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Articles

BLEDTA: Tumor Localization by a Bleomycin Analogue Containing a Metal-Chelating Group

Leslie H. DeRiemer, Claude F. Meares,*

Chemistry Department, University of California, Davis, California 95616

David A. Goodwin, and Carol I. Diamanti

Stanford University School of Medicine and Veterans Administration Medical Center, Palo Alto, California 94304.
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Two different Co(III) complexes of the antitumor antibiotic bleomycin have been prepared, and their in vivo distribution in mice has been investigated. The more thermodynamically stable of the Co(III)–bleomycin complexes has been modified by reaction with the bifunctional chelating agent 1-(*p*-bromoacetamidophenyl)ethylenedinitrilotetraacetic acid, to give a bleomycin derivative (BLEDTA) containing a powerful metal-chelating group. BLEDTA was radiolabeled with ^{111}In(III) and its in vivo distribution in mice was examined. The potential of ^{111}In-labeled BLEDTA as a tumor-visualizing agent was also investigated in humans with biopsy-proven cancers, predominantly (70%) squamous carcinoma of the head and neck. All of the 29 patients studied had at least one clinically proven site of the disease visualized with ^{111}In–BLEDTA. These clinical results are significantly better than results we obtained in a comparable group of patients using directly labeled ^{111}In–bleomycin and are similar to those reported by Nouel for ^{57}Co–bleomycin [*GANN Monogr. Cancer Res.*, **19**, 301 (1976)].

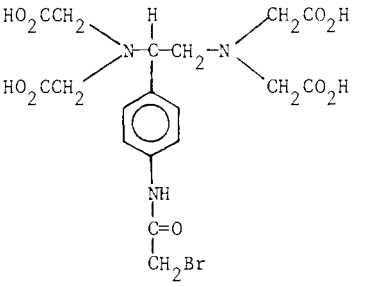
The antineoplastic antibiotic bleomycin has been extensively investigated as an in vivo carrier of γ-emitting metal ions.[1-5] Because bleomycin is selectively accumulated in some cancer cells,[6] radiolabeled bleomycin is potentially useful in locating tumors. Bleomycin is able to bind a number of metal ions, but most bleomycin–metal ion complexes have been shown to be unstable in vivo and unsuitable for diagnostic use.[1-5] ^{57}Co–bleomycin is stable in vivo, and the excellent clinical results obtained with this compound have shown that a stable radiolabeled bleomycin can be a very useful diagnostic tool.[5] However, the long half-life of ^{57}Co (270 days) poses contamination problems which preclude its widespread clinical use.

The resistance to decomposition exhibited by ^{57}Co–bleomycin is characteristic of Co(III) compounds. ^{57}Co–bleomycin is prepared by the addition of Co(II) salts to aqueous solutions of bleomycin; in the presence of nitrogen-containing ligands such as bleomycin, complexed Co(II) is easily air-oxidized to the Co(III) complex.[14] Due to the d^6 electronic configuration of Co(III), most of its complexes undergo ligand exchange extremely slowly; Taube[15] has classified them as inert. Co(III)–bleomycin has been shown to be kinetically inert both in vivo and for prolonged periods in vitro in the presence of ethylenedinitrilotetraacetic acid.[16,17]

It has also been observed that the uptake of bleomycin by mouse Ehrlich solid ascites tumor is enhanced when bleomycin is bound to cobalt.[16] The kinetic inertness and higher tumor uptake of cobalt–bleomycin suggested that it would be a suitable starting material for chemical-modification studies. The goal of these studies was the addition of a powerful metal-chelating group at a non-essential site on bleomycin, such that its tumor-localizing properties would be retained. Such a compound could be used as a carrier of radionuclides more useful clinically than ^{57}Co—in particular those which emit only γ radiation, with energy between 100 and 400 keV and with half-lives ranging from several hours to a few days.[5]

