Notes

Mixed Bis(thiosemicarbazone) Ligands for the Preparation of Copper Radiopharmaceuticals: Synthesis and Evaluation of Tetradentate Ligands Containing Two Dissimilar Thiosemicarbazone Functions

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A series of four "mixed" bis(thiosemicarbazone) keto aldehyde derivatives containing dissimilar thiosemicarbazone functions were synthesized and evaluated as ligands for preparation of radiocopper-labeled radiopharmaceuticals. The pyruvaldehyde-based mixed bis(thiosemicarbazone) ligands $CH_3C[{=}NNHC(S)NH_2]CH[{=}NNHC(S)NHMe]$ (**4a**), $CH_3C[{=}NNHC(S)NHMe]CH[{=}NNHC(S)NH_2]$ (**4b**), $CH_3C[{=}NNHC(S)NH_2]CH[{=}NNHC(S)NMe_2]$ (**4c**), and $CH_3C[{=}NNHC(S)NHMe]CH[{=}NNHC(S)NMe_2]$ (**4d**) were obtained by reaction of thiosemicarbazide, N^4-methylthiosemicarbazide, or N^4,N^4-dimethylthiosemicarbazide with pyruvaldehyde 2-thiosemicarbazones that had been generated by oxidative cleavage of the appropriate pyruvic aldehyde dimethyl acetal 2-thiosemicarbazone. The ^{67}Cu-labeled complexes of ligands **4a**−**d** were prepared and screened in a rat model to assess the potential of each chelate as a ^{62}Cu radiopharmaceutical for imaging with positron emission tomography. In the rat model the ^{67}Cu complexes of ligands **4a**−**d** exhibit significant uptake into the brain and heart after intravenous injection, following trends similar to those previously reported for the related bis(thiosemicarbazone) complexes, Cu−PTS, Cu−PTSM, and Cu−$PTSM_2$ (derived from pyruvaldehyde bis(thiosemicarbazone), pyruvaldehyde bis(N^4-methylthiosemicarbazone), and pyruvaldehyde bis(N^4,N^4-dimethylthiosemicarbazone), respectively). Ultrafiltration studies using solutions of dog and human serum albumin reveal that the ^{67}Cu complexes of ligands **4a**−**d**, like the Cu(II) complex of pyruvaldehyde bis(N^4-methylthiosemicarbazone), interact more strongly with human albumin than dog albumin.

Introduction

Copper-62-labeled pyruvaldehyde bis(N^4-methylthiosemicarbazonato)copper(II), Cu−PTSM (Figure 1, $R_1 = R_2 = NHCH_3$), has been widely investigated as a generator-based radiopharmaceutical for evaluation of regional tissue perfusion with positron emission tomography (PET).[1−10] The ^{62}Cu−PTSM radiopharmaceutical is attractive as a marker of both cerebral and myocardial perfusion, since following intravenous administration it affords reasonably high first-pass extraction accompanied by prolonged microsphere-like tissue retention of the copper radiolabel. Thus, Cu−PTSM allows the 9.3 min ^{62}Cu half-life to be exploited with relatively long image acquisition periods designed to afford good counting statistics, while the physical decay of the radiolabel remains sufficiently rapid to permit repeat imaging without excessive delay between scans. Unfortunately, contrary to experience with the dog model,[3] in humans it is found that myocardial perfusion is underestimated with Cu−PTSM at high rates of flow.[4−8]

The interspecies variability in the performance of ^{62}Cu−PTSM as a perfusion tracer appears to result from species-dependent binding of the radiopharmaceutical to serum albumin, with the radiotracer's interaction being especially strong with human serum albumin.[11,12] An extensive number of related bis(thiosemicarbazone) complexes derived from β-keto aldehydes have been studied.[13] Two related agents, Cu−ETS and Cu−*n*-PrTS, have been identified that do not exhibit the problem of interspecies variability in albumin binding that has been problematic with Cu−PTSM.[12] This has led to interest in exploration of copper(II) radiopharmaceutical chemistry with bis(thiosemicarbazone) ligands exhibiting greater structural diversity.

