STUDIES ON β-LACTAM ANTIBIOTICS

VIII.† STRUCTURE-ACTIVITY RELATIONSHIPS OF 7β-[(Z)-2-CARBOXYMETHOXYIMINO-2-ARYLACETAMIDO]-3-CEPHEM-4-CARBOXYLIC ACIDS

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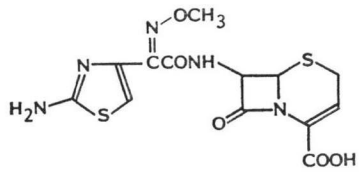
The synthesis, antimicrobial activity and oral absorptivity of 7β-[(Z)-2-carboxymethoxyimino-2-arylacetamido]-3-cephem-4-carboxylic acids are described. The [(Z)-2-(2-amino-4-thiazolyl)-2-carboxymethoxyimino]acetyl group was selected as the most suitable 7-substituent from seven 7-acyl groups for our further investigation of orally active cephalosporins.

Recently, extensive studies have been undertaken on a new family of cephalosporins. On the contrary, there has not been any report about an orally active cephalosporin possessing the same antibacterial activity and significant resistance to β-lactamases as those new injectable cephalosporins at all.

Orally active cephalosporins such as cephalexin and cephalexin-analogs are much less active against Gram-negative bacteria and less stable to β-lactamases than those new injectable cephalosporins. In addition, the continuous increase of β-lactamase-producing strains in clinical practice have necessitated a new orally active cephalosporin possessing high stability to β-lactamases and far more potent antibacterial activity against a wide range of Gram-negative bacteria than the existing oral β-lactam antibiotics.

Since ceftizoxime (**1**) (Fig. 1),[1,2,3] one of the new injectable cephalospoins, was found in our laboratories, we have intensively focused our attention on searching for a new oral cephalosporin possessing the same antimicrobial activity as ceftizoxime.

Fig. 1.

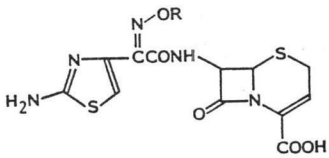


Ceftizoxime (**1**)

During the course of our research on ceftizoxime and its related compounds, we found remarkable evidence that several cephem antibiotics having a 2-(2-amino-4-thiazolyl)-(Z)-2-alkoxyiminoacetyl group at the 7-position of a cephem nucleus were excreted in bile (*e.g.* **2a**, 22.8%; **2b**, 33.3%: Table 1) without detectable amounts in the urine after oral administration in rats.[4] Improvement in oral absorptivity of **2a** and **2b** in comparison with ceftizoxime (**1**) was supposed to be due to increased lipophilicity. From the standpoint of evaluating the potential utility of a cephalosporin, it was a rather undesirable chemotherapeutic property that **2a** and **2b** modified in the alkyl chain length of the oxime

† Paper VII. See ref 5).

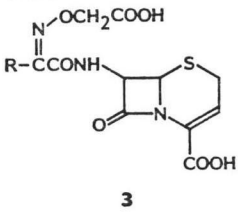
Table 1. The 24-hour urinary and biliary recovery after oral administration in rats.



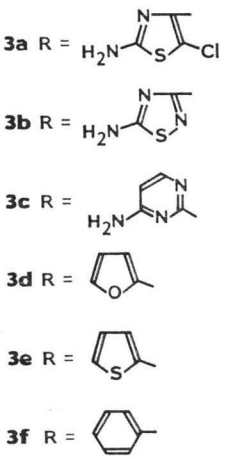
Compounds R	Recovery (%) Urine	Bile
1 $-CH_3$ (Ceftizoxime)	8.53	0.33
2a $-CH_2(CH_2)_3CH_3$	n.d.	22.84
2b $-CH_2(CH_2)_4CH_3$	n.d.	33.25
2c $-CH_2CH_2OH$	4.77	n.d.
2d $-CH_2CH_2NH_2$	7.05	0.56
2e $-CH_2COOH$	41.0	3.80
2f $-CH_2COOC_2H_5$	3.59	3.45
2g $-CH_2CN$	4.24	n.d.

n.d.: Not detected.

Fig. 2.



3



3a R =

3b R =

3c R =

3d R =

3e R =

3f R =

ether group were poorly excreted in the urine. Therefore, we have concentrated our research on chemical modification of alkoxyimino moiety in order to find such a cephem antibiotic exhibiting a high excretion rate in the urine.

