Pyrylium-mediated Transformations of Natural Products. Part 7.[1] Displacement of the *N*-Substituents of Pyridinium Ions in Aqueous Solution: Replacement of the ω-Amino Group of Lysine, and of the Terminal Amino Group of Glycylglycine

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In two-step sequences, lysine has been converted into the thio-substituted derivatives $RS[CH_2]_4CH(NH_2)CO_2H$ (R = Ph or $PhCH_2$), and glycylglycine into $PhSCH_2CONHCH_2CO_2H$. All reactions proceeded in aqueous solution at ≤75 °C; they thus provide models for the selective conversion of proteins at the ω-amino groups of lysine side chains and at the terminal amino groups, respectively.

Previous papers of this series have described the use of water-soluble pyrylium salts to convert amines, particularly lysine [2] and aminoglycosides,[1] preparatively into pyridinium ions in aqueous solution. We now describe experiments which define conditions for nucleophilic displacement of the *N*-substituents of pyridinium ions in aqueous solution and which have permitted the two-step conversions of lysine into 2-amino-6-(phenylthio)hexanoic acid and 2-amino-6-(benzylthio)-hexanoic acid, and of glycylglycine into *N*-[(phenylthio)-acetyl]glycine in water at 70—75 °C. This work thus provides models for the conversion in proteins of ω-amino groups of lysine side chains and amino terminals, respectively, into other functionalities.

Preparation of Pyrylium and Pyridinium Salts.—As pyrylium salts we utilised (1a)—(4a), previously described,[1,2] and (6a) which was prepared from the benzenesulphonate (5) and α-tetralone.

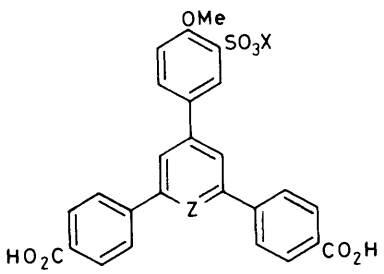
The pyrylium salts were treated with amines in aqueous solution to give the previously described pyridinium salts (1c), (1d), (3d), (4c), and (4d),[2] and the new pyridinium salts (2d), (2e), (6d), (6e), and (6f) (Table 1). During the formation of the pyridinium salts, the pH was controlled at 9 by addition of sodium hydrogen carbonate and sodium carbonate. Exceptionally, for the pentacyclic pyrylium salt (6a), the pH was raised to 10—10.5 by addition of sodium hydroxide: the tendency for pseudobase formation is less in the pentacyclic case and a higher pH can be used.

The pyridinium salts were characterized by their ^1H n.m.r. spectra (Table 2): the general patterns and chemical shifts were in line with those previously reported [2] for the monocyclic and tricyclic series. In the pentacyclic series (6d—f) the 1′-H signals were at still lower field, as would be expected.

Displacement Reactions with Sulphur Nucleophiles.—Initial experiments were carried out in D_2O solution and the reactions were followed by ^1H n.m.r. In this way we showed that at 100 °C the *N*-n-butyl group was transferred to give n-butyl phenyl sulphide from the monocyclic pyridinium ions in fair yields for (3d), (1d), and (2d) in 17, 10, and 6 h respectively (Table 3). The tricyclic pyridinium ion (4d) reacted, as expected,[3] more quickly and gave a high yield of product at 100 °C in 3 h.

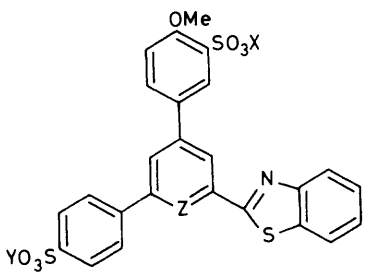
Preparative experiments were then carried out in H_2O. The *N*-benzyl tricyclic derivative (4c) reacted even at 25 °C. The reaction of the pentacyclic n-butyl compound (6d) preceded in quantitative yield at 70 °C and the reactions with the lysine and glycylglycine analogues (6e) and (6f) were similar. Exclusion of oxygen from reaction systems while heating increases the yield of product. The known thioethers $PhSBu^n$ and $PhCH_2SPh$ were characterized by comparison of m.p.s, refractive indices, and spectra with literature data.