1-Phenyl derivatives of ethylenedinitrilotetraacetic acid form thermodynamically stable complexes with a large number of metal ions;[7] these complexes are also kinetically inert to some degree, because of the steric constraints imposed by substitution on the ethylene carbons of EDTA.[8-11] The stability of para-substituted 1-phenylethylenedinitrilotetraacetic acid chelates of ^{111}In(III) has been demonstrated in vitro and in vivo.[10,11]

A new member of this class of "bifunctional" chelating agents, 1-(*p*-bromoacetamidophenyl)ethylenedinitrilotetraacetic acid (1), has been synthesized and coupled to



1

the "terminal amine" region of cobalt–bleomycin (Scheme I). The properties of the product "BLEDTA" [4 with

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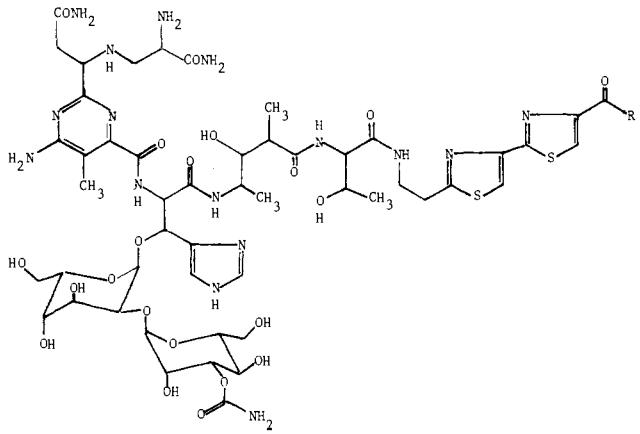
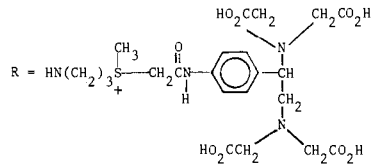
Scheme I

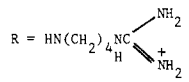


2, R = $NH(CH_2)_3\overset{+}{S}(CH_3)_2$

3, R = $HN(CH_2)_3SCH_3$



4, R = $HN(CH_2)_3\overset{+}{S}(CH_3)CH_2CONH$



5 R = $HN(CH_2)_4NC(=NH_2^+)NH_2$

stable cobalt(III) complexed to the bleomycin moiety] are described.

Results

The commercially available bleomycin, Blenoxane, is a mixture of bleomycins differing only in the terminal amine (R, Scheme I). A neutral aqueous solution containing Blenoxane and a slight excess of $CoCl_2$ was saturated with O_2 to generate Co(III)-bleomycin. Co(III)-bleomycin A_2 (**2**, Scheme I) was isolated by cation-exchange chromatography in two distinct peaks having different colors, green and orange. Green Co(III)-**2** was unstable with respect to the orange form. If left at room temperature, aqueous solutions of green Co(III)-**2** would convert to a mixture of orange and green Co(III)-**2**; the conversion could be accelerated by heating. ^1H NMR (100 MHz) spectra of the compounds showed differences in the aliphatic region corresponding to protons on the threonine and valeric acid residues and on the terminal amine group.[20] The properties of the orange and green compounds will be discussed in detail elsewhere. All further chemistry was done with the thermodynamically stable orange compound.

Co(III)-**2** was demethylated to Co(III)-**3** by reaction with sodium methyl mercaptide in methanolic solution[29] and purified by cation-exchange chromatography to give Co(III)-**3** as shown in Scheme I. As proof of identity, Co(III)-**3** was methylated with CH_3I to yield Co(III)-**2**;[6b] LC and TLC confirmed that Co(III)-**2** was produced.

As shown in Figure 1, the sharp singlet in the proton magnetic resonance spectrum corresponding to the sulfur-bound methyl group on the terminal amine (R) of Co(III)-**3** has less intensity and is upfield (δ 2.10) from the resonance in Co(III)-**2** corresponding to the two sulfur-bound methyl groups (δ 2.92) on its terminal amine. The multiplet at δ 1.94 has been assigned to one of the terminal amine methylene resonances and is also upfield from the corresponding resonance in Co(III)-**2** (δ 2.20). These observations are in agreement with previously reported NMR data for metal-free bleomycins[20] and confirm that demethylation has taken place.

