博士学位論文

非全身曝露型化合物を指向した 腸管 NaPi 阻害薬の創製

前本　道寛

北海道大学大学院生命科学院

生命科学専攻 生命医薬科学コース

創薬有機化学研究室

令和 5 年 3 月

目次

略語表

本報では以下の略語を用いた。

CKD: chronic kidney disease

DMA: *N*,*N*-dimethylacetamide

ESRD: end-stage renal disease

FGF23: fibroblast growth factor 23

HBA: H-bond acceptor

HBD: H-bond donor

HEPES: 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid

HMDS: 1,1,1,3,3,3-hexamethyldisilazane

HPLC: high-performance liquid chromatography

IPA: isopropyl alcohol

IS: internal standard

LC: lanthanum carbonate

MES: 2-(*N*-morpholino)ethanesulfonic acid

Ms: methanesulfonyl

MW: molecular weight

NaH: sodium hydride

NaI: sodium iodide

NaPi2b: sodium-dependent phosphate transport protein 2b

nROT: number of rotatable bonds

PAMPA: parallel artificial membrane permeability assay

PBS: phosphate-buffered saline

PEG: polyethylene glycol

PTH: parathyroid hormone

Ro5: rule of 5

SD: Sprague-Dawley

SH: sevelamer hydrochloride

SLC: solute carrier

TEA: triethylamine

TFA: trifluoroacetic acid

TFAA: trifluoroacetic anhydride

tPSA: topological polar surface area

緒言

リン酸塩は生体内で最も豊富な化学種の 1 つであり、骨、歯、細胞膜および核酸の必要不可欠な構成成分である。加えて、細胞内シグナル伝達の調節、組織におけるエネルギー産生、そして生体内 pH の維持に不可欠である。[1-3] したがって、適切な生体内リン酸塩濃度の維持は、恒常性のために非常に重要である。生体内リン酸塩濃度は、食事に由来する無機リン酸塩の腸管からの取り込み、骨と軟部組織間での交換、腎臓での再吸収と排出、および消化管からの便を介した排泄によって一定の範囲に保たれている。[4, 5]

腸管からのリン酸塩の吸収機構に関しては完全には解明されていないものの、二つの独立した経路を介していると広く認識されている。一つはナトリウム依存的な能動経路であり、溶質キャリア（solute carrier, SLC）トランスポーターであるナトリウム依存性リン酸トランスポーター2b（sodium-dependent phosphate transport protein 2b, NaPi2b; SLC34A2）、Pit1（SLC20A1）、そして Pit2（SLC20A2）を介した取り込みが知られている。もう一つはナトリウム非依存的な細胞間隙を介した受動輸送である。[6] 一方で、NaPi2b コンディショナルノックアウトマウスにおいては、Pit1 および Pit2 を介した腸管からのリン酸塩の吸収はほとんどないと報告されており、能動輸送には、NaPi2b が大きく関与していると推察されている。[7]

NaPi2 ファミリーを介したリン酸塩の恒常性の制御について、**Figure 1** に示す。NaPi2 ファミリーは、NaPi2a（SLC34A1）、NaPi2b および NaPi2c（SLC34A3）の 3 種類で構成されており、いずれもリン酸塩の恒常性の維持に重要な役割を果たしている。これらのうち、NaPi2a および NaPi2c は、主に腎臓の近位尿細管の頂端膜側に発現しており、糸球体濾液からの無機リン酸塩の再吸収を担っている。一方、NaPi2b は小腸の頂端膜側に発現しており、食事性無機リン酸塩の吸収を担っている。[8] これらのトランスポーターの発現にはいくつかのホルモンが関わっている。NaPi2a および NaPi2c は、線維芽細胞増殖因子 23（fibroblast growth factor 23, FGF23）および副甲状腺ホルモン（parathyroid hormone, PTH）によって発現抑制さ

れ、近位尿細管における無機リン酸塩の再吸収抑制に伴い、尿中への無機リン酸塩の分泌を増加させる。[9, 10] 一方で、NaPi2b は 1,25(OH)$_2$-ビタミン D$_3$ によって発現亢進され、腸からの食事性無機リン酸塩の吸収を増加させる。[9, 10]

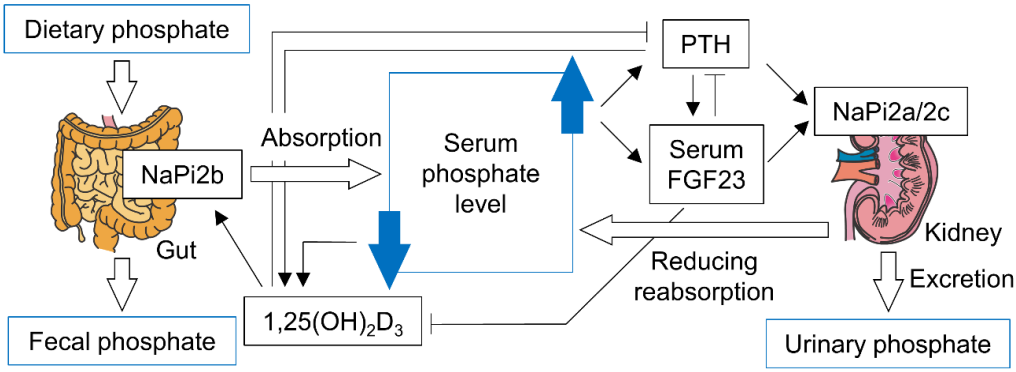


Figure 1. Diagram of phosphate homeostasis. The secretion of FGF23 and PTH is increased in response to high serum phosphate levels, and renal phosphate excretion is increased due to the downregulated expression of NaPi2a and NaPi2c. On the other hand, 1,25(OH)$_2$D$_3$ is increased at low phosphate serum levels, which upregulates NaPi2b expression and increases intestinal phosphate absorption.[9, 10] Reprinted with permission from reference.[11] Copyright 2022 American Chemical Society.

慢性腎臓病（Chronic kidney disease, CKD）が進行すると骨・ミネラルの代謝異常が起こり、その病態は CKD-mineral and bone disorder と呼ばれている。[12] 多くの研究において、CKD 患者における血清リン酸塩濃度の上昇は、心血管疾患および死亡リスクの上昇に繋がることが示唆されている。[13-17] それゆえ、CKD 患者、特に末期腎不全患者（end-stage renal disease, ESRD）においては、リン制限食事療法や透析により過剰なリン酸塩を低減し、血清リン酸塩濃度を正常に保つことが重要である。[18] これらに加えて、食事から得られる過剰な無機リン酸塩の吸収を妨げる治療薬として、塩酸セベラマーや炭酸ランタンなどの経口リン酸塩吸着剤が末期腎不全患者に使用されている。[19-21] しかしながら、経口リン酸塩吸着剤は 1 日の服薬量が多いことや（炭酸ランタン：0.5－4.5 g/day、塩酸セベラマー：0.4－9.6 g/day）、CKD 患者は処方されている薬剤数が多い傾向があることから、服薬アドヒアランスの低下を招いている。[22] これに加え、経口リン酸塩吸着剤による下痢や便秘など

の消化管に対する副作用も大きな課題である。[23-27]

これらの課題に対し、筆者は腸管からの食事性無機リン酸塩の吸収を阻害する低分子薬の開発を目指した。すなわち、高用量の服薬を回避し、また経口リン酸塩吸着剤の有する副作用を示さない低分子高リン血症治療薬の創製に取り組んだ。先述の通り、腸管に発現しているNaPi2bは食事性無機リン酸塩の吸収に大きく関与していることから、腸管のNaPi2bを阻害する治療薬は、経口リン酸塩吸着剤よりも低用量で血清リン酸塩濃度の低下が期待できると考えた。[7,28] 一方で、NaPi2bは腸管だけでなく肺や精巣にも発現しており、NaPi2b遺伝子の変異は肺胞小石症や精巣小石症に関連していると報告されている。[29-31] それゆえ、血中曝露型[a]のNaPi2b阻害薬を用いた際には、異所性石灰化のようなオンターゲットの副作用が懸念される。[31]

これまでに、NaPi2aもしくはNaPi2b阻害薬として化合物**1, 2**が報告されている（**Figure 2**）。[32-35] NaPi2a阻害薬**1**（IC_{50} = 380 nM）は、NaPi2bに対する親和性と比較して65倍以上のNaPi2a選択性を示しており、その誘導体はヒト近位尿細管細胞においてリン酸塩の取り込み阻害を示している。一方で、*in vivo*におけるリン酸塩取り込み阻害活性は報告されていない。NaPi2b阻害薬**2**（IC_{50} = 120 nM）は、ラットにおいて、十二指腸内投与（10 mg/kg）で、門脈における食事性無機リン酸塩の吸収抑制を示した最初の化合物である。しかしながら、化合物**2**の膜透過性や経口吸収性については報告されていない。

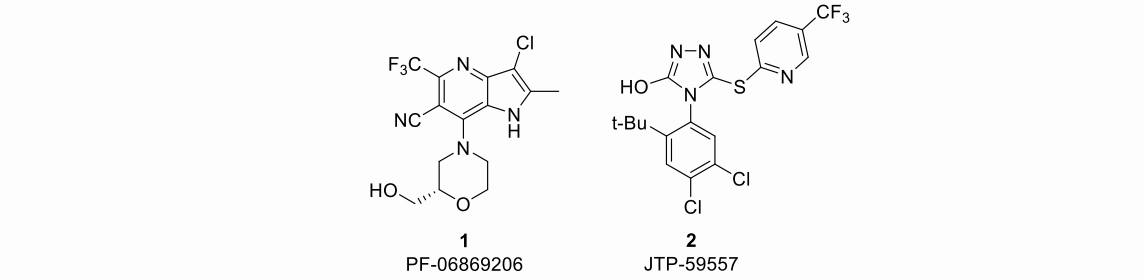


Figure 2. Chemical structures of the reported NaPi inhibitors.
Reprinted with permission from reference.[11] Copyright 2022 American Chemical Society.

a 経口投与により腸管から吸収されて、全身に分布する。

高リン血症治療薬の開発を目指した創薬において、能動輸送経路の阻害薬である ASP3325（構造不明）や EOS789、受動輸送経路の阻害薬である Tenapanor を用いた腸管からの食事性無機リン酸塩吸収阻害薬の臨床試験が報告されている（**Figure 3**）。[36-39] ASP3325 は、血中曝露型 NaPi2b 阻害薬であり、アデニン誘発性腎不全モデルラットおよび高リン酸塩食を与えた正常ラットに対する経口投与試験において、血清リン酸塩濃度の低減に有効であることと安全性が確認されている。しかしながら、ESRD 患者において、経口投与による血清リン酸塩濃度の低下は確認できなかった。[38] EOS789 は、低吸収性を示すリン酸トランスポーター阻害薬であり、経口投与によって健康なボランティアおよび血液透析患者の糞便中リン酸塩排泄量を有意に増加させた。[37] Tenapanor は、腸管からほとんど吸収されない非血中曝露型の Na^+/H^+交換輸送体 3（sodium-hydrogen exchanger 3, NHE3）阻害薬であり、経口投与によって ESRD 患者の血清リン酸塩濃度を有意に低下させた。[39] 最近の高リン血症治療薬の臨床開発動向から、NaPi2b 阻害薬以外の作用機序の研究も実施されてきているものの、依然として経口リン酸塩吸着剤が高リン血症治療薬として唯一の臨床使用可能な治療薬である。

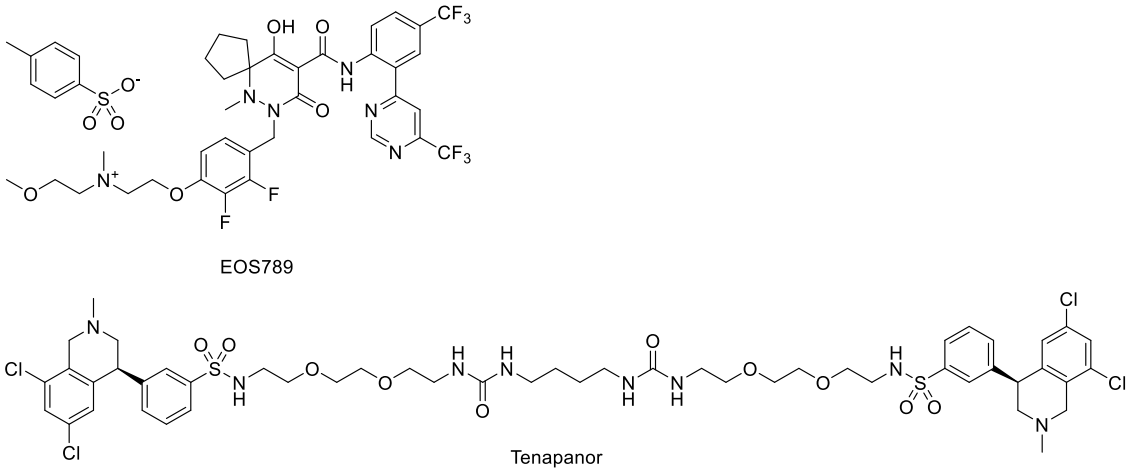


Figure 3. Chemical structures of phosphate absorption inhibitors.

以上の研究動向を考慮し、筆者は、経口リン酸塩吸着剤に比べて低用量かつ副作用の懸念なく使用できる高リン血症治療は、経口吸収されて血中に分布することなく腸管局所で作

用する強力なリン酸塩取り込み阻害活性を示すNaPi2b阻害薬により実現できるのではないかと考え、創薬研究を実施した。本論文において、スクリーニングヒット化合物**3**からのリード化合物の創出、副作用抑制を目的とした肺移行性低減のための構造最適化、副作用が懸念されるアシルヒドラゾン構造回避に向けた誘導体展開、および*in vivo*でのリン酸塩吸収阻害作用に関して報告する。

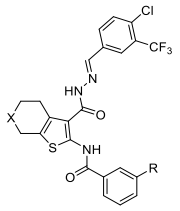
第 1 章　リード化合物の創出と最適化研究

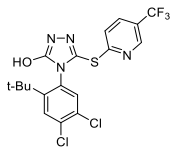
1.1　低膜透過性 NaPi2b 阻害薬としてのテトラヒドロチエノピリジン誘導体 **7** の同定

NaPi2b は腸管の頂端膜側に主に発現している。それゆえ、非血中曝露型の化合物であっても腸管の NaPi2b に作用することが可能であり、十分なリン酸塩吸収阻害活性を示すことが可能であろうと推測した。[7,8] 筆者は、非経口吸収性を示す腸管の NaPi2b 選択的阻害薬の取得に向けた手法として、膜透過性を限りなく下げることによりバイオアベイラビリティを低減させる戦略を取ることとした。リード化合物取得に向けて、ヒト NaPi2b 発現 KJMGER8 細胞 [40] を作製し、^{33}P-ラジオ標識リン酸塩の取り込み測定によるスクリーニングを行った。協和キリンの化合物ライブラリーの中から、化合物 **3**（IC_{50} = 87 nM）[41] が非常に強いリン酸塩取り込み阻害活性を示すヒット化合物として取得された。ヒト NaPi2b 非発現 KJMGER8 細胞においては、化合物 **3** によるリン酸塩吸収取り込み阻害活性は観察されてない。化合物 **3** の課題として低溶解性（0.1 μM in PBS at pH 7.4）があることから、溶解性向上に向け化合物に極性官能基を導入することとした。加えて、電荷を有する官能基を導入することで、脂質二重膜との親和性の低減が可能であり、結果として低膜透過性にもつながるのではないかと期待し、実施した。

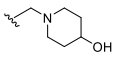
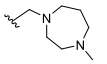
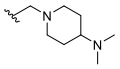
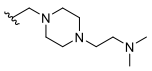
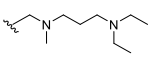
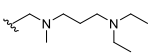
低膜透過性かつ高溶解性の化合物の取得を目指し、化合物 **3** の 4,5,6,7-テトラヒドロベンゾチオフェン部位（X）へシクロプロピルアミノ基を導入し、末端ベンズアミドの *m* 位（R）に環状あるいは鎖状アミノ基を置換し、*in vitro* における NaPi2b 阻害活性、溶解性、そして膜透過性を評価した。合成した 6-シクロプロピル-4,5,6,7-テトラヒドロチエノピリジン誘導体と評価（リン酸塩取り込み阻害活性、溶解性、人工膜透過性）結果に関して **Table 1** に示す。報告されている NaPi2b 阻害薬である化合物 **2** を陽性対照として用いた。

Table 1. Structure-activity relationship of 6-cyclopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine and 6-hydroxy-6-methyl-4,5,6,7-tetrahydrobenzo[b]thiophene derivatives by introducing a basic functional group at the *m*-position of benzamide





2
JT-59557

Compound	X	R	NaPi2b IC_{50}[a] (nM)	Sol.[b] (μM)	Pe[c] (10^{-6} cm/s)
2[d]			838	1.9	2.1
3	CH_2		87	0.1	ND[e]
4			455	7.9	0.12
5			404	17	0.068
6			249	45	0.068
7			354	66	0.051
8			997	82	0.26

[a]50% inhibitory concentration against $H_2[^{33}P]O_4$ uptake in human NaPi2b-transfected KJMGER8 cells. The values are shown as a mean of three determinations. [b]Solubility in PBS at pH 7.4. [c]Passive permeability from the apical to basolateral direction was measured using PAMPA. [d]Compound 2 was synthesized according to the literature.[35] [e]Not determined. Reprinted with permission from reference.[11] Copyright 2022 American Chemical Society.

R 部位に *N*-メチルジアゼピルメチル基を導入した化合物 **4**（IC_{50}＝455 nM）は、ヒット化合物 **3** と比較してリン酸塩取り込み阻害活性が 5 倍程度減弱した。一方で、溶解性は向上した（7.9 μM in PBS）。ピペリジン誘導体を導入した化合物 **5**、ピペラジン誘導体を導入した化合物 **6** は、それぞれリン酸塩取り込み阻害活性が 3–5 倍程度減弱した（**5**, IC_{50}＝404 nM;

6, IC_{50} = 249 nM)。これら誘導体の溶解性は更に向上した（**5**, 17 μM in PBS; **6**, 45 μM in PBS)。プロパンジアミン鎖を導入した化合物 **7** は、リン酸塩取り込み阻害活性が 4 倍程度減弱し（IC_{50} = 354 nM)、溶解性は 66 μM であった。化合物の溶解性に関して、疎水性および p*K*a 計算値（calculated p*K*a, cp*K*a）を比較した。化合物 **4** および **7** の疎水性は、逆相高速液体クロマトグラフィー（HPLC）の保持時間から同程度であった。一方で、cp*K*a は化合物 **7** の方が高値を示した（**4**, cp*K*a = 8.91; **7**, cp*K*a = 10.39)。高 cp*K*a 値を示したプロパンジアミン体 **7** は中性付近でイオン化しやすく、高溶解性をもたらしたと考えられる。

更なる誘導体展開に向け、4,5,6,7-テトラヒドロベンゾチオフェンの X 部位にヒドロキシ基を導入した。しかしながら、**Table 1** に示すように、化合物 **8** のリン酸塩取り込み阻害活性（IC_{50} = 997 nM）は、化合物 **7** よりも低減した。したがって、4,5,6,7-テトラヒドロベンゾチオフェンの X 部位への水素結合供与体（H-bond donor, HBD）の導入は許容されにくいと判断した。

これらの化合物が非経口吸収性を示すかどうかの指標として、parallel artificial membrane permeability assay （PAMPA）[42] を用いた膜透過性評価を実施した。これまでに、ラットにおける経口吸収性と PAMPA を用いた膜透過性に相関関係があるとの研究が報告されている。[43] それらの研究結果では、PAMPA 膜透過性が 0.1×10^{-6} cm/s 未満の化合物のラットにおける平均バイオアベイラビリティは 13%であり、0.1×10^{-6} cm/s を超える化合物の平均バイオアベイラビリティは 35%と示されている。筆者は、低経口吸収性を示す閾値として 0.1×10^{-6} cm/s を設定した。その値と比較し、塩基性官能基を有するジアミノおよびトリアミノ体 **4**–**7**（$Pe^b < 0.12 \times 10^{-6}$ cm/s）は、閾値と同程度もしくは低い膜透過性を示した。中でもプロパンジアミン鎖を側鎖に有する化合物 **7**（$Pe = 0.051 \times 10^{-6}$ cm/s）は、非常に低い膜透過性を示した。

結果として、化合物 **7** はこれまでに報告されていた化合物 **2**（IC_{50} = 838 nM）と比較して

b PAMPA を用いた膜透過性評価における apical から basolateral への膜透過係数

NaPi2b 阻害活性が 2 倍程度向上し、膜透過性（Pe = 2.1×10^{-6} cm/s）は 40 倍程度低減した。また、ヒット化合物 **3** と比較して 660 倍程度の溶解性向上を示した。

先述の通り、NaPi2a や NaPi2c は腎臓におけるリン酸塩の再吸収に関与している。筆者の誘導体展開している腸管選択的化合物は非経口吸収性であり、血中曝露をほとんど示さない化合物を目指している。NaPi サブタイプである NaPi2a および NaPi2c は腎臓に発現しているものの、筆者が目指す低吸収性化合物が実現すれば、腎臓への分布はほとんどないと予想される。したがって、本低膜透過化戦略においては、NaPi サブタイプ間の選択性は無視することができると考えている。

化合物 **2** を用いた *in vivo* 試験において、十二指腸への化合物投与後ラット門脈におけるリン酸塩濃度低下が観察されいることから [32]、化合物 **2** と同程度の NaPi2b 阻害活性を有する化合物であれば、*in vivo* でのリン酸塩取り込み阻害が期待できると考えた。本誘導体展開における NaPi2b 阻害活性および膜透過性の結果は、ヒット化合物 **3** から更なる構造最適化を実施するための十分な裏付けとなった。

1.2　肺への化合物分布低減に向けた塩基性低減展開

種々の研究結果から、塩基性化合物は、肺、肝臓、腎臓などのリソソーム豊富な組織へ移行性が高いことが知られている。[44,45] 先述したように、肺には NaPi2b が発現しており、化合物が経口吸収されて血中に分布すると肺での副作用につながる懸念がある。そこで、塩基性化合物の中で最も低膜透過性を示した化合物 **7** の肺移行性と経口吸収性を評価した。**Table 2** に示すように、化合物 **7**（10 mg/kg）のラットにおけるバイオアベイラビリティは非常に低く、0.96%と算出された。

Table 2. Pharmacokinetic properties of compound 7 following 10 mg/kg oral administration in rats

Compound	C_{max}[a] (ng/mL)	$AUC_{0\rightarrow t}$[a] (ng·h/mL)	F[a,b] (%)
7	3.59	10.4	0.96

[a]The values are shown as a mean of three determinations. [b]The compound was administered at a dose of 10 mg/kg (po) and 1 mg/kg (iv). Reprinted with permission from reference.[11] Copyright 2022 American Chemical Society.

一方で、**Table 3** に示すように、化合物 **7**（30 mg/kg）の経口投与後 6 時間の血漿中濃度は 1.86 ng/mL であり、肺濃度は 6350 ng/g であった。その際の化合物の肺－血漿中濃度比（$K_{p,lung}$）[c]は 4180 と算出され、文献報告のある様々な塩基性化合物の移行性と比較し高値を示した。[45, 46]

Table 3. Plasma and lung pharmacokinetic properties of compound 7 following 30 mg/kg oral administration in rats

Compound	Plasma conc[a] (ng/mL)	Lung conc[a] (ng/g)	$K_{p,lung}$[a,b]
7	1.86	6350	4180

[a]The values are shown as a mean of three determinations, and were measured 6 hours after oral administration. [b]The values were calculated using the ratio of plasma concentration to lung concentration. Reprinted with permission from reference.[11] Copyright 2022 American Chemical Society

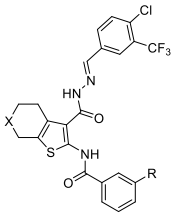
一般的に肺移行性と化合物の塩基性が相関することは知られていることから、筆者は、肺移行性の低減を目指して誘導体の塩基性アミノ基を減らしつつ、NaPi2b 阻害活性と低膜透

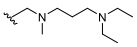
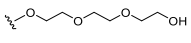
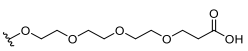
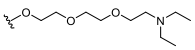
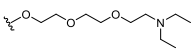
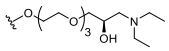
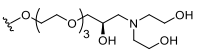
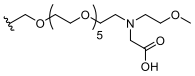
c ラットにおける経口投与後の肺と血漿中の化合物濃度比から算出した値。化合物の血漿中から肺への移行性比較に用いた。

過性を維持しようと考えた。加えて、更なる膜透過性の低下を目指した展開も実施した。リピンスキーの法則（Lipinski's rule of 5, Ro5）は、一般的な経口薬のドラッグライクネスを示す指標として腸管からの吸収や膜透過率の予測に広く用いられている。[47] 他にも一般則として、10 もしくはそれ以下の回転可能結合数（number of rotatable bonds, nROT）および 140Å 以下のトポロジカル極性表面積（topological polar surface area, tPSA）の性質を有する化合物は、良好なバイオアベイラビリティを示す可能性が高い。[43] 筆者は、低膜透過型の化合物取得を目指し、これらの一般的な経口薬としてのドラッグライクネスから意図的に脱却する化学的特徴を有する化合物をデザインし合成することとした。すなわち、分子量（molecular weight, MW）が 500 より大きく、tPSA が 140Å より大きく、nROT が 10 を超え、水素結合受容体（H-bond acceptor, HBA）が 10 個を超える化合物の創製を目指した。

まずは高 tPSA 化と nROT 数の増加による低膜透過化を指向し、テトラヒドロチエノピリジン骨格からシクロプロピルアミノ基を除去することで塩基性を低減し、非塩基性の PEG 鎖を R 部位に導入した。合成した化合物とそれらの化学的特徴および評価結果を **Table 4** に示す。

Table 4. Structure-activity relationship of basicity-reduced derivatives of compound 7 with a PEG side chain at the *m*-position of the benzamide moiety



Compd.	X	R	NaPi2b IC_{50}[a] (nM)	Sol.[b] (μM)	Pe[c] (10^{-6} cm/s)	MW[d]	tPSA[d]	nROT[d]	HBD[d]	HBA[d]
7			354	66	0.051	703	108.52	15	2	6
9			<100	3.3	ND[e]	682	146.72	15	3	7
10			<100	81	0.56	754	173.02	19	3	9
11			40	55	<0.025	737	129.73	18	2	7
12			315	82	0.23	750	132.96	19	2	8
13			33	94	<0.025	811	159.19	22	3	9
14			83	76	ND[e]	843	199.64	24	5	11
15			64	122	<0.025	943	203.95	31	3	13

[a]50% inhibitory concentration against $H_2[^{33}P]O_4$ uptake in human NaPi2b-transfected KJMGER8 cells. The values are shown as a mean of three determinations. [b]Solubility in PBS at pH 7.4. [c]Passive permeability from the apical to basolateral direction was measured using PAMPA. [d]Calculated value using BIOVIA, Dassault Systèmes, BIOVIA Pipeline Pilot, 2019.1.0.1964, San Diego: Dassault Systèmes, 2019. [e]Not determined. Reprinted with permission from reference.[11] Copyright 2022 American Chemical Society

化合物 **7** と比較し、アルコール体 **9**、カルボン酸体 **10**、ジエチルアミノ体 **11** ともに 3–9 倍程度の強力なリン酸塩取り込み阻害活性を示した（**9** and **10**, IC_{50} < 100 nM; **11**, IC_{50} = 40

nM)。アルコール体 **9** は低溶解性（3.3 μM in PBS）であった一方で、カルボン酸体 **10** とジエチルアミノ体 **11** は良好な溶解性を示した（**10**, 81 μM in PBS; **11**, 55 μM in PBS)。膜透過性に関して、カルボン酸体 **10**（Pe = 0.56 × 10^{-6} cm/s）は筆者の設定した低膜透過性の閾値より大きな値を示した一方で、ジエチルアミノ体 **11**（Pe < 0.025 × 10^{-6} cm/s）は閾値を満たし低膜透過性を示した。tPSA、nROT、HBA を比較すると、カルボン酸体 **10** の方が低膜透過化に有利と期待されたが、PAMPA を用いた膜透過試験結果からは塩基性化合物であるジエチルアミノ体 **11** の方が 20 倍程度の低膜透過性を示した。アルコール体 **9** およびカルボン酸体 **10** は強力なリン酸塩取り込み阻害活性を示したものの、溶解性や膜透過性の点で好ましくなかった。R 部位への PEG 鎖の導入により強活性は保持された一方で、シクロプロピルアミノ基を X 部位に有する化合物 **12**（IC_{50} = 315 nM）は、ジエチルアミノ体 **11** と比較してリン酸塩取り込み阻害活性が約 1/8 に低下した。したがって、4,5,6,7-テトラヒドロベンゾチオフェン骨格の X 部位への脂溶性官能基の導入は、NaPi2b 結合能の増強に有効と考えらた。これらの結果を受け、化合物 **11** から更なる構造最適化を実施することとした。