The novel lysine derivatives $PhS[CH_2]_4CH(NH_2)CO_2H$ and $PhCH_2S[CH_2]_4CH(NH_2)CO_2H$, and the glycylglycine product $PhSCH_2CONHCH_2CO_2H$, crystallized from water in yields of 77, 63, and 66%, respectively. They were characterized by their



(1)

a; Z = $\overset{+}{O}$ ClO_4^-, X = H
b; Z = N, X = Na
c; Z = $\overset{+}{N}CH_2Ph$, X = negative charge
d; Z = $\overset{+}{N}Bu^n$ X = negative charge



(2)

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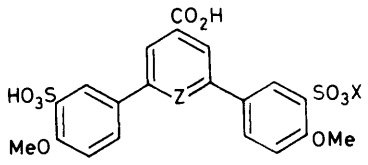
b; Z = N, X = Y = Na
d; Z = $\overset{+}{N}Bu^n$, X = H, Y = negative charge
e; Z = $\overset{+}{N}[CH_2]_4\underset{\displaystyle \overset{+}{N}H_3}{\underset{|}{C}}H{-}CO_2H$, X = Y = negative charge



(3)

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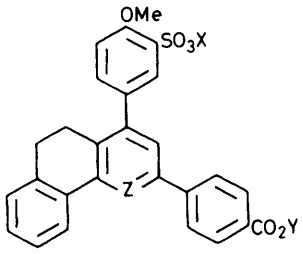
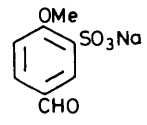


(4)

a; Z = $\overset{+}{O}$ ClO_4^-, X = Y = H
b; Z = $\overset{+}{N}$, X = Y = Na
c; Z = $\overset{+}{N}CH_2Ph$
X = negative charge
Y = H
d; Z = $\overset{+}{N}Bu^n$, Y = H
X = negative charge



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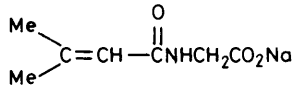

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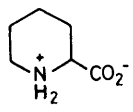

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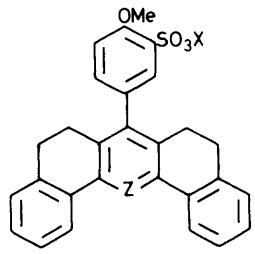

(6)

a; Z = $\overset{+}{O}$ ClO_4^-, X = H
b; Z = $\overset{+}{N}H$ ClO_4^-, X = H
d; Z = $\overset{+}{N}Bu^n$ ClO_4^-, X = H
e; Z = $\overset{+}{N}[CH_2]_4$–CH(+NH$_3$)–CO_2H ClO_4^-,
X = negative charge
f; Z = $\overset{+}{N}CH_2CONHCH_2CO_2^-$,
X = Na

Table 1. Preparation of *N*-substituted pyridinium salts

Compd. no.	*N*-Substituent	Anion	Recryst. solvent	Crystal form	M.p. (°C)	Yield (%)
(2d)	Bu^n	*a*	H_2O–CH_3COCH_3 [b]	Pale yellow prisms	>300	66
(2e)	$[CH_2]_4CH(\overset{+}{N}H_3)CO_2H$	*a*	EtOH–H_2O [b]	Pale yellow prisms	>300	61
(6b)	H	ClO_4^-	EtOH	Pale yellow needles	>300	80—100
(6d)	Bu^n	ClO_4^-	MeOH–Et_2O [b]	Yellow prisms	240—242	64
(6e)	$[CH_2]_4CH(\overset{+}{N}H_3)CO_2H$	*c*	EtOH [d]	Yellow prisms	>300	73
(6f)	$CH_2CONHCH_2CO_2^-$		H_2O–EtOH	Yellow prisms	>300	39

Compd. no.	Found (%) C	H	N	Molecular formula	Required (%) C	H	N
(2d)		*e*		$C_{29}H_{26}N_2O_7S_3 \cdot H_2O$	57.1	4.6	4.6
(2e)	49.5	5.5	5.2	$C_{31}H_{29}N_3O_9S_3 \cdot 4H_2O$	49.2	5.1	5.5
(6b)	60.6	4.3	2.3	$C_{28}H_{24}ClNO_8S$	61.1	4.4	2.5
(6d)	57.7	5.0		$C_{32}H_{32}ClNO_8S \cdot 2H_2O$	58.0	5.4	2.1
(6e)	57.2	5.2	3.9	$C_{34}H_{35}ClN_2O_{10}S \cdot H_2O$	56.9	5.2	3.9
(6f)	59.5	5.1	4.1	$C_{32}H_{27}N_2NaO_7S \cdot H_2O$	59.8	4.8	4.4

[a] Betaine. [b] Dissolved and precipitated again. [c] Zwitterionic perchlorate. [d] Triturated at boiling temperature. [e] Too hygroscopic to be analysed.

m.p.s, elemental analyses, i.r. spectra, and ^1H and ^{13}C n.m.r. spectra.

