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SYNTHESIS AND BIOLOGICAL EVALUATION OF TWO NOVEL DAT-BINDING TECHNETIUM COMPLEXES CONTAINING A PIPERIDINE BASED ANALOGUE OF COCAINE

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Abstract: Two new technetium complexes containing a piperidine template have been synthesized and evaluated as possible leads for the development of dopamine transporter (DAT) imaging agents. Binding data for the corresponding rhenium complexes containing either a monoaminomonoamide (MAMA') or a diaminodithiol (DADT) chelating unit exhibited significant affinity for the DAT. Initial biodistribution studies in rats revealed only a low brain uptake.

Introduction

The increase in life expectancy in highly developed countries has led to an increased public awareness of several insidious neurodegenerative diseases like Alzheimer's dementia and Parkinson's disease (PD). At this point in time, there is no satisfactory medication for PD in sight. Thus, the ability to readily monitor disease progression may facilitate the discovery of PD medications.

Parkinson's disease is known to be associated with a significant loss of dopaminergic neurons in the basal ganglia.[1] Hence, the measurement of the depletion of these neurons through radioactive labeling of the dopamine transporter (DAT) in dopaminergic nerve terminals appears to be a suitable way of diagnosing and assessing the progression of this disease. Technetium-99m is the preferred radionuclide due to its favorable properties,[2] and it is used for single photon emission computed tomography (SPECT) for routine diagnosis in nuclear medicine. Several technetium complexes like TRODAT-1, Technepine, or TROTEC-1 (Figure 1) appear to be promising candidates for the development of a SPECT imaging agent of dopaminergic neuronal function in the basal ganglia.[3–7] Today, TRODAT-1 appears to be the most advanced of these SPECT agents. However, this molecule is still not perfect. There are important features that could be improved including binding affinity and brain

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uptake. Optimization of pharmacodynamic and pharmacokinetic parameters could result in an improved background to target ratio, thereby reducing the exposure of the patients to radioactivity.

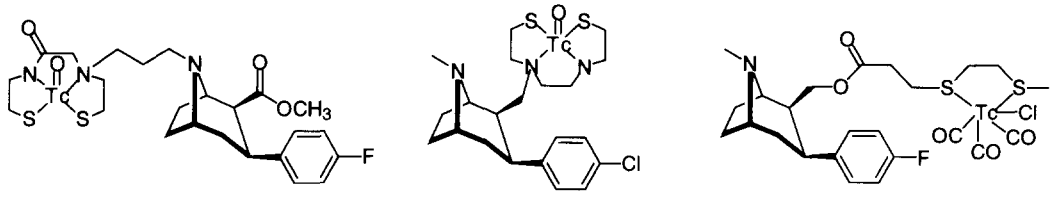


Figure 1. Tropane technetium conjugates.

Common to these available ligands is their derivation from naturally occurring cocaine; thus, all three ligands contain a tropane ring system. Recently, however, our group has shown that the tropane skeleton is not a prerequisite for high binding affinity at the DAT.[8] Thus, a number of piperidine derivatives bearing the same functional groups as those contained in tropanes belonging to the WIN series, but lacking the 2-carbon bridge, were shown to exhibit high affinity to the DAT.

The rationale of the present study was to design a piperidine technetium conjugate and to investigate whether it is possible to maintain its binding affinity for the DAT and possibly to improve the brain uptake in comparison to TRODAT-1.

Results and Discussion

A 3-propyl substituted piperidine derivative with excellent binding affinity (Figure 2) was chosen as the lead structure for the synthesis of a technetium complex for exploration as a potential SPECT ligand.

