THE SYNTHESIS AND BIOLOGICAL EVALUATION OF 7β-[2-(2-AMINOOXAZOL-4-YL)-2-*Z*-METHOXIMINOACETAMIDO]-CEPHALOSPORIN ANTIBIOTICS

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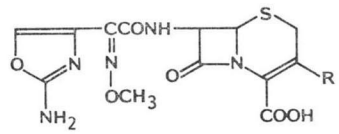
7β-[2-(2-Aminooxazol-4-yl)-2-*Z*-methoximinoacetamido]-3-cephem-4-carboxylic acids **12** and **13** were synthesized. The microbiological activity of **12** and **13** as well as the β-lactamase stability of **12** were discussed. Both **12** and **13** were quite active against a wide variety of microorganisms although usually less active than cefotaxime.

Within the past few years several new cephalosporin antibiotics have been studied clinically which bear the 2-(2-aminothiazol-4-yl)-2-*Z*-methoximinoacetamido side chain in the 7-position of the cephem nucleus[1~4]. These compounds have similar antimicrobial spectra with changes in the structure at C-3 usually having only minor effects on the *in vitro* activity. In some cases the C-3 substituent has had rather profound effects on the pharmacokinetics of these compounds, however[5].

Recent reports in the literature concerning the synthesis and antimicrobial evaluation of novel heterocyclic methoxime cephalosporins[6,7] have prompted us to share the results of our investigations on aminooxazole methoxime cephalosporin antibiotics of the general structure shown in **1**.



