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Carbapenem Antibiotics: Displacement of C-3 Sulfur Side Chain by Another Thiol Moiety, and Quaternization of a Weaker Pyridine Nitrogen over a Stronger Primary Amine (Preparation of BMY 40732 and BMY 40886)

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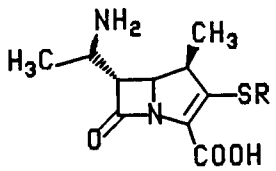
ABSTRACT

Displacement of sulfoxide moiety by a thiol group is shown to be useful in the preparation of 6-aminoethyl carbapenem derivatives. Also described is a simple strategy to mask a stronger basic primary amine as a nonbasic, nonnucleophilic imine in order to preferentially alkylate (quaternize) a weaker nitrogen of a pyridine in the same molecule. A discussion on the conformational aspects of the β-lactam-opened products is also presented.

INTRODUCTION

Imipenem, [1] a β-lactam antibiotic with a carbapenem ring structure is extremely active against both gram-positive and gram-negative aerobic and anaerobic species. However, its susceptibility toward mammalian renal dipeptidase, dehydropeptidase I (DHP-I), requires the coadministration of sodium cilastatin, an inhibitor of this enzyme to increase its efficacy against urinary tract infections. A great deal of attention has been focused on preparing compounds with the same basic skeleton but with different chemical entities around the ring in order to improve its stability against DHP-I and to achieve yet better antimicrobial activity. Our own efforts in this regard are focused on preparing compounds with the general structure **1** in which the C-6 substituent is a 1-aminoethyl moiety with both (*R*) and (*S*) configuration at C-8. The synthetic details of these compounds are described elsewhere [2].

The C-3 substituent has been generally introduced by the well-known C-3-0-phosphate or triflate displacement by a thiol group, and a number of thiol derivatives have been prepared by this technique. However, we were in search of a method in which a common intermediate may be used and thus were drawn to the "sulfoxide approach" [3]. We now describe the usefulness of the latter in the preparation of **7** requiring the C-3 sulfenyl derivative **2**, the C-6 substituent of which is the 1-azidoethyl group. At the outset it appears that the basic reaction conditions employed for the displacement of the sulfoxide moiety from **3** by a thiol may be incompatible with azido groups as they are well known [4] to be reduced to amines. Although the



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*To whom correspondence should be addressed.

Dedicated to Professor Ernest L. Eliel on the occasion of his seventieth birthday.

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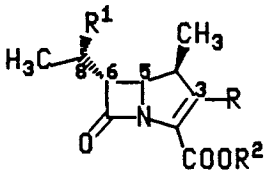
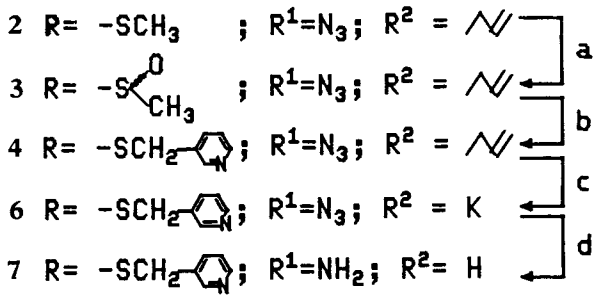
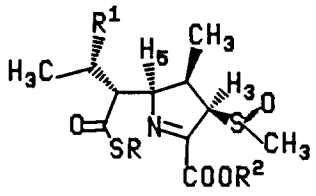


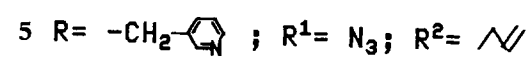


azido group is intended as a precursor for the amino group in **7**, its reduction at an early stage is undesirable and is incompatible with the rest of the sequence. Also described is a simple strategy to mask a stronger basic primary amine as a nonbasic, nonnucleophilic imine in order to preferentially alkylate (quaternize) a weaker nitrogen of a pyridine in the same molecule. A discussion on the conformational aspects of the β-lactam-opened products is also presented.

RESULTS AND DISCUSSION

The C-3 sulfenyl derivative (**2**) was oxidized with mCPBA to the *S*-oxide which was a better leaving group. The diastereomeric *S*-oxides (**3**) were treated with 3-picolylthiol in the presence of diisopropyl ethyl amine to give **4** along with the opened β-lactam **5**. It is noteworthy that the azide was resistant to reduction during this step as no amine was isolated. The formation of **5** may be attributed to a sluggish rate of displacement of the sulfoxide by the thiol and a comparable rate of its attack on the carbonyl group of the already strained β-lactam ring. The stereochemistry at C-3 in **5** is assigned on the basis of proton 2D-NMR and decoupling experiments. The H-3 proton exhibits long-range coupling with H-5 with a magnitude of 1.82 Hz that can only occur through a "W" path between them.

