SYNTHESIS AND *IN VITRO* ACTIVITY OF C-2 QUATERNARY HETEROCYCLIC ALKYLTHIO CARBAPENEMS

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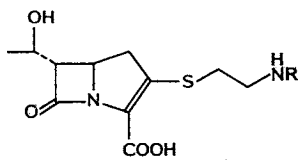
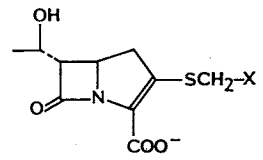
The synthesis of new carbapenems having various (substituted) quaternary heterocyclic alkylthio groups at the C-2 position is described. The *in vitro* antibacterial activity and the dehydropeptidase-I susceptibility were examined. Some of these compounds (*e.g.*, **11**, **16**, **26** and **27**) showed an excellent wide spectrum of *in vitro* antibacterial activity including activity against *Pseudomonas aeruginosa* and greater stability than imipenem toward the dehydropeptidase-I.

The discovery of the potent broad spectrum antibiotic, thienamycin and its relatives have stimulated considerable interest in the synthesis of carbapenem analogs.[1,2] Extensive structural modification studies of thienamycin resulted in the discovery of *N*-formimidoyl thienamycin (MK-0787, imipenem)[3] which possesses much improved chemical stability and more potent antibacterial activities than thienamycin. However, the great susceptibility of imipenem toward dehydropeptidase-I (DHP-I) results in extensive renal metabolism in man which leads to very low urinary recovery.[4] To prevent this metabolic degradation during therapy, cilastatin (MK-0791)[5,6], a substituted aminopropionate inhibitor of DHP-I, was developed to be co-administered with imipenem. In search of carbapenem antibiotics with the broad antibacterial spectrum of imipenem and with greater stability toward DHP-I, we undertook a chemical modification program of the carbapenem molecule, focusing our attention on the C-2 side chain.

Chemical modification of the amide side chain at the C-7 position of cephalosporins and introduction of quaternary heterocyclic methyl groups at the C-3 position had led to a number of clinically useful new class of cephalosporins, *e.g.* cefsulodin,[7] ceftazidime.[8] The conceptual operation of incorporating the quaternary heterocyclic side chains of cephalosporins into the C-2 position of the carbapenem molecules as quaternary heterocyclic alkylthio groups gave rise to a novel class of carbapenem antibiotics **3**. This new class of carbapenems retained the broad antibacterial activity of imipenem including the excellent anti-pseudomonal activity, and also showed an improved stability toward hydrolysis by DHP-I.



