calculated. A standard curve of histidylleucine was always prepared with each assay.

Activity was designated in terms of the IC_{50}, which was the molar concentration of test inhibitor causing 50% inhibition of the control converting enzyme activity.

Inhibitory Effect on Angiotensin I Induced Pressor Response in Anesthetized Rats. Male Wistar slc rats, weighing 300–400 g, were used after fasting for 18–20 h. Under urethane anesthesia (1.2 g/kg, sc) the arterial cannula inserted into the left carotid was connected to a pressure transducer (Nihon Kohden, MPU-0.5), and the blood pressure was recorded by carrier amplifier (Nihon Kohden, RP-3 and RM-150). Angiotensin I (300 ng/kg) dissolved in 0.9% physiological saline was injected through a cannula which had been inserted into the left femoral vein. After the constant elevation of blood pressure by angiotensin I was confirmed, the test compounds dissolved in distilled pure water were administered intravenously or orally. Angiotensin I induced pressor responses were measured, at fixed intervals, up to 6 h after oral administration of the test compounds. The inhibitory percentages of the test compounds were calculated by the following formula: [1 – (mean blood pressure induced by angiotensin I after the test compound/mean blood pressure induced by angiotensin I before the test compound)] × 100. ID_{50} (50% inhibitory dose) was graphically calculated by the linear regression curve.

Antihypertensive Effect in SHRs. Eighteen to 22 week old male NCrj SHRs (Charles River Japan, Inc.), weighing about 350 g, with 180–200 mmHg of systolic blood pressure were used. Systolic blood pressure was measured by a rat tail plethysmograph (Ueda, USM-105-R). The test compounds were dissolved in distilled pure water and administered orally after fasting for 18–20 h.

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Registry No. **2a**, 117581-62-1; **2a** (protected), 117560-20-0; **2b**, 117560-21-1; **2b** (protected), 117560-26-6; **3a**, 117560-10-8; **3b**, 89460-21-9; **3b** (free base), 117605-20-6; **3c**, 89384-26-9; **3d**, 89371-74-4; **3e**, 89371-44-8; **3f**, 89371-87-9; **3g**, 89708-53-2; **3h**, 89371-62-0; **3i**, 89371-52-8; **3j**, 89371-71-1; **3k**, 89371-67-5; **3l**, 39396-97-4; **3l** (free base), 97549-58-1; **3m**, 89371-51-7; **3n**, 89371-76-6; **3o**, 89371-41-5; **3p**, 89396-94-1; **3p** (free base), 89371-37-9; **3q**, 117605-19-3; **3q** (free base), 117676-68-3; **3r**, 89371-49-3; **3s**, 89371-48-2; **5**, 59760-01-9; **6a**, 77999-24-7; **6b**, 89384-29-2; **7a**, 83056-78-4; **7b**, 89371-89-1; **7c**, 89371-94-8; **7d**, 83057-00-5; **7e**, 89371-88-0; **8a**, 83056-79-5; **8b**, 89371-35-7; **8c**, 89371-95-9; **8d**, 83057-01-6; **8e**, 89371-46-0; **8f**, 117560-19-7; **9a** (isomer 1), 117605-24-0; **9a** (isomer 2), 117605-25-1; **9b** (isomer 1), 117605-26-2; **9b** (isomer 2), 117605-27-3; **9c** (isomer 1), 117605-28-4; **9c** (isomer 2), 117605-29-5; **9d** (isomer 1), 117605-30-8; **9d** (isomer 2), 117605-31-9; **10a** *p*-toluenesulfonate, 16652-76-9; **10b** *p*-toluenesulfonate, 117560-22-2; **10c** *p*-toluenesulfonate, 117560-23-3; **10d** *p*-toluenesulfonate, 1738-78-9; **10e** *p*-toluenesulfonate, 117560-24-4; **10f**·HCl, 113889-70-6; **10f** (acid), 84277-81-6; (±)-**10f** (acid), 35237-35-5; **10g**·HCl, 90891-21-7; **10g** (acid), 943-73-7; **11a**, 89397-13-7; **11b**, 89371-81-3; **11c**, 89371-40-4; **11d**, 89371-73-3; **11e**, 117605-21-7; **11f**, 117605-22-8; **11g**, 117605-23-9; **11g** (free base), 89371-38-0; **11h**, 89371-86-8; **11i**, 89655-62-9; **11j**, 89371-60-8; **11k**, 89384-28-1; **11l**, 89384-27-0; **11m**, 89371-69-7; **11n**, 89371-65-3; **11o**, 89396-95-2; **11p**, 89371-55-1; **11q**, 89371-47-1; **11r**, 89371-75-5; **11s**, 117560-18-6; **12** (R = CH_2Ph), 117560-25-5; **12** (R = Bu-*t*), 32821-07-1; **13a**, 117560-12-0; **13a** (free base), 117560-11-9; **13b**, 117560-13-1; **13b** (free base), 89371-90-4; **13c**, 117560-15-3; **13c** (free base), 117560-14-2; **13d**, 111542-00-8; **13d** (free base), 93841-86-2; **13e**, 97457-39-1; **13e** (free base), 82717-95-1; **13f**, 117560-16-4; **13f** (free base), 90315-81-4; **14a**, 89371-45-9; **14b**, 89371-39-1; **14c**, 89371-42-6; **14d**, 85196-26-5; **14d** (succinimidyl ester), 89371-34-6; **14e**, 82717-96-2; *N*-succinimidyl *N*-(benzyloxycarbonyl)-L-alaninate, 3401-36-3; 2-bromopropionyl chloride, 71425-59-7; 2-bromobutyryl chloride, 38188-35-1; 2-bromodecanoic acid, 2623-95-2; (2*S*)-2-aminodecanoic acid, 84277-81-6; (±)-*N*-acetyl-2-aminodecanoic acid, 5440-41-5; benzyl L-alaninate, 17831-01-5; ethyl 2-bromo-4-phenylbutyrate, 82586-61-6; (4*S*)-1-benzyl-3-[(2*S*)-[*N*-(1*S*)-(benzyloxycarbonyl)-3-phenylpropyl]-amino]propionyl]-2-oxoimidazolidine-4-carboxylic acid, 89371-56-2.

