carboxy groups of a *m*-dioxamic acid. By displacing the acidic protons, E_1 and E_2 provide a strong ionic component to the receptor-binding energy. These receptor features are considered to underlie the tremendous enhancement in PCA activity represented by factors δ_3–δ_5. Differences in activity among the aa, sa, and ss structures may be explained by the positions of the carboxy oxygen atoms relative to E_1 and E_2 in the complexes of the three conformers. Receptor moieties, A_1 and A_2, with electron-accepting capacity are located near the positions occupied by C(3) and C(5) of the aromatic ring. A rationale for the role of δ_1 and δ_8 is provided by the existence of A_1 and A_2, respectively. If the drug molecule contains atoms with lone-pair electrons in juxtaposition to these entities, the stability of the complex will be increased through the resultant donor–acceptor interactions.

Conclusion

In an effort to discover which structural features affect the activity of drugs in the rat PCA assay, an investigation has been carried out involving a series of 51 molecules from several classes of compounds. The theoretical model employed in the study takes into account the following factors: (1) charge-transfer interactions between the receptor and the essential pharmacophore of the drug, where the latter may possess a manifold of acceptor orbitals; (2) effects of specific modifications in drug structure; (3) entropy contributions due to molecular size, shape, and symmetry; and (4) the distribution of bound drug among different conformational states of the molecule. These considerations led to the development of a nonlinear regression equation relating the biological activity index, $A = -\ln ED_{50}$, to a variety of calculable structural indexes. The ab initio SCF–MO molecular fragment method was employed to determine the electronic and geometric properties required by the theoretical model. The statistical findings lend support to the model and establish the importance of nine structural features for activity in the PCA assay. The magnitude of A_{calcd} for 50 drugs proved to be the same as A_{obsd} within the 95% confidence limits established by the experimental error. The existence of the single low-activity outlier, molecule **48**, suggests that bulky substituents could be used as probes to explore the extent of the binding site. Finally, inferences have been made regarding the cause of certain substituent effects based upon calculated indicators of reactivity, such as the density distribution in the higher filled molecular orbitals. From these inferences, a schematic receptor map has been created that may prove useful in designing further studies of this system.

Acknowledgment. The synthesis and biological testing of the compounds discussed in this work were carried out under the auspices of Drs. W. J. Wechter, J. B. Wright, C. M. Hall, and H. G. Johnson. Structural data for the oxamic acids were based upon the X-ray crystallographic results for *N,N'*-(*m*-phenylene)dioxamic acid and *N,N'*-(2-chloro-5-cyano-*m*-phenylene)dioxamic acid obtained by Dr. D. J. Duchamp. Assistance with the statistical computations was provided by Dr. G. L. Schooley. The authors express their appreciation for these contributions.

Registry No. **1**, 500-72-1; **2**, 58446-15-4; **3**, 61068-77-7; **4**, 58446-13-2; **5**, 58446-17-6; **6**, 58446-11-0; **7**, 58446-19-8; **8**, 67283-71-0; **9**, 52930-06-0; **10**, 52980-10-6; **11**, 13593-94-7; **12**, 67283-72-1; **13**, 30095-78-4; **14**, 101-09-7; **15**, 72269-26-2; **16**, 84944-23-0; **17**, 58446-09-6; **18**, 52979-88-1; **19**, 53882-08-9; **20**, 67451-36-9; **21**, 53882-32-9; **22**, 84944-24-1; **23**, 58763-12-5; **24**, 53882-12-5; **25**, 49635-52-1; **26**, 49635-47-4; **27**, 63920-88-7; **28**, 25201-04-1; **29**, 53882-30-7; **30**, 53882-14-7; **31**, 53882-05-6; **32**, 53882-10-3; **33**, 79808-23-4; **34**, 60494-53-3; **35**, 56216-25-2; **36**, 60494-55-5; **37**, 60722-37-4; **38**, 60722-36-3; **39**, 60722-35-2; **40**, 67451-34-7; **41**, 67451-33-6; **42**, 53882-17-0; **43**, 84944-25-2; **44**, 53882-22-7; **45**, 84944-26-3; **46**, 54046-97-8; **47**, 84944-27-4; **48**, 84944-28-5; **49**, 84944-29-6; **50**, 84944-30-9; **51**, 84944-31-0.

Supplementary Material Available: Geometrical data; variation of E, ϵ_u*, and c_{au}' with rotation of certain substituents (Table II); and coefficients in the cosine expansion (eq 16) used to fit calculated values of $E(l, \theta)$ for molecules with rotating substitutents (Table III) (9 pages). Ordering information is given on any current masthead page.

