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SYNTHESIS AND BIOLOGICAL EVALUATION OF 4-ALKOXY SUBSTITUTED TRINEMS. PART I

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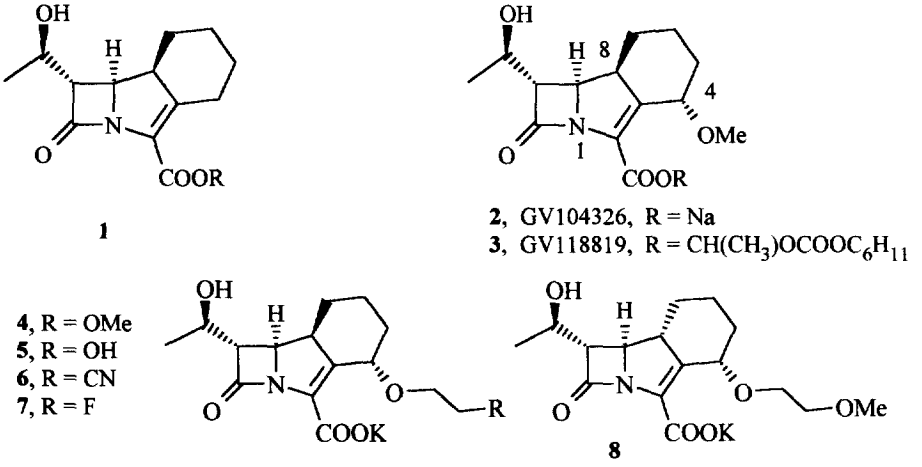
Abstract. Synthesis of new 4-alkoxy substituted trinems **4**, **5**, **6**, **7** and **8** together with their antibacterial profiles compared to imipenem and GV104326 (**2**) are described. The good antibacterial profile observed for derivatives **4-7** encouraged further exploration of these derivatives.

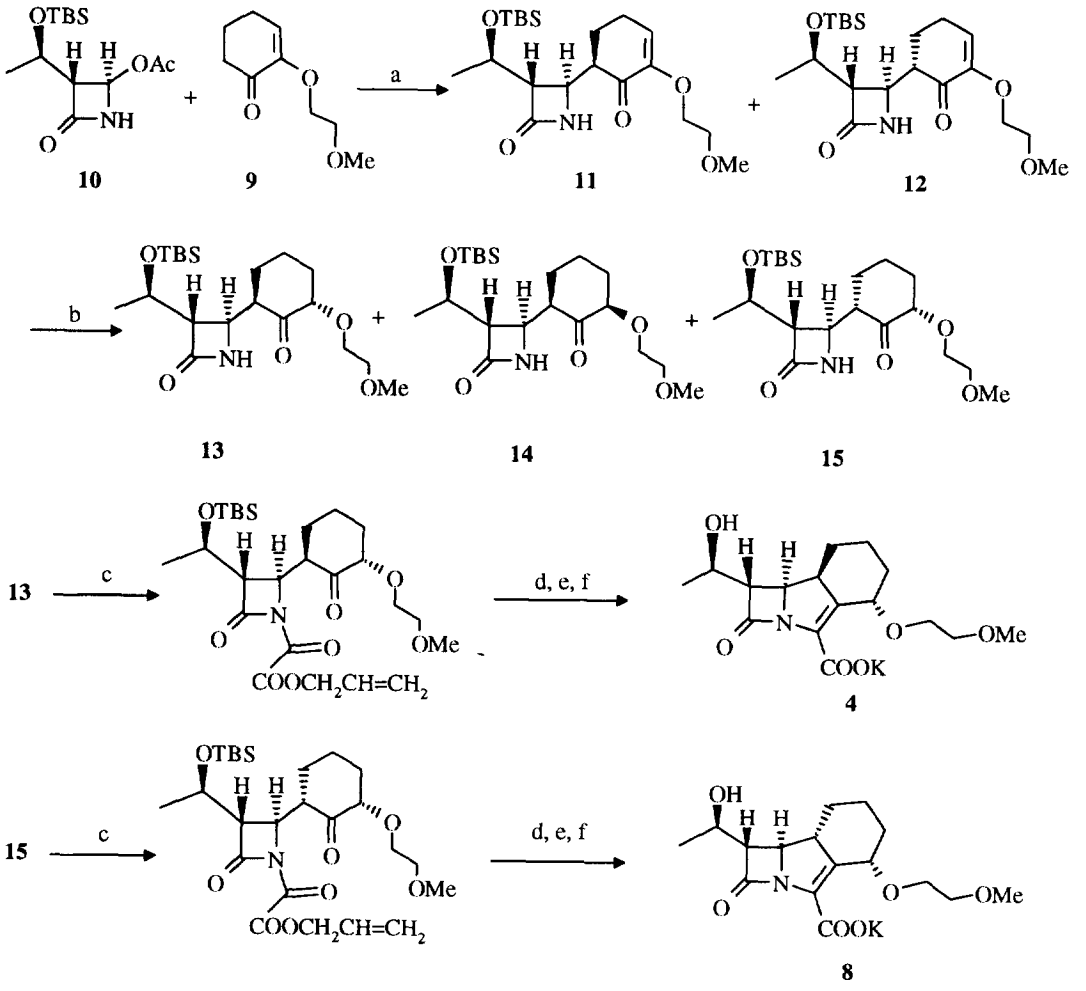
The intense interest in the study of β-lactam antibiotics has led, in the last fifteen years, to the continue introduction of new classes of compounds[1] endowed with a broad spectrum of activity associated with very low toxicity levels which ensure them an outstanding role in antibacterial chemotherapy.

