CEPHALOSPORIN ANTIBIOTICS. SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 7β-[2-(5-AMINO-1,2,4-THIADIAZOL-3-YL)-2-OXYIMINOACETAMIDO]CEPHALOSPORIN DERIVATIVES

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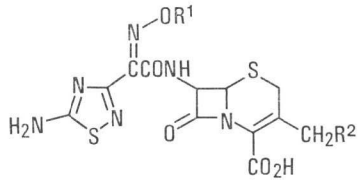
Cephalosporins with a 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2-oxyiminoacetamido] side chain were synthesized and their *in vitro* inhibitory potency was established. The compounds exhibit a strong antibacterial activity against various Gram-positive and Gram-negative bacteria. The antimicrobial activity is related to the oxime-substituent R^1 and the C-3 substituent R^2. Selected amino-1,2,4-thiadiazolyl-cephems **1** show a prolonged half-life in mice.

In recent years several new cephalosporin antibiotics with a broad spectrum of activity and increased resistance against bacterial β-lactamases have been developed[1~5]. Most of them bear a 2-(2-aminothiazol-4-yl)-2-*syn*-alkoxyiminoacetamido substituent at the C-7 position of the cephem nucleus. Structure-activity studies among this series of derivatives revealed the importance of the *syn*-oxyimino moiety and of the unsubstituted amino group attached to the hetero-aromatic ring for the outstanding antibacterial potency showed by many derivatives of this new generation of cephalosporin antibiotics[6,7].

As part of our research program on novel cephalosporin antibiotics we therefore became interested in the synthesis of derivatives with new five-membered hetero-aromatic α-substituents in the 2-*syn*-oxyiminoacetic acid side chain.

We describe herein the synthesis of 2-(5-amino-1,2,4-thiadiazol-3-yl)acetic acid intermediates, their conversion to cephalosporin antibiotics and the antimicrobial activity of the new derivatives with the general structure **1***.



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Chemistry

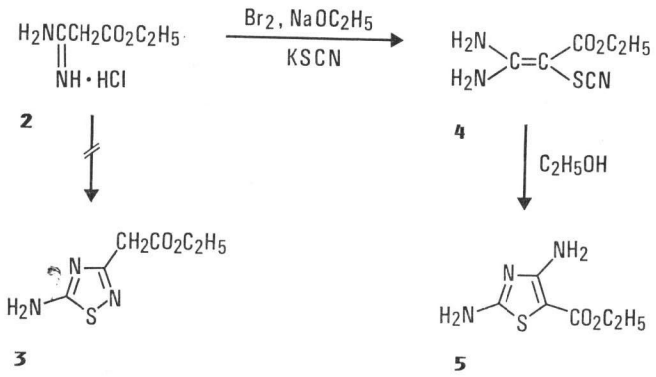
Our initial target was the synthesis of ethyl 2-(5-amino-1,2,4-thiadiazol-3-yl)acetate (**3**), considered to be a valuable intermediate in the preparation of α-alkoxyiminoacetic acid derivatives **6**. The attempted approach, ring closure reaction of ethyl amidinoacetate **2**[9] following GOERDELER's general procedure for the synthesis of 5-amino-1,2,4-thiadiazoles (Br_2; NaOEt; KSCN)[10] failed to give the expected product **3**. Instead, the ethyl acrylate derivative **4** was formed, which upon heating in ethanol yielded 2,4-diamino-5-carbethoxythiazole (**5**) (see Scheme 1).

In order to avoid the use of a compound like **2** with an active methylene group, the acetic acid side chain was built up after the ring closure step, as shown in Scheme 2.

Treatment of acetamidine hydrochloride **7** with potassium thiocyanate gave 3-methyl-5-amino-1,2,4-thiadiazole (**8**)[10] in 75% yield. Heating of **8** with excess BOC-anhydride afforded the *N*-protected

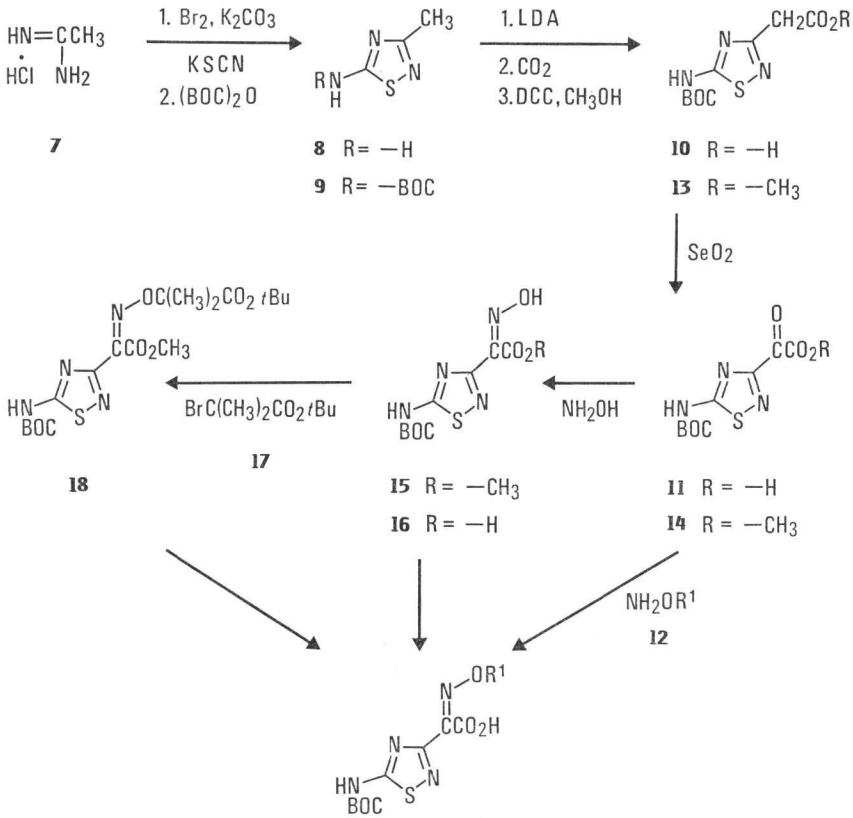
* Amino-1,2,4-thiadiazolyl cephems were studied independently in the Laboratories of Fujisawa[5].

Scheme 1.



