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γ-LACTAM ANALOGUES OF CARBAPENICILLANIC ACIDS

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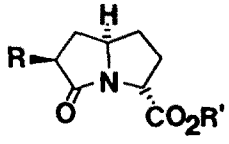
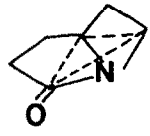
Abstract - The syntheses and biological activities of the γ-lactam analogues (endo-25), (exo-25) and (26) of carbapenicillanic acids are described. The strategy employed for the synthesis of 26 effected construction of a γ-lactam onto a preformed azetidine, followed by direct amination of the lactam enolate (20) with diphenylphosphinoyl-hydroxylamine. X-ray crystallographic studies demonstrated that the analogues bore a strong morphological resemblance to carbapenicillanic acids; however, they were shown to be devoid of antibacterial and beta-lactamase inhibitory activity.[1]

Since the recent discoveries of the non-traditional beta-lactam antibiotics,[2] it now appears that the minimum structural requirement for a member of this class of antibiotic to show antibacterial activity is a suitably activated beta-lactam ring. In an attempt to develop a novel class of antibiotics we recently reported the synthesis of the beta-lactam analogue (1) and demonstrated it was devoid of both antibacterial and beta-lactamase inhibitory activity.[3,4] X-Ray crystallographic studies showed that the sum of the bond angles about the nitrogen atom [5] of 2 was 356°.

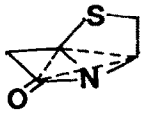
Molecular modelling studies indicated that fused γ-lactam-azetidine analogues, such as 3, would have similar pyramidal distortions to those observed for the lactam-nitrogen in penicillins. As it has been hypothesised that the degree of pyramidal distortion of the lactam-nitrogen of beta-lactam antibiotics, as in 4, is related to antibacterial activity[6] we undertook a synthesis of the analogue, 5.

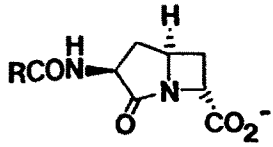




1 R=PhOCH$_2$CONH, R^1=H
2 R=OH, R^1=Me

3

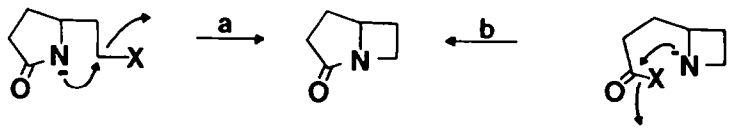




4

5

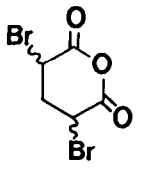
Two possible strategies to the target (5), a and b were explored (Scheme 1). In initial studies we were unable to construct an azetidine onto a preformed lactam as in a, however the alternative b was sucessful.



Scheme 1

Thus, bromination of glutaric anhydride gave crude α,α'-dibromoglutaric anhydride (6),[7] which was ring opened with formic acid. The crystalline α,α'-dibromoglutaric acid (7) which resulted, was reconverted into pure 6, with a dl:meso ratio of 3:1[8], by refluxing in acetyl chloride. Solvolysis of 6 ($MeOH$, Na_2CO_3) gave the monomethyl ester (8). Formation of acid chloride (9) (oxalyl chloride, N,N-dimethylformamide) followed by reaction with t-butanol and pyridine at -15°C, gave diester (10) (diastereomeric ratio 3:2). Formation of the diester (10) at 20°C gave a reversal in the diastereomeric ratio, such that the previously minor isomer became predominant (4:1), in accordance with proposals that the base catalysed solvolysis of acid chlorides can proceed via two distinct mechanisms.[9]

Cromwell has reported the preparation of azetidines in good yield by the reaction of benzylamine with 2,4-dibromobutanoates.[10] Thus, reaction of the diester (10) with 3 equivalents of benzylamine in dimethylformamide at 80°C gave the diastereomeric azetidines (cis-11) and (trans-11) (84% from 6, trans:cis 3:2). Separation of the diastereomers was achieved by careful chromatography, however a more facile separation of the cis and trans series was produced by reduction ($NaBH_4$) to give cis-12 and trans-12 (80%), from which cis-12 could be fractionally crystallised. The structure of cis-12 was unequivocally established by X-ray crystallographic analysis (Figure 1).



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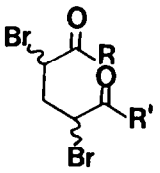
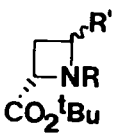


7 R, R^1=OH
8 R=OH, R^1=OMe
9 R=Cl, R^1=OMe
10 R=O^{t}Bu, R^1=OMe



11 R=CH_2Ph, R^1=CO_2Me
12 R=CH_2Ph, R^1=CH_2OH
13 R=H, R^1=CH_2OH
14 R=CO_2CH_2Ph, R^1=CH_2OH

Hydrogenation of the mother liquors (containing almost pure trans-12), followed by reprotection of the resultant amine (trans-13) gave the N-benzyloxycarbonylazetidine (trans-14), which was recrystallised to homogeneity. A similar sequence applied to pure cis-12 gave cis-14 as an oil.

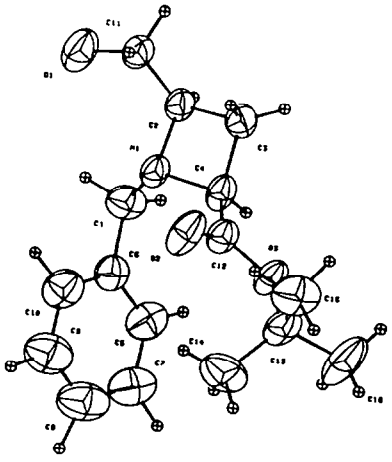
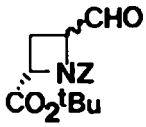


Figure 1. Structure of cis-12



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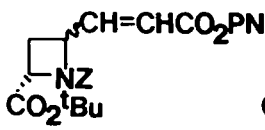
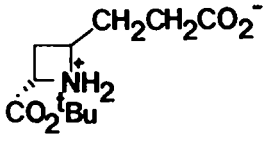


16 17

[Z=$PhCH_2OCO$-]

[PNB=$O_2NC_6H_4CH_2$-]

The individual alcohols (trans-14) and (cis-14) were converted into the bicyclic-γ-lactams (exo-19) and (endo-19) by standard methodologies. Thus, Moffatt oxidation of cis-14 gave the aldehyde (cis-15) which was reacted with (triphenylphosphoranylidene)acetic acid p-nitrobenzyl ester to give cis-16, as a mixture of olefins. Similarly, aldehyde trans-15 gave trans-16, though in this case only the E isomer was isolated. Reduction of cis-16 using 10% palladium on carbon as catalyst led to cleavage of the azetidine ring.[11] However, a two stage reduction using Wilkinson's catalyst for the initial conversion of cis-16 to cis-17, followed by hydrogenation using palladium on carbon gave the amino acid (cis-18). Cyclisation with 2,2'-dipyridyldisulphide and triphenylphosphine[12] gave the bicyclic γ-lactam (endo-19) (55% from cis-14). An analogous sequence applied to trans-16 gave the bicyclic lactam, (exo-19) (58% from trans-14). The structures as endo-and exo-19 were unequivocally established by X-ray crystallographic analysis.