筆者は、cp*K*a を指標とし、化合物 **11**（cp*K*a = 9.72）と比較して塩基性の低減した化合物をデザインした。PEG 側鎖末端のジエチルアミノ基の分子内プロトン化を指向して、PEG 側鎖に β-アミノアルコールを導入した。合成したジエチルアミノエタノール体 **13**（cp*K*a = 9.66）は、化合物 **11** と同程度の強力なリン酸塩取り込み阻害活性（IC_{50} = 33 nM）を示し、膜透過性は非常に低かった（Pe < 0.025 × 10^{-6} cm/s)。更なる塩基性の低減と tPSA、HBD、HBA の増加を指向し、PEG 側鎖末端にヒドロキシ基を導入した。合成したトリエタノールアミン体 **14** は cp*K*a が 7.59 と低かったものの、化合物 **11** と比較して 2 倍程度リン酸塩取り込み阻害活性が減弱（IC_{50} = 83 nM）した。化合物 **13** と **14** を比較すると、PEG 側鎖末端へのヒドロキシ基の導入はリン酸塩取り込み阻害活性の減弱に寄与する可能性が示唆された。それゆえ、PEG 側鎖末端にメトキシ基を導入した。更に、脂質二重膜との相互作用低減および塩基性の低減を指向して、PEG 鎖を伸長し、両性イオン基を導入した。両性イオン体 **15**

（cp*K*a＝8.37）は強力なリン酸塩取り込み阻害活性を示し（IC_{50}＝64 nM）、ほとんど膜透過性を示さなかった（Pe＜0.025×10^{-6} cm/s）。合成展開を実施した 4,5,6,7-テトラヒドロチエノピリジンおよび 4,5,6,7-テトラヒドロベンゾチオフェン誘導体の構造活性相関のまとめを **Figure 4** に示す。4,5,6,7-テトラヒドロベンゾチオフェン部位へのシクロプロピルアミノ基およびヒドロキシ基の導入、ベンズアミド *m* 位への環状および鎖状アミノ基の導入は、NaPi2b 阻害活性を減弱させた。一方で、6,6-ジメチル-4,5,6,7-テトラヒドロベンゾチオフェン骨格への変換では強力な NaPi2b 阻害活性を示し、脂溶性官能基の導入が NaPi2b 阻害活性の向上に寄与する可能性が示唆された。ベンズアミド *m* 位から伸長した PEG 鎖の末端は、ヒドロキシ基よりもメチル基やメトキシ基の方が好まれる傾向であった。

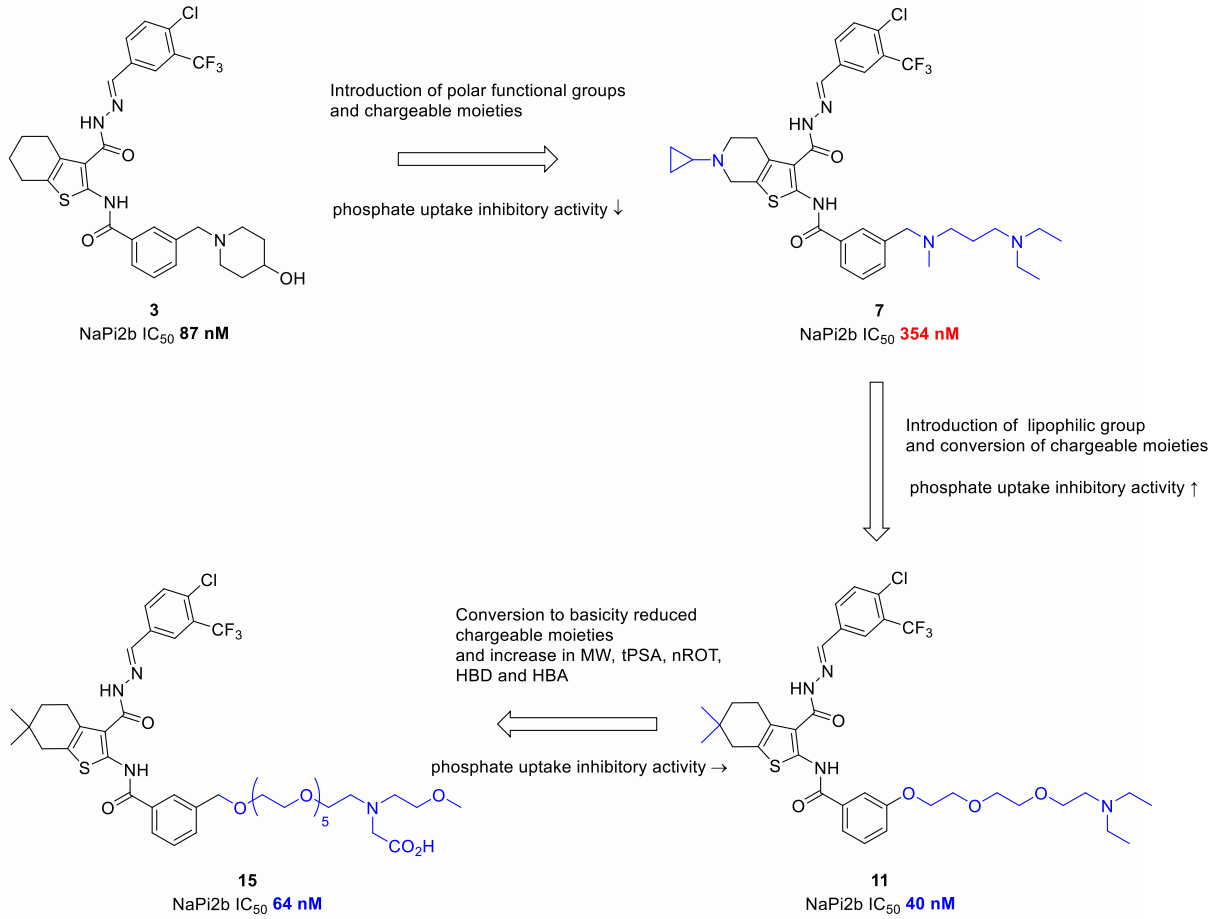


Figure 4. Structure-activity relationships of 4,5,6,7-tetrahydrothieno[2,3-c]pyridine and 4,5,6,7-tetrahydrobenzo[b]thiophene derivatives for the in vitro phosphate uptake inhibitory effect. Reprinted with permission from reference.[11] Copyright 2022 American Chemical Society

所望のリン酸塩取り込み阻害活性、溶解性、低膜透過性を示した化合物 **11** および **13**–**15** の血漿－肺移行性（$K_{p,lung}$）を評価した。cp*K*a と $K_{p,lung}$ の値を **Table 5** に示す。

Table 5. Comparison between the calculated p*K*a and $K_{p,lung}$ values for the basicity-reduced derivatives of compound 7

Compound	Calculated p*K*a[a]	$K_{p,lung}$[b]
7	10.39	4180[c]
11	9.72	130
13	9.66	428
14	7.59	44
15	8.37	-[d]

[a]The highest value of each compound calculated using ACD/Percepta, version 2017, Advanced Chemistry Development, Inc., Toronto, On, Canada, www.acdlabs.com, 2021. [b]The values were calculated using the ratio of plasma concentration to lung concentration. The values were measured 7 hours after oral administration in rats (30 mg/kg). [c]The values were measured 6 hours after oral administration in rats (30 mg/kg). [d]Incalculable. Reprinted with permission from reference.[11] Copyright 2022 American Chemical Society

期待通り、化合物 **11**（$K_{p,lung}$ = 130）、**13**（$K_{p,lung}$ = 428）、**14**（$K_{p,lung}$ = 44）の経口投与後 7 時間の $K_{p,lung}$ は、化合物 **7**（$K_{p,lung}$ = 4180）と比較して低値を示した。化合物 **15** は、経口投与後 7 時間の血漿中濃度（< 0.3 ng/mL）及び肺濃度（< 1.5 ng/g）が検出限界以下であった。

1.3　両性イオン体 **15** の薬物動態

上述の誘導体展開の結果から、強力な NaPi2b 阻害活性を示すだけでなく、最も低 $K_{p,lung}$ を示した両性イオン体 **15** を用いて、ラットにおける PK 試験を実施した。**Table 6** に示すよ

うに、30 mg/kg 経口投与後の最大血漿中濃度（C_{max}）は 0.444 ng/mL であり、バイオアベイラビリティは 0.1%と算出された。両性イオン体 **15** は、ほとんど経口吸収性を示さないと推察され、望みの動態プロファイルを示す化合物の取得に至った。

Table 6. Pharmacokinetic properties of compound 15 following 30 mg/kg oral administration in rats

Compound	Plasma conc[a,b] (ng/mL)	Lung conc[a,b] (ng/g)	C_{max}[a] (ng/mL)	$T_{1/2}$[a] (h)	$AUC_{0\rightarrow t}$[a] (ng·h/mL)	$AUC_{0\rightarrow \infty}$[a] (ng·h/mL)	MRT[a] (h)	F[a,c] (%)
15	<0.3	<1.5	0.444	3.53	0.665	2.62	5.25	0.1

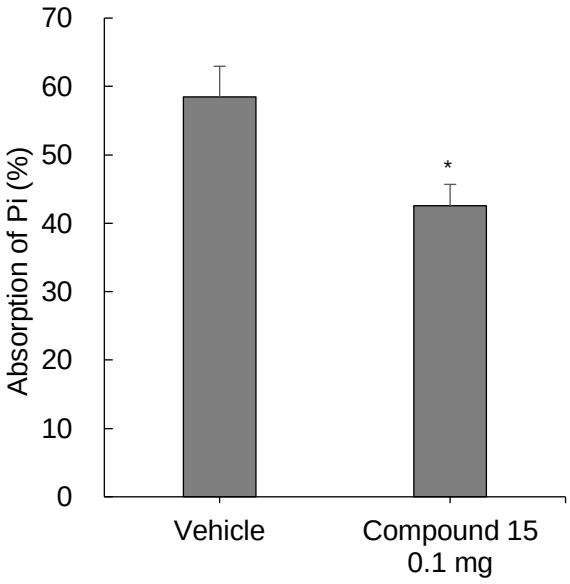
[a]The values are shown as a mean of two determinations. [b]The values were measured 7 hours after oral administration. [c]The compound was administered at a dose of 30 mg/kg (po) and 0.5 mg/kg (iv). Reprinted with permission from reference.[11] Copyright 2022 American Chemical Society

1.4　両性イオン体 **15** の腸管ループ試験

膜透過性がほとんど確認されず経口吸収性を示さない両性イオン体 **15** が、*in vivo* で NaPi2b 阻害によるリン酸塩吸収阻害作用を示すかどうか、ラット腸管ループ試験を用いて検証した。[48] 臨床で経口リン酸塩吸着剤として使用されている塩酸セベラマー（SH）を陽性対照として用いた。リンの 1 日の摂取量は 700―2000 mg と報告されており、リン酸塩として細胞膜を通過して輸送されている（31 mg/L 元素リン ＝ 1 mM リン酸塩）。[5] そこで、腸管ループ試験におけるリン酸塩濃度を生理的条件に相当すると考えられる 12.5 mM と設定した。ラットの体重に応じて、塩酸セベラマーの投与量を 1 日の臨床投与相当量（5.8 mg）と過剰量（7.8 mg）の 2 群に設定した。評価結果を **Figure 5A, B** に示す。両性イオン体 **15**（0.1 mg）を小腸内で NaPi2b が高発現しているとされる空腸に投与したところ、ビークル群と比較してリン酸塩の吸収作用を 17%阻害した。本結果は、臨床で使用されている塩酸セベラマーを投与した際のリン酸塩吸収阻害作用とほとんど同程度であった（16%, 5.8 mg;

19%, 7.8 mg)。低膜透過性で非経口吸収性を示す NaPi2b 阻害薬を用いた腸管におけるリン酸塩吸収阻害のコンセプト確認に至った。

(A)



(B)

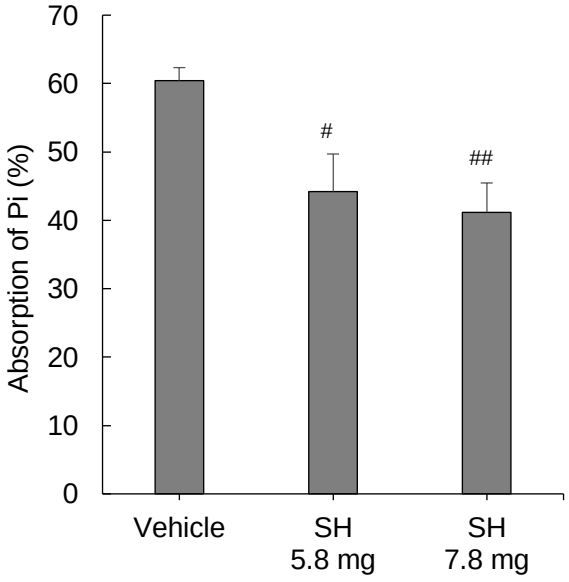


Figure 5. Inhibition of phosphate uptake in the small intestine of rats using an in situ closed-loop method after the intrajejunal administration of the 12.5 mM phosphate buffer solution (pH 6.5) with **(A)** compound **15** at a dose of 0.1 mg, and **(B)** sevelamer hydrochloride (SH) at the doses of 5.8 mg or 7.8 mg. Each value represents the mean ± S.E.M. of six rats. $^{*}p < 0.05$ comparison of the vehicle and compound **15** (Student's *t*-test). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ comparison of the vehicle and SH (Dunnett test). Reprinted with permission from reference.[11] Copyright 2022 American Chemical Society

第２章　アシルヒドラゾン構造からの脱却

2.1　アシルヒドラゾン構造を回避した誘導体展開戦略

両性イオン体 **15** はアシルヒドラゾン構造を有しており、容易に加水分解を受けアシルヒドラジドを生成する可能性がある。アシルヒドラジドは、ミクロソームの酵素により肝毒性のリスク因子となる反応性代謝物を生成しうる。[49] それゆえ、低膜透過性のため経口吸収性がほとんど無視できるほど少ないとしても、血中に分布した少量の反応性代謝物による毒性の懸念がある。両性イオン体 **15**（F = 0.1%）はほとんど経口吸収性を示さないものの、リスクの低減に向け、アシルヒドラゾン構造を回避した化合物の取得を目指した。筆者の実施してきた低膜透過性を指向した誘導体展開における構造活性相関の概要は、以下の通りである。中心部の 6,6-ジメチル-4,5,6,7-テトラヒドロベンゾチオフェン骨格および上部に位置する 4-クロロ-3-トリフルオロメチルフェニル基に代表される脂溶性置換基は、強力なリン酸塩吸収阻害活性を示す為に必要であると考えられる。ベンズアミド *m* 位から伸長する PEG 側鎖への極性官能基や電荷を有する官能基の導入は、低膜透過化に有効である。[11] 以上を踏まえ、変換にあたり中心部の 2-アミノチオフェンのアミノ部位とアシルヒドラゾンのカルボニル部位との相互作用を環状化により代替すること、または類似の置換基を導入して維持することはリン酸塩吸収阻害活性を示す為に必要と考え、デザインした。非アシルヒドラゾン構造で NaPi2b 阻害活性を示す誘導体取得に向けた研究戦略を **Figure 6** に示す。両性イオン体 **15** のアシルヒドラゾン構造を有する 2-アミノチオフェン-3-カルボヒドラジド部位を、チエノオキサジン-4-オン（環化体）および 2-アミノチオフェン-3-カルボキサミド（アニリド体）へ変換することとした。

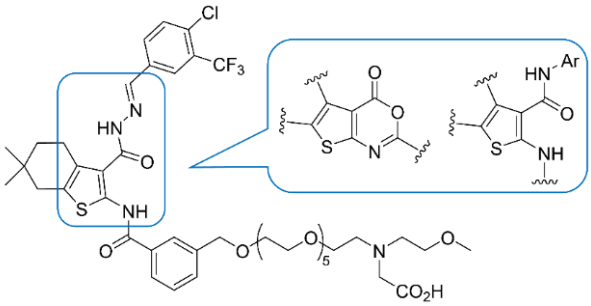
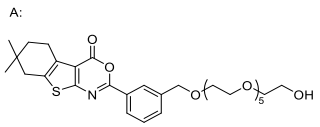


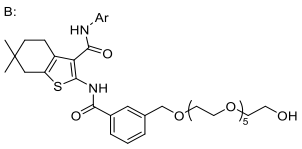
Figure 6. Synthetic strategies to obtain the derivatives of non-acylhydrazone structures.

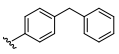
2.2　非アシルヒドラゾン構造であるアニリド誘導体の取得

Table 7 に三環性チエノオキサジン-4-オンおよびアニリド誘導体の構造活性相関を示す。三環性化合物 **16**（IC_{50} = 2881 nM）は、両性イオン体 **15** と比較してリン酸塩取り込み阻害活性が減弱した。続いて、アシルヒドラゾン構造の代替としてアニリド構造への変換を実施した。フェニル体 **17**（IC_{50} = 1404 nM）およびメトキシフェニル体 **18**（IC_{50} = 1140 nM）ではリン酸塩取り込み阻害活性が減弱し、フェニルアセトアミドおよびメトキシフェニルアセトアミドへの変換許容性は低いと考えられた。一方で、エチルフェニル体 **19**、ベンジルフェニル体 **20** は強力なリン酸塩取り込み阻害活性を示した（IC_{50} < 100 nM）。以上の結果から、NaPi2b との結合を促進するには、アニリド誘導体の上部ベンゼン環部位から伸長した箇所に、芳香族のような脂溶性置換基が必要であることが示唆された。

Table 7. Structure–activity relationship of tricyclic thieno[2,3-d][1,3]oxazin-4-one (A) and anilide conversion of 2-aminothiophene-3-carboxamide (B) derivatives



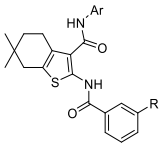


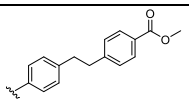
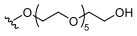
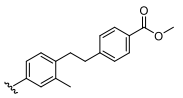
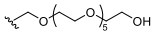
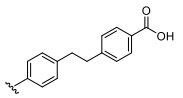
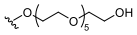
Compound	Structure	Ar	NaPi2b IC_{50}[a] (nM)	Sol.[b] (μM)
16	A		2881	36
17	B		1404	81
18	B		1140	2.3
19	B		<100	0.39
20	B		<100	0.48

[a]Half-maximal (50%) inhibitory concentration against $H_2[^{33}P]O_4$ uptake in human NaPi2b-transfected KJMGER8 cells. The data are presented as the mean value of three determinations. [b]Solubility in phosphate-buffered saline at pH 7.4.

続いて、アニリド構造を有する誘導体の上部ベンゼン環側鎖を伸長した。**Table 8** に構造活性相関を示す。期待通り、メチル 4-フェネチルベンゾエイト体 **21** およびメチル 4-(2-メチルフェネチル)ベンゾエイト体 **22** は、強力なリン酸塩取り込み阻害活性を示した（IC_{50} < 100 nM）。しかしながら、これらの化合物は低溶解性であった（**19**－**22,** < 1.6 μM in PBS at pH 7.4）。それゆえ、上部ベンゼン環の末端に極性官能基を導入し、溶解性向上を試みた。4-フェネチルカルボン酸体 **23**（IC_{50} < 100 nM）は強力なリン酸塩取り込み阻害活性を有し、期待通りに高溶解性を示した（115 μM in PBS）。アニリド構造において、極性官能基の導入が溶解性の改善に有効である可能性が示唆された。

Table 8. Structure–activity relationship of anilide derivatives with elongated side chains



Compound	Ar	R	NaPi2b IC_{50}[a] (nM)	Sol.[b] (μM)
21			<100	1.6
22			<100	0.72
23			<100	115

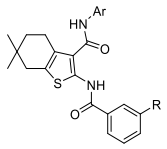
[a]Half-maximal (50%) inhibitory concentration against $H_2[^{33}P]O_4$ uptake in human NaPi2b-transfected KJMGER8 cells. The data are presented as the mean value of three determinations. [b]Solubility in phosphate-buffered saline at pH 7.4.

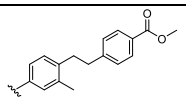
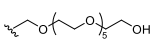
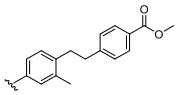
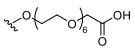
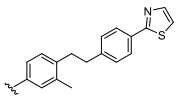
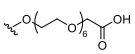
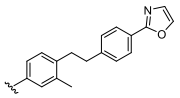
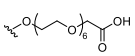
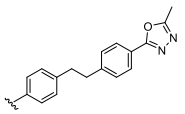
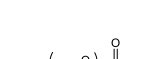
2.3　上部ベンゼン環へのヘテロ環導入による構造活性相関

アニリド部位（Ar）の末端ベンゼン環にエステル基およびヘテロ環、ベンズアミド *m* 位に PEG 側鎖（R）を有する誘導体の構造活性相関を **Table 9** に示す。化合物 **23** の溶解性向上の結果を受けて、極性官能基を PEG 側鎖の末端に導入した。期待通りに、PEG 側鎖の末端にカルボン酸を有するメチルエステル体 **24** は高溶解性を示し（116 μM in PBS）、強力なリン酸塩取り込み阻害活性を示した（IC_{50} = 71 nM）。強力なリン酸塩取り込み阻害活性かつ高溶解性を示す誘導体の取得に向けて、PEG 側鎖の末端へのカルボン酸の導入が有効である可能性が示唆された。メチルエステル体のバイオアイソスターとして、チアゾール体 **25**、オキサゾール体 **26**、1,3,4-オキサジアゾール体 **27** を合成した。一連の化合物はメチルエステル体 **24** と比較して、同程度から 4 倍程度の活性減弱を示した（**25**: IC_{50} = 288 nM; **26**: IC_{50} = 43 nM; and **27**: IC_{50} = 103 nM）。以上の結果から、アニリド誘導体の上部に位置する末端ベ

ンゼン環のメチルエステル基を環化した安定なバイオアイソスターへの変換は許容された。

Table 9. Structure–activity relationship of derivatives with a heterocyclic ring and a PEG-carboxylic acid side chain at the *m*-position of the benzamide moiety



Compound	Ar	R	NaPi2b IC_{50}[a] (nM)	Sol.[b] (μM)
22			<100	0.72
24			71	116
25			288	87
26			43	79
27			103	62

[a]Half-maximal (50%) inhibitory concentration against $H_2[^{33}P]O_4$ uptake in human NaPi2b-transfected KJMGER8 cells. The data are presented as the mean value of three determinations. [b]Solubility in phosphate-buffered saline at pH 7.4.

2.4　両性イオン体 **30** の創製

メチルエステル基を環化したバイオアイソスターへの変換が許容されたことから、1,3,4-オキサジアゾール体 **27** を用いてベンズアミド *m* 位から伸長する PEG 側鎖（R）の変換を実施した。**Table 10** に 1,3,4-オキサジアゾール誘導体の構造活性相関を示す。PEG 側鎖を伸長した化合物 **28**（IC_{50} = 24 nM）、短縮した化合物 **29**（IC_{50} = 61 nM）において、化合物 **27**（IC_{50} = 103 nM）と比較して 1.5–4 倍程度のリン酸塩取り込み阻害活性の向上が確認された。これ

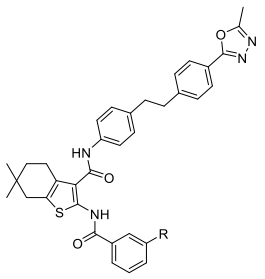
らの結果は、更なる PEG 側鎖の変換を後押しするものとなった。

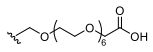
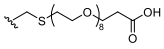
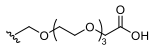
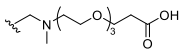
筆者の先行研究の結果から、両性イオン体 **15**（IC_{50} = 64 nM）は強力なリン酸塩取り込み阻害活性かつ非常に低い経口吸収性（F = 0.1%）を示していた。[11] それゆえ、強力なリン酸塩取り込み阻害活性と低膜透過性の両立を期待し、化合物 **27** の PEG 側鎖に両性イオン基を導入した。化合物 **27** と比較して、両性イオン体 **30**（IC_{50} = 14 nM）は、7 倍程度リン酸塩取り込み阻害活性が向上し、かつ高溶解性（96 μM in PBS）を示した。加えて、期待通りに低膜透過性を示した（$Pe < 0.090 \times 10^{-6}$ cm/s）。

非アシルヒドラゾン構造で強力な NaPi2b 阻害活性を示す誘導体取得を目指した化合物展開のまとめを以下に示す。三環性チエノオキサジン-4-オンは NaPi2b 阻害活性が大幅に減弱した。アニリド誘導体では、フェニルアセトアミド体やメトキシフェニルアセトアミド体において NaPi2b 阻害活性が大幅に減弱した一方で、上部ベンゼン環の側鎖を伸長することで強力な NaPi2b 阻害活性を示した。これらの結果を踏まえると、NaPi2b との結合を促進するには、芳香族のような脂溶性官能基を上部ベンゼン環を伸長した箇所に導入することが望ましいと推察された。上部ベンゼン環の末端エステル基のバイオアイソスターや、ベンズアミド *m* 位から伸長する PEG 鎖の許容性は比較的広く、複数の誘導体で強力な NaPi2b 阻害活性を保持した。

強力なリン酸塩取り込み阻害活性かつ低膜透過性を示した両性イオン体 **30** を用いて、PK 試験を実施した。ラットへの経口投与において低バイオアベイラビリティを示し（FaFg = 5.9%、**Table 11**）、ほとんど経口吸収性のない化合物であった。以上の結果から、筆者の目指す腸管局所のみで薬理作用を示すコンセプトに合致するプロファイルを有する非アシルヒドラゾン構造の誘導体を取得するに至った。

Table 10. Structure–activity relationship of 1,3,4-oxadiazole derivatives



Compound	R	NaPi2b IC_{50}[a] (nM)	Sol.[b] (μM)
27		103	62
28		24	91
29		61	156
30		14	96

[a]Half-maximal (50%) inhibitory concentration against $H_2[^{33}P]O_4$ uptake in human NaPi2b-transfected KJMGER8 cells. The data are presented as the mean value of three determinations. [b]Solubility in phosphate-buffered saline at pH 7.4.

Table 11. Intestinal availability (FaFg) of compound 30 following oral administration in rats

Compound	Dose (mg/kg)	$AUC_{0\rightarrow\infty}$[a] (ng·h/mL)	FaFg (%)
30	3	30.0	5.85[b]
Antipyrine	1	171	

AUC, area under the curve

[a]Values are presented as the mean value of three determinations by oral cassette dosing. [b]Values were calculated by comparing with the Fa value of antipyrine as 100%.