Synthesis and Biological Distribution of Radiolabeled Ammineruthenium(III)–Amino Acid Complexes as Potential Pancreatic Imaging Agents

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Complexes of ammine[^{103}Ru]ruthenium(III) with L-histidine, β-(4-pyridyl)-α-alanine, and *S*-[β-(4-pyridyl)ethyl]-L-cysteine were synthesized in low specific activity and evaluated in mice as potential radiodiagnostic agents for pancreatic imaging. The biological distribution of each complex was determined in normal mice at 15 min, 1 h, and 2 h following intravenous administration. All four complexes were rapidly cleared through the kidneys, with 50% of the injected dose concentrated in the urine within 15 min. None of the complexes showed a tendency to accumulate in any major organ. Major differences in distribution were found in lungs, heart, spleen, stomach, intestine, bone, and soft tissues. A significant relative difference in pancreatic uptake was observed. Only the β-(4-pyridyl)-α-alanine complex exhibited pancreas to liver ratios significantly greater than 1. The pancreas to liver ratio of 17 was reached 1 h following injection of this ruthenium complex, which is considerably higher than commonly reported values of 2.5 for [^{75}Se]selenomethionine. The β-(4-pyridyl)-α-alanine complex is therefore a promising candidate for evaluation as a pancreatic imaging agent when labeled with cyclotron-produced ruthenium-97.

The rapid protein turnover in the pancreas relative to other organs formed the basis for the development of L-[^{75}Se]selenomethionine as a pancreatic imaging agent.[1-5] However, this radiopharmaceutical is far from ideal due

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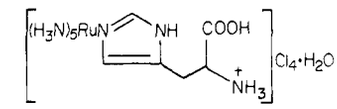
0022-2623/83/1826-0737$01.50/0

to concomitant high liver uptake, suboptimal and variable pancreatic specificity, and a long effective half-life resulting from a physical half-life of 120 days for ^{75}Se and a biological half-life of >200 days for 40% of the injected dose.[6] These adverse physical and biological characteristics severely limit the levels of activity that can be administered to patients, leading to poor counting statistics and low-quality images.[7] The numerous anatomical variants of the shape of the normal pancreas make interpretation of such poor contrast images particularly difficult and further contribute to the 30% false positive rate presently associated with diagnosis of pancreatic disease by L-[^{75}Se]-selenomethionine radionuclide scintigraphy.[8] With the exception of some Third World and emerging countries, the technique has been almost totally replaced by ultrasonography and computed body tomography.[9]

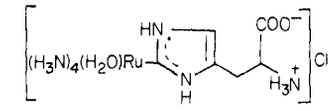
Reports that ^{14}C- or ^{11}C-labeled lysine, valine, and aromatic amino acids exhibit pancreas to liver uptake ratios considerably higher than selenomethionine[2,10-13] have led to an extensive synthesis program in our laboratories aimed at improving image contrast through development of new radiopharmaceuticals based on ^{75}Se-labeled analogues of amino acids having greater specificity for the pancreas.[14,15]

We are currently interested in investigating the utility of other γ-emitting radionuclides for pancreatic imaging. Ruthenium-97, a pure γ-emitting isotope (216 keV, 88%), with a half-life of 2.9 days, is inherently superior to ^{75}Se as a radionuclide for nuclear medicine applications. In addition, the varied organometallic chemistry of this group 8 transition metal makes it possible to exploit known biochemistry in the rational design of potential ^{97}Ru radiopharmaceuticals.[16-21]

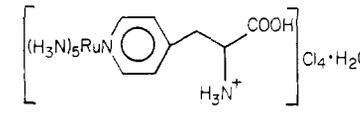
Available literature methods for synthesizing ammineruthenium(III) complexes of histidine[22] and para-substituted pyridines[23,24] raised the possibility of exploring these complexes of ^{97}Ru as potential pancreatic imaging agents. Since natural aromatic amino acids show high pancreatic uptake in several species,[25-28] the ammineruthenium complexes of histidyl- and pyridyl-amino acid ligands may also retain their pancreatic specificity. To test this hypothesis, we employed the more readily available ^{103}Ru isotope for evaluation of potential ^{97}Ru radiopharmaceuticals. Ruthenium-103 does not have good physical properties (β, α emitter, $t_{1/2}$ = 39.5 days; 497 keV) but is valuable as a radiolabel for developmental work. We have prepared ^{103}Ru analogues of known histidine complexes 1 and 2 and previously unreported pyridyl-amino acid complexes 3 and 4. The synthesis (Scheme I) and biological distribution of complexes 1-4 are reported below and in Table I.



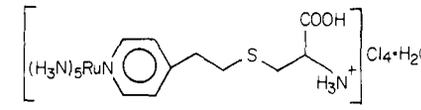
1



2



3



4

Chemistry. Chloropentaammineruthenium(III) dichloride (6) was prepared from ruthenium trichloride (5) by a published method.[29] Zinc amalgam reduction of complex 6 in aqueous solution under argon produced an air-sensitive solution of pentaammineaquoruthenium(II), complex 7, which was further reacted with ligands 9-11 by a slight modification of a literature procedure[22] to yield aromatic amino acid complexes 1-4. The complexes were separated by ion-exchange column chromatography, isolated by crystallization, and characterized by infrared (IR) and ultraviolet (UV) spectroscopy and elemental analyses.

