

Structure-activity Relationships of 1 β -Methyl-carbapenems to Antimicrobial Activity: Effect of C-6 Substituent

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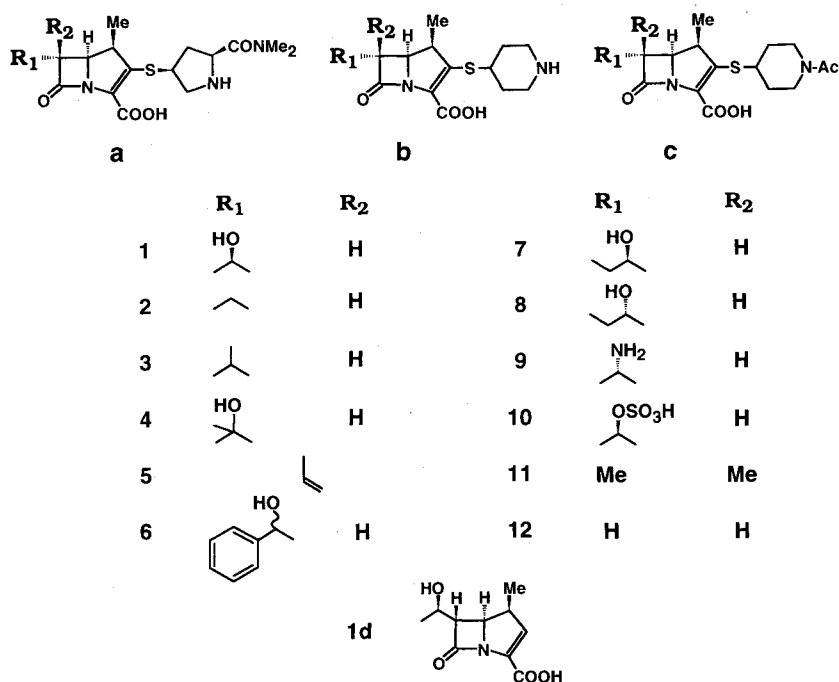
We have been investigating the synthesis and biological properties of 6-hydroxyethyl carbapenem compounds^{1~4)} and demonstrated that meropenem (MEPM, **1a** in Fig. 1) having a 1 β -methyl group and the 5'-dimethylaminocarbonylpyrrolidin-3'-ylthio (DMAP) group exhibits an extended antimicrobial spectrum including anti-pseudomonal activity and high stability to renal dehydropeptidase-I (DHP-I)¹⁾. From the structure-activity relationship studies, we found that the basicity of the C-2 side chain is important for exhibiting antimicrobial activity especially against *Pseudomonas aeruginosa* by supporting good permeability through the outer membrane (OM)⁵⁾. However, the strength of the basicity does not directly correlate with the anti-pseudomonal activity⁵⁾. We also found that the 1 β -methyl group of carbapenem compounds not only im-

proves their DHP-I stability, but also variably affects their antimicrobial activity^{1,5,6)}.

Regarding the effect of C-6 substituent, there are several studies on natural occurring or synthesized carbapenems which have a different substituent on C-6 from thienamycin (*i.e.*, hydroxyethyl group)^{7~11)}. Stereochemical properties of the C-6 substituent have also been investigated with naturally occurring products, such as epithienamycins¹²⁾. *Trans* configuration of the β -lactam ring and the *R*-absolute configuration of the 8-hydroxyl group appears to be preferred for the antimicrobial activity and stability against β -lactamases and DHP-I¹²⁾. However, there are no reports investigating the systematic structure-activity relationships between the antimicrobial activity and the C-6 substituent.

On the basis of these studies, we investigated the effect of C-6 substituent, focusing on 5,6-*trans* 1 β -methyl carbapenems, on the antimicrobial activity and the stability against DHP-I. In terms of activity against *P. aeruginosa*, not only the affinities of antimicrobial agents to the targets, penicillin binding proteins (PBPs), but also the permeability through the OM influence their effectiveness¹³⁾. Therefore, we determined the affinity of carbapenem compounds for the PBPs of *P. aeruginosa*, expecting this would lead to additional information concerning permeability through the OM of the tested compounds.