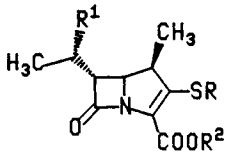


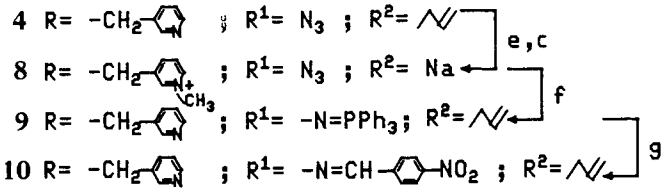


Since the stereochemistry of C-5 is predetermined, this establishes the relative configuration at C-3, as depicted in **5**. Following the palladium-catalyzed allyl deprotection of **4** and catalytic reduction of the azido group, the amine **7** was isolated.

Quaternization of the Pyridine Nitrogen of **7**

Quaternization of the pyridine nitrogen of **4** with methyltriflate and allyl deprotection gave **8**. Attempted catalytic reduction of the azido group in **8** resulted in extensive decomposition. This failure led us to develop a simple strategy by which preferential quaternization of the weaker pyridine nitrogen may be achieved over the stronger primary amine. This was accomplished by temporarily masking the primary amine as a less basic, less nuclophilic imine moiety, as compared to the pyridine nitrogen. Thus conversion of the azido group of **4** to the *p*-nitrobenzylideneimine (**10**) via the phosphinimine intermediate (**9**), treatment with methyl triflate followed by allyl deprotection, and finally pH adjustment to 6.0 gave **11** along with a minor amount of β-lactam-cleaved product **12**. Isolation of **12** from the reaction reaffirms the unstable nature of **11**. The amine derivative **7** was also obtained from the imine **10** by allyl deprotection followed by pH adjustment to 6.0.

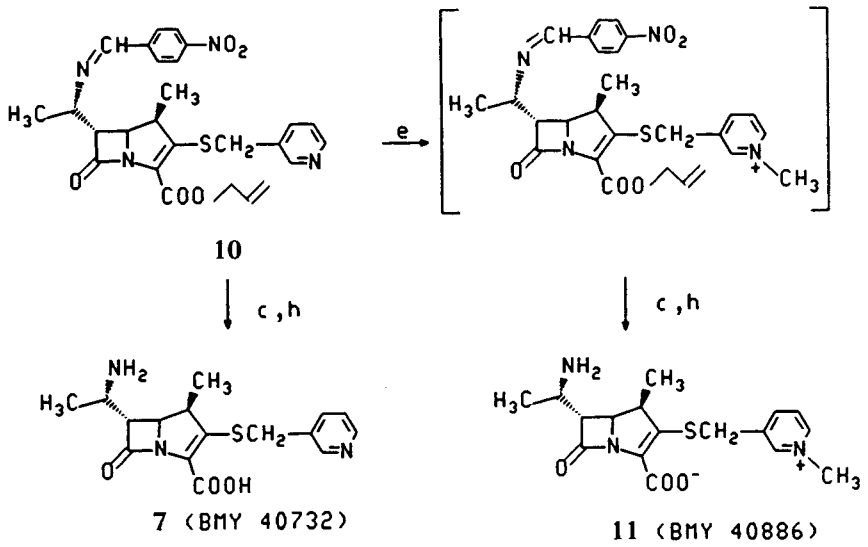




Conformational Analysis of **12**, **13**, **14**, *and* **15**

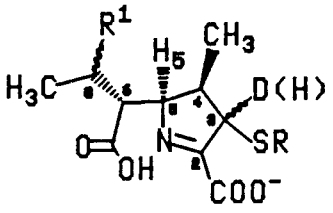
The irreversible opening of the β-lactam ring by transpeptidase enzymes is generally considered responsible for the bactericidal activity of β-lactam-containing compounds [5]. In this context it appeared relevant to study the conformational aspects of the product of alkaline hydrolysis of the β-lactam ring by NMR. The geometry of this product, in particular the relationship of N_1 to the carbonyl group, may have a bearing on the extent of difficulty of enzyme reactivation (i.e., the reverse of the β-lactam ring opening) and the sustained antibacterial activity of the parent compound, *in vitro* and/or *in vivo*.

