The Chemistry of Penicillanic Acids. Part III.[1] A Route to 1,2-Seco-Penicillins

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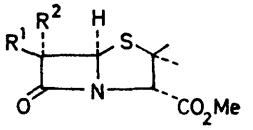
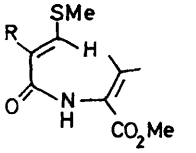
Treatment of 6β-(triphenylmethylamino)penicillanates with certain alkylating agents in the presence of strong anhydrous bases causes *S*-alkylation and cleavage of the thiazolidine ring between the sulphur atom and C-2. The scope and possible mechanism of the reaction are discussed. A typical product, (3*R*,4*R*)-1-(1-*p*-methoxybenzyloxycarbonyl-2-methylprop-1-enyl)-4-methylthio-3-(triphenylmethylamino)azetidin-2-one, has been converted into analogues of penicillins and cephalosporins containing a non-fused β-lactam ring.

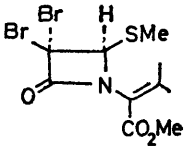
We reported in Part II that treatment of methyl penicillanate (1) or its 6α-bromo-derivative (2) with methyl iodide and strong anhydrous base cleaved both the thiazolidine and β-lactam rings to give compounds (4) and (5), respectively, whereas similar treatment of the 6,6-dibromo-derivative (3) gave the non-fused azetidinone (7). We have now extended these model investigations to a penicillin ester and related penicillanates containing a substituted 6β-amino-group.[2]



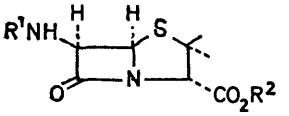
(1) $R^1 = R^2 = H$
(2) $R^1 = H, R^2 = Br$
(3) $R^1 = R^2 = Br$



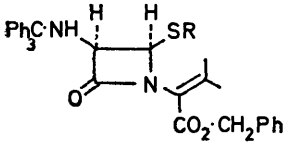
(4) R = H
(5) R = Br
(6) R = PhO·CH$_2$·CO·NH



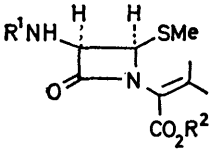
(7)



(8) R^1 = PhO·CH$_2$·CO, R^2 = Me
(9) R^1 = Ph$_3$C, R^2 = CH$_2$Ph
(10) R^1 = Ph$_3$C, R^2 = C$_6$H$_4$·NO$_2$(*p*)
(11) R^1 = Ph$_3$C, R^2 = CH$_2$·C$_6$H$_4$·OMe(*p*)
(12) R^1 = Ph$_3$C, R^2 = CO$_2$Et
(13) R^1 = PhCH(NH$_2$)·CO, R^2 = H



(14) R = Me
(15) R = CH$_2$CO$_2$CMe$_3$
(16) R = CH$_2$·C$_6$H$_4$·NO$_2$(*p*)
(17) R = CH(CH$_2$·C$_6$H$_4$·NO$_2$(*p*))·C$_6$H$_4$·NO$_2$(*p*)
(18) R = H



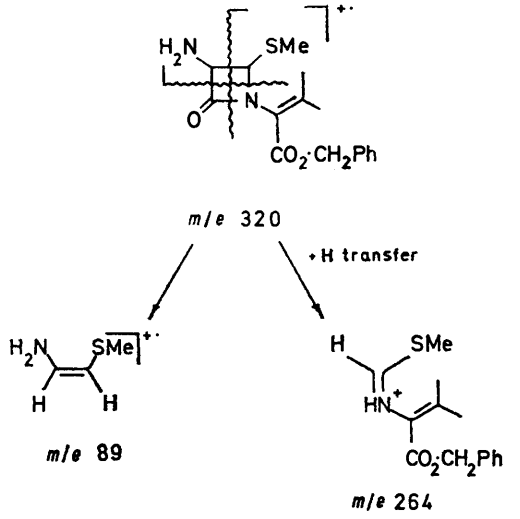
(19) R^1 = H, R^2 = CH$_2$Ph
(20) R^1 = Ph$_3$C, R^2 = CH$_2$·C$_6$H$_4$·OMe(*p*)
(21) R^1 = H, R^2 = CH$_2$·C$_6$H$_4$·OMe(*p*)
(22) R^1 = PhO·CH$_2$·CO, R^2 = H
(23) R^1 = PhCH(NH$_2$)·CO, R^2 = H
(24) R^1 = PhCH(NH·C(Me)=CH·CO$_2$Me)·CO, R^2 = CH$_2$·C$_6$H$_4$·OMe(*p*)

When phenoxymethylpenicillin methyl ester (8) was treated with methyl iodide and sodium hydride in tetrahydrofuran no β-lactam-containing transformation

[1] Part II, J. P. Clayton, J. H. C. Nayler, M. J. Pearson, and R. Southgate, *J.C.S. Perkin I*, 1974, 22.

[2] Preliminary report, J. P. Clayton, J. H. C. Nayler, R. Southgate, and P. Tolliday, *Chem. Comm.*, 1971, 590.

products could be identified, but the ester (6) was isolated in low yield. The stereochemistry of the methylthioacrvloyl system was assigned by analogy with the evident



SCHEME Characteristic fragmentations in the mass spectrum of compound (19)

trans-arrangement of the hydrogen atoms in the similarly prepared ester (4).[1] The compound is probably identical with one prepared by Kukolja and his co-workers [3] by a route which did not indicate the configuration.

