Synthesis and Characterization of Radioiodinated N-(3-Iodopropen-1-yl)-2β-carbomethoxy-3β-(4-chlorophenyl)tropanes: Potential Dopamine Reuptake Site Imaging Agents

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Received March 5, 1993®

Methods have been developed for the preparation of radioiodinated N-substituted 2β-carbomethoxy-3β-(4-chlorophenyl)tropanes. The syntheses, physical properties, radiolabeling, and characterization of the pharmacological properties of *N*-(3(*Z*)-iodopropen-1-yl)-2β-carbomethoxy-3β-(4-chlorophenyl)tropane (**12**) and *N*-(3(*E*)-iodopropen-1-yl)-2β-carbomethoxy-3β-(4-chlorophenyl)tropane (**13**) are described. 2β-Carbomethoxy-3β-(*p*-substituted-phenyl)tropanes are potent ligands for the dopamine transporter. The radioiodinated derivatives are of interest because of the high uptake and prolonged striatal retention that may result from specific binding to low-capacity, high-affinity, dopamine reuptake sites. Radioiodine was introduced into the 3*Z* and 3*E*-position of *N*-(3-iodopropen-1-yl)-2β-carbomethoxy-3β-(4-chlorophenyl)tropane by iododemetalation of the corresponding 3-(tri-*n*-butylstannyl) derivatives. Competition binding data of various dopamine reuptake ligands with rat striatal tissue preparation for either [^{125}I]-**12** or [^{125}I]-**13** exhibited the following order of potency: *E*-**13** > *Z*-**12** > GBR 12909 ≫ mazindol >>> (−)-cocaine. Tissue distribution studies in rats showed that the *E*-**13** was the best analogue. *E*-**13** showed high striatal uptake (60 min, 1.23% dose/g; 120 min, 0.61% dose/g) and high striatal to cerebellum ratios (60 min, 15.9/1; 120 min, 16.5/1). These studies indicate that iodine-123-labeled *E*-**13** is a potentially useful agent for imaging the dopamine reuptake sites by single-photon-emission computerized tomography.

Introduction

Crack cocaine is one of the most powerful positively reinforcing psychoactive drugs. The binding properties of (−)-cocaine and a variety of analogues have been extensively evaluated over the past decade to delineate the locus within the brain and the neurochemical mechanism that is responsible for the reinforcing properties of this drug. Binding studies with [^{3}H]-(−)-cocaine under *in vivo* and *in vitro* conditions have demonstrated that the striatum is the primary binding site for cocaine in rodents and nonhuman and human primates.[1-6] Competitive binding studies and uptake assays with [^{3}H]-(−)-cocaine and several ligands that bind at the dopamine transport site have shown that cocaine's reinforcing properties are associated with central nervous system (CNS) dopaminergic neurotransmission.[7] Dopamine is synthesized in a vesicle of a presynaptic neuron, where it is stored. When a nerve terminal in the presynaptic neuron is stimulated dopamine is released and diffuses across a synaptic gap where it interacts with the D2 receptor on the postsynaptic neuron. The interaction of dopamine with the D2 receptor initiates a series of intracellular biochemical reactions which includes adenyl cyclase activity. This neurotransmission of dopamine constitutes a signal which ends with the reuptake of dopamine by dopamine transporters into the presynaptic neuron. Cocaine has been hypothesized to bind to the dopamine transporter. This results in the inhibition of dopamine transport which affords an increase of dopamine at the synapse. The excess of dopamine at the synapse leads to a transient but significant increase in dopaminergic transmission creating the euphoric feeling believed to be responsible for cocaine's reinforcing properties.[8]

Abnormalities in CNS dopaminergic neurotransmission have also been implicated in psychomotor disorders such as Parkinson's disease,[9] tardive dyskinesia,[10] Huntington's chorea,[11] and schizophrenia.[12] Regional dopamine receptor density measurements in postmortem schizophrenics show an increase in dopamine receptor density in the striatum. These results have led to the hypothesis that schizophrenia is the result of a greater than normal synthesis, transport, and binding of dopamine in the mesolimbic dopaminergic neurons. Regional dopamine receptor density measurements in postmortem brain samples from patients with movement disorders such as Parkinson's disease show a decrease in dopamine receptor density in the striatum. In contrast to schizophrenia, these disorders have been shown to be caused by a significant decrease in the synthesis and transmission of dopamine which results from a degeneration of dopamine neurons in the substantia nigra and striatum. Because the dopamine transporter plays a pivotal role in regulating dopamine transmission by removing dopamine from the synapse, the development of cocaine receptor ligands radiolabeled with γ-emitting isotopes which exhibit pronounced brain uptake, very high selectivity and affinity for the transporter, and low nonspecific binding would be excellent biochemical probes for the measurement of the density of presynaptic dopamine transporter sites by emission tomography. This could be a potentially valuable technique for the diagnosis,

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® Abstract published in *Advance ACS Abstracts*, April 15, 1994.

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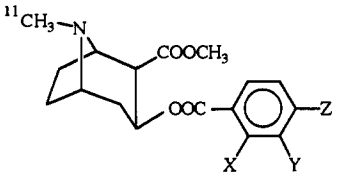


compound	X	Y	Z
(-)-cocaine	H	H	H
4-fluorococaine	H	H	F
2-iodococaine	I	H	H
3-iodococaine	H	I	H
4-iodococaine	H	H	I

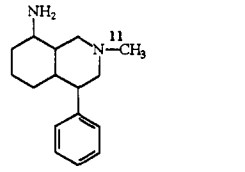
Figure 1. Chemical structures of carbon-11-labeled halogenated cocaines.

management of treatment, and study of the pathogenesis of addictive and psychomotor disorders in humans.

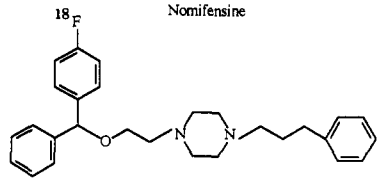
Dynamic imaging studies of the dopamine uptake site in nonhuman and human primates with a series of [*N-methyl*-^{11}C]-(−)-cocaine analogues (Figure 1) by positron emission tomography (PET) have been reported.[13,14] Serial PET images demonstrate that [*N-methyl*-^{11}C]-(−)-cocaine accumulates in the dopamine transporter rich striatum. The time course of [*N-methyl*-^{11}C]-(−)-cocaine in humans showed that the release of radioactivity from the striatum was rapid, $T_{1/2}$ = 25 min, with striatum to cerebellum ratios of only 2:1. Cocaine binding and kinetic studies in nonhuman primates with [*N-methyl*-^{11}C]-(−)-4-fluorococaine and [*N-methyl*-^{11}C]-(−)-2-iodococaine, [*N-methyl*-^{11}C]-(−)-3-iodococaine, and [*N-methyl*-^{11}C]-(−)-4-iodococaine were also measured to determine whether these agents were suitable for labeling with fluorine-18 for PET and iodine-123 for single-photon-emission tomography (SPECT) studies, respectively.[15,16] [*N-methyl*-^{11}C]-(−)-4-Fluorococaine gave values that were nearly identical to those of [*N-methyl*-^{11}C]-(−)-cocaine. However, the [*N-methyl*-^{11}C]-(−)-iodococaines did not show specific binding to the dopamine transporter. The rapid dissociation of radiolabeled (−)-cocaine from the dopamine transporter complex and the resulting low target (striatum) to nontarget (cerebellum) ratios demonstrates that these radioligands are not suitable PET imaging agents.

