N-Substituted Analogs of 2β-Carbomethoxy-3β-(4′-iodophenyl)tropane (β-CIT) with Selective Affinity to Dopamine or Serotonin Transporters in Rat Forebrain

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This report concerns the synthesis and chemical characterization of novel series of N-substituted 2β-carbomethoxy-3β-(4′-iodophenyl)tropane (β-CIT, **2**) analogs and their neuropharmacological evaluation for affinity at dopamine (DA_T), serotonin ($5\text{-}HT_T$), and norepinephrine membrane transporters in rat brain tissue. N-Substituted analogs of β-CIT with a 2β-carbomethoxy ester moiety showed lower DA_T affinity than β-CIT for the DA_T, and some were more selective for the $5\text{-}HT_T$ over the DA_T. 2β-Carbomethoxy(iodophenyl)nortropane analogs of β-CIT with the N-substituents difluoroethyl, mesoxypropyl, iodopropyl, and methylpropionyl all yielded >10-fold lower DA_T affinity than β-CIT itself, whereas the *N*-(fluoropropyl)-2β-isopropyl ester analog (**1**) of β-CIT exceeded β-CIT (**2**, an *N*-methyl-2β-carbomethoxy ester) in DA_T affinity. Several *N*-haloalkyl-substituted β-CIT analogs yielded high $5\text{-}HT_T$ affinity (K_i < 0.6 nM), ranking: *N*-fluoropropyl (**5**) > *N*-chloropropyl (**4**) ≥ *N*-bromopropyl (**3**) > β-CIT (**2**) > *N*-3′-phthalimidopropyl (**11**), with particularly high (ca. 30-fold) $5\text{-}HT_T$-over-DA_T selectivity found in the *N*-fluoropropyl (**5**) and *N*-fluoroethyl (**6**) compounds, compared to only 3.0-fold $5\text{-}HT_T$ selectivity in β-CIT itself. Highly $5\text{-}HT_T$ selective agents such as **5** and **6** may be useful as brain-imaging ligands for serotonin neurons or as mood-elevating drugs, while the high affinity and selectivity for the DA transporter found in *N*-(fluoropropyl)-2β-(carboxyisopropyl)-3β-(4′-iodophenyl)-nortropane (**1**) and *N*-(fluoropropyl)-2β-carboxymethoxy-3β-(4′-iodophenyl)nortropane (FP-β-CIT, **5**) support their use as improved markers for DA neurons.

The benzoyltropane cocaine is one of the most powerful central nervous system stimulants, and its illicit consumption is a major public health and social problem. Intensive efforts have been devoted to elucidating its mode of action. At a molecular level, the natural active isomer of the benzoyltropane, (−)-cocaine (Figure 1), preferentially binds to dopamine transporter (DA_T) proteins in the cell membranes of DA neurons, with lesser interactions with amine transporters for norepinephrine (NE_T) and serotonin (5-hydroxytryptamine, $5\text{-}HT_T$).[1−5] This selective interaction with DA_T inhibits the major physiological process for inactivation of DA by neuronal reuptake and potentiates dopaminergic neurotransmission in the forebrain.[6−8] Saturable binding sites for (−)-cocaine at the DA_T associated with DA-containing nerve terminals have been identified in striatal (caudate-putamen) tissue in the forebrain of rodents,[4] nonhuman primates,[5] and man.[9]

The synthetic (halophenyl)tropane analogs of cocaine, 2β-carbomethoxy-3β-(4′-fluorophenyl)tropane (CFT, also designated WIN 35,428, **14**) and its 4′-iodophenyl congener β-CIT (also designated RTI-55, **2**), have high affinity at DA_T and are lead compounds for a growing number of relatively stable, long-acting ligands for the DA_T. In the phenyltropanes, the aromatic ring is attached directly to the tropane system (Figure 1), thus avoiding hydrolysis of the 3β-benzoyl ester that produces

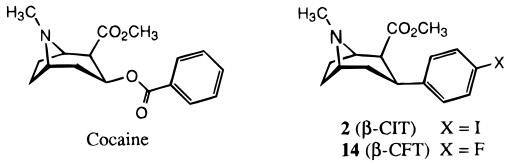

Figure 1.

rapid metabolic inactivation of cocaine.[10] These compounds bind avidly to the DA_T but also interact with the $5\text{-}HT_T$.[10−16] Despite their shared affinity for $5\text{-}HT_T$ as well as DA_T, they appear to label selectively the transporter molecules on DA neurons which are particularly densely distributed in the basal ganglia.[2,4,5,9] Radiolabeled [^{11}C]β-CFT[15,17] and [^{123}I]β-CIT[10−14] are useful imaging agents for positron emission tomography (PET) and single-photon emission-computed tomography (SPECT), respectively. This technique has especially important potential for clinical applications in the diagnosis of Parkinson's disease (paralysis agitans) and in monitoring the progressive loss of DA cells in that common idiopathic neurodegenerative disorder.[18]

