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Preliminary communication

Synthesis and structure–activity relationship of C-3 substituted triazolylthiomethyl cephems

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Summary — A series of C-3 substituted triazolylthiomethyl cephems with an aminothiazolemethoxyiminoacetamido side chain at C-7 were synthesized and tested for antimicrobial activity against clinically-relevant isolates. The compound with 3-pyridyl at C-5 and methyl at N-4 of the triazole moiety exhibited good antibacterial activity against both Gram-positive and Gram-negative bacteria, with the exception of *pseudomonas* spp against which none of the derivatives exhibited favorable activity.

β-lactam antibiotic / C-3 substituted cephem / structure–activity relationship / antimicrobial activity

Introduction

Cephalosporins constitute an important class of clinically useful β-lactam antibiotics with diverse chemical, microbiological and pharmacokinetic properties [1–5]. Extensive structural modifications of the cephem nucleus at the C-7 and C-3 positions have been achieved in order to identify compounds with improved antimicrobial activity against clinical isolates resistant to the earlier compounds and compounds with desirable pharmacokinetic profiles [6–8]. Many heterocyclic moieties containing more than one heteroatom have been linked via a 3'-thioether bridge and some compounds of this nature are either currently in the clinics or under development [1, 2]. Only a few compounds with a triazolyl side chain at C-3 and either a phenyl or *p*-hydroxyphenyl acetamido chain at C-7 have been prepared and tested [3–5]. The chemical nature of the side chain present at the C-7 position of cephems has been found to have a prominent effect on their penicillin-binding protein (PBP) binding profile, the permeability through cell membrane and also their β-lactamase susceptibility [5, 8]. The substitution at C-3 is thought primarily to affect the pharmacokinetic properties, although some effect on antimicrobial activity can also result [5, 7].

The nature of the substitution at the C-3 position influences the charge density on the β-lactam carbonyl by electronic effects and thus tends to alter its reactivity towards various bacterial as well as host enzymes [9]. The presence of an aminothiazolylmethoxyiminoacetamido side chain in compounds like cefotaxime, cefpirome, cefmenoxime and cefodizime has been known to broaden their usefulness against resistant bacteria [3, 10]. We have prepared a series of compounds **2** with novel triazolyl substitutions at the C-3 position and aminothiazolyl methoxyimino acetamido side chain at C-7 in order to study the structure–activity relationship of this class of cephalosporins. The nature of the substituents on the triazolyl moiety profoundly affected the antibacterial activity of the compounds and some of these compounds were found to exhibit comparable antibacterial activity in vitro to that of cefodizime. Some of these data were presented at the 33rd ICAAC held in New Orleans [11].

Chemistry

Substituted 1,2,4-triazoles, required as starting material, were prepared by the procedure described in the literature [12–15] and scheme 1, with modification of the method of Sandstrom and Wennerbeck [16]. The aroyl or acylated thiosemicarbazides were prepared by reaction of acid chloride **3** with desired substituted

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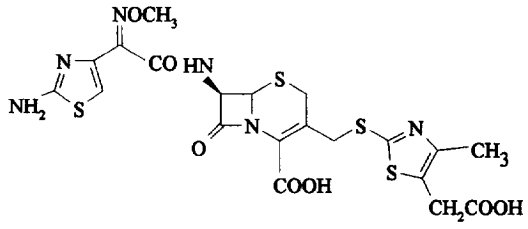
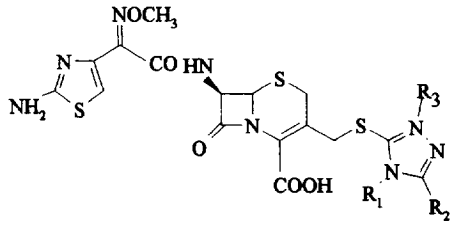
thiosemicarbazide **7**, acetic anhydride **4** with thiosemicarbazide, acetic hydrazide **5** with methyl isothiocyanate **8**, or picolinic hydrazide **6** with methyl isothiocyanate. Thus the crude aroyl or acylated thiosemicarbazides obtained were cyclized in refluxing aqueous sodium bicarbonate or sodium hydroxide to yield the desired substituted 1,2,4-triazole-5-thiones (**9**, table I).

C-3 substituted triazolylthiomethyl cephems were synthesized following the synthetic route depicted in scheme 2. The nucleophilic displacement of the acetoxy group of (6*R*,7*R*)-7-amino-3-acetoxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid **10** was achieved with various substituted 1,2,4-triazoles **9** by heating either in an acetone/water



1(Cefodizime, THR-221)



