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PEPTIDIC PRODRUGS OF NOVEL AMINOMETHYL-THF 1β-METHYLCARBAPENEMS

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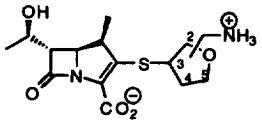
Abstract: Peptidic prodrugs of the five most active aminomethyl-THF 1β-methylcarbapenems were synthesized. Of these, only L-amino acid derivatives from **1a** demonstrated an improved oral activity. These results indicate that the L-amino acid derivatives from **1a** are orally absorbed most likely through the dipeptide and tripeptide transport mechanism. © 1997 Elsevier Science Ltd.

In previous publications[1] we reported the synthesis and antimicrobial activity of novel aminomethyl-THF 1β-methylcarbapenems **1**, of which CL191,121 (**1a**) is a representative member. These carbapenems had the spectrum of activity against Gram-positive and Gram-negative organisms, comparable to those of imipenem and meropenem with the exception of only moderate antipseudomonal activity. Most importantly, they demonstrated some intrinsic oral activity (ED_{50} = 2-4 mg/kg) against an *E. coli* infection in mice. However, the effective oral dose (ED_{50}) was about 11 to 14 times higher than the effective subcutaneous dose (ED_{50}). Ideally, the ratio of ED_{50} values obtained from SOD (single oral dose) and SSC (single subcutaneous dose) should approach 1.0 showing bioequivalence. Therefore, efforts were directed toward improving this ratio. Since β-lactam antibiotics could be absorbed by active transport through a carrier mechanism,[2] we prepared peptidic prodrugs of dipeptide-like aminomethyl-THF 1β-methylcarbapenems **1** in order to improve absorption through di/tripeptide transport mechanism by increasing their resemblance to tripeptides. We report here the synthesis, antimicrobial activity and oral activity of peptidic prodrugs of the aminomethyl-THF 1β-methylcarbapenems (**3**).

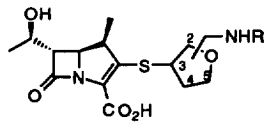
Chemistry

Synthesis of aminomethyl-THF 1β-methylcarbapenems **1** was previously described.[1(a)] The carbapenems, **1a**, **1d** and **1e**, are optically pure, and the carbapenems, **1b** and **1c**, are each a mixture of diastereomers. Peptidic derivatives **3** were synthesized in two steps in 40–50% overall yields by reaction of the carbapenems **1** with the PNZ-protected O-Su ester of an amino acid (**6**) at pH 8.5, followed by catalytic hydrogenation with 10% palladium on charcoal at pH 6.5 (Scheme 1). These THF carbapenems, **1** and **3**, are

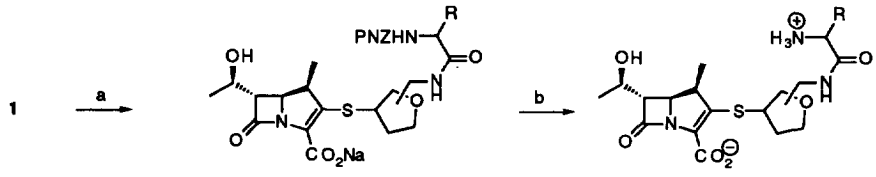
quite stable at room temperature between pH 6 and 8 with a half life of ca. 200 to ca. 400 h but their stability steeply declines outside of this range.[3] Therefore, the reactions in 0.1 M buffer solution (NaH_2PO_4-Na_2HPO_4) at pH 8.5 were carried out at 0 °C, and the reactions at pH 6.5 were carried out at room temperature. Synthesis of the PNZ-protected O-Su ester of an amino acid (**6**) is shown in Scheme 2.



