高リン血症治療薬の開発を目的とした
腸管選択的 NaPi2b 阻害物質の創薬研究

2023 年

宇敷　康信

目次

序論 1

本論 8

第 1 章　チオフェン骨格の NaPi2b 阻害物質の研究

第1節　ドラッグデザイン.....8

第2節　チオフェン化合物（**15at-36ct**）の合成.....10

第3節　チオフェン化合物（**15at-36ct**）のNaPi2b阻害活性.....15

第4節　チオフェン化合物（**5t**、**36at**、**36bt**）の薬物動態.....19

第5節　チオフェン化合物（**36at**、**36bt**）のリン吸収抑制作.....20

第6節　まとめ.....22

第2章　ピリジン骨格のNaPi2b阻害物質の研究

第1節　ドラッグデザイン.....23

第2節　ピリジン化合物（**10ap-20bp**）の合成.....25

第3節　ピリジン化合物（**10ap-20bp**）のNaPi2b阻害活性.....29

第4節　ピリジン化合物（**19p**、**20bp**）の薬物動態.....32

第5節　ピリジン化合物（**20bp**）のリン吸収抑制作.....34

第6節　まとめ.....35

第3章　インドール骨格のNaPi2b阻害物質の研究

第1節　ドラッグデザイン.....36

第2節　インドール化合物（**5i-27i**）の合成.....38

第3節　インドール化合物（**5i-27i**）のNaPi2b阻害活性.....43

第4節　インドール化合物（**5i、27i**）の薬物動態.....46

第5節 インドール化合物（**5i、27i**）のリン吸収抑制作用.....48

第6節　まとめ.....50

結論 51

謝辞 54

実験の部 55

参考文献 112

主論文目録 117

論文審査の主査及び副査名 118

略語表

本論文において以下に示す略語及び略号を用いた。

Ac	acetyl
APCI	atmospheric pressure chemical ionization
Ar	aryl
Bn	Benzyl
Boc	*tert*-butoxycarbonyl
$CHCl_3$	chloroform
CKD	chronic kidney disease
DFT	density functional theory
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
EDC・HCl	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide Hydrochloride
ELSD	evaporative light-scattering detector
ESI	electrospray ionization
Et	ethyl
EtOAc	ethyl acetate
F	bioavailability
FaSSIF	fasted state simulated intestinal fluid
FeSSIF	fed state simulated intestinal fluid
FGF23	Fibroblast growth factor 23
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate
HCl	hydrochloride
$HOBt \cdot H_2O$	1,2,3-benzotriazol-1-ol monohydrate
HPLC	high performance liquid chromatography
HR-MS	high-resolution mass spectrometry
IC_{50}	half-maximal inhibitory concentration

IPE	diisopropyl ether
*i*Pr	isopropyl
LCMS	liquid chromatography mass spectrometry
Me	methyl
MeCN	acetonitrile
$NaBH(OAc)_3$	sodium triacetoxyborohydride
NaI	sodium iodide
NaOH	sodium hydroxide
NaPi2b	Sodium-dependent transport protein 2b (SLC34A2)
n-BuLi	*n*-butyllithium
NMR	nuclear magnetic resonance
PK	pharmacokinetics
PTH	parathyroid hormone
Ph	phenyl
Pr	propyl
SAR	structure–activity relationship
SD rats	Sprague-Dawley rats
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMS	tetramethylsilane
tPSA	topological polar Surface Area

序論

慢性腎臓病における高リン血症

リンは食事中に豊富に含まれ、1 日の摂取量は 1200mg ほどに達する。摂取されたリンの約 80%が小腸から吸収され、吸収されたリンのほとんどが腎臓から尿中へと排泄される。生体内のリンは主に骨に貯蔵されており、細胞外液に存在するリンは全体の 1 %以下と考えられている。小腸からの吸収、腎臓からの排泄、骨からの流出入のバランスにより、生体内のリンの恒常性が維持されている（**Figure 1**）[1)]。

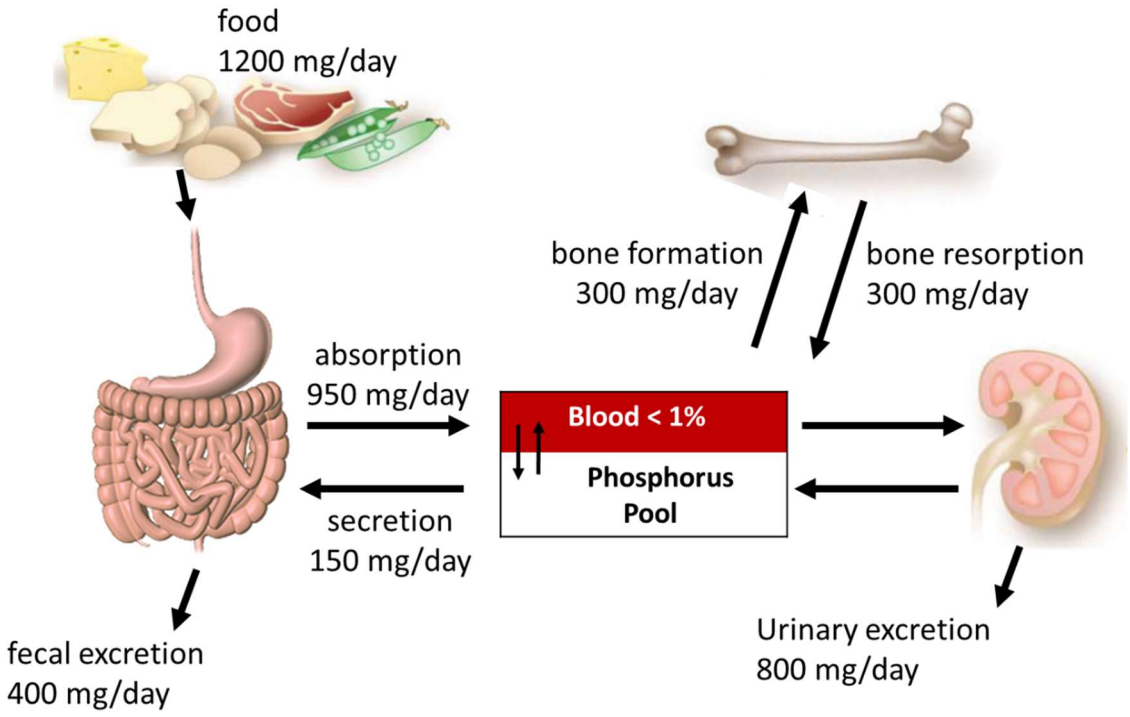


Figure 1. Phosphorus balance in normal physiology（文献 1 を一部改変）

リンの除去は腎臓による尿中排泄に依存しているため、腎機能が低下した場合には体内にリンが滞留する。軽度の腎機能低下であれば、Fibroblast growth factor 23（FGF23）あるいは Parathyroid hormone（PTH）などのリン利尿因子が代償的に増加し、尿へのリン排泄量を維持することで、腎機能低下による血清リン濃度の上昇を抑制することができる。一方で、腎機能が極度に低下した慢性腎不全（Chronic kidney disease、CKD）や腎機能が廃絶した透析患者におい

ては、リン利尿因子による代償作用も破綻し、高リン血症が発症する [2]。腎機能の廃絶により発症した高リン血症は、FGF23 や PTH の分泌亢進とも関連し、骨代謝異常、異所性石灰化などを合併する。このような、CKD に付随して起こる全身性の骨・ミネラル代謝異常や血管石灰化を含む病態は、CKD-Mineral and Bone Disorder（CKD-MBD）と定義される [3]。日本透析医学会のガイドライン「慢性腎臓病に伴う骨・ミネラル代謝異常の診療ガイドライン」では、CKD-MBD の治療予後の検討に基づき、高リン血症を優先して是正することが推奨されている（**Figure 2**）[4]。

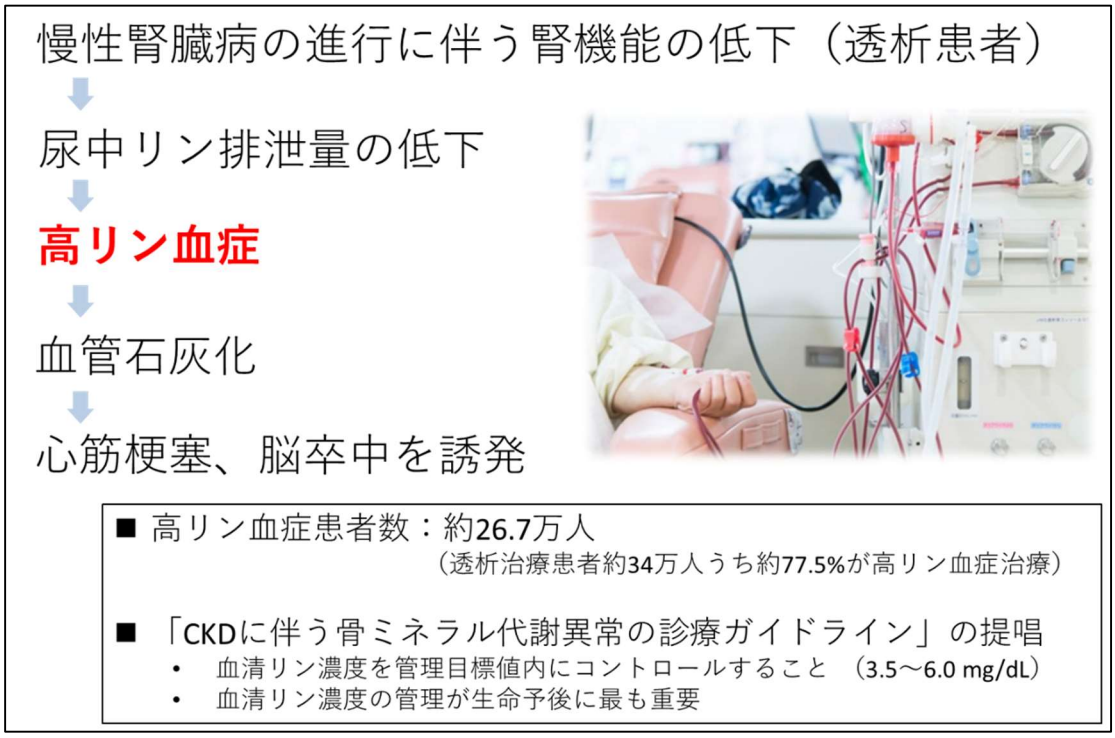


Figure 2. 高リン血症

腎機能が廃絶している透析患者の場合、透析によるリンの除去が行われるが、除去できるリンの量は十分ではなく、食事療法によるリン摂取制限が課せられる。しかし、食事から摂取されるリンは摂取蛋白質量と正の相関を示すことから、リン制限食は患者の栄養状態を悪化させ、逆に予後不良となる可能性がある。このように、透析及び食事制限のみではリンの適切なバランスを維持できず、高リン血症是正のためのリン吸着薬を用いた薬物治療が必要となる [5]。使用可能なリン吸着薬は複数種類あり、金属系リン吸着薬と樹脂系リン吸着薬に

大別できる。いずれの薬剤も消化管内でリンを吸着し、糞便とともにリンを体外へ排泄させる薬剤であるが、概して服薬量が多いという問題点を抱えている[6]。服薬量の多さは飲水制限のある透析患者においては重要な問題であり、リン吸着薬療法における服薬アドヒアランスの悪化と関連する[7]。また、金属系の吸着薬では金属が一部吸収され、体内蓄積することで、長期安全性の懸念がある。特に、カルシウム含有製剤ではカルシウム非含有製剤と比較して、全死亡率が増加することが報告されている[8]。更に、金属系、樹脂系薬剤ともに下痢あるいは便秘を中心とする消化器系の副作用が報告されている。特に樹脂系薬剤は、消化管内の水分を吸収し膨潤するため、腹部膨満感などの消化管症状が生じやすい[9]。そのため、新規高リン血症治療薬には、安全性面、利便性面が改善された、アドヒアランスの良い薬物が望まれている（**Figure 3**）。

薬剤 （成分名）	タイプ	安全性の懸念	1日最低～最高用量 （服用数）	製剤
カルタン	金属系	血中Ca上昇	3 g （6錠）	
ホスレノール		成分の体内蓄積 嘔吐 悪心	0.75-2.25 g （3-6錠）	
リオナ		鉄過剰 下痢	1.5～6 g （6-24錠）	
ピートル		鉄過剰 下痢	0.75～3 g （3-6錠）	
レナジェル/ フォスブロック	樹脂系	便秘 腹部膨満	3～9 g （12-36錠）	
キックリン		便秘	1.5～7.5 g （6-30ｶﾌﾟｾﾙ）	

Figure 3. リン吸着薬

ナトリウム依存性リン酸輸送タンパク質 2b（SLC34A2: NaPi2b）

腸管からのリンの吸収経路は受動拡散による吸収とトランスポーターを介した吸収に大別され、トランスポーターを介した吸収はナトリウム依存的な経路とナトリウム非依存的な経路が存在する。ナトリウム依存性リン酸輸送タンパク質 2b は、消化管でのリン酸吸収において主要な役割を担っていると考えら

れており、ノックアウトマウス（腸管特異的コンディショナルノックアウト）を用いた研究から、腸管でのリン酸吸収の約 40%を担っていると報告されている [10),11)]。従って、腸管において NaPi2b を阻害する化合物は、消化管からのリン吸収を阻害することで高リン血症を是正できる可能性がある。また前述したように、リン吸着薬にはさまざまな副作用が認められているが、NaPi2b 阻害物質はリン吸着薬とは異なる新規メカニズムの分子標的薬であることから、リン吸着薬の副作用を回避しつつ、服薬量も低減して、腎不全患者の服薬アドヒアランスを改善できる可能性がある（**Figure 4**）。

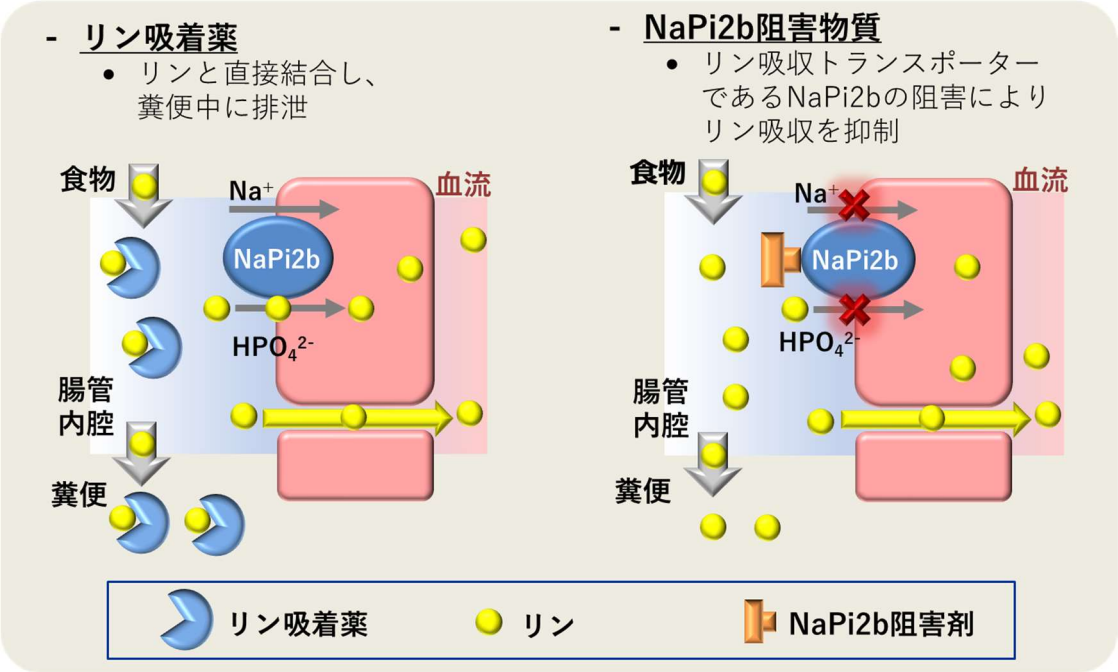


Figure 4. リン吸着薬と NaPi2b 阻害物質の作用機序

しかし、NaPi2b は腸管以外でも発現が確認されており、特に肺においては NaPi2b の機能不全型変異と肺胞微石症との関連が示唆されている [12),13)]。また、精巣微小石灰化症患者では、NaPi2b のヘテロ接合性変異が確認されている [14)]。以上のことから、低経口吸収性で全身曝露を可能な限り低減させ、腸管における NaPi2b を選択的に阻害する化合物が望ましいと考えられる。

これまでに幾つかの化合物が NaPi2b を阻害する化合物として報告されている（**Figure 5**）。Ardelyx のグループは、全身曝露の少ない腸管選択的な NaPi2b 阻害剤を報告しているが [15)]、その log half-maximal inhibitory concentration（pIC_{50}）

値は約 6 である（化合物 **1**）。アステラス製薬及び REO Pharma のグループは、NaPi2b 阻害剤として化合物 **2a**、**2b** 及び化合物 **3** を報告しているが、これらの化合物が腸管選択的であることは示されていない [16),17),18)]。**ASP3325**（アステラス製薬の全身曝露型 NaPi2b 阻害剤、構造不明）の臨床試験では、健常人及び高リン血症の CKD 患者のいずれにおいても有意な臨床効果が得られなかったため中止された [19)]。前臨床評価では良好な結果が得られていたにも関わらず、**ASP3325** の臨床効果が得られなかった理由は明らかにされていないが、臨床試験デザインの要因、種差、腸管組織への曝露不足、全身への曝露の影響、細胞間隙からのリン吸収の影響などが考えられる。また最近では、協和キリン（株）が腸管選択的な 4,5,6,7-tetrahydrobenzo[b]thiophene 誘導体の前臨床における検討結果を報告している [20),21)]。 このように幾つかの化合物が検討されているが、良好な NaPi2b 阻害活性と腸管選択性（低い全身曝露）を両立している化合物は乏しく、腸管選択的 NaPi2b 阻害物質の高リン血症治療薬としての可能性は十分に検討されていない。

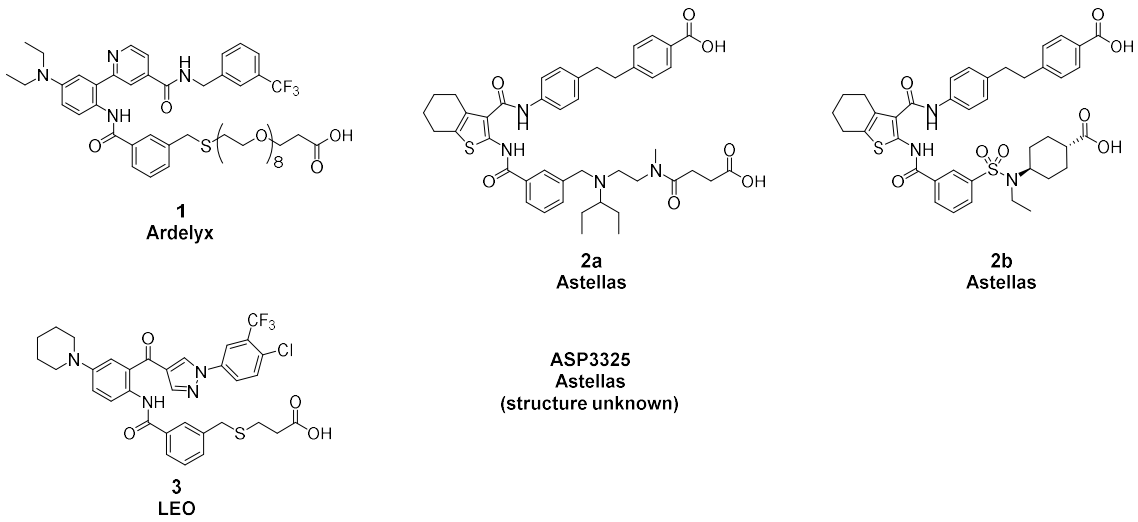


Figure 5. NaPi2b 阻害物質

本研究の目的と概要

リン吸着薬とは異なる作用機序の高リン血症治療薬を創出すべく、良好な NaPi2b 阻害活性と腸管選択性を両立した化合物の創出を目指して研究を実施した（**Figure 6**）。既知の NaPi2b 阻害物質を参考に、水素結合などの分子内相互作用を有するコア骨格について合成展開することで、新規化合物であるチオフェン誘導体、ピリジン誘導体、インドール誘導体を見出し、その高リン血症治

療薬としての可能性について検討した。

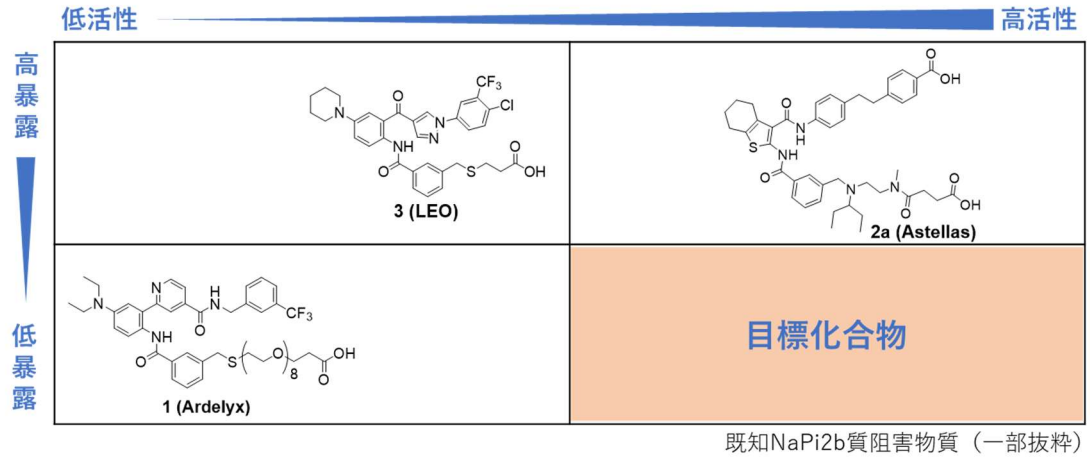


Figure 6. 目標化合物のイメージ

第 1 章ではチオフェン骨格の化合物の検討結果について述べる。NaPi2b 阻害活性を指標に構造活性相関を探索し、優れた NaPi2b 阻害活性を示す化合物 **5t** を取得した。次に経口吸収性を低下させるため、極性表面積（topological Polar Surface Area、tPSA）の増加を目指し、高極性置換基の導入を検討した。一般的に tPSA と経口吸収性には負の相関があることが報告されている。その結果、化合物 **5t** のカルボン酸部位において様々な高極性置換基の導入が許容されることを見出し、良好な NaPi2b 阻害活性を有し、Sprague-Dawley ラット（SD ラット）においてバイオアベイラビリティ（F）が低く低吸収性を示す化合物 **36at** 及び **36bt** を取得した。しかし、化合物 **36at**、**36bt** は SD ラットを用いた in vivo 薬効試験においてリン吸収抑制効果を示さなかった。化合物の薬物動態や物性を精査した結果、高い疎水性（高い CLogP 値）が原因で腸管表面近傍の非攪拌水層の透過性が低下すること等により、腸管上皮細胞管腔側に存在する NaPi2b へのアクセシビリティが低下する可能性が一因として考えられた。

第 2 章ではピリジン骨格の化合物の検討結果について述べる。第 1 章の考察に基づいて、疎水性を低減した新たなコア構造を探索し、ピリジン骨格を有する誘導体を見出した。NaPi2b 阻害活性を指標に構造活性相関を探索し、良好な NaPi2b 阻害活性を示す化合物 **5p** を見出した。次に経口吸収性を低下させるために、tPSA を増加させる高極性置換基の導入を検討した結果、ピリジン骨格の化合物についてもカルボン酸部位において様々な高極性置換基の導入が許容されることを見出し、化合物 **20bp** を創出した。化合物 **20bp** の SD ラットにおけ

るリン吸収抑制作用を評価した結果、10 mg/kg の経口投与において、有意に腸管からのリン吸収を抑制した。この結果から、化合物の疎水性が in vivo 薬効に影響を及ぼす可能性が示された。

第 3 章ではインドール骨格の化合物の検討結果について述べる。第 2 章では、ラットにおいてリン吸収抑制作用を示すピリジン骨格の化合物を見出した。本章では、より低い経口吸収性で in vivo 薬効を示す化合物の取得を目指して検討した結果、インドール骨格を有する誘導体を見出した。NaPi2b 阻害活性を指標に構造活性相関を探索し、良好な NaPi2b 阻害活性を示す化合物 **5i** を見出した。次に腸管からの吸収性を低下させるために、tPSA を増加させる高極性置換基の導入を検討した結果、インドール骨格の化合物についてもカルボン酸部位において様々な高極性置換基の導入が許容されることを見出し、化合物 **27i** を創出した。化合物 **27i** の SD ラットにおけるリン吸収抑制作用を評価した結果、10 mg/kg の経口投与において、有意に腸管からリン吸収を抑制した。化合物 **27i** は、最も低いバイオアベイラビリティを示したことから、非常に低い経口吸収性でラットにおいてリン吸収抑制作用を示す化合物であると考えられた。

本論

第 1 章　チオフェン骨格の NaPi2b 阻害物質の研究

第 1 節　ドラッグデザイン

ドラッグデザインをするにあたり、既知 NaPi2b 阻害剤 **2a** のコア構造について、density functional theory（DFT）により算出した最安定コンフォメーションを参考にした。算出された最安定コンフォメーションにおける原子間距離の解析から、コア構造は O···HN の水素結合と O···S 相互作用の 2 種類の分子内相互作用を形成していることが示唆された [22]。分子内相互作用によりコア構造が固定化されていることが NaPi2b 阻害活性の発現に重要である可能性が示唆されたため、その維持が期待できるコア骨格（core structure of **15at**）をデザインした。このコア骨格についても、DFT により算出した最安定コンフォメーションで 2 種類の分子内相互作用を形成していることが示唆されたため、このコア骨格を起点に誘導体合成を展開した（**Figure 1t**）。

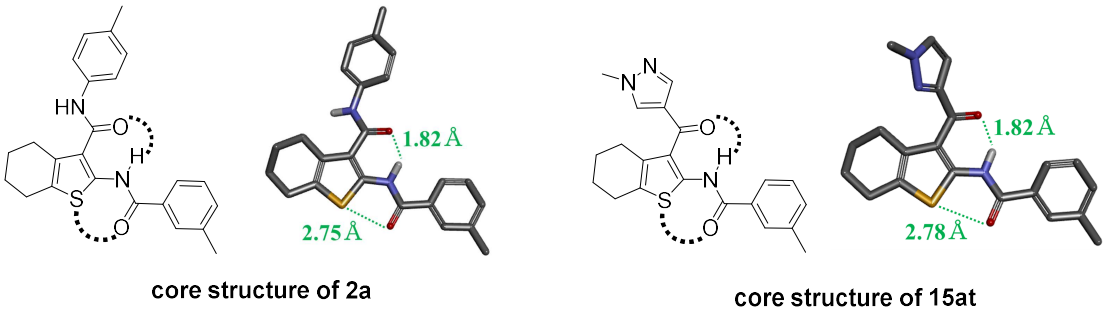


Figure 1t. The most stable conformations of core structures of these compounds based on DFT calculations in a water environment.[23),24)] The calculated O···H and O···S interatomic distances of these conformations are also shown. The estimated intramolecular hydrogen bond and O···S interaction are shown by the dotted lines.

誘導体の合成戦略を **Figure 2t** に示す。core structure の R^{1t}、R^{2t} 及び X 部位について、NaPi2b 阻害活性を指標に構造活性相関を探索し、活性向上を図りつつ、tPSA を増加させて経口吸収性の低減が期待できる高極性基（highly polar

functional group）が導入可能な部位を探索した。一般に、tPSA が 140 Å^2 を超える薬物は低い経口吸収性を示すことが報告されていることから [25),26)]、誘導体合成において、経口吸収性を予測するパラメータとして使用した。

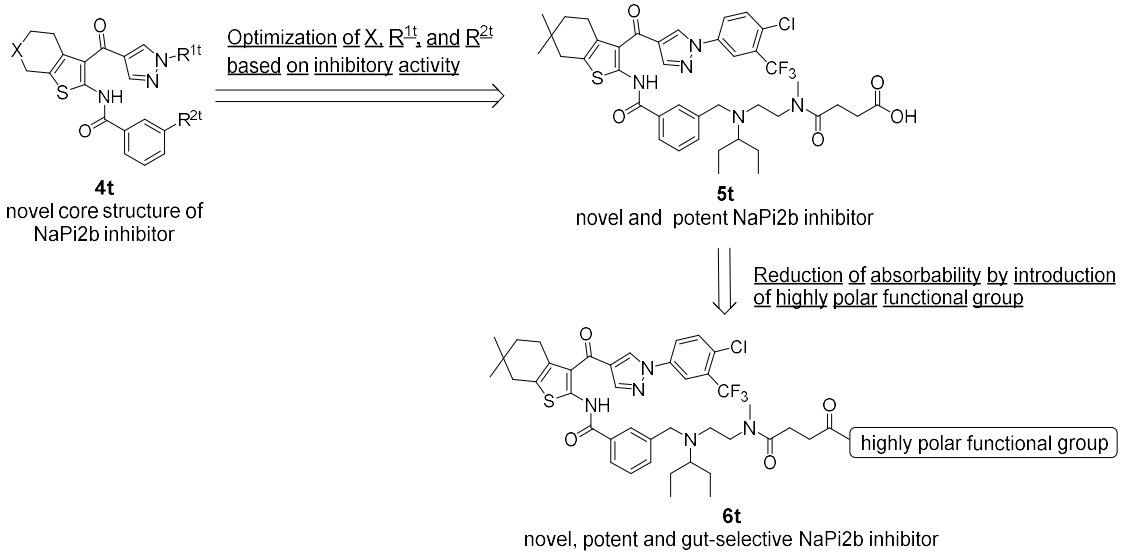
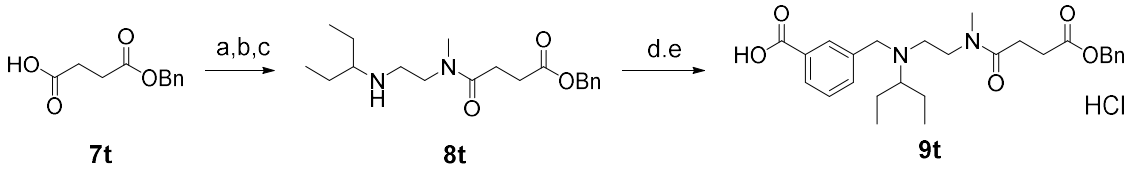


Figure 2t. 誘導体の合成戦略

第 2 節　チオフェン化合物（**15at-36ct**）の合成

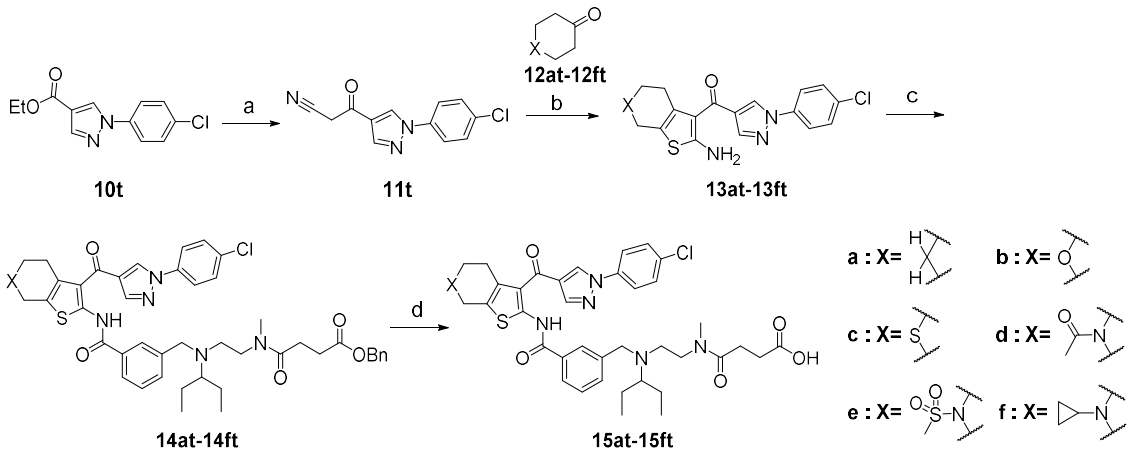
チオフェン化合物の合成法を **Scheme 1t-8t** に示す。化合物 **7t** を *tert*-butyl [2-(methylamino)ethyl]carbamate と縮合した後、*tert*-ブチルカルボニル（Boc） 基を塩酸で脱保護して得られたアミン塩酸塩を pentan-3-one を用いて還元的アミノ化し、化合物 **8t** を得た。化合物 **8t** を *tert*-butyl 3-(chloromethyl)benzoate でアルキル化した後、塩酸を加えて *tert*-ブチル基を除去し、中間体 **9t** を得た（**Scheme 1t**）。



7t　　**8t**　　**9t**

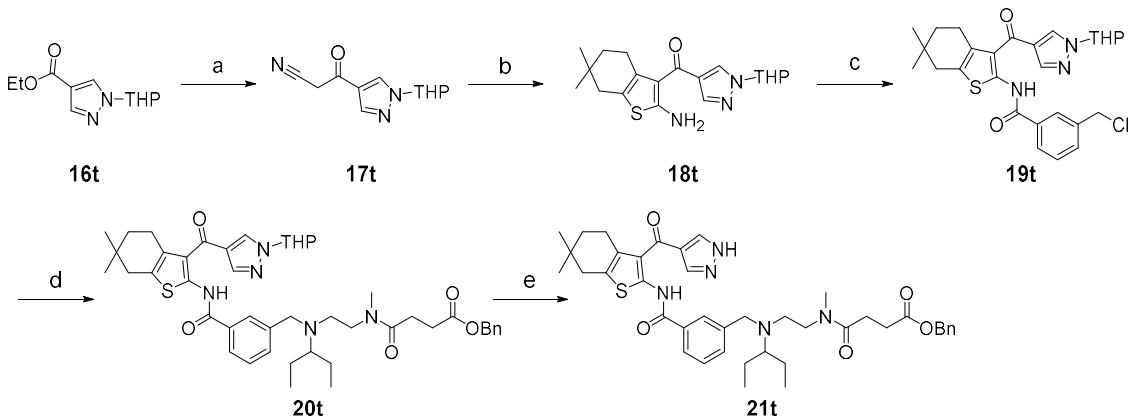
Scheme 1t. Preparation of benzoic acid **9t**. Reagents and conditions: (a) *tert*-butyl [2-(methylamino)ethyl]carbamate, EDC, HOBt, MeCN, rt; (b) 4M-HCl/dioxane, rt; (c) pentan-3-one, AcOH, $NaBH(OAc)_3$, rt, 31% in 3 steps; (d) *tert*-butyl 3-(chloromethyl)benzoate, DIPEA, NaI, MeCN, 85 °C; (e) 4M-HCl/dioxane, rt, 89% in 2 steps.

アセトニトリルと *n*-Butyllithium （*n*-BuLi） から調製したリチウム試薬に化合物 **10t** を加えて化合物 **11t** を得た。化合物 **13at-13ft** は、**11t** に硫黄と化合物 **12at-12ft** を用いて反応させることにより合成した。安息香酸 **9t** を塩化チオニル（$SOCl_2$）で酸クロリドにし、その後化合物 **13at-13ft** とアミド化することで化合物 **14at-14ft** が得た。その後、水酸化ナトリウム（NaOH）水溶液で加水分解することにより、化合物 **15at-15ft** を合成した（**Scheme 2t**）。



Scheme 2t. Preparation of **15at-15ft**. Reagents and conditions: (a) MeCN, *n*-BuLi, THF, -78 °C, 76%; (b) **12at-12ft** sulfur, morpholine, EtOH, 80 °C, 53%-84%; (c) **9t**, $SOCl_2$, $CHCl_3$, 60 °C -75 °C, then **13at-13ft**, DIPEA, $CHCl_3$, rt, 50%-quantitative yield; (d) 1M-4M-NaOH aq., THF, rt-60 °C, 33%-84%.

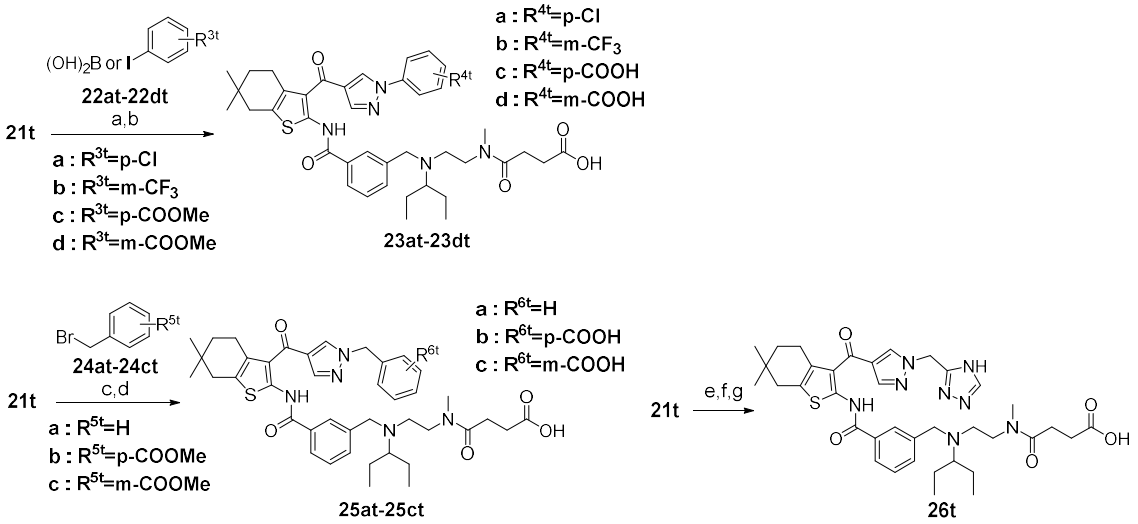
化合物 **13at** の合成と同様の方法で、化合物 **16t** から **17t** を合成後 4,4-dimethylcyclohexan-1-one、硫黄と反応させることにより化合物 **18t** を合成した。化合物 **18t** を 3-(chloromethyl)benzoyl chloride でアミド化し、化合物 **19t** を得た。続いて、化合物 **8t** を化合物 **19t** でアルキル化することにより化合物 **20t** を得て、その後、テトラヒドロピランを酸で除去して **21t** を得た（**Scheme 3t**）。



Scheme 3t. Preparation of **21t**. Reagents and conditions: (a) MeCN, *n*-BuLi, THF, -67 °C, 84%; (b) 4,4-dimethylcyclohexan-1-one, sulfur, ethylenediamine, EtOH, 80 °C, 70%; (c) 3-(chloromethyl)benzoyl chloride, pyridine, $CHCl_3$, rt, 70%; (d) **8t**, DIPEA, NaI, MeCN, reflux, 51%; (e)TFA, 1,4-dioxane, H_2O, 60 °C, 59%.

化合物 **21t** と **22at-22dt** を用いてカップリング反応を実施後、ベンジルエステ

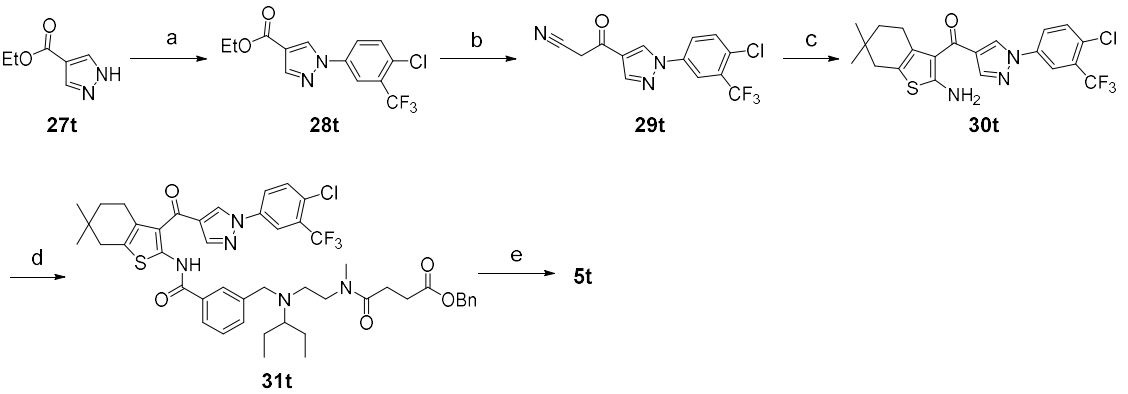
ルを加水分解することで化合物 **23at-23dt** を得 た。化合物 **21t** と化合物 **24at-24ct** とのアルキル化反応後、ベンジルエステルを加水分解することにより化合物 **25at-25ct** を得た。化合物 **21t** を 2-iodoacetonitrile でアルキル化し、hydrazinecarboxaldehyde で環化反応させることによりトリアゾール環を構築し、その後ベンジルエステルを加水分解することにより化合物 **26t** を得た（**Scheme 4t**）。



Scheme 4t. Preparation of **23at-23dt, 25at-25ct** and **26t**. Reagents and conditions: (a) **22at-22ct** (boronic acids), pyridine, $Cu(OAc)_2$, $CHCl_3$, rt or **22dt** (methyl-3-iodobenzoate), (1R,2R)-N1,N2-dimethylcyclohexane-1,2-diamine, cuprous iodide, Cs_2CO_3, DMF, 60 °C; (b) NaOH aq., THF, rt-60 °C, 10%-55% in 2 steps; (c) **24at-24ct**, Cs_2CO_3 or DIPEA, MeCN, rt; (d) 1M-NaOH aq., THF, rt, 28%-52% in 2 steps; (e) 2-iodoacetonitrile, DIPEA, MeCN, 100 °C; (f) Cs_2CO_3, MeOH, rt, then hydrazinecarboxaldehyde, *n*-BuOH, 120 °C; (g) 2M-NaOH aq., THF, 60 °C, 10% in 3 steps.

