2 位に硫黄官能基を有する
1,6-ジアザビシクロ[3.2.1]オクタン系
新規経口 β-ラクタマーゼ阻害剤の創製

2022

藤生　基弘

Abbreviations

Ac	acetyl	*E. coli*	*Escherichia coli*
AVI	avibactam	EDC	1-ethyl-3-(3-dimethylaminopropyl)- carbodiimide
BLI	β-lactamase inhibitor	ESBL	extended-spectrum β-lactamase
Bn	benzyl	Et	ethyl
Boc	*tert*-butoxycarbonyl	EUCAST	European Committee on Antimicrobial Susceptibility Testing
Bzh	benzhydryl	FDA	Food and Drug Administration
CAMHB	cation-adjusted Mueller Hinton broth	^{i}Pr	isopropyl
CAZ	ceftazidime	*K. pneumoniae*	*Klebsiella pneumoniae*
CFM	cefixime	LED	light-emitting diode
C. freundii	*Citrobacter freundii*	MBL	metallo-β-lactamase
CFU	colony forming unit	*m*-CPBA	*m*-chloroperoxybenzoic acid
CL	clearance	Me	methyl
CLSI	Clinical Laboratory Standards Institute	MHB	Mueller Hinton broth
CRAB	carbapenem-resistant *Acinetbacter baumannii*	MIC	minimum inhibitory concentration
CRE	carbapenem-resistant *Enterobacteriaceae*	MPC	minimum potentiating concentration
CRPA	carbapenem-resistant *Pseudomonus aeruginosa*	NAC	nacubactam
CSI	chlorosulfonyl isocyanate	NMR	nuclear magnetic resonance
CTB	ceftibuten	NOAEL	no observed adverse effect level
DABCO	1,4-diazabicyclo[2.2.2]octane	NOE	nuclear Overhauser effect
DBO	1,6-diazabicyclo[3.2.1]octane	NOESY	nuclear Overhauser effect spectroscopy
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene	PBP	penicillin-binding protein
DIAD	diisopropyl azodicarboxylate	Ph	phenyl
DMAP	4-dimethylaminopyridine	PK	pharmacokinetics
DMB	2,4-dimethoxybenzyl	PMB	*p*-methoxybenzyl
DMEAD	di-2-methoxyethyl azodicarboxylate	PNB	*p*-nitrobenzyl
DMF	*N*,*N*-dimethylformamide	Py	2-pyridyl
dr	diastereomeric ratio	QD	quaque die
DUR	durlobactam	REL	relebactam
E. cloacae	*Enterobacter cloacae*	SAR	structure-activity relationship

SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SFC	supercritical fluid chromatography
Su	succinimide
TASF	tris(dimethylamino)sulfonium difluorotrimethylsilicate
TBAF	tetra-*n*-butylammonium fluoride
TBS	*tert*-butyldimethylsilyl
^{t}Bu	*tert*-butyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMS	trimethylsilyl
Ts	*p*-toluenesulfonyl
VAB	vaborbactam
WHO	World Health Organization
ZID	zidebactam

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緒論

第１節　薬剤耐性菌とβ-ラクタマーゼ

第1項 抗生物質と薬剤耐性菌

アレクサンダー・フレミングによるペニシリンの発見以来，数々の抗生物質が発見・開発され人類の健康に貢献してきた．中でもβ-ラクタム系抗菌薬は，高い有効性と安全性を兼ね備えた抗生物質として，ペニシリン系（ペナム）をはじめとして，セフェム，モノバクタム，カルバペネムといった複数の骨格をもったβ-ラクタム系抗菌薬が臨床現場で使われている (Figure 1)．このように数多くのβ-ラクタムが開発された背景には，抗菌スペクトルの拡大や投与経路の変更，血中半減期の改善による利便性の向上を志向した研究がされてきたことと共に，耐性菌への対応に主眼を置いた数多くの研究がなされてきたことがある．耐性菌との戦いは，新薬の開発と耐性菌の出現を繰り返す「いたちごっこ」であり，この中でモノバクタムやカルバペネムといった異なる骨格のβ-ラクタム抗菌薬が見いだされてきた．特にカルバペネム系抗菌薬は他の抗生物質に対する耐性菌に対して有効であり，現在，耐性菌に対する「最後の手段」として使われている．[1]

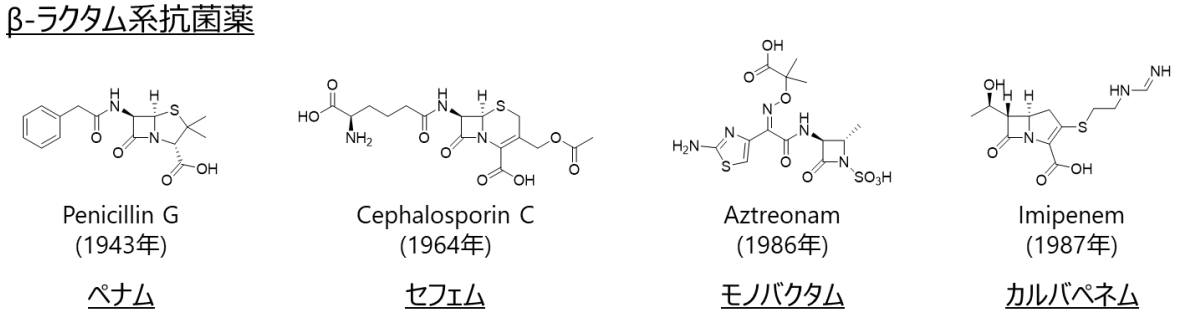

Figure 1. A series of β-lactam antibiotics.

一方で，これらの抗生物質の過剰な使用や誤った使用により，多剤耐性菌の問題が深刻化しており, 依然として新たな抗生物質の開発が求められている. 世界保健機関 (WHO) が 2017 年に発表した “Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics.”[2] によると，カルバペネム耐性アシネトバクター (CRAB, carbapenem-resistant *Acinetbacter baumannii*) ，カルバペネム耐性緑膿菌 (CRPA, carbapenem-resistant *Pseudomonus aeruginosa*) , および, カルバペネム耐性腸内細菌科細菌 (CRE, carbapenem-resistant *Enterobacteriaceae*) への対応が “Priority 1: Critical” に位置付けられている (Table 1). これらの耐性菌はβ-ラクタム系抗菌薬だけでなくほとんど全ての抗生物質が効かない多剤耐性菌であり，その対応は喫緊の課題である．[3]

Table 1. Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics[2]

Priority 1 : CRITICAL	***Acinetobacter baumannii***, carbapenem-resistant	***Pseudomonas aeruginosa***, carbapenem-resistant	***Enterobacteriaceae****, carbapenem-resistant, 3rd generation cephalosporin-resistant
Priority 2: HIGH	***Enterococcus faecium***, vancomycin-resistant	***Staphylococcus aureus***, methicillin-resistant, vancomycin intermediate and resistant	***Helicobacter pylori***, clarithromycin-resistant
	Campylobacter, fluoroquinolone-resistant	***Salmonella*** *spp.*, fluoroquinolone-resistant	***Neisseria gonorrhoeae***, 3rd generation cephalosporin-resistant, fluoroquinolone-resistant
Priority 3: MEDIUM	***Streptococcus pneumoniae***, penicillin-non-susceptible	***Haemophilus influenzae***, amipicillin-resistant	***Shigella*** *spp.*, fluoroquinolone-resistant

*Enterobacteriaceae include: *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter* spp., *Serratia* spp., *Proteus* spp., *Providencia* spp., and *Morganella* spp.

第2項　細菌の構造と薬剤耐性機構

ハンス・グラムによって考案されたグラム染色法により，細菌はグラム陽性菌とグラム陰性菌の２つに大別される. 前項で言及した CRE や CRPA, CRAB は全てグラム陰性菌である. グラム陰性菌の構造を以下に示す (Figure 2). グラム陰性菌は細胞壁の外側に外膜と呼ばれる特長的な膜構造を有している．外膜は細胞膜と同様に脂質二重膜で形成されているが，外側にリポ多糖体の糖鎖を伸ばしている．薬剤の透過性の観点から見ると，脂質二重膜を透過するためには脂溶性分子である必要があるが，外側のリポ多糖体を透過するためには親水性分子である必要がある. つまり, 外膜は脂溶性分子も親水性分子も通さない構造になっている. 一方で，栄養素（親水性物質）を取り込むために，外膜にはポーリンと呼ばれるタンパク構造があり，β-ラクタム等の親水性の抗生物質はポーリンを経由することでグラム陰性菌の菌体内に到達することができると言われている．[4]

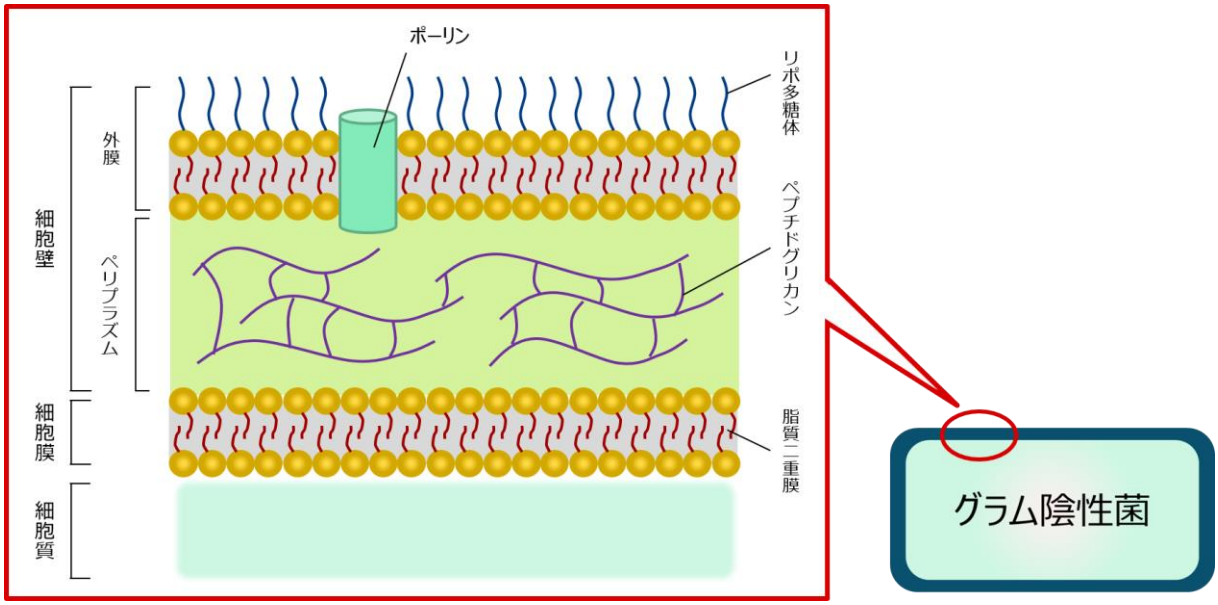

Figure 2. Structure of Gram-negative bacteria.

細菌が細胞レベルで獲得する耐性化のメカニズムは，1）抗生物質を不活性化する酵素の産生，2）標的分子の変異や代替ルートの獲得，3）薬物の膜透過性の変化の３つに大別できる (Figure 3). [5] このうち，1) 抗生物質を不活性化する酵素にはβ-ラクタムを分解するβ-ラクタマーゼやアミノグリコシド系抗生物質を不活性化するアミノグリコシド修飾酵素がある．また，2）標的分子の変異や代替ルートの代表的なものとしては，β-ラクタムの標的分子である PBP2 (penicillin-binding protein 2) の PBP2'への変異がある．3）薬物の膜透過性の変化に関しては，薬物を細胞外に排出する薬剤排出ポンプの発現やポーリンの欠損がある．これらのうち，CRE を含むグラム陰性菌における主要な耐性化機構はβ-ラクタマーゼの産生である．

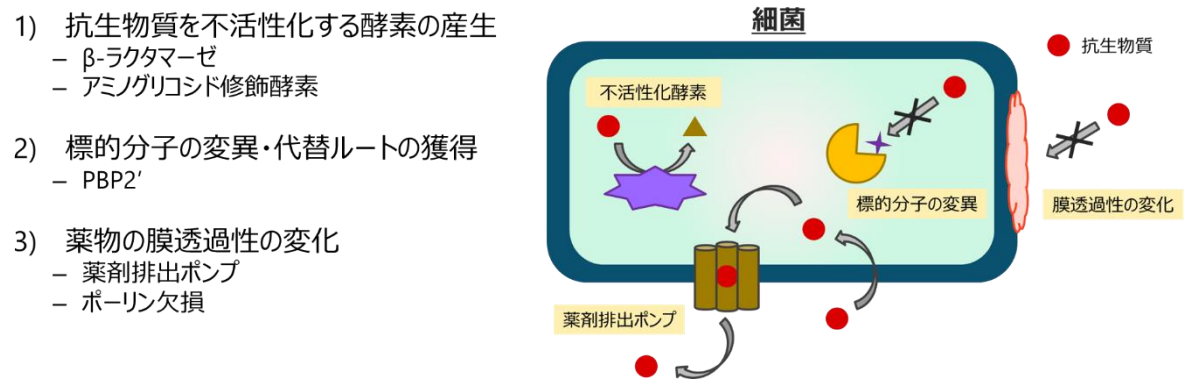

Figure 3. Principal bacterial mechanism of antibiotic resistance.

第3項　β-ラクタマーゼ

β-ラクタマーゼはβ-ラクタム系抗菌薬を加水分解し，不活性化する酵素である．これらは多様な基質特異性をもった一連の酵素群であり，様々な観点から分類されている．[6] 最もよく使われている分類方法は，アミノ酸配列の相同性に基づいた Ambler の分類である．この分類によると，β-ラクタマーゼは４つのクラス（クラス A, B, C, D）に分類できる．クラス A, C, D に属するβ-ラクタマーゼは活性中心にセリン残基をもったセリン-β-ラクタマーゼであり，クラス B は活性中心に亜鉛イオンをもったメタロ-β-ラクタマーゼである．

セリン-β-ラクタマーゼによるβ-ラクタムの加水分解機構を以下に示す (Figure 4A)．まずはじめに，活性中心のセリンがβ-ラクタムを求核攻撃することで，四面体中間体 (**TI1**) を形成する．続いて，**TI1** からラクタム環の開環が進行し，セリン残基と共有結合した酵素複合体 (enzyme adduct) が形成される．この酵素複合体が水分子の求核攻撃を受けて四面体中間体 (**TI2**) を経由して加水分解されることで，β-ラクタムの加水分解が完了し，β-ラクタマーゼが再生する．

続いて，メタロ-β-ラクタマーゼの反応機構を説明する (Figure 4B)．まずはじめに Zn^{2+}に結合した水酸化物イオン (OH^-) がβ-ラクタムのカルボニル炭素を求核攻撃し，Zn^{2+}に配位することによって安定化された四面体中間体 (**TI3**) を形成する．続いて，**TI3** からβ-ラクタムの開環が進行し，アニオン中間体 (anionic intermediate) を経由した後，アニオンがプロトン化を受けることによって，β-ラクタムの加水分解が完了し，触媒が再生する．

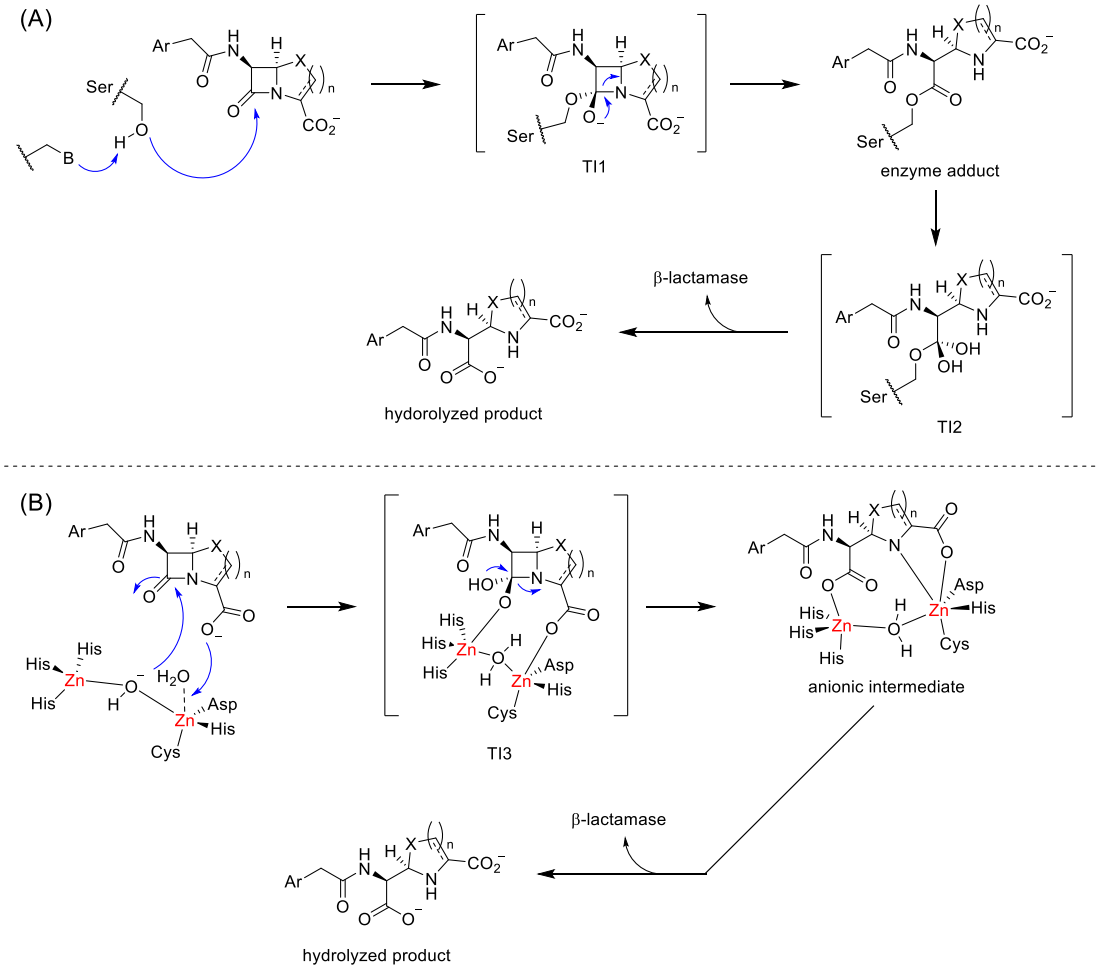

Figure 4. Hydrolysis mechanism of β-lactams by serine-β-lactamase (A) and metallo-β-lactamase (B).

各クラスのβ-ラクタマーゼとその基質の範囲，および，代表的な酵素について以下に示す(Table 2)．クラス A に属するβ-ラクタマーゼは基質の範囲の観点から，いくつかのグループに分けられる．グラム陰性菌が産生する酵素として代表的なものは，腸内細菌科細菌（*Enterobacteriaceae*）においては TEM や SHV であり，大腸菌（*E. coli*）や肺炎桿菌（*K. pneumoniae*）においては CTX-M である．これらのβ-ラクタマーゼの多くは基質特異性拡張型β-ラクタマーゼ（ESBL, extended-spectrum β-lactamase）と呼ばれ，ペニシリン系の他にセファロスポリンやモノバクタムも加水分解できる．[7] また，近年，KPC に代表されるようなカルバペネムも分解できるセリンカルバペネマーゼも報告されている．[8] クラス B はメタロ-β-ラクタマーゼであり，モノバクタムを除く全てのクラスのβ-ラクタムを分解する．IMP や VIM，NDM が知られている．[9] クラス C のβ-ラクタマーゼはセリンセファロスポリナーゼとも呼ばれ，セフタジジム，セフォタキシム，セフトリアキソン等の第三世代セファロスポリンやセファマイシンを含むほぼ全てのセフェムを分解する．クラス D に属するβ-ラクタマーゼは，ペニシリン，セファロスポリン，モノバクタム系β-ラクタムを分解する．また，OXA-23, 24/40, 48 といったいくつかの酵素はカルバペネマーゼ活性を有しており臨床上問題視されている．[10]

Table 2. Substrate Scope and Relevant Examples of β-Lactamases

class	type	substrates				most relevant examples
		penicillins	cephalosporins	carbapenems	monobactams	
A	Serine-	○	×	×	×	penicillinases from Gram-positive bacteria
		○	△	×	×	TEM-1, TEM-2, SHV-1
	(ESBL)	○	○	×	△	SHV-2, TEM-10, CTX-M, GES-1
	(serin carbapenemase)	○	○	○	△	KPC, SME, NMC-A, GES-2
B	Metallo-	○	○	○	×	IMP, VIM, NDM
C	Serine-	○	○	×	○	AmpC, CMY, ACT-1, DHA
D	Serine-	○	○	△	○	OXA-1/30, OXA-10, OXA-23, OXA-24/40, OXA-48

β-ラクタマーゼの名称について

KPC や OXA といったβ-ラクタマーゼの名称は固有名詞であり，略語として扱わないのが一般的である． 肺炎桿菌（*K. pneumoniae*）から発見されたカルバペネムを分解するβ-ラクタマーゼであることから *K. pneumoniae* carbapenemase の頭文字をとって KPC と命名されているように、略語のように見える名称も存在するが，本論文では前述の一般則に従ってβ-ラクタマーゼの名称は略語ではなく固有名詞として扱う．

第２節　β-ラクタマーゼ阻害剤

β-ラクタマーゼ産生菌に対しては，β-ラクタマーゼ阻害剤 (BLI) をβ-ラクタム系抗菌薬と併用するというアプローチが有効である．1982 年，米国にてクラブラン酸がアモキシシリンとの併用で臨床使用されて以降，いくつかのβ-ラクタム系 BLI は臨床現場で今もなお使用されている．一方で，これらの古典的な BLI はカルバペネム耐性腸内細菌科細菌 (CRE) が産生するようなβ-ラクタマーゼを阻害することは困難である．このような中，CRE にも有効な新たな BLI の開発が行われ，1,6-ジアザビシクロ[3.2.1]オクタン (DBO) 骨格をもったアビバクタムが 2015 年に FDA から承認された．その後，アビバクタムと同様に DBO 系 BLI のレレバクタムやデュルロバクタム，ボロン酸系 BLI であるバボルバクタムといった新規 BLI が次々と登場しており，これら２骨格を中心に新規 BLI の研究が盛んに行われている．[5] 古典的な BLI であるβ-ラクタム系，及び，DBO 系，ボロン酸系の BLI について以下で詳細を述べる．

第1項　β-ラクタム系β-ラクタマーゼ阻害剤

β-ラクタム系 BLI は古くから臨床使用されている BLI であり，クラブラン酸，スルバクタム，タゾバクタムがある (Figure 5)．このうち，クラブラン酸は経口薬として経口β-ラクタムであるアモキシシリンとの併用薬で使われている．また，スルバクタムはアンピシリンと互いにエステル結合で結びついた “mutual prodrug” というユニークなプロドラッグで経口投与に用いられている (スルタミシリン).

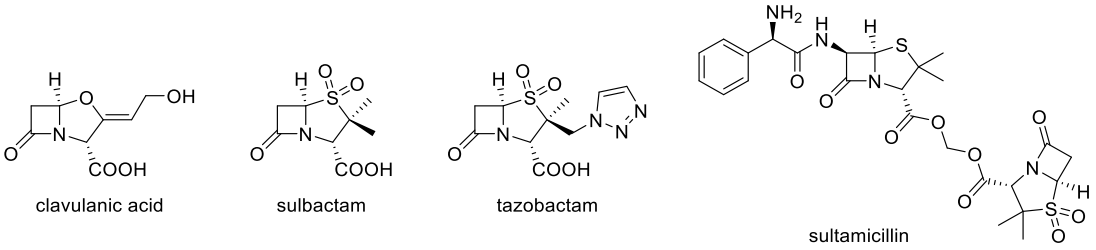

Figure 5. A series of β-lactam type β-lactamase inhibitors.

これらの BLI は，基質拡張型β-ラクタマーゼ (ESBL) を含むクラス A のβ-ラクタマーゼを阻害可能であるが，KPC をはじめとしたセリンカルバペネマーゼやクラス C, D のセリンβ-ラクタマーゼはほとんど阻害できない．また、活性中心のセリン残基と共有結合を形成するという阻害様式から，クラス B のメタロ-β-ラクタマーゼも阻害できない.

β-ラクタム系 BLI の阻害メカニズムとして，クラブラン酸の SHV-1 阻害メカニズムの例を以下に示す (Figure 6)．[11], [12] SHV-1 の Ser^{70}-OH がクラブラン酸のカルボニルを求核攻撃し，四面体中間体を経由して，酵素複合体 **EA-1** を形成する．続いて，オキサゾリジンの開環により **EA-2** となった後，イミンーエナミンの異性化やエナミンの *cis-trans* の異性化と続く脱炭酸によって，**EA-5**, **6** が形成する．**EA-6** はα,β-不飽和エステルであり，Ser^{130} が 1,4-付加することで架橋複合体 **EA-7** を形成する．この架橋複合体 **EA-7** は安定性が高く，酵素阻害に大きく寄与していると考えられる．**EA-7** は Ser^{130} の加水分解を経て，アルデヒド **EA-10** を形成する．このアルデヒドは水和体 **EA-9** との平衡状態にあることで，Ser^{70} の加水分解に使われる水分子をトラップし，**EA-10** の加水分解によって酵素が再生するのを妨げている．こ

れらの酵素複合体は，いくつかの工程を経て最終的には加水分解され酵素が再生するが，上記のようないくつかの安定なものを含め，複数の酵素複合体を形成することで，結果としてβ-ラクタマーゼを阻害することができている．

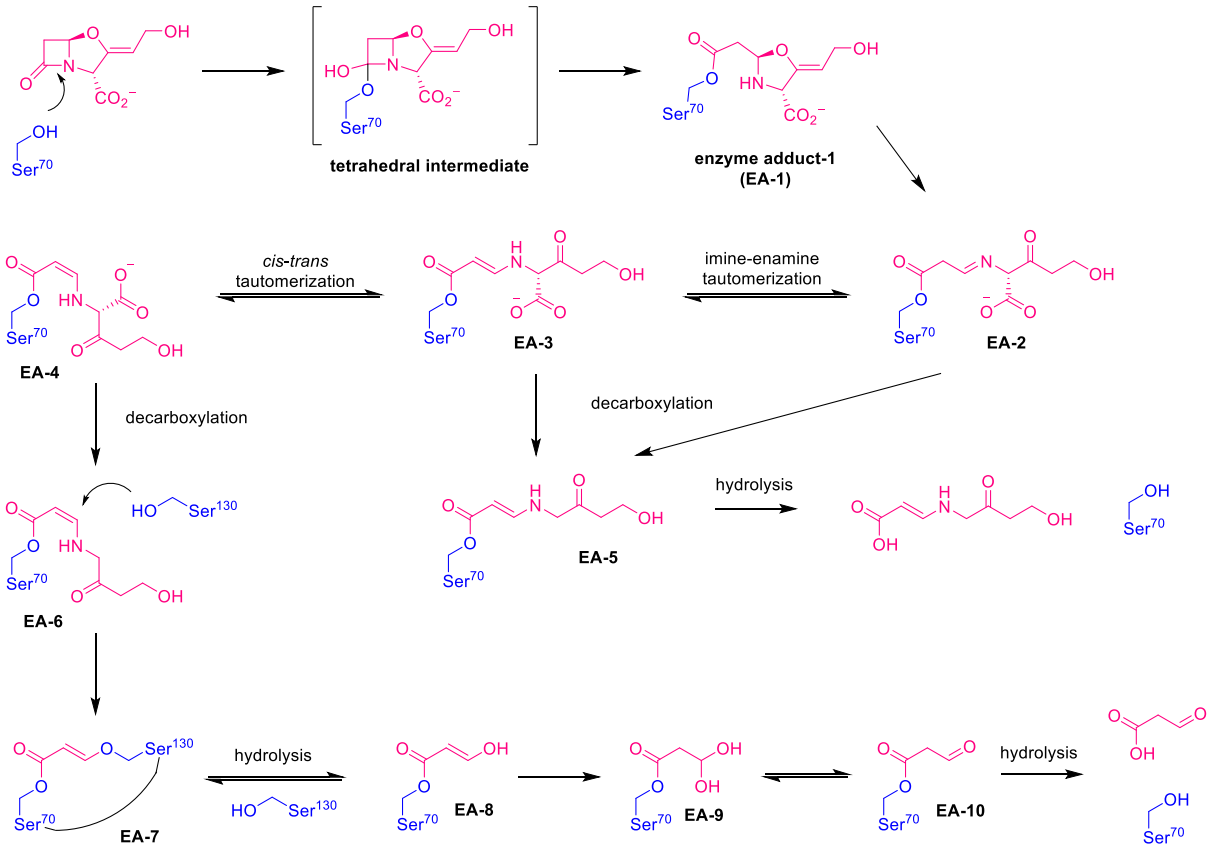

Figure 6. Pathway of clavulanic acid-mediated serine β-lactamase inhibition.

第2項　1,6-ジアザビシクロ[3.2.1]オクタン系β-ラクタマーゼ阻害剤

ジアザビシクロオクタン (DBO) は，非β-ラクタム系新規抗菌薬としてデザイン・合成された化合物である．[13] 当初の期待に反して DBO 化合物の抗菌活性は弱かったが，KPC 等のセリンカルバペネマーゼを含むセリンβ-ラクタマーゼに対して強力な阻害活性を示したことから，[14] β-ラクタマーゼ阻害剤 (BLI) としての可能性が見いだされ，研究～上市まで数多くの誘導体が知られている (Figure 7).

アビバクタム (AVI) は最初に上市された DBO 系 BLI であり，セフタジジムと組み合わせた静注薬として使用されている．[15] AVI はクラス A，C のβ-ラクタマーゼに対する阻害能が高い。一方で，クラス D に対しては OXA-48 等の一部のβ-ラクタマーゼを除いては阻害活性が弱い．[16], [17] DBO 系 BLI はクラス B のメタロ-β-ラクタマーゼ (MBL) を阻害できないため，クラス B に対して安定なモノバクタム系抗菌薬のアズトレオナムとの併用効果も研究されており，[18] この組み合わせの臨床試験も実施されている．レレバクタム (REL) は，イミペネム-シラスタチンとの併用で承認されている．AVI と同様に REL もクラス A，C のβ-ラクタマーゼに対する阻害能が高いが，クラス D に対しては阻害活性を持たない．[19] OprD ポーリン欠損や AmpC 高産生によるイミペネム耐性緑膿菌に対する有効性が報告されている点が特徴である．[20] デュルロバクタム (DUR) は母核に二重結合をもったユニークな DBO 誘

導体である．[21] 多剤耐性緑膿菌/アシネトバクターに対応するために，クラス A, C に対する阻害活性を維持しつつ，クラス D に対する活性を強化する目的でデザインされている．二重結合の導入は，分子に歪みを与えることで β-ラクタマーゼの活性中心のセリンとの反応性を高めることが目的であり (DBO 系 BLI の阻害メカニズムは Figure 9 参照)，それによってクラス D に対する阻害活性の強化に成功している．また，DUR は PBP2 (penicillin-binding protein 2) を選択的に阻害することで，BLI であると同時に抗菌活性も示す．この PBP2 阻害作用に着目して，同じく BLI でありながらアシネトバクターに抗菌作用を有するスルバクタム[22]との併用により，カルバペネム耐性アシネトバクターに特化して使用されている．ナクバクタム (NAC) はメロペネムとの併用で臨床開発中の DBO 誘導体である．NAC の β-ラクタマーゼ阻害における活性やスペクトルは AVI と比較してやや劣る～同程度であるが，デュルロバクタムと同様に PBP2 選択的な阻害作用により抗菌活性を示すことで β-ラクタムとの併用効果は高い．[23] また， MBL に対して安定な傾向にあることで，MBL を阻害することはできないにもかかわらず，各種 β-ラクタムとの組み合わせで MBL 産生菌に対して併用効果を示す．[24] ジデバクタム (ZID) も同様に抗菌活性を併せ持った BLI であり，セフェピムとの併用で現在臨床試験中である．[19], [25] WCK 4234 は他の DBO 系 BLI と異なり，2 位にアミドではなくシアノ基を持っていることが特徴である．シアノ基の強い電子求引性の影響のためか，各種ラクタマーゼとの反応性 (acylation rate, k_{acyl}) が向上しており，AVI 等が苦手とするクラス D に対する阻害活性が向上している．[19], [26]

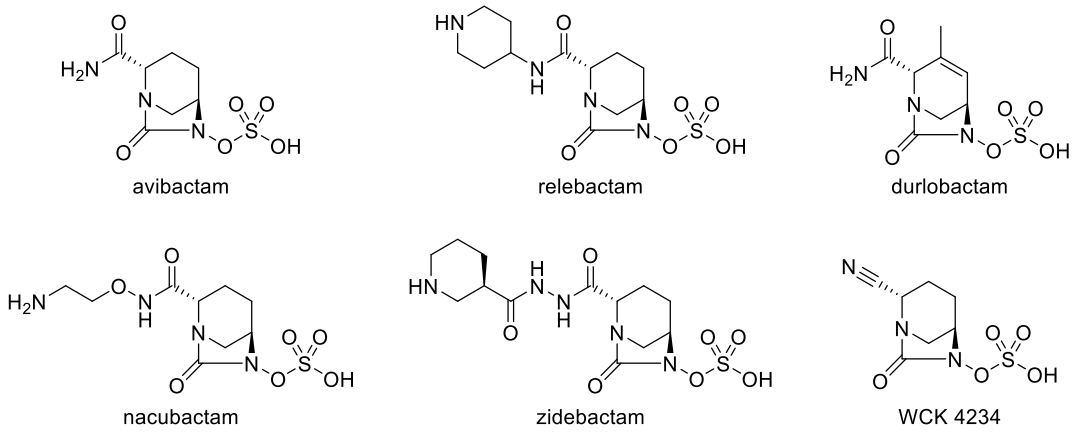

Figure 7. A series of DBO type β-lactamase inhibitors.

前述した DBO 系 BLI は全て静脈注射によって投与されているが，最近，プロドラッグ化によって経口吸収性を付与した DBO 誘導体が報告されている (Figure 8A)． ARX-1796 は AVI の経口プロドラッグであり，末端のエステルがエステラーゼによって加水分解された後，β-ラクトン環の形成によってスルホン酸が遊離するというユニークなメカニズムが報告されている (Figure 8B)．[27] ETX0282 は ETX1317 の 6 位フルオロ酢酸構造をエステル化した経口プロドラッグである．[28], [29] DUR と同様に母核に二重結合を導入によって分子の歪みを増大させることで，β-ラクタマーゼの活性中心のセリンとの反応性を高めている (阻害メカニズムは Figure 9 参照)．また，他の DBO 系 BLI と異なり 6 位がフルオロ酢酸であるという特徴を有している．

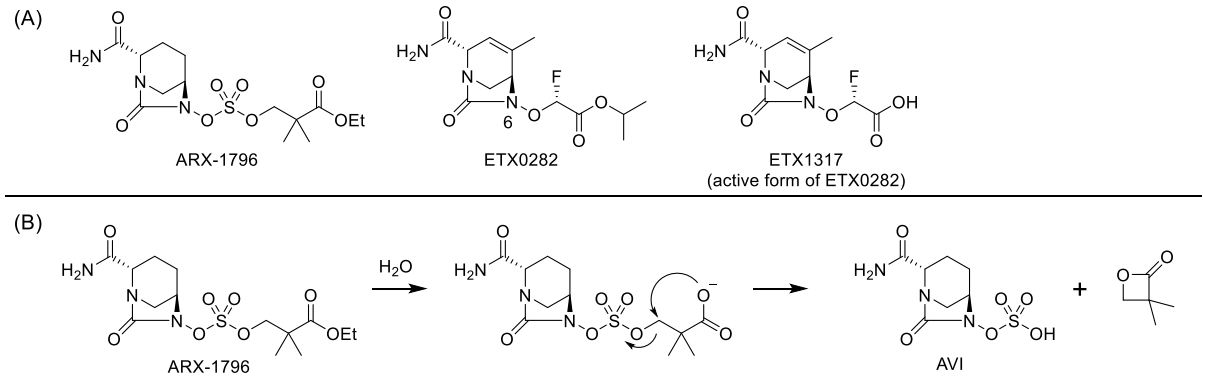

Figure 8. (A) A series of orally available DBO type β-lactamase inhibitor and its active form. (B) Mechanism of AVI release from ARX-1796.

DBO 系 BLI はセリン β-ラクタマーゼの活性中心のセリンと安定なカルバモイル - 酵素複合体 (carbamoyl-enzyme complex) を形成することによって，β-ラクタマーゼを阻害する．(Figure 9). [30] なお，DBO と β-ラクタマーゼとの反応はアシル化ではなくカルバモイル化であるが，アシル化 (acylation) と表現されることが一般的であるため，Figure 9 においても acylation と表記している．β-ラクタム系 BLI と異なり，DBO 系 BLI の場合は，この酵素複合体から DBO の再環化により阻害剤と酵素が共に再生する逆反応 (deacylation) が存在し，β-ラクタマーゼを可逆的に阻害する．この逆反応以外の β-ラクタマーゼの再生プロセスとしては，酵素複合体が直接加水分解されるのではなく(direct hydrolysis)，脱硫酸化 (desulfation) が進行した後に，カルバモイル基の加水分解によってラクタマーゼが再生することが報告されている．[31] また，DBO の再環化による逆反応の進行速度は酵素によって異なり，クラス A および C においては非常に早く（分単位），クラス D においては遅い（数日単位）．[32]

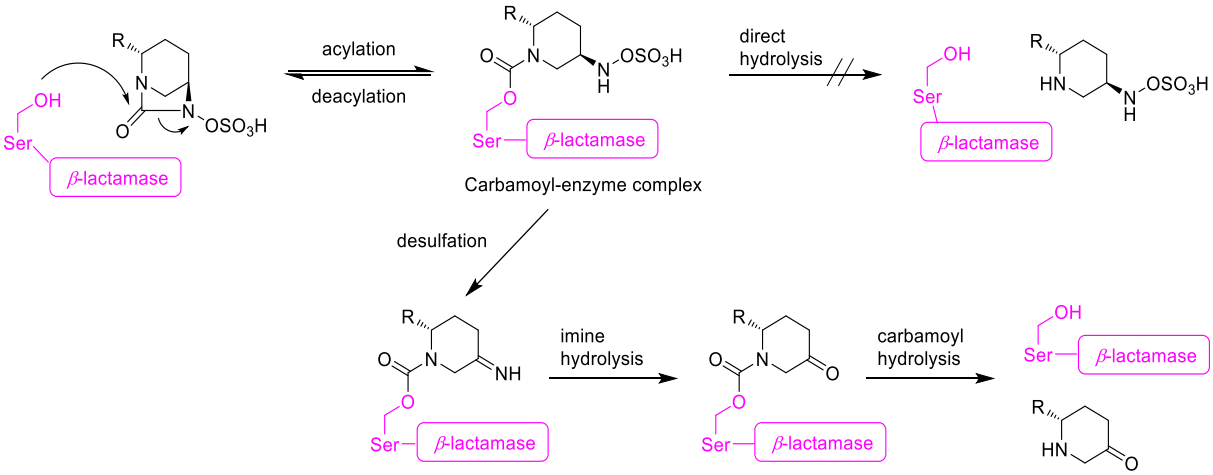

Figure 9. Pathway of DBO-mediated serine β-lactamase inhibition.

第3項　ボロン酸系 β-ラクタマーゼ阻害剤

ボロン酸誘導体は，β-ラクタム系抗菌薬の β-ラクタマーゼによる加水分解の中間体である四面体中間体を模倣することで，活性中心に対して親和性を示し，β-ラクタマーゼを阻害する (Figure 10A). [33] 現在知られているボロン酸系 BLI を以下に示す (Figure 10B). バボルバクタム (VAB) は 2017 年に FDA によって承認された最初のボロン酸系 BLI であり，メロペネムとの併用で使用されている. [34] VAB は KPC を含むクラス A およびクラス C のラクタマーゼに対して阻害活性を持っているが，クラス B, D に対する阻害能はない. より広域な阻害スペクトルを持ったボロン酸系 BLI の探索が行われる中で見出されたのがタニボルバクタムである. [35] タニボルバクタムはクラス A, C に加えて，クラス B, D に対しても阻害活性を持っており, セフェピムとの併用でカルバペネム耐性腸内細菌科細菌 (CRE) だけでなくカルバペネム耐性緑膿菌 (CRPA) に対しても有効であることが確認されている. VNRX-7145 は VNRX-5236 のカルボン酸構造をエステル化した経口プロドラッグであり，クラス A, C, D の β-ラクタマーゼを阻害することができる. [36] 経口セフェムであるセフチブテンとの併用でアビバクタム/セフタジジムと同等の有効性を示す. QPX7728 は非常に広域な阻害スペクトルを持ったボロン酸系 BLI であり，全クラスの β-ラクタマーゼに対して阻害活性を示す. [37] QPX7728 はタニボルバクタムが有効でないクラス B β-ラクタマーゼの 1 つである IMP も阻害することができる. 各種 β-ラクタムと組み合わせることで，CRE だけでなくカルバペネム耐性アシネトバクター (CRAB) および CRPA に対する有効性も確認されている. また，QPX7728 はプロドラッグ化なしでも経口吸収性を示し，プロドラッグ化によるさらなる経口吸収性の向上も期待できるとされている.

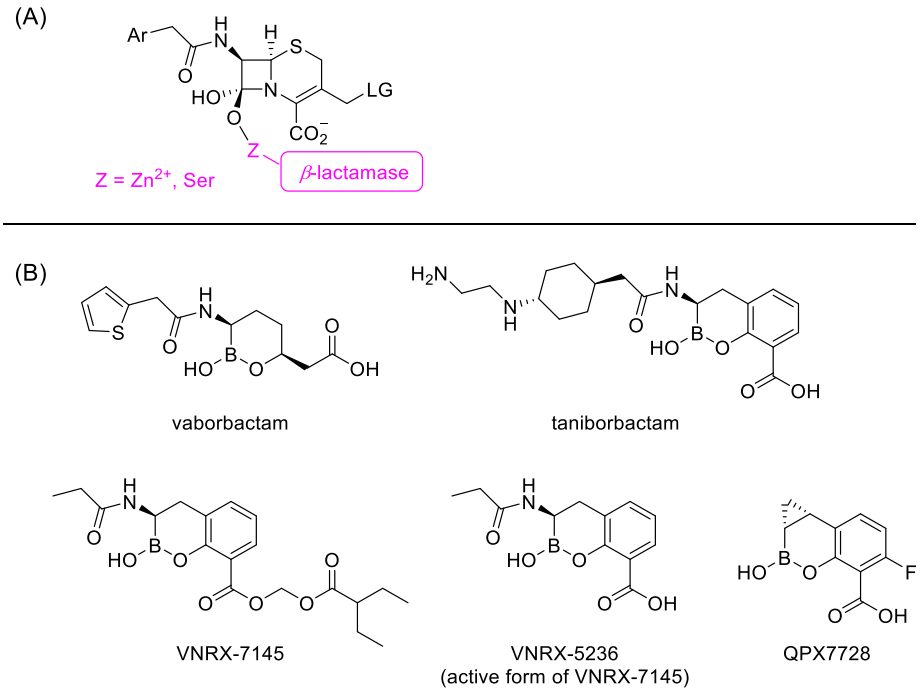

Figure 10. (A) Tetrahedral intermediate of cefem. (B) A series of cyclic boronate type β-lactamase inhibitors.

第３節　本研究について

第1項　本研究の目的

前節で述べたように，現在数多くの新規 BLI が開発されている．しかしながら，患者や医療従事者の負担軽減，医療費の削減の観点から，在宅でも治療可能な経口薬にニーズはあるものの，CRE にも有効な経口 BLI は未だ上市に至っていない．また，DBO 系 BLI に着目すると，DBO 骨格が化学的に不安定で誘導体の合成難易度が高いこともあり，既存の化合物の 2 位置換基はアミド誘導体もしくはシアノ基に限定されていることから (Figure 7)，新たな官能基の探索余地が残されている． さらに, 現在開発段階にある経口 DBO 系 BLI は ARX-1796 と ETX0282 であるが， ARX-1796 の活性体である AVI に対して感受性が低下する β-ラクタマーゼの変異がすでに報告され始めている．[38], [39] また，ETX0282 は環内に二重結合を有するため, 他の DBO 系 BLI と同じ出発物質から合成することができず, 市販されている原料から 12 工程という長い工程を経て合成する必要がある．[28]

このような背景の下，本研究では新規経口 DBO 系 β-ラクタマーゼ阻害剤の創出を目的とし，DBO 骨格 2 位の新たな置換基探索を行うこととした.

第2項　新規経口 DBO 系 BLI のデザイン

新規 DBO 系 BLI をデザインするにあたっては，既存薬よりも優れた薬効が期待できることが重要であると考えた．DBO 系 BLI による β-ラクタマーゼの阻害を速度論の観点から考えると，Michaelis-Menten 機構で考えることができる (Figure 11)．すなわち，DBO 化合物，および，反応前の β-ラクタマーゼ (Apo-enzyme) は，非共有結合性の Michaelis 複合体 (E:I) を経由した後に，共有結合性のカルバモイル - 酵素複合体 (E-I) を形成し，酵素の活性が阻害される．それぞれの反応の速度定数を k_1, k_2, k_{-1}, k_{-2}，アシル化の速度定数を k_{acyl} とすると，アシル化速度 V_{acyl} は eq 1 のようになる．この式を k_{acyl} について解くと eq 2 のようになり，k_{acyl} は Michaelis 複合体の結合乖離定数 K，および，Michaelis 複合体から β-ラクタマーゼの活性中心のセリン残基と DBO のカルボニルが反応し共有結合が形成される反応の速度定数 k_2 で表すことができる．すなわち，アシル化速度を向上させる方法は，① DBO と β-ラクタマーゼの親和性を向上させる（K を小さくする），② DBO のカルボニル基の化学的反応性を向上させる（k_2 を大きくする）の２通りが考えられる．しかしながら，構造の異なるあらゆる β-ラクタマーゼに対して親和性を向上させることは現実的ではないと考えられるため，新規 DBO のデザインにおいては化学的反応性を高めることに主眼を置くべきであると考えた.

$$V_{acyl} = k_{acyl}[E][I] = k_2[E{:}I] \tag{1}$$

$$k_{acyl} = k_2[E{:}I]/[E][I] = k_2/K \qquad \text{ただし、} K = [E][I]/[E{:}I] \tag{2}$$

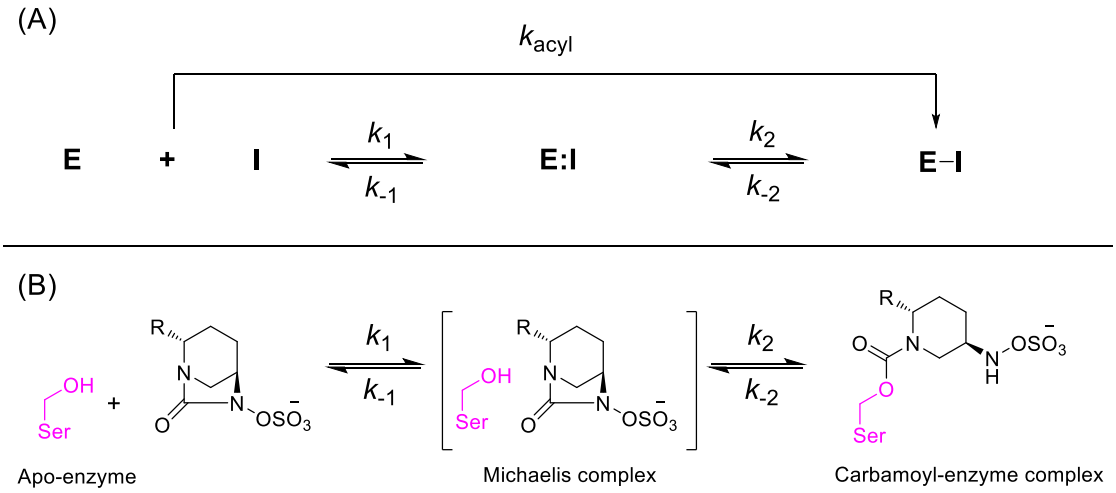

Figure 11. (A) Scheme representing the interaction of β-lactamases (E) with DBOs (I). (B) Chemical representation of (A).

DBO 骨格の化学的反応性を高めることで阻害活性の改善に成功している例として WCK 4234 がある．[19], [26] WCK 4234 は AVI の 2 位置換基をアミドからシアノ基へと変換したものであるが，両官能基の電子求引性の指標である Hammett 則の置換基定数σ_m値はそれぞれ，σ_m = 0.28 (-$CONH_2$), 0.56 (-CN) であり，[40] シアノ基の方が強力な電子求引性基である．この電子求引性の増強によって，WCK 4234 は AVI と比較して 10~100 倍以上のアシル化速度の向上に成功している (Figure 12)．この現象が構造的に大きく異なるクラス A, C, D それぞれのラクタマーゼに対して確認されていることからも，シアノ基への変換が構造的に親和性を高めたのではなく，カルボニル基の化学的反応性を向上させることでアシル化速度の改善につながっていると考えられる．

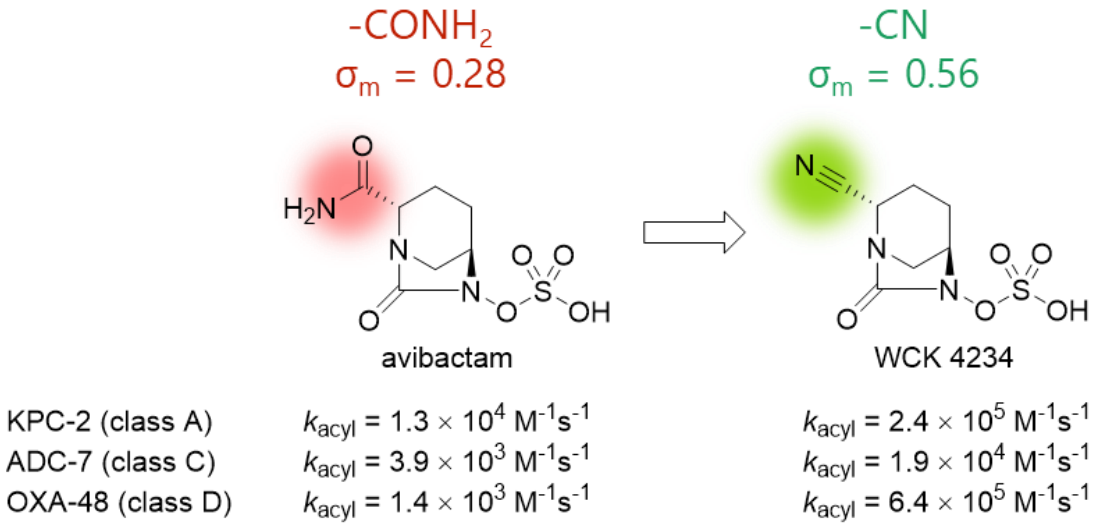

Figure 12. Relationship between inductive effect of functional group at the C2 position and acylation rate.

前述のシアノ基は強力な電子求引性基として，DBO 化合物の β-ラクタマーゼ阻害活性を向上させるのに有用であった．しかしながら，アミドと異なり，シアノ基は側鎖を持たないため，側鎖の変換によって化合物の動態特性や物性を調整することができないという欠点がある．そこで，シアノ基と同様に強力な電子求引性を有するスルホン、スルホキシド，スルホンアミドといった硫黄系官能基に着目した (Figure 13)．これらの官能基のσ_m 値はシアノ基と同程度であるため，強力な β-ラクタマーゼ阻害活性が期待でき，多様な側鎖を持った化合物の合成も可能である．一方で，DBO 骨格の 2 位にこのような硫黄官能基を導入する方法は知られておらず，合成法の探索から研究を開始する必要がある．

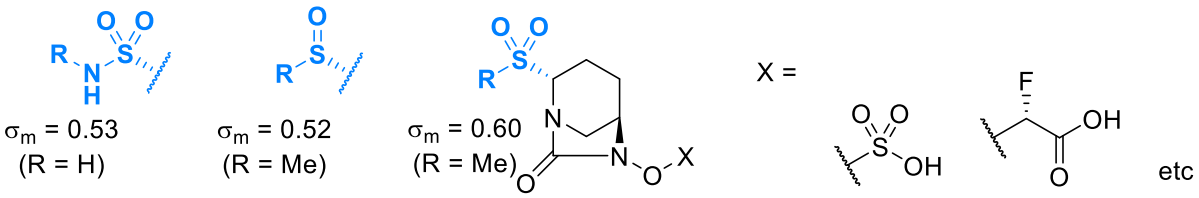

Figure 13. Design of 2-thio-substituted novel DBO compounds.

第４節　本論文について

本論文は緒論，本論３章および結論から成っている．

第１章は「2 位に硫黄官能基を有する 1,6-ジアザビシクロ[3.2.1]オクタン誘導体の合成」と題し，脱炭酸的ラジカル反応による 2 位へのスルフィドの導入を起点に，スルホン，スルホキシド，スルホンアミドを有する DBO 系 BLI の合成研究について述べる．

第２章は「2 位に硫黄官能基を有する 1,6-ジアザビシクロ[3.2.1]オクタン誘導体の活性・動態評価」と題し，第１章で合成した DBO 誘導体の β-ラクタマーゼ阻害活性やセフィキシムと併用した場合の効果，薬物動態について述べる．また，この研究の過程で見出した本 DBO 誘導体のユニークな抗菌活性についても併せて述べる．

第３章は「2 位にスルホキシドを有する 1,6-ジアザビシクロ[3.2.1]オクタン誘導体の構造最適化」と題して，第２章で見出した活性と動態を両立できる 2 位スルホキシド，6 位フルオロ酢酸の組み合わせに対して，2 位側鎖の構造最適化を実施した結果を述べる．また，最適化した誘導体の経口プロドラッグ化検討，および，セフチブテンとの併用経口投与による *in vivo* 薬効試験を実施した結果についても併せて述べる．

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本論

第 1 章　2 位に硫黄官能基を有する 1,6-ジアザビシクロ[3.2.1]オクタン誘導体の合成

第 1 節　2 位に硫黄官能基を有する DBO 誘導体の合成戦略

目的とする 2 位に硫黄官能基を有する DBO 誘導体は，2 位にスルフィドをもった中間体 A から酸化反応等の官能基変換を経ることで合成できると考えた (Figure 1-1)．また，スルフィド中間体 A は，市販の DBO 骨格を持ったカルボン酸 **1** から脱炭酸的ラジカル反応によって合成できると考えた．なお，カルボン酸 **1** は入手性の良いピログルタミン酸から合成することも可能である．[1]

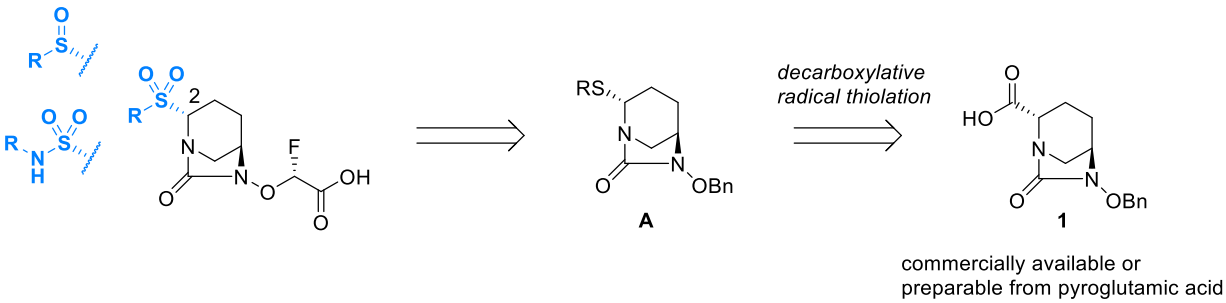


Figure 1-1. Synthetic strategy for 2-thio-substituted DBO derivatives.

DBO 化合物は立体的なひずみによって 1 位の窒素原子の孤立電子対とカルボニル炭素が共鳴していない (Figure 1-2)．そのため，ウレア構造であるにもかかわらず，カルボニルの求電子性は高い．また，孤立電子対がカルボニルと共鳴していないために，1 位の窒素原子は塩基性を有しているが，これがプロトン化を受けることによってカルボニルの求電子性はさらに向上する. そのため, DBO 化合物は酸や塩基, 熱によって容易に開環するという性質があり, DBO 環に対して-SR 基を導入する場合には温和な反応条件を設定する必要がある.

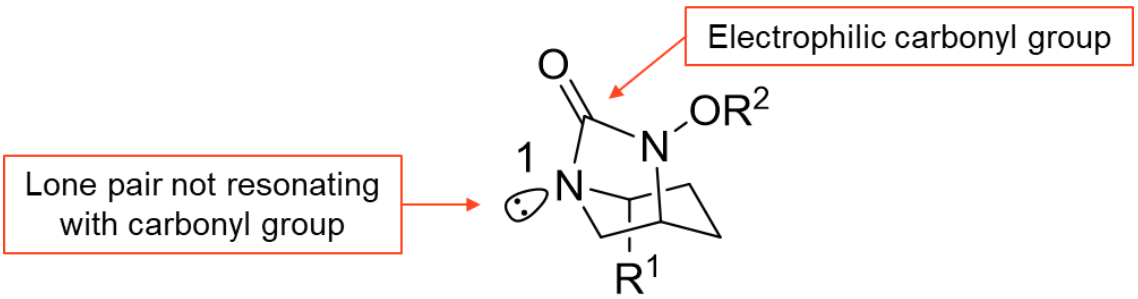
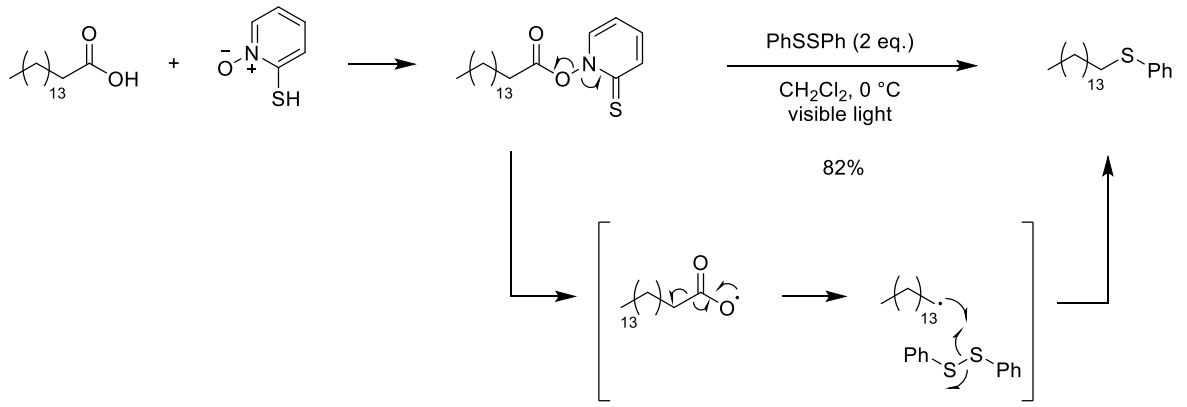


Figure 1-2. Structural feature of DBO compound.

カルボン酸をスルフィドに変換する反応例としては，チオヒドロキサム酸エステルの光照射による脱炭酸的ラジカル反応が古くから知られている (Scheme 1-1). [2] すなわち，カルボン酸と 2-メルカプトピリジン-*N*-オキシドの縮合によって得られる 2-チオキソピリジニルエステル(Barton エステル) は，光照射によって N-O 結合が開裂し，カルボキシラジカルを生じる．このカルボキシラジカルは脱炭酸を伴って 1 炭素減炭したアルキルラジカルを生成し，系中のジスルフィドと反応することでスルフィドが得られる．この反応は光照射下，0 ˚C かつ中性という温和な条件で進行するため, 酸や塩基に対して不安定な DBO 骨格をもった化合物の合成に適している．

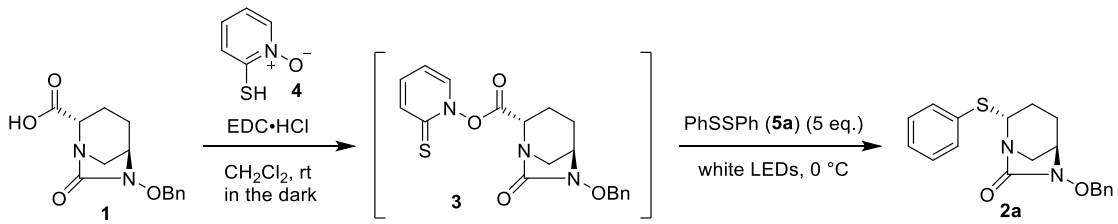
Scheme 1-1. Photo-Induced Decarboxylative Thiolation



第２節　脱炭酸的ラジカル反応による硫黄官能基の導入

前述の脱炭酸的ラジカル反応を用いて DBO 骨格 2 位への硫黄置換基の導入を試みた (Scheme 1-2)．カルボン酸 **1** を遮光条件下，EDC·HCl を用いて 2-メルカプトピリジン-*N*-オキシド **4** と縮合し，ラジカル前駆体の Barton エステル **3** を調製した．この Barton エステル **3** を単離することなく系中に 5 当量のジフェニルジスルフィド **5a** を加え，氷冷下，LED ランプを用いて白色光を照射したところ，目的の **2a** を 1,1,2,2-テトラクロロエタンを内部標準として用いた NMR 収率で 61%，単離収率 46%で得ることが出来た．NMR 収率と比較して単離収率が低い原因は，スルフィド **2a** が酸に対して不安定であるためと考えられる．後述のスルフィド **2b** を TLC で 2 次元展開したところ，シリカゲル上での分解が確認されている (Figure 1-5)．この反応において，**2a** は単一のジアステレオマーとして得られた．その相対配置については，8 位の axial 位のプロトン H_a と-SPh 基のオルト位プロトン H_d との間に NOE 相関が観測されたことから決定した (Figure 1-3a)．立体選択的に目的物が得られたのは，ラジカル中間体 **7** が convex 面からジスルフィドと反応したためであると考えている (Figure 1-3b)．

Scheme 1-2. Introduction of -SPh Group via Photo-Reactive Ester 3



NMR yield[a] 64%
Isolated yield 46%

[a]NMR yield was determined by ^{1}H NMR using 1,1,2,2-tetrachloroethane as an internal standard.