Synthesis and Radioprotective Activity of Dipeptide Cysteamine and Cystamine Derivatives

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Some *N*-(dipeptidyl)-*S*-acetylcysteamine and *N,N'*-(dipeptidyl)cystamine salt derivatives were synthesized and evaluated as canditate radioprotector agents. Toxicity and radioprotective activity as the dose reduction factor (DRF) were determined in vivo on mice and compared to *N*-glycyl-*S*-acetylcysteamine trifluoroacetate. One of the most interesting compounds of this series was *N*-glycylglycyl-*S*-acetylcysteamine trifluoroacetate (**8**).

We have recently shown[1] that conjugation of an amino acid with *S*-acetylcysteamine and with cystamine lead us to a class of low-toxicity radioprotectors.

Furthermore the lead compound of this series, i.e., *N*-glycyl-*S*-acetylcysteamine trifluoroacetate (**1**), was shown to afford preferential radioprotection for certain normal tissues as opposed to tumors.[2]

TFA, $H_2NCH_2CONH(CH_2)_2SCOCH_3$
1

These data prompted us to extend this approach to some dipeptide derivatives in order to evaluate the influence of the extension of the amino acid conjugation on the biological response.

Chemistry

As the most promising amino acids have been shown to be glycine and L-alanine,[1] we focused first on some dipeptides corresponding to those two amino acids.

The synthesis of **5–7** (Table I) was accomplished by coupling reactions between N-protected dipeptide (glycylglycine (**2**), glycyl-L-alanine (**3**), L-alanylglycine (**4**)) and *S*-acetylcysteamine. These coupling reactions can be

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Table I. Physical Properties of *N*-(Boc-dipeptidyl)-*S*-acetylcysteamines: Boc-AA_1-AA_2-NH-$(CH_2)_2$-S-CO-CH_3