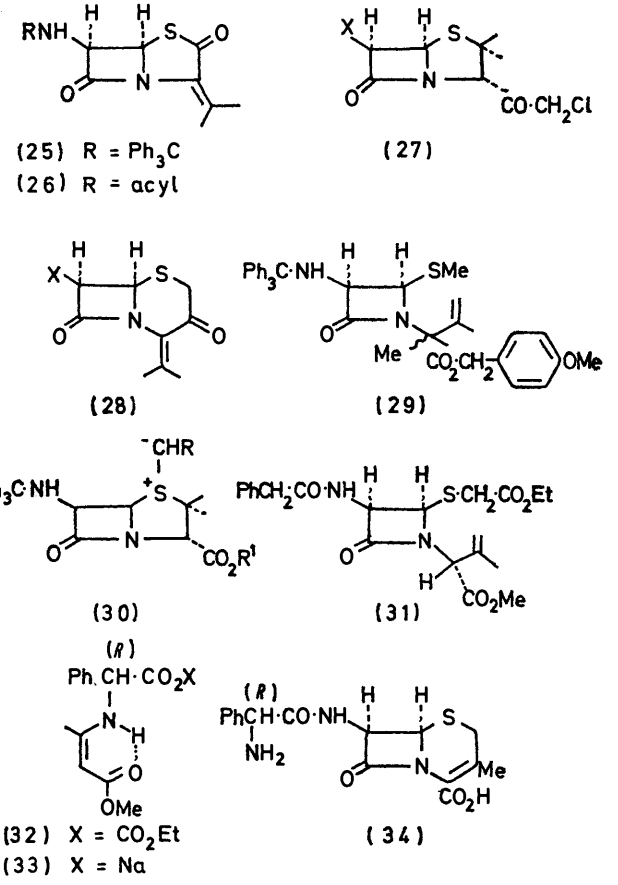
When benzyl 6β-triphenylmethylaminopenicillanate (9) was similarly treated with methyl iodide and sodium hydride only the thiazolidine ring was cleaved, and the azetidinone (14) was isolated in reasonable yield. The stereochemistry of this product was established by n.m.r. studies, which showed the characteristic *cis*-coupling of the β-lactam hydrogen atoms.[4] Detritylation with toluene-*p*-sulphonic acid in acetone gave the crystalline primary amine (19). The mass spectrum of this product showed the fragmentation pattern indicated in the Scheme, and similar patterns proved to be highly characteristic of all the non-fused azetidinones reported here.

The novel and selective cleavage of the 1,2-bond in 6β-(triphenylmethylamino)penicillanates appeared to be a promising starting point for the conversion of penicillins into related β-lactam-containing molecules. We therefore studied the reaction in some detail, varying the ester group, the base, and the alkylating agent.

The methyl and *p*-methoxybenzyl esters of 6β-(triphenylmethylamino)penicillanic acid behaved like the benzyl ester in yielding 4-(methylthio)azetidin-2-ones, but activated esters such as the *p*-nitrophenyl ester (10) underwent preferential intramolecular rearrangement in which the methyl iodide took no part and could be omitted. The rearrangement product proved to be the anhydropenicillin analogue (25), previously obtained [5] by the action of base on 6β-(triphenylmethylamino)-penicillanoyl chloride. In fact activated esters of penicillins generally give better yields of anhydropenicillins (26) than do the previously utilised [6] acid chlorides or mixed anhydrides, either inorganic or organic bases being used for the conversion.

Organic bases are also known to be useful in the intramolecular *S*-alkylation whereby the chloro-ketones (27; X = phthalimido or acylamino) are converted into 3-oxocephams (28).[7] Nevertheless organic bases, even such a strong one as 1,5-diazabicyclo[4.3.0]non-5-ene (DBN), proved unsuitable for converting 6β-(triphenylmethylamino)penicillanates into 4-(alkylthio)azetidin-2-ones by intermolecular *S*-alkylation. In these reactions, however, sodium hydride could be successfully replaced by other strong inorganic bases such as potassium t-butoxide and also, surprisingly in view of the susceptibility of fused β-lactams to nucleophilic attack, by powdered sodium hydroxide.

Attempts to effect thiazolidine cleavage of the penicillanate (9) with other alkylating agents succeeded only



with certain of the more reactive halides. Thus no reaction occurred when methyl iodide was replaced by methyl toluene-*p*-sulphonate. Ethyl iodide and ethyl β-bromo-

[3] S. Kukolja, R. D. G. Cooper, and R. B. Morin, *Tetrahedron Letters*, 1969, 3381.
[4] K. D. Barrow and T. M. Spotswood, *Tetrahedron Letters*, 1965, 3325.
[5] S. Wolfe, *Canad. J. Chem.*, 1968, **46**, 459.
[6] S. Wolfe, J. C. Godfrey, C. T. Holdrege, and Y. G. Perron, *J. Amer. Chem. Soc.*, 1963, **85**, 643; *Canad. J. Chem.*, 1968, **46**, 2549.
[7] B. G. Ramsay and R. J. Stoodley, *Chem. Comm.*, 1970, 1517; *J. Chem. Soc.* (*C*), 1971, 3859, 3864.

propionate also failed to give 4-(alkylthio)azetidin-2-ones, possibly owing to olefin formation from the halides. On the other hand, esters of bromoacetic acid were used successfully and t-butyl bromoacetate gave particularly good yields of the azetidinone (15). Satisfactory results were also obtained with allylic and benzylic halides, including allyl, β-methylallyl, cinnamyl, benzyl, *p*-methoxybenzyl, and *p*-nitrobenzyl bromides. Most of these reactions were conducted at room temperature, but reflux temperature was used with allyl bromide. When the reaction with *p*-nitrobenzyl bromide was carried out at reflux, the normal product (16) was accompanied by a by-product formulated on spectroscopic evidence as (17), apparently resulting from further alkylation in the *S*-substituent.

A different type of further alkylation was observed when the *p*-methoxybenzyl ester (11) was treated at room temperature with methyl iodide and potassium t-butoxide, both present in excess. The normal product (20) was accompanied by another with spectroscopic properties indicative of the βγ-unsaturated α-methyl ester structure (29). The dimethylated product (29) was also obtained when the monomethyl derivative (20) was further treated with methyl iodide and strong base.

The finding that base-promoted cleavage of the thiazolidine ring in structure (9) can be brought about only with certain alkyl halides suggests that the reaction probably does not proceed by β-elimination followed by alkylation of an intermediate thiol (18). It is equally unlikely that *S*-alkylation precedes β-elimination, since sulphonium salts do not appear to be formed in the absence of base. Another possible mechanism would involve attack on the sulphur atom by a carbene, generated by the action of base on the alkyl halide, followed by 1,2-bond cleavage in the sulphonium ylide (30). Indeed, subsequent to our work, it has been demonstrated [8] that carbenes generated by heating diazoacetates with copper compounds attack penicillin esters in the absence of base to give 1,2-secopenicillin esters such as (31), the double bond being shifted into conjugation with the ester function on subsequent treatment with base. However, if a carbene mechanism were operative in our process, reaction might be expected to proceed even if the C-3 proton were not activated by a neighbouring ester group. We therefore treated the mixed anhydride (12) with diethylamine to give the diethylamide, but found that no 1,2-bond cleavage occurred when this was treated with methyl iodide and sodium hydride in tetrahydrofuran, even on prolonged refluxing. It is therefore concluded that removal of the C-3 proton by base is an integral part of our ring-cleavage reaction, and that attack of the alkyl halide on sulphur then results in β-elimination as part of a concerted process.

