phosphonate oxalate etherate (7.42 g, 0.02 mol) in dry chloroform (40 mL) containing triethylamine (6.0 mL) was added, and the mixture was slowly heated to boiling, cooled to room temperature, and allowed to stand overnight. Then it was washed successively as in method A, yielding crude diethyl [*N*-(benzyloxycarbonyl)-L-alanylamino]cyclopropylmethanephosphonate, which was deblocked by acidolysis with 45% hydrogen bromide in glacial acetic acid solution. Workup procedure as in method A yielded the crystalline dipeptide **15**: yield 2.2 g (44%); mp 270–274 °C dec; ^{1}H NMR (D_2O + D_2SO_4, HMDS) δ 0.2–1.25 (m, J = 7 Hz, 5 H, cyclopropyl protons), 1.50 (d, J = 7 Hz, 3 H, CH_3), 3.36 and 3.39 (dd, J = 8 Hz, J = 175 Hz, 0.5 H, CHP), 4.08 (q, J = 7 Hz, 1 H, CHCO).

Microbiology. All organisms were obtained as lyophilized preparations from the Polish Collection of Microorganisms (PCM) and are indicated by the appropriate accession numbers.

The IC_{50} values of each peptide for each strain were determined on a defined liquid peptide medium free from antagonists to small peptide mimetics (broth) as described by Atherton et al.[5]

Inocula of all strains were prepared by growing the test organisms overnight in the liquid peptide medium at 37 °C and diluting the cultures to approximately 4 × 10^6 cfu (colony-forming units) per milliliter.

A doubling dilution series was prepared in the liquid peptide medium for each compound. A 0.5-mL amount of each concentration was added to 9.4 mL of the peptide medium to give, after addition of 0.1 mL of inoculum, a final concentration range of from 0.065 to 512 μg/mL. The incubation was carried out overnight at 37 °C. The turbidity of each culture was then measured.

IC_{50} values were defined as the concentration required to reduce the growth of the 24-h culture in the liquid peptide medium to 50% of the control value.

The samples in which no turbidity was observed were used for MIC determination. Thus, 0.1 mL of such sample was transferred from the wells onto the plates containing solid peptide medium[5] (agar) and incubated overnight at 37 °C.

MIC was defined as the lowest concentration of the peptide that either completely inhibited growth or permitted 10 or fewer microcolonies to grow.

Registry No. (L,L)-1, 60668-24-8; (L,D)-1, 66023-94-7; (L,L)-**2**, 60668-50-0; (L,D)-**2**, 84340-65-8; (L,L)-**3**, 97993-89-0; (L,D)-**3**, 97993-87-8; (L,L)-**4**, 84340-73-8; (L,D)-**4**, 84340-74-9; (L,L)-**5**, 104155-45-5; (L,D)-**5**, 104131-05-7; (L,L)-**6**, 84340-79-4; (L,D)-**6**, 84340-80-7; (L,L)-**7**, 60668-65-7; (L,D)-**7**, 66024-04-2; (L,L)-**8**, 104130-76-9; (L,D)-**8**, 104131-06-8; (L,L)-**9**, 104130-77-0; (L,D)-**9**, 104131-07-9; (L,L)-**10**, 104130-78-1; (L,D)-**10**, 104131-08-0; (L,L)-**11**, 104130-79-2; (L,D)-**11**, 104155-03-5; (L,L)-**12**, 104130-80-5; (L,D)-**12**, 104131-09-1; (L,L)-**13**, 98820-97-4; (L,D)-**13**, 98820-98-5; (L,L)-**14**, 97993-91-4; (L,D)-**14**, 97993-88-9; (L,L)-**15**, 104130-81-6; (L,D)-**15**, 104131-10-4; (L,L)-**16**, 104130-82-7; (L,D)-**16**, 104131-11-5; (L,L)-**17**, 104130-83-8; (L,D)-**17**, 104131-12-6; (L,L)-**18**, 104130-84-9; (L,D)-**18**, 104131-13-7; (L,L)-**19**, 104130-85-0; (L,D)-**19**, 104131-14-8; (L,L)-**20**, 104130-86-1; (L,D)-**20**, 104131-15-9; (L,L)-**21**, 104130-87-2; (L,D)-**21**, 104131-16-0; (L,L)-**22**, 104130-88-3; (L,D)-**22**, 104131-17-1; (L,L)-**23**, 104130-89-4; (L,D)-**23**, 104131-18-2; (L,L)-**24**, 104130-90-7; (L,D)-**24**, 104131-19-3; (L,L)-**25**, 104130-91-8; (L,D)-**25**, 104131-20-6; (L,L)-**26**, 104130-92-9; (L,D)-**26**, 104131-21-7; **27**, 104130-93-0; **28**, 104130-94-1; **29**, 84139-32-2; **30**, 98188-76-2; (L,L)-**31**, 104130-95-2; (L,D)-**31**, 104131-22-8; (L,L)-**32**, 97993-96-9; (L,D)-**32**, 97993-95-8; **33**, 104130-96-3; **34**, 88024-19-5; **35**, 104130-97-4; **36**, 104130-99-6; (L,L)-Z-Ala-NHCH(CH_2Ph)P(O)(OPh)$_2$, 104131-00-2; (L,D)-Z-Ala-NHCH(CH_2Ph)P(O)(OPh)$_2$, 104131-23-9; (L,L)-Z-Ala-NHCH(CH_2Ph)P(O)(OMe)$_2$, 104131-01-3; (L,D)-Z-Ala-NHCH-(CH_2Ph)P(O)(OMe)$_2$, 104131-24-0; triphenyl phosphite, 101-02-0; phenylacetaldehyde, 122-78-1; benzyl carbamate, 621-84-1; diethyl phthalate, 84-66-2; carbobenzoxy-L-alanine, 1142-20-7; diethyl amino(cyclopropyl)methanephosphonate oxalate, 104131-03-5; (L,L)-diethyl [*N*-(benzyloxycarbonyl)alanylamino]cyclopropylmethanephosphonate, 104131-04-6; (L,D)-diethyl [*N*-(benzyloxycarbonyl)alanylamino]cyclopropylmethanephosphonate, 104131-25-1.

Synthesis and Radioprotective Activity of New Cysteamine and Cystamine Derivatives

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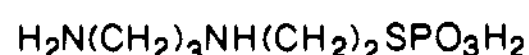
A variety of *N*-(aminoalkanoyl)-*S*-acylcysteamine and *N,N'*-bis(aminoalkanoyl)cystamine salt derivatives were synthesized. Toxicity and radioprotective activity (as the dose reduction factor DRF) were determined in vivo on mice and compared to WR 2721 and *S*-acetylcysteamine hydrochloride. One of the most interesting compounds of this series was *N*-glycyl-*S*-acetylcysteamine trifluoroacetate (**16**, I 102). Structure–activity relationships are discussed.

Since the 1950s, numerous radioprotectors have been described. However, there has been renewed interest in the area since it has been shown that the most promising radioprotector WR 2721 (**1**)[1] appears to afford preferen-



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tially radioprotection for certain normal tissues[2–4] as opposed to tumors.[4–7] The origin of such differentiation has not been firmly established although some correlations between the activity and the hydrophilicity of the drug have been presented.[8]

Regarding the mechanisms of action of such phosphorothioates, it is postulated that they act through their

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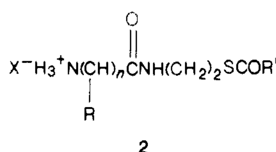
aminothiol derivative, which is liberated in vivo.[9,10] The different mechanisms of action of amino thiols have been reviewed by Foye[11] and Klayman and Copeland.[12] Lowering of intracellular oxygen tension, radical scavenging, formation of mixed disulfides, decrease in DNA degradation, and lowering of lipidic peroxydation of cell membrane can be mentioned.

Furthermore, the nucleophilic thiol group could also trap xenobiotic electrophilic intermediates originating from alkylating agents by formation of covalent bonds. The DNA affinity of **1**,[13] which can be explained by consideration of the closely related spermidine structure,[14] is also noteworthy.

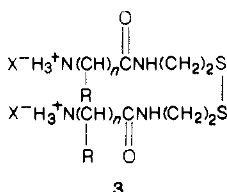
These features then constitute the rationale for an eventual use of such drugs in radio- and chemotherapy. Unfortunately, WR 2721 has some limitations; it is not very stable,[10] and it shows some toxic side effects.[15] We have therefore decided to look for new cysteamine derivatives that might overcome these problems while maintaining selectivity in cellular response.