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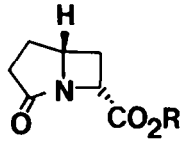
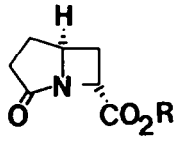
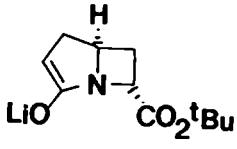


endo-19, R=^{t}Bu
endo-23, R=H
endo-25, R=K
endo-27, R=Me



exo-19, R=^{t}Bu
exo-23, R=H
exo-25, R=K
exo-27, R=Me

Reaction of exo-19 with lithium hexamethyldisilazide gave the monoanion (20) which was quenched with O-(diphenylphosphinoyl)hydroxylamine[13] to give the 3-aminobicyclic-γ-lactam (21), which was coupled with phenoxyacetic acid to give 22 (40% from exo-19). X-Ray crystallographic analysis (Figure 2) demonstrated that the amine function was introduced trans- to the t-butyl ester (trans:cis addition ca > 10:1), probably indicating that the steric bulk of the t-butyl group overode that of the convex nature of the bicyclic γ-lactam (20) during the amination reaction.



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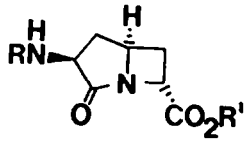


21 R=H, R^1=^{t}Bu
22 R=$PhOCH_2CO$, R^1=^{t}Bu
24 R=$PhOCH_2CO$, R^1=H
26 R=$PhOCH_2CO$, R^1=K
29 R=$PhOCH_2CO$, R^1=K

The analogues (exo-19), (endo-19) and (22) were deprotected (trifluoroacetic acid) to give exo-23, endo-23 and 24 respectively, then dissolved in pH 7.6 phosphate buffer to give the respective carboxylates (exo-25), (endo-25) and (26). These were shown to be stable in phosphate buffer for greater than 12 hours (by ^{1}H NMR, 300MHz) and by subsequent re-esterification to their corresponding methyl esters (exo-27), (endo-27) and (29).

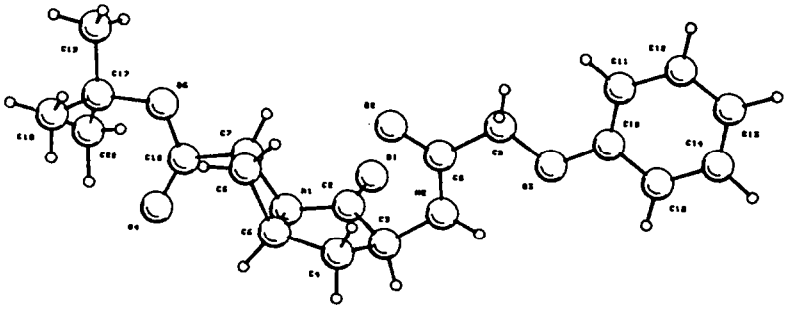
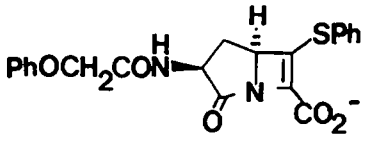


Figure 2. Structure of 22

The X-ray crystallographic studies demonstrated that in common with antibacterial bicyclic β-lactams, compounds endo-19, exo-19 and 22 have pyramidal lactam-nitrogen atoms. The degree of pyramidality was of a similar order to that of the penicillins, the sum of the angles[5] around nitrogen for endo-19, exo-19, 22 and penicillin G being 334, 330, 326 and 337° respectively. However, Cohen has pointed out that a more significant parameter for antibacterial activity may be the interatomic distance between the β-lactam oxygen and the carboxylate carbon, values of between 3.0 and 3.9 Å being observed for active compounds.[14] The analogous distances for exo-19 and 22 were both 4.1 Å.

The analogues (endo-25), (exo-25) and (26) were tested against a representative panel of Gram positive and Gram negative organisms (Pfizer, Sandwich). All were found to be inactive up to a concentration of 0.1mg ml^{-1}. In separate tests of the three analogues for β-lactamase inhibitory activity against β-lactamase I (from Bacillus cereus), no significant activity was detected.



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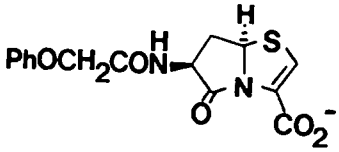

30

Recently, the preparation and biological evaluation of the bicyclic azete (28)[15] and the γ-lactam analogue of the penems (30)[16] have been reported. These compounds have been shown to possess antibacterial activity against both gram positive and gram negative bacteria.

EXPERIMENTAL

Standard laboratory practice as previously described[17] was observed. Melting points were recorded on a Büchi 510 apparatus and are uncorrected. Infra red spectra were recorded on a Perkin-Elmer Model 681 spectrophotometer. All ^{1}H NMR spectra were recorded at 300MHz on a Bruker WH 300 NMR spectrometer using deuteriochloroform as solvent referenced to residual $CHCl_3$ = 7.27 p.p.m. unless otherwise stated. Coupling constants, J, were recorded to the nearest (±) 0.5Hz. All ^{13}C NMR spectra were recorded at 62.85MHz or 75.4MHz using either a Bruker AM 250 or Bruker WH 300 spectrometer respectively, with deuteriochloroform as solvent referenced to $CDCl_3$ = 77.00 p.p.m. unless otherwise stated. Only selected I.R. and ^{13}C NMR signals are assigned. Mass spectra in the electron impact (E.I.) or chemical ionisation (C.I.) modes were recorded on a VG Micromass 16F spectrometer. Samples requiring desorption chemical ionisation (D.C.I.) and accurate mass measurement (E.I.) were run on VG Micromass 30F or ZAB IF spectrometers.

Preparation of α,α'-Dibromoglutaric acid methyl, t-butyl ester (10).

The following four reactions were performed without purification of the intermediates. An overall yield is given.

α,α'-Dibromoglutaric anhydride (6).

A solution of 7[7] (50.0g, 172 mmol) in acetyl chloride (65 ml) was refluxed until the solid had dissolved and evolution of gas had ceased (ca 2.5 hours). The excess acetyl chloride was removed in vacuo to give crude 6, as an oil (dl:meso, ca 3:1).[8] ν_{max} (film) 1823 s, 1782 s, 1242 m, 1035 s, 688 m and 615 s cm^{-1}; δH (meso) 2.68-2.98(1H, m, CH_2), 3.11-3.19(1H, m, CH_2), 4.78-4.89 (2H, m, CHBr); (dl) 2.86(2H, t, J 6Hz, CH_2), 4.91(2H, t, J 6Hz, CHBr); m/e (NH_3, C.I.) 275/273/271 (M^++1), 274/272/270 (M^+), 149(17), 147(17), 122(80), 120(80), 39(100).

α,α'-Dibromoglutaric acid monomethyl ester (8).

To the solution of crude 6 in dichloromethane at 0°C was added, dropwise (over 10 minutes), a saturated solution of sodium carbonate in dry methanol (100 ml). The solution was then allowed to warm to room temperature (ca 30 minutes) and all volatiles were removed in vacuo (30°C, 1mm Hg), to give crude 8 as an oil (diastereomer ratio, ca 3:1). ν_{max} (film) 3220 b, 1740 s, 1430 s, 1280 b, 1165 s. δH (major) 2.58-2.79(2H, m, CH_2), 3.82(3H, s, CH_3), 4.48-4.62(2H, m, CHBr), 10.1 [2H, bs, OH of both (major) and (minor)]; (minor): 2.58-2.79 [1H, m, CH_2, obscured by CH_2 of (major)], 2.81-2.96(1H, m, CH_2), 3.85(3H, s, CH_3), 4.38-4.45(2H, m, CHBr), 10.1(2H, bs, OH); m/e (E.I.) 305/303/301 (M^+-1), 289/287/285 (M^+-OH), 288/286/284 (M^+-H_2O), 225/223 (M^+-Br), 163(50), 99(100).

