2-Substituted Penems with Amino Acid-Related Side Chains: Synthesis and Antibacterial Activity of a New Series of β-Lactam Antibiotics

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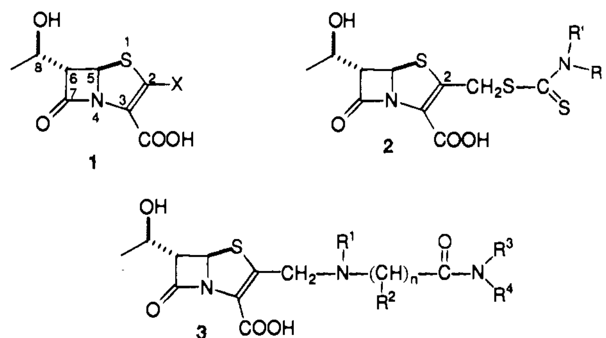
A new series of 6-(hydroxyethyl)penems 2-substituted with amino acid-related side chains was synthesized. The nature of the amino acyl derivative proved to be crucial both from a synthetic point of view, as β-lactam ring opening can compete with C-2 nucleophilic substitution, and for antibacterial activity. Primary amino acid amides emerged as the most suitable side chains for enhancing permeability through a Gram-negative outer membrane. *In vitro* activity of the new 2-[(aminoamido)methyl]penems **3a–u** was influenced by the nature and position of the amide moiety, the ring size for cyclic amides, and the configuration of the amino acid. Compounds bearing amides derived from small *N*-methyl amino acids (such as **3a**) or from cyclic amino acids (such as prolinamide **3p** and 4-hydroxyprolinamide **3r**) showed broad spectrum *in vitro* activity against both Gram-positive and Gram-negative microorganisms.

Introduction

The wide spectrum of activity of the new classes of β-lactam antibiotics,[1] associated with their very low toxicity levels, is still the basis of a continuous interest in the synthesis of new derivatives. Among the "nonclassic" β-lactams, penem antibiotics **1**,[2] first designed by the Woodward group[3] as a hybrid between penicillins and cephalosporins, retain the unique feature of being totally synthetic ones, lacking a natural counterpart. A number of penem derivatives, such as ritipenem,[4] furopenem,[5] and sulopenem,[6] are now in clinical trials, and some general structure–activity relationships have been defined. While the presence of a free carboxy group at C-3 and a 1(*R*)-hydroxyethyl group at C-6 proved to be crucial[2d] to ensure good activity levels and β-lactamase stability, a large differentiation is possible in the nature of the C-2 group.

Following our interest in the synthesis of 2-substituted penems,[7] we focused our attention on 2-CH_2Y derivatives (**1**, X = CH_2Y), in which a heteroatom Y is linked to the bicyclic skeleton through a methylene spacer. The presence of a heteroatom in the C-2 side chain has been related in the past[8] to an enhanced chemical reactivity of the β-lactam linkage and therefore to a higher biological activity. However, the synthesis of a series of sulfur-substituted penems, the dithiocarbamates **2**,[9] allowed us to obtain a number of compounds of only limited therapeutic interest; in fact, compounds **2** exhibited potent *in vitro* antibacterial activity against Gram-positive bacteria, including heterogeneous methicillin resistant *Staphylococcus aureus* strains, but their Gram-negative activity was considerably lower. In this series, the *N*-methyl glycinamido (sarcosinamido) derivative **2a** (R′ = CH_3, R″ = CH_2CONH_2) showed among other dithiocarbamate compounds one of the best antibacterial profiles. While other piperazino-substituted penem dithiocarbamates **2** actually displayed the best overall properties, results obtained with **2a** encouraged us to further explore the use of amino acid-derived side chains directly linked to the methylene spacer in position 2 through the amino acid nitrogen. We reasoned that the insertion, at the C-2 carbon atom on the penem skeleton, of amino acid-derived moieties as small polar groups should improve the penetration through the outer membrane of Gram-negative bacteria. In addition, natural and unnatural amino acids provide a large pool of side chains with tunable biological and chemical features; incorporation of amino acid-derived moieties in other series of β-lactam compounds has been used to improve the spectrum of activity[10] or pharmacokinetic properties, such as oral absorption.[11] In this account, we describe the synthesis, antibacterial activity, and structure–activity relationships of the new, amido-substituted, amino acid-derived penem derivatives **3**.[12]



Chemistry

The general synthetic strategy is summarized in Scheme 1. The 2-(hydroxymethyl)penem **4**[13] was trans-

† This paper is dedicated to the memory of Prof. Giuseppe Satta, who passed away on October 9, 1994.
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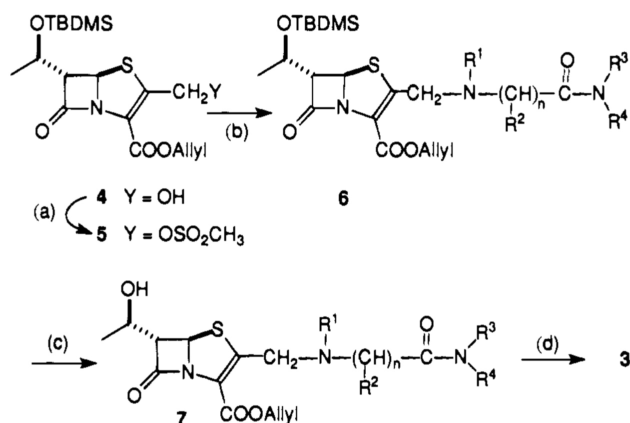
Scheme 1[a]



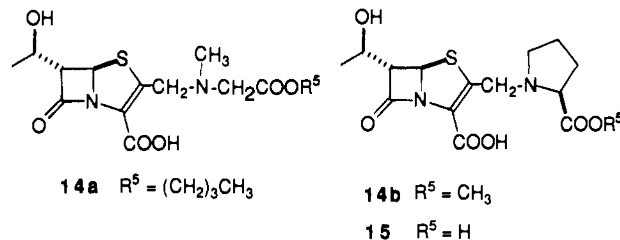
[a] (a) CH_3SO_2Cl, TEA, THF, 0 °C, 1 h; (b) $HNR^1(CHR^2)_nCONR^3R^4$, DMSO, room temperature, 20 h; (c) TBAF, AcOH, THF, room temperature, 24 h; (d) $Pd(PPh_3)_4$, PPh_3, AcOH, THF or ethyl acetate, 20–40 °C, 30 min.

formed into the corresponding mesylate **5**[7] and allowed to react with the amino acid derivative; deprotection at C-8 to give **7** and removal of the allyl ester moiety by palladium catalysis[14] followed by reverse phase column chromatography gave final compounds **3a–u**.

Amino acid-derived amides **8** (Scheme 2) were prepared by different methods (Tables 1 and 2); simple *N*-alkylglycine (**8a,b**) or N-alkyl-β-alanine (**8c**) derivatives were obtained by allowing the primary amine to react with chloro-substituted amides[15] (Method A). In the other cases, the N-protected amino acid was activated as its *N*-hydroxysuccinimido ester[16] (Method B) or acylisobutyl carbonate[17] (Method C) or transformed into the corresponding methyl ester (CH_3I, K_2CO_3, DMF (Method D) or Amberlyst 15,[18] CH_3OH (Method E)) and then allowed to react with the amine; final NH deprotection gave amino acid amides **8d–m** and **8r–u**. Synthetic work on amino acid amides included preparation of all four 4-hydroxyproline isomers **8r–u** from the corresponding *N*-Cbz amino acids **9r–u** (PG = Cbz); (4*S*)-isomers **9t** and **9u** were in turn obtained by inversion of the 4-hydroxy group of natural isomers **9r** and **9s**.[19]

Nucleophilic displacement by amine reagents on 2-[(mesyloxy)methyl]penem **5** can in principle suffer competition from β-lactam ring opening by the same reagent. In fact, when we allowed **5** to react with a primary nitrogen nucleophile (e.g. glycinamide), 5-methylthiazole **13** ($R^1 = R^2 = R^3 = R^4$ = H) was obtained[20] (Scheme 3) as the sole product.[22] Evidently, steric factors influence reaction outcome (Table 3); the use of a more hindered amide, such as leucinamide (entry 2) instead of glycinamide (entry 1), still allowed for recovery of some penem compound. However, nucleophilic substitution at C-2 became the prevalent reaction only with the use of *N*-alkyl (entries 3–12) or cyclic (entries 13–21) amino acid derivatives, bearing in any case a secondary nucleophilic nitrogen. Different alkyl substitutions on the nitrogen atom or α-substitutions on the amino acid slightly influenced the reaction outcome (compare entries 3 *vs* 4, 1 *vs* 2, and 3 *vs* 9), as did insertion of a different moiety on the carboxy group (entries 3 *vs* 10 and 11 and 14 *vs* 17). For α-substituted cyclic compounds (entries 13, 16, and 19), a larger ring size seemed to increase penem-coupling yields. The influence of steric hindrance on reaction outcome was also evident in the six-membered ring series, where α- and β-amino acid amides (entries 19 and 20) gave a more selective reaction than the γ-analogue (entry 21). Finally, amino acid configuration (entries 14–16) also showed some influence on the penem:thiazole ratio.

Some other penem syntheses were performed in order to ascertain the influence of the amino acid C-terminal group on antibacterial activity; sarcosine *n*-butyl ester **12a** (Scheme 2, $R^1 = CH_3$, R^2 = H, $R^5 = (CH_2)_3CH_3$, n = 1) and L-proline methyl ester **12b** (R^1 and $R^2 = (CH_2)_3$, $R^5 = CH_3$, n = 1) were coupled with penem mesylate **5** in the usual manner, giving, after deprotection, penem derivatives **14a** and **14b**. Enzymatic hydrolysis of ester moiety in **14b** (pig liver esterase, pH = 7 phosphate buffer, 36–48 h, 25 °C) gave in turn the free acid **15**.



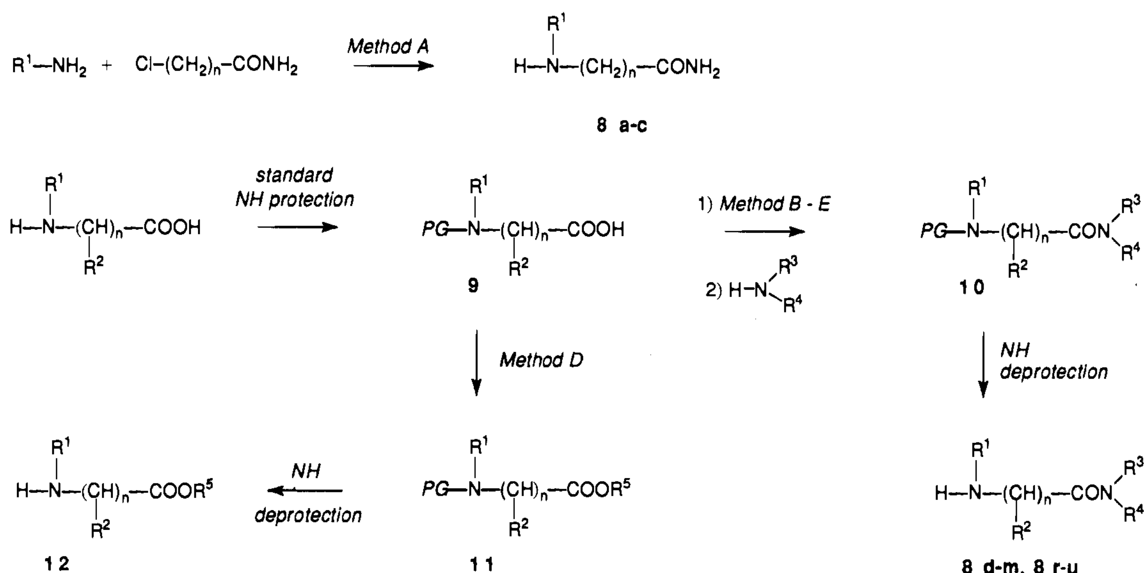
Finally, in order to determine the effect of the free hydroxy group in position 4 on the proline ring of **3r**, the corresponding acetyloxy (**17a**, Scheme 4) and carbamoyloxy (**17b**) derivatives were synthesized by allowing the protected penem **6r** to react directly with acetyl chloride or trichloroacetyl isocyanate and performing the ordinary deprotection steps.

Antibacterial Activity: Results and Discussion

Antibacterial activity of penem compounds **3a–u**, **14a,b**, **15**, and **17a,b** was first tested against a panel of standard and modified Gram-positive and Gram-negative strains (Tables 4 and 5). Tested microorganisms included Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) standard ATCC strains, methicillin resistant *S. aureus* strains, hyperpermeable β-lactamase producer and β-lactamase-lacking *E. coli* strains, and hyperpermeable and β-lactamase-lacking *P. aeruginosa* strains. We chose as standard compounds a known, highly active, broad spectrum penem compound (ritipenem[4]), the carbapenem imipenem,[24] as one of the most active broad spectrum β-lactam antibiotics now available, and the commonly used association ampicillin–sulbactam.

The advantages of the introduction of amino acid-derived side chains linked to the penem skeleton through the amino acid nitrogen were made evident at first by comparing the data for sarcosinamido derivative **3a** with the data for the corresponding sarcosinamido dithiocarbamate compound **2a**; while both compounds showed potent antistaphylococcal activity, the activity against the standard *E. coli* strain was considerably higher for the new derivative **3a**. In addition, the difference in minimum inhibitory concentrations between standard and hyperpermeable (Ec DC2) *E. coli* strains, previously shown for **2a**, and indicating in that case a reduced permeability through the outer Gram-negative bacterial membrane, was not observed for **3a**, supporting the hypothesis of enhanced permeability of the compound bearing the small, polar, sarcosinamide-

Scheme 2[a]



[a] Method A: (i) H_2O, 0–5 °C, 4–6 h; (ii) KOH, MeOH, 20 °C, 15 min. Method B: DCCI, HONSu, CH_2Cl_2, 5 °C, overnight. Method C: *i*-BuOCOCl, TEA, $CHCl_3$, 0 °C, 15 min. Method D: K_2CO_3, R_5I, DMF, room temperature, overnight. Method E: Amberlyst 15, CH_3OH, room temperature, 48 h. NH deprotection for *N*-Boc derivatives: HCl (1 M in ethyl acetate), 1 h, 20 °C. NH deprotection for *N*-Cbz derivatives: H_2, Pd/C (10%), CH_3OH, 1 h, room temperature.

