A QD 1-9 treatment schedule was used. Tumored, untreated control mice died between days 8-15 (B16), 8-11 (L1210), and 9-13 (P388).

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Synthesis, Characterization and Myocardial Uptake of Cationic Bis(arene)technetium(I) Complexes

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A series of bis(arene)technetium(I) complexes has been synthesized from $^{99m}TcO_4^-$ in order to study their organ distribution. Syntheses using either ultrasound/Al/$AlCl_3$ or Zn/HCl gave products relatively free from transalkylation. The identity of the complexes was verified by comparison to the ^{99}Tc complexes. Equivalence of the ^{99}Tc and ^{99m}Tc complexes was demonstrated by HPLC techniques. Biodistribution studies in rats reveal substantial myocardial uptake for many members of the series, especially those containing benzene rings substituted with about four to six carbon atoms. The myocardial uptake is related to the lipophilicity of the complexes as measured by octanol/buffer partition ratios (OBPR). Optimal ranges of lipophilicity for maximal myocardial uptake occur for OBPR from 2 to 9. Rat and human plasma binding of the complexes increases with lipophilicity after a threshold value is exceeded.

Introduction

Since the demonstration that cationic complexes of technetium can show substantial uptake in myocardial tissue,[2] several classes of complexes have been discovered to have such properties. Among the more notable cationic classes are the hexakisisonitriles,[3,4] the hexakis(trimethylphosphite) and related complexes,[5,6] and the 1,2-bis(dimethylphosphino)ethane series.[2,7] For these classes there usually exists a compound which accumulates in the myocardium at a much higher level than closely related complexes. Drawbacks of these classes include high plasma-binding values, especially for the complexes of ligands containing phosphorus, and the lack of a correlation between myocardial uptake in animals and humans. Moreover, the nature of these classes is such that when the ligand is changed slightly, this change is multiplied over the six identical ligands and results in gross changes in physical properties. Thus, solution of the clinical problems through structure–distribution studies is hindered due to the inability to vary subtly the properties of the complexes.

Neutral BATO complexes of technetium also show myocardial uptake.[8,9] In these complexes the substituent on the capping boron atom of the BATO complexes can be varied to give complexes with finely tuned biodistributions. Until recently, a series of cationic complexes with such versatility has not been available.

$Tc(arene)_2^+$ complexes have been known for years.[10-12] The benzene and hexamethylbenzene complexes are stable in aqueous solution as cations. Since only two arene rings are coordinated to the metal, the class is much more amenable to subtle variations in structure than other cationic systems since changing one substituent on the arene ring introduces only two changes in the complex.

This work reports in detail the synthesis, characterization, biodistribution in rats, and plasma binding for a wide range of $Tc(arene)_2^+$ complexes with subtle structural variations. Significant progress has been made in the synthesis of isomerically pure $Tc(arene)_2^+$ complexes in order to gain access to this class of complexes. The structure–distribution relationships reveal that heart up-

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(2) Deutsch, E.; Bushong, W.; Glavan, K. A.; Elder, R. C.; Sodd, V. J.; Scholz, K. L.; Fortman, D. L.; Lukes, S. J. Heart Imaging with Cationic Complexes of Technetium. *Science* **1981**, *214*, 85-86.
(3) Holman, L. B.; Jones, A. G.; Lister-James, J.; Davison, A.; Abrams, M. J.; Kieshenbaum, J. M.; Tumeh, S. S.; English, R. J. A New Tc-99m-Labeled Myocardial Imaging Agent, Hexakis(*t*-Butylisonitrile)Technetium(I) [Tc-99m TBI]: Initial Experience in the Human. *J. Nucl. Med.* **1984**, *25*, 1350-1355.
(4) Holman, B. L.; Sporn, V.; Jones, A. G.; Benjamin Sia, S. T.; Perez-Balino, N.; Davison, A.; Lister-James, J.; Kronauge, J. F.; Mitta, A. E. A.; Camin, L. L.; Campbell, S.; Williams, S. J.; Carpenter, A. T. Myocardial Imaging with Technetium-99m CPI: Initial Experience in the Human. *J. Nucl. Med.* **1987**, *28*, 13-18.
(5) Jehangir, M.; Subramanian, G.; McAfee, J. G.; Thomas, F. D.; Gagne, G.; Zapf-Longo, C.; Ritter-Hrncirik, C. An Evaluation of ^{99m}Tc-TMP as a Myocardial Imaging Agent. *J. Nucl. Med.* **1985**, *26*, P83 (Abstract No. 347).
(6) Gerundini, P.; Deutsch, E.; Savi, A.; Gilardi, M. C.; Zecca, L.; Hirth, W.; Libson, K.; Fazio, F. Evaluation in Man and Dog of Three New Cationic Technetium-99m Complexes for Myocardial Perfusion Imaging. *J. Nucl. Med.* **1985**, *26*, 824 (Abstract).
(7) Deutsch, E.; Glavan, K. A.; Sodd, V. J.; Nishiyama, H.; Ferguson, D. L.; Lukes, S. J. Cationic Tc-99m Complexes as Potential Myocardial Imaging Agents. *J. Nucl. Med* **1981**, *22*, 897-907.
(8) Coleman, R. E.; Maturi, M.; Nunn, A. D.; Eckelman, W. C.; Juri, P. N.; Cobb, F. R. Imaging of Myocardial Perfusion with Tc-99m SQ 30217: Dog and Human Studies. *J. Nucl. Med.* **1986**, *27*, 893-894 (Abstract).
(9) Nunn, A. D.; Treher, F. T.; Feld, T. Boronic Acid Adducts of Technetium Oxime Complexes (BATOs) A New Class of Neutral Complexes with Myocardial Imaging Capabilities. *J. Nucl. Med.* **1986**, *27*, 893 (Abstract).
(10) Baumgartner, F.; Fischer, E. O.; Zahn, U. Arene complexes of metals. XLVI. Chemistry of nuclear processes. 3. Dibenzenetechnetium(I) cation by element conversion from dibenzenemolybdenum(I). *Chem. Ber.* **1961**, *94*, 2198-2203.
(11) Palm, C.; Fischer, E. O.; Baumgartner, F. Aromatic complexes of metals. LX. Dibenzenetechnetium(I) cation in ponderable amounts. *Tetrahedron Lett.* **1962**, 253-254.

