Synthesis and Biological Evaluation of New Aminothiazolyl Cephalosporins with Elongated Side Chains

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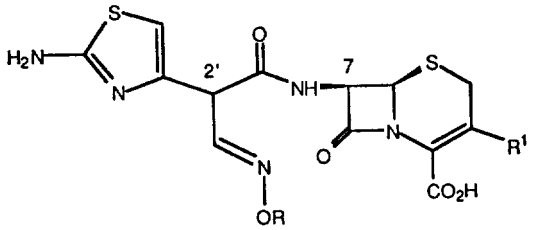
The elongation of the chain length at 2'position of aminothiazolylacetamido group of cephalosporin antibiotics was achieved. The derivatives with elongated side chain at 2'position were successfully synthesis and their *in vitro* activities were evaluated. It is indicated that there is an optimum range of the chain length in order to exhibit higher biological activities. This result could open a possibility for the development of the cephalosporin antibiotics with higher activities by further variation of the substituents at other positions. The cephalosporin derivatives with a simple (relatively non-functionalized) side chain as well as with a linear extended side chain were also prepared. Low activities of these derivatives led to a conclusion that either simple (non-functionalized) change or linear elongation of the side chain does not enhance the antibacterial activities.

Introdution

Recently, we reported a series of cephalosporin antibiotics with aminothiazolylacetamido group as a 7-position substituent, typical structures of which are represented by the following structure **1**.[1] Since these new aminothiazolyl cephalosporins exhibited promising *in vitro* activities, it was desirable to study the effect on the biological activities upon further variation of the structures of these new cephalosporins. Naturally, we were led to investigate the structure-activity relationship upon elongation of the side chain at 2'position in order to determine the optimum length and structure.



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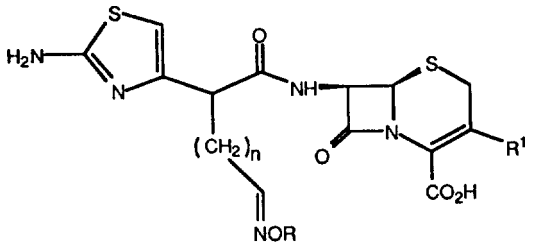
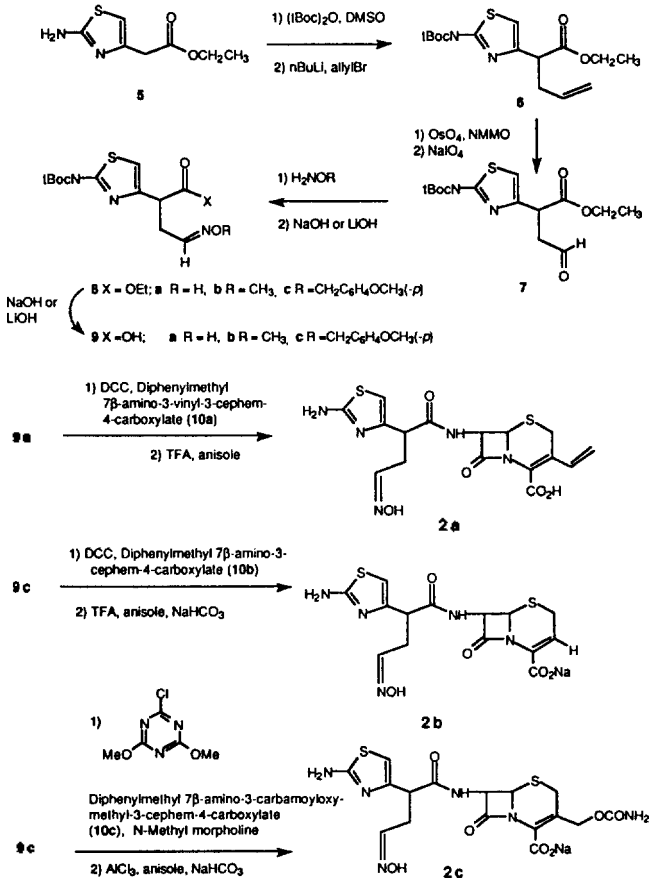
Thus, the development of the synthetic routes to the cephalosporin derivatives shown in the following formula **2** and **3** was required. In this paper we wish to disclose the details of our effort to synthesize these derivatives and of the considerations on their biological activities.



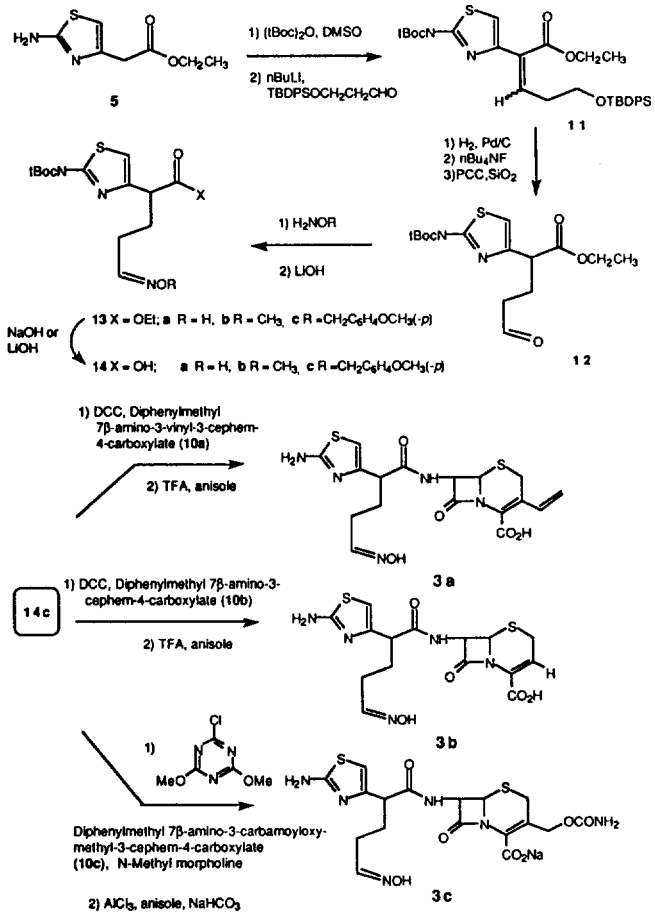
2 ($n=1$)
3 ($n=2$)

Result and discussion



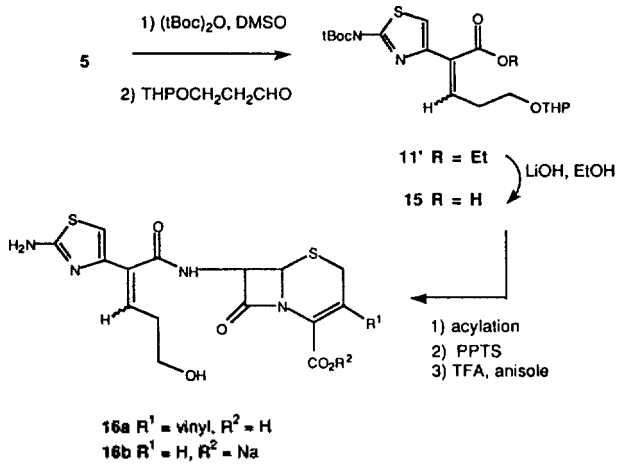
Scheme 1

Synthesis of Cephalosporins 2. A successful synthesis of the cephalosporin derivative **2** was summarized in Scheme 1. Handling of **2** as well as **3** during the synthesis was anticipated more straightforward than that of **1**, because the synthesis route would not involve the structural complication derived from the enamine-imine tautomerization, which was possible in the intermediates employed in the synthetic route for the preparation of **1**.[1] Starting from commercially available



