博士論文

経口吸収性改善を指向した
新規セファロスポリン系抗菌薬の探索研究

Exploratory research on novel prodrugs for enhancement of oral bioavailability of cephalosporin antibiotics

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略語表

IV	Intravenous
PO	Per Oral
AUC	Area Under the Curve
ACLE·HCl	7-Amino-3-chloromethyl-3-cephem-4-carboxylic acid *p*-methoxybenzyl ester, hydrochloride
BSA	*N,O*-Bis(trimethylsilyl)acetamide
NOESY	The Nuclear Overhauser Enhancement Spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation
PAMPA	Parallel Artificial Membrane Permeability Assay
HBD	Hydrogen Bond Donor
MIC	Minimum Inhibitory Concentration
PPI	Protein-Protein Interaction
GPCR	G-Protein-Coupled Receptor
ANP	Atrial Natriuretic Peptide
HBA	Hydrogen Bond Acceptor

緒論

人類は感染症との闘いを繰り返してきた。ペスト、天然痘、コレラ、スペインかぜ、新型インフルエンザなどパンデミックを起こした感染症に加え、エイズやウイルス性肝炎など慢性感染症と呼ばれるものも数多く知られている。感染症は病気の一種であるが、感染症が及ぼす影響は単に病気の範疇に収まらず、時として戦争を上回るほどの大量死を招き、社会構造の変化を起こし、また一つの文明を滅亡させることもあった。

感染症はいわゆる病原微生物の体内増殖、或いは体内での毒素産生によって生じる病態である。病原微生物としてウイルス、細菌、真菌、原虫等が知られておりこれまでにこれら病原微生物を殺す、或いは増殖を抑制する様々な薬剤が感染症治療薬として見出されてきた[1]。

細菌性感染症は研究が進展している感染症の一つで、1930 年代の A. Fleming らのペニシリンの発見や 1940 年代の S. A. Waksman らのストレプトマイシンの発見以降、多くの人々が細菌性感染症の脅威から救われており、本邦においても 1940 年までは死因別死亡率の上位を占めていた結核や肺炎の死亡率が急激に低下した[2]。しかしながら、新規治療薬の創出による期待とは裏腹に、その薬剤に対する感受性が低下したいわゆる薬剤耐性菌の出現はいわば必然であり、薬剤耐性菌と薬剤との闘いが今もなお続いている。

従来ペニシリンやストレプトマイシンのように微生物が産生する物質の内、他の微生物の発育を阻害する化学物質を抗生物質と定義していた[3]。その後マイトマイシン C 等の抗腫瘍性抗生物質の発見から現在では微生物に留まらず癌細胞等もその対象に含まれている。一方でサルファ剤やキノロン系抗菌薬などを合成抗菌薬と呼称し、抗細菌性抗生物質と合成抗菌薬の総称として抗菌薬という呼称が用いられている(Figure 0-1)。

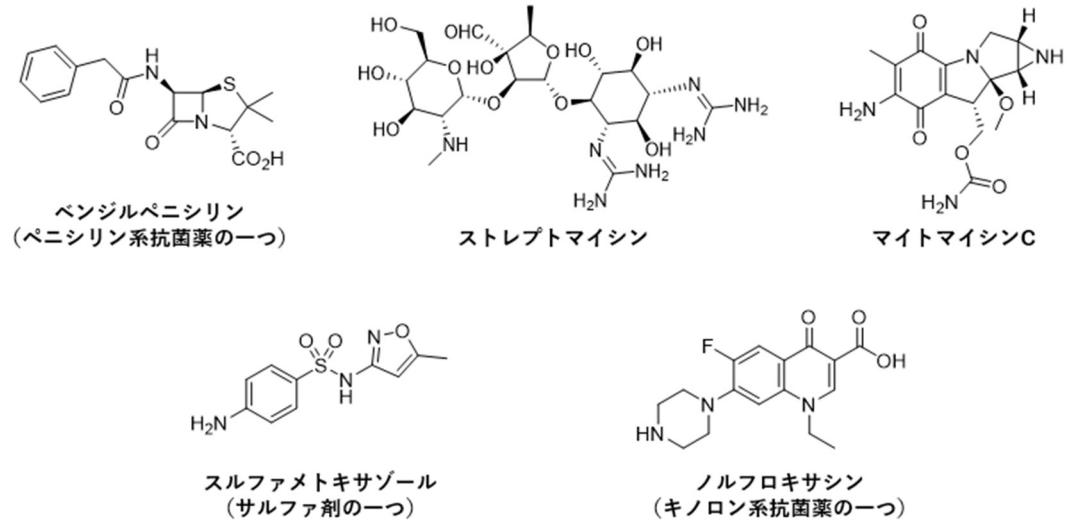


Figure 0-1. 各種化合物の構造

細菌細胞の微細構造には、一般に細胞壁、細胞質膜、リボソーム及び核酸などがあり、抗菌薬はこれらの微細構造を標的部位とし、その構造物の形成阻害、複製阻害または機能阻害等を起こし、結果として細菌細胞に対し殺作用や増殖抑制作用を起こす。細胞壁合成阻害を起こす抗菌薬にはβ-ラクタム系、ホスホマイシン系、グリコペプチド系抗菌薬がある、細胞膜に作用する抗菌薬にはポリペプチド系抗菌薬がある。リボソームに作用し、タンパク合成阻害を起こす抗菌薬にはアミノグリコシド系、マクロライド系、テトラサイクリン系、オキサゾリジノン系抗菌薬などがある。核酸に作用し、核酸合成阻害を起こす抗菌薬にはリファマイシン系、キノロン系抗菌薬などがある(Figure 0-2)[1]。

- **作用機序及び化学構造に基づく分類**
  - a. 細胞壁合成阻害剤
    - a. βラクタム系（ペニシリン系，セファロスポリン系など）
    - b. ホスホマイシン系
    - c. グリコペプチド系
  - b. 細胞膜合成阻害剤
    - a. ポリペプチド系
  - c. 蛋白合成阻害剤
    - a. アミノグリコシド系
    - b. マクロライド系
    - c. テトラサイクリン系
    - d. オキサゾリジノン系
  - d. RNA合成阻害剤
    - a. リファマイシン系
  - e. DNA合成阻害剤
    - a. キノロン系

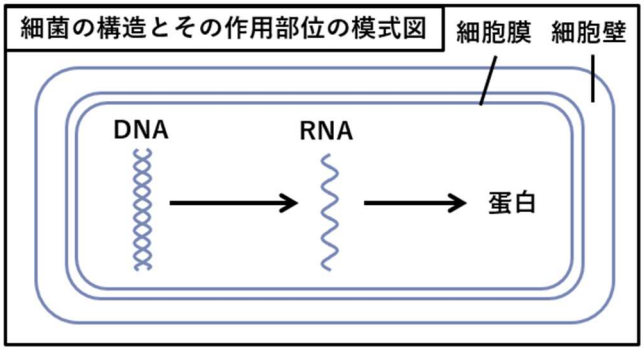
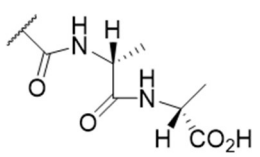
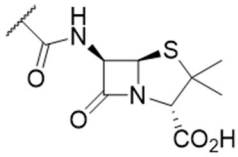


Figure 0-2.　各種抗菌薬の作用部位

β-ラクタム系抗菌薬は標的タンパクであるペニシリン結合タンパク(penicillin-binding-protein：PBP)と結合し細胞壁合成を阻害する。PBP は細胞壁におけるペプチドグリカン合成の最終段階であるペプチド架橋反応を触媒するが、その際の基質認識において重要な D-Ala-D-Ala 構造とβ-ラクタム系抗菌薬の構造が類似するため、β-ラクタム系抗菌薬は競合的にペプチド架橋反応を阻害し、細菌の増殖を抑制、或いは死滅させる(Figure 0-3)。



Acyl-D-Ala-D-Ala 構造



β-ラクタム系抗菌薬の構造
（ペニシリン系抗菌薬）

Figure 0-3. D-Ala-D-Ala 構造とβ–ラクタム系抗菌薬の構造

β-ラクタム系抗菌薬は抗菌薬の中でも最も広く臨床で使用されている抗菌薬の一つであり、ペニシリン系、カルバペネム系、モノバクタム系、セファロスポリン系、セファマイシン系などの分類が知られている(Figure 0-4)。

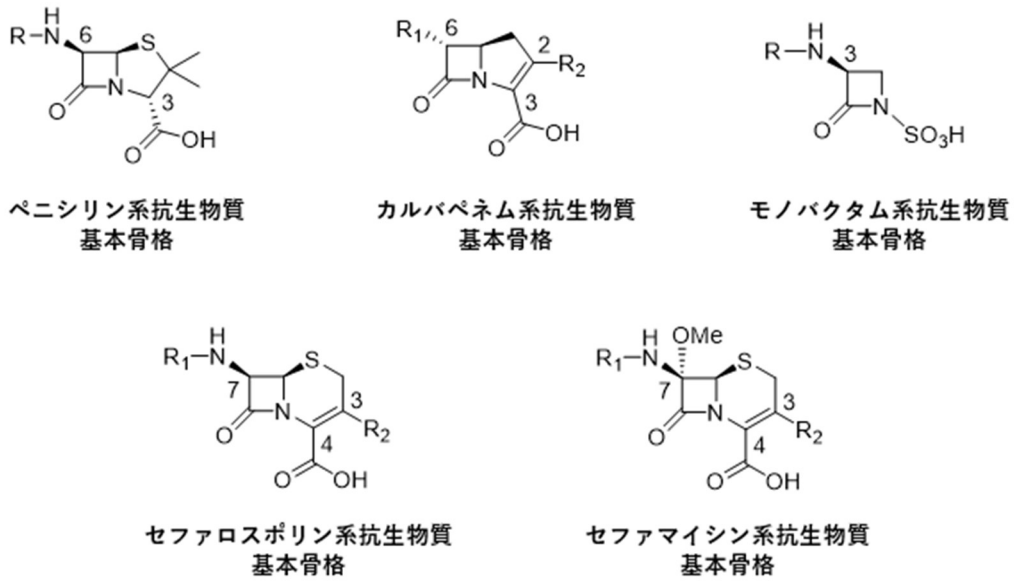


Figure 0-4. β-ラクタム系抗菌薬の基本骨格

これらのうちセファロスポリン系抗菌薬は、*Cephalosporium chrysogenum* の産生するセファロスポリン C[4] が 1955 年に単離、1961 年に構造決定されたのを端緒とし、ペニシリン耐性の細菌に対しても有効であることが示唆されると、ペニシリンの化学修飾をモデルとしてこれまでに活発なセフェム骨格の化学修飾が進められてきた(Figure 0-5)。

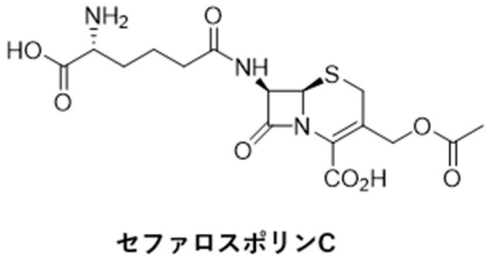


Figure 0-5. セファロスポリン C の構造

その結果、臨床現場に送り出されたセファロスポリンの数はβ-ラクタム系抗菌薬の中では最も多く、現在も治療において欠かせない存在であることは明白である。注射剤、経口剤いずれも多数の研究が行われ、注射剤は 1960 年代に登場したセファロチン、セファロリジン等以降数多くの薬剤が開発され、近年では特に多剤耐性グラム陰性菌に対して非常に強力な抗菌活性を有するセフィデロコル[5]や、セフタジジム・アビバクタム[6]等のβ-ラクタマーゼ阻害剤との合剤が研

究開発及び臨床使用されている(Figure 0-6)。

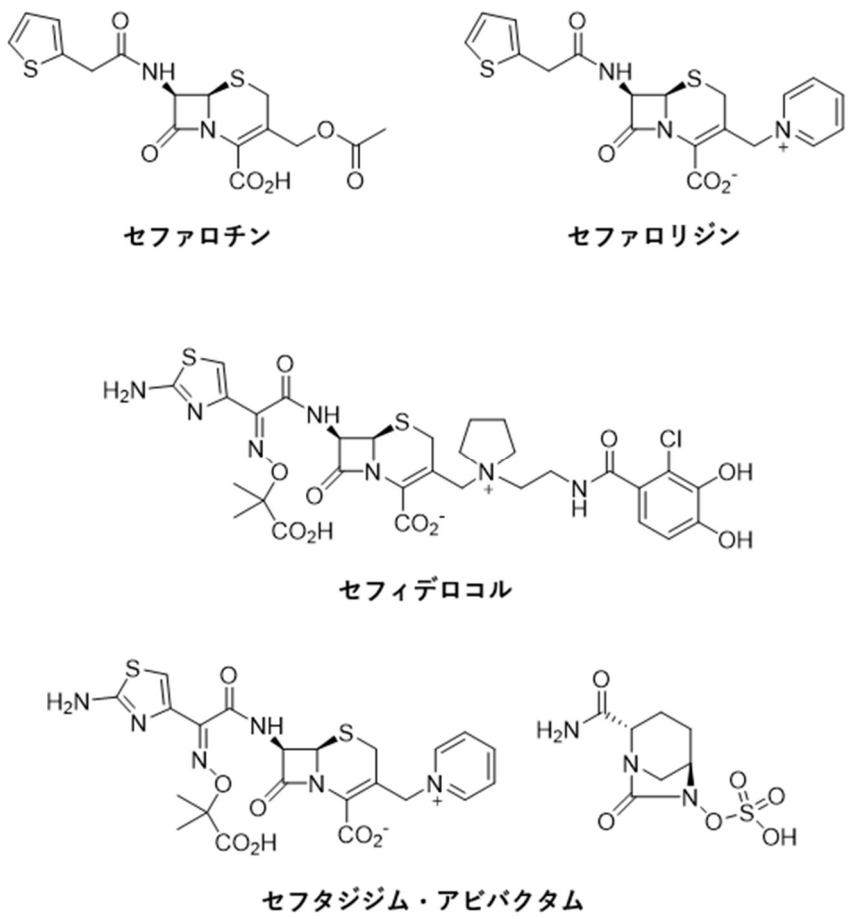
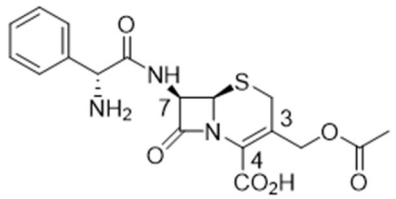


Figure 0-6. 注射用セファロスポリンの構造例

また経口剤は 1965 年にセファログリシン[7](Figure 0-7)が開発されて以来、セファトリジン[8]、セファクロル[9]などの経口剤が相次いで創出された。これらの経口セファロスポリンは第一世代と呼ばれ、7 位側鎖として*(R)*-フェニルグリシン及びその類縁体を有している(Figure 0-8)。これら第一世代はグラム陰性桿菌の産生するβ-ラクタマーゼによって分解される、或いはグラム陰性桿菌の抗菌スペクトルが狭い等の弱点が知られており、これら弱点を克服したセフチブテン[10]、セフィキシム[11]、セフテラムピボキシル[12]、セフジトレンピボキシル[13]等のいわゆる第三世代経口セファロスポリンが現在までに創出されている。しかしながら注射剤と比較した場合、強力な抗菌活性を有する経口剤の数は少なく、良好な抗菌活性と経口吸収性を両立することの難しさが窺い知れる。またこれら第三世代経口セファロスポリンはセフェム核の 7 位側鎖にアミノチアゾリル-アルコキシイミノ酢酸誘導体を有しており、これにより抗菌スペクトルの拡大が

認められていることが特徴の１つとして挙げられるが、このアミノチアゾリル-アルコキシイミノ酢酸誘導体を有するセファロスポリンは一般にそれまでの 7 位側鎖を有するセファロスポリンと比較して経口吸収性が低く、セフチブテン、セフィキシム等の一部を除いて経口剤への適用が困難である。そのため第三世代セファロスポリンの多くは 4 位カルボン酸をエステル化したプロドラッグとして開発されたが、経口吸収性には未だ改善の余地があり、第三世代以降のセファロスポリンに対して経口吸収性改善が期待できる新規修飾法の開発や、十分な経口吸収性と強力な抗菌活性を両立する新規セファロスポリンの創出が望まれている。



セファログリシン

Figure 0-7. セファログリシンの構造

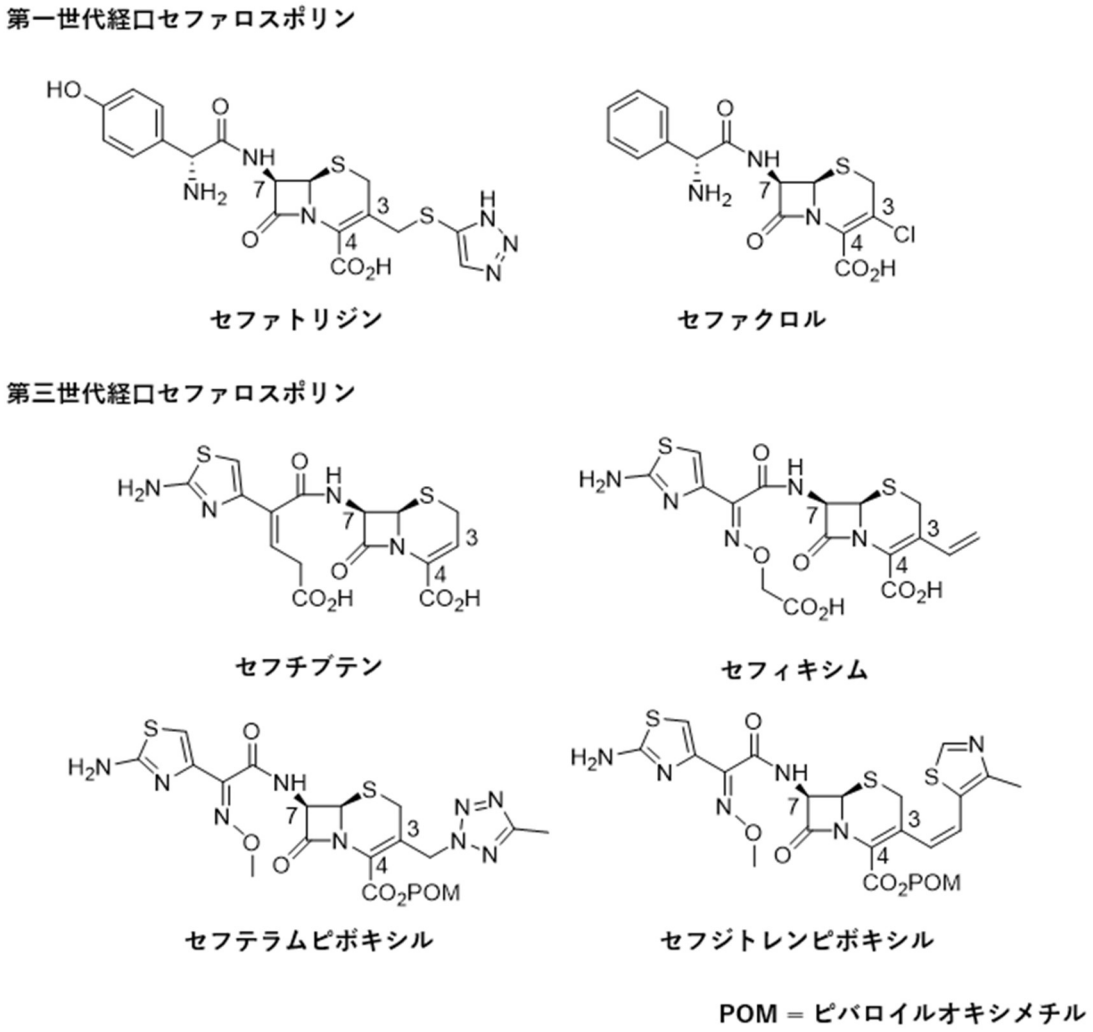


Figure 0-8. 経口用セファロスポリンの構造例

細菌はグラム陰性菌とグラム陽性菌に大別される。グラム陽性菌は細胞壁のペプチドグリカン層がグラム陰性菌と比較して厚いことが特徴として挙げられる一方で、グラム陰性菌は薄いペプチドグリカン層の外側に細胞外膜と呼ばれる外壁層が存在する(Figure 0-9)。この層はタンパク質、脂質、リポタンパク質やリポ多糖からなる脂質二重膜である。またペリプラズムには外膜形成のため水溶性タンパク質が存在している。このようにグラム陰性菌の細胞表層はグラム陽性菌の細胞表層と比較してペプチドグリカン層が薄く水溶性が高いことから、グラム陰性菌に対して強力な抗菌活性を有する薬剤の多くはこの細胞表層を透過するのに有利な水溶性の高い注射剤である。

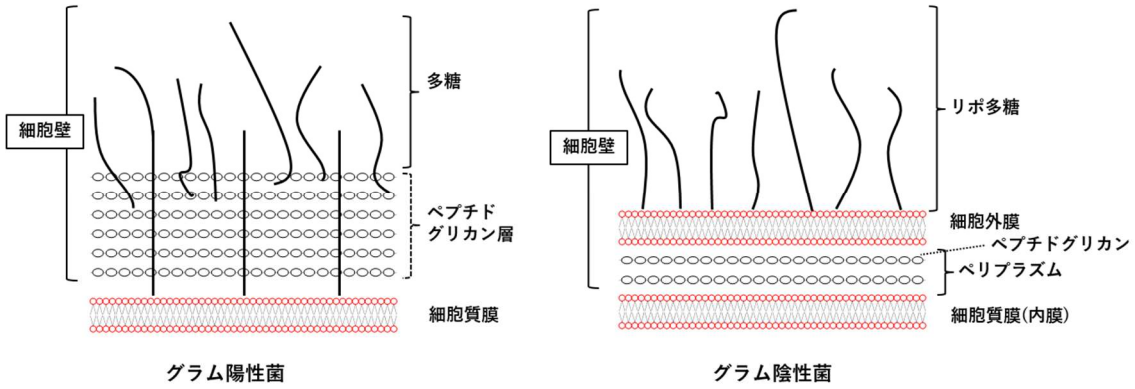


Figure 0-9. グラム陽性菌と陰性菌との細胞構造の違い

近年抗菌薬治療において、早期に静脈内投与による薬剤治療から経口投与による薬剤治療への切り替え(経口ステップダウン療法)を行うことによって、治療効果は維持しつつも入院期間と入院関連費用を低減できる点に注目が集まっている [14]。この経口剤への早期切り替えによる治療法がより広く普及することによって、抗菌薬の適正使用を更に推進できる可能性があり、各種細菌に対して強力な抗菌活性を有する経口剤の創出が望まれている。また一般に経口剤による薬物治療は静脈内注射剤による治療と比較して、利便性が高く侵襲性も低いため患者負担が少ないことから服薬コンプライアンスの改善に寄与する [15, 16] ことも早期の経口ステップダウン療法を求める理由の一つであると考えられる。

生体内利用率或いは生物学的利用能(バイオアベイラビリティ：F)は、静脈内以外の経路で投与された場合に全身循環に到達する割合を示す [17]。経口バイオアベイラビリティ(F_{po})は、静脈内(iv)投与後の血漿中薬物量と経口(po)投与後の血漿中薬物量の比率として定義され、血中薬物濃度-時間曲線下面積(AUC)を用いた以下の式(1)によって表される [18]。

$$F_{po}\,(\%) = \frac{\text{経口投与時のAUC}\ (\mathrm{AUC_{po}})}{\text{経口投与量}\ (\mathrm{dose_{po}})} \times \frac{\text{静脈内投与量}\ (\mathrm{dose_{iv}})}{\text{静脈内投与時のAUC}\ (\mathrm{AUC_{iv}})} \times 100 \qquad (1)$$

経口投与された化合物は、全身循環に至る過程で多くの障害に直面する。主に消化管内での唾液、胃液、十二指腸や小腸中の消化酵素による代謝や各種 pH 条件下における化学的な分解、また胃や小腸での吸収、肝臓での初回通過効果等がそれにあたる [18]。また近年ではこれら代謝酵素の構成や活性に対するマイクロバイオームの影響も指摘されており、経口投与後のバイオアベイラビリティを考える上で従来あまり考慮されてこなかった腸内共生細菌の研究も今後重要になると考えられる。

表面積の大きさや pH 環境などから、経口投与された薬剤のほとんどは胃ではなく空腸と回腸からなる小腸で吸収される。複数の取り込み機構が存在し、受動輸送、能動輸送、小胞輸送が知られている(Figure 0-10)。受動輸送とはエネルギーを必要としない取り込み過程を指し、そのほとんどは単純拡散である。単純拡散は腸全体の濃度勾配に従った吸収経路であり、パラセルラー経路とトランスセルラー経路がある。パラセルラー経路は 8〜18 Å ほどの小腸上皮細胞間の密着結合の間隙を透過する経路であり、トランスセルラー経路は小腸細胞膜を透過する経路を指す[19]。能動輸送とは ATP による駆動力を直接的または間接的に利用し、輸送担体を利用し濃度勾配に逆らって輸送する取り込み過程を指し、小腸での吸収においてそれを担う多くの膜貫通トランスポーターが知られている。小胞輸送とは小胞形成、エンドサイトーシス、エキソサイトーシスからなる小腸細胞を経由する機構であり、いくつかのペプチドなどで小胞輸送による取り込みが認められている[20, 21]。

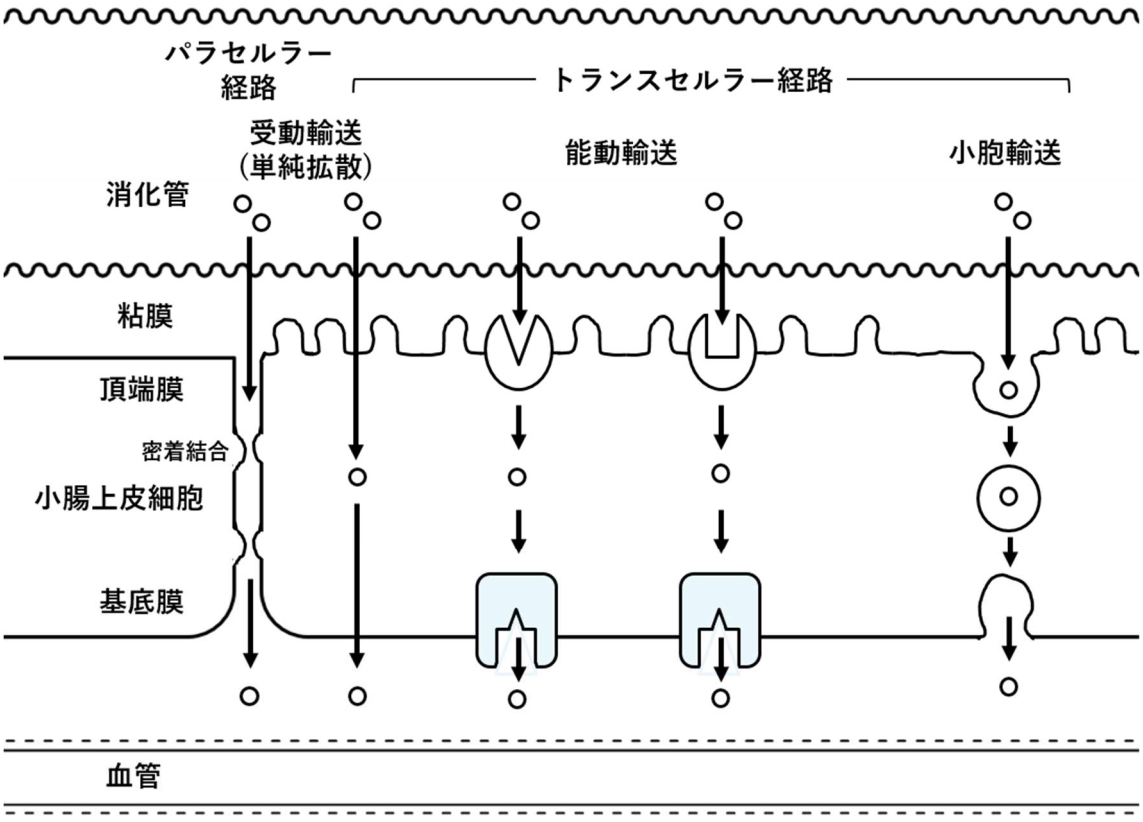


Figure 0-10. 経口投与化合物の小腸からの吸収過程

パラセルラー経路による受動輸送や、能動輸送を介した経口吸収はこれまでに多数報告があるものの、分子量や官能基による制約を受けやすい可能性が指摘

されている。一方でセファロスポリン系抗菌薬を含む従来の市販経口剤の大多数がトランスセルラー経路による受動輸送によって経口吸収されることから[22]、著者は本経路を利用し、経口ステップダウン療法可能な高い経口吸収性と強力な抗菌活性を両立した新規セファロスポリンの創出、またグラム陽性菌或いはグラム陰性菌に対して強力な抗菌活性を有する第三世代以降のセファロスポリンにおける経口吸収性改善のための一般的な新規修飾法の開発を目指し探索研究に着手した。

著者はまず、第三世代以降のセファロスポリンの共通構造であるセフェム核 7 位側鎖のアミノチアゾール部位アミノ基に着目した。アミノ基は脂溶性を低減させ，水素結合供与体(hydrogen bond donor: HBD)となり得るため単純拡散による小腸上皮細胞膜の透過において不利に働くと考えられるためである。この着想に基づき、第一章ではいくつかの第三世代セファロスポリンを用いアミノチアゾールをメチルチアゾール、*N,N*-ジメチルアミノチアゾールへと変換した化合物、またアミノ基をプロドラッグ修飾した化合物を合成、評価した結果についてその詳細を記載する(Figure 0-11)。

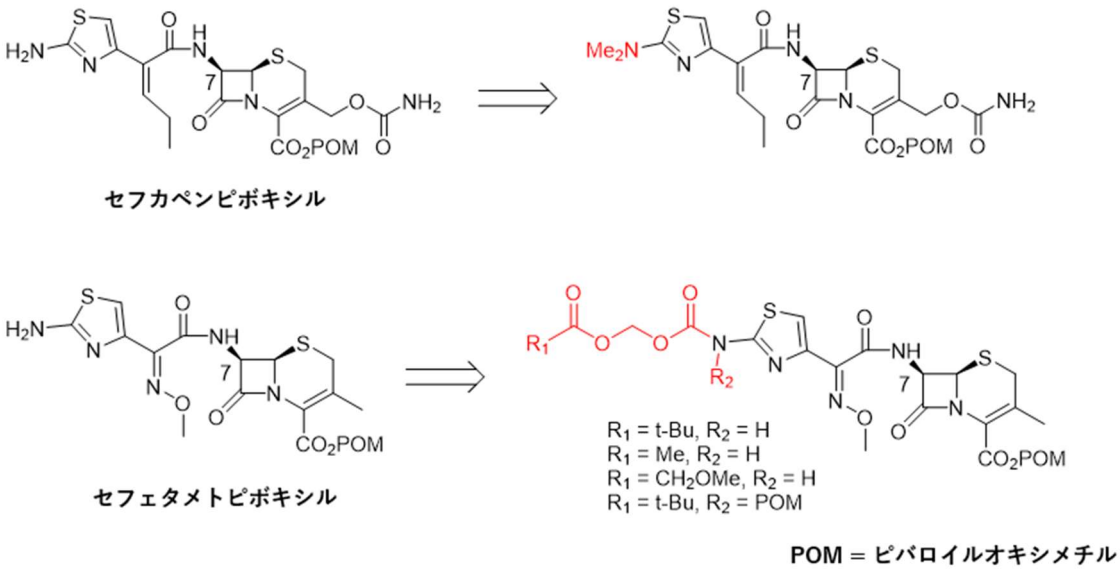


Figure 0-11. 第一章における構造変換例

次いで、近年特に分子量 500 以上の中分子医薬品における経口吸収性改善の手法の一つとして期待されているマクロサイクル化[23]に着目した。従来、医薬品の経口吸収性を規定する物理化学プロファイルの特徴として、Lipinski らによって提唱された経験則の一つであるルールオブファイブ(Rule of Five: Ro5)が知られていた。一方で経口剤として市販・開発されている分子量 500 以上のマク

ロサイクルではこの経験則の複数の項目を逸脱している化合物が多く、この手法をセファロスポリンにおいても適用できないかと考えた。この着想を基に第二章では、第三世代セファロスポリンの一つであるセフトリアキソンを用いてセフェム核 3 位と 4 位を閉環したいくつかの新規セファロスポリンを合成、評価した結果についてその詳細を記述する(Figure 0-12)。

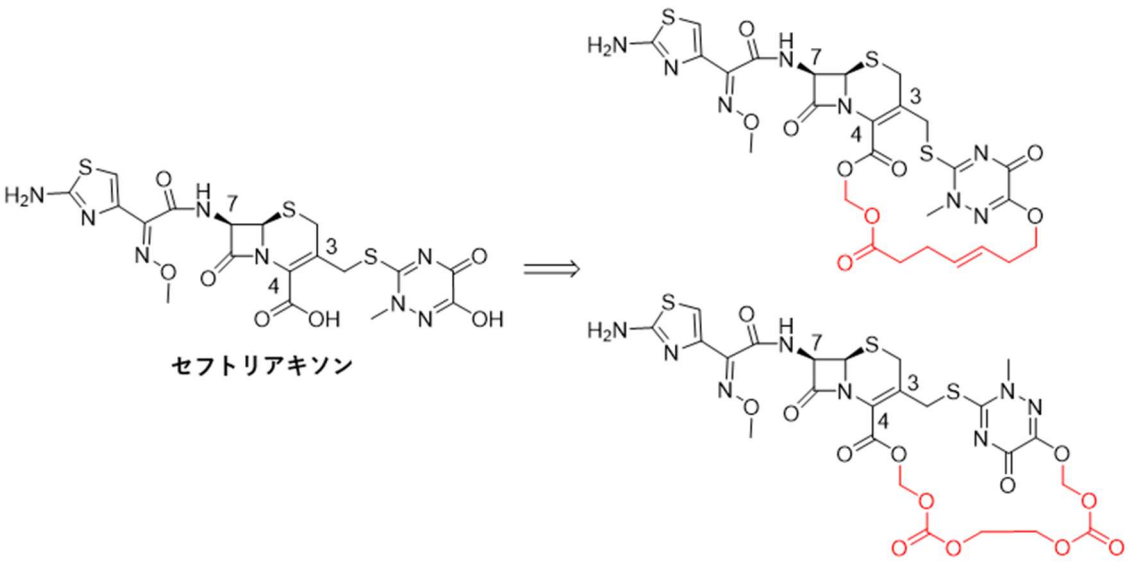


Figure 0-12. 第二章における構造変換例

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本論

第一章

セフェム核 7 位側鎖アミノチアゾール部位アミノ基のプロドラッグ修飾

第一節 経口セファロスポリンの吸収機構

経口セファロスポリンは二つの吸収機構が知られている。

一つはトランスポーターを介した能動輸送であり、活性体である遊離酸のまま吸収される。フェニルグリシン系経口セファロスポリンとアミノチアゾール系経口セファロスポリンがこれらの基質として広く認知されており、フェニルグリシン系経口セファロスポリンはセファトリジン(cefatrizine: CFT)、セファクロル(cefaclor: CCL)、セフロキサジン(cefroxadine: CXD) [1] などが、アミノチアゾール系経口セファロスポリンはセフチブテン(ceftibuten: CETB)、セフィキシム(cefixime: CFIX)、セフジニル(cefdinir: CFDN)[2] などが例として挙げられる(Figure 1-1)。フェニルグリシン系経口セファロスポリンや CFIX、CETB は小腸における吸収過程おいて PEPT1 等のオリゴペプチドトランスポーターを介していることが報告されており [3]、CFDN は PEPT1 による吸収に加え、MCT1 等のモノカルボン酸輸送系を介して吸収されることが報告されている [4]。

