Potential Tissue-Imaging Agents: 23-(Trimethyl[^{117m}Sn]stannyl)-24-nor-5α-cholan-3β-ol

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Tin-117m-labeled 23-(trimethylstannyl)-24-nor-5α-cholan-3β-ol (2) has been prepared by reaction of trimethyl[^{117m}Sn]tin lithium with 3β-acetoxy-23-bromo-24-nor-5α-cholane (1). Tin-117m (2) shows pronounced adrenal uptake (2.5% injected dose) in female rats 1 day after injection. Furthermore, the adrenal to liver (9.1:1) and adrenal to blood (33.7:1) ratios are high after this period. The absorbed radiation dose values from [^{117m}Sn]2 to human organs have also been estimated by using rat tissue distribution and excretion data. [^{117m}Sn]2 is the first reported tissue-specific organic radiopharmaceutical labeled with this nuclide and may have potential as an adrenal imaging agent.

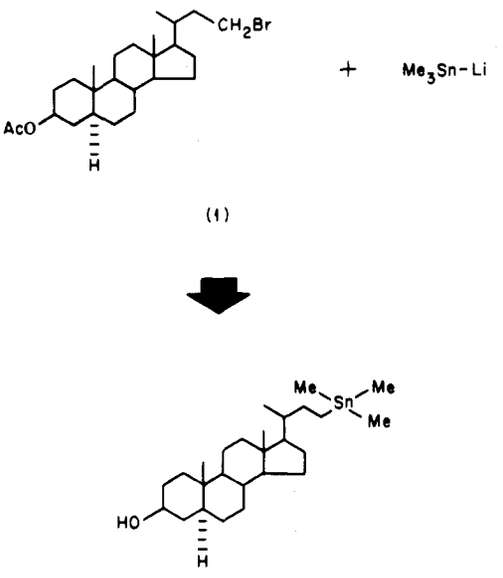
A variety of steroids labeled with γ-emitting radionuclides have been developed as potential adrenal imaging agents.[1] Iodine-131-labeled 6β-(iodomethyl)-19-norcholest-5(10)-en-3β-ol (NP-59) is presently the most widely used agent for the clinical evaluation of various adrenal disorders.[2] Recent clinical results with 6β-[(methyl-[^{75}Se]seleno)methyl]-19-norcholest-5(10)-en-3β-ol (Scintidren) indicate that this new agent compares favorably with NP-59 for the detection of unilateral adrenal aldosteromas, for the localization of adrenal remnants in patients with persistent Cushing's symptoms after adrenalectomy, and for the diagnosis of virilizing adrenal tumors.[3]

We have recently prepared and tested several Te-123m-labeled steroids as potential alternatives to NP-59 and Scintidren.[4,5] Two of these agents, 23-(isopropyl-[^{123m}Te]telluro)-24-nor-5α-cholan-3β-ol ([^{123m}Te]-23-ITC) and 24-(isopropyl[^{123m}Te]telluro)chol-5-en-3β-ol ([^{123m}Te]-24-ITC) show pronounced adrenal uptake in rats.[4,5] In addition, the absorbed radiation dose values to human organs from [^{123m}Te]-23-ITC and [^{123m}Te]-24-ITC are within the same range as values calculated for [^{131}I]-NP-59 and [^{75}Se]Scintidren.[6] Unfortunately, the enriched Te-122 target material required for reactor production of Te-123m is expensive, which limits the availability and cost effectiveness of the Te-123m-labeled steroids for potential adrenal imaging in human subjects.

In this paper we describe the synthesis of 23-(trimethyl[^{117m}Sn]stannyl)-24-nor-5α-cholan-3β-ol ([^{117m}Sn]2) and the results of initial tissue distribution studies in rats with this new adrenal imaging agent. Our interest in the preparation of Sn-117m-labeled radiopharmaceuticals was stimulated because of the attractive properties of this radionuclide. These properties include the emission of a single 158-keV γ-photon in 87% abundance and a 14-day physical half-life. In addition, Sn-117m can be reactor produced by neutron irradiation of readily available enriched Sn-116 target material. These properties, coupled with the versatility of organotin chemistry,[7] suggested that a variety of tissue-specific radiopharmaceuticals labeled with Sn-117m could be prepared. We have found that [^{117m}Sn]2 shows pronounced adrenal uptake in rats. This agent represents the first reported tissue-specific organic radiopharmaceutical labeled with the Sn-117m nuclide.