Several nontropane dopamine transporter ligands (Figure 2) have been labeled with carbon-11 and fluorine-18 as potential PET CNS dopamine transporter imaging agents. Imaging studies with [^{11}C]nomifensine in nonhuman and human primates have been reported.[17] This radioligand is rapidly taken up in the brain but exhibits low specificity for the dopamine transporter, which results in low striatum to cerebellum ratios (1.53 at 50 min). A fluorine-18 analogue of a class of aryl 1,4-dialkylpiperazine drugs has also been reported.[18-20] Imaging studies with [^{18}F]GBR 13119 in nonhuman primates showed that the specificity and resulting striatum to cerebellum ratios (1.51 at 50 min) were comparable to those of [^{11}C]nomifensine. The imaging data for these ligands appears to show affinity and nonspecific binding only slightly better than those measured with radiolabeled (−)-cocaine.

Recently, a series of C-3 tropanes has been developed in which the 3β-benzoyl substituent has been replaced by 3β-aryl moieties.[21-23] These 2β-carbomethoxy-3β-(*p*-



Nomifensine



GBR 13119

Figure 2. Chemical structures of radioactive dopamine reuptake inhibitors.

substituted-phenyl)tropanes are potent ligands which show subnanomolar to low nanomolar potency for the dopamine transporter. The 4-chloro and 4-iodo derivatives were found to be the most potent from competition binding data of the C-3β series of analogues with rat striatal tissue preparation for [^{3}H]-2β-carbomethoxy-3β-(*p*-fluorophenyl)tropane (CFT).[23] *In vitro* characterization and *in vivo* imaging studies of dopamine reuptake sites in nonhuman primates with [^{123}I]-2β-carbomethoxy-3β-(*p*-iodophenyl)-tropane ([^{123}I]CIT) in conjunction with SPECT and [^{11}C]-CFT have recently been reported.[24-29] [^{123}I]CIT showed very high specific uptake in dopamine transporter rich striatal tissue with low nonspecific uptake in the cerebellum.

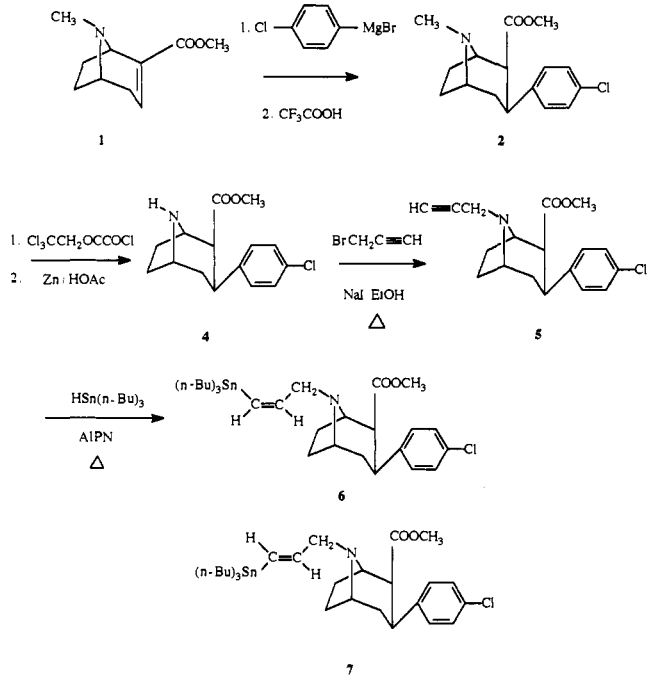
However, an undesired property accompanying very high striatal uptake of [^{123}I]CIT was high uptake of radioactivity in the hypothalamus which was associated with serotonin uptake sites. Recent structure–activity studies to determine the effect of alkyl and alkenyl substitution of CFT at the bridgehead nitrogen demonstrated that the *N*-allyl analogue was nearly as potent as C-β-CFT.[30,31] Thus, an *N*-allyl pharmacophore does not decrease the potency of C-β-CFT. These studies suggest that *N*-propen-1-yl-2β-carbomethoxy-3β-(*p*-substituted-phenyl)tropanes in which an iodine-123-labeled *N*-(3-iodopropen-1-yl) moiety replaces the bridgehead methyl group are also attractive candidates to map dopamine reuptake sites by SPECT.

The goals of the present investigation were to develop methods for the synthesis of the *E*- and *Z*-isomers of *N*-(3-iodopropen-1-yl)-2β-carbomethoxy-3β-(*p*-chlorophenyl)-tropane and to characterize the *in vitro* and *in vivo* properties of these agents in order to develop new iodine-123-labeled CNS dopamine transporter SPECT imaging agent agents which exhibit pronounced striatal uptake with high affinity for the transporter complex and low nonspecific binding.

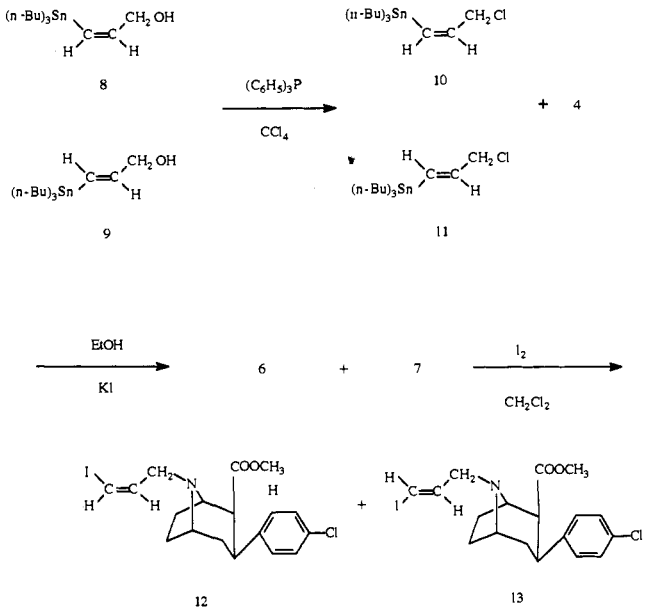
Chemistry

The synthetic route chosen for the preparation of the *N*-(3-iodopropen-1-yl)-2β-carbomethoxy-3β-(4-chlorophenyl)tropanes involved introduction of the iodine at the 3-position of the *N*-propen-1-yl substituent by iododestannylation of an trialkyltin precursor. With use of this approach, the iodine can be selectively introduced in either the trans-(*E*) or cis-(*Z*) stereochemical configuration to enable a study of the structure–activity relationships for the (*E*)-3-iodopropen-1-yl and (*Z*)-3-iodopropen-1-yl derivatives. The key substrate *N*-propyn-1-yl-2β-car-