For this purpose we have synthesized derivatives **2**. These compounds possess a thioacyl group, which is more



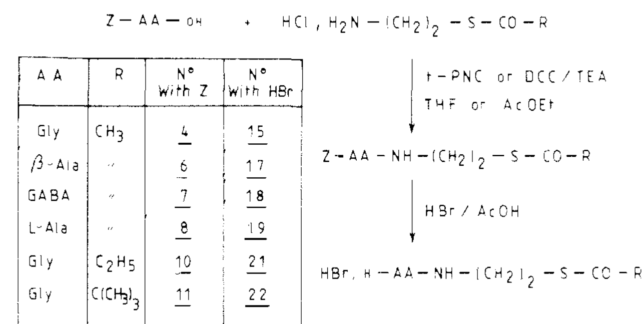
stable than a phosphorothioate (cf. **1**), and can liberate in situ the thiol group through either hydrolysis or the well-known S→N transfer reaction. Additionally, conjugation with amino acids instead of the aminopropyl group might facilitate detoxification of the drug[16] with retention of the "polyamine-like" structure. It is worth mentioning that such compounds can be converted to salts by quaternization of the terminal amino group.

In related work the corresponding cystamine amino acid derivatives (e.g., **3**) were prepared.



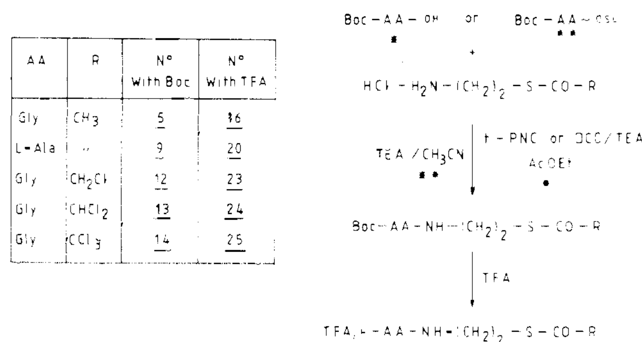
These analogues may be considered metabolically equivalent to the previous compounds (**2**) after reduction by glutathione reductase. Some of these compounds have been mentioned before (see Table V),[17-18] but no biological data have been reported.

Scheme I[a]



[a] Z = benzyloxycarbonyl; AA = amino acid.

Scheme II[a]



[a] Boc = *tert*-butyloxycarbonyl.

Chemistry

The synthesis of **2** was achieved by coupling reactions between N-protected amino acids and *S*-acylcysteamines.

Condensation of (benzyloxycarbonyl)amino acids [Z-AA-OH; Z = $PhCH_2OC(O)$] with *S*-acylcysteamine hydrochlorides in tetrahydrofuran (THF) or ethyl acetate (AcOEt), using phosphonitrilic chloride (*t*-PNC)[19] or *N*,*N'*-dicyclohexylcarbodiimide (DCC) in the presence of triethylamine (TEA) as activating agents, gave rise to the expected compounds **4**, **6**, **7**, **8**, **10**, and **11** (Scheme I; Table I). Removal of the benzyloxycarbonyl (Z) protecting group by treatment with hydrobromic acid (HBr) in glacial acetic acid (AcOH) gave the desired derivatives **15**, **17–19**, **21**, and **22** (Scheme I; Table II).

Alternatively, the reaction of (*tert*-butyloxycarbonyl)amino acid [Boc-AA-OH; Boc = $(H_3C)_3COC(O)$] or the (*tert*-butyloxycarbonyl)amino acid *N*-hydroxysuccinimide ester (Boc-AA-OSu) and an *S*-acylcysteamine in the presence of TEA and condensing agents (*t*-PNC, DCC) in acetonitrile or AcOEt gave the expected derivatives **5**, **9**, **12–14** (Scheme II; Table I). The corresponding trifluoroacetates **16**, **20**, **23–25** (Scheme II; Table II) were obtained after deprotection of the *tert*-butyloxycarbonyl (Boc) group with trifluoroacetic acid (TFA).

The corresponding *N*,*N'*-bis(aminoalkanoyl)cystamine dihydrobromide derivatives (Scheme III; Table V) **36** and **38–40** were obtained from **4** and **6–8** (see Scheme I) by the following sequence: deprotection of the thiol function with sodium methylate and hydrochloric acid, iodine oxidation of free thiols **26–29** (Table III), and benzyloxycarbonyl (Z) elimination from **30** and **32–34** (Table IV) in the usual manner.

These compounds can also be obtained by direct coupling in ethyl acetate of free cystamine with (benzyloxycarbonyl)glycine in the presence of *t*-PNC and TEA or

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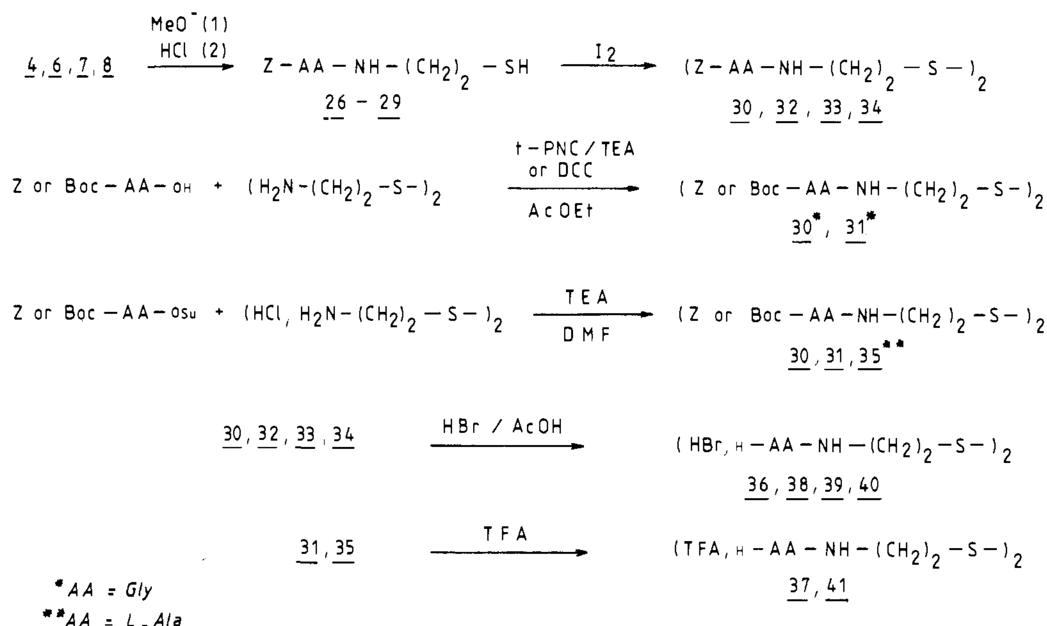
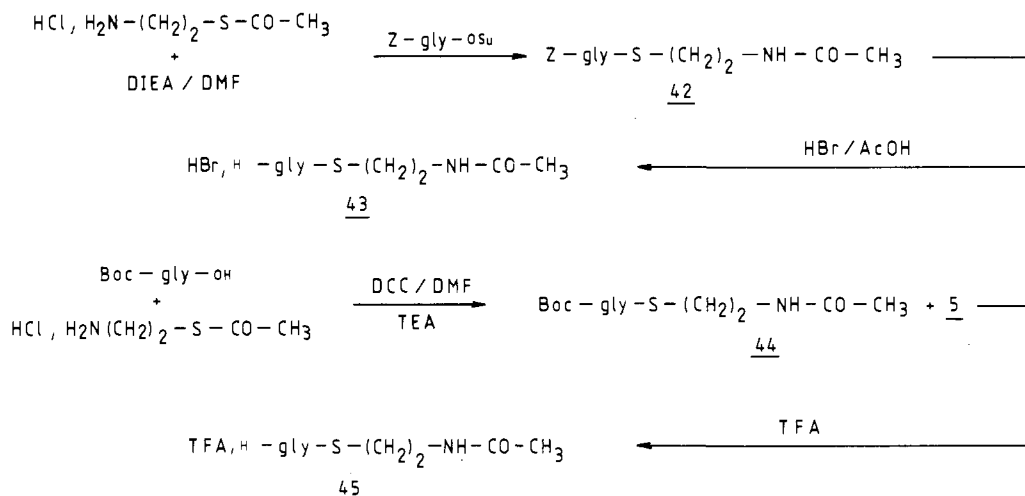
Scheme III



Scheme IV



DCC, or by coupling of cystamine dihydrochloride in *N,N*-dimethylformamide (DMF) and TEA with succinimido (benzyloxycarbonyl)glycinate (Scheme III; Table IV).

Two *N,N'*-bis(aminoalkanoyl)cystamine bis(trifluoroacetates) **37** and **41** (diglycyl, di-L-alanyl) have also been obtained (Scheme III; Table V). Compound **37** was synthesized by direct coupling of free cystamine with (*tert*-butyloxycarbonyl)glycine or by condensation of the succinimido (*tert*-butyloxycarbonyl)glycinate with cystamine dihydrochloride and then *tert*-butyloxycarbonyl (Boc) elimination from *N,N'*-bis[(*tert*-butyloxycarbonyl)-glycyl]cystamine (**31**; Table IV). Compound **41** was obtained by condensation of succinimido (*tert*-butyloxycarbonyl)-L-alaninate and cystamine dihydrochloride in the presence of DMF/TEA followed by *tert*-butyloxycarbonyl (Boc) elimination from *N,N'*-bis[(*tert*-butyloxycarbonyl)-L-alanyl]cystamine (**35**; Table IV).

