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Structure–Activity Relationships of *trans*-3,5-Disubstituted Pyrrolidinylthio-1β-methylcarbapenems. Part 1: J-111,347 and Related Compounds

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Abstract—1β-Methylcarbapenems having various 3,5-disubstituted pyrrolidinylthio-side chains at C-2 were designed and synthesized. Evaluation of their antibacterial activities indicated that J-111,347 (**1a**) is the first example of an extremely broad spectrum antibiotic showing activity against methicillin-resistant *Staphylococcus aureus* (MRSA) as well as *Pseudomonas aeruginosa*.

Recently, the incidence of mixed infection by *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* has been increasing, especially in immunodeficient patients. To date, an antibiotic that can be used as monotherapy against these two pathogens has not been developed. Vancomycin has been used for MRSA infections in spite of its adverse effect profile.[1] However, the emergence of vancomycin-resistant MRSA has raised concerns about its over use.[2] Meanwhile, broad spectrum β-lactam antibiotics, including 1β-methylcarbapenems active against Gram-positive and -negative bacteria such as *P. aeruginosa*, uniformly lack anti-MRSA activity. Therefore, our efforts have been focused on the identification of new carbapenems with anti-MRSA and antipseudomonal activities superior to those of existing agents.

We have already reported on BO-2727,[3,4] a broad spectrum carbapenem effective against *P. aeruginosa*, and BO-3482,[5,6] an anti-MRSA carbapenem. In the course of studies of BO-2727, we found that introduction of an aminoalkyl substituent into the C-5 position on the pyrrolidine ring improved activity against *P. aeruginosa* and also an increased lipophilicity enhanced the activity against MRSA. In addition, through the discovery of BO-3482 and related compounds, we learned that an increase in the lipophilicity of the molecule resulted in significant improvement of in vitro anti-MRSA activity which, however, did not correspond to the expected in vivo efficacy due to low solubility and high serum binding.

Based on these findings, we designed and synthesized new carbapenems differing in lipophilicity and basicity, that is, carbapenems possessing a phenyl ring as a hydrophobic structure between the pyrrolidine ring and aminoalkyl substituent. In this study, we evaluated *trans*-3,5-disubstituted pyrrolidine as a C-2 side chain in addition to the traditional *cis*-3,5-disubstituted pyrrolidine utilized in meropenem,[7,8] S-4661,[9] MK-826 (L-749,345),[10] BO-2727 and so on. Here, we report the structure–activity relationships of such new carbapenems including J-111,347 (**1a**), which showed an extremely broad antibacterial spectrum, inhibiting MRSA as well as *P. aeruginosa* due to its unique (3*S*,5*R*)-5-(4-aminomethylphenyl)pyrrolidine-3-ylthio C-2 side chain distinguishable by the stereochemistry of the pyrrolidine ring from those of known 1β-methylcarbapenems having a *cis*-3,5-disubstituted pyrrolidine ring.

Synthesis

Carbapenems (**1a**–**n**) having various S-linked pyrrolidinyl side-chains were synthesized as shown in Scheme 1. Allyloxycarbonyl(Alloc)-protected 4-mercaptopyrrolidines (**3a**–**n**) having various kinds of spacers between

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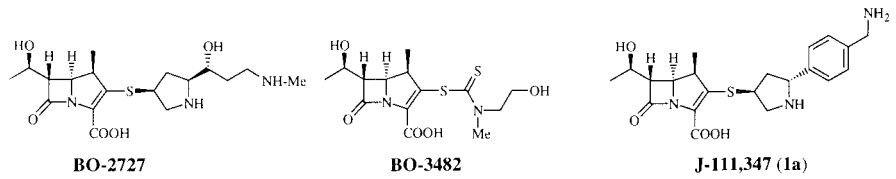
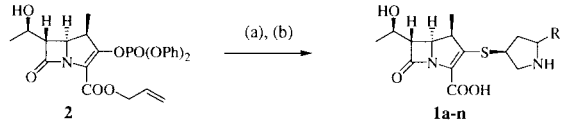


