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TROTEC-1: A New High-Affinity Ligand for Labeling of the Dopamine Transporter

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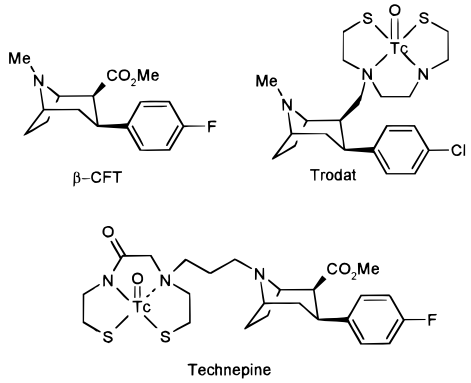
Parkinson's disease is characterized by a significant loss of dopaminergic neurons in the basal ganglia. A reduction in the dopamine transporter (DAT) located at the presynaptic nerve terminals in the striatum (ST) can be measured by selective binding of DAT ligands containing a radioactive label. A decreased specific striatal uptake of such a radiolabeled DAT ligand may therefore indicate early pathological states and allow a diagnosis.[1] Due to its favorable properties, technetium-99m is the preferred nuclide for routine diagnosis by means of single photon computed tomography (SPECT) in nuclear medicine. Considerable efforts have been undertaken toward its incorporation into a variety of central nervous system (CNS) receptor ligands.[2,3]

The first technetium complexes derived from a 2β-carbomethoxy-3β-(halophenyl)tropane useful for in vivo imaging of dopaminergic neuronal function were recently reported.[4,5] Technepine was the first reported technetium tracer crossing the blood−brain barrier and accumulating selectively in the striatum.[4] Also [^{99m}Tc]-Trodat with the chelate moiety in the 2-position of the tropane ring shows high affinity to the dopamine transporter in vivo.[5] Both compounds contain a tetradentate N_2S_2 chelating unit. This chelating unit forms a very stable complex with the oxometal(V) core.



One of the disadvantages of the tetradentate N_2S_2 ligands is the occurrence of additional stereoisomers due to the complexation reaction. These stereoisomers can exhibit different binding affinities and therefore require separation by chromatographic methods, complicating their clinical use. Another problem is the low brain uptake of the complexes known so far, due in part to the polar coordination sphere[6] and its large size. To enable metal complexes to cross the blood−brain barrier by nonspecific transport, the complexes should be nonpolar and lipophilic.

To improve the brain uptake, we are investigating new ligand/metal systems which are nonpolar, neutral, and small in size. One of the most promising approaches seemed to be the use of a dithioether/metal carbonyl(I) unit: $[TcClS_2(CO)_3]$. This unit is available by reduction of $[TcO_4]^-$ under a low-pressure carbon monoxide atmosphere.[7] In solution the $(NEt_4)_2[TcCl_3(CO)_3]$ obtained undergoes a rapid exchange of two of the chloro ligands by the solvent. At this point, the solvent in the coordination sphere can be exchanged with a dithioether chelate. The remaining chlorine atom is necessary for the formation of neutral octahedral complexes of the common formula [TcCl"S_2"$(CO)_3$] ("S_2" = $RS(CH_2)_2SR'$).[8] While this system does not overcome the stereoisomer formation upon complex-

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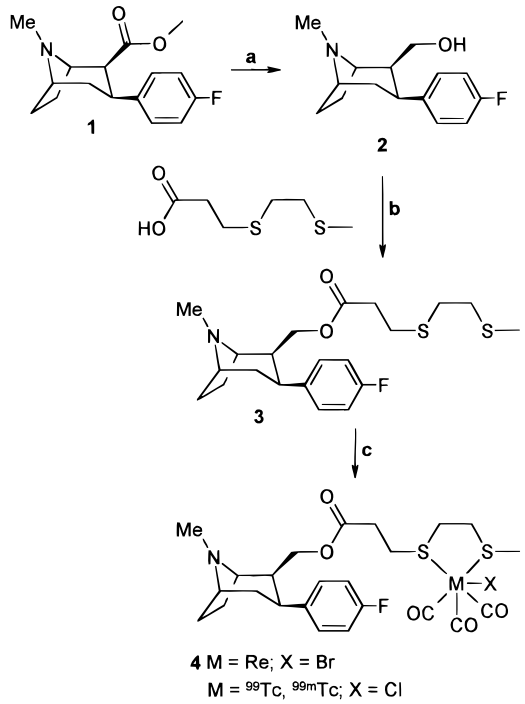
Scheme 1[a]



