Cycloaddition Reactions of a 3-(1,3-Butadienyl)cephalosporin and Antibacterial Activity of New Cephem Derivatives

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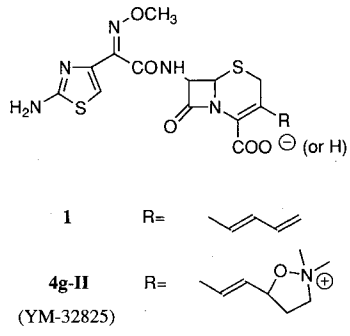
In our preceding papers, we described a facile synthesis of a 3-(1,3-butadienyl)cephem **1** and its biological evaluations[1,2]. Although its antibacterial activity and oral absorbability was insufficient for clinical application, we considered compound **1** still attractive as a unique intermediate for new derivatives manifoldly substituted at C-3. Therefore, we planned to examine [3+2] and [4+2] cycloaddition reactions using **1**. Herein, we wish to report the outcomes of cycloaddition reactions and the antibacterial activity of the thus-obtained new cephem derivatives.

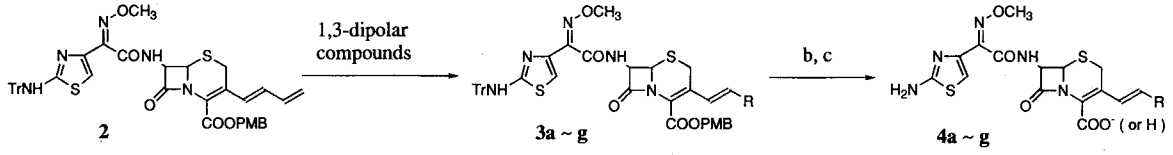
Chemistry

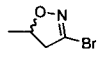
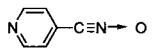
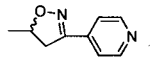
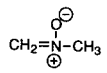
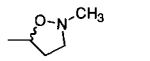
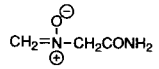
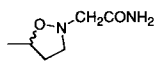
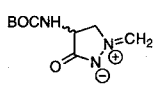
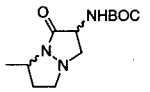
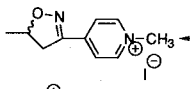
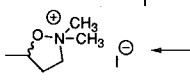
First we examined [3+2] cycloaddition using nitrile oxide, nitrone and azomethine imine (Scheme 1). Conversion of the C-3 moiety using nitrile oxide or nitrone has been known for some time[3~8]. Cycloaddition of nitrile oxides, **5a**[9] and **5b**[10], with **2** occurred only at the terminal olefin to afford 3-[(*E*)-2-(3-bromo-2-isoxazolin-

Fig. 1. Structure of YM-32825 and its corresponding butadienyl compound.



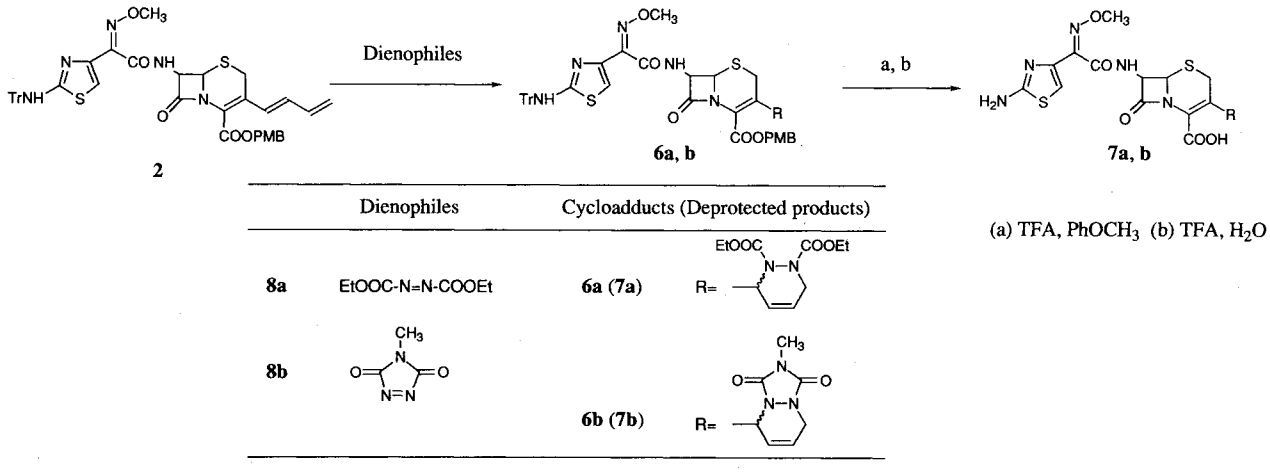
Scheme 1. Synthesis of new cephem derivatives *via* [3+2] cycloaddition.



