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Enantioselective Synthesis of *S*-(+)-2β-Carboalkoxy-3α-[bis(4-fluorophenyl)methoxy]tropanes as Novel Probes for the Dopamine Transporter

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Abstract—Synthesis of a series of pure *S*-(+)-2β-carboalkoxy-3α-[bis(4-fluorophenyl)methoxy]tropanes (>99% ee) was achieved by employing a chiral amine-induced asymmetric reaction of tropinone with methyl cyanoformate as the key step. In this series, all of the *S*-(+)-enantiomers were 2-fold more potent than their racemic mixtures and all displayed high-affinity binding for DAT (K_i = 13–40 nM). These data support previous findings of significant divergence in structural requirements for high-affinity DAT binding among tropane-based inhibitors. Furthermore, the 2-substituent in the 3α-[bis(4-fluorophenyl)methoxy]tropane series is well tolerated at the DAT but not at SERT (K_i = 690–2040 nM), or muscarinic M_1 receptors (K_i = 133–4380 nM) resulting in highly selective DAT ligands that may provide new leads toward a cocaine-abuse therapeutic.

Identifying molecular mechanisms that are associated with the pharmacological and reinforcing effects of cocaine has been deemed essential toward the discovery of a medication to be used in the treatment of cocaine abuse. Cocaine binds with moderate affinity to all three monoamine transporters, dopamine (DAT), serotonin (SERT), and norepinephrine (NET). However, the reinforcing actions of cocaine are primarily associated with increases in basal dopamine, as a result of blockade at the DAT (for review, see refs 1–4). Hence the investigation of dopaminergic agents and in particular, selective dopamine transporter ligands, has received considerable attention toward the development of a cocaine-abuse medication (for review, see refs 1 and 5–8).

The 3-phenyltropane class of dopamine uptake inhibitors has been investigated extensively[9,10] and has provided SAR for the 'cocaine' binding site on the DAT. Another class of tropane-based dopamine uptake inhibitors, the 3α-(diphenylmethoxy)tropane (Benztropine) analogues, have been investigated in our laboratory and others[11–14] in recent years. Our studies have shown that many of the 3α-(diphenylmethoxy)tropane analogues are potent inhibitors of dopamine uptake, and yet they do not produce significant locomotor stimulation or cocaine-like subjective effects, in rodents.[15–17] Furthermore, several of these compounds have been evaluated and are not appreciably self administered, in monkeys that readily self administer cocaine.[18,19] SAR for this series of compounds revealed a striking divergence from cocaine and related tropane analogues at the DAT.[11–13,17,20–23] Furthermore, immunologic and peptide mapping studies using the photoaffinity label, [^{125}I]*N*-[*n*-butyl-4-(4‴-azido-3‴-iodophenyl)]-bis-(4-fluorophenyl)methoxytropane (GA 2–34), suggested that these ligands bind to transmembrane domains on the DAT that differ from those at which cocaine analogues bind.[24,25] In total, these studies suggest that the binding domain on the DAT, that is accessed by the 3α-(diphenylmethoxy)tropane analogues, is not identical with that of cocaine, and further that these binding differences may, in part, result in different pharmacological actions of these drugs.

The *R*-configuration at the 2-position of (−)-cocaine and all of the 3-phenyltropane analogues is required for high affinity binding at the DAT.[9] In contrast, when all eight isomers of 2-carbomethoxy-3α-[bis(4-fluorophenyl)-methoxy]tropane were synthesized, it was discovered

