Fluorescence-Enhanced Europium-Diethylenetriaminepentaacetic (DTPA)-Monoamide Complexes for the Assessment of Renal Function

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Real-time, noninvasive assessment of glomerular filtration rate (GFR) is essential not only for monitoring critically ill patients at the bedside, but also for staging and monitoring patients with chronic kidney disease. In our pursuit to develop exogenous luminescent probes for dynamic optical monitoring of GFR, we have prepared and evaluated Eu^{3+} complexes of several diethylenetriamine pentaacetate (DTPA)-monoamide ligands bearing molecular "antennae" to enhance metal fluorescence via intramolecular ligand–metal fluorescence resonance energy transfer process. The results show that Eu-DTPA-monoamide complex **18b**, which contains a quinoxanlinyl antenna, exhibits large (ca. 2700-fold) Eu^{3+} fluorescence enhancement. Indeed, complex **18b** exhibits the highest fluorescent enhancement observed thus far in the DTPA-type metal complexes. The renal clearance property was assessed using the corresponding radioactive ^{111}In complex **18a**, and the data suggest that this complex clears via a complex mechanism that includes glomerular filtration.

Introduction

It is well recognized that the degree of glomerular filtration rate (GFR) represents the best overall measure of kidney function in the state of health or illness.[1] In the past three decades, many endogenous markers such as creatinine[2] and cystatin C,[3] as well as exogenous agents such as inulin, sinistrin, ^{99m}Tc-DTPA, and iothalamate have been developed to determine GFR,[4–14] but all of them require either radiometric, HPLC, or X-ray fluorescence methods for detection and quantification. Theoretical methods for estimating GFR from body cell mass and plasma creatinine concentration have also been developed,[15,16] but these methods depend upon many clinical and anthropometric variables, such as age, renal perfusion, muscle mass, and so on, that would require some correction to the theoretically estimated GFR value. Thus, it is not surprising that the measurement of GFR by endogenous serum creatinine level continues to be widely used clinically despite its inadequacies. The availability of exogenous markers that absorb and fluoresce in the visible region and that provide rapid, dynamic (i.e., continuous, real-time), and accurate measure of renal excretion rate would represent a substantial improvement over any currently available or widely practiced method. Moreover, because such a method would depend solely on the renal elimination of the exogenous chemical entity, the measurement would be absolute and would not involve subjective interpretation based on age, muscle mass, blood pressure, and so on. Indeed, such a dynamic renal function monitoring at the bedside is of high value because it will guide the type of rapid intervention that is necessary for the critically ill or injured patients who face the risk of acute renal failure that may eventually result in multiple organ failure (MOF).[17]

Small, hydrophilic, anionic substances are generally recognized to be excreted by the kidneys.[18] Substances that are filtered by glomerulus (referred to as "GFR agents") comprise inulin (**1**), creatinine (**2**), iothalamate (**3**), and ^{99m}Tc-DTPA (**4a**;[2,4,8,12,13

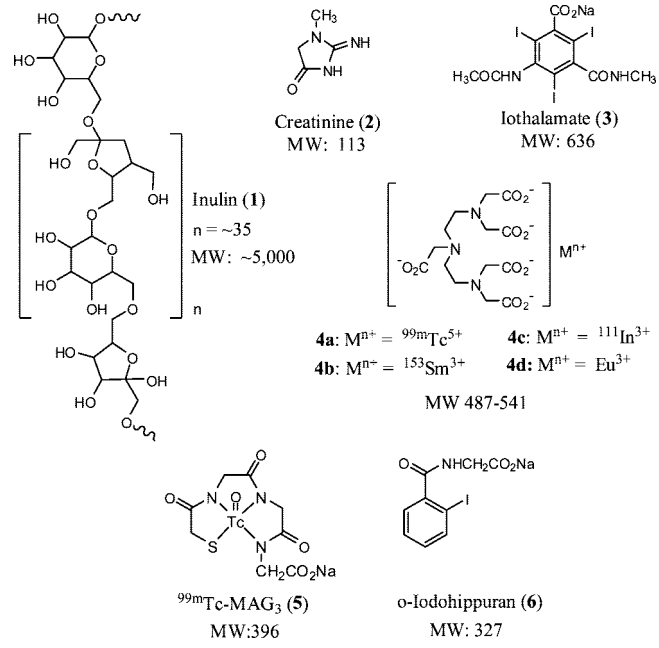

Figure 1. Exogenous renal function markers.

Figure 1). Among these, inulin is regarded as the "gold standard" for GFR measurement. Those undergoing clearance by tubular secretion via organic anion transport pathway (OAT) include include ^{99m}Tc-MAG$_3$ (**5**) and *o*-iodohippuran (**6**;[19,20] Figure 1). Indeed, ^{99m}Tc-MAG$_3$ (**5**) is currently being used for dynamic radioscintigraphic measurement renal blood flow. Cursory inspection of structures **1**–**6** does not provide a reliable pharmacophore model for the rational design for exogenous markers that clear via a particular pathway. Gross physicochemical features such as charge, molecular weight, or lipophilicity are inadequate in predicting or even explaining the mode of clearance. For instance, inulin (**1**, MW ~ 5000) and creatinine (**2**, MW 113) are both filtered through the glomerulus. On the other hand, the anionic technetium complex **4a** (MW 362) is cleared via glomerular filtration, whereas the complex **5** (MW 396) is