Fig.1



Some years ago, we at Glaxo[2] have identified a novel class of tricyclic β-lactam antibiotics, trinems (**1**, Fig. 1), formerly referred to as tribactams, which are characterised by high potency, high stability to both most relevant β-lactamases and to renal dehydropeptidases, associated with a good chemical stability. As a result GV104326, (**2**, Fig.1), and its metabolically labile ester GV118819 (**3**, Fig.1) were selected for development and are currently in phase II clinical trials.

Scheme 1



a) LHMDA, -78°C, THF; b) Pd/Al_2O_3, H_2 4.5 atm., EtOH; c) TEA, $ClCOCOOCH_2CH{=}CH_2$, CH_2Cl_2; d) $P(OEt)_3$, xylene, 120-140°C; e) TBAF, AcOH, THF; f) $Pd(PPh_3)_4$, potassium 2-ethylhexanoate.

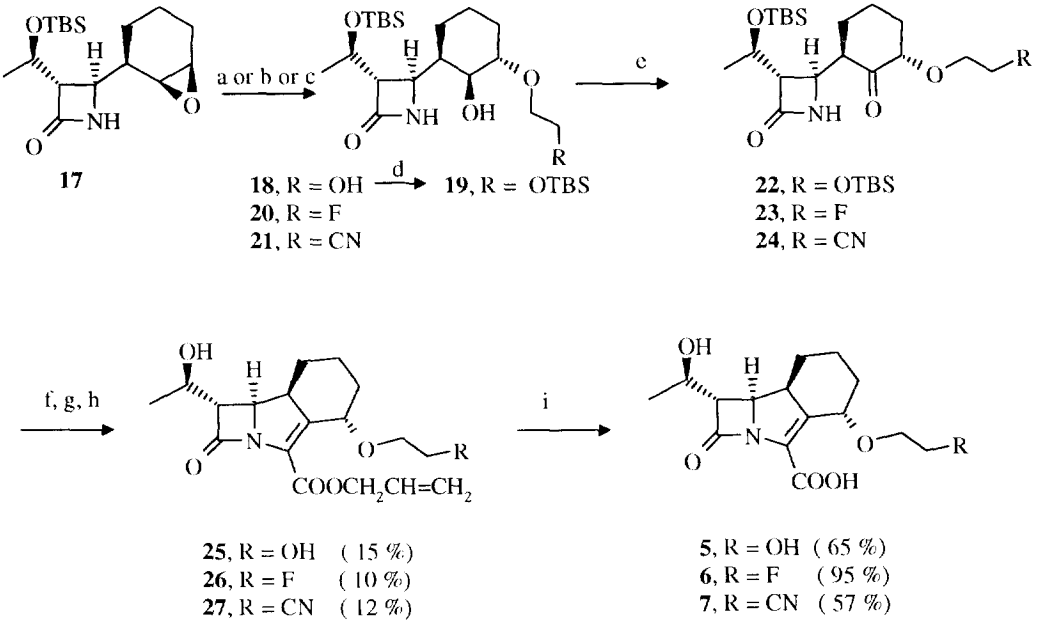
With the aim to investigate biological properties of others 4-alkoxy derivatives, the synthesis of a series of analogues of **2** was undertaken in our laboratories, and this paper describes the synthesis and the preliminary antibacterial profile of compounds **4-8** (Fig. 1).

