IMPROVED SYNTHESIS OF AN ESTER-TYPE PRODRUG, 1-ACETOXYETHYL 7-[(*Z*)-2-(2-AMINOTHIAZOL-4-YL)-2-HYDROXYIMINOACETAMIDO]-3-[(*Z*)-1-PROPENYL]-3-CEPHEM-4-CARBOXYLATE (BMY-28271)

HAJIME KAMACHI, TAKAAKI OKITA, SATSUKI OKUYAMA, HIDEAKI HOSHI and TAKAYUKI NAITO

Bristol-Myers Squibb Research Institute,
2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan

(Received for publication June 15, 1990)

1-Acetoxyethyl 7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylate (BMY-28271) is an ester-type prodrug of cephalosporin for oral use. Methods suitable for large scale preparation were investigated. The yield was improved by esterification of 7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-trityloxyiminoacetamido]cephem-4-carboxylic acid (**11**) followed by removal of the trityl group and, in addition, column chromatographic purification at each step was eliminated by optimization of the reaction conditions.

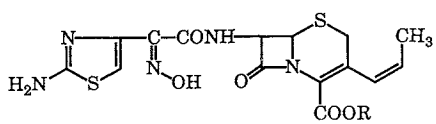
7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylic acid (**1**, BMY-28232) showed good activity against both Gram-positive and Gram-negative bacteria except *Pseudomonas aeruginosa* and its 1-acetoxyethyl ester (**2**, BMY-28271) exhibited potent *in vivo* activity and bioavailability after oral administration[1,2]. In our original synthesis[1], **1** was prepared by *N*-acylation of the 7-aminocephem diphenylmethyl ester with the *N*,*O*-dityrityl-substituted aminothiazole acid followed by simultaneous deblocking of the trityl and diphenylmethyl groups and subsequent purification by reversed phase column chromatography. **2** was derived by esterification of **1** with 1-acetoxyethyl bromide followed by purification with silica gel column chromatography. The deblocking and esterification steps were in rather low yield (33% and 12% (after crystallization), respectively) and required tedious column chromatographic purification.

This report describes an improved synthesis of **2** in higher yield and without column chromatographic purification, suitable for large scale preparation.

Synthesis of the C-7 Side Chain Acid (**8**)

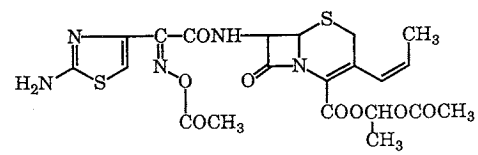
In the course of the study, we found that esterification of **1** with an excess of the 1-acetoxyethyl bromide did not increase the formation of **2**, but gave a considerable amount of the acetoxyimino ester **3**, apparently produced by transesterification with the reagent. This showed that the protection of the hydroxyimino group in the C-7 side chain would be desirable for the esterification.

Fig. 1. Structure of **1** and **2**.



BMY-28232 (**1**) R=H
BMY-28271 (**2**) R=CH(CH$_3$)–O–COCH$_3$

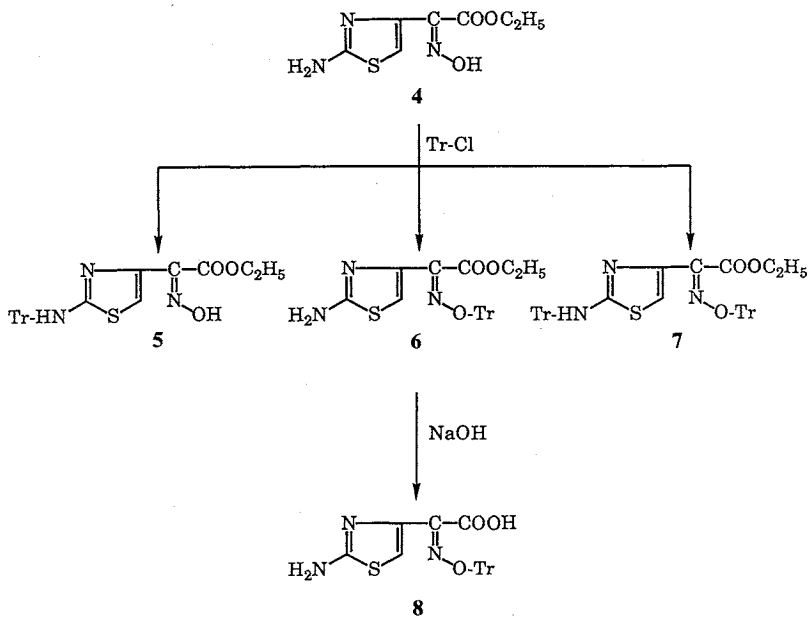
Fig. 2. Structure of the by-product, **3**.



On the other hand, BLUMBACH *et al.*[3] showed that protection of the amino group was not necessary for (*Z*)-2-(2-aminothiazol-4-yl)-2-alkoxyiminoacetic acids when used as their 1-hydroxybenzotriazole active esters in acylation of 7-amino-cephem-carboxylic acids.