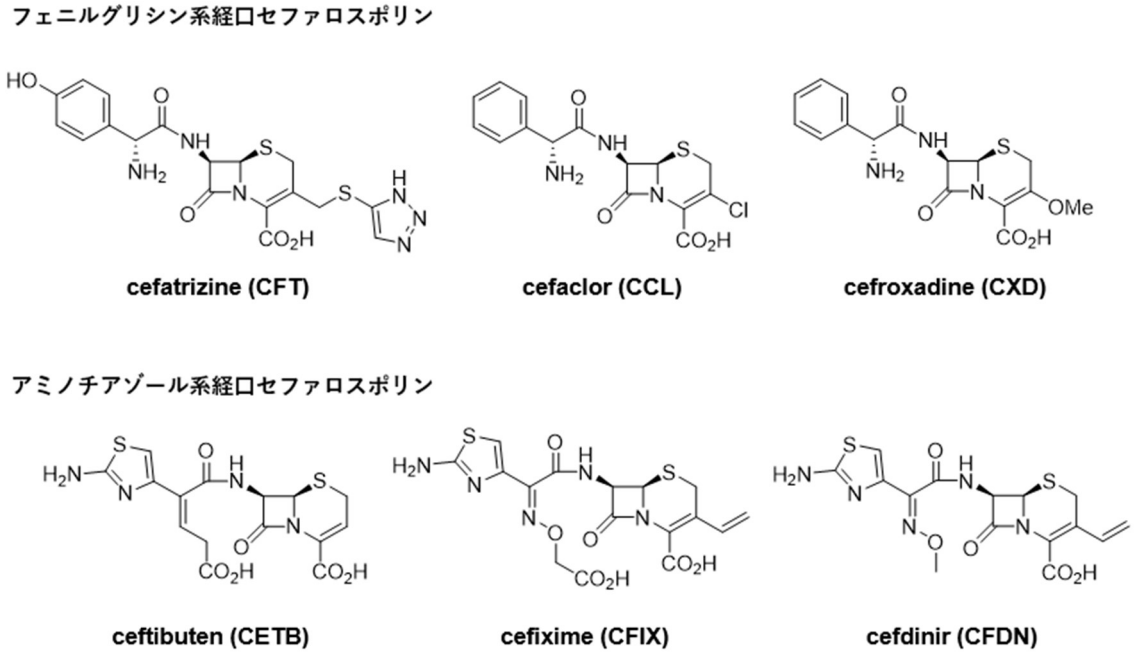


Figure 1-1. トランスポーターを介して吸収されるセファロスポリンの例

一方単純拡散による吸収では、主にセフェム核 4 位のカルボキシル基をエステル基へと変換することで脂溶性の高い非解離型分子とする。本機構を用いる例としてセフテラムピボキシル(cefteram pivoxil: CFTM-PI)、セフカペンピボキシル(cefcapene pivoxil: CFPN-PI)[5]、セフジトレンピボキシル(cefditoren pivoxil: CDTR-PI)、セフェタメトピボキシル(cefetamet pivoxil: CEMT-PI)[6]等のエステル型プロドラッグが知られており、これらは腸管吸収後直ちに腸管壁に存在するエステラーゼにより脱エステル化され、活性体へと再生されて循環血中に入ると考えられる(Figure 1-2)[7]。

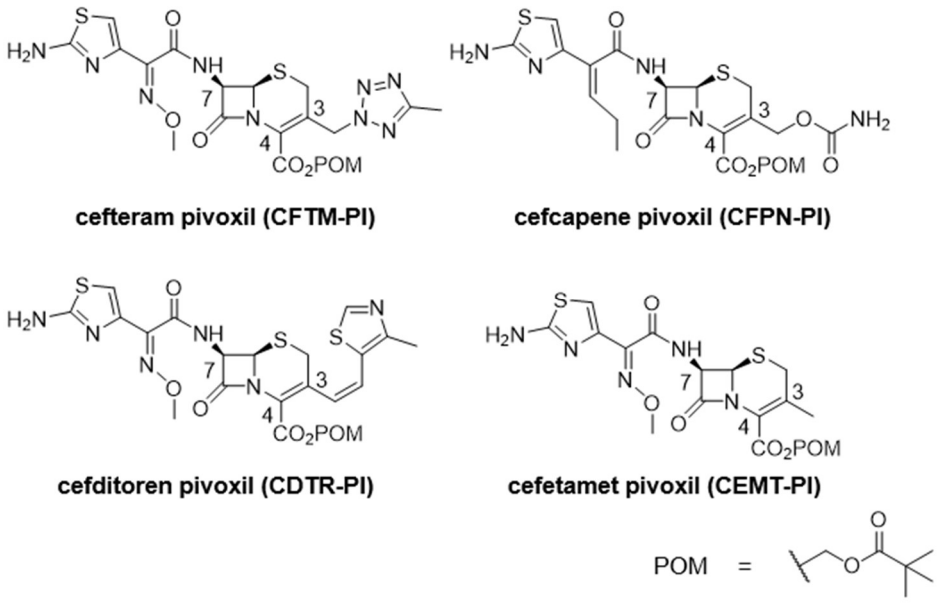


Figure 1-2. 単純拡散によって吸収されるセファロスポリンの例

今回、経口ステップダウン療法が可能な高い経口吸収性と強力な抗菌活性を両立した新規セファロスポリンの創出、またグラム陽性菌或いはグラム陰性菌に対して強力な抗菌活性を有する第三世代以降のセファロスポリンにおける経口吸収性改善のための一般的な新規修飾法の開発を目指すにあたって、これまでの構造経口吸収性相関研究から PEPT1 等のトランスポーターを介した吸収機構と比較し、単純拡散による吸収機構の方が 3 位、7 位側鎖の構造変換に対する許容度が高いことが推測された。そのため本研究では、単純拡散による吸収機構に適した新規セファロスポリンの創出を目指した。

第二節 アミノチアゾール部位変換化合物の合成

第一項　合成化合物の検討

これまでに Lipinski らは良好な経口吸収性或いは膜透過性を示す化合物の指標として、経験則として分子量、脂溶性、水素結合供与体の数、水素結合受容体の数が重要であることを報告している[8]。またいくつかの報告において脂溶性の増大や水素結合供与体数の低減により良好な吸収性と膜透過性を獲得できたことが示唆されている[9]。市販経口剤の大部分が単純拡散による吸収機構を介していることを念頭に、今回著者は第三世代以降のセファロスポリンの共通構造であるアミノチアゾール部位アミノ基に着目した。アミノ基は化合物の脂溶性を低減させ水素結合供与体となり得ることから、アミノ基の変換により経口吸収性の向上が見込めると考えた。アミノチアゾール基の代替構造としてメチルチアゾール基や *N,N*-ジメチルアミノチアゾール基を選択した。また第三世代セファロスポリン系抗菌薬として CFPN-PI、セフトリアキソン(ceftriaxone: CTRX)[10]、CEMT-PI、CFTM-PI を選択し、CTRX においては他のセファロスポリンで汎用されているピバロイルオキシメチル(POM)基[11]の導入化合物も含め、Figure 1-3 に示す 7 位側鎖アミノチアゾール変換化合物を合成することとした。

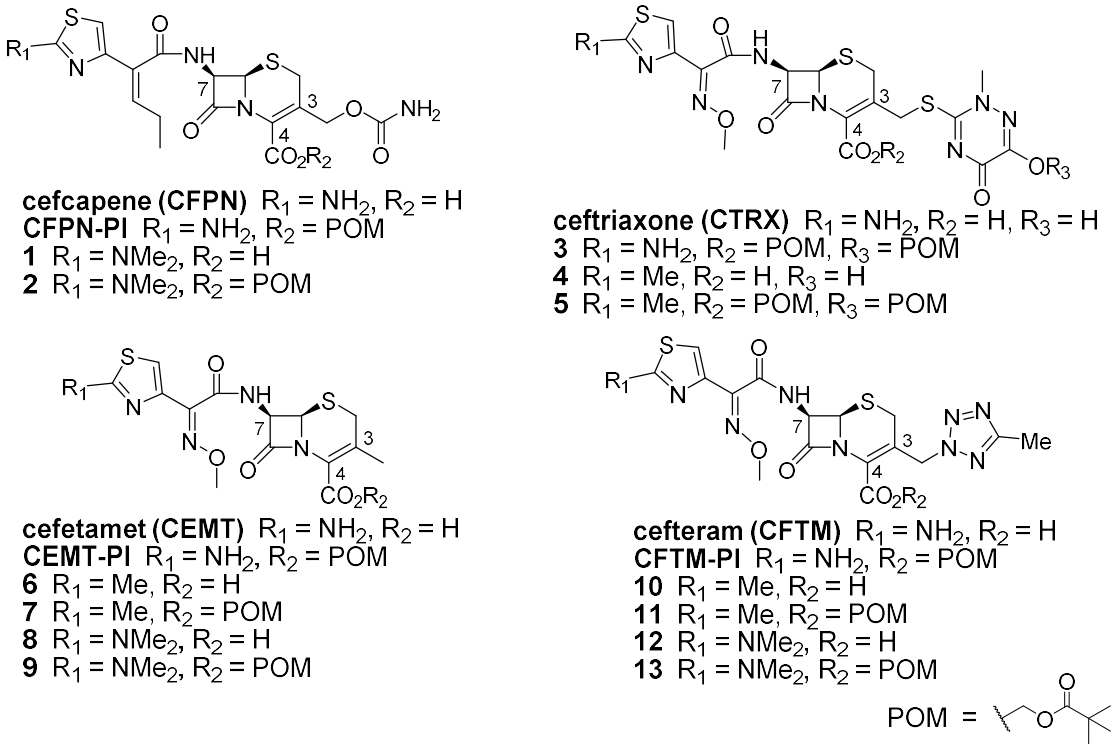
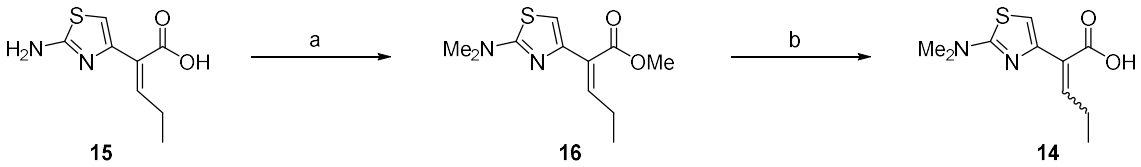


Figure 1-3. 第三世代セファロスポリンとアミノ基変換化合物の構造

第二項　各種 7 位側鎖カルボン酸の合成

まず *N,N*-ジメチルアミノチアゾリル-2-ペンテン酸 **14** の合成を Scheme 1 に記す。**15**[12] のアミノ基をヨウ化メチルを用い *N,N*-ジメチルアミン **16** とし、**16** のエチルエステルを加水分解することで **14** へと導いた。

Scheme 1-1. 7 位側鎖 **14** の合成

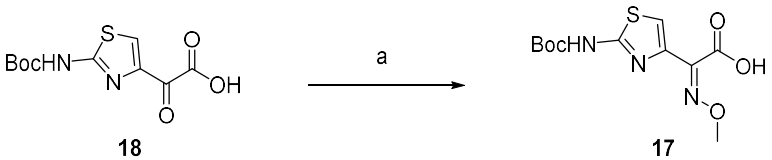


15 **16** **14**

Reagents and conditions: (a) MeI, NaOH, DMF, 60 °C, 44%; (b) 2 M NaOH aq, EtOH, 60 °C, 93% (*E*:*Z* ~ 1:1).

次に *N*-(*tert*-ブトキシカルボニル)-アミノチアゾリル-メトキシイミノ酢酸 **17** はケト酸 **18**[13] をメトキシイミノエステルとした後、エステルの加水分解により合成した(Scheme 1-2)。

Scheme 1-2. 7 位側鎖 **17** の合成

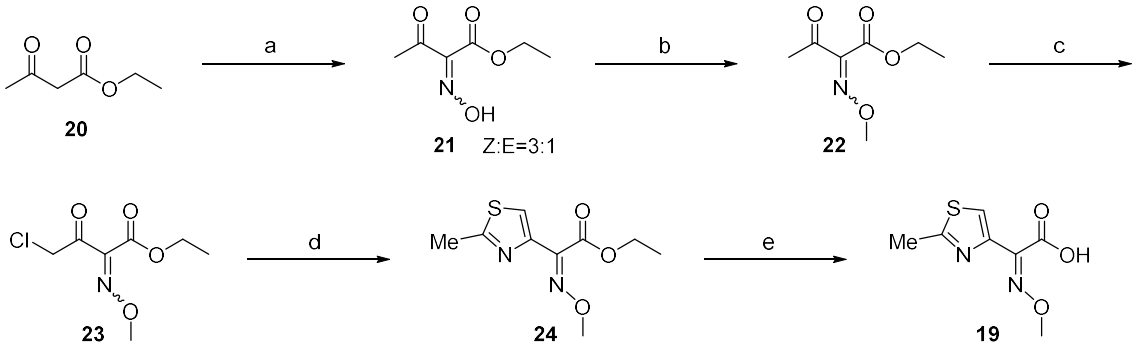


18 **17**

Reagents and conditions: (a) (i) methoxyamine hydrochloride, Et_3N, MeOH, rt; (ii) DIPEA, rt; (iii) 2 M HCl aq. rt, 80% in 3 steps.

次いでメチルチアゾリル-メトキシイミノ酢酸 **19** の合成を Scheme 1-3 に記す。アセト酢酸エステル **20** に亜硝酸ナトリウムを作用させることでオキシム **21** を得た。次いで **21** のオキシム基をジメチル硫酸を用いメチル化することでメトキシイミン **22** を得た。**22** のアセチル基を塩化スルフリルによりクロロ化することで **23** とし、その後チオアセトアミド存在下 50°C に加熱することでメチルチアゾール **24** を得た。最後に **24** のエステル部位を加水分解することで **19** へと導いた。

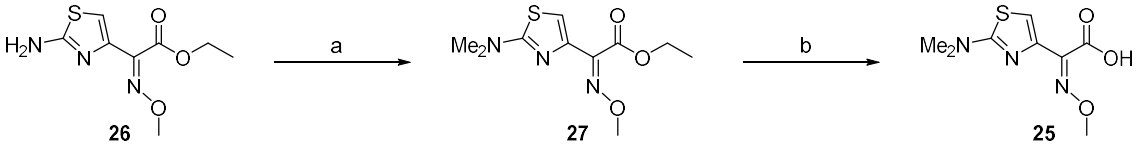
Scheme 1-3. 7 位側鎖 **19** の合成



Reagents and conditions: (a) $NaNO_2$, AcOH, H_2O, 0 °C, quant.; (b) Me_2SO_4, K_2CO_3, acetone, reflux, quant.; (c) SO_2Cl_2, AcOH, rt, quant.; (d) thioacetamide, DMA, 50 °C, 39%; (e) 2 M NaOH aq., MeOH, quant..

次に *N,N*-ジメチルアミノチアゾリル-メトキシイミノ酢酸 **25** の合成を Scheme 1-4 に記す。**26**[14] のアミノ基をヨウ化メチルを用い *N,N*-ジメチル化することでジメチルアミン **27** とし、**27** のエチルエステルを加水分解することで **25** へと導いた。

Scheme 1-4. 7 位側鎖 **25** の合成

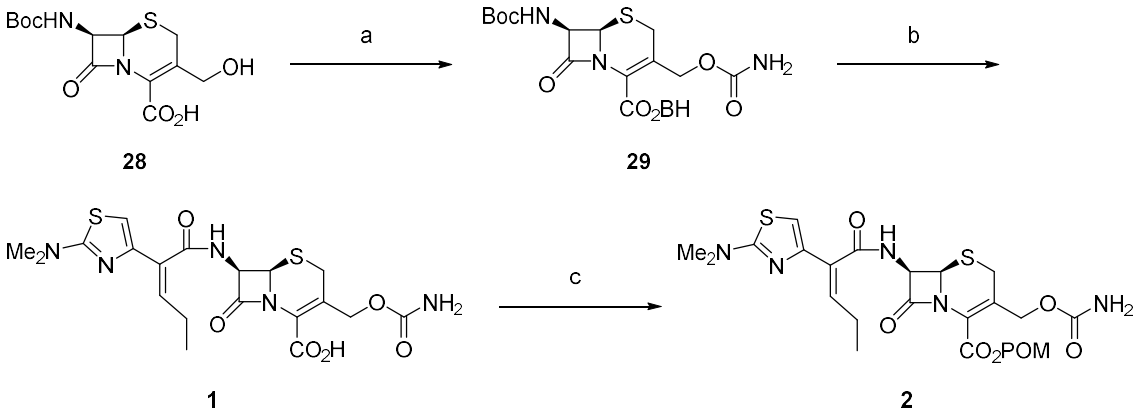


Reagents and conditions: (a) MeI, sodium hydroxide, DMF, 45 °C, 71%; (b) 2 M NaOH aq, MeOH, 0 °C, 88%.

第三項　各種セファロスポリンの合成

7 位側鎖に *N,N*-ジメチルチアゾール基を導入したセフカペン誘導体 **1**, **2** の合成を Scheme 1-5 に記す。既知の方法を用い合成した **28**[15] にイソシアン酸クロロスルホニルを作用させヒドロキシ基をカルバモイル基への変換し、次いでジフェニルジアゾメタンを用いカルボン酸をベンスヒドリルエステル **29** とした。得られた **29** の *tert*-ブトキシカルボニル基を除去し、カルボン酸 **14** と縮合することでアミドとし、ベンズヒドリル基を除去することでセフカペン誘導体 **1** を得た。**1** にヨウ化ピバロイルオキシメチルを作用させることでセフカペン誘導体 **2** へと導いた。

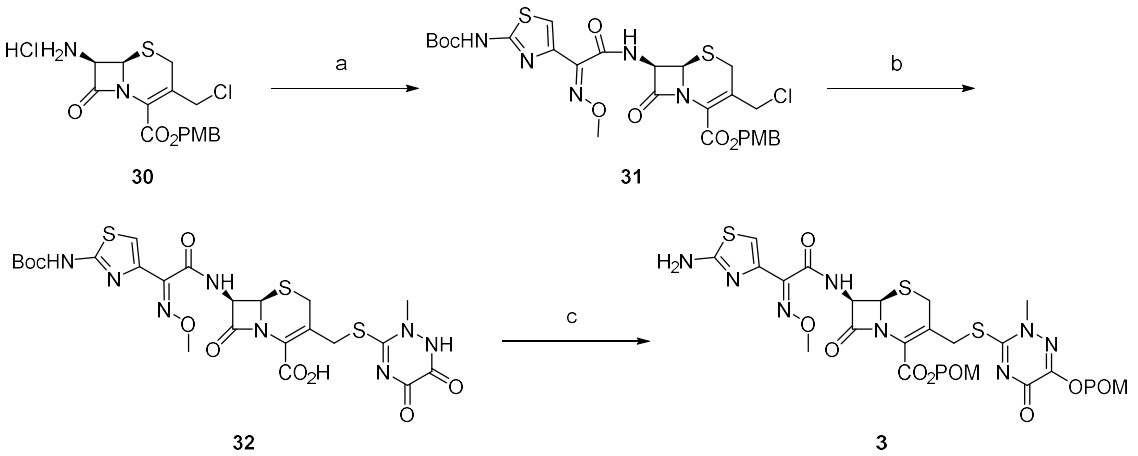
Scheme 1-5. セフカペン誘導体 **1**, **2** の合成



Reagents and conditions: (a) (i) $ClSO_2NCO$, THF, -30 °C; $NaHCO_3$, H_2O rt, quant.; (ii) diphenyl diazomethane, THF, rt, 66%; (b) (i) $TsOH \cdot H_2O$, acetonitrile, rt; (ii) 4 M HCl/EtOAc, rt, 42% in 2 steps; (iii) **14**, MsCl, Et_3N, CH_2Cl_2, -20 °C, 16%; (iv) TFA, anisole, CH_2Cl_2, 0 °C; 2 M NaOH aq., 76%; (c) iodomethyl pivalate, DMF, -40 °C, 41%

3 位側鎖と 4 位カルボン酸にピバロイルオキシメチル基を導入したセフトリアキソン誘導体 **3** の合成を以下に記す(Scheme 1-6)。7-アミノ-3-クロロメチル-3-セフェム-4-*p*-メトキシベンジルエステル塩酸塩(ACLE·HCl, **30**)[16]を用い、上記合成したカルボン酸 **17** と縮合させることにより **31** を得た。次いで **31** の *p*-メトキシベンジル基を除去し、*N,O*-ビス(トリメチルシリル)アセトアミド(BSA)存在下、6-ヒドロキシ-2-メチル-3-チオオキソ-3,4-ジヒドロ-1,2,4-トリアジノンを作用させることでカルボン酸 **32** へと導いた。カルボン酸 **32** にヨウ化ピバロイルオキシメチルを作用させ 3 位側鎖と 4 位カルボン酸にピバロイルオキシメチル基を導入し、*tert*-ブトキシカルボニル基を除去することでセフトリアキソン誘導体 **3** へと導いた。

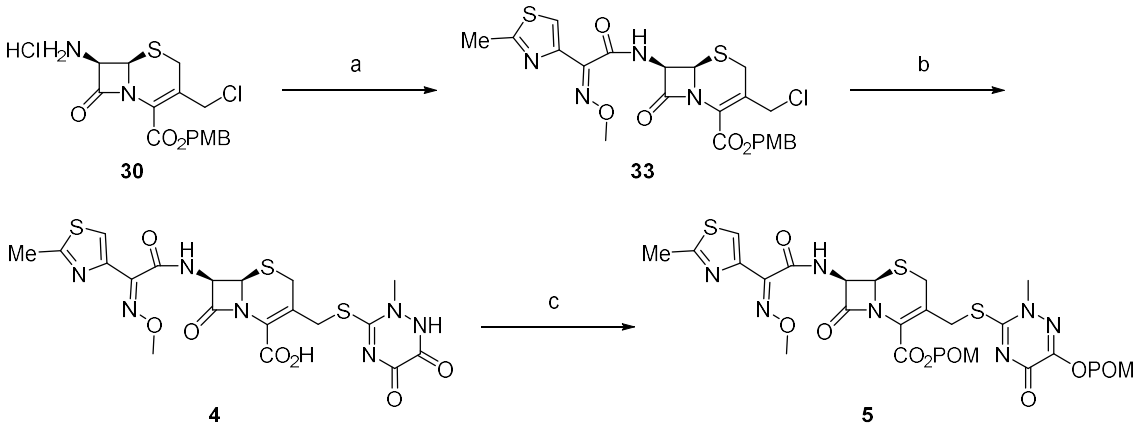
Scheme 1-6. セフトリアキソン誘導体 **3** の合成



Reagents and conditions: (a) **17**, MsCl, Et_3N,NMM, CH_2Cl_2, -30 °C, 100%; (b) (i) TFA, CH_2Cl_2, 0 °C, quant.; (ii) 6-hydroxy-2-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one, Na_2CO_3, BSA, acetonitrile, 0 °C; 2 M NaOH aq., 55%;(c) (i) iodomethyl pivalate, DMF, -40 °C to -20 °C, 79%; (ii) 2 M $AlCl_3$/nitromethane, anisole, CH_2Cl_2, -40 °C, 58%.

7 位側鎖にメチルチアゾール基を導入したセフトリアキソン誘導体 **4**、**5** の合成を Scheme 1-7 に記す。上記の合成と同様に **30** を用い、カルボン酸 **19** と縮合させることにより **33** を得た。次いで **33** のクロロ基を 6-ヒドロキシ-2-メチル-3-チオオキソ-3,4-ジヒドロ-1,2,4-トリアジノンで求核置換し、*p*-メトキシベンジル基を除去することでセフトリアキソン誘導体 **4** へと変換した。得られた **4** にヨウ化ピバロイルオキシメチルを作用させ 3 位側鎖と 4 位カルボン酸にピバロイルオキシメチル基を導入し、セフトリアキソン誘導体 **5** へと導いた。

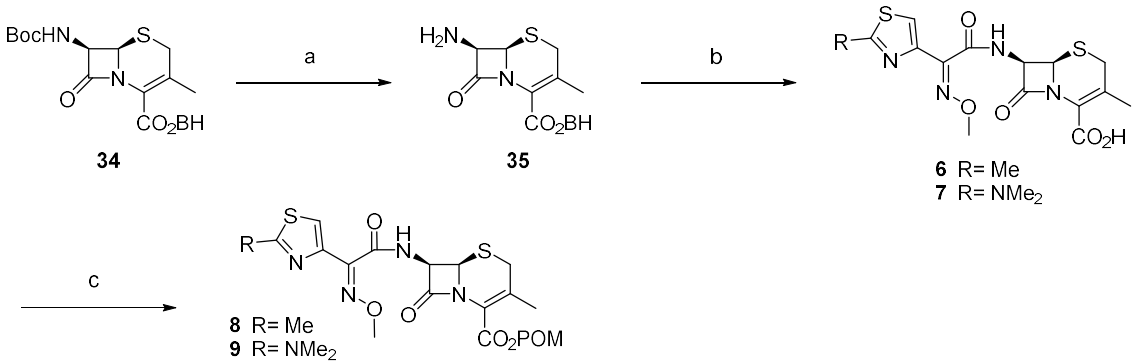
Scheme 1-7. セフトリアキソン誘導体 **4, 5** の合成



Reagents and conditions: (a) **19**, pheny dichlorophosphate, NMM, CH_2Cl_2, -20 °C, 62%; (b) (i) 6-hydroxy-2-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one, NaI, K_2CO_3, DMF, 0 °C, 72%; (ii) 2 M $AlCl_3$/nitromethane, anisole, CH_2Cl_2, -30 °C to -20 °C; 0.2 M NaOH aq., 6%; (c) iodomethyl pivalate, DMF, -30 °C to -5 °C, 30%.

それぞれ 7 位側鎖にメチルチアゾール基、*N,N*-ジメチルチアゾール基を導入したセフェタメト誘導体 **6**, **7, 8, 9** の合成を Scheme 1-8 に記す。まず既知の方法を用い合成した **34**[17] の *tert*-ブトキシカルボニル基を除去しアミン **35** とし、アミン **35** とカルボン酸 **19** もしくは **25** を縮合し、ベンズヒドリル基を除去することでアミド **6**、**7** をそれぞれ得た。**6**、**7** をそれぞれピバロイルオキシメチル化することで **8**、**9** へと導いた。

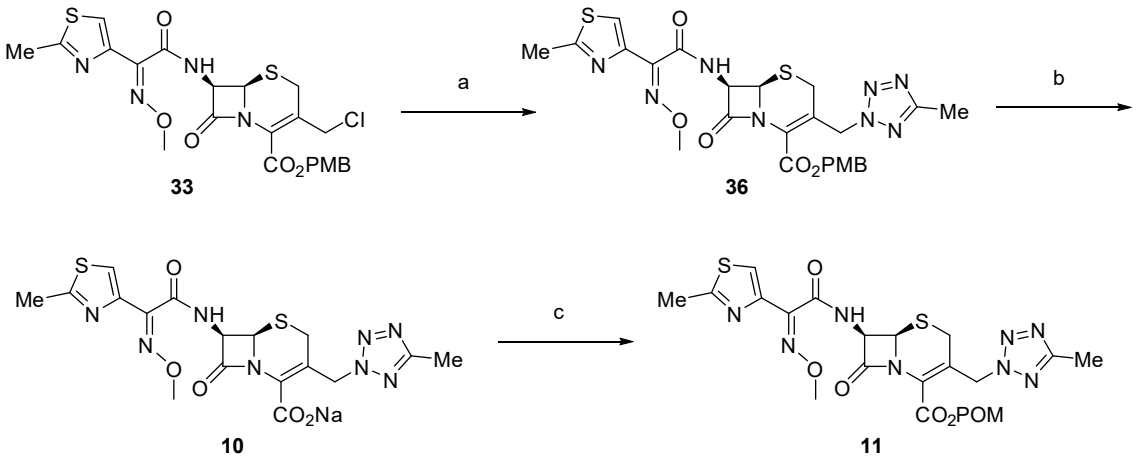
Scheme 1-8. セフェタメト誘導体 **6**、**7**、**8**、**9** の合成



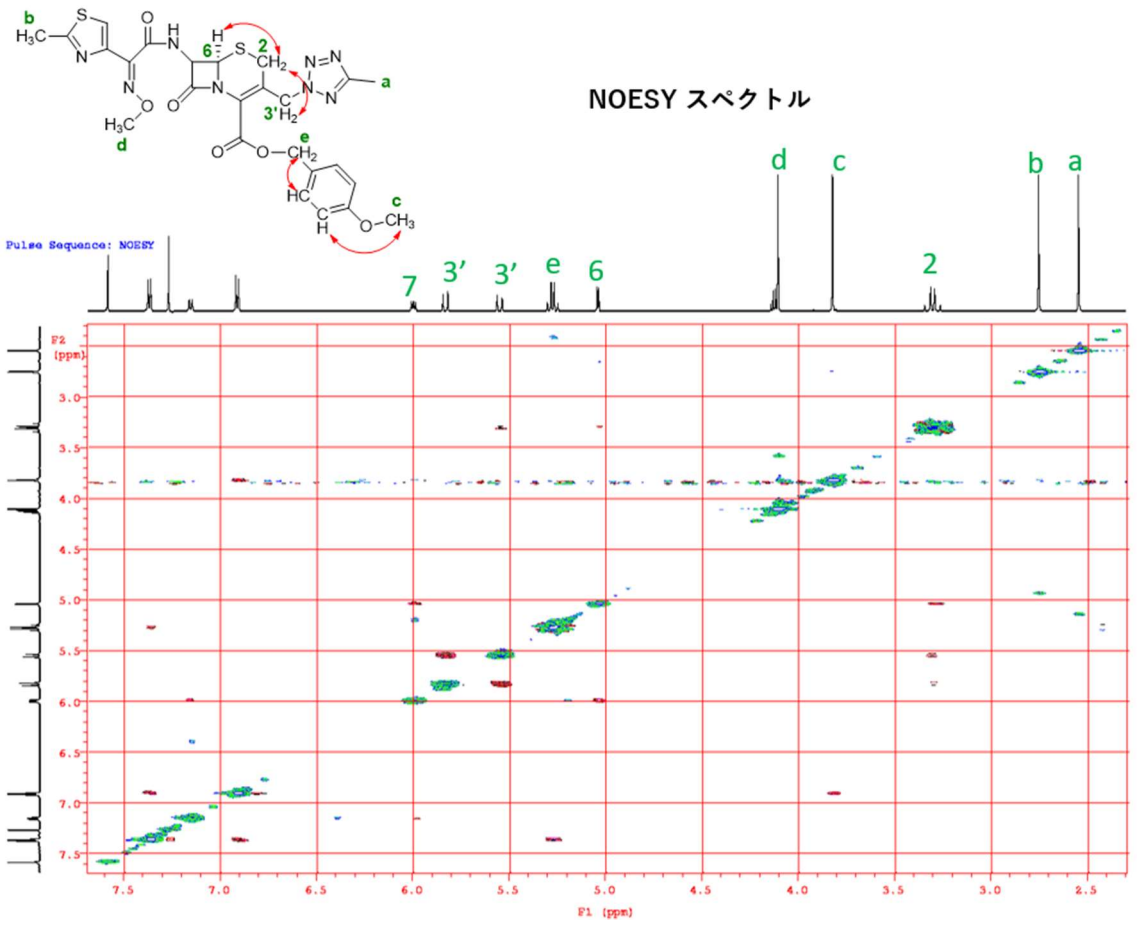
Reagents and conditions: (a) $TsOH \cdot H_2O$, acetonitrile, rt, 74%; (b) (i) pheny dichlorophosphate, NMM, **19** or **25**, -30 °C to 0 °C, 46% or quant.; (ii) 2 M $AlCl_3$/nitromethane, anisole, CH_2Cl_2, -20 °C; 2 M NaOH aq., **6**：76%, **7**：61%; (c) **8**：iodomethyl pivalate, DMF, -20 °C, 94%. **9**：iodomethyl pivalate, K_2CO_3, DMF, -30 °C, 59%

7 位側鎖にメチルチアゾール基を導入したセフテラム誘導体 **10**、**11** の合成を Scheme 1-9 に記す。セフトリアキソン誘導体 **4**、**5** の合成で用いた化合物 **33** を用い、クロロ基をヨード基へと変換した後、5-メチルトリアゾールを用いた求核置換反応、続くメタクロロ過安息香酸による酸化と三臭化リンによる還元によって **36** へと変換した。得られた **36** の *p*-メトキシベンジル基を除去しセフテラム誘導体 **10** を得た。得られた **10** にヨウ化ピバロイルオキシメチルを作用させセフテラム誘導体 **11** へと導いた。得られた **10**、**11** のメチル基の位置は中間体 **36** における NOESY と ^{1}H-^{15}N HMBC スペクトルから推定した(Figure 1-4)。

Scheme 1-9. セフテラム誘導体 **10**、**11** の合成



Reagents and conditions: (a) (i) NaI, acetone, rt, quant., (ii) 5-methyl-1*H*-tetrazole, K_2CO_3, DMF, -30 °C, quant.; (iii) *m*CPBA, CH_2Cl_2, -40 °C, 17%; (iv) PBr_3, DMF, -30 °C, quant.; (b) 2 M $AlCl_3$/nitromethane, anisole, CH_2Cl_2, -20 °C; 2 M NaOH aq., 82%; (c) iodomethyl pivalate, DMF, -20 °C, 76%.



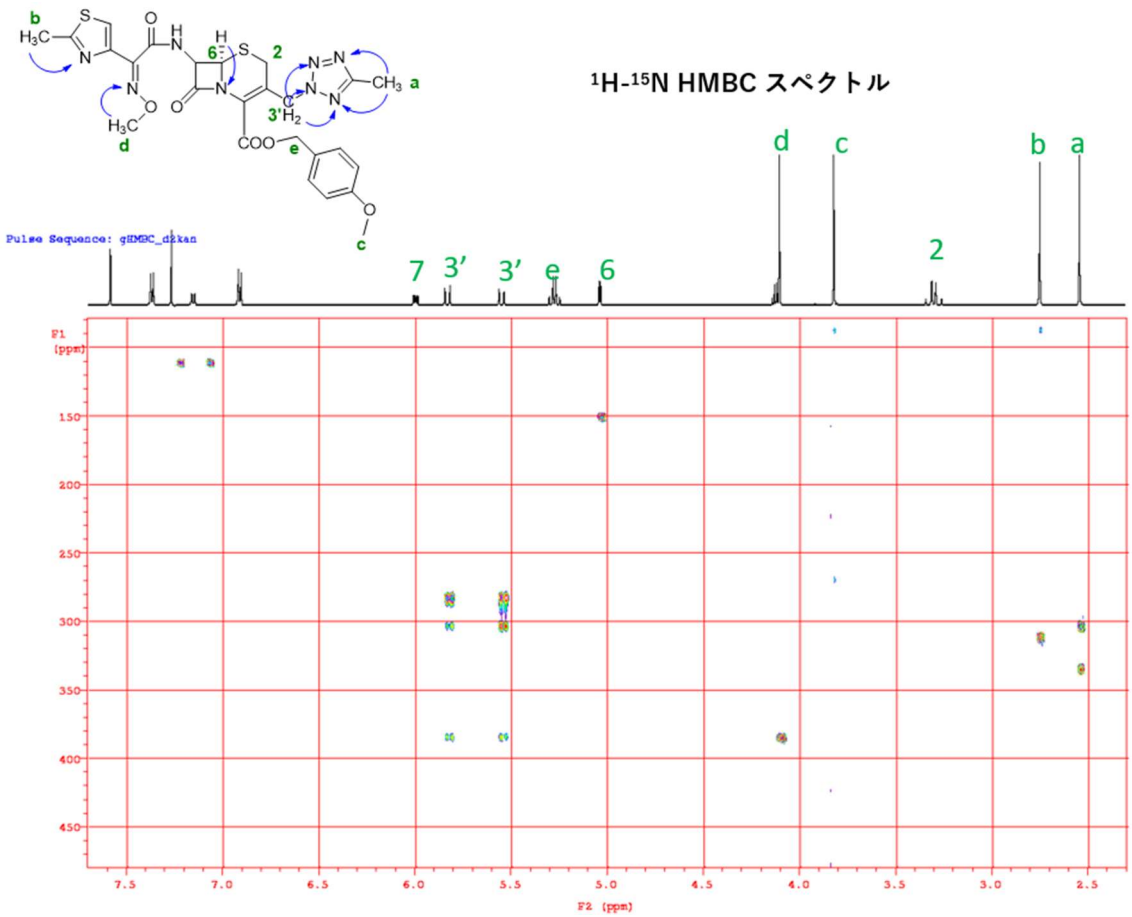
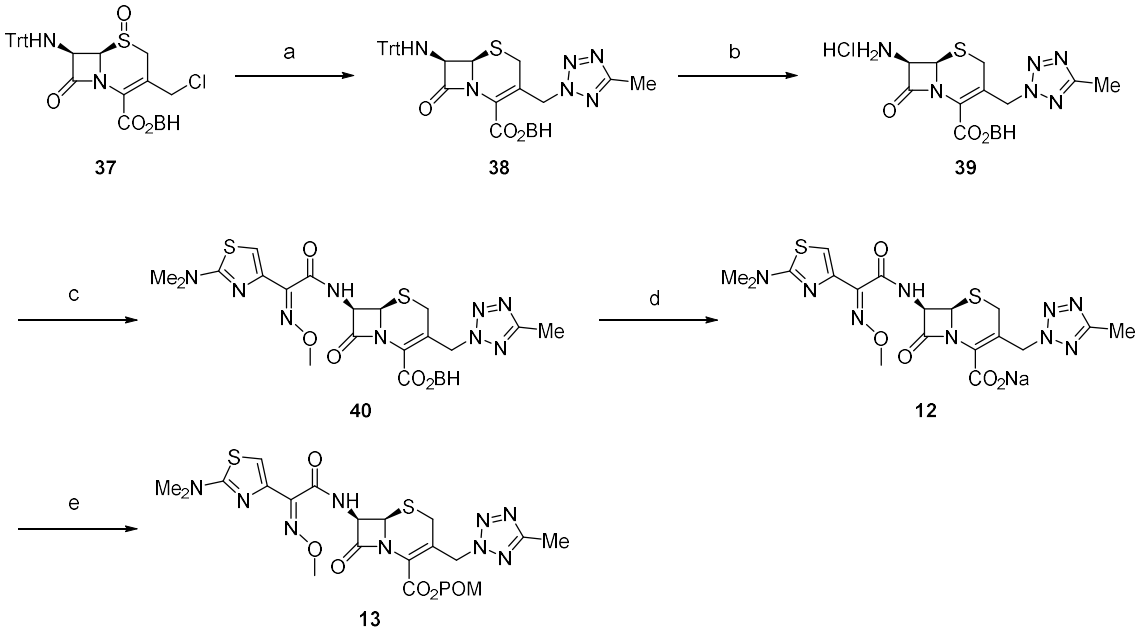


Figure 1-4. 化合物 **36** の NOESY スペクトルと ^{1}H-^{15}N HMBC スペクトル

7 位側鎖に *N,N*-ジメチルチアゾール基を導入した基を導入したセフテラム誘導体 **12**、**13** の合成を Scheme 1-10 に記す。既知の方法を用い合成した **37**[18] のクロロ基をヨード基へと変換した後、5-メチルトリアゾールの導入、次いで三臭化リンを用いて還元することでスルフィド **38** を得た。トリフェニルメチル基を除去し **39** へと変換した後、カルボン酸 **25** を作用させ **40** とし、ベンズヒドリル基の除去することで 12 を合成した。得られた **12** をピバロイルオキシメチル化することにより **13** へと導いた。得られた **12**、**13** のメチル基の位置は中間体 **38** における ^{1}H-^{13}C HMBC スペクトルから推定した(Figure 1-5)。

Scheme 1-10. セフテラム誘導体 **12**、**13** の合成



Reagents and conditions: (a) (i) NaI, acetonitrile, rt; (ii) 5-methyl-1*H*-tetrazole, 0 °C; (iii) PBr_3, -40 °C, 25%; (b) 6 M HCl aq., acetone, rt, quant.; (c) **25**, MsCl, Et_3N, DMA, -20 °C, 76%; (d) TFA, anisole, CH_2Cl_2, 0 °C; 0.2 M NaOH aq., 95%; (e) iodomethyl pivalate, DMF, -20 °C, 96%.

