Synthesis and Biological Activity of Quaternary Ammoniopropenyl Cephalosporins having Two Vinyl Groups

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During the last decades, the antibacterial activity of cephalosporins has been steadily increased through side-chain modifications at C-3 and C-7. Recently, 3-vinyl cephalosporins, such as TOC-39[1], E-1077[2,3] and YM-40220[4,5], have been intensively studied. They show well-balanced antibacterial activity against Gram-positive bacteria including *S. aureus* and Gram-negative bacteria including *P. aeruginosa*. Thus we were interested in the synthesis of quaternary ammoniopropenyl cephalosporins having two vinyl groups at C-3 side chain.

In this paper, we report the synthesis and biological activity of these novel cephalosporins.

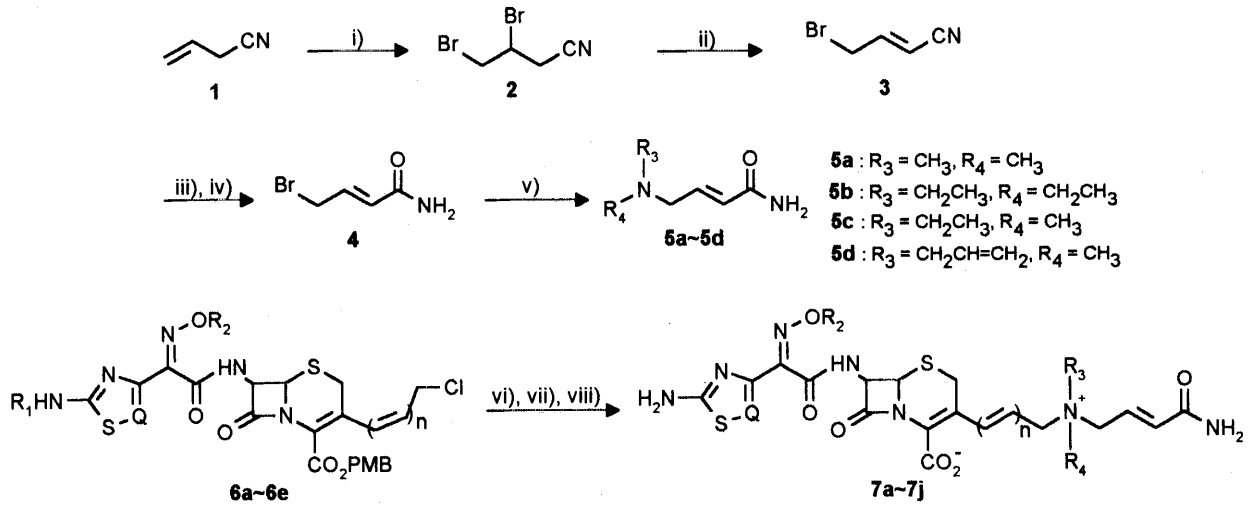
Chemistry

The new cephalosporins (**7a**~**7j**) were prepared by quaternization of 3-(*E*)-iodopropenylcephalosporin derivatives, which were prepared from 3-(*Z*)-chloro-propenylcephalosporin[6,7] derivatives (**6a**~**6e**), with tertiary amine followed by the removal of protecting group. The general procedure is as follows:

To a solution of allylcyanide (**1**; 4 g, 59.62 mmol) in *tert*-butanol (6 ml) and petroleum ether (27 ml) was added bromine (9.5 g, 59.62 mmol) at 15, and then stirred at room temperature for 20 minutes. A solution of 21% sodium ethoxide (19.3 g, 59.62 mmol) was added dropwise to the mixture and the solid was filtered off. The filtrate was distilled under reduced pressure to give 4-bromo-2-butenenitrile (**3**)[8] as a liquid (4.6 g, 52.8%).

After hydrolysis of **3** by sulfuric acid, **4** was obtained

Scheme 1. Synthesis of quaternary ammoniopropenyl cephalosporins.