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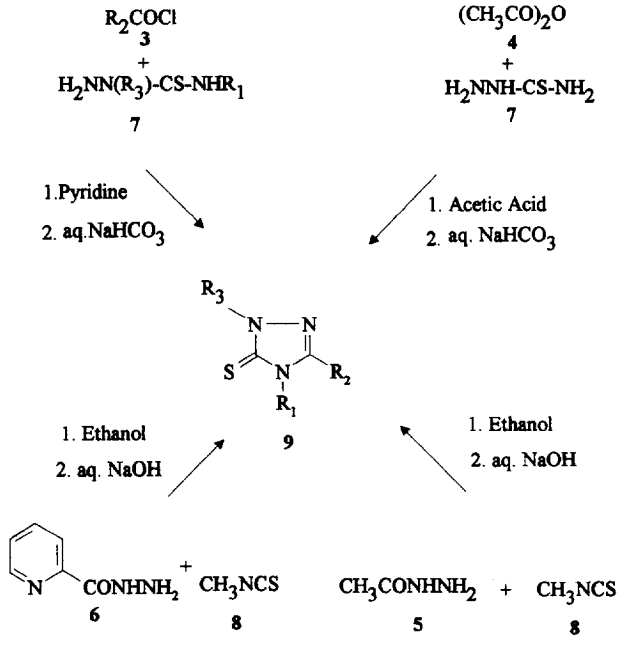
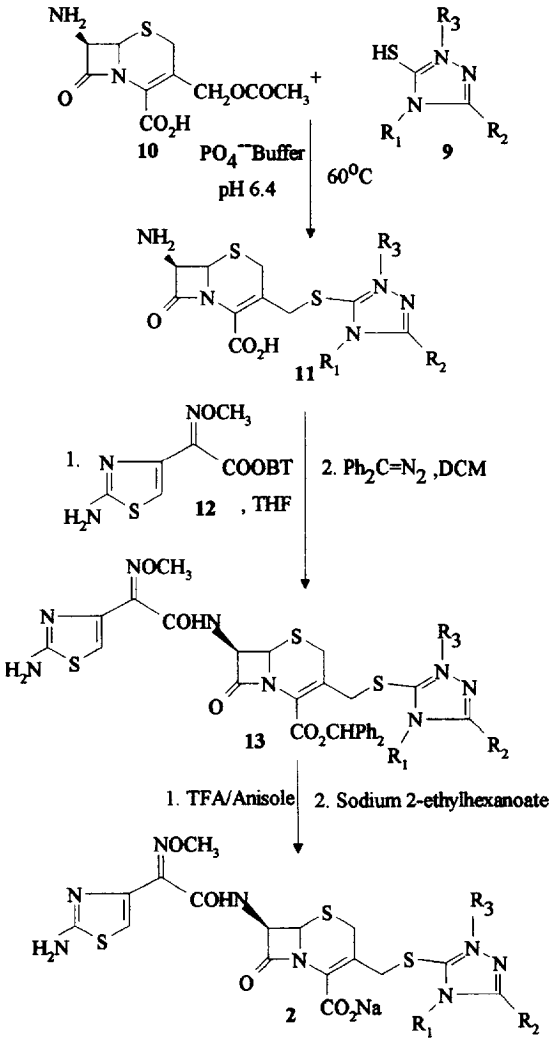
R_1 and R_3= H, CH_3

R_2= CH_3, 4-fluorophenyl, 4-methoxyphenyl, phenyl, and pyridyl



Scheme 1. Synthesis of substituted 1,2,4-triazoles.

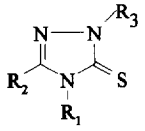


R_1 and R_3 = Hydrogen, Methyl

R_2 = Methyl, Phenyl, 4-methoxyphenyl, 4-fluorophenyl 3-Pyridyl, 2-pyridyl

Scheme 2. Synthesis of C-3 substituted triazolyl cephems.

Table I. Substituted triazoles and their physical properties.



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Compound	R_1	R_2	R_3	*Yield (%)*	*Mp (°C)*
a	H	Methyl	H	68	276–278
b	H	Phenyl	H	65	268–270 (lit [14] 256)
c	H	4-Fluorophenyl	H	71	277–279 (dec) (lit [12] 269–272)
d	H	4-Methoxyphenyl	H	68	255–256 (lit [14] 257)
e	Methyl	Methyl	H	64	283–285
f	Methyl	Phenyl	H	65	164–166 (lit [12] 166)
g	Methyl	4-Fluorophenyl	H	70	138–140
h	Methyl	4-Methoxyphenyl	H	69	196–198
i	Methyl	3-Pyridyl	H	59	184–186 (lit [15] 183–184)
j	Methyl	2-Pyridyl	H	50	234–236 (lit [13] 255–260)
k	H	Phenyl	Methyl	83	172–174
l	H	4-Fluorophenyl	Methyl	78	ND
m	H	4-Methoxyphenyl	Methyl	86	175–177 (lit [14] 176–178)

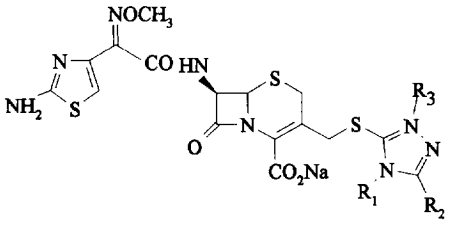
ND: not determined.

mixture (3:2) at reflux temperature or in a phosphate buffer of pH 6.4 at 60 °C following the procedure described in the literature [10, 17]. The resulting adducts **11** were acylated with 1-benzotriazolyl[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyimino]acetate **12** [18] and esterified with diphenyldiazomethane to give the crude compounds **13**. The crude compounds **13** were purified by column chromatography on silica gel using ethyl acetate and dichloromethane as gradient eluant. The yield and NMR spectra of compounds **13** thus synthesized are described in the *Experimental protocols*. The ester hydrolysis of pure compound **13** with trifluoroacetic acid/anisole afforded the desired compound **2** (table 2).

Result and discussion

The C-3 substituted triazolylthiomethyl cephems representing a new class of cephems were prepared by the methods described in the *Chemistry* section (table II). The minimal inhibitory concentrations (MICs) of these compounds against selected bacterial isolates are given in table III. In general, the sodium-(6*R*,7*R*)-7-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(substituted-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate exhibited poor activity against Gram-positive bacteria but moderate to good activity against Gram-negative bacteria, with the exception

Table II. List of synthesized cephems.