Reaction of histidine (9) with ruthenium complex 6 afforded solid complexes 1 and 2 exhibiting UV/vis absorption spectral properties identical with those reported in the literature.[22] However, the solid-state IR spectrum

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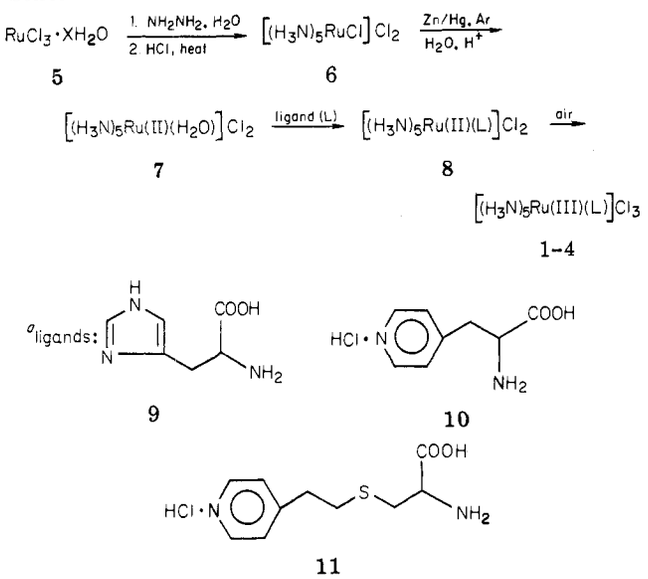
Scheme I[a]



9 10 11

of 1, not reported by previous workers, showed strong absorbances at 1725 and 1205 cm^{-1} due to C=O and C—O stretching frequencies of a protonated carboxy group. Complex 1 analyzed as a hydrochloride monohydrate, contrary to literature data[22] for a zwitterionic histidine monohydrate complex. Complex **2** had no IR absorptions corresponding to a protonated carboxy group and analyzed as a simple 2-histidinyl adduct.

Ruthenium(II) complexes **7** and **8** were formed by reaction of pentaammine complex **6** with either β-(4-pyridyl)-α-alanine (**10**) or *S*-[β-(4-pyridyl)ethyl]cysteine (**11**). Both **7** and **8** were air and light sensitive and were kept covered with aluminum foil. Acidification, followed by air oxidation and isolation, afforded **3** and **4** as crystalline solids. Elemental analysis confirmed the formation of amino acids **3** and **4** as hydrochloride monohydrates. Coordination of ruthenium to the pyridine nitrogen rather than the α-amino group was assigned on the basis of spectral evidence and known ruthenium chemistry. A strong metal to ligand charge transfer band in the visible region (λ_{max} 413 and 410 nm, respectively) for ruthenium(II) complexes of **3** and **4** was absent after oxidation to the ruthenium(III) state. Several intense UV bands at λ_{max} 268, 260, and 253 nm observed for **3** and **4** were closely analogous to those reported at λ_{max} 261, 255, and 248 nm for pentaammine(pyridyl)ruthenium(III) trichloride.[24] These bands are considerably different from the bands at λ_{max} 320 and 275 nm reported for hexaammineruthenium(III),[23] thus providing clear evidence favoring coordination of the ruthenium with the ring nitrogen rather than the α-amino nitrogen in **3** and **4**. Infrared bands for **3** and **4** at 1760 and 1180 cm^{-1}, characteristic of a free carboxylic acid moiety, precluded coordination of ruthenium to the carboxy group of the amino acid. Since pyridine is known to exhibit a higher affinity than dimethyl sulfide for pentaammineruthenium(III),[30] the cysteine complex **4** is also best formulated as an *N*-pyridine-bound complex.

Tissue Distribution. The biodistribution of ^{103}Ru complexes of **1–4** in mice and the pancreas to liver ratios at various time intervals are listed in Tables I–III. For all four compounds, the concentration of the radioactivity was similar at early time intervals. All were rapidly cleared through the kidneys; up to 50% of the injected dose concentrated in the urine within 15-min postinjection. Highest uptake [percent injected dose (% ID) per organ] was found in bone, blood, and soft tissue. None of the complexes showed a tendency to concentrate in the major organs, which collectively accounted for only 4–8% of the injected dose after 15 min and steadily declined thereafter. Histidine complex **2**, in which the tetraammineaquoruthenium(III) is bound to C-2 of the imidazole ring system, showed significantly higher initial lung uptake than the other compounds and exhibited the slowest rate of clearance from lung, heart, bone, and soft tissue. After 1 h, comparatively low levels of **4** in spleen, heart, stomach, large intestine, and muscle indicated that **4** was more rapidly eliminated. Overall, the pyridine complexes **3** and **4** tended to clear slightly faster than did the histidine complexes **1** and **2**.