Scheme 1



Scheme 2



bomethoxy-3β-(4-chlorophenyl)tropane (**5**) was prepared as outlined in Scheme 1. 2β-Carbomethoxy-3β-(4-chlorophenyl)tropane (**2**) was prepared in 55% yield from (*R*)-(−)-anhydroecognine methyl ester by treatment with (4-chlorophenyl)magnesium bromide as previously reported by Carroll.[23] The Michael addition of (4-chlorophenyl)-magnesium bromide to (*R*)-(−)-anhydroecognine methyl ester at −40 °C in ethyl ether followed by trifluoroacetic acid gave 2β-carbomethoxy-3β-(4-chlorophenyl)tropane (**2**) and 2α-carbomethoxy-3β-(4-chlorophenyl)tropane (**3**), the ^{1}H NMR of which displayed two sets of singlets in the NCH_3 region at δ 2.24 and 2.44, respectively. A configurational study of the orientation of the carbomethoxy ester group at the 2-position of a series of 2-carbomethoxy-3β-(*p*-substituted-phenyl)tropanes by ^{1}H NMR has been reported.[21] This study demonstrated that the chemical shift of the 2α-carbomethoxy epimer appears at a region approximately δ 0.2 more dishielded than the 2β-epimer, thus indicating that **2** and **3** were formed in a ratio of 7:3. The mixture of 2-carbomethoxy esters was purified by both fractional crystallization and flash chromatography. Desmethylation of 2β-carbomethoxy-3β-(4-chlorophenyl)-tropane (**2**) was achieved by treatment with 2,2,2-trichloroethyl chloroformate to give an 2,2,2-trichloroethyl carbamate intermediate. The crude carbamate was reduced with zinc and 95% acetic acid to afford 2β-carbomethoxy-3β-(4-chlorophenyl)nortropane (**4**) in 80% overall yield. Alkylation of nortropane **4** with propargyl bromide gave N-substituted alkynyltropane *N*-propyn-1-yl-2β-carbomethoxy-3β-(4-chlorophenyl)tropane (**5**).

The key step in the synthesis of the (*E*/*Z*)-*N*-(3-iodopropen-1-yl)-2β-carbomethoxy-3β-(4-chlorophenyl)-tropanes involved introduction of iodine into the *N*-propynyl group *via* iododestannylation of the tri-*n*-butylstannyl substrates **6** and **7**. Two routes were developed for the preparation of the *E* and *Z* tri-*n*-butylstannyl substrates. In one route, *N*-propyn-1-yl-2β-carbomethoxy-3β-(4-chlorophenyl)tropane (**5**) was hydrostannylated with (n-Bu)$_3$SnH and AIPN to give a 3:1 mixture of *N*-[(*E*)-3-(tri-*n*-butylstannyl)propen-1-yl]-2β-carbomethoxy-3β-(4-chlorophenyl)tropane (**7**) and *N*-[(*Z*)-3-(tri-*n*-butylstannyl)propen-1-yl]-2β-carbomethoxy-3β-(4-chlorophenyl)tropane (**6**). The stannyltropanes **6** and **7** were separated by silica gel flash chromatography. The *Z*- and *E*-confirmations were assigned to **6** and **7** respectively, by 400-MHz ^{1}H NMR spectral analysis of the purified tropanes. The *N*-[(*E*)-3-(tri-n-butylstannyl)propen-1-yl]-2β-carbomethoxy-3β-(4-chlorophenyl)tropane (**7**) exhibited a doublet centered at δ 6.01, for the proton geminal to the tri-*n*-butyltin group, with a large coupling constant J = 18.9 Hz, and a doublet of triplets centered at δ 5.89, for the trans-vinylic proton, with coupling constants J = 19.0 and 5.34 Hz. In comparison, the *N*-[(*Z*)-3-(tri-*n*-butylstannyl)propen-1-yl]-2β-carbomethoxy-3β-(4-chlorophenyl)tropane (**6**) exhibited a doublet centered at δ 5.87 for the proton geminal to the tri-*n*-butyltin group, with an expected smaller coupling constant J = 13 Hz, and a doublet of doublets centered at δ 6.47, for the cis-vinylic proton, with coupling constants J = 7.35 and 0.2 Hz. The assigned configurations were consistent with proton nuclear magnetic resonance spectral properties of reported 3-(tri-*n*-butylstannyl)propen-1-yl derivatives.[32]

The second synthetic route developed for the preparation of tri-*n*-butylstannyl tropanes **6** and **7** involved the alkylation of nortropane **4** with (*Z*)-3-(tri-n-butylstannyl)-propen-1-yl chloride (**10**) and (*E*)-3-(tri-n-butylstannyl)-propen-1-yl chloride (**11**) (Scheme 2). This route was pursued in order to obtain a larger quantity of the (*Z*)-3-iodopropen-1-yl isomer required for the characterization studies and to develop a synthetic method which did not entail a separation of the chromatographically similar stannyltropanes **6** and **7**. Using this synthetic strategy, we converted propargyl alcohol to (*Z*)-3-(tri-*n*-butylstannyl)propen-1-yl alcohol (**8**) and (*E*)-3-(tri-*n*-butylstannyl)-propen-1-yl alcohol (**9**) by the method of Jung.[32] Chlorination of alcohols **8** and **9** with triphenylphosphine and carbon tetrachloride gave the corresponding halides **10** and **11** in 90% and 92% yield, respectively. Alkylation of 2β-carbomethoxy-3β-(4-chlorophenyl)nortropane (**4**) with (*Z*)-3-(tri-n-butylstannyl)propen-1-yl chloride (**10**) and (*E*)-3-(tri-*n*-butylstannyl)propen-1-yl chloride (**11**) gave *N*-[(*Z*)-3-(tri-*n*-butylstannyl)propen-1-yl]-2β-carbomethoxy-3β-(4-chlorophenyl)tropane (**6**) and *N*[(E)-3-(tri-*n*-butylstannyl)propen-1-yl]-2β-carbomethoxy-3β-(4-chlo-

Table 1. Inhibition Constants of Various Ligands on [^{125}I]-13[a] Binding to Rat Striatal Membranes

ligand	K_i, nM (mean ± SEM)	Hill coeff
13	3.2 ± 0.1	1.07
12	6.3 ± 0.5	1.03
GBR 12909	10.4 ± 1.0	1.30
mazindol	41.6 ± 3.7	1.02
cocaine	642 ± 61	0.97

[a] K_d value for [^{125}I]-13 was 0.80 nM.