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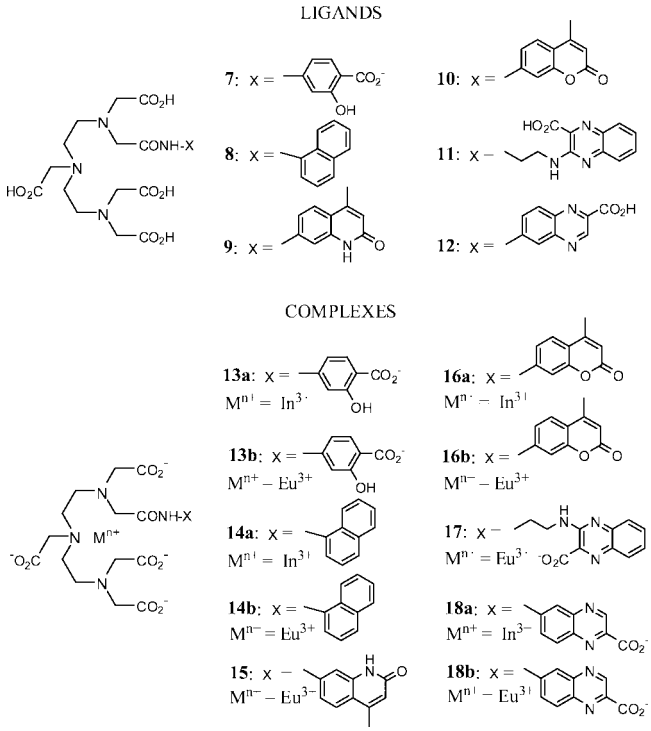

Figure 2. DTPA-monoamide ligands and complexes.

cleared by OAT pathway. Similarly, iothalamate (**3**) and iodohippuran (**6**) are cleared by glomerlular filtration and tubular secretion, respectively. It is apparent that subtle factors that are responsible for enabling a molecule to clear via a particular pathway cannot be ascertained from this limited set of data. Therefore, one has to rely on the empirical approach for designing and developing new GFR agents, and each new compound needs to be compared against a known GFR marker.

Considerable effort is now being directed at developing exogenous GFR agents for rapid real-time assessment of specific renal function using nonradioactive methods.[21–26] In particular, the effort is focused on luminescent agents that absorb and emit light in the visible or NIR regions. In principle, two general approaches for designing fluorescent renal agents can be considered: the first method involves enhancing the fluorescence of known renal agents that are intrinsically poor emitters such as lanthanide metal complexes, and the second involves transforming highly fluorescent dyes (which are intrinsically lipophilic) into hydrophilic, anionic species to force them to clear via the kidneys. This paper focuses on the former approach. The method of enhancing the fluorescence via intramolecular fluorescence resonance energy transfer (FRET) process by the use of molecular "antenna" to boost the fluorescence of lanthanide ions such as europium, terbium, and dysprosium, has been reported previously.[27–30] Europium complexes of diethylenetriaminepentaacetae (DTPA) ligands endowed with benzopyranone antennae have been shown to enhance europium fluorescence by three orders of magnitude compared to Eu-DTPA.[27] Also, gadolinium complexes derived from polyaminocarboxylate ligands, such as DTPA, have been shown to clear through via GFR pathway.[31,32] Recently, Eu-DTPA containing carbostyryl antennae have been used to assess GFR.[21] As a part of our continuing efforts to develop exogenous fluorescent GFR markers that absorb and emit in the visible region, we wish to report our recent findings on the fluorescence and renal clearance properties of some novel DTPA complexes **13–18** endowed with aromatic antennae (Figure 2). As will be demonstrated later, one of the complexes, **18b**, exhibited high fluorescence enhancement and rapid renal clearance.

Scheme 1

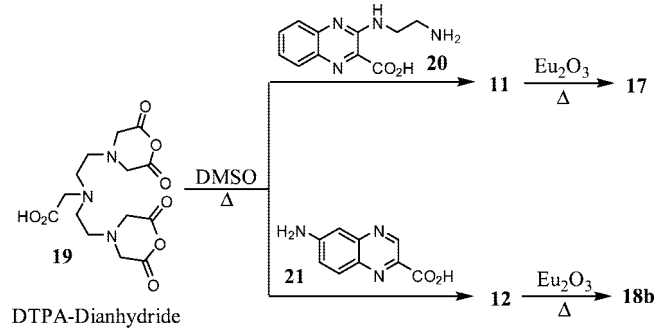

Table 1. HPLC Retention Times and Stability of Metal Complexes

		radiochemical purity (%)	
complex	R_t (min)	0 h	6 h
^{99m}Tc-DTPA (**4a**)	3.1	99[a]	97[a]
^{153}Sm-DTPA (**4b**)	3.9	97[a]	98[a]
^{111}In-DTPA (**4c**)	3.0	100	99
^{111}In-salicylamide complex **13a**	3.9	92	99
^{111}In-naphthylamide complex **14a**	13.2	97	97
^{111}In-coumarylamide complex **16a**	13.0	98	97
^{111}In-quinoxalinylamide complex **18a**	11.6	97	97

[a] Radiochemical purity determined by ITLC-SG.

Table 2. Fluorescence Enhancement of Eu^{3+} Emission by Aromatic Antennae

europium complex	relative fluorescence
Eu-DTPA (**4d**)	1
salicylamide **13b**	3
naphthylamide **14b**	12
carbostyrylamide **15**	200[a]
coumarylamide **16b**	900[b]
quinoxalinyl-6-amide **17**	20
quinoxalinyl-3-amide **18b**	2700[c]

[a] Ref. 28. [b] Ref 26. [c] Average of three independent measurements.

Results and Discussion

Ligand Syntheses and Metal Complexation. Preparation and Eu^{3+} complexation of DTPA monoamides **7–10** were reported previously.[21,27,29] Ligands **11** and **12** were prepared by standard method of reacting DTPA-bis(anhydride) (**19**) with 1 equiv of desired aminoquinoxalines **20** or **21** (Scheme 1). Europium complexation of ligands **11** and **12** were carried out by heating a mixture of europium oxide with the respective ligands in water. Radiolabeling of the ligands **7–12** with indium, samarium, and technetium were carried out by either known or slightly modified procedures.[33–35] In most cases, complex formation was quite rapid under ambient conditions, and the diluted radiolabeled complexes remained stable over time (Table 1). However, ^{111}In labeling of the salicylamide ligand **8** resulted first in the formation of a kinetic product (R_t = 11.4 min), which underwent, under the reaction condition, to a stable thermodynamic product in about 30 min (R_t = 3.9 min) and remained unchanged thereafter.