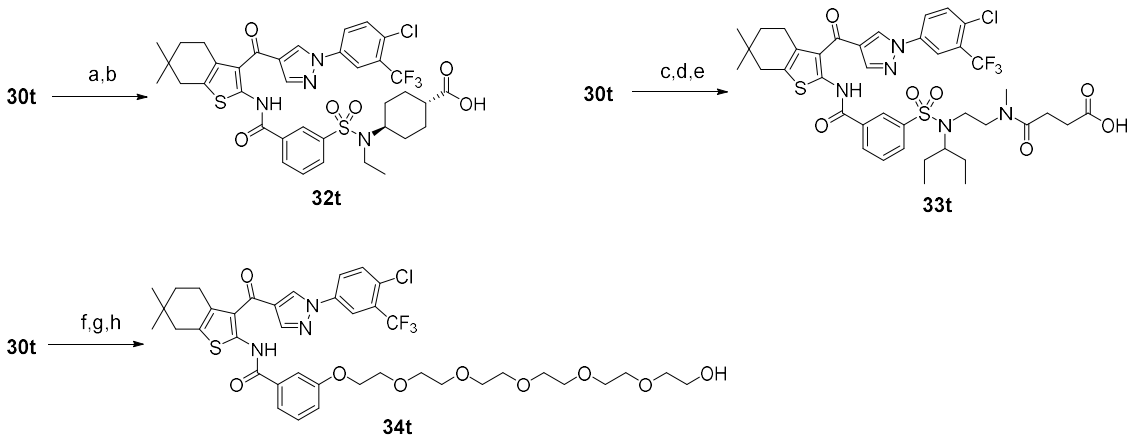
化合物 **27t** と 1-chloro-4-iodo-2-(trifluoromethyl)benzene のカップリング反応により化合物 **28t** を得た。その後、化合物 **15at** と同様の合成方法で、4,4-dimethylcyclohexan-1-one を用いて化合物 **5t** を合成した（**Scheme 5t**）。

Scheme 5t. Preparation of **5t**. Reagents and conditions: (a) 1-chloro-4-iodo-



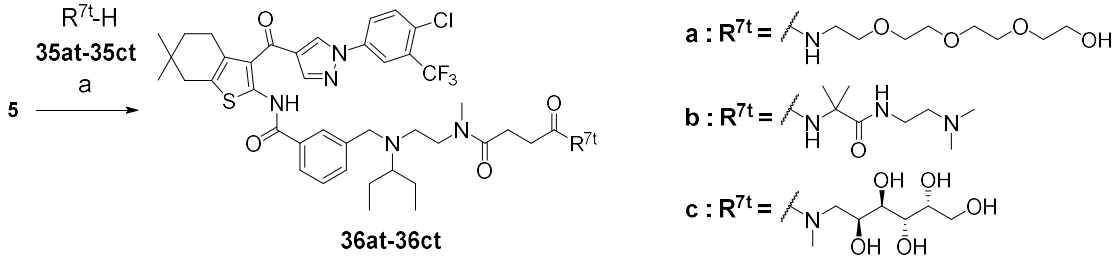
2-(trifluoromethyl)benzene, (1R,2R)-N1,N2-dimethylcyclohexane-1,2-diamine, cuprous iodide, sodium ascorbate, Na_2CO_3, DMSO, 150 °C, 50%; (b) MeCN, *n*-BuLi, THF, -40 °C, 89%; (c) 4,4-dimethylcyclohexan-1-one, sulfur, morpholine, EtOH, 70 °C, 73%; (d) **9t**, $SOCl_2$, $CHCl_3$, 60 °C, then **30t**, DIPEA, $CHCl_3$, rt, 63%; (e) 4M-NaOH aq., THF, rt, 62%.

既知化合物である 3-{ethyl[(1r,4r)-4-(methoxycarbonyl)cyclohexyl]sulfamoyl} benzoic acid[17]を塩化オキサリルで酸クロリドにし、化合物 **30t** とアミド化した後、メチルエステルを加水分解して化合物 **32t** を得た。化合物 **30t** を 3-(chlorosulfonyl)benzoyl chloride でアミド化した後、得られたスルホニルクロリドを化合物 **8t** と反応させてスルホンアミドを得た後、ベンジルエステルを加水分解して化合物 **33t** を得た。化合物 **30t** を 3-(chlorocarbonyl)phenyl acetate でアミド化後、NaOH 水溶液でアセチル基を除去し、17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate でアルキル化することにより **34t** が得た（**Scheme 6t**）。



Scheme 6t. Preparation of **32t, 33t** and **34t**. Reagents and conditions: (a) 3-{ethyl[(1r,4r)-4-(methoxycarbonyl)cyclohexyl]sulfamoyl}benzoic acid, oxalyl chloride, $CHCl_3$, 70 °C, then **30t**, pyridine, $CHCl_3$, rt; (b) 4M-NaOH aq., THF, MeOH, rt, 82% in 2 steps; (c) 3-(chlorosulfonyl)benzoyl chloride, Et_3N, $CHCl_3$, rt; (d) **8t**, Et_3N, $CHCl_3$, rt; (e) 2M-NaOH aq., THF, rt, 6.2% in 3 steps; (f) 3-(chlorocarbonyl)phenyl acetate, $SOCl_2$, $CHCl_3$, 70 °C, then **30t**, Pyridine, $CHCl_3$, rt, 98%; (g)1M-NaOH aq., MeOH, THF, rt; (h) 17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate, Cs_2CO_3, DMF, 100 °C, 63% in 2 steps.

化合物 **36at-36ct** は、化合物 **5t** と化合物 **35at-35ct** のアミド化により合成した（**Scheme 7t**）。

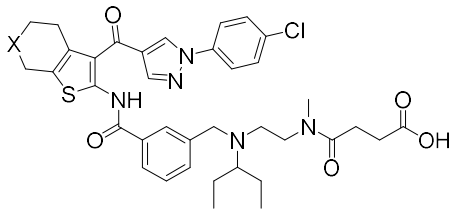


Scheme 7t. Preparation of **36at-36ct**. Reagents and conditions: (a) **35at-35ct**, HATU, DIPEA, MeCN, rt, 39%-58%.

第 3 節　チオフェン化合物（**15at-36ct**）の NaPi2b 阻害活性

DFT により算出した最安定構造において、分子内相互作用によりコア構造が固定化されていることが想定されたチオフェン骨格を有する化合物 **15at** を合成したところ、NaPi2b 阻害活性が認められ、このコア骨格が有効であることが示された。次に、X 部位の構造活性相関を検討した。その結果、化合物 **23at** に強い NaPi2b 阻害活性が認められた。化合物 **15ft** は **23at** と比較してやや活性が減弱し、化合物 **15bt-15et** では阻害活性の大幅な減弱が認められた。これらの結果から、極性の高い官能基は X 部位では許容されないことが示唆された（**Table 1t**）。

Table 1t. Human NaPi2b inhibitory activities of compounds **15at-15ft** and **23at**

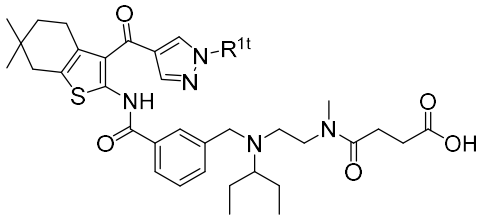


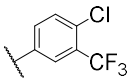
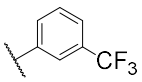
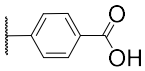
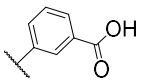
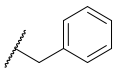
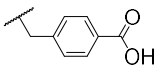
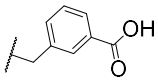
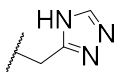
Compound	X	human NaPi2b IC_{50} (nM)[a]
15at		118
15bt		437
15ct		713
15dt		>1000
15et		2486
15ft		80
23at		36

[a] The IC_{50} values for human NaPi2b activity represent the mean values of at least two experiments (with the exception of that for **15dt**, which represents the value of one experiment)

次に化合物 **23at** について R^{1t} 部位の構造活性相関を検討した（**Table 2t**）。その結果、化合物 **23bt** と **5t** に優れた NaPi2b 阻害活性が認められた。他の化合物では阻害活性の減弱が認められ、特に高極性基や窒素原子を含む **23ct**、**23dt**、**25bt**、**25ct**、**26t** では大幅に活性は減弱した。これらの結果から、R^{1t} 部位では高極性基が許容されないことが示唆された。

Table 2t. Human NaPi2b inhibitory activities of compounds **5t**, **23at-23dt**, **25at-25ct** and **26t**

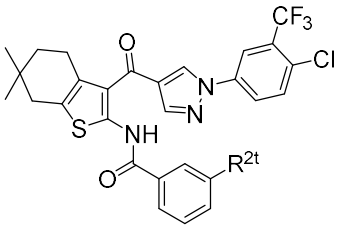


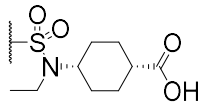
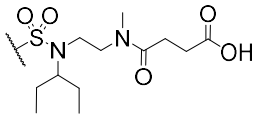
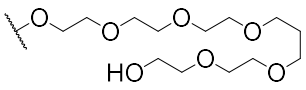
Compound	R^{1t}	human NaPi2b IC_{50} (nM)[a]
5t		38
23bt		46
23ct		>1000
23dt		>1000
25at		179
25bt		>1000
25ct		>1000
26t		>1000

[a] The IC_{50} values for human NaPi2b activity represent the mean values of at least two experiments.

次に、化合物 **5t** について R^{2t} 部位の構造活性相関を検討した（**Table 3t**）。化合物 **32t** と **33t** の阻害活性は、化合物 **5t** と同程度であった。また化合物 **34t** は中程度の阻害活性を示した。これらの結果から、R^{2t} 部位には様々な高極性基を導入できることが示された。

Table 3t. Human NaPi2b inhibitory activities of compounds **32t**, **33t**, and **34t**

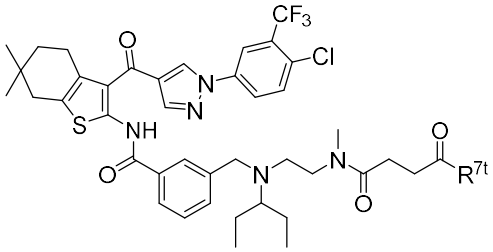


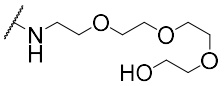
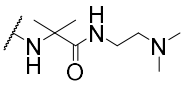
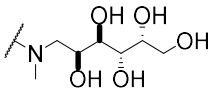
Compound	R^{2t}	human NaPi2b IC_{50} (nM)[a]
32t		42
33t		39
34t		170

[a] The IC_{50} values for human NaPi2b activity represent the mean values of at least two experiments.

次に更なる検討として、化合物 **5t** の R^{7t} 部位について検討した（**Table 4t**）。化合物 **36at-36ct** の human NaPi2b 阻害活性は、化合物 **5t** と同程度であった。また、化合物 **36at** 及び **36bt** は rat NaPi2b に対しても優れた阻害活性を示した。化合物 **5t**、**36at**、**36bt** の tPSA 値はいずれも 140Å^2 以上で、低い経口吸収性が期待された。そこで、化合物 **5t**、**36at** 及び **36bt** について SD ラットを用いた pharmacokinetics（PK）試験で薬物動態を評価した。

Table 4t. *In vitro* activity and tPSA of compounds **5t** and **36at-36ct**



Compound	R^{7t}	human/rat NaPi2b IC_{50} (nM)[a]	tPSA[b] ($Å^2$)
5t		38/ND[c]	153
36at		15/2.1	193
36bt		48/7.6	177
36ct		87/NT[d]	237

[a] The IC_{50} values for human/rat NaPi2b activity represent the mean values of at least two experiments

[b] The tPSA value was calculated using software from ACD/Percepta, version 2019, Advanced Chemistry Development, Inc.

[c] Not determined.

[d] Not tested.

第 4 節　チオフェン化合物（**5t**、**36at**、**36bt**）の薬物動態

化合物 **5t**、**36at** 及び **36bt** の PK 試験の結果を **Table 5t** に示す。化合物 **5t** のバイオアベイラビリティは予想外に高い値であった。一方、化合物 **36at** 及び **36bt** の F は、想定通り極めて低かった。分子内水素結合の形成は化合物の経口吸収性を向上させることがあるとの報告がある[27),28)]。**Figure 1t** に示すように、化合物 **5t**、**36at** 及び **36bt** のコア構造は分子内相互作用を形成することが示唆されている。この影響により、経口吸収性を低下させるために一般的に必要な tPSA 値である 140Å^2 よりも高い、>170Å^2 の tPSA 値が必要となった可能性が考えられた。また **Table 6t** に示すように、化合物 **36at** 及び **36bt** は経口投与後、腸管内を通過して大部分が未変化体として糞便中に排泄されることが確認された。薬物動態試験の結果、望むプロファイルを示した化合物 **36at** 及び **36bt** について SD ラットを用いたリン吸収抑制作用を評価した。

Table 5t. Pharmacokinetic parameters after a single intravenous (i.v.) or oral (p.o.) administration of compounds **5t**, **36at**, or **36bt** to fasted male SD rats.

Compound	i.v.[a]		p.o.[b]			
	Dose (mg/kg)	$AUC_{0\text{-}t}$[c] (h*ng/mL)	Dose (mg/kg)	C_{max}[c] (ng/mL)	$AUC_{0\text{-}t}$[c] (h*ng/mL)	F (%)
5t	1	3070 ± 330	10	625 ± 160	2190 ± 550	7.1
36at	1	1570 ± 160	10	28.1 ± 12.0	32.3 ± 12.0	0.2
36bt	1	2580 ± 280	10	15.3 ± 7.9	163 ± 57	0.6

[a] Dosing vehicle was PEG400.

[b] Dosing vehicle was 0.5% MC400.

[c] Results are presented as the mean ± standard deviation (S.D.) of three animals.

Table 6t. Cumulative excretion of unchanged forms into feces within 24 hours after oral administration of compounds **36at** or **36bt** to fasted male SD rats

Compound	p.o.[a]	
	Dose (mg/kg)	Fecal excretion (%)[b]
36at	10	61.7
36bt	10	76.8

[a] Dosing vehicle was 0.5% MC400.

[b] Results are presented as the mean of three animals.

第 5 節　チオフェン化合物（**36at**、**36bt**）のリン吸収抑制作用

化合物**36at**及び**36bt**のリン吸収抑制作用の結果を**Figure 3t**に示す。化合物**36at**及び**36bt**は、10 mg/kgの経口投与において、リン吸収を有意に抑制しなかった。ラットの腸管容積を論文[29),30)]を参考に算出すると20.5-51.7 mLとなる。この腸管容積を用いて、ラットの体重を300 gとして10 mg/kg経口投与後の推定腸管内化合物濃度を算出すると58〜146 μg/mLとなり、化合物**36at**及び**36bt**のrat NaPi2b IC_{50}値を大きく上回っていると考えられた。また、未変化体の糞中への排泄率を腸管内における未変化体の存在率と想定して補正しても、化合物**36at**及び**36bt**の推定腸管内濃度はそれぞれ36-90 μg/mL（58-146 μg/mL×0.617），45-112 μg/mL（58-146 μg/mL×0.768) であり、いずれもIC_{50}値を十分に超えていた。また**Table 7t**に示すように、化合物**36at**及び**36bt**の人工腸液中（FaSSIF、fasted state simulated intestinal fluid、あるいはFeSSIF、fed state simulated intestinal fluid）の溶解度は、それぞれのIC_{50}値を十分に超えていた。その一方で、化合物**36at**及び**36bt**のClogP値は非常に高かった。疎水性の高い化合物は、腸管表面近傍の非撹拌水層を通過し難いことが報告されている[31)]。また、主に胆汁酸により形成されるミセルとの親和性の影響を受け、ミセルから放出され難くなる可能性も報告されている[32)]。化合物**36at**及び**36bt**がリン吸収抑制作用を示さなかったのは、疎水性が高いために、非撹拌水層の影響やミセルへの親和性の影響を受けることによって、腸管上皮細胞管腔側に存在するNaPi2bへのアクセシビリティが低下することが一因として考えられた。

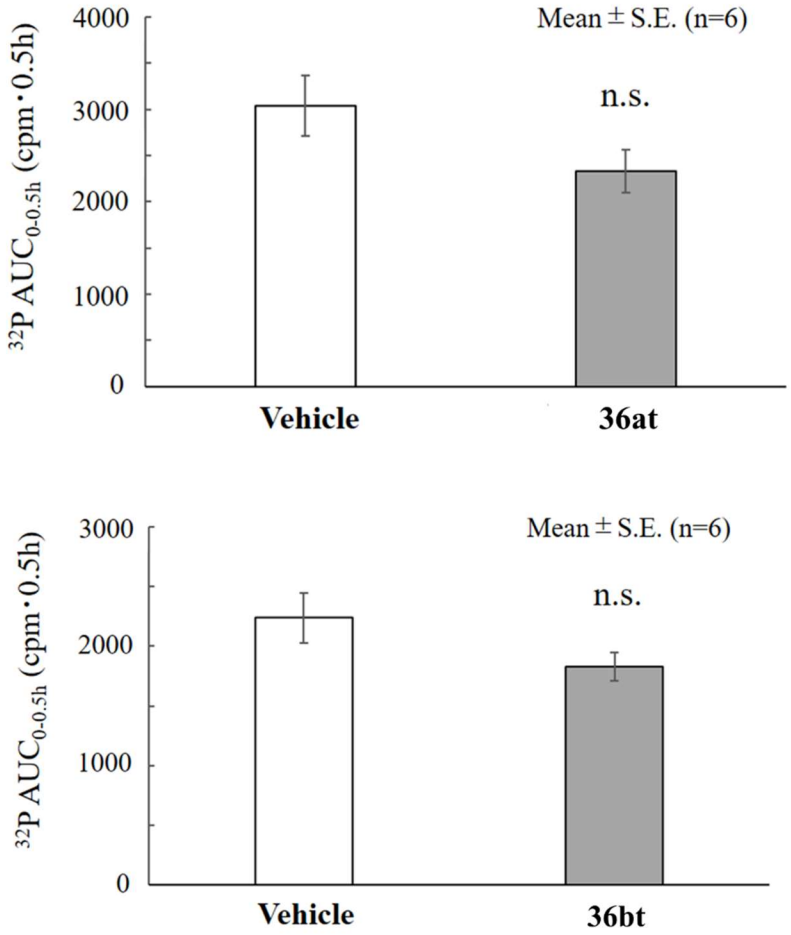


Figure 3t. ^{32}P phosphate was administered to SD rats 5 minutes after oral dosing with **36at** or **36bt** hydrochloride salt (10 mg/kg, respectively). The radioactivity in the plasma was then measured for 0.5 hours (the approximate time it takes for phosphate to reach the maximum concentration in the plasma - data not shown).

Table 7t. Solubility (FaSSIF, FeSSIF) and ClogP values of compounds **36at** and **36bt**

Compound	Solubility (µg/mL) FaSSIF (pH 6.6)	Solubility (µg/mL) FeSSIF (pH 5.1)	ClogP[a]
36at	107[b]	>384[b]	9.28
36bt	242[b]	>434[b]	10.6

[a] The ClogP value was calculated using ChemDraw Professional, ver.19.1.1.21, PerkinElmer Informatics, Inc.

[b] Hydrochloride salt data.

第 6 節　まとめ

本章では、既知 NaPi2b 阻害物質である化合物 **2at** のコア骨格に関する DFT 計算による最安定構造情報を参考にして、分子内相互作用により固定化されたコア骨格に着目し、同様の分子内相互作用を有すると考えられるチオフェン誘導体をデザイン・合成・評価した。その結果、化合物 **15at** に良好な human NaPi2b 阻害活性が認められ、この分子内相互作用を有するコア骨格が有効であることが示された。全身曝露の低い腸管選択的な化合物の取得を目指して、チオフェン化合物の X、R^{1t}、R^{2t} 部位の変換を実施したところ、X 部位については化合物 **23at** のジメチル構造が最も良好な阻害活性を示し、高極性基の導入は許容されなかった。R^{1t} についても、化合物 **5t** のように疎水性置換基を有するフェニル環で良好な阻害活性を示し、高極性基の導入は許容されなかった。一方、R^{2t} 部位については高極性基の導入が許容され、良好な阻害活性と高い tPSA 値を示す **36at**、**36bt** を得た。薬物動態試験の結果、**36at**、**36bt** はバイオアベイラビリティが低く、また大部分が未変化体として糞便中から排泄されることから、低い経口吸収性で腸管選択的な作用が期待できる化合物であった。そこで **36at**、**36bt** のリン吸収抑制作用を評価したが、薬効は認められなかった。化合物の物性を精査したところ、疎水性が非常に高いことが判明した。化合物 **36at**、**36bt** がリン吸収抑制作用を示さなかったのは，疎水性が高いために、非撹拌水層の影響やミセルへの親和性の影響を受けることによって、腸管上皮細胞管腔側に存在する NaPi2b へのアクセシビリティが低下することが一因として考えられた。

第 2 章　ピリジン骨格の NaPi2b 阻害物質の研究

第 1 節　ドラッグデザイン

第 1 章のチオフェン骨格の検討で示した通り、NaPi2b 阻害活性の発現には分子内相互作用で固定化されたコア骨格が重要であることが示唆されていたことを考慮し、既知 NaPi2b 阻害物質の構造情報を参考にして、チオフェン骨格と同様に 2 つの分子内相互作用（水素結合）による固定化が期待できるピリジン骨格（core structure of **11ap**）をデザインした。DFT により算出した最安定コンフォメーションを解析したところ、2 つの分子内水素結合を形成していることが示唆されたため（**Figure 1p**）、このコア骨格を起点に誘導体合成を展開した。また、チオフェン化合物がリン吸収抑制作用を示さなかった一因として、疎水性が高いことが考えられたことから、チオフェン骨格をピリジン骨格に変えることにより、疎水性の低減を期待した。

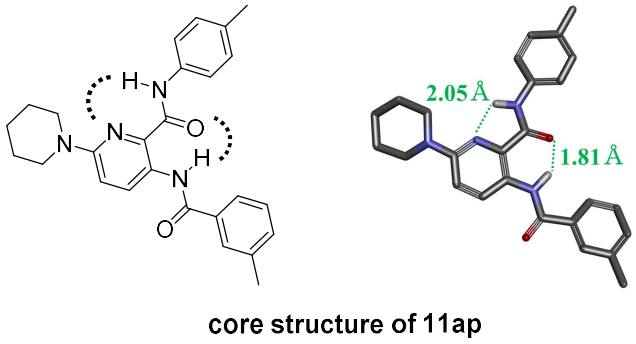


Figure 1p. The most stable conformations of core structures of **11ap** based on DFT calculations in a water environment. The calculated O···H and N···H interatomic distances of these conformations are also shown. The estimated intramolecular hydrogen bonds are shown by the dotted lines.

誘導体の合成戦略を **Figure 2p** に示す。core structure の R^{1p}、R^{2p} 及び R^{3p} 部位について、NaPi2b 阻害活性を指標に構造活性相関を探索し、活性向上を図りつつ、tPSA を増加させて経口吸収性の低減が期待できる高極性基の導入可能な部位を探索した。

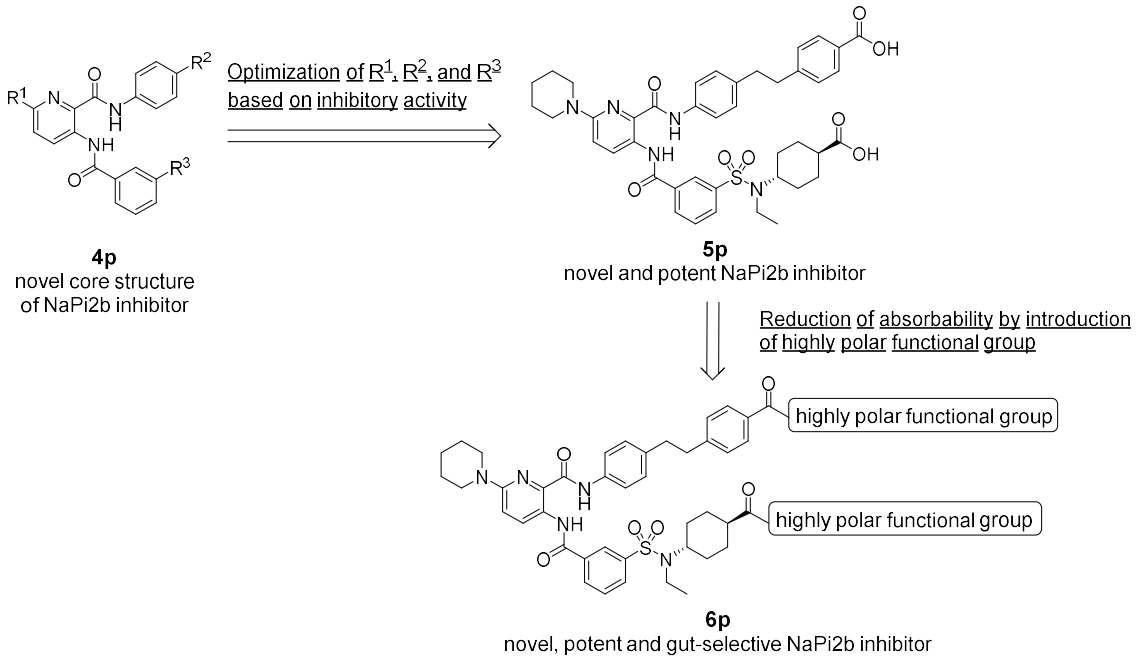
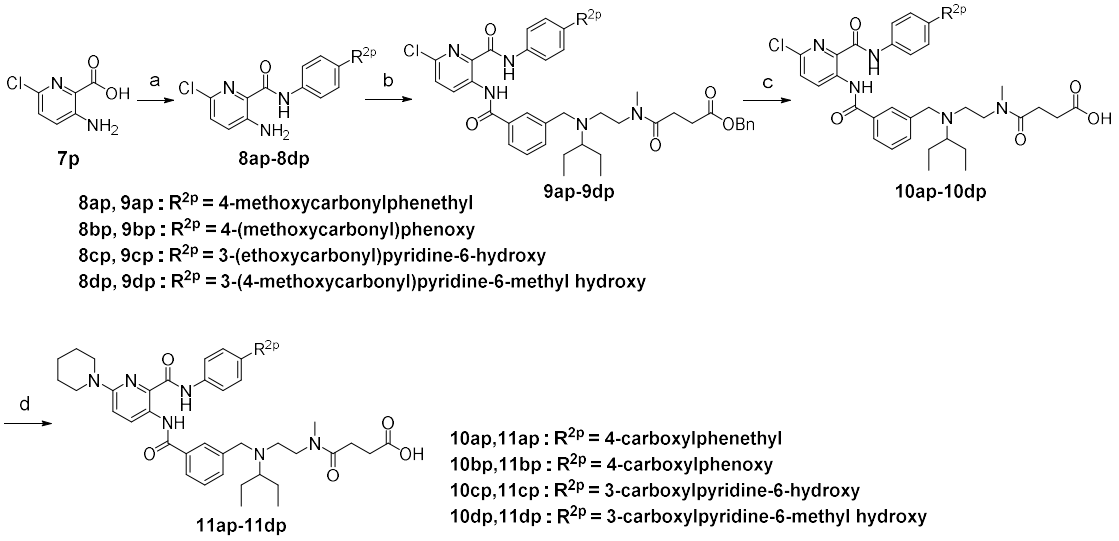


Figure 2p. 誘導体の合成戦略

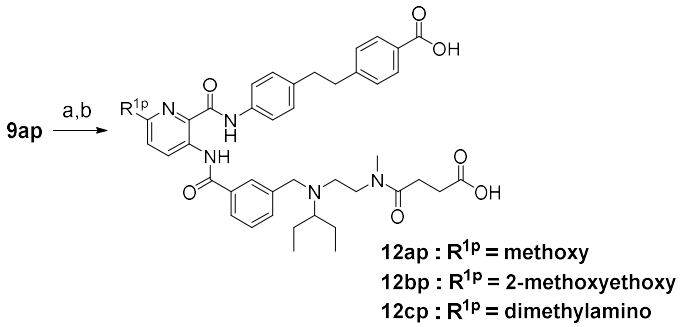
第 2 節　ピリジン化合物（**10ap-20bp**）の合成

ピリジン化合物の合成法を **Scheme 1p-5p** に示す。カルボン酸 **7p** と市販のアニリン類を縮合した後、チオフェン誘導体の合成でも使用していた塩化ベンゾイルを用いて化合物 **8ap-8dp** のアミド化を行い、化合物 **9ap-9dp** を得た。その後、水酸化ナトリウム水溶液で加水分解することで化合物 **10ap-10dp** を得ており、更にピペリジンで芳香族求核置換反応することで化合物 **11ap-11dp** を合成した（**Scheme 1p**）。



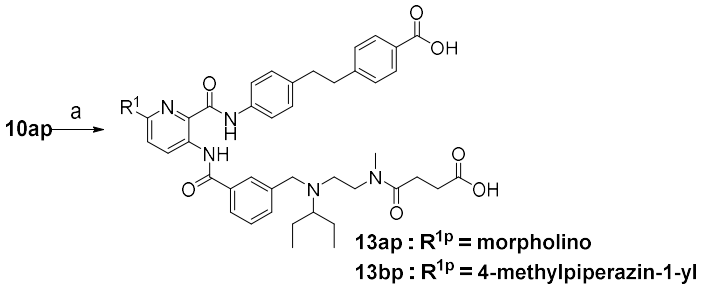
Scheme 1p. Preparation of **11ap-11dp**. Reagents and conditions: (a) aniline, EDC, HOBt･H_2O, rt-100 °C, 40%-77%; (b) benzyl 4-[2-[(3-chlorocarbonylphenyl)methyl-(1-ethylpropyl) amino]ethyl-methyl-amino]-4-oxo-butanoate, pyridine, $CHCl_3$, rt-85 °C; (c) 1M-NaOH aq., THF or THF/MeOH, rt, 24%-40% in 2 steps; (d) piperidine, microwave, 100 °C-160 °C, 28%-78%.

化合物 **9ap** を市販のアルコールまたはアミンとカップリング反応させた後、NaOH 水溶液で加水分解して化合物 **12ap-12cp** を得た（**Scheme 2**）。



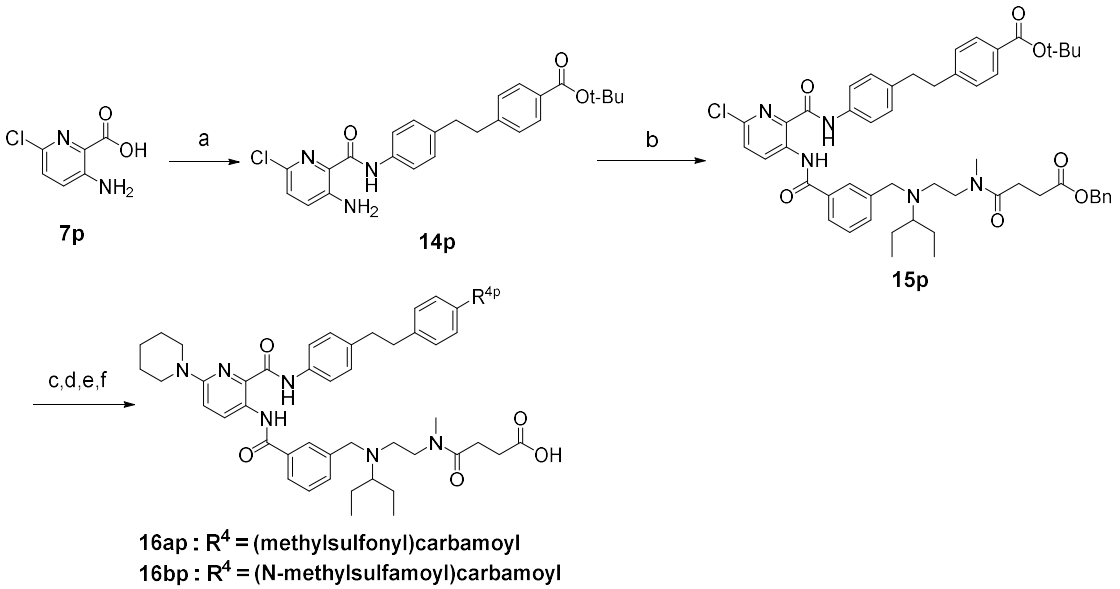
Scheme 2p. Preparation of **12ap**-**12cp**. Reagents and conditions: (a) alcohol or dimethylamine, (1R,2R)-N,N'-dimethyl-1,2-cyclohexanediamine, sodium carbonate, cuprous iodide, DMSO, rt-150 °C; (b) 1M-NaOH aq., THF, MeOH, rt, 17%-57% in 2 steps.

化合物 **13ap** 及び **13bp** は、**Scheme 3p** に示すように，化合物 **10ap** と市販のアミンとの芳香族求核置換反応により合成した。



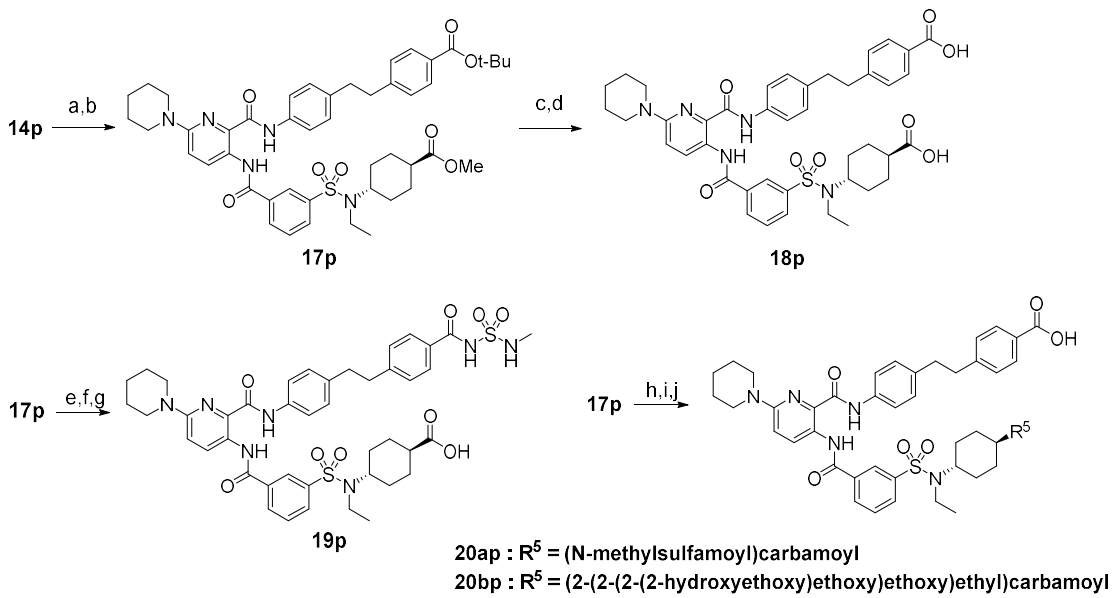
Scheme 3p. Preparation of **13ap**-**13bp**. Reagents and conditions: (a) amine, microwave, 160 °C, 34%-38%.

化合物 **7p** を 4-(4-aminophenethyl)benzoate *tert*-butyl と縮合した後、化合物 **9ap** の合成で使用した塩化ベンゾイルを用いて化合物 **14p** のアミド化を行い、化合物 **15p** を得た。化合物 **15p** とピペリジンの芳香族求核置換反応、TFA による *tert*-butyl の脱保護、スルホンアミドとの縮合、ベンジルエステルの加水分解により化合物 **16ap** 及び **16bp** を得た（**Scheme 4p**）。



Scheme 4p. Preparation of **16ap** and **16bp**. Reagents and conditions: (a) *tert*-butyl 4-(4-aminophenethyl)benzoate, EDC, HOBt・H_2O, rt, DMF, 60%; (b) benzyl 4-[2-[(3-chlorocarbonylphenyl)methyl-(1-ethylpropyl)amino]ethyl-methyl-amino]-4-oxo-butanoate, pyridine, $CHCl_3$, rt, 58%. (c) piperidine, microwave, 150 °C, 34%; (d) TFA, $CHCl_3$, rt; (e) sulfonamides, EDC, DMAP, $CHCl_3$, rt-60 °C; (f) 1M-NaOH aq., THF, rt, 23%-25% in 3 steps.

チオフェン誘導体の合成でも使用していた 3-{ethyl[(1r,4r)-4-(methoxycarbonyl)cyclohexyl]sulfamoyl} benzoyl chloride を用いて化合物 **14p** をアミド化し、ピペリジンとの 芳香族求核置換反応により、化合物 **17p** が得られた。化合物 **17p** のメチルエステルの加水分解と TFA による *tert*-ブチル基の脱保護により、化合物 **18p** を得た。化合物 **17p** の *tert*-ブチル基を TFA で脱保護し、(sulfamoylamino)methane と縮合し、加水分解することにより化合物 **19p** を合成した。化合物 **17p** のメチルエステルの加水分解、(sulfamoylamino)methane または 2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}ethanol との縮合、及び TFA による *tert*-ブチルの脱保護により化合物 **20ap** 及び **20bp** を得た（**Scheme 5**）。

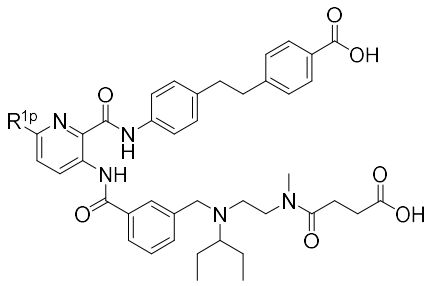


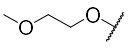
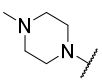
Scheme 5p. Preparation of **18p**-**20bp**. Reagents and conditions: (a) methyl (1r,4r)-4-{[3-(chlorocarbonyl)benzene-1-sulfonyl](ethyl)amino}cyclohexane-1-carboxylate, pyridine, $CHCl_3$, rt ; (b) piperidine, microwave, 140 °C, 53% in 2 steps; (c) 1M-NaOH aq., THF, MeOH, rt, (d) TFA, $CHCl_3$, rt, 24% in 2 steps; (e) TFA, $CHCl_3$, rt; (f) (sulfamoylamino)methane, EDC, DMAP, $CHCl_3$, DMF, 60 °C; (g) 1M-NaOH aq., THF, MeOH, rt, 30% in 3 steps. (h) 1M-NaOH aq., THF, MeOH, rt; (i) for **20ap**, (sulfamoylamino)methane, EDC, DMAP, $CHCl_3$, DMF, rt; for **20bp**, 2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}ethanol, DIPEA, HATU, DMF, rt; (j) TFA, $CHCl_3$, rt, 20%-quant. in 3 steps.

第 3 節　ピリジン化合物（**10ap-20bp**）の NaPi2b 阻害活性

DFT により算出した最安定構造において、分子内水素結合によりコア構造が固定化されていることが想定されたピリジン骨格を有する化合物 **11ap** を合成・評価したところ、NaPi2b 阻害活性が認められ、このコア骨格が有効であることが示された。次に、R^{1p} 部位の構造活性相関を検討した（**Table 1p**）。その結果、ピペリジン誘導体 **11ap** が最も優れた阻害活性を示した。化合物 **11ap** と比較して、化合物 **10ap**、**12ap-12cp**、**13ap**、**13bp** の阻害活性は減弱しており、経口吸収性を低減させるための高極性基の導入は許容されないことが示唆された。

Table 1p. Human NaPi2b inhibitory activities of compounds **10ap, 11ap, 12ap-12cp, 13ap,** and **13bp**

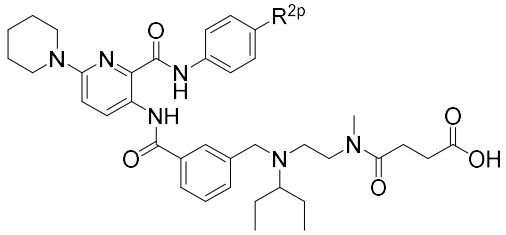


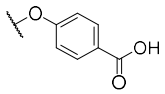
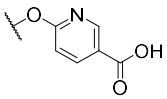
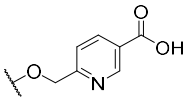
Compound	R^{1p}	human NaPi2b IC_{50} (nM)[a]
10ap		271
12ap		107
12bp		201
12cp		161
11ap		36
13ap		205
13bp		1342

[a] The IC_{50} values for the human NaPi2b activities represent the mean values of at least two experiments.