Cocaine and its phenyltropane analogs bear a tertiary amino nitrogen which is sufficiently basic to be protonated at physiological pH. Since the interaction between cocaine and monoamine reuptake sites may involve electrostatic or hydrogen-bonding interactions, N-substituents that change electron density at the nitrogen atom and the lipophilicity of phenyltropanes should affect their transporter-binding properties, possibly with gains in selectivity for specific transporters. We recently reported that *N*-fluoroalkyl analogs of such phenyltropanes, *N*-(3-fluoropropyl)-2β-carbomethoxy-3β-

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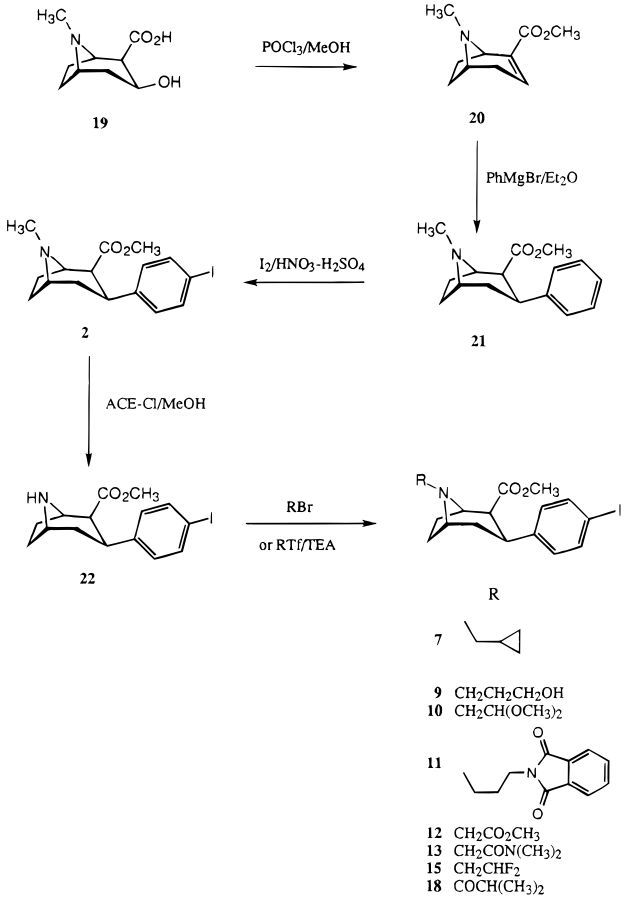
Scheme 1



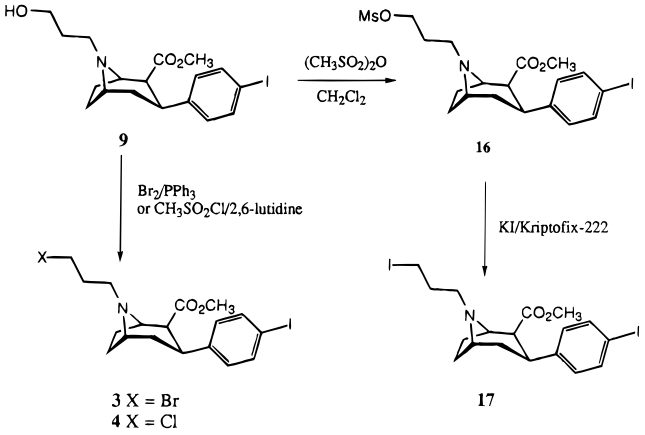
(4′-iodophenyl)nortropane (FP-β-CIT, **5**), *N*-(2-fluoroethyl)-2β-carbomethoxy-3β-(4′-iodophenyl)nortropane (FE-β-CIT, **6**), and their isopropyl ester congeners **1** and **8**, showed high DA_T affinity.[19] These halogenated compounds are promising agents for PET when labeled with ^{18}F[20] or ^{11}C,[20,21] as well as for SPECT when labeled with ^{123}I.[22,23] In a program to develop additional probes of monoamine transporters and the sites of action of cocaine, we have synthesized and measured the binding affinity of a series of phenyltropane analogs in which the *N*-methyl group was systematically modified.

Chemistry

N-Substituted analogs (**3**, **4**, **7**, **9**−**13**, **15**−**18**) of β-CIT (**2**) were prepared by the general routes shown in Schemes 1 and 2. Nor-β-CIT (**22**) was prepared as described previously.[10,19] Thus, ecgonine (**19**) was reacted with phosphorus oxychloride followed by treatment with methanol to give anhydroecgonine methyl ester (**20**). This intermediate was reacted with phenylmagnesium bromide in diethyl ether to produce the 2β-carbomethoxy-3β-phenyltropane (**21**) using an established quenching procedure.[10] Iodination of compound **21** was achieved efficiently with iodine dissolved in HNO_3/H_2SO_4 to yield (**2**), which was N-demethylated with α-chloroethyl chloroformate (ACE-Cl) to yield the desired starting material **22**.