Thienamycin (**1**) R = H
Imipenem (**2**) R = CH=NH

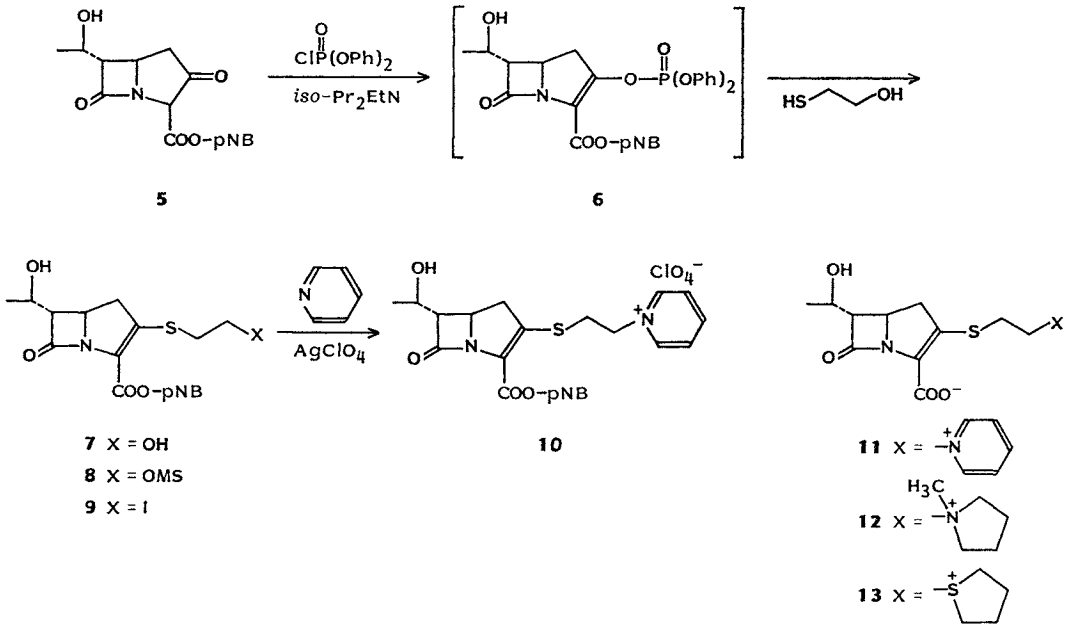


3 X = Quaternary amines, quaternary heterocycles, sulfonium

Chemistry

The bicyclic keto compound **5**, first synthesized by the Merck group,[9] was used as a common intermediate for the synthesis of new carbapenems. As shown in Scheme 1, treatment of **5** with diphenylphosphoryl chloride in the presence of diisopropylethylamine generated *in situ* the phosphonate **6**,[9] which was further converted into the crystalline intermediate **7** in 74% overall yield by addition of 2-hydroxyethylmercaptan. Conversion of **7** into the mesylate **8** followed by treatment with sodium iodide yielded the iodide **9** in high yeild. Although direct displacement of the iodide **9** with pyridine

Scheme 1.



pNB=*p*-Nitrobenzyl
MS=Mesyl

Scheme 2.

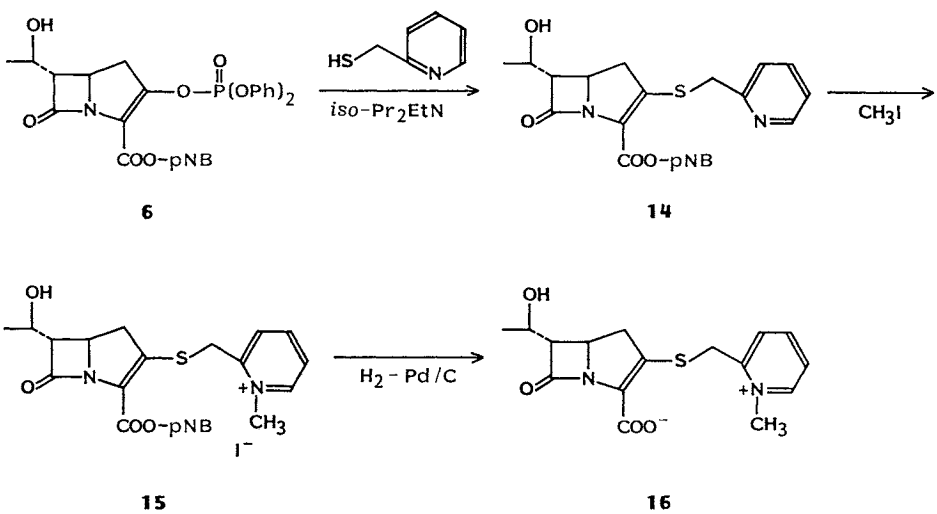
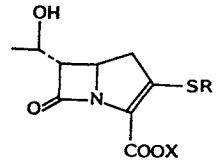


Table 1. *In vitro* activity.



