SYNTHESES AND BIOLOGICAL ACTIVITIES OF NEW PENEM DERIVATIVES WITH SIDE CHAINS DERIVED FROM 4-HYDROXYPROLINE

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New penem derivatives with various substituted, enantiomerically pure pyrrolidine-thio side chains at the C-2 position were synthesized and their chemotherapeutic potentials assessed in comparison with Sch 29482. The following criteria were used for preliminary evaluation: Antibacterial activity *in vitro*, β-lactamase inhibition and apparent hydrolysis rates by crude murine and human kidney enzyme preparations. The most active compounds, **16e**, **16f** and **18** exhibit properties typical of this substance class with a tendency towards greater antibacterial potency in comparison with Sch 29482, especially against *Pseudomonas aeruginosa*. No clear-cut structure-activity relationships could be found with respect to β-lactamase inhibition and stability against degrading renal enzymes.

A considerable number of penem derivatives have already been described in the literature which exhibit powerful antibiotic activities *in vitro* against a wide range of bacteria. However, in many cases activity has not been satisfactory under *in vivo* conditions[1]. For example, sodium (5*R*,6*S*,8*R*)-2-ethylthio-6-hydroxyethylpenem-3-carboxylate (Sch 29482), the first representative to be tested clinically, was found to be rapidly metabolized in rodents and in man, and urinary recovery was rather low[2]. In addition, Sch 29482 was hydrolyzed by hog renal dipeptidase at a rate of about 1/3 of that of *N*-formimidoylthienamycin (imipenem)[3].

From the few structure-activity relationships already worked-out for penems it appears that a side chain at C-2 bearing a basic functionality at a slightly variable distance range relative to the nucleus is essential for achieving activity against *Pseudomonas aeruginosa*[4]. Bearing this in mind we were interested to investigate the extent to which an additional negative charge or a polar group with defined distance and stereochemistry, relative to the amino group in the side chain, would influence the chemotherapeutic properties of new penems.

To this end, we synthesized a series of new and appropriately substituted chiral pyrrolidine-thiols starting from optically active hydroxyprolines and transformed them into the penem derivatives **16a**~**g** and **18**. We wish to report here the synthetic routes that led to the desired penems. Their antibacterial activities *in vitro* were determined with a small but representative collection of bacterial strains. In addition, their inhibitory activities against various β-lactamases and the relative stabilities against kidney homogenates were compared with the corresponding data for Sch 29482 as standard. This set of criteria together with preliminary information on *in vivo* activity in mouse infection models was used for estimating the chemotherapeutic potential of compounds **16a**~**g** and **18**.

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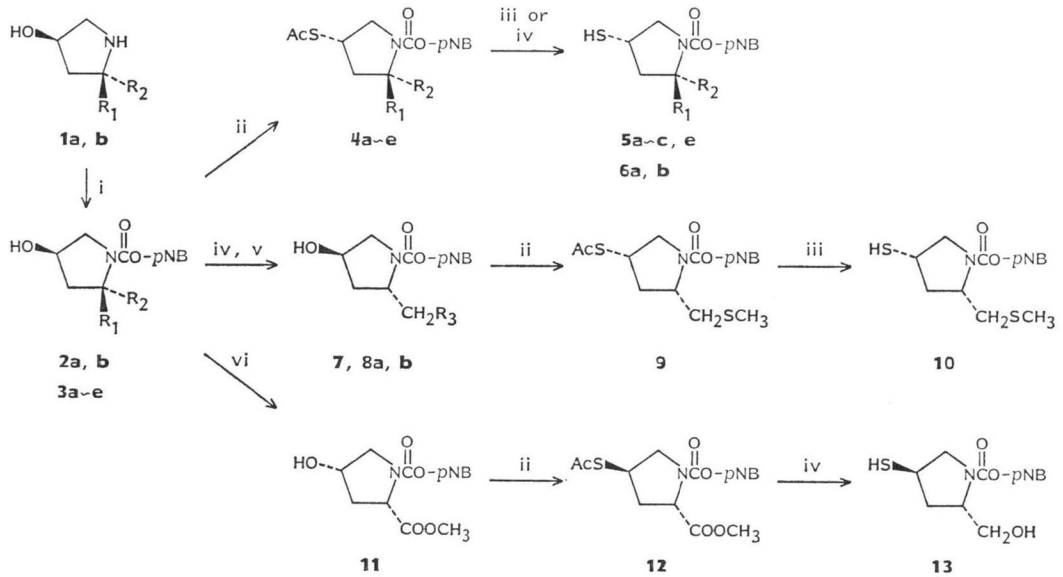
Chemistry

In order to avoid undue repetition in the multistep synthesis of each penem derivative we looked for a suitable synthetic intermediate which allowed the introduction of the side chain on C-2 of the penem nucleus at a late stage of the synthesis. It has been reported that sulfinyl groups in carbapenem-*S*-oxides[5,6] and penem-*S*-oxides[7] can be displaced by different sulfenyl groups. For our purpose,

Scheme 1.



1a	R_1=H,	R_2=COOH
1b	R_1=COOH,	R_2=H
2a	R_1=H,	R_2=COOH
2b	R_1=COOH,	R_2=H
3~5a	R_1=H,	R_2=COO-allyl
3~5b	R_1=COO-allyl,	R_2=H
3~5c	R_1=H,	R_2=$COOCH_3$
3~4d	R_1=$COOCH_3$,	R_2=H
3~5e	R_1=H,	R_2=$CONHCH_3$
6a	R_1=H,	R_2=CH_2OH
6b	R_1=CH_2OH,	R_2=H
7	R_3=OH	
8a	R_3=OSO_2CH_3	
8b	R_3=SCH_3	

i	**1a, 1b**	→ **2a, 2b:**	ClCOO*p*NB, NaOH, ether
	2a, 2b	→ **3a, 3b:**	CH_2=CH-CH_2OH, *p*-toluenesulfonic acid
	2a, 2b	→ **3c, 3d:**	MeOH, $SOCl_2$
	2a	→ **3e:**	BSA, dioxane, ClCOOEt, Et_3N, THF; CH_3NH_2
ii	**3a~d, 8b, 11**	→ **4a~d, 9, 12:**	PPh_3, DEAD, CH_3COSH
	3e	→ **4e:**	CH_3SO_2Cl, pyridine; CH_3COSK, DMF
iii	**4a~c+e, 9**	→ **5a~c+e, 10:**	NH_3, MeOH
iv	**4c, 4d, 3c, 12**	→ **6a, 6b, 7, 13:**	$NaBH_4$, $CaCl_2$
v	**7**	→ **8a:**	CH_3SO_2Cl, pyridine
	8a	→ **8b:**	$NaSCH_3$
vi	**3c**	→ **11:**	PPh_3, DEAD, HCOOH; NaOH (1 eq)

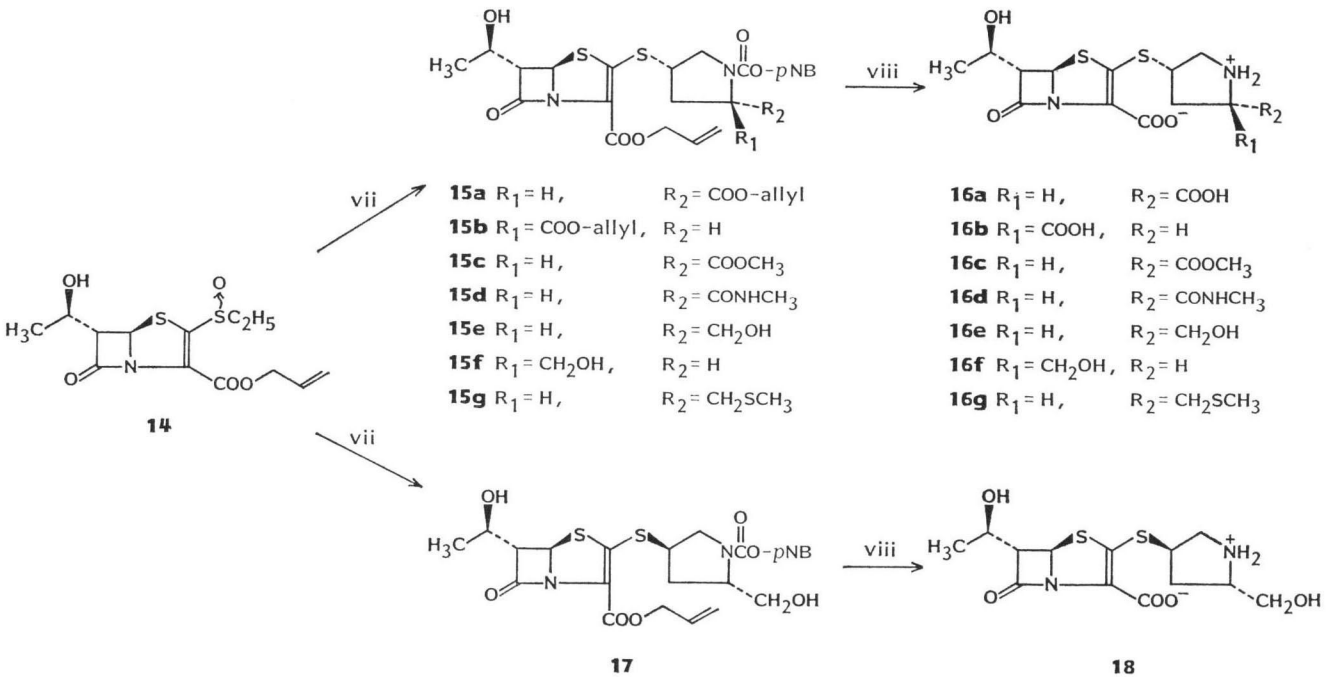
(*p*NB: *p*-Nitrobenzyloxycarbonyl)

new side chains not yet reported in the literature were required bearing a free thiol function and protected amino and carboxyl groups. The choice of protective groups was thereby critical, as deprotection after attachment to the penem nucleus had to occur under extremely mild conditions.