1

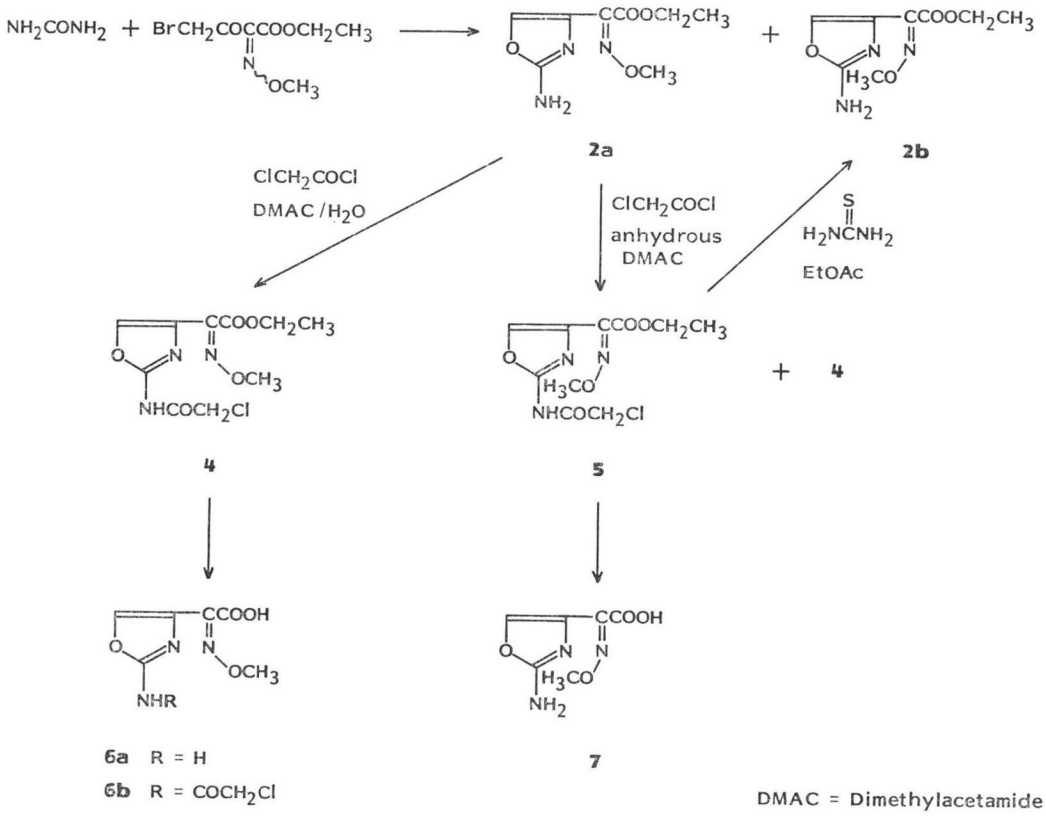
Chemistry

Initial approaches directed towards the synthesis of ethyl 2-(2-aminooxazol-4-yl)-2-*Z*-methoximinoacetate (**2a**) involved reaction of ethyl 4-bromo-2-methoximinoacetoacetate (**3**) with urea, both with and without catalysis by silver salts and cuprous oxide. These were unsuccessful, although there were ample precendents in the literature for the synthesis of aminooxazoles by the reaction of urea with α-haloketones[8]. The low reactivity of **3** towards urea was overcome by conducting the reaction in the presence of zinc oxide[9], although the yield of **2** was still far from ideal (Scheme 1). Chromatography of the crude reaction mixture over silica gel yielded the desired **2a** as a crystalline solid.

The assignment of the structure of one of the methoxime geometrical isomers in the absence of the other is difficult. When both isomers are available, however, there are significant differences observed in the NMR resonances of the methoxime methyl as well as the 5H of the aminooxazole methoxime isomers†. The chemical shift for the resonance of the *Z*-methoxime methyl occurs at

† Similar differences in chemical shifts have been reported for the methoxime methyl and thiazole methine of the corresponding aminothiazole methoxime acetic acids[10].

Scheme 1.

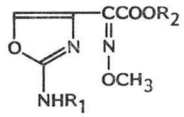


lower field than the corresponding *E*-methoxime methyl. In addition the 5H-oxazole resonance for the *Z*-methoxime is farther upfield than that of the *E*-methoxime.

Base hydrolysis of the unprotected aminoester, **2a**, resulted in substantial decomposition[11]. Protection of **2a** by reaction with tritylchloride - triethylamine was unsuccessful. Acylation of **2a** with chloroacetyl chloride in anhydrous dimethylacetamide (DMAC) resulted in significant scrambling of the methoxime geometry, yielding both the *Z*- and *E*-isomers (**4** and **5**); however, acylation in wet DMAC proceeded smoothly yielding only **4** (and unreacting starting material, **2a**). Subsequent hydrolysis with 2 equivalents of sodium hydroxide yielded the desired amino acid **6a**. Attempted hydrolysis of **4** to the chloroacetyl protected amino acid **6b** by using only one equivalent of base yielded a mixture of **6a** and unreacted starting material. Further attempts to synthesize **6b** were not made. Removal of the chloroacetyl-protecting group from **5** with thiourea provided a reference sample of the *E*-aminoester **2b**. Subsequent hydrolysis of **2b** was unsuccessful; however, hydrolysis of **5** with 2 equivalents of sodium hydroxide yielded the *E*-amino acid **7**.

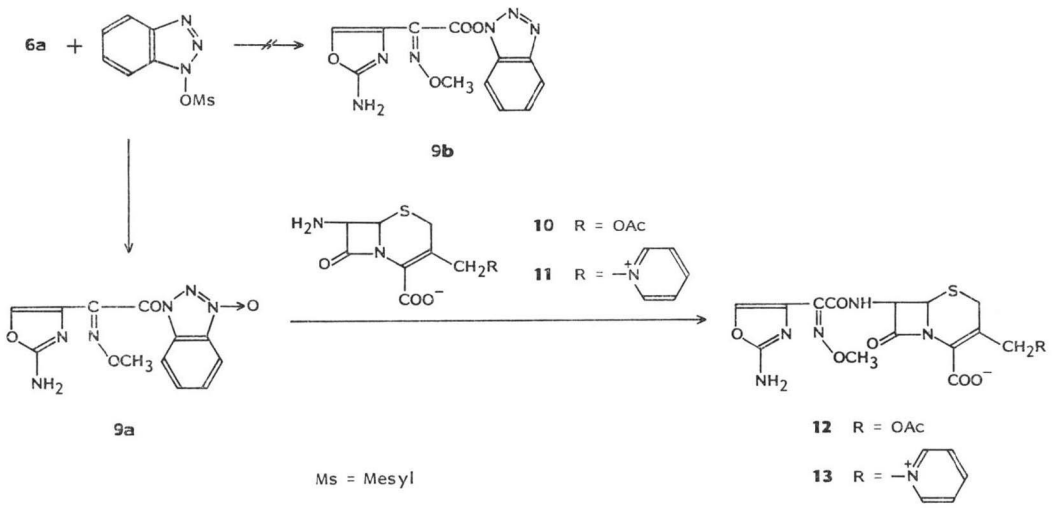
The unprotected amino acid **6a** was activated by reaction with HBT-mesylate (**8**) (prepared *in situ* by the reaction of 1-hydroxybenzotriazole (HOBT) with mesyl chloride in the presence of triethylamine) in DMAC. Acylation of HOBT can potentially occur at either nitrogen or oxygen. HORIKI established that isomerization of the resulting *N*-acyl and *O*-acyl products occurs in solution and the equilibrium was determined by the nature of the solvents used[12]. HORIKI further showed that with several different protected amino acids, one regio-isomer was exclusively obtained when reacted with