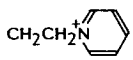
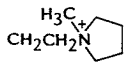
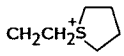
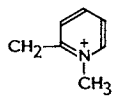
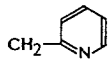
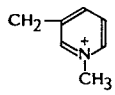
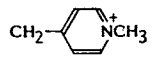
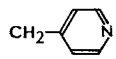
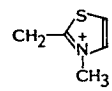
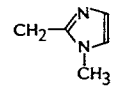
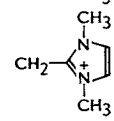
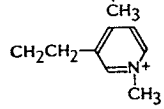
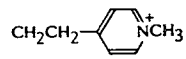
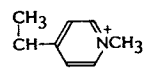
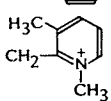
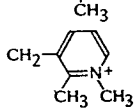
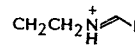
Compound	R	X	Antibacterial activity[a] (μg/ml)						Renal dipeptidase hydrolysis[b] (relative rate)
			S.a.	*E.c.*	*E.cl.*	*S.m.*	*P.v.*	*P.a.*	
11		—	0.016	0.03	0.25	0.06	0.03	0.13	0.37
12		—	0.016	0.016	0.13	0.06	0.016	0.25	—
13		—	0.016	0.003	0.25	0.06	0.03	0.25	—
16		—	0.016	0.016	0.03	0.03	0.016	0.13	0.17
17		Na	0.03	0.03	0.5	0.13	0.03	32	18.6
18		—	0.016	0.03	0.13	0.016	0.015	0.5	—
19		—	0.016	0.03	0.13	0.13	0.03	0.5	0.94
20		Na	0.16	0.03	0.13	0.13	0.25	32	3.84
21		—	0.03	0.016	0.13	0.13	0.13	0.25	—
22		Na	0.06	0.03	0.13	0.13	0.25	1	—
23		—	0.008	0.008	0.25	0.03	0.03	0.13	—
24		—	0.008	0.016	0.06	0.03	0.016	2	—
25		—	0.008	0.016	0.13	0.06	0.13	32	—
26		—	0.03	0.016	0.13	0.06	0.03	2	0.18
27		—	0.016	0.004	0.016	0.008	0.008	0.25	0.28
28		—	0.016	0.008	0.06	0.03	0.03	0.5	—

Table 1. (Continued)

Compound	R	X	Antibacterial activity[a] (μg/ml)						Renal dipeptidase hydrolysis[b] (relative rate)
			S.a.	*E.c.*	*E.cl.*	*S.m.*	*P.v.*	*P.a.*	
Imipenem		—	0.008	0.016	0.06	0.03	0.03	0.25	1.00

[a] Determined by serial 2-fold dilution of compound in Mueller-Hinton agar and inoculation of the agar surface or broth with an appropriately diluted 18~24 hours broth culture. Agar plates and tubes of broth were incubated at 37°C for 17 hours, and the lowest concentration causing inhibition of visible growth was considered to be the MIC.

[b] Pure hog renal dipeptidase was prepared as described.[11] Solutions of carbapenem (0.10 mM) in buffer (50 mM MOPS, pH 7.1) were freshly prepared. The UV/VIS spectrum of a 2.5-ml aliquot was measured and then 0.025 ml 1 M NH_2OH was added to degrade the β-lactam bond. The spectrum was again measured after 30 minutes at 25°C and again at 5~10 minutes intervals until no further decrease in absorbance was observed. The different spectrum between intact and degraded β-lactam was used to calculate a λ_{max} and ε. A similar aliquot of carbapenem solution was incubated at 25°C and the rate of change in absorbance at λ_{max} was determined. Enzyme was then added to give a rate of at least 10^{-4} absorbance units per second. The rate of enzymatic hydrolysis was corrected for spontaneous hydrolysis and then converted using ε to nmol/minute/ml of enzyme. All rates are reported relative to that observed with imipenem.

S.a.: *Staphylococcus aureus*, pen-res A-9606, *E.c.*: *Escherichia coli* A-15119, *E.cl.*: *Enterobacter cloacae* A-9695, *S.m.*: *Serratia marcescens* A-20019, *P.v.*: *Proteus vulgaris* A-21559, *P.a.*: *Pseudomonas aeruginosa*.

failed under a variety of reaction conditions, the quaternized intermediate **10** was obtained in high yield by addition of one equivalent of $AgClO_4$. Catalytic hydrogenation of **10** over Pd/C and purification of the crude product by a medium pressure C_{18} μBondapak column (Waters Associates) gave the zwitterionic compound **11** as a white amorphous solid. Similarly, compounds **12** and **13** were prepared by the reaction sequence described above. Alternatively, **11** could be obtained in one pot reaction by treatment of **7** with $(CF_3SO_2)_2O$ - pyridine at −20°C. Many other carbapenem analogs possessing the C-2 quaternized heterocyclic alkylthio groups were prepared according to the Scheme 2 as exemplified by the synthesis of **16**. Treatment of the phosphate **6** with 2-mercaptomethyl pyridine gave the carbapenem **14** which was then quaternized with methyl iodide or methyl trifluoromethanesulfonate to produce the quaternized intermediate **15** in high yield. Similar treatment of **6** with other heterocyclic alkylthiols followed by quaternization and hydrogenolysis produced carbapenems possessing the C-2 quaternized heterocyclic alkylthio groups (Table 1).

