C-Nucleosides. Part 1. Preparation of Tiazofurin and N-Substituted Tiazofurins from Benzyl (2',3',5'-Tri-O-benzoyl-β-D-ribofuranosyl)penicillinate

David C. Humber,[a] Keith R. Mulholland,[b] and Richard J. Stoodley[b,*]
[a] *Microbiological Chemistry Department, Glaxo Group Research Ltd., Greenford, Middlesex UB6 0HE*
[b] *Department of Chemistry, UMIST, P.O. Box, 88, Manchester M60 1QD*

The title penicillinate (**11a**)—assembled from 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-allonic acid (**12e**) and benzyl 6β-aminopenicillanate (**13a**)—has been transformed into tiazofurin (**6a**), *N*-[(1*R*)-1-carbamoyl-2-methylprop-2-enyl]tiazofurin (**6c**), and *N*-(1-methoxycarbonyl-2-methylprop-1-enyl)-tiazofurin (**6d**).

C-Nucleosides—a family of compounds in which a sugar is linked at position 1 to a heterocycle by way of a C–C bond—are of considerable interest because of the antibacterial, antitumour, and antiviral properties of certain representatives. In the biologically active compounds, the sugar is D-ribofuranose and it is β-linked to a 5- or 6-membered heterocycle. Naturally occurring members include pyrazofurin (**1**), formycin (**2**), formycin B (**3**), showdomycin (**4**), and oxazinomycin (**5**).[1] Tiazofurin (**6a**),[2,3] which possesses significant antitumour properties and broad-spectrum antiviral activity, is a synthetic representative which has been subjected to clinical evaluation.[4]

The synthesis of *C*-nucleosides has attracted much attention and two main strategies have been devised. In one strategy, the heterocycle is assembled on a sugar derivative bearing a functionalised *C*-appendage at position 1; in the other, a preformed heterocycle is condensed with a sugar.

Potassium benzylpenicillinate (**7a**) has been converted into a wide range of heterocyclic systems by relatively short reaction sequences.[5] For example, it has been transformed into the thiazole (**8a**),[6] the oxazole (**9**),[7] and the imidazole (**10**).[8] It occurred to us that if these processes could be extended to β-D-ribofuranosylpenicillinates, *e.g.* (**11a**), a new method for the synthesis of *C*-nucleosides would emerge. In particular, from a common intermediate, it should be possible to derive *C*-nucleosides incorporating a range of heterocycles, each with appendages which should permit extensive structural variation.

In this paper, we show the feasibility of our plan by describing the conversion of the penicillin (**11a**)—assembled from the ribofuranosyl cyanide (**12a**)[9] and benzyl 6β-aminopenicillanate (**13a**)[10]—into tiazofurin (**6a**) and *N*-substituted tiazofurins.

Prior to this work, two syntheses of tiazofurin (**6a**) had been reported, both stemming from the ribofuranosyl cyanide (**12a**).[2,3,11] In one route, [2,3] in which the thiazole ring was constructed by the classical Hantzsch method, the overall yield of tiazofurin (**6a**) was *ca.* 26%. Thus, the nitrile (**12a**) was converted into the thioamide (**12b**) by the action of hydrogen sulphide and thence into the thiazole (**14a**) by treatment with ethyl bromopyruvate; compound (**14a**) was then transformed into tiazofurin (**6a**) by reaction with ammonia. A complication arose in the reaction of the thioamide (**12b**) with ethyl bromopyruvate; compound (**14a**) was accompanied by its α-anomer and by the furan derivative (**15a**).[3] In the second route,[11] which was effected in *ca.* 19% overall yield, the nitrile (**12a**) was converted by the action of sodium methoxide into the imino ether (**12c**), which was treated with hydrogen sulphide to give the thioester (**12d**); condensation of the last-cited compound with ethyl 2-amino-2-cyanoacetate afforded the thiazole (**6b**), which was transformed into tiazofurin (**6a**) by a reductive deamination (HNO_2–H_3PO_2) and ammonolysis sequence.

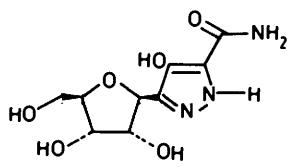
Results and Discussion

Our initial objective was to prepare the penicillinate (**11a**). It was envisaged that the compound would be accessible from the acid (**12e**) and the amine (**13a**). The acid (**12e**)[9] [prepared in 62% yield by hydrolysis of the nitrile (**12a**)] failed to react in a satisfactory manner with the amine (**13a**)[10] in the presence of some standard coupling reagents (dicyclohexylcarbodi-imide; 1,1-carbonyldi-imidazole; Et_3N–$ClCO_2Et$). However, the acid chloride (**12f**), generated *in situ* by treatment of the acid (**12e**) with oxalyl chloride–*N*,*N*-dimethylformamide (DMF) in dichloromethane, did react in the prescribed way with the amine (**13a**) in the presence of 4-dimethylaminopyridine (DMAP).[12] Following silica-gel chromatography, the penicillinate (**11a**) was isolated as a pure amorphous solid in 77% yield.

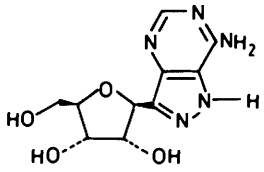
The structure of the penicillinate (**11a**) was established by its analytical and spectroscopic properties. In particular, the 300 MHz ^{1}H n.m.r. spectrum featured a doublet (*J* 4 Hz) at δ 5.54 and a double doublet (*J* 10 and 4 Hz) at δ 5.64 for the β-lactam hydrogen atoms, and a doublet (*J* 5 Hz) at δ 4.82 attributed to the anomeric hydrogen atom of the ribofuranose moiety.

Having accomplished the synthesis of the penicillin (**11a**), attention was turned to effecting its conversion into tiazofurin (**6a**) and *N*-substituted tiazofurins. Several groups have reported the transformation of penicillin esters into thiazoles of type (**8**). Thus, Wolfe *et al.*[6] found that methyl benzylpenicillinate (**7b**) was converted into the thiazole (**8a**) by the action of lithium 2-methylpropanethiolate in hexamethylphosphoramide (HMPA) followed by an acidic work-up. Cooper and José developed a three-step process[13] in which a penicillin ester, *e.g.* (**7c**), was oxidised to a sulphoxide, *e.g.* (**7d**), which was then reductively rearranged in the presence of trimethyl phosphite to a thiazoline-azetidinone, *e.g.* (**16a**); isomerisation to a thiazole, *e.g.* (**8b**), was achieved in the presence of trifluoroacetic acid (TFA). Mercaptoazetidinones, *e.g.* (**17a**) [generated by acidic hydrolysis of thiazoline-azetidinones, *e.g.* (**16b**)], have also been shown[14] to act as forerunners of thiazoles, *e.g.* (**8c**). It was decided, initially, to examine the Cooper process for effecting the conversion of the penicillin (**11a**) into the thiazole (**14c**).

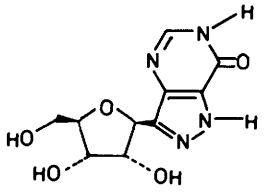
The transformation of the penicillin (**11a**) into the sulphoxide (**11b**) was achieved by using either sodium periodate (5 mol equiv.) in aqueous methanol or *m*-chloroperbenzoic acid (MCPBA) (1 mol equiv.) in dichloromethane. Following silica-gel chromatography, compound (**11b**) was isolated as an amorphous solid in 65% yield from the former reaction and in 87% yield from the latter.



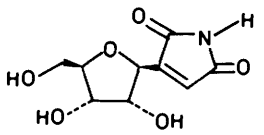
(1)



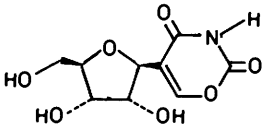
(2)



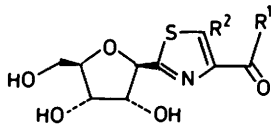
(3)



(4)



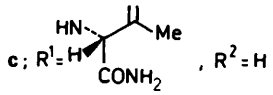
(5)



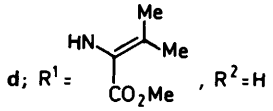
(6)

a; $R^1 = NH_2$, $R^2 = H$

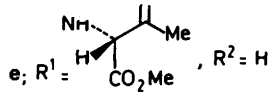
b; $R^1 = OEt$, $R^2 = NH_2$



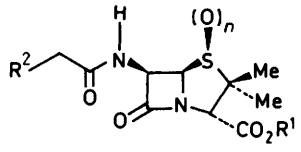
c; R^1 = , $R^2 = H$



d; R^1 = , $R^2 = H$



e; R^1 = , $R^2 = H$



(7)

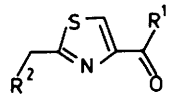
a ; $R^1 = K$, $R^2 = Ph$, $n = 0$

b ; $R^1 = Me$, $R^2 = Ph$, $n = 0$

c ; $R^1 = CH_2CCl_3$, $R^2 = OPh$, $n = 0$

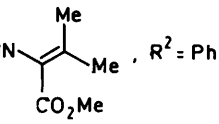
d ; $R^1 = CH_2CCl_3$, $R^2 = OPh$, $n = 1$

e ; $R^1 = CH_2Ph$, $R^2 = Ph$, $n = 0$

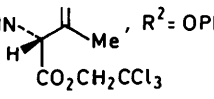


(8)

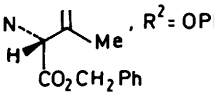
a ; R^1 = , $R^2 = Ph$



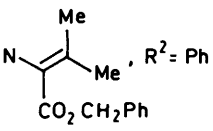
b ; R^1 = , $R^2 = OPh$



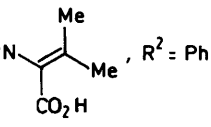
c ; R^1 = , $R^2 = OPh$



d ; R^1 = , $R^2 = Ph$



e ; R^1 = , $R^2 = Ph$



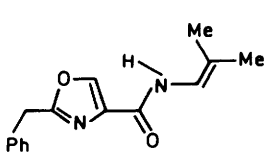
The structure of the sulphoxide (**11b**) was founded upon elemental and spectroscopic analysis, and upon the well established tendency of the oxidants to deliver oxygen to the sulphur atom of penicillins from the β-face.[15] In comparison with that of its precursor, the 300 MHz ^{1}H n.m.r. spectrum of the sulphoxide (**11b**) displayed a doublet (J 4 Hz) at δ 5.00 and a double doublet (J 10 and 4 Hz) at δ 6.00 ascribed to the β-lactam hydrogen atoms, and a doublet (J 5 Hz) at δ 4.80 for the anomeric hydrogen atom of the ribofuranose entity. Moreover, the geminal dimethyl group resonated as two singlets at δ 1.05 and 1.45 in the sulphoxide (**11b**) and at δ 1.38 and 1.48 in the sulphide (**11a**).

