

A Computer-Aided Radiopharmaceutical Drug Design Study Using *Ab Initio* and Molecular Mechanics Methods

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Abstract

This is an investigation of technetium ligands and their complexes with $[\text{TcO}]^{3+}$ using *ab initio* population analysis and molecular mechanics conformational searching methods. Calculated atomic electronic populations on the technetium atom in complexes with a number of ligands gauge the degree of covalent bonding between technetium and these ligands. Here a reduction in the positive charge on the $[\text{TcO}]^{3+}$ moiety by complexation with a given ligand is correlated with covalent bonding. Our *ab initio* results suggest that ligands with more sulphur atoms have better covalent bonding to technetium than do other ligands. A conformational analysis of the uncomplexed ligands indicates that conformational reorganization before complexation correlates inversely with stable complex formation. This conformational analysis shows that ligands with ethylene carbonyl bridges have low energy conformations closer to the final complexation geometries than do ligands with ethylene, propylene or propylene carbonyl bridges. The presence of these low energy conformations facilitates a faster complexation of the ethylene carbonyl $[\text{TcO}]^{3+}$ moiety. This result produces a kinetic explanation why ethylene carbonyl bridged ligands form stable complexes while many other ligands do not [1]. The conclusion is that kinetic and thermodynamic considerations play a role in stable complex formation between these ligands and technetium.

Keywords: Technetium ligands, radiopharmaceuticals, drug design, conformational analysis, *ab initio* population analysis

Introduction

Technetium has in the last several years become an important tool in medical diagnostic imaging [2] and this has energized the search for suitable technetium complexing ligands [3]. Technetium has atomic number 43 and when complexed

with oxygen as a $[\text{TcO}]^{3+}$ cation it has the electronic configuration $[\text{Kr}]5s^2$ which is a closed shell system and representable at the Hartree-Fock level of theory. In this computational study our objective has been to understand some of the experimental trends observed in the differing abilities of certain ligands to form stable complexes with technetium. One such trend is the relative selectivity of the $[\text{TcO}]^{3+}$ cation

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for ligands having two nitrogen and two sulphur atoms above those having four nitrogen atoms [1]. Also, we have tried to understand the experimental result that the $[\text{TcO}]^{3+}$ cation readily forms stable complexes with ligands having ethylene bridges with a carbonyl group in the bridge [4] while it does not do the same with a number of other apparently similar ligands.

There are no studies to date in the literature which have used computational methods to evaluate technetium selective ligands although this has been suggested [5]. As described in reference 5 the lack of work in this area is probably due to the inherent difficulties associated with doing calculations on a metal so far down the periodic table. Some of these problems include the lack of parameters for semi-empirical or empirical methods even though the number of atoms in typical technetium complexes indicate the use of these lower level calculations would be appropriate. At the *ab initio* level one encounters the computational difficulties associated with handling molecules containing thirty or more atoms. Also, at this higher level of theory there is the problem of representing the influence of a relativistic effects [6] for nuclei down the periodic table such as technetium. *ab initio* studies on technetium complexes also encounter the problem of balancing basis sets which must describe both the metal center and group II or III elements such as carbon, nitrogen, oxygen and sulfur [7].

However, in recent years a number of methods for representing nuclei in the lower part of the periodic table have been described [8-10]. The result of this has been an increase in the number of properties being calculated for both technetium and other transition metal [11] compounds. In this study we use *ab initio* atomic population analysis to measure a ligand's capacity for complexing technetium by looking at the ability of the ligand to reduce the high positive charge on the $[\text{TcO}]^{3+}$ cation. When any ligand reduces this charge, then we conclude that a degree of covalent bonding is taking place between the ligand donor atoms and the technetium atom. When ligands exhibit the same capacity for reducing this positive charge, then it is concluded that their complexes have approximately the same thermodynamic stability.

Those ligands selected for study herein have three characteristics which are varied. These include the actual donor atoms which are either nitrogen or sulphur, the number of carbons which link these donor atoms together and the presence or absence of a carbonyl functionality on the linker car-

bon atoms. For the purpose of this study we have developed a method for naming our ligands and their corresponding technetium complexes. This nomenclature designates the ligand or complex by the number of nitrogen (N) or sulfur (S) atoms and uses the number of atoms which enclose rings having two donor atoms and the complexed technetium atom. When a linker has a carbonyl group then the complex is designated by Tc and when the carbonyl oxygen is replaced by two hydrogen atoms then the compound is denoted as TcH. Figure 1 demonstrates the use of this naming convention for two structures which we therefore name $\text{TcS}_2\text{N}_2(555)$ and $\text{TcHS}_2\text{N}_2(565)$ respectively.

