The Chemistry of Some 2-Aminothiazol-4-ylacetic Acid Derivatives and the Synthesis of Derived Penicillins

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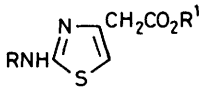
The *N*-protected 2-aminothiazol-4-ylacetic acid derivatives (**6**), (**11**), (**14**), (**16**), (**18**), (**26**), (**27**), (**30**), (**33**), and (**34**) have been prepared. They are readily available by reaction of the corresponding 2-amino ethyl esters such as (**1**) with the appropriate acylating agents, generally giving a bis-carbamate of type (**5**), followed by saponification. The process is general for α-methylene, α-oximino, and α-amino substituents in the side-chain affording in the last case a route to differentially protected α-amino-α-(2-aminothiazol-4-yl)acetic acids such as (**30**). The optimum conditions for the acylations are described; the course of the saponification of the bis-carbamate esters is discussed and physical evidence for the structures of the derivatives is presented. The use of the protected acids (**11**) and (**34**) in the synthesis of the piperacillin analogue (**38**) is described.

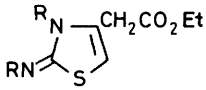
A (2-aminothiazol-4-yl)acetyl unit is a common feature of certain broad-spectrum antibacterial cephalosporins which have been recently reported.[1–4] Some penicillins containing a thiazole side-chain but without the 2-amino substituent had been reported earlier.[5] In the case of the cephalosporins the methylene group of the 7-acyl substituent may be unsubstituted[3] or include an alkyl or hydroxy substituent,[2] an oxime substituent,[1] or an amino substituent.[3] Protection of the heterocyclic amino group was achieved using trityl,[1] 2,2,2-trichloroethoxycarbonyl,[2–4] or 2-chloroacetyl[2,4] groups.

We should now like to report our own observations on the chemistry of this heterocyclic system. We were particularly interested in the possibility of introducing a urethane-type protecting group onto the ring amino function. In particular, the 2-benzyloxycarbonylaminothiazol-4-ylacetic acids or the corresponding 4-nitro derivatives should allow later deprotection under very mild conditions (hydrogenolysis). Such procedures appeared necessary in view of the acidic deprotection conditions required for the removal of trityl or 2,2,2-trichloroethoxycarbonyl groups, which would not be compatible with a penicillin (as opposed to a cephalosporin) β-lactam. 2-Chloroacetyl protection, removable with thiourea,[6] might be satisfactory, but the evidence from the cephalosporin series[2,4] suggested that yields might be low.

The acylation of aminothiazoles has recently been reviewed.[7] Little is known, however, about the acylation of 2-aminothiazoles with halogenoformates, though it has been shown that the reaction of ethyl chloroformate with 2-amino-4-methylthiazole may give a mono- or a di-carbamate according to the conditions.[8] We have found the production of a dicarbamate to be quite general; moreover, one of the protecting groups in the resulting molecule is very labile to base and is removed under the conditions of basic hydrolysis of an ester side-chain in the 4-position. The overall procedure affords a convenient and high-yielding synthesis of *N*-protected 2-aminothiazol-4-ylacetic acids. It is convenient to list our results according to the substitution in the α-position of the acetic acid unit.

A α-Methylene Series.—Our first observations were made on ethyl 2-aminothiazol-4-ylacetate (**1**), readily available as its HCl salt by the standard Hantzsch synthesis[9] from thiourea and ethyl 4-chloroacetoacetate.[10] The hydrochloride of (**1**) could be used satisfactorily in the acylations in the presence of an extra mole of base; (**1**) itself decomposed very slowly at room temperature. Acylation of the weakly basic 2-amino group (pK_a 5.39[11]) with benzyl chloroformate proved to be no easy matter. Thus, reaction in organic solvents in the presence of pyridine or triethylamine, even at elevated temperatures, gave only low yields of (**2**) with much unchanged starting material. Nor could acylation be achieved under aqueous (Schotten–Baumann) conditions, in contrast to the above-mentioned findings with 2-amino-4-methylthiazole.[8]





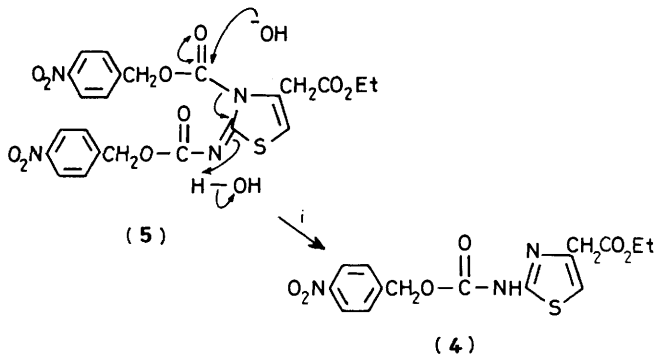
(**1**) R = H, R^1 = Et
(**2**) R = Z,* R^1 = Et
(**4**) R = Z(NO$_2$),† R^1 = Et
(**6**) R = Z(NO$_2$), R^1 = H

(**3**) R = Z
(**5**) R = Z(NO$_2$)

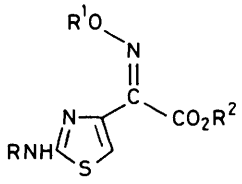
* Z = $PhCH_2OCO$. † Z(NO$_2$) = 4-$O_2NC_6H_4CH_2OCO$

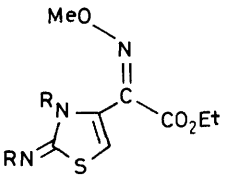
However, when (**1**) was treated with an excess (2.5 equiv.) of benzyl chloroformate under two-phase conditions (dichloromethane–saturated aqueous sodium hydrogen carbonate), the starting material was completely consumed after overnight reaction. The product appeared as a mixture of two compounds by t.l.c.; trituration with ether afforded the pure mono derivative (**2**) which was fully characterised, in about 50% yield. From the mother-liquors, a semi-solid could be obtained which by n.m.r. appeared to be a mixture of compound (**2**) and the bis derivative (**3**). When the mixture was subjected to silica-gel chromatography only the mono derivative (**2**) could be eluted; the overall yield of (**2**) was about 75%.

When 4-nitrobenzyl chloroformate was used instead as the acylating agent, we were pleased to find a very clear-cut result which confirmed the above indications in the benzyl series. Treatment of the hydrochloride of (**1**) with 2.5 equiv. of 4-nitrobenzyl chloroformate under the two-phase conditions described above gave predominantly the bis derivative (**5**), which separated from the reaction mixture as a solid in 77% yield. When this material was subjected to base-catalysed hydrolysis, the eventual product was the mono-protected acid (**6**); in fact the sodium salt of (**6**) precipitated from the aqueous ethanolic solution employed if insufficient water was present. However, if the hydrolysis was stopped at an intermediate stage, the mono-protected ester (**4**) could be isolated. The clear implication of this observation was that the second protecting group in the 2-imino-2,3-dihydrothiazole (**5**) was extremely labile to base and was indeed lost before saponification of the ester. This is predictable in view of the regeneration of the aromatic ring (Scheme 1). The observation recalls the finding[12,13] that *N*(im)-benzyloxycarbonylhistidine derivatives readily lose the



Scheme 1. Postulated mechanism for the base-catalysed cleavage of the 3-(4-nitrobenzyloxycarbonyl) group in (**5**). *Reagents:* i, NaOH–aq.EtOH





(**7**) R = R^1 = H, R^2 = Et
(**8**) R = H, R^1 = Me, R^2 = Et
(**10**) R = Z(NO$_2$), R^1 = Me, R^2 = Et
(**11**) R = Z(NO$_2$), R^1 = Me, R^2 = H
(**12**) R = Z, R^1 = Me, R^2 = Et
(**14**) R = Z, R^1 = Me, R^2 = H
(**15**) R = ClCH$_2$CO, R^1 = Me, R^2 = Et
(**16**) R = ClCH$_2$CO, R^1 = Me, R^2 = H
(**18**) R = Cl$_3$CCH$_2$OCO, R^1 = Me, R^2 = H
(**19**) R = R^1 = Z, R^2 = Et

(**9**) R = Z(NO$_2$)
(**13**) R = Z
(**17**) Cl$_3$CCH$_2$OCO

ring-protecting group under basic conditions, even in the presence of a free amino acid ester.

