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# Synthesis and radioprotective activity of new *N*-(amino acid)-*S*-acetylcysteamine and cystamine derivatives

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**Summary** — In order to evaluate the influence of an amino acid conjugation (Sar, Ser, Phe, Pro, Thz) on *S*-acetylcysteamine, cystamine, *N*-(amino acid)-*S*-acetylcysteamine (**14–18**) and *N,N'*-bis (amino acid) cystamine (**24–28**) derivatives have been synthesized and evaluated as potential radioprotectors. Their toxicity and radioprotective activity, as the dose reduction factor (DRF) have been determined (*in vivo*; ip) and compared with cysteamine and cystamine parent compounds: *N*-glycyl-*S*-acetylcysteamine trifluoroacetate **1** and *N,N'*-bis (glycyl)cystamine bis (trifluoroacetate) **2**. Among these compounds, **14** (Sar), **15** (Ser), **15a** [Ser (Ac)], **16** [Phe], **24** (Sar) had significant radioprotective activity.

***N*-(amino acid)-*S*-acetylcysteamines / *N,N'*-bis(amino acid) cystamines / thiazolidine-4 carboxylic acid / radioprotectors**

We have previously reported [1, 2] that conjugation of an amino acid or dipeptide with *S*-acylcysteamine or cystamine leads us to a class of low-toxicity radioprotectors such as the glycine and glycylglycine derivatives (**1–3**).

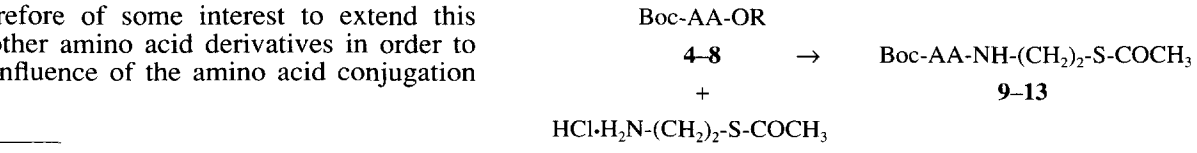
$TFA{\cdot}H\text{-}Gly\text{-}NH(CH_2)_2SCOCH_3$ **1**
$[TFA{\cdot}H\text{-}Gly\text{-}NH(CH_2)_2S\text{-}]_2$ **2**
$TFA{\cdot}H\text{-}Gly\text{-}Gly\text{-}NH(CH_2)_2SCOCH_3$ **3**

Furthermore, compounds **1**, **3** were shown to afford a significant preferential radioprotection to most normal tissues as compared to tumours [3, 4] and metabolic studies have shown that **1**, labelled with $^{14}C$ [5], is rapidly cleaved in the cell to glycylcysteamine and then to cysteamine which represents the essential metabolite involved in the radioprotective process [6, 7].

It was therefore of some interest to extend this approach to other amino acid derivatives in order to evaluate the influence of the amino acid conjugation on the biological response. For this purpose, five amino acids have been studied (sarcosine, L-serine, L-phenylalanine, L-proline, L-thiazolidine-4 carboxylic acid) and the toxicity and the radioprotective activity in mice of the corresponding derivatives have been determined and compared with one of the parent compounds.

## Chemistry

The coupling reactions can be achieved by various procedures previously described [1, 2], but in this work, we will report only on the methods which give the highest yields for obtaining the *N*-[(Boc-amino)-acid]-*S*-acetylcysteamines **9–13** required for the preparation of **14–18** (table I).




R = H or Su; AA = Sar: **4**, Ser: **5**, Phe: **6**, Pro: **7**, Thz: **8**

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**Table I.** Physicochemical properties of *N*-[(Boc-amino)acid]-*S*-acetylcysteamines: Boc-AA-NH-$(CH_2)_2$-S-CO-$CH_3$.

