An approach to the synthesis of 5,5-*trans*-fused lactam analogues of β-lactam antibiotics

Paul W. Smith,* Andrew R. Whittington, Kevin N. Cobley, Albert Jaxa-Chamiec and Harry Finch

Department of Medicinal Chemistry, Glaxo Wellcome Limited, Gunnels Wood Road, Stevenage, UK SG1 2NY

Received (in Cambridge, UK) 8th September 2000, Accepted 27th October 2000
First published as an Advance Article on the web 5th December 2000

A racemic synthesis of two diastereoisomeric α-benzyloxycarbonylamino substituted *trans*-fused bicyclic lactams (**4** and **5**), was achieved from cyclopentene oxide. These lactams are useful intermediates to investigate the possibility of using a *trans*-lactam template as a replacement for the β-lactam ring found in conventional antibacterial agents. One of the intermediates (**4**) was further elaborated to an analogue of the antibacterial agent ceftazidime.

Introduction

Over recent years the continuing problem of emerging resistance to chemotherapeutic agents has intensified the search for new and novel antibacterial drugs. In particular, the emergence of broad spectrum β-lactamases has reduced the effectiveness of many of the older β-lactam antibiotics and indeed rendered some of them obsolete. The situation can be retrieved by the co-administration of β-lactamase inhibitors *e.g.* Augmentin,[1] but there are already reports of β-lactamases resistant to clavulanic acid.[2] The new carbapenems such as imipenem[3] are potent antibacterials and useful inhibitors of β-lactamases which suggests that it is possible to combine these two properties in a single molecule. The ideal drug, however, would be a powerful inhibitor of the bacterial transpeptidases whilst displaying no affinity for β-lactamases. Towards this goal, in addition to synthesising β-lactam antibiotics with improved stability to β-lactamases, many groups have attempted to replace the β-lactam nucleus with appropriately substituted novel ring systems capable of selectively acylating the bacterial serine transpeptidases. Notable successes have been few, but the appreciable activity of the acyl hydrazides **1**[4] and Lactivicin **2** (Fig. 1)[5,6] suggests that other compounds of this type with useful antibacterial properties will be found.

The highly strained *trans*-fused 5,5 lactam and lactone ring systems **3a** and **b** recently reported by co-workers at Glaxo-Wellcome represent novel templates for the design of compounds which can potentially acylate a wide range of serine proteases. Selective inhibitors of both thrombin and elastase have already been designed based on these templates.[7–12] The current manuscript describes a useful racemic synthesis of the diastereoisomeric Cbz ($CO_2CH_2C_6H_5$) protected *trans*-lactams **4** and **5** which are suitable starting materials to investigate the potential of the 5,5-*trans*-lactam template to mimic the penicillin and cephalosporin β-lactam antibiotics. Transformation of compound **4** to the aminothiazolyl derivative **6**, structurally related to the antibiotic ceftazidime **7**, is also described.

