Eur J Med Chem (1995) 30, 47–52

Short communication

Synthesis and radioprotective activity of WR-1065 derivatives: *N*-(2-acetylthioethyl)-1,3-propanediamine and *N*,*N*'-bis(2-acetylthioethyl)-1,3-propanediamine

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(Received 30 June 1994; accepted 6 September 1994)

Summary — *N*-(2-Acetylthioethyl)-1,3-propanediamine (**13a**, as its 2HBr salt, or **13b**, as its $2CF_3CO_2H$ salt) and *N*,*N*'-bis(2-acetylthioethyl)-1,3-propanediamine (**16**, as its $2CF_3CO_2H$ salt) have been prepared and evaluated as potential radioprotectors in mice. Their toxicity and radioprotective activity (survival rate) have been determined and compared with that of WR-2721. Intermediate *N*-(2-acetylthioethyl)-*N*,*N*'-bis(*Z* or Boc)-1,3-propanediamines were prepared in two ways from the corresponding alcohols. The most convenient method (the Mitsunobu procedure) was used to obtain the *N*,*N*'-bis(2-acetylthioethylated) derivative from the corresponding diol. Surprisingly, none of these compounds possesses radioprotective activity.

***N*-(2-acetylthioethyl)-1,3-propanediamine / *N*,*N*'-bis(2-acetylthioethyl)-1,3-propanediamine / WR-2721 / WR-1065 / radioprotector**

Introduction

The compound *S*-2-(3-aminopropylamino)ethylphosphorothioic acid (**1**, ethiofos, WR-2721) [1] was found to be the most effective drug for protection against the cytotoxicity of ionizing radiation and alkylating agent chemotherapy. This phosphorylated aminothiol is dephosphorylated to its active form (**2**, WR-1065) on entering the cell [2-4]. The conversion is a result of enzymatic hydrolysis by alkaline phosphatase [5-8]. WR-2721 preferentially protects normal tissues [9-12] as opposed to tumors [13-15]. The compound is now used clinically as an adjunct to both radiotherapy and/or chemotherapy for cancer treatment [14, 16-21]. A new perspective for the treatment of hyperparathyroidism and hypercalcemia has also been investigated [22-26]. However, WR-2721 has some limitations; it is not very stable [3], and in humans, it induces certain toxic side effects [17, 18, 27].

$H_2N(CH_2)_3NH(CH_2)_2SPO_3H_2 \cdot 1.5\ H_2O$
1: Ethiofos (WR-2721)

$H_2N(CH_2)_3NH(CH_2)_2SH$
2: WR-1065

H-Gly-$NH(CH_2)_2SCOCH_3 \cdot$TFA
3: I-102

H-Gly-$NH(CH_2)_2SH$
4

$H_2N(CH_2)_2SH$
5

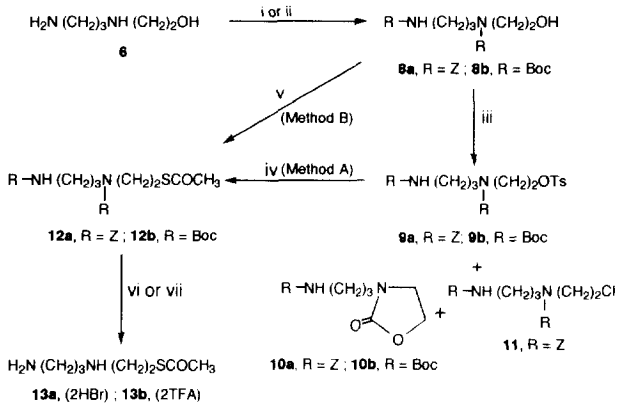
Some years ago, we reported that conjugation of an amino acid or dipeptide with *S*-acylcysteamine leads to a class of low-toxicity radioprotectors [28-30]. Furthermore, in mice, some of these compounds were shown to afford a significant preferential radioprotec-

tion of normal tissues in comparison with tumors [31-32]. Of the tested drugs, *N*-glycyl-*S*-acetylcysteamine (**3**, I-102) has been developed and metabolic studies have shown that this compound is rapidly deacetylated and then undergoes a cleavage of the amide bond leading to *N*-glycylcysteamine **4** and cysteamine **5** which are the two essential radioprotector metabolites [33, 34].

