Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 95–99

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Antibacterial Activity of New Carbapenems Containing Isoxazole Moiety

Yong Koo Kang,[a] Kye Jung Shin,[a] Kyung Ho Yoo,[a] Kyung Jae Seo,[a] Chang Yong Hong,[b] Chang-Seok Lee,[b] Seung Yong Park,[c] Dong Jin Kim[a,*] and Sang Woo Park[a,*]

[a]*Medicinal Chemistry Research Center, Korea Institute of Science and Technology, Seoul 130-650, South Korea*
[b]*Biotech Research Institute, LG Chemical Ltd., PO Box 61, Yusung, Daejeon 305-380, South Korea*
[c]*Korea Research Institute of Chemical Technology, PO Box 107, Yusung, Daejeon 305-606, South Korea*

Received 31 August 1999; accepted 1 November 1999

Abstract—The synthesis and biological activity of a series of new 1β-methylcarbapenems **1a–g** containing 5′-isoxazolopyrrolidin-3′-ylthio derivatives as C-2 side chain are described. Most compounds exhibited potent and well-balanced antibacterial activity as well as high stability to DHP-I comparable to that of meropenem. **1e** and **1c** showed the best combination of antibacterial activity and stability to DHP-I, respectively.

1β-Methylcarbapenems exhibit a broad antibacterial spectrum against both Gram-positive and Gram-negative organisms and high stability to dehydropeptidase-I (DHP-I).[1] Meropenem,[2] which has a 1β-methyl group in carbapenem nucleus, is stable to renal DHP-I and it has successively been launched on the market. In recent years, several analogues such as BO-2727,[3] S-4661,[4] ZD-4433,[5] ER-35786,[6] FR-21818,[7] and IH201[8] are under clinical or preclinical stage.

In the preceding papers,[8–10] we reported the synthesis and biological evaluation of new 1β-methylcarbapenems having a 5′-substituted pyrrolidin-3′-ylthio group as C-2 side chain.

As a continuation of these studies, we carried out the chemical modification on the pyrrolidine side chain of BO-2727, showing the potent antibacterial activity and high stability to DHP-I. To this end we tried the introduction of cyclic isoxazolidine, isoxazoline, and isoxazole derivatives via 1,3-dipolar cycloaddition reaction of 2-vinylpyrrolidine with nitrone and nitrile oxide instead of acyclic side chain of BO-2727 to give the rigid conformation. It was known[11,12] that carbapenem derivatives directly linked with isoxazolidine or isoxazoline ring at C-2 position showed potent antibacterial activities.

In this study, we wish to describe the synthesis of the 1β-methylcarbapenems **1a–g** containing 5′-isoxazolopyrrolidin-3′-ylthio derivatives as C-2 side chain and their biological properties.

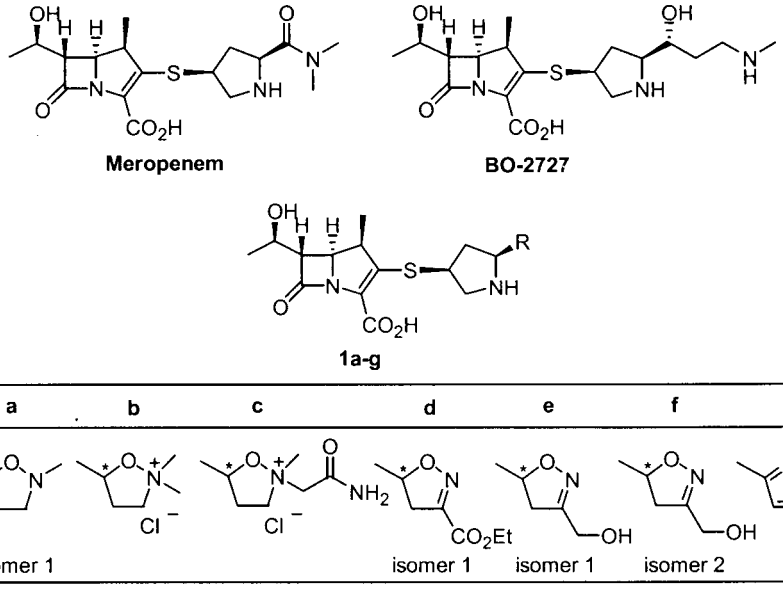
Chemistry

2-Vinylpyrrolidine **8**, a key dipolarophile for 1,3-dipolar cycloaddition reaction, was prepared by the reaction sequence shown in Scheme 1.