Chemistry. The new tin steroid (2) was prepared (Scheme I) by coupling Me_3SnLi, generated in situ by the reaction of lithium metal in dry THF with commerical Me_3SnCl, with 3β-acetoxy-23-bromo-24-nor-5α-cholestane (1). The structure of 2 was confirmed by elemental analysis and NMR and MS studies. The ^{117m}Sn-labeled steroid (2) was synthesized by reaction of $Me_3{}^{117m}SnLi$ with 1. The $Me_3{}^{117m}SnCl$ was prepared by the comproportionation reaction[7] of $^{117m}SnCl_4$ with Me_4Sn as shown below.

Scheme I



$$*SnO_2 + H_2 \xrightarrow{450\ ^\circ C} *Sn + Cl_2 \xrightarrow{150\ ^\circ C} *SnCl_4 + 3Me_4Sn \xrightarrow{37\text{–}45\ ^\circ C} Me_3{*}SnCl$$

The SnO_2 was reduced at high temperature[8] to elemental tin prior to chlorination to $SnCl_4$ and distillation into a conical reaction vessel. In developmental studies with tin

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Table I. Distribution of Radioactivity in Female Rat Tissues at 1, 3, 7, 14 and 21 Days After Intravenous Administration of 23-(Trimethyl[^{117m}Sn]stannyl)-24-nor-5α-cholan-3β-ol[a]

Days After Injection	% dose/g (range)				
	Tissue				
	Adrenals	Blood	Liver	Ovaries	Kidneys
1	47.13 (37.13-53.47)	1.40 (1.33-1.54)	5.18 (4.74-5.71)	18.22 (11.88-21.73)	0.71 (0.66-0.75)
3	50.88 (45.80-59.54)	0.80 (0.77-0.85)	2.13 (1.81-2.49)	10.99 (10.24-11.70)	0.79 (0.74-0.80)
7	19.37 (17.54-21.26)	0.19 (0.15-0.29)	0.44 (0.33-0.61)	4.90 (3.82-6.66)	0.54 (0.41-0.76)
14	7.46 (5.83-9.47)	0.15 (0.11-0.17)	0.20 (0.16-0.23)	2.53 (1.31-3.48)	0.38 (0.28-0.43)
21	2.33 (2.25-2.44)	0.10 (0.09-0.10)	0.14 (0.14-0.15)	1.26 (0.89-1.78)	0.26 (0.23-0.27)

[a] Percent dose/gram values are the mean and range for three female rats. The radioactive contents of the following tissues were also analyzed: heart, lungs, pancreas, spleen, and small and large intestines. The uptake of [^{117m}Sn]-23-TSC in these tissues was less than 5% dose/g.

Table II. Adrenal uptake (Percent Injected Dose) and Adrenal/Blood and Adrenal/Liver Ratios[a] Determined after Intravenous Injection of 23-(Trimethyl[^{117m}Sn]stannyl)-24-nor-5α-cholan-3β-ol

days after iv injection	adrenal uptake: % injected dose, mean (range)	adrenal/blood ratio	adrenal/liver ratio
1	2.51 (2.28-2.72)	33.66	9.10
3	2.86 (2.64-3.26)	63.60	23.89
7	1.10 (0.86-1.29)	101.91	44.02
14	0.34 (0.26-0.43)	49.73	37.30
21	0.16 (0.15-0.18)	23.30	16.64

[a] Adrenal/tissue ratios calculated from the percent dose per gram of tissue values tabulated in Table I.

metal, the $SnCl_4$ was regularly obtained in ~90% yield. Addition of 3 equiv of Me_4Sn lead to the formation of Me_3SnCl exclusively. The homogeneity of the Me_3SnCl was determined by NMR, and no traces of Me_2SnCl_2 or $MeSnCl_3$ were detected. Following the coupling of $Me_3{}^{117m}SnCl$ with the steroid stubstrate (1), the ^{117m}Sn-labeled steroid (2) was purified by column chromatography and exhibited a single radioactive spot on TLC that co-chromatographed with the unlabeled standard.

Biological Studies. Tissue distribution studies with [^{117m}Sn]**2** demonstrated pronounced uptake in rat adrenals 1 day after injection (Table I). After 3 days the radioactive contents of the adrenal glands were still high and then decreased steadily over the 21-day period. In contrast, the radioactive contents of the nontarget tissues, such as blood, liver, and kidneys, had decreased significantly after 3 days. The absolute adrenal uptake values expressed as percent of the injected dose are compared in Table II over the 21-day period. The adrenal/blood and adrenal/liver values (Table II), calculated from the percent dose per gram of tissue data in Table I, clearly illustrate the pronounced and specific uptake of [^{117m}Sn]**2** in rat adrenal glands.