1,3-Dipolar compounds		Cycloadducts		Deprotected products
5a	Br–C≡N→O	**3a**	R= 	**4a**#
5b		**3b**	R= 	**4b**#
5c		**3c**	R= 	**4c-I***, **4c-II****
5d		**3d**	R= 	**4d-I***, **4d-II****
5e		**3e**	R= 	**4e-I**, **4e-II**, **4e-III*****
		3f	R= 	**4f**#
		3g	R= 	**4g-I***, **4g-II****

(a) CH_3I, DMF (b) TFA, $PhOCH_3$ (c) TFA, H_2O Tr= trityl, PMB= *p*-methoxybenzyl # diastereomers not separated.
* more polar isomer. ** less polar isomer. *** mixture of two isomers.

Scheme 2. Synthesis of new cephem derivatives *via* [4+2] cycloaddition.



5-yl)vinyl]cephem **3a** and 3-[(*E*)-2-[3-(pyridin-4-yl)-2-isoxazolin-5-yl]vinyl]cephem **3b**. Because no isoxazolin-4-yl isomer was detected in these reactions, the cycloaddition was exclusively regiospecific. Similarly, nitrones **5c** and **5d** gave the 3-vinyl cephems **3c** and **3d**, respectively. The diastereoselectivity regarding the 5-position of the isoxazolidine ring was poor (55:45~50:50). Compounds **3b** and **3c** were treated with methyl iodide to give quaternary ammonio cephems **3f** and **3g**. Deprotection of **3a**~**3d**, **3f** and **3g** was performed using TFA/anisole and then TFA/H_2O systems to yield **4a**~**4d**, **4f** and **4g**. Diastereomers of both **4c** and **4g** were separated by preparative HPLC: more polar isomer, **4c-I**, **4g-I**, less polar isomer, **4c-II**, **4g-II**. **4g-II**: IR (KBr) cm^{-1} 1772, 1672, 1540, 1038; ^1H NMR (DMSO-d_6) δ 2.50 (1H, m, NCH_2CHH), 2.73 (1H, m, NCH_2CHH), 3.5 (1H, m, 2-C*H*H), 3.51 and 3.55 (6H, each s, $NCH_3 \times 2$), 3.80 (1H, m, 2-CH*H*), 3.84 (3H, s, OCH_3), 4.05~4.18 (2H, m, NCH_2), 5.04 (1H, d, J=5 Hz, 6-CH), 5.18 (1H, dt, J=6 and 8 Hz, CH=CHC*H*), 5.55~5.61 (2H, m, 7-CH+C*H*=CH), 6.74 (1H, s, thiazole), 7.10 (1H, d, J=16 Hz, CH=C*H*), 7.23 (2H, s, NH_2), 9.56 (1H, d, J=8 Hz, CONH); FAB-MS (positive) m/z 509 $(M+H)^+$. We tried several 1,3-dipolar compounds other than nitrile oxide and nitrone. Azomethine imine **5e**[11] afforded a mixture of adducts, **3e**. Removal of the protecting groups and the subsequent HPLC analysis revealed that the mixture was made up of four isomers (in the ratio of 1.4:1.2:1.0:2.1), the latter two of which were incompletely separated. Three fractions, which consisted of two single isomers (**4e-I** and **-II**) and a mixture of two other isomers (**4e-III**), were collected. They were shown by ^1H NMR to possess a 3-vinyl structure, which indicated that the reaction occurred only at the terminal olefin. The direction of cycloaddition was determined for **4e-I** by the COSY technique. Thus, the existence of methylene protons at δ 1.98 and 2.55 ppm and the observation of the cross peak between the signals of δ 1.98 and 3.25 ppm were consistent with a (6-amino-7-oxo-tetrahydropyrazolo[1,2-*a*]pyrazol-1-yl)vinyl structure. **4e-I**: IR (KBr) cm^{-1} 1766, 1540; ^1H NMR (DMSO-d_6) δ 1.98 (1H, m, 2-tetrahydropyrazolopyrazole(Tpp)), 2.41 (1H, 3-Tpp), 2.55 (1H, m, 2-Tpp), 2.64 (1H, m, 5-Tpp), 3.25 (1H, m, 3-Tpp), 3.47 (1H, d, J=17 Hz, 2-C*H*H), 3.60 (1H, d, J=17 Hz, 2-C*H*H), 3.78 (1H, m, 5-Tpp), 3.84 (3H, s, OCH_3), 4.05 (1H, m, 6-Tpp), 4.25 (1H, m, 1-Tpp), 5.08 (1H, d, J=5 Hz, 6-CH), 5.65 (2H, m, 7-CH+C*H*=CH), 6.74 (1H, s, thiazole), 6.90 (1H, d, J=16 Hz, CH=C*H*), 7.21 (2H, s, NH_2), 9.58 (1H, d, J=8 Hz, CONH); FAB-MS (positive) m/z 549 $(M+H)^+$. Because the ^1H NMR spectra of **4e-II** and **4e-III** were very similar to that of **4e-I**, it was deduced that the additions were regiospecific and those four isomers were diastereomers derived from the 1- and the 6-positions of the tetrahydropyrazolopyrazole ring.