trans-4-Hydroxy-L-proline (**2**) was treated with *p*-nitrobenzyl chloroformate to give *N*-protected 4-hydroxyproline **3**, which was esterified to afford *N*-protected ester **4**. Protection of hydroxyl group of **4** with *t*-butyldimethylsilyl chloride in the presence of imidazole followed by reduction of the resulting ester **5** with sodium borohydride provided the alcohol **6**. This was oxidized using pyridine sulfur trioxide complex in the presence of triethyl amine to give formylpyrrolidine **7**. 2-Vinylpyrrolidine **8** was obtained by Wittig reaction of **7** with methyltriphenylphosphonium bromide in the presence of sodium bis(trimethylsilyl)amide.

*Corresponding authors. Fax: +82-2-958-5189; e-mail: djk2991@kistmail.kist.re.kr.

0960-894X/00/$ - see front matter
PII: S0960-894X(99)00646-0



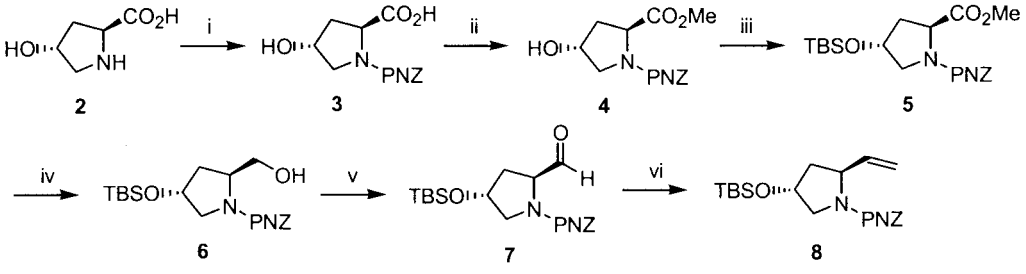
1,3-Dipolar cycloaddition reaction of 2-vinylpyrrolidine **8** with nitrone, generated in situ from *N*-methylhydroxylamine and formaldehyde in the presence of sodium acetate, gave the isoxazolidines **9a,b** as a diastereomeric mixture, which were separable by column chromatography (Scheme 2). Isoxazolidine **9a**, which was exclusively obtained, was deprotected readily with tetrabutylammonium fluoride in THF to give alcohol **10a**, which was converted to the thioacetate **11a**[13] by Mitsunobu reaction.[14] Thiol **12a**, applicable for the coupling with carbapenem enolphosphate **28**,[1] was prepared by deacetylation under basic condition.

Isoxazoline thiol **17a** was prepared by similar procedure to those described above. Nitrile oxide which was generated in situ by dropwise addition of triethylamine to ethyl chlorooximidoacetate was subjected to the 1,3-dipolar cycloaddition reaction to give isoxazolines **13a,b** as a mixture of diastereomers.