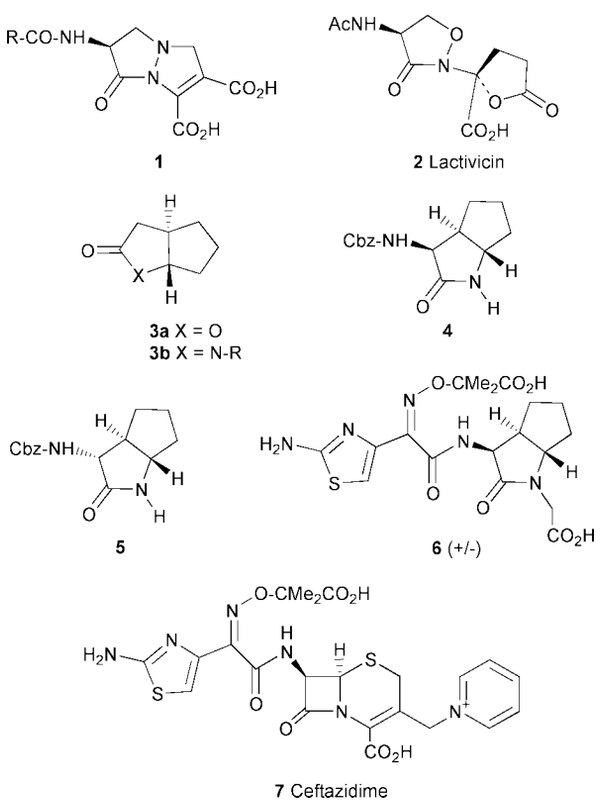


Fig. 1

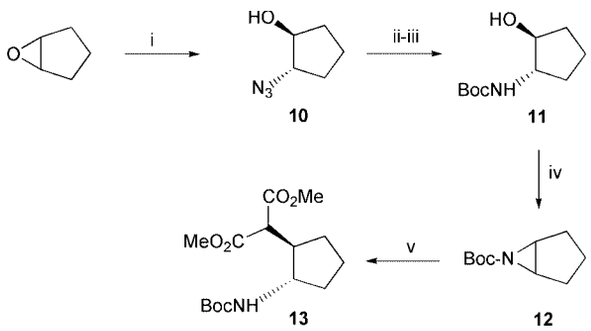
Results and discussion

Synthesis of the lactams 4 and 5

For the synthesis of derivatives **4** and **5** we required a reliable method for the stereoselective formation of the protected amino acids **8** and **9** (see Scheme 2) which fixed the relative stereochemistry of the two adjacent chiral centres on the cyclopentane ring *trans* to each other. Synthesis of **8** and **9** was accomplished in 8 stages from the azido alcohol **10**, which was obtained from cyclopentene oxide by ring opening with sodium azide (Scheme 1).[13] Triphenylphosphine reduction of **10** and treatment with di-*tert*-butyl dicarbonate afforded the protected aminol **11** (41% yield) as a crystalline solid. Under Mitsunobu conditions **11** was cleanly converted to the Boc protected aziridine **12** in high yield (79%). The aziridine ring was then opened by heating **12** with the sodium salt of dimethyl malonate in DMF at 155 °C. This afforded **13** as a crystalline solid after flash chromatography in good yield (73%). On a larger scale **13** was isolated directly by crystallisation. By ring opening the aziridine in this fashion the required ring junction *trans*-stereochemistry has been fixed. The initial stages outlined

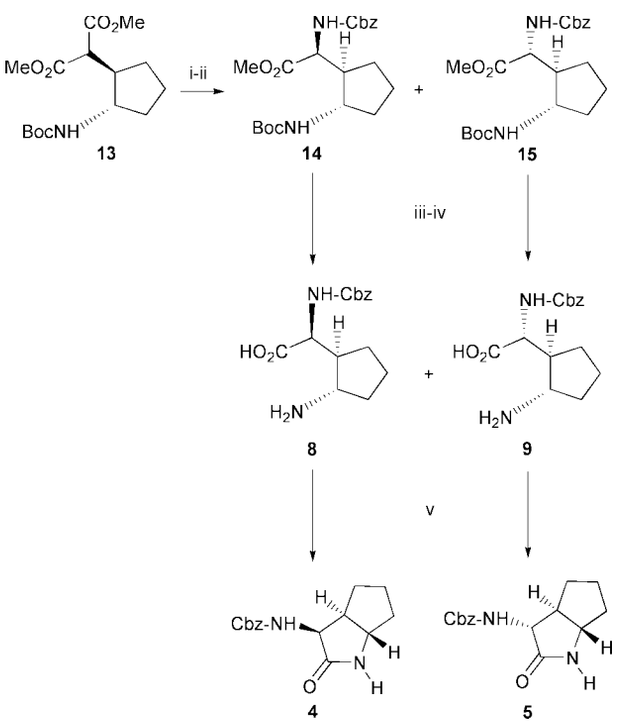
DOI: 10.1039/b007286i

above were readily carried out on 50 g scale affording **13** in 24% overall yield from **10** (Scheme 1).



Scheme 1 *Reagents:* i) NaN_3, NH_4Cl, aq. EtOH; ii) Ph_3P; iii) Boc_2O; iv) Ph_3P–diisopropylazodicarboxylate; v) $CH_2(CO_2Me)_2$, NaH, DMF.

Selective hydrolysis of **13** with aqueous KOH afforded a diastereomeric mixture of monoacid esters in quantitative yield (Scheme 2). The isomers were not separated at this stage, but the mixture subjected to a Curtius rearrangement with diphenylphosphoryl azide $(PhO)_2P(O)N_3$ (DPPA) and then adding benzyl alcohol to trap the intermediate isocyanate[14] affording a mixture of the Cbz protected diastereomers **14** and **15** (1 : 1 ratio, 37% combined yield) (Scheme 2) which were



Scheme 2 *Reagents:* i) KOH; ii) DPPA, $PhCH_2OH$, heat; iii) NaOH; iv) CF_3CO_2H; v) DPPA, DMF.

separable by flash chromatography. Products **14** and **15** now contain both the nitrogens required for the final *trans*-lactam products in a differentially protected form.

Hydrolysis of the remaining methyl ester in **14** was achieved by treatment with aqueous sodium hydroxide and the Boc group subsequently removed with trifluoroacetic acid to afford the required amino acid **8** (91%). A similar sequence of reactions starting with **15** produced **9** also in high overall yield (91%). Finally **8** and **9** were cyclised to the required *trans*-lactams **4** and **5** respectively using DPPA in DMF (69% yield **8**→**4**, 76% yield **9**→**5**).[6] In this reaction the initial acyl azide formed by the DPPA is trapped by the nucleophilic amine in an intramolecular reaction. The relative stereochemistry of **4** and

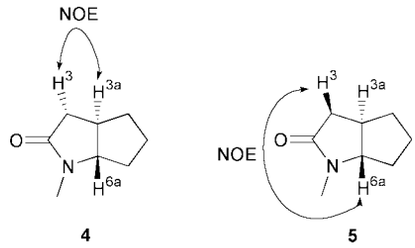


Fig. 2 NOE's observed in isomers **4** and **5**.

5 was deduced from their proton NMR spectra by examining the coupling constants and NOE's between the H-3 proton adjacent to the amide carbonyl group and the ring junction protons H-3a and H-6a (Fig. 2). There is an NOE observed in **4** between H-3 and H-3a, but not between H-3 and H-6a, whereas in **5** there is an NOE between H-3 and H-6a but not between H-3 and H-3a. The relative stereochemistry of the earlier intermediates **14**, **15**, **8** and **9** was also inferred from this experiment.