2.5　両性イオン体 **30** を用いたラットにおけるリン酸塩吸収阻害作用評価

強力なリン酸塩取り込み阻害活性かつ高溶解性を示し、経口吸収性も無視できるほど低い両性イオン体 **30** を用いて、ラットにおけるリン酸塩吸収阻害作用を評価した。臨床で有効性を示す経口リン酸塩吸着剤である炭酸ランタンを陽性対照として使用した。本試験においては、リン酸塩濃度 10 mM を生理的なリン酸塩濃度として、リン酸塩濃度 100 mM を高濃度条件と設定して評価した。炭酸ランタンの投与量はラットの体重換算から二つ設定した。一つは臨床相当投与量である 10 mg/kg、もう一つは高投与量の 20 mg/kg とした。炭酸ランタンの投与量低減が可能であるかどうかを検証するため、炭酸ランタンと両性イオン体 **30** の併用投与も実施した。

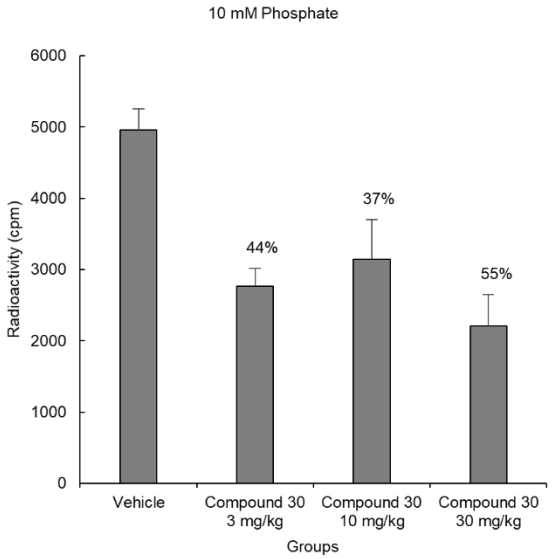
被試験化合物を経口投与後、ラットの尾静脈から血液を回収し、血清 100 μL 中の放射活性を評価した。評価結果を **Figure 7A, B, C** に示す。10 mM リン酸塩条件下では、ビークル群と比較して、化合物 **30** は 44%（3 mg/kg, p.o.）、37%（10 mg/kg, p.o.）、55%（30 mg/kg, p.o.）のリン酸塩吸収阻害作用を示した（**Figure 7A**）。一方で、100 mM リン酸塩条件下では、ビークル群と比較して、11%（3 mg/kg, p.o.）、14%（10 mg/kg, p.o.）と両性イオン体 **30** によるリン酸塩吸収阻害作用が低減した（**Figure 7B**）。

本結果から、両性イオン体 **30** は、腸管の NaPi2b 阻害によるリン酸塩吸収阻害作用を示すものの、高濃度のリン酸塩条件下では NaPi2b 阻害作用が低減する可能性が示唆された。言い換えると、リン酸塩濃度 10 mM 条件下では NaPi2b を介した経細胞輸送が優勢であると考えられる一方で、リン酸塩濃度 100 mM 条件下では傍細胞輸送が優勢であると推察された。これらの結果は、高リン酸塩濃度条件下では傍細胞輸送がリン酸塩吸収の大部分を占めるという報告と一致していた。[50-52]

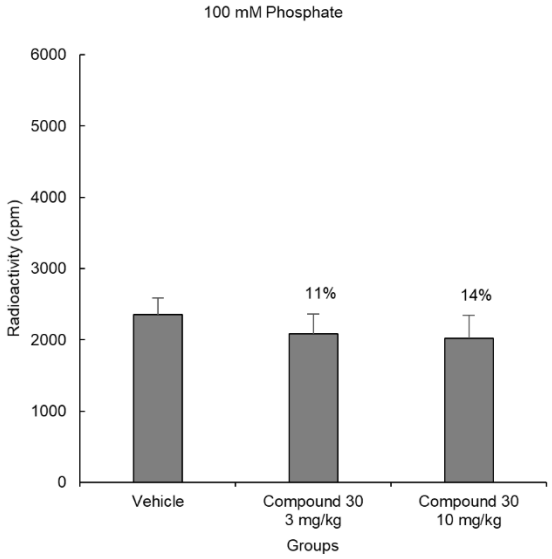
続いて、炭酸ランタンとの比較および、炭酸ランタンと両性イオン体 **30** の併用試験を実施した（**Figure 7c**）。10 mM リン酸塩条件下において、ビークル群と比較して、両性イオン体 **30**（10 mg/kg, p.o.）は炭酸ランタン（10 mg/kg, p.o.）と同等のリン酸塩吸収阻害作用（両

性イオン体 **30**: 23%, 炭酸ランタン: 20%）を示した。両性イオン体 **30** は、臨床で使用されている治療薬と同等の薬理作用を発揮する可能性が示唆された。高用量の炭酸ランタン投与（20 mg/kg, p.o.）においては、更なるリン酸塩吸収阻害作用（62%）を示し、本評価系における炭酸ランタンの用量依存性が示唆された。両性イオン体 **30**（10 mg/kg, p.o.）と炭酸ランタン（10 mg/kg, p.o.）の併用条件下では、ビークル群と比較して 51%のリン酸塩吸収阻害作用が確認された。これらの結果から、両性イオン体 **30** と炭酸ランタンとの併用によるリン酸塩吸収阻害作用の上乗せ効果が示唆された。[53]

(A)


10 mM Phosphate
Radioactivity (cpm)
6000
5000
4000
3000
2000
1000
0
44%
37%
55%
Vehicle
Compound 30
3 mg/kg
Compound 30
10 mg/kg
Compound 30
30 mg/kg
Groups

(B)


100 mM Phosphate
Radioactivity (cpm)
6000
5000
4000
3000
2000
1000
0
11%
14%
Vehicle
Compound 30
3 mg/kg
Compound 30
10 mg/kg
Groups

(C)

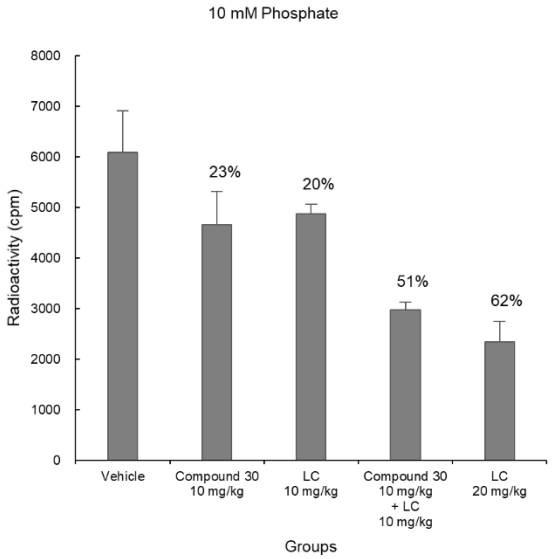


Figure 7. Measurement of [^{32}P]-phosphate in the phosphate absorption inhibition assay in rats after oral administration of 10 mM and 100 mM phosphate buffer solution (pH 6.4) with (**A**) compound **30** (doses of 3, 10, and 30 mg/kg), (**B**) compound **30** at doses of 3 and 10 mg/kg, and (**C**) compound **30** (10 mg/kg), lanthanum carbonate (LC; doses of 10 and 20 mg/kg), and the combination of compound **30** (10 mg/kg) and LC (10 mg/kg). For (**A**) and (**B**), the data are presented as mean ± standard error of the mean (SEM) measurement from five rats. For (**C**), the data are presented as mean ± SEM measurement from four rats, and the test was conducted twice independently. The number at the top of each bar indicates the reduction percentage of phosphate absorption when compared with the vehicle group.

第３章　化合物の合成

3.1　化合物 **4**–**15** の合成

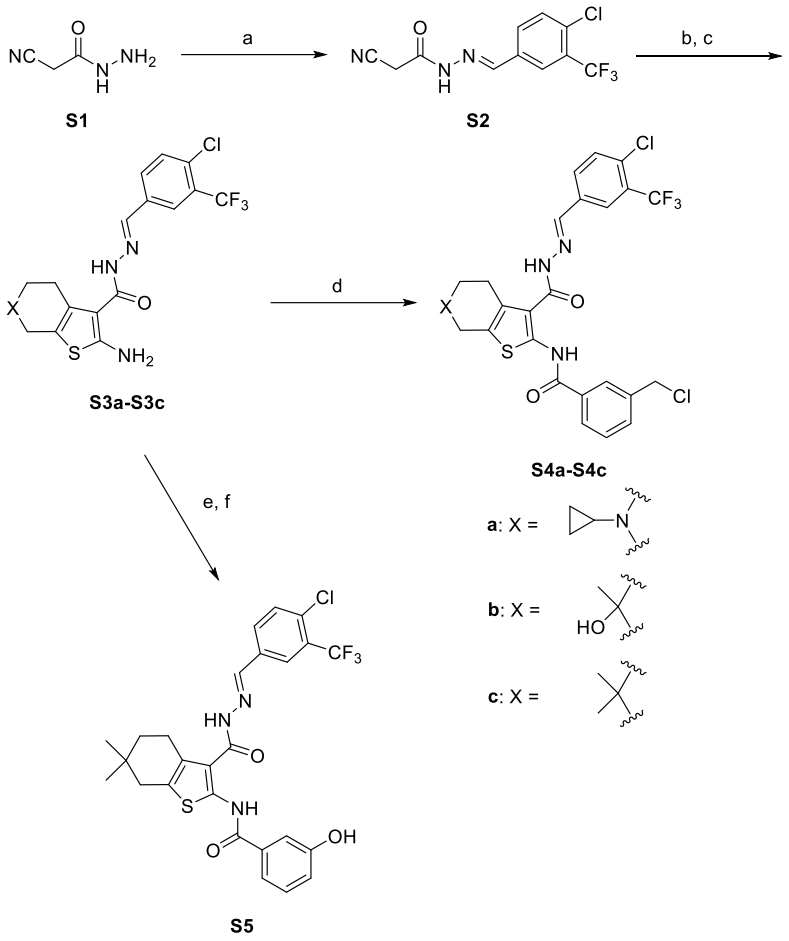
化合物 **4**–**8** は、*m*-クロロメチルベンズアミド誘導体 **S4a** および **S4b** と対応する第二級アミンを用いてそれぞれ合成した（**Schemes 1**, **2**）。

化合物 **9**–**11**, **13** および **14** は、*m*-ヒドロキシベンズアミド誘導体 **S5** を用いて合成した（**Schemes 3**, **4**）。アルコール体 **9** は、2-(2-(2-クロロエトキシ)エトキシ)エタノールと *m*-ヒドロキシベンズアミド誘導体 **S5** を用いて合成した。アルコール体 **9** は化合物 **11, 13** および **14** の合成中間体としても用いた。ヨウ素化体 **S8** は、アルコール体 **9** のメシル体と続くヨウ素化を経て取得し、ジエチルアミンと反応させることでジエチルアミノ体 **11** を得た。アルコール体 **9** と(S)-エピクロロヒドリンの反応によりエポキシド体 **S9** を取得し、アミノ化により化合物 **13** および **14** を合成した。フェノール体 **S5** と PEG 体 **S7** を用いて、*tert*-ブチルエステル体 **S10** を取得し、TFA で処理することでカルボン酸体 **10** を取得した。

化合物 **12** は、2-アミノ-4,5,6,7-テトラヒドロチエノピリジン誘導体 **S3a** と対応するカルボン酸体 **S12** を用いて合成した（**Schemes 5**, **6**）。

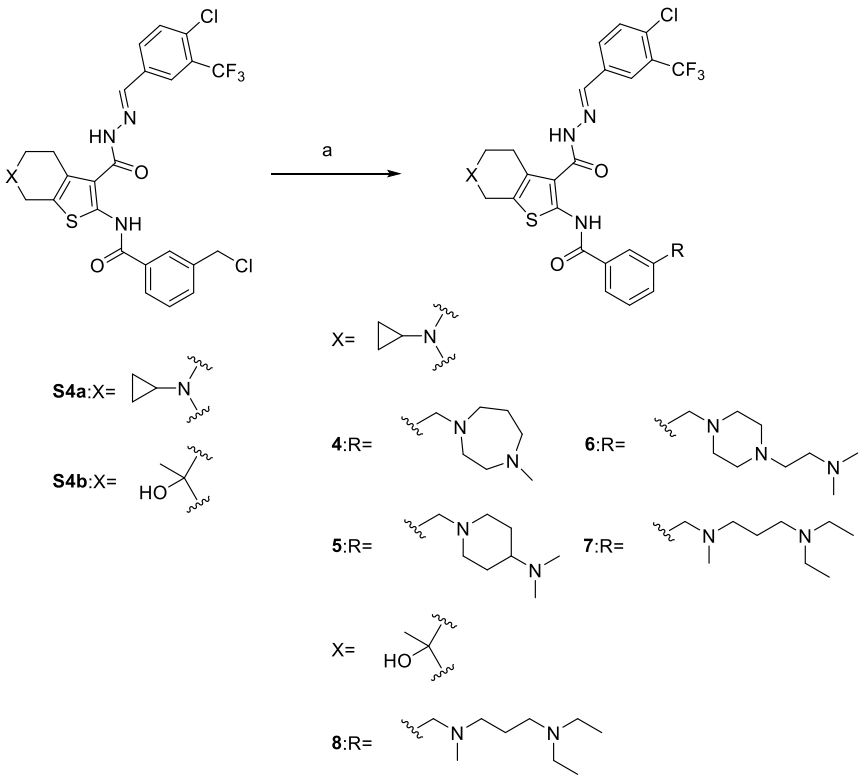
化合物 **15** は、*m*-クロロメチルベンズアミド誘導体 **S4c** から合成した（**Schemes 7**, **8**）。**S4c** とヘキサエチレングリコール、NaH との反応によりアルコール体 **S15** を取得し、メシル体 **S16** へと導いた。両性イオン体 **15** は、メシル体 **S16** へのグリシン誘導体 **S14** の S_N2 置換反応、続く加水分解により取得した。

Scheme 1. Synthesis of *m*-chloromethyl benzamide and *m*-hydroxy benzamide derivatives (S4a–S4c, and S5)[a]



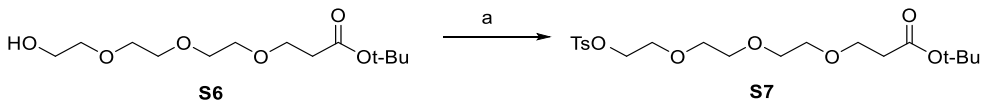
[a]Reagents and conditions: (a) 4-chloro-3-(trifluoromethyl)benzaldehyde, EtOAc, 50 °C, 96%; (b) 1-cyclopropylpiperidin-4-one (for **S3a**), 4-hydroxy-4-methylcyclohexanone (for **S3b**) or 4,4-dimethylcyclohexan-1-one (for **S3c**), HMDS, AcOH, THF, rt; (c) sulfur, TEA, THF, rt, 19−53% over 2 steps; (d) 3-(chloromethyl)benzoyl chloride, py., DCE (for **S4a**) or DCM (for **S4b** and **S4c**), rt, 84−91%; (e) (*i*) 3-acetoxybenzoic acid, $SOCl_2$, DMF, DCM, rt; (*ii*) **S3c**, py., DCM, rt; (f) K_2CO_3, MeOH, reflux, 87% over 2 steps.

Scheme 2. Synthesis of 6-cyclopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine and 6-hydroxy-6-methyl-4,5,6,7-tetrahydrobenzo[b]thiophene derivatives with a substituent at the *m*-position of benzamide[a]



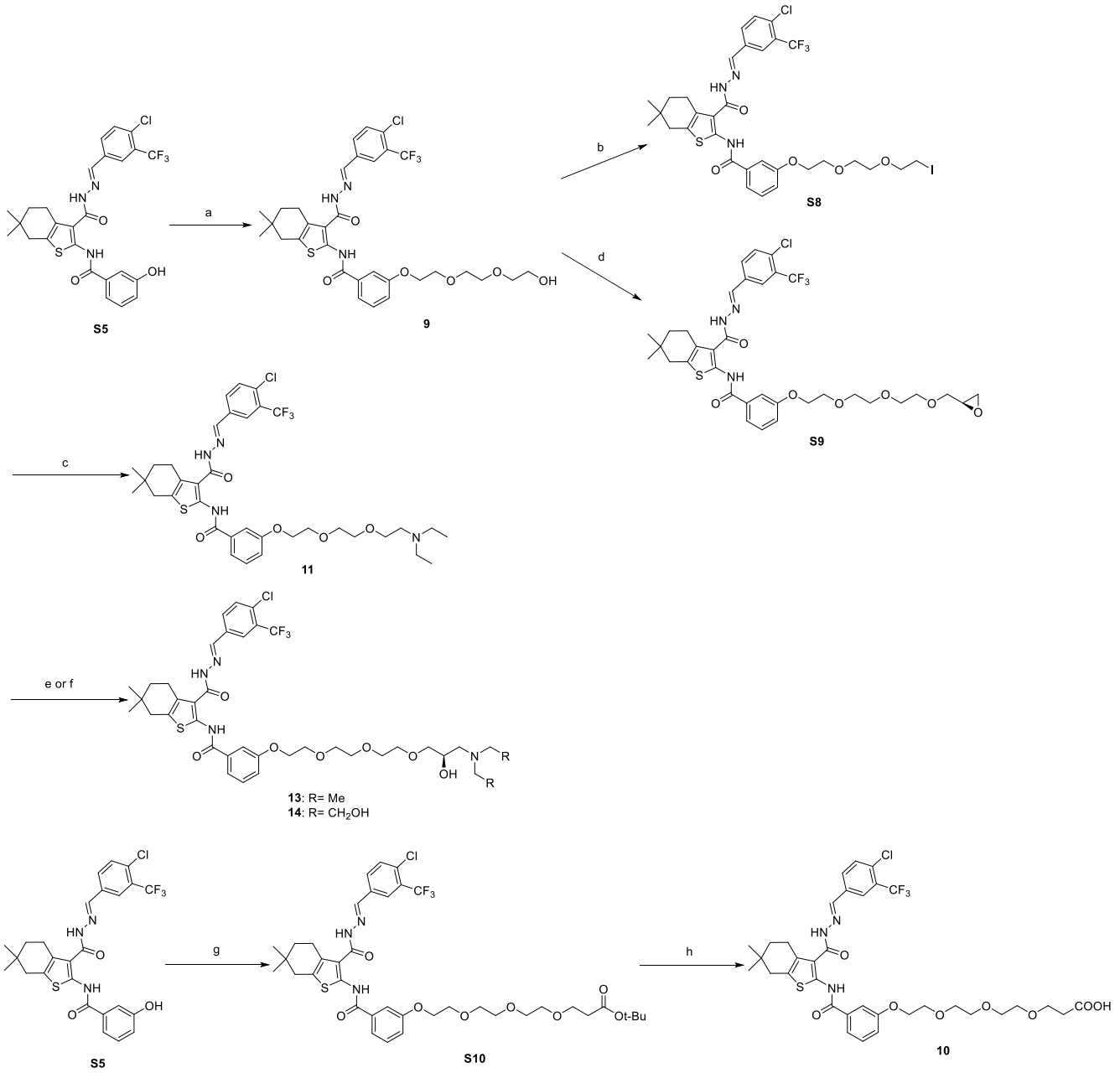
[a]Reagents and conditions: (a) corresponding secondary amine, TEA, THF, rt, 49−94%.

Scheme 3. Synthesis of S7[a]



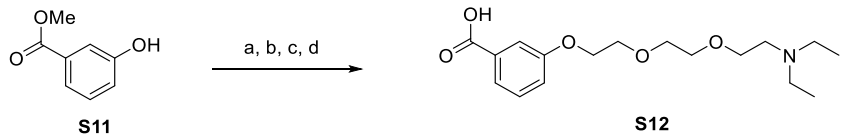
[a]Reagents and conditions: (a) *p*-TsCl, TEA, DCM, rt, 98%.

Scheme 4. Synthesis of 6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophen derivatives with a substituent at the *m*-position of benzamide[a]



[a]Reagents and conditions: (a) 2-(2-(2-chloroethoxy)ethoxy)ethanol, K_2CO_3, DMF, 100 °C, 54%; (b) (*i*) MsCl, TEA, DMF, rt; (*ii*) NaI, 100 °C, 74%; (c) diethylamine, K_2CO_3, DMF, 100 °C, 87%; (d) NaH, (*S*)-epichlorohydrin, DMF, rt, 29%; (e) diethylamine, THF, rt, 18% (for **13**); (f) 2,2'-azanediyldiethanol, IPA, 70 °C, 50% (for **14**); (g) *tert*-butyl 3-(2-(2-(2-(tosyloxy)ethoxy)ethoxy)ethoxy)propanoate (**S7**), K_2CO_3, NaI, DMF, 100 °C, 64%; (h) TFA, DCM, rt, 32%.

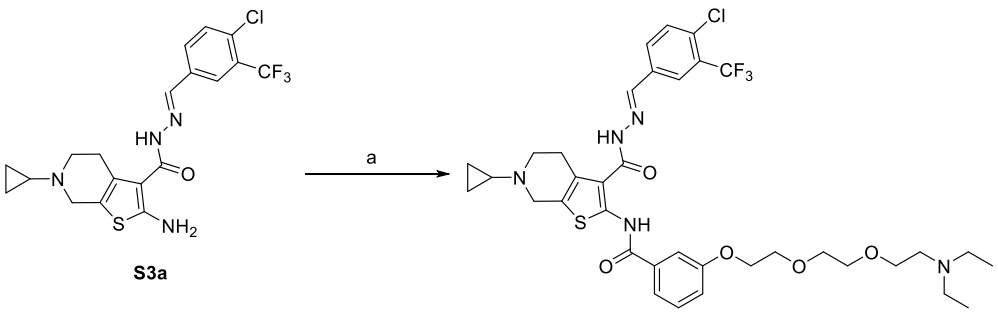
Scheme 5. Synthesis of S12[a]



S11 **S12**

[a]Reagents and conditions: (a) potassium carbonate, 2-(2-(2-chloroethoxy)ethoxy)ethanol, DMF, 100 °C; (b) (*i*) MsCl, TEA, DMF, rt,; (*ii*) NaI, 100 °C, 42% over 2 steps; (c) K_2CO_3, diethylamine, DMF, 100 °C; (d) lithium hydroxide monohydrate, 50% aqueous EtOH, rt, 74% over 2 steps.

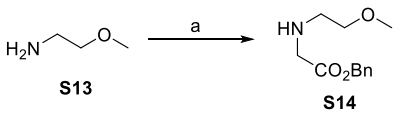
Scheme 6. Synthesis of 6-cyclopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine derivative with a PEG side chain[a]



S3a **12**

[a]Reagents and conditions: (a) (*i*) 3-(2-(2-(2-(diethylamino)ethoxy)ethoxy)ethoxy)benzoic acid (**S12**), $SOCl_2$, DMF, DCM, rt,; (*ii*) py., DCM, rt, 20%.

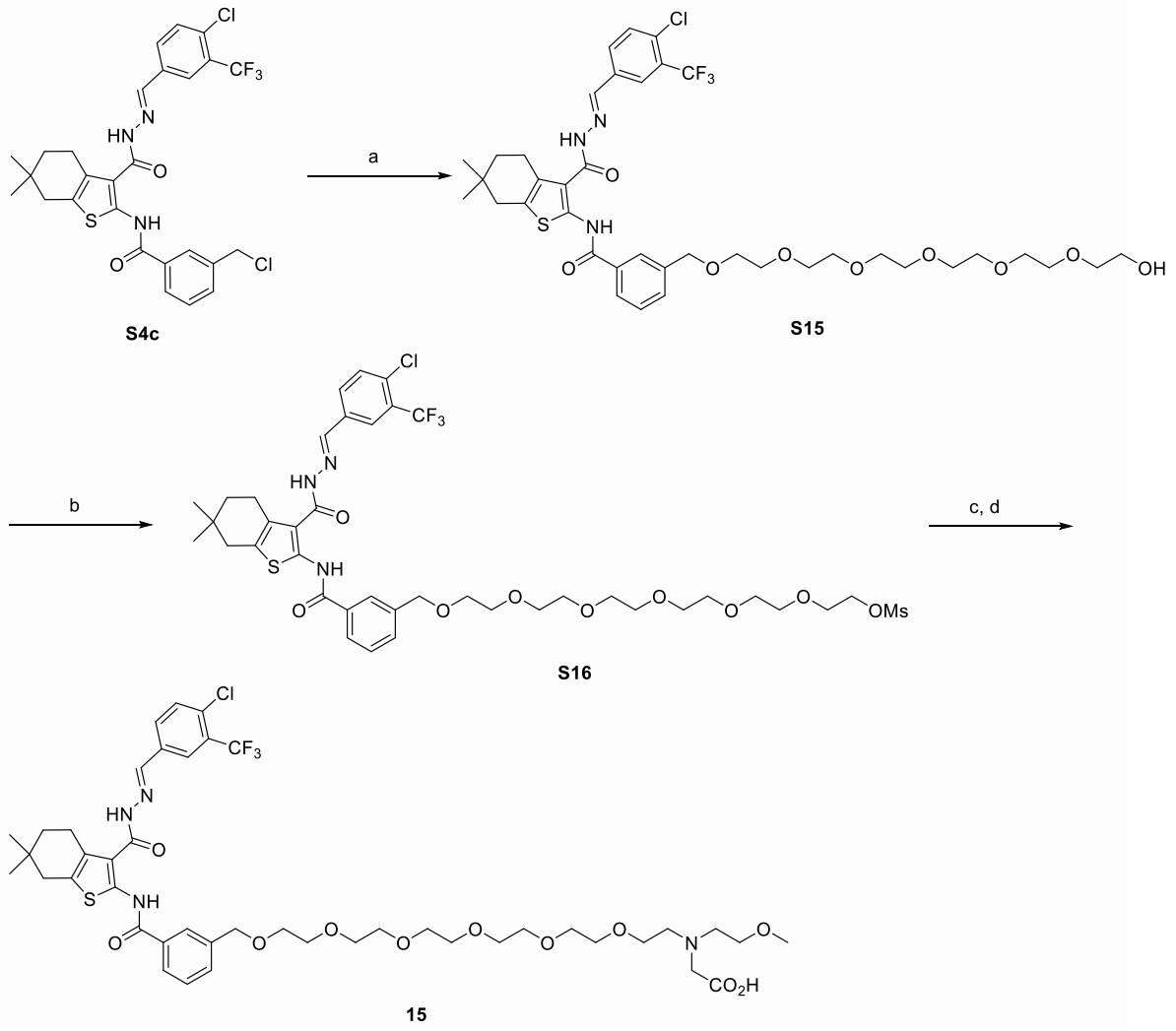
Scheme 7. Synthesis of S14[a]



S13 **S14**

[a]Reagents and conditions: (a) benzyl 2-bromoacetate, DCM, 0 ℃, 90%.

Scheme 8. Synthesis of zwitterionic compound 15[a]



[a]Reagents and conditions: (a) hexaethylene glycol, NaH, DMF, rt, 79%; (b) MsCl, TEA, DCM, rt, 79%; (c) benzyl (2-methoxyethyl)glycinate (**S14**), DMA, 120 °C, microwave; (d) 1M NaOH aqueous solution, EtOH, rt, 23% over 2 steps.

