# Effect of Stereochemistry on the Clearance Mechanism of $^{111}$In(III)-Labeled D- or L-Benzyldiethylenetriaminepentaacetic Acid

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The diethylenetriaminepentaacetic acid (DTPA) derivatives L-Bn-DTPA and D-Bn-DTPA were synthesized and radiolabeled with $^{111}In^{3+}$. The uptake and clearance of the compounds were determined through biodistribution and excretion studies in Wistar rats. Both isomers readily cleared from the animal. The D isomer showed relatively high kidney uptake and predominately renal clearance. The L isomer showed substantial kidney and liver uptake with equal biliary and renal clearance. Clearance was also evaluated in TR- Wistar rats, which are defective in the liver canalicular multispecific organic anion transporter (cMOAT) protein. cMOAT mediates hepatobiliary clearance of many organic anions. Both compounds were excreted through the urine in TR- Wistar rats, suggesting that cMOAT is important in the clearance of the compounds from the liver.

The design of improved metal based radiopharmaceuticals is assisted by an understanding of the uptake and clearance mechanism of the metal complex. In many instances, the uptake can be predetermined by receptor systems, like in galactose BSA,[1−3] Octreotide,[4] and monoclonal antibody conjugate systems.[5,6] Uptake of low molecular weight complexes appears to be governed by characteristics of the metal complex, such as charge[7] and lipophilicity.[8] The clearance of both receptor mediated conjugates[9] and metal complexes[8] seems to be influenced by the charge, lipophilicity, and conformation of the metal complex. The effect of charge and lipophilicity on clearance has been addressed for both protein/peptide conjugates[10] and metal complexes;[11] however, the effect of stereochemistry needs further evaluation.

The role of stereochemistry on clearance has been addressed for a series of $^{99m}$Tc-mercaptoacetyltripeptides,[12] but few studies have looked at $^{111}In^{3+}$-labeled chiral DTPA (diethylenetriaminepentaacetic acid) derivatives. Camera and co-workers have studied the clearance of a series of CHX-DTPA [(2-(*p*-SCN-Bz)-cyclohexyl)diethylenetriaminepentaacetic acid] isomers conjugated to monoclonal antibodies.[13] CHX-DTPA is a DTPA derivative with three stereochemical centers and a cyclohexyl group substituted on the DTPA backbone. Analysis of the $^{88}$Y-labeled antibody conjugates showed significant differences in biodistribution and stability between the antibody conjugates.[13] The thermodynamic stability of the [$^{88}$Y]CHX-DTPA complexes were affected by the conformation of the three stereochemical centers. Similar blood clearance profiles for all of the conjugates, and different biodistributions, were assigned to the different handling of the $^{88}$Y-chelate metabolite products.[13] The $^{88}$Y study suggests that stereochemistry can play a crucial role in the stability and biological activity of metal-based radiopharmaceuticals.

To explore the role of stereochemistry on uptake and clearance, the D and L isomers of a benzyl-derivatized DTPA have been synthesized (D- or L-Bn-DTPA). The DTPA derivatives were radiolabeled with $^{111}In^{3+}$ and the biological activity evaluated. Normal Wistar rats were initially used to evaluate the biodistribution and clearance. The clearance was evaluated in TR- Wistar rats, a mutant rat lacking the hepatocyte canalicular multispecific organic anion transport protein (cMOAT).[14] cMOAT is a general purpose transport protein, which works with gluthathione-*S*-transferase to remove toxins from hepatocyte lysosomes to the bile.[15] Clearance of [$^{111}$In]D-Bn-DTPA and [$^{111}$In-]L-Bn-DTPA from TR- rats, which are characterized by jaundice, were performed to elucidate the clearance mechanism.

## Results

**Synthesis.** Application of the stereospecific methodology of Williams and Rapoport[16] to the amino acids L-phenylalanine and D-phenylalanine resulted in the preparation of D-Bn-DTPA and L-Bn-DTPA (Scheme 1). Initially, the amino acid carboxylate was protected as the benzyl ester and the amine tosylated. The benzyl ester amino acid tosylated salt was N-alkylated with [*N*-(bromoethyl)amino]diacetic acid ester to give the benzyl-protected DTPA ester. Deprotection of the carboxylate groups by hydrogenation resulted in the pure benzyl DTPA derivatives, L-Bn-DTPA and D-Bn-DTPA. Optical rotation measurements gave D-Bn-DTPA as $[\alpha_D] = 31°$ and L-Bn-DTPA as $[\alpha_L] = -33°$; indicating D and L forms are enantiomers.