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Compound	R_1	R_2	R_3
a	H	Methyl	–
b	H	Phenyl	–
c	H	4-Fluorophenyl	–
d	H	4-Methoxyphenyl	–
e	Methyl	Methyl	–
f	Methyl	Phenyl	–
g	Methyl	4-Fluorophenyl	–
h	Methyl	4-Methoxyphenyl	–
i	Methyl	3-Pyridyl	–
j	Methyl	2-Pyridyl	–
k	–	Phenyl	Methyl
l	–	4-Fluorophenyl	Methyl
m	–	4-Methoxyphenyl	Methyl

of *Pseudomonas* and *Serratia* species. This class of triazolyl cephems appeared to be much more active than the previously reported compounds with phenyl or *p*-hydroxyphenyl groups at the C-7 position [4, 5].

Amongst all the compounds tested, compounds **2a–d**, with no methyl group at either the N-1 or N-4 positions of the triazole substituted at C-3 of the cephem nucleus, exhibited moderate activity against both Gram-positive and Gram-negative bacteria. Compounds **2e–i**, with a methyl group at N-4 of the triazole, exhibited one to three times better activity than their counterparts **2a–d**, with no methyl group at the N-4 position, against most of the Gram-negative bacteria except the *Pseudomonas* and *Serratia* species. The presence of a methyl group at N-4 either had no effect or only a marginal adverse effect on the antibacterial activity against Gram-positive isolates. Compounds **2k–m**, with a methyl group at N-1, exhibited equivalent or one- to twofold poorer activity against both Gram-negative and Gram-positive bacteria than compounds **2a–d** (with no methyl group at either N-1 or N-4) and compounds **2e–i** (with a methyl group at N-4). These results indicate that the introduction of a methyl group at N-4 of the triazole increased the antibacterial activity against Gram-negative bacteria, whereas introduction of a methyl group at N-1 did not favour either Gram-negative or Gram-positive activity (table III).

Amongst the compounds with either alkyl, aryl or heteroaryl substitution at C-3 of the triazole, the compounds **2a** and **2e** with methyl substitution, and compound **2i** with pyridyl substitution at C-3 of the triazole, exhibited good activity against Gram-negative bacteria, whereas the compounds **2b–h** with aryl substitution at C-3 of the triazole exhibited decreased antibacterial activity. Furthermore, the introduction of an electron-withdrawing group (compounds **2c**, **2l**, **2g**) or electron-donating group (compounds **2d**, **2m**, **2h**) on the phenyl of the triazole reduced the activity against both Gram-negative and Gram-positive bacteria (table III).

Table III. Antibacterial activity (MIC μg/mL) of C-3 substituted triazolylthiomethyl cephems **2**.

Test organism	*THR-221*	***2a***	***2b***	***2c***	***2d***	***2e***	***2f***	***2g***	***2h***	***2i***	***2k***	***2l***	***2m***
Sa ATCC29213	8.0	4.0	1.0	1.0	2.0	8.0	2.0	2.0	2.0	4.0	2.0	1.0	2.0
Sa JHHD178	8.0	4.0	1.0	1.0	2.0	2.0	2.0	2.0	2.0	4.0	1.0	0.5	1.0
Sa JHH241	64	64	64	8.0	16	32	64	64	32	32	4.0	4.0	4.0
Sa H	8.0	4.0	0.5	1.0	1.0	2.0	2.0	2.0	2.0	2.0	1.0	0.5	1.0
Ec ATCC25922	0.25	0.5	1.0	1.0	1.0	0.12	0.5	1.0	2.0	0.12	2.0	4.0	1.0
Ec DCO	0.12	0.25	0.25	0.5	1.0	0.12	0.25	0.25	0.5	0.06	1.0	2.0	0.5
Ec DC2	0.06	<0.06	0.06	0.06	0.25	<0.06	0.06	0.06	0.06	<0.06	0.06	0.06	<0.06
Ec SHV1	0.12	0.25	0.25	0.5	1.0	<0.06	0.25	0.25	0.5	0.06	1.0	2.0	0.50
Ec TEM1	0.12	0.12	0.25	0.5	0.5	<0.06	0.12	0.25	0.50	0.06	0.5	0.5	0.50
Ec TEM2	0.06	0.12	0.12	0.25	0.06	<0.06	0.06	0.06	0.06	0.03	0.12	0.25	<0.06
Ec O XA1	0.5	0.5	0.5	1.0	1.0	0.25	0.25	0.5	1.0	0.12	1.0	2.0	1.0
Ec OXA3	0.12	0.12	0.5	0.5	1.0	<0.06	0.12	0.25	0.5	0.06	0.5	0.5	0.50
Kp ATCC13883	0.25	0.5	1.0	2.0	2.0	0.25	1.0	1.0	1.0	0.25	2.0	2.0	1.0
Pr ATCC29944	0.06	<0.06	0.25	0.5	0.5	<0.06	0.12	0.25	0.5	<0.06	0.25	0.5	0.12
Pm ATCC2675	0.06	0.12	0.5	0.5	1.0	<0.06	1.0	2.0	2.0	0.5	0.25	2.0	0.25
Ecl ATCC23355	0.5	0.5	1.0	2.0	2.0	0.25	0.5	1.0	2.0	0.12	2.0	2.0	1.0
Ecl (multi resist)	>128	>128	ND	ND	>128	>128	>128	>128	>128	>128	128	128	>128
Cf (R-Ceph)	1.0	0.5	1.0	2.0	2.0	0.12	0.5	1.0	1.0	0.12	2.0	4.0	2.0
Sm ATCC29882	16	64	4.0	4.0	16	64	64	64	64	64	64	64	64
Pa ATCC27853	32	64	32	64	128	64	128	>128	128	64	128	128	64
PaPSE1	64	128	64	128	128	128	128	>128	128	128	128	128	128
PaPSE2	64	128	64	64	128	128	128	>128	128	128	128	64	64

Sa: *Staphylococcus aureus*; Ec: *Escherichia coli*; Kp: *Klebsiella pneumoniae*; Pr: *Providencia rettgeri*; Pm: *Proteus mirabilis*; Ecl: *Enterobacter cloacae*; Cf: *Citrobacter freundii*; Sm: *Serratia marcescens*; Pa: *Pseudomonas aeruginosa*; ND: not determined.