Fig. 1. Structure of 1 β -methyl carbapenem compounds.

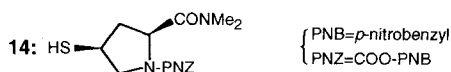
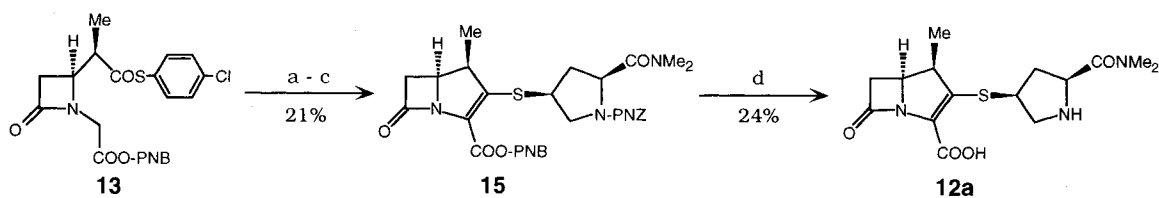


Chemistry

The carbapenem compounds were prepared in our laboratories by applying the Dieckmann-type cyclization method reported previously¹⁴). A typical synthetic procedure is shown in Scheme 1. Thioester **13** was treated with sodium hydride and then diphenyl chlorophosphate to give an activated phosphate. Then, mercaptan **14** was added into the above reaction mixture to afford the protected carbapenem **15**; IR (neat) cm^{-1} 1776, 1709, 1657, 1521, 1346, 1112; ^1H NMR (270 MHz, CDCl_3) δ 1.29 (3H, d, $J=7.3$ Hz), 1.96 (1H, m), 2.66 (1H, m), 2.93

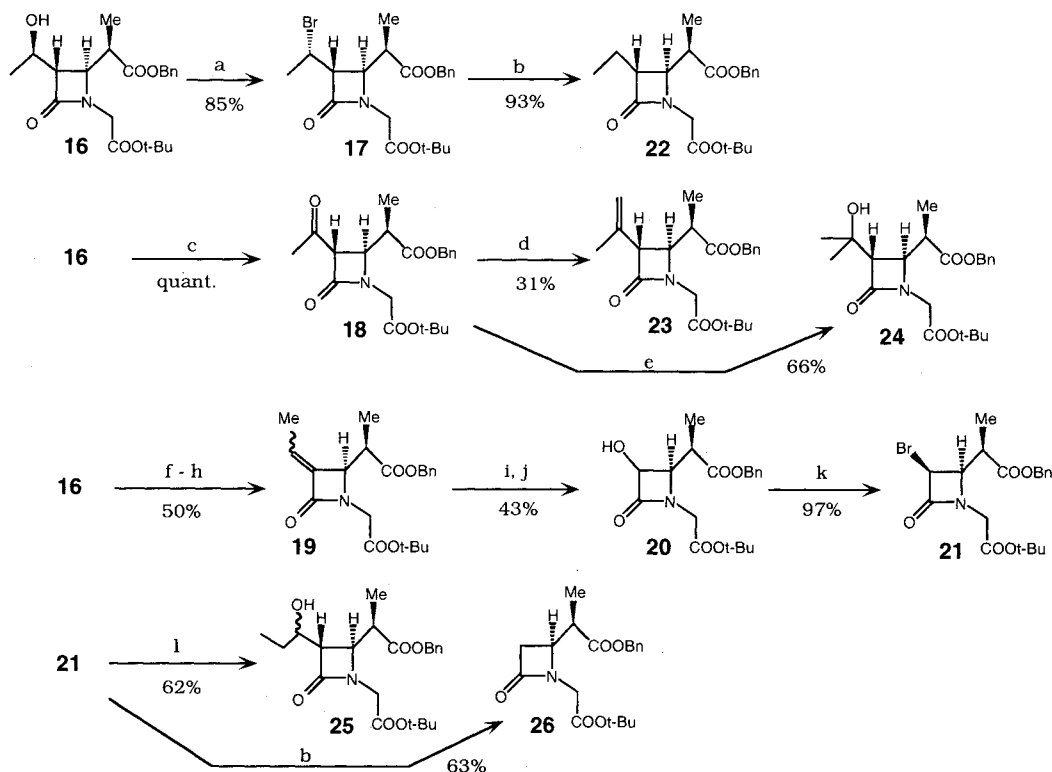
(3/3H, s), 2.98 (6/3H, s), 3.00 (3/3H, s), 3.11 (6/3H, s), 3.33 (2H, m), 3.52 (1H, m), 3.70 (1H, m), 3.95~4.25 (2H, m), 4.28 (1H, m), 4.76 (1H, m), 5.08 (1/3H, d, $J=13.5$ Hz), 5.20~5.35 (3H, m), 5.08 (2/3H, d, $J=13.5$ Hz), 7.44 (2/3H, d, $J=8.6$ Hz), 7.51 (4/3H, d, $J=8.9$ Hz), 7.65 (2H, d, $J=8.2$ Hz), 8.21 (4H, m). The deprotection of **15** by hydrogenolysis over 10% palladium-carbon gave the desired product **12a**; IR (KBr) cm^{-1} 3421, 1734, 1653, 1388; ^1H NMR (270 MHz, D_2O) δ 1.21 (3H, d, $J=7.3$ Hz), 1.93 (1H, ddd, $J=13.9, 8.9$ and 6.3 Hz), 3.01 (2H, m), 3.01 (3H, s), 3.08 (3H, s), 3.16 (1H, dd, $J=16.8$ and 2.5 Hz), 3.34 (1H, dd, $J=16.8$ and 5.3 Hz), 3.41 (1H,