Displacement Reactions with Sodium 2-Nitropropan-2-ide.—Initial experiments were carried out in D_2O solution and the reactions were followed by ^1H n.m.r. The reactions with the *N*-benzyl monocyclic pyridinium ion (1c) at 80 °C gave the *C*-alkylated product $PhCH_2CMe_2NO_2$. Although the expected products, pyridine and $Bu^nCMe_2NO_2$, were not obtained from the *N*-n-butyl monocyclic derivative (1d) even after heating at 120 °C for 16 h (Table 4), the *N*-n-butyl group was transferred from the tricyclic pyridinium ion (4d) at 100 °C in 18 h to give 2-methyl-2-nitrohexane.

Preparative experiments were carried out in Me_2SO and H_2O. The pentacyclic n-butyl derivative (6d) reacted at 70 °C to give 2-methyl-2-nitrohexane (54%). However, the reaction with the lysine analogue (6e) both in Me_2SO and in H_2O gave the intramolecular displacement product piperidine-2-carboxylic acid (8) (in yields of 58 and 64%, respectively), which was characterized by comparison of ^1H and ^{13}C n.m.r. spectra with literature data. Sodium *N*-(3-methyl-1-oxobut-2-enyl)-glycinate (7) (42%) was obtained from the reaction of the glycylglycine analogue (6f) with sodium 2-nitropropan-2-ide in H_2O. Presumably (7) arose from the normal *C*-alkylated product $O_2NCMe_2CH_2CONHCH_2CO_2Na$ through elimination of HNO_2 by base.

Conclusions and Implications for Further Work.—The foregoing reactions, which proceed in aqueous solution at 70 °C in good yield, offer models for the selective conversions of amino groups in polypeptides and proteins, both in the lysine side chains and at the glycine amino-terminals of the main chains.

Table 2. ^{1}H Chemical shifts (δ) of new pyridines and pyridinium salts

Compd. no.	*N*-Substituent	Heterocycle 3-, 5-H	α-Aryl	γ-Aryl 2′-H	γ-Aryl 5′-H	γ-Aryl 6′-H
(2a) [a]	Bu^n $^+$	8.05 [c]	8.16—7.80 [d]	8.30 [e]	7.30 [f]	[g]
(2e) [a]	$(CH_2)_4CH(NH_3)CO_2H$ ClO_4^-	8.03 [c]	8.20—7.60 [d]	8.30 [e]	7.30 [f]	[g]
(6b) [b]			7.9—7.4 [d]	8.2 [e]	7.4 [f]	[g]
(6d) [i]	Bu^n $^+$		8.10—7.10 [d]	[g]	[g]	[g]
(6e) [b]	$[CH_2]_4CH(NH_3)CO_2H$ ClO_4^-		8.30—7.10 [d]	[g]	7.20 [f]	[g]
(6f) [b]	$CH_2CONHCH_2CO_2^-$		8.20—7.30 [d]	8.10 [e]	[g]	[g]

Compd. no.	OMe	$CHCH_2$	1′-H	*N*-Substituents 2′-H	3′-H	4′-H	5′-H
(2a) [a]	3.97 [c]		4.40—4.00 [d]	1.50—1.20 [d]	1.00—0.50 [d]	0.37 [h]	
(2e) [a]	3.95 [c]		4.70—4.40 [d]		1.40—0.60 [k]		3.50—3.20 [d]
(6b) [b]	4.10 [c]	2.97 [c]					
(6d) [i]	3.90 [c]	3.10—2.60 [d]	5.50—5.20 [d]	1.50—1.20 [d]	1.10—0.80 [d]	0.63 [h]	
(6e) [b]	4.10 [c]	3.10—2.60 [d]	5.60—5.40 [d]		2.10—0.90 [k]		3.40—3.10 [d]
(6f) [b]	4.10 [c]	3.10—2.60 [d]	6.27 [c]		[g]	3.87 [j]	

[a] In D_2O, ref. DSS. [b] In CF_3CO_2H, ref. DSS. [c] Singlet. [d] Multiplet. [e] Doublet, *J* 2.0 Hz. [f] Doublet, *J* 9.0 Hz. [g] Concealed. [h] Triplet, *J* 5.5 Hz. [i] In CF_3CO_2H–$CDCl_3$, ref. Me_4Si. [j] Doublet, *J* 6.0 Hz. [k] Multiplet, for 2′-H, 3′-H, and 4′-H.