α,α'-Dibromoglutaric monoacid chloride monomethyl ester (9).

To the solution of crude 8 (as obtained from the previous reaction) and oxalyl chloride (25.0 g, 195 mmol) in dichloromethane (125 ml) was added pyridine (ca 0.5 ml). The solution was stirred until gas evolution had ceased (ca 3 hours). All volatiles were removed in vacuo (30°C, 1mm Hg) to give crude 9 as a colourless oil (diastereomer ratio, ca 3:1); ν_{max} (film) 1784 s, 1745 s, 1440 m, 1280 m, and 1164 m; δH (major): 2.59-2.85(2H, m, CH_2), 3.80(3H, s, CH_3), 4.49(1H, dd, J 10,4Hz, $CHCO_2$), 4.80(1H, dd, J 10,4Hz, CHCOCl); (minor): 2.59-2.65 [1H, m, CH_2, obscured by CH_2 of (major)], 2.89-3.00(1H, m, CH_2), 3.78(3H, s, CH_3), 4.37-4.42(1H, m, $CHCO_2$), 4.67-4.72(1H, m, CHCOCl); m/e (NH_3, C.I.) 289/287/285 (M^+-COCl), 207(10), 205(10), 179(26), 177(26), 151(17), 149(17), 39(100).

α,α'-Dibromoglutaric acid methyl, t-butyl ester (10).

A stirred solution of crude 9 from the previous reaction in dichloromethane (150 ml) and t-butanol (50 ml) was cooled to -25°C. Pyridine (13.8 ml, 0.17 mol) in dichloromethane (30 ml) was then added dropwise over 15 minutes, whilst maintaining the temperature of the reaction mixture below -15°C. After the addition was complete the reaction mixture was allowed to warm to room temperature and was stirred for a further 1 hour. The solution was then washed (1N hydrochloric acid, aqueous saturated sodium bicarbonate and brine, dried (Na_2SO_4), filtered and evaporated to dryness to give crude 10 [59.4 g, 90% from 7 (50 g)] (diastereomer ratio, ca 3:2); ν_{max} (film) 2990 m, 1740 s, 1440 m, 1372 s, 1280 b and 1151 cm^{-1}; δH (major): 1.44(9H, s, t-Bu), 2.43-2.65(2H, m, CH_2), 3.76(3H, s, CH_3), 4.29-4.43(2H, m, CHBr); (minor): 1.49(9H, s, t-Bu), 2.50-2.60(1H, m, CH_2), 2.70-2.74(1H, m, CH_2), 3.77(3H, s, CH_3), 4.22(1H, t, J 7.5Hz, CHBr), 4.45-4.53(1H, m, CHBr); m/e (E.I.) 289/287/285 (M^+-C_4H_9), 179(3), 177(3), 57(100).

1-Benzyl-2-(carboxylic acid t-butyl ester)-4-carboxylic acid methyl ester)azetidine (11).

A solution of the crude diester (10) (56.4 g, 157 mmol) in dimethylformamide (200 ml) containing benzylamine (50.5 g, 471 mmol) was heated at 80°C for 2 hours. All volatiles were removed in vacuo (35°C, 0.1mm Hg) and the residue dissolved in ethyl acetate (100 ml). The organic solution was washed (1N hydrochloric acid, aqueous saturated sodium bicarbonate and brine), dried (Na_2SO_4), filtered and evaporated to dryness to give the title compound (11) (42.6 g, 89%) (trans-11:cis-11, ca 3:2). A small amount (20 mg) of the mixture was chromatographed [P.L.C., two elutions with ethyl acetate/hexane, 1:19] to give the isolated azetidines (trans-11) and (cis-11) as colourless oils. For trans-11: t.l.c. [2 x ethyl acetate/hexane, 1:19] Rf 0.5; ν_{max} 1740 s, 1735 s, 1370 m and 1156 m cm^{-1}; δH 1.35(9H, s, t-Bu), 2.39(2H, ca t, J 7Hz, 3-H), 3.56(3H, s, OCH_3), 3.82(2H, s, NCH_2), 4.02(1H, ca t, J 7Hz, CHN), 4.13(1H, ca t, J 7Hz, CHN), 7.16-7.32(5H, m, Ph); m/e (F.D.) 305 (M^+) [Found M^+ 305.1627. $C_{17}H_{23}NO_4$ requires 305.1626]. For cis-11: t.l.c. [2 x ethyl acetate/hexane, 1:19] Rf 0.45; ν_{max} 1740 s, 1370 m, 1230 s and 1158 cm^{-1}; δH 1.36(9H, s, t-Bu), 2.22-2.50(2H, m, 3-H), 3.48(1H, ca t, J 8Hz, CHN), 3.56(1H, ca t, J 8Hz, CHN), 3.61(3H, s, OCH_3), 3.65, 3.85(2H, ABq, J 13Hz, NCH_2), 7.20-7.37(5H, m, Ph); m/e (F.D.) 305 (M^+) [Found 305.1627. $C_{17}H_{23}NO_4$ requires 305.1626].

1-Benzyl-2-(carboxylic acid t-butyl ester)-4-(hydroxymethyl)azetidine (12).

The crude 11 (10.0 g, 32.8 mmol) was reduced with sodium borohydride (10.0 g, 263 mmol) at 0°C in methanol (150 ml) for 3h. The excess borohydride was destroyed with aqueous ammonium chloride, after which work up as described for the preparation of 11 gave crude 12 (5.85 g, 80%) (trans:cis, ca 3:2). Fractional crystallisation (at -30°C from hexane) gave pure cis-12 leaving trans-12 in the mother liquors. A small amount of trans-12 was purified for spectroscopic analysis by chromatography [P.L.C., ether/hexane, 1:1]. For trans-12: t.l.c. [ether/hexane, 1:1] Rf 0.4; ν_{max} 3420 b, 2980 m, 1736 s, 1368 m and 1153 s cm^{-1}; δH 1.38(9H, s, t-Bu), 1.94-2.20(1H, m, 3-H), 2.36(1H, ca dt, J 11, 8Hz, 3-H), 2.40(1H, b, OH), 3.17(2H, d, J 3Hz, CH_2O), 3.66, 3.88 (2H, ABq, J 13Hz, NCH_2), 3.87(1H, dd, J 8, 3Hz, 2-H), 3.89-3.96(1H, m, 4-H), 7.13-7.23(5H, m, Ph); m/e (NH_3, C.I.) 278(35, M^++1), 222(45), 176(81), 91(100). For cis-12: m.p. 93-95°C (from hexane); ν_{max} 3420 b, 1717 s, 1745 s and 1702 s, 1360 s cm^{-1}; δH 1.30(9H, s, t-Bu), 2.11-2.23 (2H, m, 3-H), 2.90(1H, b, OH), 3.09-3.20(2H, bm, CH_2O), 3.23-3.33(1H, m, 4-H), 3.47(1H, t, J 8.5Hz, 2-H), 3.58, 3.71(2H, ABq, J 12.5Hz, NCH_2), 7.16-7.23(5H, m, Ph); m/e (NH_3, C.I.) 278(100, M^+), 222(20), 176(40), 91(13) [Found C, 69.00; H, 8.34; N, 4.89. $C_{16}H_{23}NO_4$ requires C, 69.27; H, 8.36; N, 5.05%].