Table IV. Antibacterial activity (MIC μg/mL) of selected C-3 triazolylthiomethyl cephems against resistant clinical isolates.

Test organism	***2f***	***2i***	*Cefotaxime*	*Cefotiam*
S aureus 54K	3.13	6.25	3.13	1.56
S aureus 157-399	>100	>100	>100	>100
S aureus (MRSA)HL-1185	>100	>100	100	>100
S aureus (MRSA) CT-10	>100	>100	>100	>100
S aureus CT-23	>100	>100	>100	>100
E coli TEM-1	0.20	0.10	0.05	0.20
E coli OXA-1	0.39	0.20	0.10	0.39
E coli CT-73	3.13	0.78	1.56	6.25
K pneumoniae 366L	0.39	0.20	0.05	0.39
K pneumoniae CTX-1	12.5	25	12.5	3.13
S marcescens 200L	0.78	0.39	0.10	12.5
S marcescens CT-98	50	50	25	>100
C freundii 2046E	0.78	1.56	0.39	0.78
C freundii CT-76	12.5	50	25	>100
E cloacae P99	50	50	100	>100
E cloacae CT-95	12.5	50	50	>100
Proteus vulgaris CT-106	>100	>100	>100	>100
Morganella morganii CT-112	3.13	3.13	6.25	50
Acinetobacter 450L	100	100	12.5	>100
Acinetobacter 553L	100	100	12.5	100
P aeruginosa PSE-1	>100	100	25	>100
P aeruginosa PSE-4	100	100	12.5	>100
P aeruginosa 46001(CRC-1)	100	100	12.5	>100
P aeruginosa 46015(CRC-15)	6.25	6.25	12.5	>100
P aeruginosa CT-137	>100	>100	>100	>100
P aeruginosa CT-144	>100	>100	>100	>100

Amongst the 12 different derivatives tested, the compound **2i** with 3-pyridyl at C-3 and a methyl group at N-4 of the triazole moiety, and compound **2e** with a methyl group at both the C-3 and N-4 positions, exhibited similar or comparable activity to cefodizime (table III). Compounds **2f** and **2i** were further tested against a panel of antibiotic-resistant clinical isolates of Gram-positive and Gram-negative bacteria. Both compounds exhibited comparable activities to those of cefotaxime (CTX) and one to two times better activity against *Morganella, Enterobacter* and *Citrobacter* resistant strains than cefotiam (CTM). The antibacterial activity results are summarized in table IV. Based on the preceding result it may be concluded that the C-7 aminothiazolylmethoxyiminoacetamido side chain goes well with the C-3 triazolyl side chain in enhancing the antibacterial activity. Further in vivo studies with some of these compounds will provide information about their pharmacokinetic profiles.

Experimental protocols

General methods

Melting points were determined on a Electrothermal digital melting point apparatus and the values are uncorrected. ^{1}H NMR spectra (δ ppm) were obtained on a Brucker AM-300 spectrometer in $CDCl_3$ or DMSO-d_6 (for esters) with tetramethylsilane and D_2O (for salts), with TSP as an internal standard. Infrared spectra were obtained using Nujol on a Shimadaju IR-460 infrared spectrophotometer. The chemical purities of the synthesized compounds were checked on thin-layer chromatography plates. Mass spectroscopic analyses (FAB) were performed on an AEI Ms-9 (modified) mass spectrometer for molecular ion peaks.

Organisms and reference compounds

Clinical isolates were collected from 1987–1992 from various medical centers in Canada and Japan. The quality control strains were obtained from the American Type Culture Collection, Rockville, MD, USA. *E coli* DCO and DC2 were obtained from BJ Wilkinson, Illinois State University, Normal, Bloomington, IL, USA. All clinical isolates were stored frozen at –80 °C in 20% glycerol. Cefodizime (THR-221) and Cefotaxime (CTX) powders were obtained from Taiho Pharmaceutical Company Ld, Tokyo, Japan. Cefotiam (CTM) powder was obtained from Sigma Chemical Company, USA.

Antibacterial activity

The in vitro antibacterial activities were determined by the standard agar dilution method as recommended by the National Committee for Clinical Laboratory Standards [19]. Briefly, plates (15 x 150 mm petri dishes) with two-fold serial dilution of the reference and test compounds in the unsupplemented Mueller Hinton Agar (BBL) medium were prepared with concentrations in the range 128–0.06 μg/mL. The cathra multipoint inoculation system (MCT Medical Inc, Saint Paul, MN, USA) was used to dispense about 10^4 cfu/spot of the test organism onto the agar plate. The minimum inhibitory concentration (MIC) was recorded as the lowest concentration of antibiotic that prevented visible growth after 18 h of incubation at 35 °C, disregarding the appearance of a single colony or very hazy growth.