Table 3. Displacement reactions of *N*-benzyl- and *N*-n-butyl-pyridinium salts with KSPh in aqueous solution

Method	Solvent (ml)	Substrate	Substrate concn. (M)	Nucleophile concn. (M) HSPh	Nucleophile concn. (M) KOH	*T*/°C	Time (h)	Yield of pyridine (%)	Product	Yield of product (%)
A	D_2O (0.5)	(4d)	0.4	1.2	1.6	100	3.0	100 [a]	$PhSBu^n$	71
A	D_2O (0.5)	(1d)	0.4	1.2	2.0	100	10.0	60 [a]	$PhSBu^n$	*b*
A	D_2O (0.5)	(2d)	0.4	1.2	1.6	100	6.0	60 [a]	$PhSBu^n$	*b*
A	D_2O (0.5)	(3d)	0.4	1.2	2.0	100	17.0	50 [a]	$PhSBu^n$	*b*
B	H_2O (5.0)	(4c)	0.4	1.2	1.6	25	2.0	100	$PhSCH_2Ph$	81
B	H_2O (10)	(6d)	0.2	0.6	0.8	70	1.5	100	$PhSBu^n$	100

[a] Calculated from ^{1}H n.m.r. spectra of the reaction mixtures. [b] Too little to calculate.

Table 4. Displacement reactions of *N*-benzyl- and *N*-n-butyl-pyridinium salts with $NaCMe_2NO_2$ in aqueous solution

Method	Solvent (ml)	Substrate	Substrate concn. (M)	Nucleophile concn. (M) $HCMe_2NO_2$	Nucleophile concn. (M) NaH	Nucleophile concn. (M) NaOH	*T*/°C	Time (h)	Yield of pyridine (%)	Product	Yield of product (%)
C	D_2O (0.5)	(1c)	0.4	1.2		2.0	80	3	100 [a]	$PhCH_2CMe_2NO_2$	*b*
C	D_2O (0.5)	(4d)	0.4	1.2		1.6	100	18	80 [a]	$Bu^nCMe_2NO_2$	*b*
C	D_2O (0.5)	(1d)	0.4	1.2		2.0	120	16	0	$Bu^nCMe_2NO_2$	0
D	Me_2SO (3)	(6d)	0.33	1.0	1.3	—	70	2	100 [c]	$Bu^nCMe_2NO_2$	54

[a] Calculated from ^{1}H n.m.r. spectra of the reaction mixtures. [b] Too little to calculate. [c] Calculated from u.v. spectra of the reaction mixture.

Different conditions are expected to be required for the conversion of amino-terminals derived from amino acids other than glycine because secondary-alkyl primary amines RR′$CHNH_2$ are known to react much less readily than primary-alkyl primary amines with pyrylium salts.[4]

Experimental

^{1}H N.m.r. spectra were recorded with a Varian EM 360L spectrometer, and a JEOL FX-100 spectrometer was used for ^{13}C n.m.r. spectra. U.v. spectra were obtained with a Pye Unicam 8-200 spectrometer and i.r. spectra with a Perkin-Elmer 297 instrument. Elemental analyses were carried out by Atlantic Microlab, Inc., Atlanta, Georgia. M.p.s were taken with a Reichert hot-stage apparatus.

Preparations of Pyrylium Salts.—The pyrylium salts and betaines (1a),[2] (2a),[1] (3a),[2] and (4a),[2] and sodium 3-formyl-6-methoxybenzenesulphonate (5) [2] were prepared according to literature procedures and characterized by their ^{1}H n.m.r. and i.r. spectra.[1,2]

5,6,8,9-Tetrahydro-7-(4-methoxy-3-sulphophenyl)dibenzo[c,h]*xanthylium Perchlorate* (6a).—Perchloric acid (70%; 9 ml) was added to sodium 3-formyl-6-methoxybenzenesulphonate (15 g, 0.059 mol), α-tetralone (30 ml), and acetic anhydride (45 ml) over 15 min, with stirring to keep the mixture at 100 °C without heating. The whole was allowed to cool to 25 °C, then the yellow solid *product* was filtered off and washed with Ac_2O and acetone; yield 14.0 g (39.0%), m.p. > 300 °C (decomp.) (Found: C, 59.1; H, 4.2. $C_{28}H_{23}ClO_9S$ requires C, 58.9; H, 4.0%); $\nu_{max.}$ ($CHBr_3$) 3 480—3 320 (OH), 1 600 (pyrylium),

1 560, 1 475, 1 420, 1 260, 1 085 (ClO_4^-), 825, and 770 cm^{-1}; δ_H (CF_3CO_2H, ref. TSP) 8.27 (1 H, d, *J* 2 Hz), 8.05 (1 H, d, *J* 2 Hz), 7.80—7.30 (9 H, m), 4.05 (3 H, s), and 3.04 (8 H s).