In Vitro Antibacterial Activity and DHP-I Stability

The MIC of these new carbapenems are shown in Table 1. The compounds tested were highly active against a wide range of Gram-positive and Gram-negative organisms with MIC values comparable to imipenem. One general trend in this series of compounds is quite clear: The positive charge in the quaternized heterocyclic ring imparts better anti-pseudomonal activity to the compounds when compared to their unquaternized partners. For example, compounds **17** and **20** which possess no positive charge on the C-2 side chain did not show any significant activity against *Pseudomonas aeruginosa* strains whereas their positively charged partners **16** and **19** did. The excellent antibacterial activity of the sulfonium derivative **13** indicates that effect of the sulfonium positive charge is equivalent to the quaternized nitrogen. Interestingly, the *N*-methyl imidazole derivative **22** which contains a

more basic nitrogen than the pyridine derivatives showed good anti-pseudomonal activity, suggesting that a more basic functionality on the C-2 side chain provides better anti-pseudomonal activity. In comparison, the activity of compounds **18** and **19** with **24** and **25** seems to indicate that the position of the positive charge plays the important role for the anti-pseudomonal activity. In contrast, an additional methyl group at various positions on the pyridine ring (compounds **27** and **28**) did not show much effect. On the other hand, an additional methyl group at the methylene of the C-2 side chain (compound **26**) resulted in the significantly reduced anti-pseudomonal activity, thus implying the importance of the steric bulkiness surrounding the C-2 side chain. The quaternized thiazole **21** and imidazole **23** also showed an excellent overall MIC.

Some of the compounds in this study were also examined for their stability toward DHP-I (Table 1) and their rate of degradation was measured relative to the rate of imipenem. A comparison of compounds **16** with **17** and **19** with **20** clearly indicates that the quaternization of the pyridine ring leads to significantly reduced DHP-I susceptibility. Additional methyl groups at the methylene of the C-2 side chain (**26** vs. **19**) also seems to lead to more DHP-I stable compounds.†

Experimental

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The UV spectra were run in EtOH; IR spectra were recorded on a Beckman 5240 spectrophotometer using KBr pellets; NMR spectra were obtained on a Varian HA-100 spectrometer using $(CH_3)_4Si$ as an internal standard. All solid compounds were characterized by UV, IR, NMR and elemental analysis (C, H, N). Unless stated otherwise, these analyses were within $\pm 0.4\%$ of the theoretical value.

p-Nitrobenzyl-3-(2-hydroxyethylthio)-6α-[1-(*R*)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate (**7**)

A solution of 1.69 g (4.85 mmol) of *p*-nitrobenzyl-6α-[1-(*R*)-hydroxyethyl]-3,7-dioxo-1-azabicyclo-(3.2.0)hept-2-ene-2-carboxylate (**5**) in 20 ml of acetonitrile was cooled at 0°C under a nitrogen atmosphere. A solution of 726 mg (7.18 mmol) of diisopropylethylamine in 2 ml of acetonitrile was added followed by a dropwise addition of 1.51 g (5.60 mmol) of diphenylchlorophosphate in 12 ml of acetonitrile over a period of 3 minutes. The resulting solution was stirred at 0°C for 20 minutes to provide *p*-nitrobenzyl-3-(diphenylphosphoryloxy)-6α-(1-(*R*)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate (**6**). To this solution was added a solution of 726 mg (7.18 mmol) of diisopropylethylamine in 2 ml of acetonitrile followed by a solution of 439 mg (5.63 mmol) of 2-mercaptoethanol in 2 ml of acetonitrile. The reaction solution was stirred at 0°C for 3 hours, diluted with 200 ml of EtOAc and washed with 200 ml of water, 100 ml of 20% aq H_3PO_4, and brine. Evaporation of the dried ($MgSO_4$) solution gave a semisolid which was triturated with methylene chloride and filtered to yield 1.2 g (61% yield) of the title product **7** as a white amorphous solid: ^{1}H NMR (DMSO-d_6) δ 1.20 (3H, d, J=6.0 Hz), 2.9~3.2 (9H, m), 5.22 (1H, d, J=8.5 Hz), 8.23 (2H, d, J=8.5 Hz); IR (KBr) cm^{-1} 3500, 1770, 1700.