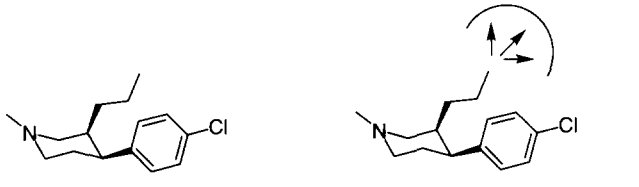
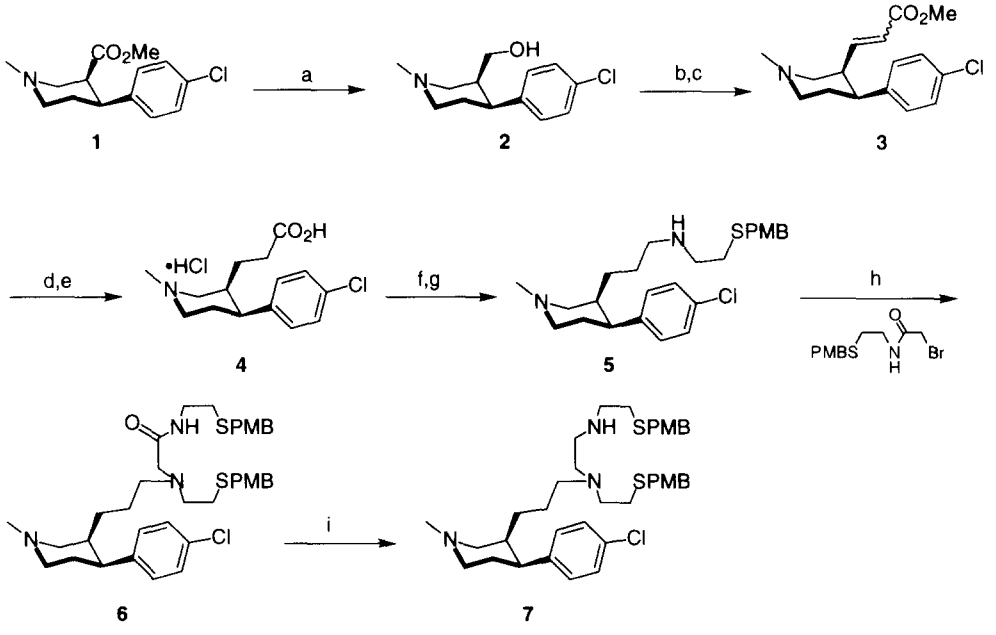


Figure 2. Piperidine derivative with high binding affinity to the DAT and expected region of bulk tolerance.

The existence of a region of bulk tolerance was concluded from the observed biological activity of tropane technetium complexes like TRODAT-1 and TROTEC-1. In both compounds the complex unit is attached to the C-2 position, which is equivalent to C-3 in piperidine (Figure 2). As chelating groups, the monoaminomonoamide

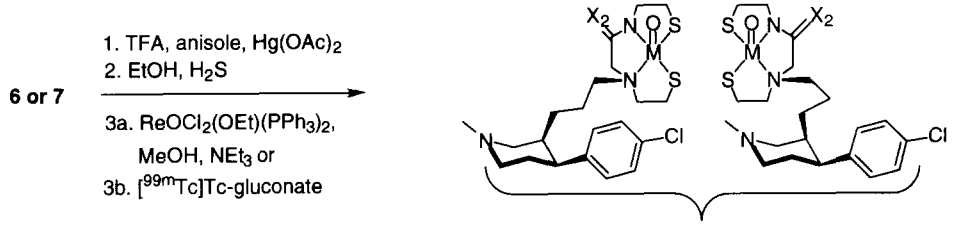
(MAMA') and the diaminodithiol (DADT) chelating units are the most likely to incorporate a $[Tc^{V}O]^{3+}N_2S_2$ core into a receptor targeted agent, in terms of biostability and biodistribution.[9] Furthermore, since both groups are easily accessible and seem to be well tolerated by the DAT, either in TRODAT-1 or Technepine, we decided to make use of them as chelator moieties. Since a longer spacer like that found in TROTEC-1 seems to be beneficial for achieving high affinity at the DAT, we chose a complete conjugate approach[10] and decided to attach a $[Tc^{V}O]^{3+}N_2S_2$ metal complex unit at the end of the propyl spacer. By maintaining the propyl group, the overall carbon atom number is exactly the same as in TRODAT-1, and therefore the biodistribution profile, in particular the brain uptake, was expected to be comparable.