Preparation of Pyridinium Salts.—The pyridinium betaines (1c), (1d), (3d), (4c), and (4d) were prepared according to ref. 2 and characterized by their ^1H n.m.r. spectra.[2]

1-*n-Butyl*-2-(4-*sulphonatophenyl*)-4-(4-*methoxy*-3-*sulphophenyl*)-6-(*benzothiazol*-2-*yl*)*pyridinium* (2d).—The pyrylium perchlorate (2a) was added to sodium hydrogen carbonate (0.2 g) and n-butylamine (0.3 g, 4 mmol) in water (20 ml). After stirring at 25 °C for 48 h, the mixture was acidified with $HClO_4$ (40%) (to pH 2) and concentrated at 60 °C and 10 mmHg to about 3 ml. It was then added dropwise to acetone (150 ml). The precipitated *product* was filtered off, purified, and characterized (Tables 1 and 2).

1-(1-*Ammonio*-1-*carboxypentyl*)-6-(*benzothiazol*-2-*yl*)-4-(4-*methoxy*-3-*sulphonatophenyl*)-2-(4-*sulphonatophenyl*)*pyridinium* (2e).—The *pyridinium disulphonate* (2e), prepared by the foregoing procedure from the pyrylium perchlorate (2a) and lysine monohydrochloride, was purified and characterized as in Tables 1 and 2.

14-*n-Butyl*-5,6,8,9-*tetrahydro*-7-(4-*methoxy*-3-*sulphophenyl*)-*dibenzo*[c,h]*acridinium Perchlorate* (6d).—The pyrylium perchlorate (6a) (0.86 g, 1.5 mmol) was added to sodium hydrogencarbonate (0.25 g), sodium carbonate (0.2 g), and n-butylamine (0.22 g, 3 mmol) dissolved in water (20 ml). After being stirred at 25 °C for 48 h, the mixture was acidified with $HClO_4$ (70%). The gum formed was dissolved in ethanol (10 ml) and was added dropwise to ether (200 ml). The purification and characterization of the precipitated *product* were carried out as indicated in Tables 1 and 2.

14-(1-*Ammonio*-1-*carboxypentyl*)-5,6,8,9-*tetrahydro*-7-(4-*methoxy*-3-*sulphonatophenyl*)*dibenzo*[c,h]*acridinium Perchlorate* (6e).—10M-Sodium hydroxide (*ca.* 8 ml) was added to lysine monohydrochloride (7.2 g, 0.04 mol) in water (20 ml) (to pH 10.0—10.5). The pyrylium perchlorate (8.5 g, 0.015 mol) was added in portions with stirring, with the pH kept at 10—10.2 by addition of 10M-sodium hydroxide. After being stirred at 25 °C for 48 h, the mixture was brought to pH 2 with $HClO_4$ (10%). The gum formed was triturated with ethanol (150 ml). The solid *product* was purified and characterized as indicated in Tables 1 and 2.

Sodium 14-(*Carboxymethylcarbamoylmethyl*)-5,6,8,9-*tetrahydro*-7-(4-*methoxy*-3-*sulphonatophenyl*)*dibenzo*[c,h]*acridinium* (6f).—The pyrylium salt (6a) (1.18 g, 2 mmol) was added in portions with stirring to glycylglycine (0.53 g, 4 mmol) and sodium hydroxide (0.2 g), in water–ethanol (4 : 1; 5 ml), with the pH kept at 10—10.2 by addition of 10M-sodium hydroxide. After stirring at 25 °C for 48 h, a small precipitate was filtered off and the filtrate was brought to pH 7 with $HClO_4$ (10%). The mixture was kept for 12 h at 0 °C. The yellow solid *product* was filtered off, purified, and characterized as indicated in Tables 1 and 2.