Table 1. Effect of methoxime geometry on the chemical shifts of the methoxime methyl and oxazole C-5 proton.



Compound	R_1	R_2	NMR δ (in ppm)		
			Solvent	Methoxime	Oxazole C-5
E-**2b**	H	C_2H_5	$CDCl_3$	4.20	7.90
Z-**2a**	H	C_2H_5	$CDCl_3$	4.95	7.27
E-**5**	$ClCH_2CO$	C_2H_5	$CDCl_3$	3.97	7.67
Z-**4**	$ClCH_2CO$	C_2H_5	$CDCl_3$	4.00	7.30
E-**7**	H	H	DMSO-d_6	3.90	7.78
Z-**6a**	H	H	DMSO-d_6	3.84	7.48

Scheme 2.



HOBT - *N,N'*-dicyclohexylcarbodiimide (DCC). The site of acylation was clearly evident from the IR spectra (*N*-acyl carbonyl occurred at ~1735 cm^{-1} while that of the *O*-acylated product occurred at ~1825 cm^{-1}). When **6a** was reacted with HBT-mesylate, only the *N*-acylated product **9a** was isolated (carbonyl 1734 cm^{-1}).

Acylation (Scheme 2) of cephem nucleus (**10**, **11**) with the activated amino acid **9a** yielded the corresponding cephalosporins (**12**, **13**).

Antimicrobial Activity

Using cefotaxime as a reference compound, the minimum inhibitory concentrations (MIC) of **12** and **13** against a variety of Gram-positive and Gram-negative bacteria were determined by an agar dilution technique[13]. These results are outlined in Table 2. Against penicillin-sensitive and penicillin-resistant *Staphylococcus aureus*, the activity of **12** and **13** were comparable to that of cefotaxime. The activities of **12** and **13** as well as cefotaxime against *Streptococcus pyogenes* and *S. pneumoniae* were extraordinary. As is the case with most cephalosporins, the activity of **12** and **13**

Table 2. *In vitro* antibacterial activity of aminooxazole methoxime cephalosporins.

		Agar dilution MIC (μg/ml)		
		Cefotaxime	**12**	**13**
Staphylococcus aureus	XI.1	2	2	1
〃	V41	4	4	2
〃	X400	128	128	32
〃	S13E	8	16	4
S. epidermidis	EPI1	8	4	2
〃	EPI2	32	64	16
Streptococcus pyogenes	C203	0.015	0.03	0.015
S. pneumoniae	PARK	0.015	0.015	0.03
Enterococcus faecalis	X66	128	>128	32
〃	9960	4	32	32
Haemophilis influenzae	BRUN	0.03	0.125	0.5
〃	251	0.03	0.125	0.5
Shigella sonnei	N9	0.06	0.25	0.25
Escherichia coli	N10	0.125	0.5	0.5
〃	EC14	0.06	0.125	0.25
〃	TEM	0.03	0.06	1
Klebsiella pneumoniae	X26	<0.008	0.03	0.25
〃	KAE	2	64	128
Enterobacter aerogenes	X68	0.06	0.125	0.5
〃	EB17	0.125	0.5	0.5
E. cloacae	EB5	0.25	1	1
〃	265A	128	>128	128
Salmonella heidelberg	X514	0.125	0.25	0.25
〃	1335	0.125	0.5	0.5
Pseudomonas aeruginosa	X528	16	128	128
〃	X239	16	64	16
〃	PS18	32	128	16
Serratia marcescens	X99	0.5	8	1
〃	SE3	1	16	1
Morganella morganii	PR15	0.25	2	4
Providencia stuartii	PR33	0.06	0.125	0.5
P. rettgeri	PR7	<0.008	0.015	0.03
〃	C24	0.25	5	1
Citrobacter freundii	CF17	2	8	4

Table 3. β-Lactamase stabilities of various cephalosporins.

