Nuclear Modification of Clavulanic Acid. The Preparation of Two 4,7-Fused β-Lactam Systems

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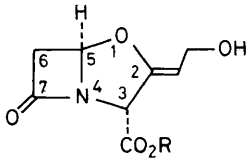
The 4,7-fused β-lactam compounds benzyl 3-methoxy-9-oxo-6-oxa-1-azabicyclo[5.2.0]non-2-ene-2-carboxylate and benzyl 3-methoxy-9-oxo-6-oxa-1-azabicyclo[5.2.0]nona-2,4-diene-2-carboxylate have been prepared by routes which utilise the total carbon–oxygen skeleton of benzyl clavulanate (2). The corresponding 4-nitrobenzyl esters were similarly prepared from 4-nitrobenzyl clavulanate and these were converted into the lithium carboxylates *via* hydrogenolysis. In an unsuccessful attempt to prepare one of these 4,7-fused ring systems, compound (2) was found to react with triethylamine to give two 14-membered ring compounds, dibenzyl (7*RS*, 16*RS*)-3,12-dihydroxy-9,18-dioxo-6,15-dioxa-1,10-diazatricyclo[14.2.0.0^{7,10}]octadeca-2,11-diene-2,11-dicarboxylate and its (7*RS*, 16*SR*)-isomer.

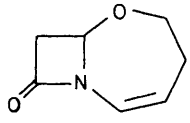
Following the discovery of the β-lactamase inhibitor clavulanic acid (1) [1] much effort has been expended in carrying out structural modifications of this important natural product.[2] Although most of this work has been concerned with modifications which leave the bicyclic nucleus of compound (1) intact, two reports have described modifications leading to nuclear analogues of β-lactam antibiotics.[2e,g] We now describe some chemical manipulations of the carbon–oxygen skeleton of compound (1) which result in the construction of the 4,7-fused ring systems (3) and (4).† These bicyclic systems were of interest to us as ring-expanded analogues of the 1-dethia-1-oxacephem ring system.[4]

In principle, the conversion of compound (1) into a 4,7-fused ring system requires the breaking of the C-5 to O-1 bond followed by the making of a new bond between C-5 and the oxygen on C-9. A useful method for breaking the C-5 to O-1 bond in derivatives of (1) involves their reaction with triethylamine, either alone or in the presence of another nucleophile.[2d,5] Thus, by analogy with the reaction of 4-nitrobenzyl 9-deoxyclavulanate with triethylamine,[2d] we might expect that treatment of benzyl clavulanate (2) with 1 equivalent or more of triethylamine would give an equilibrium mixture of the clavem ‡ (5) and the betaine (6). It was of interest to see if this betaine (6) would cyclise to the required ring system (7).

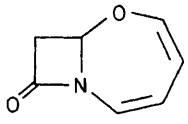
In practice, when the ester (2) was allowed to react with 1 equivalent of triethylamine in dichloromethane during 24 h, only non-mobile (on t.l.c.) products were obtained following an acidic work-up. When the amount of triethylamine was reduced to a catalytic amount (0.06 equiv.), however, the reaction yielded two new β-lactam compounds which were assigned structures (8) (13% yield) and (10) (11%) on the basis of their spectroscopic properties.§ These isomeric compounds were distinguished by their n.m.r. spectra. Thus, in the n.m.r. spectrum of compound (8) pairs of protons such as 7-H and 16-H, 5-H and 14-H, *etc.* are chemically equivalent, whereas in the spectrum of its isomer (10) these pairs (*e.g.* 7-H and 16-H) have different chemical shifts. These compounds were further characterised by the formation of the methyl ethers (9) and (11) on reaction with diazomethane. The possibility that compounds (8) and (10) had also been formed (and then destroyed) when ester (2) reacted with 1 equivalent of triethylamine was discounted, when it was found that compounds (8) and (10) were unaffected by an equivalent of triethylamine in dichloromethane during 24 h.



(1) R = H
(2) R = CH_2Ph



(3)



(4)

In fact, the presence of triethylamine is not necessary for the formation of compounds (8) and (10). A solution of the clavem (5) was produced by treating the ester (2) with triethylamine, and the triethylamine was then removed by washing with HCl. The clavem (5) was then converted into compounds (8) and (10) simply by allowing this solution to stand at room temperature for one day.

It is not difficult to see how the clavem (5) can react with itself to form the large-ring compounds (8) and (10). It is, however, curious that the same compounds are not formed in the presence of 1 equivalent of triethylamine. In this case, the clavem (5) must react preferentially with triethylamine to form the betaine (6). It appears that this betaine does not react with itself to give compounds (8) and (10) nor does it cyclise to compound (7).