The sulphoxide (**11b**) was heated in the presence of triethyl phosphite (2 mol equiv.) both in boiling benzene and in boiling toluene. Following silica-gel purification, a syrupy product was isolated (in 64% yield from the former reaction and in 70% yield from the latter), which comprised a 5:1 mixture of the thiadiazabicycloheptenones (**18a** and **b**) according to ^{1}H n.m.r. spectroscopy. Treatment of the mixture with triethylamine effected its conversion into compound (**18b**) isolated as an amorphous solid in 91% yield.

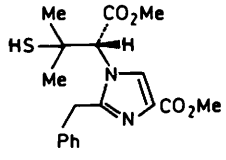
The thiadiazabicycloheptenone (**18b**) was characterised by its elemental composition and by its spectroscopic properties. In particular, the 300 MHz ^{1}H n.m.r. spectrum featured two singlets at δ 1.63 and 2.12 for the geminal dimethyl group, two doublets (J 4 Hz) at δ 5.70 and 5.93 for the β-lactam hydrogen atoms, and a doublet (J 6.5 Hz) at δ 5.23 attributed to the anomeric proton of the ribofuranose ring.

In the presence of TFA, the thiadiazabicycloheptenone (**18b**) was transformed into a syrupy product (73% yield after SiO_2 chromatography) which was not the desired thiazole (**14c**). Spectroscopic considerations left little doubt that the material was the furan derivative (**19**). Notably, the 300 MHz ^{1}H n.m.r. spectrum incorporated two doublets (J 4 Hz) at δ 5.91 and 6.11 for the β-lactam hydrogen atoms, and two doublets (J 3 Hz) at δ 6.60 and 6.95 ascribed to the furan hydrogen atoms.

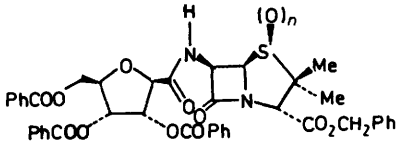
Clearly, TFA had induced the elimination of two molecules of benzoic acid from the ribofuranose ring of compound (**18b**)



(9)

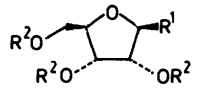


(10)



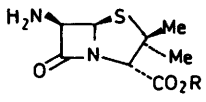
(11)

a; n = O
b; n = 1



(12)

a; R^1 = CN, R^2 = PhCO
b; R^1 = C(S)NH_2, R^2 = PhCO
c; R^1 = C(NH)OMe, R^2 = H
d; R^1 = C(S)OMe, R^2 = H
e; R^1 = CO_2H, R^2 = PhCO
f; R^1 = COCl, R^2 = PhCO



(13)

a; R = CH_2Ph
b; R = H

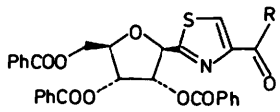
rather than the hoped-for isomerisation. Presumably, the ribofuranosyl anomeric hydrogen atom is acidified by the adjacent imino thioether function, enabling the species (**20**) to be generated which then undergoes an elimination to give the dihydrofuran (**21**) and thence the furan (**19**).

The thiadiazabicycloheptenone (**18b**) was subjected to a range of other acidic conditions in the hope of deriving the thiazole (**14c**). The use of toluene-*p*-sulphonic acid (PTSA) monohydrate (1 mol equiv.) in boiling methanol afforded a syrupy product (36% recovery by mass after SiO_2 chromatography) which, although homogeneous by t.l.c., comprised a 6:2:1 mixture of compounds (**14c**), (**15b**), and (**19**) according to 300 MHz ^{1}H n.m.r. spectroscopy. When the thiadiazabicycloheptenone (**18b**) was heated in boiling ethanol containing PTSA monohydrate (1 mol equiv.), the syrupy product (46% recovery by mass after SiO_2 chromatography) consisted of a 4:1 mixture of compounds (**14c**) and (**15b**).

In view of the difficulties experienced in effecting the (**18b**) ⟶ (**14c**) transformation in a direct and efficient manner, it was decided to proceed by way of the thiol (**17b**). Thus, the thiazoline-azetidinone (**18b**) was treated with PTSA in dichloromethane–acetone–water and the crude product [presumed to contain the thiol (**17b**)] was heated briefly in xylene; following silica-gel chromatography, the thiazole (**14c**) was isolated in 48% yield as an amorphous white solid.

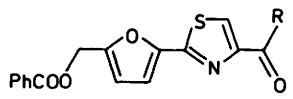
The thiazole (**14c**) was analytically and spectroscopically characterised. Its 300 MHz ^{1}H n.m.r. spectrum incorporated a singlet at δ 8.10 for the thiazole hydrogen atom, and a broad singlet at δ 8.41 attributed to the amide hydrogen atom; the ribofuranosyl anomeric hydrogen atom resonated as a doublet (J 5 Hz) at δ 5.60.

Having accomplished the synthesis of the thiazole (**14c**), attention was turned to the preparation of the related compounds (**14b** and **d**). Thus, the 5:1 mixture of the thiazoline-azetidinones (**18a** and **b**) was subjected to the hydrolytic conditions (*p*-$MeC_6H_4SO_3H$–CH_2Cl–Me_2CO–water) and the crude product was heated in xylene; following silica-gel



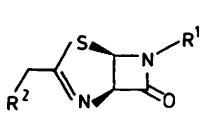
(14)

a; R = OEt
b; R = HN–CH(Me)–CO_2CH_2Ph
c; R = HN–C(Me)=C(Me)–CO_2CH_2Ph
d; R = NH_2



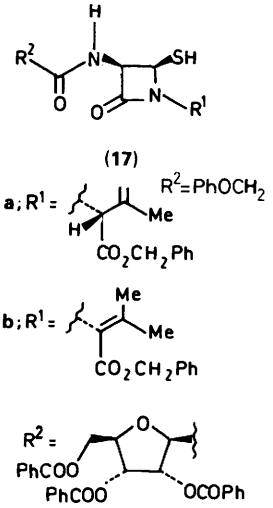
(15)

a; R = OEt
b; R = HN–C(Me)=C(Me)–CO_2CH_2Ph



(16)

a; R^1 = CH(Me)–$CO_2CH_2CCl_3$; R^2 = OPh
b; R^1 = CH(Me)–CO_2CH_2Ph; R^2 = OPh



(17)

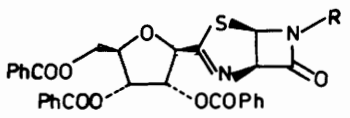
a; R^1 = CH(Me)–CO_2CH_2Ph; R^2 = $PhOCH_2$
b; R^1 = C(Me)=C(Me)–CO_2CH_2Ph
R^2 = PhCOO, PhCOO, OCOPh

chromatography, the thiazole (**14b**) was isolated in 54% yield as an analytically pure amorphous white solid.

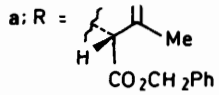
The thiazole (**14b**) was characterised by its spectroscopic properties. Although the signals for the thiazole and amide hydrogen atoms overlapped with those of six aromatic hydrogen atoms (appearing as a multiplet at δ 7.84—8.00) in the 300 MHz ^{1}H n.m.r. spectrum, the prop-2-enyl moiety appeared as a three-proton singlet at δ 1.70 and two one-proton broad singlets at δ 4.95 and 5.04; furthermore, the ribofuranosyl hydrogen atom resonated as a one-proton doublet (J 5 Hz) at δ 5.54.

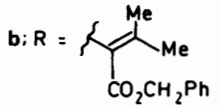
Two methods were examined to effect the removal of the β-lactam substituent from the thiazoline-azetidinone (**18b**). The one-pot procedure, developed by Beecham chemists and involving the use of potassium permanganate in aqueous acetone,[16] was unsatisfactory. However, the ozonolysis–methanolysis sequence, introduced by Cooper and José,[17] proved to be effective. Thus, a solution of compound (**18b**) in dichloromethane at −78 °C was saturated with ozone and then the mixture was allowed to warm up to room temperature; evaporation of the solvent and treatment of the residue with methanol–triethylamine led, after silica-gel chromatography, to the isolation of compound (**18c**) in 76% yield as a crystalline solid.

The constitution of the thiazoline-azetidinone (**18c**) followed from its analytical and spectral properties. In particular, the 300 MHz ^{1}H n.m.r. spectrum showed the presence of a one-proton doublet (J 4 Hz) at δ 5.45 for one β-lactam hydrogen atom, a

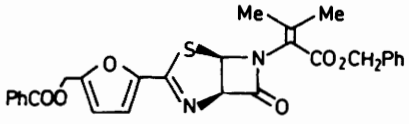


(18)

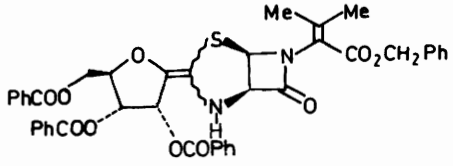
a; R = 

b; R = 

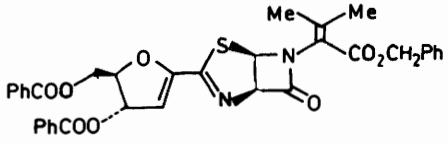
c; R = H



(19)



(20)



(21)

two-proton doublet (separation 4 Hz) at δ 5.83 for the other β-lactam hydrogen atom and for the ribofuranosyl anomeric hydrogen atom, and a broad one-proton singlet at δ 6.20 for the amide hydrogen atom.