The most significant difference between the four complexes was the pancreas to liver concentration ratio. In order to fully evaluate the potential of amino acids **1–4** as radiodiagnostic agents for pancreatic imaging, uptake by pancreas and liver was also determined as percent injected dose per gram of tissue (% ID/g). As shown in Table III, the β-(4-pyridyl)-α-alanine complex **3** was concentrated to a greater extent in the pancreas at all time periods. Within experimental error, values determined for pancreas uptake of **1**, **2**, and **4** were equivalent. Liver uptake was approximately 1% ID/g in all cases, but pyridine complexes **3** and **4** were more rapidly cleared. The higher pancreatic uptake of **3**, combined with more rapid liver clearance, resulted in uniquely high target to nontarget ratios for **3**. The initial pancreas to liver concentration ratio for **3** of 7.4 increased to an average value of 17 over the course of an hour, but the other three complexes consistently exhibited pancreas to liver ratios close to 1. No significant further increase in the ratio for **3** was observed during the 2nd h, and after 24 h, the ratio was found to have fallen to 4.42 ± 1.04. The most favorable combination of pancreatic uptake and target to nontarget ratio occurred between 30 and 60 min following injection.

Discussion

Practical considerations governed the selection of pentaammineruthenium(III) complexes for initial research into tissue-specific ^{97}Ru radiopharmaceuticals. Key among these was the known synthetic chemistry of ammineruthenium(III)–histidine and –pyridine complexes[22-24] prepared from readily available ruthenium trichloride (**5**) according to the reaction sequence shown in Scheme I. Since the only intermediate that must be isolated and purified in the procedure is chloropentaammineruthenium(III) chloride (**6**), the method provides a simple two-step procedure for radioactive synthesis. Established chemical and physical properties of known pentaammineruthenium(III) complexes also make this class of compounds appealing to apply in biological studies. In addition, these complexes are readily purified by ion-exchange chromatography and isolated as crystalline solids, which are highly water soluble, stable at neutral to acidic pH, and inert to further substitution at the metal center.[31] This latter property is particularly important in the rational design of ^{97}Ru radiopharmaceuticals, for it suggests that the heterocyclic ligand will remain coordinated in vivo and be used to predictably target ^{97}Ru. The amount of isomerization from **1** to **2** is first order with respect to pH. Sundberg and Gupta[22] obtained nearly equal amounts of the two complexes using 0.075 M HCl, whereas the ratio of complexes **2** to **1** in our study was 4:1 using 0.02 M HCl. Our results are consistent with those recently reported by Tweedle and Taube.[32] Tweedle and Taube also reported

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Table I. Percent Injected Dose per Tissue of Ruthenium-103 Labeled Ammineruthenium(III)-Amino Acid Complexes in Mice 15 min, 1 h, and 2 h Following Intravenous Administration[a]