Scheme 1.



(a) NaH, allylbromide, THF, -40°C . (b) ClP(O)(OPh)_2 , -20°C . (c) **14**, iPrEt_2N , DBU, MeCN, 0°C . (d) H_2 , Pd-C, THF-Phosphate buffer.

Scheme 2.



(a) CBR_4 , PPh_3 , THF. (b) Zn, HCOOH , DMF, 100°C . (c) Jones oxidation. (d) Zn, CH_2Br_2 , TiCl_4 , CH_2Cl_2 . (e) $\text{Me}_2\text{Tl(OiPr)}_2$, Et_2O . (f) MsCl , Et_3N , CH_2Cl_2 . (g) NaI , Acetone, reflux. (h) DBU, CH_2Cl_2 . (i) O_3 , PPh_3 , CH_2Cl_2 , -20°C . (j) NaBH_4 , MeOH. (k) CBR_4 , PPh_3 , THF. (l) CuI , BuLi , EtCHO , THF, -78°C .

dd, $J=12.2$ and 5.0 Hz), 3.66 (1H, dd, $J=12.2$ and 6.3 Hz), 4.02 (1H, m), 4.30 (1H, m), 4.68 (1H, t, $J=8.6$ Hz); UV (H_2O) λ_{max} 295 nm. MS (FAB, positive, glycerol) m/z 340 (MH^+), 362 (MNa^+).

The key intermediates **22**~**26** were prepared from hydroxyethyl compound **16**¹⁴) as shown in Scheme 2. Compound **16** was converted into bromide **17** by treating with carbon tetrabromide and triphenyl phosphine¹⁵), then **17** was reduced by zinc powder under acidic condition to afford ethyl derivative **22**. Compound **16** was oxidized by Jones reagent to afford the ketone **18**, which was used in the Tebbe-type reaction¹⁶) to give isopropenyl derivative **23**. 1-Hydroxyisopropyl derivative **24** was also prepared from **18** by methylation with diisopropoxydimethyltitanium¹⁷). Bromo compound **21**, which is useful for the preparation of several intermediates, was derived from **16** as follows. Compound **16** was dehydrated *via* mesyl intermediate to obtain **19** which was used in ozone oxidation and sodium borohydride reduction to give hydroxy derivative **20**. This was converted into **21** by treatment with carbon tetrabromide and triphenylphosphine. 1-Hydroxypropyl derivative **25** was obtained from **21** by treatment with cuprus iodide and *n*-butyllithium, and then propionaldehyde¹⁸). The zinc reduction of **21** afforded 6-nor-derivative **26**, which was converted to thioester **13** by the combination of deprotection reactions of ester group, thioesterification, and *p*-nitrobenzylation of the acid group¹⁴). **13**: IR (neat) cm^{-1} 1765, 1692, 1523, 1346, 1187; 1H -NMR (270 MHz, $CDCl_3$) δ 1.32 (3H, d, $J=6.9$ Hz), 2.90 (1H, br. d, $J=15.0$ Hz), 3.14 (1H, m), 3.15 (1H, dd, $J=15.0$ and 5.3 Hz), 3.92 (1H, d, $J=18.2$ Hz), 4.23 (1H, m), 4.38 (1H, d, $J=18.2$ Hz), 5.20 (1H, d, $J=13.2$ Hz), 5.25 (1H, d, $J=13.2$ Hz), 7.30 (2H, d, $J=8.9$ Hz), 7.39 (2H, d, $J=8.9$ Hz), 7.46 (2H, d, $J=8.9$ Hz), 8.19 (2H, d, $J=8.9$ Hz). The other compounds were prepared by applying for similar manner to previously reported procedures^{11,19~21}). The spectral data of the key intermediates **22**~**26** were listed below.

