

FUNCTIONALIZED ISONITRILE COMPLEXES OF TECHNETIUM
AS RADIOPHARMACEUTICALS

by

JAMES FREDRIC KRONAUGE, JR.

Submitted to the Department of Chemistry
in May, 1987 in partial fulfillment of the
requirements for the Degree of Doctor of Philosophy

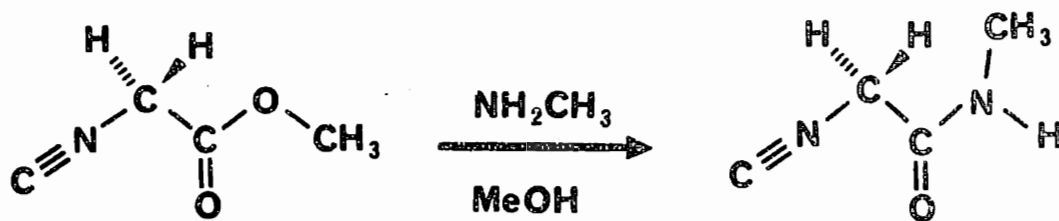
ABSTRACT

Various carboxy, carboalkoxy and aminocarbonyl substituted isonitrile ligands and their respective hexakis(isonitrile)technetium(I) complexes have been synthesized with the long lived isotope ^{99}Tc . These compounds were characterized by IR, FAB mass spectrometry, RP-HPLC, and ^1H , ^{13}C , ^{99}Tc NMR. Synthesis was extended to the "no carrier added" concentration of 10^{-9}M for production of complexes with the isotope $^{99\text{m}}\text{Tc}$ and their subsequent evaluation as radiopharmaceuticals. Radiochemical identity, yield, and purity of all compounds were established by reverse-phase HPLC. Initial screening by dynamic Anger scintillation camera studies identified one complex, hexakis(2-carbomethoxyisopropyl isocyanide)technetium(I), $\text{Tc}(\text{CPI})_6^+$, as a prospective myocardial imaging agent. Subsequent biodistribution studies confirmed this agent's biological properties, culminating in successful human clinical trials.

To evaluate in vivo integrity of this potential radiopharmaceutical, synthesis and characterization of the possible metabolic hydrolysis products was performed. Identification of these products allowed HPLC analysis of in vitro enzymatic hydrolysis in plasma of various animal species. Differences in enzyme systems was established as the source of interspecies biodistribution variation. These studies also confirm the rabbit as an optimistic animal model for screening these ester isonitrile complexes.

Cellular kinetics and binding characteristics of $\text{Tc}(\text{CPI})_6^+$ were evaluated in chick embryo heart cells grown in monolayer cultures. Myocytes showed uptake of $\text{Tc}(\text{CPI})_6^+$ to a plateau level with a half-time ($t_{1/2}$) of 4.1 ± 0.7 min; $t_{1/2}$ appeared independent of extracellular $\text{Tc}(\text{CPI})_6^+$ concentration. Plateau level $\text{Tc}(\text{CPI})_6^+$ uptakes were a linear function of extracellular $\text{Tc}(\text{CPI})_6^+$ concentration. Tracer $^{99\text{m}}\text{Tc}(\text{CPI})_6^+$ uptake (binding) was not competitively displaced by carrier $^{99}\text{Tc}(\text{CPI})_6^+$. Uptake was temperature-sensitive; however, several inhibitors of cationic membrane transport showed no significant effect. The data do not demonstrate a specific mechanism for uptake of $\text{Tc}(\text{CPI})_6^+$, however, results show preferential binding to myocytes in a manner proportional to the delivery of the complex to the extracellular spaces. Such properties would be desirable for a myocardial perfusion imaging agent.