Modification of the synthetic method described previously for the preparation of *N*-(fluoroalkyl)phenyltropanes **1**, **5**, **6**, and **8**[19] employed alkyl bromides to synthesize the *N*-alkyl-substituted derivatives **7**, **9**−**13**, and **18**. Attempts to N-alkylate compound **22** with

Scheme 2



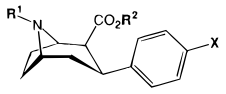
commercially available 2,2′-difluoroethyl iodide failed to give compound **15**; however, with triflate as the leaving group, compound **15** was obtained in 46% yield. When compound **9** was treated with methanesulfonyl chloride in the presence of 2,6-lutidine (Scheme 2) for 48 h, *N*-(chloropropyl)nor-β-CIT (**4**) was isolated. Apparently, mesylate formed during the reaction continued to react with the chloride generated from mesyl chloride. However, we found that the mesylate **16** was readily prepared by treatment of the alcohol **9** with methanesulfonic anhydride. The *N*-bromopropyl analog **3** was prepared from **9** by treatment with Br_2/PPh_3 at 0−5 °C. Displacement of the mesyl group of **16** with iodine gave the corresponding *N*-(iodopropyl)nor-β-CIT (**17**).

Results and Discussion

The transporter-binding affinity of test compounds was evaluated by competitive *in vitro* radioaffinity assays (see the Experimental Section) for DA_T,[19,24,25] 5-HT_T,[26] and NE_T[27] sites in rat forebrain tissue. The results are summarized in Table 1, with compounds ranked in descending order of affinity at the DA_T site. The observed pattern of binding affinities generally indicated a closer correlation of affinities for DA_T and 5-HT_T than of either of these with NE_T affinity.

The novel N-substituted (iodophenyl)tropane compound with the greatest DA_T affinity (K_i = 1.2 nM) was the *N*-fluoropropyl carboisopropyl analog of β-CIT, compound **1**. It was also the most DA_T-selective agent over both NE_T (8300-fold) and 5-HT_T (41-fold). In contrast, while β-CIT (**2**) itself (*N*-methyl substituted) had slightly lower affinity than **1** at DA_T sites (K_i = 1.4 nM), it was only weakly selective for DA_T over NE_T sites (2-fold) and actually 3-fold less selective for DA_T than 5-HT_T binding (Table 1). The presence of a *N*-fluoropropyl moiety apparently did not assure high DA_T affinity or selectivity since the *N*-fluoropropyl congener of β-CIT (FP-β-CIT, **5**) had somewhat lower DA_T affinity than β-CIT itself and was 32 times *less* selective for DA_T than for 5-HT_T (Table 1). Replacement of the carbomethoxy moiety of *N*-(fluoroethyl)nor-β-CIT (FE-β-CIT, **6**) with a carboisopropyloxy group, as in compound **8**, had little effect on DA_T affinity (K_i = 4.4 vs 4.0 nM) but did diminish 5HT_T selectivity over DA_T. In contrast, replacement of the carbomethoxy moiety of *N*-(fluoropropyl)nor-β-CIT (**5**) with a carboisopropyloxy group (compound **1**) not only yielded greatly improved DA_T selectivity (in particular, 41-fold over the 5-HT_T) but also somewhat higher DA_T affinity (K_i = 1.2 vs 3.5 nM; Table 1).

Table 1. Binding Affinities of N-Substituted Nor-β-CITs and Comparison Agents at DA, 5-HT, and NE Transporters *in Vitro*



				affinity (K_i, nM)[a]			selectivity: DA_T vs	
compd	R^1	R^2	X	DA_T	5-HT_T	NE_T	5-HT_T	NE_T
1[b]	fluoropropyl	isopropyl	I	1.20 ± 0.29	48.7 ± 8.4	ca. 10 000	40.6	ca. 8300
2 (β-CIT)[b,c]	methyl	methyl	I	1.40 ± 0.20	0.46 ± 0.06	2.80 ± 0.40	0.320	2.00
3	bromopropyl	methyl	I	2.56 ± 0.57	0.35 ± 0.08	164 ± 47	0.137	64.1
4	chloropropyl	methyl	I	3.10 ± 0.57	0.32 ± 0.06	96.0 ± 29.0	0.100	31.0
5[b] (FP-β-CIT)	fluoropropyl	methyl	I	3.50 ± 0.39	0.110 ± 0.02	63.0 ± 4.0	0.032	18.0
6[b] (FE-β-CIT)	fluoroethyl	methyl	I	4.00 ± 0.73	0.140 ± 0.02	93.0 ± 17.0	0.035	23.2
7	cyclopropylmethyl	methyl	I	4.30 ± 0.87	1.30 ± 0.25	198 ± 9.6	0.302	45.6
8[b]	fluoroethyl	isopropyl	I	4.40 ± 0.35	21.7 ± 8.3	>10 000	4.90	>2300
9	hydroxypropyl	methyl	I	5.39 ± 0.21	2.50 ± 0.20	217 ± 19	0.463	40.3
10	2′,2′-dimethoxyethyl	methyl	I	6.80 ± 1.10	1.69 ± 0.09	110 ± 7.7	0.249	16.2
11	3-phthalimidopropyl	methyl	I	9.10 ± 1.10	0.59 ± 0.07	73.7 ± 11.6	0.065	8.10
12	carbomethoxymethyl	methyl	I	11.9 ± 1.4	0.81 ± 0.10	29.1 ± 1.0	0.068	2.50
13	(*N*′,*N*′-dimethylamino)acetyl	methyl	I	12.2 ± 3.8	6.40 ± 1.70	522 ± 145	0.524	42.8
14 (CFT)[c]	methyl	methyl	F	14.7 ± 2.9	181 ± 21	635 ± 110	12.3	43.2
15	2′,2′-difluoroethyl	methyl	I	15.1 ± 3.7	9.6 ± 1.5	>5000	0.636	>330
16	(mesyloxy)propyl	methyl	I	36.3 ± 2.1	17.3 ± 1.2	ca. 5000	0.477	ca. 138
17	iodopropyl	methyl	I	38.9 ± 6.3	8.84 ± 0.53	ca. 5000	0.227	ca. 129
18	2′-methylpropionyl	methyl	I	2,100 ± 140	102 ± 23	>10 000	0.049	>4.80
GBR-12909[c]				0.060 ± 0.020	52.8 ± 4.4	>20 000	880	>330 000
(−)-cocaine[c]				350 ± 80	>10 000	>30 000	>29	>86