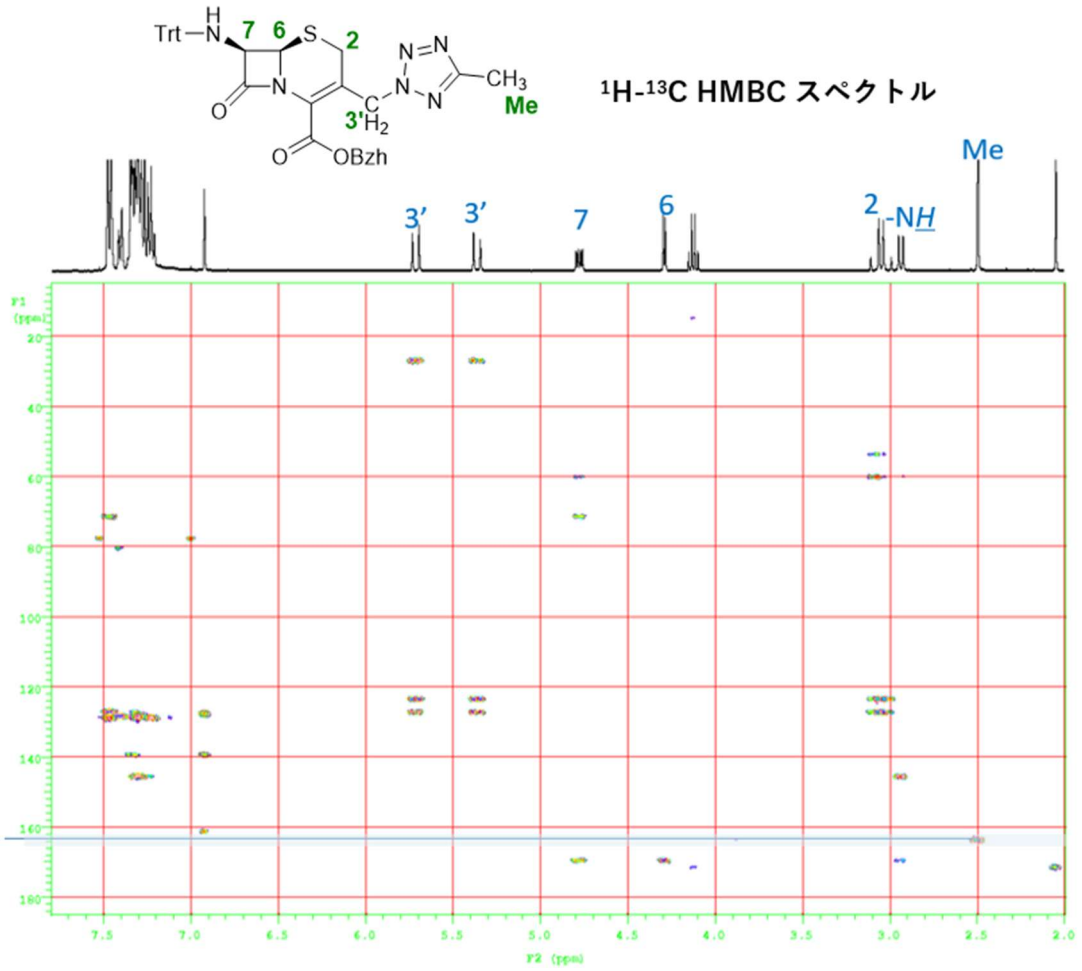


Figure 1-5. 化合物**38**の^{1}H-^{13}C HMBCスペクトル

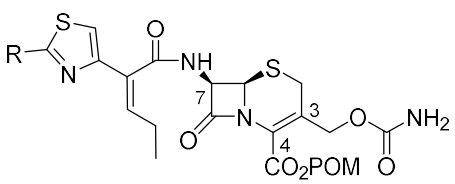
第三節 アミノチアゾール部位変換化合物の評価結果

第一項　物性・薬物動態関連評価結果

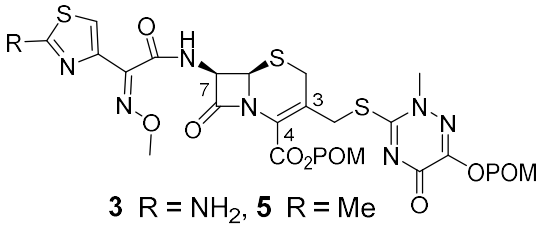
合成化合物の各種評価結果を Table 1-1 に記す。物理化学パラメータとして、脂溶性の指標となる ClogP[19]、水素結合供与体の数として HBD を記載した。また受動拡散による膜透過性の指標となる人工膜透過性試験(PAMPA)[20] の評価結果とラットにおける経口投与時のバイオアベイラビリティ(F_{po})、またそのときの血中曝露(AUC_{po})の評価結果を記載した。

はじめにいずれのセファロスポリンを用いた場合においても 7 位側鎖を変換することで脂溶性を増大させ、水素結合供与体の数が低減した。次いで CTRX 誘導体 **3**、**5** を除いたセファロスポリンにおいて、いずれも 7 位側鎖の変換により膜透過性が改善することが判明した。CTRX 誘導体 **3**、**5** のいずれも膜透過性が認められなかったことは、リン酸緩衝液(pH 6.8)に対していずれも 0.1 μmol/L 以下の低い溶解度しか示さなかったためであると考えている [21]。またバイオアベイラビリティについて CEMT、CFTM 誘導体では確認できていないが、CFPN 誘導体や CTRX 誘導体においてはアミノチアゾールの変換によって大幅な改善が認められた(CFPN-PI に対して **2**、**3** に対して **5**)。またこのとき **2**、**5** の血中曝露もそれぞれ向上していることを確認している。CEMT や CFTM においては、化合物 **9**, **11** はそれぞれ CEMT-PI や CFTM-PI に対して AUC_{po} が低くなったものの、**7**、**13** ははそれぞれ CEMT-PI や CFTM-PI に対して血中曝露の向上が認められており、以前他のセファロスポリンにおいて経口吸収性の改善が認められた結果 [22] と同様に、ここに示す 7 位側鎖の変換は経口吸収性を改善するための有用な方法となり得ることが示唆された。一方で化合物 **9**, **11** の CEMT-PI や CFTM-PI に対して AUC_{po} が低くなった理由は活性体であるカルボン酸 **8**, **10** 自体の血中からの消失が CEMT や CFTM と比較して早かったためであると考えている。

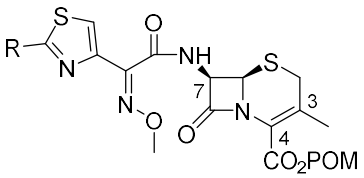
Table 1-1. アミノ基変換化合物の評価結果



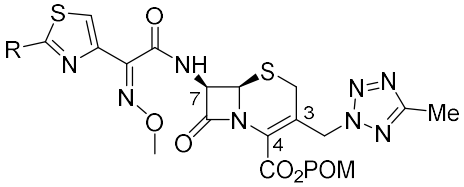
CFPN-PI R = NH_2, **2** R = NMe_2



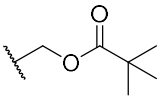
3 R = NH_2, **5** R = Me



CEMT-PI R = NH_2,
7 R = Me, **9** R = NMe_2



CFTM-PI R = NH_2
11 R = Me,**13** R = NMe_2

POM = 

Compounds	CLogP[a]	HBD[b]	PAMPA coefficient[c]	PAMPA category[d]	F_{po} (%)[e]	AUC_{po} (μg·hr/mL)
CFPN-PI	0.48	5	0.09	medium	15[f]	7.04
2	1.50	3	1.99	high	43	20.5
3 (CTRX-di-POM)	1.56	3	N.D.[g]	-	1	1.34
5	2.29	1	N.D.[g]	-	14	6.38
CEMT-PI	2.22	3	0.63	medium	22	6.50
7	2.95	1	7.75	high	N.T.[h]	8.48
9	3.18	1	6.58	high	N.T.[h]	5.33
CFTM-PI	1.31	3	0.06	low	N.T.[h]	19.1
11	2.05	1	2.31	high	N.T.[h]	14.0
13	2.27	1	2.14	high	N.T.[h]	26.3

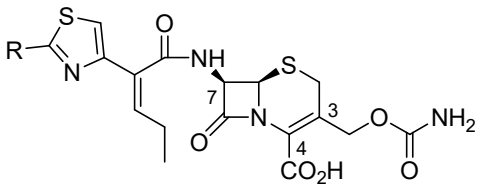
[a] Reference 19. [b]The number of hydrogen bond donors. [c]Permeability coefficient in PAMPA (10^(¹6)*cm/s). [d]Permeation category in PAMPA. [e]Calculated by area under the curve (AUC) of each carboxylic acid formed from each compound administrated orally/AUC of of each carboxylic acid administrated intravenously. [f]Reference 23. [g]Not Detected. [h]Not Tested.

第二項　*in vitro* 抗菌活性評価結果

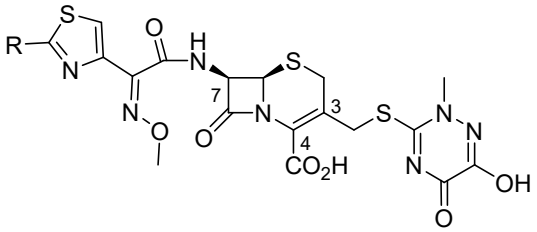
次にこれら化合物の活性体であるカルボン酸 **1**, **4**, **6**, **8**, **10**, **12** の *in vitro* 抗菌活性を Table 1-2 に示す。今回評価したグラム陽性菌もしくはグラム陰性菌いずれの菌株に対しても、7 位側鎖にメチルチアゾール或いは *N,N*-ジメチルアミノ

チアゾールを有するすべてのセファロスポリン **1**, **4**, **6**, **8**, **10**, **12** は、7 位側鎖にアミノチアゾールを有するセファロスポリンと比較して、最小発育阻害濃度(Minimum Inhibitory Concentration: MIC)が同等以上となり、抗菌活性が低下する傾向を示した。以前他のセファロスポリンにおいてアミノチアゾールからメチルチアゾールへの変換により *S.aureus* 等のグラム陽性菌、*E.coli* 等のグラム陰性菌に対する抗菌活性が減弱することが報告されており、今回の結果と一致している[24]。

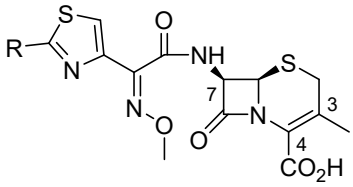
Table 1-2. アミノ基変換化合物の *in vitro* 抗菌活性



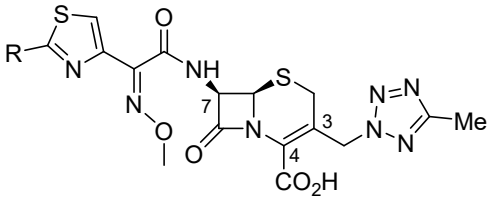
cefcapene (CFPN) R = NH_2, **1** R = NMe_2



ceftriaxone (CTRX) R = NH_2, **4** R = Me



cefetamet (CEMT) R = NH_2
6 R = Me, **8** R = NMe_2



cefteram (CFTM) R = NH_2
10 R = Me, **12** R = NMe_2

		MIC[a] (μg/mL)					
species	strain ID	CFPN	**1**	CTRX	**4**		
E. coli	NIHJ JC-2[b]	0.5	>32	0.25	2		
S. pneumoniae	SR16675[c]	0.5	>32	2	16		
H. influenzae	SR11435[d]	2	>32	0.25	2		
B. subtilis	ATCC6633[e]	0.25	16	1	8		
E. cloacae	ATCC13047[e]	8	>32	32	>32		
S. marcescens	ATCC13880[e]	1	>32	1	4		
		MIC[a] (μg/mL)					
species	strain ID	CEMT	**6**	**8**	CFTM	**10**	**12**
E. coli	NIHJ JC-2[b]	0.5	>32	32	0.25	16	>32
S. pneumoniae	SR16675[c]	>32	>32	>32	2	16	>32
H. influenzae	SR11435[d]	16	>32	>32	0.5	8	>32
B. subtilis	ATCC6633[e]	32	>32	>32	0.25	8	32
E. cloacae	ATCC13047[e]	>32	>32	>32	32	>32	>32
S. marcescens	ATCC13880[e]	1	>32	>32	1	>32	>32

[a]Minimum Inhibitory Concentration values. [b]Reference 25. [c]A penicillin-resistant *Streptococcus pneumoniae* strain. [d]A beta-lactamase non-producing ampicillin-resistant *Haemophilus influenzae* strain. [e]These strains were Purchased from American Type Culture Collection (ATCC).

第四節 アミノチアゾール部位アミノ基のプロドラッグ修飾

第一項 プロドラッグ化合物の検討

上記の結果から、アミノチアゾールからメチルチアゾール或いは *N,N*-ジメチルアミノチアゾールへの変換により CFPN-PI や CTRX 等の第三世代セファロスポリンの経口吸収性が改善されるものの、それらの抗菌活性が減弱するため、抗菌活性を犠牲にしない変換としてプロドラッグ化による経口吸収性向上と良好な抗菌活性の両立を目指すこととした。脂溶性の増大と水素結合供与体数の低減を指向し、基質として第三世代セファロスポリンの一つである CEMT-PI を選択し、以下の化合物 **41~45** の新規プロドラッグを考案した。アミノチアゾールの保護基の報告はほとんどないものの、*N*-アシルオキシメチルカルバメート型プロドラッグは脂肪族アミンやいくつかの芳香族アミンの保護基として知られており [26]、今回新しく設計した化合物は経口吸収された後に修飾部位が切断されると考えた。またセフチゾキシムピボキシル(ceftizoxime pivoxil: CZX-PI)のアミノチアゾール部位アミノ基への L-アラニル基の導入は、セフチゾキシム(ceftizoxime: CZX)の経口吸収性を向上させることが報告されている [27] ことから、参照化合物として L-アラニル基をアミノ基に導入した化合物 **46** を合成することとした(Figure 1-6)。

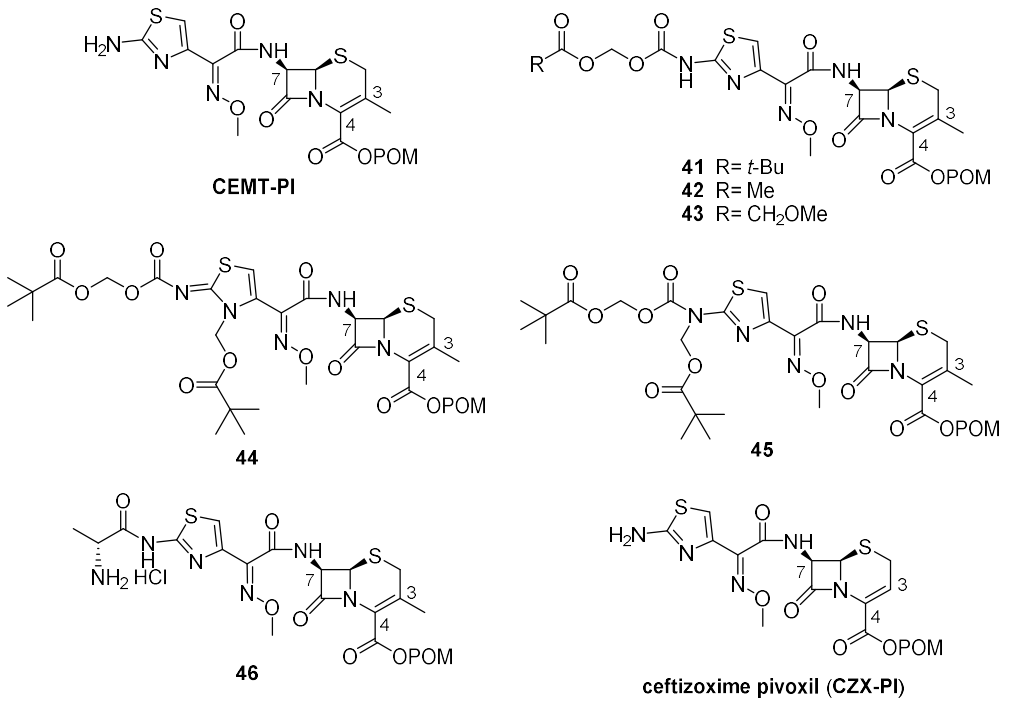
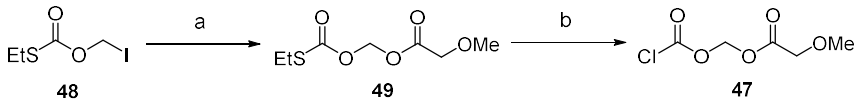


Figure 1-6. CEMT-PI、CZX-PI と新規プロドラッグ化合物 **41~46** の構造

第二項 プロドラッグ化合物の合成

はじめに化合物 **47** の合成を Scheme 1-11 に示す。既知の手順[28]で調製されたヨードメチル **48** に対し、メトキシ酢酸を作用させチオエステル **49** とした。次いで **49** を塩化スルフリルおよび三フッ化ホウ素エチルエーテルで処理することで **47** へと導いた。

Scheme 1-11. 化合物 **47** の合成

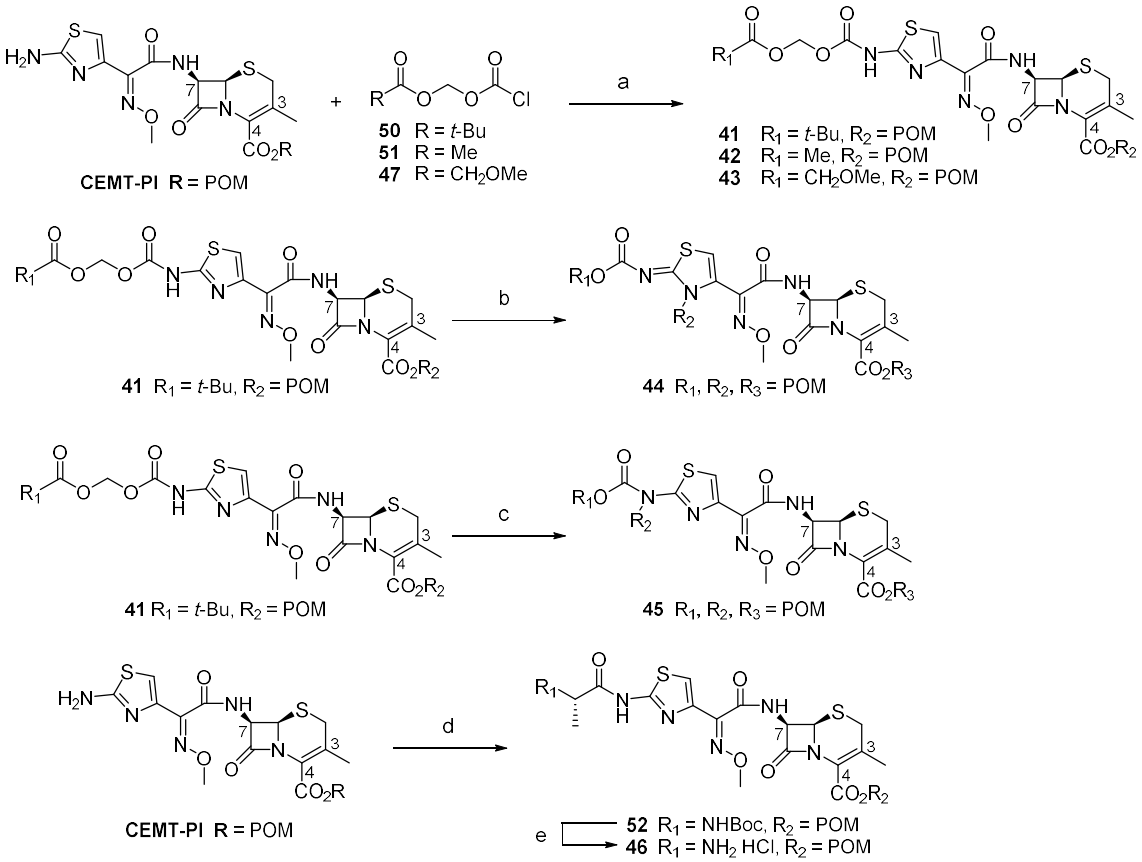


48 **49** **47**

Reagents and conditions: (a) RCO_2H, tetrabutylammonium hydrogensulfate, $NaHCO_3$, CH_2Cl_2, H_2O, rt, quant.; (b) SO_2Cl_2, $BF_3{\cdot}OEt_2$, 130 °C to rt, 87%.

CEMT-PI[29] に対し、既知の手順[30]で調整されたクロロホルメート **50**、**51** また上述した **47** をそれぞれ作用させることで、対応するカルバメート **41~43** へと変換した(Scheme 1-12)。化合物 **41** は、80 °C でヨウ化ピバロイルオキシメチルを使用し、室温で水素化ナトリウムの存在下で塩化ピバロイルオキシメチルとヨウ化ナトリウムを使用して、それぞれ **44** と **45** へと導いた。CEMT-PI と Boc-L-Ala-OH の縮合反応によりアミド **52** を得た後に、**52** の *tert*-ブトキシカルボニル基をギ酸溶液中塩酸で除去して **46** へと変換した。

Scheme 1-12. 化合物 **41~46** の合成



Reagents and conditions: (a) **41**: **50**, pyridine, DMA/ CH_2Cl_2, 0 °C, 61%; **42** or **43**: **51** or **47**, pyridine, DMA, 0 °C, 25% or 18%; (b) iodomethyl pivalate, DMF, 80 °C, 31%; (c) chloromethyl pivalate, NaH, DMF, 0 °C to rt; NaI, rt, 34%; (d) Boc-L-Ala-OH, EDC·HCl, DMAP, CH_2Cl_2, 64%; (e) 4 M HCl/EtOAc, HCO_2H, 0 °C, 79%.

合成した**44**と**45**の構造は以下のように^{1}H-^{13}C HMBCスペクトルにより決定した(Figure 1-7、Figure 1-8)。

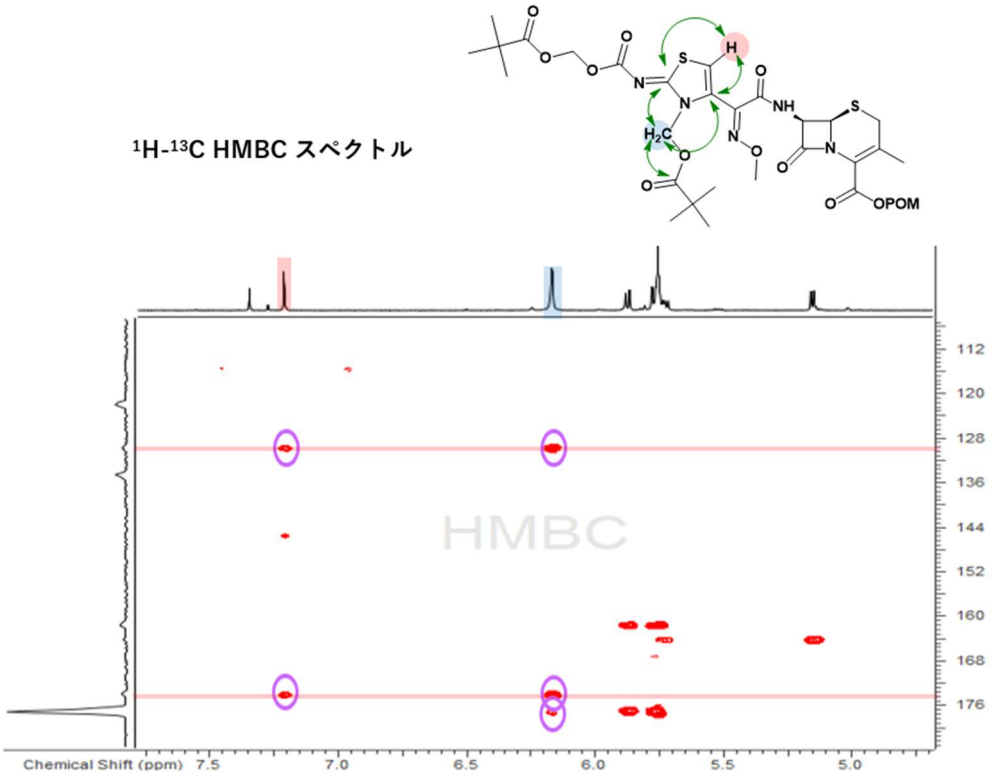


Figure 1-7. 化合物**44**の^{1}H-^{13}C HMBCスペクトルによる構造決定

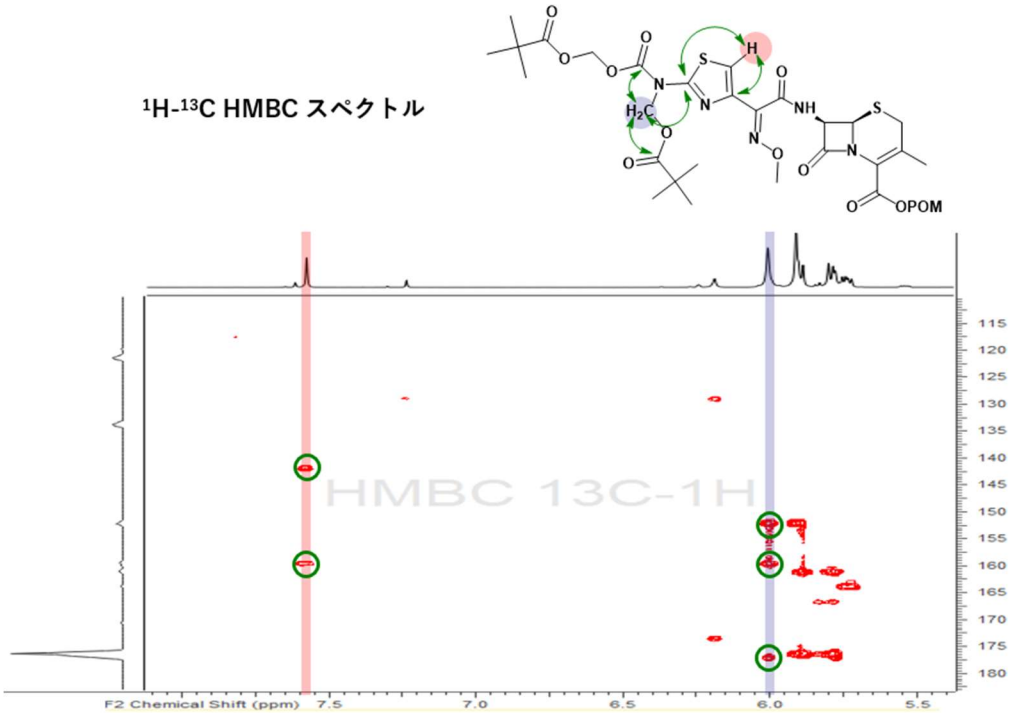


Figure 1-8. 化合物**45**の^{1}H-^{13}C HMBCスペクトルによる構造決定

第三項 プロドラッグ化合物の評価結果

ラットに経口投与した場合のこれらの化合物のバイオアベイラビリティを Table 1-3 に示す。**41** の F_{po}(%)は CEMT-PI よりも比較的低かったものの、**42** および **43** の F_{po}(%)は CEMT-PI のそれとほぼ同等であり、**46** よりも高かった。一方で **44** と **45** は、CEMT-PI や **46** と比較してバイオアベイラビリティが低下しそれぞれ 5%と 6%であった。化合物 **41**、**44**、**45** のバイオアベイラビリティが CEMT-PI よりも低い理由は、リン酸緩衝液(pH 6.8)への溶解性が低いためであると考えられる。またこれらの化合物は溶解性が低いために PAMPA 評価が実施できていない。**46** が CEMT-PI に比べて低い経口吸収性を示した理由として、脂溶性が低く水素結合供与体の数が多いため、低い膜透過性を示したためであると考えられる。**42** と **43** の経口吸収性は CEMT-PI と同等程度であったが、膜透過性は **42** と **43** の方が CEMT-PI よりも高かった。脂溶性の増大と水素結合供与体数の低減による膜透過性の改善が経口吸収性の改善を示さなかった理由として **42** と **43** が胃腸液中に存在する酵素に対して安定性が低かったためと考えられる。その理由として **41** や **45** が日本薬局方崩壊試験第 1 液(JP1, pH 1.2)や第 2 液(JP2, pH 6.8)において 3 時間後も安定であり(**41**：98.2%, 98.2%, **45**：99.1%, 96.4%)、類似する構造を有する **42**, **43** も **41**, **45** と同様に各 pH の水溶液中では安定であると考えられるためである。また考案した **41**～**45** はいずれも血中において CEMT へと変換されたことから、*N*-アシルオキシメチルカルバメートがアミノチアゾール部位アミノ基のプロドラッグとして機能することが認められた。

Table 1-3. 各種プロドラッグ化合物の評価結果

Compounds	CLogP[a]	HBD[b]	PAMPA coefficient[c]	PAMPA category[d]	Kinetic solubility (μM)	F_{po} (%)[e]	AUC_{po}[f]
41	3.64	2	N.T.[g]	N.T.[g]	N.D.[h]	21	4.82[i]
42	2.40	2	0.95	High	>50	30	6.82[i]
43	2.35	2	2.53	High	>50	27	6.16[i]
44	5.97	1	N.T.[g]	N.T.[g]	N.D.[h]	5	0.69[i]
45	4.38	1	N.T.[g]	N.T.[g]	N.D.[h]	6	0.91[i]
46	1.77	4	0.00	low	>50	21	4.70[i]
CEMT-PI	2.22	3	0.63	medium	>50	29	6.50

[a] Reference 19. [b]The number of hydrogen bond donors. [c]Permeability coefficient in PAMPA (10^(-6)*cm/s). [d]Permeation category in PAMPA. [e]Calculated by AUC of Cefetamet formed from prodrugs administrated orally / AUC of Cefetamet administrated

intravenously. [f]AUC of Cefetamet formed from prodrugs administrated orally. [g]Not Tested. [h]Not Detected. [i]Corrected AUC when the same dose as Cefetamet pivoxil is administrated.

膜透過性を改善し、消化管内での酵素に対する安定性を高めるため、*N*-アシルオキシメチルカルバメート型プロドラッグの構造最適化が有用であると考える。具体的には以下に示す R1, R2 部位の構造を変換することにより、良好な膜透過性と消化管での安定性を示し、経口吸収性を改善するプロドラッグを創出できると考えている(Figure 1-9)。

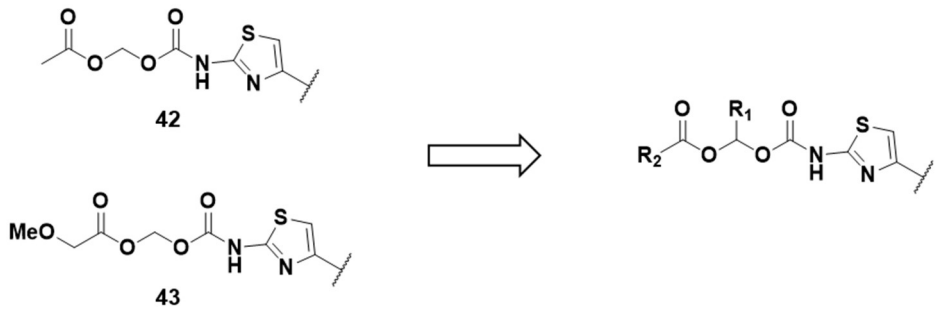


Figure 1-9. 経口吸収性改善に向けた構造修飾案

第四節　小括

第三世代セファロスポリンの経口吸収性を改善するための一般的な方法論を確立することを目的として、第三世代セファロスポリン系抗菌薬として CFPN-PI、CTRX、CEMT-PI、CFTM-PI を用いて 7 位側鎖のアミノチアゾールをメチルチアゾールや *N,N*-ジメチルアミノチアゾールに変換した化合物を合成した。

合成した複数の新規セファロスポリンは経口吸収性が向上し、特に **2** や **5** は CFPN-PI や **3** (CTRX-di-POM)と比較して経口バイオアベイラビリティが大きく向上することが確認された。

一方で **2** や **5** を含む 4 位 POM エステル(**2**、**5**、**7**、**9**、**11**、**13**)の活性体である 4 位カルボン酸 **1**、**4**、**6**、**8**、**10**、**12** はそれぞれ CFPN、CTRX、CEMT、CFTM と比較して各種グラム陽性菌、グラム陰性菌に対する抗菌活性が大きく減弱した。

経口吸収性の改善とグラム陽性菌及びグラム陰性菌に対する良好な抗菌活性との両立を目指し、アミノ基を *N*-アシルオキシメチルカルバメート型のプロドラッグへと変換した CEMT の新規プロドラッグ **41**～**45** を考案し合成した。

考案した **41**～**45** はいずれも血中において CEMT へと変換されたことから、*N*-アシルオキシメチルカルバメートがアミノチアゾール部位アミノ基のプロドラッグとして機能することが明らかとなった。

脂溶性の増大と水素結合供与体数の低減によって膜透過性を改善した新規プロドラッグ **42** 及び **43** を見出すことができた。一方でそれらのバイオアベイラビリティは CEMT-PI とほぼ同等程度であった。

膜透過性を改善した **42**、**43** の経口吸収性が CEMT-PI とほぼ同等程度に留まった理由として消化管に存在する酵素に対して安定性が低かったためであると考えており、この点を改善するためにカーバメートプロドラッグにおけるメチレン部位やアシル部位の構造変換が有用であると考えている。

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Experimental Section

General Remarks.

The reagents and solvents were purchased from commercial sources and used without purification. Unless otherwise noted, reactions were performed under nitrogen atmosphere with anhydrous solvents. “Brine” refers to a saturated aqueous sodium chloride solution. Analytical thin-layer chromatography (TLC) was run on silica gel F254 precoated plates. Visualization of the developed chromatogram was performed by fluorescence quenching or Vaughn’s reagent. Column chromatography was carried out using silica gel. Reverse phase column chromatography was performed using HP20SS and octadecylsilyl (ODS) Silica Gel (Yamazen Ultra Pack). Preparative high performance liquid chromatography (HPLC) was performed using Phenomenex Gemimi-NX. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV400 spectrometer (400 MHz). Chemical shifts (δ) are reported in parts per million (ppm) from tetramethylsilane (in $CDCl_3$, DMSO-d_6 and D_2O) as an internal standard. Multiplicities are reported as s (singlet), d (doublet), t (triplet), m (multiplet), br (broad) or combinations of those. Coupling constants (J) are in hertz. High-resolution mass spectral data was acquired on Orbitrap Q Exactive Plus (ESI). Low-resolution mass spectral data was collected on a Waters ZQ mass detector or Shimadzu LCMS-2010EV (ESI). Elemental analysis was performed using Micro Corder JM11 (J-SCIENCE LAB CO., Ltd.). All studies with animals were approved by the Institutional Animal Care and Use Committee of Shionogi & Co., Ltd.

Experimental for Chapter 1

Synthesis

Compound **16**

To a solution of **15** (1.76 g, 8.88 mmol) in *N*,*N*-dimethylformamide (5.28 mL) were added sodium hydroxide (1.56 g, 39.1 mmol) and iodomethane (3.66 ml, 58.6 mmol) and the mixture was stirred at 60 °C for 3 h. To this resulting mixture were added *N*,*N*-dimethylformamide (5.28 mL), sodium hydroxide (1.56 g, 39.1 mmol) and iodomethane (3.66 ml, 58.6 mmol) and the reaction mixture was stirred at 60 °C for 3 h. After being cooled to room temperature, the mixture was added water followed by extraction with ethyl acetate. The organic layer was washed with brine and then dried over $MgSO_4$, filtered, and evaporated under reduced pressure. This resulting residue was subjected to silica gel column chromatography, being eluted with hexane/ethyl acetate. The fractions containing the desired compound were concentrated to obtain **16** (0.94 g, 44%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.86 (1H, t, J = 7.8 Hz), 6.48 (1H, s), 3.84 (3H, s), 3.09 (6H, s), 2.38 (2H, dt, J = 7.8, 7.7 Hz), 1.11 (3H, t, J = 7.5 Hz). MS (ESI) *m/z*: 241$[M+H]^+$.