The observed coupling between H-6 and H-8 in



Reagents and reaction conditions: a. mCPBA, CH_2Cl_2, $-70° \rightarrow -45°C$, 100%; b. 3-picolylthiol, CH_3CN, Hunig's base (DIPEA), DMAP (cat.) 0°C, 31%; c. Pd $[Ph_3P]_4$, Ph_3P, CH_3CN, 2-ethylhexanoate (EtOAc) or pyrrolidine, 0°C, 34.3%; d. 30% Pd/celite, H_2 (40 psi), 0°C, 24.8%; e. $CF_3SO_3CH_3$, CH_3CN, 0°C; f. Ph_3P, C_6H_6, 80°C; g. *p*-NO_2–C_6H_4–CHO, RT, 87.4%; h. 0.2 M pH 6.0 phosphate buffer, 0°C, 41.5%

12 was 3.12 Hz, suggesting a restricted rotation around the C_6–C_8 bond. On the other hand the coupling between H-6 and H-5 was 7.46 Hz, indicating free rotation around the C_5–C_6 bond. A conformational model in agreement with these data may be formulated in a hydrogen-bonded (H-bonded) structure, such as **16**, in which the dihedral angle between H-6 and H-8 is near 60°. This angle can easily be flipped into 120° by a mere twist around the C_8–N bond. However, it may be argued that the indicated coupling may arise from a slow rotation to give one conformer followed by a rapid rotation to give another short lived one and so on. Nevertheless, on the NMR time scale this angle must lie near 60° or 120° to give rise to a coupling of 3.12 Hz, and the choice for the 60° angle becomes evident later in the discussion.



12 R= $-CH_2$–(N-picolyl, $^+CH_3$) ; R^1= NH_2(S)

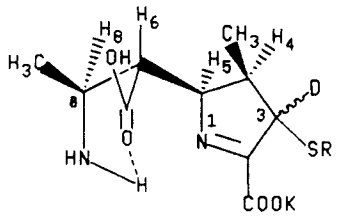
13 R= $-CH_2$–(N-picolyl, $^+CH_3$) ; R^1= OH(R)

14 R= $-CH_3$; R^1= NH_2(S)

15 R= $-CH_3$; R^1= NH_2(R)

The β-lactam-cleaved product **13** in which the C-8 amino group of **12** is replaced by a hydroxyl group of the opposite configuration showed a coupling of 8.71 Hz between H-6 and H-8 and 9.01 Hz between H-6 and H-5, indicating different rotamer populations around the C–C bonds bearing these protons. These differences may be attributed to the changes in their H-bonding characteristics, NH---O=C (**12**) versus OH---O=C (**13**). Incidentally, it may be pointed out that the H-3 proton in **13** exhibited a long-range coupling of 1.26 Hz with H-5 indicating a "W" arrangement between them, thus indirectly establishing the relative configuration at C-3.

The observed small coupling (3.12 Hz) between H-6 and H-8 in **12** appeared interesting enough to study by ^1H NMR a pair of β-lactam-opened diastereomers (**14** and **15**, epimeric at C-8) for their conformational behavior. The H-6 proton in **14** is a triplet, implying that it is coupled to H-8 and H-5; an equal magnitude of 3.83 Hz indicates its gauche arrangement (~60°) with them. The value of coupling between H-4 and H-5 is 10.79 Hz, which is in agreement with a dihedral angle of ~0° between them. This data is consistent with a restricted conformation possessing two 6-membered ring H-bonds



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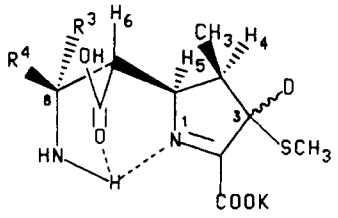
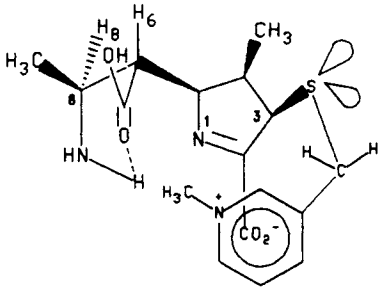


17 $R^3 = H_8$; $R^4 = CH_3$ (S)
18 $R^3 = CH_3$; $R^4 = H_8$ (R)



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that involve the C-8 amino group as the donor and the carbonyl oxygen of the C-6 carboxyl group and the N-1 nitrogen as acceptors for the same donor in a bifurcated fashion, as depicted in **17**. In this model the dihedral angle between H-4 and H-5 is near 0° and is consistent with the magnitude of coupling observed between them (10.79 Hz).

Breaking the H-bond involving the N-1 nitrogen allows free rotation around the C_5–C_6 bond while retaining the restriction around the C_6–C_8 bond. This can account for the small coupling (3.12 Hz) between H-6 and H-8 and a larger value (7.46 Hz) between H-5 and H-6 (**16**), and at the same time provides the basis for choosing the dihedral angle arrangement of 60° instead of 120° between H-6 and H-8 in **12**. It may be noted that **12** and **14** differ in their C-3 side chains: The former contains a quaternary pyridine ring that can interact with the C-2 carboxylate anion in a stacked fashion (**19**). A detailed discussion appeared in a previous publication [6].

