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SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL TRICYCLIC β-LACTAMS

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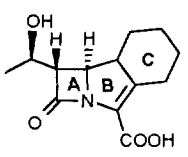
Abstract: The synthesis and the preliminary data on microbiological activity of some tricyclic β-lactam derivatives (**4**), analogues of synthetic "trinems", is described. Compound **14**, key intermediate in the synthesis of **18**, was stereoselectively obtained from its unsubstituted analogue, **12b**, in 4 steps through enolphosphate **13**.

β-Lactams are agents of choice in the current therapeutical approach to most of the infectious diseases induced by bacteria both for their high efficacy and their extremely safe toxicological profile.[1] However the rapid emergence of bacterial strains resistant to the most generally used members of this class of compounds has stimulated, over the years, research for novel β-lactam structures that could overcome the problem of resistance.

Tricyclic β-lactams, trinems **1** (formerly tribactams) first described by our group,[2] are new, totally synthetic antibacterial agents, stable to clinically isolated β-lactamases, whose potency and breadth of spectrum of action have been demonstrated both *in vitro* and *in vivo*. In particular, **2a** (GV104326) showed a particularly good biological profile[3] and, together with its metabolically labile ester **2b** (GV118819) has been selected for development studies.

Fig. 1



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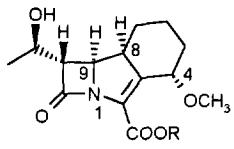
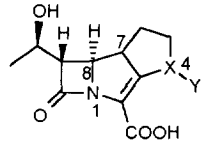


2a GV104326, R=Na
2b GV118819, R=CH(Me)$O_2COC_6H_{11}$



3 X=S
4 X=CH, Y=H, OMe

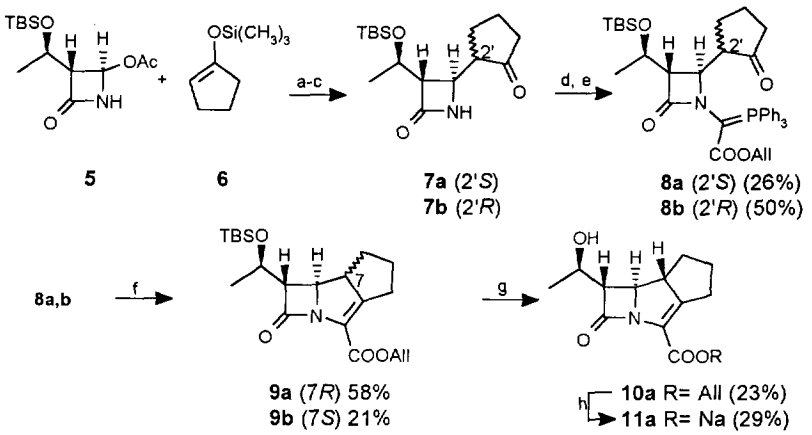
As a part of an ongoing programme aimed at studying how structure activity relationships, among trinems, are influenced by the size of ring **C**, we became interested in the synthesis of the 5-membered ring derivatives of general structures **3** and **4**. We have already reported our results on the synthesis of 4-thia-1-azatricyclo[6.2.0.$0^{3,7}$]dec-2-enes (**3**).[4] Although the preliminary data on both chemical stability and biological activity on this class of compounds were not very encouraging, we decided to undertake the synthesis of the carbocyclic analogues with general structure **4**.

The commercially available 4-acetoxyazetidinone **5**[5] (Scheme 1) was protected at the nitrogen with a TMS derivative then successfully reacted with 1.5 equivalents of 1-trimethylsilyloxy-1-cyclopentene to give, after removal of the nitrogen protecting group, a 2:1 mixture of epimers **7a** and **7b** in 75% overall yield.

Separation of isomers by flash chromatography was not possible at this stage but could be achieved after transformation of the mixture of **7a** and **7b** to the corresponding phosphoranes **8a** and **8b** according to the protocol established by Woodward.[6] Phosphorane **8a** was transformed into fully protected trinem **9a** in moderate

yield by heating it in toluene at 100 °C. The silyl protecting group was then removed with TBAF and acetic acid in THF[7] and the allyl group with sodium 2-ethylhexanoate in the presence of catalytic amounts of $Pd(PPh_3)_4$.[8] The crude sodium salt **11a** could be purified by reverse phase preparative HPLC (Lichrosorb C-18, 10μm, 20× 250mm, water as eluant).