The synthesis of the piperidine technetium conjugate was rather straightforward (Scheme 1). Starting from arecoline, (-)-methyl 4β-(chlorophenyl)-1-methylpiperidine-3β-carboxylate **1** was prepared by addition of the appropriate Grignard reagent, followed by optical resolution of the resulting racemic mixture with dibenzoyltartaric acid according to a literature procedure.[8] Next, LAH was used to reduce the methoxycarbonyl group to hydroxymethyl to give **2**. Swern oxidation and subsequent reaction of the aldehyde with methoxycarbonylmethylenetriphenylphosphorane afforded an *E,Z*-mixture of the olefins **3**. This crude mixture was subjected directly to reduction of the double bond, and the ester group was hydrolyzed in turn to the acid **4**. Reaction with *S-para*-methoxybenzyl (*S*-PMB) protected cysteamine and subsequent borane reduction gave amine **5**. By alkylation of the secondary amino group with *S*-PMB-protected *N*-bromoacetylcysteamine, the piperidine-MAMA' conjugate **6** was generated. Finally, the piperidine-DADT conjugate **7** was obtained by reduction of the amide function using borane in THF.



Scheme 1. Synthesis of the MAMA' and the DADT ligands. (a) $LiAlH_4$, THF, 84–100%; (b) $(COCl)_2$, NEt_3, DMSO, CH_2Cl_2; (c) $Ph_3P{=}CHCOOCH_3$, toluene, 53–63%; (d) Mg, MeOH, 100%; (e) 6 N HCl, 100%; (f) *S*-PMB-cysteamine, NEt_3, EDCI, DMAP, CH_2Cl_2, 75%; (g) BH_3•THF, THF; 1 N HCl, 60%; (h) NEt_3, CH_2Cl_2, 44%; (i) BH_3•THF, THF; 1 N HCl, 40%.

Deprotection of either **6** or **7** with mercuric acetate in trifluoroacetic acid and removal of the mercury ions with hydrogen sulfide afforded the free bisthiols as trifluoroacetate salts, which were used without further purification to prepare the corresponding rhenium complexes (Scheme 2), which have been characterized by HRMS and IR.[11] Due to the formation of a quarternary nitrogen upon complexation and the stereogenic nature of the metal atom itself, four diastereoisomers, a *syn* and an *anti* pair, exist theoretically. Complex **8** was actually obtained as a mixture of two diastereoisomers which have been separated by preparative TLC. In accordance with previously published results, we assume that both *syn* diastereoisomers have been formed.[5,6] However, the absolute configuration at the quarternary nitrogen of both complexes still awaits assignment through the use of X-ray structural analysis. Complex **9** was obtained in an analogous fashion; however, in this case, separation of the diastereomers has not proven possible.



X_2 = O, M = Re : **8a, b**
X_2 = H, M = Re : **9a, b**
X_2 = O, M = ^{99m}Tc : **10a, b**
X_2 = H, M = ^{99m}Tc : **11a, b**

Scheme 2. Preparation of the rhenium/technetium complexes **8**, **9**, **10**, and **11**.