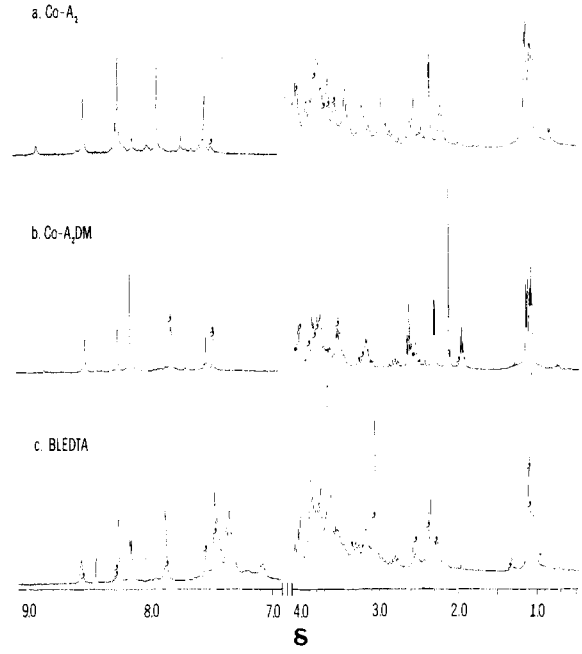


Figure 1. 360-MHz ^1H NMR spectra in D_2O: (A) Co-A_2 [Co(III)-**2**] at a pH meter reading of 5.2; (B) Co-A_2DM [Co(III)-**3**], pH 5.2; (C) BLEDTA [Co(III)-**4**], pH 4.9. In all spectra, the signal amplification is greater in the aromatic region than in the aliphatic region. Spectra were taken at room temperature.

Studies of zinc- and copper-bleomycin by NMR and X-ray diffraction implicate the imidazole and pyrimidine moieties in binding to the metal ions.[21,22] In the NMR spectrum of Co(III)-**3** (Figure 1b) there appear to be two different resonances for each of the imidazole protons (δ 8.25, 8.52 and δ 7.48, 7.52) and for the protons of the methyl group on the pyrimidine residue (δ 2.28, 2.31).[20] This may be accounted for if orange Co(III)-**3** is a mixture of species differing slightly in coordination about the cobalt ion or by a rotational motion which is slow on the NMR time scale. The possibility that the product may contain mercaptide coordinated to cobalt was eliminated by the finding that demethylation with CH_3SNa or with C_2H_5SNa leads to the same product, as determined by 360-MHz ^1H NMR. Column chromatography on Sephadex C-25 at pH 4.5, which separates the copper complexes of epibleomycin and isobleomycin from that of bleomycin, results in elution of Co(III)-**3** as a single peak; this suggests that neither epimerization nor isomerization has occurred.[26] Further investigation is in progress.

Co(III)-**3** was alkylated with **1** in aqueous solution; fractionation of the reaction mixture gave the final product BLEDTA [**4**, Scheme I, in which Co(III) is complexed with the bleomycin moiety]; the ethylenedinitrilotetraacetic acid moiety remains free to chelate other metal ions as demonstrated by in vitro experiments, studies with mice, and clinical results.