3.2　化合物 **16**–**30** の合成

三環性化合物 **16** は、*m*-クロロメチルベンズアミド誘導体 **S19** および市販のヘキサエチレングリコールと NaH との反応、続く TFAA と TFA との反応により取得した。アニリド誘導体 **17**–**20** および **22** は、三環性化合物 **16** と対応するアニリノ体との反応で取得した (**Schemes**

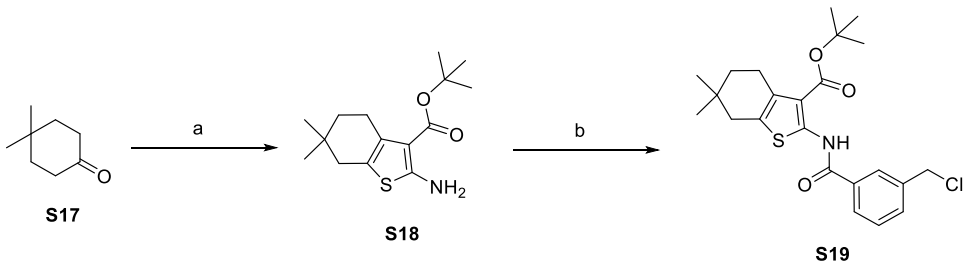
9–**11**)。

化合物 **21** および **23** は、化合物 **S25** を用いて合成した(**Schemes 12**–**14**)。メチルエステル体 **21** は、化合物 **S25** と 3-アセトキシ安息香酸、塩化チオニル、および DMF を用いたアミド化、続く加水分解によりフェノール化合物を取得後、PEG 体 **S27** と炭酸セシウムと反応することで得た。カルボン酸体 **23** は、メチルエステル体 **21** の加水分解により取得した。

化合物 **24**–**26** は、三環性誘導体 **S31** と対応するアニリノ体との反応により合成した (**Schemes 15**–**17**)。

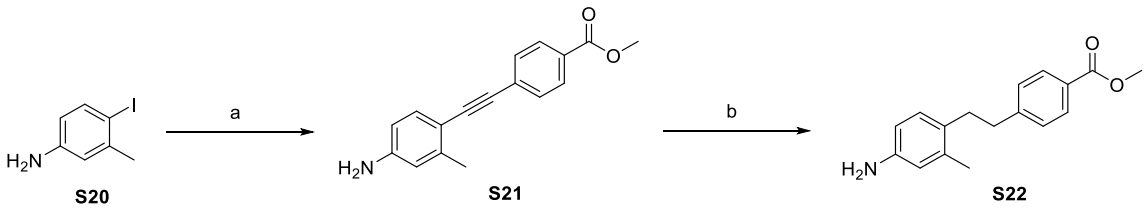
1,3,4-オキサジアゾール誘導体 **27**–**30** は、*m*-クロロメチルベンズアミド誘導体 **S42** を用いて取得した (**Schemes 13**, **18**–**20**)。化合物 **S42** と PEG 体 **S29** および PEG 体 **S44** と NaH との反応により化合物 **27** と **29** をそれぞれ取得した。化合物 **28** は、化合物 **S42** と両末端にメルカプト基およびカルボン酸を有する市販の PEG 体および炭酸セシウムを用いて合成した。両性イオン体 **30** は、化合物 **S42** と両末端にアミノ基および *tert*-ブチルエステル基を有する市販の PEG 体、テトラエチルアンモニウムブロミド、およびフッ化カリウムを用いてアミノ体を取得し、ホルムアルデヒドを用いた還元的アミノ化によりメチル化したのち、TFA と反応させることで合成した。

Scheme 9. Synthesis of compound S19[a]



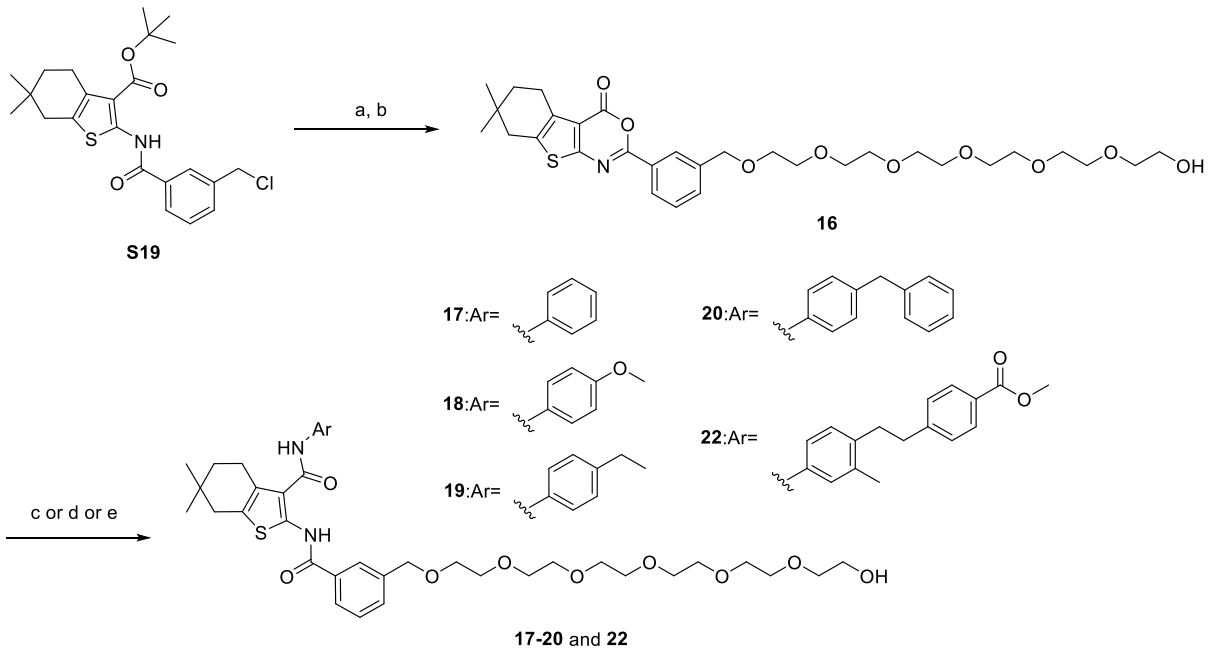
[a]Reagents and conditions: (a) *tert*-butyl 2-cyanoacetate, sulfur, ethylenediamine, AcOH, DMF, rt, quant.; (b) 3-(chloromethyl)benzoyl chloride, py., DCM, rt, 86%.

Scheme 10. Synthesis of compound S22[a]



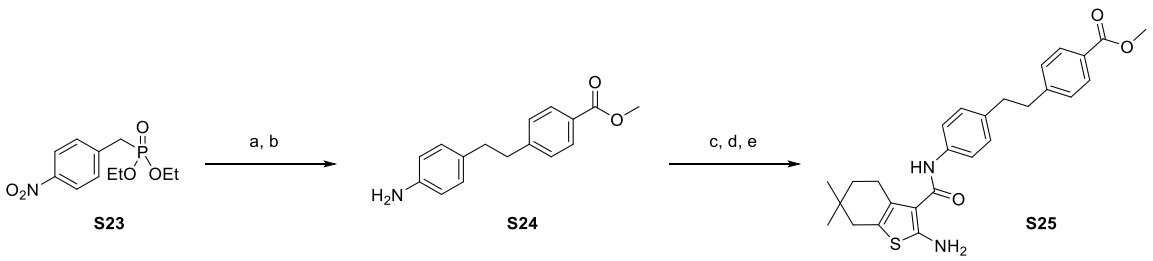
[a]Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, CuI, PPh_3, methyl 4-ethynylbenzoate, diethylamine, DMF, 120 °C, 50%; (b) H_2, $Pd(OH)_2/C$, EtOH, 40 °C, 94%.

Scheme 11. Synthesis of tricyclic compound 16 and anilide compounds 17–20 and 22[a]



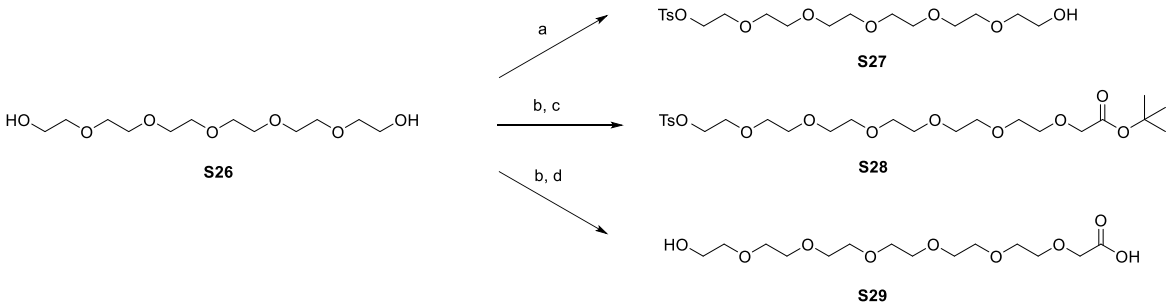
[a]Reagents and conditions: (a) hexaethylene glycol, NaH, DMF, rt; (b) TFAA, TFA, rt, 27% over 2 steps; (c) (*i*) corresponding aniline derivative, pivalic acid, 120 °C, 47% (for compound **22**); (*ii*) 1 M NaOH aqueous solution, MeOH, 50 °C, 67% (for compound **17**), 70 °C, 49% (for compound **18**); (d) 4-ethylaniline, sodium acetate, AcOH, 90 °C, 43% (for compound **19**); (e) (*i*) 4-benzylaniline, sodium acetate, AcOH, 90 °C; (*ii*) potassium carbonate, MeOH, rt, 21% (for compound **20**).

Scheme 12. Synthesis of compound S25[a]



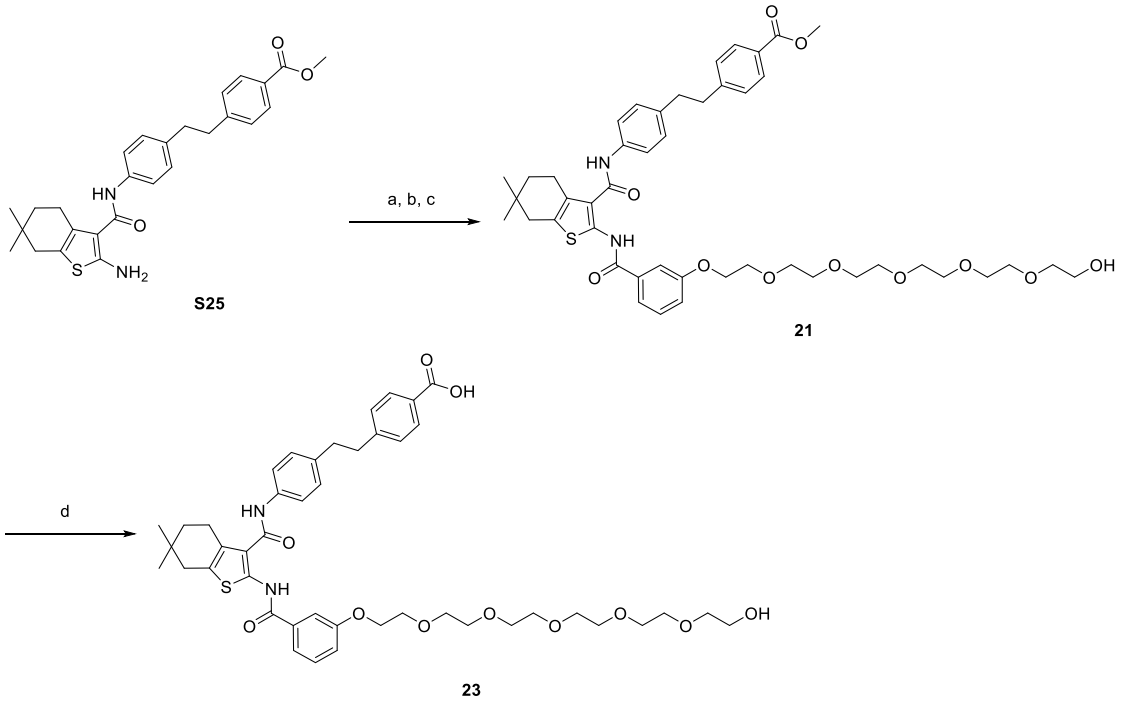
[a]Reagents and conditions: (a) 28% sodium methoxide-MeOH solution, methyl 4-formylbenzoate, MeOH, rt; (b) H_2, Pd/C, THF, DMF, 35 ℃, 94% over 2 steps; (c) 2-cyanoacetic acid, HOBt monohydrate, WSC・HCl, DMF, rt; (d) AcOH, HMDS, 4,4-dimethylcyclohexanone, THF, rt; (e) morpholine, sulfur, EtOH, reflux, 34% over 3 steps.

Scheme 13. Synthesis of compounds S27–S29[a]



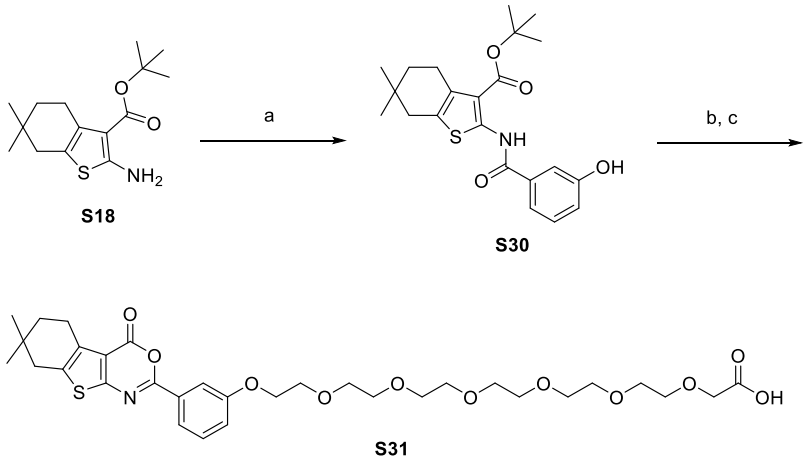
[a]Reagents and conditions: (a) TsCl, Ag_2O, potassium iodide, DCM, 0 ℃, 85%; (b) *tert*-butyl 2-bromoacetate, potassium carbonate, NaOH, THF, rt; (c) TsCl, TEA, DCM, rt, 32% over 2 steps; (d) TFA, DCM, rt, 50% over 2 steps.

Scheme 14. Synthesis of phenethylphenyl acetamide compounds 21 and 23[a]



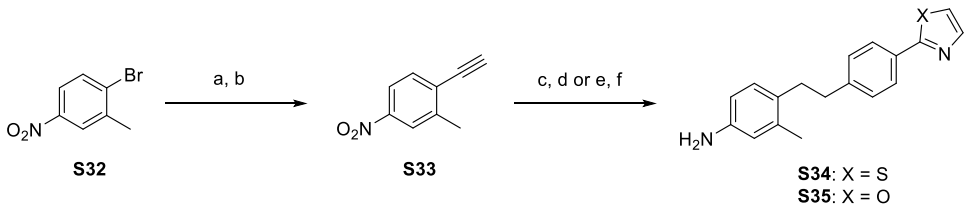
[a]Reagents and conditions: (a) (*i*) 3-acetoxybenzoic acid, thionyl chloride, DMF, $CHCl_3$, 50 °C; (*ii*) **S25**, py., $CHCl_3$, rt; (b) 1 M NaOH aqueous solution, MeOH, 0 °C; (c) 17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (**S27**), cesium carbonate, DMF, 70 °C, 92% over 3 steps; (d) 1 M NaOH aqueous solution, MeOH, 70 °C, quant..

Scheme 15. Synthesis of compound S31[a]



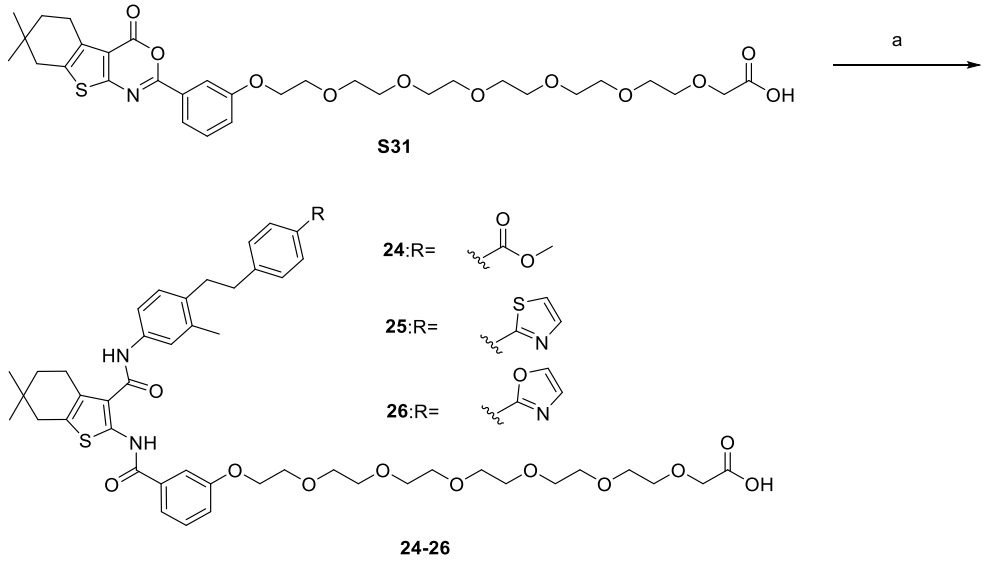
[a]Reagents and conditions: (a) (*i*) 3-hydroxybenzoic acid, thionyl chloride, DMF, DCM, 45 °C; (*ii*) **S18**, py., DCM, rt; (*iii*) 1 M NaOH aqueous solution, MeOH, rt, 66%; (b) *tert*-butyl 20-(tosyloxy)-3,6,9,12,15,18-hexaoxaicosanoate (**S28**), cesium carbonate, DMF, 85 °C; (c) TFA, TFAA, rt, 68% over 2 steps.

Scheme 16. Synthesis of compounds S34 and S35[a]



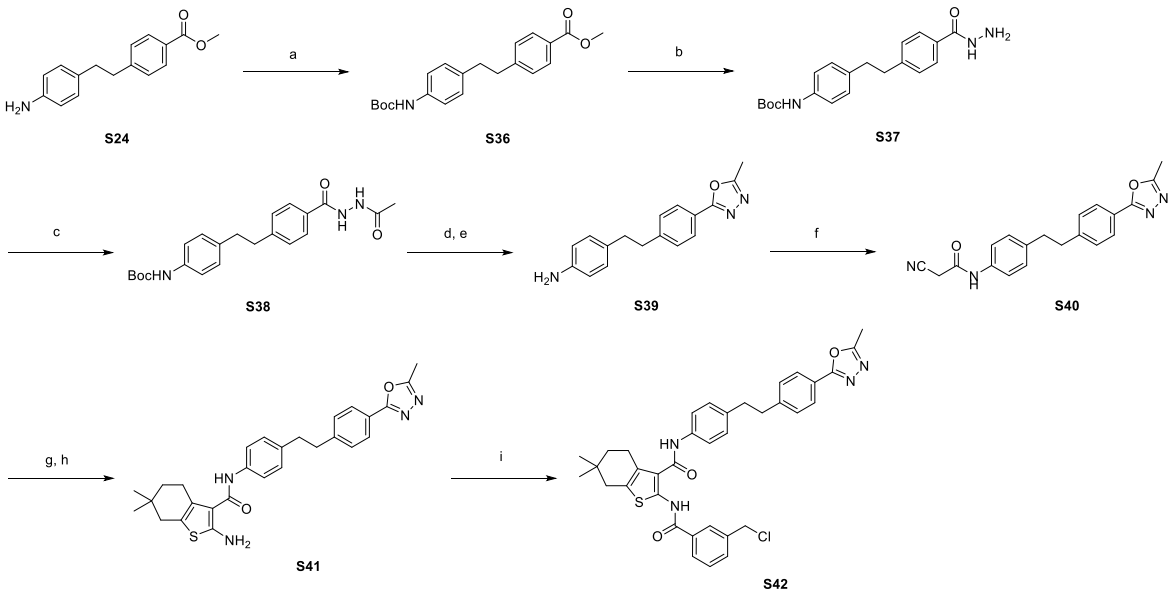
[a]Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, CuI, TEA, trimethylsilylacetylene, DMF, rt; (b) potassium carbonate, DCM, MeOH, rt, 97% over 2 steps; (c) $PdCl_2(PPh_3)_2$, CuI, TEA, PPh_3, 2-(4-bromophenyl)thiazole, DMF, 120 °C; (d) H_2, Pd/C, THF, rt, 25% over 2 steps; (e) $PdCl_2(PPh_3)_2$, CuI, TEA, PPh_3, 2-(4-bromophenyl)oxazole, DMF, 70 °C; (f) H_2, $Pd(OH)_2$/C, THF, 40 °C, 15% over 2 steps.

Scheme 17. Synthesis of phenethylphenyl acetamide derivatives (compounds 24–26) with a poly(ethylene glycol) carboxylic acid side chain[a]



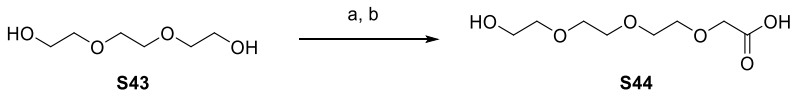
[a]Reagents and conditions: (a) pivalic acid, corresponding aniline derivative, 120 °C, 16−57%.

Scheme 18. Synthesis of compound S42[a]



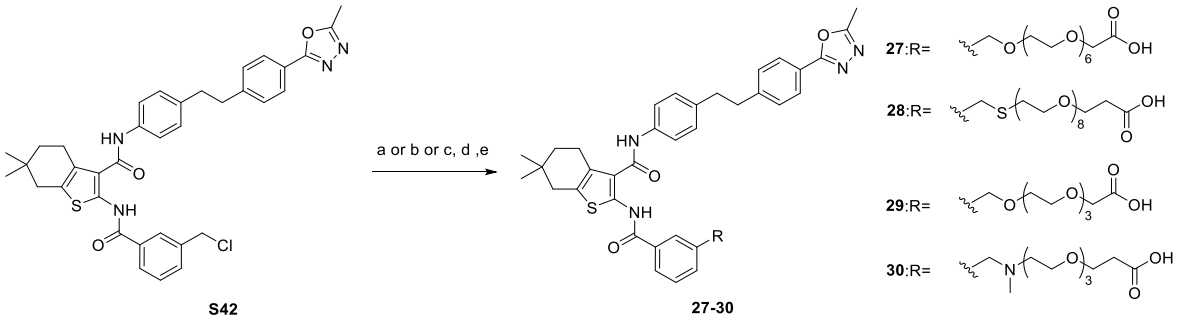
[a]Reagents and conditions: (a) $(Boc)_2O$, DIPEA, THF, rt, 73%; (b) hydrazine monohydrate, EtOH, THF, reflux, 95%; (c) AcCl, py., DCM, rt, quant.; (d) thionyl chloride, toluene, reflux; (e) TFA, DCM, rt, 71% over 2 steps; (f) 2-cyanoacetic acid, HOBt monohydrate, WSC·HCl, DMF, rt, 64%; (g) 4,4-dimethylcyclohexanone, AcOH, HMDS, THF, reflux; (h) sulfur, morpholine, EtOH, reflux, 62% over 2 steps; (i) 3-(chloromethyl)benzoyl chloride, py., DCM, rt, 93%.

Scheme 19. Synthesis of compound S44[a]



[a]Reagents and conditions: (a) *tert*-butyl 2-bromoacetate, potassium carbonate, NaOH, THF, rt; (b) TFA, DCM, rt, 17% over 2 steps.

Scheme 20. Synthesis of 1,3,4-oxadiazole phenethylphenyl acetamide compounds 27–30[a]



[a]Reagents and conditions: (a) NaH, 20-hydroxy-3,6,9,12,15,18-hexaoxaicosanoic acid (**S29**) (for compound **27**) or 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)acetic acid (**S44**) (for compound **29**), DMF, rt, 20−62%; (b) cesium carbonate, 1-mercapto-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid, DMF, 70 °C, 40% (for compound **28**); (c) tetraethylammonium bromide, potassium fluoride on celite, *tert*-butyl 3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propanoate, propionitrile, reflux; (d) 37% formaldehyde solution, $NaBH(OAc)_3$, DCM, and MeOH, rt; (e) TFA, DCM, rt, 14% over 3 steps (for compound **30**).

結論

筆者は、ヒット化合物 **3** をプロトタイプ化合物とし、Ro5 を意識的に脱却する戦略により低膜透過性の獲得を目指した。極性官能基および電荷を有する官能基を導入した新規な 4,5,6,7-テトラヒドロチエノピリジンおよび 6,6-ジメチル-4,5,6,7-テトラヒドロベンゾチオフェン骨格をデザインし、合成した。

検討の結果、中程度の NaPi2b 阻害活性を示し、低膜透過性かつ低バイオアベイラビリティを示すリード化合物 **7** を取得した。しかしながら、化合物 **7** は肺移行性が高く、長期投与による肺への蓄積性の懸念が持たれた。リード化合物 **7** の最適化研究を実施し、MW、tPSA、nROT および HBA を増加させるべく、ベンズアミド *m* 位に非塩基性で電荷を有する PEG 側鎖を導入した。加えて、塩基性の低下による肺移行性の低減を目指し、p*K*a 計算値を指標とした化合物展開も実施した。本展開で得られた両性イオン体 **15** は、強力な NaPi2b 阻害活性と非常に低い膜透過性を示し、低肺移行性であった。PK 試験の結果から、両性イオン体 **15** は経口投与により腸管からほとんど吸収されず、腸管局所のみで活性を示す可能性が示唆された。ラット腸管ループ試験において、両性イオン体 **15** はビークル群と比較してリン酸塩吸収阻害作用を示し、その効果は塩酸セベラマーの臨床用量と同程度であった。一方で、両性イオン体 **15** はアシルヒドラゾン構造を有していることから、毒性の懸念を回避すべく非アシルヒドラゾン誘導体の取得に着手した。

種々検討の結果、強力なリン酸塩取り込み阻害活性かつ低膜透過性を示す化合物として、アニリド構造を有する両性イオン体 **30** を取得した。両性イオン体 **30** は、ラットへの経口投与試験において低バイオアベイラビリティを示した。ラットを用いた *in vivo* 薬理試験において、両性イオン体 **30** は、リン酸塩濃度 10 mM 条件下で腸管におけるリン酸塩吸収阻害作用を示した。本結果は、炭酸ランタンの臨床用量と同等のリン酸塩吸収阻害作用であった。一方で、リン酸塩濃度 100 mM 条件下ではリン酸塩吸収阻害作用は確認されなかった。これらの結果から、リン酸塩の経細胞輸送は低濃度条件下で優勢であることが示唆された。両性

イオン体 **30** と炭酸ランタンの併用試験では、リン酸塩吸収阻害機序の異なる薬剤の組み合わせによるリン酸塩吸収阻害作用の上乗せ効果が確認された。本結果から、併用療法における炭酸ランタンの服薬量低減の可能性が示唆された。

以上より、筆者は低膜透過性で非腸管吸収性を示し、腸管局所で強力なリン酸塩吸収阻害作用を有する新規な高リン血症治療薬の候補化合物 **30** を創製することに成功した。両性イオン体 **30** は、単剤で炭酸ランタンの臨床用量と同等のリン酸塩吸収阻害作用を示し、併用療法において炭酸ランタンの服薬量低減の可能性が示唆された。加えて、併用療法による炭酸ランタンの服薬量低減に伴う下痢や便秘などの経口リン酸塩吸着剤に関連する消化管の副作用の低減も期待され、新たな治療薬としての展開が期待される。

試験方法

Phosphate uptake measurements in human NaPi2b-transfected KJMGER8 cells.

The assay cells were established by transfecting human NaPi2b into KJMGER8 cells.[40] The transfected cells were maintained in a serum-free RPMI 1640–ITPSG medium as previously described.[54] The RPMI 1640–ITPSG medium was prepared by adding 0.188% $NaHCO_3$, 6 mmol/L l-glutamine, 10 mmol/L HEPES (pH 7.0), 3 mg/L insulin, 5 mg/L transferrin, 5 mmol/L sodium pyruvate, 125 nmol/L sodium selenite, 1 mg/ml galactose, 100 U/mL penicillin–streptomycin, and 2 μg/mL blasticidin S to RPMI 1640 medium. After treatment with 10 nmol/L 17β-estradiol for 24 hours in phosphate-free DMEM-ITPSG medium, the cells were seeded in 96-well plates with the following assay buffer: 115 mmol/L NaCl, 5.4 mmol/L KCl, 0.8 mmol/L $MgCl_2$ $6H_2O$, 1.8 mmol/L $CaCl_2$, and 10 mmol/L HEPES pH 7.0. The test compounds were treated for 60 minutes at room temperature. The uptake reaction was performed by adding 5 μCi/mL $H_2[^{33}P]O_4$ for 30 min at room temperature and stopped by washing three times with ice-cold assay buffer through a Whatman GF/B unifilter. The radioactivity was measured by TopCount (Perkin-Elmer Japan).

PAMPA method and analysis.

The assay was carried out using a BD Gentest™ Pre-coated PAMPA Plate System, as previously described.[55] In brief, the compound solutions were prepared by diluting 50 mM MES (pH 6.0), to afford a final concentration of 100 μM. The compound solutions were added to the wells (350 μL/well)

of the donor plate, and 50 mM HEPES (pH 7.4) was added to the wells (200 µL/well) of the acceptor plate. The acceptor plate was then coupled with the donor plate, and the plate assembly was incubated at 37°C for 5 h. At the end of the incubation, the plates were separated and the solution from each well was collected. The concentration of the compounds was measured by LC-UV.

Method for measuring the solubility of compounds.

Solubility was measured in PBS (pH 7.4) in the presence of 1% DMSO solvent.

Animals.

All animal studies were performed in accordance with the Standards for Proper Conduct of Animal Experiments at Kyowa Kirin Co., Ltd. under the approval of the company's Institutional Animal Care and Use Committee.

Prediction of distribution of compounds in the lungs.

The test compounds were suspended with 0.5 w/v% methyl cellulose 400 to afford a 6 mg/mL administration solution. Male Crl:CD (SD) rats at 8 weeks of age (Charles River Laboratories Japan, Inc., Kanagawa, Japan) were orally administered with 5 mL/kg of the test compounds. Under isoflurane anesthesia, blood was collected from the portal vein and inferior vena cava after 6 or 7 hours dosing. The rats were sacrificed, and their lungs were collected. The homogenate lung samples were prepared by homogenizing the collected lungs with water. The blood and homogenate lung samples were centrifuged and used as analysis samples. The concentration of analysis samples was determined

by LC/MS/MS compared to IS. The $K_{p,lung}$ values were calculated as a ratio of plasma concertation to lung concentration.

