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# SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SOME THIO TRINEMS.

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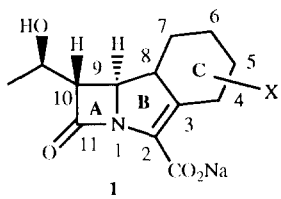
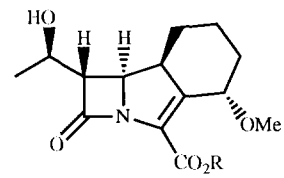
**Abstract:** The synthesis and antibacterial activity of some trinems having a sulfur atom in various positions of ring C are presented. © 1997 Elsevier Science Ltd.

The trinems[1] **1** (formerly referred to as tribactams) represent a novel class of antibacterial agents[2] discovered at GlaxoWellcome. The extremely good biological profile shown by sanfetrinem **2a** and its metabolically labile ester sanfetrinem cilexetil **2b**[3,4] (Fig.1), together with the novelty of the structure and the commercial availability of the starting material **3**,[5] prompted us to undertake a further exploration on the class.

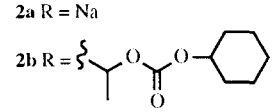
**Fig.1**

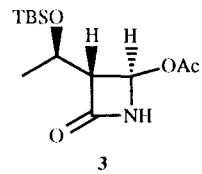



**1**




**2a** R = Na

**2b** R = 




**3**

As part of our research programme, we were particularly interested at exploring the effect of the introduction of an heteroatom at different positions in ring C. Preliminary results have already been reported on compounds bearing a five-membered ring C containing a sulfur atom[6] showing a promising effect on the biological profile, and here we wish to report the synthesis and the antibacterial activity of some trinems bearing a six-membered ring C containing a sulfur atom at different positions[7]. The aim of the present work was to evaluate the antibacterial profile of thio trinems and to verify the possibility to improve the activity against Gram negative strains including possibly *Pseudomonas* spp.

In Scheme 1 the synthesis of the 4-thiotrinem **9a** is reported. The thiolactone **4** was converted into the silylthioketene acetal **5** using trimethylsilylchloride and triethylamine in refluxing dimethylformamide according to procedure of House.[8] Reaction of **5** with the commercially available (*3R,4R*)-4-acetoxy-[(*1R*)-tert-butyldimethylsilyloxyethyl]-azetidin-2-one **3** in the presence of a catalytic amount of boron trifluoride etherate afforded a mixture of the two epimers **6a** and **6b** in a 80/20 ratio. The isomers were not separated at this stage but reacted with allyl oxalyl chloride and triethylamine in dichloromethane to give the oxalimide intermediates[9] that were immediately converted into the two trinems **7a** and **7b** in moderate yield using triethylphosphite in refluxing xylene. The two isomers were separated by flash chromatography, then treated with

