Bioorganic & Medicinal Chemistry Letters, Vol.3, No.11, pp. 2205-2210, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

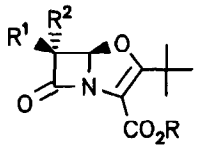
Substituted 6-Alkyloxapenems: Potent ß-Lactamase Inhibitors; Synthesis and Biological Characterization

Hanno Wild[1*] and Karl-Georg Metzger[2]
Bayer AG, [1]Chemistry Science Laboratories Pharma, [2]Institute for Chemotherapy,
P.O.Box 101709, D-5600 Wuppertal 1, Germany

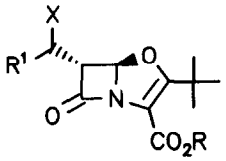
(*Received* 1 *April* 1993)

Summary: The synthesis of 6-alkyloxapenems bearing hydroxy, fluorine, amino, acylamino and sulfonamido substituents in the 1'-position is described. Several of the compounds are potent inhibitors of ß-lactamases from *Staphylococcus aureus* and *Proteus vulgaris* and have an appreciable stability against chemical hydrolysis.

The oxapenem class of ß-lactams was first described in 1977[1]. Although some derivatives showed activity as ß-lactamase inhibitors[2], the instability of the highly strained oxapenem ring system towards chemical hydrolysis precluded their use in biological systems. Recently, it was discovered that 2-*tert*-alkyl-oxapenems **1** are much more stable than expected[3] and it was even possible to prepare free 6-methylene



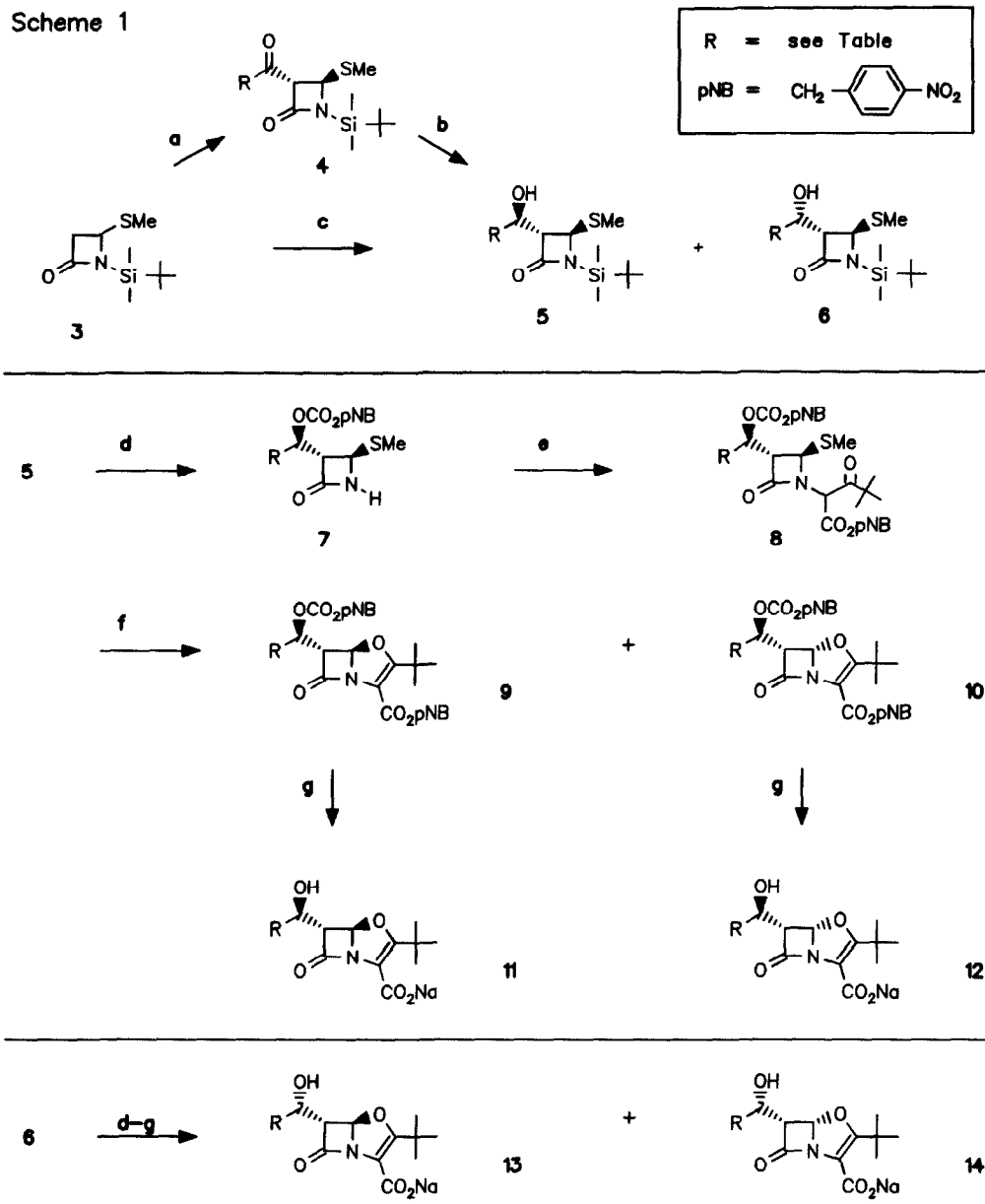
1a R^1, R^2 = H, CH_3
1b R^1, R^2 = R^3N
1c R^1 = H, R^2 = (R)–CH_3CHOH
1d R^1 = H, R^2 = CH_2OH