5a : $R_3 = CH_3$, $R_4 = CH_3$
5b : $R_3 = CH_2CH_3$, $R_4 = CH_2CH_3$
5c : $R_3 = CH_2CH_3$, $R_4 = CH_3$
5d : $R_3 = CH_2CH{=}CH_2$, $R_4 = CH_3$

6a : n = 0, Q = CH, R_1 = Tr, $R_2 = CH_3$
6b : n = 1, Q = CH, R_1 = Tr, $R_2 = CH_3$
6c : n = 1, Q = CH, R_1 = Tr, $R_2 = CH_2CH_2F$
6d : n = 1, Q = N, R_1 = H, $R_2 = CH_2CH_2F$
6e : n = 1, Q = N, R_1 = H, $R_2 = CH_3$

7a : n = 0, Q = CH, $R_2 = CH_3$, $R_3 = CH_3$, $R_4 = CH_3$
7b : n = 0, Q = CH, $R_2 = CH_3$, $R_3 = CH_2CH_3$, $R_4 = CH_2CH_3$
7c : n = 0, Q = CH, $R_2 = CH_3$, $R_3 = CH_2CH_3$, $R_4 = CH_3$
7d : n = 0, Q = CH, $R_2 = CH_3$, $R_3 = CH_2CH{=}CH_2$, $R_4 = CH_3$
7e : n = 1, Q = CH, $R_2 = CH_3$, $R_3 = CH_3$, $R_4 = CH_3$
7f : n = 1, Q = CH, $R_2 = CH_3$, $R_3 = CH_2CH_3$, $R_4 = CH_3$
7g : n = 1, Q = CH, $R_2 = CH_2CH_2F$, $R_3 = CH_2CH_3$, $R_4 = CH_3$
7h : n = 1, Q = N, $R_2 = CH_3$, $R_3 = CH_2CH_3$, $R_4 = CH_3$
7i : n = 1, Q = N, $R_2 = CH_2CH_2F$, $R_3 = CH_3$, $R_4 = CH_3$
7j : n = 1, Q = N, $R_2 = CH_2CH_2F$, $R_3 = CH_2CH_3$, $R_4 = CH_3$

(i) Br_2, tert-butanol, petroleum ether; (ii) NaOEt; (iii) H_2SO_4; (iv) NH_4OH; (v) alkyl amine, CH_3CN; (vi) NaI, Acetone; (vii) **5a~5d**, EA; (viii) HCOOH, Tr = trityl, PMB = *p*-methoxybenzyl.

by treatment with ammonia solution. To a solution of **4** (600 mg, 3.66 mmol) in acetonitrile (6 ml) was added *N*-ethylmethylamine (432 mg, 7.32 mmol) at 0°C. The reaction mixture was stirred for an hour and the resulting precipitate was collected by filtration to give **5c** as a white solid (280 mg, 54%): ^{1}H NMR (DMSO-d_6) 0.98 (3H, t, NCH_2CH_3), 2.12 (3H, s, NCH_3), 2.35 (2H, q, NCH_2CH_3), 3.05 (2H, d, NCH_2CH=), 5.94 (1H, d, J=16 Hz, CH=C*H*), 6.55 (1H, dt, J=16 Hz, C*H*=CH), 6.93 (1H, s, $CONH_2$), 7.40 (1H, s, $CONH_2$).

To a stirred solution of *p*-methoxybenzyl 7[(z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-methoxyiminoacetamido]-3-[(*Z*)-3-chloro-1-propen-1-yl]-3-cephem-4-carboxylate (**6e**, 650 mg, 1.12 mmol) in acetone (10 ml) was added sodium iodide (505 mg, 3.37 mmol) in one portion at room temperature followed by stirring for 2 hours. The reaction mixture was evaporated and dissolved in ethyl acetate.

