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Triazene Drug Metabolites. Part 11. Synthesis of *S*-Cysteinyl and Related Derivatives of *N*-Hydroxymethyltriazenes

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S-Cysteinyl-, *S*-(*N*-acetyl)cysteinyl-, *S*-glutathionyl and some related thioether derivatives of anticancer triazenes have been synthesised in high yield. The synthesis involves reaction between *N*-alkyl-*N*-hydroxymethyltriazenes with cysteine, *N*-acetylcysteine, glutathione or appropriate thiol in trifluoroacetic acid. The cysteinyl derivative is not a substrate for a mammalian β-lyase. Consequently the cysteinyl, *N*-acetylcysteinyl and glutathionyl derivatives lack anticancer activity against the Walker, L1210 and V79 tumour cell lines, mutagenic activity against Salmonella TA 100 and TA 2638 cell lines and cytotoxicity against renal proximal tubule cells.

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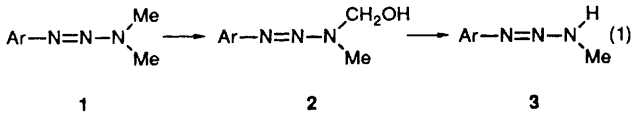
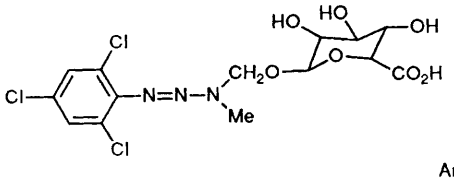
Dimethyltriazenes, **1**, have anti-cancer activity and are used to treat malignant melanoma and other soft tissue tumours.[1] They exert their therapeutic effect by acting as pro-drugs for the corresponding monomethyltriazenes **3**.[2] Monomethyltriazenes are known cytotoxic compounds that are able to alkylate nucleic acids.[3] Implicit in the *in vivo* conversion of **1** into **3** (eqn. 1)



1 **2** **3**

is the *N*-hydroxymethyltriazene **2**,[4] and one such compound has been identified in blood[5] and urine.[6] The *N*-hydroxymethyl derivatives are thought to have a role in the transport of the drug, decomposing with loss of formaldehyde to form the monomethyltriazene. Such a process does occur, but in aqueous solutions it is too fast for *N*-hydroxymethyltriazenes to have a lifetime sufficient to be transported.[7] Alternatively, the *N*-hydroxymethyl moiety may undergo biological conjugation, and one such conjugate, the glucuronide **4**, has been isolated from rat urine.[8] Moreover, it has been suggested that the interaction of such *N*-hydroxymethyl derivatives with biomolecules such as DNA, RNA, protein *etc.*, [eqn. (2)] may be



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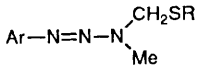

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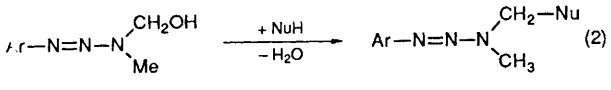
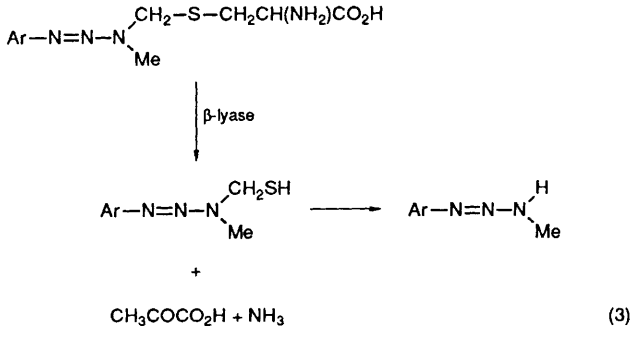


responsible for the antimitotic action of these compounds.[9] However, no evidence for such a role has been yet adduced. Indeed, biological conjugates of **2** other than the glucuronide remain to be identified, though derivatives of the mercapturic acid pathway of metabolism seem prime candidates.