Trinems **4** and **8** have been prepared according to the procedure[3] utilised for compounds **2**, as outlined in Scheme 1. 2-(Methoxyethoxy)-cyclohex-2-en-1-one[4] **9** was reacted with commercially available

(+)-(3*R*,4*R*,1'*R*)-4-Acetoxy-3-[1'-(*tert*-butyldimethylsilyl)oxy]-ethyl]-2-azetidinone (**10**)[5] using LHMDA as base at -78°C in anhydrous THF yielding an inseparable 3:7 mixture of diasteroisomers **11** and **12** which was purified by flash chromatography in 52% overall yield.

During our attempts of double bond hydrogenation a number of catalysts as well as various reaction conditions were tried. Hydrogenation of the above mentioned mixture was found to give the best result using Pd/Al_2O_3 as catalyst, and separation by flash chromatography gave isomers **13**, **14** and **15** in 5, 11 and 35% yield respectively from **10**. Both azetidinones **13** and **15** were progressed to the corresponding trinems **4** and **8**, through intramolecular cyclisation of the corresponding oxalimide derivatives in the presence of $P(OEt)_3$ (Scheme 1)[6]. Oxalimides were obtained according to well estabilished procedures[6] and were used without any purification.

Scheme 2



a) R = OH, $HOCH_2CH_2OH$ as solvent and pTSA 10 % as catalyst; b) R = F, $HOCH_2CH_2F$ as solvent; c) R = CN, $HOCH_2CH_2CN$ as solvent and 0.25 eq of CAN; d) TBSCl, Imidazole DMF; e) i) DMSO, $(COCl)_2$, CH_2Cl_2, -78°C; ii) TEA, f) TEA, $ClCOCOOCH_2CH{=}CH_2$, CH_2Cl_2; g) $P(OEt)_3$, xylene, 120-140°C; h) TBAF, AcOH, THF; i) $Pd(PPh_3)_4$, potassium 2-ethylhexanoate.

Previous works[7] have shown that trinems with absolute configuration 8*S*,4*R* are generally the most promising isomers in terms of both antibacterial profile and biological stability. This prompted us to define a new and stereoselective route[8] for the synthesis of relevant compounds. The epoxide **17**, previously utilised in the synthesis of **2**[8], was therefore selected as a key intermediate in the preparation of 4-alkoxy trinems **5**, **6** and **7** as shown in Scheme 2.

When the epoxide ring of intermediate **17** was regioselectively opened, using ethylene glycol and dichloromethane 20:1 as solvent in the presence of catalytic amount of pTSA, the alcohol **18** was obtained and then directly converted into the corresponding silylated compound **19** using TBSCl and imidazole in dimethylformamide as solvent (75% overall yield from **17**). However, the same opening reaction using 3-hydroxyproprionitrile as solvent gave the desired product **21** in very low yield, which increased to 20% by using CAN[9] instead of pTSA as acidic catalyst.

Finally, in the case of 2-fluoroethanol as solvent, the epoxide **17** was opened in the absence of catalyst to give the desired product **20** in moderate yield (21%).

Oxidation of secondary alcohols **19**, **20** and **21** under Swern conditions (Scheme 2) provided the corresponding ketones **22**, **23** and **24** in good yields. Their conversion into the corresponding trinems **5**, **6** and **7** was achieved by the same procedure reported in Scheme 1.

