1,3-Dipolar Addition Reactions of 6-Ethylideneolivanic Acid Derivatives with Diazomethane and Acetonitrile Oxide

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p-Nitrobenzyl (5*R*)-3-[(*E*)-2-acetamidovinylthio]-6-[(*Z*)-ethylidene]-7-oxo-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylate (**1**) undergoes a 1,3-dipolar addition reaction with diazomethane to afford *p*-nitrobenzyl(4'*S*,5*R*,6*S*)-3-[(*E*)-2-acetamidovinylthio]-4'-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-6-spiro-5'-(4',5'-dihydro-3'*H*-pyrazole)-2-carboxylate (**14**) and the (4'*R*,5*R*,6*R*)-isomer (**12**). The analogous 6-(*E*)-ethylideneolivanic acid derivative similarly gives the corresponding (4'*R*,5*R*,6*S*)- and (4'*S*,5*R*,6*R*)-spirodihydropyrazoles. ^{1}H N.m.r. chemical-shift correlations have been used to verify that the major diastereoisomer in each case arises from α-face addition of the dipole. Related spirodihydropyrazole-carbapenems with a 3-ethylthio substituent are similarly prepared. Thermolytic elimination of nitrogen results in the corresponding 6-isopropylidene carbapenem derivatives. Hydrogenolysis of (**12**) and (**14**) gives the respective sodium salts, whilst modification of the C-3 substituent affords 3-aminoethylthio and 3-*N*,*N*-dimethylamidinomethylthio spirodihydropyrazole-carbapenems.

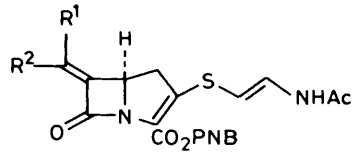
The (*Z*)-ethylidene (**1**) also undergoes cycloaddition with acetonitrile oxide to furnish *p*-nitrobenzyl (5*R*,5'*S*,6*S*)-3-[(*E*)-2-acetamidovinylthio]-3',5'-dimethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-6-spiro-4'-(4',5'-dihydroisoxazole)-2-carboxylate (**33**), as the major product. Addition in the opposite regio-sense gives the (4'*S*,5*R*,6*S*)- and (4'*R*,5*R*,6*R*)-isomers of *p*-nitrobenzyl 3-[(*E*)-2-acetamidovinylthio]-3',4'-dimethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-6-spiro-5'-(4',5'-dihydroisoxazole)-2-carboxylate (**37**) and (**35**), respectively. The sodium salts derived from (**33**) and (**35**) are also described.

Since the original discovery of the olivanic acids[1] and thienamycin,[2] many related β-lactam antibiotics containing the 7-oxo-1-azabicyclo[3.2.0]hept-2-ene (carbapenem) nucleus have been isolated from natural sources.[3] Many of these carbapenems possess potent antibacterial activity but suffer from problems of chemical[4] or metabolic instability.[5] It has thus been of particular interest to investigate the synthesis of new structural types which retain certain features of the parent molecules in order to gain a fuller understanding of the requirements necessary for optimum bioactivity. Both the chemical modification of the natural products and the total synthesis of the carbapenem system have been employed to provide a wide range of derivatives.[4] As part of our investigations into the chemistry of the naturally occurring olivanic acids, we now report[6] the synthesis of some novel spirocyclic carbapenem derivatives by the 1,3-dipolar addition reactions of diazomethane and acetonitrile oxide to the 6-ethylidene compounds (**1**) and (**2**).

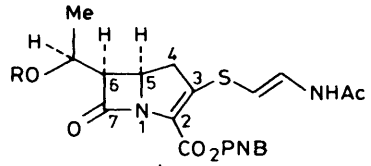
Ethylidene derivatives (**1**) and (**2**) are readily prepared from olivanic acids by several methods. The sulphates (**3**) and (**4**), derived from MM 13902, react with suitable bases to give mixtures of (**1**) and (**2**), whilst elimination of ethyl hydrogen sulphate occurs in a *trans*-coplanar manner from the diester (**5**) to afford the (*E*)-isomer (**2**) only.[1] Similar stereospecific elimination reactions of the mesylates (**6**) and (**7**), derived in turn from the naturally occurring olivanic acids MM 22382 and MM 22383, give rise to the (*E*)- and (*Z*)-ethylidenes (**2**) and (**1**), respectively.[1] The reaction of the *p*-nitrobenzyl ester of MM 22383 (**8**) with triphenylphosphine and diethyl azodicarboxylate provides the most efficient synthesis of the (*Z*)-ethylidene (**1**).[7,8]

Despite the presence of three olefinic functionalities in compounds such as (**1**) and (**2**), it has been found possible to react selectively at the 6-ethylidene double bond. Catalytic hydrogenation or sodium borohydride reduction results in the formation of analogues of the carbapenem metabolite PS-5, bearing a 6-ethyl substituent,[9] whilst nucleophilic addition of thiolates affords 8-thioethers.[7] We have been unable to perform similar additions of oxygen or nitrogen nucleophiles to this system. However, the electron-deficient crotonamide nature of the 6-ethylidene double bond in (**1**) and (**2**) suggested that it might be susceptible to 1,3-dipolar addition reactions. Such reactions have been performed with a variety of α,β-unsaturated carbonyl systems,[10] including the acrylate component of the simple carbapenem system (**9**).[6,11] For instance, reaction of (**9**) with diazomethane yields mainly the pyrazoline (**10**) which undergoes thermolysis to the fused cyclopropane (**11**). It has now been found that the 6-ethylidene double bond in derivatives (**1**) and (**2**) undergoes analogous cycloaddition reactions with diazomethane, but that the resulting pyrazolines behave differently on thermolysis. Nitrile oxides also react with the C-6 double bond in (**1**) to afford isoxazolines.

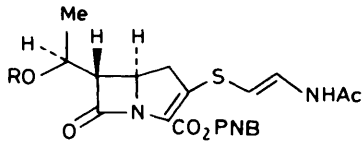
Reaction of the (*Z*)-ethylidene (**1**) with ethereal diazomethane resulted in the formation of two diastereoisomeric pyrazolines (**12**) and (**14**). The two isomers, formed in a ratio of *ca.* 4.5 to 1, were separated by silica-gel chromatography and each was obtained crystalline. The major isomer was assigned structure (**12**), and the minor isomer structure (**14**), on the basis of mechanistic considerations and spectroscopic analysis. The regiochemistry of addition of diazomethane to electron-deficient dipolarophiles is well established,[10,12] and the ^{1}H n.m.r. spectra of the two products (**12**) and (**14**) clearly supported the formation of the one favoured regioisomer. The two diastereoisomers formed were therefore the respective products of α- and β-face attack at the 6-ethylidene bond, and the major isomer was expected to be that arising by approach of the dipole from the least hindered α-face of the molecule, *i.e.* isomer (**12**). The stereochemical assignments were substantiated by examination of ^{1}H n.m.r. spectra of the cycloadducts (Table). The C-5 proton of the major diastereoisomer (**12**) has a relative chemical shift of 0.7 p.p.m. downfield from that of the minor isomer (**14**), and this can be attributed to the deshielding effect of the proximate C-6α N=N–system in (**12**). In the minor isomer (**14**) the 6-azo group occupies the β-position and deshielding of the 4β-proton can now occur, as both of these moieties lie



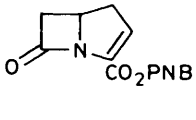
(1) R^1 = H, R^2 = Me
(2) R^1 = Me, R^2 = H



(3) R = $SO_3^-Na^+$
(4) R = $SO_3^-{}^+NMe_2(PhCH_2)(C_{16}H_{33})$
(5) R = SO_3Et
(6) R = SO_2Me



(7) R = SO_2Me
(8) R = H



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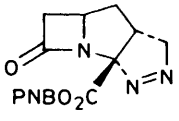
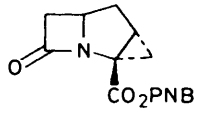


(10)