Table 2. Inhibition Constants of Various Ligands on [^{125}I]-12[a] Binding to Rat Striatal Membranes

ligand	K_i,[b] nM (mean ± SEM)	Hill coeff
13	0.69 ± 0.12	0.90
12	1.27 ± 0.38	0.76
GBR 12909	0.88 ± 0.14	0.88
mazindol	32.0 ± 9.8	0.95
cocaine	603 ± 66	1.03
paroxetine	400 ± 52	1.12
dopamine	>5000	
haloperidol	>1000	
despiramine	>4000	

[a] 0.2–0.3 nM [^{125}I]-13 was incubated in the presence of the indicated ligand in 7–11 concentrations and membrane preparation from rat striatum. Each value represents the mean ± SEM of three to five determinations. [b] K_d value for [^{125}I]-12 was 1.1 nM.

rophenyl)tropane (7), respectively. The stannyltropanes 6 and 7 from both synthetic routes (Schemes 1 and 2) possessed identical physical and spectral properties. Iododestannylation of 6 and 7 by treatment with I_2 in methylene chloride gave *N*-[(*Z*)-3-iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)tropane (12) and *N*-[(*E*)-3-iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)-tropane (13), respectively.

The radioiodinated iodopropen-2-yltropanes *N*-[(*Z*)-3-[^{125}I]iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)tropane (12) and *N*-[(*E*)-3-[^{125}I]iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)tropane (13) were prepared by no-carrier-added Na^{125}I treatment of their corresponding stannyl derivatives with I^+ generated in situ by H_2O_2/HCl oxidation of radioiodide.

Biological Studies

The affinities of *N*-[(*Z*)-3-iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)tropane (12) and *N*-[(*E*)-3-iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)-tropane (13) for the dopamine transporter were determined using *in vitro* competitive binding assays. Competition binding data using [^{125}I]-12 and [^{125}I]-13 are shown in Tables 1 and 2, respectively. The rank of order of potency for binding is *E*-13 > *Z*-12 > GBR 12909 ≫ mazindol ≫ nomifensine > (−)-cocaine. The results from binding studies of [^{125}I]-12 and [^{125}I]-13 demonstrate that the *E*-configuration of the iodine atom in the 3-iodopropen-1-yl group is 2 times more effective than the *Z*-configuration for increasing the competitive binding. Competition binding data to further evaluate the effects of bulk substitution for a methyl group at the bridgehead nitrogen using [^{3}H]CFT and a series of N-substituted unsaturated derivatives were examined. The IC_{50} values for alkynyl 5, alkenyl 14, *E*-13, *Z*-12, nomifisene, and (−)-cocaine are 0.1, 0.1, 0.1, 0.1, 36.9, and 46.2 nM, respectively (data not shown). These data suggest that bulk tolerance exists in this part of the molecule and that additional studies of a series of N-substituted-fluoroalkyl and -fluoroaryl derivatives should be pursued to determine whether these agents are suitable for labeling with fluorine-18 for PET studies.

Table 3. Distribution of Radioactivity in Tissues of Unfasted Male Sprague-Dawley Rats following Intravenous Administration of *N*-[(*E*)-3-[^{125}I]Iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)tropane (13)

	mean % injected dose/organ (average of values for three rats ± SD)			
organ	2 min	30 min	60 min	120 min
blood	2.98 ± 0.15	7.12 ± 0.70	5.24 ± 0.45	4.77 ± 0.73
heart	1.05 ± 0.19	0.29 ± 0.09	0.19 ± 0.02	0.11 ± 0.01
muscle	28.27 ± 2.75	18.54 ± 4.46	12.14 ± 0.36	9.04 ± 0.83
lung	1.19 ± 0.34	0.91 ± 0.11	0.40 ± 0.03	0.31 ± 0.11
kidney	3.11 ± 0.60	0.86 ± 0.16	0.58 ± 0.05	0.46 ± 0.06
spleen	0.41 ± 0.15	0.23 ± 0.08	0.12 ± 0.02	0.09 ± 0.01
liver	11.77 ± 1.53	3.72 ± 0.74	1.97 ± 0.24	1.44 ± 0.14
skin	5.14 ± 1.81	23.76 ± 1.43	23.9 ± 1.60	20.0 ± 1.36
thyroid	0.06 ± 0.01	0.76 ± 0.24	0.97 ± 0.54	1.06 ± 0.79
brain	1.62 ± 0.27	0.58 ± 0.17	0.28 ± 0.11	0.13 ± 0.02
	regional uptake (% dose/g)			
region	2 min	30 min	60 min	120 min
cerebellum	0.97 ± 0.16	0.23 ± 0.12	0.077 ± 0.02	0.037 ± 0.001
striatum	1.15 ± 0.26	1.67 ± 0.29	1.23 ± 0.19	0.61 ± 0.07
hippocampus	0.84 ± 0.10	0.26 ± 0.10	0.095 ± 0.026	0.038 ± 0.001
cortex	1.15 ± 0.14	0.22 ± 0.13	0.08 ± 0.004	0.05 ± 0.02
ST/CB	1.18	6.91	15.9	16.5

The degree of specific to nonspecific binding for each ligand to rat striatal tissue was determined. The specific binding for [^{125}I]-12 and [^{125}I]-13 was found to be saturable and exhibited a K_d of 1.1 and 0.80 nM, respectively. The *Z*-isomer, [^{125}I]-12, exhibits a high degree of nonspecific binding. The *E*-isomer, [^{125}I]-13, on the other hand, exhibits a significantly lower nonspecific binding (<10% at K_d). The binding of both iodinated ligands toward 5-HT uptake sites were examined in the rat frontal cortical homogenates using [^{3}H]citalopram[33] as the labeled ligand. The K_i values obtained were 28.6 and 6.5 nM for *Z*- and *E*-isomers, respectively (data not shown). The results indicated that these iodinated ligands, similar to other tropane derivatives reported, displayed *in vitro* binding toward 5-HT uptake sites.

The distribution of radioactivity expressed as percent dose per organ in tissues of male rats at 2, 30, and 60 min and 2 h after intravenous administration of *N*-[(*E*)-3-[^{125}I]-iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)-tropane (13) is shown in Table 3. The initial level of accumulation of radioactivity in the brain after injection of [^{125}I]-13 was high. The agent, however, exhibited rapid global washout from the brain. The brain uptake exhibited a maximum at 2 min (1.62% dose/organ) and exhibited a 65% decrease at 30 min (0.58% dose/organ). After 2 h the brain uptake (0.13% dose/organ) had decreased 92% when compared to the peak uptake at 2 min. The magnitude and kinetics of [^{125}I]-13 washout from the brain is expected for a low-capacity high-affinity binding selective receptor agent.