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take is related to lipophilicity. Portions of this work have been reported earlier.[13-15]

Results and Discussion

Syntheses. The ^{99}Tc complexes of benzene and hexamethylbenzene were synthesized by previously published methods. The syntheses proceeded satisfactorily as published, producing milligram quantities of the ^{99}Tc product as the hexafluorophosphate salt. The benzene complex was analyzed satisfactorily by FABMS. Microgram quantities of the tri- and tetramethylbenzene complexes were prepared by adding carrier amounts of ^{99}Tc to the ^{99m}Tc preparations. These were analyzed by high-resolution FABMS. The measured exact masses of the parent peaks (339.0947 and 367.1255 for $C_6H_3(CH_3)_3$ and $C_6H_2(CH_3)_4$, respectively) agreed within 3 millimass units of the calculated values. Such agreement is generally accepted as an analytical elemental analysis. Thus, by the techniques of high-resolution FABMS, the products of the syntheses were identified as the ^{99}Tc complexes.

A significant hindrance to the study was encountered since a synthetic procedure of much shorter duration than that reported for the ^{99}Tc complexes was required for the ^{99m}Tc complexes due to the short half-life (6.0 h). For instance, the syntheses of the ^{99}Tc complexes required $TcCl_4$, which was synthesized by chlorination of Tc metal (~6 h),[16] and reaction of $TcCl_4$ with reducing agent and ligand (~24 h). We investigated the use of $^{99m}TcO_4^-$ (dried $NaTcO_4$) as starting material instead of $TcCl_4$ in order to circumvent the delays which would be required to synthesize $^{99m}TcCl_4$ and react this with the arenes. A product with paper chromatographic (saline and MeOH) and electrophoretic behavior similar to that of the ^{99}Tc material was isolated in good yield. In addition, it was observed that reaction times as short as 1 h gave substantial quantities of product. Thus, the first major obstacle had been overcome.

A second complication arose when the HPLC traces for products prepared using method 1 showed multiple products that appeared to arise from transalkylation of the benzene rings. This was especially evident for the methyl derivatives. Further refinement of the procedure was necessary since for the partially substituted benzene rings the Freidel–Crafts conditions led to substantial amouunts of alkyl transfer. For example, reaction of tetramethylbenzene gave products containing tri- and pentamethylbenzene. The presence of the transmethylated benzenes was demonstrated by GC analysis of the cyclohexane layer after the residual ^{99m}Tc activity had decayed to insignif-

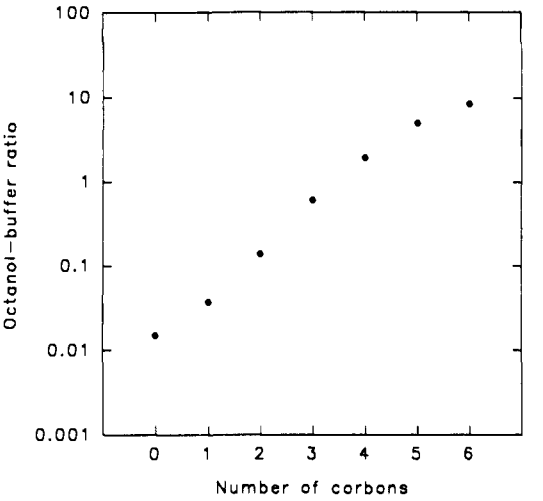

Figure 1. Plot of octanol/buffer partition ratio as a function of the number of methyl groups on the ring for benzenes substituted only with methyl groups.

icant amounts. The transmethylation also occurred, as expected, in blank experiments that did not contain dried $NaTcO_4$. The alkyl transfer was least with meta-substituted starting materials.

The transalkylation was successfully suppressed by two different methods. The first method simply involved substituting Zn dust for Al powder with production of $ZnCl_2$ by addition of HCl gas. Use of these reagents effectively obviated the Friedel–Crafts conditions that led to alkyl transfer. However, this generally resulted in a lower yield than that seen with method 1. The second method took advantage of low-intensity ultrasonic irradiation. Ultrasound has been shown recently to give products that are sometimes quite different from those of thermal reactions.[17-26] In our case, transalkylation was nearly undetectable by GC after reaction for 1 h using ultrasound. Either of these methods produced compounds that were pure enough for pharmacologic testing without extensive additional separation of isomers. It is interesting to note that the ultrasonic method worked only where $Al/AlCl_3$ or Al/HCl was used.

Purification of the Complexes. Reaction of the starting materials in nonaqueous solvent under anaerobic conditions gave the bis(arene) complexes as tetrachloroaluminate salts. Hydrolysis of the $AlCl_4^-$ salt led to water-soluble species which were readily extracted from