	1			2			3			4		
tissue	15 min (n = 4)	1 h (n = 4)	2 h (n = 2)	15 min (n = 2)	1 h (n = 2)	2 h (n = 2)	15 min (n = 5)	1 h (n = 5)	2 h (n = 2)	15 min (n = 2)	1 h (n = 2)	2 h (n = 2)
pancreas	0.10 ± 0.08	0.09 ± 0.05	0.14 ± 0.06	0.27 ± 0.18	0.14 ± 0.08	0.09 ± 0.06	0.48 ± 0.10	0.37 ± 0.12	0.23 ± 0.01	0.26 ± 0.06	0.04 ± 0.02	0.07 ± 0.01
liver	1.20 ± 0.50	0.74 ± 0.28	0.72 ± 0.04	1.60 ± 0.20	1.10 ± 0.20	0.96 ± 0.29	1.70 ± 0.30	0.47 ± 0.22	0.10 ± 0.05	2.20 ± 0.20	0.55 ± 0.09	0.40 ± 0.08
spleen	0.09 ± 0.06	0.06 ± 0.03	0.04 ± 0.02	0.18 ± 0.10	0.16 ± 0.06	0.05 ± 0.06	0.12 ± 0.05	0.08 ± 0.06	0	0.14 ± 0.01	0.02 ± 0.02	0.05 ± 0.03
lung	0.39 ± 0.06	0.18 ± 0.05	0.18 ± 0.01	1.10 ± 0.10	0.64 ± 0.06	0.44 ± 0.06	0.48 ± 0.13	0.16 ± 0.11	0.04 ± 0.01	0.78 ± 0.01	0.10 ± 0.03	0.06 ± 0.02
heart	0.20 ± 0.08	0.07 ± 0.04	0.02 ± 0.02	0.32 ± 0.02	0.18 ± 0.02	0.11 ± 0.06	0.16 ± 0.06	0.04 ± 0.03	0	0.16 ± 0.03	0.04 ± 0.01	0.06 ± 0.01
stomach	0.27 ± 0.07	0.21 ± 0.08	0.24 ± 0.06	0.52 ± 0.07	0.26 ± 0.04	0.30 ± 0.19	0.51 ± 0.23	0.24 ± 0.10	0.08 ± 0.04	0.31 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
kidney	2.60 ± 0.40	2.20 ± 0.80	2.80 ± 0.30	2.50 ± 0.30	1.80 ± 0.10	1.50 ± 0.09	1.10 ± 0.90	1.50 ± 0.80	0.62 ± 0.07	3.80 ± 0.60	2.00 ± 0.20	3.00 ± 0.06
sm intest	1.60 ± 0.30	0.96 ± 0.27	0.91 ± 0.08	1.20 ± 0.10	1.10 ± 0.10	1.10 ± 0.60	2.70 ± 0.80	1.80 ± 0.80	0.64 ± 0.02	1.50 ± 0.10	0.46 ± 0.01	0.24 ± 0.06
lg intest	0.89 ± 0.37	0.40 ± 0.13	0.55 ± 0.09	1.00 ± 0.50	0.50 ± 0.01	1.00 ± 0.40	0.91 ± 0.43	0.69 ± 0.42	0.76 ± 0.31	0.50 ± 0.03	0.26 ± 0.15	0.51 ± 0.18
muscle	8.30 ± 3.20	6.40 ± 5.30	4.50 ± 0.60	13.00 ± 8.00	7.60 ± 0.30	9.00 ± 2.70	11.00 ± 4.00	2.40 ± 0.90	1.00 ± 0.40	21.00 ± 8.00	1.80 ± 0.50	4.30 ± 2.00
fat and fur	8.20 ± 1.20	3.70 ± 1.50	1.80 ± 0.10	16.00 ± 2.00	10.00 ± 2.00	7.20 ± 3.30	12.00 ± 6.00	2.60 ± 1.30	0.87 ± 0.76	18.00 ± 4.00	3.10 ± 0.30	2.50 ± 0.10
bone	3.10 ± 1.20	3.00 ± 1.50	0.69 ± 0.38	13.00 ± 2.00	4.10 ± 3.30	5.60 ± 4.40	5.70 ± 2.10	1.60 ± 0.80	0	7.70 ± 2.00	2.00 ± 1.10	3.60 ± 3.00
blood	2.60 ± 0.50	1.20 ± 0.40	0.54 ± 0.05	3.80 ± 0.70	3.90 ± 1.50	1.80 ± 0.70	3.30 ± 1.30	0.43 ± 0.34	0	5.60 ± 3.80	2.30 ± 1.70	0.94 ± 0.30
urine	55.00 ± 7.00			29.00 ± 18.00			47.00 ± 9.00			40.00 ± 6.00		

[a] Mean plus or minus standard deviation for n mice.

that the coordinated water in *trans*-tetraammineaquo(2-imidazolium)ruthenium(III) could be readily substituted by NCS^-. This trans labilizing effect is not significant for our studies, since the authors reported the affinity of the compound for nitrogen heterocycles and halides to be too low to measure.

Complexes 1–4 illustrate three conceptually different approaches to designing ^{97}Ru radiopharmaceuticals based on the well-known requirement of the pancreas for exogenous amino acids. Complexes 1 and 2 are examples of the direct attachment of a heterocyclic amino acid to ruthenium, while complex 4 may be viewed as a derivative of methionine, made functional with a strongly coordinating pyridine ligand. Complex 3 illustrates the possibility of preparing an analogue of the naturally occurring compound in which a benzene ring is replaced by pyridine in order to allow, in this instance, direct attachment of a phenylalanine analogue to ruthenium.

Results obtained in this investigation establish the feasibility of developing tissue-specific radiopharmaceuticals based on ammineruthenium(III) complexes of small, biologically active compounds. Although pentaammineruthenium(III) complexes of β-(4-pyridyl)-α-alanine and *S*-[β-(4-pyridyl)ethyl]cysteine had not been reported previously, a slight modification of reaction conditions[22–24] employed in the synthesis of other pentaammineruthenium(III) complexes did indeed provide practical radioactive syntheses.

Pancreatic uptake for all four complexes was markedly lower than that of [^{75}Se]selenomethionine. However, this finding is not surprising in view of the rapid urinary clearance associated with high ionic charge and hydrophilicity. However, the pancreas to liver ratio of 17 ± 5 found 1 h after intravenous administration of **3** is considerably higher than the values of about 2–3 commonly reported for [^{75}Se]selenomethionine. This significantly greater ratio was unexpected and indicates the importance of considering how the structural and chemical properties of small, biologically active compounds are likely to be altered by attachment to a pentaammineruthenium(III) moiety.