The skeletal muscle, liver, and kidneys also showed high initial uptake of radioactivity. The accumulation of radioactivity in the skeletal muscle, liver, and kidneys reached a maximum of 28.27, 11.77, and 3.11% dose/organ respectively at 2 min after intravenous injection. The skeletal muscle, liver, and kidneys exhibited an elimination of 67, 75, and 75%, respectively, at 2 h compared with their uptake at 2 min. The accumulation of radioactivity in the thyroid was initially low. However, the thyroid radioactivity showed a gradual increase from 0.06% dose/organ at 2 min to 1.06% dose/organ at 2 h, which

Table 4. Distribution of Radioactivity in Tissues of Unfasted Male Sprague-Dawley Rats following Intravenous Administration of *N*-[(*Z*)-3-[^{125}I]Iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)tropane (**12**)

	mean % injected dose/organ (average of values for three rats ± SD)			
organ	2 min	30 min	60 min	120 min
blood	5.01 ± 0.22	8.00 ± 1.11	5.77 ± 0.50	5.16 ± 0.70
heart	0.85 ± 0.13	0.22 ± 0.05	0.16 ± 0.03	0.11 ± 0.01
muscle	29.15 ± 2.75	17.43 ± 3.58	12.22 ± 0.29	9.22 ± 0.91
lung	1.23 ± 0.21	0.78 ± 0.09	0.50 ± 0.04	0.40 ± 0.10
kidney	2.65 ± 0.43	0.78 ± 0.13	0.56 ± 0.05	0.46 ± 0.05
spleen	0.40 ± 0.10	0.19 ± 0.05	0.11 ± 0.02	0.09 ± 0.01
liver	12.13 ± 1.13	2.78 ± 0.60	1.60 ± 0.29	1.28 ± 0.12
skin	7.27 ± 1.61	26.73 ± 1.02	24.81 ± 1.39	19.30 ± 1.20
thyroid	0.08 ± 0.003	1.02 ± 0.27	0.94 ± 0.54	1.29 ± 0.96
brain	1.27 ± 0.17	0.21 ± 0.08	0.07 ± 0.02	0.04 ± 0.004
	regional uptake (% dose/g)			
region	2 min	30 min	60 min	120 min
cerebellum	0.82 ± 0.10	0.19 ± 0.06	0.04 ± 0.007	0.02 ± 0.002
striatum	0.92 ± 0.17	0.33 ± 0.11	0.11 ± 0.03	0.05 ± 0.004
hippocampus	0.69 ± 0.06	0.12 ± 0.05	0.04 ± 0.009	0.03 ± 0.001
cortex	0.91 ± 0.13	0.11 ± 0.04	0.04 ± 0.009	0.03 ± 0.001
ST/CB	1.12	3.0	2.7	1.9

demonstrated the expected stability of the iodovinyl group to significant *in vivo* deiodination.

The regional distribution of radioactivity expressed as percent dose per gram in brain of male rats at 2, 30, and 60 min and 2 h after intravenous administration of *N*-[(*E*)-3-[^{125}I]iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)tropane (**13**) is also shown in Table 3. The level of accumulation of radioactivity in the striatum, a region rich in dopamine reuptake sites, was significant. The *E*-isomer exhibited a prolonged retention in the striatum. The striatum uptake reached a maximum at 30 min (1.67% dose/g) and exhibited a 25% decrease at 60 min (1.23% dose/g). After 120 min, the striatum uptake decreased 64% when compared to the peak activity at 30 min. The striatum to cerebellum (ST/CB) ratio exhibited a pronounced increase with time. The ST/CB ratio was 1.18, 6.91, 15.9, and 16.5 at 2, 30, 60, and 120 min, respectively. In contrast to the striatum, the cerebellum (CB), hippocampus (HP), and cortex (CX) regions of low dopamine receptor density displayed a rapid washout of radioactivity; HP/CB and CX/CB ratios at 120 min were 1 and 1.5, respectively. These data strongly suggest that iodine-123-labeled *N*-[(*E*)-3-iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)tropane (**13**) is a potentially useful agent for mapping dopamine reuptake sites with single-photon tomography.

Tissue distribution studies in male rats (Table 4) after intravenous administration of *N*-[(*Z*)-3-[^{125}I]iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)tropane (**12**) demonstrated that the *Z*-isomer did not exhibit receptor affinity. The initial accumulation of radioactivity in the whole brain and striatum after injection of this agent was high (1.27% dose/organ and 0.92% dose/g at 2 min, respectively), similar to that of *N*-[(*E*)-3-[^{125}I]iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)tropane (**13**). However, the ST, CB, HP, and CX regions displayed a rapid washout of radioactivity with time. The ST/CB ratio was 1.12, 3.0, 2.7, and 1.9 at 2, 30, 60, and 120 min, respectively. The higher striatal uptake and retention of the *E*-isomer in comparison to the *Z*-isomer is consistent with the results from the saturation curves which show that the *E*-isomer exhibits a higher affinity with lower nonspecific binding. Even though the differences in the selectivity for the dopamine transporter by the *E*- and *Z*-isomers can be explained from the *in vitro binding studies*, it is still surprising that the subtle translocation of the iodine from the *Z*- to the *E*-configuration gives rise to such a dramatic enhancement in selectivity. Further evaluation of *N*-alkenyl-substituted derivatives of this series of 3β-(*p*-substituted-phenyl)tropane-2β-carboxylic acid esters should be pursued to gain further insight into the structural requirements for binding to the dopamine transporter.

Conclusion

Synthetic routes have been developed for the syntheses of *N*-[(*E*)-3-[^{125}I]iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)tropane and *N*-[(*Z*)-3-[^{125}I]iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)tropane. *In vitro* and *in vivo* studies demonstrated that the *E*-radioiodinated isomer showed high affinity for the dopamine transporter along with significant striatal uptake and retention in rats. The very high striatal to cerebellum ratios and *in vivo stability to* deiodination suggest that iodine-123-labeled *N*-[(*E*)-3-iodopropen-1-yl]-2β-carbomethoxy-3β-(*p*-chlorophenyl)tropane is an excellent candidate for imaging dopamine reuptake sites in humans in conjunction with SPECT to study neurogenerative disease and aid in the development of new agents for the treatment of cocaine abuse.

Experimental Section

All chemicals and solvents were analytical grade and were used without purification. The iodine-125 (^{125}I) and iodine-123 (^{123}I) were obtained commercially. The melting points (mp) were determined in capillary tubes using an Electrothermal apparatus and are uncorrected. The thin-layer chromatographic (TLC) analyses were performed using 250-μm-thick layers of silica gel G PF-254 coated on aluminum plates (Whatman). The proton nuclear magnetic resonance (NMR) spectra were obtained at 90 and 400 MHz with a Varian 390 instrument and a Brooker instrument, respectively. Carbon-13 nuclear magnetic resonance spectra were obtained at 400 MHz with a Brooker instrument. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA) and, unless noted otherwise, were within ±0.4% of the calculated values. High-resolution voltage scans were performed at 70 EV with reference scans over narrow mass, ±0.0006. Analyses were within 0.002 of the theoretical values. Mass spectra (MS) were determined on a ZAB-EQ (VG Analytical) hybrid high-resolution, double-focusing mass spectrometer with collision and analyzing quadrupole.