Computational Methods

The initial geometries for the ligands and complexes in this study were generated by using MM+ calculations in the HyperChem [12] molecular modeling package. Final geometries were obtained by cycles of energy minimization and molecular dynamics equilibration at 400 °C. These dynamics based conformational searches were done for a minimum of 100 ps to ensure that all important conformations were visited. The low energy geometries were obtained by minimization of conformations found in the dynamics analysis and the lowest energy conformers were selected for further study. The geometries found using this search method were then geometry optimized at the STO-3G level of theory. Here, depending upon the flexibility of the ligand either of the energetically close trigonal bipyramid or square pyramidal [13] geometries were obtained around the metal center. The geometries obtained using this approach were found to be similar to obtained from X-ray studies [2] on technetium complexes. The geometries of the complexes obtained in this manner were then used as input for single point *ab initio* level calculations using the Gaussian 92 set of programs [14]. A published 33333/333/33 gaussian basis set for technetium [15] was broken up to generate a 33333/333/2121 gaussian basis set on technetium. A polarization function was added to this by energy minimization varying coefficients and exponents using the $[\text{TcO}]^{3+}$ moiety to produce a 33333/333/2121/11 basis set on technetium. Nonmetal atoms were represented by the D95* basis set as implemented in Gaussian 92 [14] and the basis set combination was shown to contain no significant basis set superposition error. Atomic population analysis used a Mullikan and NBO population scheme as implemented in the Gaussian92 program. Conformational searches on the uncomplexed ligands were done using the QUANTA/CHARMM [16], Sybyl [17] and Catalyst [18] molecular modelling packages. These searches used the method of molecular dynamics in an aqueous box as implemented in Sybyl, the conformational search algorithm as implemented in Catalyst, the Boltzman Jump Method in QUANTA/CHARMM and molecular dynamics in an aqueous box as implemented in QUANTA/CHARMM. *ab initio* geometry optimizations and energies of interactions were

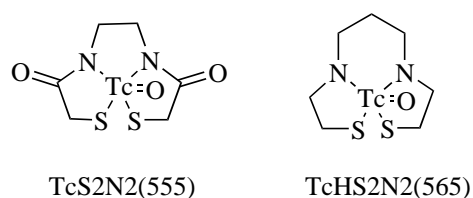


Figure 1. Two technetium complexes and their nomenclature.

Table 1. Mullikan and Lowden populations and charges for the Tc atoms in the STO-3G optimized geometries of the ligands investigated in this study.

Complex	Mull.pop.	charge	Low.Pop.	charge
TcS2N2555	41.214844	1.785156	42.350929	0.649071
TcHS2N2555	41.205875	1.794125	42.325451	0.674549
TcS2N2565	41.215859	1.784141	42.336444	0.663556
TcS2N2H565	41.208380	1.791620	42.346675	0.653325
TcS2N2656	41.249999	1.750001	42.356292	0.643708
TcHS2N2656	41.262293	1.737707	42.369468	0.630532
TcS2N2666	41.385017	1.714983	42.396777	0.503223
TcHS2N2666	41.307691	1.692309	42.448602	0.551398
TcSN3555	41.116917	1.883083	42.155955	0.844045
TcHSN3555	41.116507	1.883493	42.132688	0.867312
TcSN3656	41.169813	1.830187	42.164649	0.835351
TcHSN3656	41.179081	1.820919	42.190675	0.809325
TcSN3666	41.205715	1.794285	42.245908	0.754092
TcHSN3666	41.182019	1.817981	42.221879	0.778121
TcN4555	40.971271	2.028729	41.976074	1.023926
TcHN4555	40.970977	2.029023	41.972482	1.027518
TcN4656	40.588045	2.411955	41.603507	1.396493
TcHN4656	41.375882	1.624118	42.324133	0.675867
TcN4666	40.268252	2.731748	41.389369	1.610631
TcHN4666	41.115844	1.884156	42.062821	0.937179

done using the GAMESS program. [19] Interaction energies were measured by comparing *ab initio* energies at the 6-31G** level for the bonded and non bonded situations.