As starting materials for these side chains we chose (2*S*,4*R*)-4-hydroxyproline (**1a**) and its 2*R*,4*R* isomer (**1b**) which are both commercially available and treated them as shown in Scheme 1. The amino functions in both compounds were protected by a *p*-nitrobenzyloxycarbonyl group and the resulting carbamates **2a** and **2b** were transformed into the allyl esters **3a** and **3b** or into the methyl esters **3c** and **3d** following standard procedures. The *N*-methylamide **3e** was prepared from **2a** and CH_3NH_2 *via* a mixed carbonic anhydride. To avoid side reactions, the hydroxyl group of **2a** was silylated by bis(trimethylsilyl)acetamide (BSA) in dioxane prior to amide formation. The protective silyl group was lost during purification of the amide on silica gel and compound **3e** was obtained in 67% yield. The 4*R* hydroxyl group in **3a**~**d** was transformed in a modified MITSUNOBU reaction[8], using triphenylphosphine (TPP), diethyl azodicarboxylate (DEAD) and thioacetic acid, into the 4*S* thiolacetate group leading to the compounds **4a**~**d**. This method failed in the case of **3e** where we could not obtain any product even under more drastic conditions and with a large excess of reagent. The desired thiolacetate **4e** was finally obtained *via* the mesylate by substitution with potassium thioacetate in DMF. Liberation of the thiol function was achieved by treatment of **4a**~**c** and **4e** with a methanolic solution of NH_3 to give compounds **5a**~**c** and **5e**. The thiolacetate and ester groups in compounds **4c** and **4d** could be simultaneously reduced by $NaBH_4/CaCl_2$ without affecting the *p*-nitrobenzyloxycarbonyl group leading to the mercaptoprolinol derivatives **6a** and **6b**. The ester function of **3c** was reduced under the same conditions giving compound **7**. The hydroxymethyl function of **7** could be selectively transformed into the methylthiomethyl function *via* substitution of the mesylate with sodium methyl sulfide. The resulting compound **8b** was further converted into compound **10** *via* the thiolacetate **9** as already described for compounds **3c** and **3d**. The preparation of the 2*S*,4*R* isomer **13** by the same method would require (2*S*,4*S*)-4-hydroxyproline as starting material which is rather difficult to obtain. Therefore we devised a strategy to start from a suitably protected precursor with 2*S*,4*R* configuration (**3c**) and to transform it into the 2*S*,4*R* isomer **12** by double inversion at C-4. For this reason compound **3c** was transformed into its formate by the MITSUNOBU reaction and saponified to the 2*S*,4*S* configurated alcohol **11** which was treated under the same conditions as **3d**, leading to **13**.

The assignment of absolute configuration at C-4 of the diastereomers **4a**~**d**, **9** and **12** is based on spectroscopic methods and mechanistic considerations. When we treated the hydroxyl compounds **3a**~**d**, **8b** and **11** with TPP, DEAD and thioacetic acid we clearly obtained only one product in each case. Even under careful examination no second diastereomer could be detected. The appearance of duplicated signals in the ^1H NMR spectra of all *N*-protected compounds reported here when recorded in $CDCl_3$ at room temperature results from the existence of two rotational isomers. To ensure that these signals can be attributed to rotamers and not to diastereomers we also ran the ^1H NMR spectra in DMSO-d_6 at elevated temperatures where the duplicated signals collapsed into single ones. We can conclude therefore that substitution at C-4 proceeds stereospecifically. This is in good agreement with the literature[9] where it is well documented that the MITSUNOBU reaction of secondary alcohols generally is a clean S_N2 process. Furthermore compounds **3d**, **4d** and **6b** are indeed optical antipodes of **11**, **12** and **13**, exhibiting identical ^1H NMR spectra and optical rotations with the same magnitudes but opposite signs for each couple.

Scheme 2.



vii **14** → **15a**~**g**, **17**: **5a**~**c**+**e**, **6a**, **6b**, **10**, **13**, *i*Pr_2NEt, MeCN, −30°C
viii **15a**~**g**, **17** → **16a**~**g**, **18**: $Pd(PPh_3)_4$, TPP, Na-ethyl hexanoate; H_2, Pd/C

The penem-*S*-oxide **14**, prepared in 12 steps from 6-aminopenicillanic acid in analogy to already established methods[7,10,11,12], was treated with thiols **5a**~**c**, **6a**, **6b**, **10** and **13** in MeCN in the presence of HÜNIG base (iPr$_2$NEt) and gave compounds **15a**~**g** and **17** (Scheme 2). The allylic esters were cleaved by Pd(PPh$_3$)$_4$ and sodium 2-ethyl hexanoate[13] while *p*-nitrobenzyloxycarbonyl groups were removed by catalytical hydrogenation. The resulting final products **16a**~**g** and **18** were purified by reversed phase chromatography (RP 18).

Materials and Methods

Antibiotics

Schering penem (Sch 29482) was prepared by Dr. A. FRANK, Biochemie Ges.m.b.H., Kundl, Austria. Nitrocefin was kindly supplied by Glaxo Group Research Ltd., U.K. Glycyldehydrophenylalanine was synthesized by Dr. H. BEER of the Sandoz Forschungsinstitut, Vienna.

Assay for Antibacterial Activity *In Vitro*

The minimum inhibitory concentrations (MICs) were determined in 50 μl Tryptic soy broth (TSB-Difco) by serial two-fold dilutions of the test compounds (Dynatech MIC-2000-system). Bacterial strains were from our stock culture collection and were stored as suspensions in liquid nitrogen until used. After rapid thawing the bacteria were diluted to 10^6 colony forming units per ml in TSB for inoculation. The lowest concentration of a compound which resulted in complete inhibition of visible bacterial growth after 20 hours incubation at 37°C is recorded as the MIC.

Assay for β-Lactamase Inhibition

The β-lactamases were isolated from exponentially growing cultures of *Enterobacter cloacae* P99, *Escherichia coli* J5/R6K (TEM 1), *E. coli* p 453 (SHV 1), *E. coli* J5/R46 (OXA 2) and *Staphylococcus aureus* NCIB 11195 (PC 1) as previously described[14].

The β-lactamases were incubated with various concentrations of compounds **16a**~**g** and **18** in 2 ml of 100 mM potassium phosphate buffer at pH 7.0 for 5 minutes at 25°C and the enzyme activities were measured as previously published[14]. The concentrations causing 50% inhibition (I_{50}) were calculated from regression lines in which percentage inhibition was plotted against the inhibitor concentration.

Assay for Stability against Crude Kidney Enzymes

Fresh kidney tissue from NMRI mice and human kidney tissue after storage in liquid nitrogen were cut into small pieces and suspended in 2.5 volumes of 25 mM 3-(*N*-morpholino)propane sulfonic acid (MOPS) buffer at pH 7.0. The suspensions were homogenized and centrifuged at 1,200$\times g$ for 20 minutes at 4°C. The supernatants were dialyzed overnight against 25 mM MOPS buffer at pH 7.0 and stored in liquid nitrogen. The protein concentrations were 28 mg/ml for the mouse and 11 mg/ml for the human preparation respectively. The dipeptidase activities were checked spectrophotometrically using glycyldehydrophenylalanine as the substrate[15].

Compounds **16a**~**g** and **18** were incubated in 25 mM MOPS buffer at pH 7.0 with mouse or human kidney homogenates (50%) for 60 minutes at 35°C in a total volume of 200 μl. Control reactions for chemical stability were run with the test compounds in 25 mM MOPS buffer (pH 7.0) without kidney enzymes. Aliquots of 10 μl were removed at time 0 and appropriate intervals thereafter and assayed for residual biological activities making use of the β-lactamase inhibitory activities of penems similar to the method described by FRERE and coworkers[16]. Briefly, the aliquots were diluted into 2 ml of 100 mM phosphate buffer (pH 7.0) and incubated with P99 β-lactamase for 5 minutes at 25°C. Nitrocefin was added to a final concentration of 25 μM and its hydrolysis by the β-lactamase measured following the increase of absorption at 486 nm[15]. The inhibition of the P99 β-lactamase was calculated as described[14]. The residual concentrations of the test compounds (% c_o) were calculated from regression lines in which the percentage of β-lactamase inhibition was plotted against the inhibitor concentrations. The crude kidney enzymes did not exhibit any β-lactamase inhibitory activity.

Table 1. Antibacterial activity *in vitro*, MIC-ranges in μg/ml against

Bacterial species	n	16a	16b	16c
Pseudomonas aeruginosa	2	25~50	>50	50
Escherichia coli	4	0.125~0.156	0.5~0.78	0.125~0.25
Enterobacter cloacae	3	0.062~1.25	0.25~6.25	0.062~1.56
Klebsiella pneumoniae	5	0.125~0.25	0.25~0.5	0.125~0.25
Serratia marcescens	3	0.25~0.625	0.78~1.56	0.25 ~0.5
Proteus vulgaris	2	0.125~1	0.25~3.12	0.125~0.25
P. mirabilis	2	1~1.56	3.12~12.5	0.5~1
Staphylococcus aureus	8	0.25~6.25	0.156~3.12	0.062~1.56
S. epidermidis	1	0.125	0.25	0.125
Streptococcus pyogenes	2	0.015	0.062~0.312	0.015
Enterococcus faecalis	2	25~50	25	25

n=No. of isolates.