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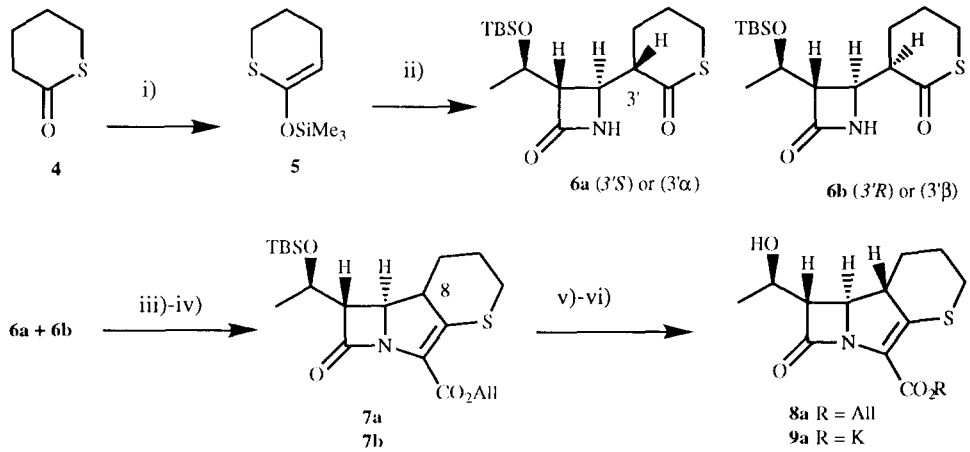
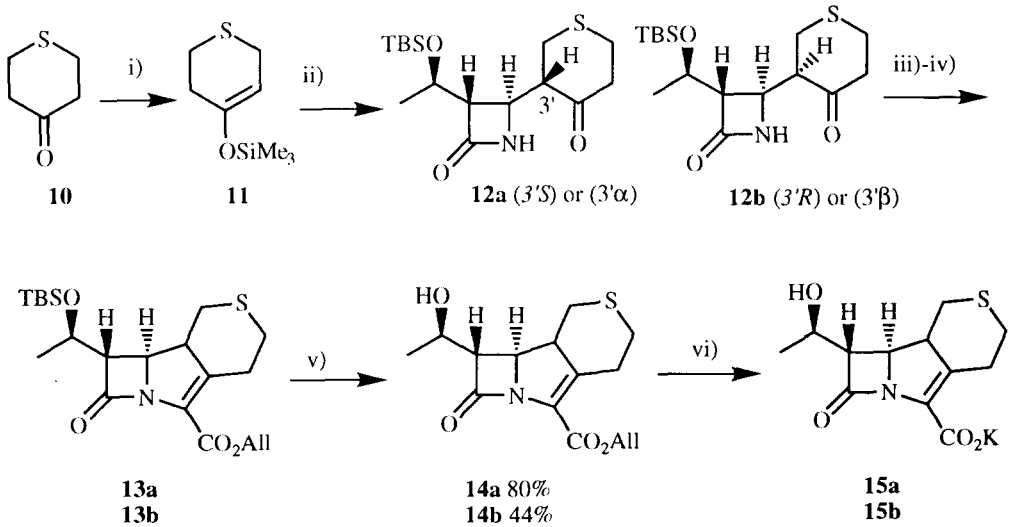
tetrabutylamonium fluoride and acetic acid to remove the tert-butyldimethylsilyl protecting group on the hydroxyethyl side chain. The isomer **7a**, gave the ester **8a** in 40% yield, while the β-isomer **7b**, gave only a mixture of unidentified products. The allyl ester of compound **8a** was removed using $Pd(Ph_3P)_4$ and potassium-2-ethylhexanoate to give the desired trinem **9a**.

**Scheme 1**



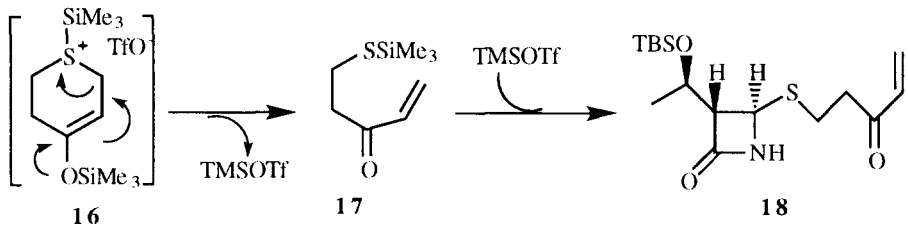

i) TMSCl, TEA, DMF; ii) **3**, $BF_3.Et_2O$, $CH_2Cl_2$, 3 days; iii) $ClCOCO_2All$, TEA, $K_2CO_3$, $CH_2Cl_2$; iv) $P(OEt_3)$, xylene, 130°C; v) TBAF, AcOH, THF, RT; vi) $Pd(Ph_3P)_4$, K-2-ethylhexanoate.

**Scheme 2**




i) TMSCl, TEA, DMF, 80%; ii) **3**, Lewis acid; iii) $ClCOCO_2All$, TEA, $K_2CO_3$, $CH_2Cl_2$; iv) $P(OEt_3)$, xylene, 130°C; v) TBAF, AcOH, THF, RT; vi) $Pd(Ph_3P)_4$, K-2-ethylhexanoate.

