N^{ω}-(2-Mercaptoethyl) Derivatives of Ornithine and Lysine as Antiradiation Agents

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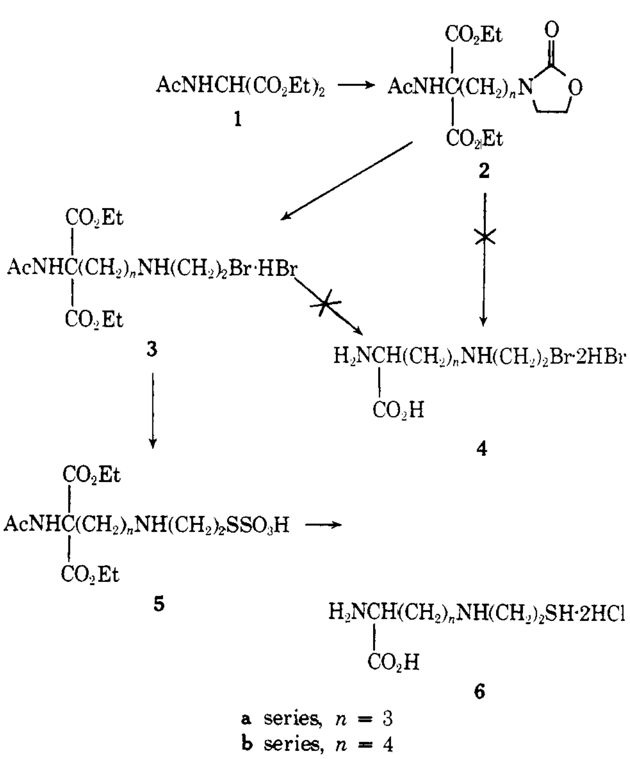
The radioprotective properties of a series of *S*-2-(ω-aminoalkylamino)ethyl dihydrogen phosphorothioates and related compounds[1,2] suggested, *inter alia*, a structural modification incorporating amino acids. An immediate goal was the preparation of N^{ω}-(2-bromoethyl) derivatives of ornithine and lysine as dihydrobromides **4a,b** from which phosphorothioates could be derived. The following exploratory sequences that involved alkylation of acylated intermediates, however, failed to produce characterizable products: (1) N^6,O-dialkylation of N^2-acetyl-N^6-(*p*-tolylsulfonyl)-L-lysine[3] with 2-bromoethyl acetate, (2) alkylation of diethyl phthalimidomalonate with 3-(3-chloropropyl)-2-oxazolidinone, (3) alkylation of 2-oxazolidinone with diethyl (4-bromobutyl)phthalimidomalonate,[4] and (4) alkylation of *N*-(2-hydroxyethyl)-*p*-toluenesulfonamide[5] with diethyl acetamido(4-chlorobutyl)malonate.[6]

The conversion of diethyl acetamidomalonate (1) to the oxazolidinones **2a,b** and the ring opening with hydrogen bromide[7] to give the bromoethylamine hydrobromides **3a,b** were the promising beginnings of the sequence (Scheme I) that eventually led to **6a,b.** The initial step is the first example of C-alkylation with 3-(ω-chloroalkyl)-2-oxazolidinones and complements the utility of previous examples of O-, N-, and S-alkylations.[7] The ring-opening step was effected with retention of the ester and amide functions. However, the attempted preparation of **4a** by treatment of **2a** with 48% hydrobromic acid under Cortese-type conditions[8] gave a crude, oily product, and attempts to prepare **4a,b** by hydrolysis and concomitant decarboxylation of **3a,b** gave deliquescent products, which could not be obtained pure enough for characterization and conversion to phosphorothioates. At this point, the thiols **6a,b** became attainable alternative targets; the thiosulfates **5a,b** were derived by the thallous thiosulfate method[9] and hydrolyzed with hydrochloric acid, the coproduced sulfate ions being removed as barium sulfate.