The binding affinities of the rhenium complexes were determined using competitive binding assays and [^{3}H]mazindol as the radioligand.[12] All complexes showed good binding affinities with the following K_i values: **8a**, 49 ± 3 nM; **8b**, 43 ± 3 nM; **9**, 96 ± 22 nM.[13] Little difference is seen between the binding affinities of the two diastereoisomers of **8**. This result demonstrates once again that, just as in the case of the TRODAT stereoisomers, differences in binding affinity based on the stereoisomerism of the complex moiety are negligible. In fact, it has previously been shown by Kung that a mixture of the ^{99m}Tc complexes of TRODAT-1 displayed the same image in a baboon brain as the better of the two diastereoisomers.[5]

The binding affinity of the DADT complex **9** is slightly poorer in comparison to the MAMA' complexes **8a** and **8b**. Apparently, the amide group in the complex moiety contributes to the binding of the whole molecule to the DAT. Therefore the MAMA' chelating unit should be the preferred chelating unit to achieve a high binding affinity at the DAT.

Radiolabeling was performed with [^{99m}Tc]technetium gluconate using the free bisthiols which had been deprotected directly before use. The yield was 65–85%, and the radiochemical purity was >95% after reverse-

phase solid-phase extraction. Preliminary biodistribution studies were performed in male Sprague–Dawley rats, and the brain uptake was measured after 60/75 and 120 min. The brain uptake of both complexes is low, 0.02% after 75 min for **10** and 0.03% after 60 min for **11**. The brain uptake for **11** is about twice that of **10**, thus confirming the superiority of the DADT chelator moiety in terms of brain uptake.

Table 1. Brain uptake of technetium complexes **10** and **11**.

Tc complex	brain uptake in rats (% dose/organ)	
	60/75 min	120 min
10	0.018 (75 min)	0.01
11	0.028 (60 min)	0.017

In comparison to the TRODAT-1 diastereoisomers, the brain uptake of **11** is about one order of magnitude lower, thus suggesting a poorer biodistribution behavior. We assume that the spacer length may have a negative effect on the biodistribution in comparison to the TRODAT complexes, since it is unlikely that the lack of the 2-carbon bridge in our complexes should be solely responsible for their poor brain uptake. Currently, little is known about the influence of the spacer length between biomolecule and complex moiety. Hence, additional investigations should reveal its influence on lipophilicity and pK_a values as the fundamental physico-chemical parameters involved in brain uptake. The low brain uptake exhibited by our compounds precluded further studies, such as regional biodistribution in the brain.

Conclusions

In summary, we have synthesized two new technetium complexes with high binding affinity to the DAT using a 4β-phenylpiperidine as the starting template. We have thus demonstrated that there is enough space around C-3 of the piperidine ring to accommodate a bulky metal complex unit, which is in good agreement with the results obtained with derivatives of the WIN series.[5,7] The low brain uptake of these complexes was certainly not expected, and further studies are necessary to overcome this problem. However, this new class of compounds offers a multitude of possibilities for further variation. Fine tuning of structural parameters should result in compounds with higher affinity and improved biodistribution. Therefore, the results presented here are considered a good starting point for further studies which could possibly afford a new class of imaging agents for the DAT in the central nervous system.

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11. Analytical data: **8**: IR (KBr) 964, 952 cm^{-1}; HRMS (EI): *m/z* 643.1082, calcd. for $C_{21}H_{31}ClReO_2N_3S_2$, 643.1103; chromatographic separation (ethyl acetate/triethylamine/ethanol = 8.5/1/0.5), R_f: **8a**, 0.36; **8b**, 0.25; **9**: IR (KBr) 979, 936 cm^{-1} (HCl salt); HRMS (EI): *m/z* 629.1301, calcd. for $C_{21}H_{33}ClReON_3S_2$, 629.1311.
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13. Data for inhibition of monoamine reuptake, K_i in nM. [^{3}H]DA uptake: **8a**, 110 ± 3, **8b**, 112 ± 8, **9**, 193 ± 16; [^{3}H]5-HT uptake: **8a**, 361.9 ± 38.4, **8b**, 888.4 ± 4.1, **9**, 242.5 ± 13.7; [^{3}H]NE uptake: **8a**, 392.6 ± 50.0, **8b**, 2030 ± 80, **9**, 231.1 ± 13.7.