Table 1. Preparation and ^1H NMR Data of Acyclic Amides **8a–k**

compound	R^1	R^2	R^3	R^4	n	config[a]	method	yield, (%)[b]	^1H NMR δ (ppm)
8a	CH_3	H	H	H	1	–	A	68	($CDCl_3$): 2.41 (3 H, s, CH_3), 3.20 (2 H, s, CH_2), 6.2 (1 H, br s, CONH), 7.0 (1 H, br s, CONH)
8b	C_2H_5	H	H	H	1	–	A	88	(D_2O): 1.00 (3 H, t, J = 7 Hz, CH_3), 2.51 (2 H, q, J = 7 Hz, CH_2CH_3), 3.23 (2 H, s, CH_2CO)
8c	CH_3	H	H	H	2	–	A	51	(D_2O):[c] 2.72 (3 H, s, CH_3), 2.72 (2 H, t, J = 7 Hz, CH_2), 3.27 (2 H, t, J = 7 Hz, CH_2)
8d	CH_3	CH_3	H	H	1	*S*	D	69	(DMSO-d_6):[d] 1.37 (3 H, d, J = 7.0 Hz, $CHCH_3$), 2.5 (3 H, s, NCH_3), 3.72 (1 H, br q, J = 7.1 Hz, $CHCH_3$), 7.6 (1 H, br s, CONH), 7.96 (1 H, br s, CONH), 8.86 (2 H, br d, NH_2)
8e	CH_3	CH_3	H	H	1	*R*	D	59	(DMSO-d_6): 1.08 (3 H, d, J = 6.9 Hz, $CHCH_3$), 2.19 (3 H, s, NCH_3), 2.86 (1 H, q, J = 6.9 Hz, $CHCH_3$), 6.92 (1 H, br s, CONH), 7.24 (1 H, br s, CONH)
8f	CH_3	CH_2OH	H	H	1	*S*	D	79	($CDCl_3$): 2.47 (3 H, s, CH_3), 3.10 (1 H, t, J = 5 Hz, CH), 3.82 (2 H, d, J = 5 Hz, CH_2), 5.45 (1 H, br s, CONH), 7.12 (1 H, br s, CONH)
8g	CH_3	CH_2Ph	H	H	1	*S*	C	82	(D_2O):[c] 2.68 (3 H, s, CH_3), 3.07–3.40 (2 H, m, CH_2), 4.04–4.21 (1 H, m, CH), 7.20–7.52 (5 H, m, ArH)
8h	CH_3	H	H	CH_2CONH_2	1	–	B	31	(D_2O): 2.33 (3 H, s, CH_3), 3.35 (2 H, s, CH_2), 3.91 (2 H, s, CH_2)
8i	CH_3	H	CH_3	CH_3	1	–	B	49	($CDCl_3$): 2.45 (3 H, s, NCH_3), 2.95 (3 H, s, $CONCH_3$), 2.96 (3 H, s, $CONCH_3$)
8j	CH_3	H	$(CH_2)_2N(CH_3)(CH_2)_2$		1	–	B	66	($CDCl_3$): 2.27 (3 H, s, CH_3), 2.36 (4 H, br, 2 × piperazine CH_2), 2.44 (3 H, s, CH_3), 3.20–3.55 (4 H, br, CH_2 sarcosine overlapped to piperazine CH_2), 3.55–3.72 (2 H, m, piperazine CH_2)
8k	CH_2CONH_2	H	H	H	1	–	D	58	(D_2O): 3.34 (4 H, s, 2 × CH_2)[e]

[a] Amino acid configuration. [b] Methods B–D: overall yield (three steps) from N-protected amino acid **9**. [c] Recorded on isolated hydrochloride. [d] Recorded on isolated trifluoroacetate. [e] ^{13}C NMR (D_2O): δ 54.8 (CH_2), 180.9 (C=O).

derived side chain. On the other hand, the importance of the terminal amido moiety was evident from the comparison of **3a** with the ester derivative **14a**; the latter compound completely lost Gram-negative activity, with a marked decrease also in Gram-positive values.

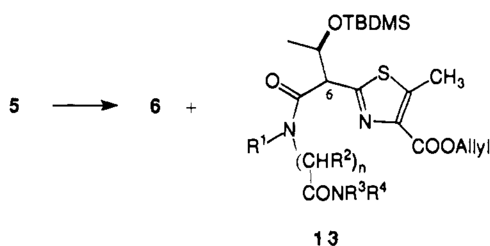
All penem compounds **3a–k** (Table 4) bearing noncyclic, amido-substituted, amino acid-derived side chains showed high Gram-positive activity, equal to the standard penem ritipenem and reaching in some cases the imipenem level. The results on Gram-negative microorganisms were however different within the series, allowing for some structure–activity considerations. Taking the *E. coli* data into consideration, the best results were obtained with amido side chains derived from small, polar amino acids, such as sarcosine (**3a**), (*S*)- and (*R*)-*N*-methylalanine (**3d** and **3e**), and (*S*)-*N*-methylserine (**3f**). In all these cases, the MIC values were in the range between the ritipenem and imipenem values. When different stereoisomers were tested, such as for **3d** and **3e**, no significant differences were noted in the results. Side chain homologation, such as for the *N*-ethyl derivative **3b** or the β-amino acid **3c**, did not

Table 2. Preparation and ^{1}H NMR Data of Cyclic Amides **8l,m** and **8r–u**

compound	$R^1NH-(CHR^2)_n-CONR^3R^4$	config[a]	method	yield (%)[b]	^{1}H NMR δ (ppm)
8l	H–N, CONH$_2$	*R,S* mixture	c	82[d]	(DMSO-d_6): 0.98 (1 H, br s, NH), 1.61 (2 H, br s), 2.22–2.44 (1 H, m), 6.70–8.30 (2 H, br, CONH$_2$)
8m	N, CONH$_2$, H	*R,S* mixture	C	45	(DMSO-d_6): 0.85–0.92 (1 H, m), 1.22–1.55 (4 H, m), 1.55–1.90 (2 H, m), 2.45–2.60 (1 H, m), 2.85–3.06 (2 H, m), 6.95 (1 H, br s, CONH), 7.14 (1 H, br s, CONH)
8r	OH, H–N, CONH$_2$	2*S*,4*R*	D,E	63	(D_2O): 1.98–2.18 (1 H, m), 2.28–2.46 (1 H, m), 3.22–3.49 (2 H, m), 4.27 (1 H, dd, *J* = 8, 10 Hz), 4.52–4.64 (1 H, m)
8s	OH, H–N, CONH$_2$	2*R*,4*R*	D,E	65	(D_2O): 1.74–1.92 (1 H, m), 2.28–2.50 (1 H, m), 2.85–3.05 (2 H, m), 3.77 (1 H, dd, *J* = 5, 10 Hz), 4.25–4.45 (1 H, m)
8t	OH, H–N, CONH$_2$	2*S*,4*S*	D,E	55	(D_2O): 1.80–2.15 (1 H, m), 2.32–2.58 (1 H, m), 2.98–3.22 (2 H, m), 3.98 (1 H, dd, *J* = 4, 11 Hz), 4.36–4.55 (1 H, m)
8u	OH, H–N, CONH$_2$	2*R*,4*S*	D,E	49	(D_2O): 1.82–2.04 (1 H, m), 2.05–2.23 (1 H, m), 2.82–3.16 (2 H, m), 3.91–4.04 (1 H, m), 4.38–4.52 (1 H, m)

[a] Amino acid configuration. [b] Overall yield (three steps) from N-protected amino acid **9**. [c] Prepared directly from the corresponding methyl ester.[23] [d] From methyl aziridine-2-carboxylic acid.

Scheme 3



improve activity, while a marked decrease of activity was noted when a bulkier, aromatic phenylalanine residue (**3g**) was inserted. Primary amides showed better results than substituted ones (**3a** *vs* **3i** and **3j**). Insertion of a second amido group such as in **3h** or **3k** did not improve activity, although MIC values remained at a good level against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) strains. Finally, all tested penems **3a–k** did not show activity against *P. aeruginosa*.

Antibacterial activity of penem compounds bearing cyclic, amino acid-derived substituents (Table 5) showed some difference according to ring size, best results being obtained with five-membered, α-amido-substituted rings (compare **3p** and **3q** with three-membered **3l** and six-membered **3m**); however, in the six-membered series, the α-, β-, and γ-substituted derivatives **3m–o** gave similar results for potency and spectrum of activity. It is really interesting to note that configuration at the amino acid α-carbon (**3p** and **3q**) and at the additional chiral center of 4-hydroxyprolinamido derivatives **3r–u** strongly influenced inhibitory activity against *E. coli* and, to a lesser extent, against other bacterial strains. In fact, the ratio between the minimum inhibitory concentrations against *E. coli* for the (*S*)-isomer and the (*R*)-isomer was 0.06:1 for the **3p/3q** couple and 0.015:1 for **3r/3s**, the (*S*)-prolinamido derivative **3p** and the

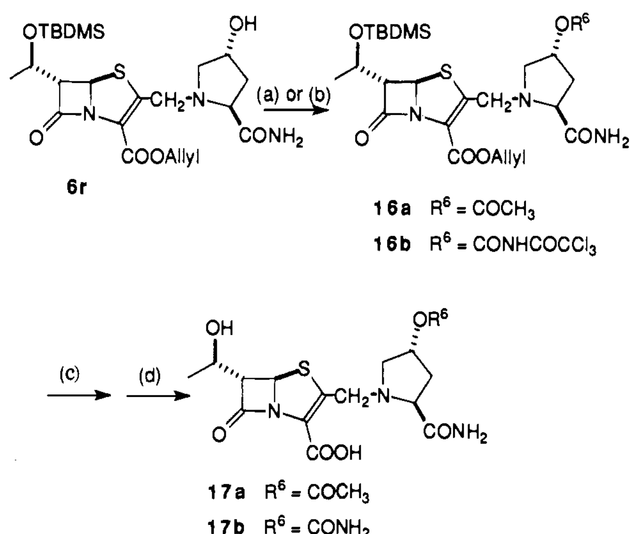
Table 3. Nucleophilic Displacement *vs* β-Lactam Ring Opening in the Reaction of Mesylate **5** with Amino Acid-Derived Compounds

entry	amino acid derivative[a]	yield (%)[b]	penem **6**: thiazole **13**[c]
1	$H_2NCH_2CONH_2$	82	0:100
2	$H_2NCH(CH_2CH(CH_3)_2)CONH_2$	35	10:90
3	**8a**	74	85:15
4	**8b**	45	93:7
5	**8c**	43	95:5
6	**8d**	56	>98:2
7	**8e**	51	>98:2
8	**8f**	39	>98:2
9	**8g**	45	>98:2
10	**8i**	68	>98:2
11	**8j**	49	97:3
12	**8k**	44	>98:2
13	**8l**	24	nd[d]
14	H-L-Pro-NH_2 (**8p**)	76	75:25
15	H-D-Pro-NH_2 (**8q**)	69	95:5
16	H-DL-Pro-NH_2	72	85:15
17	H-L-Pro-OCH_3 (**12b**)	80	>98:2
18	**8r**	61	95:5
19	H-DL-Pip-(α-$CONH_2$) (**8m**)	70	>98:2
20	H-DL-Pip-(β-$CONH_2$) (**8n**)	84	>98:2
21	H-DL-Pip-(γ-$CONH_2$) (**8o**)	70	68:32

[a] Pro = proline; Pip = piperidine. [b] Total isolated yield of penem **6** and thiazole **13** derivatives after column chromatography (silica gel). [c] Calculated on ^{1}H NMR spectra of the crude reaction product. Values agreed with yields of isolated products. [d] nd = not detected.

(2*S*,4*R*)-4-hydroxyprolinamido derivative **3r** being by far the most active against *E. coli* among all of the tested compounds, including ritipenem and imipenem. On the other hand, the (*R*)-isomers **3q** and **3s** were the only ones to show a minimum of antipseudomonal activity, while all other derivatives were completely inactive. Taking into consideration the cyclic series on the whole, the (*S*)-prolinamido derivative **3p** and the (2*S*,4*R*)-4-hydroxyprolinamido derivative **3r** can be regarded as the best compounds, showing, when compared with the

Scheme 4[a]



[a] (a) CH_3COCl, py, CH_2Cl_2, 2 h, room temperature; (b) $Cl_3CCONCO$, anhydrous THF, 0 °C, 10 min; (c) TBAF, AcOH, THF, room temperature, 24 h; (d) $Pd(PPh_3)_4$, PPh_3, AcOH, THF or ethyl acetate, room temperature, 30 min.

corresponding isomers **3q** and **3s–u**, a better balance between Gram-positive and Gram-negative activity. Finally, tests on ester **14b** and carboxylic acid **15** confirmed the decrease in antibacterial potency in the absence of the amido moiety yet noted for the noncyclic series. Moreover, data for **17a** and **17b**, in which the hydroxy substituent in position 4 on the proline ring was masked as its acetyloxy or carbamoyloxy derivative, allowed us to point out the importance of the free hydroxy group for antibacterial activity.

A more detailed investigation of the significance of synthesized compounds as potent, broad spectrum antibacterial agents was done for the most promising ones. On the basis of the previous tests, we selected in the noncyclic series (Table 4) the sarcosinamido derivative **3a** and the (*S*)-*N*-methylalaninamido derivative **3d**; in the cyclic series (Table 5), the (*S*)-prolinamido derivative **3p** and the (2*S*,4*R*)-4-hydroxyprolinamido derivative **3r** appeared to be most interesting ones. We also included the (*R*)-prolinamido derivative **3q** in order to allow for a broader comparison between the two optical isomers **3p** and **3q** and to check, on a major number of strains, the real significance of unexpected, although very low, antipseudomonal activity of the latter compound.