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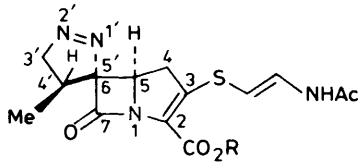
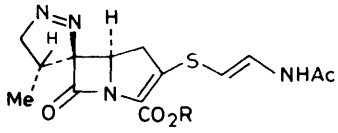
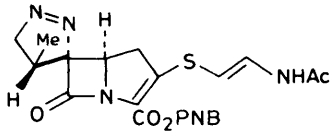


(12) R = PNB
(13) R = Na



(14) R = PNB
(15) R = Na



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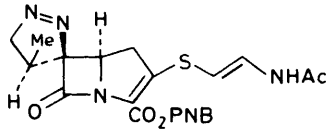

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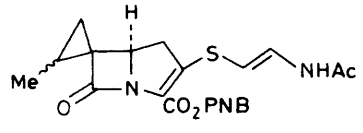

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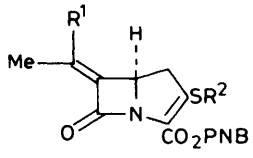
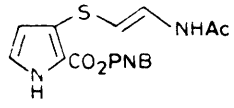


(19) R^1 = Me, R^2 = $CH=\overset{t}{=}CHNHAc$
(20) R^1 = H, R^2 = Et
(21) R^1 = Me, R^2 = Et



(22)

Table. ^{1}H N.m.r chemical shifts of dihydropyrazoles and dihydroisoxazoles in ($CDCl_3$)* (δ/p.p.m.)

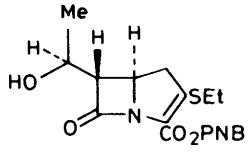
Compound	*Me*CH	C*H*Me	4α-CH	4β-CH	5-CH
(12)	1.37	2.41	3.21	3.29	4.94
(14)*	1.25	2.36	3.09	3.94	4.24
(16)	0.78	2.61	3.13	3.32	5.04
(17)	0.92	2.67	3.13	4.11	4.50
(24)	1.28	2.29	3.21	3.21	4.90
(27)	1.27	2.35	3.13	4.10	4.26
(33)	1.50	4.74	3.05	3.05	4.29
(35)	1.35	3.48	3.00	3.10	4.43
(37)	1.40	3.53	2.93	3.39	4.21

* A drop of $(CD_3)_2NCDO$ was required to solubilise derivative (14).

within the fold of the bicyclic system. Indeed, the 4β-proton in the ^{1}H n.m.r. spectrum of (14) resonates 0.65 p.p.m. downfield from the corresponding proton in that of (12).

Further evidence for these assignments came from the corresponding reaction of diazomethane with the (*E*)-ethylidene (2). Again, two diastereoisomeric pyrazolines (16) and (17) (*ca.* 3.5:1) were isolated. The ^{1}H n.m.r. spectra of the new derivatives revealed similar chemical shift differences of the C-5 and C-4β protons (Table) consistent with addition of diazomethane from the α-face of the molecule in the major isomer (16). It was further noted that in the pyrazolines (12) and (14), derived from ethylidene (1) with (*Z*)-geometry, the 4′-methyl groups resonate 0.3—0.5 p.p.m. downfield from similar protons in (16) and (17), obtained from the (*E*)-ethylidene (2). The respective C-4′ protons showed relative upfield shifts of 0.2—0.3 p.p.m. These effects are due to deshielding of the 4′-methyl groups in (12) and (14), and the C-4′ protons in (16) and (17), by the β-lactam carbonyl moiety, and confirm that the configuration at C-4′ in the products reflects the geometry of the starting ethylidene.

In view of the facile thermal elimination of nitrogen from the pyrazoline (10) to afford the cyclopropane (11),[6,11] it was expected that the olivanic acid spiropyrazolines might undergo a similar transformation to the spirocyclopropane system (18). However, no reactions were observed when (16) or (17) were heated in refluxing ethyl acetate, whilst thermolysis in toluene produced the isopropylidene derivative (19) as the only



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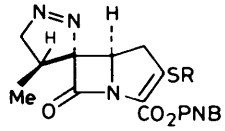
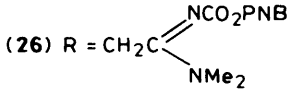
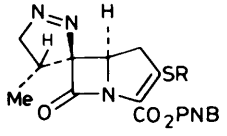
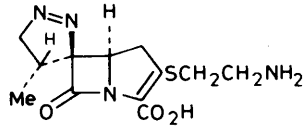


(24) R = Et
(25) R = H





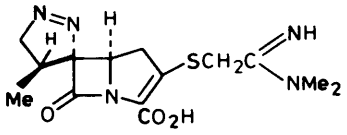
(27) R = Et
(28) R = H
(29) R = $CH_2CH_2NHCO_2PNB$



(30)

derivative (23)[15] with triphenylphosphine and diethylazodicarboxylate. The ethylidene double-bond of (20) underwent a 1,3-dipolar addition with diazomethane to afford a 2.5:1 mixture of the pyrazolines (24) and (27). The ^1H n.m.r. spectra of these products (Table) were in accord with previous observations. When the major isomer (24) was heated in refluxing toluene, the only isolated product consisted predominantly of the olefin (21), thus parallelling the earlier results.

Catalytic hydrogenolysis of the *p*-nitrobenzyl esters of pyrazolines (12) and (14) afforded aqueous solutions of the sodium salts (13)* and (15),* respectively. These two products displayed only weak antibacterial activity, and it was therefore decided to undertake the synthesis of analogous systems with basic 3-substituents which are known to confer superior biological activity on carbapenem derivatives.[4] A pyrazoline derivative with the C-3 thienamycin side chain was prepared by utilising the versatile methodology of modification of the natural product side-chain *via* the C-3 thiol.[15] Thus, the pyrazoline (14) was treated with *N*-bromoacetamide to give the crude thiol (28) which was alkylated with 2-(*p*-nitrobenzyloxycarbonylamino)ethyl bromide to afford the *N*-protected



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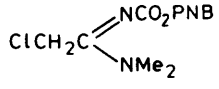

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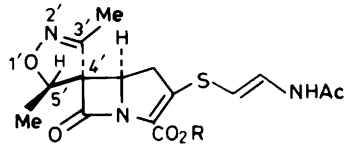
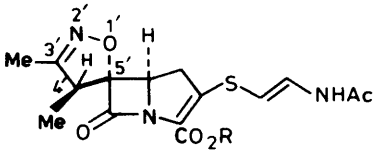
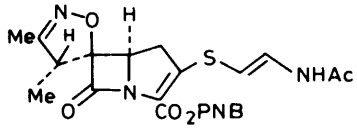


(33) R = PNB
(34) R = Na



(35) R = PNB
(36) R = Na



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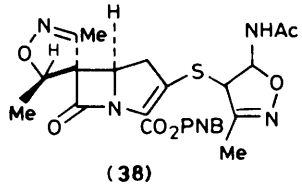

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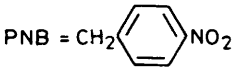

recognisable product. The pyrazoline (12) similarly gave the olefin (19) together with the pyrrole (22). These reactions were not high-yielding and were accompanied by tar formation. Competitive olefin formation in the thermolysis of pyrazolines to cyclopropanes is well documented, and can be minimised by employing a photolytic procedure.[13] A single attempt to photolyse the pyrazoline (12) in a quartz vessel with a medium-pressure lamp resulted in a multitude of products. Failure to isolate the cyclopropane (18) from these reactions indicates either that the preferred reaction pathway is olefin formation or that the spirotricyclic system (18) is inherently unstable under the conditions employed. It is interesting to note that the pyrolysis of a related pyrazoline-penam system afforded a spirocyclopropane with ease, but direct comparisons are inappropriate owing to a different substitution pattern on the pyrazoline ring.[14]

In order to determine any possible effect of a different C-3 substituent on the course of these reactions, the 3-ethylthio (*Z*)-ethylidene derivative (20) was utilised. This compound was prepared from the reaction of the C-3 modified MM 22383 derivative (29). Hydrogenolysis of (29) at neutral pH then furnished the zwitterion (30).*

The major pyrazoline isomer (12) was similarly converted into the *N*-protected carbamimidoyl derivative (26) by alkylation of the C-3 thiol (25) with the chloroacetamidine (32). Although compound (26) could not be obtained free from an impurity, hydrogenolysis followed by fractionation of the product on Diaion HP20 resulted in the isolation of a clean sample of the carbamimidoyl derivative (31).* Disappointingly, both zwitterionic derivatives (30) and (31) possessed poor antibacterial activity, but displayed good stability to renal dehydropeptidase, the enzyme responsible for the rapid metabolism of thienamycin and other carbapenem antibiotics.[5]

* In each case the product consisted of predominantly one component by h.p.l.c. and possessed a characteristic u.v. spectrum in accord with the proposed structure. In some cases confirmatory i.r. and n.m.r. spectra were also obtained. Owing to the limited quantities involved and consequent difficulties in purification, analytically pure samples of the sodium salts and amino acids were not isolated.