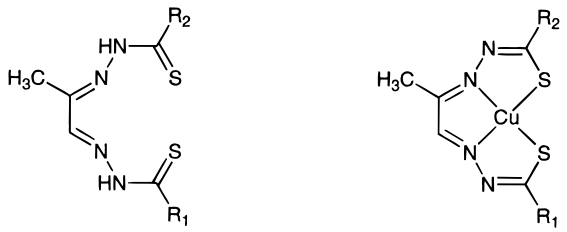

Figure 1. General structural formula of (A) the pyruvaldehyde bis(thiosemicarbazone) ligands and (B) the corresponding bis(thiosemicarbazonato)copper(II) complexes.

The properties of the copper(II) bis(thiosemicarbazone) radiopharmaceuticals are known to be quite sensitive to substitution patterns at the R_1 and R_2 sites (Figure 1, where $R_1, R_2 = NH_2$, $NHCH_3$, or $N(CH_3)_2$).[13−15] Unfortunately, conventional synthetic methods (involving condensation of a β-ketoaldehyde with two equivalents of a thiosemicarbazide derivative) generate only ligands in which R_1 and R_2 cannot be independently varied (*i.e.*, $R_1 = R_2$). To gain access to ligands in which

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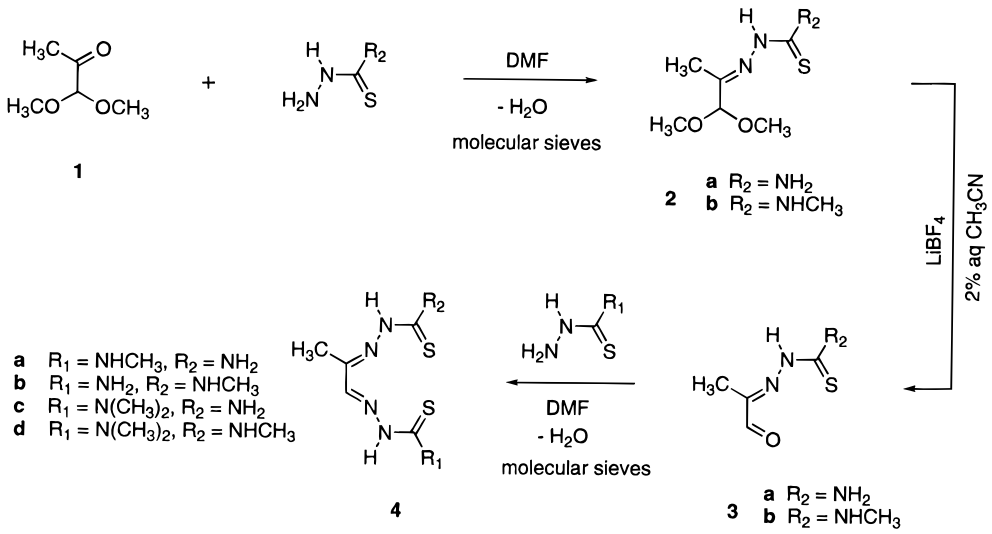

Figure 2. Synthetic route successfully employed for preparation of the mixed bis(thiosemicarbazone) ligands and copper(II) chelates.

the properties of the final Cu complex might be more finely-tuned to optimize radiopharmaceutical biodistribution and pharmacokinetics, it is desirable to develop methods for preparation of "mixed" bis(thiosemicarbazone) ligands containing dissimilar thiosemicarbazone functions (*i.e.*, $R_1 \neq R_2$). We report here the development of synthetic methods that provide access to such mixed bis(thiosemicarbazone) radiopharmaceuticals.

