Synthesis of (5*S*)-Tricyclic Penems as Novel and Potent Inhibitors of Bacterial Signal Peptidases

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Abstract: (5*S*)-Tricyclic penems were synthesized for the first time via intramolecular cyclization of penem epoxy amides catalyzed by a weak Lewis acid [$Mg(ClO_4)_2$]. Due to the high degree of ring strain in these molecules, mild reaction conditions were developed to successfully construct penem intermediates and the tricyclic penem final products. These tricyclic penems and other newly synthesized (5*S*)-penem esters and amides exhibited good-to-high potency when tested as inhibitors of bacterial signal peptidases.

Key words: tricyclic penems, β-lactams, cyclization, epoxidation, signal peptidase, inhibitors

Over recent years, drug researchers have increasingly sought to find new antibiotics that inhibit novel targets or possess novel mechanisms of antibacterial action.[1] Bacterial signal peptidase (SPase) as a protease involves in protein translocation through the cytoplasmic membrane in the final step of the bacterial protein secretion pathway.[2] In this process, an amino-terminal signal peptide is cleaved and the release of mature protein is facilitated into the outer medium or periplasm.[3,4] They are essential for cell viability,[5] and therefore represent a class of novel antibacterial targets, providing an alternative approach to conquer bacterial infections, including those caused by drug resistant pathogens. Recently, (5*S*)-penems **2**, which are stereochemical isomers and structural analogs of the known β-lactam antibiotics such as (5*R*)-penems **1**,[6] appeared in the literature as SPase inhibitors (Figure 1). In this context, we wish to report our endeavors toward the design and synthesis and biological evaluation of novel (5*S*)-tricyclic penems SPase inhibitors.

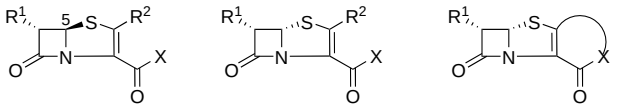
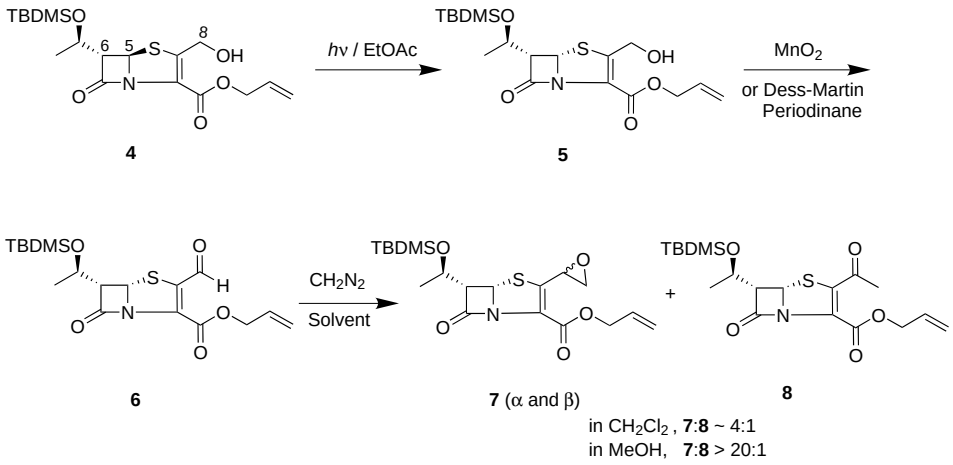


Figure 1 Structures of (5*S*)-Penems **1**–**3**

Our design strategy for the synthesis of tricyclic penems was based on the construction of an additional ring to an existing penem bicyclic ring system. Due to the sensitivity of the penem functionality toward nucleophiles, we sought to develop mild reaction conditions, so as to minimize the destruction of penem molecules during the synthesis. As shown in Scheme 1, the (5*S*)-penem **5** was readily derived from the corresponding (5*R*)-counterpart **4**[7] via photochemical isomerization.[3,8] The process typically yielded a mixture of nearly 3:2 ratio of 5*S*/5*R*-isomers in 1.5 hours, which were readily separated by flash chromatography.[9] Extended reaction time did not consid-