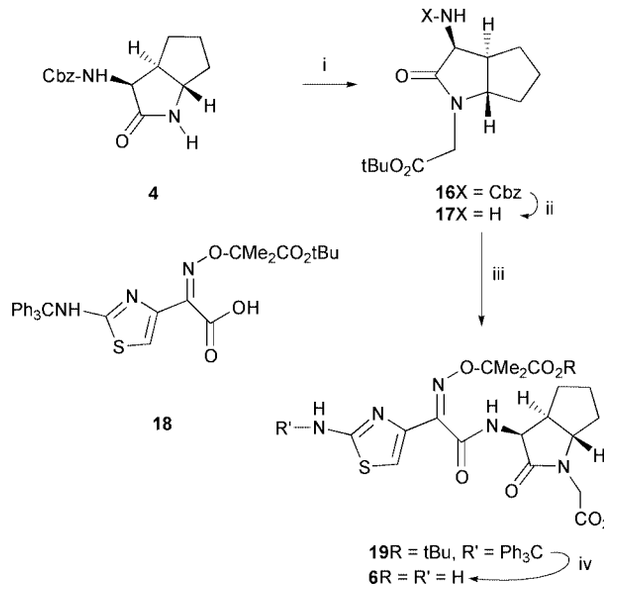
Design and synthesis of compound 6

In order to display antibacterial properties the β-lactam antibiotics require a suitably positioned acid moiety and an appropriately activated β-lactam nucleus.[15] Once these features are in place, then the choice of the appropriate sidechain is critical in order to optimise binding affinity and specificity for the target penicillin binding proteins.[16–18] Activation of the β-lactam is achieved by creating strain in the amide bond either through fusion to another ring (penicillins and cephalosporins *etc.*) or by introducing electron withdrawing substituents onto the amide nitrogen (monobactams such as aztreonam). An indication of ring strain can often be inferred from the IR stretching frequency of the amide carbonyl group.[19,20] The carboxylic acid of the antibiotic forms a salt bridge with the lysine residue of the highly conserved Lys-X-X-Ser motif found in the penicillin binding proteins.[21,22] In the initial *trans*-lactam target compound **6**, the acetic acid extension from the amide nitrogen was chosen to allow **6** the possibility of forming this key interaction. Simple molecular modelling overlays (not shown) indicated that similar positions of the amide carbonyl groups and carboxylic acids of the *trans*-lactam **6** and cephalosporin nucleus of ceftazidime **7** were likely. The aminothiazolyl derived amide sidechain was chosen since this group has proved highly successful in the cephalosporin and monobactam series of antibiotics.[23,24]

N-Alkylation of the amide in **4** was achieved with *tert*-butyl bromoacetate in acetonitrile with caesium carbonate[25] producing **16** (79%) (Scheme 3). The Cbz group was next removed by hydrogenolysis liberating the amine **17** (94%). Conventional coupling of **17** with the acid **18**[26] afforded the fully protected intermediate **19** (60%). Finally, TFA deprotection of **19** gave the required target **6** (78%).

Disappointingly compound **6** showed no whole cell antibacterial activity when evaluated *in vitro* against a screen of commonly encountered bacteria comprising both Gram positive and Gram negative organisms (MIC's all > 128 μg cm^3).[27] Neither did it demonstrate any significant *in vitro* inhibition of penicillin G binding to penicillin binding proteins, or any inhibition of TEM-1 β-lactamase.[28] Thus it appears likely that the *trans*-lactam ring in compound **6** is not sufficiently activated to acylate the bacterial enzymes. (The observed IR spectra may provide some evidence for this. Whilst the highly strained penicillins and cephalosporins typically show carbonyl stretches between 1780 and 1790 cm^{-1} the *trans*-lactam amide carbonyl group in compound **6** stretches only at 1718 cm^{-1}.)

In conclusion, synthesis of further *trans*-lactams with increased activation would be of interest in order to determine



Scheme 3 *Reagents*: i) $BrCH_2CO_2tBu$, Cs_2CO_3, MeCN; ii) H_2, Pd; iii) Acid **18** + $(COCl)_2$; iv) CF_3COOH.

whether a useful *trans*-lactam antibacterial agent can be achieved. Compounds **4** and **5** will be ideal starting materials to pursue this investigation.

Experimental

General methods

FTIR spectra were recorded using a Nicolet 20SXB or a Bio-Rad FTS-7. ^1H NMR spectra were recorded either at 250 MHz using a Bruker AC or AM 250 or at 400 MHz with a Varian VXR 400. Mass spectra were measured on a HP Engine (thermospray positive) or VG Autospec Q (LSIMS). Routine microanalyses were performed on a Leco CHNS-932 or Carlo-Erba instrument. Fluorine analyses were carried out with a Phillips PW 9415 ion selective meter and water analyses using a Mitsubishi CA-05. Flash chromatography was performed with Merck Kieselgel 9385.

trans-*N*-(2-Hydroxycyclopentyl)carbamic acid *tert*-butyl ester 11

Cyclopentene oxide (50.0 g, 0.595 mol) was dissolved in 50% aqueous ethanol (800 cm^3) and ammonium chloride (31.9 g, 0.595 mol) and sodium azide (38.7 g, 0.595 mol) added. The resulting mixture was refluxed for 48 h then concentrated to a small volume and partitioned between water (200 cm^3) and ethyl acetate (200 cm^3). The organic layer was dried (anhydrous $MgSO_4$) and evaporated to a yellow oil (64.4 g, 85%) which was used directly without further purification. (Crude azide ν_{max} $(CHBr_3)/cm^{-1}$ 3430 and 2105; δ_H $(CDCl_3)$: 4.2 (1H, m, H-1), 3.7 (1H, m, H-2), 2.2–2.0 (2H, m), 1.9–1.5 (5H, m).)