On the basis of the above considerations, coupled with our interest in antiradiation agents, we decided to prepare some *S*-acetyl derivatives of 2-[(3-aminopropyl)amino]ethanethiol (**2**, WR-1065) in order to determine and compare their toxicity and radioprotective activity with WR-2721. For this purpose, and by analogy with **3** (I-102), compounds **13a,b** (scheme 1) have been synthesized and tested. In addition, the symmetrical compound **16** containing two 2-acetylthioethyl groups has also been investigated.

Chemistry

The ring opening of ethylene oxide with 1,3-propanediamine by an adaptation of the procedure of Steck *et al* [35] gave a mixture of **6** and **7**. These mono and dihydroxyethylated derivatives were isolated by fractional distillation and obtained in 60 and 20% yield, respectively. The benzyloxycarbonyl *(Z)* and *tert*-butyloxycarbonyl (Boc) groups were introduced by treatment of **6** with benzyl chloroformate or with 2-(*tert*-butyloxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON) [36] to afford **8a** or **8b**.



Scheme 1. *Z* = benzyloxycarbonyl; Boc = *tert*-butyloxycarbonyl; Ts = tosyl. Reagents: i) benzyl chloroformate; ii) 2-(Boc-oxyimino)-2-phenylacetonitrile (Boc-ON); iii) tosyl chloride; iv) H_3CCOSH, HNa; v) triphenylphosphine, diisopropyl azodicarboxylate (DIAD), H_3CCOSH; vi) HBr/H_3CCO_2H; vii) F_3CCO_2H (TFA).

The two methods that led to the *S*-acetyl derivatives **12a, b** are outlined in scheme 1. The first involved classic activation, under specific conditions, of the hydroxyethylated derivatives **8a,b** by convertion to the corresponding tosylates **9a,b**, which were subsequently displaced with thioacetic acid in the presence of sodium hydride to give the expected compounds **12a** or **12b** *(Method A)*. However, tosylation of **8a** or **8b** was experimentally difficult, requiring careful attention to reaction conditions (see *Experimental protocols*). In fact, the synthesis of **9a,b** can be accompanied by a large amount of 3-substituted-2-oxazolidinones **10a,b** and the *N*-(2-chloroethylated) derivative **11**.

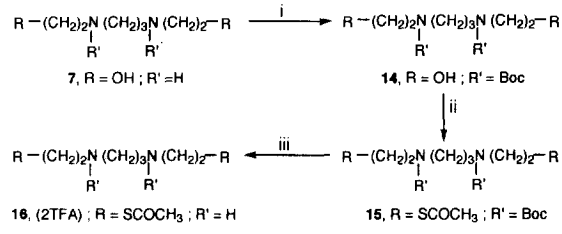
The initial two-step preparation of **12a,b** was replaced by a more convenient method. Using *Method B*, we easily obtained the thioesters (in good yields) directly from alcohols **8a** or **8b** according to the procedure of Mitsunobu as described by Volante [37]. The method consists of treating the preformed adduct of triphenylphosphine and diisopropyl azodicarboxylate (DIAD) in THF, with a mixture of the appropriate alcohol and thioacetic acid to afford the expected compounds.

The corresponding bis(hydrobromide) **13a** and bis(trifluoroacetate) **13b** salts were obtained after deprotection of the *Z* group with HBr in glacial acetic acid or of the Boc group with TFA in CH_2Cl_2. These two different salts have been prepared in order to evaluate the influence of the anion on the biological response.

Compound **16** was prepared from *N,N'*-bis(2-hydroxyethyl)-1,3-propanediamine **7** according to a procedure analogous to that described for **13b** by the following sequence as shown in scheme 2.

The *N,N'*-protection of **7** with Boc-ON gave **14**, which was thioesterified with thioacetic acid in the presence of triphenylphosphine and DIAD to afford the corresponding bis(thioacetate) **15**. The Boc groups were then removed in the usual manner to give the expected bis(trifluoroacetate) **16**.

The IR, NMR, MS spectra and elemental analyses of the compounds were consistent with their proposed structures.



Scheme 2. Reagents: i) Boc-ON; ii) triphenylphosphine, DIAD, H_3CCOSH; iii) TFA.

Biological results and discussion

The compounds listed in table I were evaluated for their radioprotective activity in mice by the intraperitoneal route, as described in the *Experimental protocols*. Their activities were compared with that of WR-2721. The results clearly indicate that the replacement of the *S*-phosphorothioate in WR-2721 by an *S*-acetate caused a suppression of activity and an increase in toxicity. Thus, compounds **13a,b** have no survival effect at 9.5 Gy 15 min or 2 h after injection of doses around half their LD_{50}. In terms of toxicity, in mmol/kg, these products are 2–3 times more toxic that the reference compound. In addition, the nature of the anion influences the toxicity: LD_{50} = 1.18 mmol for the bis(hydrobromide) salt and 1.98 mmol for the bis(trifluoroacetate) salt.

