Novel Hexakis(areneisonitrile)technetium(I) Complexes as Radioligands Targeted to the Multidrug Resistance P-Glycoprotein

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Transport substrates and modulators of the human multidrug resistance (*MDR1*) P-glycoprotein (Pgp) are generally lipophilic cationic compounds, many with substituted aryl moieties. We sought to synthesize aromatic technetium–isonitrile complexes to enable functional detection *in vivo* of Pgp expression in tissues. A series of substituted aromatic isonitrile analogs were synthesized from their corresponding amines by reaction with dichlorocarbene under phase transfer-catalyzed conditions, and the non-carrier-added hexakis(areneisonitrile)Tc-99m(I) complexes were produced by reaction with pertechnetate in the presence of sodium dithionite. Cellular accumulation *in vitro*, whole body biodistribution, and the imaging properties of these lipophilic, monocationic organometallic complexes were determined in Chinese hamster lung fibroblasts expressing MDR Pgp, in normal rats, and in rabbits, respectively. For this initial series, verapamil (50 μM), the classical Pgp modulator, significantly enhanced cellular accumulation or displaced binding of Tc complexes of **1b**, **1d**, **1h**, **2a**, **2d**, **3a**, and **3b**, indicative of targeted interactions with Pgp. Most complexes, despite their modestly high lipophilicity, were excluded by the blood/brain barrier, and several complexes displayed simultaneously high hepatobiliary and renal excretion *in vivo*, consistent with the physiological expression pattern of Pgp in these tissues. Selected Tc– and Re–areneisonitrile complexes of this class have potential applicability to the functional imaging and modulation, respectively, of MDR Pgp in human tissues.

Introduction

The ability of tumors to develop resistance to chemotherapeutic agents is one of the greatest barriers to successful treatment of human cancers. One form of acquired resistance, characterized by resistance to a primary chemotherapeutic agent and concordant cross-resistance to structurally unrelated agents, is known as multidrug resistance (MDR) and correlates with overexpression of a small family of related mammalian MDR and multidrug resistance-associated (MRP) genes.[1–4] The best characterized of these, the human *MDR1* gene product, P-glycoprotein (Pgp; M_r ~170 000), is an integral membrane protein expressed on the plasma membrane of MDR cells.[5,6] Drug resistance conferred by *MDR1* Pgp is thought to be due to its action as an ATP-dependent drug efflux pump.[7] Thus, net accumulation of a variety of chemotherapeutic agents included in the MDR phenotype, such as vincristine, vinblastine, taxol, and adriamycin, is reduced in cells overexpressing *MDR1* Pgp.

Strategies designed to block expression or to circumvent this form of drug resistance are being actively sought by many laboratories in cancer research.[8–12] Particularly desirable are agents that inhibit Pgp activity at concentrations displaying little or no intrinsic cytotoxic or pharmacological side effects. These drugs, termed MDR reversal or modulation agents,[9] could be used in overcoming multidrug resistance when they are co-administered in combination with other anticancer drugs during clinical chemotherapy. Previously, it was believed that MDR substrates and reversal agents, generally lipophilic cations, structurally require aromatic groups and a quaternizable ammonium functionality.[9,13–17] These features are found in derivatives of colchicine, reserpine, quinidine, acridonecarboxamide, and verapamil, all of which contain quaternary nitrogens and methoxy-substituted aromatic rings. Beyond these rather simplistically defined similarities, what is striking about known candidate reversal agents is their structural diversity.

It has recently been established by our research group that hexakis Tc complexes of 2-methoxyisobutaneisonitrile (Tc-99m–MIBI), a lipophilic monocationic radiopharmaceutical, is a transport substrate recognized by *MDR1* Pgp.[18,19] Baculoviral expression of recombinant human *MDR1* in insect cells confers decreased accumulation of Tc-99m–MIBI,[19] and furthermore, MDR cells expressing Pgp accumulate Tc-99m–MIBI in inverse proportion to the amount of immunodetectable transporter.[18] However, relative to other compounds that interact with the *MDR1* Pgp, nonspecific lipid bilayer partitioning of Tc-99m–MIBI is extremely low,[20–22] and the agent shares few of the structural features thought essential for recognition by *MDR1* Pgp, other than the cationic hydrophobic properties.

Technetium-99m, the most widely used radioisotope in nuclear medicine, offers the advantages of high specific activity (labeled molar fraction ~0.8) and a 140 keV γ photon—sufficient in energy to minimize tissue adsorption by organs deep in the body, but readily collimated and detected by NaI(Tl) scintillation cameras

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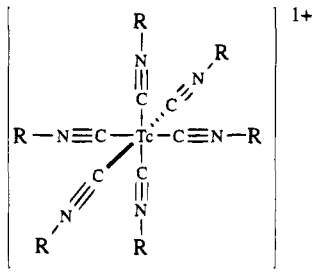

Figure 1. General structure of hexakis(R-isonitrile)technetium(I) complexes.

for diagnostic applications. Tc-99m and nonradioactive Re are known to readily form monomeric, octahedral complexes with monodentate isonitriles[23] as well as mono- and polymeric complexes with multidentate isonitrile ligands.[24] Isonitrile complexes of Tc-99m or isostructural complexes of nonradioactive Re may be further developed as organometallic cations for the potential imaging and modulation of MDR Pgp, respectively. Our strategy was to combine potent functionalities of known MDR transport substrates and reversal agents (such as the methoxyaryl moieties of colchicine, verapamil or reserpine analogs) with the lipophilic cationic character of the hexakis metal(I)–isonitrile complexes (Figure 1) to enable functional detection and modulation *in vivo* of MDR Pgp. Survey of the literature reveals no data pertaining to this class of compounds in relation to MDR, thus synthesis of areneisonitriles containing a variety of functional groups was undertaken to begin exploring structure–activity relationships of their corresponding metal complexes. MDR Pgp interactions of Tc complexes were investigated by determination of tracer accumulation in cells overexpressing Pgp, by biodistribution analysis, and by whole body imaging.

Results and Discussion

Chemistry. Isonitriles, known for decades to possess characteristic repulsive odors, have been extensively described.[25,26] The most widely used method for the synthesis of isonitriles from amines has been via a two-step formylation–dehydration process. Amines are readily converted to formamides using formic acid in the presence of acetic anhydride, or with ethyl formate at high pressure. Formamides are converted to isonitriles by a number of methods;[25,26] the most efficient uses phosgene to effect dehydration. Yields are high, but phosgene must be used with caution due to its extreme toxicity. More recently, trifluoromethanesulfonic anhydride[27] was demonstrated to be a useful dehydrating agent for the conversion of formamides to isonitriles. Other useful reagents include toluenesulfonyl chloride, thionyl chloride in DMF,[28] phosphorous oxychloride, triphenylphosphine–carbon tetrachloride, and oxomethylenebis(imidazolium)bis(methanesulfonate).[29]

The method of choice, in terms of speed and ease of preparation, is the phase-transfer-catalyzed conversion of amines to isonitriles in the presence of base and chloroform.[30–34] It is believed that the reaction proceeds via the formation of trichloromethyl carbanion, which undergoes α-elimination to give dichlorocarbene. Reaction of this carbene with the amine, and elimination of two molecules of HCl, gives an isonitrile in yields