Anal Calcd for $C_{18}H_{20}N_2O_7S$: C 52.93, H 4.94, N 6.86.
Found: C 52.83, H 4.90, N 6.52.

p-Nitrobenzyl-3-(2-methanesulfonyloxyethylthio)-6α-[1-(*R*)-hydroxyethyl]-7-oxo-1-azabicyclo-(3.2.0)-hept-2-ene-2-carboxylate (**8**)

To a solution of 4.2 g (10.3 mmol) of **7** in 200 ml of THF, there was added at −40°C 1.3 g (11.3 mmol) of methanesulfonyl chloride followed by a dropwise addition of 1.26 g (12.4 mmol) of triethylamine in 5 ml of THF. The reaction mixture was stirred for 5 hours at −40°C, then for 2 hours at −30°C under a nitrogen atmosphere and poured into a mixture of EtOAc (700 ml) and 5% aq phos-

† Similar results have been presented by B. G. CHRISTENSEN at the North American Medicinal Symposium, Univ. of Toronto, Toronto, Canada, 1982.

phoric acid (1,000 ml). The organic layer was washed with brine dried over $MgSO_4$, filtered and condensed to a syrup. This material was purified by silica gel column chromatography (elution with methylene chloride - EtOAc (3 : 1)) to give 3.55 g (75% yield) of the title compound **8** as a white amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, d, J=6.0 Hz), 3.05 (3H, s), 3.06~3.40 (5H, m), 4.05~4.40 (4H, m), 5.25 (1H, d, J=14.0 Hz), 5.50 (1H, d, J=14.0 Hz), 7.70 (2H, d, J=8.5 Hz), 8.23 (2H, d, J=8.5 Hz); IR (KBr) cm^{-1} 3400, 1770, 1600.

Anal Calcd for $C_{19}H_{22}N_2O_9S_2$: C 46.90, H 4.56, N 5.76.
Found: C 46.52, H 4.35, N 5.91.

p-Nitrobenzyl-3-(2-iodoethylthio)-6α-[1-(*R*)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate (**9**)

A solution of 350 mg (0.72 mmol) of the mesylate **8** and 216 mg (1.4 mmol) of sodium iodide in 20 ml of acetone was heated at reflux for 4 hours. Evaporation of the acetone gave a white amorphous solid which was suspended in ether (10 ml) - water (10 ml). Filtration of the white solid and vacuum drying produced 300 mg (80% yield) of the title compound **9** as a white amorphous powder. ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, d, J=6.0 Hz), 3.20~3.60 (7H, m), 3.80~4.25 (2H, m), 5.10 (1H, d, J=5.5 Hz), 5.25 (1H, d, J=12.0 Hz), 5.45 (1H, d, J=12.0 Hz), 7.70 (2H, d J=8.5 Hz), 8.27 (2H, d, J=8.5 Hz); IR (KBr) cm^{-1} 3500, 1768, 1700.

Anal Calcd for $C_{18}H_{19}N_2O_6I$: C 41.71, H 4.56, N 5.76.
Found: C 42.10, H 3.75, N 5.80.

3-[2-(1-Pyridinium)ethylthio]-6α-[1-(*R*)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate (**11**)