Chemistry

(6R,7R)-7-Amino-3-[(3-methyl-1,2,4-trizol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid ***11a***

A mixture of (6*R*,7*R*)-7-amino-3-acetoxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (2.7 g, 0.01 mol), 3-methyl-1,2,4-triazol-5-thione (**9a**, 1.12 g, 0.0097 mol) and sodium bicarbonate (1.2 g, 0.0143 mol) in phosphate buffer (60 mL) of pH 6.4 was heated at 60 °C overnight under stirring. The orange-color reaction mixture was cooled and acidified to pH 2–3 with 1 N HCl. The separated solid was filtered, washed with water and dried under vacuum. This crude product (80% pure) was obtained in 70% (2.3 g) yield and was used further for coupling with active ester **12**.

Following a similar procedure, other adducts **11b–m** were prepared by reaction of (6*R*,7*R*)-7-amino-3-acetoxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid **11a** with substituted 1,2,4-triazoles (**9b–m**), which were coupled with active ester **12** without purification and further identification.

1-Benzotriazolyl-(Z)-2-(2-aminothiazol-4-yl)-2-methoxyimino-acetate ***12***

To a solution of *N,N'*-dicyclohexyldicarbodiimide (5.12 g, 0.0249 mol) in THF (80 mL), 1-hydroxy benzotriazole (3.35 g, 0.0249 mol) was added and stirred vigorously at room temperature for 15 min, then (*Z*)-2-(aminothiazol-4-yl)-2-methoxyimino acetic acid (5.0 g, 0.029 mol) was added all in one portion and the suspension stirred at room temperature for 2 h under nitrogen. The reaction mixture was cooled and the separated *N,N'*-dicyclohexyl urea removed by filtration. The filtrate was diluted with *n*-heptane at 0 °C. The solid separated was filtered, washed with cold heptane and dried under vacuum. Yield 6.5 g (82%): IR (KBr) cm^{-1} 3394, 3263, 3115, 2934, 1812, 1624, 1541, 1101, 1087, 1004, 936, 746.

Diphenylmethyl(6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(3-methyl-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***13a***

(6*R*,7*R*)-7-Amino-3-[(3-methyl-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**11a**, 1.11 g, 0.0034 mol) was dissolved in ice-cold water (20 mL) at pH 7.5–8.0 by the addition of 1 N NaOH under stirring. THF (50 mL) was added to this solution and the temperature was raised to 8–10 °C followed by addition of 1-benzotriazolyl-(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyimino acetate (**9**, 1.70 g, 0.0051 mol) in five equal portions over a period of 15–20 min. The resulting reaction mixture was stirred at room temperature for 3 h and extracted with methylisobutyl ketone (5 x 50 mL). The aqueous portion was cooled, acidified with 1 N HCl. The separated solid was filtered and dried to give 1.2 g crude cephem carboxylic acid.

The crude product (1.2 g) was dissolved in a mixture of dichloromethane/methanol (4:1). A solution of diphenyldiazomethane in dichloromethane (25 mL) was added dropwise to the above solution until the color persisted. The resulting solution was stirred for an additional hour and was then

concentrated under vacuum. The residue was diluted with ethyl acetate and the separated solid was filtered off. The filtrate was washed with sodium bicarbonate, water and brine successively, dried over Na_2SO_4 (anhydrous) and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using ethyl acetate and dichloromethane as gradient eluants, which gave the pure title compound as a foam. Yield 700 mg (44%); NMR ($CDCl_3$) δ: 2.31 (s, 3H), 3.50 and 3.70 (abq, 2H, J = 18 Hz), 4.00 and 4.35 (abq, 2H, J = 12.8 Hz), 4.03 (s, 3H), 5.05 (d, J = 4.8 Hz, 1H), 5.34 (bs, 2H), 5.99 (dd, J_1 = 8.5 Hz, J_2 = 4.8 Hz, 1H), 6.88 (s, 1H), 7.00 (s, 1H), 7.30–7.42 (m, 11H).

The following additional compounds were prepared following the procedure described above.

Diphenyl methyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(3-phenyl-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***13b***. Yield 38%; NMR ($CDCl_3$) δ: 3.55 and 3.70 (abq, 2H, J = 18 Hz), 3.90 (s, 3H), 4.10 and 4.30 (abq, 2H, J = 12.5 Hz), 5.04 (d, J = 5 Hz, 1H), 5.45 (bs, 2H), 5.93 (dd, J_1 = 8.8 Hz, J_2 = 5 Hz, 1H), 6.81 (s, 1H), 6.96 (s, 1H), 7.30 (m, 13H), 7.63 (d, J = 8.8 Hz, 1H), 7.89 (m, 2H).

Diphenyl methyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[{3-(4-fluoromethyl)-1,2,4-triazol-5-yl}-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***13c***. Yield 49%; NMR ($CDCl_3$) δ: 3.62 (bs, 2H), 3.85 (s, 3H), 4.10 and 4.25 (abq, J = 12.5 Hz, 2H,), 5.05 (d, J = 4.0 Hz, 1H), 5.55 (bs, 2H), 5.90 (dd, J_1 = 8.8 Hz, J_2 = 4 Hz, 1H), 6.78 (1H, s), 6.90 (s, 1H), 7.10 (m, 2H), 7.30 (m, 11H), 7.75 (d, J = 8.8 Hz, 1H), 7.90 (m, 2H).

Diphenyl methyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[{3-(4-methoxyphenyl)-1,2,4-triazol-5-yl}-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***13d***. Yield 33%; NMR ($CDCl_3$) δ: 3.45 and 3.55 (abq, J = 18 Hz, 2H), 3.65 (s, 3H), 3.75 (s, 3H), 3.95 and 4.25 (abq, J = 13.5 Hz, 2H), 4.90 (d, J = 5 Hz, 1H), 5.62 (bs, 2H), 5.85 (dd, J_1 = 8.8 Hz, J_2 = 5 Hz, 1H), 6.65 (s, 1H), 6.80 (m, 3H), 7.25 (m, 11H), 7.72 (d, J = 9.5 Hz, 2H), 7.85 (d, J = 8.8 Hz, 1H).