Amidation of Esterisonitrile



Experimental:

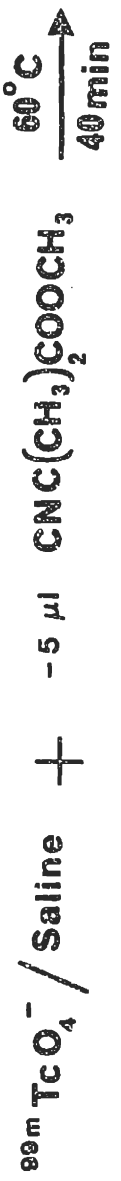
Materials and methods. Technetium-99 in aqueous solution as the NH_4 salt of $[\text{}^{99}\text{TcO}_4]^-$ was obtained from NEN Products / DuPont Medical Products, Billerica, MA. All manipulations were carried out in laboratories approved for low-level radioactivity (^{99}Tc is a weak beta emitter with a half-life of 2.15×10^5 years and a particle energy of 0.292 MeV). Precautions for handling were detailed previously.(21) The metastable radionuclide $^{99\text{m}}\text{Tc}$, as sodium pertechnetate, was obtained from a commercial NEN/DuPont ($^{99}\text{Mo}/^{99\text{m}}\text{Tc}$) generator in a 0.15 M saline solution. Technetium- $^{99\text{m}}$ decays primarily by isomeric transition from the metastable spin 1/2 state to the spin 9/2 ground state of ^{99}Tc with emission of a single gamma photon of 140 KeV. All manipulations involving the high specific activity $^{99\text{m}}\text{Tc}$ isotope were performed under guidelines established by the NRC in a laboratory approved for intermediate level radioactivity at the Harvard Medical School.

Infrared spectra, obtained as KBr pellets or as CHCl_3 solutions, were recorded in the range of $4000\text{--}600\text{ cm}^{-1}$ on a model 1420 Perkin-Elmer spectrophotometer. Elemental analyses were performed by Atlantic Microlab Inc., Atlanta, GA. Fourier transform ^1H NMR spectra were obtained on a Bruker WM 250 MHz instrument with TMS as the internal standard, ^{13}C NMR spectra were obtained on a Varian 400 MHz instrument at 100 MHz, with CDCl_3 as the lock solvent/standard. Fast-Atom-Bombardment mass spectra were run on a Varian MAT 731 mass spectrometer from a glycerol matrix.(17) Doubly distilled, deionized water was used; all other solvents and chemicals were of reagent grade and used as received.

Reverse-Phase thin-layer chromatography (RPTLC) was carried out on Whatman MK C18 plates developed in (2:3:3:2) tetrahydrofuran, methanol,

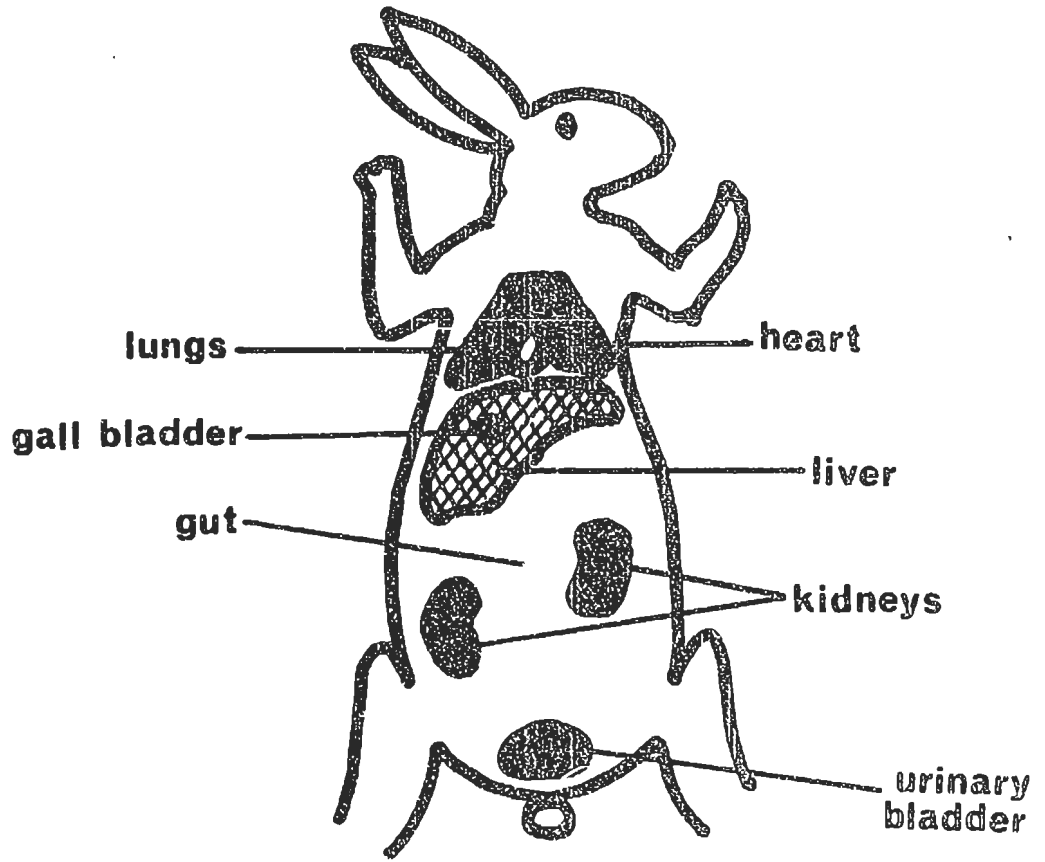
^{99m}Tcnetiumhexakis(2-carbomethoxyisopropylisotrile)