The crude azido alcohol (33.4 g, 0.263 mol) was dissolved in toluene (500 cm^3) and refluxed with triphenylphosphine (103.2 g, 0.394 mol, 1.5 eq.) for 1.5 h. The solution was cooled to 0 °C and diluted with ethyl acetate (50 cm^3) and saturated sodium bicarbonate solution (50 cm^3). The resulting biphasic mixture was then stirred rapidly as di-*tert*-butyl dicarbonate (57.3 g, 0.263 mol) in ethyl acetate (500 cm^3) was added over several minutes. The mixture was allowed to warm to room temperature and stirred for 12 h. The organic phase was then separated, dried (anhydrous $MgSO_4$) and evaporated to a yellow oil. This was treated with ether (500 cm^3) to precipitate Ph_3PO which was removed by filtration. The filtrate was concentrated to an oil which was purified by flash chromatography (eluted with ethyl acetate–petroleum ether 1:1) to afford the title compound **11** (21.4 g, 41%) as a white crystalline solid. Mp 106 °C (Found C: 59.5; H, 9.25; N, 6.8. $C_{10}H_{19}NO_3$ Requires C, 59.7; H, 9.5; N, 7.0%). ν_{max} $(CHBr_3)/cm^{-1}$ 3437, 2972, 1690, 1498 and 1366; δ_H $(CDCl_3)$: 4.7 (1H, br s, NH), 4.0 (2H, m, OH, H-1), 3.6 (1H, m, H-2), 2.2–1.3 (6H, m, H-3,4,5), 1.4 (9H, s, tBu).

cis-6-Azabicyclo[3.1.0]hexane-6-carboxylic acid *tert*-butyl ester 12

To a stirred solution of triphenylphosphine (114.0 g, 0.435 mol, 1.5 eq.) in THF (800 cm^3) at −78 °C under nitrogen was added diisopropyl azodicarboxylate (85.6 cm^3, 0.435 mol, 1.5 eq.) dropwise over 30 min. The reaction mixture was stirred for 30 min until a pale yellow suspension was formed. A solution of the alcohol **11** (58.3 g, 0.290 mol) in THF (200 cm^3) was added dropwise and the reaction stirred for 60 min at −78 °C then allowed to warm to RT. After a further 21 h at RT the solvent was evaporated and the residue treated with ether. The precipitated triphenylphosphine oxide was removed by filtration and the filtrate evaporated to a pale yellow residue which was purified by flash chromatography (eluting with petroleum ether–ether, 4:1) to give the title compound **12** (41.9 g, 79%) as a clear oil (Found C: 65.5; H, 9.35; N, 7.6. $C_{10}H_{17}NO_2$ Requires C, 65.1; H, 9.2; N, 7.6%); ν_{max} $(CHBr_3)/cm^{-1}$ 2973, 1699 and 1367; δ_H $(CDCl_3)$: 2.9 (2H, s, H-1,5), 2.05 (2H, dd, H-2,4), 1.6 (3H, m, H-2,3,4), 1.4 (9H, s, tBu), 1.2 (1H, m, H-3). *m*/*z* $C_{10}H_{17}NO_2$ M^+ 183.26.

trans-2-(2-*tert*-Butoxycarbonylaminocyclopentyl)malonic acid dimethyl ester 13

Dimethyl malonate (90.3 g, 0.684 mol, 3 eq.) in DMF (1.5 l) was treated with sodium hydride (60% dispersion in oil, 9.3 g, 0.232 mol, 1.02 eq.) at RT under nitrogen. After effervescence had stopped and the solution had cleared a solution of the aziridine **12** (41.8 g, 0.228 mol, 1 eq.) in DMF (25 cm^3) was added. The solution was stirred with heating (155 °C) for 18 h then cooled, filtered and evaporated to a solid. The crude product was partitioned between ether (1.5 l) and water (1.5 l). The organic layer was separated and the aqueous layer further extracted with ether (1.5 l) and the combined organic extracts washed with brine (2 × 1 l) and then dried ($MgSO_4$). Evaporation gave a solid which was triturated with petrol 40–60°C then filtered and dried *in vacuo* to give a white solid **13** (52.5 g, 0.167 mol, 73%). Mp 104–105 °C (Found C, 57.05; H, 8.1; N, 4.6. $C_{15}H_{25}NO_6$ Requires C, 57.1; H, 8.0; N, 4.4); ν_{max} $(CHBr_3)/cm^{-1}$: 3429, 2950, 1750, 1729, 1705 and 1503; δ_H $(CDCl_3)$: 4.5 (1H, br s, NH), 3.75 (6H, s, MeO), 3.7 (1H, m, H-2′), 3.45 (1H, d, H-2, $J_{2,1'}$ 7.5 Hz), 2.3 (1H, m, H-1′), 2.15–1.2 (6H, m, H-3′,4′,5′), 1.4 (9H, s, tBu); *m*/*z* 333 (MNH_4^+), 316 (MH^+), 216.