次に化合物 **11ap** について、R^{2p}部位における構造活性相関を検討した（**Table 2p**）。ヘテロ原子を導入した化合物 **11bp-11dp** は阻害活性が低下する傾向が見られ、好ましくないことが示唆された。

Table 2p. Human NaPi2b inhibitory activities of compounds **11bp-11dp**



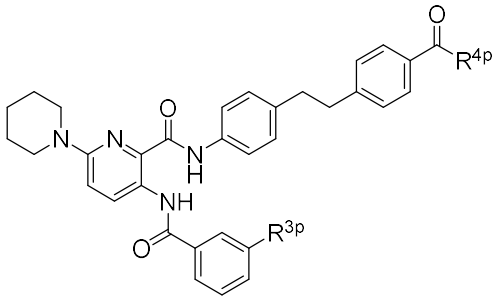
Compound	R^{2p}	human NaPi2b IC_{50} (nM)[a]
11bp		162
11cp		715
11dp		744

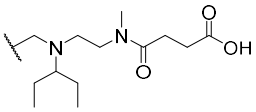
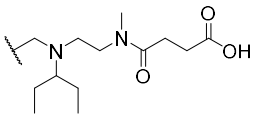
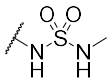
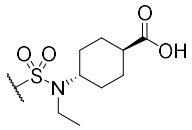
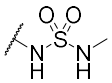
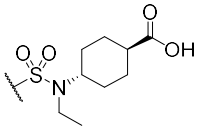
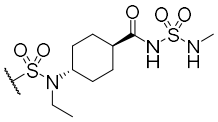
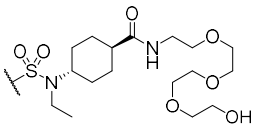
[a] The IC_{50} values for the human NaPi2b activities represent the mean values of at least two experiments.

次に、化合物 **11ap** の R^{3p}及び R^{4p}部位の構造活性相関を検討した（**Table 3p**）。R^{4p} 部位では、カルボン酸、或いはその生物学的等価体であるスルホンアミド誘導体とスルファモイル誘導体で、いずれも良好な阻害活性を示す傾向が認められた。R^{3p} 部位では、エチル-（4-カルボキシルシクロヘキシル）スルファモイル誘導体 **19p** 及び **18p** が優れたヒト NaPi2b 阻害活性を示した。R^{3p}部位の末端では、スルファモイル誘導体 **20ap**、エチレングリコール誘導体 **20bp** でも良好な阻害活性が認められた。これらの結果から、R^{3p}及び R^{4p}部位への高極性基の導入は許容されることが示唆された。human NaPi2b 阻害活性が最も強いスルファモイル誘導体 **19p** と、構造的にそれとは異なるタイプのエチレングリコール誘導体 **20bp** について、rat NaPi2b 阻害活性を評価したところ、どちらも良好な阻害活性を示した。また、**19p** と **20bp** はいずれも高い tPSA 値を示した。そ

こで、**19p** と **20bp** について SD ラットを用いた PK 試験で評価した。

Table 3p. *In vitro* activities and tPSA of compounds **16ap-16bp,** and **18p-20bp**



Compound	R^{3p}	R^{4p}	human / rat NaPi2b IC_{50} (nM)[a]	tPSA[b](Å2)
16ap			237 / NT[c]	206
16bp			192 / NT[c]	219
19p			21 / 10	241
18p			77 / NT[c]	195
20ap			147 / NT[c]	241
20bp			141 / 121	234

[a] The IC_{50} values for the human/rat NaPi2b activities represent the mean values of at least two experiments.

[b] The tPSA value was calculated using software from ACD/Percepta, version 2019, Advanced Chemistry Development, Inc.

[c] Not tested.

第 4 節　ピリジン化合物（**19p**、**20bp**）の薬物動態

化合物 **19p**、**20bp** の PK 試験の結果を **Table 4p** に示す。化合物 **19p** は tPSA 値が高いにも関わらず 10%を超えるバイオアベイラビリティを示した。一方で、化合物 **19p** とほぼ同じ tPSA 値を持つ化合物 **20b** は、比較的低いバイオアベイラビリティを示した。**Figure 1p** に示すように化合物 **19p** と **20bp** のコア骨格は分子内水素結合していることが示唆されており、その影響によって、経口吸収性を低下させるために、より高い tPSA が必要となった可能性が考えられた。また、回転可能結合数の増加による分子の柔軟性の増加は、バイオアベイラビリティを低下させる傾向があることが報告されている [24]。化合物 **20bp** の回転可能結合数は 25、化合物 **19p** の回転可能結合数は 16 であり、この差が両化合物のバイオアベイラビリティの違いの一因だと考えられた。**Table 5p** に示すように、化合物 **20bp** の大部分は、経口投与後、腸管内を通過して未変化体として糞中に排泄された。**Table 6p** に示すように、化合物 **20bp** の人工腸液中の溶解度は rat NaPi2b に対する IC_{50} 値を十分に超えていた。また化合物 **20bp** の ClogP 値は、リン吸収抑制作用を示さなかったチオフェン誘導体（ClogP>9）と比較して低い値を示した。化合物 **20b** は腸管選択的な傾向が認められ、かつ疎水性についても低減されていたため、SD ラットを用いてリン吸収抑制作用を評価した。

Table 4p. Pharmacokinetic parameters after a single intravenous (i.v.) or oral (p.o.) administration of compound **19p** or **20bp** to fasted male SD rats

Compound	i.v.[a]		p.o.[b]			
	Dose (mg/kg)	$AUC_{0\text{-}t}$[c] (h*ng/mL)	Dose (mg/kg)	C_{max}[c] (ng/mL)	$AUC_{0\text{-}t}$[c] (h*ng/mL)	F (%)
19p	1	1200 ± 130	11.9	1540 ± 570	1480 ± 450	10.4
20bp	1	319 ± 52	10	70.6 ± 30.9	84.5 ± 88.9	2.7

[a] Dosing vehicle was PEG400.

[b] Dosing vehicle was 0.5% MC400.

[c] Results are presented as the mean ± standard deviation (S.D.) of three animals.

Table 5p. Cumulative excretion of unchanged forms into feces within 24 hours after oral administration of compound **20bp** to fasted male SD rats

Compound	p.o.[a]	
	Dose (mg/kg)	Fecal excretion (%)[b]
20bp	10	99.1

[a] Dosing vehicle was 0.5% MC400.

[b] Results are presented as the mean of three animals.

Table 6p. Solubility (FaSSIF, FeSSIF) and ClogP of compound **20bp**

Compound	Solubility (μg/mL) FaSSIF	Solubility (μg/mL) FeSSIF	ClogP[a]
20bp	20.5 (pH 6.6)	15.9 (pH 4.9)	6.50

[a] The ClogP value was calculated using ChemDraw Professional ver.19.1.1.21, PerkinElmer Informatics, Inc.

第 5 節　ピリジン化合物（**20bp**）のリン吸収抑制作用

SD ラットを用いた化合物 **20bp** のリン吸収抑制作用の評価結果を **Figure 3p** に示す。化合物 **20bp** は 10 mg/kg の経口投与によって、有意なリン吸収抑制作用を示した。チオフェン誘導体の考察（第 1 章の第 4 節）で記載しているように 10 mg/kg の経口投与後の推定腸管内濃度は 58-146 μg/mL と考えられるので、化合物 **20bp** の rat NaPi2b IC_{50} 値を十分に超えていることが想定される。この点はチオフェン誘導体もピリジン誘導体も条件はほぼ同等である。また、経口投与された化合物のほとんどが未変化体で糞便中から排泄されること、及び化合物の溶解度がそれぞれの IC_{50} 値を十分に超えている点も条件はほぼ同様と考えられる。一方で化合物の疎水性に関しては、チオフェン誘導体（CLogP>9）と比較してピリジン誘導体（CLogP<7）で低減されており、このことがピリジン誘導体でリン吸収抑制作用が認められた一因と考えられた。すなわち、ピリジン化合物では疎水性が低減したことにより、非攪拌水層の透過性が向上、あるいはミセルとの親和性が低減して腸管上皮細胞管腔側に存在する NaPi2b へのアクセシビリティが向上した可能性が考えられた。また今回の結果で、化合物 **20bp** の 10mg/kg 経口投与時の血漿中ピーク濃度（C_{max}）は NaPi2b 阻害活性の IC_{50} 値以下であったことから、全身曝露に伴う NaPi2b 阻害の影響は小さく、腸管の NaPi2b を選択的に阻害することでリン吸収を抑制できることが示された。

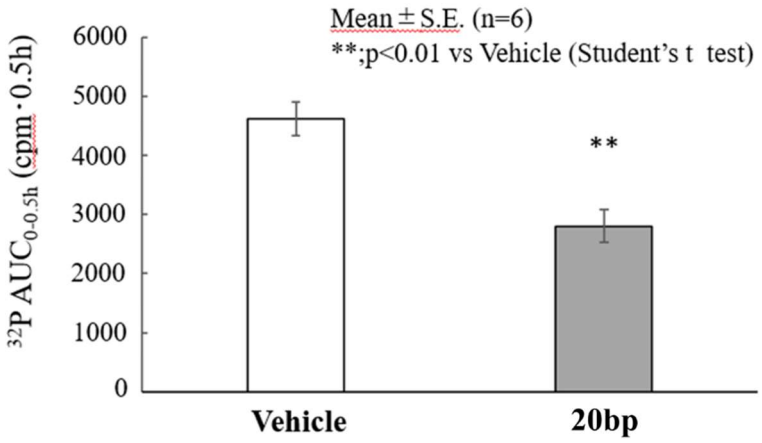


Figure 3p. ^{32}P phosphate was administered to SD rats 5 minutes after oral dosing with compound **20bp** (10 mg/kg), and radioactivity in the plasma was measured for 0.5 hours (the approximate time it takes for phosphate to reach the maximum concentration in the plasma - data not shown).

第 6 節　まとめ

本章では SD ラットにおいてリン吸収抑制作用を示す化合物を取得すべく、ピリジン骨格を有する誘導体のデザイン・合成・評価を行った。NaPi2b 阻害活性の発現には、分子内相互作用により固定化されたコア骨格が重要であることが示唆されていたので、ピリジン骨格についても、同様の分子内水素結合の形成ができるようにデザインした。その結果、化合物 **10ap** に良好な human NaPi2b 阻害活性が認められ、このコア骨格が有効であることが示された。全身曝露の低い腸管選択的な化合物の取得を目指して、R^{1p}、R^{2p}、R^{3p} 及び R^{4p} 部位の変換を実施したところ、R^{1p} 及び R^{2p} 部位については高極性基の導入は許容されなかったが、R^{3p} 及び R^{4p} 部位については高極性基の導入が可能であった。良好な阻害活性と高い tPSA 値を示した化合物 **19p**、**20bp** の PK 試験の結果、**20bp** は低い経口吸収性が認められたが、**19p** は 10%を超える経口吸収性が認められ、経口吸収性の予測においては tPSA 値だけでなく、回転可能結合数も考慮する必要性が示唆された。疎水性が低減された化合物 **20bp** は、ラットにおいて 10mg/kg の経口投与でリン吸収抑制作用を示したことから、化合物の疎水性を低減することが薬効発現に有効である可能性が示された。また、10mg/kg 経口投与時の化合物 **20bp** の C_{max} は NaPi2b 阻害活性の IC_{50} 値以下であったことから、全身曝露に伴う NaPi2b 阻害の影響は小さい状況であったと考えられ、腸管の NaPi2b を選択的に阻害することでリン吸収を抑制できることが示された。

第 3 章　インドール骨格の NaPi2b 阻害物質の研究

第 1 節　ドラッグデザイン

第 2 章のピリジン骨格の検討では、ラットにおいてリン吸収抑制作用を示す化合物を創出した。本章ではより高い腸管選択性（より低い全身曝露）でリン吸収抑制作用を示す化合物を創出すべく、インドール骨格の化合物について検討した。NaPi2b 阻害活性の発現には分子内相互作用で固定化されたコア骨格が重要であることが示唆されていたが、インドール骨格であれば同様の分子内相互作用を維持できると考えた（core structure of **5i**）。DFT により算出した最安定コンフォメーションを解析したところ、想定したとおり 2 つの分子内水素結合を形成していることが示唆されたため（**Figure 1i**）。この骨格を起点に誘導体合成を展開した。リン吸収抑制作用を発揮するためには、疎水性を低減することが有効であることが示めされたことから、インドール骨格によって更なる疎水性の低減も期待した。

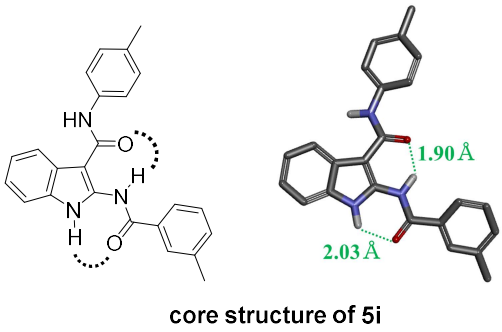


Figure 1i. The most stable conformations of core structures of **5i** based on DFT calculations in a water environment. The calculated O···H and N···H interatomic distances of these conformations are also shown. The estimated intramolecular hydrogen bonds are shown by the dotted lines.

誘導体の合成戦略を **Figure 2i** に示す。チオフェン骨格やピリジン骨格の検討と同様に、core structure の R^{1i} 及び R^{2i} 部位について、NaPi2b 阻害活性を指標に構造活性相関を探索し、活性向上を図りつつ、tPSA を増加させて経口吸収性の低減が期待できる高極性基の導入可能な部位を探索した。

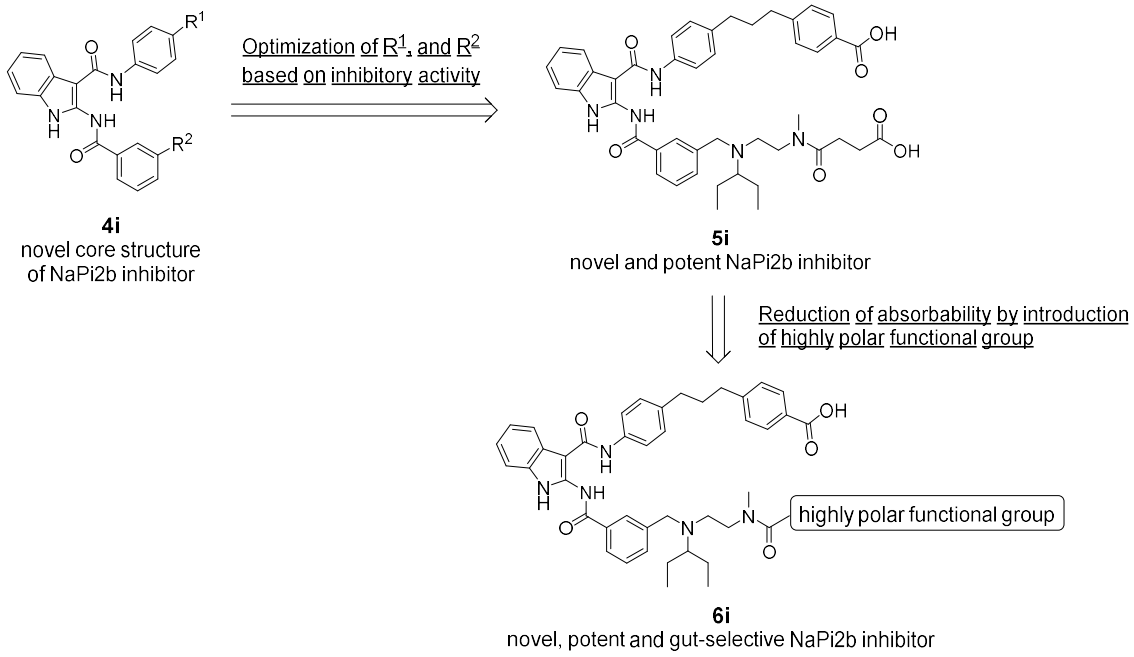
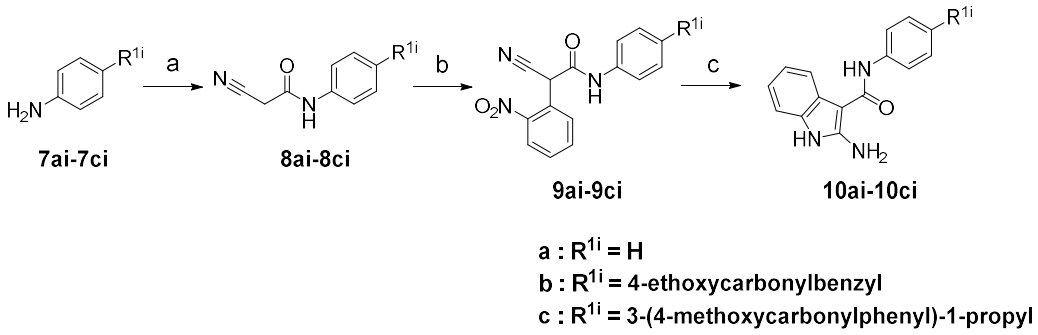


Figure 1i. 誘導体の合成戦略

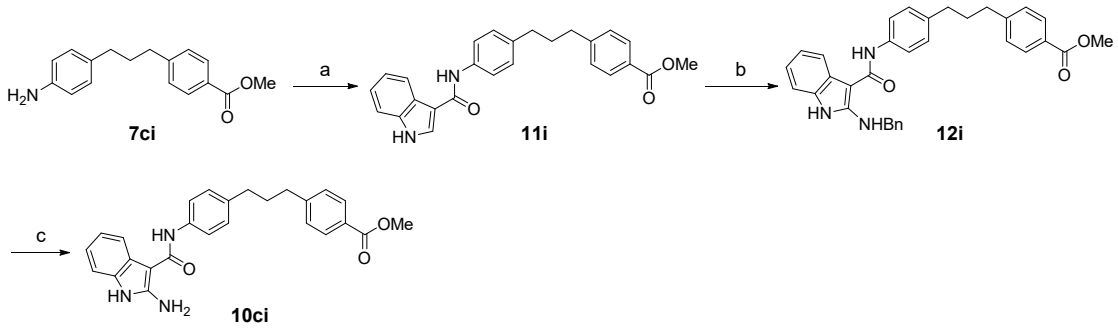
第 2 節　インドール化合物（**5i-31i**）の合成

インドール化合物の合成法を **Scheme 1i-5i** に示す。アニリン **7ai-7ci** を 2-シアノ酢酸と縮合し、得られた **8ai-8ci** を *N*,*N*-ジメチルホルムアミド（DMF）中で水素化ナトリウム（NaH）と反応させた後、 1-フルオロ-2-ニトロベンゼンに対して求核的芳香族置換反応を行い **9ai-9ci** を得た。次に亜鉛（Zn）を用いたニトロ基の還元反応を行い化合物 **10ai-10ci** を合成した。



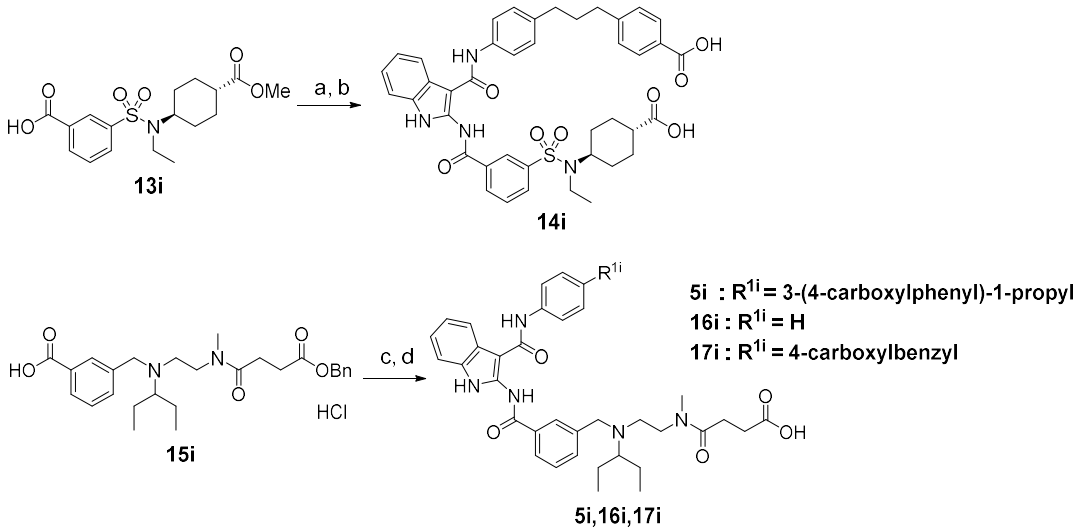
Scheme 1. Preparation of **10ai-10ci**. Reagents and conditions: (a) 2-Cyanoacetic acid, EDC･HCl, DMF, rt, 77%-94%; (b) NaH, DMF, 0 °C-rt, then 1-Fluoro-2-nitrobenzene, rt, 51%-71%; (c) Zn, AcOH, toluene, 90 °C, 13%-59%.

化合物 **10ci** の合成の別法を **Scheme 2i** に示す。アニリン **7ci** とインドール-3-カルボン酸の縮合によりアミド **11i** を得た。インドールの 2 位を *N*-クロロスクシンイミド（NCS）で塩素化した後、ベンジルアミンと芳香族求核置換反応することにより、化合物 **12i** を得た。水素雰囲気下、パラジウムカーボンを用いた接触水素添加反応によりベンジル基を除去して **10ci** を合成した。



Scheme 2i. Preparation of **10ci**. Reagents and conditions: (a) indole-3-carboxylic acid, HATU, DIPEA, MeCN, rt, 78%; (b) NCS, Et_3N, $CHCl_3$, 0 °C, then benzylamine, 0 °C, 88%; (c) Pd/C, H_2, MeOH, rt, quantitative yield.

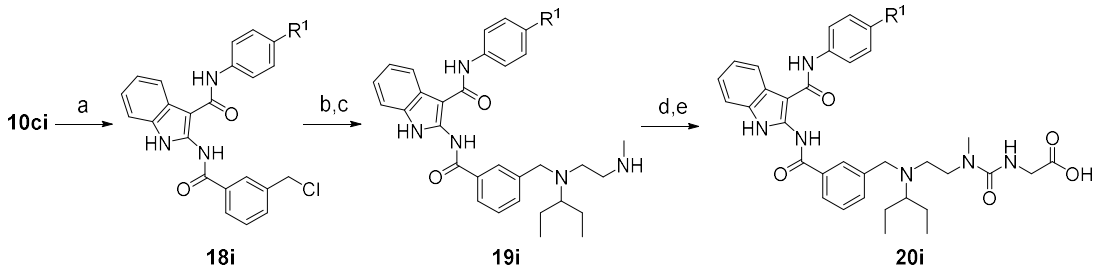
インドール誘導体 **5i**、**14i**、**16i** 及び **17i** の合成法を **Scheme 3i** に示す。安息香酸 **13i** を塩化チオニルを用いて酸クロリドとし、アミン **10ci** とアミド化、メチルエステルを水酸化ナトリウム水溶液で加水分解してカルボン酸 **14i** を得た。安息香酸 **15i** を塩化チオニルを用いて酸クロリドとし、化合物 **10ci**、**10ai** 及び **10bi** とアミド化、メチルエステルを水酸化ナトリウム水溶液で加水分解して、化合物 **5i**、**16i** 及び **17i** をそれぞれ調製した。



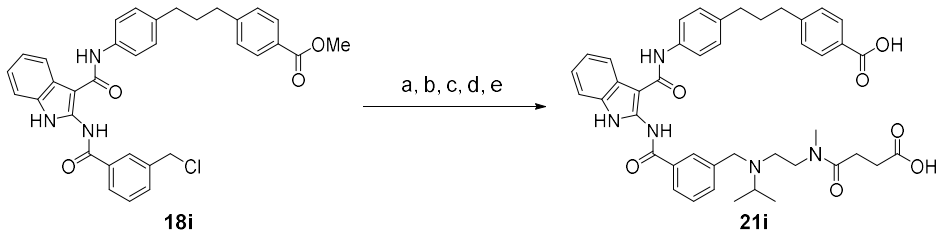
Scheme 3i. Preparation of **14i**, **5i**, **16i**, and **17i**. Reagents and conditions: (a) $SOCl_2$, $CHCl_3$, reflux, then **10ci**, DIPEA, $CHCl_3$, rt-reflux; (b) 1M-NaOH aq., THF, MeOH, 80 °C, 34% in 2 steps; (c) $SOCl_2$, $CHCl_3$, 60 °C-85 °C, then **10ai**-**10ci**, DIPEA or pyridine, $CHCl_3$, rt-70 °C, 8.0%-58%; (d) 1M-NaOH aq. or 2M-NaOH aq., THF or MeOH or THF/MeOH, rt-60 °C, 36%-56%.

インドール誘導体 **20i** の合成を **Schem 4i** に示す。化合物 **10ci** を 3-(chloromethyl)benzoyl chloride でアミド化し、化合物 **18i** を合成した。*tert*-butyl *N*-methyl-*N*-{2-[（pentane-3-yl）amino]ethyl}carbamate を化合物 **18i** でアルキル化、その後 Boc 基を脱保護して、化合物 **19i** を得た。化合物 **19i** をイソシアナート酢酸エチルと反応させ、水酸化ナトリウム水溶液で加水分解して、カルボン酸 **20i** を得た。*tert*-butyl *N*-methyl-*N*-(2-aminoethyl)carbamate を化合物 **18i** でアルキル化した後、得られた 2 級アミンをアセトンで還元アミノ化して 3 級アミンが得られた。得られた 3 級アミンの Boc 基をトリフルオロ酢酸（TFA）で脱保護

した後、無水コハク酸でアミド化し、NaOH 水溶液で加水分解して、化合物 **21** を得た（**Scheme 5i**）。

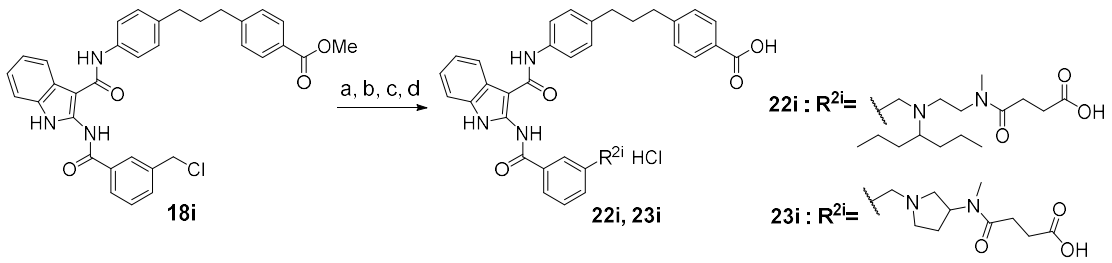


Scheme 4i. Preparation of **20i**. Reagents and conditions: (a) 3-(chloromethyl)benzoyl chloride, Pyridine, $CHCl_3$, rt, 72%; (b) *tert*-butyl *N*-methyl-*N*-{2-[（pentane-3-yl）amino]ethyl}carbamate, DIPEA, NaI, toluene, 95-100 °C, 64 %; (c) TFA, $CHCl_3$, rt, 99%; (d) ethyl isocyanatoacetate, THF, rt; (e) 2M-NaOH aq., MeOH, 65 °C, 73% in 2 steps.

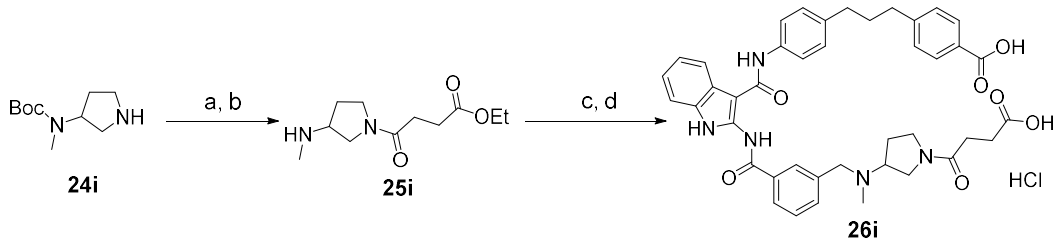


Scheme 5i. Preparation of **21i**. Reagents and conditions: (a) *tert*-butyl *N*-methyl-*N*-(2-aminoethyl)carbamate, DIPEA, NaI, toluene, rt-50 °C, 40%; (b) acetone, AcOH, 1,2-dichloroethane, 75 °C, then $NaBH(OAc)_3$, rt, 54%; (c) TFA, $CHCl_3$, rt, 98%; (d) succinic anhydride, THF, rt; (e) 2M-NaOH aq., MeOH, 65 °C, 58% in 2 steps.

インドール誘導体 **22i** 及び **23i** の合成経路を **Scheme 6i** に示す。市販のアミンを化合物 **18i** でアルキル化し、トリフルオロ酢酸で Boc 基を脱保護、得られた第 2 級アミンを無水コハク酸でアミド化した後、NaOH 水溶液でエステルを加水分解し、塩酸（HCl）で処理して化合物 **22i** 及び **23i** の塩酸塩を得た。化合物 **24i** を ethylsuccinylchloride でアミド化した後、Boc を酸で脱保護して化合物 **25i** を得た。化合物 **25i** を化合物 **18i** でアルキル化し、エステルを NaOH 水溶液で加水分解して、化合物 **26i** を合成した（**Scheme 7i**）。



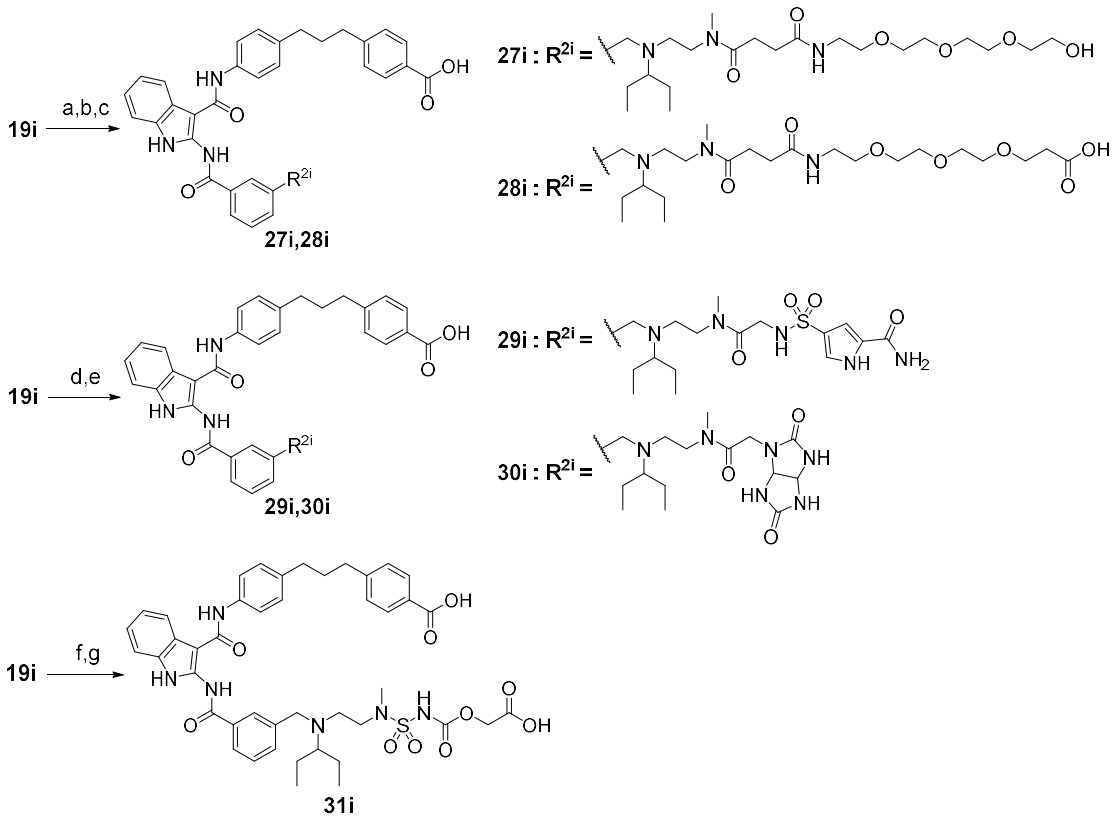
Scheme 6i. Preparation of **22i** and **23i**. Reagents and conditions: (a) for **22i**, *tert*-butyl *N*-methyl-*N*-{2-[(heptane-4-yl)amino]ethyl}carbamate, DIPEA, NaI, toluene, 95 °C, 64%; for **23i**, 3-(*tert*-butoxycarbonylamino)pyrrolidine, DIPEA, NaI, toluene, 50 °C - 100 °C, 94%; (b) TFA, $CHCl_3$, rt-45 °C; (c) succinic anhydride, THF, rt; (d)1M-NaOH aq., MeOH, 65 °C, then 2M-HCl aq. THF, rt, 67%-78% in 3 steps.



Scheme 7i. Preparation of **26i**. Reagents and conditions: (a) ethylsuccinylchloride, DIPEA, THF, rt, 98%; (b)TFA, $CHCl_3$, rt, 77%; (c) DIPEA, NaI, Toluene, **18i**, 80 °C, 97%; (d) 1M-NaOH aq., THF, MeOH, 60 °C, then 2M-HCl aq. THF, rt, 66%.

インドール誘導体 **27i-31i** の合成法を **Scheme 8i** に示す。化合物 **19i** を無水コハク酸でアミド化した後、2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}ethanol でアミド化、エステルを NaOH 水溶液で加水分解してカルボン酸 **27i** を得た。化合物 **19i** を無水コハク酸でアミド化した後、*tert*-butyl 3-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}propanoate でアミド化し、TFA で *tert*-ブチル基を脱保護、エステルを NaOH 水溶液で加水分解して化合物 **28i** を得た。化合物 **19i** を 2-(5-carbamoyl-1h-pyrrole-3-sulfonamido)acetic acid でアミド化した後、NaOH 水溶液で加水分解して化合物 **29i** を得た。化合物 **19i** と(2,5-dioxo-hexahydro-imidazo[4,5-d]-imidazol-1-yl)-acetic acid を用いて、化合物 **29i** と同様な方法でカルボン酸 **30i** を合成した。2-ヒドロキシ酢酸ベンジルとクロロスルホニルイソシアネートの反応混合液に 2 級アミン **19i** を添加し、対応するスルファモイル

カルバメートを得た。その後、得られたスルファモイルカルバメート中のベンジルエステルを NaOH 水溶液で加水分解して、カルボン酸 **31i** を得た。

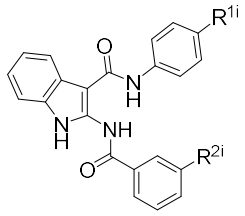


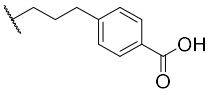
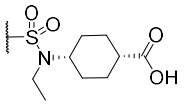
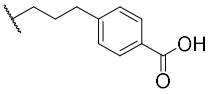
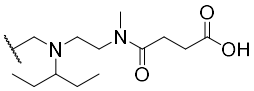
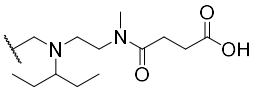
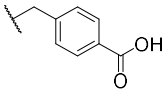
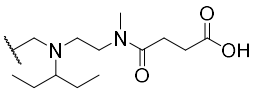
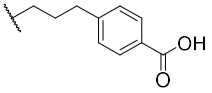
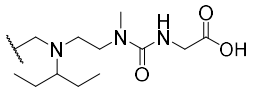
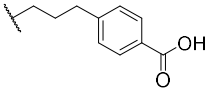
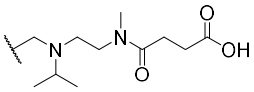
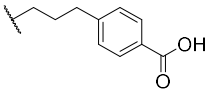
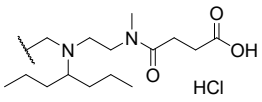
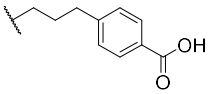
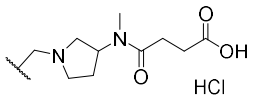
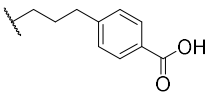
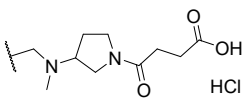
Scheme 8i. Preparation of **27i**-**31i**. Reagents and conditions: (a) succinic anhydride, DIPEA, THF, rt, quantitative yield; (b) for **27i**, 2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}ethanol, HATU, DIPEA, MeCN, rt; for **28i**, *tert*-butyl 3-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}propanoate, HATU, DIPEA, MeCN, rt; (c) for **27i**, 4M-NaOH aq., MeOH, THF, 60 °C, 51% in 2 steps; for **28i**, TFA, rt then 4M-NaOH aq., MeOH, THF, 60 °C, 80% in 3 steps; (d) for **29i**, 2-(5-carbamoyl-1h-pyrrole-3-sulfonamido)acetic acid, HATU, DIPEA, DMF, rt; for **30i**, (2,5-dioxo-hexahydro-imidazo[4,5-d]-imidazol-1-yl)-acetic acid, HATU, DIPEA, DMF, rt; (e) 4M-NaOH aq., MeOH, THF, rt-60 °C, 16%-22% in 2 steps; (f) benzyl 2-hydroxyacetate, chlorosulfonyl isocyanate, 1,2-dichloroethane, -15 °C-rt, then **19i**, Et_3N, -15 °C-rt, 44%; (g) 2M-NaOH aq., MeOH, THF, 65 °C, 75%.

第 3 節　インドール化合物（**5i-31i**）の NaPi2b 阻害活性

DFT により算出した最安定構造において、分子内水素結合によりコア構造が固定化されていることが想定されたインドール骨格を有する化合物 **5i** を合成・評価したところ、NaPi2b 阻害活性が認められ、このコア骨格が有効であることが示された。次に、R^{1i} 部位の構造活性相関を検討した（**Table 1i**）。その結果、化合物 **5i** が最も優れた阻害活性を示し、化合物 **16i**, **17i** では阻害活性の減弱が認められた。R^{2i} 部位では、ウレア誘導体 **20i** とイソプロピル誘導体 **21i** は化合物 **5i** よりも低い活性を示した。一方、1-プロピルブチル誘導体 **22i** の阻害活性は、化合物 **5i** と同程度であった。これらの結果から、N-メチル部分はウレアよりもアミドの方が優れた阻害活性を示すことが示唆され、3 級アミン部位は嵩高い置換基の方が優れた阻害活性を示す傾向があることが示唆された。また、環化により立体構造を固定させた化合物 **23i** 及び **26i** は、阻害活性が著しく低下していた。これらの結果を踏まえ、化合物 **5i** を中心に更なる誘導体展開を実施した。

Table 1i. Human NaPi2b inhibitory activities of compounds **5i**, **14i**, **16i**, **17i**, **20i-23i**, and **26**



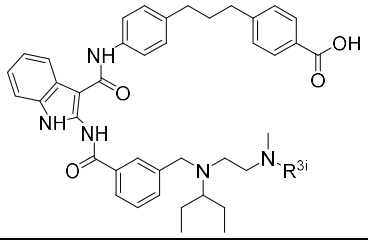
Compound	R^{1i}	R^{2i}	human NaPi2b IC_{50} (nM)[a]
14i			257
5i			71
16i			538
17i			448
20i			369
21i			356
22i			78
23i			1015
26i			2582

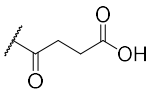
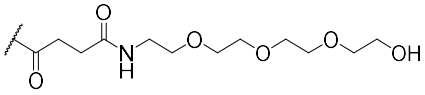
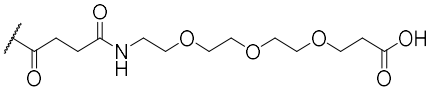
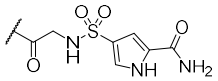
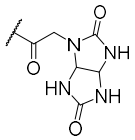
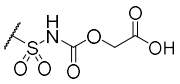
[a] The IC_{50} values for human NaPi2b activity represent the mean values of at least two experiments.

