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

MAKOTO SUNAGAWA, HARUKI MATSUMURA, AKIRA SASAKI, HIROSHI YAMAGA, YUTAKA KITAMURA, YOSHIHIRO SUMITA and HIROSHI NOUDA

Sumitomo Pharmaceuticals Research Center, 3-1-98 Kasugade-naka, Konohana-ku, Osaka 554, Japan

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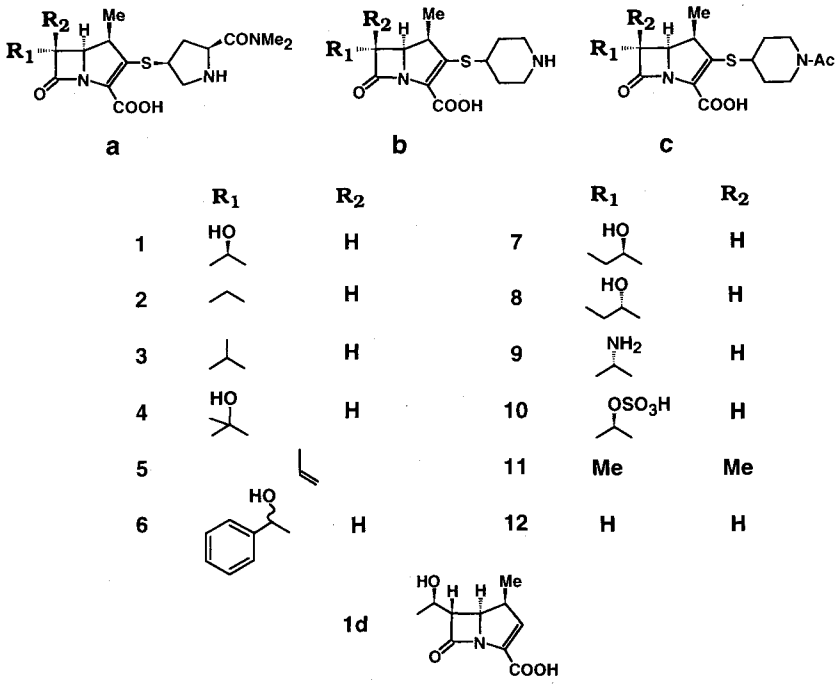
We have been investigating the synthesis and biological properties of 6-hydroxyethyl carbapenem compounds[1~4] and demonstrated that meropenem (MEPM, **1a** in Fig. 1) having a 1β-methyl group and the 5'-dimethylaminocarbonylpyrrolidin-3'-ylthio (DMAP) group exhibits an extended antimicrobial spectrum 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 activity[5]. We also found that the 1β-methyl group of carbapenem compounds not only improves 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 epithienamycins[12]. *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-I[12]. 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* 1β-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 effectiveness[13]. 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.

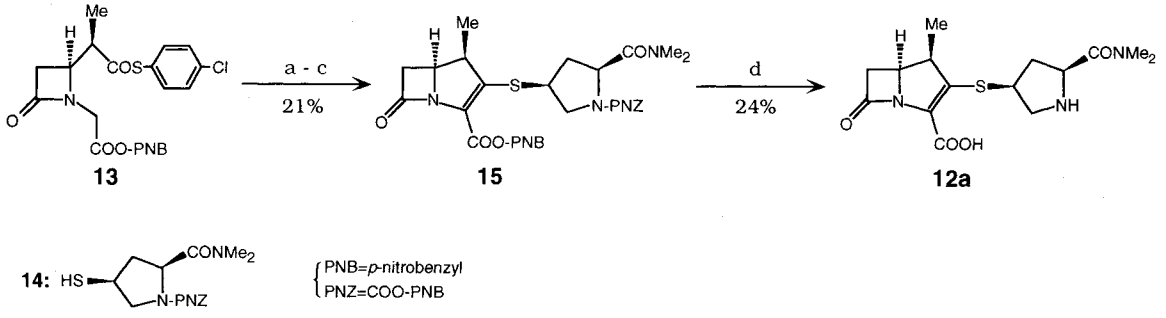
Fig. 1. Structure of 1β-methyl carbapenem compounds.