Compound **14**

To a solution of **16** (3.17 g, 13.2 mmol) in ethanol (22.2 mL) was added 2 mol/L aqueous sodium hydroxide solution (26.4 ml, 52.8 mmol) and stirred at 60 °C for 30 min. To this resulting mixture were added ethanol (10.0 mL) and 2 mol/L aqueous sodium hydroxide solution (26.4 ml, 52.8 mmol) and the reaction mixture was stirred at 60 °C for 1 h. After being cooled to room temperature, the mixture was poured into water and ethyl acetate, and the layers were separated. The organic layer was extracted with water and the combined aqueous layer were added tetrahydrofuran/ethyl acetate (1:1) solution and 2 mol/L aqueous hydrochloric acid solution and adjusted the pH of the mixture to around 4.3. The layers were separated and the aqueous layer was extracted with tetrahydrofuran/ethyl acetate (1:1) solution twice, and the combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure to give **14** and its *E*-olefin isomer (2.78 g, 93%, *E*:*Z* ~ 1:1) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.25-6.60 (1H, m), 6.45-6.44 (1H, m), 3.15-3.06 (6H, m), 2.83-2.43 (1H, m), 1.19-1.10 (2H, m).

MS (ESI) *m/z*: 227 $[M+H]^+$.

Compound **17**

To a solution of **18** (100 g, 367 mmol) in methanol (1000 mL) were added triethylamine (53.5 ml, 386 mmol) and *O*-methylhydroxylamine hydrochloride (32.2 g, 386 mmol) at 0 °C and the mixture was stirred at the same temperature for 90 min, and then the solvent was removed under reduced pressure. To this resulting mixture were added ethyl acetate (300 mL), tetrahydrofurane (150 mL) and 2 mol/L aqueous hydrochloric acid solution (1000 ml) and the layers were separated and the organic layer was washed with water and brine and then dried over $MgSO_4$, filtered, added diisopropylamine (62.8 ml, 441 mmol) and the solvent was removed under reduced pressure. This residue was filtered and ethyl acetate, tetrahydrofuran and 2 mol/L aqueous hydrochloric acid solution were added to this filtration residue and the layers were separated. The organic layer was washed with water and brine, and then dried over $MgSO_4$, filtered and evaporated under reduced pressure to give **17** (88.5 g, 80%) as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ: 11.73 (1H, s), 7.40 (1H, s), 3.88 (3H, s), 1.47 (9H, s). $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d_6) δ: 163.6, 160.5, 153.0, 147.9, 140.7, 113.9, 81.5, 62.4, 27.9. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{11}H_{15}N_3O_5S$ 302.0805; found 302.0803.

Compound **21**

To a solution of **20** (29.0 g, 223 mmol) in acetic acid (31.9 mL) was added sodium nitrite (18.5 g, 267 mmol) in water (46.4 mL) dropwise over 40 min under 0 °C and then stirred at the same temperature for 2 h. To this resulting mixture were poured into water (250 mL) and ethyl acetate (250 mL) and then added sodium carbonate (4.72 g, 44.6 mmol) to adjust pH of aqueous solution to ~7. To this resulting mixture was sepearted and the aqueous layer was extracted with ethyl acetate and the combined organic layer was washed with brine, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The resulting residue was concentrated in vacuo to obtain **21** (41.5 g) as a mixture of geometric isomers.

Compound **22**

To a solution of **21** (35.5 g, 223 mmol) in acetone (284 mL) were added potassium carbonate (61.6 g, 446 mmol) and dimethyl sulfate (42.6 ml, 446 mmol) dropwise over 20 min under 10 °C and the mixture was stirred at reflux temperature for 2 h. The mixture was cooled to room temperature and filtered. The filtrate was poured into water and ethyl acetate, and the layers were separated. The organic layer was washed with brine, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure to obtain **22** (38.6 g, 100%) as a yellow oil . ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.35 (2H, q, *J* = 7.2 Hz), 4.10 (3H, s), 2.40 (3H, s), 1.33 (3H, t, *J* = 7.1 Hz).

Compound **23**

To a solution of **22** (38.6 g, 223 mmol) in acetic acid (38.6 mL) was added sulfuryl chloride (21.8 ml, 268 mmol) dropwise over 20 min under 10 °C and the mixture was stirred at reflux temperature for 2 h and then stirred at room temperature overnight. The mixture was poured into water and ethyl acetate, and the layers were separated. The organic layer was washed with saturated aqueous sodium bicarbonate solution and water, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure to obtain **23** (11.00 g, 74%) as a mixture of geometric isomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.58-3.96 (7H, m), 1.37-1.32 (3H, m).

Compound **24**

To a solution of **23** (18.7 g, 38.9 mmol) in *N*,*N*-dimethylacetamide (43 mL) was added thioacetamide (4.00 g, 53.2 mmol) and the mixture was stirred at 50 °C for 4 h. The mixture was poured into water and ethyl acetate, and the layers were separated. The organic layer was washed with water, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **24** (3.60 g, 39%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.02 (1H, s), 4.41 (2H, q, *J* = 7.2 Hz), 4.12 (3H, s), 2.71 (3H, s), 1.38 (3H, t, *J* = 7.2 Hz). MS (ESI): *m/z* 229 $[M+H]^+$.

Compound **19**

To a solution of **24** (3.60 g, 15.8 mmol) in methanol (25 mL) was added 2 mol/L aqueous sodium hydroxide solution (23.7 ml, 47.3 mmol) at 0 °C and

the mixture was stirred at the same temperature for 2 h. The mixture was poured into 1 mol/L aqueous hydrochloric acid solution and ethyl acetate, and the layers were separated. The aqueous layer was extracted with ethyl acetate and the combined organic layer was washed with brine, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure and then concentrated in vacuo to obtain **19** (3.20 g, quant.) as a yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.82 (1H, s), 3.91 (3H, s), 2.67 (3H, s).

Compound **27**

To a solution of **26** (20.0 g, 87.0 mmol) in *N*,*N*-dimethylformamide (100 mL) were added sodium hydroxide (15.4 g, 384 mmol) and iodomethane (36.0 ml, 576 mmol) and the mixture was stirred at 45 °C for 4 h. The mixture was poured into water and ethyl acetate, and the layers were separated. The organic layer was washed with brine, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **27** (15.9 g, 71%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.76 (1H, s), 4.40 (2H, q, J = 7.2 Hz), 4.01 (3H, s), 3.11 (6H, s), 1.37 (3H, t, J = 7.1 Hz). MS (ESI) *m/z*: 258$[M+H]^+$.

Compound **25**

To a solution of **27** (8.00 g, 31.1 mmol) in methanol (48 mL) was added 2 mol/L aqueous sodium hydroxide solution (46.6 ml, 93.0 mmol) at 0 °C and the mixture was stirred at the same temperature for 2 h. The solvent was removed under reduced pressure and added tetrahydrofuran and The mixture was concentrated and poured into 1 mol/L aqueous hydrochloric acid solution and tetrahydrofuran, and the layers were separated. The aqueous layer was extracted with tetrahydrofuran twice and the combined organic layer was washed with brine, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure and then concentrated in vacuo to obtain **25** (6.30 g, 88%) as a yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.00 (1H, s), 3.86 (3H, s), 3.03 (6H, s). MS (ESI) *m/z*: 230$[M+H]^+$.

Compound **29**

To a solution of **28** (30.0 g, 91 mmol) in tetrahydrofuran (300 mL) was

added chlorosulfonyl isocyanate (7.88 ml, 91 mmol) at -30 °C. After being stirred for 30 min at -30 °C, the mixture was added water (50 mL) and sodium bicarbonate (7.63 g, 91 mmol) and stirred for 30 min at room temperature. The mixture was poured into 2 mol/L aqueous hydrochloric acid solution and ethyl acetate, and the layers were separated. The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure to give the corresponding carbamate (33.9 g, quant.) as a white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.00 (1H, d, J = 8.9 Hz), 5.47 (1H, dd, J = 8.8, 4.8 Hz), 5.06 (1H, d, J = 4.9 Hz), 4.87 (1H, d, J = 12.8 Hz), 4.62 (1H, d, J = 12.8 Hz), 3.56 (1H, d, J = 19.4 Hz), 3.44 (1H, d, J = 18.1 Hz), 1.41 (9H, s). MS (ESI) *m/z*: 372 $[M-H]^-$

To a solution of carbamate obtained above (30.0 g, 47.4 mmol) in tetrahydrofuran (300 mL) was added diphenyl diazomethane (9.21 g, 47.4 mmol). After being stirred for 1 h at room temperature, the solvent was removed under reduced pressure, and the crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **29** (16.8 g, 66%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44-7.28 (11H, m), 6.95 (1H, s), 5.66 (1H, dd, J = 9.6, 4.8 Hz), 5.27 (1H, d, J = 9.7 Hz), 5.05 (1H, d, J = 13.7 Hz), 4.96 (1H, d, J = 4.8 Hz), 4.79 (1H, d, J = 13.6 Hz), 4.63 (2H, s), 3.58 (1H, d, J = 18.7 Hz), 3.42 (1H, d, J = 18.6 Hz), 1.46 (9H, s). MS (ESI) *m/z*: 540 $[M+H]^+$.

Compound **1**

To a solution of **29** (19.0 g, 35.2 mmol) in acetonitrile (190 mL) was added *p*-toluenesulfonic acid monohydrate (33.5 g, 176 mmol) at 0 °C and stirred at room temperature for 1 h. The mixture was poured into aqueous sodium bicarbonate solution and ethyl acetate, and the layers were separated. The organic layer was washed with water and brine, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The resulting mixture was dissolved in ethyl acetate, and then poured into 4 mol/L hydrochloric acid/ethyl acetate (17.6 mL, 70.4 mmol). The resulting residue was filtered and washed with ethyl acetate and then concentrated in vacuo to obtain corresponding amine (7.00 g, 42%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.45-7.20 (12H, m), 6.97 (1H, s), 5.01 (1H, d, J = 13.4 Hz), 4.94 (1H, d, J = 5.1 Hz), 4.78 (1H, d, J = 5.3 Hz), 4.75 (1H, d, J = 13.4 Hz), 4.53 (2H, s), 3.58 (1H, d, J = 18.7 Hz), 3.41 (1H, d, J = 18.7 Hz). MS (ESI) *m/z*: 440 $[M+H]^+$.

To a solution of **14** (2.78 g, 12.3 mmol) in dichloromethane (90.0 mL) was added triethylamine (1.97 ml, 14.2 mmol) and methanesulfonyl chloride (1.03 ml, 13.2 mmol) at -20 °C and stirred at -20 °C for 30 min. To the reaction mixture was added a solution of amine obtained above (4.50 g, 9.45 mmol) and *N*-methylmorphiline (2.60 ml, 23.6 mmol) in dichloromethane (90.0 mL) at -20 °C and stirred at -20 °C for 1 h. The mixture was poured into water and ethyl acetate, and the layers were separated. The organic layer was washed with water, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure, and the crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain corresponding amide (1.00 g, 16%) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.70 (1H, d, *J* = 8.3 Hz), 7.45-7.28 (10H, m), 6.95 (1H, s), 6.44 (1H, t, *J* = 7.5 Hz), 6.39 (1H, s), 5.97 (1H, dd, *J* = 8.3, 4.9 Hz), 5.08-5.04 (2H, m), 4.81 (1H, d, *J* = 13.7 Hz), 4.52 (2H, br s), 3.58 (1H, d, *J* = 18.8 Hz), 3.40 (1H, d, *J* = 18.7 Hz), 3.08 (6H, s), 2.59 (2H, dq, *J* = 7.7, 7.5 Hz), 1.10 (3H, t, *J* = 7.5 Hz). MS (ESI) *m/z*: 648 $[M+H]^+$.

To a solution of amide obtained above (600 mg, 0.926 mmol) in dichloromethane (0.2 mL) was added anisole (1.01 ml, 9.26 mmol) and trifluoroacetic acid (7.14 ml, 93.0 mmol) at 0 °C and stirred at 0 °C for 1 h. The reaction mixture was poured into diisopropyl ether and the resulting residue was filtered and washed with diisopropyl ether. The resulting residue was applied onto the HP20SS resin and subjected to ODS column chromatography (acetonitrile/water) to afford **1** (340 mg, 76%) as a white amorphous solid after lyophilization. ^{1}H NMR (400 MHz, D_2O) δ: 6.50 (1H, s), 6.45 (1H, t, *J* = 7.9 Hz), 5.81 (1H, d, *J* = 4.6 Hz), 5.24 (1H, d, *J* = 4.6 Hz), 4.87 (1H, d, *J* = 12.5 Hz), 4.68 (1H, d, *J* = 12.4 Hz), 3.69 (1H, d, *J* = 17.8 Hz), 3.42 (1H, d, *J* = 17.8 Hz), 3.11 (6H, s), 2.28 (2H, dq, *J* = 7.5, 7.7 Hz), 1.09 (3H, t, *J* = 7.5 Hz). MS (ESI) *m/z*: 482 $[M+H]^+$.

Compound **2**

To a solution of **1** (160 mg, 0.318 mmol) in *N,N*-dimethylacetamide (1.60 mL) was added iodomethyl pivalate (85.0 mg, 0.350 mmol) at -40 °C and stirred at -40 °C for 1 h. The reaction mixture was poured into water and ethyl acetate, and the layers were separated. The organic layer was washed with water, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure, and the crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **2** (85.0 mg, 41%) as a white

solid after lyophilization using dioxane. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.72 (1H, d, *J* = 8.0 Hz), 6.44 (1H, t, *J* = 7.6 Hz), 6.38 (1H, s), 5.95-5.87 (3H, m), 5.11 (1H, d, *J* = 13.8 Hz), 5.04 (1H, d, *J* = 4.9 Hz), 4.83 (1H, d, *J* = 13.6 Hz), 4.67 (2H, br s), 3.59 (1H, d, *J* = 18.6 Hz), 3.41 (1H, d, *J* = 18.7 Hz), 3.07 (6H, s), 2.58 (2H, dq, *J* = 8.0, 7.5 Hz), 1.23 (9H, s), 1.10 (3H, t, *J* = 7.5 Hz). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 176.8, 166.9, 164.8, 160.2, 155.8, 149.6, 146.2, 144.0, 129.0, 127.6, 124.8, 102.8, 80.0, 63.3, 59.6, 57.6, 40.1, 38.6, 26.7, 26.4, 23.0, 13.8. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{25}H_{33}N_5O_8S_2$ 596.1843; found 596.1841. Anal. Calcd for $C_{25}H_{33}N_5O_8S_2(H_2O)_{0.6}(C_4H_8O_2)_{0.1}$: C, 49.58; H, 5.73; N, 11.38; S, 10.42. Found: C, 49.53; H, 5.67; N, 11.33; S, 10.32.

Compound **31**

To a solution of **17** (200 g, 664 mmol) in dichloromethane (1200 mL) was added triethylamine (107 ml, 774 mmol) and methanesulfonyl chloride (56.0 ml, 719 mmol) at -30 °C and stirred at -30 °C for 30 min. To the reaction mixture was added a solution of **30** (224 g, 553 mmol) and *N*-methylmorphiline (152 ml, 1.38 mol) in dichloromethane (400 mL) at -30 °C and stirred at -30 °C for 1 h. The mixture was poured into aqueous 1 mol/L hydrochloric acid solution and the layers were separated. The organic layer was washed with saturated aqueous sodium bicarbonate solution, water and brine, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **31** (359 g, 100%) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.31 (1H, s), 7.35 (2H, d, *J* = 8.6 Hz), 7.29 (1H, s), 7.17 (1H, d, *J* = 8.6 Hz), 6.91 (2H, d, *J* = 8.8 Hz), 6.00 (1H, dd, *J* = 9.0, 4.9 Hz), 5.30-5.20 (2H, m), 5.07 (1H, d, *J* = 5.1 Hz), 4.56 (1H, d, *J* = 11.9 Hz), 4.44 (1H, d, *J* = 11.9 Hz), 4.08 (3H, s), 3.82 (3H, s), 3.68 (1H, d, *J* = 18.4 Hz), 3.51 (1H, d, *J* = 18.2 Hz), 1.54 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 164.8, 162.4, 161.1, 160.7, 160.1, 152.3, 147.1, 141.3, 130.7, 127.0, 126.7, 125.7, 115.6, 114.2, 83.1, 68.4, 63.4, 58.9, 57.9, 55.4, 43.3, 28.3, 27.6. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{27}H_{30}N_5O_8S_2$ 652.1297; found 652.1296.

Compound **32**

To a solution of **31** (15.0 g, 23.0 mmol) in dichloromethane (75.0 mL) was added trifluoroacetic acid (45.0 mL, 588 mmol) at 0 °C and stirred at 0 °C for 30 min. The mixture was poured into water and diisopropyl

ether/dichloromethane (1/1) solution and the layers were separated. The organic layer was washed with water twice and brine, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure to give corresponding carboxylic acid (12.24 g, quant.) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.71 (1H, d, *J* = 7.3 Hz), 7.52 (1H, s), 5.07 (1H, d, *J* = 4.8 Hz), 4.60 (1H, d, *J* = 11.6 Hz), 4.53 (1H, d, *J* = 11.6 Hz), 4.13 (3H, s), 3.78-3.66 (2H, m), 3.54 (1H, d, *J* = 18.7 Hz), 1.57 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 164.8, 163.8, 162.9, 162.9, 161.2, 152.5, 146.0, 128.8, 125.8, 113.5, 83.2, 63.7, 59.8, 57.9, 43.1, 28.2, 27.9.

To a solution of carboxylic acid obtained above (8.0 g, 15.0 mmol) in acetonitrile (64.0 mL) was added *N*,*O*-bis(trimethylsilyl)acetamide (4.90 mL, 15.0 mmol) at 0 °C. To the reaction mixture was added a solution of 6-hydroxy-2-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one (1.44 g, 9.02 mmol) and sodium carbonate (956 mg, 9.02 mol) at 0 °C and stirred at 0 °C for 4 h. The mixture was poured into water/acetonitrile (10/1) solution, and then 2 mol/L aqueous sodium hydroxide solution was added to the mixture to adjust the pH to around 6.5. The solvent of the resulting mixture was removed under reduced pressure to give **32** (5.40 g, 55%) as an orange solid after lyophilization. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 11.76 (1H, br s), 9.57 (1H, d, *J* = 8.3 Hz), 7.26 (1H, s), 5.57 (1H, dd, *J* = 8.1, 4.8 Hz), 5.00 (1H, d, *J* = 4.5 Hz), 4.37 (1H, d, *J* = 12.6 Hz), 4.14 (1H, d, *J* = 12.6 Hz), 3.86 (3H, s), 3.56-3.47 (5H, m), 1.47 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, DMSO-D_6) δ: 171.5, 164.7, 164.3, 162.7, 162.1, 161.1, 156.5, 153.2, 148.7, 141.8, 133.3, 115.8, 114.5, 81.4, 62.0, 58.0, 57.5, 44.4, 42.2, 27.8, 26.4. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{23}H_{26}N_8O_9S_3$ 655.1058; found 655.1056.

Compound **3**

To a solution of **32** (300 mg, 0.429 mmol) in *N*,*N*-dimethylformamide (3.00 mL) was added iodomethyl pivalate (312 mg, 1.29 mmol) at -40 °C. The mixture was stirred at -40 °C to -20 °C for 2 h and standed at -20 °C overnight. The mixture was poured into water and ethyl acetate, and then the layers were separated. The organic layer was washed with water and brine, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain pivaloyloxymethyl ester (300 mg, 79%) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.04 (1H, br s), 7.34 (1H, s), 6.90

(1H, d, *J* = 8.1 Hz), 6.02-5.90 (5H, m), 5.08 (1H, d, *J* = 5.1 Hz), 4.63 (1H, d, *J* = 12.9 Hz), 4.16-4.08 (6H, m), 3.83-3.64 (6H, m), 1.55 (9H, s), 1.25-1.22 (18H, m). MS (ESI) *m/z*: 883 $[M+H]^+$.

To a solution of pivaloyloxymethyl ester obtained above (250 mg, 0.283 mmol) in dichloromethane (2.50 mL) were added anisole (0.216 ml, 1.98 mmol) and 2 mol/L aluminium chloride solution in nitromethane (0.991 ml, 1.98 mmol) at -40 °C and stirred at -40 °C for 30 min. To this resulting mixture was added diisopropyl ether and water, and the resulting residue was filtered and washed with diisopropyl ether and water, and then concentrated in vacuo to obtain **3** (128 mg, 58%) as a white solid after lyophilization using dioxane. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.62 (1H, d, *J* = 6.8 Hz), 6.89 (1H, s), 6.00-5.89 (9H, m), 5.07 (1H, d, *J* = 4.1 Hz), 4.61 (1H, d, *J* = 13.6 Hz), 4.14-4.05 (5H, m), 3.76-3.63 (2H, m), 1.22 (9H, s), 1.21 (9H, s).δ: 9.59 (1H, d, *J* = 7.9 Hz), 7.26 (2H, br s), 6.74 (1H, s), 6.01 (1H, d, *J* = 5.9 Hz), 5.84-5.79 (4H, m), 5.15 (1H, d, *J* = 4.8 Hz), 4.53 (1H, d, *J* = 13.7 Hz), 3.99 (1H, d, *J* = 13.8 Hz), 3.85-3.77 (4H, m), 3.63-3.58 (4H, m), 1.16-1.16 (18H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 177.1, 176.9, 168.5, 164.3, 162.9, 161.7, 160.6, 155.0, 151.2, 145.7, 140.6, 130.6, 125.0, 111.8, 83.4, 80.4, 63.8, 59.2, 57.5, 42.8, 39.0, 38.9, 33.1, 28.4, 27.1, 27.0. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{27}H_{36}N_5O_{11}S_2$ 783.1895; found 783.1892.

Compound **33**

To a solution of **19** (2.06 g, 10.3 mmol) in *N,N*-dimethylacetamide (22.4 mL) was added triethylamine (1.64 ml, 11.8 mmol) and methanesulfonyl chloride (0.861 ml, 11.1 mmol) at -20 °C and stirred at -20 °C for 30 min. To the reaction mixture was added a solution of **30** (3.20 g, 7.90 mmol) and triethylamine (2.74 ml, 19.7 mol) in *N,N*-dimethylacetamide (9.60 mL) at -20 °C and stirred at -20 °C for 1 h. The mixture was poured into water and ethyl acetate, and then the layers were separated. The organic layer was washed with water and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **33** (2.70 g, 62%) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.60 (1H, s), 7.36 (2H, d, *J* = 8.7 Hz), 7.14 (1H, d, *J* = 9.0 Hz), 6.91 (2H, d, *J* = 8.8 Hz), 5.99 (1H, dd, *J* = 9.0, 5.0 Hz), 5.24 (2H, s), 5.07 (1H, d, *J* = 5.0 Hz), 4.55 (1H, d, *J* = 11.8 Hz), 4.45 (1H, d, *J* = 11.8 Hz), 4.12 (3H, s), 3.82 (3H, s), 3.68 (1H, d, *J* = 18.3 Hz), 3.51 (1H,

d, *J* = 18.3 Hz), 2.76 (3H, s). MS (ESI) *m/z*: 551 $[M+H]^+$.

Compound **4**

To a solution of **30** (2.50 g, 4.54 mmol) in *N*,*N*-dimethylformamide (7.50 mL) were added 6-hydroxy-2-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one (0.722 g, 4.54 mmol), sodium iodide (1.360 g, 9.07 mmol) and potassium carbonate (0.627 g, 4.54 mmol) at 0 °C and stirred at 0 °C for 30 min. The mixture was poured into ethyl acetate and 1 mol/L aqueous hydrochloric acid solution to adjust the pH to around 4.3 and the layers were separated. The organic layer was washed with water, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure to give corresponding thioether (2.20 g, 72%) as an orange oil. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 9.65 (1H, d, *J* = 8.1 Hz), 7.67 (1H, s), 7.38 (2H, d, *J* = 8.3 Hz), 6.92 (2H, d, *J* = 8.3 Hz), 5.84 (1H, dd, *J* = 8.0, 4.9 Hz), 5.24 (2H, s), 5.17 (1H, d, *J* = 4.8 Hz), 4.48 (1H, d, *J* = 13.4 Hz), 3.88 (3H, s), 3.80-3.75 (4H, m), 3.65-3.55 (5H, m), 2.65 (3H, d, *J* = 8.3 Hz). MS (ESI) *m/z*: 674 $[M+H]^+$.

To a solution of thioether obtained above (1.32 g, 1.96 mmol) in dichloromethane (20.0 mL) were added anisole (2.13 ml, 19.6 mmol) and 2 mol/L aluminium chloride solution in nitromethane (3.90 ml, 7.84 mmol) at -30 °C and stirred at -30 °C to -20 °C for 40 min. The reaction mixture was poured into diisopropyl ether and water and then phosphate buffer solution (pH 6.8) was added to the mixture to adjust the pH to around 4.0 The resulting residue was applied onto the HP20SS resin and subjected to ODS column chromatography (acetonitrile/water) and then 2 mol/L aqueous sodium hydroxide solution was added to the mixture to adjust the pH to around 5.5. The solvent of the resulting mixture was removed under reduced pressure to afford **4** (61 mg, 6%) as a white amorphous solid after lyophilization. ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ: 9.57 (1H, d, *J* = 8.3 Hz), 7.65 (1H, s), 5.57 (1H, dd, *J* = 8.2, 4.6 Hz), 5.00 (1H, d, *J* = 4.5 Hz), 3.99-3.89 (4H, m), 3.63-3.51 (2H, m), 3.44 (3H, s), 3.29 (1H, d, *J* = 18.1 Hz), 2.66 (3H, s). ^{13}C{^{1}H} NMR (100 MHz, DMSO-*d*$_6$) δ: 166.3, 164.3, 164.3, 162.8, 161.7, 161.1, 153.2, 148.7, 146.1, 134.1, 119.8, 119.7, 62.1, 58.0, 57.4, 42.2, 33.6, 26.2, 18.8. MS (ESI) *m/z*: 554 $[M+H]^+$.

Compound **5**

To a solution of **4** (280 mg, 0.469 mmol) in *N*,*N*-dimethylformamide (1.00

mL) were added iodomethyl pivalate (0.225 ml, 1.45 mmol) and potassium carbonate (0.129 g, 0.933 mmol) at -30 °C and stirred at -30 °C to -10 °C for 4 h. To the reaction mixture was added iodomethyl pivalate (0.112 ml, 0.724 mmol) at -10 °C and stirred at -10 °C to -5 °C for 2 h. The mixture was poured into water and ethyl acetate, and then the layers were separated. The organic layer was washed with water and brine, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **5** (110 mg, 30%) as a white amorphous solid after lyophilization. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.59 (1H, s), 7.05 (1H, d, J = 8.9 Hz), 6.03-5.90 (5H, m), 5.08 (1H, d, J = 4.9 Hz), 4.63 (1H, d, J = 13.6 Hz), 4.16-4.10 (4H, m), 3.77-3.65 (5H, m), 2.75 (3H, s), 1.24 (9H, s), 1.23 (9H, s). MS (ESI) *m/z*: 782 $[M+H]^+$.

Compound **35**

To a solution of **34** (18.7 g, 38.9 mmol) in acetonitrile (187 mL) was added *p*-toluenesulfonic acid monohydrate (33.6 g, 177 mmol) at 0 °C and the mixture was stirred at room temperature for 1 h. The mixture was poured into aqueous sodium bicarbonate solution and ethyl acetate, and the layers were separated. The aqueous layer was extracted with ethyl acetate twice and the combined organic layer was washed with brine, and then dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The resulting mixture was concentrated in vacuo to obtain **35** (11.00 g, 74%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.46 (2H, d, J = 7.4 Hz), 7.39-7.27 (8H, m), 6.94 (1H, s), 4.93 (1H, d, J = 4.9 Hz), 4.73 (1H, d, J = 4.9 Hz), 3.50 (1H, d, J = 18.3 Hz), 3.21 (1H, d, J = 18.2 Hz), 2.09 (3H, s), 1.77 (2H, br s).

Compound **6**

To a solution of **19** (0.981 g, 4.90 mmol) in dichloromethane (40.0 mL) were added **35** (1.86 g, 4.90 mmol), *N*-methymorpholine (2.69 ml, 24.5 mmol) and phenyl dichlorophosphate (1.10 ml, 7.35 mmol) at -30 °C and then stirred at -30 °C to 0 °C for 90 min. This resulting mixture was poured into water and ethyl acetate and the organic layer was washed with 5% aqueous citric acid solution, saturated aqueous sodium bicarbonate solution and brine in turn, and then dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The resulting residue was concentrated in vacuo to obtain

amide (1.28 g, 46%) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.61-7.27 (11H, m), 7.17 (1H, d, *J* = 8.8 Hz), 6.93 (1H, s), 5.95 (1H, dd, *J* = 8.8, 4.8 Hz), 5.07 (1H, d, *J* = 4.6 Hz), 4.12 (3H, s), 3.52 (1H, d, *J* = 18.9 Hz), 3.25 (1H, d, *J* = 18.4 Hz), 2.76 (3H, s), 2.13 (3H, s). MS (ESI): *m/z* 563 $[M+H]^+$.

To a solution of amide obtained above (0.65 g, 1.26 mmol) in dichloromethane (6.50 mL) were added anisole (0.825 ml, 7.55 mmol) and 2 mol/L aluminium chloride solution in nitromethane (3.77 ml, 7.55 mmol) at -20 °C and then stirred at -20 °C for 30 min. To this resulting mixture were poured into diisopropyl ether, 0.2 mol/L aqueous hydrochloric acid solution and acetonitrile, and then the resulting mixture was extracted with water. The aqueous layer was applied onto the HP20SS resin and subjected to ODS column chromatography (acetonitrile/water), and then 2 mol/L aqueous sodium hydroxide solution was added to the fractions to adjust the pH to around 5.2. The reslting solution was lyophilized to afford **6** (340 mg, 76%) as a white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.53 (1H, d, *J* = 8.3 Hz), 7.65 (1H, s), 5.52 (1H, dd, *J* = 8.2, 4.6 Hz), 4.94 (1H, d, *J* = 4.6 Hz), 3.89 (3H, s), 3.41 (1H, d, *J* = 16.9 Hz), 3.05 (1H, d, *J* = 17.3 Hz), 2.67 (3H, s), 1.87 (3H, s). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ: 166.3, 165.3, 165.3, 162.8, 161.6, 148.8, 146.2, 130.7, 119.8, 116.4, 79.3, 79.0, 78.7, 62.1, 57.8, 57.0, 28.7, 19.3, 18.8. MS (ESI): *m/z* 397 $[M-Na+2H]^+$.

Compound **7**

To a solution of **25** (91 mg, 0.40 mmol) in dichloromethane (4.0 mL) were added **35** (152 mg, 0.40 mmol), *N*-methymorpholine (220 μl, 2.00 mmol) and phenyl dichlorophosphate (292 μl, 0.600 mmol) at -30 °C and then stirred at -30 °C to 0 °C for 90 min. To this resulting mixture were poured into water and ethyl acetate and the organic layer was washed with 5% aqueous citric acid solution, saturated aqueous sodium bicarbonate solution and brine in turn, and then dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The resulting residue was concentrated in vacuo to obtain amide (250 mg, quant.) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.46-7.28 (11H, m), 7.06 (1H, s), 6.92 (1H, s), 5.79 (1H, dd, *J* = 8.7, 4.6 Hz), 5.04 (1H, d, *J* = 4.6 Hz), 4.17 (2H, s), 3.38 (3H, s), 2.16 (3H, s), 2.01 (6H, s). MS (ESI): *m/z* 592 $[M+H]^+$.

To a solution of amide (1.40 g, 2.37 mmol) in dichloromethane (7.00 mL) were added anisole (2.58 ml, 23.7 mmol) and trifluoroacetic acid (7.00 ml, 91.5

mmol) at 0 °C and then stirred at 0 °C for 50 min. To this resulting mixture were poured into ethanol, water and methanol. The layers were separated and the organic layer was extracted with water and the combined aqueous layer was washed with diethylether and the resulting aqueous layer was applied onto the HP20SS resin and subjected to ODS column chromatography (acetonitrile/water) to afford **7** (618 mg, 57%) as a white amorphous solid after lyophilization. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.57 (1H, d, J = 8.1 Hz), 6.90 (1H, s), 5.69 (1H, dd, J = 8.1, 4.5 Hz), 5.09 (1H, d, J = 4.5 Hz), 3.86 (3H, s), 3.55 (1H, d, J = 17.9 Hz), 3.34 (1H, d, J = 18.2 Hz), 3.04 (6H, s), 2.01 (3H, s). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ: 171.5, 164.1, 164.1, 162.5, 147.2, 143.1, 133.3, 122.9, 110.2, 63.2, 58.9, 57.5, 40.7, 30.7, 20.3. Anal. Calcd for $C_{16}H_{19}N_5O_5S_2(H_2O)_{1.5}$: C, 42.47; H, 4.90; N, 15.48; S, 14.17. Found: C, 42.39; H, 4.87; N, 15.59; S, 14.08. MS (ESI): *m/z* 426 $[M+H]^+$.

Compound **8**

To a solution of **6** (200 mg, 0.478 mmol) in *N*,*N*-dimethylformamide (1.00 mL) was added iodomethyl pivalate (116 mg, 0.478 mmol) at -20 °C. The mixture was stirred at -20 °C for 2 h and the mixture was poured into water and ethyl acetate, and then the layers were separated. The organic layer was washed with water and brine, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **8** (230 mg, 94%) as a yellow powder after lyophilization. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.60 (1H, s), 7.12 (1H, d, J = 8.8 Hz), 5.95-5.91 (2H, m), 5.86 (1H, d, J = 5.5 Hz), 5.07 (1H, d, J = 4.8 Hz), 4.11 (3H, s), 3.56 (1H, d, J = 18.4 Hz), 3.26 (1H, d, J = 18.6 Hz), 2.75 (3H, s), 2.15 (3H, s), 1.23 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 177.1, 167.3, 163.7, 161.8, 160.8, 146.6, 146.2, 133.2, 122.2, 121.2, 80.0, 63.7, 59.2, 57.1, 38.9, 30.6, 27.0, 20.2, 19.5. MS (ESI): *m/z* 511 $[M+H]^+$.

Compound **9**

To a solution of **7** (200 mg, 0.470 mmol) in *N*,*N*-dimethylformamide (2.00 mL) were added iodomethyl pivalate (114 mg, 0.470 mmol) and potassium carbonate (65 mg, 0.470 mmol) at -40 °C. The mixture was stirred at -30 °C for 40 min and the mixture was poured into water and ethyl acetate, and then the layers were separated. The organic layer was washed with water and brine, and then dried over $MgSO_4$, filtered, and the solvent was removed

under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **9** (150 mg, 52%) as a yellow powder after lyophilization. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.18 (1H, d, *J* = 8.7 Hz), 6.92 (1H, s), 5.93-5.90 (2H, m), 5.86 (1H, d, *J* = 5.5 Hz), 5.05 (1H, d, *J* = 4.6 Hz), 4.06 (3H, s), 3.55 (1H, d, *J* = 18.4 Hz), 3.24 (1H, d, *J* = 18.4 Hz), 3.12 (6H, s), 2.14 (3H, s), 1.23 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 177.1, 171.2, 163.9, 162.1, 160.9, 147.1, 143.4, 133.1, 122.1, 110.1, 80.0, 63.4, 59.1, 57.1, 40.5, 38.9, 30.6, 27.0, 20.2. Anal. Calcd for $C_{22}H_{29}N_5O_7S_2(H_2O)_{0.5}(C_4H_8O_2)_{0.4}$: C, 48.55; H, 5.73; N, 11.99; S, 10.98. Found: C, 48.51; H, 5.65; N, 12.13; S, 10.80. MS (ESI): *m/z* 540 $[M+H]^+$.

Compound **36**

To a solution of **33** (2.00 g, 3.63 mmol) in acetone (20.0 mL) was added sodium iodide (1.63 g, 10.9 mmol) at room temperature and then stirred at the same temperature for 2 h. To this resulting mixture were poured into 5% aqueous sodium thiosulfate solution and ethyl acetate and the organic layer was separated and washed with 5% aqueous sodium thiosulfate solution and water in turn, and then dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The resulting residue was concentrated in vacuo to obtain iodomethyl (2.40 g, quant.) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.59 (1H, s), 7.37 (2H, d, *J* = 8.7 Hz), 7.10 (1H, d, *J* = 9.0 Hz), 6.91 (2H, d, *J* = 8.8 Hz), 5.94 (1H, dd, *J* = 9.0, 5.0 Hz), 5.25 (2H, dd, *J* = 12.4, 11.5 Hz), 5.04 (1H, d, *J* = 5.0 Hz), 4.43 (1H, d, *J* = 9.3 Hz), 4.33 (1H, d, *J* = 9.4 Hz), 4.12 (3H, s), 3.82-3.77 (4H, m), 3.54 (1H, d, *J* = 18.1 Hz), 2.76 (3H, s). MS (ESI): *m/z* 643 $[M+H]^+$.