It is noteworthy that the nature of the solvent as well as the base used to liberate *S*-acetylcysteamine hydrochloride from its salt was important. Using diisopropylethylamine (DIEA) in DMF instead of TEA, S→N acetyl transfer occurred and an inverted product (**43**) was readily obtained as shown in Scheme IV. Similarly, trifluoroacetate **45** resulted from coupling (*tert*-butyloxycarbonyl)glycine with *S*-acetylcysteamine hydrochloride in DMF in the presence of DCC and TEA, followed by *tert*-butyloxycarbonyl (Boc) elimination as in the usual manner.

All the synthesized products have been fully characterized by the usual analytical methods. S→N transfer reactions were easily detected from the acetyl signal in the ^{1}H NMR.

For the structure–activity relationship study we synthesized a series of model compounds successively changing two parameters: (a) The amino acid was changed while keeping the *S*-acetyl function. For this purpose, glycine, β-alanine, 4-aminobutyric acid (GABA), and L-alanine were used in order to approximate the polyamine-like structure of spermidine. (b) Conversely, the acyl group was changed while glycine was kept as the amino acid in order to modulate the probable liberation of the thiol group either via transposition or enzymatic hydrolysis.

Physical and analytical data for these derivatives are given in Tables I–V.

Biological Results and Discussion

The compounds synthesized in the present study were evaluated for radioprotective activity, in vivo, in mice. Results are shown in Table VI. Their activities were

Table I. Physical Properties of *N*-[[(Z or Boc)amino]alkanoyl]-*S*-acylcysteamines: R_1-AA-NH$(CH_2)_2$SC(O)R_2

no.	AA	R_1	R_2	yield, %	mp,[a] °C	R_f (solvent[b])	formula[c]	IR: $\nu^{d,e}$ cm^{-1} NH	C=O	CNH	^{1}H NMR: δ (solvent *f* or *g*)
4	Gly	Z	CH_3	45	100–103	0.5	$C_{14}H_{18}N_2O_4S$	3320 3280	1665 1625[d]	1515	(*f*) 2.30 (s, 3 H, acetyl CH_3), 2.70–3.60 (m, 4 H, NCH_2CH_2S), 3.82 (d, 2 H, Gly CH_2), 5.10 (s, 2 H, benzylic CH_2), 5.75 and 6.75 (2 m, 2 H, 2 NH), 7.33 (s, 5 H, aromatic)
5	Gly	Boc	CH_3	40,[h] 48[i]	59–60	0.8	$C_{11}H_{20}N_2O_4S$	3330 3280	1685 1655[d]	1535	(*g*) 1.47 (s, 9 H, *t*-Bu), 2.35 (s, 3 H, acetyl CH_3), 3.15 (m, 2 H, SCH_2), 3.57 (m, 2 H, NCH_2), 4.07 (d, 2 H, Gly CH_2), 5.45 and 7.20 (2 m, 2 NH)
6	β-Ala	Z	CH_3	35	114–116	0.75	$C_{15}H_{20}N_2O_4S$	3320 3280	1660 1620[d]	1530	(*g*) 2.30 (s, 3 H, acetyl CH_3), 2.36 (m, 2 H, β-Ala CH_2), 2.95 (m, 2 H, SCH_2), 3.43 (m, 4 H, 2 NCH_2), 5.08 (s, 2 H, benzylic CH_2), 5.64 and 6.44 (2 m, 2 H, 2 NH), 7.31 (s, 5 H, aromatic)
7	GABA	Z	CH_3	33	100–101	0.45	$C_{16}H_{22}N_2O_4S$	3320 3290	1670 1625[d]	1525	(*g*) 1.60–2.30 (m, 4 H, GABA CH_2CH_2), 2.35 (s, 3 H, acetyl CH_3), 2.80–3.66 (m, 6 H, 2 NCH_2, SCH_2), 5.10 (s, 2 H, benzylic CH_2), 5.32 and 6.30 (2 m, 2 H, 2 NH), 7.31 (s, 5 H, aromatic)
8	L-Ala	Z	CH_3	80	78–80	0.5	$C_{15}H_{20}N_2O_4S$	3320 3280	1662 1620[d]	1520	(*g*) 1.36 (d, 3 H, L-Ala CH_3), 2.30 (s, 3 H, acetyl CH_3), 3.0 (m, 2 H, SCH_2), 3.40 (m, 2 H, NCH_2), 4.23 (m, 1 H, L-Ala CH), 5.11 (s, 2 H, benzylic CH_2), 5.90 (d, 1 H, L-Ala NH), 7.0 (m, 1 H, NH), 7.36 (s, 5 H, aromatic)
9	L-Ala	Boc	CH_3	43	71–73	0.5	$C_{12}H_{22}N_2O_4S$	3320 3300	1680 1660[d]	1520	(*g*) 1.35 (d, 3 H, L-Ala CH_3), 1.48 (s, 9 H, *t*-Bu), 2.33 (s, 3 H, acetyl CH_3), 3.02 (m, 2 H, SCH_2), 3.43 (m, 2 H, NCH_2), 4.17 (m, 1 H, L-Ala CH), 5.25 (d, 1 H, L-Ala NH), 6.84 (t, 1 H, NH)
10	Gly	Z	C_2H_5	46	94–95	0.8	$C_{15}H_{20}N_2O_4S$	3280	1675 1635[d]	1520	(*g*) 1.15 (t, 3 H, propionyl CH_3), 2.58 (q, 2 H, propionyl CH_2), 3.0 (m, 2 H, SCH_2), 3.46 (m, 2 H, NCH_2), 3.86 (d, 2 H, Gly CH_2), 5.16 (s, 2 H, benzylic CH_2), 5.50 and 6.50 (2 m, 2 H, 2 NH), 7.37 (s, 5 H, aromatic)
11	Gly	Z	$C(CH_3)_3$	50	oil	0.6	$C_{17}H_{24}N_2O_4S$	3300	1715 1670[e]	1530	(*g*) 1.22 (s, 9 H, pivaloyl CH_3), 2.94 (m, 2 H, SCH_2), 3.33 (m, 2 H, NCH_2), 3.80 (d, 2 H, Gly CH_2), 5.10 (s, 2 H, benzylic CH_2), 6.05 and 7.0 (2 m, 2 H, 2 NH), 7.30 (s, 5 H, aromatic)
12	Gly	Boc	CH_2Cl	35	83–86	0.55	$C_{11}H_{19}ClN_2O_4S$	3285	1670 1620[d]	1520	(*g*) 1.46 (s, 9 H, *t*-Bu), 3.10 (m, 2 H, SCH_2), 3.44 (m, 2 H, NCH_2), 3.78 (d, 2 H, Gly CH_2), 4.20 (s, 2 H, CH_2Cl), 5.50 and 6.90 (2 m, 2 H, 2 NH)
13	Gly	Boc	$CHCl_2$	60	111–113	0.85	$C_{11}H_{18}Cl_2N_2O_4S$	3320	1680	1530[d]	(*g*) 1.45 (s, 9 H, *t*-Bu), 3.13 (m, 2 H, SCH_2), 3.54 (m, 2 H, NCH_2), 4.05 (d, 2 H, Gly CH_2), 6.0 (s, 1 H, CH), 5.47 and 7.22 (2 m, 2 H, 2 NH)
14	Gly	Boc	CCl_3	20	111–113	0.65	$C_{11}H_{17}Cl_3N_2O_4S$	3330	1690 1670[d]	1530	(*g*) 1.48 (s, 9 H, *t*-Bu), 3.20 (m, 2 H, SCH_2), 3.62 (m, 2 H, NCH_2), 4.08 (d, 2 H, Gly CH_2), 5.35 and 7.36 (2 m, 2 H, 2 NH)

[a] All compounds were crystallized from an ethyl acetate/petroleum ether mixture or dichloromethane/petroleum ether mixture. [b] CH_2Cl_2/MeOH, 9:1. [c] All compounds gave satisfactory C, H, N analyses (±0.4%). [d] As KBr disks. [e] Dispersed in Nujol mull. [f] Me_2SO-d_6. [g] $CDCl_3$. [h] Experimental Section (method 1). [i] Experimental Section (method 2). AA = amino acid; Boc = *t*-butyloxycarbonyl; Z = benzyloxycarbonyl.

compared with that of *S*-acetylcysteamine hydrochloride and WR-2721.

It is apparent that conjugation of the simplest amino acid glycine via a peptide bond to *S*-acetylcysteamine gives rise, as expected, to a decrease of toxicity and also to an increase of radioprotection. In addition, the nature of the anion does not influence the biological response (compare **15** and **16**).