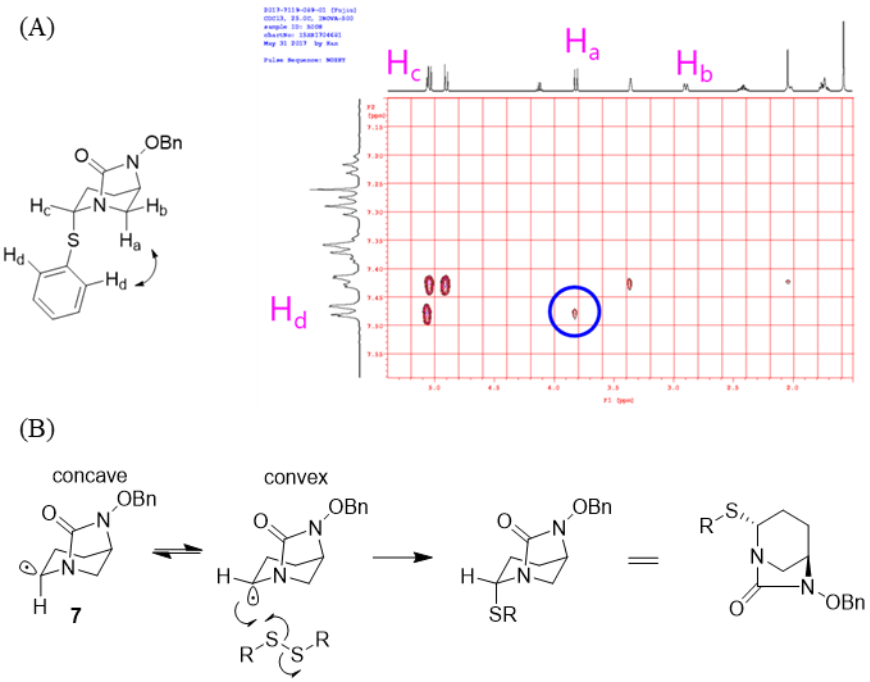
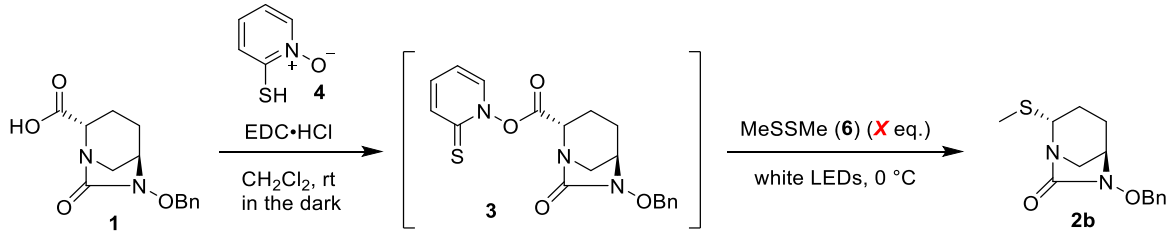


Figure 1-3. (A) NOESY spectrum of **2a**. (B) Proposed mechanism of diastereoselective thiolation.

比較的良好な収率でジアステレオ選択的にスルフィド **2a** を合成することができたため，続いて，ジメチルジスルフィド **6** を用いて同様の条件で-SMe 基の導入を試みた (Scheme 1-3). その結果, ジメチルジスルフィドを 5 当量用いた場合では収率は 30% (NMR yield) に留まり，ジフェニルジスルフィド **5a** を用いた場合 (Scheme 1-2) と比較して収率が低下した．ジメチルジスルフィド **6** を 45 当量用いることで 52% (NMR yield) まで収率が改善することは確認できたものの，今後，構造活性相関 (SAR, structure-activity relationship) 研究のために，多様なアルキル側鎖をもった化合物を合成することを考えると，より少ない試薬量で収率を改善する必要がある．アルキル系のジスルフィドを用いる場合に過剰量の試薬が必要であることは過去の報告とも一致し，未だに解決されていない課題である (Scheme 1-4). [2],[3]

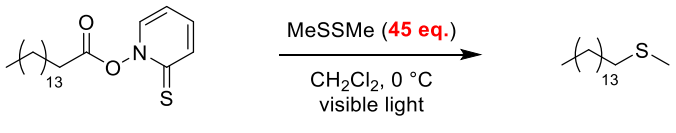
Scheme 1-3. Introduction of -SMe Group via Photo-Reactive Ester 3

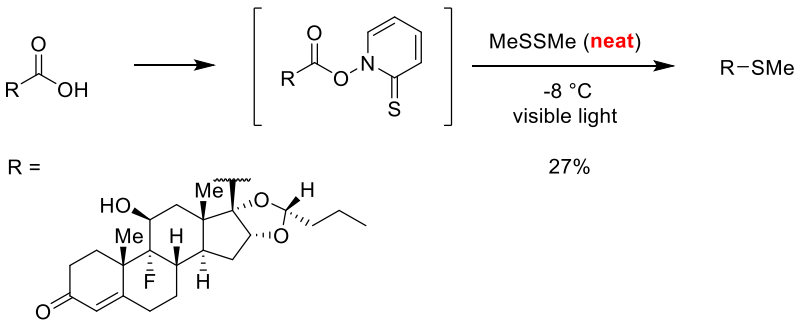


X = 5 30%[a]
45 52%[a]

[a]NMR yield was determined by ^{1}H NMR using 1,1,2,2-tetrachloroethane as an internal standard.

Scheme 1-4. Reported Examples of Photo-Induced Decarboxylative Thiolation using MeSSMe

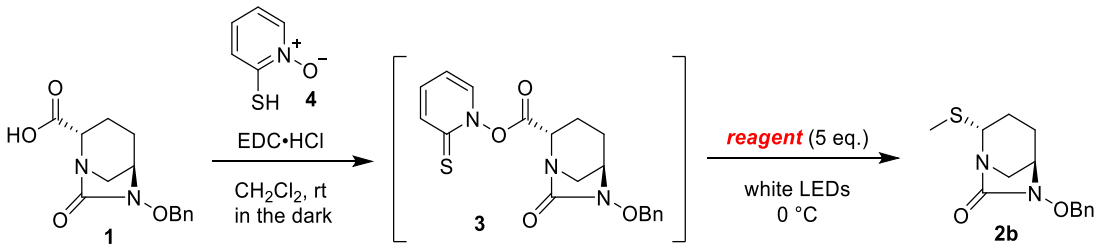




-SMe 基の導入効率を改善するために-SMe 化試薬の検討を行った (Table 1-1). まず, $MeSO_2SMe$ (**8**)を 5 当量用いたところ，ジメチルジスルフィド **6** を 45 当量用いた場合と同等の 47%収率で，スルフィド **2b** を得ることができた．また，非対称ジスルフィド PhSSMe (**5b**) を用いた場合にも同様に，NMR 収率 48%，単離収率 33%で目的の **2b** を得ることができた．単離収率と NMR 収率に乖離が見られるのは，**2b** が弱酸性のシリカゲルに対して不安定であるからだと考えている．スルフィド **2b** を EtOAc/hexane 系で 2 次元展開した TLC を Figure 1-4 に示すが，1 度 y 軸方向に展開された **2b** が TLC 上で分解した分解物が，2 度目の x 軸方向への展開時に観測されている. 非対称ジスルフィド **5b** を用いた場合, -SMe 基だけでなく-SPh 基も導入される可能性があるが，crude の ^{1}H NMR の積分比から判断すると，-SPh 基が導入された化合物の生成は 8%であった.

筆者の知る限り，-SMe 源となる試薬を変更することで収率を改善した例はなく，本知見は DBO 誘導体の合成研究だけでなく，様々な硫黄含有化合物の合成に対して応用できるものであると考えられる.

Table 1-1. Optimization of Reaction Condition for Introduction of -SMe Group



entry	*reagent*	yield (%)[a]
1	$MeSO_2SMe$ (**8**)	47
2	PhSSMe (**5b**)	48 (33)[b]

[a]Yield was determined by ^{1}H NMR using 1,1,2,2-tetrachloroethane as an internal standard.
[b]Isolated yield.

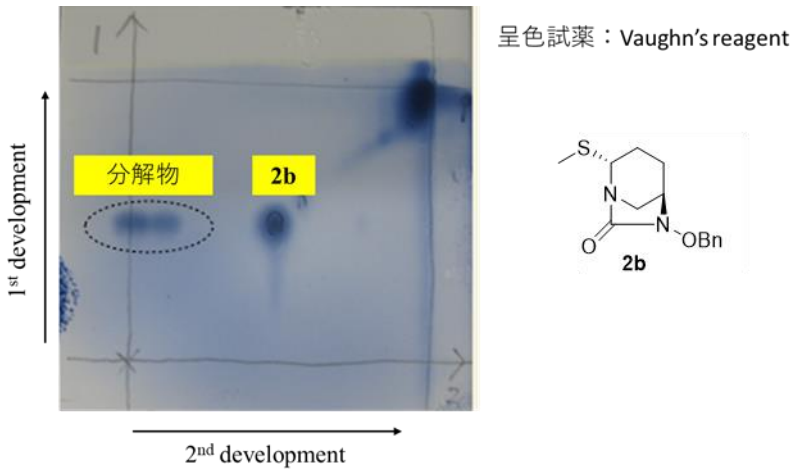
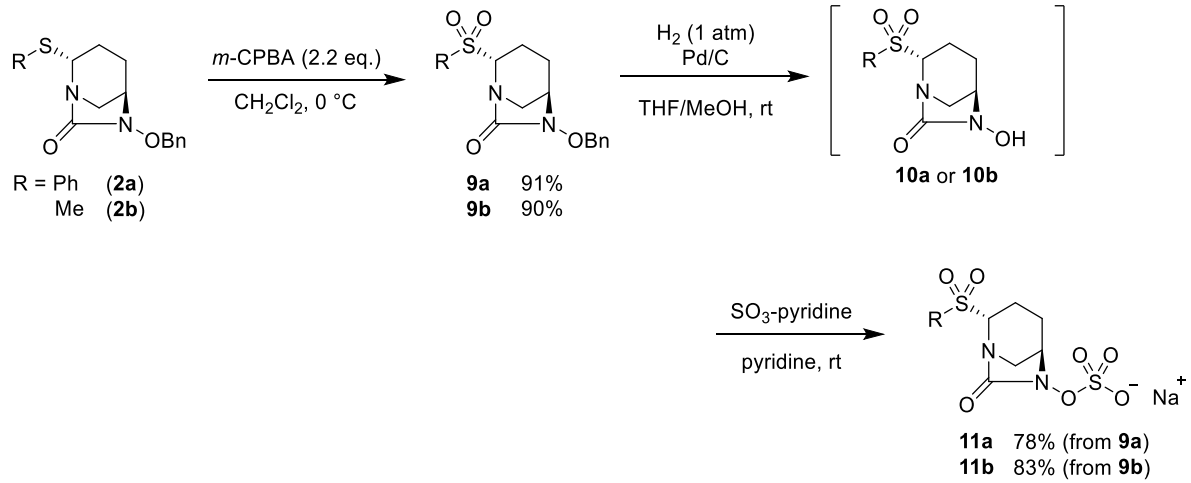


Figure 1-4. 2D developed TLC of **2b**.

第３節　スルホン型 BLI の合成

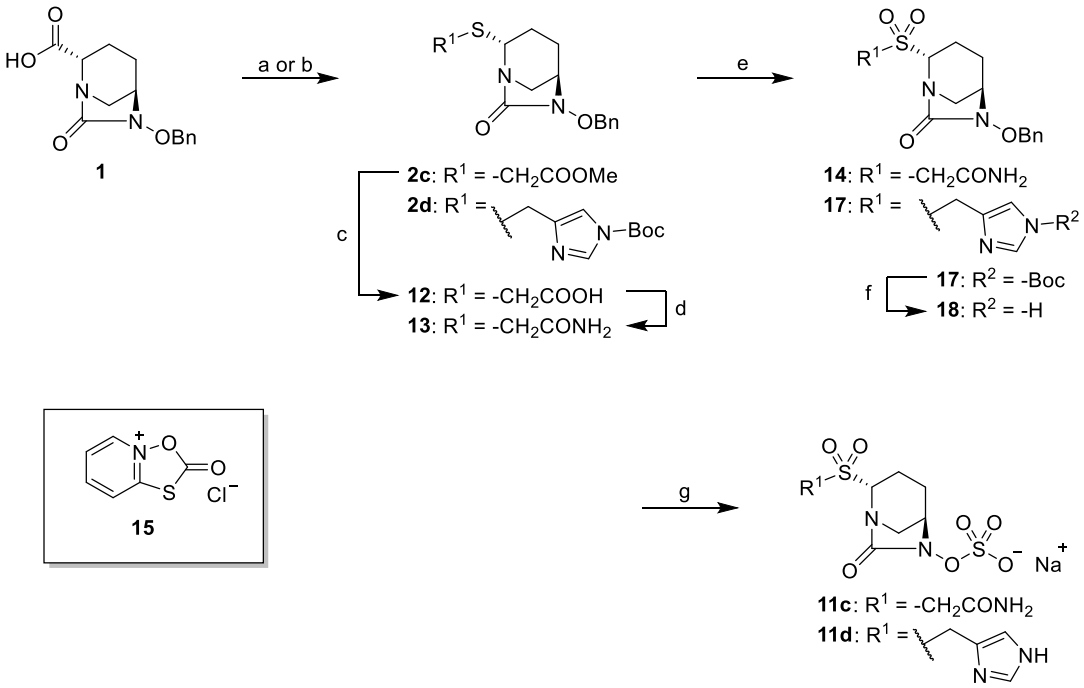
フェニルスルフィド **2a**, および, メチルスルフィド **2b** が合成できたため, それぞれの化合物をスルホンへと酸化し, 目的のβ-ラクタマーゼ阻害剤 (BLI) に誘導できるかを検討した (Scheme 1-5). スルフィド **2a, 2b** をそれぞれ 2.2 当量の *m*-CPBA で酸化したところ, 収率良くスルホン **9a, 9b** を得ることが出来た. 続いてアビバクタムの合成法[1]を参考に, 6 位ベンジルオキシアミンの Bn 基を Pd/C を用いた水素添加反応で脱保護し, SO_3-pyridine を用いて硫酸化することで, 目的の **11a**, **11b** を良好な収率で得た.

Scheme 1-5. Synthesis of Sulfone-Type BLI 11a and 11b



目的としていたスルホン型の DBO 化合物 **11a**, **11b** が合成できたため, 異なる側鎖を持った誘導体の合成も行った. アミドをもった **11c** およびイミダゾリル基をもった **11d** の合成法を以下に示す (Scheme 1-6). これまでと同様に, カルボン酸 **1** を Barton エステルへと変換し, 脱炭酸的ラジカル反応によってスルフィドを合成した. 活性エステルの調製は, これまで通り 2-メルカプトピリジン-*N*-オキシド **4** との縮合による方法（工程 b）だけでなく, オキサチアゾロピリジニウム塩[4]**15** を用いることも可能であった（工程 a）. -SR 源としては, エステル基をもった非対称ジスルフィド **16** およびイミダゾリル基をもったチオスルホネート **19** をそれぞれ使用し, 白色 LED 照射下, 反応を行うことで, 中程度の収率で目的のスルフィドを得た（工程 a, b）. この反応を利用してカルボン酸 **1** からアミド **13** を直接合成しようと試みたが, 反応が進行しなかったため, エステル体 **2c** を経由して合成を行っている. 化合物 **2c** のメチルエステルを LiOH で加水分解し（工程 c）, アンモニア源として炭酸水素アンモニウムを用いてアミド **13** へと変換した（工程 d）. 化合物 **13** および **2d** を Scheme 1-5 と同様の条件で, スルホンへの酸化, 脱 Bn 化, 硫酸化を行い, 目的の **11c**, **11d** を合成することができた.

Scheme 1-6. Synthesis of Sulfone-Type BLI 11c and 11d[a]

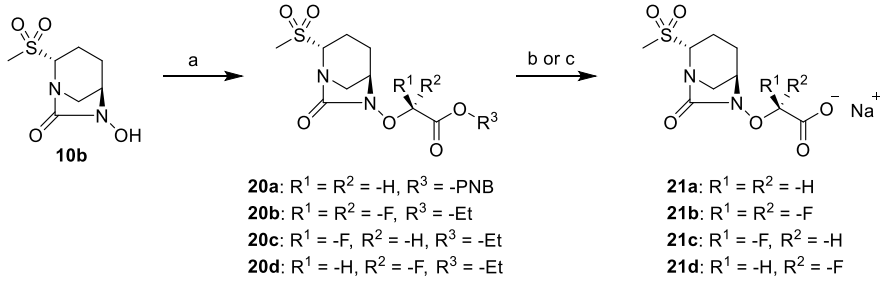


[a]Reagents and conditions: (a) 2-oxo-[1,4,2]oxathiazolo[2,3-*a*]pyridin-4-ium chloride (**15**), Et_3N, CH_2Cl_2, rt, in the dark, then $PhSSCH_2COOMe$ (**16**), rt, white LEDs, 35% for **2c**; (b) 2-mercaptopyridine 1-oxide (**4**), EDC·HCl , CH_2Cl_2, rt, in the dark, then *tert*-butyl 4-[(tosylthio)methyl]-1*H*-imidazole-1-carboxylate (**19**), rt, white LEDs, 45% for **2d**; (c) LiOH, THF/H_2O, 0 ˚C, 85%; (d) NH_4HCO_3, Boc_2O, pyridine, 1,4-dioxane/H_2O, rt, 18%; (e) *m*-CPBA, CH_2Cl_2, 0 ˚C, 60–74%; (f) TFA, CH_2Cl_2, rt, 92%; (g) (i) Pd/C, H_2 (1 atm), rt, (ii) SO_3-pyridine, pyridine, rt, 7–52% over 2 steps.

DBO 系 BLI においては、6 位に硫酸構造をもった誘導体だけでなく，カルボン酸型の誘導体も知られている．[5]-[7] 経口プロドラッグ化を考慮すると 6 位カルボン酸型の β-ラクタマーゼ阻害活性にも興味がもたれるため，それらの合成も試みた (Scheme 1-7). 塩基として K_2CO_3 を用いて，ヒドロキシルアミン **10b** をそれぞれ対応する α-ハロ酢酸エステル **22a-22c** でアルキル化することで，6 位に酢酸構造が導入された **20a-20d** を合成した（工程 a）．この時使用した 2-ブロモ-2-フルオロ酢酸エチル **22c** はラセミ体であり，ジアステレオマーの関係にある **20c**，**20d** が得られるが，これらはシリカゲルカラムクロマトグラフィーにて分離することができた．なお，後述の立体選択的な合成 (Scheme 1-8) によって得られた **20d** との比較から，**20c** と **20d** の相対配置を決定している．化合物 **20a** のパラニトロベンジル (PNB) 基を水素添加反応で脱保護し，$NaHCO_3$ でナトリウム塩とすることで化合物 **21a**（工程 b），NaOH を用いてエチルエステルを加水分解（工程 c）することで **21b-21d** をそれぞれナトリウム塩として得た．

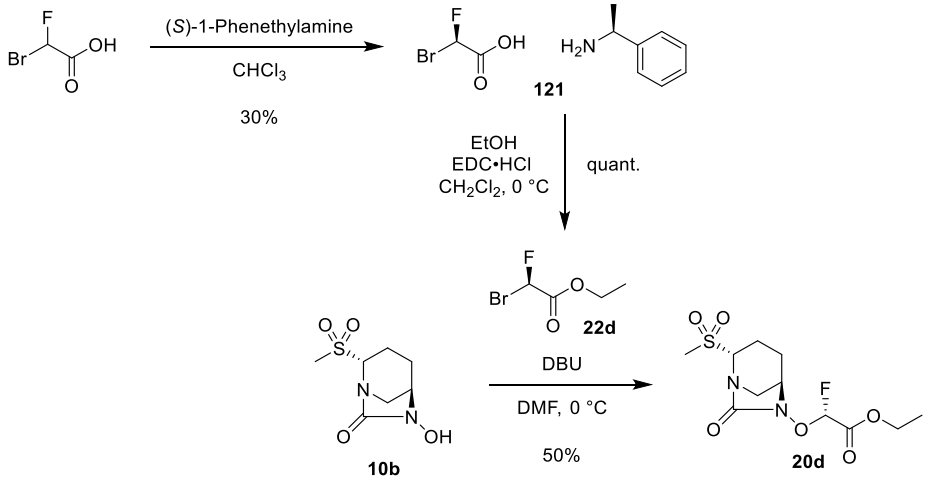
ラセミ体のブロモフルオロ酢酸はキラルなフェネチルアミンを用いたジアステレオマー塩分割によって光学分割できることが報告されている．[7], [8] そこで，光学活性なブロモフルオロ酢酸エチル[7] **22d** を合成し，これを用いて **20d** の立体選択的な合成を試みた (Scheme 1-8). 6 位にキラルなフルオロ酢酸構造をもった ETX0282 の合成法[7]に従って，0 ˚C にて塩基として DBU を用いて反応を行うと，顕著なラセミ化を伴うことなく立体反転で置換反応が進行し，**20d** を単一の異性体として合成することができた．なお，本反応が S_N2 機構で進行することは，同様の反応条件によって合成した化合物 **119** の X 線結晶構造にて確認した (Figure 1-5). [17]

Scheme 1-7. Synthesis of Sulfone-Type BLI 21a-21d[a]



[a]Reagents and conditions: (a) 4-nitorobenzyl 2-iodoacetate (**22a**), ethyl 2-bromo-2,2-difluoroacetate (**22b**), or ethyl 2-bromo-2-fluoroacetate (**22c**), K_2CO_3, DMF, rt, 42–47%; (b) Pd/C, H_2 (1 atm), then $NaHCO_3$, DMF, 0 ˚C, quant for **21a**; (c) NaOH, THF/H_2O, 0 ˚C, 79–92% for **21b**–**21d**.

Scheme 1-8. Stereoselective Synthesis of 20d



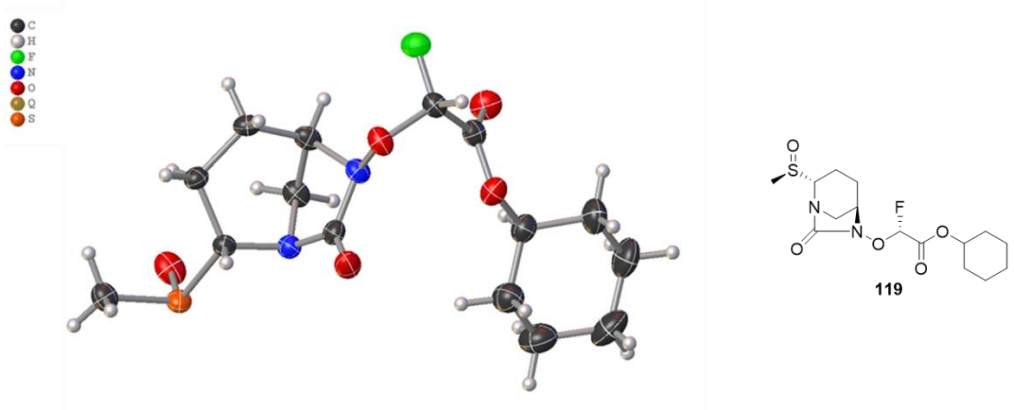


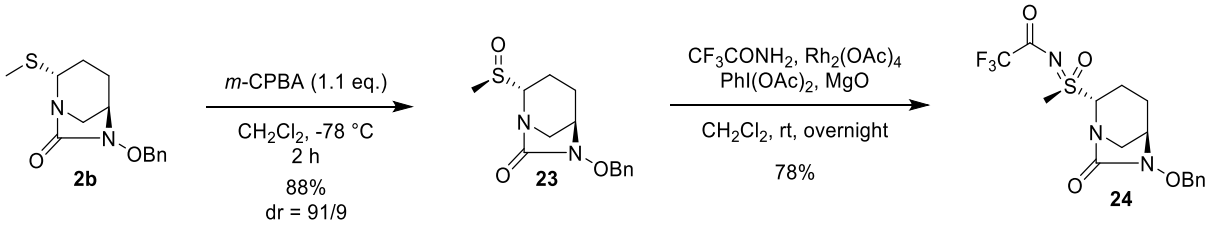
Figure 1-5. X-ray structure of compound **119**. Thermal ellipsoids are set at 30% probability.

第４節　スルホキシド型 BLI の合成

続いてスルホキシド誘導体の合成を行った (Scheme 1-9). スルフィド **2b** を－78 ˚C，ジクロロメタン溶媒中，1.1 eq.の *m*-CPBA で酸化したところ，スルホキシド **23** が良好な収率で得られた．スルホキシドの合成においては，2 種類のジアステレオマーが生成する可能性があるが，本反応においては立体選択性も良好であった (dr = 91/9). なお，ジアステレオマー比は ^{1}H NMR の積分比から決定している．得られた **23** の相対立体配置は，X 線結晶構造解析から決定した (Figure 1-6A). [9] また，ここで得られたスルホキシド **23** は近年メディシナルケミストリーの分野で注目を集めている置換基であるスルホキシイミン **24** にも誘導できることが確認できた．[10], [11]

立体選択的にスルホキシドへの酸化反応が進行した理由は以下の様に考察している (Figure 1-6B). すなわち，Me 基の立体反発および，硫黄原子と 1 位の窒素原子上の孤立電子対の静電的な反発によって，**2b** は Figure 1-6B に示すような安定配座をとると考えられる．この配座において，硫黄原子上の立体障害の影響を受けにくい側の孤立電子対が *m*-CPBA による酸化を受けることで，**23** が立体選択的に得られたと考察している．

Scheme 1-9. Oxidation to Sulfoxide 23 and Sulfoximine 24



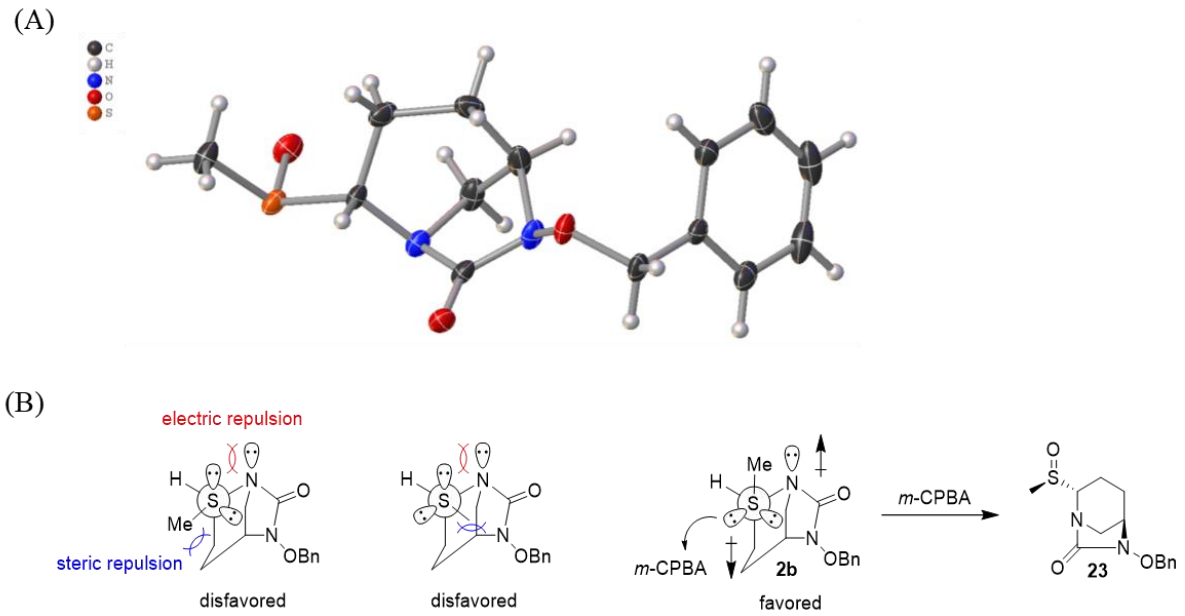
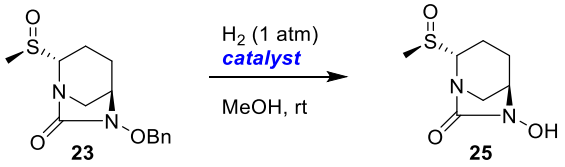


Figure 1-6. (A) X-ray structure of sulfoxide **23**. Thermal ellipsoids are set at 30% probability. (B) Presumable mechanism for diastereoselective oxidation of sulfide **2b**.

続いて，**23** の脱 Bn 化を検討した (Table 1-2)．スルホン体 **9b** においては，特に問題なく Pd/C 触媒を用いて脱 Bn 化が進行したが (Scheme 1-5)，**23** においてはスルホキシドが触媒毒として働くためか Pd/C や $Pd(OH)_2$ を単独で用いた条件では反応がほとんど進行しなかった (entries 1 and 2)．一方で，DBO 誘導体であるレレバクタムの合成において，触媒量の DABCO を添加することで Bn 基の脱保護が促進されることが報告されている．[12] この条件における DABCO の役割は明らかにはなっていないものの，アミノ酸等，DBO 骨格以外の基質を用いた場合にもその効果が確認されており，スルホキシドをもった基質に対しても反応を促進することができるのではないかと考えた．そこで，この条件を本反応へ応用したところ，Pd/C と DABCO の組み合わせでは依然として反応は進行しなかったものの (entry 3)，$Pd(OH)_2$ と組み合わせた条件では反応がスムーズに進行し，収率良く目的の脱 Bn 体 **25** を得ることができた (entry 4).

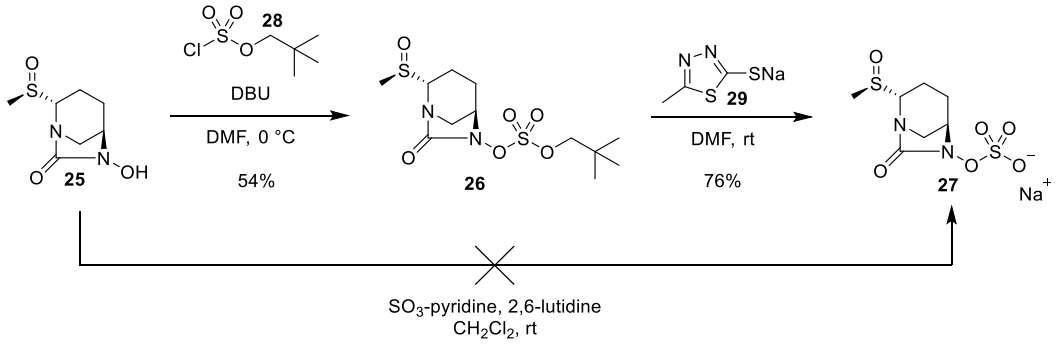
Table 1-2. Optimization of Reaction Conditions for Pd-Catalyzed Debenzylation



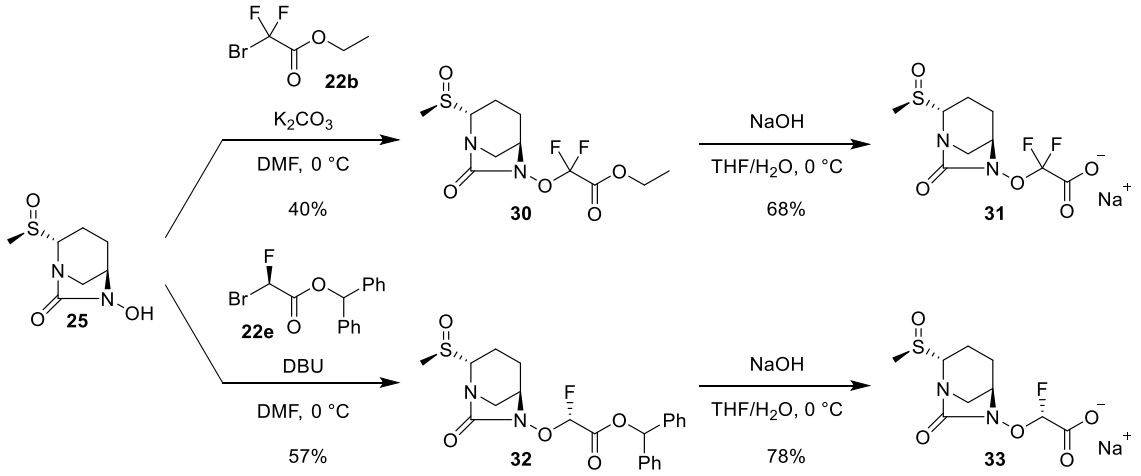
entry	*catalyst*	yield (%)
1	Pd/C (5 mol%)	trace
2	$Pd(OH)_2$ (5 mol%)	trace
3	Pd/C (5 mol%), DABCO (2 mol%)	trace
4	$Pd(OH)_2$ (5 mol%), DABCO (2 mol%)	89

脱 Bn 化の問題を解決できたために，目的のスルホキシドタイプの BLI **27** に向かって合成を進めた (Scheme 1-10)．しかしながら，化合物 **25** から目的の BLI **27** の合成においては，スルホン体 **10b** と同様の SO_3-pyridine 条件 (Scheme 1-5) では目的物を得ることができなかった．この条件においては，基質である **25** の消失が確認されるため，SO_3-pyridine が 2 位のスルホキシドを活性化することで **25** が分解したものと推測している．一方で，試薬としてクロロ硫酸エステル **28** を用いると，スルホキシドではなくヒドロキシルアミン選択的に **25** と反応し，目的の硫酸エステル体 **26** を得ることができた．硫酸エステル **26** のネオペンチル位をナトリウムチオラート **29** で求核置換することで，**27** が良好な収率で得られ，目的の 2 位にスルホキシド基をもった BLI の合成を達成できた．また，スルホンタイプと同様の条件 (Scheme 1-7) にて，6 位カルボン酸タイプの **31**，**33** も合成した (Scheme 1-11)．ここでは，キラルなブロモフルオロ酢酸エステルとして，エチルエステル **22d** より沸点が高く取り扱いが容易なベンズヒドリル (Bzh) エステル **22e** を用いている．

Scheme 1-10. Synthesis of Sulfoxide-Type BLI 27

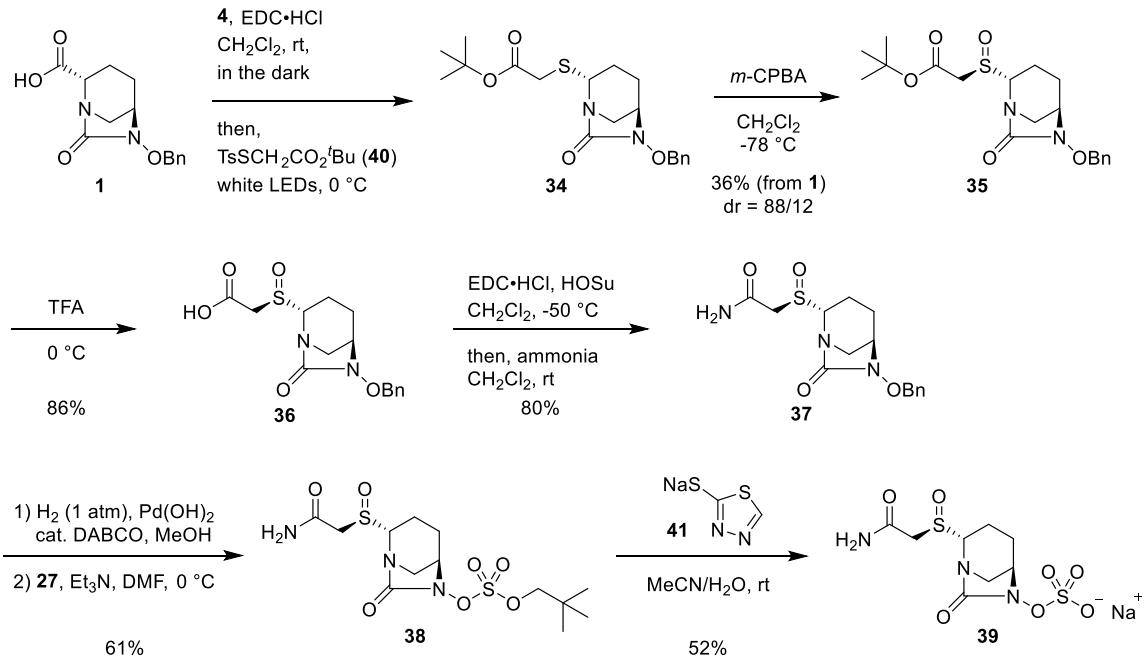


Scheme 1-11. Synthesis of Sulfoxide-Type BLI 31 and 33



目的としていたスルホキシド型 BLI **27** が合成できたため，異なる側鎖をもった誘導体の合成も行った．アミドをもった BLI **39** の合成法を以下に示す (Scheme 1-12)．チオスルホネート **40** を用いた脱炭酸的ラジカル反応で **34** を得た後，スルホキシドへと酸化し **35** を合成した．スルホキシドへの酸化におけるジアステレオ選択性は dr = 88/12 であり，側鎖メチル基の場合と同程度であった．また，側鎖を先にアミドへと変換してから酸化するとジアステレオ選択性の低下が確認されたため (dr = 55/45)，先にスルホキシドへと酸化した後に，^{t}Bu エステルを脱保護し，アミド **37** へと変換している．DABCO を添加した水素添加反応条件において Bn 基を脱保護した後，Scheme 1-10 と同様に硫酸エステル **38** を経由して，目的の BLI **39** を合成した．

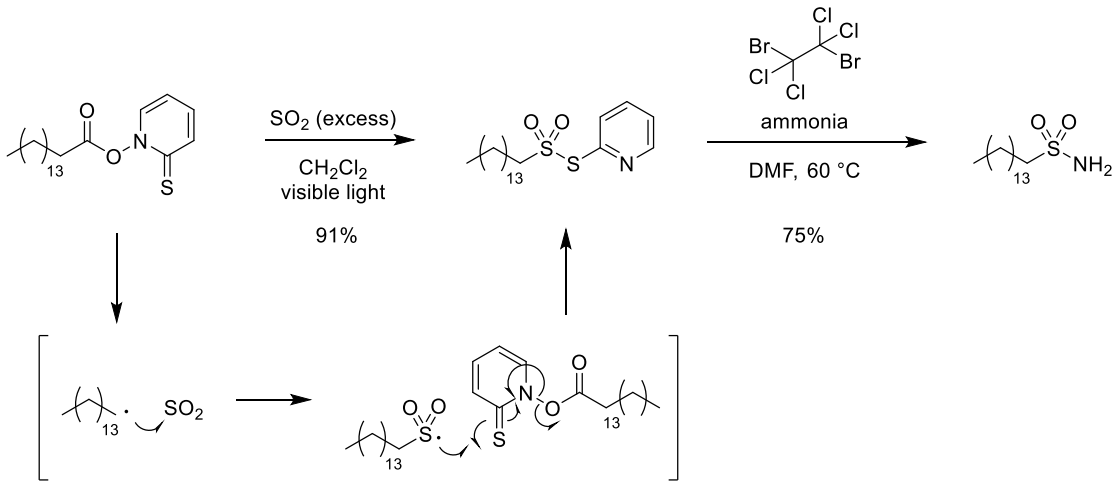
Scheme 1-12. Synthesis of Sulfoxide-Type BLI 39



第５節　スルホンアミド型 BLI の合成

ここまで 2 位への硫黄置換基導入に用いてきた脱炭酸的ラジカル反応は，スルフィドのみならずチオスルホネートを介したスルホンアミドの合成にも応用できることが報告されている (Scheme 1-13). [13] すなわち，過剰の SO_2 存在下，光照射によって Barton エステルから生じたアルキルラジカルは SO_2 によってトラップされ，スルホニルラジカルを生成する．このスルホニルラジカルが Barton エステルのチオピリドン構造と反応することで，新たなアルキルラジカルが生じるとともに, チオスルホネートが生成する. 得られたチオスルホネートは，アンモニアの存在下，1,2-ジブロモ 1,1,2,2-テトラクロロエタンのようなハロゲン化試薬と反応することで，スルホニルハライドを経由してスルホンアミドへと変換できる．

Scheme 1-13. Synthesis of Sulfonamide from Carboxylic Acid via Thiosulfonate Derived from Photo-Induced Decarboxylative Thiolation

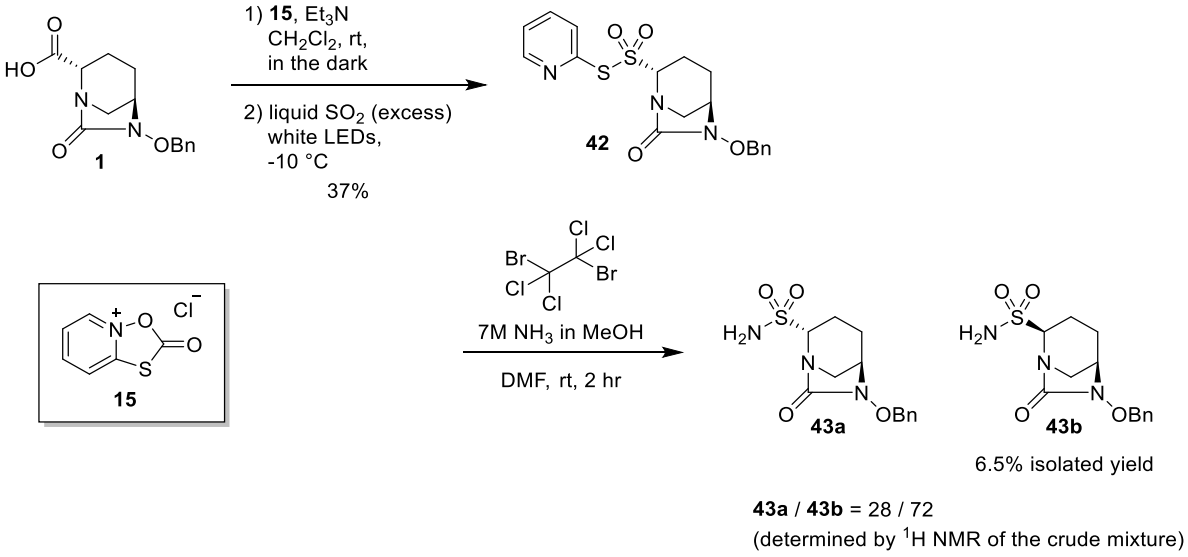


そこで, 本反応を応用してスルホンアミドを有する DBO 誘導体の合成を試みた (Scheme 1-14). 報告例と同様の条件で，カルボン酸 **1** を Barton エステルに変換後，白色 LED 照射下，−10 ˚C にて過剰量の SO_2 と反応させたところ，37%収率にて目的のチオスルホネート **42** を得た．このチオスルホネート **42** をアンモニア存在下，1,2-ジブロモ 1,1,2,2-テトラクロロエタンと反応させ，スルホンアミドへの変換を試みた．しかしながら，得られたスルホンアミドは目的の相対配置を持った **43a** ではなく，2 位がエピ化した **43b** が主生成物であり，crude の ^{1}H NMR から算出した **43a** と **43b** の比率は，**43a** / **43b** = 28 / 72 であった．さらに，主生成物である **43b** の単離収率もわずか6.5%と非常に低い結果となった. なお, チオスルホネート **42**, および，スルホンアミド **43b** の相対配置は NOESY によって確認している (実験の部：p.97, 99).

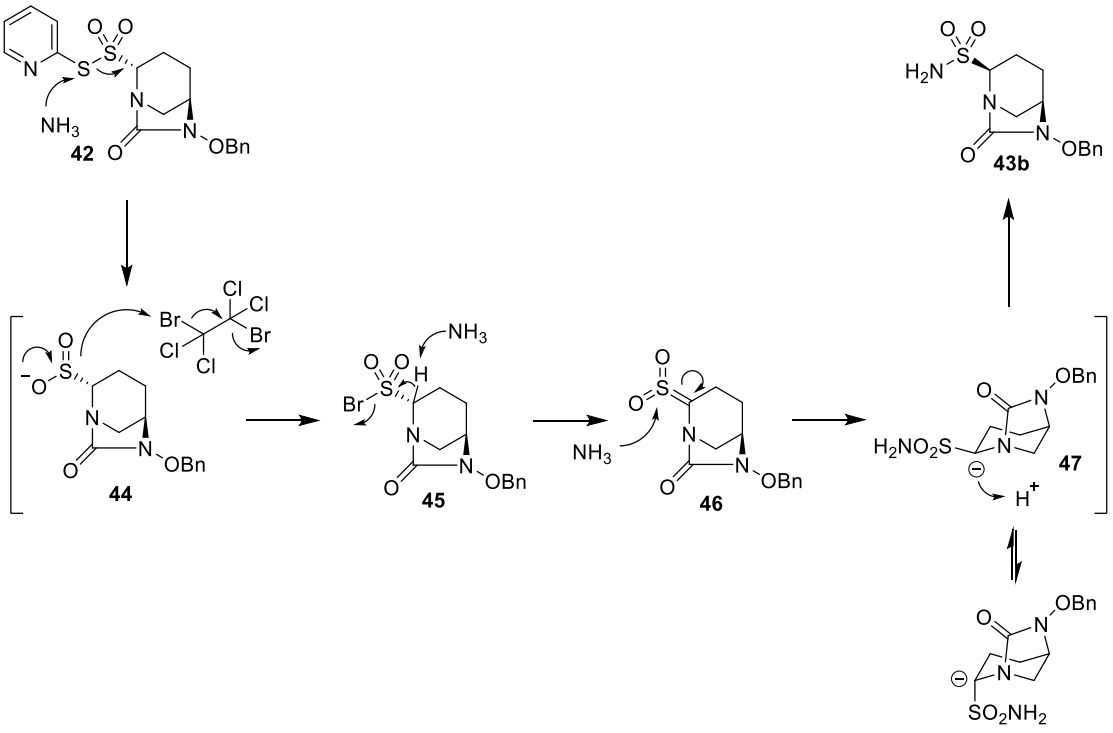
目的の **43a** ではなく，2 位がエピ化した **43b** が得られた理由は以下のように考えている (Scheme 1-15). すなわち，チオスルホネート **42** からスルホンアミドへの変換においては，アンモニアの求核攻撃によってチオスルホネート **42** からスルフィン酸 **44** が生成した後，スルホニルハライド **45** へと酸化され，これがアンモニアと反応していると考えられる．アンモニアとの反応時には, スルホニルハライド **45** から α 位の脱プロトン化が進行したスルフェン **46** が反応活性種であると考えられるが，この時点で 2 位の立体情報が消失してしまう．スルフ

ェンがアンモニアと反応すると 2 位にアニオンが生じ，このアニオン **47** が convex 面からプロトン化されることでエピ体 **43b** が主生成物として得られたと考察している．

Scheme 1-14. Synthesis of Sulfonamide 43a via Thiosulfonate 42

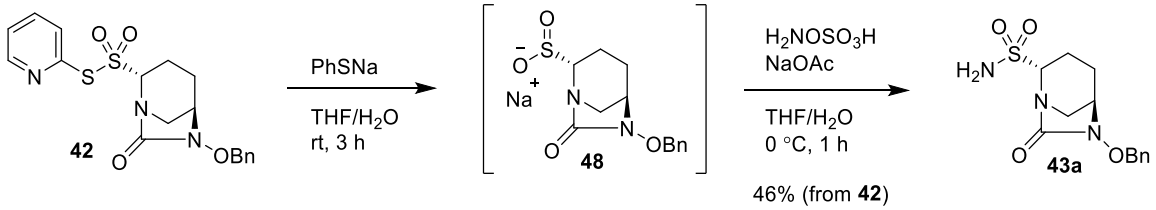


Scheme 1-15. Presumable Mechanism for Epimerization at the C2 Position



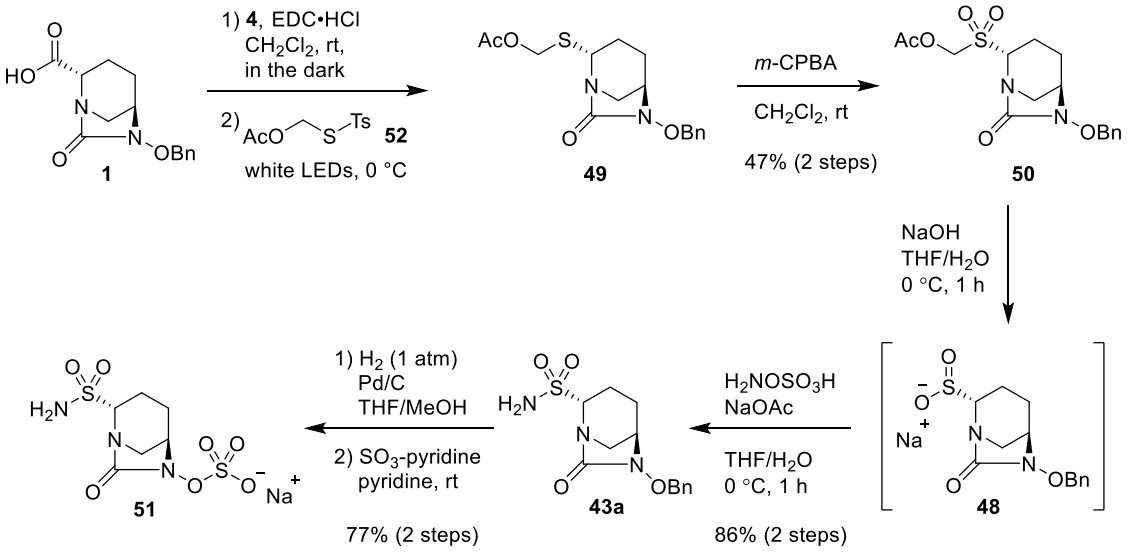
前述の考察より，スルホニルハライドを経由せずにスルホンアミドへと変換できれば，目的の立体化学を持った **43a** が選択的に得られると考えた．そこで，チオスルホネート **42** を PhSNa と反応させることでスルフィン酸 **48** を遊離させ, H_2NOSO_3H を用いて求電子的にアミノ化を行ったところ，目的のスルホンアミド **43a** を単一の異性体として得ることができた (Scheme 1-16)．**43a** の相対配置も同様に NOESY によって確認している (実験の部：p.98).

Scheme 1-16. Conversion of Thiosulfonate 42 to Sulfonamide 43a without Epimerization at the C2 Position



スルフィン酸 **48** の求電子的アミノ化反応によって目的のスルホンアミド体が得られることが分かった．しかしながら，スルフィン酸 **48** の前駆体であるチオスルホネート **42** の合成において，有害な気体である二酸化硫黄を過剰量使う必要があったため (Scheme 1-14)，より良い合成ルートの検討を行った (Scheme 1-17)．合成ルートの改良にあたっては，アセチル基の脱保護で生じるヒドロキシメチル基からの脱離によってスルフィン酸 **48** を生じるアセトキシメチルスルホン[14]**50** をスルフィン酸 **48** の前駆体に設定し，ルート検討を行った．カルボン酸 **1** を脱炭酸的ラジカル反応にてチオスルホネート **52** と反応させることで，アセトキシメチル基を持ったスルフィド **49** へと変換し，*m*-CPBA による酸化によってスルフィン酸前駆体 **50** を合成した．化合物 **50** のアセチル基を加水分解条件で脱保護すると，生じたヒドロキシメチル基からスルフィン酸 **48** が遊離し，続く求電子的アミノ化でスルホンアミド **43a** を得た．化合物 **43a** は Pd/C 条件における 6 位の脱 Bn 化と，続く SO_3-pyridine による硫酸化によって，2 位にスルホンアミドをもった DBO 型 BLI **51** へと変換した．

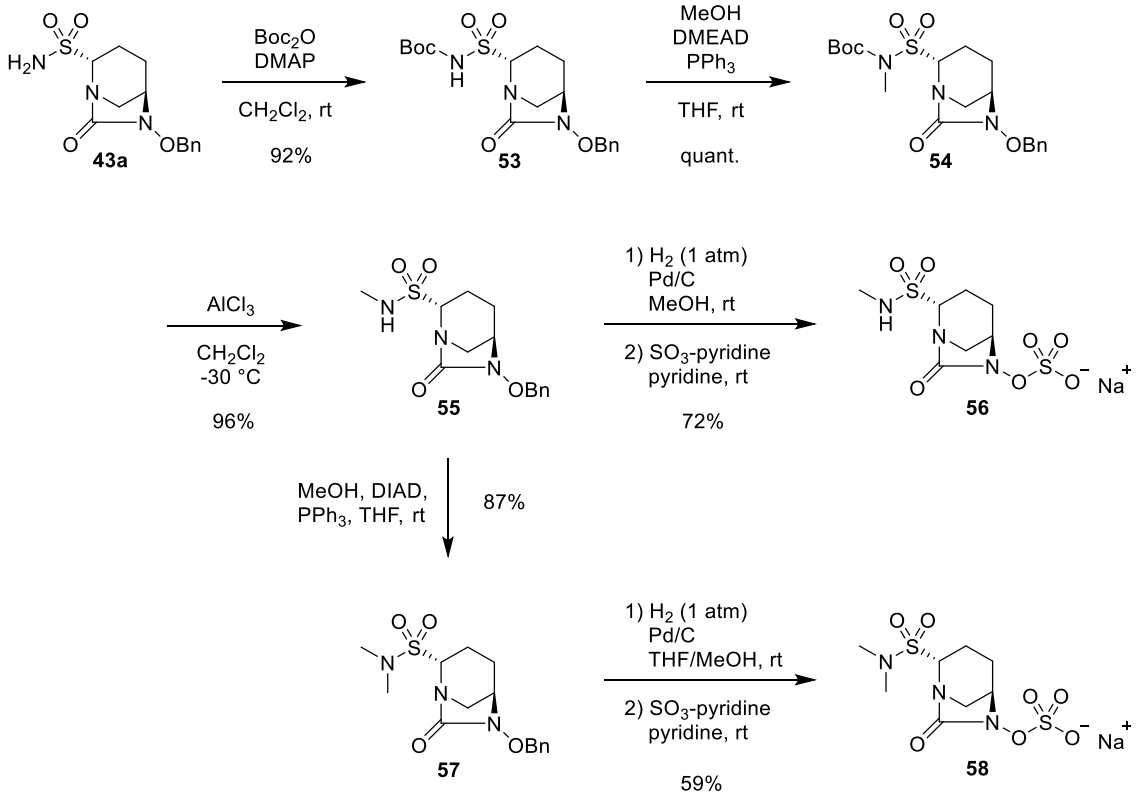
Scheme 1-17. Toxic SO_2-Free Synthetic Route to Sulfonamide-Type BLI 51



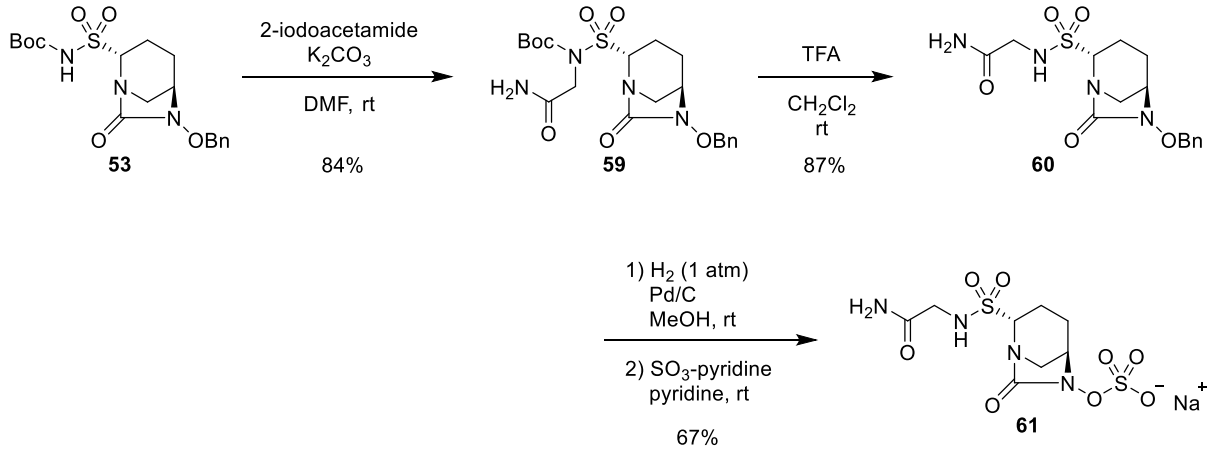
さらに，**43a** のスルホンアミドを Boc 化した後，光延条件でアルキル化することで *N*-メチル化が可能であった (Scheme 1-18)．メチル化された化合物 **54** は，$AlCl_3$ を用いた脱 Boc 化と続く脱 Bn 化，硫酸化によって BLI **56** へと導いた．また，**55** を再び光延条件にてメチル化することでジメチル化された **57** に誘導することができ，**56** と同様の条件にて BLI **58** を得た．

光延反応だけでなく，アルキルハライドを用いた条件でも Boc 体 **53** のアルキル化は可能であり，塩基として K_2CO_3 を用いた 2-ヨードアセトアミドとの反応でアルキル化体 **59** を得ることができた (Scheme 1-19)．化合物 **59** は同様の方法で BLI **61** へと誘導した．

Scheme 1-18. Synthesis of *N*-Methylated Sulfonamide-Type BLI 56 and 58

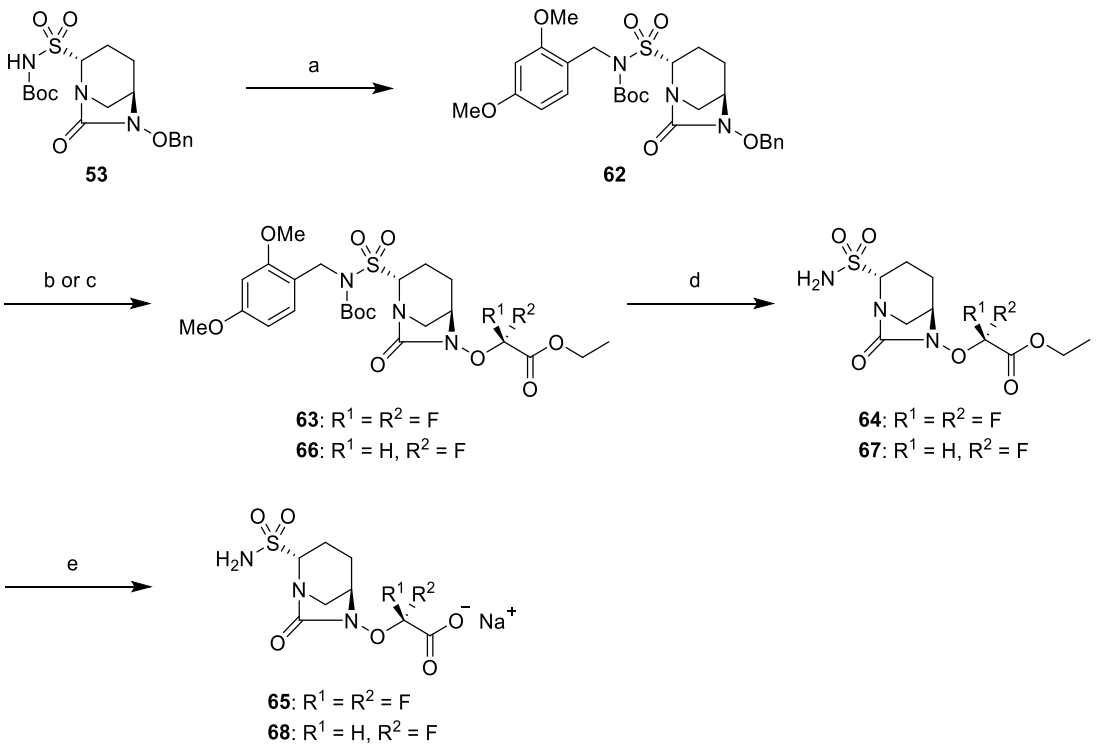


Scheme 1-19. Synthesis of *N*-Alkylated Sulfonamide-Type BLI 61



スルホンアミド型においても 6 位カルボン酸タイプの合成を行った (Scheme 1-20). 2 位スルホンアミドが無保護の **43a** やモノ Boc 体 **53** の 6 位を脱 Bn 化してアルキル化を試みたが, 6 位のアルキル化だけでなく, 2 位のスルホンアミドのアルキル化も併発してしまい収率が低下した. そこで, **53** のスルホンアミド NH をジメトキシベンジル (DMB) 基で保護してから（工程 a）, 6 位の脱 Bn 化とアルキル化によって酢酸ユニットを導入し, ジフルオロ酢酸体 **63** およびフルオロ酢酸体 **66** を得た（工程 b, c）. キラルなブロモフルオロ酢酸 **22d** を用いる場合には, 前述の通り, 0 °C にて塩基として DBU を用いることで, 顕著にラセミ化することなくフルオロ酢酸ユニットを導入できるが (Scheme 1-13), 温度を−20 °C にコントロールすることで塩基として K_2CO_3 を用いた場合にもラセミ化を抑制してフルオロ酢酸を導入することができた（工程 c）. 得られた **63** および **66** は, $AlCl_3$ を用いて Boc 基と DMB 基を一挙に脱保護した後（工程 d）, エチルエステルの加水分解によって最終物 **65** および **68** へと誘導した（工程 e）.