[a] Reagents: (a) DIBAL-H, toluene, 0 °C, 85−95%; (b) EDCI, DMAP, CH_2Cl_2, 25 °C, quant; (c) $(NEt_4)_2[MX_3(CO)_3]$ (M = Re, X = Br; M = ^{99m}Tc or ^{99}Tc, X = Cl), MeOH, 25 °C, 83%.

ation, it is nevertheless promising because of its small size and high lipophilicity. While it has recently been shown that different stereoisomers of SPECT ligands for the DAT indeed displayed different properties,[9] it is also worth mentioning that in this special case the mixture of the diastereomers gave the same image in a baboon brain as the better one of the separated diastereomers. For different receptors, a different behavior cannot be ruled out, but for the first we decided to place back the problem of diastereoisomerism.

Recent structure−activity relationship (SAR) studies revealed the possibility of accommodating bulky substituents in the 2β-position of the tropane ring.[10] Introduction of the nonbasic thioether carbonyl moiety into this position of β-CFT should afford a SPECT ligand exhibiting high affinity at the DAT with improved biodistribution properties. Originally, the existence of one or two hydrogen-bonding sites for the 2β-substituent had been postulated.[11] More recently, it has been shown that the DAT tolerates a large variety of 2β-substituents, including weakly polar or nonpolar[12] and also rather polar substituents.[13] By connecting the dithioether via an ester group, one potential H-bonding interaction was retained, and the two sulfur atoms could potentially increase the affinity by additional hydrophobic interactions.

In the present study, we report the synthesis of the DAT ligand 2β-[(4,7-dithiaoctanoyloxy)methyl]-3β-(4-fluorophenyl)tropane (**3**) as well as its complexation to the tricarbonylmetal(I) centers of Re and ^{99m}Tc (TROTEC-1). We also report in vitro competition binding studies at the DAT, serotonin transporter (5-HTT), and norepinephrine transporter (NET).

Chemistry. The synthesis was carried out according to Scheme 1. β-CFT obtained by common methods[14] was reduced with DIBAL-H in toluene.[12] After purification by flash chromatography, 2β-(hydroxymethyl)-3β-(4-fluorophenyl)tropane (**2**) was obtained in a yield of 85−95%. Esterification of **2** with 4,7-dithiaoctanoic acid using *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDCI) and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) was performed in methylene chloride at room temperature. The desired product **3** was obtained in excellent yield. In the ^{1}H NMR spectrum of **3,** the chemical shift of the *N*-methyl group is consistent with 2β,3β-stereochemistry.[11]

The synthesis of the rhenium complex was performed by reaction of **3** with $(NEt_4)_2[ReBr_3(CO)_3]$ in methanol. After workup, the complex **4** was obtained in 83% yield.

Elemental analysis, mass spectra, and IR spectra clearly confirm the proposed structure. The tricarbonylrhenium core gives rise to the strong vibration bands at 2032, 1940, and 1904 cm^{-1}. An additional C=O band at 1728 cm^{-1} is due to the ester group. The mass spectrum (FAB$^+$) shows the molecular peak at *m*/*z* 762. Three routes of fragmentation are observed. First, there is a loss of all three carbonyl ligands and the methyl group (*m*/*z* 663). The second route is the cleavage of the bond between the aromatic unit and the tropane ring followed by loss of one carbonyl ligand (*m*/*z* 531, 503). Last, the sulfur−carbon bond connecting the chelate unit with the tropane ester unit is cleaved (*m*/*z* 457). The fragment *m*/*z* 443 can be assigned to a following loss of the *S*-methyl group.

The ^{99m}Tc complex (TROTEC-1) was synthesized by a modification of the literature procedure[7] and purified by HPLC (t_R = 6.2 min).