To a solution of iodomethyl obtained above (2.33 g, 3.63 mmol) in *N*,*N*-dimethylformamide (11.6 mL) were added 5-methyltetrazole (0.396 g, 4.71 mmol) and potassium carbonate (0.601 g, 4.35 mmol) at -30 °C and then stirred at the same temperature for 1 h. To this resulting mixture was added 5-methyltetrazole (0.091 g, 1.08 mmol) at -10 °C and then stirred at -10 °C to 0 °C for 1 h. To this resulting mixture were poured into ethyl acetate and water and the organic layer was separated and washed with brine, and then dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The resulting residue was concentrated in vacuo to obtain tetrazole (2.18 g, quant.) as a crude product.

To a solution of tetrazole obtained above (2.18 g) in dichloromethane (21.0

mL) was added *m*-chloroperoxybenzoic acid (69.0~75.0%, 1.00 g) at -40 °C and then stirred at the same temperature for 1 h. To this resulting mixture were added dichloromethane (10.0 mL) and *m*-chloroperoxybenzoic acid (69.0~75.0%, 500 mg) at -40 °C and then stirred at -40 °C for 1 h. To this resulting mixture were poured into ethyl acetate and 0.1 mol/L aqueous hydrochloric acid solution and the organic layer was separated and washed with saturated aqueous sodium bicarbonate solution and brine, and then dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain sulfoxide (373 mg, 17%) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.56 (1H, s), 7.50 (1H, d, *J* = 10.3 Hz), 7.37 (2H, d, *J* = 8.7 Hz), 6.92 (2H, d, *J* = 8.7 Hz), 6.24 (1H, dd, *J* = 10.1, 4.8 Hz), 5.82 (1H, d, *J* = 15.6 Hz), 5.35-5.22 (3H, m), 4.61 (1H, dd, *J* = 4.9, 1.6 Hz), 4.10 (3H, s), 3.83 (3H, s), 3.71 (1H, d, *J* = 18.7 Hz), 3.18 (1H, d, *J* = 18.3 Hz), 2.75 (3H, s), 2.46 (3H, s). MS (ESI): *m/z* 615 $[M+H]^+$.

To a solution of sulfoxide obtained above (370 mg, 0.602 mmol) in *N*,*N*-dimethylformamide (3.7 mL) was added phosphorus tribromide (95.0 μl, 1.01 mmol) at -30 °C and then stirred at the same temperature for 30 min. To this resulting mixture were poured into ethyl acetate and water and the organic layer was separated and washed with saturated aqueous sodium bicarbonate solution and brine, and then dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **36** (366 mg, quant.) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.58 (1H, s), 7.36 (2H, d, *J* = 8.7 Hz), 7.11 (1H, d, *J* = 9.0 Hz), 6.91 (2H, d, *J* = 8.7 Hz), 5.99 (1H, dd, *J* = 9.0, 5.0 Hz), 5.83 (1H, d, *J* = 15.3 Hz), 5.55 (1H, d, *J* = 15.3 Hz), 5.29 (1H, d, *J* = 11.8 Hz), 5.25 (1H, d, *J* = 11.5 Hz), 5.03 (1H, d, *J* = 5.0 Hz), 4.10 (3H, s), 3.82 (3H, s), 3.33 (1H, d, *J* = 18.4 Hz), 3.27 (1H, d, *J* = 18.4 Hz), 2.75 (3H, s), 2.54 (3H, s). MS (ESI): *m/z* 599 $[M+H]^+$.

Compound **10**

To a solution of **36** (350 mg, 0.585 mmol) in dichloromethane (3.50 mL) were added anisole (0.383 ml, 3.51 mmol) and 2 mol/L aluminium chloride solution in nitromethane (1.75 ml, 3.51 mmol) at -20 °C and then stirred at the same temperature for 30 min. To this resulting mixture were poured into diisopropyl ether, acetonitrile and water and the aqueous layer was separated

and applied onto the HP20SS resin and subjected to ODS column chromatography (acetonitrile/water). The fractions containing the desired product were collected and the pH of the solution was adjusted to around 6.0 using 0.2 mol/L aqueous sodium hydroxide solution. The resulting solution was concentrated and lyophilized to give **10** (240 mg, 82%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.60 (1H, d, *J* = 8.3 Hz), 7.66 (1H, s), 6.01 (1H, d, *J* = 14.3 Hz), 5.63 (1H, dd, *J* = 8.2, 4.8 Hz), 5.47 (1H, d, *J* = 14.3 Hz), 5.01 (1H, d, *J* = 4.8 Hz), 3.89 (3H, s), 3.25 (1H, d, *J* = 17.2 Hz), 3.16 (1H, d, *J* = 17.2 Hz), 2.67 (3H, s), 2.44 (3H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 166.3, 163.1, 162.8, 162.2, 162.1, 148.8, 146.1, 135.9, 119.7, 110.3, 62.2, 58.1, 57.4, 53.9, 25.7, 18.8, 10.5. MS (ESI): *m/z* 479 $[M\text{-}Na+2H]^+$.

Compound **11**

To a solution of **10** (200 mg, 0.400 mmol) in *N*,*N*-dimethylformamide (1.00 mL) was added iodomethyl pivalate (97 mg, 0.400 mmol) at -20 °C and then stirred at the same temperature for 1 h. To this resulting mixture were poured into ethyl acetate and water and the organic layer was separated and washed with water and brine, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **11** (179 mg, 76%) as a white amorphous solid after lyophilization. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.59 (1H, s), 7.11 (1H, d, *J* = 8.9 Hz), 6.03 (1H, dd, *J* = 8.9, 5.0 Hz), 5.98 (1H, d, *J* = 5.5 Hz), 5.92 (1H, d, *J* = 5.5 Hz), 5.84 (1H, d, *J* = 15.4 Hz), 5.54 (1H, d, *J* = 15.3 Hz), 5.07 (1H, d, *J* = 5.0 Hz), 4.11 (3H, s), 3.35 (1H, d, *J* = 18.6 Hz), 3.28 (1H, d, *J* = 18.6 Hz), 2.75 (3H, s), 2.55 (3H, s), 1.24 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 177.1, 168.7, 164.5, 163.7, 162.2, 160.1, 146.6, 141.8, 126.2, 124.7, 111.9, 80.5, 63.4, 59.3, 57.7, 53.1, 38.9, 27.3, 27.0, 11.0. MS (ESI): *m/z* 593 $[M+H]^+$.

Compound **38**

To a solution of **37** (4.00 g, 5.94 mmol) in *N*,*N*-dimethylformamide (20.0 mL) were added sodium iodide (1.78 g, 11.9 mmol), 5-methyltetrazole (0.599 g, 7.13 mmol) and potassium carbonate (0.985 g, 7.13 mmol) at 10 °C and then stirred at the same temperature overnight. To this resulting mixture was added phosphorus tribromide (1.68 ml, 17.8 mmol) at -40 °C and then stirred at the same temperature for 30 min. To this resulting mixture were poured

into ethyl acetate and water and the organic layer was separated and washed with saturated aqueous sodium bicarbonate solution and water, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **38** (373 mg, 25%) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.47-7.20 (25H, m), 6.92 (1H, s), 5.71 (1H, d, *J* = 15.4 Hz), 5.36 (1H, d, *J* = 15.4 Hz), 4.77 (1H, dd, *J* = 9.9, 4.8 Hz), 4.29 (1H, d, *J* = 4.8 Hz), 3.09 (1H, d, *J* = 18.4 Hz), 3.02 (1H, d, *J* = 18.4 Hz), 2.94 (1H, d, *J* = 9.9 Hz), 2.49 (3H, s).

Compound **39**

To a solution of **38** (0.800 g, 1.14 mmol) in acetone (4.80 mL) was added 6.0 mol/L aqueous hydrochloric acid solution at room temperature and then stirred at the same temperature for 4 h. The solvent was removed under reduced pressure. The resulting residue was concentrated in vacuo to obtain **39** (620 mg, quant.) as a clude product.

Compound **40**

To a solution of **25** (417 mg, 1.82 mmol) in *N,N*-dimethylacetamide (4.89 mL) were added triethylamine (0.291 ml, 2.10 mmol), methanesulfonyl chloride (0.153 ml, 1.96 mmol) at -20 °C and then stirred at the same temperature for 30 min. To this resulting mixture were added triethylamine (0.485 ml, 3.50 mmol) and **39** (417 mg, 1.82 mmol) at -20 °C and then stirred at the same temperature for 1 h. To this resulting mixture were poured into ethyl acetate and water and the organic layer was separated and washed with water, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **40** (720 mg, 76%) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.45-7.28 (11H, m), 6.98 (1H, s), 6.91 (1H, s), 6.03 (1H, dd, *J* = 4.8, 8.8 Hz), 5.78 (1H, d, *J* = 15.3 Hz), 5.48 (1H, d, *J* = 15.6 Hz), 5.06 (1H, d, *J* = 4.8 Hz), 4.06 (3H, s), 3.27 (2H, s), 3.12 (6H, s), 2.53 (3H, s). MS (ESI): *m/z* 674 $[M+H]^+$.

Compound **12**

To a solution of **40** (350 mg, 0.519 mmol) in dichloromethane (3.50 mL)

were added anisole (0.567 ml, 5.19 mmol) and trifluoroacetic acid (4.00 ml, 51.9 mmol) at 0 °C and then stirred at the same temperature for 30 min. To this resulting mixture were poured into diisopropyl ether and water, and then filtered and rinsed with diisopropyl ether and water to obtain **12** (260 mg, 95%) as a white solid. ^{1}H NMR (400MHz, D_2O) δ: 7.00 (1H, s), 5.85 (1H, d, *J* = 4.6 Hz), 5.65 (1H, d, *J* = 14.9 Hz), 5.49 (1H, d, *J* = 14.9 Hz), 5.23 (1H, d, *J* = 4.8 Hz), 4.01 (3H, s), 3.50 (1H, d, *J* = 17.8 Hz), 3.33 (1H, d, *J* = 17.8 Hz), 3.12 (6H, s), 2.52 (3H, s). MS (ESI): *m/z* 508 $[M\text{-}Na+2H]^+$.

Compound **13**

To a solution of **12** (220 mg, 0.415 mmol) in *N*,*N*-dimethylformamide (1.10 mL) was added iodomethyl pivalate (101 mg, 0.415 mmol) at -20 °C. The mixture was stirred at -20 °C for 1 h and the mixture was poured into water and ethyl acetate, and then the layers were separated. The organic layer was washed with water and brine, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **13** (247 mg, 96%) as a white solid after lyophilization. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.25 (1H, d, *J* = 10.0 Hz), 6.91 (1H, s), 6.00 (1H, dd, *J* = 5.1, 8.9 Hz), 5.98 (1H, d, *J* = 5.8 Hz), 5.92 (1H, d, *J* = 5.5 Hz), 5.83 (1H, d, *J* = 15.4 Hz), 5.53 (1H, d, *J* = 15.3 Hz), 5.05 (1H, d, *J* = 5.0 Hz), 4.06 (3H, s), 3.32 (1H, d, *J* = 18.6 Hz), 3.26 (1H, d, *J* = 18.7 Hz), 3.11 (6H, s), 2.55 (3H, s), 1.24 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 177.1, 171.2, 164.1, 163.7, 162.0, 160.2, 146.9, 143.4, 126.4, 124.1, 109.7, 80.5, 63.4, 59.4, 57.6, 53.1, 40.5, 38.9, 27.0, 26.9, 11.0. MS (ESI): *m/z* 622 $[M+H]^+$.

Compound **49**

To a solution of methoxyacetic acid (3.66 ml, 47.9 mmol) in water (53.5 mL) and dichloromethane (53.5 mL) were added tetrabutylammonium hydrogen sulfate (16.3 g, 47.9 mmol) and sodium bicarbonate (8.05 g, 96.0 mmol), and the mixture was stirred at room temperature for 1 h. To this resulting mixture was added **48** (10.7 g, 39.9 mmol), and the mixture was stirred at room temperature for 3 h. To this mixture was added water followed by extraction with dichloromethane. The organic layer was washed with water, and then dried over $MgSO_4$, filtered, and evaporated under reduced pressure. To this resulting mixture was added diethyl ether, followed by

filtration and washing with diethyl ether. The resulting filtrate was evaporated under reduced pressure and concentrated in vacuo to give **49** (9.91 g, quant.) as a yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.88 (2H, s), 4.10 (2H, s), 3.47 (3H, s), 2.90 (2H, q, J = 7.4 Hz), 1.33 (3H, t, J = 7.4 Hz).

Compound **47**

To **49** (9.91 g, 39.9 mmol) was added sulfuryl chloride (3.25 ml, 39.9 mmol) at -30 °C, and the mixture was stirred at -30 °C for 15 min. To this resulting mixture was added BF_3·OEt_2 (0.176 ml, 1.36 mmol), and the mixture was stirred at 0 °C for 1 h and then at room temperature for 1 h. The resulting mixture was evaporated under reduced pressure and the resulting residue was distilled (1 Torr, 90 °C) to give **47** (6.34 g, 87%) as a light-yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.89 (2H, s), 4.14 (2H, s), 3.48 (3H, s).

Compound **50**

Compound **50** was prepared by a procedure similar to that used for compound **47**. A light-yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.83 (2H, s), 1.25 (9H, s).

Compound **51**

Compound **51** was prepared by the procedure similar to that used for compound **47**. A light-yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.82 (2H, s), 2.18 (3H, s).

Compound **41**

To a solution of cefetamet pivoxil (3.00g, 5.86 mmol) in *N*,*N*-dimethylacetamide (30.0 mL) and dichloromethane (30.0 mL) were added **50** (3.42 g, 17.6 mmol) and pyridine (1.42 mL, 17.6 mmol) at 0 °C divided into three portions, and the mixture was stirred at 0 °C for 90 min. To this resulting mixture was added water followed by extraction with ethyl acetate. The organic layer was washed with 0.2 mol/L aqueous hydrochloric acid, water and brine, and then dried over $MgSO_4$, filtered, and evaporated under reduced pressure. This resulting residue was subjected to silica gel column chromatography being eluted with hexane/ethyl acetate. The fractions containing the desired compound were concentrated under reduced pressure to obtain **41** (2.40 g, 61%) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.71

(1H, br s), 7.38 (1H, s), 7.21 (1H, d, J = 8.6 Hz), 5.96-5.92 (2H, m), 5.88-5.87 (3H, m), 5.08 (1H, d, J = 4.8 Hz), 4.08 (3H, s), 3.56 (1H, d, J = 18.7 Hz), 3.27 (1H, d, J = 18.7 Hz), 2.15 (3H, s), 1.23 (9H, s), 1.22 (9H, s). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ: 177.4, 177.2, 164.2, 162.2, 160.8, 159.9, 149.7, 146.7, 141.7, 133.7, 122.1, 116.5, 80.8, 80.1, 63.5, 59.0, 57.4, 41.4, 39.0, 38.9, 30.7, 27.0, 27.0, 20.2. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{27}H_{36}N_5O_{11}S_2$ 670.1847; found 670.1848.

Compound **42**

To a solution of cefetamet pivoxil (1.00 g, 1.96 mmol) in *N*,*N*-dimethylacetamide (7.00 mL) were added **51** (1.19 g, 7.82 mmol) and pyridine (0.632 mL, 7.82 mmol) at 0 °C divided into four portions, and the mixture was stirred at 0 °C for 2 h. To this resulting mixture was added water followed by extraction with ethyl acetate. To this resulting mixture was added 0.2 mol/L aqueous hydrochloric acid followed by extraction with ethyl acetate. The organic layer was washed with water, and then dried over $MgSO_4$, filtered, and evaporated under reduced pressure. This resulting residue was subjected to silica gel column chromatography, being eluted with hexane/ethyl acetate. The fractions containing the desired compound were concentrated and lyophilized to obtain **42** (300 mg, 25%) as a colorless foam. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.42 (1H, s), 7.12 (1H, d, J = 8.7 Hz), 5.94-5.91 (2H, m), 5.88-5.86 (3H, m), 5.08 (1H, d, J = 4.6 Hz), 4.08 (3H, s), 3.57 (1H, d, J = 19.4 Hz), 3.27 (1H, d, J = 18.6 Hz), 2.16-2.15 (6H, m), 1.23 (9H, s). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ: 177.2, 170.1, 164.2, 162.1, 160.8, 160.1, 152.2, 146.7, 141.7, 134.0, 122.2, 116.5, 80.4, 80.1, 63.4, 59.1, 57.4, 38.9, 30.7, 27.0, 20.8, 20.3. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{24}H_{30}N_5O_{11}S_2$ 628.1378; found 628.1381.

Compound **43**

To a solution of cefetamet pivoxil (0.350 g, 0.684 mmol) in *N*,*N*-dimethylacetamide (3.50 mL) were added **47** (0.125 g, 0.684 mmol) and pyridine (0.055 mL, 0.684 mmol) at 0 °C, and the mixture was stirred at 0 °C for 30 min. To this resulting mixture was added water followed by extraction with ethyl acetate. The organic layer was washed with water and then dried over $MgSO_4$, filtered, and evaporated under reduced pressure. This resulting residue was subjected to silica gel column chromatography, being eluted with hexane/ethyl acetate. The fractions containing the desired compound were

concentrated and lyophilized to obtain **43** (80.0 mg, 18%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.36 (1H, s), 7.30 (1H, d, *J* = 8.7 Hz), 5.97-5.87 (5H, m), 5.09 (1H, d, *J* = 4.8 Hz), 4.15-4.06 (6H, m), 3.56 (1H, d, *J* = 18.9 Hz), 3.49-3.47 (3H, m), 3.27 (1H, d, *J* = 18.4 Hz), 2.16 (3H, s), 1.23 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 177.2, 169.2, 164.2, 162.1, 160.8, 159.6, 151.9, 146.7, 141.7, 134.1, 122.1, 116.6, 80.6, 80.1, 69.5, 63.5, 59.7, 59.0, 57.4, 38.9, 30.7, 27.0, 20.3. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{25}H_{31}N_5O_{12}S_2$ 658.1483; found 658.1483.

Compound **44**

To a solution of **41** (350 mg, 0.523 mmol) in *N*,*N*-dimethylformamide (1.40 mL) was added iodomethyl pivalate (434 mg, 1.79 mmol), and the mixture was stirred at 80 °C for 2 h. To this resulting mixture was added water followed by extraction with ethyl acetate. The organic layer was washed with water and brine, and then dried over $MgSO_4$, filtered, and evaporated under reduced pressure. This resulting residue was subjected to silica gel column chromatography, being eluted with hexane/ethyl acetate. The fractions containing the desired compound were concentrated under reduced pressure to obtain **44** (0.126 g, 31%) as a colorless foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.57 (1H, d, *J* = 8.6 Hz), 6.98 (1H, s), 6.31 (2H, dd, *J* = 14.8, 10.2 Hz), 5.93-5.91 (3H, m), 5.86 (1H, d, *J* = 5.6 Hz), 5.80 (1H, dd, *J* = 8.3, 4.5 Hz), 5.05 (1H, d, *J* = 4.8 Hz), 4.08 (3H, s), 3.56 (1H, d, *J* = 18.4 Hz), 3.25 (1H, d, *J* = 18.4 Hz), 2.15 (3H, s), 1.23-1.21 (18H, m), 1.12 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 178.4, 177.4, 177.2, 173.2, 163.0, 161.5, 160.8, 160.4, 143.3, 133.2, 129.2, 128.5, 122.1, 113.5, 81.7, 79.9, 68.2, 64.3, 59.3, 56.9, 39.0, 38.9, 30.5, 27.2, 27.1, 27.0, 20.3. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{33}H_{45}N_5O_{13}S_2$ 784.2528; found 784.2531.

Compound **45**

To a solution of **41** (250 mg, 0.373 mmol) in *N*,*N*-dimethylformamide (2.50 mL) were added chloromethyl pivalate (0.334 ml, 2.24 mmol) and sodium hydride (60% dispersion in mineral oil, 15.0 mg, 0.373 mmol) at 0 °C, and the mixture was stirred at 0 °C for 20 min and then at room temperature for 1 h. To this resulting mixture was added sodium iodide (0.224 g, 1.49 mmol), followed by stirring at room temperature overnight. To this resulting mixture was added 0.2 mol/L aqueous hydrochloric acid followed by extraction with

ethyl acetate. The organic layer was washed with water and brine, and then dried over $MgSO_4$, filtered, and evaporated under reduced pressure. This resulting residue was subjected to silica gel column chromatography, being eluted with hexane/ethyl acetate. The fractions containing the desired compound were concentrated under reduced pressure to obtain **45** (100 mg, 34%) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.50 (1H, s), 6.97 (1H, d, *J* = 8.3 Hz), 6.19-6.11 (2H, m), 5.99-5.80 (5H, m), 5.07 (1H, d, *J* = 4.8 Hz), 4.06 (3H, s), 3.55 (1H, d, *J* = 18.4 Hz), 3.27 (1H, d, *J* = 18.7 Hz), 2.15 (3H, s), 1.23-1.22 (18H, m), 1.15 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 177.5, 177.1, 176.9, 163.7, 161.6, 160.9, 159.6, 152.4, 146.5, 142.0, 133.2, 122.3, 117.2, 113.5, 81.7, 80.1, 70.4, 64.3, 63.6, 59.3, 57.1, 39.0, 30.6, 27.1, 27.0, 27.0, 20.2. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{33}H_{45}N_5O_{13}S_2$ 784.2528; found 784.2527.

Compound **52**

To a solution of cefetamet pivoxil (500 mg, 0.977 mmol) in dichloromethane (15.0 mL) were added *N*-(*tert*-butoxycarbonyl)-L-alanine (740 mg, 3.91 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (375 mg, 1.96 mmol) and *N*,*N*-dimethyl-4-aminopyridine (12.0 mg, 98.0 μmol) with stirring at room temperature for 4 h. To this resulting mixture was added saturated aqueous sodium bicarbonate solution followed by extraction with ethyl acetate. The organic layer was washed with 0.2 mol/L aqueous hydrochloric acid, water and brine, and then dried over $MgSO_4$, filtered, and evaporated under reduced pressure. This resulting residue was subjected to silica gel column chromatography, being eluted with hexane/ethyl acetate. The fractions containing the desired compound were concentrated under reduced pressure to obtain **52** (427 mg, 64%) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.58 (1H, br s), 7.79 (1H, br s), 7.29 (1H, s), 5.94-5.87 (3H, m), 5.48 (1H, br s), 5.09 (1H, d, *J* = 4.5 Hz), 4.54-4.48 (1H, m), 4.05 (3H, s), 3.57 (1H, d, *J* = 18.7 Hz), 3.27 (1H, d, *J* = 18.7 Hz), 2.16 (3H, s), 1.47-1.44 (12H, m), 1.24 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 177.3, 172.2, 164.5, 163.0, 160.7, 159.0, 155.7, 147.9, 140.7, 133.9, 122.0, 115.8, 80.9, 80.3, 63.1, 58.9, 57.7, 51.0, 38.9, 30.8, 28.5, 27.0, 20.3, 18.5. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{28}H_{38}N_6O_{10}S_2$ 683.2164; found 683.2159.

Compound **46**

To a solution of **52** (500 mg, 0.732 mmol) in formic acid (2.81 mL, 73.2

mmol) was added 4 mol/L hydrochloric acid/ethyl acetate (0.732 mL, 2.93 mmol) at 0 °C followed by stirring at 0 °C for 15 min. To this resulting mixture was added diisopropyl ether, and the resulting residue was filtered and washed with diisopropyl ether and then concentrated in vacuo to obtain **46** (400 mg, 79%) as a white solid after lyophilization using dioxane. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.05 (1H, s), 9.68 (1H, d, J = 8.3 Hz), 8.41 (3H, s), 7.49 (1H, s), 5.89 (1H, d, J = 5.8 Hz), 5.81-5.77 (2H, m), 5.16 (1H, d, J = 4.8 Hz), 4.13-4.04 (1H, m), 3.89 (3H, s), 3.64 (1H, d, J = 18.4 Hz), 3.44 (1H, d, J = 10.0 Hz), 2.02 (3H, s), 1.47 (3H, d, J = 7.1 Hz), 1.15 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ: 176.0, 168.8, 163.5, 162.5, 160.7, 157.6, 148.4, 141.7, 133.3, 121.0, 114.8, 79.4, 62.1, 58.5, 57.2, 48.4, 38.2, 29.3, 26.5, 19.4, 16.6. HRMS (ESI) *m/z*: [M-Cl]$^{+}$ calcd for $C_{23}H_{30}N_6O_8S_2$ 583.1639; found 583.1636. Anal. Calcd for $C_{23}H_{30}N_6O_8S_2(HCl)_{1.6}(H_2O)_{2.3}(C_4H_8O_2)_{0.1}$: C, 41.03; H, 5.48; N, 12.17; S, 9.28; Cl, 7.95. Found: C, 41.04; H, 5.22; N, 12.20; S, 9.18; Cl, 7.93.

Compound Evaluation

Pharmacokinetics in Rats after Intravenous Administration of Compounds.

Pharmacokinetics in intravenous administration was investigated in rats at a dose of 1 or 5 mg/kg formulated in saline or saline/7% sodium bicarbonate (9/1) solution. Blood samples were taken using a syringe pre-treated by 0.89 M aqueous EDTA·2K solution and 20% heparin at 0.033, 0.083, 0.25, 0.5, 1, 2, 4 and 6 h or 0.033, 0.083, 0.25, 0.5, 1, 2, 4, 6 and 8 h after administration. Blood samples were centrifuged at 3000 rpm for 10 min at 4 ˚C to obtain plasma samples. Plasma samples were transferred to cluster tubes and stored at -20 ˚C prior to analysis.

Pharmacokinetics in Rats after Oral Administration of Compounds.

Pharmacokinetics in oral administration were investigated in rats at doses of 15, 20 and 30 mg/kg formulated in 0.5% Methyl cellulose (400cP). Blood samples were taken using a syringe pre-treated by 0.89 M aqueous EDTA·2K solution and 20% heparin at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after administration. Blood samples were centrifuged at 3000 rpm for 10 min at 4 ˚C. Plasma samples were transferred to cluster tubes and stored at -20 ˚C prior to analysis.

LC/MS/MS Determination of Test Compounds.

An aliquot (40 μL) of each sample was transferred to a 96-well plate, mixed with 500 μL of acetonitrile, and centrifuged at 3000 rpm for 10 min at 4 ˚C. A portion of supernatant was injected to Shimadzu Nexera HPLC system interfaced with an API-5000 mass spectrometer (AB Sciex, Framingham, MA). Plasma concentrations of test compounds were determined by LC/MS/MS with electrospray ionization in the positive ion mode using an external standard method.

Pharmacokinetic Analysis.

The area under the concentration-time curve from 0 h to the last time point ($AUC_{0\text{-}tlast}$) was calculated by linear trapezoidal approximation. The oral bioavailability was calculated by AUC of each carboxylic acid derived from each test compound administrated orally / AUC of each carboxylic acid administrated intravenously.

Antibiotic Susceptibility Testing.

The minimum inhibitory concentrations (MICs) were determined using the broth microdilution method in accordance with Clinical Laboratory Standards Institute (CLSI) guidelines.[1] Briefly, two-fold serial dilution of test compounds were prepared and transferred to a 96 well plate including cation-adjusted Mueller Hinton broth (CAMHB). Haemophilus Test Medium broth (HTM broth) and CAMHB containing laked horse blood were used for *Haemophilus influenzae* and *Streptococcus pneumoniae*, respectively. The bacterial suspension for inoculum was prepared based on optical density of 625 nm to be approximately 5×10^4 CFU/well as final inoculum size. The 96 well plates were incubated at 35°C for 16 to 20 hours. The MIC endpoint was defined as the lowest concentration of the compound that inhibited bacterial growth as detected by the unaided eye.

Kinetic Solubility Assay.

Compounds in dimethylsulfoxide (10 mmol/L) were diluted into the aqueous phosphate buffer solution (pH 6.8) with the final dimethylsulfoxide concentration being 1%. After 1 h of incubation, precipitates produced was filtered through a membrane filter and the filtrate was quantified with LC/MS.

Parallel Artificial Membrane Permeability Assay (PAMPA).

PAMPA was applied to measure compounds for passive diffusion. This study employed the Gentest Precoated PAMPA Plate System (Corning Life Sciences). The system is composed of a 96-well plate/insert system. Two fluidfilled compartments (donor well and receiver well) are separated by a polyvinylidene fluoride (PVDF) filter plate precoated with a phospholipid-oil-phospholipid trilayer primarily consisting of dioleoyl phosphatidylcholine (DOPC) phospholipids. Compounds of interest were dissolved in 2% dimethylsulfoxide in aqueous phosphate buffer solution (pH 6.8). Solutions were added to donor wells at a volume of 0.3 mL. The filter plate, containing acceptor wells filled with 0.2 mL 2% dimethylsulfoxide in PBS solution (pH 7.4), was placed on top of the donor plate. The entire system was incubated for 4 h at 25 °C. Following incubation, solution was removed from donor and acceptor wells. The removed solution was analyzed by ultra performance

liquid chromatography (UPLC), and permeability of compound was calculated. Using these measured experimental permeability rates, as well as permeability data from literature, we are able to divide our data into three groups; high permeability, medium permeability, low permeability. The boundaries between these regions are defined by using the permeability coefficient midpoints between different model compounds of known permeability categories.[2, 3]

Compound	permeability coefficient (10^(-6)*cm/s)	Permeation category
Ketoprofen	2.19	high
Metoprolol tartrate	0.91	high
Atenolol	0.09	medium
Furosemide	0.19	medium
Sulpiride	0.03	low
Lisinopril hydrate	0.00	low

References

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第二章
第三世代セファロスポリンセフトリアキソンのマクロサイクル化

第一節 マクロサイクル化による経口吸収性改善の例

大環状化合物(マクロサイクル)は主に医薬品、香料、あるいは機能性材料等の分野でその用途が報告されており、狭義には 12 以上の原子からなる環構造を有する化合物と定義されている[1]。

近年医薬品化学において、これまでの医薬品の中心的位置付けであった低分子医薬品とタンパク・抗体医薬品が標的分子に関して各々大きな課題を有することが明らかとなっている。低分子医薬品は、クラス B G タンパク質共役受容体(G-protein-coupled receptor: GPCR)やタンパク質間相互作用(Protein-protein interaction: PPI)あるいは一部の酵素などの所謂結合部位が拡張された標的分子に対して対応するのが困難である場合があり、タンパクや抗体は高分子構造であるため細胞内や中枢神経系に移入することができず、それらに局在する標的分子を狙うことが難しい場合がある。これらの課題を解決し得る医薬品モダリティとして分子量 500~5000 程度の中分子医薬品が注目されており、中でもマクロサイクルは上記課題である標的分子の拡張および細胞内送達の二点を解決し得る可能性があることから多くの研究が報告されている(Figure 2-1)[2]。

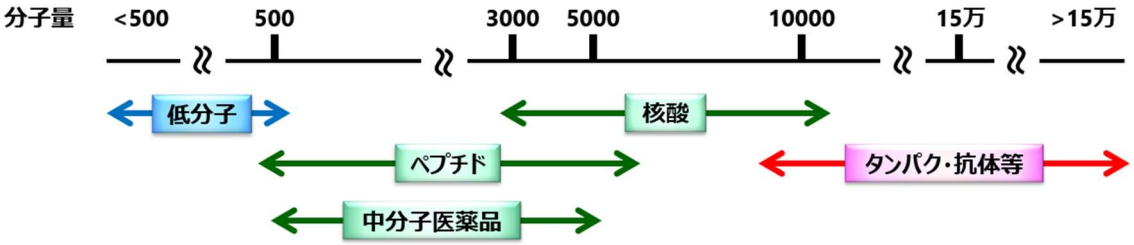


Figure 2-1. 医薬品モダリティの分子量範囲

従来、マクロサイクル医薬品あるいは臨床開発候補品は、エリスロマイシン[3]、シロリムス[4]、バンコマイシン[5]、シクロスポリン[6]、タクロリムス[7]、エリブリン[8]等の天然物や構造的に特徴のあるそれらの骨格を利用した誘導体、並びにソマトスタチン[9]、オクトレオチド[10]、インスリン[11]、カルシトニン[12]、心房性ナトリウム利尿ペプチド(atrial natriuretic peptide: ANP)[13]等の内因性ペプチド或いはそれらの誘導体など数多く知られてきた(Figure 2-2)。一方最近では、臨床開発候補品として、計算化学的手法による de novo デザインあるいは進化分子工学的手法と化学合成を組み合わせて創製した非天然物もしくは非内因性ペ

プチド由来のマクロサイクルの例が増加している。

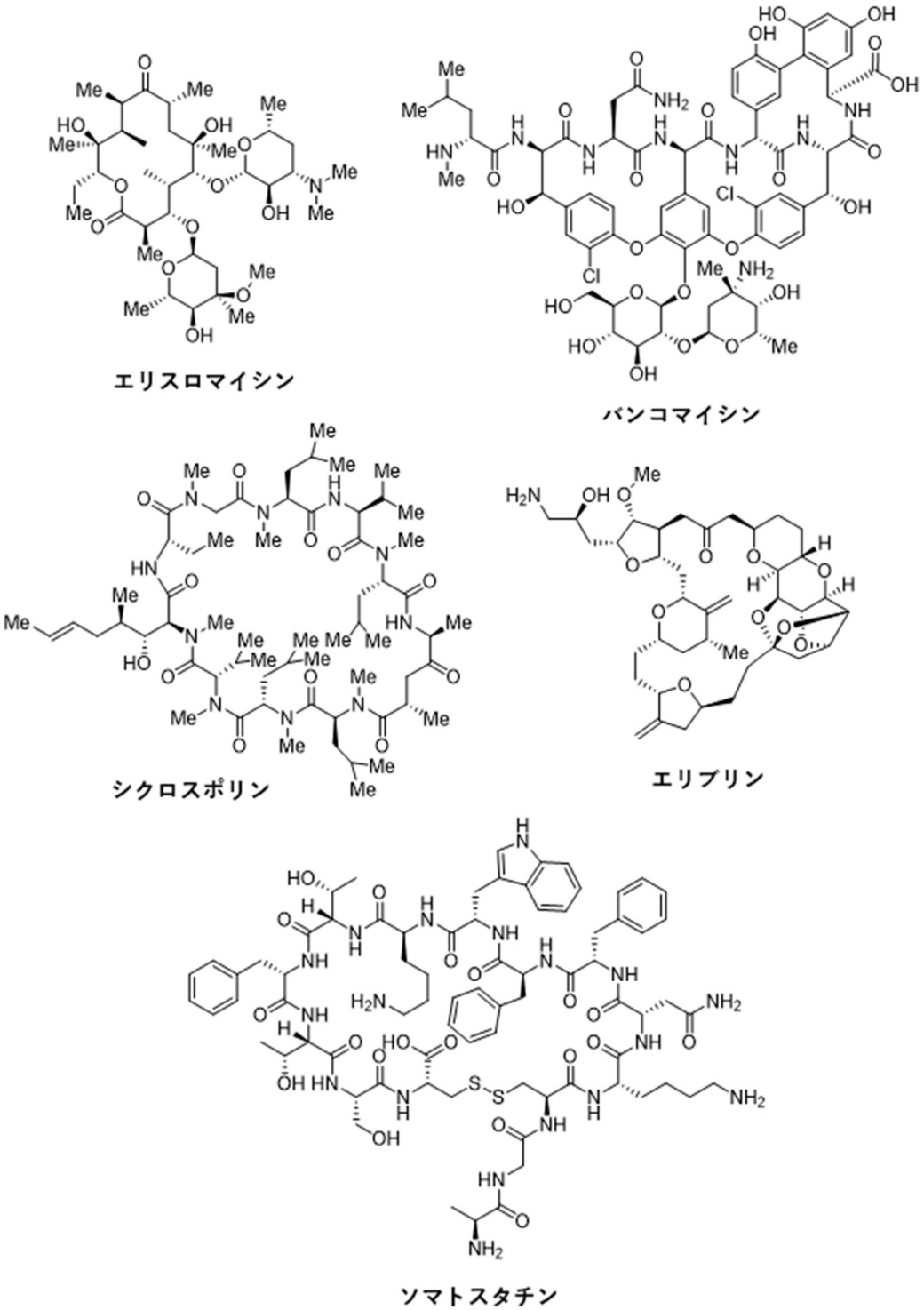


Figure 2-2. 中分子医薬品の構造例

医薬品におけるマクロサイクル化の利点は複数知られている。先にも述べた通

り、従来のいわゆる「鍵と鍵穴」で形容されるような狭く、深い結合サイトだけではなく、リガンド表面積を場合に依っては 1000 Å² まで占めるような広い結合サイト、もしくは浅く平らな、あるいは柔軟な結合サイトに対しても高親和性結合が可能となる場合がある[14]。また分子量 1203 のシクロスポリンがそうであるように、低分子化合物と比較してかなり大きい分子量であるにも関わらず、in vivo で細胞内標的に対して十分結合することが可能なレベルで細胞透過性を有する場合もある。それ以外にも、一般にマクロサイクルは直鎖状の化合物と比較して構造的自由度が低く、標的との結合においてエントロピー損失を低減することができ、結果として高い親和性を獲得できる例も報告されている。またリガンドのコンフォメーションを固定すること、もしくは変化を加えられることから、他の標的分子との間の選択性を獲得した例や代謝安定性もしくは安全性プロファイルの改善を成し得た例も報告されている。