[a] Transporter affinity assays are detailed in the methods section: rat striatal homogenates with [^{3}H]GBR-12935 (blank = 30 μM methylphenidate) for DA_T,[24,25] cerebral cortical homogenates with [^{3}H]paroxetine (blank = 1 μM fluoxetine) for 5-HT_T,[26] and [^{3}H]nisoxetine (blank = 2 μM desipramine) for NE_T.[27] Concentration−inhibition curves were computer-fit to determine IC_{50} ± SEM and converted to the inhibition constant, K_i, from the relationship: $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is radioligand concentration and K_d is the independently determined radioligand affinity constant.[25,31−33] Selectivity for the DA_T is indicated as the ratio of K_i for the 5-HT_T or NE_T to that for the DA_T (ratios < 1.0 indicate preference for the *non*-DA_T site). Test compounds are ranked by DA_T affinity. [b] Reported previously. [c] Standard reference compounds reported previously[19] and replicated independently.

N-Substituted analogs of β-CIT (**2**) with a carbomethoxy ester moiety displayed either relatively weak DA_T affinity or selectivity for 5-HT_T over DA_T. Interestingly, *N*-(haloalkyl)nor-β-CIT substituents (R^1) with highest 5-HT_T affinity (K_i all < 0.5 nM) ranked fluoropropyl > fluoroethyl > chloropropyl ≥ bromopropyl > methyl (β-CIT itself). Such highly 5-HT_T-selective agents might be useful as brain-imaging ligands for serotonin neurons or perhaps as mood-elevating clinical drugs by homology to other serotonin-reuptake inhibitors.[28]

In the series of *N*-haloalkyl-substituted phenyltropane analogs, only compound **1** exceeded β-CIT (**2**) in DA_T affinity, and within the series, only minor differences were observed in DA_T affinity, except that the *N*-mesoxypropyl and *N*-iodopropyl compounds **16** and **17** were very weak. The N-substituted methyl ester analogs ranked by DA_T affinity as methyl > bromopropyl ≥ chloropropyl ≥ fluoropropyl ≥ fluoroethyl ≥ cyclopropylmethyl > hydroxypropyl > dimethoxyethyl > phthalimidopropyl > carbomethoxymethyl ≥ (dimethylamino)acetyl > difluoroethyl > (mesyloxy)propyl ≥ iodopropyl ≫ 2′-methylpropionyl (Table 1). The low affinities of the (mesyloxy)propyl and iodopropyl N-substituted derivatives **16** and **17** may, in part, reflect their chemical instability in aqueous solution, since spontaneous partial decomposition of both compounds was detected by HPLC after several hours in solution (unpublished observations).

An interesting finding was the striking loss of DA_T affinity [by a factor of ca. 1500 vs β-CIT (**2**)] with the *N*-2′-methylpropionyl derivative **18**. In a previous report,[29] the replacement of the *N*-methyl group of cocaine by an acetyl moiety also greatly decreased DA_T affinity. These observations may support the conclusion that a basic amine function is required for optimal affinity at monoamine transporters.[29] However, several N-sulfonylated analogs of cocaine retained moderate DA_T affinity.[30] Moreover, on the basis of the effects of the present series of functionally diverse substituents, it is clear that the basicity of the nitrogen is only one factor in the interactions between monoamine transporter sites and phenyltropane ligands. N-Substituents, such as dimethoxyethyl, carbomethoxymethyl, (*N*′,*N*′-dimethylamino)acetyl, and fluoroethyl, which contain moderate to strong electron-withdrawing groups, did not result in a sharp reduction in DA_T-binding affinity. Small losses in DA_T affinity with these compounds may be due to other factors, such as lipophilicity or steric effects. It is likely that any accessory interaction of the substituents with the transporter proteins may also contribute to DA_T binding. In fact, a nonpolar cyclopropylmethyl group provided a similar steric feature as the more polar hydroxypropyl group, with only minor reduction in DA_T affinity (K_i = 4.3 vs 5.4 nM).