In the present synthesis, therefore, esterification was intended with the compound in which only the hydroxyimino group of the side chain was protected. For this purpose, the *O*-tritylated C-7 side chain acid **8** was prepared from the commercially available hydroxyiminoacetate **4** by *O*-tritylation followed by hydrolysis, as shown in Scheme 1. Optimum conditions for the *O*-tritylation of **4** were investigated by HPLC. An equimolar mixture of **4** and trityl chloride in a solvent in the presence of a base was stirred at room temperature and the reaction mixture was examined by HPLC. Table 1 shows the results of the HPLC study. The reaction mixture contained the *N*-trityl (**5**)[4], *O*-trityl (**6**) and *N*,*O*-ditrityl derivatives (**7**)[4] together with the starting compound (**4**). Their ratio varied depending on the reaction conditions employed. BUCOURT *et al.*[4] reported that tritylation of **4** in the presence of 1 equivalent of triethylamine (TEA) in *N*,*N*-dimethylformamide (DMF) gave predominantly **5**. We confirmed their results (Expt 1). Replacement of the solvent with EtOAc made the reaction very sluggish with most of **4** unreacted, although

Scheme 1. Preparation of the *O*-tritylated 7-side chain acid (**8**).



Tr: $C(C_6H_5)_3$.

Table 1. Tritylation of **4** (1 mmol).

Expt	Base (1 mmol)	Solvent	Period	Yield (%) estimated by HPLC			
				4	**5**	**6**	**7**
1	TEA	DMF	3 hours	7.2	86.3	0.4	6.0
2	TEA	EtOAc	3 days	74.0	23.2	Trace	2.2
3	DBN	EtOAc	3 days	62.0	10.9	13.3	13.3
4	NaH	EtOAc	4.5 hours	14.6	1.5	66.1	9.9
5	NaH	THF	4.5 hours	14.2	2.0	63.7	14.3
6	NaH	DMF	4.5 hours	28.9	19.9	22.5	16.0

5 was predominant among the products (Expt 2). Replacement of TEA with a stronger base (DBN) did not accelerate the reaction, but nearly equal amounts of **5**, **6** and **7** were generated in the reaction mixture (Expt 3). The reaction proceeded rapidly when DBN was replaced with sodium hydride (NaH) and the desired product **6** was predominant (Expt 4). This may be due to generation of the O^- ion by NaH which was a much stronger base than DBN or TEA. The reaction in tetrahydrofuran (THF) (Expt 5) gave similar results with somewhat decrease of **6** and increase of **7**, but the reaction in DMF (Expt 6) gave comparable amounts of **4**, **5**, **6**, and **7**. In conclusion, the reaction conditions of Expt 4 were selected and used for the preparation of **6** in 72% yield as a crystalline powder by simply concentrating the reaction mixture and subsequently washing the residue with water. Compound **6** was heated in 50% aqueous dioxane with NaOH (3 equivalents) for 1.5 hours and then acidified with HCl to afford **8** in 90% yield as colorless prisms.

7-*N*-Acylation of the 7-Aminocephem (**10**)

Scheme 2 shows the preparation process of BMY-28271 (**2**), in which *N*-acylation of the 7-amino-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylic acid (**10**)[5] was carried out by the active ester method. For the acylation, the side chain acid **8** was treated with 1-hydroxybenzotriazole and dicyclohexylcarbodiimide (DCC) in THF to give the active ester **9** in quantitative yield. The 7-aminocephem **10** (containing a small amount of the *E*-propenyl isomer; $Z/E=12/1$) was trimethylsilylated and subsequently *N*-acylated with **9** at room temperature overnight. The reaction mixture was checked by HPLC with the results shown in Table 2. Trimethylsilylation with *N*,*O*-bis(trimethylsilyl)acetamide (BSA)[6] in CH_2Cl_2 followed by treatment with **9** gave a mixture of several components, in which the *N*-acyl derivative **11** was predominant (Expt 1). Trimethylsilylation with trimethylchlorosilane (TMCS) and TEA[6] in THF increased the relative peak area of **11** (Expt 2). Excess of TEA (Expt 3) resulted in formation of a considerable amount of the Δ^2-isomer, whereas a reduced amount of TEA (Expt 4) yielded an increase of **11** and

Scheme 2. Improved synthesis of BMY-28271.