Scheme 1

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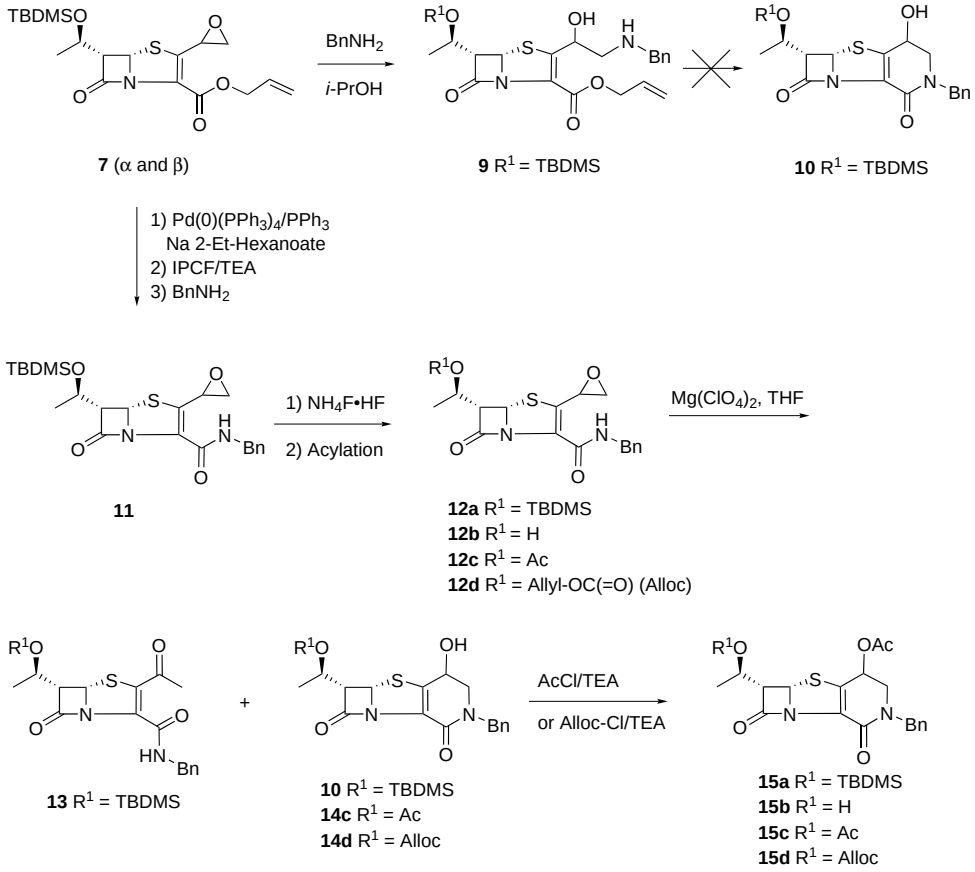
erably improve the isomerization ratio, but only resulted in a lower yield.[10] Oxidation of the (5*S*)-penem alcohol to aldehyde **6** by conventional conditions such as Swern and Jones oxidation methods were impractical, due to sensitivity of the β-lactam toward the reaction conditions. However, two mild methods using MnO_2[11] and Dess–Martin Periodinane[12] were applied to produce the aldehyde in >98% yield. Subsequent epoxidation of the aldehyde with diazomethane[13] in CH_2Cl_2 cleanly provided a mixture, identified as diastereomeric epoxide isomers **7α**, **7β**, and methyl ketone **8** in a ratio of 4:1 of **7**:**8**. The latter was likely derived from rearrangement of an initially formed diazonium species. The ratio of the designed epoxides and the unwanted ketone was increased to >20:1, when the epoxidation took place in MeOH. The epoxides **7α** and **7β** in ca.1:4 ratio were readily separated by flash chromatography, but the assignment of the relative stereochemistry by 2D NMR analyses remained inconclusive. Therefore, the mixed epoxides (obtained in 95% optimized yield) were utilized to carry out the synthesis of the tricyclic penems.