Results and Discussions

Ab initio Mullikan and Lowden populations on the central technetium atoms as a function of different ligands are documented in Table 1 at the STO-3G level for the geometry optimized complexes. Table 2 has the Mullikan Charges and the NBO populations on the Technetium atoms as a function of different chelators for the D95* basis set on all atoms except technetium and the 33333/333/2121/11 basis set on technetium. Here the ligands best able to reduce the high positive charge on the $[\text{TcO}]^{3+}$ moiety and therefore to produce the lowest positive charge on the central technetium atoms are the S2N2 ligands. The highest technetium charges are for the N4 systems and the SN3 ligands show intermediate technetium charges. The presence of sulfur atoms in these ligands

therefore correlates with a lowering of the positive charge on the metal center in the $[\text{TcO}]^{3+}$ moiety. This result is consistent with conventional chemical wisdom which states that a more polarizable sulfur atom will be better than a harder nitrogen atom at donating electron density onto a soft metal center such as technetium. This is also consistent with experiment since a number of S2N2 ligand complexes have been isolated while the N4 systems rarely form stable complexes with $[\text{TcO}]^{3+}$ [6].

The difference between the lower level STO-3G basis set on all atoms and the D95* basis set on the ligands and the 33333/333/2121 gaussian basis set on technetium is that the charges on technetium are lowered. This is as expected since a more flexible basis set should permit electron density to move from the negatively charged donor atoms to the positively charged metal center. When a set of ligand complexes have the same donor atoms, such as is the case in the S2N2 series, then the variability in the total technetium atomic charge for these ligands is small and this is evident in Table 2. One might expect that more flexible ligands should be

Table 2. The Mullikan charge and NBO population analysis for the Tc atoms complexed in the ligands in this study at basis sets as described.

Molecule	Mullikan			Charge	Natural	Population		
	Atom	No	Charge Total		Core	Valence	Rydberg	Total
TcS2N2555	1	Tc	1.560144	0.83077	35.89253	6.19724	0.07946	42.16923
TcHS2N2555	1	Tc	1.577966	0.82805	35.89156	6.19874	0.08165	42.17195
TcS2N2656	1	Tc	1.584530	0.89226	35.89062	6.13523	0.08190	42.10774
TcHS2N2656	1	Tc	1.581060	0.88520	35.88834	6.14307	0.08340	42.11480
TcS2N2666	1	Tc	1.561482	0.88709	35.92948	6.09721	0.08622	42.11291
TcHS2N2666	1	Tc	1.540141	0.85234	35.88973	6.17614	0.08179	42.14766
TcHSN3555	1	Tc	1.698596	0.97395	35.87033	6.07235	0.08336	42.02605
TcSN3656	1	Tc	1.693619	1.02089	35.86808	6.02882	0.08222	41.97911
TcHSN3656	1	Tc	1.693670	1.01911	35.86789	6.02901	0.08399	41.98089
TcHSN3666	1	Tc	1.686340	1.00270	35.86774	6.04656	0.08301	41.99730
TcSN3666	1	Tc	1.665009	0.98514	35.87095	6.06270	0.08122	42.01486
TcHSN3666	1	Tc	1.686340	1.00270	35.86774	6.04656	0.08301	41.99730
TcN4555	1	Tc	1.813344	1.08914	35.86624	5.96503	0.07959	41.91086
TcHN4555	1	Tc	1.824623	1.07908	35.86346	5.97570	0.08177	41.92092
TcN4656	1	Tc	1.786551	1.10981	35.86561	5.95413	0.08087	41.91141
TcHN4656	1	Tc	1.807861	1.09011	35.85661	5.95674	0.08161	41.90167
TcN4666	1	Tc	1.821453	1.10897	35.86541	5.96712	0.07987	41.89741
TcHN4666	1	Tc	1.771747	1.14385	35.85517	5.92076	0.08022	41.85615

better at reducing the technetium charge since they can better accommodate the geometries dictated by empty orbitals on technetium. This, however, is not the case and from Table 2 the more flexible propylene ligands do not appear to be better at reducing the technetium positive charge than are the more rigid ethylene or ethylene carbonyl ligands. The NBO population analysis in Table 2 shows that the technetium charge is different for the three different kinds of ligands. The trend that does emerge from this population analysis is that as one increases the amount of sulphur content in the donor ligands then the electron population in the valence shell of technetium increases. This results in a lowering of the positive charge on technetium and contributes a covalent nature to the interaction between the chelators atoms and the metal center.