Nitrile oxides also undergo dipolar cycloaddition reactions with suitably activated olefins,[10,16] and the reaction of acetonitrile oxide with the ethylidene (**1**) was consequently investigated. The nitrile oxide was generated[17] by treating a chloroform solution of acetaldoxime with chlorine, and adding the resulting chloro-oxime to triethylamine in the presence of the substrate (**1**). The ensuing reaction required 5—7 days for completion, and as the nitrile oxide undergoes competitive dimerisation,[10,18] it was found expedient to add fresh reagent after *ca.* 3 days. Repeated chromatography of the reaction product revealed the presence of four new compounds in an overall yield of *ca.* 50%, and as expected they were all identified as 2-isoxazolines resulting from the 1,3-dipolar addition of acetonitrile oxide to the ethylidene double bond.

The major product (55% of the total) was found to be the spiro-isoxazoline (**33**) resulting from attack of the dipole oxygen atom at the terminal position of the olefin. The regio-isomer (**35**) was the next most abundant product (30%) and could be clearly differentiated from (**33**) by virtue of the respective ^{1}H n.m.r. spectra, in particular the chemical shifts of the isoxazoline methine protons. The configuration at C-6 in (**33**) and (**35**) was assigned on the basis of the expected α-face addition of the nitrile oxide, by analogy with the predominant mode of addition of diazomethane. Further support for the stereochemistry assigned to (**35**) came from identification of the third product as the diastereoisomer (**37**), (10%) arising from β-face addition. The ^{1}H n.m.r. chemical shifts of 5-CH and 4β-CH in (**35**) and (**37**) have the same relationship to those of the corresponding pair of the pyrazoline isomers (**12**) and (**14**) (Table) although the shift differences are less pronounced.

The fourth possible isoxazoline isomer, namely the C-6 epimer of the major product (**33**), was not observed. This is presumably because β-face addition in this regio-sense is prevented by severe steric crowding between the methyl terminal of the nitrile oxide and the β-face of the bicyclic system. The most polar and minor product (5%) was in fact identified as the bis-isoxazoline (**38**) where the nitrile oxide has also undergone cycloaddition to the double-bond in the 3-substituent. The regiochemistry of addition in the side chain was deduced from the ^{1}H n.m.r. spectrum of (**38**), but the stereochemistry is uncertain. The observed lack of regioselectivity in the reaction of acetonitrile oxide with the ethylidene double bond of (**1**) is in accord with previous studies on such cycloadditions to α,β-unsaturated carbonyl compounds.[18]

The N–O bond of isoxazolines can be reductively cleaved by methods which include catalytic hydrogenation.[19] However, under the conditions employed for hydrogenolysis of the *p*-nitrobenzyl ester (5% Pd–C, dioxane, water, ambient temperature, and pressure, 3 h) the isoxazoline ring in compounds (**33**) and (**35**) remained intact. In this way, sodium salts (**34**)* and (**36**)* were prepared, the structure of the former being verified by ^{1}H n.m.r. spectroscopy. Although neither of these isoxazolines possessed useful antibacterial activity, the salt (**34**) was remarkably stable to renal dehydropeptidase. In contrast, the regio-isomer (**36**) was only moderately stable to this enzyme.

Experimental

M.p.s were determined using a Kofler hot-stage apparatus and are uncorrected. U.v. spectra were recorded on a Pye Unicam SP7-500 spectrophotometer, and i.r. spectra on a Perkin-Elmer 197 or 983 machine. ^{1}H N.m.r. spectra were all obtained at 250 MHz on a Bruker WM 250 spectrometer with tetramethylsilane as internal standard except where otherwise stated. Mass spectra were determined with a VG 7070F instrument and optical rotations were measured on a Perkin-Elmer 141 polarimeter. The homogeneity of all esters was established by t.l.c. on Merck precoated silica gel 60 F_{254} plates. Except where otherwise stated, preparative chromatography was carried out on columns of Merck silica gel 60 (1:1 mixture of <230 mesh and 230—400 mesh ASTM) using slightly increased pressure for elution. Sodium salts and amino acids were checked by h.p.l.c. on a Waters instruments or Altex analytical machine using a C_{18} μ-Bondapak reverse-phase column, eluting with mixtures of pH 4.7 ammonium dihydrogen orthophosphate buffer and acetonitrile and monitoring by u.v. absorption at the appropriate wavelength. Organic solutions were dried with anhydrous magnesium sulphate and solvents removed under reduced pressure using a rotary evaporator. Organic solvents were either of AnalaR purity, or were dried and/or distilled before use.

p-*Nitrobenzyl* (5R)-6-[(Z)-*Ethylidene*]-3-*ethylthio*-7-*oxo*-1-*azabicyclo*[3.2.0]*hept*-2-*ene*-2-*carboxylate* (**20**).—A stirred solution of the 3-ethylthio derivative (**23**) (0.5 g, 1.28 mmol) in dry tetrahydrofuran (30 ml) was treated sequentially with triphenylphosphine (0.669 g, 2.55 mmol) and diethyl azodicarboxylate (0.444 g, 2.55 mmol) at ice-bath temperature. The solution was allowed to warm to room temperature and after 15 min was diluted with ethyl acetate (100 ml). The solution was washed with water (100 ml) and brine (50 ml), dried, and evaporated. The residue was chromatographed on silica gel using a gradient elution of 50 to 70% ethyl acetate in hexane. The product, obtained as a single component by t.l.c. and isolated as a yellow solid (0.458 g), consisted of a mixture (*ca.* 4:1) of the ethylidene (**20**) and $EtO_2CNHNHCO_2Et$. Further chromatography using chloroform followed by 2% ethanol in chloroform as eluant afforded the pure title *ethylidene* (**20**) as a pale yellow solid (0.2 g, 42%); m.p. 128—129 °C (from ethyl acetate) (Found: C, 57.4; H, 4.75; N, 7.35%; M^+, 374.0938. $C_{18}H_{18}N_2O_5S$ requires C, 57.75; H, 4.85; N, 7.5%; *M*, 374.0936); $\lambda_{max.}$(MeCN) 300 (ε 11 620 dm^3 mol^{-1} cm^{-1}) and 269 nm (11 725); $\nu_{max.}$(CH_2Cl_2) 1 765 and 1 700 cm^{-1}; δ_H($CDCl_3$) 1.32 (3 H, t, *J* 7 Hz, *Me*CH_2), 2.10 (3 H, dd, *J* 7.5 and 1 Hz, *Me*CH=), 2.85 (2 H, m, CH_2S), 3.13 (1 H, dd, *J* 8 and 18 Hz, 4-H_a), 3.23 (1 H, dd, *J* 9.5 and 18 Hz, 4-H_b), 4.73 (1 H, br dd, *J* 8 and 9.5 Hz, 5-H), 5.25 and 5.54 (each 1 H, d, *J* 14 Hz, CH_2Ar), 5.98 (1 H, dq, *J* 1 and 7 Hz, =C*H*Me), and 7.69 and 8.23 (each 2 H, d, *J* 9 Hz, Ar).