Scheme 1-20. Synthesis of Sulfonamide-Type BLI 65 and 68[a]



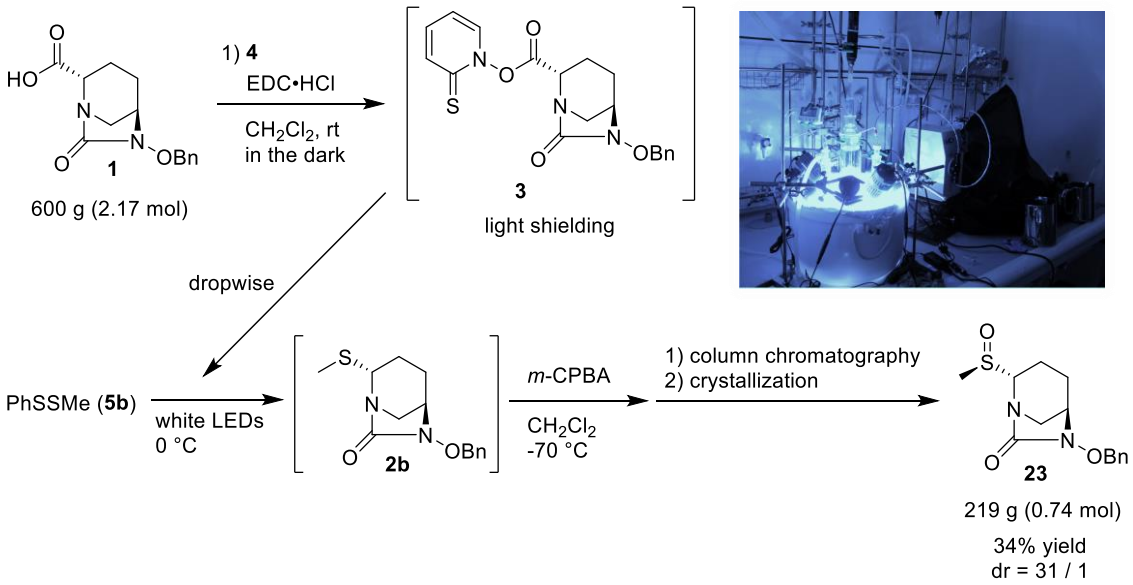
[a]Reagents and conditions: (a) 2,4-dimethoxybenzylalcohol, DMEAD, PPh_3, THF, rt, 89%; (b) (i) H_2 (1 atm), Pd/C, MeOH, rt, (ii) **22b**, K_2CO_3, DMF, rt, 75% for **63**; (c) (i) H_2 (1 atm), Pd/C, MeOH, rt, (ii) **22d**, K_2CO_3, DMF, −20 °C, 55% for **66**; (d) $AlCl_3$, anisole, CH_2Cl_2, −30 to 0 °C, 68-91%; (e) NaOH, THF/H_2O, 0 °C, 82–88%

第６節　スケールアップ合成

一般的に，光反応は量子収率や反応制御の観点からスケールアップ合成に課題があり，flow reactor 等の特殊な装置を使う必要があるケースが多い．[15] 一方で，今回用いた Barton エステルを経由する脱炭酸的ラジカル反応はラジカル連鎖反応であり，[16] 量子収率の観点からは一般的なバッチ合成設備でスケールアップが可能であると考えられた．また，連鎖反応においては反応の制御も問題となるが，別途，遮光条件にて調製した Barton エステルの溶液を，送液ポンプを用いて光照射されている反応容器に滴下していくことで，反応の制御が可能であると考えた．

実際に 600 g のカルボン酸 **1** を用いたスケールアップ合成の例を以下に示す (Scheme 1-21). カルボン酸 **1** から遮光条件にて Barton エステル **3** を調製し，あらかじめ PhSSMe (**5b**) が投入されている反応容器へ，氷冷下，光照射しながら Barton エステル **3** の溶液を送液ポンプにて滴下した．滴下速度を調整し、内温を 10 ˚C 以下に保つことで反応の制御が可能であり，反応の成績体 **2b** を得ることができた．第２節で述べた通り，スルフィド **2b** はシリカゲルに対して不安定であるため，crude のままスルホキシドへと酸化し，カラムクロマトグラフィーによる精製後，EtOAc 中から結晶化させることで，収率 34% (dr = 31 / 1)で **23** を 219 g 得ることができた．スルフィド **2b** を単離せずにスルホキシド **23** へと酸化したが，顕著な収率やジアステレオ選択性の低下は観察されなかった．

Scheme 1-21. Scale-up Synthesis of Sulfoxide 23



第 7 節　本章のまとめ

本章では 2 位に硫黄置換基をもった新規 DBO 誘導体の合成法を開発した (Figure 1-7)．入手性の良いカルボン酸 **1** を出発物質とし，Barton エステルを経由する脱炭酸的ラジカル光反応によって，酸や塩基，熱に対して不安定な DBO 骨格を維持したまま，2 位のカルボキシル基をスルフィドに変換することに成功した．本反応において，アルキルスルフィドの合成は芳香族のものと比較して収率が低く，必要な試薬量が多い傾向にあったが，-SR 源の試薬を非対称ジスルフィドやチオスルホネートへと変更することで，より効率的にアルキルスルフィドを合成できることを見出した．こうして合成が確立された 2 位スルフィド体から各種変換を行うことで，2 位にスルホン，スルホキシド，スルホンアミドをもった DBO 型 BLI の合成を達成した．

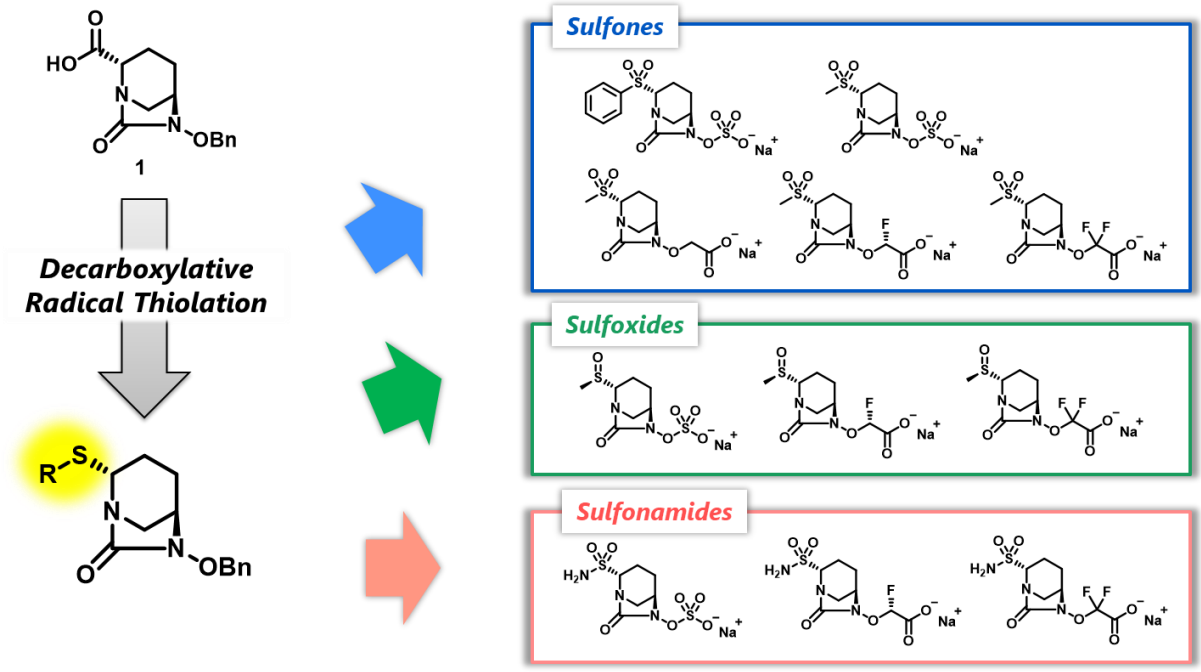


Figure 1-7. Variation of 2-thio-substituted DBO-type BLIs derived from commercially available carboxylic acid **1**.

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第２章　２位に硫黄官能基を有する 1,6-ジアザビシクロ[3.2.1]オクタン誘導体の活性・動態評価

第１節　２位の構造活性相関

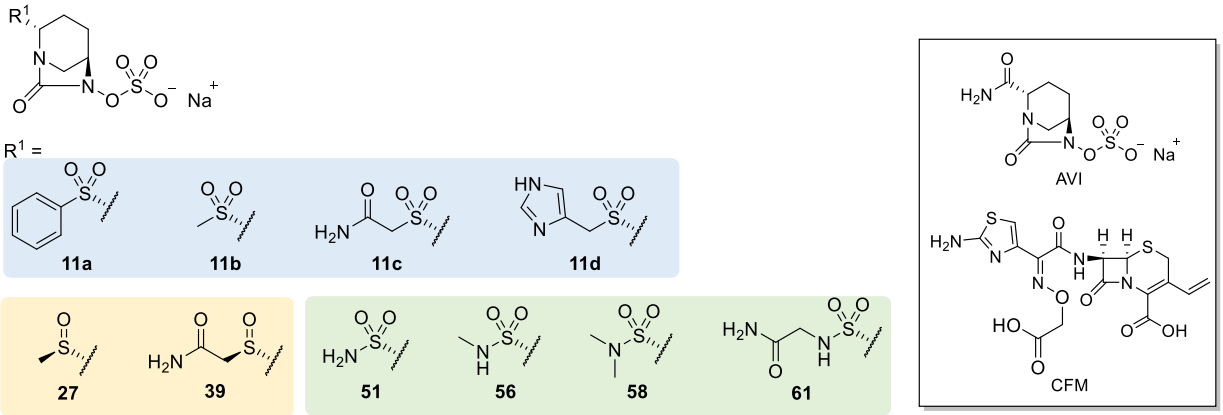
前章で合成したジアザビシクロオクタン (DBO) 誘導体のβ-ラクタマーゼ阻害剤 (BLI) としてのポテンシャルを確認するために，β-ラクタマーゼ阻害活性 (IC_{50})，および，β-ラクタム系抗菌薬との併用効果を評価した．IC_{50}は，セファロスポリンに色素原を結合させた人工基質ニトロセフィンを用い，β-ラクタマーゼによるニトロセフィンの分解に伴って遊離した色素の吸収波長 (492 nm) 観測することで算出した．共有結合性の阻害剤の評価においては，β-ラクタマーゼと阻害剤をプレインキュベーションする条件等によってIC_{50}の値が異なる可能性があり，阻害活性の指標として IC_{50} を用いることは必ずしも適切ではない．しかしながら，注意深く条件を設定することで簡便におおまかな阻害剤のポテンシャルを評価できる方法として有用であり，広く用いられている．[1] 今回は阻害活性を過大評価することが無いよう，BLI とβ-ラクタマーゼをプレインキュベーションせずに評価を実施した．（共有結合性の BLI の場合，プレインキュベーションによって，あらかじめラクタマーゼを阻害しておくことで, プレインキュベーションなしの場合と比較して IC_{50} の値が小さくなる場合がある.）β-ラクタム系抗菌薬との併用効果の評価にあたっては，β-ラクタムとして経口セフェムであるセフィキシム (CFM) を用い，4 μg/mL の BLI 存在条件における CFM の最小発育阻止濃度 (MIC) を測定し，BLI 非存在下の MIC からの回復の程度を指標として，BLI のポテンシャルを確認した.

各種評価結果を以下に示す (Table 2-1). 酵素阻害活性においては，スルホン (**11a**–**11d**)、および, スルホキシド (**27**, **39**) はアビバクタム (AVI) と同等以上であり, 特にスルホンは KPC-2 および CMY-2 に対する IC_{50} の顕著な改善が確認された．一方で，スルホンアミド (**51**–**61**) は，KPC-2 および CMY-2 に対する活性は同等であるものの，CTX-M-15 に対する阻害活性が AVI と比較して減弱傾向にあった．程度の差はあるものの，官能基の種類（スルホン，スルホキシド，スルホンアミド）が同じであれば，側鎖が異なる場合でも，酵素阻害活性の強さの傾向は類似していた.

また，CFM の抗菌活性を回復させる効果については，BLI が存在しない条件では MIC > 32 μg/mL である２つのβ-ラクタマーゼ産生株において，優位に CFM の抗菌活性を回復させており，**11a** を除く全ての化合物においてその効果は AVI 以上であった．一方で， CFM との併用効果は必ずしも酵素阻害活性 (IC_{50}) と連動する結果とはならなかった．第１章第１節で述べた通り，グラム陰性菌は外膜という特徴的な膜に覆われており，薬剤が効果を発揮するためにはこの外膜を透過する必要がある．[2] 親水性の抗菌薬は，ポーリンと呼ばれるタンパク質で形成された透過孔を通って外膜を透過することが知られており，AVI もいくつかのポーリンを経由して外膜を透過することが報告されている．[3] また，β-ラクタム系抗菌薬において，外膜の透過速度は分子の大きさや脂溶性と関わっており，より小さく親水性の高い構造が好まれることも知られている．[4] このような観点から結果を見ると， **11a** は IC_{50} がほぼ同程度の他のスルホンタイプ (**11b**–**11d**) と比較して CFM との併用効果が低いが，これは分子量が大きく，脂溶性が高いために，十分な外膜透過性を得られなかったためであると考え

られる．また，2 位の官能基の種類によって IC_{50} の傾向が類似しているが，同じ種類の官能基であるスルホンタイプ (**11b**–**11d**) やスルホンアミドタイプ (**51**–**61**) の間で比較すると，それぞれのタイプの中で最も分子量の小さい **11b** および **51** が最も効果的に CFM の抗菌活性を回復させている．ただし，**11b** と **51** はβ-ラクタマーゼ阻害活性だけでなく，比較的強い抗菌活性 (MIC) をもっているために，この抗菌活性の効果が上乗せされて高い併用効果が観察されている可能性を考慮する必要がある（Table 2-1 カッコ内の値）．スルホンアミド **51**–**61** においては，CTX-M-15 に対する阻害活性が AVI と比較して減弱しているにもかかわらず，CTX-M-15 産生株 SR34100 における CFM との併用効果は AVI を上回るものであった．この理由は定かではないが，スルホンアミド系の置換基は AVI のもつアミド構造よりも外膜透過性の観点で優れている可能性がある．

Table 2-1. Structure-Activity Relationship at the C2 Position[a]



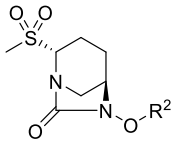
comp.	Mw[f]	clogP[f]	IC_{50} (μM)[b]			MIC (μg/mL) of CFM in the presence of 4 μg/mL of BLI[c]	
			KPC-2 (class A)	CTX-M-15 (class A)	CMY-2 (class C)	*K. pneumoniae* ATCC-BAA-1705[d] KPC-2[e]	*E. coli* SR34100[d] CTX-M-15[e]
11a	362	0.04	0.052	0.031	<0.001	1 (>32)[g]	4 (>32)[g]
11b	300	−1.65	0.004	0.017	0.003	0.125 (16)[g]	≤0.031 (4)[g]
11c	343	−2.35	0.007	0.102	0.013	0.25 (>32)[g]	0.25 (>32)[g]
11d	366	−2.75	0.020	0.055	0.007	0.25 (>32)[g]	0.5 (>32)[g]
27	284	−1.56	0.006	0.012	0.041	0.25 (16)[g]	0.5 (8)[g]
39	327	−2.30	0.028	0.008	0.014	0.25 (no data)[g]	0.25 (16)[g]
51	301	−1.89	0.044	0.140	0.116	0.125 (8)[g]	0.125 (8)[g]
56	315	−1.22	0.015	0.198	0.018	0.25 (>32)[g]	0.25 (>32)[g]
58	329	−0.80	0.013	0.360	0.036	0.25 (>32)[g]	0.5 (>32)[g]
61	358	−2.12	0.044	0.361	0.048	0.25 (>32)[g]	0.5 (>32)[g]
AVI	265	−1.63	0.072	0.013	0.059	1 (32)[g]	1 (16)[g]
alone	-	-	-	-	-	>32[h]	>32[h]

[a]Blue: ≥8-fold reduction compared to AVI, Red: ≥8-fold increase compared to AVI. AVI: avibactam. CFM: cefixime. [b]*N* = 1 determination. The values of IC_{50} were determined without enzyme-inhibitor preincubation. [c]*N* = 1 determination. [d]Strain ID. [e]Expressed β-lactamase on each strain. [f]Calculated as a free form. [g]MIC of BLI. [h]MIC of CFM alone.

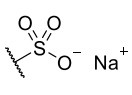
第２節　6 位の構造活性相関

続いて，2 位をメチルスルホンに固定して，6 位の変換による活性の変化を確認した (Table 2-2)．6 位のスルホン酸 (**11b**) を酢酸構造に変換すると IC_{50} は大きく減弱した (**21a**)．しかしながら，カルボン酸のα位に電子求引性基であるフッ素を導入したジフロロ酢酸構造へと変換すると，スルホン酸型 (**11b**) と遜色ない活性を示すことが分かった (**21b**)．また，フッ素を一つ削減したモノフルオロ酢酸構造へと変換すると，２つの立体異性体の内，*R* 体 (**21d**) においてジフロロ酢酸体 (**21b**) と同等の活性を示した．6 位を硫酸構造からフルオロ酢酸に変換しても強力なβ-ラクタマーゼ阻害活性を示す例として，ETX1317 の報告がある．[6], [9] この例においても，フルオロ酢酸の立体化学が *R* 体の方が活性が強いことが報告されており，今回の傾向と一致している．6 位をスルホン酸からフルオロ酢酸へ変換すると分子量の大きな変化はないものの，clogP は上昇する傾向にある．しかしながら，β-ラクタマーゼ産生菌に対するセフィキシム (CFM) の抗菌活性回復効果についても，ジフルオロ酢酸 **21b** および *R* 体のフルオロ酢酸である **21d** は，強力な IC_{50} 値を反映して，6 位スルホン酸体 **11b** と同等の効果を示した．このことから，6 位のスルホン酸からフルオロ酢酸への変換は，clogP の上昇はあるものの，分子の外膜透過性に大きな影響を及ぼさないことが示唆された．

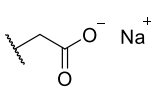
Table 2-2. Structure-Activity Relationship at the 6-Position[a]



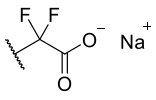
R^2 =



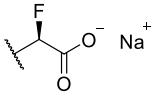
11b



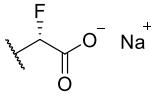
21a



21b



21c



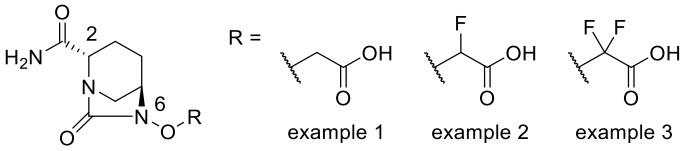
21d

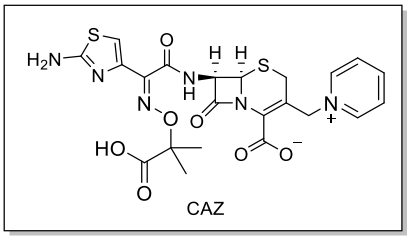
comp.	Mw[f]	clogP[f]	IC_{50} (μM)[b]			MIC (μg/mL) of CFM in the presence of 4 μg/mL of BLI[c]	
			KPC-2 (class A)	CTX-M-15 (class A)	CMY-2 (class C)	*K. pneumoniae* ATCC-BAA-1705[d] KPC-2[e]	*E. coli* SR34100[d] CTX-M-15[e]
11b	300	−1.65	0.004	0.017	0.003	0.125 (16)[g]	≤0.031 (4)[g]
21a	278	−0.46	0.536	2.24	1.05	no data	no data
21b	314	0.99	0.007	0.022	0.042	0.25 (32)[g]	≤0.031 (8)[g]
21c	296	−0.39	0.217	0.378	1.06	0.5 (no data)[g]	1 (no data)[g]
21d	296	−0.39	0.031	0.055	0.042	0.25 (32)[g]	≤0.031 (8)[g]
AVI	265	−1.63	0.072	0.013	0.059	1 (32)[g]	1 (16)[g]
alone	-	-	-	-	-	>32[h]	>32[h]

[a]Blue: ≥8-fold reduction compared to AVI, Red: ≥8-fold increase compared to AVI. AVI: avibactam. CFM: cefixime. [b]*N* = 1 determination. The values of IC_{50} were determined without enzyme-inhibitor preincubation. [c]*N* = 1 determination. [d]Strain ID. [e]Expressed β-lactamase on each strain. [f]Calculated as a free form. [g]MIC of BLI. [h]MIC of CFM alone.

アビバクタム (AVI) の 6 位硫酸構造をジフルオロ酢酸に変換した化合物は，比較的強いβ-ラクタマーゼ阻害活性を有し，セフタジジム (CAZ) の抗菌活性を十分に回復させることが報告されている (Table 2-3). [5] 一方で，ジフルオロ酢酸構造をモノフルオロ酢酸に変換すると，酵素阻害活性、および，CAZ の抗菌を回復させる効果が減弱することも併せて報告されており，2 位がアミドの場合には，2 位にスルホンを有する **21d** と異なり，6 位モノフルオロ酢酸構造で強力な活性を示すことは困難である.

Table 2-3. Reported IC_{50}s and Restoration of MICs of DBO Derivatives Pocessing Acetic Acid Moiety at the 6-Position[5]



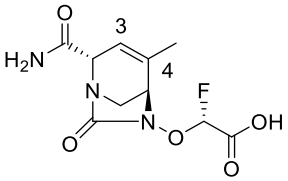


comp.	IC_{50} (μM)		MIC (μg/mL) of CAZ in the presence of 4 μg/mL of BLI			
	TEM-1 (class A)	P99 (class C)	*E. coli* 250BE6[a] TEM-3[b]	*E. cloacae* 293HT6[a] P99, AmpC[b]	*E. cloacae* 293HT4[a] AmpC[b]	*C. freundii* 261GR6[a] AmpC[b]
ex. 1	0.72	54	2	>32	>32	>32
ex. 2[c]	0.002	4.6	0.25	8	8	4
ex. 3	0.0008	2.2	0.25	1	0.5	1
alone	-	-	>32[d]	>32[d]	>32[d]	>32[d]

CAZ: ceftazidime. [a]Strain ID. [b]Expressed β-lactamases on each strain. [c]Mixture (1:1) of the two diastereomers on fluorine stereochemistry. [d]MIC of CAZ alone.

また，DBO 骨格 3 位と 4 位の間に二重結合を導入した ETX1317 においては，6 位モノフルオロ酢酸構造で強いβ-ラクタマーゼ阻害活性，および，β-ラクタム系抗菌薬の抗菌活性回復効果を示すことが報告されている (Table 2-4). [6] この理由は，二重結合の導入によって分子のひずみが増大し，DBO がβ-ラクタマーゼの活性中心のセリンを反応しやすくなったためだと考えられる．今回，2 位ヘスルホンを導入したことで DBO 骨格の反応性が向上し，母核に二重結合を導入した場合と同等のβ-ラクタマーゼ阻害活性に対する効果が観測されたと言える．

Table 2-4. Reported IC_{50}s and Restoration of MICs of ETX1317[6]



ETX1317

comp.	IC_{50} (μM)			MIC (μg/mL) of CFM in the presence of ETX1317 at a fixed 1:2 ratio (CFM:ETX1317)	
	KPC-2 (class A)	CTX-M-15 (class C)	AmpC (class C)	*E. coli* ARC6074[a] AmpC, KPC-3[b]	*K. pneumoniae* ARC6100[a] KPC-2, TEM-1, SHV-11[b]
ETX1317	0.043	0.002	0.16	0.13	0.25
alone	-	-	-	32[c]	>64[c]

CFM: cefixime. [a]Strain ID. [b]Expressed β-lactamases on each strain. [c]MIC of CFM alone.

第３節　臨床分離株に対する有効性

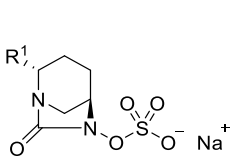
2 位に硫黄置換基をもった複数の DBO 誘導体が BLI として優れた有効性を示すことが分かったため，多様な臨床分離株に対する有効性の評価結果を行った (Table 2-5)．全体的な傾向としては，今回テストした大部分の菌株に対して，2 位に硫黄置換基をもった DBO 誘導体はアビバクタム (AVI) より優れた有効性を示し，特に SR200030，SR09603，SR200487 に対して併用効果の差が顕著であった．一方で，SR201218 のように AVI と比較して併用効果がやや減弱する菌株も存在することが認められた．

また，硫黄官能基の種類に応じて，セフィキシム (CFM) の抗菌活性回復効果における菌株間の傾向が異なることが観察された．例えば，OXA-48 と CTX-M-15 を産生する SR201218 に対して，スルホン (**11c**, **11d**)，および，スルホンアミド (**56**, **58**) は，BLI 存在下の CFM の抗菌活性は，MIC = 2–16 μg/mL と AVI よりも低い併用効果を示す一方で，スルホキシド (**27**, **39**) は，MIC = 0.5 μg/mL と AVI と同等の併用効果を示した．これは，CTX-M-15 に対する阻害活性がスルホン (**11c**, **11d**)，および，スルホンアミド (**56**, **58**) と比較して，スルホキシド (**27**, **39**) の方が強力であるためだと考えられる (Tabel 2-1 参照)．

側鎖に着目すると，スルホン，スルホキシドにおいては，シンプルな側鎖 (**11b**, **27**) が優れた BLI ポテンシャルを示した．後述の通り，これらの化合物は，化合物自身が比較的強い抗菌活性を持っていることも考慮しなければならないが (Table 2-6 参照)，分子量が小さいことが外膜透過性に有利であったことが影響していると考えている．また，スルホンアミドにおいては，最もシンプルな **51** と比較して N-Me 化された **56** の方が，SR200030 や SR09603，SR200487 といったいくつかの株に対して優れた併用効果を示した．スルホンアミド **51** と **56** の β-ラクタマーゼ阻害活性を比較すると，KPC-2 と CMY-2 に対しては **56** の方が強い阻害活性を有している (Table 2-1)．今回の評価に用いた株が産生する全ての β-ラクタマーゼに対する阻害活性を評価しているわけではないが，スルホンアミド **56** は多くの β-ラクタマーゼに対して **51** よりも阻害活性が強く，これが菌株に対する CFM の抗菌活性回復効果につながっている可能性がある．

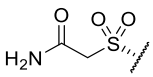
複数の臨床分離株に対しても，6 位フルオロ酢酸タイプの **21b**，**21d** は 6 位硫酸タイプ **11b** と遜色のない有効性を示した．一部の菌株に対し，**21b** より **21d** の方が有効性が高いのは，脂溶性がより低いことが外膜透過性に有利に働いたためであると考察している．

Table 2-5. MICs of CFM against Selected Clinical Isolates in the Presence of BLI[a]

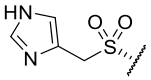


R¹ =

11b

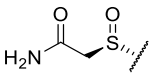


11c

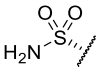


11d

27



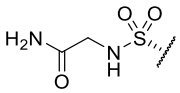
39



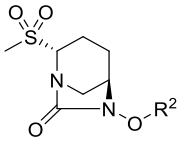
51

56

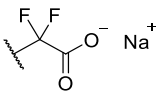
58



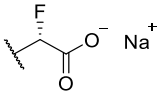
61



R² =



21b



21d

species	strain ID	characteristics[b]	MIC (μg/mL) of CFM in the presence of 4 μg/mL of BLI[c]			
			11b	**11c**	**11d**	**27**
K. pneumoniae	ATCC700603	SHV-18	0.063	0.25	0.25	0.125
E. cloacae	SR200030	PER-2, ACT, ΔompF	1	2	4	1
E. cloacae	SR36276	AmpC	<0.031	0.25	0.25	0.125
K. pneumoniae	SR09635	DHA-1	<0.031	0.25	0.25	0.25
K. pneumoniae	SR09603	CMY-8 type	<0.031	0.25	0.25	0.5
K. pneumoniae	SR200263	OXA-48, CTX-M-1 group	<0.031	0.125	0.25	0.063
K. pneumoniae	SR201218	OXA-48, CTX-M-15	n.t.[c]	2	8	0.5
K. pneumoniae	SR200487	VEB-8, CTX-M-15, SHV-110, CMY-16, OXA-48, OXA-10	0.125	0.5	0.5	0.25

species	strain ID	characteristics[b]	MIC (μg/mL) of CFM in the presence of 4 μg/mL of BLI[c]			
			39	**51**	**56**	**58**
K. pneumoniae	ATCC700603	SHV-18	0.25	0.125	0.125	0.125
E. cloacae	SR200030	PER-2, ACT, ΔompF	1	4	1	16
E. cloacae	SR36276	AmpC	0.25	0.25	0.063	0.5
K. pneumoniae	SR09635	DHA-1	0.5	0.25	0.25	0.25
K. pneumoniae	SR09603	CMY-8 type	4	1	0.25	0.5
K. pneumoniae	SR200263	OXA-48, CTX-M-1 group	0.125	0.063	0.125	0.25
K. pneumoniae	SR201218	OXA-48, CTX-M-15	0.5	n.t.[d]	2	16
K. pneumoniae	SR200487	VEB-8, CTX-M-15, SHV-110, CMY-16, OXA-48, OXA-10	0.5	2	0.5	1

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species	strain ID	characteristics[b]	MIC (μg/mL) of CFM in the presence of 4 μg/mL of BLI[c]			
			21b	**21d**	AVI	alone
K. pneumoniae	ATCC700603	SHV-18	0.125	0.63	0.5	16[e]
E. cloacae	SR200030	PER-2, ACT, ΔompF	32	2	32	>32[e]
E. cloacae	SR36276	AmpC	0.5	0.25	2	>32[e]
K. pneumoniae	SR09635	DHA-1	<0.031	<0.031	1	>32[e]
K. pneumoniae	SR09603	CMY-8 type	<0.031	0.063	32	>32[e]
K. pneumoniae	SR200263	OXA-48, CTX-M-1 group	<0.031	n.t.[d]	0.5	>32
K. pneumoniae	SR201218	OXA-48, CTX-M-15	n.t.[d]	n.t.[d]	0.5	>32
K. pneumoniae	SR200487	VEB-8, CTX-M-15, SHV-110, CMY-16, OXA-48, OXA-10	0.5	0.5	16	>32

[a]Blue: ≥8-fold reduction compared to AVI, Red: ≥8-fold increase compared to AVI. AVI: avibactam. CFM: cefixime. [b]The expressed β-lactamases and the deletion of porin on each strain. [c]*N* = 1 determination. [d]Not tested. [e]MIC of CFM alone.

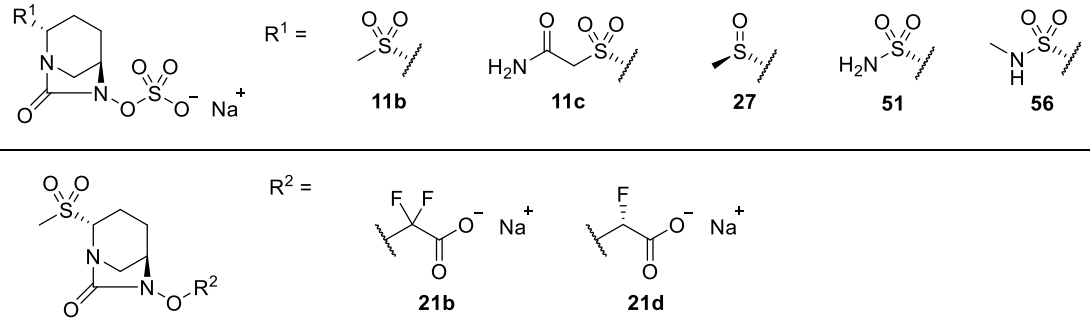
第４節　DBO 誘導体自身の抗菌活性

これまでβ-ラクタマーゼ阻害剤 (BLI) としての有効性について見てきたが，その中で本 DBO 誘導体自身が抗菌活性を有することが見えていた．そこで，これらの化合物が有する抗菌薬としての性質についてもその詳細を調べることとした．各種菌株に対する MIC をまとめた結果を以下に示す (Table 2-6)．スルホン，スルホキシド，スルホンアミド，いずれの誘導体においても一定の抗菌活性を示すものの，側鎖がかさ高くなることで活性が減弱する傾向にあった (**11b** vs **11c**, **51** vs **56**)．また，6 位フルオロ酢酸タイプにおいても抗菌活性は維持していたことから (**21b**, **21d**)，6 位よりも 2 位の側鎖の構造が抗菌活性においては重要であることが示唆された．さらに，DBO 誘導体では阻害できないメタロ-β-ラクタマーゼの一種である NDM-1 を産生する FAU に対しても抗菌活性を示したことから，これらの化合物はメタロ-β-ラクタマーゼに対して安定である可能性が示唆された．

他の DBO 誘導体としては，ナクバクタム (NAC)，および，ジデバクタム (ZID) が比較的強い抗菌活性を持つことが知られている．AVI も含めこれらの化合物は，PBP2 (penicillin-binding protein 2) を選択的に阻害することで抗菌活性を示すことが報告されている．[7], [8] このような PBP2 選択的阻害剤は，菌種や菌株間でその抗菌活性が異なる場合が多いと言われているが，[6] 今回評価した菌株セットにおいても，NAC や ZID は抗菌活性を示す株とそうでないものの差が顕著であった．一方で，2 位に硫黄置換基を導入した誘導体は，今回評価した全ての株に対して活性を示す傾向にあり，抗菌活性の発現メカニズムが NAC，ZID と異なる可能性が示唆された．

抗菌活性の発現メカニズムを検証する目的で，**21b** の PBP 選択性を確認した (Table 2-7)．実験としては，３つの異なる濃度（1, 10, 100 μM）の **21b** で各種 PBP を処理したのちに，蛍光標識されたペニシリンと反応させ，未反応の PBP を検出することで，各濃度における阻害率を算出した．その結果，**21b** は PBP2 よりも PBP4, 5/6 に対する親和性が高く，高濃度域においては全 PBP のサブタイプを阻害することが明らかとなった．これは，PBP2 を選択的に阻害するというこれまでの DBO 誘導体とは大きく異なる PBP 選択性であり，非常に興味深い知見であるとともに，本化合物を起点とした非β-ラクタム系抗菌薬が開発できる可能性を示唆するものである．

Table 2-6. MICs of 2-Thio-Substituted DBOs[a]



species strain ID β-lactamase[b]	MIC (μg/mL)[c]								
	11b	**11c**	**27**	**51**	**56**	**21b**	**21d**	**NAC**	**ZID**
K. pneumoniae ATCC700603 SHV-18	8	>32	8	8	>32	32	32	>32	>32
E. coli SR34100 CTX-M-15	4	>32	8	8	>32	8	8	2	1
K. pneumoniae ATCC-BAA-1705 KPC-2	16	>32	16	8	>32	32	32	2	1
E. cloacae SR36276 AmpC	8	>32	16	16	>32	16	8	2	1
K. pneumoniae SR09635 DHA-1	8	>32	8	16	32	8	8	>32	8
K. pneumoniae SR09603 CMY-8 type	8	>32	16	16	>32	8	16	>32	>32
K. pneumoniae FAU NDM-1	8	n.t.[d]	n.t.[d]	16	n.t.[d]	8	8	>32	>32

[a]NAC: nacubactam. ZID: zidebactam. [b]Expressed β-lactamase on each strain. [c]*N* = 1 determination. [d]n.t. = not tested.

Table 2-7. PBP Inhibition Rate of 21b

concentration (μM)	Inhibition rate (%)[a]				
	PBP1	PBP2	PBP3	PBP4	PBP5/6
1	2	11	9	62	37
10	23	32	21	82	84
100	69	72	86	92	98

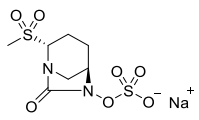
[a]*N* = 1 determination. Inhibition rates were determined by the relative fluorescence value of Bocillin FL penicillin after the incubation of membrane fraction with **21b**.

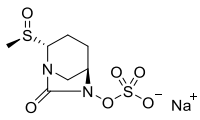
第５節　薬物動態

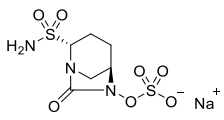
2 位に硫黄置換基を有する DBO 誘導体のラットにおける PK (Pharmacokinetics) 評価を行った (Table 2-8). 2 位の官能基別に比較すると，スルホン **11b** とスルホキシド **27** はトータルクリアランス (CL_{tot}) が高かった．化合物 **11b** においては，ラットの血漿中安定性が極めて低く，この影響で高 CL となったと考えられる．一方で，スルホンアミド **51** は，血漿中安定性が他の誘導体と比較して良好であり，アビバクタムを含む既知の DBO 誘導体と同等の低い CL を示した．[9]-[11] スルホンタイプにおいては，6 位をジフルオロ酢酸 **(21b)** やフルオロ酢酸 **(21d)** に変換したが，血漿中安定性は低いままであり，PK プロファイルの改善には至らなかった．ジフロロ酢酸 **(21b)** においては，pH 7.0 リン酸 buffer 中においても不安定であることから，化学的に不安定であると考えられるが，**21d** は buffer 中では安定であることから，血漿中に存在するエステラーゼ等の加水分解酵素によって化合物が分解されている可能性が示唆された.

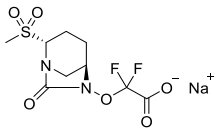
血漿中安定性が低い要因として加水分解酵素の関与が示唆されたため，血漿中安定性における種差を確認した (Table 2-9). その結果，本 DBO 誘導体はラットをはじめとする齧歯類においては不安定な傾向があったものの，ヒトを含む非齧歯類の血漿に対しては十分に安定であった．このことから，齧歯類の血漿中に含まれる加水分解酵素などの酵素の影響によって本 DBO 誘導体が分解されていることが確認された.

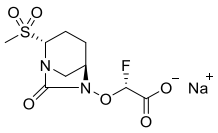
Table 2-8. PK of the DBOs in Rats after Intravenous Administration[a]











compound	AUC (μg·h/mL)	$T_{1/2}$ (h)	CL_{tot} (mL/min/kg)	rat serum stability (%)[b]	solution stability (%)[c]
11b	0.425	0.1	39.8	<1	44
27	0.188	0.2	88.7	no data	no data
51	0.953	0.2	17.6	58	51
21b	0.315	0.1	53.3	2	2
21d	n.c.[d]	n.c.[d]	n.c.[d]	<1	87

[a]AUC, area under the plasma concentration-time curve from time zero to infinity; $T_{1/2}$, apparent terminal elimination half-life; CL_{tot}, total clearance. Dose = 1 mg/kg. N = 2 determination. [b]Remaining amount in rat serum after 30 min incubation. [c]Remaining amount in pH 7.0 phosphate buffer at 40 ˚C after 16 h. [d]n.c. = not calculated because of its low stability in rat serum.

Table 2-9. Compound Stabilities in Serum

	residual ratio in serum after 30 min incubation (%)				
compound	rat	mouse	dog	monkey	human
11b	<1	55	>99	94	89
51	58	94	>99	>99	90

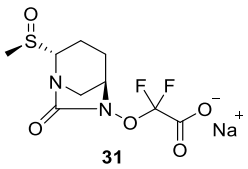
第 6 節　2 位と 6 位の組み合わせ最適化

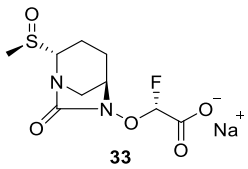
ラットも含めてすべての動物種の血漿中安定性が改善された化合物を見出すべく，2 位と 6 位の置換基の最適な組み合わせの探索を実施した (Table 2-10)．前節の Table 2-8 で未実施であった 2 位スルホキシドまたはスルホンアミドと 6 位フルオロ酢酸タイプの組み合わせを行ったところ，6 位ジフルオロ酢酸体 **(31**, **65)** については buffer 中での化学的な安定性が低く，それと連動して血漿中安定性の低下と CL の増大が観測された．一方で，モノフルオロ酢酸体 **(33**, **68)** は buffer 中，血漿中ともに良好な安定性を示し，CL が低下した．

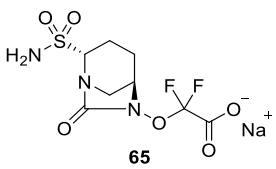
良好な PK プロファイルをもった化合物 **33**，および，**68** の活性を Table 2-11 に示す．スルホキシド **33** は評価した 3 種類の β-ラクタマーゼ全てに対して良好な阻害活性を示すとともに，これらの β-ラクタマーゼ産生株に対してセフィキシム (CFM) と併用することで，CFM の抗菌活性を大きく回復させた．一方で，スルホンアミド **68** は，**33** と比較すると酵素阻害活性が弱く，CFM の活性回復効果も低かった．

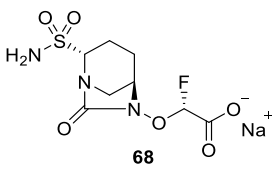
これらの結果から，活性と動態の両立が可能である，2 位スルホキシドと 6 位モノフルオロ酢酸が組み合わせられた化合物 **33** をリードとして選抜した．第 3 章にてこの化合物の構造最適化の詳細を述べる．

Table 2-10. PK of 31, 33, 65, and 68 in Rats after Intravenous Administration[a]









comp.	AUC (μg·h/mL)	$T_{1/2}$ (h)	CL_{tot} (mL/min/kg)	rat serum stability (%)[b]	solution stability (%)[c]
31	0.060	0.1	289	-	17
33	0.618	0.2	27.0	85	95
65	0.228	0.1	84.5	39	55
68	0.851	0.3	19.8	78	91

[a]AUC, area under the plasma concentration-time curve from time zero to infinity; $T_{1/2}$, apparent terminal elimination half-life; CL_{tot}, total clearance. Dose = 1 mg/kg. *N* = 2 determination. [b]Remaining amount in rat serum after 30 min incubation. [c]Remaining amount in pH 7.0 phosphate buffer at 40 ˚C after 16 h. [d]n.c. = not calculated because of its low stability in rat serum.

Table 2-11. IC_{50}s and MICs of CFM in the Presence of 33 or 68[a]

comp.	Mw[f]	clogP[f]	IC_{50} (μM)[b]			MIC (μg/mL) of CFM in the presence of 4 μg/mL of BLI[c]	
			KPC-2 (class A)	CTX-M-15 (class A)	CMY-2 (class C)	*K. pneumoniae* ATCC-BAA-1705 KPC-2	*E. coli* SR09613 CMY-2 type
33	280	−0.30	0.014	0.012	0.028	0.25 (>32)[d]	0.125 (>32)[d]
68	297	−0.63	0.977	0.249	1.11	0.25 (32)[d]	1 (>32)[d]
alone	-	-	-	-	-	>32[e]	>32[e]

[a]CFM: cefixime. [b]*N* = 1 determination. The values of IC_{50} were determined without enzyme-inhibitor preincubation. [c]*N* = 1 determination. [d]MIC of BLI. [e]MIC of CFM alone. [f]Calculated as a free form.

第７節　本章のまとめ

本章では，2 位に各種硫黄系官能基をもった DBO 誘導体の BLI としての有効性について，活性，および，薬物動態の観点から評価した．その結果，2 位への硫黄置換基の導入はβ-ラクタマーゼ産生株を用いた評価においてセフィキシムの抗菌活性を大幅に改善し，その効果は既存の DBO 系 BLI であるアビバクタムを上回るものであった．β-ラクタマーゼ阻害活性 (IC_{50}) の観点からは，スルホン，スルホキシドの導入が特に有効であり，この変換によって 6 位をフルオロ酢酸に変更しても活性を維持することが明らかになった (Figure 2-1)．さらに，これらの DBO 誘導体は multi-PBP 阻害のメカニズムによって抗菌活性を示し、PBP2 選択的なナクバクタムやジデバクタムといった既存の DBO 誘導体とは異なり，幅広い菌株に対して抗菌活性を示した．

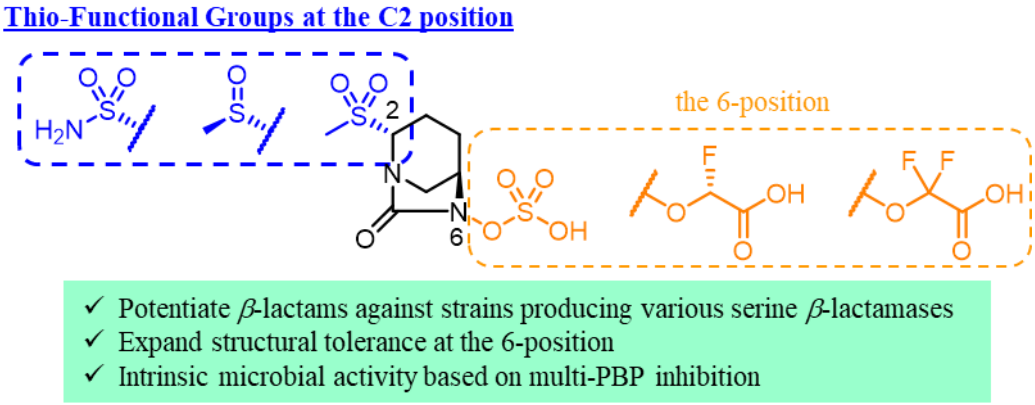


Figure 2-1. Summary of the effect of thio-functinal group at the C2 position on the biological activity.

また，これらの誘導体の多くにおいて血漿中安定性が低いことが原因で十分な血中暴露を維持できないという課題があったが，2 位と 6 位の置換基の組み合わせを網羅的に検討した結果，2 位スルホキシド，6 位フルオロ酢酸の誘導体が活性と動態を両立できることを見出した (Figure 2-2).

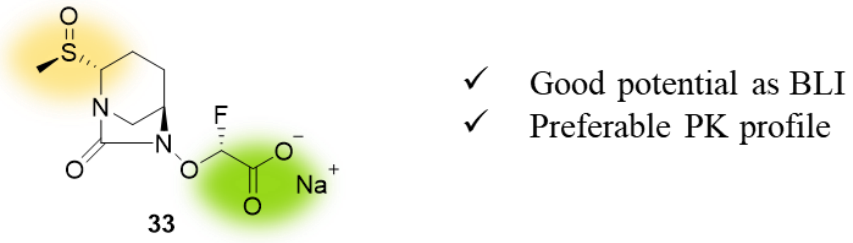


Figure 2-2. Structure of the lead compound **33** which balances BLI potential and PK profile.

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第３章　2 位にスルホキシドを有する 1,6-ジアザビシクロ[3.2.1]オクタン誘導体の構造最適化

第１節　多様な側鎖を持った 2-スルフィニル-DBO の合成

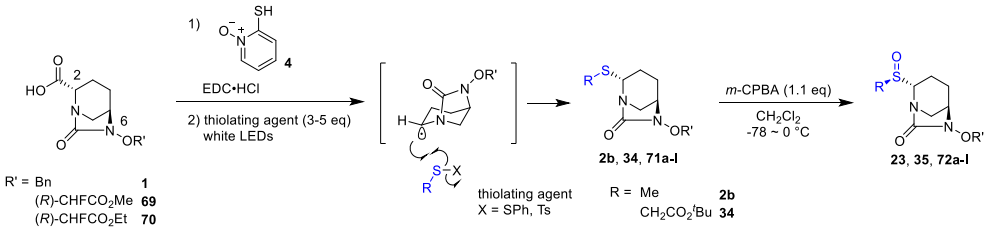
前章で見出したリード化合物 **33** の 2 位側鎖の最適化を行うために多様な側鎖をもった 2-スルフィニル-DBO 誘導体の合成を行った．これらの化合物の合成にあたっては，脱炭酸的ラジカル反応によるスルフィド側鎖の導入とジアステレオ選択性なスルホキシドへの酸化が重要な工程であるため，これらを Table 3-1 にまとめた．なお，中間体であるスルフィド **(2b, 34, 71a**–**71l)** はシリカゲルに対して不安定な可能性があるため，いずれもカルボン酸からスルフィドへの変換とスルホキシドへの酸化の２工程を経て単離した結果を示している．

第２章第１節において分子量が小さく脂溶性の低い側鎖が菌に対する活性に重要であることが示唆されていたため，シンプルな極性基を中心に合成を行った．これらの側鎖はアルキル系の置換基であるため，第１章第２節で見出した非対称ジスルフィドやチオスルホネートを-SR 源として用いることで，試薬の当量数を 3–5 当量まで削減し，効率的に合成を行った．Barton エステルを用いた脱炭酸的なスルフィド構築反応は古くから知られているが，[1] -SPh や-SMe の導入が大部分であり，多様な-SR 基の導入例としては筆者が知る限り本報告が初めてである．

脱炭酸的ラジカル反応においては，どの試薬を用いた場合にも反応は立体選択的に covex 面から進行し，いずれの反応においてもスルフィド **(2b**, **34**, **71a**–**71l)** の立体異性体は観測されなかった．本工程においては側鎖の立体障害が収率に影響することが確認され，立体障害が最も小さい-SMe **(23)** と比較して，側鎖が伸長した **72c**, **72e**, **72g** では収率が低下し，最もかさ高い ‘Bu 基 **(72k)** では目的物が得られなかった（6 位-OBn での比較）．目的物が得られなかったり，収率が低い場合には，Barton エステル由来の-SPy 基と再結合した副生成物 **85** の生成が増加することが観測された (Scheme 3-1)．すなわち，この再結合反応が目的の反応と競合しており，-SR 源となる試薬の反応性が低い場合には，副生成物 **85** が優先的に生成することで，目的物の収率が低下していると考えられる．立体障害とは別に，側鎖の電子求引性も収率に影響を与えており，電子求引基を持った **35**, **72h** は同様に硫黄原子の α 位がメチレンとなっている **72c**, **72e**, **72g** と比較して収率が高かった（6 位-OBn での比較）．なお，電子求引基であるものの末端にアミドをもった **72l** に関しては合成することができなかった．また，基質の観点からは 6 位にフルオロ酢酸エステルが導入されている **69**，**70** と比較して，Bn 保護体 **1** の方が収率が良い傾向にあった．

スルフィドからスルホキシドへの酸化反応については，側鎖によらず良好な選択性で酸化反応が進行した．第２章において X 線結晶構造解析により **23** の相対配置を決定しているが，^{1}H NMR における 2 位プロトンの化学シフトの類似性から，今回合成した他の側鎖に関しても同様のジアステレオマーが主生成物で得られていると推定している．

Table 3-1. Substrate Scope and Limitation of Photo-Induced Decarboxylative Thiolation and Diastereoselectivity of Oxidation to Sulfoxides



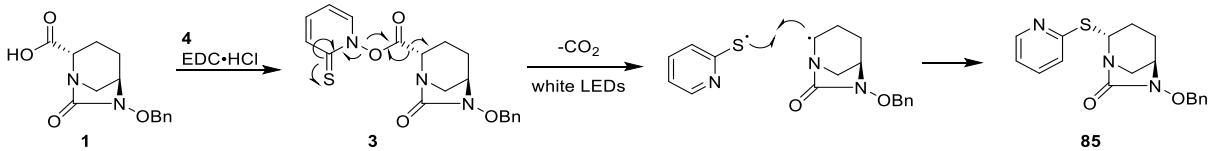
	23	72a	35	72b
Yield	29%	26%	36%	23%
dr of sulfoxide[b]	>10/1	>10/1	7/1	7/1
thiolating agent	PhS**SMe** (**5b**)	Ts**SCH$_2$F** (**73**)	Ts**SCH2CO$_2$^{t}Bu** (**40**)	Ts**SCH$_2$CN** (**74**)

	72c	72d	72e	72f[c]
Yield	24%	12%	22%	21%
dr of sulfoxide[b]	>10/1	>10/1	>10/1	>10/1
thiolating agent	Ts**S(CH$_2$)$_2$OTBS** (**75**)	PhS**S(CH$_2$)$_3$OTBS** (**76**)	PhS**SCH$_2$-((S)-2,2-diMe-1,3-dioxolan-4-yl)** (**77**)	Ts**SCH$_2$(C=NOPMB)CO$_2$(CH$_2$)$_2$TMS** (**78**)

	72g	72h	72i	72j
Yield	16%	59%	14%	18%
dr of sulfoxide[b]	>10/1	dr = 7/1	7/1	5/1
thiolating agent	Ph**S(CH$_2$)$_2$NHBoc** (**79**)	Ts**SCH$_2$(5-Me-1,3,4-oxadiazol-2-yl)** (**80**)	Ts**SCH$_2$(1-Boc-imidazol-4-yl)** (**19**)	Ts**SCH$_2$(2-Boc-amino-thiazol-5-yl)** (**82**)

	72k	72l
Yield	not obtained	not obtained
thiolating agent	Bs**S^{t}Bu** (**83**)	Ts**SCH$_2$CONH$_2$** (**84**)

Scheme 3-1. Mechanism for the Formation of Byproduct 85

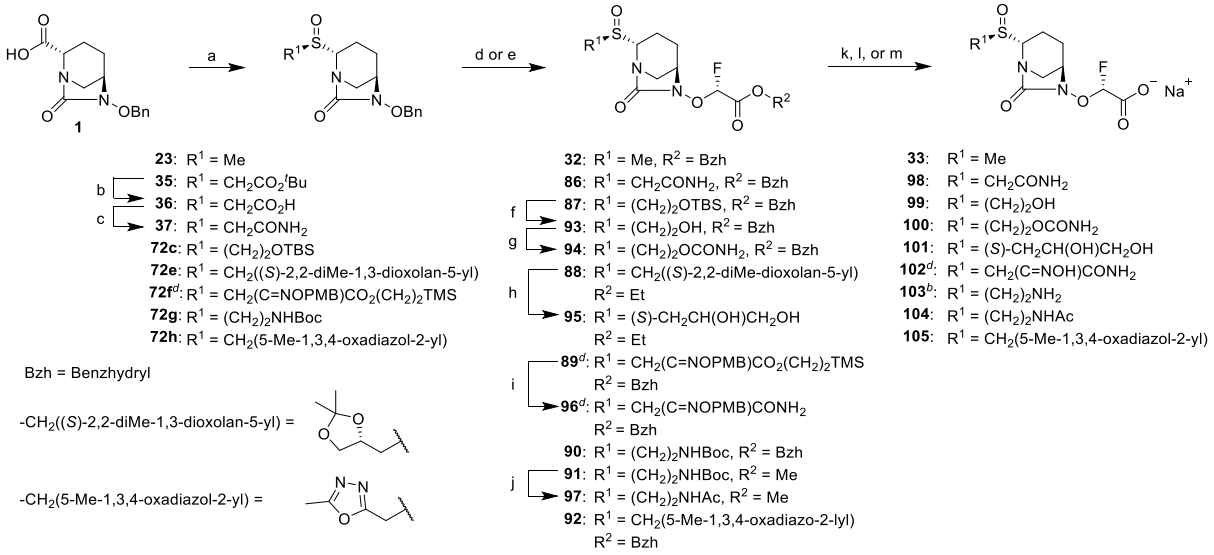


ここまでは，鍵工程となるスルフィドの合成とジアステレオ選択的な酸化について述べてきたが，目的のβ-ラクタマーゼ阻害剤 (BLI) の合成の全工程について以下に示す．脱炭酸的ラジカル反応による-SR 基の導入を鍵反応として，２つの合成ルートで目的の 2-スルフィニル-DBO 誘導体を合成した．第一のルートは，2 位に-SR 基を導入した後に 6 位をフルオロ酢酸ユニットに変換するルートであり，単一の化合物の合成においては最短の工程数で目的物を合成することができる．もう一方のルートは，あらかじめ 6 位にフルオロ酢酸ユニットを導入した後に，2 位を変換するルートであり，多様な 2 位側鎖を持った誘導体を効率的に合

成できる．

第一の合成ルートによる BLI の合成を以下に示す (Scheme 3-2)．Table 3-1 の条件にてスルホキシドを構築した後に (工程 a)，**35** の ^{t}Bu エステルを脱保護し，アンモニアと縮合することでアミド **37** を合成した (工程 b, c)．続いて，触媒量の DABCO を添加した条件で，$Pd(OH)_2$ 触媒下，水素添加反応にて脱 Bn 化を行い，得られたヒドロキシルアミンに対して，DBU (工程 d) または K_2CO_3 (工程 e) を塩基として用いてフルオロ酢酸ユニットを導入した．ヒドロキシエチル基を側鎖にもった **87** は，TBAF 条件にて TBS 基の除去を行い (工程 f)，生じた第一級ヒドロキシ基をクロロスルホニルイソシアネート (CSI) と反応させた後，$NaHCO_3$ 水溶液で処理することでカルバモイル基へと変換した (工程 g)．側鎖にジオール構造を持った **88** は，保護基であるアセトナイドを $AlCl_3$/アニソールの条件で脱保護した (工程 h)．化合物 **89** の側鎖のトリメチルシリルエチルエステルを，ジフルオロトリメチルケイ酸トリス(ジメチルアミノ)スルホニウム (TASF) にて脱保護した後に，クロロギ酸イソブチルを用いた混合酸無水物を経由してアミド **96** へと変換した (工程 i)．側鎖にアミノエチル基をもった **91** は，TFA にて脱 Boc 化した後，Ac_2O を用いてアセチル化し，**97** を合成した (工程 j)．ベンズヒドリル (Bzh) またはエチルエステルの加水分解によって，目的の化合物 **33**, **98**, **101**, **105** を得た (工程 k)．また，Bzh エステルは $AlCl_3$ を用いた条件でも脱保護可能であり，側鎖の保護基と同時に脱保護することで **99**, **100**, **102** を得た (工程 l)．さらに，Bzh エステルと Boc 基を TFA で同時に脱保護することで，**90** から **103** を側鎖のアミンの TFA 塩として得た (工程 m)．

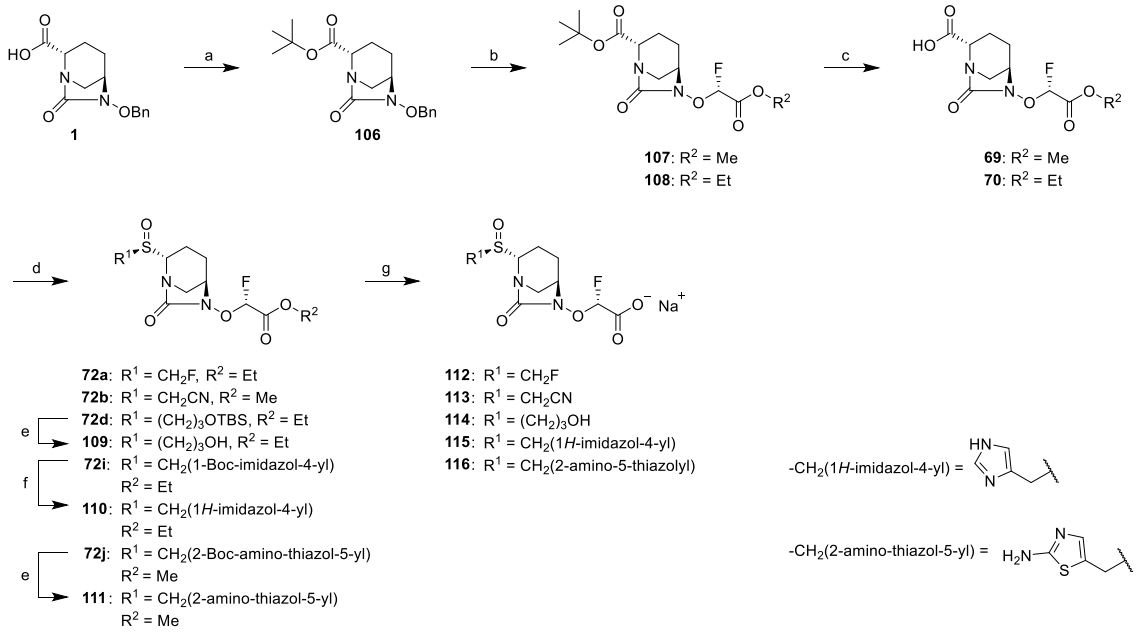
Scheme 3-2. Synthesis of BLI 33 and 98-105



[a]Reagents and conditions: (a) Conditions are shown in **Table 4-1**; (b) TFA, 0 ˚C, 86%; (c) EDC·HCl , HOSu, CH_2Cl_2, −50 ˚C, then aq NH_3, rt, 80%; (d) (i) $Pd(OH)_2$ on carbon, DABCO, H_2 (1 atm), rt; (ii) (*R*)-$BrCHFCO_2Bzh$ (**22e**), DBU, DMF, 0 ˚C, 9–78% over two steps for **32**, **86**, **87**, **89**, **90**, and **92**; (e) (i) $Pd(OH)_2$ on carbon, DABCO, H_2 (1 atm), rt; (ii) (*R*)-$BrCHFCO_2R$, R = Me (**22f**), Et (**22d**), K_2CO_3, DMF, −40 to −20 ˚C, 20–68% over two steps for **88** and **91**; (f) TBAF, AcOH, MS3A, THF, rt, 10%; (g) CSI, THF, −30 ˚C, then aq $NaHCO_3$, 84%; (h) $AlCl_3$, anisole, $MeNO_2/CH_2Cl_2$, −30 ˚C, 78%; (i) (i) TASF, DMF, rt; (ii) isobutyl chloroformate, Et_3N, CH_2Cl_2, 0 ˚C, then aq NH_3, 0 ˚C, 64% over two steps; (j) (i) TFA, CH_2Cl_2, 0 ˚C; (ii) Ac_2O, CH_2Cl_2, −78 ˚C to rt, quant. over two steps; (k) NaOH, THF/H_2O, 0 ˚C, 43–78% for **33**, **98**, **101**, **104**, and **105**; (l) $AlCl_3$, (anisole), $MeNO_2/CH_2Cl_2$, −30 ˚C, then aq $NaHCO_3$ 49–79% for **99** (from **87**), **100**, and **102**; (m) TFA, CH_2Cl_2, 0 ˚C, quant. for **103** (from **90**). [b]Compound **103** is not Na salt but TFA salt of amine on the side chain. [d]The oxime was obtained as a single geometrical isomer, but the geometry (*E*/*Z*) is unknown.

2 位の変換体を効率的に合成するために，先に 6 位にフルオロ酢酸を導入し，後から 2 位を変換するルートでの誘導体合成も行った (Scheme 3-3)．化合物 **1** のカルボン酸を EDC/DMAP 条件にて，^{t}BuOH と縮合することで ^{t}Bu エステル **106** へと変換し (工程 a)，6 位の脱 Bn 化とフルオロ酢酸ユニットの導入によって **107**, **108** を合成した (工程 b). これらの化合物の ^{t}Bu 基を $TiCl_4$ によって脱保護することで，共通中間体であるカルボン酸 **69**, **70** を合成した (工程 c). 続いて, Table 3-1 の条件にて 2 位をスルホキシドへと変換した後に (工程 d), **72d** の TBS 基および **72j** の Boc 基を $AlCl_3$ で除去して **109** および **111** を得た (工程 e)．また，**72i** のイミダゾールの Boc 基を TFA にて除去して，**110** を得た (工程 f)．最後に 6 位のフルオロ酢酸エステルを NaOH にて加水分解して，目的の **112**–**116** を得ることができた (工程 g).

Scheme 3-3. Synthesis of BLI 112-116



[a]Reagents and conditions: (a) ^{t}BuOH, pyridine, EDC·HCl , DMAP, CH_2Cl_2, rt, 66%; (b) (i) $Pd(OH)_2$ on carbon or Pd/C, H_2 (1 atm), (DABCO), MeOH or DMF, rt; (ii) (*R*)-$BrCHFCO_2R$, R = Me (**22f**), Et (**22d**), K_2CO_3, DMF, −40 to −30 ˚C, 21–55% over 2 steps; (c) $TiCl_4$, $MeNO_2/CH_2Cl_2$, −30 ˚C, 78–89%; (d) Conditions are shown in **Table 4-1**; (e) $AlCl_3$, $MeNO_2/CH_2Cl_2$, −30 ˚C, 66–72%; (f) TFA, CH_2Cl_2, rt, quant.; (g) NaOH, THF/H_2O, 0 ˚C, 50–66%.

第２節　２位側鎖の構造活性相関

合成した 2-スルフィニル-DBO 誘導体の評価を行った (Table 3-2)．酵素阻害活性 (IC_{50}) と共に，本章では minimum potentiating concentration (MPC) を評価の指標とする．今回併用した β-ラクタム系抗菌薬は経口セフェムであるセフチブテン (CTB) であり，その腸内細菌科細菌に対する EUCAST (The European Committee on Antimicrobial Susceptibility Testing) の定める 1 日 1 回 (QD, quaque die) ，400 mg，経口投与時の breakpoint は 1 μg/mL である．[2] ここでは CTB の最小発育阻止濃度 (MIC) を 1 μg/mL まで回復させるために必要な最小の β-ラクタマーゼ阻害剤濃度を MPC_1 と定義した．MPC_1 の値が小さいほど，β-ラクタマーゼ阻害剤 (BLI) としてのポテンシャルが高いということになる．

第２章第１節でも述べた通り，グラム陰性菌の外膜を透過するためには低分子量であることや親水性が高いことが重要であるため，シンプルな極性基を中心に SAR の検討を行った

(**99**–**101**, **103**, **104**, **114**). また，ラクタマーゼとの反応性を上げるために電子求引基が有効である可能性があるので，フッ素(**112**)，アミド(**98**)，ニトリル(**113**)，オキシム(**102**)といった電子求引基も導入した．これらに加えて，親水性の芳香環としてヘテロ環をもった化合物もデザインした (**105**, **115**, **116**).