Fluorescence Studies. The relative fluorescence enhancements of various Eu^{3+} complexes in water compared to Eu-DTPA are shown in Table 2. The samples were excited at 350–400 nm, depending on their respective excitation maxima. The emission intensities were recorded at the typical Eu^{3+} emission bands at 594, 616, and 692 nm. All three bands showed the same degree of enhancement, but the intensities at 692 nm

Table 3. Clearance Profile of ^{99m}Tc-DTPA (**4a**)

	percent injected dose per organ (mean ± SE)[a]			
	15 min	1 h	2 h	24 h
blood	7.5 ± 0.9	0.9 ± 0.1	0.1 ± 0.0	0.0 ± 0.0
liver	1.6 ± 0.2	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
left kidney	1.8 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.2 ± 0.0
right kidney	2.0 ± 0.2	0.6 ± 0.0	0.5 ± 0.1	0.2 ± 0.0
urine[b]	38.4 ± 4.1	76.8 ± 13.9	83.0 ± 19.2	100.9 ± 11.5
feces[b]				4.1 ± 2.6

[a] SE, standard error. [b] Fecal values were determined only at 24 h post injection.

Table 4. Clearance Profile of ^{153}Sm-DTPA (**4b**)

	percent injected dose per organ (mean ± SE)[a]			
	15 min	1 h	2 h	24 h
blood	5.5 ± 0.7	0.8 ± 0.3	0.1 ± 0.0	0.0 ± 0.0
liver	1.1 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.1 ± 0.0
left kidney	1.1 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
right kidney	1.1 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
urine[b]	62.0 ± 0.8	89.5 ± 16.6	99.6 ± 0.2	96.4 ± 19.3
feces[b]				3.5 ± 2.6

[a] SE, standard error. [b] Fecal values were determined only at 24 h post injection.

were used for the purpose of calculating relative fluorescence. As would be expected on the basis of cross-sectional area, complexes with bicyclic aromatic antennae, namely, **14b**, **15**, **16b**, **17**, and **18b**, exhibited greater enhancement than the corresponding monocyclic derivative **13b**. Within the three types of bicyclic antennae (naphthyl, coumaryl, and quinoxalinyl), there is a remarkable difference in fluorescence enhancement. Courmaryl and quinoxalinyl derivatives **16b** and **18b**, respectively, exhibited a large (900- and 2700-fold, respectively) fluorescence enhancement, whereas the carboystyryl and quinoxalinyl derivatives **15** and **17**, respectively, showing modest or weak enhancement. Substantial difference in fluorescent property between the two quinoxaline derivatives **17** and **18b** is also remarkable. Cursory inspection of the bicyclic antenna in structures **17** and **18b** indicates that the carbocyclic ring is electron rich and the heterocyclic ring is electron deficient, whereas in **17**, both electron donation and withdrawal takes place in the heterocyclic pyrazine ring. The large difference in fluorescence enhancement between **15** versus **16b** and **19b** can be qualitatively rationalized by the differences in the nature, the distance, and the relative orientation of electric dipoles between donor antenna and the acceptor metal ion in these complexes, but high level theoretical studies will be needed to elucidate the underlying electronic effects.

Biodistribution Studies. ^{111}In was selected as the preferred radionuclide for biodistribution studies due to ready availability of ^{111}In in a carrier-free form, ease of complexation with all the ligands, and due unexpected stability problems encountered with the labeling of ligands **7–12** with ^{153}Sm. Indeed, the selection of ^{111}In did prove to be appropriate due to the fact that the biodistributions of ^{99m}Tc-DTPA (**4a**), ^{153}Sm-DTPA (**4b**), and ^{111}In-DTPA (**4c**) are nearly identical (Tables 3−5), and that ^{111}In-DTPA is known to be a GFR marker.[36] The salicylamide complex **13a** and, surprisingly, the quinoxalinyl complex **18a** exhibited the best renal clearance property, akin to that of ^{111}In-DTPA itself (Tables 6 and 7). Complexes **14a** and **16a** also exhibited good renal clearance but with significantly higher (ca. 25%) clearance through the hepatobiliary route compared to **14a** and **16a** (Tables 8 and 9).

To ascertain whether the complex **18a** clears via glomerular filtration, the rats were first treated with probenecid[19,37,38] to block the tubular secretion pathway and then were administered

Table 5. Clearance Profile of ^{111}In-DTPA (**4c**)

	percent injected dose per organ (mean ± SE)[a]			
	15 min	1 h	2 h	24 h
blood	6.2 ± 0.5	0.6 ± 0.2	0.1 ± 0.0	0.0 ± 0.0
liver	1.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.1 ± 0.0
left kidney	1.5 ± 0.2	0.5 ± 0.3	0.3 ± 0.0	0.2 ± 0.0
right kidney	1.7 ± 0.4	0.7 ± 0.4	0.3 ± 0.0	0.2 ± 0.0
urine[b]	43.8 ± 8.1	87.9 ± 15.5	102.1 ± 3.4	94.8 ± 12.4
feces[b]				8.6 ± 9.0

[a] SE, standard error. [b] Fecal values were determined only at 24 h post injection.

Table 6. Clearance Profile of ^{111}In-DTPA-Salicylamide Complex **15a**

	percent injected dose per organ (mean ± SE)[a]			
	15 min	1 h	2 h	24 h
blood	8.5 ± 0.2	2.6 ± 0.3	0.6 ± 0.1	0.0 ± 0.0
liver	1.5 ± 0.1	0.7 ± 0.1	0.2 ± 0.0	0.1 ± 0.0
left kidney	1.2 ± 0.1	0.6 ± 0.1	0.3 ± 0.0	0.2 ± 0.0
right kidney	1.3 ± 0.1	0.6 ± 0.1	0.3 ± 0.0	0.2 ± 0.0
urine[b]	24.2 ± 1.0	65.5 ± 7.7	83.2 ± 5.9	94.9 ± 4.0
feces[b]				6.7 ± 3.2

[a] SE, standard error. [b] Fecal values were determined only at 24 h post injection.