Enzyme source	Relative hydrolysis rates		
	Cephaloridine	Cefotaxime	**12**
Staphylococcus aureus S13E	100	10.80	18.57
Escherichia coli TEM	100	0.18	0.43
Klebsiella pneumoniae KAE	100	2.50	12.18
Enterobacter cloacae 265A	100	0.06	0.06
Pseudomonas aeruginosa Ps18	100	0.09	0.31

against methicillin-resistant *S. aureus* and enterococci was negligible. This is also the case with cefotaxime.

While **12** and **13** were quite active against *Haemophilus influenzae* and the enterobacteriaceae, cefotaxime was generally 2~3 times more active. The activity of **12** and **13** against *Pseudomonas aeruginosa* was disappointing when compared to that of cefotaxime.

The aminooxazole cephalosporin **12** was found to be stable against the action of β-lactamases from several bacteria (Table 3). Similar trends in β-lactamase stability were observed with cefotaxime.

Experimental

IR spectra were measured on a Nicolet MX-10 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian EM-390 (90 MHz) spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are reported in parts per million relative to TMS. UV spectra were determined on a Carry Model 219 spectrophotometer in the solvent indicated. Mass spectral data was determined on a CEC 21-110 spectrometer. All melting points are reported uncorrected. Agar dilution MIC's were determined by the method described by KIRST *et al.*[13]. β-Lactamase stability was determined according to method of MAHONEY *et al.*[14].

Ethyl 2-(2-Aminooxazol-4-yl)-2-*Z*-methoximinoacetate (**2a**)

A methylethylketone solution (3,000 ml) of **3** (100 g, 0.397 mol), urea (91 g, 1.98 mol), and zinc oxide (16 g, 0.198 mol) was stirred under reflux for 48 hours, then allowed to cool to room temp. The mixture was filtered to remove excess urea and concentrated to a dark oil *in vacuo*. The residual oil was redissolved in EtOAc and filtered to remove the last traces of urea. The filtrate was evaporated and the residue was chromatographed over activity III Alumina (Mallinckrodt). After eluting the impurities with cyclohexane (1,000 ml), EtOAc - cyclohexane, 1:9 (1,000 ml), EtOAc - cyclohexane, 2:8 (2,000 ml) and EtOAc - cyclohexane, 3:7 (500 ml), the product was eluted with EtOAc - cyclohexane, 1:1. The crude product was crystallized from Et_2O to yield **2** (10.4 g, 12.3%), mp 138~140°C. *Anal* Calcd for $C_8H_{11}N_3O_4$: C 45.07, H 5.20, N 19.71. Found: C 45.05, H 5.37, N 19.40. UV λ_{max}^{MeOH} 217 (ε 18,873), 275 nm (3,370); IR ($CHCl_3$) 1870 cm^{-1}; MS, 213 (M^+); NMR ($CDCl_3$) δ 1.40 (3H, t, CH_3 ester), 4.00 (3H, s, OCH_3), 4.40 (2H, q, OCH_2), 5.67 (2H, br s, NH_2), and 7.3 (1H, s, aromatic).

Ethyl 2-(2-Chloroacetylaminooxazol-4-yl)-2-*E*-methoximinoacetate (**5**)

Neat chloroacetyl chloride (3.57 ml, 41.8 mmol) was added dropwise to a DMAC solution (90 ml) of **2** (8.9 g, 41.8 mmol) and triethylamine (TEA, 5.82 ml, 41.8 mmol) which was chilled to 0°C. After the addition was complete, stirring was continued in the cold for 0.5 hour and 2 additional hours at room temp. The reaction mixture was poured into H_2O and extracted with EtOAc. The EtOAc solution was washed with H_2O, dried ($MgSO_4$) and concentrated *in vacuo*. Crystallization from EtOAc yield **5** (2.1 g, 17.5%): mp 153~154°C. *Anal* Calcd for $C_{10}H_{13}N_3O_5$: C 41.32, H 4.51, N 14.46. Found: C 41.35, H 4.07, N 14.34. NMR ($CDCl_3$) δ 1.31 (3H, t, ester CH_3), 3.97 (3H, s, OCH_3), 4.32 (2H, q, OCH_2), 4.22 (2H, s, CH_2Cl), 5.30 (1H, br s, NH), and 7.62 (1H, s, aromatic).