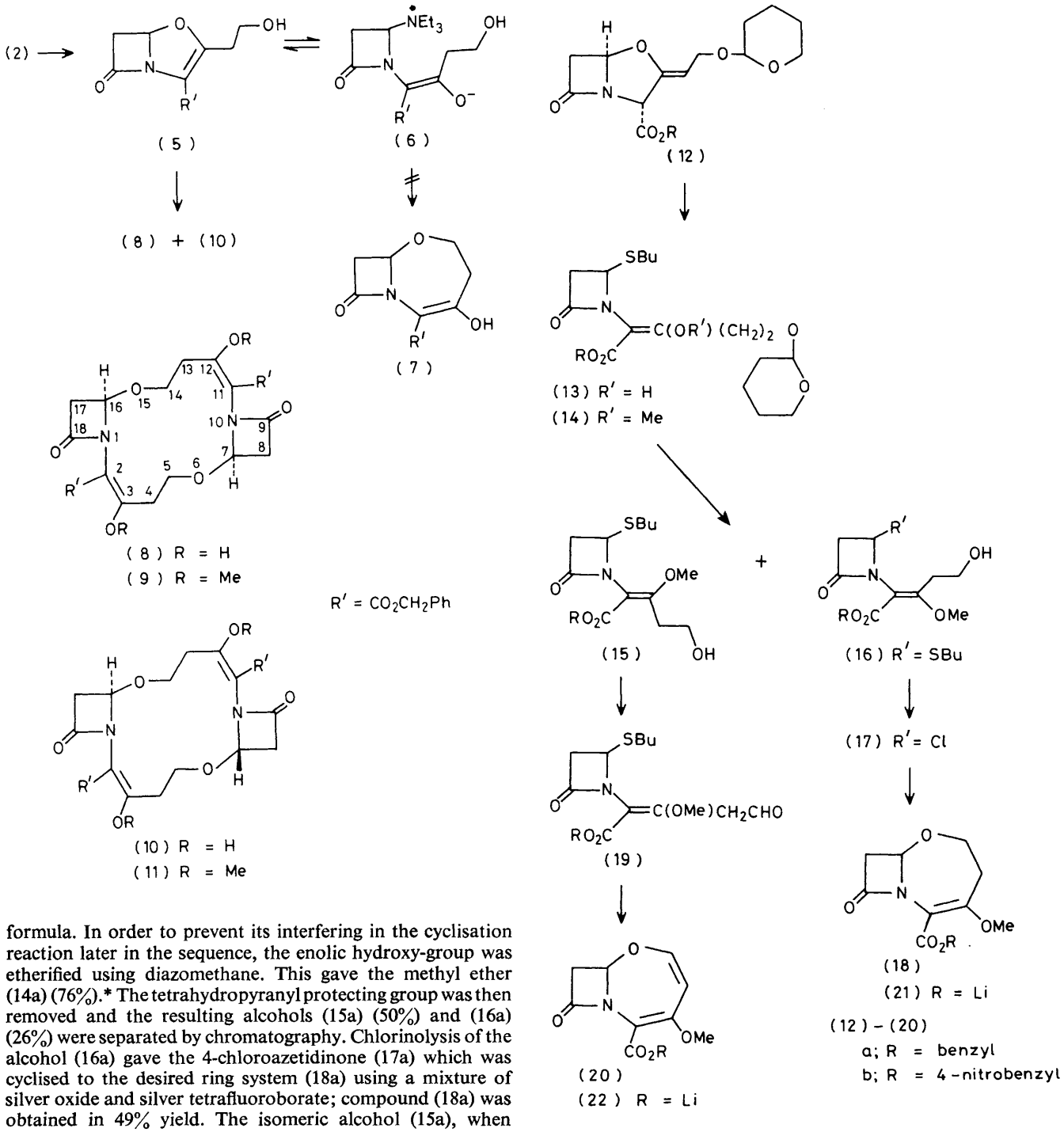
We were therefore forced to consider a less direct approach to the required ring systems. Starting with benzyl clavulanate (2), the C-9 hydroxy-group was protected by formation of the tetrahydropropyranyl ether (12a). The O-1 to C-5 bond was now cleaved, by reaction with triethylamine and n-butanethiol,[2e] to give the monocyclic β-lactam (13a) in 45% yield.¶ The n.m.r. spectrum of this compound showed that in chloroform it is fully enolised, as shown in the structural

† The related 9-oxo-6-oxa-1-azabicyclo[5.2.0]non-4-ene system has previously been reported as arising in by-products obtained during the total synthesis of analogues of compound (1); [3a] a total synthesis of t-butyl 9-oxo-6-oxa-1-azabicyclo[5.2.0]non-2-ene-2-carboxylate, which contains ring system (3), has recently been reported.[3b]

‡ The name clavem (ref. 2*h*) is used for the 4-oxa-1-azabicyclo-[3.2.0]hept-2-en-7-one nucleus.

§ These compounds were optically inactive, and hence presumably racemic; for convenience structural formulae (8)—(11) show only one enantiomer.

¶ Compounds (13), (14), and (19) were obtained as mixtures of geometric isomers.



Scheme

formula. In order to prevent its interfering in the cyclisation reaction later in the sequence, the enolic hydroxy-group was etherified using diazomethane. This gave the methyl ether (14a) (76%).* The tetrahydropyranyl protecting group was then removed and the resulting alcohols (15a) (50%) and (16a) (26%) were separated by chromatography. Chlorinolysis of the alcohol (16a) gave the 4-chloroazetidinone (17a) which was cyclised to the desired ring system (18a) using a mixture of silver oxide and silver tetrafluoroborate; compound (18a) was obtained in 49% yield. The isomeric alcohol (15a), when subjected to this reaction sequence, gave no bicyclic product, thus confirming the assignment of double bond geometries to compounds (15a) and (16a).

Oxidation of the alcohol (15a), or (16a), or a mixture of the two gave the aldehyde (19a) in 83% yield.* This aldehyde, without purification, was chlorinolysed and then treated with triethylamine to give the second 4,7-fused compound (20a) (46%).