The Scheme 2 illustrates the general route to 6-thio trinems. The silylenolether **11** was formed using the House procedure, from the corresponding 4-thiopyranone **10**, in 80% yield after distillation and allowed to react with **3** in the presence of a Lewis acid catalyst to yield a mixture of the isomers **12a** and **12b**. A limited study undertaken demonstrated that the nature of the catalyst affected both the isomeric ratio and the overall yield. Using strongly acidic conditions such as trimethylsilyltriflate the yield was moderate and no selectivity was observed even at low temperatures. Moreover, an unwanted side reaction occurred resulting in the formation of compounds **17** and **18**, that could be ascribed to the β-elimination of 4-thiopyranone and its subsequent addition to the 4-acetoxy-azetidin-2-one (Fig.2).




**16** **17** **18**

Fig. 2 Proposed mechanism of formation of compound **18**

The use of boron trifluoride diethyl ether complex gave 80% overall yield and 8/2 isomer ratio, with the preferential formation of the compound having absolute configuration (*3'S*) **12a**, in analogy to the results already described for trimethylsilyloxycyclohexene[10]. Using the softer Lewis acid zinc chloride a better **12a/12b** isomer ratio (9/1) and 85% yield after 5 days at room temperature, were obtained. Therefore as we were interested in obtaining both isomers, trimethylsilyltriflate was the catalyst of choice in order to obtain enough material of isomer **12b** to complete the synthesis.

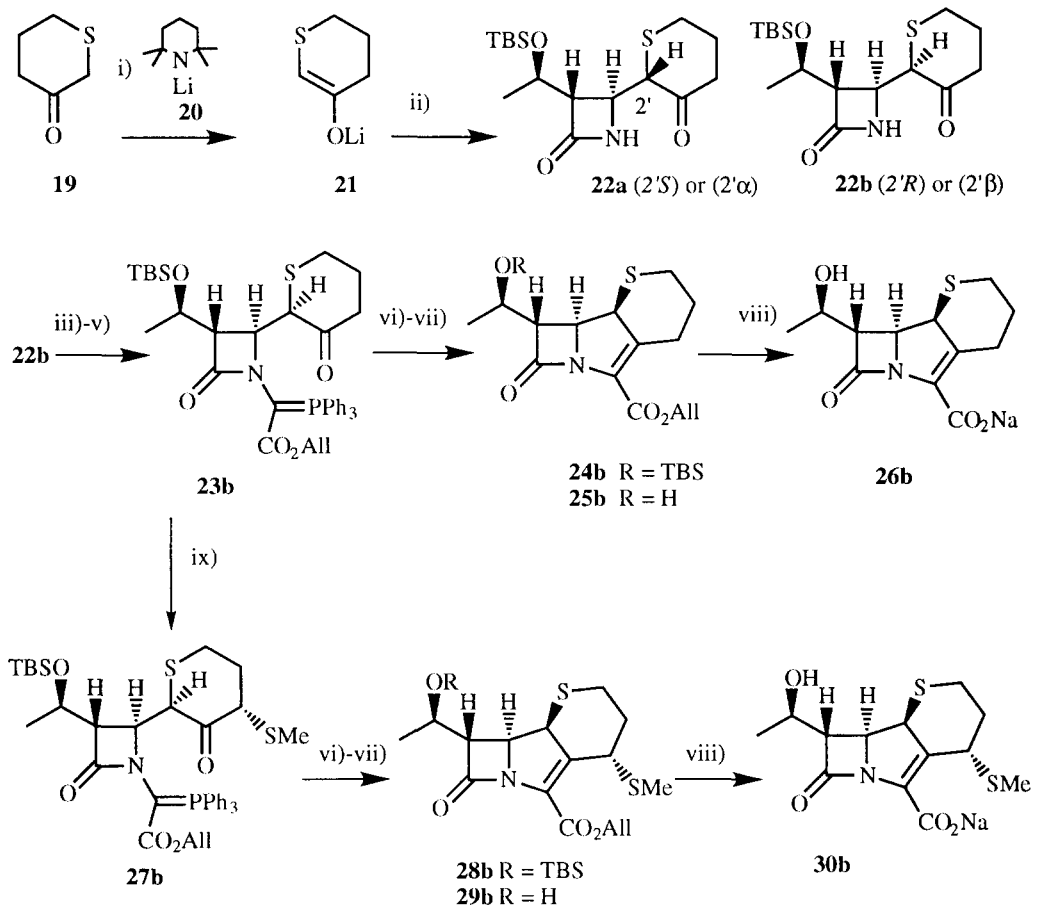
Compounds **12a** and **12b** were separated by flash chromatography and converted to the fully protected trinems **13a** and **13b** in 28% and 41% yield respectively using the oxalimide cyclisation procedure that has already been described in Scheme 1. The fully protected trinems **13a** and **13b** were converted into the potassium salts **15a** and **15b** by sequential removal of the tert-butyldimethylsilyl group on the hydroxyethyl side chain to give intermediates **14a** and **14b**, and deallylation of the ester moiety as shown in Scheme 2.