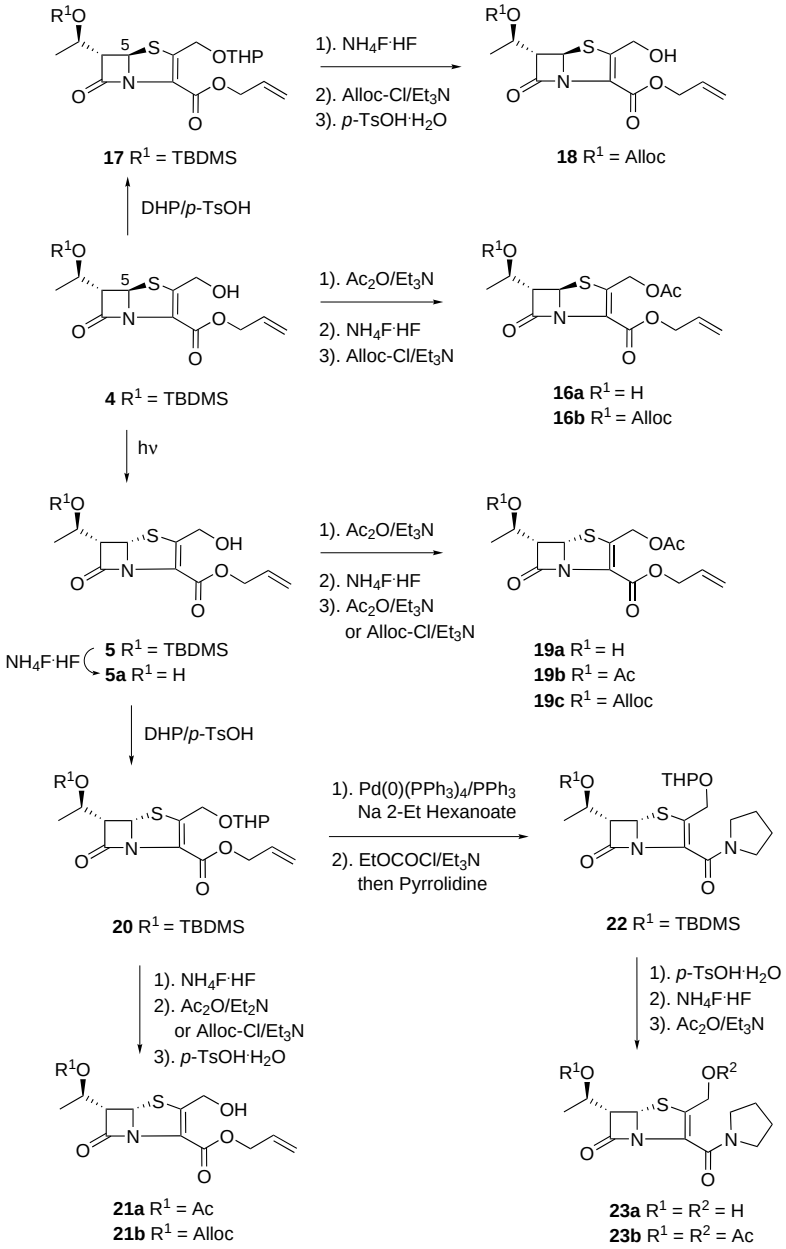
The robust preparation of the penem epoxy ester provided a viable building block for adding another ring to the existing penem skeleton. Our initial attempt to form an additional ring on the β-lactam scaffold was carried out by benzylamine nucleophilic addition to the epoxide **7** in hot *i*-PrOH to give the hydroxylamine intermediate **9** in 55% yield. However, the final cyclization failed to produce the desired tricyclic penem **10** under basic conditions including K_2CO_3 and NaH. Instead, only an unidentified mixture of degradation products was obtained. Alternatively, we decided to focus on an approach involving amide formation followed by ring construction. This structural elaboration included the conversion of the epoxy ester to the corresponding amide **11** in 75% yield through a one-pot three-step sequence: deallylation, mixed anhydride formation and subsequent amidation. Attempt to isolate the acid intermediate was unsuccessful, due largely to its apparent instability.[14] Finally, the lactam ring closure via with the epoxide ring-opening took place in refluxing THF in the presence of a weak Lewis acid, $Mg(ClO_4)_2$[15] to form tricyclic penem **10** in only 5% yield after purification. It was noticed that the complete consumption of the starting epoxide under the reaction conditions (in refluxing THF for 3 h) gave only low yield of the desired product. The poor chemical yield in the cyclization step was due partially to the formation of methyl ketone **13** (isolated and characterized), potentially from rearrangement of the initially formed tricyclic penem alcohol. The mechanism of this rearrangement is not completely understood, but we suspect that the free hydroxyl group generated in the cyclization might be involved in the rearrangement process. Therefore, the procedure was modified such that the cyclized product was isolated and immediately acylated to give the more stable acetate **15a** in 30% yield in a shorter reaction time (in refluxing THF for 1.5 h) with recovery of **12a**. Surprisingly, the removal of the silyl group



Scheme 2

in **15a** in the final step failed to give the expected product **15b** under the conditions of $NH_4F{\cdot}HF$, a very mild procedure used successfully in carbapenem[16] and penem[17] desilylation. This problem was circumvented by utilizing the acetylated precursor **12c** and **12d**, generated from desilylation of **11** followed by acylation, prior to the ring closure in the identical overall process. Thus, the final (5*S*)-tricyclic penem diacetates **15c** and **15d** (ca.1:4 ratio of diastereomers) were obtained using the aforementioned cyclization procedure in slightly improved chemical yield (38%).

We also prepared substituted (5*R*)-penem and (5*S*)-penem esters and amides as shown in Scheme 3. Again, we utilized conventional mild reaction conditions to embark on the functional group transformation to prevent degradation of the penem structure. The (5*R*)-penem alcohol **4** was converted to the corresponding alcohol **16a** and acetate **16b** via a three-step sequence: acylation, desilylation and then acylation. The (5*R*)-penem ester was protected with 3,4-dihydro-2*H*-pyran (DHP) to give intermediate **17**. The silyl protecting was removed and then acylated with Alloc-Cl. The deprotection of the THP group resulted in the (5*R)*-penem alcohol **18**. The similar procedure was used to prepare the corresponding alcohol **19a**, acetate **19b** and allyloxycarbonyl **19c** starting from the (5*S*)-penem alcohol **5**. In all cases, satisfactory chemical yields were achieved in a range of 62–75%. The DHP protected (5*S*)-penem ester **20** was converted to the amide **22** using the palladium(0) catalysis conditions as previously described followed by mixed anhydride coupling reaction. Subsequently, the silyl and THP protecting groups of the compound **22** were removed to give the diol **23a**, which was acylated to afford the amide **23b**. Alternatively, the



Scheme 3

(5*S*)-penem diol **5a** was obtained by deprotection of the silyl ether **5**, and the hydroxyl acylates **21a** and **21b** were prepared from **20** by sequential desilylation, acylation and then removal of the THP protecting group.[18]

Inhibition of SPase

A gene encoding SPase was isolated from methicillin-resistant *Staphylococcus aureus* (MRSA, strain MI339) using reported techniques.[19] The gene was cloned in a pET vector (Novagen) and overexpressed as a His-tagged protein in *Escherichia coli*. The SPase was isolated using a described protocol.[20] Determination of IC50 values for the test compounds against MRSA SPase and *E. coli*[21] was performed by a fluorescence polarization assay using a fluorescent preprotein as the substrate and FPM-2 (Jolley Inc.) as the reader. These two SPase enzymes were selected to evaluate new analogs as representatives of a broad spectrum profile for their in vitro activity.