To a solution of 327 mg (0.63 mmol) of the iodide **9** in 20 ml of THF there was added at 0°C 100 mg (1.26 mmol) of pyridine followed by a solution of 139 mg (0.67 mmol) of silver perchlorate in 1 ml of THF. The mixture was stirred for 1 hour at 0°C and then for 2 hours at room temp. The solvent was evaporated *in vacuo* affording compound **10** as a slightly yellow gum which was digested with 300 mg of Celite to give an amorphous solid. IR (KBr) cm^{-1} 3400, 1770, 1700, 1100. Without any further purification, compound **10** was hydrogenated. Thus, to a suspended mixture of compound **10** in 50 ml of ether and 50 ml of THF there was added a solution of 126 mg (1.26 mmol) of potassium bicarbonate and 110 mg (0.63 mmol) of dibasic potassium phosphate in 50 ml of water. Then, 350 mg of 10% palladium on charcoal was added and the mixture was hydrogenated at 2.8 kg/cm^2 on the Parr shaker for 60 minutes. The mixture was filtered and the catalyst was washed with water (2×10 ml). The combined filtrate and washings were extracted with ether (2×100 ml) and lyophilized to give a yellow powder. The crude yellow powder was purified on a C_{18} μBondapak reverse phase column (8 g) (Waters Associates), eluting with water under 0.56 kg/cm^2 pressure. Each 15 ml fraction was assayed by high pressure liquid chromatography, and fractions having an UV absorption at λ_{max} 300 nm were collected and lyophilized to give 40 mg (19% yield based on compound **9**) of the title product **11** as a white amorphous solid: ^{1}H NMR (D_2O) δ 1.20 (3H, d, J=6.0 Hz), 2.90~3.70 (7H, m), 3.75~4.20 (2H, m), 7.70~8.80 (5H, m); IR (KBr) cm^{-1} 3400, 1760, 1590; UV λ_{max} (EtOH) nm (ε) 296 (7,696).

Anal Calcd for $C_{16}H_{18}N_2O_4S \cdot 2H_2O$: C 51.84, H 5.40, N 7.56.
Found: C 50.91, H 5.08, N 7.17.

3-[2-(1-Tetrahydrothiophenium)ethylthio]-6α-[1-(*R*)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**13**) and 3-[2-(*N*-Methylpyrrolidinium)ethylthio]-6α-[1-(*R*)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**12**)

These compounds were prepared as amorphous solids in the same manner as that described in detail for **11** in yields of 18 and 23%, (based on compound **9**), respectively.

Compound **13**: ^{1}H NMR (D_2O) δ 1.23 (3H, d, J=6.0 Hz), 2.25~2.45 (4H, m), 3.0~3.70 (11H, m), 3.95~4.30 (2H, m); IR (KBr) cm^{-1} 3400, 1760, 1590; UV λ_{max} (EtOH) nm (ε) 289 (6,200).

Compound **12**: ^{1}H NMR (D_2O) δ 1.23 (3H, d, J=6.0 Hz), 2.1~2.4 (4H, m), 3.10 (3H, s), 3.1~3.4 (12H, m), 3.95~4.30 (2H, m); IR (KBr) cm^{-1} 3400, 1760, 1590; UV λ_{max} (EtOH) nm (ε)

297 (6,877).

p-Nitrobenzyl-3-(pyridine-2-yl-methanethio)-6α-[1-(*R*)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)-hept-2-ene-2-carboxylate (**14**)

To a cooled (0°C) solution of 925 mg (2.65 mmol) of the keto intermediate **5** in 14 ml of acetonitrile was added a solution of 377 mg (2.92 mmol) of diisopropylethylamine in 1 ml of acetonitrile followed by 786 mg (2.90 mmol) of diphenylchlorophosphate in 1 ml of acetonitrile under nitrogen atmosphere. The resulting solution was stored for 15 minutes at 0°C, there was then added a solution of 377 mg (2.92 mmol) of diisopropylethylamine in 1 ml of acetonitrile followed by 350 mg (3.0 mmol) of 2-mercaptomethylpyridine[10] in 1 ml of acetonitrile. The reaction solution was stirred for 2 hours at −10°C. The precipitate was collected by filtration and washed with 20 ml of methylene chloride to give 650 mg (54% yield) of the title product **14** as a yellow powder: ^{1}H NMR (DMSO-d_6) δ 1.26 (3H, d, J=7.0 Hz), 2.7~3.5 (4H, m), 3.9~4.3 (2H, m), 4.2 (2H, s), 5.42 (2H, ABq, J=14.4 Hz), 7.2~8.8 (8H, m); IR (KBr) cm^{-1} 3400, 1775, 1690.

Anal Calcd for $C_{22}H_{21}N_3O_6S$: C 58.01, H 4.65, N 9.23, S 7.04.
Found: C 57.56, H 4.92, N 8.94, S 7.03.