The ethyl acetate solution was washed successively with aq $Na_2S_2O_3$ solution and brine and then dried (Na_2SO_4). After filtration of Na_2SO_4, the chilled (0°C) and stirred filtrate was treated with a solution of 4-ethylmethylamino-2-butenamide (**5c**; 240 mg, 1.68 mmol) in ethyl acetate. The reaction mixture was stirred for 2 hours at 0°C. The resulting precipitate was treated with formic acid, and then purified by Diaion HP-20 column and lyophilization to give 7-[(z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-methoxyiminoacetamido]-3-[(*E*)-3-[(*E*)-(1-carbamoyl-1-propen-3-yl)-3-ethylmethylammonio]-1-propen-1-yl]-3-cephem-4-carboxylate (**7h**) as a white solid (140 mg, 22.2%): IR (KBr) cm^{-1} 1768, 1680, 1520, 1039; ^{1}H NMR (DMSO-d_6) 1.26 (3H, t, NCH_2CH_3), 2.94 (3H, s, NCH_3), 3.38 (1H, d, 2-CH_2), 3.40 (2H, m, NCH_2CH_3), 3.66 (2H, m, NCH_2CH=), 3.81 (2H, m, =$CHCH_2N$), 3.85 (1H, d, 2-CH_2), 3.90 (3H, s, OCH_3), 5.03 (1H, d, J=5 Hz, 6-CH), 5.56~5.64 (2H, m, 7-C*H*+CH=C*H*CH_2N), 6.43~6.59 (2H, m, CH=CHCO), 7.02 (1H, d, J=16 Hz, C*H*=$CHCH_2N$), 7.26 (1H, s, $CONH_2$), 8.16 (2H, s, NH_2), 8.64 (1H, s, $CONH_2$), 9.53 (1H, d, J=8 Hz, CONH).

Biological Activity

The *in vitro* activities of the new cephalosporins (**7a**~**7j**) against selected Gram-positive and Gram-negative organisms are summarized in Table 1. MICs were determined by the 2-fold serial agar dilution method in Muller-Hinton agar (Difco) at 37 for 18 hours with an inoculum size of 10^6 cfu/ml. For comparison, the MICs of cefotaxime (CTX) and cefpirome (CPR) are also shown.

All synthesized compounds in this study showed good antibacterial activity against Gram-positive and Gram-negative bacteria. In the case of cephalosporins having one vinyl group at the C-3 side chain such as **7a**, **7b**, **7c** and **7d**, the antibacterial activity of **7c** against *Staphylococcus aureus* and Gram-negative bacteria was similar to that of cefotaxime. In the series of cephalosporins having two vinyl groups (**7e**, **7f**, **7g**, **7h**, **7i** and **7j**), **7g**, **7h** and **7j** showed the most potent *in vitro* activity. Replacement of the aminothiazolyl group (**7f**) by aminothiadiazolyl

Table 1. *In vitro* antibacterial activity (MIC, μg/ml) of the cephalosporins (**7a**~**7j**).

Organism	*S.p.*	*S.f.*	*S.a.1*	*S.a.2*	*E.c.1*	*E.c.2*	*P.a.1*	*P.a.2*	*K.a.*	*En.c.*
7a	0.025	>100	6.25	6.25	0.013	0.05	6.25	12.5	0.025	6.25
7b	0.025	>100	12.5	6.25	0.05	0.05	12.5	12.5	0.05	6.25
7c	0.006	>100	3.13	1.56	0.025	0.05	3.13	6.25	0.05	0.78
7d	0.025	>100	6.25	3.13	0.025	0.1	6.25	6.25	0.78	1.56
7e	0.025	>100	0.78	0.78	0.025	0.05	6.25	6.25	0.025	3.13
7f	0.006	>100	0.39	0.78	0.025	0.05	0.78	3.13	0.05	1.56
7g	0.025	>100	0.78	0.39	0.025	0.05	0.78	3.13	0.025	1.56
7h	0.013	>100	0.39	0.39	0.05	0.025	0.39	0.78	0.025	1.56
7i	0.025	>100	0.78	0.39	0.025	0.05	1.56	3.13	0.025	1.56
7j	0.025	>100	0.78	0.39	0.013	0.025	3.13	3.13	0.012	0.78
CTX	0.006	>100	1.56	0.78	0.013	0.025	12.5	12.5	0.025	100
CPR	<0.006	>100	0.78	0.39	0.013	0.025	3.13	3.13	0.025	3.13

