化合物**7m**のメチルエーテルと*t*-ブチルエステル基を同時に切断することで合成し，化合物**8t**については，化合物**7l**のCO挿入，続くMeOH処理とその後の加水分解により合成した **(Scheme 2)**。

1b: $R^1, R^2, R^3 = H$
1c: $R^1, R^3 = H, R^2 = Cl$
1d: $R^1, R^3 = H, R^2 = Br$
1e: $R^1, R^3 = H, R^2 = I$
1f: $R^1, R^2 = H, R^3 = Cl$
1g: $R^1, R^3 = H, R^2 = OMe$
1h: $R^1, R^3 = H, R^2 = i\text{-Pr}$
1i: $R^1, R^3 = F, R^2 = H$
1j: $R^1, R^3 = Cl, R^2 = H$

2b: $R^1, R^2, R^3, R^4, R^5 = H$
2c: $R^1, R^3, R^4, R^5 = H, R^2 = F$
2d: $R^1, R^3, R^5 = H, R^2 = F, R^4 = CF_3$
2e: $R^1, R^3 = H, R^2 = F, R^4 = Br, R^5 = F$
2f: $R^1, R^2, R^3, R^5 = H, R^4 = CF_3$
2g: $R^1, R^2, R^3, R^5 = H, R^4 = Br$
2h: $R^1, R^2, R^3 = H, R^4 = Br, R^5 = F$
2i: $R^1, R^2, R^4, R^5 = H, R^3 = Cl$
2j: $R^1, R^3, R^4, R^5 = H, R^2 = Cl$
2k: $R^1, R^3, R^4, R^5 = H, R^2 = Br$
2l: $R^1, R^3, R^4, R^5 = H, R^2 = I$
2m: $R^1, R^3, R^4, R^5 = H, R^2 = OMe$
2n: $R^1, R^3, R^4, R^5 = H, R^2 = i\text{-Pr}$
2o: $R^1, R^3 = F, R^2, R^4, R^5 = H$
2p: $R^1, R^3 = Cl, R^2, R^4, R^5 = H$

2q: $R^1, R^2, R^3 = H, R^4 = Ph$
2r: $R^1, R^3 = F, R^2 = H, R^4 = Phenylethyl$

6b: $R^1, R^2, R^3 = H\ R^4 = Bn$
6c: $R^1, R^3 = H, R^2 = F, R^4 = Bn$
6d: $R^1, R^3 = H, R^2 = F, R^4 = 4\text{-CF}_3Bn$
6e: $R^1, R^3 = H, R^2 = F, R^4 = 4\text{-Br, 2-FBn}$
6f: $R^1, R^2, R^3 = H, R^4 = 4\text{-CF}_3Bn$
6g: $R^1, R^2, R^3 = H, R^4 = 4\text{-BrBn}$
6h: $R^1, R^2, R^3 = H, R^4 = 4\text{-Br, 2-FBn}$
6i: $R^1, R^2 = H, R^3 = Cl, R^4 = Bn$
6j: $R^1, R^3 = H, R^2 = Cl, R^4 = Bn$
6k: $R^1, R^3 = H, R^2 = Br, R^4 = Bn$
6l: $R^1, R^3 = H, R^2 = I, R^4 = Bn$
6m: $R^1, R^3 = H, R^2 = OMe, R^4 = Bn$
6n: $R^1, R^3 = H, R^2 = i\text{-Pr}, R^4 = Bn$
6o: $R^1, R^3 = F, R^2 = H, R^4 = Bn$
6p: $R^1, R^3 = Cl, R^2 = H, R^4 = Bn$
6q: $R^1, R^2, R^3 = H, R^4 = Ph$
6r: $R^1, R^3 = F, R^2 = H, R^4 = Phenylethyl$

7b: $R^1, R^2, R^3 = H\ R^4 = Bn, R = t\text{-Bu}$
7c: $R^1, R^3 = H, R^2 = F, R^4 = Bn, R = t\text{-Bu}$
7d: $R^1, R^3 = H, R^2 = F, R^4 = 4\text{-CF}_3Bn, R = t\text{-Bu}$
7e: $R^1, R^3 = H, R^2 = F, R^4 = 4\text{-Br, 2-FBn}, R = t\text{-Bu}$
7f: $R^1, R^2, R^3 = H, R^4 = 4\text{-CF}_3Bn, R = t\text{-Bu}$
7g: $R^1, R^2, R^3 = H, R^4 = 4\text{-BrBn}, R = t\text{-Bu}$
7h: $R^1, R^2, R^3 = H, R^4 = 4\text{-Br, 2-FBn}, R = t\text{-Bu}$
7i: $R^1, R^2 = H, R^3 = Cl, R^4 = Bn, R = t\text{-Bu}$
7j: $R^1, R^3 = H, R^2 = Cl, R^4 = Bn, R = t\text{-Bu}$
7k: $R^1, R^3 = H, R^2 = Br, R^4 = Bn, R = t\text{-Bu}$
7l: $R^1, R^3 = H, R^2 = I, R^4 = Bn, R = Me$
7m: $R^1, R^3 = H, R^2 = OMe, R^4 = Bn, R = Me$
7n: $R^1, R^3 = H, R^2 = i\text{-Pr}, R^4 = Bn, R = t\text{-Bu}$
7o: $R^1, R^3 = F, R^2 = H, R^4 = Bn, R = t\text{-Bu}$
7p: $R^1, R^3 = Cl, R^2 = H, R^4 = Bn, R = Me$
7q: $R^1, R^2, R^3 = H, R^4 = Ph, R = t\text{-Bu}$
7r: $R^1, R^3 = F, R^2 = H, R^4 = Phenylethyl, R = Me$

8b: $R^1, R^2, R^3 = H\ R^4 = Bn$
8c: $R^1, R^3 = H, R^2 = F, R^4 = Bn$
8d: $R^1, R^3 = H, R^2 = F, R^4 = 4\text{-CF}_3Bn$
8e: $R^1, R^3 = H, R^2 = F, R^4 = 4\text{-Br, 2-FBn}$
8f: $R^1, R^2, R^3 = H, R^4 = 4\text{-CF}_3Bn$
8g: $R^1, R^2, R^3 = H, R^4 = 4\text{-BrBn}$
8h: $R^1, R^2, R^3 = H, R^4 = 4\text{-Br, 2-FBn}$
8i: $R^1, R^2 = H, R^3 = Cl, R^4 = Bn$
8j: $R^1, R^3 = H, R^2 = Cl, R^4 = Bn$
8k: $R^1, R^3 = H, R^2 = Br, R^4 = Bn$
8l: $R^1, R^3 = H, R^2 = I, R^4 = Bn$
8m: $R^1, R^3 = H, R^2 = OMe, R^4 = Bn$
8n: $R^1, R^3 = H, R^2 = i\text{-Pr}, R^4 = Bn$
8o: $R^1, R^3 = F, R^2 = H, R^4 = Bn$
8p: $R^1, R^3 = Cl, R^2 = H, R^4 = Bn$
8q: $R^1, R^2, R^3 = H, R^4 = Ph$
8r: $R^1, R^3 = F, R^2 = H, R^4 = Phenylethyl$

Scheme 2. Synthesis of **8b-8t**.

13

得られた化合物の酵素阻害活性評価を行った (**Table 2**)。AKR1B1阻害活性について構造活性相関から得られた知見を以下に示す (**Figure 4**.)。

Table 2. Inhibitory potency and selectivity of tricyclic carboxylic acids for AKR1A1, AKR1Bl and AKR1B10.

Compound	IC$_{50}$ (μM)			selectivity index	
	AKR1A1	AKR1B1	AKR1B10	(AKR1A1/AKR1B1)	(AKR1B10/AKR1B1)
8b	9.5	0.22	5.1	43	23
8c	19	0.23	3.3	83	14
8d	(27%)	10	1.6	-	0.2
8e	(45%)	1.7	0.28	-	0.2
8f	(45%)	14	3.8	-	0.3
8g	14	5.9	0.85	2.4	0.1
8h	(45%)	4.2	0.68	-	0.2
8i	21	0.24	1.4	88	5.8
8j	15	0.35	1.4	43	4
8k	11	0.32	1.4	34	4.4
8l	11	0.19	1.4	58	7.4
8m	**12**	**0.15**	**2.9**	**80**	**19**
8n	16	0.20	3.4	80	17
8o	10	0.19	6.1	53	32
8p	4.7	1.3	0.48	3.6	0.4
8q	(2%)	31	21	-	0.6
8r	(39%)	1.8	0.4	-	0.2
8s	(28%)	0.2	1.9	-	10
8t	**53**	**0.17**	**40**	**312**	**253**
Epalrestat	2.6	0.10	0.33	26	3.3

ND: Not determined. The values in parentheses are inhibition percentages by 20μM
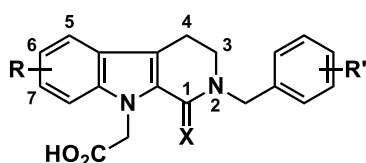
R = halogen, alkyl, alkoxy, OH, CO$_2$H
R' = halogen, CF$_3$
X = O, S

Figure 4. Structure of (1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acids.

　i) ラクタム (X = O, 化合物**4**) よりもチオアミド (X = S) の方が阻害活性は強く, 硫黄原子の導入が活性向上に大きく寄与する, ii) C-6位の誘導体 (**8b**, **8c**, **8j-8l**, **8m-8n**, **8s-8t**) においてはその阻害活性に大きな差は認められない, iii) 合

成した誘導体の中ではOMe体**8m**, CO$_2$H体**8t** が特に強力な阻害活性を示す, iv) チオアミド窒素上 (2位) 側鎖のベンゼン環上, R'置換基としてCF$_3$基を有する**8f** やhalogenを有する**8g**, **8h**においては, 無置換体**8b**と比較して阻害活性の減弱が認められた, v) チオアミド窒素上 (2位) にPh基をもつ**8q**, phenylethyl基をもつ**8r** では, 対応するベンジル体**8b**, **8o**と比較して阻害活性の低下が認められた。

　次に酵素阻害選択性について着目すると, まず, AKR1A1については, 数十倍以上の高い選択性を示す化合物が多く見出された。一方, AKR1B10については予想していた通り, AKR1B1の阻害剤は同時にAKR1B10の阻害剤として機能し, AKR1B1よりもむしろAKR1B10に対しより強く阻害能を示す化合物が発見された (**8d-8h**, **8p-8r**)。しかしながら, 唯一, 化合物**8t**は優れた酵素阻害選択性を示すことが明らかになった (AKR1A1/AKR1B1 = 312, AKR1B10/AKR1B1 = 253)。本化合物はepalrestatと比べて数十倍以上の極めて高い選択性を獲得しており, 副作用の少ない薬剤として期待される。

　最も選択性に優れた化合物**8t**のbinding conformationについてコンピュータを用いてdocking studyを行った (**Figure 5**)。AKR1B1においてはベンジル基が結合部位の奥深くに入り込み疎水性相互作用を形成し, 三環系骨格がW219と π−π stacking, CH-π 相互作用を形成していることが明らかになった (**Figure 5** (A))。また, インドール窒素上酢酸ユニットのカルボン酸がS302と強力な水素結合を形成していることが示された。一方, AKR1B10とのbinding conformationはAKR1B1のそれと大きく異なり, ベンジル基がF123と疎水性相互作用, V301との CH-π 相互作用を形成し, C-6位上のカルボン酸がY49と弱い水素結合を形成していることが示された (**Figure 5** (B))。両酵素それぞれの結合エネルギーは AKR1B1 (Glide Score: -8.60 kcal/mol), AKR1B10 (Glide Score: -7.76 kcal/mol) を示し, binding conformationの違いがエネルギー差をもたらしていることが明らかになった。
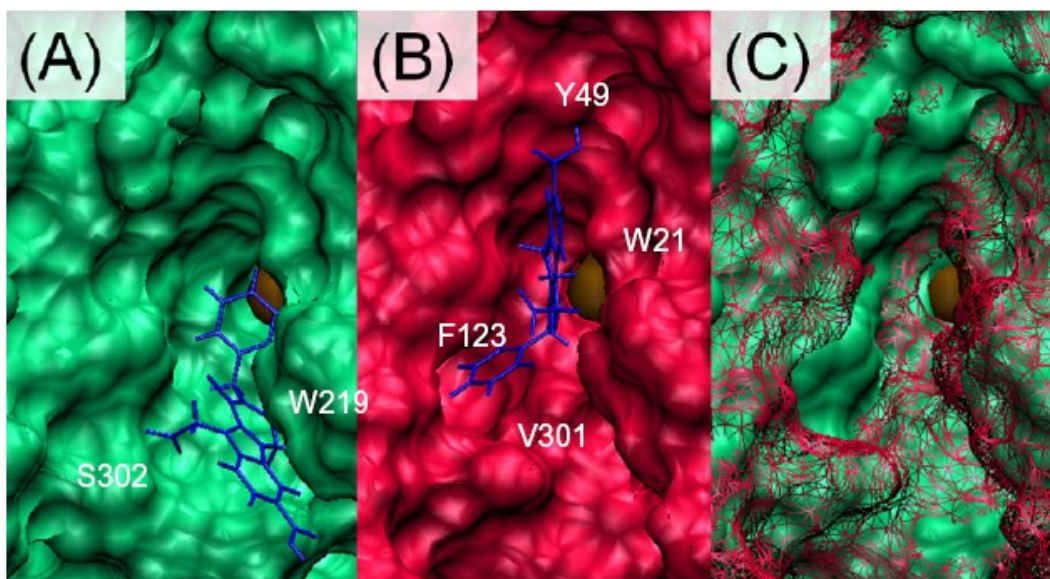
Figure 5. The binding conformation of the **8t** (blue stick) for AKR1B1 (A) and for AKR1B10 (B), and the difference of binding pocket (C) between AKR1B1 (green solid surface) and AKR1B10 (red wireframe).

第二章　GLP-1 分泌促進作用を有する新規三環系化合物の創製

第一節　GLP-1 及び関連する研究について

　人体は多様なメカニズムによって血糖恒常性を維持している。膵臓から分泌されるインスリンは骨格筋及び脂肪組織におけるグルコース吸収亢進を介して血糖を降下させる重要なホルモンの一種である。

　一方，糖を経口投与すると経静脈投与時よりもインスリンの作用が強く発現することが報告され，その原因としてインクレチンホルモンとして知られる消化管ホルモンの関与が明らかにされた。インクレチンホルモンは主に，下部小腸及び大腸を中心に存在するL細胞から分泌される GLP-1，上部小腸を中心に存在するK細胞から分泌される Glucose-dependent insulinotropic polypeptide (GIP) に分類されている。このインクレチンホルモンは，食事の摂取による血糖値の上昇を引き金にその分泌が促され，インスリンの生合成を亢進する。続いてGLP-1について以下記述する。

　GLP-1 はその受容体である GLP-1 receptor (GLP-1R) を介して多彩な生理作用を示すことが知られている。この GLP-1R は class B1 に分類される G タンパク質共役受容体 (G-protein-coupled receptors, GPCRs) であり，ペプチドホルモンに応答する細胞表面タンパク質である。本受容体はグルカゴン前駆体 (プログルカゴン) より生成される 4 種の GLP-1 peptides (GLP-1 (1-37), GLP-1 (7-37), GLP-1 (1-36)NH$_2$, GLP-1 (7-36)NH$_2$) 及び類似構造を有するオキシントモジュリンを内因性リガンドとしている [25)-26)]。膵臓, 心臓, 脳, 腎臓, 腸管といった主要臓器に分布し [27)-28)], 活性化を受けると以下に示す生理作用を発現する (**Figure 6**)。

　膵臓においては, i) グルコース依存的インスリン分泌の促進, ii) グルカゴンの過剰分泌抑制, iii) 膵β細胞保護, 複製, 新生, 分化促進, アポトーシス抑制作用 (動物モデル) を示す。また, 膵外作用 [29)-31)]としては, i) 胃内容排出速度の遅延, ii) 食欲抑制作用, iii) 脳神経保護, 形成誘導, iv) 学習, 記憶への関与, v) 心保護作用を示す。
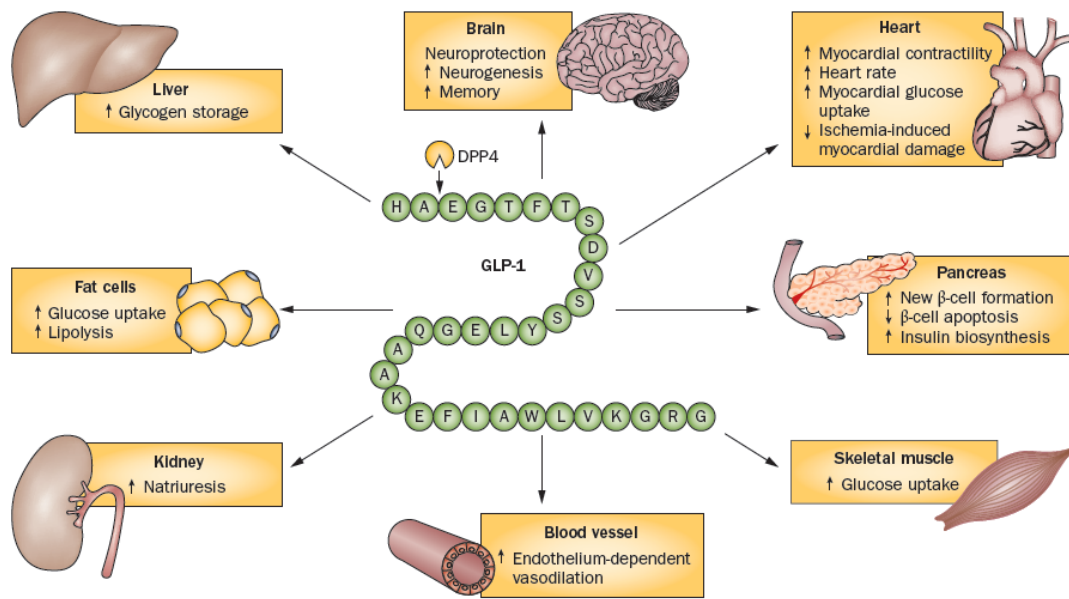
Figure 6. Physiological functions of GLP-1. (*Nat. Rev. Endocrinol.* **2012**, *8*, 728-742.より抜粋)

　膵臓におけるインスリン分泌促進, グルカゴン分泌抑制は高血糖の是正につながることから GLP-1 は 2 型糖尿病治療の標的として多くの製薬企業, 研究機関により応用研究が展開されてきた (もう一つのインクレチンホルモンである GIP は GLP-1 と比較するとインスリン分泌能が低く, 肥満を助長することから治療標的から外れている)。その結果, インクレチン関連薬として分類される i) 内因性 GLP-1 の代謝安定性を向上させたペプチド製剤である GLP-1R 作動薬, ii) GLP-1 の分解酵素である DPP-IV 阻害薬の 2 種が上市に至っている。これらのうち, GLP-1R 作動薬は優れた血糖降下作用を示すだけでなく, 他の薬剤と比較して低血糖リスクが低く, 糖尿病の指標の一つである HbA1c の数値を低下させることが明らかになっている。また, 上述したように GLP-1 が食欲抑制作用を有することから単剤で過食傾向, 肥満を伴う 2 型糖尿病患者に対しより良い治療効果をもたらすことが期待される。加えて, 2 型糖尿病モデルマウスやラットにおいては GLP-1 や GLP-1R 作動薬を投与すると膵β細胞を保護することが報告されており, 2 型糖尿病のみならず, 発症時既に膵β細胞の著しい減少が認められる 1 型糖尿病への適応を目指した応用研究も展開されている [32)-33)]。しかしながら, これまでに開発されている GLP-1R 作動薬はペプチド製剤であるために, 投与方法が注射に限られ, 投与に際し医療施設, 医療従事者の関与が不可欠である。ま

た，糖尿病の治療は長期にわたることが多く，患者への身体的負担が問題点として挙げられる。従って，経口投与を可能とする薬剤を開発することは臨床利用上の利便性向上につながる。

　一方，GLP-1 の分泌に関するメカニズムについても徐々に明らかにされつつある。GLP-1 の分泌に関与する生体内分子 [34)-40)] としては，i) K-ATP channel, ii) Na$^+$/glucose co-transporter 1, iii) glucose transporter 2, iv) sweet taste receptor, v) TGR-5 (GPR131), vi) G protein-coupled receptors (GPR40, GPR43, GPR119, GPR120), vii) 5-hydroxytryptamine (5-HT)$_4$ receptor が報告されている。このような生体内分子を標的とする低分子化合物を創製することは GLP-1 分泌促進に基づく経口糖尿病治療薬になることが期待され，先に述べた GLP-1R 作動薬の投与における問題点を改善することにつながる。以下に関連する研究の一端を記述する。

　GPR119 は膵β細胞と腸内分泌細胞に高発現している class A に属する GPCR であり，膵β細胞においてはグルコース依存的にインスリン分泌を亢進し，腸内分泌細胞ではインクレチンホルモン分泌に関与する。従って，GPR119 アゴニストを開発することは，単剤で厳格な血糖コントロールを行うだけでなく，膵臓の保護や体重の抑制を同時に行うことを可能とする。以下に既存の GPR119 アゴニストを示す **(Figure 7)**[41)-42)]。
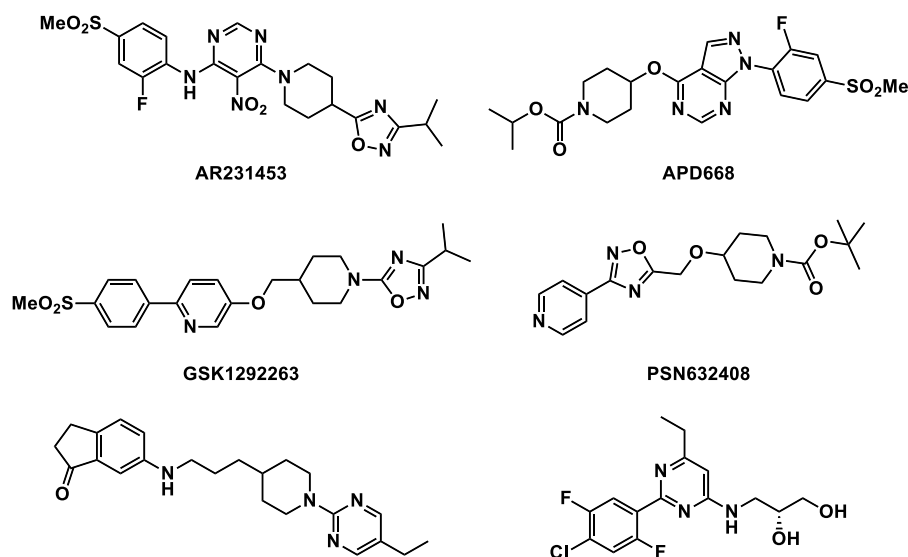
Figure 7. Structures of synthetic GPR119 agonists.

またごく最近，リガンドの結合部位とは異なる部位 (アロステリック部位) に結合することでその受容体の挙動に影響を与える positive allosteric modulator (PAM) が報告され，GLP-1R を直接的に活性化する化合物が開発されている (**Figure 8**)[43]。
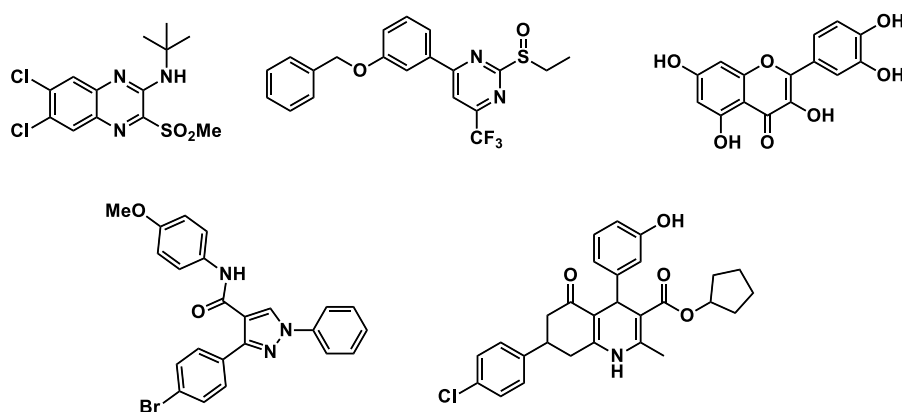
Figure 8. Structures of GLP-1 PAMs.

　以上のように，GLP-1 の優れた抗糖尿病作用が注目され，関連する研究が展開されている。しかしながら，依然としてこれらの分子を標的とした薬剤は上市されていない。従って，GLP-1R 作動薬を投薬する際には，注射薬であるため患者に身体的な負荷がかかり，医療施設や医療従事者の関与が不可欠であるという臨床利用上の課題は未解決のままである。

　次節では GLP-1 分泌作用を有する低分子化合物の探索について説明する。

第二節　GLP-1 分泌促進活性を有する低分子化合物の探索

　第一章でも述べた選択的 AKR1B1 阻害剤を含め，これまでに著者らは，rhetsinine の構造をモチーフとして i) 糖尿病性合併症治療の標的酵素 AKR1B1 阻害剤, ii) 膵臓がん細胞の栄養飢餓耐性を解除する化合物, iii) インスリン抵抗性改善に基づく糖尿病治療の標的受容体 peroxisome proliferator-activated receptor γ (PPARγ) 活性化剤の合成とその活性評価を展開してきた (**Figure 9**)[44)-48)]。その過程では数百種以上に及ぶ化合物合成を行っており，それら化合物は独自に研究を展開した結果得られたものであるため，これまでに著者ら以外に合成例がなくその構造は極めて新規性が高いといえる。従って，この化合物群を応用し GLP-1 分泌促進活性を有する化合物を見出すことができれば構造的に全く新しい分子の創製につながり，GLP-1 分泌促進に基づく経口糖尿病薬の開発に貢献することが可能となる。
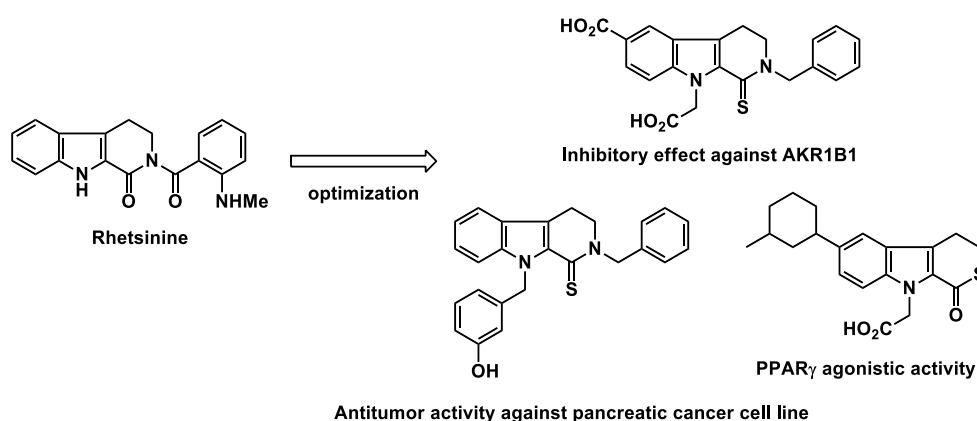
Figure 9. Structures of bioactive tricyclic compounds based on rhetsinine.

　そこで，この化合物群を用いて GLP-1 分泌促進活性を有する化合物の探索を行った。評価についてはヒト腸管細胞株である NCI-H716 細胞を用いて ELISA 法により GLP-1 の分泌量を測定した[49)]。その結果，幸いにも C-6 位上にシクロヘキシル基を有する化合物 **10** (**Scheme 3**) が濃度依存的な GLP-1 分泌促進活性を示すことが明らかになった (**Figure 10**)。以下，化合物 **10** が得られた経緯を記述する。
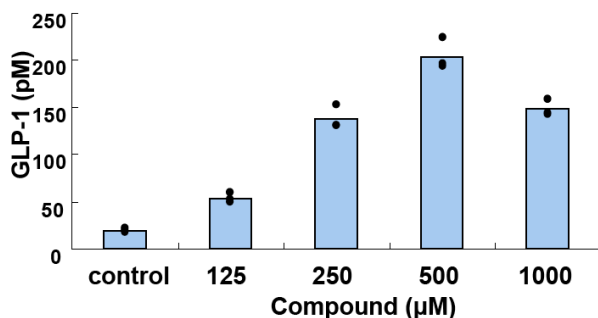
Figure 10. Effect of **10** on GLP-1 release by NCI-H716 cells. NCI-H716 cells (1×10^5/well) were incubated with **10** for 1 hour, after which the GLP-1 level in the culture medium was measured. Mean ± SEM., n=3.

　第一章でも記述したように, AKR1B1 に対する阻害活性は硫黄原子の導入が活性向上に大きく貢献していた。そこで著者は, 硫黄原子を含み, かつチオアミド誘導体と部分構造の異なる新規三環系骨格をデザインし, その誘導体の一つである化合物 **15** の AKR1B1 阻害活性の評価を行うためその合成に着手した (**Scheme 3**)。

　Scheme 2 同様, 三環系ラクトン **12** を構築し, 次に, Lawesson's 試薬を用いてチオカルボニル基に変換後, 酢酸エステルユニットを導入し化合物 **14** とした。 最後にエステルの切断を行うことで目的とする **15** が得られると考えていたが, 実際には化合物 **10** が主生成物として得られた。化合物 **10** の構造はその中間体 **14** とのスペクトルデータによる違いから決定した。すなわち, 化合物 **10** では ^{1}H NMR スペクトルにおいて C-3 位のプロトンが 3.43 ppm に観測され, 化合物 **14** の 4.67 ppm に比べ高磁場側にシフトしている。また, 両者の IR スペクトルを比較すると, 化合物 **14** のチオカルボニル基が 1228 cm^{-1} に観測されたのに対し, 化合物 **10** のカルボニル基は 1619 cm^{-1} に観測された (**Scheme 3**)。 これらの観測結果から構造を決定した。この反応のメカニズムは以下のように考察している。すなわち, エステルの切断と同時にヨウ素アニオンによる開環と続く硫黄原子による再閉環が起こることでチオエステル環が構築される。ヨウ素アニオン, 硫黄アニオンの高い求核性とチオカルボニル基よりもカルボニル基の方がエネルギー的に安定であることが driving force となっていると考えている。
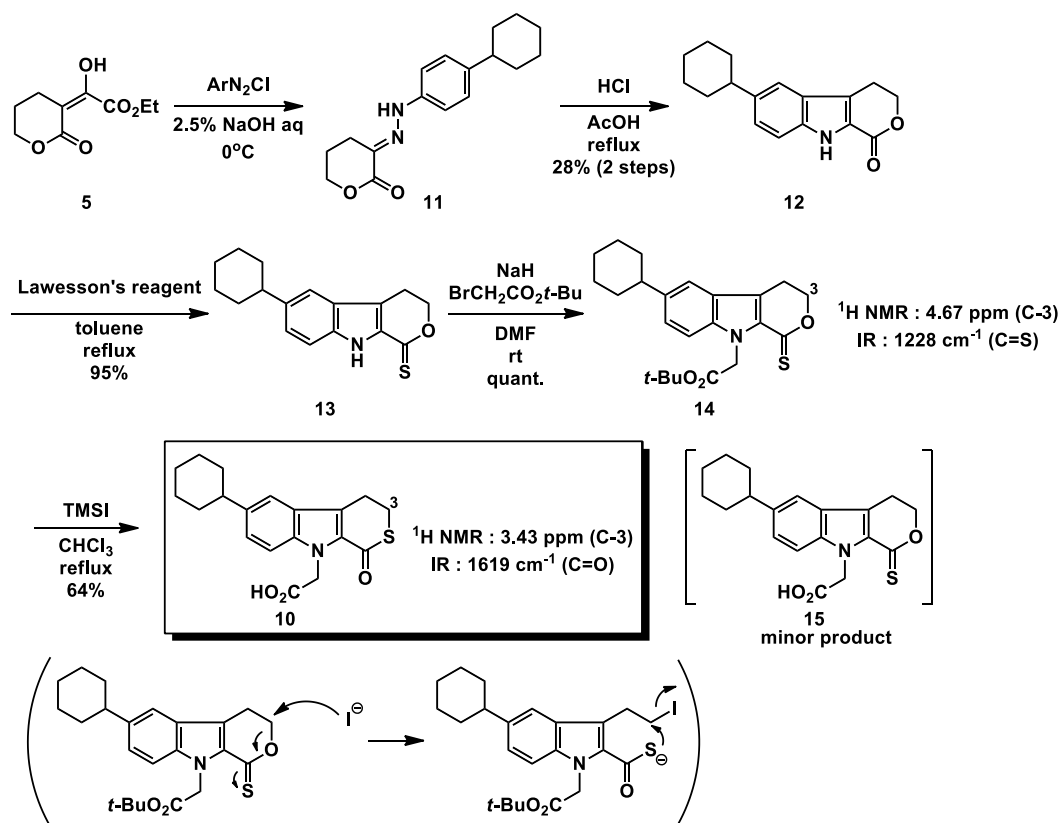
Scheme 3. Formation of unexpected product **10**.

　詳細については割愛するが，C-6 位に鎖状のアルキル基やハロゲン，アルコキシといった置換基を導入した化合物ではその活性が消失したことから，活性発現にはシクロヘキサン環が重要であると判断した。続いて，GLP-1 の分泌促進活性の向上を目的としてこのシクロヘキサン環上の様々な位置に，様々な置換基を導入した化合物を合成しその活性を評価した結果，化合物 **9** が最も強力にGLP-1 の分泌を促進することが明らかになった **(Figure 11)**。この化合物 **9** を見出した経緯やその合成方法，活性については次節にて記述する。
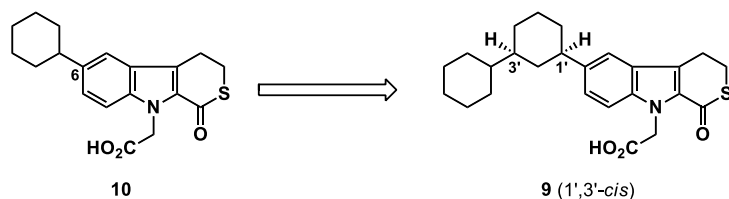
Figure 11. Structure of **9** having the most potent GLP-1 secretion activity.