Diphenyl methyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(3,4-dimethyl-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***13e***. Yield 25%; NMR (DMSO-d_6) δ: 2.30 (s, 3H), 3.40 (s, 3H), 3.62 and 3.75 (abq, J = 18 Hz, 2H), 3.86 (s, 3H), 4.00 and 4.12 (abq, J = 13 Hz, 2H), 5.22 (d, J = 5 Hz, 1H), 5.87 (dd, J_1 = 8.5 Hz, J_2 = 5 Hz, 1H), 6.76 (s, 1H), 6.87 (s, 1H), 7.22–7.60 (m, 12H), 9.70 (d, J = 8.5 Hz, 1H).

Diphenyl methyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(3-phenyl-4-methyl-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***13f***. Yield 32%; NMR (DMSO-d_6) δ: 3.30 (s, 3H), 3.52 (s, 3H), 3.80 (m, 5H), 4.10 and 4.20 (abq, J = 13 Hz, 2H), 5.25 (d, J = 5 Hz, 1H), 5.95 (dd, J_1 = 8.5 Hz, J_2 = 5 Hz, 1H), 6.78 (s, 1H), 6.87 (s, 1H), 7.20–7.80 (m, 15H), 9.75 (d, J = 8.5 Hz, 1H).

Diphenyl methyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[{3-(4-fluorophenyl)-4-methyl-1,2,4-triazol-5-yl}-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***13g***. Yield 37%; NMR (DMSO-d_6) δ: 3.35 (s, 2H), 3.52 (s, 3H), 3.80 (m, 5H), 4.10 and 4.20 (abq, J = 12.5 Hz, 2H), 5.25 (d, J = 5 Hz, 1H), 5.90 (dd, J_1 = 8.5 Hz, J_2 = 5 Hz, 1H), 6.78 (s, 1H), 6.87 (s, 1H), 7.20–7.65 (m, 12H), 7.75 (m, 2H), 9.72 (d, J = 8.5 Hz, 1H).

Diphenyl methyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[3-(4-methoxyphenyl)-4-methyl-1,2,4-triazol-5-yl}-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***13h***. Yield 33%; NMR (DMSO-d_6) δ: 3.36 (bs, 2H), 3.53 (s, 3H), 3.76–4.25 (m, 8H), 5.26 (d, J = 5 Hz, 1H), 5.93 (dd, J_2 = 8.5 Hz, J_2 = 5 Hz, 1H), 6.73 (s, 1H), 6.90 (s, 1H), 7.03–7.80 (m, 14H), 7.85 (d, J = 8.5 Hz, 1H).

Diphenyl methyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(3-pyrid-3-yl-4-methyl-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***13i***. Yield 29%; NMR (DMSO-d_6) δ: 3.60 (s, 3H), 3.87 (m, 5H), 4.12 and 4.25 (abq, J = 13 Hz, 2H), 5.25 (d, J = 5 Hz, 1H), 5.90 (dd, J_1 = 8.5 Hz, J_2 = 5 Hz, 1H), 6.76 (s, 1H), 6.87 (s, 1H), 7.23–7.70 (m, 12H), 8.12 (m, 1H), 8.78 (m, 1H), 8.90 (d, J = 2 Hz, 1H), 9.72 (d, J = 8.5 Hz, 1H).

Diphenyl methyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(3-pyrid-2-yl-4-methyl-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***13j***. Yield 32%; NMR (DMSO-d_6) δ: 3.35 (s, 3H), 3.88 (m, 5H), 4.10 and 4.32 (abq, J = 13 Hz, 2H), 5.20 (d, J = 5 Hz, 1H), 5.90 (dd, J_1 = 8.5 Hz, J_2 = 5Hz, 1H), 6.76 (s, 1H), 6.82 (s, 1H), 7.15–7.60 (m, 12H), 8.05 (m, 2H), 8.75 (d, J = 4 Hz, 1H), 9.68 (d, J = 8.5 Hz, 1H).

Diphenyl methyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(3-phenyl-1-methyl-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***13k***. Yield 19%; NMR ($CDCl_3$) δ: 3.70 (m, 5H), 4.00 (s, 3H), 4.20 and 4.40 (abq, J = 13 Hz, 2H), 5.10 (d, J = 5 Hz, 1H), 5.65 (bs, 2H), 6.10 dd, J_1 = 8.8 Hz, J_2 = 5 Hz, 1H), 6.68 (s, 1H), 6.95 (s, 1H), 7.30 (m, 13H), 8.00 (m, 3H).

Diphenyl methyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[{3-(4-fluorophenyl)-1-methyl-1,2,4-triazol-5-yl}-thiomethyl]-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylate ***13l***. Yield 18%; NMR ($CDCl_3$) δ: 3.75 (m, 5H), 4.02 (s, 3H), 4.20 and 4.40 (abq, J = 13 Hz, 2H), 5.10 (d, J = 5 Hz, 1H), 5.62 (bs, 2H), 6.10 (dd, J_1 = 9 Hz, J_2 = 5 Hz, 1H), 6.75 (s, 1H), 6.92 (s, 1H), 7.05 (m, 2H), 7.37 (m, 10H), 8.00 (m, 3H).