Hydrogenolysis of the benzyl ester (20a) over a palladium catalyst, followed by neutralisation of the resulting acid with lithium hydroxide gave a mixture of lithium salts. It was evident from the n.m.r. spectrum of this mixture that partial hydrogenation of the diene system had occurred at the same time as hydrogenolysis of the benzyl ester. The sequence shown in the Scheme was therefore repeated starting with 4-nitrobenzyl clavulanate, but with the difference that the isomeric alcohols (15b) and (16b) were not separated. This gave the two crystalline 4-nitrobenzyl esters (18b) and (20b). Hydrogenolysis of the ester (18b) followed by neutralisation of the resulting acid with lithium hydroxide gave the lithium salt (21), which was isolated as a monohydrate. Similarly, the ester (20b) was converted into the lithium salt (22).

* (19) were obtained as mixtures of

Experimental

M.p.s were determined using a Kofler hot-stage apparatus. Except where stated otherwise, i.r. spectra and specific rotations were recorded for solutions in chloroform, u.v. spectra were recorded for solutions in ethanol, and ^{1}H n.m.r. spectra were recorded at 90 MHz for solutions in $CDCl_3$ with $SiMe_4$ as internal standard. Mass spectra were determined using a V.G. Micromass 70-70F instrument. Merck silica gel 60 was used for t.l.c. and for column chromatography with ethyl acetate–light petroleum (b.p. 60—80 °C) mixtures as eluant. Solutions were dried using magnesium sulphate and solvents were removed by evaporation under reduced pressure using a rotary evaporator with bath temperature below 30 °C.

Reaction of Benzyl Clavulanate (2) *with Triethylamine* (0.06 *Equiv.*).—Benzyl clavulanate (1.45 g) in dry dichloromethane (25 ml) was treated with triethylamine (30 mg) and the solution was kept at room temperature for 20 h. The dichloromethane was removed, the residue was dissolved in ethyl acetate (50 ml), and the resulting solution was washed with dilute HCl and saturated brine. The solution was dried, the solvent was removed, and the residue was chromatographed to give, in order of elution, *dibenzyl* (7RS, 16RS)-3,12-*dihydroxy*-9,18-*dioxo*-6,15-*dioxa*-1,10-*diazatricyclo*-[14.2.0.0^{7,10}]*octadeca*-2,11-*diene*-2,11-*dicarboxylate* (8) and its (7RS, 16SR)-*isomer* (10). Compound (8) was obtained as a colourless gum (190 mg) which crystallised from ethyl acetate–light petroleum as fine, colourless needles, m.p. 167—168 °C; $[\alpha]_D^{20}$ 0° (*c* 0.5); $\lambda_{max.}$ 254 nm (ε 20 500); $\nu_{max.}$ 1 770, 1 660, and 1 620 cm^{-1}; δ 2.41 (2 H, dt, *J* 13 and 6 Hz), 2.78 (2 H, dd, *J* 14 and 1.5 Hz), 3.03 (2 H, dd, *J* 14 and 4 Hz), 3.12 (2 H, dd, *J* 13 and 7 Hz), 3.45—3.95 (4 H, m), 5.02 (2 H, dd, *J* 4 and 1.5 Hz), 5.12 (2 H, d, *J* 12 Hz), 5.31 (2 H, d, *J* 12 Hz), 7.33 (10 H, s), and 12.30 (2 H, s); *m/z* 578 (M^+, 0.4), 536(0.2), 487 (0.8), 470 (0.4), 217 (12), 108(30), 107 (24), and 91 (100) (Found: C, 62.1; H, 5.2; N, 4.8%; M^+, 578.186. $C_{30}H_{30}N_2O_{10}$ requires C, 62.3; H, 5.2; N, 4.8%; *M*, 578.190). Compound (10) was obtained as a colourless gum (160 mg) which crystallised from ethyl acetate–light petroleum as prisms, m.p. 171—173 °C; $[\alpha]_D^{20}$ 0° (*c* 0.7); $\lambda_{max.}$ 252 nm (ε 19 300); $\nu_{max.}$ 1 765, 1 660, and 1 620 cm^{-1}; δ 2.4 (2 H, m), 2.65—3.25 (6 H, complex m), 3.4—3.9 (4 H, complex m), 5.0—5.5 (6 H, complex m), 7.33 (10 H, s), and 12.60 (2 H, s); *m/z* (ammonia chemical ionisation) 579 ([*M* + H]$^+$), 218 (100), 174 (33), 108 (40), and 91 (42) (Found: C, 62.2; H, 5.2; N, 4.9. $C_{30}H_{30}N_2O_{10}$ requires C, 62.3; H, 5.2; N, 4.8%).

Dimerisation of the Clavem (5).—Benzyl clavulanate (2.9 g) in dry dichloromethane (40 ml) ($\nu_{max.}$ 3 590, 3 150, 1 805, 1 750, and 1 695 cm^{-1}) was treated with triethylamine (1.0 g) and the solution was kept at room temperature for 0.5 h. The solution was diluted with dichloromethane (60 ml) and washed with 1M-HCl (10 ml) and water (2 × 50 ml). The solution was dried and concentrated to *ca.* 30 ml to give a solution containing the clavem (5) ($\nu_{max.}$ 3 590, 3 150, 1 810, 1 705, and 1 615 cm^{-1}). The solution was allowed to stand for 23 h. The solvent was removed and the residue was chromatographed to give compound (8) as colourless needles (230 mg) and compound (10) as colourless prisms (210 mg). The products had i.r. and n.m.r. spectra as previously described.