良好な活性と高い tPSA を有するインドール誘導体の創出を目指して、化合物 **5i** の R^{3i} 部位の SAR を検討した。その結果を **Table 2i** に示す。エチレングリコール型誘導体 **27i** は、化合物 **5i** と比較して活性はやや劣るものの、良好な阻

害活性を示した。様々な高極性置換基を導入した化合物 **28i-31i** は、中程度の NaPi2b 阻害活性を示した。化合物 **5i** と **27i** の rat NaPi2b に対する阻害活性を評価したところ、human NaPi2b に対する阻害活性とほぼ同等であった。また、化合物 **5i** 及び **27i** の tPSA 値はそれぞれ 172Å^2 及び 211Å^2 であった。そこで、化合物 **5i** 及び **27i** を SD ラットを用いた PK 試験で評価した。

Table 2i. *In vitro* activities and tPSAs of compounds **5i** and **27i-31i**



Compound	R^{3i}	human/rat NaPi2b IC_{50} (nM)[a]	tPSA[b] (Å2)
5i		71/28	172
27i		166/98	212
28i		535/NT[c]	229
29i		401/NT[c]	248
30i		401/NT[c]	208
31i		267/ND[d]	234

[a] The IC_{50} values for human/rat NaPi2b activity represent the mean values of at least two experiments

[b] The tPSA value was calculated using a software from ACD/Percepta, version 2019, Advanced Chemistry Development, Inc.

[c] Not tested.

[d] Not determined.

第 4 節　インドール化合物（**5i**、**27i**）の薬物動態

化合物 **5i**、**27i** の PK 試験の結果を **Table 3i** に示す。SD ラットにおける化合物 **5i**（tPSA=172Å^2）のバイオアベイラビリティは 13.1%であった。一方で **5i** よりも高い tPSA（=211Å^2）を持つ化合物 **27i** のバイオアベイラビリティは 1.1%と低い値を示した。チオフェン化合物及びピリジン化合物と同様に、**Figure 1i** に示したような分子内水素結合による影響により、吸収性を低下させるために通常よりも高い tPSA が必要であった傾向が認められた。**Table 4i** に示すように、経口投与後、化合物 **5i** 及び **27i** の大部分は未変化体として糞中に排泄された。また、**Table 5i** に示すように、化合物 **5i** 及び **27i** の人工腸液中の溶解度は、rat NaPi2b に対する IC_{50} 値を十分に上回っていた。第 2 章において疎水性が比較的低い（CLogP<7）腸管選択的ピリジン誘導体がラットにおいてリン酸吸収阻害作用を示すことを示したが、化合物 **5i** と **27i** の ClogP 値はピリジン誘導体でリン吸収抑制作用が認められた化合物 **20bp**（ClogP=6.50）よりも更に低い値であった。バイオアベイラビリティ及び全身曝露に違いがあるが、それ以外のプロファイルはほぼ同等の化合物 **5i** と **27i** を取得できたので、両化合物について、SD ラットを用いたリン吸収抑制作用を評価した。

Table 3i. Pharmacokinetic parameters after a single intravenous (i.v.) or oral (p.o.) administration of **5i** or **27i** to fasted male SD rats

Compound	i.v.[a]		p.o.[b]			
	Dose (mg/kg)	$AUC_{0\text{-}t}$[c] (h*ng/mL)	Dose (mg/kg)	C_{max}[c] (ng/mL)	$AUC_{0\text{-}t}$[c] (h*ng/mL)	F (%)
5i	1	834 ± 353	10	568 ± 261	1090 ± 430	13.1
27i	1.17	170 ± 4	10	15.5 ± 6.5	15.4 ± 5.7	1.1

[a] Dosing vehicle was PEG400.

[b] Dosing vehicle was 0.5%MC400.

[c] Results are presented as the means ± standard deviation (S.D.) of three animals.

Table 4i. Cumulative excretion of unchanged forms into feces within 24 hours after oral administration of **5i** or **27i** to fasted male SD rats

Compound	p.o.[a]	
	Dose (mg/kg)	Fecal excretion (%)[b]
5i	10	68.1
27i	10	58.8

[a] Dosing vehicle was 0.5%MC400.

[b] Results are presented as the mean of three animals.

Table 5i. Solubility (FaSSIF, FeSSIF) and ClogP of **5i** and **27i**

Compound	Solubility (μg/mL) FaSSIF (pH 6.5)	Solubility (μg/mL) FeSSIF (pH 5.0)	ClogP[a]
5i	103[b]	264[b]	6.09
27i	>482[b]	>479[b]	4.99

[a] The ClogP value was calculated using ChemDraw Professional ver.19.1.1.21, PerkinElmer Informatics, Inc.

[b] Hydrochloride salt data.

第 5 節　インドール化合物（**5i**、**27i**）のリン吸収抑制作用

化合物 **5i**、**27i** の SD ラットを用いたリン吸収抑制作用を評価した結果を **Figure 3i** に示す。文献を参考に算出した 10 mg/kg 経口投与後の推定腸管内濃度は 58-146 μg/mL であり、第 1 章及び第 2 章での検討と同様に、化合物 **5i** 及び **27i** の rat NaPi2b IC_{50} 値を大きく上回っている条件で評価を実施した。また、未変化体の糞中への排泄率（化合物 **5i**=68.1%，化合物 **27i**=58.8%）を用いて補正しても、化合物 **5i** 及び **27i** の推定腸管内濃度はそれぞれ 39-99 μg/mL（58-146 μg/mL×0.681）、34-86 μg/mL（58-146 μg/mL×0.588 ）となり、それぞれの IC_{50} 値を十分に上回っていると考えられた。この条件下で、化合物 **5i** 及び **27i** はいずれも有意なリン吸収抑制作用を示した。第 1 章で、高疎水性（CLogP>9）かつ腸管選択的なチオフェン化合物は SD ラットにおけるリン吸収を抑制しなかった。また第 2 章では、疎水性を低減した腸管選択的なピリジン化合物（ClogP<7）がリン吸収抑制作用を示した。今回、更に疎水性の低いインドール誘導体 **5i** 及び **27i** を検討した結果、両化合物は SD ラットにおいてリン吸収抑制作用を示し、化合物の疎水性を低減することがリン吸収抑制作用の発現に有効であることが再確認された。化合物 **5i** と **27i** は、全身曝露（$AUC_{0\text{-}t}$）が 50 倍以上異なるが、同等の *in vivo* 有効性が認められたことから、リン吸収抑制作用は全身曝露に影響されないことが示された。この結果から考察すると、*in vitro* での阻害活性、物性（溶解性、疎水性）、及び未変化体の糞中への排泄率が同等の化合物は、全身曝露にかかわらず、同等のリン吸収抑制作用を発揮することが期待できることが考えられた。一方、全身暴露による副作用リスクを低減する観点から考察すると、化合物 **27i** は NaPi2b 阻害活性の IC_{50} 値を下回る C_{max} で有意なリン吸収抑制作用を示しており、腸管選択的に NaPi2b を阻害することで *in vivo* 薬効を示す望ましい化合物であると考えられた。検討の結果、第 2 章で見出したピリジン誘導体（化合物 **20bp**）よりも更に低いバイオアベイラビリティと低い全身曝露を示し、かつリン吸収抑制作用を示す化合物 **27i** の取得を達成した。

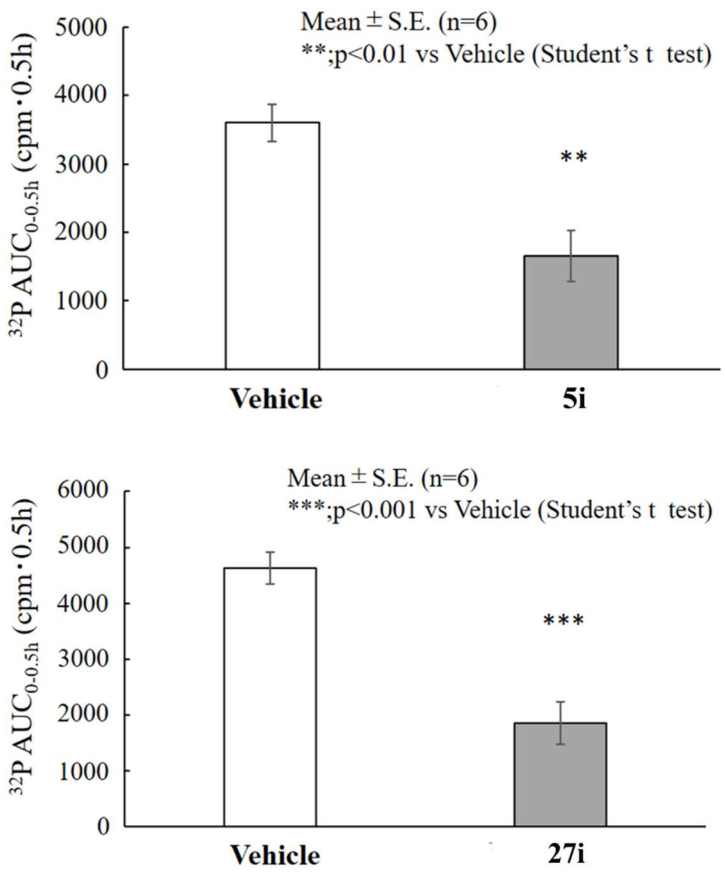


Figure 3i. ^{32}P phosphate was administered to SD rats 5 minutes after oral dosing with of compound **5i** hydrochloride salt or compound **27i** (10 mg/kg, respectively), and then the radioactivity in the plasma was measured for 0.5 hours (the approximate time it takes for phosphate to reach the maximum concentration in the plasma - data not shown).

第 6 節　まとめ

本章ではより低い全身曝露で SD ラットにおいてリン吸収抑制作用を示す腸管選択的な化合物を取得すべく、インドール骨格を有する誘導体のデザイン・合成・評価を行った。NaPi2b 阻害活性の発現には、分子内相互作用により固定化されたコア骨格が重要であることが示唆されていたので、インドール骨格についても、同様の分子内水素結合の形成ができるようにデザインした。その結果、化合物 **5i** に良好な human NaPi2b 阻害活性が認められ、このコア骨格が有効であることが示された。R^{1i}、R^{2i} 部位の構造活性相関を探索したところ、化合物 **5i** が阻害活性の面で優れていた。R^{2i} 部位の末端部分に相当する R^{3i} 部位については高極性基の導入が可能であり、良好な阻害活性、高い tPSA 値を示す化合物 **27i** を取得した。化合物 **5i** 及び **27i** の PK 試験の結果、化合物 **5i** は 10%を超える経口吸収性が認められ、化合物 **27i** は低い経口吸収性が認められた。化合物 **5i** 及び **27i** は SD ラットにおいてリン吸収抑制作用を示したことから、化合物の疎水性を低減することが薬効発現に有効であることが再確認できた。また、化合物 **5i** 及び **27i** が同等のリン吸収抑制作用を示した結果から考察すると、*in vitro* での阻害活性、物性（溶解性、疎水性）、及び未変化体の糞中への排泄率が同等の化合物であれば、全身曝露にかかわらず、同等のリン吸収抑制作用を発揮することが期待できることが示唆された。化合物 **27i** は腸管選択的な化合物で、最も低いバイオアベイラビリティと全身曝露で良好なリン吸収抑制作用を示した。

結論

慢性腎不全患者や透析患者は高リン血症を発症し、骨代謝異常、異所性石灰化などを合併する。「慢性腎臓病に伴う骨・ミネラル代謝異常の診療ガイドライン」では、高リン血症を優先して是正することが推奨されている。しかし、高リン血症治療薬としてはリン吸着薬という種類の薬剤しかなく、安全性や服薬負担の面で課題を抱えており、それらの課題が改善された新しい治療薬が望まれている。

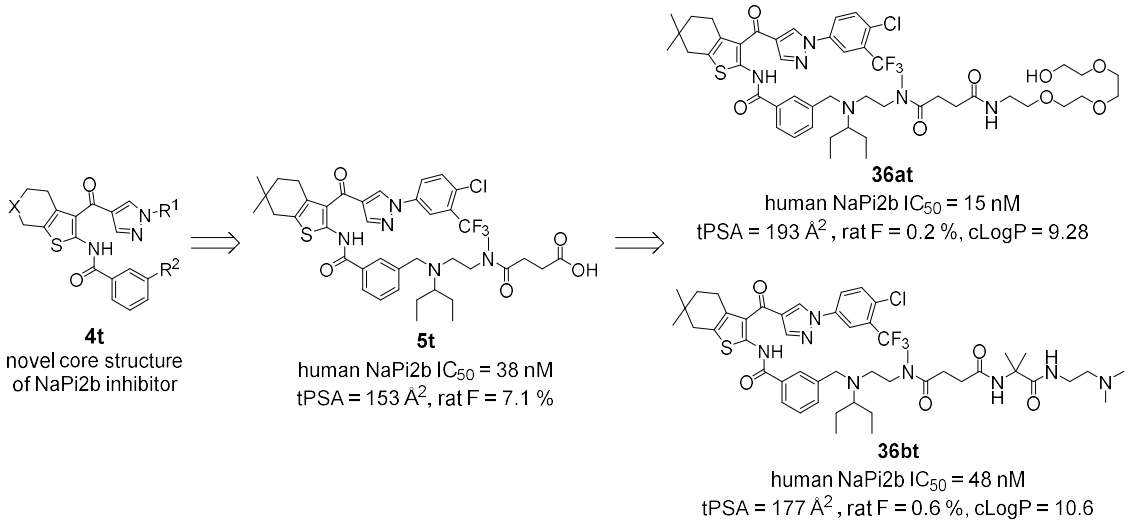
腸管に発現しているトランスポーターの NaPi2b は、食事由来のリンを吸収する役割を担っていることが報告されている。従って、その阻害物質はリン吸着薬とは異なる新規メカニズムの分子標的薬になり得ると考えられ、リン吸着薬の副作用を回避しつつ、服薬量も低減して、腎不全患者の服薬アドヒアランスを改善する可能性があると考えられる。しかし NaPi2b は肺や精巣にも発現しており、それらを阻害することによる副作用のリスクが報告されていることから、高い組織選択性を有する薬剤の創出が望ましいと考えられた。そこで、化合物の経口吸収性を可能な限り低下させ、腸管選択的に作用する NaPi2b 阻害物質の創出研究を行った。

第 1 章では、分子内相互作用によりコア構造が固定化されていることが NaPi2b 阻害活性の発現に重要である可能性が示唆されていたことを踏まえ、その分子内相互作用の維持が期待できるチオフェン骨格をデザインし、誘導体合成を展開した。NaPi2b 阻害活性を指標に構造活性相関を探索し、優れた NaPi2b 阻害活性を示す化合物 **5t** を取得した。次に腸管選択的な化合物の取得を目指し、経口吸収性を低下させるため tPSA を指標に高極性置換基の導入を検討した。その結果、化合物 **5t** のカルボン酸部位において様々な高極性置換基の導入が許容されることを見出した。その構造活性相関に基づいて誘導体展開を実施し、良好な NaPi2b 阻害活性を有し、ラットにおいて低いバイオアベイラビリティを示す化合物 **36at** 及び **36bt** を創出した。しかし、化合物 **36at**、**36bt** は SD ラットを用いた in vivo 薬効評価においてリン吸収抑制作用を示さなかった。化合物の薬物動態や物性を精査した結果、高い疎水性が原因で、腸管表面近傍の非攪拌水層の透過性が低下すること、あるいは腸管内でミセルとの親和性が上がってしまうことにより、腸管上皮細胞管腔側に存在する NaPi2b へのアクセシビリティが低下する可能性が一因として考えられた。

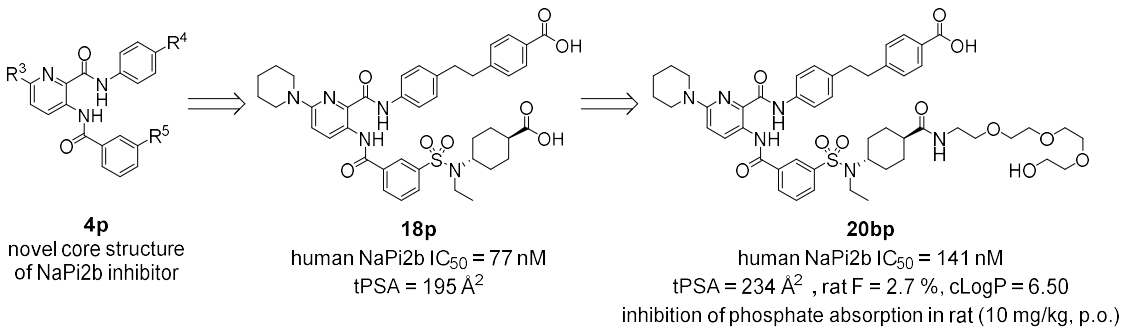
第 2 章では、第 1 章の考察に基づいて、分子内相互作用によりコア構造が固定化されており、かつ疎水性の低減が期待できる新たなコア構造として、ピリジン骨格について検討した。NaPi2b 阻害活性を指標に構造活性相関を探索し、良好な NaPi2b 阻害活性を示す化合物 **18p** を見出した。次に経口吸収性を低下させるために、tPSA を増加させる高極性置換基の導入を検討した結果、カルボン酸部位において高極性置換基の導入が許容されることを見出し、バイオアベイラビリティの低い化合物 **20bp** を創出した。化合物 **20bp** のラットにおけるリン吸収抑制作用を評価した結果、10 mg/kg の経口投与において、有意な薬効が認められた。この結果から、化合物の疎水性が in vivo 薬効に影響を及ぼす可能性が示された。

第 3 章では、より低い経口吸収性でリン吸収抑制作用を示す化合物の取得を目指し、分子内相互作用によりコア構造が固定化されており、かつ疎水性の低減が期待できるインドール骨格の化合物について検討した。NaPi2b 阻害活性を指標に構造活性相関を探索し、良好な NaPi2b 阻害活性を示す化合物 **5i** を見出した。次に腸管からの吸収性を低下させるために、tPSA を増加させる高極性置換基の導入を検討した。その結果、インドール骨格の化合物についてもカルボン酸部位において様々な高極性置換基の導入が許容されることを見出し、化合物 **27i** を創出した。化合物 **27i** のラットにおけるリン吸収抑制作用を評価した結果、10 mg/kg の経口投与において、有意に腸管からリン吸収を抑制した。化合物 **27i** は、最も低いバイオアベイラビリティで、ラットにおいてリン吸収抑制作用を示した化合物であり、全身曝露による副作用のリスクが少ない高リン酸血症治療薬の候補として期待できる。

第 1 章　チオフェン骨格の NaPi2b 阻害物質の研究



第 2 章　ピリジン骨格の NaPi2b 阻害物質の研究



第 3 章　インドール骨格の NaPi2b 阻害物質の研究

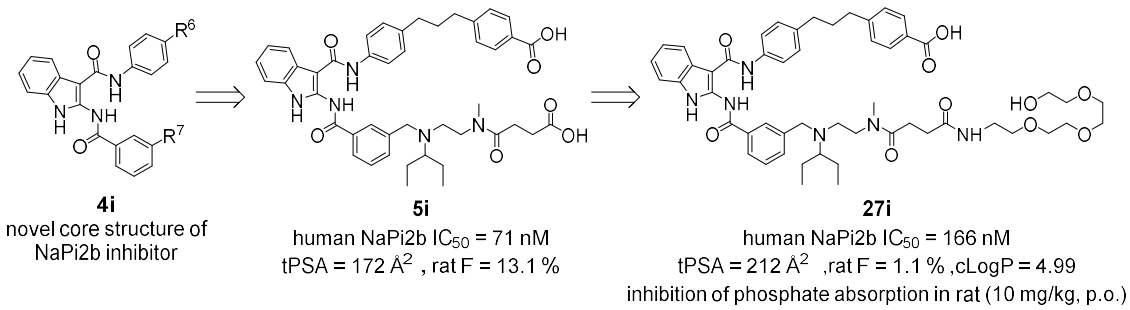


Figure 7. 本研究の概要

謝辞

本論文を纏めるにあたり、終始御懇切なる御指導と御鞭撻を賜りました千葉大学大学院薬学研究院教授 根本哲宏先生に深甚なる謝意を表します。また、本論文の審査にあたり、適切な御教示と御高閲を賜りました千葉大学大学院薬学研究院教授 石川勇人先生、千葉大学大学院薬学研究院准教授 北島満里子先生、千葉大学大学院薬学研究院講師 原田慎吾先生に深謝致します。

また、本研究の機会、及び本論文発表の許可を与えて頂きました大正製薬株式会社社長 上原茂氏、同医薬研究本部本部長 ロドニー W．スティーブンス博士、同 Discovery 研究所所長 野田昌邦博士に厚く御礼申し上げます。

また、終始御指導と温かい激励を賜りました大正製薬株式会社研究本部 柿沼浩行博士、同化学第 2 研究室室長 小橋陽平博士に謹んで感謝致します。

本研究を遂行するにあたり、御指導と御協力を頂きました大正製薬株式会社化学第 2 研究室 宇根内史氏、同化学第 1 研究室 岡田久美子氏、山口千歳氏、同知的財産 川部憲一氏、同品質保証 太田裕之博士、同プロセス化学研究室 柴田剛氏、同医薬安全管理 奥村理沙氏、同開発管理部 小佐井有紀氏、同創薬技術研究室 遠藤真弓博士、樋口彰氏、岡田敦司博士、同研究推進室 宗友栄二氏、同薬理第 2 研究室 高橋禎介博士、同製剤・分析研究室 野副晶子氏、並びに、大竹克昌氏、阿部智大氏、長南具通博士に深く感謝致します。

最後に、教育の機会を与えてくださった両親、本論文の作成にあたり心の支えとなってくれました妻 由紀子と家族に感謝致します。

実験の部

Chemistry

All the solvents and reagents were obtained from commercial suppliers and were used without further purification or were prepared according to published procedures. The ^{1}H NMR and ^{13}C NMR spectra were recorded using a JOEL JNM-ECA 600 or BRUKER AVANCE III HD 400, and all the chemical shifts were reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded using a Shimadzu LCMS 2010 EV spectrometer. High-resolution mass spectral data were acquired using a Shimadzu LCMS-IT-TOF equipped with an electrospray ionization (ESI)/atmospheric pressure chemical ionization (APCI) dual ion source. ESI mass spectra, the retention time (Rt) and Purity of high-performance liquid chromatography mass spectra (LCMS) were recorded using an Agilent 6130 or 6150 Quadrupole LC/MS connected to an Agilent 1290 Infinity HPLC instrument under the following conditions: column, Waters Acquity CSH C18 (1.7 μm, 2.1×50 mm); mobile phase A, H_2O containing 0.1% formic acid; mobile phase B, CH_3CN containing 0.1% formic acid; gradient (NM mode), 20% B to 99% over 1.2 min followed by 99% B over 0.2 min; gradient (HP mode), 5% B to 40% over 0.8 min then 40% B to 99% over 0.28 min followed by 99% B over 0.3 min; gradient (LP mode), 30% B to 99% over 0.8 min followed by 99% B over 0.6 min; flow rate, 0.8 mL/min. The wavelengths of detection were 210 nm and 254 nm.

第 1 章

Benzyl 4-(methyl{2-[(pentan-3-yl)amino]ethyl}amino)-4-oxobutanoate (8t)

4-benzyloxy-4-oxo-butanoic acid (15.0 g, 72.0 mmol), 1,2,3-benzotriazol-1-ol monohydrate (HOBt・H_2O) (13.2 g, 86.5 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC・HCl) (16.6 g, 86.5 mmol) were added to a solution of *tert*-butyl [2-(methylamino)ethyl]carbamate (12.6 g, 72.0 mmol) in MeCN (50 mL). The mixture was stirred overnight at room temperature and concentrated. The residue was diluted with $CHCl_3$ and washed with saturated ammonium chloride

(NH_4Cl) aqueous solution and saturated sodium hydrogen carbonate ($NaHCO_3$) aqueous solution. The aqueous layer was separated and extracted with $CHCl_3$ using a phase separator. The combined organic layer was concentrated to yield benzyl 4-[{2-[(*tert*-butoxycarbonyl)amino]ethyl}(methyl)amino]-4-oxobutanoate (26.1 g) as a crude product, which was used without further purification. The product (26.1 g) was taken up in 1,4-dioxane (100 mL). Then, 4M 1,4-dioxane solution of hydrochloric acid (HCl) (53.8 mL, 215 mmol) was added to this solution, and the reaction mixture was stirred overnight at room temperature. The resulting precipitates were collected by filtration, washed with IPE, and dried to yield benzyl benzyl 4-[(2-aminoethyl)(methyl)amino]-4-oxobutanoate hydrochloride (26.0 g) as a crude product, which was used without further purification. The product (30.5 g) was taken up in $CHCl_3$ (330 mL). Then, 3-pentanone (12.2 mL, 115 mmol) and AcOH (19.8 mL, 346 mmol) were added to the solution, and the reaction mixture was stirred at room temperature for 2.5 h. Sodium triacetoxyborohydride ($NaBH(OAc)_3$) (48.9 g, 231 mmol) was added and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was quenched with saturated $NaHCO_3$ aqueous solution. The aqueous layer was separated and extracted with $CHCl_3$ using a phase separator. The combined organic layer was concentrated. The residue was purified using column chromatography on an NH-silica gel and eluted with 5-15% EtOAc/*n*-hexane to yield **8t** as a colorless oil (11.8 g, 31% over 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (td, J = 7.5, 1.7 Hz, 6H) 1.36 - 1.43 (m, 4H), 2.31 – 2.39 (m, 1H), 2.60 - 2.67 (m, 1H), 2.69 - 2.79 (m, 5H), 2.92 - 3.07 (m, 3H), 3.36 - 3.50 (m, 2H), 5.13 (s, 2H), 7.28 - 7.38 (m, 5H). LCMS (ESI) *m/z* 335 $[M+H]^+$. Rt 0.528 min (NM mode).

3-{[(2-{[4-(Benzyloxy)-4-oxobutanoyl](methyl)amino}ethyl)(pentan-3-yl)amino]methyl}benzoic acid hydrochloride (9t)

Compound **8t** (8.11 g, 24.3 mmol), *N*,*N*-diisopropylethylamine (DIPEA) (5.76 mL, 33.1 mmol) and sodium iodide (NaI) (3.31 g, 22.1 mmol) were added to a solution of *tert*-butyl 3-(chloromethyl)benzoate (5.00 g, 22.1 mmol) in MeCN (150 mL). After stirring at 85 °C for 4 h, the reaction mixture was cooled to room temperature and water was added. After concentrating the MeCN, the aqueous layer was separated and

extracted with $CHCl_3$ using a phase separator. The combined organic layer was then concentrated. The residue was purified using column chromatography on silica gel and eluted with 5%-15% EtOAc/*n*-hexane to yield *tert*-butyl 3-{[(2-{[4-(benzyloxy)-4-oxobutanoyl](methyl)amino}ethyl)(pentan-3-yl)amino]methyl}benzoate as a colorless oil. Next, 4M 1,4-dioxane solution of HCl (10 mL) was added to the product (5.00 g), and the mixture was stirred at room temperature for 3 h. After concentration, the residue was added to Et_2O, then the mixture was concentrated and dried to yield **9t** (4.70 g, 89% over 2 steps) as a colorless amorphous substance. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 - 1.10 (m, 6H), 1.48 - 1.72 (m, 2H), 2.61 - 2.76 (m, 4H), 2.84 - 2.92 (m, 1H), 3.12 – 3.40 (m, 4H), 3.71 (s, 2H), 4.07 (br s, 2H), 4.52 (br s, 2H), 5.14 (s, 2H), 7.27 - 7.41 (m, 5H), 7.51 - 7.60 (m, 1H), 8.03 - 8.12 (m, 1H), 8.24 - 8.33 (m, 2H), 11.29 - 11.53 (m, 1H). LCMS (ESI) *m/z* 469 $[M+H]^+$, 467 $[M-H]^-$. Rt 0.655 min (NM mode).

3-[1-(4-Chlorophenyl)-1*H*-pyrazol-4-yl]-3-oxopropanenitrile (11t)

A 2.6M *n*-butyllithium (*n*-BuLi) solution in *n*-hexane (47.0 mL, 121 mmol) was added dropwise to a THF (250 mL) solution of MeCN (5.80 mL, 110 mmol) at −78 °C in a nitrogen atmosphere, and the mixture was stirred for 15 min at the same temperature. Then, a THF (100 mL) solution of ethyl 1-(4-chlorophenyl)-1H-pyrazole-4-carboxylate **10t** (13.8 g, 55.1 mmol) was added dropwise over 30 min, and the mixture was stirred for 1 h at the same temperature. The reaction mixture was quenched with saturated NH_4Cl aqueous solution. The aqueous layer was extracted with EtOAc, and the combined organic layer was concentrated. The resulting solid was washed with EtOAc to yield **11t** as a pink solid (10.3 g, 76%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.54 (br s, 2H), 7.64 (d, J = 8.8 Hz, 2H), 7.94 (d, J = 8.8 Hz, 2H), 8.29 (s, 1H), 9.31 (br s, 1H). LCMS (ESI) *m/z* 246 $[M+H]^+$, 244 $[M-H]^-$. Rt 0.896 min (NM mode).

(2-Amino-4,5,6,7-tetrahydro-1-benzothiophene-3-yl)[1-(4-chlorophenyl)-1*H*-pyrazol-4-yl]methanone (13at)

To a suspension of **11t** (3.00 g, 12.2 mmol) in EtOH (20 mL) was added cyclohexanone **12at** (1.26 mL, 12.2 mmol), morpholine (1.06 mL, 12.2 mmol) and sulfur (0.189 g, 12.2 mmol). The reaction mixture was stirred at 80 °C for 2 h. The

solvent was distilled off under reduced pressure, and the residue was purified using column chromatography on a silica gel and eluted with 33% EtOAc/*n*-hexane to yield **13at** (2.34 g, 53%) as a yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58 - 1.65 (m, 2H), 1.77 - 1.86 (m, 2H), 2.19 - 2.29 (m, 2H), 2.48 - 2.65 (m, 2H), 6.30 (br s, 2H), 7.44 - 7.52 (m, 2H), 7.65 - 7.69 (m, 2H), 7.91 (s, 1H), 8.20 (s, 1H). LCMS (ESI) *m/z* 358 $[M+H]^+$, 356 $[M-H]^-$. Rt 1.214 min (NM mode).

(2-Amino-4,7-dihydro-5*H*-thieno[2,3-c]pyran-3-yl)[1-(4-chlorophenyl)-1*H*-pyrazol-4-yl]methanone (13bt)

Compound **13bt** (74%) was prepared from tetrahydropyran-4-one **12bt** in a manner similar to that described for compound **13at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29 - 2.47 (m, 2H), 3.76 (t, J = 5.3 Hz, 2H), 4.58 - 4.68 (m, 2H), 6.47 (br s, 2H), 7.40 - 7.49 (m, 2H), 7.67 (d, J = 8.8 Hz, 2H), 7.94 (s, 1H), 8.21 (s, 1H). LCMS (ESI) *m/z* 360 $[M+H]^+$, 358 $[M-H]^-$. Rt 0.753 min (LP mode).

(2-Amino-4,7-dihydro-5*H*-thieno[2,3-c]thiopyran-3-yl)[1-(4-chlorophenyl)-1*H*-pyrazol-4-yl]methanone (13ct)

Compound **13ct** (65%) was prepared from tetrahydrothiopyran-4-one **12c** in a manner similar to that described for compound **13at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.57 - 2.65 (m, 2H), 2.66 - 2.72 (m, 2H), 2.87 - 2.94 (m, 1H), 3.69 - 3.76 (m, 1H), 6.19 (br s, 2H), 7.46 (d, J = 8.6 Hz, 2H), 7.67 (d, J = 8.6 Hz, 2H), 7.86 - 7.99 (m, 1H), 8.17 - 8.29 (m, 1H). LCMS (ESI) *m/z* 376 $[M+H]^+$, 374 $[M-H]^-$. Rt 0.856 min (LP mode).

1-{2-Amino-3-[1-(4-chlorophenyl)-1*H*-pyrazole-4-carbonyl]-4,7-dihydrothieno[2,3-c]pyridin-6(5*H*)-yl}ethan-1-one (13dt)

Compound **13dt** (77%) was prepared from 1-acetyl-4-piperidone **12dt** in a manner similar to that described for compound **13at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.06 - 2.19 (m, 3H), 2.37 - 2.50 (m, 2H), 3.51 - 3.73 (m, 2H), 4.42 - 4.61 (m, 2H), 6.37 - 6.51 (m, 2H), 7.40 - 7.51 (m, 2H), 7.60 - 7.73 (m, 2H), 7.89 (s, 1H), 8.10 - 8.27 (m, 1H). LCMS (ESI) *m/z* 401 $[M+H]^+$, 399 $[M-H]^-$. Rt 0.863 min (NM mode).

[2-Amino-6-(methanesulfonyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-3-yl][1-(4-chlorophenyl)-1*H*-pyrazol-4-yl]methanone (13et)

Compound **13et** (84%) was prepared from 1-methylsulfonylpiperidin-4-one **12et** in a manner similar to that described for compound **13at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 - 2.58 (m, 2H), 2.87 (s, 3H), 3.32 - 3.45 (m, 2H), 4.24 - 4.36 (m, 2H), 6.40 (s, 2H), 7.42 - 7.51 (m, 2H), 7.67 (d, J = 8.8 Hz, 2H), 7.87 - 7.94 (m, 1H), 8.16 - 8.25 (m, 1H). LCMS (ESI) *m/z* 437 $[M+H]^+$, 435 $[M-H]^-$. Rt 0.939 min (NM mode).

(2-Amino-6-cyclopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-3-yl)[1-(4-chlorophenyl)-1*H*-pyrazol-4-yl]methanone (13ft)

Compound **13ft** (77%) was prepared from cyclopropylpiperidin-4-one **12ft** in a manner similar to that described for compound **13at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.43 - 0.58 (m, 4H), 1.78 - 1.88 (m, 1H), 2.31 - 2.43 (m, 2H), 2.76 (t, J = 5.6 Hz, 2H), 3.61 - 3.66 (m, 2H), 6.46 (s, 2H), 7.45 (d, J = 8.8 Hz, 2H), 7.62 - 7.72 (m, 2H), 7.88 - 7.97 (m, 1H), 8.15 - 8.22 (m, 1H). LCMS (ESI) *m/z* 399 $[M+H]^+$, 397 $[M-H]^-$. Rt 0.569 min (NM mode).

Benzyl 4-[{2-[{[3-({3-[1-(4-chlorophenyl)-1*H*-pyrazole-4-carbonyl]-4,5,6,7-tetrahydro-1-benzothiophene-2-yl}carbamoyl)phenyl]methyl}(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoate (14at*)*

Thionyl chloride ($SOCl_2$) (1.23 mL, 16.8 mmol) was added to a solution of **9t** (1.31 g, 2.80 mmol) in $CHCl_3$ (5.0 mL) and the mixture was stirred at 75 °C for 1 h. The reaction mixture was concentrated under reduced pressure, and the residue was azeotroped twice with $CHCl_3$ to yield the corresponding carboxylic acid chloride as a crude product, which was used without further purification. The resulting carboxylic acid chloride in $CHCl_3$ (5.0mL) was then added to a solution of **13at** (500 mg, 1.40 mmol) and pyridine (0.680 mL, 8.38 mmol) in $CHCl_3$ (5.0 mL). The reaction mixture was stirred overnight at room temperature, then quenched with saturated NH_4Cl aqueous solution. The aqueous layer was extracted with $CHCl_3$, and the combined organic layer was then concentrated under reduced pressure. The residue was purified using column chromatography on a silica gel and eluted with 50% EtOAc/*n*-hexane to

yield **14at** (952 mg, 84%) as a yellow amorphous substance. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 - 1.03 (m, 6H), 1.30 - 1.53 (m, 4H), 1.63 - 1.72 (m, 2H), 1.83 - 1.93 (m, 2H), 2.22 - 2.45 (m, 4H), 2.51 - 2.69 (m, 5H), 2.74 - 2.94 (m, 5H), 3.14 - 3.22 (m, 1H), 3.36 (br t, J = 7.1 Hz, 1H), 3.71 (s, 2H), 5.02 - 5.14 (m, 2H), 7.28 - 7.50 (m, 7H), 7.53 - 7.72 (m, 3H), 7.81 - 8.19 (m, 4H), 8.26 - 8.33 (m, 1H), 12.06 - 12.19 (m, 1H). LCMS (ESI) *m/z* 808 $[M+H]^+$, 806 $[M-H]^-$. Rt 1.149 min (NM mode).

Benzyl 4-[{2-[{[3-({3-[1-(4-chlorophenyl)-1*H*-pyrazole-4-carbonyl]-4,7-dihydro-5*H*-thieno[2,3-c]pyran-2-yl}carbamoyl)phenyl]methyl}(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoate (14bt)

Compound **14bt** (62%) was prepared from **13bt** in a manner similar to that described for compound **14at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 - 0.98 (m, 6H), 1.34 - 1.52 (m, 4H), 1.58 - 1.68 (m, 2H), 2.24 - 2.39 (m, 2H), 2.52 - 2.69 (m, 5H), 2.78 - 2.90 (m, 3H), 3.14 - 3.24 (m, 1H), 3.33 - 3.41 (m, 1H), 3.66 - 3.74 (m, 2H), 3.77 - 3.87 (m, 2H), 4.82 (s, 2H), 5.05 - 5.15 (m, 2H), 7.30 - 7.37 (m, 5H), 7.43 - 7.61 (m, 4H), 7.66 - 7.72 (m, 2H), 7.82 - 7.95 (m, 1H), 7.98 - 8.05 (m, 2H), 8.31 (d, J = 9.8 Hz, 1H), 12.20 - 12.34 (m, 1H). LCMS (ESI) *m/z* 810 $[M+H]^+$, 808 $[M-H]^-$. Rt 0.771 min (LP mode).

Benzyl 4-[{2-[{[3-({3-[1-(4-chlorophenyl)-1*H*-pyrazole-4-carbonyl]-4,7-dihydro-5*H*-thieno[2,3-c]thiopyran-2-yl}carbamoyl)phenyl]methyl}(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoate (14ct)

Compound **14ct** (50%) was prepared from **13ct** in a manner similar to that described for compound **14at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 - 0.98 (m, 6H), 1.30 - 1.51 (m, 4H), 2.25 - 2.39 (m, 2H), 2.52 - 2.76 (m, 9H), 2.78 - 2.91 (m, 3H), 3.16 - 3.22 (m, 1H), 3.32 - 3.39 (m, 1H), 3.67 - 3.74 (m, 2H), 3.86 (s, 2H), 5.04 - 5.13 (m, 2H), 7.30 - 7.38 (m, 5H), 7.41 - 7.51 (m, 3H), 7.54 - 7.59 (m, 1H), 7.66 - 7.72 (m, 2H), 7.80 - 7.86 (m, 1H), 7.94 - 8.03 (m, 2H), 8.32 (d, J = 9.5 Hz, 1H), 11.87 - 12.01 (m, 1H). LCMS (ESI) *m/z* 826 $[M+H]^+$, 824 $[M-H]^-$. Rt 0.821 min (LP mode).

Benzyl 4-[{2-[{[3-({6-acetyl-3-[1-(4-chlorophenyl)-1*H*-pyrazole-4-carbonyl]-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl}carbamoyl)phenyl]methyl}(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoate (14dt)

Compound **14dt** (94%) was prepared from **13dt** in a manner similar to that described for compound **14at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 - 1.08 (m, 6H), 1.25 - 1.42 (m, 4H), 2.00 - 2.26 (m, 5H), 2.47 - 2.72 (m, 7H), 2.77 - 3.40 (m, 5H), 3.53 - 3.98 (m, 4H), 4.62 - 4.83 (m, 2H), 5.07 - 5.15 (m, 2H), 7.27 - 7.39 (m, 5H), 7.43 - 7.75 (m, 4H), 7.82 - 8.45 (m, 6H), 12.13 - 12.35 (m, 1H). LCMS (ESI) *m/z* 851 $[M+H]^+$, 849 $[M-H]^-$. Rt 0.932 min (NM mode).

Benzyl 4-[{2-[{[3-({3-[1-(4-chlorophenyl)-1*H*-pyrazole-4-carbonyl]-6-(methanesulfonyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl}carbamoyl)phenyl]methyl}(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoate (14et)

Compound **14et** (quantitative yield) was prepared from **13et** in a manner similar to that described for compound **14at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 - 1.09 (m, 6H), 1.33 - 1.47 (m, 4H), 2.30 - 2.81 (m, 9H), 2.91 (s, 3H), 3.04 - 3.25 (m, 5H), 3.38 - 3.53 (m, 2H), 3.86 - 3.94 (m, 2H), 4.45 - 4.56 (m, 2H), 5.08 - 5.15 (m, 2H), 7.30 - 7.40 (m, 5H), 7.43 - 7.90 (m, 4H), 7.96 - 8.13 (m, 3H), 8.16 - 8.43 (m, 3H), 11.81 - 12.25 (m, 1H). LCMS (ESI) *m/z* 887 $[M+H]^+$, 885 $[M-H]^-$. Rt 0.977 min (NM mode).