In Vitro Binding Studies. The binding affinities of the compounds **3** and **4** for the DAT, NET, and 5-HTT were determined, using competitive binding assays.[15,16]

Results and Discussion. Starting from β-CFT, a new chelating ligand (**3**) was prepared by introduction of a dithioether unit into the 2β-position. Binding data to cloned human DAT revealed a high binding affinity. For both compounds **3** and **4** a significantly improved fit in the estimation of the IC_{50} values was obtained, if the existence of both a low- and a high-affinity binding site was postulated. Interestingly, the rhenium complex **4** binds as strongly to the high-affinity site as the metal-free precursor **3** but, on the other hand, exhibits decreased binding to the low-affinity site. The calculated affinity with assumption of a single binding site is better for **4** (IC_{50} = 1.5 ± 0.4 nM) than it is for **3** (IC_{50} = 3.2 ± 0.3 nM). High- and low-affinity binding of β-CFT has previously been described on membranes of rat[15] and monkey[18] striatum, as well as for the cloned human[19] DAT. Our IC_{50} values obtained on cloned human DAT expressed in CHO cells are about 4-fold lower than those found by Pristupa et al.[19] using COS-7 cells. Cell type differences may explain this discrepancy, but differences in the assay procedures cannot be excluded as a possible source. Thus, Gracz and Madras found 10-fold differences in the high- and low-affinity binding between fresh or frozen and soluble striatal membrane preparations of monkeys.[18]

It is known that β-CFT also exhibits a high selectivity over the 5-HTT and the NET (Table 1). Introduction of our chelate moiety increases the affinity toward the 5-HTT and NET and consequently decreases the selectivity.

Table 1. Binding Data (IC_{50} Values) of β-Cft, **3**, and **4** to the Monoamine Transporters[a]

	DAT high affinity (nM)	DAT low affinity (nM)	NET (nM)	5-HTT (nM)
β-CFT	2.62 ± 1.06	139 ± 72	834 ± 45[17]	759 ± 47[17]
3	0.227 ± 0.202	5.69 ± 1.20	16.3 ± 0.6	28.5 ± 4.6
4	0.146 ± 0.042	20.3 ± 16.1	7.3 ± 1.1	71.8 ± 1.4

[a] Data are means ± SEM (n = 3−4).

The most important result is the markedly enhanced affinity of the tricarbonylrhenium(I) complex **4** in comparison with β-CFT. Although other metal complexes exhibit a very high affinity to the DAT, for example, a "3 + 1" complex derived from IPT,[20] a cyclopentadienyl carbonyl complex derived from β-CIT,[21] and technepine again derived from β-CFT,[4] the affinity was more or less retained in the same order of magnitude compared to the starting ligand. With complex **4**, we achieved an affinity of about 1 order of magnitude higher than that of β-CFT. This can only be explained by participation of the complex unit in the binding to the transporter in the sense that the assumed hydrogen bonding between the C-2 ester group and the DAT is complemented by an additional lipophilic interaction exerted by the sulfur atoms of the dithioether. This lipophilic interaction can slightly be enhanced by complexation. This interpretation is consistent with previously reported results from Kozikowski et al. regarding a 2-cocaine analogue bearing a 2-vinyl substituent[22] and of Carroll et al. regarding cocaine analogues with heterocyclic substituents in position 2.[23] Both reports suggest a weak hydrophobic contribution to binding at the DAT.

Conclusions. TROTEC-1 (**4**) represents the first example of a dithioether/carbonyl complex with exceptional high affinity to the DAT. We have thus demonstrated that the Tc-complexed chelator moiety, rather than necessarily representing an obstacle to binding as a consequence of its steric bulk, may in favorable cases even improve binding through additional interactions if its position within the ligand molecule is chosen judiciously.

Additional studies focused on the biodistribution behavior of the ^{99m}Tc complex and the question of which structural features influence the pharmacological activity of further dithioether/carbonyl complexes are currently in progress.

Supporting Information Available: Experimental descriptions of the chemistry and radioligand binding assays (4 pages). Ordering information is given on any current masthead page.

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