またマクロサイクルの経口吸収性に関しても数多くの研究報告がある。1997 年に Lipinski らは、低分子化合物が溶解性と膜透過性によって経口吸収可能となる可能性が高いケミカルスペースに該当するかを事前に予測できる簡単な経験則「Lipinski's Rule of Five (Ro5)」を報告した(Figure 2-3)[15]。Ro5 によれば、それまでに市販された経口低分子化合物の約 90%は、分子量が 500 以下、オクタノール－水分配係数($LogP_{ow}$)は 0 から 5 の間の値、水素結合受容体(hydrogen bond acceptor: HBA)の数は 10 以下、水素結合供与体の数は 5 以下であった(Figure 17)。この指標が報告されて以降、極性表面積や回転可能結合数等の別の物理化学パラメータが他の研究機関によって更に追加され、これらが創薬研究において経口剤を目指す上で創薬の初期段階からユビキタスに用いられる「戒め」として知られるようになった。一方で、これらの指標を逸脱したいわゆる Beyond Ro5 化合物も多数市販もしくは開発されており、その代表例の一つがマクロサイクルである。

- **Lipinski's Rule of Five**
  - 分子量 (MW)：500以下
  - 脂溶性パラメータ ($LogP_{ow}$)：5以下
  - 水素結合受容体 (HBA)数：10個以下
  - 水素結合供与体 (HBD)数： 5 個以下

Figure 2-3. Lipinski's Rule of Five

Kihlberg らは市販医薬品もしくは開発医薬品の解析から、従来の低分子化合物と比較して多くの経口用のマクロサイクル化合物の分子量や水素結合受容体

数、水素結合供与体数等の物理化学パラメータが Ro5 から外れていることを報告した [16]。例として上述したエリスロマイシンやシクロスポリンはそれぞれ分子量が 700、1200 程度にも関わらず良好な経口吸収性を示している。また Chen や Lamarre らは HCV/NS3A プロテアーゼ阻害剤において、マクロサイクルを導入することで類似する他のプロテアーゼ阻害剤ボセプレビル(boceprevir)と比較して大きく経口吸収性を改善した **53** やシルプレビル(ciluprevir)を創出している(Figure 2-4)[17]。また Singh らはカルバペネム系抗菌薬の一つであるエルタペネムを用いたマクロサイクル化により、エルタペネムと比較してイヌでの十二指腸内投与時のバイオアベイラビリティを改善した **54** を創出することに成功している [18]。

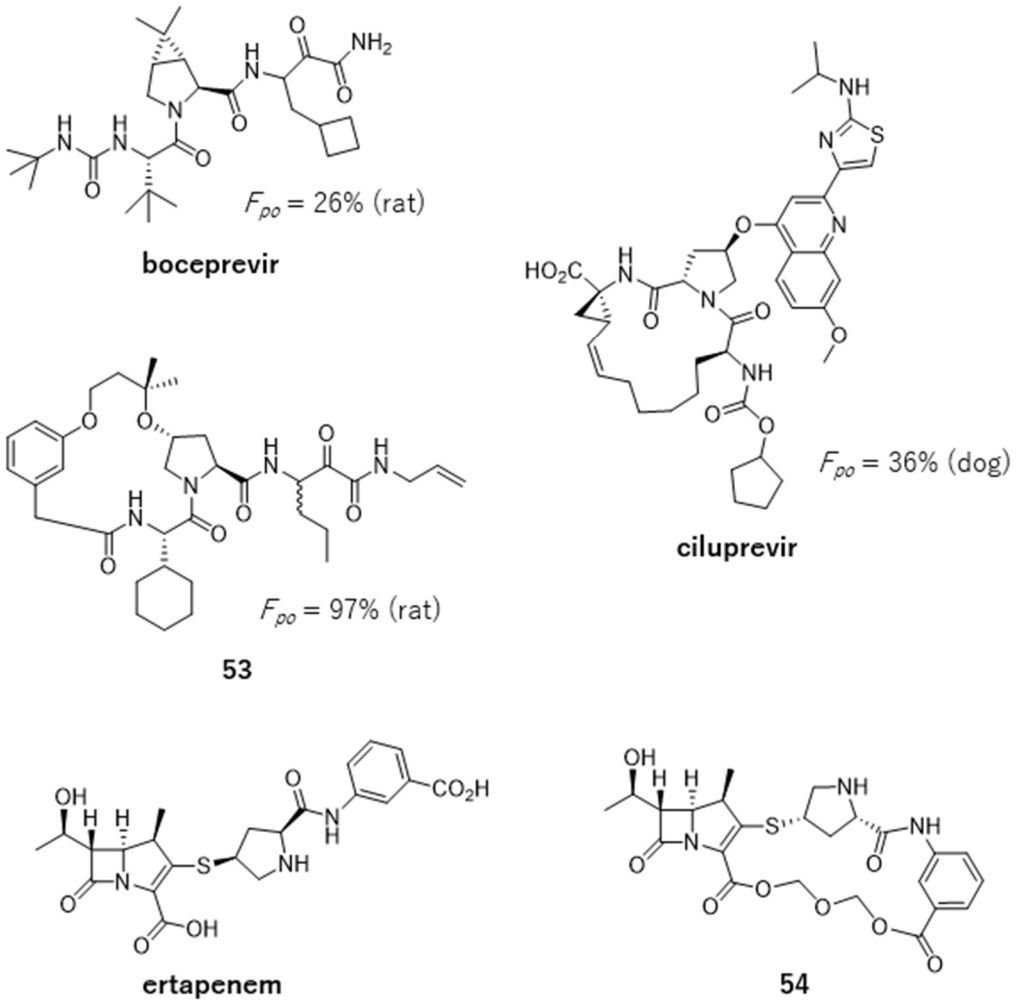


Figure 2-4. マクロサイクル化合物の例

これらのことからマクロサイクルを形成させることにより、従来の Ro5 が示す物理化学的なパラメータを逸脱したケミカルスペースにおいても経口吸収性

を改善できる可能性があると考えた。そのため経口ステップダウン療法が可能な高い経口吸収性と強力な抗菌活性を両立した新規セファロスポリンの創出、またグラム陽性菌或いはグラム陰性菌に対して強力な抗菌活性を有する第三世代以降のセファロスポリンにおける経口吸収性改善のための一般的な新規修飾法の開発を目指すにあたって、マクロサイクル化の適用可否を検討することとした。

またマクロサイクル化による経口吸収性の改善を検討するにあたり、C-3 位に 2,5-ジヒドロキシ-6-ヒドロキシ-2-メチル-5-オキソ-アズ-トリアジン-3-イル基を有する第三世代セファロスポリン系抗菌薬の一つである CTRX を選択した。CTRX はグラム陽性菌やグラム陰性菌に対する幅広い抗菌スペクトルと良好な抗菌活性を示し臨床上有用であるが、一日に一回または二回の静脈内注射による点滴が必要であり院内での使用等に限定される。そのため CTRX のプロドラッグ化による経口バイオアベイラビリティの改善検討が報告されているが、その効果は限定的であることから[19]、本研究に適した基質であると考えた。

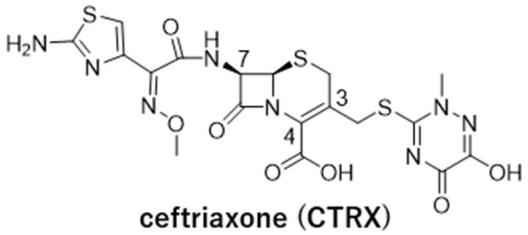


Figure 2-5. ceftriaxone (CTRX)の構造

第二節 マクロサイクル化合物の環員数の検討

第一項 マクロサイクル化合物の環員数の検討

マクロサイクル化を実施するにあたり、まずその環員数を検討した。大環状化合物の膜透過性と経口吸収に関するこれまでの報告[16, 20]から、合成される大環状化合物の分子量は 1000 以下であることがより望ましいと考えられた。一方で環員数が小さすぎる場合、環化反応が進行しない或いは得られた生成物が十分な化学的安定性を持たない可能性が考えられる。これらの点を考慮し、まず 15 員環から 20 員環を有する以下の 4 つの化合物(**55~58**)の合成を検討することとした(Figure 2-6)。

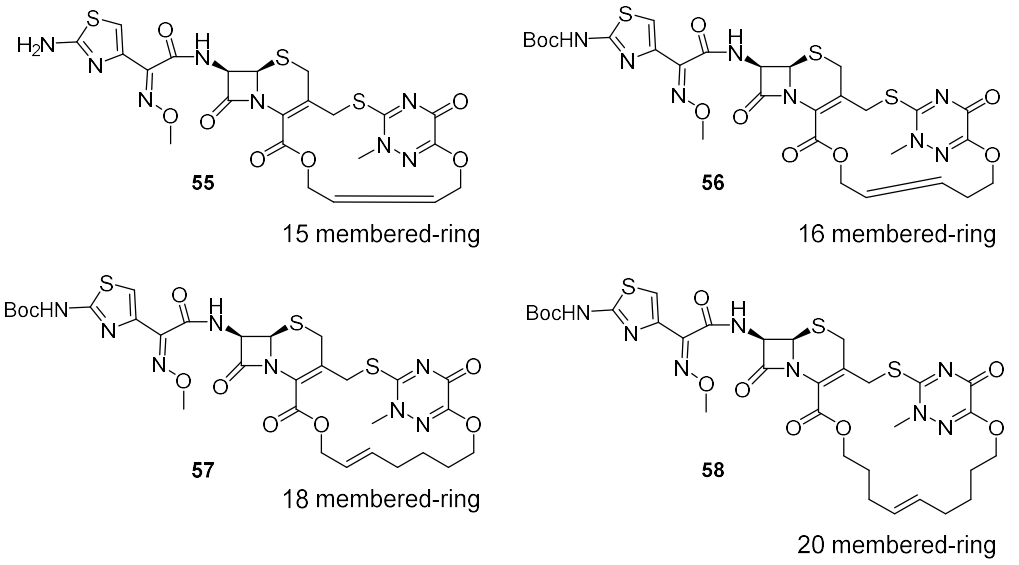
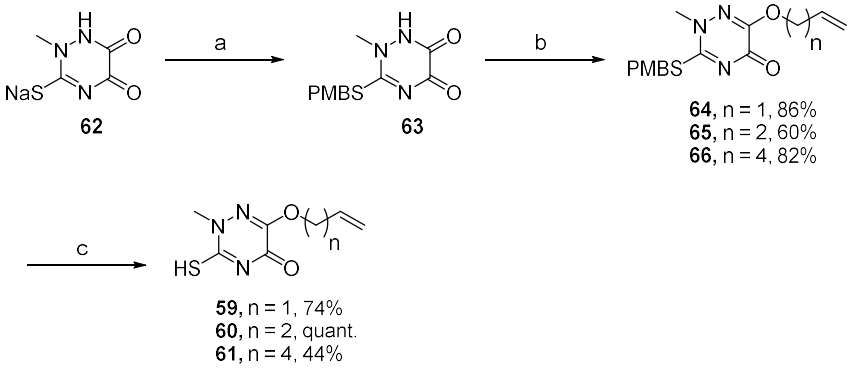


Figure 2-6. 合成検討化合物

化合物 **55~58** の 3 位側鎖 **59~61** の合成を以下の Scheme 2-1 に示す。市販の化合物 **62** に塩化 *p*-メトキシベンジルと臭化ナトリウムを作用させ化合物 **63** とした後、各種臭化アルキルを用い対応する *O*-アルキル **64~66** を得た。過塩素酸銀により *p*-メトキシベンジル基を除去し、化合物 **59~61** を合成した。

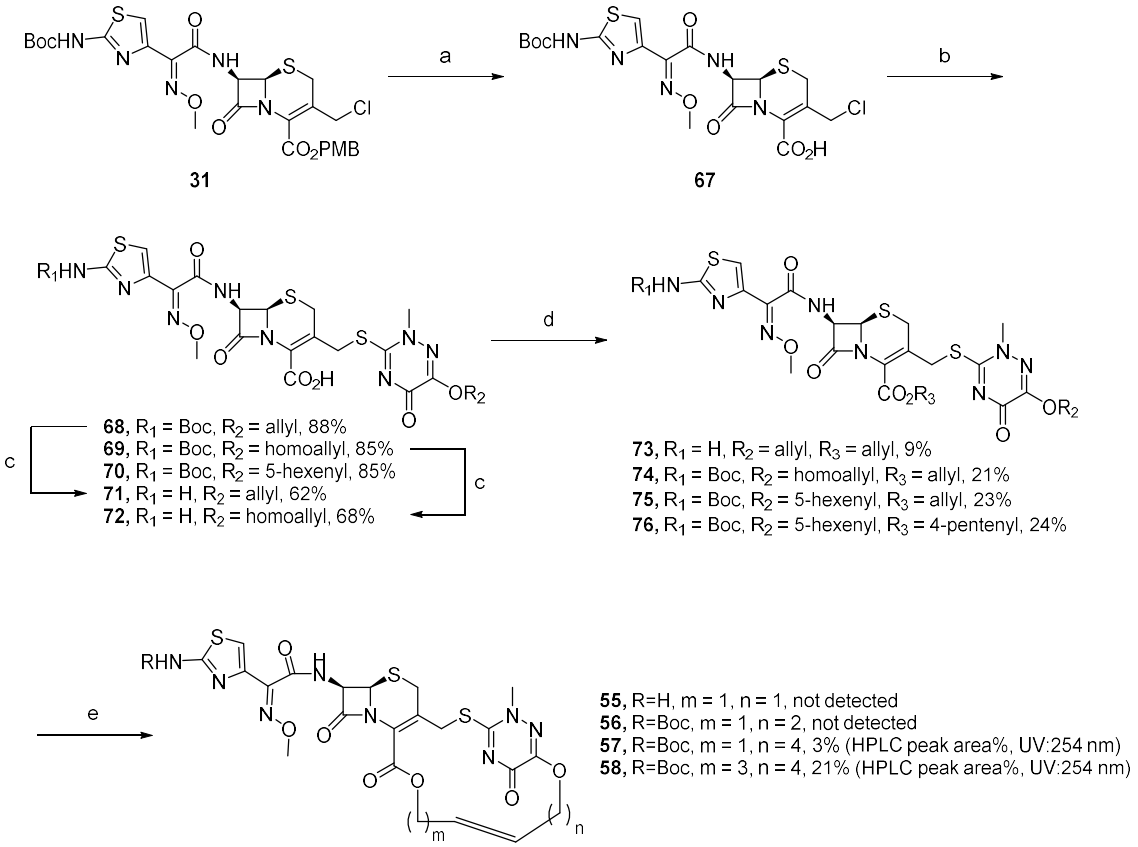
Scheme 2-1. 3 位側鎖 **59~61** の合成



Reagents and conditions: (a) PMBCl, NaBr, DMF, 99%; (b) 4-bromobut-1-ene, K_2CO_3, NaI, DMF, 65 °C; (c) $AgClO_4$, EtOAc, rt.

トリフルオロ酢酸を用いクロロメチル **31** の *p*-メトキシベンジル基を除去しカルボン酸 **67** とし、先の各種 3 位側鎖 **59~61** を作用させることで **68~70** を得た (Scheme 2-2)。得られた **68~70** に対してそれぞれ対応するアルコールを用い縮合し、続くメタクロロ過安息香酸による酸化と三臭化リンによる還元によって **74~76** へと導いた。化合物 **73** は **68** を用いアリルアルコールと縮合し、メタクロロ過安息香酸による酸化と三臭化リンによる還元後、アニソール存在下、塩化アルミニウムを用い *tert*-ブトキシカルボニル基を除去することで得た。得られた **73~76** を閉環メタセシス反応条件に晒したところ、20 員環マクロサイクル **58** は低収率ながら HPLC において生成が認められた一方で、**55** や **56** はその生成を確認することができなかった。また 18 員環マクロサイクル **57** もほとんど生成を確認できなかった。*in vitro* 抗菌活性を確認するため、化合物 **68** と **69** の *tert*-ブトキシカルボニル基を除去した化合物 **71**、**72** を別途調製した。

Scheme 2-2.　化合物 **71**～**76** の合成と環化反応の検討



Reagents and conditions: (a) TFA, CH_2Cl_2, 0 °C, 89%; (b) **59**~**61**, K_2CO_3, BSA, acetonitrile, 0 °C; (c) 2 M $AlCl_3$/nitromethane, anisole, CH_2Cl_2, -30 °C; (d) (i) corresponding alcohol, EDC·HCl, HOBt, CH_2Cl_2, rt; (ii) *m*CPBA, CH_2Cl_2, -40 °C; (iii) PBr_3, DMF, -40 °C; (iv) 2 M $AlCl_3$/nitromethane, anisole, CH_2Cl_2, -30 °C; (e) Grubbs II, CH_2Cl_2, reflux.

第二項 合成化合物の *in vitro* 抗菌活性

今回環化を検討した中間体である化合物 **71**、**72** の *in vitro* 抗菌活性を Table 2-1 に記す。**71** や **72** は 3 位側鎖上にアルキル基を持たない CTRX と比較して各種グラム陽性菌・グラム陰性菌に対して同程度の抗菌活性を示した。一方で、セフカペンピボキシルはセフカペンと比較して活性が大きく減弱する。このことは従来より報告があるように 4 位エステルでは抗菌活性があまり認められないことと一致する [21]。そのため、良好な抗菌活性を示すためには生体内で 4 位カルボン酸へ速やかに変換されるような 4 位アシルオキシメチルエステル構造を導入する必要があると考えられる。

Table 2-1. 各種化合物の *in vitro* 抗菌活性

		MIC[a] (μg/mL)				
species	strain ID	**71**	**72**	CTRX	CFPN-PI	CFPN
E. coli	NIHJ JC-2[b]	0.5	2	0.25	>32	0.5
S. pneumoniae	SR16675[c]	1	0.5	2	>32	0.5
H. influenzae	SR11435[d]	0.25	0.25	0.25	>32	2
B. subtilis	ATCC6633[e]	1	1	1	>32	0.25
E. cloacae	ATCC13047[e]	16	8	32	>32	8
S. marcescens	ATCC13880[e]	1	2	1	>32	1

[a]Minimum Inhibitory Concentration values. [b]Reference 22. [c]A penicillin-resistant *Streptococcus pneumoniae* strain. [d]A beta-lactamase non-producing ampicillin-resistant *Haemophilus influenzae* strain. [e]These strains were Purchased from American Type Culture Collection (ATCC).

以上より合成の実現可能性を考慮した上で、経口吸収性の向上と良好な抗菌活性を両立するため、生体内で 4 位カルボン酸に変換し得ると考えられるアシルオキシメチレン或いはアルコキシカルボニルオキシメチレン構造を導入した 20 員環以上のマクロサイクルを合成することとした。またこれまでにセファロスポリンを用いたマクロサイクル化の報告がないことから、閉環メタセシス、ジアルキル化、マクロラクトン化、ジスルフィド化など種々の環化反応を用い、以下の 4 つの化合物 **77~80** の合成を検討することとした(Figure 2-7)。

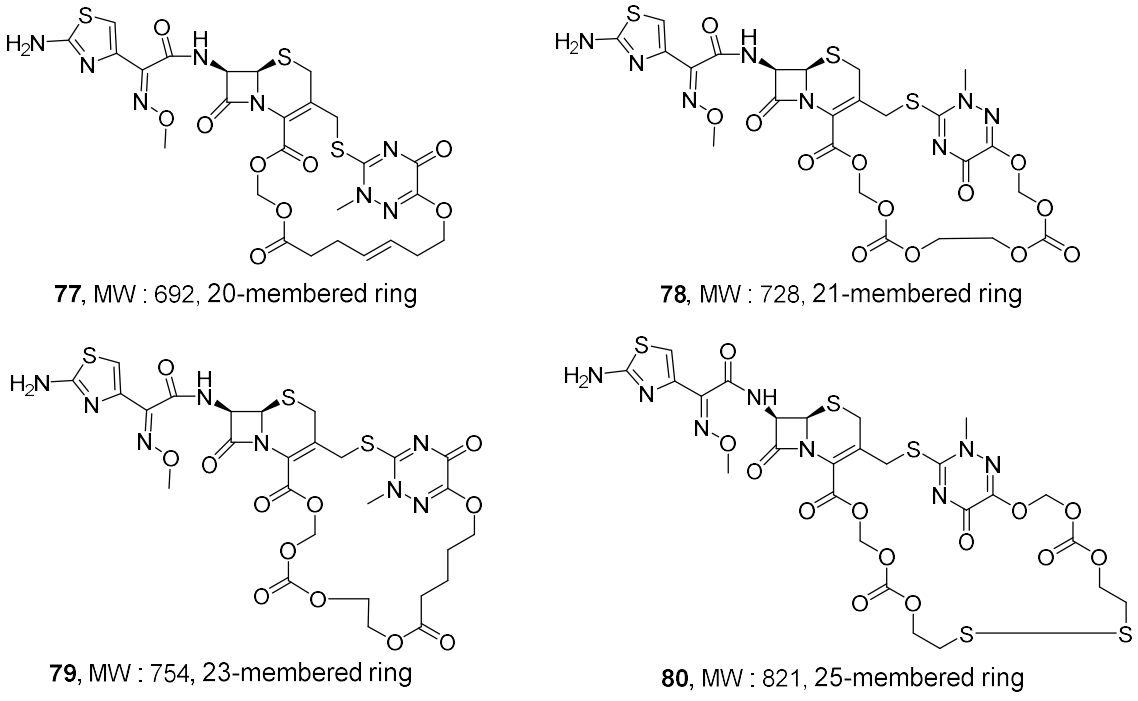


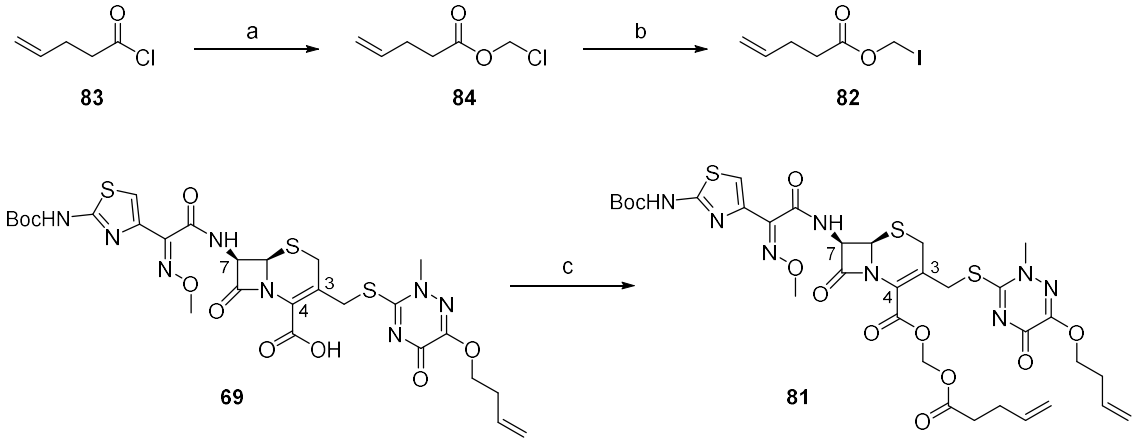
Figure 2-7. 考案したマクロサイクル **77~80** の構造

第三節 各種マクロサイクルの合成

第一項 閉環メタセシス反応によるマクロサイクルの合成

閉環メタセシス反応を用いたマクロサイクル **77** の合成にあたり、はじめにその前駆体であるジエン **81** を合成した(Scheme 2-3)。ヨウ化アシルオキシメチル **82** は、酸クロリド **83** に対するパラホルムアルデヒドおよび塩化亜鉛によるエステル **84** への変換とヨウ化ナトリウムによるヨウ素化により調製した。化合物 **69** と調製した **82** の求核置換反応によりジエン **81** を合成した。

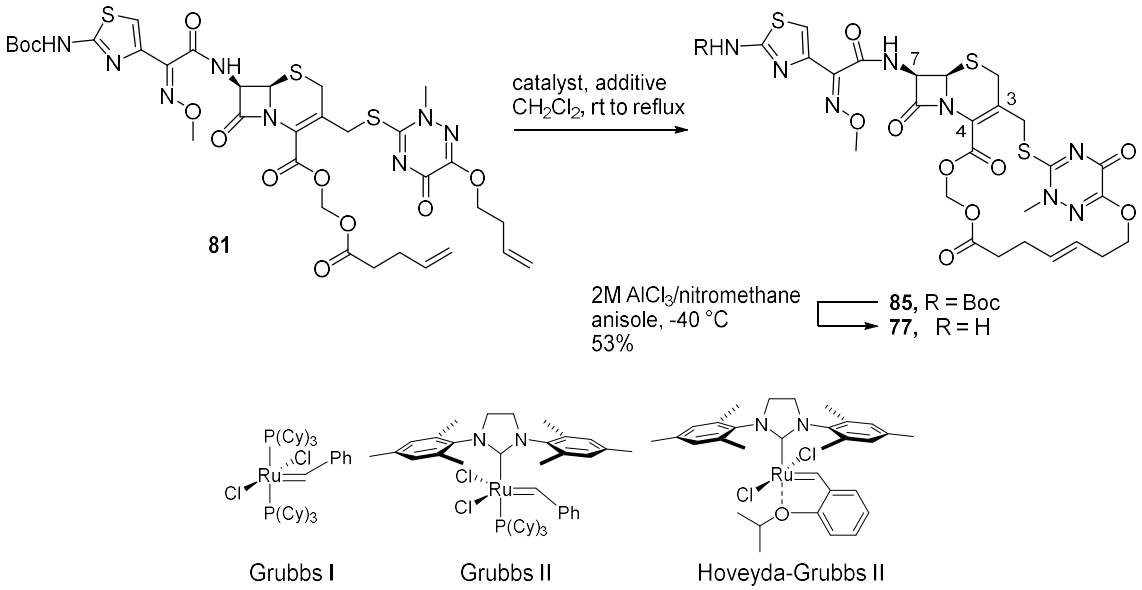
Scheme 2-3.　環化前駆体 **81** の合成



Reagents and conditions: (a) paraformaldehyde, $ZnCl_2$, 0 °C to rt, 41%; (b) NaI, acetone, rt, 91%; (c) **82**, $NaHCO_3$, K_2CO_3, DMF, -40 °C, 38%.

上述のジエン **81** を用いて、閉環メタセシス反応を検討した(Table 2-2)。第一世代グラブス触媒を用いた場合、痕跡量の **85** を得た(entry 1)。第二世代グラブス触媒または第 2 世代ホベイダグラブス触媒で処理した場合、低い収率ながら **85** を得た(entry 2, 3)。反応が遅く、二量体と推定される化合物等複数の望まない生成物が得られたため、低収率に留まったと考えている。LCMS 分析からオルトチタン酸テトライソプロピルを添加することで反応効率が向上し(entry 4)、最終的にオルトチタン酸テトライソプロピル存在下で第 2 世代グラブス触媒を使用することで **85** を 25%の収率で得ることができた(entry 5)。**85** の *tert*-ブトキシカルボニル基をアニソールの存在下塩化アルミニウムで除去し、マクロサイクル **77** を得た。

Table 2-2. 閉環メタセシス反応を用いた化合物 **77** の合成



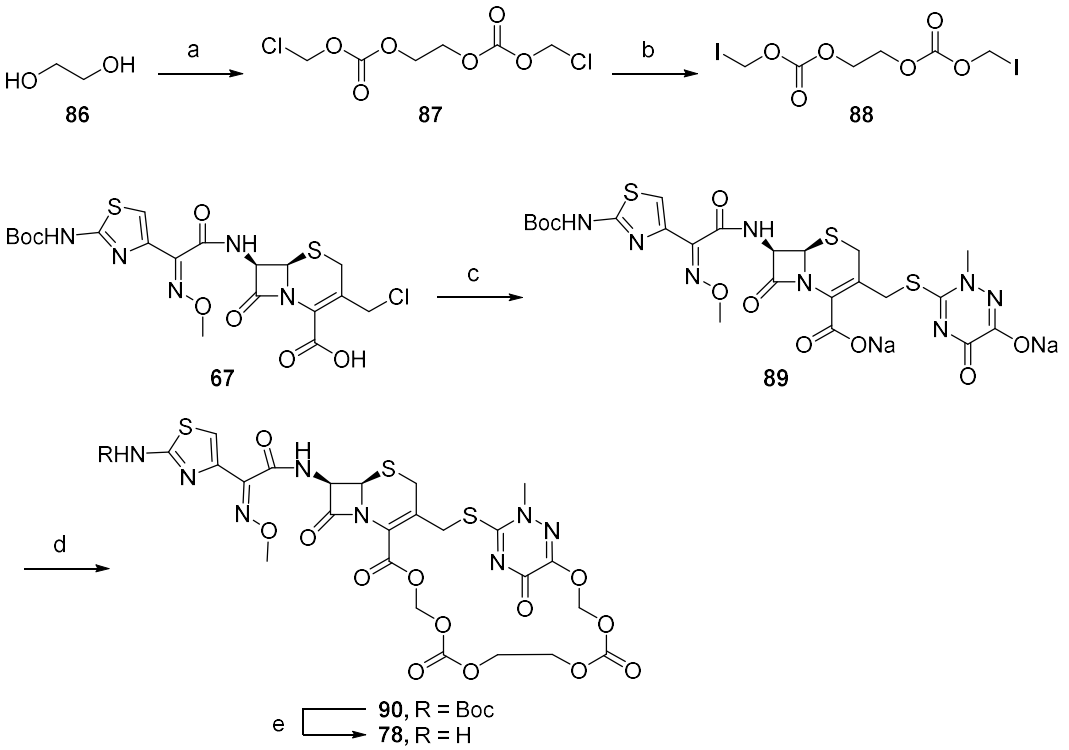
entry	catalyst	additive	yield (%)[a]
1	Grubbs I[b]	None	trace.
2	Grubbs II[c]	None	15%
3	Hoveyda-Grubbs II[d]	None	9%
4	Hoveyda-Grubbs II[d]	Ti(O*i*Pr)$_4$	4%
5	Grubbs II[c]	Ti(O*i*Pr)$_4$	25%

[a]weight yield, [b]The first-generation Grubbs catalyst, [c]The second-generation Grubbs catalyst, [d]The second generation Hoveyda–Grubbs catalyst.

第二項 ジアルキル化反応によるマクロサイクルの合成

ジアルキル化反応によるマクロサイクル **78** の合成を Scheme 2-4 に記載した。エチレングリコール **86** をクロロギ酸クロロメチルで処理し **87** へと変換した後、ヨウ化ナトリウムを用いてヨードメチル **88** とした。化合物 **67** に対し BSA 存在下、6-ヒドロキシ-2-メチル-3-チオオキソ-3,4-ジヒドロ-1,2,4-トリアジノンを作用させることでカルボン酸 **89** へと導いた。得られた **89** に上述の **88** を作用させ化合物 **90** を得たのち、*tert*-ブトキシカルボニル基を除去することでマクロサイクル **78** を合成した。

Scheme 2-4.　化合物 **78** の合成

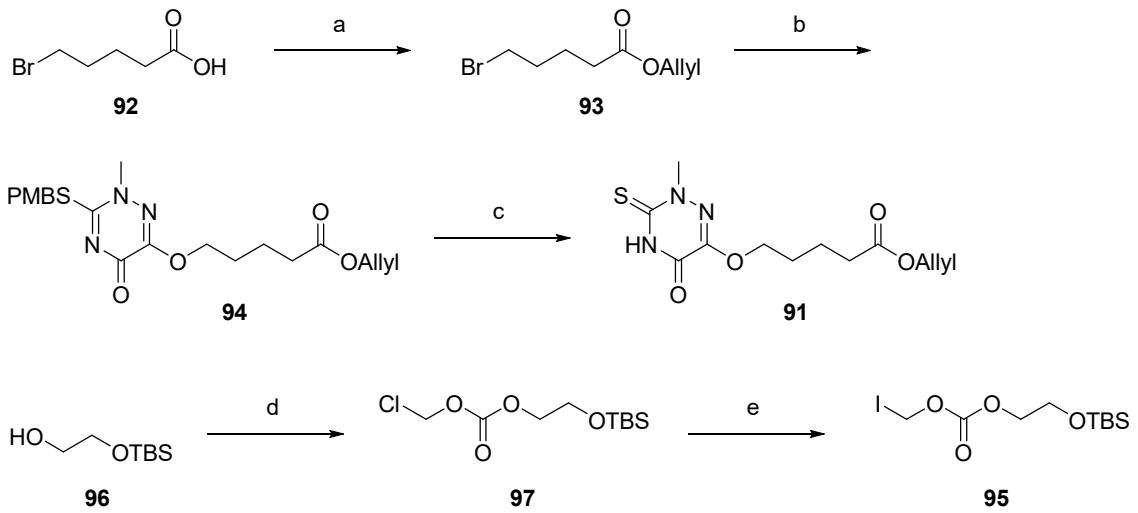


Reagents and conditions: (a) chloromethyl chloroformate, pyridine, CH_2Cl_2, 0 °C to rt, 80%; (b) NaI, acetone, rt, 91%; (c) 6-hydroxy-2-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one, K_2CO_3, BSA, acetonitrile, 0 °C, 61%; (d) **88**, DMF, -40 °C to 0 °C, 18%; (e) 2 M $AlCl_3$/nitromethane, anisole, CH_2Cl_2, -40 °C, 75%.