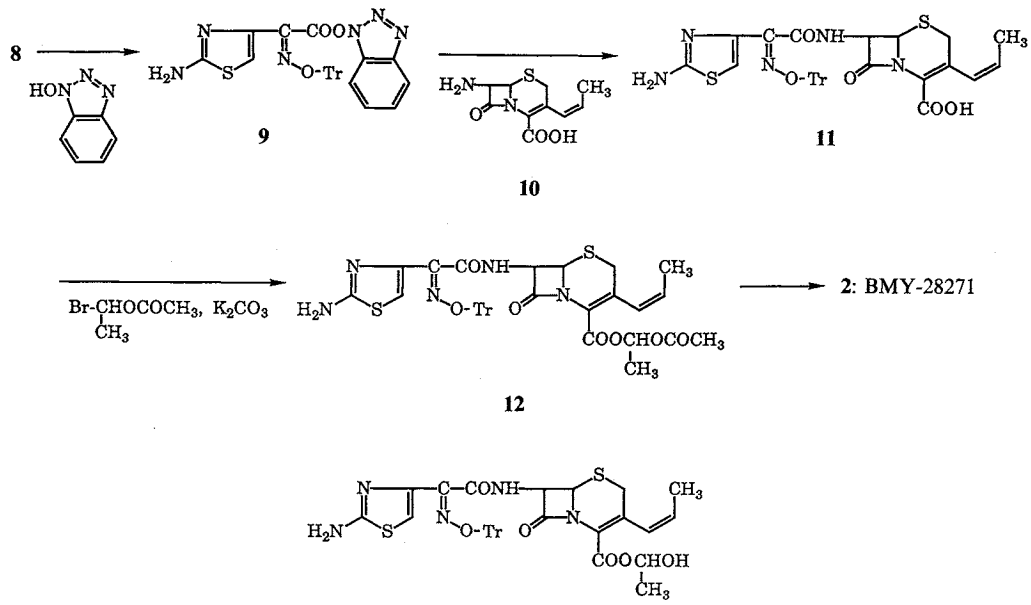


Table 2. Acylation of **10** (1 mmol) with **9** (1 mmol).

Expt	Trimethylsilylation		Acylation				
	Reagent (mmol)	Solvent	% Relative peak area in HPLC				
			11[a]	**8**	Δ^2-Isomer	A[b]	B[b]
1	BSA (4)	CH_2Cl_2	51	2.3	0.9	2.9	31
2	TMCS-TEA (2.5:2.5)	THF	85	0.5	8.9	1.3	0.7
3	TMCS-TEA (2.5:2.8)	THF	54	Trace	41	0.6	None
4	TMCS-TEA (2.5:2.2)	THF	95	None	None	1.1	Trace

[a] Area including the *E*-isomer of **11**.
[b] Unidentified impurity. Rt: A (19.7 minutes), B (22.3 minutes).

Table 3. Esterification of **11** (1 mmol) with 1-acetoxyethyl bromide in DMF (5 ml).

Expt	$BrCH(CH_3)OAc$ (mmol)	K_2CO_3 (mmol)	Period (hour)	% Relative peak area in HPLC			
				11	**12**[a]	**13**	Δ^2-Isomer
1	1	0.5	1	40	44	2	<5
2	2	1	1	1	80	6	<5
3	3	1.5	1	1	76	10	<5
4	2	1.5	1	ND	58	4	20
5	2	1	2	2	66	5	12

[a] Area including the *E*-isomer of **12**.
ND: Not detected.

decrease of the Δ^2-isomer to afford a clean reaction mixture. Thus **10** was acylated under the conditions of Expt 4 and the reaction mixture was poured into water after removal of THF to give **11** in quantitative yield.

Esterification of *O*-Tritylated Cephem Acid (**11**)

The esterification of **11** with 1-acetoxyethyl bromide[7] and K_2CO_3 in DMF was also investigated by HPLC with the results shown in Table 3. Esterification with 1 equivalent each of the bromide and K_2CO_3 for 1 hour (Expt 1) gave 44% relative peak area of **12** and 40% of **11** remained. Increase of the reagents to 2 equivalents each (Expt 2) increased the peak area of the desired product to 80% but 3 equivalents each of the reagents (Expt 3) reduced the formation of **12** with increase of a by-product which was presumed to be the 1-hydroxyethyl ester (**13**) from the ^1H NMR spectrum. An increase of K_2CO_3 (Expt 4) and prolongation of the reaction period (Expt 5) reduced the formation of **12** with increase of the Δ^2-isomer. Therefore, **11** was esterified under the reaction conditions of Expt 2 and the reaction mixture was poured into ice water to precipitate **12** as an amorphous powder (purity 80%, estimated by HPLC) in 97% yield. Crystallization of **12** was carried out from benzene-cyclohexane though the recovery was rather low.

Deblocking of *O*-Tritylated Cephem Ester (**12**)

Deblocking of the 80% pure **12** with formic acid or trifluoroacetic acid (TFA) was investigated with the results summarized in Table 4. Deblocking in 98% formic acid was rather slow. Even after 2 hours at room temperature 17% of **12** still remained (Expt 1). Deblocking in 90% formic acid and 80% formic acid (Expts 2 and 3) gave satisfactory results. After 1 hour at room temperature, 93% peak area of BMY-28271 (**2**) was observed. Deblocking with TFA (Expts 4 and 5) showed more than 10% peak area

Table 4. Deblocking of **12**.

Expt	Acid	Period (hour)	% Relative peak area in HPLC		
			BMY-28271	BMY-28232	**12**
1	98% HCOOH	1	65	3	32
		2	79	4	17
2	90% HCOOH	0.5	92	4	4
		1	93	5	2
3	80% HCOOH	0.5	90	4	6
		1	93	5	2
4	TFA	0.5	84	11	4
		1	81	12	4
5	90% TFA	0.5	85	11	4
		1	82	13	4

of BMY-28232 (**1**) generated by hydrolysis of the acetoxyethyl ester group of **2**. Thus, the ester **12** was deblocked under the reaction conditions of Expt 2 (1 hour) and the mixture was poured into ice water. After adjustment to pH 6 with NaOH, the mixture was extracted with EtOAc. Evaporation of the solvent *in vacuo* gave an amorphous product ($Z/E=8/1$). Crystallization from BuOAc - $CHCl_3$ - MeOH gave a 51% yield of **2**, of which the Z/E ratio was improved to 16/1.