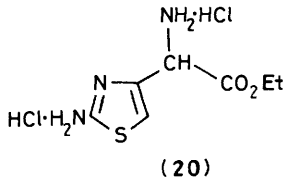
*B α-Oximino Series.**—Our starting materials in this series were ethyl α-hydroxyimino-α-(2-aminothiazol-4-yl)acetate (**7**) and the corresponding α-methoxyimino derivative (**8**). These were prepared by literature procedures[1,5] with slight modifications (see Experimental section).

The stereochemistry of these oximes has been studied and assigned by Bucourt *et al.*,[1] and our findings on the preparation of the intermediates concurred with theirs. To simplify the following discussion, the thermodynamically more stable (*E*)- or *anti*-oximes, as shown above, were used. When on one occasion the (*Z*)- or *syn*-version of (**8**) was employed, the course of the acylation and subsequent saponification were not significantly altered;† no equilibration of isomers was ever observed.

Acylation of the oxime (**8**) with 4-nitrobenzyl chloroformate, as described for the conversion of compound (**1**) into products (**4**) and (**5**), gave a similar mixture of products, (**9**) and (**10**), which on saponification yielded the same acid (**11**), in high yield. Again, in the benzyloxycarbonyl series crystalline derivatives were not so easily obtained, but n.m.r. again indicated a mixture of mono and bis derivatives, (**12**) and (**13**), which were converted into (**14**).

Other protecting groups may be attached to the ring amino group of compound (**8**): monochloroacetylation[14] has been described, employing chloroacetyl chloride in dimethylformamide to give (**15**) without any of the bis-adduct; we were able to repeat this, and also used *N,N'*-dicyclohexylcarbodiimide–chloroacetic acid. Thus, the acid (**16**) was available. A 2,2,2-trichloroethoxycarbonyl group has previously been built into similar compounds *via* the substituted urea,[3] but we found that the two-phase acylation procedure on (**8**) was again valid, giving here very largely the bis-carbamate (**17**) and, by saponification, the acid (**18**) in good yield.

In the case of the hydroxyimino derivative (**7**), the acylation behaviour was complicated by the formation of the *N,O*-diadduct (**19**). Apparently this is not a problem on tritylation[1] of (**7**), when either the *N*- or the *N,O*-derivatives may be formed according to the molar ratio of trityl chloride present. Even with just one equivalent of benzyloxycarbonyl chloride, however, much product (**19**) was formed, together with (by n.m.r.) a mono-benzyloxycarbonyl species, probably acylated on oxygen.



(**20**)

C α-Amino Series.—Various procedures were considered for the possible reduction of the oxime derivatives (**7**) and (**8**) in order to obtain the α-amino series. Attempted reduction with sodium borohydride or sodium cyanoborohydride met with no success. Previously,[4] reduction with zinc–formic acid had been used, but in our hands yields by this procedure were variable and premature loss of the ester a problem; reduction with zinc–HCl at pH 4 was found to be better. Much more satisfactory proved to be catalytic hydrogenation, described for related cases in an earlier patent.[14] Provided that at least two equivalents of HCl were present, the uptake of hydrogen proceeded very smoothly to the theoretical value to give the dihydrochloride ester (**20**).

It was generally convenient to proceed immediately with the acylation by basification of the aqueous solution of compound (**20**) and acylation *in situ*. However, formation of (**20**) was corroborated by t.l.c. analysis and by n.m.r. It is possible to isolate the free base form of (**20**), and then to diacylate it to give derivatives such as the *N,N'*-dichloroacetyl ester (**21**).[14]

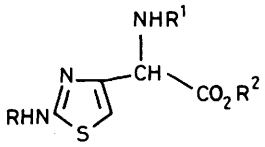
However, the free base form of (**20**) is unstable on standing, and we generally proceeded directly with (**20**). Schotten–Baumann acylation at about pH 8 with halogenoformates gave the α-acylated derivatives (**22**) and (**23**), and with di-t-butyl dicarbonate[15] gave similarly the product (**24**). Under these conditions, the ring amino group was unchanged. The two-step yield of (**24**) from (**8**) was 75%, much superior to that from the previously reported procedure.[4,14]

Differentially protected derivatives were then available by the two-phase acylation of (**22**) to give (**24**) with various electrophiles as in sections *A* and *B*. In this series the reaction was noticeably slower, probably reflecting the electron-withdrawing effect of the side-chain. When (**23**) was treated with an excess (2.5 equiv.) of benzyl chloroformate a modest yield of (**25**) was obtained; this was readily saponified to the acid (**26**). Some exchange of the α-4-nitrobenzyloxycarbonyl group (n.m.r.) appeared to take place during the acylation, however. Alternatively, reaction of compound (**23**) with 2.5 equiv. of 4-nitrobenzyl chloroformate gave a mixture of products, probably corresponding to mono- and di-acylation of the ring, which on saponification gave the acid (**27**) in high yield.

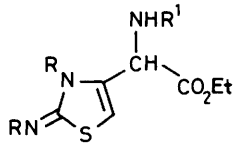
More interesting and versatile derivatives were available from the α-Boc derivative (**24**) which allowed the combination of mild acid-labile and hydrogenolysable protection. No exchange of the Boc group was observed, perhaps owing to its greater bulk.

* Including both α-hydroxyimino and α-methoxyimino derivatives.

† A. V. Stachulski, unpublished observations.



(21) R = R^1 = $ClCH_2CO$, R^2 = Et
(22) R = H, R^1 = Z, R^2 = Et
(23) R = H, R^1 = $Z(NO_2)$, R^2 = Et
(24) R = H, R^1 = Boc,* R^2 = Et
(25) R = Z, R^1 = $Z(NO_2)$, R^2 = Et
(26) R = Z, R^1 = $Z(NO_2)$, R^2 = H
(27) R = R^1 = $Z(NO_2)$, R^2 = H
(28) R = Z, R^1 = Boc, R^2 = Et
(30) R = Z, R^1 = Boc, R^2 = H
(31) R = $Z(NO_2)$, R^1 = Boc, R^2 = Et
(33) R = $Z(NO_2)$, R^1 = Boc, R^2 = H
(34) R = Z, R^1 = R^2 = H
(35) R = Z, R^1 = H, R^2 = Et



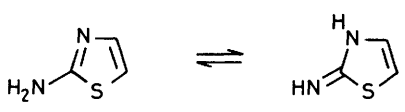
(29) R = Z, R^1 = Boc
(32) R = $Z(NO_2)$, R^1 = Boc

* Boc = t-Butoxycarbonyl.

Thus, the reaction of compound (**24**) with benzyl chloroformate under the two-phase conditions gave mainly the mono-benzyloxycarbonyl Boc ester (**28**), which was readily isolated in a pure state, together with the bis derivative (**29**). The latter could not be obtained as a crystalline solid in this series, but its presence was clear by t.l.c. and n.m.r.; further, both compounds (**28**) and (**29**) gave the same acid product (**30**) in high yield on sponification. Similarly, with 4-nitrobenzyl chloroformate the main product was (**31**) plus some (**32**), both of which saponified to (**33**). Again, in both cases the crude acylation mixture could be saponified perfectly well without isolation of intermediates to give the products (**30**) and (**33**) in good yield. An example of the value of the differential protection was given by the partial deprotection of (**30**) using 90% trifluoroacetic acid to give the zwitterion (**34**).*

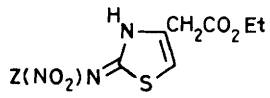
Another possible entry into the 2-amino series was provided by the *N,O*-bis(benzyloxycarbonyl) derivative (**19**) previously referred to. This material was reduced quite cleanly with zinc–HCl to give the ring-protected, free 2-amino ester (**35**) which by ester hydrolysis again gave the amino acid (**34**). However, this procedure suffered from the usual drawbacks of working with amino acid esters in basic solution (dioxopiperazine formation, *etc.*). In general terms, the 2-methoxyimino derivatives are the preferred precursors of the 2-amino series, as the initial thiourea closure gives a much higher yield and the succeeding steps of hydrogenation and acylation of the 2-amino group are also high-yielding.