Note The following reactions involving azetidines were performed on pure cis or trans isomers. A single procedure followed by the individual yields and analytical data is given where applicable.

2-(Carboxylic acid t-butyl ester)-4-hydroxymethyl)azetidine (13).

A solution of crude trans-12 (12.5 g, 45.1 mmol) in methanol (10 ml) was hydrogenated over 10% palladium on carbon (0.70 g) until the required amount of hydrogen (ca 1090 ml) was consumed (ca 4 hours). The catalyst was removed by filtration (celite) and the filtrate evaporated to dryness. The residue was then crystallised (ether/hexane) to give trans-13 as colourless prisms (7.6 g, 90%). m.p. 64-66°C; ν_{max} (KBr disc) 3305 s, 3240 b, 1733 s, 1400 m, 1369 s, 1230 s, 1160 s, 1105 s, 1034 s, 839 s and 770 m cm^{-1}; δH 1.41(9H, s, t-Bu), 2.16-2.29(1H, m, 3-H), 2.36-2.50(1H, m, 3-H), 3.30(2H, b, OH), 3.42(1H, dd, J 12, 4Hz, CH_2O), 3.51(1H, dd, J 12, 3.5Hz, CH_2O), 3.80-3.93 (1H, m, 4-H), 4.19(1H, dd, J 9, 7.5Hz, 2-H); δC 26.4(t, 3-C), 27.9(q, CCH_3), 55.3(d, CN) 57.2(d, CN), 64.8(t, CH_2O), 81.3(CCH_3), 174.4(s, CO); m/e (E.I.) 188(6, M^++1), 91(100) [Found C, 57.45; H, 9.08; N, 7.41. $C_9H_{17}NO_3$ requires C, 57.73; H, 9.15; N, 7.48%]. For trans-13: Yield [636 mg, 93% from trans-12 (1.01 g, 3.65 mmol)]. m.p. 80-81°C (from ether, hexane); ν_{max} (KBr disc) 3320 m, 3165 b, 1725 s, 1372 m, 1317 s, 1237 s, 1163 s, 1070 s and 950 m cm^{-1}; δH 1.46(9H, s, t-Bu), 2.01-2.22(1H, m, 3-H), 2.43-2.65(1H, m, 3-H), 3.46(1H, dd, J 11.5, 5Hz, CH_2O), 3.51 (1H, dd, J 11.5, 3Hz, CH_2O), 3.38-3.73(2H, b, NH, OH), 3.87(1H, dd, J 9.5, 4Hz, 2-H), 3.93-5.03(1H, m, 4-H);m/e (NH_3, C.I.) 188(100, M^++1), 132 (35), 86(25); [Found C, 57.80; H, 9.06; N, 7.73. $C_9H_{17}NO_3$ requires C, 57.73; H, 9.15; N, 7.48%].

N-(Benzyloxycarbonyl)-2-(carboxylic acid t-butyl ester)-4-(hydroxymethyl)azetidine (14).

To a solution of trans-13 (9.63 g, 51.5 mmol) in dichloromethane (100 ml) and benzyl chloroformate (85% w/w, 9.79 g, 48.9 mmol) at 0°C was added pyridine (3.90 g, 51.5 mmol). The solution was allowed to warm to room temperature and was stirred for 1 hour. Work up as described for the preparation of 11, followed by chromatography [flash silica, ether:hexane (0:1-1:0)] and crystallisation (ether/hexane) gave trans-14 (15.8 g, 91%): m.p. 51-53°C (from ether); t.l.c. (ether) Rf 0.6; ν_{max} (film) 3480 b, 1735 s, 1714 s, 1690 s, 1425 s, 1365 s, 1150 m, 1090 m and 770 m cm^{-1}; δH (showed rotational isomers in a ca 4:1 ratio, only the major is described) 1.39(9H, s, t-Bu), 2.08-2.20(1H, m, 3-H), 2.24-2.38(1H, m, 3-H), 3.57-3.88(3H, m, CH_2OH), 4.40(1H, dd, J 9.5, 4Hz, 2-H), 4.61-4.73(1H, bm, 4-H), 5.08, 5.15 (2H, ABq, J 12.5Hz, CH_2Ph), 7.15-7.25(5H, m, Ph); δC 23.3(t, 3-C), 27.8(q, CH_3), 59.5(d), 62.6(d), 65.7(t, CH_2OH), 67.1(t, CH_2Ph), 81.9(s, CCH_3), 127.9, 128.1, 128.4(3 x d, Ph), 135.9(s, Ph), 156.6(s, CO), 170.0(s, CO); m/e (NH_3, D.C.I.) 339(15, $M+NH_4^+$), 322(100, M^++1), 266(30), 220(42), 91(28); [Found C, 63.62; H, 7.01; N, 4.24. $C_{17}H_{23}NO_5$ requires C, 63.54; H, 7.21; N, 4.36%]. For cis-14: Yield [9.20 g, 88% from cis-13 (6.10 g, 32.6 mmol)]; as a colourless oil [purified by chromatography (flash silica, ether:hexane (0:1-1:0)]; t.l.c. (ether) Rf 0.6; ν_{max} (film) 3450 b, 1740 s, 1700 s, 1415 m, 1350 m and 1160 s cm^{-1}; δH 1.42(9H, s, t-Bu), 2.03-2.39(1H, bm, 3-H), 2.54(1H, ca dt, J 11.5, 9Hz, 3-H), 3.73(2H, bm, CH_2OH), 3.88(1H, bd, OH), 4.45-4.47(1H, bm, 4-H), 4.49(1H, dd, J 9.5, 6Hz, 2-H), 5.02, 5.11(2H, ABq, J 12.5Hz, CH_2Ph), 7.25-7.43(5H, m, Ph); δC 22.5(t, 3-C), 27.5(q, CH_3), 58.0(d), 61.3(d), 63.7(t, CH_2OH), 66.6(t, CH_2Ph), 81.7(s, CCH_3), 127.4, 127.7, 128.1(3 x d, Ph), 135.8(s, Ph), 155.6(s, CO), 170.1(s, CO); m/e (NH_3, D.C.I.) 339(10, $M+NH_4^+$), 322(100, M^++1), 266(35), 220(25), 91(20); [Found C, 63.41; H, 6.99; N, 4.52. $C_{17}H_{23}NO_5$ requires C, 63.54; H, 7.21; N, 4.36%].

N-(Benzyloxycarbonyl)-2-(carboxylic acid t-butyl ester)-4-(formyl)azetidine (15).

Alcohol (cis-14) (3.90 g, 12.1 mmol) and dicyclohexylcarbodiimide (7.57 g, 36.6 mmol) were dissolved in benzene (100 ml) containing dimethyl sulphoxide (21 ml) and pyridine (438 mg, 6.10 mmol). The solution was cooled to 0°C and trifluoroacetic acid (695 mg, 6.10 mmol) was added dropwise. After stirring for 30 minutes at 0°C and for 3 hours at 20°C, water (50 ml) was added and the reaction mixture stirred for a further 1 hour at 20°C. The solution was filtered (celite), washed with ether, the organic layer separated, dried and kept at -30°C for 15 hours. After filtration (celite) and evaporation the residue was chromatographed [flash silica (ether)] to give cis-15 (2.92 g, 75%). The ^1H NMR spectra of both cis-15 and trans-15 were broad, complex and indicated less than 50% of the free aldehyde was present. These aldehydes polymerised on standing, however, even after prolonged storage they reacted as the free aldehydes. For cis-15: δH (only major resonances are reported) 1.38, 1.45(9H, 2 x s, t-Bu), 2.00-2.59(2H, m, 3-H), 4.42(ca 1H, dd, J 9, 4Hz, 2-H), 4.33-4.71(ca 1H, m, 4-H), 5.01, 5.03(2H, ABq, J 12.5Hz, CH_2Ph), 7.28-7.34(5H, m, Ph), 9.8(ca 0.3H, s, CHO). For trans-15: Yield [1.93 g, 78% from trans-14 (2.02 g, 7.73 mmol)]; δH 1.33(9H, s, t-Bu), 2.11-2.68 (2H, m, 3-H), 4.40-4.51(2H, m, 2-H, 4-H), 5.02-5.12(2H, m, CH_2Ph), 7.19-7.23(5H, m, Ph), 9.85(ca 0.5H, s, CHO).