The microbiological results obtained at that time for the thio trinems, were in agreement with the general observation that compounds having (8β) configuration have a better activity than the corresponding (8α) and therefore we concentrated our efforts on the synthesis of such derivatives.

In Scheme 3, the synthesis of trinems having a sulfur atom at position 7 and 8β stereochemistry is depicted. This route involves the formation of the advanced intermediate **23b**, that can be easily functionalized at position 4. The 3-thiopyranone **19** was regioselectively[11] converted into its lithium enolate **21** using lithium-2,2,6,6-tetramethylpiperidine **20** at -70°C[12], and this reacted with the acetoxyazetidinone **3** to afford a mixture of isomers **22a** and **22b** in 70/30 ratio and 36% yield that were separated by flash chromatography. Compound **22b** was converted into the trinem **24b** using a multistep procedure that involved the formation of the phosphorane **23b** in 49% yield using the Woodward procedure[13]. Removal of tert-buthyldimethylsilyl group

was accomplished in 20% yield giving derivative **25b** that was treated with $Pd(Ph_3P)_4$ and Na-2-ethylhexanoate in THF affording the trinem **26b** in 68% yield .

**Scheme 3**




i) **20**,THF, -78°C, 30'; ii) **3**, THF, -78°C; iii) $AllO_2CCHO$, Toluene, reflux; iv) $SOCl_2$, 2,6-lutidine, THF, 0°C; v) $PPh_3$, 2,6-lutidine, RT; vi) o-xylene, 100°C;vii) TBAF, AcOH, THF, RT; viii) $Pd(Ph_3P)_4$, Na-2-ethylhexanoate, THF; ix) LDA, $MeSO_2SMe$, THF.

Among the unsubstituted thio trinems synthesised, compound **26b** showed a promising antibacterial profile, therefore its basic skeleton was selected for chemical derivatization. We have already shown in the carbocyclic analogues,[14] that introduction of a 4α substituent results in an increase of potency and stability to hydrolytic enzymes. The beneficial effect of functionalization may depend upon the nature of the substituent and the stereochemistry of the newly formed stereocentre. We sought in the phosphorane **23b** an useful advanced intermediate for the introduction of the methylthio side chain,[15] already successfully used on the carbocyclic

analogue. The phosphorane **23b** was treated with lithium bis trimethylsilyl amide in THF at -70°C to regioselectively form the less substituted enolate, which was reacted with methanethiomethylsulfonate to yield the 4-thiomethyl phosphorane **27b** as a single product. In this case the presence of a bulky substituent on the six membered ring of the enolate derived from **23b** explains the addition of methanethiomethylsulfonate from the less hindered *si* face. Cyclization under the standard reaction conditions produced the 4-thiomethyl trinem **28b** in 73% overall yield. Desilylation and subsequent removal of the allyl ester were achieved in 44%[16] and 85% yield respectively to afford compound **30b** as sodium salt.