Therefore, we set out to synthesise thioether derivatives, **5**, of *N*-hydroxymethyltriazenes based upon the mercapturic acid pathway. Such derivatives comprise compounds containing the *S*-glutathionyl, *S*-cysteinyl and *S*-(*N*-acetylcysteinyl) moieties. We wished to assess both their involvement in dimethyltriazene metabolism, and also their potential as novel anticancer pro-drugs. Unlike the hydroxymethyltriazenes **2**, the ether and thioether derivatives are stable in aqueous solutions at *ca.* pH 7.4. They therefore present themselves as more suitable candidates as pro-drugs, provided that a system for releasing the cytotoxic monomethyltriazene from them is available. One potential system is the β-lyase enzyme, which cleaves *S*-substituted cysteine derivatives to liberate pyruvate, ammonia and a thiol.[10] For the thioether derivatives of hydroxymethyltriazenes, the liberated thiol derivative is expected to decompose spontaneously to a monomethyltriazene [eqn. (3)].



We have previously developed a synthesis of ether derivatives of **2**, using acid catalysed solvolysis of the *N*-hydroxymethyltriazenes in the appropriate alcohol solvent.[11] This was an extremely surprising result, in that, generally, triazenes are exceptionally acid labile. Unfortunately, the method fails to work in pure thiol solvents, and is not applicable to non-liquid alcohols or thiols. However, we reasoned that in trifluoroacetic acid solvent the hydroxymethyltriazenes **2** could potentially form the same iminium ion intermediate **6** that is generated in the corresponding acid catalysed ether synthesis. Such an intermediate should be able to be trapped by sulphur nucleophiles, such as the amino acids of the mercapturic acid pathway and related compounds [(eqn. (4)]. Our expectations were realised and we now describe our results and some preliminary biological data for these compounds.

At the outset of our work, no synthesis of such compounds had been reported. However, after our programme was well advanced we were made aware of one other synthesis of an

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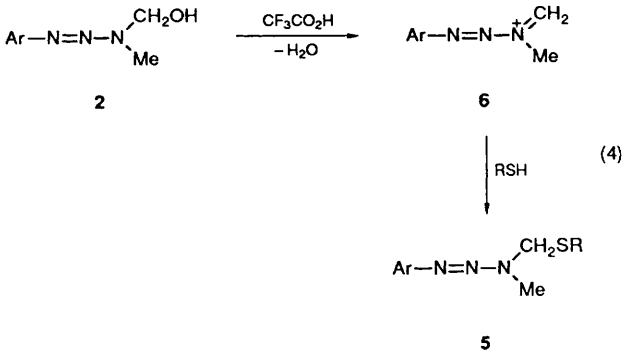
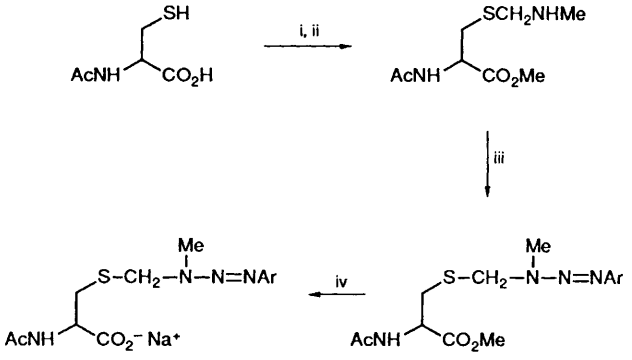


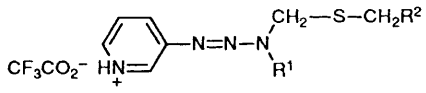
S-(*N*-acetylcysteinyl)methyltriazene.* The strategy for this alternative synthesis is outlined in Scheme 1, and involves the coupling of *S*-(methylaminomethyl)-*N*-acetylcysteine methyl ester, itself synthesised in two steps from *N*-acetylcysteine, with 2,4,6-trichlorobenzene diazonium ion, followed by alkaline hydrolysis of the ester.[12] Clearly, there is a need for a concise synthesis of such compounds, as provided by the method described herein.