3-(*N*-Methylpyridine-2-yl-methanethio)-6α-[1-(*R*)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate (**16**)

To a solution of 650 mg (1.39 mmol) of compound **14** in 100 ml of acetone was added 4 ml of methyl iodide. The reaction mixture was stirred for 3 days at room temp. The precipitate was collected by filtration and washed with acetone (10 ml) to give 500 mg (60% yield) of the quaternized pyridine (**15**) as a slightly yellow solid: ^{1}H NMR (DMSO-d_6) δ 1.26 (3H, d, J=7.0 Hz), 3.9~4.2 (2H, m), 4.4 (3H, s), 4.78 (2H, s), 5.2 (1H, d, J=3.9 Hz), 5.50 (2H, ABq, J=14 Hz), 7.8~9.4 (8H, m); IR (KBr) cm^{-1} 3400, 1765, 1690.

Anal Calcd for $C_{23}H_{24}N_3O_6SI$: C 46.24, H 4.05, N 7.03, S 5.37.
Found: C 46.62, H 4.27, N 6.80, S 5.30.

To a solution of 1.0 g (1.167 mmol) of compound **15** in 90 ml of THF and 90 ml of ether was added 215 mg (2.15 mmol) of $KHCO_3$ and 374 mg (2.1 mmol) of K_2HPO_4 in 90 ml of water followed by 1.0 g of 10% palladium on charcoal. The mixture was hydrogenated at 3.2 kg/cm^2 on the Parr shaker for 45 minutes. The mixture was filtered through a Celite pad and the catalyst was washed with water (2×10 ml). The combined filtrate and washings were extracted with ether (2×200 ml) and lyophilized to give a yellow solid which was purified on a C_{18} μBondapak (Waters Associates) reverse phase column (10 g), eluting with 5% acetonitrile in water under 0.56 kg/cm^2 pressure. Each 15 ml fraction was assayed by high pressure liquid chromatography and fractions having UV absorption at λ_{max} 300 nm were collected and lyophilized to give 390 mg (44% yield) of the title product. Recrystallization of this material from water - acetone - EtOH produced fine needles: MP 194~196°C (dec); ^{1}H NMR (D_2O) δ 1.30 (3H, d, J=6.2 Hz), 3.2 (2H, q, J=9.0 and 3.6 Hz), 3.46 (1H, q, J=6.0 and 2.7 Hz), 4.1~4.6 (3H, m), 4.60 (3H, s), 7.9~8.9 (4H, m); IR (KBr) cm^{-1} 3400, 1755, 1590; UV λ_{max} (H_2O) nm (ε) 292 (8,092).

Anal Calcd for $C_{16}H_{18}N_2O_4S\cdot 2H_2O$: C 51.87, H 5.44, N 7.56.
Found: C 51.37, H 5.69, N 7.37.

In a similar manner, **18**, **19**, **21**, **23**, **24**, **25**, **26**, **27** and **28** were prepared and the spectroscopic data are as follows:

18: ^{1}H NMR (D_2O) δ 1.25 (3H, d, J=7.0 Hz), 3.12 (2H, dd, J=7.9 Hz), 3.42 (1H, q, J=7.2 and 1.6 Hz), 3.9~4.6 (3H, m), 4.25 (2H, s), 8.0~9.0 (4H, m); IR (KBr) cm^{-1} 3400, 1750, 1580; UV λ_{max} (H_2O) nm (ε) 298 (8,058).

Anal Calcd for $C_{16}H_{18}N_2O_4S\cdot 2H_2O$: C 51.87, H 5.44, N 7.56.
Found: C 51.95, H 5.66, N 7.56.

19: ^{1}H NMR (D_2O) δ 1.25 (3H, d, J=6.0 Hz), 2.7~3.2 (2H, m), 3.40 (1H, q, J=9.0 and 2.5 Hz), 3.9~4.2 (2H, m), 4.40 (3H, s), 4.72 (2H, s), 8.10 (2H, d, J=6.0 Hz), 8.72 (2H, d, J=6.0 Hz); IR (KBr) cm^{-1} 3400, 1755, 1640; UV λ_{max} (H_2O) nm (ε) 296 (7,782), 258 (6,913).