(12) Fischer, E. O.; Schmidt, M. W. Aromatic complexes of metals. CX. Five and six ring aromatic π-complexes of technetium-99. *Chem. Ber.* **1969**, *102*, 1954–1960.
(13) Dean, R. T.; Wester, D. W.; Nosco, D. L.; Adams, M. D.; Coveney, J. R.; Robbins, M. S.; McElvany, K. D.; DeJong, R. Progress in the Design, Evaluation and Development of Tc-99m Radiopharmaceuticals. In *Technetium in Chemistry and Nuclear Medicine 2*; Nicolini, M. Bandoli, G., Mazzi, U., Eds.; Cortina International: Verona, 1986, pp 147–154.
(14) Nosco, D. L.; Tofe, A. J.; Dunn, T. J.; Lyle, L. R.; Wolfangel, R. G.; Bushman, M. J.; Grummon, G. D.; Helling, D. E.; Marmion, M. E.; Miller, K. M.; Pipes, D. W.; Strubel, T. W.; Wester, D. W. New Developments in Radiopharmaceuticals at Mallinckrodt. In *Technetium and Rhenium in Chemistry and Nuclear Medicine 3*; Nicolini, M., Bandoli, G., Mazzi, U., Eds.; Cortina International: Verona, in press.
(15) Nowotnik, P. Quantitative Structure–Distribution Relationships (QSDRs) of Radiopharmaceuticals. In *Radiopharmaceuticals Using Radioactive Compounds in Pharmaceutics and Medicine*; Theobald, A. E., Ed.; Ellis Horwood Limited: Chichester, 1989; pp 28–56.
(16) Colton, R. Technetium Chlorides. *Nature* **1962**, *193*, 872–873.
(17) Bates, R. S.; Wright, A. H. Ruthenium–Arene Complexes and Clusters via Ultrasound. *J. Chem. Soc., Chem. Commun.* **1990**, 1129–1131.
(18) Rehorek, D.; Schoffauer, S.; Hennig, H. Influence of ultrasound on chemical reactions. *Z. Chem.* **1989**, *29*, 389–396.
(19) Lee, J.; Snyder, J. K. Ultrasound-Promoted Diels–Alder Reactions: Syntheses of Tanshinone IIA, Nortanshinone, and (±)-Tanshindiol B. *J. Am. Chem. Soc.* **1989**, *111*, 1522–1524.
(20) Suslick, K. S.; Doktycz, S. J. The Sonochemistry of Zn Powder. *J. Am. Chem. Soc.* **1989**, *111*, 2342–2344.
(21) Suslick, K. S. Ultrasound in Synthesis. In *Modern Synthetic Methods 1986, Vol. 4*; Scheffold, R., Ed.; Springer-Verlag: Berlin and Heidelberg, 1989; pp 1–60.
(22) Boudjouk, P. Synthesis with Ultrasonic Waves. *J. Chem. Educ.* **1986**, *63*, 427–429.
(23) Suslick, K. S. Organometallic Sonochemistry. In *Advances in Organometallic Chemistry, Vol. 25*; Academic Press Inc.: New York, 1986; pp 73–119.
(24) Abdulla, R. F. Ultrasound in Organic Synthesis. *Aldrichimica Acta* **1988**, *21*, 31–42.
(25) Begley, M. J.; Puntambekar, S. G.; Wright, A. H. A Di-iron-Anthracene Complex via Ultrasonics. *J. Chem. Soc., Chem. Commun.* **1987**, 1251–1252.
(26) Suslick, K. S., Ed. *Ultrasound*; VCH: Weinheim, 1988.

Table I. Physical and Chemical Data for $^{99m}Tc(arene)_2^+$ Complexes

arene	% yield	HPLC data			octanol/buffer partition ratio
		t_r, min	column[a]	mobile phase[b]	
benzene	76	14.6	II	1	0.015
	90	10.2	II	2	
toluene	83	24.1	II	1	0.037
	89	13.4	II	2	
m-xylene	91	24.4	II	2	0.14
1,2,3-trimethylbenzene	83[c]	6.0	I	4	
1,2,4-trimethylbenzene	70[c]	6.0	I	4	
	89[c]	19.7	II	4	
	59[c]	25.5	II	3	
1,3,5-trimethylbenzene	49[c]	6.4	I	4	0.61
	82	15.9	II	4	
1,2,3,4-tetramethylbenzene	95[c]	28.0	II	4	
1,2,3,5-tetramethylbenzene	77	7.0	I	4	
	76[c]	27.8	II	4	
1,2,4,5-tetramethylbenzene	57[c]	28.2	II	4	1.97
pentamethylbenzene	68	11.9	I	4	5.0
hexamethylbenzene	50	20.8	I	4	8.6
1,2-diethylbenzene	60	12.1	I	4	
1,3-diethylbenzene	68	10.5	I	4	5.2
	83	32.4	II	4	
1,4-diethylbenzene	64	11.2	I	4	5.4
	59	34.0	II	4	
1-methyl-4-(2-propyl)benzene	79	26.2	II	4	
5-isopropyl-*m*-xylene	54	18.5	II	4	
1,3,5-triethylbenzene					19
1,3,5-tri-*tert*-butylbenzene					~23
hexaethylbenzene					>39
indan	95	14.1	II	4	
4,6-dimethylindan	30	8.9	I	4	
	33	~16	II	5	7.8
1,1,4,6-tetramethylindan	~5	~9.5	II	5	~78
1,1,4,6,7-pentamethylindan	<5	~9	II	5	~250
1,2,3,4-tetrahydronaphthalene	92	25.6	II	4	
1,2,3,4,5,6,7,8-octahydroanthracene	14	6.6	I	4	2.66
dodecahydrotriphenylene	6	22.6	I	4	30.9
s-indacene	12	13.6	I	4	13.4
trindan	55	7.2	I	4	2.4
tetrahydroacenaphthene	77	23.7	II	4	

[a] I = 250 × 4.1 mm, 10 μm, PRP-1; II = 300 × 7 mm, 10 μm, PRP-1. [b] Mobile phases contained KH_2PO_4 (2 mM) and were buffered to pH 3.5 with EtOH percentages of 15% (1), 25% (2), 33% (3), 38% (4), and 46% (5). [c] Synthesized by method 2 as explained in text. All others synthesized by method 3.

the organic solvent. Filtration through 0.2-μm Telfon filters separated any metal powder from the product. Aluminum ion was removed by HPLC. The HPLC conditions were kept acidic to prevent precipitation of $Al(OH)_3$. Initially, the benzene and hexamethylbenzene complexes of ^{99m}Tc were coeluted with the ^{99}Tc complexes in order to demonstrate equivalence. Other complexes were then identified by their retention times based on the expected lipophilic properties as related to the two standards. Figure 1 illustrates the OBPR as a function of the number of methyl groups on the benzene ring for derivatives containing only methyl groups. The partitioning of the complexes, which is related to lipophilicity, clearly is directly related to the amount of substitution on the ring. This relation holds for derivatives other than methyl and can serve as a guideline for estimating the HPLC retention time of the desired complex. In some cases, especially those of the more highly substituted ligands, the retention times of the products were unexpectedly low. The reason for this is not readily apparent, although it is possible that complexes with structures other than the bis(arene) formed.