Scheme 2

ethyl (2-aminothiazol-4-yl) acetate **5**, 2-allyl derivative **6** was prepared by protection of both amino and imino group with *t*-butoxycarbonyl group followed by allylation (*n*-BuLi, allyl bromide). Oxidative cleavage of the carbon-carbon double bond in allylated product **6** was efficiently achieved by O_3O_4-$NaIO_4$. Ozonolysis was not efficient for the cleavage of the double bond in this case. The resultant aldehyde **7** was subjected to the oxime ether (or oxime) preparation by reacting with the corresponding hydroxylamine derivatives. These were obtained as mixtures of syn- and anti- isomers and used without purification. Subsequent hydrolysis of the oxime (or oxime ether) provided the corresponding acid **9** which was used for the next acylation step. Usually the required acylation was effectively carried out using DCC without further activation of the reacting acids. Thus, in case that 3-substituent of the cephem-4-carboxylic acid derivatives is vinyl (**10a**) or H (**10b**), acylation underwent smoothly without any incident. When 3-position substituent is carbamoyl, however, further activation of the corresponding carboxylic acid (**10c**) to an active ester with the aid of 2-chloro-4,6-dimethoxy-1,3,5-triazine[2] was necessary. Deprotection with acids such as trifluoroacetic acid or aluminum chloride afforded the desired cephalosporins with the elongated chains substituent at 2'-position of aminothiazolylacetamido group. The cephalosporin derivatives prepared for the biological evaluation were **2a**, **2b** and **2c** (Scheme 1). On the other hand, synthesis of other oxime ether derivatives (*e.g.*, R=CH_3 at=N-OR) which could reveal the activity trend with the variation of



Scheme 3

7-position substituents was not actively pursued at this time. Oxime ether derivative **9b** was successfully acylated with DCC and the cephem-4-carboxylic acids, but difficulty in the final deprotection step prevented us from isolating the desired cephalosporin derivatives. Although more derivatives would reveal the clearer activity trend upon alteration of the side chain length, the aforementioned three derivatives, we believe, could provide enough information to decide whether elongation of the side chain length would result in favorable effect in antibacterial activity along with the results from the following series of cephalosporins (*vide infra*).

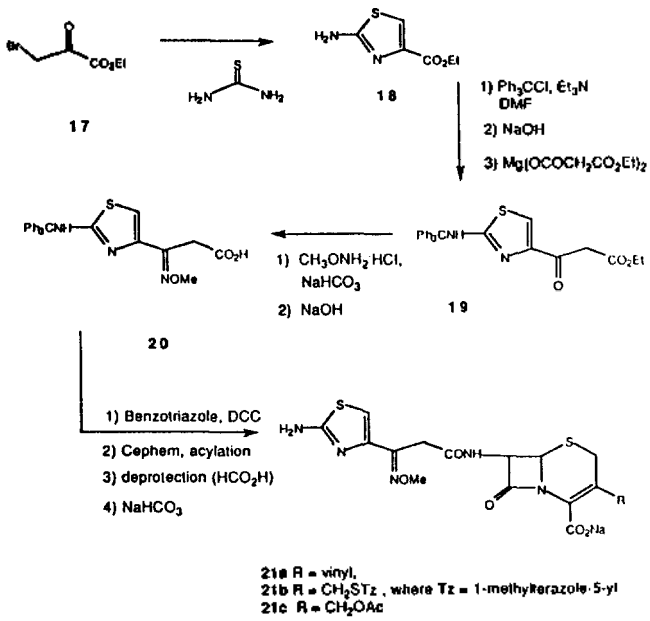
Synthesis of Cephalosporins 3. Further elongation of the side chain length in order to prepare the cephalosporin derivatives **3** was intended. A successful synthetic route is summarized in Scheme 2. The alkylation of **5** with homoallyl halide after protection of amino and imino groups, the success of which would make the whole synthesis equivalent to that described above for the preparation of **2**, met with failure. Such unsuccessful alkylation led us to investigate other routes. A practical route to synthesize **3** involved condensation with an aldehyde followed by catalytic hydrogenation (Scheme 2). Thus, after protection of amine in aminothiazole, reaction of the corresponding enolate and a silyl protected aldehyde generated **11** as a mixture of (*E*)- and (*Z*)-isomers in 70% yield. Without separation of (*E*)-, (*Z*)-mixture, catalytic hydrogenation (Pd/C) of the double bond was proceeded in 77% yield. Deprotection of *tert*-butyldiphenylsilyl group followed by oxidation (PCC) provided the desired aldehyde **12** without any incident. The synthetic sequence from this point to **3** is essentially identical to that explained for the synthesis of **2**. After preparation of oxime ethers (or an oxime) by treatment with the corresponding alkoxyamines (or hydroxylamine), acylation with cephem-4-carboxylic acids followed by deprotection completed the synthesis. Again syn- and anti-isomers of oxime derivatives were used without purification. The acylations were performed either by DCC or 2-chloro-4,6-dimethoxy-1,3,5-triazine.

Other Derivatives. We have also prepared the cephalosporin derivatives **16**, since it would provide, we believe, another opportunity to evaluate the activity change upon variation of the side chain length at 2' position. The synthesis of **16** was straightforward and shown in Scheme 3. A THP ether instead of a silyl ether for the protection of hydroxyl

Table 1. *In Vitro* Antibacterial Activities of the Cephalosporins Prepared

Strains	MIC (μg/ml)										
	2a	**2b**	**2c**	**3a**	**3b**	**3c**	**16a**	**16b**	**21a**	**21b**	**21c**
Streptococcus pyogenes A308	0.049	1.563	1.563	0.781	1.563	3.125	1.563	1.563	ND	ND	ND
Streptococcus pyogenes A77	0.049	0.391	0.391	0.195	0.781	1.563	0.781	0.391	0.025	0.013	0.049
Staphylococcus aureus SG511	0.781	12.5	12.5	12.5	25	25	100	50	0.391	0.391	0.391
Staphylococcus aureus 285	1.563	12.5	25	12.5	25	50	100	100	0.391	0.391	0.391
Staphylococcus aureus 503	0.781	6.25	12.5	6.25	12.5	25	50	50	0.391	0.391	0.391
Escherichia coli O 55	1.563	0.781	6.25	12.5	3.125	25	25	3.125	50	12.5	50
Escherichia coli DC 0	1.563	0.781	12.5	12.5	3.125	100	50	3.125	50	12.5	100
Escherichia coli DC 2	1.563	0.781	12.5	12.5	3.125	25	12.5	1.563	50	3.125	50
Escherichia coli TEM	1.563	0.781	12.5	50	6.25	100	50	3.125	50	12.5	100
Escherichia coli 1507E	1.563	0.781	12.5	50	6.25	100	50	3.125	100	25	100
Pseudomonas aeruginosa 1771M	25	25	25	50	12.5	100	100	12.5	50	25	25
Salmonella typhimurium	0.781	0.391	12.5	25	6.25	50	50	1.563	50	12.5	100
Klebsiella aerogenes 1522E	0.391	0.391	3.125	25	3.125	25	25	0.781	12.5	6.25	50
Enterobacter cloacae 1321E	0.391	0.195	3.125	12.5	0.781	25	50	0.781	25	12.5	50

ND=no data available.



Scheme 4

group was employed in the preparation of **11′** considering the practicality of scale-up. The rest of the synthetic route was achieved successfully without incident to provide **16a** and **16b** eventually. Syn- and anti- isomers with respect to the double bond (formed in approximately identical ratio) were not separated.