Results

Antibacterial Properties

The *in vitro* antimicrobial activity of the compounds **16a~g** and **18** against a selection of representative Gram-positive and Gram-negative bacteria is reported in Table 1. In general, these derivatives show excellent antibacterial properties comparable to or better than those of Sch 29482. Against Gram-positive bacteria the compounds **16e~g** and **18** are more active than the compounds **16a~d** but this trend is not found with activities against Gram-negative bacteria. In comparison with **16a**, **16c**, **16d** and **16g**, the compounds **16b**, **16e**, **16f** and **18** show decreased activity against *Proteus mirabilis*, but on the other hand have enhanced activity against *P. aeruginosa*. In the case of the other Gram-negative bacterial strains no significant difference is found between MIC values for the test compounds, with exception of **16b**, which is generally weaker against all Gram-negative bacteria. It should be noted that the isomers **16e**, **16f** and **18** have a similar spectrum and MICs, whereas **16a** is significantly more active than **16b** against Gram-negative bacteria while having equal activity against Gram-positive bacteria.

In two of our standard septicemic mouse models using strains of *Streptococcus pyogenes* and *E. coli* as pathogens, only **16e** appeared to be superior to Sch 29482 with respect to ED_{50} values after sc and po administration. Although, serum levels and urinary excretion of **16e** were significantly lower than those of Sch 29482 using either form of administrations (data not shown).

β-Lactamase Inhibition

It has been reported that the penem derivative Sch 29482 inhibits β-lactamases of the cephalosporinase type as well as the OXA 2 and OXA 3 β-lactamases, but does not inactivate the plasmid mediated β-lactamases TEM, SHV 1 or most of the PSE enzymes[17]. We tested the inhibitory effect of our penem derivatives against a set of clinically important β-lactamases. All compounds inhibited the P99 β-lactamase from *E. cloacae*. The I_{50} values after a 5-minute preincubation of the enzyme with the penems ranged between 0.2 and 0.7 μM. Similar activities were calculated for the inhibition of the OXA 2 β-lactamase from *E. coli* R46. Most of the compounds tested did not inhibit the TEM 1 and SHV 1 enzymes from *E. coli* R6K and *E. coli* p 453, respectively, at concentrations below 50 μM. However, **16a** and **16f** differ from the remaining test compounds as they exhibit a broader spectrum

several bacterial species (test medium: Tryptic soy broth).

Compound					
16d	**16e**	**16f**	**16g**	**18**	Sch 29482
50～>50	10～25	12.5～50	50～>50	12.5～50	50
0.125～0.25	0.312	0.5	0.25～0.5	0.5～1	0.625
0.125～1	0.156～2.5	0.5～1.56	0.125～1.56	0.25～1	0.156～5
0.125～0.25	0.312～0.625	0.25～0.5	0.5	0.25～1	0.312～0.625
0.25～0.5	0.625	1	0.5～1	1	1.25～5
0.125～0.25	0.156～2.5	0.25～3.125	0.25～1	0.25～1.56	0.125～0.5
0.5	5	3.125～12.5	1～3.125	3.125～6.25	1.25
0.062～1.56	0.02～1.25	0.02～1.56	0.02～0.5	0.02～0.5	0.06～5
0.125	0.02	0.03	0.03	0.03	0.06
0.008～0.016	≤0.01	0.004	0.004	0.004	0.031
6.25～12.5	10	6.25～12.5	6.25～12.5	3.125～6.25	6.25

of β-lactamase inhibitory activity. These two compounds inhibited also the TEM 1 and SHV 1 β-lactamases with I_{50} values ranging from 6 to 14 μM. It should be noted that the corresponding isomers, **16b** and **16e** did not show these broad β-lactamase inhibitory activities. In our test system Sch 29482 inhibited the TEM 1 and SHV 1 enzymes, although 20～50 times weaker than the P99 and OXA 2 β-lactamases and this resembles to the activities of **16a** and **16f**. None of the penems tested inactivated the staphylococcal β-lactamase PC 1. Lack of inhibition of some of the β-lactamases cannot be ascribed to a rapid hydrolysis of the penems during the 5-minute preincubation period in our assay. Penems have been reported to be stable against the hydrolysis by β-lactamases[17,18] and likewise our test compounds were completely stable towards a concentrated mixture of the 5 β-lactamases P99, TEM 1, SHV 1, OXA 2, and PC 1 for at least 2 hours at 25°C (data not shown).

Stability towards Crude Kidney Enzymes

The test compounds **16a～g** and **18** were susceptible towards hydrolysis by renal enzymes although to a varying degree. After 60 minutes incubation in mouse kidney homogenate the residual concentrations of biologically active penems ranged between 6% for **16d** and 69% for **16b** of the initial concentrations at time 0. Using human kidney homogenate the respective values were between 54% for **18** and 84% for **16b**. The values for Sch 29482 are 16% in mouse and 14% in human kidney homogenates. Among the test compounds described herein, **16b** is thus the most stable one against hydrolyses by mouse and human kidney homogenates. It should be noted that **16a**, **16c** and especially **16d** were more readily hydrolyzed by the mouse than by the human kidney homogenate, whereas with compounds **16b**, **16e**, **16f**, **16g** and **18** this effect was much less pronounced. In control experiments it was shown that all compounds were stable in buffer at 35°C for 1 hour, therefore chemical instability during the test can be excluded. In addition, reversible binding of the penems to proteins of the homogenate cannot be responsible for the decrease in biological activity, since the aliquots taken from these incubation mixtures were immediately diluted 1: 200 with buffer. This procedure should liberate reversibly bound penems. With the crude enzyme preparations we cannot, however, exclude the possibility that the penems were inactivated by proteins or enzymes other than the renal dipeptidase. The inactivation of Sch 29482 by purified hog kidney dipeptidase[2] makes this possibility rather unlikely, but it would have to be proven with purified mouse and human renal dipeptidases in which the

differences in the substrate specificities could also be elucidated.

Discussion

All compounds reported in this paper exhibit excellent antibacterial activities *in vitro* against a representative set of pathogenic bacterial strains, with a tendency towards lower MICs than those for Sch 29482. **16e** showed even modest activity *in vitro* against *P. aeruginosa*. From our results, it appears that this improvement in spectrum is brought about by the hydroxymethyl group in α-position to the amino function at the side chain. Replacement of this substructure by carboxylate (**16a, 16b**), ester (**16c**) or methylamido (**16d**) functions always results in significant loss of activity against *P. aeruginosa*.

When correlating stereochemical relationship with antibacterial activity we must take into account that our C-2 side chains possess two chiral centers which bear a 1,3-relationship. Therefore we have to consider the absolute configurations of the two asymmetric carbon atoms and their relative configurations. The C-2 side chains of the compounds **16a**, **16c**, **16d**, **16e** and **16g** have all *SS* configuration and differ only in the nature of the functional group in α-position to the amino groups. Furthermore these functional groups are in *cis* 1,3-relationship to the thioether groups which link the side chains to the penem nuclei. The side chains of **16b**, **16f** and **18** are *RS*(*SR*) configurated and their substituents are in *trans* 1,3-orientation. In addition the C-2 side chains of **16a**, **16c**, **16d**, **16e**, **16g** and **18** are derivatives of L-proline, in contrast to those ones of **16b** and **16f** which are derivatives of D-proline.

We were surprised to find that there is no clear correlationship between antibacterial activity *in vitro* and relative configuration of side chains with identical functional groups. This picture becomes much more complex when also taking into account our data on inhibition of β-lactamases and degradation by kidney enzymes.

Compounds **16e**, **16f** and **18** with similar MICs against bacteria are also degraded by renal enzymes at a similar rate. **16a**, in comparison with **16b** is somewhat more active *in vitro* against Gram-negative bacteria but also metabolized faster than **16b**. Compounds **16a** and **16f** showed good inhibition of TEM 1 and SHV 1 β-lactamases, a property not shared by the other compounds. At present we cannot offer an explanation for this divergent behavior.

This conflicting set of information did not predict the superiority of compound **16e** over Sch 29482 in septicemic infection models. The basis of this superiority is not clear as serum levels and urinary excretions turned out to be substantially lower after iv and po treatment than those obtained for Sch 29482 under identical conditions (data not shown).

Experimental

General

Melting points were taken on a Kofler hot stage melting point apparatus (Reichert) and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter (concentration in g/100 ml). IR and UV spectra were obtained with Perkin-Elmer 198 and Beckman DK-2A spectrophotometers, respectively. NMR spectra were recorded in solutions at 90 MHz (Bruker WH 90) or 250 MHz (Bruker WM 250) using TMS or sodium 3-(trimethylsilyl)propionate-d_4 as internal standard. Most of the *N*-protected proline derivatives exist in $CDCl_3$ solution at room temp in two rotameric forms. In these cases the ^1H NMR spectra of the mixtures of the rotamers are described. The purity of the products was checked by HPLC (pump: Waters 6000) on a column (250×4.6 mm) of RP 18 (0.01 mm) using phosphate buffer (0.01 N, pH 7) in H_2O - MeCN gradient and a Schoeffel SF 770 UV detector. Commercially available THF was distilled just prior to use, under argon, from $LiAlH_4$.