no.	AA_1	AA_2	yield, % (method prepn[a])	recrystn solvent	mp, °C	R_f	formula[b]	IR (KBr): ν, cm^{-1} NH	C=O	CNH	^1H NMR: δ (solvent $CDCl_3$)
5	Gly	Gly	16.4 (A) 60 (B) 39 (C)	EtOAc or Et_2O/ petroleum ether	109–111	0.5[c]	$C_{13}H_{23}N_3O_5S$	3290	1680 1650	1540	1.44 (s, 9 H, *t*-Bu), 2.34 (s, 3 H, acetyl CH_3), 2.81–3.58 (m, 4 H, NCH_2CH_2S), 3.84 and 3.92 (2 d, $J = 2 \times 5.5$ Hz, 2 H, 2 H, Gly CH_2), 5.80 and 7.33 (2 m, 1 H, 2 H, NH*)
6	Gly	L-Ala	20 (A) 10 (B) 41 (C)	Et_2O/ petroleum ether	112–115	0.4[d]	$C_{14}H_{25}N_3O_5S$	3330 3260	1680 1650	1520	1.35 (d, $J = 7$ Hz, 3 H, L-Ala CH_3), 1.44 (s, 9 H, *t*-Bu), 2.33 (s, 3 H, acetyl CH_3), 2.81–3.60 (m, 4 H, NCH_2CH_2S), 3.80 (d, $J = 5.5$ Hz, 2 H, Gly CH_2), 4.46 (m, 1 H, L-Ala CH), 5.55 and 7.09 (2 m, 1 H, 2 H, NH*)
7	L-Ala	Gly	22 (A) 40 (B) 33 (C)	Et_2O/ petroleum ether	60–65 dec	0.36[e]	$C_{14}H_{25}N_3O_5S$	3300 3240	1680 1650	1530	1.36 (d, $J = 7$ Hz, 3 H, L-Ala CH_3), 1.43 (s, 9 H, *t*-Bu), 2.32 (s, 3 H, acetyl CH_3), 2.81–3.56 (m, 4 H, NCH_2CH_2S), 3.90 (d, $J = 5.5$ Hz, 2 H, Gly CH_2), 4.13 (m, 1 H, L-Ala CH), 5.38 and 7.13 (2 m, 1 H, 2 H, NH*)

[a] Experimental Section and Scheme I. [b] All compounds gave satisfactory C, H, N analyses (±0.4%). [c] In CH_2Cl_2/MeOH, 9:1. [d,e] In EtOAc/Et_2O, 8:2. Boc = *tert*-butyloxycarbonyl; AA = amino acid; AA-AA = dipeptide. (*) Disappearing on deuteriation.

Table II. Physical Properties of *N*-(Dipeptidyl)-*S*-acetylcysteamine Trifluoroacetates: TFA, H-AA_1-AA_2-NH-$(CH_2)_2$-S-CO-CH_3

no.	AA_1	AA_2	yield, %	mp,[a] °C	α^{20}_D, deg (*c*, H_2O)	formula[b]	IR (KBr): ν, cm^{-1} NH	C=O	CNH	^1H NMR: δ (solvent D_2O)
8	Gly	Gly	95	119–121		$C_{10}H_{16}F_3N_3O_5S$	3320 3295	1695 1680 1640	1550	2.36 (s, 3 H, acetyl CH_3), 2.84–3.60 (m, 4 H, NCH_2CH_2S), 3.88 and 3.91 (2 s, 2 H, 2 H, Gly CH_2)
9	Gly	L-Ala	89	169–171	−43.2 (0.74)	$C_{11}H_{18}F_3N_3O_5S$	3300	1700 1680 1640	1520	1.31 (d, $J = 7$ Hz, 3 H, L-Ala CH_3), 2.33 (s, 3 H, acetyl CH_3), 2.80–3.54 (m, 4 H, NCH_2CH_2S), 3.79 (s, 2 H, Gly CH_2), 4.22 (q, $J = 7$ Hz, 1 H, L-Ala CH)
10	L-Ala	Gly	80	147–149 dec	+17.0 (1.17)	$C_{11}H_{18}F_3N_3O_5S$	3320 3280	1690 1670 1650	1530	1.46 (d, $J = 7$ Hz, 3 H, L-Ala CH_3), 2.26 (s, 3 H, acetyl CH_3), 2.80–3.55 (m, 4 H, NCH_2CH_2S), 3.81 (s, 2 H, Gly CH_2), 4.05 (q, $J = 7$ Hz, 1 H, L-Ala CH)

[a] All compounds were crystallized from a methanol/ether mixture. [b] All compounds gave satisfactory C, H, N analyses (±0.4%).

achieved by three methods (A–C, Scheme I).