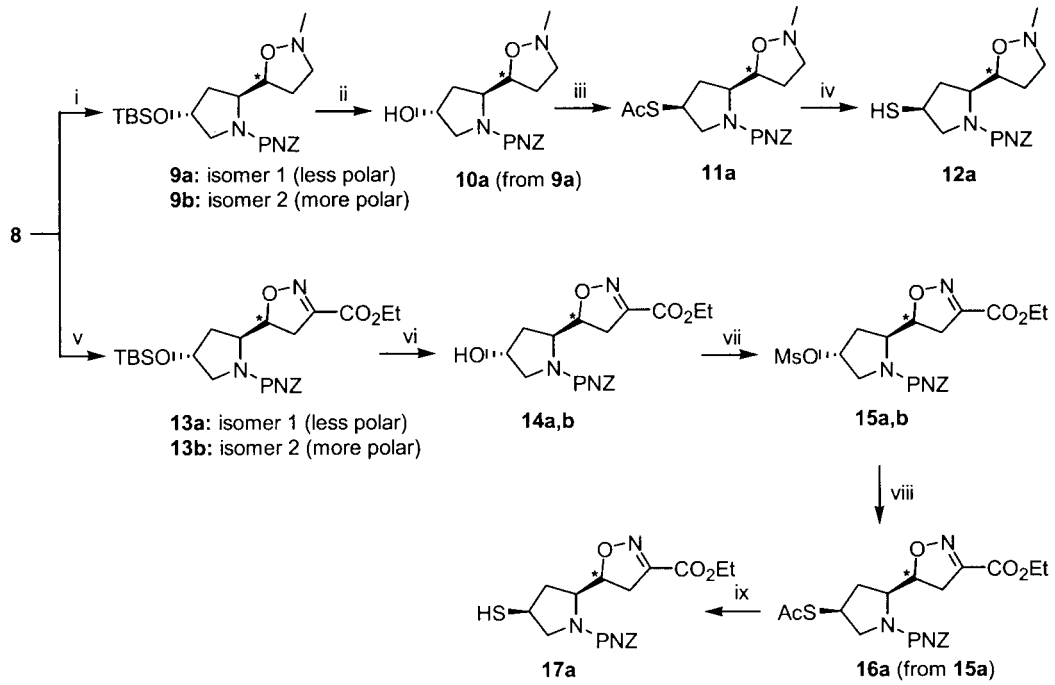
On the other hand, esters **15a,b**, which were separated by column chromatography, were reduced with sodium borohydride to the corresponding alcohols **18a,b**, respectively (Scheme 3). Thiol derivatives **20a,b** were prepared according to the same procedure as used above.

The synthesis of thiol **27** having isoxazole moiety is depicted in Scheme 4. Reaction of the aldehyde **7** with hydroxylamine followed by cycloaddition reaction of imine **21** with ethyl propiolate in the presence of *N*-chlorosuccinimide and triethylamine in DMF gave the isoxazole **22**, which was deprotected with tetrabutylammonium fluoride in THF to give alcohol **23**. Mesylation of **23** with methanesulfonyl chloride provided the mesylate **24**, which was reduced with sodium borohydride to afford alcohol **25**. Thioacetylation and subsequent deacetylation were carried out in the usual manner to give the desired thiol **27**.

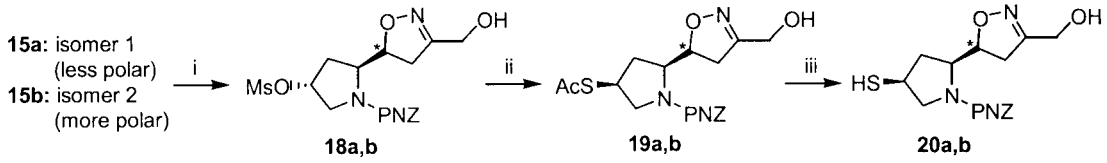
Treatment of the enolphosphate **28**[1] with freshly prepared thiols **12a**, **17a**, **20a,b**, **27** afforded the protected 1β-methylcarbapenems **29a,d–g**, respectively (Scheme 5). Deprotection of **29a,d–g** by zinc powder in the presence of phosphate buffer (pH 6.0)[15] provided the target carbapenems **1a,d–g**[16] as an amorphous solid by lyophilization, after purification by column chromatography on Diaion HP-20. Quaternized isoxazolidine



