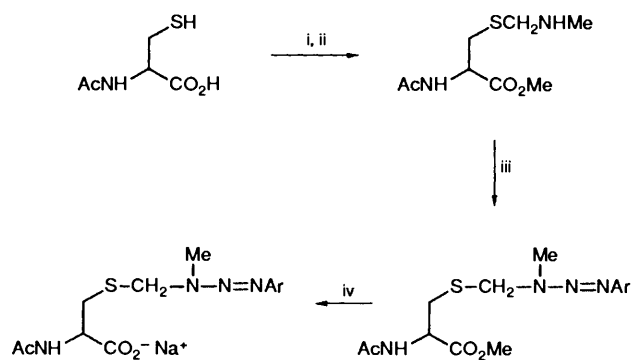


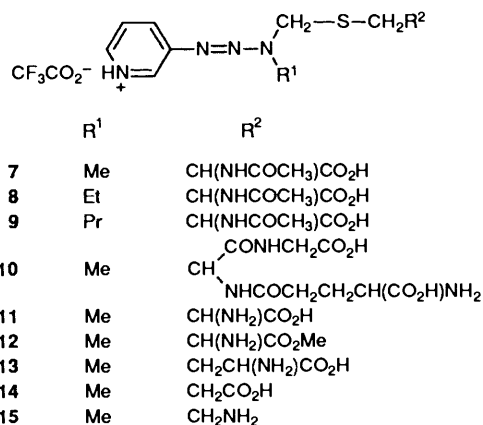
S-(*N*-acetylcysteinyl)methyltriazenes.* The strategy for this alternative synthesis is outlined in Scheme 1, and involves the coupling of *S*-(methylaminomethyl)-*N*-acetylcysteine methyl ester, itself synthesised in two steps from *N*-acetylcysteine, with 2,4,6-trichlorobenzene diazonium ion, followed 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.



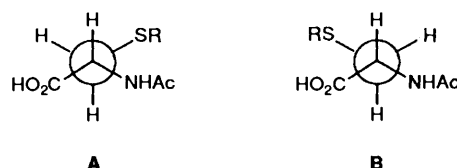
Scheme 1 Reagents and conditions: i, MeOH/H⁺; ii, MeNH₃⁺Cl⁻, HCHO/EtOH; iii, 2,4,6-Cl₃C₆H₂N₂⁺Cl⁻; iv, NaOH

Dissolution of *N*-acetylcysteine in anhydrous trifluoroacetic acid solvent followed by addition of an equimolar amount of the *N*-hydroxymethyl-*N*-methyltriazenes **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-pyridyltriazenes **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 Krebsforschungszentrum, Heidelberg for bringing this to our attention.



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 ¹³C NMR spectra of compounds **7, 9–11** which exhibit signals that can be attributed to the CF₃CO₂⁻ 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 CF₃CO₂⁻. 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 ArN₂⁺. 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 ¹³C 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 NCH₂S 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 NCH₂S 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 NCH₂S 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 NCH₂S protons in the derivative of *RS*(±)-homocysteine, **13**, is not evident in the ¹H NMR spectrum, the signal appearing as a singlet. The S-CH₂-CH- 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

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*-cysteinyll 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*-cysteinyll and related derivatives of *N*-hydroxymethyltriazenes appear not to be important to the transport and cytotoxic action of *N,N*-dimethyltriazenes. Their lack of cytotoxicity 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. ¹H NMR spectra were obtained using JEOL FX90Q (90 MHz) or Bruker AC250 (250 MHz) spectrometers. ¹³C 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. [α]_D Values are given in 10⁻¹ mol dm² 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 cm³). 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*-Acetylcysteinyll)methyl]-3-methyltriazeno}pyridinium trifluoroacetate **7**: m.p. 118–120 °C; $\nu_{\max}/\text{cm}^{-1}$ 3250, 2315, 1735, 1672, 1555 and 1211; $\delta_{\text{H}}[(\text{CD}_3)_2\text{CO}]$ 2.20 (3 H, s, *N*-COCH₃), 2.95 (1 H, dd, *J* 8, 15, *cys* CH₂), 3.07 (1 H, dd, *J* 4.5, 15, *cys* CH₂), 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, NCH₂S), 5.17 (1 H, d, *J* 14, NCH₂S), 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); $\delta_{\text{C}}(\text{D}_2\text{O})$ 23.7 (q, NAc), 33.3 (t, SCH₂), 36.3 (q, NMe), 54.3 (d, *cys* α -C), 60.8 (t, NCH₂S), 118.3 (q, CF₃), 129.6 (d), 135.9 (d), 138.9 (d), 151.5 (s), 164.8 (q, CF₃CO₂) and 175.7 (s) and 176.1 (s) (NHCO and CO₂H); [α]_D²⁵ -71.7 (H₂O); *m/z* (FAB+) 312 (M + H), 207 (M + H - HSCH₂CO₂H) and 106 (ArN₂) (Found: C, 39.4; H, 4.2; N, 16.4. C₁₄H₁₈F₃N₅O₅S requires C, 39.52; H, 4.27; N, 16.47%).