Displacement Reactions of Pyridinium Ions in Aqueous Solution.—Method A: Reaction of N-*n-butylpyridinium salts with potassium benzenethiolate in* D_2O. The pyridinium salt was dissolved in a solution of benzenethiol and potassium hydroxide in D_2O. The solution was heated at 100 °C (Table 3) and the reaction followed by ^1H n.m.r. until there was no further change in the spectrum. After cooling the solution was extracted with $CDCl_3$; the product $PhSBu^n$ was pure as shown by its ^1H n.m.r. spectrum: δ_H ($CDCl_3$; ref. Me_4Si) 7.33 (5 H, s), 2.94 (2 H, t, *J* 7.5 Hz), 1.80—1.20 (4 H, m), and 0.93 (3 H, t, *J* 7.0 Hz).

Method B: Reaction of N-*benzyl- and* N-*n-butyl-pyridinium salts* (4c) *and* (6d) *with potassium benzenethiolate in water.* Benzenethiol was added to potassium hydroxide in water under nitrogen and the mixture was stirred at 25 °C for 15 min. The pyridinium salt was added and dissolved, and the solution was stirred at 25 °C for 2 h [for (4c)] or heated at 70 °C for 1.5 h [for (6d)]. The precipitated pyridine [after acidification of the mixture in the case of (4c)] was filtered off and washed with water and ether. The filtrate [after basification in the case of (4c)] was extracted with ether. The combined ethereal solution was dried and evaporated *in vacuo*. The residual products were characterized as follows: $PhSCH_2Ph$, m.p. 39.5—41 °C (lit.,[5] 40—41 °C), δ_H (CCl_4; ref. Me_4Si) 7.20 (10 H, s) and 4.06 (2 H, s); $PhSBu^n$, n_D^{23} 1.5465 (lit.,[6] n_D^{21} 1.5472), δ_H ($CDCl_3$, ref. Me_4Si) 7.33 (5 H, s), 2.93 (2 H, t, *J* 7.5 Hz), 1.80—1.20 (4 H, m), and 0.93 (3 H, t, *J* 7.0 Hz).

Method C: Reaction of N-*benzyl- and* N-*n-butyl-pyridinium salts with sodium* 2-*nitropropan*-2-*ide in* D_2O. Sodium hydroxide and 2-nitropropane were dissolved in D_2O by heating at 60 °C for 10 min and then the pyridinium salt was dissolved in the solution. The reaction mixture was heated (Table 4), and the reaction followed by ^1H n.m.r. until there was no further change in the spectra. After cooling, the solution was extracted with $CDCl_3$ and the products were characterized by ^1H n.m.r. (ref. Me_4Si): $PhCH_2CMe_2NO_2$, δ 7.00—7.40 (5 H, m), 3.22 (2 H, s), and 1.56 (6 H, s); $Bu^nCMe_2NO_2$, δ 1.90—1.65 (2 H, m), 1.56 (6 H, s), 1.45—1.15 (4 H, m), and 0.93 (3 H, t, *J* 6.0 Hz).

Method D: Reaction of the N-*n-butylpyridinium salt* (6d) *with sodium* 2-*nitropropan*-2-*ide in* Me_2SO. To sodium hydride (0.1 g, 4 mmol) in methanol (4 ml) was added 2-nitropropane (0.267 g, 3 mmol) with stirring at 25 °C for 20 min. Methanol was evaporated off (20 °C and 1 mmHg) at 50 °C for 20 min. To the residue in Me_2SO (3 ml) was added the pyridinium salt (6d) (0.735 g, 1 mmol), and the mixture was heated (see Table 4). After cooling, water (75 ml) was added and the whole was acidified ($HClO_4$) and extracted with ether (3 × 40 ml). After drying, ether was evaporated off *in vacuo*. The pale-yellow oily product $Bu^nCMe_2NO_2$ was characterized by ^1H n.m.r.: δ ($CDCl_3$, ref. Me_4Si) 1.90—1.65 (2 H, m), 1.56 (6 H, s), 1.45—1.15 (4 H, m), and 0.93 (3 H, t, *J* 6.0 Hz).