When subjected to the aforecited hydrolysis–thermolysis conditions, the thiazoline-azetidinone (**18c**) was transformed into the thiazole (**14d**) (62% yield after SiO_2 chromatography), isolated as a crystalline solid. The 300 MHz ^1H n.m.r. spectrum of the product incorporated two broad singlets at δ 5.53 and 7.04 for the amide hydrogen atoms, a singlet at δ 8.10 for the thiazole hydrogen atom, and a doublet (*J* 4.5 Hz) at δ 5.59 ascribed to the ribofuranosyl anomeric hydrogen atom.

With the protected tiazofurin derivatives (**14b—d**) in hand, efforts were focussed upon the removal of their *O*-benzoyl groups.

Treatment of compound (**14d**) with methanolic ammonia gave tiazofurin (**6a**) (46% yield after SiO_2 chromatography and crystallisation) in an analytically pure state. The m.p. and optical rotation of the sample were in excellent agreement with the literature values.

Compound (**14b**) reacted with methanolic ammonia to give the crystalline *N*-substituted tiazofurin derivative (**6c**) (45% yield after SiO_2 chromatography) and with methanol–triethylamine–water[18] to afford the amorphous *N*-substituted tiazofurin derivative (**6d**) (54% yield after SiO_2 chromatography). Clearly, the benzyl ester function undergoes ammonolysis under the former conditions and methanolysis under the latter conditions [probably to give compound (**6e**) which then undergoes a base-induced isomerisation of the double bond]. The novel *N*-substituted tiazofurins (**6c** and **d**) were analytically and spectroscopically characterised; the chemical shifts of the thiazole hydrogen atoms (δ 8.14 *versus* 8.10) and the ribofuranosyl anomeric hydrogen atoms (δ 5.07 *versus* 5.13) were very similar in the two compounds (despite the fact that the spectra were recorded in D_2O *versus* $CDCl_3$).

Surprisingly, intractable mixtures resulted when compound (**14c**) was treated with methanolic ammonia, methanol–triethylamine–water, and methanolic sodium methoxide. However, the use of potassium cyanide in methanol[19] gave compound (**6d**) in excellent yield (91% after SiO_2 chromatography). Again, the transesterification conditions had resulted in an exchange of the benzyl ester function in addition to the removal of the *O*-benzoyl groups.

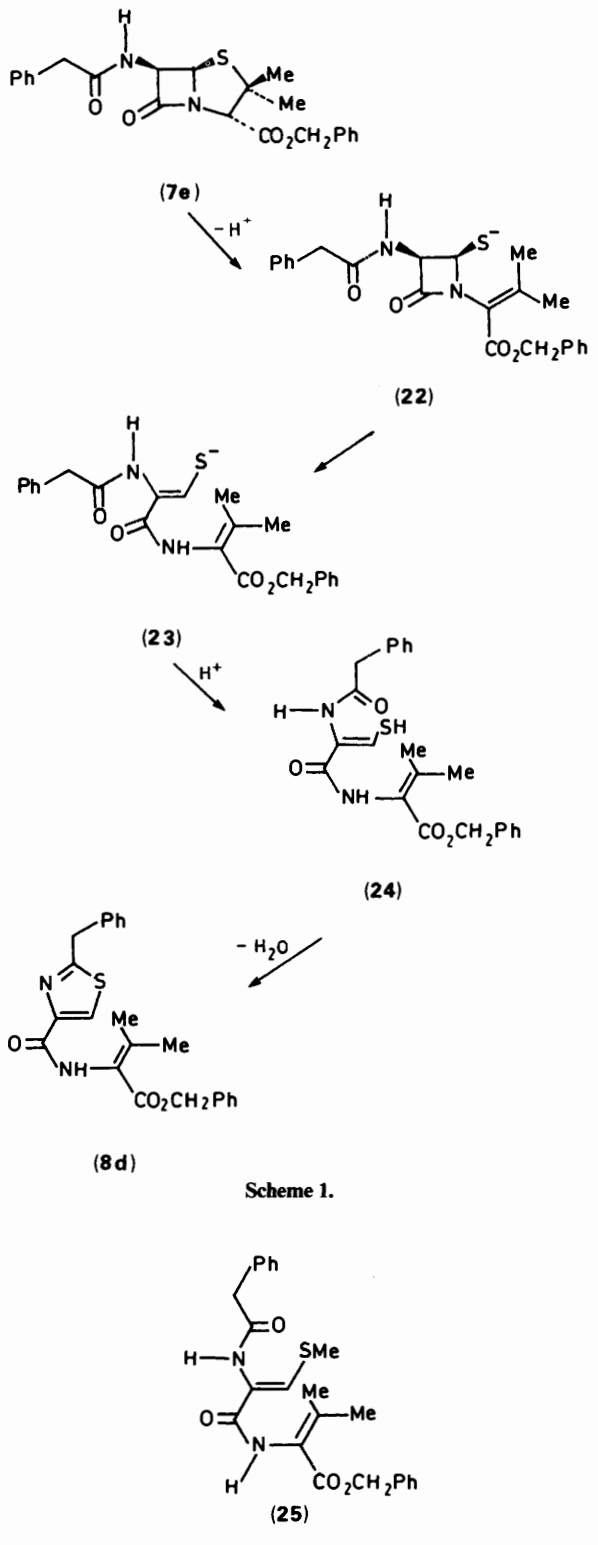
To summarise the position, the penicillin (**11a**) has been converted into tiazofurin (**6a**) in *ca.* 12% overall yield by a seven-step sequence, into the *N*-substituted tiazofurin (**6c**) in *ca.* 15% overall yield by a five-step sequence, and into the *N*-substituted tiazofurin (**6d**) in *ca.* 24% yield by a five-step sequence.

The inducement to shorten the synthesis of tiazofurin (**6a**) prompted us to subject the penicillin (**11a**) to the Wolfe conditions[6] ($LiSCMe_3$–HMPA). Disappointingly, an acidic work-up led to a complex array of products; chromatographic fractionation of the mixture led to a 10% recovery of the starting material but no other identifiable compound. To satisfy ourselves that the reaction had been performed correctly, benzyl benzylpenicillinate (**7e**)[20] was subjected to the Wolfe conditions. Following an acidic work-up and fractionation of the product by silica-gel chromatography, the thiazole (**8d**) was isolated as an oil in 15% yield. In accord with its structure, the thiazole (**8d**) displayed two three-proton singlets at δ 1.92 and 2.23 for the geminal dimethyl group, a one-proton singlet at δ 7.94 for the thiazole hydrogen atom, and a broad one-proton singlet at δ 8.57 for the amide hydrogen atom. The (**7e**) ⟶ (**8d**) transformation proceeded in much poorer yield than the (**7b**) ⟶ (**8a**) isomerisation (reported to occur in 80% yield[6]), suggesting that the nature of the ester substituent may be influential.

A consideration of the probable course of the (**7e**) ⟶ (**8d**) transformation, outlined in Scheme 1, suggested that lithium 2-methylpropanethiolate–HMPA was acting as a basic medium. Accordingly, there seemed to be a reasonable prospect of effecting the rearrangement using other base–solvent combinations. It was therefore decided to investigate this possibility, initially with benzyl benzylpenicillinate (**7e**).

Treatment of compound (**7e**) with 1M-sodium hydroxide (1 mol equiv.) in pyridine gave only starting material after an acidic work-up. However, repeating the reaction with two molar equivalents of the base led to the isolation of the thiazole (**8d**) (26% yield after SiO_2 chromatography). The use of 1M-sodium hydroxide (1 mol equiv.) or powdered sodium hydroxide (1 mol equiv.) in dimethyl sulphoxide (DMSO) was also effective in promoting the rearrangement; following an acidic work-up and silica-gel purification of the product, the thiazole (**8d**) was isolated in respective yields of 30 and 35%. Interestingly, the employment of two molar equivalents of 1M-sodium hydroxide in DMSO led to the isolation of the acid (**8e**) (38% yield after recrystallisation).

It seems likely, in the aforecited reactions, that the enethiolate (**23**) is generated *via* the thiolate (**22**) by the action of sodium hydroxide; acidification then affords the enethiol (**24**) which cyclises to the thiazole (**8d**). In accord with this notion, the



Scheme 1.

(25)

methylthio derivative (**25**) was obtained (24% yield after SiO_2 chromatography) when the penicillin (**7e**) was treated in tetrahydrofuran (THF) with potassium t-butoxide followed by iodomethane. The 300 MHz ^1H n.m.r. spectrum of compound (**25**) featured three singlets at δ 1.80, 2.09, and 2.32 for the geminal dimethyl and methylthio groups, a broad singlet at δ 6.78 for the amide hydrogen atom, and a singlet at δ 7.58 for the olefinic hydrogen atom. Compounds analogous to the methylthio derivative (**25**) have been isolated previously from reactions of penicillinate esters with sodium hydride–iodomethane.[10b]

Gratifyingly, treatment of the penicillin (**11a**) with 1M-sodium hydroxide (1 mol equiv.) in DMSO gave, after acidification and chromatography, the thiazole (**14c**) in 23% yield. The yield of the product was subsequently improved to 36% yield by using 5M-hydrochloric acid (rather than 1M-HCl) in the acidification step and heating the crude product in boiling xylene.

The transformation of the thiazole (**14c**) into tiazofurin (**6a**) was readily accomplished (61% yield after SiO_2 chromatography and crystallisation) by an ozonolysis–ammonolysis sequence. In consequence, the penicillin (**11a**) is convertible into tiazofurin (**6a**) in three steps in *ca.* 22% overall yield.

Compounds (**6c** and **d**) were inactive as antiviral agents.