Table 7. Clearance Profile of ^{111}In-DTPA-Quinoxalinylamide Complex **18b**

	percent injected dose per organ (mean ± SE)[a]			
	15 min	1 h	2 h	24 h
blood	15.6 ± 2.7	4.7 ± 0.5	1.5 ± 0.3	0.1 ± 0.0
liver	2.2 ± 0.1	0.8 ± 0.1	0.3 ± 0.1	0.1 ± 0.0
left kidney	1.3 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.1 ± 0.0
right kidney	1.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.2 ± 0.0
urine[b]	19.6 ± 2.9	69.3 ± 5.2	79.7 ± 6.8	101.5 ± 2.0
feces[b]				6.3 ± 1.4

[a] SE, standard error. [b] Fecal values were determined only at 24 h post injection.

Table 8. Clearance Profile of ^{111}In-DTPA-Naphthylamide Complex **16a**

	percent injected dose per organ (mean ± SE)[a]			
	15 min	1 h	2 h	24 h
blood	6.6 ± 0.6	1.6 ± 0.4	0.4 ± 0.1	0.1 ± 0.0
liver	5.8 ± 0.7	1.4 ± 0.3	0.6 ± 0.3	0.4 ± 0.1
left kidney	3.1 ± 0.2	1.6 ± 0.2	0.8 ± 0.2	0.3 ± 0.0
right kidney	3.0 ± 0.2	1.6 ± 0.2	0.8 ± 0.2	0.3 ± 0.0
urine[b]	36.7 ± 8.8	53.8 ± 10.2	68.3 ± 21.8	82.7 ± 5.3
feces[b]				21.1 ± 1.9

[a] SE, standard error. [b] Fecal values were determined only at 24 h post injection.

Table 9. Clearance Profile of ^{111}In-DTPA-Coumarylamide Complex **16a**

	percent injected dose per organ (mean ± SE)[a]			
	15 min	1 h	2 h	24 h
blood	7.1 ± 1.7	1.1 ± 0.2	0.2 ± 0.0	0.0 ± 0.0
liver	4.4 ± 0.3	0.8 ± 0.3	0.3 ± 0.0	0.2 ± 0.1
left kidney	2.4 ± 0.5	0.7 ± 0.3	0.4 ± 0.2	0.2 ± 0.1
right kidney	3.0 ± 1.1	0.8 ± 0.3	0.5 ± 0.1	0.2 ± 0.1
urine[b]	33.1 ± 12.3	69.9 ± 8.9	78.9 ± 15.2	70.6 ± 6.5
feces[b]				25.9 ± 7.9

[a] SE, standard error. [b] Fecal values were determined only at 24 h post injection.

with **18a**. The comparative clearance rates of the complexes from untreated and probenecid-treated rats with compounds **4c**, **5**, and **18a** are shown in Table 10. As would be expected, ^{99m}Tc-MAG$_3$ (**5**) exhibited reduced clearance upon treatment with probenecid,[39] whereas ^{111}In complexed **4c** showed no change in its clearance rate. Although the clearance rate of **18a** is slower

Table 10. Effect of Probenecid on the Clearance Rates[a] of Metal Complexes

	probenacid (mg/kg)		
complex	0	35	70
^{99m}Tc-MAG$_3$ (**5**)	11.8 ± 2.1	6.4 ± 1.0	4.6 ± 0.6
^{111}In-DTPA (**4c**)	4.3 ± 0.2	4.4 ± 0.7	not determined
^{111}In-complex **18a**	4.5 ± 0.5	8.1 ± 1.4	6.4 ± 0.5

[a] The units are expressed as mL/min/kg.

than that of **4c**, this complex is cleared almost exclusively through the renal system, as indicated by the low radioactivity in the feces. The enhanced clearance of **18a** (ca. 80%) under probenecid treatment was somewhat surprising, and this behavior is also dose-dependent. Such an increase in renal clearance rate upon probenecid-treatment has been observed previously with the antitumor agent cisplatin.[40] In this case, it was suggested that probenecid could competitively inhibit the reabsorption of Pt or Pt species, thus promoting a net secretion.[40] Based on the reported pK_a values of the carboxyl groups in DTPA and derivatives,[41] the pK_a values of the carboxyl groups in ligands **7**−**12** should be below 3. Thus, complex **18a**, like ^{99m}Tc-MAG$_3$ (**5**), would be in anionic form in the kidneys and would be expected to undergo slower clearance. Also, since it has been suggested that passive tubular reabsorption requires the molecules to be in the undissociated state,[42] complex **18a** would not be expected to undergo tubular reabsorption, but would remain in the lumen and be eliminated in the urine. Furthermore, because probenecid is known to inhibit OAT pathway, complex **18a**, if it is secreted by anion transport mechanism, would be expected to undergo slower clearance, akin to that of complex **5**, but the opposite effect was observed. On the other hand, complex **18a** clears more slowly and exhibits higher blood retention compared to ^{99m}Tc-DTPA (**4a**) and ^{111}In-DTPA (**4c**). Thus, these apparently divergent behaviors of **18a** suggest that this compound is cleared by the kidneys by a complex mechanism that includes glomerular filtration.

Conclusion. Large Eu^{3+} fluorescence enhancement via ligand to metal energy transfer mechanism was achieved with the europium complex **18b**. Indeed, complex **18b** exhibits the highest fluorescent enhancement observed thus far in the DTPA-type metal complexes. Furthermore, the corresponding indium complex **18a** exhibited excellent renal clearance property mimicking the known metal complex-based GFR agents, albeit at a slower rate. Although the excitation maximum for **18b** is at 357 nm, the large fluorescence enhancement would allow this compound to be excited in the visible region to be useful as an exogenous optical renal function monitoring agent. Pharmacokinetic, protein binding, and toxicological properties of **18b** and other compounds in this series are in progress and will be reported elesewhere.