Thus, crystalline **2** was obtained in 49% overall yield from **10** without using column chromatography in any step of the synthesis, according to the synthetic route shown in Scheme 2.

Experimental

MP's were determined using a Yanagimoto micro-hot stage apparatus and are uncorrected. IR spectra were recorded on a Jasco IRA-1 and UV-spectra on a Shimadzu UV-200 spectrophotometer. NMR spectra were recorded on a Jeol CL-60HL (60 MHz) or a Jeol GX-400 (400 MHz) spectrometer and mass spectra on a Jeol AX505H mass spectrometer.

HPLC Study on Tritylation of **4** (Table 1)

To a mixture of **4** (1 mmol) and a base (1 mmol) in a solvent (3 ml) was added trityl chloride (1 mmol) and the mixture was stirred at room temperature until the reaction ceased. The yield of each component in the reaction mixture was determined by the following gradient HPLC system using reference compounds **4**, **5**, **6** and **7** and the results are summarized in Table 1.

Column: SSC-ODS-262 (Senshu pak, 6 i.d. × 100 mm). Flow rate: 1 ml/minute. Detection: UV absorption at 254 nm. Mobile phase: Acetonitrile - water. Gradient: 25 : 75 ~ 68 : 32 from 0 ~ 2 minutes, 68 : 32 from 2 ~ 12 minutes, 68 : 32 ~ 100 : 0 from 12 ~ 14 minutes and 100 : 0 from 14 ~ 25 minutes. Rt: **4**, 4.5 minutes; **5**, 11.3 minutes; **6**, 13.3 minutes; **7**, 21.5 minutes.

Ethyl (*Z*)-2-(2-Aminothiazol-4-yl)-2-trityloxyiminoacetate (**6**)

Sodium hydride (60% dispersion in mineral oil, 9.90 g, 248 mmol) was added portionwise to a cold (0°C) mixture of ethyl (*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetate (**4**; 48.5 g, 225 mmol) in dry EtOAc (750 ml) with vigorous stirring and the mixture was stirred for 80 minutes at 0°C to 20°C. Then, the mixture was cooled again to 5°C. To the mixture was added a solution of trityl chloride (69.1 g, 248 mmol) in dry EtOAc (400 ml) with stirring and cooling. The mixture was stirred for 2 hours at room temperature and concentrated *in vacuo* to 400 ml. The concentrate was mixed with water (400 ml) and filtered to afford 76.2 g of crystalline **6**. ^1H NMR spectra and elemental analysis showed that this product was solvated with 0.5 mol of EtOAc. MP 184 ~ 187°C; IR ν_{max} (KBr) cm^{-1} 3450, 1735, 1620; ^1H NMR (60 MHz, $CDCl_3$) δ 1.30 (3H, t, $J=7$ Hz, CH_3), 4.37 (2H, q, $J=7$ Hz, CH_2), 5.93 (2H, s, NH_2), 6.42 (1H, thiazole-H), 7.3 (15H, s, phenyl-H). Along with the above signals, the CH_3-C (1.2 ppm, 1.5H, t), CH_2O

(4.1, 1H, q) and CH_3CO (2.0, 1.5H, s) signals due to 0.5 mol of EtOAc were observed.

Anal Calcd for $C_{26}H_{23}N_3O_3S \cdot \frac{1}{2}$EtOAc: C 67.05, H 5.43, N 8.38, S 6.39.
Found: C 66.56, H 5.31, N 8.17, S 6.44.

The organic layer of the filtrate was evaporated *in vacuo* and the residue was dissolved in a small amount of CH_2Cl_2. The solution was filtered to remove the starting material and then evaporated. Trituration of the residue with toluene gave 5.1 g of **6** as a second crop. Total yield 81.3 g (72%).

(*Z*)-2-(2-Aminothiazol-4-yl)-2-trityloxyiminoacetic Acid (**8**)

A mixture of the ester **6** (73.9 g, 0.147 mol) and sodium hydroxide (19.4 g, 0.485 mol) in 50% aq dioxane (540 ml) was heated under reflux for 1.5 hours. After cooling, the mixture was mixed with EtOAc (670 ml) and then, acidified to pH 4 with conc HCl (41 ml). The organic layer was separated and washed with water (670 mg × 4). During the second washing, **8** was crystallized out as colorless prisms, which were collected by filtration, washed with water (200 ml) and EtOAc (200 ml), successively and dried *in vacuo* over phosphorous pentoxide. Yield: 56.7 g (90%). MP 179~182°C; IR ν_{max} (KBr) cm^{-1} 3450, 1710, 1610, 1535; ^{1}H NMR (60 MHz, DMSO-d_6) δ 6.80 (1H, s, thiazole-H), 8.30 (15H, s, phenyl-H).