Another mode of the chain elongation would be the insertion of carbon atom(s) to the position between amide carbonyl carbon and oxime carbon. This intercalation of carbon atom(s) would give cephalosporin derivatives with interesting substituents at 7 position, that is, stretched carbon chain substituents. Although various derivatives are feasible, we limited our effort to insert only a carbon atom so that the synthetic target was **21**, and the successful route we followed is summarized in Scheme 4. Starting with pyruvic acid, esterification followed by bromination gave 3-bromo-2-oxo propanoate (**17**). Treatment with thiourea successfully installed an aminothiazole ring and furnished carboxylate **18**. Protection of amino group with trityl group and subsequent hydrolysis (aq. NaOH/EtOH) resulted in trityl protected aminothiazolyl carboxylic acid which set the stage for homologation. This step was achieved by employing Masamune's protocol which utilize a magnesium enolate of monoalkyl ester of malonic acid.[3] The resulting homologated ester **19** was reacted with methoxylamin to furnish 3-methoxyiminopropanoic acid **20** after hydrolysis. This oxime ether produced was a single isomer and believed to be an anti isomer according to the precedent of the usual oxime ether derivatives of aminothiazolylacetates. Acylation with the corresponding cephem-4-carboxylates was achieved by active ester-DCC procedure (see Experimental Section for details). Remaining steps for obtaining samples suitable for biological evaluations were deprotection (formic acid) and treatment with sodium bicarbonate. Substituents employed at 3-position of cephem-4-carboxylate were vinyl (**21a**), (1-methyltetrazol-5-yl)thiomethyl (**21b**), and acetoxymethyl (**21c**).

The MIC values of the cephalosporin derivatives prepared are summarized in Table 1. Since the number of derivatives evaluated was limited, a clear-cut conclusion could not be made. The trend in activities upon variation of the length of the side chains, however, can be realized. Fixing other variables in substituents (R^1=vinyl, R=H in **1**, **2** and **3**), the biological *in vitro* antibacterial activies (MIC values) of **1** and **3** were lower than those of **2**. Although the activities of all the cephalosporins evaluated in Table 1 (and of a series of compounds belong to **1**)[1] were not in superior level, it can be concluded that the optimum length of the side chain at 2′ position if that represented in the structure **2**. Further elongation of the side chain beyond this length doesn't seem to do any good on the biological activities. Accepting that the optimum length of the side chain at 2′ position, that is, $n=1$ as in **2**, effect on the activities upon change of 3-position substituent in the cephem ring was of interest. Comparison of the activities of **2a** (**R=vinyl**), **2b** (**R=H**) and **2c** (**R=carbamoyl**) revealed that **2a** and **2b** are better than **2c**.

This trend is also shown in $n=2$ series (**3a**, **3b** and **3c**). Further conclusions should await for more detailed investigations on the structure-activity relationships.

Simple elongation of the side chain at 2′ position was not sufficient to manifest the acceptable biological activities, which is clearly supported by the MIC values of **16a** and **16b**. It is also concluded that a linear extension of the chain length was ineffective in enhancing the biological activities as shown in the MIC values of **21a**, **21b**, and **21c**.

In summary our effort to optimize the side chain length to enhance the biological activities in cephalosporin antibiotics has been described. It seems to indicate that there is an optimum range of the length of the side chain at 2′ position of aminothiazolylacetamido group. Further simple extension of the length of the chain should be carefully planned before actual trial, since our results seem to indicate that elongations with too much structural modification does not affect the biological activities in a favorable way.

Experimental

General. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained with one of the following: a Jeol PMX 60SI, a Varian FT-80A, or a Bruker AM 200 spectrometer. Infrared (IR) spectra were obtained with a Perkin-Elmer 1310 spectrometer. The *in vitro* biological activity of the cephalosporins prepared was determined by conventional agar dilution precedures.

Ethyl 2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)pent-4-enoate (6). To a cooled (−78℃) solution of 15 g (39 mmol) of ethyl (2-*tert*-butoxycarbonylimino-3-tert-butoxycarbonylthiazol-4-yl)acetate[1] in THF (200 m*l*) was added *n*-BuLi (1.6 M solution in hexane, 26.7 m*l*, 42.8 mmol) and allyl bromide (4.0 m*l*, 47 mmol). The solution was stirred for 1 h at the temperature lower than −60℃, then slowly warmed to room temperature. After addition of 10% aqueous citric acid solution (42 m*l*), the solution was stirred for 10 min at room temperature and then concentrated to remove THF. The residue was extracted with ethyl acetate and dried ($MgSO_4$). After concentration of the solution purification by silica gel column chromatography (hexane: ethyl acetate = 8 : 1) afforded **6** (5.45 g, 43%) as an oil. ^{1}H-NMR ($CDCl_3$, δ): 1.23 (t, 3H, CH_3), 1.56 (s, 9H, $(CH_3)_3OC(O)$-), 2.68 (t, 2H, $-CH_2CH$-, 4.08 (m, 3H, $-OCH_2CH_3$ and -COC*H*-), 4.88-6.00 (m, 3H, $-CH=CH_2$), 6.73 (s, 1H, thiazole-*H*).

Ethyl 2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)-4-oxobutanoate (7). To a solution of **6** (5.45 g, 16.7 mmol) in water (10 m*l*) and acetone (40 m*l*) was added 60% *N*-methylmorpholine *N*-oxide (4.9 m*l*, 21.7 mmol) and OsO_4 (catalytic amount). The solution was stirred for 1.5 h at room temperature. Excess of $NaHSO_3$ was added and stirred for 30 min at room temperature and then the solution was filtered. After concentration to remove acetone, the resulting residue was extracted with ethyl acetate. The extract was washed with saturated aqueous sodium chloride, dried ($MgSO_4$), and concentrated. The residue was dissolved into 50% aqueous methanol (160 m*l*), and to this solution was added $NaIO_4$ (5.72 g, 26.8 mmol). After stirring for 1 h at room temperature, the solution was filtered and concentrated. The residue was extracted with ethyl acetate and the organic layer was washed with water and saturated aqueous NaCl and dried ($MgSO_4$). Filtration and concentration provided **7** (3.31 g, 60%) as a yellow solid which is used for the next step without further purification. ^{1}H-NMR ($CDCl_3$, δ): 1.23 (t, 3H, $-CH_3$), 1.56 (s, 9H, $(CH_3)_3OC(O)$-), 3.07 (t, 2H, $-CH_2CHO$), 4.18 (m, 3H, $-OCH_2CH_3$ and $-CHCO_2$), 6.68 (s, 1H, thiazole-*H*), 9.73 (s, 1H, *H*CO-).

2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-4-hydroxyiminobutanoic acid (9a). To a slution of hydroxyamine hydrochloride (169 mg, 2.44 mmol) in water (1 m*l*) and acetonitrile (3 m*l*) was added triethylamine to adjust the pH to 4-5. A solution of **7** (200 mg, 0.61 mmol) in acetonitrile (2 m*l*) was added and the resultant solution was stirred for 1.5 h at room temperature. The solution was concentrated and the residue was extracted with ethyl acetate. The organic layer was washed with water and saturated aqueous NaCl solution and dried ($MgSO_4$). Filtration followed by concentration provided ethyl ester **8a** as a light yellow colored solid (205 mg, 98%). ^{1}H-NMR ($CDCl_3$, δ): 1.23 (t, 3H, CH_3), 1.58 (s, 9H, $(CH_3)_3OC(O)$-), 3.0 (m, 2H, $N=C-CH_2$), 4.18 (m, 3H, $-OCH_2CH_3$ and -CO-*CH*), 6.83 (s, 1H, thiazole-*H*), 7.45 (t, 1H, N=CH), 9.73 (s, 1H, HCO).

To a solution of the resultant ester (205 mg, 0.60 mmol) in THF (2 m*l*) was added 1.0 m*l* of 2 N aqueous NaOH solution and the solution was stirred for 1 h. After concentration followed by adjusting the pH of the residue to 4-5, the residue was extracted with ethyl acetate. The extract was washed with water and saturated sodium chloride solution. Drying ($MgSO_4$), filtration, and concentration of the filtrate provided **9a** as a light yellow solid (184 mg, 97%). ^{1}H-NMR ($CDCl_3$, δ): 1.5 (s, 9H, $(CH_3)_3OC(O)$-), 3.0 (m, 2H, $N=C-CH_2$-), 4.0 (m, 1H, -CO-*CH*), 6.77 (s, 1H, thiazole-*H*), 7.47 (m, 1H, N=CH-).