Pharmacokinetics analysis of compound 15 in rats.

Male Crl:CD (SD) rats at 7 or 8 weeks of age (Charles River Laboratories Japan, Inc., Kanagawa, Japan) were used. The test compounds were administered orally (10 or 30 mg/kg, suspension in 0.5% methyl cellulose 400) or intravenously (0.5 or 1 mg/kg in DMA) to rats under fed conditions. After administration, blood samples were collected and centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with ice-cold acetonitrile containing IS. The pretreated samples were analyzed with the calibration curve sample by LC/MS/MS. The compound concentrations were determined from the peak area ratios of the test compounds to the IS using the calibration curve.

Rat intestinal loop assay of compound 15.

Male Crl:CD (SD) rats at 7 weeks of age (Charles River Laboratories Japan, Inc., Kanagawa, Japan) were used. The rats were kept at 19–25°C and 30–70% humidity under a 12-h light–dark cycle and given free access to tap water and commercial chow (FR-2; Funabashi Farm, Chiba, Japan) before the experiments. Intestinal closed loop procedures were performed, as previously described.[56] In brief, male Crl:CD (SD) rats fasted for 24 hours were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). An abdominal incision was carefully made to expose the 10 cm length of the jejunum. The intestinal segment was injected with 1 mL of a prewarmed (37°C) injection solution of compound **15**

or sevelamer hydrochloride (SH) in 0.5 w/v% methyl cellulose and 12.5 mmol/L phosphate solution (pH 6.5), and the ends of the segment were ligated. The intestinal segment was held inside the body for 30 minutes during the absorption experiments using a heating plate (37°C). After 30 min, the luminal solution in the loop was collected, and the loop was rinsed with saline. The concentration of phosphate in the luminal solution was measured, and the absorption rate of phosphate was calculated by the following formula:

Absorption of Pi (%) = (administered amount−remaining amount)/administered amount ×100

Statistical analysis was performed using Statistical Analysis Software (SAS, release 9.4; SAS Institute Japan Ltd.). The difference between the vehicle group and the compound **15** group (**Figure 5A**) was assessed by Student's *t*-test, and the difference between the vehicle group and SH group (**Figure 5B**) was assessed by the Dunnett test. A difference was considered statistically significant at $p < 0.05$.

Intestinal availability of compound 30 in rats.

Intestinal availability (FaFg) of compound **30** in rats was determined according to the modified Hoffman method.[57] Compound **30** (3 mg/kg) was orally administered concomitantly with reference compounds (antipyrine, atenolol, and nadolol at 1 mg/kg) by cassette dosing to rats. Blood samples were simultaneously collected from portal and postcaval veins at 10, 20, 30, 45, 60, 90, and 120 min after oral administration. The compounds of interest were determined using LC/MS/MS. The area

under the curve ($AUC_{0-\infty}$) values of the compounds were calculated using differential concentrations between plasma from the portal and postcaval veins. A ratio of the dose-corrected $AUC_{0-\infty}$ value of compound **30** to that of antipyrine, which was completely absorbed from the gastrointestinal tract and whose Fa was considered 100%, was obtained as the FaFg of compound **30**.

Phosphate absorption inhibition assay of compound 30 and LC in rats.

Six-week-old male Crl:CD (Sprague-Dawley [SD]) rats (Charles River Laboratories Japan, Inc., Kanagawa, Japan) were used for this assay. The rats were kept at 19°C–25°C and 30%–70% humidity under a 12-h light–dark cycle and were given free access to tap water and 0.2% phosphate-containing feed before the experiments. Briefly, male Crl:CD (SD) rats were maintained under fasting for 24 h. Compound **30** or LC was suspended in 0.5 w/v% methyl cellulose solution and orally administered at a dose of 5 mL/kg. After 5 min, 10 mL/kg of 10 or 100 mmol/L phosphate buffer solution (pH 6.4) containing [^{32}P]-phosphate ($H_3[^{32}P]O_4$) was orally administered to the rats. After 15 min, a 400 μL blood sample was collected from the tail vein of the treated rats, and radioactivity was measured in 100 μL of serum. [^{32}P]-phosphate concentration and [^{32}P]-phosphate absorption inhibition rate were calculated.

実験項

General methods.

Unless otherwise indicated, all reagents and solvents were purchased from commercial sources and used without further purification. Moisture- or oxygen-sensitive reactions were conducted under an atmosphere of argon or nitrogen gas. Microwave reactions were conducted using Biotage Initiator 2.5. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 or 400 MHz using JNM-AL300, JNM-AL400, Bruker Ascend 400 or instruments operating at the indicated frequencies. Chemical shifts were reported in δ values (ppm) relative to trimethylsilane. The following abbreviations are used: br = broad signal, s = singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet. Purification by silica gel column chromatography was carried out using MORITEX systems (MORITEX Corporation) with prepacked cartridges (Yamazen Hi-Flash Column Silica gel or Wako Presep Silica Gel). Liquid chromatography with tandem mass spectrometry (LC-MS) analyses were carried out using Waters Micromass ZQ (electrospray ionization [ESI]) (Nihon Waters K.K., Japan). High-resolution (HR)-LC-MS was conducted using SYNAPT G2 (ESI) (Nihon Waters K.K., Japan). Chemical purities were assessed by high-performance liquid chromatography (HPLC) at an ultraviolet wavelength of 254 nm.

HPLC analyses of compounds **4** to **15** were performed under the following conditions, and the purity of the final compounds was confirmed to be >95% unless otherwise stated.

Method A: Waters ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 × 50 mm); linear mobile phase gradient of 10%−90% B over 7 min, hold for 4 min at 90% B (mobile phase A, 0.1% TFA in water; mobile phase B, acetonitrile); 40 °C column temperature; 0.4 mL/min flow rate; photodiode array detection (254 nm).

Method B: Waters ACQUITY UPLC BEH C18 column (1.7 μm, 2.1×75 mm); linear mobile phase gradient of 10%−90% B over 7 min, hold for 4 min at 90% B (mobile phase A, 0.1% TFA in water; mobile phase B, acetonitrile); 40 °C column temperature; 0.4 mL/min flow rate; photodiode array detection (254 nm).

Method C: Waters ACQUITY UPLC BEH C18 column (1.7 μm, 2.1×75 mm); linear mobile phase gradient of 50%−90% B over 7 min, hold for 4 min at 90% B (mobile phase A, 0.1% TFA in water; mobile phase B, acetonitrile); 40 °C column temperature; 0.4 mL/min flow rate; photodiode array detection (254 nm).

(E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6-cyclopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-3-((4-methyl-1,4-diazepan-1-yl)methyl)benzamide **(4).** To a solution of **S4a** (0.047 g, 0.079 mmol) in THF (0.79 mL) were added 1-methyl-1,4-diazepane (0.029 mL, 0.237 mmol) and TEA (0.017 mL, 0.118 mmol), and the solution was stirred at room temperature overnight. A saturated aqueous $NaHCO_3$ solution was added to the reaction mixture and

extracted twice with $CHCl_3$. The combined organic layer was washed with the saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by amino silica gel column chromatography (100:0 to 90:10 $CHCl_3$/MeOH) to afford **4** (0.026 g, 49%) as an amorphous compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.55–0.60 (m, 4H), 1.79–1.87 (m, 2H), 1.89–1.96 (m, 1H), 2.36 (s, 3H), 2.63–2.76 (m, 8H), 2.98–3.05 (m, 4H), 3.73 (s, 2H), 3.81 (s, 2H), 7.45 (t, J = 7.5 Hz, 1H), 7.56–7.60 (m, 2H), 7.91 (d, J = 7.5 Hz, 1H), 7.95 (dd, J = 8.4, 1.8 Hz, 1H), 8.01 (s, 1H), 8.04 (d, J = 1.8 Hz, 1H), 8.20 (s, 1H). The protons of amide were not observed. ^{13}C NMR (100 MHz, $CDCl_3$): δ 6.4, 27.5, 29.7, 37.5, 46.7, 50.3, 51.5, 54.3, 56.5, 58.1, 62.4, 112.1, 122.5 (q, J = 272.0 Hz), 124.9, 126.1, 126.5, 126.8 (q, J = 5.1 Hz), 128.0, 128.9, 129.1 (q, J = 31.4 Hz), 131.2, 132.1, 132.4, 133.1, 134.4, 140.8, 145.3, 149.0, 164.0. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 673.2339; found, 673.2383. HPLC: purity 96%. R_T: 4.69 min (method A).

***(E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6-cyclopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-3-((4-(dimethylamino)piperidin-1-yl)methyl)benzamide* (5).** To a solution of **S4a** (0.050 g, 0.084 mmol) in THF (0.84 mL) were added *N*,*N*-dimethylpiperidin-4-amine (0.032 g, 0.251 mmol) and TEA (0.017 mL, 0.125 mmol), and the solution was stirred at room temperature overnight. A saturated aqueous $NaHCO_3$ solution was added to the reaction mixture and extracted twice with $CHCl_3$. The combined organic layer was washed with the saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The

residue was purified by amino silica gel column chromatography (100:0 to 90:10 $CHCl_3$/MeOH) to afford **5** (0.035 g, 60%) as an amorphous compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.55–0.59 (m, 4H), 1.50–1.61 (m, 2H), 1.76–1.80 (m, 2H), 1.92–2.00 (m, 3H), 2.10–2.18 (m, 1H), 2.27 (s, 6H), 2.90–3.05 (m, 6H), 3.57 (s, 2H), 3.81 (s, 2H), 7.45 (t, J = 7.5 Hz, 1H), 7.56–7.58 (m, 2H), 7.91–7.97 (m, 3H), 8.04 (s, 1H), 8.18 (s, 1H). The protons of amide were not observed. ^{13}C NMR (100 MHz, $CDCl_3$): δ 6.4, 27.5, 28.2, 37.5, 41.6, 50.3, 51.6, 53.0, 62.3, 62.5, 112.0, 122.5 (q, J = 271.2 Hz), 124.8, 126.0, 126.5, 126.8 (q, J = 5.1 Hz), 128.4, 128.8, 129.1 (q, J = 31.4 Hz), 131.2, 132.0, 132.1, 132.4, 133.3, 134.4, 139.9, 145.3, 149.0, 163.0, 164.0. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 687.2496; found, 687.2507. HPLC: purity 98%. R_T: 4.78 min (method B).

***(E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6-cyclopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-3-((4-(2-(dimethylamino)ethyl)piperazin-1-yl)methyl)benzamide* (6).** To a solution of **S4a** (0.054 g, 0.090 mmol) in THF (0.90 mL) were added *N*,*N*-dimethy-2-(piperazin-1-yl)ethanamine (0.042 g, 0.270 mmol) and TEA (0.019 mL, 0.135 mmol), and the solution was stirred at room temperature for 4 h. A saturated aqueous $NaHCO_3$ solution was added to the reaction mixture and extracted twice with $CHCl_3$. The combined organic layer was washed with the saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by amino silica gel column chromatography (100:0 to 80:20 $CHCl_3$/MeOH) to afford **6** (0.034 g, 54%) as an amorphous compound. ^{1}H NMR (300 MHz, $CDCl_3$):

δ 0.54–0.59 (m, 4H), 1.88–1.95 (m, 1H), 2.27 (s, 6H), 2.45–2.51 (m, 12H), 2.98–3.04 (m, 4H), 3.59 (s, 2H), 3.80 (s, 2H), 7.45 (t, J = 7.7Hz, 1H), 7.55–7.58 (m, 2H), 7.91 (d, J = 7.7 Hz, 1H), 7.95 (dd, J = 8.4, 1.8 Hz, 1H), 7.99 (s, 1H), 8.03 (d, J = 1.8 Hz, 1H), 8.21 (s, 1H). The protons of amide were not observed. ^{13}C NMR (100 MHz, $CDCl_3$): δ 6.4, 27.5, 37.5, 45.8, 50.3, 51.6, 53.0, 53.6, 56.6, 56.8, 62.5, 112.0, 122.5 (q, J = 272.0 Hz), 124.8, 126.1, 126.6, 126.8 (q, J = 5.1 Hz), 128.5, 128.8, 129.1 (q, J = 31.4 Hz), 131.2, 132.10, 132.12, 132.4, 133.4, 134.4, 139.5, 145.3, 149.0, 163.0, 164.0. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 716.2761; found, 716.2820. HPLC: purity 95%. R_T: 4.93 min (method B).

(E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6-cyclopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-3-(((3-(diethylamino)propyl)(methyl)amino)methyl)benzamide **(7).** To a solution of **S4a** (1.15 g, 1.93 mmol) in THF (20 mL) were added *N*,*N*-diethyl-*N'*-methyl-1,3-propanediamine (0.33 g, 2.32 mmol) and TEA (0.40 mL, 2.90 mmol), and the solution was stirred at room temperature overnight. The residue was concentrated and purified by silica gel column chromatography (100:0 to 80:20 $CHCl_3$/MeOH) to afford **7** (0.707 g, 52%) as an amorphous compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.55–0.60 (m, 4H), 1.02 (t, J = 7.1 Hz, 6H), 1.64–1.74 (m, 2H), 1.91–1.95 (m, 1H), 2.19 (s, 3H), 2.38–2.57 (m, 8H), 2.98–3.07 (m, 4H), 3.57 (s, 2H), 3.82 (s, 2H), 7.45 (t, J = 7.7 Hz, 1H), 7.55–7.60 (m, 2H), 7.90–7.97 (m, 3H), 8.04 (d, J = 1.8 Hz, 1H), 8.20 (s, 1H). The protons of amide were not

observed. ^{13}C NMR (100 MHz, $CDCl_3$): δ 6.4, 11.4, 27.5, 29.7, 37.5, 42.1, 46.8, 50.3, 50.7, 51.6, 55.7, 62.0, 112.0, 122.5 (q, J = 272.7 Hz), 124.9, 126.0, 126.5, 126.8 (q, J = 5.1 Hz), 128.3, 128.8, 129.1 (q, J = 32.0 Hz), 131.2, 132.1, 132.4, 133.2, 134.4, 140.5, 145.3, 149.0, 163.1, 164.0. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 703.2809; found, 703.2857. HPLC: purity 96%. R_T: 4.72 min (method A).

***(E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6-hydroxy-6-methyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-3-(((3-(diethylamino)propyl)(methyl)amino)methyl)benzamide* (8).** To a solution of **S4b** (0.177 g, 0.303 mmol) in THF (3.03 mL) were added *N*,*N*-diethyl-*N'*-methyl-1,3-propanediamine (0.219 g, 1.52 mmol) and TEA (0.085 mL, 0.606 mmol), and the solution was stirred at room temperature overnight. The resulting mixture was washed with a saturated aqueous $NaHCO_3$ solution, extracted twice with ethyl acetate, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0:100 to 30:70 ca.4% ammonia in MeOH/$CHCl_3$) to afford **8** (0.198 g, 94%) as an amorphous compound. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.03 (t, J = 7.1 Hz, 6H), 1.45 (s, 3H), 1.67–1.75 (m, 4H), 1.79–2.07 (m, 2H), 2.20 (s, 3H), 2.39–2.56 (m, 8H), 2.77–2.92 (m, 2H), 3.57 (s, 2H), 7.46 (t, J = 7.7 Hz, 1H), 7.56–7.59 (m, 2H), 7.90–7.97 (m, 3H), 8.05 (d, J = 1.8 Hz, 1H), 8.21 (s, 1H). The protons of amide and alcohol were not observed. ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.6, 24.4, 24.8, 29.1, 35.3, 38.7, 42.2, 46.9, 50.8, 55.8, 62.0, 68.7, 112.3, 122.5 (q, J = 271.2 Hz), 125.5, 126.0, 126.2, 126.8 (q, J = 5.1 Hz), 128.3, 128.8, 129.1 (q, J = 31.4 Hz), 131.2, 132.06, 132.12, 132.5, 133.2,

134.3, 140.6, 145.3, 149.0, 163.2, 164.0. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 692.2649; found, 692.2720. HPLC: purity 98%. R_T: 5.42 min (method B).

***(E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-3-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)benzamide* (9).**

To a solution of **S5** (3.0 g, 5.45 mmol) in DMF (30 mL) were added potassium carbonate (1.81 g, 13.1 mmol) and 2-(2-(2-chloroethoxy)ethoxy)ethanol (2.38 mL, 16.4 mmol), and the solution was stirred at 100°C for 6 h. After cooling to room temperature, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. After stirring for 1 h in MeOH, the precipitate was collected by filtration to afford **9** (2.01 g, 54%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.02 (s, 6H), 1.54 (t, J = 6.0 Hz, 2H), 2.74 (d, J = 6.0 Hz, 2H), 3.42–3.62 (m, 10H), 3.75 (t, J = 4.8 Hz, 2H), 4.16 (t, J = 4.8 Hz, 2H), 4.24 (br s, 1H), 7.17–7.21 (m, 1H), 7.42–7.45 (m, 3H), 7.76 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 8.4, 1.5 Hz, 1H), 8.10 (d, J = 1.5 Hz, 1H), 8.41 (s, 1H), 11.22 (br s, 1H), 11.42 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.0, 28.0, 30.4, 35.6, 37.8, 60.7, 67.8, 69.3, 70.2, 70.4, 72.8, 113.9, 118.8, 120.1, 123.1 (q, J = 271.2 Hz), 126.0, 127.4, 127.6 (q, J = 32.1 Hz), 128.9, 130.5, 132.1, 132.7, 132.8, 134.5, 141.9, 145.2, 159.0, 162.3, 163.5. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 682.1965; found, 682.2020. HPLC: purity 92%. R_T: 6.77 min (method C).

(E)-3-(2-(2-(2-(3-((3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6,6-

dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)phenoxy)ethoxy)ethoxy)ethoxy)propanoic acid **(10).** To a solution of **S10** (0.80 g, 0.987 mmol) in DCM (3.0 mL) was added TFA (1.5 mL, 19.5 mmol), and the solution was stirred at room temperature for 0.5 h. The reaction mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (99:1 to 95:5 $CHCl_3$/MeOH) to afford **10** (0.235 g, 32%) as an amorphous compound. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.03 (s, 6H), 1.54 (t, J = 6.3 Hz, 2H), 2.42 (t, J = 6.3 Hz, 2H), 2.74 (br s, 2H), 3.66–3.81 (m, 12H), 3.75 (t, J = 4.9 Hz, 2H), 4.15 (t, J = 4.9 Hz, 2H), 7.17–7.20 (m, 1H), 7.43–7.47 (m, 3H), 7.75–7.78 (m, 1H), 7.96 (d, J = 7.8 Hz, 1H), 8.10 (s, 1H), 8.41 (s, 1H), 11.2 (br s, 1H), 11.4 (br s, 1H). The proton of carboxylic acid was not observed. ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.0, 28.0, 30.4, 35.2, 35.6, 37.8, 66.7, 67.8, 69.3, 70.1, 70.17, 70.23, 70.3, 113.9, 118.9, 120.0, 123.1 (q, J = 271.2 Hz), 126.0, 127.4, 127.6 (q, J = 29.9 Hz), 128.9, 130.5, 132.1, 132.7, 132.8, 134.5, 141.9, 145.2, 159.0, 162.3, 163.5, 173.1. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 754.2177; found, 754.2175. HPLC: purity 97%. R_T: 6.85 min (method C).

(E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-3-(2-(2-(2-(diethylamino)ethoxy)ethoxy)ethoxy)benzamide **(11).** To a solution of **S8** (0.80 g, 1.01 mmol) in DMF (15 mL) were added diethylamine (3.17 mL, 30.3 mmol) and potassium carbonate (0.698 g, 5.05 mmol), and the solution was stirred at 100°C for 5 h. After cooling to room temperature, the reaction

mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10% MeOH in $CHCl_3$) to afford **11** (0.648 g, 87%) as an amorphous compound. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.02 (t, J = 6.8 Hz, 6H), 1.08 (s, 6H), 1.69 (t, J = 6.3 Hz, 2H), 2.54–2.58 (m, 6H), 2.68 (t, J = 6.3 Hz, 2H), 2.88 (t, J = 6.3 Hz, 2H), 3.58 (t, J = 6.3 Hz, 2H), 3.64–3.67 (m, 2H), 3.71–3.75 (m, 2H), 3.89 (t, J = 4.4 Hz, 2H), 4.21 (t, J = 4.4 Hz, 2H), 7.14 (dd, J = 7.8, 2.9 Hz, 1H), 7.40 (t, J = 7.8 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.62 (m, 2H), 7.98 (dd, J = 8.4, 2.0 Hz, 1H), 8.05 (d, J = 2.0 Hz, 1H), 8.24 (s, 1H). The protons of amide were not observed. ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.4, 24.8, 27.6, 29.9, 35.7, 37.9, 47.6, 52.0, 67.7, 69.4, 69.7, 70.5, 70.9, 112.4, 113.5, 119.4, 119.6, 122.5 (q, J = 272.0 Hz), 125.0, 126.7 (q, J = 5.1 Hz), 128.2, 129.0 (q, J = 32.1 Hz), 129.9, 131.2, 132.0, 132.5, 133.7, 134.2 (d, J = 1.4 Hz), 145.3, 148.5, 159.3, 163.3, 163.6. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 737.2751; found, 737.2760. HPLC: purity 97%. R_T: 5.48 min (method C).

***(E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6-cyclopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-3-(2-(2-(2-(diethylamino)ethoxy)ethoxy)ethoxy)benzamide* (12).** To a solution of **S12** (0.15 g, 0.461 mmol) in DCM (5.0 mL) were added thionyl chloride (0.135 mL, 1.84 mmol) and DMF (0.024 mL, 0.307 mmol), which was stirred at room temperature overnight. The reaction mixture was azeotropically evaporated

with toluene. The residue was dissolved in DCM (5.0 mL), and pyridine (0.037 mL, 0.461 mmol) and **S3a** (0.136 g, 0.307 mmol) were added. The reaction mixture was stirred at room temperature overnight. To the reaction mixture was added a saturated aqueous $NaHCO_3$ solution, and it was extracted twice with $CHCl_3$. The combined organic layer was washed with the saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by amino silica gel column chromatography (100:0 to 80:20 $CHCl_3$/MeOH) to afford **12** (0.045 g, 20%) as an amorphous compound. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.55–0.59 (m, 4H), 1.02 (t, J = 7.2 Hz, 6H), 1.89–1.94 (m,1H), 2.57 (q, J = 7.2 Hz, 4H), 2.67 (t, J = 6.4 Hz, 2H), 2.97–3.05 (m, 4H), 3.58 (t, J = 6.4 Hz, 2H), 3.63–3.67 (m, 2H), 3.72–3.81 (m, 2H), 3.81 (s, 2H), 3.87–3.91 (m, 2H), 4.19–4.23 (m, 2H), 7.11–7.15 (m, 1H), 7.39 (t, J = 8.1 Hz, 1H), 7.55–7.61 (m, 3H), 7.95 (dd, J = 8.6, 2.0 Hz, 1H), 8.03 (d, J = 2.0 Hz, 1H), 8.20 (s, 1H). The protons of amide were not observed. LC−MS (ESI, $[M + H]^+$, *m/z*) 750. HPLC: purity 96%. R_T: 5.44 min (method B).

(R,E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-3-((13-ethyl-11-hydroxy-3,6,9-trioxa-13-azapentadecyl)oxy)benzamide **(13).** To a solution of **S9** (0.020 g, 0.027 mmol) in THF (1.0 mL) was added diethylamine (0.028 mL, 0.271 mmol), which was stirred at room temperature overnight. The reaction mixture was concentrated *in vacu*o. The residue was purified by silica gel column chromatography (20% MeOH in $CHCl_3$) to afford **13** (0.004 g, 18%) as an amorphous compound. ^{1}H

NMR (400 MHz, $CDCl_3$): δ 0.99 (t, J = 7.3 Hz, 6H), 1.05 (s, 6H), 1.66 (t, J = 6.0 Hz, 2H), 2.36–2.65 (m, 8H), 2.86 (t, J = 6.0 Hz, 2H), 3.43–3.52 (m, 2H), 3.66–3.74 (m, 8H), 3.79 (dd, J = 9.8, 4.9 Hz, 1H), 3.88 (t, J = 4.8 Hz, 2H), 4.19 (t, J = 4.8 Hz, 2H), 7.11 (dd, J = 8.4, 2.4 Hz, 1H), 7.38 (t, J = 8.4 Hz, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.59–7.60 (m, 2H), 7.94 (dd, J = 8.4, 1.5 Hz, 1H), 8.02 (d, J = 1.5 Hz, 1H), 8.23 (s, 1H). The protons of amide and alcohol were not observed. ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.9, 24.9, 27.6, 29.9, 35.7, 37.9, 47.2, 55.9, 66.6, 67.7, 69.7, 70.63, 70.65, 70.9, 74.1, 112.2, 113.5, 119.5, 119.6, 122.5 (q, J = 272.0 Hz), 124.8, 126.8 (q, J = 5.1 Hz), 128.3, 129.1 (q, J = 32.1 Hz), 129.9, 131.2, 132.1, 132.5, 133.7, 134.3 (d, J = 2.1 Hz), 145.2, 148.6, 159.3, 163.3, 163.6. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 811.3119; found, 811.3102. HPLC: purity 96%. R_T: 5.27 min (method C).

(R,E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-3-((11,15-dihydroxy-13-(2-hydroxyethyl)-3,6,9-trioxa-13-azapentadecyl)oxy)benzamide **(14).** To a solution of **S9** (0.272 g, 0.368 mmol) in IPA (1.8 mL) was added 2,2'-azanediyldiethanol (0.177 mL, 1.84 mmol) and stirred at 70°C for 3 h. The reaction mixture was concentrated *in vacu*o. The residue was purified by silica gel column chromatography (100:0 to 80:20 $CHCl_3$/MeOH) to afford **14** (0.184 g, 50%) as an amorphous compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.06 (s, 6H), 1.67 (t, J = 6.0 Hz, 2H), 2.57–2.64 (m, 6H), 2.77–2.83 (m, 4H), 3.40–3.75 (m, 17H), 3.86–3.93 (m, 3H), 4.20 (t, J = 4.6 Hz, 2H), 7.11 (dd, J = 7.8, 2.2 Hz, 1H), 7.39

(t, J = 7.8 Hz, 1H), 7.56–7.60 (m, 3H), 7.95 (dd, J = 8.4, 1.6 Hz, 1H), 8.03 (d, J = 1.6 Hz, 1H), 8.24 (s, 1H), 9.37 (br s, 1H), 12.8 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.8, 27.6, 29.9, 35.7, 37.9, 57.5, 57.8, 57.9, 59.7, 67.7, 68.40, 68.43, 69.6, 70.6, 70.65, 70.71, 70.8, 73.7, 112.4, 113.5, 119.5, 119.6, 122.5 (q, J = 272.0 Hz), 124.9, 126.8 (q, J = 5.1 Hz), 128.3, 129.1 (q, J = 31.3 Hz), 129.9, 131.2, 132.1, 132.5, 133.7, 134.3, 145.3, 148.4, 159.2, 163.3, 163.7. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 843.3017; found, 843.2972. HPLC: purity 97%. R_T: 4.86 min (method C).

(E)-1-(3-((3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)phenyl)-20-(2-methoxyethyl)-2,5,8,11,14,17-hexaoxa-20-azadocosan-22-oic acid **(15).** To a solution of **S16** (0.20 g, 0.221 mmol) in DMA (0.5 mL) was added **S14** (0.246 g, 1.10 mmol) and irradiated with a microwave at 120°C for 2 h. The reaction mixture was quenched with 1M HCl aqueous solution and extracted twice with 20% MeOH in $CHCl_3$. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was dissolved in EtOH (2.0 mL), and 1M NaOH aqueous solution was added (0.884 mL, 0.884 mmol). The reaction mixture was stirred at room temperature overnight. The reaction mixture was quenched with a 2M HCl aqueous solution and extracted twice with 20% MeOH in $CHCl_3$. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (100/0 to 80/20 $CHCl_3$/MeOH) to afford **15** (0.049 g, 23%) as an amorphous compound. ^{1}H NMR (300 MHz, $CDCl_3$):

δ 1.05 (s, 6H), 1.64 (t, J = 6.4 Hz, 2H), 2.50 (s, 2H), 2.89–2.91 (m, 6H), 3.29–3.32 (m, 5H), 3.53–3.64 (m, 24H), 4.63 (s, 2H), 7.47 (t, J = 7.4 Hz, 1H), 7.54–7.57 (m, 2H), 7.94–7.97 (m, 3H), 8.06 (s, 1H), 8.40 (s, 1H), 10.00 (br s, 1H), 12.71 (br s, 1H). The proton of carboxylic acid was not observed. ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.6, 27.6, 30.0, 35.7, 37.9, 55.1, 55.2, 58.1, 58.8, 68.3, 69.76, 69.84, 70.39, 70.43, 70.46, 70.49, 70.52, 70.6, 70.7, 72.6, 122.6 (q, J = 271.3 Hz), 124.4, 125.5, 126.7, 126.8, 127.8, 128.1, 129.01, 129.03 (q, J = 32.1 Hz), 131.2, 131.5, 131.7, 132.0, 132.5, 132.7, 134.1, 139.4, 145.5, 163.4, 163.7, 172.3. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 943.3542; found, 943.3586. HPLC: purity 97%. R_T: 5.32 min (method C).