Abbreviation: *S.p.*, *Streptococcus pyogenes* 308A; *S.f.*, *Streptococcus faecium* MD8b; *S.a.1*, *Staphylococcus aureus* SG511; *S.a.2*, *Staphylococcus aureus* 503; *E.c.1*, *Escherichia coli* 078; *E.c.2*, *Escherichia coli* 1507E; *P.a.1*, *Pseudomonas aeruginosa* 9027; *P.a.2*, *Pseudomonas aeruginosa* 1771; *K.a.*, *Klebsiella aerogenes* 1522E; *En.c.*, *Enterobacter cloacae* P99; CTX, cefotaxime; CPR, cefpirome.

Table 2. Pharmacokinetic parameters of **7h** and reference antibiotics in mice (40 mg/kg)[a].

Parameters	cefotaxime	cefpirome	**7h**
$t_{1/2}$ (hours)	0.52±0.05	0.78±0.05	0.94±0.07
AUC (μg·hours/ml)	32.84±5.12	30.67±4.81	37.26±3.99

[a] sc administration.
Values are mean±standard error.

(**7h**) led to increased antibacterial activity against *Pseudomonas aeruginosa*. However, the fluoroethoxyimino cephem (**7j**) was 4- to 8-fold less active than the methoxyimino cephem (**7h**) against *Pseudomonas aeruginosa*. Among them, **7h** having two vinyl groups at the C-3 position showed the most well-balanced antibacterial activity over a wide range of Gram-positive and Gram-negative bacteria. The pharmacokinetic parameters of **7h** in mice after sc injection are indicated in Table 2. **7h** showed longer plasma elimination half-life ($T_{1/2}$) and higher area under the curve (AUC) than those of reference compounds.

After sc administration in mouse, **7h** showed an excellent *in vivo* efficacy against systemic infections (Table 3). The PD_{50} values of **7h**, CTX and CPR were as follows: *S. pyogenes* A77 (0.21, 0.15 and 0.27 mg/kg), *S. aureus* Y-80-1953 (1.09, 4.06 and 1.47 mg/kg), *E. coli* 078 (0.07, 0.20 and 0.09 mg/kg), *P. aeruginosa* 1771M (5.21, 3.52 and 11.13 mg/kg).

In conclusion, **7h** having two vinyl groups at C-3 sidechain showed good *in vitro* antibacterial activity and excellent *in vivo* efficacy against systemic infections.

Table 3. *In vivo* activity of **7h** and reference antibiotics against systemic infections caused by pathogenic bacteria.

Pathogens	compound	MIC (μg/ml)[b]	PD_{50} (mg/kg)
S. pyogenes A77 (7.2×10^1)[a]	cefotaxime	0.007	0.15
	cefpirome	0.013	0.27
	7h	0.025	0.21
S. aureus Y-80-1953 (2.9×10^7)	cefotaxime	N.T.[c]	4.06
	cefpirome	N.T.	1.47
	7h	N.T.	1.09
E. coli 078 (7.2×10^7)	cefotaxime	0.013	0.20
	cefpirome	0.013	0.09
	7h	0.049	0.07
P. aeruginosa 1771M (6.0×10^9)	cefotaxime	0.049	3.52
	cefpirome	0.195	11.13
	7h	0.195	5.21

[a] Infective challenge dose.
[b] Inoculum size (10^4 cfu/ml).
[c] Not tested.

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