The DTPA derivatives were readily labeled with $^{111}In^{3+}$, and in each case, one product was obtained with a greater than 95% radiochemical purity. The $^{111}In^{3+}$-labeled complexes were stable; free $^{111}In^{3+}$ was not detected by radio TLC over 24 h. The labeling conditions were similar to those for labeling DTPA[2] and LDTPA.[3] Because the radiolabeled D-Bn-DTPA and L-Bn-DTPA differ only in stereochemistry, the two $^{111}In^{3+}$-labeled complexes are predicted to have identical charge and lipophilicity.

**In Vivo Clearance.** The uptake and clearance of D-Bn-DTPA and L-Bn-DTPA were determined through excretion and biodistribution studies. The whole body clearance of the $^{111}In^{3+}$-labeled compounds were evaluated in normal and jaundiced (TR-) Wistar rats. Application of the Student's *t*-test at the 95% confidence

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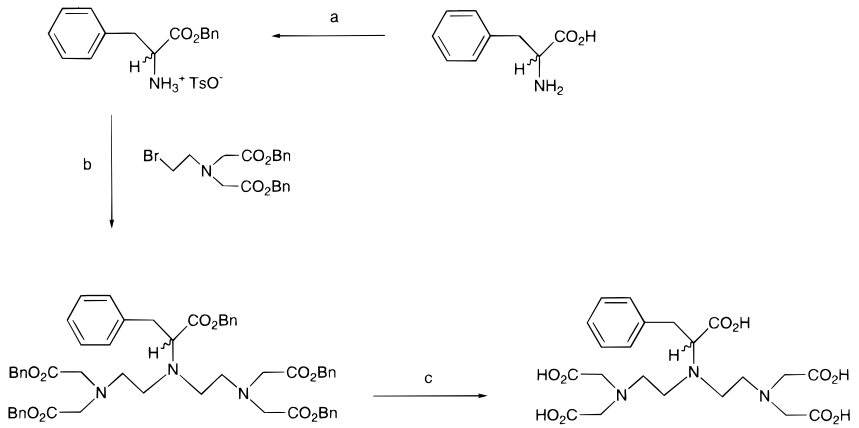
**Scheme 1**[a]




[a] (a) TsOH, BnOH, benzene; (b) phosphate buffer, acetonitrile; (c) Pd/C, $H_2$, ethanol, HCl.

**Table 1.** $^{111}$In−Chelate Clearance from Normal and TR- Wistar Rats at 72 h[a]

| chelate | animal | % urine | % feces |
|---|---|---|---|
| D-Bn-DTPA | normal | 62.0 ± 5.1 | 29.2 ± 3.8 |
| | TR- | 85.0 ± 5.0 | 3.3 ± 1.4 |
| L-Bn-DTPA | normal | 51.9 ± 4.1 | 42.8 ± 4.1 |
| | TR- | 80.4 ± 6.9 | 10.6 ± 2.7 |

[a] Data expressed as %ID/organ with five animals per study.