Scheme 1 *Reagents and conditions:* i, MeOH/H$^+$; ii, $MeNH_3^+Cl^-$, HCHO/EtOH; iii, 2,4,6-$Cl_3C_6H_2N_2^+Cl^-$; iv, NaOH

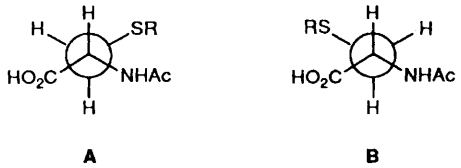
Dissolution of *N*-acetylcysteine in anhydrous trifluoroacetic acid solvent followed by addition of an equimolar amount of the *N*-hydroxymethyl-*N*-methyltriazene **2** (Ar = 3-pyridyl) and immediate removal of excess of solvent by evaporation under reduced pressure afforded the product **7** in high yield (87%). The formation of a pyridinium salt appears crucial to the success of the synthesis. Other hydroxymethyltriazenes **2** in which Ar is a substituted phenyl group form intractable tars from which no compounds with an intact triazene moiety can be isolated. Most likely, protonation of the pyridine nitrogen stabilises the triazene towards acid catalysed decomposition in the strongly acidic medium. Indeed, we have shown that 1-methyl-3-pyridyltriazene **3** (Ar = 3-pyridyl) is more stable than similar 3-phenyltriazenes towards metal ion-catalysed decomposition because of co-ordination of the triazene to the metal ion *via* the pyridyl nitrogen atom rather than *via* the triazene nitrogen atom.[13] The synthesis also succeeds in affording the ethyl and propyl homologues, **8** and **9**, from the corresponding *N*-hydroxymethyl-*N*-alkyltriazenes. Moreover, other sulphur nucleophiles can be used in place of *N*-acetylcysteine, and the glutathionyl, **10**, cysteinyl, **11**, cysteinyl methyl ester, **12**, and *RS*-homocysteinyl, **13**, derivatives are formed in yields of 80–95% from the appropriate amino acid, as are the corresponding compounds **14** and **15**, derived from 3-mercaptoacetic acid and 2-aminoethanethiol, respectively.

* We are grateful to Dr. G. F. Kolar, Institut für Toxicologie und Chemotherapie, Deutsches Krebforschungszentrum, Heidelberg for bringing this to our attention.



	R^1	R^2
7	Me	$CH(NHCOCH_3)CO_2H$
8	Et	$CH(NHCOCH_3)CO_2H$
9	Pr	$CH(NHCOCH_3)CO_2H$
10	Me	$CH(CONHCH_2CO_2H)NHCOCH_2CH_2CH(CO_2H)NH_2$
11	Me	$CH(NH_2)CO_2H$
12	Me	$CH(NH_2)CO_2Me$
13	Me	$CH_2CH(NH_2)CO_2H$
14	Me	CH_2CO_2H
15	Me	CH_2NH_2