Minimum inhibitory concentration values for **3a**, **3d**, and **3p–r** against over 500 clinical isolates are reported in Table 6 as MIC_{90} against different species. Tested microorganisms included Gram-positive, Gram-negative, aerobic, and anaerobic strains. All tested compounds exhibited potent activity against Staphylococci and Streptococci; among them, **3a** distinguished itself for its potency, which was quite constantly superior to imipenem, including the case of methicillin resistant Staphylococci. Against other Gram-positive species, tested compounds confirmed their broad spectrum activity, being however somewhat inferior to imipenem. All penem derivatives tested exhibited high activity against Gram-negative species, with **3a**, **3p**, and **3r** showing the best overall properties. In particular, **3a** was constantly more potent than ampicillin–sulbactam and amoxicillin, reaching imipenem MIC_{90} values against *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Escherichia coli*, *Yersinia* spp., *Proteus* spp., and *Providencia* spp. Especially noteworthy is the activity against *Enterobacter*, generally considered a highly resistant nosocomial pathogen. Among the other compounds, **3r** distinguishes itself for the value of *E. coli* activity (MIC_{90} was 10 times superior to imipenem), while of the two prolinamido isomers, **3p** was far superior to **3q**. The activity of the latter against standard *P. aeruginosa* was not confirmed on a major number of clinical isolates.[25]

In conclusion, we have carried out the synthesis of a new series of 2-substituted penems with amino acid-related side chains. Amido substitution on an amino acid allowed us to obtain a number of highly active, broad spectrum antibacterial agents, especially when small polar amino acids were used. Amino acid configuration markedly influenced antibacterial activity, as did the nature and position of amide moiety and ring size for cyclic amides.

Further evaluations are now in progress in order to establish the *in vivo* efficacy of the new compounds.

Experimental Section

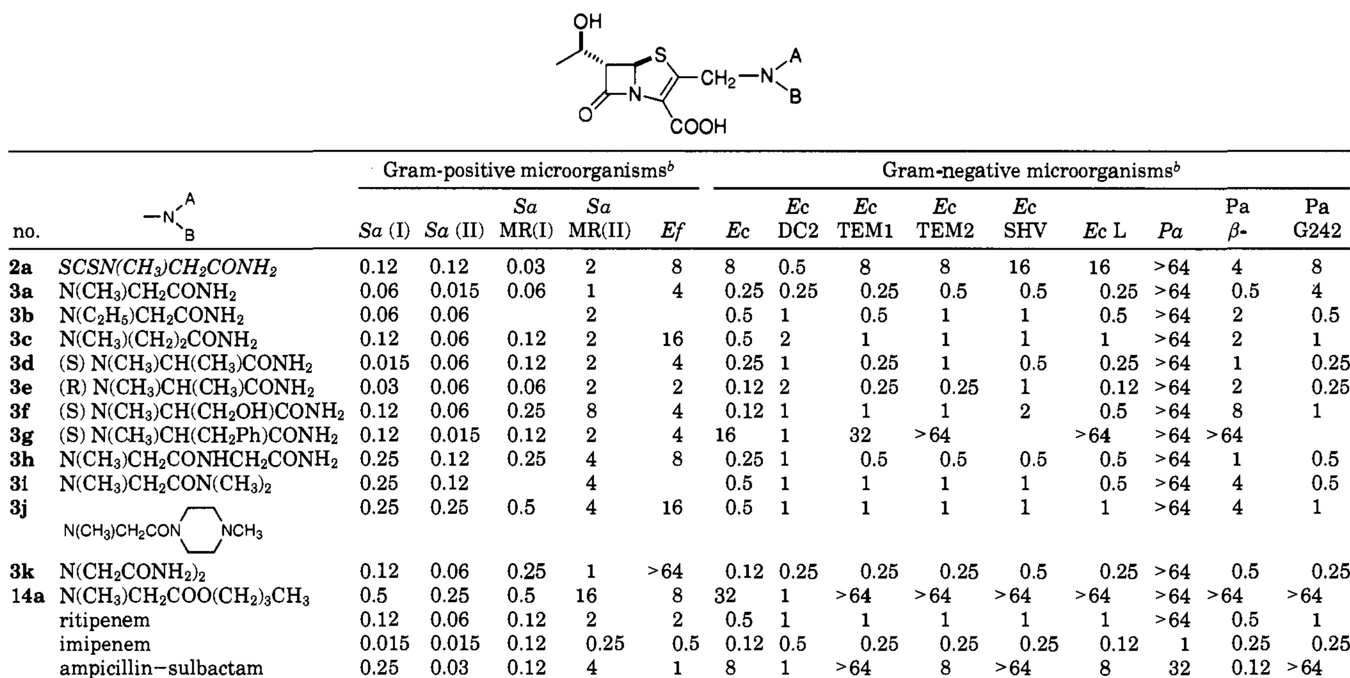
Pig liver esterase (PLE, EC 3.1.1.1, 176 U/mg) was purchased from Fluka Chemie AG as a suspension in a 3.2 M ammonium sulfate solution. Enzymic hydrolysis was performed using a Metrohm pH-stat. Column chromatography was performed using E. Merck Kieselgel 60 (70–230 mesh). Reverse phase column chromatography was carried out on Matrex silica C18 (Amicon Co.). Preparative HPLC separations were performed on a Shimadzu LC-10A liquid chromatograph using a 10 μm C_{18} Hypersil 10 ODS column (20 mm id × 250 mm), eluting at a flow rate of 20 mL/min with 95:5 v/v water/acetonitrile (UV monitoring at 220 and 320 nm).

Analytical HPLC and UV analyses were performed with a Perkin-Elmer Analyst liquid chromatograph equipped with a Perkin-Elmer 235 diode array detector and workstation. HPLC assay of final compounds **3a–u** was performed with a 5 μm C_{18} Hypersil 5 ODS column (4.6 mm id × 250 mm), eluting at a flow rate of 1.0 mL/min with a 95:5 v/v mixture of 0.02 M phosphate buffer (pH = 3) and acetonitrile (UV monitoring at 220 and 320 nm), except where otherwise indicated.

NMR spectra were obtained using a Varian Gemini 200 spectrometer at 200 and 50 MHz for ^{1}H and ^{13}C spectra, respectively. FAB MS spectra were recorded on a VG Quattro mass spectrometer (Fisons Instruments, Altrincham, U.K.), equipped with a FAB ion source. Ionization was achieved using a cesium gun (8 keV, 2.3 A), and a 1:1 mixture of glycerol–thioglycerol was used as the matrix compound. ES MS spectra were recorded on the VG Quattro mass spectrometer equipped with standard Electrospray ion source operated in the positive ion mode at a capillary voltage of 4.5 kV with nitrogen as the nebulizing gas at a flow of 20 L/h. Ten milliliters of sample solution (about 10 mg/mL in methanol) was injected *via* a Rheodyne 7125 valve. The mobile phase was a 1:1 mixture (v/v) of acetonitrile and water and was delivered by a Waters MS 600 LC pump (Waters, Milford, MA) at a flow of 10 mL/min. TS MS spectra were recorded on a HP 5988A mass spectrometer (Hewlett-Packard, Palo Alto, CA) equipped with a standard Thermospray ion source in the positive ion mode with the filament on. The source and the stem temperatures were 276 and 90 °C, respectively. Ten milliliters of sample solution (about 100 mg/mL in methanol) was injected *via* a Rheodyne 7125 valve. The mobile phase was a 75:25 v/v mixture of 0.1 M ammonium acetate and methanol and was delivered by a HP 1050 LC pump at a flow of 1 mL/min.

General Procedure for the Synthesis of 2-(Nitrogen-substituted methyl)penems (6). Amino acid amide **8** (5

Table 4. *In Vitro* Antibacterial Activity[a] (Minimum Inhibitory Concentration, μg/mL) of Penem Compounds (**3a–k** and **14a**) Bearing Noncyclic, Amino Acid-Derived Side Chains



no.	$-N(A)(B)$	Gram-positive microorganisms[b]					Gram-negative microorganisms[b]								
		Sa (I)	*Sa* (II)	*Sa* MR(I)	*Sa* MR(II)	*Ef*	*Ec*	*Ec* DC2	*Ec* TEM1	*Ec* TEM2	*Ec* SHV	*Ec* L	*Pa*	Pa β-	Pa G242
2a	*$SCSN(CH_3)CH_2CONH_2$*	0.12	0.12	0.03	2	8	8	0.5	8	8	16	16	>64	4	8
3a	$N(CH_3)CH_2CONH_2$	0.06	0.015	0.06	1	4	0.25	0.25	0.25	0.5	0.5	0.25	>64	0.5	4
3b	$N(C_2H_5)CH_2CONH_2$	0.06	0.06		2		0.5	1	0.5	1	1	0.5	>64	2	0.5
3c	$N(CH_3)(CH_2)_2CONH_2$	0.12	0.06	0.12	2	16	0.5	2	1	1	1	1	>64	2	1
3d	(S) $N(CH_3)CH(CH_3)CONH_2$	0.015	0.06	0.12	2	4	0.25	1	0.25	1	0.5	0.25	>64	1	0.25
3e	(R) $N(CH_3)CH(CH_3)CONH_2$	0.03	0.06	0.06	2	2	0.12	2	0.25	0.25	1	0.12	>64	2	0.25
3f	(S) $N(CH_3)CH(CH_2OH)CONH_2$	0.12	0.06	0.25	8	4	0.12	1	1	1	2	0.5	>64	8	1
3g	(S) $N(CH_3)CH(CH_2Ph)CONH_2$	0.12	0.015	0.12	2	4	16	1	32	>64		>64	>64	>64	
3h	$N(CH_3)CH_2CONHCH_2CONH_2$	0.25	0.12	0.25	4	8	0.25	1	0.5	0.5	0.5	0.5	>64	1	0.5
3i	$N(CH_3)CH_2CON(CH_3)_2$	0.25	0.12		4		0.5	1	1	1	1	0.5	>64	4	0.5
3j	$N(CH_3)CH_2CON$ (piperazine) NCH_3	0.25	0.25	0.5	4	16	0.5	1	1	1	1	1	>64	4	1
3k	$N(CH_2CONH_2)_2$	0.12	0.06	0.25	1	>64	0.12	0.25	0.25	0.25	0.5	0.25	>64	0.5	0.25
14a	$N(CH_3)CH_2COO(CH_2)_3CH_3$	0.5	0.25	0.5	16	8	32	1	>64	>64	>64	>64	>64	>64	>64
	ritipenem	0.12	0.06	0.12	2	2	0.5	1	1	1	1	1	>64	0.5	1
	imipenem	0.015	0.015	0.12	0.25	0.5	0.12	0.5	0.25	0.25	0.25	0.12	1	0.25	0.25
	ampicillin–sulbactam	0.25	0.03	0.12	4	1	8	1	>64	8	>64	8	32	0.12	>64

[a] Minimum inhibitory concentrations (MIC) determined in Mueller Hinton 2 Medium, bioMerieux. Inoculum: 10^4 cells/mL. Incubation: 24 h at 37 °C. [b] Microorganism: *Sa* (I), *S. aureus* ATCC 29213; *Sa* (II), *S. aureus* ATCC 25923; *Sa* MR(I), heterogeneous methicillin resistant *S. aureus*; *Sa* MR(II), homogeneous methicillin resistant *S. aureus*; *Ef*, *E. facecalis* ATCC 29212; *Ec*, *E. coli* ATCC 25922; *Ec* DC2, hyperpermeable *E. coli* strain; *Ec* TEM1, *Ec* TEM2, and *Ec* SHV, *E. coli* strains producting known β-lactamases; *Ec* L, β-lactamase-lacking strain; *Pa*, *P. aeuruginosa* ATCC 27853; *Pa* β-, β-lactamase-lacking hyperpermeable *P. aeruginosa* strain; *Pa* G242, hyperpermeable *P. aeruginosa* strain.

mmol) dissolved in DMSO (10 mL) was added under stirring, at room temperature, to a solution of freshly prepared[7] allyl (5*R*,6*S*)-2-[[(methylsulfonyl)oxy]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (**5**) (2.17 g, 4.54 mmol) in DMSO (50 mL). Triethylamine (0.70 mL, 5 mmol) was added to the mixture.[26] The resulting solution was stirred for about 20 h and then poured into ice (200 mL). The mixture was allowed to warm to room temperature and extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with water (2 × 100 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated *in vacuo* at 30 °C. This procedure allowed for the preparation of the following compounds.

Allyl (5*R*,6*S*)-2-[[*N*-(2-Acetamido)-*N*-methylamino]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6a). Yield: 63% after crystallization from 5:1 v/v *n*-hexane/cyclohexane. White solid. Mp: 118–119 °C. ^1H NMR ($CDCl_3$): δ 0.07 (6 H, s), 0.87 (9 H, s), 1.24 (3 H, d, *J* = 6.2 Hz), 2.36 (3 H, s), 3.09 (2 H, s), 3.68 (1 H, dd, *J* = 1.6, 4.5 Hz), 3.76 and 3.88 (2 H, ABq, *J* = 16 Hz), 4.17–4.31 (1 H, m), 4.57–4.58 (2 H, m), 5.18–5.48 (2 H, m), 5.55 (1 H, d, *J* = 1.6 Hz), 5.70 (1 H, br s), 5.75–6.00 (1 H, m), 6.90 (1 H, br s).

Allyl (5*R*,6*S*)-2-[[*N*-(2-Acetamido)-*N*-ethylamino]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6b). Yield: 42% after column chromatography (3:1 v/v ethyl acetate/cyclohexane). White solid. ^1H NMR ($CDCl_3$): δ 0.06 (6 H, s), 0.87 (9 H, s), 1.07 (3 H, t, *J* = 7 Hz), 1.23 (3 H, d, *J* = 6 Hz), 2.59 (2 H, q, *J* = 7 Hz), 3.10 (3 H, s), 3.67 (1 H, dd, *J* = 2.6 Hz), 3.84 (2 H, s), 4.12–4.32 (1 H, m), 4.56–4.78 (2 H, m), 5.17–5.46 (2 H, m), 5.53 (1 H, d, *J* = 2 Hz), 5.78–6.02 (1 H, m), 5.95 (1 H, br s), 6.91 (1 H, br s).