Compound **15** showed similar behavior to **14**, except that the magnitudes of coupling of H-6 with H-5 and H-8 are slightly larger (4.84 Hz) and the value of coupling between H-4 and H-5 is slightly lower (9.32 Hz). In spite of these differences, the arguments advanced to arrive at a conformational model for **14** may still be considered true for **15** as well. The marginal coupling variations may be attributed to the distortions arising from the different orientations of the C-8 methyl group, which are either pseudoequatorial or pseudoaxial in their respective conformational models, and from minor contributions of other conformers.

EXPERIMENTAL

Solutions were evaporated below 30°C under diminished pressure. ^{1}H NMR spectra were recorded with a Bruker-AC200SY instrument operating at 200 MHz for protons. Chemical shifts are expressed in ppm relative to internal $CDCl_3$ (7.24 ppm) or HOD (4.81 ppm). IR spectra were recorded on a Perkin-Elmer 781 instrument. UV spectra were recorded on a Hewlett Packard 8451A diode array spectrometer. Solvents were reagent grade and used directly or dried over 4 Å molecular sieves wherever the use of dry solvents is indicated. Preparative μBondapak C_{18} reverse phase silica, 125 Å, 55–105 μ was obtained from Waters, Chromatography Division/Millipore Corporation.

Allyl (4R,5S,6S)-6-[1′(S)-Azidoethyl]-4-methyl-3-methylthiomonooxide-7-oxo-1-azabicylo(3.2.0)hept-2-ene-2-carboxylate (**3**)

To a solution of **2** (5 g, 15.55 mmol) in dry CH_2Cl_2 (200 mL) at −70°C was added *m*-chloroperbenzoic acid (3.52 g, 17.06 mmol) under argon. The milky mixture was stirred at −70°C for 1 h and at −45°C for an additional hour after which it was poured into cold 1 M $NaHSO_3$ (100 mL). The aqueous phase was extracted with CH_2Cl_2 (100 mL). The combined organic solvent was washed successively with cold 1 M $NaHCO_3$, brine, and dried over Na_2SO_4. Removal of the solvent in vacuo gave a syrup (5.59 g). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.84 (m), 2.90 (M) (CH_3 of sulfoxides, diastereomeric mixture, major (M): minor (m) ~55:45), 3.49 (m), 3.52 (M) (H-6, $J_{5,6}$ = 3.36 Hz, $J_{6,1'}$ = 5.17 Hz), 3.69 (m), 3.84 (M) (H-4, $J_{4,CH3}$ = 7.2 Hz), 3.97 (M), (m) (H-1′), 4.23 (m), 4.31 (M) (H-5, $J_{4,5}$ = 10.37 Hz).

Allyl (4R,5S,6S)-6-[1′(S)-Azidoethyl]-4-methyl-3-[(pyridin-3-yl)methylthio]-7-oxo-1-azabicylo(3.2.0)hept-2-ene-2-carboxylate (**4**)

A solution of **3** (2.5 g, 7.4 mmol) in dry CH_3CN (50 mL) was cooled to 0°C while bubbling argon through the solution. After 15 min 3-mercaptomethylpyridine (2.05 g, 16.25 mmol) in dry CH_3CN (5 mL) was added dropwise followed by *N,N*-diisopropylethylamine (2.83 mL, 16.25 mmol) and a trace of *N,N*-4-

dimethylaminopyridine. Argon bubbling was discontinued and the mixture was stirred at 0°C for 4 h under argon after which it was poured into saturated NH_4Cl (200 mL) and extracted with EtOAC (2 × 100 mL). The combined organic solvent was washed with brine and dried over Na_2SO_4, and the solvent was removed in vacuo to give a syrup thatwas flash chromatographed on silica (7.5 × 13 cm) packed in CH_2Cl_2. The product was eluted with a mixture of CH_2Cl_2 and CH_3CN (1:1, gradient elution). IR (nujol) 2105 (azide), 1770 cm^{-1} (C=O, β-lactam). ^1H NMR (200 MHz, $CDCl_3$) δ: 1.24 (d, 3 H, CH_3-4), 1.42 (d, 3 H, CH_3), 3.28 (m, 1 H, H-4, $J_{4,5}$ = 9.17 Hz, $J_{4,CH3}$ = 7.34 Hz), 3.36 (dd, 1 H, H-6, $J_{6,1'}$ = 5.3 Hz), 3.93 (m, 1 H, H-1', $J_{1',CH3}$ = 6.66 Hz), 4.00–4.08 (AB, 2 H, SCH_2, $J_{A,B}$ = 13.63 Hz), 4.02 (dd, 1 H, H-5, $J_{5,6}$ = 2.72 Hz), 4.65, 4.81 (m, 2 H, OCH_2, allyl), 5.23, 5.42 (m, 2 H, $=CH_2$, allyl), 5.94 (m, 1 H, CH=, allyl), 7.27 (dd, 1 H, H-5, py, $J_{5,6}$ = 4.83 Hz), 7.69 (d, 1 H, H-4, py, $J_{4,5}$ = 7.91 Hz), 8.53 (d, 1 H, H-6, py), 8.55 (s, 1 H, H-2, py).