2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-4-methoxyiminobutanoic acid (9b). To a solution of methoxyamine hydrocholoride (5.37 g, 64.3 mmol) in CH_2Cl_2 (250 m*l*) was added triethylamine to adjust the pH of the solution to 7. A solution of **7** (3.31 g, 10.1 mmol) in dichloromethane (10 m*l*) was added at 0-5℃ and the resultant solution was stirred for 1.5 h at room temperature. The solution was concentrated. The same workup procedure described in the preceding for the synthesis of **9a** provided ester **8b** as a viscous oil (3.5 g, 98%) and **9b** (2.70 g, 8.4%) as a yellow solid (after hydrolysis with aq. NaOH). Ethyl ester **8b**: ^{1}H-NMR ($CDCl_3$, δ): 1.20 (t, 3H, CH_3), 1.55 (s, 9H, $(CH_3)_3OC(O)$), 2.90 (t, 2H, $N=CHCH_2$), 3.81 (d, 3H, $-NOCH_3$), 4.15 (m, 3H, OCH_2CH_3 and COC*H*-), 6.70 (s, 1H, thiazole-*H*), 7.32 (t, N=CH).

9b: ^{1}H-NMR ($CDCl_3$, δ): 1.55 (s, 9H, $(CH_3)_3OC(O)$-), 2.95 (m, 2H, $N=CHCH_2$), 3.83 (m, 3H, $NOCH_3$), 3.83 (m, 1H, -COC*H*-), 6.77 (s, 1H, thiazole-*H*), 7.40 (t, 1H, N=CH), 10.47 (s, 1H, CO*OH*).

7-[2-(2-Aminothiazol-4-yl)-4-(hydroxyimino)butanoyl]amino-3-vinyl-3-cephem-4-carboxylic acid (2a). To a solution of **9a** (600 mg, 1.9 mmol) in CH_2Cl_2 (2 m*l*) was added dicyclohexylcarbodiimide (510 mg, 2.47 mmol), diphenylmethyl 7-β-amino-3-vinyl-3-cephem-4-carboxylate (**10a**) (699 mg, 1.9 mmol), and 4-dimethylaminopyridine (23 mg, 0.19 mmol). The resulting solution was stirred at room temperature for 2 h and concentrated. Filtration, concentration, followed by purification by silica gel column chromatography (ethyl acetate : hexane = 1 : 3) provided the desired coupled

product (500 mg, 40%) as a solid. ^{1}H-NMR ($CDCl_3$, δ): 1.58 (s, 9H, $(CH_3)_3OC(O)$), 3.03 (t, 2H, N=CHCH_2), 3.45 (s, 2H, -SCH_2), 4.03 (t, 1H, COCH), 4.93-5.67 (m, 5H, C6-H, C7-H, and vinyl), 6.58 (s, 1H, thiazole-*H*, 6.87-7.90 (m, 12H, $CHPh_2$, N=CH).

A solution of the coupled product (23 mg, 0.035 mmol) in CH_2Cl_2 (1 m*l*) was added to a solution of trifluoroacetic acid (400 μ*l*, 5.2 mmol) in anisole (56 μ*l*, 0.52 mmol) at 0-5℃ with stirring. The solution was stirred at room temperature for 40 min and then concentrated. Ethyl acetate was added and stirred for 20 min. filtration provided **2a** as a solid (10 mg, 68%). ^{1}H-NMR (DMSO-d_6δ): 2.98 (m, 2H, N=CHCH_2), 3.5 (m, 2H, -SCH_2), 5.15 (m, 2H, C6-H and C7-H), 6.48 (s, 1H, thiazole-*H*), 9.15 (m, 1H, NH).

2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-4-(*p*-methoxybenzyloxy)iminobutanoic acid (9c). To a solution of 7 (115 mg, 0.32 mmol) in dichloromethane (2 m*l*) was added *p*-methoxybenzyloxyamine (50 mg, 0.33 mmol). The resultant solution was stirred for 30 min at room temperature. The solution was concentrated. Thes ame workup procedure described in the preceding procedure for the synthesis of **8a** followed by purification by column chromatography provided ethyl ester **8c** as a viscous oil (145 mg, 94%). ^{1}H-NMR ($CDCl_3$, δ): 1.30 (t, 3H, CH_3), 1.55 (s, 9H, $(CH_3)_3OC(O)$), 3.0 (t, 2H, $CHCH_2$-), 3.4 (s, 3H, OCH_3), 3.6-3.8 (d, 3H, OCH_2-CH_3 and $COCH$-), 4.0 (m, 1H, $COCH$), 4.95 (d, 3H, $NOCH_2$), 6.6-7.5 (m, 6H, thiazole-*H*, N=CH, and Ph-H's).

To a solution of **8c** prepared (145 mg, 0.30 mmol) in ethanol (3 m*l*) was added 1 N aqueous LiOH (0.90 m*l*) at 0-5℃. The solution was stirred for 17 h at room temperature. After concentration to remove ethanol and adjusting the pH by adding 1 N HCl to 5, it was extracted with ethyl acetate and the extract was dried ($MgSO_4$). Concentration provided acid **9** (123 mg, 88%) as a pale yellow solid. This acid was used for the next acylation step.

Sodium 7[2-(2-aminothiazol-4-yl)-4-(hydroxyimino) butanoyl]amino-3-cephem-4-carboxylate (2b). To a solution of **9c** (210 mg, 0.50 mmol) and diphenylmethyl 7-β-amino-3-cephem-4-carboxylate (**10b**) (200 mg, 0.50 mmol) in CH_2Cl_2 (5 m*l*) was added decyclohexylcarbodiimide (143 mg, 0.70 mmol) and stirred at room temperature for 1 h. Filtration and centration followed by purification by column chromatography (silica gel, ethyl acetate : hexane = 1 : 1) provided the desired coupled product (140 mg, 36%) as a viscous oil. ^{1}H-NMR ($CDCl_3$, δ): 1.56 (s, 9H, $(CH_3)_3OC(O)$), 3.03 (m, 2H, N=CHCH_2), 3.45 (dd, 2H, -SCH_2-), 3.80 (t, 3H, OCH_3), 4.03 (t, 1H, $COCH$), 4.82-5.24 (m, 3H, C6-H and $NOCH_2$), 5.90 (m, 1H, C7-H), 6.50 (t, 1H, C3-H), 6.78 (s, 1H, thiazole-*H*), 6.92-7.70 (m, 15H, $CHPh_2$ and Ph).

Deprotection to obtain the desired cephalosporin **2b** was achieved by the following procedure. The coupled product described above (120 mg, 0.155 mmol) was dissolved in anisole (0.50 m*l*) and trifluoroacetic acid (6.0 m*l*) at 0℃. The solution was stirred at room temperature for 2 h, and then to this solution was added ethyl ether. The solid was filtered and then it was dissolved in water. Aqueous $NaHCO_3$ was added dropwise until all the solid was dissolved. It was necessary to maintain the pH of the solution lower than 8. Purification by column chromatography (SEPRALYTE®; eluent=water, then 10% aqueous methanol) followed by freeze drying provided **2b** as a solid (60 mg, 77%). ^{1}H-NMR (D_2O, δ): 2.95-2.98 (m, 2H, N=CH-CH_2), 3.45-4.05 (m, 3H, -SCH_2 and $COCH$), 5.03 (m, 1H, C6-H), 5.67 (m, 1H, C7-H), 6.23 (t, 1H, C3-H), 6.4 (m, 1H, N=CH), 6.6 (s, 1H, thiazole-*H*).

