Physical Parameters and Biological Stability of Yttrium(III) Diethylenetriaminepentaacetic Acid Derivative Conjugates

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The solution equilibria, acid dissociation, and serum stability of a series of Y(III) complexes of DTPA ligands functionalized with *p*-nitrobenzyl, methyl, and *trans*-cyclohexyl substituents were studied. The thermodynamic stability of the complexes studied ranged from log $K = 21.53$ to 24.7. Acid dissociation rates were found to decrease as the substitution on the carbon backbone increased, and significant differences in dissociation rates were observed for the Y(III) complexes of a pair of diasteriomeric cyclohexyl-DTPA ligands. While one diastereomer was found to have the slowest acid dissociation rate of the entire DTPA series, it was remarkably labile in both serum stability and in vivo studies.

New roles continue to be found for coordination complexes in the diagnosis and treatment of disease. Notable examples include the increasing use of Gd(III) chelates as contrast agents in magnetic resonance imaging (MRI)[1] and the expanding employment in nuclear medicine[2] of complexes of metallic radionuclides such as ^{64}Cu, ^{67}Ga, ^{99m}Tc, and ^{111}In. Current clinical applications rely on low molecular weight molecules. The chemical modification of monoclonal antibodies (mAb) by linkage of bifunctional chelating agents is providing for the selective delivery of radiometals to malignancies.[3] Diagnostic imaging is being performed by labeling these conjugates with the γ- or β^+-emitting radionuclides listed above, while α or β^- emitters such as ^{67}Cu, ^{90}Y, and ^{213}Bi are in use in clinical trials of cancer radioimmunotherapy.[4,5] Such applications strictly demand that the metallic radionuclide remain intimately associated with the targeting protein to minimize the toxicity derived from the dissociation and delivery of radiation to normal, nontargeted body tissues. Similar constraints apply to MRI, in which case toxicity would result from the release of substantial quantities of Gd(III), Mn(II), or Fe(III) from coordination complexes.

Ligand structure and physical characteristics, such as thermodynamic stability, dissociation rates, and serum stability, have been invoked to rationalize the in vivo dissociation of radiolabeled coordination complexes.[3,6] The series of backbone-substituted, bifunctional DTPA[7] ligands shown in Figure 1 provides a unique opportunity to investigate the correlation of the in vivo stability of conjugated coordination complexes with the above-mentioned physical characteristics. If such a correlation exists, it will provide a useful tool for screening novel metalloradiopharmaceuticals, while minimizing the use of animal models. An understanding of these relationships will contribute to improved therapy and assist in the further development of metalloradiopharmaceuticals.

Results and Discussion

Physical Parameters. For each DTPA derivative shown in Figure 1, the protonation constants, Y(III) stability constant, and acid dissociation rate constant were measured, and the results are summarized in Table 1.

The sum of the protonation constants for each DTPA derivative provides information on the overall basicity of the ligand relative to DTPA. Increased functionalization of the carbon backbone generally results in an increased basicity of the ligand, making the cyclohexyl (CHX) derivatives 3 to 4.5 log units more basic than the parent molecule. By analogy to C-functionalized EDTA derivatives,[8] the introduction of a bulky substituent on the ethylenic backbone of DTPA would be expected to alter the most stable conformation about the carbon−carbon bond from an anti arrangement to the gauche conformation.[9] The gauche conformation will allow for the closer proximity of the iminodiacetato or diacetato, causing increased hydrogen bonding between the carboxyls. The above effect will increase the basicity of log K_1, and this was observed for all the DTPA derivatives (Table 1). The high log K_1 observed for CHX-A and CHX-B is presumably due to constraint of the nitrogens to the trans conformation, effectively promoting a proton to be shared between the two basic nitrogens.

