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Synthesis of 2-Thio Substituted 1,6-Diazabicyclo[3.2.1]octane Derivatives, Potent β-Lactamase Inhibitors

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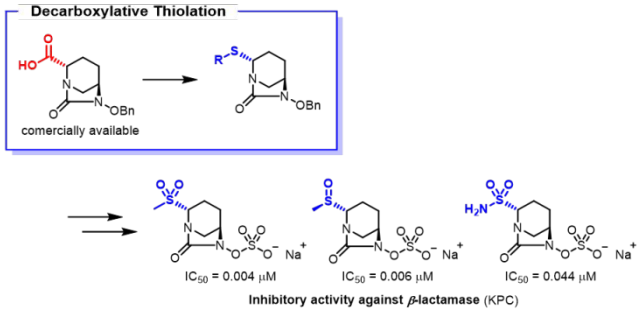


ABSTRACT: Approval of avibactam by the FDA has led to the recognition of 1,6-diazabicyclo[3.2.1]octane (DBO) derivatives as attractive compounds for β-lactamase inhibition. We achieved concise and collective synthesis of 2-thio substituted DBO derivatives. The synthesis involves diastereoselective photo-induced Barton decarboxylative thiolation, which can be applied to large-scale synthesis. The DBO analogues exhibited strong inhibitory activities against serine β-lactamases and acceptable solution stabilities for clinical development.

INTRODUCTION

β-Lactam antibiotics have played an important to release people from bacterial infectious diseases since the discovery of penicillin. In contrast with our expectation, however, a lot of resistant strains to those drugs have emerged even though new antibiotics have been approved by authorities in many countries.[1] One of the main mechanisms for resistance to β-lactam is production of β-lactamase which catalyzes hydrolysis of β-lactams.[2] Fortunately another antibiotic, carbapenem, is inert to most of β-lactamases and is used to treat β-lactam resistant strains,[3] but the emergence of carbapenem-resistant enterobacteriaceae (CRE) producing "carbapenamase" has been reported worldwide in recent year.[4]

In order to cope with the emergence of CREs, a novel serine β-lactamase inhibitor, avibactam (**1**),[5, 6] was approved by the FDA in 2015 as a combination with cephalosporin, ceftazidime. Avibactam (**1**) has a unique 1,6-diazabicyclo[3.2.1]octane (DBO) skeleton, unlike existing β-lactam-based β-lactamase inhibitors such as clavulanic acid (**5**) and tazobactam (**6**). The reaction between 2-imidazolone in DBO and an active center serine of β-lactamase results in the formation of a relatively stable carbamoyl-enzyme complex. This enables avibactam (**1**) to inhibit serine β-lactamases and this mechanism is distinct from other β-lactamse inhibitors such as clavulanic (**5**) acid and tazobactam (**6**) (**Figure 1b**).

For inhibitory activities against β-lactamases, DBO derivatives need an electron-withdrawing group at the C2 position to increase the reactivity of the 2-imidazolone backbone. Several DBOs utilize an amide electron withdrawing group including avibactam (**1**), relebactam (**2**),[7, 8] nacubactam (**3**)[9] and zidebactam (**4**).[10] Other C2 electron-withdrawing groups including nitrile and heterocycles have been reported as well as a novel 2,3-ring-fused DBO.[5, 10a, 11] The structural strain also enhances β-lactamase inhibitory activities. ETX2514 (**7**)[12] and ETX1317 (**8**)[13] have an unsaturated bond on the DBO skeleton. However, introducing a double bond seems to be more complex, and it is difficult to prepare these molecules. Herein, we explore the synthetic route of DBO derivatives possessing a sulfone, sulfoxide and sulfonamide as a strong electron-withdrawing group and report their β-lactamase inhibitory activity.

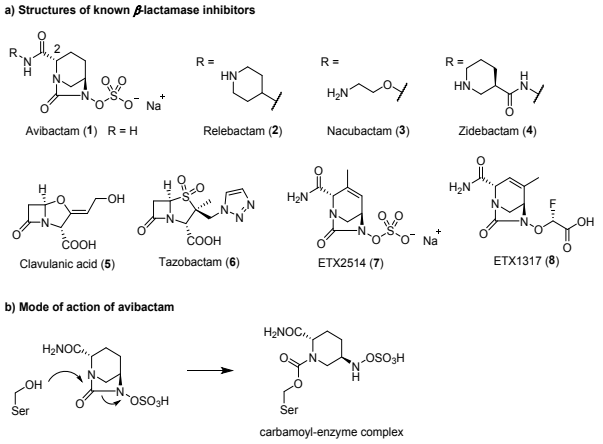


Figure 1. a) Structures of known *β*-lactamase inhibitors. b) Mode of action of avibactam.