The urinary and biliary excretion of several kinds of cephem antibiotics (**2c**~**g**) having hydrophilic functions such as 1-hydroxyethyl, 1-aminoethyl and carboxymethyl groups or relatively lipophilic functions such as ethoxycarbonylmethyl and cyanomethyl groups in the oxime ether moiety (R) in rats were listed in Table 1. The synthesis and *in vitro* antimicrobial activity of these cephems antibiotics were reported in our previous paper.[5] A cephem antibiotic (**2e**) having an acidic function such as carboxymethyl group was excreted in the urine (41.0%) and bile (3.8%) respectively. The excretion rate of **2e** was much greater than that of **1** (Table 1). On the other hand, all of remaining cephem antibiotics (**2c, d, f, g**) were excreted to a lesser extent in the urine than **1**.

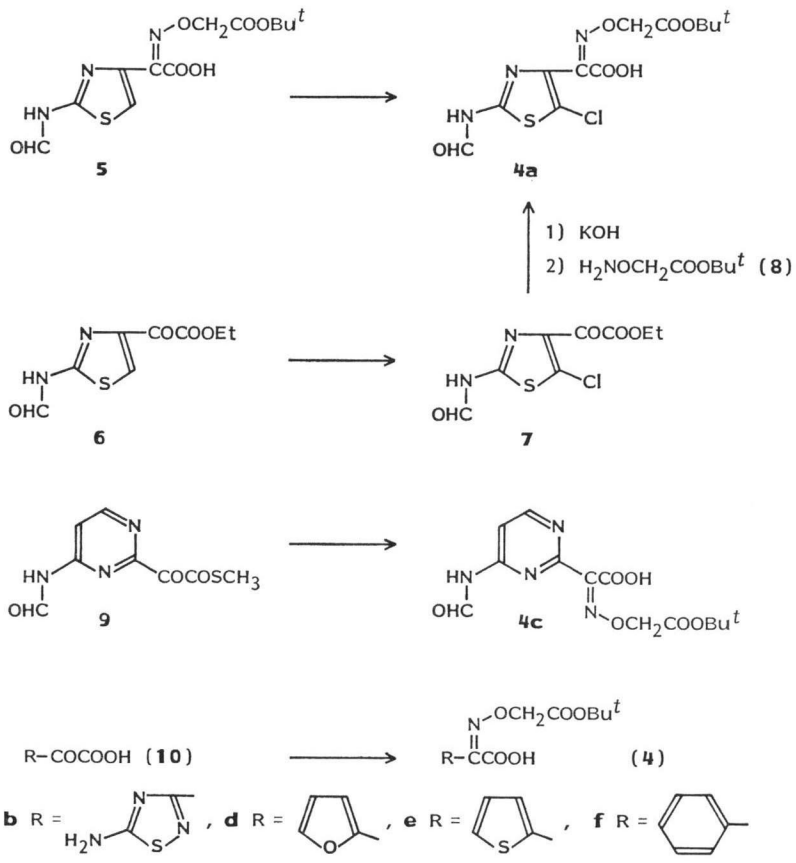
As mentioned above, we found that the new cephem antibiotic (**2e**) possessed remarkable oral absorptivity. Moreover, the structure of **2e** is completely distinct from those of the cephalexin-analogs. Therefore, we directed our main efforts toward both the modification of the heterocyclic ring of the 7-acyl side chain in **2e** and the change of the C-3 substituent in **2e** in order to find new cephalosporins with even better oral absorptivity.

In this paper we describe the effect of replacing the heterocyclic ring in the 7-acyl side chain in compounds represented by structure **3** (Fig. 2) on antibacterial activity and oral absorptivity.