Method A. The condensation of (*tert*-butyloxycarbonyl)dipeptide [Boc-AA_1-AA_2-OH; Boc = $(H_3C)_3COC(O)$] with *S*-acetylcysteamine hydrochloride[3] in dry tetrahydrofuran (THF), using phosphonitrilic chloride (t-PNC)[4] as the activating agent in the presence of triethylamine (TEA), gave the expected compounds.

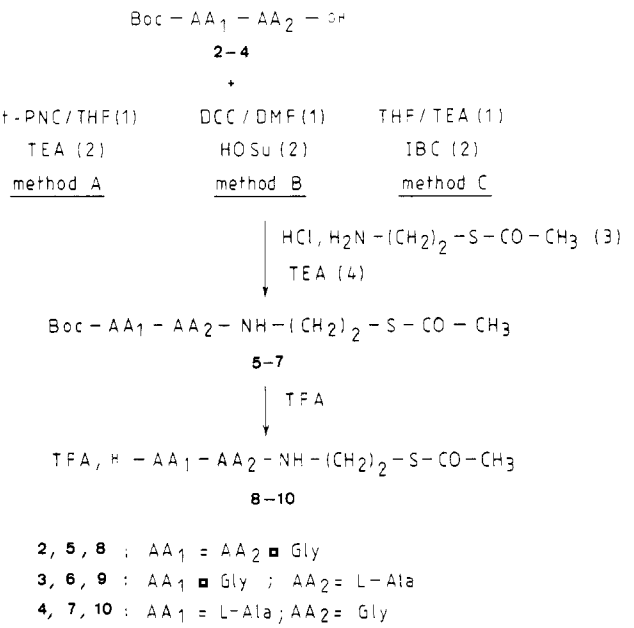
Method B. The (*tert*-butyloxycarbonyl)dipeptide succinimido ester was prepared by reaction of (*tert*-butyloxycarbonyl)dipeptide with *N*-hydroxysuccinimide and *N,N'*-dicyclohexylcarbodiimide (DCC), as coupling reagent, in *N,N*-dimethylformamide (DMF) and was condensed with *S*-acetylcysteamine hydrochloride in the presence of TEA.

Method C. The condensation of (*tert*-butyloxycarbonyl)dipeptide with *S*-acetylcysteamine hydrochloride was realized by the mixed-anhydride method[5] employing isobutyl chloroformate (IBC) in the presence of TEA in dry THF at −15 °C. The resulting crude products were purified either by column chromatography or by several recrystallizations giving crystalline solids in yields ranging from 10 to 60%.

The corresponding trifluoroacetates **8–10** (Table II; Scheme I) were obtained after deprotection of the Boc group with trifluoroacetic acid (TFA) in dichloromethane, giving after crystallization good yields (80–95%) of white crystals. Compounds **9** and **10** were recrystallized to constant rotation. ^1H NMR studies of **8–10** (Table II) indicated one multiplet signal for the methylenes (NCH_2CH_2S). This system is due to the thioester environment; the protons in the α position of S are not equivalent.

The same N-protected dipeptides have also been coupled with cystamine hydrochloride according to the methods already described (methods B and C, Scheme I).

Scheme I[a]



[a] Boc = *tert*-butyloxycarbonyl; AA = amino acid; AA-AA = dipeptide.

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Table III. Physical Properties of *N,N'*-Bis(Boc-dipeptidyl)cystamines: [Boc-AA_1-AA_2-NH-$(CH_2)_2$-S-]$_2$