In Table 1, the *in vitro* antimicrobial activity of these compounds in comparison with imipenem is summarised. The MIC values suggest that all the compounds have an overall spectra of activity which is inferior to Imipenem, although it is balanced and not profoundly affected from permeability problems (compare the MIC data for strains 1850 and 1919 of *E.coli.*). Comparison of MIC data for *Staphylococcus aureus* 663E and *Staphylococcus aureus* 853E (β-lactamases producing strain) is indicative that all the compounds tested are significantly stable to β-lactamases. Compound **30b** (4-thiomethyl derivative of **26b**) showed higher potency on Gram positive strains but a decrease of activity versus Gram negatives compared to **26b**. Moreover, compound **30b** was found significantly more stable to human renal DHP-I of the corresponding unsubstituted thio trinem **26b** as showed an half life which is about 3.5-4 times longer than imipenem. Despite this good result, the lack of a significant activity against *Pseudomonas aeruginosa* (MIC≥32) has suggested us to not further investigate this class of compounds.

**Table1.** *In vitro* antibacterial activity* (MIC mg/ml) of trinems in comparison with Imipenem

| Compound | S.a. 663 | S.a. 853 | E.coli 1850 | E.coli 1919 | C.per. 615 | B.frag. 2017 | DHP-I t1/2(min) |
|---|---|---|---|---|---|---|---|
| 8a | 1 | 1 | 1 | 2 | 0.5 | NT | 10 |
| 15a | 1 | 1 | 4 | 2 | 1 | 0.12 | 12 |
| 15b | 1 | 0.5 | 4 | 1 | 0.25 | 0.5 | <7 |
| 26b | 1 | 1 | 0.5 | 0.5 | 0.03 | 0.25 | n.d.# |
| 30b | 0.2 | 0.5 | 2 | 1 | ≤0.01 | 0.2 | 386 |
| Imipenem | 0.06 | 0.12 | 0.5 | 0.5 | 0.03 | 0.12 | 100 |

* Minimum Inhibitory Concentration (MIC) determined in Mueller Hinton broth; Anaerobes Schadler broth inoculum = $5x10^5$CFU/ml. S.a. 663 = Staphylococcus aureus 663E; S.a. 853 = Staphylococcus aureus 853E β-lactamases producing strain; E.coli 1850 = Escherichia coli 1850E; E.coli 1919 = Escherichia coli 1919 β-lactamases producing strain; B.frag. 2017 = Bacteroides fragilis 2017; C.per. 615 = Clostridium perfringens 615E; DHP-I = human renal dehydropeptidase I.

#n.d. = not determined.

**Acknowledgement**

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**References and notes**

**1.** a) Tamburini, B.; Perboni, A.; Rossi, T.; Donati. D.; Andreotti, D.; Gaviraghi; G.; Carlesso, R.; Bismara, C.; Eur Pat. Appl. EP 0416953 A2, 1991; *Chem. Abstr.* **1992**, 116, 235337t. b) Perboni, A.; Rossi, T.; Gaviraghi, G.; Ursini, A.; Tarzia, G.; WO 9203437, 1992; *Chem; Abstr.* **1992**, 117, 7735m.

**2.** For a recent review on trinems see: Biondi, S.; In *ANTI-INFECTIVES Recent Advances in Chemistry and structure activity Relationships*; Bentley, P.H.; O'Hanlon, P.J.; Eds.; RSC: Cambridge; Special Publication No.198; **1997**, pp 86-100.

**3.** a) Di Modugno, E.; Erbetti, I.; Ferrari, L.; Galassi, G.; Hammond, S.M.; Xerri, L.; *Antimicrob. Agents Chemother.* **1994**, 38, 2362. b) Andreotti, D.; Rossi, T.; Gaviraghi, G.; Donati, D.; Marchioro, C.; Di Modugno, E.; Perboni, A.; *Bioorg. Med. Chem. Lett.* **1996**, 6, 491.