N-(Benzyloxycarbonyl)-2-(carboxylic acid t-butyl ester)-4-(3-propenoic acid p-nitrobenzyl ester) azetidine (16).

To a solution of trans-15 (7.10 g, 22.3 mmol) in dichloromethane (40 ml) was added a solution of triphenylphosphoranylidene(acetic acid)p-nitrobenzyl ester[18] (10.2 g, 22.3 mmol) in dichloromethane (20 ml). The solution was left to stand overnight, then was evaporated to dryness and the residue chromatographed [flash silica, ether/hexane (0:1-1:0)] to give trans-16 (10.3 g, 93%) as a colourless oil (E:Z, > 10:1). For E-trans-16: t.l.c. (ether) Rf 0.45; ν_{max} (film) 1739 s, 1723 s, 1716 s, 1523 s, 1350 s, 1158 s, 853 s, 748 m and 700 w cm^{-1}; δH (showed rotational isomers in a ca 1:1 ratio) 1.37, 1.45(9H, 2 x s, t-Bu), 2.35(2H, b, ca t, 3-H), 4.45(1H, b, ca t, 2-H), 5.01-5.23(1H, m, 4-H), 5.05-5.07(2H, m, CH_2Ar), 5.23-5.25(2H, m, CH_2Ar), 6.04, 6.12(1H, 2 x d, J 16, 15.5Hz, C=CHCO), 7.09, 7.10(1H, 2 x dd, J 16, 5.5Hz and J 15.5, 5.5Hz, CH=CO), 7.18-7.37(5H, m, Ph), 7.50(2H, d, J 8.5Hz, $ArNO_2$), 8.17(2H, d, J 8.5Hz, $ArNO_2$); δC 27.9(q, CH_3), 27.9(t, 3-C), 58.9(d) and 59.6(d) (2-C and 4-C), 64.8(t) and 67.1(t) (2 x CH_2Ar), 82.2(s, CCH_3), 121.2(d, C=CCO), 123.7(d, $ArNO_2$), 127.9, 128.0, 128.1(3 x d, Ph), 128.1(d, $ArNO_2$), 136.5(s, Ph), 143.0(s, $ArNO_2$), 146.9(d, C=CCO), 148.1(s, $ArNO_2$), 153.0(s, CO), 165.4(s, CO), 170.0(s, CO); m/e (NH_3, D.C.I.) 514(2, $M+NH_4^+$), 497(5, M^++1), 458(22), 441(10), 400(30), 323(50), 306(60), 122(80), 91(100); [Found C, 63.04; H, 5.62; N, 5.81. $C_{26}H_{28}N_2O_8$ requires C, 62.90; H, 5.68; N, 5.64]. For cis-16: Yield [1.39 g, 93% from cis-15 (970 mg, 3.04 mmol)]. A ca 1:1 mixture of E:Z isomers was obtained. A small sample was separated for analytical purposes. For Z-cis-16: m.p. 72-74°C (from ether/hexane); ν_{max} (KBr disc) 1739 s, 1723 s, 1703 s, 1520 s, 1420 m, 1347 s, 1160 s, 842 m, 812 m, 768 m, 741 m and 701 m cm^{-1}; δH 1.43(9H, s, t-Bu), 1.94(1H, ca dt, J 12, 5.5Hz, 3-H), 2.98(1H, ca dt, J 12, 5.5Hz, 3-H), 4.30(1H, dd, J 9, 5.5Hz, 2-H), 5.07, 5.11(2H, ABq, J 12.5Hz, CH_2Ar), 5.21(2H, s, CH_2Ar), 5.53-5.63(1H, m, 4-H), 5.91(1H, dd, J 11.5, 1.5Hz, CH=CHCO), 6.72(1H, dd, J 11.5, 6.5Hz, CH=CHCO), 7.27-7.35(5H, s, Ph), 7.49(2H, d, J 8.5Hz, $ArNO_2$), 8.21(2H, d, J 8.5Hz, $ArNO_2$); m/e (NH_3, D.C.I.) 514(23, $M+NH_4^+$), 497(3, M^++1), 458(100), 441(27), 323(31), 306(15), 122(20), 108(35), 91(38); [Found C, 63.19; H, 5.48; N, 5.45. $C_{26}H_{28}N_2O_8$ requires C, 62.90; H, 5.68; N, 5.64%]. For E-cis-16, m.p. 95.5-97.5°C (from ether/hexane); ν_{max} (KBr disc) 1736 s, 1723 s, 1714 s, 1501 s, 1414 s, 1314 s, 1159 s, 852 m, 731 m and 695 m cm^{-1}; δH 1.44(9H, s, t-Bu), 2.05(1H, ca dt, J 11.5, 6Hz, 3-H), 4.56(1H, dd, J 9, 6Hz, 2-H), 4.80-4.88(1H, m, 4-H), 5.10,5.14(2H, ABq, J 12.5Hz, CH_2Ar), 5.28(2H, s, CH_2Ar), 6.25(1H, dd, J 15.5, 1.5Hz, CH=CHCO), 7.14(1H, dd, J 15.5, 6Hz, CH=CHCO), 7.31(5H, s, Ph), 7.53(2H, d, J 9Hz, $ArNO_2$), 8.22(2H, d, J 9Hz, $ArNO_2$); m/e (NH_3, D.C.I.) 497(5, M^++1), 458(22), 441(18), 323(18), 306(15), 122(100), 108(58), 91(53); [Found C, 62.87; H, 5.58; N, 5.59. $C_{26}H_{28}N_2O_8$ requires C, 62.90; H, 5.68; N, 5.64%].

N-(Benzyloxycarbonyl)-2-(carboxylic acid t-butyl ester)-4-(3-propanoic acid p-nitrobenzyl ester) azetidine (17).