Sodium 7-[2-(2-aminothiazol-4-yl)-4-(hydroxylimino) butanoyl]amino-3-cephem-3-carbamoyloxymethyl-3-cephem-4-carboxylate (2c). To a solution of **9c** (100 mg, 0.23 mmol) in CH_2Cl_2 (2m*l*) was added 2-chloro-4,6-dimethoxy-1,3,5-triazine (40 mg, 0.23 mmol) and *N*-methylmorpholine (25.2 μ*l*, 0.23 mmol) and stirred at −5℃ for 40 h. A solution of dephenylmethyl 7-β-amino-3-carbamoyloxymethyl-3-cephem-4-carboxylate (**10c**) (101 mg, 0.23 mmol) and *N*-methylmorpholine (25.2 μ*l*, 0.23 mmol) was added and the resulting solution was stirred at −5℃ for 2 h, then warmed to room temperature. The stirring was continued for additional 17 h. The reaction mixture was washed with dilute HCl and saturated aqueous sodium bicarbonate solution, dried ($MgSO_4$), and concentrated. Purification by column chromatography (silica gel, ethyl acetate : hexane = 1 : 1) provided the desired coupled product (60 mg, 30%) as a viscous oil. ^{1}H-NMR ($CDCl_3$, δ): 1.50 (s, 9H, $(CH_3)_3OC(O)$), 2.98 (t, 2H, N=CH-CH_2), 3.77 (s, 4H, OCH_3 and $COCH$), 4.5 (s, 2H, CH_2O), 4.73-5.57 (m, 4H, C6-H, C7-H and $NOCH_2$), 6.53−7.30 (m, 17H, $CHPh_2$, thiazole-*H*, -*N*=CH, and Ph-H's).

Deprotection to obtain the desired cephalosporin **2c** was achieved by the following procedure. The coupled product described above (55 mg, 0.064 mmol) was dissolved in anisole (350 μ*l*) and the solution was cooled to −30℃. To this was added a solution of $AlCl_3$ (51 mg, 0.038 mmol) in anisole (0.30 m*l*) and the solution was stirred at −30-−15℃ for 3 h, then warmed to 0℃, saturated aqueous $NaHCO_3$ solution was added to neutralize (pH 7). After filtration, the filtrate was washed with ethyl acetate and concentrated. Purification by column chromatography (SEPRALYTE®; eluent=water, then 10% aqueous methanol) followed by freeze drying provided **2c** as a solid (10 mg, 32%). ^{1}H-NMR (D_2O, δ): 2.95−2.98 (m, 2H, N=CH-CH_2), 3.56-4.12 (m, 3H, -SCH_2 and $COCH$), 4.7 (m, 2H, CH_2O), 5.1-5.7 (m, 2H, C6-H and C7-H), 6.4 (t, 1H, N=CH), 6.6 (s, 1H, thiazole-*H*).

Ethyl 2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)-5-*tert*-butyldiphenylsilyloxypent-2-enoate (11). To a cooled (−78℃) solution of 1.0 g (2.6 mmol) of Ethyl (2-*tert*-butoxycarbonylimino-3-tert-butoxycarbonylthiazol-4-yl)acetate[1] in THF (10 m*l*) was added *n*-BuLi (1.6 M solution in hexane, 1.8 m*l*, 2.9 mmol) under nitrogen atmosphere. Then, a solution of 3-*tert*-butyldiphenylsilyloxypropanal (0.9 g, 2.9 mmol) in THF (5 m*l*) was added slowly at −78℃ and the resultant solution was warmed slowly to room temperature with stirring. After the solution was stirred at room temperature for 17 h, then aqueous 10% citric acid solution (7 m*l*) was added. After 10 min it was concentrated to remove THF, then extracted with ethyl acetate and dried ($MgSO_4$). Filtration, concentration followed by purification by silica gel column chromatography provided ester **11** (1.23 g, 79%) as an oil. ^{1}H-NMR ($CDCl_3$, δ): 1.03 (s, 9H, $SiC(CH_3)_3$), 1.28 (t, 3H, OCH_2CH_3), 1.5 (s, 9H, $(CH_3)_3OC(O)$), 2.68 (q, 2H, CH_2CH_2OSi), 3.75 (t, 2H, CH_2CH_2OSi), 4.23 (q, 2H, OCH_2), 6.88 (s, 1H, thiazole-*H*), 7.20-7.78 (m, 10H, $SiPh_2$).

Ethyl 2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)-5-(tetrahydropyran-2-yloxy)pent-2-enoate (11′). THP protected ester **11′** was prepared as the same precedure as

described above using 2-tetrahydropyranoxypropanal. Yield (60%). ^{1}H-NMR ($CDCl_3$, δ): 1.3 (t, 3H, OCH_2CH_3), 1.5 (s, 9H, $(CH_3)_3OC(O)$), 1.5-1.7 (s, 6H, THP), 2.65 (q, 2H, C=CH-CH_2), 3.4-4.0 (m, 4H, CH_2CH_2O and THF(OCH_2)), 4.24 (q, 2H, -OCH_2CH_3), 4.65 (bs, 1H, THP (C2-H)), 7.0 (s, 1H, thiazole-*H*), 7.1-7.3 (t, 1H, C=CH).

Ethyl 2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)-5-oxopentanoate (12). A mixture of ester **11** (100 mg, 0.17 mmol) in ethyl acetate (5 m*l*) and 5% palladium on carbon (catalytic amount) was stirred under H_2 (1 atm) for 2 days. Filtration, concentration followed by purification (silica gel column chromatography, hexane : ethyl acetate = 6 : 1) afforded the desired hydrogenated ester (78 mg, 77%) as an oil. ^{1}H-NMR ($CDCl_3$, δ): 1.03 (s, 9H, $SiC(CH_3)_3$), 1.28 (t, 3H, OCH_2CH_3), 1.5 (s, 9H, $(CH_3)_3OC(O)$), 2.68 (q, 2H, CH_2CH_2OSi), 3.64 (m, 3H, CH_2CH_2OSi, CHC(O)), 4.12 (q, 2H, OCH_2CH_2), 6.65 (s, 1H, thiazole-*H*), 7.23-7.73 (m, 10H, $SiPh_2$).

The above prepared ester was dissolved in THF (2 m*l*). After addition of tetra-*n*-butylammonium fluoride (in THF, 1 M solution, 0.43 m*l*, 1.5 mmol), it was stirred at room temperature for 2 h, then concentrated. The residue was partitioned ethyl acetate and saturated aqueous ammonium chloride, and stirred for 10 min. The aqueous layer was separated, washed with water and satruated aqueous sodium chloride, and dried ($MgSO_4$). Filtration, concentration, and purification (silica gel column chromatography) gave the desired alcohol (26 mg, 58%) as an oil. ^{1}H-NMR ($CDCl_3$, δ): 1.18 (t, 3H, CH_3), 1.55 (s, 9H, $(CH_3)_3OC(O)$), 3.53 (m, 3H, CH_2OH and CH C(O)), 4.1 (q, 2H, OCH_2CH_3), 6.67 (s, 1H, thiazole-H).

Into CH_2Cl_2 (5 m*l*) was suspended a well-ground pyridinium chlorochromate (427 mg, 1.98 mmol)-silica gel (427 mg) mixture. A solution of the alcohol prepared in the preceding procedure (340 mg, 0.99 mmol) in CH_2Cl_2 (1 m*l*) was added and the resultant solution was stirred at room temperature for 2 h. Ethyl ether (10 m*l*) was added and stirred. The mixture was filtered. The filtrate was washed with ethyl ether (20 m*l*). Concentration and purification by passing through a short column of Florisil with the aid of ethyl ether provided **12** (235 mg, 69%) as an oil. ^{1}H-MNR ($CDCl_3$, δ): 1.20 (t, 3H, CH_3), 1.5 (s, 9H,$(CH_3)_3OC(O)$), 2.27 (m, 4H, CH_2CH_2CHO), 3.72 (t, 1H, CHC(O)), 4.13 (q, 2H, -OCH_2CH_3), 6.57 (s, 1H, thiazole-H), 8.43 (m, 1H, HCO).

Ethyl 2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)-5-(p-methoxybenzyloxy)iminopentanoate (13c). To a solution of aldehyde **12** (850 mg, 2.49 mmol) in CH_2Cl_2 (5 m*l*) was added *p*-methoxybenzyloxyamine (351 mg, 2.49 mmol) and the resultant solution was stirred at room temperature for 30 min. Concentration followed by purification (silica gel, ethyl acetate : hexane = 1 : 2) furnished **13c** (1.14 g, 96%) as an oil. ^{1}H-NMR ($CDCl_3$, δ): 1.3 (t, 3H, CH_3), 1.53 (s, 9H, $(CH_3)_3OC(O)$), 2.0-2.3 (m, 4H, CH_2CH_2), 3.8 (s, 4H, CHC(O) and OCH_3), 4.17 (q, 2H, -OCH_2CH_3), 5.0 (d, 2H, $NOCH_2$), 6.67-7.43 (m, 5H, thiazole-H and Ph-H's).