The bis(*N,N'*-substitution) of the 1,3-diaminopropane by a 2-acetylthioethyl group (**16**) does not improve the radioprotective power, which is very weak: 10% of survival at 9.5 Gy at 0.5 LD_{50} and 20% at 0.25 LD_{50}. This compound is also less toxic than WR-2721.

In summary, at present it is difficult to explain the lack of activity of these products as compared with our previous results with *S*-acetylcysteamine derivatives [28-30, 33, 34]. Numerous possibilities can be considered: half-lives, times of administration before irradiation, or formation of metabolites responsible for radioprotective properties. Consequently, additional research on these *S*-acylated series will not be pursued.

Experimental protocols

Chemistry

Melting points were determined on a Büchi capillary melting point apparatus, and are uncorrected. IR spectra were obtained on a Beckman Acculab 4 spectrophotometer; absorbances are reported in ν cm^{-1}. NMR spectra were recorded on a Varian EM 390 spectrometer unless otherwise stated, in which case a Brüker AC 250 instrument was used. Chemical shifts are reported in ppm and given in δ units relative to TMS as an internal standard. Mass spectra were obtained on a Jeol JMS-DX 300 mass spectrometer in the positive-ion fast atom bombardment mode using glycerol/thioglycerol (GT) as the matrix. Elemental analyses were performed by the Service de Microanalyse de l'Ecole Nationale Supérieure de Chimie de Montpellier and were within ± 0.4% of calculated values. Analytical thin-layer chromatography (TLC) was carried out on Merck silica-gel 60 F_{254} 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 60 (230-400 mesh ASTM). All the solvents used were purified in the usual manner.

N-(2-Hydroxyethyl)-1,3-propanediamine **6** *and N,N'-Bis(2-hydroxyethyl)-1,3-propanediamine 7*

The title compound **6** was prepared from the corresponding 3-aminopropylamine and ethylene oxide in methanol at 0°C by an adaptation of the procedure of Steck *et al* [35]. The monohydroxyethylated product was isolated by fractional distillation *in vacuo*. The yield of **6** was 60%, bp 130–140°C/2 mmHg (lit [35]: 105–107°C/1 mmHg). ^{1}H-NMR ($CDCl_3$): 1.36–1.92 (m, 2H, CCH_2C); 2.08–2.47 (m, 4H, ex-D_2O, NH_2, NH, OH); 2.50–2.93 (m, 6H, NCH_2); 3.48–3.77 (apparent t, 2H, CH_2O). The by-product **7** was also formed and isolated (20% yield), bp 180–190°C/2 mmHg. ^{1}H-NMR ($CDCl_3$): 1.37–1.90 (m, 2H, CCH_2C); 2.33–2.90 (m, 8H, NCH_2); 3.05–3.45 (m, 4H, ex-D_2O, NH, OH); 3.45–3.78 (m, 4H, CH_2O).

N-(2-Hydroxyethyl)-N,N'-bis(benzyloxycarbonyl)-1,3-propanediamine ***8a***

A solution of **6** (3.13 g, 26.5 mmol) in water (5 ml) was stirred and cooled at 0°C. Benzyl chloroformate (7.61 ml, 54 mmol) and 1 N NaOH (60 ml) were added alternately in about 5 portions. The reaction mixture was stirred for 1 h at 0°C and at room temperature for 24 h. The alkaline solution was then acidified to pH 4.5 with 2 N HCl and extracted with CH_2Cl_2 (3 x 100 ml). The combined extracts were washed with water (neutral pH), dried over sodium sulfate, filtered and evaporated to dryness *in vacuo*. The crude product was purified on a silica-gel column (eluent: ethyl acetate/petroleum ether, 7:3) to afford

Table I. Toxicity (LD_{50}) and radioprotective activity for compounds **13a,b** and **16**.