Dibenzyl (7RS, 16RS)-3,12-*Dimethoxy*-9,18-*dioxo*-6,15-*dioxa*-1,10-*diazatricyclo*[14.2.0.0^{7,10}]*octadeca*-2,11-*diene*-2,11-*dicarboxylate* (9).—Compound (8) (180 mg) was dissolved in dichloromethane (10 ml) and the solution was treated with small portions of an ether solution of diazomethane until t.l.c. indicated that reaction was complete (3 h). The solvent was removed and the resulting gum was chromatographed to give the *bis-methyl ether* (9) as a colourless gum (150 mg); $\lambda_{max.}$ 250 nm (ε 21 500); $\nu_{max.}$ 1 765, 1 730, and 1 610 cm^{-1}; δ 2.4—3.2 (8 H, complex m), 3.4—3.8 (4 H, m), 3.75 (6 H, s), 5.0—5.3 (6 H, complex m), and 7.32 (10 H, s); *m/z* 606 (M^+, 0.5), 575 (0.5), 515 (2), 271 (25), and 91 (100) (Found: M^+, 606.216. $C_{32}H_{34}N_2O_{10}$ requires *M*, 606.221).

Dibenzyl (7RS, 16SR)-3,12-*Dimethoxy*-9,18-*dioxo*-6,15-*dioxa*-1,10-*diazatricyclo*[14.2.0.0^{7,10}]*octadeca*-2,11-*diene*-2,11-*dicarboxylate* (11).—Using the process described in the preceding experiment, compound (10) (110 mg) was brought into reaction with diazomethane. The *bis-methyl ether* (11) was obtained as colourless prisms (65 mg), m.p. 211—213 °C (ethyl acetate–light petroleum); $\lambda_{max.}$ 251 nm (ε 22 300); $\nu_{max.}$ 1 760, 1 720, and 1 610 cm^{-1}; δ 2.5—2.9 (6 H, complex m), 3.06 (2 H, dd, *J* 14 and 4 Hz), 3.4—4.1 (10 H, m overlapped by s at δ 3.77), 5.0—5.35 (6 H, m), and 7.33 (10 H, s); *m/z* 606 (M^+, 0.5), 575 (1), 515 (1), 271 (30), and 91 (100) (Found: C, 63.15; H, 5.6; N, 4.6%; M^+, 606.220. $C_{32}H_{34}N_2O_{10}$ requires C, 63.35; H, 5.65; N, 4.6%; *M*, 606.221).

Tetrahydropyranyl Ether (12a) *of Benzyl Clavulanate*.—Benzyl clavulanate (5 g), dihydropyran (2.5 ml), and toluene-*p*-sulphonic acid monohydrate (0.1 g) were dissolved in ethyl acetate (150 ml) and the mixture was stirred at room temperature for 3 h. The solution was washed with 1M-sodium hydrogen carbonate (50 ml), and was then dried. The solvent was removed and the resulting residue was chromatographed to give benzyl 9-*O*-(tetrahydropyran-2-yl)clavulanate, containing some polymer from the dihydropyran, as a pale yellow oil (4.9 g); $[\alpha]_D^{20}$ +33.6° (*c* 1.8); $\nu_{max.}$ 1 800, 1 750, and 1 695 cm^{-1}; δ 1.3—2.0 (12 H, m, includes protons of polymer), 3.02 (1 H, d, *J* 17 Hz), 3.3—4.3 (8 H, complex m, includes protons of polymer), 4.5—4.7 (1 H, m), 4.89 (1 H, t, *J* 8 Hz), 5.11 (1 H, s), 5.21 (2 H, s), 5.70 (1 H, d, *J* 2.5 Hz), and 7.38 (5 H, s); *m/z* (ammonia chemical ionisation) 391 ([*M* + NH$_4$]$^+$) and 374 ([*M* + H]$^+$).

Conversion of (12a) *into the Monocyclic Azetidinone* (13a).—The tetrahydropyranyl ether (12a) (4.7 g) in dichloromethane (100 ml) was treated with triethylamine (1.75 ml) and the solution was kept at room temperature for 0.5 h. The dichloromethane was removed and the resulting residue was dissolved in ethyl acetate (200 ml). The solution was washed with water (2 × 100 ml), dried, and concentrated to *ca.* 50 ml. n-Butanethiol (10 ml) was added to the solution which was then refluxed for 4 h. The solution was cooled, the solvent was removed, and the residue was chromatographed to give benzyl 2-(4-butylthio-2-oxoazetidin-1-yl)-3-hydroxy-5-(tetrahydropyran-2-yloxy)pent-2-enoate (13a) as a yellow gum (2.65 g); $\lambda_{max.}$ 265 nm (ε 6 680); $\nu_{max.}$ 1 760, 1 650, and 1 605 cm^{-1}; δ 0.7—1.0 (3 H, m), 1.1—2.0 (10 H, m), 2.43 (2 H, t, *J* 7 Hz), 2.7—3.0 (3 H, m), 3.2—4.2 (5 H, m), 4.60br (1 H, s), 4.8—5.0 (1 H, m), 5.0—5.5 (2 H, m), 7.34 (5 H, s), and 12.45 (1 H, s); *m/z* (ammonia chemical ionisation) 481 ([*M* + NH$_4$]$^+$, 5), 464 ([*M* + H]$^+$, 5), 397 (25), 380 (35), 263 (30), 246 (40), 218 (75), 102 (55), and 85 (100).