(2*S*,4*R*)-4-Hydroxy-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxylic Acid (**2a**)

To a solution of **1a** (10.4 g, 80 mmol) in 4 N NaOH (50 ml), water (100 ml) and ether (100 ml) were added followed by dropwise addition of a solution of *p*-nitrobenzyl chloroformate (21.6 g, 100

mmol) in ether (100 ml) at 0°C and the resulting mixture was stirred overnight at room temp. The precipitate which formed was filtered off, the organic layer was washed with EtOAc, acidified with 1 N HCl and extracted with EtOAc. This extract was washed with H_2O and dried over Na_2SO_4. After evaporation of the solvent *in vacuo* **2a** (24.1 g, 97%) was obtained as a white solid which was used for the next reaction without further purification. An analytical sample was recrystallized from EtOAc and diisopropyl ether: mp 135~136°C, $[\alpha]_D^{25}$ −47.9° (*c* 1.0, CH_2Cl_2), ^{1}H NMR (90 MHz, $CDCl_3$) δ 8.24+7.56 and 8.22+7.46 (4H, each AA'BB', J=8 Hz, aromatic), 5.25 and 5.32+5.13 (2H, s and ABq, J=13.5 Hz, CH_2), 4.54 (1H, dd, J_1=15 Hz, J_2=7.5 Hz, 2-H), 4.54 (1H, br s, 4-H), 3.68 (2H, m, 5a-H, 5b-H), 2.40 (1H, m, 3a-H), 2.18 (1H, m, 3b-H).

(2*R*,4*R*)-4-Hydroxy-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxylic Acid (**2b**)

^{1}H NMR (90 MHz, $CDCl_3$) δ 8.24+7.54 and 8.22+7.48 (4H, each AA'BB', J=8 Hz, aromatic), 6.18 (2H, br s, OH, COOH), 5.28 and 5.35+4.97 (s and ABq, J=16 Hz, CH_2), 4.48 (2H, m, 2-H, 4-H), 3.68 (2H, m, 5a-H, 5b-H), 2.32 (2H, 3a-H, 3b-H).

Allyl (2*S*,4*R*)-4-Hydroxy-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxylate (**3a**)

The *N*-protected amino acid **2a** (15.5 g, 50 mmol) was heated in benzene (350 ml) with allylic alcohol (100 ml) and a catalytical amount of *p*-toluenesulfonic acid. The water which was formed during the reaction was separated by azeotropic distillation. After 4 hours the reaction mixture was cooled to room temp, washed with satd $NaHCO_3$ solution followed by H_2O. The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel with a mixture of hexane - EtOAc (1 : 2) to yield **3a** (15.5 g, 96%, colorless oil): ^{1}H NMR (90 MHz, $CDCl_3$) δ 8.16+7.54 and 8.16+7.50 (4H, each AA'BB', J=8 Hz, aromatic), 5.86 (1H, m, vinylic), 5.34 (2H, m, vinylic), 5.26 and 5.37+5.15 (2H, s and ABq, J=12.5 Hz, CH_2), 4.60 (4H, m, 2-H, 4-H, allylic), 3.70 (2H, m, 5a-H, 5b-H), 2.30 (1H, m, 3b-H), 2.14 (2H, m, 3a-H, OH).

Allyl (2*R*,4*R*)-4-Hydroxy-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxylate (**3b**)

The esterification of **2b** was performed in the same way as described in the preparation of **3a** with the exception that the mixture was refluxed for 18 hours to get complete consumption of **2b**. After work up **3b** was obtained as a colorless oil in 97% yield: IR ($CHCl_3$) cm^{-1} 3460, 2950, 1705, 1610, 1520, 1425, 1400, 1342; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.22+7.55 and 8.20+7.47 (4H, each AA'BB', J=8 Hz, aromatic), 5.89 (1H, m, vinylic), 5.31 (2H, m, vinylic), 5.27 and 5.32+5.13 (2H, s and ABq, J=13.5 Hz, CH_2), 4.62 (2H, m, allylic), 4.49 (1H, dd, J_1=9.5 Hz, J_2=2 Hz, 2-H), 4.44 (1H, br, s, 4-H), 3.69 (2H, m, 5a-H, 5b-H), 3.31 and 3.12 (1H, each br s, OH), 2.41 and 2.39 (1H, each ddd, J_1=14 Hz, J_2=11 Hz, J_3=9.5 Hz, 3a-H), 2.23 and 2.17 (1H, each ddd, J_1=14 Hz, J_2=3.5 Hz, J_3=2 Hz, 3b-H).

Methyl (2*S*,4*R*)-4-Hydroxy-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxylate (**3c**)

To a stirred solution of **2a** (24.8 g, 80 mmol) in MeOH (400 ml), $SOCl_2$ (34.8 ml, 480 mmol) was added dropwise at 0°C. After addition was complete the reaction was refluxed for 4 hours. Afterwards the solution was cooled, concentrated *in vacuo*, diluted with MeOH and evaporated again. The residue was chromatographed on silica gel with a mixture of hexane - EtOAc (1 : 5) followed by EtOAc to give **3c** (23.4 g, 90%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 8.24+7.52 and 8.22+7.48 (4H, each AA'BB', J=8 Hz, aromatic), 5.26 and 5.29+5.13 (2H, s and ABq, J=14.5 Hz, CH_2), 4.54 (2H, m, 2-H, 4-H), 3.76 and 3.70 (5H, 2s+m, OCH_3, 5a-H, 5b-H), 2.40 (1H, m, 3b-H), 2.14 (2H, m, 3a-H, OH).

Methyl (2*R*,4*R*)-4-Hydroxy-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxylate (**3d**)

$[\alpha]_D^{25}$ +12.6° (*c* 1.0, CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.25+7.53 and 8.24+7.48 (4H, each AA'BB', J=8 Hz, aromatic), 5.24 and 5.32+5.13 (2H, s and ABq, J=13.5 Hz, CH_2), 4.52 (1H, dd, J_1=9.5 Hz, J_2=2 Hz, 2-H), 4.43 (1H, m, 4-H), 3.80 and 3.71 (3H, each s, OCH_3), 3.70 (2H, m, 5a-H, 5b-H), 3.35 and 3.10 (1H, each d, J=8 Hz, OH), 2.40 and 2.38 (1H, each ddd, J_1=14 Hz, J_2=11 Hz, J_3=9.5 Hz, 3a-H), 2.22 and 2.18 (1H, each ddd, J_1=14 Hz, J_2=3.5 Hz, J_3=2 Hz, 3b-H).

N-Methyl (2*S*,4*R*)-4-Hydroxy-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxamide (**3e**)

BSA (4.41 ml, 18 mmol) was added to a solution of **2a** (1.86 g, 6 mmol) in anhydrous dioxane

(60 ml) and the resulting mixture refluxed for 6 hours. After cooling, MeOH (60 ml) was added, the solvents were evaporated *in vacuo*, the residue was taken up in toluene and the solvent evaporated again. The remaining residue was redissolved in THF (80 ml) and treated with Et_3N (0.83 ml, 6 mmol) and ethyl chloroformate (2.86 ml, 30 mmol) at −5°C. The mixture was stirred for 1 hour at room temp and afterwards the solvent and the excess of reagent were evaporated *in vacuo*. The residue was redissolved in THF (80 ml) and added dropwise to a 33% (w/v) solution of $MeNH_2$ in EtOH (3 ml, 24 mmol). After stirring overnight and concentration *in vacuo* the residue was purified by column chromatography on silica gel using a mixture of CH_2Cl_2 - MeOH (9: 1) to yield **3e** (1.3 g, 67%, colorless solid): mp 180°C (EtOAc, Et_2O); $[\alpha]_D^{25}$ −35.5° (*c* 1.0, CH_2Cl_2); ^1H NMR (90 MHz, $CDCl_3$ - DMSO-d_6 (4: 1)) δ 8.24+7.52 (4H, AA'BB', J=8 Hz, aromatic), 6.74 (1H, br s, CONH), 5.24 and 5.29+5.19 (2H, s and ABq, J=14.5 Hz, CH_2), 4.44 (2H, m, 2-H, 4-H), 4.24 (1H, m, OH), 3.65 (2H, m, 5a-H, 5b-H), 2.83 and 2.78 (3H, each s, $CONCH_3$), 2.50~2.10 (2H, m, 3a-H, 3b-H).

Allyl (2*S*,4*S*)-4-Acetylthio-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxylate (**4a**)

TPP (2.1 g, 8 mmol) and **3a** (2.2 g, 6.3 mmol) were dissolved in THF (50 ml) and cooled to −10°C. A solution of DEAD (1.26 ml, 8 mmol) in THF (20 ml) was added dropwise under stirring followed by a solution of thioacetic acid (0.57 ml, 8 mmol) in THF (10 ml). Stirring was continued for 30 minutes at −10°C and for 1 hour at room temp. The solvent was evaporated *in vacuo* and the residue purified by column chromatography on silica gel with mixtures of hexane - EtOAc (4: 1 and 1: 1) to yield **4a** (2.78 g, 85%): $[\alpha]_D^{25}$ −34.6° (*c* 1.03, CH_2Cl_2); IR (CH_2Cl_2) cm^{-1} 2939, 1750, 1710, 1608, 1525, 1403, 1346; ^1H NMR (250 MHz, $CDCl_3$) δ 8.26+7.52 and 8.22+7.47 (4H, each AA'BB', J=8 Hz, aromatic), 5.88 (1H, m, vinylic), 5.28 (2H, m, vinylic), 5.25 and 5.32+5.13 (2H, s and ABq, J=13.5 Hz, CH_2), 4.63 (2H, m, allylic), 4.47 and 4.46 (1H, each dd, J_1=8 Hz, J_2=6.5 Hz, 2-H), 4.10 (1H, dd, J_1=10.5 Hz, J_2=7 Hz, 5a-H), 4.03 (1H, dddd, J_1=7 Hz, J_2=7 Hz, J_3=7 Hz, J_4=7 Hz, 4-H), 3.45 (1H, dd, J_1=10.5 Hz, J_2=7 Hz, 5b-H), 2.80 (1H, m, 3b-H), 2.57 and 2.35 (3H, each s, $SCOCH_3$), 2.05 (1H, m, 3a-H).

Anal Calcd for $C_{18}H_{20}N_2O_7S$: C 52.93, H 4.94, N 6.86, S 7.85.
Found: C 52.38, H 5.07, N 7.27, S 7.81.

