Synthesis and Biologic Distribution of Mercapto Derivatives of Palmitic Acid

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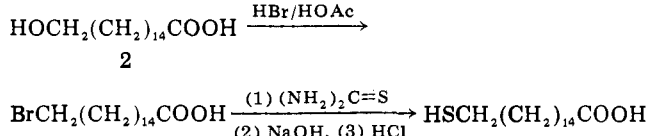
Mercapto derivatives of palmitic acid are capable of binding ^{99m}Tc. Based on the hypothesis that ^{99m}Tc-labeled palmitic acid derivatives would behave biologically like palmitic acid and thus could be used as myocardial imaging agents, three mercaptopalmitic acid derivatives have been prepared. The synthesis of 2-mercaptopalmitic acid, 2-mercapto-1,16-hexadecanedioic acid, and 16-mercaptopalmitic acid was accomplished by reaction of the corresponding bromo compound with thiourea. The ^{35}S-labeled compounds and [16-^{14}C]palmitic acid were evaluated in rats with a heat-inflicted myocardial infarction to study the effect of the introduction of the mercapto group. The organ distribution of 2-[^{35}S]mercaptopalmitic acid was most similar to that of [16-^{14}C]palmitic acid.

Nonesterified long-chain fatty acids (C_{14}–C_{18}) serve as the major energy source for the normal functioning myocardium.[2a] Evans and co-workers[2b] suggested that regions of the heart with diminished arterial blood flow or impaired metabolism might be detected externally through the use of [^{131}I]oleic acid. Reduced uptake of γ-emitting radiolabeled fatty acids in areas of myocardial ischemia or infarction was later demonstrated with [^{131}I]oleic acid,[3a-c] 16-[^{123}I]iodo-9-hexadecenoic acid,[4] and [1-^{11}C]palmitic acid.[5] The above fatty acids suffer from a number of drawbacks. The stability in vivo of radioiodinated compounds is limited because of dehalogenating enzymes; in addition, ^{131}I (β,γ emitter, γ-ray energy of 364 keV, half-life = 8.04 days) has poor physical decay properties for external imaging with a γ-scintillation camera, while ^{123}I is expensive and not readily available. The use of ^{11}C-labeled compounds is limited to medical centers having cyclotrons and positron cameras due to its 20-min half-life and its mode of decay (positron emission) resulting in a penetrating γ radiation of 511 keV.

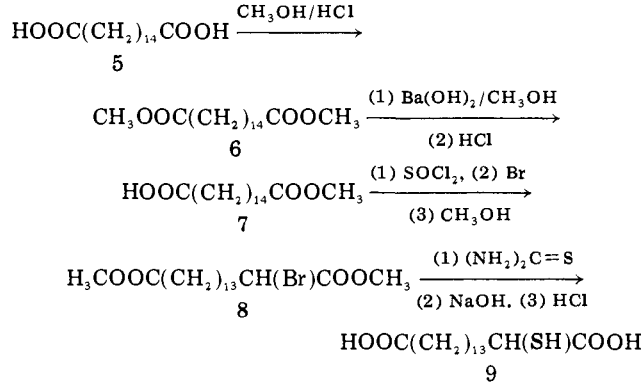
Technetium-99m is the isotope of choice in nuclear medicine because of its excellent physical properties (γ-ray energy of 140 keV, $t_{1/2}$ = 6 h) and its ready availability from ^{99}Mo–^{99m}Tc generators.[6] However, binding of technetium to fatty acids can be achieved only by first attaching a chelating group to the biologic substrate. Such an approach has been taken by Eckelman et al.,[7] who labeled with ^{99m}Tc derivatives of palmitic acid containing poly(amino)poly(carboxylic acids) as chelating groups. The myocardial uptake of these derivatives[8] was lower than that of [^{3}H]palmitic acid. This was attributed to the difference in polarity between [^{3}H]palmitic acid and the ^{99m}Tc-labeled fatty acid derivatives. A similar approach was taken by Loberg et al.,[9] who labeled undecanoic acid and a mixture of undecane and undecanoic acid with ^{99m}Tc using carbamoylmethyl iminodiacetate as the chelating group. The biologic distribution of these compounds was different from that of [^{3}H]palmitic acid, and this difference was thought to be due to the lower lipophilicity of the ^{99m}Tc-labeled compounds.

