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SYNTHESIS AND BIOLOGICAL EVALUATION OF 4-HETEROTRIBACTAMS

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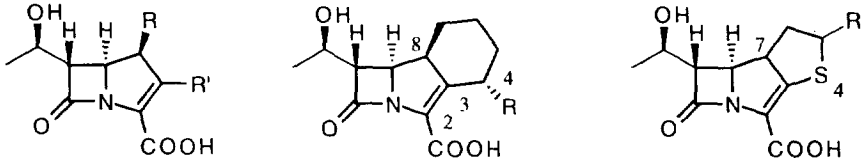
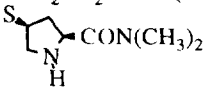
Abstract: The synthesis and the preliminary microbiological activity of some tryciclic β–lactam derivatives designed combining the structural features of the natural carbapenems and the synthetic "*tribactams*" are described.

The carbapenem thienamycin **1**, isolated in 1976 from *Streptomyces cattleya*[1], was found to exhibit a broad spectrum antibacterial activity, including *Pseudomonas aeruginosa,* and remarkable stability towards bacterial β–lactamases. Its discovery resulted in an increased synthetic effort devoted to the identification of new carbapenems[2] with an improved chemical stability and decreased susceptibility to renal dehydropeptidase-I (DHP-I). In this regard, the N-formimidoyl analogue of thienamycin, imipenem **2** (MK-0787), showed a greater chemical stability[3], but was susceptible to DHP-I, whereas the 1β–methyl derivative, meropenem **3** (SM-7338) was found to be fairly insensitive to DHP-I, while retaining excellent antibacterial activity[4].

Figure 1



1 R = H R' = $SCH_2CH_2NH_2$
2 R = H R' = $SCH_2CH_2NHCH(NH)$
3 R = CH_3 R' = 

4

5

Some years ago researchers at Glaxo[5] designed the tricyclic derivatives (*tribactams*) of type **4** (Fig. 1), with the aim of further increasing the metabolic stability and to investigate the consequence of a more constrained β–lactam derivative in enhancing the electrophilicity of the β-lactam carbonyl and the antibacterial activity.

In particular, GG-326 **4** (Fig. 1, R=OCH_3) is a clinically promising β-lactam antibacterial agent, with a particularly broad-spectrum, resistance to β-lactamases and stability to dihydropeptidases.

In view of the efficacy of these novel antibacterial agents, it was of interest to consider similar tricyclic derivatives of general structure **5** (Fig.1), bearing a suitable heteroatom at the vinylic position, as in the case of some carbapenems and some synthetically available cephalosporins[6]. Considering the highly strained nature of these derivatives, a potential problem could be their chemical instability. Therefore, before introducing suitable substituents to mimic the side chain of thienamycin, the unsubstituted derivative **5** (Fig.1, R=H), was synthesized to test the chemical feseability of this novel class of 4-heterosubstituted tribactams.

The first synthetic approach followed is showed in Scheme 1. Addition of two equivalents of the lithium enolate derived from the γ–thiobutyrolactone[7] **7** to the commercially available acetoxyazetidinone derivative[8] **6,** gave a 1:1 mixture of the 7α and 7β epimers in 91% yield . The mixture of **8** and **9** was smoothly transformed into the corresponding phosporane derivatives **10** and **11**, according to the Woodward's three step procedure[9] and submitted to a Wittig-type cyclization,[10] without any attempt to separate the two isomers. The ring closure occurred in moderate yield by heating the mixture of phosphoranes in *o*-xylene at reflux for 5 hrs,[11] giving the highly strained tricyclic derivatives **12** and **13**[12], which were easily separable by MPLC.

It is worth underlining that the more strained 7β isomer **13** was found to be unstable on silica gel and its isolation necessitated buffering the silica gel with AcOEt-TEA 95:5 before the purification of the reaction mixture.