22: IR (neat) cm^{-1} 1760, 1738, 1368, 1230, 1160; 1H -NMR (270 MHz, $CDCl_3$) δ 1.00 (3H, d, $J=7.3$ Hz), 1.23 (3H, d, $J=6.9$ Hz), 1.46 (9H, s), 1.60~1.88 (2H, m), 2.86 (1H, qd, $J=7.0$ and 5.0 Hz), 2.92 (1H, ddd, $J=11.8$, 9.3 and 2.3 Hz), 3.56 (1H, dd, $J=17.8$ and 1.0 Hz), 4.05 (1H, dd, $J=5.0$ and 2.3 Hz), 5.11 (1H, ABq, $J=12.2$ Hz), 7.35 (5H, m). **23**: IR (neat) cm^{-1} 1765, 1737, 1369, 1229, 1157; 1H -NMR (270 MHz, $CDCl_3$) δ 1.24 (3H, d, $J=7.3$ Hz), 1.46 (9H, s), 1.83 (3H, d, $J=0.7$ Hz), 2.92 (1H, m), 3.57 (1H, d, $J=17.8$ Hz), 3.68 (1H, m), 4.05 (1H, dd, $J=4.3$ and 2.6 Hz), 4.17 (1H, d, $J=$

17.8 Hz), 4.93 (2H, m), 5.12 (2H, s), 7.36 (5H, m). **24**: IR (neat) cm^{-1} 3453, 1732; 1H -NMR (270 MHz, $CDCl_3$) δ 1.27 (3H, d, $J=7.5$ Hz), 1.34 (3H, s), 1.37 (3H, s), 1.45 (9H, s), 2.89 (1H, m), 3.09 (1H, d, $J=2.1$ Hz), 3.58 (1H, d, $J=18.6$ Hz), 4.10 (1H, m), 4.16 (1H, d, $J=18.6$ Hz), 5.11 (2H, s), 7.36 (5H, m). **25**: IR (neat) cm^{-1} 3469, 1732, 1394, 1229, 1160; 1H -NMR (270 MHz, $CDCl_3$) δ 0.97 (3H, t, $J=7.3$ Hz), 1.25 (3H, d, $J=7.3$ Hz), 1.46 (9H, s), 1.55 (2H, m), 2.38 (0.3H, d, $J=4.0$ Hz), 2.41 (0.7H, $J=0.5$ Hz), 2.86 (1H, m), 3.13 (1H, m), 3.62 (1H, d, $J=17.8$ Hz), 3.80 (0.7H, m), 3.94 (1H, m), 4.00 (1H, dd, $J=5.0$ and 2.6 Hz), 4.10 (0.3H, m), 4.11 (1H, d, $J=17.8$ Hz), 5.12 (2H, s), 7.36 (5H, m). **26**: 1H -NMR (270 MHz, $CDCl_3$) δ 1.22 (3H, d, $J=6.9$ Hz), 1.44 (9H, s), 2.78~2.87 (2H, m), 3.05 (1H, dd, $J=14.8$ and 5.2 Hz), 3.58 (1H, d, $J=18.8$ Hz), 4.10 (1H, d, $J=18.8$ Hz), 4.15 (1H, m), 5.11 (2H, ABq, $J=12.2$ Hz), 7.35 (5H, m).