A control reaction was run with orange Co(III)-bleomycin B_2 (**5**, Scheme I) and **1**. The reaction was monitored by TLC and LC; even after 46 h there was no alkylation of Co(III)-bleomycin B_2. Because bleomycin B_2 is identical with bleomycin A_2DM except for the terminal amine moiety, this result implies that it is the thioether sulfur

Table I. Distribution and Uptake in BALB/c Mice with KHJJ Tumor

	% of injected radioactivity/g[a]						
	^{57}Co-B$_2$[d]		^{57}Co-A$_2$[b]				
organ	green	orange	green	orange	^{111}In-BLEDTA[c]	^{111}In-bleomycin[e]	^{111}In-1-Ph-EDTA
blood	0.024 ± 0.002	0.006 ± 0.005	0.013 ± 0.004	0.003 ± 0.001	0.55 ± 0.05	0.48 ± 0.20	0.19 ± 0.029
lungs	0.18 ± 0.05	0.10 ± 0.003	0.10 ± 0.04	0.075 ± 0.008	0.42 ± 0.05	1.22 ± 0.58	0.029 ± 0.045
liver	0.65 ± 0.04	0.23 ± 0.004	0.40 ± 0.11	0.20 ± 0.03	0.35 ± 0.03	2.77 ± 1.03	0.66 ± 0.43
spleen	0.19 ± 0.03	0.12 ± 0.02	0.15 ± 0.03	0.093 ± 0.011	0.27 ± 0.03	1.93 ± 0.40	0.83 ± 0.65
kidneys	2.12 ± 0.66	2.80 ± 0.82	1.43 ± 0.46	1.69 ± 0.33	1.72 ± 0.26	8.98 ± 2.24	0.22 ± 0.18
tumor	0.54 ± 0.05	0.26 ± 0.04	0.55 ± 0.14	0.19 ± 0.03	0.68 ± 0.16	1.98 ± 0.38	0.098 ± 0.088
muscle	0.017 ± 0.003	0.011 ± 0.003	0.020 ± 0.008	0.015 ± 0.008	0.063 ± 0.010	0.31 ± 0.026	0.006 ± 0.010
bone	0.094 ± 0.013	0.031 ± 0.009	0.089 ± 0.048	0.035 ± 0.016	0.077 ± 0.060	1.26 ± 0.087	0.015 ± 0.026
skin	0.066 ± 0.026	0.209 ± 0.228	0.059 ± 0.016	0.064 ± 0.009	0.21 ± 0.03	1.18 ± 1.02	0.037 ± 0.036

[a] Mean ± SD for three mice. [b] Co(III)-2. [c] Co(III)-4. [d] Co(III)-5. [e] Directly labeled Blenoxane (ref 4b); six mice at 24 h.

in the terminal amine moiety of Co(III)–**3** which reacts with **1** to give Co(III)–**4**. In the 360-MHz ^{1}H NMR spectrum of Co(III)–**4** the terminal amine methyl resonance (δ 3.05) is downfield from the corresponding resonance in Co(III)–**3** (δ 2.10). The multiplet at δ 2.24, which has been assigned to a methylene group in the terminal amine, is downfield from the corresponding resonance (δ 1.94) in the NMR spectrum of Co(III)–**3** and is very near the position of the corresponding resonance in Co(III)–**2** (δ 2.20).[20] Both of these observations are consistent with alkylation of the terminal amine thioether to generate a sulfonium ion. In the aromatic region, the resonance (δ 8.15) assigned to the bithiazole proton nearest the terminal amine consists of two closely spaced peaks; this is consistent with the formation of diasteromers upon alkylation of Co(III)–**3** by **1**. The proton resonances of the disubstituted benzene ring of Co(III)–**4** are also evident in the aromatic region of the spectrum (δ 7.35, 7.45).

Radiolabeling the product was straightforward; a citric acid solution containing $^{111}InCl_3$ was combined with an aqueous solution of Co(III)–**4** at room temperature. Labeling was monitored by TLC and was complete within 5 min.

Tumor-Bearing Mice. BALB/c mice with "KHJJ" tumor implanted in the flank were injected with the orange and green ^{57}Co(III) complexes of bleomycins A_2 (**2**) and B_2 (**5**) and with ^{111}In-labeled Co(III)–**4**. As control experiments, the complex formed by addition of $^{111}In^{3+}$ to Blenoxane (^{111}In–bleomycin) and the chelate of $^{111}In^{3+}$ with 1-Ph-EDTA were also studied. The organ distribution and tumor uptake of radioactivity in the mice are shown in Table I.