The newly synthesized (5*R*)-, (5*S*)-penems and tricyclic penems were tested against the isolated *E. coli* and MRSA enzymes and exhibited interesting biological activity as shown in Table 1. The stereochemical effect at the C-5 position of the penems was evident in examples of (5*R*)-penem **16a** and (5*S*)-penem **19a**, the latter showed at least 4-fold more potent against MRSA. This effect was more pronounced when the counterparts were compared between the Alloc derivatives **18** and **19c**; greater than 33-fold potency discrepancy observed in favor of the (5*S*)-penem isomer. When both R^1 and R^2 substituents were kept as free hydroxyl groups, compound **5a** showed only moderate in vitro inhibitory activity against both *E. coli* and MRSA SPases. However, enhanced activity was seen in mono acetates **19b** and **21a** against *E. coli* with only weak activity against MRSA, and it was also true for the diacetate **21b**. One of the most intriguing finding in substitution variations was the incorporation of the Alloc group at the R^1 position in analog **19c** and the activity was significantly increased in both *E. coli* (IC50 at 0.5 μM) and MRSA (IC50 at ≤6 μM) assays. Our structure-activity learnings were further expanded in the case of (5*S*)-penem amides. The free hydroxyl amide **23a** displayed about 25-fold greater potency than the acetyl amide **23b** in *E. coli* assay, but possessed similarly low activity in the MRSA assay. When the amides were compared with the esters having the same variations, unsubstituted diol **23a** exhibited 47-fold increase in its potency against *E. coli* vs. its counterpart ester analog **5a**, although the activity against MRSA SPace was worsened by about 4-fold. In contrast, the diacetate amide **23b** was less active than the ester compound **18b**. Again, the Alloc substitution displayed the tendency of enhancing the in vitro activity in the case of epoxides, in which the Alloc analog **12d** was over 60-fold more active than the acetate **12c** against *E. coli* SPase and over 4-fold more active against MRSA. The same trend was sustained in the case of tricyclic penems, but with more pronounced potency. The Alloc substituted tricyclic penem **15d** exhibited IC50 at 0.2 μM and 5 μM against *E. coli* and MRSA SPases, respectively, the best compound evaluated in our isolated enzyme assays, whereas the acetate **15c** showed similar activity to that of the diacetate **21b**. Overall, the Alloc group presented a significant influential enhancement in the SPase inhibi-

Table 1 Biological and Stability Data of New (5*S*)-Penems and (5*S*)-Tricyclic Penems[a]

Product	Config. at C5	R^1	R^2	SPase IC50 (μM)		MIC MRSA[b]	Stability
				E. coli	MRSA	(μg/mL)	$T_{1/2}$ (h)
16a	*R*	H	Ac	n.d.	>200	n.d.	4.6
19b	*S*	H	Ac	12	50	>250	1.6
5a	*S*	H	H	47	50	90	n.d.
21a	*S*	Ac	H	3	50	90	n.d.
21b	*S*	Ac	Ac	6	100	>256	<1
19c	*S*	Alloc	H	0.5	≤6	120	n.d.
18	*R*	Alloc	H	n.d.	>200	n.d.	n.d.
23a	*S*	H	H	1	>200	>128	21
23b	*S*	Ac	Ac	25	>100	>128	n.d.
12c	*S*	Ac	–	38	>100	n.d.	n.d.
12d	*S*	Alloc	–	0.6	25	n.d.	n.d.
15c	*S*	Ac	–	12	>100	>128	0.5
15d	*S*	Alloc	–	0.2	5	>128	n.d.

[a] Ac = Acetyl, Alloc = Allylxoycarbonyl, n.d. = Not determined.
[b] MRSA = strain MI339.

tory assays, which was especially pronounced in the (5*S*)-tricyclic penem.

When the newly synthesized (5*S*)-penems and tricyclic penems were tested for MIC measurement in whole cell assay, only the free diol **5a**, the mono acetate **21a** and Alloc ester **19c** exhibited weak antibacterial activity against MRSA. We subsequently determined solution stability of these analogs at 37 °C in 50 mM pH 7.4 potassium phosphate buffer. The half-life for disappearance of the test compounds was measured by reverse phase HPLC. As shown in Table 1, most of the compounds displayed short half-life property, which may explain their poor antibacterial activity.