Benzyl 4-[{2-[{[3-({3-[1-(4-chlorophenyl)-1*H*-pyrazole-4-carbonyl]-6-cyclopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl}carbamoyl)phenyl]methyl}(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoate (14ft)

Compound **14ft** (quantitative yield) was prepared from **13ft** in a manner similar to that described for compound **14at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 - 1.13 (m, 10H), 1.37 - 1.59 (m, 5H), 1.94 - 2.15 (m, 4H), 2.53 - 2.98 (m, 7H), 3.05 - 3.31 (m, 4H), 3.47 - 3.53 (m, 2H), 3.98 - 4.12 (m, 2H), 4.38 - 4.49 (m, 1H), 5.06 - 5.23 (m, 2H), 7.29 - 7.42 (m, 5H), 7.45 - 7.72 (m, 4H), 7.96 - 8.75 (m, 6H), 11.52 - 11.87 (m, 1H). LCMS (ESI) *m/z* 849 $[M+H]^+$, 847 $[M-H]^-$. Rt 0.976 min (NM mode).

4-[{2-[{[3-({3-[1-(4-Chlorophenyl)-1H-pyrazole-4-carbonyl]-4,5,6,7-tetrahydro-1-benzothiophene-2-yl}carbamoyl)phenyl]methyl}(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoic acid (15at)

A 1M sodium hydroxide (NaOH) aqueous solution (5.00 mL, 5.00 mmol) was added to a solution of **14at** (952 mg, 1.18 mmol) in THF (10 mL) and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified using column chromatography on a silica gel and eluted with 10% MeOH/$CHCl_3$ to yield **15at** (707 mg, 84%) as a yellow amorphous substance. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 - 1.02 (m, 6H), 1.30 - 1.49 (m, 4H), 1.63 - 1.72 (m, 2H), 1.85 - 1.93 (m, 2H), 2.27 - 2.44 (m, 4H), 2.51 - 2.67 (m, 4H), 2.75 - 2.92 (m, 5H), 3.12 - 3.21 (m, 1H), 3.36 - 3.43 (m, 1H), 3.65 - 3.74 (m, 2H), 3.98 - 4.07 (m, 1H), 7.41 - 7.57 (m, 4H), 7.67 - 7.74 (m, 2H), 7.83 (br d, J = 7.8 Hz, 1H), 7.96 - 8.02 (m, 2H), 8.33 (d, J = 5.9 Hz, 1H), 12.07 (s, 1H). LCMS (ESI) *m/z* 718 $[M+H]^+$, 716 $[M-H]^-$. Rt 0.958 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{38}H_{44}ClN_5O_5S$ $[M+H]^+$ 718.2824, found 718.2815.

4-[{2-[{[3-({3-[1-(4-Chlorophenyl)-1*H*-pyrazole-4-carbonyl]-4,7-dihydro-5*H*-thieno[2,3-c]pyran-2-yl}carbamoyl)phenyl]methyl}(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoic acid (15bt)

Compound **15bt** (64%) was prepared from **14bt** in a manner similar to that described for compound **15at**. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 0.68 - 0.89 (m, 6H), 1.07 - 1.34 (m, 4H), 2.04 - 2.13 (m, 1H), 2.23 - 2.43 (m, 6H), 2.61 - 2.81 (m, 5H), 2.99 - 3.40 (m, 4H), 3.81 (br t, J = 5.5 Hz, 2H), 4.62 (br s, 2H), 7.13 - 7.33 (m, 2H), 7.46 - 7.64 (m, 4H), 7.86 - 7.96 (m, 2H), 8.07 (d, J = 2.4 Hz, 1H), 8.96 - 9.03 (m, 1H). LCMS (ESI) *m/z* 720 $[M+H]^+$, 718 $[M-H]^-$. Rt 0.827 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{37}H_{42}ClN_5O_6S$ $[M+H]^+$ 720.2617, found. 720.2614.

4-[{2-[{[3-({3-[1-(4-Chlorophenyl)-1*H*-pyrazole-4-carbonyl]-4,7-dihydro-5*H*-thieno[2,3-c]thiopyran-2-yl}carbamoyl)phenyl]methyl}(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoic acid (15ct)

Compound **15ct** (76%) was prepared from **14ct** in a manner similar to that described for compound **15at**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.69 - 0.90 (m, 6H), 1.09 - 1.39 (m, 4H), 2.07 - 2.43 (m, 6H), 2.61 - 2.90 (m, 6H), 3.01 - 3.44 (m, 6H), 3.80 (br s, 2H), 7.14 - 7.67 (m, 6H), 7.86 - 7.99 (m, 2H), 8.06 (s, 1H), 9.01 (d, J = 2.2 Hz, 1H). LCMS (ESI) *m/z* 736 $[M+H]^+$, 734 $[M-H]^-$. Rt 0.671 min (LP mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{37}H_{42}ClN_5O_5S_2$ $[M+H]^+$ 736. 2389, found. 736.2358.

4-[{2-[{[3-({6-Acetyl-3-[1-(4-chlorophenyl)-1*H*-pyrazole-4-carbonyl]-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl}carbamoyl)phenyl]methyl}(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoic acid (15dt)

Compound **15dt** (33%) was prepared from **14dt** in a manner similar to that described for compound **15at**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.71 - 0.90 (m, 6H), 1.16 - 1.42 (m, 5H), 2.07 - 2.43 (m, 10H), 2.63 - 2.86 (m, 4H), 3.09 - 3.24 (m, 2H), 3.44 - 3.56 (m, 2H), 3.60 - 3.70 (m, 2H), 4.61 - 4.73 (m, 2H), 7.34 - 7.63 (m, 6H), 7.86 - 8.01 (m, 2H), 8.07 - 8.16 (m, 1H), 9.07 (d, J = 2.4 Hz, 1H), 11.34 (br s, 1H), 11.80 - 11.99 (m, 1H). LCMS (ESI) *m/z* 761 $[M+H]^+$, 759 $[M-H]^-$. Rt 0.744 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{39}H_{45}ClN_6O_6S$ $[M+H]^+$ 761. 2883, found. 761.2881.

4-[{2-[{[3-({3-[1-(4-Chlorophenyl)-1H-pyrazole-4-carbonyl]-6-(methanesulfonyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl}carbamoyl)phenyl]methyl}(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoic acid (15et)

Compound **15et** (34%) was prepared from **14et** in a manner similar to that described for compound **15at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 - 1.04 (m, 6H), 1.34 - 1.56 (m, 6H), 2.26 - 2.38 (m, 2H), 2.52 (br d, J = 6.4 Hz, 1H), 2.58 - 2.83 (m, 7H), 2.91 (s, 3H), 3.14 - 3.25 (m, 1H), 3.33 - 3.48 (m, 3H), 3.71 (br d, J = 4.9 Hz, 2H), 4.52 (s, 2H), 7.44 - 7.60 (m, 3H), 7.66 - 7.75 (m, 2H), 7.81 - 8.04 (m, 4H), 8.33 (d, J = 3.9 Hz, 1H), 12.01 - 12.15 (m, 1H). LCMS (ESI) *m/z* 797 $[M+H]^+$, 795 $[M-H]^-$. Rt 0.795 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{38}H_{45}ClN_6O_7S_2$ $[M+H]^+$ 797.2552, found. 797.2566.

4-[{2-[{[3-({3-[1-(4-Chlorophenyl)-1*H*-pyrazole-4-carbonyl]-6-cyclopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl}carbamoyl)phenyl]methyl}(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoic acid (15ft*)*

Compound **15ft** (33%) was prepared from **14ft** in a manner similar to that described for compound **15at**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.37 - 0.53 (m, 4H), 0.75 - 0.90 (m, 6H), 1.13 - 1.44 (m, 5H), 1.87 - 1.94 (m, 1H), 2.10 - 2.19 (m, 1H), 2.24 - 2.43 (m, 6H), 2.63 - 2.86 (m, 5H), 3.06 - 3.23 (m, 3H), 3.46 - 3.53 (m, 2H), 3.73 (s, 2H), 7.33 - 7.61 (m, 6H), 7.91 - 7.98 (m, 2H), 8.06 - 8.13 (m, 1H), 9.04 (s, 1H), 11.28 - 11.36 (m, 1H), 11.90 - 12.02 (m, 1H). LCMS (ESI) *m/z* 759 $[M+H]^+$, 757 $[M-H]^-$. Rt 0.630 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{40}H_{47}ClN_6O_5S$ $[M+H]^+$ 759.3090, found. 759.3069.

3-[1-(Oxan-2-yl)-1*H*-pyrazol-4-yl]-3-oxopropanenitrile (17t)

Compound **17t** (84%) was prepared from ethyl 1-(oxan-2-yl)-1H-pyrazole-4-carboxylate **16t** in a manner similar to that described for compound **11t**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.64 - 1.75 (m, 3H), 1.97 - 2.06 (m, 2H), 2.12 - 2.19 (m, 1H), 3.70 - 3.76 (m, 1H), 3.81 (s, 2H), 4.06 - 4.12 (m, 1H), 5.42 (dd, J = 9.3, 2.7 Hz, 1H), 7.99 (s, 1H), 8.25 (s, 1H).

(2-Amino-6,6-dimethyl-4,5,6,7-tetrahydro-1-benzothiophene-3-yl)[1-(oxan-2-yl)-1*H*-pyrazol-4-yl]methanone (18t)

Compound **18t** (70%) was prepared from **17t** and 4,4-dimethylcyclohexan-1-one in a manner similar to that described for compound **13at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (s, 6H), 1.36 (t, J = 6.2 Hz, 2H), 1.59 - 1.75 (m, 3H), 1.99 - 2.13 (m, 3H), 2.21 - 2.27 (m, 2H), 2.32 (t, J = 1.6 Hz, 2H), 3.67 - 3.76 (m, 1H), 4.03 - 4.11 (m, 1H), 5.38 - 5.44 (m, 1H), 6.06 (br s, 2H), 7.74 (s, 1H), 7.95 (s, 1H). LCMS (ESI) *m/z* 360 $[M+H]^+$. Rt 0.718 min (LP mode).

3-(Chloromethyl)-N-{6,6-dimethyl-3-[1-(oxan-2-yl)-1*H*-pyrazole-4-carbonyl]-4,5,6,7-tetrahydro-1-benzothiophene-2-yl}benzamide (19t)

Pyridine (1.10 mL, 14.2 mmol) and 3-(chloromethyl)benzoyl chloride (2.02 mL, 14.2

mmol) were added to a solution of **18t** (5.00 g, 13.9 mmol) in $CHCl_3$ (25 mL) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was washed with water. The aqueous layer was separated and extracted with $CHCl_3$ using a phase separator. The combined organic layer was concentrated. The resulting residue was washed with *n*-hexane and then purified using column chromatography on a silica gel and eluted with 5%-20% EtOAc/*n*-hexane and 0%-20% MeOH/$CHCl_3$ to yield **19t** as a yellow powder (5.00 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04 (s, 6H), 1.44 (t, J = 6.2 Hz, 2H), 1.62 - 1.79 (m, 3H), 2.01 - 2.17 (m, 3H), 2.39 (br t, J = 6.4 Hz, 2H), 2.52 (s, 2H), 3.69 - 3.78 (m, 1H), 4.04 - 4.13 (m, 1H), 4.63 - 4.69 (m, 2H), 5.44 (dd, J = 8.9, 3.1 Hz, 1H), 7.46 - 7.54 (m, 1H), 7.62 (d, J = 8.1 Hz, 1H), 7.81 (s, 1H), 7.89 - 7.93 (m, 1H), 7.98 - 8.02 (m, 1H), 8.05 (s, 1H), 11.94 - 12.05 (m, 1H). LCMS (ESI) *m/z* 510 $[M-H]^-$. Rt 0.904 min (LP mode).

Benzyl 4-[{2-[{[3-({6,6-dimethyl-3-[1-(oxan-2-yl)-1*H*-pyrazole-4-carbonyl]-4,5,6,7-tetrahydro-1-benzothiophene-2-yl}carbamoyl)phenyl]methyl}(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoate (20t)

Compound **8t** (3.92 g, 11.7 mol), NaI (2.20 g, 14.7 mmol) and DIPEA (4.25 mL, 24.4 mmol) were added to a solution of **19t** (5.00 g, 9.76 mmol) in MeCN (50 mL). The mixture was stirred under reflux for 1 h, then quenched with water. The aqueous layer was separated and extracted with $CHCl_3$ using a phase separator, and the combined organic layer was concentrated. The resulting residue was washed with *n*-hexane and then purified using column chromatography on a silica gel and eluted with 3% MeOH/$CHCl_3$ to yield **20t** as a yellow amorphous substance (4.00 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 - 0.98 (m, 6H), 1.04 (s, 6H), 1.25 - 1.78 (m, 11H), 2.00 - 2.16 (m, 3H), 2.24 - 2.41 (m, 4H), 2.48 - 2.69 (m, 5H), 2.75 - 2.90 (m, 3H), 3.14 - 3.22 (m, 1H), 3.35 (t, J = 7.1 Hz, 1H), 3.67 - 3.77 (m, 3H), 4.07 (br d, J = 12.2 Hz, 1H), 5.04 - 5.12 (m, 2H), 5.42 (dd, J = 8.7, 2.1 Hz, 1H), 7.27 - 7.37 (m, 5H), 7.38 - 7.45 (m, 1H), 7.52 - 7.59 (m, 1H), 7.78 - 7.85 (m, 2H), 7.94 - 8.05 (m, 2H), 11.93 (s, 1H). LCMS (ESI) *m/z* 810 $[M+H]^+$, 808 $[M-H]^-$. Rt 1.083 min (NM mode).

Benzyl 4-[(2-{[(3-{[6,6-dimethyl-3-(1*H*-pyrazole-4-carbonyl)-4,5,6,7-tetrahydro-1-benzothiophene-2-yl]carbamoyl}phenyl)methyl](pentan-3-yl)amino}ethyl)(methyl)amino]-4-oxobutanoate (21t)

Trifluoroacetic acid (TFA) (6.70 mL, 87.1 mmol) was added to a solution of **20t** (4.00 g, 4.90 mmol) in 1,4-dioxane (40 mL) and water (6.7 mL). The mixture was stirred at 60 °C for 2 h, then quenched with saturated $NaHCO_3$ aqueous solution at 0 °C. The aqueous layer was extracted with EtOAc, and the combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using column chromatography on a silica gel and eluted with 5% MeOH/$CHCl_3$ to yield **21t** as a yellow amorphous substance (2.10 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 - 0.98 (m, 6H), 1.04 (s, 6H), 1.29 - 1.51 (m, 6H), 2.25 - 2.45 (m, 4H), 2.51 - 2.69 (m, 7H), 2.78 - 2.88 (m, 3H), 3.17 - 3.23 (m, 1H), 3.34 - 3.40 (m, 1H), 3.70 (d, J = 6.6 Hz, 2H), 5.07 - 5.13 (m, 2H), 7.28 - 7.38 (m, 5H), 7.39 - 7.46 (m, 1H), 7.50 - 7.56 (m, 1H), 7.80 - 8.00 (m, 4H), 10.66 - 10.82 (m, 1H), 11.57 - 12.03 (m, 1H). LCMS (ESI) *m/z* 726 $[M+H]^+$, 724 $[M-H]^-$. Rt 0.929 min (NM mode).

4-[{2-[{[3-({3-[1-(4-Chlorophenyl)-1*H*-pyrazole-4-carbonyl]-6,6-dimethyl-4,5,6,7-tetrahydro-1-benzothiophene-2-yl}carbamoyl)phenyl]methyl}(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoic acid (23at*)*

4-Chlorophenylboronic acid **22at** (6.00 mg, 38.4 μmol), pyridine (3.30 μL, 41.3 μmol), and $Cu(OAc)_2$ (8.00 mg, 40 μmol) were added to a solution of **21t** (10.0 mg, 13.7 μmol) in $CHCl_3$ (0.10 mL). The mixture was stirred at room temperature for 5 h, then concentrated under reduced pressure. THF (0.10 mL) and 1M NaOH aqueous solution (0.10 mL, 0.10 mmol) were then added to the resulting residue, and the mixture was stirred at 50 °C for 25 min. The mixture was quenched by acetic acid (AcOH) and diluted with THF and DMSO, and the resulting precipitate was filtered. The resulting filtrate was purified using reverse-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=80:20 → 5:95 → 1:99, 40 mL/min) to yield **23at** (3.50 mg, 35%) as a yellow powder. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.92 (t, J = 7.4 Hz, 3H), 0.98 (t, J = 7.4 Hz, 3H), 1.06 (s, 6H), 1.32 - 1.56 (m, 6H), 2.28 - 2.37 (m, 2H), 2.40 - 2.45 (m, 2H), 2.52 - 2.66

(m, 7H), 2.81 - 2.92 (m, 3H), 3.14 - 3.19 (m, 1H), 3.37 - 3.41 (m, 1H), 3.68 - 3.73 (m, 2H), 7.42 - 7.56 (m, 4H), 7.70 (dd, *J* = 8.9, 1.0 Hz, 2H), 7.82 (br d, *J* = 7.8 Hz, 1H), 7.94 - 8.00 (m, 2H), 8.34 (d, *J* = 8.7 Hz, 1H), 11.94 - 12.02 (m, 1H). LCMS (ESI) *m/z* 746 $[M+H]^+$, 744 $[M-H]^-$. Rt 1.080 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{40}H_{48}ClN_5O_5S$ $[M+H]^+$ 746. 3137, found 746.3156.

4-[{2-[({3-[(6,6-Dimethyl-3-{1-[3-(trifluoromethyl)phenyl]-1*H*-pyrazole-4-carbonyl}-4,5,6,7-tetrahydro-1-benzothiophene-2-yl)carbamoyl]phenyl}methyl)(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoic acid (23bt)

Compound **23bt** (55% over 2 steps) was prepared from **21t** and **22bt** in a manner similar to that described for compound **23at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 - 1.00 (m, 6H), 1.06 (s, 6H), 1.32 - 1.56 (m, 6H), 2.28 - 2.45 (m, 4H), 2.52 - 2.66 (m, 7H), 2.80 - 2.91 (m, 3H), 3.14 - 3.20 (m, 1H), 3.39 (br t, *J* = 6.6 Hz, 1H), 3.68 - 3.73 (m, 2H), 7.41 - 7.57 (m, 2H), 7.62 - 7.68 (m, 2H), 7.81 - 7.86 (m, 1H), 7.91 - 8.01 (m, 3H), 8.07 (s, 1H), 8.41 - 8.45 (m, 1H), 11.98 - 12.06 (m, 1H). LCMS (ESI) *m/z* 780 $[M+H]^+$, 778 $[M-H]^-$. Rt 1.103 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{41}H_{48}F_3N_5O_5S$ $[M+H]^+$ 780.3401, found 780.3423.

4-{4-[2-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-6,6-dimethyl-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonyl]-1*H*-pyrazol-1-yl}benzoic acid (23ct)

Compound **23ct** (27% over 2 steps) was prepared from **21t** and **22ct** in a manner similar to that described for compound **23at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 - 1.03 (m, 6H), 1.06 (s, 6H), 1.32 - 1.59 (m, 6H), 2.28 - 2.47 (m, 5H), 2.51 - 2.69 (m, 6H), 2.81 - 2.93 (m, 3H), 3.15 - 3.21 (m, 1H), 3.37 - 3.43 (m, 1H), 3.68 - 3.74 (m, 2H), 7.40 - 7.59 (m, 3H), 7.82 - 7.89 (m, 2H), 7.94 - 8.03 (m, 2H), 8.15 - 8.24 (m, 2H), 8.41 - 8.46 (m, 1H), 12.02 - 12.09 (m, 1H). LCMS (ESI) *m/z* 756 $[M+H]^+$, 754 $[M-H]^-$. Rt 0.880 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{41}H_{49}N_5O_7S$ $[M+H]^+$ 756.3425, found 756.3442.

3-{4-[2-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-6,6-dimethyl-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonyl]-1*H*-pyrazol-1-yl}benzoic acid (23dt)

Ethyl 3-iodobenzoate (38.0 mg, 138 μmol), cuprous iodide (2.62 mg, 13.8 μmol), (1R,2R)-N1,N2-dimethylcyclohexane-1,2-diamine (3.92 mg, 27.6 μmol), and cesium carbonate (Cs_2CO_3) (48.9 mg, 138 μmol) were added to a solution of **21t** (50.0 mg, 68.9 μmol) in DMF (0.50 mL). The mixture was stirred at 60 °C for 6 h and filtered, then the resulting filtrate was purified using column chromatography on a silica gel and eluted with 2%-10% MeOH/$CHCl_3$. THF (0.20 mL), MeOH (0.20 mL) and a 4M NaOH aqueous solution (0.10 mL, 0.40 mmol) were added to the resulting product. The mixture was stirred at 60 °C for 30 min and then concentrated under reduced pressure. The residue was purified using reverse-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=80:20→5:95→1:99, 40 mL/min) to yield **23dt** (5.00 mg, 10% over 2 steps) as a yellow amorphous substance. ^{1}H NMR (400 MHz, CD_3OD) δ 0.86 - 0.99 (m, 6H), 1.07 (s, 6H), 1.34 - 1.64 (m, 6H), 2.24 - 2.32 (m, 1H), 2.40 - 2.45 (m, 1H), 2.49 - 2.60 (m, 7H), 2.68 - 2.95 (m, 4H), 3.14 - 3.21 (m, 1H), 3.35 - 3.43 (m, 2H), 3.63 (s, 1H), 3.91 (br s, 1H), 7.38 - 7.82 (m, 6H), 7.96 - 8.08 (m, 3H), 8.39 - 8.46 (m, 1H), 8.73 - 8.79 (m, 1H). LCMS (ESI) *m/z* 756 $[M+H]^+$, 754 $[M-H]^-$. Rt 0.880 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{41}H_{49}N_5O_7S$ $[M+H]^+$ 756.3425, found 756.3454.

4-[(2-{[(3-{[3-(1-Benzyl-1H-pyrazole-4-carbonyl)-6,6-dimethyl-4,5,6,7-tetrahydro-1-benzothiophen-2-yl]carbamoyl}phenyl)methyl](pentan-3-yl)amino}ethyl)(methyl)amino]-4-oxobutanoic acid (25at)

Cs_2CO_3 (18.0 mg, 55.1 μmol) and benzyl bromide (6.60 μL, 55.1 mmol) were added to a solution of **21t** (40.0 mg, 55.1 μmol) in MeCN (0.20 mL) at 0 °C. The mixture was stirred at room temperature for 12 h and then purified using column chromatography on a silica gel and eluted with 0%-100% EtOAc/hexane. The resulting product was taken up in THF (0.50 mL), and 1M NaOH aqueous solution (0.50 mL, 0.50 mmol) was added to the solution. The mixture was stirred at room temperature for 2 h and filtered to remove the insoluble material. The filtrate was purified using reverse-phase

preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=90:10 → 20:80 → 5:95, 40 mL/min) to yield **25at** (21 mg, 52% over 2 steps) as a pale yellow amorphous substance. ^{1}H NMR (600 MHz,$CDCl_3$) δ 0.88 - 1.02 (m, 12H), 1.32 - 1.57 (m, 6H), 2.27 - 2.35 (m, 4H), 2.46 - 2.54 (m, 3H), 2.56 - 2.69 (m, 4H), 2.80 - 2.91 (m, 3H), 3.13 - 3.18 (m, 1H), 3.38 - 3.43 (m, 1H), 3.69 - 3.73 (m, 2H), 5.34 (s, 2H), 7.28 - 7.45 (m, 6H), 7.50 - 7.55 (m, 1H), 7.70 - 7.73 (m, 1H), 7.77 - 7.81 (m, 1H), 7.82 - 7.84 (m, 1H), 7.93 - 7.98 (m, 1H), 11.82 - 11.92 (m, 1H). LCMS (ESI) *m/z* 726 $[M+H]^+$, 724 $[M-H]^-$. Rt 0.920 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{41}H_{51}N_5O_5S$ $[M+H]^+$ 726.3684, found 726.3689.

4-({4-[2-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-6,6-dimethyl-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonyl]-1*H*-pyrazol-1-yl}methyl)benzoic acid (25bt)

Compound **25bt** (28% over 2 steps) was prepared from **21t** in a manner similar to that described for compound **25at.** ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.83 - 1.03 (m, 12H), 1.27 - 1.53 (m, 6H), 2.24 - 2.38 (m, 4H), 2.45 - 2.65 (m, 7H), 2.75 - 2.90 (m, 3H), 3.14 - 3.21 (m, 1H), 3.34 - 3.42 (m, 1H), 3.70 (br s, 2H), 5.41 (br s, 2H), 7.29 - 7.33 (m, 2H), 7.37 - 7.45 (m, 1H), 7.49 - 7.55 (m, 1H), 7.76 - 7.85 (m, 3H), 7.91 - 7.97 (m, 1H), 7.99 - 8.05 (m, 2H), 11.79 - 11.90 (m, 1H). LCMS (ESI) *m/z* 770 $[M+H]^+$, 768 $[M-H]^-$. Rt 0.823 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{42}H_{51}N_5O_7S$ $[M+H]^+$ 770.3582, found 770.3601.

3-({4-[2-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-6,6-dimethyl-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonyl]-1*H*-pyrazol-1-yl}methyl)benzoic acid (25ct)

Compound **25ct** (42% over 2 steps) was prepared from **21t** in a manner similar to that described for compound **25at**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.87 - 1.03 (m, 12H), 1.30 - 1.58 (m, 6H), 2.27 - 2.40 (m, 4H), 2.47 - 2.66 (m, 7H), 2.76 - 2.88 (m, 3H), 3.12 - 3.17 (m, 1H), 3.30 - 3.36 (m, 1H), 3.69 - 3.73 (m, 2H), 5.42 (d, J = 6.2 Hz, 2H), 7.38 - 7.59 (m, 4H), 7.65 - 7.92 (m, 5H), 7.99 - 8.04 (m, 1H), 11.72 - 11.77 (m, 1H). LCMS

(ESI) *m/z* 770 $[M+H]^+$, 768 $[M-H]^-$. Rt 0.830 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{42}H_{51}N_5O_7S$ $[M+H]^+$ 770.3582, found 770.3605.

4-[{2-[({3-[(6,6-Dimethyl-3-{1-[(4H-1,2,4-triazol-3-yl)methyl]-1*H*-pyrazole-4-carbonyl}-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)carbamoyl]phenyl}methyl)(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoic acid (26t)

2-Iodoacetonitrile (1.24 g, 7.44 mmol) and DIPEA (424 μL, 2.48 mmol) were added to a solution of **21t** (180 mg, 248 μmol) in MeCN (4.5 mL). The mixture was stirred at 100 °C for 3 h and concentrated, and the resulting residue was purified using column chromatography on a silica gel and eluted with 0%-95% EtOAc/*n*-hexane to yield the corresponding nitrile (150 mg, 78%) as a brown amorphous substance. Cs_2CO_3 (1.20 mg, 3.53 μmol) was added to a solution of the nitrile (13.5 mg, 17.7 μmol) in MeOH (0.50 mL), and the mixture was stirred at room temperature for 10 h. The solvent was removed under reduced pressure, and *n*-BuOH (0.50 mL) and hydrazinecarboxaldehyde (1.30 mg, 21.2 μmol) were added to the residue; the mixture was then stirred at 120 °C for 3 h. The solvent was removed under reduced pressure, and THF (0.50 mL) and 2M NaOH aqueous solution (0.10 μL, 200 μmol) were added to the residue; the mixture was then stirred at 60 °C for 2 h. The solvent was removed under reduced pressure, and the residue was purified using reverse-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=90:10 → 20:80 → 5:95, 40 mL/min) to yield **26t** (1.70 mg, 13% over 2 steps) as a pale yellow amorphous substance. ^1H NMR (600 MHz, $CDCl_3$) δ 0.87 - 0.91 (m, 3H), 0.95 - 0.99 (m, 3H), 1.01 - 1.05 (m, 6H), 1.36 - 1.57 (m, 6H), 2.26 - 2.46 (m, 5H), 2.49 - 2.66 (m, 5H), 2.77 - 3.00 (m, 4H), 3.22 - 3.25 (m, 1H), 3.49 - 3.52 (m, 1H), 3.70 - 3.83 (m, 2H), 5.55 (br d, J = 7.0 Hz, 2H), 7.37 - 7.44 (m, 1H), 7.46 - 7.51 (m, 1H), 7.78 - 7.83 (m, 2H), 7.90 - 7.97 (m, 1H), 8.02 - 8.09 (m, 2H), 8.14 - 8.20 (m, 1H), 11.52 - 11.74 (m, 1H). LCMS (ESI) *m/z* 717$[M+H]^+$, 715 $[M-H]^-$. Rt 0.701 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{37}H_{48}N_8O_5S$ $[M+H]^+$ 717.3541, found 717.3547.

Ethyl 1-[4-chloro-3-(trifluoromethyl)phenyl]-1*H*-pyrazole-4-carboxylate (28t)

1-Chloro-4-iodo-2-(trifluoromethyl)benzene (43.7 g, 142 mmol), (1R,2R)-N1,N2-dimethylcyclohexane-1,2-diamine (3.05 g, 21.4 mmol), cuprous iodide (2.72 g, 14.3 mmol), sodium ascorbate (2.83 g, 14.3 mmol), and sodium carbonate (Na_2CO_3) (30.3 g, 285 mmol) were added to a solution of **27t** (20.0 g, 142 mmol) in DMSO (80 mL) and the mixture was stirred at 150 °C for 6 h. Next, 10% NaCl aqueous solution and toluene were added, and the resulting mixture was filtered to remove the insoluble material. The filtrate was filtered again to remove the insoluble material. The filtrate was extracted with toluene, and the combined organic layer was washed with 10% NaCl aqueous solution; then, the organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified using column chromatography on a silica gel and eluted with 10%-25% EtOAc/*n*-hexane to yield **28t** as a pale red solid (22.6 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, J = 7.1 Hz, 3H), 4.31 - 4.40 (m, 2H), 7.60 - 7.66 (m, 1H), 7.81 - 7.87 (m, 1H), 8.09 (d, J = 2.7 Hz, 1H), 8.12 (s, 1H), 8.43 (s, 1H). LCMS (ESI) *m/z* 319 $[M+H]^+$. Rt 0.786 min (LP mode).

3-{1-[4-Chloro-3-(trifluoromethyl)phenyl]-1*H*-pyrazol-4-yl}-3-oxopropanenitrile (29t)

Compound **29t** (89%) was prepared from **28t** in a manner similar to that described for compound **11t**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.88 (s, 2H), 7.67 (d, J = 8.8 Hz, 1H), 7.86 (dd, J = 8.7, 2.6 Hz, 1H), 8.10 (d, J = 2.4 Hz, 1H), 8.19 (s, 1H), 8.57 (s, 1H). LCMS (ESI) *m/z* 312 $[M+H]^+$. Rt 0.673 min (LP mode).

3-{1-[4-Chloro-3-(trifluoromethyl)phenyl]-1*H*-pyrazol-4-yl}-3-oxopropanenitrile (30t)

Compound **30t** (73%) was prepared from **29t** and 4,4-dimethylcyclohexan-1-one in a manner similar to that described for compound **13at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (s, 6H), 1.38 (t, J = 6.4 Hz, 2H), 2.21 - 2.27 (m, 2H), 2.34 (t, J = 1.6 Hz, 2H), 6.33 - 6.39 (m, 2H), 7.59 - 7.65 (m, 1H), 7.80 - 7.85 (m, 1H), 7.90 (s, 1H), 8.10 (d, J = 2.4 Hz, 1H), 8.25 (s, 1H). LCMS (ESI) *m/z* 454 $[M+H]^+$. Rt 0.916 min (LP mode).

Benzyl 4-[{2-[({3-[(3-{1-[4-chloro-3-(trifluoromethyl)phenyl]-1*H*-pyrazole-4-carbonyl}-6,6-dimethyl-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)carbamoyl]phenyl}methyl)(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoate (31t)

Compound **31t** (63%) was prepared from **30t** in a manner similar to that described for compound **14at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 - 0.99 (m, 6H), 1.05 (s, 6H), 1.30 - 1.53 (m, 6H), 2.24 - 2.45 (m, 4H), 2.51 - 2.69 (m, 7H), 2.77 - 2.90 (m, 3H), 3.16 - 3.22 (m, 1H), 3.36 (t, J = 7.0 Hz, 1H), 3.68 - 3.74 (m, 2H), 5.03 - 5.12 (m, 2H), 7.28 - 7.36 (m, 5H), 7.40 - 7.47 (m, 1H), 7.54 - 7.58 (m, 1H), 7.62 - 7.66 (m, 1H), 7.82 - 7.88 (m, 2H), 7.95 - 8.02 (m, 2H), 8.13 (d, J = 2.4 Hz, 1H), 8.36 (d, J = 10.0 Hz, 1H), 12.04 - 12.17 (m, 1H). LCMS (ESI) *m/z* 904 $[M+H]^+$. Rt 0.832 min (LP mode).

4-[{2-[({3-[(3-{1-[4-Chloro-3-(trifluoromethyl)phenyl]-1*H*-pyrazole-4-carbonyl}-6,6-dimethyl-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)carbamoyl]phenyl}methyl)(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoic acid (5t)

Compound **5t** (62% over 2 steps) was prepared from **30t** in a manner similar to that described for compound **15at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 - 1.00 (m, 6H), 1.06 (s, 6H), 1.30 - 1.55 (m, 6H), 2.29 - 2.44 (m, 4H), 2.48 - 2.66 (m, 7H), 2.79 - 2.92 (m, 3H), 3.13 - 3.19 (m, 1H), 3.38 (br t, J = 6.6 Hz, 1H), 3.67 - 3.73 (m, 2H), 7.43 - 7.56 (m, 2H), 7.65 (d, J = 8.8 Hz, 1H), 7.80 - 7.85 (m, 1H), 7.88 (dd, J = 8.8, 2.7 Hz, 1H), 7.94 - 8.00 (m, 2H), 8.15 (d, J = 2.7 Hz, 1H), 8.40 - 8.44 (m, 1H), 11.98 - 12.05 (m, 1H). LCMS (ESI) *m/z* 814 $[M+H]^+$. Rt 0.726 min (LP mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{41}H_{47}ClF_3N_5O_5S$ $[M+H]^+$ 814. 3011, found 814.3035.

(1r,4r)-4-[{3-[(3-{1-[4-Chloro-3-(trifluoromethyl)phenyl]-1*H*-pyrazole-4-carbonyl}-6,6-dimethyl-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)carbamoyl]benzene-1-sulfonyl}(ethyl)amino]cyclohexane-1-carboxylic acid (32t)

Oxalyl chloride (0.224 mL, 2.64 mmol) was added to a solution of 3-{ethyl[(1r,4r)-4-(methoxycarbonyl)cyclohexyl]sulfamoyl}benzoic acid (0.489 g, 1.32 mmol) in $CHCl_3$ (5.0 mL), and the mixture was stirred at room temperature for 2 h; then, oxalyl chloride

(0.224 mL, 2.64 mmol) was again added, and the mixture was stirred at 70 °C for 1 h. The reaction mixture was concentrated under reduced pressure. Pyridine (0.430 mL, 5.29 mmol) and **30t** (400 mg, 0.881 mmol) were then added to a solution of the resulting residue in $CHCl_3$ (5.0 mL). The reaction mixture was stirred overnight at room temperature, then concentrated under reduced pressure. The residue was washed with *n*-hexane to yield the corresponding methyl ester. Next, 1M NaOH aqueous solution (15.0 mL, 15.0 mmol) was added to a solution of the methyl ester in THF (15 mL) and MeOH (15 mL), and the mixture was stirred at room temperature for 2 h. The organic solvents were removed under reduced pressure, and the resulting precipitate was collected by filtration and washed with isopropyl ether to yield **32t** (700 mg, 82%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (s, 6H), 1.26 (t, J = 7.1 Hz, 3H), 1.47 - 1.68 (m, 7H), 1.77 - 1.83 (m, 2H), 2.02 - 2.10 (m, 2H), 2.18 - 2.27 (m, 1H), 2.40 - 2.46 (m, 2H), 2.53 - 2.57 (m, 2H), 3.25 - 3.32 (m, 2H), 7.63 - 7.68 (m, 2H), 7.87 - 7.91 (m, 1H), 7.94 - 7.97 (m, 1H), 8.04 - 8.08 (m, 1H), 8.14 - 8.18 (m, 2H), 8.43 - 8.45 (m, 1H), 8.46 (s, 1H), 12.31 - 12.34 (m, 1H). LCMS (ESI) *m/z* 791 $[M+H]^+$, 789 $[M-H]^-$. Rt 1.038 min (LP mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{37}H_{38}ClF_3N_4O_6S_2$ $[M+H]^+$ 791.1946, found 791.1957.

4-[{2-[{3-[(3-{1-[4-Chloro-3-(trifluoromethyl)phenyl]-1*H*-pyrazole-4-carbonyl}-6,6-dimethyl-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)carbamoyl]benzene-1-sulfonyl}(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoic acid (33t)

Triethylamine (34.0 μL, 225 μmol) and 3-(chlorosulfonyl)benzoyl chloride (53.0 mg, 223 μmol) in $CHCl_3$ (1.0 mL) were added to a solution of **30t** (101 mg, 223 μmol) in $CHCl_3$ (2.0 mL), and the mixture was stirred overnight at room temperature. Triethylamine (68.0 μL, 450 μmol) and **8t** (89.0 mg, 267 μmol) in $CHCl_3$ (1.0 mL) were then added to the resulting mixture. The mixture was stirred at room temperature for 7 h and concentrated under reduced pressure. The residue was purified using column chromatography on a silica gel and eluted with 10%-60% EtOAc/*n*-hexane to yield the corresponding benzyl ester as a pale yellow amorphous substance (15.0 g, 7.0% over 2 steps). Compound **33t** (88%) was then prepared from the benzyl ester in a manner similar to that described for compound **15at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.73 - 0.81

(m, 6H), 1.06 (s, 6H), 1.22 - 1.54 (m, 6H), 2.39 - 2.45 (m, 2H), 2.55 (s, 2H), 2.63 - 2.68 (m, 2H), 2.75 - 2.90 (m, 2H), 2.99 - 3.18 (m, 3H), 3.19 - 3.31 (m, 2H), 3.47 - 3.53 (m, 1H), 3.63 - 3.74 (m, 2H), 7.64 - 7.69 (m, 2H), 7.85 - 8.06 (m, 3H), 8.11 - 8.15 (m, 1H), 8.20 - 8.25 (m, 1H), 8.40 (s, 2H), 12.21 (s, 1H). LCMS (ESI) *m/z* 864 $[M+H]^+$, 862 $[M-H]^-$. Rt 1.156 min (LP mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{40}H_{45}ClF_3N_5O_7S_2$ $[M+H]^+$ 864. 2474, found 864.2479.

_N_-(3-{1-[4-Chloro-3-(trifluoromethyl)phenyl]-1_H_-pyrazole-4-carbonyl}-6,6-dimethyl-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)-3-[(17-hydroxy-3,6,9,12,15-pentaoxaheptadecan-1-yl)oxy]benzamide (34t)

$SOCl_2$ (43.0 μL, 590 μmol) was added to a solution of 3-acetoxybenzoic acid (36.0 mg, 200 μmol) in $CHCl_3$ (1.0 mL). The mixture was then stirred at 70 °C for 2 h and concentrated under reduced pressure. $CHCl_3$ (1.0 mL) and **30t** (45.0 mg, 100 μmol) were then added to the resulting residue. The mixture was stirred overnight at room temperature and concentrated under reduced pressure. The residue was purified using preparative TLC on a silica gel and eluted with 33% EtOAc/*n*-hexane to yield the corresponding acetate as a yellow amorphous substance (60.0 mg, 98%). A 1M NaOH aqueous solution (500 μL, 500 μmol) was then added to a solution of the acetate (60.0 mg, 100 μmol) in THF (0.25 mL) and MeOH (0.25 mL). The mixture was stirred at room temperature for 10 min. The mixture was concentrated under reduced pressure and quenched with 1M HCl aqueous solution. The aqueous layer was separated and extracted with $CHCl_3$ using a phase separator. The combined organic layer was concentrated under reduced pressure. To a solution of the resulting residue in DMF (2.0 mL) was added Cs_2CO_3 (95.0 mg, 290 μmol) and 17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (128 mg, 290 μmol). The mixture was stirred at 100 °C for 2 h and purified using reverse-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=80:20 → 5:95 → 1:99, 40 mL/min) to yield **34t** (52.0 mg, 64% over 2 steps) as a yellow amorphous substance. ^1H NMR (400 MHz, $CDCl_3$) δ 1.06 (s, 6H), 1.46 (t, J = 6.4 Hz, 3H), 2.38 - 2.44 (m, 2H), 2.52 - 2.56 (m, 2H), 2.65 - 2.72 (m, 1H), 3.57 - 3.62 (m, 2H), 3.63 - 3.75 (m, 17H), 3.86 - 3.91 (m, 2H), 4.18 - 4.24 (m, 2H),

7.12 - 7.17 (m, 1H), 7.40 (t, *J* = 7.9 Hz, 1H), 7.52 - 7.58 (m, 2H), 7.66 (d, *J* = 8.8 Hz, 1H), 7.86 (dd, *J* = 8.8, 2.7 Hz, 1H), 7.97 (s, 1H), 8.11 - 8.14 (m, 1H), 8.36 (s, 1H), 12.06 - 12.13 (m, 1H). LCMS (ESI) *m/z* 838 $[M+H]^+$. Rt 1.446 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{40}H_{47}ClF_3N_3O_9S$ $[M+H]^+$ 838. 2746, found 838.2775.