**Table 2.** Biodistribution of [$^{111}$In]D-Bn-DTPA and [$^{111}$In]L-Bn-DTPA in Normal Wistar Rats[a]

| | 5 min | 15 min | 30 min | 60 min |
|---|---|---|---|---|
| | | D-Bn-DTPA | | |
| blood | 12.7 ± 0.5 | 6.36 ± 0.92 | 2.66 ± 0.48 | 0.86 ± 0.16 |
| liver | 13.2 ± 2.2 | 6.84 ± 1.06 | 2.37 ± 0.43 | 0.82 ± 0.09 |
| kidney | 4.74 ± 0.48 | 3.04 ± 0.24 | 1.96 ± 0.34 | 1.10 ± 0.02 |
| bone | 4.59 ± 0.12 | 3.76 ± 0.64 | 2.17 ± 0.23 | 2.43 ± 0.46 |
| liver/blood | 1.04 ± 0.15 | 1.10 ± 0.20 | 0.90 ± 0.09 | 0.99 ± 0.20 |
| kidney/blood | 0.37 ± 0.02 | 0.48 ± 0.10 | 0.74 ± 0.07 | 1.30 ± 0.25 |
| | | L-Bn-DTPA | | |
| blood | 10.7 ± 0.7 | 4.67 ± 1.11 | 2.03 ± 0.12 | 0.71 ± 0.20 |
| liver | 15.6 ± 1.4 | 7.63 ± 1.70 | 3.15 ± 0.26 | 1.10 ± 0.13 |
| kidney | 3.45 ± 0.22 | 1.63 ± 0.17 | 0.92 ± 0.06 | 0.57 ± 0.13 |
| bone | 3.28 ± 0.50 | 3.81 ± 0.96 | 2.31 ± 0.85 | 2.40 ± 0.74 |
| liver/blood | 1.46 ± 0.20 | 1.69 ± 0.30 | 1.55 ± 0.10 | 1.60 ± 0.40 |
| kidney/blood | 0.33 ± 0.02 | 0.36 ± 0.05 | 0.46 ± 0.02 | 0.82 ± 0.20 |

[a] Data expressed as %ID/organ with four animals per time point.

level indicated a statistical difference was observed between $^{111}In^{3+}$-labeled D- and L-Bn-DTPA for the urine and feces of the normal rat. Both isomers readily cleared from the two animal models. In normal rats, the D isomer showed 62% clearance through the urine and 30% clearance through the feces at 72 h. The same compound in the TR- animal showed exclusive renal clearance (85%). The L isomer demonstrated approximately equal clearance in the urine and the feces in the normal rat at 72 h. The clearance pathway of the L isomer shifted to the urine (80%) in the TR- rat (Table 1). In comparison, [$^{111}$In]DTPA had predominately (88%) renal clearance to the urine in the normal rat.

The biodistributions of $^{111}In^{3+}$-labeled D-Bn-DTPA and L-Bn-DTPA were evaluated in normal Wistar rats (Table 2). To determine if the observed differences between the D-Bn-DTPA and L-Bn-DTPA were significant, a Student's *t*-test was performed. At the 95% confidence level, a statistical significant difference was observed for the kidney (all times), liver (30 and 60 min), blood (5 and 30 min), and bone (5 min). The blood clearance is similar for the two compounds, but [$^{111}$In]-L-Bn-DTPA has a slightly higher liver uptake while [$^{111}$In]D-Bn-DTPA has a higher kidney uptake. The increased kidney uptake observed for [$^{111}$In]D-Bn-DTPA ranged from 30% (5 min) to 50% (60 min) greater than the [$^{111}$In]L-Bn-DTPA kidney uptake. The increased liver uptake observed for [$^{111}$In]L-Bn-DTPA is 25% greater than the [$^{111}$In]D-Bn-DTPA liver uptake at 30 and 60 min.

The biodistribution data was normalized to the blood, and liver/blood and kidney/blood ratios were calculated (Table 2). The [$^{111}$In]D-Bn-DTPA liver/blood ratio was constant at 1.0 for all time points. The liver/blood ratio for [$^{111}$In]L-Bn-DTPA was constant at approximately 1.6. The [$^{111}$In]D-Bn-DTPA kidney/blood ratio was steadily increasing from 0.4 at 5 min to 1.3 at 60 min, while the kidney/blood ratio was increasing from 0.3 at 5 min to 0.8 at 60 min for [$^{111}$In]L-Bn-DTPA.

## Discussion

**Synthesis.** To determine the effect, if any, that stereochemistry had on the uptake and clearance of metal based radiopharmaceuticals, two enantiomertic DTPA derivatives were synthesized and characterized. The ideal method for incorporation of a chiral center into DTPA is to utilize amino acids in the synthesis. Most amino acids are commercially available as the D or L isomer. Williams and Rapoport[16] have developed a methodology to introduce *p*-nitro-L-phenylalanine at the central carboxylate of DTPA. Their synthetic method showed complete retention of the asymmetric center as the L isomer. Since racemization was not observed, it seemed reasonable to attempt the incorporation of a different amino acid at the central carboxylate. For this study, D-phenylalanine and L-phenylalanine were incorporated into the synthesis, which resulted in the preparation of D-Bn-DTPA and L-Bn-DTPA.