Chemistry

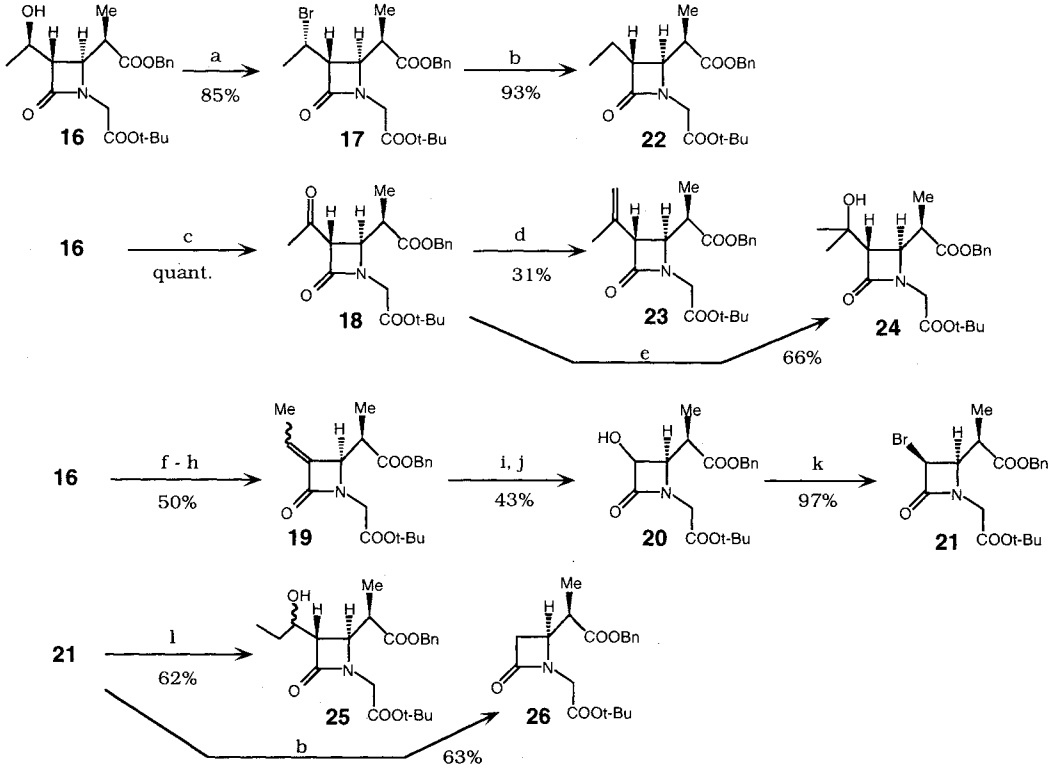
The carbapenem compounds were prepared in our laboratories by applying the Dieckmann-type cyclization method reported previously[14]. A typical synthetic procedure is shown in Scheme 1. Thioester **13** was treated with sodium hydride and then diphenyl chlorophosphate to give an activated phosphate. 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) $Me_2Ti(OiPr)_2$, 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 phosphine[15], 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 reaction[16] to give isopropenyl derivative **23**. 1-Hydroxyisopropyl derivative **24** was also prepared from **18** by methylation with diisopropoxydimethyltitanium[17]. 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 propionaldehyde[18]. 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 group[14]. **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 method[1]. *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 previously[22]. Since the PBPs 1a/1b, 2, and 3 of *P. aeruginosa* were reported as the targets of the β-lactam antibiotics[23], 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 kidney[1]. The results were expressed as relative to MEPM (**1a**) in terms of time required for hydrolysis of the compound to 80% of initial.