N^1-{2-[({3-[(3-{1-[4-Chloro-3-(trifluoromethyl)phenyl]-1*H*-pyrazole-4-carbonyl}-6,6-dimethyl-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)carbamoyl]phenyl}methyl)(pentan-3-yl)amino]ethyl}-N^4-(2-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}ethyl)-N^1-methylbutanediamide (36at and 36at hydrochloride)

DIPEA (128 μL, 0.737 mmol), **35at** (85.4 mg, 0.442 mmol) and 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) (210 mg, 0.553 mmol) were added to a solution of **5t** (300 mg, 0.368 mmol) in MeCN (1.2 mL). The mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated under reduced pressure, and the residue was purified using column chromatography on an NH-silica gel and eluted with 5%-90% EtOH/*n*-hexane to yield **36at** (250 mg, 58%) as a yellow amorphous substance. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 - 0.98 (m, 6H), 1.00 - 1.09 (m, 6H), 1.27 - 1.55 (m, 7H), 2.27 (td, *J* = 6.7, 3.8 Hz, 1H), 2.40 - 2.50 (m, 5H), 2.52 - 2.67 (m, 5H), 2.76 - 2.92 (m, 3H), 3.21 - 3.27 (m, 1H), 3.32 - 3.44 (m, 3H), 3.49 - 3.55 (m, 2H), 3.58 - 3.67 (m, 8H), 3.67 - 3.75 (m, 5H), 7.41 - 7.48 (m, 1H), 7.54 - 7.59 (m, 1H), 7.65 (d, *J* = 8.8 Hz, 1H), 7.81 - 7.90 (m, 2H), 7.95 - 7.99 (m, 2H), 8.12 - 8.17 (m, 1H), 8.40 - 8.45 (m, 1H), 12.02 - 12.09 (m, 1H). LCMS (ESI) *m/z* 989 $[M+H]^+$,987$[M-H]^-$. Rt 0.796 min (LP mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{49}H_{64}ClF_3N_6O_8S$ $[M+H]^+$ 989.4220, found 989.4232. 4M Hydrogen chloride in 1,4-dioxane (27.8 μL, 111 μmol) was added to a solution of **36at** (100 mg, 97.5 μmol) in 1,4-dioxane (0.40 mL), and the mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure to yield **36at** hydrochloride (100 mg, 96%) as a yellow amorphous substance. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.04 (s, 1H), 11.73 - 11.81 (m, 1H), 8.39 - 8.44 (m, 2H), 8.11 - 8.14 (m, 1H), 7.98 - 8.05 (m, 3H), 7.85 - 7.89 (m, 1H), 7.62 - 7.67 (m, 2H), 4.38 - 4.45 (m, 1H), 4.21 - 4.28 (m, 1H), 3.99 - 4.08 (m, 1H), 3.83 - 3.92 (m, 1H), 3.71 - 3.76

(m, 3H), 3.60 - 3.67 (m, 7H), 3.51 - 3.55 (m, 2H), 3.41 (br d, *J* = 3.9 Hz, 2H), 3.16 - 3.29 (m, 2H), 3.08 (s, 3H), 2.93 - 3.00 (m, 1H), 2.58 - 2.64 (m, 2H), 2.41 - 2.55 (m, 5H), 1.87 - 2.15 (m, 5H), 1.67 (td, *J* = 14.4, 7.1 Hz, 2H), 1.47 (br t, *J* = 6.2 Hz, 2H), 0.95 - 1.18 (m, 12H). LCMS (ESI) *m/z* 989 $[M+H]^+$,987$[M-H]^-$. Rt 0.798 min (LP mode).

2.44. N^1-{2-[({3-[(3-{1-[4-Chloro-3-(trifluoromethyl)phenyl]-1*H*-pyrazole-4-carbonyl}-6,6-dimethyl-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)carbamoyl]phenyl}methyl)(pentan-3-yl)amino]ethyl}-N^4-(1-{[2-(dimethylamino)ethyl]amino}-2-methyl-1-oxopropan-2-yl)-N^1-methylbutanediamide (36bt and 36bt hydrochloride)

Compound **36bt** (48%) (**36bt** hydrochloride [quantitative yield]) was prepared from **5t** and **35bt** in a manner similar to that described for compound **36at** (**36at** hydrochloride). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (t, *J* = 7.3 Hz, 3H), 0.96 (t, *J* = 7.3 Hz, 3H), 1.02 - 1.09 (m, 6H), 1.34 - 1.51 (m, 12H), 2.16 - 2.25 (m, 8H), 2.38 - 2.48 (m, 5H), 2.51 - 2.66 (m, 5H), 2.74 - 2.94 (m, 4H), 3.23 - 3.27 (m, 1H), 3.38 - 3.44 (m, 1H), 3.69 - 3.76 (m, 4H), 7.31 - 7.56 (m, 3H), 7.63 - 7.69 (m, 1H), 7.77 - 7.91 (m, 2H), 7.98 (s, 1H), 8.11 - 8.17 (m, 1H), 8.38 - 8.45 (m, 1H), 12.04 - 12.14 (m, 1H). LCMS (ESI) *m/z* 969 $[M+H]^+$. Rt 0.719 min (LP mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{49}H_{64}ClF_3N_8O_5S$ $[M+H]^+$ 969.4434, found 969.4457. **36bt** hydrochloride : ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.95 (s, 1H), 10.92 - 11.52 (m, 1H), 8.41 - 8.48 (m, 1H), 8.12 - 8.28 (m, 3H), 7.86 - 8.02 (m, 3H), 7.56 - 7.68 (m, 2H), 4.66 - 4.77 (m, 1H), 4.42 - 4.52 (m, 1H), 4.29 - 4.37 (m, 1H), 3.72 - 3.77 (m, 1H), 3.62 - 3.69 (m, 2H), 3.34 - 3.48 (m, 2H), 3.20 - 3.30 (m, 2H), 2.90 - 3.12 (m, 4H), 2.68 - 2.87 (m, 7H), 2.41 - 2.57 (m, 5H), 2.05 - 2.26 (m, 2H), 1.62 - 1.67 (m, 6H), 1.46 - 1.55 (m, 6H), 0.81 - 1.08 (m, 12H). LCMS (ESI) *m/z* 969 $[M+H]^+$. Rt 0.979 min (NM mode).

1-[{4-[{2-[({3-[(3-{1-[4-Chloro-3-(trifluoromethyl)phenyl]-1*H*-pyrazole-4-carbonyl}-6,6-dimethyl-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)carbamoyl]phenyl}methyl)(pentan-3-yl)amino]ethyl}(methyl)amino]-4-oxobutanoyl}(methyl)amino]-1-deoxy-D-glucitol (36ct)

Compound **36ct** (39%) was prepared from **5t** and **35ct** in a manner similar to that described for compound **36at**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 - 1.11 (m, 12H), 1.33 - 1.68 (m, 6H), 2.00 (s, 1H), 2.38 - 2.55 (m, 5H), 2.64 - 2.78 (m, 3H), 2.96 - 3.21 (m, 5H), 3.52 - 4.11 (m, 15H), 7.43 - 7.67 (m, 3H), 7.81 - 8.03 (m, 4H), 8.11 - 8.18 (m, 1H), 8.39 - 8.48 (m, 1H),11.98 - 12.10 (m, 1H). LCMS (ESI) *m/z* 991 $[M+H]^+$,989$[M-H]^-$. Rt 1.009 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{48}H_{62}ClF_3N_6O_9S$ $[M+H]^+$ 991.4012, found 991.3993.

第 2 章

Methyl 4-(2-{4-[(3-amino-6-chloropyridine-2-carbonyl)amino]phenyl}ethyl) benzoate (8ap)

Methyl 4-[2-(4-aminophenyl)ethyl]benzoate (124 mg, 487 μmol), EDC･HCl (117 mg, 608 μmol) and HOBt･H_2O (93.2 mg, 608 μmol) were added to a solution of **7p** (70.0 mg, 406 μmol) in DMF (2.0 mL). The mixture was stirred at room temperature for 2 d and then at 100 °C for 14 h. The reaction mixture was washed with water and extracted with EtOAc. The combined organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified using column chromatography on silica gel and eluted with 50% EtOAc/*n*-hexane to yield **8ap** (100 mg, 60%) as a yellow amorphous substance. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.87 - 3.02 (m, 4H), 3.90 (s, 3H), 6.09 (br s, 2H), 7.04 (d, J = 8.6 Hz, 1H), 7.14 (d, J = 8.3 Hz, 2H), 7.18 - 7.24 (m, 3H), 7.55 - 7.63 (m, 2H), 7.94 (d, J = 8.1 Hz, 2H), 9.61 - 9.71 (m, 1H). LCMS (ESI) *m/z* 410 $[M+H]^+$. Rt 1.295 min (LP mode).

Methyl 4-{4-[(3-amino-6-chloropyridine-2-carbonyl)amino]phenoxy}benzoate (8bp)

Compound **8bp** (77%) was prepared from **7p** and methyl 4-(4-aminophenoxy)benzoate in a manner similar to that described for compound **8ap**. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.90 (s, 3H), 6.01 - 6.17 (m, 2H), 6.94 - 7.02 (m, 2H),

7.04 - 7.12 (m, 3H), 7.22 (d, *J* = 8.6 Hz, 1H), 7.69 - 7.75 (m, 2H), 7.97 - 8.04 (m, 2H), 9.70 - 9.76 (m, 1H). LCMS (ESI) *m/z* 398 $[M+H]^+$. Rt 1.231 min (NM mode).

Ethyl 6-{4-[(3-amino-6-chloro-pyridine-2-carbonyl)amino]phenoxy}pyridine-3-carboxylate (8cp)

Compound **8cp** (62%) was prepared from **7p** and ethyl 6-(4-aminophenoxy)pyridine-3-carboxylate in a manner similar to that described for compound **8ap**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 - 1.43 (m, 3H), 4.38 (q, *J* = 7.2 Hz, 2H), 6.09 (br s, 2H), 6.93 (d, *J* = 8.7 Hz, 1H), 7.03 - 7.08 (m, 1H), 7.14 - 7.23 (m, 3H), 7.77 (d, *J* = 8.7 Hz, 2H), 8.28 (dd, *J* = 8.7, 2.2 Hz, 1H), 8.84 (d, *J* = 2.2 Hz, 1H), 9.70 - 9.80 (m, 1H). LCMS (ESI) *m/z* 413 $[M+H]^+$. Rt 1.182 min (NM mode).

Methyl 6-[[4-[(3-amino-6-chloro-pyridine-2-carbonyl)amino]phenoxy] methyl]pyridine-3-carboxylate (8dp)

Compound **8dp** (40%) was prepared from **7p** and methyl 6-[(4-aminophenoxy)methyl]pyridine-3-carboxylate in a manner similar to that described for compound **8ap**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97 (s, 3H), 5.27 (s, 2H), 6.04 - 6.12 (m, 2H), 6.97 - 7.06 (m, 3H), 7.17 - 7.22 (m, 1H), 7.59 - 7.67 (m, 3H), 8.30 - 8.35 (m, 1H), 9.20 (d, *J* = 1.5 Hz, 1H), 9.58 - 9.64 (m, 1H). LCMS (ESI) *m/z* 413 $[M+H]^+$. Rt 1.182 min (NM mode).

Methyl 4-[2-(4-{[3-(3-{[(2-{[4-(benzyloxy)-4-oxobutanoyl](methyl) amino}ethyl)(pentan-3-yl)amino]methyl}benzamido)-6-chloropyridine-2-carbonyl]amino}phenyl)ethyl]benzoate (9ap)

Pyridine (180 μL, 2.21 mmol) and benzyl 4-[2-[(3-chlorocarbonylphenyl)methyl-(1-ethylpropyl)amino] ethyl-methyl-amino]-4-oxo-butanoate (647 mg, 1.33 mmol) were added to a solution of **8ap** (200 mg, 443 μmol) in $CHCl_3$ (2.0 mL). The mixture was stirred overnight at room temperature, then washed with a saturated $NaHCO_3$ aqueous solution, a saturated ammonium chloride (NH_4Cl) aqueous solution and extracted with $CHCl_3$. The combined organic layer was concentrated under reduced pressure, and the residue was purified using column chromatography on silica gel and eluted with 50%

EtOAc/*n*-hexane to yield **9ap** (230 mg, 58%) as a pale brown amorphous substance. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 - 1.07 (m, 6H), 1.28 - 1.55 (m, 4H), 2.21 - 2.71 (m, 7H), 2.76 - 2.89 (m, 3H), 2.92 - 3.02 (m, 4H), 3.15 - 3.25 (m, 1H), 3.39 (t, *J* = 7.1 Hz, 1H), 3.74 (d, *J* = 3.4 Hz, 2H), 3.90 (s, 3H), 5.09 (d, *J* = 17.1 Hz, 2H), 7.11 - 7.37 (m, 9H), 7.42 - 7.69 (m, 5H), 7.95 (d, *J* = 8.3 Hz, 3H), 8.06 (br d, *J* = 11.7 Hz, 1H), 9.33 - 9.45 (m, 1H), 9.98 (s, 1H), 12.85 - 13.02 (m, 1H). LCMS (ESI) *m/z* 860 $[M+H]^+$. Rt 1.169 min (NM mode).

Ethyl 6-(4-{[3-(3-{[(2-{[4-(benzyloxy)-4-oxobutanoyl](methyl)amino}ethyl)(pentan-3-yl)amino]methyl}benzamido)-6-chloropyridine-2-carbonyl]amino}phenoxy)pyridine-3-carboxylate (9cp)

Compound **9cp** (75%) was prepared from **8cp** in a manner similar to that described for compound **9ap**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 - 1.00 (m, 6H), 1.34 - 1.53 (m, 7H), 2.27 - 2.67 (m, 7H), 2.79 - 2.89 (m, 3H), 3.15 - 3.23 (m, 1H), 3.37 - 3.43 (m, 1H), 3.72 - 3.77 (m, 2H), 4.33 - 4.44 (m, 2H), 5.05 - 5.11 (m, 2H), 6.96 (d, *J* = 8.6 Hz, 1H), 7.19 - 7.24 (m, 2H), 7.30 - 7.35 (m, 5H), 7.45 - 7.58 (m, 3H), 7.78 - 7.84 (m, 2H), 7.91 - 7.97 (m, 1H), 8.05 - 8.13 (m, 1H), 8.26 - 8.32 (m, 1H), 8.83 (d, *J* = 2.2 Hz, 1H), 9.41 (dd, *J* = 8.9, 4.8 Hz, 1H), 10.06 (s, 1H), 12.90 - 12.96 (m, 1H). LCMS (ESI) *m/z* 863 $[M+H]^+$. Rt 1.134 min (NM mode).

4-[2-(4-{[3-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-6-chloropyridine-2-carbonyl]amino}phenyl)ethyl]benzoic acid (10ap)

A 1M NaOH aqueous solution (3.00 mL, 3.00 mmol) was added to a solution of **9ap** (85.0 mg, 98.8 μmol) in THF (6.0 mL). The mixture was stirred overnight at room temperature and purified using reverse-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=90:10 → 20:80 → 5:95, 40 mL/min) to yield **10ap** (30.0 mg, 40%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 - 0.98 (m, 6H), 1.25 - 1.55 (m, 4H), 2.23 - 2.31 (m, 2H), 2.45 - 2.50 (m, 1H), 2.57 - 2.72 (m, 5H), 2.82 - 3.01 (m, 6H), 3.20 - 3.26 (m, 1H), 3.37 - 3.44 (m, 1H), 3.72 - 3.78 (m, 2H), 6.91 - 7.01 (m, 2H), 7.08 (br d, *J*

= 8.1 Hz, 2H), 7.43 - 7.66 (m, 5H), 7.89 - 8.03 (m, 4H), 9.34 - 9.40 (m, 1H), 9.92 - 9.95 (m, 1H), 12.96 (s, 1H). LCMS (ESI) *m/z* 756 $[M+H]^+$. Rt 0.925 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{41}H_{46}ClN_5O_7$ $[M+H]^+$ 756.3159, found 756.3165.

Methyl 6-[(4-{[3-(3-{[(2-{[4-(benzyloxy)-4-oxobutanoyl](methyl)amino}ethyl)(pentan-3-yl)amino]methyl}benzamido)-6-chloropyridine-2-carbonyl]amino}phenoxy) methyl]pyridine-3-carboxylate (10bp)

Compound **10bp** (36% over 2 steps) was prepared from **8bp** in a manner similar to that described for compounds **9ap** and **10ap**. LCMS (ESI) *m/z* 744 $[M+H]^+$. Rt 0.890 min (NM mode).

6-(4-{[3-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-6-chloropyridine-2-carbonyl]amino}phenoxy)pyridine-3-carboxylic acid (10cp)

Compound **10cp** (53%) was prepared from **9cp** in a manner similar to that described for compound **10ap**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 - 1.06 (m, 6H), 1.34 - 1.58 (m, 4H), 2.29 - 2.38 (m, 2H), 2.50 - 2.74 (m, 5H), 2.80 - 2.91 (m, 3H), 3.16 - 3.21 (m, 1H), 3.42 - 3.47 (m, 1H), 3.73 - 3.79 (m, 2H), 6.96 - 7.02 (m, 1H), 7.21 - 7.24 (m, 1H), 7.46 - 7.83 (m, 5H), 7.92 - 7.99 (m, 1H), 8.01 - 8.09 (m, 2H), 8.24 - 8.35 (m, 1H), 8.79 - 8.87 (m, 1H), 9.36 - 9.43 (m, 1H), 10.01 - 10.10 (m, 1H), 12.90 - 12.96 (m, 1H). LCMS (ESI) *m/z* 745 $[M+H]^+$, 743 $[M-H]^-$. Rt 0.825 min (NM mode).

Methyl 6-[(4-{[3-(3-{[(2-{[4-(benzyloxy)-4-oxobutanoyl](methyl)amino}ethyl)(pentan-3-yl)amino]methyl}benzamido)-6-chloropyridine-2-carbonyl]amino}phenoxy) methyl]pyridine-3-carboxylate (10dp)

Compound **10dp** (34% over 2 steps) was prepared from **8dp** in a manner similar to that described for compounds **9ap** and **10ap**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75 - 1.01 (m, 6H), 1.25 - 1.58 (m, 4H), 2.29 - 2.77 (m, 7H), 2.80 - 2.99 (m, 3H), 3.25 - 3.34 (m, 1H), 3.49 - 3.57 (m, 1H), 3.72 - 3.85 (m, 2H), 5.30 - 5.40 (m, 2H), 6.94 - 7.04 (m, 2H), 7.43 - 7.71 (m, 5H), 7.87 - 8.00 (m, 2H), 8.30 - 8.39 (m, 1H), 9.17 - 9.24 (m, 1H), 9.34 -

9.40 (m, 1H), 9.86 - 9.95 (m, 1H), 12.91 - 13.06 (m, 1H). LCMS (ESI) *m/z* 759 $[M+H]^+$, 757 $[M-H]^-$. Rt 0.816 min (NM mode).

4-[2-(4-{[3-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-6-(piperidin-1-yl)pyridine-2-carbonyl]amino}phenyl) ethyl]benzoic acid (11ap)

A solution of **10ap** (30.0 mg, 39.7 μmol) in piperidine (1.0 mL) was stirred at 100 °C under microwave irradiation for 30 min and then at 120 °C for 3 h. The mixture was purified using reverse-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN = 90:10 → 20:80 → 5:95, 40 mL/min) to yield **11ap** (23.0 mg, 72%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 - 1.00 (m, 6H), 1.25 - 1.57 (m, 6H), 1.70 (br s, 4H), 2.38 - 2.45 (m, 3H), 2.57 - 2.61 (m, 2H), 2.64 - 2.71 (m, 3H), 2.82 - 3.02 (m, 7H), 3.18 - 3.25 (m, 1H), 3.41 - 3.46 (m, 1H), 3.53 - 3.59 (m, 3H), 3.71 - 3.80 (m, 2H), 6.95 - 7.16 (m, 4H), 7.42 - 7.61 (m, 4H), 7.90 - 8.06 (m, 5H), 9.08 - 9.14 (m, 1H), 10.14 (s, 1H),12.56 - 12.65 (m, 1H). LCMS (ESI) *m/z* 805 $[M+H]^+$. Rt 1.037 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{46}H_{56}N_6O_7$ $[M+H]^+$ 805.4283, found 805.4290.

4-(4-{[3-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-6-(piperidin-1-yl)pyridine-2-carbonyl]amino}phenoxy) benzoic acid (11bp)

Compound **11bp** (78%) was prepared from **10bp** in a manner similar to that described for compound **11ap**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 - 1.04 (m, 6H), 1.30 - 1.61 (m, 6H), 1.71 (br s, 4H), 2.29 - 2.36 (m, 2H), 2.54 - 2.68 (m, 5H), 2.80 - 2.91 (m, 3H), 3.12 - 3.20 (m, 1H), 3.39 - 3.45 (m, 1H), 3.54 - 3.59 (m, 3H), 3.71 - 3.77 (m, 3H), 6.95 - 7.16 (m, 5H), 7.38 - 7.59 (m, 2H), 7.72 (t, J = 8.8 Hz, 2H), 7.89 - 8.09 (m, 4H), 9.14 (d, J = 9.3 Hz, 1H), 10.21 - 10.29 (m, 1H), 12.53 - 12.61 (m, 1H). LCMS (ESI) *m/z* 793 $[M+H]^+$. Rt 1.014 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{44}H_{52}N_6O_8$ $[M+H]^+$ 793.3919, found 793.3924.

6-(4-{[3-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-6-(piperidin-1-yl)pyridine-2-carbonyl]amino}phenoxy) pyridine-3-carboxylic acid (11cp)

Compound **11cp** (38%) was prepared from **10cp** in a manner similar to that described for compound **11ap**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 - 1.05 (m, 6H), 1.28 - 1.58 (m, 6H), 1.71 (br s, 4H), 2.31 - 2.35 (m, 2H), 2.51 - 2.70 (m, 6H), 2.79 - 2.90 (m, 3H), 3.14 - 3.21 (m, 1H), 3.40 - 3.46 (m, 1H), 3.56 (br s, 3H), 3.74 (s, 2H), 6.94 - 7.04 (m, 2H), 7.16 - 7.23 (m, 1H), 7.44 - 7.55 (m, 2H), 7.70 - 7.79 (m, 2H), 7.90 - 7.97 (m, 1H), 8.01 - 8.08 (m, 2H), 8.25 - 8.34 (m, 1H), 8.80 - 8.86 (m, 1H), 9.08 - 9.18 (m, 1H), 10.22 - 10.30 (m, 1H), 12.54 - 12.63 (m, 1H). LCMS (ESI) *m/z* 794 $[M+H]^+$. Rt 0.945 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{43}H_{51}N_7O_8$ $[M+H]^+$ 794.3872, found 794.3872.

6-(4-{[3-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-6-(piperidin-1-yl)pyridine-2-carbonyl]amino}phenoxy) pyridine-3-carboxylic acid (11dp)

Compound **11dp** (28%) was prepared from **10dp** in a manner similar to that described for compound **11ap**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.71 - 0.96 (m, 6 H), 1.20 - 1.54 (m, 6H), 1.56 - 1.67 (m, 4H), 2.18 - 2.43 (m, 6H), 2.60 - 2.67 (m, 2H), 2.69 - 2.89 (m, 3H), 3.10 - 3.19 (m, 2H), 3.55 - 3.64 (m, 3H), 3.69 - 3.78 (m, 2H), 5.23 (s, 2H), 7.01 - 7.07 (m, 1H), 7.21 - 7.26 (m, 1H), 7.45 - 7.68 (m, 4H), 7.77 - 7.82 (m, 1H), 7.91 - 8.00 (m, 1H), 8.24 (s, 3H), 8.89 - 9.00 (m, 2H), 10.31 (s, 1H), 12.44 - 12.51 (m, 1H). LCMS (ESI) *m/z* 808 $[M+H]^+$, 806 $[M-H]^-$. Rt 0.931 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{44}H_{53}N_7O_8$ $[M+H]^+$ 808.4028, found 808.4033.

4-[2-(4-{[3-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-6-methoxypyridine-2-carbonyl]amino}phenyl)ethyl] benzoic acid (12ap)

MeOH (0.3 mL), (1R,2R)-N,N'-dimethyl-1,2-cyclohexanediamine (1.86 mg, 13.1 μmol), Na_2CO_3 (3.70 mg, 34.9 μmol) and cuprous iodide (1.66 mg, 8.72 μmol) were added to a solution of **9ap** (15.0 mg, 17.4 μmol) in DMSO (1.0 mL). The mixture was

stirred at 150 °C for 2.5 h, then washed with a saturated NH_4Cl aqueous solution and brine and extracted with EtOAc. The combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified using column chromatography on an NH-silica gel and eluted with 50% EtOAc/*n*-hexane to yield methyl 4-[2-(4-{[6-methoxy-3-(3-{[{2-[(4-methoxy-4-oxobutanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)pyridine-2-carbonyl]amino}phenyl)ethyl]benzoate (9.90 mg, 73%) as a colorless oil. LCMS (ESI) *m/z* 780 $[M+H]^+$, 778 $[M-H]^-$. Rt 1.026 min (NM mode). Next, a solution of the resulting methoxypyridine (9.90 mg, 13.0 μmol) in THF (1.0 mL) and MeOH (0.3 mL) was prepared and a 1M NaOH aqueous solution (300 μL, 300 μmol) was added. The mixture was stirred overnight at room temperature and concentrated under reduced pressure. The residue was purified using reverse-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=90:10 → 20:80 → 5:95, 40 mL/min) to yield **12ap** (7.4 mg, 78%) as a colorless solid. ^1H NMR (400 MHz, DMSO-d_6) δ 0.80 - 0.97 (m, 6H), 1.24 - 1.35 (m, 2H), 1.45 - 1.55 (m, 2H), 2.19 - 2.42 (m, 8H), 2.69 - 3.00 (m, 8H), 3.69 - 3.79 (m, 2H), 4.05 (s, 3H), 7.19 - 7.38 (m, 5H), 7.51 - 7.72 (m, 4H), 7.81 - 7.87 (m, 2H), 7.97 - 8.05 (m, 1H), 8.15 - 8.19 (m, 1H), 9.08 - 9.15 (m, 1H), 10.36 - 10.44 (m, 1H), 12.53 - 12.60 (m, 1H). LCMS (ESI) *m/z* 752 $[M+H]^+$, 750 $[M-H]^-$. Rt 0.871 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{42}H_{49}N_5O_8$ $[M+H]^+$ 752.3654, found 752.3653.

4-[2-(4-{[3-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-6-(2-methoxyethoxy)pyridine-2-carbonyl]amino}phenyl)ethyl]benzoic acid (12bp)

Compound **12bp** (25% over 2 steps) was prepared from **9ap** and 2-methoxyethanol in a manner similar to that described for compound **12ap**. ^1H NMR (400 MHz, CD_3OD) δ 0.89 - 1.06 (m, 6H), 1.39 - 1.49 (m, 2H), 1.58 - 1.69 (m, 2H), 2.32 - 2.53 (m, 5H), 2.72 - 3.15 (m, 9H), 3.36 - 3.52 (m, 6H), 3.79 - 3.92 (m, 3H), 4.57 - 4.63 (m, 2H), 7.15 - 7.32 (m, 4H), 7.48 - 7.69 (m, 4H), 7.88 - 7.98 (m, 3H), 8.09 - 8.15 (m, 1H), 8.33 (s, 1H), 9.19 - 9.24 (m, 1H). LCMS (ESI) *m/z* 796 $[M+H]^+$, 794 $[M-H]^-$. Rt 0.849 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{44}H_{53}N_5O_9$ $[M+H]^+$ 796.3916, found.

4-[2-(4-{[3-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-6-(dimethylamino)pyridine-2-carbonyl]amino}phenyl) ethyl]benzoic acid (12cp)

Compound **12cp** (17% over 2 steps) was prepared from **9ap** and dimethylamine in a manner similar to that described for compound **12ap**. ^{1}H NMR (400 MHz, CD_3OD) δ 0.89 - 1.07 (m, 6H), 1.40 - 1.51 (m, 2H), 1.58 - 1.72 (m, 2H), 2.51 (s, 5H), 2.72 - 2.80 (m, 2H), 2.88 - 3.05 (m, 7H), 3.17 (s, 6H), 3.44 - 3.55 (m, 2H), 3.81 (s, 1H), 3.94 - 4.01 (m, 1H), 7.00 (d, J = 9.5 Hz, 1H), 7.13 - 7.30 (m, 4H), 7.55 - 7.68 (m, 3H), 7.87 - 7.99 (m, 3H), 8.07 - 8.15 (m, 1H), 8.24 (s, 1H), 8.97 - 9.03 (m, 1H). LCMS (ESI) *m/z* 765 $[M+H]^+$, 763 $[M-H]^-$. Rt 0.898 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{43}H_{52}N_6O_7$ $[M+H]^+$ 765.3970, found 765.3979.

4-[2-(4-{[3-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-6-(morpholin-4-yl)pyridine-2-carbonyl]amino}phenyl) ethyl]benzoic acid (13ap)

Compound **13ap** (34%) was prepared from **10ap** and morpholine in a manner similar to that described for compound **11ap**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 - 1.01 (m, 6H), 1.29 - 1.44 (m, 2H), 1.47 - 1.60 (m, 2H), 2.31 - 2.35 (m, 1H), 2.42 - 2.45 (m, 1H), 2.58 - 2.72 (m, 5H), 2.77 - 3.02 (m, 6H), 3.18 - 3.29 (m, 1H), 3.37 - 3.46 (m, 2H), 3.48 - 3.56 (m, 3H), 3.57 - 3.69 (m, 2H), 3.70 - 3.81 (m, 2H), 3.86 - 3.95 (m, 3H), 6.89 - 7.22 (m, 5H), 7.41 - 7.63 (m, 4H), 7.90 - 8.07 (m, 4H), 9.17 - 9.25 (m, 1H), 10.01 - 10.07 (m, 1H), 12.62 - 12.73 (m, 1H). LCMS (ESI) *m/z* 807 $[M+H]^+$, 805 $[M-H]^-$. Rt 0.877 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{45}H_{54}N_6O_8$ $[M+H]^+$ 807.4076, found 807.4104.

4-[2-(4-{[3-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-6-(4-methylpiperazin-1-yl)pyridine-2-carbonyl]amino}phenyl)ethyl]benzoic acid (13bp)

Compound **13bp** (38%) was prepared from **10ap** and 1-methylpiperazine in a manner similar to that described for compound **11ap**. ^{1}H NMR (400 MHz, $CDCl_3$) δ

0.73 - 1.10 (m, 6H), 1.29 - 1.59 (m, 4H), 2.38 - 2.40 (m, 1H), 2.54 (s, 8H), 2.75 - 3.05 (m, 8H), 3.16 - 3.24 (m, 1H), 3.49 (s, 6H), 3.59 - 3.81 (m, 5H), 6.84 - 7.18 (m, 5H), 7.35 - 7.63 (m, 4H), 7.86 - 8.02 (m, 3H), 8.15 (s, 1H), 9.18 (br dd, J = 9.2, 2.1 Hz, 1H), 9.97 (s, 1H), 12.65 (s, 1H). LCMS (ESI) *m/z* 807 $[M+H]^+$, 805 $[M-H]^-$. Rt 0.877 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{46}H_{57}N_7O_7$ $[M+H]^+$ 820.4392, found 820.4378.

***tert*-Butyl 4-(2-{4-[(3-amino-6-chloropyridine-2-carbonyl)amino]phenyl}ethyl) benzoate (14p)**

Compound **14p** (60%) was prepared from **7** and *tert*-butyl 4-(4-aminophenethyl)benzoate in a manner similar to that described for compound **8ap**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H), 2.35 - 3.00 (m, 4H), 6.08 (br s, 2H), 7.00 - 7.25 (m, 6H), 7.55 - 7.65 (m, 2H), 7.85 - 7.95 (m, 2H), 9.69 (s, 1H).

***tert*-Butyl 4-[2-(4-{[3-(3-{[(2-{[4-(benzyloxy)-4-oxobutanoyl](methyl)amino} ethyl)(pentan-3-yl)amino]methyl}benzamido)-6-chloropyridine-2-carbonyl]amino} phenyl)ethyl]benzoate (15p)**

Compound **15p** (58%) was prepared from **14p** in a manner similar to that described for compound **9ap**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 - 1.00 (m, 6H), 1.30 - 1.51 (m, 4H), 1.59 (s, 9H), 2.26 - 2.41 (m, 2H), 2.54 - 2.68 (m, 5H), 2.78 - 2.89 (m, 3H), 2.91 - 3.00 (m, 4H), 3.12 - 3.24 (m, 1H), 3.32 - 3.43 (m, 1H), 3.66 - 3.78 (m, 2H), 5.05 - 5.15 (m, 2H), 7.15 - 7.23 (m, 4H), 7.28 - 7.37 (m, 5H), 7.45 - 7.67 (m, 5H), 7.86 - 7.97 (m, 3H), 8.02 - 8.16 (m, 1H), 9.40 (dd, J = 8.9, 4.5 Hz, 1H), 9.89 - 10.03 (m, 1H), 12.97 (br d, J = 9.8 Hz, 1H). LCMS (ESI) *m/z* 902 $[M+H]^+$. Rt 0.992 min (LP mode).

4-[(2-{[(3-{[2-{[4-(2-{4-[(Methanesulfonyl)carbamoyl]phenyl}ethyl)phenyl] carbamoyl}-6-(piperidin-1-yl)pyridin-3-yl]carbamoyl}phenyl)methyl](pentan-3-yl)amino}ethyl)(methyl)amino]-4-oxobutanoic acid (16ap)

Tert-butyl 4-[2-(4-{[3-(3-{[(2-{[4-(benzyloxy)-4-oxobutanoyl](methyl)amino}ethyl) (pentan-3-yl)amino]methyl}benzamido)-6-(piperidin-1-yl)pyridine-2-carbonyl]amino} phenyl)ethyl]benzoate (34%) was prepared from **15p** in a manner similar to that

described for compound **11ap**. LCMS (ESI) *m/z* 951 $[M+H]^+$. Rt 1.319 min (NM mode). The resulting intermediate (232 mg, 244 μmol) in $CHCl_3$ (3.0 mL) was then used. TFA (2.0 mL) was added, and the mixture was stirred at room temperature for 1 h and concentrated under reduced pressure; then, the residue was azeotroped twice with toluene and 3 times with IPE to yield a mono-carboxylic acid intermediate (306 mg) as a yellow amorphous substance, which was used without further purification. LCMS (ESI) *m/z* 895 $[M+H]^+$, 893 $[M-H]^-$. Rt 1.141 min (NM mode). The resulting intermediate (30.0 mg, 33.5 μmol) in $CHCl_3$ (170 μL) was used in the next step. Methanesulfonamide (6.38 mg, 67.0 μmol), EDC·HCl (15.4 mg, 80.4 μmol) and *N*,*N*-dimethyl-4-aminopyridine (DMAP) (9.83 mg, 80.4 μmol) were added, and the mixture was stirred overnight at room temperature and at 60 °C for 30 min. Next, methanesulfonamide (6.38 mg, 67.0 μmol), EDC·HCl (15.4 mg, 80.4 μmol) and *N*,*N*-dimethyl-4-aminopyridine (DMAP) (9.83 mg, 80.4 μmol) were added to the reaction mixture. The mixture was stirred at 60 °C for 20 min and washed with a saturated NH_4Cl aqueous solution and extracted with $CHCl_3$. The combined organic layer was concentrated under reduced pressure to yield a methanesulfonyl amide intermediate, which was used without further purification. **16ap** (25% over 3 steps) was prepared from the resulting methanesulfonyl amide intermediate in a manner similar to that described for compound **10ap**. ^1H NMR (400 MHz, $CDCl_3$) δ 0.85 - 1.06 (m, 6 H), 1.29 - 1.77 (m, 10H), 2.25 - 2.47 (m, 3H), 2.50 - 2.72 (m, 4H), 2.76 - 2.89 (m, 3H), 2.92 - 3.04 (m, 4H), 3.17 - 3.25 (m, 1H), 3.34 - 3.46 (m, 4H), 3.55 (br s, 4H), 3.69 - 3.78 (m, 2H), 6.92 - 7.12 (m, 3H), 7.13 - 7.23 (m, 2H), 7.36 - 7.66 (m, 4H), 7.71 - 7.82 (m, 2H), 7.87 - 7.97 (m, 1H), 8.04 (br d, *J* = 6.6 Hz, 1H), 9.04 - 9.17 (m, 1H), 10.17 (s, 1H), 12.45 - 12.65 (m, 1H). LCMS (ESI) *m/z* 882 $[M+H]^+$, 880 $[M-H]^-$. Rt 0.953 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{47}H_{59}N_7O_8S$ $[M+H]^+$ 882.4219, found 882.4204.

4-[Methyl(2-{[(3-{[2-{[4-(2-{4-[(methylsulfamoyl)carbamoyl]phenyl}ethyl) phenyl]carbamoyl}-6-(piperidin-1-yl)pyridin-3-yl]carbamoyl}phenyl)methyl](pentan-3-yl)amino}ethyl)amino]-4-oxobutanoic acid (16bp)

Compound **16bp** (7.8% over 4 steps) was prepared from **15p** and (sulfamoylamino)methane in a manner similar to that described for compound **16ap**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 - 1.04 (m, 6H), 1.37 - 1.74 (m, 10H), 2.33 - 2.75 (m, 10H), 2.78 - 2.89 (m, 3H), 2.91 - 3.03 (m, 4H), 3.16 - 3.24 (m, 1H), 3.43 (br t, J = 6.5 Hz, 1H), 3.55 (br s, 4H), 3.68 - 3.78 (m, 2H), 6.94 - 7.23 (m, 5H), 7.39 - 7.62 (m, 4H), 7.72 - 7.80 (m, 2H), 7.87 - 7.96 (m, 1H), 8.03 (br d, J = 8.6 Hz, 1H), 9.05 - 9.16 (m, 1H), 10.17 (s, 1H), 12.49 - 12.64 (m, 1H). LCMS (ESI) *m/z* 897 $[M+H]^+$, 895 $[M-H]^-$. Rt 0.944 min (NM mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{47}H_{60}N_8O_8S$ $[M+H]^+$ 897.4328, found 897.4314.

***tert*-Butyl 4-[2-(4-{[3-(3-{ethyl[(1r,4r)-4-(methoxycarbonyl)cyclohexyl] sulfamoyl}benzamido)-6-(piperidin-1-yl)pyridine-2-carbonyl]amino}phenyl) ethyl]benzoate (17p)**

Compound **17p** (53% over 2 steps) was prepared from **14p** and methyl (1r,4r)-4-{[3-(chlorocarbonyl)benzene-1-sulfonyl](ethyl)amino}cyclohexane-1-carboxylate in a manner similar to that described for compounds **9ap** and **11ap**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19 - 1.34 (m, 4H), 1.42 - 1.53 (m, 4H), 1.57 - 1.62 (m, 9H), 1.67 - 1.77 (m, 7H), 1.96 - 2.02 (m, 2H), 2.11 - 2.20 (m, 1H), 2.91 - 3.01 (m, 4H), 3.29 - 3.38 (m, 2H), 3.54 - 3.72 (m, 7H), 7.00 (d, J = 9.5 Hz, 1H), 7.14 - 7.23 (m, 4H), 7.53 - 7.69 (m, 3H), 7.89 (d, J = 8.3 Hz, 2H), 7.98 - 8.28 (m, 3H), 8.53 (s, 1H), 9.11 (d, J = 9.3 Hz, 1H), 10.14 - 10.22 (m, 1H), 12.83 - 12.92 (m, 1H). LCMS (ESI) *m/z* 874 $[M+Na]^+$. Rt 1.308 min (LP mode).

4-[2-(4-{[3-{3-[(4-Carboxycyclohexyl)(ethyl)sulfamoyl]benzamido}-6-(piperidin-1-yl)pyridine-2-carbonyl]amino}phenyl)ethyl]benzoic acid (18p)

A mono-carboxylic acid intermediate was prepared from **17p** in a manner similar to that described for compound **10ap**. LCMS (ESI) *m/z* 860 $[M+Na]^+$, 836 $[M-H]^-$. Rt 1.253 min (LP mode). The resulting intermediate (30.0 mg, 35.8 μmol) in $CHCl_3$ (1.0 mL) was used in the next step. TFA (500 μL) was added, and the mixture was stirred at room temperature for 30 min and concentrated under reduced pressure; then, the residue was purified using reverse-phase preparative HPLC (column, YMC-Actus Triart 5 μm

C18 50×30 mm; mobile phase, 0.1% formic acid in H2O:0.1% formic acid in MeCN=80:20 → 5:95 → 1:99, 40 mL/min) to yield **18p** (6.70 mg, 24% over 2 steps) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 - 1.21 (m, 3H), 1.25 - 1.68 (m, 11H), 1.79 - 1.95 (m, 2H), 2.05 - 2.17 (m, 1H), 2.85 - 3.04 (m, 4H), 3.24 - 3.28 (m, 4H), 3.62 (br s, 4H), 7.14 - 7.39 (m, 5H), 7.59 - 7.70 (m, 2H), 7.78 - 7.89 (m, 3H), 8.03 - 8.13 (m, 1H), 8.15 - 8.21 (m, 1H), 8.29 - 8.37 (m, 1H), 8.85 (d, J = 9.3 Hz, 1H), 10.36 (s, 1H), 12.49 - 12.59 (m, 1H). LCMS (ESI) *m/z* 782 $[M+H]^+$, 780 $[M-H]^-$. Rt 1.077 min (LP mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{42}H_{47}N_5O_8S$ $[M+H]^+$ 782.3218, found 782.3219.