Scheme 1



TBS= *t*-$BuMe_2Si$, All= $CH_2CH{=}CH_2$

a. $(CH_3)_3SiCl$, $(C_2H_5)_3N$, THF, 0°C; b. $(CH_3)_3SiOTf$, 0°C, CH_2Cl_2, c. KF, CH_3OH, 75% overall from **5**; d. OHCCOOAll, toluene, ref.; e. $SOCl_2$, lutidine, THF, -20 °C then PPh_3, lutidine, THF; f. toluene, 100°C; g. TBAF, AcOH, THF, 25% from **9a**; g. $Pd(PPh_3)_4$, Na 2-ethylhexanoate

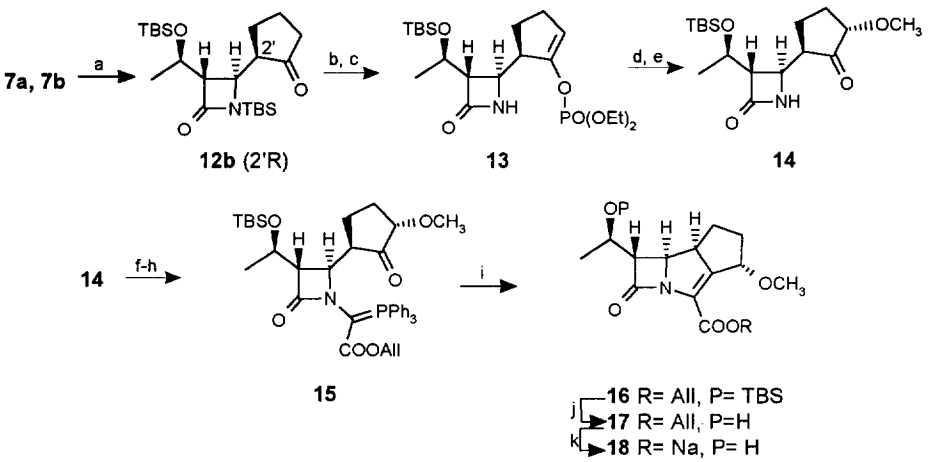
When phosphorane **8b** was heated under similar conditions applied for **8a**, **9b** could be isolated in low yield (21%). Particular care was taken in the isolation of the product. As compound **9b** is unstable on silica gel, its isolation necessitated buffering the silica gel with AcOEt:TEA 95:5 before the purification of the reaction mixture. Compound **9b** undergoes spontaneous decomposition on standing at -30 °C and extensive degradation occurred during our attempts to remove the silyl protecting group.

Previous experience with the 6-membered ring **C** (**1**) homologues led us to hypothesise that the introduction of a substituent at position C-4 of **4** should lead to the best compromise in terms of chemical and enzymatic stability, antibacterial potency, breadth of spectrum and *in vivo* properties. Bearing these considerations in mind we directed our efforts towards the synthesis of 4-methoxy trinem **4** with (4*S*,7*S*) absolute configuration at the corresponding stereogenic centres.

The mixture of isomers **7a** and **7b** (Scheme 2) was protected at the amide nitrogen with *t*-butyldimethylsilylchloride and TEA. At this stage, the two epimers could be separated by MPLC. Isomer **12b** was then converted into the enolphosphate **13** by treatment of the lithium enolate of **12b** with diethylphosphorochloridate[9] at -78°C followed by removal of the silyl protecting group on the nitrogen then transformed into ketoazetidinone **14** according to a procedure established in our laboratories.[10] Oxidation with 3-chloroperoxybenzoic acid followed by treatment of the crude reaction mixture after work-up with methanol gave ketoazetidinone **14**. It is worth mentioning that the presence of the isomer of **14** with inverted configuration at the newly formed stereogenic centre could not be detected by NMR analysis of the reaction mixture after work-

up. The conversion of **14** to the final compound **18** was accomplished following conventional methods; compounds **16**, **17** and **18** were found chemically stable and could be stored for months at -30°C.[11]

Scheme 2



a. TBSCl, TEA, DMF, flash chromatography; b. $LiN(TMS)_2$ (1.5 eq), THF, -78°C then $ClPO(OEt)_2$ (1.5 eq); c. KF, MeOH, 0°C, 49% from **12b**; d. m-CPBA, CH_2Cl_2; e. MeOH, lutidine, 51% from **13**; f. OHCCOOAll, toluene, ref.; g. $SOCl_2$, lutidine, THF; h. PPh_3, lutidine, THF, 47%; i. toluene, Δ, 58%; j. TBAF, AcOH, THF, 39%; k. $Pd(PPh_3)_4$, Na 2-ethylhexanoate, 43% (after prep. HPLC).

Table 1. *In vitro* antibacterial activity* (MIC μg/ml) of trinems **11a** and **18** in comparison with Imipenem.