2-(3-(19-Hydroxy-2,5,8,11,14,17-hexaoxanonadecyl)phenyl)-7,7-dimethyl-5,6,7,8-tetrahydro-4H-benzo[4,5]thieno[2,3-d][1,3]oxazin-4-one **(16).** To a solution of hexaethylene glycol (14.6 g, 51.8 mmol) in DMF (40 mL), NaH (2.28 g, 57.0 mmol) was added at 0°C, and the solution was stirred at room temperature for 1.5 h. A solution of **S19** (9.00 g, 20.7 mmol) dissolved in DMF (50 mL) was added to the reaction mixture, and the solution was stirred for 3 h at room temperature. Saturated aqueous NH_4Cl solution was added, and the reaction mixture was extracted with $CHCl_3$. The organic layer was washed with brine and water, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resultant residue was purified by silica gel column chromatography ($CHCl_3$/MeOH 100:0–94:6). To a stirred solution of the obtained residue in TFA (24 mL), TFAA (10.1 mL, 71.4 mmol) was added, and the solution was stirred for 2 h at room temperature. The reaction mixture was azeotropically

evaporated with toluene. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH 100:0–96:4) to obtain **16** (3.44 g, 27%) as an amorphous compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.06 (s, 6H), 1.63 (t, J = 6.8 Hz, 2H), 2.58 (s, 2H), 2.97 (t, J = 6.8 Hz, 2H), 3.60–3.80 (m, 24H), 4.64 (s, 2H), 7.48 (t, J = 7.8Hz, 1H), 7.58 (d, J = 7.8 Hz, 1H), 8.18 (d, J = 7.8 Hz, 1H), 8.22 (s, 1H). The proton of hydroxide was not observed. LC−MS (ESI, $[M + H]^+$, *m/z*) 606. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 606.2737; found, 606.2748. HPLC: 99% purity.

2-(3-(19-Hydroxy-2,5,8,11,14,17-hexaoxanonadecyl)benzamido)-6,6-dimethyl-N-phenyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide **(17).** To a solution of **16** (0.071 g, 0.117 mmol) in pivalic acid (0.70 mL), aniline (0.016 mL, 0.176 mmol) was added, and the solution was stirred for 9 h at 120°C. After cooling to room temperature, the resulting mixture was washed with saturated aqueous $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was dissolved in MeOH (1.5 mL), and 4 M NaOH solution (0.293 mL, 1.17 mmol) was added to this solution. The reaction mixture was stirred for 3 h at 50°C. The reaction mixture was quenched with 1 M HCl solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH 95:5) to obtain **17** (0.055 g, 67%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (s, 6H), 1.70 (t, J = 6.2 Hz, 2H), 2.55 (s, 2H), 2.91 (t, J = 6.0 Hz, 2H), 3.58–3.73 (m, 25H), 4.66 (s, 2H), 7.19 (t, J = 7.5 Hz, 1H),

7.41 (t, J = 8.1 Hz, 2H), 7.49 (t, J = 7.5 Hz, 1H), 7.58–7.62 (m, 3H), 7.78 (s, 1H), 7.92 (d, J = 7.7 Hz, 1H), 8.00 (s, 1H), 13.05 (s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 699. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 699.3315; found, 699.3360. HPLC: 93% purity.

2-(3-(19-Hydroxy-2,5,8,11,14,17-hexaoxanonadecyl)benzamido)-N-(4-methoxyphenyl)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide **(18).** To a solution of **16** (0.074 g, 0.122 mmol) in pivalic acid (0.74 mL), 4-methoxyaniline (0.023 g, 0.183 mmol) was added, and the solution was stirred for 9 h at 120°C. After cooling to room temperature, the resulting mixture was washed with saturated aqueous $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was dissolved in MeOH (1.5 mL), after which 4 M NaOH solution (0.306 mL, 1.22 mmol) was added. The solution was stirred for 1 h at 70°C. The reaction mixture was quenched with 1 M HCl solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH 95:5) to obtain **18** (0.044 g, 49%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (s, 6H), 1.69 (t, J = 6.2 Hz, 2H), 2.54 (s, 2H), 2.90 (t, J = 6.4 Hz, 2H), 3.58–3.73 (m, 25H), 3.83 (s, 3H), 4.65 (s, 2H), 6.94 (dd, J = 6.6, 2.2 Hz, 2H), 7.49 (d, J = 8.8 Hz, 3H), 7.58 (d, J = 7.7 Hz, 1H), 7.67 (s, 1H), 7.91 (d, J = 7.7 Hz, 1H), 7.99 (s, 1H), 13.09 (s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 729. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 729.3421; found, 729.3409. HPLC: 95% purity.

***N-(4-Ethylphenyl)-2-(3-(19-hydroxy-2,5,8,11,14,17-hexaoxanonadecyl)benzamido)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide* (19).** To a solution of **16** (0.100 g, 0.165 mmol) in AcOH (1.0 mL), sodium acetate (0.068 g, 0.825 mmol) and 4-ethylaniline (0.103 mL, 0.825 mmol) were added, and the solution was stirred for 2 h at 90°C. After cooling to room temperature, saturated aqueous $NaHCO_3$ solution was added, and the reaction mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by preparative HPLC to obtain **19** (0.051 g, 43%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.09 (s, 6H), 1.26 (t, J = 7.8 Hz, 3H), 1.70 (t, J = 5.9 Hz, 2H), 2.56 (s, 2H), 2.67 (q, J = 7.5 Hz, 2H), 2.74 (t, J = 6.3 Hz, 1H), 2.91 (t, J = 6.3 Hz, 2H), 3.59–3.71 (m, 24H), 4.66 (s, 2H), 7.24 (d, J = 7.8 Hz, 3H), 7.50 (d, J = 8.8 Hz, 2H), 7.59 (d, J = 7.8 Hz, 1H), 7.73 (s, 1H), 7.93 (d, J = 7.8 Hz, 1H), 8.00 (s, 1H), 13.09 (s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 727. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 727.3628; found, 727.3690. HPLC: 99% purity.

***N-(4-Benzylphenyl)-2-(3-(19-hydroxy-2,5,8,11,14,17-hexaoxanonadecyl)benzamido)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide* (20).** To a solution of **16** (0.100 g, 0.165 mmol) in AcOH (1.0 mL), sodium acetate (0.163 g, 1.98 mmol) and 4-benzylaniline (0.303 g, 1.65 mmol) were added, and the solution was stirred overnight at 90°C. After cooling to room temperature, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and

concentrated *in vacuo*. The residue was dissolved in MeOH (1.0 mL), and potassium carbonate (0.100 g, 0.724 mmol) was added. The solution was stirred for 0.5 h at room temperature. The resulting mixture was purified by amino silica gel column chromatography ($CHCl_3$/MeOH 100:0–95:5) to obtain **20** (0.027 g, 21%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.07 (s, 6H), 1.68 (t, J = 6.2 Hz, 2H), 2.53 (s, 2H), 2.88 (t, J = 6.2 Hz, 2H), 3.05 (br s, 1H), 3.56–3.73 (m, 24H), 3.99 (s, 2H), 4.65 (s, 2H), 7.18–7.33 (m, 7H), 7.44–7.53 (m, 3H), 7.58 (t, J = 7.8 Hz, 2H), 7.73 (s, 1H), 7.91 (t, J = 7.8 Hz, 1H), 13.04 (brs, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 789. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 789.3785; found, 789.3841. HPLC: 91% purity.

***Methyl 4-(4-(2-(3-((17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl)oxy)benzamido)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamido)phenethyl)benzoate* (21).** To a solution of 3-acetoxybenzoic acid (1.95 g, 10.8 mmol) in $CHCl_3$ (36 mL), thionyl chloride (2.37 mL, 32.4 mmol) and DMF (0.042 mL, 0.540 mmol) were added at 0°C, and the solution was stirred for 1 h at 50°C. The reaction mixture was azeotropically evaporated with toluene. The residue was dissolved in $CHCl_3$ (30 mL), and **S25** (2.50 g, 5.40 mmol) and pyridine (2.62 mL, 32.4 mmol) were added at 0°C. The reaction mixture was stirred for 1.5 h at room temperature, followed by addition of saturated aqueous $NaHCO_3$ solution and extraction with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Diisopropyl ether (40 mL) was added to the residue, and the solution was stirred for 1 h before filtration. To a stirred solution of the obtained residue in

MeOH (103 mL), 2 M NaOH solution (2.59 mL, 5.17 mmol) was added, and the solution was stirred for 0.5 h at 0°C. The reaction mixture was quenched with 1 M HCl solution and then filtered. To a stirred solution of the obtained residue in DMF (25 mL), cesium carbonate (3.23 g, 9.92 mmol) and 17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (**S27**) (4.33 g, 9.92 mmol) were added; the solution was stirred for 1 h at 70°C. After cooling to room temperature, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate/MeOH 100:0–80:20) to obtain **21** (4.20 g, 92%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.03 (s, 6H), 1.56 (s, 2H), 2.81 (s, 2H), 2.93–2.98 (m, 6H), 3.45–3.57 (m, 21H), 3.76 (s, 2H), 3.83 (s, 3H), 4.16 (s, 2H), 7.15–7.18 (m, 3H), 7.34 (d, J = 7.8 Hz, 2H), 7.43 (s, 3H), 7.56 (d, J = 7.8 Hz, 2H), 7.85 (d, J = 8.8 Hz, 2H), 9.18 (s, 1H), 11.60 (s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 847. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 847.3840; found, 847.3895. HPLC: 99% purity.

Methyl 4-(4-(2-(3-(19-hydroxy-2,5,8,11,14,17-hexaoxanonadecyl)benzamido)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamido)-2-methylphenethyl)benzoate **(22).** To a solution of **16** (0.073 g, 0.121 mmol) in pivalic acid (1.0 mL), methyl 4-(4-amino-2-methylphenethyl)benzoate (**S22**) (0.065 g, 0.241 mmol) was added, and the solution was stirred overnight at 120°C. After cooling to room temperature, the reaction mixture was quenched with 1 M

NaOH solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by preparative HPLC to obtain **22** (0.049 g, 47%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.07 (s, 6H), 1.69 (t, J = 6.0 Hz, 2H), 2.30 (s, 3H), 2.54 (s, 2H), 2.87–2.95 (m, 6H), 3.56–3.72 (m, 25H), 3.91 (s, 3H), 4.65 (s, 2H), 7.10 (d, J = 8.1 Hz, 1H), 7.24 (d, J = 8.4 Hz, 2H), 7.33–7.39 (m, 2H), 7.48 (d, J = 7.7 Hz, 1H), 7.58 (d, J = 7.7 Hz, 1H), 7.70 (s, 1H), 7.90–8.01 (m, 4H), 13.05 (s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 875. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 875.4153; found, 875.4213. HPLC: 95% purity.

***4-(4-(2-(3-((17-Hydroxy-3,6,9,12,15-pentaoxaheptadecyl)oxy)benzamido)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamido)phenethyl)benzoic acid* (23).** To a solution of **21** (0.033 g, 0.039 mmol) in MeOH (1.0 mL), 1 M NaOH solution (0.156 mL, 0.156 mmol) was added, and the solution was stirred for 8 h at 70°C. After cooling to room temperature, the reaction mixture was quenched with 2 M HCl solution and extracted with $CHCl_3$/MeOH. The organic layer was washed with brine and water, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to obtain **23** (0.032 g, quant.) as an amorphous compound. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.07 (s, 6H), 1.68 (t, J = 6.0 Hz, 2H), 2.53 (s, 2H), 2.92–2.99 (m, 6H), 3.58–3.70 (m, 21H), 3.88–3.89 (m, 2H), 4.21 (t, J = 4.6 Hz, 2H), 7.10–7.15 (m, 3H), 7.23 (d, J = 8.3 Hz, 2H), 7.33–7.41 (m, 2H), 7.49 (d, J = 8.5 Hz, 2H), 7.57–7.58 (m, 2H), 7.74 (s, 1H), 7.99 (d, J = 8.1 Hz, 2H). The proton of carboxylic acid was not observed.

LC−MS (ESI, $[M + H]^+$, *m/z*) 833. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 833.3683; found, 833.3741. HPLC: 98% purity.

***20-(3-((3-((4-(4-(Methoxycarbonyl)phenethyl)-3-methylphenyl)carbamoyl)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)phenoxy)-3,6,9,12,15,18-hexaoxaicosanoic acid* (24).** To a solution of **S31** (0.050 g, 0.077 mmol) in pivalic acid (0.50 mL), methyl 4-(4-amino-2-methylphenethyl)benzoate (**S22**) (0.062 g, 0.231 mmol) was added, and the solution was stirred for 1 h at 120°C. After cooling to room temperature, the reaction mixture was purified by preparative HPLC to obtain **24** (0.012 g, 17%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (s, 6H), 1.70 (t, J = 6.0 Hz, 2H), 2.30 (s, 3H), 2.54 (s, 2H), 2.85–2.94 (m, 6H), 3.55–3.76 (m, 20H), 3.87–3.94 (m, 7H), 4.22 (t, J = 4.6 Hz, 2H), 7.07–7.14 (m, 2H), 7.23 (d, J = 8.4 Hz, 2H), 7.34 (s, 1H), 7.37–7.44 (m, 2H), 7.56–7.63 (m, 2H), 7.74 (s, 1H), 7.96 (d, J = 8.4 Hz, 2H), 13.04 (s, 1H). The proton of carboxylic acid was not observed. LC−MS (ESI, $[M + NH_4]^+$, *m/z*) 937. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 919.4051; found, 919.4052. HPLC: 99% purity.

***20-(3-((6,6-Dimethyl-3-((3-methyl-4-(4-(thiazol-2-yl)phenethyl)phenyl)carbamoyl)-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)phenoxy)-3,6,9,12,15,18-hexaoxaicosanoic acid* (25).** To a solution of **S31** (0.180 g, 0.277 mmol) in pivalic acid (2.0 mL), 3-methyl-4-(4-(thiazol-2-yl)phenethyl)aniline (**S34**) (0.163 g, 0.554 mmol) was added, and the solution was stirred for 1 h at 120°C. After cooling to room temperature, the reaction mixture was diluted with $CHCl_3$ and purified

by silica gel column chromatography (ethyl acetate/MeOH 90:10–60:40) to obtain **25** (0.150 g, 57%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (s, 6H), 1.69 (t, J = 6.0 Hz, 2H), 2.33 (s, 3H), 2.54 (s, 2H), 2.87–2.95 (m, 6H), 3.60–3.78 (m, 20H), 3.88 (t, J = 4.6 Hz, 2H), 4.09–4.13 (m, 2H), 4.21 (t, J = 4.6 Hz, 2H), 7.10–7.14 (m, 2H), 7.24 (s, 1H), 7.27 (s, 1H), 7.32 (d, J = 3.3 Hz, 1H), 7.34–7.41 (m, 3H), 7.58 (d, J = 7.8 Hz, 1H), 7.60–7.62 (m, 1H), 7.72 (s, 1H), 7.85–7.91 (m, 3H), 13.05 (s, 1H). The proton of carboxylic acid was not observed. LC−MS (ESI, $[M + H]^+$, *m/z*) 944. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 944.3826; found, 944.3812. HPLC: 90% purity.

20-(3-((6,6-Dimethyl-3-((3-methyl-4-(4-(oxazol-2-yl)phenethyl)phenyl)carbamoyl)-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)phenoxy)-3,6,9,12,15,18-hexaoxaicosanoic acid **(26).** To a solution of **S31** (0.100 g, 0.154 mmol) in pivalic acid (1.0 mL), 3-methyl-4-(4-(oxazol-2-yl)phenethyl)aniline (**S35**) (0.089 g, 0.320 mmol) was added, and the solution was stirred for 1 h at 120°C. After cooling to room temperature, the reaction mixture was purified by preparative HPLC to obtain **26** (0.023 g, 16%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.07 (s, 6H), 1.69 (t, J = 5.8 Hz, 2H), 2.31 (s, 3H), 2.53 (s, 2H), 2.86–2.98 (m, 6H), 3.56–3.75 (m, 20H), 3.86–3.93 (m, 4H), 4.21 (br s, 2H), 7.09–7.13 (m, 2H), 7.22 (s, 1H), 7.25 (s, 1H), 7.34–7.42 (m, 3H), 7.57 (d, J = 7.8 Hz, 1H), 7.61 (s, 1H), 7.70 (s, 1H), 7.77 (s, 1H), 7.96 (d, J = 7.8 Hz, 2H), 13.04 (s, 1H). The protons of amide and carboxylic acid were not observed. LC−MS (ESI, $[M + H]^+$, *m/z*) 928. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 928.4054; found, 928.4086. HPLC: 99% purity.

***1-(3-((6,6-Dimethyl-3-((4-(4-(5-methyl-1,3,4-oxadiazol-2-yl)phenethyl)phenyl)carbamoyl)-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)phenyl)-2,5,8,11,14,17,20-heptaoxadocosan-22-oic acid* (27).** To a solution of 20-hydroxy-3,6,9,12,15,18-hexaoxaicosanoic acid (**S29**) (0.527 g, 1.55 mmol) in DMF (5.0 mL), NaH (0.206 g, 5.16 mmol) was added at 0°C, and the solution was stirred for 0.5 h at 0°C. Next, **S42** (0.330 g, 0.516 mmol) and NaH (0.206 g, 5.16 mmol) were added to the reaction mixture at 0°C, and the solution was stirred for 5 h at room temperature. The reaction mixture was quenched with 2 M HCl solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate/MeOH 93:7) to obtain **27** (0.304 g, 62%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.07 (s, 6H), 1.68 (t, J = 6.2 Hz, 2H), 2.53 (br s, 2H), 2.60 (s, 3H), 2.86–2.92 (m, 2H), 2.94–3.00 (m, 4H), 3.53–3.75 (m, 24H), 4.14 (s, 2H), 4.64 (s, 2H), 7.16 (d, J = 8.4 Hz, 2H), 7.28 (d, J = 8.4 Hz, 2H), 7.45–7.57 (m, 4H), 7.82–8.01 (m, 5H), 13.02 (br s, 1H). The proton of carboxylic acid was not observed. LC−MS (ESI, $[M + H]^+$, *m/z*) 943. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 943.4163; found, 943.4133. HPLC: 97% purity.

***1-(3-((6,6-Dimethyl-3-((4-(4-(5-methyl-1,3,4-oxadiazol-2-yl)phenethyl)phenyl)carbamoyl)-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)phenyl)-5,8,11,14,17,20,23,26-octaoxa-2-thianonacosan-29-oic acid* (28).** To a solution of **S42** (0.032 g, 0.050 mmol) in DMF (1.0 mL), 1-mercapto-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid (0.023 g, 0.050 mmol) and cesium

carbonate (0.049 g, 0.150 mmol) were added, and the solution was stirred for 3 h at 70°C. The reaction mixture was quenched with 1 M HCl solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was then purified by preparative thin-layer chromatography ($CHCl_3$/MeOH 90:10) to obtain **28** (0.021 g, 40%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (s, 6H), 1.69 (t, J = 6.0 Hz, 2H), 2.53 (s, 2H), 2.57–2.63 (m, 7H), 2.89 (t, J = 5.9 Hz, 2H), 2.95–2.98 (m, 4H), 3.58–3.61 (m, 30H), 3.75 (t, J = 6.0 Hz, 2H), 3.83 (s, 2H), 7.16 (d, J = 8.4 Hz, 2H), 7.28 (d, J = 8.1 Hz, 2H), 7.42–7.56 (m, 4H), 7.75 (s, 1H), 7.86 (d, J = 7.7 Hz, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.97 (s, 1H), 13.04 (s, 1H). The proton of carboxylic acid was not observed. LC−MS (ESI, $[M + H]^+$, *m/z*) 1061. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 1061.4616; found, 1061.4709. HPLC: 99% purity.

1-(3-((6,6-Dimethyl-3-((4-(4-(5-methyl-1,3,4-oxadiazol-2-yl)phenethyl)phenyl)carbamoyl)-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)phenyl)-2,5,8,11-tetraoxatridecan-13-oic acid **(29).** To a solution of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)acetic acid (**S44**) (0.177 g, 0.849 mmol) in DMF (5.0 mL), NaH (0.227 g, 5.66 mmol) was added at 0°C, and the solution was stirred for 0.5 h at 0°C. Next, **S42** (0.362 g, 0.566 mmol) and NaH (0.227 g, 5.66 mmol) were added to the reaction mixture at 0°C, and the solution was stirred for 4.5 h at room temperature. The reaction mixture was quenched with 2 M HCl solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified

by silica gel column chromatography (ethyl acetate/MeOH 93:7) to obtain **29** (0.090 g, 20%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.03 (s, 6H), 1.55 (t, J = 6.2 Hz, 2H), 2.48 (s, 2H), 2.56 (s, 3H), 2.82 (t, J = 6.2 Hz, 2H), 2.94–2.99 (m, 4H), 3.54–3.56 (m, 8H), 3.60 (s, 4H), 3.98 (s, 2H), 4.58 (s, 2H), 7.19 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 8.1 Hz, 2H), 7.51 (t, J = 7.3 Hz, 2H), 7.59 (d, J = 8.4 Hz, 2H), 7.79 (d, J = 7.0 Hz, 1H), 7.86 (d, J = 8.1 Hz, 3H), 9.61 (s, 1H), 11.80 (s, 1H). The proton of carboxylic acid was not observed. LC−MS (ESI, $[M + H]^+$, *m/z*) 811. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 811.3377; found, 811.3422. HPLC: 94% purity.

1-(3-((6,6-Dimethyl-3-((4-(4-(5-methyl-1,3,4-oxadiazol-2-yl)phenethyl)phenyl)carbamoyl)-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)phenyl)-2-methyl-5,8,11-trioxa-2-azatetradecan-14-oic acid **(30).** To a solution of **S42** (0.729 g, 1.14 mmol) in propionitrile (5.7 mL), tetraethylammonium bromide (0.240 g, 1.14 mmol), potassium fluoride on celite (0.265 g, 2.28 mmol), and *tert*-butyl 3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propanoate (0.791 g, 2.28 mmol) were added, and the solution was refluxed for 1 h. After cooling to room temperature, the reaction mixture was filtered through a celite pad, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH 97:3–93:7). To a stirred solution of the obtained residue in DCM (2.0 mL) and MeOH (2.0 mL), 37% formaldehyde solution (0.356 mL, 4.78 mmol) and $NaBH(OAc)_3$ (0.507 g, 2.39 mmol) were added to the solution; the solution was stirred again overnight at room temperature. The reaction mixture was quenched with saturated aqueous $NaHCO_3$

solution and extracted with ethyl acetate. The organic layer was washed with aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH 94:6). To a stirred solution of the obtained residue in DCM (3.0 mL), TFA (0.773 mL, 10.0 mmol) was added, and the solution was stirred again for 2 h at room temperature. The reaction mixture was azeotropically evaporated with toluene. The residue was dissolved in ethyl acetate and washed with saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH 94:6) to obtain **30** (0.130 g, 14%) as an amorphous compound. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.08 (s, 6H), 1.69 (t, J = 6.3 Hz, 2H), 2.40 (s, 3H), 2.50–2.54 (m, 4H), 2.61 (s, 3H), 2.82 (t, J = 6.3 Hz, 2H), 2.88–2.92 (m, 2H), 2.98 (m, 4H), 3.63–3.68 (m, 8H), 3.77 (t, J = 5.4 Hz, 2H), 3.82 (t, J = 5.4 Hz, 2H), 3.94 (s, 2H), 7.17 (d, J = 7.8 Hz, 2H), 7.29 (d, J = 7.8 Hz, 2H), 7.50–7.52 (m, 3H), 7.67 (d, J = 7.8 Hz, 1H), 7.77 (s, 1H), 7.91–7.95 (m, 3H), 7.99 (s, 1H), 13.0 (s, 1H). The proton of carboxylic acid was not observed. LC−MS (ESI, $[M + H]^+$, *m/z*) 838. HR−LC−MS (ESI, $[M + H]^+$, *m/z*) calcd, 838.3850; found, 838.3834. HPLC: 99% purity.

***(E)-N'-(4-Chloro-3-(trifluoromethyl)benzylidene)-2-cyanoacetohydrazide* (S2).** To a stirred solution of 2-cyanoacetohydrazide (**S1**) (2.0 g, 20.2 mmol) in ethyl acetate (70 mL) was added 4-chloro-3-(trifluoromethyl)benzaldehyde (5.05 g, 24.2 mmol), and the solution was stirred at 50°C for 3 h. After cooling to room temperature, the resulting mixture was concentrated *in vacuo*. Diethyl ether

(60 mL) was added to the residue and stirred for 1 h, and then filtered to afford **S2** (5.64 g, 96%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.94 (s, 2H), 7.58 (d, J = 8.4 Hz, 1H), 7.80 (dd, J = 8.4, 1.6 Hz, 1H), 7.93 (s, 1H), 7.98 (d, J = 1.6 Hz, 1H), 9.89 (br s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 290.

***(E)-2-Amino-N'-(4-chloro-3-(trifluoromethyl)benzylidene)-6-cyclopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carbohydrazide* (S3a).** To a solution of **S2** (5.0 g, 17.3 mmol) in THF (57.5 mL) were added AcOH (3.95 mL, 69.1 mmol), HMDS (7.24 mL, 34.5 mmol), and 1-cyclopropylpiperidin-4-one (2.88 g, 20.7 mmol) in THF (10 mL), and the solution was stirred at room temperature overnight. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. To the residue was added MeOH and stirred for 1 h, and then filtered. To a stirred solution of obtained residue in THF (20 mL) was added sulfur (0.195 g, 6.09 mmol) and TEA (1.7 mL, 12.2 mmol), and the solution was stirred at room temperature overnight. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was added to 15 mL of 1:1 *n*-hexane/ethyl acetate solution and stirred for 1 h, and then filtered to afford **S3a** (1.44 g, 19%) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.53−0.58 (m, 4H), 1.88−1.93 (m, 1H), 2.85−2.86 (m, 2H), 2.99 (t, J = 5.9 Hz, 2H), 3.65 (s, 2H), 6.11 (br s, 2H), 7.53 (d, J = 8.8 Hz, 1H), 7.90 (dd, J = 8.8, 1.6 Hz, 1H), 7.99 (d, J = 1.6 Hz, 1H), 8.14 (s, 1H), 8.89 (s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 443.

(E)-2-Amino-N'-(4-chloro-3-(trifluoromethyl)benzylidene)-6-hydroxy-6-methyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbohydrazide **(S3b)** To a solution of **S2** (1.25 g, 4.32 mmol) in THF (30 mL) were added AcOH (0.989 mL, 17.3 mmol), HMDS (1.81 mL, 8.63 mmol), and 4-hydroxy-4-methylcyclohexanone (0.664 g, 5.18 mmol) in THF (4.0 mL), and the solution was stirred for 3h at room temperature. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. To the residue was added MeOH and stirred for 1h, and then filtered. To a stirred solution of obtained residue in THF (20 mL) were added sulfur (0.128 g, 4.00 mmol) and TEA (0.588 mL, 4.00 mmol), and the solution was stirred at room temperature overnight. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was added diethyl ether and stirred for 1h, and then filtered to afford **S3b** (0.983 g, 53%) as a white solid. ^{1}H NMR (270 MHz, DMSO-d_6): δ 1.21 (s, 3H), 1.53−1.69 (m, 2H), 2.47 (s, 2H), 2.59−2.76 (m, 2H), 4.51 (s, 1H), 6.66 (br s, 2H), 7.79 (d, J = 8.2 Hz, 1H), 7.95 (dd, J = 8.2, 1.3 Hz, 1H), 8.10 (s, 1H), 8.30 (s, 1H), 10.79 (s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 432.