| *Compd* | *AA* | *Yield % (method prepn)*[a] | *mp*[b] *(°C)* | *Formula*[c] | *IR: ν*[d,e] *($cm^{-1}$)* | | | *$^1$H-NMR: δ (solvent $CDCl_3$)* |
|---|---|---|---|---|---|---|---|---|
| | | | | | *NH* | *C=O* | *CNH* | |
| **9** | Sar | 38 *(A)* | 77–79 | $C_{12}H_{22}N_2O_4S$ | 3270 | 1700<br>1655 | 1560[d] | 1.48 (s, 9H, *t*-Bu), 2.35 (s, 3H, $SCOCH_3$), 2.92 (s, 3H, Sar $CH_3$), 2.80–3.18 (m, 2H, $SCH_2$), 3.23–3.63 (m, 2H, $NCH_2$), 3.82 (s, 2H, Sar $CH_2$), 6.39 (m, 1H, NH*) |
| **10** | Ser | 36 *(B)* | 97–99 | $C_{12}H_{22}N_2O_5S$ | 3330<br>3300<br>(br: OH, NH) | 1690<br>1670 | 1560[d]<br>1535 | 1.46 (s, 9H, *t*-Bu), 2.36 (s, 3H, acetyl $CH_3$), 2.86–3.21 (m, 2H, $SCH_2$), 3.23–4.41 (m, 6H, $NCH_2$, Ser CH-$CH_2OH$), 5.64 (d, *J* = 7 Hz, 1H, Sar NH*), 7.02 (m, 1H, NH*) |
| **10a** | Ser (Ac) | 95 | Oil | $C_{14}H_{24}N_2O_6S$ | 3320 | 1730<br>1675 | 1515[e] | 1.45 (s, 9H, *t*-Bu), 2.06 (s, 3H, Ser $COCH_3$), 2.35 (s, 3H, $SCOCH_3$), 2.84–3.20 (m, 2H, $SCH_2$), 3.22–3.72 (m, 2H, $NCH_2$), 4.12–4.56 (m, 3H, Ser CH-$CH_2$), 5.53 (d, *J* = 7 Hz, 1H, Sar NH*), 6.96 (m, 1H, NH*) |
| **11** | Phe | 59 *(C)* | 102–104 | $C_{18}H_{26}N_2O_4S$ | 3340 | 1685<br>1650 | 1525[d] | 1.41 (s, 9H, *t*-Bu), 2.29 (s, 3H, $SCOCH_3$), 2.71–3.13 (m, 4H, $SCH_2$, Phe $CH_2$), 3.16–3.58 (m, 2H, $NCH_2$), 4.10–4.58 (m, 1H, Phe CH), 5.16 (d, *J* = 7 Hz, 1H, Phe NH*), 6.42 (m, 1H NH*), 7.22 (s, 5H, Phe aromatic H) |
| **12** | Pro | 50 *(C)* | 77–79 | $C_{14}H_{24}N_2O_4S$ | 3280 | 1685<br>1660 | 1530[d] | 1.46 (s, 9H, *t*-Bu), 2.36 (s, 3H, $SCOCH_3$), 1.65–2.23 (m, 4H, Pro $CH_2$3,4), 2.83–3.16 (m, 2H, $SCH_2$), 3.21–3.70 (m, 4H, $NCH_2$, Pro $CH_2$5), 4.06–4.33 (m, 1H, Pro H2), 6.80 (m, 1H, NH*) |
| **13** | Thz | 87 *(A)* | Oil | $C_{13}H_{22}N_2O_4S_2$ | 3305 | 1690<br>1670 | 1520[e] | 1.40 (s, 9H, *t*-Bu), 2.36 (s, 3H, $SCOCH_3$), 2.82–3.64 (m, 6H, $NCH_2CH_2S$, Thz $CH_2$5), 4.13–4.80 (m, 3H, Thz $CH_2$2, H4), 6.65 (m, 1H, NH*) |

[a]*Experimental protocols*; [b]all compounds were crystallized from an EtOAc/petroleum ether mixture; [c]all compounds gave satisfactory C, H, N analyses (± 0.4%); [d]as KBr disks; [e]dispersed in Nujoll mull; *disappearing on deuteriation. AA = amino acid; Boc = *t*-butyloxycarbonyl; Thz = thiazolidine-4 carboxylic acid.

*Method A*

Compounds **9**, **13** were obtained by coupling reactions between the appropriate N-protected amino acids [(*tert*-butyloxycarbonyl) sarcosine **4**, *N*-(*tert*-butyloxycarbonyl) thiazolidine-4 carboxylic acid **8**], and *S*-acetylcysteamine using phosphonitrilic chloride (*t*-PNC) [8], as an activating agent, in the presence of triethylamine. The N-protected thiazolidine-4 carboxylic acid, previously described by Barber and Jones [9] (azide method), was obtained by using a di-*tert*-butyl dicarbonate in the presence of aqueous sodium hydroxyde.

*Method B*

The (*tert*-butyloxycarbonyl) serine **5** was condensed with *N*-hydroxysuccinimide and *N,N'*-dicyclohexylcarbodiimide, as coupling reagent, to form the succinimido (*tert*-butyloxycarbonyl) serinate. This, non-isolated active ester was then coupled with *S*-acetylcysteamine in the presence of triethylamine to

give **10**. The corresponding *O*-acetyl derivative **10a** was obtained by acetylation with acetic anhydride of the side chain hydroxyl group.

*Method C*

The N-protected active esters **6**, **7** which were commercially available were coupled with *S*-acetylcysteamine and triethylamine, in a similar manner as that described in *Method B*, to give the corresponding compounds **11**, **12**.

$$\text{Boc-AA-NH-(CH}_2)_2\text{-S-COCH}_3 \rightarrow \text{TFA,H-AA-NH-(CH}_2)_2\text{-S-COCH}_3$$

**9–13** **14–18**

Removal of the *tert*-butyloxycarbonyl groups of **9–13** with trifluoroacetic acid in dichloromethane (table II) gave the *N*-(amino acid)-*S*-acetylcysteamines **14–18** as trifluoroacetate salts. For **13**, the N-deprotection was performed in the presence of thioanisole, as scavenger of carbocation, to avoid any S-alkylation of thiazolidine ring **18.**

**Table II.** Physicochemical properties of *N*-(amino acid)-*S*-acetylcysteamine trifluoroacetates: TFA•H-AA-NH-$(CH_2)_2$-S-CO-$CH_3$.