Compound	*Structure*	*Approximate LD_{50}, mg/kg (mmol)*	*Drug dose, mg/kg, ip (t, min[a])*	*γ-Radiation dose, Gy*	*30-d survival (%)*
WR2721[b]	$H_2N(CH_2)_3NH(CH_2)_2SPO_3H_2 \cdot 1.5H_2O$	1000 (4.67)	600 (30)	10	100
			300 (30)	10	100
13a	$H_2N(CH_2)_3NH(CH_2)_2SCOCH_3 \cdot 2HBr$	400 (1.18)	200 (15)	9.5	0
13b	$H_2N(CH_2)_3NH(CH_2)_2SCOCH_3 \cdot 2CF_3CO_2H$	800 (1.98)	400 (15)	9.5	0
16	$H_3CCOS(CH_2)_2NH(CH_2)_3NH(CH_2)_2SCOCH_3 \cdot 2CF_3CO_2H$	500 (0.98)	250 (15)	9.5	10
			125 (15)	9.5	20

[a]Time interval between injection of compound and irradiation; [b]listed for comparison.

6.6 g (64%) of pure **8a**, mp 58–60°C (ethyl acetate/petroleum ether); R_f (ethyl acetate/petroleum ether, 7:3) 0.4; IR (KBr): 3480–3240, 3070, 3040, 2940, 2880, 1700, 1680, 1540, 1520, 1250, 1050. ^{1}H-NMR ($CDCl_3$): 1.38–1.92 (m, 2H, CCH_2C); 2.81–3.50 (m, 8H, NCH_2, ex-D_2O, NH, OH); 3.50–3.84 (apparent t, 2H, CH_2O); 5.02, 5.06 (2s, 4H, benzylic CH_2); 7.26 (s, 10H, aromatic H). Anal $C_{21}H_{26}N_2O_5$ (C, H, N).

N-(2-Tosyloxyethyl)-N,N'-bis(benzyloxycarbonyl)-1,3-propanediamine ***9a***

To a cold (0°C) stirred solution of **8a** (5.44 g, 14 mmol) in 25 ml of pyridine was added 4 g (21 mmol) of toluene-4-sulfonyl chloride (tosyl chloride). The reaction conditions (time and temperature) must be carefully controlled after the addition of the tosyl chloride. For example, with **8a** (2.08 g, 5.41 mmol), tosyl chloride (1.22 g, 6.4 mmol) in pyridine (40 ml), when the reaction mixture was stirred for 24 h to room temperature, three products were detected by TLC (R_f, in the same eluent as above: 0.77:0.65:0.15). After the addition was complete, the ice bath was removed and the reaction stirred for 1.15 h at room temperature and at 40°C for 8 min. The solution was then poured into ice-water (300 ml) and filtered on celite. The viscous oil formed was extracted from the celite with CH_2Cl_2 (4 x 50 ml). The CH_2Cl_2 extracts were pooled, washed with water (100 ml), ice-cold 10% HCl (75 ml), water (100 ml), ice-cold 5% sodium bicarbonate (75 ml), and water (3 x 75 ml). The organic phase was dried over sodium sulfate, filtered and evaporated to dryness *in vacuo*. Compound **9a** was obtained as an oil with a yield of 6.1 g (80%). Thin-layer chromatography (R_f (ethyl acetate/petroleum ether, 7:3) 0.65) and NMR analysis indicated that the purity of the product was in excess of 95% and could be used without further purification.

An analytical sample was isolated by chromatography on a silica-gel column (eluent: ethyl acetate/petroleum ether, 4:6). IR (film): 3320, 3040, 3020, 2930, 2860, 1720, 1700, 1680, 1520, 1350, 1250, 1170. ^{1}H-NMR ($CDCl_3$): 1.38–1.95 (m, 2H, CCH_2C); 2.42 (s, 3H, tosyl CH_3); 2.94–3.67 (m, 6H, NCH_2); 3.92–4.31 (m, 3H, CH_2O, ex-D_2O, NH); 5.03, 5.07 (2s, 4H, benzylic CH_2); 7.07–7.46, 7.55–7.90 (2m, 14H, aromatic H). Anal $C_{28}H_{32}N_2O_7S$ (C, H, N).

These three compounds were separated by chromatography on a silica-gel column (eluent: stepwise gradient of ethyl acetate (20–100%) in petroleum ether).