Aliphatic compounds containing a sulfhydryl function α to a carboxy group and aromatic mercaptans are known to bind technetium and are used in analytical chemistry to determine the element quantitatively.[10a,b] The above chelating groups are also present in several ^{99m}Tc-labeled radiopharmaceuticals.[11a,b] In our laboratory, three mercapto derivatives of palmitic acid, **1**, **4**, and **9**, have been synthesized, two of which (**4** and **9**) are new compounds. In order to study the effect that the introduction of a mercapto group has on the biologic distribution of palmitic acid, the ^{35}S-labeled derivatives **11**–**13** were prepared. The biologic distribution of the ^{35}S-labeled analogues was compared with that of [16-^{14}C]palmitic acid (**10**) in rats with heat-induced myocardial lesions.[12]

Scheme I



Scheme II



Chemistry. 2-Mercaptopalmitic acid (**1**) had been previously prepared.[13] The synthesis of 16-mercaptopalmitic acid (**4**) is shown in Scheme I. The synthesis of 2-mercapto-1,16-hexadecanedioic acid (**9**) is shown in Scheme II.

The [^{35}S]mercapto analogues were formed by the reaction of [^{35}S]thiourea with the corresponding bromo-substituted compounds.

Tissue Distribution. The concentrations of the radiolabeled fatty acids in normal and damaged rat myocardium and their ratios at various time intervals are listed in Table I. In all of the compounds tested, concentration of the radioactivity in the normal heart tissue decreased over a 60-min period. The temporal change of percent injected dose per gram in the damaged myocardium varied from compound to compound.

Except for **13**, the concentration ratio of normal to infarct tissue at all time intervals was greater than one. The highest concentration ratio was obtained with **11** at 5-min postinjection. Tables II and III show the biodistribution of **10**–**13** in various rat tissues as a function of time. The blood level of radioactivity of the [^{35}S]mercapto derivatives was higher than that of **10** at all times. The percent of injected dose per gram of liver decreased between 5 and 15 min in all of the compounds except **12**. The kidney uptake of **13** was higher at all times than uptake of the other compounds. Radioactivity concentration of **10** in muscle was similar to that of **11** and **12** and two to

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Table I. Normal and Infarcted Myocardial Uptake in Rats and Their Ratios Following Intravenous Administration of Radiolabeled Fatty Acids

compd	5 min[a]			15 min			60 min		
	N[b]	I[b]	N/I	N	I	N/I	N	I	N/I
[16-^{14}C]10	2.65 ± 0.75[c]	1.29 ± 0.23	2.25 ± 0.69	2.04 ± 0.79	0.59 ± 0.38	4.95 ± 3.07	0.89 ± 0.22	0.59 ± 0.24	1.67 ± 0.61
[^{35}S]11	3.76 ± 0.09	0.90 ± 0.55	5.15 ± 3.25	3.32 ± 0.23	0.99 ± 0.03	3.32 ± 0.13	3.09 ± 0.70	1.19 ± 0.09	2.57 ± 0.38
[^{35}S]12	1.85 ± 0.61	1.04 ± 0.24	1.76 ± 0.45	1.47 ± 0.69	1.22 ± 0.60	1.12 ± 0.01	0.72 ± 0.19	0.59 ± 0.21	1.24 ± 0.36
[^{35}S]13	0.63 ± 0.18	0.16 ± 0.19	0.54 ± 0.12	0.29 ± 0.08	0.58 ± 0.16	0.50 ± 0.08	0.16 ± 0.04	0.31 ± 0.20	0.60 ± 0.27

[a] Three to five rats sacrificed at each time. [b] N, normal myocardium; I, infarcted myocardium. [c] Expressed as percent injected dose per gram of fresh tissue ± SD.