2 X = OH, NH_2, $NHCOR^2$, $NHSO_2R^3$

oxapenems **1b** as their sodium salts[4]. The interesting antibacterial and ß-lactamase inhibitory activity of the 6-(1-hydroxyethyl)[5] and the 6-hydroxymethyl[6] derivatives (**1c,d**) prompted us to investigate in depth the synthesis and biological properties of 2-*tert*-butyloxapenems **2** bearing various substituted alkyl residues in the 6-position.

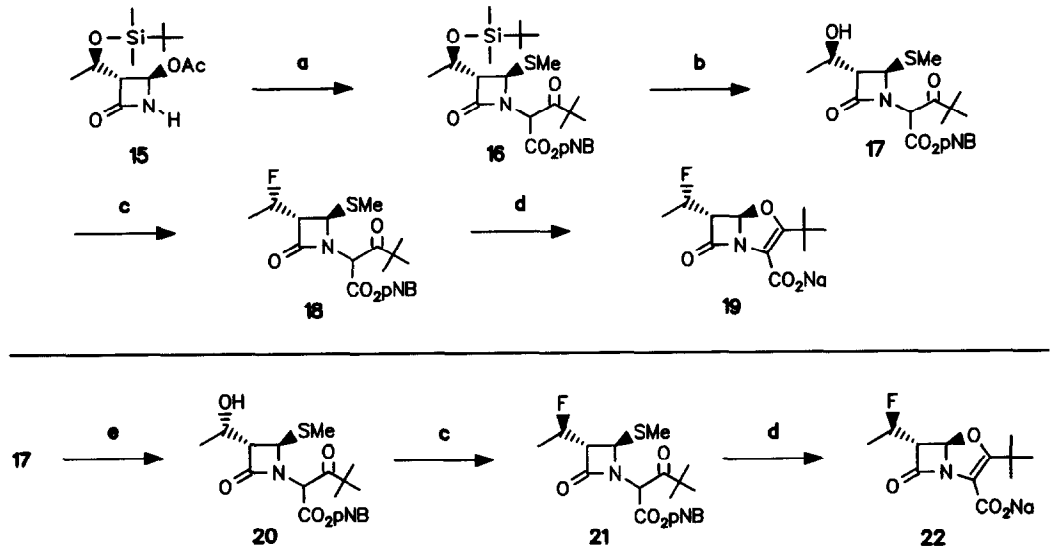
Scheme 1



(a) LDA, RCO_2Me, THF, −78°C; (b) $NaBH_4$, THF/EtOH, 0°C or L-Selectride, THF, −78°C or K-Selectride, THF, −78°C (see text); (c) LDA, R-CHO, THF, −78°C; (d) $ClCO_2pNB$, DMAP, CH_2Cl_2, −10°C –> rt; n-Bu_4NF, HOAc, THF, 0°C; (e) t-BuCOCHBrCO_2pNB, Cs_2CO_3, CH_3CN; (f) Cl_2, CH_2Cl_2, −78°C; KOt-Bu, THF, −15°C; (g) H_2, Pd-C, EtOAc/aq. $NaHCO_3$, 0°C.