With an increase in the length of the amino acid chain, the activity decreases rapidly as does the LD_{50} (compare **15** with **17** and **18**). The best compounds are those bearing only one carbon atom on the amino acid chain (i.e., glycine, L-alanine). This finding is quite surprising since we expected those analogues bearing β-alanine or 4-aminobutyric acid moieties to be more active.

From the various acyl protections introduced on the model compounds **15** or **16**, it is obvious that the acetyl is the most satisfactory. This may indicate that in vivo thiol deprotection resulted from enzymatic hydrolysis rather than S→N transfer, which should have been facilitated with the haloacyl derivatives.

Furthermore, the cystamine analogues show, as expected, the same activity as the corresponding *S*-acetyl compounds (compare **36** and **15** or **38** and **17**). This indicates that under the experimental conditions a reductive bioactivation has occurred.

On the basis of these initial data, it appears that the best compound of this series is the glycine derivative **16**, known under the code number of I 102. In terms of radioprotection, using standard experimental conditions, this de-

Table II. Physical Properties of *N*-(Aminoalkanoyl)-*S*-acylcysteamine Hydrobromides: X^-H_2-$^+$AA-NH$(CH_2)_2$SC(O)R

no.	AA	X⁻	R	yield, %	mp,[a] °C	formula[b]	¹H NMR: δ (solvent D_2O)
15	Gly	Br	CH_3	90	oil	$C_6H_{13}BrN_2O_2S$	2.35 (s, 3 H, acetyl CH_3), 3.05 (m, 2 H, SCH_2), 3.46 (m, 2 H, NCH_2), 3.78 (s, 2 H, Gly CH_2)
16	Gly	F_3CCO_2	CH_3	92	93–95	$C_8H_{13}F_3N_2O_4S$	2.29 (s, 3 H, acetyl CH_3), 2.98 (t, 2 H, SCH_2), 3.37 (t, 2 H, NCH_2), 3.70 (s, 2 H, Gly CH_2)
17	β-Ala	Br	CH_3	88	oil	$C_7H_{15}BrN_2O_2S$	2.35 (s, 3 H, acetyl CH_3), 2.66 (m, 2 H, β-Ala CH_2), 2.82–3.60 (m, 6 H, 2 NCH_2, SCH_2)
18	GABA	Br	CH_3	90	c		2.31 (s, 3 H, acetyl CH_3), 1.66–2.50 (m, 4 H, GABA CH_2CH_2), 2.76–3.17 and 3.17–3.47 (2 m, 6 H, 2 NCH_2, SCH_2)
19	L-Ala	Br	CH_3	92	c, d		1.50 (d, 3 H, L-Ala CH_3), 2.38 (s, 3 H, acetyl CH_3), 3.10 (m, 2 H, SCH_2), 3.50 (m, 2 H, NCH_2), 4.10 (m, 1 H, L-Ala CH)
20	L-Ala	F_3CCO_2	CH_3	90	oil[e]	$C_9H_{15}F_3N_2O_4S$	1.38 (d, 3 H, L-Ala CH_3), 2.31 (s, 3 H, acetyl CH_3), 2.98 (m, 2 H, SCH_2), 3.40 (m, 2 H, NCH_2), 3.95 (q, 1 H, L-Ala CH)
21	Gly	Br	C_2H_5	80	oil	$C_7H_{15}BrN_2O_2S$	1.08 (t, 3 H, propionyl CH_3), 2.62 (q, 2 H, propionyl CH_2), 3.03 (m, 2 H, SCH_2), 3.40 (m, 2 H, NCH_2), 3.73 (s, 2 H, Gly CH_2)
22	Gly	Br	$C(CH_3)_3$	78	oil	$C_9H_{19}BrN_2O_2S$	1.14 (s, 9 H, pivaloyl CH_3), 2.98 (m, 2 H, SCH_2), 3.38 (m, 2 H, NCH_2), 3.70 (s, 2 H, Gly CH_2)
23	Gly	F_3CCO_2	CH_2Cl	70	51–53	$C_8H_{12}ClF_3N_2O_4S$	2.97–3.66 (2 m, 4 H, NCH_2CH_2S), 3.75 (s, 2 H, Gly CH_2), 4.40 (s, 2 H, CH_2Cl)
24	Gly	F_3CCO_2	$CHCl_2$	75	c		3.0–3.68 (2 m, 4 H, NCH_2CH_2S), 4.07 (s, 2 H, Gly CH_2), 6.15 (s, 1 H, CH)
25	Gly	F_3CCO_2	CCl_3	85	131–133	$C_8H_{10}Cl_3F_3N_2O_4S$	3.24 (m, 2 H, SCH_2), 3.57 (m, 2 H, NCH_2), 4.11 (s, 2 H, Gly CH_2)

[a] All compounds were crystallized from an ethyl acetate/petroleum ether mixture. [b] All compounds gave satisfactory C, H, N analyses (±0.4%). [c] Hygroscopic compound. [d] $[\alpha]^{20}_D$ −5.2° (*c* 1.5, H_2O). [e] $[\alpha]^{20}_D$ −2.9° (*c* 1.4, H_2O).

Table III. Physical Properties of *N*-(Z-aminoalkanoyl)cysteamines: Z-AA-NH$(CH_2)_2$SH

no.	Z-AA	yield, %	mp,[a] °C	R_f (solvent[b])	formula[c]	IR (KBr): ν, cm⁻¹ NH	C=O	CNH	¹H NMR: δ (solvent $CDCl_3$)
26	Gly	85	95–96	0.5	$C_{12}H_{16}N_2O_3S$	3320	1680, 3280 1650	1540	1.38 (t, 1 H, SH), 2.62 (m, 2 H, SCH_2), 3.40 (m, 2 H, NCH_2), 3.86 (d, 2 H, Gly CH_2), 5.15 (s, 2 H, benzylic CH_2), 5.86 (m, 1 H, Gly NH), 6.83 (m, 1 H, cysteamine NH), 7.36 (s, 5 H, aromatic)
27	β-Ala	40	120–122	0.7	$C_{13}H_{18}N_2O_3S$	3290	1680, 1645	1540	1.36 (t, 1 H, SH), 2.40 (m, 2 H, β-Ala CH_2), 2.93 (m, 2 H, SCH_2), 3.46 (m, 4 H, 2 NCH_2), 5.09 (s, 2 H, benzylic CH_2), 5.70 and 6.45 (2 m, 2 H, 2 NH), 7.31 (s, 5 H, aromatic)
28	GABA	60	90–93	0.6	$C_{14}H_{20}N_2O_3S$	3300	1680, 1635	1540	1.38 (t, 1 H, SH), 1.55–2.30 (m, 4 H, GABA CH_2CH_2), 2.70–3.58 (m, 6 H, 2 NCH_2, SCH_2), 5.10 (s, 2 H, benzylic CH_2), 5.40 and 6.50 (2 m, 2 H, 2 NH), 7.30 (s, 5 H, aromatic)
29	L-Ala	60	114–116	0.85	$C_{13}H_{18}N_2O_3S$	3280	1680, 1640	1530	1.37 (t, 1 H, SH), 1.36 (d, 3 H, L-Ala CH_3), 2.60 (m, 2 H, SCH_2), 3.40 (q, 2 H, NCH_2), 4.26 (m, 1 H, L-Ala CH), 5.13 (s, 2 H, benzylic CH_2), 5.77 (d, 1 H, L-Ala NH), 6.92 (m, 1 H, cysteamine NH), 7.35 (s, 5 H, aromatic)

[a] All compounds were crystallized from an ethyl acetate/petroleum ether mixture. [b] $CHCl_3$/MeOH, 9:1. [c] All compounds gave satisfactory C, H, N analyses (±0.4%).

rivative is less toxic but less active than WR 2721 (LD_{50} 950 mg/kg, DRF 2.7). However, its stability is excellent. No changes were observed in buffered solutions up to 26 days at pH 3.8, 7.2, or 9.0, as monitored by ¹H NMR spectroscopy. Under these conditions, WR 2721 has been partially dephosphorylated.[10] Further experiments with **16** have shown that it exhibits the same selectivity in cellular response as the reference product 1. With EMT6 tumors the same DRF as WR 2721 was observed based on a 60-min time interval, and on a 20-min basis the same gain factor was again obtained.[20] Furthermore, preliminary data suggest that **16** also possesses excellent DNA affinity.

Due to the potential practical importance of such compounds as adjuvant drugs in radio- and chemotherapy, various studies are in progress and will be reported elsewhere. Additional synthetic work is also in progress to study further the influence of amino acid conjugation on cysteamine derivatives with the aim of improving the biological response to such promising drugs.

It is important to note that little or no radioprotective activity was observed when compounds were injected 2 h before irradiation or when the dose was equal to $LD_{50/8}$.