Biodistribution Studies. Uptake in the myocardium is the most interesting property of these complexes. The percent dose/g or mL of tissue values for myocardium (whole heart) and other major tissues are reported in Table II. The myocardial uptake varies widely for the different complexes. Structure–distribution relationships can be discerned.

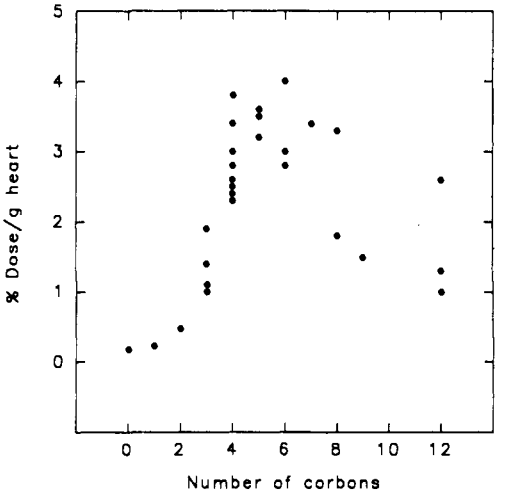

Figure 2. Heart uptake (whole heart) for $^{99m}Tc(arene)_2^+$ complexes as a function of the number of carbon atoms in the substituents on the ring.

Figure 2 illustrates the relation between heart uptake and the total number of carbon atoms in the substituents on the benzene ring. The data clearly illustrate the trend toward higher myocardial uptake with substitution increasing up to about four to six carbon atoms. As the number of carbon atoms rises above six, the heart uptake begins to fall. Lipophilicity in excess of the optimum for

Table II. Biodistribution Data for $^{99m}Tc(arene)_2^+$ Complexes

arene	% dose/g or mL of tissue at 5 min						
	heart	blood	liver	lung	kidney	muscle	brain
benzene	0.18	0.40	2.9	0.46	2.4	0.072	0.03
toluene	0.22	0.20	2.6	0.51	3.4	0.069	0.01
m-xylene	0.47	0.10	2.0	0.59	8.0	0.17	0.01
1,2,3-trimethylbenzene	1.0	0.13	1.0	0.66	9.0	0.27	N/A[a]
1,2,4-trimethylbenzene	1.4	0.38	0.80	0.78	7.0	0.32	N/A
1,3,5-trimethylbenzene	1.9	0.096	0.98	0.82	7.9	0.35	0.10
1,2,3,4-tetramethylbenzene	2.4	0.094	0.73	1.1	8.2	0.66	N/A
1,2,3,5-tetramethylbenzene	3.4	0.10	0.67	1.3	6.7	0.61	0.02
1,2,4,5-tetramethylbenzene	2.3	0.28	0.90	1.3	9.8	0.72	0.02
pentamethylbenzene	3.5	0.14	0.45	1.6	5.5	0.66	0.02
hexamethylbenzene	3.0	0.16	0.79	1.2	6.2	0.55	0.03
1,2-diethylbenzene	3.0	0.10	0.62	1.3	7.8	0.58	0.03
1,3-diethylbenzene	2.8	0.09	0.46	1.2	5.1	0.64	0.03
1,4-diethylbenzene	3.8	0.13	0.58	1.4	5.4	0.66	0.03
1-methyl-4-(2-propyl)benzene	2.5	0.16	0.42	1.1	5.0	0.56	N/A
5-isopropyl-*m*-xylene	3.2	0.18	0.38	1.3	6.5	0.70	N/A
1,3,5-triethylbenzene	2.8	0.24	1.4	1.1	7.3	0.63	0.04
1,3,5-tri-*tert*-butylbenzene	1.0	0.49	2.0	1.3	8.0	0.38	0.04
hexaethylbenzene	1.3	0.40	8.4	5.7	10.0	0.33	0.06
indan	1.1	0.077	1.7	0.98	11	0.23	N/A
4,6-dimethylindan	3.6	0.12	0.66	1.4	8.4	0.42	0.03
1,1,4,6-tetramethylindan	3.4	0.35	0.73	1.5	6.4	0.66	0.04
1,1,4,6,7-pentamethylindan	3.3	0.49	1.2	2.5	5.0	0.56	0.06
1,2,3,4-tetrahydronaphthalene	2.6	0.24	0.65	1.0	9.1	0.51	N/A
1,2,3,4,5,6,7,8-octahydroanthracene	1.8	0.16	0.79	0.9	9.1	0.48	0.02
dodecahydrotriphenylene	2.6	0.16	1.0	1.0	12	0.55	0.02
trindan	1.5	0.09	1.0	1.0	9.3	0.49	0.02
tetrahydroacenaphthene	4.0	0.23	0.45	1.4	6.0	0.82	N/A

[a] Not available.