21: ^{1}H NMR (D_2O) δ 1.28 (3H, d, J=7.0 Hz), 3.12 (2H, d, J=7.0 Hz), 3.44 (1H, dd, J=1 and

3.0 Hz), 4.20 (3H, s), 4.24 (2H, m), 4.76 (3H, m), 8.12 (1H, d, J=4 Hz), 8.24 (1H, d, J=4 Hz); IR (KBr) cm^{-1} 3400, 1740, 1580; UV λ_{max} (H_2O) nm (ε) 292 (7,285).

23: ^1H NMR (D_2O) δ 1.12 (3H, d, J=7.0 Hz), 3.08 (1H, dd, J=13.0 and 6.4 Hz), 3.15 (1H, dd, J=13.0 and 6.4 Hz), 3.45 (1H, dd, J=3.2 and 4.5 Hz), 3.85 (6H, s), 4.1~4.3 (2H, m), 4.40 (1H, d, J=13.5 Hz), 4.52 (1H, d, J=13.5 Hz), 7.40 (2H, s); IR (KBr) cm^{-1} 3500, 1750, 1590; UV λ_{max} (H_2O) nm (ε) 296 (8,411).

Anal Calcd for $C_{15}H_{19}N_3O_4S \cdot H_2O$: C 51.68, H 5.67, N 12.06, S 9.50.
Found: C 49.43, H 5.94, N 11.46, S 9.03.

24: ^1H NMR (D_2O) δ 1.30 (3H, d, J=3.0 Hz), 3.0~3.5 (7H, m), 4.3 (3H, s), 4.0~4.5 (3H, m), 7.90 (2H, d), 8.70 (2H, d); IR (KBr) cm^{-1} 1750, 1640.

25: ^1H NMR (D_2O) δ 1.25 (3H, d, J=6.2 Hz), 3.1~3.6 (7H, m), 4.0~4.3 (2H, m), 4.32 (3H, s), 7.8~8.9 (4H, m); IR (KBr) cm^{-1} 3400, 1750, 1590; UV λ_{max} (H_2O) nm (ε) 300 (8,108).

26: ^1H NMR (D_2O) δ 1.32 (3H, d, J=7.0 Hz), 1.63 (3H, d, J=7.2 Hz), 2.5~4.6 (6H, m), 4.32 (3H, s), 8.2~8.9 (4H, m); IR (KBr) cm^{-1} 3400, 1750, 1590; UV λ_{max} (H_2O) nm (ε) 296 (7,573).

Anal Calcd for $C_{17}H_{20}N_2O_4S \cdot H_2O$: C 54.38, H 5.77, N 7.46.
Found: C 54.39, H 5.98, N 7.68.

27: ^1H NMR (D_2O) δ 1.24 (3H, d, J=7.0 Hz), 2.62 (3H, s), 3.2~3.5 (3H, m), 4.2~4.4 (2H, m), 4.45 (3H, s), 4.50 and 4.59 (1H, each ABq, J=12.6 Hz), 7.82 (1H, q, J=7.0 and 6.5 Hz), 8.35 (1H, d, J=7.0 Hz), 8.65 (1H, d, J=6.5 Hz), 7.82 (1H, q); IR (KBr) cm^{-1} 3400, 1750, 1580; UV λ_{max} (H_2O) nm (ε) 296 (8,014).

Anal Calcd for $C_{17}H_{20}N_2O_4S \cdot \frac{1}{4}H_2O$: C 57.85, H 5.85, N 7.94.
Found: C 58.60, H 5.86, N 7.87.

28: ^1H NMR (D_2O) δ 1.28 (d, 3H, J=7.0 Hz), 2.86 (3H, s), 3.20 (2H, dd, J=10 and 3.5 Hz), 3.42 (1H, dd, J=5.4 and 3.5 Hz), 4.20 (3H, m), 4.32 (3H, s), 4.35 (2H, s), 9.88 (1H, dd, J=7.2 and 6.5 Hz), 8.5 (1H, d, J=8 Hz), 8.70 (1H, d, J=8 Hz); IR (KBr) cm^{-1} 3400, 1760, 1590; UV λ_{max} (H_2O) nm (ε) 298 (8,391).

Anal Calcd for $C_{17}H_{20}N_2O_4S \cdot H_2O$: C 55.73, H 5.41, N 7.65, S 8.74.
Found: C 55.50, H 6.05, N 7.74, S 8.68.

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