**In Vivo Clearance.** The differences observed between the [$^{111}$In]D-Bn-DTPA and [$^{111}$In]L-Bn-DTPA biodistribution and excretion studies can be correlated with the changes in stereochemistry. The two complexes have a formal charge of −2 and were more lipophilic than [$^{111}$In]DTPA. The more hydrophobic character is a reflection of the benzyl group on the central carboxylate of DTPA. The increased lipophilicity was reflected by the dramatic differences in the biodistribution and excretion pathway of [$^{111}$In]D-Bn-DTPA and [$^{111}$In]L-Bn-DTPA compared to [$^{111}$In]DTPA.[2] Both the D- and L-Bn-DTPA radiolabeled complexes showed significant fecal

excretion compared to DTPA. [$^{111}$In]DTPA biodistribution has been previously reported in Sprague−Dawley rats.[2] Generally, [$^{111}$In]DTPA showed low liver uptake and high kidney uptake (2.5% ID/liver, 5.2% ID/kidney at 5 min). The kidney uptake for [$^{111}$In]D-Bn-DTPA was similar to that for [$^{111}$In]DTPA, but the liver uptake was greater (13% ID/liver at 5 min). In contrast, [$^{111}$In]L-Bn-DTPA showed lower kidney (3.5% ID/kidney at 5 min) uptake and greater liver uptake (16% ID/liver at 5 min) than [$^{111}$In]DTPA.

It was possible that the differences in the $^{111}$In-labeled D- and L-Bn-DTPA are due to differences in the stability of the In(III) complex for the two isomers. This does not seem likely since minor changes at the methylene carbon of the central carboxylate have been shown to have only minor effects on the metal stability constant.[3] The stability constant of [In]D- or -L-Bn-DTPA are expected to be similar to [In]DTPA. Free $^{111}In^{3+}$, represented by $^{111}$In(III) acetate, is known to bind transferrin and deposit in the bone.[17] The biodistribution of $^{111}$In(III) acetate in Sprague−Dawley rats showed slow blood clearance with 52% ID/blood at 1 h.[2] Both the $^{111}$In-labeled D-Bn-DTPA and L-Bn-DTPA showed less than 1% ID/blood at 1 h (Table 2). The bone accumulation (Table 2) of the $^{111}In^{3+}$ isomers (2.5% ID/bone at 1 h) was considerably lower than $^{111}$In(III) acetate accumulation of 15% ID/bone at 1 h. Finally, $^{111}$In(III) acetate whole body clearance from Sprague-Dawley rats was slow, with 25% of the injected dose remaining in the bone at 48 h.[2] The whole body clearance of $^{111}$In-labeled D- and L-Bn-DTPA was nearly complete with approximately 85% of the injected dose excreted in 24 h.

The differences in [$^{111}$In]D-Bn-DTPA and [$^{111}$In]L-Bn-DTPA biodistribution and clearance are subtle. Statistical analysis at a 95% confidence level indicated that a significant difference was observed between the $^{111}$In-labeled D- and L-Bn-DTPA for liver (30 and 60 min) and kidney (all time points). There appears to be a preference for the L isomer in the liver. This was reflected by the increased liver/blood ratios observed at all time points and the increased %ID in the liver at 30 and 60 min. The increased liver uptake corresponded to an increase in activity excreted in the feces for the L isomer. These differences suggest that the liver has different recognition systems for stereochemical centers. The L isomer may be considered biologically useful, and was retained for recycling. The D isomer was recognized as foreign and excreted quickly through the kidney.[18]

The clearance of [$^{111}$In]D-Bn-DTPA and [$^{111}$In]L-Bn-DTPA from TR- Wistar rats suggested that cMOAT was involved in the liver clearance.[19] cMOAT is an adenosine triphosphate dependent transport system responsible for the excretion of negatively charged compounds from hepatocytes.[14] If cMOAT was involved in the clearance of a compound, the excretion route will change from the feces to the urine.[20] This was observed for both [$^{111}$In]D-Bn-DTPA and [$^{111}$In]L-Bn-DTPA. It is interesting that some residual activity remains in the feces for [$^{111}$In]L-Bn-DTPA, suggesting liver uptake is not limited to hepatocytes.