Radiolabeled methionine and histidine are known to have pancreas to liver uptake ratios close to 3,[2] while considerably higher values of 8.7 and 5.6, respectively, have been found for tyrosine and phenylalanine. On this basis alone, compound **3** is expected to exhibit a higher target to nontarget ratio. However, the magnitude of the difference and the ratios of about 1 for compounds 1, 2, and 4 remain unexplained. It seems more likely that the enhanced relative pancreatic uptake of **3** is largely due to the fact that this complex bears a much closer structural and chemical resemblance to tyrosine than complexes 1, 2, and 4 have to any naturally occurring amino acid. Direct attachment of pentaammineruthenium(III) to histidine not only represents a gross perturbation in structure, but also significantly affects the chemical properties of the coordinated imidazole ring.[22] In compound **4**, the incorporation of ruthenium requires the substitution of a large hydrophilic group for the small lipophilic methyl group. We are currently investigating the possibility that indirect attachment, as in **4**, could be successfully employed to introduce ^{97}Ru in cases where the ruthenium moiety replaces an already existing hydrophilic group in the native molecule. In cases such as **3**, where direct attachment of the ruthenium moiety to an organic molecule produces a complex that is fundamentally similar in structural fea-

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Table II. Pancreas Uptake of Ruthenium-103 Labeled Ammineruthenium(III)-Amino Acid Complexes 1-4 in Mice[a]

	% ID/g			
	1 (n = 3)	2 (n = 2)	3 (n = 5)	4 (n = 2)
15 min	0.58 ± 0.26	2.30 ± 1.10	4.00 ± 2.00	0.94 ± 0.24
30 min	0.31 ± 0.16	0.60 ± 0.01	2.50 ± 0.90	0.45 ± 0.13
1 h	0.58 ± 0.35	0.94 ± 0.42	2.60 ± 0.20	0.16 ± 0.07
2 h	0.76 ± 0.32	0.54 ± 0.41	1.50 ± 0.10	0.26 ± 0.04

[a] Mean plus or minus standard deviation for n mice.

Table III. Pancreas to Liver Concentration Ratios of Ruthenium-103 Labeled Ammineruthenium(III)-Amino Acid Complexes 1-4[a]

	1 (n = 3)	2 (n = 2)	3 (n = 5)	4 (n = 2)
15 min	1.40 ± 0.70	2.00 ± 0.70	7.40 ± 2.40	1.00 ± 0.30
30 min	1.60 ± 0.60	0.76 ± 0.12	14.00 ± 6.00	1.40 ± 0.10
1 h	1.40 ± 0.20	1.10 ± 0.40	17.00 ± 5.00	0.63 ± 0.32
2 h	1.90 ± 0.90	1.10 ± 0.80	16.00 ± 5.00	1.20 ± 0.10

[a] Mean plus or minus standard deviation for n mice.

tures and polarity to a naturally occurring molecule having the desired tissue specificity, this approach to the design of potential radiopharmaceuticals could prove useful. It must be noted, however, that pancreatic uptake of amino acids is very species dependent. There are numerous reports showing that high uptake of radiolabeled amino acids in rodents does not assure similar uptake in higher species, such as dogs, pigs, and primates.[25-27,33]

In conclusion, minor differences found among whole-body distribution and clearance rates of complexes 1–4 in this investigation, along with a major difference in pancreas to liver ratios, demonstrate that the biological fate of radiolabeled pentaammineruthenium(III) complexes can be controlled through proper choice of heterocyclic ligands. The significant target to nontarget ratio of complex **3** in conjunction with its more rapid whole-body clearance and the superior radiophysical properties of ^{97}Ru show that complex **3** has definite potential as a pancreatic imaging agent. Further evaluation of the clinical utility of this agent is being pursued in our laboratories and will be reported subsequently.

Experimental Section

Ruthenium trichloride hydrate (40% Ru) was obtained from Johnson Matthey, Inc., Winslow, NJ. [^{103}Ru]Ruthenium trichloride (0.5 mCi/mg of Ru) dissolved in dilute HCl was obtained from New England Nuclear Corp., Billerica, MA. Histidine (**9**) from Nutritional Biochemicals Corp. and *S*-[β-(4-pyridyl)-ethyl]cysteine (**11**) from Sigma Chemical Co. were used as received. Chloropentaammineruthenium(III) chloride (**6**) was prepared by literature procedure.[29] β-(4-Pyridyl)-α-alanine (**10**) was synthesized from 4-pyridinecarboxaldehyde by literature methods.[34] Analytical-grade AG 50W-X2, 200–400 mesh cation exchange resin (Bio-Rad) was washed successively with three times its volume of H_2O, 1 N NaOH, H_2O, 1 N HCl, H_2O, acetone, and H_2O and equilibrated in 0.1 N HCl. Columns were approximately 1.5 × 2 cm^3, and elution was monitored by recording the UV spectrum of each fraction. Purified products were characterized by electronic (Beckmann-G grating spectrophotometer) and IR (Beckmann IR-10 spectrophotometer using KBr pellets) spectra, molar extinction coefficients (Gilford 240 spectrophotometer), and elemental analyses (Schwarzkopf Microanalytical Laboratory, Inc., Woodside, NY.