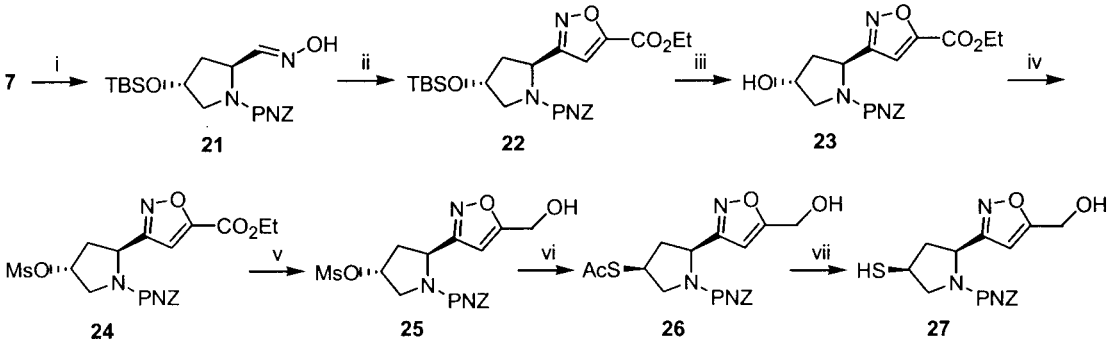
Scheme 1. Reagents and reaction conditions: (i) *p*-Nitrobenzyl chloroformate, 2*N* NaOH, CH_2Cl_2, 0 °C, 2 h (98%); (ii) AcCl, MeOH, reflux, 8 h (99%); (iii) TBSCl, imidazole, DMF, 0 °C to rt, 1.5 h (85%); (iv) $NaBH_4$, LiCl, THF-EtOH, 0 °C to rt, 4 h (95%); (v) $Py.SO_3$, TEA, DMSO, 0 °C to rt, 30 min (84%); (vi) Ph_3PCH_3Br, sodium bis(trimethylsilyl)amide, THF, −70 °C to rt, 1.5 h (57%).



Scheme 2. Reagents and reaction conditions: (i) *N*-Methylhydroxylamine, 35% HCHO, AcONa, 80% EtOH, reflux, 5 h (**9a**: 43%, **9b**: trace); (ii) TBAF, THF, rt, 30 min (86%); (iii) PPh_3, DEAD, AcSH, THF, 0 °C to rt (65%); (iv) 2N NaOH, MeOH, rt, 30 min; (v) ethyl chlorooximidoacetate, TEA, dioxane, CH_2Cl_2, rt (47%); (vi) TBAF, THF, ice-bath, 30 min (85%); (vii) MsCl, TEA, CH_2Cl_2 (**15a**: 58%, **15b**: 36%); (viii) AcSK, MeCN, reflux, 5 h (73%); (ix) 1N NaOH, MeOH, rt, 30 min.



Scheme 3. Reagents and reaction conditions: (i) $NaBH_4$, LiCl, THF-EtOH, 0 °C to rt, 3 h (**18a**: 71%, **18b**: 78%); (ii) AcSK, MeCN, reflux, 5 h (**19a**: 84%, **19b**: 86%); (iii) 1N NaOH, MeOH, rt, 30 min.