第三項 マクロラクトン化反応によるマクロサイクルの合成

はじめに 3 位側鎖 **91** の合成を Scheme 2-5 に記す。5-ブロモペンタン酸 **92** をアリルエステル **93** とし、**63** との求核置換反応により **94** を得た。得られた **94** の *p*-メトキシベンジル基を過塩素酸銀により除去することで 3 位側鎖 **91** へと導いた。また 4 位側鎖 **95** は市販の 2-*tert*-ブチルジメチルシリルオキシエタノール **96** にクロロギ酸クロロメチルを作用させ、クロロメチル **97** とした後、ヨウ素化することで合成した。

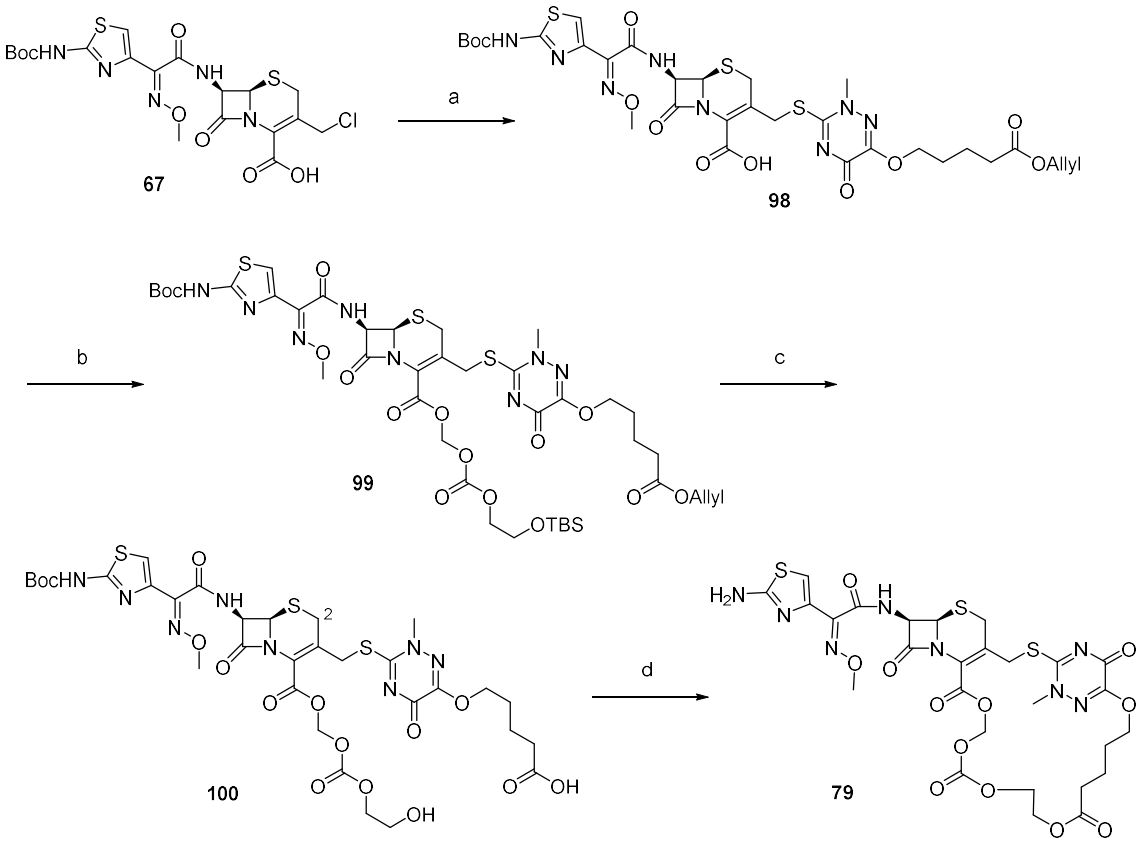
Scheme 2-5. 3 位側鎖 **91** と 4 位側鎖 **95** の合成



Reagents and conditions: (a) oxalyl chloride, cat. DMF, 0 °C to rt; allyl alcohol, rt, 87%; (b) **63**, K_2CO_3, NaI, DMF, 65 °C, 69%; (c) $AgClO_4$, EtOAc, rt, 82%; (d) chloromethyl chloroformate, pyridine, CH_2Cl_2, 0 °C to rt, quant.; (e) NaI, acetone, rt, quant.;

クロロメチル **67** に対して上述した 3 位側鎖 **91** を導入することでカルボン酸 **98** を得た。得られた **98** に対して 4 位側鎖 **95** を導入しエステル **99** とした (Scheme 2-6)。*tert*-ブチルジメチルシリル基とアリル基を 1 段階で除去し、セコ酸 **100** とした。得られた **100** に対し高希釈条件下 PyBOP を用いたラクトン化によりマクロラクトンを得たが、セフェム核内のオレフィンの異性化(Δ2 異性体の生成)も伴ったため、メタクロロ過安息香酸による酸化、三臭化リンにより還元した後、塩化アルミニウムによる *tert*-ブトキシカルボニル基を除去を経てマクロラクトン **79** を合成した。

Scheme 2-6. 化合物 **79** の合成

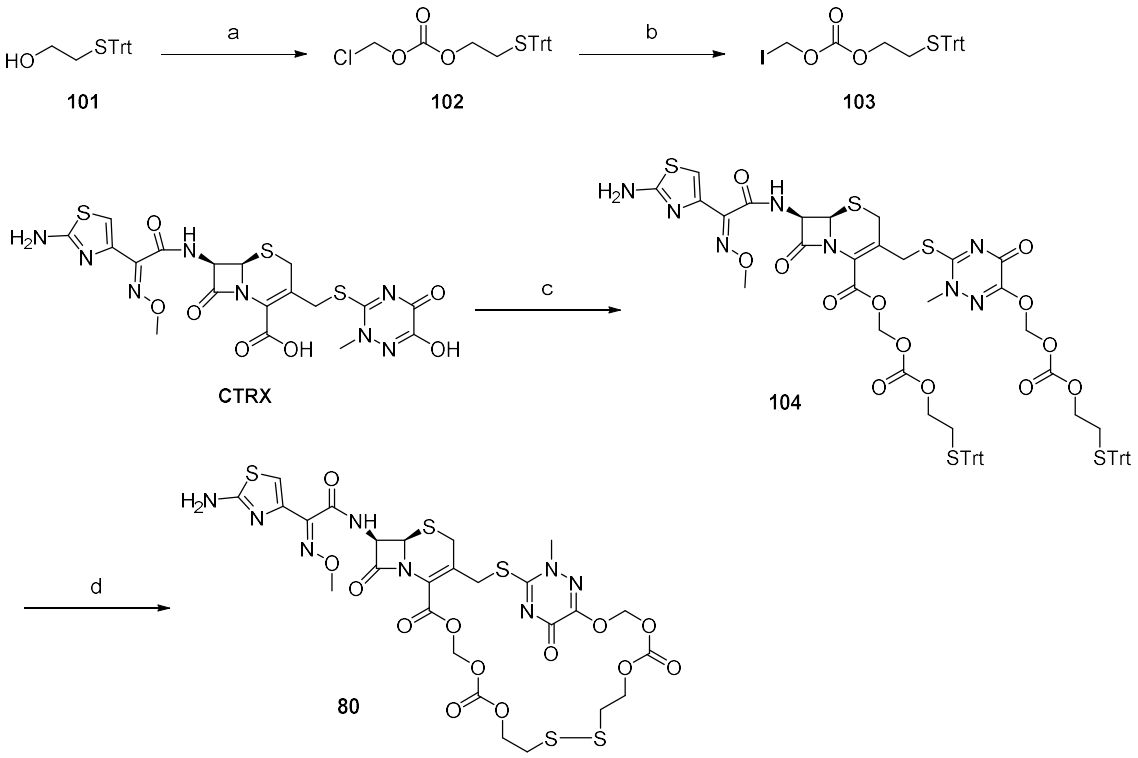


Reagents and conditions: (a) **91**, K_2CO_3, BSA, acetonitrile, 100%; (b) **95**, $NaHCO_3$, K_2CO_3, DMF, -40 °C, 49%; (c) HCO_2H, THF, H_2O(3/6/1), rt; $Pd(PPh_3)_4$, rt, 86%; (d) (i) PyBOP, DMAP, CH_2Cl_2, rt, 63% (a mixture of Δ2 isomer); (ii) *m*CPBA, CH_2Cl_2, -40 °C; (iii) PBr_3, DMF, -40 °C; (iv) 2 M $AlCl_3$/nitromethane, anisole, CH_2Cl_2, -30 °C, 74% in 3 steps.

第四項 ジスルフィド化反応によるマクロサイクルの合成

既知の方法 [23] で合成したアルコール **101** に対してクロロギ酸クロロメチルを用いクロロメチル **102** とした後、ヨウ素化することでヨウ化メチル **103** とした(Scheme 2-7)。CTRX に先のヨウ化メチル **103** を反応させエステル **104** とした後、ヨウ素存在下ジスルフィド化させることでマクロサイクル **80** を得た。

Scheme 2-7. 化合物 **80** の合成

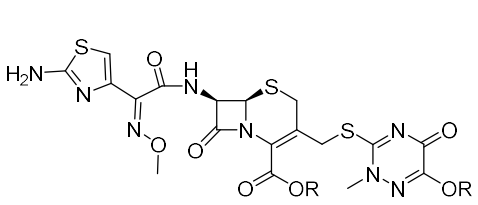


Reagents and conditions: (a) chloromethyl chloroformate, pyridine, CH_2Cl_2, 89%; (b) NaI, acetone, quant.; (c) **103**, K_2CO_3, DMF, -15 °C to 0 °C, 61%; (d) iodine, MeOH, H_2O, rt, 15%.

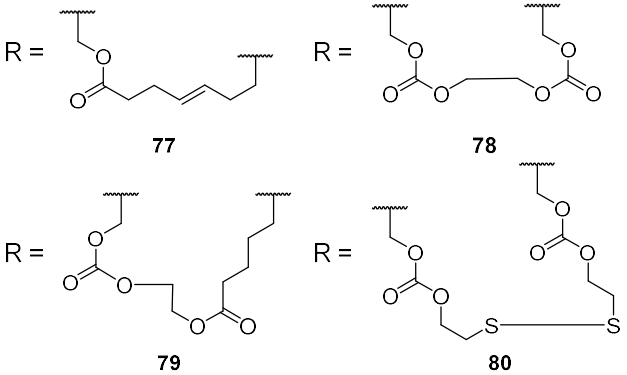
第四節 マクロサイクルの評価結果

化合物 **77~80** の PAMPA 評価結果、リン酸緩衝液(pH 6.8)中の溶解度、並びにラットの経口バイオアベイラビリティ(F_{po})を Table 2-3 に示す。今回合成した化合物は **80** を除いて低い膜透過性を示した。また **77**、**78** は経口バイオアベイラビリティも低かった。また化合物 **80** は中程度の膜透過性を示したが、経口吸収性は対照化合物である **3** や CTRX と比較して同程度であった。膜透過性の改善が不十分であったことに加え、**80** が日本薬局方崩壊試験第 2 液(JP2, pH 6.8)において 3 時間後に半分程度の残存率となっており、消化管における安定性が低かったことも原因として考えられる(**80**:JP1:91.2%, JP2:50.1%)。また化合物 **77** は血中において CTRX へと変換されず滞留している可能性も考えられたが、静脈内投与による薬物動態試験や *in vivo* 代謝物検索の結果から **77** は生体内で静脈内投与 2 分後にはほぼ消失されることが確認された。

Table 2-3. 各種マクロサイクル化合物の評価結果



ceftriaxone (CTRX) R= H, **3** R = POM



77 **78** **79** **80**

Compounds	MW	PAMPA coefficient[a]	PAMPA category[b]	Kinetic solubility (μM)	F_{po} (%)[c]
77	692	0.00	low	46.4	N.D.[d]
78	728	N.D.[d]	-	N.T.[e]	0.1
79	754	N.D.[d]	-	>50	N.T.[e]
80	821	0.11	medium	7.5	0.7
3 (CTRX-di-POM)	782	N.D.[d]	-	3.3	1.0
CTRX	555	-	-	>50	0.7

[a]Permeability coefficient in PAMPA (10^(-6)*cm/s). [b]Permeation category in PAMPA. [c]Calculated by AUC of Ceftriaxone formed from prodrugs administrated orally / AUC of Ceftriaxone administrated intravenously. [d]Not Detected. [e]Not Tested.

また **77** や **79** は良好な溶解度を示しているにも関わらず **3** に対して膜透過性

の改善が認められていない。経口吸収性の向上を達成するにあたり、例えば第一章におけるアミノ基のプロドラッグ化の知見を組み合わせること、或いは他のマクロサイクル化合物において指摘されているように分子内非共有結合を活用し、細胞膜を透過する際と水溶液中とで異なる安定配座をとるような分子設計をすることにより、膜透過性を向上させることが重要であると考えている[24](Figure 2-8)。

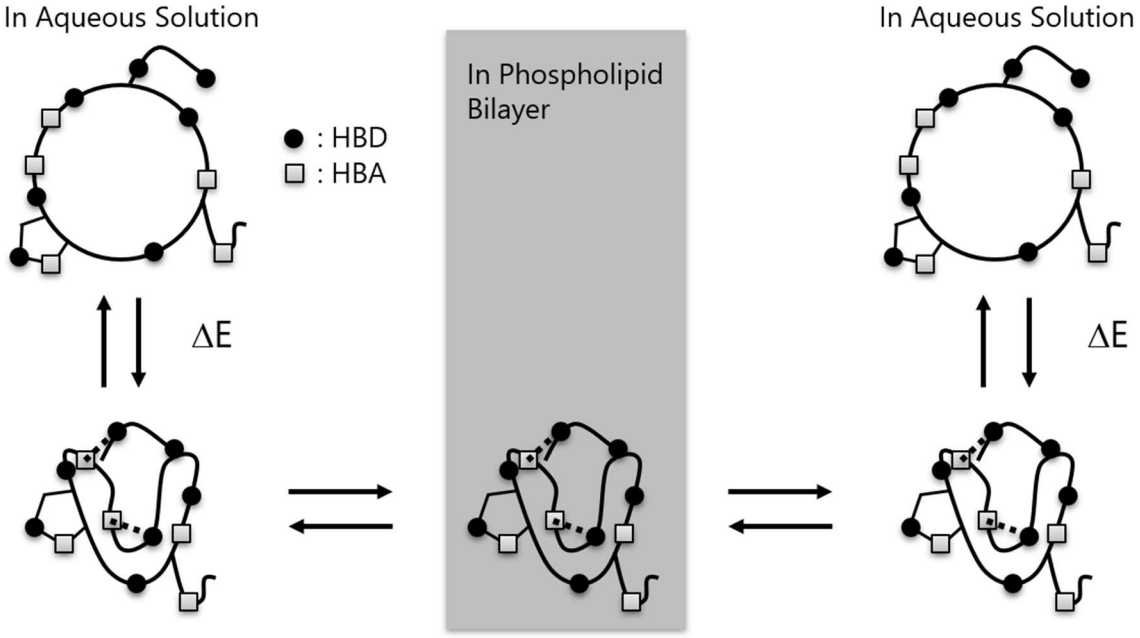


Figure 2-8. 水溶液中と脂質二重膜中で異なる安定配座についての概略図

また化合物 **77** の *in vitro* 抗菌活性を確認した(Table 2-4)。予想された通りセフカペンピボキシルで見られるように 4 位エステルである化合物 **77** のグラム陽性菌やグラム陰性菌に対する抗菌活性は **71** や **72** と比較して大きく減弱した。

Table 2-4. 各種化合物の *in vitro* 抗菌活性

		MIC[a] (μg/mL)			
species	strain ID	**77**	**71**	**72**	CTRX
E. coli	NIHJ JC-2[b]	16	0.5	2	0.25
S. pneumoniae	SR16675[c]	32	1	0.5	2
H. influenzae	SR11435[d]	4	0.25	0.25	0.25
B. subtilis	ATCC6633[e]	>32	1	1	1
E. cloacae	ATCC13047[e]	>32	16	8	32
S. marcescens	ATCC13880[e]	16	1	2	1

[a]Minimum Inhibitory Concentration values. [b]Reference 22. [c]A penicillin-resistant *Streptococcus pneumoniae* strain. [d]A beta-lactamase non-producing ampicillin-resistant *Haemophilus influenzae* strain. [e]These strains were Purchased from American Type Culture Collection (ATCC).

第五節 小括

第三世代セファロスポリン系抗菌薬としてCTRXを選択し、マクロサイクル化を実施した。

はじめにマクロサイクルの環員数を閉環メタセシス反応を用いて検討し、20員環以上のマクロサイクルを合成することとした。

閉環メタセシス、ジアルキル化、マクロラクトン化、ジスルフィド化により4種類の新規マクロサイクル**77~80**の合成に成功した。

これまでにセファロスポリンにおけるマクロサイクル化の報告例はなく、セファロスポリンにおいて初のマクロサイクル化を実現した。

残念ながら**77~79**は膜透過性が低く、測定した**77, 78**はCTRXより経口バイオアベイラビリティが低下した。また**80**はCTRXと比較し膜透過性の改善傾向が認められたが、その経口バイオアベイラビリティはCTRXや**3**と比較し同程度であった。

マクロサイクル化による経口吸収性の改善に向けて、消化管の安定性を高めるとともに、第一章でのアミノチアゾール部位アミノ基のプロドラッグ化を組み合わせたり、分子内非共有結合を活用した分子設計により膜透過性を向上させることが有用であると考えている。

またマクロサイクル**77**と合成中間体である**71**、**72**の抗菌活性を確認したところ、**71**、**72**はCTRXと同程度の抗菌活性を示したものの、マクロサイクル**77**は抗菌活性が大きく減弱した。

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Experimental for Chapter 2

Synthesis

Compound **63**

To a solution of **62** (9.06 g, 50.0 mmol) in *N*,*N*-dimethylformamide (91.0 mL) were added *p*-methoxybenzyl chloride (8.17 ml, 60.0 mmol) and sodium bromide (6.17 g, 60.0 mmol) at 0 °C, and then the mixture was stirred at room temperature for 2 h. After removal of *N*,*N*-dimethylformamide, to the mixture were added ethyl acetate and water with stirring for 10 min. The resulting precipitate was filtered and washed with water and dried under reduced pressure to give **63** (13.8 g, 99%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.35 (2H, d, J = 8.5 Hz), 6.88 (2H, d, J = 8.7 Hz), 4.37 (2H, s), 3.73 (3H, s), 3.54 (3H, s). HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{12}H_{13}N_3O_3S$ 280.0750; found 280.0746.

Compound **65**

To a solution of **63** (8.98 g, 30.6 mmol) in *N*,*N*-dimethylformamide (53.9 mL) were added 4-bromobut-1-ene (15.4 ml, 153 mmol), potassium carbonate (5.08 g, 36.7 mmol) and sodium iodide (4.59 g, 30.6 mmol), and then the mixture was stirred at 65 °C for 6 h. After removal of *N*,*N*-dimethylformamide, water was added to the mixture followed by extraction with ethyl acetate. The combined organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and evaporated. Diisopropyl ether was added to the residual solid, which was collected by filtration and dried under reduced pressure to give **65** (6.10 g, 60%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 (2H, d, J = 8.6 Hz), 6.85 (2H, d, J = 8.3 Hz), 5.91-5.81 (1H, m), 5.18-5.09 (2H, m), 4.46 (2H, s), 4.22 (2H, t, J = 7.1 Hz), 3.79 (3H, s), 3.65 (3H, s), 2.59 (2H, dt, J = 6.8, 6.8 Hz). HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{16}H_{19}N_3O_3S$ 334.1220; found 334.1214.

Compound **64**

Compound **64** was prepared by a procedure similar to that used for compound **65**. A yellow solid, 86%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 (2H, d, J = 8.6 Hz), 6.85 (2H, d, J = 8.8 Hz), 6.06 (1H, ddt, J = 16.4, 10.6, 5.8 Hz), 5.41 (1H, dd, J = 17.3, 1.4 Hz), 5.31 (1H, dd, J = 10.4, 1.0 Hz), 4.71 (2H, d, J = 5.8

Hz), 4.45 (2H, s), 3.79 (3H, s), 3.65 (3H, s). MS (ESI): *m/z* 320 $[M+H]^+$.

Compound **66**

Compound **66** was prepared by a procedure similar to that used for compound **64**. A pale yellow solid, 82%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 (2H, d, J = 8.6 Hz), 6.85 (2H, d, J = 8.8 Hz), 5.80 (1H, ddt, J = 17.2, 10.7, 6.5 Hz), 5.04-4.95 (2H, m), 4.45 (2H, s), 4.17 (2H, t, J = 6.7 Hz), 3.79 (3H, s), 3.65 (3H, s), 2.10 (2H, dt, J = 6.5, 6.5 Hz), 1.84 (2H, tt, J = 6.5, 6.5 Hz), 1.58-1.50 (2H, m). MS (ESI): *m/z* 362 $[M+H]^+$.

Compound **60**

To a solution of **65** (500 mg, 1.50 mmol) in ethyl acetate (5.00 mL) was added silver perchlorate (311 mg, 1.50 mmol). The mixture was stirred at room temperature for 2 h, then filtered. To the resulting precipitate were added ethyl acetate and water. To this mixture was added 2 mol/L aqueous hydrochloric acid with stirring at room temperature for 10 min. The mixture was extracted with ethyl acetate and the aqueous layer was extracted with tetrahydrofuran / ethyl acetate (1/1) twice. The combined organic layer was dried over $MgSO_4$, filtered, evaporated and dried under reduced pressure to give **60** (320 mg, quant.) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.37 (1H, br s), 5.89-5.79 (1H, m), 5.20-5.12 (2H, m), 4.26 (2H, t, J = 6.8 Hz), 3.84 (3H, s), 2.58 (2H, dt, J = 7.3, 6.8 Hz). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 170.4, 151.0, 148.1, 133.2, 118.2, 68.2, 45.2, 32.6. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_8H_{11}N_3O_2S$ 214.0645; found 214.0644.

Compound **59**

Compound **59** was prepared by a procedure similar to that used for compound **60**. A white solid, 74%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.61 (1H, s), 6.03 (1H, ddt, J = 17.4, 10.4, 5.8 Hz), 5.45 (1H, dd, J = 17.4, 1.5 Hz), 5.36 (1H, dd, J = 10.4, 1.5 Hz), 4.73 (2H, ddd, J = 5.9, 1.3, 1.3 Hz), 3.84 (3H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 170.4, 151.0, 148.3, 130.7, 120.4, 69.5, 45.1. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_7H_9N_3O_2S$ 200.0488; found 200.0489.

Compound **61**

Compound **61** was prepared by a procedure similar to that used for compound **59**. A yellow oil, 44%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.47 (1H, s),

5.80 (1H, ddt, J = 17.2, 10.7, 6.5 Hz), 5.05-4.97 (2H, m), 4.21 (2H, t, J = 6.6 Hz), 3.84 (3H, s), 2.12 (2H, dt, J = 6.5, 6.5 Hz), 1.84 (2H, tt, J = 6.5, 6.5 Hz), 1.59-1.51 (2H, m). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ: 170.2, 151.6, 148.2, 138.2, 115.2, 69.1, 45.2, 33.3, 27.7, 25.1. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{10}H_{15}N_3O_2S$ 242.0958; found 242.0958.

Compound **67**

To a solution of **31** (45.0 g, 69.0 mmol) in dichloromethane (225 mL) was added trifluoroacetic acid (135 mL) at 0 °C , and the mixture was stirred at 0 °C for 30 min. To this resulting mixture were added diisopropyl ether (100 mL), dichloromethane (100 mL) and water (300 mL) and the mixture was stirred at 0 °C for 5 min, followed by extraction. The organic layer was washed with water twice and brine, and then dried over $MgSO_4$, filtered and evaporated under reduced pressure. To this resulting residue was added diisopropyl ether (200 mL) with stirring at room temperature for 10 min. The mixture was filtered, and the residue was washed with diisopropyl ether and concentrated in vacuo to give **67** (32.5 g, 89%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.16 (1H, d, J = 7.1 Hz), 7.65 (1H, s), 5.79 (1H, dd, J = 5.3, 6.8 Hz), 5.05 (1H, d, J = 5.3 Hz), 4.57 (1H, d, J = 11.9 Hz), 4.44 (1H, d, J = 11.6 Hz), 4.18 (3H, s), 3.66 (1H, d, J = 18.4 Hz), 3.53 (1H, d, J = 18.2 Hz), 1.58 (9H, s). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ: 164.8, 163.8, 162.9, 162.9, 161.2, 152.5, 146.0, 128.8, 125.8, 113.5, 83.2, 63.7, 59.8, 57.9, 43.1, 28.2, 27.9.

Compound **68**

To a solution of **67** (6.38 g, 12.0 mmol) in acetonitrile (51.1 mL) was added *N,O*-bis(trimethylsilyl)acetamide (3.91 ml, 12.00 mmol) at 0 °C, and the mixture was stirred at 0 °C for 5 min. To this resulting mixture were added compound **59** (1.91 g, 9.60 mmol) and potassium carbonate (1.66 g, 12.00 mmol) and acetonitrile (12.8 mL), and the mixture was stirred at 0 °C for 20 min. 2 mol/L aqueous sodium hydroxide solution was added to the mixture to adjust the pH to around 4.0, with stirring for 1 h at 0 °C. To this resulting mixture were added diisopropyl ether, ethyl acetate, water and 2 mol/L aqueous sodium hydroxide solution followed by extraction. The aqueous layer was added sodium chloride and extracted with tetrahydrofuran twice and the combined organic layer was dried over $MgSO_4$, filtered, evaporated under reduced pressure and concentrated in vacuo. to give **68** (7.35 g, 88%) as an

orange foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.75 (1H, s), 9.61 (1H, d, J = 8.1 Hz), 6.66 (1H, s), 7.25 (1H, br s), 6.05 (1H, ddt, J = 10.6, 16.2, 5.6 Hz), 5.65 (1H, dd, J = 8.0, 4.7 Hz), 5.42 (1H, dd, J = 17.3, 1.4 Hz), 5.30 (1H, dd, J = 10.6, 1.5 Hz), 5.04 (1H, d, J = 4.8 Hz), 4.65 (2H, d, J = 5.8 Hz), 4.38 (1H, d, J = 12.9 Hz), 4.22 (1H, d, J = 12.6 Hz), 3.86 (3H, s), 3.70-3.57 (5H, m), 1.47 (9H, s). MS (ESI): *m/z* 695 $[M+H]^+$.

Compound **69**

Compound **69** was prepared by a procedure similar to that used for compound **68**. A pale yellow solid, 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.78 (1H, s), 9.60 (1H, d, J = 8.0 Hz), 7.23 (1H, s), 5.84 (1H, ddt, J = 17.1, 10.3, 6.5 Hz), 5.60 (1H, dd, J = 8.0, 4.4 Hz), 5.18-5.07 (2H, m), 5.01 (1H, d, J = 4.5 Hz), 4.33 (1H, d, J = 12.3 Hz), 4.20 (1H, d, J = 35.9 Hz), 4.14 (2H, t, J = 6.8 Hz), 3.88-3.81 (4H, m), 3.67-3.53 (5H, m), 2.50-2.43 (2H, m), 1.46 (9H, s). MS (ESI): *m/z* 709 $[M+H]^+$.

Compound **70**

Compound **70** was prepared by a procedure similar to that used for compound **68**. A pale yellow solid, 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.73 (1H, br s), 9.57 (1H, d, J = 8.2 Hz), 7.30-7.22 (2H, m), 6.65 (1H, br s), 5.81 (1H, ddt, J = 17.0, 10.5, 6.6 Hz), 5.60 (1H, dd, J = 7.8, 4.4 Hz), 5.05-4.95 (3H, m), 4.35 (1H, d, J = 12.7 Hz), 4.22 (1H, d, J = 12.4 Hz), 4.10 (2H, t, J = 6.5 Hz), 3.86 (3H, s), 3.62-3.61 (4H, m), 2.07 (2H, dt, J = 6.8, 6.8 Hz), 1.76-1.67 (2H, m), 1.50-1.42 (11H, m). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ: 171.5, 164.3, 162.8, 162.5, 161.9, 154.8, 152.2, 148.8, 141.8, 138.5, 138.4, 134.1, 115.0, 114.9, 114.4, 81.3, 67.1, 62.0, 58.1, 57.2, 42.4, 34.0, 32.7, 27.9, 27.3, 26.2, 24.6. MS (ESI): *m/z* 737 $[M+H]^+$.

Compound **71**

Compound **71** was prepared by a procedure similar to that used for compound **10**. A white solid, 53%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.63 (1H, d, J = 8.3 Hz), 7.20 (2H, br s), 6.73 (1H, s), 6.05 (1H, ddt, J = 16.8, 10.8, 5.6 Hz), 5.76 (1H, dd, J = 7.7, 4.4 Hz), 5.43 (1H, d, J = 17.2 Hz), 5.31 (1H, d, J = 10.4 Hz), 5.13 (1H, d, J = 4.8 Hz), 4.59 (2H, d, J = 5.3 Hz), 4.35 (1H, d, J = 12.7 Hz), 4.11 (1H, d, J = 13.3 Hz), 3.82 (3H, s), 3.74-3.55 (5H, m). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ: 168.4, 163.5, 163.1, 163.0, 161.3, 154.6, 151.8, 149.0,

142.5, 132.1, 131.8, 119.1, 119.0, 108.9, 67.7, 61.9, 58.6, 57.5, 42.5, 32.8, 27.8. MS (ESI): *m/z* 595 $[M+H]^+$. Anal. Calcd for $C_{21}H_{22}N_8O_7S_3(H_2O)_{5.4}(C_4H_8O_2)_{0.1}$: C, 36.68; H, 4.83; N, 15.99; S, 13.73. Found: C, 36.79; H, 4.28; N, 15.68; S, 13.54.

Compound **72**

To a solution of **69** (80.0 mg, 0.113 mmol) in dichloromethane (1.00 mL) were added anisole (99.0 μl, 0.903 mmol) and 2 mol/L aluminium chloride solution in nitromethane (0.451 ml, 0.903 mmol) at -30 °C, and the mixture was stirred at -30 °C for 30 min. To this resulting mixture were added diisopropylether and water followed by filtration. This resulting residue was dissolved using water and acetonitrile, and to the aqueous crude solution was added HP20SS. The mixture was concentrated, charged onto an HP20SS column, and chromatographed with aqueous acetonitrile. The fractions containing the desired product were collected and the pH of the solution was adjusted to around 6.0 using aqueous sodium bicarbonate solution. The resulting solution was concentrated and lyophilized to give **72** (56.0 mg, 68%) as a white powder. ^{1}H NMR (400 MHz, D_2O) δ: 7.00 (1H, s), 5.94 (1H, ddt, *J*= 17.2, 10.6, 6.8 Hz), 5.79 (1H, d, *J*= 4.5 Hz), 5.22-5.13 (3H, m), 4.41-4.33 (3H, m), 4.11 (1H, d, *J*= 13.6 Hz), 3.99 (3H, s), 3.79-3.75 (4H, m), 3.49 (1H, d, *J*= 18.2 Hz), 2.58 (2H, dt, *J*= 6.3, 6.6 Hz). MS (ESI) *m/z*: 609 $[M+H]^+$. Anal. Calcd for $C_{22}H_{23}N_8NaO_7S_3(H_2O)_{3.9}(NaHCO_3)_{0.4}$: C, 36.63; H, 4.28; N, 15.26; Na, 4.38; S, 13.09. Found: C, 36.70; H, 4.30; N, 15.22; Na, 4.50; S, 13.04.

Compound **73**

To a solution of **68** (4.20 g, 6.05 mmol) in dichloromethane (16.8 mL) were added 1-hydroxybenzotriazole (1.63 g, 12.09 mmol), allyl alcohol (12.4 ml, 181 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.32 g, 12.1 mmol) and the mixture was stirred at room temperature for 2 h. To this resulting mixture were added 0.1 mol/L aqueous hydrochloric acid solution and dichloromethane followed by extraction. The organic layer was washed with saturated aqueous sodium bicarbonate solution, water and brine, dried over $MgSO_4$, filtered, and then evaporated under reduced pressure and concentrated in vacuo. to give allyl ester (3.18 g) as an orange foam. MS (ESI): *m/z* 735 $[M+H]^+$.

To a solution of allyl ester obtained above (3.18 g, 4.33 mmol) in

dichloromethane (31.8 mL) was added *m*-chloroperoxybenzoic acid (~70%, 1.17 g, 4.76 mmol) at -40 °C, and the mixture was stirred at the same temperature for 45 min. To this resulting mixture were added saturated sodium bisulphite solution and extracted with dichloromethane. The organic layer was washed with saturated aqueous sodium bicarbonate solution, water and brine, dried over $MgSO_4$, filtered, and then evaporated under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain sulfoxide (2.20 g, 68%) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.75 (1H, br s), 9.08 (1H, d, J = 7.8 Hz), 6.10-5.91 (3H, m), 5.43 (2H, d, J = 15.9 Hz), 5.29 (2H, dd, J = 16.3, 10.7 Hz), 4.98-4.79 (3H, m), 4.66-4.62 (3H, m), 4.06-3.94 (3H, m), 3.89 (3H, s), 3.78 (1H, d, J = 18.4 Hz), 3.64 (3H, s), 1.47 (9H, s). MS (ESI): *m/z* 751 $[M+H]^+$.

To a solution of sulfoxide obtained above (2.20 g, 6.05 mmol) in *N,N*-dimethylformamide (22.0 mL) was added phosphorus tribromide (0.553 ml, 5.86 mmol) at -40 °C and the mixture was stirred at the same temperature for 20 min. To this resulting mixture were added water and ethyl acetate followed by extraction. The organic layer was washed with saturated aqueous sodium bicarbonate solution, water and brine, dried over $MgSO_4$, filtered, and then evaporated under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain sulfide (2.12 g, 99%) as a colorless foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.35 (1H, br s), 7.24 (1H, s), 7.17 (1H, d, J = 8.3 Hz), 6.10-5.93 (3H, m), 5.42 (2H, d, J = 17.2 Hz), 5.32 (2H, dd, J = 10.6, 1.8 Hz), 5.09 (1H, d, J = 4.8 Hz), 4.84-4.72 (4H, m), 4.62 (1H, d, J = 13.4 Hz), 4.20 (1H, d, J = 13.4 Hz), 4.07 (3H, s), 3.76-3.65 (5H, m), 1.54 (9H, s).

To a solution of sulfide obtained above (2.10 g, 2.86 mmol) in dichloromethane (21.0 mL) were added anisole (1.87 ml, 17.2 mmol) and 2 mol/L aluminium chloride solution in nitromethane (8.57 ml, 17.2 mmol) at -30 °C, and the mixture was stirred at -30 °C for 30 min. To this resulting mixture were added diisopropylether and water followed by filtration. This resulting residue was dissolved using water and acetonitrile, and to the aqueous crude solution was added HP20SS. The mixture was concentrated, charged onto an HP20SS and ODS column chromatography (acetonitrile/water) to afford **73** (245 mg, 12%) as a white amorphous solid after lyophilization. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.72 (1H, d, J = 9.0 Hz), 6.77 (1H, s), 6.09-5.91 (3H, m), 5.55 (2H, br s), 5.40 (2H, dt, J = 17.3, 1.8 Hz),

5.30 (2H, dd, J = 9.7, 5.1 Hz), 5.08 (1H, d, J = 4.8 Hz), 4.83-4.70 (4H, m), 4.59 (1H, d, J = 13.3 Hz), 4.16 (1H, d, J = 13.6 Hz), 4.03 (3H, s), 3.74-3.63 (5H, m). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ: 168.3, 164.9, 162.6, 162.1, 161.5, 155.8, 152.6, 147.1, 142.3, 131.3, 131.3, 129.7, 125.2, 120.0, 119.8, 112.0, 68.9, 67.1, 63.5, 58.8, 57.5, 42.7, 32.9, 28.3. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{24}H_{26}N_8O_7S_3$ 635.1159; found 635.1162. Anal. Calcd for $C_{24}H_{26}N_8O_7S_3(H_2O)_{0.8}(C_4H_8O_2)_{0.2}$: C, 44.68; H, 4.41; N, 16.81; S, 14.43. Found: C, 44.64; H, 4.39; N, 16.90; S, 14.36.

Compound **74**

Compound **74** was prepared by a procedure without Boc deprotection used for compound **73**. A colorless foam, 21%. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.82 (1H, br s), 7.57 (1H, d, J = 7.7 Hz), 7.10 (1H, s), 6.10 (1H, dd, J = 8.8, 4.7 Hz), 6.02-5.92 (1H, m), 5.89-5.79 (1H, m), 5.40 (1H, d, J = 17.2 Hz), 5.30 (1H, d, J = 10.3 Hz), 5.17-5.09 (3H, m), 4.82-4.74 (2H, m), 4.61 (1H, d, J = 13.4 Hz), 4.24-4.17 (3H, m), 4.06 (3H, s), 3.78-3.64 (5H, m), 2.58 (2H, dt, J = 6.8, 6.8 Hz), 1.53 (9H, s). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ: 164.9, 162.4, 162.0, 161.5, 160.6, 155.7, 153.1, 152.3, 147.2, 141.3, 133.5, 131.3, 129.7, 125.3, 119.7, 117.8, 115.9, 83.1, 67.5, 67.1, 63.5, 58.9, 57.6, 42.7, 32.9, 32.7, 28.3, 28.3. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{30}H_{36}N_8O_9S_3$ 749.184; found 749.1836.

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Compound **75**

Compound **75** was prepared by a procedure similar to that used for compound **74**. A colorless foam, 23%. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.66 (1H, br s), 7.45 (1H, br s), 7.15 (1H, s), 6.09 (1H, dd, J = 9.0, 4.6 Hz), 5.98 (1H, ddt, J = 16.8, 10.3, 6.3 Hz), 5.79 (1H, ddt, J = 17.2, 10.6, 6.5 Hz), 5.41 (1H, dd, J = 17.2, 1.1 Hz), 5.31 (1H, d, J = 10.5 Hz), 5.09 (1H, d, J = 5.0 Hz), 5.01 (1H, ddt, J = 17.2, 1.5, 1.5 Hz), 4.96 (1H, ddt, J = 10.3, 1.5, 1.5 Hz), 4.83-4.74 (2H, m), 4.62 (1H, d, J = 13.3 Hz), 4.20-4.16 (3H, m), 4.06 (3H, s), 3.75-3.64 (5H, m), 2.10 (2H, dt, J = 6.5, 6.5 Hz), 1.84 (2H, tt, J = 6.5, 6.5 Hz), 1.57-1.49 (11H, m). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ: 164.8, 162.3, 161.8, 161.5, 160.5, 155.9, 153.2, 152.2, 147.1, 141.3, 138.3, 131.3, 129.6, 125.2, 119.8, 116.0, 115.1, 83.1, 68.4, 67.1, 63.6, 58.9, 57.5, 42.7, 32.9, 28.3, 28.2, 27.8, 25.2. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{32}H_{40}N_8O_9S_3$ 777.2153; found 777.2158.

Compound **76**

Compound **76** was prepared by a procedure similar to that used for compound **74**. A white solid, 24%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.72 (1H, br s), 7.48 (1H, br s), 7.14 (1H, s), 6.09 (1H, dd, J = 9.2, 4.9 Hz), 5.86-5.74 (2H, m), 5.10-4.95 (5H, m), 4.60 (1H, d, J = 13.3 Hz), 4.35-4.24 (2H, m), 4.20-4.16 (3H, m), 4.06 (3H, s), 3.75-3.64 (5H, m), 2.19-2.07 (4H, m), 1.87-1.79 (4H, m), 1.53 (11H, t, J = 14.9 Hz). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 164.8, 162.3, 161.9, 161.8, 160.5, 155.9, 153.2, 152.3, 147.1, 141.3, 138.3, 137.3, 129.2, 125.4, 116.0, 115.7, 115.1, 83.1, 68.3, 66.0, 58.8, 57.5, 53.6, 42.7, 33.4, 32.9, 30.1, 28.3, 28.2, 27.8, 27.7, 25.2. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{34}H_{44}N_8O_9S_3$ 777.2153; found 777.2158.

Compound **84**

To paraformaldehyde (3.34 g, 111 mmol) was added **83** (11.0 g, 93.0 mmol) dropwise at 0 °C for 5 min, followed by the addition of zinc chloride (0.126 g, 0.928 mmol). The mixture was stirred below 30 °C for 2 h and quenched with water. The mixture was extracted with dichloromethane, and dried over $MgSO_4$, filtered, and evaporated. The resulting residue was distilled (20 Torr, 100 °C) to give **84** (5.69 g, 41%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.87-5.77 (1H, m), 5.71 (2H, s), 5.10-5.02 (2H, m), 2.52-2.38 (4H, m).