Experimental Section

Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Le Service Central d'Analyse du CNRS (Vernaison, France). IR spectra were determined on a Beckman Acculab 4 spectrophotometer. Proton nuclear magnetic resonance spectra were recorded on a Varian EM 390 and are expressed as δ relative to tetramethylsilane as internal standard. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel GF 254 plates. Spots were visualized by UV, iodine vapor, or ninhydrin spray. Column chromatography was conducted with Merck silica gel, 60–230 mesh, ASTM.

General Method of Coupling Involving a (Benzyloxycarbonyl)amino Acid and *S*-Acylcysteamine. *N*-[(Benzyloxycarbonyl)glycyl]-*S*-acetylcysteamine (4). A solution of (benzyloxycarbonyl)glycine (4.598 g, 22 mmol) in tetrahydrofuran (THF; 80 mL) was stirred at 0 °C with phosphonitrilic chloride (*t*-PNC; 3.88 g, 11 mmol) [*N,N'*-dicyclohexylcarbodiimide (DCC) could be also used] previously dissolved in THF (20 mL) or ethyl acetate. After a 30-min stirring at 0 °C, triethylamine (TEA; 2.4 mL) was added and the mixture was again left stirring for 30-min. After this time, a solution of *S*-acetylcysteamine hydrochloride [21] (3.421 g, 22 mmol) in THF (50 mL) or ethyl

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Table IV. Physical Properties of N,N'-Bis[[(Z or Boc)amino]alkanoyl]cystamines: $(R\text{-}AA\text{-}NH(CH_2)_2S\text{-})_2$

no.	AA	R	yield, % (method[a-c])	mp,[d] °C	R_f (solvent[e])	formula[f]	IR (KBr): ν, cm^{-1} NH	C=O	CNH	^1H NMR: δ (solvent g or h)
30	Gly	Z	40 (a) 34 (b) 65 (c)	168–170 (DMF/H_2O)	0.6	$C_{24}H_{30}N_4O_6S_2$	3320	1680 1635	1535	(g) 2.58–3.10 and 3.10–3.50 (2 m, 8 H, NCH_2CH_2S), 3.62 (d, 4 H, Gly CH_2), 5.05 (s, 4 H, benzylic CH_2), 7.38 (s, 10 H, aromatic), 7.44 and 8.12 (2 m, 4 H, NH)
31	Gly	Boc	37 (b) 60 (c)	97–98 (ethyl acetate)	0.4	$C_{18}H_{34}N_4O_6S_2$	3360	1700 1660	1535	(h) 1.42 (s, 18 H, t-Bu), 2.77 (t, 4 H, SCH_2), 3.55 (m, 4 H, NCH_2), 3.82 (d, 4 H, Gly CH_2), 7.24 (m, 4 H, NH)
32	β-Ala	Z	65 (a)	177 (methanol)	0.5	$C_{26}H_{34}N_4O_6S_2$	3320 3300	1670 1625	1525	(h) 2.36–3.40 and 3.40–4.0 (4 m, 16 H, β-Ala CH_2, SCH_2, NCH_2), 5.10 (s, 4 H, benzylic CH_2), 7.31 (s, 10 H, aromatic), 6.10 and 7.35 (2 m, 4 H, NH)
33	GABA	Z	66 (a)	162–164 (methanol)	0.75	$C_{28}H_{38}N_4O_6S_2$	3320 3280	1680 1635	1525	(g) 1.33–2.35 (m, 8 H, GABA CH_2CH_2), 2.60–3.70 (m, 12 H, NCH_2, SCH_2), 5.03 (s, 4 H, benzylic CH_2), 7.40 (s, 10 H, aromatic), 7.26 and 8.05 (2 t, 4 H, NH)
34	L-Ala	Z	65 (a)	145–147 (ethyl acetate/petroleum ether)	0.8	$C_{26}H_{34}N_4O_6S_2$	3295	1680 1640	1535	(h) 1.33 (d, 6 H, L-Ala CH_3), 2.73 (t, 4 H, SCH_2), 3.50 (q, 4 H, NCH_2), 4.31 (m, 2 H, L-Ala CH), 5.10 (s, 4 H, benzylic CH_2), 6.0 (d, 2 H, L-Ala NH), 7.20 (t, 2 H, cystamine NH), 7.32 (s, 10 H, aromatic)
35	L-Ala	Boc	91 (c)	109–111 (acetone/hexane)	0.5	$C_{20}H_{38}N_4O_6S_2$	3340	1690 1660	1525	(h) 1.30 (d, 6 H, L-Ala CH_3), 1.40 (s, 18 H, t-Bu), 2.79 (m, 4 H, SCH_2), 3.49 (m, 4 H, NCH_2), 4.20 (m, 2 H, L-Ala CH), 5.68 (d, 2 H, L-Ala NH), 7.36 (t, 2 H, cystamine NH)

[a] Oxidation of corresponding N-(aminoalkanoyl)cysteamine derivatives. [b] Condensation between the Z- or Boc-amino acid and free cystamine with phosphonitrilic chloride (t-PNC) or N,N'-dicyclohexylcarbodiimide (DCC) as activating agent. [c] Condensation between the Z- or Boc-amino acid succinimide ester and cystamine hydrochloride. [d] Crystallization solvent. [e] $CHCl_3$/MeOH, 9:1. [f] All compounds gave satisfactory C, H, N analyses (±0.4%). [g] $Me_2SO\text{-}d_6$. [h] $CDCl_3$.

Table V. Physical Properties of N,N'-Bis(aminoalkanoyl)cystamine Dihydrobromides or Bis(trifluoroacetates): $(X^-H_2\text{-}^+AA\text{-}NH(CH_2)_2S\text{-})_2$

no.	AA	X^-	yield, %	mp, °C	formula[a]	^1H NMR: δ (solvent D_2O)
36	Gly	Br	85	183–185[b]	$C_8H_{20}Br_2N_4O_2S_2$	2.83 (t, 4 H, SCH_2), 3.55 (t, 4 H, NCH_2), 3.78 (s, 4 H, Gly CH_2)
37[f]	Gly	F_3CCO_2	98	oil	$C_{12}H_{20}F_6N_4O_6S_2$	2.66 (t, 4 H, SCH_2), 3.38 (t, 4 H, NCH_2), 3.61 (s, 4 H, Gly CH_2)
38[f]	β-Ala	Br	89	c		2.50–3.0 and 3.0–3.55 (2 × 2 m, 16 H, β-Ala CH_2, SCH_2, NCH_2)
39	GABA	Br	85	c		1.55–2.45 (m, 8 H, GABA CH_2CH_2), 2.45–3.53 (2 m, 12 H, NCH_2, SCH_2)
40	L-Ala	Br	85	c, d		1.52 (d, 6 H, L-Ala CH_3), 2.90 (t, 4 H, SCH_2), 3.60 (m, 4 H, NCH_2), 4.10 (q, 2 H, L-Ala CH)
41	L-Ala	F_3CCO_2	90	oil[e]	$C_{14}H_{24}F_6N_4O_6S_2$	1.46 (d, 6 H, L-Ala CH_3), 2.76 (t, 4 H, SCH_2), 3.48 (m, 4 H, NCH_2), 3.98 (m, 2 H, L-Ala CH)

[a] All compounds gave satisfactory C, H, N analyses (±0.4%). [b] This compound was crystallized from a methanol/ether mixture. [c] Hygroscopic compound. [d] $[\alpha]^{20}_D$ +13.3° (c 1.5, H_2O). [e] $[\alpha]^{20}_D$ +20.1° (c 1.3, H_2O). [f] Compounds **37** and **38** have been previously obtained by other methods as hydrochlorides.[17,18]

acetate was added. Stirring was maintained at 0 °C for 30 min, and the mixture was then allowed to return to room temperature, the basic pH being maintained by addition of TEA. The reaction was followed by TLC in a chloroform/methanol (9:1) eluent, and the time needed for coupling was approximately 2–3 h.

The mixture was evaporated to dryness under reduced pressure and then taken up in ethyl acetate (500 mL), and the solution was washed as follows: ice-cold saturated aqueous sodium bicarbonate, water, ice-cold 5% aqueous hydrochloric acid, water (neutral pH). The organic phase was dried over sodium sulfate and evaporated to dryness under vacuum. A pale yellow oil (5 g) was collected. The crude product was purified by chromatography on a silica gel column (eluent ethyl acetate/petroleum ether, 8:2) and was crystallized from ethyl acetate: yield 45%; mp 100–103 °C; R_f (dichloromethane/methanol, 9:1) 0.5.

Yields, physical characteristics, and spectroscopic features of N-[[(benzyloxycarbonyl)amino]alkanoyl]-S-acylcysteamines **4**, **6–8**, **10**, and **11** are recorded in Table I.