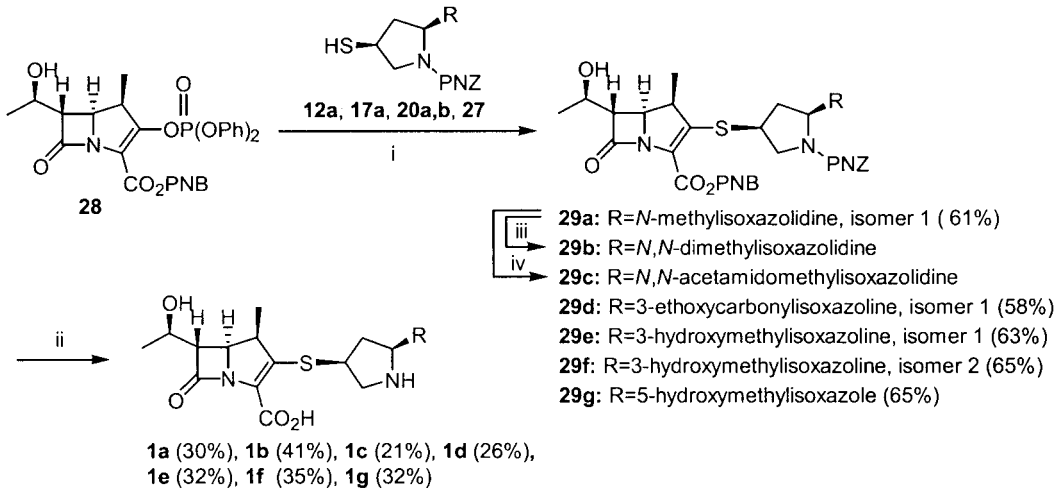


Scheme 4. Reagents and reaction conditions: (i) NH_2OH-HCl, pyridine, EtOH (60%); (ii) ethyl propiolate, NCS, TEA, DMF (50%); (iii) TBAF, THF, ice-bath, 30 min (95%); (iv) MsCl, TEA, CH_2Cl_2, ice-bath, 2 h (85%); (v) $NaBH_4$, LiCl, THF-EtOH, 0 °C to rt, 3 h (74%); (vi) MeCOSK, MeCN, reflux, 5 h (80%); (vii) 1N NaOH, MeOH, rt, 30 min.

carbapenems **1b,c**[16] were obtained as a chloride form by quaternization of **29a** with iodomethane and iodoacetamide followed by deprotection and ion-exchange with Amberlyst A-26, respectively.

Biological Properties

The in vitro antibacterial activity and stability to porcine renal DHP-I of new carbapenems are listed in



Scheme 5. Reagents and reaction conditions: (i) DIPEA, CH_3CN, rt, 1 h; (ii) Zn, phosphate buffer (pH 6.0), THF, rt, Diaion HP-20; (iii) iodomethane, acetone, rt; (iv) iodoacetamide, acetone, rt.

Table 1. In vitro antibacterial activity and DHP-I stability of carbapenem compounds **1a–g**

Organism	MIC (μg/mL)[a]							
	1a	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**MEM**[b]
S. pyogenes 308A	0.007	0.013	0.007	0.025	0.013	0.013	0.013	0.013
S. aureus SG 511	0.098	0.098	0.049	0.195	0.098	0.098	0.098	0.195
S. aureus 285	0.098	0.195	0.098	0.391	0.098	0.098	0.098	0.195
S. aureus 503	0.049	0.049	0.025	0.098	0.049	0.049	0.049	0.098
E. coli 078	0.098	0.195	0.098	0.013	0.025	0.049	0.025	0.025
E. coli 1507E	0.049	0.098	0.049	0.013	0.025	0.049	0.025	0.025
P. aeruginosa 9027	0.391	0.391	0.195	0.781	0.391	0.391	1.563	0.195
P. aeruginosa 1592E	0.391	0.391	0.391	0.781	0.391	0.391	1.563	0.195
P. aeruginosa 1771M	0.195	0.195	0.195	0.391	0.195	0.098	0.391	0.049
S. typhymurium	0.098	0.195	0.195	0.049	0.049	0.098	0.049	0.049
K. aerogenes 1522E	0.098	0.195	0.195	0.049	0.049	0.098	0.049	0.049
E. cloacae 1321E	0.025	0.098	0.049	0.025	0.025	0.049	0.025	0.025
DHP-I stability[c]	1.27	1.50	1.66	0.94	0.78	1.09	0.80	1.00

[a]MIC was determined by agar dilution method using Mueller–Hinton.
[b]MEM = meropenem.
[c]Relative $t_{1/2}$ of hydrolysis to meropenem by partially purified porcine renal DHP-I.