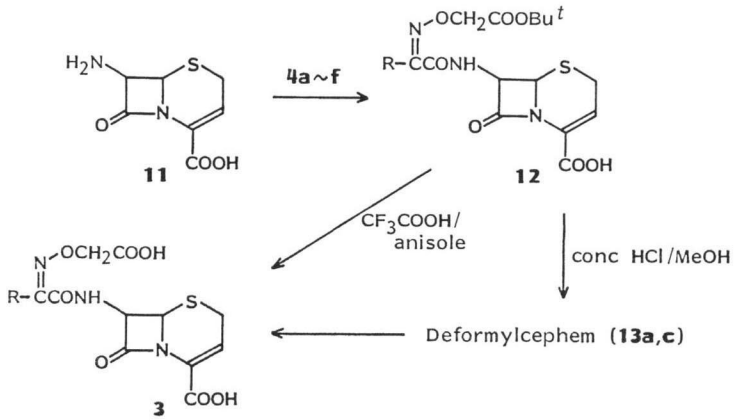
Chemistry

Scheme 1 summarizes the synthesis of [(*Z*)-2-*tert*-butoxycarbonylmethoxyimino-2-aryl]acetic acids (**4a**~**f**). The 5-chlorothiazol acid (**4a**) was prepared from **5**[5] by treatment with chlorine or from **6**[5] by chlorination with trichloroisocyanuric acid followed by subsequent alkaline hydrolysis, and treatment with *tert*-butoxycarbonylmethoxyamine (**8**). The thiadiazole acid (**4b**)[6] and the pyrimidine derivative (**9**)[7] were prepared according to the synthetic procedure described by GOTO. **4c** was similary prepared from **9** by alkaline hydrolysis and treatment with **8**. The acids (**4d, e, f**) were obtained in a similar manner as reported by the Glaxo group.[8]

Scheme 1.

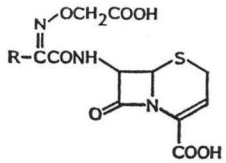


Scheme 2.

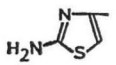
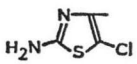
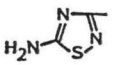
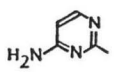


The synthetic route of the novel cephalosporins (**3a~f**) is outlined in Scheme 2. The acylation of **11**[9] was carried out in good yield (85~95%) under non-aqueous conditions by trimethylsilylation using *N*-(trimethylsilyl)acetamide (MSA). The acids (**4a~f**) were activated with Vilsmeier reagent prepared from *N*,*N*-dimethylformamide (DMF) and phosphoryl chloride ($POCl_3$) or converted to the

Table 2. Antimicrobial activity and urinary and biliary excretion of cephalosporins.

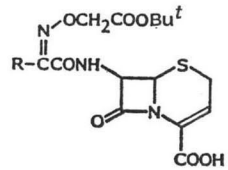


Inoculum size 10^6 cfu/ml

Compounds No.	R	MIC (μg/ml) *Staphylococcus aureus* 209P JC-1	*Escherichia coli* NIHJ JC-2	*Escherichia coli* 28*	*Klebsiella pneumoniae* 12	*Proteus mirabilis* 1	*Proteus vulgaris* 1	Recovery (%) Urine	Recovery (%) Bile
2e		25	0.20	0.20	0.10	≦0.025	0.05	41.0	3.8
3a		>100	0.20	0.20	0.39	0.05	0.05	15.4	7.5
3b		>100	0.05	0.10	0.05	≦0.025	0.05	3.8	0.8
3c		>100	0.20	0.05	0.10	0.05	0.10	10.3	2.8
3d		>100	0.39	1.56	0.39	0.20	0.20	40.4	23.6
3e		50	0.78	1.56	1.56	0.20	0.20	48.4	30.0
3f		25	0.78	1.56	12.5	0.39	0.39**	22.3	24.3
1 (Ceftizoxime)		6.25	≦0.025	0.05	0.05	≦0.025	≦0.025	8.5	0.3
Cephalexin		3.13	12.5	12.5	3.13	12.5	100	77.3	13.3

* Cephalosporinase producer, ** *Proteus vulgaris* IAM 1095.

Table 3. NMR and IR spectral data of **12** and **13a**.