2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-5-(p-methoxybenzyloxy)iminopentanoic acid (14c). To a solution of **13c** (1.49 g, 2.39 mmol) in ethanol (5 m*l*) was added 1 N aqueous LiOH (7 m*l*). This solution was stirred at room temperature for 19 h and concentrated. Water (5 m*l*) was added and pH of the solution was adjusted to 4 with 1 N HCl, It was extracted with ethyl acetate, washed with saturated aqueous sodium chloride solution, and dried ($MgSO_4$). Filtration and concentration provided **14c** (930 mg, 87%) as a light yellow colored solid. ^{1}H-NMR ($CDCl_3$, δ): 1.53 (s, 9H, $(CH_3)_3OC(O)$), 2.0-2.5 (m, 4H, CH_2CH_2), 3.77 (s, 4H, OCH_3 and CHC(O)), 4.99 (d, 2H, $NOCH_2$), 6.6-7.4 (m, 6H, CH=N, thiazole-*H*, and Ph-H's).

7-[2-(2-Aminothiazol-4-yl)-5-(hydroxyimino)pentanoyl]amino-3-vinyl-3-cephem-4-carboxylic acid (3a). To a solution of **14c** (310 mg, 0.69 mmol) in CH_2Cl_2 (5 m*l*) was added a solution of dicyclohexylcarbodiimide (510 mg, 2.47 mmol) in CH_2Cl_2 (1 m*l*), diphenylmethyl 7-β-amino-3-vinyl-3-cephem-4-carboxylate (**10a**) (270 mg, 0.69 mmol). The resultant solution was stirred at room temperature for 17 h and concentrated. To this residue was added a small amount of ethyl acetate and the mixture was stirred at 0-5℃. Filtration, concentration, followed by purification by column chromatography (silica gel, ethyl acetate : hexane = 1 : 2) provided the desired coupled product (350 mg, 61%) as a solid. ^{1}H-NMR ($CDCl_3$, δ): 1.53 (s, 9H, $(CH_3)_3OC(O)$), 2.0-2.5 (m, 4H, CH_2CH_2), 3.4 (bs, 2H, SCH_2), 3.77 (s, 4H, CHC(O) and OCH_3), 4.99 (d, 2H, $NOCH_2$), 5.1-6.1 (m, 5H, C6-H, C7-H, and vinyl), 6.5-7.5 (m, 17H, thiazole-*H*, $CHPh_2$, N=CH, and Ph).

A solution of the coupled product (60 mg, 0.073 mmol) in CH_2Cl_2 (1 m*l*) was added to a solution of trifluoroacetic acid (1.68 m*l*, 21.9 mmol) in anisole (238 μ*l*, 2.19 mmol) at 0-5℃ with stirring. The solution was stirred at room temperature for 70 min and then concentrated. Ethyl acetate was added and the mixture was stirred for 20 min. Filtration provided **3a** as a solid (18 mg, 55%). ^{1}H-NMR (DMSO-d_6, δ): 1.8-2.4 (m, 4H, CH_2CH_2), 3.5 (m, 3H, SCH_2, C(O)CH), 5.0-5.5 (m, 5H, C6-H, C7-H, and vinyl), 6.5 (d, 1H, thiazole-*H*), 6.8-7.4 (m, 3H, N=CH and NH_2), 8.8-9.0 (m, 1H, NH).

7-[2-(2-Aminothiazol-4-yl)-5-(hydroxyimino)pentanoyl]amino-3-cephem-4-carboxylic acid (3b). To a solution of **14c** (30 mg, 0.067 mmol) in CH_2Cl_2 (1 m*l*) was added a solution of dicyclohexylcarbodiimide (18 mg, 0.087 mmol) in CH_2Cl_2 (1 m*l*), diphenylmethyl 7-β-amino-3-cephem-4-carboxylate (**10b**) (25.3 mg, 0.067 mmol). The resulting solution was stirred at room temperature for 17 h and concentrated. To this residue was added a small amount of ethyl acetate and the mixture was stirred at 0-5℃. Filtration, concentration, followed by purification by column chromatography (silica gel, ethyl acetate : hexane = 1 : 2) provided the desired coupled produt (30 mg, 55%) as a solid. ^{1}H-NMR ($CDCl_3$, δ): 1.53 (s, 9H, $(CH_3)_3OC(O)$), 2.0-2.5 (m, 4H, CH_2CH_2), 3.4 (bs, 2H, SCH_2), 3.77 (s, 4H, C(O)CH, OCH_3), 4.93 (d, 2H, $NOCH_2$), 6.5-7.4 (m, 18H, thiazole-*H*, $CHPh_2$, N=CH, Ph, and C3-H).

A solution of the coupled product (90 mg, 0.111 mmol) in CH_2Cl_2 (2.5 m*l*) was added to a solution of trifluoroacetic acid (2.53 m*l*, 33.0 mmol) in anisole (240 μ*l*, 2.21 mmol) at 0-5℃ with stirring. The solution was stirred at room temperature for 1 h and then concentrated. Ethyl acetate was added and the mixture was stirred for 20 min. Filtration provided **3b** as a solid (15 mg, 33%). ^{1}H-NMR (DMSO-d_6, δ): 2.0 (m, 4H, CH_2CH_2), 3.5 (m, 3H, -SCH_2 and C(O)CH), 5.0 (d, 1H, C6-H), 5.7 (d, 1H, C7-H), 6.4 (d, 1H, thiazole-*H*), 6.5 (m, 1H, C3-H), 6.8-7.5 (m, 3H, N=CH and NH_2).

Sodium 7-[2-(2-aminothiazol-4-yl)-5-(hydroxyimino) pentanoyl]amino-3-carbamoyloxymethyl-3-cephem-4-

carboxylate (3c). To a solution of **14c** (100 mg, 0.22 mmol) in CH_2Cl_2 (2 m*l*) was added 2-chloro-4,6-dimethoxy-1,3,5-triazine (39 mg, 0.22 mmol) and *N*-methylmorpholine (25 μ*l*, 0.22 mmol) and stirred at −5℃ for 1 h. A solution of diphenylmethyl 7-β-amino-3-carbamoyloxymethyl-3-cephem-4-carboxylate (**10c**) (98 mg, 0.22 mmol) and *N*-methylmorpholine (25 μ*l*, 0.22 mmol) was added and the resulting solution was stirred at −5℃ for 1 h, then warmed to room temperature. The stirring was continued for additional 17 h. The reaction mixture was washed with dilute HCl and saturated aqueous sodium bicarbonate solution, dried ($MgSO_4$), and concentrated. Purification by column chromatography (silica gel, ethyl acetate : hexane = 1 : 1) provided the desired coupled product (76 mg, 39%) as a solid. ^{1}H-NMR ($CDCl_3$, δ): 1.5 (s, 9H, $(CH_3)_3OC(O)$), 2.0-2.5 (m, 4H, CH_2CH_2), 3.4(bs, 2H, $-SCH_2$), 3.77 (s, 4H, OCH_3 and $CHC(O)$), 4.7 (s, 2H, CO_2CH_2), 4.9-5.2 (m, 4H, C6-H, C7-H, and $NOCH_2$), 6.5 (s, 1H, thiazole-H), 6.8-7.3 (m, 16H, $CHPh_2$, N=CH and Ph-H's).