Results and Discussion

Cold Chemistry. Attempts to generate pure samples of the mixed bis(thiosemicarbazone) ligands by methods described in the literature[16] produced unsatisfactory results. Our attempts to selectively synthesize mixed bis(thiosemicarbazones) by stepwise reaction of dissimilar thiosemicarbazides with pyruvic aldehyde failed because we were unable to cleanly isolate the β-keto imine intermediate; reaction of pyruvaldehyde with 1 equiv of thiosemicarbazide or *N* [4]-methylthiosemicarbazide resulted in condensation at both the aldehyde and ketone sites to generate a mixture of both possible monothiosemicarbazones and the "unmixed" bis(thiosemicarbazone). We were also unsuccessful with the second reported synthetic route in which a thiosemicarbazide is first condensed onto pyruvic aldehyde 1-oxime to generate the thiosemicarbazone 1-oxime. Selective hydrolysis of the oxime moiety, followed by reaction with a dissimilar thiosemicarbazide, reportedly[16] provides the desired mixed bis(thiosemicarbazone) ligands; however, in our hands this method invariably resulted in the formation of a mixture of both unmixed and mixed bis(thiosemicarbazones). NMR studies of the reaction products showed four imine C−H proton resonances in approximate 1:1:1:1 ratios, indicating nearly random mixed and unmixed product formation (assignments subsequently confirmed by comparison to the authentic products). Therefore, an alternative strategy was devised for the synthesis of the desired mixed bis(thiosemicarbazone) ligands.

The route successfully employed for selective synthesis of the desired mixed bis(thiosemicarbazone) ligands is outlined in Figure 2. One equivalent of a thiosemicarbazide was condensed with pyruvic aldehyde dimethyl acetal, **1**, to form the pyruvic aldehyde dimethyl acetal 2-thiosemicarbazone, **2**. Our attempts to hydrolyze the acetal in protic acid media resulted in the concomitant hydrolysis of the imine as well, ultimately yielding a mixture of bis(thiosemicarbazones) analogous to that obtained by the thiosemicarbazone 1-oxime route. This problem was resolved through the oxidative cleavage of the acetal with a mild Lewis acid, lithium tetrafluoroborate, in 2% aqeous acetonitrile at roomtemperature over 30 min to cleanly and efficiently generate the pyruvaldehyde 2-thiosemicarbazones **3a** and **3b**. The final step in the synthesis involved a second condensation reaction of **3** with a dissimilar thiosemicarbazide to yield the desired mixed bis(thiosemicarbazone) ligands **4a**−**d**.

Radiochemistry. The no-carrier-added ^{67}Cu complexes of the tetradentate mixed bis(thiosemicarbazone) ligands, **4a**−**d**, were prepared by reaction of the $^{67}Cu^{2+}$ ion with the thiosemicarbazone ligand in an ethanolic solution of acetate buffer. The radiochemical purity of the radiotracers was always found to exceed 98% by thin layer chromatography. As expected, the lipophilicity of these compounds increases with increasing methyl substitution at the terminal amine groups of the ligand backbone. Measured octanol/water partition coefficients, *P*, for the ^{67}Cu complexes of mixed bis(thiosemicarbazone) ligands **4a**−**d**[17] fall between the values previously reported for the corresponding unmixed complexes, Cu−PTS, Cu−PTSM, and Cu−$PTSM_2$.[13] Complex lipophilicity was found to depend somewhat on the position of the *N*-alkyl group relative to the pyruvaldehyde methyl; for example, the copper complex of ligand **4b** is slightly more lipophilic than the isomeric complex of ligand **4a** ($\Delta \log P = 0.16$).