Ethyl 2-(2-Chloroacetylaminooxazol-4-yl)-2-*Z*-methoximinoacetate (**4**)

Chloroacetyl chloride (0.29 ml, 3.14 mmol) was dissolved in anhydrous DMAC and added dropwise to a 90% aqueous DMAC solution (8 ml) of **2** (0.5 g, 2.85 mmol) and TEA (0.44 ml, 3.14 mmol); the resulting solution was stirred at room temp overnight. The mixture was poured into ice and extracted twice with EtOAc. The combined EtOAc extracts were combined, washed with H_2O, dried ($MgSO_4$) and concentrated *in vacuo*. The crystalline residue was triturated with Et_2O and filtered. The filtrate was concentrated and recrystallized from CCl_4 to yield **4**; mp 91~92°C. *Anal* Calcd for $C_{10}H_{13}ClN_3O_5$: C 41.32, H 4.51, N 14.46. Found: C 41.27, H 4.45, N 14.54. NMR ($CDCl_3$) δ 1.32 (3H, t, ester CH_3), 4.0 (3H, s, OCH_3), 4.1 (2H, s, CH_2Cl), 4.37 (2H, q, OCH_2), and 7.25 (1H, s, aromatic).

Ethyl 2-(2-Aminooxazol-4-yl)-2-*E*-methoximinoacetate (**2b**)

An EtOAc solution (25 ml) of **5** (0.250 g, 0.86 mmol) and thiourea (0.054 g, 0.86 mmol) was mixed and stirred under reflux for 4 hours during which time a precipitate of 2-amino-4-thiazalone formed. The mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was charcoal treated and recrystallized twice from 2-PrOH to yield **2b** as a white solid: 0.078 g, mp 105~108°C. *Anal* Calcd for $C_8H_{11}N_3O_4$: C 45.07, H 5.20, N 19.71. Found: C 45.63, H 5.25, N 18.93. NMR

($CDCl_3$) δ 1.34 (3H, t, ester CH_3), 4.2 (3H, s, OCH_3), 4.32 (2H, q, OCH_2), 4.68 (2H, br s NH_2), and 7.90 (1H, s, aromatic).

2-(2-Aminooxazol-4-yl)-2-*Z*-methoximinoacetic Acid (**6a**)

An aqueous suspension (50 ml) of **4** (1.74 g, 5.95 mmol) was chilled in an ice bath and sodium hydroxide (2.6 ml, 13.1 mmol) was added; the material slowly went into solution. After 1.5 hours the mixture was allowed to come to room temp. The aqueous solution was treated with XAD resin (8 ml, 1.7 mmol/ml) and stirred for 2 minutes. The solution was filtered and the resin was washed with 5 ml of H_2O. The filtrate was lyophilized to yield **6a**; 0.812 g (74%). NMR (DMSO-d_6) δ 3.84 (3H, s OCH_3), 6.77 (2H, br s, NH_2), and 7.48 (1H, s, aromatic).

2-(2-Aminooxazol-4-yl)-2-*E*-methoximinoacetic Acid (**7**)

An aqueous solution (25 ml) of **5** (0.9 g, 3.1 mmol) and sodium hydroxide (5 N, 1.25 ml) was stirred at room temp for 3 hours. Dowex resin (4 ml, 1.7 mmol/ml) was added and stirring was continued for 0.5 hour; then the mixture was filtered and concentrated *in vacuo*. The residue was crystallized from EtOAc to yield **7** as a white crystalline solid, 0.175 g (31%); mp 160~162°C: NMR (DMSO-d_6) δ 3.90 (3H, s, OCH_3), 6.77 (2H, br s, NH_2), and 7.48 (1H, s, aromatic).

2-(2-Aminooxazol-4-yl)-2-*Z*-methoximinoacetic Acid-HOBT Adduct (**9a**)

A mixture of HOBT monohydrate (1.02 g 6.68 mmol) and TEA (1.138 ml, 8.16 mmol) in DMAC (8 ml) was cooled in an ice-acetone bath and a DMAC solution (2 ml) of methanesulfonyl chloride (0.57 ml, 7.3 mmol) was added dropwise. Stirring at 0 to 10°C was continued for 90 minutes.