**Summary.** To explore the role of stereochemistry on clearance mechanisms, D-Bn-DTPA and L-Bn-DTPA have been synthesized and labeled with $^{111}In^{3+}$. The biodistribution and excretion of the $^{111}$In-labeled complexes demonstrated that the L isomer has slightly greater liver activity. The clearance of the two isomers from TR- Wistar rats suggested that the cMOAT protein was involved in the liver clearance of both isomers. Further studies are necessary to determine the uptake mechanism and role of cMOAT in the metabolism of metal-based radiopharmaceuticals.

## Experimental Section

Sodium acetate was purchased from Fluka. $^{111}InCl_3$ was supplied by Mallinckrodt Medical, Inc. All other chemicals were purchased from Aldrich Chemical Co. and used without further purification. Water was purified by filtration through a Waters-Millipore purification system.

Silica gel 60 and Silica gel 60 F-254 thin-layer chromatography plates were purchased from EM Science. $^1$H and $^{13}$C NMR were obtained on a 300 MHz Gemini or Varian NMR. The optical rotation was measured on a Perkin-Elmer 241 polarimeter with a sodium lamp. Elemental analysis was performed by Galbriath Laboratories. Radioactivity was measured with either a Capintec Radioisotope Dose Calibrator or a Beckman 8000 Gamma Counter. Radio thin-layer chromatography plates were read on a Bioscan System 200 Imaging Scanner with an Autochanger 4000 and PC control.

Female Wistar rats were purchased from Charles River Laboratory and TR- Wistar rats were obtained from a colony maintained at Washington University. The metabolism cages were purchased from Nalgene. All animal experiments were performed in compliance with the Washington University Animal Care Committee guidelines.

**D- or L-Phenylalanine Benzyl Ester Tosylate Salt**. The tosylate salt was prepared by modification of the method described by Williams and Rapoport.[16] Specifically, D- or L-phenylalanine (5 g, 30.4 mmol) was combined with *p*-toluenesulfonic acid monohydrate (6.9 g, 36.1 mmol) in benzyl alcohol (65 mL) and benzene (45 mL). The reaction mixture was heated at reflux overnight and about 5 mL of water was collected in a Dean−Stark trap. The colorless solution was cooled, 100 mL of ether was added, and the product was collected by filtration. The waxy solid was rinsed with ether and dried under vacuum to give the crude product (11.7 g, 90% yield). Recrystallization from benzene gave the product as a white, waxy solid. Both isomers were characterized; similar spectra and elemental analysis were obtained. Data shown is for the D isomer: $^1$H NMR (DMSO-$d_6$) $\delta$ 2.28 (s, 3H), 3.09 (m, 2H), 4.37 (t, 1H), 5.13 (d, 2H), 7.11−7.48 (m, 10H), 7.51 (d, 2 H), 8.52 (s, 1H); $^{13}$C NMR $\delta$ 20.9, 36.1, 53.4, 67.1, 125.6, 127.3, 128.3, 128.4, 128.5, 128.7, 129.5, 134.6, 134.8, 138.1, 145.1, 163.6, 168.9, 216.5, 226.6. Anal. Calcd for $C_{23}H_{23}NO_5S$: C, 64.92; H, 5.45; N, 3.29. Found: C, 65.25; H, 6.03; N, 3.34.

***N,N*-Bis[2-[*N′,N″*-bis[(benzyloxycarbonyl)methyl]amino]-ethyl]-D- or -L- phenylalanine Benzyl Ester**. *N,N*−Bis-[(benzyloxycarbonyl)methyl]-2-bromoethylamine[16] (2.7 g, 6.45 mmol), D- or L-tosylate salt (1.3 g, 3.1 mmol), $CH_3CN$ (10 mL), and phosphate buffer (30 mL, 1 M, pH 8.0) were combined in a 100 mL flask and vigorously stirred for 2 h. The lower buffer layer was removed and extracted with $CH_3CN$ (10 mL). The $CH_3CN$ extract was added to the reaction mixture with new buffer (30 mL, 1 M pH 8.0) and stirred vigorously overnight. The $CH_3CN$ layer was separated and evaporated to give a pale yellow residue which was partitioned between buffer (36 mL) and ethyl acetate (36 mL). The ethyl acetate layer was washed with saturated NaCl solution (12 mL × 2), dried, and evaporated to dryness to give 2.5−3.0 g of crude oil. Pure material was obtained after chromatography on silica (gradient 3/1 to 2/1 hexane/ethyl acetate 1% methanol) as a pale yellow oil (1.1−1.3 g, 30−45% yield): $R_f$ = 0.49 (2/1 hexane/ethyl acetate 1% methanol). Both isomers were characterized, and similar spectra and elemental analysis were obtained. Data shown is for the D isomer: $^1$H NMR ($CDCl_3$) $\delta$ 2.71 (m, 10H), 3.00 (m, 1H), 3.51 (s, 8H), 3.77 (t, 1H), 5.01 (s, 2H), 5.08 (s, 8H), 7.10−7.30 (m, 30 H); $^{13}$C NMR $\delta$ 35.9, 49.9, 53.0, 54.9, 64.9, 65.2, 65.8, 66.0, 126.1, 126.8, 127.3, 127.9, 128.0, 128.1, 128.3,