Allyl (5*R*,6*S*)-2-[[*N*-Methyl-*N*-(3-propionamido)amino]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6c). Yield: 41% after column chromatography (ethyl acetate). Pale yellow solid. ^1H NMR ($CDCl_3$): δ 0.07 (6 H, s), 0.88 (9 H, s), 1.23 (3 H, d, *J* = 6 Hz), 2.29 (3 H, s), 2.40 (2 H, t, *J* = 6 Hz), 2.74 (2 H, t, *J* = 6 Hz), 3.64–3.86 (4 H, dd overlapped to AB_q lines, *J* not detectable), 4.08–4.28 (1 H, m), 4.56–4.87 (2 H, m), 5.15–5.50 (2 H, m), 5.58 (1 H, d, *J* = 2 Hz), 5.82–6.08 (1 H, m), 5.92 (1 H, br s), 7.32 (1 H, br s).

Allyl (5*R*,6*S*)-2-[[*N*-Methyl-*N*-(2*S*)-(2-propionamido)amino]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6d). Yield: 56% after column chromatography (3:2 v/v ethyl acetate/cyclohexane). Yellow solid foam. ^1H NMR ($CDCl_3$): δ 0.07 (6 H, s), 0.87 (9 H, s), 1.24 (6 H, two d, *J* = 6.2, 7.0 Hz), 2.29 (3 H, s), 3.30 (1 H, q, *J* = 7.0 Hz), 3.67 (1 H, dd, *J* = 1.6, 4.6 Hz), 3.68 and 3.93 (2 H, ABq, *J* = 16 Hz), 4.23 (1 H, dq, *J* = 4.7, 6.3 Hz), 4.58–4.78 (2 H, m), 5.21–5.44 (2 H, m), 5.54 (1 H, d, *J* = 1.6 Hz), 5.68 (1 H, br s), 5.83–6.03 (1 H, m), 6.94 (1 H, br s).

Allyl (5*R*,6*S*)-2-[[*N*-Methyl-*N*-(2*R*)-(2-propionamido)amino]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6e). Yield: 51% after column chromatography (7:3 v/v ethyl acetate/cyclohexane). Yellow solid foam. ^1H NMR ($CDCl_3$): δ 0.07 (6 H, s), 0.89 (9 H, s), 1.24 (6 H, two d, *J* = 6.2, 7.0 Hz), 2.28 (3 H, s), 3.31 (1 H, q, *J* = 7.0 Hz), 3.67 (1 H, dd, *J* = 1.6, 4.6 Hz), 3.69 and 3.94 (2 H, ABq, *J* = 16 Hz), 4.24 (1 H, dq, *J* = 4.6, 6.2 Hz), 4.58–4.78 (2 H, m), 5.21–5.43 (2 H, m), 5.48 (1 H, br s), 5.54 (1 H, d, *J* = 1.6 Hz), 5.82–6.02 (1 H, m), 6.94 (1 H, br s).

Allyl (5*R*,6*S*)-2-[[*N*-Methyl-*N*-(2*S*)-(3-hydroxy-2-propionamido)amino]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6f). Yield: 39% after column chromatography (ethyl acetate). Orange solid foam. ^1H NMR ($CDCl_3$): δ 0.03 (6 H, s), 0.84 (9 H, s), 1.20 (3 H, d, *J* = 6.2 Hz), 2.37 (3 H, s), 2.95–3.50 (1 H, br), 3.22–3.32 (1 H, m), 3.65 (1 H, dd, *J* = 1.6, 4.4 Hz), 3.74–4.10 (4 H, m), 4.10–4.28 (1 H, m), 4.52–4.76 (2 H, m), 5.14–5.42 (2 H, m), 5.51 (1 H, d, *J* = 1.6 Hz), 6.53 (1 H, br), 6.98 (1 H, br).

Allyl (5*R*,6*S*)-2-[[*N*-Methyl-*N*-(2*S*)-(3-phenyl-2-propionamido)amino]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6g). Yield: 45% after column chromatography (3:1 v/v ethyl acetate/cyclohexane). White solid. ^1H NMR ($CDCl_3$): δ 0.07 (6 H, s), 0.88 (9 H, s), 1.23 (3 H, d, *J* = 6.2 Hz), 2.37 (3 H, s), 2.86 (1 H, four lines, part of AMX spin system, *J* = 6.5, 14 Hz), 3.29 (1 H, four lines, part of AMX spin system, *J* = 6.5, 14 Hz), 3.51 (1 H, three lines, part of AMX spin system, *J* = 6.5, 6.5 Hz), 3.64

Table 5. *In Vitro* Antibacterial Activity[a] (Minimum Inhibitory Concentration, μg/mL) of Penem Compounds (**3l–u**, **14b**, **15**, and **17a,b**) Bearing Cyclic, Amino Acid-Derived Side chains

OH S CH_2–N A B N O COOH

		Gram-positive microorganisms[b]					Gram-negative microorganisms[b]								
no.	–N(A)(B)	*Sa* (I)	*Sa* (II)	*Sa* MR(I)	*Sa* MR(II)	*Ef*	*Ec*	*Ec* DC2	*Ec* TEM1	*Ec* TEM2	*Ec* SHV	*Ec* L	*Pa*	*Pa* β-	*Pa* G242
3l	CONH$_2$ [c]	0.5	0.25	0.25	8	16	2	4	2	2	4	4	>64	1	2
3m	CONH$_2$ [c]	0.12	0.06	0.12	2	16	1	1	1	2	2	1	>64	4	1
3n	CONH$_2$ [c]	0.06	0.06	0.12	2	32	4	4	4	4	4	8	>64	1	4
3o	–CONH$_2$ [c]	0.06	0.015	0.12	2	16	2	2	2	2	2	2	>64	1	2
3p	CONH$_2$	0.12	0.015	0.5	2	2	0.06	0.25	0.12	0.5	0.25	0.12	>64	1	1
3q	CONH$_2$	0.06	0.015	0.12	4	16	1	0.5	1	1	1	1	32	0.25	1
3r	OH, CONH$_2$	0.12	0.12	0.25	8	2	0.03	0.12	0.12	0.25	0.25	0.03	>64	0.25	0.06
3s	OH, CONH$_2$	0.25	0.25	0.25	8	32	2	2	2	2	2	2	64	0.5	2
3t	OH, CONH$_2$	0.25	0.12	0.25	8	16	0.5	2	1	2	2	2	>64	8	1
3u	OH, CONH$_2$	0.12	0.06	0.25	16	32	1	1	1	1	1	1	>64	0.5	1
14b	COOCH$_3$	0.25	0.12	0.25	4	8	4	2	8	8	8	4	>64	4	8
15	COOH	0.03	0.5	1	32	>64	2	16	8	32	8	8	>64	32	4
17a	OCONH$_2$, CONH$_2$	0.5	0.5	0.5	16	32	0.25	2	2	1	1	1	>64	8	16
17b	OCOCH$_3$, CONH$_2$	0.25	0.25	0.5	16	2	1	4	2	32	32	1	>64	16	16
	ritipenem	0.12	0.06	0.12	2	2	0.5	1	1	1	1	1	>64	0.5	1
	imipenem	0.015	0.015	0.12	0.25	0.5	0.12	0.5	0.25	0.25	0.25	0.12	1	0.25	0.25
	ampicillin–sulbactam	0.25	0.03	0.12	4	1	8	1	>64	8	>64	8	32	0.12	>64

[a] Minimum inhibitory concentrations (MIC) determined in Mueller Hinton 2 Medium, bioMerieux. Inoculum: 10^4 cells/mL. Incubation: 24 h at 37 °C. [b] Microorganism: *Sa* (I), *S. aureus* ATCC 29213; *Sa* (II), *S. aureus* ATCC 25923; *Sa* MR(I), heterogeneous methicillin resistant *S. aureus*; *Sa* MR(II), homogeneous methicillin resistant *S. aureus*; *Ef*, *E. faecalis* ATCC 29212; *Ec*, *E. coli* ATCC 25922; *Ec* DC2, hyperpermeable *E. coli* strain; *Ec* TEM1, *Ec* TEM2, and *Ec* SHV, *E. coli* strains producing known β-lactamases; *Ec* L, β-lactamase-lacking strain; *Pa*, *P. aeruginosa* ATCC 27853; *Pa* β-, β-lactamase-lacking hyperpermeable *P. aeruginosa* strain; *Pa* G242, hyperpermeable *P. aeruginosa* strain. [c] Data refer to *R* and *S* isomer mixture. The mixture could be separated by preparative HPLC (see the Experimental Section). Microbiological tests on separated compounds did not show any significant difference between the two isomers.

Table 6. *In Vitro* Antibacterial Activity (MIC$_{90}$)[a] of Penem Derivatives against Clinical Isolates[b]

microorganism (no. of strains)	MIC$_{90}$ (μg/mL) 3a	3d	3p	3q	3r	IMI	AMP-SUL	AMX
Staphylococcus aureus MS (6)[c]	0.0075	0.12	0.06	0.06	0.12	0.015	1	1
Staphylococcus aureus MR (20)[c]	4	8	8	8	>32	>32	32	>32
Staphylococcus epidermidis (13)[c]	0.015	0.25	0.12	0.12	1	0.25	4	4
Streptococcus pyogenes A (31)[d]	0.06	0.06	0.06	0.5	0.03	0.06	0.03	0.015
Streptococcus pneumoniae (23)[d]	0.06	0.06	0.06	0.5	0.03	0.06	0.03	0.015
Streptococcus agalactiae B (21)	0.06	0.06	0.03	0.12	0.06	0.015	0.06	0.06
Clostridium perfringens (27)	0.5	0.5	1	1	0.25	0.03	0.12	0.06
Enterococcus faecalis (47)	8	8	8	32	8	2	2	0.5
Enterococcus faecium (47)	>64	>64	>64	>64	>64	>64	>64	32
Listeria monocytogenes (20)	0.5	0.5	0.5	2	0.12	0.03	0.5	0.5
Branhamella catarrhalis (27)	0.25	0.5	0.25	2	1	0.03	0.5	2
Haemophilus influenzae (21)[e]	2	4	4	2	4	1	1	1
Bacteroides fragilis (15)	2	8	16	16	32	0.25	4	32
Aeromonas spp. (21)	1	2	0.5	0.25	2	0.06	>32	>32
Klebsiella pneumoniae (20)	0.5	1	0.5	1	0.5	0.25	16	>64
Acinetobacter anitratus (20)	16	32	16	32	16	0.5	32	nt[f]
Pseudomonas aeruginosa (11)	>64	>64	>64	>64	>64	8	>64	nt
Xanthomonas maltophilia (9)	>64	>64	>64	>64	>64	>64	>64	nt
Enterobacter aerogenes (19)	8	32	16	16	16	4	>64	nt
Escherichia coli (21)	0.25	0.25	0.12	1	0.015	0.12	8	nt
Citrobacter freundii (20)	1	1	0.5	1	1	0.5	>64	nt
Yersinia spp. (20)	0.5	0.5	0.25	1	0.15	0.25	32	>64
Proteus mirabilis (16)	1	nt	4	nt	4	2	32	nt
Proteus vulgaris (10)	2	nt	4	nt	4	2	8	nt
Providencia rettgeri (10)	2	nt	4	nt	4	1	32	nt
Providencia stuartii (10)	2	nt	4	nt	4	2	64	nt
Morganella morganii (10)	2	nt	4	nt	8	2	16	nt

[a] Minimum inhibitory concentration against 90% of tested clinical isolates. Experimental reference compounds: imipenem (IMI), ampicillin–sulbactam (AMP-SUL), and amoxicillin (AMX). [b] MICs determined in Mueller Hinton 2 Medium, bioMerieux (aerobes), inoculum of 10^4 cells/mL, incubation for 24 h at 37 °C. MICs determined in Wilkins-Chalgren Agar, Oxoid (anaerobes), inoculum of 10^5 cells/mL, incubation for 24–48 h at 37 °C. [c] MICs determined in Mueller Hinton 2 + 5% NaCl, inoculum of 10^5 cells/mL, incubation for 48 h at 25 °C. [d] MICs determined in Tryptone Soya Agar, Oxoid + 1% Supplement B, Bacto; inoculum of 10^4 cells/mL, incubation for 24–48 h at 37 °C. [e] MICs determined in Haemophilus Test Medium Base, Oxoid + Haemophilus Test Medium Supplement, Oxoid; inoculum of 10^4 cells/mL, incubation for 24–48 h at 37 °C. [f] nt = not tested.

(1 H, dd, J = 1.5, 4.6 Hz), 3.74 and 3.88 (2 H, AB$_q$, J = 16 Hz), 4.12–4.20 (1 H, m), 4.54–4.78 (2 H, m), 5.16–5.46 (2 H, m), 5.49 (1 H, d, J = 1.5 Hz), 5.74 (1 H, br s), 5.78–6.02 (1 H, m), 6.63 (1 H, br s), 7.08–7.35 (5 H, m).

Allyl (5*R*,6*S*)-2-[[*N*-Methyl-*N*-[*N*′-(2-acetamido)-2-acetamido]amino]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6h). Yield: 41% after column chromatography (3:1 v/v ethyl acetate/cyclohexane). Yellow foam. ^{1}H NMR (CDCl$_3$): δ 0.07 (3 H, s), 0.88 (9 H, s), 1.24 (3 H, d, J = 5.9 Hz), 2.38 (3 H, s), 3.15 (2 H, s), 3.69 (1 H, dd, J = 1.6, 4.2 Hz), 3.76 and 3.92 (2 H, ABq, J = 19 Hz), 3.99 (2 H, d, J = 6 Hz), 4.12–4.31 (1 H, m), 4.56–4.78 (2 H, m), 5.16–5.50 (3 H, m overlapped to br s), 5.56 (1 H, d, J = 1.6 Hz), 5.80–6.02 (1 H, m), 6.10 (1 H, br s), 7.58 (1 H, br s).