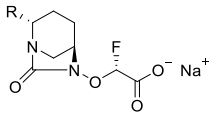
前章で見出した化合物 **33** は，IC_{50}，MPC_1 ともにアビバクタム (AVI) と同等以上の結果であった. 一方で, スルホキシドの立体化学が **33** と反対のジアステレオマーである **117** は，IC_{50} の大きな減弱が確認された．酵素阻害活性の減弱にも関わらず，**117** は ATCC BAA-1705 および SR34100 に対して **33** と同等の有効性を示し，SR09613 に対してのみ有効性の減弱が見られた．この結果は，セフチブテン (CTB) の各 β-ラクタマーゼに対する安定性の差から考察できる．各種ラクタマーゼを発現させた形質転換株に対する CTB の MIC を Table 3-3 にまとめたが，この結果から，CTB は class A の β-ラクタマーゼである KPC-2 や CTX-M-15 の発現による MIC の減弱よりも，class C の CMY-2 の発現による MIC の減弱の方が大きいことが分かる．つまり，CTB は KPC-2 や CTX-M-15 よりも CMY-2 に対して不安定であると可能性が高い．Table 3-2 で評価した３つの菌株はこれらの β-ラクタマーゼの産生株であるが，CTB の β-ラクタマーゼに対する安定性の差から，CMY-2 産生株である SR09613 においてのみ IC_{50} の減弱の影響が顕著に表れたと考えている.

スルホキシドのジアステレオマーである **117** を除いて，多くの誘導体では顕著な IC_{50} の減弱は見られなかったが，いくつかの化合物において阻害活性の減弱が確認された．オキシム構造を導入した **102** については，今回評価した 3 つのラクタマーゼ全てにおいて>5 倍の活性減弱が認められ，塩基性のアミノ基をもった **103** においては，CTX-M-15 と CMY-2 に対して >10 倍の活性減弱が観測された．これらの化合物を除いては、顕著な IC_{50} の減弱はなかったものの，ほとんどの化合物において **33** と比較して 4 倍以上の MPC_1 の悪化が確認された．これは、分子量の増大に起因した外膜透過性の悪化が一因であると考えられ，今回評価した化合物の中でも，分子量が小さく，親水性も高い **98** と **113** のみが化合物 **33** と同等の MPC_1 を示した. 同様の好ましい物性値をもった **103** についても, 酵素阻害活性が減弱している CMY-2 産生の SR09613 を除いた２つの株 (ATCC BAA-1705, SR34100) に対して，化合物 **33** と同等の併用効果を示した．反対に，強い酵素阻害活性を持っていたとしても，分子量が 350 を超える化合物は全て ATCC BAA-1705 および SR34100 に対する MPC_1 が 4 倍以上悪化した (**100**, **102**, **104**, **105**, **115**, **116**).

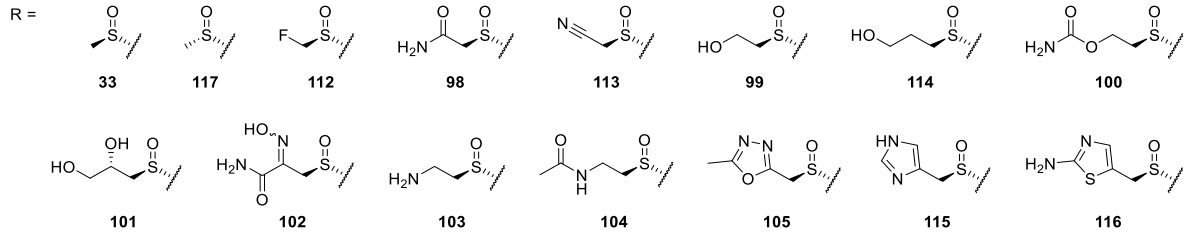
第２章第４節において，2 位に硫黄置換基をもった DBO 誘導体は，multi-PBP 阻害に基づくユニークな抗菌活性を示すことを報告したが，今回の一連の化合物は抗菌活性をほとんど示さなかった．このことから，これらの化合物は自身のもつ抗菌活性ではなく，β-ラクタマーゼ阻害活性によって CTB の抗菌活性を回復させていると考えられる.

以上の検討より，今回検証した３種類の β-ラクタマーゼに対して良好な IC_{50} を示し，β-ラクタマーゼ産生株を用いた MPC_1 の評価においても全ての株に対して良好な結果を与えた化合物 **33** が最も有望な化合物であると結論し，さらなる評価を行うこととした.

Table 3-2. SAR of Side Chain of Sulfoxide[a]



R =



comp.	Mw[h]	clogP[h]	IC_{50} (μM)[b]			MPC_1 (μg/mL)[c] / MIC (μg/mL)[d]		
			KPC-2 (class A)	CTX-M-15 (class A)	CMY-2 (class C)	*K. pneumoniae* ATCC BAA-1705[e] 8 μg/mL[f]	*E. coli* SR34100[e] >32 μg/mL[f]	*E. coli* SR09613[e] >32 μg/mL[f]
33	280	−0.30	0.014	0.012	0.028	0.063 / 32	0.125 / 32	0.125 / 32
117	280	−0.30	0.443	0.445	0.655	0.125 [≤0.031][g] / 32	0.125 / 16	0.5 / 32
112	298	−0.13	0.090	0.006	0.113	0.5 / 32	0.25 / 32	0.25 / 32
98	323	−1.04	0.035	0.050	0.057	0.063 / 32	0.25 / 32	0.125 / 32
113	305	−0.52	0.039	0.007	0.096	≤0.031 / >32	0.25 / >32	0.125 /32
99	310	−0.55	0.036	0.016	0.057	1 / >32	0.5 / >32	0.125 / >32
114	324	−0.30	0.038	0.010	0.039	0.5 / >32	1 / >32	0.063 / >32
100	353	−0.51	0.027	0.014	0.064	1 / >32	1 / >32	0.125 / >32
101	340	−0.45	0.056	0.010	0.052	0.25 / >32	1 / >32	0.125 / >32
102	366	−0.86	0.132	0.070	0.143	2 / >32	2 / >32	0.25 / >32
103	309	−2.75	0.069	0.159	0.431	≤0.031 / >32	0.125 / >32	0.5 / >32
104	351	−0.59	0.007	0.101	0.101	0.25 / >32	0.5 / >32	0.063 / >32
105	362	−1.88	0.016	0.024	0.036	1 / >32	1 / >32	0.125 / >32
115	346	−0.77	0.078	0.008	0.059	1 / 32	1 / 32	0.125 / 32
116	378	−0.51	0.088	0.010	0.041	0.25 / >32	1 / >32	0.5 / >32
AVI	265	−1.63	0.072	0.013	0.059	0.125 / 32	0.125 / 16	0.5 / 16

[a]Red: >5-fold increase on IC_{50} value compared to **33**. Pink: ≥4-fold increase on MPC_1 value compared to **33**. AVI: avibactam. CTB: ceftibuten. [b]The values of IC_{50} were determined without enzyme-inhibitor preincubation. *N* = 1 determination. [c]MPC_1 to reduce the MIC of CTB to 1 μg/mL. *N* = 1 determination. [d]MIC of BLI. *N* = 1 determination. [e]Strain ID. The followings are the expressed *β*-lactamases and the deletion of porin on each strain: ATCC BAA-1705, KPC-2 and ΔompK35; SR34100, CTX-M-15; SR09613, CMY-2 type. [f]The MIC of CTB alone. [g]MPC_1 did not converge on an identical value. "X [Y]" means a compound reduced MIC of CTB to 1 μg/mL at its concentration of X and Y skipping values between them. [h]Calculated as a free form.

Table 3-3. MICs of CTB against Isogenic Strains of *E. coli* Expressing β-Lactamase

β-lactamase	parent	KPC-2	CTX-M-15	CMY-2	OXA-48
MIC (μg/mL)[a]	0.031	0.25	0.25	8	0.031

[a]*N* = 1 determination.

第３節　化合物 33 の多数株評価

最も有望な化合物と考えられた **33** について，多数の臨床分離株に対する有効性の評価を行った (Table 3-4)．その結果，化合物 **33** は class A, C, D 全てのクラスのセリン β-ラクタマーゼ産生株に対して優れた有効性を示すことが明らかとなった．特に，複数の株において優位にアビバクタム (AVI) を上回る併用効果を示した点は注目に値する．SR100758 は，AVI による阻害効率が低下する D179Y 変異の KPC-2 産生株であり，[3] AVI の MPC_1 は変異の無い KPC-2 産生株である ATCC BAA-1705 (Table 3-2) と比較して 16 倍低下していたが，**33** の有効性の低下は 4 倍にとどまり，変異の β-ラクタマーゼに対しての有効性を維持していた．また，いくつかの class C に属する β-ラクタマーゼ産生株に対して，**33** は AVI を大きく上回る有効性を示した (SR100108, SR09603)．今回の評価で用いた臨床分離株は、高度に耐性化した株のセットであるにもかかわらず，全ての株に対して $MPC_1 \leq 4$ μg/mL を達成することができており，化合物 **33** は BLI として高いポテンシャルを有することが明らかになった．

Table 3-4. MPC_1 and MIC of 33 and AVI against a Set of Clinical Isolates[a]

species	strain ID	characteristics[b]	MIC of CTB (μg/mL)	MPC_1 (μg/mL)[c] / MIC (μg/mL)[d]	
				33	**AVI**
E. cloacae	SR200030	PER-2, ACT, ΔompF	>32	4 / >32	16 / 32
K. pneumoniae	SR100758	KPC-2 (D179Y)	16	0.25 / >32	2 / >32
K. pneumoniae	SR201325	KPC-3	32	2 / >32	2 / 32
E. cloacae	SR36276	AmpC	>32	2 / >32	4 / 32
E. cloacae	SR100108	AmpC	>32	1 / >32	8 / 32
K. pneumoniae	SR09635	DHA-1	>32	2 / 32	0.5 / >32
K. pneumoniae	SR09603	CMY-8 type	>32	0.125 / 32	8 / >32
K. pneumoniae	SR201218	OXA-48, CTX-M-15	>32	2 / >32	1 / >32
K. pneumoniae	SR200263	OXA-48, CTX-M-1 group	>32	0.125 / 32	0.063 / >32
K. pneumoniae	SR200487	VEB-8, CTX-M-15, SHV-110, CMY-16, OXA-48, OXA-10	>32	0.5 / >32	4 / >32

[a]Blue: ≥8-fold reduction compared to AVI, Red: ≥8-fold increase compared to AVI. AVI: avibactam. CTB: ceftibuten. [b]The expressed β-lactamases and the deletion of porin on each strain. [c]MPC_1 to reduce the MIC of CTB to 1 μg/mL. N = 1 determination. [d]MIC of BLI. N = 1 determination.

ここまで触れてこなかったが，class D に分類される OXA-48 に対する酵素阻害活性を以下に示す (Table 3-5)．化合物 **33** は，OXA-48 に対する IC_{50} では AVI を>10 倍上回わっており，この点からも優れた BLI であることが示唆される．なお，今回 β-ラクタムとして用いたセフチブテンは OXA-48 発現株に対して MIC の減弱が無いため (Table 3-3)，主薬としてセフチブテンを用いる場合には，BLI の OXA-48 に対する阻害活性を考慮する必要性は低く，OXA-48 産生株 (Table 3-4: SR201218, SR200263) に対する効果も AVI と比較して大きな差は見られなかった．

Table 3-5. Inhibition Activity against OXA-48

compound	**33**	**116**	**117**	AVI
IC_{50} against OXA-48 (μM)[a]	0.443	0.981	32.6	5.70

AVI: avibactam. [a]The values of IC_{50} were determined without enzyme-inhibitor preincubation. *N* = 1 determination.

第 4 節　化合物 33 の動態プロファイル

化合物 **33** を iv 投与した時の各種動物種における動態パラメータを以下に示す (Table 3-6)．この化合物は中程度のクリアランス (CL_{tot}) と短い血中半減期 ($T_{1/2}$) を示したが，これらの値はアビバクタムを含む他の DBO 誘導体と同程度であった．[4], [5], [6] また，全ての動物種において血漿中フリー濃度（PB % unbound）は高く，この傾向はヒトの血漿を用いた試験においても確認された（PB in human = 81% unbound）．β-ラクタム系抗菌薬や BLI においては，薬物の血中フリー濃度が *in vivo* 薬効と相関することが報告されているため，[9] この結果は薬効試験において良い結果を与える可能性を示唆するものである．一方で，脂溶性が低い (clogP = −0.30) ことから予想されるように，化合物 **33** を経口投与した場合の経口吸収性は低かった（*F* = 1.4% in rat）．

Table 3-6. PK Parameter of 33 after Intravenous Administration[a]

Animal	Dose (mg/kg)	AUC (μg·h/mL)	$T_{1/2}$ (h)	CL_{tot} (mL/min/kg)	PB (% unbound)
Mouse	10	4.92 ± 0.94	0.4 ± 0.2	34.7 ± 6.8	74
Rat	1	0.618[b]	0.2[b]	27.0[b]	100
Dog	5	19.4 ± 1.1	0.9 ± 0.1	4.30 ± 0.24	86
Monkey	5	13.8 ± 2.2	0.5 ± 0.1	6.15 ± 1.06	84

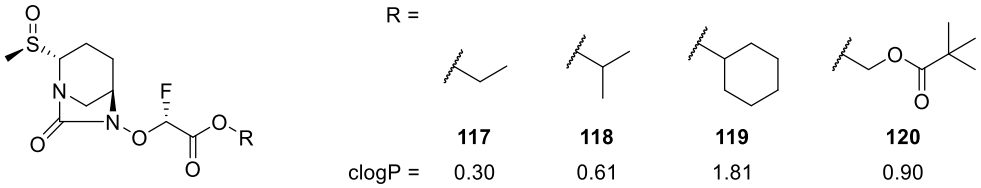
[a]*N* = 3 determination. AUC, area under the plasma concentration-time curve from time zero to infinity. $T_{1/2}$, apparent terminal elimination half-life. CL_{tot}, total clearance. PB, serum protein binding. [b]*N* = 2 determination.

第５節　経口プロドラッグの探索

化合物 **33** 自身の経口吸収性は低かったことから，プロドラッグ化による経口吸収性の改善を試みた (Table 3-7). 上市されているエステルプロドラッグの構造を参考に，[7] いくつかのアルキルエステル **117**–**119** を合成した．また，β-ラクタム系抗菌薬のプロドラッグとして実績のあるピボキシル[8] **120** の合成も試みたが，カルボン酸側が反応性の高いフルオロ酢酸構造であることにより，**120** は非常に不安定であり単離することができなかった．

プロドラッグ **117**–**119** は，いずれも化合物 **33** の経口吸収性を大きく改善した．また，エステル構造は経口吸収後速やかに活性本体 **33** へと加水分解され，血中において未反応のプロドラッグは検出されなかった．エステルの構造間において経口吸収性の差は見られたが，T_{max} をはじめとしたその他の動態パラメータには違いはなかった．一方で，pH 7.4 の buffer 中での安定性は分枝型のエステル **(118**, **119)** の方が直線型のもの **(117)** よりも高かった．このように溶液安定性が良好で，非齧歯類（サル，イヌ）における経口吸収性が高かったシクロヘキシルエステル **119** を選抜し，次の経口投与での *in vivo* 薬効試験を行うこととした．

Table 3-7. PK Parameter of 33 after Oral Administration of Prodrugs[a]



comp.	Rat (fed, 5 mg/kg, *N* = 2)				Dog (fasted, 10 mg/kg, *N* = 2)			
	C_{max} (μg/mL)	AUC_{po} (μg·h/mL)	T_{max} (h)	*F* (%)	C_{max} (μg/mL)	AUC_{po} (μg·h/mL)	T_{max} (h)	*F* (%)
117	2.15	2.30	0.5	74.4	10.6	26.0	1.0	68.3
118	1.70	2.09	0.5	67.6	7.39	18.4	0.8	48.1
119	0.62	1.17	0.5	37.8	15.8 ± 1.4[b]	41.2 ± 3.8[b]	1.0 ± 0.0[b]	106.1 ± 7.4[b]

comp.	Monkey (fasted, 10 mg/kg, *N* = 3)				solution stability at pH 7.4 (%)[c]
	C_{max} (μg/mL)	AUC_{po} (μg·h/mL)	T_{max} (h)	*F* (%)	
117	4.51 ± 0.33	8.32 ± 1.96	1.0 ± 0.0	29.9 ± 2.8	11
118	4.38 ± 1.10	7.06 ± 1.26	1.0 ± 0.0	25.5 ± 0.7	56
119	7.94 ± 2.74	15.2 ± 2.7	1.0 ± 0.0	54.8 ± 3.7	56

[a]C_{max}, peak concentration. AUC, area under the plasma concentration-time curve from time zero to infinity. T_{max}, time at peak concentration. *F*, bioavailability calculated from AUC_{po} and AUC_{iv} of **33**. [b]*N* = 3 determination. [c]Remaining amount in pH 7.4 phosphate buffer at 40 ˚C after 16 h.

第６節　経口投与による *in vivo* 薬効

マウス尿路感染モデルにおいて，セフチブテン (CTB) とプロドラッグ **119** を併用した *in vivo* 薬効評価を行った (Figure 3-1)．このモデルは，マウスの尿路に KPC 産生株である *K. pneumoniae* SR08667 を感染させたものであり，この株に対する CTB 単剤での抗菌活性を調べたところ，MIC = 32 μg/mL であった．また，この株に対してプロドラッグ **119** の活性本体である **33** が，CTB の MIC を 1 μg/mL まで回復させるのに必要な最小濃度である MPC_1 は 1 μg/mL であった．この試験において，CTB 単剤を 30 mg/kg で投与した場合には，投与開始前と比較して腎内の菌数は減少せずに 2 log (100 倍) 以上も増加する．これに対して，CTB の投与量を 10 mg/kg に固定した上で，**119** を 3, 10, 30 mg/kg と投与量を増やしていったところ，10 mg/kg の投与量で併用した場合において，投与開始時点の菌数と比較して－0.8 log (ca. 1/6) の腎臓内の菌数の減少が確認された．一方で，**119** を 30 mg/kg まで増量してもこれ以上の菌数の減少にはつながらなかった．また，CTB と **119** を共に 30 mg/kg 投与した場合においては，−1 log (1/10) を超える菌数の減少が認められた．以上の結果より，**119** と併用することで CTB の抗菌活性を回復させていることが *in vivo* 試験で確認できた．なお，薬効試験に先立って，マウスにおける **33** の血中濃度の用量依存性を確認したところ，プロドラッグ **119** は 100 mg/kg までの投与量において，用量依存的に血中暴露が増加することを確認している (Table 3-8).

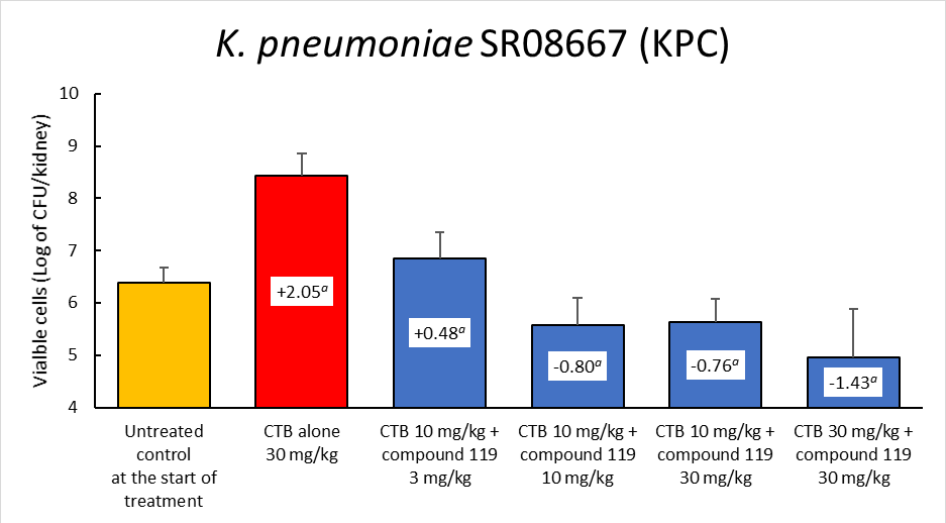


Figure 3-1. *In vivo* efficacy in a murine urinary tract infectious model after oral administration of CTB and prodrug **119**. [a]Change in Log CFU/kidney compared to the untreated control at the start of treatment. *N* = 5 determination. CTB: ceftibuten. CFU: colony forming unit.

Table 3-8. PK Parameter of 33 after Oral Administration of 119 in Mice[a]

Dose (mg/kg)	C_{max} (μg/mL)	AUC (μg·h/mL)	*F* (%)
10	5.6	3.9	80
30	21.4	18.1	122
100	52.0	50.2	102

[a]*N* = 3 determination. C_{max}, peak concentration. AUC, area under the plasma concentration-time curve from time zero to infinity. *F*, bioavailability calculated from AUC_{po} and AUC_{iv} of **33**.

第７節　安全性

薬効を示した化合物 **119** は、ラットを用いた 2 週間連続投与の毒性試験において安全性を確認した．その結果，無毒性量 (NOAEL, no observed adverse effect level) は 600 mg/kg/day であり，致死量 (lethal dose) は>2000 mg/kg/day であった．

第８節　本章のまとめ

本章では，強力な活性と良好な薬物動態プロファイルが両立した 2-スルフィニル-DBO 誘導体の側鎖の最適化を実施し，側鎖に Me 基をもった化合物 **33** が BLI として最も優れた有効性を示すことを見出した．この化合物は多数の臨床分離株を用いた *in vitro* の有効性評価において，既存の DBO 型 BLI であるアビバクタムと同等以上の有効性を示した．化合物 **33** 自身には十分な経口吸収性はなかったが，プロドラッグ化を検討したところ，経口吸収性が大きく改善できることを見出した．この中でも非げっ歯類における経口吸収性が高かったシクロヘキシルエステル **119** を選抜し，*in vivo* 薬効試験を実施した結果，KPC 産生株を用いたマウス尿路感染モデルにおいて，経口投与にてセフチブテンの抗菌活性を回復させることが確認された．この結果から，本研究の目的であるカルバペネム耐性腸内細菌科細菌 (CRE) に有効新規経口 β-ラクタマーゼ阻害剤を見出すことができたと言える．

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結語

本論文では，1,6-ジアザビシクロ[3.2.1]オクタン (DBO) 骨格を有する新規経口 β-ラクタマーゼ阻害剤 (BLI) の探索研究について述べた. DBO 骨格において 2 位の置換基の電子求引性基が β-ラクタマーゼ阻害活性を向上させるという仮説の元，2 位に硫黄置換基をもった新規 DBO 系 BLI に着目し，脱炭酸的ラジカル反応を鍵反応として，この新規 DBO 誘導体の合成法を確立した．このような硫黄置換基の導入は，β-ラクタマーゼ阻害活性を向上させるとともに，6 位の構造的許容性を拡張し，2 位と 6 位の構造の組み合わせの中から，活性だけでなく，医薬品として必要な動態，物性特性を兼ね備えた経口 BLI を同定した．本研究成果は，DBO 系 BLI や DBO 骨格を持った新規抗菌薬のデザインに有用な知見を与えるものである.

本研究により得られた成果を以下に示す.

1. 酸や塩基，熱に対して不安定な DBO 化合物の誘導体合成において，中性・室温条件で進行する光照射による脱炭酸的ラジカル反応が活用できると考え，この反応を利用して， DBO 骨格 2 位に対してジアステレオ選択的に-SR 基を導入することに成功した．また，これまでの報告例では，本反応においては芳香族ジスルフィドと比較して脂肪族ジスルフィドの反応性が低く，過剰量の試薬が必要であることが知られていたが，脂肪族-SR 化試薬を対称なジスルフィドではなく，芳香族チオールと組み合わせた非対称ジスルフィドやチオスルホネートとすることで，反応性を改善し，試薬の当量数を減少させることに成功した．こうして合成したスルフィドから，酸化等の官能基変換を行うことで，目的とした 2 位に硫黄置換基を有する DBO 系 BLI の合成を達成した.

2. 2 位に硫黄置換基を有する DBO 系 BLI が，既存薬であるアビバクタムと同等以上の β-ラクタマーゼ阻害活性を有し，β-ラクタム系抗菌薬であるセフェキシム (CFM) に耐性を持った β-ラクタマーゼ産生菌に対して，CFM と併用することで，その抗菌活性を十分に回復させることを明らかにした．さらに，阻害活性が向上したことで 6 位の構造許容性が拡大し，硫酸構造だけでなく，フルオロ酢酸構造においても強力な活性を示すことを見出した．これらの DBO 誘導体は，ラットにおいて血漿中安定性が低く，血中濃度を十分に維持できないものが多かったが，2 位と 6 位の構造の組み合わせを探索したところ，2 位にスルホキシド，6 位にフルオロ酢酸構造を持った誘導体が，阻害活性とラット血漿中安定性を両立できることを見出した.

3. 上記で見出した DBO 化合物の 2 位スルホキシド側鎖の最適化を検証した結果，メチル基を側鎖とした化合物が最も良い活性を示すことを確認した．この化合物は脂溶性が低く，経口吸収性を示さなかったが，6 位のフルオロ酢酸をエステルプロドラッグへと変換することで経口吸収性が改善できることを見出した．さらに，β-ラクタマーゼ産生の耐性菌を用いた *in vivo* マウス尿路感染モデルにおいて，経口セフェムであるセフチブテン (CTB) の単剤投与では腎臓内の菌数を減少させることはできなかったが，前述のプロドラッグを併用経口投与することで菌数を減少させることを確認した.

業績リスト

論文発表

1) Synthesis of 2-Thio-Substituted 1,6-Diazabicyclo[3.2.1]octane Derivatives, Potent β-Lactamase Inhibitors
Motohiro Fujiu, Katsuki Yokoo, Toshiaki Aoki, Satoru Shibuya, Jun Sato, Kazuo Komano, Hiroki Kusano, Soichiro Sato, Masayoshi Ogawa, and Kenji Yamawaki
J. Org. Chem. **2020**, *85*, 9650–9660.

2) Introduction of a Thio Functional Group to Diazabicyclooctane: An Effective Modification to Potentiate the Activity of β-Lactams against Gram-Negative Bacteria Producing Class A, C, and D Serine β-Lactamases
Motohiro Fujiu, Katsuki Yokoo, Jun Sato, Satoru Shibuya, Kazuo Komano, Hiroki Kusano, Soichiro Sato, Toshiaki Aoki, Naoki Kohira, Satoshi Miyagawa, Tomoyuki Kawachi, and Kenji Yamawaki
ACS Infect. Dis. **2020**, *6*, 3034–3047.

3) Discovery of 2-Sulfinyl-Diazabicyclooctane Derivatives, Potential Oral β-Lactamase Inhibitors for Infections Caused by Serine β-Lactamase-Producing Enterobacterales
Motohiro Fujiu, Katsuki Yokoo, Jun Sato, Satoru Shibuya, Kazuo Komano, Hiroki Kusano, Soichiro Sato, Toshiaki Aoki, Naoki Kohira, Sachi Kanazawa, Ryosuke Watari, Tomoyuki Kawachi, Yuya Hirakawa, Daiki Nagamatsu, Emi Kashiwagi, Hideki Maki, and Kenji Yamawaki
J. Med. Chem. **2021**, *64*, 9496–9512.

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2022 年 12 月

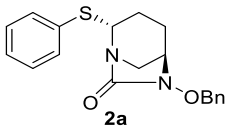
実験の部

General. Unless otherwise noted, reactions were performed under a nitrogen atmosphere. Solvents and commercial reagents were used without purification. Carboxylic acid **1** was purchased from PharmaBlock R&D Co. Avibactam, [1] nacubactam,[2] and zidebactam[3] were synthesized via analogous method as reported. 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). ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on a Bruker AV400 spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) from tetramethylsilane (TMS) (in CD_3Cl, DMSO-d_6, methanol-d_4), sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS-d_6) (in D_2O) or the solvent residual peak (in D_2O δ 4.79) as an internal reference. High-resolution mass spectral data were acquired on Orbitrap Q Exactive Plus (ESI). Low-resolution mass spectral data were collected on a Waters ZQ mass detector (ESI), a Shimadzu LCMS-8030 (ESI), or a Shimadzu LCMS-2020 (ESI). Purity was determined on a Shimadzu Prominence HPLC system using a COSMOSIL 5C18-AR-II column. Mobile phase A was 0.1% trifluoroacetic acid in water, and mobile phase B was 0.1% trifluoroacetic acid in acetonitrile. One of the following methods (A–D) was used: method-A, a gradient from 2% to 100% B in 10 min and held at 100% B for 5 min; method-B, a gradient from 10% to 100% B in 10 min and held at 100% B for 5 min; method-C, an isocratic 5% B; method-D, an isocratic 7% B; method-E, an isocratic 10% B; method-F, an isocratic 4% B. Elemental analysis was performed using Micro Corder JM11 (J-SCIENCE LAB CO., Ltd.). Light promoted reactions were carried out using a Kessil A 160WE Tuna Blue 40 W lamp with the white light mode. LED lamps were placed 2–10 cm away from the reaction vessel without any filters. Purities for all tested compounds were determined by HPLC analysis and described along with other characteristic data.

All studies with animals were approved by the Institutional Animal Care and Use Committee of Shionogi & Co., Ltd.

第１章の合成

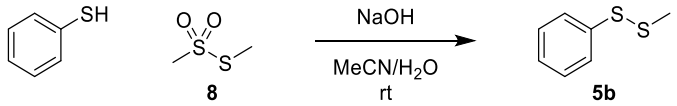
第２節



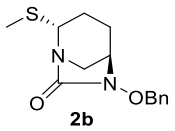
2a

(2R,5R)-6-(Benzyloxy)-2-(phenylthio)-1,6-diazabicyclo[3.2.1]octan-7-one (2a). To a solution of carboxylic acid **1** (300 mg, 1.09 mmol) and 2-mercaptopyridine 1-oxide **4** (145 mg, 1.14 mmol) in CH_2Cl_2 (3.0 mL) was added EDC·HCl (219 mg, 1.14 mmol). The solution was stirred for 1.5 hours at room temperature in the dark. Diphenyldisulfide **5a** (1.19 g, 5.43 mmol) was added and stirred under white light irradiation using two LED lamps for 30 min at 0 ˚C. The reaction mixture was poured into water and the layers were separated. The organic layer was dried over $MgSO_4$ and concentrated under

reduced pressure. The crude product was purified by flash column chromatography to obtain **2a** (171 mg, 46%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.48–7.20 (10H, m), 5.06–5.04 (2H, m), 4.90 (1H, d, *J* = 11.5 Hz), 3.82 (1H, d, *J* = 11.5 Hz), 3.37–3.34 (1H, br m), 2.90 (1H, d, *J* = 11.5 Hz), 2.47–2.36 (1H, m), 2.05–2.01 (1H, m), 1.78–1.69 (2H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 168.6, 135.9, 133.8, 130.1, 129.2, 129.0, 128.7, 128.5, 127.0, 78.2, 66.3, 59.0, 44.0, 24.7, 20.9. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{19}H_{21}N_2O_2S$ 341.1318; Found 341.1317.



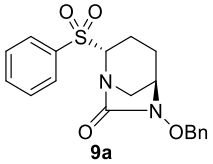
1-Methyl-2-phenyldisulfane (5b).[4] To a solution of NaOH (486 g, 12.1 mol) in H_2O (1220 mL) was added benzenthiol (1.25 L, 12.1 mol) at 0 ˚C. After stirring for 10 minutes at room temperature, methyl methanethiosulfonate **8** (1.25 L, 12.1 mol) was added at 0 ˚C and the mixture was stirred for 1 hour at room temperature. The reaction mixture was extracted with EtOAc (600 mL) and the organic layer was died over $Na_2S_2O_3$. After the solvent was removed, the crude product was distilled under reduced pressure (10 mmHg, 105–115 ˚C) to afford **5b** (1710 g, 90%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.53–7.54 (2H, m), 7.34 (2H, t, *J* = 7.6 Hz), 7.24 (1H, t, *J* = 7.4 Hz), 2.45 (3H, s).



2b

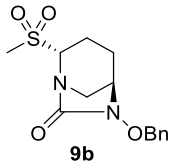
(2R,5R)-6-(Benzyloxy)-2-(methylthio)-1,6-diazabicyclo[3.2.1]octan-7-one (2b). To a solution of carboxylic acid **1** (300 mg, 1.09 mmol) and 2-mercaptopyridine 1-oxide **4** (145 mg, 1.14 mmol) in CH_2Cl_2 (3.0 mL) was added EDC·HCl (219 mg, 1.14 mmol). The solution was stirred for 1.5 hours at room temperature in the dark. PhSSMe **5b** (848 mg, 5.43 mmol) was added and stirred under white light irradiation using two LED lamps for 30 min at 0 ˚C. The reaction mixture was poured into water and the layers were separated. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography to obtain **2b** (99.3 mg, 33%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44–7.36 (5H, m), 5.06 (1H, d, *J* = 11.5 Hz), 4.91 (1H, d, *J* = 11.5 Hz), 4.58 (1H, d, *J* = 7.3 Hz), 3.74 (1H, d, *J* = 11.5 Hz), 3.33–3.31 (1H, br m), 2.82 (1H, d, *J* = 11.5 Hz), 2.34–2.24 (1H, m), 2.11 (3H, s), 1.97–1.94 (1H, m), 1.68–1.53 (2H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 169.4, 136.0, 129.2, 128.7, 128.5, 78.2, 65.6, 59.2, 43.4, 24.4, 20.7, 13.9. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{14}H_{19}N_2O_2S$ 279.1162; Found 279.1160.

第３節



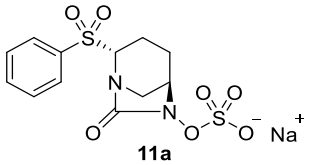
9a

(2R,5R)-6-(Benzyloxy)-2-(phenylsulfonyl)-1,6-diazabicyclo[3.2.1]octan-7-one (9a). To a solution of **2a** (353 mg, 1.04 mmol) in CH_2Cl_2 (10 mL) was added 72 wt% *m*-CPBA (547 mg, 2.28 mmol) at 0 ˚C. After stirred for 1 hour at room temperature, the mixture was poured into aqueous sodium thiosulfate solution and the layers were separated. The aqueous layer was extracted with ethyl acetate (20 mL × 2) and the combined organic layers were dried over Na_2SO_4. After the solvent was removed under vacuum, the crude product was purified by flash column chromatography (20–50% EtOAc/hexane) to obtain **9a** (353 mg, 91%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.91 (2H, *J* = 7.5 Hz), 7.67 (1H, *J* = 7.4 Hz), 7.56 (2H, *J* = 7.8 Hz), 7.37–7.35 (5H, m), 4.94 (1H, *J* = 11.5 Hz), 4.81 (1H, *J* = 11.5 Hz), 4.41 (1H, *J* = 7.5 Hz), 3.73 (1H, *J* = 11.8 Hz), 3.43–3.42 (1H, m), 3.02 (1H, *J* = 12.0 Hz), 2.48–2.44 (1H, m), 2.22–2.09 (2H, m), 1.91–1.86 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 167.2, 136.2, 135.5, 134.1, 129.2, 129.2, 129.0, 128.8, 128.6, 78.1, 74.2, 57.8, 43.2, 18.7, 15.6. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{19}H_{21}N_2O_4S$ 373.1217; Found 373.1214.



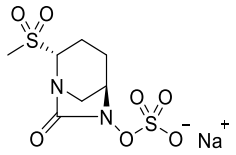
9b

(2R,5R)-6-(Benzyloxy)-2-(methylsulfonyl)-1,6-diazabicyclo[3.2.1]octan-7-one (9b). To a solution of **2b** (1.00 g, 3.59 mmol) in CH_2Cl_2 (20 mL) was added 72 wt% *m*-CPBA (2.16 g, 9.00 mmol) at 0 ˚C. After stirred for 1 hour at 0 ˚C, the mixture was poured into a mixture of aqueous sodium thiosulfate solution and sodium bicarbonate solution and the layers were separated. The aqueous layer was extracted with dichloromethane (20 mL × 1) and the combined organic layers were dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude product was purified by flash column chromatography (33–67% EtOAc/hexane) to obtain **9b** (1.01 g, 90%) as a white amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39 (5H, t, *J* = 5.5 Hz), 5.01 (1H, d, *J* = 11.5 Hz), 4.88 (1H, d, *J* = 11.3 Hz), 4.33 (1H, t, *J* = 7.8 Hz), 3.60 (1H, d, *J* = 12.0 Hz), 3.45–3.44 (1H, m), 3.06–3.02 (4H, m), 2.36–2.26 (1H, m), 2.15–2.03 (2H, m), 1.84–1.79 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 167.3, 135.4, 129.2, 128.9, 128.6, 78.3, 72.7, 57.7, 43.0, 37.3, 18.2, 13.8. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{14}H_{19}N_2O_4S$ 311.1060; Found 311.1058.



11a

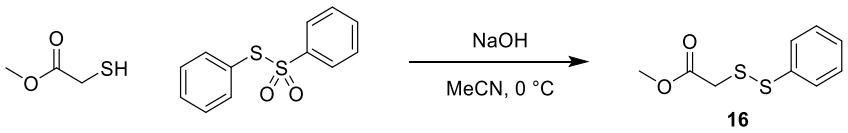
Sodium (2R,5R)-7-oxo-2-(phenylsulfonyl)-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (11a). To a solution of **9a** (200 mg, 0.54 mmol) in THF/MeOH (v/v = 2/1, 6 mL) was added 5% Pd/C (57.1 mg, 0.29 wt equiv). After stirred at room temperature under H_2 (1 atm) atmosphere for 1 hour, the mixture was filtered. The solvent was removed *in vacuo*. The crude product was dissolved to pyridine (4 mL) and SO_3-pyridine (514 mg, 3.23 mmol) was added. After stirred for 3.5 hours, 8.4% $NaHCO_3$ aq (20 mL) was added at 0 ˚C and the aqueous layer was washed with CH_2Cl_2 (20 mL × 3). To the aqueous layer was added CH_2Cl_2 (20 mL) and tetrabutylammonium hydrogen sulfate (183 mg, 0.54 mmol) at 0 ˚C. After stirred at room temperature for 15 minutes, the aqueous layer was extracted with CH_2Cl_2 (20 mL × 3) and the solvent was removed under reduced pressure. The crude product was applied onto a Dowex® sodium form column (Dowex® 50WX8 hydrogen form treated with 1N NaOH aq and washed until neutral pH with H_2O) and subjected to ODS column chromatography (H_2O only). The fractions containing the desired compound were combined, frozen and lyophilized to afford **11a** (162 mg, 78%) as a white amorphous. ^{1}H NMR (400 MHz, D_2O) δ: 7.99 (2H, d, *J* = 7.8 Hz), 7.85 (1H, t, *J* = 7.5 Hz), 7.72 (2H, t, *J* = 7.8 Hz), 4.84–4.80 (1H, m), 4.26–4.26 (1H, m), 3.56 (1H, d, *J* = 12.5 Hz), 3.32 (1H, dd, *J* = 12.4, 2.6 Hz), 2.39–2.33 (1H, m), 2.18–2.03 (3H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 167.9, 135.4, 133.8, 129.7, 129.1, 75.0, 59.3, 43.4, 17.7, 16.3. HPLC: 93.0%. Anal. Calcd for $C_{12}H_{13}N_2O_7S_2Na(H_2O)_{1.3}$: C, 35.35; H, 3.86; N, 6.87; S, 15.72; Na, 5.64. Found: C, 35.48; H, 4.13; N, 6.84; S, 15.88; Na, 5.48.



11b

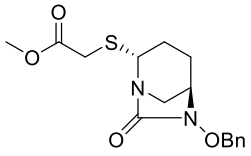
Sodium (2R,5R)-2-(methylsulfonyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (11b). To a solution of **9b** (200 mg, 0.54 mmol) in THF/MeOH (v/v = 1/1, 4 mL) was added 5% Pd/C (68.6 mg, 0.34 wt equiv). After stirred at room temperature under H_2 (1 atm) atmosphere for 1 hour, the mixture was filtered. The solvent was removed *in vacuo* and the crude product was used without any purification. The crude product was dissolved to pyridine (4 mL) and SO_3-pyridine (514 mg, 3.23 mmol) was added. After stirred for 3.5 hours, 8.4% $NaHCO_3$ aq (20 mL) was added at 0 ˚C and the aqueous layer was washed with CH_2Cl_2 (20 mL × 3). To the aqueous layer was added CH_2Cl_2 (20 mL) and tetrabutylammonium hydrogen sulfate (183 mg, 0.54 mmol) at 0 ˚C. After stirred at room temperature for 15 minutes, the aqueous layer was extracted with CH_2Cl_2 (20 mL × 3) and the solvent was removed under reduced pressure. The crude product was applied onto a Dowex® sodium form column (Dowex® 50WX8 hydrogen form treated with 1N NaOH aq and washed until neutral pH with H_2O) and subjected to ODS column chromatography (H_2O only). The fractions containing the desired compound were combined, frozen and lyophilized to afford **11b** (172 mg, 83%) as a white amorphous. ^{1}H NMR (400 MHz, D_2O) δ: 4.77 (1H, t, *J* = 8.3 Hz), 4.31–4.31 (1H, br m), 3.69 (1H, d, *J* = 12.3 Hz), 3.42 (1H, dd, *J* = 12.4, 2.6 Hz), 3.19 (3H,

s), 2.38–2.28 (1H, m), 2.21–2.07 (3H, m). $^{13}C\{^{1}H\}$ NMR (100 MHz, D_2O) δ: 168.2, 73.1, 59.4, 43.3, 36.5, 17.4, 14.5. HPLC: 99.3% (method-C). Anal. Calcd for $C_7H_{11}N_2O_7S_2Na(H_2O)_{1.9}$: C, 23.58; H, 4.18; N, 7.86; S, 17.99; Na, 6.45. Found: C, 23.64; H, 4.27; N, 7.89; S, 17.88; Na, 6.64.



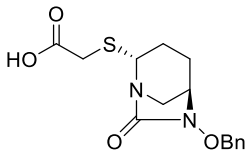
16

Methyl 2-(phenyldisulfaneyl)acetate (16). To a solution of methyl 2-mercaptoacetate (100 mg, 0.94 mmol) in MeCN (2 mL) was added 1 N aq NaOH (0.94 mL, 0.94 mmol) at 0 ˚C. After being stirred for 10 min at 0 ˚C, *S*-phenyl benzenesulfonothioate[5] was added, and the mixture was stirred for 1 h at room temperature. The mixture was poured into water, and the aqueous layer was extracted with EtOAc. After the organic layer was dried over $MgSO_4$, the solvent was removed under reduced pressure. The crude was purified by flush column chromatography (0–10%, EtOAc/hexane) to give **16** (80 mg, 40%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.55 (2H, d, *J* = 7.3 Hz), 7.34 (2H, t, *J* = 7.5 Hz), 7.26 (2H, t, *J* = 7.3 Hz), 3.58 (3H, s), 3.50 (2H, s). MS-ESI (*m/z*): 215 $[M + H]^+$.



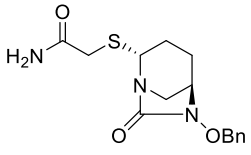
2c

Methyl 2-[[(2R,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]thio]acetate (2c). To a solution of carboxylic acid **1** (138 mg, 0.50 mmol) in CH_2Cl_2 (1.4 mL) were added Et_3N (104 μL, 0.75 mmol) and 2-oxo-[1,4,2]oxathiazolo[2,3-*a*]pyridin-4-ium chloride **15** (114 mg, 0.60 mmol). The mixture was stirred for 1 h in the dark (solution A). To another solution of methyl 2-(phenyldisulfaneyl)acetate **16** (332 mg, 1.55 mmol) in CH_2Cl_2 (1.4 mL) was added the solution A dropwise under white LEDs irradiation. After 1 h, the solvent was removed under reduced pressure, and the crude was purified by flush column chromatography (5–35%, EtOAc/hexane) to give **2c** (58.0 mg, 35%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44–7.35 (5H, m), 5.04 (1H, d, *J* = 11.4 Hz), 4.90 (1H, d, *J* = 11.4 Hz), 4.75 (1H, d, *J* = 6.9 Hz), 3.72–3.71 (4H, m), 3.50 (1H, d, *J* = 15.6 Hz), 3.33–3.32 (1H, m), 3.28 (1H, d, *J* = 15.6 Hz), 2.81 (1H, dt, *J* = 11.5, 3.0 Hz), 2.35–2.25 (1H, m), 2.01–1.95 (1H, m), 1.69–1.55 (2H, m). MS-ESI (*m/z*): 337 $[M + H]^+$.



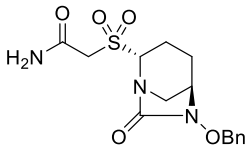
12

2-[[(2R,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]thio]acetic acid (12). To a solution of **2c** (1.22 g, 3.63 mmol) in THF/H_2O (v/v = 1/1, 24 mL) was added 4 M aq LiOH (1.09 mL, 4.35 mmol) at 0 ˚C. After being stirred for 2 h at 0 ˚C, 10% aq citric acid was added. The mixture was extracted with EtOAc three times, and the combined organic layers were washed with water and brine before being dried over $MgSO_4$. The solvent was removed under reduced pressure to give **12** (988 mg, 85%) as pale yellow oil, which was used without further purification.



13

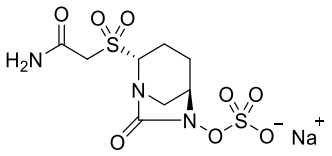
2-[[(2R,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]thio]acetamide (13). To a solution of **12** (943 mg, 2.93 mmol) in 1,4-dioxane/H_2O (v/v = 9/1, 9.4 mL) were added ammonium hydrogencarbonate (289 mg, 3.66 mmol), Boc_2O (1.02 mL, 4.39 mmol) and pyridine (118 μL, 1.46 mmol). After being stirred overnight, water was added, and the mixture was extracted EtOAc three times. The combined organic layers were washed with water and brine before being dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude was purified by flush column chromatography (50–100%, EtOAc/hexane) to give **13** (170 mg, 18%) as a pale colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44–7.36 (5H, m), 6.69 (1H, br s), 5.40 (1H, br s), 5.04 (1H, d, *J* = 11.5 Hz), 4.90 (1H, d, *J* = 11.5 Hz), 4.73–4.71 (1H, m), 3.65 (1H, d, *J* = 11.7 Hz), 3.41–3.37 (2H, m), 3.12 (1H, d, *J* = 14.8 Hz), 2.90–2.87 (1H, m), 2.35–2.24 (1H, m), 2.03–1.99 (1H, m), 1.71–1.65 (2H, m). MS-ESI (*m/z*): 322 $[M + H]^+$.



14

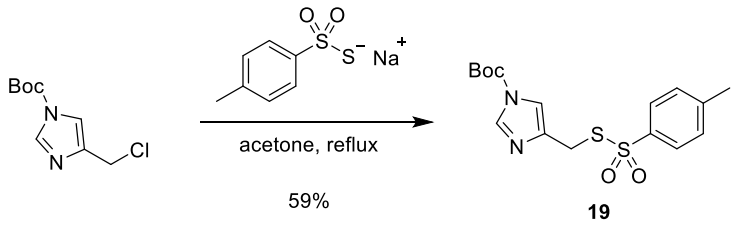
2-[[(2R,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]sulfonyl]acetamide (14). To a solution of **13** (170 mg, 0.53 mmol) in CH_2Cl_2 (1.7 mL) was added 69 wt % *m*-CPBA (331 mg, 1.32 mmol) at 0 ˚C. After being stirred for 30 min at room temperature, 10% aq $Na_2S_2O_3$ was added, and the layers were separated. After the organic solvent was removed under reduced pressure, EtOAc was added. The organic layer was washed with 5% aq $NaHCO_3$ twice and brine before being dried over $MgSO_4$. After the removal of solvent, the crude was purified by flush column chromatography (10–50%, EtOAc/hexane) to give **14** (138 mg, 74%) as a pale colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42–7.38 (5H, m), 6.91 (1H, br s), 5.63 (1H, br s), 4.99 (1H, d, *J* = 11.4 Hz), 4.88 (1H, d, *J* = 11.4 Hz), 4.64–4.60 (1H, m), 4.43 (1H, d, *J* = 14.2 Hz), 3.86 (1H, d, *J* = 14.2 Hz), 3.54–3.49 (2H, m), 3.10 (1H, dd, *J*

= 12.2, 3.1 Hz), 2.39–2.26 (1H, m), 2.17–2.09 (2H, m), 1.91–1.84 (1H, m). MS-ESI (*m/z*): 354 [M + H]$^+$.



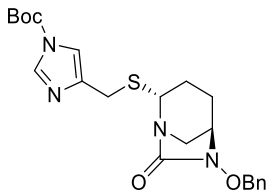
11c

Sodium (2R,5R)-2-[(2-amino-2-oxoethyl)sulfonyl]-7-oxo-1,6-diazabicy-clo[3.2.1]octan-6-yl sulfate (11c). To a solution of **14** (500 mg, 1.42 mmol) in THF/MeOH (v/v = 1/1, 10 mL) was added 5% Pd/C (151 mg, 0.30 wt equiv). After being stirred under a H_2 atmosphere (1 atm) for 45 min, DMF was added to dissolve the precipitation of debenzylated product. The mixture was filtered, and the solvent was removed under reduced pressure to give the product, which was used without further purification. To a solution of the crude debenzylated product in pyridine (5 mL) was added SO_3-pyridine (292 mg, 1.84 mmol). After being stirred for 5 h, the mixture was poured into aq $NaHCO_3$, and the aqueous layer was washed with CH_2Cl_2 twice. The aqueous solvent was removed under reduced pressure before the crude was applied onto the HP20SS resin and subjected to ODS column chromatography (0–3%, MeCN/H_2O) to give **11c** (34.2 mg, 6.6%) as a white amorphous solid after lyophilization. ^{1}H NMR (400 MHz, D_2O) δ: 4.96 (1H, t, *J* = 8.3 Hz), 4.32–4.32 (1H, m), 3.70 (1H, d, *J* = 12.4 Hz), 3.46–3.43 (1H, m), 2.43–2.33 (1H, m), 2.23–2.09 (3H, m). HPLC: 93.7% (method-C). Anal. calcd for $C_8H_{12}N_3O_8S_2Na(H_2O)_{2.3}$: C, 23.62; H, 4.11; N, 10.33; S, 15.76; Na, 5.65. found: C, 23.68; H,3.88; N, 10.40; S, 15.48; Na, 5.58.



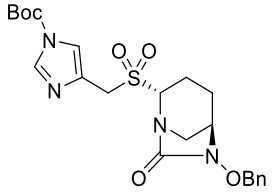
19

tert-Butyl 4-[(tosylthio)methyl]-1H-imidazole-1-carboxylate (19). To a solution of *tert*-butyl 4-(chloromethyl)-1*H*-imidazole-1-carboxylate[6] (4.0 g, 18.5 mmol) in acetone (40 mL) was added potassium 4-methylbenzenesulfonothioate (4.4 g, 19.4 mmol). After being stirred for 5 h at reflux, the solvent was removed under reduced pressure, and the crude mixture was dissolved to EtOAc. The organic layer was washed with water and brine before being dried over $MgSO_4$. After the removal of solvent, the crude was purified by flush column chromatography (5–40%, EtOAc/hexane) to give **19** (4.0 g, 59%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.87 (1H, s), 7.77 (2H, d, *J* = 8.3 Hz), 7.29 (2H, d, *J* = 8.3 Hz), 7.15 (1H, s), 4.19 (2H, s), 2.43 (3H, s), 1.61 (9H, s). MS-ESI (*m/z*): 369 [M + H]$^+$.



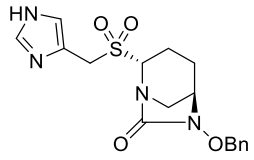
2d

tert-Butyl 4-[[[(2R,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]thio]methyl]-1H-imidazole-1-carboxylate (2d). To a solution of carboxylic acid **1** (120 mg, 0.43 mmol) in CH_2Cl_2 (0.6 mL) were added 2-mercaptopyridine 1-oxide **4** (58 mg, 0.46 mmol) and EDC·HCl (87.0 mg, 0.46 mmol). The mixture was stirred for 1 h in the dark (solution A). To another solution of *tert*-butyl 4-((tosylthio)methyl)-1*H*-imidazole-1-carboxylate **19** (320 mg, 0.87 mmol) in CH_2Cl_2 (0.6 mL) was slowly added the solution A under white LEDs irradiation. After being stirred for 30 min, the mixture was directly purified by flush column chromatography (0–40%, EtOAc/hexane) to give **2d** (87 mg, 45%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.02 (1H, d, *J* = 0.9 Hz), 7.43 (2H, dd, *J* = 7.5, 1.9 Hz), 7.40–7.35 (3H, m), 7.32 (1H, s), 5.05 (1H, d, *J* = 11.4 Hz), 4.91 (1H, d, *J* = 11.4 Hz), 4.61 (1H, d, *J* = 6.8 Hz), 3.82 (1H, d, *J* = 14.2 Hz), 3.77 (1H, d, *J* = 11.5 Hz), 3.61 (1H, d, *J* = 14.2 Hz), 3.34–3.34 (1H, m), 2.86–2.84 (1H, m), 2.32–2.21 (1H, m), 1.99–1.93 (1H, m), 1.69–1.51 (11H, m). MS-ESI (*m/z*): 445 $[M + H]^+$.



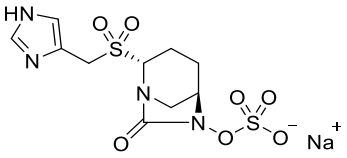
17

tert-Butyl 4-[[[(2R,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]sulfonyl]methyl]-1H-imidazole-1-carboxylate (17). To a solution of **2d** (87 mg, 0.20 mmol) in CH_2Cl_2 (1.3 mL) was added 69 wt % *m*-CPBA (98 mg, 0.39 mmol) at 0 ˚C. After being stirred for 2 h at 0 ˚C, aq $NaHCO_3$ and aq $Na_2S_2O_3$ were added, and the layers were separated. The organic layer was washed with aq $NaHCO_3$ and brine before being dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude was purified by flush column chromatography (10–60%, EtOAc/hexane) to give **17** (56 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.07 (1H, s), 7.70 (1H, s), 7.42–7.37 (5H, m), 5.00 (1H, d, *J* = 11.4 Hz), 4.88 (1H, d, *J* = 11.4 Hz), 4.69 (1H, d, *J* = 14.7 Hz), 4.57 (1H, t, *J* = 7.8 Hz), 4.29 (1H, d, *J* = 14.6 Hz), 3.66 (1H, d, *J* = 12.0 Hz), 3.45–3.44 (1H, m), 3.09 (1H, d, *J* = 10.9 Hz), 2.34–2.26 (1H, m), 2.17–2.00 (2H, m), 1.86–1.78 (1H, m), 1.62 (9H, s). MS-ESI (*m/z*): 477 $[M + H]^+$.



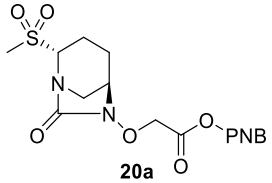
18

(2R,5R)-2-[[(1H-Imidazol-4-yl)methyl]sulfonyl]-6-(benzyloxy)-1,6-diazabicyclo[3.2.1]octan-7-one (18). To a solution of **17** (55 mg, 0.12 mmol) in CH_2Cl_2 (0.55 mL) was added TFA (0.55 mL). After 3 h, the mixture was poured into aq $NaHCO_3$, and the aqueous layer was extracted with EtOAc three times. The combined organic layers were washed with aq $NaHCO_3$ and brine before being dried over Na_2SO_4. After the solvent was removed, the crude was purified by flush column chromatography (50–100%, EtOAc/hexane) to give **18** (40 mg, 92%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.69 (1H, s), 7.42–7.40 (6H, br m), 5.01 (1H, d, *J* = 11.4 Hz), 4.91–4.89 (2H, m), 4.45–4.42 (1H, br m), 4.20–4.17 (1H, br m), 3.63 (1H, d, *J* = 11.5 Hz), 3.51–3.49 (1H, br m), 3.12 (1H, d, *J* = 12.5 Hz), 2.35–2.25 (1H, m), 2.14–1.96 (2H, m), 1.87–1.84 (1H, m). MS-ESI (*m/z*): 377 $[M + H]^+$.



11d

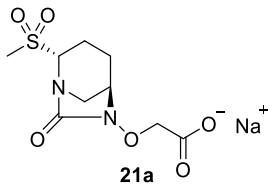
Sodium (2R,5R)-2-[[(1H-imidazol-4-yl)methyl]sulfonyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (11d). To a solution of **18** (39 mg, 0.10 mmol) in MeOH (0.59 mL) was added 5% Pd/C (22 mg, 0.56 wt equiv.). After being stirred under a H_2 atmosphere (1 atm) for 1 h, the mixture was filtered, and the solvent was removed to give the crude product, which was used without further purification. To a solution of the crude debenzylated product in pyridine (1.5 mL) was added SO_3-pyridine (99 mg, 0.62 mmol). After being stirred overnight, the mixture was poured into aq $NaHCO_3$ and the aqueous layer was washed with CH_2Cl_2 twice. The aqueous solvent was removed under reduced pressure, and the crude was applied onto the HP20SS resin and subjected to ODS column chromatography (H_2O only) to give **11d** (21 mg, 52%) as a white amorphous solid after lyophilization. ^{1}H NMR (400 MHz, D_2O) δ: 7.80 (1H, s), 7.42 (1H, s), 4.82–4.79 (2H, m), 4.64 (1H, d, *J* = 14.8 Hz), 4.32 (1H, d, *J* = 2.9 Hz), 3.73 (1H, d, *J* = 12.3 Hz), 3.45 (1H, dd, *J* = 12.4, 3.1 Hz), 2.39–2.28 (1H, m), 2.16–2.07 (3H, m). HPLC: >99.9% (method-C). MS-ESI (*m/z*): 365 $[M - H]^-$.



20a

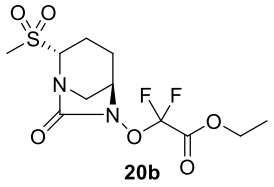
4-Nitrobenzyl 2-[[(2R,5R)-2-(methylsulfonyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-acetate (20a). To a solution of **10b** (179 mg, 0.814 mmol) in DMF (5 mL) were added 4-nitrobenzyl 2-iodoacetate **22a** (340 mg, 1.06 mmol) and K_2CO_3 (146 mg, 1.06 mmol). After being stirred overnight, the mixture was poured into 10% aq citric acid, and the aqueous layer was extracted with EtOAc twice.

The combined organic layers were washed with water and dried over Na_2SO_4 before the solvent was remover under reduced pressure. The crude was purified by flash column chromatography (30–70% EtOAc/hexane) to give **20a** (294 mg, 87%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.25 (2H, d, *J* = 8.5 Hz), 7.54 (2H, d, *J* = 8.8 Hz), 5.32 (1H, d, *J* = 13.1 Hz), 5.28 (1H, d, *J* = 13.1 Hz), 4.66 (1H, d, *J* = 17.1 Hz), 4.62 (1H, d, *J* = 17.1 Hz), 4.38 (1H, t, *J* = 8.2 Hz), 4.18–4.16 (1H, m), 3.70 (1H, d, *J* = 12.0 Hz), 3.13 (1H, d, *J* = 12.3 Hz), 3.04 (3H, s), 2.41–2.31 (1H, m), 2.25–2.07 (2H, m), 2.00–1.97 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 168.6, 168.5, 148.0, 142.0, 128.7, 124.0, 73.3, 72.5, 65.5, 59.1, 42.8, 37.3, 18.1, 14.1. HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{16}H_{20}N_3O_8S$ 414.0966; found 414.0962.



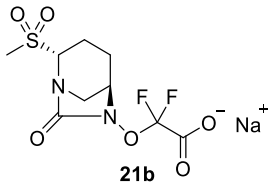
21a

Sodium 2-[[(2R,5R)-2-(methylsulfonyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]acetate (21a). To a solution of **20a** (146 mg, 0.352 mmol) in DMF (2 mL) was added 5% Pd/C (37.5 mg, 0.26 wt equiv). After being stirred under a H_2 atmosphere (1 atm) for 2 h at 0 ˚C, the mixture was filtered and poured into aq $NaHCO_3$. After the aqueous layer was washed with EtOAc, the resulting solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–10%, MeCN/H_2O) to give **21a** (116 mg, quant.) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 4.70 (1H, t, *J* = 8.0 Hz), 4.43 (1H, d, *J* = 15.3 Hz), 4.35 (1H, d, *J* = 15.1 Hz), 4.26–4.24 (1H, m), 3.60 (1H, d, *J* = 12.3 Hz), 3.31 (1H, d, *J* = 11.8 Hz), 3.17 (3H, s), 2.38–2.27 (1H, m), 2.21–2.01 (3H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 178.4, 170.9, 77.3, 75.5, 60.6, 46.7, 39.4, 20.6, 17.4. HPLC: >99.9%.



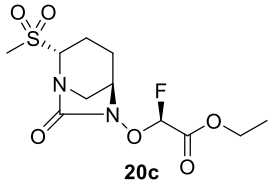
20b

Ethyl 2,2-difluoro-2-[[(2R,5R)-2-(methylsulfonyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy] acetate (20b). To a solution of **10b** (523 mg, 2.38 mmol) in DMF (10 mL) were added ethyl 2-bromo-2,2-difluoroacetate **22b** (723 mg, 3.56 mmol) and K_2CO_3 (427 mg, 2.09 mmol). After being stirred for 2.5 h, the mixture was poured into 10% aq citric acid, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with water and brine before being dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude product was purified by flash column chromatography (20–60% EtOAc/hexane) to give **20b** (354 mg, 45%) as a white amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.46–4.38 (3H, m), 4.08 (1H, dd, *J* = 5.5, 2.9 Hz), 3.79 (1H, d, *J* = 12.4 Hz), 3.32 (1H, d, *J* = 12.4 Hz), 3.05 (3H, s), 2.41–2.33 (1H, m), 2.23–2.00 (3H, m), 1.40 (3H, t, *J* = 7.2 Hz).