第三節　強力な GLP-1 分泌促進活性を有する化合物の合成及びその活性評価

　前節でも記述したとおり，初期スクリーニングより得られたモノシクロヘキシル体 **10** の構造をもとに活性の増強を目的として更なる誘導体の合成を行った。詳細については省略するが，その一連の誘導体化の過程で合成したシクロヘキサン環上にさらにシクロヘキシル基が置換した bi-cyclohexyl 体 **16 (Figure 12)** について以下詳細に記述する。
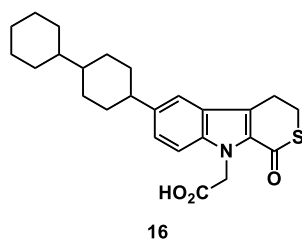
Figure 12. Structure of **16**.

　Scheme 3 で示した合成経路を用いて化合物 **16** を合成するためには対応するジアゾニウム塩を入手できればよいと考え，まず容易にジアゾニウム塩への変換ができるアニリン **19** の合成に着手した。

　市販の置換シクロヘキサノールを Friedel-Crafts 条件に付し，アルキル化体 **17, 18** を得たのち，ニトロ化，続く接触還元により化合物 **19** が得られると考えた。しかしながら，実際に実験を行ってみると予期に反し得られた主生成物は転位が伴った **20** (1,3-*cis*) であった **(Scheme 4)**。この転位反応の詳細については後述するが，この合成を行っていた当時は主成績体が転位生成物であり，かつ 1,3-*cis* 置換の幾何異性体であるという認識はなく，アニリン **19** が得られているものとして以降の合成を行った。

24
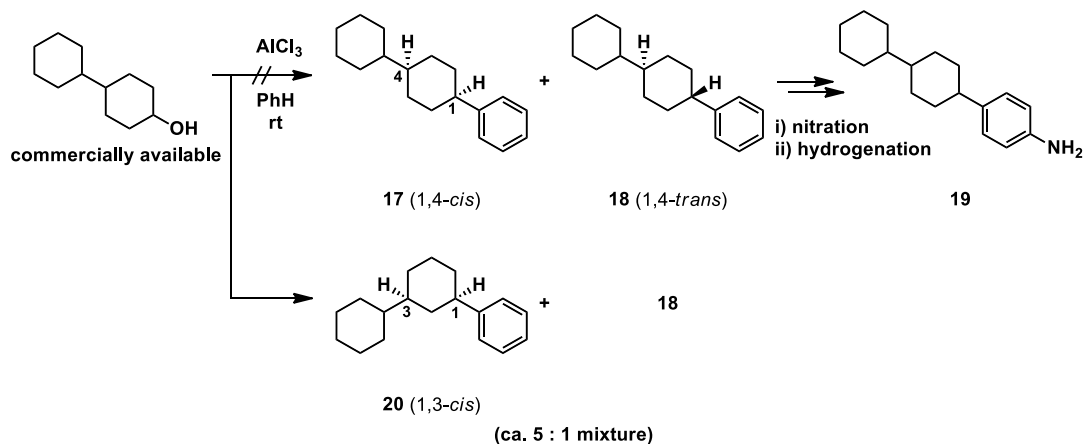
Scheme 4. Formation of unexpected rearrangement product **20**.

 初期の合成過程においては以下に示す合成中間体を含め, 2 つの位置異性体を分離することは困難であったため, 混合物のまま合成を進めることで評価化合物であるカルボン酸 **9**, **30** (以下, **A** とする) へ変換し, その活性評価を検討することにした。

 化合物 **20**, **18** の混合物 (約 5 : 1) に対し, ニトロ化, 続く接触還元を行いアニリン **23**, **24** とした。以降は **Scheme 3** と同様に化合物 **27** とした。**Scheme 3** で示したモノシクロヘキシル体の合成では最終過程でチオエステル環とカルボン酸を構築しているが, その手法では生成物がカルボン酸であり TLC による反応終点の見極めが困難であったため, TMSI, 加熱条件下, チオエステル環を先に構築することで化合物 **28** とし, **A** を合成した (**Scheme 5**)。
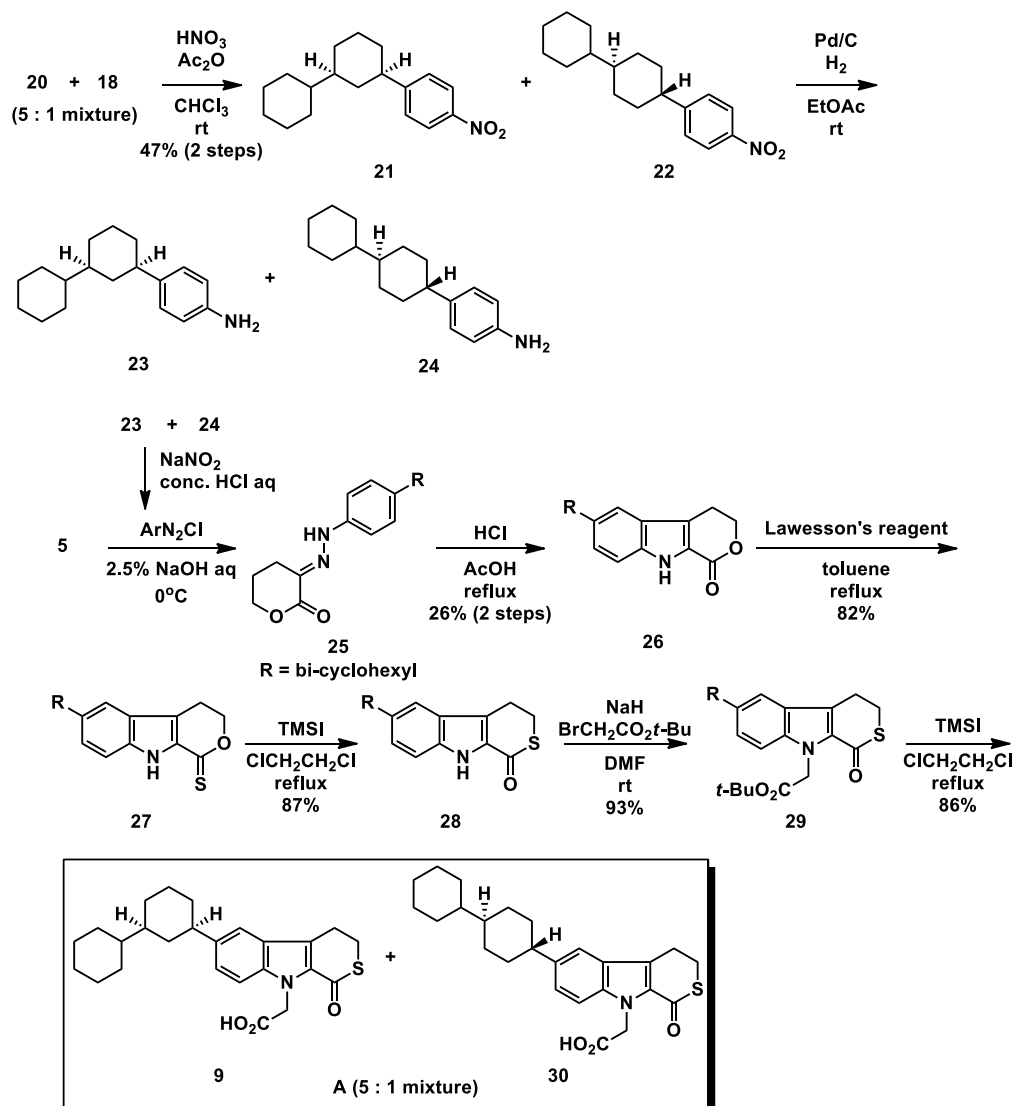
Scheme 5. Synthesis of **A**.

　続いて, *in vitro* 系における化合物 **A** の GLP-1 分泌活性評価を行った。その結果, EC$_{50}$ 100 μM, Emax 約 1300 pM を示し, 強力に GLP-1 の分泌を亢進することが明らかになった **(Figure 13)**。
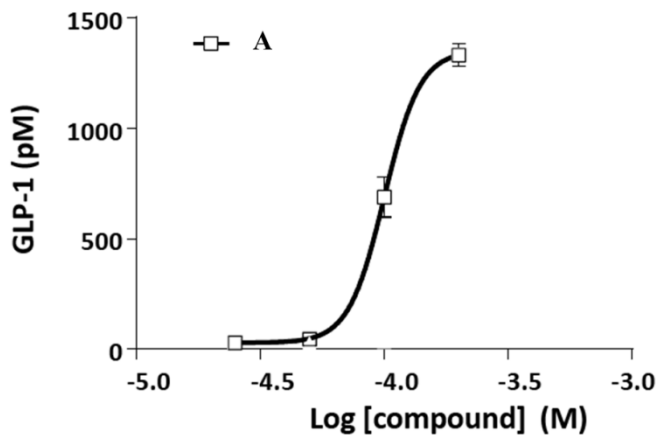
Figure 13. Effect of **A** on GLP-1 release by NCI-H716 cells. NCI-H716 cells (1×10^5/well) were incubated with **A** for 1 hour, after which the GLP-1 level in the culture medium was measured. Mean ± SEM., n=3.

　次に，*in vivo* 系，経口投与での血糖降下作用を検討した。まず，正常マウス C57BL/6J マウスを用いて単回投与時における血中グルコース濃度の経時変化を観測した **(Figure 14)**。化合物 **A** 単独 (100 mg/kg) の投与では vehicle 群と比較して有意な血糖降下作用が認められた。また，GLP-1 アンタゴニスト Exedin (9-39) **(Ex9)** [49)-50)]との同時投与においてはその作用の減弱が認められ，本化合物が GLP-1 分泌に基づいて血中グルコース濃度を抑えていることが明らかになった。
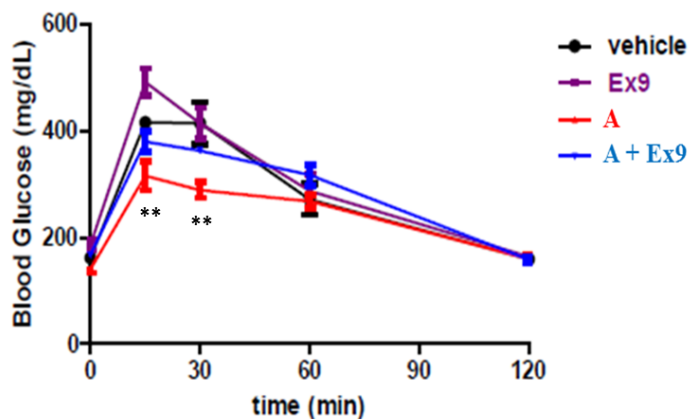
Figure 14. Hypoglycemic effect of **A** after an oral glucose load and the influence of a GLP-1 antagonist (Exendin (9-39)) on it in C57BL/6J mice. After an overnight fast, either the vehicle, or 100 mg/kg of **A** was administered by oral gavage with or without subcutaneous injection of exendin (9-39) (24 nmol/kg). Then, 2 g/kg of glucose was given orally immediately after **A** administration. Blood samples were collected from the

tail vein, and the blood glucose levels were measured. Mean ± SEM., n=6. **p<0.01 vs vehicle (Dunnett's test).

　さらに，糖尿病モデルマウス KKAy マウスを用いて抗糖尿病作用を評価した **(Table 3)**。化合物 **A** (100 mg/kg) を 3 週間継続的に投与した後の体重，空腹時血中グルコース濃度，食後血中グルコース濃度，HbA1c，空腹時インスリン濃度，空腹時グルカゴン濃度それぞれについて測定を行った。また，既存薬である DPP-IV 阻害薬 sitagliptin (10 mg/kg), インスリン抵抗性改善薬 pioglitazone (10 mg/kg) を比較対照として同時に評価した。その結果，体重や食事の摂取量については あまり影響が認められなかったが，空腹時，食後ともに血中グルコース濃度の低下が認められた。また，3 週間という比較的短い投与間隔にもかかわらず，HbA1c の数値を低下させることが明らかになり，加えてインスリン，グルカゴン濃度の低下が認められた。総合的に化合物 **A** は優れた抗糖尿病作用を示すことが明らかになったが，実際に医薬品としての応用を考えると投与量 **(A**: 100 mg/kg, sitagliptin: 10 mg/kg, pioglitazone: 10 mg/kg) を減らす必要があることも併せて示された。

	Body weight (g)	Fasting blood glucose (mg/dl)	Non-fasting blood glucose (mg/dl)	HbA1c (%)	Fasting plasma insulin (ng/ml)	Fasting plasma glucagon (pg/ml)
Normal	25.5±0.3	166.0±12.9	156.0±6.4	3.9±0.1	2.9±0.4	155.7±17.9
Vehicle	39.5±1.8	204.8±16.5	353.2±37.5	7.5±0.3	20.6±10.4	257.8±34.5
A	41.0±1.9	124.6±6.6**	231.6±13.5	6.3±0.3	8.7±3.3	217.1±38.5
Sitagliptin	39.9±1.4	194.6±18.5	457.6±35.1	7.9±0.3	5.8±0.4	249.8±33.2
Pioglitazone	42.6±1.1	151.6±11.1	256.4±49.4	6.8±0.4	7.7±1.4	241.6±29.4

Table 3. Long-term effect of **A** treatment for 3 weeks on blood glucose control in KKAy mice.

　混合物ではあるが，動物における有効性が明らかになったので 次に著者はそれぞれ単一の化合物を合成することで活性本体を特定することにした。合成経路の各段階では 2 種類の位置異性体をカラムクロマトグラフィーや再結晶にて分離することは困難であったため，より立体選択性の高い合成経路の構築を目指し，別途合成法を検討した。すなわち，共通の出発物質である置換シクロヘキ

サノールに対し PCC 酸化を行いケトン **31** とし, PhMgBr の付加により 3 級アル
コール **32** を得た。最後にルイス酸存在下, Et₃SiH により還元反応を行った。期
待に反し選択性の向上は認められなかったが **18** と **17** の混合物が約 3 : 1 で得ら
れた (**Scheme 6**)。
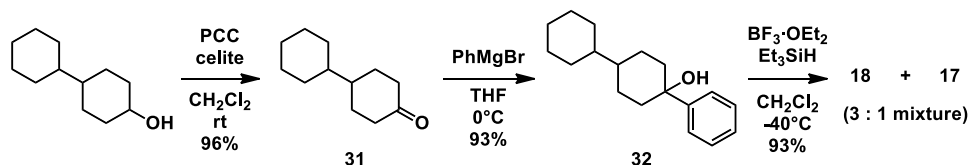
Scheme 6. Synthesis of **18** and **17** via silane reduction.

Scheme 4 において, Friedel-Crafts 反応における主成績体は転位が伴った **20** で
あることは先に述べたが, **Scheme 6** での検討によって転位が起きたことが判明
した。以下に詳細を記述する。

Friedel-Crafts 反応における生成物 **20**, **18** 及びシラン還元により得られた生成
物 **18**, **17** の ¹H NMR スペクトルにおけるベンジル位のプロトンを以下に示した
(**Figure 15**)。この両者は当初, 同一の化合物であると思っていたため, 生成物の
含有比率に違いが観察されたとしてもそのケミカルシフトは一致するはずであ
ったが, 実際に両者を比較すると, 高磁場側のケミカルシフトは一致したが, 低
磁場側のそれは一致しなかった。そこで, Friedel-Crafts 反応において転位反応が
起きている可能性が強く疑われ, 化合物 **20**, **18** それぞれの構造を X 線結晶構造
解析により決定することにした。
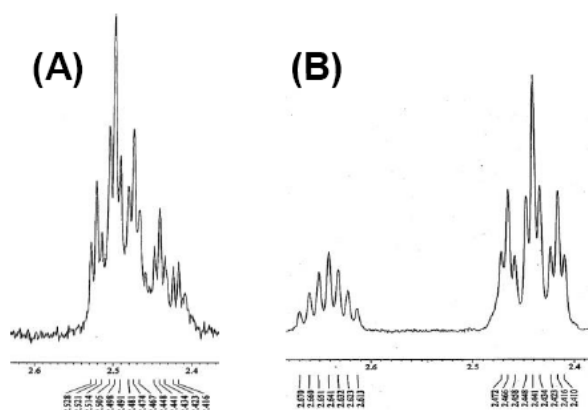
Figure 15. ¹H NMR spectrum on the benzylic position. **(A)** indicates the product from
Friedel-Crafts reaction. **(B)** indicates the product from silane reduction.

まず, 単結晶を得るために誘導体化を行った (Scheme 7)。位置異性体の分離が困難であることは先に述べたが, 初期の合成過程においては置換アニリンにおけるカラムクロマトグラフィーでの分離を行っておらず, 精製条件を精査したところ, 幸いにもアニリン誘導体においてカラムクロマトグラフィーでの分離が可能であることが判明した。そこで, 得られた **23** についてはスルホンアミド **33** に変換した。一方, **24** は Friedel-Crafts 反応を用いるとマイナー成分となり効率的に得ることができないため, この化合物はシラン還元を用いて量的供給を実現し, トシル酸塩 **36** に変換した。
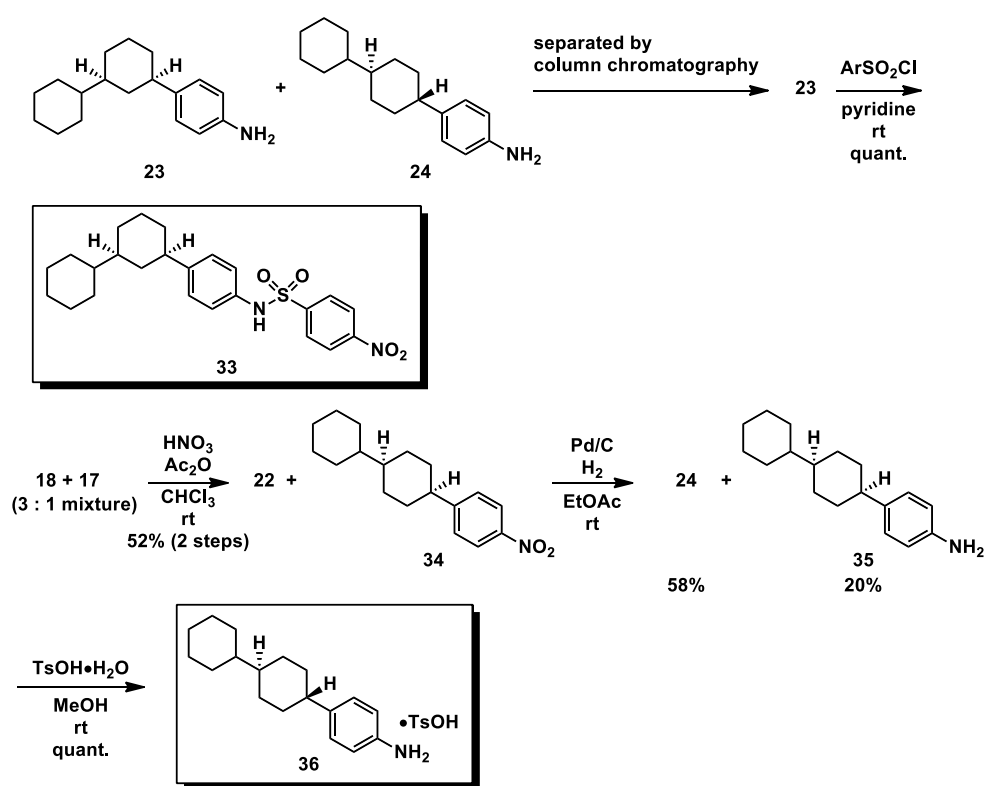
Scheme 7. Synthesis of **33** and **36**.

　スルホンアミド **33**, トシル酸塩 **36** の X 線結晶構造解析の結果を以下に示す (**Figure 16-17**)。この結果から Friedel-Crafts 反応における主成績体は 1,3-*cis* 置換の **20**, マイナー生成物は 1,4-*trans* 置換の **18** であることが判明した。
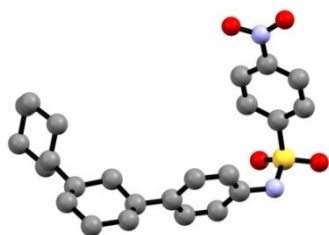
Figure 16. X-ray crystal structure of compound **33**.
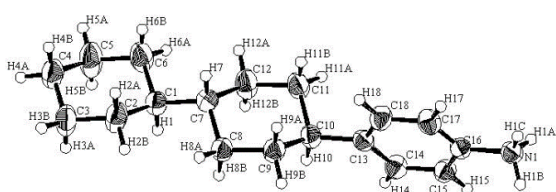
Figure 17. X-ray crystal structure of compound **36**.

　続いて，この反応のメカニズムについて考察した。ルイス酸により発生する第二級カルボカチオンの安定性を PM6 法により計算したところ，最初に生じるカチオンの最安定配座であるいす型配座 B よりも，転位後の最安定配座であるねじれ舟形配座 A の方がより安定であることがわかった **(Figure 18)**。このカチオン中間体の安定性の違いによって主成績体は **20** になると考えられる。
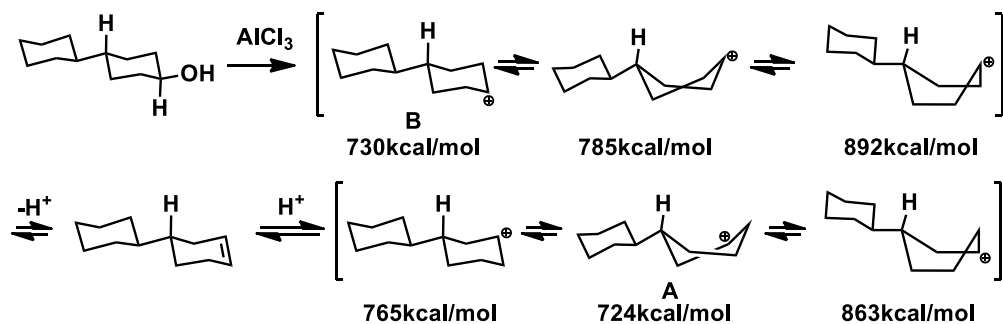
Figure 18. PM6 Calculations of the intermedially carbocations on the Friedel-Crafts reaction.

それぞれ単一の置換アニリン **23**, **24** を得ることができたので, **Scheme 5** と同様の経路でカルボン酸 **9**, **30** を合成した。ヒドラゾン **37** を構築する際に用いるジアゾニウム塩はシクロヘキシル基が二つあることで水への溶解性が著しく低く, それに伴い収率の低下が認められたため, **Scheme 5** と異なり THF を補助溶媒として用いることで収率の改善を図った (**Scheme 8**)。化合物 **30** についても同様に合成した (**Scheme 9**)。
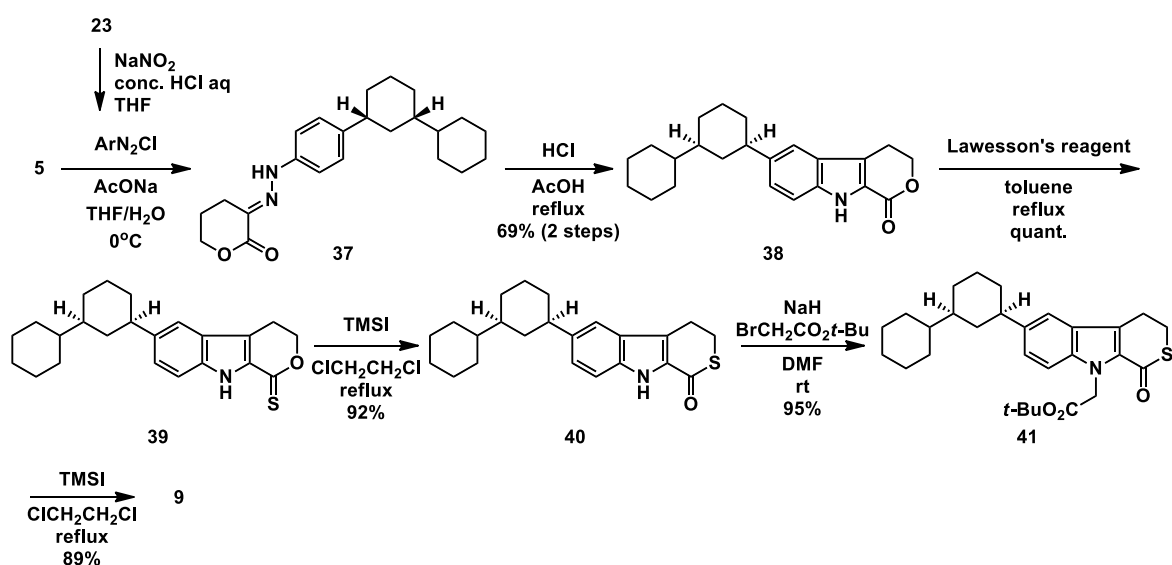
Scheme 8. Synthesis of **9**.
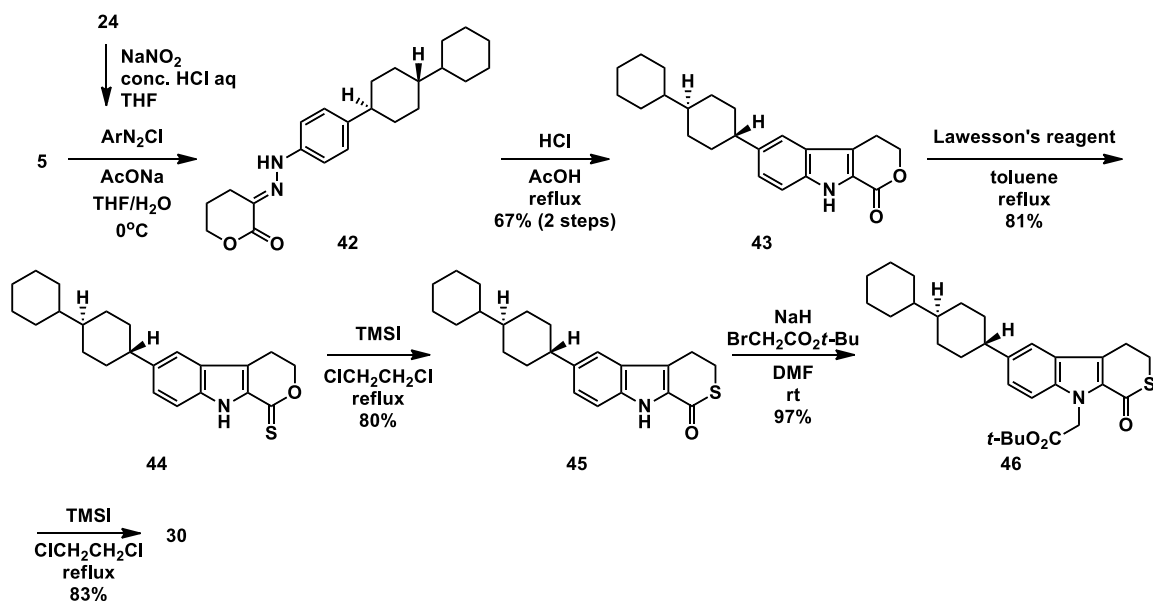
Scheme 9. Synthesis of **30**.

合成した **9**, **30** それぞれの位置異性体の GLP-1 分泌促進活性について評価を行った **(Figure 19)**。その結果, 活性本体は転位体である 1',3'-*cis* 置換体 **9** であることが明らかになった。化合物 **30** については全く GLP-1 分泌活性に影響を示さなかったことから, 予期せず得られた転位体の 1',3'-*cis* 置換による部分構造がその活性に大きく寄与していることが示唆された。
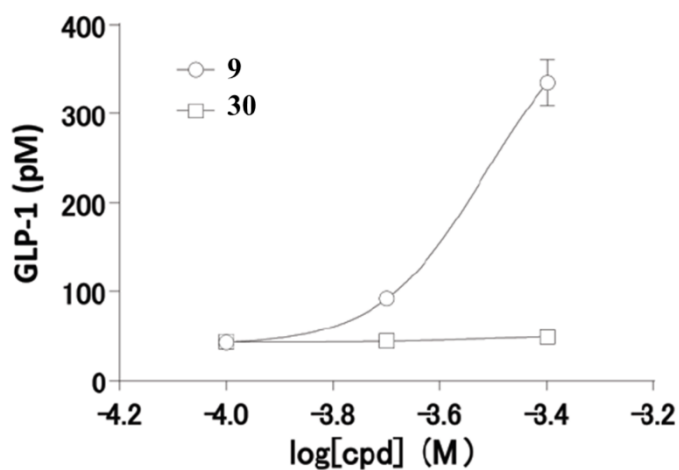
Figure 19. Comparison of the effect of regioisomers of **9** and **30** on GLP-1 secretion in NCI-H716 cells. NCI-H716 cells (1×10^5/well) were incubated with **9** or **30** for 1 hour, after which the GLP-1 level in the culture medium was measured. Mean ± SEM., n=3.

第三章　グリコシダーゼ阻害剤開発を目指したイミノ糖誘導体の合成

第一節　既存のグリコシダーゼ阻害剤及び標的分子のデザイン

　糖加水分解酵素群であるグリコシダーゼは，細胞内での糖タンパク質，糖脂質プロセシング，小腸での糖質消化をはじめとして生体内において非常に重要な役割を果たしている。これらの多様な生理作用は，糖尿病，AIDS，がん転移，リソソーム蓄積症といった疾患に密接に関連しており，各種グリコシダーゼに焦点を当てた研究が行われてきた [51)-56)]。小腸粘膜に存在するα-グルコシダーゼ阻害剤, voglibose, miglitol は食後過血糖の是正につながるため 2 型糖尿病治療薬として臨床利用されている。本薬剤は膵β細胞を標的とする他の糖尿病治療薬とメカニズムの点で大きく異なり，治療の選択肢を拡張するとともに，併用薬としての貢献度が高い。また，インフルエンザ治療薬である oseltamivir や zanamivir もまたグリコシダーゼに分類されるノイラミニダーゼの阻害剤である (**Figure 20**)。

Vogribose　　**Miglitol**　　**Oseltamivir**　　**Zanamivir**
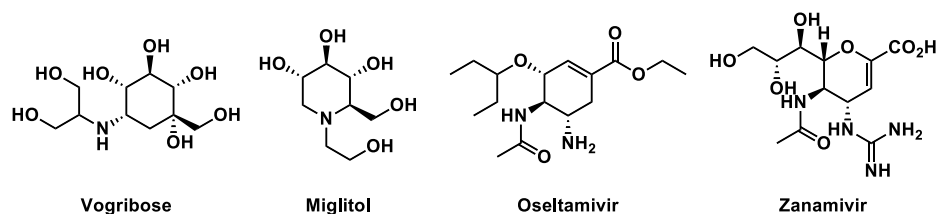
Figure 20. Structures of drugs based on glycosidase inhibition.

　グリコシダーゼおよび関連する酵素研究の第一人者, Asano は, 1965 年頃からのグリコシダーゼ阻害剤開発の変遷を以下の様に総説している [57)]。
第 I 期:
古典的グルコシダーゼ阻害剤ノジリマイシンの発見と酵素学研究への応用
第 II 期:
α-グルコシダーゼ阻害剤の糖尿病治療薬としての臨床利用
第 III 期:

糖蛋白質糖鎖プロセシング・グリコシダーゼ阻害剤による糖鎖の生理学的意義の解明および抗ウィルス・癌転移抑制への応用

第Ⅳ期:

グルコシルセラミド (GlcCer : Glucosylceramide) 合成酵素 (糖転移酵素) 阻害剤による先天的なスフィンゴ糖脂質 (GSL : Glycosphingolipid) 蓄積症の症状改善, リソソーム・グリコシダーゼ阻害剤による先天的 GSL 蓄積症の分子治療 (ファーマコロジカル・シャペロン療法) への応用

　このようにグリコシダーゼ阻害剤に関する研究は約 50 年にわたり, 現在なお精力的に取り組まれている。グリコシダーゼ阻害剤としては糖類似化合物であるイミノ糖が広く知られているが, 近年, ピロリチジンやインドリチジンといった二環性のイミノ糖がグリコシダーゼ阻害作用のほか優れた生物活性を示すことが報告されている (**Figure 21**)[58)-60)]。その中でもピロリチジンを母核として有するイミノ糖は **Scheme 10** に示すように続々と天然物が単離され興味深い活性を有している。以下, ピロリチジンを母核として有する化合物の歴史について簡単に紹介する。

Casuarine　　　　**Swainsonine**　　　　**6-*O*-Butanoyl-castanospermine**

(乳がん, **AIDS**)　　(進行性悪性腫瘍)　　(デング熱, **C**型肝炎)
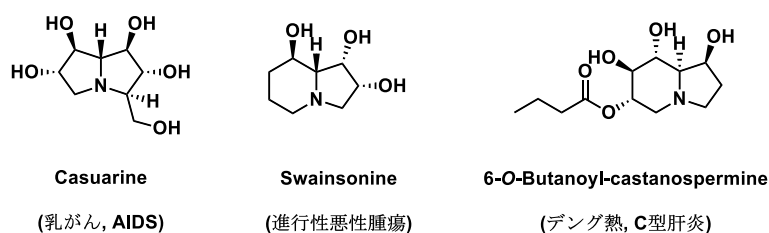
Figure 21. Promising compounds based on bicyclic core skeleton.