Physical Data.—In view of the tautomeric nature of the 2-aminothiazole system[7] (Scheme 2), we now give some evidence based on u.v. spectroscopy that our mono-protected derivatives, such as (**4**), are indeed acylated on the exocyclic nitrogen.



Scheme 2. Tautomerism of 2-aminothiazoles

The bis derivatives, such as (**5**), can only reasonably have the 2-imino-2,3-dihydrothiazole structure as shown.† On the other hand, it could be argued that compound (**4**) may exist either as shown or in the 2-imino formulation (**4'**).



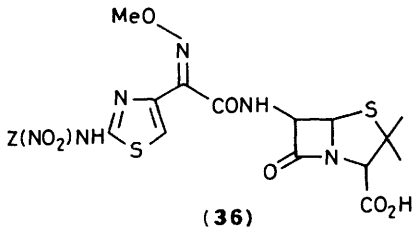
(**4'**)

We found that (**4**) has a very simple chromophore, $\lambda_{max.}$ 262 nm (ε 18 200) (2% $CHCl_3$–EtOH), and is virtually transparent to wavelengths above 300 nm. On the other hand, (**5**), though possessing a similar absorption maximum, $\lambda_{max.}$ 263 nm (ε 22 500) (2% $CHCl_3$–EtOH), still absorbs significantly at 320 nm, a pronounced 'shoulder' appearing at about 300 nm. This strongly suggests that (**4**) does not possess the 2-imino-2,3-dihydrothiazole chromophore of (**5**). The fact that saponification of (**4**) cleanly gives the acid (**6**) in high yield without loss of the urethane protecting group is also significant, in view of the lability to base of such urethanes when they lack an –NH– moiety (see above and refs. 12 and 13). The simple chromophore of the type shown by (**4**) is shared by the final acid products after saponification of the esters, and we are therefore confident that they are acylated on the exocyclic nitrogen as formulated. A similar conclusion was reached by Zugravescu and Carp.[8]

Penicillin Synthesis.—The simplest approach was provided by coupling of the (*E*)-α-methoxyimino and (**11**) to 6-aminopenicillanic acid (APA) which was achieved in high yield *via* the acid chloride of (**11**). The product (**36**), isolated as its sodium salt, could be converted on prolonged hydrogenation into the diastereoisomeric α-aminopenicillin (**37**), which by reaction with 4-ethyl-2,3,-dioxopiperazin-1-ylcarbonyl chloride was transformed into the piperacillin[16] analogue (**38**).[17] Material produced in this way, however, was invariably

* This compound has been described previously [A. G. Bayer, W. Germany, U.S.P. 4 235 774 (*Chem. Abstr.*, 1980, **92**, 76 493)] but no details of its preparation were given.

† An alternative 2-diacylaminothiazole structure would be expected to show two equivalent aryloxycarbonyl groups. In fact, the ^1H n.m.r. of all our bis derivatives show two $ArCH_2O$ signals, and compounds (**5**) and (**9**) clearly show two double doublets for the aryl protons.



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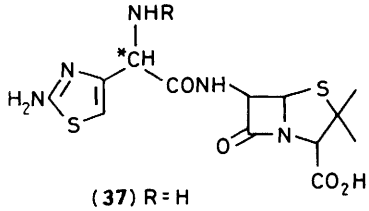
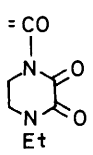
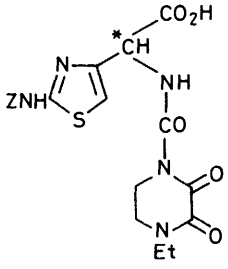
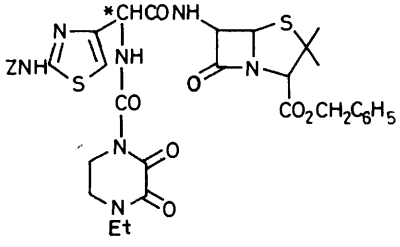


(37) R = H

(38) R = CO







(39) (40)

Table 1. Comparative antibacterial activities of piperacillin and compound (**38**) against selected organisms. Minimum inhibitory concentration values (M.I.C.s) are quoted in μg per ml and were measured by serial dilution in nutrient agar. We are grateful to Mr. Brian Slocombe and other members of our biological team for these measurements

Organism	M.I.C. values	
	Piperacillin	(**38**)
Escherichia coli ESS*	<0.02	<0.02
Escherichia coli NCTC10418	1.2	1.0
Pseudomonas aeruginosa NCTC10662	5.0	5.0
Enterobacter cloacae N1	1.2	0.5
Proteus mirabilis C977	0.2	0.2
Proteus morganii I580	0.5	0.5
Staphylococcus aureus Oxford NCTC6511	0.5	1.0
Neisseria catarrhalis NCTC3622	<0.02	<0.02

* Outer cell-wall deficient mutant; see P. H. Bentley and A. V. Stachulski, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1187.

contaminated with 4-ethyl-2,3-dioxopiperazine, which was not easy to remove, and in any case the yield was modest (21%).

A more satisfactory synthesis, which also gave the possibility of isomeric separation, was provided by acylation of the zwitterion (**34**) with the above-mentioned carbonyl chloride to give the acid (**39**).* This material, a fully characterised solid, was coupled to APA benzyl ester to afford, in high yield, a separable mixture of the diastereoisomeric penicillins (**40**).†

Finally, hydrogenolysis of the separated isomers gave the isomers of the piperacillin analogue (**38**). The more biologically active isomer of (**38**) came from the more polar protected isomer (**40**), and its biological activity against selected organisms compared to that of piperacillin itself is shown in Table 1. They can be seen to be very similar. Against β-lactamase producing strains of Gram-positive and -negative bacteria both (**38**) and piperacillin itself exhibited M.I.C. values ⩾100, in common with most 6*H*-penicillins.

Experimental

M.p.s were determined in a Büchi oil immersion apparatus. N.m.r. spectra were recorded on a Perkin-Elmer R32 instrument at 90 MHz using tetramethylsilane as an internal standard unless otherwise stated. All the compounds for which elemental analyses are quoted were obtained in a chromatographically homogeneous state and had i.r. spectra consistent with the structures quoted, including a strong absorption at 1 770—1 780 cm^{-1} for the penicillins. Thin-layer chromatograms were run on Merck Kieselgel $60F_{254}$ plates in one of the following systems: A, methanol–chloroform (1:9); B, chloroform–methanol–acetic acid (17:2:1); C, n-butyl alcohol–acetic acid–water, 4:1:1, unless otherwise stated. Final organic extracts were, in general, washed with brine and dried (Na_2SO_4) prior to rotary evaporation at <35 °C. Ether refers to diethyl ether, and light petroleum to the fraction with b.p. 60—80 °C.

Ethyl 2-Aminothiazol-4-ylacetate (**1**).—Thiourea (1.52 g, 20 mmol) was added to a solution of ethyl 4-chloroacetoacetate (3.29 g, 20 mmol) in ethanol (20 ml). The mixture was warmed briefly on the steam-bath until all the solid had dissolved, then stoppered and left at room temperature for 16 h. Concentration to a small volume and addition of an equal volume of ethyl acetate afforded crystals, which were filtered off and recrystallised from propan-2-ol to give the *hydrochloride* of the title compound (3.53 g. 79%), m.p. 156—158 °C; R_F (A) 0.45; δ([CD_3]$_2$SO) 1.20 (3 H, t, CH_3CH_2), 3.73 (2 H, s, CH_2CO), 4.10 (2 H, qt, CH_3CH_2), 6.71 (1 H, s, 5-H), and 9.45 (3 H, br s, D_2O exchanged, NH_3^+) (Found: C, 37.8; H, 4.7; N, 12.8. $C_7H_{11}ClN_2O_2S$ requires C, 37.8; H, 4.9; N, 12.6%). The free base had m.p. 93—94 °C (lit.,[18] 94—95 °C).