Benzotriazol-1-yl (*Z*)-2-(2-Aminothiazol-4-yl)-2-trityloxyiminoacetate (**9**)

To a stirred solution of **8** (143.6 g, 0.32 mol) and 1-hydroxybenzotriazole monohydrate (55.5 g, 0.36 mol) in THF (3.2 liters) was added dicyclohexylcarbodiimide (74.8 g, 0.36 mol). The mixture was stirred at room temperature for 1 hour and filtered. The filtrate was concentrated to *ca.* 500 ml. Isopropyl ether (600 ml) was added to the concentrate to precipitate a crystalline solid, which was filtered, washed with isopropyl ether and dried to give 174.9 g (100%) of **9**. MP 186~190°C; IR ν_{max} (KBr) cm^{-1} 1815, 1620, 1540; ^{1}H NMR (60 MHz, DMSO-d_6) δ 7.0~8.5 (aromatic, 19H).

HPLC Study on Acylation of **10** with **9** (Table 2)

To a mixture of **10** (1 mmol) in a solvent (5 ml) was added trimethylsilylating reagents and the mixture was stirred for 30 minutes at room temperature. A solution of **9** (1 mmol) in DMF (2 ml) was added to the mixture, which was stirred overnight at room temperature. The % relative peak areas of products in HPLC were determined under the following conditions and the results are summarized in Table 2. The reference sample of the Δ^2-isomer of **11** was prepared from the Δ^2-isomer of **10** by procedure similar to the preparation of **11** (see the Experimental part for the Δ^2-isomer of **11**).

Column: SSC-ODS-262 (Senshu pak, 6 i.d. × 100 mm). Flow rate: 1 ml/minute. Detection: UV absorption at 254 nm. Mobile phase: Acetonitrile-water. Gradient: 40:60 from 0~7 minutes, 40:60~80:20 from 7~20 minutes and 80:20~85:15 from 20~25 minutes. Rt: **11** (*Z*-isomer), 6.32 minutes; **11** (*E*-isomer) 7.64 minutes; Δ^2-isomer, 6.99 minutes.

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-trityloxyiminoacetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylic Acid (**11**)

TEA (44.6 g, 441 mmol) was added to a suspension of 7-amino-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylic acid (**10**, *Z*/*E*=12/1) (48.1 g, 200 mmol) in dry THF (960 ml). TMCS (54.6 g, 503 mmol) was added dropwise to the cold mixture (10°C) during a period of 5 minutes. The mixture was stirred for 30 minutes at room temperature and cooled again to *ca.* 10°C. To the mixture was added a solution of the active ester **9** (109.5 g, 200 mmol) in DMF (400 ml) over 2 minutes. The mixture was stirred overnight at room temperature and concentrated under reduced pressure to remove most of the THF. The concentrate was poured into ice water (5 liters) with vigorous stirring to separate **11**, which was collected by filtration, washed with 2 liters of water, and dried *in vacuo*. Yield 130 g (100%, purity 96%). IR ν_{max} (KBr) cm^{-1} 1782, 1684, 1617; UV λ_{max}^{EtOH} nm (ε) 290 (14,700); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.65 (3H, dd, *J*=2 and 7 Hz, =C−CH_3), 3.53 (1H, d, *J*=17 Hz, 2-H), 3.61 (1H, d, *J*=17 Hz, 2-H), 5.28 (1H, d, *J*=5 Hz, 6-H), 5.66 (1H, d, *J*=7 and 11 Hz, CH=C*H*−CH_3), 5.91 (1H, dd, *J*=5 and 8 Hz, 7-H), 6.19 (1H, d, *J*=11 Hz, C*H*=CH−CH_3), 6.64 (1H, s, thiazole-H), 7.2~7.3 (15H, br s, phenyl-H).

HPLC Study on Esterification of **11** with 1-Acetoxyethyl Bromide (Table 3)

To a cold solution of **11** (1 mmol) and an indicated amount of K_2CO_3 in DMF (5 ml) was added an

appropriate amount of 1-acetoxyethyl bromide at 0°C and the mixture was stirred at 5°C. The % relative peak areas of products in HPLC were determined by the following conditions and the results are summarized in Table 3. The reference sample of the Δ^2-isomer of **12** was prepared from the Δ^2-isomer of **11** by a procedure similar to the preparation of **12** (see the Experimental part for the Δ^2-isomer of **12**).

Column: SSC-ODS-262 (Senshu pak, 6 i.d. × 100 mm). Flow rate: 1 ml/minute. Detection: UV absorption at 254 nm. Mobile phase: Acetonitrile - pH 7.0 buffer. Gradient: 40:60~65:35 from 0~3 minutes, 65:35~70:30 from 3~15 minutes, 70:30~80:20 from 15~20 minutes, 80:20~85:15 from 20~25 minutes and 85:15 from 25~30 minutes. Rt: **11**, 5.04 minutes; **12** (*Z*-isomer), 14.1 minutes; **12** (*E*-isomer), 14.8 minutes; Δ^2-isomers, 14.6 minutes (diastereoisomer A) and 14.8 minutes (diastereoisomer B); **13**, 27.1 minutes.