The synthesis of 6-(1-hydroxyalkyl)oxapenems shown in scheme 1 follows standard oxapenem methodology[7]. The lithium enolate of ß-lactam **3** is hydroxyalkylated to yield a separable mixture of the alcohols **5** and **6**. Alternatively **3** can be acylated followed by reduction of the derived ketone **4** with sodium borohydride to a 1:1-mixture of **5** and **6**. Selective formation of **5** is possible with K-Selectride, whereas reduction with L-Selectride gives alcohol **6** as the major product. The stereochemistry of alcohols **5** and **6** was determined by conversion into the corresponding chlorides under inversion of configuration (N-chlorosuccinimide, triphenylphosphine), subsequent E_2-elimination using DBU, and analysis of the geometry of the derived double bond by ^{1}H-NMR following literature procedures[4,8]. The hydroxy group of the separated isomer **5** is then protected as a *para*-nitrobenzylcarbonate, the ß-lactam nitrogen is deprotected and the N-side chain is built up in one step[3,9]. The methylthio substituent is transformed into a chloride leaving group followed by smooth cyclization of the potassium enolate of the ß-ketoester. The *trans/cis*-isomeric mixture of oxapenems **9** and **10** is then separated by careful chromatography on silica gel at -20°C. This low temperature is necessary because of the high elimination tendency of 1'-substituents in the 6-position of oxapenems[4]. In many cases only the major and more stable *trans*-isomer **9** is isolated. The separated isomers are then deprotected to give free oxapenems **11** and **12** as their sodium salts. The second alcohol diastereomer **6** is converted into the final products **13** and **14** following the same synthetic scheme.

Scheme 2

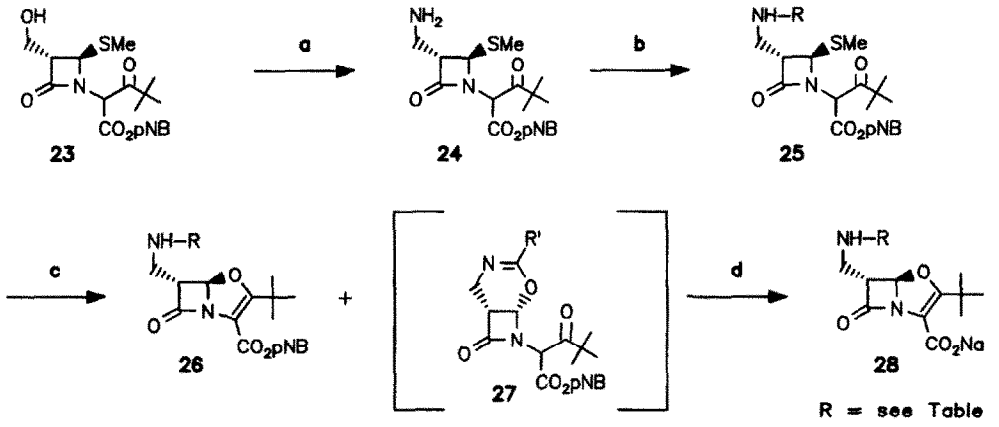


(a) NaSMe, H_2O/CH_3CN, 0° C (81 %); t-BuCOCHBrCO$_2$pNB, Cs_2CO_3, CH_3CN (80 %); (b) n-Bu_4NF, HOAc, THF, 45°C (65 %); (c) DAST, CH_2Cl_2, -78°C -> rt (18: 95 %; 21: 65 %); (d) see f-g scheme 1; (e) CH_3SO_2Cl, py (86 %); DMF, 120°C (35 %); 2N HCl, MeOH (82 %).

The synthesis of the 6-(1-fluoroethyl) substituted oxapenems **19** and **22** is shown in scheme 2[7]. The alcohol **17** is readily available from the commercial enantiomerically pure ß-lactam **15**. **17** is converted into fluoride **18** under inversion of configuration using diethylamino sulfurtrifluoride (DAST)[10]. The diastereoisomeric fluoride **21** is prepared by fluorination of alcohol **20**, which is obtained from **17** by inverting the stereochemistry in a 3-step sequence. Cyclization of **18** and **21** to the oxapenems and final deprotection is uneventful.