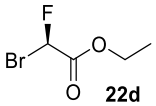
21b

Sodium 2,2-difluoro-2-[[(2R,5R)-2-(methylsulfonyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy] acetate (21b). To a solution of **20b** (315 mg, 0.920 mmol) in THF/H_2O (v/v = 2/1, 9.5 mL) was added 1 N aq NaOH (1.01 mL, 1.01 mmol) at 0 ˚C. After being stirred for 30 min at 0 ˚C, the reaction was quenched by dry ice. The resulting solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–10%, MeCN/H_2O) to give **21b** (284 mg, 92%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 4.84–4.82 (1H, m), 4.26–4.23 (1H, m), 3.69 (1H, d, *J* = 12.5 Hz), 3.43 (1H, dd, *J* = 12.4, 2.8 Hz), 3.19 (3H, s), 2.38–2.28 (1H, m), 2.23–2.05 (3H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 172.4, 166.3 (t, $^3J_{C\text{-}F}$ = 31.9 Hz), 120.1 (t, $^2J_{C\text{-}F}$ = 280.6 Hz), 76.5, 63.8, 46.0, 39.4, 20.3, 17.6. ^{19}F NMR (377 MHz, D_2O) δ: −83.2 (1H, d, $^3J_{F\text{-}F}$ = 134.9 Hz), −83.9 (1H, d, $^3J_{F\text{-}F}$ = 136.2 Hz). HPLC: >99.9% (method-E). Anal. calcd for $C_9H_{11}F_2N_2O_6SNa(H_2O)_{0.9}$: C, 30.67; H, 3.66; F, 10.78; N, 7.95; S, 9.10; Na, 6.52. Found: C, 30.85; H, 3.81; F, 10.47; N, 7.94; S, 8.86; Na, 6.78.



20c

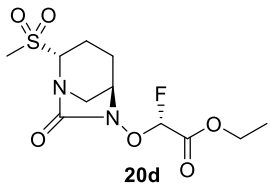
Ethyl (2S)-2-fluoro-2-[[(2R,5R)-2-(methylsulfonyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy] acetate (20c). To a solution of **10b** (6.83 g, 31.0 mmol) in DMF (70 mL) were added ethyl 2-bromo-2-fluoroacetate **22c** (8.03 mg, 43.4 mmol) and K_2CO_3 (5.57 g, 40.3 mmol). After being stirred for 3 h, the mixture was poured into 10% aq citric acid, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with water and brine before being dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude product was purified by flash column chromatography (30–100% EtOAc/hexane) to give **20c** (4.74 g, 47%) and **20d** (4.18 g, 42%) as a white amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.80 (1H, d, $^3J_{H\text{-}F}$ = 59.7 Hz), 4.42 (1H, t, *J* = 8.2 Hz), 4.39–4.32 (2H, m), 4.08 (1H, dd, *J* = 5.5, 3.0 Hz), 3.76 (1H, d, *J* = 12.3 Hz), 3.26–3.23 (1H, m), 3.05 (3H, s), 2.41–2.31 (1H, m), 2.25–2.09 (2H, m), 2.06–1.98 (1H, m), 1.37 (3H, t, *J* = 7.2 Hz). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 167.8, 162.4 (d, $^3J_{C\text{-}F}$ = 29.3 Hz), 106.4 (d, $^2J_{C\text{-}F}$ = 239.9 Hz), 73.5, 63.0, 60.7, 42.6, 37.3, 18.0, 14.1, 14.0. ^{19}F NMR (377 MHz, $CDCl_3$) δ: −133.9 (d, $^3J_{H\text{-}F}$ = 59.9 Hz). HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{11}H_{18}FN_2O_6S$ 325.0864; found 325.0860.



22d

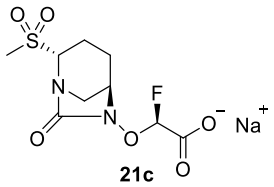
Ethyl (R)-2-bromo-2-fluoroacetate (22d).[8] To a solution of (*R*)-2-bromo-2-fluoroacetic acid[7,8] (25.7 g, 100 mmol) in CH_2Cl_2 (257 mL) were added EtOH (29.2 mL, 500 mmol) and EDC·HCl (21.1 g, 110 mmol) at 0 ˚C. After the mixture was stirred for 30 min at 0 ˚C, diluted aq H_2SO_4 was added. The organic

layer was separated and washed with water before being dried over $MgSO_4$. The solvent was carefully removed under reduced pressure (200 torr, 20 ˚C) to give **22d** (47.7 g, 38.7 wt%, quant.) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.56 (1H, d, $^3J_{H\text{-}F}$ = 50.6 Hz), 4.35 (2H, q, J = 7.1 Hz), 1.35 (3H, t, J = 7.1 Hz). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 164.7 (d, $^3J_{C\text{-}F}$ = 25.7 Hz), 80.9 (d, $^2J_{C\text{-}F}$ = 263.4 Hz), 63.1, 13.9. ^{19}F NMR (377 MHz, $CDCl_3$) δ: −150.69 (d, $^3J_{H\text{-}F}$ = 50.4 Hz).



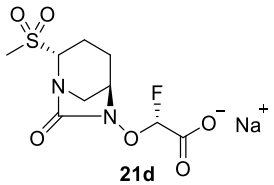
20d

Ethyl (2R)-2-fluoro-2-[[(2R,5R)-2-(methylsulfonyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy] acetate (20d). To a solution of **10b** (142 mg, 0.645 mmol) in DMF (2 mL) were added 38.7 wt% ethyl (*R*)-2-bromo-2-fluoroacetate **22d** (370 mg, 0.774 mmol) and DBU (0.117 mL, 0.774 mmol) at 0 ˚C. After being stirred for 1.5 h at 0 ˚C, the mixture was poured into 10% aq citric acid, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with water and dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude product was purified by flash column chromatography (20–50% EtOAc/hexane) to give **20d** (104 mg, 50%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.86 (1H, d, $^3J_{H\text{-}F}$ = 52.5 Hz), 4.41 (1H, t, J = 8.1 Hz), 4.37–4.28 (2H, m), 4.09 (1H, dd, J = 5.2, 2.9 Hz), 3.75 (1H, d, J = 12.4 Hz), 3.22 (1H, dt, J = 12.3, 1.4 Hz), 3.04 (3H, s), 2.39–2.31 (1H, m), 2.23–1.99 (3H, m), 1.34 (3H, t, J = 7.2 Hz). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 167.4, 162.8 (d, $^3J_{C\text{-}F}$ = 35.2 Hz), 105.2 (d, $^2J_{C\text{-}F}$ = 239.2 Hz), 73.6, 62.8, 60.9, 42.6, 37.3, 17.9, 14.1, 14.0. ^{19}F NMR (377 MHz, $CDCl_3$) δ: −136.7 (d, $^3J_{H\text{-}F}$ = 51.8 Hz). HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{11}H_{18}FN_2O_6S$ 325.0864; found 325.0861.



21c

Sodium (2S)-2-fluoro-2-[[(2R,5R)-2-(methylsulfonyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] oxy]acetate (21c). To a solution of **20c** (90.0 mg, 0.278 mmol) in THF/H_2O (v/v = 2/1, 3 mL) was added 1 N aq NaOH (0.278 mL, 0.278 mmol) at 0 ˚C. After being stirred for 30 min at 0 ˚C, the reaction was quenched by dry ice. The aqueous layer was washed with diisopropyl ether, and the resulting solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–15%, MeCN/H_2O) to give **21c** (69.3 mg, 79%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 5.80 (1H, d, $^3J_{H\text{-}F}$ = 57.0 Hz), 4.79–4.76 (1H, m), 4.19 (1H, t, J = 4.0 Hz), 3.64 (1H, d, J = 12.4 Hz), 3.34 (1H, dt, J = 12.3, 1.4 Hz), 3.18 (3H, s), 2.37–2.26 (1H, m), 2.21–2.02 (3H, m). HPLC: >99.9%. Anal. calcd for $C_9H_{12}FN_2O_6SNa(H_2O)_{1.8}$: C, 30.83; H, 4.48; F, 5.42; N, 7.99; S, 9.14; Na, 6.56. Found: C, 30.81; H, 4.62; F, 5.22; N, 8.10; S, 8.96; Na, 6.63.



21d

Sodium (2R)-2-fluoro-2-[[(2R,5R)-2-(methylsulfonyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] oxy]acetate (21d). To a solution of **20d** (102 mg, 0.315 mmol) in THF/H_2O (v/v = 2/1, 3 mL) was added 1 N aq NaOH (0.315 mL, 0.315 mmol) at 0 ˚C. After being stirred for 30 min at 0 ˚C, the reaction was quenched by dry ice. The aqueous layer was washed with diisopropyl ether, and the resulting solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–15%, MeCN/H_2O) to give **21d** (86.2 mg, 86%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 5.80 (1H, d, $^3J_{H\text{-}F}$ = 58.6 Hz), 4.79–4.75 (1H, m), 4.27–4.26 (1H, m), 3.66 (1H, d, *J* = 12.4 Hz), 3.37–3.34 (1H, m), 3.18 (3H, s), 2.38–2.27 (1H, m), 2.21–2.05 (3H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 169.0 ($^3J_{C\text{-}F}$ = 27.1 Hz), 168.7, 107.7 ($^2J_{C\text{-}F}$ = 240.6 Hz), 73.2, 59.9, 43.2, 36.4, 17.3, 14.5. ^{19}F NMR (377 MHz, D_2O) δ: −130.2 ($^3J_{H\text{-}F}$ = 58.6 Hz). HPLC: >99.9%. Anal. calcd for $C_9H_{12}FN_2O_6SNa(H_2O)_{0.8}$: C, 32.50; H, 4.12; F, 5.71; N, 8.42; S, 9.64; Na, 6.91. Found: C, 32.60; H, 4.28; F, 5.58; N, 8.61; S, 9.15; Na, 6.75.

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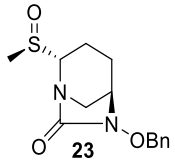
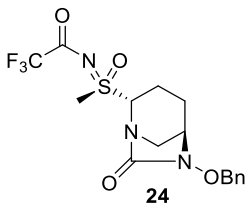


23

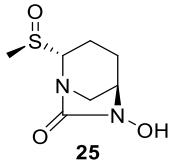
(2R,5R)-6-(Benzyloxy)-2-[(R)-methylsulfinyl]-1,6-diazabicyclo[3.2.1]octan-7-one (23). To a solution of **2b** (2.47 g, 8.89 mmol) in CH_2Cl_2 (25 mL) was added 72 wt% *m*-CPBA (2.34 g, 9.78 mmol) at −78 ˚C. After stirred for 2 hour at −78 ˚C, the mixture was poured into aqueous sodium thiosulfate solution and the layers were separated. The aqueous layer was extracted with ethyl acetate (30 mL × 3) and the combined organic layers were dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude product was purified by flash column chromatography (0–10% MeOH/EtOAc) to obtain **23** (2.31 g, 88%, dr = 91/9) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43–7.37 (5H, m), 5.04 (1H, d, *J* = 11.3 Hz), 4.90 (1H, d, *J* = 11.5 Hz), 4.02 (1H, dd, *J* = 7.5, 4.3 Hz), 3.39–3.39 (1H, m), 3.18 (1H, d, *J* = 11.8 Hz), 3.03 (1H, d, *J* = 11.8 Hz), 2.68 (3H, s), 2.39–2.36 (1H, m), 2.17–2.11 (2H, m), 1.80–1.77 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 166.9, 135.5, 129.3, 128.9, 128.6, 78.4, 76.3, 57.5, 45.7, 37.2, 19.6, 15.9. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{14}H_{19}N_2O_3S$ 295.1111; Found 295.1108. Melting point: 96-99 ˚C.



24

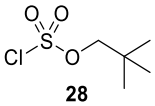
N-[(1R)-[(2R,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl](methyl)(oxo)-λ^6-sulfaneylidene]-2,2,2-trifluoroacetamide (24). To a solution of **23** (592 mg, 2.01 mmol) in CH_2Cl_2 (10

mL) were added 2,2,2-trifluoroacetamide (445 mg, 4.02 mmol), magnesium oxide (324 mg, 8.04 mmol), $Rh_2(OAc)_4$ (44.4 mg, 0.101 mmol) and $PhI(OAc)_2$ (971 mg, 3.02 mmol). After stirred overnight, the reaction mixture was filtered and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (20–50% EtOAc/hexane) to obtain **24** (632 mg, 78%) as a white amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39 (5H, s), 4.99 (1H, d, J = 11.5 Hz), 4.87 (1H, d, J = 11.5 Hz), 4.49–4.47 (1H, m), 3.70 (1H, d, J = 12.3 Hz), 3.55 (3H, s), 3.47–3.46 (1H, m), 3.06 (1H, d, J = 12.0 Hz), 2.55–2.50 (1H, m), 2.21–2.08 (2H, m), 1.95–1.90 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 166.6, 164.4 (q, $^3J_{C\text{-}F}$ = 38.1 Hz), 135.1, 129.3, 129.1, 128.7, 115.9 (q, $^2J_{C\text{-}F}$ = 288.1 Hz), 78.4, 75.2, 57.7, 42.6, 35.7, 17.9, 14.4. ^{19}F NMR (377 MHz, $CDCl_3$) δ: −76.12 (3F, s). HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{16}H_{19}F_3N_3O_4S$ 406.1043; Found 406.1039.



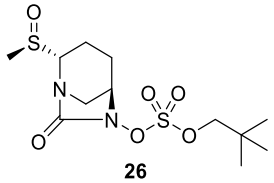
25

(2R,5R)-6-Hydroxy-2-[(R)-methylsulfinyl]-1,6-diazabicyclo[3.2.1]octan-7-one (25). To a solution of **23** (1.53 g, 5.20 mmol) in MeOH (15 mL) were added 10% $Pd(OH)_2$ on carbon (365 mg, 0.24 wt equiv) and DABCO (11.7 mg, 0.10 mmol). After stirred at room temperature under H_2 atmosphere (1 atm) for 1 hour, the mixture was filtered. The solvent was removed *in vacuo* to afford **25** (941 mg, 89%) as a white solid, which was used to the next step without any purification. ^{1}H NMR (400 MHz, D_2O) δ: 4.20 (1H, t, J = 5.9 Hz), 3.95–3.94 (1H, m), 3.34–3.31 (2H, m), 2.77 (3H, s), 2.39–2.29 (1H, m), 2.19–2.14 (2H, m), 1.98–1.94 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 166.4, 74.4, 58.9, 46.3, 34.9, 18.6, 15.2. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_7H_{13}N_2O_3S$ 205.0641; Found 205.0642.



28

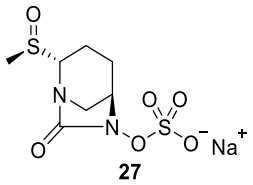
Neopentyl chlorosulfate (28).[9] To a solution of sulfuryl chloride (2.00 mL, 24.6 mmol) in Et_2O (2 mL) was added a solution of neopentyl alcohol (2.39 g, 27.1 mmol) and pyridine (1.99 mL, 24.6 mmol) in Et_2O (3 mL) at −78 ˚C. After stirred at room temperature for 2 hours, the mixture was diluted with Et_2O (30 mL) and the organic phase was washed with 10% citric acid aq, water and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford **28** (3.96 g, 86%) as a colorless oil, which was used without a further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.18 (2H, s), 1.05 (9H, s).



26

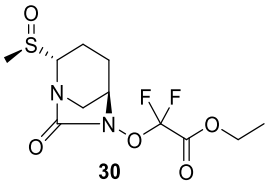
(2R,5R)-2-[(R)-Methylsulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl neopentyl sulfate (26). To a solution of **25** (300 mg, 1.47 mmol) in DMF (3 mL) were added DBU (0.288 mL, 1.91 mmol) and chlorosulfate **28** (274 mg, 1.47 mmol) at 0 ˚C and stirred for 15 minutes. The reaction mixture was

poured into 10% aqueous citric acid solution and the aqueous layer was extracted with ethyl acetate (20 mL × 4). The combined organic layers were washed with water, dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography (0–10% MeOH/EtOAc) to afford **26** (281 mg, 54%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.42 (1H, d, *J* = 8.8 Hz), 4.26–4.26 (1H, br m), 4.18 (1H, d, *J* = 8.8 Hz), 4.11 (1H, t, *J* = 6.3 Hz), 3.45 (1H, d, *J* = 12.3 Hz), 3.34 (1H, d, *J* = 12.3 Hz), 2.69 (3H, s), 2.52–2.43 (1H, m), 2.29–2.12 (2H, m), 2.06–2.01 (1H, m), 1.00 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 166.4, 85.2, 76.9, 59.9, 44.9, 37.1, 31.9, 25.8, 19.3, 15.9. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{12}H_{23}N_2O_6S_2$ 355.0992; Found 355.0989.



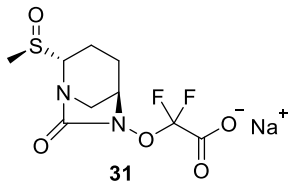
27

Sodium (2R,5R)-2-[(R)-methylsulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (27). To a solution of **26** (286 mg, 0.807 mmol) in DMF (3 mL) was added sodium thiolate **29** (187 mg, 1.21 mmol). After stirred overnight, the solvent was removed *in vacuo*. The crude product was applied onto the HP20SS resin and subjected to ODS column chromatography (H_2O only) to afford **27** (188 mg, 76%) as a white amorphous after lyophilization. ^{1}H NMR (400 MHz, D_2O) δ: 4.34–4.31 (2H, m), 3.47 (1H, d, *J* = 12.0 Hz), 3.39 (1H, d, *J* = 12.3 Hz), 2.79 (3H, s), 2.38–2.34 (1H, m), 2.20–2.16 (2H, m), 2.05–2.01 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 167.9, 75.3, 59.2, 45.3, 34.8, 18.5, 15.5. Anal. Calcd for $C_7H_{11}N_2O_6S_2Na(H_2O)_{1.3}$: C, 25.50; H, 4.16, N, 8.50; S, 19.45; Na, 6.97. Found: C, 25.86; H, 4.02; N, 8.25; S, 18.95; Na, 7.24.



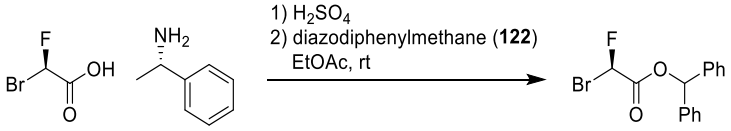
30

Ethyl 2,2-difluoro-2-[[(2R,5R)-2-[(R)-methylsulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] oxy]acetate (30). To a solution of **25** (402 mg, 1.97 mmol) in DMF (10 mL) were added a solution of ethyl 2-bromo-2,2-difluoroacetate **22b** (599 mg, 2.95 mmol) and K_2CO_3 (354 mg, 2.56 mmol). After being stirred for 3 h, the mixture was poured into 10% aq citric acid, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with water and dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude product was purified by flash column chromatography (0–10% MeOH/EtOAc) to give **30** (258 mg, 40%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.44–4.37 (2H, m), 4.15–4.12 (1H, m), 4.06–4.04 (1H, br m), 3.42 (1H, d, *J* = 12.2 Hz), 3.30 (1H, d, *J* = 12.3 Hz), 2.69 (3H, s), 2.49–2.41 (1H, m), 2.29–2.11 (2H, m), 2.06–1.98 (1H, m), 1.40 (3H, t, *J* = 7.2 Hz). MS-ESI (*m/z*): 327 $[M + H]^+$



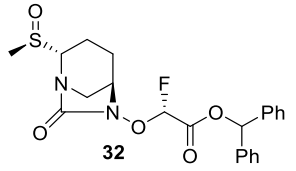
31

Sodium 2,2-difluoro-2-[[(2R,5R)-2-[(R)-methylsulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] oxy]acetate (31). To a solution of **30** (258 mg, 0.791 mmol) in THF/H_2O (v/v = 2/1, 4.5 mL) was added 1 N aq NaOH (0.791 mL, 0.791 mmol) at 0 ˚C. After being stirred for 30 min at 0 ˚C, the reaction was quenched by dry ice. The organic solvent was removed under reduced pressure, and the resulting solution was washed with EtOAc. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–10%, MeCN/H_2O) to give **31** (173 mg, 68%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 4.38 (1H, t, *J* = 6.0 Hz), 4.24–4.21 (1H, br m), 3.48 (1H, d, *J* = 12.3 Hz), 3.39 (1H, d, *J* = 12.0 Hz), 2.78 (3H, s), 2.38–2.31 (1H, m), 2.24–2.14 (2H, m), 2.08–2.05 (1H, m). ^{13}C{1H} NMR (100 MHz, D_2O) δ: 172.0, 166.1 (t, $^3J_{C\text{-}F}$ = 31.9 Hz), 120.0 (t, $^2J_{C\text{-}F}$ = 281.0 Hz), 78.4, 63.5, 47.7, 37.6, 21.3, 18.4. ^{19}F NMR (377 MHz, D_2O) δ: −83.1 (d, $^3J_{F\text{-}F}$ = 136.2 Hz), −83.7 (d, $^3J_{F\text{-}F}$ = 134.9 Hz). HPLC: 88.1% (method-A). Anal. calcd for $C_9H_{11}F_2N_2O_5SNa(H_2O)_{1.3}$: C, 31.46; H, 3.99; F, 11.06; N, 8.15; S, 9.33; Na, 6.69. found: C, 31.69; H, 4.04; F, 11.20; N, 8.33; S, 9.03; Na, 6.71.



121 22e

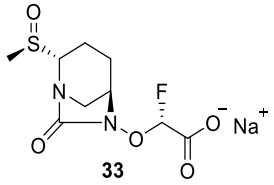
Benzhydryl (R)-2-bromo-2-fluoroacetate (22e). To a suspension of **121**[7, 8] (50.0 g, 180 mmol) in EtOAc (500 mL) and H_2O (250 mL) was added 64% aq H_2SO_4 (21.5 mL, 216 mmol) at 0 ˚C. After the mixture was stirred at room temperature for 15 min, the organic layer was separated and washed with water and brine. To the EtOAc solution was slowly added diazodiphenylmethane **122** (35.7 g, 184 mmol) in EtOAc (40 mL) at 0 ˚C. After the mixture was stirred at room temperature for 1 h, aq $NaHCO_3$ was added. The aqueous layer was extracted with EtOAc twice, and the combined organic layers were washed with brine before being dried over $MgSO_4$. The solvent was removed under reduced pressure to give **22e** (62.6 g, 90.2 wt%, 97%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.37–7.32 (10H, m), 6.98 (1H, s), 6.66 (1H, d, $^3J_{H\text{-}F}$ = 50.4 Hz). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 163.6 (d, *J* = 25.7 Hz), 138.5, 138.5, 128.7, 128.7, 128.6, 128.4, 127.3, 126.9, 80.9 (d, $^2J_{C\text{-}F}$ = 264.8 Hz). ^{19}F NMR (377 MHz, $CDCl_3$) δ: −150.69 (d, $^3J_{H\text{-}F}$ = 50.4 Hz).



32

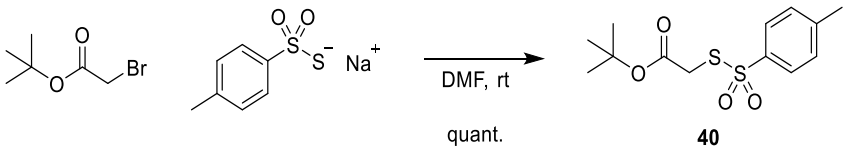
Benzhydryl (2R)-2-fluoro-2-[[(2R,5R)-2-[(R)-methylsulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]acetate (32). To a solution of **23** (15.1 g, 51.1 mmol) in MeOH (150 mL) were added 10% $Pd(OH)_2$ on carbon (3.59 g, 0.24 wt eq) and DABCO (229 mg, 2.05 mmol, 0.04 eq). After being stirred at room temperature under H_2 atmosphere (1 atm) for 1 h, the mixture was filtered. The solvent was

removed *in vacuo* to afford debenzylated product **25** as a white solid. To a solution of the debenzylated product and DBU (8.48 mL, 56.2 mmol, 1.1 eq) in DMF (50 mL) was added a solution of **22e** (19.8 g, 61.3 mmol, 1.20 eq) in DMF (30 mL) at 0 ˚C. After being stirred for 1 h at 0 ˚C, the mixture was poured into 5% aq citric acid, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a yellow solid. The solid was washed with ^{i}PrOAc to give **32** (13.1 g, 57% over 2 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.37–7.31 (10H, m), 6.95 (1H, s), 5.98 (1H, d, $^{3}J_{H\text{-}F}$ = 53.2 Hz), 4.08 (1H, t, *J* = 6.1 Hz), 3.97–3.95 (1H, br m), 3.19 (1H, d, *J* = 12.0 Hz), 2.68–2.65 (1H, m), 2.65 (3H, s), 2.41–2.33 (1H, m), 2.23–2.07 (2H, m), 1.97–1.89 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 167.0, 162.1 (d, $^{3}J_{C\text{-}F}$ = 35.2 Hz), 138.6, 138.5, 128.7, 128.5, 128.4, 128.4, 127.6, 127.1, 105.4 (d, $^{2}J_{C\text{-}F}$ = 238.4 Hz), 79.7, 76.7, 60.8, 44.5, 37.0, 19.1, 15.8. ^{19}F NMR (377 Hz, $CDCl_3$) δ: −134.1 (d, $^{3}J_{H\text{-}F}$ = 53.1 Hz). HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{22}H_{24}N_2O_5FS$ 447.1384; found 447.1385.



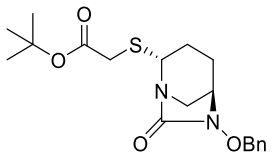
33

Sodium (2R)-2-fluoro-2-[[(2R,5R)-2-[(R)-methylsulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]acetate (33). To a solution of **32** (50.0 g, 112 mmol) in THF/H_2O (v/v = 2/1, 915 mL) was added 1 N aq NaOH (112 mL, 112 mmol) at 0 ˚C. After being stirred for 20 min at 0 ˚C, the reaction was quenched by dry ice. The organic solvent was removed under reduced pressure, and the resulting solution was washed with EtOAc. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–10%, MeCN/H_2O) to give **33** (26.5 g, 78%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 5.81 (1H, d, *J* = 58.5 Hz), 4.32 (1H, t, *J* = 6.3 Hz), 4.24–4.22 (1H, br m), 3.42 (1H, d, *J* = 12.0 Hz), 3.30 (1H, d, *J* = 12.0 Hz), 2.77 (3H, s), 2.38–2.29 (1H, m), 2.23–2.12 (2H, m), 2.07–1.99 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 171.9 (d, $^{3}J_{C\text{-}F}$ = 27.1 Hz), 171.4, 110.7 (d, $^{2}J_{C\text{-}F}$ = 241.4 Hz), 78.3, 62.6, 48.1, 37.7, 21.4, 18.5. ^{19}F NMR (377 MHz, D_2O) δ: −130.2 (d, $^{3}J_{H\text{-}F}$ = 59.9 Hz). HPLC: >99.9% (method-A). Anal. calcd for $C_9H_{12}FN_2O_5SNa(H_2O)_{2.1}$: C, 31.79; H, 4.80; F, 5.59; N, 8.24; S, 9.43; Na, 6.76. found: C, 31.81; H, 4.83; F, 5.40; N, 8.45; S, 9.28; Na, 6.64.



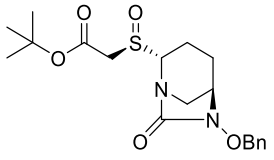
40

tert-Butyl 2-(tosylthio)acetate (40). To a solution of *tert*-butyl 2-bromoacetate (20.0 g, 103 mmol) in DMF (100 mL) was added potassium 4-methylbenzenesulfonothioate (23.2 g, 103 mmol), and the mixture was stirred for 1 h. The mixture was diluted with EtOAc, and the organic layer was washed with water twice before being dried over Na_2SO_4. The solvent was removed under reduced pressure to give **40** (31.5 g, quant.) as a pale yellow oil, which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.82 (2H, d, *J* = 8.3 Hz), 7.35 (2H, d, *J* = 8.2 Hz), 3.72 (2H, s), 2.45 (3H, s), 1.39 (9H, s). MS-ESI (*m/z*): 303 $[M + H]^+$.



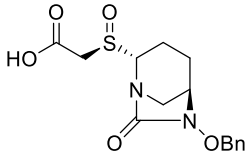
34

tert-Butyl 2-[[(2R,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]thio]acetate (34). To a solution of carboxylic acid **1** (41.5 g, 150 mmol) in CH_2Cl_2 (415 mL) were added 2-mercaptopyridine 1-oxide **4** (19.1 g, 150 mmol) and EDC·HCl (28.8 mg, 150 mmol). The mixture was stirred for 1 h in the dark (solution A). To another solution of **40** (136 g, 451 mmol) in CH_2Cl_2 (415 mL) was slowly added the solution A at 0 ˚C under white LEDs irradiation. After being stirred for 30 min at 0 ˚C, diluted aq HCl was added, and the layers were separated. The organic layer was washed with water and brine before being dried over $MgSO_4$. The solvent was removed under reduced pressure to give the crude product. To a slurry of silica-gel (100 g) in EtOAc/Hexane (v/v = 1/4, 500 mL) was added a solution of the crude product in EtOAc (100mL) at 0 ˚C. After being stirred for 15 min, the mixture was filtered, and the silica-gel was washed with EtOAc/hexane (v/v = 2/1) three times. The combined solvents were removed to give the roughly purified crude **34**, which was used without further purification.



35

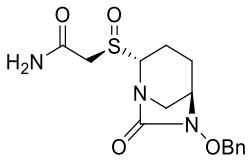
tert-Butyl 2-[(R)-[(2R,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]sulfinyl]acetate (35). To a solution of the crude **34** in CH_2Cl_2 (200 mL) was slowly added a solution of 65 wt % *m*-CPBA (39.8 g, 150 mmol) in CH_2Cl_2 (400 mL) at −78 ˚C. After being stirred for 30 min at −78 ˚C, aq $NaHCO_3$ and aq $Ns_2S_2O_3$ were added, and the organic solvent was removed under reduced pressure. The aqueous layer was extracted with EtOAc, and the organic layer was washed with brine before being dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude was purified by flush column chromatography (0–70%, EtOAc/hexane) to give **35** (21.5 g, 36%, dr = 88/12) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42–7.37 (5H, m), 5.03 (1H, d, *J* = 11.4 Hz), 4.89 (1H, d, *J* = 11.4 Hz), 4.31–4.28 (1H, m), 3.89 (1H, d, *J* = 14.7 Hz), 3.59 (1H, d, *J* = 14.4 Hz), 3.40–3.39 (1H, m), 3.17 (1H, d, *J* = 11.9 Hz), 3.03–3.00 (1H, m), 2.39–2.32 (1H, m), 2.19–2.10 (2H, m), 1.83–1.74 (1H, m), 1.49 (9H, s). MS-ESI (*m/z*): 395 $[M + H]^+$.



36

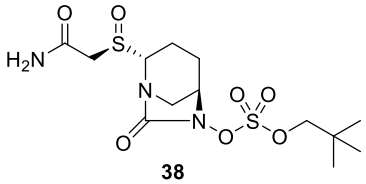
2-[(R)-[(2R,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]sulfinyl]acetic acid (36). To **35** (21.5 g, 54.6 mmol) was added TFA (150 mL) at 0 ˚C. After being stirred for 3 h at 0 ˚C, the solvent was removed under reduced pressure. The crude was triturated with EtOAc to give **36** (15.9 g,

86%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.21 (1H, br s), 7.45–7.35 (5H, m), 4.94 (1H, d, *J* = 12.0 Hz), 4.91 (1H, d, *J* = 11.6 Hz), 4.30 (1H, t, *J* = 6.4 Hz), 3.97 (1H, d, *J* = 14.9 Hz), 3.78–3.75 (1H, br m), 3.60 (1H, d, *J* = 14.7 Hz), 3.27 (1H, d, *J* = 11.9 Hz), 2.98 (1H, d, *J* = 11.6 Hz), 2.30–2.20 (1H, m), 1.95–1.80 (3H, m). MS-ESI (*m/z*): 339 $[M + H]^+$.



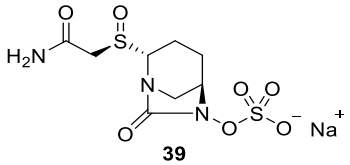
37

2-[(R)-[(2R,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]sulfinyl]acetamide (37). To a solution of **36** (15.6 g, 46.2 mmol) in CH_2Cl_2 (109 mL) were added HOSu (6.4 g, 55.5 mmol) and EDC·HCl (10.6 g, 55.5 mmol) at 0 ˚C. After being stirred for 2.5 h at room temperature, the mixture was cooled to −50 ˚C (solution A). To another solution of 28% aq ammonia (9.4 mL, 139 mmol) in CH_2Cl_2 (47 mL) was added the solution A at −50 ˚C. After being stirred for 1 h at room temperature, the mixture was poured into 10% aq citric acid, which was saturated with NaCl, and the aqueous layer was extracted with CH_2Cl_2 three times. The combined organic layers were washed with aq $NaHCO_3$, which was saturated with NaCl, before being dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude was purified by flush column chromatography (0–10%, MeOH/EtOAc) to give **37** (12.5 g, 80%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43–7.37 (5H, m), 6.60 (1H, br s), 5.60 (1H, br s), 5.03 (1H, d, *J* = 11.5 Hz), 4.89 (1H, d, *J* = 11.4 Hz), 4.31 (1H, dd, *J* = 7.6, 5.2 Hz), 3.86 (1H, d, *J* = 14.1 Hz), 3.57 (1H, d, *J* = 14.2 Hz), 3.42–3.40 (1H, br m), 3.10 (1H, d, *J* = 11.8 Hz), 3.06–3.03 (1H, m), 2.36–2.12 (3H, m), 1.84–1.75 (1H, m). MS-ESI (*m/z*): 338 $[M + H]^+$.



38

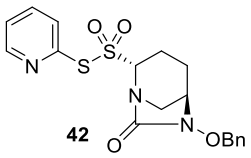
(2R,5R)-2-[(R)-(2-Amino-2-oxoethyl)sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl neopentyl sulfate (38). To a solution of **37** (1.00 g, 2.96 mmol) in MeOH (40 mL) were added 10% $Pd(OH)_2$ on carbon (0.83 g, 0.83 wt equiv) and DABCO (13 mg, 0.12 mmol). After being stirred under a H_2 atmosphere (1 atm) for 1 h, the mixture was filtered, and the solvent was removed to give the crude product, which was used without further purification. To a solution of the crude debenzylated product in DMF (5 mL) were added Et_3N (0.82 mL, 5.93 mmol) and neopentyl chlorosulfate **27** (0.83 g, 4.45 mmol) at 0 ˚C. After being stirred for 1 h at 0 ˚C, the mixture was poured into brine, and the aqueous layer was extracted with EtOAc three times. The combined organic layers were dried over Na_2SO_4 before the solvent was removed under reduced pressure. The crude was purified by flush column chromatography (0–10%, MeOH/EtOAc) to give **38** (0.72 g, 61%) as a white solid. ^{1}H NMR (400 MHz, methanol-d_4) δ: 4.56 (1H, t, *J* = 6.6 Hz), 4.40 (1H, d, *J* = 8.8 Hz), 4.27–4.24 (1H, br m), 4.16 (1H, d, *J* = 8.8 Hz), 3.92 (1H, d, *J* = 13.9 Hz), 3.67 (1H, d, *J* = 13.9 Hz), 3.56 (1H, d, *J* = 12.6 Hz), 3.33–3.31 (1H, m), 2.49–2.38 (1H, m), 2.21–2.03 (3H, m), 0.99 (9H, s). MS-ESI (*m/z*): 398 $[M + H]^+$.



39

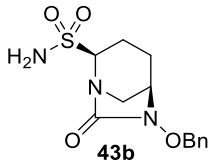
Sodium (2R,5R)-2-[(R)-(2-amino-2-oxoethyl)sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (39). To a solution of **38** (100 mg, 0.252 mmol) in MeCN/H_2O (v/v = 1/1, 2 mL) was added sodium 1,3,4-thiadiazole-2-thiolate (35.3 mg, 0.252 mmol). After being stirred overnight, water was added, and the aqueous layer was washed with EtOAc twice. The aqueous solution was applied onto the HP20SS resin and subjected to ODS column chromatography (0–3%, MeCN/H_2O) to give **39** (46.0 mg, 52%) as a white amorphous solid after lyophilization. ^{1}H NMR (400 MHz, D_2O) δ: 4.59 (1H, t, *J* = 6.3 Hz), 4.33–4.31 (1H, br m), 4.16 (1H, d, *J* = 14.4 Hz), 3.88 (1H, d, *J* = 14.1 Hz), 3.47–3.39 (2H, m), 2.41–2.32 (1H, m), 2.26–2.15 (2H, m), 2.10–2.02 (1H, m). HPLC: 98.6% (method-F). Anal. Calcd for $C_8H_{12}N_3O_7S_2Na(H_2O)_{1.1}$: C, 26.03; H, 3.88; N, 11.38; S, 17.37; Na, 6.23. Found: C, 26.03; H, 3.93; N, 11.72; S, 17.23; Na, 5.69.

第５節



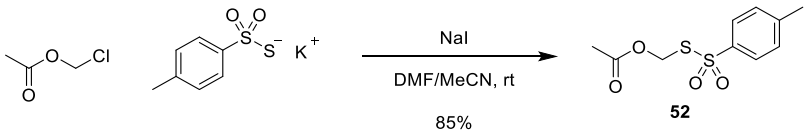
42

S-(Pyridin-2-yl) (2R,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-sulfonothioate (42). To a solution of the carboxylic acid **1** (2.00 g, 7.24 mmol) in CH_2Cl_2 (20 mL) were added Et_3N (1.51 mL, 10.9 mmol) and salt **15** (1.65 g, 8.69 mmol). The reaction mixture was stirred at room temperature for 1 hour in the dark (solution A). To another flask was added CH_2Cl_2 (20 mL) and cooled to −78 ˚C. After liquid sulfur dioxide (20 mL, 7.24 mmol) was added, an inner temperature was raised to −15 ˚C. Under white light irradiation using four LED lamps, the solution A was slowly added by a syringe wrapped in aluminum foil while maintaining the inner temperature under −10 ˚C. After the reaction mixture was stirred at 0 ˚C for 2 hours, the solvent was removed and water was added. The aqueous layer was extracted with EtOAc (30 mL × 2) and the combined organic layers were washed with $NaHCO_3$ aq and brine. After dried over $MgSO_4$, the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography (10–70% EtOAc/hexane) to obtain **42** (1.07 g, 37%) as a white amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.67 (1H, d, *J* = 4.0 Hz), 7.78–7.75 (2H, m), 7.41–7.35 (6H, m), 5.09 (1H, t, *J* = 8.2 Hz), 5.00 (1H, d, *J* = 11.5 Hz), 4.88 (1H, d, *J* = 11.5 Hz), 3.53 (1H, d, *J* = 12.3 Hz), 3.48–3.47 (1H, m), 3.13 (1H, d, *J* = 12.0 Hz), 2.37–2.31 (1H, m), 2.25–2.08 (2H, m), 1.88–1.80 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 166.8, 151.2, 150.7, 138.0, 135.4, 131.1, 129.3, 128.9, 128.6, 124.8, 79.6, 78.2, 57.8, 43.6, 18.5, 17.4. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{18}H_{20}N_3O_4S_2$ 406.0890; Found 406.0885.



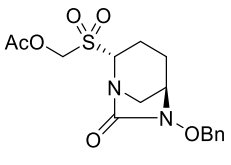
43b

(2S,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-sulfonamide (43b). To a solution of **42** (40.0 mg, 0.099 mmol) in DMF (0.40 mL) were added 1,2-dibromo-1,1,2,2-tetrachloroethane (257 mg, 0.789 mmol) and 7 M methanol solution of ammonia (42.3 μL, 0.296 mmol). After stirred for 2 hours, 0.5 N HCl aq was added. The aqueous layer was extracted with EtOAc (10 mL × 2) and the combined organic layers were washed with water and brine. After dried over $MgSO_4$, the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography (0–60% EtOAc/hexane) to obtain **43b** (2.00 mg, 6.5%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40–7.38 (5H, m), 5.12 (2H, s), 5.04 (1H, d, *J* = 11.5 Hz), 4.91 (1H, d, *J* = 11.3 Hz), 4.20 (1H, dd, *J* = 12.9, 4.1 Hz), 3.44 (1H, d, *J* = 11.5 Hz), 3.38–3.38 (1H, br m), 2.89 (1H, d, *J* = 11.5 Hz), 2.31–2.28 (2H, m), 2.14–2.07 (1H, m), 1.80–1.75 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 166.3, 135.1, 129.4, 129.1, 128.7, 78.5, 78.3, 58.2, 54.8, 23.2, 21.1. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{13}H_{18}N_3O_4S$ 312.1013; Found 312.1010.



52

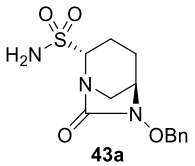
(Tosylthio)methyl acetate (52). To a white suspension of potassium 4-methylbenzenesulfonothioate (5.00 g, 22.1 mmol) in MeCN (20 mL) were added chloromethyl acetate (3.36 g, 30.9 mmol), NaI (3.31 g, 22.1 mmol) and DMF (15 mL). After stirred overnight, water was added and the mixture was extracted with EtOAc (40 mL × 2). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (0-33% EtOAc/hexane) to obtain **52** (4.86 g, 85%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.84 (2H, d, *J* = 8.3 Hz), 7.35 (2H, d, *J* = 8.3 Hz), 5.58 (2H, s), 2.46 (3H, s), 1.87 (3H, s). ^{13}C{^{1}H} NMR (100 MHz, CDCl3) δ: 169.6, 145.1, 142.9, 129.8, 127.2, 67.0, 21.7, 20.4. HRMS (ESI) *m/z*: [M + Na]+ Calcd for $C_{10}H_{12}O_4S_2Na$ 283.0069; Found 283.0066.



50

[[(2R,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]sulfonyl]methyl acetate (50). To a solution of carboxylic acid **1** (20.0 g, 72.4 mmol) and 2-mercaptopyridine 1-oxide **4** (9.20 g, 72.4 mmol) in CH_2Cl_2 (200 mL) was added EDC·HCl (13.9 g, 72.4 mmol). The solution was stirred for 1 hours at room temperature in the dark (solution A). Under white light irradiation using four LED lamps, solution A was slowly added to a solution of thiosulfonate **52** (56.5 g, 217 mmol) in CH_2Cl_2 (200 mL) at 0 ˚C over 20 minutes by a liquid feeding pump. After the reaction mixture was stirred at 0 ˚C for 20

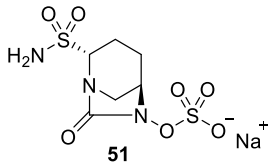
minutes, the organic layer was washed with water (300 mL) and dried over $MgSO_4$. After the solvent was removed *in vacuo*, the crude product was purified by flash column chromatography (0–40% EtOAc/hexane) to obtain **49** as a mixture of thiosulfonate **52** (25.6 g, 49 wt% purity). The product was used without any further purification. To a solution of **49** (25.6 g, 49 wt% purity) in CH_2Cl_2 (256 mL) was added 70 wt% *m*-CPBA (23.0 g, 93.3 mmol) at 0 ˚C. After stirred at room temperature for 2 hours, an aqueous solution of $Na_2S_2O_3$ and $NaHCO_3$ was added at 0 ˚C and the organic solvent was removed under reduced pressure. The aqueous layer was extracted with EtOAc (200 mL × 2) and the combined organic layers were washed with $NaHCO_3$ aq and dried over $MgSO_4$. After the solvent was removed *in vacuo*, the crude product was purified by flash column chromatography (25–50% EtOAc/hexane) to obtain **50** as a white amorphous (12.6 g, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39–7.38 (5H, m), 5.57 (1H, d, *J* = 12.0 Hz), 5.00 (2H, dd, *J* = 11.7, 2.6 Hz), 4.87 (1H, d, *J* = 11.5 Hz), 4.61 (1H, t, *J* = 8.3 Hz), 3.52 (1H, d, *J* = 12.3 Hz), 3.44–3.43 (1H, m), 3.06 (1H, d, *J* = 12.0 Hz), 2.31–2.28 (1H, m), 2.23 (3H, s), 2.17–2.03 (2H, m), 1.88–1.81 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 168.7, 166.9, 135.3, 129.3, 129.0, 128.7, 78.3, 70.1, 69.4, 57.6, 42.9, 20.4, 18.0, 13.3. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{16}H_{21}N_2O_6S$ 369.1115; Found 369.1113.



43a

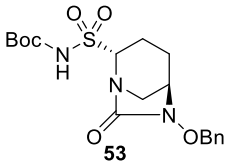
(2R,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-sulfonamide (43a). **Method A** (Scheme 1-16): To a solution of thiosulfonate **42** (75.9 mg, 0.187 mmol) in THF/H_2O (v/v = 10/1, 0.84 mL) was added NaSPh (27.5 mg, 0.187 mmol). After the mixture was stirred at room temperature for 3 hours, H_2O (10 mL) was added and the aqueous layer was washed with EtOAc (10 mL × 2). The aqueous solvent was partially removed under reduced pressure, the resulting aqueous sodium sulfinate **48** solution was equally divided into two flasks and one of the solution was used to the next step. To the aqueous solution of **48** (ca. 1.5 mL) were added NaOAc (19.2 mg, 0.23 mmol) and (aminooxy)sulfonic acid (13.2 mg, 0.12 mmol). After stirred at room temperature overnight, $Na_2S_2O_3$ aq was added and the aqueous layer was extracted with EtOAc (10 mL × 2). The combined organic layers were washed with 1 N HCl aq, $NaHCO_3$ aq, water and brine. After dried over $MgSO_4$, the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (0–60% EtOAc/hexane) to obtain **43a** as a white amorphous (13.3 mg, 46%). **Method B** (Scheme 1-17): To a solution of sulfone **50** (1.24 g, 3.37 mmol) in THF/H_2O (v/v = 1/1, 25 mL) was added 1 N NaOH aq (6.74 mL, 6.74 mmol) at 0 ˚C. After stirred at 0 ˚C for 1 hour, NaOAc (1.11 g, 13.5 mmol) and (aminooxy)sulfonic acid (1.22 g, 10.8 mmol) were added and the mixture was stirred at room temerature for 6 hours. After the organic solvent was removed under reduced pressure, the aqueous layer was extracted with EtOAc (20 mL × 2) and the combined organic layers were dried over $MgSO_4$. After the solvent was removed *in vacuo*, the crude product was purified by flash column chromatography (0–60% EtOAc/hexane) to obtain **43a** as a white amorphous (0.90 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39–7.38 (5H, m), 5.00 (1H, d, *J* = 11.5 Hz), 4.96 (2H, s), 4.87 (1H, d, *J* = 11.5 Hz), 4.39 (1H, t, *J* = 8.2 Hz), 3.55 (1H, d, *J* = 12.0 Hz), 3.45–3.44 (1H, m), 3.07 (1H, d, *J* = 11.8 Hz), 2.32–2.07 (3H, m), 1.84–1.77 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 167.7, 135.3, 129.3, 129.0, 128.7, 78.3, 74.0, 57.9, 43.3, 18.2, 17.1. HRMS (ESI)

m/z: $[M + H]^+$ Calcd for $C_{13}H_{18}N_3O_4S$ 312.1013; Found 312.1010.



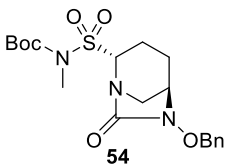
51

Sodium (2R,5R)-7-oxo-2-sulfamoyl-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (51). To a solution of **43a** (168 mg, 0.54 mmol) in MeOH (6.7 mL) was added 5% Pd/C (115 mg, 0.68 wt equiv). After stirred at room temperature under H_2 atmosphere (1 atm) for 1 hour, the mixture was filtered. The solvent was removed *in vacuo* and the crude product was used without any purification. The crude product was dissolved to pyridine (6 mL) and SO_3-pyridine (514 mg, 3.23 mmol) was added. After stirred for 3.5 hours, 8.4% $NaHCO_3$ aq (20 mL) was added at 0 ˚C and the aqueous layer was washed with CH_2Cl_2 (20 mL × 3) and the solvent was removed under reduced pressure. The crude product was applied onto the HP20SS resin and subjected to ODS column chromatography (H_2O only) to afford **51** (133 mg, 77%) as a white amorphous after lyophilization. ^{1}H NMR (400 MHz, D_2O) δ: 4.60 (1H, t, *J* = 8.1 Hz), 4.32–4.29 (1H, br m), 3.72 (1H, d, *J* = 12.3 Hz), 3.41 (1H, dd, *J* = 12.0, 2.1 Hz), 2.30–2.24 (2H, m), 2.10–2.04 (2H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 168.7, 74.2, 59.6, 55.4, 43.4, 17.7. HPLC: 94.4% (method-C). Anal. Calcd for $C_6H_{10}N_3O_7S_2Na(H_2O)_{2.0}(NaHCO_3)_{0.3}$: C, 19.68; H, 3.75; N, 10.93; S, 16.68; Na, 7.77. Found: C, 19.52; H, 3.75; N, 11.10; S, 16.89; Na, 7.72.



53

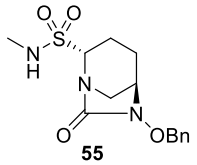
tert-Butyl [[(2R,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]sulfonyl]carbamate (53). To a solution of **43a** (2.58 g, 8.27 mmol) in CH_2Cl_2 (13 mL) were added DMAP (1.21 g, 9.93 mmol) and Boc_2O (2.11 mL, 9.10 mmol) at 0 ˚C. After being stirred overnight at room temperature, the mixture was diluted with EtOAc. The organic layer was washed with 10% aq citric acid, 8.4% aq $NaHCO_3$, water, and brine, before being dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flush column chromatography (0–65%, EtOAc/hexane) to give **53** (3.14 g, 92%) as a white sticky foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41–7.36 (5H, m), 6.96 (1H, brs), 5.01 (1H, d, *J* = 11.4 Hz), 4.93 (1H, t, *J* = 8.4 Hz), 4.85 (1H, d, *J* = 11.4 Hz), 3.48 (1H, d, *J* = 12.2 Hz), 3.41–3.39 (1H, m), 3.09–3.05 (1H, m), 2.33–2.07 (3H, m), 1.85–1.78 (1H, m), 1.54 (9H, s). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 167.0, 149.1, 135.4, 129.3, 128.9, 128.6, 85.0, 78.3, 71.5, 57.8, 43.3, 27.9, 18.3, 16.6. HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{18}H_{26}N_3O_6S$ 412.1537; found 412.1533.



54

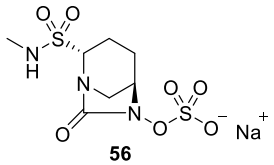
tert-Butyl [[(2R,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]sulfonyl](methyl) carbamate (54). To a solution of **53** (400 mg, 0.97 mmol) in THF (8 mL) were added MeOH (59 μL, 1.46

mmol), PPh_3 (433 mg, 1.65 mmol) and di-2-methoxyethyl azodicarboxylate (DMEAD) (387 mg, 1.65 mmol) at 0 ˚C. After being stirred for 1 h at room temperature, the solvent was removed under reduced pressure. The crude was purified by flush column chromatography (0–50%, EtOAc/hexane) to give **54** (412 g, quant.) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41–7.36 (5H, m), 5.13 (1H, t, *J* = 8.2 Hz), 5.00 (1H, d, *J* = 11.5 Hz), 4.85 (1H, d, *J* = 11.5 Hz), 3.50 (1H, d, *J* = 12.0 Hz), 3.41–3.39 (1H, m), 3.24 (3H, s), 3.04 (1H, dt, *J* = 12.0, 1.5 Hz), 2.35–2.24 (1H, m), 2.21–2.03 (2H, m), 1.86–1.77 (1H, m), 1.56 (9H, s). MS-ESI (*m/z*): 426 $[M + H]^+$.



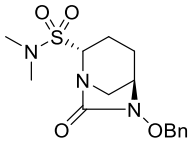
55

(2R,5R)-6-(Benzyloxy)-N-methyl-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-sulfonamide (55). To a solution of **54** (202 mg, 0.474 mmol) in CH_2Cl_2 (4 mL) was added 2 M $AlCl_3$ in $MeNO_2$ (0.47 mL, 0.949 mmol) at −30 ˚C. After being stirred for 1 h at −30 ˚C, 1 N aq HCl was added, and the mixture was extracted with EtOAc. The organic layer was washed with aq $NaHCO_3$ before being dried over Na_2SO_4. The solvent was removed to give **55** (148 mg, 96%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40–7.37 (5H, m), 4.99 (1H, d, *J* = 11.4 Hz), 4.87 (1H, d, *J* = 11.4 Hz), 4.51 (1H, q, *J* = 5.1 Hz), 4.41 (1H, t, *J* = 8.0 Hz), 3.55 (1H, d, *J* = 11.9 Hz), 3.47–3.46 (1H, m), 3.05–3.03 (1H, m), 2.89 (3H, d, *J* = 5.3 Hz), 2.33–2.22 (1H, m), 2.17–2.04 (2H, m), 1.83–1.80 (1H, m). MS-ESI (*m/z*): 326 $[M + H]^+$.



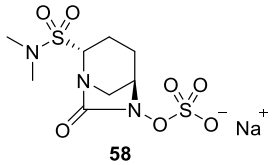
56

Sodium (2R,5R)-2-(N-methylsulfamoyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (56). To a solution of **55** (148 mg, 0.455 mmol) in MeOH (3 mL) was added 5% Pd/C (97 mg, 0.65 wt equiv). After being stirred under a H_2 atmosphere (1 atm) for 40 min, the mixture was filtered, and the solvent was removed to give the debenzylated product, which was used without further purification. To a solution of the crude product in pyridine (3 mL) was added SO_3-pyridine (181 mg, 1.14 mmol). After being stirred overnight, the mixture was poured into aq $NaHCO_3$ and the aqueous layer was washed with CH_2Cl_2 twice. To the aqueous layer were added CH_2Cl_2 (20 mL) and tetrabutylammonium hydrogen sulfate (176 mg, 0.519 mmol) at 0 ˚C. After being stirred for 15 min at room temperature, the aqueous layer was extracted with CH_2Cl_2 (20 mL × 3), and the solvent was removed under reduced pressure. The crude product was applied onto a Dowex sodium form column (Dowex 50WX8 hydrogen form treated with 1 N aq NaOH and washed until neutral pH with H_2O) and subjected to ODS column chromatography (H_2O only). The fractions containing the desired compound were combined, frozen and lyophilized to afford **56** (115 mg, 75%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 4.75–4.71 (1H, m), 4.32–4.29 (1H, br m), 3.71 (1H, d, *J* = 12.3 Hz), 3.40 (1H, d, *J* = 12.0 Hz), 2.82 (3H, s), 2.34–2.13 (2H, m), 2.09–2.04 (2H, m). HPLC 89.5% (method-C). Anal. calcd for $C_7H_{12}N_3O_7S_2Na(H_2O)_{1.0}$: C, 23.66; H, 3.97; N, 11.83; S, 18.05; Na, 6.47. found: C, 23.58; H, 4.04; N, 11.48; S, 18.21; Na, 6.79.



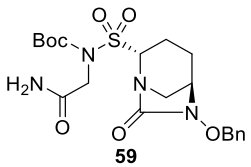
57

(2R,5R)-6-(Benzyloxy)-N,N-dimethyl-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-sulfonamide (57). To a solution of **55** (142 mg, 0.435 mmol) in THF (2.8 mL) were added MeOH (27 μL, 0.653 mmol), PPh_3 (194 mg, 0.740 mmol) and diisopropyl azodicarboxylate (DIAD) (144 μL, 0.740 mmol) at 0 ˚C. After being stirred for 30 min at room temperature, the solvent was removed. The crude was purified by flush column chromatography (0–70%, 25% EtOAc in CH_2Cl_2 / hexane) to give **57** (129 mg, 87%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42–7.36 (5H, m), 4.99 (1H, d, *J* = 11.4 Hz), 4.87 (1H, d, *J* = 11.5 Hz), 4.51 (1H, t, *J* = 7.6 Hz), 3.60 (1H, d, *J* = 11.9 Hz), 3.45–3.43 (1H, m), 3.00–2.99 (7H, m), 2.31–2.21 (1H, m), 2.15–2.04 (2H, m), 1.85–1.76 (1H, m). MS-ESI (*m/z*): 340 $[M + H]^+$.



58

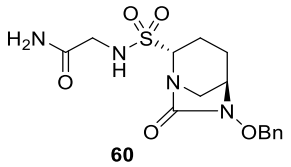
Sodium (2R,5R)-2-(N,N-dimethylsulfamoyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (58). To a solution of **57** (255 mg, 0.750 mmol) in MeOH/THF (v/v = 4/1, 12.5 mL) was added 5% Pd/C (160 mg, 0.63 wt equiv). After being stirred under a H_2 atmosphere (1 atm) for 1 h, the mixture was filtered, and the solvent was removed to give the crude product, which was used without further purification. To a solution of the crude debenzylated product in pyridine (9.3 mL) was added SO_3-pyridine (716 mg, 4.50 mmol). After being stirred overnight, the mixture was poured into aq $NaHCO_3$, and the aqueous layer was washed with CH_2Cl_2 twice. The aqueous solvent was removed under reduced pressure, and the crude was applied onto the HP20SS resin and subjected to ODS column chromatography (0–7%, MeCN/H_2O) to give **58** (156 mg, 59%) as a white amorphous solid after lyophilization. ^{1}H NMR (400 MHz, D_2O) δ: 4.85 (1H, t, *J* = 8.2 Hz), 4.31–4.28 (1H, br m), 3.72 (1H, d, *J* = 12.4 Hz), 3.40 (1H, d, *J* = 12.6 Hz), 3.01 (6H, s), 2.35–2.25 (1H, m), 2.21–2.11 (1H, m), 2.08–2.04 (2H, m). HPLC: 98.9%. MS-ESI (*m/z*): 328 $[M - H]^-$.



59

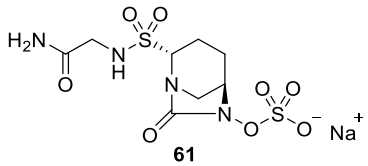
tert-Butyl (2-amino-2-oxoethyl)[(2R,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl] sulfonyl]carbamate (59). To a solution of **53** (483 mg, 1.17 mmol) in DMF (4.8 mL) were added 2-iodoacetamide (651 mg, 3.52 mmol) and K_2CO_3 (389 mg, 2.82 mmol). After being stirred overnight, the mixture was poured into water, and the aqueous layer was extracted with EtOAc. The organic layer was washed with water and brine before being dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flush column chromatography (30–80%, EtOAc/hexane) to give **59** (460 mg, 84%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40–7.38 (5H, br m), 7.33 (1H, br s), 5.39–5.35 (2H, m), 5.02 (1H, d, *J* = 11.4 Hz), 4.85 (1H, d, *J* = 11.3 Hz), 4.48 (1H,

d, *J* = 17.4 Hz), 4.11 (1H, d, *J* = 17.4 Hz), 3.49 (1H, d, *J* = 12.2 Hz), 3.45 (1H, dd, *J* = 6.0, 3.1 Hz), 3.06 (1H, d, *J* = 11.5 Hz), 2.39–2.22 (2H, m), 2.17–2.08 (1H, m), 1.89–1.83 (1H, m), 1.55 (9H, s). MS-ESI (*m/z*): 469 $[M + H]^+$.



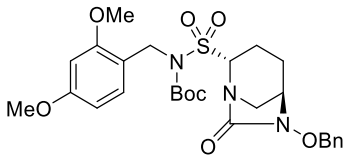
60

2-[[(2R,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane]-2-sulfonamido]acetamide (60). To a solution of **59** (220 mg, 0.47 mmol) in CH_2Cl_2 (1.1 mL) was added TFA (1.1 mL) at 0 ˚C. After being stirred for 2 h at room temperature, the mixture was poured into water, and the aqueous layer was extracted with EtOAc. The organic layer was washed with water and brine before being dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flush column chromatography (0–8%, MeOH/EtOAc) to give **60** (150 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41–7.37 (5H, m), 6.81 (1H, br s), 5.75 (1H, br s), 5.36 (1H, t, *J* = 6.1 Hz), 4.99 (1H, d, *J* = 11.3 Hz), 4.86 (1H, d, *J* = 11.4 Hz), 4.41 (1H, t, *J* = 8.1 Hz), 3.93 (2H, d, *J* = 6.3 Hz), 3.53 (1H, d, *J* = 12.0 Hz), 3.46–3.44 (1H, m), 3.08–3.05 (1H, m), 2.32–2.03 (3H, m), 1.85–1.77 (1H, m). MS-ESI (*m/z*): 369 $[M + H]^+$.



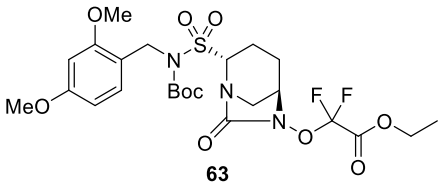
61

Sodium (2R,5R)-2-[N-(2-amino-2-oxoethyl)sulfamoyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (61). To a solution of **60** (150 mg, 0.407 mmol) in MeOH (2.3 mL) was added 5% Pd/C (435 mg, 2.9 wt equiv). After stirred under a H_2 atmosphere (1 atm) for 1 h, the mixture was filtered, and the solvent was removed to give the crude product, which was used without further purification. To a solution of the crude debenzylated product in pyridine (5.7 mL) was added SO_3-pyridine (388 mg, 2.44 mmol). After being stirred overnight, the mixture was poured into aq $NaHCO_3$, and the aqueous layer was washed with CH_2Cl_2 twice. The aqueous solvent was removed under reduced pressure, and the crude was applied onto the HP20SS resin and subjected to ODS column chromatography (H_2O only) to give **61** (103 mg, 67%) as a white amorphous solid after lyophilization. ^{1}H NMR (400 MHz, D_2O) δ: 4.70 (1H, t, *J* = 8.1 Hz), 4.31–4.29 (1H, br m), 3.92 (2H, s), 3.71 (1H, d, *J* = 12.4 Hz), 3.42–3.39 (1H, m), 2.35–2.17 (2H, m), 2.08–2.05 (2H, m). HPLC: 92.9% (method-C). MS-ESI (*m/z*): 357 $[M - H]^-$.



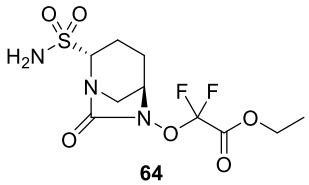
62

tert-Butyl [[(2R,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]sulfonyl](2,4-dimethoxybenzyl)carbamate (62). To a solution of **53** (8.09 g, 19.7 mmol) in THF (162 mL) were added (2,4-dimethoxyphenyl)methanol (4.96 g, 29.5 mmol), PPh_3 (8.77 g, 33.4 mmol), and di-2-methoxyethyl azodicarboxylate (DMEAD) (7.83 g, 33.4 mmol) at 0 ˚C. After the mixture was stirred for 2 h at room temperature, the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (0–50%, EtOAc/hexane) to give **62** (9.77 g, 89%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39–7.36 (5H, m), 7.21 (1H, d, *J* = 8.3 Hz), 6.43 (1H, dd, *J* = 8.2, 2.1 Hz), 6.39 (1H, d, *J* = 2.0 Hz), 5.17 (1H, t, *J* = 8.1 Hz), 4.98 (1H, d, *J* = 11.4 Hz), 4.87–4.78 (3H, m), 3.78 (3H, s), 3.77 (3H, s), 3.35–3.32 (2H, m), 2.77 (1H, d, *J* = 11.9 Hz), 2.28–2.02 (3H, m), 1.80–1.72 (1H, m), 1.51 (9H, s).