The absolute stereochemistry of the final compounds was confirmed by spectroscopic studies and a more detailed description will be reported elsewhere.

The antibacterial activities of **4**, **5**, **6**, **7** and **8** tested against several bacterial strains[10] are reported in Tab. 1 confirming the superior overall good antibacterial profile of the trinem class with *S* absolute configuration at position C-8 (compare **8** to **4**).

Table 1. *In vitro* antibacterial activity of trinems **4, 5, 6, 7** and **8** compared to Imipenem and **2**,

MIC (μg/ml)

	S.aureus **853**	***S.pneumoniae*** **3512**	***E.faecalis*** **850**	***E.coli*** **1850**	***E.coli*** **1919**	***P.aeruginosa*** **1911**	***C.perfringens*** **615**	***B.fragilis*** **2017**
Imipenem	**0.1**	**<=0.01**	**2**	**0.5**	**0.5**	**4**	**0.03**	**0.06**
2	**0.2**	**<=0.01**	**1**	**0.5**	**0.5**	**>32**	**0.03**	**0.06**
4	**0.5**	**0.2**	**2**	**2**	**0.5**	**>32**	**0.03**	**0.1**
5	**0.5**	**0.1**	**8**	**0.5**	**0.5**	**>32**	**0.06**	**0.1**
6	**0.5**	**0.06**	**2**	**4**	**1**	**>32**	**0.03**	**0.2**
7	**0.2**	**0.03**	**4**	**4**	**0.5**	**>32**	**<=0.01**	**0.2**
8	**8**	**8**	**>32**	**>32**	**32**	**>32**	**>32**	**32**

***S. aureus* 853** = *Staphylococcus aureus* 853E, Penicillinase (PC1) producing strain; ***S. pneumoniae* 3512** = *Streptococcus pneumoniae 3512*; ***E. faecalis* 850** = *Enterobacter faecalis* 850; ***E. coli* 1850** = *Escherichia coli* 1850E; ***E. coli* 1919** = *Escherichia coli* 1919, β-lactamase producing strain (TEM 1) with permeable outer membrane; ***P.aeruginosa* 1911** = *Pseudomonas aeruginosa* 1911; ***C. perfringens* 615** = *Clostridium perfringens* 615E; ***B. fragilis* 2017** = *Bacteroides fragilis* 2017.

Compounds **4-7** have shown a good activity against Gram-positives anaerobes and aerobes and Gram-negative anaerobes but moderate activity Gram-negative aerobes. It is worth highlighting that trinems **4-8** have been proven to be notably more stable to DHP-I enzyme than Imipenem.

In conclusion, the described chemical modifications made on the remote position of alkoxy side chain of **2**, demonstrated promising antibacterial profile confirming the need for further and more detailed studies on this class of compounds.

Acknowledgements

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4) 2-(Methoxyethoxy)-cyclohex-2-en-1-one was prepared refluxing for 16 hr a mixture of 2-methoxyethanol and 1,2-cyclohexanedione in toluene using Dowex resin as catalyst, purification by chromatography on alumina gave the desired keton in 35 % yield.

5) (+)-(3*R*,4*R*,1'*R*)-4-Acetoxy-3-[1'-(*tert*-butyldimethylsilyl)oxy]-ethyl]-2-azetidinone (**10**) is commercially available from Aldrich Chemical Company Inc, Milwaukee, WI

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10) Minimal inhibitory concentrations (MIC) were determined according to the procedures recommended by NCCLS for aerobes (M7-A2, **10**, N8) and anaerobes (M11-A2, **10**, N 15).
Mueller Hinton broth (MHB), MHB supplemented with 5% of bovine serum and Schadler broth were used as test medium for aerobes, *S. pneumoniae* and anaerobes, respectively. The final bacterial inoculum was 10^5 CFU/ml.
The MIC was defined as the lowest drug concentration that resulted in no visible growth after 20 hours for aerobes and 48 hours for anaerobes of incubation at 37°C .
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