Methylation of (13a) *using Diazomethane*.—Compound (13a) (2.55 g) in ethyl acetate (100 ml) was stirred while an ether solution of diazomethane was added in small portions. When t.l.c. indicated that there was no starting material remaining, the solvent was removed and the residue was chromatographed to give a mixture of the *E*- and *Z*-isomers of benzyl 2-(4-butylthio-2-oxoazetidin-1-yl)-3-methoxy-5-(tetrahydropyran-2-yloxy)-pent-2-enoate (14a) as a colourless gum (2.0 g); $\lambda_{max.}$ 263 nm (ε 8 790); $\nu_{max.}$ 1 760, 1 710, and 1 600 cm^{-1}; δ 0.7—1.0 (3 H, m), 1.1—1.9 (10 H, m), 2.46 (2 H, t, *J*

7 Hz), 2.7—4.0 (11 H, m, including s at δ 3.89), 4.58br (1 H, s), 4.9—5.4 (3 H, m), and 7.32 (5 H, s); *m/z* (ammonia chemical ionisation) 495 ($[M + NH_4]^+$, 55), 478 ($[M + H]^+$, 20), 411 (75), 394 (85), 377 (60), 362 (30), 321 (20), 286 (45), 259 (30), 108 (40), and 85 (100).

Removal of the Tetrahydropyranyl Group from (14a).—Compound (14a) (1.9 g) and pyridinium toluene-*p*-sulphonate (125 mg) were dissolved in absolute ethanol (40 ml) and the mixture was stirred at 50—60 °C for 3 h. The solution was diluted with ethyl acetate (200 ml), washed with water, and dried. The solvent was removed and the residue was chromatographed to give, in order of elution, *benzyl* (E)-2-(4-*butylthio*-2-*oxoazetidin*-1-*yl*)-5-*hydroxy*-3-*methoxypent*-2-*enoate* (16a) as a colourless gum (410 mg) and *benzyl* (Z)-2-(4-*butylthio*-2-*oxoazetidin*-1-*yl*)-5-*hydroxy*-3-*methoxypent*-2-*enoate* (15a) also as a colourless gum (790 mg).

The alcohol (15a) had $\lambda_{max.}$ 264 nm (ε 9 410); $\nu_{max.}$ 3 400, 1 750, 1 710, and 1 610 cm^{-1}; δ 0.7—1.0 (3 H, m), 1.1—1.7 (4 H, m), 2.45 (2 H, t, *J* 7 Hz), 2.6—3.4 (5 H, m, reduced to 4 H on D_2O exchange), 3.7—4.0 (5 H, m, including s, at δ 3.88), 4.93 (1 H, dd, *J* 5 and 3 Hz), 5.18 (2 H, ABq, *J* 12 Hz), and 7.33 (5 H, s); *m/z* 393 (M^+, 0.5), 303 (5), 277 (70), 247 (45), 116 (15), and 91 (100) (Found: M^+, 393.162. $C_{20}H_{27}NO_5S$ requires *M*, 393.161).

Alcohol (16a) had $\lambda_{max.}$ 260 nm (ε 9 470); $\nu_{max.}$ 3 400, 1 740, and 1 600 cm^{-1}; δ 0.7—1.0 (3 H, m), 1.1—1.7 (4 H, m), 2.45 (2 H, d, *J* 7 Hz), 2.5—3.2 (4 H, m, reduces to 3 H on D_2O exchange), 3.37 (1 H, dd, *J* 15.5 and 5.5 Hz), 3.7—4.0 (5 H, m, including s, at δ 3.88), 4.9—5.4 (3 H, m), and 7.33 (5 H, s); *m/z* 393 (M^+, 0.5), 304 (0.5), 277 (25), 247 (15), 169 (10), 116 (20), and 91 (100) (Found: M^+, 393.160. $C_{20}H_{27}NO_5S$ requires *M*, 393.161).

Chlorinolysis of (16a) *and Cyclisation to* (18a).—Compound (16a) (100 mg) in dichloromethane (4 ml) was stirred at −60 °C while a solution of chlorine (18 mg) in carbon tetrachloride (0.3 ml) was added. After 5 min, the solvent was removed to give the chloride (17a) as a yellow oil. The chloride was dissolved in dichloromethane (5 ml) and to the stirred solution silver oxide (60 mg) and silver tetrafluoroborate (50 mg) were added. The mixture was stirred for 15 min, diluted with dichloromethane (20 ml), and filtered. The filtrate was washed with 1M-sodium hydrogen carbonate and dried. The solvent was removed and the residue was chromatographed to give *benzyl* 3-*methoxy*-9-*oxo*-6-*oxa*-1-*azabicyclo*[5.2.0]*non*-2-*ene*-2-*carboxylate* (18a) as a colourless gum (38 mg); $\lambda_{max.}$ 265 nm (ε 8 220); $\nu_{max.}$ 1 770, 1 715, and 1 620 cm^{-1}; δ 2.53 (1 H, ddd, *J* 16, 5, and 1.5 Hz), 2.7—3.1 (2 H, m), 3.1—3.6 (2 H, m), 3.65 (3 H, s), 4.03 (1 H, ddd, *J* 12.5, 6, and 2.5 Hz), 4.92 (1 H, dd, *J* 4 and 1.5 Hz), 5.21 (2 H, s), and 7.34 (5 H, s); *m/z* 303 (M^+, 15), 261 (5), 154 (5), 124 (5), 111 (5), 98 (10), and 91 (100) (Found: M^+, 303.111. $C_{16}H_{17}NO_5$ requires *M*, 303.111).