Allyl (4R,5S,6S)-6-[1'(S)-p-Nitrobenzylideneaminoethyl]-4-methyl-3-[(pyridin-3-yl)methylthio]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate (**10**)

A mixture of **4** (0.75 g, 1.87 mmol) and triphenylphosphine (0.54 g, 2.06 mmol) in dry benzene (20 mL) was heated gradually, under argon, to 80°C and kept there for 3.5 h. The reaction mixture was cooled to room temperature, and the crude phosphinimine was treated with *p*-nitrobenzaldehyde (0.29 g, 1.89 mmol). The mixture was stirred at room temperature while monitoring the course of the reaction by ^1H NMR. After 20 h, the solvent was removed in vacuo and the resulting syrup was dried briefly in vacuum and then dissolved in CH_2Cl_2 (3 mL). The product was oiled out by gradual addition of petroleum ether (30–60°C). The solvent was decanted and the oil was dried in vacuum to give a foam (0.83 g, 87%). ^1H NMR (200 MHz, $CDCl_3$) δ: 1.28 (d, 3 H, CH_3-4), 1.4 (d, 3 H, CH_3), 3.32 (m, 1 H, H-4, $J_{4,5}$ = 9.32 Hz, $J_{4,CH3}$ = 7.27 Hz), 3.50 (dd, 1 H, H-6, $J_{6,1'}$ = 6.63 Hz), 3.87 (m, 1 H, H-1', $J_{1',CH3}$ = 6.44 Hz), 4.0, 4.09 (AB, 2 H, SCH_2, $J_{A,B}$ = 13.80 Hz), 4.05 (dd, 1 H, H-5, $H_{5,6}$ = 2.75 Hz), 4.69, 4.82 (m, 2 H, OCH_2, allyl), 5.25, 5.44 (m, 2 H, $=CH_2$, allyl), 5.97 (m, 1 H, CH=, allyl), 7.92–8.26 (A_2B_2, 4 H, Ph, $J_{A,B}$ = 8.82 Hz), 8.43 (s, 1 H, N=CH), 8.53 (dd, 1 H, H-6, py, J_{meta} = 1.62 Hz, J_{ortho} = 4.81 Hz), 8.57 (d, 1H, H-2, py, J_{meta} = 1.87 Hz).

(4R,5S,6R)-6-[1'(S)-Aminoethyl]-4-methyl-3-[(pyridin-3-yl)methylthio]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylic acid (**7**)

A solution of **10** (0.81 g, 1.6 mmol) in CH_3CN (10 mL) was cooled to 0°C under argon and treated successively with triphenylphosphine (0.06 g), tetrakis(triphenylphosphine)palladium(O) (0.15 g) in CH_2Cl_2 (5 mL) and potassium 2-ethyl hexanoate (0.32 g, 1.76 mmol) in EtOAC (5 mL). The mixture was stirred at 0°C for 1 h and then 0.2 M pH 6.0 phosphate buffer (50 mL) was added followed by CH_2Cl_2 (25 mL). The bilayer mixture was stirred vigorously at 0°C for 1 h. After phase separation, the organic phase was extracted with deionized distilled H_2O (15 mL). The combined aqueous phase was washed with CH_2Cl_2 (20 mL) and passed through a column (3.5 × 8.5 cm) of reverse-phase silica (μBondapak C_{18}). The title compound was eluted with a mixture of H_2O and CH_3CN (9:1, gradient elution) and was obtained as a white fluffy solid after lyophilization (0.22 g, 41.5%). Purity 98.99% by HPLC (UV detection at 304 nm) on μBondapak C_{18} column (4 mm × 30 cm), eluant: 15% CH_3CN in pH 7.4 phosphate buffer, flow rate: 1 mL/min, retention time: 4.46 min, UV (pH 7.4) 266 (5461), 304 (8011). IR (nujol) 1760 cm^{-1} (C=O, β-lactam). Chemical half-life (*t* 1/2) 37°C, pH 7.4 = 1.7 h, pH 2.0 = 31 min. ^1H NMR (200 MHz, D_2O) δ: 1.17 (d, 3 H, CH_3-4), 1.39 (d, 3 H, CH_3), 3.36 (m, 1 H, H-4, $J_{4,5}$ = 8.05 Hz, $J_{4,CH3}$ = 7.22 Hz), 3.58 (dd, 1 H, H-6, $J_{5,6}$ = 2.41 Hz, $J_{6,1'}$ = 7.6 Hz), 3.83 (m, 1 H, H-1', $J_{1',CH3}$ = 6.67 Hz), 4.04, 4.19 (AB, 2 H, SCH_2, $J_{A,B}$ = 14.07 Hz), 4.07 (dd, 1 H, H-5), 7.44 (dd, 1 H, H-5, py, $J_{4,5}$ = 7.93 Hz, $J_{5,6}$ = 3.85 Hz), 7.89 (d, 1 H, H-4, py), 8.43 (d, 1 H, H-6, py), 8.52 (d, 1 H, H-2, py, J_{meta} = 1.71 Hz).