Allyl (2*R*,4*S*)-4-Acetylthio-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxylate (**4b**)

$[\alpha]_D^{25}$ +17.5° (*c* 1.03, CH_2Cl_2); ^1H NMR (250 MHz, $CDCl_3$) δ 8.26+7.51 and 8.24+7.46 (4H, each AA'BB', J=8 Hz, aromatic), 5.90 (1H, m, vinylic), 5.30 (2H, m, vinylic), 5.26 and 5.32+5.13 (2H, s and ABq, J=13.5 Hz, CH_2), 4.62 (2H, dd, J_1=19 Hz, J_2=5.5 Hz, allylic), 4.50 and 4.48 (1H, each dd, J_1=9 Hz, J_2=4.5 Hz, 2-H), 4.08 (2H, m, 4-H, 5a-H), 3.51 (1H, m, 5b-H), 2.56 and 2.35 (3H, each s, $SCOCH_3$), 2.40 (2H, m, 3a-H, 3b-H).

Methyl (2*S*,4*S*)-4-Acetylthio-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxylate (**4c**)

$[\alpha]_D^{25}$ −38.7° (*c* 1.08, CH_2Cl_2); IR (CH_2Cl_2) cm^{-1} 2950, 1752, 1709, 1525, 1403, 1346; ^1H NMR (250 MHz, $CDCl_3$) δ 8.24+7.53 and 8.23+7.47 (4H, each AA'BB', J=8 Hz, aromatic), 5.25 and 5.32+5.13 (2H, s and ABq, J=13.5 Hz, CH_2), 4.45 (1H, dd, J_1=8.5 Hz, J_2=6 Hz, 2-H), 4.09 (1H, dd, J_1=10.5 Hz, J_2=7 Hz, 5a-H), 4.03 (1H, dddd, J_1=7 Hz, J_2=7 Hz, J_3=7 Hz, J_4=7 Hz, 4-H), 3.78 and 3.69 (3H, each s, OCH_3), 3.45 (1H, dd, J_1=10.5 Hz, J_2=7 Hz, 5b-H), 2.79 (1H, m, 3b-H), 2.35 (3H, s, $SCOCH_3$), 2.06 (1H, m, 3a-H).

Anal Calcd for $C_{16}H_{18}N_2O_7S$: C 50.26, H 4.74, N 7.33.
Found: C 50.11, H 4.69, N 7.16.

Methyl (2*R*,4*S*)-4-Acetylthio-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxylate (**4d**)

$[\alpha]_D^{25}$ +15.5° (*c* 0.55, CH_2Cl_2); IR (CH_2Cl_2) cm^{-1} 2952, 1748, 1708, 1524, 1403, 1346; ^1H NMR (250 MHz, $CDCl_3$) δ 8.24+7.52 and 8.23+7.47 (4H, each AA'BB', J=8 Hz, aromatic), 5.25 and 5.32+5.13 (2H, s and ABq, J=13.5 Hz, CH_2), 4.48 and 4.46 (1H, dd, J_1=9 Hz, J_2=4.5 Hz, 2-H), 4.07 (2H, m, 4-H, 5a-H), 3.77 and 3.69 (3H, each s, OCH_3), 3.48 (1H, dd, J_1=16 Hz, J_2=4.5 Hz, 5b-H), 2.34 (5H, s, $SCOCH_3$; m, 3a-H, 3b-H).

(3*S*,5*S*)-5-Methylthiomethyl-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-3-thiol Acetate (**9**)

IR (KBr) cm^{-1} 3480, 3275, 3100, 2950, 1690, 1650, 1520, 1403, 1340; ^1H NMR (90 MHz, $CDCl_3$)

δ 8.26+7.54 (4H, AA'BB', J=8 Hz, aromatic), 5.24 (2H, s, CH_2), 4.20~3.70 (3H, m, 2a-H, 3-H, 5-H), 3.28 (1H, dd, J_1=11 Hz, J_2=6.5 Hz, 2b-H), 3.10~2.60 (3H, m, 4b-H, CH_2S), 2.35 (3H, s, $SCOCH_3$), 2.12 (3H, s, SCH_3), 1.90 (1H, m, 4a-H).

Methyl (2*S*,4*R*)-4-Acetylthio-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxylate (**12**)

IR and ^{1}H NMR identical with **4d**.

N-Methyl (2*S*,4*S*)-4-Acetylthio-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxamide (**4e**)

To a solution of **3e** (488 mg, 1.5 mmol) in pyridine (2 ml), CH_3SO_2Cl (0.14 ml, 1.8 mmol) was added dropwise at 0°C and the solution was kept at 4°C overnight. The reaction mixture was worked up by pouring it on a mixture of ice and 0.1 N HCl followed by extraction with EtOAc. The organic layer was washed with H_2O and dried with Na_2SO_4 leading to 636 mg of a crude product in form of a brownish powder which was used without further purification. It was dissolved in DMF (14 ml) and stirred with potassium thioacetate (856 mg, 7.5 mmol) at 50°C for 10 hours. After cooling, the reaction mixture was diluted with EtOAc, washed 5 times with H_2O and dried over Na_2SO_4. After evaporation of the solvent *in vacuo* the residue was chromatographed by silica gel with EtOAc to give **4e** (390 mg, 68%): mp 181°C (EtOAc, Et_2O); $[\alpha]_D^{25}$ −69.4° (*c* 1.0, CH_2Cl_2); IR (CH_2Cl_2) cm^{-1} 3447, 2996, 1691, 1524, 1402, 1347; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.25+7.51 (4H, each AA'BB', J=8 Hz, aromatic), 6.55 and 6.03 (1H, each br s, CONH), 5.27 and 5.29+5.23 (2H, s and ABq, J=12.5 Hz, CH_2), 4.37 (1H, dd, J_1=8 Hz, J_2=5.5 Hz, 2-H), 4.14 (1H, dd, J_1=11.5 Hz, J_2=7.5 Hz, 5a-H), 4.00 (1H, dddd, J_1=7.5 Hz, J_2=7.5 Hz, J_3=6.5 Hz, J_4=6.5 Hz, 4-H), 3.20 (1H, dd, J_1=11.5 Hz, J_2=6.5 Hz, m, 5b-H), 2.84 and 2.82 (3H, each s, NCH_3), 2.20~2.50 (2H, m, 3a-H, 3b-H), 2.35 (3H, s, $SCOCH_3$).

Anal Calcd for $C_{16}H_{19}N_3O_6S$: C 50.38, H 5.02, N 11.01.
Found: C 50.36, H 5.00, N 10.84.

Allyl (2*S*,4*S*)-4-Mercapto-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxylate (**5a**)

18 ml of a satd solution of NH_3 in MeOH were added to a solution of **4a** (950 mg, 2.33 mmol) in MeOH (80 ml) under an atmosphere of argon at 0°C. After 1 hour the solution was concentrated *in vacuo* and the residue was purified by column chromatography on silica gel using mixtures of hexane - EtOAc (2:1 and 1:1) to yield **5a** (530 mg, 62%): $[\alpha]_D^{25}$ −33.7° (*c* 1.09, CH_2Cl_2); IR (CH_2Cl_2) cm^{-1} 2939, 1746, 1709, 1522, 1405, 1347; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.24+7.52 and 8.21+7.46 (2H, each AA'BB', J=8 Hz, aromatic), 5.88 (1H, m, vinylic), 5.29 (2H, m, vinylic), 5.25 and 5.32+5.13 (2H, s and ABq, J=13.5 Hz, CH_2), 4.63 (2H, m, allylic), 4.42 (1H, dd, J_1=8 Hz, J_2=8 Hz, 2-H), 4.07 (1H, dd, J_1=10 Hz, J_2=4 Hz, 5a-H), 3.39 (2H, m, 4-H, 5b-H), 2.78 (1H, m, 3b-H), 2.02 (1H, m, 3a-H), 1.84 (1H, d, J=6.5 Hz, SH).

Allyl (2*R*,4*S*)-4-Mercapto-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxylate (**5b**)

$[\alpha]_D^{25}$ +35.6° (*c* 0.7, CH_2Cl_2); IR (CH_2Cl_2) cm^{-1} 2942, 1744, 1709, 1522, 1405, 1347; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.26+7.54 and 8.21+7.48 (4H, each AA'BB', J=8 Hz, aromatic), 5.86 (1H, m, vinylic), 5.29 (2H, m, vinylic), 5.25 and 5.32+5.13 (2H, s and ABq, J=13.5 Hz, CH_2), 4.62 (2H, dd, J_1=20 Hz, J_2=5.5 Hz, allylic), 4.54 (1H, dd, J_1=9 Hz, J_2=4 Hz, 2-H), 4.05 and 4.00 (1H, each dd, J_1=11 Hz, J_2=6.5 Hz, 5a-H), 3.57 (1H, m, 4-H), 3.44 and 3.39 (1H, each dd, J_1=11 Hz, J_2=6.5 Hz, 5b-H), 2.41 (1H, m, 3b-H), 2.24 (1H, m, 3a-H), 1.74 and 1.62 (1H, d, J=7.5 Hz, and s, SH).

Methyl (2*S*,4*S*)-4-Mercapto-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxylate (**5c**)

^{1}H NMR (90 MHz, $CDCl_3$) δ 8.16+7.54 and 8.16+7.48 (4H, each AA'BB', J=8 Hz, aromatic), 5.13 and 5.32+5.14 (2H, s and ABq, J=13.5 Hz, CH_2), 4.40 (1H, dd, J_1=7.5 Hz, J_2=7.5 Hz, 2-H), 4.04 (1H, m, 5a-H), 3.76 and 3.68 (3H, 2s, OCH_3), 3.40 (2H, m, 4-H, 5b-H), 2.76 (1H, m, 3a-H), 2.04 (1H, m, 3b-H), 1.82 (1H, d, J=7 Hz, SH).