An intriguing result was observed with the rather bulky *N*-phthalimidopropyl analog (**11**) of nor-β-CIT. A large sterically bulky group such as a benzyl function near the nitrogen can markedly reduce DA_T affinity of phenyltropanes.[29] However, the *N*-phthalimidopropyl compound **11** showed moderate DA_T affinity (K_i = 9 nM), suggesting that there is additional space available at the putative amino docking site on the DA_T protein. Accordingly, further exploration of the N-substituent region may be worthwhile.

In conclusion, a series of N-substituted derivatives of β-CIT, a relatively metabolically stable (iodophenyl)-tropane analog of (−)-cocaine, were synthesized and evaluated for their affinities at monoamine transporters for DA, NE, and 5-HT in rat forebrain tissue. N-

Substitution with haloalkyl groups (containing F, Cl, or Br) yielded compounds with high affinity at DA_T and 5-HT_T. Moreover, the inclusion of more polar, larger, or more electronegative N-substituents did not lead to substantial loss of DA_T affinity. The present findings suggest that the basicity of the tropane amino nitrogen is only one factor for binding to the DA_T. Additionally, a large sterically bulky group separated from the nitrogen atom by three carbon atoms failed to diminish DA_T affinity markedly, indicating that, with an appropriate spacer arm, the DA_T can accommodate a sterically demanding functional group at the tropane nitrogen. This observation may broaden the scope of suitable ligands for probing monoamine transporters.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary tube apparatus and are reported uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Varian XL-300 spectrometer using tetramethylsilane as an internal reference. Mass spectra were obtained on a Varian EM-360 spectrometer. All optical rotations were measured at the sodium D line using a Rudolph polarimeter (Model DP1A31, 10 cm cell). Elemental analyses, performed by Atlantic Microlabs, Atlanta, GA, were within ±0.4% of theoretical values. Analytical thin layer chromatography (TLC) was carried out on 0.2 mm thick Kieselgel 60F_{254} silica gel TLC plastic sheets (EM Science, Newark, NJ), and visualization was with 254 nm UV light or by exposure to iodine vapor. Flash chromatography was used for the routine purification of reaction products. The HPLC apparatus consisted of a Rainin-Rabbit-HP pump, a Rheodyne injector, a Phenomenex Bondclone C18 (3.9 × 300 mm) or E. Merck Aluspher RP select B250-4 column, and a variable wavelength UV detector.

General Procedure for N-Alkylation of Nor-β-CIT (22). N-Alkylation reactions typically were carried out with 0.27 mmol of nor-β-CIT (**22**).[10] The appropriate alkyl bromide (0.4 mmol) and KI (10 mg) were added to a solution of nor-β-CIT (**22**) and triethylamine (TEA; 46 mmol) in absolute EtOH (10 mL). The mixture was refluxed under nitrogen from 1 to 24 h, depending on the requirements of individual alkyl bromides, and the progress of the reaction was monitored with TLC. The solvent was then removed under reduced pressure, and the residue was passed through a silica gel column (eluted with a mixture of hexane/ether/triethylamine in varying vol ratios stated below) to yield the pure compounds.

***N*-(Cyclopropylmethyl)-2β-carbomethoxy-3β-(4′-iodophenyl)nortropane (7).** Compound **7** was prepared from nor-β-CIT (**22**) and cyclopropylmethyl bromide as described by the preceding general procedure to obtain a white solid (43%): mp 75−77 °C. $[\alpha]^{20}_D$ −27.6° (*c* 0.3, MeOH). ^1H NMR (250 MHz, $CDCl_3$): δ 7.57 (d, *J* = 8.4 Hz, 2H), 7.02 (d, *J* = 8.4 Hz, 2H), 3.95 (m, 1H), 3.59 (s, 3H), 3.43 (m, 1H), 3.58 (s, 3H), 2.90 (m, 2H), 2.55 (dd, *J* = 12.1, 2.8 Hz, 1H), 2.39 (dd, *J* = 12.3, 5.3 Hz, 1H), 1.96 (m, 3H), 1.64 (m, 4H), 0.78 (m, 1H), 0.43 (m, 2H), 0.06 (m, 2H). MS (FAB, NBA): 427 (25), 426 (100, M + H^+), 425 (8), 424 (11), 300 (8). Anal. ($C_{19}H_{23}NO_2I$): C,H,N.

***N*-(3-Hydroxypropyl)-2β-carbomethoxy-3β-(4′-iodophenyl)nortropane (9).** A solution of nor-β-CIT (**22**) (250 mg, 0.67 mmol), 3-bromopropanol (300 mg, 2.13 mmol), and triethylamine (0.5 mL) in toluene (20 mL) was refluxed under a dry nitrogen atmosphere for 4 h, cooled, and filtered. The separated residue was washed twice with toluene (2 mL). The combined filtrate and washings were concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel and eluted with hexane/ether/triethylamine (10/7/0.1, v/v) to give 168 mg (58%) of **9** as a liquid: $[\alpha]^{20}_D$ −5.6° (*c* 0.3, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 1.62−1.80 (m, 5H), 1.98−2.18 (m, 2H), 2.36−2.42 (m, 2H), 2.51−2.63 (m), 2.90−3.02 (m, 2H), 3.40 [s (br), m, 1H], 3.48 (s, 3H), 3.70 [s (br), 1H], 4.44−4.59 (m, 2H), 7.00−7.03 and 7.57−7.60 (m, 4H). Anal. ($C_{18}H_{24}NO_3I$): C,H,N.