Experimental Section

Unless otherwise indicated, all commercial starting materials, reagents, and solvents were used as such without further purification. The two quinoxaline starting materials, 6-amino-quinoxaline-2-carboxylic acid and 3[(2-aminoethyl)-amino]quinoxaline-2-carboxylic acid, were procured by custom synthesis from Curragh Chemistries, Cleveland, OH. All NMR spectra were recorded using Varian Gemini 300 MHz spectrometer. Mass spectra were obtained using Shimadzu LCMS-2010 A liquid chromatograph/mass spectrometer. UV spectra were obtained using Shimadzu UV-3101 spectrometer. Fluorescence spectra were recorded by photon counting method using Jobin Yvon Fluorolog 3 spectrofluorometer. Flash chromatography was carried out using Arganaut Flash Master Solo System. All animals were handled according to the guidelines of the National Institutes of Health, *Guide for Use and Care of Laboratory Animals*, NIH publication No. 85-23, and the animal studies were approved by Covidien's IACUC. Standard analyses were performed using Micosoft Excel spreadsheet program.

Ligand 11. A mixture of DTPA-bis(anhydride) (199.5 mg, 0.6 mmol), 3[(2-aminoethyl)-amino]quinoxaline-2-carboxylic acid (302.0 mg, 1.1 mmol), and triethylamine (0.5 mL) in DMSO (5 mL) was stirred under nitrogen at 60 °C for 16 h. Upon cooling to room temperature, the reaction mixture was poured onto a stirring solution of acetone (100 mL). The liquid portion of the mixture was decanted and the solid was dissolved in water (15 mL). The solution was adjusted to about pH 3.0 with HCl and then evaporated in vacuo. The crude product (0.33 g) was purified by reverse phase (C_{18}) flash chromatography (25 g prepacked column) using water−acetonitrile gradient elution (20 mL/min flow rate). The desired fractions were pooled and lyophilized to give 31.8 mg of **13** as a pale tan solid. ^{1}H NMR (300 MHz, D_2O, δ, ppm): 3.11 (t, 2H), 3.19 (t, 2H), 3.22 (t, 2H), 3.38 (t, 2H), 3.53 (t, 2H), 3.57 (s, 2H), 3.58 (t, 2H), 3.64 (s, 2H), 3.76 (s, 4H), 3.79 (s, 2H), 7.39 (t, 1H), 7.47 (d, 1H), 7.58 (t, 1H), 7.70 (d, 1H). ^{13}C NMR (75.4 MHz, D_2O, δ, ppm): 37.5, 41.2, 50.6, 51.5, 51.9, 52.1, 54.5, 56.2, 56.9, 118.0, 127.2, 129.9, 130.9, 133.2, 134.4, 139.6, 147.0, 166.7, 169.5, 170.5, 171.9, 172.4. LRMS *m*/*z* MH^+: 608.

Ligand 12. A mixture of DTPA-bis(anhydride) (494.5 mg, 1.4 mmol), sodium 6-amino-quinoxaline-2-carboxylate (78.7 mg, 0.4 mmol), and triethylamine (1 mL) in DMSO (4 mL) was stirred under nitrogen at 60 °C for 16 h. Upon cooling to room temperature, the reaction mixture was poured onto a stirring solution of acetone (200 mL). The solvent was decanted, the solid was dissolved in water (5 mL), and the pH was adjusted to about 2.0 with HCl. The solution was then lyophilized, and the crude solid (1.0 g) was purified by reverse phase (C_{18}) flash chromatography (25 g prepacked column) using water−acetonitrile gradient elution. The desired fractions were pooled and lyophilized to give 145.7 mg of **12** as a pale yellow solid. ^{1}H NMR (300 MHz, D_2O, δ, ppm): 3.22 (t, 2H), 3.26 (t, 2H), 3.38 (t, 2H), 3.45 (t, 2H), 3.65 (s, 2H), 3.71 (s, 2H), 3.81 (s, 4H), 4.03 (s, 2H), 7.34 (d, 1H), 7.43 (d, 1H), 7.55 (s, 1H), 8.71 (s, 1H). ^{13}C NMR (75.4 MHz, D_2O, δ, ppm): 50.6, 51.5, 52.2, 52.4, 55.0, 56.8, 57.1, 57.7, 115.0, 125.1, 130.0, 137.4, 140.3, 142.0, 142.3, 144.8, 166.9, 167.3, 170.6, 172.2, 172.7. LRMS *m*/*z* MH^+: 565.

Eu^{3+} Complex 17. A mixture of the ligand **11** (11.5 mg, 0.02mmol) and europium(III) oxide (3.3 mg, 0.01mmol) in deionized, distilled water (5 mL) was heated at 70 °C for 16 h. Upon cooling to room temperature, the solution was filtered through a 0.2 μm filter to remove insoluble material and then lyophilized to give 22.2 mg of complex **17**. HRMS (negative ion mode) calcd for $C_{25}H_{29}N_7O_{11}Eu$: 754.1118, 756.1132. Found: 754.1120, 756.1133. Anal. Calcd for $C_{23}H_{24}N_6O_{11}Eu \cdot 7.0H_2O$: C, 34.02; H, 5.02; N, 11.11; Eu, 17.22. Found: C, 34.40; H, 5.06; N, 11.11; Eu, 17.20.

Eu^{3+} Complex 18b. A mixture of the ligand **12** (27.0 mg, 0.05mmol) and europium(III) oxide (8.0 mg, 0.02mmol) in deionized, distilled water (5 mL) was heated at 65 °C for 16 h. Upon cooling to room temperature, the solution was filtered through a 0.2 μm filter to remove insoluble material and then lyophilized to give 38.0 mg of complex **18b**. HRMS (negative ion mode) calcd for $C_{23}H_{24}N_6O_{11}Eu$: 711.0696, 713.0710. Found: 711.0698, 713.0711. Anal. Calcd. for $C_{23}H_{24}N_6O_{11}Eu \cdot 5.5H_2O$: C, 34.02; H, 4.32; N, 10.36; Eu, 18.74. Found: C, 34.19; H, 4.35; N, 10.73; Eu, 17.80.