Allyl (5*R*,6*S*)-2-[[*N*-(*N*′,*N*′-Dimethyl-2-acetamido)-*N*-methylamino]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6i). Yield: 68% after column chromatography (3:1 v/v ethyl acetate/cyclohexane). Yellow solid. Mp: 71–72 °C. ^{1}H NMR (CDCl$_3$): δ 0.07 (6 H, s), 0.88 (9 H, s), 1.24 (3 H, d, J = 6.2 Hz), 2.37 (3 H, s), 2.95 (3 H, s), 3.06 (3 H, s), 3.29 (2 H, s), 3.66 (1 H, dd, J = 1.6, 4.5 Hz), 3.84 (2 H, s), 4.12–4.32 (1 H, m), 4.56–4.78 (2 H, m), 5.18–5.46 (1 H, m), 5.50 (1 H, d, J = 1.6 Hz), 5.80–6.04 (1 H, m).

Allyl (5*R*,6*S*)-2-[[*N*-[*N*′-(1-Piperazino-4-methyl)-2-acetamido]-*N*-methylamino]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6j). Yield: 48% after column chromatography (95:5 v/v dichloromethane/methanol). Yellow wax. ^{1}H NMR (CDCl$_3$): δ 0.07 (6 H, s), 0.88 (9 H, s), 1.25 (3 H, d, J = 6.3 Hz), 2.15–2.60 (10 H, m), 3.29 (2 H, s), 3.45–3.78 (5 H, m), 3.81 (2 H, s), 4.12–4.32 (1 H, m), 4.55–4.88 (2 H, m), 5.12–5.45 (1 H, m), 5.50 (1 H, d, J = 1.4 Hz).

Allyl (5*R*,6*S*)-2-[[*N*,*N*-Bis(2-acetamido)-*N*-methylamino]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6k). Yield: 44% after column chromatography (92:8 v/v ethyl acetate/methanol). ^{1}H NMR (CDCl$_3$): δ 0.07 (6 H, s), 0.88 (9 H, s), 1.23 (3 H, d, J = 6.2 Hz), 3.28 (4 H, s), 3.72 (1 H, dd, J = 1.7, 4 Hz), 3.98 (2 H, s), 4.16–4.33 (1 H, m), 4.53–4.78 (2 H, m), 5.18–5.50 (2 H, m), 5.58 (1 H, d, J = 1.7 Hz), 5.79–6.15 (1 H, m), 6.06 (2 H, br s), 6.78 (2 H, br s).

Allyl (5*R*,6*S*)-2-[[(2-Carbamoyl)aziridin-1-yl]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6l). Yield: 24% after column chromatography (3:1 v/v ethyl acetate/cyclohexane) ^{1}H NMR (CDCl$_3$): δ 0.08 (6 H, s), 0.88 (9 H, s), 1.25 (3 H, d, J = 5.3 Hz), 1.76–1.88 (1 H, m), 2.01–2.12 (1 H, m), 2.14–2.26 (1 H, m), 3.57–3.93 (3 H, m), 4.18–4.32 (1 H, m), 4.58–4.76 (2 H, m), 5.12–5.48 (3 H, m), 5.60 (1 H, s), 5.78–6.10 (1 H, m), 6.23 (1 H, br s).

Allyl (5*R*,6*S*)-2-[[(2-Carbamoyl)piperidin-1-yl]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6m). Yield: 70% after column chromatography (ethyl acetate). White solid. ^{1}H NMR (CDCl$_3$): δ 0.07 (6 H, s), 0.88 (9 H, s), 1.23 (3 H, d, J = 6.3 Hz), 1.13–1.81 (5 H, m), 1.87–2.26 (2 H, m), 2.82–2.98 (1 H, m), 3.03–3.22 (1 H, m), 3.57–4.00 (3 H, m), 4.13–4.32 (1 H, m), 4.55–4.81 (2 H, m), 5.17–5.48 (3 H, m), 5.52 (1 H, s), 5.78–6.03 (1 H, m), 6.53 (2 H, br s).

Allyl (5*R*,6*S*)-2-[[(3-Carbamoyl)piperidin-1-yl]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6n). Yield: 84% after column chromatography (3:1 v/v ethyl acetate/cyclohexane). Yellow wax. ^{1}H NMR (CDCl$_3$): δ 0.07 (6 H, s), 0.87 (9 H, s), 1.23 (3 H, d, J = 6.3 Hz), 1.48–1.97 (4 H, m), 2.13–2.37 (1 H, m), 2.40–2.64 (2 H, m), 2.64–3.01 (2 H, m), 3.51–3.89 (3 H, m), 4.14–4.32 (1 H, m), 4.53–4.81 (2 H, m), 5.17–5.49 (2 H, m), 5.53 (1 H, d, J = 1.5 Hz), 5.73–6.06 (2 H, br s, overlapped to multiplet), 7.22 (1 H, br).

Allyl (5*R*,6*S*)-2-[[(4-Carbamoyl)piperidin-1-yl]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6o). Yield: 70% after column chromatography (3:1 v/v ethyl acetate/cyclohexane). Yellow solid. ^{1}H NMR (CDCl$_3$): δ 0.07 (6 H, s), 0.88 (9 H, s), 1.24 (3 H, d, J = 6.2 Hz), 1.55–1.93 (4 H, m), 2.00–2.24 (3 H, m), 2.83–3.13 (2 H, m), 3.64 (1 H, dd, J = 1.6, 4.7 Hz), 3.70 (2 H, s), 4.10–4.31 (1

H, m), 4.55–4.80 (2 H, m), 5.12–5.45 (4 H, m), 5.47 (1 H, d, J = 1.6 Hz), 5.80–6.14 (1 H, m).

Allyl (5*R*,6*S*)-2-[((2*S*)-1-Prolinamido)methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6p). Yield: 57% after column chromatography (ethyl acetate). ^{1}H NMR (DMSO-d_6): δ 0.05 (6 H, s), 0.79 (9 H, s), 1.20 (3 H, d, J = 6.3 Hz), 1.50–1.76 (3 H, m), 1.95–2.18 (1 H, m), 2.30–2.52 (1 H, m), 3.07–3.26 (2 H, m), 3.76 and 4.04 (2 H, AB$_q$, J = 18 Hz), 3.92 (1 H, dd, J = 2.0, 4.5 Hz), 4.13–4.31 (1 H, m), 4.49–4.82 (2 H, m), 5.12–5.47 (2 H, m), 5.61 (1 H, d, J = 2.0 Hz), 5.78–6.03 (1 H, m), 7.01 (1 H, br s), 7.19 (1 H, br s).

Allyl (5*R*,6*S*)-2-[((2*R*)-1-Prolinamido)methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6q). Yield: 66% after column chromatography (ethyl acetate). Mp: 107–108 °C. ^{1}H NMR (CDCl$_3$): δ 0.07 (6 H, s), 0.88 (9 H, s), 1.24 (3 H, d, J = 6.0 Hz), 1.64–2.02 (3 H, m), 2.12–2.31 (1 H, m), 2.31–2.52 (1 H, m), 3.10–3.32 (2 H, m), 3.68 (1 H, d, J = 4.7 Hz), 3.78 and 4.10 (2 H, AB$_q$, J = 16 Hz), 4.13–4.32 (1 H, m), 4.56–4.80 (2 H, m), 5.18–5.49 (1 H, m), 5.43 (1 H, br s), 5.54 (1 H, s), 5.77–6.02 (1 H, m), 7.12 (1 H, br s).

Allyl (5*R*,6*S*)-2-[((2*S*,4*R*)-4-Hydroxy-1-prolinamido)methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6r). Yield: 58% after column chromatography (95:5 v/v ethyl acetate/methanol). White solid foam. ^{1}H NMR (CDCl$_3$): δ 0.07 (6 H, s), 0.88 (9 H, s), 1.24 (3 H, d, J = 6.3 Hz), 1.93 (1 H, br s), 1.98–2.12 (1 H, m), 2.15–2.36 (1 H, m), 2.51–2.66 (1 H, m), 3.40–3.66 (2 H, m), 3.66 (1 H, dd overlapped to AB$_q$ lines, J not detectable), 3.76 and 4.28 (2 H, AB$_q$, J = 16 Hz), 4.14–4.30 (1 H, m, overlapped to AB$_q$ lines), 4.35–4.50 (1 H, m), 4.56–4.82 (2 H, m), 5.18–5.45 (2 H, m), 5.50 (1 H, br s), 5.57 (1 H, d, J = 1.7 Hz), 5.79–6.04 (1 H, m), 7.04 (1 H, br s).

Allyl (5*R*,6*S*)-2-[((2*R*,4*R*)-4-Hydroxy-1-prolinamido)methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6s). Yield: 55% after column chromatography (95:5 v/v ethyl acetate/methanol). White solid foam. ^{1}H NMR (CDCl$_3$): δ 0.07 (6 H, s), 0.87 (9 H, s), 1.23 (3 H, d, J = 6.3 Hz), 1.94–2.09 (1 H, m), 2.25 (1 H, br s), 2.36–2.66 (2 H, m), 3.12–3.36 (2 H, m), 3.70 (1 H, dd, J = 1.6, 4.5 Hz), 3.85 and 4.12 (2 H, AB$_q$, J = 16 Hz), 4.39–4.46 (1 H, m), 4.57–4.80 (2 H, m), 5.18–5.43 (2 H, m), 5.55 (1 H, d, J = 1.6 Hz), 5.58 (1 H, br s), 5.78–6.02 (1 H, m), 7.19 (1 H, br s).

Allyl (5*R*,6*S*)-2-[((2*S*,4*S*)-4-Hydroxy-1-prolinamido)methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6t). Yield: 60% after column chromatography (95:5 v/v ethyl acetate/methanol). White solid foam. ^{1}H NMR (CDCl$_3$): δ 0.08 (6 H, s), 0.88 (9 H, s), 1.25 (3 H, d, J = 6.1 Hz), 1.90–2.20 (1 H, m), 2.28–2.78 (2 H, m), 3.22–3.39 (2 H, m), 3.68 (1 H, dd, J = 1.6, 4.4 Hz), 3.78 and 4.18 (2 H, AB$_q$, J = 16 Hz), 4.15–4.30 (1 H, m), 4.31–4.45 (1 H, m), 4.58–4.78 (2 H, m), 5.18–5.46 (3 H, br s overlapped to m), 5.59 (1 H, d, J = 1.6 Hz), 7.07 (1 H, br s).

Allyl (5*R*,6*S*)-2-[((2*R*,4*S*)-4-Hydroxy-1-prolinamido)methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (6u). Yield: 64% after column chromatography (95:5 v/v ethyl acetate/methanol). White solid foam. ^{1}H NMR (CDCl$_3$): δ 0.06 (6 H, s), 0.86 (9 H, s), 1.23 (3 H, d, J = 6.4 Hz), 1.85–2.06 (1 H, m), 2.17–2.36 (1 H, m), 2.44–2.61 (1 H, m), 2.70 (1 H, br), 3.25–3.42 (1 H, m), 3.51–3.64 (1 H, m), 3.68 (1 H, dd, J = 1.6, 5 Hz), 3.76 and 4.27 (2 H, AB$_q$, J = 16 Hz), 4.12–4.28 (1 H, m), 4.32–4.43 (1 H, m), 4.53–4.74 (2 H, m), 5.16–5.45 (2 H, m), 5.52 (1 H, d, J = 11.6 Hz), 5.78–6.00 (1 H, m), 6.05 (1 H, br s), 7.12 (1 H, br s).

Synthesis of the 5-Methylthiazole Derivative (13). Compound **13** was isolated by allowing the mesylate **5** (2.17 g, 4.54 mmol) to react with glycinamide hydrochloride (0.55 g, 5 mmol) and triethylamine (1.4 mL, 10 mmol) in DMSO, as described above in the general procedure for compounds **6**. Column chromatography purification (95:5 v/v ethyl acetate/methanol) gave **13** as a yellow foam. Yield: 72%. ^{1}H NMR (CDCl$_3$): δ −0.01 (3 H, s, SiCH$_3$), 0.08 (3 H, s, SiCH$_3$), 0.85 (9 H, s, C(CH$_3$)$_3$), 1.25 (3 H, d, J = 6.0 Hz, C*H*$_3$CH), 2.75 (3 H, s, 5-CH$_3$ on the thiazole ring), 3.78–4.13 (3 H, m, NHC*H*$_2$CO and H-6), 4.42–4.52 (1 H, m, CH$_3$C*H*), 4.77–4.92 (2 H, m, COOCH$_2$), 5.22–5.55 (3 H, br s overlapped to m, one of CONH$_2$ and CH=C*H*$_2$), 5.89–6.18 (1 H, m, C*H*=CH$_2$), 6.55 (1 H, br s, one of CONH$_2$), 6.98 (1 H, br t, CONHCH$_2$). The reaction led to a partial epimerization at C-6. Pure C-6 epimer was isolated from column chromatography as a yellow foam (11% yield). ^{1}H NMR (CDCl$_3$): δ −0.24 (3 H, s, SiCH$_3$), 0.22 (3 H, s, SiCH$_3$), 0.79 (9 H, s, C(CH$_3$)$_3$), 1.38 (3 H, d, J = 6.4 Hz, C*H*$_3$CH), 2.75 (3 H, s, 5-CH$_3$ on the thiazole ring), 3.66 (1 H, four lines, part of AMX spin system, J = 4.5, 18 Hz, NHC*H*$_2$CO), 3.92 (1 H, d, J = 3.2 Hz, H-6), 4.20–4.37 (1 H, m, CH$_3$C*H*), 4.49 (1 H, four lines, part of AMX spin system, J = 8, 18 Hz, NHC*H*$_2$CO), 4.70–4.88 (2 H, m, COOCH$_2$), 5.26–5.50 (2 H, m, CH=C*H*$_2$), 5.53 (1 H, br s, one of CONH$_2$), 5.88–6.12 (1 H, m, C*H*=CH$_2$), 7.89 (1 H, br, four lines, part of AMX spin system, J = 4.5, 8 Hz, CON*H*CH$_2$), 8.18 (1 H, br s, one of CONH$_2$).