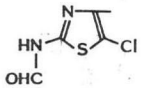
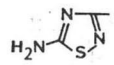
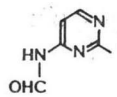
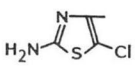
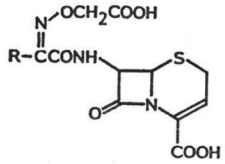
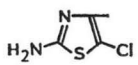
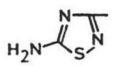
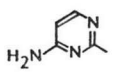
Compounds		NMR δ value (DMSO-d_6)								IR (Nujol, cm^{-1})	
No.	R	CONH 1H, d J=8 Hz	C(3)-H 1H m	C(7)-H 1H, dd J=5, 8 Hz	C(6)-H 1H, d J=5 Hz	C(2)-CH_2 2H m	CH_2COO 2H, s	Bu^t 9H, s	R	Lactam	CONH
12a		9.50	6.47	5.87	5.09	3.60	4.60	1.45	8.50 (1H, s)	1775	1670
12b		9.50	6.47	5.83	5.07	3.57	4.60	1.40	8.13 (2H, s)	1790	1680
12c		9.50	6.46	5.90	5.10	3.54	4.70	1.40	7.2~9.2 (3H, m)	1780	1660
12d		9.63	6.43	5.80	5.08	3.56	4.56	1.43	6.63 (2H, m) 7.76 (1H, m)	1788	1685
12e		9.63	6.45	5.81	5.10	3.57	4.54	1.42	6.9~7.3 (2H, m) 7.58 (1H, m)	1785	1680
12f		9.65	6.37	5.87	5.32	3.60	4.61	1.43	7.50 (5H, m)	1780	1680
13a		9.33	6.46	5.82	5.07	3.56	4.56	1.44	—	1775	1680

Table 4. Yield, NMR and IR spectral data of **3**.



Compounds		NMR δ value (DMSO-d_6)							IR (Nujol, cm^{-1})		Yield (%)
No.	R	CONH 1H, d J=8 Hz	C(3)-H 1H m	C(7)-H 1H, dd J=5, 8 Hz	C(6)-H 1H, d J=5 Hz	C(2)-CH_2 2H m	CH_2COO 2H, s	R	Lactam	CONH	
3a		9.50	6.49	5.83	5.08	3.66	4.62	—	1760	1670	79.2
3b		9.50	6.45	5.85	5.07	3.53	4.63	8.13 (2H, s)	1770	1680	41.5
3c		9.27	6.43	5.87	5.07	3.57	4.63	8.10 (1H, d, J=7 Hz) 6.40 (1H, d, J=7 Hz)	1770	1650	21.5
3d	 *	9.60	6.41	5.82	5.27	3.58	4.61	6.63 (2H, m) 7.75 (1H, m)	1770	1673	74.9
3e		9.63	6.44	5.84	5.11	3.61	4.61	6.97 (2H, m) 7.62 (1H, m)	1770	1670	74.0
3f		9.63	6.46	5.90	5.13	3.60	4.60	7.50 (5H, m)	1770	1675	65.0

* Disodium salt.

corresponding acid chloride with phosphorus pentachloride (PCl_5) for the above coupling reaction. Deprotection of the *N*-formyl group in cephem antibiotics (**12a, c**) proceeded at room temperature in a methanolic solution containing conc hydrochloric acid. The *tert*-butyl ester group in cephem antibiotics (**12b, d, e, f** and **13a, c**) was cleaved at room temperature by treatment with trifluoroacetic acid and anisole.

Structures of the acids (**4a, c**), and the intermediates (**12** and **13a**) and **3** were confirmed on the basis of IR and NMR spectral data as shown in Tables 3 and 4 and the experimental section.

Biological Activity

The minimum inhibitory concentration (MIC) values of the cephalosporins (**2e** and **3**) possessing several kinds of aromatic ring in the acyl side chain at the 7-position of the cephem nucleus against one Gram-positive and five Gram-negative bacteria are shown in Table 2. For comparison, the MIC values of ceftizoxime (**1**) and cephalexin are listed at the bottom of Table 2. The urinary and biliary recovery (%) of the cephem antibiotics (**2e** and **3**) after oral administration in rats are also given in the last column of Table 2.

The MIC values of the cephem antibiotics (**3a**~**c**) against *Staphylococcus aureus* were found to be two to four times less active than that of **2e**. All cephem antibiotics (**2e** and **3**) with hydrophilic function such as carboxymethyl group showed lower inhibitory potency against *S. aureus* than ceftizoxime and cephalexin. On the other hand, these the same cephem antibiotics (**2e** and **3**) displayed high activity against Gram-negative bacteria when compared with cephalexin. The activity of the cephem antibiotics (**3a, b** and **c**) with an amino group on their heteroaromatic ring against Gram-negative bacteria was similar to that of **2e**, but was slightly decreased compared with ceftizoxime. The cephem antibiotics (**3d, 3e** and **3f**) were three times less active than **2e** against *Escherichia coli* 28 which is a cephalosporinase-producing strain.

In contrast to the correlation between the heteroaromatic ring and antibacterial activity, the urinary recovery of **3a, 3b** and **3c** which are structurally similar to **2e** was much lower than that of **2e**. On the other hand, the urinary excretion of **3d** and **3e** was similar to that of **2e** but the biliary recovery was greater.