Reaction of N-(5-*Ammonio*-5-*carboxypentyl*)*pyridinium Salt* (6e) *with Potassium Benzenethiolate in Water.*—The pyridinium salt (6e) (2.2 g, 3 mmol) was added to benzenethiol (0.99 g, 9 mmol) and potassium hydroxide (1.0 g, 15 mmol) in water (8 ml) as in Method B. The mixture was heated at 70 °C under N_2 for 2 h. After the precipitated pyridine (6b) had been filtered off, the filtrate was neutralized with hydrochloric acid (10%). The precipitated *product* $PhS[CH_2]_4\overset{+}{C}H(\overset{+}{N}H_3)CO_2^-$ (77%) was filtered off, washed with water (5 ml), acetone (10 ml), and ether (10 ml), and recrystallized from EtOH–H_2O (1 : 1); m.p. 233—235 °C (Found: C, 60.45; H, 7.2; N, 5.7. $C_{12}H_{17}NO_2S\cdot H_2O$ requires C, 60.25; H, 7.1; N, 5.85%); $\nu_{max.}$ ($CHBr_3$) 2 920—2 900, 2 860sh, 2 600sh, and 2 120 ($\overset{+}{N}H_3$), 1 575 (CO_2^-), 1 500, 1 445, 1 405, 1 338, 1 318, 1 185, 937, 841, 822, and 732 cm^{-1}; δ_H [CF_3CO_2H, ref. sodium 3-trimethylsilylpropane-1-sulphonate (DSS)] 7.30 (5 H, s, Ph), 4.60—4.25 (1 H, m, 2-H), 3.00 (2 H, t, *J* 7.5 Hz, 6-H), and 2.35—1.60 (6 H, m, 3-, 4-, 5-H); δ_C (D_2O, ref. DSS) 185.0 (CO_2^-), 137.9, 131.1, 130.6, and 128.0 (Ph), 58.0 (C-2), 36.7 (C-6), 34.6 (C-3), and 30.6 and 26.6 (C-4 and C-5).

Reaction of the N-(5-*Ammonio*-5-*carboxypentyl*)*pyridinium Salt* (6e) *with Potassium Phenylmethanethiolate in Water.*—The *product* $PhCH_2S[CH_2]_4CH(\overset{+}{N}H_3)CO_2^-$ (63%) was obtained similarly from the pyridinium salt (6e) (2.2 g, 3 mmol), phenylmethanethiol (1.12 g, 9 mmol), and potassium hydroxide (1.0 g, 15 mmol) after heating under N_2 at 75 °C for 1 h; m.p. 215—217 °C (Found: C, 57.5; H, 7.5; N, 5.3. $C_{13}H_{19}NO_2S\cdot H_2O$ requires C, 57.6; H, 7.7; N, 5.2%); $\nu_{max.}$ ($CHBr_3$) 2 920—2 900, 2 860sh, 2 600sh, and 2 120 ($\overset{+}{N}H_3$), 1 575 (CO_2^-), 1 500, 1 443, 1 408, 1 340, 1 317, 1 182, 1 085, 1 071, 932, 849, 768, and 734 cm^{-1}; δ_H (D_2O, ref. DSS) 7.33 (5 H, s, Ph), 3.67 (2 H, s, SCH_2), 3.30—3.00 (1 H, m, 2-H), 2.42 (2 H, t, *J* 6 Hz, 6-H), and 1.75—1.00 (6 H, m, 3-, 4-, 5-H); δ_C (D_2O, ref. DSS) 185.4 (CO_2^-), 140.8, 131.2, 130.9, and 129.3 (Ph), 58.3 (C-2), 37.8 (SCH_2), 37.1 (C-6), 33.1 (C-3), and 30.9 and 27.0 (C-4 and C-5).

Reaction of the N-(*Carboxymethylcarbamoylmethyl*)*pyridinium Salt* (6f) *with Potassium Benzenethiolate in Water.*—The pyridinium salt (6f) (2.8 g, 4 mmol) was heated under N_2 with benzenethiol (1.32 g, 16 mmol) and potassium hydroxide (1.34 g, 20 mmol) in water (20 ml) at 75 °C for 1 h as just described. After the precipitated pyridine (6b) was filtered off, the filtrate was neutralized with hydrochloric acid (10%), extracted with ether (2 × 30 ml), acidified with HCl (10%) (to pH 1—2), then concentrated *in vacuo* (0.1 mmHg) at 50 °C (to 10 ml), and extracted with ethyl acetate (2 × 20 ml). After drying the ethyl acetate was evaporated off at 50 °C and 1 mmHg. The solid *product* $PhSCH_2CONHCH_2CO_2H$ (66%) had m.p. 120—121.5 °C (Found: C, 53.5; H, 5.0; N, 6.15. $C_{10}H_{11}NO_3S$ requires C, 53.35; H, 4.9; N, 6.2%); $\nu_{max.}$ ($CHBr_3$) 3 330 (NH), 2 972, 2 922, and 1 720 (CO_2H), 1 610—1 540 and 1 520 (CONH), 1 478, 1 425, 1 400, 1 342, 1 340—1 310, 980, 895, 786, and 735 cm^{-1}; δ_H (CF_3CO_2H, ref. DSS) 8.26 (1 H, t, *J* 5.5 Hz, NH), 7.40 (5 H, s, Ph), 4.27 (2 H, d, *J* 5.5 Hz, 2-H), and 3.87 (2 H, s, SCH_2); δ_C (CF_3CO_2H–D_2O, ref. DSS) 177.5 (CO_2H), 172.6 (CONH), 135.3, 131.1, 130.8, and 128.7 (Ph), 68.0 (C-2), and 45.0 (SCH_2).