A mixture of **6a** (1.24 g, 6.68 mmol) together with 1.01 ml of TEA was then added dropwise and stirring in the cold was continued for an additional 90 minutes. H_2O (21 ml) was then added dropwise. After half the H_2O was added a precipitate began to form. Within 10 minutes after the addition was complete, the precipitation was collected by filtration, washed with cold water and dried to yield 1.28 g (63%) of **8a** as an off-white crystalline solid, mp 155~157°C. *Anal* Calcd for $C_{12}H_{10}N_6O_4 \cdot H_2O$: C 45.13, H 3.78, N 26.24. Found: C 45.95, H 3.34, N 26.15. NMR ($CDCl_3$ + DMSO-d_6) δ 3.90 (3H, s, OCH_3), 5.95 (2H, s, NH_2), 7.43 (1H, s, oxazole H), 7.45~8.1 (3H, m, 1′, 2′, and 3′H aromatic), and 9.45 (1H, d, 4′ aromatic).

7-[2-(2-Aminooxazol-4-yl)-2-*Z*-methoximinoacetyl]amino-3-acetoxymethyl-3-cephem-4-carboxylic Acid (**12**)

7-ACA (0.43 g, 1.58 mmol) was suspended in 25 ml of cold 50% aqueous acetone. TEA (0.2 ml 1.43 mmol) was added dropwise to the stirred solution. After all of the 7-ACA was in solution, **9a** (0.5 g, 1.66 mmol) was added portionwise. The pH was adjusted as needed with 45% aqueous potassium phosphate to maintain the pH at 7.5. After 2 hours the mixture was allowed to warm to room temp and stirring was continued overnight.

The acetone was removed and the resulting aqueous solution was diluted with 25 ml of H_2O, layered with EtOAc, and the pH was adjusted to 2.5 with 1 N HCl. The EtOAc extract was washed with H_2O, dried ($MgSO_4$) and evaporated to a partially crystalline residue. The solid was triturated with EtOAc and filtered to yield **11** (0.3 g, 41.1%). *Anal* Calcd for $C_{16}H_{17}N_5O_8S$: C 43.74, H 3.90, N 15.94. Found: C 44.01, H 3.97, N 15.75. UV λ_{max}^{MeOH} 217 (ε 19,254), 265 nm (10,200). NMR (DMSO-d_6) δ 2.00 (3H, s, OAc), 3.32 and 3.61 (2H, ABq, J=9 Hz, C-2 methylene), 4.85 (3H, s, OCH_3), 4.7 and 5.0 (2H, ABq, J=6 Hz, C-3), 5.08 (1H, d, J=2.25 Hz, C-6), 5.72 (1H, q, J=2.25 and 4.5 Hz, C-7), 6.6 (2H, br s, amino), 7.38 (1H, s, aromatic), and 9.5 (1H, J=4.5 Hz, amido).

7-[2-(2-Aminooxazol-4-yl)-2-*Z*-methoximinoacetyl]amino-3-pyridiniummethyl-3-cephem-4-carboxylic Acid (**13**)

An aqueous acetone solution (2.6 ml) of **11** (0.134 g, 0.364 mmol) was stirred and the pH was adjusted to ~7.5 by the dropwise addition of 45% aqueous potassium phosphate. The HOBT-activated side chain **9a** (0.1 g, 0.33 mmol) was added. The resulting suspension was stirred at 0°C for 2 hours, then allowed to slowly warm to room temp. As needed potassium phosphate was added to keep the

pH at 7.5. The acetone was removed *in vacuo*; the resulting aqueous solution was further diluted with H_2O and acidified to pH 2 by the addition of 1 N HCl. After washing twice with EtOAc, the aqueous mixture was concentrated *in vacuo*. The resulting solid was purified by reversed-phase preparative HPLC [C-18, eluted by Me_3CN - AcOH - H_2O (20: 2: 78) to yield **13** (0.025 g): NMR (DMSO-d_6) δ 3.42 (2H, m, C-2), 3.80 (3H, s, OCH_3), 5.03 (1H, d, C-6), 5.08 and 5.70 (2H, ABq, C-3 methylene), 5.64 (1H, q, C-7), 6.80 (2H, s, NH_2), 7.42 (1H, s, C-5 oxazole), and 8.00~9.6 (6H, m, 7-amido and pyridinium protons).

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