Scheme I



a series, $n = 3$
b series, $n = 4$

The 2-(ω-aminoalkylamino)ethanethiols[10] corresponding to **6a,b** were among a group of compounds described[11] as having "demonstrated activity in protecting animals against the harmful effects of ionizing radiation," but specific data that would enable a comparison were not reported. The thiols **6a,b** and the intermediate thiosulfates **5a,b** were screened in mice according to a previously described[12] method. Only intraperitoneally (ip) injected **6a** was significantly protective; **6b** was slightly protective (survival rates up to 20% in some tests) when administered both ip (90 and 180 mg/kg, LD_{50} ~350 mg/kg) and orally (po, 500 and 1000 mg/kg, LD_{50} >1250 mg/kg); and **5a,b** were essentially nonprotective (**5b** was not tested po). The protective properties of **6a** were demonstrated with variations in solution pH and radiation rate. Doses of 300 and 150 mg/kg of **6a** (LD_{50} >600 mg/kg), administered ip as an aqueous 3% solution (unadjusted pH 2.0) 15 min prior to irradiation (849 rads from ^{137}Cs, 141.5 rads/min), each gave a 70% survival rate (seven of ten mice surviving 30 days with no control mice surviving). Similar results were obtained with a solution of adjusted pH (6.2) and 975 rads from ^{60}Co (230 rads/min): 100% survival (dose, 300 mg/kg), 47% (150 mg/kg); a dose of 200 mg/kg (pH 5.9) gave a 53% survival against 950 rads from ^{60}Co at a slow radiation rate (30–50 rads/min). But 5% solutions of **6a** when administered po at doses of 1000 and 500 mg/kg (LD_{50} >1750 mg/kg) 30 and 60 min prior to irradiation were nonprotective against 975 rads (^{60}Co, 230 rads/min).

Experimental Section

Melting points were determined with a Mel-Temp apparatus and are uncorrected. Ir spectra were determined with Perkin-Elmer 521 and 621 spectrophotometers and pmr spectra with a Varian A-60A spectrometer. Analytical results indicated by element or function symbols were within ±0.4% of the theoretical values. Microanalyses were performed, for the most part, by Galbraith Laboratories, Knoxville, Tenn. Spectral determinations and some of the C, H, and N analyses were performed in the Molecular Spectroscopy Section of Southern Research Institute under the direction of Dr. W. C. Coburn, Jr.

Diethyl Acetamido[3-(2-oxo-3-oxazolidinyl)propyl]malonate (2a). A solution of 1 (65.2 g, 0.300 mol) in anhydrous *N,N*-dimethylacetamide (DMAC, 300 ml) was added dropwise to a mechanically stirred mixture of NaH (12.0 g of 60% dispersion in oil, 0.300 mol) and DMAC (130 ml) at 25–30°. The mixture was stirred until frothing had virtually ceased (~1 hr). Freshly distilled 3-(3-chloropropyl)-2-oxazolidinone [49.1 g, 0.300 mol; bp 126–128° (0.09 mm); purchased from Asta-Werke AG, Brackwede (Westf.), West Germany] and dry NaI (2.5 g) were added. The stirred mixture was gradually heated to 130°, kept at 120–135° (mainly 125°) for 4 hr, cooled, treated with Norit, and filtered (Celite). DMAC was removed by distillation *in vacuo* (<1 mm, bath up to 50°). The residual dark-red oil was dissolved in EtOAc (300 ml), and the Norit-treated solution was filtered through a compressed mat of silica gel (Silica Gel H, Stahl) of 3-cm thickness on a 10-cm diameter Büchner funnel. The undisturbed mat was washed with EtOAc (~400 ml), and the filtrate and wash solution were combined. Removal of the EtOAc left crude **2a** as an orange-colored oil, which was redissolved in EtOAc (1 ml/g), and the seeded solution was refrigerated overnight. (The oily, crude product from an earlier run partially crystallized when left to stand for several days.) The first crop of **2a**, mp 77–79°, amounted to 34.2 g. Two additional crops of 9.5 and 1.1 g were obtained from EtOAc in the same manner, and a final crop of 8.8 g was obtained from EtOAc-

Et_2O (3:2 v/v): total yield 53%. The later crops each had mp 77–78°. A sample recrystallized twice from C_6H_6–cyclohexane had mp 79–80°. *Anal.* ($C_{15}H_{24}N_2O_7$) C, H, N.