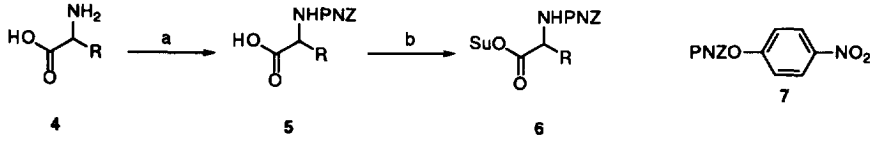
1: THF Carbapenems
1a: 3*R*, 2*R* (CL191,121)
1b: 3*S*, 5*R* and 3*R*, 5*S* (*trans*)
1c: 3*R*, 5*R* and 3*S*, 5*S* (*cis*)
1d: 3*R*, 4*R*
1e: 3*S*, 4*S*



3: Monopeptide Derivatives
3aa: R = L-Ala; 3*R*, 2*R*
3ab: R = L-Val; 3*R*, 2*R*
3ac: R = L-Ile; 3*R*, 2*R*
3ad: R = L-Phe; 3*R*, 2*R*
3ae: R = Gly; 3*R*, 2*R*
3af: R = D-Ala; 3*R*, 2*R*
3ag: R = D-Phe; 3*R*, 2*R*
3ba: R = L-Ala; 3*S*, 5*R* and 3*R*, 5*S* (*trans*)
3ca: R = L-Ala; 3*R*, 5*R* and 3*S*, 5*S* (*cis*)
3da: R = L-Ala; 3*R*, 4*R*
3ea: R = L-Ala; 3*S*, 4*S*



Scheme 1: (a) 6/pH 8.5 buffer/dioxane/0°C, 75%; (b) H_2/10% Pd/C/pH 6.5 buffer/dioxane/r t, 65%



Scheme 2: (a) 7/TEA/EtOH/H_2O, 80%; (b) NHS/DCC/dioxane/rt, 93%.

Results and Discussion

L-Alanine derivatives, **3aa**, **3ba**, **3ca**, **3da**, and **3ea**, from the five most active aminomethyl-THF carbapenems **1a–e** were synthesized. Of these, only the L-alanine derivative **3aa** demonstrated improved oral activity against acute lethal bacterial infections in mice (Table 1). This was followed by the synthesis of other L- and D-amino acid derivatives of **1a**. The D-alanine and D-phenylalanine derivatives, **3af** and **3ag**, of **1a** both failed to demonstrate improved oral activity. However, three other L-amino acid derivatives, **3ab**, **3ac**, and **3ad**, and the glycyl derivative **3ae** of **1a** also demonstrated improved oral activity. Among the L-amino acid derivatives of **1a**, L-Val derivative **3ab** demonstrated the best oral activity. The SOD/SSC ratio decreased from 11 to 1.1 and 22 to 1.4, respectively, against an *E. coli* infection and a *S. aureus Smith* infection in mice. These results indicate that all four L-amino acid derivatives and the glycyl derivative of **1a** are orally absorbed

Table 1. ED_{50} (mg/kg)[a] for THF Carbapenems, 1 and 3, Against Acute Lethal Bacterial Infections in Mice

		S. aureus Smith			*E. coli #311*		
Compound	Clog P[b]	SOD[c]	SSC[d]	SOD/SSC	SOD	SSC	SOD/SSC
1a	-3.02	0.88	0.04	22	3.8	0.34	11
3aa	-3.39	0.09	0.04	2.2	0.55	0.28	2.0
3ab	-2.46	0.10	0.07	1.4	0.31	0.29	1.1
3ac	-1.93	0.21	0.28	0.75	0.58	1.1	0.53
3ad	-1.97	0.28	0.06	4.7	1.2	0.48	2.5
3ae	-3.70	0.41	0.15	2.7	1.4	0.56	2.5
3af	-3.39	>6.6	0.42-0.83	>16-8.0	NT	NT	
3ag	-1.97	NT	NT		24	13	1.8
1b	-3.02	1.7	0.11	16	3.7	0.27	14
3ba	-3.39	0.83-1.7	0.10-0.21	8.3	NT	NT	
1c	-3.02	1.1	0.05	22	3.5	0.28	12
3ca	-3.39	0.83-1.7	0.06-0.12	14	NT	NT	
1d	-3.80	4-8	0.12-0.25	33	NT[e]	0.5-1.0	
3da	-4.43	4.5	0.11	41	NT	NT	
1e	-3.80	NT	NT		NT	NT	
3ea	-4.43	4.0	0.11	36	NT	NT	
Primaxin[f]		3.3	0.03	110	79	0.70	113

