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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF (1'R, 5R, 6R)-2-TERT-BUTYL-6-(1'-HYDROXYETHYL)PENEM-3-CARBOXYLIC ACID

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Abstract: *As with the oxapenems, the 2-tert-butyl substituents substantially increased the hydrolytic stability of penems. The corresponding penems* ***1*** *and* ***2*** *were prepared and found to be extremely stable compounds.* ***2*** *showed good in vitro activity against gram-positive bacteria.*

ß-Lactam antibiotics are known for the unusual reactivity of their ß-lactam system toward nucleophilic agents. So the ß-lactam ring of the first penemcarboxylic acid, the 6-phenoxyacetylamino derivative, which was synthesized by *Woodward et al.*[1] in 1975, is too easily hydrolyzed to find any clinical use. A second generation of penems[2,3], lacking the 6-phenoxyacetamido substituent, was chemically more resistant. As these compounds were not sufficiently stable against bacterial ß-lactamases a third generation of 6-(1'-hydroxyethyl)penems was prepared[4,5]. They have a broad antibacterial spectrum including anaerobic and penicillin resistant strains. However, similar to thienamycin but unlike to the penicillins and cephalosporins, the penems suffer from renal inactivation precluding their use as clinically useful antibiotics. We thought that the low in vivo stability of the penems eventually could be improved by generating derivatives which are chemically more stable.

The stabilizing influence of certain side chains on ß-lactam antibiotics has often been demonstrated. 2-alkylsubstituted penems, for example, are known to be more stable than their unsubstituted derivatives. Whereas the 2-unsubstituted, racemic penemcarboxylic acid salt **3** has a hydrolysis half life in phosphate buffer (pH 7.4 at 37°C) of only 20 hours[6], the 2-methylsubstituted representative **4** with a half life of 100 hours[5], is already about five times more stable. Similar correlations can be found for the oxapenems, although neither the 2-un-

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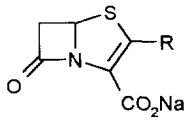
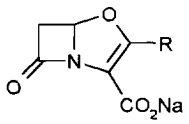
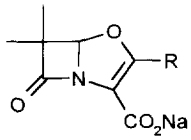
substituted nor the 2-methylsubstituted derivatives **5** and **6** could be prepared in pure form in our laboratory.



3 R = H; **4** R = Me



5 R = H; **6** R = Me

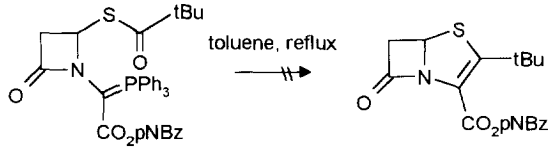


7 R = tBu; **8** R = Me

In 1987 *Pfaendler* and *Hendel* [7] were able to show, that the introduction of the *tert*-butyl group in the 2-position of the 6,6-dimethylsubstituted oxapenem **7** provided a remarkable 30 fold stabilization, relative to the corresponding 2-methylsubstituted derivative **8**.

The stabilizing effect of the *tert*-butyl group on the parent penem system has not yet been investigated, probably because of the difficulties encountered in the preparation of 2-*tert*-butylpenemcarboxylic ester **10**[8].

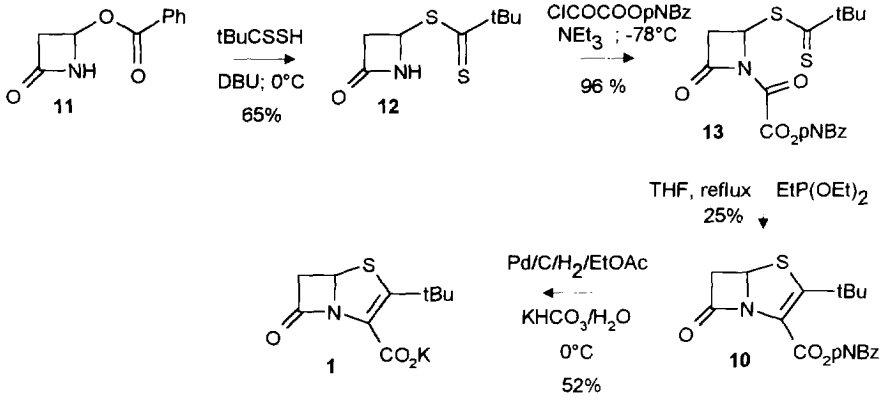
The most versatile method for preparing 2-unsubstituted or 2-alkylpenems is the intramolecular Wittig olefination of suitable phosphoranes. However, in the preparation of sterically demanding 2-*tert*-butylpenemcarboxylic ester **10** this method was not suitable. No change occured when **9** was heated in toluene for 14 days! At higher temperatures the ß-lactam of **9** was cleaved.