Animal Tissue Distribution Experiments. The distribution of radioactivity was determined in tissues of male Sprague-Dawley rats (225–300 g) after intravenous administration of the radioiodinated tropane. The animals were allowed food and water *ad libitum* prior to the course of the experiment. The radioiodinated tropane [^{125}I]-**12** and [^{125}I]-**13** (8–10 μCi) in 0.2 mL of 0.9% NaCl was injected directly into the femoral vein of rats under ether anesthesia. The animals were sacrificed at various time points postinjection by cardiac excision under halothane anesthesia and the organs excised, rinsed, and blotted dry. The organs were weighed and the radioactivity of the contents was determined with a Packard δ automatic counter (Model 5000). The percent dose per organ was calculated by a comparison of the tissue counts to suitably diluted aliquots of injected material. Total activities of blood and muscle were calculated under the assumption that they were 7% and 40% of the total body weight, respectively.

Regional brain distribution was obtained in male Sprague-Dawley rats (225–300 g) after intravenous administration of [^{125}I]-**12** and [^{125}I]-**13**. The cortex, striatum, hippocampus, and cerebellum were dissected and placed in tared test tubes. The test tubes were weighed, and the radioactivity of the contents

was determined with a δ automatic counter. The percent dose per gram was calculated by a comparison of the tissue counts with the counts of the diluted initial injected dose. The uptake ratio of each brain region was obtained by dividing the percent/gram of each region by that of the cerebellum.

Tissue Preparation. Male Sprague-Dawley rats (200–250 g) were decapitated under ether anesthesia and the brains excised and placed in ice. Striatal and cortical tissues were dissected, pooled, and homogenized in 100 volumes (w/v) of ice cold Tris-HCl buffer (50 mM, pH 7.4). The tissue homogenates were centrifuged at 20000*g* for 20 min and the resultants pellets were rehomogenized in the same buffer and recentrifuged. The final pellets were resuspended in a buffer composed of 50 mM Tris buffer, pH 7.4; 120 mM NaCl; 5 mM KCl; 2 mM $CaCl_2$; and 1 mM $MgCl_2$ and kept at −20 °C for the binding assay detailed below.

Binding Assays. For dopamine reuptake site assay striatal tissue preparations (50 μL, 40–60 μg protein) were incubated with appropriate amounts of iodine-125-labeled ligand and competitors in a total volume of 0.2 mL of the assay buffer. The assay mixture was incubated for 60 min at room temperature with stirring, and the resulting samples were rapidly filtered through Whatman GF/B glass-fiber filters pretreated with 0.2% protamine base and washed with 3 × 5 mL of cold (4 °C) 50 mM Tris-HCl buffer, pH 7.4. The nonspecific binding was obtained in the presence of 10 μM (−)-cocaine. The filters were counted in a γ-counter (Packard 5000) at an efficiency of 70%. Saturation binding, Scatchard, and competition experiments were analyzed with the iterative nonlinear-least-squares curve-fitting program Ligand.[34] Binding of [^{3}H]citalopram (specific activity, 86.8 Ci/mmol) was performed according to the procedure described by D'Amato.[33] Nonspecific binding was defined in the presence of 0.5 μM paroxetine.

Chemistry. 2β-Carbomethoxy-3β-(4-chlorophenyl)tropane (2). (*R*)-(−)-Anhydroecognine methyl ester (1; 1.48 g, 8 mmol) was dissolved in 25 mL of anhydrous Et_2O and added to (4-chlorophenyl)magnesium bromide (8.64 g, 40 mmol) at −40 °C as described earlier.[21] The resulting mixture was cooled to −78 °C and treated dropwise with a solution of 5 mL of trifluoroacetic acid and 25 mL of anhydrous Et_2O while a temperature of −78 °C was maintained. The reaction mixture was warmed to 0 °C and worked up as previously reported.[23] The dried Et_2O layer was evaporated in vacuo to yield 2.24 g of a yellow solid. The crude product was crystallized from petroleum ether (30–60 °C) to give **2** as yellow rosettes (1.56 g, 55%). Mp: 123.5–125.5 °C. A single component was detected by TLC (CH_3CN:H_2O:NH_4OH 90:10:1): R_f 0.40. ^{1}H NMR (90 MHz, $CDCl_3$): 1.5–1.9 (m, 4 H, CH_2CH_2), 2.24 (s, 3 H, NCH_3), 2.85–2.95 (m, 2 H, C2eq, C3ax), 3.52 (s, 3 H, CO_2CH_3), 7.23 (s, 4 H, ArH).

2β-Carbomethoxy-3β-(4-chlorophenyl)nortropane (4). A mixture of the tropane (2, 587 mg, 2 mmol) and 1.5 mL of 2,2,2-trichloroethyl chloroformate (11 mmol) was heated at 120 °C and stirred for 75 min. The resulting solution was cooled to room temperature and vacuum distilled (bath temperature, 60 °C) to remove the unreacted 2,2,2-trichloroethyl chloroformate. The resulting crude carbamate **3** was used without further purification. The carbamate was dissolved in 15 mL of 95% acetic acid and treated with zinc dust (1.6g, 54 mmol). The mixture was stirred at room temperature for 16 h, treated with Celite and filtered. The salts were washed with 15 mL of 95% acetic acid and the combined filtrates extracted with $CHCl_3$ (4 × 50 mL). The combined $CHCl_3$ extracts were washed with 10% NaOH until the aqueous layers remained basic. The combined aqueous layers were extracted with 50 mL of $CHCl_3$, and the $CHCl_3$ fractions (250 mL) were washed with H_2O (2 × 50 mL) and dried over anhydrous $MgSO_4$, and the solvent was removed in vacuo to afford 494 mg of a viscous oil which crystallized on standing. The crude solid was purified by flash chromatography (silica, Et_2O:triethylamine, 90:10) to afford 0.45g of 4 (80%) as a white solid. Mp: 120–121 °C. A single component was detected by TLC (CH_3CN:H_2O:NH_4OH 90:10:1): Rf 0.23. ^{1}H NMR (90 MHz, $CDCl_3$): 1.5–1.9 (m, 4 H, CH_2CH_2), 2.85–2.95 (m, 2 H, C2eq, C3ax), 3.52 (s, 3 H, CO_2CH_3), 7.24 (s, AB q, 4 H, J = 12 Hz, ArH).