The in vivo distributions of green ^{57}Co–**2** and green ^{57}Co–**5** do not differ significantly; orange ^{57}Co–**2** and orange ^{57}Co–**5** also have similar organ distributions. One day after injection, the concentration of green ^{57}Co–**2** in blood and in tumor is more than twice that of orange ^{57}Co–**2**; the same is true for the distribution of green and orange ^{57}Co–**5**. Tumor uptake of radioactivity after injection of ^{111}In-labeled Co(III)–**4** (^{111}In–BLEDTA) is more than double that observed with orange ^{57}Co–**2**; the blood, lungs, spleen, muscle, and skin also showed higher uptake of radioactivity for ^{111}In–BLEDTA. The organ distribution of ^{111}In–bleomycin shows very high uptake of radioactivity in the kidneys, liver, spleen, and bone, which suggests dissociation of this complex in vivo.[4b] In comparison, the organ distribution of ^{111}In–1-Ph-EDTA shows little uptake of radioactivity in any organ except the liver and spleen, reflecting its rapid excretion.

Human Subjects. Tumor localization by ^{111}In–BLEDTA was investigated in a series of patients with biopsy-proven cancer.[25] Of the 29 patients studied, 21 had squamous carcinomas of the head and neck; scans following

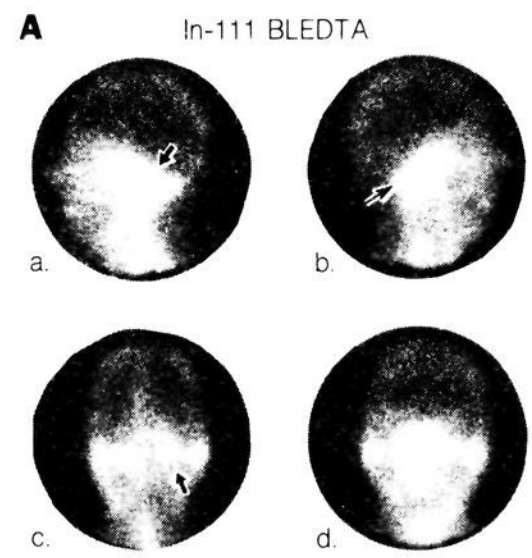


Figure 2. (A) Right lateral view of the head of a 40-year old white male patient with squamous cell carcinoma of the larynx, metastatic to the base of the skull (arrow). This tumor was not visualized on a CAT scan or by routine X-ray studies. (B) Posterior view of the lungs of a 36-year-old white male patient with anaplastic thyroid carcinoma, metastatic to the lungs (arrow). These tumors were not visualized with $^{131}I^-$. Each scan was taken 24 h after intravenous injection of 2 mCi of ^{111}In–BLEDTA.

injection of ^{111}In-labeled Co(III)–**4** showed all the disease present for 17 of these patients. Each of the other four patients with squamous carcinoma of the head and neck had only some disease visualized. Also studied were individuals with lung cancer (3), unknown primary tumors (2), metastatic adenocarcinomas (1), thyroid cancer (1), and mucoepidermoid cancer (1). All disease was visualized in one lung-cancer patient; ^{111}In–BLEDTA revealed some, but not all, of the disease in each of the other patients. The smallest tumor localized was 1-cm in diameter. Typical scans are shown in Figure 2. TLC of 24-h urine showed 87% of the total radioactivity on the TLC plate moved with R_f 0.5, identical with that of ^{111}In-labeled Co(III)–**4**.