The least polar (R_f 0.77) was isolated and identified as *N*-(2-chloroethyl)-*N,N'*-bis(benzyloxycarbonyl)-1,3-propanediamine **11** (301 mg of a colorless oil). IR (film): 3340, 3060, 3020, 2940, 2920, 2880, 1720, 1690, 1520, 1250. ^{1}H-NMR (250 MHz, $CDCl_3$): 1.65 (m, 2H, CCH_2C); 3.0–3.17 (m, 2H, propyl $NHCH_2$); 3.24–3.40 (m, 2H, propyl NCH_2); 3.40–3.66 (m, 4H, NCH_2CH_2Cl); 5.02, 5.06 (2s, 4H, benzylic CH_2); 5.39–5.49 (m, 1H, ex-D_2O, NH); 7.28 (s, 10H, aromatic H). MS (FAB$^+$, GT): 405 $[M + H]^+$. Anal $C_{21}H_{25}ClN_2O_4$ (C, H, Cl, N).

The product (R_f 0.65), was identified as **9a** (181 mg, 6.2%).

The most polar compound (R_f 0.15) was collected as an oil (542 mg) which crystallized from ethyl acetate/petroleum ether mixture and was identified as *N*-[3-(*N'*-benzyloxycarbonylamino)propyl]oxazolidinone **10b**: mp 61–62°C. IR (KBr): 3340, 3040, 3020, 2940, 2910, 1740, 1710, 1530, 1270, 1250. ^{1}H-NMR (250 MHz, $CDCl_3$): 1.68 (m, 2H, CCH_2C); 3.15 (q, 2H, propyl $NHCH_2$); 3.26 (t, 2H, propyl NCH_2); 3.49 (t, 2H, oxazolidinone NCH_2); 4.26 (t, 2H, oxazolidinone CH_2O); 5.02 (s, 2H, benzylic CH_2); 5.22–5.35 (m, 1 H, ex-D_2O, NH); 7.28 (s, 5H, aromatic H). Anal $C_{14}H_{18}N_2O_4$ (C, H, N).

N-(2-Acetylthioethyl)-N,N'-bis(benzyloxycarbonyl)-1,3-propanediamine ***12a***

Method A. A suspension of hexane-washed NaH ((50% suspension), 3.85 g, 80 mmol) in 20 ml of dry acetonitrile was added over a period of 30 min to a stirred solution of thioacetic acid (5.7 ml, 80 mmol) in 20 ml of acetonitrile at 0°C. The mixture was stirred for 30 min followed by the dropwise addition of 5.4 g (10 mmol) of **9a** in acetonitrile (20 ml). After the addition was completed, the stirred mixture was heated on a steam bath (40°C). The progress of the reaction was monitored by TLC, and when all the tosylate had disappeared the reaction mixture was filtered and evaporated to dryness *in vacuo*. The residue was dissolved in 300 ml of CH_2Cl_2, washed with water (2 x 100 ml), ice-cold 10% HCl (100 ml), and water (3 x 100 ml). The organic phase was dried over sodium sulfate, filtered and evaporated to dryness *in vacuo*. The crude product (oil, 5.7 g) was then purified on a silica-gel column (eluent: ethyl acetate/petroleum ether, 2:8) to afford 1.95 g (44%) of **12a** as an oil. R_f (ethyl acetate/petroleum ether, 3:7) 0.3; IR (film): 3330, 3040, 3020, 2940, 2860, 1700, 1690, 1680, 1515, 1250. ^{1}H-NMR ($CDCl_3$): 1.42–1.97 (m, 2H, CCH_2C); 2.27 (s, 3H, acetyl CH_3); 2.77–3.61 (m, 8H, NCH_2, SCH_2); 5.07, 5.12 (2s, 4H, benzylic CH_2); 5.35–5.68 (m, 1H, ex-D_2O, NH); 7.30 (s, 10H, aromatic H). Anal $C_{23}H_{28}N_2O_5S$ (C, H, N).

Method B. To a cold (0°C) stirred solution of triphenylphosphine (5.25 g, 20 mmol) in THF (50 ml) was added diisopropyl azodicarboxylate (DIAD; 4.06 ml, 20 mmol). The mixture was stirred at 0°C for 45 min. A white precipitate resulted. Compound **8a** (3.86 g, 10 mmol) and thioacetic acid (1.43 ml, 20 mmol) in 25 ml THF were added dropwise over 5 min and the reaction mixture was stirred for 1 h at 0°C and at room temperature for 12 h. A clear yellow solution resulted. The solution was evaporated to dryness *in vacuo* and the residue was then chromatographed on a silica-gel column (eluent: stepwise gradient of ethyl acetate (20–40%) in petroleum ether) to afford 3.1 g (70%) of the expected product **12a**.