The Y(III) stability constants were measured using Arsenazo III dye complexes by the methodology originally developed by Sherry and co-workers for gadolinium(III).[10] The methodology was verified by measuring the Y(III) stability constants for previously reported ligands.[8] Since yttrium and the lanthanides form well-defined Arsenazo III complexes, the dye was titrated with YCl_3 to determine the conditional stability constants. Titration of the resulting Y(III) Arsenazo III complex with the DTPA derivatives resulted in a decrease in the absorbance at 652 nm, and the conditional stability constant was calculated on the basis of the absorbance difference, followed by conversion to log *K* values (Table 1). The p*M* values (Table 1) were also

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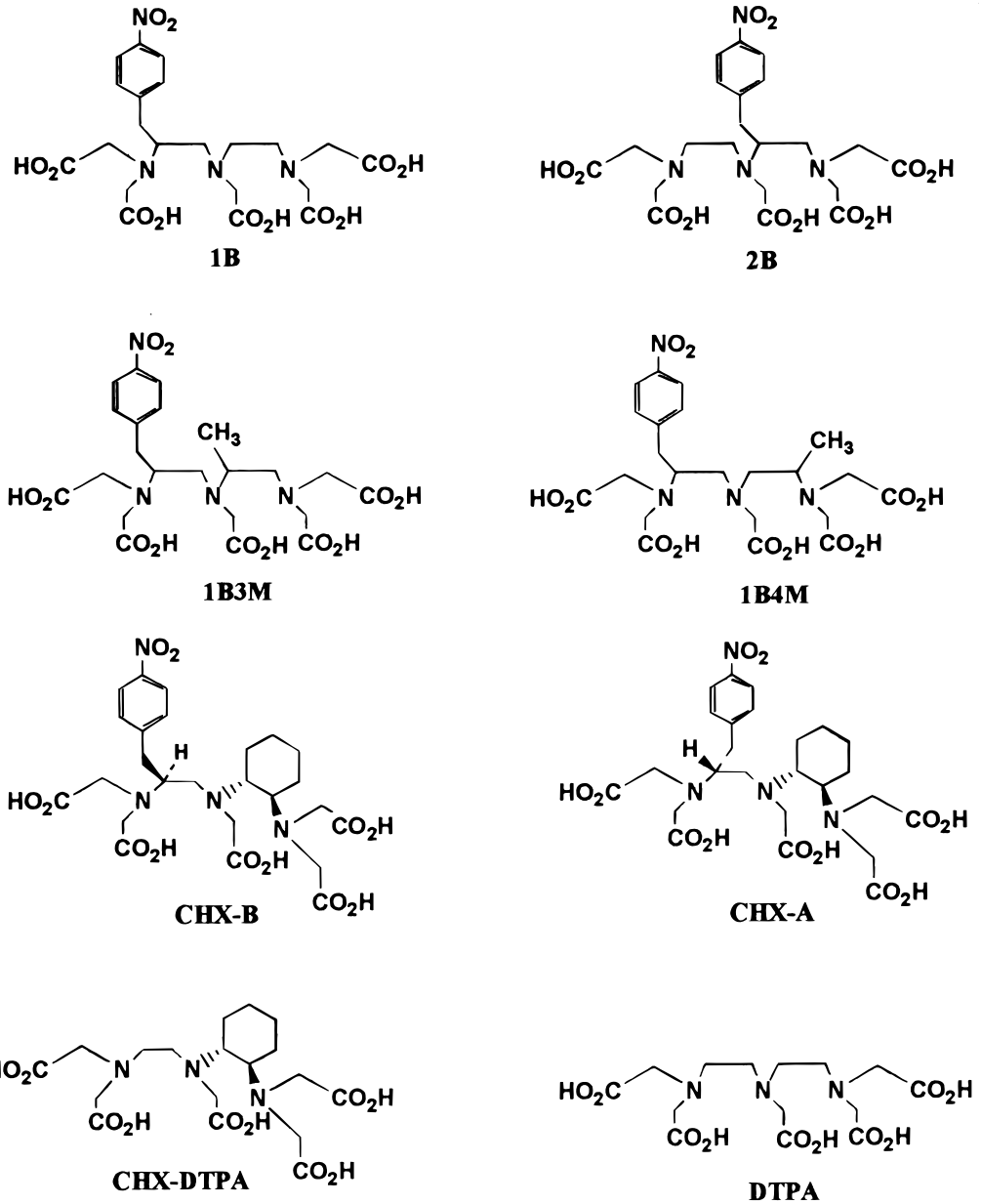