Experimental

Dry solvents, referred to in the ensuing experiments, were prepared in the following manner: acetone was left over anhydrous calcium sulphate, decanted, distilled, and stored over 3 Å molecular sieves; dichloromethane, benzene, and xylene were left over anhydrous calcium chloride, decanted, and distilled; methanol was allowed to stand over 3 Å molecular sieves; DMSO was distilled under reduced pressure and stored over 4 Å molecular sieves; HMPA was distilled under reduced pressure from lithium aluminium hydride; THF was distilled from sodium-benzophenone. Light petroleum refers to that fraction boiling in the range 40—60 °C.

Column chromatography was effected under pressure using either Merck 9385 or Sorbsil C60 'flash' silica. The progress of reactions was followed by t.l.c. using either Merck 5735 (plastic) or 5715 (glass) plates; the plates were initially examined under u.v. light and then developed with iodine vapour, aqueous potassium permanganate, or 5M-sulphuric acid (followed by heating in an oven). Ozone was generated using a Wallace and Tieman ozonator. Evaporations were conducted under reduced pressure (using a water-pump or an oil-pump) with a Buchi rotary evaporator. M.p.s were determined by using either a Kofler hot-block apparatus or a Buchi 512 apparatus. Optical rotations were measured at *ca.* 20 °C with a Thorn Automation Type 243 polarimeter. I.r. spectra were recorded with a Perkin-Elmer 783 or 298 spectrometer. Either a Cary 118 or a Perkin-Elmer Lambda 15 spectrometer was employed to determine u.v. spectra. ^1H N.m.r spectra were measured at 60 MHz with a Varian EM360, at 250 MHz with a Bruker AM250, and at 300 MHz with either a Varian XL300 or a Bruker AC300 spectrometer. Electron impact (e.i.) and chemical ionisation (c.i.) mass spectra were determined using a Kratos MS45 instrument; fast-atom bombardment (f.a.b.) mass spectra were recorded on a VG ZAB-E instrument. Elemental analyses were performed with a Carlo-Erba Model 1106 analyser.

Preparation of 2,5-*Anhydro*-3,4,6-*tri*-O-*benzoyl*-D-*allonic Acid* (**12e**).—D-Ribose (25.0 g) was converted into 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose by the method of Recondo and Rinderknecht;[21] the recrystallised product (48.1 g, 57%) showed m.p. 128—129 °C (lit.,[21] 130—131 °C). The latter compound (42.8 g) was transformed into 2,5-anhydro-2,3,5-tri-*O*-benzoyl-D-allonitrile (**12a**) by the procedure of Bobek and Farkaš;[9a] the recrystallised material (30.3 g, 76%) possessed m.p. 78—79 °C (lit.,[9a] 78.5—80 °C). The allonitrile (**12a**) (25.0 g) was converted into the title compound (**12e**) by the literature method;[9a] after recrystallisation from cyclohexane–ethyl acetate, the product (16.0 g, 62%) showed m.p. 100—102 °C (lit.,[9b] 101—103.5 °C); δ(300 MHz; $CDCl_3$) 4.65—4.82 (3 H, m, 5-H and 6-H_2), 4.90 (1 H, br d, *J* 5 Hz, 2-H), 5.75 (1 H, br t, *J* 5 and 5 Hz, 3-H), 6.00 (1 H, br t, *J* 5 and 5 Hz, 4-H), and 7.32—7.62 and 7.89—8.13 (9 and 6 H, each m, together 3 × C_6H_5).

Preparation of Benzyl 6β-Aminopenicillanate Toluene-p-sulponic Acid Salt.—To a stirred ice-cooled suspension of 6β-aminopenicillanic acid (**13b**) (40.0 g, 185 mmol) in dry acetone (150 cm^3) under nitrogen was added triethylamine (25.4 cm^3, 182 mmol) followed by benzyl bromide (22.1 cm^3, 186 mmol). After 1.5, 3.0, and 4.5 h, further quantities of triethylamine (22.8 cm^3, 164 mmol) and benzyl bromide (19.9 cm^3, 167 mmol) were added. After a further 2 h, the mixture was poured into diethyl ether (1 dm^3) and the filtered solution was washed with aqueous sodium hydrogen carbonate followed by brine. To the dried ($MgSO_4$) organic layer was added a solution of PTSA monohydrate (41.0 g, 216 mmol) in acetone (100 cm^3). The white precipitate (60.1 g, 68%), which was collected by filtration and dried (*in vacuo*, $CaCl_2$), was the title compound m.p. 152—154 °C (decomp.) [lit.,[10a] 153—154 °C (decomp.);[10b] 155—158 °C (decomp.)]; δ(60 MHz; CD_3SOCD_3) 1.14 and 1.35 each 3 H, s, 2-Me_2), 2.00 (3 H, s, MeC_6H_4), 3.5 (3 H, br s, NH_3), 4.30 (1 H, s, 3-H), 4.84 and 5.26 (each 1 H, d, *J* 4 Hz, 5- and 6-H), 4.93 (2 H, s, PhCH_2O), 6.84 and 7.28 (each 2 H, d, *J* 7 Hz, C_6H_4), and 7.15 (5 H, s, C_6H_5).

Preparation of Benzyl (2′,3′,5′-Tri-O-benzoyl-β-D-ribofuranosyl)penicillinate (**11a**).—The allonic acid (**12e**) (18.1 g, 36.9 mmol) was dissolved in dry dichloromethane (110 cm^3) and to the cooled (ice–NaCl) stirred solution was added oxalyl chloride (4.10 cm^3, 47 mmol) followed by a few drops of DMF. The mixture was then allowed to warm up to room temperature and, after 1 h, the solvent was removed by evaporation to leave the allonyl chloride (**12f**), which was dissolved in dry dichloromethane (55 cm^3).

A suspension of benzyl 6β-aminopenicillanate PTSA salt (20.0 g, 41.8 mmol) in dichloromethane (20 cm^3) was washed with aqueous sodium hydrogen carbonate. Evaporation of the dried (Na_2SO_4) organic layer gave the amine (**13a**) (12.6 g, 41.1 mmol), which was dissolved in dry dichloromethane (55 cm^3) containing DMAP (4.52 g, 37 mmol).

The acid chloride solution was added in drops to the stirred amine solution. After 0.75 h, the mixture was diluted with dichloromethane and washed with 1M-hydrochloric acid (×2) followed by water (×2). Evaporation of the dried (Na_2SO_4) organic layer and purification of the resultant yellow foam by silica-gel chromatography [hexane–EtOAc (2:1) as eluant] gave the *title compound* (**11a**) (22.1 g, 77%) as an amorphous solid with $[\alpha]_D$ −18° (1.2% in $CHCl_3$); $\nu_{max.}$(KBr) 3 380 (NH), 1 785 (β-lactam C=O), 1 730 (ester C=O), and 1 695 cm^{-1} (amide C=O); $\lambda_{max.}$(EtOH) 228 (44 000), 272 (3 200), and 320 nm (760); δ(300 MHz; $CDCl_3$) 1.38 and 1.48 (each 3 H, s, CMe_2), 4.40 (1 H, s, 3-H), 4.60 (3 H, br s, 4′-H and 5′-H_2), 4.82 (1 H, d, *J* 5 Hz, 1′-H), 5.20 (2 H, s, PhCH_2O), 5.54 (1 H, d, *J* 4 Hz, 5-H), 5.64 (1 H, dd, *J* 10 and 4 Hz, 6-H), 5.68—5.73 (1 H, m, 2′-H), 5.90 (1 H, t, *J* 5 and 5 Hz, 3′-H), and 7.30—7.60 and 7.85—8.10 (15 and 6 H, each m, 4 × C_6H_5 and CONH) [addition of D_2O caused the dd at δ 5.64 to collapse to a d (*J* 4 Hz)] (Found: C, 64.5; H, 5.0; N, 3.6; S, 4.3. $C_{42}H_{38}N_2O_{11}S$ requires C, 64.7; H, 4.9; N, 3.5; S, 4.1%).

Preparation of Benzyl (2′,3′,5′-Tri-O-benzoyl-β-D-ribofuranosyl)penicillinate 1β-Oxide (**11b**).—(*a*) To a stirred solution of the penicillinate (**11a**) (1.30 g, 1.67 mmol) in methanol (30 cm^3) was added a solution of sodium periodate (1.79 g, 8.37 mmol) in water (20 cm^3). The resulting cloudy solution was warmed gently and a further quantity of methanol (15 cm^3) was added. After 3 days, the mixture was filtered and the filtrate extracted with dichloromethane (×3). Evaporation of the dried (Na_2SO_4) organic layer and purification of the product by silica-gel chromatography [hexane–EtOAc) (1:2) as eluant] gave the *title compound* (**11b**) (0.860 g, 65%) as an amorphous solid with $[\alpha]_D$ +68° (1.2% in $CHCl_3$); $\nu_{max.}$(KBr) 3 360 (NH), 1 795 (β-lactam C=O), 1 730 (ester C=O), and 1 695 cm^{-1} (amide C=O); $\lambda_{max.}$(EtOH) 227 (37 500) and 282 nm (2 600); δ(300 MHz; $CDCl_3$) 1.05 and 1.45 (each 3 H, s, 2-Me_2), 4.50 (1 H, s, 3-H), 4.58—4.77 (3 H, m, 4′-H and 5′-H_2), 4.80 (1 H, d, *J* 5 Hz, 1′-H), 5.00 (1 H, d, *J* 4 Hz, 5-H), 5.23 (2 H, AB q, *J* 12 Hz, separation of inner lines 21 Hz, PhCH_2O), 5.72 (1 H, t, *J* 5 and 5 Hz, 2′-H), 5.88 (1 H, t, *J* 5 and 5 Hz, 3′-H), 6.00 (1 H, dd, *J* 10 and 4 Hz, 6-H), 7.27—7.60 and 7.85—8.10 (14 and 6 H, each m, 4 × C_6H_5), and 8.36 (1 H, d, *J* 10 Hz, CONH) [addition of D_2O caused the signal at δ 8.36 to disappear and that at δ 6.00 to collapse to a d (*J* 4 Hz)] (Found: C, 63.2; H, 4.8; N, 3.4; S, 3.6. $C_{42}H_{38}N_2O_{12}S$ requires C, 63.45; H, 4.8; N, 3.55; S, 4.05%).