N-(2-Acetylthioethyl)-1,3-propanediamine bis(hydrobromide) ***13a***

A solution of **12a** (1.8 g, 4.0 mmol) in glacial acetic acid (5 ml) saturated with HBr was stirred at a temperature below –5°C under nitrogen. The reaction, followed by TLC, was over in 1 h . The bis(hydrobromide) was precipitated from the mixture by adding ice-cold anhydrous ether (100 ml), and the ether phase was then decanted. This procedure of washing with ether was performed 3 times and the crude product (1.25 g, 92%) was crystallized from ethanol to afford 980 mg (72%) of **13a** as white crystals; mp 203–205°C; IR (KBr): 3500–3100, 3020, 3000, 2920, 2750, 2660, 2540, 1690, 1560. ^{1}H-NMR (250 MHz, D_2O): 1.86–2.02 (m, 2H, CCH_2C); 2.26 (s, 3H, acetyl CH_3); 2.95 (apparent t, 2H, propyl H_2NCH_2); 3.02– 3.10 (m, 4H, CH_2NHCH_2); 3.10–3.19 (m, 2H, SCH_2). Anal $C_7H_{16}N_2OS{\cdot}2HBr$ (C, H, N).

N-(2-Hydroxyethyl)-N,N'-bis(tert-butyloxycarbonyl)-1,3-propanediamine ***8b***

A solution of Boc-ON [36] (24.6 g, 100 mmol) in 100 ml THF was added dropwise to a solution of **6** (5.9 g, 50 mmol) in 100 ml THF at 0°C. After the addition was complete, the ice-bath was removed and the reaction stirred for 12 h at room temperature. The solvent was then evaporated to dryness *in vacuo* and the crude product was chromatographed on a silica-gel column (eluent: stepwise gradient of ethyl acetate (0–80%) in CH_2Cl_2) to afford 9.3 g (58%) of product as an oil. R_f (ethyl

acetate/CH_2Cl_2, 5:5): 0.32; IR (film): 3590–3200, 3000, 2970, 2920, 2880, 1700, 1675, 1520, 1500, 1270, 1240, 1050. ^{1}H-NMR ($CDCl_3$): 1.47 (s, 18H, *t*-Bu H); 1.47–1.97 (m, 2H, CCH_2C); 2.82–3.49 (m, 7H, NCH_2, ex-D_2O, OH); 3.56–3.92 (apparent t, 2H, CH_2O); 4.66–5.10 (m, 1H, ex-D_2O, NH). Anal $C_{15}H_{30}N_2O_5$ (C, H, N).

N-(2-Tosyloxyethyl)-N,N'-bis(tert-butyloxycarbonyl)-1,3-propanediamine ***9b***

To a stirred solution of **8b** at – 30°C (1 g, 3.14 mmol) in 5 ml pyridine was added 0.9 g (3.14 mmol) of tosyl chloride. The reaction mixture was then stirred for 2 h at –30 to 0°C and, at room temperature for 1 h. As described for **9a** (with the same quantities of reagents used above), if the reaction mixture was left stirring for 12 h at room temperature and, if after treatment the organic phase (CH_2Cl_2) was evaporated to dryness *in vacuo* at more than 40°C, two spots were detected by TLC. The two products were separated by chromatography on a silica-gel column (eluent: ethyl acetate/petroleum ether, 6:4). The mixture was poured into ice-water (100 ml) and filtered on celite. The viscous oil formed was extracted from the celite with CH_2Cl_2 (4 x 30 ml). The CH_2Cl_2 extracts were pooled, washed with water (75 ml). The organic phase was dried over sodium sulfate, filtered and evaporated at 20°C to dryness *in vacuo*. Compound **9b** was obtained as an oil with a yield of 1.02 g (69%). Thin-layer chromatography (R_f (ethyl acetate/petroleum ether, 5:5): 0.7) and NMR analysis indicated that the purity of the product was in excess of 95% and could be used without further purification.

An analytical sample was obtained by chromatography on a silica-gel column (eluent: ethyl acetate/petroleum ether, 3:7). IR (film): 3340, 3060, 3020, 3000, 2940, 2920, 2880, 1730, 1700, 1680, 1520, 1300, 1250, 1230, 1170. ^{1}H-NMR ($CDCl_3$): 1.38, 1.42 (2s, 18H, *t*-Bu H); 1.33–1.88 (m, 2H, CCH_2C); 2.42 (s, 3H, tosyl CH_3); 2.87–3.56 (m, 6H, NCH_2); 4.10 (t, 2H, CH_2O); 4.58–4.85 (m, 1H, ex-D_2O, NH); 7.33, 7.77 (2d, 4H, tosyl H). Anal $C_{22}H_{36}N_2O_7S$ (C, H, N).