The structure of the derivatives **7**–**15** follows from their spectroscopic and analytical data. Elemental analyses are consistent with their formulation as trifluoroacetate salts, and this is confirmed by the ^{13}C NMR spectra of compounds **7**, **9**–**11** which exhibit signals that can be attributed to the $CF_3CO_2^-$ carbon atoms. Further, the negative ion fast atom bombardment (FAB) mass spectra of **11** and **14** reveal a peak at *m*/*z* 113 due to $CF_3CO_2^-$. The negative ion FAB MS spectra also contain $(M - H)^+$ peaks. Positive ion FAB mass spectrometry produce spectra that contain peaks corresponding to $(M + H)^+$ and ArN_2^+. The latter is consistent with the presence of a triazene group. The presence of the *N*-alkyl group is most apparent in the ^1H and ^{13}C NMR spectra. In particular, the signal at δ_H 3.2–3.4 is as expected for a triazene *N*Me group. The presence of the NCH_2S functionality is apparent from the signal at *ca.* 5.1 and δ_C *ca.* 60. The ^1H NMR signals for this methylene group in compounds **7**–**15** lie in the region previously established for similar derivatives of simple thiols, *e.g.* EtSH,[11] though they tend to lie to higher frequency presumably because of the presence of electron withdrawing substituents in the *S*-alkyl group. The NCH_2S ^1H NMR signal for compounds **14** and **15** are singlets, like the corresponding *S*-alkyl derivatives. However, for compounds **7**–**12** the protons of the NCH_2S group are diastereotopic, due to the presence of a chiral centre in the *S*-alkyl group, and the ^1H NMR signal is split into a pair of doublets, *J* *ca.* 15, due to geminal coupling. Interestingly, the diastereotopic nature of the NCH_2S protons in the derivative of *RS*(±)-homocysteine, **13**, is not evident in the ^1H NMR spectrum, the signal appearing as a singlet. The SCH_2CH-system of **7** and **11** form an ABX system in which the protons of the methylene group are also diastereotopic. The geminal coupling constant is *ca.* 15 Hz, and the two vicinal coupling constants are *ca.* 4.5, 8 Hz. These are consistent with the derivatives preferentially adopting conformations **A** or **B**.



The enzyme β-lyase requires substrates that are derivatives of cysteine which contain a free amino group, although *N*-acetyl derivatives will often suffice because the cytosolic enzyme preparation usually contains a deacetylase. However, whereas *S*-(2-benzothiazolyl)cysteine was a substrate and was used to quantify enzyme activity, compound **11** proved not to be a substrate for the rat liver β-lyase under any conditions, there

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being no 3-aminopyridine or pyruvate formed on incubation with the enzyme. To date only *S*-aryl, *S*-haloalkenyl and *S*-haloalkyl derivatives of cysteine have been found to be substrates for mammalian β-lyases.[14,15] There is clearly a strong specificity of the enzyme for the *S*-substituent, apparently related to the nucleofugacity of the leaving group.[10,14] Not surprisingly therefore, when tested for anticancer activity against the Walker, L1210 and V79 tumour cell lines, the compounds **7**, and **10** and **11** proved inactive up to 10^{-4} mol dm^{-3}. Moreover, of these compounds, only compound **7** was found to have any mutagenic activity in the Ames test against *Salmonella typhimurium* strains TA100 and TA2638, and that only very weak. These Salmonella strains contain a β-lyase enzyme and have been shown to express mutagenicity for several *S*-cysteinyl derivatives.[14,16] Further, compounds **7**, **10** and **11** were subjected to cytotoxicity testing using freshly isolated renal proximal tubule cells, which is a good system for examining nephrotoxicity. Whereas *S*-haloalkenyl and *S*-haloalkyl cysteine conjugates induce toxicity at concentrations between 0.1 and 0.5 mmol dm^{-3}, compounds **7**, **10** and **11** were not cytotoxic up to 5 mmol dm^{-3}. Thus, these *S*-cysteinyl and related derivatives of *N*-hydroxymethyltriazenes appear not to be important to the transport and cytotoxic action of *N*,*N*-dimethyltriazenes. Their lack of cytoxicity is an excellent criterion for a pro-drug. However, their inability to act as substrates for the β-lyase system means that they are unsuitable pro-drug candidates of dimethyltriazenes. Whether or not they are excreted metabolites that lack cytotoxicity remains to be demonstrated.