Ethyl 2-Benzyloxycarbonylaminothiazol-4-ylacetate (**2**).—Ethyl 2-aminothiazol-4-ylacetate (0.93 g, 5 mmol), freshly prepared from its hydrochloride salt, was vigorously stirred in a mixture of dichloromethane (10 ml) and saturated aqueous sodium hydrogen carbonate (20 ml) while benzyl chloroformate (2.14 g, 12.5 mmol) was added dropwise. After the addition, vigorous stirring was continued for 16 h. Ethyl acetate (70 ml) was added, the aqueous phase was run off, and the organic phase washed twice more with water. Following evaporation (see general experimental details above) an oil resulted, which on trituration with ether–light petroleum deposited a white solid (1.64 g). T.l.c. showed this to be a mixture of two major products which were faster-running than starting material, of which none remained; n.m.r. strongly suggested a mixture of the mono- and di-acylated species (δ 5.30, 5.45, $PhCH_2O$-type). Chromatography on silica gel (140 g), eluting with chloroform, afforded after pooling and evaporation of the appropriate fractions, the title *benzyloxycarbonyl derivative* (1.16 g, 73%), m.p. 105—106 °C; R_F (A) 0.70; δ($CDCl_3$) 1.18 (3 H, t, CH_3CH_2),

* The corresponding 2-(4-nitrobenzyloxycarbonylamino) acid could be prepared similarly in two steps from (**33**). This variant allowed easier hydrogenolysis in the final step, but the acid corresponding to (**39**) was difficult to obtain in a pure state.

† Interestingly, coupling of the acid (**27**) to APA benzyl ester gave an inseparable diastereoisomeric mixture.

3.58 (2 H, s, CH_2CO), 4.11 (2 H, qt, CH_3CH_2), 5.26 (2 H, s, $PhCH_2O$), 6.74 (1 H, s, 5-H), 7.37 (5 H, s, $C_6H_5CH_2$), and 11.27 (1 H, br s, D_2O exchanged, NH) (Found: C, 56.2; H, 5.0; N, 8.6%; M^+, 320.082 08. $C_{15}H_{16}N_2O_4S$ requires C, 56.3; H, 5.0; N, 8.8%; M^+, 320.082 11).

Ethyl 2-(4-*Nitrobenzyloxycarbonylamino*)*thiazol*-4-*ylacetate* (**4**) *and Ethyl* 3-(4-*Nitrobenzyloxycarbonyl*)-2-(4-*nitrobenzyloxycarbonylimino*)-2,3-*dihydrothiazol*-4-*ylacetate* (**5**).—Ethyl 2-aminothiazol-4-ylacetate hydrochloride (11.5 g, 50 mmol) was converted into the free base form, then subjected to two-phase acylation in dichloromethane (100 ml) and saturated aqueous sodium hydrogen carbonate (75 ml) with 4-nitrobenzyl chloroformate (25 g, 116 mmol) as described above for the preparation of (**2**). After 2 h the precipitated solid was filtered off, washed with water and acetone, and dried to afford the *bis*(4-*nitrobenzyloxycarbonyl*) *derivative* (21 g, 77%), m.p. 169—170 °C (decomp.); δ[$(CD_3)_2SO$] 1.14 (3 H, t, CH_2CH_3), 3.92 (2 H, s, $COCH_2$), 4.05 (2 H, q, CH_3CH_2), 5.33 and 5.62 (4 H, 2 s, OCH_2Ar), 6.95 (1 H, s, 5-H), and 7.5—8.5 (8 H, 2 dd, aryl H) (Found: C, 49.9; H, 3.6; N, 10.1; S, 5.45. $C_{23}H_{20}N_4O_{10}S{\cdot}0.5H_2O$ requires C, 49.9; H, 3.8; N, 10.1; S, 5.8%).

This material (5.44 g, 10 mmol) was added to a solution of sodium hydroxide (0.8 g, 20 mmol) in water (5 ml) and diluted with ethanol (50 ml). The mixture was stirred for 3 h at room temperature after which insoluble material was filtered off, washed with ethanol, and dried to give sodium 2-(4-nitrobenzyloxycarbonylamino)thiazol-4-ylacetate (2 g, 56%), which was converted into the acid (*vide infra*). The filtrate was evaporated to dryness, diluted with water, and extracted exhaustively with ethyl acetate, the aqueous phase being retained. The organic extracts after evaporation were recrystallised from ethyl acetate to give the *mono*(4-*nitrobenzyloxycarbonyl*) *derivative* (0.75 g, 21%), m.p. 155—157 °C; δ[$(CD_3)_2SO$] 1.17 (3 H, t, CH_2CH_3), 3.63 (2 H, s, $COCH_2$), 4.08 (2 H, q, CH_2CH_3), 5.38 (2 H, s, OCH_2Ar), 6.93 (1 H, s, 5-H), and 7.68 and 8.28 (4 H, dd, aryl H) (Found: C, 49.1; H, 4.1; N, 11.3; S, 8.2. $C_{15}H_{15}N_3O_6S$ requires C, 49.3; H, 4.1; N, 11.5; S, 8.8%).

2-(4-*Nitrobenzyloxycarbonylamino*)*thiazol*-4-*ylacetic Acid* (**6**).—The sodium salt isolated from the preceding preparation was dissolved in warm water and acidified to pH 4 using 5M-hydrochloric acid, when the N-*protected acid* precipitated; it was filtered, washed with water, and dried (1.1 g, 33%). Further product resulted from the acidification of the aqueous phase remaining from the isolation of (**4**) (*vide supra*) giving a total yield of 1.7 g (50%), m.p. 208—209 °C; δ[$(CD_3)_2SO$] 3.58 (2 H, s, $COCH_2$), 5.40 (2 H, s, OCH_2Ar), 6.97 (1 H, s, 5-H), 7.70 and 8.28 (4 H, dd, aryl H), and 9—12 (2 H, vbr s, D_2O exchanged, NH and OH) (Found: C, 45.9; H, 3.3; N, 12.2; S, 9.1. $C_{13}H_{11}N_3O_6S$ requires C, 46.3; H, 3.3; N, 12.45; S, 9.5%).

Ethyl (E)-α-(2-*Aminothiazol*-4-*yl*)-α-*hydroxyiminoacetate* (**7**).—Ethyl α-oximinoacetoacetate[19] (7.95 g, 50 mmol) was chlorinated according to the procedure of Hatanaka.[5] The disappearance of the methyl group signal at δ 2.4 and the appearance of a methylene at δ 4.7 showed when the reaction had reached completion. After evaporation the crude ethyl γ-chloro-α-oximinoacetoacetate (9.68 g, quantitative) which was unstable, was immediately dissolved in ethanol (50 ml) and treated with thiourea (3.8 g, 50 mmol) at room temperature. After 3 h the solution was evaporated, the residue was redissolved in water and washed once with ether. The aqueous phase was evaporated to near dryness, the residue was taken up in hot ethanol (20 ml) and ethyl acetate (20 ml) was added. Crystals of the title compound hydrochloride separated (4.81 g, 38%), m.p. 185—187.5 °C (decomp.); R_F (B) 0.55; δ(D_2O) 1.20 (3 H, t, CH_2CH_3), 4.30 (2 H, q, CH_2CH_3), and 7.70 (1 H, s, 5-H). The appearance of only one thiazole C-5 proton showed the material to be probably a single isomer; this was confirmed by conversion into the free base form, giving material identical (m.p., n.m.r.) with that prepared previously.[1]

Ethyl (E)-α-(2-*Aminothiazol*-4-*yl*)-α-*methoxyiminoacetate* (**8**).—This was prepared by a slight modification of the procedure of Bucourt *et al.* Ethyl γ-bromo-α-methoxyiminoacetoacetate[1] (10.1 g, 40 mmol), freshly prepared, was immediately dissolved in ethanol (40 ml) and heated at reflux for 2 h with thiourea (6.4 g, 84 mmol). Much solid had already separated. The bulk of the ethanol was removed by evaporation and water (25 ml) was added. The mixture was cooled to complete crystallisation, then the crystals were filtered, washed with a little cold water, and dried to give the hydrobromide of the title compound (9.1 g, 73%), m.p. 203—204 °C (decomp.); δ[$(CD_3)_2SO$] 1.12 (3 H, t, CH_2CH_3), 3.93 (3 H, s, OCH_3), 4.10 (2 H, q, CH_2CH_3), 7.42 (1 H, s, 5-H), and 8.10 (3 H, br s, D_2O exchanged, NH_3^+). The material was suspended in a mixture of ethyl acetate (100 ml) and a solution of sodium carbonate (6.5 g) in water (100 ml) and stirred till all had dissolved. The organic phase was separated and the aqueous layer extracted with further ethyl acetate (50 ml); then the total extract was washed with water. After evaporation the residue was recrystallised from ethyl acetate–light petroleum to afford the (*E*)-oxime (5.53 g, 60% overall), m.p. 114—115 °C (lit.,[1] m.p. 115 °C); R_F (A) 0.45.