The finding that the 1,2-bond in 6β-(triphenylmethylamino)penicillanates can be cleaved without disturbing the integrity of the β-lactam ring or the stereochemistry of the substituents attached thereto has facilitated the preparation of non-fused β-lactams which in other structural respects are direct analogues of the antibacterially active penicillins and cephalosporins. Thus treatment of compound (20) with toluene-*p*-sulphonic acid in acetone at 0° gave the toluene-*p*-sulphonate salt of the amine (21). The action of phenoxyacetyl chloride on the free base then gave the amide, and removal of the *p*-methoxybenzyl group with trifluoroacetic acid finally afforded the penicillin V analogue (22).

The amine (21) was also acylated with the mixed anhydride (32), prepared [9] from the condensation product (33) of sodium (*R*)-α-amino(phenyl)acetate and methyl acetoacetate. Removal of the amine and carboxy-protecting groups from (24) with trifluoroacetic acid gave the amorphous amino-acid (23), analogous to the important antibiotics ampicillin (13) and cephalexin (34).

Tests carried out by Mr. R. Sutherland and his colleagues showed that neither 1,2-secopenicillin [(22) or (23)] had significant antibacterial activity. The antibiotic effect of penicillins and cephalosporins is due to inhibition of membrane-bound enzymes involved in the later stages of the completion of the bacterial cell wall.[10] At least in some cases the inhibition appears to be irreversible, and has been attributed to acylation of the enzymes by the antibiotic, with opening of the β-lactam ring. The inactivity of 1,2-secopenicillins could be due to adoption of a conformation, different from that of the more rigid fused β-lactams, which is not accommodated at the active site, or it could be a result of the much reduced acylating power of non-fused β-lactams. Evidence of the latter is provided by the failure of 1,2-secopenicillins to give hydroxamic acids when treated with neutral hydroxylamine under the conditions of a widely-used penicillin assay procedure.[11]

EXPERIMENTAL

Sodium hydride was used in the form of a 50% dispersion in oil. Tetrahydrofuran was distilled from CaH_2. Other general procedures were as described in Part II.[1]

Methylation of Phenoxymethylpenicillin Methyl Ester.—The ester (8) (2 g) in tetrahydrofuran (20 ml) was treated with methyl iodide (0·91 g) and sodium hydride (0·15 g) and stirred at room temperature for 3 h. The mixture was then diluted with ethyl acetate (150 ml), washed with water, dried, and evaporated *in vacuo*. Chromatography of the residual gum (1·3 g) gave methyl 3-methyl-2-[3-methylthio-2-(phenoxyacetamido)acrylamido]crotonate (6) (0·14 g), m.p. 158—160° (from ethyl acetate) (lit.,[3] 153°); $\lambda_{max.}$ 222 (ε 14,100) and 285 nm (15,440); $\nu_{max.}$ (Nujol) 3270, 1718, 1678, 1638, and 1593 cm^{-1}; δ 1·84 (3H, s), 2·14 (3H, s), 2·41 (3H, s), 3·71 (3H, s), 4·64 (2H, s), 6·85—7·50 (6H, m, aromatic and olefinic), 7·60br (1H, exch.), and 7·88br (1H, exch.); *m/e* 378 (M^+) (Found: C, 57·1; H, 5·9; N, 7·3. Calc. for $C_{18}H_{22}N_2O_5S$: C, 57·1; H, 5·9; N, 7·4%).

Derivatives of 6β-(Triphenylmethylamino)penicillanate.—(a) *Benzyl ester.* 6β-Aminopenicillanic acid (216 g) in

[8] M. Yoshimoto, S. Ishihara, E. Nakayama, E. Shoji, H. Kuwano, and N. Soma, *Tetrahedron Letters*, 1972, 4387; M. Numata, Y. Imashiro, I. Minamida, and M. Yamaoka, *ibid.*, p. 5097.

[9] G. R. Fosker, J. H. C. Nayler, and J. A. Wilcox, B.P. 991,586/1965.

[10] J. L. Strominger, P. M. Blumberg, H. Suginaka, J. Umbreit, and G. G. Wickus, *Proc. Roy. Soc.* (*B*), 1971, **179**, 369.

[11] J. H. Ford, *Ind. and Eng. Chem.* (*Analytical*), 1947, **19**, 1004.

acetone (220 ml) was cooled (0°) and treated with triethylamine (140 ml). After stirring for 30 min, and while maintaining the mixture at 0°, benzyl bromide (120 ml) in acetone (350 ml) was added in portions, and the mixture was stirred at 0° for 4 h more, then poured into dry ether (3 l). The precipitate was filtered off and the filtrate washed with sodium hydrogen carbonate solution followed by water. The organic phase was treated with acetone (1 l) containing toluene-*p*-sulphonic acid (190 g) to give a crystalline precipitate of benzyl 6β-aminopenicillanate toluene-*p*-sulphonate salt (149 g), m.p. 155—158° (decomp.) (from methanol–ether) [lit.,[12] 153—154° (decomp.)]; $\nu_{max.}$ (Nujol) 1790 and 1740 cm^{-1}; δ [$(CD_3)_2SO$] 1·40 (3H, s), 1·63 (3H, s), 2·32 (3H, s), 4·60 (1H, s), 5·15 (1H, d, *J* 4·5 Hz), 5·25 (2H, s), 5·58 (1H, d, *J* 4·5 Hz), 7·15 (2H, d, *J* 8 Hz), 7·42 (5H, s), 7·55 (2H, d, *J* 8 Hz), and 8·63br (3H, exch.). This salt (12 g) was slurried with ethyl acetate and shaken with 5% sodium hydrogen carbonate solution. The organic layer was separated, dried, and evaporated to give benzyl 6β-aminopenicillanate (7·04 g) as an oil; $\nu_{max.}$ 3500, 3400, 1780, and 1750 cm^{-1}; δ 1·42 (3H, s), 1·60 (3H, s), 1·85br (2H, exch.), 4·40 (1H, s), 4·53 (1H, d, *J* 4 Hz), 5·16 (2H, s), 5·48 (1H, d, *J* 4 Hz), and 7·53 (5H, s). This ester (7 g) in dry methylene chloride (40 ml) was treated with triphenylmethyl chloride (7·7 g) and triethylamine (4·0 ml). After stirring for 1 h at room temperature the solution was washed with water, dried, and evaporated. Crystallisation of the residue from chloroform–ether gave benzyl 6β-(triphenylmethylamino)-penicillanate (9·35 g), m.p. 210° (lit.,[13] 210°); $\nu_{max.}$ 1780 and 1750 cm^{-1}; δ 1·27 (3H, s), 1·53 (3H, s), 3·03br (1H, exch.), 4·40 (1H, s), 4·47 (2H, s, β-lactam protons), 5·08 (2H, s), and 7·1—7·65 (20H, m, aromatic).