　1900 年代初頭, ピロリチジンの C-1, C-7 位に置換基を有しかつ C-1, C-2 位にオレフィン構造を有するアルカロイドが単離されたが, 一連の化合物は肝毒性を示すことが報告されたため, 医薬品化学的な価値はあまりなかった。一方, 1990 年代に入ると australine, alexine などのピロリチジン型イミノ糖が単離された [61)-62)]。これらのイミノ糖は高度に酸素官能基化されグリコシダーゼ阻害作用を示すとともに合成化学的な興味より全合成研究やエピ体の合成が行われてきた [63)-64)]。さらに, 2000 年代に入ると hyacinthacine B$_2$ や broussonetine N といったイミノ糖が単離された [65)-66)]。これらのイミノ糖は従来の化合物と異なりピロリチジン環の C-5 位にヒドロキシメチル基や非常に長い側鎖を有するという構造

的な特徴が挙げられ，グリコシダーゼ阻害作用を示すことが明らかになっている。

　天然から供給されるピロリチジン型イミノ糖は有望な活性を示すものが多いが，天然からの供給量が少なく，また，天然物の構造決定に誤りがあり，その構造が訂正される場合や立体異性体を合成してもそのグリコシダーゼ阻害活性評価を行っていないなど，効率的に研究が進められているとはいえないのが現状である。従って，誘導体化を可能とする天然型ならびに非天然型ピロリチジン型イミノ糖の合成方法の開発は構造活性相関研究を進めるだけでなく，医薬品開発に応用可能な分子の創出へとつながることが期待される。そこで著者はaustraline, alexine がα-グルコシダーゼ阻害活性を有し，hyacinthacine B_1 やbroussonetine N がβ-グルコシダーゼ, β-ガラクトシダーゼ阻害活性を示すことは既に知られていたため，ピロリチジン環の C-3 位にアルキル側鎖を有する化合物およびその光学異性体を標的分子として定め **(Scheme 10)**，併せてそのグリコシダーゼ阻害活性についても評価を行った。
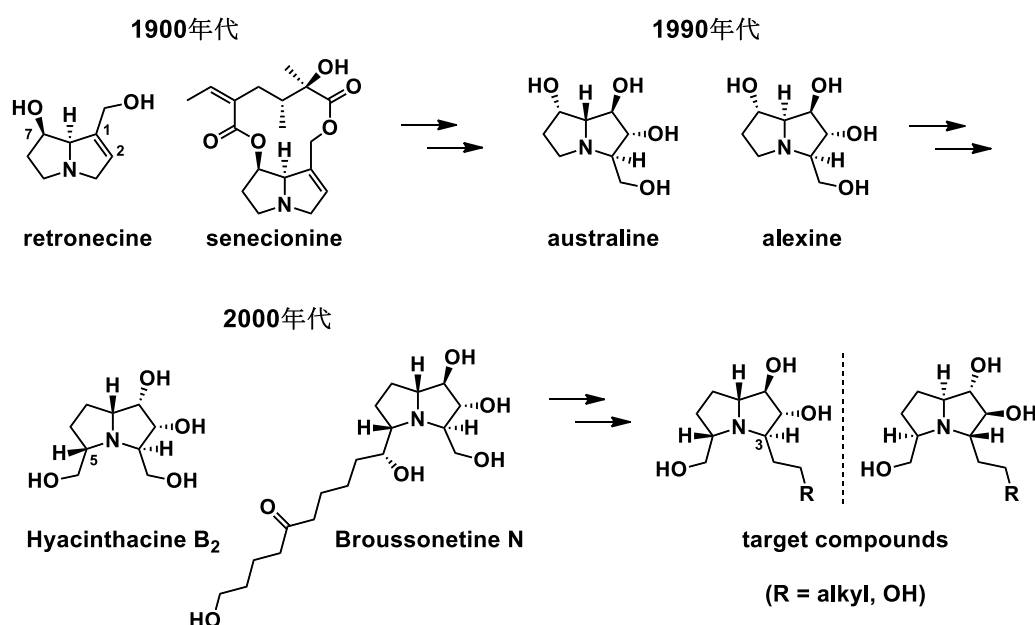
Scheme 10. History of pyrrolizidine alkaloids and structures of target compounds

第二節　C-3 位にアルキル側鎖を有する非天然型ピロリチジン型イミノ糖の合成法の確立

　一般にイミノ糖は複数の水酸基を有していることが構造的特徴として挙げられるが，第二級の水酸基などにより不斉炭素が多く，イミノ糖誘導体を合成する際には以下の点を考慮する必要がある。

i) 連続する不斉炭素を如何に構築するか，ii) 如何に高度に立体化学を制御するか，iii) 水酸基を複数含むため，如何に適切な保護基を選択するか，iv) 如何に効率的に合成するかという点である。

　以上の点を踏まえ著者は両対掌体を効率的に得るために，単一の光学活性体から両対掌体の合成が可能な enantio divergent strategy[67] に基づき目的化合物を合成することとした。併せて合成経路終盤での誘導体化を可能とする経路の構築を目指した。以下に詳細を示す。
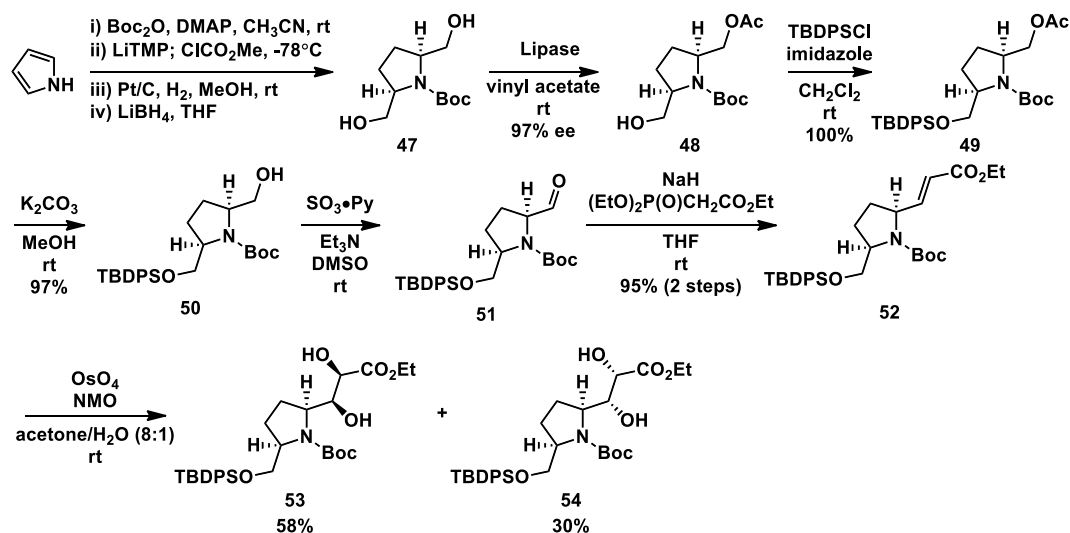
Scheme 11. Synthesis of intermediate diols **53** and **54**.

　ピロールから 4 工程で得られるジオール **47** に対し，文献既知の方法 [68]に従い非対称化を行い光学活性なモノアセテート **48** とした。次に，第一級アルコールを TBDPS 基により保護し，アセチル基の脱保護と続く SO$_3$・Py を用いた DMSO

酸化によりアルデヒド **51** とした。得られた **51** に対し，HWE 反応を行い，*trans*-α,β-不飽和エステル **52** としたのち (オレフィン部の ¹H NMR よりトランスと判断した，*J* = 15.6 Hz)，立体選択的なジオール化を試みた。まず化合物 **52** に対し，AD-mix αを用いて反応を行ったところ，反応は全く進行せず原料が回収される結果となった。これは，AD-mix に含まれる触媒の嵩高さに起因していると考えている。そこで，substrate control による通常条件を適用しジヒドロキシル化を行ったところ，中程度の立体選択性 (2：1) にて目的の化合物を合成することができた (**Scheme 11**)。この 2 種類のジアステレオマーは容易にカラムクロマトグラフィーでの分離が可能であった。
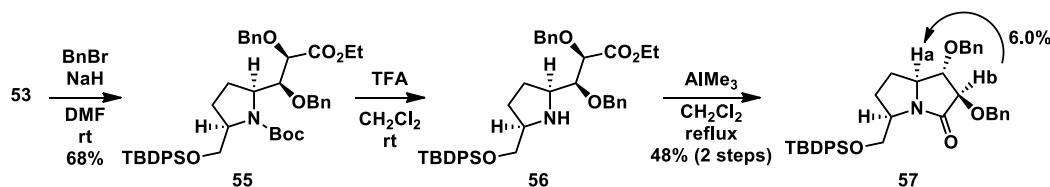
Scheme 12. Synthesis of **57**.

次に，このジヒドロキシル化において新たに生成した不斉炭素の立体化学を決定した (**Scheme 12**)。主生成物である **53** のジオールをベンジル基で保護し，Boc 基の脱保護，続くルイス酸条件下でラクタム化を行い **57** とした。この化合物について差 NOE を測定したところ，Ha と Hb 間に NOE が観測されたことから化合物 **53** の立体配置は *S,S* 配置であると判断した。
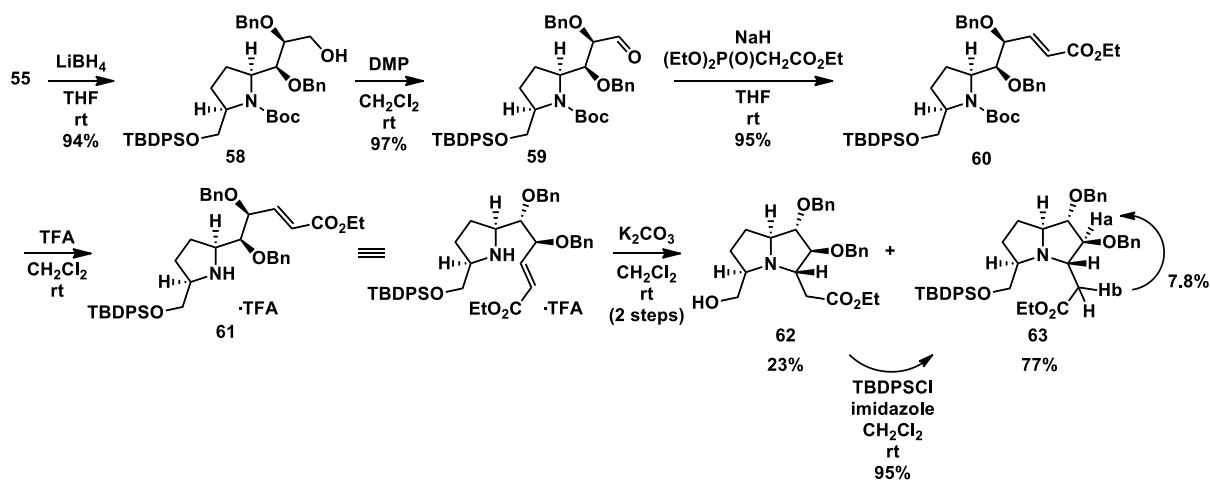
Scheme 13. Synthesis of key intermediate **63**.

続いて，鍵中間体である化合物 **63** の合成に着手した **(Scheme 13)**。化合物 **55** のエステル部を還元し，第一級アルコールとしたのち，Dess-Martin 酸化，続く HWE 反応により α,β-不飽和エステル **60** を得た。次に，Boc 基を脱保護し，立体選択的分子内 aza-Michael 環化反応を行い **63** とした。この反応において新たに生成した不斉炭素については，先と同様，差 NOE を測定し Ha と Hb 間に NOE が観測されたことから *S* 配置と判断した。また，本反応では TBDPS 基が脱保護された **62** が副生成物として得られるが，再度 TBDPSCl を用いて保護すると化合物 **63** が得られることを確認している。環化の際の選択性については速度論的に有利な配座から反応が進行し，生成物としてもより安定な **63** が得られると考えている [69] **(Figure 22)**。

favor (*Si*-face attack)　　　　vs　　　　disfavor (*Re*-face attack)
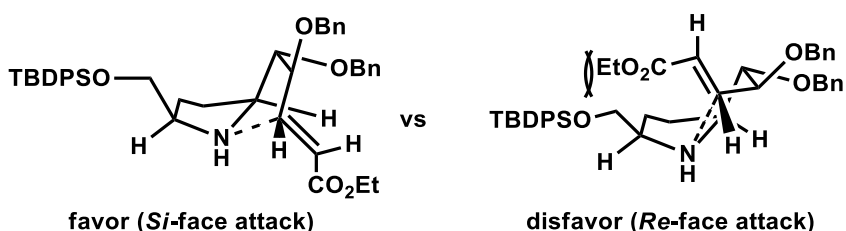
Figure 22. Kinetic control on pyrrolizidine formation.

　次に，環化体 **62**, **63** を用いて誘導体の合成を行った**(Scheme 14)**。化合物 **62** について LiAlH$_4$ 還元を行いジオール **64** とし，最後にベンジル基の脱保護を行った。まず，接触還元による脱保護を行ったが，常圧，中圧条件下においても効率的に脱保護は進行しなかった。そのため，ルイス酸である BCl$_3$ を用いて反応を行ったところ，目的とするテトラオール **65** を定量的に得ることができた。
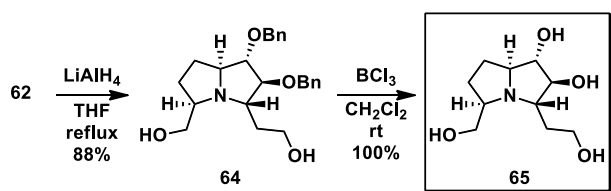
Scheme 14. Synthesis of **65**.
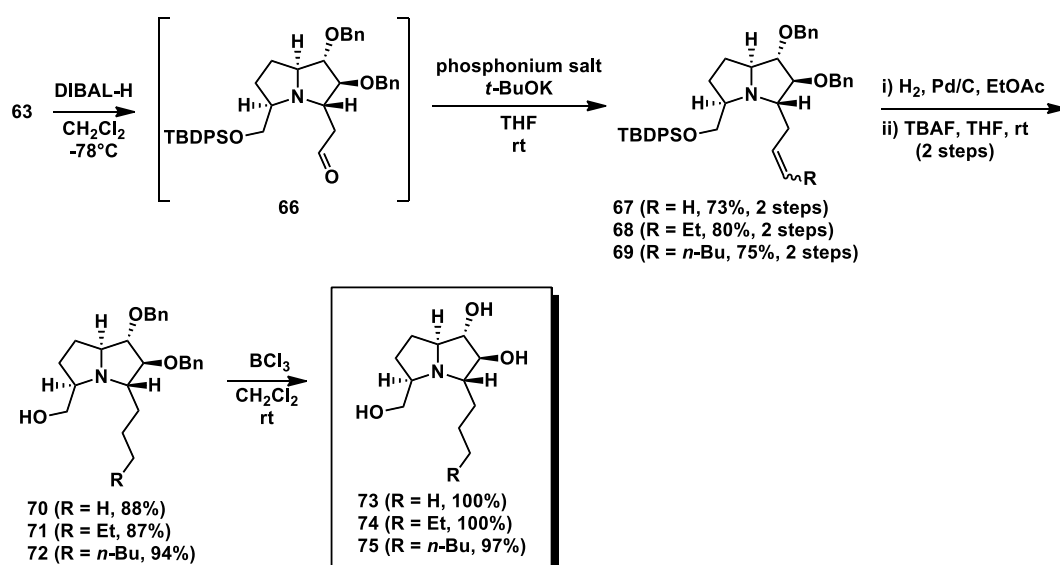
Scheme 15. Synthesis of **73-75**.

　また，側鎖を伸張したアルキル化体についても合成を行った (**Scheme 15**)。化合物 **63** について DIBAL-H 還元，続く Wittig 反応により側鎖を導入し，オレフィンの還元，TBDPS 基，ベンジル基の脱保護を行い目的とするアルキル化体 3 種を合成した。

　Enantio divergent strategy に基づき **65**，**73**，**74**，**75** それぞれのエナンチオマーについても合成を行った。共通の中間体 **48** について 4 工程にて保護，脱保護を行い，***ent*-50** を合成し，上記合成経路同様，***ent*-65**，***ent*-73**，***ent*-74**，***ent*-75** を合成した (**Scheme 16**)。

Scheme 16. Synthesis of *ent-65*, *ent-73-75*.

第三節　グリコシダーゼ阻害活性評価

　　光学異性体を含む計 8 種類の化合物の各種グリコシダーゼ阻害活性について評価を行った **(Table 4)**。

Table 4. Concentration of 73, 74, 75, 65 and their enantiomers giving 50 % inhibition of various glycosidases

enzyme	73	74	75	65	ent-73	ent-74	ent-75	ent-65
				IC_{50} (μM)				
α-Glucosidase								
Rat intestinal maltase	0%[a]	7.6%	10.2%	10.6%	0%	6.5%	3.9%	9.0%
β-Glucosidase								
Almond	5.3%	0.9%	0.2%	0%	7.1%	3.6%	2.1%	7.7%
α-Galactosidase								
Coffee beans	3.1%	9.2%	7.2%	1.1%	0%	0%	0%	3.9%
β-Galactosidase								
Bovine liver	49.7%	48.7%	49.0%	23.6%	47.7%	970	587	792
α-Mannosidase								
Jack beans	1.7%	0%	0%	5.0%	0%	0%	0%	0%
β-Mannosidase								
Snail	0%	0%	0%	0.6%	0%	0%	0%	4.5%
α-L-Fucosidase								
Bovine kidney	0%	3.0%	2.0%	**34**	0%	0%	0%	0%
			a ; inhibition % at 1000 μM.					

　　評価化合物の中で唯一化合物 **65** だけがα-L-fucosidase に対し阻害作用を示すことが明らかになった。その IC_{50} 値は 34 μM を示し，中程度の阻害能ではあるが，他の酵素群に対してほぼ作用しないことからα-L-fucosidase に選択的な阻害剤であるといえる。pyrrolizidine 型イミノ糖において選択的なα-L-fucosidase 阻害剤の報告例は少ない。また，α-L-fucosidase は細菌細胞壁の強度維持に関与していることが近年明らかになっており [70]，化合物 **65** が同酵素阻害剤のリード化合物として期待できることが示唆された。一方，*ent-73*，*ent-74*，*ent-75* はβ-galactosidase に対し弱いながらも選択的な阻害作用を示すことが明らかになった。

結論

著者は糖代謝関連疾患治療を目指し，医薬品候補化合物として期待できる低分子化合物の合成とその活性評価を行った。

第一章では，rhetsinine の構造をモチーフとして強力な AKR1B1 阻害剤の創製に成功した。阻害活性の向上には硫黄原子の導入が大きく寄与し，中でも化合物 **8t** は市販薬である epalrestat に匹敵する阻害活性を示すだけでなく (**8t** : 0.17 μM, epalrestat : 0.10 μM)，副作用の発症の抑制につながる酵素阻害選択性も極めて高いことを明らかにした (AKR1A1/AKR1B1 = 312, AKR1B10/AKR1B1 = 253)。

第二章においては，第一章同様 rhetsinine を基盤として合成展開を行い，強力な GLP-1 分泌促進活性を有する **9** を見出した。合成過程においては Friedel-Crafts 反応時に立体選択的な転位反応が生じることを明かにした。活性面では正常マウスのみならず，糖尿病モデルマウスにおいても優れた抗糖尿病活性を有することが示され，加えて，転位成績体の 1',3'-*cis* 置換の部分構造がその活性に重要であることが示唆された。

第三章では，非天然型のピロリチジン型イミノ糖の柔軟な合成手法を構築し，光学異性体を含む計 8 種類の誘導体の合成に成功した。合成過程では enantio divergent startegy に基づき効率的に合成展開を行い，所望の立体化学を高度に制御することができた。活性評価では化合物 **65** がα-L-fucosidase 選択的な阻害作用を示すことを明らかにした。

以上の研究結果が今後の創薬研究発展の一助となれば幸いである。

謝辞

　本研究に際し，終始ご懇篤なるご指導，ご鞭撻を賜りました富山大学附属病院教授　足立　伊佐雄　先生に謹んで感謝致します。また，研究室配属当時から，本研究の計画，実施，考察にわたりご指導頂きました富山大学附属病院准教授　加藤　敦　先生，約2年間，特に有機合成に関してご指導を頂きました富山大学附属病院助教授　友原　啓介　先生に深く感謝致します。

　また，本研究は富山大学工学部教授　豊岡　尚樹　先生の多大なるお力添えに基づくものであり，ここに厚くお礼申し上げます。

　約1か月間，東北薬科大学での合成研究に携わる機会を提供して頂きました東北薬科大学教授　故　高畑　廣紀　先生，同助教 名取　良浩　先生に深く感謝申し上げます。

　本研究実施に際し，有益なご助言を頂きました富山大学薬学部教授，松谷 裕二　先生，同准教授，杉本　健士　先生，同助教，湊　大志郎 先生に深く感謝いたします。

　博士論文作成及び発表に際し，有益なご助言を頂きました富山大学薬学部教授，矢倉　隆之 先生に深く感謝いたします。

　本研究に際し，ご助力を頂きました岐阜薬科大学薬学部教授，原　明　先生，同准教授，松永　俊之　先生，同助教授，遠藤　智史　先生，北里大学薬学部教授，広野　修一　先生，同准教授，合田　浩明　先生，同講師，山乙　教之　先生，味の素株式会社，北原　吉郎　氏，三浦　恭子　氏に深く感謝いたします。

　研究室生活において多くの面でご協力を頂きました富山大学附属病院臨床薬剤学研究室　中川　進平　氏，富山大学工学部生体機能性分子工学研究室諸氏に感謝申し上げます。

　最後に、これまで様々な面で支援して頂き、9 年間という長い学生生活を終始温かい目で見守って下さいました家族，親戚一同に心から感謝致します。

実験の部

General

Flash chromatography was performed with Kanto Kagaku silica gel 60N (63-210 mm). NMR spectra were recorded on a Varian Gemini300 or JEOL ECX400 spectrometer in the solvent indicated. Chemical shifts (δ) are given in ppm downfield from TMS and referenced with CHCl$_3$ (7.26 ppm) as an internal standard. Peak multiplicities are designated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad and coupling constants are given in (*J*) Hz. High resolution mass spectral data was obtained on a JEOL JMS-GC MATE Ⅱ or JEOL JMS-AX505HAD. All commercial reagents were used as received unless otherwise noted.

第一章

Typical procedure for lactones (**1a–1j**): The known lactones (**1a**, **1b**, **1c**, **1d**, **1f**, and **1g**) and other lactones (**1e**, **1h**, **1i**, and **1j**) were prepared by the literature procedure[71].

6-Iodo-4,9-dihydro-3H-pyrano[3,4-b]indol-1-one (1e)
Yield: 52%; mp: 228-230 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 9.13 (1H, br), 8.00 (1H, s), 7.63 (1H, dd, *J* = 1.5 Hz, 8.4 Hz), 7.27 (1H, d, *J* = 8.4 Hz), 4.71 (2H, t, *J* = 6.3 Hz), 3.13 (2H, t, *J* = 6.3 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 160.93, 137.01, 134.99, 129.84, 128.99, 126.95, 121.90, 114.66, 84.16, 69.44, 21.27; IR (KBr): 3283, 1705 cm^{-1}; MS (EI) m/z 313 (M+); HRMS (EI) calcd for C$_{11}$H$_8$NO$_2$I: 312.9600 (M+), found: 312.9620.
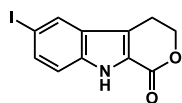
6-Iso-propyl-4,9-dihydro-3H-pyrano[3,4-b]indol-1-one (1h)
Yield: 62%; ^{1}H NMR (300 MHz, CDCl$_3$): δ 9.64 (1H, br), 7.45 (2H, d, *J* = 8.8 Hz), 7.31-7.28 (1H, m), 4.71 (2H, t, *J* = 6.3 Hz), 3.17 (2H, t, *J* = 6.3 Hz), 3.03 (1H, sept, *J* = 6.9 Hz), 1.32 (6H, d, *J* = 6.9 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 161.72, 141.28, 137.08, 126.33, 124.21, 122.79, 122.00, 116.87, 112.79, 69.48, 34.08, 24.37, 21.46; IR (neat): 3276, 1689 cm^{-1}; MS (EI) m/z 229 (M+); HRMS (EI) calcd for C$_{14}$H$_{15}$NO$_2$: 229.1103 (M+), found: 229.1096.
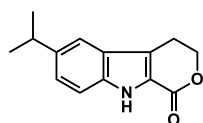
5,7-Difluoro-4,9-dihydro-3H-pyrano[3,4-b]indol-1-one (1i)
Yield: 40%; mp: 186-187 °C; ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 7.03 (1H, dd, *J* = 2.1 Hz, 9.4 Hz), 7.00-6.96 (1H, m), 4.63 (2H, t, *J* = 6.2 Hz),
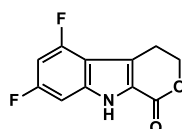
45

3.19 (2H, t, J = 6.2 Hz); ^{13}C NMR (125 MHz, CDCl$_3$): δ 162.11 (dd, J = 12.5 Hz, 246.3 Hz), 160.60, 157.70 (dd, J = 15.3 Hz, 253.0 Hz), 139.25 (dd, J = 12.5 Hz, 15.3 Hz), 22.77 (d, J = 3.8 Hz), 121.47 (d, J = 1.9 Hz), 111.36 (d, J = 21.1 Hz), 97.03 (dd, J = 24.0 Hz, 29.7 Hz), 94.98 (dd, J = 4.8 Hz, 26.8 Hz), 69.48, 22.28; IR (KBr): 3275, 1697 cm^{-1}; MS (EI) m/z 223 (M+); HRMS (EI) calcd for C$_{11}$H$_7$NO$_2$F$_2$: 223.0445 (M+), found: 223.0417.
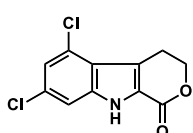
Dichloro-4,9-dihydro-3H-pyrano[3,4-b]indol-1-one (1j)

Yield: 42%; mp: 137-139 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 9.17 (1H, br), 7.38 (1H, d, J = 1.6 Hz), 7.17 (1H, d, J = 1.6 Hz), 4.71 (2H, t, J = 6.3 Hz), 3.44 (2H, t, J = 6.3 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 168.47, 139.60, 135.20, 128.49, 124.21, 122.08, 117.91, 111.32, 69.51, 24.60, 22.64; IR (KBr): 3267, 1712 cm^{-1}; MS (EI) m/z 255 (M+); HRMS (EI) calcd for C$_{11}$H$_7$NO$_2$Cl$_2$: 254.9854 (M+), found: 254.9856.

Typical procedure for lactams (**2a-2r**): The known lactams (**2b**, **2c**, **2i**, **2j**, **2k**, **2m**, and **2q**) and other lactams (**2a**, **2d**, **2e**, **2f**, **2g**, **2h**, **2l**, **2n**, **2o**, **2p**, and **2r**) were prepared by the literature procedure[24), 82)].
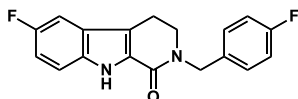
6-Fluoro-2-(4-fluorobenzyl)-2,3,4,9-tetrahydro-β-carbolin-1-one (2a)

Yield: 34%; mp: 211-213 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 11.8 (1H, brs), 7.40-7.36 (4H, m), 7.18 (2H, t-like, J = 9.0 Hz), 7.08 (1H, td, J = 2.6 Hz, 9.2 Hz), 4.68 (2H, s), 3.60 (2H, t, J = 7.0 Hz), 2.95 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 160.1, 133.9, 129.5 & 129.4, 128.4, 124.7, 117.4 & 117.3, 115.3, 115.0, 113.7 & 113.5, 112.8 & 112.4, 104.6 & 104.3, 48.0, 47.2, 20.0; IR (KBr) 3216, 1635 cm^{-1}; MS 312 (M+); HRMS calcd for C$_{18}$H$_{14}$N$_2$OF$_2$: 312.1074, found: 312.1046.
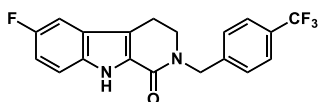
6-Fluoro-2-(4-trifluoromethylbenzyl)-2,3,4,9-tetrahydro-β-carbolin-1-one (2d)

Yield: 35%; mp: 237-240 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.74 (1H, br), 7.61 (2H, d, J = 8.0 Hz), 7.48 (2H, d, J = 8.0 Hz), 7.35-7.32 (1H, m), 7.19 (1H, dd, J = 1.4 Hz, 9.2 Hz), 7.04 (1H, td, J = 2.3 Hz, 9.2 Hz), 4.86 (2H, s), 3.67 (2H, t, J = 6.9 Hz), 3.02 (2H, t, J = 6.9 Hz); ^{13}C NMR (100 MHz, CDCl$_3$): δ 161.37, 157.97 (d, J = 236.7 Hz), 141.52, 134.07, 129.92 (q, J = 32.6 Hz), 128.09, 127.77, 125.71 (q, J =

3.8 Hz), 125.39, 125.28, 118.25 (d, J = 4.8 Hz), 113.96 (d, J = 26.8 Hz), 113.35 (d, J = 9.6 Hz), 104.67 (d, J = 24.0 Hz), 49.36, 47.72, 20.61; IR (KBr): 3223, 1635 cm^{-1}; MS (EI) m/z 362 (M+); HRMS (EI) calcd for $C_{19}H_{14}N_2OF_4$: 362.1042 (M+), found: 362.1027.
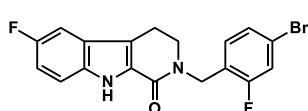
2-(4-Bromo-2-fluorobenzyl)-6-fluoro-2,3,4,9-tetrahydro-β-carbolin-1-one (2e)

Yield: 33%; mp: 235-238 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.53 (1H, br), 7.36-7.26 (4H, m), 7.19 (1H, dd, J = 2.3 Hz, 9.2 Hz), 7.05 (1H, td, J = 2.3 Hz, 9.2 Hz), 4.79 (2H, s), 3.71 (2H, t, J = 6.9 Hz), 3.01 (2H, t, J = 6.9 Hz); ^{13}C NMR (100 MHz, CDCl$_3$): δ 161.50, 160.86 (d, J = 251.2 Hz), 157.95 (d, J = 236.8 Hz), 134.14, 131.53 (d, J = 4.8 Hz), 128.12, 127.83 (d, J = 3.8 Hz), 125.28 (d, J = 9.6 Hz), 123.58 (d, J = 14.4 Hz), 121.61 (d, J = 9.6 Hz), 119.14 (d, J = 24.9 Hz), 118.27 (d, J = 4.8 Hz), 113.93 (d, J = 26.8 Hz), 113.40 (d, J = 9.6 Hz), 104.64 (d, J = 24.0 Hz), 47.97, 43.08 (d, J = 2.9 Hz), 20.64; IR (KBr): 3217, 1637 cm^{-1}; MS (EI) m/z 390 (M+); HRMS (EI) calcd for $C_{18}H_{13}N_2OF_2Br$: 390.0179 (M+), found: 390.0175.
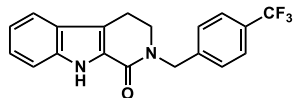
2-(4-Trifluoromethylbenzyl)-2,3,4,9-tetrahydro-β-carbolin-1-one (2f)

Yield: 37%; mp: 224-227 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 9.15 (1H, br), 7.62-7.57 (3H, m), 7.48 (2H, d, J = 7.7 Hz), 7.44 (1H, d, J = 8.2 Hz), 7.31 (1H, t, J = 7.1 Hz), 7.16 (1H, t, J = 7.1 Hz), 4.85 (2H, s), 3.66 (2H, t, J = 7.1 Hz), 3.06 (2H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, CDCl$_3$): δ 161.61, 141.72, 137.50, 129.83 (q, J = 32.6 Hz), 128.10, 126.55, 125.67 (q, J = 3.8 Hz), 125.24, 125.10, 122.74, 120.34, 120.20, 118.50, 112.45, 49.29, 47.76, 20.71; ; IR (KBr): 3230, 1636 cm^{-1}; MS (EI) m/z 344 (M+); HRMS (EI) calcd for $C_{19}H_{15}N_2OF_3$: 344.1137 (M+), found: 344.1156.
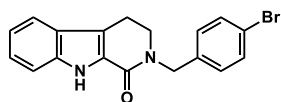
2-(4-Bromobenzyl)-2,3,4,9-tetrahydro-β-carbolin-1-one (2g)

Yield: 40%; mp: 249-252 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.96 (1H, br), 7.57 (1H, d, J = 7.7 Hz), 7.48-7.42 (3H, m), 7.34-7.22 (3H, m), 7.15 (1H, t, J = 7.7 Hz), 4.73 (2H, s), 3.63 (2H, t, J = 7.0 Hz), 3.03 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 161.25, 137.22, 136.49, 131.70, 129.57, 126.59, 125.22, 124.99, 121.30, 120.28, 120.12, 118.32, 112.29, 49.04, 47.54, 20.78; IR (KBr): 3218, 1635 cm^{-1}; MS (EI) m/z 354 (M+); HRMS (EI) calcd for $C_{18}H_{15}N_2OBr$: 354.0368 (M+), found: 354.0323.
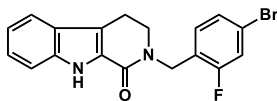
2-(4-Bromo-2-fluorobenzyl)-2,3,4,9-tetrahydro-β-carbolin-1-one (2h)

Yield: 33%; mp: 217-219 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.66 (1H, s), 7.60-7.56 (2H, m), 7.43-7.32 (3H, m), 7.22 (1H, t, J = 8.1 Hz), 7.06 (1H, t, J = 8.1 Hz), 4.71 (2H, s), 3.65 (2H, t, J = 7.0 Hz), 3.00 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 160.09 (d, J = 249.0 Hz), 160.46, 137.21, 131.21 (d, J = 4.9 Hz), 127.57 (d, J = 3.7 Hz), 126.53, 124.56, 124.33, 124.10 (d, J = 4.9 Hz), 120.30 (d, J = 9.8 Hz), 120.01, 119.39, 118.59 (d, J = 25.6 Hz), 117.66, 112.44, 47.64, 42.61, 20.15; IR (KBr): 3223, 1635 cm^{-1}; MS (EI) m/z 371 (M+); HRMS (EI) calcd for $C_{18}H_{14}N_2OFBr$: 372.0274 (M+), found: 372.0299.
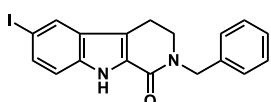
2-Benzyl-6-iodo-2,3,4,9-tetrahydro-β-carbolin-1-one (2l)

Yield: 45%; mp: 227-229 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ 10.57 (1H, br), 7.78 (1H, s), 7.35 (1H, dd, J = 1.4 Hz, 8.7 Hz), 7.27-7.15 (5H, m), 7.08 (1H, d. J = 8.7 Hz), 4.73 (2H, s), 3.55 (2H, t, J = 7.1 Hz), 2.87 (2H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, CDCl$_3$): δ 161.43, 137.30, 136.73, 132.91, 128.97, 128.77, 127.88, 127.84, 127.60, 127.44, 117.07, 114.70, 83.29, 49.74, 47.47, 20.51; IR (KBr): 3202, 1631 cm^{-1}; MS (EI) m/z 402 (M+); HRMS (EI) calcd for $C_{18}H_{15}N_2OI$: 402.0230 (M+), found: 402.0228.
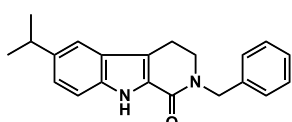
2-Benzyl-6-iso-propyl-2,3,4,9-tetrahydro-β-carbolin-1-one (2n)