1-Acetoxyethyl 7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-trityloxyiminoacetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylate (**12**)

To a cooled solution of **11** (34.4 g, 52.8 mmol) and well milled K_2CO_3 (7.24 g, 52.8 mmol) in dry DMF (344 ml) was added 1-acetoxyethyl bromide (17.64 g, 105.6 mmol) at 0°C under argon atmosphere. The mixture was stirred at about 5°C for 65 minutes. The reaction mixture was poured into ice water (1,720 ml) with stirring, adjusted to pH 7 with aq $NaHCO_3$ and stirred for 30 minutes. The resulting precipitate was filtered off, washed with water (100 ml) and dried to give 37.78 g of **12** (97%, purity 80%, estimated by HPLC) as an amorphous powder.

Five g of 80% pure **12** was chromatogtaphed on a column of silica gel (Merck, Kieselgel 60, 100 g). The column was eluted with CH_2Cl_2 and then CH_2Cl_2 containing 1% MeOH. The fractions containing **12** were evaporated to give 2.23 g of the product as an amorphous powder, which was crystallized from benzene - cyclohexane to give 1.54 g of crystalline **12**. MP 136~138°C; IR ν_{max} (KBr) cm^{-1} 1788, 1767, 1685; UV λ_{max}^{EtOH} nm (ε) 293 (14,800); ^1H NMR (400 MHz, $CDCl_3$) δ 1.53 (1.5H, d, J=6 Hz, CH−CH_3), 1.56 (1.5H, d, J=6 Hz, CH−CH_3), 1.65 (1.5H, dd, J=2 and 7 Hz, =C−CH_3), 1.67 (1.5H, dd, J=2 and 7 Hz, =C−CH_3), 2.08 (1.5 H, s, OAc), 2.09 (1.5H, s, OAc), 3.20 (0.5H, d, J=18 Hz, 2-H), 3.25 (0.5H, d, J=18 Hz, 2-H), 3.41 (0.5H, d, J=18 Hz, 2-H), 3.42 (0.5H, d, J=18 Hz, 2-H), 5.08 (0.5 H, d, J=5 Hz, 6-H), 5.09 (0.5H, d, J=5 Hz, 6-H), 5.68~5.81 (1H, m, −CH=CH−CH$_3$), 5.90 (0.5 H, dd, J=5 and 8 Hz, 7-H), 5.94 (1H, dd, J=5 and 8 Hz, 7-H), 6.16 (0.5 H, d, J=11 Hz, CH=CH−CH$_3$), 6.18 (0.5H, d, J=11 Hz, CH=CH−CH$_3$), 6.60 (0.5H, s, thiazole-H), 6.62 (0.5H, s, thiazole-H), 6.98 (0.5 H, q, J=6 Hz, CH−CH$_3$), 7.04 (0.5H, q, J=6 Hz, CH−CH$_3$), 7.25~7.38 (15H, m, phenyl-H).

1-Hydroxyethyl 7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-trityloxyiminoacetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylate (**13**)

To a cold solution of **11** (5.0 g, 7.7 mmol) and K_2CO_3 (1.6 g, 11.6 mmol) in dry DMF (50 ml) was added 1-acetoxyethyl bromide (3.86 g, 23.1 mmol) at 0°C under argon atmosphere. The mixture was stirred at about 5°C for 60 minutes. The reaction mixture was poured into ice water with stirring, adjusted to pH 7 with aq $NaHCO_3$ and stirred for 30 minutes. The resulting precipitate was filtered off, washed with water and dried to give 5.5 g of crude product which contained **13** as a minor product.

The product was chromatographed on a column of silica gel (Merck, Kieselgel 60, 100 g) and the column was eluted with CH_2Cl_2 containing 3~5% MeOH. The fractions were checked by HPLC, and the desired fractions containing **13** were combined and concentrated. The concentrate was again chromatographed on a column (Prep C_{18}, Waters, 20 × 200 mm). Elution with acetonitrile - water (8:2~9:1) and subsequent concentration of the desired fractions afforded 203 mg of the product (**13**). IR ν_{max} (KBr) cm^{-1} 1780, 1720, 1680; UV $\lambda_{max}^{CH_2Cl_2}$ nm (ε) 294 (15,500); FAB-MS m/z 696 $(M+H)^+$, 652 $(M-CH_3CHO+H)^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 1.36 (1.5H, d, J=6 Hz, CH−CH_3), 1.41 (1.5H, d, J=6 Hz, CH−CH_3), 1.63 (3H, d, J=7 Hz, =C−CH$_3$), 3.56 (1H, d, J=18 Hz, 2-H), 3.63 (0.5H, d, J=18 Hz, 2-H), 3.66 (0.5 H, d, J=18 Hz, 2-H), 5.34 (0.5H, d, J=5 Hz, 6-H), 5.35 (0.5H, d, J=5 Hz, 6-H), 5.65~5.75 (1H, m, −CH=CH−CH$_3$), 6.0 (1H, dd, J=5 and 8 Hz, 7-H), 6.12 (0.5H, d, J=11 Hz, −CH=CH−CH$_3$), 6.16 (0.5H, d, J=11 Hz, −CH=CH−CH$_3$), 6.26 (1H, q, J=6 Hz, CH−CH$_3$), 6.62 (1H, s, thiazole-H), 7.2~7.4 (15H, m, phenyl-H), 9.88 (0.5 H, d, J=8 Hz, CONH), 9.93 (0.5H, d, J=8 Hz, CONH).