2 3 4 5

Scheme 2.



7 8 R = —H 9 R = —BOC 10 R = —H 13 R = —CH3 11 R = —H 14 R = —CH3 15 R = —CH3 16 R = —H 17 18 12

6a $R^1 = -CH_3$
6b $R^1 = -C_2H_5$
6c $R^1 = -n-C_3H_7$
6d $R^1 = -i-C_3H_7$
6e $R^1 = -CH_2CH=CH_2$
6f $R^1 = -n-C_4H_9$
6g $R^1 = -CONHCH_3$
6h $R^1 = -THP$
6i $R^1 = -C(CH_3)_2CO_2t-Bu$

compound (**9**) in 55% yield. The methyl group on the 1,2,4-thiadiazole ring was then metallated (LDA; −78°C; THF) and the 3-lithiomethyl intermediate carboxylated with CO_2 to afford the acetic acid (**10**).

Subsequent conversion of **10** into the α-oxyiminoacetic acids **6a**~**6i** was achieved in two ways: Heating of **10** with selenium dioxide (dioxane; 95°C) resulted in the formation of the α-keto acid (**11**) in 60% yield. Elaboration of **11** into the α-alkoxyimino acids (**6a**~**6f**) proceeded smoothly by treatment of **11** with the corresponding alkoxyamines (**12**). In all cases only the *syn*-isomer was isolated. A different approach was used for the synthesis of the acids **6g** and **6h**.

Conversion of the acid **10** into its methyl ester **13** by the method of HASSNER[11] (methanol; 4-*N*,*N*-dimethylaminopyridine; 82%), followed by selenium dioxide oxidation to the α-keto ester **14** and oximination with hydroxylamine hydrochloride in ethanol afforded the α-hydroxyimino ester **15** (73%). Ester hydrolysis with aqueous KOH and treatment of the α-hydroxyimino acid **16** with methylisocyanate in tetrahydrofurane gave the α-*N*-methylcarbamoyloxyimino acid (**6g**) in good yield (75%).

The α-tetrahydropyranyloxyimino acid (**6h**) was prepared either by treatment of the α-hydroxyiminoacid (**16**) with dihydropyrane in the presence of *p*-toluenesulfonic acid or, more conveniently, by reacting the α-keto acid (**11**) with *O*-tetrahydropyranyloxyamine[12].

2-Carbo-*tert*-butoxy-2-propyloxyimino acid (**6i**) was generated by alkylating the α-hydroxyimino ester (**15**) with *tert*-butyl-2-bromoisobutyrate (**17**) (K_2CO_3; DMSO; 89%), followed by ester cleavage of **18** with aqueous base (2 N NaOH; 70%).

The coupling of the α-oxyiminoacetic acids **6a**~**6i** to the 7β-aminocephalosporin nuclei **19** or **20** was accomplished *via* their acid chlorides, formed with VILSMEYER-reagent[13] at ice-bath temperature (see Scheme 3). The protecting groups were removed with trifluoroacetic acid/anisole (from **21**) or with trifluoroacetic acid alone (from **22**). Nucleophilic displacement of the 3-acetoxymethyl group in **1a** with heterocyclic thiols **23** or with substituted pyridines **24** was performed in the usual way[14] to afford the thiolo compounds **1b** and the pyridinio compounds **1c**, respectively. Cephalosporin derivatives with a *N*-methylcarbamoyloxyimino substituent were only partly stable under the thiolisation conditions. The products of such reactions consisted of mixtures of the *N*-methylcarbamoyloxyimino and the hydroxyimino compounds.

Antimicrobial Activity

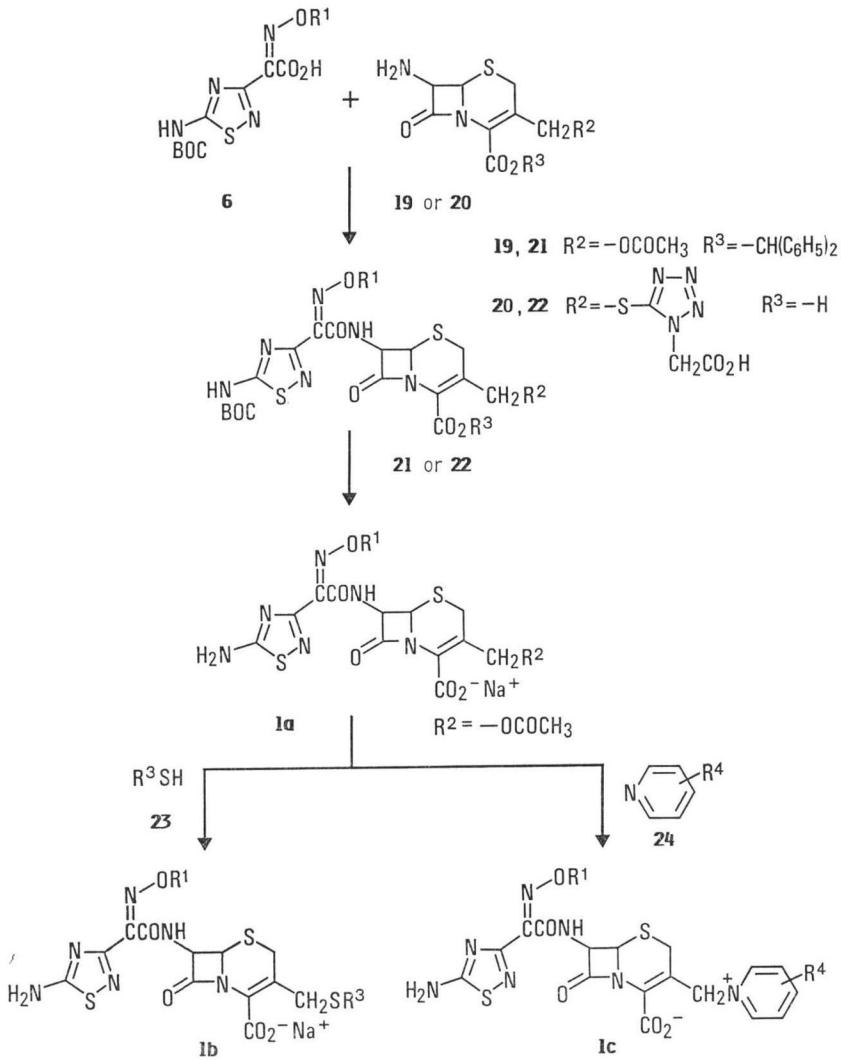
The minimum inhibitory concentration (MIC) values of the 5-amino-1,2,4-thiadiazol-3-yl cephalosporins **1** against selected strains of Gram-positive and Gram-negative bacteria were determined by the agar dilution technique[15]. Cefotaxime and ceftazidime were used as reference compounds.