The coupled product described above (50 mg, 0.057 mmol) was dissolved in anisole (0.40 m*l*) and the solution was cooled to −30℃. To this was added a solution of $AlCl_3$ (46 mg, 0.034 mmol) in anisole (0.40 m*l*) and the solution was stirred at −30-−15℃ for 3 h, then warmed to 0℃, saturated aqueous $NaHCO_3$ solution was added to neutralize (pH 7). After filtration, the filtrate was washed with ethyl acetate and concentrated. Purification by column chromatography (SEPRALYTE®; eluent = water, then 10% aqueous methanol) followed by freeze drying provided of **3c** as a solid (13 mg, 45%). ^{1}H-NMR (D_2O, δ): 2.0-2.5 (m, 4H, CH_2CH_2), 3.5-4.0 (m, 3H, C2-H and $CHC(O)$), 4.6 (s, 2H, CH_2O), 5.2-5.6 (m, 2H, C6-H and C7-H), 6.6 (d, 1H, thiazole-*H*), 6.7-7.5 (m, 1H, N=CH).

2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-5-(tetrahydropyran-2-yloxy)pent-2-enoic acid (15). Acid **15** was obtained by hydrolysis of **11**'with LiOH according to the same procedure as described for the preparation of **14c**. Yield (62%). ^{1}H-NMR ($CDCl_3$, δ): 1.5(s, 9H, $(CH_3)_3OC(O)$), 1.5-1.6 (bs, 6H, THP), 2.8 (q, 2H, C=CH-CH_2), 3.4-4.0 (m, 4H, CH_2O and THP(OCH_2)), 4.65 (bs, 1H, THP (CH)), 7.0 (s, 1H, thiazole-*H*), 6.6-7.1 (m, 1H, C=CH).

7-[2-(2-Aminothiazol-4-yl)-5-hydroxypentanoyl] amino-3-vinyl-3-cephem-4-carboxylic acid (16a). To a solution of **15** (1.0 g, 2.51 mmol) in CH_2Cl_2 (2 m*l*) was added 2-chloro-4,6-dimethoxy-1,3,5-triazine (440.5 mg, 2.51 mmol) and *N* -methylmorpholine (0.28 m*Cl*, 2.5 mmol) and the solution was stirred at −5℃ for 40 h. A solution of diphenylmethyl 7-β-amino-3-vinyl-3-cephem-4-carboxylate (**10a**) (980 mg, 2.51 mmol) and *N*-methylmorpholine (0.28 m*l*, 2.5 mmol) was added and the resulting solution was stirred at −5℃ for 2 h, then warmed to room temperature. The stirring was continued for additional 17 h. The reaction mixture was washed with dilute HCl and saturated aqueous sodium bicarbonate solution, dried ($MgSO_4$), and concentrated. Purification by column chromatography (silica gel, ethyl acetate : hexane = 1 : 3) provided the desired coupled product (800 mg, 41%) as a solid. ^{1}H-NMR ($CDCl_3$, δ): 1.5 (s, 9H, $(CH_3)_3OC(O)$), 1.2-1.7 (m, 6H, THP), 2.6 (q, 2H, C=CH-CH_2), 3.2-4.0 (m, 6H, $-SCH_2$, THP(OCH_2), and CH_2OTHP), 4.5 (m, 1H, THP(CH)), 4.85 (d, 1H, C6-H), 5.0-5.6 (m, 3H, C7-H and CH=CH_2), 6.6-6.9 (t, 1H, C=CH-), 7.0 (s, 1H, thiazole-*H*), 7.0-7.5 (m, 12 h, $CHPh_2$ and CH=CH_2).

A solution of the coupled product prepared (800 mg, 1.04 mmol) and pyridinium *p*-toluene sulfonate (26 mg, 0.104 mmol) in anhydrous EtOH (5 m*l*) was stirred at 55℃ for 10 h and concentrated to remove EtOH. The residue was extracted with ethyl acetate and the resultant extract was washed with water and saturated aqueous sodium chloride and dried ($MgSO_4$). Filtration, concentration followed by purification (silica gel chromatography, hexane : ehtyl acetate = 1 : 1 or 1 : 3) provided the desired alcohol (586 mg, 82%) as a solid. ^{1}H-NMR ($CDCl_3$, δ): 1.55 (s, 9H, $(CH_3)_3OC(O)$), 2.6 (q, 2H, C=CHCH_2), 3.22 (d, 2H, $-SCH_2$), 3.75 (m, 2H, CH_2OH), 4.9 (d, 1H, C6-H), 5.1-5.6 (m, 3H, C7-H, CH=CH_2), 6.8-6.9 (m, 2H, thiazole-*H*, C=CH), 7.0-7.5 (m, 12H, $CHPH_2$ and CH=CH_2), 8.0 (d, 1H, NH).

The coupled product described above (40 mg, 0.058 mmol) in CH_2Cl_2 (1 m*l*) was added to a solution of trifluoroacetic acid (0.67 m*l*, 8.72 mmol) and anisole (94.6 μ*l*, 0.87 mmol) at 0℃. After the solution was stirred at room temperature for 1 h, it was concentrated. The residue was dried under vaccum and dissolved into ethyl acetate (1 m*l*) and solidified by adding isopropyl ether. Filtration provided **16a** as a yellow solid (23 mg, 94%). ^{1}H-NMR (DMSO-d_6, δ): 2.52 (m, 2H, C=CH-CH_2), 3.53 (m, 4H, $-SCH_2$ and CH_2OH), 4.73 (t, 1H, OH), 4.94-5.20 (q, 2H, CH=CH_2), 5.07 (d, 1H, C6-H), 5.67 (q, 1H, C7-H), 6.57 (s, 1H, thiazole-H), 6.76 (t, 1H, C=CH), 7.08 (q, 1H, CH=CH_2=), 7.21 (s, 2H, NH_2), 9.09 (d, 1H, NH).

Sodium 7-[2-(2-aminothiazol-4-yl)-5-hydroxypentanoyl]amino-3-cephem-4-carboxylate (16b). Sodium salt **16b** was prepared according to the same procedure as described in the preceding procedure. This acid was treated with $NaHCO_3$ to provide a sodium salt. NMR data of the salt are as follows: ^{1}H-NMR (D_2O, δ): 2.54 (q, 2H, C=CH-CH_2), 3.4-3.7 (m, 4H, $-SCH_2$, CH_2OH), 5.14 (d, 1H, C6-H), 5.68 (d, 1H, C7-H), 6.28 (m, 1H, C3-H), 6.71 (s, 1H, thiazole-H), 6.80 (t, 1H, C=CH).

Ethyl 2-aminothiazol-4-yl carboxylate (18). A mixture of thiourea (27 g, 0.35 mol) and ethyl 3-bromo-2-oxopropanoate (57.6 g, 0.30 mol) in EtOH (400 m*l*) was stirred for 18 h. It was filtered and concentrated until the total volume of the solution to ca. 50 m*l*. The solid formed was filtered and dried. This salt was dissolved into wated (100 m*l*) and to this was added 24% ammonia water (13 m*l*) to precipitate solid. Filtration and drying produced ester **18** as a white solid (30.4 g, 59%; mp. 176-178℃).

Ethyl 2-triphenylmethylaminothiazol-4-yl carboxylate. To a solution of **18** in DMF (50 m*l*) was added triethyl amine (7.1 m*l*) and triphenylmethyl chloride (13 g, 47 mol) at 0℃. This solution was stirred at 50-55℃ for 2 h. To this reaction mixture water (400 m*l*) was added. Viscous product was dissolved in ethyl acetate (300 m*l*), washed with water (100 m*l*×3), and dried ($MgSO_4$). After filtration and concentration, the residue was recrystallized from EtOH. The desired ester was obtained as a light yellow solid (16.5 g, 86%; mp. 136-137℃). ^{1}H-NMR ($CDCl_3$, δ): 1.33 (t, 3H, OCH_2CH_3), 4.37 (q, 2H, OCH_2CH_3), 7.07 (br s, 1H, NH), 7.25 (s, 1H, C5-H), 7.33 (s, 15H, Ph_3).