RESULTS AND DISCUSSION

We attempted to convert carboxylic acid (**9**),[6] a commercially available starting material, into a sulfide (**11**) by photo-induced Barton decarboxylative thiolation[14] (**Table 1**). First, 2-mercaptopyridine (**12**) was condensed onto **9** to form intermediate *N*-hydroxy-2-thiopyridone ester (**10**) with EDC hydrochloride. After an addition of diphenyl disulfide, irradiation under white LED gave sulfide (**11a**) as a single isomer (entry 1). The relative configuration of **11a** was determined by NOE correlations. We suppose the radical intermediate stereoselectively reacted with diphenyl disulfide from the convex side (**Figure 2**).

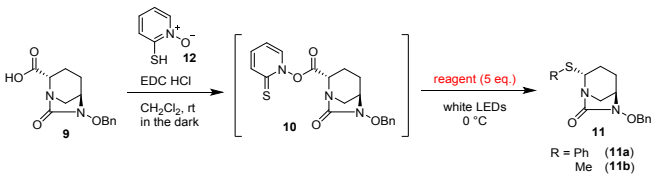
Subsequently, attempts were made to obtain a methyl sulfide (**11b**) by the same method (**Table 1**). However, the yield was low when dimethyldisulfide was used as a radical trapping reagent (entry 2). Our investigation suggested that excess of dimethyldisulfide was necessary to obtain the product in good yield (entry 3). This result is consistent with the previous report.[14, 15] We speculated that dimethyl disulfide might be less reactive. Then, methyl methanethiosulfonate (**34**) and unsymmetrical disulfide PhSSMe (**35**) were tested to afford **11b** with acceptable yield (entry 4, 5). Interestingly, we observed high chemoselectivity although both phenylthio and methylthio groups might be introduced when PhSSMe (**35**) was used. The unsymmetrical disulfide (**35**) is preferred because it is less polar and easily removed by silica-gel column chromatography. To the best of our knowledge, this is the first example of utilizing an unsymmetrical disulfide to improve radical thiolation, although the resulting sulfide (**11**) was unstable on silica-gel column chromatography[16] and the isolated yield was low.

The oxidation of sulfide (**11**) using 2.2 eq. of *m*-CPBA gave sulfone (**13**) in a good yield (**Scheme 1**). Sulfone (**13**), after catalytic hydrogenolysis of the benzyl group, led to sulfonate (**15**) in a similar manner to the synthesis of avibactam.[6]

The sulfide (**11b**) was also oxidized to sulfoxide (**16**) by using 1.1 eq. of *m*-CPBA at -78 ℃ (**Scheme 2**). Diastereoselective oxidation was observed and the ratio was improved up to 31/1 after recrystallization from EtOAc at -30 ℃.[17] The relative configuration of sulfoxide (**16**) was established by X-ray crystallography.[18] The diastereoselectivity can be explained by the conformation of the substrate (**11b**). Steric repulsion of the methyl group and electric repulsion between the lone pair on sulfur and that on nitrogen at the bridge-head would give the stable conformation shown in **Figure 3**. It is assumed that the sterically less hindered lone pair on sulfur is preferentially oxidized by *m*-CPBA to give the sulfoxide (**16**) with high diastereoselectivity.

In contrast to the case of sulfone (**13**), deprotection of the benzyl group by Pd/C was not successful because the sulfoxide presumably acted as a catalytic poison. Yasuda and coworkers have reported that addition of a catalytic amount of DABCO significantly accelerated Pd-catalyzed hydrogenolysis in the synthesis of relebactam.[8c] We applied this procedure and the result is shown in **Table 2**. The combination of DABCO and $Pd(OH)_2$ successfully gave the debenzylated intermediate (**17**) (entry 4) although no improvement was observed in the case of $Pd(OH)_2$ alone and a combination of Pd/C and DABCO (entry 2, 3). Conversion to sulfate (**19**) using SO_3-pyridine was also unsuccessful and neither **19** was obtained nor **17** was recovered. On the other hand, neopentyl chlorosulfate (**20**) chemoselectively reacted with the hydroxyl amino group and **17** was successfully led to a neopenthyl sulfate (**18**). Nucleophilic substitution at the neopentyl position released sulfate (**19**) in good yield.

Table 1. Optimization of Reaction Conditions for Barton Decarboxylative Thiolation



entry	reagent	R	yield (%)[a]
1	PhSSPh	Ph	61 (50)[b]
2	MeSSMe	Me	30
3	MeSSMe[c]	Me	52
4	$MeSO_2SMe$ (**34**)	Me	47
5	PhSSMe (**35**)	Me	48 (33)[b]

[a]Yield was determined by 1H NMR using 1,1,2,2-tetrachloroethane as an internal standard. [b]Isolated yield. [c]45 eq. of MeSSMe was used.