A solution of cis-16 (12.2 g, 24.6 mmol) in benzene (120 ml) containing tris-triphenylphosphine rhodium chloride (1.2 g, 1.3 mmol) was hydrogenated until the required amount of hydrogen was consumed (3-16 hours). The solvent was removed in vacuo and the residue dissolved in ether (50 ml) and allowed to stand for 2 hours. The solution was then filtered (celite) and evaporated to dryness. The residue was chromatographed [flash silica, ether/hexane (0:1-1:0)] to give cis-17 (11.8 g, 96%). m.p. 80-81°C (from ether); t.l.c. (ether/hexane, 1:1) Rf 0.3; ν_{max} ($CHCl_3$) 1748 s, 1739 s, 1705 s, 1600 s, 1515 s, 1345 s, 1177 s, 835 m, 765 m and 732 m cm^{-1}; δH 1.44(9H, s, t-Bu), 1.85-1.93(1H, m, CH_2), 2.06-2.29(1H, m, CH_2), 2.58-2.67(3H, m, CH_2), 4.27-4.34(1H, m, 4-H), 4.49(1H, dd, J 9.5, 6Hz, 2-H), 5.07, 5.13(2H, ABq, J 12.5Hz, CH_2Ar), 5.19(2H, s, CH_2Ar), 7.28-7.33(5H, m, Ph), 7.50(2H, d, J 8.5Hz, $ArNO_2$), 8.21(2H, d, J 8.5Hz, $ArNO_2$); δC 26.7(t, CH_2CH_2CO), 27.9(q, CH_3), 29.9(t, 3-C), 30.6(t, CH_2CO_2), 58.5(d), 59.2(d) (2-C, 4-C), 64.7(t, CH_2Ar), 66.8(t, CH_2Ar), 123.7(d, $ArNO_2$), 127.7, 128.0, 128.2, 128.4(4 x d, Ar), 136.6(s, Ph), 143.3(s, $ArNO_2$), 147.6(s, $ArNO_2$), 156.3(s, CO), 170.2(s, CO), 172.6(s, CO); m/e (NH_3, D.C.I.) 516(40, $M+NH_4^+$), 499(10, M^++1), 460(60), 443(100), 353(35), 303(36), 91(90). [Found C, 62.91; H, 5.94; N, 5.61. $C_{26}H_{30}N_2O_8$ requires C, 62.64; H, 6.07; N, 5.62%]. For trans-17: Yield [5.01 g, 92% from trans-16 (5.41 g, 10.9 mmol)]; m.p. 46-51°C (from ether); ν_{max} (film) 1740 s, 1715 s, 1522 m, 1345 s, 1155 bs, 849 m and 739 m cm^{-1}; δH (showed rotational isomers in a ca 1:1 ratio) 1.37, 1.45(9H, 2 x s, t-Bu), 1.99-2.13(1H, m, CH_2), 2.14-2.34(3H, m, CH_2), 2.43(1H, ca t, CH_2), 2.58(1H, ca t, CH_2), 4.42(1H, dd, J8.5, 5.5Hz, 2-H), 4.45-4.53(1H, m, 4-H), 5.06(2H, s, CH_2Ar), 5.10-5.28(2H, m, CH_2Ar), 7.23-7.40(5H, m, Ph), 7.50(2H, d, J 8.5Hz, $ArNO_2$), 8.20(2H, d, J 8.5Hz, $ArNO_2$); m/e (NH_3, D.C.I.) 516(1, $M+NH_4^+$), 499(5, M^++1), 460(1), 230(40), 122(60), 91(100). [Found C, 62.85; H, 5.98; N, 5.28. $C_{26}H_{30}N_2O_8$ requires C, 62.64; H, 6.07; N, 5.62%].

2-(Carboxylic acid t-butyl ester)-4-(3-propanoic acid)azetidine (18).

A solution of trans-17 (1.40 g, 2.81 mmol) in methanol (20 ml) was hydrogenated over 10% palladium on charcoal (200 mg) at 20°C until the required amount of hydrogen (ca 340 ml) was absorbed (2-4 hours). The catalyst was removed (filtration, celite) and the filtrate evaporated to dryness. The residue was triturated with ether until a precipitate formed. The solid was then recrystallised (methanol/ether) to give trans-18 (560 mg, 87%); m.p. 111-113°C (from methanol/-ether); ν_{max} (KBr disc) 3425 b(OH), 2655 b, 2470 b, 1743 s, 1740 s, 1602 m, 1554 s, 1259 s, 1165 s and 1018 m cm^{-1}; δH 1.46(9H, s, t-Bu), 1.91-2.60(6H, m, CH_2), 4.20-4.23(1H, m, 4-H), 4.31(1H, dd, J 9.5, 6Hz, 2-H); m/e (NH_3, D.C.I.) 230(1, M^++1), 174(3), 128(100), 85(55). [Found C, 57.21; H, 8.45; N, 6.31. $C_{11}H_{19}NO_4$ requires C, 57.63; H, 8.65; N, 6.11%]. For cis-18: Yield [2.7 g, 97% from cis-17 (5.73 g, 11.5 mmol)]; m.p. 134-136°C (from methanol/ether); ν_{max} (KBr, disc) 1747 s, 1739 s, 1704 s, 1515 m, 1416 s, 1408 s, 1345 s, 1256 s, 1155 s, 832 m, 765 m, 731 s and 697 m cm^{-1}; δH 1.46(9H, s, t-Bu), 1.75-1.88(1H, m, CH_2), 2.07-2.29(3H, m, CH_2), 2.39-2.42(1H, m, CH_2), 2.80-2.85(1H, m, CH_2), 4.23-4.27(1H, m, 4-H), 4.63(1H, dd, J 9.5, 8Hz, 2-H); m/e (NH_3, D.C.I.) 230(100, M^++1), 174(10), 128(10). [Found C, 57.30; H, 8.54; N, 5.91. $C_{11}H_{19}NO_4$ requires C, 57.63; H, 8.65; N, 6.11%].

t-Butyl-2-oxo-1-azabicyclo[3.2.0]heptane-7-exo-carboxylate (exo)-19) and t-Butyl-2-oxo-1-azabicyclo[3.2.0]heptane-7-endo-carboxylate (endo-19).

To a solution of trans-18 (689 mg, 3.01 mmol) in acetonitrile (40 ml) were added 2,2'-dipyridyldisulphide (729 mg, 3.31 mmol) and triphenylphosphine (868 mg, 3.31 mmol). The solution was stirred for 3h at 20°c and then evaporated to dryness. The residue was dissolved in ether (20ml), filtered, evaporated to dryness and chromatographed (flash silica, ether) to give exo-19 (575 mg, 91%); m.p. 73.5-74.5°C (prisms from ether); t.l.c. (ether) Rf 0.6; ν_{max} (KBr disc) 1740 s, 1694 s, 1362 s, 1277 m, 1239 s, 1168 s, 1104 s, 976 m and 853 m cm^{-1}; δH 1.48(9H, s, t-Bu), 1.98-2.14(1H, m, 4-H), 2.26(1H, ddd, J 16, 9, 1Hz, 3-H), 2.39-2.45(1H, m, 4-H), 2.53-2.74(3H, m, 3-H, 2 x 6-H), 4.48(1H, dd, J 9.5, 4.5Hz, 7-H), 4.51-4.55(1H, m, 5-H). A 2D-COSY spectrum was consistent with the connectivity as specified; δC 27.9(q, CH_3), 30.9(t), 32.1(t) (4-C, 6-C), 35.5(t, 3-C), 59.9(d), 61.5(d) (5-C, 7-C), 82.0(s, CCH_3), 170.3(s, CO), 183.8(s, CO); m/e (E.I.) 211(2, M^+), 155(15), 110(100); 57(80); [Found C, 62.77; H, 7.80; N, 6.73. $C_{11}H_{17}NO_3$ requires C, 62.54; H, 8.11; N, 6.63%]. For endo-19: Yield [254 mg, 75% from cis-18 (368 mg, 1.61 mmol)]; m.p. 75.0-75.5°C (prisms from ether). t.l.c. (ether) Rf 0.6; ν_{max} (KBr disc) 1736 s, 1704 s, 1372 s, 1279 s, 1238 s, 1158 s, 1120 m and 848 m cm^{-1}; δH 1.49(9H, s, t-Bu), 2.22-2.55(5H, m, CH_2), 2.75(1H, ca dt J 11.5, 7.5Hz, 6-H), 4.26-4.32(1H, m, J 7.5Hz, 5-H), 4.60(1H, ca t, J 7Hz, 7-H); A 2D-COSY spectrum was consistent with the connectivity as specified; δC 27.7(q, CH_3), 31.5(t), 31.7(t) (4-C, 6-C), 32.6(t, 3-C), 58.7(d), 62.6(d) (5-C, 7-C), 82.2(s, CCH_3), 177.7(s, CO), 182.9(s, CO); m/e (E.I.) 211(2, M^+), 155(18), 110(100), 57(95); [Found C, 62.36; H, 7.91; N, 6.81. $C_{11}H_{17}NO_3$ requires C, 62.54; H, 8.11; N, 6.63%].

t-Butyl-3-endo-amino-2-oxo-1-azabicyclo[3.2.0]heptane-7-exo-carboxylate (21).