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General Method of Coupling Involving a (*tert*-Butyloxycarbonyl)amino Acid or (*tert*-Butyloxycarbonyl)amino Acid Active Ester and *S*-Acylcysteamine. *N*-[(*tert*-Butyloxycarbonyl)glycyl]-*S*-acetylcysteamine (5). Method 1. A solution of (*tert*-butyloxycarbonyl)glycine (9 g, 50 mmol) in ethyl acetate (150 mL) was stirred at 0 °C with t-PNC (18 g, 50 mmol) (DCC could be also used) in ethyl acetate (100 mL). After a 30-min stirring at 0 °C, TEA (7.25 mL) was added and the mixture was left stirring again for 15 min. After this time, S-acetylcysteamine hydrochloride (9.1 g, 50 mmol) was added to the mixture, followed by the dropwise addition of TEA (11 mL). The reaction, followed by TLC in a dichloromethane/methanol (9:1) mixture, was finished in 5 h.

The reaction mixture was then washed with water, ice-cold saturated aqueous sodium bicarbonate, water, ice-cold 1 N aqueous citric acid solution, and water (neutral pH). The organic phase was dried over sodium sulfate and evaporated to dryness under vacuum. The crude product was obtained in the form of an oil, 12.5 g. This product was purified by chromatography on a silica gel column (eluent dichloromethane/methanol, 9.4:0.6) and was crystallized from dichloromethane/petroleum ether: yield 40%; mp 59–60 °C; R_f (dichloromethane/methanol, 9:1) 0.8.

Table VI. Toxicity (LD_{50}) and Radioprotective Activity of Compounds Intraperitoneally Injected with a Dose Equal to Half or One-Eighth of Their LD_{50}, 15 min or 2 h before Whole-Body Irradiation

compd	LD_{50}, mg/kg	DRF obtained with 1/2 LD_{50} 15 min before irrad	DRF obtained with 1/2 LD_{50} 2 h before irrad	DRF obtained with 1/8 LD_{50} 15 min before irrad
WR 2721	950	2.7	>1.3	1.5
HCl, $H_2N(CH_2)_2SCOCH_3$	500	1.05	1.05	1
15	1500	1.4	1	1
16	1500	1.4	1	1
17	400	1	1	1
18	800	1.1		1
19	1500	1.4	1.2	1
20	1250	1.4	1	1
21	1500	1.2	1	1
22	1200	1.05	1	1
23	550	1	1	1
24	800	1.1	1	1
25	700	1.05	1	1
36	1500	1.4	1	1
37	1500	1.55	1.1	1.2
38	350	1	1	1
39	300	1	1	1
40	800	1	1	1
41	1200	1.4	1.1	1
43	600	1.1	1	1
45	1000	1.2	1	1

Yields, physical characteristics, and spectroscopic features of *N*-[[(*tert*-butyloxycarbonyl)amino]alkanoyl]-*S*-acylcysteamines **5**, **9**, **12–14** are recorded in Table I.

Method 2. A solution of succinimido (*tert*-butyloxycarbonyl)glycinate (6.8 g, 25 mmol) in acetonitrile (100 mL) was stirred at 0 °C with *S*-acetylcysteamine hydrochloride (3.88 g, 25 mmol), and a solution of TEA (3.48 mL, 25 mmol) in acetonitrile (10 mL) was added dropwise. After the addition was complete, the reaction mixture was stirred for 3 h at 0 and 20 °C for an additional 12 h. *N*-Hydroxysuccinimide was removed by filtration, and the filtrate was evaporated to dryness. The residue was dissolved in dichloromethane (300 mL) and washed with water, ice-cold saturated aqueous sodium bicarbonate, water, ice-cold 1 N aqueous citric acid solution, and water (neutral pH). The organic phase was dried over sodium sulfate and evaporated to dryness under vacuum. The crude product was purified by chromatography on a silica gel column (eluent ethyl acetate/petroleum ether, 7:3) and was crystallized; yield 48% (physicochemical criteria identical with those above).

General Method for Deprotecting the Amine with Formation of the Hydrobromide or Trifluoroacetate. Removal of the Benzyloxycarbonyl Protecting Group. *N*-Glycyl-*S*-acetylcysteamine Hydrobromide (15). A mixture of *N*-[(benzyloxycarbonyl)glycyl]-*S*-acetylcysteamine (**4**; 1.49 g, 4.8 mmol) and glacial acetic acid (AcOH; 4 mL) saturated with hydrobromic acid (HBr) was stirred at a temperature below -5 °C under nitrogen, protecting the mixture from the light. The reaction time was 30 min. The hydrobromide was precipitated from the mixture by adding ice-cold anhydrous ether (80 mL), and the ether phase was then decanted. This procedure of washing with ether was performed five times. The residual oil was then taken up in distilled water (30 mL) and lyophilized. The expected derivative was collected in 90% yield in the form of a colorless, very hygroscopic paste. It must be stored under nitrogen or under vacuum.

Yields, physical characteristics, and spectroscopic features of *N*-(aminoalkanoyl)-*S*-acylcysteamine hydrobromides **15**, **17–19**, **21** and **22** are recorded in Table II.

Removal of the *tert*-Butyloxycarbonyl Protecting Group. *N*-Glycyl-*S*-acetylcysteamine Trifluoroacetate (16). A solution of *N*-[(*tert*-butyloxycarbonyl)glycyl]-*S*-acetylcysteamine (**5**; 2.03 g, 7 mmol) was stirred at room temperature with trifluoroacetic acid (TFA; 10 mL) while being protected from moisture. The reaction, followed by TLC, was finished in 4 h. The trifluoroacetate was precipitated from the mixture in the form of an oil by adding anhydrous ether (150 mL), washed with ether (150 mL, two times), and then dried in a vacuum desiccator containing phosphoric anhydride and potasium hydroxide flakes. The yield was almost quantitative. Additional purification was obtained by dissolving the salt in distilled water (10 mL), followed by lyophilization.

Since they are hygroscopic, these salts are generally stored under vacuum or nitrogen. Nevertheless, this particular compound was crystallized from ethyl acetate/petroleum ether (3:1): mp 93–95 °C; IR (KBr, cm^{-1}) several bands indicating the presence of an amine salt, 3340, 3220, 3080, 2995, 2960, 2800, 2740, 2620, 2540; 1700, 1670 (C=O); 1590 (amide).

Yields, physical characteristics, and spectroscopic features of *N*-(aminoalkanoyl)-*S*-acylcysteamine trifluoroacetates **16**, **20** and **23–25** are recorded in Table II.

General Method for the Synthesis of *N*-[[(Benzyloxycarbonyl)amino]alkanoyl]cysteamine. *N*-[(Benzyloxycarbonyl)glycyl]cysteamine (26). A solution of *N*-[(benzyloxycarbonyl)glycyl]-*S*-acetylcysteamine (**4**; 2.5 g, 8 mmol) in methanol (40 mL) was treated with sodium methylate [sodium (0.263 g) in methanol (5 mL)]. The reaction mixture was stirred at room temperature for 1 h and then brought to pH 1.2 with concentrated hydrochloric acid. The solution was evaporated to dryness under vacuum, and the paste obtained was chromatographed on silica gel column (eluent dichloromethane containing 5% of methanol).

The colorless oil (1.5 g) was collected and was crystallized from ethyl acetate/petroleum ether: mp 95–96 °C; R_f (chloroform/methanol, 9:1) 0.5.

Yields, physical characteristics, and spectroscopic features of *N*-[[(benzyloxycarbonyl)amino]alkanoyl]cysteamines **26–29** are recorded in Table III.

General Methods for the Synthesis of *N*,*N*′-Bis[[(benzyloxycarbonyl- or -(*tert*-butyloxycarbonyl)amino]alkanoyl]cystamine (Three Methods of Synthesis). Method 1 [(a) Table IV]. Oxidation of *N*-[[(Benzyloxycarbonyl)amino]alkanoyl]cysteamine. *N*,*N*′-Bis[(benzyloxycarbonyl)glycyl]cystamine (30). To a stirred solution of *N*-[(benzyloxycarbonyl)glycyl]cysteamine (**26**; 3.6 g, 13 mmol) in acetic acid (15 mL) and distilled water (15 mL) at room temperature was added acetic acid (20 mL) containing iodine (250 mg). An immediate precipitate formed. The reaction mixture was maintained for 15 min at room temperature, and the precipitate was filtered, washed with distilled water, and dried in a vacuum desiccator over phosphoric anhydride. The product was crystallized from a mixture of DMF and distilled water. The collected crystals were washed with a v/v mixture of ethanol and ether and then with ether. After drying under vacuum over phosphoric anhydride, the expected derivative (**30**) was collected: yield 40%; mp 168–170 °C; R_f (chloroform/methanol, 9:1) 0.5.

Yields, physical characteristics, and spectroscopic features of *N*,*N*′-bis[[(benzyloxycarbonyl)amino]alkanoyl]cystamines **30** and **32–34** are recorded in Table IV.