Table II. Biodistribution of Palmitic Acid and Three Mercapto Derivatives in Rat Tissues (Expressed as Percent Injected Dose per Gram).

tissue	[16-^{14}C]10: time, min			[^{35}S]11: time, min			[^{35}S]12: time, min			[^{35}S]13: time, min		
	5[a]	15	60	5	15	60	5	15	60	5	15	60
blood	0.09 ± 0.03	0.10 ± 0.04	0.09 ± 0.01	0.21 ± 0.12	0.46 ± 0.02	0.54 ± 0.01	0.28 ± 0.14	0.33 ± 0.16	0.27 ± 0.01	1.13 ± 0.28	0.47 ± 0.16	0.14 ± 0.03
liver	4.84 ± 1.13	3.33 ± 1.44	2.01 ± 1.02	5.36 ± 0.27	4.69 ± 0.05	2.42 ± 0.01	4.10 ± 2.61	4.27 ± 0.64	2.59 ± 0.63	3.18 ± 1.22	2.95 ± 0.90	1.27 ± 0.15
lung	2.78 ± 1.56	2.26 ± 0.32	1.89 ± 0.12	2.24 ± 0.95	1.98 ± 0.24	1.05 ± 0.18	1.17 ± 0.52	0.90 ± 0.15	0.63 ± 0.19	0.94 ± 0.48	0.43 ± 0.16	0.88 ± 0.52
spleen	0.43 ± 0.16	0.42 ± 0.14	0.39 ± 0.18	0.61 ± 0.14	0.76 ± 0.09	0.49 ± 0.05	0.43 ± 0.22	0.42 ± 0.16	0.66 ± 0.46	0.37 ± 0.09	0.22 ± 0.04	0.14 ± 0.00
kidney	1.75 ± 0.83	1.17 ± 0.54	0.73 ± 0.33	1.15 ± 0.15	1.62 ± 0.11	1.36 ± 0.05	1.49 ± 0.58	1.75 ± 0.35	1.03 ± 0.38	2.31 ± 0.44	4.69 ± 2.32	1.54 ± 0.31
muscle	0.34 ± 0.09	0.26 ± 0.12	0.20 ± 0.11	0.22 ± 0.03	0.22 ± 0.01	0.18 ± 0.00	0.32 ± 0.11	0.30 ± 0.08	0.26 ± 0.18	0.13 ± 0.05	0.13 ± 0.04	0.07 ± 0.01

[a] Three to five rats sacrificed at each time.

Table III. Biodistribution of Palmitic Acid and Three Mercapto Derivatives in Rat Tissues (Expressed as Percent Injected Dose per Organ)