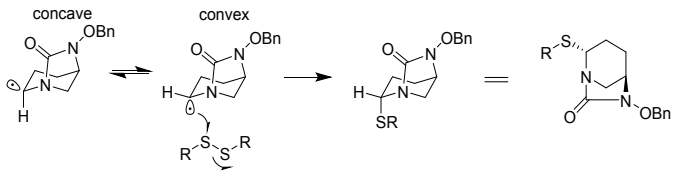
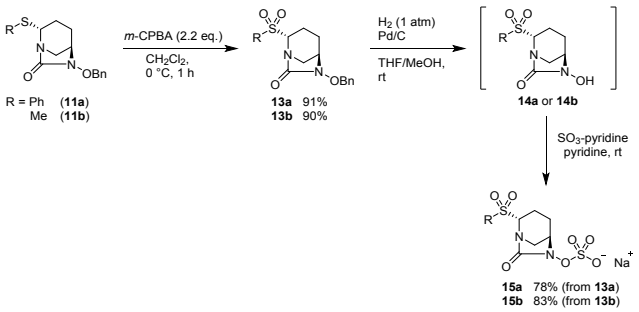


Figure 2. Proposed mechanism of diastereoselectivity.

Scheme 1. Synthesis of Sulfone (15)



Scheme 2. Synthesis of Sulfoxide (19)

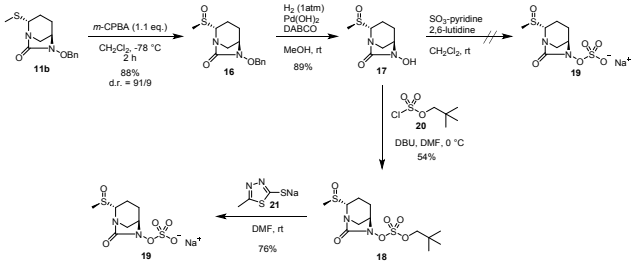


Table 2. Optimization of Debenzylation of Sulfoxide 16

entry	condition	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

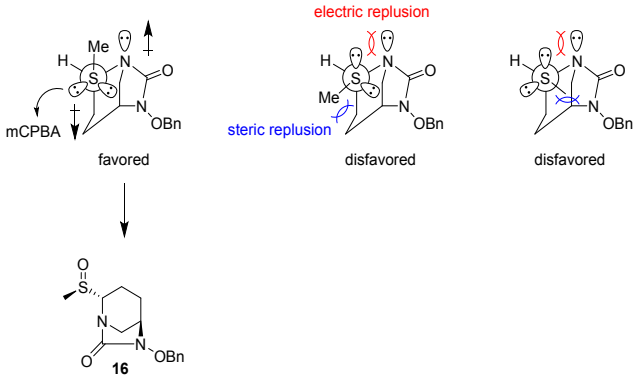
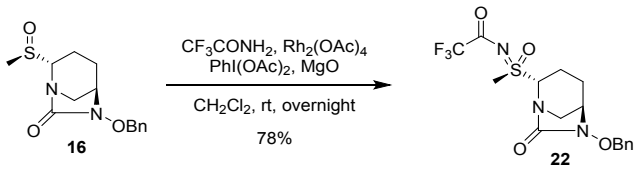


Figure 3. Presumable mechanism for diastereoselective oxidation of **11b**.

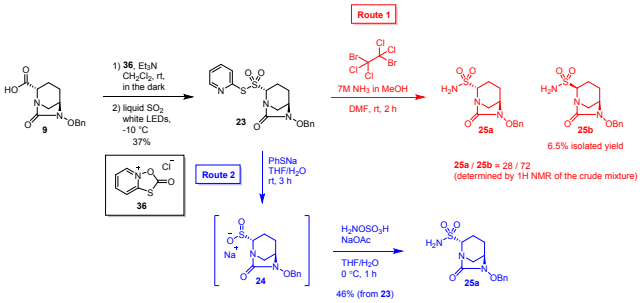
The sulfoxide (**16**) was also led to a sulfoximine (**22**),[19] a functional group attracting attention in the field of medicinal chemistry because its tetrahedral structure offers unique properties such as favorable physicochemical properties, hydrogen bond functionalities and structural diversity[20] (**Scheme 3**).