Pentaammine(histidine)ruthenium(III) tetrachloride (**1**) **and tetraammineaquo(2-histidinyl)ruthenium(III) trichloride** (**2**) were synthesized by slight modification of Sundberg and Gupta's procedure.[22] Pentaammine complex **6** (80 mg, 0.273 mmol) in 10 mL of 0.5 N HCl was degassed by passing argon through the mixture for 15 min. Freshly prepared zinc dust amalgam (250 mg) was added, and the reaction mixture was stirred under a continuous argon purge for 30 min to form a solution of pentaammineaquoruthenium(II)chloride (**7**). Histidine (**9**; 260 mg, 1.676 mmol) was then added and allowed to react for 3 h to form a pale yellow solution of **8** in the ruthenium(II) oxidation state. Partial isomerization of complex **1** to **2** was achieved by filtering this solution under argon into 100 mL of degassed 0.02 N HCl and stirring under argon for 4 h. The resulting pale yellow solution was oxidized by a vigorous air stream for 1 h to form a red solution, which was acidified with 4 mL of concentrated HCl and allowed to stand overnight.

Complexes **1** and **2** were separated and isolated by ion-exchange chromatography. Elution with 100 mL each of 1.2, 1.3, and 1.4 N HCl removed unreacted starting material. The carbon-bound isomer was then eluted by 200 mL of 1.5 N HCl collected in 25-mL fractions. Evaporation of the solvent under reduced pressure, followed by recrystallization from water/ethanol, afforded **2** as a cherry red powder in 5–10% yield. Anal. ($C_6H_{23}N_7Cl_3O_3Ru$) C, H, N.

Continued elution of the column with 100 mL each of 2, 2.2, 2.4, and 2.6 N HCl removed the nitrogen-bound isomer as a bright orange band. Solvent removal as above, followed by recrystallization from water/acetone, afforded **1** as bright orange needles in 49–60% yield: (KBr) 1725, 1205 cm^{-1}. Anal. ($C_6H_{25}N_8Cl_4O_2Ru\cdot H_2O$) C, H, N.

Pentaammine[β-(4-pyridyl)-α-alanine]ruthenium(III) tetrachloride (**3**) was prepared by a modified literature procedure.[22] A mixture of 58.5 mg (0.2 mmol) of pentaammine complex **6** and 10 mL of 0.05 N HCl was degassed under argon for 10 min and reduced with 100 mg of zinc dust amalgam (freshly prepared) over 30 min. To the reaction mixture covered with aluminum foil was added 100 mg (0.6 mmol) of β-(4-pyridyl)-α-alanine (**10**).[34] The reaction mixture was then stirred in the dark under argon for 3 h. The bright orange solution was filtered under argon pressure into 20 mL of degassed 0.1 N HCl and oxidized in the dark by passing a stream of air through the solution overnight. The pale yellow solution was eluted through an ion-exchange column with 200 mL each of 1.5 and 2 N HCl to remove unreacted complex **6**. Elution with 300 mL of 3 N HCl separated a fraction containing the product **3**. Evaporation of the solvent in a rotary evaporator gave an off-white solid residue, which was dissolved in a few drops of water. The solution was transferred into a crystallizing flask and diluted with absolute ethanol until turbidity appeared. Upon cooling in a freezer overnight, off-white crystals were formed. The solid was collected by filtration, washed with ethanol, and dried to give 38 mg (37%) of pure product **3**: UV λ_{max} (0.1 N $HClO_4$) 268 nm (ε 4.6), 260 (5.06), 252 (5.44); IR (KBr) 1760, 1180 cm^{-1}. Anal. ($C_8H_{26}N_7Cl_4O_2Ru\cdot H_2O$) C, H, N.

Pentaammine[*S*-[β-(4-pyridyl)ethyl]cysteine]ruthenium(III) Tetrachloride (**4**). By [^{103}Ru]chloropentaammineruthenium procedure used for **3**, a pale yellow solution of **4** was prepared from pentaammine complex **6** (40 mg, 0.136 mmol) and *S*-[β-(4-pyridyl)ethyl]cysteine (**11**; 150 mg, 0.66 mmol). Purifi-

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cation was achieved on an ion-exchange column eluted with 200 mL each of 1.5, 2, and 2.5 N HCl prior to removal of the product with 250 mL of 3 N HCl. Solvent evaporation, followed by recrystallization as described for **3**, produced pale yellow needles of **4** in 40–50% yield: UV λ_{max} (0.1 N $HClO_4$) 268 nm (ϵ 5.44), 260 (6.35), 254 (7.02); IR (KBr) 1760, 1180 cm^{-1}. Anal. ($C_{10}H_{30}N_7Cl_4O_2SRu \cdot H_2O$) C, H, N, S.

Ruthenium-103 Complexes. Radiolabeled [^{103}Ru]chloropentaammineruthenium dichloride was synthesized according to a literature procedure[29] from 400 mg of $RuCl_3 \cdot H_2O$ containing 1.5 mCi ($^{103}RuCl_3$). After two recrystallizations from boiling 0.1 N HCl, 80–100 mg of product was obtained as bright orange crystals with a calculated specific activity of 3.0 μCi/mg of complex. Complexes 1–4 were then prepared according to the above procedures from equal amounts of radioactive and cold pentaammine complex. The identity of each crystalline complex obtained was confirmed by comparison of IR and electronic spectra with those of previously prepared unlabeled samples having satisfactory elemental analyses. Final specific activities of the ^{103}Ru-labeled complexes were approximately 1.0 μCi/mg of complex.