Ethyl (E)-α-*Methoxyimino*-α-[2-(4-*nitrobenzyloxycarbonylamino*)*thiazol*-4-*yl*]*acetate* (**10**) *and Ethyl* (E)-α-*Methoxyimino*-α-[3-(4-*nitrobenzyloxycarbonyl*)-2-(4-*nitrobenzyloxycarbonylimino*)-2,3-*dihydrothiazol*-4-*yl*]*acetate* (**9**).—Ethyl (*E*)-α-methoxyimino-α-(2-aminothiazol-4-yl)acetate (1.6 g, 7 mmol) in purified dichloromethane (15 ml) was subjected to two-phase acylation with saturated aqueous sodium hydrogen carbonate (25 ml) and 4-nitrobenzylchloroformate (3.66 g, 17 mmol) as described for the preparation of (**2**). After 2 h a little ether was added and the precipitated solid filtered, washed with water and ether, and dried to give the *bis*(4-*nitrobenzyloxycarbonyl*) *oxime* (**9**) (1.88 g, 47%), m.p. 110—115 °C; R_F (A) 0.7; δ[$(CD_3)_2SO$] 1.2 (3 H, t, CH_2CH_3), 4.0 (3 H, s, OCH_3), 4.25 (2 H, q, CH_2CH_3), 5.35 and 5.60 (4 H, 2 s, 2 × OCH_2Ar), 7.40 (1 H, s, 5-H), and 7.5—8.5 (8 H, 2 dd, aryl H) (Found: C, 49.0; H, 3.7; N, 11.8. $C_{24}H_{21}N_5O_{11}S$ requires C, 49.1; H, 3.6; N, 11.9%). The mother-liquors contained a mixture of products by t.l.c.; chromatography on silica gel, eluting with chloroform afforded firstly a little more (*ca.* 10%) of the above derivative, then, on further elution, the *mono*(4-*nitrobenzyloxycarbonyl*) *oxime* (**10**) (0.69 g. 24%), m.p. 126—133 °C; R_F (A) 0.60; δ[$CDCl_3$ + $(CD_3)_2CO$] 1.35 (3 H, t, CH_2CH_3), 4.18 (3 H, s, OCH_3), 4.42 (2 H, q, CH_2CH_3), 5.50 (2 H, s, OCH_2Ar), 7.77 and 8.40 (4 H, dd, aryl H), 8.04 (1 H, s, 5-H), 11.0 (1 H, br s, D_2O exchanged, NH) (Found: C, 47.2; H, 4.0; N, 13.7. $C_{16}H_{16}N_4O_7S$ requires C, 47.1; H, 3.9; N, 13.7%).

(E)-α-*Methoxyimino*-α-[2-(4-*nitrobenzyloxycarbonylamino*)-*thiazol*-4-*yl*]*acetic Acid* (**11**).—The preceding bis-protected ester (**9**) (6.48 g, 11 mmol) in ethanol (66 ml) was stirred with a solution of potassium hydroxide (2.46 g, 44 mmol) in water (44 ml) at room temperature. A clear solution was obtained in 1 h. After 4 h the solution was washed with ethyl acetate (2 × 50 ml), backwashing each time with a little water. The total aqueous phase was acidified to pH 2 with 5M-hydrochloric acid and the crude product was filtered, washed with water and ether, and dried. Recrystallisation from tetrahydrofuran–light petroleum afforded the *protected acid* (3.37 g, 88%) (see Table 2). The preceding mono-protected ester could be processed in a similar way to give further acid of equivalent purity.

Table 2. α-(*E*)-Oximino acids

Compound	Intermediate	Yield (%) from (**8**)	M.p. (°C) (Solvent)	R_F (B)	δ[$(CD_3)_2SO$] OCH_3	δ[$(CD_3)_2SO$] 5-H	Analysis: Found (%) C	H	N	Formula	Required (%) C	H	N
(**11**)	(**9**) + (**10**)	88[a]	>200[b] (THF–light petroleum)	0.30	4.00	8.00	44.2	3.2	14.4	$C_{14}H_{12}N_4O_7S$	44.2	3.2	14.7
(**14**)	(**12**) + (**13**)	75	155—156 (aq.EtOH)	0.40	4.01	7.99	50.1	3.9	12.3	$C_{14}H_{13}N_3O_5S$	50.1	3.9	12.5
(**16**)	(**15**)[c]	80	194 (EtOAc–light petroleum)		4.00	8.07							
(**18**)	(**17**)	83	152—154[d] (EtOAc–light petroleum)	0.35	4.02	8.03							

[a] Yield from (**9**), see text. [b] Ill-defined with decomposition. [c] Prepared either according to the literature procedure[14] or by reaction with *N,N'*-dicyclohexylcarbodi-imide and chloroacetic acid. Saponification time 0.4. h. [d] Lit.,[14] m.p. 162—163 °C (oxime stereochemistry not specified).

Other N-*Protected* (E)-α-(2-*Aminothiazol*-4-*yl*)-α-*methoxyiminoacetic Acids*, (**14**), (**16**), and (**18**).—These were prepared essentially according to the procedures described for the preparation of (**11**) from (**8**); the results are summarised in Table 2. The intermediate species, whether mono- or di-acylated or a mixture, was regarded as sufficiently characterised by t.l.c. and n.m.r. behaviour by analogy with (**9**) and (**10**). Saponification of the intermediate mixtures gave in all cases good yields of single isomers of the protected acids.

Ethyl (E)-α-*Benzyloxycarbonyloxyimino*-α-(2-*benzyloxycarbonylaminothiazol*-4-*yl*)*acetate* (**19**).—Ethyl (*E*)-α-(2-aminothiazol-4-yl)-α-hydroxyiminoacetate (1.07 g, 5 mmol) in dichloromethane (50 ml) was treated with saturated aqueous sodium hydrogen carbonate solution (15 ml) and benzyl chloroformate (1.88 g, 11 mmol) under the two-phase conditions described for the preparation of (**2**). After being stirred for 24 h at room temperature the phases were separated, the aqueous phase further extracted with dichloromethane, and the combined extracts washed with water. Evaporation afforded a yellow oil which was subjected to chromatography on silica gel 60 (<230 mesh ASTM), eluting with 20% ethyl acetate in cyclohexane, to afford the bis(benzyloxycarbonyl) ester as a yellow oil (1.53 g, 65%); R_F (A) 0.80; δ($CDCl_3$) 1.29 (3 H, t, CH_2CH_3), 4.33 (2 H, q, OCH_2CH_3), 5.28 and 5.32 (4 H, 2 s, 2 × OCH_2Ph), 7.40 (10 H, br s, aryl H), 7.99 (1 H, s, 5-H), and 9.83 (1 H, br s, D_2O exchanged, NH). The material could not be induced to crystallise, but its conversion into (**34**) (*q.v.*) provided further support for its structure.

The use of just 1 equiv. of benzyl chloroformate in the above reaction gave a mixture of products including (**19**), unchanged starting material, and a material more polar than (**19**) which was eluted from a silica column, probably an *O*-benzyloxycarbonyl derivative, obtained as an oil, R_F (A) 0.60; δ($CDCl_3$) 1.41 (3 H, t, CH_2CH_3), 4.50 (2 H, q, OCH_2CH_3), 5.46 (2 H, s, OCH_2Ph), 5.65 (2 H, br s, D_2O exchanged, 2-NH_2), 7.55 (5 H, s, aryl H), and 7.85 (1 H, s, 5-H).