In conclusion, considering the reduced antibacterial activity of **3d, 3e** and **3f** against *E. coli* 28 and other Gram-negative bacteria, we selected the 2-amino-4-thiazol ring as the most suitable heterocyclic ring for our further investigation of modifications at the 3-position.

Experimental

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and uncorrected. NMR spectra were recorded at 60 MHz on a JNM-PMX 60 NMR spectrometer and at 100 MHz on a Jeor-MH 100 MHz spectrometer using Me_4Si as an internal standard. IR spectra were taken on a Hitachi 260-10 spectrophotometer or a Shimadzu IR-420 spectrophotometer.

Antibiotic Susceptibility

All the *in vitro* antibacterial activities are given as the MIC in μg/ml required to prevent growth of the bacterial culture. MIC's were determined by agar dilution method using heart infusion agar (Difco) after incubation at 37°C for 20 hours with inoculum size of about 10^6 cfu/ml. *E. coli* 28 is a cephalosporin-resistant strain.

Urinary and Biliary Excretion

Sprague Dawley rats were fasted overnight and orally dosed with 100 mg/kg of the test drugs. Urine samples were collected for 24 hours after dosing. For bile collection another group of rats was

cannulated with a polystyrene tube into the bile duct and the test drugs were given orally at doses of 100 mg/kg. The samples were assayed by a disc-plate diffusion method using *E. coli* NIHJ JC-2 or *E. coli* ATCC 39188 as test organism and nutrient agar (Difco) as the test medium.

Preparation of [2-(5-Chloro-2-formamido-4-thiazolyl)-(*Z*)-2-*tert*-butoxycarbonylmethoxyimino]-acetic Acid (**4a**)

Method A: To a suspension of **5** (20.0 g, 60.7 mmol) in $CHCl_3$ (500 ml) was added a solution of chlorine (4.8 g, 68.3 mmol) in acetic acid (68 ml) at 0°C. The mixture was stirred at this temp for 30 minutes, and poured into 5% $NaHCO_3$ solution. The resultant mixture was adjusted to pH 7.5 with 10% NaOH solution. The separated aqueous layer was acidified to pH 2.0 with 10% HCl and extracted with EtOAc. The EtOAc layer was washed with brine, dried ($MgSO_4$), and concd under reduced pressure to give 13.3 g (60.3%) of **4a**; mp 105~109°C (dec); IR (Nujol) 3130, 1725, 1690, 1648 cm^{-1}; NMR (DMSO-d_6) δ 1.45 (9H, s), 4.67 (2H, s), 8.55 (1H, s), 12.87 (1H, br s).

Method B: To a solution of **6** (6.9 g, 30 mmol) in DMF (40 ml) was added a solution of trichloroisocyanuric acid (2.8 g, 12 mmol) in DMF (10 ml) at 60°C over 15 minutes. After being stirred at the same temp for 1 hour, the mixture was poured into ice-water (400 g). The precipitate was collected by filtration, washed with H_2O, and dried (P_2O_5) to give 7.1 g (89.3%) of **7** as a crystal; mp 151~153°C. IR (Nujol) 3150, 1740, 1675 cm^{-1}; NMR (DMSO-d_6) δ 1.67 (3H, t, J=8 Hz), 4.40 (2H, q, J=8 Hz), 8.67 (1H, s), 13.05 (1H, br s).

A suspension of **7** (20.0 g, 76.1 mmol) in 1 N KOH (152.3 ml) was stirred at room temp for 10 minutes. The resultant solution was acidified to pH 2.0 with 10% HCl under ice-cooling. To the suspension was added a solution of *tert*-butoxycarbonylmethoxyamine (14.6 g, 99 mmol) in THF (75 ml) and pyridine (27.7 ml). After being stirred at room temp for 6 hours, the mixture was acidified to pH 2.0 with 10% HCl, and extracted with EtOAc. The separated organic layer was washed with brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was triturated with Et_2O to give 9.3 g (33.6%) of **4a**.