no.	AA_1	AA_2	yield, % (method prepn[a])	recrystn solvent	mp, °C	R_f	formula[b]	IR (KBr): ν, cm^{-1} NH	C=O	CNH	^1H NMR: δ (solvent Me_2SO-d_6)
11	Gly	Gly	74 (B) 35 (C)	EtOAc/ petroleum ether	118–120	0.8[c]	$C_{22}H_{40}N_6O_8S_2$	3300	1685 1650	1540	1.44 (s, 18 H, *t*-Bu), 2.95 (t, J = 6.6 Hz, 4 H, SCH_2), 3.53 (m, 4 H, NCH_2), 3.97 and 4.03 (2 d, J = 2 × 5.5 Hz, 4 H, 4 H, Gly CH_2), 5.80 and 7.40 (2 m, 2 H, 4 H, NH*)
12	Gly	L-Ala	79 (B) 46 (C)	CH_2Cl_2/ petroleum ether or MeOH/ Et_2O	182–183[f]	0.4[d]	$C_{24}H_{44}N_6O_8S_2$	3280 3260	1695 1685 1645	1535	1.24 (d, J = 7 Hz, 6 H, L-Ala CH_3), 1.45 (s, 18 H, *t*-Bu), 2.86 (t, J = 6.7 Hz, 4 H, SCH_2), 3.44 (m, 4 H, NCH_2), 3.66 (d, J = 5.5 Hz, 4 H, Gly CH_2), 4.40 (m, 2 H, L-Ala CH), 7.15 and 8.25 (2 m, 2 H, 4 H, NH*)
13	L-Ala	Gly	82 (B) 44 (C)	EtOAc/ Et_2O or CH_2Cl_2/ petroleum ether	120–123[g] dec	0.5[e]	$C_{24}H_{44}N_6O_8S_2$	3300	1680 1650	1530	1.22 (d, J = 7 Hz, 6 H, L-Ala CH_3), 1.45 (s, 18 H, *t*-Bu), 2.86 (t, J = 6.7 Hz, 4 H, SCH_2), 3.43 (m, 4 H, NCH_2), 3.81 (d, J = 5.5 Hz, 4 H, Gly CH_2), 4.09 (m, 2 H, L-Ala CH), 7.26 and 8.26 (2 m, 2 H, 4 H, NH*)

[a] Experimental Section and Scheme II. [b] All compounds gave satisfactory C, H, N analyses (±0.4%). [c] In *n*-BuOH/EtOH/H_2O, 2:1:1. [d,e] In CH_2Cl_2/MeOH, 9:1. [f] $[\alpha]^{20}_D$ −6.9° (*c* 0.87, EtOH). [g] $[\alpha]^{20}_D$ +11.0° (*c* 1.09, EtOH). (*) Disappearing on deuteriation.

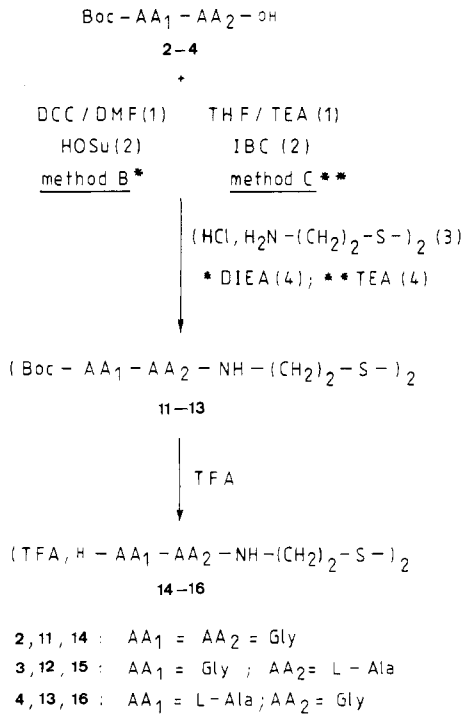
Table IV. Physical Properties of *N,N'*-Bis(dipeptidyl)cystamine Bis(trifluoroacetates): [TFA, H-AA_1-AA_2-NH-$(CH_2)_2$-S-]$_2$

no.	AA_1	AA_2	yield, %	mp,[a] °C	α^{20}_D, deg (*c*, H_2O)	formula[b]	IR: ν,[c,d] cm^{-1} NH	C=O	CNH	^1H NMR: δ (solvent D_2O)
14	Gly	Gly	93	oil		$C_{16}H_{26}F_6N_6O_8S_2$	3295	1680 1650	1540[c]	2.82 (t, J = 6.5 Hz, 4 H, SCH_2), 3.51 (t, J = 6.5 Hz, 4 H, NCH_2), 3.86 and 3.95 (2 s, 4 H, 4 H, Gly CH_2)
15	Gly	L-Ala	95	a	−48.9 (0.92)		3340	1655	1530[d]	1.36 (d, J = 7 Hz, 6 H, L-Ala CH_3), 2.82 (t, J = 6.5 Hz, 4 H, SCH_2), 3.48 (t, J = 6.5 Hz, 4 H, NCH_2), 3.81 (s, 4 H, Gly CH_2), 4.28 (q, J = 7 Hz, 2 H, L-Ala CH)
16	L-Ala	Gly	96	a	+11.5 (0.78)		3300	1670 1650	1540[d]	1.51 (d, J = 7 Hz, 6 H, L-Ala CH_3), 2.81 (t, J = 6.5 Hz, 4 H, SCH_2), 3.50 (t, J = 6.5 Hz, 4 H, NCH_2), 3.93 (s, 4 H, Gly CH_2), 4.10 (q, J = 7 Hz, 2 H, L-Ala CH)