Figure 1. Backbone-substituted DTPA derivatives. 1B, 2B, 1B3M, 1B4M, CHX-A, and CHX-B can be modified for protein conjugation (see text).

Table 1. Selected in Vitro and in Vivo Properties of Polyamino Carboxylate Derivatives

ligand	pK_a[a]	β[b]	log K[c]	pM[d]	k_D (s^{-1})[e]	% ^{88}Y-serum[f]	% ID/g femur[g]
CHX-A	**12.3**; 9.21; 5.58; 3.37; 2.46	32.92	24.7	19.0	0.462	3.79 ± 0.49	3.6 ± 0.2
CHX-B	**12.3**; 8.99; 4.99; 2.84; 2.35	31.47	24.4	18.8	0.047	18.5 ± 1.31	6.1 ± 0.7
IB4M	**11.31**; 8.80; 4.77; 2.87; 2.61	30.36	22.5	18.2	6.62	6.27 ± 0.97	2.5 ± 0.3
IB3M	**11.46**; 9.07; 4.61; 3.19; 2.33	30.60	22.5	17.7	13.5	4.84 ± 0.62	3.0 ± 1.2
2B	**10.75**; 8.57; 4.46; 2.83; 2.63	29.24	21.7	18.1	41.8	7.00 ± 0.43	5.2 ± 1.2
1B	**11.16**; 8.30; 4.44; 2.75; 2.53	29.18	21.5	17.8	37.4	not measured	4.3 ± 0.6
CHX-DTPA	**12.3**; 9.24; 5.23; 3.32; 2.18	32.27	24.2	18.4	0.75	*h*	*h*
DTPA	**10.48**; 8.64; 4.28; 2.6; 2.0[i]	28.00	22.4[i]	18.4	144	*h*	*h*

[a] Log protonation constants at 25 °C, μ = 0.1 M $N(CH_3)_4Cl$, pK_{a1}. [b] $\beta = \sum pK_a$'s. [c] Yttrium(III) stability constant KML = [ML]/[M][L] (25 °C, μ = 0.1 M $NaClO_4$). [d] p$M = -\log[M(H_2O)_x]$ calculated at pH 7.4. [e] Acid-catalyzed dissociation rate constant at [YL] = 1.00×10^{-5} M and [AAIII] = 5.00×10^{-5}M. [f] Fraction of ^{88}Y (%) released from chelate after incubation in serum for 15 h. [g] Percent injected dose per g at 168 h for mice injected with ^{88}Y−Ligand-B72.3. Reference 12. [h] Not measured. No site available for protein conjugation. See Figure 1. [i] Reference 8

calculated to estimate the relative ability of the DTPA derivatives to sequester a metal under physiological conditions (pH 7.4). The p*M* was insensitive to ligand structure and followed the same pattern as the stability constants.

The Y(III) stability constants for the *p*-nitrobenzyl-substituted complexes tended to increase with additional substituents on the carbon backbone. The highest stability constant was observed for the cyclohexyl derivatives CHX-A and CHX-B, suggesting that

the cyclohexyl ring possibly preorganized the DTPA ligand for metal binding. It was surprising that the 1B and 2B derivatives formed marginally less thermodynamically stable Y(III) complexes compared to DTPA. This may be due to the desolvation energy of the complex, which is expected to be lower for the more lipophilic derivatives. As noted previously,[10,11] the ligand basicity and stability constant exhibit a linear free energy relationship, with CHX-A showing both the greatest overall basicity and stability.