(*b*) To an ice-cooled stirred solution of the penicillinate (**11a**) (6.26 g, 8.04 mmol) in dry dichloromethane (70 cm^3) was added *ca.* 80% MCPBA (2.10 g, *ca.* 9.7 mmol). After 5 min, the mixture was allowed to warm up to room temperature. Evaporation of the solvent, after 1 h, left a residue, which was dissolved in ethyl acetate. The solution was washed successively with aqueous sodium sulphite (×2), aqueous sodium hydrogen carbonate (×2), and water. Evaporation of the dried (Na_2SO_4) organic layer and purification of the residue by silica-gel chromatography [hexane–EtOAc (1:2) as eluant] gave an amorphous solid (5.56 g, 87%) which was identical with the sulphoxide (**11b**) by 300 MHz ^1H n.m.r. spectroscopy.

Reaction of the Sulphoxide (**11b**) *with Triethyl Phosphite.*—(*a*) A solution of the sulphoxide (**11b**) (1.00 g, 1.26 mmol) and triethyl phosphite (0.43 cm^3, 2.53 mmol) in dry benzene (25 cm^3) was heated under reflux for 9 h. Evaporation and purification of the product by silica-gel chromatography [hexane–EtOAc (3:1) as eluant] gave a syrup (0.600 g, 64%) which comprised a 5:1 mixture of benzyl (2*R*)-3-methyl-2-{(1*R*,5*R*)-3-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-7-oxo-4-thia-2,6-diazabicyclo-[3.2.0]hept-2-en-6-yl}but-3-enoate (**18a**) and its double-bond isomer (**18b**); δ(300 MHz; $CDCl_3$) [for (**18a**)] 1.70 (3 H, s, CMe), 4.56 (1 H, dd, *J* 12 and 4 Hz, 5′-H), 4.67 (1 H, apparent q, separation 4 Hz, 4′-H), 4.78, 4.82, and 4.91 (1.5, 1.5, and 1 H, each br s, C=CH_2 and NCHCO_2), 5.15 (2 H, AB q, *J* 12 Hz, separation of inner lines 8 Hz, PhCH_2O), 5.18 (1 H, d, *J* 6.5 Hz, 1′-H), 5.69 (1 H, t, separation 5.5 Hz, 2′-H), 5.82 (1 H, t, separation 5 Hz, 3′-H), and 5.88 and 5.96 (each 1 H, d, *J* 4 Hz, 2 × β-lactam-H).

(*b*) A solution of the sulphoxide (**11b**) (6.00 g, 7.56 mmol) and triethyl phosphite (2.60 cm^3, 15.1 mmol) in dry toluene (150 cm^3) was heated under reflux for 2.5 h. Evaporation and purification of the residue as above gave a syrup (3.99 g, 70%) which comprised a 5:1 mixture of compounds (**18a** and **b**) by 300 MHz ^1H n.m.r. spectroscopy.

Preparation of Benzyl 3-Methyl-2-{(1R,5R)-3-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-7-oxo-4-thia-2,6-diazabicyclo-[3.2.0]hept-2-en-6-yl}but-2-enoate (**18b**).—Triethylamine (10 drops) was added to a 5:1 mixture of compounds (**18a** and **b**) (1.10 g) dissolved in dichloromethane (10 cm^3). After 0.75 h, the mixture was diluted with dichloromethane and washed successively with 1M-hydrochloric acid (×2) and water. Evaporation of the dried (Na_2SO_4) organic layer gave the *title compound* (**18b**) (1.00 g, 91%) as a foam with $[\alpha]_D$ −23° (0.7% in CH_2Cl_2); $\nu_{max.}$(KBr) 1 775 (β-lactam C=O) and 1 725 cm^{-1} (ester C=O); $\lambda_{max.}$(EtOH) 228 (50 000) and 317 nm (400); δ(300 MHz; $CDCl_3$) 1.63 and 2.12 (each 3 H, s, 2-Me_2), 4.44 (1 H, dd, *J* 12 and 4 Hz, 5′-H), 4.63 (1 H, apparent q, separation 4 Hz, 4′-H), 4.76 (1 H, dd, *J* 12 and 3 Hz, 5′-H), 5.12 (2 H, AB q, *J* 12 Hz, separation of inner lines 23 Hz, PhCH_2O), 5.23 (1 H, d, *J* 6.5 Hz, 1′-H), 5.54 (1 H, dd, *J* 6.5 and 5 Hz, 2′-H), 5.70 and 5.93 (each 1 H, d, *J* 4 Hz, 2 × β-lactam-H), 5.78 (1 H, dd, *J* 5 and 4 Hz, 3′-H), and 7.25—7.52 and 7.82—8.10 (14 and 6 H, each m, 4 × C_6H_5) (Found: C,

66.1; H, 4.7; N, 3.5; S, 4.2. $C_{42}H_{36}N_2O_{10}S$ requires C, 66.3; H, 4.75; N, 3.7; S, 4.2%).

Preparation of Benzyl 2-{(1R,5R)-3-(5-*Benzoyloxymethyl*-2-*furyl*)-7-*oxo*-4-*thia*-2,6-*diazabicyclo*[3.2.0]*hept*-2-*en*-6-*yl*}-3-*methylbut*-2-*enoate* (**19**).—A solution of compound (**18b**) (0.100 g, 0.13 mmol) in deuteriochloroform (0.5 cm^3) was treated with TFA (2 drops). When the reaction was complete (^1H n.m.r. spectroscopy), the solution was diluted with dichloromethane and washed with aqueous sodium hydrogen carbonate (× 2). Evaporation of the dried (Na_2SO_4) organic layer and purification of the residue by silica-gel chromatography (light petroleum–EtOAc; gradient elution) gave the title compound (**19**) (0.050 g, 73%) as a syrup with $[\alpha]_D$ −30° (1.4% in CH_2Cl_2); $\nu_{max.}$(film) 1 770 (β-lactam C=O) and 1 730 cm^{-1} (ester C=O); $\lambda_{max.}$(EtOH) 229 (29 000) and 281 nm (17 200); δ(300 MHz; $CDCl_3$) 1.88 and 2.26 (each 3 H, s, CMe_2), 5.20 (2 H, AB q, *J* 12 Hz, separation of inner lines 20 Hz, PhCH_2O), 5.34 (2 H, s, CH_2OCOPh), 5.91 and 6.11 (each 1 H, d, *J* 4 Hz, 2 × β-lactam-H), 6.60 and 6.95 (each 1 H, d, *J* 3 Hz, 2 × furan-H), and 7.33—7.60 and 8.04—8.08 (8 and 2 H, each m, 2 × C_6H_5); *m/z* (f.a.b., *m*-nitrobenzyl alcohol as matrix) 517 (MH^+, 20%), 285 (12), 105 ($C_7H_5O^+$, 100), and 91 ($C_7H_7^+$, 70).

Reaction of the Thiadiazabicycloheptenone (**18b**) *with PTSA*.—(*a*) A solution of compound (**18b**) (0.147 g, 0.19 mmol) and PTSA (0.037 g, 0.19 mmol) in methanol (5 cm^3) was heated under reflux for 7 h. Evaporation and purification of the residue by silica-gel chromatography [hexane–EtOAc (4:1) as eluant] gave a syrup (0.050 g, 36% by mass) which comprised a 6:2:1 mixture of compounds (**14c**), (**15b**), and (**19**) by ^1H n.m.r. spectroscopy; δ(300 MHz; $CDCl_3$) [for (**14c**)] *inter alia* 1.84 and 2.18 (each 3 H, s, CMe_2), 4.60 (1 H, dd, *J* 12 and 4 Hz, 5′-H), 4.80—4.90 (2 H, m, 4′- and 5′-H), 5.18 (2 H, s, PhCH_2O), 5.60 (1 H, d, *J* 5 Hz, 1′-H), 5.87 and 6.06 (each 1 H, t, *J* 5 and 5 Hz, 2′- and 3′-H), 8.10 (1 H, s, thiazole-H), and 8.42 (1 H, br s, CONH); [for (**15b**)] *inter alia* 1.97 and 2.25 (each 3 H, s, CMe_2), 5.23 (2 H, s, PhCH_2O), 5.38 (2 H, s, CH_2OCOPh), 6.66 and 7.00 (each 1 H, d, *J* 3 Hz, 2 × furan-H), 8.13 (thiazole-H), and 8.60 (1 H, br s, CONH); [for (**19**)] *inter alia* 1.89 and 2.27 (each 3 H, s, CMe_2), 5.21 (2 H, AB q, *J* 12 Hz, separation of inner lines 20 Hz, PhCH_2O), 5.35 (2 H, s, CH_2OCOPh), 5.82 and 6.13 (each 1 H, d, *J* 4 Hz, 2 × β-lactam-H), and 6.62 and 6.97 (each 1 H, d, *J* 3 Hz, 2 × furan-H).

(*b*) A solution of compound (**18b**) (0.153 g, 0.20 mmol) and PTSA (0.038 g, 0.20 mmol) in ethanol (5 cm^3) was heated under reflux for 4 h. Evaporation and purification of the residue as above gave a syrup (0.070 g, 46% by mass) which comprised a 4:1 mixture of compounds (**14c**) and (**15b**) by 220 MHz ^1H n.m.r. spectroscopy.