Experimental

IR spectra were recorded as Nujol mulls (solids) or oils using a Perkin-Elmer 1710 Fourier Transform spectrometer. ^{1}H NMR spectra were obtained using JEOL FX90Q (90 MHz) or Bruker AC250 (250 MHz) spectrometers. ^{13}C NMR spectra were recorded using the JEOL FX90Q spectrometer. *J* Values are given in Hz. Mass spectra were obtained using either a Kratos MS80RFA or VG Micromass 20–250 mass spectrometer. $[\alpha]_D$ Values are given in 10^{-1} mol dm^2 g^{-1}. The hydroxymethyltriazenes were available from our previous work.[12] Trifluoroacetic acid (Aldrich) was used as supplied.

Typical S-Conjugate Synthesis.—1-Hydroxymethyl-1-methyl-3-(3′-pyridyl)triazene (0.1 mol) was added with rapid stirring at 0 °C to a solution of the appropriate thiol compound (0.1 mol) in trifluoroacetic acid (3 cm^3). After 1 min the solvent was removed rapidly under reduced pressure. The residual gum was then subjected to column chromatography (silica gel 60 H) using methanol as eluent. The following compounds were synthesised in this way:

3-{3-[S-(N-*Acetylcysteinyl*)*methyl*]-3-*methyltriazeno*}-*pyridinium trifluoroacetate* **7**: m.p. 118–120 °C; ν_{max}/cm^{-1} 3250, 2315, 1735, 1672, 1555 and 1211; δ_H[CD_3OD/$(CD_3)_2$-CO] 2.20 (3 H, s, *N*-$COCH_3$), 2.95 (1 H, dd, *J* 8, 15, cys CH_2), 3.07 (1 H, dd, *J* 4.5, 15, cys CH_2), 3.38 (3 H, s, NMe), 4.52 (1 H, dd, *J* 4.5, 8, cys α-H), 5.10 (1 H, d, *J* 14, NCH_2S), 5.17 (1 H, d, *J* 14, NCH_2S), 8.01 (1 H, dd, *J* 6, 9), 8.53 (1 H, d, *J* 6), 8.59 (1 H, d, *J* 9) and 8.78 (1 H, s); δ_C(D_2O) 23.7 (q, NAc), 33.3 (t, SCH_2), 36.3 (q, NMe), 54.3 (d, cys α-C), 60.8 (t, NCH_2S), 118.3 (q, CF_3), 129.6 (d), 135.9 (d), 138.9 (d), 151.5 (s), 164.8 (q, CF_3CO_2) and 175.7 (s) and 176.1 (s) (NHCO and CO_2H); $[\alpha]_D^{25}$ −71.7 (H_2O); *m*/*z* (FAB+) 312 (M + H), 207 (M + H − $HSCH_2CO_2H$) and 106 (ArN_2) (Found: C, 39.4; H, 4.2; N, 16.4. $C_{14}H_{18}F_3N_5O_5S$ requires C, 39.52; H, 4.27; N, 16.47%).

3-{3-[S-(N-*Acetylcysteinyl*)*methyl*]-3-*ethyltriazeno*}-*pyridinium trifluoroacetate* **8**: oil; ν_{max}/cm^{-1} 3288, 2939, 2361, 1733, 1666, 1557 and 1201; δ_H[$(CD_3)_2CO$] 1.34 (3 H, t), 2.12 (3 H, s, $NCOCH_3$), 2.8–3.2 (2 H, m, cys CH_2), 4.02 (2 H, q, NCH_2), 4.6–4.9 (1 H, m, cys α-H), 5.12 (1 H, d, *J* 15, NCH_2S), 5.28 (1 H, d, *J* 15, NCH_2S), 8.12 (1 H, dd, *J* 6, 9), 8.65 (1 H, d, *J* 9), 8.76 (1 H, d, *J* 6) and 9.01 (1 H, s); $[\alpha]_D^{25}$ − 75.4 (H_2O); *m*/*z* (FAB+) 326 (M + H), 221 (M + H − $HSCH_2CHCO_2H$) and 106 (ArN_2) (Found: C, 40.5; H, 5.0; N, 15.9. $C_{15}H_{20}F_3N_5O_5S$ requires C, 41.0; H, 4.59; N, 15.94%).