Table 1 lists the activity of a series of α-methoxyimino cephems **1** with commonly used C-3 substituents. These compounds showed a high intrinsic antibacterial activity, especially against Gram-negative bacteria, and good stability against β-lactamases.

Replacement of the acetoxy-residue in **1a-1** by five-membered hetero-aromatic thiols did not effect significantly the activity against Gram-negative bacteria. However, acidic substituents on the heterocyclic ring tended to decrease the anti-staphylococcal activity. Cephalosporins **1c** featuring a 3-pyridiniomethyl substituent had a well balanced spectrum of activity. They showed exceptionally high inhibitory potency against *Pseudomonas aeruginosa*, comparable to that of ceftazidime and substantially higher than that of cefotaxime.

Introduction of a hydroxymethyl or halogen group into the pyridine ring had a beneficial effect

Scheme 3.



on the anti-staphylococcal activity. Compounds **1c-4**, **1c-5** as well as the isonicotinamide derivative **1c-2** showed a higher overall *in vitro* activity than cefotaxime and ceftazidime.

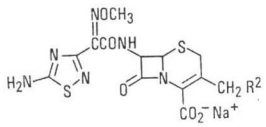
The effect of different oxime oxygen substituents R^1 on the *in vitro* antibacterial potency of the cephems **1** was also examined. The results are shown in Table 2.

In general, both the α-hydroxyimino cephems and the α-*N*-methylcarbamoyloximes (which slowly decomposed to the former compounds) were less active than the corresponding α-methoximes in Table 1.

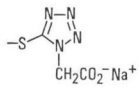
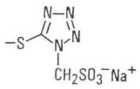
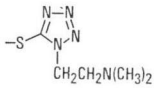
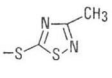
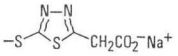
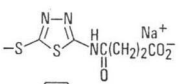
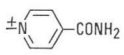
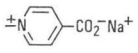
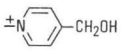
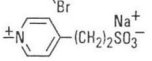
Cephalosporins **1** with an isobutyric acid substituent R^1 displayed only moderate activity against *Staphylococcus aureus*. **1c-7** showed high inhibitory potency against *P. aeruginosa*.

Recent interest in the exploitation of the pharmacokinetic properties of β-lactam antibiotics led us to examine the plasma half-life in mice of selected aminothiadiazolyl cephems **1** (Table 3). Compounds **1** bearing C-3 substituents known to produce high and prolonged serum concentrations in

Table 1. *In vitro* antibacterial activity of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2-*syn*-methoxyimino-acetamido]cephalosporins.

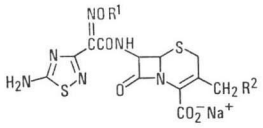


1

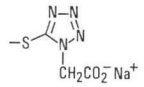
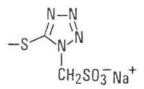
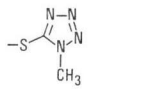
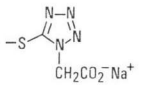
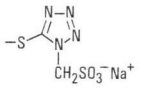
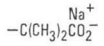
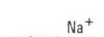
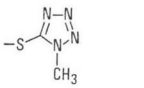
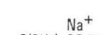
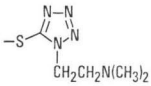
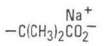
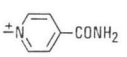
1	R^2	MIC (μg/ml)					
		S. aureus 10 B	*Streptococcus pyogenes* Aronson	*Escherichia coli* 205	*E. coli* 16	*Proteus morganii* 2359	*P. aeruginosa* ATCC 12055
a-1		4	0.05	0.05	0.05	0.05	4
b-1		4	0.05	0.01	0.05	0.05	8
b-2		32	0.5	0.05	0.5	0.05	2
b-3		16	n.d.	0.05	0.5	0.1	2
b-4		4	0.05	0.01	0.1	0.1	4
b-5		2	0.05	0.05	0.5	0.5	16
b-6		4	0.01	0.05	0.2	0.1	16
b-7		8	0.1	0.2	0.5	0.2	8
c-1		8	0.1	0.5	1	2	0.5
c-2		4	0.05	0.05	2	0.5	0.5
c-3		8	0.01	0.05	2	0.5	1
c-4		2	0.1	0.05	0.5	0.1	0.1
c-5		2	0.1	0.1	1	0.2	0.1
c-6		8	0.03	0.5	2	0.1	0.5
	Cefotaxime	2	0.05	0.05	0.1	0.1	4
	Ceftazidime	8	n.d.	0.1	0.2	0.05	0.5

n.d.=not determined.

Table 2. *In vitro* antibacterial activity of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2-oxyiminoacetamido]-cephalosporins.



1

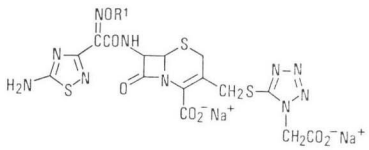
1	R^1	R^2	MIC (μg/ml)					
			S. aureus 10 B	*S. pyogenes* Aronson	*E. coli* 205	*E. coli* 16	*P. morganii* 2359	*P. aeruginosa* ATCC 12055
a-2	$-H$	$-OCOCH_3$	32	1	1	8	2	64
b-8	$-H$		8	0.05	0.05	2	0.1	8
b-9	$-H$		8	0.5	0.1	1	0.1	4
b-10	$-H$		16	0.5	1	4	2	128
a-3	$-CONHCH_3$	$-OCOCH_3$	2	0.05	0.1	0.5	0.5	16
b-11	$-CONHCH_3$		8	0.5	0.05	2	0.01	8
b-12	$-CONHCH_3$		4	0.5	0.1	2	0.1	4
a-4		$-OCOCH_3$	32	1	1	n.d.	2	4
b-13			16	0.1	0.5	n.d.	0.5	4
b-14			64	0.1	0.5	n.d.	0.5	4
c-7			32	0.5	0.5	1	0.5	0.5
	Cefotaxime		2	0.05	0.05	0.1	0.1	4

n.d.: not determined.