Diphenyl methyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[{3-(4-methoxyphenyl)-1-methyl-1,2,4-triazol-5-yl}-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***13m***. Yield 21%; NMR ($CDCl_3$) δ: 3.76 (m, 8H), 4.00 (s, 3H), 4.20 and 4.38 (abq, J = 13 Hz, 2H), 5.08 (d, J = 5 Hz, 1H), 5.68 (bs, 2H), 6.05 (dd, J_1 = 8.8 Hz, J_2 = 5 Hz, 1H), 6.70 (s, 1H), 6.90 (m, 3H), 7.32 (m, 10H), 7.86 (m, 3H).

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(3-methyl-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***2a***

Trifluoroacetic acid (8 mL) was added to a solution of compound **13a** (0.5 g, 0.00074 mol) in anisole (2 mL) at 0 °C under stirring. The resulting solution was stirred for an additional 20 min under cooling and then diluted with hexane/ether (2:1). The separated colloidal mixture was centrifuged and

filtered. The filtered solid was washed with ether and dried under vacuum. The crude solid product was purified by reverse phase C_{18} preparative thin layer chromatography plates using water/acetonitrile (95:5) as developing solvent. Yield 200 mg (51%); mp 130 °C (dec); IR (Nujol) cm^{-1}: 3400, 3195, 2730, 1759, 1650, 1604, 1535. NMR (D_2O) δ: 2.40 (s, 3H), 3.38 and 3.82 (abq, J = 18 Hz, 2H), 3.76 and 4.30 (abq, J = 13 Hz, 2H), 4.00 (s, 3H), 5.20 (d, J = 4 Hz, 1H), 5.78 (d, J = 4 Hz, 1H), 7.05 (s, 1H); MS (FAB) *m/z*: 532.8 calc for $C_{17}H_{17}N_8O_5S_3Na$ 532.54.

The following additional compounds as sodium salt were prepared from corresponding esters by the procedure described above for compound **2a**.

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyimino-acetamido]-3-[(3-phenyl-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***2b***. Yield 52%; mp 85 °C (dec); IR (Nujol) cm^{-1}: 3275, 2730, 1759, 1670, 1632, 1611, 1579, 1539; NMR (D_2O) δ: 3.50 and 3.62 (abq, J = 17 Hz, 2H), 3.92 (s, 3H), 4.10 and 4.20 (abq, J = 13 Hz, 2H), 5.17 (d, J = 4 Hz, 1H), 5.70 (d, J = 4 Hz, 1H), 6.95 (s, 1H), 7.50 (m, 3H), 7.85 (m, 2H); MS (FAB) *m/z*: 595.0 calc for $C_{22}H_{19}N_8O_5S_3Na$ 594.61.

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyimino-acetamido]-3-[{3-(4-fluorophenyl)-1,2,4-triazol-5-yl}-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***2c***. Yield; 74%; mp 150 °C (dec); IR (Nujol) cm^{-1}: 3320, 3200, 2730, 1761, 1644, 1608, 1528; NMR (D_2O) δ: 3.55 and 3.75 (abq, J = 18 Hz, 2H), 3.90 (s, 3H), 4.10 and 4.20 (abq, J = 12.5 Hz, 2H), 5.22 (d, J = 4 Hz, 1H), 5.75 (d, J = 4 Hz, 1H), 6.90 (s, 1H), 7.25 (m, 3H), 7.80 (m, 2H); MS (FAB): 613.1 calc for $C_{22}H_{18}N_8O_5S_3FNa$ 612.60.

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyimino-acetamido]-3-[{3-(4-methoxyphenyl)-1,2,4-triazol-5-yl}-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***2d***. Yield 51%; mp 220 °C (dec); IR (Nujol) cm^{-1}: 3310, 3200, 2730, 1760, 1671, 1610, 1529; NMR (D_2O) δ: 3.45 and 3.60 (abq, J = 17 Hz, 2H), 3.75 (s, 3H), 3.85 (s, 3H), 3.90 and 4.20 (abq, J = 13 Hz, 2H), 5.12 (d, J = 4 Hz, 1H), 5.68 (d, J = 4 Hz, 1H), 6.80 (m, 3H), 7.60 (m, 2H); MS (FAB) *m/z*: 625.0 calc for $C_{23}H_{21}N_8O_6S_3Na$ 624.64.

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyimino-acetamido]-3-[(3,4-dimethyl-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***2e***. Yield 54%; mp 80 °C (dec); IR (Nujol) cm^{-1}: 3355, 3195, 2725, 1767, 1669, 1611, 1579, 1540; NMR (D_2O) δ: 2.40 (s, 3H), 3.37 (d, J = 18 Hz, 1H), 3.60 (bs, 4H), 3.95 (bs, 4H), 4.32 (d, J = 13 Hz, 1H), 5.12 (d, J = 4 Hz, 1H), 5.70 (d, J = 4 Hz, 1H), 7.00 (s, 1H); MS (FAB) *m/z*: 546.7 calc for $C_{18}H_{19}N_8O_5S_3Na$ 546.57.

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyimino-acetamido]-3-[(3-phenyl-4-methyl-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***2f***. Yield 75%; mp 70 °C (dec); IR (Nujol) cm^{-1}: 3305, 3200, 2730, 1761, 1670, 1605, 1534; NMR (D_2O) δ: 3.37 and 3.80 (abq, J = 18 Hz, 2H), 3.70 (s, 3H), 4.00 (s, 3H), 3.95 and 4.35 (abq, J = 12.5 Hz, 2H), 5.12 (d, J = 4 Hz, 1H), 5.70 (d, J = 4 Hz, 1H), 7.00 (s, 1H), 7.62 (m, 5H); MS (FAB) *m/z*: 608.9 calc for $C_{23}H_{21}N_8O_5S_3Na$ 608.64.