Yield: 40%; mp: 205-207 °C; 1H NMR (400 MHz, CDCl$_3$): δ 9.42 (1H, br), 7.38-7.26 (7H, m), 7.19 (1H, dd, J = 1.6 Hz, 8.5 Hz), 4.82 (2H, s), 3.64 (2H, t, J = 6.9 Hz), 3.03-2.97 (3H, m), 1.30 (6H, d, J = 6.9 Hz); ^{13}C NMR (100 MHz, CDCl$_3$): δ 161.64, 140.91, 137.64, 136.14, 128.66, 127.92, 127.42, 127.01, 125.31, 124.49, 118.00, 116.59, 112.28, 49.48, 47.44, 34.17, 24.49, 20.71; IR (KBr): 3214, 1635 cm^{-1}; MS (EI) m/z 318 (M+); HRMS (EI) calcd for $C_{21}H_{22}N_2O$: 318.1732 (M+), found: 318.1780.
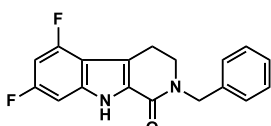
2-Benzyl-5,7-difluoro-2,3,4,9-tetrahydro-β-carbolin-1-one (2o)

Yield: 40%; mp: 167-169 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ 10.62 (1H, br), 7.37-7.28 (5H, m), 6.89 (1H, dd, J = 1.8 Hz, 8.7 Hz), 6.58 (1H, dt, J = 1.8 Hz, 10.5 Hz), 4.83 (2H, s), 3.67 (2H, t, J = 7.1 Hz), 3.15 (2H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, CDCl$_3$): δ 161.17 (dd, J = 11.5 Hz, 242.5 Hz), 159.96, 157.49 (dd, J =

15.3 Hz, 251.1 Hz), 139.19 (dd, J = 13.4 Hz, 15.3 Hz), 137.51, 137.07, 129.12 (d, J = 3.8 Hz), 128.16, 127.54 (d, J = 3.8 Hz), 116.86, 111.94 (d, J = 21.1 Hz), 96.32 (dd, J = 22.5 Hz, 29.2 Hz), 95.13 (dd, J = 4.8 Hz, 25.9 Hz), 49.98, 47.78, 21.85; IR (KBr): 3193, 1626 cm^{-1}; MS (EI) m/z 312 (M+); HRMS (EI) calcd for $C_{18}H_{14}N_2OF_2$: 312.1074 (M+), found: 312.1069.
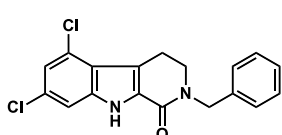
2-Benzyl-5,7-dichloro-2,3,4,9-tetrahydro-β-carbolin-1-one (2p)

Yield: 30%; mp: 248-250 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ 10.04 (1H, br), 7.37-7.28 (6H, m), 7.09 (1H, d, J = 1.4 Hz), 4.81 (2H, s), 3.66 (2H, t, J = 7.1 Hz), 3.31 (2H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, CDCl$_3$): δ 160.96, 138.36, 137.04, 130.25, 128.81, 128.18, 128.00, 127.88, 127.68, 121.90, 121.21, 118.07, 111.23, 49.67, 47.38, 21.83; IR (KBr): 3188, 1633 cm^{-1}; MS (EI) m/z 344 (M+); HRMS 360 (EI) calcd for $C_{18}H_{14}N_2OCl_2$: 344.0483 (M+), found: 344.0474.
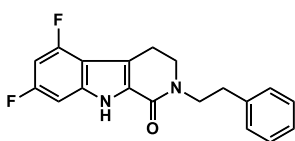
5,7-Difluoro-2-phenylethyl-2,3,4,9-tetrahydro-β-carbolin-1-one (2r)

Yield: 33%; mp: 220-222 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 10.51 (1H, br), 7.35-7.22 (5H, m), 6.98 (1H, dd, J = 1.9 Hz, 9.1 Hz), 6.60 (1H, dd, J = 1.9 Hz, 10.2 Hz), 3.84 (2H, t, J = 7.4 Hz), 3.58 (2H, t, J = 7.0 Hz), 3.07 (2H, t, J = 7.0 Hz), 3.01 (2H, t, J = 7.4 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 160.95 160.76 (dd, J = 11.5 Hz, 241.5 Hz), 157.23 (dd, J = 15.3 Hz, 251.1 Hz), 139.17 (dd, J = 12.9 Hz, 14.9 Hz), 138.92, 128.85, 128.66, 127.49 (d, J = 1.9 Hz), 126.57, 116.37 (d, J = 3.8 Hz), 111.52 (d, J = 1.9 Hz, 21.1 Hz), 95.82 (dd, J = 22.5 Hz, 29.2 Hz), 94.90 (dd, J = 4.8 Hz, 25.9 Hz), 49.03, 48.86, 34.64, 21.56; IR (KBr): 3161, 1629 cm^{-1}; MS (EI) m/z 326 (M+); HRMS (EI) calcd for $C_{19}H_{16}N_2OF_2$: 326.1231 (M+), found: 326.1241.
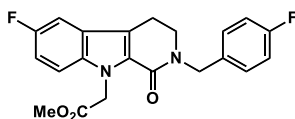
[6-Fluoro-2-(4-fluorobenzyl)-1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid methyl ester (3)

To a stirred solution of **2a** (193 mg, 0.62 mmol) in DMF (5 ml) was added NaH (60%, 37 mg, 0.93 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min. To the mixture was added BrCH$_2$CO$_2$Me (87 μl, 0.93 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 24 h. The reaction was quenched with H$_2$O (10 mL), and the aqueous mixture was extracted with Et$_2$O (10 ml×3). The organic extracts were combined, dried over MgSO$_4$, and evaporated. The residue was chromatographed on SiO$_2$ (Hexane :

Acetone = 8: 1) to give **3** (173 mg, 73%). mp: 87-89 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.31-7.28 (2H, m), 7.22-7.19 (2H, m), 7.10 (1H, td, J = 2.6 Hz, 9.0 Hz), 7.02 (2H, t, J = 8.5 Hz), 5.44 (2H, s), 4.70 (2H, s), 3.77 (3H, s), 3.61 (2H, t, J = 7.0 Hz), 2.97 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 169.3, 160.9, 135.3, 133.0 & 132.9, 129.4 & 129.2, 126.9, 124.3 & 124.2, 118.9 & 118.8, 115.4 & 115.1, 113.8 & 113.5, 110.6 & 110.4, 105.0 & 104.7, 52.3, 48.6, 46.9, 45.7, 20.4; IR (KBr) 1737, 1650 cm^{-1}; MS (EI) m/z 384 (M+); HRMS (EI) calcd for C$_{21}$H$_{18}$N$_2$O$_3$F$_2$: 384.1286 (M+), found: 384.1260.
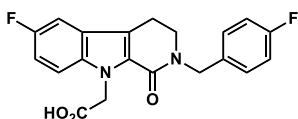
[2-(4-Fluorobenzyl)-6-fluoro-1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid (4)

To a stirred solution of **3** (173 mg, 0.45 mmol) in MeOH (3 ml) and H$_2$O (1 ml) was added LiOH・H$_2$O (37.2 mg, 0.90 mmol), and the resulting mixture was refluxed for 1 h. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with AcOEt (10 ml×3). The organic extracts were combined, dried over MgSO$_4$, and evaporated. The residue was chromatographed on SiO$_2$ (Hexane : Acetone = 2: 1) to give **4** (0.44 mmol, 162.9 mg, 98%).

mp: 148-150 °C; 1H NMR (300 MHz, DMSO-d$_6$): δ 7.61-7.58 (1H, m), 7.44-7.42 (1H, dd, J = 2.6 Hz, 9.4 Hz), 7.37-7.34 (2H, m), 7.19-7.14 (3H, m), 5.35 (2H, s), 4.65 (2H, s), 3.59 (2H, t, J = 7.0 Hz), 2.97 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 170.2, 160.2, 158.4, 155.7, 135.3, 133.9, 129.5 & 129.4, 126.9, 123.7, 118.6, 115.3 & 115.0, 113.2 & 112.9, 112.1 & 112.0, 104.8 & 104.5, 48.0, 47.0, 45.8, 19.9; IR (KBr) 2929, 1724, 1647 cm^{-1}; MS 370 (M+); HRMS calcd for C$_{20}$H$_{16}$N$_2$O$_3$F$_2$: 370.1129, found: 370.1124.

Typical procedure for thiolactams (**6b-6r**): To a stirred solution of lactam (1.00 mmol) in toluene (5 mL) was added Lawesson's reagent (0.55 equiv), and the resulting mixture was refluxed for 20-24 h. After cooling, the solvent was removed, and the residue was chromatographed on SiO$_2$ (Hexane : Acetone = 25 : 1) to give thiolactam (**6b-6r**).
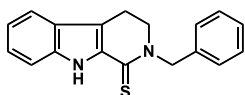
2-Benzyl-2,3,4,9-tetrahydro-β-carboline-1-thione (6b)

Yield: 98%; mp: 197-198 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 11.32 (1H, br), 7.60-7.22 (8H, m), 7.05 (1H, t, J = 7.5 Hz), 5.38 (2H, s), 3.77 (2H, t, J = 7.0 Hz), 3.00 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 182.90, 138.31, 136.50, 132.32, 128.44, 127.39, 127.23, 124.52, 124.47, 120.61, 119.70, 112.66, 111.47, 54.94, 49.55, 19.65; IR (KBr): 3319, 1556 cm^{-1}; MS

(EI) m/z 292 (M+); HRMS (EI) calcd for $C_{18}H_{16}N_2S$: 292.1034 (M+), found: 292.1054.
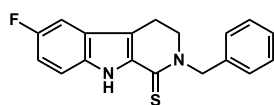
2-Benzyl-6-fluoro-2,3,4,9-tetrahydro-β-carboline-1-thione (6c)

Yield: 53%; mp: 127-130 °C; ^1H NMR (300 MHz, DMSO-d$_6$): δ 11.43 (1H, br), 7.47 (1H, m), 7.41-7.26 (6H, m), 7.11 (1H, dt, J = 2.5 Hz, 9.4 Hz), 5.37 (2H, s), 3.77 (2H, t, J = 7.2 Hz), 2.97 (2H, t, J = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 182.70, 156.84 (d, J = 233.2 Hz), 136.35, 135.01, 133.75, 128.44, 127.39, 127.26, 124.47 (d, J = 9.8 Hz), 113.96 (d, J = 9.8 Hz), 113.31 (d, J = 26.9 Hz), 111.43 (d, J = 6.1 Hz), 104.84 (d, J = 23.2 Hz), 55.03, 49.60, 19.55; IR (KBr): 3320, 1555 cm^{-1}; MS (EI) m/z 310 (M+); HRMS (EI) calcd for $C_{18}H_{15}N_2FS$: 310.0940 (M+), found: 310.0914.
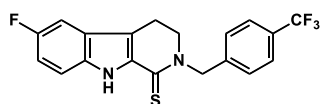
6-Fluoro-2-(4-trifluoromethylbenzyl)-2,3,4,9-tetrahydro-β-carboline-1-thione (6d)

Yield: 90%; mp: 180-182 °C; ^1H NMR (300 MHz, CDCl$_3$): δ 9.08 (1H, br), 7.62 (2H, d, J = 8.1 Hz), 7.51 (2H, d, J = 8.1 Hz), 7.38-7.34 (1H, m), 7.19 (1H, d, J = 9.1 Hz), 7.08 (1H, dt, J = 2.5 Hz, 9.1 Hz), 5.45 (2H, s), 3.78 (2H, t, J = 7.4 Hz), 3.02 (2H, t, J = 7.4 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 184.11, 157.88 (d, J = 236.8 Hz), 140.10, 134.68, 133.48, 130.03 (q, J = 33.0 Hz), 127.92, 125.69 (q, J = 3.7 Hz), 122.11, 118.50, 114.60 (d, J = 26.9 Hz), 113.10 (d, J = 9.8 Hz), 111.43 (d, J = 4.9 Hz), 105.19 (d, J = 23.2 Hz), 55.56, 49.80, 20.36; IR (KBr): 3309, 1558 cm^{-1}; MS (EI) m/z 378 (M+); HRMS (EI) calcd for $C_{19}H_{14}N_2F_4S$: 378.0814 (M+), found: 378.0838.
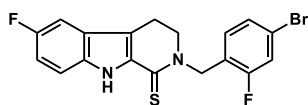
2-(4-Bromo-2-fluorobenzyl)-6-fluoro-2,3,4,9-tetrahydr-β-carboline-1-thione (6e)

Yield: 90%; mp: 128-131 °C; ^1H NMR (300 MHz, CDCl$_3$): δ 9.07 (1H, br), 7.43 (1H, t, J = 8.1 Hz), 7.36-7.24 (3H, m), 7.19 (1H, dd, J = 1.6 Hz, 9.1 Hz), 7.07 (1H, td, J = 2.5 Hz, 9.1 Hz), 5.38 (2H, s), 3.82 (2H, t, J = 7.4 Hz), 3.01 (2H, t, J = 7.4 Hz); ^{13}C NMR (100 MHz, CDCl$_3$): δ 184.23, 160.51 (d, J = 278.0 Hz), 158.08 (d, J = 264.6 Hz), 134.70, 133.60, 131.34 (d, J = 3.8 Hz), 127.78 (d, J = 3.8 Hz), 125.55 (d, J = 9.6 Hz), 122.29 (d, J = 15.3 Hz), 121.86 (d, J = 9.6 Hz), 119.15 (d, J = 24.9 Hz), 114.55 (d, J = 26.8 Hz), 113.13 (d, J = 9.6 Hz), 111.48 (d, J = 5.8 Hz), 105.17 (d, J = 23.0 Hz), 49.99, 49.02 (d, J = 2.9 Hz), 20.19; IR (KBr): 3310, 1554 cm^{-1}; MS (EI) m/z 406 (M+); HRMS (EI) calcd for $C_{18}H_{13}N_2F_2SBr$: 405.9951 (M+), found: 405.9924.
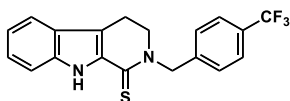
2-(4-Trifluoromethylbenzyl)-2,3,4,9-tetrahydro-β-carboline-1-thione (6f)

Yield: 95%; mp: 187-190 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 9.08 (1H, br), 7.62 (2H, d, J = 8.5 Hz), 7.58 (1H, d, J = 8.2 Hz), 7.51 (2H, d, J = 8.5 Hz), 7.43 (1H, d, J = 8.2 Hz), 7.33 (1H, t, J = 7.5 Hz), 7.14 (1H, t, J = 7.5 Hz), 5.47 (2H, s), 3.79 (2H, t, J = 7.4 Hz), 3.06 (2H, t, J = 7.4 Hz); ^{13}C NMR (100 MHz, CDCl$_3$): δ 184.54, 140.40, 138.26, 132.27, 130.08 (q, J = 32.6 Hz), 128.00, 125.77 (q, J = 3.8 Hz), 125.50, 125.37, 122.66, 120.93, 120.74, 112.20, 118.78, 55.45, 49.78, 20.36; IR (KBr): 3340, 1557 cm^{-1}; MS (EI) m/z 360 (M+); HRMS (EI) calcd for C$_{19}$H$_{15}$N$_2$F$_3$S: 360.0908 (M+), found: 360.0861.
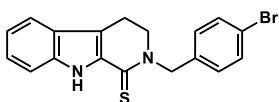
2-(4-Bromobenzyl)-2,3,4,9-tetrahydro-β-carboline-1-thione (6g)

Yield: 86%; mp: 171-174 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 9.08 (1H, br), 7.57 (1H, d, J = 8.0 Hz), 7.48 (2H, d, J = 8.2 Hz), 7.42 (1H, d, J = 8.5 Hz), 7.33 (2H, d, J = 8.2 Hz), 7.29 (1H, d, J = 8.5 Hz), 7.14 (1H, m), 5.35 (2H, s), 3.76 (2H, t, J = 7.4 Hz), 3.03 (2H, t, J = 7.4 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 184.03, 138.09, 135.28, 132.21, 131.80, 129.47, 125.56, 125.41, 121.66, 120.81, 120.59, 112.11, 111.61, 55.29, 49.59, 20.44; IR (KBr): 3334, 1558 cm^{-1};MS (EI) m/z 370 (M+); HRMS (EI) calcd for C$_{18}$H$_{15}$N$_2$SBr: 370.0139 (M+), found: 370.0139.
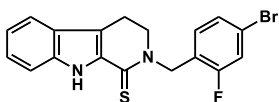
2-(4-Bromo-2-fluorombenzyl)-2,3,4,9-tetrahydro-β-carboline -1-thione (6h)

Yield: 68%; mp: 160-162 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 11.32 (1H, brs), 7.62-7.57 (2H, m), 7.49 (1H, d, J = 8.2 Hz), 7.42-7.21 (3H, m), 7.06 (1H, t, J = 7.4 Hz), 5.35 (2H, s), 3.86 (2H, t, J = 7.4 Hz), 3.05 (2H, t, J = 7.4 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 184.18, 160.42 (d, J = 250.3 Hz), 137.98, 132.11, 131.15 (d, J = 3.7 Hz), 127.62 (d, J = 3.7 Hz), 127.48, 125.27, 122.31 (d, J = 14.6 Hz), 121.62 (d, J = 8.5 Hz), 120.72, 120.49, 118.97 (d, J = 24.4 Hz), 112.00, 111.59, 49.98, 48.87 (d, J = 3.7 Hz), 20.36; IR (KBr): 3321, 1555 cm^{-1}; MS (EI) m/z 389 (M+); HRMS (EI) calcd for C$_{18}$H$_{14}$N$_2$FSBr: 388.0045 (M+), found: 388.0075.
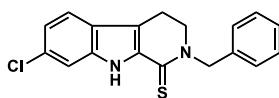
2-Benzyl-7-chloro-2,3,4,9-tetrahydro-β-carboline-1-thione (6i)

Yield: 99%; mp: 162-163 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 11.45 (1H, brs), 7.62-7.30 (6H, m), 7.06 (2H, d-like, J = 6.6 Hz), 5.37 (2H, s), 3.79 (2H,

52

t, *J* = 6.9 Hz), 3.00 (2H, t, *J* = 6.9 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.40, 138.09, 136.02, 132.79, 131.19, 128.69, 127.76, 124.02, 121.66, 121.45, 111.90, 111.35, 55.87, 49.33, 20.21; IR (KBr): 3318, 1551, cm^{-1}; MS (EI) m/z 326 (M+); HRMS (EI) calcd for C$_{18}$H$_{15}$N$_2$SCl: 326.0644 (M+), found: 326.0645.
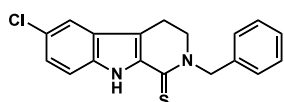
2-Benzyl-6-chloro-2,3,4,9-tetrahydro-β-carboline-1-thione (6j)

Yield: 99%; mp: 194-196 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 11.53 (1H, brs), 7.69 (1H, s), 7.49 (1H, d, *J* = 9.4 Hz), 7.40-7.31 (4H, m), 7.30 (1H, t, *J* = 9.4 Hz), 7.23 (1H, d, *J* = 9.4 Hz), 5.37 (2H, s), 3.78 (2H, t, *J* = 7.3 Hz), 2.99 (2H, t, *J* = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 182.61, 136.58, 136.30, 133.42, 128.44, 127.39, 127.26, 125.45, 124.52, 124.12, 119.78, 114.27, 110.97, 55.03, 49.55, 19.42; IR (KBr): 3317, 1551 cm^{-1}; MS (EI) m/z 326 (M+); HRMS (EI) calcd for C$_{18}$H$_{15}$N$_2$SCl: 326.0645 (M+), found: 326.0678.
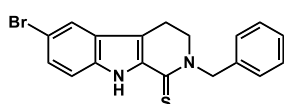
2-Benzyl-6-bromo-2,3,4,9-tetrahydro-β-carboline-1-thione (6k)

Yield: 85%; mp: 176-178 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 11.94 (1H, brs), 7.82 (1H, s), 7.47-7.27 (7H, m), 5.36 (2H, s), 3.76 (2H, t, *J* = 7.2 Hz), 2.98 (2H, t, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 182.59, 136.79, 136.29, 133.23, 128.41, 127.39, 127.24, 126.97, 126.17, 122.87, 114.65, 112.05, 110.82, 55.03, 49.53, 19.44; IR (KBr): 3220, 1550 cm^{-1}; MS (EI) m/z 370 (M+); HRMS (EI) calcd for C$_{18}$H$_{15}$N$_2$SBr: 370.0139 (M+), found: 370.0178.
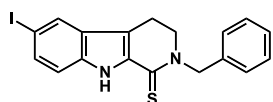
2-Benzyl-6-iodo-2,3,4,9-tetrahydro-β-carboline-1-thione (6l)

Yield: 99%; mp: 185-187 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 11.50 (1H, s), 8.01 (1H, s), 7.50-7.29 (7H, m), 5.36 (2H, s), 3.77 (2H, t, *J* = 7.6 Hz), 2.98 (2H, t, *J* = 7.6 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 182.56, 137.13, 136.33, 132.76, 132.31, 129.13, 128.46, 127.42, 127.27, 127.08, 115.04, 110.51, 83.40, 55.03, 49.56, 19.42; IR (KBr): 3318, 1551 cm^{-1}; MS (EI) m/z 418 (M+); HRMS (EI) calcd for C$_{18}$H$_{15}$N$_2$SI: 418.0001 (M+), found: 418.0062.
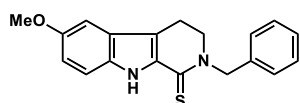
2-Benzyl-6-methoxy-2,3,4,9-tetrahydro-β-carboline-1-thione (6m)

Yield: 94%; mp: 173-174 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 11.18 (1H, br), 7.41-7.28 (6H, m), 7.04 (1H, s), 6.90 (1H, dd, *J* = 2.6 Hz, 9.0 Hz), 5.37

(2H, s), 3.75 (3H, s), 3.76 (2H, t, J = 7.5 Hz), 2.97 (2H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.82, 153.57, 136.54, 133.76, 132.74, 128.42, 127.40, 127.23, 124.60, 116.08, 113.62, 111.14, 100.63, 55.23, 54.90, 49.60, 19.73; IR (KBr): 3318, 1548 cm^{-1}; MS (EI) m/z 322 (M+); HRMS (EI) calcd for $C_{19}H_{18}N_2OS$: 322.1140 (M+), found: 322.1131.
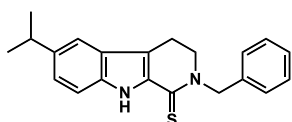
2-Benzyl-6-iso-propyl-2,3,4,9-tetrahydro-β-carboline-1-thione (6n)

Yield: 86%; mp: 161-164 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 9.03 (1H, br), 7.42-7.20 (8H, m), 5.41 (2H, s), 3.76 (2H, t, J = 7.4 Hz), 3.04-2.95 (3H, m), 1.30 (6H, d, J = 6.9 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 184.06, 141.35, 136.90, 136.41, 132.61, 128.77, 127.83, 127.75, 125.59, 125.27, 117.26, 111.96, 111.46, 55.80, 49.49, 34.16, 24.41, 20.36; IR (KBr): 3326, 1558 cm^{-1}; MS (EI) m/z 334 (M+); HRMS (EI) calcd for $C_{21}H_{22}N_2S$: 334.1504 (M+), found: 334.1500.
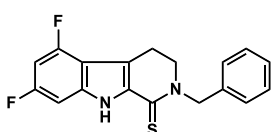
2-Benzyl-5,7-difluoro-2,3,4,9-tetrahydro-β-carboline-1-thione (6o)

Yield: 93%; mp: 109-111 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.20 (1H, br), 7.39-7.30 (4H, m), 7.07 (1H, dd, J = 2.2 Hz, 9.6 Hz), 6.89 (1H, t, J = 10.6 Hz), 5.36 (2H, s), 3.79 (2H, t, J = 7.4 Hz), 3.09 (2H, t, J = 7.4 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.13, 161.30 (dd, J = 11.5 Hz, 244.4 Hz), 157.97 (dd, J = 15.3 Hz, 252.1 Hz), 139.05 (d, J = 1.9 Hz), 136.07, 132.70 (d, J = 3.8 Hz), 128.84, 127.91, 127.87, 109.74 (d, J = 1.9 Hz), 96.55 (dd, J = 22.5 Hz, 29.2 Hz), 94.45 (dd, J = 4.8 Hz, 26.8 Hz), 55.84, 49.29, 21.07; IR (KBr): 3264, 1578 cm^{-1}; MS (EI) m/z 328 (M+); HRMS (EI) calcd for $C_{18}H_{14}N_2F_2S$: 328.0846 (M+), found: 328.0829.
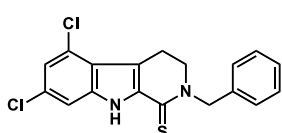
2-Benzyl-5,7-dichloro-2,3,4,9-tetrahydro-β-carboline-1-thione (6p)

Yield: 98%; mp: 185-187 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 9.19 (1H, br), 7.39-7.30 (6H, m), 7.09 (1H, d, J = 1.6 Hz), 5.38 (2H, s), 3.76 (2H, t, J = 7.4 Hz), 3.30 (2H, t, J = 7.4 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.13, 138.56, 135.95, 133.29, 130.93, 128.86, 128.79, 127.95, 127.89, 122.26, 121.66, 111.30, 110.73, 55.92, 49.32, 21.37; IR (KBr): 3272, 1557 cm^{-1}; MS (EI) m/z 360 (M+); HRMS (EI) calcd for $C_{18}H_{14}N_2Cl_2S$: 360.0255 (M+), found: 360.0273.
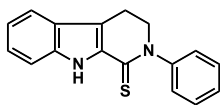
2-Phenyl-2,3,4,9-tetrahydro-β-carboline-1-thione (6q)

Yield: 99%; mp: 175-176 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 11.31 (1H, brs), 7.66 (1H, d, J = 8.0 Hz), 7.52-7.32 (6H, m), 7.26 (1H, t, J = 8.0 Hz), 7.08 (1H, t, J = 8.0 Hz), 4.11 (2H, t, J = 7.3 Hz), 3.18 (2H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 184.11, 146.04, 138.41, 132.95, 128.99, 127.14, 126.90, 125.36, 124.80, 120.85, 119.85, 113.33, 112.73, 54.01, 20.23; IR (KBr): 3395, 1556 cm^{-1}; MS (EI) m/z 277 (M+); HRMS (EI) calcd for C$_{17}$H$_{14}$N$_2$S: 278.0878 (M+), found: 278.0890.
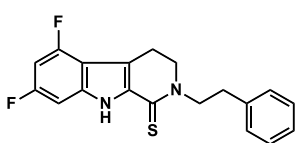
5,7-Difluoro-2-phenylethyl-2,3,4,9-tetrahydro-β-carboline-1-thione (6r)

Yield: 99%; mp: 150-153 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 9.18 (1H, br), 7.36-7.22 (5H, m), 6.87 (1H, dd, J = 1.9 Hz, 9.1 Hz), 6.58 (1H, dd, J = 1.9 Hz, 10.4 Hz), 4.29 (2H, t, J = 7.7 Hz), 3.61 (2H, t, J = 7.4 Hz), 3.12 (2H, t, J = 7.7 Hz), 3.00 (2H, t, J = 7.4 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 181.95, 161.07 (dd, J = 11.5 Hz, 243.5 Hz), 157.84 (dd, J = 15.3 Hz, 252.1 Hz), 138.85 (dd, J = 12.9 Hz, 14.9 Hz), 138.46, 132.88 (d, J = 2.9 Hz), 128.90, 128.63, 126.68, 112.00 (d, J = 21.1 Hz), 109.52, 96.38 (dd, J = 22.5 Hz, 29.2 Hz), 94.45 (dd, J = 4.8 Hz, 24.9 Hz), 55.61, 51.35, 33.06, 20.87; IR (KBr): 3336, 1551 cm^{-1}; MS (EI) m/z 342 (M+); HRMS (EI) calcd for C$_{19}$H$_{16}$N$_2$F$_2$S: 342.1002 (M+), found: 342.0992.

Typical procedure for esters (**7b-7r**): To a stirred solution of thiolactam (1.0 mmol) in DMF (5 ml) was added NaH (60%, 1.2 equiv) at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min. To the mixture was added BrCH$_2$CO$_2$$t$-Bu or BrCH$_2CO_2$Me (1.2 equiv) at 0 °C, and the resulting mixture was stirred at room temperature for 20-24 h. The reaction was quenched with H$_2$O (10 ml), and the aqueous mixture was extracted with Et$_2$O (10 ml×3). The organic extracts were combined, dried over MgSO$_4$, and evaporated. The residue was chromatographed on SiO$_2$ (Hexane : Acetone = 20 : 1) to give t-butyl ester (**7b-7j**, **7k**, **7n-7o**, and **7q**) or methyl ester (**7l-7m**, **7p**, and **7r**).
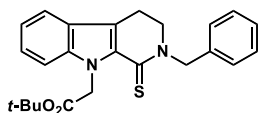
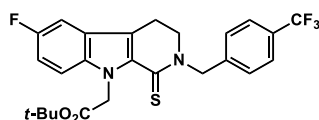### (2-Benzyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid t-butyl ester (7b)

Yield: 66%; mp: 63-64 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.56 (1H, d, J = 9.0 Hz), 7.39-7.27 (7H, m), 7.16 (1H, t, J = 7.5 Hz), 5.73 (2H, br), 5.47 (2H, s), 3.75 (2H, t, J = 7.3 Hz), 2.97 (2H, t, J = 7.3 Hz), 1.47 (9H, s); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.11, 167.91, 140.41, 136.22, 132.03, 128.31, 127.32, 127.19, 125.33,

122.89, 120.59, 120.51, 115.02, 109.88, 81.47, 55.22, 49.07, 47.28, 28.01, 20.37; IR (KBr): 1742 cm^{-1}; MS (EI) m/z 406 (M+); HRMS (EI) calcd for C$_{24}$H$_{26}$N$_2$O$_2$S: 406.1715 (M+), found: 406.1745.
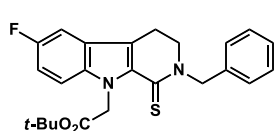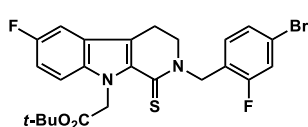
(2-Benzyl-6-fluoro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid *t*-butyl ester (7c)
Yield: 93%; mp: 54-56 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.37-7.18 (7H, m), 7.10 (1H, dt, *J* = 2.5 Hz, 9.4 Hz), 5.71 (2H, br), 5.46 (2H, s), 3.75 (2H, t, *J* = 7.2 Hz), 2.92 (2H, t, *J* = 7.2 Hz), 1.47 (9H, s); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.17, 167.96, 157.98 (d, *J* = 237.5 Hz), 137.07, 136.19, 133.27, 128.49, 127.46, 127.41, 123.09 (d, *J* = 10.1 Hz), 114.70 (d, *J* = 5.5 Hz), 114.19 (d, *J* = 26.7 Hz), 111.04 (d, *J* = 9.2 Hz), 104.90 (d, *J* = 23.9 Hz), 81.72, 55.31, 49.07, 47.37, 27.92, 20.22; IR (KBr): 1742 cm^{-1};MS (EI) m/z 424 (M+); HRMS (EI) calcd for C$_{24}$H$_{25}$N$_2$O$_2$FS: 424.1621 (M+), found: 424.1602.

[6-Fluoro-1-thioxo-2-(4-trifluoromethylbenzyl)-1,2,3,4-tetrahydro-β-carbolin-9-yl]-acetic acid *t*-butyl ester (7d)
Yield: 76%; mp: 53-55 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.59 (2H, d, *J* = 8.0 Hz), 7.47 (2H, d, *J* = 8.0 Hz), 7.24-7.05 (3H, m), 5.68 (2H, br), 5.51 (2H, s), 3.76 (2H, t, *J* = 6.9 Hz), 2.96 (2H, t, *J* = 6.9 Hz), 1.47 (9H, s); ^{13}C NMR (100 MHz, CDCl$_3$): δ 184.00, 168.16, 158.30 (d, *J* = 237.7 Hz), 140.46, 137.37, 133.39, 129.89 (q, *J* = 32.6 Hz), 128.11, 128.00, 125.66 (q, *J* = 3.8 Hz), 114.99 (d, *J* = 4.8 Hz), 114.74 (d, *J* = 26.8 Hz), 113.15 (d, *J* = 9.6 Hz), 111.28 (d, *J* = 9.6 Hz), 105.17 (d, *J* = 23.0 Hz), 82.14, 55.32, 49.65, 47.58, 28.10, 20.58; IR (KBr): 1740 cm^{-1}; MS (EI) m/z 492 (M+); HRMS (EI) calcd for C$_{25}$H$_{24}$N$_2$O$_2$F$_4$S: 492.1495 (M+), found: 492.1518.