***N*-(2′,2′-Dimethoxyethyl)-2β-carbomethoxy-3β-(4′-iodophenyl)nortropane (10).** Compound **10** was prepared from nor-β-CIT (**22**) and 2′,2′-dimethoxyethyl bromide to give a white solid (32%): mp 126−128 °C. $[\alpha]^{20}_D$ −36.6° (*c* 0.3, MeOH). ^1H NMR (250 MHz, $CDCl_3$): δ 7.66 (d, *J* = 8.3 Hz, 2H), 7.02 (d, *J* = 8.3 Hz, 2H), 4.32 (t, *J* = 5.2 Hz, 1H), 4.48 (m, 1H), 3.78 (m, 1H), 3.51 (s, 3H), 3.42 (m, 1H), 3.37 (s, 3H), 3.35 (s, 3H), 2.88 (m, 2H), 2.57 (td, *J* = 2.7, 12.1 Hz, 1H), 2.41 (m, 2H), 2.03 (m, 2H), 1.66 (m, 4H). MS (FAB, NBA): 461 (21), 460 (100, M + H^+), 459 (2), 428 (12), 245 (23). Anal. ($C_{19}H_{26}NO_4I$): C,H,N.

***N*-(3-Phthalimidopropyl)-2β-carbomethoxy-3β-(4′-iodophenyl)nortropane (11).** Compound **11** was similarly prepared from nor-β-CIT (**22**) and 3-phthalimidopropyl bromide to give a white solid (59%) which was converted to HCl salt with HCl/ether: mp 136−138 °C (HCl salt). $[\alpha]^{20}_D$ −119.8° (*c* 0.31, MeOH) (free base). ^1H NMR (250 MHz, $CDCl_3$): δ 7.83 (m, 2H), 7.70 (m, 2H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.00 (d, *J* = 8.4 Hz, 2H), 3.79 (m, 1H), 3.68 (m, 1H), 3.52 (s, 3H), 3.41 (m, 1H), 2.89 (m, 2H), 2.51 (m, 3H), 2.32 (m, 3H), 2.03 (m, 2H), 1.67 (m, 5H). MS (FAB, NBA): 559 (27), 445 (22), 444 (100), 417 (27). Anal. ($C_{26}H_{26}N_2O_4I\cdot HCl\cdot H_2O$): C,H,N.

***N*-(Carbomethoxymethyl)-2β-carbomethoxy-3β-(4′-iodophenyl)nortropane (12).** Compound **12** was prepared from nor-β-CIT (**22**) and carbomethoxymethyl bromide to give a white solid (56%): mp 120−122 °C. $[\alpha]^{20}_D$ −58.7° (*c* 0.3, MeOH). ^1H NMR (250 MHz, $CDCl_3$): δ 7.58 (d, *J* = 8.4 Hz, 2H), 7.02 (d, *J* = 8.4 Hz, 2H), 3.74 (m, 1H), 3.68 (s, 3H), 3.51 (s, 3H), 3.58 (s, 3H), 3.45 (m, 1H), 3.14 (dd, *J* = 16.5, 13.3 Hz, 2H), 2.90 (m, 2H), 2.75 (t, *J* = 9.8 Hz, 1H), 2.12 (m, 1H), 2.01 (m, 1H), 1.68 (m, 3H). MS (FAB, NBA): 445 (20), 444 (100, M + H^+), 443 (16), 412 (5), 385 (9), 384 (45). Anal. ($C_{18}H_{22}NO_4I$): C,H,N.

***N*-[(*N*′,*N*′-Dimethylamino)acetyl]-2β-carbomethoxy-3β-(4′-iodophenyl)nortropane (13).** Compound **13** was prepared from nor-β-CIT (**22**) and (*N*′,*N*′-dimethylamino)acetyl bromide to give a white solid (40%): mp 194−196 °C. $[\alpha]^{20}_D$ −45.3° (*c* 0.3, MeOH). ^1H NMR (250 MHz, $CDCl_3$): δ 7.58 (d, *J* = 8.3 Hz, 2H), 7.00 (d, *J* = 8.3 Hz, 2H), 3.70 (m, 1 H), 3.45 (s, 3H), 3.12 (m, 1H), 3.11 (m, 2H), 2.90 (s, 3H), 2.55 (m, 1H), 2.18 (m, 2H), 1.65 (m, 4H). Anal. ($C_{19}H_{24}N_2O_3I$): C,H,N.