Method 2 [(b) Table IV]. Reaction between (Benzyloxycarbonyl)- or (*tert*-Butyloxycarbonyl)amino Acid and Cystamine Involving a Coupling Agent According to the General Method Already Described. Synthesis of 30. Reagents used: (benzyloxycarbonyl)glycine (7.2 g, 34 mmol) in ethyl acetate (150 mL); *t*-PNC (12 g, 34 mmol) in ethyl acetate (50 mL) (DCC could be also used); TEA (5.10 mL); free cystamine (3.04, 20 mmol) in ethyl acetate (30 mL). On addition of the cystamine, a precipitate formed immediately and the reaction mixture was stirred at room temperature for 6 h. The precipitate was collected, washed with ethyl acetate (100 mL) and water (100 mL), and recrystallized at approximately 80 °C from DMF containing 10% of distilled water. The product, after filtration and drying in a vacuum desiccator over phosphoric anhydride, was collected; yield 34% (physicochemical criteria identical with those above).

Yield, physical characteristics, and spectroscopic features of *N,N'*-bis[(*tert*-butyloxycarbonyl)glycyl]cystamine (31) are recorded in Table IV.

Method 3 [(c) Table IV]. General Method of Coupling Involving an Activated Ester of an N-Protected Amino Acid and an Amine. Synthesis of 30. To a cold (0 °C) stirred solution of cystamine hydrochloride (2.25 g, 10 mmol) in DMF (20 mL) was added dropwise TEA (1.70 mL). After the addition of the base (15 min), succinimido (benzyloxycarbonyl)glycinate (3.06 g, 20 mmol) in DMF (30 mL) was added to the mixture at 0 °C, and the mixture was allowed to return to room temperature. The reaction, followed by TLC (eluent chloroform containing 10% of methanol), was finished in 6 h.

The solvent was evaporated to dryness under vacuum. The residual paste was stirred in ethyl acetate, inducing formation of a colorless powder that was filtered and washed with distilled water. The residue was crystallized as above. After filtration and drying, 3.5 g of a product was collected having physicochemical criteria identical with those above.

Yield, physical characteristics, and spectroscopic features of *N,N'*-bis[[(*tert*-butyloxycarbonyl)amino]alkanoyl]cystamines 31 and 35 are recorded in Table IV.

***N,N'*-Bis(aminoalkanoyl)cystamine Dihydrobromide or Bis(trifluoroacetate).** Reactions were performed according to the general methods described for deprotecting the amines with formation of dihydrobromide or bis(trifluoroacetate).

Yields, physical characteristics, and spectroscopic features of *N,N'*-bis(aminoalkanoyl)cystamine dihydrobromides 36 and 38–40 or bis(trifluoroacetates) 37 and 41 are recorded in Table V.

***S*-[(Benzyloxycarbonyl)glycyl]-*N*-acetylcysteamine (42).** A suspension of *S*-acetylcysteamine hydrochloride (1.24 g, 8 mmol) in DMF (20 mL) was stirred, and diisopropylethylamine (DIEA; 1.72 mL) was added. The solution was then cooled to 0 °C and succinimido (benzyloxycarbonyl)glycinate (1.53 g, 5 mmol) was added with stirring. Stirring was continued at 0 °C for 3 h and at 20 °C for 10 h. The mixture was evaporated to dryness under reduced pressure and then taken up in ethyl acetate and washed as follows: water, ice-cold 5% strength aqueous hydrochloric acid, water, ice-cold saturated aqueous sodium bicarbonate, and water (neutral pH). The organic phase was dried over sodium sulfate and evaporated to dryness. The crude product was crystallized from ethyl acetate/petroleum ether: yield 65%; mp 98–100 °C; R_f (dichloromethane/methanol, 9:1) 0.4; IR (KBr cm^{-1}) 3320, 3250 (NH); 1670, 1655, 1625 (C=O); 1540, 1520 (thioamide and amide I); 750, 690 (benzyl); ^{1}H NMR ($CDCl_3$) δ 7.30 (s, 5 H, aromatic), 6.08 and 5.72 (2 m, 2 H, NH), 5.12 (s, 2 H, benzylic CH_2), 4.06 (d, 2 H, Gly CH_2), 3.60–2.85 (m, 4 H, NCH_2CH_2S), 1.90 (s, 3 H, acetyl CH_3). Anal. ($C_{14}H_{18}N_2O_4S$) C, H, N.

***S*-Glycyl-*N*-acetylcysteamine Hydrobromide (43).** 43 was prepared according to the general method described for deprotecting the amine with formation of hydrobromide. Reagents used: *S*-[(benzyloxycarbonyl)glycyl]-*N*-acetylcysteamine (42; 1.49 g, 4.8 mmol); AcOH saturated with HBr (6 mL).

The reaction, followed by TLC, was finished in 30 min. The hydrobromide precipitated from the mixture on addition of anhydrous ether (80 mL). The crude product was collected by filtration, washed with anhydrous ether, and dried under vacuum over phosphoric anhydride: yield 90%; mp 170–172 °C; ^{1}H NMR (D_2O) δ 4.20 (s, 2 H, Gly CH_2), 3.50–2.90 (m, 4 H, NCH_2CH_2S), 1.90 (s, 3 H, acetyl CH_3). Anal. ($C_6H_{13}BrN_2O_2S$) C, H, N.

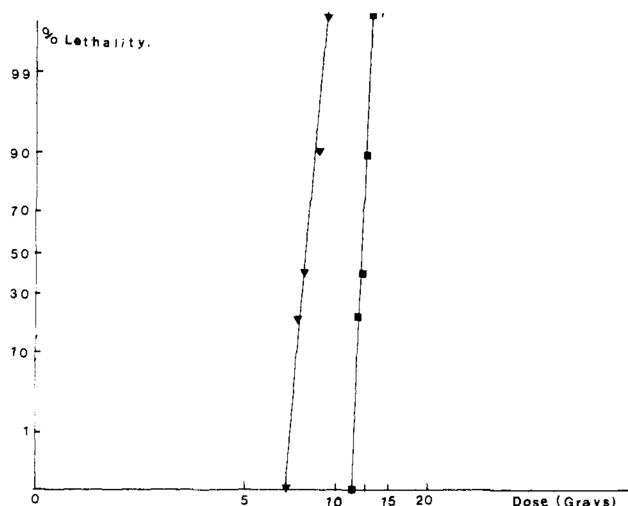

Figure 1. Lethality curves (log–probit coordinates): ▼, control mice; ■, mice receiving an intraperitoneal injection of 750 mg/kg of compound 37, 15 min before irradiation. The graphically determined irradiation LD_{50} values are, respectively, equal to 8.15 and 12.6 Gy.

***S*-[(*tert*-Butyloxycarbonyl)glycyl]-*N*-acetylcysteamine (44).** To a cold (0 °C) stirred solution of (*tert*-butyloxycarbonyl)glycine (7 g, 40 mmol) in DMF (60 mL) were added DCC (8.24 g, 40 mmol), *S*-acetylcysteamine hydrochloride (6.22 g, 40 mmol), and dropwise TEA (5.56 mL) in DMF (20 mL). The mixture was allowed to return to room temperature, and stirring was continued for 2 h. The resulting precipitate of *N,N'*-dicyclohexylurea was filtered, and the filtrate was concentrated to dryness under vacuum. The residual paste was dissolved in ethyl acetate and washed with water, ice-cold saturated aqueous sodium bicarbonate, water, 1 N aqueous citric acid, and water (neutral pH). The organic phase was dried over sodium sulfate and evaporated to dryness under vacuum. TLC (eluent ethyl acetate) showed two spots with R_f 0.4 and 0.25. The two products were separated by chromatography on a silica gel column (eluent dichloromethane containing 2% of methanol). The less polar compound (R_f 0.4; 3 g) was isolated and identified as *N*-[(*tert*-butyloxycarbonyl)glycyl]-*S*-acetylcysteamine (5).

The more polar compound (R_f 0.25) was collected as an oil (2.55 g) and crystallized from ethyl acetate and petroleum ether mixture: mp 120 °C; IR (KBr, cm^{-1}) 3340, 3260 (NH); 3060, 3000, 2960 (CH, CH_2, CH_3); 1695, 1660 (C=O); 1550 (amide I); ^{1}H NMR ($CDCl_3$) δ 7.22 and 6.28 (2 m, 2 H, NH), 4.14 (d, 2 H, Gly CH_2), 3.48 (m, 2 H, NCH_2), 3.26 (m, 2 H, SCH_2), 2.10 (s, 3 H, acetyl CH_3), 1.58 (s, 9 H, *t*-Bu). Anal. ($C_{11}H_{20}N_2O_4S$) C, H, N.

***S*-Glycyl-*N*-acetylcysteamine Trifluorocetate (45).** 45 was prepared according to the general method already described for deprotecting the amine with formation of trifluoroacetate. Reagents used: *S*-[(*tert*-butyloxycarbonyl)glycyl]-*N*-acetylcysteamine (44; 2.072 g, 7.5 mmol); TFA (3 mL).