Oxidation of the Alcohols (15a) *and* (16a).—A 1 : 1 mixture of the alcohols (15a) and (16a) (315 mg) was dissolved in a mixture of benzene (2 ml) and dry dimethyl sulphoxide (4 ml). To the solution were added pyridine [0.065 ml; in benzene (1 ml)], trifluoroacetic acid [0.031 ml; in benzene (1 ml)], and dicyclohexylcarboxi-imide (495 mg) in that order. The mixture was stirred for 2 h, diluted with ethyl acetate, and filtered. The filtrate was washed with water and dried. The solvent was removed and the resulting residue was taken up into ethyl acetate–light petroleum (1 : 1) and filtered. The solvent was removed from the filtrate to give a 2 : 1 mixture of the geometric isomers of benzyl 2-(4-butylthio-2-oxoazetidin-1-yl)-3-methoxy-5-oxopent-3-enoate (19a) as a pale yellow gum (260 mg); $\lambda_{max.}$ 253 nm (ε 10 120); $\nu_{max.}$ 1 760, 1 665, and 1 630 cm^{-1}; δ 0.7—1.0 (3 H, m), 1.1—1.9 (4 H, m), 2.3—2.7 (2 H, m), 2.9—3.6 (2 H, m), 3.63 (*ca.* 2 H, s), 3.70 (*ca.* 1 H, s), 4.8—5.4 (3 H, m), 5.50 (*ca.* 0.65 H, d, *J* 6 Hz), 5.56 (*ca.* 0.35 H, d, *J* 6 Hz), 5.74 (*ca.* 0.35 H, s), 5.94 (*ca.* 0.65 H, s), 7.32 (5 H, s), 9.77 (*ca.* 0.35 H, d, *J* 6 Hz), and 9.84 (*ca.* 0.65 H, d, *J* 6 Hz); *m/z* 391 (M^+, 0.5), 275 (3), 231 (4), 167 (5), 143 (5), 116 (25), and 91 (100) (Found: M^+, 391.147. $C_{20}H_{25}NO_5S$ requires *M*, 391.145).

Chlorinolysis of (19a) *and Cyclisation to* (20a).—Compound (19a) (50 mg) in carbon tetrachloride (5 ml) was stirred and ice-cooled while chlorine (9 mg) in carbon tetrachloride (0.2 ml) was added. After 5 min, the solvent was removed. The residue was dissolved in tetrahydrofuran (2 ml) and the stirred, ice-cooled solution was treated with triethylamine (0.018 ml) in tetrahydrofuran (1 ml). After 1.5 h, the mixture was filtered and the solvent was removed from the filtrate. The resulting residue was chromatographed to give *benzyl* 3-*methoxy*-9-*oxo*-6-*oxa*-1-*azabicyclo*[5.2.0]*nona*-2,4-*diene*-2-*carboxylate* (20a) as a colourless gum (18 mg); $\lambda_{max.}$ 235infl. (ε 5 290) and 314 nm (8 460); $\nu_{max.}$ 1 785, 1 710, 1 625, and 1 580 cm^{-1}; δ 2.87 (1 H, dd, *J* 15.5 and 1.5 Hz), 3.44 (1 H, dd, *J* 15.5 and 4.5 Hz), 3.69 (3 H, s), 5.0—5.2 (2 H, m), 5.26 (2 H, s), 6.68 (1 H, d, *J* 9 Hz), and 7.2—7.5 (5 H, m); *m/z* 301 (M^+, 15), 232 (20), 182 (10), 166 (10), 152 (5), 138 (30), 110 (10), and 91 (100) (Found: M^+, 301.094. $C_{16}H_{15}NO_5$ requires *M*, 301.095).

Tetrahydropyranyl Ether (12b) *of* 4-*Nitrobenzyl Clavulanate*.—4-Nitrobenzyl clavulanate (15 g), dihydropyran (6.4 ml), and toluene-*p*-sulphonic acid (0.3 g) were dissolved in ethyl acetate (350 ml) and the mixture was stirred at room temperature for 45 min. The solution was washed with 1M-sodium hydrogen carbonate solution (200 ml) and then dried. The solvent was removed and the resulting residue (19.3 g) was used without further purification.

Conversion of (12b) *into Monocyclic Azetidinone* (13b).—The crude tetrahydropyranyl ether (12b) (19.3 g) in dichloromethane (300 ml) was treated with triethylamine (5.83 ml) and the solution was stirred 20 min at room temperature. The dichloromethane was removed and the resulting residue was dissolved in ethyl acetate (200 ml). The solution was washed with water (2 × 100 ml), dried, and the solvent removed. The residue was redissolved in ethyl acetate (200 ml) and the solution was treated with n-butanethiol (25 ml) under reflux for 3 h. The mixture was cooled and the solvent was removed; the residue was chromatographed to give 4-nitrobenzyl 2-(4-butylthio-2-oxoazetidin-1-yl)-3-hydroxy-5-(tetrahydropyran-2-yloxy)pent-2-enoate (13b) as a yellow gum (8.42 g); $\lambda_{max.}$ 274 nm (ε 19 250); $\nu_{max.}$ 1 760, 1 660, and 1 610 cm^{-1}; δ 0.7—1.0 (3 H, m), 1.1—1.9 (10 H, m), 2.51 (2 H, t, *J* 7 Hz), 2.7—3.1 (3 H, m), 3.2—4.1 (5 H, m), 4.59br (1 H, s), 4.8—5.0 (1 H, m), 5.32 (2 H, s), 7.53 (2 H, d, *J* 9 Hz), 8.23 (2 H, d, *J* 9 Hz), and 12.34 (1 H, s); *m/z* (ammonia chemical ionisation) 526 ($[M + NH_4]^+$, 1), 509 ($[M + H]^+$, 1), 347 (3), 330 (3), 272 (40), 263 (30), 246 (40), 156 (20), 122 (25), 118 (30), 102 (50), and 85 (100).