The kinetic inertness of the Y(III)-DTPA derivative complexes was evaluated through measurement of the acid dissociation rate constant, k_D, tabulated in Table 1. The measured k_D progressively decreased as alkyl substituents were added to the DTPA backbone. The rates group closely with substitution; monofunctionalized ligands dissociate about $^1/_3$ as fast as DTPA, while 1B3M and 1B4M dissociate more slowly by a factor of more than 10. As anticipated, the *trans*-cyclohexyl ring conferred the greatest kinetic inertness. Surprisingly, the CHX-B complex is the most kinetically inert DTPA derivative evaluated.

Biological Stability. Each *p*-nitrobenzyl-DTPA derivative was converted to its *p*-isothiocyanato derivative, conjugated to the B72.3 antibody, and radiolabeled with ^{88}Y(III) for in vivo stability[12] and in vitro serum stability studies. The dissociation of ^{88}Y from the radiolabeled DTPA derivative conjugates CHX-A, CHX-B, 1B4M, 1B3M, and 2B in serum was evaluated by size exclusion HPLC. The ^{88}Y-bound serum albumin eluted at 7.7 min postinjection and was assigned by comparison to a known molecular weight standard. CHX-A was found to be the most stable DTPA derivative evaluated, while the stereoisomer, CHX-B, was the most labile (Table 1).

The importance of the choice of bifunctional chelate for ^{90}Y radioimmunotherapy has been demonstrated.[13] Y(III) is a bone-seeking metal[14] and premature release of ^{90}Y from the radiolabled antibody conjugate in vivo will result in deposition of excess radiation in the bone, irradiating bone marrow, a critical organ for radiotoxicity.[5] The relative lability of the ^{88}Y-DPTA derivative antibody conjugate can thus be inferred from tissue distribution studies measuring the radioactivity that accumulates in the bone of mice injected with the radiolabeled conjugate.

In two recent studies,[12] Roselli et al. evaluated the in vivo biodistribution of the DTPA derivatives, and the results for 168 h bone accumulation are given in Table 1. The in vivo bone accumulation data provides an opportunity to find a correlation between the physical parameters and the in vivo stability. The in vivo inertness of ^{88}Y-labeled CHX-A, 1B4M, and 1B3M conjugated to B72.3 are similar. This suggests that the differences in the measured thermodynamic stability and ligand basicity may be insignificant in a biological system. The significant differences in the acid dissociation rates for these three ligands, along with the similar bone uptake, implies that acid-catalyzed dissociation is not a dominant pathway for the release of ^{88}Y(III) from B72.3 in vivo. The ^{88}Y(III) complexes of 1B and 2B both have a slightly greater release of the radiometal in vivo compared to that of CHX-A, 1B3M, and 1B4M. *The instability of CHX-B in vivo was not predicted by the physical parameters. In fact, on the basis of the measured log K and acid dissociation rate, one would predict this derivative to have high in vivo stability, while the serum stability predicted low in vivo stability.* The low stability of CHX-B radiolabeled conjugate is likely due to stereochemical considerations of the chelate and of the Y(III) complex(es) formed.[15]

Conclusion. The series of backbone functionalized DTPA ligands with one site available for protein conjugation, shown in Figure 1, have provided a unique opportunity to examine both the physical parameters and the in vivo stabilities for a series of acyclic ^{88}Y-labeled chelates. Examination of the measured parameters and the release of ^{88}Y in vivo failed to elucidate an obvious correlation between in vitro and in vivo studies. Careful biological studies in animal models still remain the most appropriate method for evaluating the in vivo inertness of radiometal-labeled chelates prior to clinical trials.