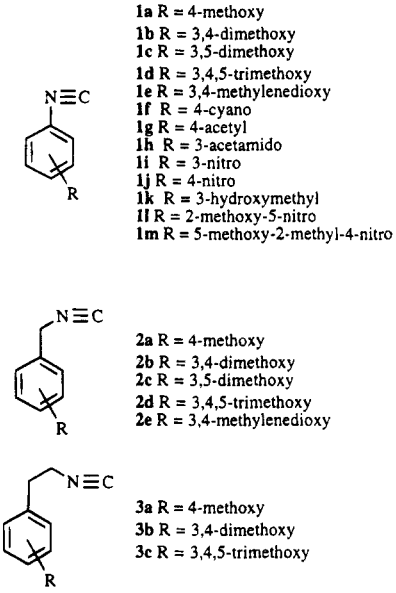

Figure 2. Substituted areneisonitrile ligands.

generally in the 30–50% range. While the yields using a two-step amidation–dehydration process are usually higher (70–90%), the phase-transfer method is fast (one step, usually under 1–2 h), and the use of potentially hazardous reagents (e.g., phosgene) is avoided. The reaction may also be carried out in the absence of phase-transfer catalyst, starting from sodium trichloroacetate.[35] Under heating conditions, CO_2 is eliminated, and the remaining trichloromethyl carbanion undergoes α-elimination to yield dichlorocarbene. This method is less mild and generally gives lower yields than phase-transfer catalysis.

Synthesis. Twenty-one benzene-, α-toluene, and phenylethaneisonitrile analogs were synthesized from the corresponding amine using chloroform and base under standard phase-transfer conditions (Figure 2 and Table 1). This method also provided easy access to the reported areneisonitriles **1a**, **1f**, **1g**, **1i**, **1j**, **1l**, **2a**, and **3c**[25,36,37] using an alternative synthetic strategy. Starting amines used for this initial series of aromatic isonitriles were inexpensive and commercially available. Phase-transfer catalysis proved advantageous in terms of avoidance of hazardous material and convenient reaction times (often under 2 h). However, for this series, the method suffered from variable yields, ranging from 4 to 54%, although generally in the 15–30% range (Table 1). Nonetheless, isolation of pure materials was facilitated by lack of byproducts and a significant increase in mobility on silica gel or basic alumina of product isonitriles, relative to starting amines. Since subsequent synthesis of non-carrier-added (NCA) hexakis Tc(1+)–isonitrile complexes required only 1–2 mg of neat ligand per experiment, only small total amounts of material were needed for analytical characterization and biological evaluation.

All synthesized isonitriles were characterized by ^{1}H NMR and GC MS while several biologically promising ligands (**1b**, **1d**, **2a**, **2d**, **3a**, **3b**, and **3c**; see below) were further analyzed by proton-decoupled ^{13}C NMR, IR spectroscopy, and HRMS (EI) (see Methods: Spectral Analysis). As reported,[38] the isonitrilic carbon yielded a small, broad ^{13}C NMR signal at 150–165 ppm relative to TMS for most ligands. Disappearance of N–H stretching and appearance of a strong, characteristic

Table 1. Synthetic Conditions of Substituted Areneisonitrile Ligands for Preparation of Hexakis Tc(I) Complexes

starting material	product	reaction time (h)	workup solvent	purification solvent (EtOAc:Hex)	isolated yield (%)	HPLC t_R[a] (min)
4-methoxyaniline	**1a**	2	ether	25:75	18	19.1
3,4-dimethoxyaniline	**1b**	18	ether	25:75	41	11.7
3,5-dimethoxyaniline	**1c**	3	EtOAc	15:85	18	22.0
3,4,5-trimethoxyaniline	**1d**	2	ether	25:75	15	13.0
3,4-methylenedioxyaniline	**1e**	2	EtOAc	12:88	15	18.3
4-cyanoaniline	**1f**	2	EtOAc	10:90, then 25:75	20	8.3
4-acetylaniline	**1g**	2	EtOAc	20:80	4	9.4
3-aminoacetanilide	**1h**	2	EtOAc	50:50	14	6.3
3-nitroaniline	**1i**	1	ether	10:90	15	8.0*
4-nitroaniline	**1j**	0.33	EtOAc	15:85	27	2.0
3-aminobenzyl alcohol	**1k**	5	EtOAc	25:75	17	8.9*
2-methoxy-5-nitroaniline	**1l**	2.5	EtOAc	50:50 CH_2Cl_2:hexane	54	7.8*
5-methoxy-2-methyl-4-nitroaniline	**1m**	4.5	EtOAc	25:75	15	8.1*
4-methoxybenzylamine	**2a**	2.5	ether	12:88	47	14.8
3,4-dimethoxybenzylamine	**2b**	18	ether	25:75	25	3.6
3,5-dimethoxybenzylamine	**2c**	2	ether	15:85	28	16.3
3,4,5-trimethoxybenzylamine	**2d**	18	ether	25:75	23	5.0
3,4-(methylenedioxy)benzylamine	**2e**	2.5	ether	25:75	18	15.4*
4-methoxyphenethylamine	**3a**	4	ether	15:85	28	17.0
3,4-dimethoxyphenethylamine	**3b**	18	ether	25:75	17	6.8
3,4,5-trimethoxyphenethylamine	**3c**	1.5	EtOAc	40:60	17	11.2*

[a] HPLC t_R is the reverse phase HPLC retention time in minutes for the corresponding hexakis Tc(I)complex; t_R of solvent front (TcO_4^-) was 1.7 min. An asterisk (*) indicates 10 min gradient ramp.

C≡N absorption between 2100 and 2145 cm^{-1} in the infrared spectra was diagnostic of isonitriles. The relatively nonpolar isonitriles were sufficiently volatile to permit convenient retention times (t_R = 6–12 min) for GC MS using nonpolar capillary columns (DB-210, SE-30, SE-54, OV-210). Methoxyl-containing aromatic isonitriles gave strong molecular ion signals using an electron impact mass detector. The molecular ion was always the parent peak in the compounds that we synthesized. Loss of a methyl group (M − 15) was characteristic of methoxyl-bearing aromatic rings. Compounds subjected to high-temperature volatilization (>225 °C) occasionally gave spurious peaks, which have been reported to correspond to the relevant nitrile, formed by a three-centered sigmatropic rearrangement.[39] In addition, seven biologically active ligands have been characterized through high-resolution MS (EI) to confirm their chemical composition. t_R values of the ligands, one measure of relative lipophilicity, are reported in Methods: Spectral Analysis. t_R values ranged between 5 and 16 min on this isocratic system.

Hexakis Tc(1+)–isonitrile complexes have been synthesized by combining appropriate neat isonitrile ligands dissolved in EtOH/saline with reducing agent and NCA $^{99m}TcO_4$, followed by heating of the resulting reaction mixture at 100 °C for 15–20 min. Abrams[23] evaluated the effectiveness of a number of reductants in formation of Tc complexes with monodentate isonitrile ligands, and found dithionite ion ($S_2O_4^-$) to be highly efficient at high pH (>11). The ease with which the hexakis Tc–isonitrile complexes could be synthesized by this general procedure contributed to the success of the synthesis of NCA Tc-99m complexes starting from all of the isonitriles reported here.