3-{3-[*S*-(*N*-Acetylcysteinyll)methyl]-3-ethyltriazeno}pyridinium trifluoroacetate **8**: oil; $\nu_{\max}/\text{cm}^{-1}$ 3288, 2939, 2361, 1733, 1666, 1557 and 1201; $\delta_{\text{H}}[(\text{CD}_3)_2\text{CO}]$ 1.34 (3 H, t), 2.12 (3 H, s, NCOCH₃), 2.8–3.2 (2 H, m, *cys* CH₂), 4.02 (2 H, q,

NCH₂), 4.6–4.9 (1 H, m, *cys* α -H), 5.12 (1 H, d, *J* 15, NCH₂S), 5.28 (1 H, d, *J* 15, NCH₂S), 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); [α]_D²⁵ -75.4 (H₂O); *m/z* (FAB+) 326 (M + H), 221 (M + H - HSCH₂CHCO₂H) and 106 (ArN₂) (Found: C, 40.5; H, 5.0; N, 15.9. C₁₅H₂₀F₃N₅O₅S requires C, 41.0; H, 4.59; N, 15.94%).

3-{3-[*S*-(*N*-Acetylcysteinyll)methyl]-3-propyltriazeno}pyridinium trifluoroacetate **9**: oil; $\nu_{\max}/\text{cm}^{-1}$ 3283, 2970, 2582, 1667, 1556 and 1196; $\delta_{\text{H}}[(\text{CD}_3)_2\text{CO}]$ 0.97 (3 H, t, *J* 7), 1.20 (2 H, m), 2.11 (3 H, s, NCOCH₃), 2.7–3.2 (2 H, m, *cys* CH₂), 3.91 (2 H, t, *J* 7, NCH₂), 4.5–4.9 (1 H, m, *cys* α -CH), 5.0 (1 H, d, *J* 14, NCH₂S), 5.32 (1 H, d, *J* 14, NCH₂S), 7.5–7.8 (2 H, m), 7.9 (1 H, m) and 8.96 (1 H, s); $\delta_{\text{C}}[(\text{CD}_3)_2\text{CO}]$ 11.7 (q, propyl CH₃), 22.5 (t, propyl CH₂), 22.6 (q, NAc), 30.0 (t, propyl NCH₂), 32.4 (t, SCH₂), 53.1 (d, *cys* α -C), 57.7 (t, NCH₂S), 117.5 (q, CF₃), 128.2 (d), 136.5 (d), 138.8 (d), 150.2 (s), 162.0 (q, CF₃CO₂), 171.4 (s) and 172.1 (s) (NHCO and CO₂H); [α]_D²⁵ -76.1 (H₂O); *m/z* (FAB+) 340 (M + H), 235 (M + H - HSCH₂CHCO₂H) and 106 (ArN₂) (Found: C, 41.7; H, 5.1; N, 14.9. C₁₆H₂₂F₃N₅O₅S requires C, 42.37; H, 4.89; N, 15.45%).

3-[3-(*S*-Glutathionylmethyl)-3-methyltriazeno]pyridinium trifluoroacetate **10**: indefinite m.p.; $\nu_{\max}/\text{cm}^{-1}$ 3245, 2925, 2302, 1641, 1565 and 1205; $\delta_{\text{H}}(\text{D}_2\text{O})$ 2.12 (2 H, m, glu β -CH₂), 2.50 (2 H, m, glu γ -CH₂), 2.84 (1 H, m, *cys* CH₂), 3.08 (1 H, dd, *J* 5, 13, *cys* CH₂), 3.37 (3 H, s, NMe), 3.81 (2 H, br s, gly CH₂), 3.88 (1 H, m, glu α -CH), 4.56 (1 H, m, *cys* α -CH), 5.11 (1 H, d, *J* 15, NCH₂S), 5.17 (1 H, d, *J* 15, NCH₂S), 8.01 (1 H, dd, *J* 6, 10), 8.52 (1 H, d, *J* 6), 8.58 (1 H, d, *J* 10, NCH₂S) and 8.78 (1 H, s); $\delta_{\text{C}}(\text{D}_2\text{O})$ 27.7 (t, glu β -CH₂), 33.1 (t, glu γ -CH₂), 33.5 (t, SCH₂), 36.4 (q, NCH₃), 4.32 (t, gly CH₂), 54.6 (d, glu α -C), 54.9 (d, *cys* α -C), 60.9 (t, NCH₂S), 118.5 (q, CF₃), 129.8 (d), 136.1 (d), 138.9 (d), 139.1 (d), 151.5 (s), 164.8 (q, CF₃CO₂), 174.4 (s), 174.6 (s), 174.8 (s) and 176.3 (s) (NHCO and CO₂H); [α]_D²⁵ -77.9 (H₂O); *m/z* (FAB+) 570 (M + H) (Found: C, 39.5; H, 4.2; N, 16.9. C₁₉H₂₆F₃N₇O₈S requires C, 40.06; H, 4.57; N, 17.2%).