Animal Studies. The biodistribution of each ^{67}Cu complex was determined in Sprague−Dawley rats at 1, 5, and 120 min following femoral vein injection of the radiotracer in order to screen for possible utility in imaging the brain and/or heart. The potential clinical utility of the ^{62}Cu−bis(thiosemicarbazone) radiopharmaceuticals as perfusion tracers derives from two characteristic factors in their biodistribution and pharmacokinetics. Specifically, the most promising of the previously studied compounds provide high first-pass tissue extraction of tracer (thereby assuring that the

Table 1. Myocardial Uptake of ^{67}Cu(II)−Bis(thiosemicarbazone) Complexes in the Rat following Intravenous Injection

	percentage of the injected dose in the heart[a]		
ligand	1 min	5 min	120 min
PTS[b]	2.8 ± 0.4	2.3 ± 0.1	
4a	2.73 ± 0.15	2.44 ± 0.14	2.03 ± 0.23
4b	2.84 ± 0.16	2.86 ± 0.01	2.59 ± 0.22
PTSM[b]	2.7 ± 0.3	3.4 ± 1.2	3.3 ± 0.9
4c	1.59 ± 0.27	1.23 ± 0.17	1.05 ± 0.09
4d	1.43 ± 0.05	1.27 ± 0.19	0.89 ± 0.13
$PTSM_2$[b]	0.97 ± 0.15	0.43 ± 0.14	

[a] Values represent the mean ± standard deviation of data obtained from three rats. [b] From ref 13.

Table 2. Brain Uptake of ^{67}Cu(II)−Bis(thiosemicarbazone) Complexes in the Rat Following Intravenous Injection

	percentage of the injected dose in the brain[a]		
ligand	1 min	5 min	120 min
PTS[b]	0.46 ± 0.15	0.29 ± 0.05	
4a	1.40 ± 0.06	1.37 ± 0.26	1.10 ± 0.24
4b	1.72 ± 0.17	1.81 ± 0.09	1.67 ± 0.17
PTSM[b]	3.0 ± 0.7	3.0 ± 0.6	3.2 ± 0.4
4c	2.79 ± 0.62	1.97 ± 0.12	1.98 ± 0.33
4d	2.76 ± 0.17	1.80 ± 0.48	1.63 ± 0.20
$PTSM_2$[b]	3.1 ± 0.4	2.2 ± 0.3	

[a] Values represent the mean ± standard deviation of data obtained from three rats. [b] From ref 13.

regional tissue distribution of radionuclide initially maps the pattern of capillary blood flow), while also exhibiting prolonged tissue retention of the extracted radiolabel. The high first-pass tissue extraction of these lipophilic ^{62}Cu agents results from high diffusibility of the blood-borne radiotracer.[3,14,18] The subsequent tissue trapping of the radiolabel results from liberation of ionic copper by reductive intracellular decomposition of the uncharged, square-planar Cu(II)−bis(thiosemicarbazone) complex.[13,15,19,20]

The ^{67}Cu complexes of **4a**, **4b**, **4c**, and **4d** are all found to clear from blood rather rapidly following intravenous administration, with only 5.7−9.0% of the injected ^{67}Cu radioactivity remaining in circulation at 1 min postinjection. Tables 1 and 2 show the heart and brain uptake of the ^{67}Cu complexes of **4a**, **4b**, **4c**, and **4d**, expressed in each case as a percentage of the injected radioactivity per organ. For the purposes of this study the tissue uptake at 1 min postinjection can be assumed to largely reflect the unidirectional first-pass tissue extraction of tracer from blood.[13] The ^{67}Cu complexes of **4a**, **4b**, **4c**, and **4d** all show significant myocardial uptake at this 1 min time point (ranging from 1.4% to 2.8% of the injected dose), as well as the ability to penetrate the blood−brain barrier (brain uptake also ranging from 1.4% to 2.8% of the injected dose at 1 min postinjection).