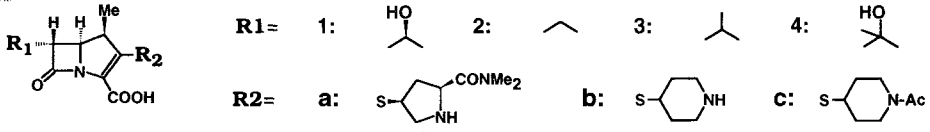
To demonstrate the effect of lipophilicity and bulkiness of the C-6 substituent on the antimicrobial activity and the susceptibility to hydrolysis by DHP-I, 1β-methyl carbapenem compounds having four types of C-6 substituents, (1-hydroxy)ethyl (**1**), ethyl (**2**), isopropyl (**3**), and (1-hydroxy)isopropyl (**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 (pKa 7.4) side chain and one of the most potent side chains in 6-hydroxyethyl 1β-methyl carbapenems[1]. 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 (Meropenem)	2a	3a	4a	1b	2b	3b	4b	1c	2c	3c	4c
						MIC (μg/ml)						
S.a. FDA209P	≤0.013	0.1	0.39	12.5	0.05	0.025	0.39	12.5	0.2	0.1	1.56	25
S.py. Cook	≤0.013	≤0.013	0.05	1.56	≤0.013	≤0.013	0.39	12.5	0.025	0.05	0.78	25
E.c. NIHJ JC-2	≤0.013	0.1	0.39	12.5	0.05	3.13	6.25	>200	0.39	6.25	100	>200
K.p. ATCC10031	≤0.013	0.05	0.1	6.25	0.05	0.78	1.56	200	0.025	0.2	1.56	25
P.m. GN2425	≤0.013	0.2	0.78	25	0.2	3.13	6.25	>200	0.2	6.25	100	200
S.m. X100	≤0.013	0.1	0.78	12.5	0.1	3.13	25	>200	3.13	25	200	>200
P.a. IFO3451	0.39	6.25	100	>200	1.56	25	>200	>200	50	>200	>200	>200
H.i. IID983	0.05	0.2	0.78	50	0.1	0.78	0.78	200	0.05	0.78	6.25	100
E.c. ML1410	≤0.013	0.1	0.78	25	0.39	3.13	25	>200	0.78	25	200	>200
E.c. ML1410 RP4*	≤0.013	0.2	0.78	12.5	0.39	3.13	12.5	>200	0.78	50	200	>200
E.clo. GN7471*	≤0.013	0.1	0.78	12.5	0.1	1.56	12.5	200	6.25	25	200	>200
S.m. 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} (μg/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 mirabillis*; *S.m.*, *Serratia marcescens*; *P.a.*, *Pseudomonas aeruginosa*; *H.i.*, *Haemophilus influenzae*; *E.clo.*, *Enterobacter cloacae*.
* ß-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.

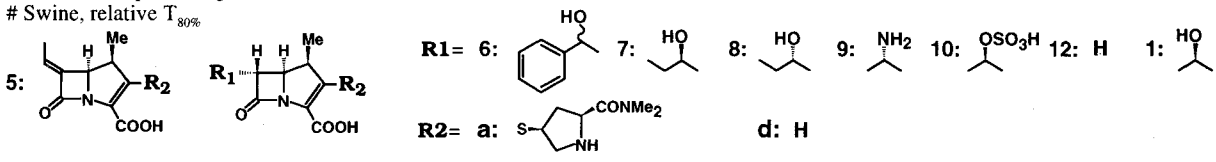
Compounds **2a** and **3a**, which do not possess a hydroxyl group on C-8, were unstable to DHP-I. On the other hand, introduction of an additional methyl group to the C-6 substituent improved the stability against DHP-I, comparing **2a** with **3a**, and **1a** with **4a**. These findings suggest that 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 piperidinylthio group (**1b**～**4b**) and an *N*-acetyl piperidinylthio 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 (μg/ml)				
S.a. FDA209P	0.78	0.39	0.1	0.78	6.25	3.13	0.05	0.2
S.py. Cook	0.2	0.2	0.1	0.2	3.13	1.56	≤0.013	0.39
E.c. NIHJ JC-2	0.78	1.56	0.05	0.78	3.13	1.56	≤0.013	0.78
K.p. ATCC10031	0.2	0.39	0.1	0.39	0.78	1.56	≤0.013	0.78
P.m. GN2425	1.56	3.13	0.2	1.56	3.13	3.13	0.025	1.56
S.m. X100	1.56	3.13	0.2	0.78	3.13	3.13	0.025	1.56
P.a. IFO3451	25	200	6.25	50	0.78	100	0.2	12.5
H.i. IID983	6.25	1.56	0.1	3.13	50	1.56	0.05	0.78
E.c. ML1410	1.56	3.13	0.2	0.78	6.25	1.56	0.05	1.56
E.c. ML1410 RP4*	1.56	3.13	0.1	0.78	6.25	1.56	3.13	1.56
E.clo. GN7471*	0.78	200	0.2	0.78	1.56	1.56	≤0.013	0.78
S.m. GN6473*	3.13	12.5	0.39	0.78	3.13	3.13	6.25	1.56
Affinities for pseudomonal PBPs				IC_{50} (μg/ml)				
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.
* ß-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 chain[5]. 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 inappropriate[12] 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 ($>12.5\,\mu g/ml$) and showed undetectable anti-pseudomonal activity ($>200\,\mu g/ml$) and affinities ($IC_{50}s$) for PBPs ($>10\,\mu g/ml$). 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 thienamycin[25].