While it is difficult to spectroscopically characterize NCA materials, reverse-phase HPLC established the purity of the Tc complexes and correlated the identity of the complexes with the structurally characterized ligands. RP HPLC radiodetection showed a single peak indicating that final radiochemical yields of >95% were achieved. t_R values of Tc complexes (determined by γ detection, Table 1) tended to be somewhat longer than, but correlated well with, t_R values of the corresponding free isonitrile ligands (by UV detection), consistent with each complex containing intact ligand. By comparison, the t_R for Tc–MIBI is 12 min, indicative of the relative range of lipophilicity of the novel complexes. Although it has been reported that ketones may be reduced by dithionite to the corresponding alcohol,[40] Tc complexes of the acetyl-substituted ligand (**1g**) exhibited the generally observed correlative trend in HPLC behavior for free ligand and complex. Furthermore, the absence of new materials with shorter retention times suggested a lack of reduction of the starting acetyl ligand, in spite of the presence of excess reducing agent. The structure of these NCA complexes could not be directly determined, although the octahedral geometry of the Tc(I) metal core could be inferred from the published crystal coordinates of analogous carrier-added Tc and isostructural Re complexes.[41]

MDR Cellular Accumulation Studies. Parental Chinese hamster V79 lung fibroblasts and the derivative adriamycin-selected 77A cells express modestly low and intermediate levels of Pgp, respectively, as determined by immunoblots of plasma membrane preparations with the anti-Pgp monoclonal antibody C219.[18] These provided convenient preparations for quantitative MDR bioassays. Cells in monolayer culture on coverslips exposed to these novel Tc-99m–areneisonitrile complexes accumulated the compounds in a time-dependent manner which could be characterized as a simple two-compartment system by computer fit of the data. One compartment corresponded to binding of complexes to the growth substrate (glass coverslips) and linearly increased with time, while the other compartment corresponded to cell-associated activity which exponentially approached a plateau generally by 30–60 min. Assuming 3 pmol of Pgp/mg of membrane protein,[42] calculations indicated that typical extracellular content of Tc complexes (10 μCi/mL; specific activity = 9 fmol/μCi) would exceed by 10-fold all available transporter sites, further indicating that the observed plateau was not a result of significant ligand depletion but rather a

Table 2. Net Accumulation of Tc-99m–Isonitrile Complexes in V79 and 77A MDR Cells[a]

ligand	cell line	control	+verapamil	verapamil index
MIBI	77A	3.1	102	32.9
SD		1.7	4.4	
MIBI	V79	18.0	70.4	3.91
SD		3.8	10.6	
1a	77A	36.4	84.7	2.33
SD		2.5	34.8	
1b	77A	113	397	3.52
SD		26.6	51.9	
1c	77A	8.3	15.3	1.84
SD		1.6	3.3	
1d	77A	268	790	2.95
SD		48.4	16.6	
1d	V79	271	455	1.68
SD		32.1	35.2	
1e	77A	50.1	93.9	1.87
SD		9.6	26.3	
1f	77A	100	161	1.61
SD		30.4	32.2	
1g	77A	478	826	1.73
SD		114	248	
1h	77A	190	147	0.77
SD		59.7	38.4	
1i	V79	14.2	14.0	0.99
SD		1.5	0.5	
1j	77A	25.2	29.1	1.15
SD		2.4	1.6	
1k	V79	108	257	2.38
SD		39.6	22.1	
1l	V79	61.0	58.3	0.96
SD		4.2	6.0	
1m	V79	36.4	31.7	0.87
SD		4.3	3.2	
2a	77A	138	359	2.60
SD		21.7	74.2	
2b	77A	236	362	1.53
SD		85.6	50.9	
2c	77A	144	315	2.19
SD		56.4	130	
2d	77A	70.3	57.0	0.81
SD		5.9	22.5	
2e	V79	259	287	1.11
SD		21.6	43.4	
3a	77A	160	494	3.09
SD		38.9	97.2	
3b	77A	44.2	146	3.30
SD		8.4	51.5	
3c	77A	94.6	101	1.07
SD	8.7	8.4		

[a] Preparations were equilibrated for 60 min in control buffer containing Tc complexes in the absence and in the presence of the MDR modulator verapamil (50 μM). Cell-associated activity, obtained by subtracting nonspecific activity binding to the glass substrate from total activity, was determined for each preparation. Accumulation values were quantified as fmol of Tc complex associated with the cells per milligram of cell protein normalized to constant extracellular concentration of Tc- complex: fmol·(mg protein)$^{-1}$·(nM$_o$)$^{-1}$. Values are the mean ± SD of four to eight preparations each. The verapamil index represents the ratio of net uptake in the presence of verapamil over control.

steady-state or equilibrium binding process. Cell-associated net uptake values at 60 min obtained in control buffer and in buffer containing a maximal concentration of the classical MDR modulator verapamil (50 μM) are presented in Table 2. The ratio of verapamil/control accumulation (verapamil index; VI) is also shown. Complexes transported by Pgp would be expected to show low accumulation in control buffer and verapamil-induced enhancement of accumulation (VI >1), as confirmed with Tc–MIBI (Table 2), a previously identified transport substrate of this general class of organometallic cations.[18] Complexes binding competitively or allosterically to a verapamil site on Pgp would be expected to show high cell accumulation, especially in 77A cells, and modulator-induced displacement (i.e., VI < 1).

Hexakis Tc complexes of the 4-acetylbenzeneisonitrile (**1g**) showed the highest 77A cell accumulation in control buffer (478 ± 114 fmol·(mg protein)$^{-1}$·(nM$_o$)$^{-1}$; mean ± SD; n = 4), while complexes of 3,5-dimethoxybenzeneisonitrile (**1c**) and 4-nitrobenzeneisonitrile (**1j**) showed the lowest (8.3 ± 1.6 and 25.2 ± 2.4 fmol·(mg protein)$^{-1}$·(nM$_o$)$^{-1}$, respectively). In V79 cells, another nitro-containing complex (**1i**) showed the lowest accumulation (14.2 ± 1.5 fmol·(mg protein)$^{-1}$·(nM$_o$)$^{-1}$). The VI values of Tc complexes of **1g** (1.73) and **1c** (1.84) suggested transport components, while **1j** and **1i** showed nonspecific cellular interaction (VI ~ 1). The three highest VI values were demonstrated by the methoxyl-containing complexes **1b**, **3a**, and **3b**, respectively (VI > 3). Also showing significantly high VI values were Tc complexes of **1a**, **1d**, **1k**, **2a**, and **2c**, each also containing methoxy groups, except **1k**, which contained a hydroxymethyl substitution. In addition, the VI for complexes of **1d** was greater with 77A cells compared to V79 cells, also consistent with expectations for a transport substrate recognized by Pgp.

These results suggested that methoxy functionalities conferred Pgp recognition to this class of organometallic complexes. To further explore effects of structural variation within the benzeneisonitrile series, 4-cyano and 4-acetyl substitutions (**1f** and **1g**) were synthesized. While demonstrating significantly higher control cell accumulation, complexes of these ligands tended to show a decrease in the VI compared to complexes with 4-methoxy substitution (**1a**). The data suggested that these functionalities tended to enhance cellular bilayer adsorption of the complexes. The 3-acetamido substitution (**1h**) also showed higher cell accumulation, but the VI value was significantly < 1, suggesting a Pgp binding displacement component for this Tc-complex. The higher cell accumulations could not be attributed to simple lipophilic physicochemical properties of these ligands or complexes, since complexes of **1a**, although showing by far the lowest control cell accumulation of the above series, possessed one of the longest reverse phase HPLC retention times (Table 1).