63

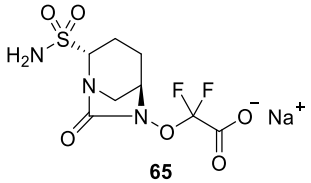
Ethyl 2-[[(2R,5R)-2-[N-(tert-butoxycarbonyl)-N-(2,4-dimethoxybenzyl)sulfamoyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2,2-difluoroacetate (63). To a solution of **62** (2.00 g, 3.56 mmol) in THF/MeOH (v/v = 1/3, 80 mL) was added 5% Pd/C (0.76 g, 0.38 wt equiv). After being stirred under a H_2 atmosphere (1 atm) for 1 h, the mixture was filtered, and the solvent was removed to give the crude product. To a solution of the crude debenzylated product in DMF (34 mL) were added ethyl 2-bromo-2,2-difluoroacetate **22b** (1.37 mL, 10.7 mmol) and K_2CO_3 (1.18 g, 8.54 mmol). After being stirred overnight for 3 h, the mixture was poured into water, and the aqueous layer was extracted with EtOAc three times. The combined organic layers were washed with water and dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (20–40%, EtOAc/hexane) to give **63** (1.58 g, 75%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.20 (1H, d, *J* = 8.4 Hz), 6.43 (1H, dd, *J* = 8.4, 2.3 Hz), 6.39 (1H, d, *J* = 2.3 Hz), 5.27 (1H, t, *J* = 8.3 Hz), 4.90 (1H, d, *J* = 16.2 Hz), 4.77 (1H, d, *J* = 16.3 Hz), 4.43–4.35 (2H, m), 3.97–3.95 (1H, m), 3.79 (3H, s), 3.78 (3H, s), 3.49 (1H, d, *J* = 12.5 Hz), 2.94 (1H, d, *J* = 12.5 Hz), 2.35–2.07 (3H, m), 2.01–1.94 (1H, m), 1.51 (9H, s), 1.39 (3H, t, *J* = 7.2 Hz). MS-ESI (*m/z*): 594 $[M + H]^+$.



64

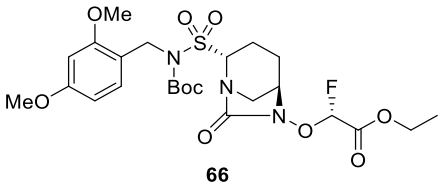
Ethyl 2,2-difluoro-2-[[(2R,5R)-7-oxo-2-sulfamoyl-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]acetate (64). To a solution of **63** (1.58 g, 2.66 mmol) in CH_2Cl_2 (32 mL) were added anisole (1.74 mL, 16.0

mmol) and 2 M solution of $AlCl_3$ in $MeNO_2$ (8.0 mL, 16.0 mmol) at −30 ˚C. After being stirred for 1 h at 0 ˚C, the mixture was poured into water, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with aq $NaHCO_3$, water, and brine before being dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (0–60%, EtOAc/hexane) to give **64** (835 mg, 91%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.78 (2H, s), 4.47 (1H, t, *J* = 8.5 Hz), 4.43–4.38 (2H, m), 4.10–4.08 (1H, m), 3.75 (1H, d, *J* = 12.4 Hz), 3.34 (1H, d, *J* = 12.6 Hz), 2.40–2.00 (4H, m), 1.39 (3H, t, *J* = 7.2 Hz).



65

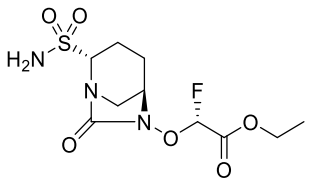
Sodium 2,2-difluoro-2-(((2R,5R)-7-oxo-2-sulfamoyl-1,6-diazabicyclo[3.2.1]octan-6-yl)oxy)acetate (65). To a solution of **64** (819 mg, 2.39 mmol) in THF/H_2O (v/v = 2/1, 24 mL) was added 1 N aq NaOH (2.39 mL, 2.39 mmol) at 0 ˚C. After the mixture was stirred for 1 h at 0 ˚C, the aqueous layer was washed with EtOAc. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–10%, MeCN/H_2O) to give **65** (706 mg, 88%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 4.65 (1H, t, *J* = 8.3 Hz), 4.25–4.22 (1H, br m), 3.71 (1H, d, *J* = 12.4 Hz), 3.40 (1H, dt, *J* = 12.4, 1.4 Hz), 2.33–2.24 (2H, m), 2.16–2.02 (2H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 172.7, 166.2 (t, $^3J_{C\text{-}F}$ = 31.9 Hz), 120.0 (t, $^2J_{C\text{-}F}$ = 280.6 Hz), 77.5, 64.0, 46.0, 20.7, 20.5. ^{19}F NMR (377 MHz, D_2O) δ: −83.1 (d, $^3J_{F\text{-}F}$ = 136.2 Hz), −83.8 (d, $^3J_{F\text{-}F}$ = 134.9 Hz). HPLC: >99.9% (method-A). Anal. calcd for $C_8H_{10}F_2N_3O_6SNa(H_2O)_{1.3}$: C, 26.64; H, 3.52; F, 10.54; N, 11.65; S, 8.89; Na, 6.37. found: C, 26.92; H, 3.27; F, 10.28; N, 11.88; S, 8.90; Na, 6.63.



66

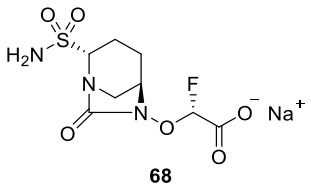
Ethyl (2R)-2-[[(2R,5R)-2-[N-(tert-butoxycarbonyl)-N-(2,4-dimethoxybenzyl)sulfamoyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (66). To a solution of **61** (9.77 g, 17.4 mmol) in MeOH (244 mL) was added 5% Pd/C (3.70 g, 0.38 wt equiv). After being stirred under a H_2 atmosphere (1 atm) for 1 h, the mixture was filtered, and the solvent was removed to give the crude product. To a solution of the crude debenzylated product in DMF (41 mL) were added **22d** (5.76 g, 19.1 mmol) and K_2CO_3 (2.65 g, 19.1 mmol) at −20 ˚C. After being stirred overnight at −20 ˚C, the mixture was poured into water, and the aqueous layer was extracted with EtOAc three times. The combined organic layers were washed with water and dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (20–50%, EtOAc/hexane) to give **66** (5.54 g, 55%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.19 (1H, d, *J* = 8.3 Hz), 6.42 (1H, dd, *J* = 8.3, 2.3 Hz), 6.38 (1H, d, *J* = 2.0 Hz), 5.82 (1H, d, $^3J_{H\text{-}F}$ = 52.8 Hz), 5.23 (1H, t, *J* = 8.3 Hz), 4.89 (1H, d, *J* = 16.2 Hz), 4.77 (1H, d, *J* = 16.2 Hz), 4.38–4.27 (2H, m), 3.99–3.98 (1H, m), 3.79 (3H, s), 3.77 (3H, s),

3.44 (1H, d, *J* = 12.4 Hz), 2.86 (1H, d, *J* = 12.4 Hz), 2.33–2.07 (3H, m), 1.97–1.91 (1H, m), 1.52 (9H, s), 1.35 (3H, t, *J* = 7.2 Hz). MS-ESI (*m/z*): 576 $[M + H]^+$.



67

Ethyl (2R)-2-fluoro-2-[[(2R,5R)-7-oxo-2-sulfamoyl-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]acetate (67). To a solution of **66** (164 mg, 0.284 mmol) in CH_2Cl_2 (3 mL) were added anisole (186 μL, 1.7 mmol) and 2 M solution of $AlCl_3$ in $MeNO_2$ (852 μL, 1.70 mmol) at −30 ˚C. After being stirred for 1 h at 0 ˚C, the mixture was poured into aq HCl, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with aq $NaHCO_3$, water, and brine before being dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (0–60%, EtOAc/hexane) to give **67** (63.2 mg, 68%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.85 (1H, d, *J* = 52.3 Hz), 4.75 (2H, br s), 4.44 (1H, t, *J* = 8.5 Hz), 4.36–4.28 (2H, m), 4.10 (1H, dd, *J* = 6.2, 3.2 Hz), 3.70 (1H, d, *J* = 12.4 Hz), 3.24 (1H, d, *J* = 12.1 Hz), 2.38–2.13 (3H, m), 2.05–1.97 (1H, m), 1.33 (3H, t, *J* = 7.1 Hz).



68

Sodium (2R)-2-fluoro-2-[[(2R,5R)-7-oxo-2-sulfamoyl-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]acetate (68). To a solution of **67** (150 mg, 0.461 mmol) in THF/H_2O (v/v = 2/1, 4.5 mL) was added 1 N aq NaOH (461 μL, 0.461 mmol) at 0 ˚C. After the mixture was stirred for 5 min at 0 ˚C, the aqueous layer was washed with EtOAc. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–10%, MeCN/H_2O) to give **68** (121 mg, 82%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 5.80 (1H, d, $^3J_{H\text{-}F}$ = 58.5 Hz), 4.60 (1H, t, *J* = 8.2 Hz), 4.25 (1H, t, *J* = 3.8 Hz), 3.67 (1H, d, *J* = 12.3 Hz), 3.33 (1H, dt, *J* = 12.3, 1.5 Hz), 2.34–2.19 (2H, m), 2.17–2.01 (2H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 172.1, 171.9 (d, $^3J_{C\text{-}F}$ = 27.1 Hz), 110.6 (d, $^2J_{C\text{-}F}$ = 240.6 Hz), 77.2, 63.1, 46.2, 20.6, 20.5. ^{19}F NMR (377 MHz, D_2O) δ: −130.1 (d, $^3J_{H\text{-}F}$ = 58.6 Hz). HPLC: >99.9% (method-A). Anal. calcd for $C_8H_{11}FN_3O_6SNa(H_2O)_{1.0}$: C, 28.49; H, 3.89; F, 5.63; N, 12.46; S, 9.51; Na, 6.82. found: C, 28.58; H, 4.12; F, 5.69; N, 12.52; S, 9.53; Na, 6.41.

第６節

Large-scale Synthesis of 23. To a solution of carboxylic acid **1** (600 g, 2.17 mol) in CH_2Cl_2 (3.0 L) were added 2-mercaptopyridine 1-oxide **4** (290 mg, 2.28 mol) and EDC·HCl (437 g, 2.28 mol) maintaining the inner temperature 10–20 ˚C, with CH_2Cl_2 (600 mL) for rinsing apparatuses. The reaction mixture was stirred at room temperature for 1.5 hours in the dark (solution A). A solution of PhSSMe **5b** (1.20 kg, 6.52 mol) in CH_2Cl_2 (1.2 L) was cooled to 0 ˚C under white light irradiation using eight

LED lamps (A 160WE Tuna Blue, Kessil®). The solution A was added over 1 hour by a liquid feeding pump, with a CH_2Cl_2 (600 mL) linewash while maintaining the inner temperature under 10 ˚C. After stirred at 0 ˚C for 1.5 hours, CO_2 gas evolution stopped and the reaction mixture was poured into water (10 L). The organic phase was separated and the aqueous layer was extracted with CH_2Cl_2 (300 mL). After dried over $MgSO_4$ (400 g), the solvent was removed under reduced pressure to afford 1.86 kg of the crude product. To a slurry of silica-gel (1.0 kg) in EtOAc/hexane (v/v = 1/2, 3.0 L) was slowly added a hexane (2.0 L) solution of the crude product while keeping the inner temperature under 10 ˚C. After stirred for 15 minutes, the mixture was filtered and the silica-gel was washed with EtOAc/hexane (v/v = 1/2, 3.0 L× 3). The solvent was removed under reduced pressure to afford a roughly purified sulfide **2b** (1.57 kg) containing PhSSMe **5b**. To a solution of the roughly purified sulfide **2b** (1.57 kg) in CH_2Cl_2 (2.4 L) was slowly added a solution of 72 wt% *m*-CPBA (448 g, 1.87 mol) in CH_2Cl_2 (4.8 L) over 1.5 hours while maintaining the inner temperature under −55 ˚C. After stirred for 15 minutes, the inner temperature was raised to −30 ˚C and the reaction mixture was poured into a stirring aqueous solution (4.3 L) of $NaHCO_3$ (225 g) and $Na_2S_2O_3 \cdot 5H_2O$ (350 g). After the organic layer was separated, NaCl (675 g) was added and the aqueous phase extracted with EtOAc (4.5 L × 2). The combined organic layers were dried over $MgSO_4$ (750 g) and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (0–10% MeOH/EtOAc) using 3.0 kg of silica-gel. To the purified product (553 g) was added EtOAc (97 mL) and the slurry was stirred at −30 ˚C for 20 minutes before filtering, washing with EtOAc (480 mL × 2) and diisopropyl ether (480 mL × 2), and drying to afford **23** (219 g, 34%, dr = 31/1) as a white crystalline solid.

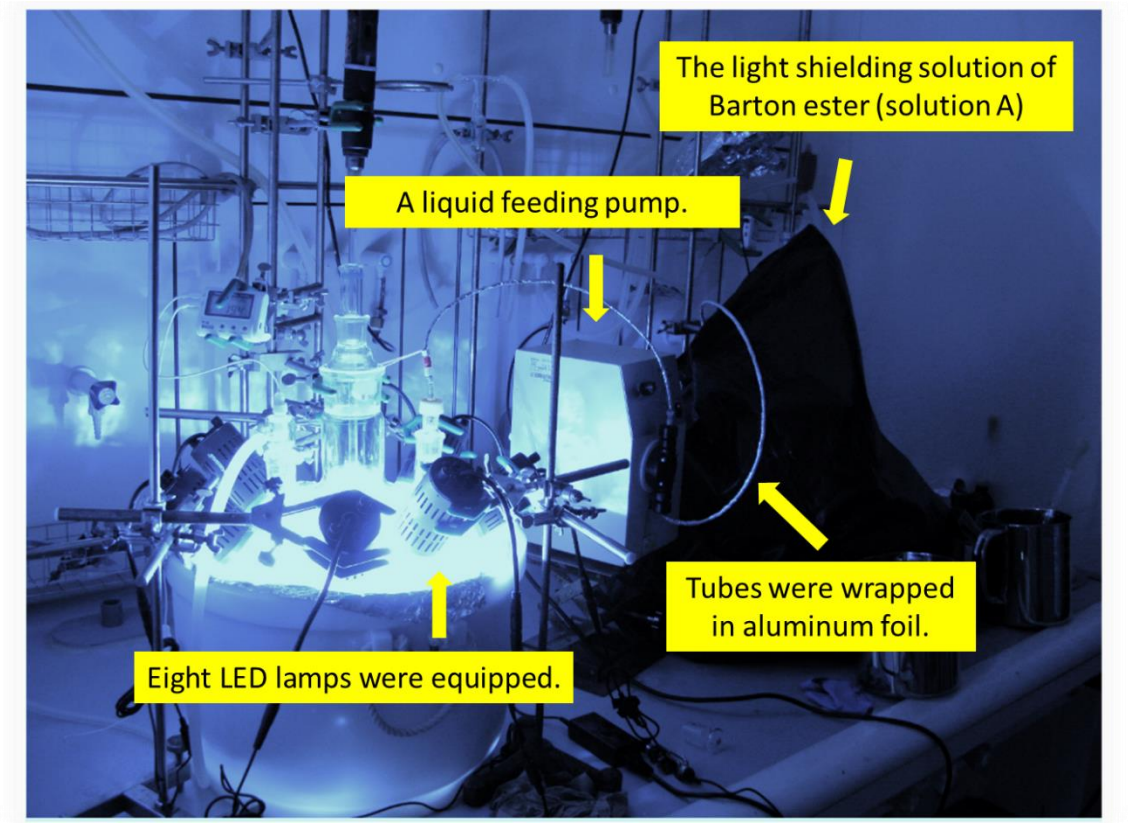
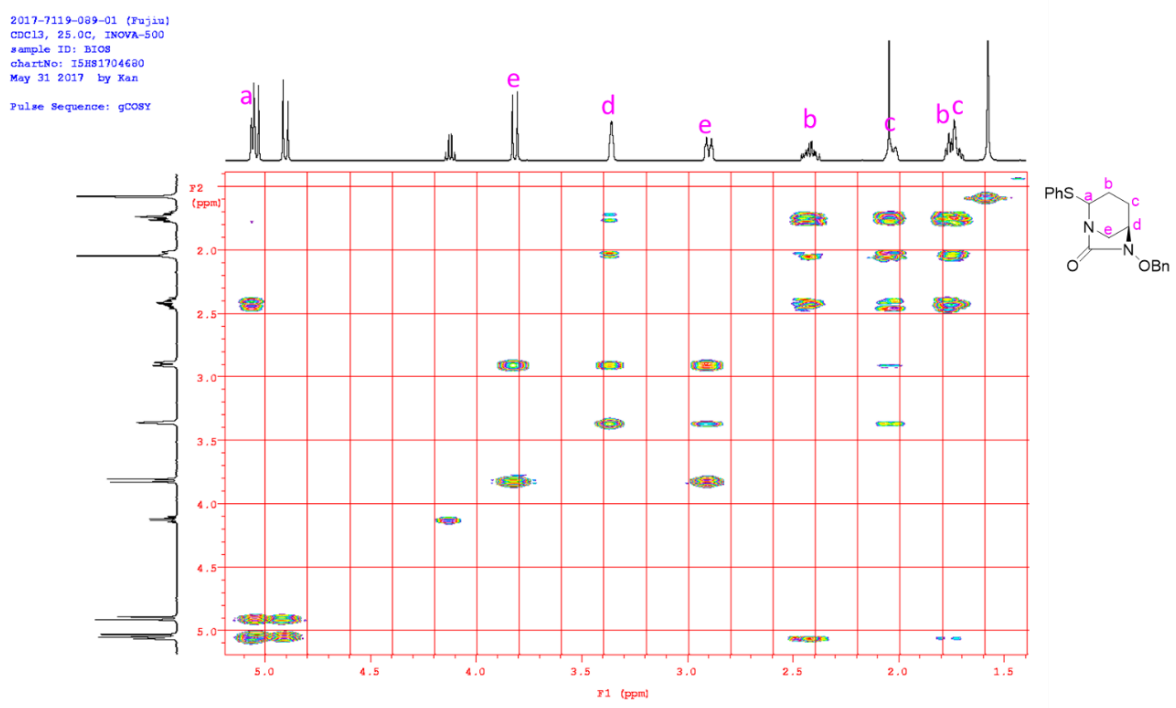


Figure S1. Reaction set up for the large-scale synthesis of **23**.

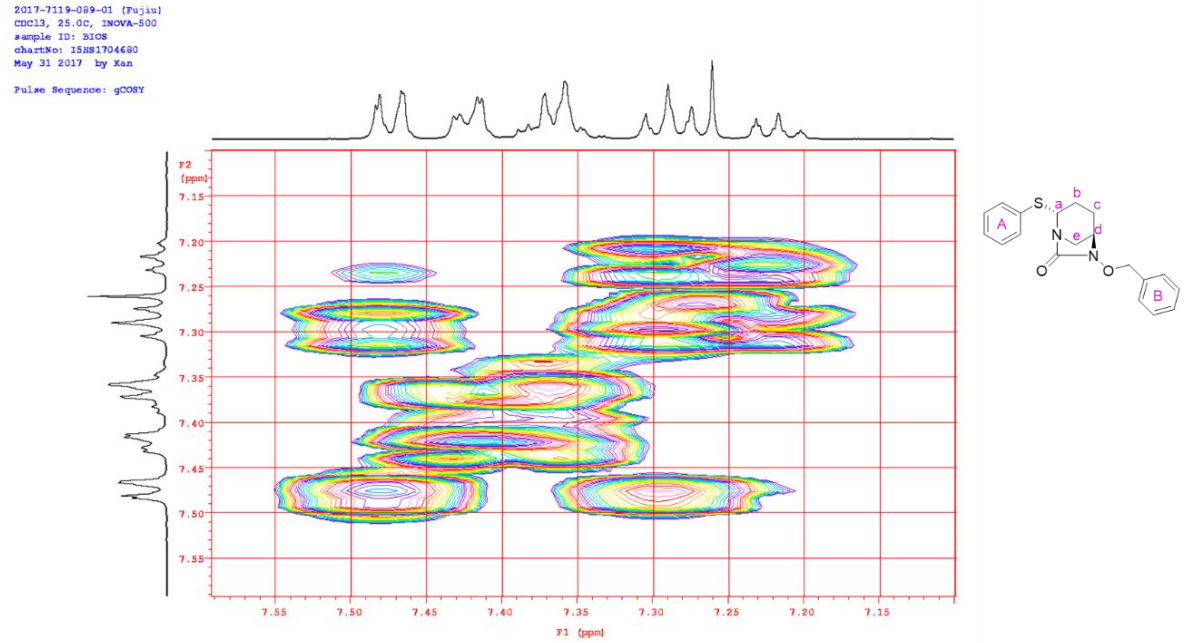
COSY and NOESY spectra of 2a, 42, 43a and 43b

COSY and NOESY of **2a**

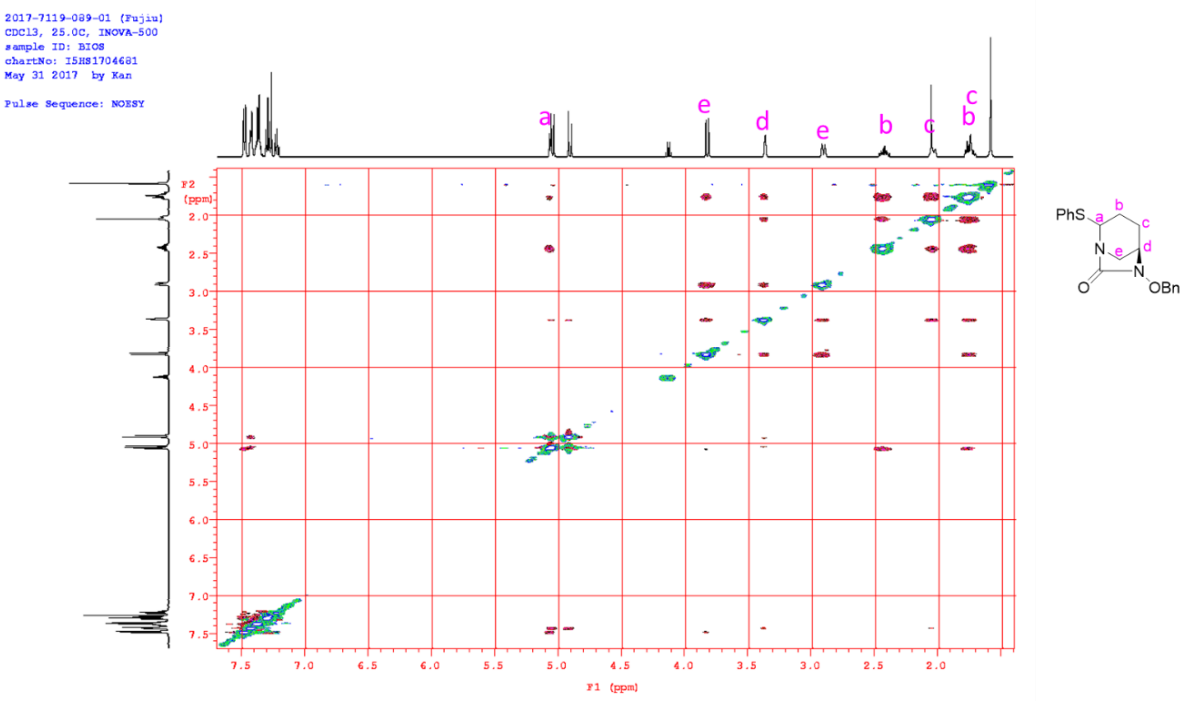
COSY-1 (500 MHz, $CDCl_3$)



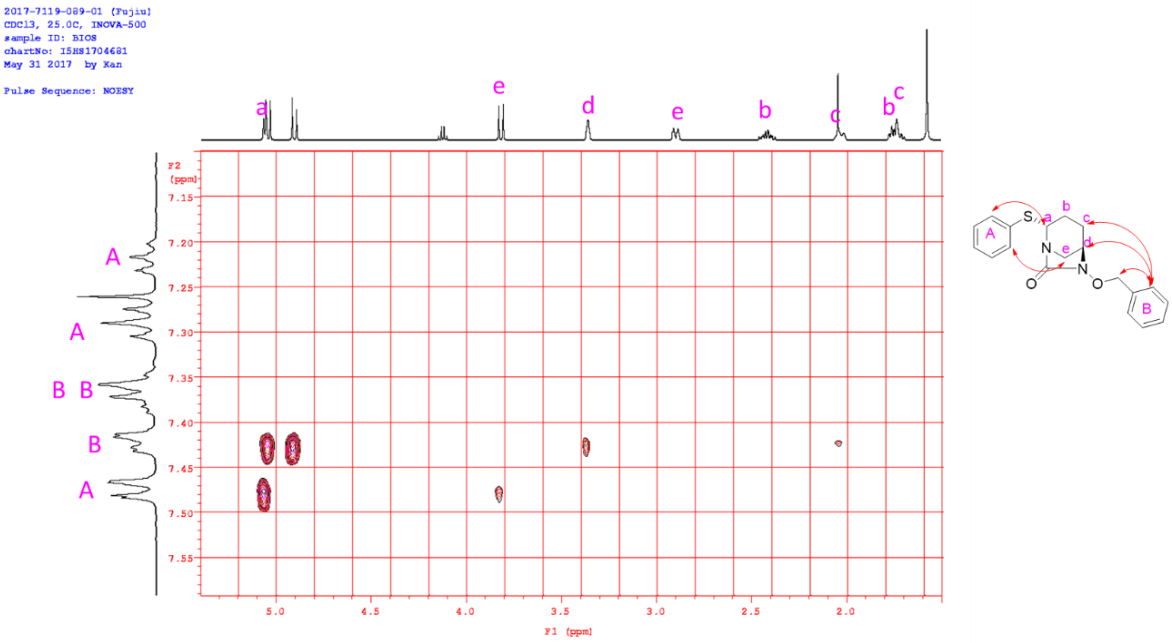
COSY-2 (500 MHz, $CDCl_3$)



NOESY-1 (500 MHz, $CDCl_3$)

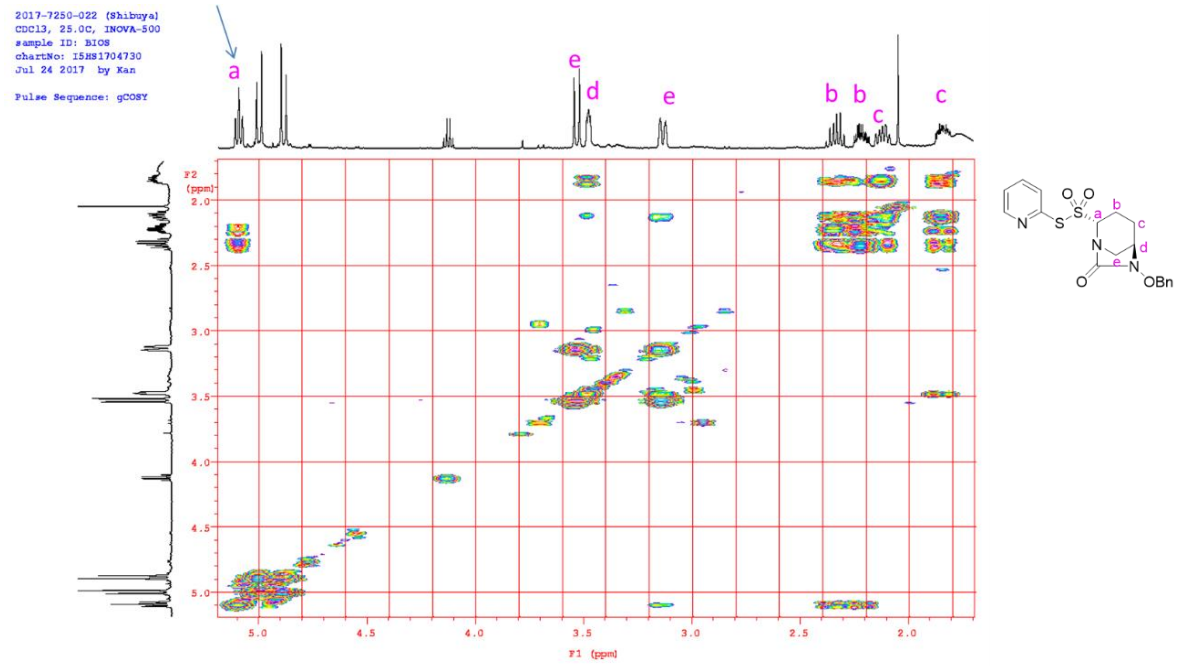


NOESY-2 (500 MHz, $CDCl_3$)

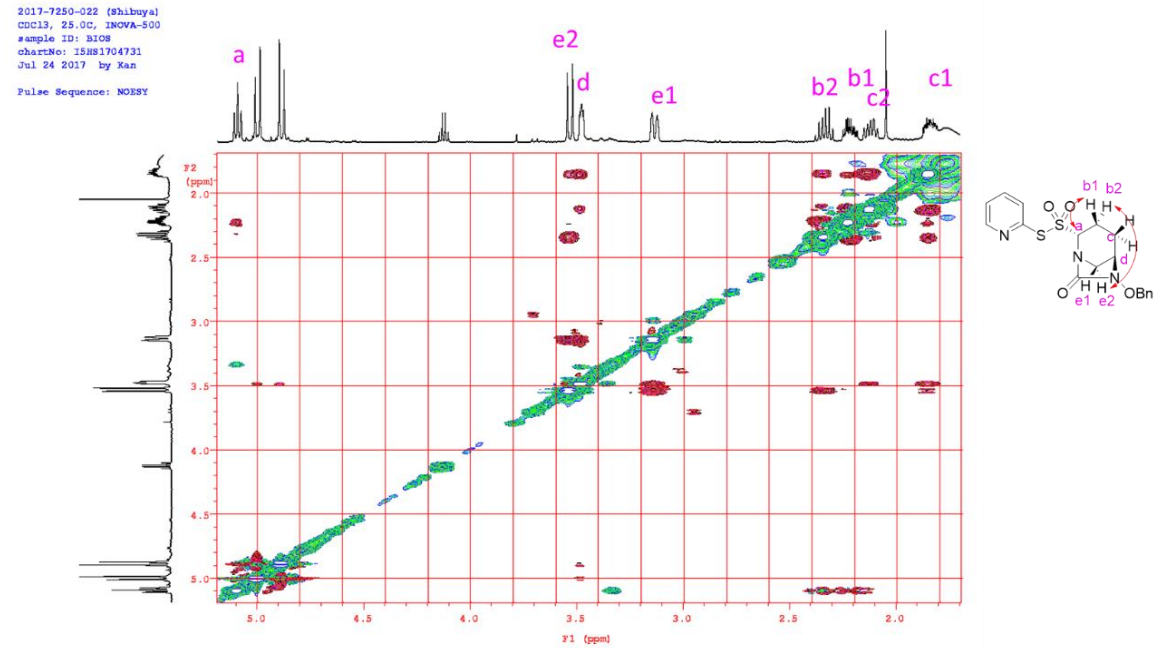


COSY and NOESY of **42**

COSY (500 MHz, $CDCl_3$)

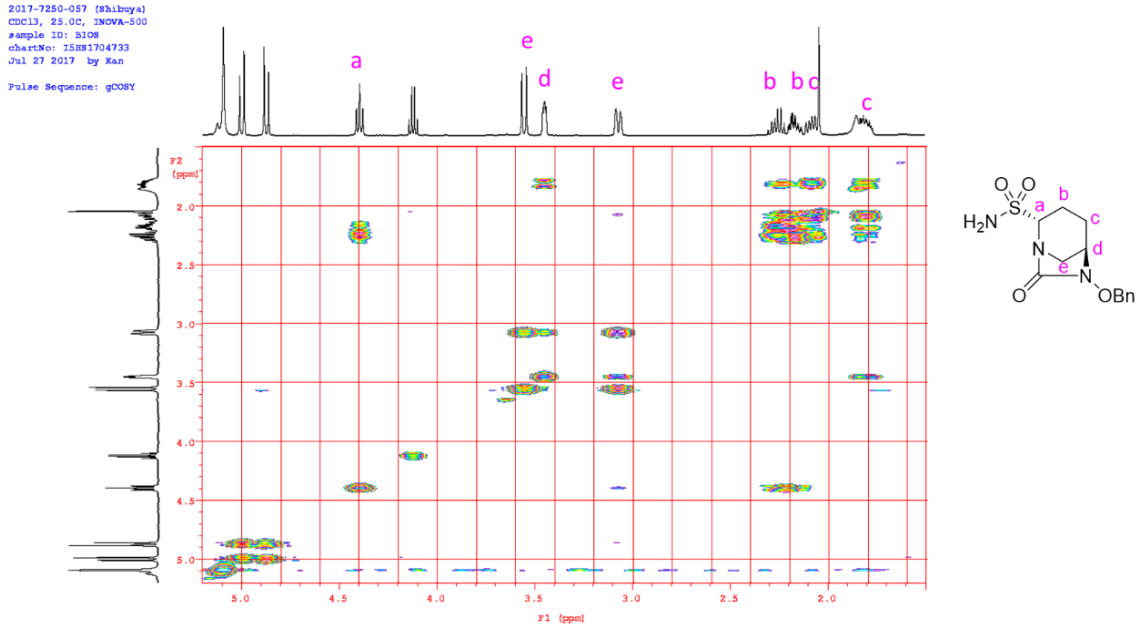


NOESY (500 MHz, $CDCl_3$)

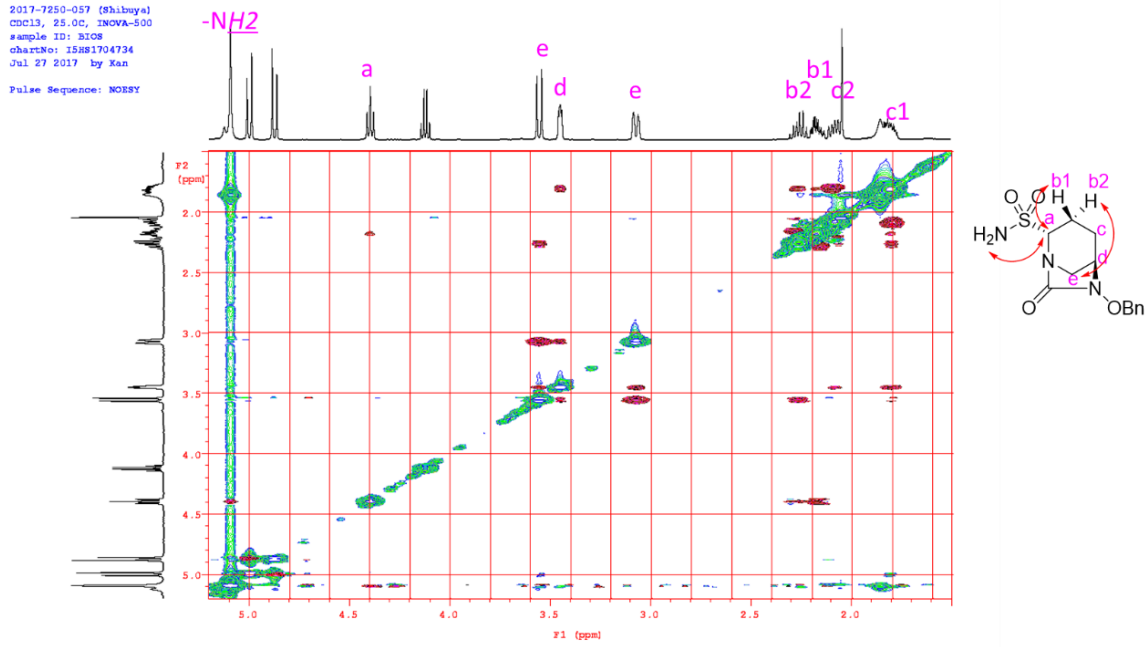


COSY and NOESY of **43a**

COSY (500 MHz, $CDCl_3$)

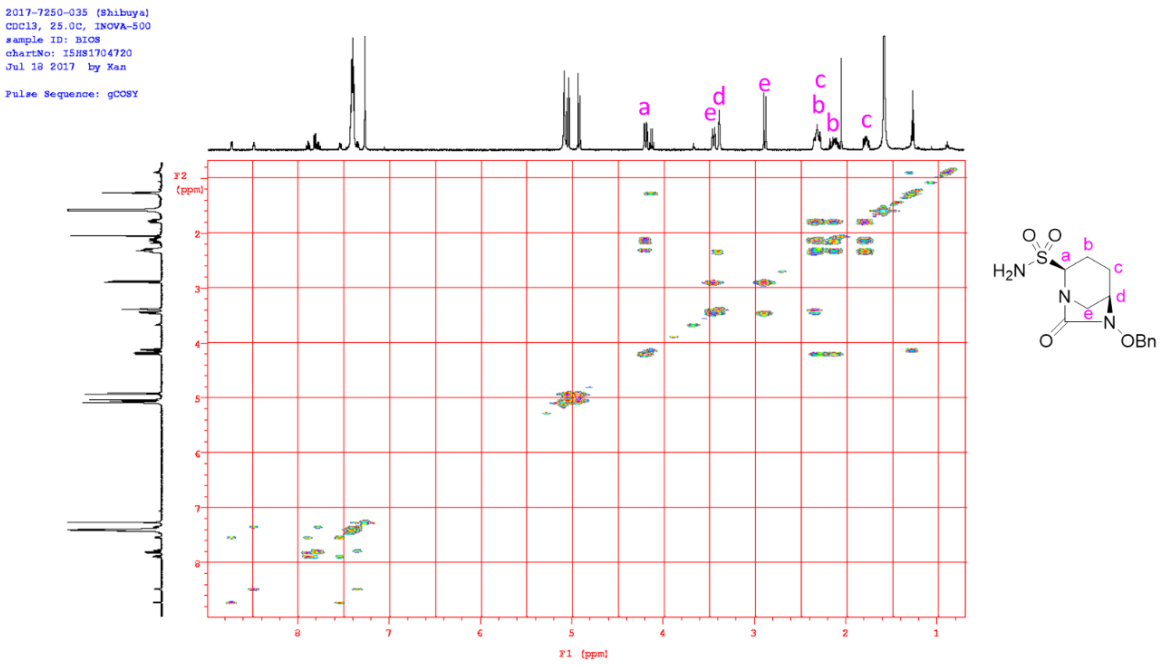


NOESY (500 MHz, $CDCl_3$)

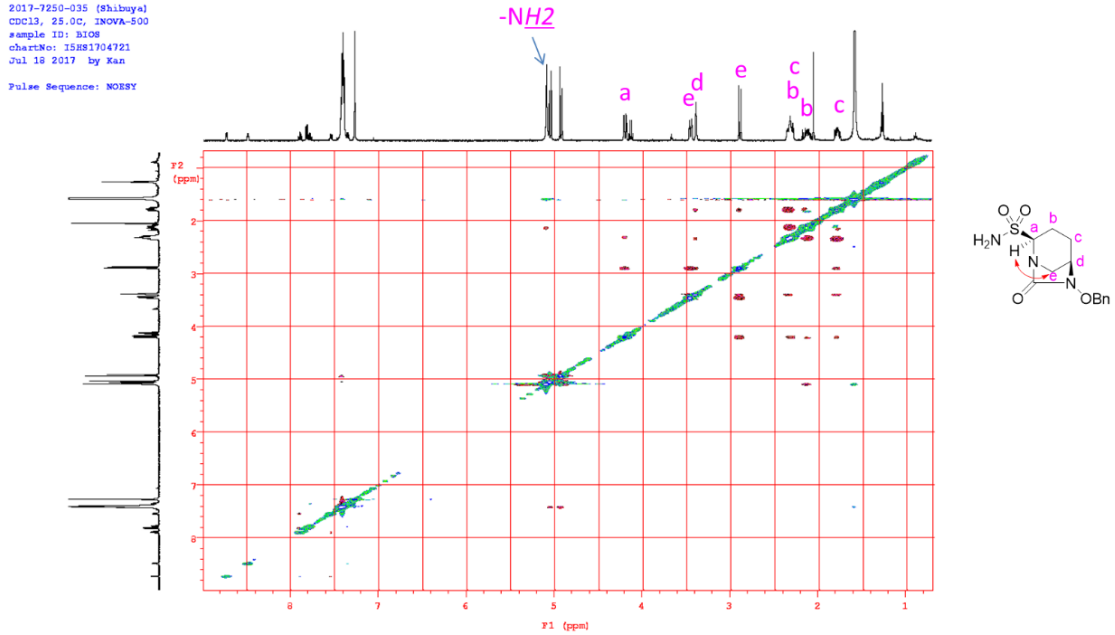


COSY and NOESY of **43b**

COSY (500 MHz, $CDCl_3$)



NOESY (500 MHz, $CDCl_3$)



X-ray crystallographic data of compound 23

X-ray crystallography:

The diffraction data of **23** were collected on an XtaLAB AFC10 (RCD3): quarter-chi single diffractometer. The crystal was kept at 100.0 K during data collection. Using Olex2,[10] the structure was solved with the ShelXT[11] structure solution program using Intrinsic Phasing and refined with the ShelXL[12] refinement package using Least Squares minimisation.

Sample preparation:

X-ray quality crystal was prepared by vapor diffusion method using EtOAc/hexane at room temperature.

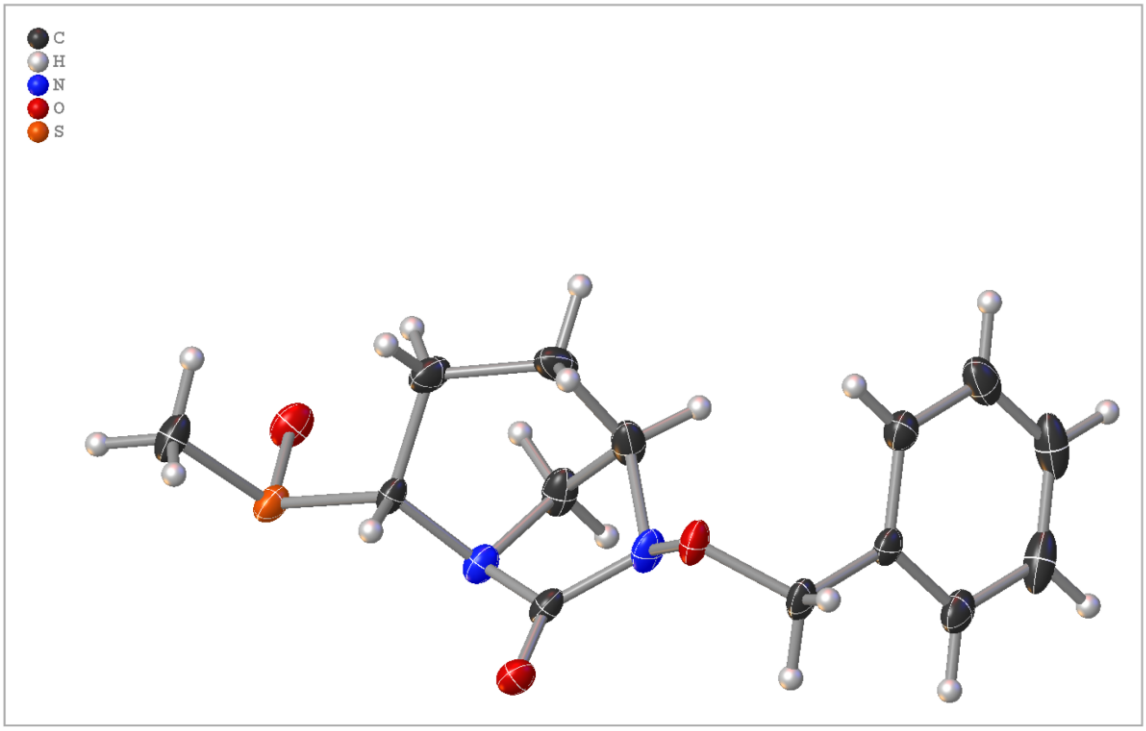
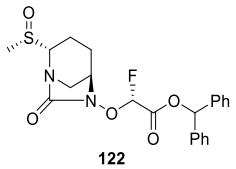


Figure S2. X-ray Structure of compound **23**. Thermal ellipsoids are set at 30% probability.

Table S1. Crystal data for 23

Formula	$C_{14}H_{18}N_2O_3S$
Formula weight	294.36
Temperature (K)	100
Crystal system	monoclinic
Space group	C2
a (Å)	20.2904 (10)
b (Å)	6.2175 (3)
c (Å)	11.6198 (5)
α (°)	90
β (°)	101.089 (4)
γ (°)	90
Volume ($Å^3$)	1438.53 (12)
Z	4
ρ (g/cm^3)	1.359
μ (mm^{-1})	2.085
F(000)	624.0
Crystal size (mm^3)	0.18 × 0.15 × 0.1
R_1	0.0441
Flack parameter	0.01(3)
CCDC Deposition Number	1993055

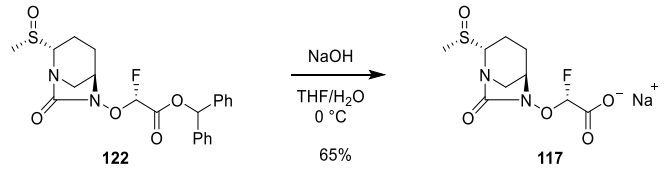
第３章の合成



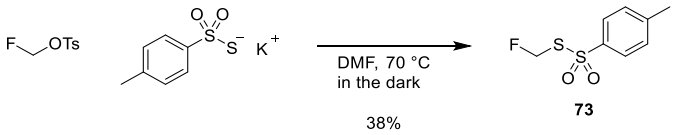
122

Benzhydryl (2R)-2-fluoro-2-[[(2R,5R)-2-[(S)-methylsulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]acetate (122). Compound **122** was isolated by preparative supercritical fluid chromatography (SFC) using CHIRALPAK IC/SFC (flow rate: 30 mL/min, mobile phase: ^{i}PrOH 38%, back pressure: 8 MPa). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41–7.28 (10H, m), 6.97 (1H, s), 5.98 (1H, d, $^3J_{H\text{-}F}$ = 53.5 Hz), 3.98–3.95 (1H, br m), 3.81 (1H, t, *J* = 8.0 Hz), 3.62 (1H, d, *J* = 12.0 Hz), 2.75 (1H, d, *J* = 12.0 Hz), 2.69 (3H, s), 2.52–2.41 (1H, m), 2.23–2.09 (2H, m), 2.04–1.94 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 168.9, 162.2 (d, $^3J_{C\text{-}F}$ = 35.2 Hz), 138.6, 138.6, 128.7, 128.5, 128.5, 128.4, 127.7, 127.1, 105.4 (d, $^2J_{C\text{-}}$

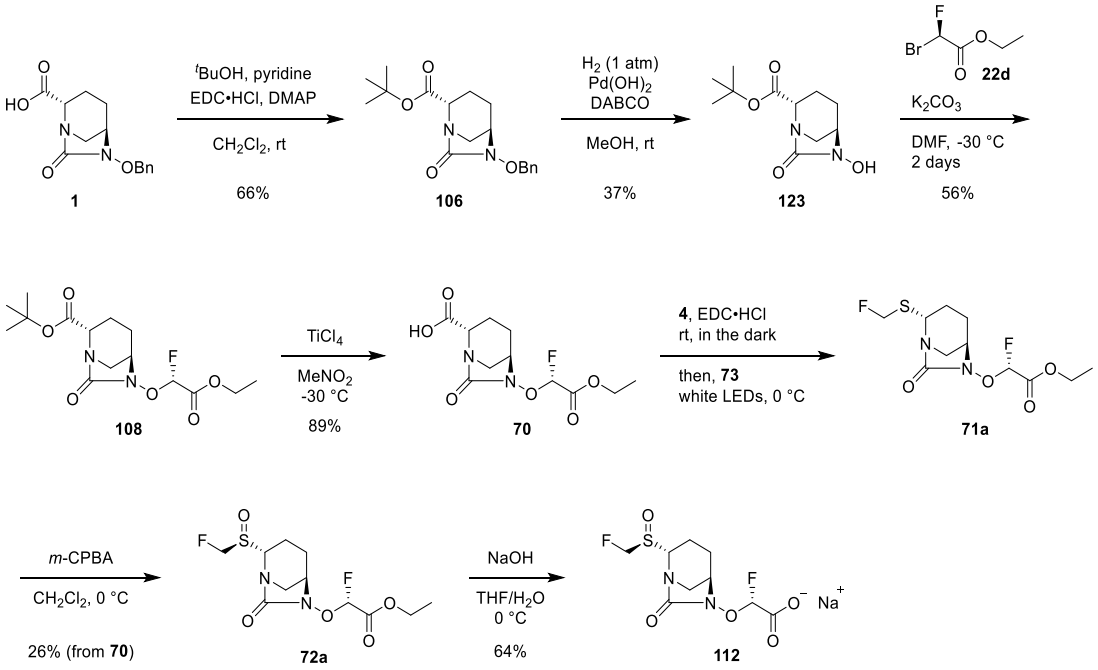
$_F$ = 238.4 Hz), 79.6, 72.4, 61.5, 44.5, 34.9, 19.1, 18.9. ^{19}F NMR (377 Hz, $CDCl_3$) δ: −134.1 (d, $^3J_{H\text{-}F}$ = 53.1 Hz). HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{22}H_{24}N_2O_5FS$ 447.1384; found 447.1382.



Sodium (2R)-2-fluoro-2-[[(2R,5R)-2-[(S)-methylsulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]acetate (117). To a solution of **122** (144 mg, 0.323 mmol) in THF/H_2O (v/v = 2/1, 3 mL) was added 1 N aq NaOH (0.323 mL, 0.323 mmol) at 0 ˚C. After being stirred for 15 min at 0 ˚C, the solution was washed with EtOAc. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–15%, MeCN/H_2O) to give **117** (63.4 mg, 64%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 5.80 (1H, d, $^3J_{H\text{-}F}$ = 59.0 Hz), 4.27–4.23 (2H, m), 3.53 (1H, d, *J* = 12.3 Hz), 3.37 (1H, dd, *J* = 12.0, 2.5 Hz), 2.75 (3H, s), 2.22–2.12 (3H, m), 2.07–1.98 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 173.0, 172.0 (d, $^3J_{C\text{-}F}$ = 27.1 Hz), 110.7 (d, $^2J_{C\text{-}F}$ = 240.6 Hz), 77.7, 63.1, 47.4, 36.3, 21.3, 21.2. ^{19}F NMR (377 MHz, D_2O) δ: −130.1 (d, $^3J_{H\text{-}F}$ = 58.6 Hz). HPLC: >99.9% (method-A). Anal. calcd for $C_9H_{12}FN_2O_5SNa(H_2O)_{2.1}$: C, 31.79; H, 4.80; F, 5.59; N, 8.24; S, 9.43; Na, 6.76. found: C, 32.03; H, 4.86; F, 5.53; N, 8.51; S, 8.84; Na, 6.27.



S-(Fluoromethyl) 4-methylbenzenesulfonothioate (73).[13] To a degassed solution of fluoromethyl 4-methylbenzenesulfonate (5.00 g, 24.5 mmol) in DMF (30 mL) was added potassium 4-methylbenzene-sulfonothioate (6.65 g, 29.4 mmol). After being stirred overnight at 70 ˚C in the dark, the mixture was poured into water, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with water and dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude product was purified by flash column chromatography (0–20% EtOAc/hexane) to give 4.83 g of 42 wt % **73** in a mixture with fluoromethyl 4-methyl-benzenesul- fonate (net. 2.02 g, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.83 (2H, d, *J* = 8.3 Hz), 7.36 (2H, d, *J* = 8.3 Hz), 6.01 (2H, d, $^3J_{H\text{-}F}$ = 50.8 Hz), 2.46 (3H, s). **Note**: Compound **73** might be light and air sensitive.



tert-Butyl (2S,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylate (106). To a solution of **1** (25.0 g, 90.0 mmol) in CH_2Cl_2 (250 mL) were added *tert*-butanol (86.0 mL, 905 mmol), pyridine (14.6 mL, 181 mmol), DMAP (1.1 g, 9.1 mmol), and EDC·HCl (20.8 g, 109 mmol) at 0 ˚C. After being stirred for 2 h, at room temperature, the mixture was washed with 1N aq HCl, water, aq $NaHCO_3$, and brine. After the solvent was removed under reduced pressure, the crude product was purified by flash column chromatography (0–30% EtOAc/hexane) to give **106** (19.9 g, 66%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42 (2H, d, J = 6.3 Hz), 7.39–7.32 (3H, m), 5.05 (1H, d, J = 11.4 Hz), 4.90 (1H, d, J = 11.4 Hz), 4.01–3.99 (1H, br m), 3.31–3.29 (1H, br m), 3.04 (1H, d, J = 12.1 Hz), 2.98 (1H, d, J = 11.6 Hz), 2.09–2.02 (3H, m), 1.71–1.62 (1H, m), 1.48 (9H, s). MS-ESI (*m/z*): 333 [M + H]$^+$.

tert-Butyl (2S,5R)-6-hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylate (123). To a solution of **106** (52.7 g, 159 mmol) were added DABCO (0.36 g, 3.17 mmol) and 10 wt % $Pd(OH)_2$ on carbon (11.1 g, 21.2 wt equiv). After being stirred under a H_2 atmosphere (1 atm) for 2 h, the mixture was filtered, and the solvent was removed under reduced pressure. The crude was triturated with diisopropyl ether to give **123** (14.4 g, 37%) as a white solid.

tert-Butyl (2S,5R)-6-[(R)-2-ethoxy-1-fluoro-2-oxoethoxy]-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylate (108). To a solution of **123** (21.8 g, 90.0 mmol) in DMF (196 mL) were added **22d** (20.0 g, 108 mmol) and K_2CO_3 (17.4 g, 126 mmol) at −40 ˚C. After being stirred for 2 days at −30 ˚C, the mixture was poured into iced water, and the aqueous layer was extracted with EtOAc. The organic layer was washed with water and brine before being dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude product was purified by flash column chromatography (20–30% EtOAc/hexane) to give **108** (17.3 g, 56%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.89 (1H, d, $^3J_{H\text{-}F}$ = 52.5 Hz), 4.35–4.26 (2H, m), 4.08 (1H, d, J = 6.3 Hz), 3.97–3.95 (1H, br m), 3.20 (1H, d, J = 12.1 Hz), 3.13 (1H, d, J = 11.9 Hz), 2.19–2.04 (3H, m), 1.89–1.81 (1H, m), 1.50 (9H, s), 1.33 (3H, t, J = 7.2 Hz). MS-

ESI (*m/z*): 347 $[M + H]^+$.

(2S,5R)-6-[(R)-2-Ethoxy-1-fluoro-2-oxoethoxy]-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylic acid (70). To a solution of **108** (17.3 g, 49.9 mmol) in $MeNO_2$ (86 mL) was added 2 M solution of $TiCl_4$ in CH_2Cl_2 (49.9 mL, 100 mmol) at −30 ˚C. After being stirred for 20 min at −30 ˚C, the mixture was poured into iced water, and the aqueous layer was extracted with EtOAc. The organic layer was washed with water and brine before being dried over $MgSO_4$. The solvent was removed under reduced pressure to give **70** (12.9 g, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.29 (1H, d, $^3J_{H\text{-}F}$ = 55.1 Hz), 4.30–4.17 (2H, m), 3.98–3.95 (2H, m), 3.07–3.00 (2H, m), 2.04–1.77 (4H, m), 1.22 (3H, t, *J* = 7.1 Hz).

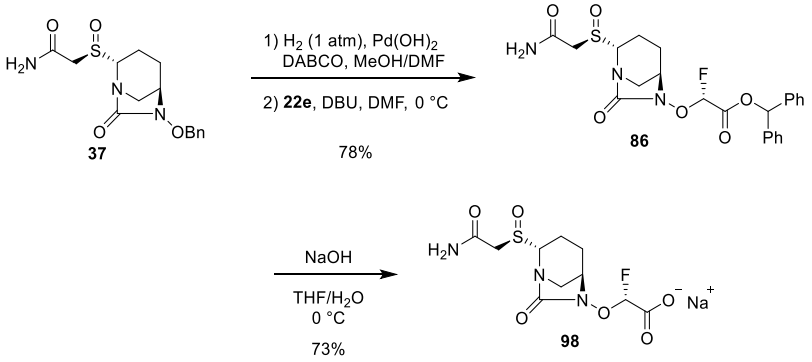
Ethyl (2R)-2-fluoro-2-[[(2R,5R)-2-[(fluoromethyl)thio]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] oxy]acetate (71a). To a solution of **70** (1.13 g, 3.89 mmol) in CH_2Cl_2 (12 mL) were added 2-mercaptopyridine 1-oxide **4** (0.52 g, 4.09 mmol) and EDC·HCl (0.78 g, 4.09 mmol). The solution was stirred for 1.5 h at room temperature in the dark. Thiosulfonate **73** (2.57 g, 11.7 mmol) was added, and the mixture was stirred under white light irradiation using two LED lamps for 40 min at 0 ˚C. After the solvent was removed under reduced pressure, the crude product was purified by flash column chromatography to give **71a** (1.02 g, 57 wt % purity). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.89 (1H, d, $^3J_{H\text{-}F}$ = 52.5 Hz), 5.65 (1H, dd, *J* = 114.4, 10.4 Hz), 5.52 (1H, dd, *J* = 110.0, 10.0 Hz), 4.96 (1H, d, *J* = 7.8 Hz), 4.37–4.28 (2H, m), 4.02–4.01 (1H, br m), 3.78 (1H, d, *J* = 12.0 Hz), 3.04–3.01 (1H, br m), 2.39–2.33 (1H, m), 2.18–2.12 (1H, m), 1.88–1.80 (1H, m), 1.68 (1H, dd, *J* = 15.7, 7.2 Hz), 1.35 (3H, t, *J* = 7.2 Hz). MS-ESI (*m/z*): 311 $[M + H]^+$.

Ethyl (2R)-2-fluoro-2-[[(2R,5R)-2-[(S)-(fluoromethyl)sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]acetate (72a). To a solution of **71a** (57 wt %, 1.02 g, 1.85 mmol) in CH_2Cl_2 (3 mL) was added 69 wt% *m*-CPBA (486 mg, 1.94 mmol) in CH_2Cl_2 (5 mL) at −78 ˚C. After being stirred for 3 h at 0 ˚C, the reaction was quenched by 10% aq $Na_2S_2O_3$, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with aq $NaHCO_3$ and dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude product was purified by flash column chromatography to give **72a** (333 mg, 26% from **70**, dr = >10/1) as a white amorphous solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.87 (1H, d, $^3J_{H\text{-}F}$ = 52.3 Hz), 5.55 (1H, dd, *J* = 46.0, 8.8 Hz), 5.33 (1H, dd, *J* = 45.6, 8.8 Hz), 4.55 (1H, t, *J* = 6.3 Hz), 4.36–4.28 (2H, m), 4.10–4.07 (1H, br m), 3.35 (1H, d, *J* = 12.4 Hz), 3.24 (1H, d, *J* = 11.9 Hz), 2.43–2.35 (1H, m), 2.31–2.14 (2H, m), 2.05–1.96 (1H, m), 1.34 (3H, t, *J* = 7.2 Hz). MS-ESI (*m/z*): 327 $[M + H]^+$.

Sodium (2R)-2-fluoro-2-[[(2R,5R)-2-[(S)-(fluoromethyl)sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1] octan-6-yl]oxy]acetate (112). To a solution of **72a** (333 mg, 1.02 mmol) in THF/H_2O (v/v = 2/1, 4.5 mL) was added 1 N aq NaOH (1.02 mL, 1.02 mmol) at 0 ˚C. After being stirred for 10 min at 0 ˚C, the reaction was quenched by dry ice, and the solution was washed with EtOAc. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–10%, MeCN/H_2O) to give **112** (208 mg, 64%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 5.82 (1H, d, $^3J_{H\text{-}F}$ = 58.0 Hz), 5.70 (1H, dd, *J* = 47.9, 9.8 Hz), 5.64 (1H, dd, *J* = 46.6, 9.8 Hz), 4.28–4.26 (1H, br m), 3.45 (1H, d, *J* = 12.3 Hz), 3.34 (1H, d, *J* = 12.3 Hz), 2.37–2.17 (3H, m), 2.10–2.04 (1H, m). ^{13}C{^{1}H} NMR (100

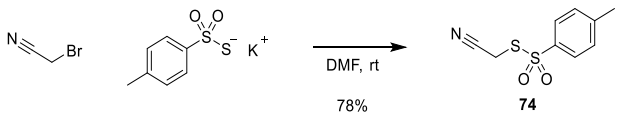
MHz, D_2O) δ: 171.8 (d, $^3J_{C\text{-}F}$ = 27.1 Hz), 170.8, 110.7 (d, $^2J_{C\text{-}F}$ = 241.4 Hz), 94.6 (d, $^2J_{C\text{-}F}$ = 220.8 Hz), 72.6 (d, $^4J_{C\text{-}F}$ = 8.1 Hz), 62.4, 48.2, 21.3, 19.5. ^{19}F NMR (377 MHz, D_2O) δ: −130.2 (d, $^3J_{H\text{-}F}$ = 58.2 Hz), −228.4 (t, $^3J_{H\text{-}F}$ = 47.2 Hz). HPLC: >99.9% (method-A). Anal. calcd for $C_9H_{11}F_2N_2O_5SNa(H_2O)_{1.3}$: C, 31.46; H, 3.99; F, 11.06; N, 8.15; S, 9.33; Na, 6.69. found: C, 31.76; H, 4.11; F, 10.88; N, 8.26; S, 8.99; Na, 6.46.