Experimental Section

Materials. All reagents and solvents were ACS reagent grade or better. Aqueous solutions were prepared with high purity deionized water (Hydroservices Picosystem). $(CH_3)_4NCl$ and $NaClO_4$ were purchased from Fluka, recrystallized from water, and vacuum-dried. Solutions of the purified salts were standardized by applying an aliquot to a AG50W-X8 cation-exchange resin, eluting with water, and titrating the liberated acid with standardized base. $(CH_3)_4NOH$ and NaOH (Baker Dilut-it) solutions (0.1000 M) were prepared with carbonate-free water and standardized using potassium hydrogen phthalate (KHP). Carbonate concentrations were measured periodically to ensure that carbonate ion concentrations of 1.2% were not exceeded. Acid solutions were prepared using Baker Dilut-it (0.1000M HCl) or G. F. Smith Ultrapure $HClO_4$ (0.1000 M) and were standardized by titration with base to a phenolphthalein endpoint. YCl_3 (>99.99%, Aldrich) and $Y(ClO_4)_3$ (prepared from Y_2O_3 (>99.99%, Aldrich and $HClO_4$) were standardized with EDTA and xylenol orange indicator. Arsenazo III (AAIII) (98%, <0.01 μmol Ca(II)/mg) was obtained from Sigma.

Ligands. The C-functionalized DTPA ligands 1B, 2B, 1B3M, 1B4M, CHX-A, CHX-B, and CHX-DTPA were synthesized, purified by ion-exchange chromatography, and fully characterized as previously described.[16]

Protonation Constants. Potentiometric pH titrations of each ligand (1 mM) were carried out from pH 2−11 in a glass-jacketed cell thermostated at 25.0 ± 0.1 °C (μ = 0.1 M $N(CH_3)_4Cl$ or $NaClO_4$) using a Fisher Accumet model 925 pH meter equipped with a Ross combination pH electrode containing either 1.75 M $N(CH_3)_4Cl$ or 4 M $NaClO_4$ fill. The electrode was calibrated to permit measurement of hydrogen ion concentration by either a 2 point calibration (pH 2.2 and 11.3) using solutions of standardized acid and base, or by a strong acid/strong base titration and then performing a linear fit of potential E versus $[H^+]$ (setting pK_w = 13.78) to the Nernst equation. Titrations were automated by using an IBM-PC-XT computer interfaced to a Metrohm Dosimat 665 buret.[17] The data were analyzed by using the nonlinear least-squares programs BETA or BEST.[18] Errors in the calculated protonation constants were estimated from triplicate, independent measurements.

Potentiometric pH titrations were repeated in the presence of a 1:1 mixture of 1 mM Y(III)/ligand (μ = 0.100 M $NaClO_4$). While concentrations of free metal ion were too low to determine stability constants, metal−ligand protonation constants were measured by treating the titration data as described above.

Stability Constants. The Y(III)−AAIII complex and the DTPA ligands were competed and concentrations of Y(III)−AAIII were measured spectrophotometrically, by modification

of the literature method.[10] Briefly, in a typical ligand competition experiment, an aliquot of Na_3DTPA (10^{-5} M) was added to a cuvette containing Y(III) (3.950×10^{-6} M) and AAIII (2.00×10^{-5} M) at pH 3.90. Following equilibration, the decrease in absorbance at 652 nm was noted and another aliquot of ligand added. Conditional equilibrium constants and extinction coefficients at $\lambda_{max} = 652$ nm were determined for the well-defined Y(III)−AAIII complexes ML and ML_2, the predominant Y(III)−AAIII complexes near pH 4, by titration of AAIII with YCl_3 (μ = 0.1 M $N(CH_3)_4Cl$, 25 °C). The data were analyzed by using the computer programs COMPETE and ARSENAZO.[10]

Acid-Catalyzed Dissociation. Acid-catalyzed dissociation measurements were made using an Applied Photophysics stopped-flow spectrophotometer (model SF17.MV). Solutions of Y(III)-DTPA−ligand complexes (2.0 μM) were prepared by the addition of equimolar quantities of $Y(ClO_4)_3$ and Na_3DTPA ligand to 0.20 M $NaClO_4$. A solution of AAIII (1×10^{-4} M) and $HClO_4$ (0.20 M) was prepared. The two solutions were mixed in the stop-flow chamber, and the reaction was monitored by the increase in absorbance at 652 nm. Data were fitted to a nonlinear least-squares method employing the Marquart algorithm to obtain rate parameters.