General Procedure for the Synthesis of 6-(Hydroxyethyl)penem Derivatives 7a–u (Cleavage of *tert*-Butyldimethylsilyl Ether). 8-(*tert*-Butyldimethylsilyl)penem derivatives **6a–u** (10 mmol) were dissolved in anhydrous THF (200 mL). Acetic acid (3.43 mL, 60 mmol) and tetrabutylammonium fluoride (30 mL of a 1 M solution in THF, 30 mmol) were added in sequence, under a nitrogen atmosphere, at 25 °C, and the resulting solution was stirred for 24 h. After concentration *in vacuo*, the residue was diluted with ethyl acetate and washed with water, 5% NaHCO$_3$, and brine. The organic phase was dried over Na$_2$SO$_4$ and concentrated *in vacuo*. Pure derivatives **7a–u** were obtained in a yield ranging from 60 to 80% by crystallization or column chromatography on silica gel, according to the following examples.

Allyl (5*R*,6*S*)-2-[[*N*-(2-Acetamido)-*N*-methylamino]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylate (7a). Yield: 64% after crystallization from 2:1 v/v ethyl acetate/*n*-hexane. White solid. Mp: 85–86 °C. ^{1}H NMR (CDCl$_3$): δ 1.36 (3 H, d, J = 6.3 Hz), 2.37 (3 H, s), 3.08 and 3.12 (2 H, AB$_q$, J = 18 Hz), 3.73 (1 H, dd, J = 1.7, 6.7 Hz), 3.80 and 3.86 (2 H, AB$_q$, J = 15 Hz), 4.17–4.22 (1 H, m), 4.58–4.84 (2 H, m), 5.21–5.46 (2 H, m), 5.53 (1 H, br s), 5.59 (1 H, d, J = 1.7 Hz), 5.84–6.03 (1 H, m), 6.86 (1 H, br s).

Allyl (5*R*,6*S*)-2-[((2*S*)-1-Prolinamido)methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylate (7p). Yield: 68% after column chromatography (95:5 ethyl acetate/methanol). White solid. Mp: 93–95 °C. ^{1}H NMR (CDCl$_3$): δ 1.34 (3 H, d, J = 6.4 Hz), 1.62–2.07 (3 H, m), 2.10–2.31 (1 H, m), 2.31–2.52 (1 H, m), 2.60 (1 H, br), 3.12–3.40 (2 H, m), 3.72 and 4.13 (2 H, AB$_q$, J = 16 Hz), 3.73 (1 H, dd overlapped to AB$_q$ lines, J not detectable), 4.12–4.34 (1 H, m), 4.55–4.90 (2 H, m), 5.20–5.49 (2 H, m), 5.61 (1 H, d, J = 1.2 Hz), 5.70 (1 H, br s), 7.09 (1 H, br s).

General Procedures for the Synthesis of 2-(Nitrogen-substituted methyl)penem Carboxylic Acids (3). Allyl esters **7a–u** (5 mmol) were dissolved, under a nitrogen atmosphere, at 25 °C, in anhydrous THF (100 mL). Triphenylphosphine (131 mg, 0.5 mmol), tetrakis(triphenylphosphine)palladium (578 mg, 0.5 mmol) and acetic acid (0.43 mL, 7.5 mmol) were added in sequence to the solution. After the solution was stirred for 30 min, diethyl ether was added to complete the precipitation of crude penem acid **3**. The obtained solid was collected by filtration under a nitrogen atmosphere, washed with diethyl ether, and purified by reverse phase column chromatography.

(5*R*,6*S*)-2-[[*N*-(2-Acetamido)-*N*-methylamino]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3a). The reaction was carried out in THF (200 mL) at 35 °C. Yield: 66% after reverse phase column chromatography (95:5 v/v water/acetone). White solid. Mp: 95–96 °C. HPLC assay: 98% (t_R = 4.75 min). UV (nm): 250, 315. ^{1}H NMR (D$_2$O): δ 1.25 (3 H, d, J = 6.2 Hz), 2.90 (3 H, s), 3.98 (2 H, s), 3.98 (1 H, dd, J = 1.4, 6.4 Hz), 4.13–4.28 (1 H, m), 4.25 (2 H, s), 5.69 (1 H, d, J = 1.4 Hz). ^{13}C NMR (D$_2$O): δ 24.9, 46.1, 57.4, 60.5, 68.0, 69.5, 75.1, 135.7, 141.1, 169.7, 173.7, 180.2. MS FAB (m/z): 316 (M + H)$^+$. Anal. (C$_{12}$H$_{17}$N$_3$O$_5$S) C, H, N.

(5*R*,6*S*)-2-[[*N*-(2-Acetamido)-*N*-ethylamino]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3b). Yield: 40% after reverse phase column chromatography (95:5 v/v water/acetone). White solid. HPLC assay: 97% (t_R = 4.8 min). UV (nm): 255, 312. ^{1}H NMR (D$_2$O): δ 1.25 (3 H, d, J

= 6.5 Hz), 1.27 (3 H, t, J = 7 Hz), 3.21 (2 H, q, J = 7 Hz), 3.88 (2 H, s), 3.95 (1 H, dd, J = 1.5, 5 Hz), 4.16 and 4.23 (2H, AB_q, J = 15 Hz), 4.12–4.32 (1 H, m), 5.68 (1 H, d, J = 1.5 Hz). ^{13}C NMR (D_2O): δ 13.6, 24.4, 54.4, 54.7, 58.1, 67.6, 69.0, 74.7, 131.8, 136.6, 168.9, 174.1, 180.1. MS FAB (m/z): 330 (M + H)$^+$. Anal. ($C_{13}H_{19}N_3O_5S$) C, H, N.

(5*R*,6*S*)-2-[[*N*-Methyl-*N*-(3-propionamido)amino]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3c). Yield: 45% after reverse phase column chromatography (95:5 v/v water/acetone). White solid. HPLC assay: 97% (t_R = 5.0 min). UV (nm): 255, 314. ^1H NMR (D_2O): δ 1.27 (3 H, d, J = 6.4 Hz), 2.81 (2 H, t, J = 6.6 Hz), 2.88 (3 H, s), 3.32–3.58 (2 H, br), 4.01 (1 H, dd, J = 2, 5 Hz), 4.05–4.34 (3 H, m), 5.72 (1 H, d, J = 2 Hz). ^{13}C NMR (D_2O): δ 25.2, 42.7, 48.1, 53.6, 59.7, 67.8, 69.3, 75.1, 132.2, 138.3, 169.1, 173.9, 180.5. MS FAB (m/z): 330 (M + H)$^+$. Anal. ($C_{13}H_{19}N_3O_5S$) C, H, N.

(5*R*,6*S*)-2-[[*N*-Methyl-*N*-(2*S*)-(2-propionamido)amino]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3d). Yield: 50% after reverse phase column chromatography (95:5 v/v water/acetone). White solid. Mp: 105 °C dec. HPLC assay: 98% (t_R = 4.2 min). UV (nm): 257, 306. ^1H NMR (D_2O): δ 1.34 (3 H, d, J = 6.4 Hz), 1.58 (3 H, d, J = 7.0 Hz), 2.82 (3 H, s), 4.04 (1 H, dd, J = 1.3, 5.8 Hz), 4.12–4.35 (4 H, m), 5.76 (1 H, d, J = 1.2 Hz). ^{13}C NMR (D_2O): δ 14.9, 22.7, 39.4, 52.6, 64.6, 66.0, 67.3, 72.9, 133.5, 138.6, 167.7, 174.0, 178.4. MS FAB (m/z): 330 (M + H)$^+$. Anal. ($C_{13}H_{19}N_3O_5S$) C, H, N.

(5*R*,6*S*)-2-[[*N*-Methyl-*N*-(2*R*)-(2-propionamido)amino]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3e). Yield: 55% after reverse phase column chromatography (95:5 v/v water/acetone). White solid. Mp: 145 °C dec. HPLC assay: 99% (t_R = 3.8 min). UV (nm): 258, 309. ^1H NMR (D_2O): δ 1.30 (3 H, d, J = 6.4 Hz), 1.59 (3 H, d, J = 7.1 Hz), 2.85 (3 H, s), 4.04 (1 H, dd, J = 1.6, 5.8 Hz), 4.04–4.39 (4 H, m), 5.74 (1 H, d, J = 1.6 Hz). ^{13}C NMR (D_2O): δ 14.7, 22.7, 40.1, 52.0, 64.6, 66.0, 67.2, 72.9, 133.1, 139.1, 167.7, 174.6, 178.5. MS FAB (m/z): 330 (M + H)$^+$. Anal. ($C_{13}H_{19}N_3O_5S$) C, H, N.

(5*R*,6*S*)-2-[[*N*-Methyl-*N*-(2*S*)-(3-hydroxy-2-propionamido)amino]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3f). Yield: 61% after reverse phase column chromatography (95:5 v/v water/acetone). White solid. Mp: 125–126 °C. HPLC assay: 98% (t_R = 3.8 min). UV (nm): 250, 314. ^1H NMR (D_2O): δ 1.25 (3 H, d, J = 6.3 Hz), 2.93 (3 H, s), 3.98 (1 H, dd, J = 1.6, 5.9 Hz), 4.01–4.41 (6 H, overlapped m), 5.69 (1 H, d, J = 1.6 Hz). ^{13}C NMR (D_2O): δ 24.8, 42.6, 54.2, 62.5, 67.3, 69.5, 71.3, 74.5, 133.5, 143.8, 166.1, 176.9, 180.7. MS FAB (m/z): 346 (M + H)$^+$. Anal. ($C_{13}H_{19}N_3O_6S$) C, H, N.

(5*R*,6*S*)-2-[[*N*-Methyl-*N*-(2*S*)-(3-phenyl-2-propionamido)amino]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3g). Yield: 51% after reverse phase column chromatography (70:30 v/v water/acetone). White solid. Mp: 105–106 °C. HPLC assay (80:20 v/v 0.02 M phosphate buffer (pH = 3)/acetonitrile): 98% (t_R = 5.7 min). UV (nm): 257, 305. ^1H NMR (D_2O): δ 1.27 (3 H, d, J = 6.2 Hz), 2.83 (3 H, s), 3.05–3.32 (2 H, m), 3.94 (1 H, dd, J = 1.2, 5.5 Hz), 3.98–4.30 (4 H, overlapped m), 5.66 (1 H, d, J = 1.2 Hz). ^{13}C NMR (D_2O): δ 24.4, 38.4, 42.1, 54.8, 67.5, 69.0, 70.8, 74.5, 132.2, 133.5, 133.6, 133.8, 138.6, 146.6, 169.6, 177.5, 180.2. MS FAB (m/z): 406 (M + H)$^+$. Anal. ($C_{19}H_{23}N_3O_5S$) C, H, N.

(5*R*,6*S*)-2-[[*N*-Methyl-*N*-[*N'*-(2-acetamido)-2-acetamido]amino]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3h). Yield: 64% after reverse phase column chromatography (95:5 v/v water/acetone). White solid. Mp: 159–161 °C. HPLC assay: 99% (t_R = 3.4 min). UV (nm): 258, 308. ^1H NMR (D_2O): δ 1.26 (3 H, d, J = 6.5 Hz), 2.86 (3 H, s), 3.96 (5 H, br s), 4.12–4.32 (3 H, m), 5.69 (1 H, s). ^{13}C NMR (D_2O): δ 24.4, 45.8, 46.3, 56.6, 60.6, 67.7, 69.0, 74.6, 134.3, 142.0, 162.2, 166.1, 172.0, 180.1. MS FAB (m/z): 373 (M + H)$^+$. Anal. ($C_{14}H_{20}N_4O_6S$) C, H, N.

(5*R*,6*S*)-2-[[*N*-(*N'*,*N'*-Dimethyl-2-acetamido)-*N*-methylamino]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3i). Yield: 55% after reverse phase column chromatography (95:5 v/v water/acetone). White-brown solid. Mp: 60 °C dec. HPLC assay: 97% (t_R = 9.1 min). UV (nm): 250, 315. ^1H NMR (D_2O): δ 1.26 (3 H, d, J = 6.3 Hz), 2.93 and 2.95 (9 H, s and s), 3.99 (1 H, dd, J = 1.5, 5 Hz), 4.0–4.28 (3 H, m), 4.30 (2 H, s), 5.70 (1 H, d, J = 1.5 Hz). ^{13}C NMR: 24.6, 36.6, 37.4, 46.2, 56.9, 61.3, 67.8, 69.4, 74.9, 134.1, 143.3, 168.6, 170.2, 180.1. MS FAB (m/z): 344 (M + H)$^+$. Anal. ($C_{14}H_{21}N_3O_5S$) C, H, N.

(5*R*,6*S*)-2-[[*N*-[*N'*-(1-Piperazino-4-methyl)-2-acetamido]-*N*-methylamino]methyl]-6-((*R*)-1-hydroxyethyl)-penem-3-carboxylic Acid (3j). Yield: 38% after reverse phase column chromatography (90:10 v/v water/acetone). White-brown solid. Mp: 60 °C dec. HPLC assay (90:10 v/v 0.02 M phosphate buffer (pH = 3)/acetonitrile): 96% (t_R = 10.1 min). UV (nm): 257, 306. ^1H NMR (D_2O): δ 1.25 (3 H, d, J = 6.2 Hz), 2.89 (6 H, s), 3.16–3.52 (4 H, br), 3.52–3.89 (4 H, br), 4.08–4.92 (5 H, br), 4.96 (1 H, d, J = 8 Hz), 5.69 (1 H, s). ^{13}C NMR (D_2O): δ 24.4, 43.6, 46.2, 46.3, 47.4, 56.9, 59.9, 67.7, 69.0, 74.6, 135.8, 140.1, 168.1, 168.4, 180.1. Anal. ($C_{17}H_{26}N_4O_5S$) C, H, N.