[2-(4-Bromo-2-fluorobenzyl)-6-fluoro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]-acetic acid *t*-butyl ester (7e)
Yield: 95%; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.45-7.07 (6H, m), 5.65 (2H, br), 5.43 (2H, s), 3.79 (2H, t, *J* = 7.1 Hz), 2.96 (2H, t, *J* = 7.1 Hz), 1.46 (9H, s); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.85, 167.98, 160.48 (d, *J* = 250.3 Hz), 158.15 (d, *J* = 238.1 Hz), 137.24, 133.32, 130.96 (d, *J* = 4.9 Hz), 127.60 (d, *J* = 3.7 Hz), 123.18 (d, *J* = 9.8 Hz), 121.49 (d, *J* = 9.8 Hz), 119.10 (d, *J* = 24.4 Hz), 119.02 (d, *J* = 24.4 Hz), 114.95 (d, *J* = 6.1 Hz), 114.65 (d, *J* = 26.9 Hz), 111.20 (d, *J* = 8.5 Hz), 105.13 (d, *J* = 23.2 Hz), 82.11, 49.03 (d, *J* = 3.6 Hz), 47.63, 31.24, 28.20, 20.71; IR (neat): 1742 cm^{-1};

MS (EI) m/z 520 (M+); HRMS (EI) calcd for $C_{24}H_{23}N_2O_2FSBr$: 520.0632 (M+), found: 520.0612.
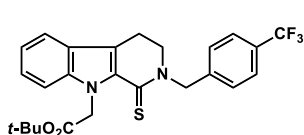
[2-(4-Trifluoromethylbenzyl)-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]-acetic acid *t*-butyl ester (7f)

Yield: 86%; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.61-7.57 (3H, m), 7.48 (2H, d, J = 8.7 Hz), 7.41-7.30 (2H, m), 7.19-7.15 (1H, m), 5.70 (2H, br), 5.52 (2H, s), 3.77 (2H, t, J = 6.9 Hz), 3.01 (2H, t, J = 6.9 Hz), 1.47 (9H, s); ^{13}C NMR (75 MHz, CDCl$_3$): δ 184.25, 168.33, 132.34, 129.86 (q, J = 32.6 Hz), 128.01, 127.82, 125.94, 125.80, 125.77, 125.66 (q, J = 3.8 Hz), 123.20, 121.09, 120.87, 115.46, 110.25, 81.94, 55.29, 49.67, 47.46, 28.14, 20.72; IR (neat): 1735 cm^{-1}; MS (EI) m/z 474 (M+); HRMS (EI) calcd for $C_{25}H_{25}N_2O_2F_3S$: 474.1589 (M+), found: 474.1571.
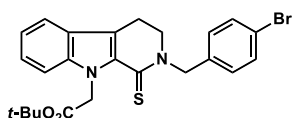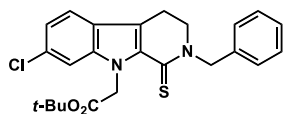
[2-(4-Bromobenzyl)-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]-acetic acid *t*-butyl ester (7g)

Yield: 92%; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.57 (1H, d, J = 8.0 Hz), 7.49-7.44 (3H, m), 7.40-7.24 (3H, m), 7.19-7.14 (1H, m), 5.70 (2H, br), 5.41 (2H, s), 3.74 (2H, t, J = 7.1 Hz), 2.98 (2H, t, J = 7.1 Hz), 1.47 (9H, s); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.74, 168.14, 140.73, 135.55, 131.80, 131.69, 129.47, 129.31, 125.75, 123.11, 120.93, 120.73, 115.31, 110.15, 81.87, 50.01, 49.43, 47.52, 28.24, 20.78; IR (neat): 1744 cm^{-1}; MS (EI) m/z 484 (M+); HRMS (EI) calcd for $C_{24}H_{25}N_2O_2SBr$: 484.0820 (M+), found: 484.0832.
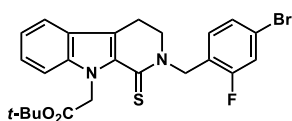
[2-(4-Bromo-2-fluorobenzyl)-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]-acetic acid methyl ester (7h)

Yield: 58%; mp: 130-132 °C; ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.59 (1H, d, J = 8.1 Hz), 7.40-7.36 (2H, m), 7.30-7.24 (3H, m), 7.18 (1H, t, J = 7.5 Hz), 5.81 (2H, brs), 5.44 (2H, s), 3.81 (2H, t, J = 6.8 Hz), 3.76 (3H, s), 3.02 (2H, t, J = 6.8 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.82, 169.53, 160.44 (d, J = 249.0 Hz), 140.63, 131.96, 131.00 (d, J = 3.7 Hz), 127.60 (d, J = 3.7 Hz), 125.94, 123.10, 122.59 (d, J = 14.7 Hz), 121.45 (d, J = 9.8 Hz), 121.11, 120.83, 118.96 (d, J = 25.6 Hz), 115.62, 110.04, 52.31, 49.87, 48.90 (d, J = 3.7 Hz), 46.84, 20.74; IR (KBr): 1739 cm^{-1}; MS (EI) m/z 460 (M+); HRMS (EI) calcd for $C_{21}H_{18}N_2O_2FSBr$: 460.0256 (M+), found: 460.0245.
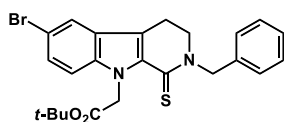
(2-Benzyl-7-chloro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid *t*-butyl ester (7i)

Yield: 52%; mp: 156-158 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.49-7.46 (1H, m), 7.38-7.27 (6H, m), 7.14-7.10 (1H, m), 5.67 (2H, s), 5.45 (2H, s), 3.74 (2H, t, *J* = 6.9 Hz), 2.94 (2H, t, *J* = 6.9 Hz), 1.49 (9H, s); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.24, 167.85, 140.93, 136.33, 132.84, 131.56, 128.63, 127.61, 121.83, 121.75, 121.62 115.09, 110.20, 82.11, 55.58, 49.22, 47.63, 28.24, 20.61; IR (KBr): 1741 cm^{-1}; MS (EI) m/z 440 (M+); HRMS (EI) calcd for C$_{24}$H$_{25}$N$_2$O$_2$SCl: 440.1325 (M+), found: 440.1358.
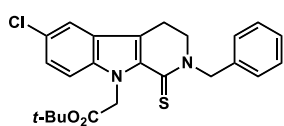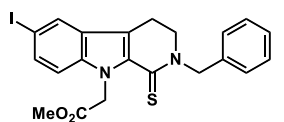
(2-Benzyl-6-chloro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid *t*-butyl ester (7j)

Yield: 65%; mp: 34-35 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.53 (1H, d, *J* = 9.0 Hz), 7.31-7.28 (7H, m), 5.70 (2H, br), 5.46 (2H, s), 3.74 (2H, t, *J* = 7.1 Hz), 2.92 (2H, t, *J* = 7.1 Hz), 1.47 (9H, s); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.07, 167.78, 136.14, 132.98, 128.60, 128.52, 127.67, 127.50, 126.19, 125.68, 123.90, 119.78, 114.26, 111.24, 81.95, 55.50, 49.19, 47.51, 28.12, 20.40; IR (KBr): 1743 cm^{-1}; MS (EI) m/z 440 (M+); HRMS (EI) calcd for C$_{24}$H$_{25}$N$_2$O$_2$SCl: 440.1325 (M+), found: 440.1313.

(2-Benzyl-6-bromo-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid *t*-butyl ester (7k)

Yield: 53%; mp: 157-158 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.69 (1H, s), 7.43-7.31 (6H, brm), 7.15 (1H, d, *J* = 8.7 Hz), 5.70 (2H, brs), 5.45 (2H, s), 3.74 (2H, t, *J* = 7.2 Hz), 2.92 (2H, t, *J* = 7.2 Hz), 1.47 (9H, s); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.11, 167.77, 139.05, 136.17, 128.65, 128.56, 128.21, 127.72, 127.55, 124.62, 122.98, 114.15, 113.71, 111.64, 82.00, 55.55, 49.20, 47.51, 28.15, 20.45; IR (KBr): 1742 cm^{-1}; MS (EI) m/z 484 (M+); HRMS (EI) calcd for C$_{24}$H$_{25}$N$_2$O$_2$SBr: 484.0820 (M+), found: 484.0800.

(2-Benzyl-6-iodo-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid methyl ester (7l)

Yield: 61%; mp: 70-72 °C; ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.92 (1H, s), 7.59 (1H, d, *J* = 9.0 Hz), 7.37-7.29 (5H, m), 7.05 (1H, d, *J* = 9.0 Hz), 5.83 (2H, brs), 5.44 (2H, s), 3.77 (3H, s), 3.74 (2H, t, *J* = 6.8 Hz), 2.92 (2H, t, *J* = 6.8 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 182.85, 169.18, 139.39, 136.14, 133.65, 132.16, 129.45, 128.56, 127.55, 125.49, 114.08, 111.96, 84.07, 55.50, 52.34, 49.06, 46.76, 20.42; IR

(KBr): 1749 cm^{-1}; MS (EI) m/z 490 (M+); HRMS (EI) calcd for C$_{21}$H$_{19}$N$_2$O$_2$SI: 490.0212 (M+), found: 490.0204.
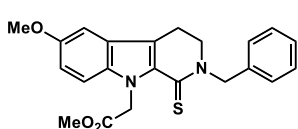
(2-Benzyl-6-methoxy-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid methyl ester (7m)

Yield: 63%; mp: 117-118 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.37-6.93 (8H, m), 5.83 (2H, brs), 5.45 (2H, s), 3.85 (3H, s), 3.75 (2H, t, J = 5.6 Hz), 2.93 (2H, t, J = 7.1 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 182.8, 169.4, 154.5, 137.3, 136.2, 135.8, 132.1, 128.3, 127.5, 127.3, 127.2, 127.0, 126.9, 123.0, 116.7, 114.7, 110.8, 100.8, 55.4, 55.1, 52.0, 48.9, 46.6, 20.3; IR (KBr) 1751 cm^{-1}; MS (EI) m/z 394 (M+); HRMS calcd for C$_{22}$H$_{22}$N$_2$O$_3$S: 394.1351, found: 394.1355.
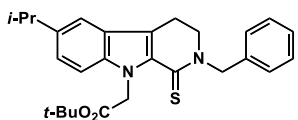
(2-Benzyl-6-iso-propyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid t-butyl ester (7n)

Yield: 80%; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.36-7.18 (8H, m), 5.70 (2H, br), 5.47 (2H, s), 3.74 (2H, t, J = 7.0 Hz), 3.04-2.94 (3H, m), 1.48 (9H, s), 1.29 (6H, d, J = 7.1 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.61, 168.30, 141.54, 139.53, 136.59, 128.58, 127.74, 127.59, 127.45, 125.34, 117.24, 115.12, 109.96, 81.74, 55.55, 49.37, 47.57, 38.67, 34.19, 28.25, 24.51, 20.81; IR (neat):1743 cm^{-1}; MS (EI) m/z 448 (M+); HRMS (EI) calcd for C$_{27}$H$_{32}$N$_2$O$_2$S: 448.2185 (M+), found: 448.2164.
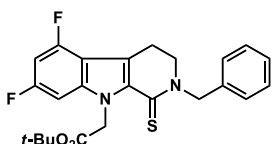
(2-Benzyl-5,7-difluoro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid t-butyl ester (7o)

Yield: 99%; mp: 118-121 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.37-7.28 (5H, m), 6.75 (1H, dd, J = 1.7 Hz, 9.4 Hz), 6.61 (1H, d, J = 10.9 Hz), 5.70 (2H, br), 5.44 (2H, s), 3.73 (2H, t, J = 7.0 Hz), 3.08 (2H, t, J = 7.0 Hz), 1.48 (9H, s); ^{13}C NMR (75 MHz, CDCl$_3$): δ 182.81, 167.76, 161.41 (dd, J = 12.5 Hz, 244.4 Hz), 158.00 (dd, J = 15.3 Hz, 252.1 Hz), 142.09 (dd, J = 12.0 Hz, 13.9 Hz), 136.31, 132.78 (d, J = 4.8 Hz), 128.81, 128.72, 127.88, 127.85, 127.68, 96.85 (dd, J = 23.0 Hz, 37.4 Hz), 92.92 (d, J = 4.8 Hz, 26.8 Hz), 82.30, 55.50, 49.40, 47.99, 28.10, 21.49; IR (KBr): 1743 cm^{-1}; MS (EI) m/z 442 (M+); HRMS (EI) calcd for C$_{24}$H$_{24}$N$_2$O$_2$F$_2$S: 442.1523 (M+), found: 442.1546.
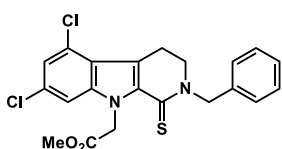
(2-Benzyl-5,7-dichloro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid methyl ester (7p)

Yield: 67%; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.36-7.32 (5H, m), 7.16-7.13 (2H, m), 5.80 (2H, br), 5.42 (2H, s), 3.79 (3H, s), 3.74 (2H, t, J = 6.9 Hz), 3.27 (2H, t, J = 6.9 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 182.57, 169.11, 141.53, 136.25, 133.34, 131.32, 129.04, 128.79, 127.80, 127.76, 122.31, 119.77, 115.54, 109.05, 55.60, 52.52, 48.96, 47.16, 21.73; IR (neat): 1754 cm^{-1}; MS (EI) m/z 432 (M+); HRMS (EI) calcd for C$_{21}$H$_{18}$N$_2$O$_2$SCl$_2$: 432.0466 (M+), found: 432.0420.
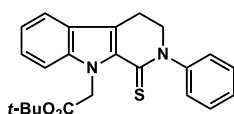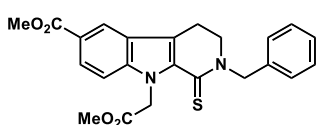

(2-Phenyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid *t*-butyl ester (7q)

Yield: 30%; mp: 165-166 °C; ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.64 (1H, d, J = 7.7 Hz), 7.50-7.25 (7H, m), 7.19 (1H, t, J = 7.5 Hz), 5.66 (2H, br), 4.09 (2H, t, J = 6.9 Hz), 3.21 (2H, t, J = 6.9 Hz), 1.45 (9H, s); ^{13}C NMR (75 MHz, CDCl$_3$): δ 184.37, 167.91, 146.17, 140.60, 132.24, 129.24, 127.38, 126.96, 125.68, 123.00, 120.78, 120.73, 115.49, 110.02, 81.56, 53.52, 47.36, 28.06, 21.05; IR (KBr): 1741 cm^{-1}; MS (EI) m/z 392 (M+); HRMS (EI) calcd for C$_{23}$H$_{24}$N$_2$O$_2$S: 392.1559 (M+), found: 392.1546.
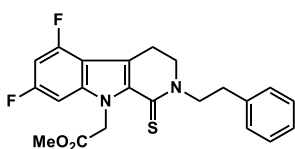
(5,7-Difluoro-2-phenylethyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid methyl ester (7r)

Yield: 60%; mp: 38–40 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.36-7.22 (5H, m), 6.72 (1H, dd, J = 1.9 Hz, 9.3 Hz), 6.62 (1H, dd, J = 1.9 Hz, 10.2 Hz), 5.77 (2H, brs), 4.32 (2H, t, J = 7.3 Hz), 3.78 (3H, brs), 3.57 (2H, t, J = 7.1 Hz), 3.10 (2H, t, J = 7.3 Hz), 2.96 (2H, t, J = 7.1 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 181.47, 169.18, 161.36 (dd, J = 11.5 Hz, 244.4 Hz), 159.18 (dd, J = 15.3 Hz, 253.0 Hz), 141.87 (dd, J = 12.5 Hz, 13.4 Hz), 138.56, 132.64 (d, J = 3.8 Hz), 128.91, 128.58, 126.60, 113.68, 109.51 (d, J = 21.1 Hz), 96.90 (dd, J = 22.5 Hz, 29.2 Hz), 92.82 (dd, J = 4.8 Hz, 24.0 Hz), 55.76, 52.39, 51.09, 47.19, 32.94, 21.22; IR (KBr): 1749 cm^{-1}; MS (EI) m/z 414 (M+); HRMS (EI) calcd for C$_{22}$H$_{20}$N$_2$O$_2$F$_2$S: 414.1214 (M+), found: 414.1241.

(2-Benzyl-6-methoxycarbonyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid methyl ester (7t)

To a stirred solution of **7l** (105 mg, 0.21 mmol) in DMF (5 ml) was added Pd (PPh$_3$)$_4$ (25.5 mg, 0.02 mmol), and the

resulting solution was stirred at room temperature under CO balloon pressure for 30 min. To the reaction mixture were added NEt_3 (60 μl, 0.84 mmol) and MeOH (0.21 ml, 8.40 mmol), and then the mixture was stirred at 80 °C under CO balloon pressure for 20 h. After cooling, the reaction mixture was diluted with H_2O (10 ml) and brine (5 ml), and the aqueous mixture was extracted with Et_2O (10 ml×5). The organic extracts were combined, dried over $MgSO_4$, and evaporated to give pale yellow oil, which was chromatographed on SiO_2 (Hexane : Acetone = 8:1) to give **7t** (72.6 mg, 81%).

mp: 63-65 °C; ^{1}H NMR (500 MHz, CDCl_3): δ 8.36 (1H, d, J = 1.3 Hz), 8.03 (1H, dd, J = 1.3 Hz, 9.0 Hz), 7.37-7.27 (6H, m), 5.87 (2H, brs), 5.45 (2H, s), 3.94 (3H, s), 3.79-3.76 (5H, m), 3.01 (2H, t, J = 7.2 Hz); ^{13}C NMR (125 MHz, CDCl_3): δ 182.86, 169.11, 167.09, 142.58, 136.14, 133.05, 128.56, 127.55, 126.38, 123.79, 123.02, 122.82, 116.32, 109.78, 55.50, 52.34, 51.95, 49.04, 47.00, 20.45; IR (KBr): 1750, 1702 cm^{-1}; MS (EI) m/z 422 (M+); HRMS (EI) calcd for $C_{23}H_{22}N_2O_4S$: 422.1300 (M+), found: 422.1311.

Typical procedure for carboxylic acids (**8b-8r**, and **8t**):

To a stirred solution of methyl ester (1 mmol) in MeOH (3 ml) and H_2O (1 ml) was added LiOH・H_2O (4 equiv), and the resulting mixture was refluxed for 1-4 h. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with $CHCl_3$ (10 ml×3). The organic extracts were combined, dried over $MgSO_4$, and evaporated. The residue was chromatographed on SiO_2 (Hexane : Acetone = 2 : 1) to give carboxylic acid (**8l-8m**, **8p, 8r** and **8t**).

To a stirred solution of NaI (4 equiv) in $ClCH_2CH_2Cl$ (3 ml) was added TMSCl (4 equiv), and the resulting mixture was stirred at room temperature for 15 min. To a solution of *t*-butyl ester (1 mmol) in $ClCH_2CH_2Cl$ (5 ml) was transferred a solution of TMSI, prepared above, via a cannula, and then the resulting mixture was refluxed for 1-2 days. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with $CHCl_3$ (10 ml×3). The organic extracts were combined, dried over $MgSO_4$, and evaporated. The residue was chromatographed on SiO_2 (Hexane : Acetone = 2 : 1) to give carboxylic acid (**8b–8j**, **8k**, **8n-8o** and **8q**).
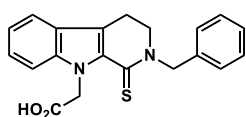
(2-Benzyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (8b)

Yield: 78%; mp: 207-209 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.66 (1H, d, J = 7.5 Hz), 7.52 (1H, d, J = 7.5 Hz), 7.37-7.28 (6H, m), 7.15 (1H, t, J = 7.5 Hz), 5.77 (2H, br), 5.43 (2H, s), 3.76 (2H, t, J = 7.2 Hz), 2.99 (2H, t, J = 7.2 Hz);

^{13}C NMR (75 MHz, DMSO-d$_6$): δ 182.14, 170.12, 140.17, 136.46, 131.51, 128.41, 127.19, 125.24, 122.48, 120.69, 120.54, 115.17, 110.90, 54.71, 49.48, 46.49, 19.81; IR (KBr): 1722 cm^{-1}; MS (EI) m/z 350 (M+); HRMS (EI) calcd for C$_{20}$H$_{18}$N$_2$O$_2$S: 350.1089 (M+), found: 350.1085.
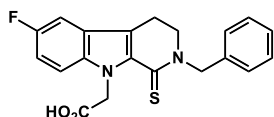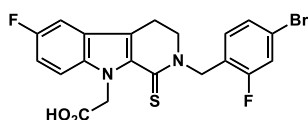
(2-Benzyl-6-fluoro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (8c)

Yield: 85%; mp: 190-191 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 7.58 (1H, dd, J = 4.3 Hz, 9.0 Hz), 7.48 (1H, dd, J = 2.5 Hz, 9.4 Hz), 7.36 (4H, m), 7.30 (1H, m), 7.20 (1H, dt, J = 2.5 Hz, 9.4 Hz), 5.76 (2H, br), 5.42 (2H, s), 3.76 (2H, t, J = 7.3 Hz), 2.98 (2H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 181.96, 170.00, 157.30 (d, J = 234.4 Hz), 136.84, 136.32, 132.69, 128.41, 127.19, 122.58 (d, J = 9.8 Hz), 114.94 (d, J = 4.9 Hz), 113.81 (d, J = 26.9 Hz), 112.49 (d, J = 9.8 Hz), 105.00 (d, J = 23.2 Hz), 54.81, 49.53, 46.68, 19.75; IR (KBr): 1722 cm^{-1}; MS (EI) m/z 368 (M+); HRMS (EI) calcd for C$_{20}$H$_{17}$N$_2$O$_2$FS: 368.0995 (M+), found: 368.0952.
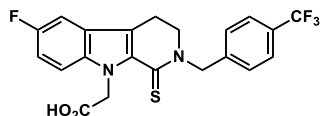
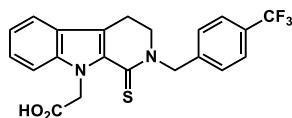**[6-Fluoro-1-thioxo-2-(4-trifluoromethylbenzyl)-1,2,3,4-tetrahydro-β-carbolin-9-yl]-acetic acid (8d)**

Yield: 98%; mp: 84-86 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.61 (2H, d, J = 8.0 Hz), 7.47 (2H, d, J = 8.0 Hz), 7.32-7.13 (3H, m), 5.78 (2H, br), 5.49 (2H, s), 3.76 (2H, t, J = 6.9 Hz), 2.96 (2H, t, J = 6.9 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.51, 173.17, 158.53 (d, J = 238.7 Hz), 140.27, 137.44, 133.04, 130.07 (q, J = 3.8 Hz), 128.10, 127.88, 125.78 (q, J = 3.8 Hz), 115.64 (d, J = 4.8 Hz), 115.29 (d, J = 26.8 Hz), 111.48 (d, J = 9.6 Hz), 111.17 (d, J = 9.6 Hz), 105.39 (d, J = 24.0 Hz), 55.39, 49.48, 47.13, 20.56; IR (KBr): 1723 cm^{-1}; MS (EI) m/z 436 (M+); HRMS (EI) calcd for C$_{21}$H$_{16}$N$_2$O$_2$ F$_4$S: 436.0869 (M+), found: 436.0916.

[2-(4-Bromo-2-fluorobenzyl)-6-fluoro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]-acetic acid (8e)

Yield: 70%; mp: 173-175 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.41-7.13 (6H, m), 5.74 (2H, br), 5.42 (2H, s), 3.80 (2H, t, J = 7.1 Hz), 2.97 (2H, t, J = 7.1 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.51, 173.74, 160.66 (d, J = 251.2 Hz), 158.51 (d, J = 238.7 Hz), 137.37, 133.06, 131.24 (d, J = 4.8 Hz), 127.83 (d, J = 3.8 Hz), 123.36 (d, J = 9.6 Hz), 122.35 (d, J = 14.4 Hz), 121.82 (d, J = 9.6 Hz), 119.19 (d, J = 24.9 Hz), 115.67 (d, J = 4.8 Hz), 115.25 (d, J = 26.8 Hz), 111.42 (d, J = 9.6 Hz), 105.40 (d, J = 23.0 Hz), 49.83, 49.09 (d, J = 3.8 Hz), 47.09, 20.56; IR (KBr): 1712 cm^{-1}; MS

(EI) m/z 464 (M+); HRMS (EI) calcd for $C_{20}H_{15}N_2O_2F_2SBr$: 464.0006 (M+), found: 464.0016.

[1-Thioxo-2-(4-trifluoromethylbenzyl)-1,2,3,4-tetrahydro-β-carbolin-9-yl]-acetic acid (8f)

Yield: 63%; mp: 148-151 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.61 (3H, m), 7.49 (2H, d, J = 8.0 Hz), 7.41 (2H, d, J = 6.0 Hz), 7.24-7.19 (1H, m), 5.77 (2H, br), 5.51 (2H, s), 3.78 (2H, t, J = 7.1 Hz), 3.02 (2H, t, J = 7.1 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.63, 173.50, 140.98, 140.47, 131.98, 129.99 (q, J = 31.6 Hz), 128.09, 127.88, 126.47, 125.75 (q, J = 3.8 Hz), 123.19, 121.55, 120.98, 116.18, 110.40, 55.32, 49.46, 47.06, 20.64; IR (KBr): 1723 cm^{-1}; MS (EI) m/z 418 (M+); HRMS (EI) calcd for $C_{21}H_{17}N_2O_2F_3SBr$: 418.0963 (M+), found: 418.0929.
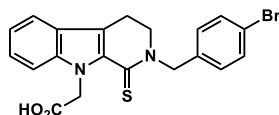
[2-(4-Bromobenzyl)-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]-acetic acid (8g)

Yield: 57%; mp: 183-185 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.58 (1H, d, J = 8.0 Hz), 7.48 (2H, d, J = 8.5 Hz), 7.40 (2H, d, J = 7.1 Hz), 7.28-7.18 (3H, m), 5.77 (2H, br), 5.39 (2H, s), 3.74 (2H, t, J = 7.0 Hz), 2.98 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.09, 173.30, 140.84, 135.33, 131.91, 131.78, 129.37, 126.32, 123.10, 121.59, 121.43, 120.88, 116.07, 110.35, 55.11, 49.27, 47.20, 20.73; IR (KBr): 1710 cm^{-1}; MS (EI) m/z 428 (M+); HRMS (EI) calcd for $C_{20}H_{17}N_2O_2SBr$: 428.0194 (M+), found: 428.0189.

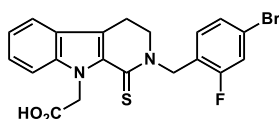**[2-(4-Bromo2-fluorobenzyl)-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]-acetic acid (8h)**

Yield: 93%; mp: 163-165 °C; ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 7.69 (1H, d, J = 8.1 Hz), 7.60 (1H, dd, J = 1.7 Hz, 9.8 Hz), 7.51 (1H, d, J = 8.5 Hz), 7.39 (1H, dd, J = 1.7 Hz, 8.5 Hz), 7.34 (1H, t, J = 7.5 Hz), 7.26 (1H, t, J = 8.3 Hz), 7.16 (1H, t, J = 7.5 Hz), 5.71 (2H, br), 5.38 (2H, s), 3.85 (2H, t, J = 7.0 Hz), 3.06 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 182.70, 170.07, 159.92 (d, J = 249.0 Hz), 140.27, 131.48, 130.36 (d, J = 6.1 Hz), 127.50, 125.43, 122.91 (d, J = 14.6 Hz), 122.50, 120.72 (d, J = 9.8 Hz), 120.32 (d, J = 9.8 Hz), 118.69 (d, J = 25.6 Hz), 115.53, 110.93, 50.20, 49.29, 46.51, 19.89; IR (KBr): 1712 cm^{-1}; MS (EI) m/z 446 (M+); HRMS (EI) calcd for $C_{20}H_{16}N_2O_2FSBr$: 446.0100 (M+), found: 446.0099.
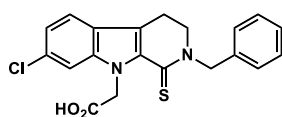
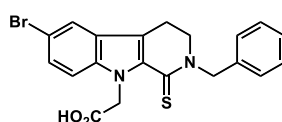**(2-Benzyl-7-chloro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (8i)**

Yield: 50%; mp: 184-186 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 7.74 (1H, d, J = 1.8 Hz), 7.69 (1H, d, J = 8.5 Hz), 7.36 (4H, m), 7.32-7.27 (1H, m), 7.16 (1H, dd, J = 1.8 Hz, 8.5 Hz), 5.74 (2H, br), 5.42 (2H, s), 3.76 (2H, t, J = 7.3 Hz), 2.98 (2H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 181.81, 169.91, 141.22, 140.48, 136.32, 132.16, 129.96, 128.41, 127.19, 122.21, 121.26, 121.06, 115.22, 110.93, 54.74, 49.42, 46.73, 19.65; IR (KBr): 1717 cm^{-1}; MS (EI) m/z 384 (M+); HRMS (EI) calcd for C$_{20}$H$_{17}$N$_2$O$_2$SCl: 384.0699 (M+), found: 384.0709.
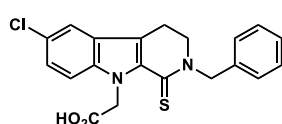
(2-Benzyl-6-chloro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (8j)

Yield: 56%; mp: 228-229 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 7.77 (1H, s), 7.59 (1H, d, J = 9.0 Hz), 7.38-7.28 (6H, m), 5.76 (2H, br), 5.42 (2H, s), 3.76 (2H, t, J = 7.3 Hz), 2.98 (2H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 181.88, 169.91, 138.52, 136.29, 132.44, 128.42, 127.23, 125.07, 124.96, 123.50, 119.85, 114.57, 112.78, 54.81, 49.52, 46.68, 19.63; IR (KBr): 1728 cm^{-1}; MS (EI) m/z 384 (M+); HRMS (EI) calcd for C$_{20}$H$_{17}$N$_2$O$_2$SCl: 384.0699 (M+), found: 384.0662.
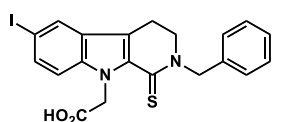
(2-Benzyl-6-bromo-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (8k)

Yield: 65%; mp: 227-229 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 7.91 (1H, d, J = 2.1 Hz), 7.54 (1H, d, J = 9.0 Hz), 7.43 (1H, dd, J = 2.1 Hz, 9.0 Hz), 7.36 (4H, m), 7.32-7.27 (1H, m), 5.76 (2H, br), 5.42 (2H, s), 3.75 (2H, t, J = 7.2 Hz), 2.98 (2H, t, J = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 181.86, 169.89, 138.76, 136.30, 132.24, 128.44, 127.57, 127.24, 124.22, 122.97, 114.48, 113.17, 112.84, 54.82, 49.52, 46.67, 19.63; IR (KBr): 1727 cm^{-1}; MS (EI) m/z 428 (M+); HRMS (EI) calcd for C$_{20}$H$_{17}$N$_2$O$_2$SBr: 428.0194 (M+), found: 428.0145.

(2-Benzyl-6-iodo-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (8l)

Yield: 99%; mp: 216-218 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 8.32 (1H, s), 8.06 (1H, d, J = 1.4 Hz), 7.56 (1H, d, J = 8.8 Hz), 7.39-7.28 (5H, m), 5.73 (2H, s), 5.42 (2H, s), 3.74 (2H, t, J = 6.9 Hz), 2.96 (2H, t, J = 6.9 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 181.91, 170.31, 139.18, 136.37, 132.81, 131.93, 129.09, 128.44, 127.26, 127.21, 125.03, 114.06, 113.54, 84.09, 54.79, 49.50,

46.91, 19.66; IR (KBr): 1726 cm^{-1}; MS (EI) m/z 476 (M+); HRMS (EI) calcd for C$_{20}$H$_{17}$N$_2$O$_2$SI: 476.0056 (M+), found: 476.0044.
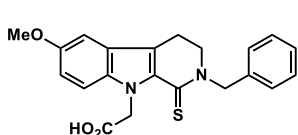
(2-Benzyl-6-methoxy-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (8m)

Yield: 81%; mp: 126-128 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 7.44 (1H, d, J = 9.0 Hz), 7.35 (4H, m), 7.34-7.27 (1H, m), 7.12 (1H, d, J = 2.6 Hz), 6.98 (1H, dd, J = 2.6 Hz, 9.0 Hz), 5.73 (2H, br), 5.42 (2H, s), 3.78 (3H, s), 3.74 (2H, t, J = 7.3 Hz), 2.96 (2H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 182.11, 170.18, 154.18, 136.53, 135.67, 131.85, 128.42, 127.23, 127.16, 122.68, 116.47, 114.77, 111.95, 100.90, 55.40, 54.69, 49.55, 46.55, 19.91; IR (KBr): 1711 cm^{-1}; MS (EI) m/z 380 (M+); HRMS (EI) calcd for C$_{21}$H$_{20}$N$_2$O$_3$S: 380.1195 (M+), found: 380.1188.
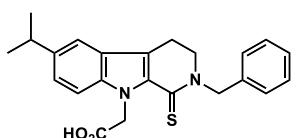
(2-Benzyl-6-iso-propyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (8n)

Yield: 63%; mp: 188-190 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.38-7.28 (8H, m), 5.76 (2H, br), 5.45 (2H, s), 3.74 (2H, t, J = 7.0 Hz), 3.05-2.93 (3H, m), 1.29 (6H, d, J = 6.9 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 183.04, 173.55, 142.22, 139.78, 136.42, 135.49, 132.24, 128.75, 127.70, 126.02, 123.19, 117.39, 116.00, 110.30, 55.59, 49.14, 47.28, 34.08, 24.35, 20.61; IR (KBr): 1723 cm^{-1}; MS (EI) m/z 392 (M+); HRMS (EI) calcd for C$_{23}$H$_{24}$N$_2$O$_2$S: 392.1559 (M+), found: 392.1533.
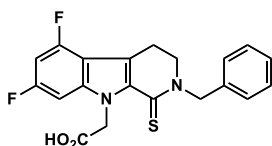
(2-Benzyl-5,7-difluoro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (8o)

Yield: 47%; mp: 219-220 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 7.41 (1H, d, J = 10.2 Hz), 7.36-7.26 (5H, m), 6.99 (1H, t, J = 10.2 Hz), 5.76 (2H, br), 5.41 (2H, s), 3.76 (2H, t, J = 7.3 Hz), 3.08 (2H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 181.62, 169.92, 160.48 (dd, J = 12.5 Hz, 240.6 Hz), 157.10 (dd, J = 15.8, 250.6 Hz), 141.72 (d, J = 14.4 Hz), 136.43, 132.37 (d, J = 2.9 Hz), 128.67, 127.44, 127.41, 113.15, 108.86 (d, J = 21.1 Hz), 96.60 (dd, J = 23.0 Hz, 29.7 Hz), 94.45 (dd, J = 4.3 Hz, 27.3 Hz), 54.84, 49.42, 47.36, 20.75; IR (KBr): 1709 cm^{-1}; MS (EI) m/z 386 (M+); HRMS (EI) calcd for C$_{20}$H$_{16}$N$_2$O$_2$F$_2$S: 386.0901 (M+), found: 386.0902.
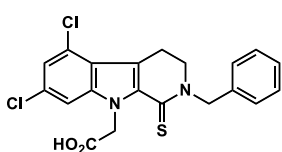
(2-Benzyl-5,7-dichloro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (8p)

Yield: 76%; mp: 208-210 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.37-7.28 (6H, m), 7.15 (1H, s), 5.75 (2H, br), 5.42 (2H, s), 3.73 (2H, t, J = 6.9 Hz), 3.27 (2H, t, J = 6.9 Hz); ^{13}C NMR (75 MHz, CD$_3$OD): δ 183.72, 172.28, 142.85, 137.78, 136.47, 134.82, 131.74, 129.59, 129.51, 128.69, 128.45, 122.58, 116.21, 110.62, 56.06, 50.09, 48.92 22.42; IR (KBr): 1727 cm^{-1}; MS (EI) m/z 418 (M+); HRMS (EI) calcd for C$_{20}$H$_{16}$N$_2$O$_2$SCl$_2$: 418.0310 (M+), found: 418.0351.
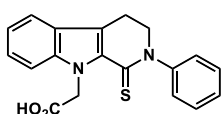
(2-Phenyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (8q)

Yield: 78%; mp: 200-202 °C; ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 7.74 (1H, d, J = 8.1 Hz), 7.53-7.46 (3H, m), 7.39-7.34 (4H, m), 7.19 (1H, t, J = 7.8 Hz), 5.75 (2H, brs), 4.04 (2H, t, J = 7.0 Hz), 3.20 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 183.38, 170.02, 146.22, 140.28, 131.58, 129.13, 127.14, 125.51, 122.53, 120.90, 120.66, 115.71, 110.95, 53.67, 46.46, 20.34; IR (KBr): 1721 cm^{-1}; MS (EI) m/z 336 (M+); HRMS (EI) calcd for C$_{19}$H$_{16}$N$_2$O$_2$S: 336.0933 (M+), found: 336.0926.
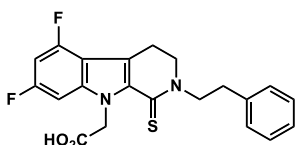

(5,7-Difluoro-2-phenylethyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (8r)

Yield: 73%; mp: 199-201 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.35-7.22 (5H, m), 6.84 (1H, dd, J = 1.9 Hz, 9.2 Hz), 6.63 (1H, dd, J = 1.9 Hz, 10.1 Hz), 5.72 (2H, br), 4.32 (2H, t, J = 7.4 Hz), 3.55 (2H, t, J = 7.1 Hz), 3.11 (2H, t, J = 7.4 Hz), 2.95 (2H, t, J = 7.1 Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 181.09, 172.47, 161.64 (dd, J = 12.5 Hz, 245.4 Hz), 157.95 (dd, J = 14.9 Hz, 253.5 Hz), 142.07 (dd, J = 12.0 Hz, 13.9 Hz), 138.45, 132.58 (d, J = 1.9 Hz), 128.96, 128.68, 126.73, 114.25 (d, J = 2.9 Hz), 109.57 (d, J = 21.1 Hz), 97.32 (dd, J = 22.5 Hz, 29.2 Hz), 93.19 (dd, J = 4.8 Hz, 26.8 Hz), 55.98, 51.17, 47.59, 33.00, 21.23; IR (KBr): 1718 cm^{-1}; MS (EI) m/z 400 (M+); HRMS (EI) calcd for C$_{21}$H$_{18}$N$_2$O$_2$F$_2$S: 400.1057 (M+), found: 400.1030.
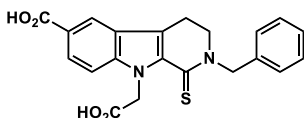
(2-Benzyl-6-carboxy-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (8t)

Yield: 94%; mp: 208-210 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 8.33 (1H, s), 7.88 (1H, d, J = 7.3 Hz), 7.60 (1H, d, J = 8.7 Hz), 7.35-7.27 (5H, m), 5.78 (2H, br), 5.41 (2H, s), 3.77 (2H, t, J = 7.0 Hz), 3.04 (2H, t,

$J = 7.0$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.83, 169.89, 167.51, 142.21, 136.33, 132.69, 127.47, 127.27, 125.79, 123.47, 123.21, 122.21, 116.35, 110.97, 54.82, 49.50, 46.86, 19.63; IR (KBr): 1704 cm^{-1}; MS (EI) m/z 394 (M+); HRMS (EI) calcd for $C_{21}H_{18}N_2O_4S$: 394.0987 (M+), found: 394.0957.
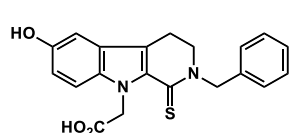
(2-Benzyl-6-hydoxy-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (8s)

To a stirred solution of **7m** (485 mg, 1.19 mmol) in CHCl$_3$ (3 ml) was added BBr$_3$ (1 M in CH$_2$Cl$_2$, 3.56 ml, 3.56 mmol), and the resulting mixture was stirred at room temperature for 4 h. The reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with CHCl$_3$ (10 ml×3). The organic extracts were combined, dried over MgSO$_4$, and evaporated. The residue was chromatographed on SiO$_2$ (Hexane : Acetone = 3 : 1) to give **8s** (343.3 mg, 73%).

mp: 200-202 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.07 (1H, s), 7.36-7.29 (6H, m), 6.87-6.84 (2H, m), 5.66 (2H, br), 5.42 (2H, s), 3.72 (2H, t, $J = 7.0$ Hz), 2.89 (2H, t, $J = 7.0$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 179.61, 170.26, 160.78, 151.69, 136.58, 135.17, 131.80, 128.42, 127.21, 123.08, 116.50, 114.18, 111.60, 103.22, 54.66, 49.53, 46.64, 19.86; IR (KBr): 1721 cm^{-1}; MS (EI) m/z 366 (M+); HRMS (EI) calcd for $C_{20}H_{18}N_2O_3S$: 366.1038 (M+), found: 336.1057.