In this new series of ^{67}Cu complexes the brain uptake at 1 min is highest for the more lipophilic complexes in which the ligand contains at least one NMe_2 group. For reasons that are unclear, in contrast to the brain results the presence of the NMe_2 group is associated with the lowest myocardial uptake of radiotracer. We find this somewhat surprising, since one might expect the efficiency of first-pass myocardial extraction to be increased by the same physicochemical properties that favor high first-pass cerebral extraction. However, the disparate effects of the NMe_2 substituent on the initial cerebral and myocardial uptake of these mixed Cu(II)−bis(thiosemicarbazone) radiotracers are fully consistent with substituent effects reported for the ^{67}Cu−PTS, ^{67}Cu−PTSM, and ^{67}Cu−$PTSM_2$ series.[13,14]

Table 3. Binding of Copper(II)−Bis(thiosemicarbazone) Complexes to Serum Albumin[a]

	free (unbound) ^{67}Cu-L determined by ultrafiltration (%)	
complex (Cu−L)	dog albumin (35 mg/mL saline)	human albumin (35 mg/mL saline)
^{67}Cu−PTSM[b]	40.2 ± 6.7	5.1 ± 1.2
^{67}Cu−**4a**	57.4 ± 1.8	10.4 ± 0.6
^{67}Cu−**4b**	46.5 ± 1.5	8.6 ± 0.5
^{67}Cu−**4c**	57.6 ± 2.9	17.2 ± 0.6
^{67}Cu−**4d**	25.7 ± 1.7	5.4 ± 0.3
^{67}Cu−ETS[b]	44.3 ± 6.4	41.5 ± 4.0
^{67}Cu−*n*-PrTS[b]	30.3 ± 2.4	37.0 ± 2.8

[a] Amicon Centrifree (30 000 Da) ultrafiltration devices centrifuged at 1000*g* for 20 min in a 45° fixed angle rotor at 20 °C. Values shown represent the mean ± standard deviation of 6−12 measurements. All values are corrected for nonspecific binding of tracer to the ultrafiltration device using binding data independently measured for tracer in protein-free saline solution. [b] From ref 12.

For both the heart and brain, levels of the ^{67}Cu radiolabel are essentially unchanged between 1 and 5 min postinjection using ligands **4a** and **4b**, while the complexes of **4c** and **4d** exhibit clearance of tissue radiotracer over the same time frame (Tables 1 and 2). As was the case in analyzing structural effects on initial cerebral and myocardial uptake of radiotracer, the observed variations in the efficiency with which the tracer is trapped by tissue correlate with whether the NMe_2 substituent is present in the ligand backbone. The present observations that the NMe_2 substituent inhibits tracer trapping by tissue is consistent with findings from previous biodistribution studies with radiolabeled Cu(II)−bis(thiosemicarbazone) complexes.[13,14] This structural effect can be explained by the known influences of the electron-releasing NMe_2 substituent on chelate redox potential and susceptibility to intracellular reductive decomposition.[13−15]

Ultrafiltration Studies. Ultrafiltration studies were performed with the ^{67}Cu complexes of ligands **4a**−**d** in order to assess the amout of free (*i.e.*, non-albumin-associated) radiotracer present in solutions of dog or human serum albumin that approximate the albumin concentration of plasma. These ultrafiltration studies demonstrate that the four new ^{67}Cu-labeled mixed bis(thiosemicarbazone) complexes, like Cu−PTSM, bind more strongly to human serum albumin than to dog serum albumin (Table 3). In the dog albumin solutions, albumin-bound tracer was in the range of 40−75% for the ^{67}Cu complexes of **4a**−**d**; while in human albumin solutions of these agents, 83−95% of the radiotracer was albumin-associated. Thus, copper radiopharmaceuticals derived from ligands **4a**−**d** are unlikely to overcome the interspecies variability in radiotracer biodistribution that somewhat hinders clinical utilization of ^{62}Cu−PTSM as a PET radiopharmaceutical for myocardial perfusion imaging. Nevertheless, this derivatization strategy may still be useful as a means of improving the properties of the related copper(II) radiopharmaceuticals, Cu−ETS and Cu−*n*-PrTS, which are based on the ethylgyloxal and *n*-propylglyoxal bis(thiosemicarbazone) ligand backbones and which do not exhibit the strong affinity for human serum albumin that has

characterized the pyruvaldehyde-based copper bis(thiosemicarbazone) complexes.[12]