Table 1, together with those of meropenem as a reference compound. Most compounds exhibited potent antibacterial activities against a wide range of Gram-positive and Gram-negative organisms and high stability to DHP-I comparable to that of meropenem. Although there was no significant difference among the antibacterial activities of isoxazolidines **1a–c**, isoxazolines **1d–f**, and isoxazole **1g** series, **1d–f** showed slightly better activity against Gram-negative bacteria except *Pseudomonas aeruginosa* isolates. And isoxazolidines **1a–c** showed slightly better anti-pseudomonal activities than those of isoxazolines **1d–f** and isoxazole **1g** because of their stronger basicities of pyrrolidine moiety as known literature.[17] All the compounds exhibited more potent antibacterial activities than meropenem against Gram-positive bacteria, particularly against *Staphylococcus aureus* isolates. Quaternized isoxazolidine **1b** showed little difference in the anti-pseudomonal activity, whereas they possessed enhanced stability to DHP-I. Especially, **1c** quaternized by acetamido group displayed excellent DHP-I stability compared to unquaternized isoxazolidine **1a** or meropenem. In case of isoxazolines, hydroxymethyl compounds **1e,f** showed better activities against Gram-positives bacteria compared to parent ester **1d**. As for the configuration at the C-5 position of isoxazolines **1e,f**, the isomer **1e** (less polar) was more potent than the corresponding isomer **1f** (more polar) against Gram-negative bacteria, although there was only a slight reduction in DHP-I stability. Isoxazole compound **1g** displayed similar activity to meropenem against most of the Gram-positive and Gram-negative bacteria except *P. aeruginosa* isolates.

Among these compounds, isoxazoline derivative **1e** exhibited the most potent antibacterial activity and quaternized isoxazolidine **1c** possessed the best stability to DHP-I.

Acknowledgement

We are grateful to the Ministry of Science and Technology (MOST) of Korea for financial support.