(E)-2-Amino-N'-(4-chloro-3-(trifluoromethyl)benzylidene)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbohydrazide **(S3c).** To a solution of **S2** (4.4 g, 15.2 mmol) in THF (44 mL) were added AcOH (3.48 mL, 60.8 mmol), HMDS (6.37 mL, 30.4 mmol), and 4,4-dimethylcyclohexan-1-one (2.3 g, 18.2 mmol) in THF (10 mL), and the solution was stirred at room

temperature overnight. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. MeOH was added to the residue and stirred for 1 h, and then filtered. To a stirred solution of obtained residue in THF (30 mL) were added sulfur (0.403 g, 12.6 mmol) and TEA (3.5 mL, 25.1 mmol), and the solution was stirred at room temperature overnight. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (100:0 to 90:10 $CHCl_3$/MeOH) to afford **S3c** (2.92 g, 44%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.99 (s, 6H), 1.17 (t, J = 6.0 Hz, 2H) , 2.27 (s, 2H), 2.60 (t, J = 6.0 Hz, 2H), 6.68 (br s, 2H), 7.79 (d, J = 8.2 Hz, 1H), 7.95 (dd, J = 8.2, 2.0 Hz, 1H), 8.11 (d, J = 2.0 Hz, 1H), 8.30 (s, 1H), 10.77 (br s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 430.

(E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6-cyclopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-3-(chloromethyl)benzamide **(S4a).** To a solution of **S3a** (0.21 g, 0.483 mmol) in 1,2-dichloroethane (4.8 mL) was added 3-(chloromethyl)benzoyl chloride (0.103 mL, 0.725 mmol) and pyridine (0.078 mL, 0.966 mmol), and the solution was stirred at room temperature for 2 h. To the reaction mixture was added 1M HCl aqueous solution and extracted twice with $CHCl_3$. The combined organic layer was washed with saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column

chromatography (5% MeOH in $CHCl_3$) to afford **S4a** (0.24 g, 84%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.56–0.63 (m, 4H), 1.90–1.97 (m, 1H), 2.99–3.09 (m, 4H), 3.83 (s, 2H), 4.67 (s, 2H), 7.49–7.65 (m, 3H), 7.97–8.04 (m, 4H), 8.19 (s, 1H), 9.11 (br s, 1H), 12.86 (br s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 595.

(E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6-hydroxy-6-methyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-3-(chloromethyl)benzamide **(S4b).** To a solution of **S3b** (0.15 g, 0.347 mmol) in DCM (3.16 mL) were added 3-(chloromethyl)benzoyl chloride (0.074 mL, 0.521 mmol) and pyridine (0.042 mL, 0.521 mmol), and the solution was stirred at room temperature for 1h. The reaction mixture was added 1M HCl solution and extracted twice with $CHCl_3$. The combined organic layer was washed with saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (7% MeOH in $CHCl_3$) to afford **S4b** (0.177 g, 87%) as a yellow solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.46 (s, 3H), 1.80−2.08 (m, 2H), 2.77−3.08 (m, 4H), 4.68 (s, 2H), 7.49−7.65 (m, 3H), 7.96−8.07 (m, 4H), 8.18 (s, 1H), 9.19 (s, 1H), 12.91 (s, 1H). The proton of alcohol was not observed. LC−MS (ESI, $[M - H]^-$, *m/z*) 582.

(E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-3-(chloromethyl)benzamide **(S4c).** To a solution of **S3c** (0.843 g, 1.96 mmol) in DCM (20 mL) was added 3-(chloromethyl)benzoyl chloride (0.418 mL, 2.94

mmol) and pyridine (0.238 mL, 2.94 mmol), and stirred at room temperature for 2 h. The reaction mixture was washed with saturated aqueous $NaHCO_3$ solution and extracted twice with $CHCl_3$. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. MeOH was added to the residue and stirred for 1 h, then filtered to afford **S4c** (1.04 g, 91%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.08 (s, 6H), 1.71 (t, J = 5.9 Hz, 2H), 1.89 (t, J = 5.9 Hz, 2H), 2.55 (s, 2H), 4.68 (s, 2H), 7.51–7.65 (m, 3H), 7.98–8.03 (m, 4H), 8.23 (s, 1H), 9.24 (br s, 1H), 13.0 (br s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 582.

(E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-3-hydroxybenzamide **(S5).** To a solution of 3-acetoxybenzoic acid (0.629 g, 3.49 mmol) in DCM (15 mL) were added thionyl chloride (1.02 mL, 14.0 mmol) and DMF (0.18 mL, 2.33 mmol), and the solution was stirred at room temperature overnight. The reaction mixture was azeotropically evaporated with toluene. The residue was dissolved in DCM (15 mL) and followed by addition of pyridine (0.282 mL, 3.49 mmol) and **S3c** (1.0 g, 2.33 mmol). The reaction mixture was stirred at room temperature for 3 h. Saturated aqueous $NaHCO_3$ solution was added to the reaction mixture and extracted twice with ethyl acetate. The combined organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was dissolved in MeOH (50 mL) followed by addition of potassium carbonate (1.52 g, 11.0 mmol), and the solution was refluxed for 2 h. After

cooling to room temperature, the reaction mixture was quenched with 1M HCl aqueous solution and extracted twice with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. After stirring for 1 h in MeOH, the precipitate was filtered off to afford **S5** (1.11 g, 87%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.03 (s, 6H), 1.53 (t, J = 6.0 Hz, 2H), 2.76 (t, J = 6.0 Hz, 2H), 3.18–3.19 (m, 2H), 3.81 (br s, 1H), 6.99–7.01 (m, 1H), 7.30–7.36 (m, 3H), 7.76 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 8.4 Hz, 1H), 8.10 (s, 1H), 8.43 (s,1H), 9.64 (br s, 1H), 11.12 (br s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 550.

***tert-Butyl 3-(2-(2-(2-(tosyloxy)ethoxy)ethoxy)ethoxy)propanoate* (S7).** To a solution of **S6** (2.76 mL, 10.4 mmol) in DCM (30 mL) were added *p*-toluenesulfonyl chloride (2.98 g, 15.6 mmol) and TEA (8.42 mL, 60.4 mmol), and the solution was stirred at room temperature overnight. The reaction mixture was washed with 10% HCl aqueous solution and extracted twice with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (80:20 to 50:50 *n*-hexane/ethyl acetate) to afford **S7** (4.4 g, 98%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (s, 9H), 2.45 (s, 3H), 2.50 (t, J = 6.4 Hz, 2H), 3.58–3.60 (m, 8H), 3.67–3.72 (m, 4H), 4.16 (t, J = 4.8 Hz, 2H), 7.34 (d, J = 8.2 Hz, 2H), 7.80 (d, J = 8.2 Hz, 2H). LC−MS (ESI, $[M + H + 18]^+$, *m/z*) 451.

***(E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-3-(2-(2-(2-iodoethoxy)ethoxy)ethoxy)benzamide* (S8).** To

a solution of **9** (1.5 g, 2.20 mmol) in DMF (15 mL) was added methanesulfonyl chloride (0.257 mL, 3.30 mmol) and TEA (0.460 mL, 3.30 mmol), and the solution was stirred at room temperature overnight. NaI (0.659 g, 4.40 mmol) was added to the reaction mixture and stirred at 100°C for 2 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (50% ethyl acetate in *n*-hexane) to afford **S8** (1.29 g, 74%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.02 (s, 6H), 1.53 (t, J = 6.3 Hz, 2H), 2.74 (br s, 2H), 3.00 (s, 2H), 3.58–3.62 (m, 4H), 3.65–3.69 (m, 4H), 3.76 (t, J = 4.9 Hz, 2H), 4.16 (t, J = 4.9 Hz, 2H), 7.17–7.19 (m, 1H), 7.44 (m, 3H), 7.75 (d, J = 8.8 Hz, 1H), 7.95 (d, J = 8.8, 1.5 Hz, 1H), 8.09 (d, J = 1.5 Hz, 1H), 8.41 (s, 1H), 11.18 (br s, 1H), 11.42 (br s, 1H). LC−MS (ESI, $[M - H]^-$, *m/z*) 790.

(R,E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-3-(2-(2-(2-(oxiran-2-ylmethoxy)ethoxy)ethoxy)ethoxy)benzamide **(S9).** To a solution of **9** (0.050 g, 0.073 mmol) in DMF (1.5 mL) were added NaH (60% in mineral oil, 0.0044 g, 0.11 mmol) and (*S*)-epichlorohydrin (0.011 mL, 0.147 mmol), and the solution was stirred at room temperature overnight. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10% ethyl acetate in heptane) to afford **S9** (0.016 g, 29%) as an amorphous

compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.07 (s, 6H), 1.69 (t, J = 6.0 Hz, 2H), 2.53 (br s, 2H), 2.60 (dd, J = 5.0, 2.9 Hz, 1H), 2.78 (dd, J = 5.0, 4.2 Hz, 1H), 2.87 (t, J = 6.0 Hz, 2H), 3.13-3.19 (m, 1H), 3.43 (dd, J = 11.4, 5.9 Hz, 1H), 3.61–3.82 (m, 9H), 3.89 (t, J = 4.8 Hz, 2H), 4.21 (t, J = 4.8 Hz, 2H), 7.13 (dd, J = 8.0, 2.0 Hz, 1H), 7.40 (t, J = 8.0 Hz, 1H), 7.55–7.63 (m, 3H), 7.97 (dd, J = 8.0, 1.8 Hz, 1H), 8.04 (d, J = 1.8 Hz, 1H), 8.23 (s, 1H), 9.24 (br s, 1H), 12.89 (br s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 738.

tert-Butyl (E)-3-(2-(2-(2-(3-((3-(2-(4-chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)phenoxy)ethoxy)ethoxy)ethoxy)propanoate **(S10).** To a solution of **S5** (1.76 g, 3.20 mmol) in DMF (15 mL) were added **S7** (4.15 g, 9.60 mmol), potassium carbonate (1.77g, 12.8 mmol), and NaI (0.24 g, 1.6 mmol), and the solution was stirred at 100°C for 2 h. After cooling to room temperature, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (99:1 to 95:5 $CHCl_3$/MeOH) to afford **S10** (1.65 g, 64%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (s, 6H), 1.45 (s, 9H), 1.69 (t, J = 5.7 Hz, 2H), 2.49–2.55 (m, 2H), 3.46–3.51 (m, 2H), 3.60–3.76 (m, 12H), 3.89 (t, J = 4.8 Hz, 2H), 4.21 (t, J = 4.8 Hz, 2H), 7.12–7.15 (m, 1H), 7.40 (d, J = 8.2 Hz, 1H), 7.56–7.64 (m, 3H), 7.99–8.06 (m, 2H), 8.24 (s, 1H), 9.24 (br s, 1H), 12.9 (br s, 1H). LC−MS (ESI, $[M - H]^-$, *m/z*) 808.

***3-(2-(2-(2-(Diethylamino)ethoxy)ethoxy)ethoxy)benzoic acid* (S12).** To a solution of **S11** (3.0 g, 19.7 mmol) in DMF (30 mL) were added potassium carbonate (6.54 g, 47.3 mmol) and 2-(2-(2-chloroethoxy)ethoxy)ethanol (8.60 mL, 59.2 mmol), and the solution was stirred at 100°C for 3 h. After cooling to room temperature, the resulting mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue in DMF (100 mL) were added TEA (8.25 mL, 59.2 mmol) and methanesulfonyl chloride (4.61 mL, 59.2 mmol), and stirred at room temperature overnight. The reaction mixture was added sodium iodide (5.91 g, 39.4 mmol) and stirred at 100°C for 2h. After cooling to room temperature, the resulting mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (100:0 to 90:10 $CHCl_3$/MeOH) to afford methyl 3-(2-(2-(2-iodoethoxy)ethoxy)ethoxy)benzoate (3.25 g, 42%). To the solution of methyl 3-(2-(2-(2-iodoethoxy)ethoxy)ethoxy)benzoate (3.25 g, 8.24 mmol) in DMF (60 mL) were added potassium carbonate (5.7 g, 41.2 mmol) and diethylamine (25.8 mL, 247 mmol), and stirred at 100°C overnight. After cooling to room temperature, the resulting mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was dissolved in 50% aqueous EtOH solutioin (60 mL). To this solution was added lithium hydroxide monohydrate (0.685 g, 16.3 mmol) and stirred at room temperature for 1h.

The resulting mixture was quenched with 3M HCl solution and extracted twice with 17% 2-propanol in $CHCl_3$. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10% MeOH in $CHCl_3$) to afford **S12** (1.97 g, 74%) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.37 (t, J = 7.2 Hz, 6H), 3.21–3.26 (m, 6H), 3.65–3.72 (m, 4H), 3.83–3.85 (m, 2H), 3.99–4.02 (m, 2H), 4.18–4.21(m, 2H), 7.11–7.15 (m, 1H), 7.36 (t, J = 7.9 Hz, 1H), 7.64–7.71(m, 2H). The proton of carboxylic acid was not observed. LC−MS (ESI, $[M + H]^+$, *m/z*) 326.

***Benzyl (2-methoxyethyl)glycinate* (S14).** To a solution of **S13** (22.8 mL, 262 mmol) in DCM (50 mL) was added benzyl 2-bromoacetate (2.77 mL, 17.5 mmol) at 0°C and stirred for 0.5 h. Saturated aqueous $NaHCO_3$ solution was added to the reaction mixture and extracted twice with ethyl acetate. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (100/0 to 80/20 $CHCl_3$/MeOH) to afford **S14** (3.5 g, 90%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.91 (br s, 1H), 2.78–2.82 (m, 2H), 3.34 (s, 3H), 3.46–3.49 (m, 4H), 5.16 (s, 2H), 7.31–7.37 (m, 5H). LC−MS (ESI, $[M + H]^+$, *m/z*) 224.

***(E)-N-(3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-3-(19-hydroxy-2,5,8,11,14,17-hexaoxanonadecyl)benzamide* (S15).** To a solution of hexaethylene glycol (6.09 mL, 31.6 mmol) in DMF (40 mL) was added NaH (60% in mineral oil, 1.26 g, 31.6 mmol) at 0°C and stirred for 0.5 h.

To the reaction mixture was added **S4c** (4.6 g, 7.90 mmol), which was then stirred at room temperature for 3 h. The reaction mixture was quenched with a saturated aqueous NH_4Cl solution and extracted twice with $CHCl_3$. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (100/0 to 80/20 $CHCl_3$/MeOH) to afford **S15** (5.16 g, 79%) as an amorphous compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.07 (s, 6H), 1.69 (t, J = 6.0 Hz, 2H), 1.84 (br s, 1H), 2.53 (s, 2H), 2.88 (br s, 2H), 3.58–3.73 (m, 24H), 4.65 (s, 2H), 7.49 (t, J = 7.8 Hz, 1H), 7.57–7.60 (m, 2H), 7.95–8.04 (m, 4H), 8.24 (s, 1H), 9.29 (br s, 1H), 12.89 (br s, 1H). LC–MS (ESI, $[M + H + 17]^+$, *m/z*) 845.

(E)-1-(3-((3-(2-(4-Chloro-3-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)phenyl)-2,5,8,11,14,17-hexaoxanonadecan-19-yl methanesulfonate **(S16).** To a solution of **S15** (1.96 g, 2.37 mmol) in DCM (12 mL) were added methanesulfonyl chloride (0.277 mL, 3.55 mmol) and TEA (0.66 mL, 4.73 mmol) at 0°C, and the solution was stirred at room temperature for 1 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (100/0 to 80/20 $CHCl_3$/MeOH) to afford **S16** (2.16 g, 79%) as an amorphous compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.04 (s, 6H), 1.65 (br s, 2H), 2.48 (br s, 2H), 2.87 (br s, 2H), 3.06 (s, 3H), 3.61–3.75 (m, 22H), 4.34–4.37 (m, 2H), 4.64 (s, 2H), 7.49–7.55 (m, 3H), 7.94–8.01 (m, 4H), 8.26 (s, 1H), 9.40 (br s, 1H) , 12.88

(br s, 1H). LC−MS (ESI, $[M + H + 17]^+$, *m/z*) 923.

tert-Butyl 2-amino-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate **(S18).** To a solution of 4,4-dimethylcyclohexanone (**S17**) (10.0 g, 79.0 mmol) in DMF (80 mL), *tert*-butyl 2-cyanoacetate (11.3 mL, 79.0 mmol), sulfur (2.53 g, 79.0 mmol), and a solution of ethylenediamine (2.64 mL, 40.0 mmol) dissolved in DMF (10 mL) and AcOH (4.5 mL) were added, and the solution was stirred for 23 h at room temperature. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine and water, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to obtain **S18** (24.0 g, quant.). This compound was used for the next reaction without further purification. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.99 (s, 6H), 1.47 (t, J = 6.4 Hz, 2H), 1.55 (s, 9H), 2.27 (s, 2H), 2.67 (t, J = 6.4 Hz, 2H), 5.86 (br s, 2H).

tert-Butyl 2-(3-(chloromethyl)benzamido)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate **(S19).** To a solution of **S18** (24.0 g, 85.0 mmol) in DCM (130 mL), 3-(chloromethyl)benzoyl chloride (12.4 mL, 87.0 mmol) and pyridine (7.00 mL, 87.0 mmol) were added, and the solution was stirred for 0.5 h at room temperature. The reaction mixture was poured into water and extracted with $CHCl_3$. The organic layer was washed with brine and water, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was washed with *n*-hexane to obtain **S19** (29.5 g, 86%), which was a yellow solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.02 (s, 6H), 1.54 (t, J = 6.4 Hz, 2H),

1.62 (s, 9H), 2.46 (s, 2H), 2.77 (t, J = 6.4 Hz, 2H), 4.67 (s, 2H), 7.54 (t, J = 7.8 Hz, 1H), 7.62 (d, J = 7.8 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 8.05 (s, 1H), 12.43 (br s, 1H).

***Methyl 4-((4-amino-2-methylphenyl)ethynyl)benzoate* (S21).** To a solution of 4-iodo-3-methylaniline (**S20**) (0.500 g, 2.15 mmol) in DMF (0.90 mL), $PdCl_2(PPh_3)_2$ (0.075 g, 0.107 mmol), CuI (0.020 g, 0.107 mmol), triphenylphosphine (0.113 g, 0.429 mmol), methyl 4-ethynylbenzoate (0.412 g, 2.57 mmol), and diethylamine (3.36 mL, 32.2 mmol) were added; this solution was irradiated in a microwave at 120°C for 1 h. After cooling to room temperature, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with aqueous ammonia solution and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (95:5 to 50:50 *n*-heptane/ethyl acetate) to obtain **S21** (0.282 g, 50%), which was a brown solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H), 3.80 (s, 2H), 3.92 (s, 3H), 6.49 (dd, J = 8.1, 2.2 Hz, 1H), 6.55 (d, J = 2.2 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.53 (d, J = 8.4 Hz, 2H), 7.99 (d, J = 8.4 Hz, 2H). LC−MS (ESI, $[M + H]^+$, *m/z*) 266.

***Methyl 4-(4-amino-2-methylphenethyl)benzoate* (S22).** A solution of **S21** (0.295 g, 1.11 mmol) in EtOH (22.2 mL) was pumped through the H-Cube® Pro (THALESNano) flow hydrogenerator using 10% $Pd(OH)_2$/C cartridges at 40°C. The residue was concentrated *in vacuo* to obtain **S22** (0.282 g, 94%), and this was used for the subsequent reaction without further purification. LC−MS (ESI, $[M + H]^+$, *m/z*) 270.

***Methyl 4-(4-aminophenethyl)benzoate* (S24).** To a solution of diethyl(4-nitrobenzyl)phosphonate (**S23**) (10.3 g, 37.7 mmol) in MeOH (94 mL), 28% sodium methoxide MeOH solution (14.6 mL, 75.0 mmol) was added at 0°C and stirred for 0.5 h at 0°C. Methyl 4-formylbenzoate (6.50 g, 39.6 mmol) was added, and the reaction mixture was stirred overnight at room temperature and then filtered. A stirred solution of the obtained residue in THF (450 mL) and DMF (150 mL) was pumped through the H-Cube® Pro flow hydrogenerator using 10% Pd/C cartridges at 35°C. The residue was concentrated *in vacuo*. Water was subsequently added to the residue, and the solution was then filtered to obtain **S24** (9.00 g, 94%), which was a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.84–2.89 (m, 4H), 3.90 (s, 3H), 6.61 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 7.21 (d, J = 7.8 Hz, 2H), 7.93 (d, J = 7.8 Hz, 2H). The protons of amine were not observed. LC−MS (ESI, $[M + H]^+$, *m/z*) 256.

***Methyl 4-(4-(2-amino-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamido)phenethyl)benzoate* (S25).** To a solution of **S24** (7.66 g, 30.0 mmol) in DMF (60 mL), 2-cyanoacetic acid (3.32 g, 39.0 mmol), HOBt monohydrate (13.8 g, 90.0 mmol), and WSC·HCl (17.3 g, 90.0 mmol) were added at 0°C, and the solution was stirred at room temperature for 2 h. Saturated aqueous $NaHCO_3$ solution was added, and the reaction mixture was extracted twice with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. To a stirred solution of the obtained residue in THF (90 mL), AcOH (5.14 mL, 90.0 mmol), HMDS (11.8 mL, 56.1 mmol), and 4,4-dimethylcyclohexanone (4.25 g, 33.7 mmol) were added, and

the solution was stirred for 2 h at room temperature. The reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (50% *n*-heptane in ethyl acetate). To a stirred solution of the obtained residue in EtOH (43 mL), morpholine (1.59 mL, 18.2 mmol) and sulfur (0.583 g, 18.2 mmol) were added, and the solution was refluxed for 2 h. After cooling to room temperature, the reaction mixture was filtered to obtain **S25** (4.67 g, 34%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.99 (s, 6H), 1.44 (t, J = 6.3 Hz, 2H), 2.27 (s, 2H), 2.64 (t, J = 6.3 Hz, 2H), 2.84–2.88 (m, 2H), 2.93–2.96 (m, 2H), 3.83 (s, 3H), 6.55 (s, 2H), 7.12 (d, J = 8.8 Hz, 2H), 7.37 (d, J = 8.8 Hz, 2H), 7.50 (d, J = 8.8 Hz, 2H), 7.87 (d, J = 8.8 Hz, 2H), 8.85 (s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 463.

***17-Hydroxy-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate* (S27).** To a solution of hexaethylene glycol (**S26**) (15.0 g, 53.1 mmol) in DCM (150 mL), Ag_2O (18.5 g, 80.0 mmol), *p*-toluenesulfonyl chloride (11.1 g, 58.4 mmol), and potassium iodide (1.76 g, 10.6 mmol) were added at 0°C, and the solution was stirred for 0.4 h at 0°C. The reaction mixture was filtered through a celite pad and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH 100:0–80:20) to obtain **S27** (19.7 g, 85%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ

2.45 (s, 3H), 2.75–2.77 (m, 1H), 3.62–3.66 (m, 22H), 4.15–4.17 (m, 2H), 7.35 (d, *J* = 8.1 Hz, 2H), 7.79 (d, *J* = 8.1 Hz, 2H).

***tert-Butyl 20-(tosyloxy)-3,6,9,12,15,18-hexaoxaicosanoate* (S28).** To a solution of hexaethylene glycol (**S26**) (4.88 mL, 19.5 mmol) in THF (100 mL), NaOH (1.17 g, 29.2 mmol), potassium carbonate (5.38 g, 39.0 mmol), and *tert*-butyl 2-bromoacetate (3.17 mL, 21.4 mmol) were added, and the solution was stirred overnight at room temperature. The reaction mixture was washed with saturated aqueous NH_4Cl solution and extracted twice with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate/MeOH 90:10). To a stirred solution of the obtained residue in DCM (39 mL), TEA (2.90 mL, 20.8 mmol) and *p*-toluenesulfonyl chloride (2.64 g, 13.9 mmol) were added at 0°C, and the solution was stirred for 3 h at room temperature. The reaction mixture was washed with 1 M HCl solution, extracted with $CHCl_3$, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH 90:10) to obtain **S28** (3.46 g, 32%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): *d* 1.50 (s, 9H), 2.48 (s, 3H), 3.61 (s, 4H), 3.64–3.76 (m, 18H), 4.05 (s, 2H), 4.19 (t, *J* = 4.8 Hz, 2H), 7.37 (d, *J* = 8.1 Hz, 2H), 7.83 (d, *J* = 8.1 Hz, 2H).

***20-Hydroxy-3,6,9,12,15,18-hexaoxaicosanoic acid* (S29).** To a solution of hexaethylene glycol (**S26**) (6.39 mL, 25.5 mmol) in THF (110 mL), NaOH (1.53 g, 38.3 mmol), potassium carbonate (7.05

g, 51.0 mmol), and *tert*-butyl 2-bromoacetate (4.15 mL, 28.1 mmol) were added, and the solution was stirred overnight at room temperature. The reaction mixture was washed with saturated aqueous NH_4Cl solution and extracted twice with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate/MeOH 95:5–75:25). To a stirred solution of the obtained residue in DCM (30 mL), TFA (10.0 mL, 130 mmol) was added at 0°C, and the solution was stirred for 1 h at room temperature. The reaction mixture was azeotropically evaporated with toluene to obtain **S29** (4.38 g, 50%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): *d* 3.68–3.70 (m, 18H), 3.76–3.80 (m, 4H), 4.18 (s, 2H), 4.48–4.51 (m, 2H), 6.22 (s, 1H). The proton of carboxylic acid was not observed. LC−MS (ESI, $[M-H]^-$, *m/z*) 339.

***tert-Butyl 2-(3-hydroxybenzamido)-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate* (S30).** To a solution of 3-hydroxybenzoic acid (5.10 g, 37.0 mmol) in DCM (80 mL), thionyl chloride (6.22 mL, 85.0 mmol) and DMF (0.440 mL, 5.69 mmol) were added, and the solution was stirred for 4 h at 45°C. The reaction mixture was azeotropically evaporated with toluene. The residue was dissolved in DCM (80 mL), followed by addition of pyridine (3.45 mL, 42.6 mmol) and **S18** (8.00 g, 2.84 mmol). The reaction mixture was stirred overnight at room temperature. Saturated aqueous $NaHCO_3$ solution was added, and the reaction mixture was extracted with $CHCl_3$. The organic layer was washed with 1 M HCl solution, water, and brine; dried over $MgSO_4$; filtered; and

concentrated *in vacuo*. The residue was dissolved in MeOH (100 mL), followed by addition of 4 M NaOH solution (14.2 mL, 56.9 mmol), and the solution was stirred for 2 h at room temperature. The reaction mixture was quenched with 5 M HCl solution and then filtered to obtain **S30** (7.49 g, 66%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): *d* 1.01 (s, 6H), 1.47–1.55 (m, 2H), 1.62 (s, 9H), 2.46 (s, 2H), 2.77 (t, *J* = 7.0 Hz, 2H), 7.07 (d, *J* = 7.0 Hz, 1H), 7.40 (t, *J* = 7.9 Hz, 1H), 7.53 (d, *J* = 7.3 Hz, 1H), 7.60 (s, 1H), 12.39 (s, 1H). The proton of alcohol was not observed. LC−MS (ESI, $[M + H]^+$, *m/z*) 402.

***20-(3-(7,7-Dimethyl-4-oxo-5,6,7,8-tetrahydro-4H-benzo[4,5]thieno[2,3-d][1,3]oxazin-2-yl)phenoxy)-3,6,9,12,15,18-hexaoxaicosanoic acid* (S31).** To a solution of **S30** (2.00 g, 4.98 mmol) in DMF (25 mL), *tert*-butyl 20-(tosyloxy)-3,6,9,12,15,18-hexaoxaicosanoate (**S28**) (3.29 g, 5.98 mmol) and cesium carbonate (2.43 g, 7.47 mmol) were added, and the solution was stirred for 0.5 h at 85°C. After cooling to room temperature, the reaction mixture was washed with brine, extracted with ethyl acetate, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH 93:7). To a stirred solution of the obtained residue in TFA (20 mL), TFAA (3.44 mL, 24.4 mmol) was added at 0°C, and the solution was stirred for 2 h at room temperature. The reaction mixture was azeotropically evaporated with toluene. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH 94:6) to obtain **S31** (2.20 g, 68%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): *d* 1.05 (s, 6H), 1.63 (t, *J* = 6.3 Hz, 2H), 2.57 (s, 2H), 2.97 (t, *J* = 6.3

Hz, 2H), 3.65–3.76 (m, 20H), 3.89–3.91 (m, 2H), 4.15 (s, 2H), 4.23 (t, *J* = 4.9 Hz, 2H), 7.11–7.13 (m, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.77 (s, 1H), 7.86 (d, *J* = 7.8 Hz, 1H). The proton of carboxylic acid was not observed. LC−MS (ESI, $[M + H]^+$, *m/z*) 650.