Reaction of the N-(5-*Ammonio*-5-*carboxypentyl*)*pyridinium Salt* (6e) *with Sodium* 2-*Nitropropan*-2-*ide in* Me_2SO.—The pyridinium salt (6e) (1.47 g, 2 mmol) was treated with 2-nitropropane (0.534 g, 6 mmol) and sodium hydride (0.2 g, 8 mmol) in Me_2SO (3 ml) at 70 °C for 1 h as in Method D. After the precipitated pyridine (6b) had been filtered off, the filtrate was neutralized with $HClO_4$ (40%). A small precipitate was filtered off and the solvents were evaporated (0.5 mmHg and 65—70 °C). The residue was extracted with methanol (5 ml) and the solution added dropwise to ether (100 ml). The precipitate was dissolved in acetone (10 ml); addition of ether (2 ml) then gave piperidine-2-carboxylic acid (8) (58%), characterized by ^1H n.m.r. (D_2O, ref. DSS): δ 3.70—3.42 (1 H, m) 3.42—3.23 (1 H, m), 3.23—2.90 (1 H, m), and 1.45—2.35 (6 H, m); and ^{13}C n.m.r. (D_2O, ref. dioxane): 176.2 (s), 59.9 (d), 44.6 (t), 27.7 (t), 22.8 (t), and 22.7 (t).[7]

Reaction of the N-(5-*Ammonio*-5-*carboxypentyl*)*pyridinium Salt* (6e) *with Sodium* 2-*Nitropropan*-2-*ide in Water.*—2-Nitropropane (0.54 g, 6 mmol), sodium hydroxide (0.40 g, 10 mmol), and water (8 ml) were stirred at 25 °C for 10 min. The pyridinium salt (6e) (1.47 g, 2 mmol) was added and dissolved by stirring for 20 min, and the solution was heated at 80 °C for 3 h. The reaction mixture was treated as in the preceding experiment, and the white product was characterized by ^1H and ^{13}C n.m.r. as piperidine-2-carboxylic acid (8) (64%).

Reaction of the N-(*Carboxymethylcarbamoylmethyl*)*pyridinium Salt* (6f) *with Sodium* 2-*Nitropropan*-2-*ide in Water.*—The pyridinium salt (6f) (1.37 g, 2 mmol) was treated similarly with 2-nitropropane (0.54 g, 6 mmol) and sodium hydroxide (0.40 g, 10 mmol) in water (10 ml) at 80 °C for 1 h. After the precipitated pyridine (6b) had been filtered off, the filtrate was acidified with perchloric acid (to pH 6.0) and concentrated *in vacuo* (10 mmHg and 60 °C, to 2 ml). Then it was added dropwise to acetone (100 ml) and the precipitate was dissolved in methanol (10 ml). The solution was passed through a column of silica gel and eluted with methanol. A solid product was purified by recrystallization from methanol–ether (1 : 5) [m.p. 205—208 °C (decomp.)], and was characterized as *N*-(3-methyl-1-oxobut-2-enyl)glycine (7) (42%) by ^1H n.m.r. (D_2O, ref. DSS): δ 5.83 (1 H, d, *J* 1.5 Hz), 3.82 (2 H, s), 2.04 (3 H, d, *J* 1.5 Hz), and 1.87 (3 H, d, *J* 1.5 Hz); ^{13}C n.m.r. (D_2O, ref. dioxane): δ 177.9 (s), 170.7 (s), 153.2 (s), 118.2 (d), 43.9 (t), 27.0 (q), and 20.1 (q); and i.r. spectra: $\nu_{max.}$ ($CHBr_3$) 3 560, 3 362, and 3 280 (CONH), 2 945 and 2 920 (CH_3 and CH_2), 1 670 (CONH), 1 600 and 1 405 (CO_2^-), 1 565sh and 1 530 (C=C), 1 430, 1 270—1 220, and 1 030 cm^{-1}.

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