Biological Studies

The MICs were measured by a twofold agar dilution method¹¹). *P. aeruginosa* cell membranes were prepared, and the affinities of the carbapenems for PBPs were determined by means of a competition assay using [^{14}C] benzylpenicillin as described previously²²). Since the PBPs 1a/1b, 2, and 3 of *P. aeruginosa* were reported as the targets of the β -lactam antibiotics²³), affinities for these PBPs are listed in the tables. The DHP-I stability was determined by a spectrophotometric method using partially purified DHP-I from swine kidney¹¹). The results were expressed as relative to MEPM (**1a**) in terms of time required for hydrolysis of the compound to 80% of initial.

To demonstrate the effect of lipophilicity and bulkiness of the C-6 substituent on the antimicrobial activity and the susceptibility to hydrolysis by DHP-I, 1 β -methyl carbapenem compounds having four types of C-6 substituents, (1-hydroxy)ethyl (**1**), ethyl (**2**), isopropyl (**3**), and (1-hydroxy)isopropyl (**4**) groups, were studied, and the results are listed in Table 1. In addition, to clarify whether or not the effect of C-6 substituent is variable depending on the C-2 side chain, a series of compounds (**1**~**4**) having three types of the C-2 side chains (**a**~**c**) were investigated. First was a DMAP group (**a**) as a weakly basic (pK_a 7.4) side chain and one of the most potent side chains in 6-hydroxyethyl 1 β -methyl carbapenems¹¹). Second was a piperidinylthio group (**b**) as a basic side chain, and last was an *N*-acetylated derivative of the piperidinyl group (**c**) as a neutralized side chain.

The 6-ethyl compound (**2a**) was still active against most organisms, but its antimicrobial activity was lower than

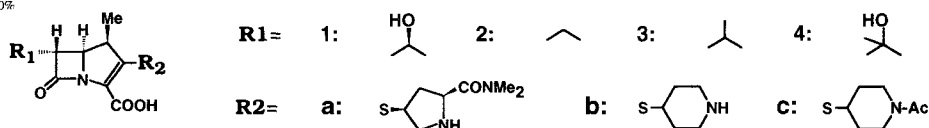
Table 1. Effect of C-6 side chain on the antimicrobial activity, affinities for the PBP's of *P. aeruginosa*, and dehydropeptidase-I stability of carbapenem compounds having three types of C-2 side chain.

Compound :	1a	2a	3a	4a	1b	2b	3b	4b	1c	2c	3c	4c
	(Meropenem)											
	MIC ($\mu\text{g/ml}$)											
<i>S.a.</i> FDA209P	≤ 0.013	0.1	0.39	12.5	0.05	0.025	0.39	12.5	0.2	0.1	1.56	25
<i>S.py.</i> Cook	≤ 0.013	≤ 0.013	0.05	1.56	≤ 0.013	≤ 0.013	0.39	12.5	0.025	0.05	0.78	25
<i>E.c.</i> NIHJ JC-2	≤ 0.013	0.1	0.39	12.5	0.05	3.13	6.25	>200	0.39	6.25	100	>200
<i>K.p.</i> ATCC10031	≤ 0.013	0.05	0.1	6.25	0.05	0.78	1.56	200	0.025	0.2	1.56	25
<i>P.m.</i> GN2425	≤ 0.013	0.2	0.78	25	0.2	3.13	6.25	>200	0.2	6.25	100	200
<i>S.m.</i> X100	≤ 0.013	0.1	0.78	12.5	0.1	3.13	25	>200	3.13	25	200	>200
<i>P.a.</i> IFO3451	0.39	6.25	100	>200	1.56	25	>200	>200	50	>200	>200	>200
<i>H.i.</i> IID983	0.05	0.2	0.78	50	0.1	0.78	0.78	200	0.05	0.78	6.25	100
<i>E.c.</i> ML1410	≤ 0.013	0.1	0.78	25	0.39	3.13	25	>200	0.78	25	200	>200
<i>E.c.</i> ML1410 RP4*	≤ 0.013	0.2	0.78	12.5	0.39	3.13	12.5	>200	0.78	50	200	>200
<i>E.clo.</i> GN7471*	≤ 0.013	0.1	0.78	12.5	0.1	1.56	12.5	200	6.25	25	200	>200
<i>S.m.</i> GN6473*	0.05	0.39	3.13	12.5	0.2	6.25	100	>200	12.5	200	>200	>200
	Affinities for pseudomonal PBPs											
	IC ₅₀ ($\mu\text{g/ml}$)											
PBP-1a	0.34	0.1	1.2	2.5	0.68	0.31	0.12	>10	7.7	0.31	0.33	>10
PBP-1b	0.58	0.42	2.8	>10	0.96	0.56	2.2	>10	1.3	0.32	3.1	>10
PBP-2	0.11	>10	>10	7.4	0.71	>10	10	>10	0.36	7.4	4.7	>10
PBP-3	0.04	6.6	4.3	1.3	0.57	5.9	1.8	>10	0.4	0.5	0.03	>10
DHP-I stability#	1	0.09	0.31	17	6.8	0.32	3.3	14	2.6	0.08	1.0	17