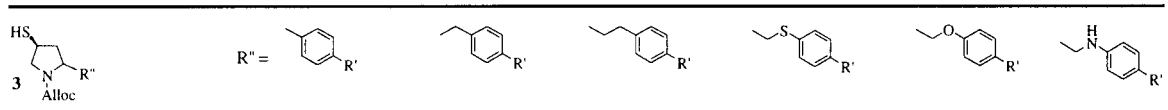
benzene and the pyrrolidine ring were coupled with carbapenem diphenylphosphate **2**[11,12] in the presence of diisopropylethylamine in CH_3CN to provide protected adducts. Subsequent deprotection by the known method yielded the crude carbapenems and purification on reversed phase column chromatography yielded carbapenem derivatives (**1a**–**n**) as amorphous solids after lyophilization.

The synthesis of thiols **3a**–**n** is outlined in Schemes 2–4. Side-chain thiols having ether-type (**3h**, **3m**) or thioether-type (**3g**, **3l**) spacers were prepared from 2-(hydroxymethyl)pyrrolidine **4**[13] through substitution with thiophenol or phenol, and removal of the trityl protection with triethylsilane in TFA-CH_2Cl_2 (Scheme 2). Thiol **3f**, a *trans* isomer of **3g**, could be obtained starting from (2*R*,4*R*)-D-hydroxyproline via the *trans* isomer of **4**.

Thiol **3j** possessing a methylene spacer was prepared from *trans*-aldehyde **6** (Scheme 3). Phenyllithium was added to the aldehyde **6** and the resulting secondary hydroxyl group was removed by Barton deoxygenation to yield 2-benzylpyrrolidine **7**.[14] Replacement of the Boc protection of **7** to the Alloc group followed by the introduction of a mercapto group using Mitsunobu reaction afforded the desired thiol **3j** in good yield. Thiols **3c** and **3d** could be obtained starting from aldehyde **6** and its *cis* isomer, respectively, using 4-substituted phenyllithium via the formation of Alloc-protected benzyl amino group in addition to the same procedures described for **3j**. Thiols **3i** and **3n** having an aminomethyl spacer were also prepared from aldehyde **6** and the corresponding anilines by reductive amination.

Thiol **3a** having no spacer between the pyrrolidine and phenyl ring was synthesized as shown in Scheme 4. Commercially available 4-hydroxyproline could not be utilized; therefore, we started the synthesis of **3a** from D-malic acid derived 3,4-dihydroxy aldehyde **8**,[15] which corresponds to the pyrrolidine ring of **3a**. An addition reaction of 4-substituted phenyllithium to aldehyde **8** provided a diastereomeric mixture of alcohol **9**, which was then converted to a separable mixture of carbamate (**10a**:**10b** = ca. 4:3) in a nine-step procedure including the introduction of two benzyl nitrogen functions by substitution with sodium azide. Diastereomers **10a** and **10b** could be separated on silica gel column chromatography. After transformation of azidomethyl to the Alloc-protected aminomethyl group, **10a** was desilylated. The resulting 1,2-diol was subsequently treated with mesyl chloride to give the corresponding dimesylate, which was then subjected to pyrrolidine formation reaction mediated by potassium *tert*-butoxide. Thus formed pyrrolidine **11** was converted to the thiol **3a** by substitution with potassium thioacetate in DMF at

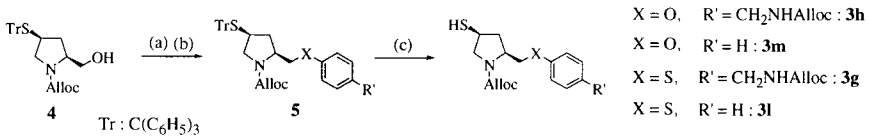




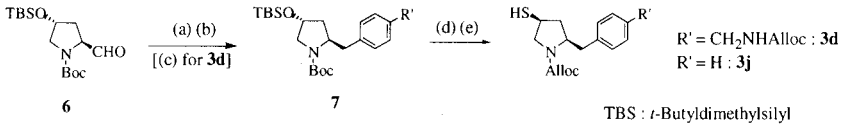
Pyrrolidine*	*trans*	*cis*	*trans*	*cis*	*cis*	*trans*	*cis*	*cis*	*cis*
R′ = $CH_2NHAlloc$	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**
R′ = H				**3j**	**3k**		**3l**	**3m**	**3n**

*Relative stereochemistry of thiol **3**.