^{99m}Tc-DTPA (4a). A draximage DTPA kit (lot #1G757A) was reconstituted with 5 mL of 0.9% NaCl containing 1.8 mCi of Tc-99m pertechnetate. The preparation was allowed to stand at room temperature and the product yield was measured by ITLC-SG in MEK. The solution was diluted to 250 μCi/mL with 0.9% NaCl for biodistribution studies, and the stability of the final preparation was monitored by ITLC-SG in MEK.

^{153}Sm-DTPA (4b). To a plastic screw top 3 mL vial was added 100 μL of DTPA solution (10 mg/mL in water or 0.05 N NaOH) followed by 240 μL of 0.05 N HCl. A total of 10 μL of Sm-153 in 0.1 N HCl was then added, followed by the addition of 10 μL of 0.5 N NaOAc. The resulting solution was allowed to stand at ambient temperature for 10–15 min. Complex formation was

checked by ITLC-SG using 1:10:20 hydroxylamine/MeOH/H_2O as the eluent. The solution was diluted to 250 μCi/mL with 0.9% NaCl for biodistribution studies, and the stability of the final preparation was monitored by ITLC-SG as described above.

General Procedure for the Preparation of ^{111}In-DTPA-Monoamide Complexes. To a plastic screw top 3 mL vial was added 100 μL of ligand solution (10 mg/mL in water or 0.05 N NaOH) followed by 250 μL of ^{111}In in 0.05 N HCl (ca. 1 mCi). Next, 10 μL of 0.5 N NaOAc was added, and the resulting solution was allowed to stand at room temperature for 10–15 min. Radiochemical purities of the ^{111}In complexes were determined by HPLC using an XTerra C-18 column (4.6 × 250 mm, 5 μ) and 0.1%TFA/acetonitrile gradient. Reaction mixtures were diluted to 125 μCi/mL with 0.9% NaCl for biodistribution studies, and the stabilities of the diluted preparations were evaluated by radiochemical purity assay at 6 h post dilution.

Normal Rat Biodistribution Protocol. Sprague–Dawley (Harlan, Indianapolis, IN) rats (n = 3; 170–273 g) were given 25 μCi (0.20 mls of 125 μCi/ml) of an ^{111}In-labeled complex via the lateral tail vein under conscious restraint. An approximately 50 μCi sample of each of ^{111}In, ^{153}Sm, and ^{99m}Tc DTPA complexes was also assayed as controls. Rats were placed in metabolism cages immediately after injection and were provided free access to water. Rats that were housed for 24 h were allowed access to food and water. Three rats per group were euthanized at 15, 60, and 120 min and 24 h post injection. Whole organs and tissue specimens were rinsed with 0.9% sodium chloride injection U.S.P., blotted dry, and weighed.

Tissues collected and weighed included blood, liver, kidney, heart, lungs, muscle, and spleen. The uptake in heart, lungs, and spleen were varied at the background levels. The uptake in muscle in all the complexes tested ranged from 8% to 13% and decreased to the background levels in about one hour. A portion of the tail where the injection was made was also assayed for radioactivity. Duplicate 0.50 mL samples of heparinized whole blood were pipetted, and two approximately 1 g samples of liver were weighed for assay of radioactivity.

Urine specimens were collected from rats sacrificed at 15, 60, and 120 min. After euthanasia, the cages were rinsed with tap water and the volume of combined urine and rinse was measured. A 0.50 mL aliquot of urine (with rinse) was assayed for radioactivity. After the feces were collected for the 24 h rats, the urine collection was the same as described above. Tissues, urine, and feces were counted by gamma scintillation (Packard Cobra B5005 Gamma Counter).

Injection solution standards were prepared by diluting the injection solutions 1:100 using 0.9% sodium chloride injection USP. The diluted standards were dispensed in 0.05 mL aliquots with 0.95 mL tap water into tubes for counting. Each sample was assayed for 1 min by gamma scintillation using a gamma counter with the windows set to detect photoelectric energies of each specific isotope assayed (80–500 keV for ^{111}In, 40–240 keV for ^{99m}Tc and 15–240 keV for ^{153}Sm). Raw data were reported in counts per minute (CPM). The instrument was set up to subtract background CPM from the samples and standards prior to any calculations. The total amount of ^{111}In, ^{153}Sm, or ^{99m}Tc administered to each rat was determined by assaying standards prepared from each injection solution. Appropriate injection solution standards were placed at the beginning and end of the tissue samples and at the beginning and end of urine and fecal samples for each rat. Decay of radioactivity in sample specimens was corrected by averaging the CPM for the standards preceding and immediately following each set of samples.

The percent injected dose (ID) per gram or mL of tissue was calculated for those tissues in which tissue weights (or volume) were measured. The percent ID per organ was calculated for all organs. For the purpose of calculation, total blood volume was assumed to represent 5.0% of the initial body weight and muscle mass was to represent 45.5%. For urine and feces, the percent ID in the entire contents was determined. Percent ID for each tissue, fluid, and excreta were normalized for the activity remaining at the injection site by subtracting CPM at the injection site from total CPM administered. The percent recovery was calculated for individual animals by adding percent ID values for all organs and excreta. Group means and standard errors were calculated for percent ID per gram and percent ID per organ at each time point using the normalized values.