3-[3-(*S*-Cysteinyllmethyl)-3-methyltriazeno]pyridinium trifluoroacetate **11**: m.p. 190–191 °C (decomp.); $\nu_{\max}/\text{cm}^{-1}$ 2924, 2361, 1738, 1673, 1552 and 1192; $\delta_{\text{H}}(\text{D}_2\text{O})$ 3.04 (1 H, dd, *J* 7, 12, *cys* CH₂), 3.08 (1 H, dd, *J* 5, 12, *cys* CH₂), 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, NCH₂S), 5.16 (1 H, d, *J* 15, NCH₂S), 7.94 (1 H, dd, *J* 6, 9), 8.30–8.60 (2 H, m) and 8.77 (1 H, s); $\delta_{\text{C}}(\text{D}_2\text{O})$ 32.5 (t, SCH₂), 36.4 (q, NMe), 55.5 (d, *cys* α -C), 60.3 (t, NCH₂S), 118.5 (q, CF₃), 129.8 (d), 136.2 (d), 139.2 (d), 151.5 (s), 169.4 (q, CF₃CO₂) and 174.2 (s, CO₂H); [α]_D²⁵ -65.1 (H₂O); *m/z* (FAB+) 270 (M + H), 165 (M + H - HSCH₂CHCO₂H), 106 (ArN₂), (FAB-) 268 (M - H), 205, 113 (CF₃CO₂⁻) (Found: C, 37.8; H, 4.1; N, 18.4. C₁₂H₁₆F₃N₅O₄S requires C, 37.59; H, 4.21; N, 18.28%).

3-{3-[(1-Amino-2-methoxycarbonyl)ethyl]thiomethyl}triazeno}pyridinium trifluoroacetate **12**: oil; $\nu_{\max}/\text{cm}^{-1}$ 3220, 2905, 1750, 1680, 1560 and 1205; $\delta_{\text{H}}[(\text{CD}_3)_2\text{CO}]$ 3.1–3.4 (2 H, m, *cys* CH₂), 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, NCH₂S), 5.30 (1 H, d, *J* 14, NCH₂S), 7.96 (1 H, dd, *J* 6, 9), 8.4–8.76 (2 H, m) and 8.90 (1 H, s); [α]_D²⁵ -62.5 (H₂O) (Found: C, 39.1; H, 5.0; N, 17.1. C₁₃H₁₈F₃N₅O₄S requires C, 39.28; H, 4.57; N, 17.6%).

(RS)-3-[3-(*S*-Homocysteinyllmethyl)-3-methyltriazeno}pyridinium trifluoroacetate **13**: oil; $\nu_{\max}/\text{cm}^{-1}$ 3215, 2920, 2320, 1705, 1675 and 1210; $\delta_{\text{H}}(\text{D}_2\text{O})$ 2.21 (2 H, dt, *J* 6, 7.5, homocys CH₂), 2.72 (2 H, t, *J* 7, homocys SCH₂), 3.31 (3 H, s, NMe), 3.83 (1 H, t, *J* 6, homocys α -CH), 5.07 (2 H, s, NCH₂S), 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. C₁₃H₁₃F₃N₅O₄S requires C, 39.28; H, 4.57; N, 17.6%).

3-{3-[(2-Methoxycarbonyl)ethyl]thiomethyl}triazeno}pyridinium trifluoroacetate **14**: m.p. 117–120 °C; $\nu_{\max}/\text{cm}^{-1}$ 2935, 1705, 1685, 1555 and 1210; $\delta_{\text{H}}[(\text{CD}_3)_2\text{CO}/\text{D}_2\text{O}]$ 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,

NCH₂S), 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 (CF₃CO₂⁻) (Found: C, 39.1; H, 4.2; N, 15.2. C₁₂H₁₅F₃N₄O₄S requires C, 39.1; H, 4.1; N, 15.2%).

3-{3-[(2-Aminoethyl)thiomethyl]-3-methyltriazeno}-pyridinium trifluoroacetate **15**: oil; $\nu_{\max}/\text{cm}^{-1}$ 2915, 2335, 1675, 1575 and 1205; $\delta_{\text{H}}[(\text{CD}_3)_2\text{CO}]$ 3.05 (2 H, m), 3.39 (3 H, s, NMe), 3.99 (2 H, m), 5.26 (2 H, s, NCH₂S), 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. C₁₁H₁₆F₃N₅O₂S 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 cm³, 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 cm³) 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 dm³) for 24 h. The dialysed supernatant (ca. 40 cm³) was frozen in 5 cm³ 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⁻¹¹⁷).

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 cm³) were taken after 5, 15 and 30 min, treated with a cooled solution of trichloroacetic acid (10% w/v, 0.2 cm³) 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 cm³ 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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