Preparation of Benzyl 3-*Methyl*-2-[2-(2′,3′,5′-*tri*-O-*benzoyl*-β-D-*ribofuranosyl*)*thiazol*-4-*ylcarbonylamino*]*but*-2-*enoate* (**14c**).—40% Aqueous PTSA (1.11 cm^3) was added to a stirred solution of compound (**18b**) (0.800 g, 1.05 mmol) in 1:1 dichloromethane–acetone (16 cm^3). After 10 h, the mixture was partitioned between dichloromethane and water. The aqueous layer was extracted with dichloromethane (× 2). Evaporation of the combined dried ($MgSO_4$) organic extracts left a syrup, which was dissolved in dry xylene (30 cm^3). The aforecited solution was heated under reflux for 6 min, when the solvent was removed by evaporation. Purification of the product by silica-gel chromatography [light petroleum–EtOAc (3:1) as eluant] gave the *title compound* (**14c**) (0.380 g, 48%) as an amorphous solid with $[\alpha]_D$ −10° (0.6% in CH_2Cl_2); $\nu_{max.}$(KBr) 3 380 (NH), 1 730 (ester C=O), and 1 680 cm^{-1} (amide C=O); $\lambda_{max.}$(EtOH) 230 nm (62 000); δ(300 MHz; $CHCl_3$) 1.84 and 2.19 (each 3 H, s, CMe_2), 4.60 (1 H, dd, *J* 12 and 4 Hz, 5′-H), 4.78—4.91 (3 H, m, 4′-H and 5′-H), 5.19 (2 H, s, PhCH_2O), 5.60 (1 H, d, *J* 5 Hz, 1′-H), 5.89 and 6.06 (each 1 H, t, *J* 5 and 5 Hz, 2′-and 3′-H), 7.21—7.62 and 7.92—8.05 (14 and 6 H, each m, 4 × C_6H_5), 8.10 (1 H, s, thiazole-H), and 8.41 (1 H, br s, CONH); *m/z* (f.a.b., thioglycerol as matrix) 761 (MH^+, 5%), 653 (5), 105 ($C_7H_7O^+$, 100), and 91 ($C_7H_7^+$, 42) (Found: C, 66.6; H, 4.7; N, 3.4; S, 3.9. $C_{42}H_{36}N_2O_{10}S$ requires C, 66.3; H, 4.75; N, 3.7; S, 4.2%).

Preparation of Benzyl (2R)-3-*Methyl*-2-[(2′,3′,5′-*tri*-O-*benzoyl*-β-D-*ribofuranosyl*)*thiazol*-4-*ylcarbonylamino*]*but*-3-*enoate* (**14b**).—40% Aqueous PTSA (1.1 cm^3) was added to a stirred 5:1 mixture of compounds (**18a** and **b**) (0.800 g, 1.05 mmol) in 1:1 dichloromethane–acetone (16 cm^3). After 15 h, the mixture was partitioned between dichloromethane and water. The aqueous layer was extracted with dichloromethane (× 2). Evaporation of the combined dried (Na_2SO_4) organic extracts left a syrup, which was dissolved in dry xylene (30 cm^3). The aforecited solution was heated under reflux for 30 min, when the solvent was removed by evaporation. Purification of the product by silica-gel chromatography [light petroleum–EtOAc (3:1 ⟶ 2:1) as eluant] gave the *title compound* (**14b**) (0.430 g, 54%) as an amorphous solid with $[\alpha]_D$ −123° (1% in CH_2Cl_2); $\nu_{max.}$(KBr) 3 400 (NH), 1 730 (ester C=O), and 1 680 cm^{-1} (amide C=O); $\lambda_{max.}$(EtOH) 230 nm (33 000); δ(300 MHz; $CDCl_3$) 1.70 (3 H, s, CMe), 4.54 (1 H, dd, *J* 12 and 3 Hz, 5′-H), 4.72 (1 H, apparent q, separation 4 Hz, 4′-H), 4.81 (1 H, dd, *J* 12 and 3.5 Hz, 5′-H), 4.95 and 5.04 (each 1 H, br s, C=CH_2), 5.10—5.14 (3 H, m, PhCH_2O and NHC*H*CO), 5.54 (1 H, d, *J* 5 Hz, 1′-H), 5.82—5.88 (2 H, m, 2′- and 3′-H), and 7.25—7.54 and 7.84—8.00 (14 and 8 H, each m, 4 × C_6H_5, CONH, and thiazole-H); *m/z* (f.a.b., thioglycerol as matrix) 761 (MH^+, 30%), 105 ($C_7H_7O^+$, 100), and 91 ($C_7H_7^+$, 37) (Found: C, 66.0; H, 4.6; N, 3.6; S, 3.8. $C_{42}H_{36}N_2O_{10}S$ requires C, 66.3; H, 4.75; N, 3.7; S, 4.2%).

Preparation of (1R,5R)-3-(2′,3′,5′,*Tri*-O-*benzoyl*-β-D-*ribofuranosyl*)-4-*thia*-2,6-*diazabicyclo*[3.2.0]*hept*-2-*en*-7-*one* (**18c**).—Ozone was passed into a cooled (Me_2CO–solid CO_2) solution of compound (**18b**) (1.00 g, 1.31 mmol) in dry dichloromethane (120 cm^3) until a deep-blue colour developed. Passage of ozone was maintained for a further 5 min whereupon the solution was aerated with oxygen and allowed to warm up to room temperature. Evaporation of the solvent left a syrup, which was dissolved in methanol (80 cm^3). Triethylamine (3 drops) was added to the solution which, after 7 h, was concentrated. Purification of the material by silica-gel chromatography [Et_2O–CH_2Cl_2 (15:1) as eluant] and trituration of the product with light petroleum gave the *title compound* (**18c**) (0.570 g, 76%) as a solid with m.p. 79–82 °C; $[\alpha]_D$ −5° (1.1% in CH_2Cl_2); $\nu_{max.}$(KBr) 3 300br (NH), 1 780 (β-lactam C=O), and 1 720 cm^{-1} (ester C=O); $\lambda_{max.}$(EtOH) 228 (44 000), 272 (1 700), and 280 nm (1 300); δ(300 MHz; $CDCl_3$) 4.57 (1 H, dd, *J* 12 and 4 Hz, 5′-H), 4.64—4.74 (1 H, m, 4′-H), 4.85 (1 H, dd, *J* 12 and 4 Hz, 5′-H), 5.17—5.22 (1 H, m, 3′-H), 5.45 (1 H, d, *J* 4 Hz, β-lactam-H), 5.83 (2 H, d, separation 4 Hz, 1′- and β-lactam-H), 6.02 (1 H, t, *J* 3 and 3 Hz, 2′-H), 6.20 (1 H, br s, CONH), and 7.31—7.62 and 7.90—8.20 (9 and 6 H, each m, 3 × C_6H_5) (Found: C, 62.7; H, 4.3; H, 5.0; S, 5.5. $C_{30}H_{24}N_2O_8S$ requires C, 62.9; H, 4.2; N, 4.9; S, 5.6%).

Preparation of 2-(2′,3′,5′-*Tri*-O-*benzoyl*-β-D-*ribofuranosyl*)-*thiazole*-4-*carboxamide* (**14d**).—40% Aqueous PTSA (0.92 cm^3) was added to a stirred solution of compound (**18c**) (0.500 g, 0.87 mmol) in dichloromethane–acetone (1:1) (13 cm^3). After 3 h, the mixture was partitioned between dichloromethane and water. The aqueous layer was extracted with dichloromethane (× 2). Evaporation of the combined dried (Na_2SO_4) organic extracts left a syrup, which was dissolved in dry xylene (5 cm^3).

The aforecited solution was heated under reflux for 30 min when the solvent was removed by evaporation. Purification of the material by silica-gel chromatography [Et_2O–CH_2Cl_2 (15:1) as eluant] and trituration of the product with light petroleum gave the *title compound* (**14d**) (0.310 g, 62%) as a crystalline solid with m.p. 68—70 °C; $[\alpha]_D$ −21° (0.8% in CH_2Cl_2); $\nu_{max.}$(KBr) 3 460 and 3 340 (NH_2), 1 730 (ester C=O), and 1 680 cm^{-1} (amide C=O); $\lambda_{max.}$(EtOH) 230 (48 000), 274 (3 700), and 280 nm (3 100); δ(300 MHz; $CDCl_3$) 4.60 (1 H, dd, *J* 12 and 4 Hz, 5′-H), 4.79—4.92 (2 H, m, 4′- and 5′-H), 5.53 (1 H, br s, CONH), 5.59 (1 H, d, *J* 5 Hz, 1′-H), 5.87 and 6.10 (each 1 H, t, *J* 5 and 5 Hz, 2′- and 3′-H), 7.04 (1 H, s, CONH), 7.34—7.62 and 7.91—8.04 (9 and 6 H, each m, 3 × C_6H_5), and 8.10 (1 H, s, thiazole-H); *m/z* (f.a.b., glycerol as matrix) 573 (MH^+, 28%) and 105 ($C_7H_5O^+$, 100) (Found: C, 63.0; H, 4.3; N, 4.6; S, 5.2. $C_{30}H_{24}N_2O_8S$ requires C, 62.9; H, 4.2; N, 4.9; S, 5.6%).

Preparation of 2-(β-D-*Ribofuranosyl*)*thiazole*-4-*carboxamide* (**6a**).—Compound (**14d**) (0.290 g, 0.51 mmol) was added to a stirred saturated solution of ammonia in methanol (19 cm^3). Evaporation after 48 h left a yellow syrup which, after being dried (*in vacuo*, $CaCl_2$), was subjected to silica-gel chromatography [the column was packed using EtOAc and eluted with EtOAc–PrOH–water (4:1:2, upper phase)]. Crystallisation of the production from ethanol–ethyl acetate gave the title compound (**6a**) (0.060 g, 46%) with m.p. 143—143.5 °C (lit.,[2] 145—146 °C;[3] 144—145 °C); $[\alpha]_D$ −9° (0.5% in EtOH) [lit.,[2] −9° (EtOH)]; $\nu_{max.}$(KBr) 3 550, 3 420, 3 340br, and 3 120 (NH and OH), 1 670 (amide C=O), and 1 650 cm^{-1} (C=N); $\lambda_{max.}$(EtOH) 241 nm (7 100); δ(250 MHz; CD_3SOCD_3) 3.45—3.65 (2 H, s, 5′-H_2), 3.90 and 4.07 (2 and 1 H, each br s, 2′-, 3′-, and 4′-H), 4.85 (1 H, t, separation 5 Hz, 5′-OH), 4.92 (1 H, d, *J* 5 Hz, 1′-H), 5.06 and 5.47 (each 1 H, br s, 2′- and 3′-OH), 7.58 and 7.70 (each 1 H, br s, $CONH_2$), and 8.20 (1 H, s, thiazole-H) [addition of D_2O caused the signals at δ 4.85, 5.06, 5.47, 7.58, and 7.70 to disappear, that at δ 3.90 to sharpen, and that at δ 4.07 to appear as a triplet (*J* 5 Hz)]; *m/z* (f.a.b., thiglycerol as matrix) 261 (MH^+, 100%) (Found: C, 41.7; H, 4.7; N, 10.7; S, 12.2. Calc. for $C_9H_{12}N_2O_5S$: C, 41.55; H, 4.65; N, 10.75; S, 12.3%).