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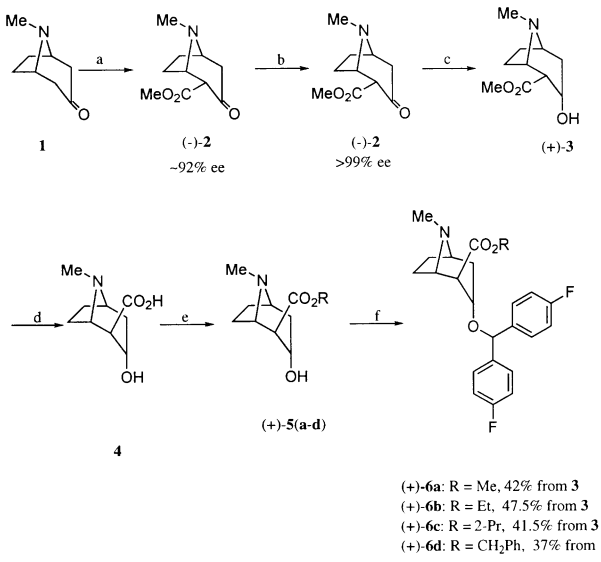
that the *S*-enantiomer, [*S*-(+)-difluoropine], was considerably more potent and selective than any of the other seven isomers.[11] The *S*-(+)-2β-carbomethoxy-3α-[bis(4-fluorophenyl)methoxy]tropane (IC_{50} = 10.9 nM),[11] appears to be equiactive to 3α-[bis(4-fluorophenyl)-methoxy]tropane (K_i = 11.8 nM),[15] although this comparison is across studies employing different assay conditions. Nonetheless, this relative equivalence of activity suggests that the 2-position, in this series, may provide an excellent opportunity for maintaining or potentially improving binding affinities at the DAT. Furthermore, it was reported that *S*-(+)-difluoropine was highly selective for DAT over SERT (IC_{50} = 3580 nM),[11] which suggests that this substituent may not be as well tolerated at the other monoamine transporters or, possibly muscarinic receptors, where many of the benztropine analogues bind with high affinity.[16,20] Thus, the discovery of chemical modifications to the parent compound that decreases binding affinity at muscarinic receptors while retaining high affinity at DAT was desirable. Herein, we report the enantioselective synthesis and binding profiles of a series of *S*-(+)-2β-carboalkoxy-3α-[bis(4-fluorophenyl)methoxy]tropane analogues. The racemates of these analogues were also prepared for biological and chemical comparison.



Scheme 1. Reagents and conditions: (a) 1. *n*-BuLi, NDPPA, THF; 2. $NCCO_2Me$, THF, 77%; (b) D-Tartaric acid, 80%; (c) H_2 (50 psi)/PtO_2, EtOH, 4 days, 86%; (d) H_2O, reflux; (e) HCl gas, ROH, rt or 50 °C; (f) 4,4′-difluorobenzhydrol, *p*-toluenesulfonic acid hydrate, benzene, reflux.

Synthesis of the racemic (±)-2β-carboalkoxy-3α-[bis(4-fluorophenyl)methoxy]tropanes was performed as a model for the esterifications and for biological comparison using literature methods.[11,26] The synthesis of the optically pure *S*-(+)-2β-carboalkoxy-3α-[bis(4-fluorophenyl)methoxy]tropanes [(+)-**6a**–**6d**] was achieved using the asymmetric deprotonation strategy of Majewski and Lanny (Scheme 1).[26] Synthesis of the chiral amine (*R*)-*N*-(2,2-dimethylpropyl)-1-phenyl-2-piperidinoethylamine (NDPPA) was prepared from commercially available *R*-phenylglycine by modifying Shirai's original procedure.[27] 3-Tropinone was asymmetrically deprotonated by the lithium salt of chiral amine NDPPA and then treated with methyl cyanoformate ($NCCO_2ME$) to give (−)-2- carbomethoxy-tropinone [(−)-**2**] with ~92% ee. Resolution with D-tartaric acid of (−)-**2**, followed by two recrystallizations gave the enantiomerically pure (−)-**2**.[28] Catalytic reduction of (−)-**2** gave (+)-**3** as the only isomer, which was converted to (+)-**4** and esterified to (+)-**5a**–**d** upon treatment with HCl gas in the respective alcohol. Conversion of (+)-**5a**–**d** to (+)-**6a**–**d** was achieved using literature methods.[11] Spectral data for the enantiomers ((+)-**6a**–**6d**) were identical to their racemates.[29] The enantiomeric analysis was conducted on the chiral HPLC column 'Klassix Chiral-A' (ChromBA, State College, PA, USA).[29]

The binding affinities of the four enantiomerically pure *S*-(+)-2-carboalkoxy-3α-[bis(4-fluorophenyl)methoxy]-tropanes analogues [(+)-**6a**–**d**] and their respective racemic mixtures, (±)-**6a**–**d**, were evaluated in radiolabeled ligand displacement assays for DAT, SERT, NET and muscarinic M_1 receptors, in rat brain, using previously described methods.[23] In addition, inhibition of dopamine uptake in rat synaptosomes was also evaluated and these data were compared to those for the unsubstituted 3α-[bis(4-fluorophenyl)methoxy]tropane (**7**; Table 1). Table 2 shows the selectivities of the enantioselective compounds for the DAT over SERT, NET, and muscarinic M_1 binding sites. All of the (±)- and enantiomerically pure *S*-(+)-2-carboalkoxy-3α-[bis(4-fluorophenyl)methoxy]tropane analogues (**6a**–**6d**) bind with high affinity and selectivity at the DAT, with the *S*-(+)-enantiomers showing approximately 2-fold

Table 1. Binding results at the monoamine transporters and muscarinic M_1 receptor