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 stability, 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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References

1) SUNAGAWA, M.; H. MATSUMURA, T. INOUE, M. FUKASAWA & M. KATO: A novel carbapenem antibiotic, SM-7338 structure-activity relationships. J. Antibiotics 43: 519~532, 1990
2) SUNAGAWA, M.; H. MATSUMURA, T. INOUE, M. FUKASAWA & M. KATO: A new series of carbapenem antibiotics with 5'-substituted pyrrolidinylthio group at C-2 position. J. Antibiotics 44: 459~462, 1991
3) SUNAGAWA, M.; H. MATSUMURA, T. INOUE, H. YAMAGA & M. FUKASAWA: Synthesis and biological properties of 1β-methyl-carbapenems with *N*-methylpyrrolidinylthio group at C-2 position. J. Antibiotics 45: 971~976, 1992
4) SUNAGAWA, M.; A. SASAKI, H. YAMAGA, H. SHINAGAWA, Y. SUMITA & H. NOUDA: Novel quaternary ammonium carbapenems: 1β-methyl-2-(5'-substituted pyrrolidinylthio)carbapenems. J. Antibiotics 47: 1337~1340, 1994
5) SUNAGAWA, M.; H. MATSUMURA, A. SASAKI, H. YAMAGA, Y. SUMITA & H. NOUDA: Structure-activity relationship of 1β-methyl-carbapenem to its antibacterial activity: Effect of the C-2 side chain and the 1β-methyl group. J. Antibiotics 49: 1175~1178, 1996
6) SUMITA, Y.; Y. EGUCHI, M. FUKASAWA, T. OKUDA, H. YAMAGA, H. MATSUMURA & M. SUNAGAWA: The effect of 1β-methyl and imidoyl substituents on the anti-pseudomonal activity of carbapenems. J. Antibiotics 46: 1629~1632, 1993
7) SAKAMOTO, M.; H. IGUCHI, K. OKAMURA, S. HORI, Y. FUKAGAWA, T. ISHIKURA & J. LEIN: PS-5, a new β-lactam antibiotic. II. Antimicrobial activity. J. Antibiotics 32: 272~279, 1979
8) NAKAYAMA, M.; A. IWASAKI, S. KIMURA, T. MIZOGUCHI, S. TANABE, A. MURAKAMI, I. WATANABE, M. OKUCHI, H.

ITOH, Y. SAINO, F. KOBAYASHI & T. MORI: Carpetimycins A and B, new β-lactam antibiotics. J. Antibiotics 33: 1388～1390, 1980

9) KIMURA, Y.; K. MOTOKAWA, H. NAGATA, Y. KAMEDA, S. MATSUURA, M. MAYAMA & T. YOSHIDA: Asparenomycins A, B and C, new carbapenem antibiotics. IV. Antibacterial activity. J. Antibiotics 35: 32～38, 1982

10) BASKER, M. J.; R. J. BOON, S. J. BOX, E. A. PRESTIGE, G. M. SMITH & S. R. SPEAR: Properties of MM13902 and other carbapenem antibiotics *in vitro* and *in vivo*. J. Antibiotics 36: 416～422, 1983