tissue	[16-^{14}C]10: time, min			[^{35}S]11: time, min			[^{35}S]12: time, min			[^{35}S]13: time, min		
	5[a]	15	60	5	15	60	5	15	60	5	15	60
blood[b]	1.19 ± 0.56	1.66 ± 0.65	1.45 ± 0.19	2.70 ± 1.06	5.90 ± 0.22	7.54 ± 0.34	4.96 ± 0.66	5.47 ± 0.07	4.67 ± 1.1	18.25 ± 4.14	13.14 ± 5.42	5.04 ± 2.69
liver	48.09 ± 4.46	36.20 ± 7.07	23.85 ± 9.09	62.42 ± 2.17	53.74 ± 1.40	30.13 ± 1.76	40.35 ± 12.32	45.95 ± 7.25	26.45 ± 2.88	51.21 ± 8.75	34.79 ± 5.15	17.05 ± 2.86
lung	2.39 ± 0.41	2.44 ± 0.69	2.33 ± 0.15	2.83 ± 1.27	2.48 ± 0.41	1.33 ± 0.18	1.76 ± 0.29	1.46 ± 0.85	0.92 ± 0.27	2.37 ± 1.44	0.76 ± 0.31	0.53 ± 0.01
spleen	0.24 ± 0.02	0.24 ± 0.06	0.23 ± 0.10	0.28 ± 0.03	0.48 ± 0.01	0.33 ± 0.02	0.27 ± 0.13	1.15 ± 0.02	1.22 ± 0.98	0.23 ± 0.03	0.16 ± 0.02	0.10 ± 0.04
kidney	2.71 ± 0.63	2.26 ± 0.70	1.53 ± 0.55	2.40 ± 0.18	3.41 ± 0.28	2.57 ± 0.07	2.29 ± 0.25	2.86 ± 0.38	1.95 ± 0.37	4.41 ± 0.39	10.48 ± 4.10	3.21 ± 0.10
muscle[c]	26.06 ± 3.02	23.99 ± 7.47	19.63 ± 7.43	19.78 ± 2.58	19.80 ± 0.90	16.54 ± 0.64	34.33 ± 5.87	28.15 ± 0.94	25.39 ± 10.10	15.27 ± 7.29	16.26 ± 3.44	9.67 ± 1.51

[a] Three to five rats sacrificed at each time. [b] Estimated as 6% of body weight. [c] Estimated as 40% of body weight.

three times higher than that of **13**.

Discussion

Of the three [^{35}S]mercapto compounds, the biologic distribution of **13** deviates most from that of **10**. This is not unexpected, since **13** is a diacid and, because of an increase in hydrophilic character, it probably does not cross cell membranes as readily as **10**. However, [^{99m}Tc]**9** is expected to be more lipophilic than **13**, since the α-mercaptocarboxy would serve as the chelating group while the other carboxy would be free to be activated to the acyl-CoA derivative in vivo.

Pande et al.[14] observed that α-bromopalmitate and α-hydroxypalmitate were activated at approximately 5 to 6% of the rate of palmitate by the microsomal fraction of rat liver. It is conceivable that **11** is also activated by the acyl-CoA synthetase at a slower rate than **10**. This would result in slower metabolism of the mercapto analogue and could explain the higher concentration in the liver of **11** compared to that of **10**. Slower activation of **11** compared to that of **10** could explain the higher concentration of the former in the normal myocardium.

Experimental Section

Melting points were determined on a Fisher Johns apparatus. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y. Column chromatography was performed using 50 g of silica gel (Baker 60–200 mesh) per 1 g of impure compound. NMR spectra were obtained with a Varian T-60 spectrometer in $CDCl_3$ using Me_4Si as internal standard. The IR spectra were obtained on a Perkin-Elmer Model 700 spectrophotometer.

[16-^{14}C]Palmitic acid and [^{35}S]thiourea were obtained from New England Nuclear, Boston, Mass. 2-Bromopalmitic acid and 16-hydroxyhexadecanoic acid were obtained from Aldrich Chemical Co., Milwaukee, Wis. 1,16-Hexadecanedioic acid was obtained from Sapon Laboratories, Oceanside, N.Y. The hexanes purchased from Mallinckrodt had a boiling range of 68–70 °C. Liquid scintillation counting was performed on a Packard Tri-Carb Model 3385 spectrometer.

2-Mercaptopalmitic Acid (1). The procedure of Koenig et al.[15] for synthesis of 2-mercaptostearic acid was followed to yield 63% of pure 1: mp 70–71 °C (lit.[13] mp 71.6–71.8 °C); IR (KBr) 2900, 2550, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.3 (s, 29 H), 2.1 (d, 1 H), 3.3 (m, 1 H), 10.9 (s, 1 H). Anal. ($C_{16}H_{32}O_2S$) C, H, S.