2-Triphenylmethylaminothiazol-4-yl carboxylic acid. To a mixture of ethyl 2-triphenylmethylaminothiazol-4-yl carboxylate (45 g, 0.11 mol) in MeOH (350 m*l*) was added 5 N NaOH (90 m*l*). The mixture was heated at reflux for 1 h. The solid produced was filtered and suspended to water

(150 m*l*). After adjusting the pH of the suspension to 4 by adding 2 N HCl, the suspension was stirred for 30 min. Filtration, washing with provided the desired acid (34 g, 81 %; mp. 235-238℃).

Ethyl 3-(2-triphenylmethylaminothiazol-4-yl)-3-oxopropanoate (19). To a solution of 2-triphenylmethylaminothiazol-4-yl carboxylic acid (23.7 g, 0.163 mmol) in THF (300 m*l*) was added 1,1'-carbonyldiimidazole (11.05 g, 68.1 mmol). The resultant solution was stirred at room temperature. To this was added magnesium salt of ethyl monomalonate [prepared by adding magnesium ethoxide (4 g, 40 mmol) to a solution of ethyl monomalonate (10.6 g, 80 mmol) in THF (200 m*l*) followed by stirring and concentration].[3] After stirring at room temperature for 18 h, the solution was heated at 45-50℃ for 6 h. After concentration the residue was partitioned into ethyl ether (400 m*l*), 3 N HCl (200 m*l*), and water (100 m*l*) and extracted. The organic layer was separated and the aqueous layer was extracted with ethyl acetate and the organic layer was combined. It was washed with saturate aqueous sodium bicarbonate (300 m*l*) and dried ($MgSO_4$). Concentration provided **19** as a light yellow colored solid (28 g, 98%). ^{1}H-NMR ($CDCl_3$, δ): 1.22 (t, 3H, OCH_2CH_3), 3.80 (s, 2H, $COCH_2$), 4.15 (q, 2H, OCH_2CH_3). 6.94 (s, 1H, thiazole-*H*), 7.33 (s, 15H, Ph_3).

3-(2-Triphenylmethylaminothiazol-4-yl)-3-methoxyimino-3-oxopropanoic acid (20). To a mixture of methoxyamine HCl (10.7 g, 128 mmol) and sodium bicarbonate (1.12 g, 129 mmol) in acetonitrile (400 m*l*)-water (100 m*l*) was added **19** (20.0 g, 42 mmol) and the mixture was stirred at room temperature for 48 h. After concentration the residue was dissolved into ethyl ether (600 m*l*) and washed with water (100 m*l*×3), and dried ($MgSO_4$). Concentration provided ethyl 3-(2-triphenylmethylaminothiazol-4-yl)-3-methoxyimono-3-oxopropanoate (20 g, 94%) as a light yellow solid. ^{1}H-NMR ($CDCl_3$, δ): 1.18 (t, 3H, OCH_2CH_3), 3.60 (s, $COCH_2$), 3.92 (s, 3H, N-OCH_3), 4.12 (q, 2H, OCH_2CH_3), 6.63 (s, 1H, thiazole-*H*), 7.33 (s, 15H, Ph_3).

A solution of the solid obtained (1.0 g, 34 mmol) and 5 N NaOH (14 m*l*) in EtOH (150 m*l*) and THF (50 m*l*) was stirred at room temperature for 15 min. To this was added 5 N NaOH (6 m*l*) additionally, and then the solution was heated at 45-50℃ for 40 min. Concentration followed by addition of 3 N HCl to adjust the pH of the solution to 4. The solid formed was filtered and dissolved into acetone (500 m*l*). Filtration and concentration provided acid **20** (11 g, 70%) as a white solid (mp. 116-117℃). ^{1}H-NMR ($CDCl_3$ +DMSO-d_6, δ): 3.47 (s, $COCH_2$), 3.88 (s, 3H, OCH_3), 6.72 (s, 1H, thiazole-H), 7.30 (s, 15H, Ph_3).

Sodium 7-[2-(2-aminothiazol-4-yl)-3-methoxyiminopropanoyl]amino-3-vinyl-3-cephem-4-carboxylate (21a). To a solution of **20** in THF (50 m*l*) was added 1-hydroxybenzotriazole (0.57 g, 4.0 mmol) and dicyclohexylcarbodiimide (0.83 g, 4.0 mmol) at 0℃. The solution was stirred at 0℃ for 10 min and 4.5 h for room temperature. Filtration and concentration provide the desired activated ester as a light yellow solid in almost quantitative yield.

A solution of the activated ester in CH_2Cl_2 (30 m*l*) and **10a** was stirred at room temperature for 18 h. Concentration and purification by column chromatography (silica gel, hexane : ethyl acetate = 1 : 1) provided the desired coupled product (0.88g, 78%) as a light yellow solid. ^{1}H-NMR ($CDCl_3$, δ): 3.37 (ABq, 2H, -SCH_2), 3.63 (d, 2H, CH_2C(O)NH), 4.03 (s, 3H, -OCH_3), 4.8-5.7 (m, 3H, CH=CH_2, C6-H), 5.97 (dd, 1H, C7-H), 6.76 (s, 1H, thiazole-H), 7.03 (s, $CHPh_2$), 7.1-7.6 (m, 26H, CH=CH_2, Ph_3, and Ph), 8.43 (d, CONH).

The coupled product (0.50 g, 0.583 mmol) was dissolved in 80% aqueous formic acid (5 m*l*) and the solution was stirred at 40-50℃ for 1 h. It was cooled to 0℃ and filtered. The solution was concentrated. To the residue was added ethyl ether to solidify the product. Drying provided a yellow solid (0.15 g). This solid was suspended into water (3 m*l*) and to this was added sodium bicarbonate (19 mg). Filtration to remove the insolubles followed by purification by reverse phase column chromatography (stationary phase: IR 120, mobile phase 15% MeOH) provided **21** (35 mg, 32%) as a light yellow colored solid [mp. 171-200℃ (dec)]. ^{1}H-NMR (D_2O, δ): 3.60 (ABq, 2H, -SCH_2), 3.62-3.71 (d, 2H, CH_2C(O)NH), 3.92 (s, 3H, OCH_3), 5.07 (d, 1H, C6-H), 5.20 (d, 1H, vinyl CH), 5.35 (d, 1H, vinyl CH), 5.77 (d, 1H, C7-H), 6.67 (dd, 1H, vinyl CH), 6.98 (s, 1H, thiazole-*H*). IR (KBr, cm^{-1}): 3349, 1758, 1606, 1535, 1370, 1048.

Sodium 7-[2-(2-aminothiazol-4-yl)-3-methoxyiminopropanoyl]amino-3-[(1-methyltetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate (21b). This compound was prepared by the similar procedure as in the preceding procedure for the preparation of **21a**. mp. 169-185℃ (dec). ^{1}H-NMR (D_2O, δ): 3.67 (ABq, 2H, -SCH_2), 3.70 (d, 2H, CH_2C(O)NH), 3.91 (s, 3H, -NCH_3), 3.97 (s, 3H, -OCH_3), 4.73 (s, 2H, CH_2S), 5.02 (d, 1H, C6-H), 5.51 (d, 1H, C7-H), 6.96 (s, 1H, thiazole-*H*). IR (KBr, cm^{-1}): 3372, 1760, 1610, 1535, 1379, 1049.

Sodium 7-[2-(2-aminothiazol-4-yl)-3-methoxyiminopropanoyl]amino-3-acetoxymethyl-3-cephem-4-carboxylate (21c). This compound was prepared by the similar procedure as in the preceding procedure for the preparation of **21a**. mp. 149-170℃ (dec). ^{1}H-NMR (D_2O, δ): 2.05 (s, 3H, OAc), 3.46 (ABq, 2H, -SCH_2), 3.71 (d, 2H, CH_2C(O)NH), 3.92 (s, 3H, -OCH_3), 5.06 (d, 1H, C6-H), 4.5-5.5 (dd, 2H, CH_2O), 5.57 (d, 1H, C7-H), 6.98 (s, 1H, thiazole-*H*). IR (KBr, cm^{-1}): 3375, 1779, 1612, 1535, 1372, 1241, 1043.

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