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MONO AND BIS DOUBLE ESTER PRODRUGS OF NOVEL AMINOMETHYL-THF 1β-METHYLCARBAPENEMS

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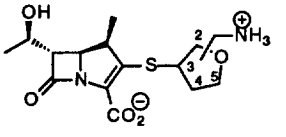
Abstract: Mono and bis double ester prodrugs of aminomethyl THF 1β-methylcarbapenems **1** were synthesized. Mono double ester derivatives (**2**, **4** and **7**) did not demonstrate significantly improved oral activity due to the presence of the charged species. However, bis double ester derivatives (**3** and **5**) demonstrated enhanced oral activity. © 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 is a representative member. These carbapenems had a spectrum of activity against Gram-positive and Gram-negative organisms, comparable to or better than that of imipenem 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* lethal 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.



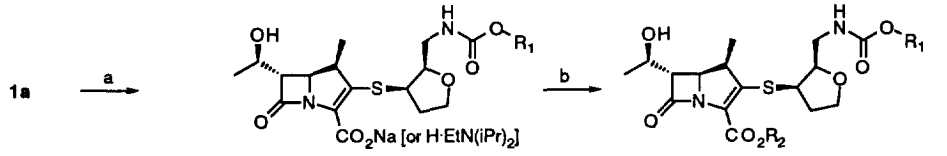
1: THF Carbapenems
1a: 3*R*, 2*R* (CL191,121)
1b: 3*R*, 5*R* and 3*S*, 5*S* (*cis*)

Oral drugs can be absorbed either by passive transport through phospholipid membranes or by active transport through a carrier mechanism. Therefore, we took both approaches for improving oral absorption of aminomethyl-THF 1β-methylcarbapenems **1**. They were: (a) to prepare L-amino acid prodrugs in order to

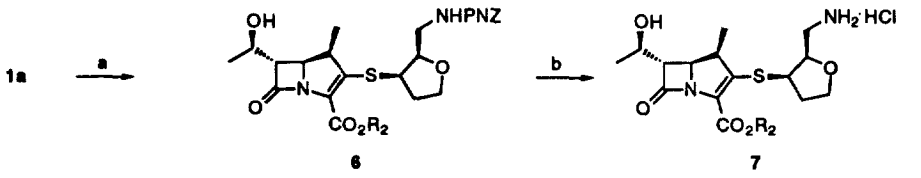
improve absorption through di/tripeptide transport mechanism by increasing their resemblance to tripeptides and (b) to prepare its double ester prodrugs in order to facilitate absorption through the phospholipid membrance by eliminating the ionic nature and increasing the lipophilicity of the parent compound. In the previous communication,[2] we reported the L-amino acid prodrug approach for improving oral activity. We report here the synthesis, antimicrobial activity and oral activity of mono and bis double ester prodrugs of the aminomethyl-THF 1β-methylcarbapenems **1**.

Chemistry

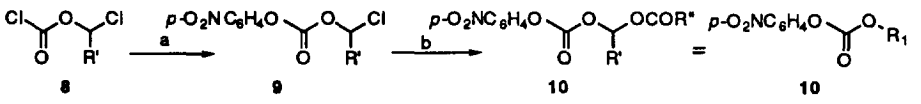
Synthesis of aminomethyl-THF 1β-methylcarbapenems (**1a** and **1b**) was previously described.[1(a)] The carbapenem **1a** is optically pure, and the carbapenem **1b** is a mixture of diastereomers. As shown in Scheme 1, mono double ester derivatives **2** were synthesized in 75–80% yields by reaction of the carbapenems **1a** with acylating agents **10** either in a mixture of 0.1 M buffer solution and *p*-dioxane at pH 8.5 or in the presence of Hunig base in a mixture of acetonitrile and *p*-dioxane. Bis double ester derivatives **3** were synthesized in about 77% yield by reaction of the mono double ester derivatives **2** with alkylating agents R_2I in acetonitrile. Mono double ester derivative **4** and bis double ester derivative **5** in Table 3 were similarly synthesized from the carbapenem **1b**. Mono double ester derivatives **7** were synthesized in three steps by reaction of the 1β-methylcarbapenem **1a** with $PNZOC_6H_4NO_2$-*p*, followed by alkylation with R_2I and catalytic hydrogenation with 10% Pd/C at pH 6.5 (Scheme 2). The THF carbapenems **1** 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 declines steeply 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 under anhydrous conditions were carried out at room temperature. Synthesis of the acylating agents **10** is shown in Scheme 3.[4]



Scheme 1: (a) **10**/pH 8.5 buffer/*p*-dioxane/0 °C, 75% or **10**/$EtN(iPr)_2$/CH_3CN/*p*-dioxane/r t, 80%; (b) R_2I/K_2CO_3/CH_3CN/r t,77%



Scheme 2: (a) (1) $PNZOC_6H_4NO_2$-*p*/EtN(*i*Pr)$_2$/CH_3CN/*p*-dioxane/r t, 80% and (2) $R_2I/K_2CO_3/CH_3CN$/r t,77%; (b) H_2/10% Pd/C/pH 6.5 buffer/*p*-dioxane/r t, 65%