16-Mercaptopalmitic Acid (4). Following the procedure for the preparation of **9**, compound **3**[16] (670 mg, 2 mmol) and thiourea (152 mg, 2 mmol) were reacted to give 500 mg of crude **4**, which was purified by column chromatography. The column was eluted with 125-mL portions of benzene–hexanes (1:1), benzene, benzene–ether (9:1), and benzene–ether (8:2), to give pure **4** (268 mg, 47%): mp 66–67 °C; IR (KBr) 2925, 1700 cm^{-1}; ^1H NMR δ 1.30 (s, 27 H), 2.61–2.27 (m, 4 H), 10.83 (s, 1 H). Anal. ($C_{16}H_{32}O_2S$) C, H, S.

15-Carbomethoxypentadecanoic Acid (7). The partial hydrolysis of **6**[17] as reported in the literature[18] resulted in **7** contaminated with **5** and **6**. It was necessary to purify the crude product by column chromatography. The column was eluted with benzene–ether (3:1). Homogeneous fractions were evaporated, and the product was crystallized from EtOH to give (42%) pure **7**: mp 71–72 °C (lit.[17] mp 67–68 °C); IR (KBr) 1700, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26 (s, 26 H), 2.16–2.50 (m, 2 H), 3.66 (s, 3 H), 9.86 (s, 1 H). Anal. ($C_{17}H_{32}O_4$) C, H.

2-Bromo-1,14-dicarbomethoxytetradecane (8). A procedure similar to that of Sweet[19] for the preparation of ethyl α-bromopalmitate was followed. Thionyl chloride (7 mL) and **7** (1.8 g, 5.99 mmol) were refluxed for 90 min in a 100-mL flask equipped with a dropping funnel and condenser which was protected with a $CaCl_2$ drying tube. Bromine (1.55 g, 9.68 mmol) was then added dropwise over a 3-h period and refluxed for an additional 3 h. The excess thionyl chloride and bromine were removed under vacuum. Methanol (30 mL) was added and the solution was refluxed for 4 h. The solution was evaporated, leaving 2.15 g of crude product, which was purified by column chromatography. The column was eluted with 330-mL portions of benzene–hexanes (1:1), benzene, benzene–ether (9:1), and benzene–ether (4:1). Homogeneous fractions were evaporated, and the product (1.47 g, 62%) was crystallized from hexanes: mp 48–49 °C; IR (KBr) 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.3 (s, 22 H), 1.90–2.46 (m, 4 H), 3.66 (s, 3 H), 3.8 (s, 3 H), 4.26 (t, 1 H). Anal. ($C_{18}H_{33}BrO_4$) C, H, Br.

2-Mercapto-1,16-hexadecanedioic Acid (9). A mixture of **8** (750 mg, 1.9 mmol) and thiourea (145 mg, 1.9 mmol) was refluxed for 4 h in 8 mL of EtOH. The solution was evaporated to dryness, and the remaining solid was stirred for 5 min with 2 × 10 mL of chloroform. The mixture was filtered and the residue was returned to the original flask; 8 mL of EtOH was added and the mixture was warmed until the solid dissolved. A solution of NaOH in 80% EtOH (180 mg/mL, 3 mL) was added slowly with stirring, and reflux continued for 44 h. The solution was cooled in an ice bath, and 4 mL of 4 N HCl was added slowly with stirring. The solution was evaporated to dryness at 40 °C under a stream of N_2. The residue was stirred with 3 × 10 mL of H_2O for 5 min, and the mixture was filtered. The residue (300 mg) was purified by column chromatography. Elution was made with 50-mL portions of benzene–ether (8:2) to give **9** (136 mg, 23%). The compound was crystallized from benzene: mp 114–116 °C; IR (KBr) 2910, 2575, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26 (s, 24 H), 2.06 (d, 1 H), 2.23–2.50 (m, 2 H), 3.16–3.60 (m, 1 H), 9.60 (s, 2 H). Anal. ($C_{16}H_{30}O_4S$) C, H, S.