To a solution of exo-19 (79 mg, 0.37 mmol) in tetrahydrofuran (1.5 ml) was added a solution of lithium bis(trimethylsilyl)amide in tetrahydrofuran(1.0 molar, 0.41 mmol). After stirring for 1 hour at -70°C, O-(diphenylphosphinoyl)hydroxylamine (96 mg, 0.41 mmol) was added. The suspension was stirred at -70°C for a further 3 hours, then allowed to warm to 20°C overnight, after which it was quenched with 1N hydrochloric acid (5 ml). The tetrahydrofuran was removed in vacuo and the aqueous solution extracted with ether (10 ml), basified to pH 10 with 5N potassium hydroxide and extracted with ethyl acetate (3 x 15 ml). The organic solution was dried and evaporated to dryness to give 21 (40 mg, 47%) (>90% pure). The ethereal washings were dried, evaporated to dryness and the residue chromatographed (P.L.C., ether) to give (25 mg, 30%) recovered starting material (exo-19). For 21: ν_{max} ($CHCl_3$) 3450 m, 3390 m, 1740 bs and 1520 cm^{-1}; δH 1.49(9H, s, t-Bu), 1.69-1.88(1H, m, 4-H), 2.02-2.54(2H, b, NH_2), 2.56-2.73(2H, m, 6-H), 2.78-2.87(1H, m, 4-H), 3.81(1H, dd, J 12.5, 8Hz, 3-H), 4.44-4.50(1H, m, 5-H), 4.59(1H, dd, J 9.5, 4.5Hz, 7-H); m/e (NH_3, D.C.I.), 453($2M^+$+1), 227(80, M^++1), 171(100), 156(60).

t-Butyl-3-endo-phenoxyacetamido-2-oxo-1-azabicyclo[3.2.0]heptane-7-exo-carboxylate (22).

To a solution of 21 (75 mg, 0.33 mmol) in dichloromethane (5 ml) was added phenoxyacetic acid (51 mg, 0.33 mmol), 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (99 mg, 0.40 mmol) and sodium sulphate (ca 5 mg). After stirring for 24 hours, work up as for 11, followed by chromatography (P.L.C., ether) gave 22 (95.4 mg, 85%); m.p. 104-105°C (prisms from ether/hexane); t.l.c. (ether) Rf 0.4; ν_{max} (KBr disc) 1747 s, 1705 s, 1700 (shoulder), 1565 s, 1525 s, 1288 s, 1233 s, 1157 m, 1108 m and 751 cm^{-1}; δH 1.51(9H, s, t-Bu), 1.94(1H, ca dt, J 12, 8Hz, 4-H), 2.60-2.79(2H, m, 6-H), 3.13-3.18(1H, m, 4-H), 4.51(2H, s, CH_2O), 4.54-4.63(1H, obscured by CH_2O, 3-H), 4.62(1H, dd, J 9.5, 4.5Hz, 7-H), 4.59-4.66(1H, m, 5-H), 6.94-7.10(3H, m, Ar), 7.24(1H, bd, J 5.5Hz, NH), 7.26-7.36(2H, m, Ar); A 2D-COSY experiment was consistent with the connectivity as specified; m/e (NH_3, D.C.I.) 361(82, M^++1), 305(100), 304(41), 259(12), 152(9), 80(28), 57(24). [Found C, 63.45; H, 6.51; N, 7.43. $C_{19}H_{24}N_2O_5$ requires C, 63.32; H, 6.71; N, 7.77%].

1-Azabicyclo[3.2.0]heptane-7-exo-carboxylate (exo-23) and 1-Azabicyclo[3.2.0]heptane-7-endo-carboxylate (endo-23).

The ester (exo-19) (20 mg, 9.5 x 10^{-2} mmol) was dissolved in freshly distilled trifluoroacetic acid (0.25 ml) and allowed to stand at room temperature for 15 minutes. The excess acid was removed in vacuo to give a gum which was titurated with ether until precipitation occured to give exo-23 (12.5 mg, 83%). m.p. 134.0-134.5°C (from dichloromethane/ether); ν_{max} (KBr disc) 3100-2800 b, 2715 m, 2590 m, 1745 s, 1665 s, 1365 s, 1280 m, 1200 s and 1105 m cm^{-1}; δH 2.03-2.21(1H, m, 4-H), 2.33-2.36(1H, m, 4-H), 2.44-2.53(1H, m, CH_2), 2.63-2.84(3H, m, CH_2), 4.54-4.63(1H, m, 5-H), 4.70(1H, dd, J 10, 4.5Hz, 7-H), 9.24(1H, s, CO_2H); m/e (E.I.) 155(10, M^+), 110(6), 84(22), 82(20). [Found 155.0583. $C_7H_9NO_2$ requires 155.0582]. For endo-23; Yield [8.8 mg, 80% from endo-19 (15 mg, 7.1 x 10^{-2} mmol]; m.p. 107-110°C (from dichloromethane/ether); ν_{max} (KBr disc) 3100-2820 b, 2700 m, 2580 m, 1738 s; δH 2.26-2.61(5H, m, CH_2), 2.82(1H, ca dt, J 11.5, 7.5Hz, 6-H), 4.33-4.39(1H, m, 5-H), 4.81(1H, ca t, J 7.5Hz, 7-H), 11.53(1H, s, CO_2H); m/e (E.I.) 155(10, M^+), 127(6), 111(17), 110(100). [Found 155.0583. $C_7H_9NO_2$ requires 155.0582].

3-Endo-phenoxyacetamido-2-oxo-1-azabicyclo[3.2.0]heptane-7-exo-carboxylate (26).