Table 3. Half-life of cephalosporins **1** in the plasma of mice after a single subcutaneous administration.

Compound	$t^1/_2$ (hours)	Compound	$t^1/_2$ (hours)
b-1	0.17	**c-2**	0.30
b-2	0.90	**c-4**	0.30
b-3	2.50	**c-7**	0.10
b-8	1.50	Cefotaxime	0.30
b-11	2.10	Ceftazidime	0.20

Table 4. Effect of the oxime-substituent R^1 on the *in vitro* antibacterial activity of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2-*syn*-oxyiminoacetamido]-3-(1-carboxymethyl-1*H*-tetrazol-5-yl) thiomethylcephalosporins.



1b

1	R^1	MIC (μg/ml)					
		S. aureus 10 B	*S. pyogenes* Aronson	*E. coli* 205	*E. coli* 16	*P. morganii* 2359	*P. aeruginosa* ATCC 12055
b-2	$-CH_3$	32	0.5	0.05	0.5	0.05	2
b-8	$-H$	8	0.1	0.1	2	0.1	8
b-11	$-CONHCH_3$	8	0.05	0.1	4	0.1	8
b-15	$-C_2H_5$	8	0.05	0.1	0.2	0.05	1
b-16	$-CH_2CH=CH_2$	8	0.02	0.1	0.5	0.05	n.d.
b-17	$-n-C_3H_7$	4	0.02	0.2	1	0.2	2
b-18	$-i-C_3H_7$	32	0.05	0.5	n.d.	0.5	4
b-19	$-n-C_4C_9$	2	0.02	0.5	1	0.5	8

n.d.: not determined.

experimental animals and in man[16,17] were found to have a much longer plasma half-life than other structural analogues examined.

Because of their excellent *in vitro* antibacterial activity against Gram-negative bacteria and their favorable pharmacokinetic profile, cephalosporins **1b** bearing a 3-(1-carboxymethyltetrazol-5-yl)-thiomethyl substituent were selected for further evaluation. In order to improve their moderate anti-staphylococcal activity, the effect of various oxime substituents was studied. The results are listed in Table 4. Similarly to observations made in the 7β-[(2-aminothiazol-4-yl)-2-alkoxyiminoacetamido]-cephem series[6,7,18], an increase in the chain length of the oxime residue R^1 had no influence on the broadness of the antibacterial spectrum. However, some noteworthy shifts in the MIC values were observed. The α-ethoxyimino compound **1b-15** and the α-allyloxyimino compound **1b-16** showed improved activity against *Staphylococcus aureus*. Against Gram-negative bacteria their inhibitory potency was comparable to the activity of the α-methoxyimino derivative **1b-2**.

Further increase in the lipophilicity of R^1 led to compounds (**1b-17** and **1b-19**) with even higher anti-staphylococcal activity. However, a simultaneous decrease of activity against Gram-negative bacteria was also noted.

Branching of the alkyl chain in immediate vicinity of the oxime oxygen (**1b-18**) had a negative effect on the activity against staphylococci.

Conclusions

Cephalosporins bearing a 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2-*syn*-oxyiminoacetamido] side chain displayed high antibacterial activity. Their *in vitro* inhibitory potency against Gram-negative bacteria was comparable to that of aminothiazolyl cephems.

Selected 5-amino-1,2,4-thiadiazol-3-yl-cephems with suitable C-3 substituents showed a prolonged plasma half-life in mice, significantly longer than that of cefotaxime and ceftazidime. Their moderate

anti-staphylococcal activity has been partly improved through variation of the oxime substituent. Lower MIC-values against *S. aureus* were, however, coupled with decreased activity against Gram-negative bacteria.

The preliminary data presented demonstrate the high overall *in vitro* antimicrobial efficacy of 3-pyridiniomethyl cephems **1c**. Their further evaluation is in progress.

Experimental

The reference cephalosporins, cefotaxime and ceftazidime, were synthesized in our laboratories for comparison purpose.

Infrared spectra were obtained using a Perkin-Elmer apparatus Model 141 (main absorptions given in cm^{-1}). The ^1H NMR spectra were recorded on a Varian HA-100 instrument (100 MHz). The signals are listed in δ values (TMS: δ 0.0). Column chromatography was performed on "Opti UPC_{12}", Antec, reversed phase silica gel, and for thin-layer chromatography Merck PF 254 plates and Opti UPC_{12} plates were used. Melting points are uncorrected.

Antibiotic Susceptibility

All the *in vitro* antibacterial activity is given as the MIC in μg/ml. MIC was determined by conventional agar dilution technique using agar plates DST agar Oxoid enriched with 1% supplement C Difco after incubation at 37°C overnight and an inoculum size of about 10^4 cfu.

Ethyl 3,3-Diamino-2-isothiocyanatoacrylate (**4**)

To a stirred solution of ethyl amidinoacetate hydrochloride (1.0 g, 6.0 mmol) in EtOH (10 ml) were added potassium thiocyanate (0.6 g, 6.0 mmol) and EtOH (20 ml). After stirring for 1 hour at room temperature, the mixture was cooled to −5°C.

After simultaneous addition of a NaOEt solution (prepared from 0.275 g Na and 10 ml EtOH) and of a solution of Br_2 (0.32 ml) in EtOH (10 ml), the resulting mixture was allowed to warm to room temperature, was neutralized with conc. HCl and stirred at room temperature for 1 hour. The separated solids were collected by filtration and washed with EtOH. The filtrate was evaporated, the residue was diluted with EtOAc, washed with H_2O, dried ($MgSO_4$) and evaporated. Trituration with Et_2O afforded the title compound **4** as colorless crystals, 0.6 g. Mp 115~120°C; IR (Nujol) cm^{-1} 3450, 3340, 1640, 1615, 1285; ^1H NMR (DMSO-d_6) δ 1.24 (3H, t, CH_3), 4.10 (2H, q, CH_2), 7.12 (4H, bs, $NH_2\times2$); ^{13}C NMR (20.1 MHz, DMSO-d_6) δ 168.3 and 54.2 (2 olefinic C), 163.9 (CO), 114.2 (SCN), 58.9 and 14.7 (C_2H_5).