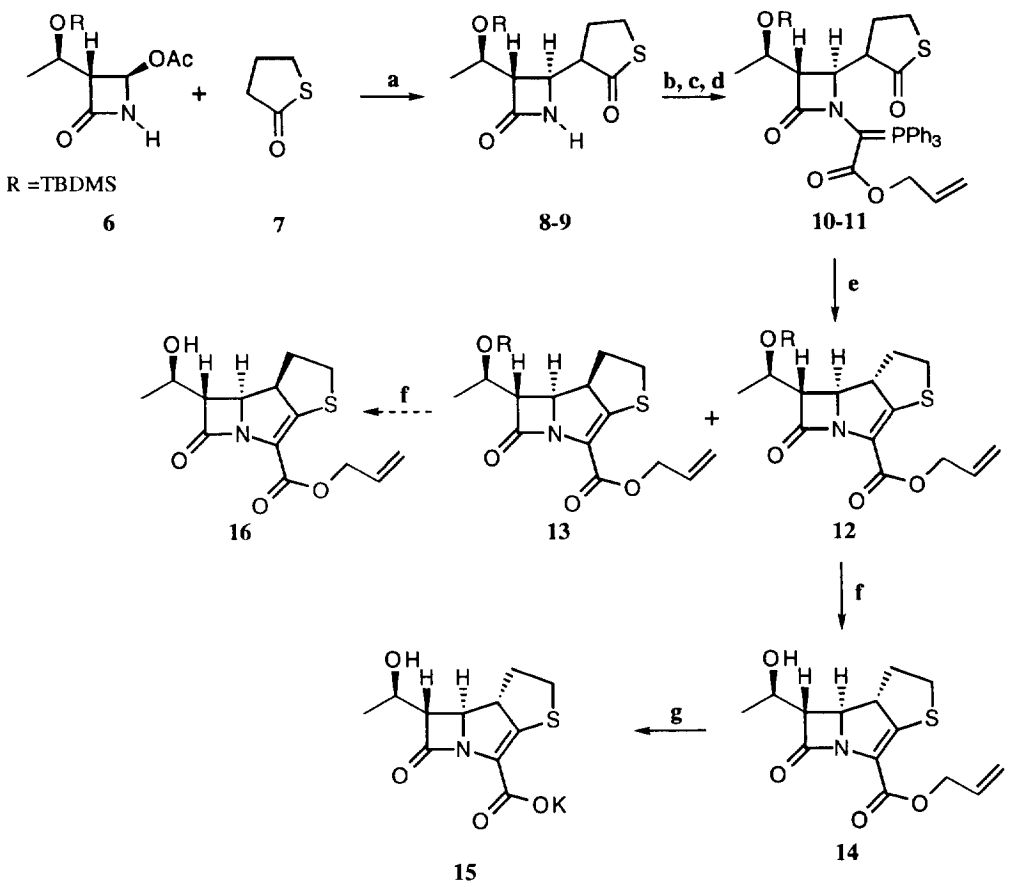
The removal of the silyl protecting group of the secondary alcohol was known to be problematic on carbapenems, due to the substrate instability under several reaction conditions. Based on a reported procedure in the synthesis of thienamycin[13], the 7α isomer **14** was isolated in 54% yield using an excess of TBAF in THF in the presence of AcOH at room temperature for 48 hrs. The final potassium salt **15** was obtained by a deallylation reaction catalyzed by $Pd(PPh_3)_4$ in the presence of potassium 2-ethylhexanoate.[14] Despite the highly strained nature of this β–lactam tricyclic system **15** was found to be completely stable in phosphate buffer at pH 7.4 at 37° C.

As far as the synthesis of the corresponding 7β analogue **16** is concerned, extensive decomposition occurred during the desilylation reaction of the intermediate **13**, using the same reaction conditions for the deprotection of **12**. However, it was possible to prepare **16** by first replacing the TBDMS protecting group with the more labile TMS (Scheme 2).

The phosphoranes **10** and **11** were separated by MPLC and the TBDMS group easily removed using hydrolytic conditions. Treatment of **11** with 1:1:2 mixture of TFA-H_2O-AcOEt at room temperature for 24 hrs gave the corresponding free hydroxy derivative **17** in 89% yield.