128.4, 129.2, 135.5, 135.7, 138.1, 168.6, 170.9, 172.1, 189.0, 221.4, 231.5. Anal. Calcd for $C_{56}H_{59}N_3O_{10}$: C, 72.03; H, 6.32; N, 4.50. Found (D): C, 72.26; H, 6.41; N, 4.05. Found (L): C, 72.03; H, 6.34; N, 4.22.

***N,N*-Bis[2-[*N′,N″*-bis[(benzyloxycarbonyl)methyl]amino]-ethyl]-D- or -L-phenylalanine Trihydrochloride Salt.** The D- or L-pentabenzyl ester (1.3 g, 1.38 mmol) was dissolved in methanol (25 mL) while adding 4 M HCl (1.4 mL). To this solution was added 10% Pd/C (150 mg). The reaction was shaken on a Parr hydrogenator at 50 psi for 50 min. The catalyst was removed by filtration through Celite, the filtrate was evaporated, and the residue was redissolved in methanol and evaporated to dryness. The off-white powder was dried under vacuum (727 mg, 89%). FAB mass spectrum showed (M+1) = 484 for both the D and L isomers. Both isomers were characterized and similar spectra were obtained. Data shown is for the D isomer: $^{13}$C NMR ($D_2O$) $\delta$ 57.6, 69.3, 77.1, 78.6, 88.0, 150.7, 152.6, 152.7, 161.3, 192.0, 198.9. D: $[\alpha]^{30} = 31°$ ($c$ = 1, $H_2O$). L: $[\alpha]^{30} = -33°$ ($c$ = 1, $H_2O$).

**[$^{111}$In]D- or L-Bn-DTPA.** The radioactive $^{111}$In(III)-labeled D- or L- Bn-DTPA complex was generated by incubating the ligand with $^{111}$In(III) acetate. In a typical reaction, a 1 $\mu$Ci/$\mu$L $^{111}$In(III) acetate stock was generated by incubating $^{111}InCl_3$ with 0.4 M sodium acetate at pH 5.5 for 15 min. The radiolabel was then added to 30 $\mu$g of ligand in 0.4 M sodium acetate at pH 5.5 and mixed at room temperature for 30 min. The purities of [$^{111}$In]D-Bn-DTPA and [$^{111}$In]L-Bn-DTPA were greater than 95%, as assessed by radio thin-layer chromatography on silica eluted with 1:1 methanol/10% aqueous sodium acetate. The $R_f$ of [$^{111}$In]D- or -L-Bn-DTPA is 0.76, and the $R_f$ of $^{111}$In(III) acetate is 0.0.

**Excretion Studies.** The whole body clearance of [$^{111}$In]D- or L-Bn-DTPA was evaluated in normal, adult, male and TR-Wistar, adult, male rats. The radiopharmaceutical was prepared as described above, and approximately 4 $\mu$Ci/rat or 5 $\mu$g/rat was injected intravenously. Five rats were used for each study. Each rat was housed individually in a metabolism cage. The urine and feces were collected periodically for 72 h, at which time greater than 90% of the injected dose was excreted. The percentage injected dose per excretion was calculated.

**Biodistribution.** $^{111}In^{3+}$-labeled D-Bn-DTPA and L-Bn-DTPA were evaluated in normal, adult, female Wistar rats. For each, 5−10 $\mu$g of radiolabeled chelate was injected, and four rats were sacrificed at predetermined time points. Samples of blood, liver, and kidney were removed. The percent injected dose per organ (%ID/organ) was calculated as previously described.[21] The liver/blood and kidney/blood ratios were calculated for each animal and than averaged.

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