Ethyl α-*Amino*-α-(2-*aminothiazol*-4-*yl*)*acetate Dihydrochloride* (**20**).—Ethyl (*E*)-α-(2-aminothiazol-4-yl)-α-methoxyiminoacetate (**8**) (2.07 g, 9 mmol) was suspended in ethanol (25 ml) and 1M-hydrochloric acid (25 ml). 10% Palladium on charcoal (0.85 g) was added and the mixture hydrogenated at S.T.P. until the theoretical volume of hydrogen had been absorbed, usually in about 2 h. The catalyst was filtered off, washed with ethanol and water, and the filtrate concentrated to remove ethanol. Generally the aqueous dihydrochloride solution was used at once for α-acylation (*vide infra*), since on storage it decomposed more quickly in the free base form. The product had R_F (A) 0.1; an aliquot on evaporation showed δ[$(CD_3)_2SO$] 1.22 (3 H, t, CH_2CH_3), 4.25 (2 H, q, CH_2CH_3), 5.38 (1 H, s, α-H), and 7.13 (1 H, s, 5-H).

Ethyl α-*Chloroacetamido*-α-(2-*chloroacetamidothiazol*-4-*yl*)*acetate* (**21**).—A portion of the aqueous solution of the above dihydrochloride was partitioned between sodium hydrogen carbonate and five portions of chloroform. Evaporation afforded ethyl α-amino-α-(2-aminothiazol-4-yl)acetate (1.07 g, 5.3 mmol) which was at once converted into the bis(chloroacetamido) ester according to the published procedure[14] (1.34 g, 71%), m.p. 102—103.5 °C (lit.,[14] 102.5—103.5 °C); R_F (methanol–chloroform, 1:19), 0.40. Saponification according to the patent procedure[14] gave the acid, m.p. 183—184 °C (lit.,[14] 184—186 °C).

Ethyl α-(2-*Aminothiazol*-4-*yl*)-α-(4-*nitrobenzyloxycarbonylamino*)*acetate* (**23**).—An aqueous solution (100 ml) of the dihydrochloride (**20**) prepared as described from (**8**) (4.58 g, 20 mmol) was cooled to 0 °C. Solid potassium carbonate was added until the solution reached pH 9, and it was then vigorously stirred while 4-nitrobenzyl chloroformate (4.47 g, 22 mmol) in solution in tetrahydrofuran (40 ml) was added dropwise during a few min. The solution was allowed to regain room temperature, the pH being maintained at 9 by the addition of further base. After 1 h ethyl acetate (50 ml) was added and the organic phase was separated. The aqueous phase was further extracted with ethyl acetate, then the total organic phase was washed with water. Evaporation gave a crude product, which on recrystallisation from ethyl acetate–light petroleum afforded, in two crops, the *mono-protected ester* (5.9 g, 77%); for characterisation see Table 3.

Other N-α-*Protected Ethyl* α-*Amino*-α-(2-*aminothiazol*-4-*yl*)*acetates* (**22**) *and* (**24**).—These were prepared according to the procedure described for the synthesis of (**23**), using benzyl chloroformate and di-t-butyl dicarbonate[15] respectively as the acylating agent for (**22**) and (**24**). The characterisation of all three derivatives (**22**)—(**24**) is summarised in Table 3.

Ethyl α-(2-*Benzyloxycarbonylaminothiazol*-4-*yl*)-α-(*t-butoxycarbonylamino*)*acetate* (**28**) *and Ethyl* α-(3-*Benzyloxycarbonyl*-2-*benzyloxycarbonylimino*-2,3-*dihydrothiazol*-4-*yl*)-α-(*t-butoxycarbonylamino*)*acetate* (**29**).—The α-(t-butoxycarbonylamino)

Table 3. Mono-protected ethyl α-amino esters

Compound	Yield (%) from (8)	M.p. (°C)	(Solvent)	R_F (A)	δ[(CD$_3$)$_2$SO] α-H	δ[(CD$_3$)$_2$SO] 5-H	Analysis Found (%) C	H	N	Formula	Required (%) C	H	N
(22)	50[a]	116	(EtOAc–ether)	0.41	5.08	6.47	53.7	5.0	12.3	$C_{15}H_{17}N_3O_4S$	53.7	5.1	12.5
(23)	79	154—156	(EtOAc–light petroleum)	0.40	5.09	6.51	47.5	4.4	14.4	$C_{15}H_{16}N_4O_6S$	47.4	4.2	14.7
(24)	76	144.5—145.5[b]	(EtOAc–light petroleum)	0.38	4.89	6.38	48.0	6.45	13.9	$C_{12}H_{19}N_3O_4S$	47.8	6.3	13.95

[a] Yield from (7). [b] Lit.,[4] m.p. 143—144 °C.

Table 4. Bis-protected ethyl α-amino esters

Compound	Yield (%)	M.p. (°C)[d]	R_F (A)	δ(CDCl$_3$) α-H	δ(CDCl$_3$) 5-H	Analysis Found (%) C	H	N	Formula	Required (%) C	H	N
(25)	54[a]	144—145	0.70	5.45	6.87	53.7	4.4	10.6	$C_{23}H_{22}N_4O_8S$	53.7	4.3	10.9
(28)	65[b]	126.5—128	0.71	5.38	6.86	55.0	5.7	9.55	$C_{20}H_{25}N_3O_6S$	55.2	5.7	9.7
(31)	77[c]	164.5—165.5	0.65	5.43	6.94	50.3	5.2	11.7	$C_{20}H_{24}N_4O_8S$	50.0	5.0	11.7

[a] From (23). Facile crystallisation on trituration of the crude product with ether was observed. N.m.r. of the mother-liquors confirmed substantial loss of the α-4-nitrobenzyloxycarbonyl group (see text), accounting in part for the relatively low yield. [b] From (24), see text. Compound (29) was also formed, combined yield of (28) + (29) = 85%. [c] From (24). Chromatography was necessary to obtain pure (31), free of bis(4-nitrobenzyl) carbonate (silica gel, $CHCl_3$ elution). A less polar material was obtained from the column which appeared consistent with the bis(4-nitrobenzyloxycarbonyl) ester (32), R_F (A) 0.75; δ(CDCl$_3$) 1.20 (3 H, t, OCH_2CH_3), 1.40 (9 H, s, CMe_3), 4.20 (2 H, q, CH_2CH_3), 5.35 and 5.40 (4 H, 2 s, 2 × OCH_2Ph), 7.20 (1 H, s, 5-H), 7.60 and 8.30 (8 H, 2 m, aryl H). [d] All from EtOAc–light petroleum.

ester (**24**) (0.9 g, 3 mmol) in dry dichloromethane (6 ml) and tetrahydrofuran (4 ml) was subjected to two-phase acylation with saturated aqueous sodium hydrogen carbonate (12 ml) and benzyl chloroformate (1.07 ml, 1.28 g, 7.5 mmol) as described for the preparation of (**2**). After being stirred vigorously for 16 h the reaction mixture was partitioned between water (50 ml) and ethyl acetate (80 ml). The organic phase was separated and washed twice more with water. After evaporation the crude, oily product was triturated with ether (5 ml), when a white solid was deposited. This was filtered, washed with a little cold ether and light petroleum and dried to give the *mono(benzyloxycarbonyl) ester* (**28**) (0.7 g, 52%). Further product (**28**) was obtained by chromatography of the mother-liquors on silica gel, eluting with 2.5% methanol in chloroform, raising the total yield to about 65%; for characterisation see Table 4.

The less polar material eluted from the column could not be induced to crystallise but its characteristics were consistent with the bis(benzyloxycarbonyl) ester (**29**), particularly as it could be saponified to the same acidic product; R_F (A) 0.8; δ($CDCl_3$) 1.07 (3 H, t, OCH_2CH_3), 1.40 (9 H, s, CMe_3), 4.08 (2 H, q, OCH_2CH_3), 5.20 and 5.30 (4 H, 2 s, 2 × OCH_2Ph), 7.07 (1 H, s, 5-H), and 7.40 (10 H, s, aryl H). The other bis-protected esters, (**25**) and (**31**), were similarly obtained.