Figure 2 below shows the published X-ray crystallographic structure of a (5*S*)-penem inhibitor covalently bound in the active site of *E. coli* SPase,[22] where it was observed that the dihydrothiazole ring formed π-stacking with Tyr143 residue. We also examined our novel (5*S*)-tricyclic penem **15d** in the same crystal structure of *E. coli* SPase using a molecular modeling approach as shown in Figure 3. Interestingly, a similar binding pattern of the tricyclic penem with the SPase to that of the penem was predicted, in which the dihydrothiazolopyridone ring also exhibited π-stacking with Tyr143. In addition, the *N*-benzyl group was positioned along the peptide cleft, and the Alloc group was extended into the S3 pocket by π-stacking with Phe84. These additional binding features may contribute to the observed high in vitro potency of the (5*S*)-tricyclic penem in bacterial SPase.

In conclusion, (5*S*)-tricyclic penems containing a highly strained, fused 4-, 5- and 6-membered ring system were synthesized for the first time by intramolecular cyclization of penem epoxy-amides. The accomplishment of the synthesis was based on careful control of reaction conditions to preserve the sensitive 5S-penem functionality. The tricyclic penems were good inhibitors of bacterial SPases from *E. coli* and MRSA. Other (5*S*)-penems were also prepared and exhibited activity against the SPases. Molecular modeling of the (5*S*)-tricyclic penems in *E. coli* crystallographic structure was used to rationalize the high potency in SPase assay. However, the disappearance of antibacterial activity for MIC determination may be related to instability of this class of molecules.

All reagents were commercial grade and were used as received without further purification, unless otherwise specified. Commercially available anhydrous solvents were used for reactions conducted under inert atmosphere. Reagent grade solvents were used in all other cases, unless otherwise specified. Flash chromatography was performed on E. Merck silica gel 60 (230–400 mesh). TLC was performed on E. Merck 60 F-254 precoated silica plates (250 mm layer thickness). ^{1}H NMR spectra were recorded at 300 MHz. Chemical shifts for ^{1}H NMR spectra are expressed in parts per mil-

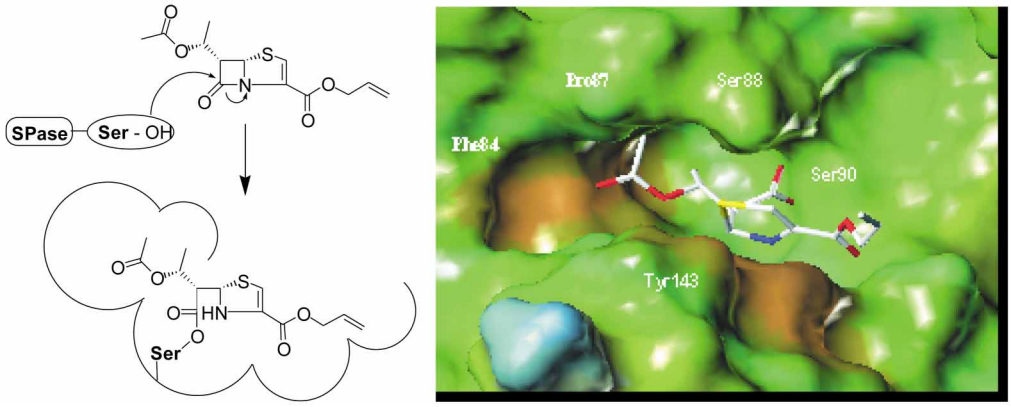


Figure 2 Crystal structure of a (5*S*)-penem in *E. coli* SPase

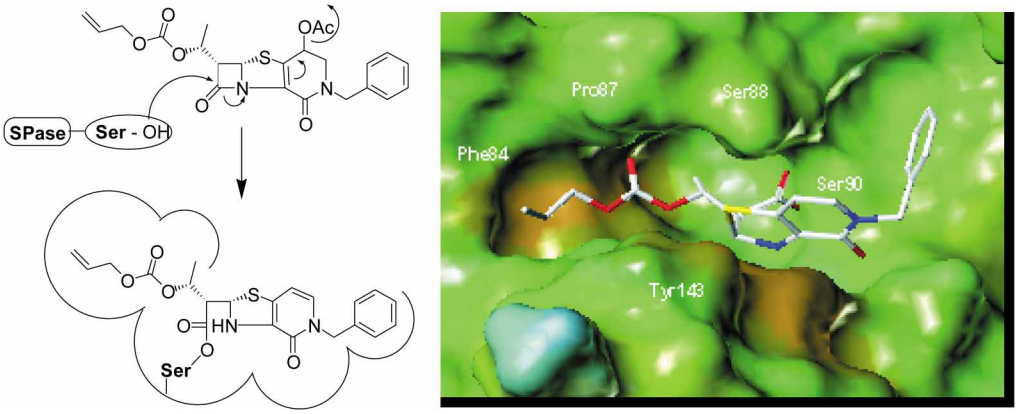


Figure 3 Molecular modeling of (5*S*)-tricyclic penem **15d** in *E. coli* SPase

lion downfield from tetramethylsilane. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet; br, broad. Coupling constants are given in Hertz (Hz). The ^{13}C NMR spectra were recorded at 75 MHz. Chemical shifts for ^{13}C NMR spectra are expressed in parts per million downfield from $CHCl_3$. Mass spectra were obtained by electrospray mass spectrometry utilizing an electrospray ionization (ESI) interface in flow injection analysis (FIA) mode equipped with an ESI interface. Melting points were taken on a capillary melting point apparatus. High resolution mass spectra were obtained by fast atom bombardment (FAB) with a *p*-nitrobenzyl alcohol matrix. IR spectra were recorded using attenuated total reflectance spectroscopy with a germanium internal reflection element. Optical rotations were measured using a 10 cm cell at 20 °C.