Ten percent of the excreted radioactivity was accounted for by a degradation product with R_f 0.9, which is typical for many small indium chelates. The plasma levels of ^{111}In-BLEDTA fell rapidly; 24 h after injection only 3 ± 0.8% of the injected radioactivity was present in the total blood volume, of which only 16% was present in the plasma fraction.[25]

Discussion

As shown in Table I for the several metallobleomycins investigated, organ distributions in tumor-bearing mice show statistically significant differences between the green and orange forms of Co(III)-**2** [and of Co(III)-**5**]. As reported previously,[17] the different terminal groups (R) of **2** and **5** do not appear to have an important effect. The structural differences between the green and orange cobalt-bleomycins almost certainly involve different coordination at cobalt; both species are diamagnetic, and the NMR spectrum of green Co(III)-**2** suggests that the methyl groups of the threonine and valeric acid residues are perturbed from their normal environments. Experiments are now under way to fully elucidate the structures of these interesting compounds.

It is striking that the blood levels of ^{111}In-BLEDTA in mice are roughly two orders of magnitude higher than those of the ^{57}Co-bleomycins studied. High blood levels in mice have been reported for other bleomycin analogues containing aromatic groups in the terminal amine.[27] In human subjects, the 24-h plasma level of ^{111}In-BLEDTA was 0.17% ± 0.07%/L of plasma, which is similar to 24-h plasma levels of ^{57}Co-Blenoxane reported by Nouel et al.[30] (0.1%/L) and by Grove et al.[28] (0.5%/L). Uptake of radioactivity by the tumor and other organs of the mouse does not differ greatly for ^{111}In-BLEDTA as compared to the ^{57}Co-bleomycins; however, the organ distributions of ^{111}In-bleomycin and ^{111}In-1-Ph-EDTA contrast markedly with that of ^{111}In-BLEDTA. These results strongly suggest that the in vivo transport properties of Co(III)-**4** are not substantially different from those of Co(III)-**2** or Co(III)-**5**. Support of this hypothesis is provided by the distribution and tissue concentrations of ^{111}In-BLEDTA in human subjects, which are similar to those previously reported for ^{57}Co-bleomycin. ^{111}In-BLEDTA gave tumor/background ratios between 3 and 11 in human subjects;[25] Nouel et al. report a mean tumor/background ratio of 3.2 for ^{57}Co-bleomycin.[5] All 21 patients with known squamous cell carcinoma of the head and neck had at least one site of disease visualized by ^{111}In-BLEDTA and 81% (17) had all disease visualized. This is a considerable improvement over our previous study of a comparable series of randomly chosen patients using the complex formed between indium(III) and Blenoxane, in which 5 of 15 biopsy-proven cases of squamous carcinoma of the head and neck failed to visualize at all.[4b]

The difference in tumor uptake between green and orange ^{57}Co-bleomycins illustrates the sensitivity of the process to the conformation of bleomycin. It may be possible to make use of this difference in studies of the mechanism by which bleomycin accumulates in tumors. It is likely that previous studies of ^{57}Co-bleomycin have involved mixtures of the green and orange forms. As shown in Table I, the uptake of ^{111}In-BLEDTA in the mouse KHJJ tumor is much higher than its closest analogue, orange ^{57}Co-bleomycin A_2. A possible explanation for this is that BLEDTA remains in the circulation longer before being excreted.

It may be possible to prepare anlogues of BLEDTA with even better tumor-localizing properties, for example, by variation of the charge on the chelate and/or on the terminal group. Furthermore, the results obtained with BLEDTA suggest that in other cases it will be possible to couple chelating groups to relatively small molecules, with retention of desired biological properties.

Experimental Section

Chemical Procedures. Reagents. Blenoxane (bleomycin sulfate) was obtained from Dr. W. T. Bradner of Bristol Laboratories. All columns were monitored at 280 nm. All Sephadex (Pharmacia) columns were equilibrated with gradient starting buffer before sample application, and only linear gradients were used. High-pressure liquid chromatographies were run using a Waters 3.9 mm × 30 cm C_{18} μ-bondapak column with 1% aqueous ammonium acetate/methanol (6:4, v/v) as the solvent. TLCs were run on Merck silica gel 60 or 60F plates developed in 10% aqueous ammonium acetate/methanol (50:50, v/v). Carrier-free $^{111}InCl_3$ (in 0.05 N HCl, 0.9% NaCl) was purchased from Medi-Physics, Emeryville, CA, and was purified as described previously.[18] Carrier-free $^{57}CoCl_2$ (in 0.5 N HCl) was purchased from ICN, Irvine, CA, and was used without further purification. All syntheses and buffers employed purest commercially available reagents. Doubly distilled water was used throughout and labware was acid washed to avoid heavy metal contamination.[19] Elemental analysis was performed by Galbraith Analytical Labs, Inc. FT ^{1}H NMR (360 MHz) spectra were taken by Mr. P. D. Burns using the Nicolet instrument at the UCD Magnetic Resonance Facility. Chemical shifts are reported relative to HDO (δ 4.8).