3-{3-[S-(N-*Acetylcysteinyl*)*methyl*]-3-*propyltriazeno*}-*pyridinium trifluoroacetate* **9**: oil; ν_{max}/cm^{-1} 3283, 2970, 2582, 1667, 1556 and 1196; δ_H[$(CD_3)_2CO$] 0.97 (3 H, t, *J* 7), 1.20 (2 H, m), 2.11 (3 H, s, $NCOCH_3$), 2.7–3.2 (2 H, m, cys CH_2), 3.91 (2 H, t, *J* 7, NCH_2), 4.5–4.9 (1 H, m, cys α-CH), 5.0 (1 H, d, *J* 14, NCH_2S), 5.32 (1 H, d, *J* 14, NCH_2S), 7.5–7.8 (2 H, m), 7.9 (1 H, m) and 8.96 (1 H, s); δ_C[$(CD_3)_2CO$] 11.7 (q, propyl CH_3), 22.5 (t, propyl CH_2), 22.6 (q, NAc), 30.0 (t, propyl NCH_2), 32.4 (t, SCH_2), 53.1 (d, cys α-C), 57.7 (t, NCH_2S), 117.5 (q, CF_3), 128.2 (d), 136.5 (d), 138.8 (d), 150.2 (s), 162.0 (q, CF_3CO_2), 171.4 (s) and 172.1 (s) (NHCO and CO_2H); $[\alpha]_D^{25}$ −76.1 (H_2O); *m*/*z* (FAB+) 340 (M + H), 235 (M + H − $HSCH_2CHCO_2H$) and 106 (ArN_2) (Found: C, 41.7; H, 5.1; N, 14.9. $C_{16}H_{22}F_3N_5O_5S$ requires C, 42.37; H, 4.89; N, 15.45%).

3-[3-(S-*Glutathionylmethyl*)-3-*methyltriazeno*]*pyridinium trifluoroacetate* **10**: indefinite m.p.; ν_{max}/cm^{-1} 3245, 2925, 2302, 1641, 1565 and 1205; δ_H(D_2O) 2.12 (2 H, m, glu β-CH_2), 2.50 (2 H, m, glu γ-CH_2), 2.84 (1 H, m, cys CH_2), 3.08 (1 H, dd, *J* 5, 13, cys CH_2), 3.37 (3 H, s, NMe), 3.81 (2 H, br s, gly CH_2), 3.88 (1 H, m, glu α-CH), 4.56 (1 H, m, cys α-CH), 5.11 (1 H, d, *J* 15, NCH_2S), 5.17 (1 H, d, *J* 15, NCH_2S), 8.01 (1 H, dd, *J* 6, 10), 8.52 (1 H, d, *J* 6), 8.58 (1 H, d, *J* 10, NCH_2S) and 8.78 (1 H, s); δ_C(D_2O) 27.7 (t, glu β-CH_2), 33.1 (t, glu γ-CH_2), 33.5 (t, SCH_2), 36.4 (q, NCH_3), 4.32 (t, gly CH_2), 54.6 (d, glu α-C), 54.9 (d, cys α-C), 60.9 (t, NCH_2S), 118.5 (q, CF_3), 129.8 (d), 136.1 (d), 138.9 (d), 139.1 (d), 151.5 (s), 164.8 (q, CF_3CO_2), 174.4 (s), 174.6 (s), 174.8 (s) and 176.3 (s) (NHCO and CO_2H); $[\alpha]_D^{25}$ −77.9 (H_2O); *m*/*z* (FAB+) 570 (M + H) (Found: C, 39.5; H, 4.2; N, 16.9. $C_{19}H_{26}F_3N_7O_8S$ requires C, 40.06; H, 4.57; N, 17.2%).