References and Notes

1. Shih, D. H.; Baker, F.; Cama, L.; Christensen, B. G. *Heterocycles* **1984**, *21*, 29.
2. Sunagawa, M.; Matsumura, H.; Inoue, T.; Fukasawa, M.; Kato, M. *J. Antibiot*. **1990**, *43*, 519.
3. Yamaji, E.; Watanabe, T.; Nakayama, I. Abstracts of Papers, H141, 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 17–20 September 1995.
4. Arakawa, S.; Kamidono, S.; Inamatsu, T.; Shimada, J. Abstracts of Papers, F218, 37th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Ontario, 28 September–1 October 1997.
5. Pelak, B. A.; Gerckens, L. S.; Scott, P. M.; Gill, C.; Pacholok, C.; Lynch, L.; Dorso, K.; Kohler, J.; Shungu, D.; Rosen, H.; Kroppe, H. Abstracts of Papers, F119, 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, 15–18 September 1996.
6. Sato, N.; Sasho, M.; Kamada, A.; Suzuki, T.; Ashizawa, K.; Sugiyama, I. Abstracts of Papers, F151, 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 17–20 September 1995.
7. Tawara, S.; Matsumoto, S.; Matsumoto, Y.; Ishiguro, K.; Maki, K.; Sasaki, K.; Matsuda, K. Abstracts of Papers, F145, 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 17–20 September 1995.
8. Shin, K. J.; Yoo, K. H.; Kim, D. J.; Park, S. W.; Ko, B. S.; Lee, S. J.; Huh, J. D.; Park, S. Y. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 1607.
9. Hwang, S. H.; Shin, K. J.; Kang, Y. K.; Kim, D. J.; Kim, D. C.; Yoo, K. H.; Park, S. W.; Lee, K. J. *Arch. Pharm. Pharm. Med. Chem*. **1998**, *331*, 139.
10. Kang, Y. K.; Shin, K. J.; Yoo, K. H.; Seo, K. J.; Park, S. Y.; Kim, D. J.; Park, S. W. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 2385.
11. Nishi, K.; Imuta, M.; Kimura, Y.; Miwa, H. *J. Antibiot*. **1995**, *48*, 1481.
12. Burton, G.; Clarke, G. J.; Douglas, J. D.; Eglington, A. J.; Frydrych, C. H.; Hinks, J. D.; Hird, N. W.; Moss, S. F.; Naylor, A.; Nicholson, N. H.; Pearson, M. J. *J. Antibiot*. **1996**, *49*, 1266.
13. **11a**: ^{1}H NMR ($CDCl_3$) δ 1.19–2.19 (m, 1H), 2.26 (s, 3H), 2.33–2.46 (m, 2H), 2.56–2.73 (m, 4H), 3.10–3.15 (m, 1H), 3.24–3.32 (m, 1H), 3.70–3.78 (m, 1H), 4.01–4.08 (m, 2H), 4.13–4.23 (m, 1H), 4.66–4.74 (m, 1H), 5.13 (s, 2H), 7.46 (d, 2H, J = 8.6 Hz), 8.15 (d, 2H, J = 8.6 Hz). **16a:** ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 3H), 1.74–1.77 (m, 1H), 2.33 (s, 3H), 2.30–2.47 (m, 1H), 2.84–3.12 (m, 1H), 3.12–3.46 (m, 2H), 3.80–3.88 (m, 1H), 4.03–4.18 (m, 2H), 4.34 (d, 2H), 5.22 (s, 2H), 5.28–5.42 (m, 1H), 7.52 (d, 2H, J = 8.8 Hz), 8.23 (d, 2H, J = 8.8 Hz). **19a**: ^{1}H NMR ($CDCl_3$) δ 1.83–1.90 (m, 1H), 2.35 (s, 3H), 2.36–2.48 (m, 1H), 2.82–2.92 (m, 1H), 3.14–3.28 (m, 2H), 3.84–3.90 (m, 1H), 4.06–4.12 (m, 1H), 4.14–4.18 (m, 1H), 4.46 (s, 2H), 5.13–5.20 (m, 1H), 5.23 (s, 2H), 7.52 (d, 2H, J = 8.7 Hz), 8.25 (d, 2H, J = 8.7 Hz). **19b**: ^{1}H NMR ($CDCl_3$) δ 1.03–1.85 (m, 1H), 2.33 (s, 3H), 2.38–2.46 (m, 1H), 2.52–2.62 (m, 1H), 2.99–3.05 (m, 1H), 3.15–3.22 (m, 2H), 3.76–3.82 (m, 1H), 4.26 (d, 4H), 4.26 (s, 2H), 4.89–4.96 (m, 1H), 5.21 (s, 2H), 7.52 (d, 2H, J = 8.2 Hz), 8.23 (d, 2H, J = 8.2 Hz). **26**: ^{1}H NMR ($CDCl_3$) δ 2.01–2.11 (m, 1H), 2.20–2.30 (m, 1H), 2.32 (s, 3H), 2.80–2.92 (m, 1H), 3.40–3.48 (m, 1H), 4.00–4.12 (m, 1H), 4.13–4.24 (m, 1H), 4.70 (s, 2H), 5.20–5.31 (m, 2H), 6.19 (s, 1H), 7.26 (d, 1H, J = 7.6 Hz), 7.50 (d, 1H, J = 7.6 Hz), 8.12 (d, 1H, J = 7.6 Hz), 8.21 (d, 1H, J = 7.6 Hz).