(b) p-*Methoxybenzyl ester.* In similar fashion 6β-aminopenicillanic acid (11·3 g) and *p*-methoxybenzyl bromide (10·26 g) furnished p-*methoxybenzyl* 6β-*aminopenicillanate toluene*-p-*sulphonate* salt (6·7 g), m.p. 130—131° (from acetone–ether); $\nu_{max.}$ (Nujol) 1795 and 1760 cm^{-1}; δ [$(CD_3)_2SO$] 1·37 (3H, s), 1·60 (3H, s), 2·30 (3H, s), 3·76 (3H, s), 4·52 (1H, s), 5·10 (1H, d, *J* 4·5 Hz), 5·15 (2H, s), 5·53 (1H, d, *J* 4·5 Hz), and 6·8—7·7 (8H, m, aromatic) (Found: C, 54·2; H, 5·6; N, 5·4; S,12·7. $C_{23}H_{28}N_2O_7S_2$ requires C, 54·3; H, 5·6; N, 5·5; S, 12·6%). This salt (38·1 g) was suspended in dry methylene chloride (200 ml) and treated with triethylamine (17·3 g), followed by triphenylmethyl chloride (25·7 g) and more triethylamine (17 g). After 2 h at room temperature the mixture was washed with water, dried, and evaporated to give a foam which was percolated through silica, with chloroform as eluant. Crystallisation from ether gave p-*methoxybenzyl* 6β-(*triphenylmethylamino*)*penicillanate* (11) (31·2 g), m.p. 137—139°; $\nu_{max.}$ 1780 and 1745 cm^{-1}; δ 1·40 (3H, s), 1·51 (3H, s), 3·78 (1H, d, *J* 13 Hz, exch.), 3·78 (3H, s), 4·37 (1H, s), 4·41—4·63 (2H, m, collapsing to singlet on D_2O exch., β-lactam protons), 5·03 (2H, s), 6·85 (2H, d, *J* 8 Hz, aromatic), and 7·1—7·7 (17H, m, aromatic) (Found: C, 72·5; H, 6·0; N, 4·6; S, 5·6. $C_{35}H_{34}N_2O_4S$ requires C, 72·7; H, 5·9; N, 4·4; S, 5·5%).

(c) p-*Nitrophenyl ester.* An ice-cold solution of 6β-(triphenylmethylamino)penicillanic acid [13] (1·61 g) and triethylamine (0·36 g) in methylene chloride (30 ml) was treated with ethyl chloroformate (0·38 g). The mixture was allowed to attain room temperature during 1 h and the resulting mixed anhydride treated *in situ* with *p*-nitrophenol (0·49 g) in tetrahydrofuran (5 ml). Next morning the solution was washed with water and sodium hydrogen carbonate solution, dried, and evaporated. Crystallisation of the residual syrup from ether gave p-*nitrophenyl* 6β-(*triphenylmethylamino*)*penicillanate* (10) (0·65 g), m.p. 176—178° (Found: C, 68·4; H, 5·4; N, 7·2. $C_{33}H_{29}N_3O_5S$ requires C, 68·4; H, 5·0; N, 7·25%).

(d) *Diethylamide.* 6β-(Triphenylmethylamino)penicillanic acid (0·916 g) was converted into the mixed ethoxyformic anhydride as in (c) and treated at 0° with diethylamine (0·146 g) in methylene chloride. The mixture was allowed to attain room temperature during 1 h, worked up as in (c), and chromatographed to give NN-*diethyl*-6β-(*triphenylmethylamino*)*penicillanamide* (0·47 g) as an amorphous white solid; $\nu_{max.}$ 3500, 1772, and 1642 cm^{-1}; δ 1·05 (3H, t, *J* 7 Hz) overlapping with 1·22 (3H, t, *J* 7 Hz), 1·33 (3H, s), 1·57 (3H, s), 2·87—3·76 (5H, m, one exch.), 4·31 (1H, d, *J* 4 Hz), 4·55 (1H, dd, *J* 4 and 6 Hz, collapsing to d, *J* 4 Hz, on D_2O exch.), 4·64 (1H, s), and 7·0—7·67 (15H, m, aromatic) (Found: C, 72·1; H, 7·0; N, 7·8; S, 6·4. $C_{31}H_{35}N_3O_2S$ requires C, 72·5; H, 6·9; N, 8·2; S, 6·2%).

Alkylation of Benzyl 6β-(*Triphenylmethylamino*)*penicillanate.*—(a) *With methyl iodide.* The ester (9) (10 g) in tetrahydrofuran (300 ml) was treated with sodium hydride (0·5 g) and methyl iodide (28 g). After stirring for 15 h at room temperature under nitrogen, the mixture was diluted with ethyl acetate (1 l) and washed with water. The dried organic layer was evaporated to a solid (11·2 g) which was separated by chromatography into unchanged penicillanate (9) (5·25 g) and (3*R*,4*R*)-1-(1-benzyloxycarbonyl-2-methylprop-1-enyl)-4-methylthio-3-(triphenylmethylamino)azetidin-2-one (14) (4·05 g), an amorphous solid, $\nu_{max.}$ 1760, 1720, and 1615 cm^{-1}; δ [$(CD_3)_2CO$] 1·58 (3H, s), 1·93 (3H, s), 2·00 (3H, s), 3·20 (1H, d, *J* 7 Hz, exch.), 4·42 (1H, dd, *J* 5 and 7 Hz collapsing to d, *J* 5 Hz on D_2O exch.), 4·56 (1H, d, *J* 5 Hz), 4·96 and 5·25 (2H, ABq, *J* 12·5 Hz), and 7·17—7·72 (20H, m, aromatic). Detritylation of this product (1 equiv. of toluene-*p*-sulphonic acid in acetone at 0° overnight) and liberation of the free base gave (3R,4R)-3-*amino*-1-(1-*benzyloxycarbonyl*-2-*methylprop*-1-*enyl*)-4-(*methylthio*)*azetidin*-2-*one* (19), m.p. 92—93°, $\nu_{max.}$ 3370, 1760, 1725, and 1630 cm^{-1}, *m/e* 320 (M^+) (Found: C, 59·9; H, 6·4; N, 8·7. $C_{16}H_{20}N_2O_3S$ requires C, 59·9; H, 6·3; N, 8·7%).