Methylation of (13b) *using Diazomethane*.—Compound (13b) (4.27 g) in ethyl acetate (100 ml) was methylated in the same manner as compound (13a). The resulting gum was not characterised.

Removal of the Tetrahydropyranyl Group from (14b).—The compound obtained from methylation of (13b) in dry ethanol (50 ml) was treated with pyridinium toluene-*p*-sulphonate (0.18 g) and the mixture was stirred at 70 °C for 1.5 h. The solution was cooled, evaporated, and the residue was taken up

in ethyl acetate (100 ml). This solution was washed with water (50 ml), dried, and the solvent removed. The residue was chromatographed to give a 1 : 1 mixture of *E*- and *Z*-isomers of 4-nitrobenzyl 2-(4-butylthio-2-oxoazetidin-1-yl)-5-hydroxy-3-methoxypent-2-enoate (15b) and (16b) as a pale yellow gum (2.29 g); $\lambda_{max.}$ 266 nm (ε 18 620); $\nu_{max.}$ 3 400, 1 750, 1 710sh, and 1 600 cm^{-1}; δ 0.7—1.0 (3 H, m), 1.1—1.7 (4 H, m), 2.51 (2 H, t, *J* 7 Hz), 2.6—3.5 (5 H, m), 3.7—4.0 (5 H, m, including s at δ 3.91), 4.9—5.1 (1 H, m), 5.26 and 5.28 (2 H, 2s), 7.54 (2 H, d, *J* 9 Hz), and 8.21 (2 H, d, *J* 9 Hz); *m/z* (ammonia chemical ionisation) 456 ($[M + NH_4]^+$, 2), 439 ($[M + H]^+$, 3), 421 (5), 303 (6), 286 (100), 187 (30), 144 (40), 122 (85), 117 (55), and 106 (100).

Chlorinolysis of (15b) *and* (16b) *and Cyclisation to* (18b).—The mixture of alcohols (15b) and (16b) (1.0 g) in dichloromethane (10 ml) was stirred and ice-cooled while a solution of chlorine (0.162 g) in carbon tetrachloride (2 ml) was added. After 5 min the solvent was removed to give a yellow oil. This was dissolved in dichloromethane (15 ml) and the stirred solution was treated with silver oxide (0.53 g) and silver tetrafluoroborate (0.45 g). After 15 min the mixture was filtered, the solvent was removed from the filtrate, and the residue chromatographed to give 4-*nitrobenzyl* 3-*methoxy*-9-*oxo*-6-*oxa*-1-*azabicyclo*[5.2.0]*non*-2-*ene*-2-*carboxylate* (18b) (0.125 g). This crystallised from ethyl acetate as colourless prisms, m.p. 138—140 °C; $\lambda_{max.}$ 268 nm (ε 18 370); $\nu_{max.}$ 1 770, 1 720, and 1 610 cm^{-1}; δ 2.5—3.6 (5 H, m), 3.72 (3 H, s), 4.07 (1 H, ddd, *J* 13, 5.5 and 3 Hz), 4.96 (1 H, dd, *J* 4 and 1.5 Hz), 5.29 (2 H, ABq, *J* 14 Hz), 7.54 (2 H, d, *J* 9 Hz), and 8.19 (2 H, d, *J* 9 Hz); *m/z* 348 (M^+, 35), 306 (60), 180 (20), 154 (50), 152 (40), 136 (80), 126 (50), 124 (45), 98 (60), and 78 (100) (Found: C, 55.1; H, 4.5; N, 8.0%; M^+, 348.097. $C_{16}H_{16}N_2O_7$ requires C, 55.2; H, 4.6; N, 8.1%; *M*, 348.096).

Hydrogenolysis of (18b).—A solution of the 4-nitrobenzyl ester (18b) (80 mg) in tetrahydrofuran (5 ml) was added to a pre-hydrogenated suspension of 10% palladium–carbon (50 mg) in tetrahydrofuran (2 ml). The mixture was shaken under hydrogen at 1 atm for 2.5 h. The mixture was filtered and the filtrate was evaporated to *ca.* 2 ml. This solution was treated with water (5 ml) and 0.1M-lithium carbonate solution (1.15 ml). The mixture was washed with ethyl acetate (2 × 10 ml) and the solvent was removed. The residue was chromatographed on cellulose powder (Whatman CC31), with n-butanol–ethanol–water (4 : 1 : 1) as eluant, to give *lithium* 3-*methoxy*-9-*oxo*-6-*oxa*-1-*azabicyclo*[5.2.0]*non*-2-*ene*-2-*carboxylate* (21) as a white powder (30 mg); $\lambda_{max.}$ (H_2O) 251 nm (ε 5 480); $\nu_{max.}$ (KBr) 1 745 and 1 605 cm^{-1}; δ(D_2O, with HOD at δ 4.61; 250 MHz), 2.44 (1 H, ddd, *J* 16.5, 5.5 and 1.5 Hz), 2.55—2.75 (2 H, m), 3.08 (1 H, dd, *J* 16 and 4 Hz), 3.3—3.5 (4 H, m, including s at δ 3.44), 3.90 (1 H, ddd, *J* 13.5, 5.5 and 2.5 Hz), and 4.88 (1 H, dd, *J* 4 and 1.5 Hz) (Found: C, 45.6; H, 4.8; N, 5.6. $C_9H_{10}LiNO_5 \cdot H_2O$ requires C, 45.6; H, 5.1; N, 5.9%).