Attempts to cyclize this deprotected phosphorane derivative in a variety of aprotic or protic solvents resulted in extensive decomposition and it was necessary to reprotect the secondary alcoholic function using TMSOTf and TEA at -78° C, giving **18** in 97% yield. Cyclization, as reported above, by heating in *o*-xylene at reflux for 3 hrs, gave the tricyclic derivative **19** in 47% yield. As expected, the next deprotection of the TMS group was found to be faster with respect to the corresponding TBMDS derivative. Instantaneous reaction occurred at -40° C using the same reagents (TBAF-AcOH), to give the unstable alcohol derivative **16** (extensive decomposition occurred just by storage overnight even at low temperature!), in moderate yield after chromatographic purification.

Scheme 1



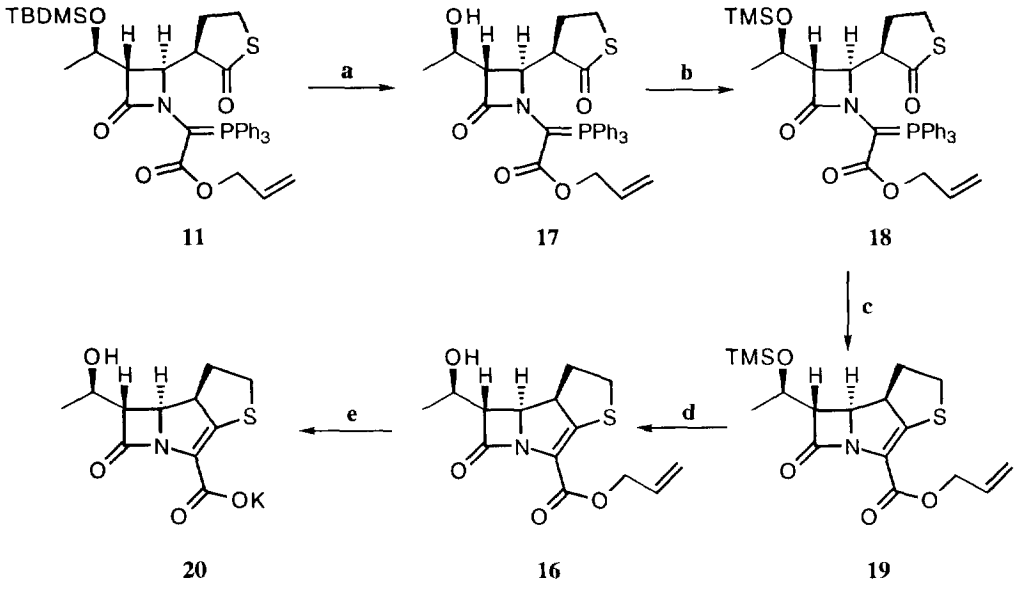
a) i) 7 (2eq), LiHMDS (2eq), -78°C, 30 min, ii) add 6, -78°C, 1h, 91%; b) Allylglyoxylate, toluene, reflux (Dean-Stark), 3h; c) $SOCl_2$, 2,6-Lutidine, -40°C, 30 min; d) PPh_3, 2,6-Lutidine, THF, 50°C, 16 h, 64% (three steps); e) *o*-xylene, reflux, 5h, 25%; f) from 11 to 13, TBAF (5eq), AcOH (5eq), THF, r.t., 48 h, 54%; g) $Pd(PPh_3)_4$, potassium 2-ethylhexanoate, PPh_3, r.t., 25 min, 87%.

Deallylation of **16** gave the target compound **20** which was difficult to purify due to its instability of the in solid phase and in buffered solution (15% yield by HPLC).

Considering the observed lability of **16** and **20** no further attempts to optimize the synthetic route were performed, trying to improve the yield of the removal of the trimethylsilyl protecting group under different reaction conditions. Therefore the initial objective of preparing suitably substituted analogues of **5** (Fig.1), as conformationally restricted analogues of thienamycin, was discontinued[15].