Next we examined a Diels-Alder type reaction (Scheme 2). When diene **2** was allowed to react with the dienophiles, **8a** and **8b**, the corresponding adducts **6a** and **6b** were smoothly afforded as a mixture of diastereomers in a ratio of 2:1 for **6a** and 10:1 for **6b**. They were transformed to **7a** and **7b** by the usual deprotection procedure.

Biological Activity

The antibacterial activity of the synthesized cephalosporins 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 Mueller-Hinton agar at 37°C for 18 hours with an inoculum size of 10^6 cfu/ml. For comparison, the MICs of ceftazidime (CAZ), cefpirome (CPR), and flomoxef (FMOX) are also shown.

In a series of [3+2] cycloadducts, 3-(isoxazolidinyl)-vinyl cephems exhibited the best activity and 3-(isoxazolinyl)vinyl cephems came second. When comparison was made among the 3-(isoxazolinyl)vinyl cephems (**4a**, **4b** and **4f**), the pyridyl derivative **4b** was less potent than the bromide **4a** against both Gram-positive and

Table 1. Comparative activity (MIC, μg/ml)[a] of new cephem compounds.

Organism	*S. a. 1*	*S. a. 2*	*S. p.*	*E. f.*	*E. co.*	*C. f.*	*E. cl.*	*S. m.*	*P. a. 1*	*P. a. 2*
4a	0.78	12.5	0.2	>25	<0.006	6.25	6.25	12.5	>25	25
4b	3.13	>25	0.2	>25	<0.006	12.5	25	25	>25	>25
4f	0.78	>25	0.05	>25	<0.006	3.13	3.13	6.25	>25	>25
4e-I	6.25	>25	0.05	>25	0.025	3.13	3.13	1.56	12.5	>25
4e-I	12.5	>25	1.56	>25	0.05	12.5	12.5	6.25	>25	>25
4e-III	12.5	>25	0.78	>25	0.2	25	12.5	>25	>25	>25
4c-I	1.56	>25	0.1	>25	<0.006	3.13	0.78	12.5	>25	>25
4c-II	1.56	>25	0.05	>25	<0.06	6.25	6.25	3.13	>25	>25
4d-I	1.56	>25	0.025	>25	0.025	6.25	12.5	3.13	>25	>25
4d-II	3.13	>25	0.025	>25	0.025	12.5	12.5	12.5	>25	25
4g-I	0.39	12.5	0.05	>25	<0.006	0.39	0.39	0.78	>25	6.25
4g-II	0.39	12.5	0.05	25	<0.006	0.39	0.39	0.78	>25	6.25
11a	>25	>25	0.39	>25	6.25	>25	>25	>25	>25	>25
11b	12.5	>25	0.1	>25	0.013	12.5	>25	6.25	>25	>25
CPR	0.39	25	0.05	25	0.013	0.78	0.78	0.78	50	1.56
CAZ	6.25	100	0.78	>100	0.025	12.5	50	0.78	50	0.78
FMOX	0.39	6.25	0.2	>100	0.1	12.5	>100	50	>100	>100