6-[1-*tert*-Butyldimethylsilyl)ethyl]-3-formyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Allyl Ester (6)

A suspension of penem alcohol **5** (1.96 g, 4.91 mmol) and MnO_2 (8.54 g, excess) in CH_2Cl_2 (6 mL) was stirred at r.t. for 1.5 h. The solid was removed by filtration and the filtrate was evaporated under reduced pressure to give a light yellow solid (1.95 g, ca. 100%). The crude product was pure by ^1H NMR analysis and was used for the next step without purification; mp 83–85 °C.

^1H NMR (300 MHz): δ = 10.42 (s, 1 H), 5.98 (m, 1 H), 5.71 (d, *J* = 4.8 Hz, 1 H), 5.47 (dd, *J* = 1.5, 17.1 Hz, 1 H), 5.38 (dd, *J* = 1.5, 10.8 Hz, 1 H), 4.81, (m, 2 H), 4.41 (m, 1 H), 3.94 (dd, *J* = 5.1, 12.3 Hz, 1 H), 1.42 (d, *J* = 6.6 Hz, 3 H), 0.85 (s, 9 H), 0.70 (s, 6 H).

ESI-MS: *m*/*z* = 398 [M + H^+], 198 [M + H^+ – (O=C=CHCH(Me)OTBDMS)].

6-[1-*tert*-Butyldimethylsilyloxy)ethyl]-3-oxiranyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2 carboxylic Acid Allyl Ester (7)

To a solution of the penem aldehyde **6** (1.95 g, 4.91 mmol) in MeOH (100 mL) was added CH_2N_2 in Et_2O dropwise at –30 °C. After the addition, the temperature was raised to r.t. in 30 min and the volatiles were evaporated under reduced pressure to afford a light yellow oil (2.018 g, ca. 100%). The crude product was pure by ^1H NMR analysis and was used for the next step without purification.

^1H NMR (300 MHz, $CDCl_3$): δ = 5.96 (m, 1 H), 5.62 (m, 1 H), 5.41 (dd, *J* = 17.1 Hz, 1 H), 5.22 (dd, *J* = 10.8 Hz, 1 H), 4.86, (m, 3 H), 4.61 (dd, *J* = 3.3, 5.7 Hz, 1 H), 4.40 (m, 1 H), 3.88 (dd, *J* = 3.3, 7.2 Hz, 1 H), 3.14 (dd, *J* = 6.0, 7.2 Hz, 1 H), 2.93 (dd, *J* = 3.3, 6.0 Hz, 1 H), 1.42 (d, *J* = 6.6 Hz, 3 H), 0.85 (s, 9 H), 0.70 (s, 6 H).

ESI-MS: *m*/*z* = 412 [M + H^+], 212 [M + H^+ – (O=C=CHCH(Me)OTBDMS)].

Acetic acid 1-(2-Benzylcarbamoyl-3-oxiranyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-en-6-yl)ethyl Ester (12c)

To a solution of the epoxy penem ester **7** (0.268 g, 0.790 mmol), sodium 3-ethylhexanoate (0.092 g, 0.790 mmol) and Ph_3P (0.062 g, 0.237 mmol) in anhyd THF (8 mL) was added $(Ph_3P)_4Pd$ at 0 °C. After stirring for 6 min, isopropyl chloroformate (IPCF, 0.95 mL, 0.948 mmol) was added followed by Et_3N at 0 °C. The stirring was continued for another 7 min at the same temperature and then $BnNH_2$ (0.121 mL, 1.185 mmol) was added. The resulting mixture was maintained at 0 °C for additional 5 min and then evaporated under reduced pressure. The residue was purified by flash chromatography using EtOAc–hexanes (1:4 → 1:2) to give the penem amide **11** as a foaming yellow solid (0.205 g, 67%). A mixture of this penem amide **11** (0.200 g, 0.435 mmol) and $NH_4F{\cdot}HF$ (0.248 g, 4.35 mmol) in anhyd DMF (20 mL) was heated at 60 °C for 1.5 h. After cooling, the mixture was partitioned between brine and EtOAc. The organic layer was separated and washed with brine (5 ×), dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography using EtOAc–hexanes (1:4 → 1:2) to give a yellow solid (0.146 g, 97%). The obtained hydroxyl penem **12b** (0.146 g, 0.422 mmol) was dissolved in anhyd CH_2Cl_2 (10 mL) and AcCl (0.045 mL, 0.633 mmol) was added followed by Et_3N (0.088 mL, 0.633 mmol) at r.t. The resulting solution was stirred for 30 min and directly purified by flash chromatography using EtOAc–hexanes (1:4 → 1:2); R_f 0.15 (EtOAc–hexanes, 1:2) to give **12c**.