Experimental Section

General. Unless otherwise noted, materials obtained from commercial suppliers were used without further purification. Pyruvic aldehyde dimethyl acetal, lithium tetrafluoroborate, thiosemicarbazide, N^4-methylthiosemicarbazide, and N^4,N^4-dimethylthiosemicarbazide were purchased from the Aldrich Chemical Co. Whatman KSF silica gel 60A plates (without Florisil indicator) were used for TLC analysis. Microanalysis were performed at the Purdue Microanalysis Laboratory, and all values were within ±0.4% of the calculated compositions. Copper-67 copper(II) chloride was obtained from Brookhaven National Laboratory and Los Alamos National Laboratory. Human serum albumin (essentially globulin and fatty acid free) and dog serum albumin (essentially fatty acid free) were purchased from Sigma Chemical Co. (St. Louis, MO). Melting points were measured on a Gallenkamp MFB-595 capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured on either a Bruker ARX 300 spectrometer (300 MHz) or a Varian XL-500 (500 MHz) spectrometer. FAB mass spectra were obtained from a Kratos MS50 mass spectrometer. All experiments involving animals were carried out following protocols approved by the Purdue Animal Care and Use Committee. Rat biodistribution studies were performed as described previously[11,13] following injection of tracer (~2 μCi in 0.2 mL) into the femoral vein of ether-anesthetized animals.

Preparation of Pyruvaldehyde Dimethyl Acetal 2-Thiosemicarbazones. To a stirred solution of pyruvic aldehyde dimethyl acetal (5.00 g, 42.3 mmol) in DMF (75 mL) was added freshly activated molecular sieves (4 Å). The appropriate thiosemicarbazide (0.95 equiv) was then added, resulting in a clear, pale yellow to deep amber solution. The reaction was allowed to stir at ambient temperature for a total of 3 days. The molecular sieves were filtered, and the DMF was removed *in vacuo*. The crude material was dissolved in DMF (1−2 mL), which was loaded onto a silica gel column (22 cm × 60 cm) packed in diethyl ether. The column was eluted with diethyl ether (flow rate 2 mL/min), and fractions corresponding to the product (TLC) were pooled and evaporated to dryness under reduced pressure. The product was then recrystallized from diethyl ether. Isolated yields of the purified material obtained were between 60 and 80%.[21]

Preparation of Pyruvaldehyde 2-Thiosemicarbazones. Pyruvaldehyde dimethyl acetal 2-thiosemicarbazone and lithium tetrafluoroborate (2 equiv) were combined, and approximately 15 mL of a 2% aqueous solution of acetonitrile was added at ambient temperature to form a clear, pale yellow solution. Approximately 30 min into the reaction, the formation of a white precipitate was observed. Completion of the reaction was confirmed by thin-layer chromatography (Et_2O, silica gel). A saturated solution of sodium carbonate (30 mL) was added to the suspension, resulting in an intense, bright yellow homogeneous solution which was extracted with diethyl ether (3 × 50 mL). The ether extracts were combined, dried over magnesium sulfate, filtered, and solvent removed *in vacuo*. The solid material was washed with diethyl ether and pentanes to leave behind white to pale yellow flaky, needle-like crystals. Isolated yields of the purified material were generally >90%.[22]