Pgp targeting properties conferred by varying the positions of multisubstituted methoxy functionalities were explored for these complexes. For the α-tolueneisonitrile series, compared to 3,4-substitution (**2b**), complexes with 3,5- substitution (**2c**) tended to enhance the VI and decrease control net uptake (Π = 0.05), while 3,4,5-substitution (**2d**) significantly decreased both VI (<1) and control net uptake (P < 0.05). The **2c** data indicated enhanced transport properties, while the **2d** data, displaying the lowest VI observed in the α-toluene series (0.81), suggested that addition of a third methoxy group conferred partial displacement of the complex by verapamil from a putative Pgp binding site. Similarly, for the benzene series, compared to 3,4-dimethoxy substitution (**1b**), complexes of 3,4,5-trimethoxy substitution (**1d**) decreased the VI while enhancing control net uptake in 77A cells (P < 0.005), suggesting increased nonspecific cellular binding. Conversely, complexes of the 3,5-dimethoxy substitution (**1c**) decreased cell binding, in spite of the high lipophilicity of the ligand. For each aryl series, complexes of 3,4-dimethoxy substitu-

Table 3. Biodistribution of Tc-99m–Isonitrile Complexes in Rat (% id/organ)[a]

ligand	blood	liver	spleen	lungs	kidney	muscle	skin	bone	adrenal	brain	heart	fat	GI
MIBI	0.33	10.8	0.60	0.71	6.26	18.5	3.95	11.0	0.16	0.05	2.36	0.73	30.3
1a	1.36	91.9	2.03	3.98	0.16	0.48	0.38	5.00	0.05	0.01	0.06	0.30	0.38
1b	3.92	87.7	4.50	1.89	0.61	4.67	1.07	4.38	0.07	0.02	0.13	0.40	2.44
1c	0.50	92.5	2.25	1.83	0.49	0.74	0.26	1.53	0.00	0.01	0.03	0.11	3.12
1d	0.40	89.5	3.98	1.92	0.18	0.50	0.15	3.09	0.01	0.03	0.01	0.24	0.16
1e	1.45	91.8	1.82	2.62	0.12	0.47	0.19	4.73	0.05	0.01	0.03	0.08	0.38
1f	9.89	75.7	2.47	2.20	1.23	3.84	1.23	4.90	0.03	0.03	0.13	0.37	2.99
1g	1.13	92.7	2.28	1.73	0.66	0.68	0.70	4.27	0.06	0.01	0.03	0.07	3.15
1h	0.87	86.9	1.50	1.45	0.38	0.53	0.17	3.48	0.02	0.01	0.03	0.03	2.12
1i	9.44	13.8	0.28	0.37	5.21	7.61	5.30	5.74	0.01	0.03	0.17	0.51	42.9
1j	19.2	38.0	1.12	1.06	4.84	6.87	3.65	8.79	0.03	0.06	0.33	1.26	10.1
1k	3.39	62.2	1.85	0.63	2.89	2.99	0.62	3.60	0.05	0.01	0.12	0.20	13.8
1l	14.1	32.5	1.25	0.51	2.74	6.82	2.88	9.58	0.04	0.04	0.35	0.55	19.4
1m	6.88	71.9	3.05	0.72	1.13	1.28	0.77	5.59	0.03	0.03	0.14	0.32	3.96
2a	4.56	85.6	2.45	1.31	0.45	1.92	0.58	2.73	0.03	0.02	0.08	0.24	3.31
2b	3.27	44.2	0.34	1.22	2.25	2.48	1.35	4.72	0.07	0.02	0.10	0.21	33.5
2c	5.06	75.9	3.35	0.87	0.30	1.82	0.43	3.63	0.04	0.02	0.12	0.20	2.22
2d	0.59	39.2	0.13	0.61	3.83	3.02	0.76	2.29	0.04	0.01	0.17	0.17	65.1
2e	4.19	73.8	2.78	3.04	0.61	3.90	0.60	4.29	0.07	0.01	0.15	0.26	5.75
3a	0.79	81.1	2.94	3.93	0.19	1.99	0.41	3.44	0.03	0.00	0.15	0.26	1.86
3b	3.00	77.5	1.91	1.10	1.26	1.77	0.82	2.96	0.04	0.02	0.07	0.14	15.7
3c	0.93	44.2	1.50	0.43	10.0	4.84	1.33	5.11	0.12	0.01	0.38	0.19	24.5

[a] Biodistribution values represent the mean % id/organ for paired rats 30 min postintravenous injection of hexakis Tc-99m–isonitrile complexes of the indicated ligands. Biodistribution of the commercial product Tc-99m–MIBI (MIBI) is shown for comparison. Values of 0.00 indicate % id/organ < 0.005.

tions gave higher VI values than complexes of 3,4,5-trimethoxy substitutions (**1b** vs **1d**, **2b** vs **2d**, and **3b** vs **3c**), but no clear trend was evident for cell accumulation in control buffer.

Nitro functionalities tested at various ligand positions (**1i**, **1j**, **1l**, and **1m**) failed to confer favorable Pgp recognition properties to the Tc complexes. In fact, compared to methoxy groups, complexes containing nitro groups tended to antagonize Pgp interactions, resulting in low net uptakes in control buffer in association with VI values of ~1. For the benzene- and α-tolueneisonitrile complexes, 3,4-methylenedioxy functionalities also did not appear to confer any significant advantage over methoxy in Pgp recognition (**1b** vs **1e**, and **2b** vs **2e**).

Biodistribution and Imaging Studies. Pgp is physiologically expressed in many organs involved in transport and excretion, including the biliary canalicular surface of hepatocytes, the brush border of renal proximal tubules, the apical surface of glandular epithelium of the skin, and the apical surface of intestinal mucosa.[43,44] Pgp has also been identified in bronchial epithelium and on the luminal surface of brain and of testicular capillary endothelium where it may be a functional component of the blood/brain and blood/testicular barriers.[45,46] Thus, biodistribution analyses in normal rat can yield relevant information on Pgp targeting for this class of metallopharmaceuticals as well as screening for favorable overall pharmacokinetics.

The distribution of each hexakis Tc–areneisonitrile complex was determined in two male rats 30 min after intravenous injection of Tc-99m–areneisonitrile complex (6–10 μCi; 30 μCi/kg; 5–9 fmol/μCi) by harvesting organs, weighing, and γ counting. This time point was chosen as a trade-off between the relatively rapid cell accumulation rates generally identified *in vitro* (Table 2) and the desire to sample animals after modest excretion times. Total recovery of injected materials was based on calculations of total counts from organs that were completely isolated (e.g. heart, kidney, and brain) and published mass estimations (based on total body weight) of organs that were only sampled (i.e., skin, fat, and muscle); total recoveries were generally 85–100%.