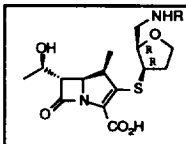
[a] For all peptidic compounds **3**, the numbers have been normalized with a factor which is the molecular weight of the parent compound **1** divided by the molecular weight of the peptidic compound **3**. [b] The Mac-Clog P program from Biobyte was used to calculate log P values of the nonionic form. [c] Single oral dose. [d] Single subcutaneous dose. [e] Not tested. [f] A 1:1 combination of imipenem and cilastatin.

efficiently probably through the dipeptide and tripeptide transport mechanism,[4] whereas the rest of the six peptidic derivatives are not orally absorbed sufficiently. This observation is further supported by the calculated partition coefficients (Clog P) of peptidic derivatives shown in Table 1. Oral drugs can be absorbed either by active transport through a carrier mechanism or by passive transport through phospholipid membranes. These THF carbapenems in water exist in zwitterionic forms and have Clog P in the range of -1.97 to -3.39 for the nonionic form. Such polar compounds can not possibly be significantly absorbed through phospholipid membranes at all physiogical pH's (1~7.4). In addition, since the L- and D-amino acid prodrug pairs (**3aa/3af** and **3ad/3ag**) as well as the three isomeric L-Ala prodrugs (**3aa**, **3ba** and **3ca**) each have the same Clog P value and molecular weight, they should be orally absorbed through phospholipid membranes by the same extent. Therefore, the dramatic difference in oral activity between the L- and D-amino acid prodrug pairs as well as between the L-Ala prodrug **3aa** and the two other isomeric derivatives (**3ba** and **3ca**) supports that only the L-amino acid prodrugs of **1a** are orally absorbed through an active transport system; most likely, the dipeptide and tripeptide transport system.

The *in vitro* antimicrobial activity of these peptidic derivatives **3** was also examined (Table 2 and 3). L-

Table 2 **2R,3R-Disubstituted THF Carbapenems**

In vitro activity (MIC; μg/mL)

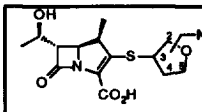


R =	H	L-Ala	L-Val	L-Ile	L-Phe	L-Gly	D-Ala	D-Phe	Imipenem
	1a	**3aa**	**3ab**	**3ac**	**3ad**	**3ae**	**3af**	**3ag**	
ORGANISM / Strain									
E. coli ATCC 25922	≤0.06	≤0.06	≤0.06	≤0.06	0.25	≤0.06	≤0.06	0.50	0.12
E. coli GC 2205	≤0.06	≤0.06	≤0.06	≤0.06	0.03	≤0.06	≤0.06	0.06	0.12
E. coli GC 1792	≤0.06	≤0.06	0.12	0.25	0.50	≤0.06	0.12	0.50	≤0.06
E. cloacae GC 2209	≤0.06	0.25	0.50	1.0	4.0	≤0.06	0.25	4.0	≤0.06
C. freundii GC 2211	≤0.06	1.0	1.0	4.0	8.0	0.50	1.0	8.0	0.25
M. morganii GC 2213	0.50	1.0	1.0	2.0	4.0	0.50	1.0	2.0	1.0
A. calcoaceticus GC 756	2.0	16	16	64	64	8.0	8.0	32	0.25
P. aeruginosa ATCC 27853	8.0	32	32	64	64	8.0	64	64	1.0
P. aeruginosa GC 1544 OprD-	16	64	128	128	128	64	64	128	16
X. maltophilia GC 562	>128	>128	>128	>128	>128	>128	>128	>128	>128
S. aureus ATCC 29213	≤0.06	0.12	0.25	0.25	0.25	0.12	≤0.06	0.25	≤0.06
S. aureus GC 2220 MRSA	1.0	8.0	8.0	32	4.0	8.0	8.0	8.0	1.0
E. faecalis GC 842	0.50	4.0	2.0	2.0	1.0	2.0	2.0	1.0	1.0
E. faecium GC 1182	64	>128	>128	>128	128	>128	>128	128	64
Rel. hydrolysis by hog DHP	8.5	12	8.4	9.2	2.9	7	3	2.4	100