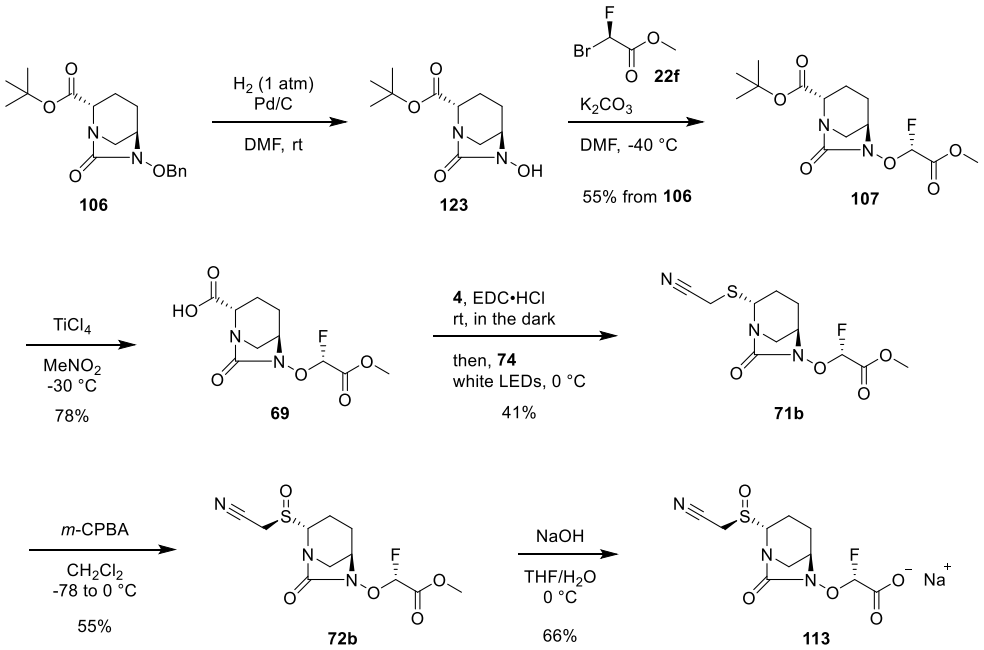
Benzhydryl (2R)-2-[[(2R,5R)-2-[(R)-(2-amino-2-oxoethyl)sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (86). To a solution of **37** (449 mg, 1.33 mmol) in MeOH/DMF (v/v = 1/1, 10 mL) were added 10% $Pd(OH)_2$ on carbon (841 mg, 1.87 wt equiv) and DABCO (3.0 mg, 0.03 mmol). After being stirred under a H_2 atmosphere (1 atm) for 1 h, the mixture was filtered, and the solvent was removed to give the crude product, which was used without further purification. To a solution of the crude debenzylated product in DMF (5 mL) were added DBU (0.20 mL, 1.33 mmol) and **22e** (473 mg, 1.46 mmol) at 0 ˚C. After being stirred for 10 min at 0 ˚C, the mixture was poured into diluted aq HCl, and the aqueous layer was extracted with EtOAc three times. The combined organic layers were washed with water end brine before being dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (0–20%, MeOH/EtOAc) to give **86** (0.51 g, 78%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41–7.29 (10H, m), 6.95 (1H, s), 6.53 (1H, br s), 5.98 (1H, d, $^3J_{H\text{-}F}$ = 53.2 Hz), 5.56 (1H, br s), 4.35 (1H, t, *J* = 6.3 Hz), 4.00–3.97 (1H, br m), 3.80 (1H, d, *J* = 14.1 Hz), 3.53 (1H, d, *J* = 14.1 Hz), 3.09 (1H, d, *J* = 12.3 Hz), 2.67 (1H, d, *J* = 12.0 Hz), 2.35–2.28 (1H, m), 2.24–2.15 (2H, m), 1.97–1.90 (1H, m).

Sodium (2R)-2-[[(2R,5R)-2-[(R)-(2-amino-2-oxoethyl)sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (98). To a solution of **86** (0.51 g, 1.04 mmol) in THF/H_2O (v/v = 2/1, 15 mL) was added 1 N aq NaOH (1.04 mL, 1.04 mmol) at 0 ˚C. After being stirred for 10 min at 0 ˚C, the reaction was quenched by dry ice, and the organic solvent was removed under reduced pressure. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0-10%, MeCN/H_2O) to give **98** (261 mg, 73%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 5.82 (1H, d, $^3J_{H\text{-}F}$ = 58.9 Hz), 4.58 (1H, t, *J* = 6.6 Hz), 4.27–4.25 (1H, br m), 3.42 (1H, d, *J* = 12.4 Hz), 3.33 (1H, d, *J* = 11.6 Hz), 2.41–2.32 (1H, m), 2.26–2.16 (2H, m), 2.09–2.03 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 171.9 (d, $^3J_{C\text{-}F}$ = 27.1 Hz), 171.6, 171.0, 110.6 (d, $^2J_{C\text{-}F}$ = 241.4 Hz), 76.9, 62.5, 48.1, 21.2, 19.1. ^{19}F NMR (377 MHz, D_2O) δ: −130.2 (d, $^3J_{H\text{-}F}$ = 58.6 Hz). HPLC: 99.4% (method-A). Anal. calcd for $C_{10}H_{13}FN_3O_6SNa(H_2O)_{1.7}$: C, 31.95; H, 4.40; F, 5.05; N, 11.18; S, 8.53; Na, 6.12. found: C,

32.03; H, 4.27; F, 4.92; N, 11.30; S, 8.08; Na, 6.49.



S-(Cyanomethyl) 4-methylbenzenesulfonothioate (74). To a solution of bromoacetonitrile (11.6 mL, 167 mmol) in DMF (200 mL) was added potassium 4-methylbenzenesulfonothioate (40 g, 177 mmol) at 0 ˚C, and the mixture was stirred for 1 h at room temperature. The mixture was diluted with EtOAc, and the organic layer was washed with water twice before being dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was dissolved to CH_2Cl_2, and diisopropyl ether was added. The solvent was partially removed under reduced pressure, and the white solid was precipitated. The precipitate was filtered to give **74** (29.6 g, 78%) as a white solid. **Note**: Thiosulfonate **74** is unstable on silica gel. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.87 (2H, d, *J* = 8.4 Hz), 7.41 (2H, d, *J* = 8.2 Hz), 3.87 (2H, s), 2.48 (3H, s). MS-ESI (*m/z*): 228 $[M + H]^+$.



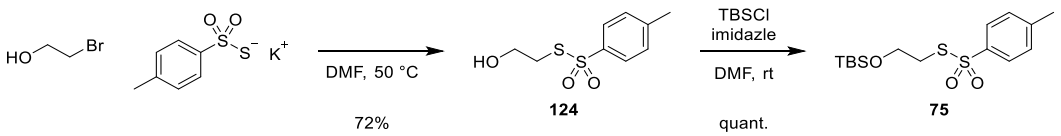
tert-Butyl (2S,5R)-6-[(R)-1-fluoro-2-methoxy-2-oxoethoxy]-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylate (107). To a solution of **106** (10.5 g, 31.7 mmol) in DMF (105 mL) were added 5 wt % Pd/C (4.72 g, 44.8 wt equiv). After being stirred under a H_2 atmosphere (1 atm) for 2 h, the mixture was filtered, and the solvent was removed under reduced pressure to give **123**. To a solution of **123** in DMF (105 mL) were added **22f** (6.51 g, 38.1 mmol) and K_2CO_3 (6.14 g, 44.4 mmol) at −40 ˚C. After being stirred for 8 h at −40 ˚C, the mixture was poured into iced water, and the aqueous layer was extracted with EtOAc. The organic layer was washed with water and brine before being dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude product was purified by flash column chromatography (25–35% EtOAc/hexane) to give **107** (5.80 g, 55%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.90 (1H, d, $^3J_{H\text{-}F}$ = 52.5 Hz), 4.09–4.08 (1H, br m), 3.96–3.94 (1H, br m), 3.86 (3H, s), 3.21 (1H, d, *J* = 12.1 Hz), 3.13 (1H, d, *J* = 12.1 Hz), 2.18–2.06 (3H, m), 1.88–1.81 (1H, m), 1.50 (9H, s).

(2S,5R)-6-[(R)-1-Fluoro-2-methoxy-2-oxoethoxy]-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylic acid (69). To a solution of **107** (3.46 g, 10.4 mmol) in $MeNO_2$ (17 mL) was added 2 M solution of $TiCl_4$ in CH_2Cl_2 (10.4 mL, 20.8 mmol) at −30 ˚C. After being stirred for 20 min at −30 ˚C, the mixture was poured into iced water, and the aqueous layer was extracted with EtOAc. The organic layer was washed with water and brine before being dried over $MgSO_4$. The solvent was removed under reduced pressure to give **69** (2.25 g, 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.30 (1H, d, $^3J_{H\text{-}F}$ = 55.5 Hz), 3.98–3.96 (2H, m), 3.78 (3H, s), 3.06–3.03 (2H, br m), 2.04–1.76 (4H, m).

Methyl (2R)-2-[[(2R,5R)-2-[(cyanomethyl)thio]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (71b). To a solution of **69** (1.00 g, 3.62 mmol) in CH_2Cl_2 (10 mL) were added 2-mercaptopyridine 1-oxide **4** (0.48 g, 3.80 mmol) and EDC·HCl (0.73 g, 3.80 mmol). The solution was stirred for 1 h at room temperature in the dark. Thiosulfonate **74** (2.47 g, 10.9 mmol) was added, and the mixture was stirred under white light irradiation for 40 min at 0 ˚C. After the solvent was removed under reduced pressure, the crude product was purified by flash column chromatography to give **71b** (0.44 g, 41%) as a yellow gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.90 (1H, d, $^3J_{H\text{-}F}$ = 52.5 Hz), 4.87 (1H, dd, *J* = 7.7, 1.9 Hz), 4.03–4.02 (1H, br m), 3.88 (3H, s), 3.78 (1H, d, *J* = 12.1 Hz), 3.50 (1H, d, *J* = 17.2 Hz), 3.28 (1H, d, *J* = 16.9 Hz), 3.10–3.06 (1H, m), 2.38–2.30 (1H, m), 2.18–2.12 (1H, m), 1.87–1.79 (1H, m), 1.66–1.60 (1H, m). MS-ESI (*m/z*): 304 $[M + H]^+$.

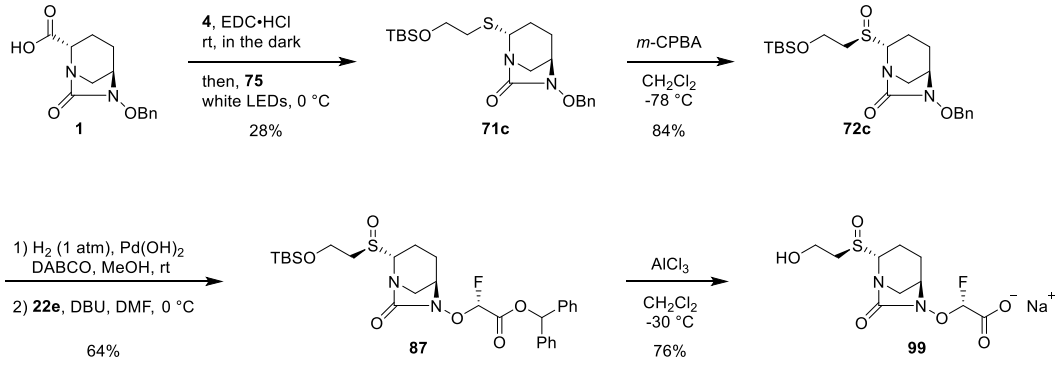
Methyl (2R)-2-[[(2R,5R)-2-[(R)-(cyanomethyl)sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (72b). To a solution of **71b** (295 mg, 0.973 mmol) in CH_2Cl_2 (8 mL) was added 72 wt% *m*-CPBA (233 mg, 0.973 mmol) at −78 ˚C. After being stirred for 3 h at 0 ˚C, the reaction was quenched by 10% aq $Na_2S_2O_3$ and aq $NaHCO_3$. The aqueous layer was extracted with EtOAc three times, and the combined organic layers were dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude product was purified by flash column chromatography to give **72b** (172 mg, 55%, dr = 7/1) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.89 (1H, d, *J* = 52.1 Hz), 4.42 (1H, t, *J* = 6.9 Hz), 4.12–4.10 (1H, m), 3.98 (1H, d, *J* = 16.1 Hz), 3.88 (3H, s), 3.74 (1H, d, *J* = 16.2 Hz), 3.29–3.26 (1H, m), 3.22 (1H, d, *J* = 12.0 Hz), 2.41–2.24 (3H, m), 2.05–1.98 (1H, m). MS-ESI (*m/z*): 320 $[M + H]^+$.

Sodium (2R)-2-[[(2R,5R)-2-[(R)-(cyanomethyl)sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (113). To a solution of **72b** (171 mg, 0.536 mmol) in THF/H_2O (v/v = 2/1, 3 mL) was added 1 N aq NaOH (0.536 mL, 0.536 mmol) at 0 ˚C. After being stirred for 15 min at 0 ˚C, the solution was washed with EtOAc. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–10%, MeCN/H_2O) to give **113** (115 mg, 66%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 5.82 (1H, d, $^3J_{H\text{-}F}$ = 58.5 Hz), 4.59 (1H, t, *J* = 6.7 Hz), 4.28–4.26 (1H, br m), 3.44–3.32 (2H, m), 2.43–2.20 (3H, m), 2.11–2.04 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 171.8 (d, $^3J_{C\text{-}F}$ = 27.1 Hz), 170.6, 115.3, 110.6 (d, $^2J_{C\text{-}F}$ = 241.4 Hz), 77.4, 62.4, 48.1, 21.1, 19.5. ^{19}F NMR (377 MHz, D_2O) δ: −130.2 (d, $^3J_{H\text{-}F}$ = 58.6 Hz). HPLC: 99.5% (method-A). Anal. calcd for $C_{10}H_{11}FN_3O_5SNa(H_2O)_{0.9}$: C, 34.97; H, 3.76; F, 5.53; N, 12.23; S, 9.33; Na, 6.69. found: C, 34.96; H, 3.71; F, 5.40; N, 12.32; S, 9.31; Na, 6.60.



S-(2-Hydroxyethyl) 4-methylbenzenesulfonothioate (124). To a solution of potassium 4-methylbenzenesulfonothioate (10.0 g, 44.2 mmol) in DMF (100 mL) was added 2-bromoethanol (4.05 mL, 57.4 mmol). After being stirred for 3 h at 50 ˚C, the mixture was poured into water, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with water and brine before dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (20–60%, EtOAc/hexane) to give **124** (7.38 g, 72%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.83 (2H, d, *J* = 8.4 Hz) , 7.36 (2H, d, *J* = 8.2 Hz), 3.87–3.86 (2H, m), 3.17 (2H, t, *J* = 5.8 Hz), 2.46 (3H, s), 1.98 (1H, br s).

S-[2-[(tert-Butyldimethylsilyl)oxy]ethyl] 4-methylbenzenesulfonothioate (75). To a solution of **124** (7.38 g, 31.8 mmol) in DMF (37 mL) were added TBSCl (5.51 g, 36.5 mmol) and imidazole (2.60 g, 38.1 mmol). After being stirred for 1.5 h, the mixture was poured into water, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with water and brine before being dried over $MgSO_4$. After the solvent was removed under reduced pressure to give **75** (11.8 g, quant.) as a pale yellow oil, which was used without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.81 (2H, d, *J* = 8.3 Hz), 7.34 (2H, d, *J* = 8.0 Hz), 3.77 (2H, t, *J* = 6.3 Hz), 3.12 (2H, t, *J* = 6.3 Hz), 2.45 (3H, s), 0.85 (9H, s), 0.01 (6H, s).

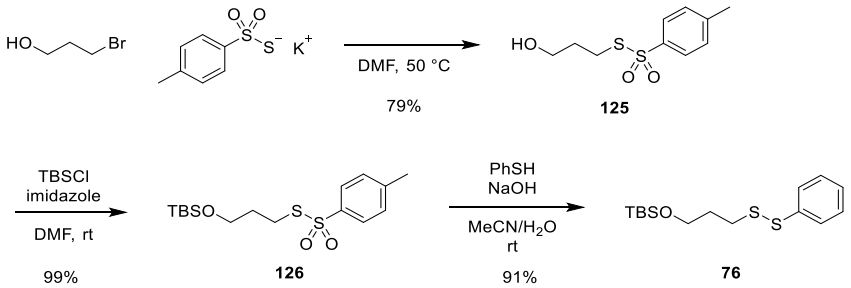


(2R,5R)-6-(Benzyloxy)-2-[[2-[(tert-butyldimethylsilyl)oxy]ethyl]thio]-1,6-diazabicyclo[3.2.1]octan-7-one (71c). To a solution of carboxylic acid **1** (44.0 g, 159 mmol) in CH_2Cl_2 (220 mL) were added 2-mercaptopyridine 1-oxide **4** (21.26 g, 167 mmol) and EDC·HCl (32.1 g, 167 mmol) at 0 ˚C. The mixture was stirred for 1 h at room temperature in the dark (solution A). To another solution of **75** (138 g, 398 mmol) in CH_2Cl_2 (88 mL) was slowly added the solution A at 0 ˚C under white Light irradiation. After being stirred for 30 min at 0 ˚C, water was added, and the layers were separated. The solvent was removed before the crude was purified by flash column chromatography (0–20%, EtOAc/hexane) to give **71c** (18.6 g, 28%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44–7.33 (5H, m), 5.05 (1H, d, *J* = 11.6 Hz), 4.90 (1H, d, *J* = 11.4 Hz), 4.68 (1H, d, *J* = 7.3 Hz), 3.83–3.72 (3H, m), 3.33–3.30 (1H, br m), 2.86–2.79 (2H, m), 2.67–2.61 (1H, m), 2.33–2.23 (1H, m), 1.98–1.92 (1H, m), 1.69–1.53 (2H, m), 0.89 (9H, s), 0.06 (3H, s), 0.06 (3H, s). MS-ESI (*m/z*): 423 $[M + H]^+$.

(2R,5R)-6-(Benzyloxy)-2-[(R)-[2-[(tert-butyldimethylsilyl)oxy]ethyl]sulfinyl]-1,6-diazabicyclo[3.2.1]octan-7-one (72c). To a solution of **71c** (18.6 g, 43.9 mmol) in CH_2Cl_2 (180 mL) was added 72 wt % *m*-CPBA (10.5 g, 43.9 mmol) at −78 ˚C. After being stirred for 1 h at −78 ˚C, aq $NaHCO_3$ and aq $Ns_2S_2O_3$ were added, and the organic solvent was removed under reduced pressure. The aqueous layer was extracted with EtOAc, and the organic layer was dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (30–70%, EtOAc/hexane) to give **72c** (16.2 g, 84%, dr = >10/1) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42–7.37 (5H, m), 5.05 (1H, d, *J* = 11.4 Hz), 4.90 (1H, d, *J* = 11.5 Hz), 4.18 (1H, dd, *J* = 7.7, 3.9 Hz), 4.12–4.00 (2H, m), 3.37-–3.36 (1H, br m), 3.24 (1H, d, *J* = 11.8 Hz), 3.19–3.16 (1H, m), 3.02 (1H, d, *J* = 10.9 Hz), 2.77 (1H, dt, *J* = 13.1, 3.9 Hz), 2.39–2.33 (1H, m), 2.17–2.07 (2H, m), 1.83–1.77 (1H, m), 0.90 (9H, s), 0.10 (3H, s), 0.09 (3H, s). MS-ESI (*m/z*): 439 $[M + H]^+$.

Benzhydryl (2R)-2-[[(2R,5R)-2-[(R)-[2-[(tert-butyldimethylsilyl)oxy]ethyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (87). To a solution of **72c** (3.99 g, 9.10 mmol) in MeOH (40 mL) were added 10% $Pd(OH)_2$ on carbon (1.28 g, 0.32 wt equiv) and DABCO (20 mg, 0.18 mmol). After being stirred under a H_2 atmosphere (1 atm) for 1 h, the mixture was filtered, and the solvent was removed to give the crude product, which was used without further purification. To a solution of the crude debenzylated product in DMF (30 mL) were added **22e** (4.41 g, 13.6 mmol) and DBU (1.65 mL, 10.9 mmol) at 0 ˚C. After being stirred for 30 min at 0 ˚C, the mixture was poured into 10% aq citric acid, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with water and dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (20–60%, EtOAc/hexane) to give **87** (3.46 g, 64%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42–7.28 (10H, m), 6.95 (1H, s), 5.98 (1H, d, $^3J_{H\text{-}F}$ = 53.3 Hz), 4.27 (1H, dd, *J* = 7.5, 4.7 Hz), 4.13–4.01 (2H, m), 3.96–3.94 (1H, br m), 3.26 (1H, d, *J* = 12.0 Hz), 3.16–3.09 (1H, m), 2.77 (1H, dt, *J* = 13.1, 3.8 Hz), 2.66 (1H, d, *J* = 12.2 Hz), 2.40–2.33 (1H, m), 2.22–2.07 (2H, m), 1.97–1.91 (1H, m), 0.91 (9H, s), 0.12 (3H, s), 0.10 (3H, s). MS-ESI (*m/z*): 591 $[M + H]^+$.

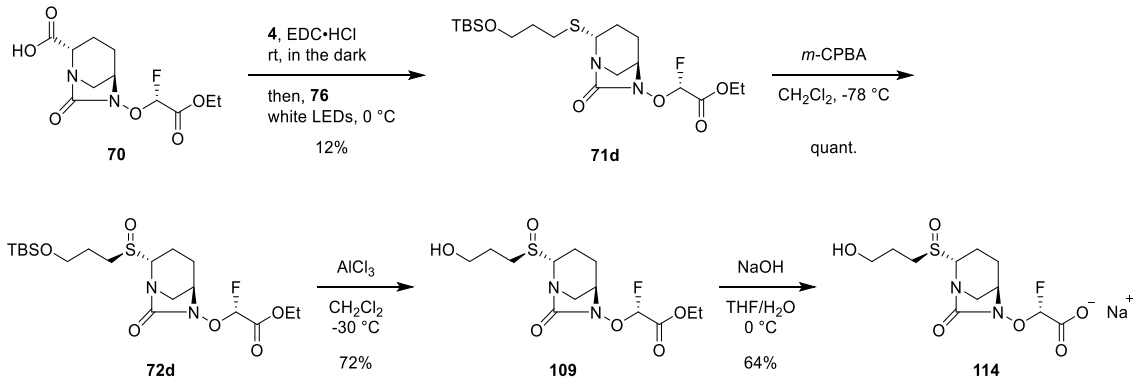
Sodium (2R)-2-fluoro-2-[[(2R,5R)-2-[(R)-(2-hydroxyethyl)sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]acetate (99). To a solution of **87** (518 mg, 0.877 mmol) were added anisole (0.383 mL, 3.51 mmol) and 2 M solution of $AlCl_3$ in $MeNO_2$ (1.75 mL, 3.51 mmol) at −30 ˚C. After being stirred for 30 min at −30 ˚C, the mixture was poured into aq $NaHCO_3$, and the aqueous layer was washed with EtOAc. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–10%, MeCN/H_2O) to give **99** (221 mg, 76%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 5.82 (1H, d, $^3J_{H\text{-}F}$ = 58.6 Hz), 4.49 (1H, t, *J* = 6.1 Hz), 4.27–4.24 (1H, br m), 4.12–4.00 (2H, m), 3.47 (1H, d, *J* = 12.4 Hz), 3.37–3.30 (2H, m), 3.06 (1H, dt, *J* = 13.9, 4.2 Hz), 2.40–2.31 (1H, m), 2.26–2.15 (2H, m), 2.07–1.99 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 171.8 (d, $^3J_{C\text{-}F}$ = 27.1 Hz), 171.2, 110.6 (d, $^2J_{C\text{-}F}$ = 240.6 Hz), 77.1, 62.5, 57.4, 55.0, 48.1, 21.4, 18.8. ^{19}F NMR (377 MHz, D_2O) δ: −130.2 (d, $^3J_{H\text{-}F}$ = 58.6 Hz). HPLC: 96.3% (method-C). Anal. calcd for $C_{10}H_{14}FN_2O_6SNa(H_2O)_{2.3}$: C, 32.14; H, 5.02; F, 5.08; N, 7.50; S, 8.58; Na, 6.15. found: C, 32.01; H, 4.77; F, 4.99; N, 7.69; S, 8.50; Na, 6.52.



S-(3-Hydroxypropyl) 4-methylbenzenesulfonothioate (125). To a solution of potassium 4-methyl-benzenesulfonothioate (26.2 g, 116 mmol) in DMF (130 mL) was added 3-bromopropan-1-ol (16.1 g, 116 mmol). After being stirred for 3 h at 50 ˚C, the mixture was poured into water, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with water and brine before being dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (30–70%, EtOAc/hexane) to give **125** (22.5 g, 79%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.82 (2H, d, *J* = 8.1 Hz), 7.35 (2H, d, *J* = 8.1 Hz), 3.71 (2H, t, *J* = 5.8 Hz), 3.12 (2H, t, *J* = 6.9 Hz), 2.46 (3H, s), 1.93–1.86 (2H, m). MS-ESI (*m/z*): 247 $[M + H]^+$.

S-[3-[(tert-butyldimethylsilyl)oxy]propyl] 4-methylbenzenesulfonothioate (126). To a solution of **125** (22.5 g, 91 mmol) in DMF (100 mL) were added TBSCl (15.8 g, 105 mmol) and imidazole (7.45 g, 109 mmol) at 0 ˚C. After stirred for 1 h at room temperature, the mixture was diluted with EtOAc/hexane (v/v = 1/1), and the organic layer was washed with 1 N aq HCl, water, and brine before being dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (0–20%, EtOAc/hexane) to give **126** (32.5 g, 99%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.81 (2H, d, *J* = 8.3 Hz), 7.33 (2H, d, *J* = 8.1 Hz), 3.60 (2H, t, *J* = 5.7 Hz), 3.07 (2H, t, *J* = 7.1 Hz), 2.45 (3H, s), 1.85–1.79 (2H, m), 0.85 (9H, s), 0.00 (6H, s). MS-ESI (*m/z*): 361 $[M + H]^+$.

tert-Butyldimethyl[3-(phenyldisulfaneyl)propoxy]silane (76). To 1 N aq NaOH (90.0 mL, 90.0 mol) was added benzenthiol (9.28 mL, 90.0 mol). After being stirring for 30 min, a solution of **126** (32.5 g, 90.0 mol) in MeCN (30 mL) was added, and the mixture was stirred for 1 h. The reaction mixture was extracted with EtOAc, and the organic layer was died over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (0–10%, EtOAc/hexane) to give **76** (25.9 g, 91%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.53 (2H, d, *J* = 7.9 Hz), 7.32 (2H, t, *J* = 7.5 Hz), 7.22 (1H, t, *J* = 7.3 Hz), 3.66 (2H, t, *J* = 5.9 Hz), 2.82 (2H, t, *J* = 7.1 Hz), 1.91–1.85 (2H, m), 0.87 (9H, s), 0.03 (6H, s). MS-ESI (*m/z*): 315 $[M + H]^+$.

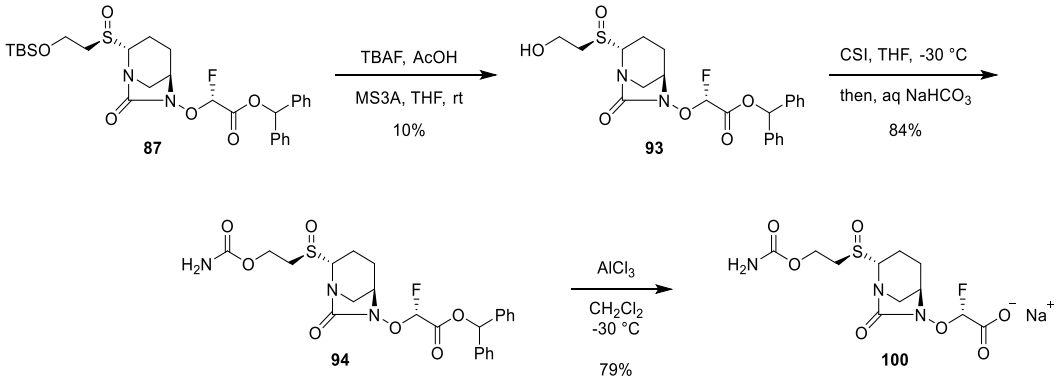


Ethyl (2R)-2-[[(2R,5R)-2-[[3-[(tert-butyldimethylsilyl)oxy]propyl]thio]-7-oxo-1,6-diazabicyclo [3.2.1]octan-6-yl]oxy]-2-fluoroacetate (71d). To a solution of **70** (1.15 g, 3.96 mmol) in CH_2Cl_2 (10 mL) were added 2-mercaptopyridine 1-oxide **4** (21.26 g, 167 mmol) and EDC·HCl (32.1 g, 167 mmol). After the mixture was stirred for 1 h at room temperature in the dark, **76** (3.73 g, 11.9 mmol) was added, and the solution was stirred for 30 min at 0 ˚C under white light irradiation. The solvent was removed before the crude was purified by flash column chromatography (0–20%, EtOAc/hexane) to give **71d** (216 mg, 12%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.88 (1H, d, $^3J_{H\text{-}F}$ = 52.6 Hz), 4.73 (1H, d, *J* = 7.3 Hz), 4.36–4.28 (2H, m), 3.96–3.93 (2H, br m), 3.71–3.64 (2H, m), 2.96–2.93 (1H, m), 2.74–2.58 (2H, m), 2.37–2.26 (1H, m), 2.11–2.07 (1H, m), 1.89–1.73 (3H, m), 1.62–1.58 (1H, m), 1.34 (3H, t, *J* = 7.2 Hz), 0.89 (9H, s), 0.05 (6H, s). MS-ESI (*m/z*): 451 $[M + H]^+$.

Ethyl (2R)-2-[[(2R,5R)-2-[(R)-[3-[(tert-butyldimethylsilyl)oxy]propyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (72d). To a solution of **71d** (216 mg, 0.479 mmol) in CH_2Cl_2 (3 mL) was added 72 wt % *m*-CPBA (115 mg, 0.479 mmol) at −78 ˚C. After being stirred for 1 h at −78 ˚C, aq $NaHCO_3$ and aq $Ns_2S_2O_3$ were added, and the aqueous layer was extracted with EtOAc twice. The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (50–100%, EtOAc/hexane) to give **72d** (240 mg, quant., dr = >10/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.87 (1H, d, $^3J_{H\text{-}F}$ = 52.3 Hz), 4.38–4.26 (2H, m), 4.18–4.15 (1H, m), 4.05–4.03 (1H, br m), 3.81–3.70 (2H, m), 3.41 (1H, d, *J* = 12.1 Hz), 3.20–3.15 (1H, m), 3.05 (1H, dt, *J* = 15.1, 6.6 Hz), 2.79–2.72 (1H, m), 2.45–2.37 (1H, m), 2.26–2.21 (1H, m), 2.14 (1H, td, *J* = 14.9, 7.4 Hz), 2.04–1.93 (3H, m), 1.33 (3H, t, *J* = 7.2 Hz), 0.88 (9H, s), 0.05 (6H, s). MS-ESI (*m/z*): 467 $[M + H]^+$.

Ethyl (2R)-2-fluoro-2-[[(2R,5R)-2-[(R)-(3-hydroxypropyl)sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1] octan-6-yl]oxy]acetate (109). To a solution of **72d** (240 mg, 0.514 mmol) in CH_2Cl_2 (2 mL) was added 2 M solution of $AlCl_3$ in $MeNO_2$ (0.771 mL, 1.54 mmol) at −30 ˚C. After being stirred for 30 min at −30 ˚C, the mixture was poured into brine, and the aqueous layer was extracted with EtOAc three times. The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (0–10%, MeOH/EtOAc) to give **109** (131 mg, 72%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.88 (1H, d, $^3J_{H\text{-}F}$ = 52.6 Hz), 4.33–4.25 (3H, m), 4.08–4.05 (1H, br m), 3.80–3.77 (2H, br m), 3.36 (1H, d, *J* = 11.8 Hz), 3.22–3.11 (2H, m), 2.93–2.86 (1H, m), 2.50 (1H, br s), 2.42–2.37 (1H, m), 2.27–1.97 (5H, m), 1.34 (3H, t, *J* = 7.0 Hz). MS-ESI (*m/z*): 353 $[M + H]^+$.

Sodium (2R)-2-fluoro-2-[[(2R,5R)-2-[(R)-(3-hydroxypropyl)sulfinyl]-7-oxo-1,6-diazabicyclo [3.2.1]octan-6-yl]oxy]acetate (114). To a solution of **109** (130 mg, 0.369 mmol) in THF/H_2O (v/v = 2/1, 3 mL) was added 1 N aq NaOH (0.369 mL, 0.369 mmol) at 0 ˚C. After being stirred for 15 min at 0 ˚C, the organic solvent was removed under reduced pressure. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–10%, MeCN/H_2O) to give **114** (81.4 mg, 64%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 5.81 (1H, d, $^3J_{H\text{-}F}$ = 58.7 Hz), 4.42 (1H, t, *J* = 6.1 Hz), 4.26–4.23 (1H, br m), 3.75 (2H, t, *J* = 6.3 Hz), 3.46 (1H, d, *J* = 12.3 Hz), 3.32 (1H, d, *J* = 12.0 Hz), 3.19–3.12 (1H, m), 2.99–2.91 (1H, m), 2.38–2.29 (1H, m), 2.25–2.13 (2H, m), 2.06–1.99 (3H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 171.8 (d, $^3J_{C\text{-}F}$ = 27.1 Hz), 171.2, 110.7 (d, $^2J_{C\text{-}F}$ = 241.4 Hz), 76.8, 62.9, 62.5, 48.6, 48.1, 27.4, 21.4, 18.8. ^{19}F NMR (377 MHz, D_2O) δ: −130.2 (d, *J* = 59.9 Hz). HPLC: 98.3% (method-A). Anal. calcd for $C_{11}H_{16}FN_2O_6SNa(H_2O)_{1.7}$: C, 35.05; H, 5.19; F, 5.04; N, 7.43; S, 8.51; Na, 6.10. found: C, 35.29; H, 5.14; F, 4.69; N, 7.60; S, 8.08; Na, 5.83.

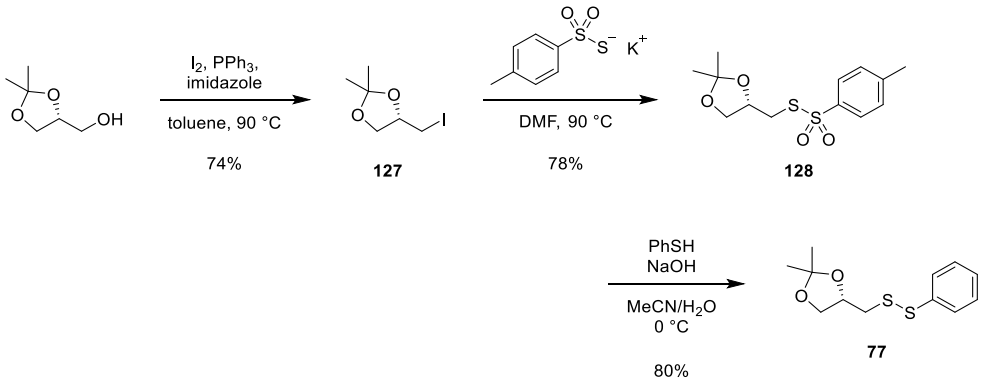


Benzhydryl (2R)-2-fluoro-2-[[(2R,5R)-2-[(R)-(2-hydroxyethyl)sulfinyl]-7-oxo-1,6-diazabicyclo[3. 2.1]octan-6-yl]oxy]acetate (93). To a solution of **87** (2.93 g, 4.96 mmol) were added 3A molecular sieves, 1 M tetrabutylammonium fluoride (TBAF) in THF (5.95 mL, 5.95 mmol) and AcOH (0.340 mL, 5.95 mmol) at 0 ˚C. After being stirred for 4 h at room temperature, the mixture was filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (0–10%, MeOH/EtOAc) to give **93** (246 mg, 10%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41–7.26 (10H, m), 6.95 (1H, s), 5.98 (1H, d, $^3J_{H\text{-}F}$ = 53.3 Hz), 4.36–4.33 (1H, m), 4.21–4.17 (2H, m), 3.98–3.96 (1H, br m), 3.21–3.14 (2H, m), 3.01–2.95 (1H, m), 2.67 (1H, d, *J* = 11.9 Hz), 2.38–2.31 (1H, m), 2.24–2.11 (2H, m), 2.09 (1H, s), 1.97–1.91 (1H, m). MS-ESI (*m/z*): 477 [M + H]$^+$.

Benzhydryl (2R)-2-[[(2R,5R)-2-[(R)-[2-(carbamoyloxy)ethyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3. 2.1]octan-6-yl]oxy]-2-fluoroacetate (94). To a solution of **93** (232 mg, 0.487 mmol) in THF (4 mL) was added chlorosulfonyl isocyanate (CSI) (0.076 mL, 0.876 mmol) at −30 ˚C. After the mixture was stirred for 30 min at −30 ˚C, 8% aq $NaHCO_3$ was added, and the mixture was stirred for 1 h at room temperature. Brine was added, and the aqueous layer was extracted with EtOAc twice. After the combined organic layers were dried over Na_2SO_4, the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (0–10%, MeOH/EtOAc) to give **94** (212 mg, 84%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40–7.29 (10H, m), 6.96 (1H, s), 5.98 (1H, d, *J* = 53.1 Hz), 4.59–4.54 (2H, m), 4.45–4.42 (1H, m), 3.99–3.97 (1H, br m), 3.24–3.17 (1H, m), 3.12 (1H, d, *J* =

12.1 Hz), 3.07–3.01 (1H, m), 2.71 (1H, d, *J* = 12.1 Hz), 2.38–2.31 (1H, m), 2.22–2.10 (2H, m), 1.97–1.90 (1H, m). MS-ESI (*m/z*): 520 $[M + H]^+$.

Sodium (2R)-2-[[(2R,5R)-2-[(R)-[2-(carbamoyloxy)ethyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1] octan-6-yl]oxy]-2-fluoroacetate (100). To a solution of **94** (212 mg, 0.408 mmol) were added anisole (0.134 mL, 1.22 mmol) and 2 M solution of $AlCl_3$ in $MeNO_2$ (0.612 mL, 1.22 mmol) at −30 ˚C. After being stirred for 15 min at −30 ˚C, the mixture was poured into aq $NaHCO_3$. The precipitate was filtered before the aqueous layer was washed with EtOAc. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–7%, MeCN/H_2O) to give **100** (121 mg, 79%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 5.82 (1H, d, $^3J_{H\text{-}F}$ = 58.5 Hz), 4.58–4.46 (3H, m), 4.27–4.24 (1H, br m), 3.51–3.43 (2H, m), 3.33 (1H, d, *J* = 10.8 Hz), 3.20 (1H, ddd, *J* = 14.4, 6.0, 3.7 Hz), 2.40–2.30 (1H, m), 2.26–2.15 (2H, m), 2.09–1.99 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 171.8 (d, $^3J_{C\text{-}F}$ = 27.1 Hz), 171.1, 161.5, 110.6 (d, $^2J_{C\text{-}F}$ = 241.4 Hz), 77.0, 62.5, 60.8, 51.5, 48.0, 21.3, 18.9. ^{19}F NMR (377 MHz, D_2O) δ: −130.2 (d, $^3J_{H\text{-}F}$ = 58.6 Hz). HPLC: >99.9% (Method-A). Anal. calcd for $C_{11}H_{15}FN_3O_7SNa(H_2O)_{2.0}$: C, 32.12; H, 4.66; F, 4.62; N, 10.22; S, 7.79; Na, 5.59. found: C, 32.07; H, 4.73; F, 4.52; N, 10.48; S, 7.67; Na, 5.62.

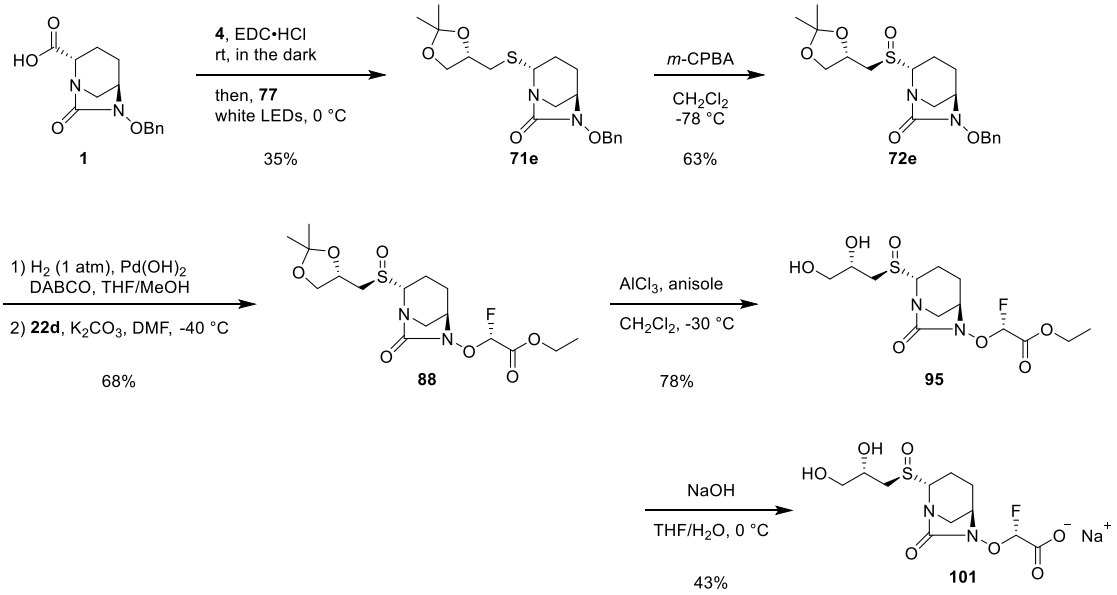


(S)-4-(Iodomethyl)-2,2-dimethyl-1,3-dioxolane (127). To a solution of (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (33.0 g, 250 mmol) in toluene (550 mL) were added imidazole (51.0 g, 749 mmol), PPh_3 (79.0 g, 300 mmol), and I_2 (82.0 g, 325 mmol). After being stirred for 2 h at 90 ˚C, the solvent was removed under reduced pressure. The residue was dissolved to $CHCl_3$, and the organic layer was washed with 10% aq $Na_2S_2O_3$ before being dried over $MgSO_4$. After the removal of solvent, the residue was triturated with diisopropyl ether to precipitate triphenylphosphine oxide. The mixture was filtered, and the solvent was removed. The crude product was purified by flash column chromatography (0–10%, EtOAc/hexane) to give **127** (45.0 g, 74%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.31–4.25 (1H, m), 4.15 (1H, dd, *J* = 8.7, 6.1 Hz), 3.79 (1H, dd, *J* = 8.6, 5.5 Hz), 3.26 (1H, dd, *J* = 9.9, 4.6 Hz), 3.14 (1H, dd, *J* = 9.8, 8.5 Hz), 1.46 (3H, s), 1.35 (3H, s).

(S)-S-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl] 4-methylbenzenesulfonothioate (128). To a solution of potassium 4-methylbenzenesulfonothioate (47.6 g, 210 mmol) in DMF (230 mL) was added **127** (46.2 g, 191 mmol). After being stirred for 1 h at 90 ˚C, the mixture was diluted with EtoAc. The organic layer was washed with water and dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (10–35%, EtOAc/hexane) to give **128**

(45.1 g, 78%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.82 (2H, d, *J* = 8.4 Hz), 7.35 (2H, d, *J* = 8.2 Hz), 4.35–4.29 (1H, m), 4.04 (1H, dd, *J* = 8.7, 6.0 Hz), 3.68 (1H, dd, *J* = 8.7, 5.6 Hz), 3.16–3.15 (2H, m), 2.46 (3H, s), 1.37 (3H, s), 1.30 (3H, s). MS-ESI (*m/z*): 303 $[M + H]^+$.

(S)-2,2-Dimethyl-4-[(phenyldisulfaneyl)methyl]-1,3-dioxolane (77). To 1 N aq NaOH (149 mL, 149 mol) was added benzenthiol (15.4 mL, 149 mol) at 0 ˚C. After being stirring for 15 min at 0 ˚C, a solution of **128** (45.1 g, 149 mol) in MeCN (20 mL) was added, and the mixture was stirred for 1 h at 0 ˚C. The reaction mixture was extracted with EtOAc, and the organic layer was died over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (0–10%, EtOAc/hexane) to give **77** (30.4 g, 80%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.54 (2H, d, *J* = 7.5 Hz), 7.33 (2H, t, *J* = 7.6 Hz), 7.24 (1H, t, *J* = 7.6 Hz), 4.34 (1H, dt, *J* = 13.0, 5.9 Hz), 4.05 (1H, dd, *J* = 8.4, 6.0 Hz), 3.74 (1H, dd, *J* = 8.4, 5.9 Hz), 2.99 (1H, dd, *J* = 13.6, 5.6 Hz), 2.81 (1H, dd, *J* = 13.5, 7.2 Hz), 1.41 (3H, s), 1.32 (3H, s).



(2R,5R)-6-(Benzyloxy)-2-[[[(S)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl]thio]-1,6-diazabicyclo[3.2.1]octan-7-one (71e). To a solution of carboxylic acid **1** (11.0 g, 39.8 mmol) in CH_2Cl_2 (70 mL) were added 2-mercaptopyridine 1-oxide **4** (5.32 g, 41.8 mmol) and EDC·HCl (8.01 g, 41.8 mmol). After the mixture was stirred for 1 h at room temperature in the dark, **77** (18.5 g, 36.2 mmol) was added, and the solution was stirred for 30 min at 0 ˚C under white light irradiation. The solvent was removed before the crude was purified by flash column chromatography (0–50%, EtOAc/hexane) to give **71e** (5.30 g, 35%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44–7.35 (5H, m), 5.04 (1H, d, *J* = 11.5 Hz), 4.90 (1H, d, *J* = 11.5 Hz), 4.70 (1H, d, *J* = 7.0 Hz), 4.37–4.31 (1H, m), 4.08 (1H, dd, *J* = 8.3, 6.2 Hz), 3.73 (1H, d, *J* = 11.5 Hz), 3.69 (1H, dd, *J* = 8.3, 6.3 Hz), 3.33–3.33 (1H, m), 2.87–2.80 (2H, m), 2.72 (1H, dd, *J* = 13.6, 6.5 Hz), 2.34–2.24 (1H, m), 1.99–1.93 (1H, m), 1.69–1.55 (2H, m), 1.42 (3H, s), 1.35 (3H, s). MS-ESI (*m/z*): 379 $[M + H]^+$.

(2R,5R)-6-(Benzyloxy)-2-[(R)-[[(S)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl]sulfinyl]-1,6-diazabicyclo[3.2.1]octan-7-one (72e). To a solution of **71e** (5.29 g, 14.0 mmol) in CH_2Cl_2 (60 mL) was added 72

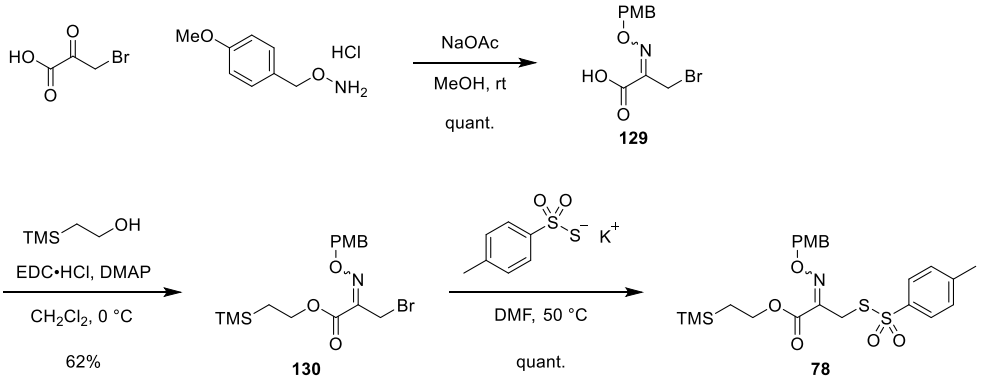
wt % *m*-CPBA (3.35 g, 14.0 mmol) at −78 ˚C. After being stirred for 1 h at −78 ˚C, aq $NaHCO_3$ and aq $Ns_2S_2O_3$ were added, and the aqueous layer was extracted with EtOAc three times. The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (0–10%, MeOH/EtOAc) to give **72e** (3.48 g, 63%, dr = >10/1) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44–7.37 (5H, m), 5.04 (1H, d, *J* = 11.5 Hz), 4.90 (1H, d, *J* = 11.5 Hz), 4.64–4.57 (1H, m), 4.18 (1H, dd, *J* = 8.5, 6.1 Hz), 4.13 (1H, dd, *J* = 7.3, 4.6 Hz), 3.73 (1H, dd, *J* = 8.5, 6.2 Hz), 3.39–3.39 (1H, br m), 3.26 (1H, dd, *J* = 13.1, 9.0 Hz), 3.19 (1H, d, *J* = 11.8 Hz), 3.04–3.02 (1H, br m), 2.80 (1H, dd, *J* = 13.1, 3.8 Hz), 2.40–2.32 (1H, m), 2.20–2.10 (2H, m), 1.82–1.76 (1H, m), 1.43 (3H, s), 1.38 (3H, s). MS-ESI (*m/z*): 395 $[M + H]^+$.

Ethyl (2R)-2-[[(2R,5R)-2-[(R)-[[(S)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (88). To a solution of **72e** (3.48 g, 8.82 mmol) in THF/MeOH (v/v = 1/1, 35 mL) were added 10% $Pd(OH)_2$ on carbon (0.619 g, 0.18 wt equiv) and DABCO (20 mg, 0.18 mmol). After being stirred under a H_2 atmosphere (1 atm) for 30 min, the mixture was filtered, and the solvent was removed under reduced pressure. Trituration with diisopropyl ether gave the debenzylated product (2.09 g, 78%). To a solution of the debenzylated product (2.09 g, 6.87 mmol) in DMF (20 mL) were added **22d** (61.5 wt%, 2.48 g, 8.24 mmol) and K_2CO_3 (1.04 g, 7.55 mmol) at −40 ˚C. After being stirred overnight at −20 ˚C, the mixture was poured into 10% aq citric acid, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with water and dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (0–10%, MeOH/EtOAc) to give **88** (2.45 g, 68%) as a colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.88 (1H, d, $^3J_{H\text{-}F}$ = 52.3 Hz), 4.61–4.59 (1H, m), 4.37–4.29 (2H, m), 4.24–4.21 (2H, m), 4.07–4.05 (1H, br m), 3.73 (1H, dd, *J* = 8.5, 6.2 Hz), 3.38 (1H, d, *J* = 12.1 Hz), 3.25–3.19 (2H, m), 2.82 (1H, dd, *J* = 13.0, 3.7 Hz), 2.46–2.38 (1H, m), 2.29–2.12 (2H, m), 2.03–1.94 (1H, m), 1.44 (3H, s), 1.38 (3H, s), 1.35 (3H, q, *J* = 7.2 Hz). MS-ESI (*m/z*): 409 $[M + H]^+$.

Ethyl (2R)-2-[[(2R,5R)-2-[(R)-[(S)-2,3-dihydroxypropyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (95). To a solution of **88** (340 mg, 0.832 mmol) in CH_2Cl_2 (3.4 mL) were added anisole (0.546 mL, 4.99 mmol) and 2 M solution of $AlCl_3$ in $MeNO_2$ (2.50 mL, 4.99 mmol) at −30 ˚C. After being stirred for 20 min at −30 ˚C, the mixture was poured into brine. The aqueous layer was extracted with THF four times and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (0–10%, MeOH/$CHCl_3$) to give **95** (240 mg, 78%) as a pale-yellow gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.87 (1H, d, $^3J_{H\text{-}F}$ = 52.3 Hz), 4.40–4.28 (4H, m), 4.09–4.06 (1H, br m), 3.77 (1H, dd, *J* = 11.4, 3.7 Hz), 3.63 (1H, dd, *J* = 11.4, 5.6 Hz), 3.33 (1H, d, *J* = 12.2 Hz), 3.26–3.20 (2H, m), 2.87 (1H, dd, *J* = 13.6, 2.8 Hz), 2.43–2.36 (1H, m), 2.30–2.16 (2H, m), 2.04–1.97 (1H, m), 1.33 (3H, t, *J* = 7.2 Hz). MS-ESI (*m/z*): 369 $[M + H]^+$.

Sodium (2R)-2-[[(2R,5R)-2-[(R)-[(S)-2,3-dihydroxypropyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (101). To a solution of **95** (64.8 mg, 0.176 mmol) in THF/H_2O (v/v = 2/1, 3 mL) was added 1 N aq NaOH (0.176 mL, 0.176 mmol) at 0 ˚C. After being stirred for 15 min at 0 ˚C, the organic solvent was removed under reduced pressure. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–10%, MeCN/H_2O) to give **101** (27.9

mg, 43%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 5.81 (1H, d, $^{3}J_{H\text{-}F}$ = 58.7 Hz), 4.45 (1H, t, *J* = 6.1 Hz), 4.26–4.23 (1H, br m), 4.19–4.16 (1H, m), 3.72 (1H, dd, *J* = 11.8, 4.0 Hz), 3.62 (1H, dd, *J* = 11.9, 5.9 Hz), 3.46 (1H, d, *J* = 12.0 Hz), 3.33 (1H, d, *J* = 11.8 Hz), 3.19 (1H, dd, *J* = 13.6, 10.8 Hz), 3.02 (1H, dd, *J* = 13.6, 2.3 Hz), 2.36–2.30 (1H, m), 2.25–2.14 (2H, m), 2.07–2.02 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 171.9 (d, $^{3}J_{C\text{-}F}$ = 27.1 Hz), 171.2, 110.7 (d, $^{2}J_{C\text{-}F}$ = 241.4 Hz), 77.3, 68.5, 67.7, 62.6, 56.2, 48.2, 21.4, 18.8. ^{19}F NMR (377 MHz, D_2O) δ: −130.1 (d, $^{3}J_{H\text{-}F}$ = 59.9 Hz). HPLC: >99.9% (method-A).

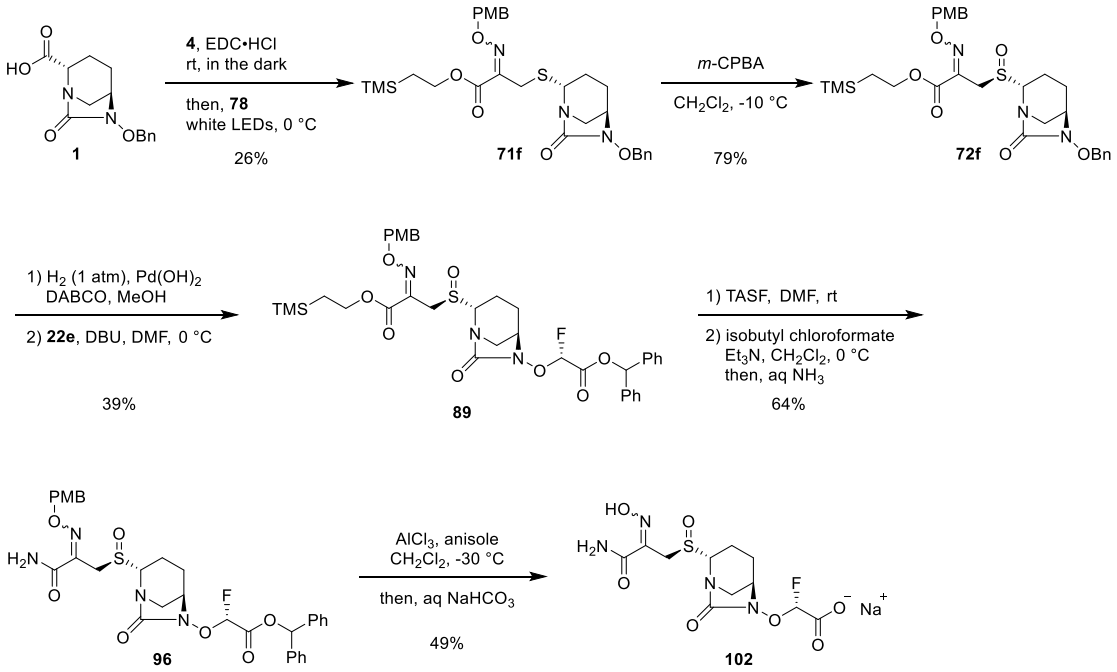


3-Bromo-2-(((4-methoxybenzyl)oxy)imino)propanoic acid (129). To a solution of 3-bromo-2-oxopropanoic acid (20.0 g, 120 mmol) in MeOH (400 mL) were added *O*-(4-methoxybenzyl)hydroxylamine hydrochloride (22.7 g, 120 mmol) and NaOAc (19.7 g, 240 mmol) at 0 ˚C. After being stirred for 6 h at room temperature, the mixture was filtered, and the solvent was removed under reduced pressure. To the residue was added 1 N aq HCl, and the aqueous layer was extracted with EtOAc twice. After the removal of the solvent, **129** (37.4 g, quant.) was recrystallized from EtOH/H_2O. **Note**: Compound **129** was obtained as a mixture of geometrical isomers (61/39). The geometry of the major isomer was unknown. ^{1}H NMR of major isomer (400 MHz, $CDCl_3$) δ: 7.32 (2H, d, *J* = 8.6 Hz), 6.92 (2H, d, *J* = 8.3 Hz), 5.32 (2H, s), 4.18 (2H, s), 3.83 (3H, s). MS-ESI (*m/z*): 300 $[M - H]^-$.

2-(Trimethylsilyl)ethyl 3-bromo-2-[[(4-methoxybenzyl)oxy]imino]propanoate (130). To a solution of **129** (36.2 g, 120 mmol) in CH_2Cl_2 (360 mL) were added 2-(trimethylsilyl)ethan-1-ol (17.0 g, 144 mmol), DMAP (4.39 g, 35.9 mmol), and EDC·HCl (27.6 g, 144 mmol) at 0 ˚C. After being stirred for 1 h at 0 ˚C, the mixture was washed with water, and the organic layer was dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (0–25%, EtOAc/hexane) to give **130** (29.6 g, 62%) as a colorless oil. **Note**: Compound **130** was obtained as a mixture of geometrical isomers (75/25). The geometry of the major isomer was unknown. ^{1}H NMR of major isomer (400 MHz, $CDCl_3$) δ: 7.32 (2H, d, *J* = 8.6 Hz), 6.90 (2H, d, *J* = 8.6 Hz), 5.30 (2H, s), 4.39 (2H, t, *J* = 8.4 Hz), 4.35 (2H, s), 3.82 (3H, s), 1.12 (2H, t, *J* = 8.4 Hz), 0.07 (9H, s). MS-ESI (*m/z*): 402 $[M + H]^+$.

2-(Trimethylsilyl)ethyl 2-[[(4-methoxybenzyl)oxy]imino]-3-(tosylthio)propanoate (78). To a solution of potassium 4-methylbenzenesulfonothioate (16.7 g, 73.7 mmol) in DMF (150 mL) was added **130** (29.6 g, 73.7 mmol). After being stirred for 1 h at 50 ˚C, the mixture was diluted with EtOAc/hexane (v/v = 1/1). The organic layer was washed with water and brine before being dried over Na_2SO_4. After

the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (0–40%, EtOAc/hexane) to give **78** (37.6 g, quant.) as a colorless oil. **Note**: Compound **78** was obtained as a single isomer. The geometry was unknown. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.78 (2H, d, *J* = 8.1 Hz), 7.30 (2H, d, *J* = 8.6 Hz), 7.26 (2H, d, *J* = 8.1 Hz), 6.90 (2H, d, *J* = 8.6 Hz), 5.22 (2H, s), 4.32 (2H, t, *J* = 8.6 Hz), 3.97 (2H, s), 3.82 (3H, s), 2.42 (3H, s), 1.10–1.05 (2H, m), 0.06 (9H, s). MS-ESI (*m/z*): 510 $[M + H]^+$.



2-(Trimethylsilyl)ethyl 3-[[(2R,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]thio]-2-[[(4-methoxybenzyl)oxy]imino]propanoate (71f). To a solution of carboxylic acid **1** (5.00 g, 18.1 mmol) in CH_2Cl_2 (25 mL) were added 2-mercaptopyridine 1-oxide **4** (2.42 g, 19.0 mmol) and EDC·HCl (3.64 g, 19.0 mmol). After the mixture was stirred for 1 h at room temperature in the dark, **78** (18.5 g, 36.2 mmol) was added, and the solution was stirred for 30 min at 0 ˚C under white light irradiation. The solvent was removed before the crude was purified by flash column chromatography (0–50%, EtOAc/hexane) to give **71f** (2.80 g, 26%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41–7.34 (7H, m), 6.88 (2H, d, *J* = 8.6 Hz), 5.26 (2H, s), 5.04 (1H, d, *J* = 11.6 Hz), 4.88 (1H, d, *J* = 11.4 Hz), 4.73 (1H, d, *J* = 7.6 Hz), 4.37–4.27 (2H, m), 3.80 (3H, s), 3.71 (1H, d, *J* = 12.9 Hz), 3.58 (2H, d, *J* = 12.4 Hz), 3.24 (1H, s), 2.71 (1H, d, *J* = 11.4 Hz), 2.28–2.18 (1H, m), 1.89–1.88 (1H, m), 1.60–1.44 (2H, m), 1.10 (2H, t, *J* = 8.6 Hz), 0.06 (9H, s). MS-ESI (*m/z*): 486 $[M + H]^+$.

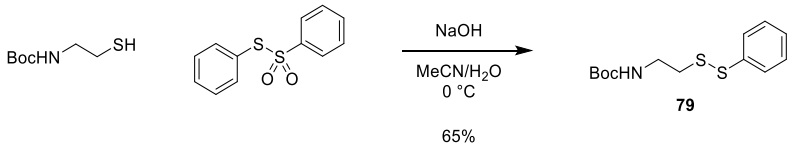
2-(Trimethylsilyl)ethyl 3-[(R)-[(2R,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl] sulfinyl]-2-[[(4-methoxybenzyl)oxy]imino]propanoate (72f). To a solution of **71f** (2.80 g, 4.06 mmol) in CH_2Cl_2 (30 mL) was added 72 wt % *m*-CPBA (0.974 g, 4.06 mmol) at −60 ˚C. After being stirred for 1 h at −10 ˚C, aq $NaHCO_3$ and aq $Ns_2S_2O_3$ were added, and the aqueous layer was extracted with EtOAc three times. The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (50–100%, EtOAc/hexane) to give **72f** (1.93 g, 79%, dr = >10/1) as a colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42–7.37 (5H,

m), 7.32 (2H, d, *J* = 8.6 Hz), 6.88 (2H, d, *J* = 8.6 Hz), 5.30 (1H, d, *J* = 11.4 Hz), 5.26 (1H, d, *J* = 11.4 Hz), 5.03 (1H, d, *J* = 11.4 Hz), 4.87 (1H, d, *J* = 11.4 Hz), 4.39–4.35 (2H, m), 4.18–4.15 (2H, m), 4.01 (1H, d, *J* = 12.1 Hz), 3.76 (3H, s), 3.28–3.27 (1H, br m), 2.98 (1H, d, *J* = 11.9 Hz), 2.85–2.82 (1H, br m), 2.35–2.29 (1H, m), 2.04–1.96 (2H, m), 1.71–1.63 (1H, m), 1.13–1.09 (2H, m), 0.06 (9H, s). MS-ESI (*m/z*): 602 $[M + H]^+$.