第二章
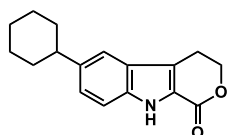
6-Cyclohexyl-4,9-dihydro-3H-pyrano[3,4-b]indol-1-one (12)

According to the literature procedure,[71)] lactone **12** was synthesized. Yield: 28%; mp: 175-176 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 9.03 (1H, br), 7.43-7.38 (2H, m), 7.30-7.26 (1H, m), 4.70 (2H, t, $J = 6.2$ Hz), 3.15 (2H, t, $J = 6.2$ Hz), 2.60-2.57 (1H, m), 1.94-1.26 (10H, m); ^{13}C NMR (125 MHz, CDCl$_3$): δ 161.74, 140.82, 137.15, 126.94, 124.43, 122.91, 122.14, 117.43, 112.72, 69.48, 44.50, 34.87, 26.93, 26.13, 21.42; IR (KBr): 3269, 2920, 2849, 1735 cm^{-1}; MS (EI) m/z 269 (M+); HRMS (EI) calcd for $C_{17}H_{19}NO_2$: 269.1416 (M+), found: 269.1414.
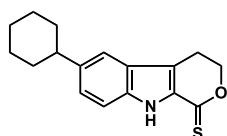
6-Cyclohexyl-4,9-dihydro-3H-pyrano[3,4-b]indole-1-thione (13)

To a stirred solution of **12** (500 mg, 1.86 mmol) in toluene (10 ml) was added Lawesson's reagent (413 mg, 1.02 mmol), and the resulting mixture was refluxed for 24 h. After cooling, the solvent

was removed, and the residue was chromatographed on SiO_2 (Hexane : Acetone = 20 : 1) to give **13** (502 mg, 95%).

mp: 40-42 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.75 (1H, br), 7.40 (1H, s), 7.31 (1H, d, *J* = 8.7 Hz), 7.27 (1H, dd, *J* = 1.4 Hz, 8.7 Hz), 4.72 (2H, t, *J* = 6.4 Hz), 3.15 (2H, t, *J* = 6.4 Hz), 2.57 (1H, tt, *J* = 3.2 Hz, 11.4 Hz), 1.92-1.84 (4H, m), 1.78-1.74 (1H, m), 1.50-1.23 (5H, m); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 197.93, 139.96, 138.34, 132.26, 127.18, 123.81, 118.34, 116.06, 112.68, 71.26, 43.82, 34.36, 26.49, 25.70, 20.57; IR (KBr): 3348, 2922, 2848, 1541, 1226 cm^{-1}; MS (EI) m/z 285 (M+); HRMS (EI) calcd for $C_{17}H_{19}NOS$: 285.1187 (M+), found: 285.1191.
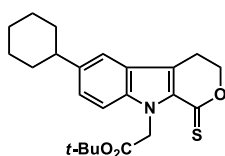
(6-Cyclohexyl-1-thioxo-3,4-dihydro-1*H*-pyrano[3,4-*b*]indol-9-yl) acetic acid *tert*-butyl ester (14)

To a stirred solution of **13** (452 mg, 1.58 mmol) in DMF (12 ml) was added NaH (60%, 76 mg, 1.90 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min. To the mixture was added BrCH$_2$CO$_2$*t*-Bu (0.28 ml, 1.90 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 24 h. The reaction was quenched with H$_2$O, and the aqueous mixture was extracted with Et$_2$O. The organic extracts were combined, dried over Na$_2$SO$_4$, and evaporated. The residue was chromatographed on SiO_2 (Hexane : Acetone = 15 : 1) to give **14** (655 mg, quant.).

mp: 123-125 °C; ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.44 (1H, s), 7.34 (1H, dd, *J* = 1.7 Hz, 9.0 Hz), 7.19 (1H, d, *J* = 9.0 Hz), 5.49 (2H, s), 4.67 (2H, t, *J* = 6.4 Hz), 3.17 (2H, t, *J* = 6.4 Hz), 2.61-2.57 (1H, m), 1.92-1.85 (4H, m), 1.78-1.76 (1H, m), 1.51-1.24 (5H, m), 1.46 (9H, s); ^{13}C NMR (75 MHz, CDCl$_3$): δ 196.63, 167.62, 141.62, 140.05, 131.72, 128.19, 122.71, 118.58, 118.50, 110.04, 82.28, 70.19, 46.92, 44.51, 34.87, 28.17, 27.01, 26.21, 21.59; IR (KBr): 2925, 2851, 1740, 1525, 1228 cm^{-1}; MS (EI) m/z 399 (M+); HRMS (EI) calcd for $C_{23}H_{29}NO_3S$: 399.1868 (M+), found: 399.1858.
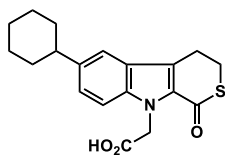
(6-Cyclohexyl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl) acetic acid (10)

To a stirred solution of NaI (939 mg, 6.28 mmol) in CHCl$_3$ (10 ml) was added TMSCl (0.8 ml, 6.28 mmol), and the resulting mixture was stirred at room temperature for 15 min. To a solution of **14** (625 mg, 1.57 mmol) in CHCl$_3$ (5 ml) was transferred a solution of TMSI, prepared above, via a cannula, and then the resulting mixture was refluxed for 24 h. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with EtOAc. The

organic extracts were combined, dried over Na_2SO_4, and evaporated. The residue was chromatographed on SiO_2 (Hexane : Acetone = 3 : 1) to give **10** (341 mg, 64%).

mp: 245-247 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.45 (1H, s), 7.33 (1H, d, J = 8.5 Hz), 7.21 (1H, d, J = 8.5 Hz), 5.26 (2H, s), 3.43 (2H, t, J = 6.2 Hz), 3.32 (2H, t, J = 6.2 Hz), 2.62-2.58 (1H, m), 1.92-1.85 (4H, m), 1.78-1.76 (1H, m), 1.50-1.38 (4H, m), 1.32-1.26 (1H, m); ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.82, 169.96, 140.06, 137.00, 127.08, 127.02, 126.14, 124.04, 117.81, 110.58, 46.31, 43.77, 34.39, 30.83, 26.49, 25.67, 21.69; IR (KBr): 2924, 2850, 1717, 1619 cm^{-1}; MS (EI) m/z 343 (M+); HRMS (EI) calcd for $C_{19}H_{21}NO_3S$: 343.1242 (M+), found: 343.1280.
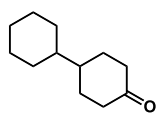
Bicyclohexyl-4-one (31)

To a suspension of 4-cyclohexylcyclohexanol (7.17 g, 39.3 mmol) and Celite (20 g) in CH_2Cl_2 (80 ml) was added PCC (12.7 g, 59.0 mmol) at 0 °C, and the resulting mixture was stirred at the room temperature for 14 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane : EtOAc = 20 : 1) to give **31** (6.78 g, 96%).[72]
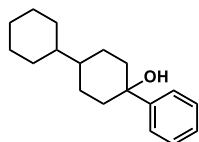
4-Phenyl-bicyclohexyl-4-ol (32)

To a freshly made solution of PhMgBr (30 mmol) in THF (30 ml) was added **23** (2.16 g, 12.0 mmol) in THF (13 ml) at 0 °C, and the resulting mixture was stirred at the same temperature for 3 h. The reaction was quenched by the addition of sat. NH_4Cl aq, diluted with EtOAc. And then, the organic phase was separated, the aqueous mixture was extracted with EtOAc. The organic extracts were combined, dried over Na_2SO_4, and evaporated. The residue was chromatographed on SiO_2 (Hexane : EtOAc = 30 : 1) to give **32** (2.88 g, 93%).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.55-7.50 (2H, m), 7.38-7.33 (2H, m), 7.29-7.22 (1H, m), 1.88-0.83 (20H, m).
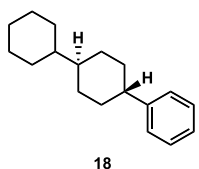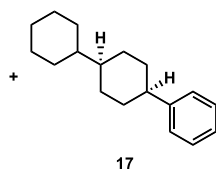
trans-4-Phenyl-1,1'-bi(cyclohexane) (18) and *cis*-4-Phenyl-1,1'-bi-(cyclohexane) (17)

To a solution of **32** (2.88 g, 11.1 mmol) was added slowly Et_3SiH (3.60 ml, 22.2 mmol) , $BF_3 \cdot OEt_2$ (1.39 ml, 11.1 mmol) at -40 °C, and the resulting mixture was stirred at the same temperature for 4 h. The reaction was diluted with CH_2Cl_2 and neutralized with sat. $NaHCO_3$ aq. The organic phase was separated, dried over Na_2SO_4 and concentrated in vacuo. The residue

was chromatographed on SiO$_2$ (Hexane) to give mixture of **18** and **17** (the ratio of compound **18** : **17** = ca. 3 : 1, 2.47 g, 92%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 8.16-8.13 (2H, m), 7.40 (1H, d, J = 8.6 Hz), 7.34 (1H, d, J = 8.5 Hz), 2.79-2.74 (**17**; 1H, m), 2.56 (**18**; 1H, tt, J = 3.1 Hz, 12.0 Hz), 1.94-0.99 (20H, m).
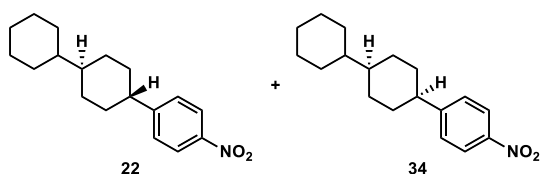
trans-4-(4-Nitrophenyl)-1,1'-bi(cyclohexane) (22) and

cis-4-(4-Nitrophenyl)-1,1'-bi(cyclohexane) (34)

To a solution of mixture of **18** and **17** (2.47 g) in CHCl$_3$ (10 ml) was added Ac$_2$O (2.9 ml) at 0 °C. At the same time HNO$_3$ (6.1 ml) was slowly added to Ac$_2$O (10 ml) at 0 °C, and this mixture was added dropwise to the solution, prepared above, at 0 °C. The resulting mixture was stirred at room temperature for 13 h. The reaction was quenched by the addition of 10% NaOH aq, and the organic layer was separated, dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was chromatographed on SiO$_2$ (Hexane) to give mixture of **22** and **34** (1.53 g, 52%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 8.16-8.13 (2H, m), 7.40 (1H, d, J = 8.6 Hz), 7.34 (1H, d, J = 8.5 Hz), 2.79-2.74 (**34**; 1H, m), 2.56 (**22**; 1H, tt, J = 3.1 Hz, 12.0 Hz), 1.94-0.99 (20H, m).
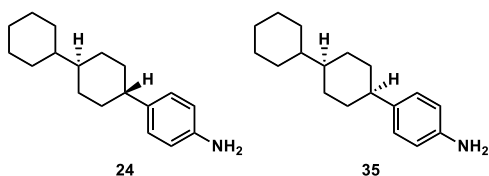
4-(trans-[1,1'-Bi(cyclohexane)]-4-yl)aniline (24) and

4-(cis-[1,1'-Bi(cyclohexane)]-4-yl)aniline (35)

To a solution of mixture of **22** and **34** (1.53 g, 5.33 mmol) in EtOAc (23 ml) was added 10% Pd/C (60 mg), and the resulting suspension was stirred under a hydrogen atmosphere at 1 atm for 3 days. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was chromatographed on SiO$_2$ (Hexane : EtOAc = 15 : 1) to give **24** (795 mg, 58%), **35** (273 mg, 20%).

24: mp: 118-120 °C; ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.00 (2H, d, J = 8.3 Hz), 6.64 (2H, d, J = 8.3 Hz), 3.53 (2H, br), 2.34 (1H, tt, J = 3.5 Hz, 12.4 Hz), 1.89-0.96 (20H, m); ^{13}C NMR (125 MHz, CDCl$_3$): δ 144.08, 138.12, 127.41, 115.09, 43.68, 43.27, 42.92, 34.80, 30.26, 30.21, 26.81; IR (KBr): 3382, 3312, 2920, 2849, 1516 cm^{-1}; MS (EI) m/z 257 (M+); HRMS (EI) calcd for C$_{18}$H$_{27}$N: 257.2143 (M+), found: 257.2141.

35: mp: 67-69 °C; ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.04 (2H, dd, J = 1.4 Hz, 6.3 Hz),

6.64 (2H, dd, J = 2.0 Hz, 6.3 Hz), 3.53 (2H, br), 2.57-2.51 (1H, m), 1.85-0.78 (20H, m); ^{13}C NMR (125 MHz, CDCl$_3$): δ 143.96, 137.68, 127.69, 115.12, 41.99, 39.19, 36.67, 30.95, 29.54, 27.41, 26.65; IR (KBr): 3464, 3371, 2927, 2846, 1520 cm^{-1}; MS (EI) m/z 257 (M+); HRMS (EI) calcd for C$_{18}$H$_{27}$N: 257.2143 (M+), found: 257.2144.
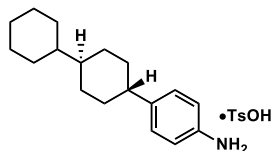
4-(*trans*-[1,1'-Bi(cyclohexane)]-4-yl)aniline *p*-toluenesulfonate (36)

To a stirred solution of **24** (63 mg, 0.25 mmol) in MeOH (3 ml) was added *p*-toluenesulfonic acid (46.6 mg, 0.25 mmol), and the resulting mixture was stirred at room temperature for 2.5 h. The solvent was concentrated in vacuo to give **36** (107 mg, quant.)

mp: 250-251 °C; ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 9.88 (1H, br), 7.48 (2H, d, J = 8.0 Hz), 7.33 (2H, d, J = 8.0 Hz), 7.25 (2H, d, J = 8.0 Hz), 7.11 (2H, d, J = 8.0 Hz), 2.47 (1H, tt, J = 3.2 Hz, 12.0 Hz), 2.28 (3H, s), 1.81-0.94 (20H, m); ^{13}C NMR (125 MHz, DMSO-d$_6$): δ 147.65, 145.35, 137.84, 129.11, 128.13, 127.98, 125.48, 123.08, 43.35, 42.72, 42.21, 33.98, 29.72, 29.60, 26.34, 26.31, 20.79. IR (KBr): 2920, 2849, 1516 cm^{-1}.
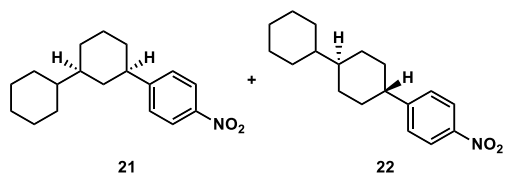
cis-3-(4-nitrophenyl)-1,1'-bi(cyclohexane) (21) and *trans*-4-(4-nitrophenyl)-1,1'-bi(cyclohexane) (22)

To a solution of 4-cyclohexylcyclohexanol (2.42 g, 13.3 mmol) in dry benzene (60 ml) was slowly added AlCl$_3$ (1.77 g, 13.3 mmol) at room temperature. The resulting mixture was stirred at the same temperature for 17 h. The reaction was quenched by the addition of ice water, and then the organic phase was separated, washed with Na$_2$CO$_3$ (saturated aqueous solution), dried over Na$_2$SO$_4$ and concentrated in vacuo to yield mixture of *cis*-3-phenyl-1,1'-bi(cyclohexane) **20** and *trans*-4-phenyl-1,1'-bi(cyclohexane) **18** (the ratio of compound **20** : **18** = ca. 5 : 1). The product was used for the next reaction without further purification. To a solution of crude (3.22 g) in CHCl$_3$ (13 ml) was added Ac$_2$O (2.7 ml) at 0 °C. At the same time HNO$_3$ (1.3 ml) was slowly added to Ac$_2$O (2.7 ml) at 0 °C, and this mixture was added dropwise to the solution, prepared above, at 0 °C. The resulting mixture was stirred at room temperature for 14 h. The reaction was quenched by the addition of 10% NaOH aq, and the organic layer was separated, dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was chromatographed on SiO$_2$ (Hexane : EtOAc = 70 : 1) to give mixture of **21** and **22** (1.78 g, 47% in two steps).

^{1}H NMR (500 MHz, CDCl$_3$): δ 8.14 (2H, dd, J = 2.0 Hz, 7.9 Hz), 7.35 (2H, dd, J = 2.0 Hz, 7.9 Hz), 2.65-2.53 (1H, m), 1.94-0.96 (20H, m)
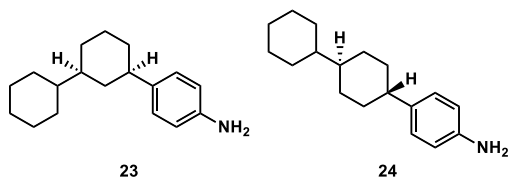
4-(*cis*-[1,1'-Bi(cyclohexane)]-3-yl)aniline (23) and

4-(*trans*-[1,1'-Bi(cyclohexane)]-4-yl)aniline (24)

To a solution of **21** and **22** (3.30 g, 11.5 mmol) in EtOAc (15 ml) was added Pd/C (59 mg), and the resulting suspension was stirred under a hydrogen atmosphere at 1 atm for 5 days. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was chromatographed on SiO$_2$ (Hexane : EtOAc = 12 : 1) to give **23** (1.80g, 61%), **24** (531mg, 18%).

23: mp: 58-60 °C; ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.01 (2H, d, J = 8.3 Hz), 6.64 (2H, d, J = 8.3 Hz), 3.54 (2H, br), 2.39 (1H, tt, J = 3.3 Hz, 11.9 Hz), 1.89-0.94 (20H, m); ^{13}C NMR (125 MHz, CDCl$_3$): δ 144.09, 138.37, 127.41, 115.10, 43.76, 43.67, 43.44, 38.35, 34.55, 30.16, 30.04, 29.53, 26.87, 26.80; IR (KBr): 3423, 3348, 2920, 2846, 1517 cm^{-1}; MS (EI) m/z 257 (M+); HRMS (EI) calcd for C$_{18}$H$_{27}$N: 257.2143 (M+), found: 257.2147.
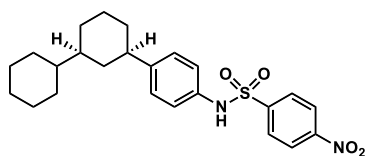
N-(*cis*-4-Bicyclohexyl-3-yl-phenyl)-4-nitro-benzenesulfonamide (33)

To a stirred solution of **23** (108 mg, 0.37 mmol) in pyridine (2 ml) was added 4-nitrobenzenesulfonyl chloride (90 mg, 0.41 mmol), and the resulting mixture was stirred at room temperature for 23 h. The mixture was diluted with CH$_2$Cl$_2$ and washed with 10% HCl aq. The organic layer was dried over Na$_2$SO$_4$, and evaporated. The residue was chromatographed on SiO$_2$ (Hexane : CH$_2$Cl$_2$ = 1 : 2) to give **33** (186 mg, quant.).

mp: 174-175 °C; ^{1}H NMR (500 MHz, CDCl$_3$): δ 8.28 (2H, d, J = 9.2 Hz), 7.90 (2H, d, J = 9.2 Hz), 7.11 (2H, d, J = 8.3 Hz), 6.96 (2H, d, J = 8.3 Hz), 6.44 (1H, br), 2.45 (1H, tt, J = 3.2 Hz, 12.0 Hz) 1.89-0.95 (20H, m); ^{13}C NMR (125 MHz, CDCl$_3$): δ 150.15, 146.78, 144.78, 132.69, 128.53, 127.96, 124.21, 122.87, 44.10, 43.59, 43.40, 37.93, 34.28, 30.16, 30.13, 29.39, 26.79, 26.70; IR (KBr): 3220, 2926, 2920, 1535, 1349 cm^{-1}; MS (EI) m/z 442 (M+); HRMS (EI) calcd for C$_{24}$H$_{30}$N$_2$O$_4$S: 442.1926 (M+), found: 442.1928.
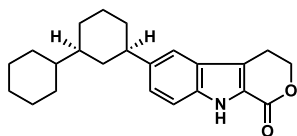
1',3'-*cis*-6-Bicyclohexyl-3-yl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indol-1-one (38)

To a suspension of **23** (105 mg, 0.41 mmol) and conc. HCl (0.1 ml) in THF (0.63 ml) and H_2O (0.42 ml) was slowly added a solution of $NaNO_2$ (31 mg, 0.44 mmol) in H_2O (0.15 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. At the same time, to a solution of enol lactone **5** (98 mg, 0.49 mmol) and sodium acetate (134 mg, 1.63 mmol) in THF (0.21 ml) and H_2O (0.11 ml) was slowly added the diazonium salt, prepared above, at 0 °C. The resulting mixture was stirred at 0 °C for 2 h. The reaction was diluted with CH_2Cl_2 and the organic phase was separated, dried over Na_2SO_4 and concentrated in vacuo to yield hydrazone **37**, which was used for the next reaction without further purification. A solution of the above **37** (138 mg) in AcOH (1.2 ml) and HCl (1.2 ml, 1.0 M in AcOH solution) was refluxed for 4 h, and cooled to room temperature. The resulting mixture was diluted with CH_2Cl_2 and the organic phase was separated, washed with H_2O, neutralized with sat. $NaHCO_3$ aq, dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane : EtOAc = 15 : 1) to give **38** (99 mg, 69% in two steps).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.76 (1H, br), 7.43 (1H, brs), 7.38 (1H, d, *J* = 8.6 Hz), 7.28 (1H, dd, *J* = 1.7 Hz, 8.6 Hz), 4.69 (2H, t, *J* = 6.3 Hz), 3.15 (2H, t, *J* = 6.3 Hz), 2.63-2.59 (1H, m), 1.92-0.96 (20H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 161.54, 140.96, 137.02, 127.02, 124.55, 122.89, 122.23, 117.55, 112.59, 69.46, 44.73, 43.77, 43.47, 38.57, 34.81, 30.21, 30.10, 29.57, 26.90, 26.80, 21.47; IR (neat): 3291, 2922, 2850, 1700 cm^{-1}; MS (EI) m/z 351 (M+); HRMS (EI) calcd for $C_{23}H_{29}NO_2$: 351.2198 (M+), found: 351.2199.
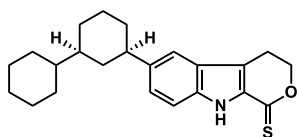
1',3'-*cis*-6-Bicyclohexyl-3-yl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indole-1-thione (39)

To a stirred solution of **38** (43mg, 0.12 mmol) in toluene (3 ml) was added Lawesson's reagent (27 mg, 0.067 mmol), and the resulting mixture was refluxed for 18 h. After cooling, the solvent was removed, and the residue was chromatographed on SiO_2 (Hexane : EtOAc = 20 : 1) to give **39** (50 mg, quant.).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.76 (1H, br), 7.42 (1H, brs), 7.32 (1H, d, *J* = 8.6 Hz), 7.29 (1H, dd, *J* = 1.5 Hz, 8.6 Hz), 4.74 (2H, t, *J* = 6.6 Hz), 3.17 (2H, t, *J* = 6.6 Hz), 2.59 (1H, tt, *J* = 3.2 Hz, 11.8 Hz), 1.92-0.96 (20H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 198.23, 141.48, 137.83, 132.30, 128.07, 124.85, 118.53, 115.74, 112.12, 71.15, 44.67,

43.69, 43.41, 38.43, 34.68, 30.16, 30.07, 29.50, 26.84, 26.77, 21.22; IR (neat): 3356, 2921, 2850, 1540, 1231 cm^{-1}; MS (EI) m/z 367 (M+); HRMS (EI) calcd for C$_{23}$H$_{29}$NOS: 367.1970 (M+), found: 367.1970.
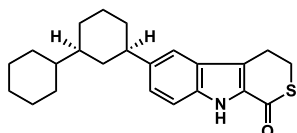
1',3'-*cis*-6-Bicyclohexyl-3-yl-4,9-dihydro-3*H*-2-thia-9-azafluoren-1-one (40)

To a stirred solution of NaI (85 mg, 0.57 mmol) in ClCH$_2$CH$_2$Cl (10 ml) was added TMSCl (72 μl, 0.57 mmol), and the resulting mixture was stirred at room temperature for 15 min. To a solution of **39** (52mg, 0.14 mmol) in ClCH$_2$CH$_2$Cl (5 ml) was transferred a solution of TMSI, prepared above, via a cannula, and then the resulting mixture was refluxed for 15 days. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with CHCl$_3$. The organic extracts were combined, dried over Na$_2$SO$_4$, and evaporated. The residue was chromatographed on SiO$_2$ (Hexane : EtOAc = 30 : 1) to give **40** (48 mg, 92%).

mp: 147-149 °C; ^{1}H NMR (500 MHz, CDCl$_3$): δ 8.74 (1H, br), 7.44 (1H, brs), 7.33 (1H, d, *J* = 8.6 Hz), 7.27 (1H, dd, *J* = 1.7 Hz, 8.6 Hz), 3.48 (2H, t, *J* = 6.2 Hz), 3.29 (2H, t, *J* = 6.2 Hz), 2.60 (1H, tt, *J* = 3.1 Hz, 11.8 Hz), 1.93-0.96 (20H, m); ^{13}C NMR (125 MHz, CDCl$_3$): δ 183.75, 140.86, 135.45, 128.69, 127.54, 125.86, 125.21, 117.67, 112.31, 44.75, 43.77, 43.48, 38.56, 34.80, 31.54, 30.22, 30.11, 29.58, 26.91, 26.82, 21.80; IR (KBr): 3306, 2909, 2848, 1606 cm^{-1}; MS (EI) m/z 367 (M+); HRMS (EI) calcd for C$_{23}$H$_{29}$NOS: 367.1970 (M+), found: 367.1970.
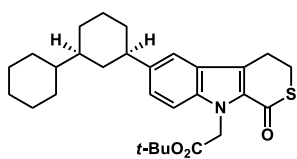
1',3'-*cis*-(6-Bicyclohexyl-3-yl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid *tert*-butyl ester (41)

To a stirred solution of **40** (104 mg, 0.28 mmol) in DMF (3 ml) was added NaH (60%, 17mg, 0.42 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min. To the mixture was added BrCH$_2$CO$_2$*t*-Bu (63 μl, 0.42 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 17 h. The reaction was quenched with H$_2$O, and the aqueous mixture was extracted with Et$_2$O. The organic extracts were combined, dried over Na$_2$SO$_4$, and evaporated. The residue was chromatographed on SiO$_2$ (Hexane : EtOAc = 30 : 1) to give **41** (130 mg, 95%).

mp: 163-165 °C; ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.44 (1H, brs), 7.30 (1H, dd, *J* = 1.4 Hz, 8.6 Hz), 7.17 (1H, d, *J* = 8.6 Hz), 5.13 (2H, s), 3.43 (2H, t, *J* = 6.2 Hz), 3.32 (2H, t, *J* = 6.2 Hz), 2.60 (1H, tt, *J* = 3.1 Hz, 11.6 Hz), 1.91-0.98 (20H, m), 1.45 (9H, s); ^{13}C

NMR (125 MHz, CDCl$_3$): δ 183.89, 167.88, 140.98, 137.30, 127.91, 127.59, 126.25, 124.85, 118.02, 109.62, 82.21, 47.11, 44.71, 43.78, 43.49, 38.51, 34.83, 31.27, 30.23, 30.13, 29.58, 28.01, 26.92, 26.83, 22.40; IR (KBr): 2922, 2851, 1742, 1627 cm^{-1}; MS (EI) m/z 481 (M+); HRMS (EI) calcd for C$_{23}$H$_{39}$NO$_3$S: 481.2651 (M+), found: 481.2652.
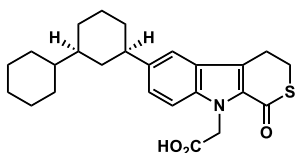
1',3'-*cis*-(6-Bicyclohexyl-3-yl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (9)

To a stirred solution of NaI (162 mg, 1.08 mmol) in ClCH$_2$CH$_2$Cl (5 ml) was added TMSCl (0.14 ml, 1.08 mmol), and the resulting mixture was stirred at room temperature for 15 min. To a solution of **41** (130mg, 0.27 mmol) in ClCH$_2$CH$_2$Cl (5 ml) was transferred a solution of TMSI, prepared above, via a cannula, and then the resulting mixture was refluxed for 19 h. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with EtOAc. The organic extracts were combined, dried over Na$_2$SO$_4$, and evaporated. The residue was chromatographed on SiO$_2$ (Hexane : EtOAc = 3 : 1) to give **9** (102 mg, 89%).

mp: 219-221 °C; ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.45 (1H, brs), 7.33 (1H, d, J = 8.6 Hz), 7.21 (1H, dd, J = 1.5 Hz, 8.6 Hz), 5.26 (2H, s), 3.42 (2H, t, J = 6.0 Hz), 3.32 (2H, t, J = 6.0 Hz), 2.61 (1H, tt, J = 3.3 Hz, 11.7 Hz), 1.90-0.96 (20H, m), ^{13}C NMR (125 MHz, CDCl$_3$): δ 184.46, 174.08, 141.39, 137.26, 128.03, 127.73, 126.79, 124.87, 118.11, 109.60, 46.28, 44.68, 43.77, 43.48, 38.52, 34.76, 31.15, 30.22, 30.12, 29.57, 26.90, 26.82, 22.31; IR (KBr): 2925, 2850, 1723, 1631 cm^{-1};MS (EI) m/z 425 (M+); HRMS (EI) calcd for C$_{25}$H$_{31}$NO$_3$S: 425.2025 (M+), found: 425.2029.
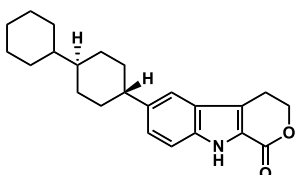
1',4'-*trans*-6-Bicyclohexyl-4-yl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indol-1-one (43)

To a suspension of **24** (120 mg, 0.47 mmol) and conc. HCl (0.12 ml) in THF (0.72 ml) and H$_2$O (0.24 ml) was slowly added a solution of NaNO$_2$ (35 mg, 0.50 mmol) in H$_2$O (0.16 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. At the same time, to a solution of enol lactone **5** (112 mg, 0.56 mmol) and sodium acetate (153 mg, 1.86 mmol) in THF (0.48 ml) and H$_2$O (0.12 ml) was slowly added the diazonium salt, prepared above, at 0 °C. The resulting mixture was stirred at 0 °C for 2 h. The reaction was diluted with CH$_2$Cl$_2$ and the organic phase was separated, dried over Na$_2$SO$_4$ and concentrated in vacuo to yield hydrazone **42**. The product was used for the next reaction

without further purification. A solution of **42** (153 mg) in AcOH (1.3 ml) and HCl (1.3 ml, 1.0 M in AcOH solution) was refluxed for 4 h, and cooled to room temperature. The resulting mixture was diluted with CH_2Cl_2 and the organic phase was separated, washed with H_2O, neutralized with sat. $NaHCO_3$ aq, dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane : EtOAc = 8 : 1) to give **43** (110 mg, 67% in two steps).

mp: 272-273 °C; ^{1}H NMR (500 MHz, CDCl$_3$): δ 8.67 (1H, br), 7.43 (1H, brs), 7.37 (1H, d, J = 8.6 Hz), 7.28 (1H, dd, J = 1.4 Hz, 8.6 Hz), 4.69 (2H, t, J = 6.2 Hz), 3.14 (2H, t, J = 6.2 Hz), 2.55 (1H, tt, J = 3.3 Hz, 12.2 Hz), 1.98-0.97 (20H, m); ^{13}C NMR (125 MHz, CDCl$_3$): δ 161.51, 140.75, 137.00, 127.00, 124.56, 122.90, 122.24, 117.55, 112.56, 69.45, 44.64, 43.29, 43.00, 35.05, 30.32, 30.27, 26.84, 21.46; IR (KBr): 3265, 2919, 2850, 1734 cm^{-1}; MS (EI) m/z 351 (M+); HRMS (EI) calcd for $C_{23}H_{29}NO_2$: 351.2198 (M+), found: 351.2201.
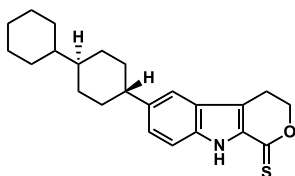
1',4'-*trans*-6-Bicyclohexyl-4-yl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indole-1-thione (44)

To a stirred solution of **43** (150 mg, 0.43 mmol) in toluene (6 ml) was added Lawesson's reagent (96 mg, 0.24 mmol), and the resulting mixture was refluxed for 17 h. After cooling, the solvent was removed, and the residue was chromatographed on SiO_2 (Hexane : EtOAc = 40 : 1) to give **44** (126 mg, 81%).