2,4-Diamino-5-carbethoxythiazole (**5**)

A solution of **4** (0.15 g) in EtOH (15 ml) was stirred for 16 hours under reflux. The solvent was evaporated and the residue triturated with Et_2O to afford the title compound **5** as colorless crystals. 0.066 g. Mp 113~116°C (decomp.); IR (Nujol) cm^{-1} 3375, 3160, 1665, 1640, 1610, 1333; ^1H NMR (DMSO-d_6) δ 1.22 (3H, t, CH_3), 4.07 (2H, q, CH_2), 6.62 (2H, bs, NH_2), 7.65 (2H, bs, NH_2); ^{13}C NMR (20.1 MHz, DMSO-d_6) δ 170.18 (CO), 163.29 and 78.6 (3-thiazole ring C), 58.32 and 14.63 (C_2H_5).

5-BOC-amino-3-methyl-1,2,4-thiadiazole (**9**)

A suspension of 5-amino-3-methyl-1,2,4-thiadiazole (**8**)[10] (100.0 g, 0.87 mol) in BOC-anhydride (500 ml) was stirred for 12 hours at 100°C and evaporated *in vacuo*. The residue was triturated with MeOH to afford the title compound **9** as colorless crystals, 122.7 g. Mp 136~137°C; IR (Nujol) cm^{-1} 1705, 1560, 1155; ^1H NMR (DMSO-d_6) δ 1.55 (9H, s, $CH_3\times3$), 2.43 (3H, s, CH_3), 11.84 (1H, bs, NH).

2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)acetic Acid (**10**)

To a stirred −78°C cold solution of lithium diisopropylamide (prepared from 26 ml diisopropylamine and 104 ml ~2 N BuLi in hexane) in THF (100 ml) was added a solution of **9** (10.0 g, 0.046 mol) in THF (50 ml). After stirring for 30 minutes at −78°C, the mixture was poured into dry ice (~200.0 g) and allowed to warm to −10°C. H_2O was added, the solution was adjusted to pH 8.5 with 2 N HCl and extracted with Et_2O. The aqueous layer was separated, adjusted to pH 2.0 with 2 N HCl and extracted with EtOAc. The organic layer was separated, washed with brine, dried

Table 5. Spectral and physical properties of compounds **6a**~**6i**.

Compound	Mp (°C)	^{1}H NMR (DMSO-d_6)	IR (Nujol)
6a	140~142	1.50 (9H, s, $CH_3\times3$), 3.96 (3H, s, CH_3), 12.42 (1H, s, NH)	3200, 1745, 1717, 1610, 1040.
6b	148~150	1.27 (3H, t, CH_3), 1.54 (9H, s, $CH_3\times3$), 4.27 (2H, q, CH_2), 12.65 (1H, s, NH)	3180, 1713, 1603, 1550, 1155.
6c	151~154	0.94 (3H, t, CH_3), 1.54 (9H, s, $CH_3\times3$), 1.64 (2H, m, CH_2), 4.17 (3H, t, CH_2), 12.62 (1H, bs, NH), 12.50 (1H, b, COOH)	3250, 1747, 1725, 1600, 1542, 1143.
6d	156~158	1.23 (3H, s, CH_3), 1.30 (3H, s, CH_3), 1.53 (9H, s, $CH_3\times3$), 4.50 (1H, m, CH), 12.71 (1H, s, NH), 13.92 (1H, b, COOH)	3200, 1745, 1610, 1140.
6e	135~136	1.55 (9H, s, $CH_3\times3$), 4.77 (2H, d, CH_2), 5.23 (2H, m, CH_2), 5.99 (1H, m, CH), 12.63 (1H, s, NH), 14.00 (1H, b, COOH)	3157, 1745, 1713, 1545, 1022.
6f	128~130	0.90 (3H, t, CH_3), 1.54 (9H, s, $CH_3\times3$), 1.17~1.70 (4H, m, $CH_2\times2$), 4.23 (2H, t, CH_2), 12.67 (1H, s, NH)	3190, 1720, 1555, 1028.
6g	124~125	1.54 (9H, s, $CH_3\times3$), 2.70 (3H, d, CH_3), 7.48 (1H, q, NH), 7.80 (1H, b, COOH), 12.75 (1H, bs, NH)	3600, 3500, 3200, 1762, 1717, 1550, 1245.
6h	133~134	1.58 (9H, s, $3CH_3$), 1.80 (6H, b, $CH_2\times3$), 3.75 (2H, b, CH_2), 5.44 (1H, s, CH), 12.20 (1H, b, COOH)	3210, 1715, 1552, 1158.
6i	180~183 (decomp.)	1.39 (6H, s, $CH_3\times2$), 1.52 (9H, s, $CH_3\times3$), 12.72 (1H, bs, NH), 13.95 (1H, b, COOH)	3230, 1740, 1722, 1615, 1555, 1150.

($MgSO_4$) and evaporated. The residue was triturated with Et_2O to afford the title compound **10** as a powder, 5.7 g. Mp 163~165°C; IR (Nujol) cm^{-1} 3200, 1735, 1710, 1550; NMR (DMSO-d_6) δ 1.55 (9H, s, $CH_3\times3$), 3.77 (2H, s, CH_2), 13.65 (1H, b, COOH).

2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-oxoacetic Acid (**11**)

To a solution of **10** (5.0 g, 19.25 mmol) in dioxane (100 ml) was added selenium dioxide (4.2 g). After stirring for 3 hours at 85°C the mixture was filtered and the filtrate was evaporated. The residue was diluted with EtOAc and extracted with 2 N $NaHCO_3$. The aqueous layer was separated, adjusted to pH 2.0 with 2 N HCl and extracted with EtOAc. The organic layer was separated, washed with brine, dried ($MgSO_4$) and evaporated. The residue was triturated with Et_2O to afford the title compound **11** as a powder, 3.4 g. Mp 180~185°C (decomp.); IR (Nujol) cm^{-1} 3200, 1720, 1625, 1555; NMR (DMSO-d_6) δ 1.56 (9H, s, $CH_3\times3$), 13.15 (1H, bs, COOH).