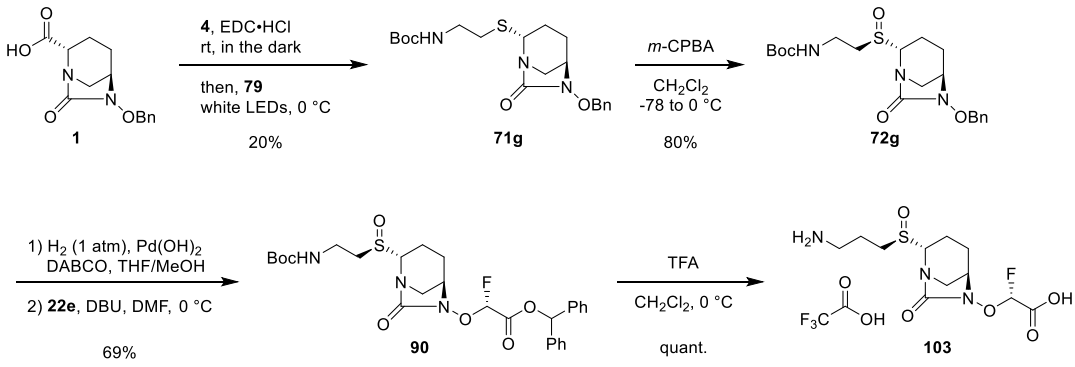
2-(Trimethylsilyl)ethyl 3-[(R)-[(2R,5R)-6-[(R)-2-(benzhydryloxy)-1-fluoro-2-oxoethoxy]-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]sulfinyl]-2-[[(4-methoxybenzyl)oxy]imino]propanoate (89). To a solution of **72f** (1.92 g, 3.19 mmol) in MeOH (20 mL) were added 10% $Pd(OH)_2$ on carbon (1.20 g, 0.63 wt equiv) and DABCO (36 mg, 0.32 mmol). After being stirred under a H_2 atmosphere (1 atm) for 15 min, the mixture was filtered, and the solvent was removed to give the crude product, which was used without further purification. To a solution of the crude debenzylated product in DMF (15 mL) were added **22e** (1.34 g, 4.15 mmol) and DBU (0.481 mL, 3.19 mmol) at 0 ˚C. After being stirred for 1 h at 0 ˚C, the mixture was poured into 10% aq citric acid, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with water and dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (20–50%, EtOAc/hexane) to give **89** (930 mg, 39%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40–7.29 (12H, m), 6.94 (1H, s), 6.89 (2H, d, *J* = 8.6 Hz), 5.96 (1H, d, $^3J_{H\text{-}F}$ = 53.3 Hz), 5.32 (1H, d, *J* = 11.4 Hz), 5.28 (1H, d, *J* = 11.4 Hz), 4.38 (2H, dd, *J* = 9.6, 7.3 Hz), 4.24–4.20 (1H, m), 4.12 (1H, d, *J* = 12.1 Hz), 3.98 (1H, d, *J* = 12.1 Hz), 3.87–3.84 (1H, br m), 3.80 (3H, s), 3.09 (1H, d, *J* = 12.1 Hz), 2.55 (1H, d, *J* = 11.9 Hz), 2.38–2.31 (1H, m), 2.09–1.94 (2H, m), 1.86–1.81 (1H, m), 1.12 (2H, t, *J* = 8.7 Hz), 0.06 (9H, s). MS-ESI (*m/z*): 755 $[M + H]^+$.

Benzhydryl (2R)-2-[[(2R,5R)-2-[(R)-[3-amino-2-[[(4-methoxybenzyl)oxy]imino]-3-oxopropyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (96). To a solution of **89** (929 mg, 1.23 mmol) in DMF (10 mL) was added tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) (407 mg, 1.48 mmol). After being stirred for 1 h, the mixture was poured into 10% aq citric acid, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure to give a crude product. To a solution of the crude carboxylic acid in CH_2Cl_2 (10 mL) were added Et_3N (0.256 mL, 1.85 mmol) and isobutyl chloroformate (0.178 mL, 1.36 mmol) at 0 ˚C. After being stirred for 15 min at 0 ˚C, 28% aq. ammonia (0.286 mL, 3.69 mmol) was added, and the mixture was stirred for 15 min at 0 ˚C. The mixture was poured into water, and the aqueous layer was extracted with CH_2Cl_2 twice. The combined organic layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (50–100%, EtOAc/hexane) to give **96** (517 mg, 64%) as a white amorphous solid. ^{1}H NMR (400MHz, $CDCl_3$) δ: 7.41–7.27 (12H, m), 6.94 (1H, s), 6.91 (2H, d, *J* = 8.7 Hz), 6.59 (1H, br s), 5.97 (1H, d, $^3J_{H\text{-}F}$ = 53.3 Hz), 5.29 (1H, br s), 5.23 (2H, s), 4.24 (1H, t, *J* = 6.4 Hz), 4.12 (1H, d, *J* = 11.9 Hz), 4.03 (1H, d, *J* = 11.9 Hz), 3.91–3.88 (1H, br m), 3.81 (3H, s), 3.22 (1H, d, *J* = 12.2 Hz), 2.63 (1H, d, *J* = 12.2 Hz), 2.42–2.35 (1H, m), 2.14–2.00 (2H, m), 1.90–1.83 (1H, m). MS-ESI (*m/z*): 653 $[M + H]^+$.

Sodium (2R)-2-[[(2R,5R)-2-[(R)-[3-amino-2-(hydroxyimino)-3-oxopropyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (102). To a solution of **96** (238 mg, 0.365 mmol) in CH_2Cl_2 (4 mL) were added anisole (0.159 mL, 1.46 mmol) and 2 M solution of $AlCl_3$ in $MeNO_2$ (0.729 mL, 1.46 mmol) at −30 ˚C. After being stirred for 1 h at −10 ˚C, the mixture was poured into aq $NaHCO_3$. The precipitate was filtered before the aqueous layer was washed with EtOAc. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–7%, MeCN/H_2O) to give **102** (69.7 mg, 49%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 5.82 (1H, d, *J* = 58.6 Hz), 4.53 (1H, t, *J* = 6.3 Hz), 4.31 (2H, s), 4.26–4.23 (1H, br m), 3.42 (1H, d, *J* = 12.4 Hz), 3.33–3.30 (1H, m), 2.39–2.32 (1H, m), 2.26–2.21 (2H, m), 2.05–2.01 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 171.9 (d, $^3J_{C\text{-}F}$ = 27.1 Hz), 170.9, 169.2, 146.9, 110.7 (d, $^2J_{C\text{-}F}$ = 240.6 Hz), 78.2, 62.4, 48.3, 46.0, 21.4, 18.9. ^{19}F NMR (377 MHz, D_2O) δ: −130.2 (d, $^3J_{H\text{-}F}$ = 58.6 Hz). HPLC: 96.1% (method-D).



tert-Butyl [2-(phenyldisulfaneyl)ethyl]carbamate (79). To 1 N aq NaOH (207 mL, 207 mmol) were added *tert*-butyl (2-mercaptoethyl)carbamate (36.7 g, 207 mmol) and a solution of *S*-phenyl benzenesulfonothioate[5] (51.9 g, 207 mmol) in MeCN (100 mL) at 0 ˚C. After being stirred for 1 h at 0 ˚C, the mixture was poured into brine, and the aqueous layer was extracted with EtOAc. After the organic layer was dried over Na_2SO_4, the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (0–20%, EtOAc/hexane) to give **79** (38.5 g, 65%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.56-7.53 (2H, m), 7.33 (2H, t, *J* = 7.6 Hz), 7.26–7.22 (1H, m), 4.79 (1H, br s), 3.43-3.42 (2H, m), 2.84 (2H, t, *J* = 6.2 Hz), 1.43 (9H, s). MS-ESI (*m/z*): 286 $[M + H]^+$.



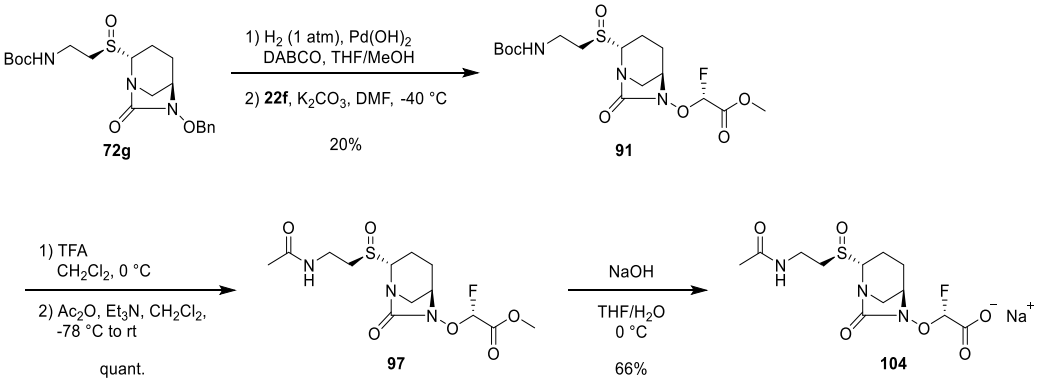
tert-Butyl [2-[(2R,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]thio]ethyl]carbamate (71g). To a solution of carboxylic acid **1** (10.0 g, 36.2 mmol) in CH_2Cl_2 (100 mL) were added 2-mercaptopyridine 1-oxide **4** (5.06 g, 39.8 mmol) and EDC·HCl (7.63 g, 39.8 mmol). The mixture was stirred for 1 h at room temperature in the dark (solution A). To a solution of **79** (31.0 g, 109 mmol) in CH_2Cl_2 (30 mL) was slowly added solution A at 0 ˚C under white light irradiation, and the mixture was stirred for 30 min. The solvent was removed before the crude was purified by flash column chromatography (10–30%, EtOAc/hexane) to give **71g** (2.88 g, 20%) as a pale yellow gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44–7.35 (5H, m), 5.05 (1H, d, *J* = 11.4 Hz), 5.01 (1H, br s), 4.90 (1H, d, *J* = 11.4 Hz), 4.64

(1H, d, *J* = 7.2 Hz), 3.74 (1H, d, *J* = 11.4 Hz), 3.39–3.27 (3H, m), 2.86–2.79 (2H, m), 2.68–2.62 (1H, m), 2.35–2.25 (1H, m), 1.99–1.93 (1H, m), 1.68–1.54 (2H, m), 1.44 (9H, s). MS-ESI (*m/z*): 408 [M + H]$^+$.

tert-Butyl [2-[(R)-[(2R,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]sulfinyl]ethyl] carbamate (72g). To a solution of **71g** (1.00 g, 2.45 mmol) in CH_2Cl_2 (20 mL) was added 72 wt % *m*-CPBA (0.588 g, 2.45 mmol) at −78 ˚C. After being stirred for 1 h at −78 ˚C, aq $NaHCO_3$ and aq $Ns_2S_2O_3$ were added, and the aqueous layer was extracted with EtOAc three times. The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (30%, acetone/toluene) to give **72g** (834 mg, 80%, dr = >10/1) as a white gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41–7.37 (5H, m), 5.12 (1H, br s), 5.04 (1H, d, *J* = 11.4 Hz), 4.89 (1H, d, *J* = 11.4 Hz), 4.16–4.13 (1H, m), 3.67–3.65 (2H, br m), 3.39–3.38 (1H, br m), 3.27–3.20 (1H, m), 3.17 (1H, d, *J* = 11.8 Hz), 3.03 (1H, d, *J* = 12.0 Hz), 2.85 (1H, dt, *J* = 13.3, 5.5 Hz), 2.38–2.28 (1H, m), 2.19–2.10 (2H, m), 1.81–1.74 (1H, m), 1.44 (9H, s). MS-ESI (*m/z*): 424 [M + H]$^+$.

Benzhydryl (2R)-2-[[(2R,5R)-2-[(R)-[2-[(tert-butoxycarbonyl)amino]ethyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (90). To a solution of **72g** (883 mg, 3.19 mmol) in MeOH (10 mL) were added 10% $Pd(OH)_2$ on carbon (146 mg, 0.17 wt equiv) and DABCO (5 mg, 0.04 mmol). After being stirred under a H_2 atmosphere (1 atm) for 1 h, the mixture was filtered, and the solvent was removed to give the crude product. A part of the product was used for the next step. To a solution of the crude debenzylated product (241 mg, 0.723 mmol) in DMF (2 mL) were added **22e** (327 mg, 1.01 mmol) and DBU (0.131 mL, 0.867 mmol) at 0 ˚C. After being stirred for 1 h at 0 ˚C, the mixture was poured into 10% aq citric acid, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with water and dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (50–100%, EtOAc/hexane) to give **90** (286 mg, 69%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41–7.29 (10H, m), 6.95 (1H, s), 5.98 (1H, d, $^3J_{H\text{-}F}$ = 53.3 Hz), 5.10 (1H, br s), 4.19 (1H, t, *J* = 6.2 Hz), 3.97–3.94 (1H, br m), 3.66–3.65 (2H, br m), 3.24–3.15 (2H, m), 2.83 (1H, dt, *J* = 13.2, 5.6 Hz), 2.65 (1H, d, *J* = 12.4 Hz), 2.37–2.30 (1H, m), 2.23–2.07 (2H, m), 1.96–1.88 (1H, m), 1.45 (9H, s). MS-ESI (*m/z*): 576 [M + H]$^+$.

(2R)-2-[[(2R,5R)-2-[(R)-(3-Aminopropyl)sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetic acid-2,2,2-trifluoroacetic acid (1/1) (103). To a solution of **90** (286 mg, 0.497 mmol) in CH_2Cl_2 (3 mL) was added TFA (3 mL) at 0 ˚C. After the mixture was stirred for 15 min at 0 ˚C, the solvent was removed under reduced pressure. The crude was triturated with ^{i}PrOAc to give **103** (107 mg, 51%). ^{1}H NMR (400 MHz, D_2O) δ: 5.81 (1H, d, *J* = 58.4 Hz), 4.52 (1H, t, *J* = 6.7 Hz), 4.26–4.24 (1H, br m), 3.59–3.47 (3H, m), 3.41 (1H, d, *J* = 12.1 Hz), 3.34–3.25 (2H, m), 2.36–2.30 (1H, m), 2.24–2.19 (2H, m), 2.07–2.01 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 171.3, 170.9 (d, $^3J_{C\text{-}F}$ = 24.9 Hz), 165.9 (q, $^3J_{C\text{-}F}$ = 35.5 Hz) (TFA), 119.3 (q, $^2J_{C\text{-}F}$ = 291.7 Hz) (TFA), 110.0 (d, $^2J_{C\text{-}F}$ = 242.1 Hz), 77.3, 63.0, 48.5, 47.9, 36.8, 21.2, 19.3. ^{19}F NMR (377 MHz, D_2O) δ: −75.6 (s) (TFA), −132.5 (d, $^3J_{H\text{-}F}$ = 55.9 Hz). HPLC: 96.7% (method-A). Anal. calcd for $C_{10}H_{16}FN_3O_5S(TFA)_{0.7}(^i PrOAc)_{0.1}(H_2O)_{0.6}$: C, 34.85; H, 4.64; F, 14.36; N, 10.25; S, 7.82. found: C, 35.04; H, 4.58; F, 13.90; N, 10.29; S, 7.67.

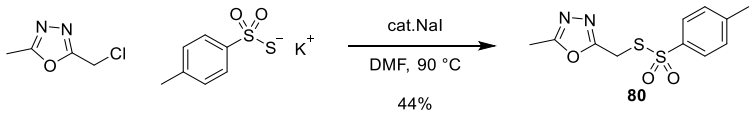


Methyl (2R)-2-[[(2R,5R)-2-[(R)-[2-[(tert-butoxycarbonyl)amino]ethyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (91). To a solution of **72g** (833 mg, 3.19 mmol) in MeOH (40 mL) were added 5% Pd/C (4.19 g, 5.03 wt equiv). After being stirred under a H_2 atmosphere (1 atm) for 0.5 h, the mixture was filtered, and the solvent was removed to give the crude product. A part of the product was used for the next step. To a solution of the crude debenzylated product (656 mg, 1.97 mmol) in DMF (6 mL) were added methyl 2-bromo-2-fluoroacetate **22f** (370 mg, 2.16 mmol) and K_2CO_3 (272 mg, 1.97 mmol) at −40 ˚C. After being stirred overnight at −40 ˚C, the mixture was poured into 10% aq citric acid, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with water and dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (25%, acetone/toluene) to give **91** (164 mg, 20%) as a white amorphous solid. **Note**: Both stereo isomers were obtained because racemic methyl 2-bromo-2-fluoroacetate was used. The polar isomer was isolated on the assumption that it would be a desired isomer described in the scheme. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.89 (1H, d, $^3J_{H\text{-}F}$ = 52.3 Hz), 5.10 (1H, br s), 4.22 (1H, dd, *J* = 7.3, 5.0 Hz), 4.06–4.04 (1H, br m), 3.87 (3H, s), 3.67–3.66 (2H, m), 3.35 (1H, d, *J* = 12.2 Hz), 3.27–3.20 (2H, m), 2.85 (1H, dt, *J* = 13.3, 5.5 Hz), 2.46–2.36 (1H, m), 2.29–2.12 (2H, m), 2.02–1.94 (1H, m), 1.44 (9H, s). MS-ESI (*m/z*): 424 $[M + H]^+$.

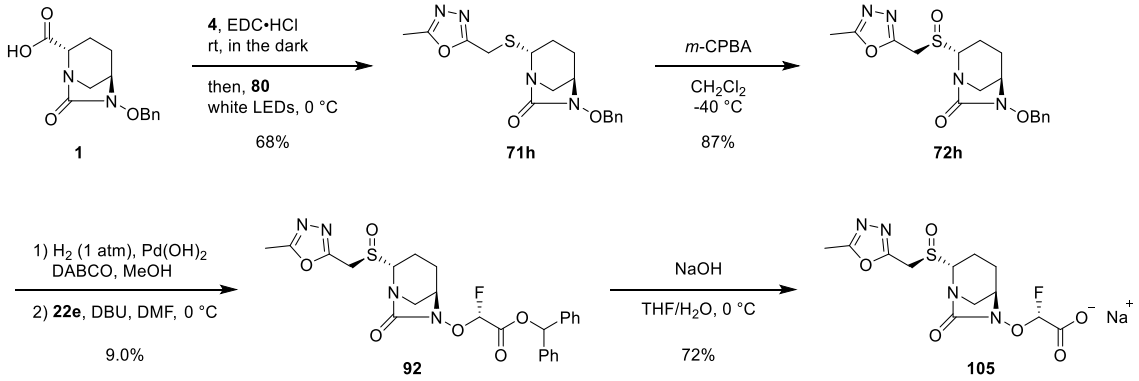
Methyl (2R)-2-[[(2R,5R)-2-[(R)-(2-acetamidoethyl)sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (97). To a solution of **91** (164 mg, 0.387 mmol) in CH_2Cl_2 (2 mL) was added TFA (2 mL) at 0 ˚C. After the mixture was stirred for 15 min at 0 ˚C, the solvent was removed under reduced pressure to give a crude product. To a solution of the crude product in CH_2Cl_2 (2 mL) were added Et_3N (0.268 mL, 1.93 mmol) and Ac_2O (0.037 mL, 0.386 mmol) at −78 ˚C. After the mixture was stirred at from −78 ˚C to room temperature, the mixture was directly purified by flash column chromatography (0–10%, MeOH/$CHCl_3$) to give **97** (142 mg, quant.) as a white gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.29 (1H, br s), 5.89 (1H, d, $^3J_{H\text{-}F}$ = 52.2 Hz), 4.25 (1H, t, *J* = 6.2 Hz), 4.08–4.05 (1H, br m), 3.91–3.72 (2H, m), 3.87 (3H, s), 3.32 (1H, d, *J* = 12.2 Hz), 3.28–3.20 (2H, m), 2.94–2.88 (1H, m), 2.42–2.34 (1H, m), 2.29–2.13 (2H, m), 2.03–1.97 (1H, m), 1.99 (3H, s). MS-ESI (*m/z*): 366 $[M + H]^+$.

Sodium (2R)-2-[[(2R,5R)-2-[(R)-(2-acetamidoethyl)sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (104). To a solution of **97** (142 mg, 0.389 mmol) in THF/H_2O (v/v = 2/1, 3 mL) was added 1 N aq NaOH (0.389 mL, 0.389 mmol) at 0 ˚C. After the mixture was stirred for 15 min at 0 ˚C, the organic solvent was removed under reduced pressure. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–20%, MeCN/H_2O) to give **104**

(96.4 mg, 66%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 5.81 (1H, d, $^{3}J_{H\text{-}F}$ = 58.6 Hz), 4.45 (1H, t, *J* = 6.3 Hz), 4.26–4.23 (1H, br m), 3.68 (2H, t, *J* = 6.2 Hz), 3.43 (1H, d, *J* = 12.3 Hz), 3.35–3.28 (2H, m), 3.05 (1H, dt, *J* = 13.7, 5.5 Hz), 2.35–2.28 (1H, m), 2.24–2.12 (2H, m), 2.06–1.97 (4H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 177.3, 171.8 (d, $^{3}J_{C\text{-}F}$ = 27.1 Hz), 171.1, 110.7 (d, $^{2}J_{C\text{-}F}$ = 241.4 Hz), 77.1, 62.5, 52.1, 48.0, 36.1, 24.7, 21.3, 18.9. ^{19}F NMR (377 MHz, D_2O) δ: −130.0 (d, $^{3}J_{H\text{-}F}$ = 58.6 Hz). HPLC: 99.8% (method-A). Anal. calcd for $C_{12}H_{17}FN_3O_6SNa(H_2O)_{1.8}$: C, 35.52; H, 5.12; F, 4.68; N, 10.36; S, 7.90; Na, 5.67. found: C, 35.49; H, 5.12; F, 4.77; N, 10.51; S, 7.69; Na, 5.39.



S-[(5-Methyl-1,3,4-oxadiazol-2-yl)methyl] 4-methylbenzenesulfonothioate (80). To a solution of 2-(chloromethyl)-5-methyl-1,3,4-oxadiazole (1.40 g, 10.6 mmol) in DMF (7 mL) was added potassium 4-methylbenzenesulfonothioate (2.30 g, 10.6 mmol). After being stirred for 2 h, the mixture was poured into water, and the aqueous layer was extracted EtOAc twice. The combined organic layers were washed with water and brine before being dried over Na_2SO_4. After the solvent was removed under reduced pressure, recrystallization from EtOAc/diisopropyl ether gave **80** (1.32 g, 44%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80 (2H, d, *J* = 8.4 Hz), 7.34 (2H, d, *J* = 8.0 Hz), 4.38 (2H, s), 2.46 (3H, s), 2.40 (3H, s).



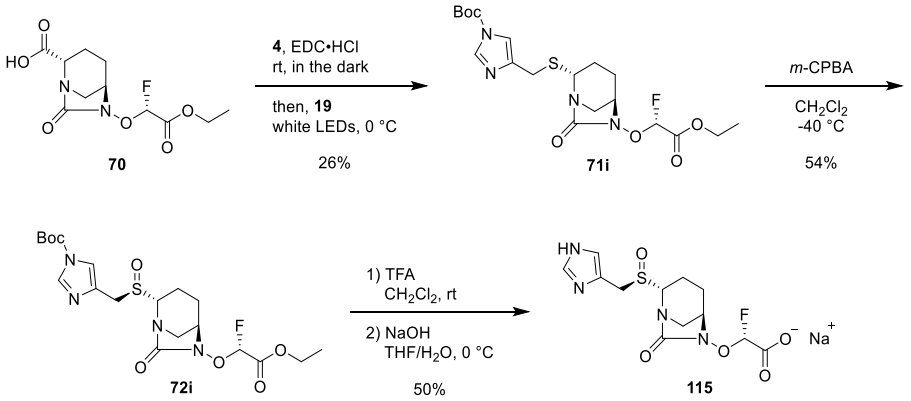
(2R,5R)-6-(Benzyloxy)-2-[[(5-methyl-1,3,4-oxadiazol-2-yl)methyl]thio]-1,6-diazabicyclo[3.2.1]octan-7-one (71h). To a solution of carboxylic acid **1** (430 mg, 1.56 mmol) in CH_2Cl_2 (2 mL) were added 2-mercaptopyridine 1-oxide **4** (208 mg, 1.63 mmol) and EDC·HCl (313 mg, 1.63 mmol). The mixture was stirred for 1 h at room temperature in the dark (solution A). To a solution of **80** (1.33 g, 4.67 mmol) in CH_2Cl_2 (2 mL) was slowly added solution A at 0 ˚C under white light irradiation, and the mixture was stirred for 30 min. The solvent was removed before the crude was purified by flash column chromatography (20–60%, EtOAc/hexane) to give **71h** (383 mg, 68%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44–7.36 (5H, m), 5.05 (1H, d, *J* = 11.4 Hz), 4.91 (1H, d, *J* = 11.5 Hz), 4.81 (1H, d, *J* = 6.7 Hz), 4.05 (1H, t, *J* = 11.1 Hz), 3.85 (1H, d, *J* = 15.2 Hz), 3.69 (1H, d, *J* = 11.7 Hz), 3.36–3.34 (1H, m), 2.87–2.84 (1H, m), 2.53 (3H, s), 2.36–2.26 (1H, m), 2.03–1.96 (1H, m), 1.69–1.57 (2H, m).

(2R,5R)-6-(Benzyloxy)-2-[(R)-[(5-methyl-1,3,4-oxadiazol-2-yl)methyl]sulfinyl]-1,6-diazabicyclo[3.2.1]octan-7-one (72h). To a solution of **71h** (375 mg, 1.04 mmol) in CH_2Cl_2 (4 mL) was slowly

added a solution of 69 wt % *m*-CPBA (273 mg, 1.09 mmol) in CH_2Cl_2 (2 mL) at −40 ˚C. After the mixture was stirred for 2 h at -40 ˚C, aq $NaHCO_3$ and aq $Ns_2S_2O_3$ were added, and the aqueous layer was extracted with EtOAc three times. The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (60–100%, EtOAc/hexane) to give **72h** (340 mg, 87%, dr = 7/1) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41–7.39 (5H, m), 5.04 (1H, d, *J* = 11.4 Hz), 4.90 (1H, d, *J* = 11.4 Hz), 4.54 (1H, d, *J* = 14.2 Hz), 4.39 (1H, t, *J* = 6.1 Hz), 4.22 (1H, d, *J* = 14.2 Hz), 3.42–3.41 (1H, m), 3.13 (1H, d, *J* = 11.9 Hz), 3.05 (1H, d, *J* = 11.8 Hz), 2.56 (3H, s), 2.36–2.27 (1H, m), 2.24–2.14 (2H, m), 1.83–1.74 (1H, m).

Benzhydryl (2R)-2-fluoro-2-[[(2R,5R)-2-[(R)-[(5-methyl-1,3,4-oxadiazol-2-yl)methyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]acetate (92). To a solution of **72h** (340 mg, 0.903 mmol) in MeOH (7 mL) were added 10% $Pd(OH)_2$ on carbon (951 mg, 2.80 wt equiv) and DABCO (2.0 mg, 0.02 mmol). After being stirred under a H_2 atmosphere (1 atm) for 8 h, the mixture was filtered, and the solvent was removed to give the crude product. To a solution of the crude debenzylated product in DMF (3 mL) were added **22e** (249 mg, 0.772 mmol) and 1 M solution of DBU in DMF (534 μL, 0.534 mmol) at 0 ˚C. After being stirred for 1 h at 0 ˚C, the mixture was poured into water, and the aqueous layer was extracted with EtOAc three times. The combined organic layers were washed with water and dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (20–100%, EtOAc/hexane) to give **92** (43.0 mg, 9.0%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.35–7.30 (10H, m), 6.95 (1H, s), 5.98 (1H, d, $^3J_{H\text{-}F}$ = 53.2 Hz), 4.50–4.43 (2H, m), 4.20 (1H, d, *J* = 14.2 Hz), 4.00-–3.97 (1H, br m), 3.14 (1H, d, *J* = 12.4 Hz), 2.92 (1H, d, *J* = 28.9 Hz), 2.57 (3H, s), 2.35–2.29 (1H, m), 2.24–2.15 (2H, m), 1.97–1.90 (1H, m).

Sodium (2R)-2-fluoro-2-[[(2R,5R)-2-[(R)-[(5-methyl-1,3,4-oxadiazol-2-yl)methyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]acetate (105). To a solution of **92** (40.0 mg, 0.076 mmol) in THF/H_2O (v/v = 2/1, 1.2 mL) was added 1 N aq NaOH (76 μL, 0.076 mmol) at 0 ˚C. After the mixture was stirred for 1 h at 0 ˚C, the reaction was quenched by dry ice, and the organic solvent was removed under reduced pressure. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–12%, MeCN/H_2O) to give **105** (21.5 mg, 72%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 5.82 (1H, d, $^3J_{H\text{-}F}$ = 58.6 Hz), 4.63–4.61 (2H, m), 4.28–4.25 (1H, br m), 3.43 (1H, d, *J* = 12.3 Hz), 3.33 (1H, d, *J* = 11.8 Hz), 2.59 (3H, s), 2.38–2.16 (3H, m), 2.08–2.05 (1H, m). ^{13}C{^{1}H} NMR (100MHz, D_2O) δ: 171.8 (d, $^3J_{C\text{-}F}$ = 26.4 Hz), 170.7, 170.2, 162.0, 110.7 (d, $^2J_{C\text{-}F}$ = 240.6 Hz), 76.8, 62.3, 48.2, 21.1, 19.2, 13.0. ^{19}F NMR (377 MHz, D_2O) δ: −130.0 (d, $^3J_{H\text{-}F}$ = 58.6 Hz). HPLC: 99.1% (method-A).

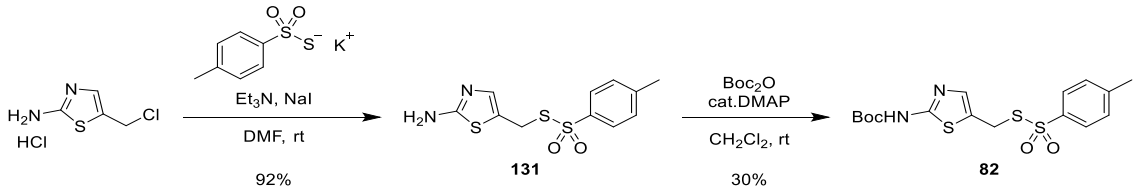


tert-Butyl 4-[[[(2R,5R)-6-[(R)-2-ethoxy-1-fluoro-2-oxoethoxy]-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]thio]methyl]-1H-imidazole-1-carboxylate (71i). To a solution of **70** (350 mg, 1.21 mmol) in CH_2Cl_2 (2 mL) were added 2-mercaptopyridine 1-oxide **4** (161 mg, 1.27 mmol) and EDC·HCl (243 mg, 1.27 mmol). The mixture was stirred for 1 h at room temperature in the dark (solution A). To a solution of **19** (1.33 g, 3.62 mmol) in CH_2Cl_2 (2 mL) was slowly added solution A at 0 ˚C under white light irradiation, and the mixture was stirred for 30 min. The solvent was removed before the crude was purified by flash column chromatography (10–50%, EtOAc/hexane) to give **71i** (145 mg, 26%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.03 (1H, s), 7.32 (1H, s), 5.88 (1H, d, $^3J_{H\text{-}F}$ = 52.7 Hz), 4.69 (1H, d, *J* = 7.5 Hz), 4.37–4.28 (2H, m), 4.00–3.98 (1H, br m), 3.94 (1H, d, *J* = 11.8 Hz), 3.81 (1H, d, *J* = 14.2 Hz), 3.62 (1H, d, *J* = 14.1 Hz), 2.99 (1H, d, *J* = 11.7 Hz), 2.33–2.23 (1H, m), 2.11–2.06 (2H, m), 1.86–1.78 (1H, m), 1.62–1.61 (9H, m), 1.35 (3H, t, *J* = 7.2 Hz).

tert-Butyl 4-[[(R)-[(2R,5R)-6-[(R)-2-ethoxy-1-fluoro-2-oxoethoxy]-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]sulfinyl]methyl]-1H-imidazole-1-carboxylate (72i). To a solution of **71i** (170 mg, 0.371 mmol) in CH_2Cl_2 (1.4 mL) was slowly added a solution of 69 wt % *m*-CPBA (93 mg, 0.371 mmol) in CH_2Cl_2 (1.2 mL) at −40 ˚C. After the mixture was stirred for 1 h at −40 ˚C, aq $NaHCO_3$ and aq $Ns_2S_2O_3$ were added, and the aqueous layer was extracted with EtOAc three times. The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (70–100%, EtOAc/hexane) to give **72i** (95.0 mg, 54%, dr = 7/1) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.06 (1H, d, *J* = 1.0 Hz), 7.45 (1H, s), 5.87 (1H, d, $^3J_{H\text{-}F}$ = 52.5 Hz), 4.40 (1H, dd, *J* = 7.2, 5.0 Hz), 4.36–4.27 (2H, m), 4.20 (1H, d, *J* = 13.7 Hz), 4.06–4.04 (1H, br m), 3.92 (1H, d, *J* = 13.8 Hz), 3.44 (1H, d, *J* = 12.2 Hz), 3.23 (1H, d, *J* = 12.2 Hz), 2.45–2.36 (1H, m), 2.29–2.11 (2H, m), 2.03–1.94 (1H, m), 1.62 (9H, s), 1.33 (3H, t, *J* = 7.2 Hz).

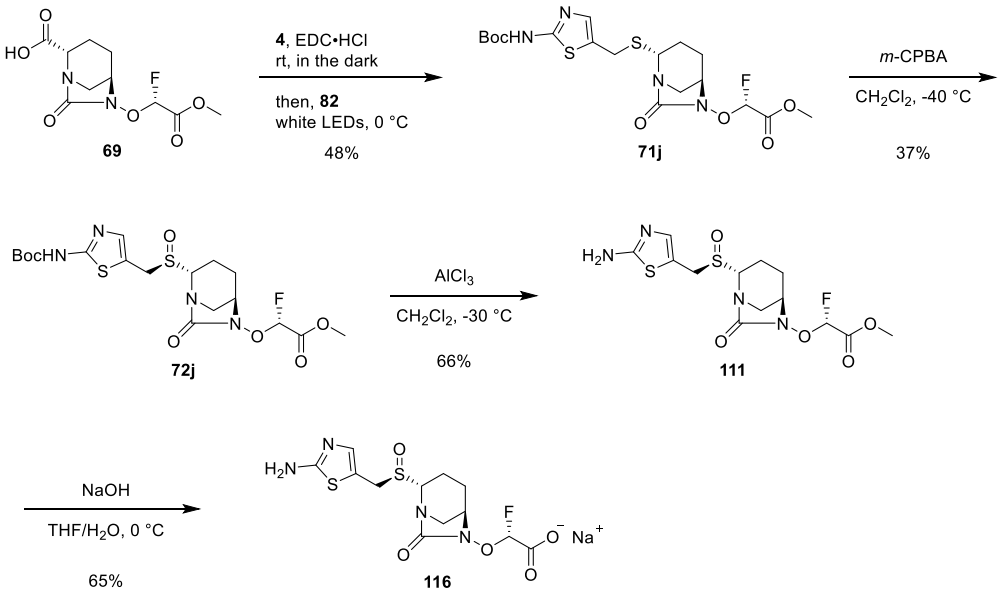
Sodium (2R)-2-[[(2R,5R)-2-[(R)-[(1H-imidazol-5-yl)methyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (115). To a solution of **72i** (90.0 mg, 0.190 mmol) in CH_2Cl_2 (1.4 mL) was added TFA (1.4 mL) at 0 ˚C. After the mixture was stirred for 1 h at room temperature, the solvent was removed under reduced pressure. To a solution of the product in THF/H_2O (v/v = 2/1, 2.8 mL) was added 1 N aq NaOH (914 μL, 0.914 mmol) at 0 ˚C. After the mixture was stirred for 1 h at 0 ˚C, the reaction was quenched by dry ice, and the organic solvent was removed under reduced pressure. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–7%, MeCN/H_2O) to give **115** (39.0 mg, 50%) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O)

δ: 7.91 (1H, s), 7.33 (1H, s), 5.83 (1H, d, $^{3}J_{H\text{-}F}$ = 58.7 Hz), 4.44–4.37 (2H, m), 4.26–4.23 (2H, m), 3.46 (1H, d, *J* = 12.3 Hz), 3.35 (1H, d, *J* = 12.0 Hz), 2.29–2.09 (3H, m), 2.06–1.99 (1H, m). $^{13}C\{^{1}H\}$ NMR (100 MHz, D_2O) δ: 171.9 (d, $^{3}J_{C\text{-}F}$ = 26.4 Hz), 171.0, 139.7, 128.7, 122.4, 110.7 (d, $^{2}J_{C\text{-}F}$ = 241.4 Hz), 75.7, 62.5, 49.1, 48.4, 21.5, 18.8. ^{19}F NMR (377 MHz, D_2O) δ: −130.1 (d, $^{3}J_{H\text{-}F}$ = 58.6 Hz). HPLC: 94.7% (method-A).



S-[(2-aminothiazol-5-yl)methyl] 4-methylbenzenesulfonothioate (131). To a solution of 5-(chloromethyl)thiazol-2-amine hydrochloride (2.00 g, 10.8 mmol) in DMF (20 mL) were added potassium 4-methylbenzenesulfonothioate (2.45 g, 10.8 mmol), NaI (1.62 g, 10.8 mmol), and Et_3N (1.50 mL, 10.8 mmol). After being stirred overnight, the mixture was poured into water, and the aqueous layer was extracted with EtOAc. The organic layer was washed with water and brine before being dried over $MgSO_4$. After the removal of solvent, the crude was triturated with CH_2Cl_2 give **131** (3.00 g, 92%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.75 (2H, d, *J* = 8.3 Hz), 7.29 (2H, d, *J* = 8.1 Hz), 6.30 (1H, s), 4.86 (2H, br s), 4.14 (2H, s), 2.43 (3H, s). MS-ESI (*m/z*): 301 $[M + H]^+$.

S-[[2-[(tert-Butoxycarbonyl)amino]thiazol-5-yl]methyl] 4-methylbenzenesulfonothioate (82). To a solution of **131** (2.64 g, 8.79 mmol) in CH_2Cl_2 (35 mL) were added Boc_2O (2.04 mL, 8.79 mmol) and DMAP (107 mg, 0.88 mmol). After the mixture was stirred overnight, the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (0–50%, EtOAc/hexane) to give **82** (1.04 mg, 30%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.45 (1H, br s), 7.68 (2H, d, *J* = 8.1 Hz), 7.25 (2H, d, *J* = 6.6 Hz), 6.61 (1H, s), 4.27 (2H, s), 2.41 (3H, s), 1.54 (9H, s). MS-ESI (*m/z*): 401 $[M + H]^+$.



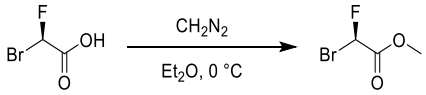
Methyl (2R)-2-[[(2R,5R)-2-[[[2-[(tert-butoxycarbonyl)amino]thiazol-5-yl)methyl]thio]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (71j). To a solution of **69** (310 mg, 0.898 mmol) in CH_2Cl_2 (3 mL) were added 2-mercaptopyridine 1-oxide **4** (114 mg, 0.898 mmol) and EDC·HCl (172 mg, 0.898 mmol). The mixture was stirred for 1 h at room temperature in the dark. To the solution was added **82** (971 mg, 2.42 mmol), and the mixture was stirred for 10 min under white light irradiation. The solvent was removed before the crude was purified by flash column chromatography (0–50%, EtOAc/hexane) to give **71j** (206 mg, 48%) as a colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.11 (1H, br s), 6.79 (1H, s), 5.89 (1H, d, $^3J_{H\text{-}F}$ = 52.6 Hz), 4.70 (1H, d, *J* = 6.4 Hz), 3.98 (1H, d, *J* = 2.8 Hz), 3.89–3.86 (2H, m), 3.88 (s, 3H), 3.63 (1H, d, *J* = 13.9 Hz), 3.02–2.99 (1H, m), 2.28–2.22 (1H, m), 2.11–2.06 (1H, m), 1.86–1.80 (1H, m), 1.58–1.55 (1H, m)1.53 (s, 9H). MS-ESI (*m/z*): 477 $[M + H]^+$.

Methyl (2R)-2-[[(2R,5R)-2-[(R)-[[2-[(tert-butoxycarbonyl)amino]thiazol-5-yl]methyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (72j). To a solution of **71j** (206 mg, 0.431 mmol) in CH_2Cl_2 (2 mL) was slowly added 69 wt % *m*-CPBA (53.9 mg, 0.216 mmol) at −40 ˚C. After the mixture was stirred for 1 h at −40 ˚C, aq $NaHCO_3$ and aq $Ns_2S_2O_3$ were added, and the aqueous layer was extracted with EtOAc three times. The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (0–20%, MeOH/EtOAc) to give **72j** (78.2 mg, 37%, dr = 5/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.07 (1H, br s), 6.93 (1H, s), 5.89 (1H, d, $^3J_{H\text{-}F}$ = 52.5 Hz), 4.54 (1H, t, *J* = 6.2 Hz), 4.20 (1H, d, *J* = 13.6 Hz), 4.06–4.03 (1H, br m), 4.01 (1H, d, *J* = 13.7 Hz), 3.87 (3H, s), 3.42 (1H, d, *J* = 12.0 Hz), 3.24 (1H, d, *J* = 12.2 Hz), 2.40–2.31 (1H, m), 2.27–2.22 (1H, m), 2.15–1.93 (2H, m), 1.54 (9H, s). MS-ESI (*m/z*): 493 $[M + H]^+$.

Methyl (2R)-2-[[(2R,5R)-2-[(R)-[(2-aminothiazol-5-yl)methyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (111). To a solution of **72j** (78.2 mg, 0.159 mmol) was added 2 M solution of $AlCl_3$ in $MeNO_2$ (318 μL, 0.636 mmol) at −30 ˚C. After being stirred for 1 h at −30 ˚C, the mixture was poured into aq $NaHCO_3$, and the aqueous layer was extracted with EtOAc three times. The combined organic layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure

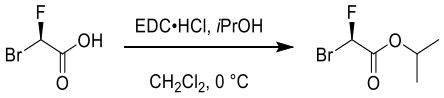
to give **111** (41.4 mg, 66%) as a white solid. MS-ESI (*m/z*): 393 $[M + H]^+$.

Sodium (2R)-2-[[(2R,5R)-2-[(R)-[(2-aminothiazol-5-yl)methyl]sulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2-fluoroacetate (116). To a solution of **111** (41.4 mg, 0.105 mmol) in THF/H_2O (v/v = 2/1, 1.5 mL) was added 1 N aq NaOH (105 μL, 0.105 mmol) at 0 ˚C. After the mixture was stirred for 1 h at 0 ˚C, the reaction was quenched by dry ice, and the organic solvent was removed under reduced pressure. The aqueous solution was applied onto HP20SS resin and subjected to ODS column chromatography (0–7%, MeCN/H_2O) to give **116** (27.5 mg, 65%, dr = 3/1) as a white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ: 6.74 (1H, s), 5.82 (1H, d, $^3J_{H\text{-}F}$ = 58.7 Hz), 4.48 (1H, t, *J* = 5.9 Hz), 4.31–4.25 (2H, m), 4.08 (1H, d, *J* = 14.1 Hz), 3.46 (1H, d, *J* = 12.3 Hz), 3.34 (1H, d, *J* = 12.0 Hz), 2.31–2.12 (3H, m), 2.08–1.99 (1H, m). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ: 173.7, 171.9 (d, $^3J_{C\text{-}F}$ = 27.1 Hz), 171.0, 140.7, 113.1, 110.7 (d, $^2J_{C\text{-}F}$ = 240.6 Hz), 76.3, 62.5, 52.6, 48.4, 21.5, 18.8. ^{19}F NMR (377 MHz, D_2O) δ: −130.1 (d, $^3J_{H\text{-}F}$ = 58.6 Hz). HPLC: 97.4% (method-A).



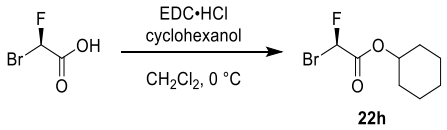
22f

Methyl (R)-2-bromo-2-fluoroacetate (22f). To a solution of (*R*)-2-bromo-2-fluoroacetic acid[7,8] (18.0 g, 115 mmol) in Et_2O (200 mL) was babbled diazomethane at 0 ˚C. After the solution turned into yellow, remaining diazomethane was removed by N_2 bubbling. The solvent was carefully removed under reduced pressure (200 torr, 20 ˚C) to give **22f** (24.9 g, 69.7 wt%, 89%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.59 (1H, d, $^3J_{H\text{-}F}$ = 50.4 Hz), 3.91 (3H, s).

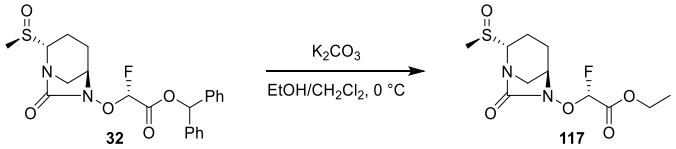


22g

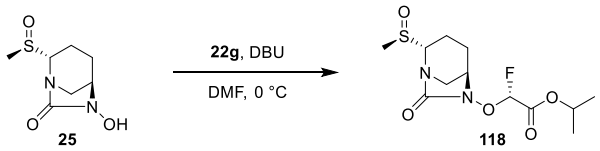
Isopropyl (R)-2-bromo-2-fluoroacetate (22g).[8] To a solution of (*R*)-2-bromo-2-fluoroacetic acid[7,8] (5.20 g, 25.8 mmol) in CH_2Cl_2 (52 mL) were added ^{i}PrOH (19.9 mL, 258 mmol) and EDC·HCl (5.45 g, 28.4 mmol) at 0 ˚C. After the mixture was stirred for 30 min at 0 ˚C, diluted aq H_2SO_4 was added. The organic layer was separated and washed with water before being dried over $MgSO_4$. The solvent was carefully removed under reduced pressure (200 torr, 20 ˚C) to give **22g** (9.72 g, 52.6 wt%, 99%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.53 (1H, d, $^3J_{H\text{-}F}$ = 50.7 Hz), 5.22–5.13 (1H, sep, *J* = 6.0 Hz), 1.34 (3H, d, *J* = 6.0 Hz), 1.33 (3H, d, *J* = 6.0 Hz). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 164.2 (d, $^3J_{C\text{-}F}$ = 25.7 Hz), 81.1 (d, $^2J_{C\text{-}F}$ = 263.4 Hz), 71.4, 21.5, 21.3. ^{19}F NMR (377 MHz, $CDCl_3$) δ: −150.53 (d, $^3J_{H\text{-}F}$ = 51.8 Hz).



Cyclohexyl (R)-2-bromo-2-fluoroacetate (22h). To a solution of (*R*)-2-bromo-2-fluoroacetic acid[7,8] (39.0 g, 102 mmol) in CH_2Cl_2 (195 mL) were added cyclohexanol (107 mL, 1019 mmol) and EDC·HCl (21.5 g, 112 mmol) at 0 ˚C. After the mixture was stirred for 30 min at 0 ˚C, diluted aq H_2SO_4 was added. The organic layer was separated and washed with water before being dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (0–5%, EtOAc/hexane) to give **22h** (35.4 g, 63 wt%, 92%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.55 (1H, d, $^3J_{H\text{-}F}$ = 50.8 Hz), 4.97–4.91 (1H, m), 1.90–1.87 (2H, m), 1.78–1.73 (2H, m), 1.56–1.51 (2H, m), 1.45-1.27 (4H, m). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 164.2 (d, $^3J_{C\text{-}F}$ = 25.7 Hz), 81.1 (d, $^2J_{C\text{-}F}$ = 263.4 Hz), 75.9, 31.1, 30.9, 25.1, 23.4, 23.3. ^{19}F NMR (377 MHz, $CDCl_3$) δ: −150.34 (d, $^3J_{H\text{-}F}$ = 50.4 Hz).

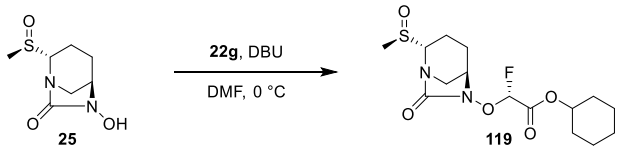


Ethyl (2R)-2-fluoro-2-[[(2R,5R)-2-[(R)-methylsulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] oxy]acetate (117). To a solution of **32** (5.00 g, 11.2 mmol) in EtOH/CH_2Cl_2 (v/v = 1/2, 150 mL) wad added K_2CO_3 (1.55g, 11.2 mmol) at 0 ˚C. After the mixture was stirred for 3 h at 0 ˚C, 10% aq citric acid and brine were added, and the layers were separated. The aqueous layer was extracted with EtOAc twice, and the combined organic layers were dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude was purified by flash column chromatography (0–10%, EtOH/EtOAc) to give **117** (2.67 g, 77%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.88 (1H, d, $^3J_{H\text{-}F}$ = 52.2 Hz), 4.38–4.26 (2H, m), 4.10 (1H, t, *J* = 6.1 Hz), 4.07–4.05 (1H, br m), 3.36 (1H, d, *J* = 12.0 Hz), 3.20 (1H, d, *J* = 12.3 Hz), 2.68 (3H, s), 2.48–2.39 (1H, m), 2.29–2.11 (2H, m), 2.03–1.94 (1H, m), 1.34 (3H, t, *J* = 7.2 Hz). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 167.1, 162.8 (d, $^3J_{C\text{-}F}$ = 35.9 Hz), 105.2 (d, $^2J_{C\text{-}F}$ = 239.2 Hz), 76.8, 62.9, 60.5, 45.1, 37.1, 19.3, 15.9, 14.0. ^{19}F NMR (377 MHz, $CDCl_3$) δ: −136.7 (d, $^3J_{H\text{-}F}$ = 51.8 Hz). HPLC: 99.9% (method-B). HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{11}H_{18}O_5N_2FS$ 309.0915; found 309.0911.



Isopropyl (2R)-2-fluoro-2-[[(2R,5R)-2-[(R)-methylsulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]acetate (118). To a suspension of **25** (150 mg, 0.734 mmol) in DMF (1 mL) were added 52.6 wt% **22g** (333 mg, 0.881 mmol) and DBU (0.133 mL, 0.881 mmol) at 0 ˚C. After being stirred for 1 h at 0 ˚C, the mixture was directly applied onto HP20SS resin and subjected to ODS column chromatography (30–50%, MeCN/H_2O) to give **118** (159 mg, 67%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$)

δ: 5.85 (1H, d, $^{3}J_{H\text{-}F}$ = 52.5 Hz), 5.19–5.10 (1H, sep, *J* = 6.3 Hz), 4.12–4.07 (2H, m), 3.37 (1H, d, *J* = 12.0 Hz), 3.19 (1H, d, *J* = 12.0 Hz), 2.68 (3H, s), 2.48–2.39 (1H, m), 2.28–2.11 (2H, m), 2.02–1.94 (1H, m), 1.33 (3H, d, *J* = 6.3 Hz), 1.30 (3H, d, *J* = 6.3 Hz). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$) δ: 167.0, 162.3 (d, $^{3}J_{C\text{-}F}$ = 35.9 Hz), 105.3 (d, $^{2}J_{C\text{-}F}$ = 237.7 Hz), 76.8, 71.2, 60.6, 45.1, 37.1, 21.6, 21.4, 19.3, 15.8. ^{19}F NMR (377 MHz, $CDCl_3$) δ: −136.5 (d, $^{3}J_{H\text{-}F}$ = 51.8 Hz). HPLC: >99.9% (method-B). HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{12}H_{20}O_5N_2FS$ 323.1071; found 323.1067.



Cyclohexyl (2R)-2-fluoro-2-[[(2R,5R)-2-[(R)-methylsulfinyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]acetate (119). To a suspension of **25** (16.0 g, 78 mmol) in DMF (112 mL) were added 92 wt% **22h** (24.4 g, 94 mmol) and 2 M DBU in DMF (43.1 mL, 86 mmol) at 0 ˚C. After being stirred for 1 h at 0 ˚C, the mixture was diluted with EtOAc (160 mL) and poured into water. The aqueous layer extracted with EtOAc twice, and the combined organic layers were washed with water and brine before being dried over $MgSO_4$. After the solvent was removed, the crude was recrystallized form CH_2Cl_2/$^{i}Pr_2O$ to give **119** (23.5 g, 83%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.87 (1H, d, $^{3}J_{H\text{-}F}$ = 52.2 Hz), 4.93–4.87 (1H, m), 4.12–4.07 (2H, m), 3.36 (1H, d, *J* = 12.0 Hz), 3.19 (1H, d, *J* = 12.3 Hz), 2.68 (3H, s), 2.47–2.39 (1H, m), 2.27–2.11 (2H, m), 2.02–1.89 (3H, m), 1.78–1.21 (8H, m). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$) δ: 167.0, 162.3 (d, $^{3}J_{C\text{-}F}$ = 35.9 Hz), 105.3 (d, $^{2}J_{C\text{-}F}$ = 238.4 Hz), 76.8, 76.1, 60.6, 45.1, 37.1, 31.4, 31.2, 25.1, 23.7, 23.7, 19.3, 15.8. ^{19}F NMR (377 MHz, $CDCl_3$) δ: −136.2 (d, $^{3}J_{H\text{-}F}$ = 53.1 Hz). HPLC: >99.9% (method-B). HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{15}H_{24}O_5N_2FS$ 363.1384; found 363.1379.

X-ray crystallographic data of compound 119

X-ray Crystallography:

The diffraction data of **119** were collected on an XtaLAB AFC10 (RCD3): quarter-chi single diffractometer. The crystal was kept at 100.0 K during data collection. Using Olex2,[10] the structure was solved with the ShelXT[11] structure solution program using Intrinsic Phasing and refined with the ShelXL[12] refinement package using Least Squares minimisation.

Sample Preparation:

X-ray quality crystal was prepared by vapor diffusion method using methyl ethyl ketone/hexane at room temperature.

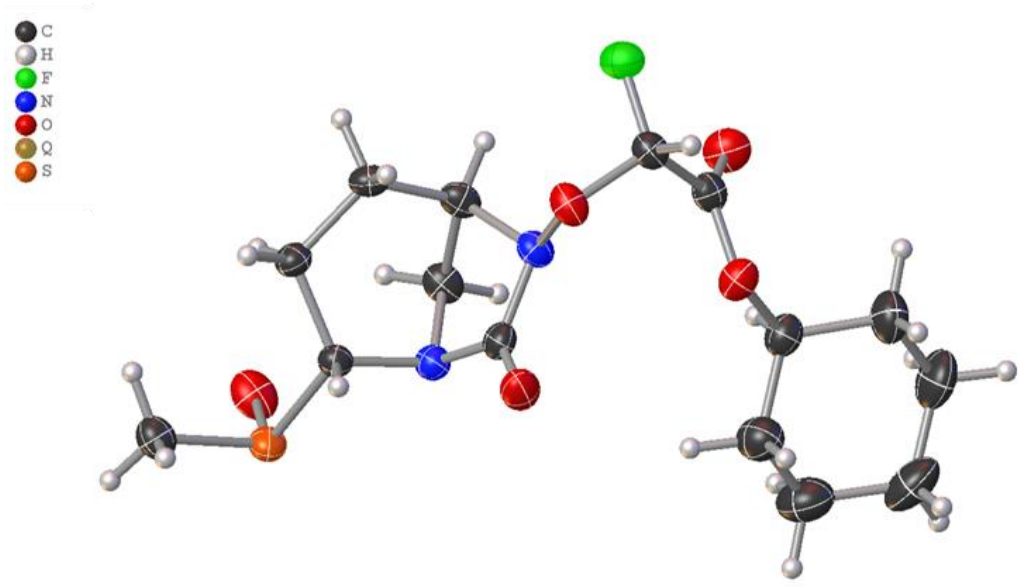


Figure S3. X-ray Structure of compound **119**. Thermal ellipsoids are set at 30% probability.

Table S2. Crystal data for 119

Formula	$C_{15}H_{23}FN_2O_5S$
Formula weight	362.41
Temperature (K)	200
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a (Å)	5.3772 (10)
b (Å)	12.7909 (2)
c (Å)	24.8398 (4)
α (°)	90
β (°)	90
γ (°)	90
Volume ($Å^3$)	1708.46 (5)
Z	4
ρ (g/cm^3)	1.409
μ (mm^{-1})	2.036
F(000)	768.0
Crystal size (mm^3)	0.2 × 0.02 × 0.01
R_1	0.0316
Flack parameter	−0.004(11)
CCDC Deposition Number	2195413

化合物の生物学的，物理学的評価

***β*-Lactamase Inhibition Assay.** The inhibition of *β*-lactamases was determined by the hydrolysis of nitrocefin. The hydrolysis of nitrocefin was recorded as absorbance at 492 nm by Envision2013 (Perkin Elmer). The concentration of *β*-lactamase enzyme (KPC-2, CTX-M-15, CMY-2, and OXA-48) used was determined by the measurement of hydrolysis of nitrocefin (50 μg/mL), where the change of the absorbance was approximately 0.2 from the negative control (no enzyme). The enzyme was added to the sample solution including nitrocefin (final concentration 50 μg /mL) and an inhibitor at the volume ratio of 1:3 (final 80 μL). The absorbance was measured at 35 ˚C after 20 min incubation. The concentration of the inhibitor needed to reduce the hydrolysis of the substrate by 50% (IC_{50}) was calculated using XLfit (IDBS, UK).

Antibiotic Susceptibility Testing. Strains with a "SR" designation are from previous surveillance which are kept in the Shionogi collection of clinical isolates and have been characterized by multiplex PCR and sequencing analysis including whole genome sequencing. All strains with an "ATCC" designation are purchased from American Type Culture Collection (ATCC). The minimum inhibitory concentrations (MICs) were determined using the broth microdilution method in accordance with Clinical Laboratory Standards Institute (CLSI) guidelines.[14] Briefly, two-fold serial dilution of test compounds were prepared and transferred to a 96-well plate including cation-adjusted Mueller Hinton broth (CAMHB). The bacterial suspension for inoculum were 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. The MPC (minimal potentiating concentration) values were used to define of BLI. The MPC_1 as defined as the concentration of BLI that was required to reduce the CTB MIC to ≤ 1 μg/mL. The MPC_1 values were determined using the broth microdilution method. Briefly, two-fold serial dilution of test BLIs were prepared and transferred to a 96-well plate including CAMHB with 1 μg/mL of CTB. The bacterial suspension for inoculum were 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 MPC_1 endpoint was defined as the lowest concentration of the compound that inhibited bacterial growth as detected by the unaided eye.

PBP Inhibitory Rates Testing. Membrane fraction (2 mg/ml) of *E. coli* NIHJ JC-2 was incubated at 30 ˚C for 10 min with diluted **21b**, and Bocillin™ FL penicillin sodium salt (final concentration was 100 μg/mL) (Life Technologies) was added and incubated at 30 ˚C for 30 min to label intact PBPs. Final concentrations of *N*-lauroylsarcosine sodium salt (Nacalai Tesque) and penicillin G sodium salt (Chem-Impex International) were adjusted to 1.7% (w/v) and 20 mg/mL, and incubated at 30 ˚C for 15 min. Incubated samples were centrifuged at 18,000g for 30 min at 8 ˚C. An aliquot (50 μL) of supernatant mixed with 10 μL of 6x sample buffer solution (Nacalai Tesque) and 1 μL of 2-mercaptoethanol was heated in boiled water for 3 min. Samples were subjected to a 7.5% mini-gel (Nacalai Tesque) for SDS-PAGE to separate PBPs and the gel was washed with water and scanned by LAS-3000 (Fuji Film) with Y515 cutoff filter. The fluorescence values were measured using Multi Gauge (Fuji Film) and PBP

inhibitory rates were calculated when fluorescence value of the control was considered to be 0%.

Pharmacokinetics Studies. Pharmacokinetics studies were performed in mice, rats, dogs, and monkeys. Briefly, 6-week old Jcl:ICR male mice, 8-week old Crl:CD (SD) male rats, male Marshal beagles, and female cynomolugus monkeys were used in these studies. Blood samples were collected after intravenous and oral administration at designed time and centrifuged to obtain the plasma samples. Plasma samples were stored at −20 ˚C until LC/MS/MS analysis.

LC/MS/MS Determination of Test Compounds. Aliquots (5–40 μL) of each samples were transferred to a 96-well plate, mixed with 250 or 500 μL of methanol, and centrifuged at 1,580*g* 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). Test compounds plasma concentration were determined by LC/MS/MS with electrospray ionization in the positive ion mode using an external standard method.

Pharmacokinetic Analysis. The elimination half-life ($T_{1/2}$) was estimated as ln 2/k_{el}, where k_{el} is the elimination rate constant derived from the slope of the log concentration versus time profile. The area under the concentration-time curve from 0 h to the last time point (AUC) was calculated by linear trapezoidal approximation. Total clearance (CL_{tot}) was estimated as Dose/AUC.

Serum Stability. Each experiment was performed in duplicate. Pooled human, rat, mouse, dog, and monkey serum were incubated with test compound for 30 min at 37 ˚C, respectively. The concentration of test compounds was 5 μmol/L. After incubation, an aliquot (20 μL) of each sample was transferred to a 96-well plate, mixed with 120 μL of methanol, and centrifuged at 1,580*g* for 10 min at 4 ˚C. The supernatant was analyzed by LC/MS/MS, and the plasma stability (%) of test compounds was calculated.

Serum Protein Binding. Serum protein binding was evaluated by ultrafiltration method. Test compounds were spiked in the serum collected from fasted mice, rats, dogs, and monkeys at 10 μg/mL. After incubation for 5 min at 37 ˚C, serum samples were transferred to Centrifree (Merck KGaA, Darmstadt, Germany) and centrifuged. Initial serum samples and filtrates were analyzed by LC/MS/MS.

***In Vivo* Efficacy Studies.** *In vivo* efficacy of CTB and prodrug **119** was evaluated in the murine urinary tract infection model against *K. pneumoniae* strains SR08667. Briefly, 5-week old ICR female mice (N = 5 per arm, CLEA Japan, Inc.) were used in this study. This animal study was approved by the Institutional Animal Care and Use Committee of Shionogi & Co., Ltd. *K. pneumoniae* SR08667 suspension was diluted with MHB to be 1.2×10^7 CFU/mL. A 0.1 mL of the diluted bacterial suspension was transurethrally inoculated (1.2×10^6 CFU/mouse) and the urethra was tightened for 4 hours for the infection. The CTB and **119** were orally administered four times at the time points of 4, 8, 28, 32 h after the infection. The both of CTB and **119** were formulated in 0.5% methylcellulose. At 4- or 48-hour post-infection, animals were euthanized, and kidneys (2 samples/mouse) were collected and homogenized in 2.7 mL of MHB. The tissue homogenates were serially diluted and the viable cells in the samples were counted by using modified Drigalski agar.

Repeated-Dose Toxicity Study. Briefly, 6-week old Crl:CD (SD) male and female rats were used in this study. Compound **119** was orally administered for 2 weeks at a daily dosage of 600 mg/kg or 2000 mg/kg. The following items were examined; clinical observations, body weight, feed consumption, urinalysis, hematology, blood chemistry, organ weight, macropathology, and histopathology.

References

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