The target organ for many members of this group of aromatic Tc–isonitrile complexes was the liver, where 80% or more of the injected dose (id) was typically found (Tables 3 and 4). Spleen and lung also exhibited relatively high per gram net uptake in some cases (Tables 3 and 4). Despite the modest to high lipophilicity of these complexes, ≤1% id/organ was found in fat. In general, methoxy- and methylenedioxy-substituted benzeneisonitrile complexes (**1a**, **1b**, **1c**, **1d**, and **1e**) demonstrated very little hepatobiliary clearance into the gastrointestinal tract, despite high hepatic accumulation. In particular, complexes of 3,4,5-trimethoxybenzeneisonitrile (**1d**) showed no significant hepatobiliary (or renal) clearance (Figure 3A). For the methoxy-substituted α-toluene- and phenylethaneisonitrile complexes, progression from 4-methoxy-substituted ligands to 3,4-dimethoxy and 3,4,5-trimethoxy ligands conferred successively enhanced hepatobiliary clearance (Tables 3 and 4 and Figure 3B), a property anticipated by *MDR1* expression on the biliary canalicular surface of hepatocytes. For the benzene series, addition of nitro groups to the complexes also resulted in significant hepatobiliary clearance (**1i**, **1j**, and **1l**). In addition to significant hepatobiliary excretion, complexes of nitrobenzeneisonitrile (**1i** and **1j**) displayed some of the highest levels of renal accumulation (~5% id/organ). Complexes of 2-(3,4,5-trimethoxyphenyl)ethaneisonitrile (**3c**) also demonstrated high combined hepatobiliary clearance and renal accumulation, but renal elimination seemed to be, on the whole, minor for most of the tested Tc–areneisonitrile complexes. Interestingly, Tc complexes of **3c** had the highest heart/blood and adrenal/blood ratios of this series (9.0 and 54, respectively, Table 4), confirming a preliminary report of the favorable heart uptake properties of complexes of **3c**,[37] while Tc complexes of **2d** and **3a** also displayed significant uptake in heart, a non-Pgp tissue.[47] Blood/brain ratios (based on % id/gm) for most of these Tc

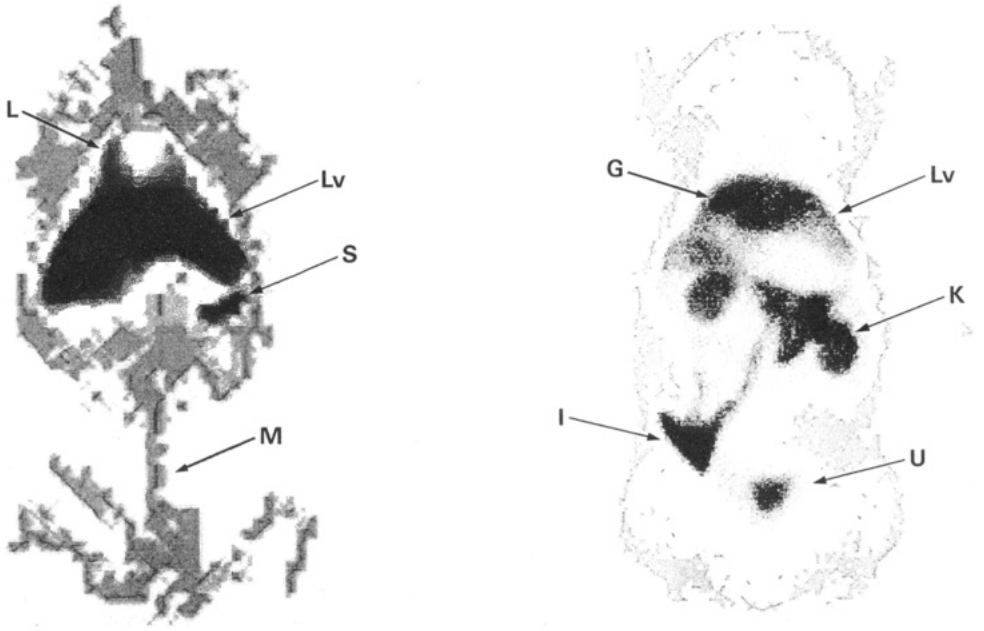

Figure 3. Whole body rabbit planar scintigraphic images 60 min postintravenous injection of hexakis Tc-99m complexes of **1d** (A, left) and **2b** (B, right). Tc-99m complexes of **1d** show high hepatic and pulmonary accumulation with moderate renal accumulation, but no significant hepatobiliary or renal clearance into the intestine or urinary bladder (A). Tc-99m complexes of **2b** demonstrate an example of a complex with abundant hepatobiliary and renal clearance (B). G, gall bladder; I, intestine; K, kidney; L, lung; Lv, liver; M, marrow; S, spleen; U, urinary bladder.

Table 4. Biodistribution of Tc-99m–Isonitrile Complexes in Rat[a]

	(% id/g)												selected organ ratios		
ligand	blood	liver	spleen	lungs	kidney	muscle	skin	bone	adrenal	brain	heart	fat	heart/blood	adrenal/blood	blood/brain
MIBI	0.02	0.93	0.72	0.57	2.61	0.16	0.14	0.26	3.15	0.03	2.40	0.05	120	158	0.7
1a	0.08	7.38	2.57	3.16	0.07	0.00	0.01	0.12	1.28	0.00	0.06	0.02	0.8	16	27
1b	0.23	8.28	5.94	1.53	0.28	0.04	0.04	0.11	1.67	0.01	0.15	0.03	0.7	7.3	26
1c	0.03	8.07	2.81	1.59	0.20	0.01	0.01	0.03	0.05	0.00	0.03	0.01	1.0	1.7	7.5
1d	0.02	8.12	8.33	1.39	0.08	0.00	0.01	0.07	0.14	0.01	0.01	0.02	0.5	7.0	1.5
1e	0.07	6.32	2.65	1.99	0.05	0.00	0.01	0.10	1.00	0.01	0.03	0.01	0.4	14	9.3
1f	0.49	5.45	3.42	1.68	0.46	0.03	0.04	0.10	0.61	0.02	0.13	0.02	0.3	1.2	31
1g	0.05	6.77	3.23	1.02	0.24	0.00	0.02	0.07	1.11	0.00	0.03	0.00	0.6	22	17
1h	0.06	9.24	2.99	1.32	0.21	0.01	0.01	0.09	0.53	0.00	0.04	0.00	0.7	8.8	15
1i	0.53	1.16	0.40	0.34	2.04	0.06	0.18	0.13	0.30	0.02	0.19	0.03	0.4	0.6	31
1j	1.21	3.47	1.57	0.94	2.23	0.07	0.14	0.22	0.73	0.03	0.40	0.10	0.3	0.6	39
1k	0.19	5.11	1.81	0.52	1.21	0.03	0.02	0.08	1.18	0.01	0.14	0.01	0.7	6.2	27
1l	0.91	3.11	1.66	0.48	1.44	0.07	0.11	0.25	0.82	0.02	0.44	0.04	0.5	0.9	38
1m	0.42	6.71	4.39	0.61	0.50	0.01	0.03	0.14	0.75	0.02	0.16	0.02	0.4	1.8	28
2a	0.24	6.70	3.31	0.97	0.17	0.02	0.02	0.06	0.59	0.01	0.08	0.02	0.3	2.5	30
2b	0.17	3.31	0.58	0.99	0.87	0.02	0.04	0.10	1.35	0.01	0.10	0.01	0.6	7.9	19
2c	0.33	8.42	6.35	0.80	0.16	0.02	0.02	0.09	1.01	0.01	0.13	0.02	0.4	3.1	28
2d	0.04	4.59	0.23	0.57	2.03	0.03	0.03	0.07	0.96	0.01	0.22	0.02	5.5	24	6.7
2e	0.29	7.01	4.47	2.73	0.29	0.04	0.03	0.12	2.01	0.01	0.18	0.02	0.6	6.9	36
3a	0.05	7.05	3.34	3.53	0.10	0.02	0.02	0.09	0.64	0.00	0.18	0.02	3.5	13	26
3b	0.16	6.27	3.03	0.94	0.55	0.01	0.03	0.06	0.66	0.01	0.07	0.01	0.4	4.1	23
3c	0.04	3.55	2.47	0.34	3.74	0.03	0.04	0.09	2.17	0.01	0.36	0.01	9.0	54	5.0