Preparation of [2-(Formamidopyrimidin-2-yl)-(*Z*)-2-*tert*-butoxycarbonylmethoxyimino]acetic Acid (**4c**)

To a suspension of *S*-methyl 2-(4-formamidopyrimidin-2-yl)thioglyoxylate (**9**) in H_2O (180 ml) was added dropwise 1 N NaOH (80 ml) at room temp and the mixture was stirred the same temp for 20 minutes. To the solution was added a solution of **8** (14.8 g, 0.101 mol) in EtOH (20 ml) and adjusted to pH 4.0 with 1 N HCl. After being stirred at room temp for 1 hour, the mixture was adjusted to pH 7.0 with 5% $NaHCO_3$ solution. After removal of EtOH, the aqueous solution was washed with EtOAc, acidified to pH 4.0 with 1 N HCl, and washed with EtOAc. The separated aqueous layer was adjusted to pH 2.0 with 1 N HCl and extracted with EtOAc. The EtOAc layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was triturated with diisopropyl ether (iPE) to give 9.7 g (33.7%) of **4c**; mp 124~127°C; IR (Nujol) 3200, 1750, 1718, 1692 cm^{-1}; NMR (DMSO-d_6) δ 1.42 (9H, s), 4.73 (2H, s), 7.2~9.2 (3H, m).

General Procedure for Acylation of **11**

Acid Chloride Method: To a suspension of PCl_5 (6 mmol) in CH_2Cl_2 (30~50 ml) was added **4(b, c)** (5 mmol) at −15°C, and the mixture was stirred at −15~−10°C for 30~60 minutes. To a solution of **11** (6 mmol) and MSA (46 mmol) in CH_2Cl_2 (30 ml) was added the acid chloride solution prepared above all at once at −15°C, and the reaction mixture was stirred at −5~0°C for 30 minutes. To the resultant solution were added H_2O (100 ml) and sodium bicarbonate (42 mmol), and stirred at room temp for 30 minutes. The separated aq solution was washed with EtOAc (30 ml), adjusted to pH 2 with 10% HCl and extracted with EtOAc. The EtOAc layer was washed with brine, dried ($MgSO_4$) and evaporated *in vacuo*. The residue was triturated with Et_2O to afford **12(b, c)**.

Vilsmeier Reagent Method: A mixture of DMF (5.5 mmol) and $POCl_3$ (5.5 mmol) in THF (5 ml) was stirred under ice-cooling for 30 minutes to prepare Vilsmeier reagent. To a solution of the above Vilsmeier reagent in THF (20 ml) was added **4(a, d, e, f)** (5 mmol) at −5°C, and the mixture was stirred at 0~5°C for 30~60 minutes to produce an activated acid solution. To a solution of **11** (5 mmol)

and MSA (30~40 mmol) in CH_2Cl_2 (20 ml) was added the above activated acid solution at −30°C, and the reaction mixture was stirred at −30~−10°C for 40~60 minutes. To the resultant mixture was added 5% $NaHCO_3$ solution (30 ml). The separated aq solution was washed with EtOAc, adjusted to pH 2.0 with 10% HCl, and extracted with EtOAc. The separated EtOAc layer was washed with brine, and dried ($MgSO_4$). The solvent was evaporated and the residue was triturated with Et_2O to afford **12(a, d, e, f)**.

General Preparation of **3**

To a suspension of ester (**12b, d, e, f** and **13a, c**) (3.1 mmol) in anisole (2 ml) was added TFA (10 ml) under ice-cooling. The mixture was stirred at room temp for 1 hour. After evaporating TFA *in vacuo*, the residue was dissolved in 5% $NaHCO_3$ solution (30 ml). After being washed with EtOAc, the aqueous solution was acidified to pH 2.0 with 10% HCl, and extracted with EtOAc. The EtOAc solution was washed with brine, dried ($MgSO_4$) and concd under reduced pressure. The residue was triturated with Et_2O to give **3**.

General Procedure for Deformylation of **12(a, c)**

To a mixture of **12(a, c)** (3.9 mmol) in MeOH (50 ml) was added conc HCl (12~16 mmol) at room temp, and the mixture was stirred at the same temp for 2~3 hours. After being neutralized with 5% $NaHCO_3$ solution, the mixture was evaporated *in vacuo* and the residue was dissolved in 5% $NaHCO_3$ solution. The solution was washed with EtOAc, and the aq layer was acidified to pH 2 with 10% HCl under ice-cooling. The resulting precipitate was collected by filtration, washed with cold H_2O, and dried (P_2O_5) to afford **13(a, c)**.

13c was treated with TFA and anisole without spectral measurements and the yield of **3c** listed in Table 4 was based on **12c**.

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