Reaction of Ethylidene Derivatives with Diazomethane: p-*Nitrobenzyl* (4'S,5R,6S)-3-[(E)-2-*Acetamidovinylthio*]-4'-*methyl*-7-*oxo*-1-*azabicyclo*[3.2.0]*hept*-2-*ene*-6-*spiro*-5'-(4',5'-*dihydro*-3'H-*pyrazole*)-2-*carboxylate* (**14**) *and the* (4'R,5R,6R)-*Isomer* (**12**).—To a solution of the (*Z*)-ethylidene (**1**)† (1.0 g, 2.33 mmol) in dichloromethane (50 ml) at 0 °C was added an excess of ethereal diazomethane (20 ml). The solution was allowed to attain room temperature and left for 24 h. Excess of diazomethane was purged with argon and the solvents were removed by evaporation. The crude product was chromatographed on silica gel eluting with chloroform followed by 2, 3, 4, and 5% ethanol in chloroform. Early fractions contained starting ethylidene and the (8*R*)-formate impurity present in the starting material†, and were discarded. The first-eluted product, obtained as a pale-yellow foam (80 mg, 7%) was the (4'S,5R,6S)-*spirodihydropyrazole* (**14**), m.p. 182—184 °C (from ethyl acetate–hexane) (Found: C, 53.3; H, 4.5; N, 14.85. $C_{21}H_{21}N_5O_6S$ requires C, 53.5; H, 4.5; N, 14.85%); $[\alpha]_D^{20}$ −558° (*c* 0.4, $CHCl_3$); $\lambda_{max.}$(MeCN) 322 (ε 16 820 dm^3 mol^{-1} cm^{-1}) and 263 nm (17 810); $\nu_{max.}$(CH_2Cl_2) 3 420, 1 780, 1 700, and 1 620 cm^{-1}; δ_H[$CDCl_3$ containing 1 drop of

† See footnote on p. 423.

† The (*Z*)-ethylidene (**1**) used in these reactions was obtained as a by-product in the preparation of a (8*R*)-formyloxy derivative.[8] It invariably contained small amounts of the latter compound and triphenylphosphine oxide. Consequently, yields obtained in reactions employing (**1**) are only approximate and not optimised.

$(CD_3)_2NCDO$] 1.25 (3 H, d, *J* 7 Hz, *Me*CH), 2.05 (3 H, s, MeCO), 2.36 (1 H, m, C*H*Me), 3.09 (1 H, dd, *J* 18.5 and 9.5 Hz, 4-H_a), 3.94 (1 H, dd, *J* 18.5 and 8.5 Hz, 4-H_b), 4.13 (1 H, dd, *J* 17.5 and 8 Hz, 3'-H_a), 4.24 (1 H, t, *J* 9 Hz, 5-H), 4.91 (1 H, dd, *J* 17.5 and 8.5 Hz, 3'-H_b), 5.30 and 5.54 (each 1 H, d, *J* 14 Hz, CH_2Ar), 5.89 (1 H, d, *J* 13.5 Hz, =CHS), 7.33 (1 H, dd, *J* 10.5 and 13.5 Hz, =C*H*NH), 7.68 and 8.22 (each 2 H, d, *J* 9 Hz, Ar), and 9.85 (1 H, br d, *J* 10.5 Hz, NH); *m/z* 443.1147 (corresponds to $M^+ - N_2$, $C_{21}H_{21}N_3O_6S$ requires *M*, 443.1151).

Further elution afforded the (4'R,5R,6R)-*spirodihydropyrazole* (**12**) (0.36 g, 33%) as a pale-yellow foam which crystallised from ethyl acetate–hexane; m.p. 124—125 °C (Found: C, 53.25; H, 4.55; N, 14.5. $C_{21}H_{21}N_5O_6S$ requires C, 53.5; H, 4.5; N, 14.85%); $[\alpha]_D^{20}$ +220° (*c* 1, $CHCl_3$); $\lambda_{max.}$(MeCN) 322 (ε 16 400 dm^3 mol^{-1} cm^{-1}) and 264 nm (18 410); $\nu_{max.}(CH_2Cl_2)$ 3 420, 1 780, 1 705, and 1 620 cm^{-1}; $\delta_H(CDCl_3)$ 1.37 (3 H, d, *J* 7 Hz, *Me*CH), 2.20 (3 H, s, MeCO), 2.41 (1 H, m, C*H*Me), 3.21 (1 H, dd, *J* 9.5 and 18.5 Hz, 4-H_a), 3.29 (1 H, dd, *J* 10 and 18.5 Hz, 4-H_b), 4.19 (1 H, dd, *J* 17.5 and 8.5 Hz, 3'-H_a), 4.94 (1 H, t, *J* 9.5 Hz, 5-H), 5.02 (1 H, dd, *J* 17.5 and 8.5 Hz, 3'-H_b), 5.42 and 5.62 (each 1 H, d, *J* 14 Hz, CH_2Ar), 6.06 (1 H, d, *J* 13.5 Hz, SCH=), 7.39 (1 H, dd, *J* 10.5 and 13.5 Hz, =C*H*NH), 7.77 and 8.34 (each 2 H, d, *J* 9 Hz, Ar), and *ca.* 7.80 (1 H, d, *J* 10.5 Hz, NH); *m/z* 443.1155 ($M^+ - N_2$, $C_{21}H_{21}N_3O_6S$ requires *M*, 443.1151).

p-*Nitrobenzyl* (4'R,5R,6S)-3-[(E)-2-*Acetamidovinylthio*]-4'-*methyl*-7-*oxo*-1-*azabicyclo*[3.2.0]*hept*-2-*ene*-6-*spiro*-5'-(4',5'-*dihydro*-3'H-*pyrazole*)-2-*carboxylate* (**17**) *and the* (4'S,5R,6R)-*Isomer* (**16**).—To a solution of the ethylidene (**2**) (0.5 g, 1.17 mmol) in dichloromethane (30 ml) at 0 °C was added an excess of ethereal diazomethane (8 ml). The solution was kept at room temperature for 20 h before being purged with argon and then washed with 5% aqueous citric acid (30 ml). The organic phase was then washed with water, dried, and evaporated to give a residue which was chromatographed on silica gel, eluting with chloroform followed by 2, 3, and 4% ethanol in chloroform. The first-eluted component was starting ethylidene (**2**) and was discarded. Later fractions contained the (4'R,5R,6S)-*spirodihydropyrazole* (**17**), which was obtained as a pale-yellow foam (55 mg, 10%); $\lambda_{max.}$(EtOH) 321 and 263 nm; $\nu_{max.}(CH_2Cl_2)$ 3 430, 1 785, 1 705, and 1 625 cm^{-1}; $\delta_H(CDCl_3)$ 0.92 (3 H, d, *J* 7 Hz, *Me*CH), 2.08 (3 H, s, MeCO), 2.67 (1 H, m, C*H*Me), 3.13 (1 H, dd, *J* 18 and 10 Hz, 4-H_a), 4.11 (1 H, dd, *J* 18 and 8.5 Hz, 4-H_b), 4.50 (1 H, t, *J* 9 Hz, 5-H), 4.60 (2 H, d, *J* 5 Hz, 3'-H_2), 5.29 and 5.51 (each 2 H, d, *J* 9 Hz, CH_2Ar), 5.94 (1 H, d, *J* 13.5 Hz, SCH=), 7.28 (1 H, dd, *J* 10.5 and 13.5 Hz, =C*H*NH), 7.70 (1 H, d, *J* 10.5 Hz, NH), and 7.64 and 8.22 (each 2 H, d, *J* 8.5 Hz, Ar); *m/z* 443.1151 ($M^+ - N_2$, $C_{21}H_{21}N_3O_6S$ requires *M*, 443.1151).