Co(III)-Bleomycin A_2 [Co(III)-2]. A 0.100 M solution of $CoCl_2$ (1.11 mL) was added to 164 mL of a 1 mg/mL solution of Blenoxane (0.11 mmol) and the pH was adjusted to 6.5 with 25 μL of 6 M NaOH. The solution was placed in a 500 mL container and saturated with O_2. The container was capped, and the solution was left at 50 °C overnight and then heated for 2 h at 110 °C to partially convert green Co(III)-**2** to orange Co(III)-**2**. This mixture was applied to a Sephadex C-25 column (NH_4^+ form, 1 × 79 cm) and eluted with a gradient of 0.01–0.5 M ammonium formate, pH 6.5. The orange and green Co(III)-**2** were eluted with ammonium formate concentrations of 0.25 and 0.28 M, and the solutions were lyophilized to remove salt. The yield was 35 mg (22 μmol) of orange Co(III)-**2** and 27 mg (17 μmol) of green Co(III)-**2**.

Only slight differences in TLC and LC properties were observed. On TLC the orange and green compounds had R_f values of 0.36 and 0.40, respectively. The LC retention time was 5.6 min for the orange compound and 7.2 min for the green compound at a flow rate of 1 mL/min. The visible absorption spectrum of orange Co(III)-**2** exhibited a maximum at 452 nm (ε 214 M^{-1} cm^{-1}).

^{57}Co-labeled bleomycins were prepared by adding carrier-free $^{57}CoCl_2$ to one vial of Blenoxane (~5 mg) dissolved in 1 mL of H_2O. Co(II) was oxidized as described above, except the solution was not heated to 110 °C. ^{57}Co-Blenoxane was fractionated by chromatography as described above. Aliquots of each fraction were counted, and radioactive fractions were lyophilized to remove salt. The identity of the compounds was established by TLC. This procedure gave only green ^{57}Co(III)-**2** and green ^{57}Co(III)-**5**. The orange compounds were prepared by heating a ^{57}Co-Blenoxane solution at 110 °C for 1 h before applying to the column. A small sample of green Co(III)-**2** was also applied to the column and absorbance of the effluent at 450 nm was monitored. The effluent was counted and identified as described; the green Co(III)-**2** eluted after the radioactive ^{57}Co(III)-**2**, indicating that the ^{57}Co(III)-**2** was the orange species. Lyophilized ^{57}Co-bleomycins were stored at −70 °C until immediately before use.

Co(III)-Bleomycin A_2DM [Co(III)-3]. The published pyrolysis procedure[6b] gave unsatisfactory results with Co(III)-**2**, leading to development of the following reaction. A 0.73 M solution of sodium methyl mercaptide (50 mL) was prepared by the addition of 1.98 g (37 mmol) of sodium methoxide dissolved in 25 mL of ice-cold methanol to 25 mL of ice-cold anhydrous methanol containing 2.40 g (50 mmol) of methyl mercaptan. Methanol and excess mercaptan were removed by rotary evaporation. A 0.313 M methanolic solution of the mercaptide (1.39 mL) was added to 46 mg (29 μmol) of the formate salt of orange Co(III)-**2** dissolved in 10.25 mL of methanol, and the mixture was incubated at 50 °C for 50 min. The reaction was monitored by LC; at a flow rate of 2 mL/min, Co(III)-**3** and Co(III)-**2** had retention times of 8.8 and 3.2 min, respectively.