^{1}H NMR (500MHz, CDCl$_3$): δ 8.76 (1H, br), 7.42 (1H, brs), 7.32 (1H, d, J = 8.6 Hz), 7.29 (1H, dd, J = 1.4 Hz, 8.6 Hz), 4.74 (2H, t, J = 6.5 Hz), 3.16 (2H, t, J = 6.5 Hz), 2.53 (1H, tt, J = 3.1 Hz, 12.0 Hz), 1.97-0.97 (20H, m); ^{13}C NMR (125 MHz, CDCl$_3$): δ 198.30, 141.32, 137.87, 132.36, 128.09, 124.92, 118.56, 115.75, 112.11, 71.18, 44.62, 43.28, 42.98, 34.96, 30.28, 26.84, 21.27; IR (neat): 3363, 2922, 2850, 1540, 1231 cm^{-1}; MS (EI) m/z 367 (M+); HRMS (EI) calcd for $C_{23}H_{29}NOS$: 367.1970 (M+), found: 367.1968.
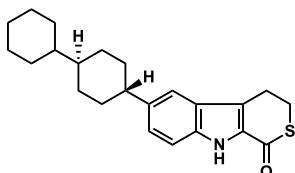
1',4'-*trans*-6-Bicyclohexyl-4-yl-4,9-dihydro-3*H*-2-thia-9-azafluoren-1-one (45)

To a stirred solution of NaI (206 mg, 1.37 mmol) in $ClCH_2CH_2Cl$ (10 ml) was added TMSCl (0.18 ml, 1.37 mmol), and the resulting mixture was stirred at room temperature for 15 min. To a solution of **44** (126 mg, 0.34 mmol) in $ClCH_2CH_2Cl$ (10 ml) was transferred a solution of TMSI, prepared above, via a cannula, and then the resulting

mixture was refluxed for 4 days. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with CHCl$_3$. The organic extracts were combined, dried over Na$_2$SO$_4$, and evaporated. The residue was chromatographed on SiO$_2$ (Hexane : EtOAc = 35 : 1) to give **45** (101 mg, 80%).

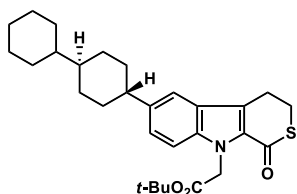mp: 275-276 °C; ^{1}H NMR (500 MHz, CDCl$_3$): δ 8.74 (1H, br), 7.44 (1H, brs), 7.33 (1H, d, J = 8.6 Hz), 7.27 (1H, dd, J = 1.4 Hz, 8.6 Hz), 3.48 (2H, t, J = 6.5 Hz), 3.28 (2H, t, J = 6.5 Hz), 2.55 (1H, tt, J = 3.4 Hz, 12.2 Hz), 1.98-0.98 (20H, m); ^{13}C NMR (125 MHz, CDCl$_3$): δ 183.61, 140.68, 135.35, 128.70, 127.52, 125.90, 125.18, 117.70, 112.18, 44.67, 43.30, 43.01, 35.05, 31.54, 30.33, 30.29, 26.85, 21.79; IR (KBr): 3288, 2915, 2848, 1628 cm^{-1}; MS (EI) m/z 367 (M+); HRMS (EI) calcd for C$_{23}$H$_{29}$NOS: 367.1970 (M+), found: 367.1971.

1',4'-*trans*-(6-Bicyclohexyl-4-yl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid *tert*-butyl ester (46)

To a stirred solution of **45** (101 mg, 0.28 mmol) in DMF (3 ml) was added NaH (60%, 17 mg, 0.41 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min. To the mixture was added BrCH$_2$CO$_2$t-Bu (61 μl, 0.41 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 15 h. The reaction was quenched with H$_2$O, and the aqueous mixture was extracted with Et$_2$O. The organic extracts were combined, dried over Na$_2$SO$_4$, and evaporated. The residue was chromatographed on SiO$_2$ (Hexane : EtOAc = 30 : 1) to give **46** (128 mg, 97%).

mp: 178-180 °C; ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.44 (1H, brs), 7.30 (1H, d, J = 8.7 Hz), 7.17 (1H, d, J = 8.7 Hz), 5.13 (2H, s), 3.42 (2H, t, J = 6.4 Hz), 3.31 (2H, t, J = 6.4 Hz), 2.54 (1H, tt, J = 3.3 Hz, 12.2 Hz), 1.97-0.98 (20H, m), 1.45 (9H, s); ^{13}C NMR (125 MHz, CDCl$_3$): δ 183.87, 167.90, 140.73, 137.31, 127.90, 127.54, 126.24, 124.84, 118.00, 109.60, 82.21, 47.11, 44.61, 43.30, 43.00, 35.02, 31.26, 30.31, 30.27, 28.01, 26.84, 22.37; IR (KBr): 2921, 2850, 1740, 1641 cm^{-1}; MS (EI) m/z 481 (M+); HRMS (EI) calcd for C$_{29}$H$_{39}$NO$_3$S: 481.2651 (M+), found: 481.2655.
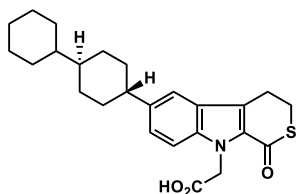
1',4'-*trans*-(6-Bicyclohexyl-4-yl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (30)

To a stirred solution of NaI (138 mg, 0.92 mmol) in ClCH$_2$CH$_2$Cl (5 ml) was added TMSCl (0.12 ml, 0.92 mmol), and the resulting mixture was stirred at room temperature for 15 min. To a solution of **46** (111 mg, 0.23 mmol) in ClCH$_2$CH$_2$Cl (5 ml) was transferred

a solution of TMSI, prepared above, via a cannula, and then the resulting mixture was refluxed for 19 h. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with EtOAc. The organic extracts were combined, dried over Na_2SO_4, and evaporated. The residue was chromatographed on SiO_2 (Hexane : EtOAc = 3 : 1) to give **30** (81 mg, 83%).

mp: 234-236 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.44 (1H, brs), 7.33 (1H, dd, J = 1.5, 8.6 Hz), 7.21 (1H, d, J = 8.6 Hz), 5.25 (2H, s), 3.42 (2H, t, J = 6.3 Hz), 3.31 (2H, t, J = 6.3 Hz), 2.55 (1H, tt, J = 3.3 Hz, 12.0 Hz), 1.96-0.97 (20H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 184.59, 172.37, 141.15, 137.36, 128.02, 127.81, 126.82, 124.87, 118.11, 109.67, 46.42, 44.60, 43.30, 43.01, 35.01, 31.16, 30.30, 29.68, 26.86, 22.32; IR (KBr): 2908, 2850, 1723, 1632 cm^{-1}; MS (EI) m/z 425 (M+); HRMS (EI) calcd for $C_{25}H_{31}NO_3S$: 425.2025 (M+), found: 425.2024.

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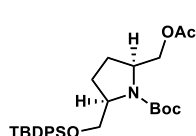
(2*S*,5*R*)-2-Acetoxymethyl-5-(*tert*-butyl-diphenyl-silanyloxymethyl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (49)

To a stirred solution of **48** (130 mg, 0.48 mmol) in CH_2Cl_2 (5 ml) were added imidazole (49 mg, 0.71 mmol), TBDPSCl (0.18 ml, 0.71 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 15 h. The reaction mixture was washed with H_2O and brine successively, dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane : EtOAc = 15 : 1) to give **49** (244 mg, 100%) as a colorless oil.

$[\alpha]_D^{25}$ +12.1 (c 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67-7.64 (4H, m), 7.42-7.35 (6H, m), 4.11-3.49 (6H, m), 2.11-1.78 (4H, m), 1.90 (3H, s), 1.45/1.35 (9H, each s), 1.06 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.31, 154.37, 135.30, 133.24, 129.43, 127.48, 79.34, 64.55, 63.47, 59.37, 56.64, 28.15, 26.64, 26.00, 25.54, 20.48, 19.00; IR (neat): 2963, 2932, 1747, 1695, 1111 cm^{-1}; MS (EI) m/z 511 (M+); HRMS (EI) calcd for $C_{29}H_{41}NO_5Si$: 511.2754 (M+), found: 511.2757.
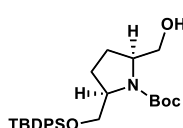
(2*R*,5*S*)-2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-5-hydroxymethyl-pyrrolidine-1-carboxylic acid *tert*-butyl ester (50)

To a stirred solution of **49** (284 mg, 0.55 mmol) in MeOH (5 ml) was added K_2CO_3 (115 mg, 0.83 mmol), and the resulting mixture was stirred for 50 min at room temperature. The reaction was quenched with H_2O, and the aqueous mixture was extracted with EtOAc. The organic extracts were combined, dried over Na_2SO_4, and

concentrated in vacuo. The residue was chromatographed on SiO$_2$ (Hexane : EtOAc = 2 : 1) to give **50** (252 mg, 97%) as a colorless oil.

$[\alpha]_D^{26}$ +2.2 (c 0.7, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.66-7.64 (4H, m), 7.43-7.37 (6H, m), 4.88 (1H, br), 3.97-3.93 (2H, m), 3.65-3.64 (2H, m), 3.54-3.53 (2H, m), 2.17-1.86 (4H, m), 1.49/1.35 (9H, each s), 1.05 (9H, s); ^{13}C NMR (100 MHz, CDCl$_3$): δ 157.06, 135.49, 133.23/133.14, 129.71, 127.70, 80.47, 68.02, 64.61, 61.76, 60.36, 28.30, 27.01, 26.81, 26.57, 19.18, 14.17; IR (neat): 2964, 2931, 1695, 1111 cm^{-1}; MS (EI) m/z 469 (M+); HRMS (EI) calcd for C$_{27}$H$_{39}$NO$_4$Si: 469.2648 (M+), found: 469.2637.
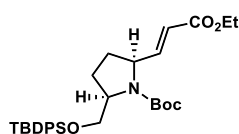

(2*R*,5*S*)-2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-5-(2-ethoxycarbonyl-vinyl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (52)

To a stirred solution of **50** (914 mg, 1.95 mmol) in DMSO (6 ml) and Et$_3$N (6 ml) was added SO$_3$・Py (1.55 g, 9.73 mmol), and the resulting mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with ether, and the resulting mixture was washed with 10% HCl aq, H$_2$O and brine successively, dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was used for the next reaction without further purification.

To a stirred solution of Triethyl Phosphonoacetate (0.78 ml, 3.89 mmol) in THF (5 ml) was added NaH (60%, 141 mg, 3.50 mmol) at 0 °C, and the resulting mixture was stirred at 0 °C for 10 min. To the mixture was added a solution of the above aldehyde **51** in THF (5 ml) via a cannula at 0 °C, and the resulting mixture was stirred at room temperature for 11 h. The reaction was quenched with sat.NH$_4$Cl aq, and the aqueous mixture was extracted with EtOAc. The organic extracts were combined, dried over Na$_2$SO$_4$, and concentrated in vacuo. The residue was chromatographed on SiO$_2$ (Hexane : EtOAc = 7 : 1) to give **52** (989 mg, 95%, 2 steps) as a colorless oil.

$[\alpha]_D^{25}$ -6.8 (c 1.2, MeOH); ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.67-7.65 (4H, m), 7.42-7.36 (6H, m), 6.82 (1H, dd, J = 6.9 Hz, 15.6 Hz), 5.87 (1H, br), 4.43-4.29 (1H, m), 4.14 (2H, q, J = 7.1 Hz), 4.03-3.54 (3H, m), 2.04-1.77 (4H, m), 1.62/1.61 (9H, each s), 1.22 (3H, t, J = 7.1 Hz), 1.05 (9H, s); ^{13}C NMR (125 MHz, CDCl$_3$): δ 166.42, 154.67, 149.29, 135.53, 133.47, 129.62, 127.65, 120.57, 79.83, 65.00, 60.17, 59.93, 59.38, 28.33, 27.49, 26.86, 26.62, 19.22, 14.19; IR (neat): 1717, 1699 cm^{-1}; MS (EI) m/z 537 (M+); HRMS (EI) calcd for C$_{31}$H$_{43}$NO$_5$Si: 537.2911 (M+), found: 537.2917.
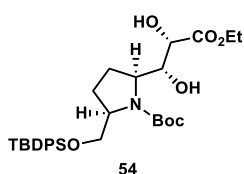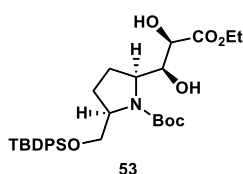
(2*R*,5*S*)-2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-5-[(1*S*,2*R*)-2-ethoxycarbonyl-1,2-dihydroxy-ethyl]-pyrrolidine-1-carboxylic acid *tert*-butyl ester (53)

(2*R*,5*S*)-2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-5-[(1*R*,2*S*)-2-ethoxycarbonyl-1,2-dihydroxy-ethyl]-pyrrolidine-1-carboxylic acid *tert*-butyl ester (54)

To a stirred solution of **52** (841 mg, 1.56 mmol) in acetone (8 ml) and H_2O (1 ml) were added NMO (4.8 M in H_2O, 0.65 ml, 3.13 mmol) and OsO_4 (1% in H_2O, 1.91 ml, 0.08 mmol), and the resulting mixture was stirred at room temperature for 13 h. The reaction was quenched with 10% $Na_2S_2O_3$ aq, and the aqueous mixture was extracted with EtOAc. The organic extracts were combined, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane : EtOAc = 3 : 1- 2 : 1) to give **53** (522 mg, 58%) and **54** (267 mg, 30%) as a colorless oil, respectively.

53: $[\alpha]_D^{23}$ -1.6 (c 0.8, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66-7.64 (4H, m), 7.45-7.35 (6H, m), 4.90 (1H, br), 4.22-4.15 (3H, m), 3.93-3.84 (3H, m), 3.72-3.70 (1H, m), 3.54 (1H, br), 2.17-2.12 (3H, m), 1.95-1.94 (1H, m), 1.32 (9H, s), 1.21 (3H, t, J = 7.1 Hz), 1.05 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.73, 157.19, 135.49/135.48, 133.27, 129.74, 127.71, 81.20, 73.68, 70.75, 65.59, 61.28, 60.74, 60.23, 28.20, 27.33, 26.85, 26.38, 19.22, 14.16; IR (neat): 3310, 1742, 1666 cm^{-1}; MS (EI) m/z 571 (M+); HRMS (EI) calcd for $C_{31}H_{45}NO_7Si$: 571.2965 (M+), found: 571.2973.

54: $[\alpha]_D^{25}$ -0.97 (c 0.7, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66-7.65 (4H, m), 7.45-7.37 (6H, m), 4.31-4.01 (5H, m), 3.79-3.77 (1H, m), 3.69-3.67 (1H, m), 3.57-3.55 (1H, m), 2.11 (2H, br), 1.98-1.89 (1H, m), 1.76-1.71 (1H, m), 1.34 (9H, s), 1.24 (3H, t, J = 7.1 Hz), 1.06 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.85, 158.41, 135.54, 133.27/133.16, 129.73/129.70, 127.74/127.72, 81.24, 77.69, 72.17, 64.67, 61.63, 61.23, 60.53, 28.22, 26.85, 26.81, 26.50, 19.17, 14.14; IR (neat): 3310, 1747, 1663 cm^{-1}; MS (EI) m/z 571 (M+); HRMS (EI) calcd for $C_{31}H_{45}NO_7Si$: 571.2965 (M+), found: 571.2956.
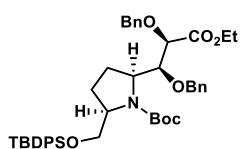
(2*S*,5*R*)-2-[(1*S*,2*R*)-1,2-Bis-benzyloxy-2-ethoxycarbonyl-ethyl]-5-(*tert*-butyl-diphenyl-silanyloxymethyl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (55)

To a stirred solution of **53** (1.12 g, 1.96 mmol) in DMF (15 ml) was added NaH (60%, 172 mg, 4.31 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 10 min. To the mixture was added BnBr (0.5 ml, 4.31 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 1.5 h. The reaction was quenched with H_2O, and the

aqueous mixture was extracted with EtOAc. The organic extracts were combined, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane : EtOAc = 8 : 1) to give **55** (1.01 g, 68%) as a colorless oil.

$[\alpha]_D^{26}$ -4.4 (c 0.7, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.58-7.52 (4H, m), 7.39-7.30 (11H, m), 7.08-7.04 (5H, m), 4.68 (1H, ABq, J = 11.5 Hz), 4.54-3.34 (11H, m), 2.04-1.75 (4H, m), 1.37 (9H, br), 1.22 (3H, t, J = 7.1 Hz), 0.99 (9H, s); ^{13}C NMR (100 MHz, CDCl$_3$): δ 170.77, 154.88, 135.51, 133.88, 129.45, 128.30, 128.25, 128.03, 127.98, 127.83, 127.57, 127.54, 127.35, 80.62, 77.21, 75.30, 72.82, 63.74, 60.97, 60.59, 59.83, 53.41, 28.44, 26.82, 26.62, 23.39, 19.20, 14.16; IR (neat): 2931, 2856, 1734, 1692, 1111cm^{-1}; MS (EI) m/z 751 (M+); HRMS (EI) calcd for $C_{45}H_{57}NO_7Si$: 751.3904 (M+), found: 751.3909.
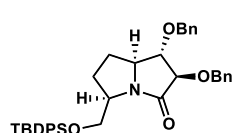
(1*S*,2*R*,5*R*,8*S*)-1,2-Bis-benzyloxy-5-(tert-butyl-diphenyl-silanyloxymethyl)-hexahydro-pyrrolizin-3-one (57)

To a stirred solution of **55** (31 mg, 0.041 mmol) in CH$_2$Cl$_2$ (0.4 ml) was added TFA (0.4 ml) at 0 °C, and the resulting mixture was stirred at 0 °C for 20 min. The solvent was removed, and the residue was dissolved in CH$_2$Cl$_2$, neutralized with K$_2$CO$_3$ and the resulting suspension was filtered off by Celite and the filtrate was concentrated in vacuo. The residue was used for the next reaction without further purification.

To a stirred solution of the above secondary amine **56** in CH$_2$Cl$_2$ (3.5 ml) was added AlMe$_3$ (1.08 M in hexane, 98 μl, 0.106 mmol) at 0 °C, and the resulting mixture was refluxed for 22 h. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with CH$_2$Cl$_2$. The organic extracts were combined, dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was chromatographed on SiO$_2$ (Hexane : EtOAc = 2 : 1) to give **57** (12 mg, 48%) as a colorless oil.

$[\alpha]_D^{25}$ +22.6 (c 0.6, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.66-7.63 (4H, m), 7.43-7.27 (16H, m), 5.12 & 4.83 (2H, ABq, J = 11.5 Hz), 4.63 & 4.58 (2H, ABq, J = 12.0 Hz), 4.43 (1H, dd, J = 1.1 Hz, 8.6 Hz), 4.14 (1H, dd, J = 5.2 Hz, 10.3 Hz), 3.94 (1H, t-like, J = 7.8 Hz), 3.90 (1H, dd, J = 2.2 Hz, 10.3 Hz), 3.73 (1H, m), 3.60-3.56 (1H, m), 2.17-2.06 (2H, m), 1.94-1.89 (1H, m), 1.67-1.59 (1H, m), 1.03 (9H, s); ^{13}C NMR (125 MHz, CDCl$_3$): δ 168.42, 138.07, 137.67, 135.79, 135.68, 135.62, 135.52, 133.43, 133.00, 129.67, 128.02, 127.67, 127.34, 85.83, 85.27, 72.33, 63.13, 61.80, 55.63, 55.32, 31.20, 26.92, 26.86, 19.23; IR (neat): 2930, 2856, 1704, 1112 cm^{-1}; MS (EI) m/z 605 (M+); HRMS (EI) calcd for $C_{38}H_{43}NO_4Si$: 605.2961 (M+), found: 605.2954.
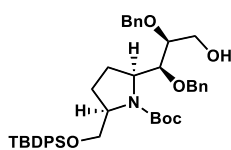
**(2*S*,5*R*)-2-[(1*S*,2*S*)-1,2-Bis-benzyloxy-3-hydroxy-propyl)-5-(*tert*-butyl-diphenyl-silanyloxymethyl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (58)**

To a stirred solution of **55** (1.06 g, 1.41 mmol) in THF (15 ml) was added $LiBH_4$ (3.0 M in THF, 0.94 ml, 2.82 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 21 h. The reaction was quenched with H_2O, and the aqueous mixture was extracted with EtOAc. The organic extracts were combined, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane : EtOAc = 2 : 1) to give **58** (940 mg, 94%) as a colorless oil.

$[\alpha]_D^{25}$ -21.5 (c 0.9, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.58-7.52 (4H, m), 7.41-7.27 (11H, m), 7.13-7.08 (5H, m), 4.74 & 4.57 (2H, ABq, J = 11.3 Hz), 4.53 (1H, ABq, J = 14.1 Hz), 4.35 (2H, br), 3.96 (1H, br), 3.81-3.39 (6H, m), 1.67 (4H, br), 1.46/1.34 (9H, each s), 1.00 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$):δ 154.94, 138.27, 135.39, 133.72, 133.57, 129.40, 128.25, 128.03, 127.76, 127.63, 127.47, 127.23, 126.78, 81.22, 79.46, 77.58, 75.19, 73.00, 63.61, 62.14, 59.67, 53.31, 28.31, 26.74, 26.58, 22.60, 19.10; IR (neat): 3434, 2931, 2855, 1692, 1112cm^{-1}; MS (EI) m/z 709 (M+); HRMS (EI) calcd for $C_{43}H_{55}NO_6Si$: 709.3799 (M+), found: 709.3796.
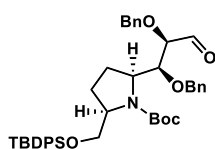
(2*S*,5*R*)-2-[(1*S*,2*R*)-1,2-Bis-benzyloxy-3-oxo-propyl)-5-(*tert*-butyl-diphenyl-silanyloxymethyl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (59)

To a stirred solution of **58** (324 mg, 0.46 mmol) in CH_2Cl_2 (5 ml) was added DMP (290 mg, 0.69 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 4 h. The reaction was quenched with 10% $Na_2S_2O_3$ aq, sat. $NaHCO_3$ aq, and the aqueous mixture was extracted with CH_2Cl_2. The organic extracts were combined, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane : EtOAc = 7 : 1) to give **59** (312 mg, 97%) as a colorless oil.

^1H NMR (400 MHz, $CDCl_3$): δ 9.56 (1H, s), 7.60-7.56 (4H, m), 7.40-7.31 (11H, m), 7.16-7.06 (5H, m), 4.64 (1H, ABq, J = 12.0 Hz), 4.57-4.55 (2H, m), 4.45-4.32 (2H, m), 3.96-3.75 (4H, m), 3.43-3.39 (1H, m), 2.05 (1H, br), 1.78 (3H, br), 1.37 (9H, s), 1.26 (3H, t, J = 7.1 Hz), 1.01 (9H, s).
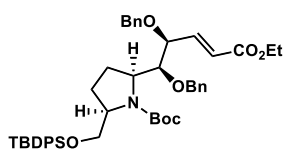
(2*S*,5*R*)-2-[(1*S*,2*S*)-1,2-Bis-benzyloxy-4-ethoxycarbonyl-but-3 -enyl)-5-(*tert*-butyl-diphenyl-silanyloxymethyl)-Pyrrolidine-1 -carboxylic acid *tert*-butyl ester (60)

To a stirred solution of Triethyl Phosphonoacetate (0.48 ml, 2.42 mmol) in THF (5 ml) was added NaH (60%, 87 mg, 2.19 mmol) at 0 °C, and the resulting mixture was stirred at 0 °C for 10 min. To the mixture was added a solution of **59** (860 mg, 1.21 mmol) in THF (5 ml) via a cannula at 0 °C, and the resulting mixture was stirred at room temperature for 13 h. The reaction was quenched with sat. NH_4Cl aq, and the aqueous mixture was extracted with EtOAc. The organic extracts were combined, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane : EtOAc = 10 : 1) to give **60** (911 mg, 97%) as a colorless oil.

$[\alpha]_D^{24}$ -3.9 (c 1.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58-7.56 (4H, m), 7.40-7.27 (11H, m), 7.12-7.04 (5H, m), 6.84 (1H, br), 6.04 (1H, d, *J* = 15.9 Hz), 4.55-4.52 (2H, m), 4.41-3.67 (9H, m), 3.43-3.38 (1H, m), 2.10 (1H, br), 1.93 (1H, m), 1.63 (2H, br), 1.42/1.35 (9H, each s), 1.30 (3H, t, *J* = 7.1 Hz), 0.99 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.94, 154.71, 144.54, 138.31, 137.97, 135.48, 133.89, 133.71, 129.46, 128.27, 128.06, 127.91, 127.71, 127.54, 127.32, 123.39, 80.51, 79.36, 78.67, 75.45, 71.28, 63.42, 60.53, 60.37, 59.72, 28.43, 26.83, 26.26, 22.78, 19.21, 14.22; IR (neat): 2931, 2857, 1721, 1694, 1109 cm^{-1}; MS (EI) m/z 777 (M+); HRMS (EI) calcd for $C_{47}H_{59}NO_7Si$: 777.4061 (M+), found: 777.4049.
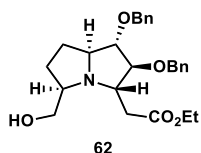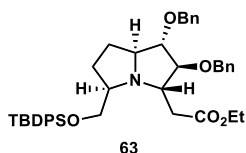
(1*S*,2*S*,3*S*,5*R*,8*S*)-[1,2-Bis-benzyloxy-5-(*tert*-butyl-diphenyl-silanyloxymethyl)-hexahydro-pyrrolizin-3-yl]-acetic acid ethyl ester (63) (1*S*,2*S*,3*S*,5*R*,8*S*)-(1,2-Bis-benzyloxy-5-hydroxymethyl-hexahydro-pyrrolizin-3-yl)-acetic acid ethyl ester (62)

To a stirred solution of **60** (777 mg, 1.00 mmol) in CH_2Cl_2 (10 ml) was added TFA (10 ml), at 0 °C, and the resulting mixture was stirred at room temperature for 1 h. The solvent was removed, and the residue was used for the next reaction without further purification.

To a stirred solution of the above TFA salt **61** in CH_2Cl_2 (30 ml) was added K_2CO_3 (1.38 g, 10.0 mmol), and the resulting suspension was stirred at room temperature for 6 days. The suspension was filtered off by Celite and the filtrate was concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane : EtOAc = 3 : 1-CH_2Cl_2 : MeOH = 20 : 1) to give **63** (524 mg, 77%) as a yellow oil along with **62** (99 mg, 23%) as a colorless

oil.

To a stirred solution of **55** (99 mg, 0.23 mmol) in CH_2Cl_2 (2 ml) were added imidazole (61 mg, 0.9 mmol), TBDPSCl (0.12 ml, 0.45 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 17 h. The reaction was quenched with H_2O and the aqueous mixture was extracted with CH_2Cl_2. The organic extracts were combined, dried over K_2CO_3, and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane : EtOAc = 3 : 1) to give **56** (145 mg, 95%) as yellow oil.

63: $[\alpha]_D^{26}$ +1.6 (c 1.05, MeOH); ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.68-7.65 (4H, m), 7.41-7.27 (16H, m), 4.68 & 4.58 (2H, ABq, J = 11.8 Hz), 4.54 & 4.47 (2H, ABq, J = 11.8 Hz), 4.03-3.98 (3H, m), 3.80-3.74 (2H, m), 3.70-3.65 (2H, m), 3.53-3.49 (1H, m), 3.23-3.20 (1H, m), 2.57 (1H, dd, J = 5.5 Hz, 14.6 Hz), 2.49 (1H, dd, J = 7.6 Hz, 14.6 Hz), 1.89-1.78 (2H, m), 1.71-1.63 (2H, m), 1.26 (3H, t, J = 7.0 Hz), 1.05 (9H, s); ^{13}C NMR (100 MHz, CDCl$_3$): δ 171.97, 138.57, 138.28, 135.68, 133.39, 129.62, 128.36, 128.21, 127.65, 127.56, 127.38, 89.52, 88.50, 72.15, 71.84, 68.19, 64.06, 61.24, 60.13, 57.84, 39.93, 29.93, 29.24, 26.91, 19.19, 14.16; IR (neat): 2930, 2855, 1734, 1113 cm^{-1}; MS (EI) m/z 677 (M+); HRMS (EI) calcd for $C_{42}H_{51}NO_5Si$: 677.3537 (M+), found: 677.3523 .

62: $[\alpha]_D^{25}$ -5.2 (c 0.2, CHCl$_3$); ^{1}H NMR (500MHz, CDCl$_3$): δ 7.36-7.28 (10H, m), 4.59 (2H, s), 4.56 & 4.47 (2H, ABq, J = 12.0 Hz), 4.13 (2H, q, J = 7.3 Hz), 3.93-3.62 (6H, m), 3.35 (1H, br), 3.24 (1H, br), 2.74 (1H, dd, J = 7.1 Hz, 15.5 Hz), 2.55 (1H, dd, J = 6.3 Hz, 15.5 Hz), 1.96-1.70 (4H, m), 1.24 (3H, t, J = 7.1 Hz); ^{13}C NMR (125 MHz, CDCl$_3$): δ 172.30, 137.89, 137.49, 128.33, 128.30, 127.73, 127.71, 127.60, 127.51, 89.38, 88.38, 71.92, 71.59, 69.34, 62.83, 62.26, 60.42, 58.81, 39.30, 29.34, 29.10, 14.08; IR (neat): 3320, 2924, 1733, 1093 cm^{-1}; MS (EI) m/z 439 (M+); HRMS (EI) calcd for $C_{26}H_{33}NO_5$: 439.2359 (M+), found: 439.2374.
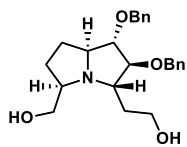
(1*S*,2*S*,3*S*,5*R*,8*S*)-2-(1,2-Bis-benzyloxy-5-hydroxymethyl-hexahydro-pyrrolizin-3-yl)-ethanol (64)

To a stirred solution of **62** (38 mg, 0.086 mmol) in THF (0.86 ml) was added LiAlH$_4$ (7 mg, 0.17 mmol) at 0 °C, and the resulting suspension was stirred at room temperature. After 30 min of stirring at the same temperature the reaction mixture was refluxed for 14 h. After cooling, the reaction was quenched with 10% NaOH aq and diluted with EtOAc, and the resulting mixture was filtered off by Celite, the filtrate was dried over K_2CO_3, and concentrated in vacuo. The residue was chromatographed on SiO_2 (CH$_2$Cl$_2$: MeOH = 10 : 1) to give 64 (30 mg, 88%) as a colorless oil.

$[\alpha]_D^{26}$ -12.7 (c 0.75, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.38-7.28 (10H, m), 4.62 & 4.51 (2H, ABq, J = 11.8 Hz), 4.59 & 4.54 (2H, ABq, J = 11.7 Hz), 3.97-3.96 (1H, m), 3.92-3.88 (1H, m), 3.82-3.69 (6H, m), 3.56-3.54 (1H, m), 3.37 (1H, br), 2.01-1.66 (6H, m); ^{13}C NMR (125 MHz, CDCl$_3$): δ 137.70, 137.33, 128.50, 128.46, 127.99, 127.86, 127.81, 127.61, 88.12, 88.01, 72.17, 71.70, 69.16, 64.85, 61.88, 61.84, 60.87, 32.80, 29.65, 29.02; IR (neat): 3299, 2926, 2854 cm^{-1}; MS (EI) m/z 397 (M+); HRMS (EI) calcd for C$_{24}$H$_{31}$NO$_4$: 397.2253 (M+), found: 397.2246.
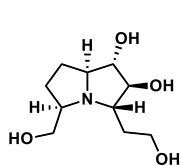
(1*S*,2*S*,3*S*,5*R*,8*S*)-3-(2-Hydroxy-ethyl)-5-hydroxymethyl-hexahydro-pyrrolizine-1,2-diol (65)

To a stirred solution of **64** (27 mg, 0.068 mmol) in CH$_2$Cl$_2$ (7 ml) was added BCl$_3$ (1.0 M in hexane, 0.34 ml, 0.34 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 19 h. The reaction was quenched with MeOH, and the solvents were evaporated. The residue was dissolved in MeOH (1 ml), added K$_2$CO$_3$ (94 mg, 0.68 mmol), and the resulting mixture was stirred at room temperature for 3 h. The resulting mixture was filtered off by Celite and the filtrate was evaporated. The residue was purified using Dowex 50Wresin (X-8, H+ form, eluent: 0.5 N aqueous NH$_3$) to give **65** (15 mg, quant.) as a colorless oil.