Further elution gave the (4'S,5R,6R)-*isomer* (**16**) as a yellow foam (0.183 g, 33%); $\lambda_{max.}$(EtOH) 332sh, 320, and 264 nm; $\nu_{max.}(CH_2Cl_2)$ 3 430, 1 780, 1 705, and 1 625 cm^{-1}; $\delta_H(CDCl_3)$ 0.78 (3 H, d, *J* 7 Hz, *Me*CH), 2.10 (3 H, s, MeCO), 2.61 (1 H, m, C*H*Me), 3.13 (1 H, dd, *J* 17.5 and 10 Hz, 4-H_a), 3.32 (1 H, dd, *J* 17.5 and 9 Hz, 4-H_b), 4.39 (1 H, dd, *J* 17 and 6 Hz, 3'-H_a), 4.71 (1 H, d, *J* 17 Hz, 3'-H_b), 5.04 (1 H, dd, *J* 9 and 10.5 Hz, 5-H), 5.27 and 5.50 (each 1 H, d, *J* 14 Hz, CH_2Ar), 5.93 (1 H, d, *J* 13.5 Hz, =CHS), 7.29 (1 H, dd, *J* 10.5 and 13.5 Hz, =C*H*NH), 7.54 (1 H, d, *J* 10.5 Hz, NH), 7.61 and 8.21 (each 2 H, d, *J* 9 Hz, Ar); *m/z* 443.1151 ($M^+ - N_2$, $C_{21}H_{21}N_3O_6S$ requires *M*, 443.1151).

p-*Nitrobenzyl* (4'S,5R,6S)-3-*Ethylthio*-4'-*methyl*-7-*oxo*-1-*azabicyclo*[3.2.0]*hept*-2-*ene*-6-*spiro*-5'-(4',5'-*dihydro*-3'H-*pyrazole*)-2-*carboxylate* (**27**) *and the* (4'R,5R,6R)-*Isomer* (**24**).—A solution of the 3-ethylthio (*Z*)-ethylidene (**20**) (0.2 g, 0.53 mmol) in dichloromethane (10 ml) and an excess of ethereal diazomethane (20 ml) were mixed at 0 °C. The solution was allowed to attain room temperature and kept for 42 h before being purged with argon to remove excess of diazomethane. The white crystalline solid now present was obtained by filtration and identified as the title (4'R,5R,6R)-*spirodihydropyrazole* (**24**) (80 mg), m.p. 160—162 °C (Found: C, 54.95; H, 4.95; N, 13.25%; M^+, 416.1153. $C_{19}H_{20}N_4O_5S$ requires C, 54.8; H, 4.85; N, 13.45%; *M*, 416.1155); $[\alpha]_D^{20}$ +260° (*c* 1, $CHCl_3$); $\lambda_{max.}$(MeCN) 326sh, 317 (ε 11 210 dm^3 mol^{-1} cm^{-1}), and 271 nm (10 410); $\nu_{max.}(CH_2Cl_2)$ 1 785 and 1 710 cm^{-1}; $\delta_H(CDCl_3)$ 1.28 (3 H, d, *J* 7 Hz, *Me*CH), 1.36 (3 H, t, *J* 7.5 Hz, *Me*CH_2), 2.29 (1 H, m, C*H*Me), 2.93 (2 H, m, CH_2S), 3.21 (2 H, d, *J* 9.5 Hz, 4-H_2), 4.08 (1 H, dd, *J* 8.5 and 17.5 Hz, 3'-H_a), 4.90 (1 H, t, *J* 9.5 Hz, 5-H), 4.93 (1 H, dd, *J* 8.5 and 17.5 Hz, 3'-H_b), 5.29 and 5.51 (each 1 H, d, *J* 14 Hz, CH_2Ar), and 7.66 and 8.22 (each 2 H, d, *J* 9 Hz, Ar).

The mother liquors were concentrated and the residue chromatographed on silica gel employing a gradient elution of 40 to 70% ethyl acetate in hexane. The first-eluted product was the (4'S,5R,6S)-*spirodihydropyrazole* (**27**), obtained as a foam (46 mg, 21%) (Found: M^+, 416.1148. $C_{19}H_{20}N_4O_5S$ requires 416.1155); $\lambda_{max.}$(EtOH) 319 and 266 nm; $\nu_{max.}(CH_2Cl_2)$ 1 780 and 1 705 cm^{-1}; $\delta_H(CDCl_3)$ 1.27 (3 H, d, *J* 7 Hz, *Me*CH), 1.36 (3 H, t, *J* 7.5 Hz, *Me*CH_2), 2.35 (1 H, m, C*H*Me), 2.93 (2 H, m, CH_2S), 3.13 (1 H, dd, *J* 17.5 and 9.5 Hz, 4-H_a), 4.10 (1 H, dd, *J* 17.5 and 8.5 Hz, 4-H_b), 4.13 (1 H, dd, *J* 17.5 and 8 Hz, 3'-H_a), 4.26 (1 H, t, *J* 9 Hz, 5-H), 4.93 (1 H, dd, *J* 17.5 and 8.5 Hz, 3'-H_b), 5.28 and 5.53 (each 1 H, d, *J* 14 Hz, CH_2Ar), and 7.66 and 8.22 (each 2 H, d, *J* 9 Hz, Ar).

Further elution gave a second batch of the (4'*R*,5*R*,6*R*)-isomer (**24**) (32 mg; total yield 112 mg, 50%).

Thermolysis of Dihydropyrazoles.—(a) The dihydropyrazole (**16**) (100 mg, 0.21 mmol) was dissolved in a little ethyl acetate and toluene was then added to give a volume of 30 ml. The solution was heated under reflux for 4 h, and the solvents were evaporated off. The residue was then chromatographed on silica gel employing a gradient elution of chloroform to 5% ethanol in chloroform. Other than recovered starting material (20 mg) the only isolated product, obtained as a slightly impure pale-yellow solid (27 mg, 29%), was *p*-nitrobenzyl (5*R*)-3-[(*E*)-2-acetamidovinylthio]-6-isopropylidene-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**19**) (Found: M^+, 443.1151. $C_{21}H_{21}N_3O_6S$ requires *M*, 443.1151); $\lambda_{max.}$(EtOH) 315 nm; $\nu_{max.}$(KBr) 1 755, 1 690, 1 675sh, and 1 620 cm^{-1}; $\delta_H[(CD_3)_2NCDO]$ 1.85, 2.02, and 2.07 (each 3 H, s, Me_2C= and MeCO), 3.11 (1 H, dd, *J* 18.5 and 7.5 Hz, 4-H_a), 3.33 (1 H, dd, *J* 18.5 and 10 Hz, 4-H_b), 4.80 (1 H, t, *J ca.* 9 Hz, 5-H), 5.39 and 5.58 (each 1 H, d, *J* 14 Hz, CH_2Ar), 6.02 (1 H, d, *J* 14 Hz, SCH=), 7.21 (1 H, dd, *J* 10.5 and 14 Hz, =C*H*NH), 7.87 and 8.30 (each 2 H, d, *J* 9 Hz, Ar), and 10.48 (1 H, d, *J* 10.5 Hz, NH). The spectrum also revealed a minor impurity.

(b) A solution of the dihydropyrazole (**12**) (100 mg, 0.21 mmol) in toluene (50 ml), containing sufficient ethyl acetate to maintain solubility, was heated under reflux for 15 h. The solution was evaporated and the crude product was chromatographed on silica gel employing a gradient elution of chloroform to 5% ethanol in chloroform. Two products were isolated, the least polar being the isopropylidene derivative (**19**) (18 mg, 20%). The more polar component, isolated as a gummy solid (16 mg, 21%), was identified as p-*nitrobenzyl* 3-[(E)-2-*acetamidovinylthio*]*pyrrole*-2-*carboxylate* (**22**) (Found: M^+, 361.0732. $C_{16}H_{15}N_3O_5S$ requires 361.0732); $\lambda_{max.}$(EtOH) 297 and 269 nm; $\nu_{max.}$(KBr) 1 690, 1 670, and 1 625 cm^{-1}; $\delta_H[(CD_3)_2NCDO]$ 2.03 (3 H, s, MeCO), 5.50 (2 H, s, CH_2Ar), 6.02 (1 H, d, *J* 13.5 Hz, =CHS), 6.07 (1 H, t, *J ca.* 2.5 Hz, 4-H), 7.18 (1 H, t, *J ca.* 2.5 Hz, 5-H), 7.24 (1 H, dd, *J* 13.5 and 10.5 Hz, =C*H*NH), 7.83 and 8.32 (each 2 H, d, *J* 9 Hz, Ar), and 12.13 (1 H, br, 1-H).

(c) The pyrazoline (**17**) (50 mg, 0.11 mmol) was similarly

heated in toluene. After 8 h, t.l.c. showed a mixture (*ca.* 1:1) of starting material and a less polar component, but insoluble polymers had also formed in the reaction. On isolation by silica-gel chromatography, the product was again found to be the 6-isopropylidene derivative (**19**) (7 mg, 15%).