Compound **82**

To a solution of **84** (4.10 g, 27.6 mmol) in acetone (12.3 mL) was added sodium iodide (8.27 g, 55.2 mmol), and the mixture was stirred at room temperature for 5 h. The mixture was quenched with water and then extracted with ethyl acetate. The organic layer was washed with 2 mol/L aqueous sodium bisulfite solution and brine, and then dried over $MgSO_4$, filtered, and evaporated to give **82** (6.00 g, 91%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.91 (2H, s), 5.86-5.76 (1H, m), 5.11-5.02 (2H, m), 2.50-2.36 (4H, m).

Compound **81**

To a solution of **69** (2.50 g, 3.53 mmol) in *N*,*N*-dimethylformamide (25.0 mL) were added compound **82** (0.915 g, 3.53 mmol) and potassium carbonate (0.292 g, 2.12 mmol) at -40 °C, and the mixture was stirred below -30 °C for 20 min. To this resulting mixture was added potassium carbonate (0.195 g,

1.41 mmol) at -30 °C, and the mixture was stirred below -30 °C for 50 min. To this resulting mixture were added ethyl acetate, water and 2 mol/L aqueous hydrochloric acid to adjust the pH around 5.0, followed by extraction with ethyl acetate. The organic layer was washed with water and brine, and then dried over $MgSO_4$, filtered and evaporated under reduced pressure. This resulting residue was subjected to silica gel column chromatography, and eluted with hexane/ethyl acetate. The fractions containing the desired compound were concentrated under reduced pressure to obtain **81** (1.11 g, 38%) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.18 (1H, br s), 7.31 (1H, s), 6.99 (1H, d, *J* = 8.8 Hz), 6.02-5.98 (2H, m), 5.93-5.77 (3H, m), 5.19-4.99 (5H, m), 4.66 (1H, d, *J* = 13.6 Hz), 4.24 (2H, t, *J* = 7.1 Hz), 4.13 (1H, d, *J* = 13.9 Hz), 4.07 (3H, s), 3.77-3.65 (5H, m), 2.59 (2H, dt, *J* = 6.6, 7.3 Hz), 2.54-2.50 (2H, m), 2.41 (2H, dt, *J* = 6.3, 7.1 Hz), 1.54 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 171.7, 168.3, 164.9, 162.7, 161.8, 160.5, 160.5, 155.8, 153.0, 147.2, 142.4, 136.4, 133.5, 131.1, 124.6, 117.8, 115.9, 112.0, 79.8, 67.5, 67.2, 63.4, 58.9, 57.6, 42.7, 33.2, 32.9, 32.7, 28.5, 28.4, 28.4. MS (ESI) *m/z*: 821 $[M+H]^+$.

Compound **85**

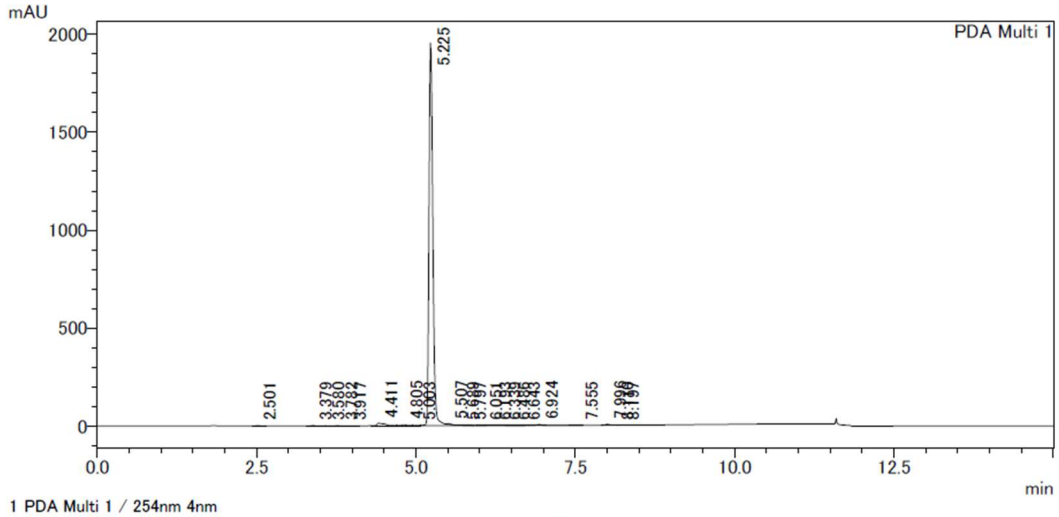
To a solution of **81** (1.00 g, 1.22 mmol) in dichloromethane (1000 mL) were added a Grubbs second-generation catalyst (0.207 g, 0.244 mmol) and tetraisopropyl titanate (0.714 ml, 2.44 mmol) at room temperature, and the mixture was stirred at 35 °C for 2 h. To this resulting mixture were added a Grubbs second-generation catalyst (0.207 g, 0.244 mmol) and tetraisopropyl titanate (0.714 ml, 2.44 mmol) at room temperature, and the mixture was stirred at 35 °C for 2 h and evaporated under reduced pressure. This resulting residue was subjected to silica gel column chromatography, and eluted with hexane/ethyl acetate. The fractions containing the desired compound were concentrated under reduced pressure to obtain **85** (0.240 g, 25%) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.16 (1H, br s), 7.33 (1H, s), 7.07-7.04 (1H, m), 6.01-3.35 (19H, m), 2.62-1.95 (6H, m), 1.54 (9H, s). MS (ESI) *m/z*: 793 $[M+H]^+$.

Compound **77**

To a solution of **85** (0.120 g, 0.151 mmol) in dichloromethane (1.20 mL) were added anisole (0.116 ml, 1.06 mmol) and 2 mol/L aluminium chloride solution in nitromethane (0.530 ml, 1.06 mmol) at -40 °C, and the mixture

was stirred at -40 °C for 30 min. To this resulting mixture were added diisopropylether and water followed by filtration. This resulting residue was dissolved using water, acetonitrile and tetrahydrofuran, and to the aqueous crude solution was added HP20SS. The mixture was concentrated, charged onto an HP20SS column, and reverse phase column chromatography was performed with aqueous acetonitrile. The fractions containing the desired product were collected, concentrated and lyophilized to give **77** (56.0 mg, 46%) as a white powder after lyophilization using dioxane. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.66-9.60 (1H, m), 7.27 (2H, br s), 6.75 (1H, s), 5.90-5.57 (3H, m), 5.44-5.14 (3H, m), 4.61-3.49 (12H, m), 2.56-1.91 (6H, m). HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{26}H_{29}N_8O_9S_3$ 693.1214; found 693.1223. Anal. Calcd for $C_{26}H_{28}N_8O_9S_3(H_2O)_{3.3}(C_4H_8O_2)_{0.3}$: C, 42.33; H, 4.87; N, 14.11; S, 12.11. Found: C, 42.40; H, 4.58; N, 14.08; S, 11.33. HPLC: 96.46pa%

HPLC data of Compound **77**



1 PDA Multi 1 / 254nm 4nm

ピークテーブル

PDA Ch1 254nm 4nm

ピーク#	保持時間	面積	高さ	面積%	高さ%
1	2.501	4117	834	0.048	0.042
2	3.379	6895	1878	0.080	0.094
3	3.580	3316	806	0.039	0.040
4	3.782	2810	453	0.033	0.023
5	3.917	2812	387	0.033	0.019
6	4.411	115349	13635	1.342	0.680
7	4.805	35839	5347	0.417	0.267
8	5.003	12811	1923	0.149	0.096
9	5.225	8293146	1955346	96.464	97.556
10	5.507	11578	3290	0.135	0.164
11	5.689	2190	622	0.025	0.031
12	5.797	4885	1136	0.057	0.057
13	6.051	7383	1406	0.086	0.070
14	6.193	8963	1529	0.104	0.076
15	6.339	15341	1919	0.178	0.096
16	6.486	6239	1088	0.073	0.054
17	6.643	8593	904	0.100	0.045
18	6.924	22889	4449	0.266	0.222
19	7.555	1642	251	0.019	0.013
20	7.996	21543	5218	0.251	0.260
21	8.110	4726	1102	0.055	0.055
22	8.197	4048	807	0.047	0.040
合計		8597115	2004330	100.000	100.000

Compound **87**

To a solution of ethane-1,2-diol (**86**) (1.08 ml, 19.4 mmol) in dichloromethane (50.0 mL) were added chloromethyl carbonochloridate (3.45 ml, 38.8 mmol) and pyridine (3.14 ml, 38.8 mmol) at 0 °C and the mixture was stirred at 0 °C for 30 min and then stirred at room temperature overnight. To this resulting mixture was added water followed by extraction with dichloromethane. The organic layer was washed with water and brine, and then dried over $MgSO_4$, filtered and evaporated under reduced pressure. This resulting residue was subjected to silica gel column chromatography, and eluted with hexane/ethyl acetate. The fractions containing the desired compound were concentrated under reduced pressure to obtain **87** (3.84 g, 80%) as a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.74 (4H, s), 4.48 (4H, s). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ: 153.3, 72.5, 66.0. MS (ESI) *m/z*: 247 $[M+H]^+$.

Compound **88**

To a solution of **87** (3.84 g, 15.6 mmol) in acetone (11.5 mL) was added sodium iodide (4.66 g, 31.1 mmol), and the mixture was stirred at room temperature for 5 h. To this resulting mixture was added 2 mol/L aqueous sodium bisulfite solution followed by extraction with ethyl acetate. The organic layer was washed with water and brine, and then dried over $MgSO_4$, filtered, evaporated under reduced pressure and concentrated in vacuo. to give **88** (6.10 g, 91%) as a yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.96 (4H, s), 4.46 (4H, s). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ: 153.1, 66.0, 33.8. MS (ESI) *m/z*: 431 $[M+H]^+$.

Compound **89**

To a solution of **67** (8.40 g, 15.8 mmol) in acetonitrile (67.2 mL) was added *N,O*-bis(trimethylsilyl)acetamide (5.15 ml, 15.8 mmol) at 0 °C, and the mixture was stirred at 0 °C for 5 min. To this resulting mixture were added 6-hydroxy-2-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one (1.54 g, 9.47 mmol) and sodium carbonate (1.00 g, 9.47 mmol) at 0 °C, and the mixture was stirred at 0 °C for 4 h and then evaporated under reduced pressure. To this resulting mixture were added diisopropylether (50.0 mL) and 10% aqueous sodium chloride solution (40.0 mL) followed by filtration. The resulting

residue was washed with diisopropylether and 10% aqueous sodium chloride solution and then concentrated in vacuo. This crude product was dissolved using water and acetonitrile and then pH of the solution was adjusted around 7.0 by 2 mol/L aqueous sodium hydroxide solution and lyophilized to give **89** (6.73 g, 61%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.76 (1H, br s), 9.57 (1H, d, J = 8.3 Hz), 7.26 (1H, s), 5.57 (1H, dd, J = 8.1, 4.8 Hz), 5.00 (1H, d, J = 4.5 Hz), 4.37 (1H, d, J = 12.6 Hz), 4.14 (1H, d, J = 12.6 Hz), 3.86 (3H, s), 3.56-3.47 (5H, m), 1.47 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ: 171.5, 164.7, 164.3, 162.7, 162.1, 161.1, 156.5, 153.2, 148.7, 141.8, 133.3, 115.8, 114.5, 81.4, 62.0, 58.0, 57.5, 44.4, 42.2, 27.8, 26.4. MS (ESI) *m/z*: 655 [M-2Na+3H]$^{+}$.

Compound **90**

To a solution of **89** (400 mg, 0.611 mmol) in *N*,*N*-dimethylformamide (40.0 mL) was added **88** (263 mg, 0.611 mmol) at -40 °C, and the mixture was stirred at -40 °C for 2 h and then stirred at 0 °C for 2 h. To this resulting mixture was added water and extracted with ethyl acetate. The organic layer was washed with water and brine, and then dried over $MgSO_4$, filtered and evaporated under reduced pressure. This resulting residue was subjected to silica gel column chromatography, and eluted with hexane/ethyl acetate. The fractions containing the desired compound were concentrated under reduced pressure to obtain **90** (90.0 mg, 18%) as a yellow foam. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.58 (1H, d, J = 8.8 Hz), 7.18 (1H, s), 6.34 (1H, d, J = 5.8 Hz), 6.15 (1H, d, J = 5.8 Hz), 6.05 (1H, dd, J = 8.9, 4.9 Hz), 5.71 (1H, d, J = 5.8 Hz), 5.67 (1H, d, J = 5.8 Hz), 5.13 (1H, d, J = 4.8 Hz), 4.61-4.57 (1H, m), 4.44-4.40 (3H, m), 4.31-4.26 (1H, m), 4.07-4.01 (5H, m), 3.68 (3H, s), 3.35 (1H, d, J = 18.6 Hz), 1.54 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 164.6, 162.3, 162.2, 160.7, 160.2, 155.4, 154.3, 153.4, 152.3, 150.2, 147.0, 141.2, 128.2, 125.0, 85.8, 83.2, 82.6, 65.9, 65.8, 63.5, 59.1, 57.6, 42.8, 33.9, 30.6, 29.8, 28.2. HRMS (ESI) *m/z*: [M+H]$^{+}$ calcd for $C_{29}H_{32}N_8O_{15}S_3$ 829.1222; found 829.1221.

Compound **78**

To a solution of **90** (110 mg, 0.133 mmol) in dichloromethane (1.10 mL) were added anisole (0.250 ml, 2.29 mmol) and 2 mol/L aluminium chloride solution in nitromethane (1.15 ml, 2.29 mmol) at -40 °C, and the mixture was stirred at -40 °C for 1 h. To this resulting mixture were added diisopropylether

and 2% aqueous sodium chloride solution followed by filtration. This resulting residue was dissolved using water and acetonitrile and to the aqueous crude solution was added HP20SS. Next, the mixture was evaporated under reduced pressure, charged onto an HP20SS and chromatographed using reverse phase column chromatography with aqueous acetonitrile. The fractions containing the desired product were collected, concentrated and lyophilized to give **78** (84.0 mg, 75%) as a white powder after lyophilization using dioxane. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.58 (1H, d, J = 8.1 Hz), 7.22 (2H, br s), 6.74 (1H, s), 5.94-5.92 (2H, m), 5.88 (1H, d, J = 6.3 Hz), 5.81 (1H, dd, J = 8.0, 4.7 Hz), 5.60 (1H, d, J = 5.8 Hz), 5.14 (1H, d, J = 4.8 Hz), 4.62 (1H, d, J = 13.9 Hz), 4.44-4.32 (4H, m), 4.02 (1H, d, J = 14.1 Hz), 3.90 (1H, d, J = 18.4 Hz), 3.84 (3H, s), 3.64-3.60 (4H, m). MS (ESI) *m/z*: 729 $[M+H]^+$. Anal. Calcd for $C_{24}H_{24}N_8O_{13}S_3(H_2O)_{1.5}(C_4H_8O_2)_{1.0}$: C, 39.84; H, 4.18; N, 13.34; S, 11.36. Found: C, 39.86; H, 4.18; N, 13.28; S, 11.40.

Compound **93**

To a solution of **92** (25.0 g, 138 mmol) in dichloromethane (150 mL) were added oxalyl chloride (12.7 ml, 145 mmol) and *N,N*-dimethylformamide at 0 °C, and the mixture was stirred at 0 °C to room temperature for 2 h. To this resulting mixture was added allyl alcohol (10.3 ml, 152 mmol), and the mixture was stirred at room temperature for 2 h. The resulting mixture was added water and extracted with dichloromethane. The organic layer was washed with 2 mol/L aqueous hydrochloric acid solution, water and brine, and then dried over $MgSO_4$, filtered, and then evaporated under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **93** (28.5 g, 87%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.92 (1H, ddt, J = 17.2, 10.2, 5.6 Hz), 5.32 (1H, dd, J = 17.2, 1.5 Hz), 5.24 (1H, dd, J = 10.2, 1.5 Hz), 4.59 (2H, d, J = 5.6 Hz), 3.42 (2H, t, J = 6.4 Hz), 2.38 (2H, t, J = 7.2 Hz), 1.95-1.88 (2H, m), 1.84-1.76 (2H, m).

Compound **94**

To a solution of **63** (12.0 g, 39.5 mmol) in *N,N*-dimethylformamide (120 mL) were added **93** (13.1 g, 59.3 mmol), sodium iodide (5.92 g, 39.5 mmol) and potassium carbonate (6.56 g, 47.4 mmol) and stirred at 65 °C overnight. To the mixture were added **93** (2.62 g, 11.9 mmol), sodium iodide (1.18 g, 7.90 mmol) and potassium carbonate (1.31 g, 9.48 mmol) and stirred at 65 °C for 8

h. The solvent was removed under reduced pressure and the resulting mixture was poured into ethyl acetate and water and the layers were separated. The aqueous layer was extracted with ethyl acetate and the combined organic layer was washed with water and brine, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The resulting white solid was filtered and washed with diisopropyl ether and ethyl acetate to give **94** (11.5 g, 69%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 (2H, d, *J* = 8.6 Hz), 6.85 (2H, d, *J* = 8.6 Hz), 5.92 (1H, ddt, *J* = 17.2, 10.4, 5.8 Hz), 5.31 (1H, dd, *J* = 17.2, 1.0 Hz), 5.23 (1H, d, *J* = 10.4 Hz), 4.58 (2H, d, *J* = 5.8 Hz), 4.46 (2H, s), 4.19 (2H, t, *J* = 6.3 Hz), 3.79 (3H, s), 3.65 (3H, s), 2.41 (2H, t, *J* = 7.2 Hz), 1.92-1.76 (4H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 173.0, 162.2, 159.4, 156.1, 152.8, 132.3, 130.7, 127.4, 118.4, 114.3, 67.6, 65.2, 55.4, 42.6, 36.0, 33.8, 27.8, 21.5. MS (ESI): *m/z* 420 $[M+H]^+$.

Compound **91**

Compound **91** was prepared by a procedure similar to that used for compound **94**. A white solid, 82%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.36 (1H, s), 5.92 (1H, ddt, *J* = 17.2, 10.5, 6.3 Hz), 5.32 (1H, dd, *J* = 17.2, 1.5 Hz), 5.24 (1H, dd, *J* = 10.5, 1.5 Hz), 4.58 (2H, dt, *J* = 6.3, 1.3 Hz), 4.22 (2H, t, *J* = 6.6 Hz), 3.84 (3H, s), 2.42 (2H, t, *J* = 7.2 Hz), 1.92-1.76 (4H, m). MS (ESI): *m/z* 300 $[M+H]^+$.

Compound **97**

To a solution of chloromethyl carbonochloridate (4.20 ml, 47.3 mmol) in dichloromethane (50 mL) were added 2-((*tert*-butyldimethylsilyl)oxy)ethanol (**96**) (10.0 g, 56.7 mmol) and pyridine (4.20 ml, 52.0 mmol) dropwise over 15 min under -10 °C and the mixture was stirred at -20 °C overnight. To this resulting mixture were added 0.4 mol/L aqueous hydrochloric acid solution and dichloromethane and the organic layer was separated and washed with water and brine, and then dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **97** (14.6 g, quant.) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.74 (2H, s), 4.29 (2H, t, *J* = 4.9 Hz), 3.85 (2H, t, *J* = 4.9 Hz), 0.89 (9H, s), 0.07 (6H, s). MS (ESI): *m/z* 269 $[M+H]^+$.

Compound **95**

To a solution of **97** (10.0 g, 32.4 mmol) in acetone (30.0 mL) was added sodium iodide (9.70 g, 64.7 mmol) at room temperature and then stirred at 40 °C for 2 h. The solvent was removed under the reduced pressure and the resulting mixture was poured into water and ethyl acetate and the organic layer was separated and washed with 5% aqueous sodium hydrogensulfite solution, water and brine, and then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure to obtain **95** (17.5 g, quant.) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.96 (2H, s), 4.28 (2H, t, *J* = 4.9 Hz), 3.84 (2H, t, *J* = 4.9 Hz), 0.89 (9H, s), 0.07 (6H, s).

Compound **98**

Compound **98** was prepared from **67** and **91** by a procedure similar to that used for compound **89**. An orange foam, 100%. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 11.78 (1H, br s), 9.66 (1H, d, *J* = 8.1 Hz), 7.26 (1H, s), 5.91 (1H, ddt, *J* = 17.3, 10.6, 5.3 Hz), 5.81 (1H, dd, *J* = 8.2, 4.7 Hz), 5.29 (1H, dt, *J* = 17.3, 1.6 Hz), 5.21 (1H, dd, *J* = 10.6, 1.3 Hz), 5.15 (1H, d, *J* = 4.8 Hz), 4.55 (2H, d, *J* = 5.3 Hz), 4.38 (1H, d, *J* = 13.4 Hz), 4.13-4.08 (3H, m), 3.87 (3H, s), 3.74-3.55 (5H, m), 2.41 (2H, t, *J* = 7.3 Hz), 1.78-1.71 (2H, m), 1.69-1.62 (2H, m), 1.47 (9H, s).δ: 9.36 (1H, s), 5.92 (1H, ddt, *J* = 17.2, 10.5, 6.3 Hz), 5.32 (1H, dd, *J* = 17.2, 1.5 Hz), 5.24 (1H, dd, *J* = 10.5, 1.5 Hz), 4.58 (2H, dt, *J* = 6.3, 1.3 Hz), 4.22 (2H, t, *J* = 6.6 Hz), 3.84 (3H, s), 2.42 (2H, t, *J* = 7.2 Hz), 1.92-1.76 (4H, m). MS (ESI): *m/z* 795 $[M+H]^+$.

Compound **99**

Compound **99** was prepared from **98** by a procedure similar to that used for compound **81**. A yellow foam, 49%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 (1H, s), 6.90 (1H, d, *J* = 9.1 Hz), 6.01-5.88 (4H, m), 5.31 (1H, d, *J* = 16.9 Hz), 5.23 (1H, d, *J* = 10.6 Hz), 5.07 (1H, d, *J* = 5.1 Hz), 4.66 (1H, d, *J* = 13.9 Hz), 4.58 (2H, d, *J* = 5.6 Hz), 4.29-4.25 (2H, m), 4.22-4.08 (7H, m), 3.85 (2H, t, *J* = 4.9 Hz), 3.73-3.69 (5H, m), 2.41 (2H, t, *J* = 7.1 Hz), 1.90-1.78 (4H, m), 1.54 (9H, t, *J* = 11.4 Hz), 0.88 (9H, s), 0.06 (6H, s).MS (ESI): *m/z* 514 $[M+2H]^{2+}$.

Compound **100**

To a solution of **99** (300 mg, 0.292 mmol) in tetrahydrofuran (18 mL) and water (3 mL) was added formic acid (8.99 ml, 235 mmol) and the mixture was

stirred at room temperature for 90 min. To this resulting mixture was added tetrakis(triphenylphosphine)palladium (404 mg, 350 mmol) and the mixture was stirred at room temperature for 40 min. To this resulting mixture were added water and ethyl acetate and the organic layer was separated. The organic layer was washed with water and brine, and then dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain **100** (220 mg, 86%) as an orange oil. MS (ESI): *m/z* 873 $[M+H]^+$.

Compound **79**

To a solution of **100** (800 mg, 0.916 mmol) in dichloromethane (400 mL) were added benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (954 mg, 1.83 mmol) and *N,N*-dimethylaminopyridine (560 mg, 4.58 mmol) and the mixture was stirred at room temperature for 2 h. To this resulting mixture was added 0.4 mol/L aqueous hydrochloric acid solution and dichloromethane and the organic layer was separated and washed with water and brine, and then dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) to obtain macrolactone (578 mg, 63%) as a mixture of Δ2 isomer. MS (ESI): *m/z* 855 $[M+H]^+$.

A compound **79** was prepared from macrolactone and its Δ2 isomer obtained above by oxidation, reduction and Boc removal procedures for **68**. A white solid, 74%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.23 (1H, d, *J* = 8.5 Hz), 6.65 (1H, s), 6.14 (1H, d, *J* = 5.5 Hz), 5.91 (1H, dd, *J* = 8.5, 4.8 Hz), 5.86 (2H, s), 5.76 (1H, d, *J* = 5.5 Hz), 4.98-4.92 (2H, m), 4.41-4.29 (4H, m), 4.22-4.11 (2H, m), 3.97 (3H, s), 3.74-3.68 (4H, m), 3.63 (3H, s), 3.26 (1H, d, *J* = 18.2 Hz), 2.51-2.38 (2H, m), 1.76-1.70 (2H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 173.4, 168.7, 164.9, 163.0, 160.9, 160.2, 155.4, 154.0, 152.8, 147.6, 142.4, 128.0, 125.4, 111.7, 83.9, 68.1, 66.9, 63.1, 61.9, 59.0, 57.9, 42.6, 34.6, 33.3, 28.9, 27.0, 22.5. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{27}H_{30}N_8O_{12}S_3$ 755.1218; found 755.1219. Anal. Calcd for $C_{27}H_{30}N_8O_{12}S_3(H_2O)_{1.8}(C_4H_8O_2)_{0.4}$: C, 41.77; H, 4.51; N, 13.62; S, 11.69. Found: C, 41.81; H, 4.46; N, 13.68; S, 11.47.

Compound **102**

Compound **102** was prepared from **101** by a procedure similar to that used for compound **97** from **96**. A colorless oil, 89%. ^{1}H NMR (400 MHz, $CDCl_3$) δ:

7.43-7.41 (6H, m), 7.31-7.20 (9H, m), 5.67 (2H, s), 3.89 (2H, t, J = 6.9 Hz), 2.54 (2H, t, J = 6.9 Hz). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ: 153.0, 144.4, 129.5, 128.0, 126.9, 72.2, 67.1, 67.0, 30.3.

Compound **103**

Compound **103** was prepared from **102** by a procedure similar to that used for compound **95** from **97**. A white solid, quant.. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.42-7.41 (5H, m), 7.31-7.20 (10H, m), 5.90 (2H, s), 3.89 (2H, t, J = 6.9 Hz), 2.53 (2H, t, J = 6.9 Hz).

Compounds **104**

Compound **104** was prepared from ceftriaxone by a procedure similar to that used for compound **90** from **89**. A white solid, 61%. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.42-7.39 (12H, m), 7.31-7.28 (12H, m), 7.24-7.21 (6H, m), 7.02 (1H, d, J = 9.0 Hz), 6.93 (1H, s), 6.00-5.78 (4H, m), 5.68 (1H, s), 5.14 (2H, br s), 5.04 (1H, d, J = 5.3 Hz), 4.60 (1H, d, J = 6.8 Hz), 4.06 (3H, s), 3.92-3.86 (4H, m), 3.74-3.62 (5H, m), 3.39 (1H, br s), 2.56-2.51 (4H, m). MS (ESI): *m/z* 1307 $[M+H]^+$.

Compounds **80**

To a solution of **104** (390 mg, 0.298 mmol) in tetrahydrofuran (78 mL) and methanol (390 mL) was added iodine (151 mg, 0.597 mmol) and the mixture was stirred at room temperature for 1 h. To this resulting mixture were added 12 mM aqueous sodium ascorbate solution and then added 0.5 mol/L aqueous hydrochloric acid solution to adjust pH around 5 and the solvent was removed. The resulting precipitate was added chloroform and the layers were seperated. The aqueous layer was extracted with chloroform and the combined organic layer was washed with brine, and then dried over MgSO4, filtered and evaporated under reduced pressure. The mixture was purified using preparative HPLC with aqueous acetonitrile added 0.1 % formic acid. The fractions containing the desired product were collected, concentrated and lyophilized to give **80** (70.0 mg, 15%) as a white powder after lyophilization. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.11 (1H, d, J = 9.1 Hz), 6.94 (1H, s), 6.16 (1H, d, J = 5.3 Hz), 6.02-5.94 (3H, m), 5.82 (1H, d, J = 5.8 Hz), 5.17 (2H, s), 5.10 (1H, d, J = 5.1 Hz), 4.58 (1H, d, J = 14.4 Hz), 4.47-4.30 (4H, m), 4.07 (3H, s), 3.93 (1H, d, J = 13.4 Hz), 3.87 (1H, d, J = 18.9 Hz), 3.71 (3H, s), 3.43 (1H, d, J

= 18.7 Hz), 3.06-2.94 (4H, m). MS (ESI): *m/z* 821 $[M+H]^+$. Anal. Calcd for $C_{26}H_{28}N_8O_{13}S_5(H_2O)_{2.5}$: C, 36.07; H, 3.84; N, 12.94; S, 18.51. Found: C, 36.39; H, 3.54; N, 13.05; S, 17.99.

<u>Compound Evaluation</u>

Pharmacokinetics in Rats after Intravenous Administration of Compounds.

Pharmacokinetics in intravenous administration was investigated in rats at a dose of 5 mg/kg formulated in distilled water. Blood samples were taken using a syringe pre-treated by 0.89 M aqueous EDTA·2K solution and 20 % heparin at 0.033, 0.083, 0.25, 0.5, 1, 2, 4 and 6h after administration. Blood samples were centrifuged at 3000 rpm for 10 min at 4 ˚C to obtain plasma samples. Plasma samples were transferred to cluster tubes and stored at -20 ˚C prior to analysis.

Pharmacokinetics in Rats after Oral Administration of Compounds.

Pharmacokinetics in oral administration were investigated in rats at doses of 15, 20 and 30 mg/kg formulated in 0.5 % Methyl cellulose (400 cP). Blood samples were taken using a syringe pre-treated by 0.89 M aqueous EDTA·2K solution and 20% heparin at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after administration. Blood samples were centrifuged at 3000 rpm for 10 min at 4 ˚C. Plasma samples were transferred to cluster tubes and stored at -20 ˚C prior to analysis.

LC/MS/MS Determination of Test Compounds.

An aliquot (40 μL) of each sample was transferred to a 96-well plate, mixed with 500 μL of acetonitrile, and centrifuged at 3000 rpm for 10 min at 4 ˚C. A portion of supernatant was injected to Shimadzu Nexera HPLC system interfaced with an API-5000 mass spectrometer (AB Sciex, Framingham, MA). Plasma concentrations of test compounds were determined by LC/MS/MS with electrospray ionization in the positive ion mode using an external standard method.

Pharmacokinetic Analysis.

The area under the concentration-time curve from 0 h to the last time point ($AUC_{0\text{-}tlast}$) was calculated by linear trapezoidal approximation. The oral

bioavailability was calculated by dividing AUC of ceftriaxone derived from each test compounds administrated orally / AUC of ceftriaxone administrated intravenously.

Antibiotic Susceptibility Testing.

The minimum inhibitory concentrations (MICs) were determined using the broth microdilution method in accordance with Clinical Laboratory Standards Institute (CLSI) guidelines.[1] Briefly, two-fold serial dilution of test compounds were prepared and transferred to a 96 well plate including cation-adjusted Mueller Hinton broth (CAMHB). Haemophilus Test Medium broth (HTM broth) and CAMHB containing laked horse blood were used for *Haemophilus influenzae* and *Streptococcus pneumoniae*, respectively. The bacterial suspension for inoculum was prepared based on optical density of 625 nm to be approximately 5×10^4 CFU/well as final inoculum size. The 96 well plates were incubated at 35°C for 16 to 20 hours. The MIC endpoint was defined as the lowest concentration of the compound that inhibited bacterial growth as detected by the unaided eye.

Kinetic Solubility Assay.

Compounds in dimethylsulfoxide (10 mmol/L) were diluted into the aqueous phosphate buffer solution (pH 6.8) with the final dimethylsulfoxide concentration being 1%. After 1 h of incubation, precipitates produced was filtered through a membrane filter and the filtrate was quantified with LC/MS.

Parallel Artificial Membrane Permeability Assay (PAMPA).

PAMPA was applied to measure compounds for passive diffusion. This study employed the Gentest Precoated PAMPA Plate System (Corning Life Sciences). The system is composed of a 96-well plate/insert system. Two fluidfilled compartments (donor well and receiver well) are separated by a polyvinylidene fluoride (PVDF) filter plate precoated with a phospholipid-oil-phospholipid trilayer primarily consisting of dioleoyl phosphatidylcholine (DOPC) phospholipids. Compounds of interest were dissolved in 2% dimethylsulfoxide in aqueous phosphate buffer solution (pH 6.8). Solutions were added to donor wells at a volume of 0.3 mL. The filter plate, containing acceptor wells filled with 0.2 mL 2% dimethylsulfoxide in PBS solution (pH

7.4), was placed on top of the donor plate. The entire system was incubated for 4 h at 25 °C. Following incubation, solution was removed from donor and acceptor wells. The removed solution was analyzed by ultra performance liquid chromatography (UPLC), and permeability of compound was calculated. Using these measured experimental permeability rates, as well as permeability data from literature, we are able to divide our data into three groups; high permeability, medium permeability, low permeability. The boundaries between these regions are defined by using the permeability coefficient midpoints between different model compounds of known permeability categories.[2, 3]

Compound	permeability coefficient (10^(-6)*cm/s)	Permeation category
Ketoprofen	2.19	high
Metoprolol tartrate	0.91	high
Atenolol	0.09	medium
Furosemide	0.19	medium
Sulpiride	0.03	low
Lisinopril hydrate	0.00	low

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2. X. Chen, A. Murawski, K. Patel, C. L. Crespi, P. V. Balimane. *Pharm. Res.* **2008**, *25*, 1511-1520.
3. A. Avdeef, S. Bendels, L. Di, B. Faller, M. Kansy, K. Sugano, Y. Yamauchi. *J. Pharm. Sci.* **2007**, *96*, 2893–2909.

結語

第三世代セファロスポリンの経口吸収性を改善するための一般的な方法論を確立することを目的として、7 位側鎖アミノチアゾールの修飾とマクロサイクル化を実施した。

7 位側鎖アミノチアゾールの修飾においては、第三世代セファロスポリン系抗菌薬としてセフカペンピボキシル(CFPN-PI)、セフトリアキソン(CTRX)、セフェタメトピボキシル(CEMT-PI)、セフテラムピボキシル(CFTM-PI)を用いて 7 位側鎖のアミノチアゾールをメチルチアゾールや *N,N*-ジメチルアミノチアゾールに変換した化合物を合成した。

2 や **5** は CFPN-PI や **3** (CTRX-di-POM)と比較して経口バイオアベイラビリティの大幅な向上することが確認された。

一方で **2** や **5** を含む 4 位 POM エステル(**2**、**5**、**7**、**9**、**11**、**13**)の活性体である 4 位カルボン酸 **1**、**4**、**6**、**8**、**10**、**12** はそれぞれ CFPN、CTRX、CEMT、CFTM と比較して各種グラム陽性菌、グラム陰性菌に対する抗菌活性が大きく減弱した。

経口吸収性の改善とグラム陽性菌及びグラム陰性菌に対する良好な抗菌活性の両立を指向して、アミノ基を *N*-アシルオキシメチルカルバメート型のプロドラッグへと変換した CEMT の新規プロドラッグ **41**～**45** を考案し合成した。

考案した **41**～**45** はいずれも血中において CEMT へと変換されたことから、*N*-アシルオキシメチルカルバメートがアミノチアゾール部位アミノ基のプロドラッグとして機能することが明らかとなった。

脂溶性の増大と水素結合供与体数の低減によって膜透過性を改善した新規プロドラッグ **42** 及び **43** を見出すことができた。一方でそれらのバイオアベイラビリティは CEMT-PI とほぼ同等程度であった。

膜透過性を改善した **42**、**43** の経口吸収性が CEMT-PI とほぼ同等程度に留まった理由として消化管に存在する酵素に対して安定性が低かったためであると考えており、この点を改善するためにカーバメートプロドラッグにおけるメチレン部位やアシル部位の構造変換が有用であると考えている。

第三世代セファロスポリン系抗菌薬としてCTRXを選択し、マクロサイクル化を実施した。

これまでにセファロスポリンのマクロサイクルの報告は無かったが、マクロサイクル化反応として閉環メタセシス、ジアルキル化、マクロラクトン化、ジスルフィド化を選択し、20員環から25員環の4種類の新規マクロサイクル**77~80**を合成を達成した。

残念ながら**77~79**は膜透過性が低く、**77, 78**はCTRXより経口バイオアベイラビリティが低下した。また**80**はCTRXと比較し膜透過性の改善傾向が認められたが、その経口バイオアベイラビリティはCTRXや**3**と比較し同程度であった。

マクロサイクル化による経口吸収性の改善に向けて、消化管の安定性を高めるとともに、第一章でのアミノチアゾール部位アミノ基のプロドラッグ化を組み合わせる、或いは分子内非共有結合を活用した分子設計により膜透過性を向上させることが有用であると考えている。

以上、これらの新しい知見は今後のセファロスポリン系抗菌薬の開発研究に有用な知見を提供すると考えられる。

学位論文の主たる部分を公表した論文

(1) 論文題目：Synthesis of Novel Orally Active Prodrugs by Introduction of an Acyloxymethyl Carbamate Moiety into Cefetamet Pivoxil
著者：Masayuki Sano, Hiroyuki Shimaoka, Naoki Kohira, Yuki Murakami, Hitoshi Murai, and Hidenori Yoshizawa
雑誌名：*Chemistry Letters*, **2020**, *49*(12), 1497-1500.

(2) 論文題目：Synthesis of Novel Macrocyclic Compounds Derived from Ceftriaxone
著者：Masayuki Sano, Hiroyuki Shimaoka, Naoki Kohira, Yuki Murakami, Hitoshi Murai, and Hidenori Yoshizawa
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