9 **10**

In 1982 a new method, called the oxalimide route, was added to the manyfold ways to synthesize penems by *Afonso et al.*[9]. The authors used $P(OEt)_3$ to cyclize azetidinone trithiocarbonates. *Yoshida et al.*[10] used $P(OEt)_3$ as well as $P(OMe)_3$ or $P(OiPr)_3$ in the cyclization of a suitable substituted oxalimide to give 2-cysteaminylpenemcarboxylic acids. More recently, this method of preparation was improved by *Budt et al.*[11] by using alkylphosphonous acid diesters $MeP(OR)_2$, allowing lower reaction temperatures during

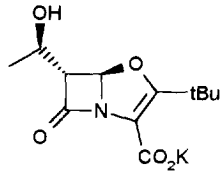
the cyclization reaction. Adopting the latter method allowed the preparation of 2-*tert*-butylpenemcarboxylic acid potassium salt **1** according to the following sequence[12].



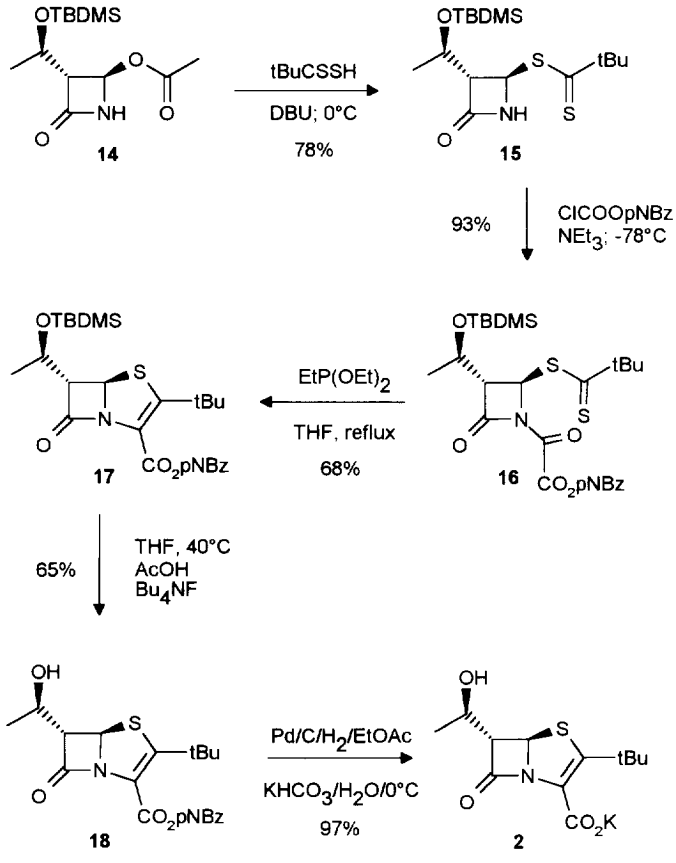
The final product **1** was obtained after lyophilisation as a colorless noncrystalline solid. We found a hydrolysis half life value of about 9 days. The increase in stability, compared with the 2-unsubstituted penem salt **3**, is approximately 10 fold[13].

The (R)-1′-hydroxyethyl substituent increased the biological activity and hydrolytic stability of oxapenems and carbapenems. Therefore we prepared also the corresponding *tert*-butyl penem **2** by a slightly modified procedure. The starting material **14** was commercially available, optically active „Azetidon-Kaneka".

(1′R,5R,6R) potassium 2-*tert*-butyl-6-(1′-hydroxyethyl)penem-3-carboxylate **2** showed a good antibacterial activity against gram-positive bacteria[14]. The minimal inhibitory concentrations (MIC, μg/ml) of the penem salt **2** in comparison with the analogous oxapenem salt **19** and ceftazidime are given in *Table 1*. Compared with **19**, **2** shows in general a slightly diminished activity[15]. In the case of *Staphylococcus epidermidis 270* its activity is better than that of the oxapenem salt **19**[16].



19



The ß-lactamase inhibiting activity of **2** was investigated with a nitrocefin test using the ß-lactamases of *Enterobacter cloacae* and *Escherichia coli 205 TEM R*$^+$ *(566)*[17] after a 15 min preincubation period at 37 °C. The IC_{50}-values of **2** are compared to those of clavulanic acid and the oxapenem salt **19** *(Table 2)*. **2** was a better inhibitor of the *E. cloacae* enzyme than clavulanic acid, but considerably less active with the TEM ß-lactamase. Compared with the oxapenem **19**, **2** was less active.