and D-amino acid derivatives (**3aa/3af** and **3ad/3ag**) demonstrated similar antimicrobial activity. In general, the peptidic derivatives **3** demonstrated little or no anti-pseudomonas activity, and against other microorganisms they were slightly less active than their corresponding parent compounds **1** except L- and D-Phe derivatives (**3ad** and **3ag**) which were 4 to >128 times less active. None of the compounds exhibited acceptable activity against methicillin-resistant *S. aureus* (GC 2220); none exhibited activity against *E. faecium* or *X. maltophilia.* As expected, these THF 1β-methylcarbapenems all demonstrated better stability than imipenem to hydrolysis by hog renal dehydropeptidase due to the presence of the 1β-methyl moiety.

Table 3 3,5- And 3,4-Disubstituted THF Carbapenems

In vitro activity (MIC; μg/mL)



		H* 3S, 5R 3R, 5S	L-Ala* 3S, 5R 3R, 5S	H* 3R,,5R 3S, 5S	L-Ala* 3R,,5R 3S, 5S	H 3R, 4R	L-Ala 3R, 4R	H 3S, 4S	L-Ala 3S, 4S	Imipenem
		1b	**3ba**	**1c**	**3ca**	**1d**	**3da**	**1e**	**3ea**	
ORGANISM	Strain									
E. coli	ATCC 25922	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	0.25	0.12	≤0.06	0.12
E. coli	GC 2205	≤0.06	0.12	≤0.06	0.12	0.12	0.25	0.12	≤0.06	0.12
E. coli	GC 1792	0.06	0.06	≤0.06	0.12	≤0.06	0.25	0.12	≤0.06	≤0.06
E. cloacae	GC 2209	≤0.06	0.25	≤0.06	0.12	≤0.06	1.0	0.12	0.25	≤0.06
C. freundii	GC 2211	0.06	1.0	≤0.06	0.50	0.25	4.0	0.50	2.0	0.25
M. morganii	GC 2213	0.25	1.0	0.5	1.0	2.0	2.0	2.0	1.0	1.0
A. calcoaceticus	GC 756	1.0	8.0	2.0	8.0	2.0	16	16	8.0	0.25
P. aeruginosa	ATCC 27853	4.0	32	8.0	32	16	64	16	8.0	1.0
P. aeruginosa	GC 1544 OprD-	8.0	64	16	64	32	128	32	32	16
X. maltophilia	GC 562	>128	>128	>128	>128	>128	>128	>128	>128	>128
S. aureus	ATCC 29213	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	0.50	0.12	0.50	≤0.06
S. aureus	GC 2220 MRSA	8.0	8.0	4.0	4.0	4.0	8.0	16	32	1.0
E. faecalis	GC 842	2.0	4.0	2.0	4.0	2.0	16	8.0	2.0	1.0
E. faecium	GC 1182	128	>128	128	>128	128	>128	>128	128	64
Rel. hydrolysis by hog DHP		19	10	10	11	<1	7	<1	5	100

* A mixture of diastereomers.

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