^1H NMR (300 MHz, $CDCl_3$): δ = 7.25 (m, 5 H), 6.95 (br, 1 H), 5.60 (d, *J* = 4.2 Hz, 1 H), 5.28 (m, 1 H), 4.73 (dd, *J* = 2.4, 4.2 Hz, 1 H), 4.52 (m, 2 H), 3.95 (dd, *J* = 3.6, 9.9 Hz, 1 H), 3.13 (dd, *J* = 4.5, 5.4 Hz, 1 H), 2.90 (dd, *J* = 2.1, 5.4 Hz, 1 H), 2.03 (s, 3 H), 1.53 (d, *J* = 6.0 Hz, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.5, 163.3, 158.5, 139.8, 133.6, 130.8, 129.9, 128.8, 127.7, 70.6, 64.3, 62.9, 58.8, 40.0, 39.2, 22.8, 19.3.

HRMS: *m*/*z* calcd for $C_{19}H_{21}N_2O_5S$, 389.1171 (M + H^+);. found, 389.1205.

Acetic Acid 1-(6-Acetoxy-4-benzyl-2,3-dioxo-1,3,4,5,6,7α-hexahydro-2*H*-7-thia-2α,4-diazacyclobuta[α]inden-1-yl)ethyl Ester (15c)

A solution of the epoxypenem amide **12c** (62 mg, 0.160 mmol) and $Mg(ClO_4)_2$ in anhyd THF (5 mL) was refluxed for 2 h. The solvent was evaporated and the residue was washed with brine. The organic layer was dried ($MgSO_4$) and evaporated. The residue was quickly loaded to a silica gel column and flash chromatographed eluting with EtOAc–hexanes (1:4 → 1:1) to yield **14c** as a pale oil (33 mg, 53%); R_f 0.15 (EtOAc–hexanes, 1:1). The purified alcohol **14c** (20 mg, 0.052 mmol) was immediately dissolved in CH_2Cl_2 (2 mL) and AcCl (6 μL, 0.078 mmol) was added followed by Et_3N (10 μL, 0.078 mmol) at r.t. The resulting solution was stirred for 10 min and then directly purified by flash chromatography using EtOAc–hexanes (1:4 → 1:2) to yield **15c** as a pale oil (16 mg, 72%); R_f 0.70 (EtOAc–hexanes, 1:2); $[\alpha]_D^{20}$ –125.3 (*c* = 0.32, $CHCl_3$).

^1H NMR (300 MHz, $CDCl_3$): δ = 7.16–7.40 (m, 5 H), 5.56 (d, *J* = 4.5 Hz, 1 H), 5.53 (t, *J* = 2.1 Hz, 1 H), 5.31 (m 1 H), 4.76 (d, *J* = 15.3 Hz, 1 H), 4.61 (d, *J* = 15.3 Hz, 1 H), 4.42 (d, *J* = 2.7 Hz, 1 H), 4.11 (m, 1 H), 2.16 (s, 3 H), 2.04 (s, 3 H), 1.53 (d, *J* = 6.0 Hz, 3 H).

HRMS: *m*/*z* calcd for $C_{21}H_{23}N_2O_6S$, 431.1377 (M + H^+); found, 431.1385.

6-[1-*tert*-Butyldimethylsilyloxy)ethyl]-2-(pyrrolidine-1-carbonyl)-3-(tetrahydropyran-2-yloxymethyl)-4-thia-1-azabicyclo-[3.2.0]hept-2-en-7-one (22)

To a solution of (5*S*)-penem ester **20** (0.500 g, 1.04 mmol) in anhyd THF (10 mL) were added Ph_3P (82 mg, 0.312 mmol, 30%), $(PPh_3)_4Pd$ (0.120 g, 0.104 mmol, 10%) and sodium 2-ethylhexanoate (0.173 g, 1.04 mmol) at 0 °C. The resulting solution was stirred for 15 min at that temperature and TLC analysis showed no starting penem ester at this point. The mixture was evaporated under reduced pressure to about 2 mL of the volume and partitioned between phosphate buffer (pH 4, 50 mL) and EtOAc (50 mL). The organic layer was separated, dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow oil. The residue was dissolved in CH_2Cl_2 (20 mL) and to it was added EtOCOCl (0.11 mL, 1.14 mmol) followed by Et_3N (0.16 mL, 1.14 mmol). The resulting solution was stirred for 0.5 h at 0 °C. To the reaction mixture was then added pyrrolidine (96 mL, 1.14 mmol). The stirring was continued for another 20 min at 5–20 °C. The reaction mixture was directly loaded onto a column of silica gel and eluted with EtOAc–hexane (1:4 → 1:3) to afford a thick yellow oil (0.306 g, 59%); R_f 0.22 (EtOAc–hexanes, 1:2).