N-Methyl (2*S*,4*S*)-4-Mercapto-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxamide (**5e**)

^{1}H NMR (90 MHz, $CDCl_3$) δ 8.25+7.52 (4H, AA'BB', J=8 Hz, aromatic), 6.40 and 6.12 (1H, each m, CONH), 5.26 and 5.29+5.23 (2H, s and ABq, J=12.5 Hz, CH_2), 4.31 (1H, dd, J_1=8 Hz, J_2=6.5 Hz, 2-H), 4.08 (1H, m, 5a-H), 3.38 (2H, m, 4-H, 5b-H), 2.85 and 2.80 (3H, each s, NCH_3), 2.80~

2.20 (2H, m, 3a-H, 3b-H), 1.90 (1H, d, J=6.5 Hz, SH).

(3*S*,5*S*)-5-Methylthiomethyl-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-3-thiol (**10**)

^{1}H NMR (90 MHz, $CDCl_3$) δ 8.26+7.54 (4H, AA'BB', J=8 Hz, aromatic), 5.24 (2H, s, CH_2), 4.08 (2H, m, 2a-H, 5-H), 3.26 (2H, m, 2b-H, 3-H), 2.92 (2H, m, CH_2S), 2.62 (1H, m, 4b-H), 2.12 (3H, s, SCH_3), 1.80 (1H, m, 4a-H), 1.72 (1H, d, J=6.5 Hz, SH).

(3*S*,5*S*)-5-Hydroxymethyl-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-3-thiol (**6a**)

At −30°C a solution of $NaBH_4$ (1.62 g, 42.8 mmol) in EtOH (25 ml) was added to a solution of powdered $CaCl_2$ (2.83 g, 15.5 mmol) in EtOH (200 ml) followed by a solution of **5a** (9.75 g, 25.5 mmol) in EtOH (100 ml). The mixture was stirred at −20°C for 20 hours followed by treatment with H_2O and 1 N HCl and extraction with EtOAc. The organic layer was washed with H_2O, dried over Na_2SO_4 and the solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel with a mixture of hexane - EtOAc (1 : 1) to yield **6a** (4.62 g, 58%): $[\alpha]_D^{25}$ −7.8° (c 1.0, CH_2Cl_2); IR (CH_2Cl_2) cm^{-1} 3406, 2995, 1685, 1609, 1523, 1427, 1406, 1348; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.25+7.53 (4H, AA'BB', J=8 Hz, aromatic), 5.24 (2H, s, CH_2), 4.23 (1H, m, OH), 4.05 (2H, m, 2a-H, 5-H), 3.75 (2H, m, CH_2O), 3.24 (2H, m, 2b-H, 3-H), 2.54 (1H, m, 4b-H), 1.71 (1H, d, J=6 Hz, SH), 1.60 (1H, m, 4a-H).

(3*S*,5*R*)-5-Hydroxymethyl-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-3-thiol (**6b**)

$[\alpha]_D^{25}$ +50.2° (c 1.0, CH_2Cl_2); IR (CH_2Cl_2) cm^{-1} 3430, 2997, 1690, 1609, 1523, 1427, 1405, 1348; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.24+7.53 (4H, AA'BB', J=8 Hz, aromatic), 5.26 (2H, s, CH_2), 4.23 (1H, m, OH), 3.78 (2H, m, 2a-H, 5-H), 3.65 (2H, m, CH_2O), 3.51 (2H, m, 2b-H, 3-H), 2.12+2.05 (2H, m, 4a-H, 4b-H), 1.73 (1H, d, J=5.5 Hz, SH).

(3*R*,5*S*)-5-Hydroxymethyl-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-3-thiol (**13**)

$[\alpha]_D^{25}$ −48.9° (c 1.01, CH_2Cl_2); IR and ^{1}H NMR identical with **6b**.

(3*R*,5*S*)-5-Hydroxymethyl-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-3-ol (**7**)

Reduction and work up were performed as described for **6a**. The obtained residue was purified by recrystallization from EtOH to yield **7** in 73%: mp 108~110°C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 8.28+7.56 (4H, AA'BB', J=8 Hz, aromatic), 5.28 (2H, s, CH_2), 4.50 (1H, m, 3-H), 4.24 (2H, m, 2b-H, 5-H), 3.82 (1H, m, 2a-H), 3.68 (2H, m, CH_2O), 2.12 (2H, m, 4a-H), 1.88 (1H, m, 4b-H), 1.70 (2H, m, OH).

(3*R*,5*S*)-5-Mesyloxymethyl-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-3-ol (**8a**)

To a solution of **7** (5 g, 16.6 mmol) in pyridine (30 ml), CH_3SO_2Cl (1.44 ml, 18.5 mmol) was added dropwise at −10°C. The reaction mixture was allowed to reach room temp and stirring was continued for 18 hours. Afterwards the mixture was poured on ice and acidified with conc HCl, extracted with CH_2Cl_2, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using EtOAc to yield **8a** (4.06 g, 64%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 8.24+7.54 (4H, AA'BB', J=8 Hz, aromatic), 5.26 (2H, s, CH_2), 4.52 (1H, m, 3-H), 4.24 (3H, m, 2a-H, 2b-H, 5-H), 3.60 (2H, m, CH_2O), 2.98 (3H, s, OSO_2CH_3), 2.12 (2H, m, 4a-H, 4b-H).

(3*R*,5*S*)-5-Methylthiomethyl-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-3-ol (**8b**)

A solution of MeSH (0.14 ml, 2.5 mmol) in THF (5 ml) was treated with 104 mg of a 55% suspension of NaH in oil at −30°C. The reaction mixture was warmed to room temp and DMF (7 ml) was added. After 20 minutes a solution of **8a** (0.6 g, 1.6 mmol) in THF (8 ml) was added dropwise and stirring was continued for 1 hour. The reaction mixture was poured on ice and 0.1 N HCl, extracted 3 times with CH_2Cl_2, washed 5 times with H_2O and dried over Na_2SO_4. After evaporation of the solvent *in vacuo* the residue was chromatographed on silica gel with a mixture of hexane - EtOAc (1 : 5) to yield **8b** (0.47 g, 90%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.25+7.53 and 8.25+7.52 (4H, each AA'BB', J=8 Hz, aromatic), 5.24 and 5.28+5.24 (2H, s and ABq, J=4.5 Hz, CH_2), 4.47 (1H, m, 3-H), 4.28 (1H, m, 5-H), 3.65 (1H, m, 2a-H), 3.53 (1H, m, 2b-H), 2.90 (2H, m, CH_2S), 2.10 (2H, m, 4a-H, 4b-H), 2.08 (3H, s, SCH_3).

Methyl (2*S*,4*S*)-4-Hydroxy-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidine-2-carboxylate (**11**)

To a solution of **3c** (5.84 g, 18 mmol) and TPP (5.25 g, 20 mmol) in THF (250 ml), DEAD (3.14 ml, 20 mmol) was added dropwise followed by HCOOH (0.75 ml, 20 mmol) at −10°C. After stirring at room temp for 3 hours the reaction mixture was concentrated *in vacuo* and chromatographed on silica gel with a mixture of hexane - EtOAc (1 : 2). The crude product which was still contaminated with some diethyl hydrazodicarboxylate was dissolved in 500 ml of a mixture of H_2O - dioxane (1 : 4) and 0.4 N NaOH (50 ml) was added at 0°C. After 15 minutes at this temp EtOAc was added, the organic layer washed with brine and dried over Na_2SO_4. The residue was purified by column chromatography on silica gel with a mixture of hexane - EtOAc (1 : 2) to yield **11** (3.35 g, 57%): $[\alpha]_D^{25}$ −11.5° (*c* 0.79, CH_2Cl_2); ^{1}H NMR is identical with **3d**.

Allyl (5*R*,6*S*,8*R*)-6-(1-Hydroxyethyl)-2-[(2′*S*,4′*S*)-2′-allyloxycarbonyl-1′-(*p*-nitrobenzyloxycarbonyl)pyrrolidinyl-4′-thio]penem-3-carboxylate (**15a**)

To a solution of **5a** (198 mg, 0.54 mmol) in MeCN (4 ml) a solution of **14** (120 mg, 0.36 mmol) in MeCN (4 ml) was added dropwise followed by iPr$_2$NEt (0.092 ml, 0.54 mmol) at −30°C. After 2 hours at this temp the reaction mixture was diluted with EtOAc, washed with H_2O and dried over Na_2SO_4. After evaporation of the solvent *in vacuo* the residue was purified by column chromatography using mixtures of hexane - EtOAc (1 : 1) and EtOAc to yield **15a** (150 mg, 67%): IR (CH_2Cl_2) cm^{-1} 1780, 1690, 1520, 1400, 1345; ^{1}H NMR (90 MHz, $CDCl_3$) δ 8.26+7.56 and 8.24+7.50 (4H, each AA′BB′, J=8 Hz, aromatic), 5.96 (2H, m, vinylic), 5.70 (1H, J=1.5 Hz, 5-H), 5.24 (6H, m, vinylic, CH_2), 4.70 (4H, m, allylic), 4.46 (1H, dd, J_1=8 Hz, J_2=4.5 Hz, 2′-H), 4.25 (2H, m, 8-H, 4′-H), 4.10 (1H, m, 5′a-H), 3.76 (1H, dd, J_1=6.5 Hz, J_2=1.5 Hz, 6-H), 3.58 (1H, m, 5′b-H), 2.80 (1H, m, 3′b-H), 2.20 (1H, m, 3′a-H), 1.84 (1H, m, OH), 1.36 (3H, d, J=6.5 Hz, CH_3).