The least polar, minor compound (R_f 0.77) was isolated and identified as **9b** (75 mg, 5%).

The most polar, major compound (R_f 0.2) was collected as an oil (583 mg) and identified as *N*-[3-(*N'-tert*-butyloxycarbonylamino)propyl]oxazolidinone **10b**. IR (film): 3360, 2980, 2940, 2920, 1735, 1715, 1510, 1270, 1250. ^{1}H-NMR (250 MHz, $CDCl_3$): 1.45 (s, 9H, *t*-Bu H); 1.50–1.97 (m, 2H, CCH_2C); 3.16 (q, 2H, propyl NHCH_2); 3.33 (t, 2H, propyl CH_2N); 3.56 (t, 2H, oxazolidinone NCH_2); 4.33 (t, 2H, oxazolidinone CH_2O); 5.0 (m, 1H, ex-D_2O, NH). Anal $C_{11}H_{20}N_2O_4$ (C, H, N).

N-(2-Acetylthioethyl)-N,N'-bis(tert-butyloxycarbonyl)-1,3-propanediamine ***12b***

Method A. This compound was prepared from NaH (50% suspension; 433 mg, 9.03 mmol) in 10 ml acetonitrile, thioacetic acid (0.64 ml, 9.03 mmol) in 10 ml acetonitrile and **9b** (1.02 g, 2.1 mmol) in 10 ml acetonitrile; according to the synthetic pathway described for **12a**. The crude product (oil) was purified by chromatography on a silica-gel column (eluent: ethyl acetate/petroleum ether, 3:7) to afford 260 mg (33%) of pure **12b**, mp 74–75°C (petroleum ether): R_f (ethyl acetate/petroleum ether, 2.5:7.5): 0.5. IR (KBr): 3360, 2980, 2940, 2880, 1700, 1690, 1680, 1515, 1260. ^{1}H-NMR ($CDCl_3$): 1.46, 1.48 (2s, 18H, *t*-Bu H); 1.49–1.97 (m, 2H CCH_2C); 2.33 (s, 3H, acetyl CH_3); 2.82–3.53 (m, 8H, NCH_2, SCH_2); 4.85–5.12 (m, 1H, ex-D_2O, NH). Anal $C_{17}H_{32}N_2O_5S$ (C, H, N).

Method B. A similar thioacetylation of **8a** was employed to obtain **12b**. The reagents used were as follows: triphenylphosphine (6.8 g, 26 mmol) in THF (60 ml), DIAD (5.12 ml, 26 mmol), **8b** (4.13 g, 13 mmol) and thioacetic acid (1.86 ml, 26 mmol) in THF (25 ml). After 12 h of stirring at room temperature, the yellow solution was evaporated to dryness *in vacuo*. The crude product was purified by chromatography on a silica-gel column (eluent: stepwise gradient of ethyl acetate (0–30%) in petroleum ether). The expected compound was obtained with a yield of 80% (3.9 g).

N-(2-Acetylthioethyl)-1,3-propanediamine bis(trifluoroacetate) ***13b***

A solution of **12b** (260 mg, 0.69 mmol) in CH_2Cl_2 (1 ml) was stirred at room temperature with trifluoroacetic acid (TFA, 1 ml) while being protected from moisture. The reaction, followed by TLC, was over in 15 min. After evaporation to dryness *in vacuo*, the residue taken up in ether (20 ml) yielded a white precipitate which was filtered and washed with ether (2 x 5 ml). The crude product (259 mg) was crystallized from ethyl acetate to afford 227 mg (82%) of **13b** as white crystals; mp 113–115°C; IR (KBr): 3500–3100, 3020, 2980, 2840, 2700, 2600, 2460, 1690, 1660, 1600; ^{1}H-NMR (250 MHz, D_2O): 1.86–2.02 (m, 2H, CCH_2C); 2.28 (s, 3H, acetyl CH_3); 2.97 (apparent t, 2H, propyl H_2NCH_2); 3.01–3.11 (m, 4H, CH_2NHCH_2); 3.12–3.21 (m, 2H, SCH_2). Anal $C_7H_{16}N_2OS \cdot 2CF_3CO_2H$ (C, H, N).