(5*R*,6*S*)-2-[[*N*,*N*-Bis(2-acetamido)-*N*-methylamino]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3k). The reaction was carried out in anhydrous THF–dichloromethane (200 mL, 50:50 v/v) at 40 °C for 10 min. Yield: 55% after reverse phase column chromatography (98:2 v/v water/acetone). White solid. Mp: 107–108 °C. HPLC assay (99:1 v/v 0.02 M phosphate buffer (pH = 3)/acetonitrile): 98% (t_R = 5.7 min). UV (nm): 258, 307. ^1H NMR (D_2O): δ 1.27 (3 H, d, J = 6.4 Hz), 3.51 (4 H, s), 3.92 (1 H, dd, J = 1.5, 4.5 Hz), 3.96 and 4.10 (2 H, AB_q, J = 15 Hz), 4.15–4.28 (1 H, m), 5.63 (1 H, d, J = 1.5 Hz). ^{13}C NMR (D_2O): δ 24.4, 56.0, 61.1, 61.3, 66.8, 69.0, 74.0, 138.9, 150.6, 167.2, 179.0, 179.2, 180.5. MS FAB (m/z): 359 (M + H)$^+$. Anal. ($C_{13}H_{18}N_4O_6S$) C, H, N.

(5*R*,6*S*)-2-[[(2-Carbamoyl)aziridin-1-yl]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3l). Yield: 35% after reverse phase column chromatography (95:5 v/v water/acetone). Pale yellow solid. HPLC assay (99:1 v/v 0.02 M phosphate buffer (pH = 3)/acetonitrile): 97% (t_R = 1.97, 2.11 min). ^1H NMR (D_2O): δ 1.26 (3 H, d, J = 6.4 Hz), 2.01–2.18 (1 H, m), 2.18–2.34 (1 H, m), 2.48–2.62 (1 H, m), 3.58 and 4.03 (2 H, AB_q, J = 15 Hz), 3.90 (1 H, d, J = 5.5 Hz), 4.10–4.32 (1 H, m), 5.63 (1 H, s). MS FAB (m/z): 314 (M + H)$^+$, 336 (M + Na)$^+$. The diastereoisomer mixture was separated by preparative HPLC (overall yield, 85%; diastereoisomer ratio, 50:50).

(5*R*,6*S*)-2-[[(2-Carbamoyl)piperidin-1-yl]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3m). The diastereoisomer mixture was separated by preparative HPLC (t_R = 9.9, 11.1 min; overall yield, 44%; diastereoisomer ratio, 50:50). ^1H NMR (diastereoisomer mixture) (D_2O): δ 1.26 (3 H, d, J = 6 Hz), 1.40–2.05 (5 H, br m), 2.05–2.30 (1 H, m), 2.75–3.10 (1 H, m), 3.55–3.75 (1 H, m), 3.75–3.92 (1 H, m), 3.98 (1 H, d, J = 5 Hz), 4.03–4.38 (3 H, m), 5.67 (1 H, s). ^{13}C NMR (D_2O): δ 24.4, 25.8, 26.9, 31.4, 32.8, 48.2, 55.0, 55.5, 61.8, 67.3, 69.0, 72.2, 74.4, 136.4, 140.9, 164.4, 170.2, 180.1. MS FAB (m/z): 356 (M + H)$^+$. Anal. ($C_{15}H_{21}N_3O_5S$) C, H, N.

(5*R*,6*S*)-2-[[(3-Carbamoyl)piperidin-1-yl]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3n). The diastereoisomer mixture was separated by preparative HPLC (t_R = 8.5, 10.2 min; overall yield, 51%; diastereoisomer ratio, 50:50). ^1H NMR (diastereoisomer mixture) (D_2O): δ 1.26 (3 H, d, J = 6.4 Hz), 1.50–2.20 (4 H, br), 2.60–3.20 (3 H, br), 3.40–3.85 (2 H, br), 3.90 (1 H, dd, J = 1, 5 Hz), 4.04–4.35 (3 H, m), 5.72 (1 H, d, J = 1 Hz). ^{13}C NMR (D_2O): δ 24.4, 26.6, 29.8, 45.5, 56.2, 67.9, 69.0, 74.6, 139.5, 140.7, 169.4, 169.5, 180.2. MS FAB (m/z): 356 (M + H)$^+$. Anal. ($C_{15}H_{21}N_3O_5S$) C, H, N.

(5*R*,6*S*)-2-[[(4-Carbamoyl)piperidin-1-yl]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3o). The diastereoisomer mixture was separated by preparative HPLC (t_R = 8.7, 10.5 min; overall yield, 55%; diastereoisomer ratio, 50:50). ^1H NMR (diastereoisomer mixture) (D_2O): δ 1.26 (3 H, d, J = 6.6 Hz), 1.60–2.22 (4 H, br m), 2.40–2.75 (1 H, m), 2.75–3.24 (2 H, m), 3.35–3.80 (2 H, br), 3.99 (1 H, d, J = 5.6 Hz), 4.17 (2 H, s), 4.10–4.32 (1 H, m), 5.70 (1 H, s). ^{13}C NMR (D_2O): δ 24.4, 29.4, 30.6, 43.5, 47.5, 54.8, 56.3, 67.8, 69.0, 74.6, 134.9, 140.0, 162.4, 169.7, 180.2, 183.2. MS FAB (m/z): 356 (M + H)$^+$. Anal. ($C_{15}H_{21}N_3O_5S$) C, H, N.

(5*R*,6*S*)-2-[((2*S*)-1-Prolinamido)methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3p). Yield: 65% after reverse phase column chromatography (95:5 v/v water/acetone). White solid. Mp: 135–136 °C. HPLC assay: 99% (t_R = 6.5 min). UV (nm): 255, 314. ^{1}H NMR (D_2O): δ 1.26 (3 H, d, J = 6.3 Hz), 1.90–2.25 (3 H, m), 2.40–2.70 (1 H, m), 3.12–3.38 (1 H, m), 3.66–3.88 (1 H, m), 3.97 (1 H, dd, J = 1.6, 5 Hz), 4.10–4.29 (1 H, m), 4.32 and 4.44 (2 H, AB_q, J = 14 Hz), 4.40–4.52 (1 H, m), 5.68 (1 H, d, J = 1.6 Hz). ^{13}C NMR (D_2O): δ 24.4, 27.6, 34.5, 54.6, 59.7, 67.7, 69.0, 71.2, 74.7, 135.9, 140.0, 169.2, 173.9, 180.1. MS FAB (*m/z*): 342 (M + H)$^+$. Anal. ($C_{14}H_{19}N_3O_5S$) C, H, N.

(5*R*,6*S*)-2-[((2*R*)-1-Prolinamido)methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3q). Yield: 68% after reverse phase column chromatography (95:5 v/v water/acetone). White solid. Mp: 148–149 °C. HPLC assay: 99% (t_R = 4.4 min). UV (nm): 259, 316. ^{1}H NMR (D_2O): δ 1.25 (3 H, d, J = 6.5 Hz), 1.85–2.30 (3 H, m), 2.35–2.65 (1 H, m), 3.15–3.45 (1 H, m), 3.70–3.90 (1 H, m), 3.92 (1 H, dd, J = 1.5, 5 Hz), 4.08–5.00 (4 H, m), 5.65 (1 H, d, J = 1.5 Hz). ^{13}C NMR (D_2O): δ 24.4, 28.1, 35.1, 55.2, 60.3, 67.5, 69.0, 71.0, 75.1, 132.9, 142.3, 160.4, 169.6, 180.0. MS FAB (*m/z*): 342 (M + H)$^+$. Anal. ($C_{14}H_{19}N_3O_5S$) C, H, N.

(5*R*,6*S*)-2-[((2*S*,4*R*)-4-Hydroxy-1-prolinamido)methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3r). Yield: 59% after reverse phase column chromatography (95:5 v/v water/acetone). White solid. Mp: 151–152 °C. HPLC assay: 98% (t_R = 3.4 min). UV (nm): 258, 307. ^{1}H NMR (D_2O): δ 1.26 (3 H, d, J = 6.4 Hz), 2.12–2.31 (1 H, m), 2.41–2.58 (1 H, m), 3.18–3.32 (1 H, m), 3.74–3.88 (1 H, m), 3.96 (1 H, dd, J = 1.6, 5.8 Hz), 4.10–4.28 (1 H, m), 4.31 and 4.48 (2 H, AB_q, J = 14 Hz), 4.51–4.68 (2 H, m), 5.67 (1 H, d, J = 1.6 Hz). ^{13}C NMR (D_2O): δ 24.4, 43.3, 56.6, 65.7, 67.5, 69.0, 70.4, 73.9, 74.6, 133.2, 141.8, 169.3, 176.1, 180.2. MS FAB (*m/z*): 358 (M + H)$^+$. Anal. ($C_{14}H_{19}N_3O_6S$) C, H, N.

(5*R*,6*S*)-2-[((2*R*,4*R*)-4-Hydroxy-1-prolinamido)methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3s). Yield: 55% after reverse phase column chromatography (95:5 v/v water/acetone). White solid. Mp: 123–124 °C. HPLC assay (98:2 v/v 0.02 M phosphate buffer (pH = 3)/acetonitrile): 99% (t_R = 3.5 min). UV (nm): 258, 307. ^{1}H NMR (D_2O): δ 1.24 (3 H, d, J = 6.4 Hz), 2.03–2.22 (1 H, m), 2.64–2.98 (1 H, m), 3.22–3.41 (1 H, m), 3.61–3.79 (1 H, m), 3.92 (1 H, dd, J = 1.5, 6 Hz), 4.15 and 4.38 (2 H, AB_q, J = 14 Hz), 4.11–4.30 (2 H, m), 4.58 (1 H, br), 5.65 (1 H, d, J = 1.5 Hz). ^{13}C NMR (D_2O): δ 24.8, 43.5, 55.7, 66.9, 67.9, 69.4, 70.7, 73.8, 74.4, 133.1, 145.4, 168.2, 175.7, 180.3. MS FAB (*m/z*): 358 (M + H)$^+$. Anal. ($C_{14}H_{19}N_3O_6S$) C, H, N.

(5*R*,6*S*)-2-[((2*S*,4*S*)-4-Hydroxy-1-prolinamido)methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3t). Yield: 60% after reverse phase column chromatography (95:5 v/v water/acetone). White solid. Mp: 103–104 °C. HPLC assay: 98% (t_R = 4.4 min). UV (nm): 255, 313. ^{1}H NMR (D_2O): δ 1.27 (3 H, d, J = 6.4 Hz), 2.04–2.22 (1 H, m), 2.67–2.89 (1 H, m), 3.20–3.35 (1 H, m), 3.61–3.78 (1 H, m), 3.96 (1 H, dd, J = 1.6, 5 Hz), 4.12–4.30 (1 H, m), 4.32 (2 H, s), 4.38–4.53 (1 H, m), 4.53–4.68 (1 H, br), 5.70 (1 H, d, J = 1.6 Hz). ^{13}C NMR (D_2O): δ 24.9, 43.3, 55.4, 66.6, 68.1, 69.6, 70.5, 73.9, 75.1, 133.6, 145.5, 169.1, 176.3, 180.1. MS FAB (*m/z*): 358 (M + H)$^+$. Anal. ($C_{14}H_{19}N_3O_6S$) C, H, N.

(5*R*,6*S*)-2-[((2*R*,4*S*)-4-Hydroxy-1-prolinamido)methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (3u). Yield: 58% after reverse phase column chromatography (95:5 v/v water/acetone). White solid. Mp: 151–152 °C. HPLC assay: 98% (t_R = 4.4 min). UV (nm): 256, 316. ^{1}H NMR (D_2O): δ 1.27 (3 H, d, J = 6.4 Hz), 2.11–2.33 (1 H, m), 2.40–2.52 (1 H, m), 3.25–3.45 (1 H, m), 3.2–4.02 (2 H, m overlapped to dd, J not detectable), 4.12–4.35 (2 H, m), 4.34 and 4.44 (2 H, AB_q, J = 14 Hz), 4.68 (1 H, br), 5.67 (1 H, d, J = 1 Hz). ^{13}C NMR (D_2O): δ 24.4, 43.7, 57.9, 66.7, 67.7, 69.0, 71.1, 74.4, 75.3, 137.8, 140.0, 169.4, 176.2, 179.9. MS FAB (*m/z*): 358 (M + H)$^+$. Anal. ($C_{14}H_{19}N_3O_6S$) C, H, N.