The reaction, followed by TLC, was finished in 2 h. After it was washed with anhydrous ether, the product was taken up in distilled water and lyophilized: 2.1 g of a yellow oil collected; yield 96%; ^{1}H NMR (D_2O) δ 4.12 (s, 2 H, Gly CH_2), 3.58–2.83 (m, 4 H, SCH_2CH_2N), 1.92 (s, 3 H, acetyl CH_3).

Radioprotective Evaluation

(1) Three-month-old albino CXVII mice were used. This inbred strain was obtained from the Institut Curie (Paris, France). Their mean weight was about 25 g.

(2) The radioprotective effect of compounds was evaluated by determining the dose reduction factor (DRF), defined as the ratio of irradiation LD_{50}/30 days of injected mice to that of control mice. Initially the survival rate was determined 30 days after irradiation in different groups of 20 mice receiving an intraperitoneal (i.p.), injection of the test compound with a dose equal to half or one-eighth of its LD_{50} 15 min or 2 h before whole-body irradiation delivered with a dose equal to the LD_{100}/30 days of control mice (9 Gy for males and 9.5 Gy for females), or with a dose equal to this dose + 2 Gy. Where necessary, other irradiation

Table VII[a]

irrad dose, Gy	N	deaths (no.)	p (death)	q (survival)	$p{\cdot}q$
9.5	20	0	0	1	0
11.5	20	0	0	1	0
12	20	4	0.2	0.8	0.16
12.5	20	8	0.4	0.6	0.24
13	20	18	0.9	0.1	0.09
13.5	20	20	1	0	0
			$\sum p = 2.5$		$\sum p{\cdot}q = 0.49$

[a] LD_{50} = 13.5–0.5(2.5–0.5) = 12.5 Gy. 13.5 Gy = lowest irradiation dose giving 100% lethality. 0.5 Gy = difference between two consecutive doses. $2.5 = \sum p$. $\sigma = (0.5^2 0.49/19)^{1/2} = 0.08$. $0.49 = \sum p{\cdot}q$. $19 = N - 1$. $LD_{50} = 12.5 \pm 0.16$ Gy $\simeq 12.5 \pm 0.2$ Gy ($p < 0.05$). LD_{50} control mice = 8.1 ± 0.2 Gy ($p < 0.05$). DRF = $(12.5 \pm 0.2)/(8.1 \pm 0.2) = 1.54 \pm 0.07 \cong 1.55 \pm 0.1$ ($p < 0.05$).

doses were tested in order to evaluate the irradiation LD_{50} of protected mice by the Karber method (calculated or graphic).[22]

An example is given for compound **37** in Appendix 1 and Figure 1.

The radiosensitivity of the strain was regularly monitored by the determination of lethality curves of males and females. The $LD_{50}/30$ days was equal to 7.7 ± 0.3 Gy for males and 8.1 ± 0.2 Gy for females ($P < 0.05$).

Under these conditions significant protection was observed with a DRF value superior to 1.15.

(3) All the compounds were easily dissolved in distilled water. The toxicity was evaluated by a Probit analysis of the LD_{50}, the dose range being determined in a preliminary study. Five groups of 10 mice were then injected with different doses within this range.

(4) Whole-body irradiation were performed with a ^{60}Co γ-ray source (6×10^{13} Bq). The dose rate was equal to 0.65 Gy/min. For the exposure, mice were positioned inside an altuglass box divided into 30 cells in a homogeneous field 28.5 cm × 28.5 cm. The dosimetry was carried out by means of ionization chamber dosimeters and lithium fluoride thermoluminescent dosimeters.

Each irradiation session included a group of 20 mice irradiated with 9 or 9.5 Gy according to their sex after intraperitoneal injection of the solvent alone. A 100% lethality was always observed with a mean survival time equal to 11 ± 1 days. Furthermore, a group of eight unirradiated mice received the test compound with a dose equal to half of its LD_{50}, in order to check for toxic lethality among the injected and irradiated mice. For all the compounds, these animals were alive 30 days after injection.

Remarks

At first sight, injection of equimolecular doses of compounds might seem preferable. However, this would lead to early deaths for the more toxic compounds or to poor protection for the less toxic ones, since radioprotection generally decreases rapidly with decreasing doses.

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Appendix

The determination of irradiation $LD_{50}/30$ days and DRF in mice receiving an ip injection of 750 mg/kg of **37**, 15 min before irradiation, calculated by the Karber method, is given in Table VII.

Registry No. **4**, 97313-99-0; **5**, 97314-04-0; **6**, 89052-99-3; **7**, 97314-02-8; **8**, 97314-00-6; **9**, 97314-14-2; **10**, 104071-71-8; **11**, 104071-72-9; **12**, 104071-73-0; **13**, 97314-06-2; **14**, 104071-74-1; **15**, 97313-68-3; **15** (free base), 97313-69-4; **16**, 97314-05-1; **17**, 104071-75-2; **17** (free base), 104071-91-2; **18**, 97314-03-9; **18** (free base), 97313-71-8; **19**, 97314-01-7; **19** (free base), 97313-70-7; **20**, 97314-16-4; **21**, 104071-76-3; **21** (free base), 104071-92-3; **22**, 104071-77-4; **22** (free base), 104071-93-4; **23**, 104071-79-6; **23** (free base), 104071-78-5; **24**, 97314-07-3; **24** (free base), 97313-72-9; **25**, 104071-81-0; **25** (free base), 104071-80-9; **26**, 97314-08-4; **27**, 104071-82-1; **28**, 104071-83-2; **29**, 97313-97-8; **30**, 97314-09-5; **31**, 97314-11-9; **32**, 104071-84-3; **33**, 104071-85-4; **34**, 104071-86-5; **35**, 104089-97-6; **36**, 97314-10-8; **36** (free base), 31060-88-5; **37**, 97314-12-0; **38**, 104071-87-6; **38** (free base), 646-08-2; **39**, 104071-88-7; **39** (free base), 73321-59-2; **40**, 97314-13-1; **40** (free base), 97313-79-6; **41**, 97313-95-6; **42**, 104071-89-8; **43**, 104071-90-1; **43** (free base), 72235-32-6; **44**, 97313-83-2; **45**, 97313-84-3; Boc-Ala-OSu, 3392-05-0; Z-β-Ala-OH, 2304-94-1; Z-GABA-OH, 5105-78-2; Z-L-Ala-OH, 1142-20-7; $H_2N(CH_2)_2SCOEt{\cdot}HCl$, 104071-94-5; $H_2N(CH_2)_2SCOBu$-*t*$\cdot$HCl, 104071-95-6; Boc-Ala-OH, 15761-38-3; $H_2N(CH_2)_2SCOCH_2Cl{\cdot}HCl$, 90587-64-7; $H_2N(CH_2)_2SCOCHCl_2{\cdot}HCl$, 97313-96-7; $H_2N(CH_2)_2SCOCCl_3{\cdot}HCl$, 104071-96-7; (benzyloxycarbonyl)glycine, 1138-80-3; *S*-acetylcysteamine hydrochloride, 17612-91-8; (*tert*-butyloxycarbonyl)glycine, 4530-20-5; succinimido (*tert*-butyloxycarbonyl)glycinate, 3392-07-2; cystamine, 51-85-4; cystamine hydrochloride, 1072-22-6; succinimido (benzyloxycarbonyl)glycinate, 2899-60-7.

In the Search for New Anticancer Drugs. 19. A Predictive Design of *N,N:N′,N′:N″,N″*-Tri-1,2-ethanediylphosphoric Triamide (TEPA) Analogues

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The nitroxyl-labeled analogues of *N,N:N′,N′:N″,N″*-tri-1,2-ethanediylphosphoric triamide (TEPA), *N,N:N′,N′*-bis(1,2-ethanediyl)-*N″*-[[(2,2,6,6-tetramethyl-1-oxypiperidin-4-yl)amino]carbonyl]phosphoric triamide (**5a**) and *N,N:N′,N′*-bis(1,2-ethanediyl)-*N″*-[[(2,2,5,5-tetramethyl-1-oxypyrrolidin-3-yl)amino]carbonyl]phosphoric triamide (**11a**), possess therapeutic indexes that are 8–12 times higher than those of thio-TEPA (**1**) and TEPA (**2**). The introduction of methyl groups into the aziridine ring, or the replacement of the nitroxyl moiety with hydroxylamine or amine derivatives, or with an adamantane moiety, results in compounds of lesser activity. An attempt is made to rationalize these results using a lipophilicity scale. A predictive design pattern is established.

N,N:N′,N′:N″,N″-Tri-1,2-ethanediylphosphorothioic triamide (thio-TEPA) (**1**) was introduced[1] more than 30 years ago into clinical oncology, and it has been applied in Hodgkin's disease,[2] metastatic carcinoma of the breast,[3,4] superficial carcinoma of the bladder,[5-9] carcinomatous meningitis,[10,11] and ovarian cancer.[12] TEPA (**2**), which

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