Scheme 3: (a) (1) $HOC_6H_4NO_2$-*p*/TEA/r t, 90%; (b) $(R"CO_2)_2Hg/R"CO_2H$/heating, 85%

Results and Discussion

1β-Methylcarbapenem **1a** has pKa values of 3.1 and 9.1 and the isoelectric point at pH 6.0.[3] The predominant species at various pH's are cationic below pH 3.1, anionic above pH 9.1 and zwitterionic between pH 3.1 and 9.1. The partition into a lipid phase (n-octanol) is less than one tenth of one percent at all physiological pH's (1~7.4)[3] and is consistent with its Clog P value of -3.02 for the nonionic form. Therefore, it can not possibly be absorbed significantly through the phospholipid bilayer at all physiological pH's, and there is a need to prepare double ester prodrugs in order to facilitate absorption through the phospholipid bilayer by eliminating the ionic nature and increasing the lipophilicity of the molecule. It is expected that the 1β-methylcarbapenem **1b** would behave similarly.

Mono double ester derivatives and bis double ester derivatives were all tested for *in vitro* activity with and without preincubation in mouse serum. All the ester derivatives were hydrolyzed to yield antibacterial activity that paralleled those of the parent compounds, **1a** and **1b**. The in vitro antimicrobial activity of some representative mono and bis double ester prodrugs is shown in Tables 1 and 2. In general, mono ester derivatives **2** were quite active whereas mono ester derivatives **7** were less active, and bis double ester derivatives **3** were inactive except against *E. coli* GC2205 (permeability mutant). These ester prodrugs all demonstrated better stability than imipenem to hydrolysis by hog renal dehydropeptidase. As is evident from the SOD value and the SOD/SSC ratio in Table 3, mono double ester derivatives (**2**, **4** and **7**) did not demonstrate

Table 1 Mono Double Ester Prodrugs

In vitro activity (MIC; μg/mL)

Compound		1a	2a	2a*	2c	2c*	2d	2d*	7a	7a*
ORGANISM	Strain									
E. coli	ATCC 25922	≤0.06	0.12	≤0.06	0.25	≤0.06	0.50	≤0.06	0.50	≤0.06
E. coli	GC 2205	≤0.06	0.12	≤0.06	0.12	≤0.06	0.06	≤0.06	1.0	≤0.06
E. coli	GC 1792	≤0.06	0.25	≤0.06	0.50	≤0.06	0.50	≤0.06	0.50	≤0.06
E. cloacae	GC 2209	≤0.06	2.0	≤0.06	1.0	0.06	2.0	0.06	2.0	0.12
C. freundii	GC 2211	≤0.06	4.0	0.25	2.0	0.25	4.0	0.12	4.0	0.25
M. morganii	GC 2213	0.50	2.0	1.0	4.0	0.50	2.0	0.50	16	0.50
A. calcoaceticus	GC 756	2.0	32	4.0	32	4.0	32	2.0	64	2.0
P. aeruginosa	ATCC 27853	8.0	64	16	64	8.0	64	8.0	128	8.0
P. aeruginosa	GC 1544 OprD-	16	>128	32	>128	16	>128	16	>128	32
X. maltophilia	GC 562	>128	>128	>128	>128	>128	>128	>128	>128	>128
S. aureus	ATCC 29213	≤0.06	0.50	≤0.06	0.25	0.03	0.12	0.03	1.0	0.03
S. aureus	GC 2220 MRSA	1.0	32	8.0	8.0	2.0	8.0	1.0	64	2.0
E. faecalis	GC 842	0.50	4.0	1.0	4.0	1.0	2.0	1.0	16	2.0
E. faecium	GC 1182	64	>128	64	>128	64	128	64	128	128
Rel. hydrolysis by hog DHP		8.5	6		2.9		5.9		11	

*Following preincubation in mouse serum. **Imipenem = 100.

Table 2 Bis Double Ester Prodrugs

In vitro activity (MIC; μg/mL)