***N*-Propyn-1-yl-2β-carbomethoxy-3β-(4-chlorophenyl)tropane (5).** 2β-Carbomethoxy-3β-(4-chlorophenyl)nortropane (4, 280 mg, 1 mmol) was added to 3 mL of absolute ethanol containing propargyl bromide, 80% in toluene (150 mg, 1.25 mmol), and a catalytic amount of KI. The reaction mixture was stirred and heated at 70 °C for 16 h. After cooling to room temperature, the mixture was added to 25 mL of H_2O containing 5 mL of 5% $NaHCO_3$ and the mixture was extracted with Et_2O (3 × 10 mL). The combined Et_2O extracts were washed with H_2O (3 × 10 mL) and dried over anhydrous $MgSO_4$, and the solvent was removed to give 200 mg (63%) of 5 as a white solid. The crude solid was purified by flash chromatography (silica, Et_2O:triethylamine, 95:5) to afford 190 mg of 5 (60%) as a white solid. Mp: 127–129 °C. A single component was detected by TLC (CH_3CN:H_2O:NH_4OH 90:10:1): R_f 0.75. ^{1}H NMR (90 MHz, $CDCl_3$): 1.5–1.9 (m, 4 H, CH_2CH_2), 2.15 (m, 1 H, C≡CH), 2.90–3.15 (m, 2 H, C2eq, C3ax), 3.45 (d, 2 H, J = 6 Hz, $CH_2C{\equiv}C$), 3.50 (s, 3 H, CO_2CH_3), 7.23 (s, 4 H, ArH). MS: m/z 317 [^{35}Cl] (M^+, 35) 317.1186, theoretical 317.1182; 319 [^{37}Cl] (M^+, 12) 319.1165, theoretical 319.1153.

1-Chloro-(*Z*)-3-(tri-*n*-butylstannyl)propen-1-yl Chloride (10). (*Z*)-3-(Tri-*n*-butylstannyl)-2-propen-1-ol (8, 405 mg, 1.15 mmol) was dissolved in 2 mL of CCl_4 containing triphenylphosphine (350 mg, 1.3 mmol) and the solution was stirred and heated in a sealed reaction vessel at 60 °C for 48 h. After cooling to room temperature, the crude mixture was purified by flash chromatography (silica, petroleum ether (30–60 °C) (PE):triethylamine 99:1) to afford 335 mg of 10 (90%) as a colorless oil. A single component was detected by TLC (benzene:PE 1:1): R_f 0.80. ^{1}H NMR (400 MHz, $CDCl_3$): 0.9–1.46 (m, $SnBu_3$), 3.97 (dd, 2 H, J = 7.35, 0.3 Hz, CH_2Cl), 6.16 (d, 1 H, J = 12.6 Hz, C=$CH(SnBu_3)_3$, 6.64 (dt, 1 H, J = 12.6, 7.3 Hz, C=$CH(CH_2Cl)$.

1-Chloro-(*E*)-3-(tri-*n*-butylstannyl)propen-1-yl Chloride (11). (*E*)-3-(Tri-*n*-butylstannyl)-2-propen-1-ol (9, 365 mg, 1.05 mmol) was dissolved in 2 mL of CCl_4 containing triphenylphosphine (340 mg, 1.28 mmol) and the mixture treated as described for **10**. The crude mixture was purified by flash chromatography (silica, PE:triethylamine 99:1) to afford 313 mg of 11 (92%) as a colorless oil. A single component was detected by TLC (benzene/PE 1:1) R_f 0.76. ^{1}H NMR (400 MHz, $CDCl_3$): 0.9–1.46 (m, $SnBu_3$), 4.06 (dt, 2 H, J = 6.1, 1.2 Hz, CH_2Cl), 6.06 (dt, 1 H, J = 18.6, 6.15 Hz, C=$CH(CH_2Cl)$), 6.30 (dt, 1 H, J = 18.6, 1.1 Hz, C=$CH(SnBu_3)$).

2β-Carbomethoxy-3β-(4-chlorophenyl)-8-[(*Z*)-3-(tributylstannyl)propen-1-yl]nortropane (6) and 2β-Carbomethoxy-3β-(4-chlorophenyl)-8-[(*E*)-3-(tributylstannyl)propen-1-yl]-nortropane (7). Alkyne **5** (63 mg, 0.02 mmol) was dissolved in 1 mL of toluene containing tributyltin hydride (110 mg, 0.04 mmol) and azobisisoproprionitrile (10 mg) and the resulting mixture was refluxed for 16 h under an argon atmosphere. After cooling to room temperature, the crude mixture was purified by flash chromatography (silica, Et_2O:triethylamine 90:10) to afford 16 mg of **6** (13%) as an oil and 21 mg of **7** (36%) as a waxy solid. A single componet was for **6** and **7** detected by TLC (CH_3CN:H_2O:NH_4OH 90:10:1): R_f 0.75 and 0.70, respectively.

Method B. 2β-Carbomethoxy-3β-(4-chlorophenyl)-8[(*Z*)-3-(tributylstannyl)propen-1-yl]nortropane (6). Nortropane **5** (56 mg, 0.20 mmol) and 1-chloro-(*Z*)-3-(tri-*n*-butylstannyl)-propen-1-yl chloride (**10**, 73 mg, 0.20 mmol) were dissolved in 2 mL of absolute ethanol containing a catalytic amount of KI, and the mixture was treated as described for **5**. The dried Et_2O extracts were concentrated, and the crude mixture was purified by flash chromatography (silica, Et_2O:triethylamine 95:5) to afford 60 mg of **6** (50%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): 0.9–1.46 (m, $SnBu_3$), 1.5–1.9 (m, 4 H, CH_2CH_2), 2.90–3.15 (m, 2 H, C2eq, C3ax), 3.45 (dd, 2 H, J = 7.35, 0.2 Hz, CH_2N), 3.50 (s, 3 H, CO_2CH_3), 5.87 (d, 1 H, J = 12.6 Hz, C=$CH(SnBu_3)$), 6.64 (dt, 1 H, J = 12.6, 7.3 Hz, C=$CH(CH_2N)$), 7.23 (s, 4 H, ArH). MS: m/z 552 ([M^+] − C_4H_9, 100) 552.1692, theoretical mass 552.1689; 550 ([M^+ − 2] − C_4H_9, 68) 550.1688, theoretical mass 550.1685; 554 ([M^+ + 2] − C_4H_9, 35) 554.1685, theoretical mass 554.1683.

2β-Carbomethoxy-3β-(4-chlorophenyl)-8[(*E*)-3-(tributylstannyl)propen-1-yl]nortropane (7). Nortropane **5** (56 mg, 0.20 mmol) and 1-chloro-(*E*)-3-(tri-n-butylstannyl)propen-1-yl chloride (**11**, 73 mg, 0.20 mmol) were dissolved in 2 mL of absolute ethanol containing a catalytic amount of KI, and the mixture

was treated as described for **5**. The dried Et_2O extracts were concentrated, and the crude mixture was purified by flash chromatography (silica, Et_2O:triethylamine, 95:5) to afford 57 mg of **7** (50%) as a waxy solid. ^1H NMR (400 MHz, $CDCl_3$): 0.9–1.46 (m, $SnBu_3$), 1.5–1.9 (m, 4 H, $CHCH_2$), 2.90–3.15 (m, 2 H, C2eq, C3ax), 3.45 (dd, 2 H, J = 7.35, 0.2 Hz, CH_2N), 3.50 (s, 3 H, CO_2CH_3), 5.89 (dt, 1 H, J = 19.0, 5.34 Hz, C=CH($SnBu_3$)), 6.01 (d, 1 H, J = 18.9 Hz, C=CH(CH_2N)), 7.23 (s, 4 H, ArH). MS: m/z 552 ([M^+] – C_4H_9, 100) 552.1692, theoretical mass 552.1689; 550 ([M^+ – 2] – C_4H_9, 72) 550.1700, theoretical mass 550.1685; 554 ([M^+ + 2] – C_4H_9, 554.1673, theoretical mass 554.1683.