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyimino-acetamido]-3-[{3-(4-fluorophenyl)-4-methyl-1,2,4-triazol-5-yl}-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***2g***. Yield 70%; mp 150 °C (dec); IR (Nujol) cm^{-1}: 3310, 3195, 2730, 1761, 1678, 1607, 1533; NMR (D_2O) δ: 3.35 (d, J = 17.5 Hz, 1H), 3.60–4.10 (m, 8H), 4.32 (d, J = 13.3 Hz, 1H), 5.20 (d, J = 4.4 Hz, 1H), 5.75 (d, J = 4.4 Hz, 1H), 7.00 (s, 1H), 7.32 (m, 2H), 7.70 (bs, 2H); MS (FAB) *m/z*: 627.3 calc for $C_{23}H_{20}N_8O_5S_3FNa$ 626.63.

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyimino-acetamido]-3-[3-(4-methoxyphenyl)-4-methyl-1,2,4-triazol-5-yl}-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***2h***. Yield 72%; mp 101 °C (dec); IR (Nujol) cm^{-1}: 3370, 3215, 1759, 1677, 1653, 1610, 1536; NMR (D_2O) δ: 3.25–4.10 (m, 12H), 4.25 (d, J = 13 Hz, 1H), 5.12 (bs, 1H), 5.72 (bs, 1H), 6.90 (s, 1H), 7.05 (bs, 2H), 7.55 (bs, 2H); MS (FAB) *m/z*: 638.9 calc for $C_{24}H_{23}N_8O_6S_3Na$ 638.66.

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyimino-acetamido]-3-[(3-pyrid-3-yl-4-methyl-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***2i***. Yield 57%; mp 71 °C (dec); IR (Nujol) cm^{-1}: 3305, 3195, 1750, 1651, 1602, 1540. NMR (D_2O) δ: 3.41 (d, J = 18 Hz, 1H), 3.72–3.97 (m, 8H), 4.36 (d, J = 13 Hz, 1H), 5.14 (d, J = 4 Hz, 1H), 5.71 (d, J = 4 Hz, 1H), 6.98 (s, 1H), 7.66 (m, 1H), 8.16 (m, 1H), 8.71 (m, 1H), 8.80 (d, J = 1.5 Hz, 1H); MS (FAB): 609.7 calc for $C_{22}H_{20}N_9O_5S_3Na$ 609.62.

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxy-iminoacetamido]-3-[(3-pyrid-2-yl-4-methyl-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***2j***. Yield 55%; mp 68 °C (dec); IR (Nujol) cm^{-1}: 3310, 3195, 2730, 1759, 1650, 1617, 1557; NMR (D_2O) δ: 3.42 (d, J = 18 Hz, 1H), 3.78–4.10 (m, 8H), 4.32 (d, J = 13 Hz, 1H), 5.20 (d, J = 4 Hz, 1H), 5.75 (d, J = 4 Hz, 1H), 7.00 (s, 1H), 7.60 (m, 1H), 7.90 (m, 1H), 8.10 (m, 1H), 8.72 (bs, 1H); MS (FAB): 609.8 calc for $C_{22}H_{20}N_9O_5S_3Na$ 609.62.

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyimino-acetamido]-3-[(3-phenyl-1-methyl-1,2,4-triazol-5-yl)-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***2k***. Yield 57%; mp 180 °C (dec); IR (Nujol) cm^{-1}: 3390, 3205, 2930, 1762, 1678, 1606, 1532; NMR (D_2O) δ: 3.30 and 3.52 (abq, J = 18 Hz, 2H), 3.70 (s, 3H), 4.00 (s, 5H), 5.08 (d, J = 4 Hz, 1H), 5.75 (d, J = 4 Hz, 1H), 6.80 (s, 1H), 7.28 (bs, 3H), 7.80 (bs, 2H); MS (FAB) *m/z*: 608.9 calc for $C_{23}H_{21}N_8O_5S_3Na$ 608.64.

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyimino-acetamido]-3-[{3-(4-fluorophenyl)-1-methyl-1,2,4-triazol-5-yl}-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***2l***. Yield 71%; mp 210 °C (dec); IR (Nujol) cm^{-1}: 3310, 3195, 2725, 1761, 1677, 1604, 1528; NMR (D_2O) δ: 3.35 and 3.55 (abq, J = 17.5 Hz, 2H), 3.68 (s, 3H), 4.00 (m, 5H), 5.10 (d, J = 4 Hz, 1H), 5.75 (d, J = 4 Hz, 1H), 6.75 (s, 1H), 6.95 (m, 2H), 7.70 (m, 2H); MS (FAB) *m/z*: 627.1 calc for $C_{23}H_{20}N_8O_5S_3FNa$ 626.63.

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyimino-acetamido]-3-[{3-(4-methoxyphenyl)-1-methyl-1,2,4-triazol-5-yl}-thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***2m***. Yield 54%; mp 212 °C (dec); IR (Nujol) cm^{-1}: 3315, 3200, 1761, 1681, 1611, 1533; NMR (D_2O) δ: 3.70 (m, 8H), 4.00 (m, 5H), 5.10 (d, J = 4 Hz, 1H), 5.75 (d, J = 4 Hz, 1H), 6.76 (m, 3H), 7.70 (d, J = 9 Hz, 2H); MS (FAB) *m/z*: 639.0 calc for $C_{24}H_{23}N_8O_6S_3Na$ 638.67.

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