Scheme 3. Synthesis of Sulfoximine (22)



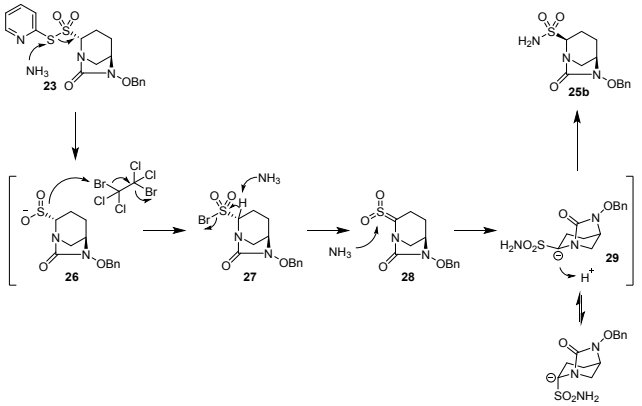
The synthetic success of the sulfone and sulfoxide analogues encouraged us to move onto synthesis of a sulfonamide analogue. It is reported that a carboxylic acid was converted into a sulfonamide via a thiosulfonate.[21] We attempted a similar synthetic route to access a sulfonamide (**Scheme 4**). In an excess amount of liquid sulfur dioxide, the Barton ester (**10**), prepared by reaction of carboxylic acid (**9**) with **36**, was irradiated with white LEDs. After sulfur dioxide trapped the radical intermediate, the resulting sulfonyl radical reacted with thiopyridone on the Barton ester (**10**) to propagate a radical chain and gave a thiosulfonate (**23**). We attempted to transform **23** into a sulfonamide (**25a**) in the presence of 1,2-dibromo tetrachloroethane and ammonia, however an epimer (**25b**) was dominantly obtained in the crude mixture (**25a**/**25b** = 28/72) and the isolated yield of **25b** was poor (Route 1).

Scheme 4. Synthesis of Sulfonamide (25a)



We hypothesized the mechanism of epimerization to be as presented in **Scheme 5**. The sulfinate (**26**), derived from **23** after cleavage of the sulfur-sulfur bond by ammonia, would be oxidized by 1,2-dibromo tetrachloroethane to the corresponding sulfonyl bromide (**27**). This sulfonyl bromide (**27**) would be immediately deprotonated and converted into the sulfene (**28**). After nucleophilic addition of ammonia to the sulfene (**28**), the resulting anion (**29**) would receive protonation from the convex side to afford the epimer (**25b**).

Scheme 5. Proposed Mechanism of Epimerization

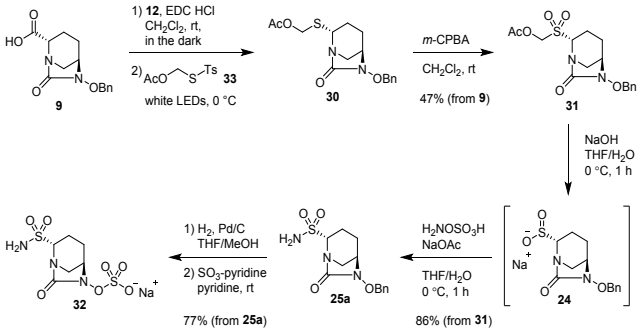


In order to suppress the epimerization, we tried an alternative route that circumvented the sulfonyl halide (**Scheme 4,** Route

2). Electrophilic amination of a sulfinate (**24**), released from the thiosulfonate (**23**), provided the desired sulfonamide (**25a**) without the epimerization.

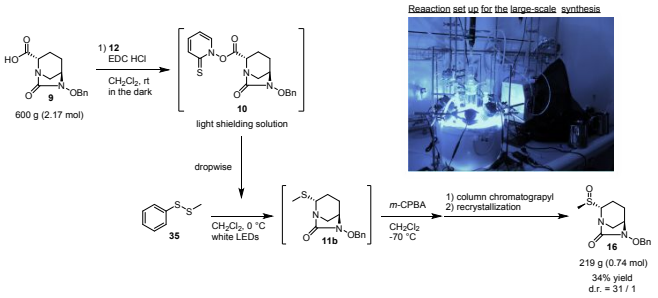
We were able to successfully synthesize the sulfonamide (**25a**), but the synthetic route required an excess amount of toxic sulfur dioxide with an unsatisfactory yield. To work around this issue, we continued our investigation and exploratory research for another synthetic route (**Scheme 6**). Conversion of acetoxymethyl sulfone into a sulfinate is known to be possible by hydrolysis of an acetoxy group.[22] We postulated that the sulfonamide (**25a**) would be effectively obtained via an acetoxymethyl sulfone (**31**). Compound **9** underwent Barton decarboxylative radical thiolation utilizing a thiosulfonate (**33**) to give a corresponding acetoxymethyl sulfide (**30**), which was oxidized to a sulfone (**31**). Compared with the case of thiosulfonate (**23**), the acetoxymethyl sulfone (**31**) could be similarly prepared from **9** in better yield without using toxic sulfur dioxide. The debenzylation of sulfonamide (**25a**), which was derived from **31** via the sulfinate (**24**), followed by sulfonation gave **32** in good yield.