4-[Ethyl(3-{[2-{[4-(2-{4-[(methylsulfamoyl)carbamoyl]phenyl}ethyl)phenyl] carbamoyl}-6-(piperidin-1-yl)pyridin-3-yl]carbamoyl}benzene-1-sulfonyl)amino] cyclohexane-1-carboxylic acid (19p)

Compound **19p** (30%, over 3 steps) was prepared from **17p** in a manner similar to that described for compound **16bp** (steps b, c, d). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 - 1.24 (m, 3H), 1.25 - 1.71 (m, 10H), 1.80 - 1.94 (m, 2H), 2.03 - 2.17 (m, 1H), 2.86 - 3.02 (m, 3H), 3.23 - 3.32 (m, 9H), 3.54 - 3.70 (m, 4H), 7.18 - 7.44 (m, 5H), 7.61 - 7.73 (m, 2H), 7.76 - 7.91 (m, 3H), 8.05 - 8.21 (m, 2H), 8.29 - 8.40 (m, 1H), 8.81 - 8.91 (m, 1H), 10.33 - 10.43 (m, 1H), 11.59 - 11.74 (m, 1H), 11.98 - 12.11 (m, 1H), 12.48 - 12.59 (m, 1H). LCMS (ESI) *m/z* 874 $[M+H]^+$, 872 $[M-H]^-$. Rt 1.004 min (LP mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{43}H_{51}N_7O_9S_2$ $[M+H]^+$ 874.3262, found 874.3258.

4-{2-[4-({3-[3-(Ethyl{4-[(methylsulfamoyl)carbamoyl]cyclohexyl}sulfamoyl) benzamido]-6-(piperidin-1-yl)pyridine-2-carbonyl}amino)phenyl]ethyl}benzoic acid (20ap)

Compound **20ap** (20%, over 3 steps) was prepared from **17p** in a manner similar to that described for compounds **10ap, 16bp** (step c), and **18p** (step d). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.07 - 1.23 (m, 3H), 1.34 - 1.83 (m, 11H), 2.04 - 2.19 (m, 2H), 2.33 - 2.44 (m, 3H), 2.88 - 3.02 (m, 3H), 3.20 - 3.30 (m, 6H), 3.52 - 3.71 (m, 4H), 7.14 - 7.42 (m, 5H), 7.60 - 7.72 (m, 2H), 7.75 - 7.93 (m, 3H), 8.04 - 8.12 (m, 1H), 8.13 - 8.22 (m, 1H), 8.30 - 8.39 (m, 1H), 8.81 - 8.92 (m, 1H), 10.37 (s, 1H), 12.55 (s, 1H). LCMS (ESI)

m/z 874 $[M+H]^+$, 872 $[M-H]^-$. Rt 1.036 min (LP mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{43}H_{51}N_7O_9S_2$ $[M+H]^+$ 874.3262, found 874.3258.

4-{2-[4-({3-[3-(ethyl{4-[(2-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}ethyl) carbamoyl]cyclohexyl}sulfamoyl)benzamido]-6-(piperidin-1-yl)pyridine-2-carbonyl}amino)phenyl]ethyl}benzoic acid (20bp)

A 1M NaOH aqueous solution (1.00 mL, 1.00 mmol) was added to a solution of **17p** (100 mg, 117 μmol) in THF (2.0 mL) and MeOH (1.0 mL). The mixture was stirred overnight at room temperature and quenched with a 2M HCl aqueous solution. The aqueous layer was extracted with $CHCl_3$ using a phase separator. The combined organic layer was concentrated to yield a mono-carboxylic acid intermediate, which was used without further purification. 2-{2-[2-(2-Aminoethoxy)ethoxy]ethoxy}ethan-1-ol (34.0 mg, 176 μmol), 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) (66.9 mg, 176 μmol) and DIPEA (61.3 μL, 352 μmol) were then added to the mono-carboxylic acid intermediate (resulting from the previous step) in DMF (1.0 mL). The mixture was stirred overnight at room temperature and washed with a saturated $NaHCO_3$ aqueous solution and a saturated NH_4Cl aqueous solution and extracted with EtOAc. The combined organic layer was concentrated under reduced pressure to yield an amide intermediate, which was used without further purification. **20bp** (quantitative yield over 3 steps) was prepared from the resulting amide intermediate in a manner similar to that described for compound **18p** (step d). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 - 1.23 (m, 3H), 1.32 - 1.75 (m, 13H), 1.93 - 2.07 (m, 1H), 2.87 - 3.02 (m, 4H), 3.10 - 3.20 (m, 2H), 3.24 - 3.51 (m, 20H), 3.62 (br s, 4H), 7.21 - 7.38 (m, 4H), 7.63 - 7.74 (m, 3H), 7.77 - 7.89 (m, 3H), 8.06 - 8.12 (m, 1H), 8.17 (br d, J = 7.8 Hz, 1H), 8.33 (s, 1H), 8.86 (d, J = 9.5 Hz, 1H), 10.37 (s, 1H), 12.55 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 17.5, 24.1, 25.0, 28.6, 29.9, 36.0, 36.9, 38.4, 38.5, 42.6, 45.9, 57.1, 60.2, 69.0, 69.5, 69.7, 69.7, 69.8, 72.3, 112.5, 121.2, 124.8, 128.2, 128.6, 128.6, 129.3, 129.7, 130.5, 130.6, 131.5, 135.1, 135.4, 137.6, 142.1, 146.6, 153.8, 162.6, 165.6, 167.3, 174.4. LCMS (ESI) *m/z* 957 $[M+H]^+$, 955 $[M-H]^-$. Rt 0.995 min (LP mode). HRMS (ESI/APCI dual) *m/z* calcd for $C_{50}H_{64}N_6O_{11}S$ $[M+H]^+$ 957.4427, found 957.4421.

第 3 章

2-Cyano-N-phenylacetamide (8ai)

To a solution of aniline (5.00 g, 53.7 mmol) in DMF (40 mL) was added 2-cyanoacetic acid (4.57 g, 53.7 mmol) and EDC･HCl (12.4 g, 64.4 mmol). The mixture was stirred at room temperature for 30 min, followed by the addition of water, and the resulting precipitate was collected by filtration to obtain **8ai** (7.70g, 90%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.56 (s, 2H), 7.16 - 7.24 (m, 1H), 7.34 - 7.40 (m, 2H), 7.50 (d, J = 7.8 Hz, 2H), 7.66 (br s, 1H). LCMS (ESI) *m/z* 159 $[M-H]^-$. Rt 0.560 min (NM mode).

Ethyl 4-{[4-(2-cyanoacetamido)phenyl]methyl}benzoate (8bi)

Compound **8bi** (77%) was prepared from **7bi** in a manner similar to that described for **8ai**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (t, J = 7.2 Hz, 3H), 3.55 (s, 2H), 4.01 (s, 2H), 4.36 (q, J = 7.1 Hz, 2H), 7.17 (d, J = 8.3 Hz, 2H), 7.23 (d, J = 8.6 Hz, 2H), 7.43 (d, J = 8.6 Hz, 2H), 7.66 (br s, 1H), 7.96 (d, J = 8.3 Hz, 2H). LCMS (ESI) *m/z* 321 $[M-H]^-$. Rt 0.997 min (NM mode).

Methyl 4-{3-[4-(2-cyanoacetamido)phenyl]propyl}benzoate (8ci)

Compound **8ci** (94%) was prepared from **7ci** in a manner similar to that described for **8ai**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91 - 2.00 (m, 2H), 2.60 - 2.72 (m, 4H), 3.55 (s, 2H), 3.90 (s, 3H), 7.16 (d, J = 8.3 Hz, 2H), 7.23 (d, J = 8.3 Hz, 2H), 7.41 (d, J = 8.3 Hz, 2H), 7.72 (br s, 1H), 7.95 (d, J = 8.3 Hz, 2H). LCMS (ESI) *m/z* 337 $[M+H]^+$, 335 $[M-H]^-$. Rt 0.781 min (LP mode).

2-Cyano-2-(2-nitrophenyl)-N-phenylacetamide (9ai)

To a solution of **8ai** (834 mg, 5.21 mmol) in DMF (10 mL) was added sodium hydride (208 mg) under ice cooling and the mixture was stirred at room temperature for 30 min. Then, 1-fluoro-2-nitrobenzene (565 mg, 5.21 mmol) was added and the mixture was stirred at the same temperature for 2 h. 1M HCl aqueous solution was added, and

the mixture was extracted with EtOAc. The combined organic layer was dried over anhydrous magnesium sulfate, and filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel and eluted with 0-50% EtOAc/*n*-hexane to afford **9ai** (795 mg, 71%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.71 (s, 1H), 7.13 - 7.21 (m, 1H), 7.33 (t, J = 7.8 Hz, 2H), 7.48 (d, J = 7.8 Hz, 2H), 7.60 - 7.67 (m, 1H), 7.79 (td, J = 7.8, 1.0 Hz, 1H), 7.92 (dd, J = 7.8, 1.0 Hz, 1H), 8.14 - 8.24 (m, 2H). LCMS (ESI) *m/z* 280 $[M-H]^-$. Rt 0.904 min (NM mode).

Ethyl 4-({4-[2-cyano(2-nitrophenyl)acetamido]phenyl}methyl)benzoate (9bi)

Compound **9bi** (51%) was prepared from **8bi** in a manner similar to that described for **9ai**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (t, J = 7.1 Hz, 3H), 3.99 (s, 2H), 4.35 (q, J = 7.1 Hz, 2H), 5.69 (s, 1H), 7.14 (d, J = 8.3 Hz, 2H), 7.20 (d, J = 8.3 Hz, 2H), 7.41 (d, J = 8.3 Hz, 2H), 7.60 - 7.67 (m, 1H), 7.75 - 7.82 (m, 1H), 7.89 - 7.97 (m, 3H), 8.06 (br s, 1H), 8.15 - 8.20 (m, 1H). LCMS (ESI) *m/z* 444 $[M+H]^+$, 442 $[M-H]^-$. Rt 1.166 min (NM mode).

Methyl 4-(3-{4-[2-cyano(2-nitrophenyl)acetamido]phenyl}propyl)benzoate (9ci)

Compound **9ci** (53%) was prepared from **8ci** in a manner similar to that described for **9ai**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.88 - 1.99 (m, 2H), 2.57 - 2.70 (m, 4H), 3.90 (s, 3H), 5.70 (s, 1H), 7.13 (d, J = 8.3 Hz, 2H), 7.22 (d, J = 8.3 Hz, 2H), 7.40 (d, J = 8.3 Hz, 2H), 7.60 - 7.67 (m, 1H), 7.79 (td, J = 7.6, 1.2 Hz, 1H), 7.89 - 7.97 (m, 3H), 8.05 (s, 1H), 8.15 - 8.20 (m, 1H). LCMS (ESI) *m/z* 458 $[M+H]^+$. Rt 1.281 min (NM mode).

2-Amino-N-phenyl-1*H*-indole-3-carboxamide (10ai)

To a solution of **9ai** (795 mg, 2.83 mmol) in toluene (2.0 mL) and AcOH (2.0 mL) was added zinc (739 mg, 11.3 mmol), and the mixture was stirred at 90 °C for 1h. it was then cooled to room temperature, followed by addition of EtOAc and filtration through a pad of Celite®, and the filtrate was concentrated under reduced pressure. The resulting solid was washed with acetone to obtain **10ai** (95.0 mg, 13%) as a light purple solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.96 - 6.05 (m, 2H), 7.05 - 7.14 (m, 2H), 7.18 - 7.22 (m, 2H), 7.34 - 7.40 (m, 2H), 7.49 (d, J = 8.1 Hz, 1H), 7.55 (br s, 1H), 7.59 - 7.64 (m, 2H), 7.75

(br s, 1H). LCMS (ESI) *m/z* 252 $[M+H]^+$, 250 $[M-H]^-$. Rt 0.873 min (NM mode).

Ethyl 4-({4-[(2-amino-1*H*-indole-3-carbonyl)amino]phenyl}methyl)benzoate (10bi)

Compound **10bi** (46%) was prepared from **9bi** in a manner similar to that described for **10ai**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (t, J = 7.1 Hz, 3H), 4.02 (s, 2H), 4.36 (q, J = 7.1 Hz, 2H), 5.96 - 6.03 (m, 2H), 7.05 - 7.10 (m, 1H), 7.15-7.29 (m, 6H), 7.46 (d, J = 7.6 Hz, 1H), 7.49 - 7.55 (m, 3H), 7.76 - 7.81 (br, s, 1H), 7.94 - 7.99 (m, 2H). LCMS (ESI) *m/z* 414 $[M+H]^+$,412 $[M-H]^-$. Rt 1.161 min (NM mode).

Methyl 4-(3-{4-[(2-amino-1*H*-indole-3-carbonyl)amino]phenyl}propyl)benzoate (10ci)

Compound **10ci** (59%) was prepared from **9ci** in a manner similar to that described for **10ai**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (s, 2H), 2.61 - 2.75 (m, 4H), 3.90 (s, 3H), 5.97 - 6.03 (m, 2H), 7.05 - 7.10 (m, 1H), 7.15 - 7.26 (m, 6H), 7.44 - 7.54 (m, 4H), 7.83 (br s, 1H), 7.96 (d, J = 8.3 Hz, 2H). LCMS (ESI) *m/z* 428 $[M+H]^+$. Rt 1.305 min (NM mode).

Methyl 4-(3-{4-[(1*H*-indole-3-carbonyl)amino]phenyl}propyl)benzoate (11i)

To a solution of 1H-indole-3-carboxylic acid (250 mg, 1.55 mmol) in MeCN (5.2 mL) was added DIPEA (811 μL, 4.65 mmol), methyl 4-[3-(4-aminophenyl) propyl]benzoate (501 mg, 1.86 mmol), and 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-Oxide Hexafluorophosphate (HATU) (885 mg, 2.33 mmol). The mixture was stirred at room temperature for 18 h. The reaction mixture was washed with saturated $NaHCO_3$ aqueous solution. The aqueous layer was extracted with EtOAc using a phase separator. The combined organic layer was concentrated, then the residue was purified by column chromatography on silica gel and eluted with 10-100% EtOAc/*n*-Hexane to obtain **11i** (500 mg, 78%) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.92 - 2.02 (m, 2H), 2.61 - 2.73 (m, 4H), 3.90 (s, 3H), 7.17 (d, J = 8.3 Hz, 2H), 7.23 - 7.33 (m, 4H), 7.42 - 7.48 (m, 1H), 7.55 - 7.60 (m, 2H), 7.71 (s, 1H), 7.83 (d, J = 2.9 Hz, 1H), 7.93 - 7.99 (m, 2H), 8.03 - 8.08 (m, 1H), 8.92 (br, s, 1H). LCMS (ESI) *m/z* 413 $[M+H]^+$. Rt 1.161 min (NM mode).

Methyl 4-[3-(4-{[2-(benzylamino)-1*H*-indole-3-carbonyl]amino}phenyl)propyl] benzoate (12i)

To a solution of **11i** (100mg, 242μmol) in $CHCl_3$ (1.0 mL) was added *N*-chlorosuccinimide (35.6 mg, 267 μmol) and triethylamine (33.8 μL, 242 μmol) under ice cooling. The mixture was stirred at the same temperature for 30 min, followed by the addition of benzylamine (53.0 μL, 485 μmol). The mixture was stirred at the same temperature for 30 min. The reaction mixture was washed with saturated aqueous ammonium chloride (NH_4Cl) solution. The aqueous layer was extracted with $CHCl_3$ using a phase separator. The combined organic layer was concentrated, then the residue was purified by column chromatography on silica gel and eluted with 5-40% EtOAc/*n*-Hexane to obtain **12i** (110 mg, 88%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.92 - 2.01 (m, 2H), 2.61 - 2.74 (m, 4H), 3.90 (s, 3H), 4.57 (d, J = 5.9 Hz, 2H), 6.98 - 7.04 (m, 1H), 7.10 (d, J = 7.6 Hz, 1H), 7.15 - 7.25 (m, 4H), 7.31 - 7.48 (m, 8H), 7.49 - 7.54 (m, 2H), 7.62 (br s, 1H), 7.94 - 7.98 (m, 2H), 8.43 (br t, J = 5.9 Hz, 1H). LCMS (ESI) *m/z* 518 $[M+H]^+$, 516 $[M+H]^+$. Rt 1.369 min (NM mode).

Methyl 4-(3-{4-[(2-amino-1*H*-indole-3-carbonyl)amino]phenyl}propyl)benzoate (10ci) (alternative method)

To a solution of **12i** (110 mg, 213 μmol) in MeOH (1.0 mL) was added 10% palladium on activated carbon (110 mg) and the mixture was stirred at room temperature for 1h under hydrogen atmosphere. The mixture was filtered through a pad of Celite®, and the filtrate was concentrated under reduced pressure to obtain **10ci** (95 mg, quantitative yield) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (s, 2H), 2.61 - 2.75 (m, 4H), 3.90 (s, 3H), 5.97 - 6.03 (m, 2H), 7.05 - 7.10 (m, 1H), 7.15 - 7.26 (m, 6H), 7.44 - 7.54 (m, 4H), 7.83 (br s, 1H), 7.96 (d, J = 8.3 Hz, 2H). LCMS (ESI) *m/z* 428 $[M+H]^+$. Rt 1.305 min (NM mode).

4-[3-(4-{[2-(3-{[(1r,4r)-4-Carboxycyclohexyl](ethyl)sulfamoyl}benzamido)-1*H*-indole-3-carbonyl]amino}phenyl)propyl]benzoic acid (14i)

To a solution of **13i** (108 mg, 292 μmol) in $CHCl_3$ (5.0 mL) was added thionyl

chloride ($SOCl_2$) (127 μL, 1.75 mmol). The mixture was stirred under reflux for 1 h and concentrated under reduced pressure. To a solution of the residue in $CHCl_3$ (5.0 mL) was added **10ci** (25.0 mg, 58.4 μmol) and DIPEA (51.0 μL, 293 μmol). The mixture was stirred overnight at room temperature and for 4 h under reflux, then washed with saturated $NaHCO_3$ aqueous solution and extracted with $CHCl_3$. The organic layer was washed with saturated aqueous NH_4Cl solution and concentrated under reduced pressure. To the residue was added MeOH (1.0 mL), THF (2.0 mL) and 1M sodium hydroxide (NaOH) aqueous solution (2.00 mL, 2.00 mmol). The mixture was stirred at 80 ℃ for 2 h and concentrated under reduced pressure, then the residue was purified by reversed-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=80:20 → 5:95 → 1:99, 40 mL/min) to obtain the **14i** (15.0 mg, 34% over 2 steps) as a yellow oil. ^1H NMR (400 MHz, DMSO-d_6) δ 1.15 (br t, J = 6.8 Hz, 3H), 1.21 - 1.55 (m, 6H), 1.80 - 1.97 (m, 4H), 2.57 - 2.73 (m, 4H), 3.14 - 3.27 (m, 3H), 3.55 - 3.70 (m, 1H), 7.07 - 7.22 (m, 3H), 7.32 - 7.47 (m, 3H), 7.64 - 7.82 (m, 3H), 7.85 - 7.90 (m, 2H), 7.93 - 8.52 (m, 5H), 12.26 - 12.53 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 17.4, 28.1, 29.5, 32.4, 34.1, 34.6, 38.3, 41.1, 56.4, 112.5, 119.0, 121.4, 125.0, 128.2, 128.3, 128.5, 129.4, 130.4, 130.6, 130.8, 132.7, 133.6, 136.5, 137.1, 140. 5, 147.3, 162.1, 164.7, 167.3,176.1. LCMS (ESI) *m/z* 428 $[M+H]^+$. Rt 1.305 min (NM mode). Purity 100%. HRMS (ESI/APCI dual) *m/z* calcd for $C_{41}H_{42}N_4O_8S$ $[M+H]^+$ 751.2796, found 751.2805.

4-[3-(4-{[2-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-1*H*-indole-3-carbonyl]amino}phenyl)propyl]benzoic acid (5i and 5i hydrochloride)

To a solution of **15i** (619 mg, 1.32 mmol) in $CHCl_3$ (2.0 mL) was added $SOCl_2$ (949 μL, 13.0 mmol) and the mixture was stirred at 80 °C for 1 h. The reaction mixture was concentrated under reduced pressure to obtain the corresponding acid chloride as a crude product, which was used without further purification. To a solution of the resulting acid chloride in $CHCl_3$ (2.0 mL) was added **10ci** (100 mg, 234 μmol) and DIPEA (407 μL, 302 μmol). The reaction mixture was stirred overnight at room temperature, then concentrated under reduced pressure. The residue was purified by

reversed-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=80:20 → 5:95 → 1:99, 40 mL/min) to obtain the corresponding amide (120mg, 58%) as a brown oil. LCMS (ESI) *m/z* 774 $[M+H]^+$. Rt 0.936 min (NM mode). To a solution of the resulting amide (120 mg, 137 μmol) in THF (3.0 mL) was added 1M NaOH aqueous solution (1.00 mL, 1.00 mmol) and the mixture was stirred overnight at room temperature, and then at 60 °C for 2 h. The reaction mixture was concentrated under reduced pressure, then the residue was purified by reversed-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=90:10 → 20:80 → 5:95, 40 mL/min) to obtain **5i** (40 mg, 38%) as a pale yellow amorphous substance. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 - 1.05 (m, 6H), 1.31 - 1.60 (m, 4H), 1.95 - 2.05 (m, 2H), 2.27 - 2.37 (m, 2H), 2.51 - 2.77 (m, 9H), 2.81 - 2.93 (m, 3H), 3.15 - 3.22 (m, 1H), 3.40 - 3.47 (m, 1H), 3.74 (s, 2H), 7.16 - 7.35 (m, 6H), 7.45 - 7.62 (m, 4H), 7.62 - 7.69 (m, 1H), 7.72 - 7.77 (m, 1H), 7.88 - 8.07 (m, 4H), 11.24 (br s, 1H), 12.23 - 12.35 (m, 1 H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 11.9, 22.2, 22.6, 22.8, 26.7, 27.7, 28.9, 32.3, 33.3, 34.2, 34.6, 35.3, 46.5, 47.7, 48.9, 53.8, 54.1, 62.8, 63.7, 67.2, 92.7, 115.6, 118.7, 120.9, 125.4, 127.2, 128.2, 128.4, 128.3, 128.9, 129.4, 132.4, 136.7, 147.4, 162.9, 165.5, 167.2, 170.3, 170.5, 173.8,173.9. LCMS (ESI) *m/z* 774 $[M+H]^+$. Rt 0.936 min (NM mode). Purity 100%. HRMS (ESI/APCI dual) *m/z* calcd for $C_{45}H_{51}N_5O_7$ $[M+H]^+$ 774.3861, found 774.3828. To a solution of **5** (40.0 mg, 51.7 μmol) in 1,4-dioxane (1.0 mL) was added 4M HCl in 1,4-dioxane (25.8 μL, 103 μmol) and the mixture was stirred at room temperature for 15 min. The mixture was concentrated under reduced pressure, and IPE was added to the residue. The mixture was stirred at room temperature for 10 min and concentrated under reduced pressure to obtain **5i** hydrochloride (40.0 mg, 96%) as a yellow amorphous substance. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.84 - 1.01 (m, 6H), 1.60 - 2.07 (m, 6H), 2.41 - 2.46 (m, 2H), 2.51 - 2.55 (m, 2H), 2.59 - 2.71 (m, 4H), 2.77 - 3.03 (m, 4H), 3.13 - 3.23 (m, 1H), 3.33 - 3.83 (m, 3H), 4.47 - 4.70 (m, 2H), 7.14 - 7.40 (m, 6H), 7.60 - 8.08 (m, 9H), 8.22 - 8.31 (m, 1H), 9.34 (s, 1H), 9.76 (br s, 1H), 12.29 - 12.44 (m, 2H). ^{13}C NMR (126 MHz, DMSO-*d6*) δ 11.1, 21.2, 21.6, 27.6 , 28.7, 32.4, 34.1, 34.6, 35.7, 44.1, 48.6, 54.0, 66.1, 93.4, 112.6, 119.0, 121.2, 121.5, 121.7, 122.4, 128.0, 128.2, 128.4, 128.5, 129.3, 129.8,

130.4, 131.6, 132.7, 133.3, 135.4, 136.5, 137.1, 140.8, 147.4, 163.7, 165.4, 167.3, 173.3, 173.8. LCMS (ESI) *m/z* 774 $[M+H]^+$. Rt 0.926 min (NM mode). Purity 97.8%. HRMS (ESI/APCI dual) *m/z* calcd for $C_{45}H_{51}N_5O_7$ $[M+H]^+$ 774.3861, found 774.3872.

4-[Methyl(2-{(pentan-3-yl)[(3-{[3-(phenylcarbamoyl)-1*H*-indol-2-yl]carbamoyl}phenyl)methyl]amino}ethyl)amino]-4-oxobutanoic acid (16i)

Compound **16i** (4.5% over 2 steps) was prepared from **15i** and **10ai** in a manner similar to that described for **5i**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.80 - 0.90 (m, 6H), 1.22 - 1.36 (m, 2H), 1.43 - 1.57 (m, 2H), 2.21 - 2.48 (m, 6H), 2.57 - 2.67 (m, 1H), 2.68 - 2.88 (m, 3H), 3.15 - 3.40 (m, 2H), 3.70 - 3.78 (m, 2H), 6.90 - 7.00 (m, 2H), 7.31 (s, 6H), 7.77 (br d, J = 8.1 Hz, 1H), 8.00 - 8.31 (m, 4H), 8.56 - 8.65 (m, 1H), 12.25 (br s, 1H). ^{13}C NMR (126 MHz, DMSO-*d6*) δ 11.9, 22.3, 22.6, 26.9, 27.7, 28.9, 33.4, 35.3, 46.5, 47.6, 48.7, 53.8, 54.1, 62.8, 63.7, 92.7, 112.6, 118.9, 121.2, 122.3, 123.4, 125.5, 127.3, 128.4, 128.9, 132.7, 132.9, 138.8, 142.0, 165.6, 170.4,173.8. LCMS (ESI) *m/z* 612 $[M+H]^+$, 610 $[M+H]^+$. Rt 0.811 min (NM mode). Purity 100%. HRMS (ESI/APCI dual) *m/z* calcd for $C_{35}H_{41}N_5O_5$ $[M+H]^+$ 612.3180, found 612.3181.

4-[Methyl(2-{(pentan-3-yl)[(3-{[3-(phenylcarbamoyl)-1*H*-indol-2-yl]carbamoyl}phenyl)methyl]amino}ethyl)amino]-4-oxobutanoic acid (17i)

Compound **17i** (12% over 2 steps) was prepared from **15i** and **10bi** in a manner similar to that described for **5**. ^{1}H NMR (400 MHz, CD_3OD) δ 0.86 - 1.05 (m, 6H), 1.31 - 1.47 (m, 2H), 1.51 - 1.65 (m, 2H), 2.29 - 2.54 (m, 6H), 2.65 - 2.93 (m, 4H), 3.39 - 3.51 (m, 2H), 3.82 (br d, J = 7.1 Hz, 2H), 3.95 - 4.08 (m, 2H), 7.09 - 7.35 (m, 6H), 7.49 - 7.66 (m, 5H), 7.83 - 7.97 (m, 4H), 8.07 - 8.14 (m, 1H). ^{13}C NMR (126 MHz, DMSO-*d6*) δ 11.9, 22.3, 22.6, 26.9, 27.7, 28.9, 28.9, 33.4, 35.3, 40.4, 46.5, 47.6, 48.7, 53.8, 54.1, 62.8, 63.7, 92.7, 118.8, 121.3, 125.5, 127.3, 128.5, 128.7, 128.8, 129.5, 132.6, 146.8, 163.0, 165.5, 167.2, 170.4, 170.5, 173.8, 173.9. LCMS (ESI) *m/z* 746 $[M+H]^+$, 744 $[M+H]^+$. Rt 0.842 min (NM mode). Purity 100%. HRMS (ESI/APCI dual) *m/z* calcd for $C_{43}H_{47}N_5O_7$ $[M+H]^+$ 746.3548, found 746.3541.

Methyl 4-{3-[4-({2-[3-(chloromethyl)benzamido]-1*H*-indole-3-carbonyl}amino)phenyl]propyl}benzoate (18i)

To a solution of **10ci** (113 mg, 264 μmol) in $CHCl_3$ (5.0 mL) was added 3-(chloromethyl)benzoyl chloride (100 mg, 529 μmol) and pyridine (42.6 μL, 529 μmol). The mixture was stirred overnight at room temperature, then concentrated under reduced pressure. Water was added to the residue, and the mixture was extracted with EtOAc. The combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel and eluted with 0%-30% EtOAc/*n*-hexane to obtain **18i** (111 mg, 72%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94 - 2.05 (m, 2H), 2.63 - 2.77 (m, 4H), 3.91 (s, 3H), 4.69 (s, 2H), 7.21 - 7.37 (m, 5H), 7.46 - 7.59 (m, 4H), 7.67 (d, J = 7.6 Hz, 2H), 7.74 (s, 1H), 7.93 - 8.11 (m, 4H), 11.17 (br s, 1H), 12.37 (s, 1H). LCMS (ESI) *m/z* 580 $[M+H]^+$, 578 $[M-H]^-$. Rt 1.115 min (LP mode).

Methyl 4-{3-[4-({2-[3-({[2-(methylamino)ethyl](pentan-3-yl)amino}methyl)benzamido]-1*H*-indole-3-carbonyl}amino)phenyl]propyl}benzoate (19i)

To a suspension of **18i** (500 mg, 862 μmol) in toluene (8.2 mL) was added *tert*-butyl *N*-[2-(1-ethylpropylamino)ethyl]-*N*-methyl-carbamate (527 mg, 2.16 mmol), NaI (775 mg, 5.17 mmol), and DIPEA (601 μL, 3.45 mmol). The mixture was stirred at 95 °C for 1 h, and then at 100 °C for 14 h. The reaction mixture was cooled to room temperature, then washed with brine and extracted with EtOAc. The combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel and eluted with 0%-70% EtOAc/*n*-hexane to obtain the corresponding tertiary amine (437 mg, 64%) as a yellow amorphous substance. LCMS (ESI) *m/z* 788 $[M+H]^+$, 786 $[M-H]^-$. Rt 0.965 min (LP mode). To a solution of the resulting tertiary amine (473 mg, 555 μmol) in $CHCl_3$ (2.0 mL) was added TFA (5.0 mL) and the mixture was stirred at room temperature for 2 h. The reaction mixture was washed with saturated $NaHCO_3$ aqueous solution. The aqueous layer was extracted with $CHCl_3$ using a phase separator. The combined organic layer was concentrated to obtain **19i** (379 mg, 99%) as a yellow

amorphous substance. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (t, J = 7.3 Hz, 6H), 1.36 - 1.48 (m, 2H), 1.52 - 1.64 (m, 2H), 1.96 - 2.03 (m, 2H), 2.26 (s, 3H), 2.31 - 2.37 (m, 1H), 2.64 - 2.82 (m, 8H), 3.71 (s, 2H), 3.91 (s, 3H), 7.21 - 7.36 (m, 5H), 7.46 - 7.57 (m, 5H), 7.63 - 7.68 (m, 1H), 7.75 (s, 1H), 7.94 - 7.99 (m, 3H), 8.04 (s, 1H), 11.12 - 11.20 (m, 1H), 12.30 - 12.40 (m, 1H). LCMS (ESI) *m/z* 688 $[M+H]^+$. Rt 0.833 min (LP mode).

4-[3-(4-{[2-(3-{[(2-{[(Carboxymethyl)carbamoyl](methyl)amino}ethyl)(pentan-3-yl)amino]methyl}benzamido)-1*H*-indole-3-carbonyl]amino}phenyl)propyl]benzoic acid (20i)

To a solution of **19i** (22.0 mg, 32.0 μmol) in THF (0.50 mL) was added ethyl isocyanatoacetate (12.4 mg, 95.9 μmol), and the mixture was stirred at room temperature for 5 min. To the mixture was added ethyl isocyanatoacetate (12.4 mg, 95.9 μmol) and the mixture was stirred at room temperature for 2 min. 2M aqueous NaOH solution (0.50 mL) and MeOH (0.50 mL) were added to the mixture, and the resulting mixture was stirred at room temperature for 5 min, and then at 65 °C for 30 min. The reaction mixture was cooled to room temperature and quenched with 2M HCl aqueous solution. The aqueous layer was extracted with a mixture solvent of EtOAc and THF using a phase separator, and the combined organic layer was concentrated under reduced pressure. The residue was purified by reversed-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=80:20 → 5:95 → 1:99, 40 mL/min) to obtain **20i** (18.0mg, 73% over 2 steps) as a yellow amorphous substance. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.83 (t, J = 7.3 Hz, 6H), 1.18 - 1.28 (m, 2H), 1.41 - 1.51 (m, 2H), 1.85 - 1.92 (m, 2H), 2.15 - 2.21 (m, 1H), 2.49 - 2.53 (m, 2H), 2.57 (t, J = 7.6 Hz, 2H), 2.65 (s, 2H), 2.71 (s, 3H), 3.19 - 3.24 (m, 2H), 3.58 (d, J = 5.7 Hz, 2H), 3.72 (s, 2H), 6.43 (t, J = 5.7 Hz, 1H), 7.10 - 7.19 (m, 4H), 7.31 (d, J = 8.4 Hz, 2H), 7.51 - 7.56 (m, 1H), 7.58 - 7.65 (m, 4H), 7.84 (d, J = 8.4 Hz, 3H), 7.88 - 7.92 (m, 1H), 8.02 - 8.05 (m, 1H), 9.24 - 9.32 (m, 1H), 12.28 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 11.9, 22.4, 32.3, 34.2, 34.4, 34.6, 42.1, 47.1, 47.6, 54.0, 63.0, 92.7, 112.5, 118.8, 121.1, 121.2, 121.4, 122.3, 125.3, 127.3, 128.2, 128.4, 128.5, 128.8, 129.4, 132.4, 132.7, 132.9, 136.6, 136.9, 141.6, 142.3, 147.4, 157.6, 163.0, 164.3, 165.5, 167.3,172.5. LCMS (ESI) *m/z* 775 $[M+H]^+$,

773 $[M-H]^-$. Rt 0.689 (LP mode). Purity 100%. HRMS (ESI/APCI dual) *m/z* calcd for $C_{44}H_{50}N_6O_7$ $[M+H]^+$ 775.3814, found 775.3832.

4-[3-(4-{[2-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(propan-2-yl)amino]methyl}benzamido)-1*H*-indole-3-carbonyl]amino}phenyl)propyl]benzoic acid (21i)

To a suspension of **18i** (100 mg, 172 μmol) in toluene (1.7 mL) was added ethyl *tert*-butyl *N*-methyl-*N*-(2-aminoethyl)carbamate (111 mg, 637 μmol), NaI (115 mg, 1.03 mmol), and DIPEA (120 μL, 690 μmol). The mixture was stirred at room temperature for 2 h 20 min, and then at 50 °C for 1 h. The reaction mixture was cooled to room temperature, washed with water, and extracted with EtOAc using a phase separator, and then the combined organic layer was concentrated under reduced pressure. The residue was purified by reversed-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=90:10 → 20:80 → 5:95, 40 mL/min) and column chromatography on silica gel and eluted with 0%-100% EtOAc/*n*-hexane and 15% MeOH/$CHCl_3$ to obtain the corresponding secondary amine (20.0 mg, 40%) as a yellow powder. LCMS (ESI) *m/z* 744 $[M+H]^+$, 742 $[M-H]^-$. Rt 0.826 (LP mode). To a solution of the resulting secondary amine (35.0 mg, 48.8 μmol) in 1,2-dichloroethane (1.8 mL) was added acetone (0.75 mL, 10.0 mmol) and AcOH (175 μL, 3.06 mmol). The mixture was stirred at 75 °C for 45 min. After cooling to room temperature, $NaBH(OAc)_3$ (51.7 mg, 244 μmol) was added and the mixture was stirred at room temperature for 5 h, then quenched with saturated $NaHCO_3$ aqueous solution. The aqueous layer was extracted with $CHCl_3$, then the combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel and eluted with 1%-50% EtOAc/*n*-hexane and reversed-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=90:10 → 20:80 → 5:95, 40 mL/min) to obtain the corresponding tertiary amine (20.0 mg, 54%) as a yellow oil. LCMS (ESI) *m/z* 760 $[M+H]^+$, 758 $[M-H]^-$. Rt 0.863 (LP mode). To a solution of the resulting tertiary amine (20 mg, 26.3 mmol) in $CHCl_3$ (1.0 mL) was added TFA (3.0

mL) and the mixture was stirred at room temperature for 1 h, and quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with $CHCl_3$, and the combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to obtain the corresponding secondary amine (17.0 mg, 98%) as a yellow amorphous substance. LCMS (ESI) *m/z* 658 $[M+H]^+$. Rt 0.953 min (LP mode). To a solution of the resulting secondary amine (16.0 mg, 24.3 μmol) in THF (1.2 mL) was added succinic anhydride (5.5 mg, 55 μmol) and the mixture was stirred at room temperature for 5 min. Then, to the mixture was added 2M NaOH aqueous solution (500 μL, 1.00 mmol) and MeOH (1.0 mL). After stirring at 65 °C for 45 min, the mixture was cooled to room temperature and the organic solvent was removed under reduced pressure. The resulting aqueous layer was washed 2M HCl aqueous solution and brine, and then extracted with EtOAc. The combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to obtain **21i** (10.5 mg, 58% over 2 steps) as a yellow amorphous substance. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.25 - 1.32 (m, 3H), 1.35 - 1.40 (m, 3H), 1.88 - 1.96 (m, 2H), 2.39 - 2.45 (m, 6H), 2.58 - 2.64 (m, 2H), 2.69 (t, J = 7.6 Hz, 2H), 2.75 - 2.96 (m, 3H), 3.06 - 3.16 (m, 1H), 3.41 - 3.47 (m, 1H), 3.56 - 3.65 (m, 1H), 3.67 - 3.76 (m, 1H), 4.48 - 4.61 (m, 1H), 7.16 - 7.24 (m, 4H), 7.35 (d, J = 8.4 Hz, 2H), 7.63 - 7.68 (m, 3H), 7.77 (br t, J = 7.5 Hz, 1H), 7.85 - 7.90 (m, 2H), 7.94 (br d, J = 7.6 Hz, 2H), 8.01 - 8.08 (m, 1H), 8.20 - 8.28 (m, 1H), 9.26 - 9.34 (m, 1H), 9.55 - 10.13 (m, 1H), 12.20 - 12.31 (m, 2H), 12.35 (br s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 15.6, 16.4, 27.7, 28.7, 29.0, 29.2, 30.4, 32.3, 34.1, 34.6, 35.4, 43.6, 46.8, 53.3, 55.0, 93.2, 112.6, 119.0, 121.2, 121.4, 121.6, 122.2, 128.0, 128.5, 129.4, 129.9, 130.3, 132.7, 133.4, 136.5, 137.0, 141.0, 147.3, 163.6, 165.4, 167.2, 173.0, 173.5, 173.8. LCMS (ESI) *m/z* 746 $[M+H]^+$, 744 $[M-H]^-$. Rt 0.653 (LP mode). Purity 98.2%. HRMS (ESI/APCI dual) *m/z* calcd for $C_{43}H_{47}N_5O_7$ $[M+H]^+$ 746.3548, found 746.3546.