Compd	[^{3}H]WIN 35, 428 DAT K_i (nM) ± SEM[a]	[^{3}H]DA UPTAKE IC_{50} (nM) ± SEM[a]	[^{3}H]Citalopram SERT K_i (nM) ± SEM[a]	[^{3}H]Nisoxetine NET K_i (nM) ± SEM[a]	[^{3}H]Pirenzepine M_1 K_i (nM) ± SEM[a]
(+)-6a	12.9 ± 1.8	1.5 ± 0.2	690 ± 58	269 ± 39	133 ± 4.2
(±)-6a	21.3 ± 3.6	2.9 ± 0.4	1750 ± 240	474 ± 65	302 ± 43
(+)-6b	16.8 ± 2.0	1.8 ± 0.2	1850 ± 270	629 ± 31	1890 ± 130
(±)-6b	26.2 ± 3.4	3.6 ± 0.4	3740 ± 490	1020 ± 120	1860 ± 190
(+)-6c	23.3 ± 3.3	6.2 ± 0.3	12,000 ± 1280	642 ± 13	2680 ± 140
(±)-6c	49.1 ± 6.4	6.8 ± 0.2	13,800 ± 680	1990 ± 380	7640 ± 1040
(+)-6d	40.2 ± 9.3	2.2 ± 0.3	2040 ± 300	2230 ± 200	4380 ± 530
(±)-6d	90.3 ± 18	3.5 ± 0.2	3320 ± 290	4110 ± 73	5100 ± 310
7	11.2 ± 1.3[b]	13.8 ± 1.7	3260 ± 110	610 ± 81	11.6 ± 0.9

[a]Each K_i value represents data from at least three independent experiments, each performed in triplicate.
[b]Data from ref 15.

Table 2. DAT selectivities of *S*-(+)-2-carboalkoxy-3-[bis(4-fluorophenyl)-methoxy]tropanes

Compd	DAT/SERT	DAT/NET	DAT/M_1
(+)-6a	53	21	10
(+)-6b	110	37	112
(+)-6c	515	28	120
(+)-6d	51	56	110
7	290	55	1

higher affinity than their corresponding racemates. These data support previously described enantioselectivity for *S*-(+)-**6a**. Interestingly, in rat brain, this analogue does not demonstrate the reported[11] >300-fold selectivity for DAT over SERT, but rather ~50-fold selectivity. This difference may reflect differing assay conditions or species differences wherein monkey SERT is less sensitive to this class of compounds than rat. However, increasing the methyl ester (**6a**) to ethyl (**6b**) or 2-propyl (**6c**), significantly decreases binding affinities to SERT, resulting in 110- and 515-fold selectivity, respectively. Likewise, muscarinic binding affinities of the larger alkyl esters (+)-**6b**, **c**, and **d** are significantly reduced resulting in >100-fold selectivity for DAT over M_1 receptors, which has previously only been achieved with a few *N*-substituted analogues, in this class of compounds.[23] All of the compounds were >20-fold selective over NET, with the benzyl ester (+)-**6d** demonstrating the highest selectivity.

The DAT binding affinities for *S*-(+)-**6a** (K_i = 12.9 nM) and *S*-(+)-**6b** (K_i = 16.8 nM), for example, are equivalent to the unsubstituted **7** (3α-[bis(4-fluorophenyl)methoxy]tropane; K_i = 11.8 nM),[15] suggesting, as we speculated previously, that this substituent may not contribute significantly to the binding interaction at DAT. As the steric bulk of the 2-position ester is increased, DAT binding affinities decreased only slightly. Equipotent inhibition of dopamine uptake in rat synaptosomes was observed for all of the S(+)-analogues and the enantiomers were ~2-fold more potent than the racemates. Likewise, binding affinities at SERT and NET were uniformly poor for the 2-carboalkoxy analogues, with the 2-propyl ester **6c** showing the lowest binding affinity at SERT (K_i = 12,000 nM). Profound losses in binding affinities were also observed at the muscarinic receptors resulting in significant increases in DAT selectivity (>100 for **6b**, **c**, **d** vs 1 for **7**).

In summary, a series of *S*-(+)-2-carboalkoxy-3α-[bis(4-fluorophenyl)methoxy]tropane analogues was prepared via an enantioselective synthetic strategy giving high yields of >99% ee products that were highly potent and selective DAT ligands. All of the analogues potently inhibited [^{3}H]dopamine uptake in synaptosomes (IC_{50} = 1.5–6.8 nM). Although all four *S*-(+)-2-carboalkoxy-analogues showed DAT selectivity, *S*-(+)-**6b** demonstrated overall the most potent and DAT selective binding profile. Further investigation into the pharmacology of this series of 2-carboalkoxy-substituted-3α-(diphenylmethoxy)tropane analogues, with an emphasis on in vivo studies, will be pursued to determine whether the unique pharmacology often observed within this class of compounds is duplicated, and if there is the possibility of developing a potential cocaine abuse pharmacotherapeutic from one of these agents.