| *Compd* | *AA* | *Yield (%)* | *mp (°C) (recrystn solvent)* | $\alpha_D^{20}$ *(deg) (c, $H_2O$)* | *Formula* [a] | *IR: ν* [b,c] *($cm^{-1}$) TFA-$H_3N^+$ NH* | *C=O* | *CNH* | *$^1$H-NMR: δ (solvent $D_2O$)* |
|---|---|---|---|---|---|---|---|---|---|
| **14** | Sar | 90 | 94–96 (EtOAc/$Et_2O$) | – | $C_7H_{14}N_2O_2S$• $CF_3COOH$ | 3350–2250 3240 | 1700 1660 | 1580 [b] | 2.36 (s, 3H, $SCOCH_3$), 2.75 (s, 3H, Sar $CH_3$), 2.85–3.19 (m, 2H, $SCH_2$), 3.24–3.65 (m, 2H, $NCH_2$), 3 . 81 (s, 2H, Sar $CH_2$) |
| **15** | Ser | 73 | 81-84 (EtOAc/$Et_2O$) | –0 14 (0.72) | $C_7H_{14}N_2O_3S$• $CF_3COOH$ | 3400–2200 (+OH) 3320 | 1680 1660 | 1560 [b] 1540 | 2.36 (s, 3H, $SCOCH_3$), 2.83–3.20 (m, 2H, $SCH_2$), 3.26–3.67 (m, 2H, $NCH_2$), 3.77–4.30 (m, 3H, Ser CH-$CH_2$) |
| **15a** | Ser (Ac) | 70 | Oil | –5.6 (1.07) | $C_9H_{16}N_2O_4S$• $CF_3COOH$ | 3450–2200 3300 | 1740 1660 | 1560 [c] 1540 | 2.10 (s, 3H, Ser $COCH_3$), 2.33 (s, 3H, $SCOCH_3$), 2.83–316 (m, 2H, $SCH_2$), 3.22–3 63 (m, 2H, $NCH_2$), 4.16–4.58 (m, 3H, Ser CH-$CH_2$) |
| **16** | Phe | 97 | Oil | +28.7 (1.29) | $C_{13}H_{18}N_2O_2S$• $CF_3COOH$ | 3450–2400 | 1680 1650 | 1560 [c] 1520 | 2.20 (s, 3H, $SCOCH_3$), 2.48–2.83 (m, 2H, $SCH_2$), 2.88–3.31 (m, 4H, $NCH_2$, Phe $CH_2$), 4.04 (t, $J$ = 7 Hz, 1H, Phe CH), 7.23 (s, 5H, Phe aromatic H) |
| **17** | Pro | 93 | Oil | –4.23 (1.04) | $C_9H_{16}N_2O_2S$• $CF_3COOH$ | 3450–2300 3250 | 1690 1650 | 1570 [c] 1555 1535 | 1.66–2.24 (m, 4H, Pro $CH_2$3,4), 2.25 (s, 3H, $SCOCH_3$), 2.76–3.10 (m, 2H, $SCH_2$), 3.12–3.51 (m, 4H, $NCH_2$, Pro $CH_2$5), 4.10–4.34 (m, 1H, Pro H2) |
| **18** | Thz | 93 | 81–84 ($CH_2Cl_2$/petroleum ether) | –5.88(1.24) | $C_8H_{14}N_2O_2S_2$• $CF_3COOH$ | 3400–2400 3295 | 1690 1670 | 1570 [b] | 2.35 (s, 3H, $SCOCH_3$), 2.88–3.70 (m, 6H, $NCH_2CH_2S$, Thz $CH_2$5), 4.36–4.71 (m, 3H, Thz $CH_2$2, H4) |

[a]All compounds gave satisfactory C, H, N analyses (±0.4%); [b]as KBr disks; [c]dispersed in Nujoll mull. TFA = trifluoroacetic acid.

The same sequences of coupling reactions have also been performed with cystamine dihydrochloride and N-protected amino acids **5**, **8** or N-protected amino acid active esters **4**, **6**, **7**; according to the methods already described (*Methods B* and *C*).

**4–8**
\+
$[HCl{\cdot}H_2N\text{-}(CH_2)_2\text{-}S\text{-}]_2$ → $[Boc\text{-}AA\text{-}NH\text{-}(CH_2)_2\text{-}S\text{-}]_2$ **19–23**

↓

$[TFA{\cdot}H\text{-}AA\text{-}NH\text{-}(CH_2)_2\text{-}S\text{-}]_2$ **24–28**

Compounds **20**, **23** were synthesized by *Method B* and **19**, **21**, **22** by *Method C* (table III). The *O*-acetyl derivative **20a** was obtained after acetylation of **20** with acetic anhydride. The corresponding *N,N'*-bis (amino acid) cystamines **24–28** were isolated after deprotection of the *tert*-butyloxycarbonyl groups as bis-trifluoroacetate salts (table IV), and the N-deprotection of **23** was also carried out in the presence of thioanisole.

All the synthesized products have been fully characterized by the usual analytical methods. Physical and analytical data for these derivatives are reported in tables I–IV.

**Table III.** Physicochemical properties of *N,N'*-bis[(Boc-amino)acid]cystamines: $[Boc\text{-}AA\text{-}NH\text{-}(CH_2)_2\text{-}S\text{-}]_2$.