To understand the reason for the reduced chemical stability of **20** with respect to the corresponding epimer **15**, the two compounds were modelled with Sybyl 6.03[16] on an Evans and Sutherland 3+, modifying

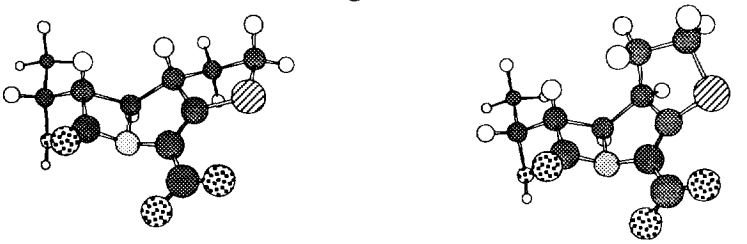
Scheme 2



a) TFA, H_2O, AcOEt 1:1:2, r.t., 12 h, 89%; b) TMSOTf, TEA, THF, -78°C, 20 min, 97%; c) *o*-xylene, reflux, 3h, 47%; d) TBAF (1 eq), AcOH (1.1eq), THF, -40°C, 5 min, 25%; e) $Pd(PPh_3)_4$, potassium 2-ethylhexanoate, PPh_3, r.t., 30 min.

the crystal structure of GG-326 **4** (R=OCH_3). After the definition of the ring A and B as an aggregate, the six membered ring of **4** was replaced with the 4-heterosubstituted five membered ring and the two epimers were minimized using a MAXMIN2 routine. Force field calculations showed that **15** lies 5.6 Kcal/mol below **20**

Figure 2



15
E = 0.0 Kcal/mol

20
E = + 5.6 Kcal/mol

(Fig. 2) and that the additional strain energy is mainly localized to the bending contributions (4 Kcal/mol and 1 Kcal/mol for the for the angle bending and out of plane bending, respectively). These results seem to be in agreement with the experimental observations of stability.

The evaluation of preliminary antibacterial activity was performed only on the stable 7α isomer **15**, using a selection of Gram positive and Gram negative bacteria. Weak activity was observed against sensitive and resistent strains of S. *aureus* (2-32 μg/mL) , S. *epidermidis* (2 μg/mL) and S. *pneumoniae* (2 μg/mL). Low level of antibacterial activity was obtained against E. *coli* 851, 1850-WT, 1852-PM and 1919-PM/TEM-1 (16 μg/mL). Moreover, as expected, **15** was found inactive against P. *aeruginosa* (>32 μg/mL). Concerning the metabolic stability towards renal DHP-I, **15** was found to be twice more sensitive to human kidney DHP-I than imipenem[17,18].

Acknowledgments

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To whom correspondence should be addressed

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12 : ^{1}H-NMR 300 MHz (Acetone-D_6) δ 5.94 (m, 1H), 5.43 (m, 1H), 5.17 (m, 1H), 4.8-4.5 (m, 2H), 4.19 (m, 1H), 4.1-3.92 (m, 2H), 3.7-3.5 (m, 1H), 3.53 (m, 1H), 3.25 (dd, 1H, J_1=6 Hz, J_2=1.7Hz), 2.48-2.38 (m, 1H), 1.87 (m, 1H), 1.24 (d, 3H, J= 6Hz), 0.89 (s,3H), 0.11 (s, 3H), 0.10 (s, 3H).

13 : ^{1}H-NMR 300 MHz (Acetone-D_6) δ 6.0–5.85 (m, 1H), 5.44 (m, 1H), 5.17 (m,1H), 4.74-4.58 (m, 2H), 4.34 (dd, 1H, J_1=11.1, J_2=4.69), 4.25 (m, 1H), 3.86 (m, 1H), 3.7-3.54 (m, 2H), 3.5-3.4 (m, 1H), 2.3-2.2 (m,1H), 2.02-1.84 (m, 1H), 1.18 (d, 3H, J=6Hz), 0.89 (s, 9H), 0.09 (s, 6H).

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