[a] Hygroscopic powder. [b] This compound gave satisfactory C, H, N analyses (±0.4%). [c] Dispersed in Nujol mull. [d] As KBr disks.

The *N,N'*-bis(Boc-dipeptidyl)cystamines **11–13** (Table III; Scheme II) have been obtained after purifications by crystallization (**11**) or by column chromatography and several recrystallizations to constant rotation (**12, 13**). Compounds **11–13** were obtained with yields ranging from 35 to 82%. The N,N'-protected (dipeptidyl)cystamines **11–13** were converted to the corresponding bis(trifluoroacetates) (Table IV; Scheme II) after deprotection of the Boc groups with TFA. The products were isolated after lyophilization in water, giving almost quantitative yields in the forms of an oil (**14**) or of a very hygroscopic powder (**15, 16**). ^1H NMR studies of **12, 13, 15,** and **16** (Tables III and IV) indicated only one doublet for Ala CH_3. This result is in good agreement with the results of Halpern et al.,[6,7] who have studied the steric purity by the chemical shift of the methyl resonances between L–L and L–D alanyl peptides, and compounds **12, 13, 15,** and **16** are optically pure.

Scheme II



Biological Results and Discussion

The compounds synthesized in the present study were tested in mice for radioprotective activity. Results are shown in Table V. Their activities were compared with that of **1**. Compounds **8–10**, which were administered 15 min before irradiation in doses around half their LD_{50}, showed significant radioprotective activity with DRF ranging from 1.20 to 1.55. These activities are in average superior to the activity obtained with **1**. With smaller doses, there is no more activity.

Among these three dipeptide derivatives, it is once more the one that contains the simplest dipeptide (glycylglycine (**8**)) that shows the best DRF and that is the less toxic.

By replacing *S*-acetylcysteamine with cystamine and keeping the same sequences, the obtained compounds (**14**,

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Table V. Toxicity (LD_{50}) and Radioprotective Activity (DRF) of Compounds Intraperitoneally Injected, 15 min or 2 h before Whole-Body Irradiation

compd	LD_{50}, mg/kg	DRF		
		15 min before irrad (dose administered, mg/kg)		2 h before irrad (dose administered, mg/kg)
1[1]	1500	1.4 (750)	1 (187)	1 (750)
8	2400[8]	1.55 (1500)	ND	ND
9	1500	1.25 (750)	ND	1 (750)
10	1500	1.55 (1000)	1 (250)	1 (1000)
14	2000	1.1 (1000)	1 (250)	1 (1000)
15	800	1.1 (400)	1.1 (100)	ND
16	1500	1.35 (1000)	1 (250)	ND

16) are not toxic ($LD_{50} \geq 1500$ mg/kg).

However, in these dipeptidylcystamine series, there is an exception for **15**, whose LD_{50} is 800 mg/kg. This is surprising because its structure is near the structure of the other products. Contrary to the previous series, only **16** showed radioprotective activity (DRF = 1.35) when it was administered 15 min before irradiation, in 1000 mg/kg.

All the compounds, which were administered 2 h before irradiation in doses near their LD_{50}, showed no activity (DRF = 1).

The radiobiological properties tested on normal tissues and on five solid tumors were studied for the compound that appears to be one of the best radioprotectors synthesized and tested. It was shown that **8** is a less effective radioprotector than WR 2721.[8] Furthermore, the importance of radioprotection on tumors and the dependence toward the time that separates the injection of **8** and irradiation is different from one tumor to another.

Nevertheless, the radioprotection afforded by **8** is comparable to that of WR 2721, but it depends on the time interval between injection and irradiation.

Due to the potential practical importance of such compounds as adjuvant drugs in radio- and/or 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 or cystamine derivatives.