The radioactive contents of the urine and feces excreted by rats administered [^{117m}Sn]**3** were also monitored over a 21-day period, and the majority of the radioactivity was excreted in the feces. These results may indicate that the steroid side chain remains intact. To estimate the human tissue distribution, we expressed the animal distribution data from Table I as percent kilogram dose per gram, and the fraction of the injected dose in organ h of man (α_h) was estimated as

$$\alpha_h = \frac{(\%\ \text{kg dose/g}) \times \text{wt of organ (g)}}{\text{kg body wt of man} \times 100}$$

Table III. Comparison of Absorbed Radiation Dose Values to Human Organs from 23-(Trimethyl[^{117m}Sn]stannyl)-24-nor-5α-cholan-3β-ol ([^{117m}Sn]-23-TSC) with Values Calculated for [^{123m}Te]-24-ITC[a] and [^{123m}Te]-23-ITC[a]

organ	absorbed dose, rd/mCi, of the following adrenal imaging agents		
	[^{117m}Sn]-23-TSC	[^{123m}Te]-23-ITC	[^{123m}Te]-24-ITC
adrenals	83	98	210
liver	5.3	1.6	2.0
lungs	4.1	1.3	1.9
ovaries	4.4	8.0	13
spleen	7.7	1.4	34
total body	0.77	0.8	1.4

[a] Values for [^{123m}Te]-23-ITC, 23-(isopropyltelluro)-24-nor-5α-cholan-3β-ol, and [^{123m}Te]-24-ITC, 24-(isopropyltelluro)-chol-5-en-3β-ol, are taken from ref 6.

Cumulated activities were calculated by using these fractional distributions and the half-times for the organs.[9] These cumulated activities were then used with the absorbed dose values S, in units of rd/μCi·h, for Sn-117m (obtained from the Metabolism and Dosimetry Group, Health and Safety Research Division, Oak Ridge National Laboratory) to calculate the radiation dose (Table III). The adrenal glands receive the highest radiation dose (83 rd/mCi), which is considerably less than the 150 rd/mCi value estimated from rat tissue distribution for [^{131}I]-NP-59.[2]

Discussion

Although [^{117m}Sn]stannous tartrate has been described as a potential bone imaging agent,[10] there have been no reports of the preparation of tissue-specific organic radiopharmaceuticals labeled with this nuclide. From the wide variety of chemical methods[7] that can be used potentially for the introduction of ^{117m}Sn into tissue-specific agents, $^{117m}SnCl_4$ is easily generated from metallic ^{117m}Sn. Although the maximum specific activity (1–2 mCi/mg) of reactor-produced Sn-117m is limited by a rather low production cross section for neutron capture by Sn-116, the

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tissue specificity of Sn-117m-labeled adrenal-specific steroids would tend to offset the limitations of low specific activity. Other radiolabeled steroids with low specific activities have been used successfully for adrenal imaging.[4,5]

In the present study the trimethyltin moiety was introduced into the steroid side chain of **2**. The pronounced adrenal uptake of [^{117m}Sn]**2** in rats further illustrates that considerable structural modification of the steroid side chain does not always decrease adrenal specificty. These encouraging results with [^{117m}Sn]**2** suggest that the preparation of other ^{117m}Sn-labeled agents should be explored. In addition, the labeling of red blood cells with ^{117m}Sn and the evaluation of the Sn-117m-labeled red blood cells as a blood pool agent for the measurement of ventricular ejection fraction is in progress.[11]

Experimental Section

General. The Oak Ridge High Flux Isotope Reactor (2.5×10^{15} n·cm^2/s) was used for production of [^{117m}Sn]SnO_2 (*SnO_2) by the ^{116}Sn(n,γ)^{117m}Sn nuclear reaction using SnO_2 isotopically enriched in Sn-116 (95.74%). The specific activity of *SnO_2 obtained after a typical 14-day irradiation of $^{116}SnO_2$ was 100 mCi/mmol (2 mCi/mg of *Sn). The published thermal neutron cross section of ^{116}Sn is 6 ± 2 mb.[12] The *SnO_2 was reduced to *Sn with hydrogen gas as described elsewhere.[8] The general analytical procedures and rat tissue distribution studies were performed as described in the preceding paper.[13]

23-(Trimethylstannyl)-24-nor-5α-cholan-3β-ol (23-TSC, 2, Scheme I). The 3β-acetoxy-23-bromo-24-nor-5α-cholane (**1**) substrate was prepared by modified Hunsdiecker degradation of 3β-acetoxy-5α-cholan-24-oic acid as described earlier.[14] Trimethyltin chloride (400 mg, 2 mmol) was dissolved in THF (25 mL) under argon. Lithium metal (140 mg, 20 mg-atoms) was cut into small pieces and cleaned by immersion in MeOH. After thorough rinsing in THF, the Li metal was dried under argon and added to the reaction mixture, which was stirred at room temperature. The reaction mixture became cloudy, turned a murky green color, and slowly changed to a brown color. After 18 h, the black-colored reaction mixture was carefully decanted from the shiny pieces of excess Li metal under argon. An aliquot of the Me_3SnLi solution (5 mL, 0.4 mmol) was added to a solution of 3β-acetoxy-23-bromo-24-nor-5α-cholane (30 mg, 0.065 mmol). The reaction mixture was stirred under argon for 1 h, and the excess reagent was destroyed by the careful addition of H_2O (2 mL). The mixture was poured into H_2O and extracted with ether (3 times), the combined extracts were washed with H_2O (3 times) and dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo to yield a white solid. The crude product was analyzed by TLC with 2% MeOH in $CHCl_3$.