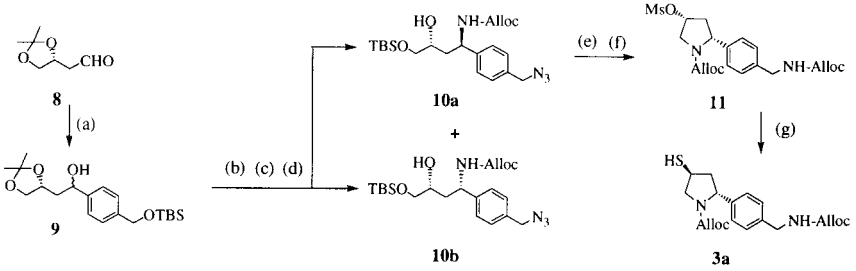
Scheme 1. Synthesis of carbapenem derivatives. Reagents: (a) **3a**–**n**, *i*-Pr_2NEt, CH_3CN, 0 °C, (b) (i) $Pd(PPh_3)_2Cl_2$, *n*-Bu_3SnH, CH_2Cl_2, H_2O; (ii) RP-18 column chromatography.



Scheme 2. Synthesis of thiol derivative-1. Reagents: (a) (i) TsCl, TEA, DMF, rt, 82%; (ii) NaI, Acetone, 50 °C, 78%, (b) 4-(AllocNHCH$_2$)C_6H_4OH (for **3h**), C_6H_5OH (for **3m**), 4-(AllocNHCH$_2$)C_6H_4SH (for **3g**), C_6H_5SH (for **3l**), NaH, THF-DMF, 50 °C, 53~68%, (c) Et_3SiH, TFA, CH_2Cl_2, 0 °C, 88~93%.



Scheme 3. Synthesis of thiol derivative-2. Reagents: (a) (i) C_6H_5Li (for **3j**), 4-$(TBSOCH_2)C_6H_4Br$/*n*-BuLi (for **3d**), THF, −78 °C, 84∼87%, (b) (i) CS_2, NaH, 50 °C then CH_3I, 75∼80%; (ii) *n*-Bu_3SnH, AIBN, toluene, 110 °C, 68∼73%, (c) (i) PPTS, MeOH, 40 °C, 75%; (ii) MsCl, TEA, 0 °C; (iii) NaN_3, DMF, 50 °C, 90%; (iv) PPh_3, THF, H_2O, rt; (v) Alloc-Cl, TEA, 0 °C, 80%, (d) (i) HCl-MeOH, rt; (ii) Alloc-Cl, TEA, 0 °C, 73∼77%, (e) (i) AcSH, DIAD, PPh_3, THF, 0 °C, 91∼94%; (ii) NaOH, MeOH, 0 °C, 88∼95%.



Scheme 4. Synthesis of thioderivatives-3. Reagents: (a) 4-$(TBSOCH_2)C_6H_4Br$/*n*-BuLi, THF, −70 °C, 88%, (b) (i) TEA, MsCl, CH_2Cl_2, 0 °C; (ii) NaN_3, DMF, 50 °C; (iii) PPh_3, THF, H_2O, rt; (iv) Alloc-Cl, TEA, THF, 0 °C, 74%, (c) (i) *n*-Bu_4NF, THF, 0 °C; (ii) MsCl, TEA, CH_2Cl_2, 0 °C; (iii) NaN_3, DMF, rt, 91%, (d) (i) *p*-TsOH, MeOH, rt; (ii) TBS-Cl, imidazole, CH_2Cl_2, rt; (**10a**: 41%, **10b**: 30%), (e) (i) PPh_3, THF, H_2O, rt; (ii) Alloc-Cl, TEA, 0 °C; (iii) HCl–MeOH, 92%, (f) (i) MsCl, TEA, CH_2Cl_2, 0 °C; (ii) *t*-BuOK, THF, −20 °C, 97%, (g) (i) AcSK, DMF, 65 °C, 87%; (ii) NaOH, MeOH, 0 °C, 95%.