Probenecid Treatment Protocol. To determine whether the complexes are cleared through glomerular filtration or tubular secretion pathway, the rats were first administered a dose of probenecid intravenously 10 min prior to the test substance. Precannulated femoral artery rats were purchased from Charles River (Indianapolis, Indiana). Either 35 or 70 mg/kg of probenecid was injected 10 min prior to the injection of the radiolabeled compound (0.2 mL of 125 uCi/mL). Arterial blood (0.3 mL) was removed from the catheter and collected in a heparinized tube at 5, 15, 30, 43, 60, 90, 120, and 180 min post injection. An aliquot of 0.2 mL of heparinized blood was assayed for radioactivity using a gamma counter (Packard Cobra B5005). Standards were made to determine the total amount of CPM injected. The weight of the rat, the counts per minute (CPM)/mL of each blood sample, and the total CPM injected was entered into the PK Solutions 2.0 (Summit Research Services; Montrose, CO) software package, which calculated clearance (mL/min). The software plotted CPM/mL versus time and the GFR clearance was calculated using the following equations:$CL = D/AUC_\infty$, $AUC_\infty = AUC_{(0-t)} + C_n/\lambda_z$ where CL is the GFR clearance rate, D is the amount of injected dose, AUC is the area under the curve of the plot of CPM/mL versus time, C_n is the last concentration, and λ_z is the elimination constant.

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References

(1) Ekanoyan, G.; Levin, N. W. In *Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification (K/DOQI)*, National Kidney Foundation: Washington, D.C., 2002; pp. 1–22.
(2) Dollan, P. D.; Alpen, E. L.; Theil, G. B. A clinical appraisal of the plasma concentration and endogenous clearance of creatinine. *Am. J. Med.* **1962**, *32*, 65–79.
(3) Reed, C. H. Diagnostic applications of cystatin C. *Br. J. Biomed. Sci.* **2000**, *57* (4), 323–329.
(4) Sturgeon, C.; Sam, A. D.; Law, William R. Rapid determination of glomerular filtration rate by single-bolus inulin: a comparison of estimation analyses. *J. Appl. Physiol.* **1998**, *84* (6), 2154–2162.
(5) Buclin, T.; Pechere-Bertschi, A.; Sechaud, R.; Decosterd, L. A.; Munafo, A.; Bumier, M.; Biollaz, J. Sinistrin clearance for determination of glomerular filtration rate: A reappraisal of various approaches using a new analytical method. *J. Clin. Pharmacol.* **1997**, *37* (8), 679–692.
(6) Brochner-Mortensen, J.; Rodbro, P. Optimum time of blood sampling for determination of glomerular filtration rate by single-injection [^{51}Cr]-EDTA plasma clearance. *Scand. J. Clin. Lab. Invest.* **1976**, *36* (8), 795–800.
(7) Nosco, D. L.; Beaty-Nosco, J. A. Chemistry of technetium radiopharmaceuticals 1: Chemistry behind the development of technetium-99m compounds to determine kidney function. *Coord. Chem. Rev.* **1999**, *184*, 91–123.
(8) Lewis, N.; Kerr, R.; Van Buren, C. Comparative evaluation of urographic contrast media, inulin, and ^{99m}Tc-DTPA clearance methods for determination of glomerular filtration rate in clinical transplantation. *Transplantation* **1989**, *48*, 790–796.
(9) Rehling, M. Stability, protein binding, and clearance studies of technetium-99m-DTPA. Evaluation of commercially available dry-kit. *Scand. J. Clin. Lab. Invest.* **1988**, *48* (7), 603–609.
(10) Rehling, M.; Nielsen, L. E.; Marqversen, J. Protein binding of ^{99m}Tc-DTPA. *Nucl. Med. Commun.* **2001**, *22* (6), 617–623.
(11) Mourad, A.; Carney, S.; Gillies, A.; Hibberd, A.; Trevillian, P.; Nanra, R. Measurement of glomerular filtration rate in renal transplant recipients: a comparison of methods. *Nephrology* **2002**, *7* (2), 77–82.
(12) Wilson, D. M.; Bergert, J. M.; Larson, T. H.; Leidtke, R. R. GFR determined by nonradiolabeled iothalamate using capillary electrophoresis. *Am. J. Kidney Dis.* **1997**, *39* (5), 646–652.
(13) Guesry, P.; Kaufman, L.; Orloff, S.; JNelson, A.; Swann, S.; Holliday, M. Measurement of glomerular filtration rate by fluorescent excitation