3-[3-(S-*Cysteinylmethyl*)-3-*methyltriazeno*]*pyridinium trifluoroacetate* **11**: m.p. 190–191 °C (decomp.); ν_{max}/cm^{-1} 2924, 2361, 1738, 1673, 1552 and 1192; δ_H(D_2O) 3.04 (1 H, dd, *J* 7, 12, cys CH_2), 3.08 (1 H, dd, *J* 5, 12, cys CH_2), 3.35 (3 H, s, N-Me), 3.98 (1 H, dd, *J* 5, 7, cys α-CH), 5.11 (1 H, d, *J* 15, NCH_2S), 5.16 (1 H, d, *J* 15, NCH_2S), 7.94 (1 H, dd, *J* 6, 9), 8.30–8.60 (2 H, m) and 8.77 (1 H, s); δ_C(D_2O) 32.5 (t, SCH_2), 36.4 (q, NMe), 55.5 (d, cys α-C), 60.3 (t, NCH_2S), 118.5 (q, CF_3), 129.8 (d), 136.2 (d), 139.2 (d), 151.5 (s), 169.4 (q, CF_3CO_2) and 174.2 (s, CO_2H); $[\alpha]_D^{25}$ −65.1 (H_2O); *m*/*z* (FAB+) 270 (M + H), 165 (M + H − $HSCH_2$-$CHCO_2H$), 106 (ArN_2), (FAB−) 268 (M − H), 205, 113 ($CF_3CO_2^-$) (Found: C, 37.8; H, 4.1; N, 18.4. $C_{12}H_{16}F_3N_5O_4S$ requires C, 37.59, H, 4.21; N, 18.28%).

3-{3-[(1-*Amino*-2-*methoxycarbonylethyl*)*thiomethyl*]-*triazeno*}*pyridinium trifluoroacetate* **12**: oil; ν_{max}/cm^{-1} 3220, 2905, 1750, 1680, 1560 and 1205; δ_H[$(CD_3)_2CO$] 3.1–3.4 (2 H, m, cys CH_2), 3.32 (3 H, s, NMe), 3.71 (3 H, s, OMe), 4.32 (1 H, dd, *J* 6, 9, cys α-CH), 4.96 (1 H, d, *J* 14, NCH_2S), 5.30 (1 H, d, *J* 14, NCH_2S), 7.96 (1 H, dd, *J* 6, 9), 8.4–8.76 (2 H, m) and 8.90 (1 H, s); $[\alpha]_D^{25}$ −62.5 (H_2O) (Found: C, 39.1; H, 5.0; N, 17.1. $C_{13}H_{18}F_3N_5O_4S$ requires C, 39.28; H, 4.57; N, 17.6%).

(RS)-3-[3-(S-*Homocysteinylmethyl*)-3-*methyltriazeno*]-*pyridinium trifluoroacetate* **13**: oil; ν_{max}/cm^{-1} 3215, 2920, 2320, 1705, 1675 and 1210; δ_H(D_2O) 2.21 (2 H, dt, *J* 6, 7.5, homocys CH_2), 2.72 (2 H, t, *J* 7, homocys SCH_2), 3.31 (3 H, s, NMe), 3.83 (1 H, t, *J* 6, homocys α-CH), 5.07 (2 H, s, NCH_2S), 7.59 (1 H, dd, *J* 5, 8), 8.0 (1 H, d, *J* 8), 8.38 (1 H, d, *J* 5) and 8.60 (1 H, s) (Found: C, 38.8; H, 4.5; N, 17.0. $C_{13}H_{13}F_3N_5O_4S$ requires C, 39.28; H, 4.57; N, 17.6%).