(d) A solution of the dihydropyrazole (**24**) (32 mg, 0.08 mmol) in toluene (15 ml) was heated under reflux for 16 h. Evaporation of the solvent, followed by chromatography on silica gel using a gradient elution of 40 to 70% ethyl acetate in hexane afforded a product consisting largely of *p*-nitrobenzyl (5*R*)-3-ethylthio-6-isopropylidene-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylate (**21**) (11 mg, 37%) (Found: M^+, 388.1098. $C_{19}H_{20}N_2O_5S$ requires *M*, 388.1093); $\lambda_{max.}$(EtOH) 306, 264, and 235 nm; $\nu_{max.}(CH_2Cl_2)$ 1 765 and 1 705 cm^{-1}; $\delta_H(CDCl_3)$ 1.32 (3 H, t, *J* 7.5 Hz, *Me*CH_2), 1.83 and 2.11 (each 3 H, s, Me_2C=), 2.86 (2 H, m, CH_2S), 3.08 (1 H, dd, *J* 17.5 and 7.5 Hz, 4-H_a), 3.22 (1 H, dd, *J* 17.5 and 10 Hz, 4-H_b), 4.72 (1 H, br t, *J ca.* 9 Hz, 5-H), 5.24 and 5.54 (each 1 H, d, *J* 14 Hz, CH_2Ar), and 7.69 and 8.23 (each 2 H, d, *J* 8.5 Hz, Ar). Although this compound was homogeneous by t.l.c., the n.m.r. spectrum revealed the presence of an unidentified impurity.

Modification of the 3-*Substituent in the Spirodihydropyrazoles* (**12**) *and* (**14**): p-*Nitrobenzyl* (4'S,5R,6S)-4'-*Methyl*-3-[2-(p-*nitrobenzyloxycarbonylamino*)*ethylthio*]-7-*oxo*-1-*azabicyclo*-[3.2.0]*hept*-2-*ene*-6-*spiro*-5'-(4',5'-*dihydro*-3'H-*pyrazole*)-2-*carboxylate* (**29**).—A solution of the dihydropyrazole (**14**) (0.2 g, 0.425 mmol) in a mixture of 1,4-dioxane (10 ml) and water (1.5 ml) was treated with a solution of *N*-bromoacetamide (59 mg, 0.43 mmol) in dioxane (0.5 ml). After being stirred for 5 min at room temperature, the solution was diluted with chloroform (50 ml) and then washed with water (50 ml) containing a little brine. Evaporation of the dried organic solution gave a foamy residue which contained the 3-thiol (**28**); $\nu_{max.}(CH_2Cl_2)$ 1 785, 1 740sh, and 1 705 cm^{-1}. This was dissolved in *N*,*N*-dimethylformamide (5 ml) and the solution was stirred with 2-(*p*-nitrobenzyloxycarbonylamino)ethyl bromide (0.257 g, 0.85 mmol) and anhydrous potassium carbonate (59 mg, 0.43 mmol). After 25 min the solution was diluted with ethyl acetate (30 ml) and washed with water (3 × 25 ml) and brine (20 ml). Evaporation of the dried organic phase gave a residue which was chromatographed on silica gel using ethyl acetate as eluant. The major isolated product, obtained as a foam (61 mg, 24%) was the title C-3 *protected aminoethylthio derivative* (**29**); $\nu_{max.}(CH_2Cl_2)$ 3 450, 1 785, 1 725, and 1 705 cm^{-1}; $\delta_H(CDCl_3)$ 1.27 (3 H, d, *J* 7 Hz, *Me*CH), 2.36 (1 H, m, C*H*Me), 3.10 (2 H, m, SCH_2), 3.22 (1 H, dd, *J* 9.5 and 17.5 Hz, 4-H_a), 3.46 (2 H, m, CH_2NH), 4.11 (2 H, m, 4-H_b and 3'-H_a), 4.27 (1 H, t, *J* 9 Hz, 5-H), 4.94 (1 H, dd, *J* 17.5 and 8.5 Hz, 3'-H_b), *ca.* 5.25 (3 H, m, CH_2Ar and NH), 5.28 and 5.53 (each 1 H, d, *J* 14 Hz, CH_2Ar), 7.49 and 7.65 (each 2 H, d, *J* 8.5 Hz, Ar), and 8.21 (4 H, d, *J* 8.5 Hz, Ar); *m/z* 582.1421 ($M^+ - N_2$, $C_{27}H_{26}N_4O_9S$ requires 582.1420).

p-*Nitrobenzyl* (4'R,5R,6R)-4'-*Methyl*-3-(N-p-*nitrobenzyloxycarbonyl*-N',N'-*dimethylcarbamimidoylmethylthio*)-7-*oxo*-1-*azabicyclo*[3.2.0]*hept*-2-*ene*-6-*spiro*-5'-(4',5'-*dihydro*-3'H-*pyrazole*)-2-*carboxylate* (**26**).—A solution of chloroacetamidine hydrochloride[20] (4 g, 23.5 mmol) in dry dichloromethane (100 ml) at 0 °C containing triethylamine (10.4 ml, 75.2 mmol) was treated dropwise with a solution of *p*-nitrobenzyl chloroformate (8.28 g, 38.4 mmol) in dichloromethane (50 ml) over 30 min. The solution was stirred at room temperature for 16 h and washed with aqueous sodium hydrogen carbonate (100 ml), water (3 × 100 ml), and brine (50 ml). Evaporation of the dried organic solution gave a residue which was chromatographed using 50 and 60% ethyl acetate in hexane as eluant. *N*,*N*-Dimethyl-*N'*-(*p*-nitrobenzyloxycarbonyl)chloroacetamidine (**32**) was obtained as a gummy solid (3.77 g, 49%); $\nu_{max.}(CHCl_3)$ 1 670, 1 590, and 1 510 cm^{-1}; δ_H ($CDCl_3$, 60 MHz) 3.17 (6 H, s, Me_2N), 4.48 (2 H, s, CH_2Cl), 5.22 (2 H, s, CH_2Ar), and 7.52 and 8.12 (each 2 H, d, *J* 9 Hz, ArCH_2).

The dihydropyrazole (**12**) (0.2 g, 0.425 mmol) was treated with *N*-bromoacetamide as described in the previous example to give the crude 3-thiol (**25**). This was dissolved in *N*,*N*-dimethylformamide (7 ml) and treated with the chloroacetamidine (**32**) (0.2 g, 0.67 mmol) and potassium carbonate (59 mg, 0.85 mmol). After being stirred for 25 min the reaction mixture was partitioned between ethyl acetate (30 ml) and water (30 ml), and the organic layer then washed with water (30 ml) and brine (20 ml). Evaporation of the dried organic solution gave a residue which was chromatographed on silica gel eluting with chloroform followed by 1, 2, 3, and 4% ethanol in chloroform. The major product obtained (97 mg) was homogeneous by t.l.c. but contained an impurity (by n.m.r.) as well as the title carbamimidoyl derivative (**26**); $\lambda_{max.}$(EtOH) 314 and 264 nm; $\nu_{max.}(CH_2Cl_2)$ 1 785, 1 705, 1 665, 1 610, and 1 580 cm^{-1}; $\delta_H(CDCl_3)$ *inter alia* 1.23 (3 H, d, *J* 7 Hz, *Me*CH), 2.25 (1 H, m, C*H*Me), 3.20 (6 H, br s, Me_2N and 1 H, m, 4-H_a), 3.31 (1 H, dd, *J* 18.5 and 9.5 Hz, 4-H_b), 4.05 (1 H, m, 3'-H_a), 4.28 and 4.43 (each 1 H, d, *J* 12 Hz, SCH_2), 4.84 (1 H, t, *J* 9 Hz, 5-H), *ca.* 4.9 (1 H, m, 3'-H_b), 5.21 (2 H, s, CH_2Ar), 5.29 and 5.50 (each 1 H, d, *J* 14 Hz, CH_2Ar), and 7.53, 7.65, 8.18, and 8.22 (each 2 H, d, *J* 8.5 Hz, Ar).