(4R,5S,6R)-6-[1'(S)-Aminoethyl]-4-methyl-3-[1-methyl-pyridinium-3-yl)methylthio]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate (**11**)

A cold (0°C) solution of **10** (0.25 g, 0.49 mmol) in CH_3CN (8 mL), under argon, was treated with methyl trifluoromethanesulfonate (0.06 mL, 0.54 mmol). After stirring for 1 h, the mixture was treated successively with triphenylphosphine (0.02 g), tetrakis(triphenylphosphine)palladium(O) (0.05 g) in CH_2Cl_2 (2 mL), and potassium 2-ethylhexanoate (0.1 g, 0.54 mmol) in EtOAc (3 mL). The mixture was stirred for 1 h and then 0.2 M pH 6.0 phosphate buffer (20 mL) was added followed by CH_2Cl_2 (10 mL). The two-phase mixture was stirred vigorously for 20 min and then allowed to warm up to room temperature. After phase separation, the organic phase was extracted with deionized distilled H_2O (10 mL). The combined aqueous phase, after washing with CH_2Cl_2 (20 mL), was passed through a column (3.5 × 6 cm) of reverse-phase silica (μBondapak C_{18}). The title compound was eluted with a mixture of H_2O and CH_3CN (95:5, gradient elution) and was obtained as a white fluffy solid after freeze drying (0.04 g, 23.4%). Purity 97.4% by hplc (UV detection at 298 nm) on μBondapak C_{18} column (4 mm × 30 cm), eluant: 15% CH_3CN in pH 7.0 buffer, flow rate: 1 mL/min, retention time: 5.05 min, UV (pH 7.4) 268 (5003), 298 (4602) nm. IR (nujol) 1760 cm^{-1}

(C=O, β-lactam), chemical half-life (*t* 1/2) 37°C, pH 7.4 = 35.4 min. ^{1}H NMR (200 MHz, D_2O) δ: 1.19 (d, 3 H, CH_3-4), 1.34 (d, 3 H, CH_3), 3.3 (m, 1 H, H-4, $H_{4,5}$ = 9.23 Hz, $J_{4,CH3}$ = 7.25 Hz), 3.57 (dd, 1 H, H-6, $J_{6,1'}$ = 7.77 Hz), 3.75 (m, 1 H, H-1', $J_{1',CH3}$ = 6.59 Hz), 4.09 (dd, 1 H, H-5, $J_{5,6}$ = 2.68 Hz), 4.14, 4.28 (AB, 2 H, SCH_2, $J_{A,B}$ = 14.65 Hz), 4.34 (s, 3 H, NCH_3), 7.96 (dd, 1 H, H-5, py, $J_{5,6}$ = 6.1 Hz), 8.45 (d, 1 H, H-4, py, $J_{4,5}$ = 8.15 Hz), 8.65 (d, 1 H, H-6, py), 8.79 (s, 1 H, H-2, py).