N,N'-Bis(2-hydroxyethyl)-N,N'-bis(tert-butyloxycarbonyl)-1,3-propanediamine ***14***

The title compound was prepared in a similar manner to that described for **8b**. The reagents used were as follows: *N,N'*-bis(2-hydroxyethyl)-1,3-propanediamine **7** (4.05 g, 25 mmol) in THF (80 ml); Boc-ON (12.31 g, 50 mmol). After a 12 h of stirring at room temperature, the reaction mixture was evaporated to dryness *in vacuo* and the crude product was chromatographed on a silica-gel column (eluent: ethyl acetate/CH_2Cl_2, 2:8) to afford 4.8 g (53%) of pure product as an oil. R_f (ethyl acetate): 0.5. IR (film): 3500–3200, 2980, 2940, 2890, 1690, 1670, 1480, 1420, 1290, 1255, 1060. ^{1}H-NMR ($CDCl_3$): 1.45 (s, 18H, *t*-Bu H); 1.49–2.05 (m, 2H, CCH_2C); 3.05–3.48 (m, 10H, NCH_2, ex-D_2O, OH); 3.50–3.88 (m, 4H, CH_2O). Anal $C_{17}H_{34}N_2O_6$ (C, H, N).

N,N'-Bis(2-acetylthioethyl)-N,N'-bis(tert-butyloxycarbonyl)-1,3-propanediamine ***15***

This compound was prepared according to *Method B* described for **12a**. The reagents used were as follows: triphenylphosphine (4.07 g, 15.5 mmol) in THF (25 ml), DIAD (3.04 ml, 15.5 mmol), **17** (1.40 g, 3.8 mmol) and thioacetic acid (1.1 ml, 15.5 mmol) in THF (15 ml). After a 12 h of stirring at room temperature, the yellow solution was evaporated to dryness *in vacuo*. The crude product was purified by chromatography on a silica-gel column (eluent: ethyl acetate/petroleum ether, 1.5:8.5) to afford 1.32 g (73%) of **15** as an oil. R_f (ethyl acetate/petroleum ether, 3:7): 0.7; IR (film): 2960, 2920, 2870, 1730, 1680, 1470, 1405, 1290, 1250. ^{1}H-NMR ($CDCl_3$): 1.46 (s, 18H, *t*-Bu H); 1.56–2.08 (m, 2H, CCH_2C); 2.31 (s, 6H, acetyl CH_3); 2.70–3.47 (m, 12H, NCH_2, SCH_2). Anal $C_{21}H_{38}N_2O_6S_2$ (C, H, N).

N,N'-Bis(2-acetylthioethyl)-1,3-propanediamine bis(trifluoroacetate) ***16***

In the same manner as for **13b**, compound **16** was prepared from **15** (622 mg, 1.30 mmol) and TFA (2 ml) in CH_2Cl_2 (2 ml). After drying, the crude product was crystallized from a

methanol and ether mixture (528 mg, 80%), mp 128–129°C. IR (KBr): 3500–3100, 3000, 2910, 2880, 2850, 2600, 2500, 1680, 1650, 1600. ^{1}H-NMR (250 MHz, D_2O): 1.91–2.10 (m, 2H, CCH_2C); 2.25 (s, 6H, acetyl CH_3); 2.94–3.08 (m, 8H, NCH_2); 3.08–3.19 (m, 4H, SCH_2). Anal $C_{11}H_{22}N_2O_2S_2 \cdot 2CF_3CO_2H$ (C, H, N).

Pharmacology

Radioprotective evaluation was performed by the Centre de Recherche du Service de Santé des Armées (La Tronche). Male CD1 mice (Charles River, France) 25 g in weight were used. The radioprotective effect of compounds was evaluated by determining the survival rate 30 d after irradiation in different groups of 20 mice receiving an intraperitoneal (ip) injection of the test compound, 15 min before whole-body irradiation delivered with a dose equal to the DL_{100}/30 d of control mice (9.5 Gy). The injected dose of compound was equal to half or a quarter of its previously LD_{50} determined.

The compounds dissolved easily 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.

In addition, a group of 8 unirradiated mice received the test compound with a dose equal to half 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 x 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 x 28.5 cm. The dosimetry was carried out by means of ionization chamber dosimeters and lithium fluoride thermoluminescent dosimeters.

Acknowledgment

We are grateful to the Direction des Recherches, Etudes et Techniques (DRET, Paris, France) for the support of this work.

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