| *Compd* | *AA* | *Yield (%) (method prepn)* [a] | *mp*[b] *(°C) (recrystn solvent)* | *Formula* [b] | *IR: ν*[c,d] *(cm⁻¹)* | | | *¹H-NMR: δ (solvent CDCl₃)* [e] |
|---|---|---|---|---|---|---|---|---|
| | | | | | *NH* | *C=O* | *CNH* | |
| **19** | Sar | 65 *(C)* | 178–180 (EtOAc/ petroleum ether) | $C_{20}H_{38}N_4O_6S_2$ | 3270 | 1700 1660 | 1560 [c] | [e]1.40 (s, 18H, *t*-Bu), 2.86 (s, 6H, Sar $CH_3$), 2.63–3.03 (m, 4H, $SCH_2$), 3.10–3.68 (m, 4H, $NCH_2$), 3.80 (s, 4H, Sar $CH_2$), 8.11 (t, *J* = 6 Hz, 2H, NH*) |
| **20** | Ser | 35*(B)* | 85–87 dec: 110 (EtOAc/ petroleum ether) | $C_{20}H_{38}N_4O_8S_2$ | 3330 (br: OH, NH) | 1685 1650 | 1525 [c] | 1.45(s, 18H, *t*-Bu), 2.83 (*t*, *J* = 6.5 Hz, 4H, $SCH_2$), 3.33–4 50 (m, 12H, $NCH_2$, Ser CH-$CH_2OH$), 6.0 (d, *J* = 7 Hz, 2H, Ser NH*), 7.53 (m, 2H, NH*) |
| **20a** | Ser (Ac) | 93 | Oil | $C_{24}H_{42}N_4O_{10}S_2$ | 3310 | 1740 1710 1655 | 1515 [d] | 1.46 (s, 18H, *t*-Bu), 2.06 (s, 6H, Ser $COCH_3$), 2.80 (t, *J* = 6.5 Hz, 4H, $SCH_2$), 3.30–3.82 (m, 4H, $NCH_2$), 4.16–4.71 (m, 6H, Ser CH-$CH_2$), 5.80 (d, *J* = 7 Hz, 2H, Ser NH*), 7.30 (t, *J* = 6 Hz, 2H, NH*) |
| **21** | Phe | 83 *(C)* | 160–162 (EtOAc) | $C_{32}H_{46}N_4O_6S_2$ | 3320 | 1670 1645 | 1510 [c] | 1.41 (s, 18H, *t*-Bu), 2.48–2.85 (m, 4H, $SCH_2$), 3.06 (d, *J* = 7 Hz, 4H, Phe $CH_2$), 3.32–3.68 (m, 4H, $NCH_2$), 4.33–4.72 (m, 2H, Phe CH), 5.74 (d, *J* = 7 Hz, 2H, Phe NH*), 7.33 (s, 10H, Phe aromatic H), 7.03–7.48 (m, 2H, NH*) |
| **22** | Pro | 90 *(C)* | 205–207 dec: 209 (MeOH/$Et_2O$) | $C_{24}H_{42}N_4O_6S_2$ | 3300 | 1680 1660 | 1540 [c] | 1.46 (s, 18H, *t*-Bu), 1.65–2.56 (m, 8H, Pro $CH_2$3,4), 2.80 (t, *J* = 6.5 Hz, 4H, $SCH_2$), 3.19–3.76 (m, 8H, $NCH_2$, Pro $CH_2$5), 4.10–4.38 (m, 2H, Pro H2), 6.96 (m, 2H, NH*) |
| **23** | Thz | 80 *(B)* | Oil | $C_{22}H_{38}N_4O_6S_4$ | 3300 | 1690 1660 | 1520 [d] | 1.43 (s, 18H, *t*-Bu), 2.76 (t, *J* = 6.5 Hz, 4H, $SCH_2$), 3.02–3.76 (m, 4H, $NCH_2$, Thz $CH_2$5), 4.15–4.81 (m, 6H, Thz $CH_2$2, H4), 6.88 (m,2H,NH*) |

[a]*Experimental protocols*; [b]all compounds gave satisfactory C, H, N analyses (± 0.4%); [c]as KBr disks; [d]dispersed in Nujoll mull; [e]solvent $Me_2SO$–$d_6$; *disappearing on deuteriation.

**Table IV.** Physicochemical properties of *N,N'*-bis(amino acid)cystamine bis(trifluoroacetates): [TFA•H-AA-NH-$(CH_2)_2$-S-]$_2$.

| *Compd* | *AA* | *Yield (%)* | *mp (°C) (recrystn solvent)* | $\alpha_D^{20}$ *(deg) (c, $H_2O$)* | *Formula* [a] | *IR: ν [c,d], ($cm^{-1}$) TFA⁻ NH* | *$H_3N^+$* | *C=O* | *CNH* | *$^1$H-NMR: δ (solvent $D_2O$)* |
|---|---|---|---|---|---|---|---|---|---|---|
| **24** | Sar | 94 | 99 | – | $C_{10}H_{22}N_4O_2S_2 \cdot 2CF_3COOH$ | 3300–2350 3250 | | 1690 1660 | 1550 [c] | 2.75 (s, 6H, Sar $CH_3$), 2.83 (t, $J$ = 6.5 Hz, 4H, $SCH_2$), 3.58 (t, $J$ = 6.5 Hz, 4H, $NCH_2$), 3.83 (s, 4H, Sar $CH_2$) |
| **25** | Ser | 80 | Oil | –0.18 (1.08) | $C_{10}H_{22}N_4O_4S_2 \cdot 2CF_3COOH$ | 3600–2400 | | 1660 | 1550 [d] 1530 | 2.84 (t, $J$ = 6.5 Hz, 4H, $SCH_2$), 3.55 (t, $J$ = 6.5 Hz, 4H, $NCH_2$), 3.77–4.20 (m, 6H, Ser CH-$CH_2$) |
| **25a** | Ser (Ac) | 85 | [b] | +1.17 (0.6) | | 3400–2400 | | 1740 1660 | 1560 [d] 1540 | 2.01 (s, 6H, Ser $COCH_3$), 2.73 (t, $J$ = 6.5 Hz, 4H, $SCH_2$), 3.41 (t, $J$ = 6.5 Hz, 4H, $NCH_2$), 4.10–4.56 (m, 6H, Ser CH-$CH_2$) |
| **26** | Phe | 98 | 201–203 ($H_2O$) | +3.80 (1.13) | $C_{22}H_{30}N_4O_2S_2 \cdot 2CF_3COOH$ | 3400–2400 3250 | 1660 | 1685 | 1540 [c] | 2.54 (*t*, $J$ = 6.5 Hz, 4H, $SCH_2$), 2.96–3.50 (m, 8H, $NCH_2$, Phe $CH_2$), 4.16 (t, $J$ = 7 Hz, 2H, Phe CH), 7.25 (s, 10H, Phe aromatic H) |
| **27** | Pro | 95 | Oil | –4.28 (0.42) | $C_{14}H_{26}N_4O_2S_2 \cdot 2CF_3COOH$ | 3600–2400 3250 | | 1720 | 1560 [d] | 1.63–2.53 (m, 8H, Pro $CH_2$3,4), 2.72 (t, $J$ = 6.5 Hz, 4H, $SCH_2$), 3.08–3.71 (m, 8H, $NCH_2$, Pro $CH_2$5), 4.03–4.39 (m, 2H, Pro H2) |
| **28** | Thz | 93 | Oil | –7.03 (0.37) | $C_{12}H_{22}N_4O_2S_4 \cdot 2CF_3COOH$ | 3400–2400 3260 | 1660 | 1680 | 1560 [d] | 2.88 (t, $J$ = 6.5 Hz, 4H, $SCH_2$), 3.18–3.77 (m, 8H, $NCH_2$, Thz $CH_2$5), 4.32–4.70 (m, 6H, Thz $CH_2$2,H4) |