11) MASTALERZ, H.; M. MENARD, E. RUEDIGER & J. FUNG-TOMC: Synthesis and antibacterial activity of some novel 6-methyl- and 6-propenyl-substituted carbapenems. J. Med Chem. 35: 953～958, 1992

12) HUTT, A. J. & J. O'GRADY: Drug chirality: a consideration of the significance of the stereochemistry of antimicrobial agents. J. Antimicrob. Chemother. 37: 7～32, 1996

13) ZIMMERMANN, W.: Penetration of β-lactam antibiotics into their target enzymes in *Pseudomonas aeruginosa*: Comparison of a highly sensitive mutant with its parent strain. Antimicrob. Agent Chemother. 18: 94～100, 1980

14) SUNAGAWA, M.; A. SASAKI, H. MATSUMURA, K. GODA & K. TAMOTO: Synthetic studies of carbapenem and penem antibiotics. V. Efficient synthesis of the 1β-methylcarbapenem skeleton. Chem. Pharm. Bull. 42: 1381～1387, 1994

15) KOCIENSKI, P. J.; G. CERNIGLIARO & G. FELDSTEIN: A synthesis of (±)-methyl *n*-tetradeca-*trans*-2,4,5-trienoate, an allenic ester produced by the male dried bean beetle *Acanthoscelides obtectus* (Say). J. Org. Chem. 42: 353～355, 1977

16) TAKAI, K.; Y. HOTTA, K. OSHIMA & H. NOZAKI: Effective methods of carbonyl methylenation using CH_2I_2-Zn-Me_3Al and CH_2Br_2-Zn-TiCl4 system. Tetrahedron Lett. 27: 2417～2420, 1978

17) REETZ, M. T.; J. WESTERMANN, R. STEINBACH, B. WENDREROTH, R. PETER, R. OSTAREK & S. MAUS: Chemoselective addition of organotitanium reagents to carbonyl compounds. Chem. Ber. 118: 1421～1440, 1985

18) LIPSHUTZ, B. H.: Review on organocopper 1,2-addition reactions. *In* Comprehensive Organic Synthesis. Vol. 1; *Ed.*, B. M. TROST & I. FLEMING, pp. 108～116, Pergamon Press, Oxford, 1991

19) CLAUB, K.; D. GRIMM & G. PROSSEL: β-Lactams bearing substituents bonded via hetero atoms. Liebigs Ann. Chem. 1974: 539～560, 1974

20) CORBETT, D. F.; S. COULTON & R. SOUTHGATE: Inversion of configuration at C-8 in the olivanic acids: Conversion into the thienamycins and other novel derivatives. J. Chem. Soc. Perkin Trans. I: 3011～3016, 1982

21) CAMA, L. D. & B. G. CHRISTENSEN: Total synthesis of thienamycin analogues. 1. Synthesis of the thienamycin nucleus and dl-descysteaminylthienamycin. J. Am. Chem. Soc. 100: 8006～8007,1978

22) SUMITA, Y.; M. FUKASAWA & T. OKUDA: Comparison of two carbapenems, SM-7338 and imipenem: affinities for penicillin-binding proteins and morphological changes. J. Antibiotics 43: 314～320, 1990

23) NOGUCHI, H.; M. MATSUHASHI & S. MITSUHASHI: Comparative studies of penicillin-binding proteins in *Pseudomonas aeruginosa* and *Escherichia coli*. Eur. J. Biochem. 100: 41～49, 1979

24) WILSON, K. E.; A. J. KEMPF, J. M. LIESCH & B. H. ARISON: Northienamycin and 8-*epi*-thienamycin, new carbapenems from *Streptomyces cattleya*. J. Antibiotics 36: 1109～1117, 1983

25) KROPP, H.; J. D. SUNDELOF, R. HAJDU & F. M. KAHAN: Metabolism of thienamycin and related carbapenem antibiotics by the renal dipeptidase: Dehydropeptidase-I. Antimicrob. Agents Chemother. 22: 62～70, 1982

26) SUMITA, Y.; H. NOUDA, H. SHINAGAWA, H. YAMAGA & M. SUNAGAWA: Structure-activity relationships for interactions between carbapenems and β-lactamases. J. Antibiotics 48: 188～190, 1995