Racemic [(1*R*,2*S*)-2-*tert*-butoxycarbonylaminocyclopentyl]-[(*S*)-benzyloxycarbonylamino]acetic acid methyl ester 14 and Racemic [(1*R*,2*S*)-2-*tert*-butoxycarbonylaminocyclopentyl]-[(*R*)-benzyloxycarbonylamino]acetic acid methyl ester 15

To a solution of the malonate **13** (10.0 g, 31.7 mmol) in methanol (150 cm^3) was added potassium hydroxide (8.6 g, 153.6 mmol, 4.8 eq.) in water (60 cm^3). The reaction mixture was stirred for 20 min then partitioned between ether (750 cm^3) and water (750 cm^3). The aqueous phase was acidified with solid potassium hydrogen sulfate and extracted with ether (2 × 750 cm^3). The combined organic extracts were dried (anhydrous $MgSO_4$) and evaporated to an off-white foam containing a mixture of the mono-acid mono-esters (9.6 g, 100%). δ_H $(CDCl_3)$: 5.1, 4.7 (1H, br d, NH), 3.75 (3H, s, MeO), 3.6–3.8 (2H, m, H-2,2′), 2.4–1.4 (7H, m, H-1′,3′4′5′), 1.4, 1.45 (9H, s, tBu). This material was used in the next stage without further purification.

A mixture of acids prepared as above (17.9 g, 59.5 mmol) was dissolved in dioxane (300 cm^3) and treated with triethylamine (8.3 cm^3, 59.5 mmol) and diphenylphosphoryl azide (14.1 cm^3, 11.8 mmol). The solution was refluxed for 4 h under nitrogen when benzyl alcohol (25 cm^3) was added and refluxing continued for a further 18 h. The reaction mixture was cooled and poured into water (2 l). The aqueous solution was extracted with ether (3 × 500 cm^3) then treated with brine and further extracted with ether (450 cm^3). The combined organic extracts were washed with brine (500 cm^3) then dried (anhydrous $MgSO_4$) and the solvent evaporated finally *in vacuo* (water bath at 90 °C) to remove the benzyl alcohol. The resulting brown residue was purified by flash chromatography (eluting first with petroleum ether–ether 2 : 1, then petroleum ether–ether, 1 : 1) to obtain racemic **14** as a white crystalline solid (1.6 g, 3.9 mmol, 7%) eluting first, and racemic isomer **15** eluting second as an off-white gum (2.0 g, 5.0 mmol, 8%). Further **14** and **15** was obtained as a mixed fraction (5.3 g, 13.1 mmol, 22%). Thus the combined overall yield of **14** and **15** was 37%. Compound **14**. (Found C, 61.8; H, 7.35; N, 7.2. $C_{21}H_{30}N_2O_6$ Requires C, 62.1; H, 7.4; N, 6.9%); ν_{max} ($CHBr_3$)/cm^{-1} 3349 (br), 1728, 1714, 1694, 1682 and 1519; δ_H ($CDCl_3$): 7.4–7.3 (5H, m, Ph), 6.4 (1H, br d, Cbz-N*H*), 5.1 (2H, s, PhCH_2), 4.4 (2H, m, Boc-N*H*, H-2), 3.72 (1H, m, H-2′), 3.7 (3H, s, MeO), 2.3–1.3 (7H, m, H-1′,3′,4′,5′), 1.4 (9H, s, tBu). Compound **15**. ν_{max} ($CHBr_3$)/cm^{-1} 3300 (br), 1721, 1712 and 1693; δ_H ($CDCl_3$): 7.4–7.3 (5H, m, Ph), 6.1 (1H, br s, Cbz-N*H*), 5.1 (2H, ABq, PhCH_2), 4.55 (1H, br s, Boc-N*H*), 4.4 (1H, br t, H-2), 3.8 (1H, m, H-2′), 3.7 (3H, s, MeO), 2.2–1.1 (7H, m, H-1′,3′,4′,5′), 1.45 (9H, s, tBu); *m*/*z* 407.2182. MH^+ $C_{21}H_{30}N_2O_6$ requires 407.2182.

Racemic [(1*S*,2*S*)-2-aminocyclopentyl][(*S*)-benzyloxycarbonylamino]acetic acid hydrochloride 8

The ester **14** (0.57 g, 1.4 mmol) was dissolved in methanol (10 cm^3) and treated with 1 M potassium hydroxide (aq.) solution (4.2 cm^3, 3 eq.). The mixture was stirred for 3 h at room temperature then partitioned between ether (150 cm^3) and water (100 cm^3). The aqueous layer was further washed with ether (150 cm^3), then acidified to pH = 3 with cooling in an ice bath. The resulting solution was extracted with ether (2 × 100 cm^3) and the combined organic extracts washed with brine (100 cm^3) and dried (anhydrous $MgSO_4$). Evaporation of the solvent gave a white foam containing the intermediate N-Boc protected acid (0.50 g, 1.3 mmol, 91.0%). This material was used in the next reaction without further purification (Found C, 60.6; H, 7.1; N, 7.2. $C_{20}H_{28}N_2O_6$ Requires C, 61.2; H, 7.1; N, 7.1%); ν_{max} (KBr)/cm^{-1} 3322 (br), 1712, 1650 and 1518; δ_H ($CDCl_3$): 7.4–7.3 (5H, m, Ph), 6.85, 6.05 (1H, 2 × br d, NHCbz, rotamers), 5.1 (2H, m, PhCH_2), 5.4, 4.9 (1H, 2 × br d, NHBoc, rotamers), 4.5 (1H, m, H-2), 3.75 (1H, m, H-2′), 2.25–1.5 (7H, m, H-1′,3′,4′,5′), 1.4 (9H, s, tBu).