[a] Same as Table 3 except biodistribution values are normalized to % id/g and selected organ ratios are calculated from the values shown.

complexes were quite high, exceeding 30 in many cases, consistent with exclusion of the compounds by the blood/brain barrier, a biological property consistent with *MDR1* Pgp targeting.[48] Tc complexes of **3a** showed the highest combined heart/blood ratio and blood/brain barrier exclusion. Conversely, Tc complexes containing 3,4,5-trimethoxy substitution (**1d**, **2d**, and **3c**) conspicuously displayed the lowest blood/brain ratios, even approaching that of the clinically available agent Tc–MIBI, indicative of either rapid clearance from plasma or accumulation within selected central nervous system structures (such as choroid plexus) in a manner analogous to Tc–MIBI.[49]

Conclusions

This work provides convenient access to selected substituted benzene-, α-toluene-, and phenylethaneisonitriles and their corresponding hexakis Tc(+1) complexes. These complexes combine lipophilic, monocationic characteristics of this class of metallopharmaceuticals with structural features of known MDR substrates which may enable functional detection *in vivo* of MDR Pgp expression with high specific activity Tc-99m complexes and modulation with isostructural Re-complexes. The results highlight methoxy functionalities for conferring Pgp recognition to this class of com-

plexes. For this initial series, complexes of **1b**, **1d**, **1h**, **2a**, **2d**, **3a**, **3b**, and **3c** show biological promise.

Experimental Section

Chemistry. General Analysis. ^{1}H NMR and ^{13}C NMR spectra were acquired on a GE GN-300 spectrometer with TMS as an internal standard; chemical shifts are given in δ (ppm). IR spectra were obtained on a Perkin-Elmer 1310 IR spectrometer in CsI plates. Mass spectrometry was performed on a Hewlett-Packard MSD GC MS, using a DB-210 capillary column (J+W Durabond FSOT, 30 m, 0.25 m diameter). The following GC parameters were used: injector temperature 250 °C; detector temperature 250 °C; column temperature, initial 120 °C, initial time 2.0 min, rate 14.0 °C/min, final temperature 240 °C, final time 5.43 min. High-resolution MS (EI) were obtained at the Washington University Resource for Biomedical and Bio-organic Mass Spectrometry. RP HPLC (Waters) of free ligands were performed with a C8 column using an isocratic system of 45% methanol:tetrahydrofuran (10:1)/55% 0.05 M $(NH_4)_2SO_4$ (flow = 1 mL/min); complexes were analyzed with a gradient system equilibrated in 60%/40% solvent followed by a linear ramp over 15 min to 95%/5%. Retention times (t_R) of most ligands (see spectral analysis) and all complexes (Table 1) are reported in minutes.

Synthesis of Aromatic Isonitriles: General Method. To a mixture of the starting amine (1 g), chloroform (3 mL), dicholoromethane (3 mL), and tetrabutylammonium bromide (50 mg; phase-transfer catalyst) under nitrogen was added 50% aqueous NaOH (3 mL). The mixture was stirred vigorously until TLC of the organic phase (EtOAc/hex on silica gel or basic alumina) indicated the presence of a significant amount of UV-detectable product in the mobile phase. Workup consisted of addition of an organic solvent (EtOAc or ether, Table 1) and water, followed by separation of the organic layer and removal of solvent under reduced pressure. Crude product was purified by column chromatography on basic alumina, generally using EtOAc/hexane as the solvent (yields given in Table 1). Acceptable purity of products was documented by analysis of NMR spectra and HRMS.

Spectral Data: 4-Methoxybenzeneisonitrile (1a). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.82 (3H, s, OC*H*$_3$), 6.87 (2H, d, J_{AB} = 9 Hz, H3 and H5), 7.31 (2H, d, J_{AB} = 9 Hz, H2 and H6). GC MS: 133 (M^+), 118 (loss of CH_3), 103 (loss of OCH_2), 90 (loss of isonitrile C, OCH_3). t_R: 15.8 min.

3,4-Dimethoxybenzeneisonitrile (1b). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (3H, s, OC*H*$_3$), 3.89 (3H, s, OC*H*$_3$), 6.81 (1H, d, J = 9 Hz, H5), 6.87 (1H, d, J = 2 Hz, H2), 6.98 (1H, dd, J_{AB} = 9 Hz, J_{AX} = 2 Hz, H6). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 57.7, 111.0, 112.4, 120.8, 150.7, 151.4, 164.0. IR (CH_2Cl_2): 2125 cm^{-1}. GC MS: 163 (M^+), 148 (loss of CH_3), 120 (loss of isonitrile C, OCH_3), 102 (loss of OCH_3, OCH_2), 92 (loss of CN, OCH_2, CH_3). HRMS (EI): calcd for $C_9H_9O_2N$ 163.0633, found 163.0638. t_R: 7.2 min.

3,5-Dimethoxybenzeneisonitrile (1c). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.78 (6H, s, OC*H*$_3$), 6.47 (1H, t, J = 2 Hz, H4), 6.51 (2H, d, J = 2 Hz, H2 and H6). GC MS: 163 (M^+), 134 (loss of OCH_2), 120 (loss of isonitrile C, OCH_3), 103 (loss of $(OCH_2)_2$).

3,4,5-Trimethoxybenzeneisonitrile (1d). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.85 (3H, s, OC*H*$_3$), 3.86 (6H, s, OC*H*$_3$), 6.62 (2H, s, H2 and H6). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 57.8, 62.6, 105.5, 141.0, 149.0, 154.9, 165.0. IR (CH_2Cl_2): 2125 cm^{-1}. GC MS: 193 (M^+), 178 (loss of CH_3), 163 (loss of OCH_2), 150 (loss of isonitrile C, OCH_3), 135 (loss of CH_3, isonitrile C, OCH_3), 120. HRMS (EI): calcd for $C_{10}H_{11}O_3N$ 193.0738, found 193.0732. t_R: 8.3 min.

3,4-(Methylenedioxy)benzeneisonitrile (1e). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.06 (2H, d), 6.79 (1H, d, J = 8 Hz), 6.86 (1H, s), 6.93 (1H, d, J = 8 Hz). GC MS: 147 (M^+), 146 (M^+ − 1), 89 (loss of isonitrile C, −OCH_2O−). t_R: 11.1 min.