Reaction of the (Z)-*Ethylidene* (**1**) *with Acetonitrile Oxide.*—Chlorine gas was bubbled into a solution of acetaldoxime (1.77 g, 30 mmol) in chloroform (45 ml) at −30 °C. The solution attained a deep-blue colour and eventually became green owing to an excess of chlorine. Argon was then flushed through the solution until the original blue colour had returned. The blue solution was added dropwise over 30 min to a stirred solution of the ethylidene (**1**) (2.62 g, 6.11 mmol) and triethylamine (3.03 g, 30 mmol) in chloroform (75 ml) at ice-bath temperature. The solution was then kept at 5 °C for 64 h before the addition of triethylamine (3.03 g, 30 mmol). A fresh solution of hydroxamic acid chloride was prepared from acetaldoxime (1.77 g, 30 mmol) and chlorine, and then was again added dropwise to the ethylidene solution. After a further 88 h at 5 °C, t.l.c. indicated that only a trace of starting material remained and that four new more-polar components had been formed in the reaction. The solution was washed with water (3 × 70 ml) and brine (50 ml), and then dried and evaporated. The crude product was chromatographed on silica gel eluting with chloroform followed by 2, 3, 4, 5, and 6% ethanol in chloroform. Partial separation of the products was achieved and fractions were combined into four separate batches (overall yield 1.45 g, 49%). After evaporation of solvents, each batch of product was individually rechromatographed employing the same eluant system. In this way, pure samples of the first three products, and a substantially pure sample of the fourth, were obtained.

The least-polar product was isolated as a foam (90 mg) which was crystallised from ethyl acetate–hexane to afford p-*nitrobenzyl* (4'S,5R,6S)-3-[(E)-2-*acetamidovinylthio*]-3',4'-*dimethyl*-7-*oxo*-1-*azabicyclo*[3.2.0]*hept*-2-*ene*-6-*spiro*-5'-(4',5'-*dihydroisoxazole*)-2-*carboxylate* (**37**), m.p. 198—201 °C (Found: C, 54.5; H, 4.85; N, 11.35%; M^+, 486.1208. $C_{22}H_{22}N_4O_7S$ requires C, 54.3; H, 4.55; N, 11.5%; *M*, 486.1209); $[\alpha]_D^{20}$ −206° (*c* 1, $CHCl_3$); $\lambda_{max.}$(MeCN) 322 (ε 15 840 dm^3 mol^{-1} cm^{-1}) and 263 nm (17 160); $\nu_{max.}(CH_2Cl_2)$ 3 420, 1 790, 1 700, and 1 625 cm^{-1}; $\delta_H(CDCl_3)$ 1.40 (3 H, d, *J* 7.5 Hz, *Me*CH), 2.00 and 2.08 (each 3 H, s, MeCO and MeC=N), 2.93 (1 H, dd, *J* 18.5 and 9.5 Hz, 4-H_a), 3.39 (1 H, dd, *J* 18.5 and 9 Hz, 4-H_b), 3.53 (1 H, q, *J* 7.5 Hz, C*H*Me), 4.21 (1 H, t, *J* 9 Hz, 5-H), 5.28 and 5.48 (each 1 H, d, *J* 13.5 Hz, CH_2Ar), 5.85 (1 H, d, *J* 13.5 Hz, =CHS), 7.26 (1 H, dd, *J* 10.5 and 13.5 Hz, =C*H*NH), 7.63 and 8.22 (each 2 H, d, *J* 8.5 Hz, Ar), and 7.97 (1 H, d, *J* 10.5 Hz, NH).

The second product (0.367 g) was identified as p-*nitrobenzyl* (5R,5'S,6S)-3-[(E)-2-*acetamidovinylthio*]-3',5'-*dimethyl*-7-*oxo*-1-*azabicyclo*[3.2.0]*hept*-2-*ene*-6-*spiro*-4'-(4',5'*dihydroisoxazole*)-2-*carboxylate* (**33**), m.p. 163—166 °C (from ethyl acetate–hexane) (Found: C, 54.4; H, 4.75; N, 11.1%; M^+, 486.1209. $C_{22}H_{22}N_4O_7S$ requires C, 54.3; H, 4.55; N, 11.5%; *M*, 486.1209); $[\alpha]_D^{20}$ −145° (*c* 1, $CHCl_3$); $\lambda_{max.}$(MeCN) 324 (16 920 dm^3 mol^{-1} cm^{-1}) and 264 nm (18 680); $\nu_{max.}$(CH_2Cl_2) 3 430, 1 785, 1 705, and 1 620 cm^{-1}; δ_H($CDCl_3$) 1.50 (3 H, d, *J* 6.5 Hz, *Me*CH), 2.11 (6 H, s, MeCO and MeC=N), 3.05 (2 H, overlapping dds, *J ca.* 19 and 10 Hz, 4-H_2), 4.29 (1 H, t, *J* 9.5 Hz, 5-H), 4.74 (1 H, q, *J* 6.5 Hz, C*H*Me), 5.27 and 5.51 (each 1 H, d, *J* 14 Hz, CH_2Ar), 5.90 (1 H, d, *J* 13.5 Hz, =CHS), 7.29 (1 H, dd, *J* 10.5 and 13.5 Hz, =C*H*NH), 7.64 and 8.23 (each 2 H, d, *J* 9 Hz, Ar), and 7.68 (1 H, br d, *J* 10.5 Hz, NH).

The third product (0.151 g) was found to consist of p-*nitrobenzyl* (4'R,5R,6R)-3-[(E)-2-*acetamidovinylthio*]-3',4'-*dimethyl*-7-*oxo*-1-*azabicyclo*[3.2.0]*hept*-2-*ene*-6-*spiro*-5'-(4',5'-*dihydroisoxazole*)-2-*carboxylate* (**35**), m.p. (from dichloromethane–ether) 165—172 °C (decomp.) (Found: C, 54.05; H, 4.65; N, 11.45. $C_{22}H_{22}N_4O_7S$ requires C, 54.3; H, 4.55; N, 11.5%); $[\alpha]_D^{20}$ −96° (*c* 1, $CHCl_3$); $\lambda_{max.}$(MeCN) 327 (ε 15 950 dm^3 mol^{-1} cm^{-1}) and 265 nm (17 630); $\nu_{max.}$(CH_2Cl_2) 1 785, 1 705, and 1 625 cm^{-1}; δ_H($CDCl_3$) 1.35 (3 H, d, *J* 7.5 Hz, *Me*CH), 1.98 and 2.09 (3 H, s, MeC=N and MeCO), 3.00 (1 H, dd, *J* 18.5 and 9.5 Hz, 4-H_a), 3.10 (1 H, dd, *J* 18.5 and 10 Hz, 4-H_b), 3.48 (1 H, q, *J* 7.5 Hz, C*H*Me), 4.43 (1 H, t, *J* 10 Hz, 5-H), 5.28 and 5.47 (each 1 H, d, *J* 14 Hz, CH_2Ar), 5.93 (1 H, d, *J* 13.5 Hz, =CHS), 7.27 (1 H, dd, *J* 10.5 and 13.5 Hz, =C*H*NH), and 7.65 and 8.22 (each 2 H, d, *J* 8.5 Hz, Ar); *m/z* 427.0832 (M^+ − $MeCONH_2$, $C_{20}H_{17}N_3O_6S$ requires *M*, 427.0838).

The fourth and most polar product, obtained as an oil (40 mg), was contaminated with a little of the third component (**35**) and was deduced to be *p*-nitrobenzyl (5*R*,5'*S*,6*S*)-3',5'-dimethyl-3-(3-methyl-5-acetamido-4,5-dihydroisoxazol-4-ylthio)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-6-spiro-4'-(4',5'-dihydroisoxazole)-2-carboxylate (**38**); $\lambda_{max.}$(EtOH) 314 and 265 nm; $\nu_{max.}$(CH_2Cl_2) 3 430, 1 790, and 1 705 cm^{-1}; δ_H($CDCl_3$) 1.50 (3 H, d, *J* 6.5 Hz, *Me*CH), 2.02, 2.12, and 2.15 (each 3 H, s, MeCO and 2 × MeC=N), 3.06 (1 H, dd, *J* 18 and 9.5 Hz, 4-H_a), 3.61 (1 H, dd, *J* 18 and 9.5 Hz, 4-H_b), 4.41 (1 H, d, *J* 1.5 Hz, SCH), 4.45 (1 H, t, *J* 9.5 Hz, 5-H), 4.78 (1 H, q, *J* 6.5 Hz, C*H*Me), 5.27 and 5.50 (each 1 H, d, *J* 13.5 Hz, CH_2Ar), 6.07 (1 H, dd, *J* 6.5 and 1.5 Hz, C*H*NH), 6.60 (1 H, d, *J* 6.5 Hz, NH), and 7.64 and 8.23 (each 2 H, d, *J* 9 Hz, Ar).