N-Boc protected acid (1.20 g, 2.9 mmol) prepared as above was dissolved in TFA (40 cm^3). After 10 min the solvent was evaporated and the residue redissolved in ethyl acetate (50 cm^3) then treated with a solution of HCl in ether (1.5 M, excess). Evaporation of the solvent gave a gum which crystallized on trituration with ether. This material was dried *in vacuo* affording the title compound **8** (0.97 g, 2.9 mmol, 100%) as a white solid. ν_{max} (film)/cm^{-1}: 3487, 2960, 1715, 1669 and 1538; δ_H (DMSO): 8.1 (3H, br s, NH_3^+), 7.7 (1H, d, NH, *J* 7.5 Hz), 7.4–7.25 (5H, m, Ph), 5.05 (2H, s, PhCH_2), 4.1 (1H, t, H-2, *J* 7.5 Hz), 3.4 (1H, m, H-2′), 2.25–1.3 (7H, m, H-1′, 3′, 4′, 5′); *m*/*z* 293.1500. MH^+ $C_{15}H_{21}N_2O_4$ requires 293.1501.

Racemic [(1*S*,2*S*)-2-aminocyclopentyl][(*R*)-benzyloxycarbonylamino]acetic acid hydrochloride 9

The methyl ester **15** (1.47 g, 3.62 mmol) was treated similarly to **14** to give the intermediate N-Boc protected acid (1.34 g, 3.41 mmol, 94%) as a white foam. This crude material (1.23 g, 3.13 mmol) was treated with TFA to give the title compound **9** (1.00 g, 3.04 mmol, 97%) as an off-white solid. ν_{max} (Nujol)/cm^{-1} 2923, 1700, 1521, 1455 and 1376; δ_H (DMSO): 8.1 (3H, br s, NH_3^+), 7.6 (1H, d, NH, *J* 8.5 Hz), 7.4–7.3 (5H, m, Ph), 5.05 (2H, s, PhCH_2), 4.4 (1H, dd, H-2, *J* 5, 8.5 Hz), 3.3 (1H, m, H-2′), 2.4–1.5 (7H, m, H-1′,3′,4′,5′); *m*/*z* 293.1502. MH^+ $C_{15}H_{20}N_2O_4$ requires 293.1501.

Racemic [(3*S*,3a*S*,6a*S*)-2-oxooctahydrocyclopenta[*b*]pyrrol-3-yl]carbamic acid benzyl ester 4

The amino acid hydrochloride salt **8** (0.86 g, 2.6 mmol) was dissolved in DMF (10 cm^3) and added *via* a syringe pump to a solution of diphenylphosphoryl azide (1.12 cm^3, 5.20 mmol) and triethylamine (1.26 cm^3, 9.09 mmol) in DMF (200 cm^3). The total addition time was 28 h. The solution was then concentrated to an oily solid and partitioned between water (300 cm^3) and ether (300 cm^3). The aqueous layer was further washed with ether (300 cm^3) and the combined organic layers washed with brine (2 × 300 cm^3). The extracts were dried (anhydrous $MgSO_4$) and then evaporated to an oil which was triturated, filtered and dried *in vacuo* to give the title compound **4** as a white solid (0.49 g, 1.80 mmol, 69%) (Found C, 65.4; H, 6.6; N, 10.3. $C_{15}H_{18}N_2O_3$ Requires C, 65.7; H, 6.6; N, 10.2%); ν_{max} ($CHBr_3$)/cm^{-1} 3418, 2974, 1712, 1506; δ_H ($CDCl_3$): 7.4–7.3 (5H, m, Ph), 6.26 (1H, br s, amide NH), 5.28 (1H, br d, Cbz-N*H*), 5.11 (2H, ABq, PhCH_2), 4.24 (1H, t, H-3), 3.21 (1H, dt, H-6a), 2.1–1.3 (7H, m, H-3a,4,5,6).

Racemic [(3*R*,3a*S*,6a*S*)-2-oxooctahydrocyclopenta[*b*]pyrrol-3-yl]carbamic acid benzyl ester 5

Using the same procedure described for preparation of **4**, the amino acid hydrochloride salt **9** (0.90 g, 2.74 mmol) in DMF (10 cm^3) was added *via* a syringe pump to a solution of diphenylphosphoryl azide (1.35 cm^3, 6.26 mmol) and triethylamine (1.52 cm^3, 10.96 mmol) in DMF (200 cm^3) to afford **5** as a white solid (0.57 g, 76%) (Found C, 65.4; H, 6.5; N, 10.2. $C_{15}H_{18}N_2O_3$ Requires C, 65.7; H, 6.6; N, 10.2%); ν_{max} ($CHBr_3$)/cm^{-1} 3419, 1707 and 1506; δ_H ($CDCl_3$): 7.4–7.3 (5H, m, Ph), 6.2 (1H, br s, NH amide), 5.4 (1H, br s, Cbz-N*H*), 5.1 (2H, ABq, PhCH_2), 3.97 (1H, dd, H-3), 3.09 (1H, m, H-6a), 2.1–1.3 (7H, m, H-3a,4,5,6).