Preparation of the Mixed Bis(thiosemicarbazones) from Pyruvaldehyde 2-Thiosemicarbazones. Freshly activated molecular sieves (4 Å) were added to a DMF solution (10 mL) of pyruvaldehyde 2-thiosemicarbazone. A clear yellow solution resulted when the appropriate dissimilar thiosemicarbazide was added. The reaction mixture was heated (60 °C) for 6 h and then cooled at room temperature for approximately 30 min prior to filtering of the solution to remove the molecular sieves. DMF was removed from the clear yellow filtrate *in vacuo*. The resulting solid product was recrystallized from DMF and washed with ethanol and pentanes. Isolated yields of the mixed bis(thiosemicarbazones) were between 50−60%.[23]

Radiotracer Synthesis. The ^{67}Cu-labeled copper(II) bis(thiosemicarbazone) complexes were prepared by evaporating a $^{67}Cu^{2+}$/HCl solution to dryness with heating under a stream of nitrogen and then reconstituting in 0.25 N acetate buffer (pH 5.5). Typically, 100 μL of ethanol and *ca.* 0.1 mg of the bis(thiosemicarbazone) ligand dissolved in 1−2 μL of DMSO was added to the aqueous ^{67}Cu−acetate (30−100 μL). The resulting solution was diluted with saline to *ca.* 5% ethanol and ≤0.5% DMSO (final ^{67}Cu concentration of ~2 μCi/μL) and then filtered through a 0.2 μm PTFE membrane before use. The radiochemical purity of the ^{67}Cu−bis(thiosemicarbazone) complexes was determined by thin-layer chromatography on silica gel plates eluted with ethanol and was always found to exceed 98%.

Partition coefficients of the ^{67}Cu radiotracers were measured following 1 min of vigorous vortex mixing of 1 mL of 1-octanol and 1 mL of isotonic TRIS-buffered saline (pH = 7)[24] with approximately 0.1 μCi of the radiolabeled copper complex. Following centrifugation at >1200*g* for 5 min, the octanol and aqueous phases were sampled and counted in an automatic well counter. A 500 μL sample of the octanol phase from this partitioning was repartitioned two to three times with fresh buffer to ensure that trace hydrophilic ^{67}Cu impurities did not alter the calculated *P* values. The reported log *P* values are the average of the second and third extractions from three to four independent measurements, so that the reported[17] log *P* values represent the mean (±standard deviation) of six to eight measurements.

Ultrafiltration Studies with ^{67}Cu Complexes. Serum albumin binding of the ^{67}Cu−bis(thiosemicarbazone) complexes was quantitatively evaluated by ultrafiltration as described previously.[12] Solutions of serum albumin were prepared fresh for each experiment at 35 mg/mL normal saline and refrigerated when not in use. Each Amicon (Beverly, MA) Centrifree ultrafiltration device (30 000 Da NMWL) was loaded with 300−600 μL of either an albumin solution (35 mg/mL saline) or normal saline (control) within 2 min of mixing with the ^{67}Cu complex. Typically, 1 mL of protein solution was mixed with 1−2 μL of ^{67}Cu−bis(thiosemicarbazone) solution. The Centrifree devices were loaded and immediately (<1 min) centrifuged in a Sorvall RC2-B refrigerated centrifuge (20 °C) with a SS-34 45° fixed angle rotor at 1000*g* for 20 min. ^{67}Cu−bis(thiosemicarbazone) complex concentrations (cpm/mL) in the unfiltered protein solutions and their ultrafiltrates were determined by counting measured aliquots in a Packard Autogamma 5530 automatic γ counter. The percentage of free (unbound) ^{67}Cu−bis(thiosemicarbazone) complex was calculated as

$$\frac{\left[\frac{(^{67}\text{Cu−L concentration in protein ultrafiltrate})}{(^{67}\text{Cu−L concentration in unfiltered protein solution})}\right]}{\left[\frac{(^{67}\text{Cu−L concentration in saline ultrafiltrate})}{(^{67}\text{Cu−L concentration in unfiltered saline solution})}\right]} \times 100$$

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Supporting Information Available: Complete tables of rat biodistribution data for each ^{67}Cu compound, calculated as both a percentage of the injected dose per gram tissue (wet weight) and as a percentage of the injected dose per organ (4 pages). Ordering information is given on any current masthead page.