Abbreviations: *S.a.1*, *Staphylococcus aureus* FDA209P JC-1; *S.a.2*, *S. aureus* CAY01-4; *S.p.*, *Streptococcus pyogenes* Cook; *E.f.*, *Enterococcus faecalis* CAY104; *E.co.*, *Escherichia coli* NIHJ; *C.f.*, *Citrobacter freundii* CAY717; *E.cl.*, *Enterobacter cloacae* CAY3207; *S.m.*, *Serratia marcescens* CAY6430; *P.a.1*, *Pseudomonas aeruginosa* ATCC 8689; *P.a.2*, *P. aeruginosa* IID5.142.

[a] Agar dilution method: Mueller-Hinton agar, 10^6 cfu/ml.

Table 2. *In vivo* antibacterial activity of **4g-I** and **4g-II** against a systemic infection in mice induced by *S. aureus* Smith.

Drug	MIC (μg/ml)[a]	ED_{50} (mg/kg)[b]	ED_{50}/MIC
4g-I	0.78	0.074	0.095
4g-II	0.78	0.085	0.11
Ceftazidime	6.25	4.5	0.72
Ceftriaxone	3.13	3.1	0.99

[a] Inoculum size: 10^6 cfu/ml.

[b] Infective challenge dose: 3×10^6 cfu/mouse.

Gram-negative bacteria. By converting its structure to betaine **4f**, the antibacterial activity was improved to somewhat surpass that of the bromide.

In the series of 3-vinylcephalosporins with an isoxazolidine ring (**4c-I**, **-II**, **4d-I**, **-II** and **4g-I**, **-II**), **4g-I** and **II** showed the most potent *in vitro* activity, being their potency against *Staphylococcus aureus* CAY01-4 comparable to that of FMOX. Moreover, they were the most effective against CAZ-resistant *Enterobacter cloacae* CAY3027 and *Citrobacter freundii* CAY717 among the compounds tested here. No significant difference was observed with regard to *in vitro* activity between the two diastereomers.

The *in vivo* efficacy of **4g-I** and **-II** against systemic infection by *S. aureus* Smith is shown in Table 2. To our excitement, they displayed excellent *in vivo* activity. The value of ED_{50}/MIC was 0.095 for **4g-I** and 0.11 for **4g-II**, whereas those of reference compounds were in the range of 0.72 to 0.99.

Derivatives with a unique tetrahydropyrazolopyrazole or tetrahydropyridazine ring at their C-3 substituents (**4e-I**, **-II**, **-III**, **11a** and **11b**) demonstrated only weak activity. Steric bulkiness of these rings probably affected their PBP binding affinity and/or membrane permeability.

In conclusion, we examined the cycloaddition reactions of a 3-(1,3-butadienyl)cephem **2** and found a promising derivative, **4g-II** (YM-32825), which showed good *in vitro* antibacterial activity and excellent *in vivo* efficacy against *S. aureus*. Disappointingly, all of the compounds synthesized here exhibited no or modest activity against *Pseudomonas aeruginosa*. In the search for compounds with more favorable activity, an SAR study was continued with 3-(isoxazolidinyl)vinyl cephems.

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