[a]All compounds gave satisfactory C, H, N analyses (± 0.4%); [b]hygroscopic compound; [c]as KBr disks; [d]dispersed in Nujoll mull. TFA: trifluoroacetic acid.

## Biological results and discussion

The compounds listed in table V were evaluated for their radioprotective activity in mice by the intraperitoneal route. Their activities were compared with that of **1** for the *S*-acetylcysteamine derivatives (**14–18**) and with **2** for the cystamine derivatives (**24–28**).

If one compares the chemical structure of the studied compounds to their activity and toxicity, it is apparent that the nature of the amino acid is very important.

In the *S*-acetylcysteamine series, the best compounds are those bearing the amino acids: sarcosine (**14**), serine (**15**), *O*-acetylserine (**15a**), phenylalanine (**16**). Thus, products **14–16**, when administered 15 min before irradiation (to the $LD_{100}$/30 days) in doses around half their $LD_{50}$, showed significant radioprotective activity with 89–100% protection for up to 30 days in mice and with DRF ranging from 1.25–1.5. With lower doses, there is no more activity except for **14** with 40% protection to the $LD_{50}$/8: 250 mg/kg.

Introduction of a cyclic amino acid suppressed the protective activity. The proline derivative **17**, and the thiazolidine-4 carboxylic acid derivative **18**, do no exhibit anti-radiation activity (10–40% protection with DRF around 1).

It is noteworthy that some previously reported thiazolidine-4 carboxylic acid [11] and 2-phenylthiazolidine-4 carboxylic acid [12] derivatives do not present radioprotective activity.

In most cases, compounds **14–18** are weakly toxic with $LD_{50}$ ranging from 600–2000 mg/kg. Nevertheless, in mmol/kg **16–18** are two and three times more toxic that the reference compound **1**.

On the basis of these initial data, it appears that the best compound of this series is the sarcosine derivative **14** as its activity is slightly superior to that

**Table V.** Toxicity ($LD_{50}$) and radioprotective activity of *S*-acetylcysteamine **14–18** and cystamine **24–28** derivatives.