The aminomethyl substituted oxapenems are synthesized as shown in scheme 3[7]. The racemic hydroxymethyl compound **23**, which is obtained in a way similar to **17**, is converted into the amine in a 3-step sequence[11]. The amine is derivatized and the oxapenems **26** are obtained in the usual way. In the case of amides (R = R'-CO) oxazines **27** are formed as byproducts in varying amounts in the cyclization step. During the final deprotection those amines which are protected as pNB-carbamates (**26a**, **26k**) are liberated to yield oxapenems **28** as betaines (**28a**: NHR = $NH_3^{\oplus}$, **28k**: NHR = Ph(CONH)$NH_3^{\oplus}$).

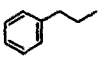
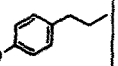
Scheme 3

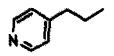
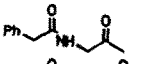
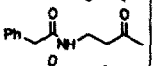
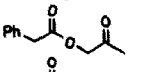
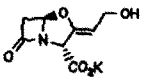


(a) CH_3SO_2Cl, py (97 %); LiN_3, DMSO, 50°C; HS–$(CH_2)_3$–SH, NEt_3, CH_2Cl_2/i–PrOH, reflux (54 %); (b) $ClCO_2pNB$, DMAP, CH_2Cl_2, –10°C –> rt (25a); $R'SO_2Cl$, NEt_3, CH_2Cl_2, 0°C (25b–d); $R'CO_2H$, DCC, HOBT, THF (25e–l); (c) see f, scheme 1; (d) see g, scheme 1.

As shown in the table the stability of the oxapenems towards chemical hydrolysis varies widely. The *trans*-substituted oxapenems **11** and **13** are more stable than the *cis*-compounds **12** and **14**. Especially the betain **28a** is a very unstable compound. On the other hand, the 1-hydroxy-3-phenylpropyl substitution (**11i,j**, **13i,j**) leads to oxapenems with an appreciably high stability even if compared with the standard clavulanic acid. The *trans*-oxapenems **11a** and **11b** - the racemates of the already known enantiomerically pure compounds **1c**[5] and **1d**[6] - possess an interesting antibacterial activity[12]. **11b** is slightly more active

Table **Hydrolytic stability and β-lactamase inhibitory activity of oxapenems 11 – 14, 19, 22 and 28**

No	R	t 1/2 (h)[a] pH 7.0 28°C	Relative protective[b] effect by inhibition of β-lactamase of Staph. aureus	Proteus vulg.
11a 12a	H [12]	5.0 2.4	1.7 0.9	1.25 0.6
11b 12b 13b	CH_3 [12]	8.1 6.4 7.1	0.5 1.7 1.5	– [c] – –
11c 13c	CH_3O_2C	6.2 6.7	< 0.2 < 0.2	< 0.1 < 0.1
11d 13d	$t\text{-}BuO_2C$	– [c] 10.7	< 0.2 < 0.2	< 0.1 < 0.1
11e 12e 13e 14e		7.0 0.5 8.9 2.2	2.3 2.3 2.3 1.25	< 0.3 < 0.3 6.0 0.3
13f	HO 	10.3	0.6	0.6
11g 13g 14g		6.8 7.0 – [c]	0.7 1.3 0.9	1.5 1.8 0.5
11h 13h		6.4 6.0	0.3 0.5	< 0.1 1.25
11i 13i		21.0 39.0	0.4 2.7	< 0.1 < 0.1
11j 12j 13j 14j	HO 	24.6 – [c] 21.2 11.8	0.75 1.0 3.0 1.9	< 0.1 < 0.1 < 0.1 < 0.1