(b) *With t-butyl bromoacetate.* The ester (9) (1·1 g) and t-butyl bromoacetate (0·43 g) in tetrahydrofuran (10 ml) and t-butyl alcohol (10 ml) was treated dropwise over 5 h with a 0·9M-solution (2·44 ml) of potassium t-butoxide in t-butyl alcohol, in tetrahydrofuran. After stirring at room temperature for 1 h more, the solution was concentrated to small volume, diluted with ethyl acetate, and washed with water. The dried organic phase was evaporated and the residual gum separated by chromatography into unchanged (9) (0·3 g) and amorphous (3R,4R)-1-(1-*benzyloxycarbonyl*-2-*methylprop*-1-*enyl*)-4-(*t-butoxycarbonylmethylthio*)-3-(*triphenylmethylamino*)*azetidin*-2-*one* (15) (0·85 g), $\nu_{max.}$ 1760, 1720br, and 1625 cm^{-1}; δ 1·40 (9H, s), 2·00 (3H, s), 2·22 (3H, s), 2·33 and 2·68 (2H, ABq, *J* 14 Hz), 2·96 (1H, d, *J* 8 Hz, exch.), 4·50 (1H, dd, *J* 5 and 8 Hz, collapsing to d, *J* 5 Hz on D_2O exch.), 4·82 (1H, d, *J* 5 Hz), 4·98 and 5·22 (2H, ABq, *J* 12 Hz), and 7·1—7·6 (20H, m, aromatic) (Found: C, 72·1; H, 6·5; N, 3·9. $C_{40}H_{42}N_2O_5S$ requires C, 72·5; H, 6·4; N, 4·2%).

(c) *With allyl bromide.* The ester (9) (5 g), allyl bromide (11·25 g), and sodium hydride (0·44 g) in tetrahydrofuran

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(90 ml) were refluxed for 22 h under nitrogen. Usual work-up gave (3R,4R)-4-*allylthio*-1-(1-*benzyloxycarbonyl*-2-*methylprop*-1-*enyl*)-3-(*triphenylmethylamino*)*azetidin*-2-*one* (1 g) as needles (from ether), m.p. 110—111°, $\nu_{max.}$ 1760, 1730, 1625, 990, and 920 cm^{-1}; δ 1·98 (3H, s), 2·20 (3H, s), 2·32 (dd, *J* 14 and 8 Hz) and 2·64 (dd, *J* 14 and 6 Hz) (2H, SCH_2), 2·96br (1H, exch.), 4·46—5·12 (6H, m, β-lactam, benzylic, and $=CH_2$ protons), 5·12—5·64 (1H, m), and 7·08—7·60 (20H, m, aromatic) (Found: C, 75·2; H, 6·2; N, 4·7. $C_{37}H_{36}N_2O_3S$ requires C, 75·6; H, 6·2; N, 4·8%).

(d) *With* β-*methylallyl bromide*. A mixture of the ester (9) (23·6 g), β-methylallyl bromide (6·45 g, 1·1 mol. equiv.) and powdered sodium hydroxide (3·82 g, 2 mol. equiv.) in tetrahydrofuran (500 ml) was stirred at room temperature overnight, then evaporated *in vacuo*. The residue was washed with water and crystallised from ethyl acetate to give (3R,4R)-1-(1-*benzyloxycarbonyl*-2-*methylprop*-1-*enyl*)-4-(β-*methylallylthio*)-3-(*triphenylmethylamino*)*azetidin*-2-*one* (18·6 g, 71%), m.p. 148—149°; $\nu_{max.}$ 3050, 2095, 1760, 1720, and 1635 cm^{-1}; δ 1·6 (3H, s), 2·02 (3H, s), 2·23 (3H, s), 2·4 and 2·75 (2H, ABq, *J* 14 Hz, SCH_2), 3·6br (1H, exch.), 4·4—4·7 (4H, m, β-lactam protons and $=CH_2$), 4·95 and 5·17 (2H, ABq, *J* 12 Hz, benzylic), and 7·1—7·7 (20H, m, aromatic) (Found: C, 76·0; H, 6·4; N, 4·7; S, 5·3. $C_{38}H_{38}N_2O_3S$ requires C, 75·8; H, 6·3; N, 4·7; S, 5·3%).

(e) *With cinnamyl bromide*. The ester (9) (4·3 g) was alkylated with cinnamyl bromide (1·71 g) by method (d) to give (3R,4R)-1-(1-*benzyloxycarbonyl*-2-*methylprop*-1-*enyl*)-4-(*cinnamylthio*)-3-(*triphenylmethylamino*)*azetidin*-2-*one* (38%), m.p. 139—140° (from ethyl acetate); $\nu_{max.}$ 1735, 1718, and 1630 cm^{-1}; δ 1·96 (3H, s), 2·15 (3H, s), 2·3—3·1 (2H, m, SCH_2), 2·8—3·1br (1H, exch.), 4·56br (2H, s, β-lactam protons), 4·81 (2H, s, benzylic), 5·5—6·2 (2H, m, CH=CH), and 7·0—7·7 (25H, m, aromatic) (Found: C, 78·1; H, 6·3; N, 4·2; S, 4·5. $C_{43}H_{40}N_2O_3S$ requires C, 77·7; H, 6·0; N, 4·2; S, 4·8%).