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 Beckmann 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. Amino acid and dipeptide derivatives were purchased from Bachem.

***N*-[(*tert*-Butyloxycarbonyl)dipeptidyl]-*S*-acetylcysteamines 5–7. General Method of Coupling Involving a (*tert*-Butyloxycarbonyl)dipeptide and *S*-Acetylcysteamine (Three Methods of Synthesis, Scheme I). Method A.** A solution of the appropriate (*tert*-butyloxycarbonyl)dipeptide [(*tert*-butyloxycarbonyl)glycylglycine, (*tert*-butyloxycarbonyl)-glycyl-L-alanine, (*tert*-butyloxycarbonyl)-L-alanylglycine (**2–4**; 15 mmol)] in tetrahydrofuran (THF; 35 mL) was stirred at 0 °C with phosphonitrilic chloride (t-PNC;[4] 5.22 g, 15 mmol) previously dissolved in THF (25 mL). After a 30 min of stirring at 0 °C, triethylamine (TEA; 2.08 mL, 15 mmol) was added and the mixture was again left stirring for 30 min. After this time, a solution of *S*-acetylcysteamine hydrochloride[3] (2.48 g, 16 mmol) in THF (35 mL) 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 (Table I), and the time needed for coupling was approximately 5–8 h.

The mixture was evaporated to dryness under reduced pressure and then taken up in ethyl acetate (400 mL), and the solution was 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 products were passed through a silica gel column (eluent: EtOAc/MeOH (from 9.9:0.1 to 9.5:0.5) for **5**, EtOAc for **6**, EtOAc/Et_2O (from 9.5:0.5 to 8:2) for **7**) and were recrystallized.

Yields, physical characteristics, and spectroscopic features of *N*-[(*tert*-butyloxycarbonyl)dipeptidyl]-*S*-acetylcysteamines **5–7** are recorded in Table I.

Method B. To a cold (0 °C) stirred solution of the appropriate (*tert*-butyloxycarbonyl)dipeptide (**2–4**; 26.2 mmol) in *N*,*N*-dimethylformamide (DMF; 50 mL) were added *N*-hydroxysuccinimide (HOSu; 3.01 g, 26.2 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (DCC; 5.4 g, 26.2 mmol). After 2 h of stirring at 0 °C, *S*-acetylcysteamine hydrochloride (6.1 g, 39.3 mmol) was added to the mixture, followed by the dropwise addition of TEA (5.4 mL, 39.3 mmol). Stirring was continued at 0 °C for 2 h and at 20 °C for 10 h. The resulting precipitate was filtered, and the filtrate was concentrated to dryness under vacuum. The residual paste was dissolved in dichloromethane 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. The crude products were purified as above. Yields are recorded in Table I (physicochemical criteria are identical with those above).

Method C. A solution of the appropriate (*tert*-butyloxycarbonyl)dipeptide (**2–4**; 10 mmol) and triethylamine (TEA; 10 mmol) in dry tetrahydrofuran (THF; 70 mL) was stirred with cooling at −15 °C. Isobutyl chloroformate (IBC; 10 mmol) was added dropwise, giving a precipitate of TEA hydrochloride. After addition, the mixture was stirred for a further 2 h at a constant temperature of −15 °C. After this time, *S*-acetylcysteamine hydrochloride (20 mmol) was added to the mixture, followed by the dropwise addition of TEA (20 mmol), and stirring was continued at −15 °C for 20 min and then at a room temperature for 20–24 h. Water (120 mL) was added, and the reaction mixture was extracted with ethyl acetate (4 × 100 mL). The extracts were 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 products were purified as above. The yield are recorded in Table I (physicochemical criteria are identical with those above).

***N*-(Dipeptidyl)-*S*-acetylcysteamine Trifluoroacetates 8–10. General Method for Deprotecting the Amine with Formation of the Trifluoroacetate.** A solution of the appropriate *N*-[(*tert*-butyloxycarbonyl)dipeptidyl]-*S*-acetylcysteamine (**5–7**; 3.5 mmol in dichloromethane (5 mL) was stirred at room temperature with trifluoroacetic acid (TFA; 5 mL) while being protected from moisture. The reaction, followed by TLC, was finished in 2–6 h.