After the plates were sprayed with ammonium molybdate–H_2SO_4 spray[15] and heated in an oven at 80–100 °C, two major spots were detected. In addition to material migrating at the solvent front, a second component at R_f 0.37 migrated with the expected mobility of the desired product (**2**). Purification was achieved by preparative TLC on 0.5-mm-thick plates prepared from silica gel H. The R_f 0.37 component was scraped from the plates and eluted with $CHCl_3$. Evaporation of the solvent gave 18 mg (48% yield) of 23-(trimethylstannyl)-24-nor-5α-cholan-3β-ol (**2**), mp 101–104 °C; IR (KBr) 3300 (OH), 1040 and 760 ($SnMe_3$) cm^{-1};[16] low-resolution mass spectrum, m/z 496 (M, 8), 481 (M – CH_3, 58), 478 (M – H_2O, 16), 332 [(M – Me_3Sn) + 1, 62], 314 [(M – Me_3Sn – H_2O) + H], 100], 299 [(M – Me_3Sn – H_2O – CH_3) + 1, 42], 285 [(M – Me_3SnCH_2 – H_2O – CH_3) + 1, 34], 255 (M – side chain – H_2O, 57); high-resolution mass spectrum, M^+ calcd for $C_{26}H_{48}O^{120}Sn$, 496.2726; found, 496.2723; NMR ($CDCl_3$) δ (downfield from the Me_4Si internal standard) 0.02 [s, 9 H, Sn-$(CH_3)_3$], 0.63 (s, 3 H, C-18 CH_3), 0.91 (s, 3 H, C-19 CH_3), 3.61 (1 H, m, C-3α H). anal. Calcd. for $C_{26}H_{48}SnO$: C, 63.02; H, 9.77. Found: C, 63.02; H, 9.81. The expected doublet for C-21 CH_3 was masked under the meethylene envelope, but this region of the spectrum did integrate for the correct number of protons.

23-(Trimethyl[^{117m}Sn]stannyl)-24-nor-5α-cholan-3β-ol ([^{117m}Sn]2). The ^{117m}Sn reactor target (60.1 mCi, 111.5 mg) was combined with carrier SnO_2 (64 mg) to give 1.2 mmol of material. This amount of material was used, since the results of a number of independent experiments indicated that $SnCl_4$ was regularly obtained in 80% yield following reduction of SnO_2 and subsequent chlorination of the Sn metal.[8]

After H_2 reduction, the *Sn metal was chlorinated, and the *$SnCl_4$ product distilled into the reaction vessel. Following the addition of Me_4Sn (420 μL, 540 mg, 3 mmol), the mixture was heated at 39–45 °C under argon for 3 h, after which a crystalline mass formed upon cooling to room temperature. The Me_3*SnCl was dissolved in 10 mL of THF (freshly distilled from $LiAlH_4$) and stirred under argon with Li (56 mg, 8 mg-atoms) overnight at room temperature. The solution rapidly turned cloudly; after 18 h, the majority of the Li had dissolved, and the solution had a murky greenish-black color. An aliquot of the Me_3*SnLi solution (1 mL, ~0.4 mmol) was decanted and added to 3β-acetoxy-23-bromo-24-nor-5α-cholane (21.5 mg, 0.055 mmol), and the mixture was stirred under argon overnight. The excess reagent was destroyed by the cautious addition of H_2O, and the crude product was obtained by solvent extraction as described above. The gummy product was dissolved in 1–2 mL of C_6H_6 and applied to a silicic acid column (acid grade, 60–200 mesh) slurried in petroleum ether. The column was eluted with increasing concentrations of ether in petroleum ether (Figure 2). Fractions 11–17 were combined to give 281 μCi of [^{117m}Sn]**2** (49% from compound **1**). Analysis by TLC (SiO_2-G) in two solvent systems indicated the presence of a single radioactive component (>99%) that cochromatographed with the 23-(trimethylstannyl)-24-nor-5α-cholan-3β-ol standard (**2**): R_f ($CHCl_3$) 0.15; R_f ($CH_3OH/CHCl_2$, 0.53. The [^{117m}Sn]**2** was stored at 4–8 °C in the column eluant.

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