Serum Stability. The DTPA derivatives with a *p*-nitrobenzyl substituent were modified for conjugation to B72.3, and the conjugate was radiolabeled with ^{88}Y by the literature methods.[19] The stabilities of the complexes were evaluated in serum. Serum samples were analyzed by size exclusion HPLC (Gilson model 230) on TSK 3000SW gel filtration column (TosoHaas, Japan) by elution with MES/Cl^- buffer (0.02 M MES, 0.15 M NaCl, 0.05% NaN_3, pH 6.5). An aliquot of each injectate was also assayed for radioactivity to measure the recovery during HPLC.

Supporting Information Available: Partial biodistribution of ^{88}Y-DTPA derivative conjugates (1 page). Ordering information is given on any current masthead page.

References

(1) (a) Lauffer, R. B. Magnetic Resonance Contrast Media: Principles and Progress. *Magn. Reson. Q.* **1990**, *6*, 65−84. (b) Rocklage, S. M.; Watson, A. D.; Carvlin, M. J. Contrast Agents in Magnetic Resonance imaging. In *Magnetic Resonance Imaging*, 2nd ed; Stark, D. M, Bradley, W. G., Eds.; Mosby Year Book, St. Louis, MO, 1992; Vol. 1.

(2) (a) Mathias, C. J.; Welch, M. J.; Perry, D. J.; McGuire, A. H.; Zhu, X.; Connett, J. M.; Green, M. J. Investigations of Copper-PTSM as a Pet Tracer for Tumor Blood-Flow. *Nucl. Med. Biol.* **1991**, *18*, 807−811. (b) Bellen, J. C.; Chattertun, B. E.; Penglis, S.; Tsopelas, C. Gallium-67 Complexes as Radioactive Markers to Assess Gastric and Colonic Transit. *J. Nucl. Med.* **1995**, *36*, 513−517. (c) Rao, T. N.; Adhikesavalu, D.; Camerman, A.; Fritzberg, A. R. Technetium(V) and Rhenium(V) Complexes of 2,3-Bis(mercaptoacetamido)propanoate. Chelate Ring Stereochemistry and Influence on Chemical and Biological Properties. *J. Am. Chem. Soc.* **1990**, *112*, 5798−5804. (d) Forsell-Aronsson, E.; Fjalling, M.; Nilsson, O.; Tisell, L.; Wangberg, B.; Ahlman, H. Indium-111 Activity Concentrations in Tissue Samples After Intravenous Injection of Indium-111-DTPA-D-Phe-1-Octreotide. *J. Nucl. Med.* **1995**, *36*, 7−12.

(3) (a) Scheinberg, D. A.; Strand, M.; Gansow, O. A. Tumor Imaging with Radioactive Metal Chelates Conjugated to Monoclonal Antibodies. *Science* **1982**, *215*, 1511−1514. (b) Parker, D. Tumour Targeting with Radiolabelled Macrocycle-Antibody Conjugates. *Chem. Soc. Rev.* **1990**, *19*, 271−291. (c) Wu, C.; Liu, Y. Radiolabeling of Monoclonal Antibodies with Metal Chelates. *Pure Appl. Chem.* **1991**, *63*, 427−463.

(4) (a) DeNardo, G. L.; Mirick, G. R.; Kroger, L. A.; O'Donnell, R. T.; Meares, C. F.; DeNardo, S. J. Antibody Responses to Macrocycles in Lymphoma. *J. Nucl. Med.* **1996**, *37*, 451−456. (b) Scheinberg, D. A.; Jurcic, J.; Finn, R.; Larson, S.; McDevitt, M. R.; Geerlings, M., Jr.; Sgouros, G.; Brechbiel, M.; Gansow, O. A. Clinical Application of Targeted Alpha Particles for Cancer Therapy. *Abstracts of Papers*, 215th National Meeting of the American Chemical Society, Dallas, TX; American Chemical Society: Washington, DC, 1998; NUCL 87.