Scheme 6. Improved Synthetic Route for Sulfonamide



Following our synthetic studies, we tried the scaling up of the synthesis for **16** (**Scheme 7**). It is generally difficult to scale up a photochemical reaction with respect to photo-efficiency. However, the Barton decarboxylative reaction is a radical chain reaction offering a high quantum yield in contrast to a catalytic photoredox reaction.[23] Thus, we assumed that the scaling up of this step would be possible without the need for a special apparatus or technique such as a flow reactor.[24] On the other hand, a radical chain reaction involves the risk of becoming a runaway reaction. To prevent this, we added the Barton ester (**10**) at an appropriate rate by a liquid feeding pump. The light shielding solution of **10** was added dropwise to the solution of **35** under white LED irradiation, maintaining the inner temperature under 10 ℃. The reaction proceeded safely and the resulting **11b**, unstable on silica-gel, was oxidized to **16** without purification by silica-gel column chromatography. The sulfoxide (**16**) was obtained with a yield and diastereo-ratio comparable to those of the small-scale synthesis.

Scheme 7. Scale-up Synthesis of 16



With the targeted 2-thio substituted DBO derivatives in hand, their *in vitro* β-lactamase inhibitory activities against KPC, CTX-M-15 and CMY-2 were evaluated using nitrocefin as a substrate[25] (**Table 3**). The inhibitory activities of sulfones (**15a, b**) and sulfoxide (**19**) were comparable or better than those of avibactam (**1**). On the other hand, sulfonamide (**32**) showed weaker activities although it was expected to have activities similar to avibactam because a sulfonamide is a bioisostere of amide. These results suggest that an electron inductive effect of the substituent at the C2 position would dominantly influence the inhibitory activities.

Table 3. Inhibitory Activities against β-Lactamases

	IC_{50} (μM)				
β-lactamase	**15a**	**15b**	**19**	**32**	Avibactam (**1**)
KPC-2	0.052	0.004	0.006	0.044	0.072
CTX-M-15	0.031	0.017	0.012	0.140	0.013
CMY-2	<0.001	0.003	0.041	0.116	0.059

The introduction of a strong electron withdrawing group, such as a sulfone, enhances the reactivity of the 2-imidazolone backbone in DBO and consequently improves the inhibitory activity against β-lactamase. On the other hand, it also means that 2-thio substituted DBO analogues might be readily hydrolyzed in an aqueous solution. Therefore, we assessed the solution stability of **15a, b** and **32** (**Table 4**). Their stability fell slightly short of avibactam (**1**) at pH 7, but they were stable at pH 2 and 5. These results led us to the conclusion that 2-thio substituted DBO derivatives offer the potential for clinical use as a β-lactamase inhibitor in terms of physical stability.

Table 4. Solution Stability

	recovery rate at 40 °C after 16 h			
pH	**15a**	**15b**	**32**	Avibactam (**1**)
pH 2	85%	81%	78%	3%
pH 5	86%	79%	87%	95%
pH 7	66%	44%	51%	89%

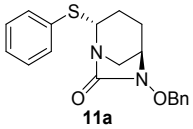
CONCLUSIONS

In summary, we have presented the synthesis and bio- and physical evaluation of 2-thio substituted DBO derivatives, which could be efficiently prepared from commercially available carboxylic acid (**9**) by Barton decarboxylative thiolation. They exhibited significant β-lactamase inhibitory activities and acceptable solution stabilities. These findings indicate that 2-thio substituted DBO derivatives have the potential to be clinically valuable β-lactamase inhibitors.

EXPERIMENTAL SECTION

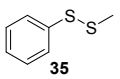
Materials and Methods

Unless otherwise noted, reactions were performed under nitrogen atmosphere. Solvents and commercial reagents were used without purification. Carboxylic acid **9**, methyl methanethiosulfonate **34** and salt **36** were purchased from PharmaBlock R&D Co., Ltd., Leap Labchem Co., Ltd. and Tokyo Chemical Industry Co., Ltd. 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 Silica Gel (YAMAZEN ULTRA PACK). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV400 spectrometer or a Varian INOVA. Chemical shifts (δ) are reported in parts per million (ppm) from trimethylsilane (in CD_3Cl) 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). Elemental analysis was performed by MICRO CORDER JM11 (J-SCIENCE LAB CO., Ltd.). Melting points were measured with a Yanagimoto melting point apparatus and were uncorrected. 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.[28] The number of lamps we employed depended on the scale of reaction and the specific number is presented in the experimental detail of each compound.



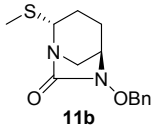
11a

(2R,5R)-6-(Benzyloxy)-2-(phenylthio)-1,6-diazabicyclo[3.2.1]octan-7-one (11a). To a solution of carboxylic acid **9** (300 mg, 1.09 mmol) and 2-mercaptopyridine 1-oxide **12** (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 (1.19 g, 5.43 mmol) was added and stirred under white light irradiation using two LED lamps (A 160WE Tuna Blue, Kessil®) for 30 min at 0 ℃. 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 **11a** (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.