**4.** For a recent total syntheses of Sanfetrinem see: Hanessian, S.; Rozema, M.J.; *J. Am. Chem. Soc.* **1996**, 118, 9884.

**5.** (3R,4R,1'R)-(+)-4-acetoxy-3[1'(*tert*-butyldimethylsilyloxy)ethyl]-azetidin-2-one **3** is commercially available from Aldrich Chemical Company Inc. Milwaukee, WI, USA.

**6.** Di Fabio, R.; Feriani, A.; Gaviraghi, G.; Rossi, T.; *Bioorg. Med. Chem. Lett.* **1996**, 6, 2589.

**7.** During our studies a patent claiming thio compounds belonging to the same class was published by Takeda Chemical Industries Ltd. : Sendai, M.; Miwa, T.; Eur. Pat. Appl. EP 0422596 A2, 1991.

**8.** House, H.O.; Czuba, L.J.; Gall, M.; Olmstead, H.D.; *J. Org. Chem.* **1969**, 34, 2324.

**9.** a) Afonso, A.; Hon, F; Weinstein, J.; Ganguli, A.K.; McPhail, A.T. *J. Am. Chem. Soc.* **1982**, 104, 6138; b) Yoshida, A.; Hayashi, T.; Takeda, N.; Oida, S.; Ohki, E. *Chem. Pharm. Bull.*, **1983**, 31, 768.

**10.** Perboni, A.; Tamburini, B.; Rossi, T.; Donati, D.; Tarzia, G.; Gaviraghi, G.; In *Recent Advances in the Chemistry of AntiInfective Agents*; Bentley, P.H.; Posford, R.; Eds.; RSC: Cambridge **1992**; pp 21-35.

**11.** We were unable to detect the presence of regioisomers of compounds **26** i.e. deriving from the lithium enolate obtained by deprotonation at position 4 of the 3-thiopyranone.

**12.** The use of LDA resulted in lower yields.

**13.** a) Woodward R. B.; In *Recent Advances in the Chemistry Of β-Lactam Antibiotics*, Elks, J., Ed.; Spec. Pub. N.28; Roy. Soc. Chem.; London; **1977**; cap. 18. b) Scartazzini, R.; Peter, H.; Bickel, H.; Heusler, K.; Woodward, R.B.; *Helv. Chim. Acta* **1972**, 55, 408.

**14.** Gaviraghi G.; In Proceedings of the XIIIth International Symposium on Medicinal Chemistry, Muller, J.C.; Ed.; Editions Scientifiques Elsevier **1995**; pp 467S-478S.

**15.** Synthesis of **28b**: To a solution of lithium bis trimethylsilyl amide (4.5 mmols) in dry THF (100 ml), cooled at -70°C under a nitrogen atmosphere, a solution of compound **23b** (1.6g) in THF (20 ml) was slowly added. The reaction mixture was stirred for 30 min. and $MeSSO_2Me$ (400 µl) added. After 1 hr the reaction mixture was poured into a saturated solution of $NH_4Cl$ and extracted with ethyl acetate. The organic phase was extracted with $NaHCO_3$ and brine, then dried over $Na_2SO_4$. The solvent was removed under reduced pressure to give a foam which was purified by flash chromatography eluting with a mixture of ethyl acetate / cyclohexane in a 20 / 80 ratio to give 700 mg of intermediate **27b.** Compound **27b** was dissolved in xylene (70 ml) and heated at 100°C for 2 hrs. The reaction mixture was cooled at room temperature and the solvent removed under reduced pressure to give an oil which was purified by flash chromatography eluting with a mixture of cyclohexane / ethyl acetate in a 95 / 5 ratio obtaining 320 mg of compound **28b.**

**16.** The yield is based on recovered starting material.

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