Abbreviations: *S.a.*, *Staphylococcus aureus*; *S.py.*, *Streptococcus pyogenes*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *P.m.*, *Proteus mirabilis*; *S.m.*, *Serratia marcescens*; *P.a.*, *Pseudomonas aeruginosa*; *H.i.*, *Haemophilus influenzae*; *E.clo.*, *Enterobacter cloacae*.

* β -lactamase producing strain

Swine, relative T_{80%}



that of 6-hydroxyethyl compound (**1a**, MEPM). The antimicrobial activity of 6-isopropyl compound (**3a**), in which the 8-hydroxyl group was substituted by a methyl group, was apparently decreased compared to **2a**. The introduction of an additional methyl group to the 6-hydroxyethyl group (**4a**) showed a more marked decrease in antibacterial activity. These findings indicated that the presence of a hydroxyl group and the bulkiness of the C-6 substituent play an important role in the antimicrobial activity. Since the antimicrobial activity of all compounds against β -lactamase producing strains was comparable to that against non-producing strains, it is suggested that the presence of a hydroxyl group and the bulkiness of the C-6 substituent does not affect the susceptibility to β -lactamases so significant as to change its antimicrobial potency.

The reduction of antimicrobial activity against *P. aeruginosa* was the most marked. The affinities for pseudomonal PBP-2 and PBP-3 of compound **2a** were much lower than that of **1a**. In addition, the affinities of compound **3a** and **4a** for all PBPs were markedly decreased. Thus, the lipophilicity and the bulkiness of

C-6 substituent obviously affect its interaction with pseudomonal PBPs. The weakened affinities for the targets may potentially reduce the anti-pseudomonal activity, although alteration of the OM permeability may also contribute to the decrease of the anti-pseudomonal activity.

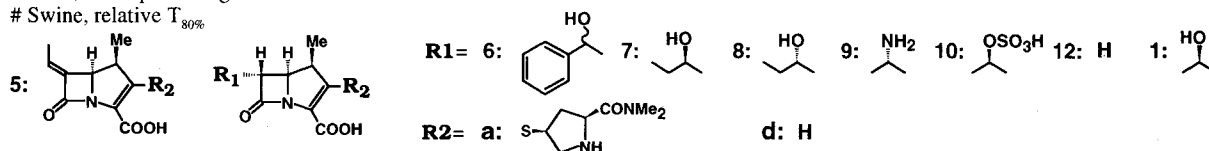
Compounds **2a** and **3a**, which do not possess a hydroxyl group on C-8, were unstable to DHP-I. On the other hand, introduction of an additional methyl group to the C-6 substituent improved the stability against DHP-I, comparing **2a** with **3a**, and **1a** with **4a**. These findings suggest that both hydrophilicity and steric bulkiness of the C-6 substituent increase its resistance to DHP-I.