(f) *With benzyl bromide*. The ester (9) (5 g), sodium hydride (0·49 g), and benzyl bromide (2·34 g) in tetrahydrofuran (110 ml) were stirred under nitrogen at room temperature for 48 h. Usual work-up gave unchanged (9) (0·6 g) and (3*R*,4*R*)-1-(1-benzyloxycarbonyl-2-methylprop-1-enyl)-4-benzylthio-3-(triphenylmethylamino)azetidin-2-one (1·9 g) as an amorphous solid, $\nu_{max.}$ 1760, 1720, and 1625 cm^{-1}; δ 1·91 (3H, s), 2·15 (3H, s), 2·98 and 3·33 (2H, ABq, *J* 13·5 Hz, SCH_2), 2·96br (1H, exch.), 4·47br (2H, sharpening to singlet on D_2O exch.), 4·91 (2H, s), and 7·0—7·7 (25H, m, aromatic). Detritylation with 1 equiv. of toluene-*p*-sulphonic acid in acetone at 0° for 4 h gave (3R,4R)-3-*amino*-1-(1-*benzyloxycarbonyl*-2-*methylprop*-1-*enyl*)-4-(*benzylthio*)*azetidin*-2-*one toluene*-p-*sulphonate salt*, m.p. 195—196° (from ethanol–ether); $\nu_{max.}$ (Nujol) 1795, 1725, and 1620 cm^{-1}; δ [$(CD_3)_2SO$] 1·88 (3H, s), 2·10 (3H, s), 2·28 (3H, s), 3·82 (2H, s), 4·91 (1H, d, *J* 5 Hz), 5·08 (2H, s), 5·22 (1H, d, *J* 5 Hz), 7·11 (2H, d, *J* 8 Hz), 7·25 (5H, s), 7·35 (5H, s), 7·57 (2H, d, *J* 8 Hz), and 8·85—9·20br (3H) (Found: C, 61·2; H, 5·6; N, 4·8; S, 11·5. $C_{29}H_{32}N_2O_6S_2$ requires C, 61·3; H, 5·7; N, 4·9; S, 11·3%).

(g) *With* p-*methoxybenzyl bromide*. Reaction of the ester (9) (46 g) with *p*-methoxybenzyl bromide (19 g) by method (d) gave (3R,4R)-1-(1-*benzyloxycarbonyl*-2-*methylprop*-1-*enyl*)-4-(p-*methoxybenzylthio*)-3-(*triphenylmethylamino*)*azetidin*-2-*one* as a foam (40 g); $\nu_{max.}$ 1760, 1720, and 1630 cm^{-1}; δ 1·91 (3H, s), 2·15 (3H, s), 2·93 and 3·29 (2H, ABq, *J* 14 Hz, covering broad NH signal at δ *ca.* 2·9), 3·27 (3H, s), 4·48br (2H, collapsing to singlet on D_2O exch., β-lactam protons), 4·94 (2H, s), 6·71 (2H, d, *J* 9 Hz, aromatic), 6·95 (2H, d, *J* 9 Hz, aromatic), and 7·1—7·8 (20H, m, aromatic) (Found: M^+, 668·2675. $C_{42}H_{40}N_2O_4S$ requires *M*, 668·2709). Detritylation gave (3R,4R)-3-*amino*-1-(1-*benzyloxycarbonyl*-2-*methylprop*-1-*enyl*)-4-(p-*methoxybenzylthio*)*azetidin*-2-*one toluene*-p-*sulphonate salt*, m.p. 172—173° (from acetone); $\nu_{max.}$ (Nujol) 1800, 1725, and 1630 cm^{-1}; δ [$(CD_3)_2SO$] 1·92 (3H, s), 2·10 (3H, s), 2·28 (3H, s), 3·70 (3H, s), 3·78 (2H, s), 4·95 (1H, d, *J* 5 Hz), 5·12 (2H, s), 5·25 (1H, d, *J* 5 Hz), 6·7—7·7 (13H, m, aromatic), and 9·0br (3H, exch.) (Found: C, 60·0; H, 5·6; N, 4·4; S, 11·3. $C_{30}H_{34}N_2O_7S_2$ requires C, 60·2; H, 5·7; N, 4·7; S, 10·7%).

(h) *With* p-*nitrobenzyl bromide*. The ester (9) (2 g) in tetrahydrofuran (60 ml) was stirred with sodium hydride (0·2 g) and *p*-nitrobenzyl bromide (0·79 g) at room temperature for 48 h. Usual work-up gave unchanged (9) (0·82 g) and (3R,4R)-1-(1-*benzyloxycarbonyl*-2-*methylprop*-1-*enyl*)-4-(p-*nitrobenzylthio*)-3-(*triphenylmethylamino*)*azetidin*-2-*one* (16) (0·6 g), m.p. 148—150° (from ether); $\nu_{max.}$ 1760, 1720, 1625, 1520, and 1340 cm^{-1}; δ 1·93 (3H, s), 2·12 (3H, s) 2·97br (1H, exch.), 3·03 and 3·36 (2H, ABq, *J* 14 Hz), 4·48br (2H, s, changing to d, δ 4·47, *J* 5 Hz, and d, δ 4·56, *J* 5 Hz on D_2O exch.), 4·81 and 5·08 (2H, ABq, *J* 12 Hz), 7·0—7·6 (22H, m, aromatic), and 8·00 (2H, d, *J* 9 Hz, aromatic) (Found: C, 72·1; H, 5·5; N, 6·1. $C_{41}H_{37}N_3O_5S$ requires C, 72·0; H, 5·5; N, 6·1%).

In another experiment the ester (9) (22 g), *p*-nitrobenzyl bromide (9 g), and sodium hydride (4 g) in tetrahydrofuran (500 ml) were refluxed for 8 h. Usual work-up gave unchanged (9) (10 g), the azetidinone (16) (1·85 g), and (3R,4R)-1-(1-*benzyloxycarbonyl*-2-*methylprop*-1-*enyl*)-4-[1,2-*bis*-(p-*nitrophenyl*)*ethylthio*]-3-(*triphenylmethylamino*)*azetidin*-2-*one* (17) (0·6 g), m.p. 210° (from ether); $\nu_{max.}$ 1760, 1720, 1630, 1525, and 1350 cm^{-1}; δ 1·81 (3H, s), 2·0 (3H, s), 2·8—3·4 (4H, m, one exch.), 4·35 (1H, d, *J* 5 Hz), 4·53 (1H, complex, sharpens to d, *J* 5 Hz, on D_2O exch.), 4·82br (2H, s, benzylic), 6·88—7·09 and 7·84—8·07 (two sets of 4H, *p*-subst. phenyls), and 7·2—7·8 (m, phenyls) (total aromatic integral *ca.* 28H) (Found: C, 70·4; H, 5·2; N, 6·9; S, 4·1. $C_{48}H_{42}N_4O_7S$ requires C, 70·4; H, 5·1; N, 6·9; S, 3·9%).