4-Cyanobenzeneisonitrile (1f). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51 (2H, d, J = 8 Hz, H3 and H5), 7.74 (2H, d, J = 8 Hz, H2 and H6). GC MS: 128 (M^+), 101 (loss of HCN). t_R: 5.9 min.

4-Acetylbenzeneisonitrile (1g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.62 (3H, s, C*H*$_3$CO), 7.48 (2H, d, J = 8 Hz, H2 and H6), 8.00 (2H, d, J = 8 Hz, H3 and H5). GC MS: 145 (M^+), 130 (loss of CH_3), 102 (loss of $COCH_3$). t_R: 7.4 min.

3-Acetamidobenzeneisonitrile (1h). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.19 (3H, s, C*H*$_3$CO), 7.11 (1H, d, J = 8 Hz, H4), 7.32 (1H, t, J = 8 Hz, H5), 7.45 (1H, d, J = 8 Hz, H6), 7.67 (1H, broad s, N*H*CO), 7.74 (1H, s, H2). GC MS: 160 (M^+), 118 (loss of $COCH_2$), 91 (loss of CH_3CO, NC). t_R: 5.5 min.

3-Nitrobenzeneisonitrile (1i). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.63 (2H, d, J = 8 Hz, H4 and H6), 7.74 (1H, dt, J_{ab} = 8 Hz, J_{ax} = 1.5 Hz, H5), 8.13 (1H, dd, J_{ab} = 8 Hz, J_{ax} = 1.5 Hz, H2). GC MS: 148 (M^+), 102 (loss of NO_2), 90 (loss of NO_2, isonitrile C).

4-Nitrobenzeneisonitrile (1j). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (2H, d, J = 9 Hz, H2 and H6), 8.29 (2H, d, J = 9 Hz, H3 and H5). GC MS: 148 (M^+), 102 (loss of NO_2), 90 (loss of NO_2, isonitrile C), 76 (loss of CN, NO_2), 75 (loss of HCN, NO_2). t_R: 10.4 min.

3-(hydroxymethyl)benzeneisonitrile (1k). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.48 (1H, broad s), 4.69 (2H, s), 7.28 (1H, d), 7.35−7.45 (3H, m). GC MS: 133 (M^+), 104 (loss of isonitrile C, OH).

2-Methoxy-5-nitrobenzeneisonitrile (1l). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.09 (3H, s, OC*H*$_3$), 7.11 (1H, d, J = 9 Hz, H3), 8.28 (1H, dd, J_{AB} = 9 Hz, J_{AX} = 3 Hz, H4), 8.32 (1H, d, J_{AX} = 3 Hz, H6). GC MS: 178 (M^+), 148 (loss of N,O), 120 (loss of isonitrile C, NO_2), 117 (loss of N,O, OCH_3).

5-Methoxy-2-methyl-4-nitrobenzeneisonitrile (1m). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.42 (3H, s, C*H*$_3$), 3.95 (3H, s, OC*H*$_3$), 7.05 (1H, s, H6), 7.76 (1H, s, H3). GC MS: 192 (M^+), 116 (loss of NO, CH_3, OCH_3).

4-Methoxy-α-tolueneisonitrile (2a). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.82 (3H, s, OC*H*$_3$), 4.57 (2H, s, benzyl C*H*$_2$), 6.92 (2H, d, J_{AB} = 9 Hz, H3 and H5), 7.27 (2H, d, J_{AB} = 9 Hz, H2 and H6). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 46.6, 56.9, 115.9, 126.2, 129.8, 159.0, 161.2. IR (CH_2Cl_2): 2140 cm^{-1}. GC MS: 147 (M^+), 132 (loss of CH_3), 121 (loss of CN), 116 (loss of OCH_3), 107 (loss of CH_2NC). HRMS (EI): calcd for C_9H_9ON 147.0684, found 147.0688. t_R: 10.3 min.

3,4-Dimethoxy-α-tolueneisonitrile (2b). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (3H, s, OC*H*$_3$), 3.91 (3H, s, OC*H*$_3$), 4.58 (2H, s, benzyl C*H*$_2$), 6.86 (2H, d, J = 4 Hz, H5 and H6), 6.87 (1H, s, H2). GC MS: 177 (M^+), 162 (loss of CH_3), 134 (loss of isonitrile C, OCH_3), 116 (loss of $(OCH_3)_2$), 107, 94. t_R: 6.2 min.

3,5-Dimethoxy-α-tolueneisonitrile (2c). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.81 (6H, s, OC*H*$_3$), 4.58 (2H, s, benzyl C*H*$_2$), 6.43 (1H, s, H4), 6.48 (2H, s, H2 and H6). GC MS: 177 (M^+), 162 (loss of CH_3), 146 (loss of OCH_3), 116 (loss of OCH_3, OCH_2), 107 (loss of $CNCH_2$, $(CH_3)_2$). t_R: 11.2 min.

3,4,5-Trimethoxy-α-tolueneisonitrile (2d). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.85 (3H, s, OC*H*$_3$), 3.88 (6H, s, OC*H*$_3$), 4.59 (2H, s, benzyl C*H*$_2$), 6.56 (2H, s, H2 and H6). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 45.8, 56.4, 61.1, 103.8, 128.1, 138.0, 153.8. IR (CH_2Cl_2): 2145 cm^{-1}. GC MS: 207 (M^+), 192 (loss of CH_3), 181 (loss of CN), 164 (loss of isonitrile C, OCH_3), 149 (loss of HNC, OCH_3), 134 (loss of isonitrile C, OCH_3, OCH_2). HRMS (EI): calcd for $C_{11}H_{13}O_3N$ 207.0895, found 207.0895. t_R: 6.3 min.

3,4-(Methylenedioxy)-α-tolueneisonitrile (2e). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.51 (2H, d, J = 1.8 Hz, benzyl C*H*$_2$), 5.97 (2H, d, J = 1.8 Hz, OCH_2O), 6.78 (1H, s, H2), 6.80 (2H, d, J = 11 Hz, H5 and H6). GC MS: 161 (M^+), 160 (M^+ − 1), 135 (loss of NC), 103 (loss of isonitrile C, OCH_2O).

2-(4-Methoxyphenyl)ethaneisonitrile (3a). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.93 (2H, broad s, αC*H*$_2$), 3.55 (1H, t, J = 2 Hz, benzyl C*H*$_2$), 3.57 (1H, s, benzyl C*H*$_2$), 3.80 (3H, d, J = 2 Hz, OC*H*$_3$), 6.83 (1H, t, J = 2 Hz, H ortho to OC*H*$_3$), 6.86 (1H, d, J = 2 Hz, H ortho to OC*H*$_3$), 7.11 (2H, d, J_{AB} = 9 Hz, H2 and H6). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 36.4, 44.9, 56.9, 115.7, 130.5, 131.3, 158.0, 160.5. GC MS: 161 (M^+), 145 (loss of CH_4), 134 (loss of HCN), 121 (loss of CH_2NC), 91 (loss of CH_2NC, OCH_2). HRMS (EI): calcd for $C_{10}H_{11}ON$ 161.0841, found 161.0846. t_R: 9.4 min.