α-(2-Benzyloxycarbonylaminothiazol-4-yl)-α-(t-butoxycarbonylamino)acetic Acid (**30**).—In general, it was easier to obtain this and the other bis-protected acids, (**26**), (**27**), and (**33**), from the appropriate 2-amino derivative (**23**) or (**24**), without isolation of the intermediate acylated esters. The crude reaction products from the two-phase acylation saponified cleanly and in good yield.

Thus, the α-(t-butoxycarbonylamino) ester (**24**) (1.5 g, 5 mmol) was subjected to two-phase acylation as described for the preparation of (**28**). The total crude reaction product was dissolved in ethanol (15 ml) and a solution of potassium hydroxide (1.4 g, 25 mmol) in water (10 ml) was added with stirring. After 1 h the solution was diluted to 50 ml with water and washed twice with ether. The aqueous phase was acidified to pH 3 with 10% aqueous citric acid, and on being scratched and cooled a solid separated. This was filtered off and washed well with water to afford the *bis-protected acid* (1.65 g, 81%); for characterisation see Table 5.

The other *bis-protected acids* (**26**), (**27**), and (**33**) were obtained similarly except that it was necessary to saponify the crystalline ester (**25**) to obtain pure (**2**); *cf.* footnote *a* to Table 4.

α-Amino-α-(2-benzyloxycarbonylaminothiazol-4-yl)acetic Acid (**34**).—*Method A*. The α-(t-butoxycarbonylamino) acid (**30**) (1.02 g, 2.5 mmol) was dissolved in neat redistilled trifluoroacetic acid (4 ml). After 0.5 h at room temperature the solution was evaporated to dryness and re-evaporated twice more with chloroform (2 × 10 ml). The resulting gum was triturated with water (20 ml) giving an immediate dense white precipitate; to ensure complete precipitation, the pH of the supernatant was adjusted to 5.5. The precipitated solid was filtered, washed with water, ethanol, and ether, and dried to give the *α-amino acid* as a dihydrate (0.80 g, 93%); m.p. 135—137 °C (decomp.); R_F (C) 0.35; δ(D_2O–NaOD), 4.44 (1 H, s, C*H*NH), 5.26 (2 H, s, OCH_2Ph), 6.77 (1 H, s, 5-H), and 7.56 (5 H, s, aryl H) (Found: C, 45.2; H, 4.9; N, 12.0. $C_{13}H_{13}N_3O_4S{\cdot}2H_2O$ requires C, 45.5; H, 4.95; N, 12.2%).

Method B. The *N,O*-bis(benzyloxycarbonyl) ester (**19**) (1.53 g, 3.3 mmol) was dissolved in tetrahydrofuran (20 ml), and water (4 ml) was added. The pH of the solution was kept at 4.5 by dropwise addition of 1M-hydrochloric acid while zinc dust was added with vigorous stirring. After 0.5 h no starting material was observable by t.l.c. and the consumption of acid had ceased. The pH was raised to 7, the solution was filtered and the solids washed well with tetrahydrofuran. The combined filtrate was partitioned between ethyl acetate and water and the organic

Table 5. Bis-protected α-amino acids

Compound	Yield (%)	M.p. (°C)	(Solvent)	R_F (B)	δ[(CD$_3$)$_2$SO] α-H	δ[(CD$_3$)$_2$SO] 5-H	Analysis Found (%) C	H	N	Formula	Required (%) C	H	N
(**26**)	99[a]	172—174	(aq. EtOH)	0.8[b]	5.00	6.97	51.4	3.7	11.4	$C_{21}H_{18}N_4O_8S$	51.85	3.7	11.5
(**27**)	59[c]	197—201	(aq. THF)	0.35	5.21	7.13	46.5	3.2	12.9	$C_{21}H_{17}N_5O_{10}S\cdot 0.5H_2O$	46.7	3.3	13.0
(**30**)	81	167—168	(EtOAC–light petroleum)	0.50	5.33[d]	7.10[d]	53.15	5.25	10.3	$C_{18}H_{21}N_3O_6S$	53.1	5.2	10.3
(**33**)	85[e]	178—179	(EtOAc–light petroleum)	0.82[b]	5.13	7.10	48.0	4.5	12.25	$C_{18}H_{20}N_4O_8S$	47.8	4.4	12.4

[a] From (**25**). [b] In system C. [c] In two steps from (**23**); see preparation of (**30**) in text. [d] In $(CD_3)_2CO$. [e] In two steps from (**24**).

phase separated and washed with water. Evaporation afforded a red oil (1.48 g), with an n.m.r. spectrum corresponding to that of the α-amino ester (**35**) plus impurities: it was unstable and used at once by being dissolved in ethanol (20 ml) and treated with 10% aqueous sodium hydroxide (3 ml). After 2 h no ester was visible by t.l.c. Concentration of the solution to remove organic solvents and acidification of the aqueous layer to pH 5 afforded a solid which was filtered, washed with water, and dried to give material (0.48 g, 48%) which appeared to be identical with that obtained by method A.

6-{(E)-α-*Methoxyimino*-α-[2-(4-*nitrobenzyloxycarbonylamino*)*thiazol*-4-*yl*]*acetamido*}*penicillanic Acid* (**36**).[17]—The protected acid (**11**) (1.14 g, 3 mmol) was stirred in dry dichloromethane (15 ml) with phosphorus pentachloride (0.68 g, 3.3 mmol) for 1 h at ambient temperature. The suspension was filtered and washed with a little cold dichloromethane, then with ether, and dried to give the acid chloride as a white solid (1.21 g, quantitative), $\nu_{max.}$(Nujol) 1 765 cm^{-1}. This material was added in small portions at 0 °C to a suspension of 6-aminopenicillanic acid (0.65 g, 3 mmol) in dichloromethane (12 ml) which had been previously stirred for 1 h with anhydrous triethylamine (1.06 ml). The pH of the vapour above the reaction mixture was maintained at 8—9 by addition of a few further drops of triethylamine. After 1 h, during which time the mixture was allowed to regain ambient temperature, the dichloromethane was evaporated and the residue partitioned between water (30 ml) and ether (2 × 20 ml). The aqueous phase was separated and acidified to pH 2 with 2.0M-hydrochloric acid with concomitant extraction into ethyl acetate (20 ml). The organic phase was separated and the aqueous layer extracted once more with ethyl acetate. The total organic extract was washed with water, then evaporated rigorously to dryness and redissolved in anhydrous ethyl acetate (30 ml). Addition of 2.0M-sodium 2-ethylhexanoate in methyl isobutyl ketone (1.5 ml) gave a white precipitate, which was filtered, well washed with ether, and dried to give the *penicillin sodium salt* (1.50 g, 83%); R_F (C) 0.70; δ[(CD$_3$)$_2$SO] 1.50 and 1.56 (6 H, 2 s, Me$_2$C), 3.95 (1 H, s, 3-H), 4.00 (3 H, s, NOC*H*$_3$), 5.36 (2 H, s, OC*H*$_2$Ar), 5.52 (2 H, br m, ABq t on D$_2$O exchange, 5-H and 6-H), 7.69 and 8.26 (4 H, dd, ArH), 7.90 (1 H, s, thiazole 5-H), and 8.92 (1 H, br d, D$_2$O exchanged, NH) (Found: C, 42.5; H, 3.8; N, 13.3. $C_{22}H_{21}N_6NaO_9S_2\cdot H_2O$ requires C, 42.7; H, 3.7; N, 13.6%).