$[\alpha]_D^{26}$ -5.2 (c 0.4, MeOH); ^{1}H NMR (500 MHz, Acetone-d$_6$): δ 4.50 (1H, br), 4.09 (1H, br), 3.91 (1H, br), 3.90-3.81 (3H, m), 3.79-3.57 (3H, m), 3.30-3.14 (3H, m), 1.82-1.67 (6H, m); ^{13}C NMR (125 MHz, Acetone-d$_6$): δ 82.91, 82.86, 69.36, 63.44, 62.72, 61.92, 60.66, 36.01, 30.21, 30.05; IR (neat): 3349 cm^{-1}; MS (EI) m/z 217 (M+); HRMS (EI) calcd for C$_{10}$H$_{19}$NO$_4$: 217.1314 (M+), found: 217.1312.

General procedure for the synthesis of (67-69)

To a stirred solution of **63** (102 mg, 0.151 mmol) in CH$_2$Cl$_2$ (1.5 ml) was added dropwise DIBAL-H (1.0 M in Hexane, 0.17 ml, 0.167 mmol) at -78 °C, and the resulting mixture was stirred at the same temperature for 30 min. The reaction was quenched with sat. NH$_4$Cl aq, diluted with EtOAc, and the resulting mixture was filtered off by Celite, the filtrate was dried over K$_2$CO$_3$, and concentrated in vacuo to yield aldehyde **66**, which was used for the next reaction without further purification.

To a stirred suspension of corresponding Wittig reagents (0.708 mmol) in THF was added *t*-BuOK (76 mg, 0.673 mmol) at 0 °C, and the resulting suspension was stirred at 0 °C for 5 min. To the suspension was added a solution of the above aldehyde in THF at 0 °C, and the resulting suspension was stirred at room temperature for 15 h. The reaction was quenched with sat. NH$_4$Cl aq, and the aqueous mixture was extracted with

EtOAc. The organic extracts were combined, dried over K$_2$CO$_3$, and concentrated in vacuo. The residue was chromatographed on SiO$_2$ (Hexane : EtOAc = 5 : 1) to give **67** and **68-69** (as a mixture of *E*-and *Z*-isomers).
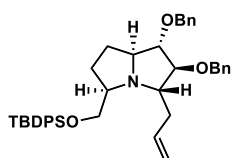
(1*S*,2*S*,3*S*,5*R*,8*S*)-3-Allyl-1,2-bis-benzyloxy-5-(tert-butyl-diphenyl-silanyloxymethyl)-hexahydro-pyrrolizine (67)
73% (2 steps); [α]$_D^{25}$ -5.0 (c 0.35, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.72-7.68 (4H, m), 7.47-7.28 (16H, m), 5.85-5.77 (1H, m), 5.03-4.97 (2H, m), 4.71 & 4.54 (2H, ABq, *J* = 11.8 Hz), 4.62 & 4.59 (2H, ABq, *J* = 10.2 Hz), 3.94 (1H, t-like, *J* = 5.7 Hz), 3.82 (1H, dd, *J* = 4.6 Hz, 10.9 Hz), 3.77 (1H, t-like, *J* = 6.0 Hz), 3.64 (1H, dd, *J* = 5.5 Hz, 10.9 Hz), 3.58-3.55 (1H, m), 3.31-3.30 (2H, m), 2.29 (2H, t-like, *J* = 6.5 Hz), 1.97-1.90 (3H, m), 1.70-1.69 (1H, m), 1.09 (9H, s); ^{13}C NMR (125 MHz, CDCl$_3$): δ 138.53, 138.26, 135.62, 133.33, 129.65, 128.50, 128.35, 128.34, 128.24, 127.66, 127.63, 127.57, 127.44, 116.63, 89.01, 88.31, 72.17, 71.85, 68.21, 64.11, 61.36, 60.38, 38.87, 29.68, 29.19, 26.88, 19.16; IR (neat): 2926, 2856, 1717, 1541 cm^{-1}; MS (EI) m/z 631 (M+); HRMS (EI) calcd for C$_{41}$H$_{49}$NO$_3$Si: 631.3482 (M+), found: 631.3494.

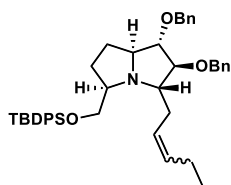**(1*S*,2*S*,3*S*,5*R*,8*S*)-1,2-Bis-benzyloxy-5-(tert-butyl-diphenyl-silanyloxymethyl)-3-pent-2-enyl-hexahydro-pyrrolizine (68)**
80% (*E*, *Z* mixtures, 2 steps); ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.67-7.64 (4H, m), 7.42-7.27 (16H, m), 5.38-5.35 (2H, m), 4.64 & 4.53 (2H, ABq, *J* = 12.0 Hz), 4.56 & 4.49 (2H, ABq, *J* = 12.0 Hz), 3.85 (1H, t-like, *J* = 5.5 Hz), 3.75 (1H, dd, *J* = 4.6 Hz, 10.3 Hz), 3.71 (1H, t-like, *J* = 5.7 Hz), 3.52 (1H, dd, *J* = 6.3 Hz, 10.3 Hz), 3.53-3.49 (1H, m), 3.27-3.21 (2H, m), 2.27-2.19 (2H, m), 1.97-1.86 (5H, m), 1.68-1.64 (1H, m), 1.04 (9H, s), 0.88 (3H, t, *J* = 7.5 Hz)
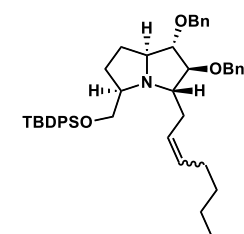
(1*S*,2*S*,3*S*,5*R*,8*S*)-1,2-Bis-benzyloxy-5-(tert-butyl-diphenyl-silanyloxymethyl)-3-hept-2-enyl-hexahydro-pyrrolizine (69)
75% (*E*, *Z* mixtures, 2 steps); ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.67-7.64 (4H, m), 7.42-7.27 (16H, m), 5.44-5.35 (2H, m), 4.64 & 4.54 (2H, ABq, *J* = 12.1 Hz), 4.56 & 4.49 (2H, ABq, *J* = 12.0 Hz), 3.85 (1H, t-like, *J* = 5.7 Hz), 3.80 (1H, dd, *J* = 4.6 Hz, 10.3 Hz), 3.71 (1H, t-like, *J* = 5.8 Hz), 3.60 (1H, dd, *J* = 6.3 Hz, 10.3 Hz), 3.54-3.50 (1H, m), 3.27-3.22 (2H, m), 2.30-1.87 (7H, m), 1.71-1.61 (1H, m), 1.36-1.22 (4H, m), 1.04 (9H,

s), 0.84 (3H, t, J = 7.2 Hz)

General procedure for the synthesis of (70-72)

To a stirred solution of **67-69** (0.125 mmol) in EtOAc (5 ml) was added Pd/C (5 mg), and the resulting suspension was stirred under a hydrogen atmosphere at 1 atm for 48 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to yield corresponding amine, which was used for the next reaction without further purification.

To a stirred solution of the crude obtained above in THF (1.5 ml) was added TBAF (1.0 M in THF, 0.38 ml 0.375 mmol), and the resulting mixture was stirred at room temperature for 15 min. The reaction was quenched with sat. NaHCO$_3$ aq, and the aqueous mixture was extracted with CH$_2$Cl$_2$. The organic extracts were combined, dried over K$_2$CO$_3$, and evaporated. The residue was chromatographed on SiO$_2$ (Hexane : EtOAc = 1 : 3) to give **70-72**.
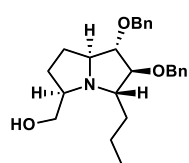
(3R,5S,6S,7S,8S)-(6,7-Bis-benzyloxy-5-propyl-hexahydro-pyrrolizin-3-yl)-methanol (70)

88% (2 steps); $[\alpha]_D^{21}$ -5.7 (c 1.0, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.37-7.28 (10H, m), 4.58 & 4.52 (2H, ABq, J = 12.0 Hz), 4.58 & 4.50 (2H, ABq, J = 12.0 Hz), 3.87 (1H, t, J = 3.2 Hz), 3.77-3.74 (2H, m), 3.58-3.54 (2H, m), 3.27-3.24 (1H, m), 3.16-3.13 (1H, m), 2.76 (1H, br), 1.97-1.86 (3H, m), 1.75-1.66 (1H, m), 1.58-1.29 (4H, m), 0.92 (3H, t, J = 7.2 Hz); ^{13}C NMR (125 MHz, CDCl$_3$): δ 138.17, 137.68, 128.43, 128.38, 127.83, 127.65, 127.63, 91.55, 88.66, 71.90, 71.67, 69.27, 62.26, 61.85, 61.75, 36.66, 29.40, 29.07, 19.86, 14.17; IR (neat): 3312, 2921, 2855 cm^{-1}; MS (EI) m/z 395 (M+); HRMS (EI) calcd for C$_{25}$H$_{33}$NO$_3$: 395.2460 (M+), found: 395.2459.
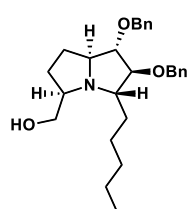
(3R,5S,6S,7S,8S)-(6,7-Bis-benzyloxy-5-pentyl-hexahydro-pyrrolizin-3-yl)-methanol (71)

87% (2 steps); $[\alpha]_D^{22}$ -6.5 (c 1.0, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.37-7.27 (10H, m), 4.58 & 4.52 (2H, ABq, J = 11.4 Hz), 4.58 & 4.49 (2H, ABq, J = 11.4 Hz), 3.87 (1H, t, J = 3.6 Hz), 3.77-3.74 (2H, m), 3.58-3.54 (2H, m), 3.27-3.23 (1H, m), 3.14-3.11 (1H, m), 2.72 (1H, br), 1.98-1.85 (3H, m), 1.75-1.66 (1H, m), 1.57-1.44 (2H, m), 1.39-1.28 (6H, m), 0.88 (3H, t, J = 6.8 Hz); ^{13}C NMR (125 MHz, CDCl$_3$): δ 138.18, 137.68, 128.43, 128.39, 127.87, 127.83, 127.64, 91.42, 88.66, 71.88, 71.68, 69.30, 62.28, 61.98, 61.86, 34.40, 31.93, 29.36, 29.09, 26.34,

22.69, 14.07; IR (neat): 3349, 2932, 2845 cm^{-1}; MS (EI) m/z 423 (M+); HRMS (EI) calcd for C$_{27}$H$_{37}$NO$_3$: 423.2773 (M+), found: 423.2775.
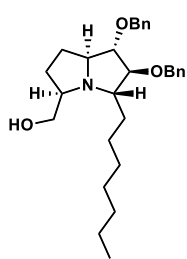
(3R,5S,6S,7S,8S)-(6,7-Bis-benzyloxy-5-heptyl-hexahydro-pyrrolizin-3-yl)-methanol (72)

94% (2 steps); [α]$_D^{26}$ -6.0 (c 1.0, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.37-7.28 (10H, m), 4.58 & 4.52 (2H, ABq, J = 12.0 Hz), 4.58 & 4.49 (2H, ABq, J = 12.0 Hz), 3.87 (1H, t, J = 2.9 Hz), 3.77-3.74 (2H, m), 3.58-3.55 (2H, m), 3.27-3.25 (1H, m), 3.14-3.10 (1H, m), 1.97-1.86 (3H, m), 1.73-1.68 (1H, m), 1.54-1.45 (2H, m), 1.35-1.26 (10H, m), 0.88 (3H, t, J = 7.2 Hz); ^{13}C NMR (125 MHz, CDCl$_3$): δ 138.16, 137.66, 128.44, 128.39, 127.87, 127.84, 127.67, 127.64, 91.37, 88.61, 71.88, 71.67, 69.30, 62.26, 62.02, 61.90, 34.41, 31.83, 29.68, 29.34, 29.31, 29.06, 26.68, 22.66, 14.09; IR (neat): 3323, 2923, 2854 cm^{-1}; MS (EI) m/z 451 (M+); HRMS (EI) calcd for C$_{29}$H$_{41}$NO$_3$: 451.3086 (M+), found: 451.3094.

General procedure for the synthesis of (73-75)

To a stirred solution of **70-72** (0.089 mmol) in CH$_2$Cl$_2$ (1.5 ml) was added BCl$_3$ (1.0 M in hexane, 0.45 ml, 0.445 mmol), at 0 °C, and the resulting mixture was stirred at room temperature for 9 h. The reaction was quenched with MeOH, and the solvents were evaporated. The residue was dissolved in MeOH (3 ml), added K$_2$CO$_3$ (62 mg, 0.445 mmol), and the resulting mixture was stirred at room temperature for 3 h. The resulting mixture was filtered off by Celite and the filtrate was evaporated. The residue was chromatographed on SiO$_2$ (CH$_2$Cl$_2$: MeOH = 1 : 1) to give **73-75**.
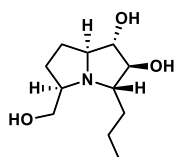
(1S,2S,3S,5R,8S)-5-Hydroxymethyl-3-propyl-hexahydro-pyrrolizine-1,2-diol (73)

100%; [α]$_D^{29}$ -2.2 (c 0.5, MeOH); ^{1}H NMR (500 MHz, Acetone-d$_6$): δ 3.99 (1H, t-like, J = 5.7 Hz), 3.89-3.87 (2H, m), 3.77-3.76 (1H, m), 3.60-3.58 (1H, m), 3.47-3.44 (1H, m), 2.09-1.77 (6H, m), 1.68-1.50 (2H, m), 1.04 (3H, t, J = 7.2 Hz); ^{13}C NMR (125 MHz, Acetone-d$_6$): δ 83.91, 82.13, 70.96, 64.82, 64.05, 61.47, 36.88, 29.04, 28.33, 20.04, 14.60; IR (neat): 3369 cm^{-1}; MS (EI) m/z 215 (M+); HRMS (EI) calcd for C$_{11}$H$_{21}$NO$_3$: 215.1521 (M+), found: 215.1513.
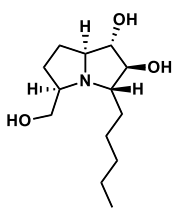
(1S,2S,3S,5R,8S)-5-Hydroxymethyl-3-pentyl-hexahydro-pyrrolizine-1,2-diol (74)

100%; $[\alpha]_D^{24}$ -2.1 (c 0.4, MeOH); ^1H NMR (500 MHz, Acetone-d_6): δ 4.00 (1H, t-like, J = 5.2 Hz), 3.99-3.83 (4H, m), 3.75-3.73 (1H, m), 3.58-3.54 (1H, m), 2.09-1.96 (3H, m), 1.92-1.84 (1H, m), 1.60-1.43 (2H, m), 1.35-1.28 (6H, m), 0.88 (3H, t, J = 6.9 Hz); ^{13}C NMR (125 MHz, Acetone-d_6): δ 82.37, 80.78, 72.30, 67.00, 66.16, 60.39, 33.31, 32.59, 27.60, 27.42, 26.63, 23.27, 14.41; IR (neat): 3369, 2922, 2854 cm^{-1}; MS (EI) m/z 243 (M+); HRMS (EI) calcd for $C_{13}H_{25}NO_3$: 243.1834 (M+), found: 243.1828.
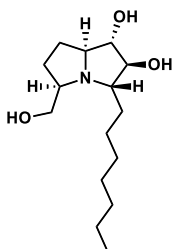
(1S,2S,3S,5R,8S)-3-Heptyl-5-hydroxymethyl-hexahydro-pyrrolizine-1,2-diol (75)

97%; $[\alpha]_D^{26}$ -2.3 (c 0.45, MeOH); ^1H NMR (500 MHz, Acetone-d_6): δ 5.40 (1H, br), 5.27 (1H, br), 4.89 (1H, br), 4.24-3.92 (6H, m), 3.77-3.73 (1H, m), 2.21-2.07 (4H, m), 1.59-1.51 (2H, m), 1.48-1.29 (10H, m), 0.87 (3H, t, J = 6.8 Hz); ^{13}C NMR (125 MHz, Acetone-d_6): δ 81.79, 80.25, 72.87, 67.84, 66.84, 59.91, 32.65, 32.52, 27.17, 27.08, 26.99, 26.93, 26.57, 23.29, 14.35; IR (neat): 3389, 2912, 2854 cm^{-1}; MS (EI) m/z 271 (M+); HRMS (EI) calcd for $C_{15}H_{29}NO_3$: 271.2147 (M+), found: 271.2153 .
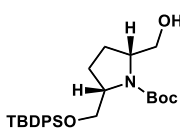
(2S,5R)2-(tert-Butyl-diphenyl-silanyloxymethyl)-5-hydroxymethyl-pyrrolidine-1-carboxylic acid tert-butyl ester (ent-50)

To a stirred solution of **48** (2.52 g, 9.22 mmol) in CH_2Cl_2 (30 ml) were added imidazole (753 mg, 11.1 mmol), TBSCl (1.53 g, 10.1 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 16 h. The reaction was quenched with H_2O and the aqueous mixture was extracted with CH_2Cl_2. The organic extracts were combined, washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was used for the next reaction without further purification.

To a stirred solution of the crude obtained above in MeOH (30 ml) was added K_2CO_3 (1.91 g, 13.8 mmol), and the resulting mixture was stirred for 50 min at room temperature. The solvent was removed, and the residue was dissolved in CH_2Cl_2, H_2O, and the aqueous mixture was extracted with CH_2Cl_2. The organic extracts were combined, dried over Na_2SO_4, and concentrated in vacuo. The residue was used for the next reaction without further purification.

To a stirred solution of the crude obtained above in CH_2Cl_2 (30 ml) were added

imidazole (871 mg, 12.8 mmol), TBDPSCl (2.8 ml, 11.0 mmol), and the resulting mixture was stirred at room temperature for 18 h. The reaction was quenched with H_2O and the aqueous mixture was extracted with CH_2Cl_2. The organic extracts were combined, washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was used for the next reaction without further purification.

  To a stirred solution of the crude obtained above in EtOH (30 ml) was added dropwise acetyl chloride (1.3 ml, 18.3 mmol) at 0 °C, and the resulting mixture was stirred at the same temperature for 2 h. The reaction mixture was neutralized with sat. $NaHCO_3$ aq and the aqueous mixture was extracted with CH_2Cl_2. The organic extracts were combined, dried over Na_2SO_4, and evaporated. The residue was chromatographed on SiO_2 (Hexane : EtOAc = 5 : 1) to give **ent-50** (2.61 g, 60%, 4 steps) as a colorless oil. $[\alpha]_D^{23}$ -2.5 (c 0.5, $CHCl_3$); Spectroscopical data identical to those of **50**.
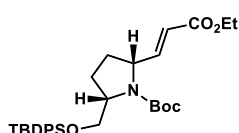
(2S,5R)-2-(tert-Butyl-diphenyl-silanyloxymethyl)-5-(2-ethoxycarbonyl-vinyl)-pyrrolidine-1-carboxylic acid tert-butyl ester (ent-52)

Preparation **ent-52** from **ent-50** is achieved by following the identical experimental procedure of **52** from **50**.
67% (2 steps); $[\alpha]_D^{25}$ +5.8 (c 1.2, MeOH) ; Spectroscopical data identical to those of **52**.
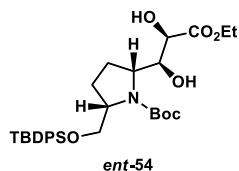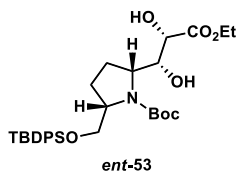
ent-53

ent-54

(2S,5R)-2-(tert-Butyl-diphenyl-silanyloxymethyl)-5-[(1R,2S)2-ethoxycarbonyl-1,2-dihydroxy-ethyl]-pyrrolidine-1-carboxylic acid tert-butyl ester (ent-53)
(2S,5R)-2-(tert-Butyl-diphenyl-silanyloxymethyl)-5-[(1S,2R)-2-ethoxycarbonyl-1,2-dihydroxy-ethyl]-pyrrolidine-1-carboxylic acid tert-butyl ester (ent-54)

Preparation **ent-53** and **ent-54** from **ent-52** is achieved by following the identical experimental procedure of **53** and **54** from **52**.
ent-53: 60%; $[\alpha]_D^{23}$ +1.6 (c 0.8, MeOH) ; Spectroscopical data identical to those of **53**.
ent-54: 34%;$[\alpha]_D^{27}$ +0.93 (c 0.5, MeOH) ; Spectroscopical data identical to those of **54**.

(2R,5S)-2-[(1R,2S)-1,2-Bis-benzyloxy-2-ethoxycarbonyl-ethyl]-5-(tert-butyl-diphenyl-silanyloxymethyl)-pyrrolidine-1-carboxylic acid tert-butyl ester (ent-55)

Preparation *ent*-**55** from *ent*-**53** is achieved by following the identical experimental procedure of **55** from **53**.

82%; $[\alpha]_D^{26}$ +4.5 (c 0.75, MeOH); Spectroscopical data identical to those of **55**.
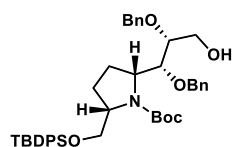
(2R,5S)-2-[(1R,2R)-1,2-Bis-benzyloxy-3-hydroxy-propyl)-5-(*tert*-butyl-diphenyl-silanyloxymethyl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (*ent*-58)

Preparation *ent*-**58** from *ent*-**55** is achieved by following the identical experimental procedure of **58** from **55**.

95%; $[\alpha]_D^{25}$ +20.1 (c 0.9, CHCl$_3$); Spectroscopical data identical to those of **58**.
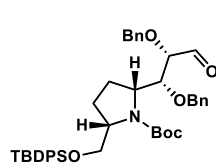
(2R,5S)-2-[(1R,2S)-1,2-Bis-benzyloxy-3-oxo-propyl)-5-(*tert*-butyl-diphenyl-silanyloxymethyl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (*ent*-59)

Preparation *ent*-**59** from *ent*-**58** is achieved by following the identical experimental procedure of **59** from **58**.

98%; ^{1}H NMR data identical to those of **59**.

(2R,5S)-2-[(1R,2R)-1,2-Bis-benzyloxy-4-ethoxycarbonyl-but-3-enyl)-5-(*tert*-butyl-diphenyl-silanyloxymethyl)-Pyrrolidine-1-carboxylic acid *tert*-butyl ester (*ent*-60)

Preparation *ent*-**60** from *ent*-**59** is achieved by following the identical experimental procedure of **60** from **59**.

98%; $[\alpha]_D^{24}$ +3.3 (c 1.4, CHCl$_3$); Spectroscopical data identical to those of **60**.
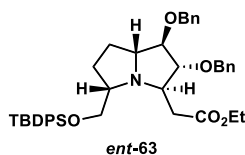
(1R,2R,3R,5S,8R)-[1,2-Bis-benzyloxy-5-(*tert*-butyl-diphenyl-silanyloxymethyl)-hexahydro-pyrrolizin-3-yl]-acetic acid (*ent*-63)

(1R,2R,3R,5S,8R)-(1,2-Bis-benzyloxy-5-hydroxymethyl-hexahydro-pyrrolizin-3-yl)-acetic acid ethyl ester (*ent*-62)

Preparation *ent*-**63** and *ent*-**62** from *ent*-**60** is achieved by following the identical experimental procedure of **63** and **62** from **60**.

ent-63: 72%; $[\alpha]_D^{26}$ -1.5 (c 0.75, CHCl$_3$) ; Spectroscopical data identical to those of **63**.

ent-62: 26%; $[\alpha]_D^{25}$ +5.4 (c 0.25, CHCl$_3$) ; Spectroscopical data identical to those of **62**.
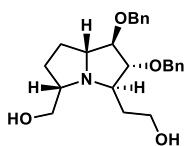
(1R,2R,3R,5S,8R)-2-(1,2-Bis-benzyloxy-5-hydroxymethyl-hexahydro-pyrrolizin-3-yl)-ethanol (ent-64)

Preparation **ent-64** from **ent-62** is achieved by following the identical experimental procedure of **64** from **62**.

71%; $[\alpha]_D^{25}$ +12.3 (c 0.55, CHCl$_3$); Spectroscopical data identical to those of **64**.
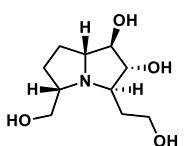
(1R,2R,3R,5S,8R)-3-(2-Hydroxy-ethyl)-5-hydroxymethyl-hexahydro-pyrrolizine-1,2-diol (ent-65)

Preparation **ent-65** from **ent-64** is achieved by following the identical experimental procedure of **65** from **64**.

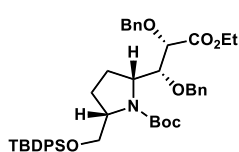69%; $[\alpha]_D^{22}$ +5.3 (c 0.2, MeOH); Spectroscopical data identical to those of **65**.
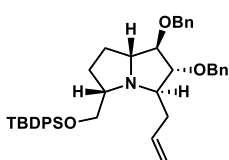
(1R,2R,3R,5S,8R)-3-Allyl-1,2-bis-benzyloxy-5-(tert-butyl-diphenyl-silanyloxymethyl)-hexahydro-pyrrolizine (ent-67)

Preparation **ent-67** from **ent-63** is achieved by following the identical experimental procedure of **67** from **63**.

77%; $[\alpha]_D^{26}$ +4.3 (c 1.0, CHCl$_3$); Spectroscopical data identical to those of **67**.
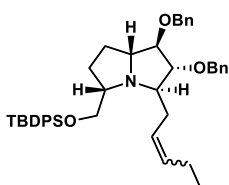
(1R,2R,3R,5S,8R)-1,2-Bis-benzyloxy-5-(tert-butyl-diphenyl-silanyloxymethyl)-3-pent-2-enyl-hexahydro-pyrrolizine (ent-68)

Preparation **ent-68** from **ent-63** is achieved by following the identical experimental procedure of **68** from **63**.

78%; ^{1}H NMR data identical to those of **68**.
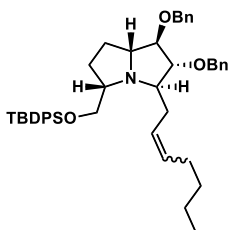
(1R,2R,3R,5S,8R)-1,2-Bis-benzyloxy-5-(tert-butyl-diphenyl-silanyloxymethyl)-3-hept-2-enyl-hexahydro-pyrrolizine (ent-69)

Preparation **ent-69** from **ent-63** is achieved by following the identical experimental procedure of **69** from **63**.

64%; ^{1}H NMR data identical to those of **69**.

(3S,5R,6R,7R,8R)-(6,7-Bis-benzyloxy-5-propyl-hexahydro-pyrrolizin-3-yl)-methanol (ent-70)

Preparation **ent-70** from **ent-67** is achieved by following the identical experimental procedure of **70** from **67**.

96% (2steps); $[\alpha]_D^{27}$ +5.6 (c 0.9, CHCl$_3$); Spectroscopical data identical to those of **70**.

(3*S*,5*R*,6*R*,7*R*,8*R*)-(6,7-Bis-benzyloxy-5-pentyl-hexahydro-pyrrolizin-3-yl)-methanol (*ent*-71)

Preparation *ent*-71 from *ent*-68 is achieved by following the identical experimental procedure of **71** from **68**.
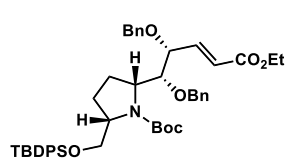
87% (2 steps); $[\alpha]_D^{24}$ +6.2 (c 1.0, CHCl$_3$); Spectroscopical data identical to those of **71**.
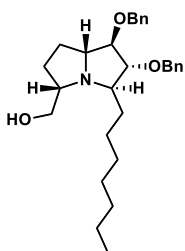
(3*S*,5*R*,6*R*,7*R*,8*R*)-(6,7-Bis-benzyloxy-5-heptyl-hexahydro-pyrrolizin-3-yl)-methanol (*ent*-72)

Preparation *ent*-72 from *ent*-69 is achieved by following the identical experimental procedure of **72** from **69**.
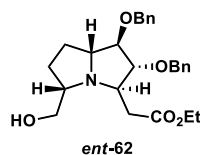
73% (2 steps); $[\alpha]_D^{26}$ +6.9 (c 1.0, CHCl$_3$); Spectroscopical data identical to those of **72**.
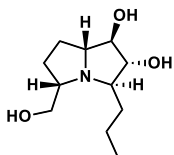
(1*R*,2*R*,3*R*,5*S*,8*R*)-5-Hydroxymethyl-3-propyl-hexahydro-pyrrolizine-1,2-diol (*ent*-73)

Preparation *ent*-73 from *ent*-70 is achieved by following the identical experimental procedure of **73** from **70**.

100%; $[\alpha]_D^{24}$ +2.0 (c 0.5, MeOH); Spectroscopical data identical to those of **73**.
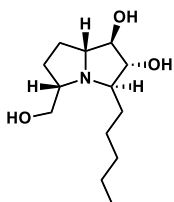
(1*R*,2*R*,3*R*,5*S*,8*R*)-5-Hydroxymethyl-3-pentyl-hexahydro-pyrrolizine-1,2-diol (*ent*-67)

Preparation *ent*-74 from *ent*-71 is achieved by following the identical experimental procedure of **74** from **71**.

100%; $[\alpha]_D^{26}$ +2.3 (c 0.35, MeOH); Spectroscopical data identical to those of **74**.
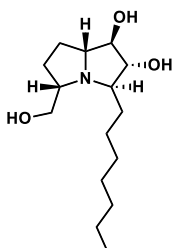
(1*R*,2*R*,3*R*,5*S*,8*R*)-3-Heptyl-5-hydroxymethyl-hexahydro-pyrrolizine-1,2-diol (*ent*-75)

Preparation *ent*-75 from *ent*-72 is achieved by following the identical experimental procedure of **75** from **72**.
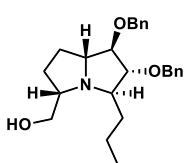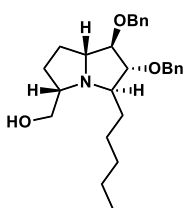
88%; $[\alpha]_D^{25}$ +2.4 (c 0.3, MeOH); Spectroscopical data identical to those of **75**.

GLP-1 secretion studies *in vitro*[49)]

Human enteroendocrine NCI-H716 cells were obtained from the American Type Culture Collection (Manassas, VA, U.S.A.), and were maintained in suspension culture as instructed by the supplier. Two days before each experiment, the cells were seeded in 96-well plates pre-coated with poly-L-lysine (1×10^5 cells /well). On the day of the experiment, the culture medium was replaced by assay buffer (146 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgSO_4$, 20 mM *N*-(2-hydroxyethyl)-piperazine-*N*-(2-ethanesulfonic acid) (HEPES), 5.6 mM glucose, 2 mg/ml bovine serum albumin, and 10 mM sitagliptin, pH 7.4) with or without test agents, and the cells were incubated for 1 hour at 37 °C. Then the GLP-1 level in the assay buffer was measured by ELISA (LINCO, Billerica, MA, U.S.A.).

Acute and long-term effect of 9 and 30 on blood glucose *in vivo*

This study was approved by the Animal Care and Use Committee of Ajinomoto. Male C57BL/6J and KKAy mice were purchased from Clea Japan (Tokyo, Japan) at 5 weeks of age, and each mouse was housed in a polycarbonate cage with wood chip bedding. Water and commercial chow were provided ad libitum. The animal room was kept on a 12-hour light/dark cycle (7:00 AM to 7:00 PM, dark; 7:00 PM to 7:00 AM, light), with a temperature range of 22°C ± 1°C and a relative humidity of 55% ± 5% throughout the experimental period. The animals were acclimatized to the laboratory condition for 4 weeks.

For evaluation of acute hypoglycemic effect of **9** and **30**, C57BL/6J mice were fasted overnight, and were administered either the vehicle (0.5% methylcellulose), or 100 mg/kg of **9** and **30** by oral gavage with or without subcutaneous injection of GLP-1 antagonist exendin (9-39) (24 nmol/kg). Then, 2 g/kg of glucose was given orally immediately after **9** and **30** administration. Blood samples were collected from the tail vein to measure the blood glucose levels.

For evaluation of long-term effect of **9** and **30** on blood glucose control, KKAy mice were divided into 4 groups, and either the vehicle, **9** and **30** (100 mg/kg), sitagliptin (10 mg/kg), or pioglitazone (10 mg/kg) was administered orally twice a day for 3 weeks. At the end of the study, blood samples were collected from the tail vein and the blood glucose, HbA1c, plasma insulin, and plasma glucagon levels were measured. Blood glucose was measured with an autoanalyzer (Fuji Dri-Chem 5500; Fujifilm, Tokyo, Japan). Plasma insulin was measured by ELISA (Morinaga, Tokyo, Japan). Plasma glucagon was also measured by ELISA (Yanaihara Institute Inc., Shizuoka, Japan).

HbA1c was measured by HPLC (TOSOH, Tokyo, Japan).

Statistical analysis

Results are expressed the mean±SEM. Statistical analysis was performed with StatView software (version 5.0, SAS institute, Cary, NC, USA). Differences were evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's test. Statistical significance was accepted at p<0.05.

Several glycosidases inhibitory assay[73]

The enzymes β-glucosidase (bovine liver), α-galactosidase (from coffee beans), β-galactosidase (from bovine liver), α-mannosidase (from jack bean), β-mannosidase (from snail), α-L-fucosidase (from bovine kidney), *p*-nitrophenyl glycosides, and various disaccharides were purchased from Sigma-Aldrich Co. Glycosidase inhibiting activities were determined by our previous methods.

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