- 5 mg Na₂S₂O₄

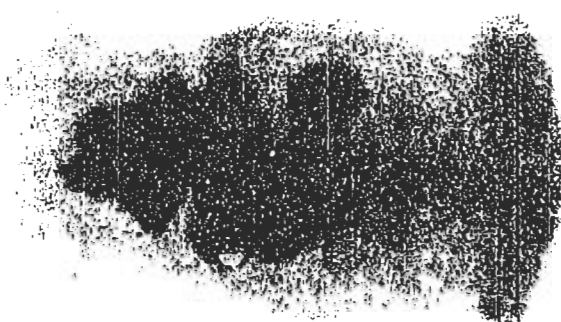


- .5 ml MeOH

> 98% by RPHPLC
and TLC

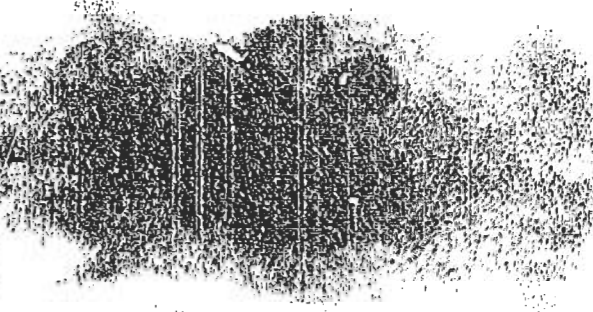


9114852 RABBITT MYO/TBI
1524 X4 PHOTO



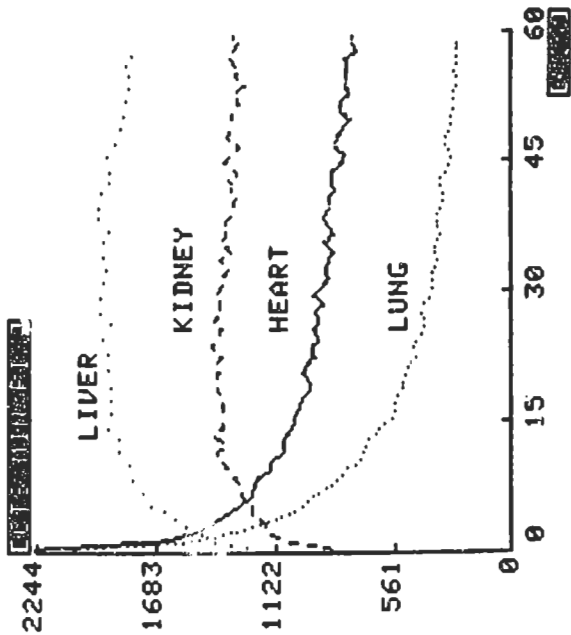
19

9114852 RABBITT MYO/TBI
1524 X4 PHOTO



60

9114852 RABBITT MYO/TBI



9114852 RABBITT MYO/TBI
1524 X4 PHOTO



30