	S.a. 663	*S.a.* 853	*S.a.* 1131	*E.faecalis* 850	*E.coli* 1850	*P.a.* 1911	*C.p.* 615
Imipenem	0.06	0.12	4	2	0.5	4	0.03
11a	1	0.5	8	16	2	>32	1
18	0.25	0.5	8	2	0.5	32	0.12

* Minimum Inhibitory Concentrations (MIC) determined in Mueller Hinton broth: Anaerobes Schadler broth Inoculum . $5x10^5$ CFU/ml ***S.a.* 663**= *Staphylococcus aureus* 663 ; ***S.a.* 853**= *Staphylococcus aureus* 853 β-lactamases producing strains; ***S.a.* 1131**= *Staphylococcus aureus* 1131 methicillin-resistant ***E.faecalis* 850**= *Enterobacter faecalis* 850E; ***E.coli* 1850**= *Escherichia coli* 1850E; ***P.a.* 1911**= *Pseudomonas aeruginosa* 1911; ***C. p.* 615** = *Clostridium perfringens* 615E.

As expected, compounds **11a** and **18** were sufficiently stable and could be tested. The antibacterial activity of trinem **18** was similar to imipenem except against *Pseudomonas aeruginosa*. Compound **18** (as most of the 4-substituted trinems synthesized) was found more stable than imipenem and meropenem to human renal DHP-I. The initial microbiological results suggest that the trinems with general structure **4** represent an extremely promising class of antibacterial agents.

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    Preparation of compound **13**: To a solution of LHMDS (12ml of a 1M solution in hexanes) in 60ml of THF cooled at -70 °C, a solution of compound **12b** (3.2g. 7.5mmol) in 60ml of dry THF was added in 30 min. The resulting mixture was stirred for 30min then diethylphosphoroylchloridate (1.6ml, 7.7mmol) was added and the reaction stirred for 30min. Aqueous NH_4Cl was added, followed by ethyl acetate. The organic layer was washed with a saturated solution of $NaHCO_3$ and brine, dried and concentrated to give an oil that was dissolved in 60ml of CH_3OH. KF (2.1g) was added and the mixture stirred for 20min at 23°C then concentrated *in vacuo*. Ethyl acetate was added and the organic layer was extracted with brine and evaporated. Purification by flash chromatography gave compound **13** (oil, 1.65g, 49%).
    Preparation of compound **14**: To a solution of compound **13** (1.6g, 3.6mmols) in dichloromethane (60ml), 3-chloroperoxybenzoic acid (3g) and sodium hydrogen carbonate (800mg) were added. The resulting mixture was stirred at 23°C for 3 hours then a 10% solution of sodium thiosulphate (100ml) was added. The organic phase was washed with a saturated solution of $NaHCO_3$ (2×100ml), dried and evaporated *in vacuo* to give a white wax (2g). Dry CH_3OH (60ml) and 2,6-lutidine (2.5ml) was added. The solution was stirred at reflux for 5 hours, cooled to 23°C and concentrated to give an oil which was dissolved in diethyl ether and washed with a pH=3 citrate buffer. The organic layer was dried, concentrated and purified by flash chromatography to give compound **14** as a colourless oil (630mg, 51%).
11. All the compounds synthesized have been characterised by routine analytical techniques.
    Compound **9b**: ^{1}HNMR 300MHz(δ, ppm, $CDCl_3$): 5.95 (m, 1H), 5.40 (m, 1H), 5.23 (m, 1H); 4.75 (m, 2H), 4.17 (m, 1H + 1H), 3.39 (m, 1H), 3.24 (dd, 1H, J_1=7.5Hz, J_2=2.7Hz), 2.82 (m, 1H), 2.40-2.20 (m, 2H), 2.20-1.90 (m, 2H), 1.90-1.70 (m, 2H), 1.22 (d, 3H, J=6.3Hz), 0.88 (s, 9H), 0.08 (s, 6H).
    Compound **10a**: IR (ν_{max}, cm^{-1}, nujol): 1759-1730 (broad, C=O), 1591 (C=C).
    ^{1}H-NMR 300MHz (δ, ppm, D_2O): 4.03 (m, 1H), 3.82 (dd, 1H, J_1=2.1Hz, J_2=8.1Hz), 3.40-3.28 (m, 1H), 3.16 (dd, 1H, J_1=2.1Hz, J_2=6.6Hz), 2.38-2.28 (m, 2H), 2.05-1.80 (m, 3H), 1.24 (m, 1H), 1.13 (d, 3H, J=6.3Hz).
    Compound **18**: IR (ν_{max}, cm^{-1}, nujol): 1755 (C=O), 1587 (C=C).
    ^{1}H-NMR 300MHz (δ, ppm, D_2O): 4.70 (m, 1H), 4.13 (dd, 1H, J_1=10Hz, J_2=3.5Hz), 4.11 (m, 1H), 3.40-3.30 (m, 2H), 3.14 (s, 3H), 2.34-2.20 (m, 1H), 1.85-1.50 (m, 2H), 1.20 (m, 1H), 1.11 (d, 3H, J=6.6Hz).

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