These population studies therefore explain the experimental result that sulfur containing ligands are better at forming stable complexes with $[\text{TcO}]^{3+}$ than are non sulfur containing ligands. However, our population analysis does not ex-

plain the experimentally observed preference that the $[\text{TcO}]^{3+}$ cation has for the carbonyl substituted ethylene ligand [4]. In terms of thermodynamic stabilities, as predicted by the *ab initio* population analysis, this should not be a preferred ligand for complexation with the $[\text{TcO}]^{3+}$ cation over other ligands such as the ethylene S2N2 analogue. Thus Table 2 shows that the TcS2N2555 and TcS2N2666 complexes have almost the same valence shell populations and total electron populations. A similar result is seen for comparisons between ligands having or lacking the carbonyl functionality where in Table 2 the TcS2N2555 and TcHS2N2555 complexes are very close in total and valence electron populations. This leads one to conclude that thermodynamic considerations resulting from differences in binding which follow from different electron distributions do not explain the differences observed for the formations of these complexes.

Kinetic considerations may therefore explain the selectivity of the $[\text{TcO}]^{3+}$ cation for ligands having the carbonyl ethylene bridge over all other ligand types. To investigate

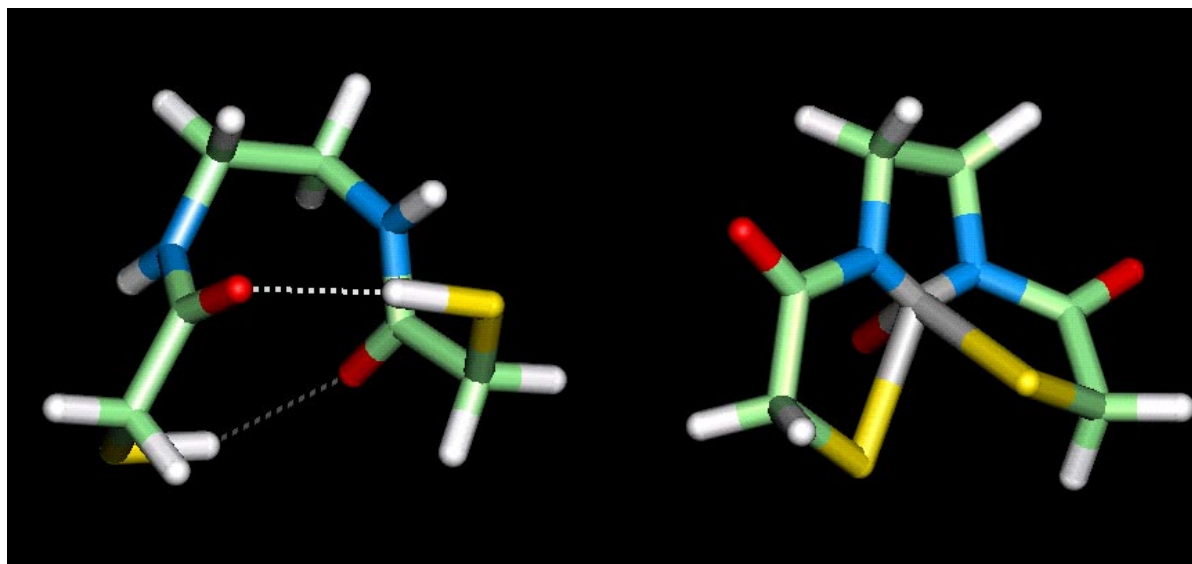


Figure 2. The low energy conformation and the complexed form of the ethylene carbonyl S2N2 ligand.

this we did conformational searches on a number of ligands in this study. In these searches any errors associated with any specific search method or force field were reduced by comparing results from a selection of conformational approaches and force fields. The consistent picture that evolved was that the low energy conformers were very different depending

upon the type of ligand. We found a number of intramolecular hydrogen bonding conformations for the ligands containing ethylene linkers between the donor atoms which predispose these ligands toward complexation with a metal center.

Figures 2 and 4 show samples of the low energy conformations of the S2N2 ethylene carbonyl and S2N2 propylene carbonyl ligands and their corresponding TcS2N2(555) and TcS2N2(666) complexes. In Figures 2 and 3 the S2N2 ethylene carbonyl ligand exhibits low energy conformations that are close to the final complexed geometry. This preferred conformation of the free ligand is due to intramolecular hydrogen bonding between the carbonyl oxygens and the terminal sulfur atoms or between these oxygens and the amide nitrogen hydrogens. We calculated the energy contributions of the two sulphur intramolecular hydrogen bonds of Figure 2 by *ab initio* calculations and found their combined contribution to be 4.4 kcal/mol. The combined NH—OC, SH—HS and SH—OC bond shown in Figure 3 gave a combined contribution of 6.7 kcal/mol to the ligand conformational energy.