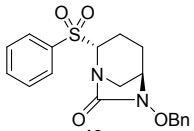
35

1-Methyl-2-phenyldisulfane (35).[26] 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 ℃. After stirring for 10 minutes at room temperature, methyl methanethiosulfonate **34** (1.25 L, 12.1 mol) was added at 0 ℃ 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 ℃) to afford **35** (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).



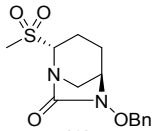
11b

(2R,5R)-6-(Benzyloxy)-2-(methylthio)-1,6-diazabicyclo[3.2.1]octan-7-one (11b). To a solution of carboxylic acid **9** (300 mg, 1.09 mmol) and 2-mercaptopyridine 1-oxide **12** (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 **35** (848 mg, 5.43 mmol) was added and stirred under white light irradiation using two LED lamps (A 160WE Tuna Blue, Kessil®) for 30 min at 0 ℃. 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 **11b** (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.



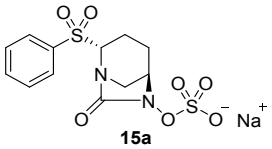
13a

(2R,5R)-6-(Benzyloxy)-2-(phenylsulfonyl)-1,6-diazabicyclo[3.2.1]octan-7-one (13a). To a solution of **11a** (353 mg, 1.04 mmol) in CH_2Cl_2 (10 mL) was added *m*-CPBA (72 wt%, 547 mg, 2.28 mmol) at 0 ℃. 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 **13a** (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), 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), 3.02 (1H, J = 12.0 Hz), 2.48-2.44 (1H), 2.22-2.09 (2H), 1.91-1.86 (1H). ^{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.



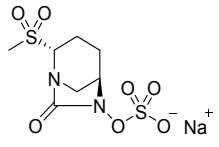
13b

(2R,5R)-6-(Benzyloxy)-2-(methylsulfonyl)-1,6-diazabicyclo[3.2.1]octan-7-one (13b). To a solution of **11b** (1.00 g, 3.59 mmol) in CH_2Cl_2 (20 mL) was added *m*-CPBA (72 wt%, 2.16 g, 9.00 mmol) at 0 ℃. After stirred for 1 hour at 0 ℃, 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 **13b** (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.



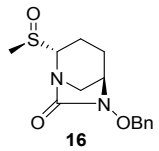
15a

Sodium (2R,5R)-7-oxo-2-(phenylsulfonyl)-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (15a). To a solution of **13a** (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 ℃ 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 ℃. 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 **15a** (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. 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.



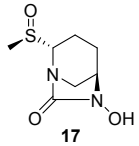
15b

Sodium (2R,5R)-2-(methylsulfonyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (15b). To a solution of **13b** (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*. 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 ℃ 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 ℃. 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 **15b** (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. 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

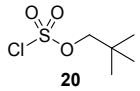
(2R,5R)-6-(Benzyloxy)-2-((R)-methylsulfinyl)-1,6-diazabicyclo[3.2.1]octan-7-one (16). To a solution of **11b** (2.47 g, 8.89 mmol) in CH_2Cl_2 (25 mL) was added *m*-CPBA (72 wt%, 2.34 g, 9.78 mmol) at -78 ℃. After stirred for 2 hour at -78 ℃, the mixture was poured into aqueous sodium thiosulfate solution 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 **16** (2.31 g, 88%, d.r. = 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 ℃.



17

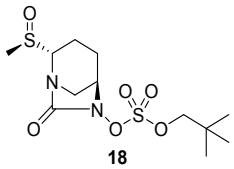
(2R,5R)-6-Hydroxy-2-((R)-methylsulfinyl)-1,6-diazabicyclo[3.2.1]octan-7-one (17). To a solution of **16** (1.53 g, 5.20 mmol) in MeOH (15 mL) were added 10% $Pd(OH)_2$ on carbon (365 mg, 0.24 wt equiv) and DABCO (11.7 mg, 0.10 mmol). After stirred at room temperature under H_2 atmosphere

(1 atm) for 1 hour, the mixture was filtered. The solvent was removed *in vacuo* to afford **17** (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.



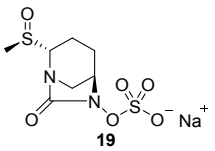
20

Neopentyl chlorosulfate (20).[27] 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 ℃. 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 **20** (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).



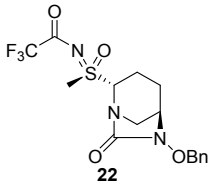
18

(2R,5R)-2-((R)-Methylsulfinyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl neopentyl sulfate (18). To a solution of **17** (300 mg, 1.47 mmol) in DMF (3 mL) were added DBU (0.288 mL, 1.91 mmol) and chlorosulfate **20** (274 mg, 1.47 mmol) at 0 ℃ 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 **18** (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.