14. Mitsunobu, O. *Synthesis* **1981**, 1.
15. Kumagai, T.; Abe, T.; Fujimoto, Y.; Hayashi, T.; Inoue, Y.; Nagao, Y. *Heterocycles* **1993**, *36*, 1729.
16. **1a**: ^{1}H NMR (D_2O) δ 1.26 (d, 3H, J = 7.1 Hz, β-methyl), 1.32 (d, 3H, J = 6.3 Hz, CH_3CHOH), 1.82–1.85 (m, 1H), 2.51–2.62 (m, 1H), 2.71 (s, 3H), 2.91–3.02 (m, 1H), 3.25–3.28 (m, 1H), 3.32–3.49 (m, 1H), 3.51–3.61 (m, 1H), 3.68–3.92 (m, 1H), 3.91–4.01 (m, 1H), 4.21–4.33 (m, 2H), 4.58–4.66 (m, 1H); FABHRMS m/z calcd for $C_{18}H_{28}N_3O_5S$ $(M+H)^+$ 398.1750, found 398.1745. **1b**: ^{1}H NMR (D_2O) δ 1.27 (d, 3H, J = 7.1 Hz, β-methyl), 1.36 (d, 3H, J = 6.3 Hz, CH_3CHOH), 1.50–1.61 (m, 1H), 1.80–1.98 (m, 1H), 2.02–2.18 (m, 1H), 2.51–2.63 (m, 1H), 2.98 (s, 3H), 3.14–3.22 (m, 1H), 3.26–3.34 (m, 1H), 3.44–3.61 (m, 3H), 3.70 (s, 3H), 3.82–3.96 (m, 1H), 4.21–4.33 (m, 2H). **1c**: ^{1}H NMR (D_2O) δ 1.23 (d, 3H, J = 7.1 Hz, β-methyl), 1.30 (d, 3H, J = 6.3 Hz, CH_3CHOH), 1.62–1.68 (m, 1H), 1.82–1.85 (m, 1H), 2.38–2.43 (m, 1H), 2.71 (s, 3H), 3.03–3.12 (m, 1H), 3.29–3.41 (m, 4H), 3.42–3.55 (m, 2H), 3.89–3.99 (m, 1H), 4.21–4.33 (m, 2H). **1d**: ^{1}H NMR (D_2O) δ 1.09 (d, 3H, J = 6.9 Hz, β-methyl), 1.24 (d, 3H, J = 6.3 Hz, CH_3CHOH), 1.28–1.38 (m, 1H), 2.30–2.42 (m, 1H), 2.76–2.82 (m, 1H), 3.02–3.18 (m, 1H), 3.21–3.34 (m, 1H), 3.33–3.40 (m, 3H), 3.41–3.52 (m, 1H), 3.60–3.70 (m, 1H), 3.76 (s, 3H). 4.02–4.18 (m, 2H), 4.90–4.98 (m, 1H). **1e**: ^{1}H NMR (D_2O) δ 1.05 (d, 3H, J = 7.0 Hz, β-methyl), 1.12 (d, 3H, J = 6.2 Hz, CH_3CHOH), 1.34–1.52 (m, 1H), 2.34–2.52 (m, 1H), 2.74–2.92 (m, 1H), 3.01–3.10 (m, 1H), 3.25–3.32 (m, 3H), 3.33–3.42 (m, 1H), 3.55–3.69 (m, 1H), 3.75–3.86 (m, 1H). 4.01–4.12 (m, 2H), 4.23 (s, 2H), 4.91–5.02 (m, 1H). **1f**: ^{1}H NMR (D_2O) δ 1.10 (d, 3H, J = 7.1 Hz, β-methyl), 1.18 (d, 3H, J = 6.3 Hz, CH_3CHOH), 1.32–1.42 (m, 1H), 2.32–2.46 (m, 1H), 2.80–2.96 (m, 2H), 3.10–3.34 (m, 4H), 3.56–3.62 (m, 3H), 4.07–4.15 (m, 2H), 4.27 (s, 2H); FABHRMS m/z calcd for $C_{18}H_{26}N_3O_6S$ $(M+H)^+$ 412.1543, found 412.1527. **1g**: ^{1}H NMR (D_2O) δ 1.09 (d, 3H, J = 7.3 Hz, β-methyl), 1.15 (d, 3H, J = 6.3 Hz, CH_3CHOH), 1.60–1.76 (m, 1H), 1.77–1.89 (m, 1H), 2.52–2.70 (m, 1H), 2.82–2.91 (m, 1H), 3.14–3.20 (m, 1H), 3.21–3.26 (m, 1H), 3.66–3.80 (m, 1H), 4.08–4.17 (m, 1H), 4.18–4.30 (m, 1H), 4.61 (s, 2H), 6.38 (s, 1H); FABHRMS m/z calcd for $C_{18}H_{24}N_3O_6S$ $(M+H)^+$ 410.1387, found 410.1390.
17. Ohtake, N.; Yamada, K.; Mano, E.; Okamoto, O.; Ushijima, R.; Nakagawa, S. *J. Antibiot*. **1995**, *50*, 567.