2-[^{35}S]Mercaptopalmitic Acid (11). [^{35}S]Thiourea (1 mCi, 8×10^{-3} mmol) was transferred with the aid of EtOH (10 mL) to the reaction flask containing 2-bromopalmitic acid (1.68 g, 5 mmol) and thiourea (0.38 g, 5 mmol), and the procedure for **1** was followed to give pure **11** (58%, 0.69 μCi/mg): mp 70–71 °C; chemical and radiochemical purity were confirmed by TLC on silica gel (Eastman 6060) with hexanes–ether–HOAc (70:30:1).

16-[^{35}S]Mercaptopalmitic Acid (12). [^{35}S]Thiourea (0.5 mCi, 0.21 mCi/mg) in 4 mL of EtOH, thiourea (80 mg, 1.05 mmol), and **3** (300 mg, 0.9 mmol) were reacted according to the procedure for **4** to give pure **12** (90 mg, 1.94 μCi/mg, 35%): mp 66–67 °C; chemical and radiochemical purity were checked by TLC on silica gel with hexanes–ether–HOAc (50:50:1).

2-[^{35}S]Mercapto-1,16-hexadecanedioic Acid (13). [^{35}S]-Thiourea (0.5 mCi, 0.21 mCi/mg) in EtOH (4 mL) was added to a flask containing **8** (370 mg, 0.94 mmol) and thiourea (76 mg, 1 mmol). The procedure described previously for **9** was followed, yielding pure **13** (73 mg, 1.71 μCi/mg, 25%): mp 114–116 °C; chemical and radiochemical purity were checked by TLC on silica gel with ether–HOAc (9:1).

Preparation of Radioactive Compounds for Tissue Distribution Studies. An aliquot (1 mL) of [16-^{14}C]palmitic acid in hexane (53 mCi/mmol) was evaporated under nitrogen. The residue was dissolved in propylene glycol (4 mL). The ^{35}S-labeled compounds were dissolved in propylene glycol (5 mg/mL).

Damaged Heart Model. The model used has been described previously.[20] Sprague–Dawley rats (210–300 g) were anesthetized with ether. A midline thoracotomy was performed and an incision made between the seventh and eighth ribs to allow access to the ventricle. The ventricle was touched for 1 s with a hot soldering iron (Wahl Iso-tip, regular size). This was repeated four or five times in adjacent areas to produce a circular pattern. The chest was then closed.

Tissue Distribution Studies. Two and a half to three hours after the infarct was made, 0.2 mL (0.5–5.0 μCi) of the radiolabeled compound was injected through the saphenous vein. The rats were sacrificed by ether asphyxiation at 5, 15, and 60 min after dosing. Before myocardial activity ceased, blood samples were obtained by cardiac puncture. The appropriate organs were then excised. Specimens were placed in liquid scintillation vials; Protosol (1 mL, New England Nuclear) was added, and the vials were placed in an oven at 50 °C overnight. Aquasol (10 mL, New England Nuclear) was added and, after a dark adaptation of 48 h, the samples were counted in a liquid scintillation spectrometer. The channels ratio method was used for quench correction.

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Potential Radiosensitizing Agents. Dinitroimidazoles[1]

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New compounds of the nitroimidazole series have been synthesized as radiosensitizers which selectively sensitize hypoxic cells to the lethal effect of radiation. The reaction of 2,4(5)-dinitroimidazole (**2**) with chloroethanol or hydrochloric acid yielded 4(5)-nitro-5(4)-chloroimidazole (**3**), which upon reaction with ethylene oxide yielded the 4-nitro-5-chloroimidazole-1-ethanol (**6**). Reaction of **2** with ethylene oxide resulted in a mixture of two compounds, the 2,4-dinitroimidazole-1-ethanol (**4**) and 2,3-dihydro-5-nitroimidazo[2,1-*b*]oxazole (**5**). The structure of the new heterocyclic compound **5** was confirmed by ^{1}H NMR, mass spectrum, and X-ray crystallography. These agents were tested for their ability to sensitize hypoxic *Escherichia coli* cells to killing by ionizing radiation. Compound **4** was found to be the most active agent of this series of compounds.