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(22) **Pyruvaldehyde 2-thiosemicarbazone (3a):** ^{1}H NMR (DMSO-d_6): mp 171 °C; TLC R_f 0.58 (Et_2O, silica gel) δ 11.12 (s, 1H, N*H*), 9.33 (s, 1H, C*H*O), 8.78 (br s, 1H, NH_2), 8.38 (br s, 1H, NH_2), 1.92 (s, 3H, CH_3). **Pyruvaldehyde 2-*N* ^{4}-methylthiosemicarbazone (3b):** mp 173 °C; TLC R_f 0.62 (Et_2O, silica gel); ^{1}H NMR (DMSO-d_6) δ 11.18 (s, 1H, N*H*), 9.35 (s, 1H, C*H*O), 9.01 (m, 1H, N*H*CH$_3$), 3.01 (d, 3H, NHCH_3), 1.93 (s, 3H, CH_3).

(23) **2-Oxopropanal 1-*N* ^{4}-methylthiosemicarbazone 2-thiosemicarbazone (4a):** mp 208−209 °C; ^{1}H NMR (DMSO-d_6) δ 11.70 (s, 1H, N*H*), 10.34 (s, 1H, N*H*), 8.41 (m, 1H, N*H*CH$_3$), 8.37 (s, 1H, NH_2), 7.88 (s, 1H, NH_2), 7.64 (s, 1H, N=C*H*), 2.98 (d, 3H, NCH_3, J = 5.0 Hz), 2.15 (s, 3H, CH_3); FAB mass spectrum, 233 (M + H). Anal. ($C_6H_{12}N_6S_2$·0.30H_2O) C, H, N. **2-Oxopropanal 2-*N* ^{4}-methylthiosemicarbazone-1-thiosemicarbazone (4b):** mp 229−230 °C; ^{1}H NMR (DMSO-d_6) δ 11.69 (s, 1H, N*H*), 10.38 (s, 1H, N*H*), 8.53 (m, 1H, N*H*CH$_3$), 8.31 (s, 1H, NH_2), 7.90 (s, 1H, NH_2), 7.65 (s, 1H, N=C*H*), 2.98 (d, 3H, NHCH_3, J = 4.0 Hz), 2.12 (s, 3H, CH_3); FAB mass spectrum, 233 (M + H). Anal. ($C_6H_{12}N_6S_2$·0.30H_2O) C, H, N. **2-Oxopropanal 1-*N* ^{4}-dimethylthiosemicarbazone 2-thiosemicarbazone (4c):** mp 192 °C; ^{1}H NMR (DMSO-d_6) δ 11.17 (s, 1H, N*H*), 10.40 (s, 1H, N*H*), 8.37 (s, 1H, NH_2), 7.81 (s, 1H, NH_2), 7.76 (s, 1H, N=C*H*), 3.24 (s, 6H, N(CH_3)$_2$), 1.94 (s, 3H, CH_3); FAB mass spectrum, 247 (M + H). Anal. ($C_7H_{14}N_6S_2$·1.10 H_2O) C, H, N. **2-Oxopropanal 1-*N* ^{4}-dimethylthiosemicarbazone 2-(*N*^{4}-methylthiosemicarbazone (4d):** mp 211−212 °C; ^{1}H NMR (DMSO-d_6) δ 11.21 (s, 1H, N*H*), 10.42 (s, 1H, N*H*), 8.47 (m, 1H, N*H*CH$_3$), 7.75 (s, 1H, N=C*H*), 3.25 (s, 6H, N(CH_3)$_2$), 2.98 (d, 3H, NHCH_3, J = 4.3 Hz), 2.08 (s, 3H, CH_3); FAB mass spectrum, 261 (M + H). Anal. ($C_8H_{16}N_6S_2$·0.60H_2O) C, H, N.

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