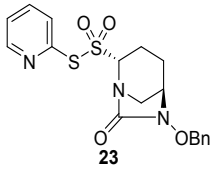
19

Sodium (2R,5R)-2-((R)-methylsulfinyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (19). To a solution of **18** (286 mg, 0.807 mmol) in DMF (3 mL) was added sodium thiolate **21** (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 **19** (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.



22

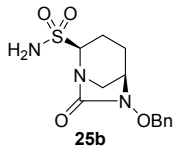
N-((1R)-((2R,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl)(methyl)(oxo)-λ^6-sulfaneylidene)-2,2,2-trifluoroacetamide (22). To a solution of **16** (592 mg, 2.01 mmol) in CH_2Cl_2 (10 mL) were added 2,2,2-trifluoroacetamide (445 mg, 4.02 mmol), magnesium oxide (324 mg, 8.04 mmol), $Rh_2(OAc)_4$ (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 **22** (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.



23

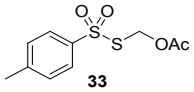
S-(Pyridin-2-yl) (2R,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-sulfonothioate (23). To a solution of the carboxylic acid **9** (2.00 g, 7.24 mmol) in CH_2Cl_2 (20 mL) were added Et_3N (1.51 mL, 10.9 mmol) and salt **36** (1.65 g, 8.69 mmol). The reaction mixture was stirred at room temperature for 1 hour in the dark (solution A). To another flask was added CH_2Cl_2 (20 mL) and cooled to -78 ℃. After liquid sulfur dioxide (20 mL, 7.24 mmol) was added, an inner temperature was raised to -15 ℃. Under white light irradiation using four LED lamps (A 160WE Tuna Blue, Kessil®), the solution A was slowly added by a syringe wrapped in aluminum foil while maintaining the inner temperature under -10 ℃. After the reaction mixture was stirred at 0 ℃ 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 **23** (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). $^{13}C\{^1H\}$ 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.



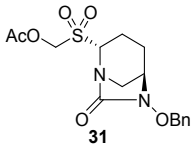
25b

(2S,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-sulfonamide (25b). To a solution of **23** (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 **25b** (2.00 mg, 6.5%) as a pale yellow oil. ^1H 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). $^{13}C\{^1H\}$ 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.



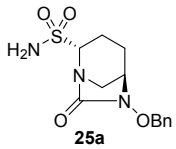
33

(Tosylthio)methyl acetate (33). 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 **33** (4.86 g, 85%) as a white solid. ^1H 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). $^{13}C\{^1H\}$ 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 C10H12O4S2Na 283.0069; Found 283.0066.



31

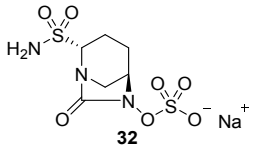
(((2R,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl)sulfonyl)methyl acetate (31). To a solution of carboxylic acid **9** (20.0 g, 72.4 mmol) and 2-mercaptopyridine 1-oxide **12** (9.20 g, 7.24 mmol) in CH_2Cl_2 (200 mL) was added EDC HCl (13.9 g, 7.24 mmol). The solution was stirred for 1 hours at room temperature in the dark (solution A). Under white light irradiation using four LED lamps (A 160WE Tuna Blue, Kessil®), solution A was slowly added to a solution of thiosulfonate **33** (56.5 g, 217 mmol) in CH_2Cl_2 (200 mL) at 0 ℃ over 20 minutes by a liquid feeding pump. After the reaction mixture was stirred at 0 ℃ 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 **30** in a mixture with thiosulfonate **33** (25.6 g, 49 wt% purity). The product was used without any further purification. To a solution of **30** (25.6 g, 49 wt% purity) in CH_2Cl_2 (256 mL) was added *m*-CPBA at 0 ℃. After stirred at room temperature for 2 hours, an aqueous solution of $Na_2S_2O_3$ and $NaHCO_3$ was added at 0 ℃ 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 **31** as a white amorphous (12.6 g, 47%). ^1H 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). $^{13}C\{^1H\}$ 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.



25a

(2R,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-sulfonamide (25a). **Method A (Scheme 4)**: To a solution of thiosulfonate **23** (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 **24** solution was equally divided into two flasks and one of the solution was used to the next step. To the aqueous solution of **24** (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 **25a** as a white amorphous (13.3 mg, 46%). **Method B (Scheme 6)**: To a solution of sulfone **31** (1.24 g, 3.37 mmol) in THF/H2O (v/v = 1/1, 25 mL) was added 1 N NaOH aq (6.74 mL, 6.74 mmol) at 0 ℃. After stirred at 0 ℃ 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 **25a** as a white amorphous (0.90 g, 86%). ^1H NMR (400 MHz, $CDCl_3$)

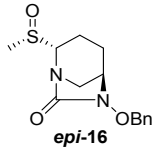
δ: 7.39-7.38 (5H, m), 5.00 (1H, d, *J* = 11.5 Hz), 4.96 (2H, s), 4.87 (1H, d, *J* = 11.5 Hz), 4.39 (1H, t, *J* = 8.2 Hz), 3.55 (1H, d, *J* = 12.0 Hz), 3.45-3.44 (1H, m), 3.07 (1H, d, *J* = 11.8 Hz), 2.32-2.07 (3H, m), 1.84-1.77 (1H, m). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ: 167.7, 135.3, 129.3, 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.