Similar results were obtained with other series of compounds having a piperidinythio group (**1b**~**4b**) and an *N*-acetyl piperidinythio group (**1c**~**4c**) at the C-2 position regarding both the antimicrobial activity and DHP-I stability. Among the three types of compounds having the same C-6 substituent, the compounds having a DMAP group at C-2 generally showed the best antimicrobial activity. Since the C-6 substituent greatly

Table 2. Effect of C-6 side chain on the antimicrobial activity, affinities for the PBPs of *P. aeruginosa*, and dehydropeptidase-I stability of carbapenem compounds.

Compound :	5a	6a	7a	8a	9a	10a	12a	1d
	MIC ($\mu\text{g/ml}$)							
<i>S.a.</i> FDA209P	0.78	0.39	0.1	0.78	6.25	3.13	0.05	0.2
<i>S.py.</i> Cook	0.2	0.2	0.1	0.2	3.13	1.56	≤ 0.013	0.39
<i>E.c.</i> NIHJ JC-2	0.78	1.56	0.05	0.78	3.13	1.56	≤ 0.013	0.78
<i>K.p.</i> ATCC10031	0.2	0.39	0.1	0.39	0.78	1.56	≤ 0.013	0.78
<i>P.m.</i> GN2425	1.56	3.13	0.2	1.56	3.13	3.13	0.025	1.56
<i>S.m.</i> X100	1.56	3.13	0.2	0.78	3.13	3.13	0.025	1.56
<i>P.a.</i> IFO3451	25	200	6.25	50	0.78	100	0.2	12.5
<i>H.i.</i> IID983	6.25	1.56	0.1	3.13	50	1.56	0.05	0.78
<i>E.c.</i> ML1410	1.56	3.13	0.2	0.78	6.25	1.56	0.05	1.56
<i>E.c.</i> ML1410 RP4*	1.56	3.13	0.1	0.78	6.25	1.56	3.13	1.56
<i>E.clo.</i> GN7471*	0.78	200	0.2	0.78	1.56	1.56	≤ 0.013	0.78
<i>S.m.</i> GN6473*	3.13	12.5	0.39	0.78	3.13	3.13	6.25	1.56
	IC ₅₀ ($\mu\text{g/ml}$)							
Affinities for pseudomonal PBPs								
PBP-1a	0.28	0.52	n.t.	n.t.	0.68	1	0.02	n.t.
PBP-1b	0.48	1.5			2.7	3.9	1.2	
PBP-2	0.24	4.2			0.8	>10	0.03	
PBP-3	0.7	1.7			10	2.9	0.34	
DHP-I stability [#]	0.04	17	n.t.	n.t.	0.08	17	0.65	0.01

Abbreviations: See a footnote in Table 1. n.t., not tested.

* β -lactamase producing strain# Swine, relative T_{80%}

affected the antimicrobial activity and the DHP-I stability in the same way irrespective of the C-2 side chain, further study was conducted using compounds having a DMAP group at C-2 (Table 2).

The dehydrated derivative **5a** showed lower antimicrobial activity against all organisms tested and lower stability against DHP-I than **1a**. This observation supports that not only hydrophilicity but also stereochemistry of the C-6 substituent are important to maintain antimicrobial activity. To clarify the effect of lipophilicity and bulkiness of the C-6 substituent, 6-(1-hydroxy)phenylmethyl compound **6a** and 6-(1-hydroxy)propyl compounds **7a** and **8a** were evaluated. Although compound **6a** was a mixture of diastereomers at C-8 (*R*:*S*=35:6), **6a** revealed a significant reduction in the activity against all tested strains and affinities for pseudomonal PBPs, and showed high resistance against DHP-I. The antimicrobial activity of **7a** was also weaker than that of **1a** but the degree of decrease was not so significant as that of **6a**. These findings show that the bulkiness and/or increased lipophilicity of C-6 substituent result in a decrease in the antimicrobial activity. The bulkiness and/or lipophilicity of C-6 substituent may

also affect the affinities for pseudomonal PBPs and the stability against DHP-I, although these data of **7a** and **8a** could not be determined due to shortage of the samples for examinations.

In the case of the (1-hydroxy)propyl substituent, a comparison of the antimicrobial activity between **7a** and its diastereomer **8a** was attempted. Compound **8a** (8-*S* configuration) was less active than **7a** (8-*R* configuration) in agreement with previous findings on the stereochemical property of 6-hydroxyethyl group^{12,24}.