Deblocking of **12** (Table 4)

A mixture of **12** (0.1 mmol) in acid (0.2 ml) was stirred for an indicated period at room temperature. The % relative peak areas of the products were determined by the following conditions and the results are summarized in Table 4.

Column: SSC-ODS-262 (Senshu pak, 6 i.d. × 100 mm). Flow rate: 1 ml/minute. Detection: UV absorption at 254 nm.

(1) Mobile phase for BMY-28271 and BMY-28232: CH_3CN - pH 3.5 buffer (40 : 60). Rt: BMY-28232, 2.47 minutes; BMY-28271, 7.10 minutes.

(2) Mobile Phase for BMY-28271 and **12**: CH_3CN - pH 3.5 buffer (70 : 30). Rt: BMY-28271, 2.10 minutes and **12**, 9.25 minutes.

1-Acetoxyethyl 7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylate (**2**, BMY-28271)

Amorphous **12** (80% pure) (1.0 g) was stirred in 90% formic acid (3 ml) for 50 minutes at 25°C. The reaction mixture was filtered, the filtrate was poured into ice water (40 ml), and the mixture was adjusted to pH 4 with 50% aq NaOH. EtOAc (40 ml) was layered and the aqueous layer was adjusted to pH 6 with aq $NaHCO_3$ with vigorous stirring. The organic layer was separated, washed with water, and dried over $MgSO_4$. Charcoal (100 mg) was added and the filtrate was evaporated under reduced pressure. The residue ($Z/E=8/1$)† was dissolved in MeOH - $CHCl_3$ (1 : 4, 12 ml). The solution was diluted with BuOAc (3 ml), concentrated to 4 ml, and seeded. From the solution, 383 mg (51% yield) of **2** ($Z/E=16/1$)† was obtained. MP 146°C (dec); IR ν_{max} (KBr) cm^{-1} 1780, 1760, 1630; UV λ_{max}^{EtOH} nm (ε) 222 (19,000), 286 (12,000).

HPLC and ^1H NMR spectra of the crystalline **2** were identical with those of an authentic sample prepared by the previously reported method[1], indicating almost 1 : 1 mixture of *R* and *S* diastereoisomers in regard to the asymmetric carbon of the ester moiety. ^1H NMR spectra and elemental analysis showed that this product was solvated with 0.5 mol of BuOAc.

Anal Calcd for $C_{19}H_{21}N_5O_7S_2 \cdot \frac{1}{2}$(BuOAc): C 47.73, H 4.92, N 12.65, S 11.58.
Found: C 47.73, H 4.82, N 12.81, S 11.66.

7-Amino-3-[(*Z*)-1-propenyl]-2-cephem-4-carboxylic Acid (the Δ^2-Isomer of **10**)

To a suspension of 960 mg (4 mmol) of **10** in 100 ml of dry CH_2Cl_2 was added 4 ml (16 mmol) of BSA and the mixture was stirred at room temperature for 30 minutes. To the resulting solution was added 5.6 ml (40 mmol) of triethylamine and the solution was stirred at room temperature for 24 hours. The reaction mixture was evaporated to dryness and the residue was dissolved in 10 ml of water. The solution was charged on a column packed with Prep C_{18} (Waters, 400 ml). The column was eluted with water and the desired fractions monitored by HPLC were collected, concentrated to 10 ml, and cooled to give the crystalline product, which was collected by filtration, washed with acetone and dried *in vacuo* over P_2O_5 to give 450 mg (47%) of the title compound. MP > 250°C (dec); IR ν_{max} (KBr) cm^{-1} 1745, 1620, 1560, 1355, 1250; UV λ_{max} (pH 7 buffer) nm (ε) 266 (9,100); ^1H NMR (400 MHz, $D_2O+Na_2CO_3$) δ 1.74 (3H, dd, $J=7$ and 2 Hz, $=C-CH_3$), 4.60 (1H, d, $J=4$ Hz, 7-H), 4.70 (1H, s, 4-H), 5.25 (1H, d, $J=4$ Hz, 6-H), 5.69 (1H, dq, $J=11$ and 7 Hz, $C=CH-CH_3$), 5.83 (1H, d, $J=11$ Hz, $CH=CH-CH_3$), 6.21 (1H, s, 2-H).

Anal Calcd for $C_{10}H_{12}N_2O_3S$: C 49.99, H 5.03, N 11.65, S 13.34.
Found: C 49.91, H 5.00, N 11.27, S 13.11.

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-trityloxyiminoacetamido]-3-[(*Z*)-1-propenyl]-2-cephem-4-carboxylic Acid (the Δ^2-Isomer of **11**)

According to the procedure described in the preparation of **11**, the Δ^2-isomer of **10** (360 mg, 1.5 mmol) was acylated and purified by column chromatography to give the title compound (650 mg, 66% yield). IR

† The ratio was determined by HPLC. Column: SSC-ODS-262. Flow rate: 1 ml/minute. Detection: UV absorption at 254 nm. Mobile phase: CH_3CN - pH 3.5 buffer (1 : 2). Rt: BMY-28271, 10.93 minutes (diastereoisomer A) and 11.30 minutes (diastereoisomer B); *E*-isomer, 14.21 minutes (The *E*-isomer was prepared by esterification of BMY-28232-*E*-isomer[1] with 1-acetoxyethyl bromide).