Diethyl Acetamido[4-(2-oxo-3-oxazolidinyl)butyl]malonate (2b). Alkylation of the Na derivative of **1** with 3-(4-chlorobutyl)-2-oxazolidinone[13] was carried out on a 0.614-mol scale as described for the preparation of **2a**. The Norit treatment and filtration (silica gel mat) of an EtOAc solution of the crude residue (from removal of the DMAC) were performed twice. Following the removal of EtOAc, the orange oil was redissolved in EtOAc, and addition of ligroine (bp 30–60°) caused partial separation of crude **2b** (85.2 g), mp 71–77°, which was recrystallized from C_6H_6–cyclohexane to give pure **2b** (69.6 g), mp 80–81°. The residue from evaporation of the EtOAc–ligroine filtrate was chromatographed on a silica gel column, and elution with EtOAc led to additional crops of crude product, which were recrystallized from C_6H_6–cyclohexane to give pure **2b** (29.2 g): mp 81–83°; total yield 45%. An analytical sample (from C_6H_6–cyclohexane) had mp 81–82°. *Anal.* ($C_{16}H_{26}N_2O_7$) C, H, N.

Diethyl Acetamido[3-(2-bromoethylamino)propyl]malonate Hydrobromide (3a). A solution of **2a** (25.0 g) in freshly prepared 30% dry HBr in AcOH solution (125 ml) was stirred at 25–30° for 43 hr. Addition of Et_2O (500 ml) precipitated **3a** as a viscous gum, which was washed with three 500-ml portions of Et_2O by decantation. The remaining gum was extracted with portions of boiling EtOAc (4 l. total), and evaporation of the clarified EtOAc solution gave a crystalline residue. Recrystallization from MeCN–Et_2O gave pure **3a**, mp 111–113°, in 63% yield (22.6 g). *Anal.* ($C_{14}H_{25}BrN_2O_5 \cdot HBr$) C, H, N.

Diethyl Acetamido[4-(2-bromoethylamino)butyl]malonate Hydrobromide (3b). A solution of **2b** (99.1 g) in 30% dry HBr in AcOH solution (500 ml) was stirred at 25–30° for 72 hr and then added in a thin stream to stirred Et_2O (2.5 l.). The clear supernatant was removed by decantation from the gummy precipitate, which was stirred with three more 2.5-l. portions of Et_2O. The still-gummy residue was dissolved in boiling EtOAc (900 ml), and a small crop (5.1 g) of **3b** separated from the cooled solution. The filtrate was evaporated, and the gummy residue was dissolved in EtOAc–EtOH solution (9:1, 750 ml). Evaporation of this solution gave a solid residue, which was combined with the small first crop and recrystallized from MeCN–Et_2O to give pure **3b**, mp 147–149°, in 65% yield (85.4 g). *Anal.* ($C_{15}H_{27}BrN_2O_5 \cdot HBr$) C, H, Br, N.

***S*-2-[4-Acetamido-4,4-bis(ethoxycarbonyl)butylamino]ethyl Hydrogen Thiosulfate (5a).** A mixture of 20.00 mmol each of $Tl_2S_2O_3$ (10.418 g) and **3a** (9.244 g) in H_2O (35 ml) was stirred at 25–30° for 64 hr, filtered from TlBr, and evaporated to dryness (bath at 25–30°, final pressure <1 mm). The residual syrup was stirred with EtOH (250 ml); the solution was clarified by filtration and evaporated to dryness. The glassy residue was again dissolved in EtOH (300 ml), and the Norit-treated and filtered (Celite) solution was evaporated as above to give **5a** as an amorphous, deliquescent, solidified foam, which was pulverized under Et_2O, collected under N_2, and dried *in vacuo* (25–30°, P_2O_5): yield 77% (6.40 g); ir (KBr) 3400 (NH), 2980, 2830 (aliphatic CH), 1730 (ester C=O), 1660 (amide I), 1500 (amide II), 1190, 1015, 620 cm^{-1} (SSO_3^-); pmr (DMSO-d_6-TMS) δ 1.15, 1.90, 0.8–2.2 (t, s, m, 13, CH_3CH_2, $COCH_3$, $CCH_2CH_2CH_2$), 2.6–3.5 (m, 6, $CH_2NCH_2CH_2S$), 4.12 (q, 4, CH_3CH_2), 8.2 (s, 1, CONH), 8.0–8.6 (2, br s, NH_2^+). *Anal.* ($C_{14}H_{26}N_2O_8S_2$) C, H, N, S. A 30.3-mmol run gave **5a** in 87% yield (12.5 g); ir spectrum identical with that of analytical sample.