Preparation of (2R)-3-*Methyl*-2-[2-(β-D-*ribofuranosyl*)*thiazol*-4-*ylcarbonylamino*]*but*-3-*enamide* (**6c**).—Compound (**14b**) (0.390 g, 0.51 mmol) was dissolved in an ice-cooled saturated solution of ammonia in methanol (20 cm^3). Evaporation after 3 days and purification of the residue by silica-gel chromatography [the column was packed using EtOAc and eluted with EtOAc–EtOH–H_2O (10:1:1, upper phase)] gave two fractions.

The first eluted material (0.030 g) was not identified. The second eluted material (0.090 g, 45%), isolated as a cream-coloured solid, was the *title compound* (**6c**). After recrystallisation from acetone–diethyl ether it showed m.p. 51—53 °C; $[\alpha]_D$ +4° (0.5% in EtOH); $\nu_{max.}$(KBr) 3 400br (OH and NH) and 1 660 cm^{-1} (amide C=O); $\lambda_{max.}$(EtOH) 204 (17 000), 223 (10 500), 230 (10 500), and 290 nm (700); δ(300 MHz; D_2O) 1.72 (3 H, s, CMe), 3.67 (1 H, dd, *J* 12 and 5 Hz, 5′-H), 3.78 (1 H, dd, *J* 12 and 3 Hz, 5′-H), 4.05—4.10 (2 H, m, 3′- and 4′-H), 4.23 (1 H, t, *J* 5 and 5 Hz, 2′-H), 4.94 (1 H, d, *J* 2 Hz, C=C*H*H), 5.07 (1 H, d, *J* 5 Hz, 1′-H), 5.07—5.13 (2 H, m, C=CH*H* and NCHCO), and 8.14 (1 H, s, thiazole-H) (Found: C, 47.4; H, 5.6; N, 11.5; S, 8.6. $C_{14}H_{19}N_3O_6S$ requires C, 47.05; H, 5.35; N, 11.75; S, 8.95%).

Preparation of Methyl (2R)-3-*Methyl*-2-[2-(β-D-*ribofuranosyl*)*thiazol*-4-*ylcarbonylamino*]*but*-3-*enoate* (**6d**).—(*a*) Compound (**14b**) (0.300 g, 0.39 mmol) was added to a stirred solution of a 5:1:1 mixture of methanol–triethylamine–water (10 cm^3). After 3 days, the solvent was removed by evaporation and the residue was partitioned between dichloromethane and water. Concentration of the aqueous layer and purification of the residue by silica-gel chromatography [the column was packed using EtOAc and eluted with EtOAc–EtOH–water (10:1:1, upper phase)] gave the *title compound* (**6d**) (0.080 g, 54%) as a hygroscopic solid with $[\alpha]_D$ −30° (0.5% in EtOH); $\nu_{max.}$(KBr) 3 300br (OH and NH), 1 720 (ester C=O), and 1 650 cm^{-1} (amide C=O); $\lambda_{max.}$(EtOH) 232 nm (14 000); δ(300 MHz; $CDCl_3$) 1.50—1.90 (3 H, br s, 3 × OH), 1.91 and 2.18 (each 3 H, s, CMe_2), 3.79 (3 H, s, CO_2Me), 3.80 (1 H, dd, *J* 12 and 4 Hz, 5′-H), 3.95 (1 H, dd, *J* 12 and 3 Hz, 5′-H), 4.15—4.19 (1 H, m, 4′-H), 4.29 and 4.38 (each 1 H, t, *J* 5 and 5 Hz, 2′- and 3′-H), 5.13 (1 H, d, *J* 5 Hz, 1′-H), 8.10 (1 H, s, thiazole-H), and 8.67 (1 H, br s, CONH); *m/z* (f.a.b., thioglycerol as matrix) 373 (MH^+, 100%) and 341 (681) (Found: C, 48·1; H, 5.6; N, 7.3; S, 8.2. $C_{15}H_{20}N_2O_7S$ requires C, 48.35; H, 5.4; N, 7.5; S, 8.6%.

(*b*) Potassium cyanide (0.155 g, 2.39 mmol) was added to a stirred solution of compound (**14c**) (0.400 g, 0.53 mmol) in dry methanol (13 cm^3). After 2.5 days, the mixture was treated with Amberlite IR 120 (H^+) ion-exchange resin (*ca.* 1.0 g). The resin was filtered off after 2 h and the filtrate was evaporated to give a light-brown oil, which was subjected to silica-gel chromatography [the column was packed using EtOAc and eluted with EtOAc–EtOH–water (10:1:1, upper phase)]. The resultant foam (0.178 g, 91%) was identified as the title compound (**6d**) by 300 MHz ^1H n.m.r. spectroscopy.

Reaction of Benzyl Benzylpenicillinate (**7e**) *with Lithium* 2-*Methylpropanethiolate*.—0.4M-Lithium 2-methylpropanethiolate [prepared by addition of 2M-BuLi in hexanes (2 cm^3; 5.0 mmol) to a solution of Me_3CSH (0.56 cm^3, 5.16 mmol) in dry HMPA (10 cm^3)] (1.25 cm^3, 0.50 mmol) was added in drops during 3 h (using a syringe pump) to a stirred solution of the benzylpenicillinate (**7e**) (0.212 g, 0.50 mmol) in dry HMPA (2 cm^3). The mixture was stirred for 3 h and then diluted with water and extracted with ethyl acetate (×2). After being washed with water (×4), the organic layer was dried (Na_2SO_4) and concentrated. Purification of the resultant oil by silica-gel chromatography [light petroleum–EtOAc (2:1) as eluant] gave *benzyl* 2-(2-*benzylthiazol*-4-*ylcarbonylamino*)-3-*methylbut*-2-*enoate* (**8d**) (0.030 g, 15%) as an oil with $\nu_{max.}$(film) 3 400 (NH), 1 720 (ester C=O), and 1 675 cm^{-1} (amide C=O); $\lambda_{max.}$(EtOH) 230sh nm (9 000); δ(60 MHz; $CDCl_3$) 1.92 and 2.23 (each 3 H, s, CMe_2), 4.27 (2 H, s, PHCH_2CO), 5.17 (2 H, s, PhCH_2O), 7.27 (10 H, s, 2 × C_6H_5), 7.94 (1 H, s, thiazole-H), and 8.57 (1 H, br s, CONH); *m/z* (c.i., NH_3 as carrier gas) 407 (MH^+, 4%) and 91 ($C_7H_7^+$, 100) (Found: MH^+, 407.1413. $C_{23}H_{23}N_2O_3S$ requires *m/z* 407.1429).

Reaction of Benzyl Benzylpenicillinate (**7e**) *with Sodium Hydroxide*.—(*a*) 1M-Sodium hydroxide (0.94 cm^3, 0.94 mmol) was added to a stirred ice-cooled solution of the benzylpenicillinate (**7e**) (0.200 g, 0.47 mmol) in pyridine (5 cm^3). The mixture was allowed to warm up to room temperature and, after 2 h, acidified with 1M-hydrochloric acid and extracted with ethyl acetate (×2). Evaporation of the dried (Na_2SO_4) organic layer and purification of the residue by silica-gel chromatography [hexane–EtOAc (3:1) as eluant] gave an oil (0.050 g, 26%) which was identified as the thiazole (**8d**) by 60 MHz ^1H n.m.r. spectroscopy.

(*b*) 1M-Sodium hydroxide (0.49 cm^3, 0.49 mmol) was added to a stirred solution of the benzyl penicillinate (**7e**) (0.207 g, 0.49 mmol) in DMSO (4 cm^3); a deep-red colour developed immediately. Work-up as above, after 0.75 h, gave the thiazole (**8d**) (0.060 g, 30%) as an oil.

(*c*) Powdered sodium hydroxide (0.021 g, 0.52 mmol) was added to a stirred solution of the benzylpenicillinate (**7e**) (0.207 g, 0.49 mmol) in dry DMSO (4 cm^3). Work-up as above, after 0.75 h, gave the thiazole (**8d**) (0.070 g, 35%) as an oil.

(*d*) 1M-Sodium hydroxide (1.01 cm^3, 1.01 mmol) was added to a stirred solution of the benzylpenicillinate (**7e**) (0.214 g, 0.504 mmol) in DMSO (4 cm^3). After 0.75 h, the mixture was acidified with 5M hydrochloric acid and extracted with ethyl acetate (× 3). The combined organic layers were washed with brine (× 5) and extracted with aqueous sodium hydrogen carbonate. The sodium hydrogen carbonate extract was acidified with 5M-hydrochloric acid and extracted with ethyl acetate (× 2). Evaporation of the dried (Na_2SO_4) organic extract and recrystallisation of the resultant solid from hexane–ethyl acetate gave 2-(2-*benzylthiazol*-4-*carbonylamino*)-3-*methylbut*-2-*enoic acid* (**8e**) (0.060 g, 38%); m.p. 155-157 °C; $\nu_{max.}$(KBr) 3 320 (NH), 3 040br (OH), 1 730 (acid C=O), and 1 630br (amide C=O); $\lambda_{max.}$(EtOH) 206 (27 000) and 232 nm (15 000); δ(300 MHz; $CDCl_3$) 1.97 and 2.27 (each 3 H, s, CMe_2), 4.33 (2 H, s, PhCH_2CO), 7.26—7.39 (5 H, m, C_6H_5), 8.05 (1 H, s, thiazole-H), and 8.56 (1 H, br s, CONH); *m/z* (e.i.) 316 (M^+, 5%), 298 (22), 272 (40), and 202 (100) (Found: C, 60.4; H, 5.1; N, 8.6; S, 9.8. $C_{16}H_{16}N_2O_3S$ requires C, 60.75; H, 5.05; N, 8.85; S, 10.1%).