ν_{max} (KBr) cm^{-1} 1760, 1670, 1620, 1540, 1360; UV λ_{max} (pH 7 buffer) nm (ε) 260 (11,000); ^1H NMR (400 MHz, DMSO-d_6) δ 1.72 (3H, dd, J=7 and 2 Hz, =C−CH_3), 4.26 (1H, s, 4-H), 5.44 (1H, d, J=4 Hz, 6-H), 5.38~5.47 (1H, m, CH=CH−CH_3), 5.50 (1H, dd, J=8 and 4 Hz, 7-H), 5.99 (1H, s, 2-H), 6.03 (1H, d, J=12 Hz, CH=CH−CH_3), 6.68 (1H, s, thiazole-H), 7.2~7.4 (15H, m, phenyl-H), 9.80 (1H, d, J=8 Hz, NH).

1-Acetoxyethyl 7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-trityloxyiminoacetamido]-3-[(*Z*)-1-propenyl]-2-cephem-4-carboxylate (the Δ^2-Isomer of **12**)

According to the procedure described in the preparation of **12**, the Δ^2-isomer of **11** (540 mg, 0.83 mmol) was esterified and purified by column chromatography to give the title compound (378 mg, 62% yield). IR ν_{max} (KBr) cm^{-1} 1783, 1767, 1685, 1618, 1542; UV λ_{max}^{MeOH} nm (ε) 235 (sh, 23,000), 265 (sh, 10,000); ^1H NMR (400 MHz, $CDCl_3$) δ 1.46 (1.5 H, d, J=5.5 Hz, CH−CH_3), 1.50 (1.5 H, d, J=6 Hz, CH-CH_3), 1.72 (3H, d, J=6 Hz, =CH−CH_3), 2.06 (1.5H, s, OAc), 2.10 (1.5H, s, OAc), 4.84 (1.5H, s, 4-H), 4.86 (1.5H, s, 4-H), 5.36 (1H, d, J=5 Hz, 6-H), 5.6~5.7 (2H, m, CH=CH−CH_3), 5.83 (0.5 H, d, J=5 Hz, after addition of D_2O, 7-H), 5.84 (0.5H, d, J=5 Hz, after addition of D_2O, 7-H), 5.98 (1H, s, 2-H), 6.67 (1H, s, thiazole-H), 6.84 (0.5H, q, J=6 Hz, CH−CH_3), 6.86 (0.5H, q, J=6 Hz, CH–CH_3), 7.3 (15H, m, phenyl-H).

Anal Calcd for $C_{38}H_{35}N_5O_7S_2$: C 61.72, H 4.78, N 9.49.
Found: C 61.31, H 4.78, N 9.05.

Acknowledgment

The authors gratefully acknowledge Dr. H. KAWAGUCHI, Consultant and Dr. T. OKI, Director of the Institute, for their valuable discussion and encouragement. The authors also express their appreciation to Dr. TSUNO and his associates of the Analytical Chemistry Laboratory of this institute for the microanalytical and spectral data.

References

1) KAMACHI, H.; Y. NARITA, T. OKITA, Y. ABE, S. IIMURA, K. TOMATSU, T. YAMASAKI, J. OKUMURA, T. NAITO, T. OKI & H. KAWAGUCHI: Synthesis and biological activity of a new cephalosporin, BMY-28232 and its prodrug-type esters for oral use. J. Antibiotics 41: 1602~1616, 1988
2) TOMATSU, K.; S. MASUYOSHI, M. HIRANO, H. KAWAGUCHI, T. OKI, J. FUNG-TOMC, J. V. DESIDERIO & R. E. KESSLER: *In vitro* and *in vivo* evaluations of a new broad-spectrum oral cephalosporin, BMY-28232, and its prodrug esters. Antimicrob. Agents Chemother. 33: 489~497, 1989
3) BLUMBACH, J.; W. DUERCKHEIMER, J. REDEN & H. SELIGER (Hoechst): Penicillins and cephalosporins. Ger. Offen. 27 58 000, July 5, 1979 [CA 91: 140858q, 1979]
4) BUCOURT, R.; R. HEYMES, A. LUTZ, L. PÉNASSE & J. PERRONNET: Cephalosporines a chaines amino-2 thiazolyl-4 acetyles. Influence de la presence et de la configuration d'un groupe oxyimino sur l'activite antibacterienne. Tetrahedron 34: 2233~2243, 1978
5) HOSHI, H.; J. OKUMURA, T. NAITO, Y. ABE & S. ABURAKI (Bristol-Myers): 7-Amino-3-propenylcephalosporanic acid and esters thereof. U.S. 4,699,979, Oct. 13, 1987
6) KRICHELDORF, H. R.: Über die Silylierung von Aminosäuren und die Peptidsynthese mit Aminosäuren-trimethylsilylestern. Liebigs Ann. Chem. 763: 17~38, 1972
7) BUCKLEY, E. & E. WHITTLE: Some reactions involved in the photobromination of simple alcohols and ketones in the vapor phase. Can. J. Chem. 40: 1611~1615, 1962