(5) Knox, S. J.; Goris, M. L.; Trisler, K.; Negrin, R.; Davis, T.; Liles, T. M.; Grillo-Lopex, A.; Chinn, P.; Varns, C.; Ning, S. C.; Fowler, S.; Deb, N.; Becker, M.; Marquez, C.; Levy, R. Yttrium-90-Labeled Anti-CD20 Monoclonal Antibody Therapy of Recurrent B-Cell Lymphoma. *Clin. Cancer Res.* **1996**, *2*, 457−470.

(6) Stimmel, J. B.; Kull, F. C., Jr. Samarium-153 and Lutetium-177 Chelation Properties of Selected Macrocyclic and Acyclic Ligands. *Nucl. Med. Biol.* **1998**, *25*, 117−125.

(7) DTPA: Diethylenetriaminepentaacetic acid.

(8) Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum Press: New York, 1974. EDTA: Ethylenediaminetetraacetic acid.

(9) Okaku, N.; Toyoda, K.; Moriguchi; Y.; Ueno, K. Synthesis of Chelating Agents. IV. Synthesis and Chelating Behavior of 1-Phenyl-ethylenedinitrilo-N,N,N′,N′-tetraacetic Acid and 1,2-Diphenyl-ethylenedinitrilo-N,N,N′,N′-tetraacetic Acid. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2326−2332.

(10) Sherry, A. D.; Cacheris, W. P.; Kah-Tiong, K. Stability Constants for Gd^{3+} Binding to Model DTPA-Conjugates and DTPA-proteins: Implications for Their Use as Magnetic Resonance Contrast Agents. *Magn. Reson. Med.* **1988**, *8*, 180−190.

(11) Deal. K. A.; Motekaitis, R. J.; Martell, A. E.; Welch, M. J. Evaluation of the Stability and Animal Biodistribution of Gadolinium(III) Benzylamine-Derivatized Diethylenetriaminepentaacetic Acid. *J. Med. Chem.* **1996**, *39*, 3096−3106.

(12) (a) Roselli, M.; Milenic, D. E.; Mirzadeh, S.; Brechbiel, M. W.; Pippin, C. G.; Gansow, O. A.; Colcher, D.; Schlom, J. Biodistribution Studies of Indium- and Yttrium-Labeled MAb B72.3 Using DPTA-Derived Bifuctional Chelators. *Tumor Targeting*, in press. (b) Roselli, M.; Milenic, D. E.; Mirzadeh, S.; Brechbiel, M. W.; Pippin, C. G.; Gansow, O. A.; Colcher, D.; Schlom, J. unpublished results.

(13) (a) Kozak, R. W.; Raubitschek, A.; Mirzadeh, S.; Brechbiel, M. W.; Junghans, R.; Gansow; O. A.; Waldmann, T. Nature of the Bifunctional Chelating Ligand Used for Radioimmunotherapy with Yttrium-90 Monoclonal Antibodies: Critical Factors in Determining in Vivo Survival and Organ Toxicity. *Cancer Res.* **1989**, *49*, 2639−2644. (b) Roselli, M.; Schlom, J.; Gansow, O. A.; Raubitschek, A.; Mirzadeh, S.; Brechbiel, M. W.; Colcher, D. Comparative Biodistributions of Yttrium- and Indium-Labeled Monoclonal Antibody B72.3 in Athymic Mice Bearing Human Colon Carcinoma Xenographs. *J. Nucl. Med.* **1989**, *30*, 672−682. (c) Huneke, R. B.; Squire, R. A.; Pippin, C. G.; Squire, R. A.; Brechbiel, M. W.; Gansow, O. A.; Strand, M. Effective α-Particle-Mediated Radioimmunotherapy of Murine Leukemia. *Cancer Res.* **1992**, *52*, 5818−5820.