Oxidation of the Mixture of Alcohols (15b) *and* (16b).—The mixture of alcohols (15b) and (16b) (1.0 g) was dissolved in a mixture of dry benzene (7.5 ml) and dry Me_2SO (7.5 ml). To the solution was added pyridine (0.18 ml), trifluoracetic acid (0.09 ml) and dicyclohexylcarboxi-imide (1.41 g) in that order. The mixture was stirred for 1.5 h, and was then filtered and the filtrate diluted with ethyl acetate (40 ml). This solution was washed with water (2 × 40 ml), dried, and the solvent was removed. The residue was taken up into ethyl acetate–light petroleum (1 : 2; 10 ml) and filtered. The solvent was removed from the filtrate and the residue was chromatographed rapidly to give a 2 : 1 mixture of the geometric isomers of 4-*nitrobenzyl* 2-(4-*butylthio*-2-*oxoazetidin*-1-*yl*)-3-*methoxy*-5-*oxopent*-3-*enoate* (19b) as a pale yellow gum (0.755 g), $\lambda_{max.}$ 260 nm (ε 17 660); $\nu_{max.}$ 1 760, 1 665, and 1 630 cm^{-1}; δ 0.7—1.0 (3 H, m), 1.1—1.8 (4 H, m), 2.4—2.8 (2 H, m), 2.8—3.6 (2 H, m), 3.71 (*ca.* 2 H, s), 3.76 (*ca.* 1 H, s), 4.8—5.1 (1 H, m), 5.1—5.5 (2 H, m), 5.5—5.7 (1 H, m), 5.90 (*ca.* 0.35 H, s), 6.00 (*ca.* 0.65 H, s), 7.4—7.6 (2 H, m), 8.22 (2 H, d, *J* 9 Hz), 9.75 (*ca.* 0.35 H, d, *J* 6 Hz), and 9.83 (*ca.* 0.65 H, d, *J* 6 Hz); *m/z* 436 (M^+, 1), 347 (4), 320 (5), 303 (15), 136 (55), and 116 (100) (Found: M^+, 436.130. $C_{20}H_{24}N_2O_7S$ requires *M*, 436.130).

Chlorinolysis of (19b) *and Cyclisation to* (20b).—Compound (19b) (0.47 g) in dichloromethane (10 ml) was stirred and ice-cooled while a solution of chlorine (0.077 g) in carbon tetrachloride (2 ml) was added. After 5 min the solvent was removed. The residue was dissolved in dry *N*,*N*-dimethylformamide (7.5 ml) and powdered potassium carbonate (0.15 g) was added. The mixture was stirred for 2 h at room temperature and was then diluted with ethyl acetate (50 ml). The solution was washed with water (20 ml), dried, and the solvent was removed. The residue was chromatographed to give 4-*nitrobenzyl* 3-*methoxy*-9-*oxo*-6-*oxa*-1-*azabicyclo*[5.2.0]-*nona*-2,4-*diene*-2-*carboxylate* (20b) (0.22 g). This crystallised from ethyl acetate as colourless prisms, m.p. 162—164 °C; $\lambda_{max.}$ 313 (ε 11 360) and 260 nm (12 110); $\nu_{max.}$ 1 780, 1 715, 1 625, 1 605, and 1 575 cm^{-1}; δ 2.87 (1 H, dd, *J* 15.5 and 1.5 Hz), 3.45 (1 H, dd, *J* 15.5 and 4.5 Hz), 3.74 (3 H, s), 5.1—5.5 (4 H, m), 6.72 (1 H, d, *J* 9 Hz), 7.56 (2 H, d, *J* 9 Hz), and 8.20 (2 H, d, *J* 9 Hz); *m/z* 346 (M^+, 35), 277 (60), 182 (70), 166 (30), and 138 (100) (Found: C, 55.8; H, 4.0; N, 7.9%; M^+, 346.080. $C_{16}H_{14}N_2O_7$ requires C, 55.5; H, 4.1; N, 8.1%; *M*, 346.081).

Hydrogenolysis of (20b).—The 4-nitrobenzyl ester (20b) (100 mg) was hydrogenolysed in a manner similar to that used for compound (18b) to give, after similar work up, *lithium* 3-*methoxy*-9-*oxo*-6-*oxa*-1-*azabicyclo*[5.2.0]*nona*-2,4-*diene*-2-*carboxylate* (22) as a pale yellow solid (50 mg), $\lambda_{max.}$ (H_2O) 297 nm (ε 6 940); $\nu_{max.}$ (KBr) 1 755, 1 630, and 1 585 cm^{-1}; δ (D_2O, with HOD at δ 4.61) 2.89 (1 H, dd, *J* 16 and 1.5 Hz), 3.4—3.7 (4 H, m, including s at δ 3.62), 5.0—5.3 (2 H, m), 6.5 (1 H, d, *J* 9.5 Hz); *m/z* (field desorption *) 211 ($[M - Li + H]^+$, 100) (Found: C, 48.1; H, 4.5; N, 6.25. $C_9H_8LiNO_5 \cdot \frac{1}{2}H_2O$ requires C, 47.8; H, 4.0; N, 6.2%); further confirmation of molecular weight was obtained from the positive-ion fast atom bombardment mass spectrum,† which, for a suspension of salt (22) in glycerol, showed *inter alia* *m/z* 310 ($[M + H +$ glycerol$]^+$) and 218 ($[M + H]^+$); the negative-ion fast atom bombardment mass spectrum showed *inter alia* *m/z* 302 ($[M - Li +$ glycerol$]^-$) and 210 ($[M - Li]^-$).

* This spectrum was obtained using a Jeol D300 instrument.

† This spectrum was obtained using a VG-ZAB mass spectrometer with high-energy xenon atoms as the fast atoms.

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