To investigate the effect of introducing a basic or acidic property into the C-6 substituent, we evaluated compound **9a** and **10a**, respectively (Table 2). Although both compounds showed markedly decreased activity against most organisms, the pattern in the antimicrobial activity against *P. aeruginosa* and *Haemophilus influenzae* of these compounds were quite different. In spite of 8-*S* configuration, the antimicrobial activity of **9a** against *P. aeruginosa* was as high as that of **1a**, while the activity against *H. influenzae* was unusually weak. In contrast, 8-sulfonated compounds **10a** showed decreased activity especially against *P. aeruginosa*. MASTALERZ *et al.*¹¹) similarly reported that carbapenem having a 6-amino-

ethyl group showed higher activity against *P. aeruginosa* than the corresponding 6-hydroxyethyl derivative. Since **9a** had significantly lowered affinities for pseudomonal PBPs than **1a**, the basic C-6 substituent contributes to the improvement of the permeability through the pseudomonal OM similarly to basicity in the C-2 side chain⁵⁾. As for DHP-I stability, the two compounds again showed exactly opposite stabilities. Compound **9a** was more unstable to DHP-I than **1a**, while **10a** was extremely stable. Thus, basic C-6 substituent seems not to be preferred for DHP-I resistance and for antimicrobial activity except against *P. aeruginosa*. On the other hand, acidic substituent seems to be favorable to DHP-I resistance but not anti-pseudomonal activity, although the effect of bulkiness of sulfonyl group should be considered.

It was predicted that the di-substitution at C-6 position led to inferior antimicrobial activity, since the *cis*-configuration was known to be inappropriate¹²⁾ and the bulkiness around the C-6 substituent would have an undesirable effect on the activity as described above. The 6,6-dimethyl carbapenem (**11a**), as predicted, showed only weak antimicrobial activity against all the tested strains ($>12.5 \mu\text{g/ml}$) and showed undetectable anti-pseudomonal activity ($>200 \mu\text{g/ml}$) and affinities ($\text{IC}_{50\text{s}}$) for PBPs ($>10 \mu\text{g/ml}$). In contrast, the stability of **11a** against DHP-I was the same as that of **1a** (relative $T_{80\%}=1.0$). It is consistent with the finding that desacetyl-epithienamycin A (*cis*-configuration) showed almost the same susceptibility to DHP-I as thienamycin²⁵⁾.

Among the tested carbapenems, the 6-hydroxyethyl group showed the best antimicrobial activity. Finally, we examined how much this group contributes to the antimicrobial activity and stability against DHP-I. 6-Nor compound **12a** as well as 2-nor compound **1d** were examined to compare with the effect of the C-6 substituent and the C-2 side chain on the biological properties. Compound **12a** showed almost the same antimicrobial activity as **1a** including against *P. aeruginosa*, and the anti-pseudomonal activity of **12a** was supported by the high affinities for the PBPs. However, the activity against β -lactamase producing strains of **12a** was lower than that against non- β -lactamase producing strains except for *Enterobacter cloacae*. It is consistent with our previous report that **12a** was hydrolyzed by some β -lactamases but not by a cephalosporinase from *E. cloacae*²⁶⁾. Since DHP-I stability of **12a** was just slightly lower than that of **1a**, it was found that the 6-hydroxyethyl group did not improve the DHP-I sta-

bility, significantly. Consequently, the 6-hydroxyethyl group is important primarily for acquiring resistance to the β -lactamases. In contrast, the 2-nor compound **1d** showed much lower antibacterial activity and DHP-I stability than those of **1a**. The reason why **1d** showed low antimicrobial activity is not unclear, because the affinities of **1d** for PBPs were not determined. However, optimization of the C-2 side chain is important in improving both antimicrobial activity and DHP-I stability.

In summary, we found the structural features of the C-6 substituent affecting the antimicrobial activity and DHP-I stability and clarified the role of the C-6 substituent. The 6-(*R*) hydroxyethyl group was optimal to maintain both antimicrobial activity and DHP-I stability among tested carbapenems and contributed to acquiring only the resistance for β -lactamases. This finding differs from the role of the C-2 side chain in relating strongly to biological properties of carbapenem antibiotics.

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