General Preparation of 2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-alkoxyiminoacetic Acids (**6a**~**6f**)

To a mixture of **11** (3.4 g, 12.4 mmol) and pyridine (1.1 ml) in 95% EtOH (70 ml) was added the appropriate *O*-alkoxyamine hydrochloride **12** (13.35 mmol). After stirring at room temperature for 2~5 hours, the mixture was evaporated. The residue was diluted with EtOAc and extracted with H_2O. The aqueous layer was separated, adjusted to pH 2.0 with 2 N HCl and extracted with EtOAc. The organic layer was separated, washed with brine, dried ($MgSO_4$) and evaporated. Trituration of the residue with CH_2Cl_2 or Et_2O afforded the title compounds **6** as a powder (Table 5).

Methyl 2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)acetate (**13**)

To a suspension of **10** (30.0 g, 0.115 mol) in CH_2Cl_2 (350 ml) were added *N*,*N*-dicyclohexylcarbodiimide (26.16 g), 4-*N*,*N*-dimethylaminopyridine (1.47 g) and MeOH (5.13 ml). After stirring for 3 hours at room temperature, further 2.6 g *N*,*N*-dicyclohexylcarbodiimide and 0.5 ml MeOH were added, the mixture was stirred for an additional hour, filtered and the filtrate was washed successively

with 2 N $NaHCO_3$, H_2O and brine, dried ($MgSO_4$) and evaporated. Trituration of the residue with Et_2O afforded **13** as a powder, 24.9 g. Mp 107~109°C; IR (CH_2Cl_2) cm^{-1} 3350, 1737, 1720, 1545, 1155. NMR ($CDCl_3$) δ 1.61 (9H, s, $CH_3\times3$), 3.75 (3H, s, OCH_3), 4.04 (2H, s, CH_2), 11.00 (1H, bs, NH).

Methyl 2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-oxoacetate (**14**)

To a solution of **13** (47.5 g, 0.174 mol) in dioxane (700 ml) was added selenium dioxide (52.13 g). After stirring for 3 hours at 95°C, the mixture was filtered and the filtrate evaporated *in vacuo*. The residue was taken up into EtOAc, washed successively with H_2O, 2 N $NaHCO_3$ and brine, dried ($MgSO_4$) and evaporated to afford **14** as a foam, 40.6 g. IR (CH_2Cl_2) cm^{-1} 3350, 1745, 1720, 1715, 1540; NMR (DMSO-d_6) δ 1.53 (9H, s, $CH_3\times3$), 3.95 (3H, s, OCH_3).

Methyl 2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-hydroxyiminoacetate (**15**)

To a solution of **14** (23.0 g, 0.08 mol) and pyridine (12.7 ml) in 95% EtOH (300 ml) was added hydroxylamine hydrochloride (9.4 g). After stirring for 3 hours at room temperature, the mixture was concentrated *in vacuo*. The residue was taken up into EtOAc and washed successively with 1 N HCl, H_2O and brine, dried ($MgSO_4$) and evaporated. The residue was triturated with Et_2O to afford **15** as colorless crystals, 18.31 g. Mp 131~132°C; IR (CH_2Cl_2) cm^{-1} 3200, 1742, 1720, 1545, 1115; NMR (DMSO-d_6) δ 1.54 (9H, s, $CH_3\times3$), 3.94 (3H, s, CH_3), 12.46 (1H, bs, NH).

2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-hydroxyiminoacetic Acid (**16**)

To a solution of **15** (30.0 g, 0.099 mol) in 95% EtOH (6.75 ml) was added a solution of potassium hydroxide (32.6 g) in H_2O (270 ml). After stirring for 6.5 hours at 40°C the mixture was concentrated *in vacuo*. The residue was diluted with H_2O, adjusted to pH 8.5 with 2 N HCl and extracted with EtOAc. The separated aqueous layer was adjusted to pH 2.0 with 2 N HCl and extracted with EtOAc. The organic layer was separated, washed with brine, dried ($MgSO_4$) and evaporated. The residue was triturated with Et_2O to afford the title compound **16** as colorless crystals, 23.85 g. Mp 158~160°C; IR (KBr) cm^{-1} 3400, 3175, 1710, 1500, 1245, 1055; NMR (DMSO-d_6) δ 1.50 (9H, s, $CH_3\times3$), 12.45 (1H, bs, NH), 12.60 (1H, b, COOH).

2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-*N*-methylcarbamoyloxyiminoacetic Acid (**6g**)

To a 0°C cold solution of **16** (23.65 g, 0.082 mmol) in THF (200 ml) was added dropwise methyl isocyanate. After stirring for 2 hours at 0°C, *n*-hexane was added, and the precipitate formed was collected by suction. Recrystallization from CH_2Cl_2 afforded the title compound **6g** as colorless crystals, 21.45 g (Table 5).

Methyl 2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-(2-*tert*-butyloxycarbonylprop-2-yloxyimino)-acetate (**18**)

To a stirred solution of *tert*-butyl 2-bromoisobutyrate (**17**, 10.8 g, 0.048 mol) and K_2CO_3 (14.95 g) in DMSO (95 ml) was added a solution of **15** (13.1 g, 0.043 mol) in DMSO (60 ml). After stirring for 4 hours at room temperature, the mixture was concentrated *in vacuo*. The residue was taken up into EtOAc, washed successively with H_2O, 1 N HCl and brine, dried ($MgSO_4$) and evaporated. The residue was triturated with Et_2O to afford the title compound **18** as colorless crystals, 9.86 g. Mp 139~141°C; IR (CH_2Cl_2) cm^{-1} 3340, 1745, 1722, 1545, 1150; NMR (DMSO-d_6) δ 1.39 (6H, s, $CH_3\times2$), 1.52 (9H, s, $CH_3\times3$), 3.89 (3H, s, CH_3), 13.22 (1H, bs, NH).

2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-(2-*tert*-butyloxycarbonylprop-2-yl-oxyimino)acetic Acid (**6i**)

To a solution of **18** (11.0 g, 22.55 mmol) in MeOH (290 ml) was added 2 N NaOH (96 ml). After stirring for 6.5 hours at 50°C the mixture was concentrated *in vacuo*. The residue was taken up in H_2O, adjusted to pH 8.5 with 2 N HCl and extracted with EtOAc. The aqueous layer was separated, adjusted to pH 2.0 with 2 N HCl and extracted with EtOAc. The organic layer was separated, washed with brine, dried ($MgSO_4$) and evaporated to afford the title compound **6i** as a powder, 9.47 g (Table 5).