No	R	t 1/2 (h)[a] pH 7.0 28°C	Relative protective[b] effect by inhibition of β-lactamase of Staph. aureus	Proteus vulg.
11k 12k 13k		13.7 7.1 15.8	0.4 1.5 1.5	< 0.1 < 0.1 < 0.1
19 22	see scheme 2	7.2 3.5	0.4 0.4	0.6 0.2
28a	H	1.2	< 0.1	< 0.1
28b	CH_3SO_2	2.2	0.75	"
28c	$PhSO_2$	– [c]	0.4	"
28d	$PhCH_2SO_2$	8.9	1.0	"
28e		< 3.0	< 0.1	"
28f		5.7	0.3	"
28g		5.5	0.3	"
28h		6.6	0.2	"
28i		5.5	0.2	"
28j		9.3	0.25	"
28k		2.2	< 0.1	"
clavulanic acid		60	1	1

(a) phosphate buffer, determination by HPLC; (b) improvement of the MIC of mezlocillin and ampicillin in the presence of 0.1 μg/ml β-lactamase inhibitor; relative rating: > 1, more active than clavulanic acid; < 1, less active than clavulanic acid; Staph. aureus: mean of 5 strains, Proteus vulgaris: 1 strain; (c) not determined.

than **11a** and has a broad spectrum of activity excluding *Pseudomonas aeruginosa*. Most of the other oxapenems prepared exhibit activity only against *Staphylococci*.

However, the inhibitory activity of the oxapenems against ß-lactamases is very high, especially against ß-lactamases from *Staphylococcus aureus* and against the TEM derived ß-lactamase from *Proteus vulgaris*. This can be shown not only against the isolated enzymes, but also in cell culture in combination experiments with mezlocillin and ampicillin (see table). The most potent compound in this respect is the α-hydroxybenzyl derivative **13e**, which is several times more active against these enzymes than clavulanic acid. The very stable phenylpropyl substituted oxapenems **13i** and **13j** are the most effective derivatives against gram positive ß-lactamases, but they are inactive against the enzyme of *Proteus vulgaris*. The fluorine substituted oxapenems **19** and **22** as well as the aminomethyl compounds **26** do not possess interesting activity.

In summary, the synthesis of various differently substituted 6-alkyloxapenems is described for the first time. These novel ß-lactams are potent inhibitors of ß-lactamases from *Staphylococcus aureus* and *Proteus vulgaris*. Especially the α-hydroxybenzyl derivative **13e** is highly active and sufficiently stable against chemical hydrolysis to be further evaluated in *in vivo* experiments.

REFERENCES AND NOTES

1. Eglington, A.J. *J.Chem.Soc.,Chem.Commun.* **1977**, 720.
2. (a) Cherry, P.C.; Newall, C.E.; Watson, N.S. *J.Chem.Soc.,Chem.Commun.* **1978**, 469.
   (b) Murakami, M.; Aoki, T.; Matsuura, M.; Nagata, W. *J.Antibiotics* **1990**, *43*, 1441.
3. (a) Bayer AG, Eur.Patent 301394; *Chem.Abstr.* **1989**, *111*, 39099.
   (b) Pfaendler, H.R.; Hendel, W.; Nagel, U. *Zeitschr.f.Naturforsch.* **1992**, *47b*, 1037.
4. Wild, H.; Hartwig, W. *Synthesis* **1992**, 1099.
5. Pfaendler, H.R.; Metzger, K.-G. *Bioorg.Med.Chem.Lett.* **1993**, submitted.
6. Pfaendler, H.R.; Neumann, Th.; Bartsch, R. *Synthesis* **1992**, 1179.
7. The compounds shown in schemes 1 and 3 are racemic mixtures of enantiomers. For convenience only one enantiomer is shown. The compounds shown in scheme 2 are pure enantiomers.
8. Foulds, C.D.; Kosmirak, M.; Sammes, P.G. *J.Chem.Soc.,Perkin Trans.I* **1985**, 963.
9. Wild, H. *Tetrahedron Lett.* **1993**, *34*, 285.
10. Yoshioka, T.; Watanabe, A.; Chida, N.; Fukagawa, Y. *J.Antibiotics* **1989**, *42*, 1520.
11. Bayley, H.; Standring, D.N.; Knowles, J.R. *Tetrahedron Lett.* **1978**, 3633.
12. The *trans/cis*-mixtures of oxapenems **11a/12a** and **11b/12b** were first prepared by Prof. Pfaendler, München. We thank Prof. Pfaendler for supplying samples of these compounds. See also ref. 5-6.