Racemic [(3*S*,3a*R*,6a*S*)-2-oxo-3-benzyloxycarbonylaminooctahydrocyclopenta[*b*]pyrrol-1-yl]acetic acid *tert*-butyl ester 16

Cbz protected *trans*-lactam **4** (0.15 g, 0.58 mmol) was dissolved in acetonitrile (10 cm^3) and caesium carbonate (0.47 g, 1.45 mmol) added. The resulting suspension was treated with *tert*-butyl bromoacetate (0.19 cm^3, 1.16 mmol) and the reaction heated at 50 °C for 4 h. After cooling, filtration and evaporation of solvent a residual gum was obtained which was purified by flash chromatography (eluant ethyl acetate–cyclohexane, 1 : 3) affording the title compound **16** (0.17 g, 79%) as a colourless gum. ν_{max} ($CHBr_3$)/cm^{-1} 1738, 1729, 1712 (*trans*-lactam), 1694; δ_H ($CDCl_3$): 7.4–7.3 (5H, m, Ph), 5.1 (3H, m, PhCH_2, N*H*), 4.35 (1H, br t, H-3), 3.9 (2H, ABq, CH_2CO_2), 3.35 (1H, dt, H-6a), 2.1–1.3 (7H, m, H-3a,4,5,6), 1.4 (9H, s, tBu).

Racemic [(3*S*,3a*R*,6a*S*)-2-oxo-3-aminohexahydrocyclopenta-[*b*]pyrrol-1-yl]acetic acid *tert*-butyl ester acetate salt 17

Cbz *trans*-lactam **16** (0.17 g, 0.44 mmol) was dissolved in acetic acid (20 cm^3) and hydrogenated with 10% palladium on carbon (50 mg) at room temperature for 18 h. The solution was filtered and evaporation afforded the title compound **17** (0.13 g, 94%) as a crystalline solid. ν_{max} (KBr)/cm^{-1} 1745, 1710 and 1554; δ_H (DMSO): 4.35 (3H, br s, NH_3^+), 3.95 (2H, ABq, CH_2CO_2), 3.5 (2H, m, H-3,6a), 2.1–1.3 (7H, m, H-3a,4,5,6), 1.4 (9H, s, tBu); *m*/*z* 509 (2M + H^+), 254 (MH^+).

Racemic 2-[(2-Aminothiazol-4-yl)-2-[(3*S*,3a*R*,6a*S*)-1-carboxymethyl-2-oxooctahydrocyclopenta[*b*]pyrrol-3-ylcarbamoyl]-methyleneaminooxy]-2-methylpropionic acid trifluoroacetate 6

Oxalyl choride (0.050 cm^3, 0.57 mmol) was added to a solution of DMF (0.066 cm^3, 0.85 mmol) in dichloromethane (5 cm^3) at −20 °C under nitrogen. Upon completion of the addition, the solution was warmed to ice bath temperature and the acid **18**[18] (0.416 g, 0.73 mmol) added as a solid. After 10 min a solution of the acetate salt of the amine **17** (0.120 g, 0.38 mmol) in dichloromethane (2 cm^3) was added with diisopropylethylamine (0.4 cm^3, 2.29 mmol). After 24 h, the mixture was diluted with ethyl acetate and water, and the organic layer washed further with 10% aqueous citric acid solution, then saturated aqueous sodium bicarbonate and finally brine. After drying (anhydrous $MgSO_4$) and evaporation the residue was purified by flash chromatography (eluant ethyl acetate–cyclohexane, 1 : 3) affording the fully protected **19** (0.183 g, 60%) as a white solid. Compound **19**: mp 103 °C; ν_{max} ($CHBr_3$)/cm^{-1} 3298 (br), 1737, 1731, 1714, 1681 and 1519; δ_H ($CDCl_3$): 7.4 (1H, d, NH amide), 7.35–7.25 (15H, m, 3 × Ph), 6.8 (1H, s, trityl NH), 6.75 (1H, s, thiazole H), 4.7 (1H, dd, H-3), 3.95 (2H, ABq, CH_2CO_2), 3.4 (1H, dt, H-6a), 1.65, 1.60 (6H, 2s, CMe_2), 1.4 (18H, s, 2 × tBu), 2.1–1.3 (7H, m, H-3a,4,5,6); *m*/*z* 808 (MH^+), 243.

Compound **19** (0.122 g, 0.031 mmol) was dissolved in trifluoroacetic acid (2 cm^3) containing a trace of anisole. After 2.5 h, the solution was diluted with ether which precipitated the title compound **6** (0.067 g, 78%) as a white solid which was collected by filtration. Mp 141 °C; ν_{max} (Nujol)/cm^{-1} 1718 and 1674; δ_H (DMSO): 8.7 (1H, d, thiazole H), 4.45 (1H, dd, H-3), 3.9 (2H, ABq, CH_2CO_2H), 3.35 (1H, m, H-6a), 1.45, 1.4 (6H, 2s, CMe_2), 2.0–1.3 (7H, m, H-3a,4,5,6); HPLC 95%; *m*/*z* 454.1397. $C_{18}H_{23}N_5O_7S$ requires MH^+ 454.1396.

Acknowledgements

The authors wish to express their thanks to Gavin Chung for performing the biological evaluation of compound **6**.