of non-radioactive meglumine iothalamate. *Clin. Nephrol.* **1975**, *3*, 134–138.
(14) Rocco, M. V.; Buckalew, V. M.; Moore, L. C. Jr.; Shihabi, Z. K. Capillary electrophoresis for the determination of glomerular filtration rate using nonradioactive iohexol. *Am. J. Kidney Dis.* **1996**, *28* (2), 173–177.
(15) Robertshaw, M.; Lai, K.; Swaminathan, R. Prediction of creatinine clearance from plasma creatinine: Comparison of five formulae. *Br. J. Clin. Pharmacol.* **1984**, *28* (3), 275–280.
(16) Sarcino, A.; Morrone, L. F.; Suriano, V.; Niccoli-Asabella, A.; Ramunni, A.; Fanelli, M.; Rubini, G.; Coratelli, P. A simple method for correcting overestimated glomerular filtration rate in obese subjects evaluated by the Cockcroft and Gault formula: A comparison with ^{51}Cr-EDTA clearance. *Clin. Nephrol.* **2004**, *62* (2), 97–103.
(17) Cerra, F. B. Multiple Organ Failure Syndrome. In *New Horizons: Multiple Organ Failure*, Bihari D. J., Cerra, F. B., Eds.; Society of Critical Care Medicine: Fullerton, CA, 1989; pp. 1–24.
(18) Roch-Ramel, F.; Besseghir K.; Murer, H. Renal excretion and tubular transport of organic anions and cations. In *Handbook of Physiology, Section 8, Neurological Physiology*; Windhager, E. E., Ed.; Oxford University Press: New York, 1992; Vol. II, pp. 2189–2262.
(19) Fritzberg, A. R.; Kasina, S.; Eshima, D.; Johnson, D. L. Synthesis and biological evaluation of technetiuim-99m-MAG_3 as a hippuran replacement. *J. Nucl. Med.* **1986**, *27*, 111–116.
(20) Tauxe, W. N. Tubular Function. In *Nuclear Medicine in Clinical Urology and Nephrology*; Tauxe, W. N., Dubovsky, E. V., Eds.; Appleton Century Crofts: East Norwalk, 1985; pp. 77–105.
(21) Rabito, C. A.; Chen, Y.; Shomacker, K. T.; Modell, M. D. Optical, real-time measurement of the glomerular filtration rate. *Appl. Opt.* **2005**, *44*, 5956–5965.
(22) Rabito C. A. Fluorescent agents for real-time measurement of organ function *U.S. Patent* 6,440,389, 2002.
(23) Rajagopalan R. Achilefu S. Bugaj J. E. Dorshow R. B. Quinoline ligands and metal complexes for diagnosis and therapy *U.S. Patent* 6,277,841, 2001.
(24) Rajagopalan, R.; Bugaj, J. E.; Dorshow, R. B.; Venkatramani, C. J.; Uetrecht, P. A.; Achilefu, S. Polyionic fluorescent bioconjugates as tracer agents for continuous monitoring of renal function. In *Molecular Imaging: Reporters, Dyes, Markers, and Instrumentation*; Priezzhev, A., Asakura, T., Briers, J. D., Eds.; Proceedings of SPIE, 2000, 3924.
(25) Dorshow, R. B.; Bugaj, J. E.; Burleigh, B. D.; Duncan, J. R.; Johnson, M. A.; Jones, W. B. Noninvasive fluorescence detection of hepatic and renal function. *J. Biomed. Opt.* **1998**, *3* (3), 340–345.
(26) Dorshow, R. B.; Bugaj, J. E.; Achilefu, S.; Rajagopalan, R.; Combs, A. H. Monitoring Physiological Function by Detection of Exogenous Fluorescent Contrast Agents. In *Optical Diagnostics of Biological Fluids IV*; Priezzhev, A., Asakura,T., Eds.; Proceedings of SPIE, 1999, *3599*, 2–8.
(27) Ozaki, H.; Suda, E.; Nagano, T.; Sawai, H. Sensitization of europium(III) luminescence by DTPA derivatives. *Chem. Lett.* **2000**, 312–313.
(28) Gunnlaugsson, T.; Parker, D. Luminescent europium tetraazamacrocyclic complexes with wide range pH sensitivity. *Chem. Commun.* **1998**, 511–512.
(29) Chen, J.; Selvin, P. R. Thiol-reactive luminescent chelates of terbium and europium. *Bioconjugate Chem.* **1999**, *10* (2), 311–315.
(30) Abusaleh, A.; Meares, C. F. Excitation and deexcitation process in lanthanide chelates bearing aromatic side chains. *Photochem. Photobiol.* **1984**, *39* (6), 763–769.
(31) Choyke, P. L.; Austin, H. A.; Frank, J. A. Hydrated clearance of gadolinium-DTPA as a measurement of glomerular filtration rate. *Kidney Int.* **1992**, *41*, 1595–1598.
(32) Tweedle, M. F.; Zhang, X.; Fernandez, M.; Wedeking, P.; Nunn, A. D.; Strauss, H. W. A noninvasive method for monitoring renal status as bedside. *Invest. Radiol.* **1997**, *32*, 802–805.
(33) Carl, K. H.; Anne, M. W.; David, R. V. Preclinical studies of ^{99m}Tc-DTPA mannosyl dextran. *Nucl. Med. Biol.* **2003**, *30*, 457–464.
(34) Arano, Y.; Uezono, T.; Akizawa, H.; Ono, M.; Wakisaka, K.; Nakayama, M.; Sakahara, H.; Konishi, J.; Yokoyama, A. Reassessment of diethylenetriaminepentaacetic acid (DTPA) as a chelating agent for Indium-111 labeling of polypeptides using a newly synthesized monoreactive DTPA derivative. *J. Med. Chem.* **1996**, *39*, 3451–3460.
(35) Stimmel, J. B.; Kull, F. C. Samarium-153 and lutetium-177 chelation properties of selected macrocyclic and acyclic ligands. *Nucl. Med. Biol.* **1998**, *25*, 117–125.
(36) Roos, J. C.; Koomans, H. A.; Boer, P.; Oei, H. Y. Determination of glomerular filtration rate by ^{111}In-DTPA. *Eur. J. Nucl. Med.* **1981**, *6* (12), 551–553.
(37) Bubeck, B.; Brandau, W.; Weber, E.; Kaelbe, T.; Parekh, N.; Georgi, P. Pharmacokinetics of technetium-99m-MAG_3 in humans. *J. Nucl. Med.* **1990**, *31*, 1285–1293.
(38) Klopper, J. F.; Hauser, W.; Atkins, H. L.; Eckelman, W. C.; Richards, P. Evaluation of ^{99m}Tc-DTPA for the measurement of glomerular filtration rate. *J. Nucl. Med.* **1972**, *13* (1), 107–110.
(39) Shikano, N.; Kanai, Y.; Kawai, K.; Ishikawa, N.; Endou, H. Transport of 99mTc-MAG3 via rat renal organic anion transporter 1. *J. Nucl. Med.* **2004**, *45* (1), 80–85.
(40) Littererst, C. L.; Osman, N. M. Effect of probenecid and N′-methylnicotinimide on renal handling of cis-dichlorodiammineplatinum-II in rats. *Cancer Lett.* **1983**, *19*, 107–111.
(41) Geraldes, C. F. G. C.; Urbano, A. M.; Alpoim, M. C.; Sherry, A. D.; Kuan, K.-T.; Rajagopalan, R.; Maton, F.; Muller, R. N. Preparation, physicochemical characterization, and relaxometry studies of various gadolinium(III)-DTPA-bis(amide) derivatives as potential magnetic resonance contrast agents. *Magn. Reson. Imaging* **1995**, *13* (3), 401–420.
(42) Roch-Ramel, F.; De Broe, M. E. Renal handling of drugs and xenobiotics. In *Clinical Nephrotoxins*, 2nd ed.; De Broe, M. E., Porter G. A., Bennett, W. M., Verpooten, G. A., Eds.; Kulwer Academic Publishers: Dordrecht, 2003; pp 21–46.

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