Tissue Distribution Studies. The ^{103}Ru-labeled complexes (10 μCi) were dissolved in 1 mL of saline solution just prior to use and injected (0.05–0.1 mL) into the tail vein of normal mice. After the desired time interval, the animals were sacrificed by ether asphyxiation, and the organs of interest were removed, weighed, and counted in a NaI(Tl) scintillation spectrometer. The activity observed in each organ was converted to percent injected dose per organ (% ID/organ) and/or per gram of organ (% ID/g). Data from the injection of different preparations of ^{103}Ru-labeled complexes were analyzed separately and were found to agree within experimental error.

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Registry No. **1**, 84800-85-1; **1**-^{103}*Ru*, 84800-88-4; **2**, 84823-40-5; **2**-^{103}*Ru*, 84809-56-3; **3**, 84800-86-2; **3**-^{103}*Ru*, 84800-89-5; **4**, 84800-87-3; **4**-^{103}*Ru*, 84800-90-8; **6**, 18532-87-1; **7**, 66402-61-7; [^{103}Ru]-chloropentaammineruthenium dichloride, 78713-16-3.

Potential Cerebral Perfusion Agents: Synthesis and Evaluation of a Radioiodinated Vinylalkylbarbituric Acid Analogue

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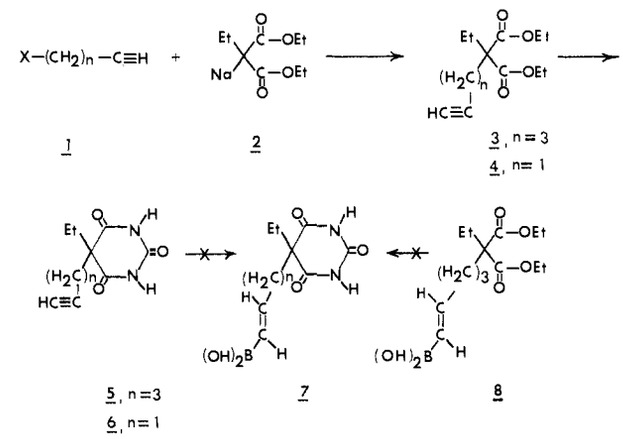
A new iodinated barbiturate has been prepared. Treatment of 5-chloropentyne and propargyl bromide with diethyl 2-ethyl-2-sodiomalonate (DESM) provided diethyl 2-ethyl-2-(1-pentyn-5-yl)malonate (**3**) and diethyl 2-ethyl-2-propargylmalonate (**4**), respectively. Similar condensation of DESM with (*E*)-(5-iodo-1-penten-1-yl)boronic acid (**9**) or the reaction of catecholborane with **3** provided diethyl (*E*)-2-ethyl-2-(1-borono-1-penten-5-yl)malonate (**8**). The direct sodium iodide–chloramine-T iodination of **8** or the treatment of (*E*)-1,5-diiodo-1-pentene (**10**) with DESM provided diethyl (*E*)-2-ethyl-2-(1-iodo-1-penten-5-yl)malonate (**11**). The condensation of functionalized malonates **3**, **4**, and **11** with urea in the presence of a base provided the corresponding barbiturates, 5-ethyl-5-(1-pentyn-5-yl)- (**5**), 5-ethyl-5-propargyl- (**6**), and (*E*)-5-ethyl-5-(1-iodo-1-penten-5-yl)barbituric acid (**12**), respectively. (*E*)-6-(Ethoxycarbonyl)-1-iodo-1-octene-6-carboxylic acid (**13**) was isolated as the hydrolytic byproduct of **11**. Compound **13** decarboxylated under vacuum to provide ethyl (*E*)-1-iodo-1-octene-6-carboxylate (**14**). The ^{125}I-labeled congeners of **12** and **13** were synthesized in the same manner and evaluated in rats. The barbiturate **12** exhibited significant brain uptake (~1% dose after 5 min), demonstrating that iodinated barbiturates freely cross the intact blood–brain barrier.

Radiolabeled agents that penetrate the blood–brain barrier[1] can potentially be used to monitor regional cerebral blood perfusion. Certain lipid-soluble[2] and pH shift agents[3-5] freely cross the intact blood–brain barrier and have been evaluated as cerebral perfusion agents. Recently, the synthesis and preliminary animal testing of *N*-isopropyl-*p*-[^{123}I]iodoamphetamine has been reported,[6,7] and this agent has been used to measure cerebral perfusion in patients with single-photon emission computed tomography.[8,9] Barbiturates are another group of lipophilic agents that freely cross the blood–brain barrier, as indicated by studying ^{11}C-labeled[10] and 5-substituted[11] ^{75}Se-

Scheme I



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