4-[3-(4-{[2-(3-{[{2-[(3-Carboxypropanoyl)(methyl)amino]ethyl}(heptan-4-yl)amino]methyl}benzamido)-1*H*-indole-3-carbonyl]amino}phenyl)propyl]benzoic acid hydrochloride (22i)

To a suspension of **18i** (66.0 mg, 114 μmol) in toluene (3.0 mL) was added *tert*-butyl

N-methyl-*N*-{2-[(heptane-4-yl)amino]ethyl}carbamate (124 mg, 455 μmol), NaI (136 mg, 910 μmol) and DIPEA (159 μL, 910 μmol). The mixture was stirred at 95 °C for 50 min. Then, to the mixture was added *tert*-butyl *N*-methyl-*N*-{2-[(heptane-4-yl)amino]ethyl}carbamate (124 mg, 455 μmol), NaI (136 mg, 910 μmol), and DIPEA (159 μL, 910 μmol). The mixture was stirred at 95 °C for 4 h, cooled to room temperature, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel and eluted with 5%-50% EtOAc/*n*-hexane to obtain the corresponding tertiary amine (60.0 mg, 64%) as a pale yellow oil. LCMS (ESI) *m/z* 816 $[M+H]^+$, 814 $[M-H]^-$. Rt 1.022 (LP mode). To a solution of the resulting tertiary amine (60.0 mg, 73.5 mmol) in $CHCl_3$ (1.0 mL) was added TFA (3.0 mL) and the mixture was stirred at room temperature for 12 h, and then quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with $CHCl_3$ using a phase separator. The combined organic layer was concentrated to obtain the corresponding secondary amine (60.0 mg, quantitative yield) as a yellow oil. LCMS (ESI) *m/z* 714 $[M-H]^-$. Rt 0.949 min (LP mode). To a solution of the resulting secondary amine (60.0 mg, 83.8 μmol) in THF (1.3 mL) was added succinic anhydride (16.8 mg, 16.8 μmol) and the mixture was stirred at room temperature for 15 min. Then, to the mixture was added 2M NaOH aqueous solution (1.3 mL) and MeOH (1.3 mL). After stirring at 65 °C for 80 min, the mixture was cooled to room temperature and concentrated under reduced pressure. The residue was washed with THF and dissolved 2M HCl aqueous solution and THF. The mixture was concentrated under reduced pressure and the residue was washed with water to obtain **22i** (55.0 mg, 78% over 3 steps) as a yellow amorphous substance. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.76 - 0.93 (m, 6H), 1.14 - 1.68 (m, 8H), 1.71 - 1.98 (m, 4H), 2.43 (br d, J = 4.6 Hz, 2H), 2.58 - 2.72 (m, 4H), 2.76 - 3.00 (m, 3H), 3.05 – 3.40 (m, 4H), 3.72 - 3.87 (m, 1H), 4.52 - 4.65 (m, 2H), 7.13 - 7.26 (m, 4H), 7.35 (d, J = 8.1 Hz, 2H), 7.61 - 7.70 (m, 3H), 7.77 (t, J = 7.7 Hz, 1H), 7.88 (d, J = 8.1 Hz, 2H), 7.92 - 8.19 (m, 3H), 8.23 - 8.33 (m, 1H), 9.33 (s, 1H), 9.72 (br s, 1H), 12.31 (br d, J = 5.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 13.6, 13.8, 19.4, 27.6, 28.7, 30.3, 31.1, 32.3, 34.1, 34.6, 35.7, 44.0, 48.4, 54.0, 62.6, 93.4, 112.6, 119.0, 121.1, 121.4, 121.6, 122.3, 127.9, 128.2, 128.4, 128.5, 129.4, 129.8, 130.4, 131.6, 132.7, 133.3, 135.3, 136.5, 137.1, 140.7, 147.3, 163.7, 165.4, 167.2, 173.2, 173.5,173.7. LCMS (ESI) *m/z*

802 $[M+H]^+$, 800 $[M-H]^-$. Rt 0.760 (LP mode). Purity 100%. HRMS (ESI/APCI dual) *m/z* calcd for $C_{47}H_{55}N_5O_7$ $[M+H]^+$ 802.4174, found 802.4193.

4-{3-[4-({2-[3-({3-[(3-Carboxypropanoyl)(methyl)amino]pyrrolidin-1-yl}methyl)benzamido]-1H-indole-3-carbonyl}amino)phenyl]propyl}benzoic acid hydrochloride (23i)

Compound **23i** (63% over 4 steps) was prepared from **18i** and 3-(*tert*-butoxycarbonylamino)pyrrolidine in a manner similar to that described for **22i**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.78 - 2.21 (m, 5H), 2.38 - 2.46 (m, 5H), 2.58 - 2.72 (m, 6H), 3.60 - 3.81 (m, 1H), 2.93 (s, 4H), 4.58 - 5.09 (m, 1H), 7.15 - 7.23 (m, 5H), 7.33 - 7.37 (m, 2H), 7.62 - 7.68 (m, 4H), 7.86 - 8.08 (m, 5H), 9.30 (br s, 1H), 12.25 - 12.34 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 27.4, 27.6, 28.2, 28.7, 28.9, 29.1, 30.7, 32.3, 34.1, 34.6, 51.7, 93.0, 112.6, 118.9, 121.2, 121.4, 121.7, 122.3, 128.2, 128.3, 128.5, 129.2, 129.4, 132.7, 136.5, 137.1, 141.1, 147.4, 163.9, 165.5, 167.2, 173.5,173.9. LCMS (ESI) *m/z* 730 $[M+H]^+$, 728 $[M+H]^+$. Rt 0.869 min (NM mode). Purity 100%. HRMS (ESI/APCI dual) *m/z* calcd for $C_{42}H_{43}N_5O_7$ $[M+H]^+$ 730.3235, found 730.3231.

Ethyl 4-[3-(methylamino)pyrrolidin-1-yl]-4-oxobutanoate (25i)

To a solution of **24i** (1.02 g, 5.90 mmol) in THF (12 mL) was added ethyl succinyl chloride (1.01 g, 6.11 mmol) and DIPEA (1.33 mL, 7.64 mmol). The mixture was stirred at room temperature for 12 h, then washed with 1M aqueous NaOH solution. The aqueous layer was extracted with EtOAc, and then the combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to obtain the corresponding amide (1.63 g, 98%) as a light brown oil. LCMS (ESI) *m/z* 329 $[M+H]^+$. Rt 0.823 min (NM mode). To a solution of the resulting amide (0.86 g, 2.6 mmol) in $CHCl_3$ (5.0 mL) was added TFA (35 mL) and the mixture was stirred at room temperature for 2 h, and then concentrated under reduced pressure. The residue was washed with saturated $NaHCO_3$ aqueous solution. The aqueous layer was extracted with $CHCl_3$, and the combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to obtain **25i** (460 mg, 77%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (t, J = 7.1 Hz, 3H), 1.65 -

2.20 (m, 2H), 2.40 - 2.50 (m, 3H), 2.52 - 2.75 (m, 4H), 3.18 - 3.38 (m, 2H), 3.43 - 3.71 (m, 3H), 4.09 - 4.20 (m, 2H). LCMS (ESI) *m/z* 229 $[M+H]^+$. Rt 0.234 min (HP mode).

4-{3-[4-({2-[3-({[1-(3-Carboxypropanoyl)pyrrolidin-3-yl](methyl)amino}methyl)benzamido]-1*H*-indole-3-carbonyl}amino)phenyl]propyl}benzoic acid hydrochloride (26i)

To a suspension of **18i** (66.0 mg, 114 μmol) in toluene (2.5 mL) was added **25i** (240 mg, 526 μmol), NaI (240 mg, 1.60 mmol), and DIPEA (120 μL, 689 μmol). The mixture was stirred at 80 °C for 9 h, and then cooled to room temperature. Brine and *n*-hexane were added to the mixture and the resulting precipitate was collected by filtration to obtain the corresponding tertiary amine (85.0 mg, 97%) as a pale yellow powder. LCMS (ESI) *m/z* 772 $[M+H]^+$, 770 $[M-H]^-$. Rt 0.774 min (LP mode). To a solution of the resulting tertiary amine (85.0 mg, 110 μmol) in THF (4.0 mL) and MeOH (4.0 mL) was added 1M aqueous NaOH solution (2.0 mL). After stirring at 60 °C for 3 h, the mixture was cooled to room temperature, and the mixture was concentrated under reduced pressure. 2M HCl was added to the residue, and the resulting precipitate was collected by filtration and washed with water. The resulting powder was purified by reversed-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=90:10 → 20:80 → 5:95, 40 mL/min). To the resulting product was added 2M HCl aqueous solution and THF, and then the mixture was concentrated under reduced pressure to obtain **26i** (56.0 mg, 66%) as a yellow amorphous substance. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.92 (quin, J = 7.6 Hz, 2H), 2.27 - 2.48 (m, 5H), 2.58 - 2.66 (m, 5H), 2.69 (t, J = 7.5 Hz, 2H), 3.19 - 3.27 (m, 1H), 3.43 - 3.81 (m, 3H), 3.84 - 4.08 (m, 2H), 4.33 - 4.44 (m, 1H), 4.58 - 4.70 (m, 1H), 7.16 - 7.24 (m, 4H), 7.35 (d, J = 8.4 Hz, 2H), 7.62 - 7.68 (m, 3H), 7.72 - 7.79 (m, 1H), 7.88 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 7.6 Hz, 1H), 7.98 - 8.07 (m, 2H), 8.20 - 8.27 (m, 1H), 9.32 (s, 1H), 11.27 - 11.43 (m, 1H), 12.25 - 12.33 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 28.2, 28.5, 28.6, 28.8, 29.2, 30.4, 32.3, 34.1, 34.6, 36.5, 37.0, 61.8, 93.3, 112.6, 119.0, 121.2, 121.4, 121.6, 122.3, 128.2, 128.4, 128.5, 129.4, 129.7, 132.7, 133.2, 135.8, 136.5, 137.1, 140.9, 147.4, 163.8, 165.4, 167.2, 169.4, 169.6, 173.5, 173.8. LCMS (ESI) *m/z* 730 $[M+H]^+$, 728 $[M-H]^-$. Rt 0.631 (LP mode). Purity 100%. HRMS

(ESI/APCI dual) *m/z* calcd for $C_{42}H_{43}N_5O_7$ $[M+H]^+$ 730.3235, found 730.3253.

4-(3-{4-[(2-{3-[21-Hydroxy-5-methyl-6,9-dioxo-2-(pentan-3-yl)-13,16,19-trioxa-2,5,10-triazahenicosan-1-yl]benzamido}-1*H*-indole-3-carbonyl)amino]phenyl}propyl) benzoic acid (27i and 27i hydrochloride)

To a solution of **19i** (809 mg, 1.18 mmol) in THF (20 mL) was added succinic anhydride (235 mg, 2.35 mmol) and DIPEA (2.05 mL, 11.8 mmol). The mixture was stirred at room temperature for 1.5 h and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel and eluted with 1-12% MeOH/$CHCl_3$ to afford a corresponding carboxylic acid to obtain (1.02g, quantitative yield) as a pale yellow amorphous substance. LCMS (ESI) *m/z* 788 $[M+H]^+$, 786 $[M-H]^-$. Rt 0.778 (LP mode). To a solution of the resulting carboxylic acid (60.0 mg, 76.2 μmol) in MeCN (3.0 mL) was added DIPEA (53.1 μL, 305 μmol), 2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}ethan-1-ol (22.1 mg, 154 μmol) and HATU (43.4 mg, 114 μmol). The mixture was stirred at room temperature for 2 h. Then, to the mixture was added DIPEA (53.1 μL, 305 μmol), 2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}ethan-1-ol (22.1 mg, 154 μmol) and HATU (43.4 mg, 114 μmol). The mixture was stirred at room temperature for 1 h and concentrated under reduced pressure. The residue was washed with water and the aqueous layer was extracted with EtOAc , and then the combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to obtain the corresponding amide as a colorless amorphous substance, which was used in the next step without further purification. LCMS (ESI) *m/z* 963 $[M+H]^+$, 961 $[M-H]^-$. Rt 0.989 (NM mode). To a solution of the resulting amide in THF (1.0 mL) and MeOH (1.0 mL) was added 4M NaOH aqueous solution (190 μL, 758 μmol). After stirring at 60 °C for 1 h, the mixture was purified by reversed-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=90:10 → 20:80 → 5:95, 40 mL/min) to obtain **27i** (36.6 mg, 51% over 2 steps) as a colorless amorphous substance. ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 - 1.00 (m, 6H), 1.29 - 1.42 (m, 2H), 1.47 - 1.58 (m, 2H), 1.98 - 2.04 (m, 2H), 2.24 - 2.33 (m, 2H), 2.44 - 2.76 (m, 11H), 2.79 - 2.92 (m, 3H), 3.27 - 3.46 (m, 5H), 3.48 - 3.56 (m, 2H), 3.57 - 3.78 (m, 11H), 7.17 - 7.35 (m, 6H), 7.44

- 7.69 (m, 5H), 7.73 (s, 1H), 7.90 - 8.06 (m, 4H), 11.21 - 11.29 (m, 1H), 12.34 - 12.39 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 11.9, 22.3, 22.6, 27.2, 28.2, 30.3, 32.4, 33.4, 34.2, 34.6, 35.4, 38.5, 46.5, 47.7, 48.8, 53.8, 54.1, 60.2, 62.7, 63.7, 69.1, 69.5, 69.7, 69.8, 72.3, 92.7, 112.6, 118.9, 121.2, 121.3, 121.5, 122.2, 125.5, 127.3, 128.2, 128.4, 128.5, 128.9, 129.4, 132.3, 132.7, 133.0, 136.5, 137.0, 147.4, 164.1, 165.5, 167.3, 170.7, 170.9, 171.4, 171.6. LCMS (ESI) *m/z* 949 $[M+H]^+$, 947 $[M-H]^-$. Rt 0.894 (NM mode). Purity 100%. HRMS (ESI/APCI dual) *m/z* calcd for $C_{53}H_{68}N_6O_{10}$ $[M+H]^+$ 949.5070, found 949.5076. To a solution of **27i** (20.0mg, 21.1 μmol) in 1,4-dioxane (2.0 mL) was added 4M HCl in 1,4-dioxane solution. The mixture was stirred at room temperature for 1 h and concentrated under reduced pressure. To the resulting residue was added 4M HCl in 1,4-dioxane solution and the mixture was stirred at room temperature for 1 h, and concentrated under reduced pressure. To the resulting residue was added water and the solution was lyophilized to obtain **27i** hydrochloride (21.1 mg, quantitative yield) as a colorless amorphous substance. ^{1}H NMR (500 MHz, DMSO-*d6*) δ 0.86 - 1.02 (m, 6H), 1.54 - 2.01 (m, 6H), 2.33 - 2.41 (m, 2H), 2.52 - 2.55 (m, 2H), 2.58 - 2.72 (m, 4H), 2.75 - 3.02 (m, 3H), 2.91 - 3.03 (m, 1H), 3.09 - 3.55 (m, 18H), 3.73 - 3.86 (m, 1H), 4.40 - 4.65 (m, 2H), 7.15 - 7.37 (m, 6H), 7.63 - 8.07 (m, 9H), 8.20 - 8.28 (m, 1H), 9.22 - 9.53 (m, 2H), 12.26 - 12.84 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 11.0, 21.1, 21.5 , 27.8, 30.0, 32.4, 34.0, 34.7, 35.9, 38.5, 44.1, 49.2, 53.8, 60.2 , 66.0, 67.6, 69.1, 69.5, 69.8, 72.3, 93.3, 112.6, 119.0, 121.2 , 121.5, 121.7, 122.3, 128.0, 128.2, 128.5, 129.4, 129.9, 130.4 , 131.6, 132.7, 133.4, 136.4, 137.1, 140.9, 147.4, 163.6, 165.5, 167.3, 171.3, 174.1. HRMS (ESI/APCI dual) *m/z* calcd for $C_{53}H_{68}N_6O_{10}$ $[M+H]^+$ 949.5070, found 949.5062.

21-[(3-{[3-({4-[3-(4-Carboxyphenyl)propyl]phenyl}carbamoyl)-1*H*-indol-2-yl]carbamoyl}phenyl)methyl]-22-ethyl-18-methyl-14,17-dioxo-4,7,10-trioxa-13,18,21-triazatetracosan-1-oic acid (28i)

Compound **28i** (80% over 3 steps) was prepared from **19i** and *tert*-butyl 3-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}propanoate in a manner similar to that described for **27i**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.82 - 0.93 (m, 6H), 1.22 - 1.36 (m, 2H), 1.44 - 1.57 (m, 2H), 1.75 - 1.95 (m 3H), 2.20 - 2.28 (m, 3H), 2.32 - 2.35 (m, 2H), 2.40 - 2.45 (m,

3H), 2.57 - 2.64 (m, 4H), 2.65 - 2.72 (m, 5H), 2.86 (s, 2H), 3.09 - 3.19 (m, 3H), 3.43 - 3.50 (m, 7H), 3.50 - 3.60 (m, 2H), 3.72 - 3.79 (m, 2H), 7.08 - 7.25 (m, 3H), 7.35 (d, J = 8.3 Hz, 2H), 7.66 (br d, J = 8.3 Hz, 5H), 7.73 - 8.00 (m, 5H), 8.03 - 8.13 (m, 1H), 9.17 - 9.38 (m, 2H), 12.28 - 12.57 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 11.9, 22.3, 22.7, 27.1, 28.1, 30.3, 32.3, 33.4, 34.1, 34.6, 34.7, 35.4, 38.5, 46.5, 46.6, 47.6, 48.7, 53.8, 54.1, 62.7, 63.7, 66.2, 69.1, 69.5, 69.6, 69.6, 92.7, 118.8, 121.2, 125.4, 127.2, 128.2, 128.4, 128.5, 128.9, 129.4, 132.6, 136.5, 147.4, 165.5, 167.3, 170.7, 170.9, 171.3, 171.5, 172.5. LCMS (ESI) *m/z* 977 $[M+H]^+$, 975 $[M+H]^+$. Rt 0.912 min (NM mode). Purity 100%. HRMS (ESI/APCI dual) *m/z* calcd for $C_{54}H_{68}N_6O_{11}$ $[M+H]^+$ 977.5019, found 977.5042.

4-[3-(4-{[2-(3-{[(2-{[N-(5-Carbamoyl-1H-pyrrole-3-sulfonyl)glycyl](methyl) amino}ethyl)(pentan-3-yl)amino]methyl}benzamido)-1*H*-indole-3-carbonyl] amino}phenyl)propyl]benzoic acid (29i)

To a solution of **19i** (20.0 mg, 29.1 μmol) in DMF (1.0 mL) was added DIPEA (20.3 μL, 15.0 μmol), 2-(5-carbamoyl-1h-pyrrole-3-sulfonamido)acetic acid (14.4 mg, 58.2 μmol) and HATU (22.1 mg, 58.2 μmol). The mixture was stirred at room temperature for 30 min, then quenched with water and extracted with EtOAc. The organic layer was washed with brine and extracted with EtOAc, and the combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to obtain the corresponding amide as a colorless amorphous substance, which was used in the next step without further purification. LCMS (ESI) *m/z* 917 $[M+H]^+$, 915 $[M-H]^-$. Rt 0.766 (LP mode). To a solution of the resulting amide in THF (1.0 mL) and MeOH (1.0mL) was added 4M NaOH aqueous solution (73.6 μL, 294 μmol). After stirring at room temperature for 30 min then at 60 °C for 4 h, the mixture was purified by reversed-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=90:10 → 20:80 → 5:95, 40 mL/min) to obtain **29i** (4.3 mg, 16% over 2 steps) as a colorless amorphous substance. ^{1}H NMR (400 MHz, CD_3OD) δ 0.75 - 0.95 (m, 6H), 1.27 - 1.39 (m, 2H), 1.44 - 1.56 (m, 2H), 1.92 - 2.02 (m, 2H), 2.20 - 2.30 (m, 1H), 2.55 - 2.88 (m, 9H), 3.19 - 3.25 (m, 1H), 3.41 - 3.48 (m, 1H), 3.59 - 3.89 (m, 4H), 6.97 - 7.39 (m, 8H), 7.43 - 7.63

(m, 5H), 7.81 - 7.99 (m, 4H), 8.15 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 11.9, 22.3, 22.5, 32.4, 33.6, 34.1, 34.4, 34.6, 43.6, 44.0, 46.3, 46.9, 47.3, 48.1, 53.8, 62.8, 63.2, 109.4, 112.5, 118.8, 121.6, 122.2, 122.8, 123.4, 123.6, 127.5, 128.2, 128.4, 128.5, 128.7, 129.4, 132.6, 136.6, 137.0, 141.4, 147.4, 161.3, 165.5, 166.7, 166.8, 167.3. LCMS (ESI) *m/z* 903 $[M+H]^+$, 901 $[M-H]^-$. Rt 0.902 (NM mode). Purity 100%. HRMS (ESI/APCI dual) *m/z* calcd for $C_{48}H_{54}N_8O_8S$ $[M+H]^+$ 903.3858, found 903.3860.

4-[3-(4-{[2-(3-{[(2-{[(2,5-Dioxohexahydroimidazo[4,5-d]imidazol-1(2H)-yl)acetyl](methyl)amino}ethyl)(pentan-3-yl)amino]methyl}benzamido)-1*H*-indole-3-carbonyl]amino}phenyl)propyl]benzoic acid (30i)

Compound **30i** (22% over 2 steps) was prepared from **19i** and (2,5-dioxohexahydroimidazo[4,5-d]imidazol-1(2H)-yl)acetic acid in a manner similar to that described for **29i**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.74 - 0.94 (m, 6H), 1.23 - 1.35 (m, 2H), 1.44 - 1.57 (m, 2H), 1.88 - 1.97 (m, 2H), 2.19 - 2.38 (m, 3H), 2.53 - 2.91 (m, 9H), 3.61 - 3.69 (m, 1H), 3.73 - 3.84 (m, 2H), 4.07 - 4.16 (m, 1H), 5.19 - 5.27 (m, 2H), 7.10 - 7.46 (m, 7H), 7.52 - 7.70 (m, 4H), 7.82 - 8.15 (m, 5H), 9.19 - 9.35 (m, 1H), 12.25 - 12.43 (m, 2H), 12.66 - 12.87 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 11.9, 22.4, 32.3, 33.6, 34.1, 34.4, 34.6, 40.9, 41.4, 46.4, 46.9, 47.4, 48.1, 54.0, 62.2, 63.1, 63.6, 68.1, 68.3, 92.7, 118.9, 121.5, 122.2, 125.4, 127.3, 128.2, 128.3, 128.5, 129.0, 129.4, 132.6, 147.4, 159.3, 161.0, 161.1, 163.0, 165.5, 167.3, 167.4, 167.5. LCMS (ESI) *m/z* 856 $[M+H]^+$, 854 $[M+H]^+$. Rt 0.869 min (NM mode). Purity 100%. HRMS (ESI/APCI dual) *m/z* calcd for $C_{47}H_{53}N_9O_7$ $[M+H]^+$ 856.4141, found 856.4171.

4-[3-(4-{[2-(3-{[{2-[{[(Carboxymethoxy)carbonyl]sulfamoyl}(methyl)amino]ethyl}(pentan-3-yl)amino]methyl}benzamido)-1H-indole-3-carbonyl]amino}phenyl)propyl]benzoic acid (31i and 31i hydrochloride)

To a solution of benzyl 2-hydroxyacetate (13.2 mg, 79.4 μmol) in 1,2-dichloroethane (0.60 mL) was added chlorosulfonyl isocyanate (5.8 μL, 67.0 μmol) at -15 °C, and the mixture was stirred at room temperature for 5 min. To the mixture was added **19i** (49.0 mg, 71.2 μmol) and triethylamine (15.0 μL, 108 μmol) at -15 °C, and the mixture was stirred at room temperature for 10 min. Then, the mixture was concentrated under

reduced pressure and the residue was purified by column chromatography on silica gel and eluted with 0%-85% EtOAc/*n*-hexane to obtain the corresponding benzyl ester (30.0 mg, 44%) as a pale yellow amorphous substance. LCMS (ESI) *m/z* 859 $[M+H]^+$. Rt 1.326 min (NM mode). To a solution of the resulting benzyl ester in THF (1.0 mL) and MeOH (1.0 mL) was added a 2M aqueous NaOH solution (1.00 mL, 2.00 mmol). After stirring at room temperature at 65 °C for 45 min, the mixture was quenched with 1M HCl aqueous solution and extracted with EtOAc. The organic layer was washed with brine and extracted with EtOAc, and the combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was washed with a mixture solvent of EtOAc and n-Hexane, and purified by reversed-phase preparative HPLC (column, YMC-Actus Triart 5 μm C18 50×30 mm; mobile phase, 0.1% formic acid in H_2O:0.1% formic acid in MeCN=90:10 → 20:80 → 5:95, 40 mL/min) to obtain **31i** (20.0 mg, 75%) as a pale yellow powder. LCMS (ESI) *m/z* 855 $[M+H]^+$. Rt 1.040 (NM mode). Purity 100%. The structure was checked in detail with the following hydrochloride. To a solution of **31i** (100 mg, 117 μmol) in THF (1.5 mL) was added 2M HCl aqueous solution (117 μL). The mixture was concentrated under reduced pressure to obtain **31i** hydrochloride (97.0 mg, 93%) as a yellow amorphous substance. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.94 - 1.02 (m, 6H), 1.63 - 1.77 (m, 2H), 1.89 - 1.95 (m, 3H), 2.02 (s, 1H), 2.60 - 2.65 (m, 5H), 2.69 (t, J = 7.6 Hz, 2H), 3.10 - 3.23 (m, 3H), 3.42 - 3.46 (m, 2H), 3.89 - 3.95 (m, 2H), 4.44 - 4.51 (m, 1H), 4.55 - 4.61 (m, 1H), 6.98 (s, 2H), 7.18 - 7.24 (m, 4H), 7.33 - 7.37 (m, 2H), 7.62 - 7.68 (m, 3H), 7.74 - 7.79 (m, 1H), 7.85 - 7.90 (m, 2H), 7.94 (d, J = 7.6 Hz, 1H), 7.99 (d, J = 7.6 Hz, 1H), 8.05 (d, J = 7.6 Hz, 1H), 8.23 - 8.27 (m, 1H), 9.32 (s, 1H), 9.43 - 9.49 (m, 1H), 12.30 (s, 1H), 12.33 - 12.36 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 11.0, 11.3, 21.6, 21.7, 30.4, 32.3, 34.1, 34.6, 36.0, 45.9, 47.8, 53.6, 59.5, 64.9, 65.5, 93.2, 112.6, 119.0, 121.2, 121.4, 121.7, 122.3, 124.9, 128.0, 128.2, 128.4, 128.5, 129.4, 129.8, 130.6, 131.4, 132.7, 133.2, 135.7, 136.5, 137.1, 141.0, 147.3, 163.6, 165.5, 167.2, 174.0. LCMS (ESI) *m/z* 855 $[M+H]^+$. Rt 1.047 (NM mode). Purity 95.1%. HRMS (ESI/APCI dual) *m/z* calcd for $C_{44}H_{50}N_6O_{10}S$ $[M+H]^+$ 855.3382, found 855.3383.

Assay of NaPi2b Inhibition: Phosphate uptake assay by CHO-K1 cells stably expressing human or rat NaPi2b

Assays of human or rat NaPi2b were performed as described below. Phosphate uptake was measured in Chinese Hamster Ovary -K1 (CHO-K1) cells stably expressing human or rat NaPi2b or non-expressing (mock) cells. The cells were seeded in 96-well plates at a density of 3×10^4 cells/well and grown overnight in a CO_2 incubator. The cells were washed with buffer A (137 mM NaCl, 5.4 mM KCl, 2.8 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM HEPES-Tris, pH 7.4), which was then replaced with buffer A containing the test compound or DMSO. After 30 minutes, the same amount of buffer A containing $^{33}PO_4$ and 20 μM KH_2PO_4 was added and the cells were allowed to react at 25 °C for 30 minutes (human NaPi2b) or 10 minutes (rat NaPi2b). After washing with ice-cold buffer B (137 mM NaCl, 10 mM HEPES-Tris, pH 7.4), the cells were lysed with 0.25 M NaOH. A portion of the lysed cell fluid and the liquid scintillator were mixed, and the ^{33}P radioactivity was measured using the TopCount system. The inhibition rate was determined using the following equation: Inhibition rate (%) = (1 − (radioactivity of added test compound in cells expressing human or rat NaPi2b − radioactivity of added test compound in mock cells) / (radioactivity of added DMSO in cells expressing human or rat NaPi2b − radioactivity of added DMSO in mock cells)) ×100. In addition, the 50% inhibitory concentration (IC_{50}) of the test compound was also calculated. Assays were run in duplicate, and compounds of interest were tested multiple times.

Rat Acute Uptake Pharmacodynamic Studies

Eight-week-old male Sprague-Dawley rats (Japan SLC, Inc.) were used as the laboratory animals. Each test compound was suspended or dissolved at a concentration of 2 mg/mL in 0.5w/v% methyl cellulose 400 solution (0.5% MC) (manufactured by Wako Pure Chemical Industries) and orally administered to each rat at the dose of 5 mL/kg body weight. 0.5%MC was administered at the same dose to the control group. Five minutes after administration of the test compound or 0.5% MC, a phosphate solution (12 mM NaH_2PO_4) containing ^{32}P-phosphate (PerkinElmer Inc.) was

administered at the dose of 5 mL/kg. Fifteen and thirty minutes after administration of the phosphate solution, blood samples were collected from the tail vein, and immediately mixed with EDTA-2K (manufactured by Dojindo Laboratories). Then, the mixtures were centrifuged at 3000 rpm at 4 °C. for 10 minutes to recover plasma. The radioactivity in 100 μL of plasma was measured using a liquid scintillation counter. The $AUC_{0\text{-}0.5h}$ calculated from the measured counts was considered as the phosphate absorption amount.

Pharmacokinetic study

The plasma concentration–time profiles of the test compounds were investigated in fasted male SD rats. After a single intravenous or oral administration of each test compound, blood was obtained from the tail vein into a tube containing EDTA-2K at each sampling time point and then centrifuged to prepare the plasma samples. The cumulative excretion of the unchanged form in the feces was investigated in fasted male SD rats. After a single oral administration of the test compounds, fecal samples were collected for a 24-hour time period. The quantitative analysis of the target analytes was performed using liquid chromatography-tandem mass spectrometry. The pharmacokinetic parameters were calculated using a non-compartmental analysis software, Phoenix WinNonlin 6.2 (Certara). Excel 2016 (Microsoft Corp.) was used to calculate excretion (% of dose) in feces.

Solubility in fasted-state simulated intestinal fluid (FaSSIF) or fed-state simulated intestinal fluid (FeSSIF)

An excess amount of each compound was added to FaSSIF (pH 6.5) or FeSSIF (pH 5.0) and shaken on a shaker (model SR-2DS; TAITEC) at 25 °C for 2 h, then kept at 37 °C for 22 h in a water bath (model LT-10F; TAITEC). The suspensions were centrifuged at 3,000 and 11,000 rpm for 10 min, and the resulting supernatant was diluted with 50% aqueous acetonitrile solution or an acetonitrile and methanol mixture (1:1). The concentrations were measured using HPLC. The HPLC analysis was performed using a Shimadzu HPLC system composed of a LC-20AD, SPD-20A and SIL-20AC. The conditions for HPLC were as follows: mobile phase, 0.1% phosphoric

acid aqueous solution/acetonitrile; flow rate, 0.8 mL/min; column, reversed-phase (Shimpack XR-ODS, 2.2 μm, 3.0×75 mm; SHIMADZU) at 40 °C; and detection wavelength, 210 nm.

Conformational stability calculations

The conformational stability calculations of these core structures were performed on all the generated conformers using the functional B3LYP with 6-311G(d,p) basis set and polarizable continuum model (PCM) of solvent. The most stable conformation was considered to be the lowest energy one among generated conformers. The initial conformers were generated using MOE software and then optimized using the B3LYP/6-311G(d,p) level theory in vacuum. All the DFT calculations were performed using the Gaussian09 package. Molecular modeling and visualization in this study were also performed with MOE.

参考文献

1. Hruska KA, Mathew S, Lund R, Qiu P, Prott R. Hyperphosphatemia of chronic kidney disease. *Kidney Int.* 2008;74:148-157.
2. Komaba H, Fukagawa M. Phosphate-a poison for humans?. *Kidney Int.* 2016;90;4:753-763.
3. Eckardt KU, Kasiske BL. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). *Kidney International.* 2009;76; Supplement113:S1-S130.
4. 日本透析医学会. 慢性腎臓病に伴う骨・ミネラル代謝異常の診療ガイドライン. 日本透析医学会雑誌. 2012;45;4:301-356.
5. 秋葉隆, 秋澤忠雄. 透析療法ネクスト XV. 2013:44-59.
6. 花房規男. 血管石灰化の基礎と臨床 Therapy 血管石灰化とリン吸着薬. *CLINICAL CALCIUM.* 2015; 25;5:87-97.
7. Mehrotra R, Martin KJ, Fishbane S, Stuart M. Sprague SM, Zeig S, Anger M. *Clin J Am Soc Nephrol* 3, 1437-1445, 2008.
8. Jamal SA, Vandermeer B, Raggi P, Mendelssohn DC, Chatterley T, Dorgan M, Lok CE, Fitchett D, Tsuyuki RT. Effect of calcium-based versus non-calcium-based phosphate binders on mortality in patients with chronic kidney disease: an updated systematic review and meta-analysis. *The Lancet.* 2013;382;9900:1268-1277.
9. 秋葉隆, 秋澤忠雄. 透析療法ネクスト XV. 2013:70-77.
10. Sabbagh Y, O'Brien SP, Song W, Boulanger JH, Stockmann A, Aebeeny C, Schiavi SC. Intestinal Npt2b Plays a Major Role in Phosphate Absorption and Homeostasis. *J. Am. Soc. Nephrol.* 2009;20:2348-2358.
11. Marks J, Debnam ES, Unwin RJ. The role of the gastrointestinal tract in phosphate

homeostasis in health and chronic kidney disease. *Curr. Opin. Nephrol. Hyperten.* 2013;22:481-487.

12. Castellana G, Castellana G, Gentile M, Castellana R, Resta O. Pulmonary alveolar microlithiasis: review of the 1022 cases reported worldwide. *Eur. Respir. Rev.* 2015;24:607-620.
13. Huqun, Izumi S, Miyazawa H, Ishii K, Uchiyama B, Ishida T, Tanaka S, Tazawa R, Fukuyama S, Tanaka T, Nagai Y, Yokote A, Takahashi H, Fukushima T, Kobayashi K, Chiba H, Nagata M, Sakamoto S, Nakata K, Takebayashi Y, Shimizu Y, Kaneko K, Shimizu M, Kanazawa M, Abe S, Inoue Y, Takenoshita S, Yoshimura K, Kudo K, Tachibana T, Nukiwa T, Hagiwara K. Mutations in the SLC34A2 gene are associated with pulmonary alveolar microlithiasis. *Am. J. Respir. Crit. Care Med.* 2007;175:263-268.
14. Corut A, Senyigit A, Ugur SA, Altin S, Ozcelik U, Calisir H, Yildirim Z, Gocmen A, Tolun A. Mutations in SLC34A2 cause pulmonary alveolar microlithiasis and are possibly associated with testicular microlithiasis. *Am. J. Hum. Genet.* 2006;79:650-656.
15. Lewis JG, Jacobs JW, Reich N, Leadbetter MR, Bell N, Chang HT, Chen T, Navre M, Charmot D, Carreras C, Labonte E. Compounds and methods for inhibiting phosphate transport. *PCT Int. Appl.* WO2012054110A2, 2012.
16. Miura M, Kaga D, Watanuki S, Hachiya S, Okuda T, Sato I, Isomura M, Terai K, Terada Y. Aminoalkyl-substituted *N*-thienyl benzamide derivatives. *PCT Int. Appl.* WO2013062065A1, 2013.
17. Hachiya S, Miura M, Imamura Y, Kaga D, Sato I, Moritomo H, Kato K, Terai K, Terada Y. Tetrahydrobenzothiophene compound. *PCT Int. Appl.*

WO2011136269A1, 2011.

18. Soerensen MD, Larsen JCH, Noerremark B, Liang X, Huang G. Phosphate transpot inhibitors I. *PCT Int. Appl.* WO2013082751A1, 2013.
19. Larsson TE, Kameoka C, Nakajo I, Taniuchi Y. Yoshida S, Akisawa T, Smulders RA. NPT-IIb Inhibition does not improve hyperphosphatemia in CKD. *Kidney Int. Rep.* 2018;3:73-80.
20. Maemoto M, Hirata Y, Hosoe S, Ouchi J, Narushima K, Akizawa E, Tsuji Y, Takeda H, Yanagisawa A, Shuto S. Discovery of gut-restricted small-molecule inhibitors of intestinal sodium-dependent phosphate transport protein 2b (NaPi2b) for the treatment of hyperphosphatemia. *J. Med. Chem*. 2022;65:3:1946-1960.
21. Maemoto M, Hirata Y, Hosoe S, Ouchi J, Uchi M, Takeda H, Akizawa E, Yanagisawa A, Shuto S. Development of potent non-acylhydrazone inhibitors of intestinal sodium-dependent phosphate transport protein 2b (NaPi2b). *Bioorg. Med. Chem.* 2022;71:116944.
22. Bnno BR, Yeung KS, Bartberger MD, Pennington LD, Meanwell NA. A survey of the role of noncovalent sulfur interactions in drug design. *J. Med. Chem.* 2015;58:4383-4438.
23. Molecular Operating Environment (MOE), 2019.01; Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2018.
24. Gaussian 09, Revision D.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A.

Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox. Gaussian, Inc., Wallingford CT, 2013.

25. Veber DF, Johnson SR, Cheng H, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem*. 2002;45:2615-2623.
26. Palm K, Stenberg P, Luthman K, Artursson P. Polar molecular surface properties predict the intestinal absorption of drugs in humans. *Pharm. Res*. 1997;14:568-571
27. Hewitt WM, Leung SSF, Pye CR, Ponkey CR, Bednarek M, Jacobson MJ, Lokey RS. Cell-permeable cyclic peptides from synthetic libraries inspired by natural products. *J. Am. Chem. Soc.* 2015;137:715-721.
28. Bockus AT, Lexa KW, Pye CR, Kalgutkar AS, Gardner JW, Hund KCR, Hewitt WM, Schwodhert JA, Glassey E, Price DA, Mathiowetz AM, Liras S, Jacobson MP, Lokey RS. Probing the physicochemical boundaries of cell permeability and oral bioavailability in lipophilic macrocycles inspired by natural products. *J. Med. Chem.* 2015;58:4581-4589.
29. Kararli TT. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm. Drug Dispos.* 1995;16;5:351-80.

30. McConnell EL, Basit AW, Murdan S. Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for *in-vivo* experiments. *J. Pharm. Pharmacol.* 2008;60;1;63-70.
31. Kimura T, Sudo K, Kanzaki Y, Miki K, Takeuchi Y, Kurosaki Y, Nakayama T. Drug absorption from large intestine: physicochemical factors governing drug absorption. *Biological and Pharmaceutical Bulletin*.1994;17;2;327-333
32. Hamada T, Goto H, Yamahira T, Sugawara T, Imaizumi K, Ikeda I. Solubility in and affinity for the bile salt micelle of plant sterols are important determinants of their intestinal absorption in rats. *Lipids.* 2006;41;551-556.

主論文目録

本学位論文内容は下記の発表論文による

(1) Yasunobu Ushiki, Kenichi Kawabe, Kumiko Yamamoto-Okada, Fumito Uneuchi, Yuta Asanuma, Chitose Yamaguchi, Hiroshi Ohta, Tsuyoshi Shibata, Tomohiro Abe, Lisa Okumura-Kitajima, Yuki Kosai, Mayumi Endo, Katsumasa Otake, Eiji Munetomo, Teisuke Takahashi, Hiroyuki Kakinuma. Design, synthesis and biological evaluation of novel 1H-pyrazole-4-carbonyl-4,5,6,7-tetrahydrobenzo [b]thiophene derivatives as gut-selective NaPi2b inhibitors. *Bioorg. Med. Chem. Lett.* **2022**;59:128572.

(2) Yasunobu Ushiki, Kenichi Kawabe, Kumiko Yamamoto-Okada, Fumito Uneuchi, Yuta Asanuma, Chitose Yamaguchi, Hiroshi Ohta, Tsuyoshi Shibata, Tomohiro Abe, Lisa Okumura-Kitajima, Yuki Kosai, Mayumi Endo, Katsumasa Otake, Eiji Munetomo, Teisuke Takahashi, Hiroyuki Kakinuma. Design, synthesis and biological evaluation of novel pyridine derivatives as gut-selective NaPi2b inhibitors. *Bioorg. Med. Chem. Lett.* **2022**;65:128700.

(3) Yasunobu Ushiki, Kenichi Kawabe, Kumiko Yamamoto-Okada, Fumito Uneuchi, Yuta Asanuma, Chitose Yamaguchi, Hiroshi Ohta, Tsuyoshi Shibata, Tomohiro Abe, Lisa Okumura-Kitajima, Yuki Kosai, Mayumi Endo, Katsumasa Otake, Eiji Munetomo, Teisuke Takahashi, Hiroyuki Kakinuma. Design, synthesis and biological evaluation of novel pyridine derivatives as gut-selective NaPi2b inhibitors. Bioorg. Med. Chem. **2022**;66;15:116783.

論文審査の主査及び副査名

本学位論文の審査は千葉大学大学院薬学研究院で指名された下記の審査委員により行われた。

主査　千葉大学大学院薬学研究院　教授　根本 哲宏
副査　千葉大学大学院薬学研究院　教授　石川勇人
副査　千葉大学大学院薬学研究院　准教授　北島満里子
副査　千葉大学大学院薬学研究院　講師　原田慎吾