Attempted Methylation of p-*Nitrophenyl* 6β-(*Triphenylmethylamino*)*penicillanate*.—The ester (10) (116 mg) in tetrahydrofuran (5 ml) was stirred with sodium hydride (20 mg) and methyl iodide (0·4 ml) for 2 h, rapidly becoming yellow. Usual work-up gave (5*R*,6*R*)-3-isopropylidene-6-(triphenylmethylamino)-2-oxopenam (25); $\nu_{max.}$ 1785, 1700, and 1640 cm^{-1}; δ 1·98 (3H, s), 2·10 (3H, s), 3·10 (1H, d, *J* 11 Hz, exch.), 4·42 (1H, d, *J* 4 Hz), 4·87 (1H, dd, *J* 4 and 11 Hz, collapsing to d on D_2O exch.), and 7·2—7·7 (15H, m); m.p. 133° (lit.,[5] 134—135°). The same product was obtained in the absence of alkylating agent, by using either sodium hydride or 1,5-diazabicyclo[4.3.0]non-5-ene as the base.

Methylation of p-*Methoxybenzyl* 6β-(*Triphenylmethylamino*)*penicillanate*.—(a) *One equivalent of potassium t-butoxide*. The ester (11) (2 g) and methyl iodide (7 ml) in tetrahydrofuran (40 ml) were stirred under nitrogen for 4 h while a 1M-solution (3·4 ml) of potassium t-butoxide in t-butyl alcohol, in tetrahydrofuran was added dropwise. The mixture was then stirred for a further 30 min, diluted with ethyl acetate (200 ml), washed with water, dried, and evaporated *in vacuo*. Purification of the residue by chromatography, followed by crystallisation from ether, gave (3R,4R)-1-(1-p-*methoxybenzyloxycarbonyl*-2-*methylprop*-1-*enyl*)-4-*methylthio*-3-(*triphenylmethylamino*)*azetidin*-2-*one* (20) (0·5 g), m.p. 126—128°; $\nu_{max.}$ 1760, 1718, and 1615 cm^{-1};

δ [$(CD_3)_2CO$] 1·55 (3H, s), 1·93 (3H, s), 2·17 (3H s), 3·18 (1H, d, *J* 8 Hz, exch.), 3·77 (3H, s), 4·40 (1H, dd, *J* 5 and 8 Hz, collapsing to d, *J* 5 Hz, on D_2O exch.), 4·53 (1H, d, *J* 5 Hz), 4·87 and 5·18 (2H, ABq, *J* 12 Hz), 6·83 (2H, d, *J* 9 Hz, aromatic), and 7·1—7·7 (17H, m, aromatic) (Found: C, 73·3; H, 6·2; N, 5·0; S, 5·3%; M^+, 592·2344. $C_{36}H_{36}N_2O_4S$ requires C, 73·0; H, 6·1; N, 4·7; S, 5·4%; *M*, 592·2396). Further crude (20) (1·2 g) recovered from the ethereal mother liquors was dissolved in the minimum volume of acetone, cooled (−20°), treated with toluene-*p*-sulphonic acid monohydrate (0·42 g) in acetone, and set aside overnight at 0° to give crystals of (3R,4R)-3-*amino*-1-(1-p-*methoxybenzyloxycarbonyl*-2-*methylprop*-1-*enyl*)-4-(*methylthio*)*azetidin*-2-*one*-p-*toluenesulphonate salt* (0·7 g), m.p. 154—155° (from acetone); $\nu_{max.}$ (Nujol) 1805, 1722, and 1630 cm^{-1}; δ [$(CD_3)_2SO$] 1·95 (3H, s), 2·05 (3H, s), 2·18 (3H, s), 2·30 (3H, s), 3·75 (3H, s), 4·88 (1H, d, *J* 5 Hz), 5·13 (1H, d, *J* 5 Hz) partially obscured by 5·18 (2H, s), 6·86—7·66 (8H, m, aromatic), and 8·75vbr (3H, exch.) (Found: C, 54·7; H, 5·8; N, 5·2; S, 12·1. $C_{24}H_{30}N_2O_7S_2$ requires C, 55·2; H, 5·8; N, 5·4; S, 12·3%). The free *base* (21) was obtained as crystals (from ether), m.p. 78—79°; $\nu_{max.}$ 1760, 1720, and 1630 cm^{-1}; δ 1·73br (2H, s, exch.), 2·00 (6H, s), 2·22 (3H, s), 3·80 (3H, s), 4·38 (1H, d, *J* 5 Hz), 4·96 (1H, d, *J* 5 Hz), 4·98 and 5·26 (2H, ABq, *J* 12 Hz), 6·86 (2H, d, *J* 9 Hz, aromatic), and 7·30 (2H, d, *J* 9 Hz, aromatic) (Found: C, 58·5; H, 6·3; N, 7·8; S, 9·1. $C_{17}H_{22}N_2O_4S$ requires C, 58·3; H, 6·3; N, 8·0; S, 9·2%).

(b) *Potassium t-butoxide in excess.* The ester (11) (0·5 g) in tetrahydrofuran (20 ml) was stirred with methyl iodide (7 ml) for 4 h while a 1M-solution (2·0 ml, 2·3 equiv.) of potassium t-butoxide in t-butyl alcohol, in tetrahydrofuran, was added dropwise. The solution was set aside overnight and worked up as in (a). Chromatography gave (20) (0·2 g) and (3R,4R)-1-(1-p-*methoxybenzyloxycarbonyl*-1,2-*dimethylprop*-2-*enyl*)-4-*methylthio*-3-(*triphenylmethylamino*)*azetidin*-2-*one* (29) (0·25 g), apparently as a single isomer, m.p. 153—156° (from ethyl acetate–light petroleum); $\nu_{max.}$ 1760 and 1738 cm^{-1}; δ 1·57 (3H, s), 1·75 (3H, s), 1·77 (3H, d, *J* 1 Hz), 2·98 (1H, d, *J* 6 Hz, exch.), 3·75 (3H, s), 4·16 (1H, d, *J* 5 Hz), 4·3 (1H, partially obscured, collapsing to d, *J* 5 Hz, δ 4·33 on D_2O exch.), 4·80 (1H, s), 5·00 (1H, d, *J* 1 Hz), 5·07 (2H, s), 6·80 (2H, d, *J* 9 Hz, aromatic), and 7·0—7·7 (17H, m, aromatic) (Found: C, 73·8; H, 6·5; N, 4·5; S, 5·5%; M^+, 606·2552. $C_{37}H_{38}N_2O_4S$ requires C, 73·2; H, 6·3; N, 4·6; S, 5·3%; *M*, 606·2552). Use of a greater excess of potassium t-butoxide (*ca.* 5 equiv.) gave (29) as the major product (75%).