65 °C and following alkaline hydrolysis. Conversion of the other diastereomer **10b** to 2,4-*cis* thiol **3b** could be easily achieved by the same method as described for 2,4-*trans* thiol **3a**.

Biological Activity

The in vitro antibacterial activities of the newly prepared carbapenems against *S. aureus*, including an MRSA strain (pMS520/Smith), methicillin-resistant *Staphylococcus epidermidis* (MRSE), *E. coli* and *P. aeruginosa* are shown in Tables 1 and 2. Both imipenem and vancomycin are included as reference drugs.

The effect of the phenyl ring attached on the C-5 position of the pyrrolidine ring by various linkages is shown in Table 1. Expectedly, these cabapenems (**1j**–**n**) showed good activity against *S. aureus* pM520/Smith (a MRSA strain) as well as *S. epidermidis* MB5181 (a MRSE strain) compared with that of imipenem, although their activity was inferior to that of vancomycin. More lipophilic compounds (**1j**–**l**) tended to show more potent activity against MRSA than did **1m** and **1n**. The antipseudomonal activity of this series was much reduced in comparison with that of imipenem; however, the more basic **1n** showed better activity than the other compounds.

Introduction of an aminomethyl group on the *cis* pyrrolidine side chain (**1d**, **1e**, **1g**, **1h**, **1i**) resulted in significantly improved activities against MRSA as well as an expected potent activity against the two strains of *P. aeruginosa* (AK109 and AKR17), as shown in Table 2. *trans* Epimers (**1c**, **1f**) showed decreased potency against all strains compared with that of the corresponding *cis* isomers (**1d**, **1g**). An aminomethylphenyl group directly attached to the pyrrolidine ring (**1a**, **1b**) provided comparable or more enhanced antibacterial activities than did **1d**, **1e**, **1g**, **1h** and **1i** against not only the MRSA strain but also the two *P. aeruginosa* strains.

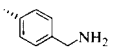
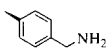
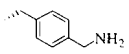
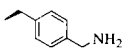
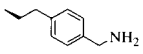
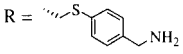
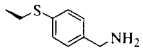
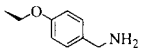
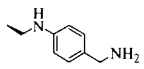
Interestingly, a *trans* diastereomer J-111,347 (**1a**) was 2-fold more active than the corresponding *cis* isomer (**1b**) against the MRSA, MRSE and *P. aeruginosa* strains.[16,17] Such ambidextrous activity of **1a** was not

Table 1. In vitro antibacterial activities[a] of aryl carbapenems

R = 				
Organism	**1j**	**1k**	**1l**	**1m**	**1n**
S. aureus 209P NIHJ JC1	≤0.006	≤0.006	≤0.006	0.012	≤0.006
S. aureus pMS520/Smith[b]	6.25	6.25	6.25	25	12.5
S. epidermidis MB5181[b]	3.13	3.13	3.13	25	6.25
E. coli NIHJ JC2	0.10	0.05	0.10	0.39	0.05
P. aeruginosa AK109	25	25	50	25	12.5
P. aeruginosa AKR17[c]	>50	>50	50	>50	25

[a]MIC (μg/mL) determined by agar dilution method.
[b]Methicillin-resistant.
[c]Ceftazidime-resistant.

Table 2. Comparative in vitro antibacterial activiteis of J-111,347 (**1a**) and related compounds[a]