2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-tetrahydropyranyloxyiminoacetic Acid (**6h**)

a) To a solution of **16** (10.0 g, 34.60 mmol) in dioxane (100 ml) was added 3,4-dihydropyrane (8 ml) and *p*-toluenesulfonic acid (0.25 g). After stirring for 24 hours at room temperature, further

Table 6. Spectral data of compounds **1a**.

Compound	Solvent	^{1}H NMR (100 MHz, δ)						IR (Nujol)		
		CH_3CO	C_2–CH_2	C_3–CH_2	C_6–H	C_7–H	Other protons	NH	β-Lactam	CO_2^-
1a-1	D_2O	2.28	3.75	4.90	5.42	6.06	4.26 (OCH_3)	3330	1770	1620
1a-2	D_2O	2.13	3.55	4.75	5.23	5.83		3300	1752	1605
1a-3	DCOOD	2.18	3.75	5.18	5.37	6.15	3.00 ($NHCH_3$)	3325	1765	1610
1a-4	DMSO-d_6	2.02	3.38	4.92	5.06	5.76	1.50 ($CH_3\times2$)	3300	1755	1600

3,4-dihydropyrane (4 ml) and *p*-toluenesulfonic acid (0.1 g) were added, and the mixture was stirred for additional 24 hours at room temperature. The mixture was concentrated *in vacuo*, the residue was taken up in EtOAc, washed with brine, dried ($MgSO_4$) and evaporated to afford the title compound **6h** as a powder (Table 5).

b) **6h** was also obtained from 2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-oxoacetic acid (**11**, 5.50 g) and *O*-tetrahydropyranyloxyamine[12] (3.50 g) following the general procedure described for the preparation of compounds **6a**~**6f**.

General Preparation of Diphenylmethyl 7β-[2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-oxyimino-acetamido]-3-acetoxymethyl-3-cephem-4-carboxylate (**21**)

To a −10°C cold suspension of oxalylchloride (4.5 ml) and *N*,*N*-dimethylformamide (4.0 ml) in EtOAc (30 ml) was added a solution of 2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-oxyiminoacetic acid (**6**, 4.30 mmol) in EtOAc (150 ml), and the mixture was stirred for 30 minutes at −10 to −5°C. Benzhydryl 7β-amino-3-acetoxymethyl-3-cephem-4-carboxylate (**19**, 4.30 mmol) was added, the mixture was stirred for 2 to 3 hours at −10 to −5°C, poured into a cold solution of 2 N $NaHCO_3$, the aqueous layer was separated, adjusted to pH 2.0 with 2 N HCl and extracted with EtOAc. The organic layer was separated, washed with brine, dried ($MgSO_4$) and evaporated to afford the title compounds **21** as a foam.

21a (R^1=–CH_3): IR (CH_2Cl_2) cm^{-1} 3400, 3270, 1775, 1725, 1690, 1045.

21b (R^1=–$CH(CH_3)_2CO_2tBu$): IR (CH_2Cl_2) cm^{-1} 3350, 1780, 1723, 1697, 1025.

21c (R^1=–$CONHCH_3$): IR (CH_2Cl_2) cm^{-1} 3375, 3190, 1765, 1723, 1695, 1042.

General Preparation of Sodium 7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-oxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylates (**1a**)

To a mixture of benzhydryl 7β-[2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-oxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylate (5.28 mmol) in CH_2Cl_2 (9 ml) were added trifluoroacetic acid (39 ml) and anisole (2.8 ml). The mixture was stirred for 30 minutes at room temperature and poured into a cold solution of diethyl ether - hexane, 1: 2. The precipitate formed was filtered, dissolved in MeOH, and the solution was adjusted to pH 7.0 with a methanolic solution of sodium 2-ethylhexanoate. The precipitate formed after addition of Et_2O was filtered and subjected to column chromatography on an Opti UPC_{12} reversed phase silica gel column. Elution with H_2O followed by lyophilization of the product-containing fractions afforded the title compounds **1a** (Table 6).

General Preparation of 7β-[2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-oxyiminoacetamido]-3-(1-carboxymethyl-1*H*-tetrazol-5-yl-thiomethyl)-3-cephem-4-carboxylic Acids (**22**)

To a −5°C cold mixture of oxalylchloride (0.6 ml) and *N*,*N*-dimethylformamide (0.54 ml) in CH_2Cl_2 (20 ml) were added 2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-oxyiminoacetic acid (**6**, 6.0 mmol) and *N*-methylmorpholine (0.84 ml). The mixture was stirred for 30 minutes at −5°C

Table 7. IR spectral data of compounds **22**.

R^1	IR (Nujol)		
	NH	β-Lactam	COOR
–CH_3	3400	1770	1725, 1600
–C_2H_5	3350	1770	1722, 1600
–$CH_2CH=CH_2$	3350	1770	1720, 1630
–*n*-C_3H_7	3350	1765	1735, 1675
–*i*-C_3H_7	3325	1770	1725, 1610
–*n*-C_4H_9	3350	1775	1727, 1615

–COOR includes: –COO-*t*-C_4H_9 and –COOH.

Table 8. Spectral data of compounds **1b**.