The ester (22) (11 mg, 3.1 x 10^{-2} mmol) was dissolved in freshly distilled trifluoroacetic acid (0.1 ml) and allowed to stand at room temperature for 15 minutes. The acid was removed in vacuo and the residue titurated with dichloromethane until precipitation occured. The solvent was decanted to give the free acid which was dissolved in deuterated pH 7.6, 50 mmolar, K_2HPO_4-KH_2PO_4-KCl buffer to give 26; δH 2.08-2.22(1H, m, 4-H), 2.54-2.83(3H, m, 4-H, 6-H), 4.37-4.49(1H, m, 5-H), 4.56(2H, s, CH_2O), 4.59-4.70(1H, m, 3-H), 4.75(1H, dd, J 14, 8Hz, 7-H), 6.38-7.28(5H, m, Ar); m/e (NH_3, C.I.) 305(10, M^++1). 26 was stable in the buffered solution for greater than 18 hours (by ^1H NMR, 300MHz). After which the solution was evaporated to dryness and the residue was resuspended in acetone (1ml). Methyl iodide (0.1ml), triethylamine (10µl, 3.6 x 10^{-2}mmol) and sodium sulphate (ca 5mg) were added. The reaction mixture was stirred for 6 hours at 20°C, when work up, as for the preparation of 11, followed by chromatography (P.L.C., ether) gave 29 as a colourless oil (2.3mg, 23%, unoptimised): t.l.c. (ether) Rf 0.4; δH 1.88-1.98(1H, m, 4-H), 2.60-2.75(2H, m, 6-H), 3.10-3.17(1H, m, 4-H), 3.84(3H, s, CH_3), 4.53(2H, s, CH_2O), 4.52-4.58(1H, m, 3-H), 4.62(1H, dd, J 9.5, 5Hz, 7-H), 4.60-4.65(1H, m, 5-H), 6.94-7.10(3H, m, Ar), 7.30(1H, bd, J 5.5Hz, NH), 7.26-7.33(2H, m, Ar); m/e (NH_3, D.C.I.) 319(74, M^++1), 259(s, $M^+-CO_2CH_3$).

Methyl-2-oxo-1-azabicyclo[3.2.0]heptane-7-exo-carboxylate (exo-27) and Methyl-2-oxo-1-azabicyclo[3.2.0]heptane-7-endo-carboxylate (endo-27).

Endo -19 (15mg, 7.1 x 10^{-2} mmol) was dissolved in freshly distilled trifluoroacetic acid (0.2 ml) and allowed to stand at room temperature for 15 minutes. The acid was removed in vacuo and the residue dissolved in pH 7.6, 50 mmolar, phosphate buffer (K_2HPO_4-KH_2PO_4-KCl). After 12 hours the solution was evaporated to dryness. The residue was re-suspended in acetone (1 ml) and methyl iodide (0.1 ml), triethylamine (19.8μl, 7.2 x 10^{-2} mmol) and sodium sulphate (ca 5 mg) were added. The reaction mixture was stirred for 2 hours at 20°C, after which work up, as for the preparation of 11, followed by chromatography (P.L.C., ether) gave endo-27 as a colourless oil (8.8 mg, 73%); t.l.c. (ether) Rf 0.35; ν_{max} ($CHCl_3$) 1743 s, 1705 s, 1475 s and 1135 m cm^{-1}; δH 2.23-2.58(5H, m, CH_2), 2.80(1H, ca dt, J 11.5, 7.5Hz, 6-H), 3.80(3H, s, CH_3), 4.31-4.37(1H, m, 5-H), 4.76(1H, bt, J 7Hz, 7-H); m/e (I.B.E.I.) 170(4, M^++1), 169(12, M^+), 110(18), 82(100); [Found 169.0738. $C_8H_{11}NO_3$ requires 169.0739]. For exo-27; Yield [2 mg, 30%) from exo-19 (8 mg, 3.8 x 10^{-2} mmol)]; ν_{max} ($CHCl_3$) 1745 s, 1702 s, 1470 s and 1032 s cm^{-1}; δH 1.99-2.60(6H, m, CH_2), 3.79(3H, s, CH_3), 4.57(1H, dd, J 9.5, 4.5Hz, 7-H), 4.60-4.65(1H, m, 5-H); m/e (NH_3, C.I.) 170(100, M^++1), 110(22). [Found 169.0738. $C_8H_{11}NO_3$ requires 169.0739].

Biological testing

The carboxylate salts exo-25, endo-25 and 26 were tested for antibacterial activity, using ampicillin as a standard of known activity, against a panel of 33 cultures in minimal nutrient agar. All were found to be biologically inactive up to a concentration of 0.1mg/1ml in pH 7.6, 50 mmolar phosphate buffer (KH_2PO_4-K_2HPO_4-KCl). The panel consisted of : Gram positive bacteria [Staphylococcus aureus, strains (222, 223, 267, 268^{-1}, 268^{-2}, 450, 451, 43, Sarcina lutea, Bacillus subtilus, Bacillus cereus, Streptomyces pyogenes, Commonas ter.,]; Gram negative bacteria [Escherichia coli, strains (116, 146, 172, 198, 603^{-1}, 603^{-2}, 604, 605, 606, 607, 608), Enterobacter (2.7.11), Klebsiella aerogenas, strains (37, 54, 175), Pseudomonas aeruginosa, strains (56, 433, 434)] and a fungus (Candida).

Crystal data for (endo-19) $C_{11}H_{17}NO_3$, M= 211.16, orthorhombic, space group Pbca, a= 10.775(3), b= 11.164(2), c= 18.807(3) Å, U= 2262.3 $Å^3$, Z= 8, D_C= 1.24 g cm^{-3}. 1985 Independent reflections were measured by four circle (CAD-4) diffractrometry using Mo-Kα radiation (λ= 0.71069 Å). The structure was solved by direct methods.[19] 1387 Reflections [$I \geq 3\sigma(I)$] uncorrected for absorption[19] [μ (Mo-Kα)= 0.98 cm^{-1}] were used in a full matrix least squares[20] refinement for all atomic parameters including one overall temperature factor for the hydrogen atoms. Restrainsts[21] were applied to the C-H bonds, and to the methyl H-C-H bond angles. The final R value was 0.072 (R_w= 0.074).

Crystal data for 22: $C_{18}H_{24}N_2O_5$, M= 360.41, monoclinic, space group $P2_1/c$, a= 15.487(2), b= 11.093(2), c= 11.931(1) Å, β= 112.42(1); U= 1894.7 $Å^3$, Z= 4, D_C= 1.26 g cm^{-3}. 4669 Independent reflections were measured with an Enraf-Nonius CAD-4 diffractometer using Cu-Kα radiation (λ= 1.5418 Å). The structure was solved by direct methods.[19] 3296 Reflections [$I \geq 3\sigma(I)$] uncorrected for absorption [μ (Cu-Kα)= 7.68 cm^{-1}] were used in a blocked least squares[20] refinement. All atomic parameters were included in the refinement, including positional parameters for the hydrogen atoms and one overall temperature factor for the hydrogen atoms. The final R value was 0.057 (R_w= 0.081).

Crystal data for cis-12: $C_{16}H_{21}NO_3$, M= 277.35, space group $P2_1/n$, a= 11.839(1), b= 9.638(5), c= 14.638(5) Å, U= 1593.0 $Å^3$, Z= 4, D_C= 1.16 g cm^{-3}. 3,791 Reflections were measured with an Enraf-Nonius CAD-4 diffractometer using Mo-Kα radiation (λ= 0.71069 Å). The structure was solved by direct methods.[19] 2046 Reflections [$I \geq 3\sigma$ (I)] uncorrected for absorption [μ (Mo-Kα)= 0.98 cm^{-1}] were used in a full matrix least squares refinement.[20] The final R value was 0.045 (R_w= 0.053).

Crystal data for (exo)-19) $C_{11}H_{17}NO_3$, M= 211.16, space group $P2_1/a$= 10.058(2), b= 10.052(1), c= 11.604(1) Å, U= 1131.6 $Å^3$, Z= 4, D_C= 1.24 g cm^{-3}. 2326 Reflections were measured with an Enraf-Nonius CAD-4 diffractometer using Cu-Kα radiation (λ= 1.5418Å). The structure was solved by direct methods.[19] 1900 Reflections [$I \geq 3\sigma$ (I)] uncorrected for absorption were used in a blocked least squares refinement.[20] The final R value was 0.055 (R_w= 0.073).

The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW.

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