(3R,4R)-1-(1-*Carboxy*-2-*methylprop*-1-*enyl*)-4-*methylthio*-3-(*phenoxyacetamido*)*azetidin*-2-*one* (22).—The amine (21) (1·25 g) in methylene chloride (30 ml) was treated with triethylamine (1·54 ml) and cooled to −20°. A solution of phenoxyacetyl chloride (0·62 g) in methylene chloride (10 ml) was added dropwise over a few minutes. After a further 5 min at −10° the mixture was washed with water, dried, and evaporated. Crystallisation of the residue (0·91 g) from ethyl acetate gave (3R,4R)-1-(1-p-*methoxybenzyloxycarbonyl*-2-*methylprop*-1-*enyl*)-4-*methylthio*-3-(*phenoxyacetamido*)-*azetidin*-2-*one*, m.p. 120—122°, $\nu_{max.}$ 3400, 1770, 1720, 1695, and 1615 cm^{-1}; δ 1·85 (3H, s), 2·00 (3H, s), 2·25 (3H, s), 3·79 (3H, s), 4·56 (2H, s), 5·00 and 5·28 (2H, ABq, *J* 12 Hz), 5·06 (1H, d, *J* 5 Hz), 5·47 (1H, dd, *J* 5 and 9 Hz), and 6·8—7·5 (10H, m, aromatic and NH) (Found: C, 61·4; H, 5·9; N, 5·7. $C_{25}H_{28}N_2O_6S$ requires C, 61·9; H, 5·8; N, 5·8%). This ester (1·1 g) in dry benzene (10 ml) was cooled (*ca.* 5°) and treated with trifluoroacetic acid (7 ml), then allowed to attain room temperature. After 90 min trifluoroacetic acid was removed by co-distillation with benzene (3 evaporations). The residue was dissolved in chloroform and extracted with sodium hydrogen carbonate solution (0·2M; 50 ml). The extracts were acidified with hydrochloric acid and themselves extracted with ethyl acetate. Evaporation of the dried organic phase gave the acid (22) as an amorphous solid (0·4 g), $\lambda_{max.}$ 235 nm (ε 5300); $\nu_{max.}$ 3400, 2600br, 1770, 1690br, and 1630 cm^{-1}; δ 2·00 (3H, s), 2·05 (3H, s), 2·30 (3H, s), 4·63 (2H, s), 5·26 (1H, d, *J* 5 Hz), 5·56 (1H, dd, *J* 8 and 5 Hz, collapsing to d, *J* 5 Hz, on D_2O exch.), 6·8—7·5 (6H, m, one exch.), and 7·65 (1H, d, *J* 8·5 Hz, exch.).

(3R,4R)-3-[(R)-α-*Amino*(*phenyl*)*acetamido*]-1-(1-*carboxy*-2-*methylprop*-1-*enyl*)-4-(*methylthio*)*azetidin*-2-*one* (23).—Sodium (*R*)-α-(1-methoxycarbonylprop-2-enylamino)phenylacetate [9] (33) (1·04 g) in ethyl acetate (15 ml) was cooled to −15° and treated with *N*-methylmorpholine (3 drops) and ethyl chloroformate (0·39 ml). The mixture was stirred at −15° for 5 min to complete formation of the mixed anhydride (32), then treated dropwise at the same temperature with the amine (21) (1·4 g) in ethyl acetate (15 ml) during 10 min. The mixture was set aside at room temperature for 30 min, then washed with sodium hydrogen carbonate solution and water. The dried organic layer was evaporated and the residue chromatographed to give amorphous (3R,4R)-1-(1-p-*methoxybenzyloxycarbonyl*-2-*methylprop*-1-*enyl*)-3-[(R)-α-(1-*methoxycarbonylprop*-2-*enylamino*)(*phenyl*)*acetamido*]-4-(*methylthio*)*azetidin*-2-*one* (24) (0·79 g), $\nu_{max.}$ 3390, 3250, 1770, 1710—1690br, and 1655 cm^{-1}; δ 1·55 (3H, s), 1·82 (3H, s), 1·90 (3H), 2·22 (3H, s), 3·63 (3H, s), 3·78 (3H, s), 4·58 (1H, s), 4·93 (1H, d, *J* 5 Hz), 5·00 and 5·25 (2H, ABq, *J* 12 Hz, covering doublet, 1H, *J* 7 Hz at δ 5·15, collapsing to singlet on D_2O exch.), 5·45 (1H, dd, *J* 9 and 5 Hz collapsing to d, *J* 5 Hz on D_2O exch.), 6·8—7·6 (10H, m, one exch., aromatic and NH), and 9·45 (1H, d, *J* 7 Hz, exch.) (Found: C, 62·0; H, 6·2; N, 7·1; S, 5·5. $C_{30}H_{35}N_3O_7S$ requires C, 61·9; H, 6·0; N, 7·2; S, 5·5%). The protected ester (24) (0·2 g) in benzene (1 ml) was cooled (ice-bath) and treated with trifluoroacetic acid (0·2 ml). The mixture was kept at room temperature for 45 min, then trifluoroacetic acid was removed by co-distillation with benzene (3 evaporations). Trituration with ether gave the amorphous trifluoroacetate salt of the amino-acid (23) (0·136 g), $\lambda_{max.}$ 240 nm (ε 4470); $\nu_{max.}$ (Nujol) 1760 and 1680br cm^{-1}; δ [$(CD_3)_2SO$] 1·67 (3H, s), 2·00 (3H, s), 2·22 (3H, s), 5·13 (1H, s), 5·16 (1H, d, *J* 4·5 Hz, partially obscured), 5·40 (1H, dd, *J* 8 and 4·5 Hz, collapsing to d, *J* 4·5 Hz on D_2O exch.), 7·56 (5H, s, covering broad 1H exch.), and 9·56 (1H, d, *J* 8 Hz, exch.).

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