| *Compd* | *AA* | *$LD_{50}$ mg/kg, ip (mmol)* | *Dose injected mg/kg, ip (t, min [a])* | *Dose of γ-radiation (Gy)* | *% Survival of mice at 30 days* | *DRF* |
|---|---|---|---|---|---|---|
| | | | TFA•H-AA-NH-$(CH_2)_2$-S-CO-$CH_3$ | | | |
| HCl•$H_2N(CH_2)_2SCOCH_3$* | | 500 (3.2) | 250 (15) | 9.5 | 20 | |
| | | | 62.5 (15) | 9.5 | 6 | |
| | | | 250 (120) | 9.5 | 40 | 1.05 |
| | | | 250 (15) | 11.5 | 0 | |
| **1** [1] | Gly | 1500 (5.17) | 750 (15) | 9 | 93 | 1.4 |
| | | | 187 (15) | 9 | 0 | |
| | | | 750 (30) | 9 | 90 | |
| | | | 750 (120) | 9 | 0 | |
| | | | 750 (15) | 11 | 50 | |
| | | | 750 (30) | 11 | 60 | 1.4 |
| **14** | Sar | 2000 (6.57) | 1000 (15) | 9 | 100 | 1.5 |
| | | | 250 (15) | 9 | 40 | |
| | | | 1000 (120) | 9 | 30 | |
| | | | 1000 (15) | 11 | 70 | |
| **15** | Ser | 2000 (6.25) | 1000 (15) | 9.5 | 89 | 1.35 |
| | | | 1000 (15) | 11.5 | 44 | |
| **15a** | Ser (Ac) | 2000 (5.52) | 1000 (15) | 9.5 | 100 | 1.25 |
| | | | 250 (15) | 9.5 | 0 | |
| | | | 1000 (15) | 11.5 | 0 | |
| **16** | Phe | 900 (2.36) | 450 (15) | 9 5 | 100 | 1.35 |
| | | | 112 (15) | 9.5 | 10 | |
| | | | 450 (120) | 9.5 | 0 | |
| | | | 450 (15) | 11.5 | 20 | |
| **17** | Pro | 600 (1.81) | 300 (15) | 9.75 | 40 | 1.1 |
| | | | 75 (15) | 9.75 | 10 | |
| | | | 300 (120) | 9.75 | 20 | |
| | | | 300 (15) | 11.75 | 0 | |
| **18** | Thz | 900 (2.58) | 450 (15) | 10 | 10 | 1 |
| | | | 112 (15) | 10 | 0 | |
| | | | 450 (120) | 10 | 10 | |
| | | | [TFA•H-AA-NH-$(CH_2)_2$-S-]$_2$ | | | |
| [HCL•$H_2N(CH_2)_2S$-]$_2$* | | 350 (1.55) | 175 (15) | 9.5 | 80 | |
| | | | 44 (15) | 9.5 | 0 | |
| | | | 175 (120) | 9.5 | 0 | |
| | | | 175 (15) | 11.5 | 20 | 1.2 |
| **2** [1] | Gly | 1500 (3.03) | 750 (15) | 9 | 100 | 1.55 |
| | | | 187 (15) | 9 | 90 | |
| | | | 94 (15) | 9 | 30 | |
| | | | 47 (15) | 9 | 0 | |
| | | | 750 (120) | 9 | 60 | |
| | | | 750 (15) | 11 | 100 | |
| | | | 187 (15) | 11 | 0 | 1.2 |
| | | | 750 (15) | 13 | 10 | |
| **24** | Sar | 1200 (2.29) | 600 (15) | 9 | 90 | 1.4 |
| | | | 150 (15) | 9 | 10 | |
| | | | 600 (15) | 11 | 40 | |
| **25** | Ser | 1500 (2.70) | 750 (15) | 9 | 100 | 1.1 |
| | | | 187 (15) | 9 | 0 | |
| | | | 750 (120) | 9 | 0 | |
| | | | 750 (15) | 11 | 0 | |
| **25a** | Ser (Ac) | 1500 (2.35) | 750 (15) | 9 | 80 | 1.1 |
| | | | 187 (15) | 9 | 0 | |
| | | | 750 (120) | 9 | 0 | |
| | | | 750 (15) | 11 | 0 | |
| **26** | Phe | 400 (0.59) | 200 (15) | 9 5 | 70 | 1.1 |
| | | | 50 (15) | 9.5 | 0 | |
| | | | 200 (120) | 9.5 | 0 | |
| | | | 200 (15) | 11.5 | 0 | |
| **27** | Pro | 400 (0.69) | 200 (15) | 9.75 | 0 | |
| | | | 50 (15) | 9 75 | 0 | 1 |
| | | | 200(120) | 9.75 | 0 | |
| **28** | Thz | 800 (1.31) | 400 (15) | 10 | 40 | 1 |
| | | | 100 (15) | 10 | 0 | |
| | | | 400 (120) | 10 | 10 | |

[a]Time interval between injection of compound and irradiation. *Listed for comparison.

compound parent **1** for a similar toxicity (DRF: 1.5, $LD_{50}$: 2000 mg/kg for **14**; DRF: 1.4, $LD_{50}$: 1500 mg/kg for **1**).

Replacing *S*-acetylcysteamine with cystamine and keeping the same amino acid conjugation, the obtained products (**24–28**) are more toxic than their cysteamine counterpart, in particular if the $LD_{50}$ are calculated in mmol/kg. These compounds present no radioprotective activity under the experimental conditions (DRF ≈ 1) except for **24**.

However its radioprotective efficacy is inferior to that of **2** which is the reference compound (DRF = 1.4 for **24** and 1.55 for **2**).

All compounds (**14–18**, **24–28**) administered 2 h before irradiation in doses around half their $LD_{50}$, showed no activity.

In conclusion, we have already shown that **1** undergoes *in vivo* rapid deacetylation giving glycylcysteamine, followed by hydrolysis of the amide bond, leading to the formation of cysteamine, which represents the main metabolite involved in the radioprotection. We can postulate that the conjugation of the amino acid must play a prominent part in the metabolism of the compounds in determining the rate of hydrolysis of the pseudopeptide bond.

In addition, due to the potential practical importance of such compounds (**1**, **3**) [3, 4, 6, 7] as adjuvant drugs in radio-and/or chemotherapy, various studies are in progress on **14** and will be subsequently reported.

## Experimental protocols

*Chemistry*

The melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. Elemental analyses were performed by the Service Central d'Analyse du CNRS (Vernaison, France). IR spectra were determined on a Beckman Acculab 4 spectrophotometer. NMR 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 polarimeter. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F254 plates. Spots were visualized by ultraviolet light (254 nm), iodine vapor or by spraying with ninhydrin solution. Column chromatography was conducted with Merck silica gel, 70-230 mesh, ASTM. All the solvents used were purified in the usual manner. Amino acids and derivatives were purchased from Bachem Inc. Thiazolidine-4 carboxylic acid and coupling reagents used in this study were obtained from Fluka. The optically active amino acids and derivatives are of the L-configuration.