1-Ethynyl-2-methyl-4-nitrobenzene **(S33).** To a solution of 1-bromo-2-methyl-4-nitrobenzene (**S32**) (2.00 g, 9.26 mmol) in DMF (5.0 mL), $PdCl_2(PPh_3)_2$ (0.195 g, 0.278 mmol), CuI (0.053 g, 0.278 mmol), TEA (19.4 mL, 139 mmol), and trimethylsilylacetylene (2.56 mL, 18.5 mmol) were added, and the solution was stirred for 2 h at room temperature. The reaction mixture was washed with saturated aqueous ammonia solution and brine, extracted with ethyl acetate, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-heptane/ethyl acetate 95:5–60:40). To a stirred solution of the obtained residue in DCM (10 mL) and MeOH (10 mL), potassium carbonate (2.49 g, 18.0 mmol) was added, and the solution was stirred for 0.2 h at room temperature. The reaction mixture was filtered and concentrated *in vacuo* to obtain **S33** (1.45 g, 97%) as a brown solid, and this was used for the next reaction without further purification.

3-Methyl-4-(4-(thiazol-2-yl)phenethyl)aniline **(S34).** To a solution of 2-(4-bromophenyl)thiazole (0.350 g, 1.46 mmol) in DMF (1.0 mL), $PdCl_2(PPh_3)_2$ (0.031 g, 0.044 mmol), CuI (0.0083 g, 0.044 mmol), TEA (3.05 mL, 21.9 mmol), triphenylphosphine (0.057 g, 0.219 mmol), and **S33** (0.329 g, 2.04 mmol) were added, and the solution was irradiated in a microwave for 0.5 h at 120°C. After cooling to room temperature, the reaction mixture was poured into water and extracted with ethyl

acetate. The organic layer was washed with aqueous ammonia solution and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (100% $CHCl_3$). To a stirred solution of the obtained residue in THF (18 mL), Pd/C (0.096 g, 0.905 mmol) was added, and the solution was filled with H_2 gas. The reaction mixture was stirred overnight at room temperature. The residue was filtered through a celite pad and concentrated *in vacuo* to obtain **S34** (0.150 g, 25%) as an orange solid, and this was used for the next reaction without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.20 (s, 3H), 2.77–2.89 (m, 4H), 3.51 (br s, 2H), 6.47–6.49 (m, 2H), 6.89 (d, J = 7.7 Hz, 1H), 7.23 (d, J = 7.7 Hz, 2H), 7.28 (d, J = 3.3 Hz, 1H), 7.84 (d, J = 3.3 Hz, 1H), 7.87 (d, J = 8.1 Hz, 2H). LC−MS (ESI, $[M + H]^+$, *m/z*) 295.

***3-Methyl-4-(4-(oxazol-2-yl)phenethyl)aniline* (S35).** To a solution of **S33** (0.350 g, 2.17 mmol) in DMF (3.0 mL), $PdCl_2(PPh_3)_2$ (0.046 g, 0.065 mmol), CuI (0.012 g, 0.065 mmol), TEA (4.54 mL, 32.6 mmol), and 2-(4-bromophenyl)oxazole (0.584 g, 2.61 mmol) were added, and the solution was stirred for 0.5 h at 70°C. The reaction mixture was washed with saturated aqueous ammonia solution and brine, extracted with ethyl acetate, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-heptane/ethyl acetate 95:5–60:40). A stirred solution of the obtained residue in THF (6.6 mL) was pumped through the H-Cube® Pro flow hydrogenerator using 10% $Pd(OH)_2$/C cartridges at 40°C. The residue was concentrated *in vacuo* to obtain **S35** (0.0899 g, 15%) as an oil, and this was used for the next reaction without further

purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.22 (s, 3H), 2.80–2.92 (m, 4H), 3.44 (br s, 2H), 6.48–6.53 (m, 2H), 6.91 (d, J = 7.7 Hz, 1H), 7.25–7.28 (m, 3H), 7.71 (s, 1H), 7.98 (d, J = 8.4 Hz, 2H). LC−MS (ESI, $[M + H]^+$, *m/z*) 279.

***Methyl 4-(4-((tert-butoxycarbonyl)amino)phenethyl)benzoate* (S36).** To a solution of **S24** (19.7 g, 77.0 mmol) in THF (386 mL), $(Boc)_2O$ (26.9 mL, 116 mmol) and DIPEA (20.2 mL, 116 mmol) were added, and the solution was stirred overnight at room temperature. The reaction mixture was quenched with 1 M HCl solution and extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Diisopropyl ether (200 mL) was added to the residue, and the resulting solution was stirred for 1 h and then filtered to obtain **S36** (20.1 g, 73%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.44 (s, 9H), 2.81 (dd, J = 8.8, 5.9 Hz, 2H), 2.90 (dd, J = 8.8, 5.9 Hz, 2H), 3.81 (s, 3H), 7.05 (d, J = 8.4 Hz, 2H), 7.30–7.33 (m, 4H), 7.84 (d, J = 8.1 Hz, 2H), 9.18 (s, 1H). LC−MS (ESI, $[M-H]^-$, *m/z*) 354.

***tert-Butyl (4-(4-(hydrazinecarbonyl)phenethyl)phenyl)carbamate* (S37).** To a solution of **S36** (10.0 g, 28.1 mmol) in EtOH (100 mL) and THF (100 mL), hydrazine monohydrate (40.9 mL, 844 mmol) was added, and the solution was refluxed overnight. After cooling to room temperature, the reaction mixture was concentrated to a quarter of its volume. The solution was poured into water and stirred for 1 h and then filtered to obtain **S37** (9.54 g, 95%) as a white solid. ^{1}H NMR (300 MHz,

DMSO-d_6): δ 1.45 (s, 9H), 2.81–2.85 (m, 4H), 4.43 (s, 2H), 7.08 (t, J = 9.5 Hz, 2H), 7.23–7.32 (m, 4H), 7.70 (d, J = 8.1 Hz, 2H), 9.18 (s, 1H), 9.65 (s, 1H).

tert-Butyl (4-(4-(2-acetylhydrazine-1-carbonyl)phenethyl)phenyl)carbamate **(S38).** To a solution of **S37** (19.1 g, 53.7 mmol) in DCM (269 mL), pyridine (4.56 mL, 56.4 mmol) and acetyl chloride (4.01 mL, 56.4 mmol) were added at 0°C, and the solution was stirred for 1 h at room temperature. The reaction mixture was concentrated. The residue was poured into water and stirred for 1 h and then filtered to obtain **S38** (21.3 g, quant.) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.45 (s, 9H), 1.89 (s, 3H), 2.81–2.88 (m, 4H), 7.06 (d, J = 8.4 Hz, 2H), 7.25–7.35 (m, 4H), 7.75 (d, J = 8.1 Hz, 2H), 9.18 (s, 1H), 9.80 (s, 1H), 10.2 (s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 398.

4-(4-(5-Methyl-1,3,4-oxadiazol-2-yl)phenethyl)aniline **(S39).** To a solution of **S38** (10.0 g, 25.2 mmol) in toluene (150 mL), thionyl chloride (9.18 mL, 126 mmol) was added, and the solution was refluxed for 1 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. To a stirred solution of the obtained residue in DCM (100 mL), TFA (38.8 mL, 503 mmol) was added at 0°C, and the solution was stirred for 1 h at room temperature. The reaction mixture was azeotropically evaporated with toluene. Saturated aqueous $NaHCO_3$ solution was added, and the reaction mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Diisopropyl ether was added to the residue and stirred for 1 h and then filtered to obtain **S39** (4.97 g, 71%) as a tan solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.57

(s, 3H), 2.73 (t, J = 7.8 Hz, 2H), 2.87 (t, J = 7.8 Hz, 2H), 4.82 (s, 2H), 6.47 (d, J = 7.8 Hz, 2H), 6.86 (d, J = 7.8 Hz, 2H), 7.40 (d, J = 7.8 Hz, 2H), 7.85 (d, J = 7.8 Hz, 2H). LC−MS (ESI, $[M + H]^+$, *m/z*) 280.

2-Cyano-N-(4-(4-(5-methyl-1,3,4-oxadiazol-2-yl)phenethyl)phenyl)acetamide **(S40).** To a solution of **S39** (4.57 g, 16.4 mmol) in DMF (82 mL), 2-cyanoacetic acid (1.81 g, 21.3 mmol), HOBt monohydrate (5.01 g, 32.7 mmol), and WSC·HCl (6.27 g, 32.7 mmol) were added at 0°C, and the solution was stirred for 1 h at room temperature. The reaction mixture was poured into water, stirred for 1 h, and then filtered to obtain **S40** (3.65 g, 64%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.57 (s, 3H), 2.87–2.88 (m, 2H), 2.94–2.95 (m, 2H), 3.87 (s, 2H), 7.18 (d, J = 8.8 Hz, 2H), 7.40–7.44 (m, 4H), 7.86 (d, J = 8.8 Hz, 2H), 10.2 (s, 1H). LC−MS (ESI, $[M + H]^+$, *m/z*) 347.

2-Amino-6,6-dimethyl-N-(4-(4-(5-methyl-1,3,4-oxadiazol-2-yl)phenethyl)phenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide **(S41).** To a solution of **S40** (3.65 g, 10.5 mmol) in THF (53 mL), 4,4-dimethylcyclohexanone (2.00 g, 15.8 mmol), AcOH (2.41 mL, 42.1 mmol), and HMDS (6.63 mL, 31.6 mmol) were added, and the solution was refluxed for 2 h. After cooling to room temperature, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. MeOH was added to the residue and stirred overnight and then filtered. To a stirred solution of the obtained residue in EtOH (36 mL), morpholine (1.24 mL, 14.2 mmol) and sulfur (0.456 g, 14.2 mmol) were added, and

the solution was refluxed for 2 h. After cooling to room temperature, the residue was concentrated *in vacuo*. MeOH (8.0 mL) was added to the residue and stirred for 1 h and then filtered to obtain **S41** (3.17 g, 62%) as a brown solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.04 (s, 6H), 1.61 (t, J = 6.4 Hz, 2H), 2.36 (s, 2H), 2.61 (s, 3H), 2.77 (t, J = 6.4 Hz, 2H), 2.92–2.97 (m, 4H), 6.00 (s, 2H), 7.11 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 8.4 Hz, 2H), 7.44 (dd, J = 6.6, 1.8 Hz, 2H), 7.52 (s, 1H), 7.92 (dd, J = 6.6, 1.8 Hz, 2H). LC–MS (ESI, $[M + H]^+$, *m/z*) 487.

2-(3-(Chloromethyl)benzamido)-6,6-dimethyl-N-(4-(4-(5-methyl-1,3,4-oxadiazol-2-yl)phenethyl)phenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide **(S42).** To a solution of **S41** (0.617 g, 1.27 mmol) in DCM (5.0 mL), 3-(chloromethyl)benzoyl chloride (0.270 mL, 1.90 mmol) and pyridine (0.154 mL, 1.90 mmol) were added, and the solution was stirred for 1 h at room temperature. The reaction mixture was quenched with 10% HCl solution and extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to obtain **S42** (0.750 g, 93%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09 (s, 6H), 1.70 (t, J = 5.9 Hz, 2H), 2.56 (s, 2H), 2.62 (s, 3H), 2.92 (t, J = 6.3 Hz, 2H), 2.99–3.03 (m, 4H), 4.68 (s, 2H), 7.19 (d, J = 8.8 Hz, 2H), 7.30 (d, J = 8.8 Hz, 2H), 7.50–7.55 (m, 3H), 7.62–7.66 (m, 1H), 7.75 (s, 1H), 7.95–7.96 (m, 3H), 8.07 (s, 1H), 13.14 (s, 1H). LC–MS (ESI, $[M + H]^+$, *m/z*) 639.

2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)acetic acid **(S44).** To a solution of triethylene glycol (**S43**) (5.00 g, 33.3 mmol) in THF (100 mL), NaOH (2.00 g, 49.9 mmol), potassium carbonate (9.20 g, 66.6 mmol), and *tert*-butyl 2-bromoacetate (5.41 mL, 36.6 mmol) were added, and the solution was stirred overnight at room temperature. The reaction mixture was washed with saturated aqueous NH_4Cl solution and extracted twice with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate/MeOH 90:10). To a stirred solution of the obtained residue in DCM (47 mL), TFA (11.0 mL, 142 mmol) was added at 0°C, and the solution was stirred for 2 h at room temperature. The reaction mixture was azeotropically evaporated with toluene to obtain **S44** (1.16 g, 17%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): *d* 3.61–3.77 (m, 12H), 4.17–4.20 (m, 2H), 4.30–4.35 (m, 1H). The proton of carboxylic acid was not observed.

謝辞

本研究結果を纏める上で以下の方々に多大なるご協力を頂いた。厚く御礼を申し上げます。

協和キリン株式会社：平田勇樹、細江慎太郎、大内潤、成島和哉、辻禎郎、高田英宜、内井雅子、秋澤絵美、柳沢新、水口美弥子、菅原正森、藤原克昭、村井美紀、萩倉敏夫、濱口晃規、小平浩史、小野寺秀幸

また、本論文を査読頂いた市川聡先生、薬師寺文華先生、渡邉瑞貴先生に厚く御礼申し上げます。

最後に、本研究を纏めるにあたって、終始ご指導頂いた周東智先生に心より感謝申し上げます。

参考文献

1. Marks, J.; Debnam, E. S.; Unwin, R. J. Phosphate homeostasis and the renal-gastrointestinal axis. *Am. J. Physiol. Renal Physiol.* **2010**, *299*, F285−296.

2. Penido, M. G. M. G.; Alon, U. S. Phosphate homeostasis and its role in bone health. Pediatr. Nephrol. **2012**, 27, 2039−2048.

3. Chande, S.; Bergwitz, C. Role of phosphate sensing in bone and mineral metabolism. *Nat. Rev. Endocrinol.* **2018**, *14*, 637−655.

4. Biber, J.; Hernando, N.; Forster, I.; Murer, H. Regulation of phosphate transport in proximal tubules. *Pflugers Arch.* **2009**, *458*, 39−52.

5. Blaine, J.; Chonchol, M.; Levi, M. Renal control of calcium, phosphate, and magnesium homeostasis. *Clin. J. Am. Soc. Nephrol.* **2015**, *10*, 1257-1272.

6. Manghat, P.; Sodi, R.; Swaminathan, R. Phosphate homeostasis and disorders. *Ann. Clin. Biochem.* **2014**, *51*, 631−656.

7. Sabbagh, Y.; O'Brien, S. P.; Song, W.; Boulanger, J. H.; Stockmann, A.; Arbeeny, C.; Schiavi, S. C. Intestinal npt2b plays a major role in phosphate absorption and homeostasis. *J. Am. Soc. Nephrol.* **2009**, *20*, 2348−2358.

8. Wagner, C. A.; Hernando, N.; Forster, I. C.; Biber, J. The SLC34 family of sodium-dependent phosphate transporters. *Pflugers Arch.* **2014**, *466*, 139−153.

9. Jacquillet, G.; Unwin, R. J. Physiological regulation of phosphate by vitamin D, parathyroid hormone (PTH) and phosphate (Pi). *Pflugers Arch.* **2019**, *471*, 83−98.

10. Bergwitz, C.; Juppner, H. Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. *Annu. Rev. Med.* **2010**, *61*, 91−104.

11. Maemoto, M.; Hirata, Y.; Hosoe, S.; Ouchi, J.; Narushima, K.; Akizawa, E.; Tsuji, Y.; Takada, 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*, 1946−1960.

12. Ketteler, M.; Block, G. A.; Evenepoel, P.; Fukagawa, M.; Herzog, C. A.; McCann, L.; Moe, S. M.; Shroff, R.; Tonelli, M. A.; Toussaint, N. D.; Vervloet, M. G.; Leonard, M. B. Executive summary of the 2017 KDIGO Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) Guideline Update: what's changed and why it matters. *Kidney Int.* **2017**, *92*, 26−36.

13. Kestenbaum, B.; Sampson, J. N.; Rudser, K. D.; Patterson, D. J.; Seliger, S. L.; Young, B.; Sherrard, D. J.; Andress, D. L. Serum phosphate levels and mortality risk among people with chronic kidney disease. *J. Am. Soc. Nephrol.* **2005**, *16*, 520−528.

14. Hruska, K. A.; Mathew, S.; Lund, R.; Qiu, P.; Pratt, R. Hyperphosphatemia of chronic kidney disease. *Kidney Int.* **2008**, *74*, 148−157.

15. Adeney, K. L.; Siscovick, D. S.; Ix, J. H.; Seliger, S. L.; Shlipak, M. G.; Jenny, N. S.; Kestenbaum, B. R. Association of serum phosphate with vascular and valvular calcification in moderate CKD. *J. Am. Soc. Nephrol.* **2009**, *20*, 381−387.

16. Eddington, H.; Hoefield, R.; Sinha, S.; Chrysochou, C.; Lane, B.; Foley, R. N.; Hegarty, J.; New, J.; O'Donoghue, D. J.; Middleton, R. J.; Kalra, P. A. Serum phosphate and mortality in patients with chronic kidney disease. *Clin. J. Am. Soc. Nephrol.* **2010**, *5*, 2251−2257.

17. Sim, J. J.; Bhandari, S. K.; Smith, N.; Chung, J.; Liu, I. L. A.; Jacobsen, S. J.; Kalantar-Zadeh, K. Phosphorus and risk of renal failure in subjects with normal renal function. *Am. J. Med.* **2013**, *126*, 311−318.

18. KDIGO. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int. Suppl.* **2009**, *76*, S1−S130.

19. Evenepoel, P.; Selgas, R.; Caputo, F.; Foggensteiner, L.; Heaf, J. G.; Ortiz, A.; Kelly, A.; Chasan-Taber, S.; Duggal, A.; Fan, S. Efficacy and safety of sevelamer hydrochloride and calcium acetate in patients on peritoneal dialysis. *Nephrol. Dial. Transplant.* **2009**, *24*, 278−285.

20. Hutchison, A. J.; Maes, B.; Vanwalleghem, J.; Asmus, G.; Mohamed, E.; Schmieder, R.; Backs, W.; Jamar, R.; Vosskuhler, A. Efficacy, tolerability, and safety of lanthanum carbonate in hyperphosphatemia: a 6-month, randomized, comparative trial versus calcium carbonate. *Nephron Clin. Pract.* **2005**, *100*, c8−c19.

21. Barreto, F. C.; Barreto, D. V.; Massy, Z. A.; Drueke, T. B. Strategies for phosphate control in patients with CKD. *Kidney Int Rep* **2019**, *4*, 1043−1056.

22. Dolores Arenas, M.; Perez-Garcia, R.; Bennouna, M.; Blanco, A.; Mauricio, O.; Prados, M. D.; Lerma, J. L.; Luisa Muniz, M.; Belen Martin, M.; Estudio, C. Improvement of therapeutic compliance in haemodialysis patients with poor phosphorus control and adherence to treatment with binders: COMQUELFOS study. *Nefrologia* **2013**, *33*, 196−203.

23. Fissell, R. B.; Karaboyas, A.; Bieber, B. A.; Sen, A.; Li, Y.; Lopes, A. A.; Akiba, T.; Bommer, J.; Ethier, J.; Jadoul, M.; Pisoni, R. L.; Robinson, B. M.; Tentori, F. Phosphate binder pill burden, patient-reported non-adherence, and mineral bone disorder markers: Findings from the DOPPS. *Hemodial Int.* **2016**, *20*, 38−49.

24. Van Camp, Y. P. M.; Vrijens, B.; Abraham, I.; Van Rompaey, B.; Elseviers, M. M. Adherence to phosphate binders in hemodialysis patients: prevalence and determinants. *J Nephrol.* **2014**, *27*, 673−679.

25. Kasai, S.; Sato, K.; Murata, Y.; Kinoshita, Y. Randomized crossover study of the efficacy and safety of sevelamer hydrochloride and lanthanum carbonate in Japanese patients undergoing hemodialysis. *Ther. Apheresis Dial.* **2012**, *16*, 341−349.

26. Hutchison, A. J.; Maes, B.; Vanwalleghem, J.; Asmus, G.; Mohamed, E.; Schmieder, R.;

Backs, W.; Jamar, R.; Vosskühler, A. Long-term efficacy and tolerability of lanthanum carbonate: results from a 3-year study. *Nephron Clin. Pract.* **2006**, *102*, c61−c71.

27. Block, G. A.; Wheeler, D. C.; Persky, M. S.; Kestenbaum, B.; Ketteler, M.; Spiegel, D. M.; Allison, M. A.; Asplin, J.; Smits, G.; Hoofnagle, A. N.; Kooienga, L.; Thadhani, R.; Mannstadt, M.; Wolf, M.; Chertow, G. M. Effects of phosphate binders in moderate CKD. *J. Am. Soc. Nephrol.* **2012**, *23*, 1407−1415.

28. Eto, N.; Miyata, Y.; Ohno, H.; Yamashita, T. Nicotinamide prevents the development of hyperphosphataemia by suppressing intestinal sodium-dependent phosphate transporter in rats with adenine-induced renal failure. *Nephrol. Dial. Transplant.* **2005**, *20*, 1378−1384.

29. Corut, A.; Senyigit, A.; Ugur, S. A.; 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.

30. 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.

31. Hernando, N.; Myakala, K.; Simona, F.; Knopfel, T.; Thomas, L.; Murer, H.; Wagner, C. A.; Biber, J. Intestinal depletion of NaPi-IIb/Slc34a2 in mice: renal and hormonal adaptation. *J. Bone Miner. Res.* **2015**, *30*, 1925−1937.

32. Matsuo, A.; Negoro, T.; Seo, T.; Kitao, Y.; Shindo, M.; Segawa, H.; Miyamoto, K. Inhibitory effect of JTP-59557, a new triazole derivative, on intestinal phosphate transport in vitro and in vivo. *Eur. J. Pharmacol.* **2005**, *517*, 111−119.

33. Filipski, K. J.; Sammons, M. F.; Bhattacharya, S. K.; Panteleev, J.; Brown, J. A.; Loria, P. M.; Boehm, M.; Smith, A. C.; Shavnya, A.; Conn, E. L.; Song, K.; Weng, Y.; Facemire, C.; Juppner, H.; Clerin, V. Discovery of orally bioavailable selective inhibitors of the sodium-phosphate cotransporter NaPi2a (SLC34A1). *ACS Med. Chem. Lett.* **2018**, *9*, 440−445.

34. Weinstock, J. Inhibitors of sodium-dependent phosphate transport. *Expert Opin. Ther. Pat.* **2004**, *14*, 81−84.

35. Kitao, Y.; Kawakami, H.; Matsuo, A. Triazole compound and medicinal use thereof. WO 2003048134 A1, **2003**.

36. Hachiya, S.; Miura, M.; Imamura, Y.; Kaga, D.; Sato, I.; Moritomo, H.; Kato, K.; Terai, K.; Terada, Y. Tetrahydrobenzothiophene compound. WO 2011136269 A1, **2011**.

37. Tsuboi, Y.; Ohtomo, S.; Ichida, Y.; Hagita, H.; Ozawa, K.; Iida, M.; Nagao, S.; Ikegami, H.; Takahashi, T.; Horiba, N. EOS789, a novel pan-phosphate transporter inhibitor, is effective for the

treatment of chronic kidney disease-mineral bone disorder. *Kidney Int.* **2020**, *98*, 343−354.

38. Larsson, T. E.; Kameoka, C.; Nakajo, I.; Taniuchi, Y.; Yoshida, S.; Akizawa, T.; Smulders, R. A. NPT-IIb inhibition does not improve hyperphosphatemia in CKD. *Kidney Int Rep.* **2018**, *3*, 73−80.

39. Block, G. A.; Rosenbaum, D. P.; Yan, A.; Chertow, G. M. Efficacy and safety of Tenapanor in patients with hyperphosphatemia receiving maintenance hemodialysis: a randomized Phase 3 trial. *J. Am. Soc. Nephrol.* **2019**, *30*, 641−652.

40. Saeki, S.; Kunitomo, H.; Narita, Y.; Mimura, H.; Nishi, T.; Sasaki, K. A reporter assay for G-protein-coupled receptors using a B-cell line suitable for stable episomal expression. *Anal. Biochem.* **2010**, *400*, 163−172.

41. Eto, N.; Nagao, R.; Miyazaki, T. Preparation of benzamide compounds as phosphorus transport inhibitors. WO 2004085382 A1, **2004**.

42. Kansy, M.; Senner, F.; Gubernator, K. Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes. *J. Med. Chem.* **1998**, *41*, 1007−1010.

43. Veber, D. F.; Johnson, S. R.; Cheng, H. Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.* **2002**, *45*, 2615−2623.

44. MacIntyre, A. C.; Cutler, D. J. The potential role of lysosomes in tissue distribution of weak bases. *Biopharm. Drug Dispos.* **1988**, *9*, 513−526.

45. Rodgers, T.; Leahy, D.; Rowland, M. Tissue distribution of basic drugs: accounting for enantiomeric, compound and regional differences amongst beta-blocking drugs in rat. *J. Pharm. Sci.* **2005**, *94*, 1237−1248.

46. Nigade, P. B.; Gundu, J.; Sreedhara Pai, K.; Nemmani, K. V. S. Prediction of Tissue-to-Plasma Ratios of Basic Compounds in Mice. *Eur. J. Drug Metab. Pharmacokinet.* **2017**, *42*, 835−847.

47. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Delivery Rev. **1997**, 23, 3−25.

48. Marks, J.; Srai, S. K.; Biber, J.; Murer, H.; Unwin, R. J.; Debnam, E. S. Intestinal phosphate absorption and the effect of vitamin D: a comparison of rats with mice. *Exp. Physiol.* **2006**, *91*, 531−537.

49. Preziosi, P. Isoniazid: metabolic aspects and toxicological correlates. *Curr Drug Metab.* **2007**, *8*, 839−851.

50. Yee, J.; Rosenbaum, D.; Jacobs, J. W.; Sprague, S. M. Small intestinal phosphate absorption: novel therapeutic implications. *Am. J. Nephrol.* **2021**, *52*, 522−530.

51. Marks, J.; Lee, G. J.; Nadaraja, S. P.; Debnam, E. S.; Unwin, R. J. Experimental and regional

variations in Na+-dependent and Na+-independent phosphate transport along the rat small intestine and colon. *Physiol Rep.* **2015**, *3*.

52. Vorland, C. J.; Biruete, A.; Lachcik, P. J.; Srinivasan, S.; Chen, N. X.; Moe, S. M.; Hill Gallant, K. M. Kidney disease progression does not decrease intestinal phosphorus absorption in a rat model of chronic kidney disease-mineral bone disorder. *J. Bone Miner. Res.* **2020**, *35*, 333−342.

53. Maemoto, M.; Hirata, Y.; Hosoe, S.; Ouchi, J.; Uchii, M.; Takada, 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.

54. Hosoi, S.; Murosumi, K.; Sasaki, K.; Satoh, M.; Miyaji, H.; Hasegawa, M.; Itoh, S.; Tamaoki, T.; Sato, S. Optimization of cell culture conditions for G-CSF (granulocyte-colony stimulating factor) production by genetically engineered Namalwa KJM-1 cells. *Cytotechnology* **1991**, *7*, 25−32.

55. Chen, X.; Murawski, A.; Patel, K.; Crespi, C. L.; Balimane, P. V. A novel design of artificial membrane for improving the PAMPA model. *Pharm. Res.* **2008**, *25*, 1511−1520.

56. Toyobuku, H.; Tamai, I.; Ueno, K.; Tsuji, A. Limited influence of P-glycoprotein on small-intestinal absorption of cilostazol, a high absorptive permeability drug. *J. Pharm. Sci.* **2003**, *92*, 2249−2259.

57. Hoffman, D. J.; Seifert, T.; Borre, A.; Nellans, H. N. Method to estimate the rate and extent of intestinal absorption in conscious rats using an absorption probe and portal blood sampling. *Pharm. Res.* **1995**, *12*, 889−894.