6-[(±)-α-*Amino*-α-(2-*aminothiazol*-4-*yl*)*acetamido*]*penicillanic Acid* (**37**).[17]—The preceding sodium salt (3.60 g, 6 mmol) was converted back into the free acid form by partition between ethyl acetate and 10% aqueous citric acid. Evaporation of the solvent gave a semi-solid which was dissolved in ethanol (50 ml), then water (50 ml) was added. To the solution was added 10% palladium on charcoal (1.0 g), then the mixture was hydrogenated at S.T.P. for 20 h, adding further catalyst (1 g) after 3 h. After this time the catalyst was filtered off and the filtrate was hydrogenated with fresh catalyst (1 g) for 5 h. The catalyst was again filtered off and washed well with water, then the combined filtrate and washings were evaporated to dryness. Traces of the catalyst were removed by redissolving the solid in water (20 ml) and filtering. The resulting solution was freeze-dried to give the *zwitterionic penicillin* (1.37 g, 61%) as a very hygroscopic solid; R_F (C) 0.10; δ[(CD$_3$)$_2$SO] 1.45 and 1.55 (6 H, 2 s, Me$_2$C), 4.10 (1 H, s, 3-H), 4.55 and 4.60 (1 H, 2 s, *ca.* 2:1, diastereoisomeric α-H), 5.44 (2 H, m, 5-H and 6-H), 6.43 (1 H, s, thiazole 5-H), and 6.92 (2 H, br s, thiazole 2-NH$_2$) (Found: C, 35.3; H, 5.2; N, 15.4. $C_{13}H_{17}N_5O_4S_2\cdot 4H_2O$ requires C, 35.2; H, 5.6; N, 15.8%).

6-[(±)-α-(2-*Aminothiazol*-4-*yl*)-α-(4-*ethyl*-2,3-*dioxopiperazin*-1-*ylcarbonylamino*)*acetamido*]*penicillanic Acid* (**38**).[17]—The zwitterionic penicillin (**37**) (0.74 g, 2 mmol) was dissolved in water (5 ml) and saturated aqueous sodium hydrogen carbonate solution (5 ml), then tetrahydrofuran (5 ml) was added. The solution was stirred at 0 °C while a solution of 4-ethyl-2,3-dioxopiperazin-1-ylcarbonyl chloride[16] (0.41 g, 2 mmol) in dry tetrahydrofuran (5 ml) was added dropwise. The pH was maintained in the range 7.0—7.5 by adding further base. The solution was allowed to regain ambient temperature and stirred for 1 h, then washed with ethyl acetate (2 × 10 ml), backwashing each time with a little water. The combined aqueous phase was acidified to pH 3.0 and a small amount of yellow solid was filtered off. The filtrate was lyophilised and the residue was stirred with dry acetone (250 ml) at ambient temperature for 1 h. The acetone solution was filtered and evaporated to dryness, then the residue was redissolved in water (10 ml), filtered and lyophilised to give the α-acylureido penicillin as a hygroscopic solid (230 mg, 21%); R_F (C) 0.20; δ[(CD$_3$)$_2$SO] 1.16 (3 H, t, *J* 7 Hz, C*H*$_3$CH$_2$N), 1.43 and 1.56 (6 H, 2 s, Me$_2$C), 3.2—4.0 (6 H, m, 3 × CH$_2$N), 4.20 (1 H, s, 3-H), 5.35 (3 H, m, approx. ABqt with superimposed s on D$_2$O exchange, 5-H + 6-H + C*H*NH), 6.50 (1 H, s, thiazole 5-H), 6.97 (2 H, br s, D$_2$O exchanged, thiazole 2-NH$_2$), 8.70 and 9.60 (2 H, 2 d, D$_2$O exchanged, 6-NH and acetamido 2-CHN*H*). Biochromatography (paper chromatography, butanol–ethanol–water, 7:1:2, with *Bacillus subtilis* as the assay organism) showed a single zone of inhibition, R_F 0.16, but the n.m.r. spectrum also revealed contamination by 4-ethyl-2,3-dioxopiperazine.

(±)-α-(2-*Benzyloxycarbonylaminothiazol*-4-*yl*)-α-(4-*ethyl*-2,3-*dioxopiperazin*-1-*ylcarbonylamino*)*acetic Acid* (**39**).[17]—The

α-amino acid (**34**) (0.74 g, 2.41 mmol) was suspended in water (10 ml) and triethylamine (0.77 ml) and acetone (5 ml) were added. The suspension was cooled to 0 °C and stirred, then a solution of 4-ethyl-2,3-dioxopiperazin-1-ylcarbonyl chloride (0.51 g, 2.5 mmol) in dry acetone (10 ml) was added dropwise. After 0.25 h dissolution was virtually complete. Further triethylamine (0.15 ml) and carbonyl chloride (100 mg) were added and the solution was allowed to regain room temperature. After 1 h a small quantity of undissolved material was filtered off and the filtrate was washed with ethyl acetate, backwashing the total organic extract with a little water. The total aqueous phase was acidified to pH 1.0 with 2M-hydrochloric acid and extracted with ethyl acetate (2 × 20 ml). The combined extracts were washed once with water (10 ml); evaporation gave the crude product, which was conveniently purified by dissolving in saturated aqueous sodium hydrogen carbonate solution and reprecipitating with 2M-hydrochloric acid. The white precipitate was filtered off, washed with water, and dried to give the *protected acid* (0.82 g, 69%); m.p. *ca.* 130 °C (ill-defined); R_F (C) 0.25; δ($CDCl_3$) 1.10 (3 H, t, *J* 7 Hz, CH_3CH_2N), 3.1—3.7 (4 H, m), 3.7—4.1 (2 H, m, 3 × CH_2N), 5.15 (2 H, s, OCH_2Ph), 5.55 (1 H, d, *J* 7 Hz, s on D_2O exchange, CHNH), 6.85 (1 H, s, 5-H), 7.28 (5 H, s, ArH), and 9.77 (1 H, br d, D_2O exchanged, CHNH) (Found: C, 49.6; H, 4.5; N, 14.3. $C_{20}H_{21}N_5O_7S·0.5H_2O$ requires C, 49.6; H, 4.5; N, 14.5%).

(±)-6-[α-(2-*Benzyloxycarbonylaminothiazol*-4-*yl*)-α-(4-*ethyl*-2,3-*dioxopiperazin*-1-*ylcarbonylamino*)*acetamidopenicillanate Diastereoisomers* (**40**).[17]—The preceding acid (0.48 g, 1 mmol) and 1-hydroxybenzotriazole monohydrate[20] (0.15 g) were dissolved in dry dichloromethane (5 ml) and added dropwise to a solution of benzyl 6-aminopenicillanate (0.30 g, 1 mmol) and *N*,*N*′-dicyclohexylcarbodi-imide (0.21 g) in dry dichloromethane (5 ml). The mixture was allowed to regain room temperature and stirred with exclusion of moisture for a further 1 h. The precipitated urea was then filtered off and washed with ethyl acetate; the organic extract was washed sequentially with 0.5M-hydrochloric acid (2 × 10 ml), water (10 ml), saturated aqueous sodium hydrogen carbonate solution (2 × 10 ml), and water. Evaporation gave a crude product (0.84 g), t.l.c. of which showed a clear separation of the diastereoisomers. Separation was achieved by chromatography on silica gel (100 g), eluting with 2% methanol–chloroform to afford the less polar penicillin (170 mg) and the more polar penicillin (180 mg) as amorphous solids which retained solvents tenaciously; R_F (5% methanol–chloroform) 0.38 and 0.34 respectively; δ($CDCl_3$) (more polar isomer) 1.19 (3 H, t, *J* 7 Hz, CH_3CH_2N), 1.28 and 1.42 (6 H, 2 s, Me_2C), 3.3—3.7 (4 H, m, 2 × CH_2N), 3.85—4.15 (2 H, m, CH_2N), 4.32 (1 H, s, 3-H), 5.15 and 5.25 (4 H, 2 s, 2 × OCH_2Ph), 5.35—5.80 (3 H, m, 5-H + 6-H + CHNH), 6.79 (1 H, s, thiazole 5-H), 7.35 (10 H, s, ArH), 7.69 (1 H, d, NHCH), 9.25 (1 H, br s, thiazole 2-NH), and 9.82 (1 H, d, NHCH). The less polar isomer showed δ 6.86 (1 H, s, thiazole 5-H); other spectral differences were minor.

Hydrogenolysis of the separated isomers of (**40**) in aqueous tetrahydrofuran gave, after freeze-drying, the separate isomers of the pencillin (**38**) described above, but without any contaminating 4-ethyl-2,3-dioxopiperazine. The products still retained solvents very tenaciously, precluding meaningful elemental analysis. The biological activity of the more active isomer of (**38**) is shown in Table 1, and this is assigned the D-configuration of the side-chain by analogy with many α-aminopenicillins.

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