Compared to earlier prepared 6-hydroxyethyl substituted penems[4] the new (1'R,5R,6R) potassium 2-*tert*-butyl-6-(1'-hydroxyethyl)penem-3-carboxylate **(2)** showed a highly increased hydrolysis half life of 16 days. In fact, to our knowledge, **2** is the most stable representative of the penem antibiotics.

Table 1	Minimal Inhibitory Concentration (MIC, μg/ml)			
Bacteria	**Strain**	**Ceftazidime**	**19 (oxapenem)**	**2 (penem)**
S. aureus	X 1.1	8	0.25	0.5
S. aureus	V 41	8	1	1
S. aureus	X 400	64	2	4
S. aureus	S 13 E	64	1	4
S. epidermidis	270	16	4	0.5
S. epidermidis	222	16	0.25	2
S. pyogenes	C 203	0.06	0.06	0.12
S. pneumoniae	Park I	0.03	0.03	0.12
Enterococcus sp.	X 66	128	2	16
Enterococcus sp.	2041	16	16	32
H. influenzae (b-lac-)	CL	0.06	0.5	8
H. influenzae (b-lac+)	76	0.03	0.5	8
E. coli	N 10	0.06	4	64
E. coli	EC 14	0.03	4	128
E. coli	TEM	0.03	4	128
Klebsiella sp.	X 26	0.06	1	8
Klebsiella sp.	KAE	0.5	4	64
Klebsiella sp.	X 68	0.12	2	64
E. aerogenes	C 32	2	8	128
E. aerogenes	EB 17	0.12	32	128
E. cloacae	EB 5	0.12	32	128
E. cloacae	265 A	32	32	128
Salmonella sp.	X 514	0.06	1	32
Salmonella sp.	1335	0.25	4	128
S. marcescens	X 99	0.12	16	128
S. marcescens	SE 3	0.06	64	128
Shigella sonnei	N 9	0.03	4	64
M. morganii	PR 15	0.25	8	32
P. stuartii	PR 33	0.06	4	32
P. rettgeri	C 24	0.03	4	8
C. freundii	CF 17	0.5	16	128
Acinetobacter sp.	AC 12	1	8	16

Table 2	ß-Lactamase Inhibition Concentration (IC_{50}, mol/l)		
	19 (oxapenem)	clavulanic acid	**2** (penem)
E. cloacae	2×10^{-9}	1×10^{-4}	3×10^{-7}
E. coli TEM	3×10^{-6}	4×10^{-8}	5×10^{-5}

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References and Notes

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8. All reported compounds **10** -**18** gave correct elemental analyses. Physical data of **2**: white powder. TLC (C_{18} reversed phase silica gel): $R_f = 0.2$ (MeCN/H_2O: 1/3). UV-spectrum in H_2O: $\lambda_{max} = 299$ nm ($\varepsilon = 2050$), 255 nm ($\varepsilon = 3400$). ^{1}H-NMR-spectrum in D_2O/ $Me_3SiCD_2CO_2Na$: δ (ppm) = 1.25 (s, 9H, tBu), 1.29 (d, 3H, CH_3, J = 6.6 Hz), 3.82 (dd, 1H, 6-H, J = 5.9 Hz, J = 1.5 Hz), 4.21 (dq, 1H, 1'-H, J = 6.6 Hz, J = 5.9 Hz), 5.55 (d, 1H, 5-H, trans, J = 1.5 Hz).
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12. Dithiopivalic acid was synthesized according to the procedure for methyl dithiopivalate, published by Westmijze, H.; Kleijn, H.; Meijer, J.; Vermeer, P. *Synthesis* **1979**, 432.
13. Interestingly, 2-tert-butyl-6,6-dimethylpenemcarboxylic acid potassium salt was found to have a reduced hydrolysis half life as compared to **1**.
14. In contrast to penems prepared earlier[2,4], no activity against gram-negative bacteria was observed, with **2**, presumably because of its lipophilic nature, arising from the *tert*-butyl group. A similar behavior can be observed with all lipophilic ß-lactam antibiotics including penicillins, cephalosporins and carbapenems.
15. As with earlier investigated penems[5] the unsubstituted compound **1** was less active than the corresponding 6-hydroxyethylpenem **2**, especially against ß-lactamase producing bacteria.
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