***N*-(2′,2′-Difluoroethyl)-2β-carbomethoxy-3β-(4′-iodophenyl)nortropane (15).** A solution of nor-β-CIT (**22**) (300 mg, 0.8 mmol), 1,1-difluoro-2-[[(trifluoromethyl)sulfonyl]oxy]ethane (300 mg, 1.4 mmol), and triethylamine (1 mL) in acetone (15 mL) was stirred at room temperature overnight. The reaction mixture was filtered and the separated residue washed twice with toluene (2 mL). The combined filtrate and washings were concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel and eluted with hexane/ether/triethylamine (10/7/0.1, v/v) to give 160 mg (46%) of **15** as a white solid: mp 113−114 °C. $[\alpha]^{20}_D$ +21.3° (*c* 0.9, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 1.62−1.80 (m, 3H), 2.01−2.18 (m, 3H), 2.53−2.55 (m, 2H), 2.62 (m, 1H), 2.91 (m, 1H), 3.43 (m, 1H), 3.51 (s, 3H), 3.80 (m, 1H), 4.36−4.52 (m, 1H), 6.99−7.02 and 7.55−7.58 (m, 4H). Anal. ($C_{17}H_{20}NO_2IF_2\cdot{}^1/_2H_2O$): C,H,N.

***N*-(2′-Methylpropionyl)-2β-carbomethoxy-3β-(4′-iodophenyl)nortropane (18).** Compounds **18** was prepared from nor-β-CIT (**22**) and 2′-methylpropionyl bromide to give a white solid (89%): mp 152−154 °C. $[\alpha]^{20}_D$ −34.06° (*c* 0.3, MeOH). ^1H NMR (250 MHz, $CDCl_3$): δ 7.59 (d, *J* = 8.4 Hz, 2H), 6.99 (d, *J* = 8.4 Hz, 2H), 5.08 (m, 1H), 4.48 (m, 1H), 3.45 (s, 3H), 3.24 (m, 1H), 2.93 (m, 1H), 2.74 (m, 2H), 2.10 (m, 1H), 1.99 (m, 1H), 1.87 (m, 1H), 1.77 (m, 2H), 1.13 (t, *J* = 6.5 Hz, 6H). MS (FAB, NBA): 443 (21), 442 (100, M + H^+), 441 (10), 372 (8), 340 (8), 312 (6), 245 (11). Anal. ($C_{19}H_{23}NO_3I$): C,H,N.

***N*-(3′-Bromopropyl)-2β-carbomethoxy-3β-(4′-iodophenyl)nortropane (3).** At 0 °C triphenylphosphine (148 mg, 0.55 mmol) was dissolved in methylene chloride and bromine (88 mg, 0.55 mmol) was added dropwise. After 10 min, *N*-(3-hydroxypropyl)nor-β-CIT (**9**) (215 mg, 0.55 mmol) was added slowly; 30 min later the solvent was removed at reduced pressure, and the residue was passed through a silica gel column eluting with ether to give 42 mg of a white solid (17%). ^1H NMR (300 MHz, $CDCl_3$): δ 7.58 (d, *J* = 8.4 Hz, 2H), 7.00

(d, J = 8.4 Hz, 2H), 3.68 (m, 1H, H_1), 3.51 (m, 5H, OCH_3, CH_2-Br), 3.36 (m, 1H, H_5), 2.95 (m, 2H), 2.57 (dd, 1H), 2.38 (t, J = 8 Hz, 2H), 1.85 (m, 7H). ^{13}C NMR ($CDCl_3$): δ 171.57, 136.73, 129.33, 90.95, 63.19, 61.16, 52.28, 50.95, 50.17, 45.86, 42.81, 39.26, 33.70, 31.70, 25.79, 8.49. MS (FAB, NBA): 495 (19), 494 (94), 493 (33), 492 (100), 491 (14), 490 (7), 412 (21), 394 (9). Anal. ($C_{18}H_{23}BrNO_2I$): C,H,N.

***N*-(3′-Chloropropyl)-2β-carbomethoxy-3β-(4′-iodophenyl)nortropane (4).** *N*-(3-Hydroxypropyl)nor-β-CIT (**9**) (1.8 g, 4.2 mmol) was dissolved in methylene chloride (150 mL) and cooled in an ice bath under nitrogen. Methanesulfonyl chloride (580 mg, 4.4 mmol) was added followed by addition of 2,6-lutidine (1 mL). The reaction mixture was stirred for 2 h, and then a second portion of methanesulfonyl chloride (580 mg, 4.4 mmol) was added. The mixture was allowed to warm to room temperature and stirred for an additional 48 h. The solvent was removed, and the residue was chromatographed on a silica gel column eluted with ether/hexane/triethylamine (50/50/5, v/v) to give 1.4 g of a white solid (75%): mp 96−98 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58 (d, J = 8.4 Hz, 2H), 7.00 (d, J = 8.4 Hz, 2H), 3.68 (m, 3H, H_1, CH_2Cl), 3.51 (s, 3H, OCH_3), 3.36 (m, 1H, H_5), 2.95 (m, 2H), 2.57 (dd, 1H), 2.38 (t, J = 8 Hz, 2H), 1.85 (m, 7H). MS (GC−MS): 449 (100), 447 (33), 384 (23). Anal. ($C_{18}H_{23}ClNO_2I$): C,H,N.