Potassium (4R,5S,6S)-6-[1'(S)-Azidoethyl]-4-methyl-3-[(pyridin-3-yl)methylthio]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate (**6**)

A cold (0°C) solution of **4** (0.4 g, 0.99 mmol) in CH_3CN (10 mL), under argon, was treated successively with triphenylphosphine (0.05 g), tetrakis(triphenylphosphine)palladium(O) (0.1 g) in CH_2Cl_2 (4 mL), and potassium 2-ethylhexanoate (0.2 g, 1.09 mmol) in EtOAC (6 mL). The mixture was stirred for 1 h, diluted with Et_2O and extracted with deionized distilled H_2O (2 × 25 mL). The combined aqueous phase was passed through a column (3.5 × 11 cm) of reverse-phase silica (μBondapak C_{18}). The title compound was eluted with a mixture of H_2O and CH_3CN (85:15, gradient elution) and was obtained as a white fluffy solid after freeze drying (0.136 g, 34.26%). Purity 99.5% by hplc (UV detection at 304 nm) on μBondapak C_{18} column (4 mm × 30 cm) eluent: 15% CH_3CN in pH 7.4 buffer, flow rate: 1 mL/min, retention time: 7.4 min, UV (H_2O) 268, 304 nm. IR (nujol) 1750 (C=O, β-lactam), 2100 cm^{-1} (azide). ^{1}H NMR (200 MHz, D_2O) δ: 1.13 (d, 3 H, CH_3-4), 1.37 (d, 3 H, CH_3), 3.31 (m, 1 H, H-4, $H_{4,5}$ = 8.73 Hz, $J_{4,CH3}$ = 7.21 Hz), 3.48 (dd, 1 H, H-6, $J_{5,6}$ = 2.33 Hz, $J_{6,1'}$ = 5.83 Hz), 3.96 (dd, 1 H, H-5), 4.01, 4.14 (AB, 2 H, SCH_2, $J_{A,B}$ = 13.98 Hz), 7.43 (dd, 1 H, H-5, py, $J_{5,6}$ = 5.02 Hz), 7.88 (d, 1 H, H-4, py, $J_{4,5}$ = 7.96 Hz), 8.41 (d, 1 H, H-6, py), 8.5 (s, 1 H, H-2, py).

(4R,5S,6S)-6-[1'(S)-Azidoethyl]-4-methyl-3-[1-methylpyridinium-3-yl)methylthio]-7-oxo-1-azabicylo(3.2.0)hept-2-ene-2-carboxylate (**8**)

A cold (0°C) solution of **4** (0.496 g, 1.24 mmol) in CH_3CN (10 mL), under argon, was treated with methyl trifluoromethanesulfonate (0.15 mL, 1.3 mmol). After stirring the mixture for 1.5 h, it was treated successively with triphenylphosphine (0.05 g), tetrakis (triphenylphosphine)palladium(O) (0.1 g) in CH_2Cl_2 (5 mL), and pyrrolidine (0.114 mL, 1.36 mmol). The mixture was stirred for 1 h, ether (50 mL) was added and was extracted with deionized distilled H_2O (2 × 30 mL). The combined aqueous phase, after washing with ether, was passed through a column (3.5 × 11 cm) of reverse-phase silica (μBondapak C_{18}). The title compound was eluted with a mixture of H_2O and CH_3CN (95:5, gradient elution) and was obtained as a yellowish fluffy solid after freeze drying (0.17 g, 37.1%). Purity 99.3% (UV detection at 298 nm) on μBondapak C_{18} column (4 mm × 30 cm), eluant: 20% CH_3CN in pH 7.4 buffer, flow rate: 1 mL/min, retention time: 5.58 min, UV (H_2O) 268, 298 nm. IR (nujol) 1750 (C=O, β-lactam), 2100 cm^{-1} (azide). ^{1}H NMR (200 MHz, D_2O) δ: 1.19 (d, 3 H, CH_3-4), 1.39 (d, 3 H, CH_3), 3.30 (m, 1 H, H-4, $J_{4,5}$ = 8.18 Hz, $J_{4,CH3}$ = 7.2 Hz), 3.57 (dd, 1 H, H-6, $H_{6,1'}$ = 5.69 Hz), 4.03 (m, 1 H, H-1', $J_{1',CH3}$ = 6.68 Hz), 4.04 (dd, 1 H, H-5, $J_{5,6}$ = 2.51 Hz), 4.14, 4.29 (AB, 2 H, SCH_2, $J_{A,B}$ = 14.77 Hz), 4.37 (s, 3 H, NCH_3), 7.99 (dd, 1 H, H-5, py, $J_{5,6}$ = 6.09 Hz), 8.47 (d, 1 H, H-4, py, $J_{4,5}$ = 8.17 Hz), 8.68 (d, 1 H, H-6, py), 8.81 (s, 1 H, H-2, py).