Hydrogenolysis of p-*Nitrobenzyl Esters: General Procedure.*— 5% Palladium on charcoal (1.5 × weight of ester) was suspended in 30% aqueous dioxane (10 ml) and shaken with hydrogen at ambient pressure and temperature for 30 min. A solution of the appropriate *p*-nitrobenzyl ester (0.04—0.15 mmol) in 1,4-dioxane (2 ml) was added to the hydrogenation vessel together with pH 7 0.05M-sodium phosphate buffer solution (2 ml) (mixture of $Na_2HPO_4 \cdot 12\,H_2O$ and NaH_2PO_4), and shaking under hydrogen was continued for 2—3 h. For the preparation of sodium salts, sodium hydrogen carbonate (1 mol equiv.) was added at this stage. The suspension was filtered over Celite and the latter then thoroughly washed with water (*ca.* 25 ml); the filtrate was then washed with ethyl acetate (3 × 25 ml). The aqueous solution was briefly concentrated further in order to remove any residual organic solvents, and was then examined by u.v. spectroscopy to determine whether further purification was necessary.

(a) *Sodium* (4'S,5R,6S)-3-[(E)-2-*Acetamidovinylthio*]-4'-*methyl*-7-*oxo*-1-*azabicyclo*[3.2.0]*hept*-2-*ene*-6-*spiro*-5'-(4',5'-*dihydro*-3'H-*pyrazole*)-2-*carboxylate* (**15**). The dihydropyrazole ester (**14**) (20 mg, 0.042 mmol) was hydrogenolysed to give an aqueous solution containing the title sodium salt (**15**); $\lambda_{max.}$(H_2O) 309 nm (yield 10 mg, based on an estimated value of ε 15 000). The solution contained largely one component by h.p.l.c.

(b) *Sodium* (4'R,5R,6R)-3-[(E)-2-*Acetamidovinylthio*]-4'-*methyl*-7-*oxo*-1-*azabicyclo*[3.2.0]*hept*-2-*ene*-6-*spiro*-(4',5'-*dihydro*-3'H-*pyrazole*)-2-*carboxylate* (**13**). The aqueous solution obtained from hydrogenolysis of the ester (**12**) (35 mg, 0.074 mmol) was concentrated to a volume of *ca.* 1 ml and subjected to chromatography on Biogel P2, eluting with water. Examination of the u.v. spectra of the column fractions suggested that considerable degradation had occurred. However, those fractions possessing a u.v. absorption at $\lambda_{max.}$(H_2O) 306 nm were collected to give an aqueous solution of the title sodium salt (**13**). H.p.l.c. of the solution showed one major peak.

(c) (4'S,5R,6S)-3-(2-*Aminoethylthio*)-4'-*methyl*-7-*oxo*-1-*azabicyclo*[3.2.0]*hept*-2-*ene*-6-*spiro*-5'-(4',5'-*dihydro*-3'H-*pyrazole*-2-*carboxylic acid* (**30**). Hydrogenolysis of the bis-*p*-nitrobenzyl derivative (**29**) (55 mg, 0.95 mmol) gave an aqueous solution which was concentrated and chromatographed on Diaion HP20, eluting with water followed by 5 and 10% ethanol in water. Those fractions having a u.v. absorption at $\lambda_{max.}$(H_2O) 293 nm and a single component by h.p.l.c. were combined and freeze-dried to afford the title amino acid (**30**) (3 mg) as a solid; $\lambda_{max.}$(H_2O) 293 nm; $\nu_{max.}$(KBr) 1 760 and 1 592 cm^{-1}.

(d) (4'R,5R,6R)-3-(N^1,N^1-*Dimethylamidinomethylthio*)-4'-*methyl*-7-*oxo*-1-*azabicyclo*[3.2.0]*hept*-2-*ene*-6-*spiro*-5'-(4',5'-*dihydro*-3'H-*pyrazole*)-2-*carboxylic acid* (**31**). The crude dihydropyrazole ester (**26**) (90 mg) was hydrogenolysed to give an aqueous solution which was concentrated to small volume and chromatographed on Biogel P2 eluting with water. Fractions possessing a u.v. absorption at $\lambda_{max.}$(H_2O) *ca.* 300 nm were found to contain two components by h.p.l.c. The combined fractions were subjected to further chromatography on Diaion HP20 eluting with water followed by 5 and 10% ethanol in water. Separation of the two components was achieved and each was obtained as a solid by freeze-drying the respective solutions. The first-eluted, more polar product (4.5 mg) was thought to possess the C-3 carbamoylmethyl side-chain resulting from hydrolysis of the amidine function in (**31**).* The least polar product (4.4 mg) was the title amidino derivative (**31**); $\lambda_{max.}$(H_2O) 299 nm; δ_H (D_2O, reference peak HOD at δ 4.80) 1.18 (3 H, d, *J* 7 Hz, *Me*CH), 2.57 (1 H, m, C*H*Me), 3.17—3.32 (8 H, m, 4-H_2 and Me_2N), 4.12 (1 H, dd, *J* 19 and 8 Hz, 3'-H_a), and 4.80—5.05 (m, 3-H_b, SCH_2 and 5-H, overlapping with HOD signal).

(e) *Sodium* (5R,5'S,6S)-3-[(E)-2-*Acetamidovinylthio*]-3',5'-*dimethyl*-7-*oxo*-1-*azabicyclo*[3.2.0]*hept*-2-*ene*-6-*spiro*-4'-(4',5'-*dihydroisoxazole*)-2-*carboxylate* (**34**). Hydrogenolysis of the dihydroisoxazole ester (**33**) (25 mg, 0.051 mmol) gave an aqueous solution which was concentrated to small volume and chromatographed on Biogel P2 eluting with water. Fractions containing a single component by h.p.l.c. and a u.v. absorption at 309 nm were combined and lyophilised to yield the title sodium salt (**34**) as a solid (18 mg); $\lambda_{max.}$(H_2O) 309 nm; $\nu_{max.}$(KBr) 1 765, 1 680, and 1 620 cm^{-1}; δ_H (D_2O, reference peak HOD at δ 4.80) 1.44 (3 H, d, *J* 6.5 Hz, *Me*CH), 2.06 and 2.09 (each 3 H, s, MeCO and MeC=N), 3.04 (1 H, dd, *J* 17 and 9 Hz, 4-H_a), 3.15 (1 H, dd, *J* 17 and 9.5 Hz, 4-H_b), 4.73 (1 H, t, *J* 9.5 Hz, 5-H), 4.90 (1 H, q, *J* 6 Hz, C*H*Me), 6.03 (1 H, d, *J* 13.5 Hz, SCH=), and 7.17 (1 H, d, *J* 13.5 Hz, NCH=).

(f) *Sodium* (4'R,5R,6R)-3-[(E)-2-*Acetamidovinylthio*]-3',4'-*dimethyl*-7-*oxo*-1-*azabicyclo*[3.2.0]*hept*-2-*ene*-6-*spiro*-5'-(4',5'-

* We have observed analogous hydrolysis products from olivanic acid derivatives with C-3 amidinomethylthio substituents. It is uncertain at which stage the hydrolysis occurs, but it may account for the impurity present in ester (**26**).

dihydroisoxazole)-2-*carboxylate* (**36**). The dihydroisoxazole ester (**35**) (40 mg, 0.082 mmol) was hydrogenolysed to give an aqueous solution of the title sodium salt (**36**). This was freeze-dried to furnish a solid (20 mg); $\lambda_{max.}(H_2O)$ 311 nm; $\nu_{max.}$(KBr) 1 760, 1 680, and 1 620 cm^{-1}. The product was largely one component by h.p.l.c.

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