Table III. Biodistribution Data as a Function of Time after Intravenous Administration for Bis(1,3,5-trimethylbenzene)[^{99m}Tc)technetium(1+)

tissue	5 min	45 min	90 min	180 min	360 min	1440 min
blood	2.67	1.24	1.02	0.67	0.52	0.13
kidneys	13.6	5.8	2.0	1.9	0.9	0.4
liver	10.4	3.1	3.6	1.9	0.8	0.3
muscle	30	26	25	30	29	25
lungs	1.15	0.86	0.60	0.48	0.34	0.20
heart	1.37	1.20	1.17	1.00	1.14	0.77
eye	0.041	0.028	0.044	0.016	0.019	0.010
bone	3.5	1.8	1.4	1.5	1.3	0.8
bone marrow	0.37	0.36	0.13	0.13	0.09	0.02
gonads	0.10	0.86	0.067	0.055	0.050	0.018
urine	N/A[a]	N/A	N/A	N/A	16	26
feces	N/A	N/A	N/A	N/A	0.4	36.2

[a] Not available.

myocardial uptake may be the cause for this. In fact, during these studies it was noted that for a given series of structurally related complexes, the myocardial uptake was maximal for the derivative with about four to six carbon atoms as substituents. For instance, the indan derivatives show maximal uptake for the dimethyl complex that contains five carbons versus only three for the parent and seven or eight for the more highly methylated derivatives. The ethyl-substituted benzenes show maximal myocardial uptake for the di- and triethyl derivatives, whereas a large decrease occurs for hexaethylbenzene and tri-*tert*-butylbenzene. In terms of OBPR, those complexes which have a value greater than about 2–3 generally show heart uptake in excess of 2.5%/g. The upper limit on OBPR was not clearly defined by this study, although a value of 9 seems to be consistent with the data. The highly annelated benzenes derived from naphthalene, anthracene, and indan show highly erratic heart uptake as a function of OBPR.

Nontarget tissue uptake is characterized by low blood values and relatively high kidney and liver values. For the very lipophilic complexes, high lung uptake is observed. This last observation parallels that for the hexakis(*tert*-butylisonitrile)technetium(I) cation, which also is known to be very lipophilic.[15]

Target to nontarget tissue ratios, which can be calculated from the data in Table II, reflect the excellent heart/blood ratio expected for compounds which have good myocardial uptake and low blood levels. In certain cases, the heart/liver ratio exceeds a value of 5.0, which is quite good when compared to other myocardial agents. Heart/lung ratios span a very tight range which approaches 3.0 as an upper limit.

The biodistribution data for various organs as a function of time are given in Tables III and IV for the 1,3,5-trimethylbenzene and the 1,2,3,5-tetramethylbenzene complexes, respectively. Note that the values in Tables III and IV are calculated for whole organs whereas those in Table II are on a per gram basis. The values for each compound are averages of data for male and female rats. The activity in the heart in both cases reaches its maximum within 45 min and slowly clears to about 50% of the maximum value over the course of 24 h. For the blood, kidneys, and liver, the compounds exhibit much more rapid clearance, falling to less than 10% of the maximum in 24 h. The activity in both cases is retained in muscle with little clearance over

Table IV. Biodistribution Data as a Function of Time after Intravenous Administration for Bis(1,2,3,5-trimethylbenzene)[^{99m}Tc]technetium(1+)

tissue	5 min	45 min	90 min	180 min	360 min	1440 min
blood	1.06	0.51	0.30	0.19	0.15	0.07
kidneys	7.1	5.4	3.9	2.0	1.5	0.2
liver	5.6	4.0	2.5	1.0	0.9	0.2
muscle	44	30	42	44	31	56
lungs	1.40	1.50	1.17	0.81	0.70	0.18
heart	2.51	2.83	2.78	2.44	2.81	1.27
eye	0.030	0.023	0.021	0.020	0.022	0.010
bone	3.8	2.6	3.5	2.2	1.4	1.7
bone marrow	0.43	0.24	0.28	0.20	0.11	0.10
gonads	0.099	0.089	0.074	0.58	0.039	0.019
urine	N/A[a]	N/A	N/A	N/A	11	20
feces	N/A	N/A	N/A	N/A	0.3	30.0

[a] Not available.

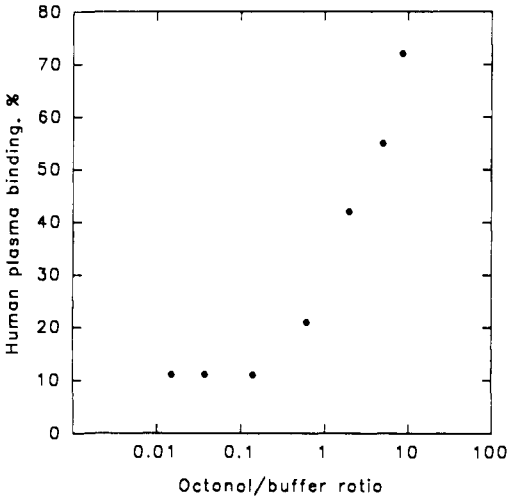

Figure 3. Binding to human plasma as a function of octanol/buffer partition ratio for ^{99m}Tc(arene)$_2^+$ complexes substituted with only methyl groups on the benzene.

24 h. These data indicate that the bis(arene) complexes have the desired properties for myocardial imaging.

Plasma Binding Studies. Figure 3 shows the relation of human plasma binding (%) to the log OBPR for the benzenes that have only methyl substituents. Table V contains rat and human plasma binding data for a number of complexes. For unsubstituted and mono- and dimethylbenzene, the plasma binding is nearly constant and relatively low. The maximal partition ratio for these complexes is 0.14. From this point, as the degree of methyl substitution is increases, the plasma binding increases. Although the compound derived from hexamethylbenzene is the most lipophilic of the simple methyl-substituted benzenes, the trend toward higher plasma binding with increasing OBPR is observed to continue as the number of carbons in the compound increases. For example, the tetra- and pentamethylindan and hexaethylbenzene complexes, with partition ratios of ~78, ~250, and >39, respectively, all have plasma binding which exceeds that of the hexamethylbenzene complex (94, 95, and 84, respectively, versus 72).

The plasma binding of the bis(arene) complexes is relatively low when compared to certain other technetium complexes, e.g., Tc(POM–POM)$_3^+$ and Tc[P(OMe)$_3$]$_6^{6+}$, that show heart uptake. This should be advantageous for myocardial imaging since the low blood values give rise to heart/blood values which consistently exceed 30.