Compound		1a	3a	3a*	3e	3e*	3h	3h*	3l	3l*
ORGANISM	Strain									
E. coli	ATCC 25922	≤0.06	32	≤0.06	4.0	≤0.06	128	0.06	128	0.06
E. coli	GC 2205	≤0.06	2.0	0.06	1.0	0.06	1.0	0.12	1.0	0.06
E. coli	GC 1792	≤0.06	16	0.06	8.0	≤0.06	>128	0.12	>128	0.06
E. cloacae	GC 2209	≤0.06	>128	0.12	64	0.12	>128	0.25	>128	0.12
C. freundii	GC 2211	≤0.06	>128	0.25	128	0.25	>128	0.50	>128	0.50
M. morganii	GC 2213	0.50	128	1.0	32	1.0	>128	4.0	>128	2.0
A. calcoaceticus	GC 756	2.0	>128	4.0	>128	4.0	>128	8.0	>128	8.0
P. aeruginosa	ATCC 27853	8.0	>128	16	>128	16	>128	32	>128	32
P. aeruginosa	GC 1544 OprD-	16	>128	32	>128	32	>128	64	>128	64
X. maltophilia	GC 562	>128	>128	>128	>128	>128	>128	>128	>128	>128
S. aureus	ATCC 29213	≤0.06	4.0	0.06	8.0	0.03	4.0	0.12	4.0	0.06
S. aureus	GC 2220 MRSA	1.0	128	4.0	>128	2.0	64	2.0	64	4.0
E. faecalis	GC 842	0.50	64	2.0	64	2.0	64	4.0	32	2.0
E. faecium	GC 1182	64	>128	128	>128	128	>128	>128	>128	64
Rel. hydrolysis by hog DHP		8.5	11		4.9		2.0		19	

*Following preincubation in mouse serum. **Imipenem = 100.

significantly improved oral activity due to the presence of the charged species. However, bis double ester derivatives, **3** and **5**, all demonstrated enhanced oral activity, indicating that the bis double ester prodrugs have high oral bioavailability in the mouse model. These results are consistent with the increased calculated partition coefficients (Clog P) of bis double ester prodrugs **3** shown in Table 3. By varying the R_2 and keeping the R_1

Table 3 ED_{50} (mg/kg)[a] for THF Carbapenems, 1, 2, 3, 4, 5 and 7, Against Acute Lethal *E. coli* Infection in Mice

				E. coli #311		
Compound	Clog P[b]	R_1	R_2	SOD[c]	SSC[d]	SOD/SSC
1a	-3.02	---	---	3.8	0.34	11
2a	0.11	CH(Me)OAc	Na	4.9	0.65	7.5
2b	0.11	CH(Me)OAc	H·EtN(*i*Pr)$_2$	4.5	0.62	7.3
2c	0.64	CH_2OCO*i*Pr	Na	5.1	0.59	8.6
2d	0.86	CH_2OCOPr	Na	3.4	0.26	13
2e	1.04	CH_2OCO*t*Bu	H·EtN(*i*Pr)$_2$	2.9	0.65	4.5
2f	1.45	$CH_2OCOC_6H_5$	Na	2.9	0.32	9.1
7a	-0.12	H·HCl	CH_2OCO_2Et	8.2	0.62	13
7b	0.19	H·HCl	$CH(Me)OCO_2Et$	25	1.8	14
7c	1.69	H·HCl	$CH(Me)OCO_2C_6H_{10}$	>3.2	1.6	---
3a	1.28	CH_2OCO*i*Pr	CH_2OCO_2Et	0.56	0.40	1.4
3b	2.04	CH_2OCO*i*Pr	CH_2OCO*t*Bu	1.6	1.9	0.84
3c	1.89	CH_2OCO*i*Pr	$CH(Me)OCO_2$*i*Pr	1.4	2.1	0.67
3d	3.09	CH_2OCO*i*Pr	$CH(Me)OCO_2C_6H_{10}$	1.5	1.9	0.79
3e	0.75	CH(Me)OAc	CH_2OCO_2Et	0.83	0.59	1.4
3f	1.50	CH_2OCOPr	CH_2OCO_2Et	1.3	0.49	2.6
3g	1.58	CH(Me)OCO*i*Pr	CH_2OCO_2Et	0.72	0.45	1.6
3h	1.67	CH_2OCO*t*Bu	CH_2OCO_2Et	0.96	0.73	1.3
3i	1.98	CH(Me)OCO*t*Bu	CH_2OCO_2Et	0.42	0.46	0.91
3j	2.08	$CH_2OCOC_6H_5$	CH_2OCO_2Et	0.76	0.54	1.4
1b	-3.02	---	---	3.5	0.28	12
4	0.64	CH_2OCO*i*Pr	H·EtN(*i*Pr)$_2$	2.3	0.18	14
5	1.28	CH_2OCO*i*Pr	CH_2OCO_2Et	0.87	0.37	2.4
Primaxin[e]				79	0.70	113

[a]For all mono and bis double ester prodrugs **2**, **3**, **4**, **5**, and **7**, the numbers have been normalized with a factor which is the molecular weight of the parent compound divided by the molecular weight of the prodrug. [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]A 1:1 combination of imipenem and cilastatin.

with the same moiety (CH_2OCO_iPr), CH_2OCO_2Et was identified as the most effective moiety for the R_2 (compounds **3a-3d**). Likewise, by changing the R_1 and keeping the R_2 with the same moiety (CH_2OCO_2Et), CH(Me)OCO*t*Bu was found to be the best moiety for the R_1 (compounds **3a** and **3f-3j**). Therefore, the best combination of the R_1 and the R_2 led to the identification of the bis double ester prodrug **3i** which had oral activity that was as good as that of the best peptidic prodrug (L-Val derivative) of carbapenem **1a**.[2]

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