R = Organism	 **J-111,347 (1a)**	 **1b**	 **1c**	 **1d**	 **1e**	
S. aureus 209P NIHJ JC1	≤0.006	≤0.006	0.05	≤0.006	≤0.006	
S. aureus pMS520/Smith[b]	0.78	1.56	12.5	3.13	3.13	
S. epidermidis MB5181[b]	1.56	3.13	12.5	3.13	25	
E. coli NIHJ JC2	0.025	0.025	0.39	0.025	0.025	
P. aeruginosa AK109	0.39	0.78	25	0.39	0.78	
P. aeruginosa AKR17[c]	3.13	6.25	>100	6.25	6.25	
R = Organism	R =  **1f**	 **1g**	 **1h**	 **1i**	VCM	IPM
S. aureus 209P NIHJ JC1	≤0.006	≤0.006	≤0.006	≤0.006	3.39	≤0.006
S. aureus pMS520/Smith[b]	3.13	1.56	3.13	3.13	0.78	50
S. epidermidis MB5181[b]	3.13	3.13	6.25	6.25	1.56	50
E. coli NIHJ JC2	0.05	0.025	0.025	0.025	>100	0.01
P. aeruginosa AK109	1.56	0.78	1.56	1.56	>100	1.56
P. aeruginosa AKR17[c]	12.5	3.13	12.5	12.5	>100	3.13

[a]MIC (μg/mL) determined by agar dilution method.
[b]Methicillin-resistant.
[c]Ceftazidime-resistant.

seen in the *trans* isomers (**1c**, **1f**) which have methylene or thiomethylene spacers between the pyrrolidine and aminomethylphenyl ring. The side chains of **1a** and **1b** without linkages between the pyrrolidine and benzene ring were likely to be more rigid than the others. These results seem to be consistent with the potent anti-MRSA activities of SM-17466, which has a biaryl side chain of conformationally restricted structure.[18,19] The anti-MRSA activities of β-lactams such as cephalosporins and carbapenems are considered to be responsible for their high affinity for PBP-2′. Indeed, good correlation between the affinity for PBP-2′ and anti-MRSA activity was observed in the case of **1a** (IC_{50} value, 2.6 μg/mL).[20] J-111,347 (**1a**) is the first example of an extremely broad spectrum antibiotic showing activity against MRSA as well as *P. aeruginosa*, however, similar to imipenem, **1a** was found to be epileptogenic by rat intracerebroventricular assay.

In summary, J-111,347 (**1a**) possessing (3*S*,5*R*)-5-aminomethylphenylpyrrolidin as an S-linked side chain was synthesized and found to have an ultra-broad antimicrobial spectrum showing activity against MRSA as well as *P. aeruginosa*.

Acknowledgement

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16. The spectral data of **1a** and **1b** were shown below. **1a**: IR ν_{max} (KBr) 3421, 1749, 1646, 1558 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.22 (3H, d, J=7.0 Hz), 1.27 (3H, d, J=6.5 Hz), 2.51 (1H, m), 2.73 (1H, m), 3.40 (3H, m), 3.86 (1H, dd, J=12.5, 6.0 Hz), 4.25 (5H, m), 5.03 (1H, dd, J=10.5, 7.0 Hz), 7.20 (4H, m); FAB–HRMS m/z calcd for $C_{21}H_{28}N_3O_4S$ $(M+H)^+$: 418.1801, found: 418.1800; UV λ_{max} 298 (ε 9520). **1b**: IR ν_{max} (KBr) 3421, 1749, 1652, 1558 cm^{-1}; ^1H NMR (300 MHz,

D_2O) δ 1.22 (3H, d, $J=7.0$ Hz), 1.27 (3H, d, $J=6.0$ Hz), 2.14 (1H, m), 3.00 (1H, m), 3.37 (1H, m), 3.46 (2H, m), 3.80 (1H, m), 4.14 (1H, m), 4.20 (2H, s), 4.24 (1H, m), 7.52 (2H, d, $J=8.0$ Hz), 7.55 (2H, d, $J=8.0$ Hz); FAB–HRMS m/z calcd for $C_{21}H_{28}N_3O_4S$ $(M+H)^+$: 418.1801, found: 418.1793; UV λ_{max} 298 (ε 9910).

17. Stereochemistry of **1a** and **1b** were determined, and this will be reported in a future paper.

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20. Affinity for PBP-2′ of MRSA was determined by a competition assay with [^{14}C] benzylpenicillin. Briefly, membrane fractions were preincubated at 30 °C for 10 min with non-labeled **1a** and postincubated with [^{14}C] benzylpenicillin for 10 min. Binding ability was expressed as the concentration of non-labeled **1a** that inhibited radio-labeling with [^{14}C] benzylpenicillin by 50% (IC_{50}) compared with the control value.