When an extra methylene unit is added to the chain joining the two donor sulfur atoms, then the intramolecular hydrogen bonds that form tend to extend the two donor atoms away from one another and thus complexation is less likely to happen. A number of low energy conformations that are noticeably different from the final complexation geometry are observed with the lowest shown in Figure 4. This implies that the free propylene carbonyl ligand must undergo considerable conformational reorganization before a complex with $[\text{TcO}]^{3+}$ can be formed. This is also true for ligands having either ethylene or propylene aliphatic linkers since these then lack the carbonyl oxygens necessary for intramolecular hydrogen bond formation.

The implications of this analysis are that a suitable ligand should spend a high proportion of its time in a conformation that is close to the complexation geometry for complexation with $[\text{TcO}]^{3+}$ to occur. This is the case for the

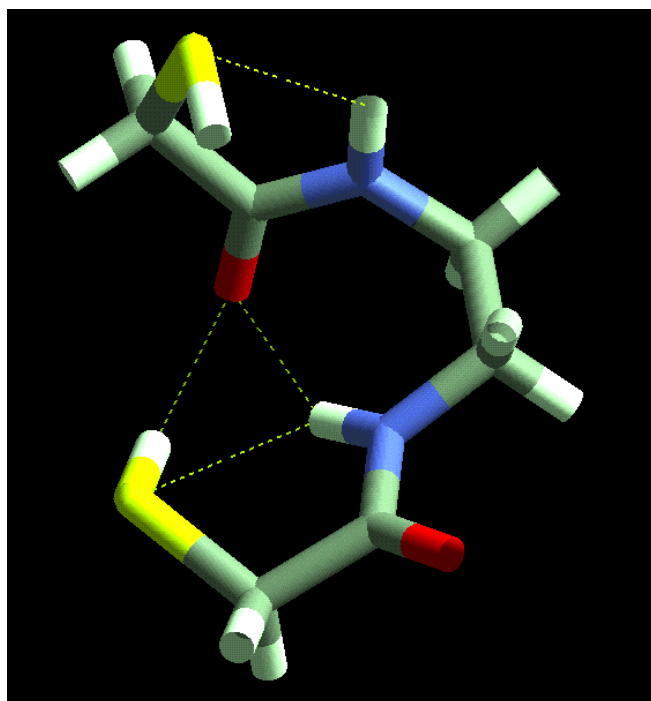


Figure 3. The S2N2 carbonyl ligand in a low energy conformation showing CO—HN hydrogen bonding.



Figure 4. The low energy conformation and the complexed form of the propylene carbonyl S2N2 ligand.

S2N2 ethylene ligands and is not the case for all other ligands investigated in this study. In effect the ligand is in a race with an oxidative process which can quickly oxidize technetium from Tc^{5+} to Tc^{7+} . Once this oxidative process occurs then the $[TcO]^{3+}$ moiety is no longer available for complexation. Ligands which do not spend a proportion of their time in a conformation close to the complexation geometry will lose the race with this further oxidative process. Thus even ligands which can produce thermodynamically stable complexes, such as the ethylene or propylene S2N2 ligands from this study, still do not necessarily form complexes with technetium. However, the addition of a carbonyl oxygen to the ethylene linkers in these ligands permits intramolecular hydrogen bonding which stabilizes conformations similar to those of the complexed ligand and improves the chances of complex formation.

The implications of this study are as follows. Designers of technetium ligands should consider both thermodynamic and kinetic contributions before proposing candidate ligands for complexation with $[TcO]^{3+}$. Good ligands are those that have relatively soft donor atoms and therefore are able to donate electron densities onto technetium but at the same time have low energy conformations which are close to the final complexation geometry.

Conclusions

This study concludes that kinetic control and thermodynamic control are both significantly influencing the formation of technetium complexes. We have used *ab initio* population analysis on a number of technetium complexes and these

suggest that many ligands which do not form stable complexes with $[TcO]^{3+}$ would have thermodynamically stable complexes if the complex formed. Our conformational analysis shows that it is possible to correlate the existence of low energy conformations that resemble the complexation geometries with the abilities of ligands to form stable complexes. Any design of new $[TcO]^{3+}$ chelators should therefore design ligands which have polarizable donating atoms and have low energy conformations which are similar to the final complexation geometry.

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