References

1 D. Jackson and D. H. Staniforth, *Acta Ther.*, 1982, **8**, 367.
2 M. Hedberg and C. E. Nord, *J. Chemother*. (*Florence*), 1996, **8**, 3.
3 S. P. Clissold, P. A. Todd and D. M. Campoli-Richards, *Drugs*, 1987, **33**, 183.
4 L. N. Jungheim, D. B. Boyd, J. M. Indelicato, C. E. Pasini, D. A. Preston and W. E. Alborn, Jr. *J. Med. Chem.*, 1991, **34**, 1732 and references cited therein.
5 Y. Nozaki, N. Katayama, H. Ono, S. Tsubotani, S. Harada, H. Okazaki and Y. Nakao, *Nature*, 1987, **325**, 179.
6 Y. Nakao, in *Recent Advances in the Chemistry of β-Lactam Antibiotics*, eds. P. H. Bentley and R. Southgate, Special Publication No. 70, RSC, London, 1989, ch. 8.
7 M. Pass, S. Abu-Rabie, A. Baxter, R. Conroy, S. J. Coote, A. P. Craven, H. Finch, S. Hindley, H. A. Kelly, A. W. Lowdon, E. McDonald, W. L. Mitchell, N. A. Pegg, P. A. Procopiou, N. G. Ramsden, R. Thomas, D. A. Walker, N. S. Watson, H. Jhoti, C. J. Mooney, C.-M. Tang, P. J. Thomas, S. Parry and C. Patel, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 1657.
8 M. Pass, R. E. Bolton, S. J. Coote, H. Finch, S. Hindley, A. Lowdon, E. McDonald, J. McLaren, M. Owen, N. A. Pegg, V. J. Mooney, C.-M. Tang, S. Parry and C. Patel, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 431.
9 H. Finch, N. A. Pegg, J. McLaren, A. Lowdon, R. Bolton, S. J. Coote, U. Dyer, J. G. Montana, M. R. Owen, M. Dowle, D. Buckley, B. C. Ross, C. Campbell, C. Dix, C. Mooney, C.-M. Tang and C. Patel, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 2955.
10 S. J. F. Macdonald, D. J. Belton, D. M. Buckley, J. E. Spooner, M. S. Anson, L. A. Harrison, K. Mills, R. J. Upton, M. D. Dowle, R. A. Smith, C. R. Molloy and C. Risley, *J. Med. Chem.*, 1998, **41**, 3919.
11 H. A. Kelly, R. Bolton, S. A. Brown, S. J. Coote, M. Dowle, U. Dyer, H. Finch, D. Golding, A. Lowdon, J. McLaren, J. G. Montana, M. R. Owen, N. A. Pegg, B. C. Ross, R. Thomas and D. A. Walker, *Tetrahedron Lett.*, 1998, **39**, 6979.
12 S. J. F. Macdonald, G. D. E. Clarke, M. D. Dowle, L. A. Harrison, S. T. Hodgson, G. G. A. Inglis, M. R. Johnson, P. Shah, R. J. Upton and S. B. Walls, *J. Org. Chem.*, 1999, **64**, 5166.
13 J. R. McCarthy, P. E. Wiedeman, A. J. Schuster, J. Whitten, R. J. Barbuch and J. C. Huffman, *J. Org. Chem.*, 1985, **50**, 3095.
14 T. Shioiri, K. Ninomiya and S. Yamada, *J. Am. Chem. Soc.*, 1972, **94**, 6203.
15 For a review see F. C. Neuhaus and N. Georgopapakadou, in *Emerging Targets in Antibacterial and Antifungal Chemotherapy*, ch. 9, eds. J. Sutcliffe and N. H. Georgopapadakou, Chapman and Hall, New York, London, 1992.
16 M. P. Singh, Y. I. Lee, R. Singh, R. G. Micetich and B. J. Wilkinson, *J. Antimicrob. Chemother.*, 1991, **27**, 459.
17 M. Nishida and Y. Shigi, *The Role of Permeability and Affinity for Penicillin-binding Proteins in the Antibacterial Activity of the Third-generation Cephalosporins*, in *Symp. Front. Pharmacol.*, 1982, vol. 1 (New β-Lactam Antibiotics), pp. 65–81.
18 N. A. C. Curtis, D. Orr, G. W. Ross and M. G. Boulton, *Antimicrob. Agents Chemother.*, 1979, **16**, 533.
19 M. Narisada, *Pure Appl. Chem.*, 1987, **59**, 459.
20 M. Takasuka, J. Nishikawa and K. Tori, *J. Antibiot.*, 1982, **35**, 1729.
21 J. M. Ghuysen, *Annu. Rev. Microbiol.*, 1991, **45**, 37.
22 M. Leyh-Bouille, J. Van Beeumen, S. Renier-Pirlot, B. Joris, M. Nguyen-Disteche and J. M. Ghuysen, *Biochem. J.*, 1989, **260**, 601.
23 R. N. Brogden and R. C. Heel, *Drugs*, 1986, **31**, 96.
24 C. E. Newall, *Chem. Br.*, 1987, **23**, 976.
25 D. Gala, M. Steinman and R. S. Jaret, *J. Org. Chem.*, 1986, **51**, 4488.
26 C. H. O'Callaghan, B. E. Ayres and D. G. H. Livermore, *Br. Pat. Appl.*, 2 040 921, Oct 26, 1979.
27 Agar MIC's were determined by a two-fold agar dilution method using Mueller–Hinton media (Becton Dickinson Ltd, USA). Exponentially growing cultures were inoculated onto the surface of agar plates using a multipoint inoculator (Denley, UK) to produce an inoculum of approximately 10^5 cfu per cm^3 (cfu = colony forming units). The plates were incubated at 37 °C for 18 hours and the minimum inhibitory concentration (MIC) value interpreted as the lowest antibiotic concentration which completely inhibited bacterial growth.
28 TEM-1 β-lactamase was pre-incubated with compound **6** before the chromogenic substrate nitrocefin (50 μg cm^{-3}) was added. The enzymatic hydrolysis of nitrocefin was followed at 495 nm. Compound **6** failed to inhibit the activity of TEM-1 at the highest concentration used (1 mM).