Preparation of Benzyl 3-*Methyl*-2-[(3-*Methylthio*-2-*phenylacetamido*)*propenamido*]*but*-2-*enoate* (**25**).—Potassium t-butoxide (0.053 g, 0.47 mmol) was added to a stirred solution of the benzylpenicillinate (**7e**) (0.200 g, 0.47 mmol) in dry THF (5 cm^3) under argon; a dark-orange colour developed rapidly. After 15 min, iodomethane (0.06 cm^3, 0.96 mmol) was added; a white precipitate formed and the solution became brown in colour. The mixture was then concentrated and the residue was partitioned between ethyl acetate and water. Evaporation of the dried (Na_2SO_4) organic layer and purification of the product by silica-gel chromatography [hexane–EtOAc (1:1) as eluant] gave the *title compound* (**25**) (0.050 g, 24%) as a white solid. After recrystallisation from hexane–ethyl acetate, the material showed m.p. 148—150 °C; $\nu_{max.}$(KBr) 3 200 and 3 160 (together NH), 1 725 (ester C=O), and 1 655 and 1 640 cm^{-1} (amide C=O); $\lambda_{max.}$(EtOH) 206 (26 500) and 285 nm (15 000); δ(300 MHz; $CDCl_3$) 1.80, 2.09, and 2.32 (each 3 H, s, Me_2C and MeS), 3.67 (2 H, s, PhCH_2CO), 5.17 (2 H, s, PhCH_2O), 6.78 (1 H, s, CONH), 7.10—7.40 (11 H, m, 2 × C_6H_5 and CONH), and 7.58 (1 H, s, C=C*H*SMe); *m/z* (e.i.) 331 (16%), 136 (25), 108 (78), and 91 ($C_7H_7^+$, 100) (Found: C, 65.9; H, 6.1; N, 6.4; S, 7.0. $C_{24}H_{26}N_2O_4S$ requires C, 65.75; H, 6.0; N, 6.4; S, 7.3%).

Reaction of the Ribofuranosylpenicillinate (**11a**) *with Sodium Hydroxide.*—(*a*) 1M-Sodium hydroxide (0.26 cm^3, 0.26 mmol) was added to a stirred solution of the ribofuranosylpenicillinate (**11a**) (0.200 g, 0.26 mmol) in DMSO (3 cm^3). After 45 min, the mixture was acidified with 1M-hydrochloric acid and extracted with ethyl acetate (× 2). The organic extracts were combined, washed with brine (× 5), dried (Na_2SO_4), and concentrated. Purification of the resultant yellow oil by silica-gel chromatography [light petroleum–EtOAc (2:1) as eluant] gave a foam (0.045 g, 23%) that was identified as the thiazole (**14c**) by 300 MHz ^{1}H n.m.r. spectroscopy.

(*b*) 1M-Sodium hydroxide (1.28 cm^3, 1.28 mmol) was added to a stirred solution of the ribofuranosylpenicillinate (**11a**) (1.00 g, 1.28 mmol) in DMSO (15 cm^3). After 45 min, the mixture was acidified with 5M-hydrochloric acid, diluted with water, and extracted with ethyl acetate (× 3). The organic extracts were combined, washed with brine (× 5), dried (Na_2SO_4), and concentrated. The resultant yellow oil was dissolved in dry xylene (10 cm^3) and the solution was heated under reflux for 20 min. Evaporation and purification of the residue by silica-gel chromatography, as above, gave the thiazole (**14c**) (0.350 g, 36%) as a foam.

Reaction of the Thiazole (**14c**) *with Ozone followed by Ammonia.*—A cooled (Me_2CO–solid CO_2) solution of the thiazole (**14c**) (0.301 g, 0.40 mmol) in dry dichloromethane (60 cm^3) was saturated with ozone. The solution was then aerated, allowed to warm up to room temperature, and evaporated. Saturated methanolic ammonia (20 cm^3) was added to the residue and the mixture was stirred for 72 h and then filtered. Evaporation of the filtrate and subjection of the residue to silica-gel chromatography [the column was packed using EtOAc and eluted with EtOAc–PrOH–water (4:1:2, upper phase)] gave a syrup, which was crystallised from ethanol–ethyl acetate. The resultant product (0.063 g, 61%) was identified as tiazofurin (**6a**) on the basis of its m.p. and ^{1}H n.m.r. spectrum.

Acknowledgements

We thank the S.E.R.C. for a C.A.S.E. studentship (to K. R. M). We are also grateful to Mr. A. Schofield for technical assistance, Mr. K. Walkling for the determination of i.r. and u.v. spectra, Messrs. C. Evans and F. Skinkis for measuring the 300 MHz ^{1}H n.m.r. spectra, Mr. A. Crompton for recording the e.i. and c.i. mass spectra, Dr. J. A. Ballantine (S.E.R.C. Mass Spectrometry Centre, University College of Swansea) for recording the f.a.b. mass spectra, Dr. R. Perry for the elemental analyses, and Dr. M. G. Boulton for the antiviral tests.

References

1 J. G. Buchanan and R. H. Wightman, in 'Topics in Antibiotic Chemistry,' ed. P. G. Sammes, Ellis Horwood Ltd., 1982, vol. 6, p. 229; J. G. Buchanan, *Prog. Chem. Org. Nat. Prod.*, 1984, **44**, 243.
2 M. Fuertes, T. Garcia-López, E. Garcia-Muñoz, and M. Stud, *J. Org. Chem.*, 1976, **41**, 4074.
3 P. C. Srivastava, M. V. Pickering, L. B. Allen, D. G. Streeter, M. T. Campbell, J. T. Witkowski, R. W. Sidwell, and R. K. Robins, *J. Med. Chem.*, 1977, **20**, 256.
4 P. J. O'Dwyer, D. D. Shoemaker, H. N. Jayaram, D. G. Johns, D. A. Cooney, S. Marsoni, L. Malspeis, J. Plowman, J. P. Davignon, and R. D. Davis, in 'Investigational New Drugs,' Martinus Nijhoff, 1984, vol. 2, p. 79.
5 R. J. Stoodley, *Tetrahedon*, 1975, **31**, 2321.
6 S. Wolfe, J.-B. Ducep, and J. D. Greenhorn, *Can. J. Chem.*, 1975, **53**, 3435.
7 D. F. Corbett. A. C. Kaura, C. D. Maycock, and R. J. Stoodley, *J. Chem. Soc., Perkin Trans. 1*, 1987, 2009.
8 M. Kovacevic, J. J. Herak, and B. Gaspert, *Croat. Chim. Acta*, 1981, **54**, 367.
9 (*a*) M. Bobek and J. Farkaš, *Collect. Czech. Chem. Commun.*, 1968, **33**, 247; (*b*) L. J. S. Knutsen, B. D. Judkins, W. L. Mitchell, R. F. Newton, and D. I. Scopes, *J. Chem. Soc., Perkin Trans. 1*, 1984, 229.
10 (*a*) A. M. Felix, J. Unowsky, J. Bontempo, and R. J. Fryer, *J. Med. Chem.*, 1968, **11**, 929; (*b*) E. G. Brain, I. McMillan, J. H. C. Nayler, R. Southgate, and P. Tolliday, *J. Chem. Soc., Perkin Trans. 1*, 1975, 562.
11 W. J. Hennen, B. C. Hinshaw, T. A. Riley, S. G. Wood, and R. K. Robins, *J. Org. Chem.*, 1985, **50**, 1741.
12 G. Hölfe, W. Steglich, and H. Vorbrüggen, *Angew. Chem. Int. Ed. Engl.*, 1978, **17**, 569.
13 R. D. G. Cooper and F. L. José, *J. Am. Chem. Soc.*, 1970, **92**, 2575.
14 R. Lattrell, *Justus Leibigs Ann. Chem.*, 1974, 1361; J. E. Baldwin and M. A. Christie, *J. Chem. Soc., Chem. Commun.*, 1978, 239; M. Narisada, H. Onoue, M. Ohtani, F. Watanabe, T. Okada, and W. Nagata, *Tetrahedron Lett.*, 1978, 1755; N. F. Osborne, *J. Chem. Soc., Perkin Trans. 1*, 1980, 146.
15 R. D. G. Cooper, P. V. DeMarco, J. C. Cheng, and N. D. Jones, *J. Am. Chem. Soc.*, 1969, **91**, 1408; D. H. R. Barton, F. Comer, and P. G. Sammes, *ibid.*, p. 1529.

16 E. G. Brain, A. J. Eglington, J. H. C. Nayler, M. J. Pearson, and R. Southgate, *J. Chem. Soc., Perkin Trans. 1*, 1976, 447.
17 R. D. G. Cooper and F. L. José, *J. Am. Chem. Soc.*, 1972, **94**, 1021.
18 K. Tsuzuki, Y. Nakajima, T. Watanabe, M. Yanagiya, and T. Matsumoto, *Tetrahedron Lett.*, 1978, 989.
19 J. Asakura and T. Tomura, *Nucleosides. Nucleotides*, 1988, **7**, 245.
20 A. W. Chow, N. M. Hall, and J. R. E. Hoover, *J. Org. Chem.*, 1962, **27**, 1381.
21 E. F. Recondo and H. Rinderknecht, *Helv. Chim. Acta*, 1959, **42**, 1172.

Paper 9/01674K
Received 20*th April* 1989
Accepted 12*th July* 1989