***S*-2-[5-Acetamido-5,5-bis(ethoxycarbonyl)pentylamino]ethyl Hydrogen Thiosulfate (5b).** Treatment of **3b** (28.57 g, 60.00 mmol) with an equimolar amount of $Tl_2S_2O_3$ (31.25 g) in H_2O (100 ml) and subsequent work-up like that described for **5a** gave deliquescent **5b** as an amorphous glass in 96% yield (24.7 g): ir (KBr) 3380 (NH), 2980, 2870 (aliphatic CH), 1740 (ester C=O), 1665 (amide I), 1515 (amide II), 1190, 1020, 625 cm^{-1} (SSO_3^-); pmr (D_2O-DSS) δ 1.23, 2.05, 0.9–2.4 (t, s, m, 15, CH_3CH_2, $COCH_3$, $CCH_2CH_2CH_2CH_2$), 3.1 (br t, 2, $CH_2CH_2CH_2N$), 3.2–3.7 (m, 4, NCH_2CH_2S), 4.27 (q, 4, CH_3CH_2). *Anal.* ($C_{15}H_{28}N_2O_8S_2$) C, H, N, S.

N^5-(2-Mercaptoethyl)ornithine Dihydrochloride (6a). A solution of **5a** (10.983 g, 26.50 mmol) in 6 *N* HCl (200 ml) was refluxed under N_2 for 4.5 hr, cooled to ~80°, treated with a solution of $Ba(OAc)_2$ (6.769 g, 26.50 mmol) in H_2O (50 ml), kept at ~80° for 30 min, cooled in an ice bath, and stored in a refrigerator overnight. The mixture was filtered from $BaSO_4$, and the filtrate was evaporated to near dryness. The remaining syrup was dissolved in H_2O (50 ml), and the solution was treated with Norit, filtered (Celite), and evaporated to ~20 ml. The colorless solution was freeze-dried to give deliquescent **6a** as a frothy glass, which was dried further *in vacuo* (25–30°, P_2O_5), broken up under Et_2O, collected under N_2, and redried as before: yield 97% (6.80 g); ir (KBr) 3300–2100 (NH_3^+), 1960 (amino acid hydrochloride), 1740 (C=O), 1580 (NH_3^+ deformation), 1200 cm^{-1} (CO_2H deformation);† pmr (DMSO-d_6-TMS) δ 1.5–2.3 (br m, 4, $CCH_2CH_2CH_2$), 2.5–3.6 (br m, 6, $CH_2NCH_2CH_2SH$), 3.9 (br m, 1, CHNH), 9 (v br s, ~7, NH_3^+, NH_2^+, OH, SH; not observed separately because of exchanging). *Anal.* ($C_7H_{16}N_2O_2S \cdot 2HCl \cdot 0.6H_2O$) C, H, Cl, N, S; SH (by iodometric titration).

N^6-(2-Mercaptoethyl)lysine Dihydrochloride (6b). Hydrolysis of **5b** (13.1 g) and subsequent treatment of the solution with $Ba(OAc)_2$ was carried out as described for the conversion of **5a** to **6a**. The filtered solution was evaporated under reduced pressure to dryness with filtrations at half-volume (to remove a small amount of crystalline $BaCl_2$) and at ~50 ml (to remove a trace of other insoluble material). The clear glassy residue was kept *in vacuo* over P_2O_5 for 66 hr, pulverized under Et_2O, collected under N_2, and dried *in vacuo* (25–30°, P_2O_5) to give deliquescent **6b** in 70% yield (6.1 g): ir (KBr) 3300–2100 (NH_3^+), 1980 (amino acid hydrochloride), 1735 (C=O), 1580 (NH_3^+ deformation), 1200 cm^{-1} (CO_2H deformation);† pmr (D_2O-DSS) δ 1.2–2.3 (m, 6, $CCH_2CH_2CH_2CH_2$), 2.7–3.6 (m, 6, $CH_2CH_2NCH_2CH_2S$), 4.10 (t, 1, $NCHCH_2$). *Anal.* ($C_8H_{18}N_2O_2S \cdot 2HCl \cdot 0.3H_2O$) C, H, Cl, N; SH: calcd, 11.62; found, 11.18 (by iodometric titration).

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† *Cf.* ref 14 for the interpretation of amino acid spectra.

Synthesis of *dl*-3-(Hydroxymethyl)tyrosine

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Dopa has been widely used in the treatment of Parkinson's disease. The efficacy of *l*-Dopa has been attributed to the penetration of *l*-Dopa and its metabolic conversion into dopamine in the extrapyramidal brain centers.[1,2]