Allyl (5*R*,6*S*,8*R*)-6-(1-Hydroxyethyl)-2-[(2′*R*,4′*S*)-2′-allyloxycarbonyl-1′-(*p*-nitrobenzyloxycarbonyl)pyrrolidinyl-4′-thio]penem-3-carboxylate (**15b**)

^{1}H NMR (90 MHz, $CDCl_3$) δ 8.25+7.51 and 8.24+7.47 (4H, each AA′BB′, J=8 Hz, aromatic), 5.88 (2H, m, vinylic), 5.71 (1H, J=1.5 Hz, 5-H), 5.28 (4H, s and m, CH_2, vinylic), 4.66 (5H, m, allylic, 2′-H), 4.32~3.60 (4H, m, 8-H, 4′-H, 5′a-H, 5′b-H), 3.74 (1H, dd, J_1=6.5 Hz, J_2=1.5 Hz, 6-H), 2.44 (2H, m, 3′a-H, 3′b-H), 1.90 (1H, m, OH), 1.36 (3H, d, J=6.5 Hz, CH_3).

Allyl (5*R*,6*S*,8*R*)-6-(1-Hydroxyethyl)-2-[(2′*S*,4′*S*)-2′-methoxycarbonyl-1′-(*p*-nitrobenzyloxycarbonyl)pyrrolidinyl-4′-thio]penem-3-carboxylate (**15c**)

^{1}H NMR (90 MHz, $CDCl_3$) δ 8.26+7.54 and 8.26+7.48 (4H, each AA′BB′, J=8 Hz, aromatic), 5.92 (1H, m, vinylic), 5.88 (1H, d, J=1.5 Hz, 5-H), 5.28 (4H, s and m, CH_2, vinylic), 4.74 (2H, m, allylic), 4.48 (1H, dd, J_1=8 Hz, J_2=6 Hz, 2′-H), 4.24 (3H, m, 8-H, 4′-H, 5′a-H), 3.89 (1H, dd, J_1= 6.5 Hz, J_2=1.5 Hz, 6-H), 3.78 and 3.70 (3H, each s, OCH_3), 2.80~2.10 (2H, m, 3′a-H, 3′b-H), 1.36 (3H, d, J=6.5 Hz, CH_3).

Allyl (5*R*,6*S*,8*R*)-6-(1-Hydroxyethyl)-2-[(2′*S*,4′*S*)-2′-*N*-methylamidocarbonyl-1′-(*p*-nitrobenzyloxycarbonyl)pyrrolidinyl-4′-thio]penem-3-carboxylate (**15d**)

^{1}H NMR (90 MHz, $CDCl_3$) δ 8.25+7.56 (4H, AA′BB′, J=8 Hz, aromatic), 7.28 (1H, br s, CONH), 5.94 (1H, m, vinylic), 5.74 (1H, d, J=1.5 Hz, 5-H), 5.40 (2H, m, vinylic), 5.28 (2H, s, CH_2), 4.72 (2H, m, allylic), 4.30 (4H, m, 8-H, 2′-H, 4′-H, 5′a-H), 3.70 (1H, dd, J_1=6.5 Hz, J_2=1.5 Hz, 6-H), 3.60 (1H, m, 5′b-H), 2.74 (3H, s, NCH_3), 2.80~2.20 (2H, m, 3′a-H, 3′b-H), 1.32 (3H, d, J=6.5 Hz, CH_3).

Allyl (5*R*,6*S*,8*R*)-6-(1-Hydroxyethyl)-2-[(3′*S*,5′*S*)-5′-hydroxymethyl-1′-(*p*-nitrobenzyloxycarbonyl)-pyrrolidinyl-3′-thio]penem-3-carboxylate (**15e**)

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.35+7.54 (4H, AA′BB′, J=8 Hz, aromatic), 5.94 (1H, m, vinylic), 5.60 (1H, d, J=1.5 Hz, 5-H), 5.34 (2H, m, vinylic), 5.25 (2H, s, CH_2), 4.72 (2H, m, allylic), 4.26 (1H, m, 8-H), 4.08 (1H, m, 3′-H), 3.85~3.60 (5H, m, 2′a-H, 2′b-H, 5′-H, CH_2O), 3.41 (1H, m, OH), 2.70 (1H, br s, OH), 2.58 (1H, ddd, J_1=15 Hz, J_2=7.5 Hz, J_3=7.5 Hz, 4′b-H), 1.77 (1H, m, 4′a-H), 1.35 (3H, d, J=6.5 Hz, CH_3).

Allyl (5*R*,6*S*,8*R*)-6-(1-Hydroxyethyl)-2-[(3′*S*,5′*R*)-5′-hydroxymethyl-1′-(*p*-nitrobenzyloxycarbonyl)-pyrrolidinyl-3′-thio]penem-3-carboxylate (**15f**)

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.36+7.52 (4H, AA′BB′, J=8 Hz, aromatic), 5.97 (1H, m, vinylic), 5.25 (2H, s, CH_2), 4.74 (2H, m, allylic), 4.24 (2H, m, 8-H, 3′-H), 4.00~3.60 (5H, m, 2′a-H, 2′b-H, 5′-H, CH_2O), 2.20 (2H, m, 4′a-H, 4′b-H), 1.85 (1H, d, J=4.5 Hz, OH), 1.37 (3H, d, J=6.5 Hz, CH_3).

Allyl (5*R*,6*S*,8*R*)-6-(1-Hydroxyethyl)-2-[(3′*S*,5′*S*)-5′-methylthiomethyl-1′-(*p*-nitrobenzyloxycarbonyl)pyrrolidinyl-3′-thio]penem-3-carboxylate (**15g**)

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.26+7.53 (4H, AA′BB′, J=8 Hz, aromatic), 5.95 (2H, m, vinylic), 5.70 (2H, m, vinylic), 5.24 (2H, s, CH_2), 4.73 (2H, m, allylic), 4.25 (1H, dq, J_1=6.5 Hz, J_2=6.5 Hz, 8-H), 4.15 (2H, m, 2′a-H, 5′-H), 3.73 (2H, dd, J_1=6.5 Hz, J_2=1.4 Hz, m, 6-H, 3′-H), 3.43 (1H, dd, J_1=11.5 Hz, J_2=7.5 Hz, 2′b-H), 2.95 (1H, m, 4′b-H), 2.70 (2H, m, SCH_2), 2.12 (3H, s, SCH_3), 1.92 (1H, OH), 1.38 (3H, d, J=6.5 Hz, CH_3).

Allyl (5*R*,6*S*,8*R*)-6-(1-Hydroxyethyl)-2-[(3′*R*,5′*S*)-5′-hydroxymethyl-1′-(*p*-nitrobenzyloxycarbonyl)-pyrrolidinyl-3′-thio]penem-3-carboxylate (**17**)

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.28+7.52 (4H, AA′BB′, J=8 Hz, aromatic), 5.98 (1H, m, vinylic), 5.69 (1H, d, J=1.5 Hz, 5-H), 5.42 (2H, m, vinylic), 5.28 (2H, s, CH_2), 4.74 (2H, m, allylic), 4.24 (2H, m, 8-H, 3′-H), 4.00~3.60 (5H, 2′a-H, 2′b-H, 5′-H, CH_2O), 3.72 (1H, dd, J_1=6.5 Hz, J_2=1.5 Hz, 6-H), 3.22 (1H, m, OH), 2.20 (2H, m, 4′a-H, 4′b-H), 1.38 (3H, d, J=6.5 Hz, CH_3).

Sodium (5*R*,6*S*,8*R*)-2-[(2′*S*,4′*S*)-2′-Carboxypyrrolidinyl-4′-thio]-6-(1-hydroxyethyl)penem-3-carboxylate (**16a**)

To a solution of **15a** (136 mg, 0.22 mmol) in 20 ml of a mixture of CH_2Cl_2 - EtOAc (2: 1), 0.88 ml of a 0.5 M solution of sodium 2-ethylhexanoate in EtOAc were added, followed by TPP (3 mg) and $Pd(PPh_3)_4$ (6 mg) at room temp. After 7 hours of stirring the reaction mixture was diluted with EtOAc and extracted with H_2O. The aq layer was lyophilized, the obtained brownish powder dissolved in THF (10 ml) and added to a stirred suspension of 400 mg of Pd/C (10%, w/w) in 0.5 M phosphate buffer pH 7.1 (20 ml). The mixture was stirred under H_2 at atmospheric pressure and the course of the reduction was carefully checked by TLC. After about 3 hours the starting material was consumed and the reaction solution was filtered. The filtrate was washed after evaporation of THF with EtOAc and lyophilized. The remaining residue was purified by column chromatography on LiChroprep RP 18 with a mixture of H_2O - MeCN (9: 1) to yield **16a** (42 mg, 47%, colorless foam): UV $\lambda_{max}^{H_2O}$ nm (ε) 253 (6,096), 316 (5,716); IR (KBr) cm^{-1} 3690, 3487, 2930, 1766, 1622; ^{1}H NMR (250 MHz, D_2O) δ 5.73 (1H, d, J=1.5 Hz, 5-H), 4.25 (1H, dq, J_1=6.5 Hz, J_2=6.5 Hz, 8-H), 4.23 (1H, dd, J_1=8 Hz, J_2=7 Hz, 2′-H), 4.10 (1H, m, 4′-H), 3.97 (1H, dd, J_1=6.5 Hz, J_2=1.5 Hz, 6-H), 3.86 (1H, dd, J_1=12.5 Hz, J_2=6 Hz, 5′a-H), 3.55 (1H, dd, J_1=12.5 Hz, J_2=5 Hz, 5′b-H), 2.87 (1H, ddd, J_1=14 Hz, J_2=8 Hz, J_3=7 Hz, 3′b-H), 2.14 (1H, ddd, J_1=14 Hz, J_2=8 Hz, J_3=7 Hz, 3′a-H), 1.31 (3H, d, J=6.5 Hz, CH_3).