2-(3,4-Dimethoxyphenyl)ethaneisonitrile (3b). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.93 (2H, t, J = 7 Hz, αC*H*$_2$), 3.59 (2H,

t, J = 7 Hz, benzyl CH_2), 3.87 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 6.74 (1H, d, J = 2 Hz, H2), 6.76 (1H, dd, J_{AB} = 8 Hz, J_{AX} = 2 Hz, H6), 6.83 (1H, d, J_{AB} = 8 Hz, H5). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 36.9, 44.8, 57.5, 112.9, 113.4, 122.3, 130.8, 149.8, 150.6, 158.0. IR (CH_2Cl_2): 2145 cm^{-1}. GC MS: 191 (M^+), 151 (loss of CH_2NC), 137 (loss of CH_2CH_2NC), 107 (loss of CH_2CH_2NC, OCH_2). HRMS (EI): calcd for $C_{11}H_{13}O_2N$ 191.0946, found 191.0953. t_R: 5.4 min.

2-(3,4,5-Trimethoxyphenyl)ethaneisonitrile (3c). ^1H NMR (300 MHz, $CDCl_3$): δ 2.93 (2H, t, J = 7 Hz, αCH_2), 3.61 (2H, t, J = 7 Hz, benzyl CH_2), 3.84 (3H, s, OCH_3), 3.87 (6H, s, OCH_3), 6.44 (2H, s, aromatic H). GC MS: 221 (M^+), 181 (loss of CH_2NC). HRMS (EI): calcd for $C_{12}H_{15}O_3N$ 221.1052, found 221.1046.

Synthesis of Hexakis(areneisonitrile)Tc(1+) Complexes. Hexakis(areneisonitrile)Tc-99m(1+) complexes were synthesized according to a method previously described.[23,50] To a solution containing the desired isonitrile (2 mg) and $Na_2S_2O_4$ (5 mg) in a mixture of 5 mL of ethanol and 5 mL of saline was added non-carrier-added $^{99m}TcO_4$ (2–10 mCi in saline; 5–9 pmol/mCi). The mixture was sealed and heated to 100 °C in a boiling water bath for 10–15 min. Completion of the reaction was monitored by radiometric HPLC. After the reaction was complete, the reaction mixture was cooled and loaded onto a C-18 Sep Pak and routinely washed with 65% EtOH/saline to remove unreacted pertechnetate and free ligand, and the product was eluted with 95% EtOH/saline. For Tc complexes of **1c**, an additional elution with 5% EtOH/saline to remove a hydrophilic species was required, followed by elution of product in 100% EtOH. Radioactive purity of final hexakis Tc-99m complexes were again confirmed by radiometric HPLC. End-of-synthesis yields (decay-corrected) were generally quantitative.

Cell Uptake Studies. Chinese hamster V79 lung fibroblasts and the adriamycin-selected 77A MDR derivative cell lines were grown as described.[18] Briefly, cells were plated in 100-mm Petri dishes containing seven 25-mm glass coverslips on the bottom and grown to confluence in α-MEM medium (GIBCO, Grand Island, NY) supplemented with L-glutamine (1%), penicillin/streptomycin (0.1%), and fetal calf serum (10%) in the presence of 0 and 0.1 μg/mL adriamycin, respectively. Control solution for transport experiments was a modified Earle's balanced salt solution (MEBSS) containing (mM) 145 Na^+, 5.4 K^+, 1.2 Ca^{2+}, 0.8 Mg^{2+}, 152 Cl^-, 0.8 $H_2PO_4^-$, 0.8 SO_4^{2-}, 5.6 dextrose, 4.0 HEPES, and 1% bovine calf serum (vol/vol), pH 7.4 ± 0.05.

Coverslips with cells were removed from culture media and pre-equilibrated for 40–60 s in control buffer. Uptake and retention experiments were initiated by immersion of coverslips with cells in 60-mm glass Pyrex dishes containing 4 mL loading solution consisting of MEBSS with 0.005–0.5 nM [Tc-isonitrile complex] (5–9 fmol/μCi; 1–50 μCi/mL) in the presence or absence of verapamil (50 μM). Coverslips were removed at various times (2, 5, 15, 30, 60, and 120 min), rinsed three times in 25 mL of ice-cold (2 °C) isotope-free solution for 8 s each to clear extracellular spaces, and extracted in 2 mL of a 1% sodium dodecyl sulfate, 10 mM sodium borate solution. To generate substrate blanks, preparations were immersed as above for 60 min, but cells were scraped off coverslips in triplicate prior to extraction. Aliquots of the loading buffer and stock solutions were then obtained for standardizing cellular data with extracellular concentrations (nM_o) of Tc–isonitrile complex. Cell and blank extracts, stock solutions, and extracellular buffer samples were assayed for γ activity in a well-type gamma counter (Packard, Minaxi Autogamma 5000). Cell samples were quantified for protein content by the method of Lowry,[51] using bovine serum albumin as the protein standard. Knowledge of the elution history of the Mo/Tc generator and activity of stock solutions allowed use of generator equilibrium equations to calculate the absolute concentration of total Tc–isonitrile complex in solutions.[52] Blank values were subtracted from total extract counts to generate corrected cell-associated agent accumulation. Values were quantified as fmol of Tc-complex associated with the cells per milligram of cell protein normalized to constant extracellular concentration of Tc complex: $fmol(mg\ protein)^{-1}(nM_o)^{-1}$.

Biodistribution Studies. Pairs of non-virus-free, nonfasted Sprague–Dawley CD male rats (250 g, Charles River) were analyzed for each complex, 30 min postinjection. Tc-99m–isonitrile complexes were diluted with sterile normal saline, until the concentration was 6–10 μC/mL. The animals were anesthetized with ethyl ether, an inguinal incision made, and a volume of 1 mL administered via the femoral vein under direct vision to ensure complete delivery of the radiolabeled compound. The inguinal incision was closed with a 9 mm wound clip, and the animal was returned to the cage. Approximately 25 min after injection, the animal was reanesthetized with ethyl ether and an incision made below the xiphoid. The diaphragm was severed 30 min postinjection, and approximately 3 mL of blood was removed via cardiac puncture using a syringe previously flushed with heparin (1000 units/mL). Each organ sample was removed, blotted well to remove traces of blood, placed in a previously tared 7 mL polyethylene scintillation vial, and weighed. Liver uptake was estimated using three samples of approximately 1g. Muscle, skin, blood, bone, and fat uptakes were estimated from samples of approximately 1 g, and extrapolating to total organ mass based on total body weight (muscle %, skin %, blood %, bone %, fat %). Samples were counted in a γ scintillation counter (Packard 5000) for 15 min, or until the sampling standard deviation was below 0.5%. Total recovery of radioactivity was >85%, and differences between the % id values determined for paired animals and organs for each Tc-complex were generally <10%.

Imaging Studies. Male nonfasted New Zealand albino rabbits (3.5–4.5 kg) were anesthetized with a mixture of ketamine (40 mg/kg) and xylezine (10 mg/kg) im. Tc-99m–isonitrile complexes (0.25–0.5 mCi/kg) were injected via an ear vein into rabbits positioned over a γ scintillation camera (Searle Pho/Gamma GE Starcam system; low energy all purpose collimator) and anterior images collected for 60 s/frame for 1 h. No scatter or attenuation corrections were made.

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