^1H NMR (300 MHz, $CDCl_3$): δ = 5.68 (d, *J* = 3.9 Hz, 1 H), 4.85 (t, *J* = 15.0 Hz, 1 H), 4.74–4.66 (m, 1 H), 4.61 (dd, *J* = 8.4, 15.0 Hz, 1 H), 4.39 (dq, *J* = 6.3, 9.9 Hz, 1 H), 3.91–3.45, (m, 7 H), 1.98–1.48

(m, 10 H), 1.40 (d, *J* = 6.3 Hz, 3 H), 0.87 (s, 9 H), 0.13 (s, 3 H), 0.11 (s, 3 H).

HRMS: *m*/*z* calcd for $C_{24}H_{40}N_2O_5SSi$, 497.2505 (M + H^+); found, 497.2438.

6-[1-*tert*-Butyldimethylsilyloxy)ethyl]-3-hydroxymethyl-2-(pyrrolidine-1-carbonyl)-4-thia-1-azabicyclo[3.2.0]hept-2-en-7-one

A solution of penem THP ether **22** (0.300 g, 0.605 mmol) and *p*-TsOH·H_2O (12 mg, 0.061 mmol) in MeOH (10 mL) was stirred at r.t. for 1.5 h. The solvent was evaporated to 1/3 of its volume under reduced pressure. The mixture was partitioned between CH_2Cl_2 (5 mL) and sat $NaHCO_3$ (5 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (5 mL). The combined extracts were dried ($MgSO_4$) and evaporated. The residue was purified by chromatography using EtOAc–hexane (1:4 → 1:1) to give 0.215 g (86%) of a yellow solid; R_f 0.33 (EtOAc–hexane, 1:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.57 (d, *J* = 4.2 Hz, 1 H), 5.34 (dd, *J* = 3.9, 9.6 Hz, 1 H), 4.40 (dd, *J* = 3.9, 14.7 Hz, 1 H), 4.32 (dq, *J* = 6.3, 10.5 Hz, 1 H), 4.12 (dd, *J* = 9.6, 14.7 Hz, 1 H), 3.70 (dd, *J* = 4.2, 9.9 Hz, 1 H), 3.57–3.34 (m, 4 H), 1.92–1.62 (m, 4 H), 1.28 (d, *J* = 6.3 Hz, 3 H), 0.75 (s, 9 H), 0.01 (s, 3 H), 0.00 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.7, 159.3, 151.9, 123.3, 67, 66.3, 65.2, 57.4, 47.1, 26.3, 25.6, 23.4, 22.2, 17.6, –3.6, –4.6;

HRMS: *m*/*z* calcd for $C_{19}H_{32}N_2O_4SSi$, 413.1930 (M + H^+); found, 413.1927.

6-(1-Hydroxylethyl)-3-hydroxymethyl-2-(pyrrolidine-1-carbonyl)-4-thia-1-azabicyclo[3.2.0]hept-2-en-7-one (23a)

A mixture of the above THP-deprotected penem silyl ether (70 mg, 0.170 mmol) and NH_4F·HF (97 mg, 1.70 mmol) in anhyd DMF (10 mL) was heated at 60 °C (oil bath temperature) for 2.5 h and cooled to r.t. The solid was removed by filtration and the filtrate was evaporated in high vacuum at 40 °C. The residue was triturated with hexane (10 mL) and the solvent layer was decanted. The solid was purified by chromatography using EtOAc–hexane (1:1 → 1:0) to yield a white solid (46 mg, 91%); R_f 0.15 (EtOAc); mp 104–106 °C; $[\alpha]_D^{20}$ –209.2 (*c* = 0.60, $CHCl_3$).

FT-IR (NaCl): 3364, 2971, 2928, 1773, 1603 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.73 (d, *J* = 4.2 Hz, 1 H), 5.53 (dd, *J* = 3.9, 9.3 Hz, 1 H), 4.54 (d, *J* = 14.1 Hz, 1 H), 4.42 (dq, *J* = 6.3, 10.2 Hz, 1 H), 4.22 (dd, *J* = 9.3, 14.1 Hz, 1 H), 3.83 (dd, *J* = 3.9, 10.2 Hz, 1 H), 3.70–3.48 (m, 4 H), 2.78–2.67 (br, 1 H), 2.06–1.76 (m, 4 H), 1.43 (d, *J* = 6.0 Hz, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.0, 159.8, 151.6, 123.8, 66.4, 66.2, 65.0, 57.7, 48.0, 47.6 (CH_2N), 26.7 (CH_2), 23.4 (CH_2), 22.5 (CH_3-9).

ESI-MS: *m*/*z* = 299 (M + H^+), 213 [M + H^+ – (O=C=CHCH(Me)OH)].

Anal. Calcd for $C_{13}H_{18}N_2O_4S$·0.25H_2O: C, 51.56; H, 6.16; N, 9.25. Found: C, 51.60; H, 5.81; N, 9.17.

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