Conclusions

The Tc(arene)$_2^+$ complexes represent another class of compounds that contains members with substantial myocardial uptake. Cationic complexes of this class have been synthesized in isomerically pure form by suppressing transalkylation through the use of ultrasound or Zn/HCl. Subsequent purification by HPLC provided solutions of ^{99m}Tc complexes suitable for biodistribution studies. Structure–distribution relationships could be discerned by varying the substituents on the benzene ring. Heart uptake was related to the lipophilicity of the complexes such that an intermediate range of lipophilicity results in optimal uptake. In rats, the target to nontarget tissue ratios showed excellent values and plasma binding of the complexes was relatively low when compared to several other classes of complexes known to have substantial heart uptake.

The properties of the ^{99m}Tc(arene)$_2^+$ complexes suggest that this class should contain members that are effective in imaging human myocardial tissue. Indeed, preliminary results indicate that the low plasma binding is also evident in human volunteers. However, for reasons that are as yet

Table V. Plasma Binding for ^{99m}Tc(arene)$_2^+$ Complexes

arene	plasma binding[a] rat	human
benzene	12	11
toluene	8.9	11
m-xylene	12	11
1,2,3-trimethylbenzene	6.6	24
1,2,4-trimethylbenzene	12	20
1,3,5-trimethylbenzene	11	21
1,2,3,4-tetramethylbenzene	22	46
1,2,3,5-tetramethylbenzene	21	36
1,2,4,5-tetramethylbenzene	18	42
pentamethylbenzene	57	55
hexamethylbenzene	57	72
1,2-diethylbenzene	21	84
1,3-diethylbenzene	19	64
1,4-diethylbenzene	16	60
1-methyl-4-(2-propyl)benzene	27	75
5-isopropyl-*m*-xylene	18	80
1,3,5-triethylbenzene	48	83
1,3,5-tri-*tert*-butylbenzene	60	81
hexaethylbenzene	50	84
indan	6.8	17
4,6-dimethylindan	54	57
1,1,4,6-tetramethylindan	75	94
1,1,4,6,7-pentamethylindan	81	95
1,2,3,4-tetrahydronaphthalene	5.8	51
1,2,3,4,5,6,7,8-octahydroanthracene	32	35
dodecahydrotriphenylene	34	84
trindan	23	27
tetrahydroacenaphthene	27	90

[a] In percent as explained in text. The number of determinations was one except for 1,2,4-trimethylbenzene, 1,2,3,4-tetramethylbenzene, pentamethylbenzene, 1,3-diethylbenzene, and 1,4-diethylbenzene (two); 1,3,5-trimethylbenzene (three); and 1,2,3,5-tetramethylbenzene (eight).

unclear, the complexes that have been tested in humans show less myocardial accumulation than in lower animal species.[27] However, the rigorously controlled synthesis conditions required for these compounds present great obstacles to their wide use in the clinic.

Experimental Section

General. Caution! ^{99}Tc is a low-energy β-emitter. ^{99m}Tc is a low-energy γ-emitter. All work with ^{99}Tc was carried out in a laboratory equipped for work with low levels of β-emitting isotopes. The long-lived isotope ^{99}Tc was handled in quantities below 50 mg to minimize exposure to bremsstrahlung. All manipulations were performed by personnel wearing gloves and labcoats to guard against contamination.

Syntheses requiring an inert atmosphere were performed with Schlenk apparatus or in an inert-atmosphere box filled with argon.

Sodium [^{99}Tc]pertechnetate was obtained by neutralization of a solution of ammonium pertechnetate (Oak Ridge, TN) with sodium hydroxide until evolution of ammonia ceased. The solid obtained after evaporation was recrystallized from water–ethanol. Sodium [^{99m}Tc]pertechnetate was obtained from a commercially available generator. Aluminum (-40 mesh), aluminum chloride (anhydrous), and zinc (dust) were stored in an inert-atmosphere box. The arenes were purchased commercially except as noted below and were purified by distillation from sodium–benzophenone for liquids and by sublimation for solids.

Fast atom bombardment mass spectra (FABMS) were obtained from Shrader Laboratories (Detroit, MI). High-pressure liquid chromatography (HPLC) was performed on Hewlett-Packard Model 1084B and Waters instruments equipped with Hamilton PRP-1 columns. Beckman Model 170 radiometric (^{99m}Tc) detectors and UV detectors operating at 254 nm (^{99}Tc) were used. Electrophoresis was carried out at 150 V for 30 min on Whatman 1MM chromatography paper wetted with 0.1 M phosphate buffer at pH ~7. Gas chromatograms were run on a Hewlett-Packard Model 5790A gas chromatograph.

Synthesis of ^{99}Tc Complexes. The complexes $(C_6H_6)_2{}^{99}Tc(PF_6)$ and $[C_6(CH_3)_6]_2{}^{99}Tc(PF_6)$ were prepared in milligram amounts by the literature method.[11,12] The white solid $(C_6H_6)_2{}^{99}Tc(PF_6)$ was analyzed by FABMS (calcd for $(C_6H_6)_2Tc$ 255, found 255 amu). The expected anionic signal for PF_6^- was observed. Complexes of 1,3,5-trimethylbenzene and 1,2,3,5-tetramethylbenzene with ^{99}Tc were obtained in microgram quantities by adding ^{99}Tc ($NaTcO_4$) to the ^{99m}Tc compounds prepared by method 1 as described below. They were purified by HPLC. High-resolution FABMS of these complexes gave 339.0947 for $[C_6(CH_3)_3H_3]_2{}^{99}Tc(PF_6)$ (calcd 339.0952 amu) and 367.1255 for $[C_6(CH_3)_4H_2]_2{}^{99}Tc(PF_6)$ (calcd 367.1255 amu).