Sodium (5*R*,6*S*,8*R*)-2-[(2′*R*,4′*S*)-2′-Carboxypyrrolidinyl-4′-thio]-6-(1-hydroxyethyl)penem-3-carboxylate (**16b**)

UV $\lambda_{max}^{H_2O}$ nm (ε) 254 (6,155), 321 (7,531); IR (KBr) cm^{-1} 1760, 1620; ^{1}H NMR (250 MHz, D_2O) δ 5.73 (1H, d, J=1.5 Hz, 5-H), 4.36 (1H, dd, J_1=7 Hz, J_2=8.5 Hz, 2′-H), 4.27 (1H, dq, J_1=6.5 Hz, J_2=6.5 Hz, 8-H), 4.11 (1H, dddd, J_1=7 Hz, J_2=4.5 Hz, J_3=6 Hz, J_4=4 Hz, 4′-H), 3.97 (1H, dd, J_1=6.5 Hz, J_2=1.5 Hz, 6-H), 3.87 (1H, dd, J_1=12.5 Hz, J_2=6 Hz, 5′b-H), 3.50 (1H, dd, J_1=12.5 Hz, J_2=4 Hz, 5′a-H), 2.57 (1H, ddd, J_1=14 Hz, J_2=7 Hz, J_3=7 Hz, 3′a-H), 2.43 (1H, ddd, J_1=14 Hz, J_2=8.5 Hz, J_3=4.5 Hz, 3′b-H), 1.33 (3H, d, J=6.5 Hz, CH_3).

(5*R*,6*S*,8*R*)-6-(1-Hydroxyethyl)-2-[(2′*S*,4′*S*)-2′-methoxycarbonylpyrrolidinyl-4′-thio]penem-3-carboxylate (**16c**)

UV $\lambda_{max}^{H_2O}$ nm (ε) 253 (5,715), 320.5 (6,822); IR (KBr) cm^{-1} 2950, 1760, 1730, 1590; ^{1}H NMR (250 MHz, D_2O) δ 5.71 (1H, d, J=1.5 Hz, 5-H), 4.26 (1H, dq, J_1=6.5 Hz, J_2=6.5 Hz, 8-H), 4.05 (1H, dd,

J_1=6.5 Hz, J_2=8.5 Hz, 2'-H), 3.92 (1H, dd, J_1=6.5 Hz, J_2=1.5 Hz, 6-H), 3.86 (1H, m, 4'-H), 3.78 (3H, s, $COOCH_3$), 3.46 (1H, dd, J_1=12 Hz, J_2=6.5 Hz, 5'a-H), 3.12 (1H, dd, J_1=12 Hz, J_2=5 Hz, 5'b-H), 2.72 (1H, ddd, J_1=14 Hz, J_2=8.5 Hz, J_3=8.5 Hz, 3'b-H), 2.01 (1H, ddd, J_1=14 Hz, J_2=7.5 Hz, J_3=6.5 Hz, 3'a-H), 1.31 (3H, d, J=6.5 Hz, CH_3).

(5*R*,6*S*,8*R*)-6-(1-Hydroxyethyl)-2-[(2'*S*,4'*S*)-2'-methylamidocarbonylpyrrolidinyl-4'-thio]penem-3-carboxylate (**16d**)

UV $\lambda_{max}^{H_2O}$ nm (ε) 251 (5,699), 320 (7,141); IR (KBr) cm^{-1} 2950, 1760, 1640, 1590; ^1H NMR (250 MHz, D_2O) δ 5.70 (1H, d, J=1.5 Hz, 5-H), 4.26 (1H, dq, J_1=6.5 Hz, J_2=6.5 Hz, 8-H), 3.92 (1H, dd, J_1=6.5 Hz, J_2=1.5 Hz, 6-H), 3.82 (2H, m, 2'-H, 4'-H), 3.45 (1H, dd, J_1=12.5 Hz, J_2=7 Hz, 5'a-H), 3.01 (1H, dd, J_1=12.5 Hz, J_2=5.5 Hz, 5'b-H), 2.77 (3H, s, NCH_3), 2.66 (1H, m, 3'b-H), 1.81 (1H, ddd, J_1=14 Hz, J_2=7 Hz, J_3=7 Hz, 3'a-H), 1.32 (3H, d, J=6.5 Hz, CH_3).

(5*R*,6*S*,8*R*)-6-(1-Hydroxyethyl)-2-[(3'*S*,5'*S*)-5'-hydroxymethylpyrrolidinyl-3'-thio]penem-3-carboxylate (**16e**)

UV $\lambda_{max}^{H_2O}$ nm (ε) 251.5 (4,939), 319.5 (5,693); IR (KBr) cm^{-1} 2950, 1760, 1580; ^1H NMR (250 MHz, D_2O) δ 5.75 (1H, d, J=1.5 Hz, 5-H), 4.29 (1H, dq, J_1=6.5 Hz, J_2=6.5 Hz, 8-H), 4.02 (1H, m, 3'-H), 3.98 (1H, dd, J_1=6.5 Hz, J_2=1.5 Hz, 6-H), 3.86 (1H, dd, J_1=12.5 Hz, J_2=8 Hz, 2'a-H), 3.81 (1H, m, 5'-H), 3.79 (2H, m, CH_2O), 3.45 (1H, dd, J_1=12.5 Hz, J_2=5 Hz, 2'b-H), 2.65 (1H, ddd, J_1=14 Hz, J_2=7 Hz, J_3=7 Hz, 4'b-H), 1.75 (1H, ddd, J_1=14 Hz, J_2=7 Hz, J_3=7 Hz, 4'a-H), 1.31 (3H, d, J=6.5 Hz, CH_3).

(5*R*,6*S*,8*R*)-6-(1-Hydroxyethyl)-2-[(3'*S*,5'*R*)-5'-hydroxymethylpyrrolidinyl-3'-thio]penem-3-carboxylate (**16f**)

UV $\lambda_{max}^{H_2O}$ nm (ε) 252 (5,000), 320 (5,169); IR (KBr) cm^{-1} 2950, 1760, 1580; ^1H NMR (250 MHz, D_2O) δ 5.72 (1H, d, J=1.5 Hz, 5-H), 4.26 (1H, dq, J_1=6.5 Hz, J_2=6.5 Hz, 8-H), 4.13 (1H, m, 3'-H), 3.98 (1H, m, 5'-H), 3.95 (1H, dd, J_1=6.5 Hz, J_2=1.5 Hz, 6-H), 3.87 (1H, dd, J_1=12.5 Hz, J_2=4 Hz, 2'b-H), 3.76 (2H, m, CH_2O), 3.47 (1H, dd, J_1=12.5 Hz, J_2=3.5 Hz, 2'a-H), 2.25 (2H, m, 4'a-H, 4'b-H), 1.32 (3H, d, J=6.5 Hz, CH_3).

(5*R*,6*S*,8*R*)-6-(1-Hydroxyethyl)-2-[(3'*S*,5'*S*)-5'-methylthiomethylpyrrolidinyl-3'-thio]penem-3-carboxylate (**16g**)

UV $\lambda_{max}^{H_2O}$ nm (ε) 253 (5,885), 320 (7,322); IR (KBr) cm^{-1} 2950, 1760, 1580; ^1H NMR (250 MHz, D_2O) δ 5.71 (1H, d, J=1.5 Hz, 5-H), 4.26 (1H, dq, J_1=6.5 Hz, J_2=6.5 Hz, 8-H), 3.99 (1H, m, 3'-H), 3.93 (1H, dd, J_1=6.5 Hz, J_2=1.5 Hz, 6-H), 3.72 (1H, m, 5'-H), 3.60 (1H, dd, J_1=12.5 Hz, J_2=7.5 Hz, 2'a-H), 3.32 (1H, dd, J_1=12.5 Hz, J_2=5 Hz, 2'b-H), 2.85 (2H, m, SCH_2), 2.70 (1H, ddd, J_1=15 Hz, J_2=7.5 Hz, J_3=7.5 Hz, 4'b-H), 2.16 (3H, s, SCH_3), 1.65 (1H, ddd, J_1=15 Hz, J_2=8.5 Hz, J_3=7.5 Hz, 4'a-H), 1.31 (3H, d, J=6.5 Hz, CH_3).

(5*R*,6*S*,8*R*)-6-(1-Hydroxyethyl)-2-[(3'*R*,5'*S*)-5'-hydroxymethylpyrrolidinyl-3'-thio]penem-3-carboxylate (**18**)

UV $\lambda_{max}^{H_2O}$ nm (ε) 250 (5,285), 320 (6,077); IR (KBr) cm^{-1} 2950, 1760, 1580; ^1H NMR (250 MHz, D_2O) δ 5.74 (1H, J=1.5 Hz, 5-H), 4.27 (1H, dq, J_1=6.5 Hz, J_2=6.5 Hz, 8-H), 4.14 (1H, m, 3'-H), 4.01 (1H, m, 5'-H), 3.97 (1H, dd, J_1=6.5 Hz, J_2=1.5 Hz, 6-H), 3.91 (1H, dd, J_1=12.5 Hz, J_2=4 Hz, 2'a-H), 3.75 (2H, m, CH_2O), 3.39 (1H, dd, J_1=12.5 Hz, J_2=3.5 Hz, 2'b-H), 2.33 (2H, m, 4'a-H, 4'b-H), 1.32 (3H, d, J=6.5 Hz, CH_3).

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