3-{3-[(2-*Methoxycarbonylethyl*)*thiomethyl*]-3-*methyltri-azeno*}*pyridinium trifluoroacetate* **14**: m.p. 117–120 °C; ν_{max}/cm^{-1} 2935, 1705, 1685, 1555 and 1210; δ_H[$(CD_3)_2CO$/D_2O] 2.37 (2 H, t, *J* 6), 2.62 (2 H, t, *J* 6), 3.16 (3 H, s, NMe), 4.92 (2 H, s,

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NCH_2S), 7.33 (1 H, dd, *J* 5, 8), 7.74 (1 H, d, *J* 8), 8.21 (1 H, d, *J* 5) and 8.43 (1 H, s); *m/z* (FAB+) 255 (M + H) (FAB−) 253 (M − H), 205, 183, 113 ($CF_3CO_2^-$) (Found: C, 39.1; H, 4.2; N, 15.2. $C_{12}H_{15}F_3N_4O_4S$ requires C, 39.1; H, 4.1; N, 15.2%).

3-{3-[(2-*Aminoethyl*)*thiomethyl*]-3-*methyltriazeno*}-*pyridinium trifluoroacetate* **15**: oil; ν_{max}/cm^{-1} 2915, 2335, 1675, 1575 and 1205; $\delta_H[(CD_3)_2CO]$ 3.05 (2 H, m), 3.39 (3 H, s, NMe), 3.99 (2 H, m), 5.26 (2 H, s, NCH_2S), 8.05 (1 H, dd, *J* 5, 8), 8.55 (1 H, d, *J* 8), 8.75 (1 H, d, *J* 5) and 9.05 (1 H, s) (Found: C, 38.6; H, 4.6; N, 20.3. $C_{11}H_{16}F_3N_5O_2S$ requires C, 38.93; H, 4.76; N, 20.65%).

Preparation of β-*Lyase*.—The livers of three Wistar rats were perfused with cold KCl solution (1.15% w/v) and homogenised in pH 7.4 phosphate buffer (40 cm^3, 0.1 mol dm^{-3}) containing sucrose (0.25 mol dm^{-3}). pH 7.4 Phosphate buffer (0.1 mol dm^{-3}) containing sucrose (0.25 mol dm^{-3}; 40 cm^3) was added and the homogenate centrifuged at 105 000 g for 1 h at 4 °C. The cytosolic supernatant was collected and dialysed against pH 7.4 phosphate buffer (0.1 mol dm^{-3}; 2 dm^3) for 24 h. The dialysed supernatant (*ca.* 40 cm^3) was frozen in 5 cm^3 fractions, stored at −40 °C and used as required. The α-lyase activity in the liver cytosol was determined using *S*-(2-benzothiazolyl)cysteine [10,17] as substrate at pH 8.6 by following the liberation of pyruvate using a commercial pyruvic acid determination kit (Boehringer Test-Combination Pyruvate, reference number 124982). The enzyme activity determined by this method was 11 nmol pyruvate min^{-1} mg^{-1} protein, which is similar to those reported elsewhere (6.2 nmol min^{-1} mg^{-1},[15] 17 nmol min^{-1} mg^{-1} [17]).

Attempted Enzyme Reactions with Compound **11**.—The cysteine conjugate **11** (final concentration 1.8 mmol dm^{-3}) was incubated at 37 °C in pH 8.6 borate buffer for 5 min. The reaction was initiated by addition of the cytosol preparation such that a ten-fold dilution of the enzyme was achieved. Aliquots (1 cm^3) were taken after 5, 15 and 30 min, treated with a cooled solution of trichloroacetic acid (10% w/v, 0.2 cm^3) and centrifuged at 3000 rpm for 10 min. The resultant supernatant was diluted to ten times the volume with pH 6.5 phosphate buffer (0.1 mol dm^{-3}) and analysed by HPLC. The HPLC conditions utilised were: column, Merck LiChrosper® 100 RP-8 250 × 4 mm, particle size 5 μm; eluent, acetonitrile (15%), 0.1 mol dm^{-3} aqueous tetraethylammonium bromide (40%), pH 6.7 0.05 mol dm^{-3} phosphate buffer (45%); flow rate, 1 cm^3 min^{-1}; detector wavelength, 297 nm. Using these conditions the substrate **11** has a retention time of 1.53 min and the product 3-aminopyridine 6.18 min.

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