Synthesis of ^{99m}Tc Complexes. The complexes $^{99m}Tc(arene)_2{}^+$ were synthesized by one of the four methods described below. In each case the eluate from a commercial generator was taken to dryness in a rotary evaporator. The resultant salt was treated twice with acetone. The acetone was removed by rotary evaporation after each treatment. The sodium [^{99m}Tc]pertechnetate was dissolved in acetone (sodium chloride dissolves to a very small extent) and placed in oven-dried (110 °C) serum vials (10 mL) which were stoppered with Teflon-coated stoppers and sealed with a closure. The acetone was evaporated, leaving no visible residue. However, the presence of ^{99m}Tc could be verified by checking with a β-γ meter (Geiger-Müller tube). The vials were filled with an atmosphere of argon by means of 18-gauge needles and a Schlenk line. By this procedure, vials containing dried, salt-free sodium [^{99m}Tc]pertechnetate under an argon atmosphere were produced. This product is referred to as "dried $NaTcO_4$".

Method 1. A vial of dried $NaTcO_4$ was opened in the inert-atmosphere box. A micro magnetic stir bar (1 × 3 mm), Al (~10 mg), and $AlCl_3$ (~10 mg) were added. The vial was stoppered and sealed as before and removed from the box. Using syringe techniques, cyclohexane (2 mL) and the appropriate arene (0.4 mL) were added through the closure. In the case of solid arenes, ~0.4 g were added in the box at the same time as the Al and $AlCl_3$. The vial was placed in an oil bath at 135 °C and heated with stirring for 60 min.

Method 2. A vial of dried $NaTcO_4$ was prepared as for method 1 except that Zn dust (~10 mg) was substituted for Al and $AlCl_3$. As a final step, the cyclohexane–arene mixture was purged with HCl gas (1 atm) for 1 min, effectively replacing the argon atmosphere with HCl. The vial was placed in an oil bath and reacted as above.

Method 3. A vial of dried $NaTcO_4$ was prepared as in method 1 with the exception that the stir bar was not added. The vial was placed in a commercially available ultrasonic bath (Bransonic 2200) and irradiated for 60 min. During this time, the temperature of the bath typically rose to 40–45 °C.

Method 4. A vial of dried $NaTcO_4$ was prepared as in method 2 with the exception that Al powder was substituted for Zn dust. The cyclohexane–arene mixture was purged with HCl gas (1 atm) for 1 min. The vial was placed in a commercially available ultrasonic bath and irradiated for 60 min, during which time the temperature typically rose to 40–45 °C.

Isolation and Purification of Complexes. The cooled vials from any of the above procedures were treated identically to obtain the air-stable $^{99m}Tc(arene)_2{}^+$ complexes. The vial was opened, H_2O (1 mL) was added, and the mixture was shaken vigorously for ~15 s. The aqueous layer was aspirated and filtered through a 0.2-μm Teflon filter. The organic layer was extracted with a second portion of H_2O (1 mL), the aqueous layer was treated as before, and the filtrates were combined. The total yield of species containing ^{99m}Tc that was isolated by this procedure varied widely from day to day and from arene to arene, approaching 80% on the high end. Sonication of the organic/aqueous mixture improved the recovery in some cases.

The aqueous filtrate from the above procedure was analyzed and purified by HPLC as necessary. Table I lists the physical and chemical characterization data of the complexes. The mobile phase consisted of ethanol–water mixtures containing 2 mM KH_2PO_4. The pH was adjusted to 3.5 by the addition of 85% H_3PO_4. A fraction collector was used to collect the products associated with the γ-emitting peaks. The $^{99m}Tc(arene)_2{}^+$ complexes were identified by electrophoretic behavior consistent with the cationic formulation and were collected at retention times consistent with the expected lipophilic properties compared with those of the benzene, tetramethylbenzene, and hexamethylbenzene standards containing ^{99}Tc.

Octanol/Buffer Partition Ratios (OBPR). The OBPR were measured on the solutions used for biodistribution studies (see below). The values for several complexes are listed in Table I. A sample of the solution of the bis(arene) complex (0.1 mL) was diluted to 1 mL with a 0.2 M phosphate buffer at pH 7. *n*-Octanol (1 mL) was added. The mixture was stirred on a vortexer for 3 min and then centrifuged for 3 min. An aliquot (0.1 mL) of each layer was assayed by γ-counting. The aliquot with the highest activity was diluted to 1 mL with the corresponding solvent and mixed with 1 mL of the opposing solvent. The extraction procedure was repeated a second and third time. The ratio was calculated as the counts in the *n*-octanol layer divided by the counts in the buffer layer averaged over at least the last two determinations. In some cases the first determination was discarded if it varied too greatly from the second.

Biodistribution Studies. The myocardial uptake of the complexes was evaluated in female Sprague–Dawley rats (150–200 g). Each bis(arene) complex was dissolved in normal saline to give an activity of ~12.5 μCi/mL. This solution was injected through a lateral tail vein at a dose of ~25 μCi/kg. Five minutes after injection the rats were killed by cervical dislocation. Tissue samples were obtained, washed with saline, blotted dry, weighed, and counted by standard γ-counting techniques to obtain the percent injected dose/g tissue values.

Plasma Binding Studies. Plasma protein binding of test substances was measured in rat plasma samples collected during the rat biodistribution. Human plasma binding was measured in 1.0 mL of heparinized plasma samples (fresh plasma obtained daily) to which 0.02 mL of the test substances had been added. Sephadex columns were prepared in advance by slurrying Sephadex G-25-80 in saline for at least 3 h. The slurry was then poured into 3-mL syringe barrels containing small cotton pledgets.

(27) Nosco, D. L.; Wester, D. W.; Fazio, F.; Verbruggen, A.; DeRoo, M. Initial Human Experience with Four Potential Tc-99m Cationic Myocardial Perfusion Imaging Agents. *J. Nucl. Med.* **1990**, *31*, 907 (Abstract No. 860).