The trifluoroacetates were precipitated from the mixture by adding anhydrous ether (100 mL), washed with ether (100 mL), and recrystallized.

Yields, physical characteristics, and spectroscopic features of compounds **8–10** are recorded in Table II.

***N*,*N*′-Bis[(*tert*-butyloxycarbonyl)dipeptidyl]cystamines 11–13.** These compounds were prepared according to the general

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methods already described (Scheme I) (two methods of synthesis, Scheme II).

Method B. The reagents used are as follows: the appropriate (*tert*-butyloxycarbonyl)dipeptide [(*tert*-butyloxycarbonyl)-glycylglycine, (*tert*-butyloxycarbonyl)glycyl-L-alanine, (*tert*-butyloxycarbonyl)-L-alanylglycine) (**2–4**; 12 mmol)] in DMF (60 mL), HOSu (1.38 g, 12 mmol), DCC (2.47 g, 12 mmol) (stirring at 0 °C for 10 h), cystamine dihydrochloride (1.35 g, 6 mmol), and diisopropylethylamine (DIEA; 2.06 mL, 12 mmol). After the addition of the base, stirring was continued at 0 °C for 3 h and at 20 °C for 10 h. The resulting precipitate was filtered, and the filtrate was concentrated to dryness under vacuum. The residual paste was dissolved in dichloromethane or ethyl acetate and washed, and the organic phase was evaporated to dryness under vacuum. The crude products were purified by crystallization (**11**) or by chromatography on a silica gel column (eluent: CH_2Cl_2/MeOH (from 9.9:0.1 to 9.5:0.5) for **12**, CH_2Cl_2/MeOH (from 9.9:0.1 to 9.4:0.6) for **13**) and recrystallizations.

Yields, physical characteristics, and spectroscopic features of *N,N'*-bis[(*tert*-butyloxycarbonyl)dipeptidyl]cystamines **11–13** are recorded in Table III.

Method C. The reagents used are as follows: the appropriate (*tert*-butyloxycarbonyl)dipeptide (**2–4**; 12 mmol) in THF (100 mL), TEA (1.66 mL, 12 mmol) (stirring at −15 °C for 1 h), IBC (1.57 mL, 12 mmol), cystamine dihydrochloride (2.02 g, 9 mmol), and TEA (2.49 mL, 18 mmol). After the addition of the base, stirring was continued at −15 °C for 2 h and at 20 °C for 10 h. The resulting precipitate was filtered, and the filtrate was concentrated to dryness under vacuum. The residual paste was dissolved in ethyl acetate and washed, and the organic phase was evaporated to dryness under vacuum. The crude products were purified as above.

Yields are recorded in Table III (physicochemical criteria are identical with those above).

***N,N'*-Bis(dipeptidyl)cystamine Bis(trifluoroacetates) 14–16.** These compounds were prepared according to the general method already described for deprotecting the amines with formation of bis(trifluoroacetate). The reagents used are as follows: the appropriate *N,N'*-bis[(*tert*-butyloxycarbonyl)dipeptidyl]-cystamine (**11–13**; 2 mmol) and TFA (5 mL).

The reaction, followed by TLC (Table III), was finished in 2–6 h. The bis(trifluoroacetate) was precipitated from the mixture in the form of an oil or a powder by adding anhydrous ether (100 mL) and was washed with ether (100 mL, five times). The product was then taken up in distilled water (30 mL), washed with dichloromethane (2 × 30 mL), and lyophilized. Since they are hygroscopic, these salts are generally stored under vacuum or nitrogen.

Yields, physical characteristics, and spectroscopic features of *N,N'*-bis(dipeptidyl)cystamine bis(trifluoroacetates) **14–16** are recorded in Table IV.

Radioprotective Evaluation. Radioprotective evaluation was performed by Le Centre de Recherche du Service de Santé des Armées (Clamart, France). 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. The radioprotective effect of the compounds was evaluated, according to the protocol already described,[1] 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 (ip) injection of the test compound, 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.

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.

Significant protection was observed with a DRF value superior to 1.15. 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.

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.

Whole-body irradiations were performed with a ^{60}CO γ-ray source (6×10^{13} Bq). The dose rate was equal to 0.65 Gy/min. The dosimetry was carried out by means of ionization chamber dosimeters and lithium fluoride thermoluminescent dosimeters.

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