Preparation of **7** *from* **6**

Compound **6** (0.12 g) was hydrogenated in 0.1 M pH 7.0 phosphate buffer (10 mL) at 40 psi on 30% palladium on celite (0.9 g) for 1 h. The catalyst was filtered and washed with H_2O, and the combined filtrate was passed through a column (3.5 × 11 cm) of reverse-phase silica (μBondapak C_{18}). The title compound was eluted with a mixture of H_2O and CH_3CN (90:10, gradient elution) and was obtained as a white fluffy solid (0.025 g, 24.7%). Purity 98.99% (UV detection at 304 nm) on μBondapak C_{18} column (4 mm × 30 cm), eluant: 15% CH_3CN in pH 7.4 buffer, flow rate: 1 mL/min, retention time: 4.46 min. UV, IR, and ^{1}H NMR data were identical to those described above for this compound, obtained by a different sequence of reactions.

^{1}H NMR data for **12** *(200 MHz, D_2O)* δ

0.99 (d, 3 H, CH_3-4), 1.38 (d, 3 H, CH_3), 2.55 (quintet, 1 H, H-4), 2.7 (dd, 1 H, H-6, $J_{5,6}$ = 7.46 Hz, $J_{6,1'}$ = 3.12 Hz), 3.66 (dq, 1 H, H-1'), 4.02, 4.11 (AB, 2 H, SCH_2, $J_{A,B}$ = 14.75 Hz), 4.37 (s, 3 H, NCH_3), 7.97 (dd, 1 H, H-5, py), 8.52 (d, 1 H, H-4, py, $J_{4,5}$ = 8.16 Hz), 8.67 (d, 1 H, H-6, py, $J_{5,6}$ = 6.12 Hz), 8.81 (s, 1 H, H-2, py).

^{1}H NMR data for **13** *(200 MHz, D_2O)* δ

0.92 (d, 3 H, CH_3-4), 1.23 (d, 3 H, CH_3), 2.47 (m, 1 H, H-4, $J_{4,5}$ = 6.22 Hz, $J_{3,4}$ = 2.87 Hz, $J_{4,CH3}$ = 7.18 Hz), 2.56 (t, 1 H, H-6, $J_{5,6}$ = $J_{6,1'}$ = 8.97 Hz), 3.66 (dd, 1 H, H-3, $J_{3,5}$ = 1.26 Hz), 3.99 (quintet, 1 H, H-1', $J_{CH3,1'}$ = 6.28 Hz), 4.07 (s, 2 H, SCH_2), 4.38 (s, 3 H, NCH_3), 7.98 (dd, 1 H, H-5, py), 8.54 (d, 1 H, H-4, py, $J_{4,5}$ = 8.22 Hz), 8.67 (d, 1 H, H-6, py, $J_{5,6}$ = 6.17 Hz), 8.83 (s, 1 H, H-2, py).

^{1}H NMR data for **14** *(200 MHz, D_2O)* δ

1.22 (d, 3 H, CH_3-4), 1.24 (d, 3 H, CH_3), 2.22 (s, 3 H, SCH_3), 2.8 (t, 1 H, $J_{5,6}$ = $J_{6,1'}$ = 3.83 Hz), 3.32 (dq, 1 H, H-4, $J_{4,5}$ = 10.79 Hz), 3.85 (dq, 1 H, H-1'), 4.44 (dd, 1 H, H-5).

^1H *NMR data for* **15** *(200 MHz, D_2O)* δ

1.18 (d, 3 H, CH_3-4), 1.28 (d, 3 H, CH_3), 2.24 (s, 3 H, SCH_3), 2.8 (t, 1 H, $J_{5,6} = J_{6,1'} = 4.84$ Hz), 3.2 (dq, 1 H, H-4, $J_{4,5} = 9.32$ Hz), 3.72 (dq, 1 H, H-1'), 4.42 (dd, 1 H, H-5).

^1H *NMR data for* **5** *(200 MHz, $CDCl_3$)* δ

1.07 (d, 3 H, CH_3-4, $J_{4,CH_3} = 7.33$ Hz), 1.46 (d, 3 H, $J = 6.75$ Hz), 2.69 (s, 3 H, S (O) CH_3), 2.96 (dd, 1 H, H-6, $J = 5.65$ Hz, $J = 9.51$ Hz), 3.31 (quintet, 1 H, H-4, $J = 1.65$ Hz, $J = 7.55$ Hz), 3.78 (t, 1 H, H-3, $J_{3,4} = J_{3,5} = 1.82$ Hz), 3.88 (quintet, 1 H, H-1', $J = 6.27$ Hz), 4.11, 4.27 (AB, 2 H, SCH_2, $J_{gem} = 14.4$ Hz), 4.74 (m), 7.23 (dd, 1 H, H-5, py, $J_{4,5} = 7.07$, $J_{5,6} = 4.15$ Hz), 7.7 (m, 1 H, H-4, py), 8.48 (d, 1 H, H-6, py), 8.55 (s, 1 H, H-2, py).

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