(5*R*,6*S*)-2-[[(2*S*)-2-(Methoxycarbonyl)pyrrolidin-1-yl]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (14b). (2*S*)-Proline methyl ester hydrochloride (**12b**, 828 mg, 5 mmol) dissolved in DMSO (20 mL) was added under stirring, at room temperature, to a solution of freshly prepared **5** (2.17 g, 4.54 mmol) in DMSO (50 mL). Triethylamine (1.4 mL, 10 mmol) was added and the resulting solution stirred for about 20 h and then poured into ice (200 mL). The mixture was allowed to warm to room temperature and extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with water (2 × 100 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated *in vacuo* at 30 °C to give, after column chromatography (1:3 v/v ethyl acetate/cyclohexane), allyl (5*R*,6*S*)-2-[[(2*S*)-2-(methoxycarbonyl)pyrrolidin-1-yl]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate. Yield: 1.94 g (75%). White solid. Mp: 101–102 °C. ^{1}H NMR ($CDCl_3$): δ 0.07 (6 H, s), 0.88 (9 H, s), 1.24 (3 H, d, J = 6.4 Hz), 1.70–2.24 (4 H, m), 2.45–2.62 (1 H, m), 3.12–3.30 (1 H, m), 3.34–3.46 (1 H, m), 3.66 (1 H, dd, J = 1.7, 4.9 Hz), 3.71 (3 H, s), 3.92 and 4.07 (2 H, AB_q, J = 16 Hz), 4.14–4.35 (1 H, m), 4.56–4.82 (2 H, m), 5.16–5.48 (2 H, m), 5.50 (1 H, d, J = 1.7 Hz), 5.70–6.05 (1 H, m). The compound was dissolved in anhydrous THF (100 mL). Acetic acid (1.30 mL, 22.8 mmol) and tetrabutylammonium fluoride (11.4 mL of a 1 M solution in THF, 11.4 mmol) were added in sequence, under a nitrogen atmosphere, at 25 °C, and the resulting solution was stirred for 24 h. After concentration *in vacuo*, the residue was diluted with diethyl ether and washed with water, 5% $NaHCO_3$, and brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (50:50 v/v ethyl acetate/cyclohexane) gave allyl (5*R*,6*S*)-2-[[(2*S*)-2-(methoxycarbonyl)pyrrolidin-1-yl]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylate (yield: 1.23 g, 3.10 mmol, 80%), which was dissolved in anhydrous THF and treated with triphenylphosphine (81 mg, 0.31 mmol), tetrakis(triphenylphosphine)palladium (358 mg, 0.31 mmol), and acetic acid (0.27 mL, 4.65 mmol) as described above for **3a–u**. Reverse phase column chromatography (90:10 v/v water/acetone) gave **14b** as a white solid. Yield: 71%. Mp: 110 °C dec. HPLC assay (90:10 v/v 0.02 M phosphate buffer (pH = 3)/acetonitrile): 99% (t_R = 6.0 min). UV (nm): 259, 311. ^{1}H NMR (D_2O): δ 1.26 (3 H, d, J = 6.5 Hz), 1.80–2.23 (3 H, m), 2.30–2.58 (1 H, m), 2.93–3.18 (1 H, m), 3.50–3.70 (1 H, m), 3.79 (3 H, s), 3.92 (1 H, dd, J = 2, 5 Hz), 4.05–4.40 (4 H, m), 5.66 (1 H, d, J = 2 Hz). ^{13}C NMR (D_2O): δ 24.4, 26.9, 33.0, 54.7, 57.9, 58.9, 67.4, 69.1, 70.4, 74.4, 131.8, 139.1, 168.2, 176.3, 180.2. MS FAB (*m/z*): 357 (M + H)$^+$. Anal. ($C_{15}H_{20}N_2O_6S$) C, H, N. The same procedure allowed for the preparation of (5*R*,6*S*)-2-[[*N*-[(*n*-butyloxycarbonyl)methyl]-*N*-methylamino]methyl]-6-((*R*)-1-hydroxyethyl)-penem-3-carboxylic acid (**14a**). Yield: 56% after reverse phase column chromatography (70:30 v/v water/acetone). Pale yellow solid. Mp: 72 °C dec. HPLC assay (90:10 v/v 0.02 M phosphate buffer (pH = 3)/acetonitrile): 98% (t_R = 6.6 min). UV (nm): 250, 315. ^{1}H NMR (D_2O): δ 0.87 (3 H, t, J = 7.3 Hz), 1.25 (3 H, d, J = 6.4 Hz), 1.20–1.42 (2 H, m), 1.55–1.72 (2 H, m), 2.93 (3 H, s), 3.98 (1 H, dd, J = 1.5, 5.9 Hz), 4.07 (2 H, s), 4.12–4.34 (5 H, m), 5.69 (1 H, d, J = 1.5 Hz). ^{13}C NMR (D_2O): δ 17.3, 22.9, 24.4, 34.1, 45.6, 56.4, 59.6, 67.8, 69.0, 71.7, 74.7, 135.2, 140.5, 171.4, 172.9, 180.1. Anal. ($C_{16}H_{24}N_2O_6S$) C, H, N.

(5*R*,6*S*)-2-[(2*S*)-2-Carboxypyrrolidin-1-yl)methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (15). Pig liver esterase (0.40 mL) was added to a suspension of **14b** (580 mg, 1.6 mmol) in 20 mL of 0.033 M phosphate buffer (pH = 7). The resulting mixture was stirred at 25 °C and maintained at the desired pH with NaOH by using a pH-stat. The hydrolysis was allowed to proceed until the NaOH consumption stopped (36–48 h). Lyophilization followed by reverse phase column chromatography (95:5 v/v water/acetone) gave **15** as a white solid. Yield: 361 mg (66%). HPLC assay: 99% (t_R = 2.2 min). UV (nm): 255, 314. ^{1}H NMR (D_2O): δ 1.25 (3 H, d, J = 6.3 Hz), 1.75–2.24 (3 H, m), 2.30–2.54 (1 H, m), 3.05–3.40 (1 H, m), 3.64–3.86 (1 H, m), 3.95 (1 H, dd, J = 2, 5 Hz), 4.01–4.12 (1 H, m), 4.12–4.50 (3 H, m), 5.67 (1 H, d, J = 2 Hz). MS FAB (*m/z*): 343 (M + H)$^+$.

(5*R*,6*S*)-2-[[(2*S*,4*R*)-4-(Acetyloxy)-1-prolinamido]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (17a). **6r** (1.53 g, 3 mmol) was dissolved in dichloromethane (100 mL). Pyridine (0.27 mL, 3.3 mmol) and acetyl chloride (0.23 mL, 3.3 mmol) were added in sequence at 5–10 °C. The solution

was allowed to warm to 25 °C and stirred for 2 h. After the solution was washed with water, 5% HCl, 5% aqueous $NaHCO_3$, and water, the organic phase was dried over Na_2SO_4 and evaporated to give 1.48 g (2.67 mmol, 89%) of allyl (5*R*,6*S*)-2-[[(2*S*,4*R*)-4-(acetyloxy)-1-prolinamido]methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (**16a**). ^1H NMR ($CDCl_3$): δ 0.08 (6 H, s), 0.89 (9 H, s), 1.25 (3 H, d, *J* = 6.3 Hz), 2.05 (3 H, s), 2.00–2.22 (1 H, m), 2.30–2.39 (1 H, m), 2.54–2.70 (1 H, m), 3.46–3.80 (4 H, m), 4.15–4.35 (2 H, m), 4.55–4.85 (2 H, m), 5.12–5.22 (1 H, m), 5.22–5.48 (3 H, br overlapped to m), 5.58 (1 H, d, *J* = 1.5 Hz), 5.80–6.03 (1 H, m), 6.98 (1 H, br). Crude **16a** was dissolved in THF (60 mL) and allowed to react with acetic acid (0.92 mL, 16 mmol) and tetrabutylammonium fluoride (8 mL of a 1 M solution in THF, 8 mmol) as described before for **7a–u**, giving, after column chromatography (95:5 v/v ethyl acetate/methanol), 0.77 g (65%) of allyl (5*R*,6*S*)-2-[[(2*S*,4*R*)-4-(acetyloxy)-1-prolinamido]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylate, which was in turn dissolved in anhydrous THF and allowed to react with triphenylphosphine (46 mg, 0.17 mmol), tetrakis(triphenylphosphine)palladium (200 mg, 0.17 mmol), and acetic acid (0.15 mL, 2.6 mmol) as described above for **3a–u**. Reverse phase column chromatography (95:5 v/v water/acetone) gave **17a** as a white solid. Yield: 51%. Mp: 88 °C dec. HPLC assay (90:10 v/v 0.02 M phosphate buffer (pH = 3)/acetonitrile): 98% (t_R = 4.7 min). UV (nm): 257, 316. ^1H NMR (D_2O): δ 1.27 (3 H, d, *J* = 6.3 Hz), 2.09 (3 H, s), 2.12–2.20 (1 H, m), 2.22–2.50 (1 H, m), 2.88–3.02 (1 H, m), 3.58–3.74 (1 H, m), 3.75–4.12 (4 H, m), 4.12–4.30 (1 H, m), 5.22 (1 H, br), 5.63 (1 H, d, *J* = 1.5 Hz). ^{13}C NMR (D_2O): δ 24.9, 25.4, 41.3, 56.4, 63.2, 67.5, 69.6, 70.5, 74.7, 78.8, 134.1, 142.6, 165.5, 168.3, 179.3, 181.1. MS FAB (*m/z*): 400 $(M + H)^+$, 422 $(M + Na)^+$. Anal. ($C_{15}H_{20}N_4O_7S$) C, H, N.

(5*R*,6*S*)-2-[[(2*S*,4*R*)-4-(Carbamoyloxy)-1-prolinamido]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylic Acid (17b). A solution of **6r** (1.02 g, 2 mmol) in anhydrous THF (40 mL) was cooled to 0 °C. Trichloroacetyl isocyanate (0.26 mL, 2.2 mmol) was added under a nitrogen atmosphere. The resulting mixture was stirred at the same temperature for 30 min and at 20 °C for 1 h.[27] Acetic acid (0.68 mL, 12 mmol) and tetrabutylammonium fluoride (6 mL of a 1 M solution in THF, 6 mmol) were added, and the solution was stirred at room temperature for 48 h. The solvent was evaporated and the residue purified by column chromatography (95:5 v/v ethyl acetate/methanol), giving 573 mg of allyl (5*R*,6*S*)-2-[[(2*S*,4*R*)-4-(carbamoyloxy)-1-prolinamido]methyl]-6-((*R*)-1-hydroxyethyl)penem-3-carboxylate (yield: 65% from **6r**). The compound was dissolved in 100 mL of ethyl acetate containing 5% water and allowed to react with triphenylphosphine (34 mg, 0.13 mmol), tetrakis(triphenylphosphine)palladium (150 mg, 0.13 mmol), and acetic acid (0.11 mL, 1.95 mmol) as described above for **3a–u**. Reverse phase column chromatography (95:5 v/v water/acetone) gave **17b** as a white solid. Yield: 48%. Mp: 125 °C dec. HPLC assay (90:10 v/v 0.02 M phosphate buffer (pH = 3)/acetonitrile): 98% (t_R = 1.7 min). UV (nm): 256, 311. ^1H NMR (D_2O): δ 1.26 (3 H, d, *J* = 6.4 Hz), 1.98–2.20 (1 H, m), 2.25 (1 H, m), 2.80–2.95 (1 H, m), 3.40–4.13 (5 H, m), 4.13–4.29 (1 H, m), 5.14 (1 H, br s), 5.60 (1 H, s). ^{13}C NMR: δ 25.7, 41.6, 56.2, 63.1, 66.9, 69.5, 70.1, 74.2, 80.4, 130.5, 148.6, 158.2, 171.1, 180.9, 182.1. MS (ES) (*m/z*): 400 (M^+).

General Methods for the Synthesis of Amino Acid-Derived Amides (8a–u). Method A. 2-Chloroacetamide (14.4 g, 0.15 mol) was added, at 0–5 °C, to a 40% aqueous methylamine solution (39 mL, 0.45 mol). The resulting mixture was stirred at the same temperature until the 2-chloroacetamide had gone into solution. Reaction required about 5 h. Traces of unreacted reagent were removed by filtration, and the aqueous solution was concentrated to a final volume of 10 mL. Absolute ethanol (100 mL) was added; solid sarcosinamide hydrochloride was collected by filtration, washed with cold ethanol, and dried *in vacuo*. The compound was dissolved at 55 °C in dry methanol. The solution was allowed to cool to room temperature, and the stoichiometric amount of KOH was added as a 3.23 M solution in dry methanol. The resulting mixture was stirred for 10 min at room temperature and then for 15 min at 0 °C, filtered to remove solid KCl, and evaporated *in vacuo* to give 8.95 g (68%) of sarcosinamide **8a**.

Method B. A solution of *N*-(benzyloxycarbonyl)sarcosine (2.23 g, 10 mmol) in dichloromethane (20 mL) was cooled to 0 °C. *N*-Hydroxysuccinimide (1.27 g, 11 mmol) and *N,N'*-dicyclohexylcarbodiimide (11 mL of 1 M solution in dichloromethane, 11 mmol) were added, and the resulting mixture was stirred for 1 h at the same temperature. After standing overnight at 5 °C, the mixture was filtered. The desired amine (10 mmol) was added to the obtained solution at 5 °C and the mixture allowed to warm to room temperature and stirred for 1 h. The mixture was filtered and washed several times with water and the organic phase dried over Na_2SO_4. Evaporation of the solvent gave compounds **8h–j**.

Method C. Triethylamine (1.53 mL, 11 mmol) was added to a solution of *N*-Cbz or *N*-Boc amino acid (10 mmol) in chloroform (30 mL). The solution was cooled to 0 °C, and isobutyl chloroformate (1.43 mL, 11 mmol) was added dropwise under stirring. Stirring was continued for 15 min at the same temperature, and then gaseous NH_3 was bubbled through the solution for 30 min. The mixture was allowed to warm to room temperature and left overnight at the same temperature. Filtration and concentration of the solvent gave a residue which was dissolved in diethyl ether or ethyl acetate, washed with water, and dried over Na_2SO_4. Evaporation of the solvent gave compounds **8g** or **8m**.

Method D. Potassium carbonate (1.52 g, 11 mmol) was added to a solution of *N*-Cbz or *N*-Boc amino acid (10 mmol) in DMF (25 mL). After the mixture was stirred for 4 h at room temperature, alkyl iodide (11 mmol) was added. Stirring was continued for about 20 h, and then the mixture was filtered, diluted with ethyl acetate, and washed with water, 5% HCl, water, 5% $NaHCO_3$, and water. The organic phase was dried over Na_2SO_4 and evaporated to give N-protected amino acid esters. Corresponding amides could be obtained by treatment with aqueous 30% ammonia solution (24–48 h, room temperature).

Method E. Amberlyst 15 (1.3 g) was added to a solution of *N*-Cbz or *N*-Boc amino acid (10 mmol) in methanol (50 mL). After stirring at room temperature for 24–48 h, the mixture was filtered, and the solvent was evaporated *in vacuo*. The residue was diluted with ethyl acetate and the organic solution washed with aqueous 5% $NaHCO_3$ and water. The organic phase was dried over Na_2SO_4 and evaporated to give N-protected amino acid esters, from which amides could be obtained as described for Method D.

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(25) Testing **3q** on 28 *P. aeruginosa* clinical isolates, we obtained MIC values in a range from 16 to >64 μg/mL (MIC_{50} = 32 μg/mL, MIC_{90} = >64 μg/mL).

(26) Amide salts, as hydrochloride or trifluoroacetate, could be used directly in the procedure by simply adding, at this time, a second equivalent of triethylamine (0.70 mL, 5 mmol).

(27) Evaporation of the solvent at this time gave allyl (5*R*,6*S*)-2-[[(2*S*,4*R*)-4-[[*N*-(trichloroacetyl)carbamoyl]oxy]-1-prolinamido]-methyl]-6-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]ethyl]penem-3-carboxylate (**16b**) as a yellow foam: MS (TS) *m/e* 701 (M + H)$^+$.

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