32

Sodium (2R,5R)-7-oxo-2-sulfamoyl-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (32). To a solution of **25a** (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*. 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 ℃ 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 **32** (133 mg, 77%) as a white amorphous after lyophilization. ^1H 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). $^{13}C\{^1H\}$ NMR (100 MHz, D_2O) δ: 168.7, 74.2, 59.6, 55.4, 43.4, 17.7. 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.

Large-scale synthesis of 16.[29] To a solution of carboxylic acid **9** (600 g, 2.17 mol) in CH_2Cl_2 (3.0 L) were added 2-mercaptopyridine 1-oxide **12** (290 mg, 2.28 mol) and EDC HCl (437 g, 2.28 mol) maintaining the inner temperature 10 ~ 20 ℃, 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 **35** (1.20 kg, 6.52 mol) in CH_2Cl_2 (1.2 L) was cooled to 0 ℃ under white light irradiation using ten 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 ℃. After stirred at 0 ℃ 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 hexane/EtOAc (v/v = 2/1, 3.0 L) was slowly added a hexane (2.0 L) solution of the crude product while keeping the inner temperature under 10 ℃. After stirred for 15 minutes, the mixture was filtered and the silica-gel was washed with hexane/EtOAc (v/v = 2/1, 3.0 L× 3). The solvent was removed under reduced pressure to afford a roughly purified sulfide **11b** (1.57 kg) containing PhSSMe **35**. To a solution of the roughly purified sulfide **11b** (1.57 kg) in CH_2Cl_2 (2.4 L) was slowly added a solution of *m*-CPBA (72 wt%, 448 g, 1.87 mol) in CH_2Cl_2 (4.8 L) over 1.5 hours while maintaining the inner temperature under -55 ℃. After stirred for 15 minutes, the inner temperature was raised to -30 ℃ 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 ℃ for 20 minutes before filtering, washing with EtOAc (480 mL × 2) and IPE (480 mL × 2), and drying to afford **16** (219 g, 34%, dr = 31/1) as a white crystalline solid.



***epi*-16**

(2R,5R)-6-(benzyloxy)-2-((S)-methylsulfinyl)-1,6-diazabicyclo[3.2.1]octan-7-one (epi-16). After recrystallization of **16**, the crude mixture of **16** and ***epi*-16** was obtained from the mother liquid. The crude was purified by preparative supercritical fluid chromatography (SFC) to afford ***epi*-16** as a white solid.[30] ^1H NMR (400 MHz, CDCl3) δ: 7.40-7.38 (5H, m), 5.02 (1H, d, *J* = 11.5 Hz), 4.88 (1H, d, *J* = 11.5 Hz), 3.75 (1H, t, *J* = 7.8 Hz), 3.70 (1H, d, *J* = 11.8 Hz), 3.43-3.43 (1H, br m), 3.12 (1H, d, *J* = 11.8 Hz), 2.71 (3H, s), 2.45-2.41 (1H, m), 2.22-2.13 (2H, m), 1.90-1.85 (1H, m). $^{13}C\{^1H\}$ NMR (100 MHz, CDCl3) δ: 169.0, 135.6, 129.2, 128.8, 128.6, 78.2, 71.3, 58.1, 45.3, 34.9, 19.4, 19.3. HRMS (ESI) m/z: $[M + H]^+$ Calcd for C14H19N2O3S 295.1111; Found 295.1108.

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

ASSOCIATED CONTENT

SUPPORTING INFORMATION

This material is available free of charge via the Internet at http://pubs.acs.org.
Experimental details and copies of NMR spectra of all new compounds (PDF)
X-ray crystallographic data of compound **16** (CIF)

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(16) 2D developed TLC showed that **11b** decomposed on silica-gel. A picture of TLC is in Supporting Information. See Figure S1.

(17) The minor isomer (***epi*-16**) was also isolated. The procedure for isolation is described in the Experimental Section.

(18) The detailed X-ray crystallographic data is presented in Supporting Information.

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(28) Typical set up for light-promoted reaction is described in Supporting Information. See Figure S2.
(29) Reaction set up for the large-scale synthesis is described in Supporting Information. See Figure S4.
(30) SFC condition and chromatogram are presented in Supporting Information.