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28. Note 1. Compound (−)-**2**: mp 107–108 °C (sublimed; mp lit. 108.5–109.5 °C); $[\alpha]_D^{27}$ −22.8° (*c* 1.0, MeOH; lit. $[\alpha]_D^{18}$ −20.2°; *c* 1, MeOH); lit: Carroll, F. I.; Lewin, A. H.; Abraham, P.; Parham, K.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem.* **1991**, *34*, 883. >99% ee by ^{1}H NMR with (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol [(*S*)-(+)-TFAE].
29. Note 2. Compound (+)-**6a**: mp 126.5–127.5 °C (mp lit.[11] 132–133 °C); $[\alpha]_D^{26}$ +19.6° (*c* 1.0, MeOH); lit.[11] $[\alpha]_D^{21}$ +21.6° (*c* 1, MeOH); analytic HPLC (Klassix Chiral-A column) eluting with hexane/2-PrOH/triethylamine (97.5:2.5:0.5) t_R 9.29 min, >99% ee; Anal. ($C_{23}H_{25}NF_2O_3$) for C,H,N. Compound (+)-**6b**: mp 60–61 °C; $[\alpha]_D^{26}$ +20.5° (*c* 1.0, MeOH); analytic HPLC (Klassix Chiral-A column) eluting with hexane/2-PrOH/triethylamine (100:1:1) t_R 6.6 min, >99% ee. IR: 1726, 1605 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.22 (3H, t, *J* = 7.0 Hz), 1.70–2.10 (6H, m), 2.18 (3H, s), 2.68 (1H, s), 3.08 (1H, m), 3.58 (1H, m), 3.98 (1H, d, *J* = 5.2 Hz), 4.0–4.2 (2H, m), 5.34 (1H, s), 6.95–7.05 (4H, m), 6.20–6.30 (4H, m); ^{13}C NMR ($CDCl_3$) δ 14.2, 24.6, 25.2, 36.2, 41.8, 60.4, 61.0, 63.2, 70.2, 80.3, 115.2, 115.4, 128.4, 138.4, 160.5, 163.7, 172.5; EIMS *m*/*z* 415 (M+). Anal. ($C_{24}H_{27}NF_2O_3$) for C, H, N. Compound (+)-**6c**: colorless oil; $[\alpha]_D^{26}$ +20.2° (*c* 1.03, MeOH); IR: 1726, 1603 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.21 (3H, d, *J* = 5.8 Hz), 1.23 (3H, d, *J* = 5.6 Hz), 1.75–2.17 (6H, m), 2.20 (3H, s), 2.66 (1H, m), 3.10 (1H, m), 3.57 (1H, m), 3.98 (1H, d, *J* = 5.1 Hz), 5.06 (1H, m), 5.36 (1H, s), 6.97–7.04 (4H, m), 7.24–7.31 (4H, m); ^{13}C NMR ($CDCl_3$) δ 22.2, 25.0, 25.6, 36.5, 42.1, 52.3, 61.4, 63.6, 67.9, 70.6, 80.7, 115.5, 115.8, 128.8, 172.3; EIMS *m*/*z* 429 (M+). Anal. ($C_{25}H_{29}NF_2O_3$) for C, H, N. Compound (+)-**6d**: colorless oil; $[\alpha]_D^{26}$ +12.6° (*c* 1.05, MeOH). Analytic HPLC (Klassix Chiral-A column) eluting with hexane/2-PrOH/triethylamine (100:1.5:1) t_R 7.4 min, >99% ee; IR: 1733, 1605 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.75–2.17 (6H, m), 2.17 (3H, s), 2.75 (1H, m), 3.09 (1H, m), 3.60 (1H, m), 4.02 (1H, d, *J* = 4.8 Hz), 5.03 (1H, d, *J* = 12.5 Hz), 5.19 (1H, d, *J* = 12.5 Hz), 5.34 (1H, s), 6.93–7.04 (4H, m), 7.21–7.39 (9H, m); ^{13}C NMR ($CDCl_3$) δ 25.0, 25.6, 36.6, 42.1, 52.3, 61.4, 63.5, 66.6, 70.5, 80.7, 115.5, 115.8, 128.4, 129.0, 136.5, 138.6, 160.8, 164.1, 172.6; EIMS *m*/*z* 477(M+). Anal. ($C_{29}H_{27}NF_2O_3$) for C, H, N.