2β-Carbomethoxy-3β-(4-chlorophenyl)-8-[(*Z*)-3-iodopropen-1-yl]nortropane (12). Stannyl ester **10** (28 mg, 0.046 mmol) was dissolved in 10 mL of CH_2Cl_2 and the resulting solution cooled to 0–5 °C. Iodine (12 mg, 0.046 mmol) was added and the resulting mixture stirred at 0–5 °C until a colorless solution resulted. The CH_2Cl_2 solution was washed with 10% $NaHSO_3$ and dried over anhydrous magnesium sulfate. The dried CH_2Cl_2 was removed in vacuo to yield an oil and the crude mixture was purified by flash chromatography (silica, Et_2O:triethylamine 95:5) to afford 14 mg of **12** (70%) as a waxy solid. A single component was detected by TLC (CH_3CN:H_2O:NH_4OH 90:10:1): R_f 0.78. ^1H NMR (90 MHz, $CDCl_3$): 1.5–1.9 (m, 4 H, CH_2CH_2), 2.90–3.15 (m, 2 H, C2eq, C3ax), 3.45 (d, 2 H, J = 6 Hz, CH_2C=C), 3.50 (s, 3 H, CO_2CH_3), 7.23 (s, 4 h, ArH). MS: m/z 445 (M^+, 70), 381 (M^+ – 127, 100), 445.0284, theoretical mass 445.0306.

2β-Carbomethoxy-3β-(4-chlorophenyl)-8-[(*E*)-3-iodopropen-1-yl]nortropane (13). Stannyl ester **11** (28 mg, 0.046 mmol) was dissolved in 10 mL of CH_2Cl_2 and the resulting solution treated with iodine (12 mg, 0.046 mmol) as described for **12**. The dried CH_2Cl_2 was removed in vacuo to yield an oil and the crude mixture was purified by flash chromatography (silica, Et_2O: triethylamine 95:5) to afford 15 mg (75%) of **13** as a waxy solid. A single component was detected by TLC (CH_3CN:H_2O:NH_4OH 90:10:1): R_f 0.78; ^1H NMR (90 MHz, $CDCl_3$): 1.5–1.9 (m, 4 H, CH_2CH_2), 2.90–3.15 (m, 2 H, C2eq, C3ax), 3.45 (d, 2 H, J = 6 Hz, CH_2C=C), 3.50 (s, 3 H, CO_2CH_3), 7.23 (s, 4 H, ArH). MS: m/z 445 (M^+, 70), 318 (M^+ – 127, 100), 445.0302, theoretical mass 445.0306.

2β-Carbomethoxy-3β-(4-chlorophenyl)-8-allylnortropane (14). Nortropane **5** (56 mg, 0.20 mmol) and allyl bromide (26 mg, 0.22 mmol) were dissolved in 2 mL of absolute ethanol containing a catalytic amount of KI, and the mixture was treated as described for **5**. The dried Et_2O extracts were concentrated, and the crude mixture was purified by flash chromatography (silica, Et_2O:triethylamine 95:5) to afford 41 mg of **14** (65%) as a white solid: Mp: 83–85 °C. A single component was detected by TLC (CH_3CN:H_2O:NH_4OH 90:10:1): R_f 0.77. ^1H NMR (90 MHz, $CDCl_3$), 1.5–1.9 (m, 4 H, CH_2CH_2), 2.90–3.15 (m, 2 H, C2eq, C3ax), 3.45 (m, 2 H, CH_2CH=CH_2), 3.50 (s, 3 H, CO_2CH_3), 4.7–5.0 (m, 3 H, CH=CH_2) 7.23 (s, 4 h, ArH). MS: m/z 319 (M^+, 55), 319.1329 theoretical mass, 319.13222 calculated mass.

Radiolabeling. [^{125}I]-2β-Carbomethoxy-3β-(4-chlorophenyl)-8-[(*Z*)-3-iodopropen-1-yl]nortropane (**12**) and [^{125}I]-2β-carbomethoxy-3β-(4-chlorophenyl)-8-[(*E*)-3-iodopropen-1-yl]nortropane (**13**) were prepared by the method reported previously for radioiodinated IBF.[35] Iodination kits were formulated by dispensing 50 μL (50 μg) of the tin substrate **10** or **11** (1 mg/mL of EtOH), in 2-mL vials. Aqueous hydrogen peroxide (50 μL, 3% w/v) was added to a septum sealed vial containing compound **10** or **11** (50 μg) in 50 μL of 0.1 N HCl, and 5 μL of [^{125}I]sodium iodide (no carrier added, sp act., 2200 Ci/mmol). The reaction was allowed to proceed at ambient temperature for 30 min. The reaction was quenched with 0.1 mL of sodium bisulfite (300 mg/mL, basified to pH 8.5 with saturated $NaHCO_3$) and extracted with ethyl acetate (1 × 3 mL). The combined ethyl acetate extracts were dried by passage through a 0.2 cm × 5 cm column of anhydrous sodium sulfate. The ethyl acetate was evaporated by a stream of nitrogen and the residue was dissolved in 50-100 μL of 100% EtOH. The radioiodinated solution was purified by HPLC using a reverse-phase column (PRP-1, Hamilton Inc.) and an isocratic solvent of 93% acetonitrile–7% pH 7.0 buffer (5 mM, 3,3-dimethylglutaric acid) and a flow rate of 1.0 mL/min. The fractions containing the desired product was collected, condensed, and extracted with ethyl acetate (3 × 1 mL). The combined ethyl acetate extracts containing the no-carrier-added product were evaporated by a stream of nitrogen, and the residue was dissolved in 50–100 μL of 100% EtOH (purity > 99%, overal yield 90%). The radioiodinated products [^{125}I]-**12** and -**13** was analyzed by sequential UV and radioactivity detectors. The products exhibited identical elution profiles when compared with chemically pure authentic product with retention times of 11–12 min. UV analysis of an aliquot of the fractions verified the absence of any UV-detectable material. The ethanolic solutions of radioiodinated **12** and **13** were diluted with 0.9% saline and used for the *in vitro* and *in vivo* studies.

Acknowledgment. The authors thank Valerie Hendrix for assistance in preparing the manuscript and Dr. Charles L. Anderson for his assistance in obtaining 400-MHz NMR spectra.

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