*N-(tert-Butyloxycarbonyl) thiazolidine-4 carboxylic acid* ***8***

A solution of thiazolidine-4 carboxylic acid (2.66 g, 20 mmol) in a mixture of dioxane (40 ml), water (20 ml) and 1 N NaOH (20 ml) was stirred and cooled at 0°C. Di-*tert*-butyl dicarbonate (4.8 g, 22 mmol) was added and stirring was continued at room temperature for 5 h. The solution was then concentrated *in vacuo* to about 20–30 ml, cooled in an ice-water bath, covered with a layer of ethyl acetate (EtOAc, 60 ml) and acidified with a diluted solution of potassium hydrogen sulfate to pH 2–3. The organic phase was decanted and the aqueous phase was extracted with EtOAc (3 x 30 ml). The EtOAc extracts were pooled, washed with water, dried over sodium sulfate, filtered and evaporated to dryness *in vacuo*. The crude product was crystallized from petroleum ether: yield 86%; mp = 133–135°C, Lit [9] 126–130°C, yield 50%. $^1$H-NMR ($CDCl_3$) δ 1.48 (s, 9H, *t*-Bu), 3.30–3.40 (m, 2H, $CH_2$ 5), 4.26–5.0 (m, 3H, $CH_2$ 2, H4), 11.30 (m, 1H, COOH). Anal $C_9H_{15}NO_4S$) (C, H, N).

*N-[(tert-Butyloxycarbonylamino)acid]-S-acetylcysteamine* ***9–13****. Three methods of synthesis*

*Method A. N-[(tert-Butyloxycarbonyl)sarcosyl]-S-acetylcysteamine* ***9****; N-(tert-butyloxycarbonyl)thiazolidine-4-[(2-acetylmercapto)ethyl]carboxamide* ***13***

A solution of the appropriate (*tert*-butyloxycarbonylamino)acid (**4**, **8**; 25 mmol) in EtOAc (90 ml) was stirred at 0°C with phosphonitrilic chloride (*t*-PNC; 8.7 g, 25 mmol) previously dissolved in EtOAc (50 ml). After 30 min stirring at 0°C, triethylamine (TEA; 3.5 ml, 25 mmol) was added and the mixture was again stirred for 15 min. After this time, *S*-acetylcysteamine hydrochloride (3.88 g, 25 mmol) was added to the mixture, followed by dropwise addition of TEA (3.8 ml, 27.5 mmol). The reaction mixture was stirred for 3 h at 0°C and then at room temperature for 10 h. The resulting precipitate was filtered and the filtrate was diluted with EtOAc (200 ml) and 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, filtered and evaporated to dryness in vacuo. The crude products were purified on a silica gel column (eluent: EtOAc/petroleum ether, 8:2 for **9**; $CH_2Cl_2/Et_2O$, 9.5: 0.5 for **13**). Compound **9** was crystallized and **13** was obtained in the oily form. Yields, physical characteristics and spectroscopic features are recorded in table I.

*Method B. N-[(tert-Butyloxycarbonyl) serinyl]-S-acetylcysteamine* ***10***

To a cold (0°C) stirred solution of (*tert*-butyloxycarbonyl) serine (**5**; 5.05 g, 25 mmol) in *N,N'*-dimethylformamide (DMF; 100 ml) were added *N*-hydroxysuccinimide (HOSu; 2.84 g, 25 mmol) and *N,N'*-dicyclohexylcarbodiimide (DCC; 5.15 g, 25 mmol). After 3 h of stirring at 0°C, the separated *N,N'*-dicyclohexylurea was removed by filtration. To this cold (0°C) stirred filtrate were added *S*-acetylcysteamine hydrochloride (5.8 g, 37. 5 mmol) and dropwise TEA (5.2 ml, 37.5 mmol) in DMF (20 ml). Stirring was continued at 0°C for 2 h and at room temperature for 10 h. The resulting precipitate was filtered and the filtrate was concentrated to dryness *in vacuo*.

The residual paste was dissolved in dichloromethane (200 ml), washed with water (2 x 20 ml), dried over sodium sulfate, filtered and evaporated to dryness *in vacuo*.

The residue was purified on a silica gel column (eluent: EtOAc/petroleum ether, 7:3) and was crystallized. Yield, physical characteristics, and spectroscopic features are recorded in table I.

*Method C. N-[(tert-Butyloxycarbonyl) phenylalanyl]-S-acetylcysteamine* ***11****; N-[(tert-butyloxycarbonyl) prolyl]-S-acetylcysteamine* ***12***

A solution of the appropriate succinimido [(*tert*-butyloxycarbonyl)amino] acid ester (**6–7**; 30 mmol) in DMF (100 ml)

was stirred at 0°C with *S*-acetylcysteamine hydrochloride (7 g, 45 mmol), and a solution of TEA (6.2 ml, 45 mmol) in DMF (25 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 *in vacuo*. The residue was dissolved in dichloromethane or EtOAc (400 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, filtered, and evaporated to dryness *in vacuo*.

The crude products were purified on a silica gel column (eluent: $CH_2Cl_2$/MeOH, 9.8:0.2 for **11**; EtOAc/petroleum ether, 5:5 for **12**) and were crystallized. Yield, physical characteristics, and spectroscopic features are recorded in table I.

*N-[(tert-Butyloxycarbonyl)-O-acetyl-serinyl]-S-acetylcysteamine* ***10a***

This compound was obtained by acetylation of **10** in pyridine with acetic anhydride according to the usual method. The crude product (oil), showed no evidence of impurities by NMR, and was used in the next step without further purification. Yields, physical characteristics, and spectroscopic features are recorded in table I.