***N*-[3′-(Mesyloxy)propyl]-2β-carbomethoxy-3β-(4′-iodophenyl)nortropane Methanesulfonate (16).** *N*-(3-Hydroxypropyl)nor-β-CIT (**9**) (223 mg, 0.52 mmol) was dissolved in methylene chloride (4 mL), and 2.2 equiv of methanesulfonic anhydride was added. The mixture was stirred for 36 h and monitored by TLC. Ether was added, and the separated oil was removed and dissolved in a minimum amount of methylene chloride; ether was added again, and the separated oil was removed. The oil was lyophilized for 24 h to give 225 mg of **16** as a white solid product (72%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.69 (d, J = 8.4 Hz, 2H), 7.05 (d, J = 8.4 Hz, 2H), 4.39 (t, J = 5.8 Hz, 2H), 4.25 (m, 1H), 4.18 (m, 1H), 3.64 (m, 1H), 3.39 (s, 3H), 3.25 (m, 1H), 3.17 (s, 3H), 2.71 (m, 4H), 2.40 (m, 1H), 2.25 (m, 4H), 2.00 (m, 1H). Anal. ($C_{20}H_{30}NO_8IS_2\cdot HCl\cdot 2H_2O$): H,N; C: calcd, 37.56; found, 37.08.

***N*-(3′-Iodopropyl)-2β-carbomethoxy-3β-(4′-iodophenyl)nortropane (17).** *N*-[3-[(methylsulfonyl)oxy]propyl]nor-β-CIT (**16**) (100 mg, 0.2 mmol) was dissolved in 3 mL of acetonitrile, and KI (60 mg, 0.39 mmol) and Kriptofix 222 (89 mg, 0.24 mmol) were added. The mixture was heated 3 h at 72 °C. The solvent was removed at reduced pressure, and the residue was passed through a silica gel column eluted with hexane/ether/triethylamine (7/3/0.5, v/v) to give 32 mg of pure **17** as a white solid (29%): mp 76−78 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.58 (d, J = 8.4 Hz, 2H), 7.00 (d, J = 8.4 Hz, 2H), 3.70 (m, 1H, H_1), 3.51 (s, 3H, OCH_3), 3.36 (m, 1H, H_5), 3.22 (m, 2H, CH_2I), 2.95 (m, 2H), 2.57 (dd, 1H), 2.38 (t, J = 8. Hz, 2H), 1.85 (m, 7H). MS (FAB, NBA): 504, 412, 319, 245. Anal. ($C_{18}H_{23}NO_2I_2$): C,H,N.

Transporter Affinity Assays. Stock solutions (1 mM) of test agents were made in 95% ethanol/DMSO (1/1, v/v) and stored at −5 °C until used for transporter affinity assays, by diluting in a large excess of each assay buffer. Agents were tested, typically, at six concentrations in duplicate, with a crude membrane fraction of homogenates of rat brain corpus striatum (for DA_T assays) in Tris-citate buffer (pH 7.4) containing Na^+ (120 nM) and Mg^{2+} (4 mM) or frontoparietal cerebral cortex (for 5-HT_T and NE_T) in 50 mM Tris-HCl buffer (pH 7.4) containing Na^+ (120 nM) and K^+ (5 mM) following methods reported previously.[19,24−27] For the DA_T assay,[19,24,25] the radioligand was [^{3}H]GBR-12935 (13 Ci/mmol; K_d = 1.0 nM) at a test concentration (L) of 0.4 nM and was incubated for 45 min at 4 °C, with or without 30 μM methylphenidate included to define nonspecific binding (blank) as recommended by Andersen;[24] nonspecific binding averaged 20−25% of total counts bound with this or alternative blanking agents included at ca. 200 times their experimentally determined IC_{50} values (GBR-13069, 100 nM; mazindol, 1 μM; nomifensine, 10 μM). For the 5-HT_T assay, L = 0.2 nM [^{3}H]paroxetine (20 Ci/mmol; K_d = 0.15 nM) assayed for 60 min at 20 °C in 50 mM Tris-HCl buffer (pH 7.4) containing Na^+ (120 nM) and K^+ (5 mM) with 1 μM fluoxetine (donated by Lilly Laboratories, Indianapolis, IN) as the blank agent.[26] For the NE_T assay, L = 0.8 nM [^{3}H]-nisoxetine (50 Ci/mmol; K_d = 0.8 nM) incubated for 180 min at 4 °C in 50 mM Tris-HCl buffer (pH 7.4) containing Na^+ (300 nM), K^+ (5 mM), and 2 μM desipramine (donated by Marion Merrell Dow, Kansas City, MO) as blank.[27] All radioligands were from DuPont-NEN (Boston, MA). Concentration−inhibition curves were microcomputer-fit with the ALLFIT program[31,32] to determine $IC_{50} \pm$ SEM and converted to K_i values from the Cheng and Prusoff[33] equation: $K_i = IC_{50}/(1 + [L]/K_d)$.[19]

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