The reaction mixture was combined with an equal volume of H_2O and applied to a Sephadex C-25 column (NH_4^+ form, 1 × 75 cm). A gradient of 0.01–0.5 M ammonium formate, pH 6.5, was applied; Co(III)-**3** eluted with 0.11 M ammonium formate. Salt and water were removed by lyophilization. The yield was determined to be 20 μmol (69%) by measuring the absorbance of an aqueous solution and using ϵ_{452} 214 M^{-1} cm^{-1}. On TLC, Co(III)-**3** had R_f 0.8. Similar results were obtained using sodium ethyl mercaptide.

1-(*p*-Bromoacetamidophenyl)ethylenedinitrilotetraacetic Acid (1). 1-(*p*-Nitrophenyl)ethylenedinitrilotetraacetic acid (100 mg, 240 μmol) was dissolved in 50 mL of aqueous NaOH (such that the final pH was 11.4) and 29 mg of 10% Pd/C was added. Reduction was carried out as previously reported to give 1-(*p*-aminophenyl)ethylenedinitrilotetraacetic acid.[12] The amino compound was dissolved in 500 μL of H_2O and neutralized; then bromoacetyl bromide (20 μL, 230 μmol) was added in 5-μL portions until the solution was negative to fluorescamine.[23] Excess bromoacetyl bromide and bromoacetic acid were removed by repetitively extracting the acidic reaction mixture with diethyl ether. The organic layer was tested with 4-(*p*-nitrobenzyl)pyridine to follow the course of the extraction.[24] On TLC, **1** had an R_f of 0.9. The pH of the aqueous layer was adjusted to 2.3 with 6 N HCl and the solution was left on ice overnight. The resulting precipitate was washed with ice-cold 0.01 M HCl and dried on a vacuum line. The yield was 69.03 mg (137 μmol, 57%). Anal. ($BrC_{18}H_{22}N_3O_9$) Br, C, H, N.

BLEDTA [Co(III)-4]. Co(III)-**3** (16.5 μmol) and **1** (165 μmol) were combined to give 5 mL of aqueous solution, which was adjusted to pH 4.3 by the addition of 15 μL of 6 N NaOH. This mixture was warmed at 37 °C for 7.5 h; 84% of Co(III)-**3** had reacted as determined by LC. The pH of the mixture was adjusted to 8.1 with 6 N NaOH and the solution was applied to a Sephadex A-25 column (1 × 67 cm, formate form). The column was washed with 0.01 M ammonium formate, pH 8.1, followed by a gradient of 0.01–0.1 M ammonium formate, pH 8.1. The product was eluted with 0.06 M ammonium formate and lyophilized 2 days to remove the salt. The yield was determined to be 4.96 μmol (30%) by measuring the absorbance at 452 nm of an aqueous solution of the product (ϵ_{452} 214 M^{-1} cm^{-1}). The product had R_f 0.50; autoradiography of a TLC plate of the ^{111}In complex of the product indicated that >88% of the radioactivity on the plate was present in a spot with R_f 0.50.

The ^{111}In chelate of the product was prepared by adding 50 μL of 0.01 M citric acid containing 4 mCi of $^{111}InCl_3$ to 50 μL of a 0.54 mM solution of Co(III)-**4**. Labeling was complete after 5 min at room temperature.

Biological Procedures. Distribution in Tumor-Bearing Mice. Following the injection of each radiolabeled compound into the tail veins of specially prepared BALB/c mice, the organ distribution and tumor uptake of radioactivity were determined by the methods described elsewhere.[12] Results are presented in Table I.

Distribution in Human Subjects. Patients were scanned 18–24 h after intravenous injection of 1–2 mCi of ^{111}In-labeled Co(III)-4. Whole-body scans and spot views were obtained with a γ camera (Searle Pho-Gamma IV). Typical results are presented in Figure 2.

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