(14) (a) Durbin, P. W. Metabolic Characteristics Within A Chemical Family. *Health Phys.* **1960**, *2*, 225−238. (b) Jowsey, J.; Rowland, R. E.; Marshall, J. H. The Deposition of the Rare Earths in Bone. *Radiat. Res.* **1958**, *8*, 490−501.

(15) (a) Wu, C.; Kobayashi, H.; Sun, B.; Yoo, T. M.; Paik, C. H.; Gansow, O. A.; Carrasquillo, J. A.; Pastan, I.; Brechbiel, M. W. Stereochemical Influence on the Stability of Radio-Metal Complexes In Vivo. Synthsis and Evaluation of the Four Stereoisomers of 2-(*p*-Nitrobenzyl)-*trans*-CyDTPA. *Bioorg. Med. Chem.* **1997**, *10*, 1925−1934. (b) Deal, K. A.; Welch, M. J. Effect of Stereochemistry on the Clearance Mechanism of ^{111}In(III)-Labeled D- or L-Benzyldiethylenetriaminepentaacetic Acid. *J. Med. Chem.* **1997**, *40*, 3986−3989.

(16) (a) Brechbiel, M. W.; Gansow, O. A. Synthesis of C-Functionalized trans-cyclohexyldiethylenetriaminepentaacetic Acids for Labeling of Monoclonal Antibodies with the Bismuth-212 α-Particle Emitter. *J. Chem. Soc., Perkin Trans I* **1992**, 1173−1179. (b) Brechbiel, W. B.; Gansow, O. A. Backbone-Substituted DTPA Ligands for ^{90}Y Radioimmunotherapy. *Bioconjugate Chem.* **1991**, *2*, 187−194. (c) Brechbiel, M. W.; Gansow, O. A.; Atcher, R. W.; Schlom J.; Esteban, J.; Simpson, D. E.; Colcher, D. Synthesis of 1-(p-Isothiocyanatobenzyl) Derivatives of DTPA and EDTA. Antibody Labeling and Tumor-Imaging Studies. *Inorg. Chem.* **1986**, *25*, 2772−2781. (d) Brechbiel, M. W.; Beitzel, P. M.; Gansow, O. A. Purification of *p*-Nitrobenzyl C-Functionalized Diethylenetriamine Pentaacetic Acids for Clinical Applications Using Anion Exchange Chromatography. *J. Chromatogr., A* **1997**, *771*, 63−69.

(17) Rocklage, S. M.; Sheffer, S. H.; Cacheris, W. P.; Quay, S. C.; Hahn, F. E.; Raymond, K. N. Structural and Thermodynamic Characterization of Manganese(II) N,N′-Dipyridoxylethylenediamine-N,N′-diacetate. A Novel Manganese(II) Chelate. *Inorg. Chem.* **1988**, *27*, 3530−3534.

(18) (a) Harris, W. R.; Raymond, K. N.; Weitl, F. R. Ferric Ion Sequestering Agents. 6. The Spectrophotometric and Potentiometric Evaluation of Sulfonated Tricatecholate Ligands. *J. Am. Chem. Soc.* **1981**, *103*, 2667. (b) Motekaitis, R. J.; Martell, A. E. BEST-A New Program for Rigorous Calculation of Equilibrium Parameters of Complex Multicomponent Systems. *Can. J. Chem.* **1982**, *60*, 2403−2409.

(19) McMurry, T. J.; Brechbiel, M. W.; Wu, C.; Gansow, O. A. Synthesis of 2-(p-NCS-Bz)NOTA: Application of the 4-methoxy-2,3,6-trimethylbenzenesulfonamide (Mtr) Protecting Group in the Synthesis of Macrocyclic Polyamines. *Bioconjugate Chem.* **1993**, *4*, 236−245.

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