Solvent	^{1}H NMR					IR (Nujol)		
	C_2–CH_2	C_3–CH_2	C_6–H	C_7–H	Other protons	NH	β-Lactam	CO
D_2O	3.64	4.18	5.19	5.82	4.02 (OCH_3), 4.08 (NCH_3)	3300	1760	15
D_2O	3.80	4.37	5.17	5.88	4.07 (OCH_3), 5.26 (NCH_2CO_2)	3335	1770	16
$CD_3OD+DCl$	3.80	4.42	5.19	5.88	4.16 (($OCH_3)_2$), 4.08 (OCH_3)	3400	1765	16
D_2O	3.72	4.22	5.20	5.81	2.90 ($N(CH_3)_2$), 4.08 (OCH_3)	3330	1770	16
D_2O	3.58	4.30	5.18	5.85	2.51 (CH_3), 4.06 (OCH_3)	3330	1760	16
D_2O	3.76	4.42	5.21	5.88	2.77 (CH_2CO_2), 4.15 (OCH_3)	3400	1760	16
D_2O	3.62	4.20	5.20	5.82	2.68 (CH_2CO_2), 4.08 (OCH_3)	3325	1765	16
D_2O	3.63	4.28	5.23	5.87	5.05 (NCH_2CO_2)	3380	1760	16
D_2O	3.90	4.59	5.46	6.15	5.82 (NCH_2SO_3)	3406	1755	16
D_2O	3.64	4.18	5.14	5.75	4.04 (NCH_3)	3350	1760	16
D_2O	3.56	4.28	5.24	5.88	2.88 (NCH_3), 5.06 (NCH_2CO_2)	3400	1760	16
DCOOD	3.90	4.58	5.47	6.15	3.13 (NCH_3), 5.79 (NCH_2SO_3)	3400	1765	16
D_2O	3.64	4.04	5.19	5.81	1.51 ($C(CH_3)_2$), 4.02 (NCH_3)	3400	1767	16
DCOOD	3.98	4.50	5.39	6.15	1.75 ($C(CH_3)_2$), 3.27 ($N(CH_3)_2$)	3325	1770	16
D_2O	3.64	4.27	5.22	5.83	1.36 (CH_3), 4.39 (CH_2)	3380	1760	16
D_2O	3.64	4.27	5.22	5.84	4.84 (OCH_2), 5.40 (CH_2), 6.15 (CH)	3320	1757	16
D_2O	3.64	4.26	5.22	5.83	0.95 (CH_3), 1.77 (CH_2), 4.29 (OCH_2)	3390	1760	16
D_2O	3.64	4.28	5.23	5.83	1.18 ($CH_3\times2$)	3420	1765	16
D_2O	3.66	4.28	5.22	5.83	0.92 (CH_3), 4.35 (OCH_2)			

(mixture A). On the other hand *N,O-bis*-trimethylsilylacetamide (6.6 ml) was added to a suspension of cephem nucleus **20** (6.6 mmol) in CH_2Cl_2 (30 ml), and the mixture was stirred for 15 minutes at room temperature to make a clear solution. This was cooled to $-10°C$ and added to mixture A. After addition of *N*-methylmorpholine (0.84 ml), the reaction mixture was stirred for 3 hours at -5 to $0°C$. The CH_2Cl_2 was evaporated, the residue was diluted with EtOAc and extracted with 2 N $NaHCO_3$. The aqueous layer was separated, adjusted to pH 2.0 with 2 N HCl and extracted with EtOAc. The organic layer was separated, washed with brine, dried ($MgSO_4$) and evaporated. The crude product **22** (see Table 7) was used without further purification.

General Preparation of Sodium 7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-alkoxyiminoacetamido]-3-(1-carboxymethyl-1*H*-tetrazol-5-yl-thiomethyl)-3-cephem-4-carboxylates (**1b-15**$\sim$**1b-19**)

To a mixture of 7β-[2-(5-BOC-amino-1,2,4-thiadiazol-3-yl)-2-alkoxyiminoacetamido]-3-(1-carboxymethyl-1*H*-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid (**22**, 2.7 mmol) in CH_2Cl_2 (13 ml) was added trifluoroacetic acid (26 ml). The mixture was stirred for 30 minutes at room temperature and poured into a solution of diethyl ether - hexane, 1 : 2. The precipitate formed was filtered, dissolved in MeOH, and the solution was adjusted to pH 7.0 with a methanolic solution of sodium 2-ethylhexanoate. The precipitate formed after addition of Et_2O was filtered and subjected to column chromatography on an Opti UPC_{12} reversed phase silica gel column. Elution with H_2O followed by lyophilization of the product-containing fractions afforded the title compounds **1b-15**$\sim$**1b-19** (Table 8).

General Preparation of Sodium 7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-oxyiminoacetamido]-3-thiomethyl-3-cephem-4-carboxylates (**1b**)

A mixture of sodium 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2-oxyiminoacetamido]-3-acetoxymethyl]-3-cephem-4-carboxylate (**1a**, 3.0 mmol) and the corresponding aromatic thiol **23** (3.6 mmol) in aqueous $NaHCO_3$ was stirred for 2.5 to 5 hours at $70°C$. The pH was kept constant at 6.0 to 6.5 by addition of solid $NaHCO_3$, as needed. The mixture was allowed to cool at room temperature, was extracted with EtOAc, the aqueous layer was separated, concentrated and subjected to column chromatography on an Opti UPC_{12} reversed phase silica gel column. Elution with H_2O followed by lyophilization of the product-containing fractions afforded the title compounds **1b** (Table 8).

General Preparation of 7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-oxyiminoacetamido]-3-pyridiniomethyl-3-cephem-4-carboxylates (**1c**)

To a solution of sodium 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2-oxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylate (**1a**, 4.0 mmol) in H_2O (4 ml) were added sodium iodide (44 mmol) and the corresponding pyridine compound **24**, and the mixture was stirred for 2 hours at $80°C$. After cooling at room temperature, the mixture was poured into cold acetone (300 ml). The precipitate formed was collected by suction and subjected to column chromatography on an Opti UPC_{12} reversed phase silica gel column. Elution with H_2O followed by lyophilization of the product-containing fractions afforded the title compounds **1c** (Table 9).

Table 9. Spectral data of compounds **1c**.

Compound	Solvent	^{1}H NMR					IR (Nujol)		
		C_2–CH_2	C_3–CH_2	C_6–H	C_7–H	Aromatic protons	NH	β-Lactam	COO^-
1c-1	D_2O	3.48	5.50	5.29	5.88	8.15, 8.62, 9.02	3380	1770	1617
1c-2	D_2O	3.42	5.55	5.27	5.89	8.37, 9.14	3350	1765	1610
1c-3	D_2O	3.44	5.52	5.30	5.90	8.30, 9.02	3325	1765	1620
1c-4	DCOOD	3.68	5.80	5.45	6.17	8.25, 9.04	3300	2775	1610
1c-5	D_2O	3.44	5.52	5.30	5.90	8.12, 8.66, 9.02, 9.25	3304	1773	1613
1c-6	D_2O	3.52	5.43	5.28	5.90	8.02, 9.02	3400	1767	1610
1c-7	D_2O	3.48	5.57	5.33	5.93	8.43, 9.22	3350	1770	1610

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