In the radiotherapy of cancer, the relative resistance of hypoxic cells present in solid tumors is a serious limitation in attempts to increase the therapeutic ratio between tumor and normal tissue damage.[2] Ionizing radiation inactivates cells in solid tumors according to a multicomponent survival curve which is related to the variation in oxygen concentration within the tumor mass.[3] Employment of hyperbaric oxygen chambers, fast neutrons, or π^- meson beams are being considered as approaches to overcome this problem of hypoxic resistance in radiotherapy. However, another logical approach which has received more attention recently is that of chemical sensitizers designed to selectively sensitize hypoxic neoplastic cells to the lethal effect of radiation.[4] The rationale for the radiosensitizing drugs is that these agents are not rapidly metabolized and can diffuse from the end of the blood capillaries to the hypoxic cells in tumors. Although the ability of these compounds to sensitize hypoxic cells is directly related to the electron affinity of the molecule, the molecular aspects of the mechanism of action are at present vague. The search for the radiosensitizing compounds was greatly facilitated by the work of Adams and his associates[5-7] and of Chapman and his co-workers[8-12] who showed that a variety of electron-affinic compounds were capable of sensitizing hypoxic bacterial and mammalian cell populations to the lethal effect of radiation.

A series of nitrobenzene analogues have been tested against Chinese hamster cells for their ability to sensitize hypoxic cells to X irradiation.[13] Cellular radiosensitization was found to correlate with drug electron affinity, as measured by the Hammett σ constant.[13] Several nitrofurans were also shown to possess significant radiosensitization properties when tested in vitro.[9] However, both the nitrobenzenes and nitrofurans failed to achieve significant radiosensitization in vivo.[4] A series of nitropyrroles have also been reported recently;[14] the most effective derivative of this class was *N*-(hydroxyethyl)-2-cyano-5-nitropyrrole. These latter studies also indicated that *N*-(hydroxyethyl) substitution decreased toxicity relative to N-CH_3, N-C_2H_5, and N-C_3H_7 substitution. The role of the partition coefficient in radiosensitization of hypoxic cells has also been examined;[15] lipophilicity seemed to have an insignificant effect on in vitro radiosensitization but may possess a greater role in vivo.

Metronidazole (Flagyl), a 5-nitroimidazole derivative, has been reported to sensitize tumor hypoxic cells both in vitro and in vivo.[11,16] Asquith et al.[17] have reported a 2-nitroimidazole (azomycin) derivative, 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol (Ro-07-0582, misonidazole), to be a most effective radiosensitizing agent. Misonidazole has since been found to be an effective radiosensitizer of hypoxic cells in at least 16 different animal tumors[18] and is currently under clinical trials.[19] However, the high doses of misonidazole required for activity were found to be a limiting factor because of resulting neurotoxicity. Convulsions and peripheral neuropathy were encountered in a relatively large number of patients.[19] We, therefore, have undertaken a systematic approach to design and synthesize various analogues of nitroimidazoles in order to study the relationship between structure and biological activity that might lead to a more advantageous sensitizing agent. Initially, a series of nitroimidazoles were examined in *E. coli* cells and compared with misonidazole for toxic side effects on end points such as mutagenesis, cell killing, and inhibition of the synthesis of the inducible enzyme β-galactosidase.[20] These results indicated that 2,4(5)-dinitroimidazole (**2**) was the most promising agent for further study because it exhibited good radiosensitization coupled with low toxicity and mutagenicity.[20] Compound **2** was initially synthesized in an effort to increase the electron affinity of the 2-nitroimidazole nucleus by inserting an additional electron-

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