*N-(Amino acid)-S-acetylcysteamine trifluoroacetates* ***14–18****. General method for deprotecting the amine with formation of the trifluoroacetate*

A solution of the appropriate *N*-[(*tert*-butyloxycarbonylamino)acid]-*S*-acetylcysteamine (**9–13**; 4 mmol) in dichloromethane (5 ml) was stirred at room temperature and treated with trifluoroacetic acid (TFA; 5 ml) while being protected from moisture. The reactions were complete in 2 h.

The trifluoroacetates (**14**, **15**, **18**) were precipitated from the mixture in the form of a powder by adding anhydrous ether (100 ml). The crude products were filtered, washed with ether and crystallized. Compound **18** was synthesized in the presence of thioanisole (0.94 ml, 8 mmol) in dichloromethane.

For **15a**, **16**, **17**, the crude products were obtained in oily form. These oils were taken up in water (30 ml), washed with dichloromethane (2 x 20 ml), and lyophilized.

All these compounds were sufficiently pure to be used directly for the biological tests. Yields, physical characteristics and spectroscopic features of **14–18** are recorded in table II.

*N,N'-bis[(tert-Butyloxycarbonylamino)acid]cystamines* ***19–23***

These compounds were prepared according to the methods already described (two methods of synthesis).

*Method B*

The reagents used were as follows: the appropriate (*tert*-butyloxycarbonylamino)acid (**5**, **8**; 15 mmol) in DMF (80 ml), HOSu (1.72 g, 15 mmol), DCC (3.09 g, 15 mmol) (stirring at 0°C for 10 h), cystamine dihydrochloride (1.68 g, 7.5 mmol), and TEA or diisopropylethylamine (DIEA, 15 mmol). After the addition of the base, stirring was continued at 0°C for 2 h and at room temperature for 10 h. The resulting precipitate was filtered, the filtrate was concentrated to dryness *in vacuo* and the compounds isolated were as follows.

The residue was chromatographed directly on a silica gel column (eluent: $CHCl_3$/MeOH, 9.5:0.5) and crystallized to give **20**.

The residual paste was dissolved in dichloromethane or EtOAc 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, filtered and evaporated to dryness *in vacuo*. The crude product was purified on a silica gel column (eluent: $CHCl_3$/MeOH, 9.8:0.2) and crystallized to give **23**. Yield, physical characteristics, and spectroscopic features are recorded in table III.

*Method C*

The reagents used were as follows: the appropriate succinimido (*tert*-butyloxycarbonylamino) acid ester (**4**, **6**, **7**; 15 mmol) in DMF (80 ml), cystamine dihydrochloride (1.68 g, 7.5 mmol), and TEA or DIEA (15 mmol). After the addition of the base, stirring was continued at 0°C for 2 h and at room temperature for 10 h. The mixture was evaporated to dryness *in vacuo* and then taken up in dichloromethane or EtOAc and washed as before. After drying, filtration and evaporation of solvent, the crude products were crystallized to give **19**, **21**, **22**. Yields, physical characteristics, and spectroscopic features are recorded in table III.

*N,N'-bis[(tert-Butyloxycarbonyl)-O-acetyl-serinyl]cystamine* ***20a***

This compound was obtained by acetylation in pyridine with acetic anhydride. The crude product was purified on a silica gel column (eluent: $CHCl_3$/MeOH, 9.75:0.25) and crystallized. Yield, physical characteristics, and spectroscopic features are recorded in table III.

*N,N'-bis(Amino acid)cystamine bis(trifluoroacetates)* ***24–28***

These compounds were prepared according to the general method already described for deprotecting the amines with formation of bis(trifluoroacetate). The reagents used were as follows: the appropriate *N,N'*-bis[(*tert*-butyloxycarbonylamino)acid] cystamine (**19–23**; 2 mmol), dichloromethane (4 ml), and TFA (4 ml). The reactions were finished in 2 h and the compounds were as isolated as follows.

**24** was precipitated from the mixture in the form of a powder by adding anhydrous ether (100 ml). It was collected by filtration, thoroughly washed with ether (3 x 100 ml) and dried *in vacuo* over phosphoric anhydride.

**26** was obtained by the same procedure described above to prepare **24**, and the crude powder was crystallized.

For **25**, **25a**, **27**, **28**, the reaction mixture was evaporated to dryness *in vacuo* and was taken up in water (30 ml). The aqueous solution was washed with dichloromethane (2 x 30 ml) and lyophilized. These products were obtained in oily form and **25a** was stored under dry nitrogen. (Compound **28** was synthesized in the presence of thioanisole (0.94 ml, 8 mmol) in dichloromethane).

All these compounds were sufficiently pure to be used directly for the biological tests.Yield, physical characteristics, and spectroscopic features of **24–28** are recorded in table IV.

## Radioprotective evaluation

Radioprotective evaluation was performed by Le Centre de Recherche du Service de Santé des Armées (La Tronche, France). Three-month-old albino CXVII male 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, 30 min, or 2 h before whole-body irradiation delivered with a dose equal to the $LD_{100}/30$ days of control mice. Where necessary, other irradiation doses were tested in order to evaluate the irradiation $LD_{50}$ of protected mice by the Karber method [1, 10].

The radiosensitivity of the strain was regularly monitored by the determination of lethality curves of mice. The $LD_{50}/30$ days was equal to $7.7 \pm 0.3$ Gy.

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 $^{60}CO\gamma$-ray source ($6 \times 10^{13}$ 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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