The excess saline was expelled from the columns by centrifuging in an IEC Model CL benchtop centrifuge at speed settings of 3 and 5 for 120 ± 10 s each (800*g* and 1300*g*, respectively) for collection of eluate. After incubation for about 15 min, duplicate plasma samples were transferred to the Sephadex desalting columns. The columns were centrifuged so that the eluate was collected in standard counting tubes. The column was removed from the counting tube. The Sephadex gel was expelled by air into a separate counting tube. The eluate and the Sephadex gel were placed adjacently in the track of the γ-counter and their radioactivity assayed for 1 min or a σ error of 1%. The percent of bound plasma protein was calculated by the following formula:

$$\% \text{ bound protein} = \frac{\text{net cpm eluate}}{\text{net cpm (eluate + gel)}} \times 100$$

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Synthesis and Evaluation of a Series of 3,5-Disubstituted Benzisoxazole-4,7-diones. Potent Radiosensitizers in Vitro

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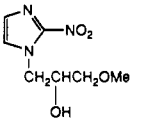
A series of 3,5-disubstituted-2,1-benzisoxazole-4,7-diones was synthesized and evaluated as radiosensitizers both in vitro and in vivo. These compounds were designed as non-nitro electron-affinic agents in an effort to alleviate some of the toxicities seen with the 2-nitroimidazole radiosensitizers evaluated in the clinic. Several compounds in this series were potent radiosensitizers in vitro, with sensitizer enhancement ratios of 2.0–2.3 at concentrations <0.5 mM. Compounds with potent in vitro activity were also evaluated in vivo. However, none of these compounds showed radiosensitizing activity in vivo. The reduction potentials of these compounds were determined by cyclic voltammetry and compared to other electron-affinic radiosensitizers. In general, the reduction potentials of this series of compounds was slightly more positive than the 2-nitroimidazoles, but they fell within the range postulated as acceptable to yield in vivo activity. The results suggest that factors other than reduction potential may be responsible for the lack of in vivo radiosensitizing activity observed for this class of radiosensitizers.

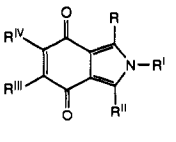
Introduction

In addition to the large number of chemical agents available for the treatment of human cancer, radiation therapy continues to be an important method for the local control of many types of tumors.[1,2] Several approaches for increasing the therapeutic benefit of radiation treatment have been examined.[3] One such approach has been the use of chemical agents which mimic the effects of oxygen.[1-3] Since oxygen mediates the lethal effects of radiation in cells, tumor cells in areas of diminished oxygen supply are radioresistant.[4] Therefore, "oxygen mimetics" have been investigated as a means to overcome this resistance.[5] Such compounds were designed to penetrate into tumors and react with radiation-produced free radicals to generate chemical species toxic to cells.[6,7] These so called "electron affinic agents" contain a reducible functionality and studies have shown that the ability of these compounds to act as radiosensitizers was related to their reduction potential.[8] If the reduction potential was too positive, the compound would be inactivated metabolically prior to reaching the tumor. If too negative, the compound would be unreactive and no sensitization would occur. It was demonstrated that nitroheterocycles with in vivo activity usually had one electron reduction potentials ($E^{1/7}$ values) in the range of −0.30 to −0.40 eV.[9]

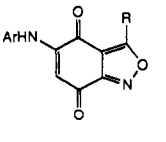
A variety of nitroheterocycles and quinones have been shown to sensitize cells to radiation in vitro by this mechanism. However, to date, only nitroimidazoles have displayed in vivo activity sufficient to conduct clinical trials.[1-3] One of the most widely studied nitroimidazoles has been misonidazole (1). Although effective as a ra-



1, Misonidazole



2, Isoindole-4,7-diones



3, Benzisoxazole-4,7-diones

(1) Bartelink, H.; Bleehen, N. M.; Phillips, T. L. Chemical Modifiers. *Int. J. Radiat. Oncol. Biol. Phys.* **1988**, *14*, 1988.
(2) Coleman, C. N.; Bump, E. A.; Kramer, R. A. Chemical Modifiers fo Cancer Treatment. *J. Clin. Oncol.* **1988**, *6*, 709–733.
(3) Nori, D.; Kim, J. H.; Hilaris, B.; Chu, F. C. Radiosensitizers and Protectors. In *Radiation Therapy of Gynecological Cancer*. Nori, D., Hilaris, B., Eds.; Alan R. Liss, Inc.: New York, 1987; pp 319–329.
(4) Revesz, L.; Palcic, B. Radiation Dose Dependence of the Sensitization by Oxygen and Oxygen Mimic Sensitizers. *Acta Radiol.* **1985**, *24*, 209–217.
(5) Adams, G. E.; Denekamp, J.; Fowler, J. F. Biological Basis of Radiosensitization by Hypoxic-Cell Radiosensitizers in Hypoxic Cell Radiosensitizers. *Chemotherapy* **1976**, *7*, 187–206.
(6) Wardman, P. The Mechanism of Radiosensitization by Electron-Affinic Compounds. *Radiat. Phys. Chem.* **1987**, *30*, 423–432.
(7) Knox, R. J.; Edwards, D. I.; Knight, R. C. The Mechanism of Nitroimidazole Damage to DNA: Coulometric Evidence. *Int. J. Radiat. Oncol. Biol. Phys.* **1984**, *10*, 1315–1318.
(8) Fowler, J. F.; Adams, G. E.; Denekamp, J. Radiosensitizers of Hypoxic Cells in Solid Tumors. *Cancer Treat. Rev.* **1976**, *3*, 227–256.
(9) Adams, G. E.; Flockhart, I. R.; Smithen, C. E.; Stratford, I. J.; Wardman, P.; Watts, M. E. Electron-Affinic Sensitization VII. A Correlation Between Structures, One-Electron Reduction Potentials, and Efficiencies of Nitroimidazoles as Hypoxic Cell Radiosensitizers. *Radiat. Res.* **1976**, *67*, 9–20. (b) Adams, G. E.; Stratford, I. J. Hypoxia Mediated Nitro-heterocyclic Drugs in Radio- and Chemotherapy of Cancer. An Overview. *Biochem. Pharm.* **1986**, *35*, 71–76.

0022-2623/91/1834-3290$02.50/0