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**MOLECULAR MODELING IN THE DEVELOPMENT OF
METAL RADIOPHARMACEUTICALS**

Final Progress Report

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Abstract

In year 1 of this project we began compilation of a structural library to serve as a data base containing descriptions of the molecular features of metal-labeled radiopharmaceuticals known to efficiently cross the blood-brain barrier. Such a data base is needed in order to identify structural features (size, shape, molecular surface areas and volumes) that are critical in allowing blood-brain barrier penetration. To date 9 metal complexes have been added to this structural library, along with 18 organic reference tracers, and numerous topological indices calculated. We also adapted the molecular modeling software in Polygen's QUANTA to allow performance of molecular mechanics calculations on metal complexes of radiopharmaceutical interest.

In year 2 of this project we completed a detailed comparison of four molecular mechanics computer programs to assess their applicability to modeling the structures of low molecular weight metal complexes. The four programs considered were QUANTA, SYBYL, BOYD, and MM2DREW. For this evaluation we tested the ability of each program to reproduce the crystallographic structures of 38 complexes between nickel(II) and saturated N-donor ligands. The programs were evaluated in terms of their ability to reproduce structural features such as bond lengths, bond angles, and torsion angles. BOYD and MM2DREW were found to best reproduce the structures of the nickel complexes, with SYBYL performing nearly as well. QUANTA was clearly poorer in structure reproduction. The susceptibility of the programs to falling into local energy minima was also investigated by using initial molecular coordinates that have been "randomized". BOYD and MM2DREW were found to easily fall into local energy minima, while SYBYL still reproduced structures well. Thus, it is concluded that SYBYL will be the most useful of these programs for reproducing the structures of metal complexes of saturated ligands.

In the third funded year of this project we investigated the synthesis and characterization of lipophilic cationic gallium complexes with hexadentate *bis*(salicylaldimine) ligands. This work identified the first gallium-68 radiopharmaceuticals that can be injected intravenously and that subsequently exhibit significant myocardial uptake followed by prolonged myocardial retention of ^{68}Ga radioactivity. Tracers of this type remain under investigation as agents for evaluation of myocardial perfusion with positron emission tomography.

Final Progress Report

One of our first goals was the development of a structural library tabulating the physicochemical properties and structural features of metal-labeled radiopharmaceuticals known to efficiently penetrate the blood-brain barrier. In addition, this structural library included other uncharged, lipophilic metal complexes previously studied by us or other investigators and found to be excluded from the brain. Finally, this structural data base also included data for organic radiopharmaceuticals from the literature for which data on blood-brain barrier permeability exists.

Table 1 presents data that was compiled and/or calculated. Compounds found in this table include several metal-labeled cerebral blood flow tracers now in clinical use or undergoing preclinical evaluation (e.g. Tc-HMPAO, Cu-PTSM); the ^{11}C -labeled alcohols and ethers studied by Dischino, et al, (1983) in a primate model that allows determination of the average brain permeability surface areas ($P \cdot S$) product; and a few other highly diffusable tracers frequently used in nuclear medicine and/or physiological research (*N*-isopropyl-*p*-iodoamphetamine (IMP, $\text{N,N,N'$ -trimethyl- N' -[2-hydroxyl-3-methyl-5-iodobenzyl]-1,3-propanediamine (HIPDM), and 4-iodoantipyrine (IAP)). Structural data for the metal complexes was obtained from published crystallographic literature. Structural data for the organic tracers was generally obtained from the Cambridge Data Base. When x-ray data was not available for the organic compounds, the molecules were constructed and minimized with the QUANTA software.

For each of the molecules in Table 1 several topological indices have been calculated using QUANTA. These indices include the molecular surface area and enclosed volume, along with the linear dimensions of the molecule based on the lengths of the sides of the minimum-sized rectangular box that can contain the molecule. Three different definitions of the molecular surface have been considered in making the surface area and volume calculations. The van der Waals surface (Figure 1a) is the surface resulting from a set of intersecting spheres centered on the atomic nuclei and with radii corresponding to the atomic van der Waals radii. The Solvent Accessible Surface (Figure 1b) is defined by the centroid of a probe sphere (radius 1.4 Å) as it rolls about the van der Waals surface. (Lee, 1971). The Molecular Surface (Figure 1c) is defined by the inner surface of the 1.4 Å probe sphere as it rolls about the van der Waals surface (Richards, 1977). It may be useful to separate the latter two surfaces into their hydrophilic and hydrophobic components, as this should have predictive value in analyzing the polarity of the molecule and its solvation in various media.

Molecular Mechanics Calculations

Molecular mechanics is a calculation method designed to give accurate structures and energies for molecules (Allinger, 1976). It is an extension of older ideas (Andrews, 1930) that bonds have "natural" lengths and angles, and that molecules adjust their atomic position in order to attain these values. Van der Waals interactions between atoms deform bond lengths and angles from their ideal values, introducing steric strain energy which can be accurately calculated. Molecular mechanics programs iteratively adjust atomic positions within a molecule to minimize the strain resulting from deformation of bond lengths and angles. This strain energy is calculated by an empirically

Table 1. Structural features of lipophilic radiopharmaceuticals.

COMPOUND	M.W.	van der WAALS VOLUME (Å ³)	Molecular Surface			Solvent Accessible Surface			Minimum Box Dimensions			
			SURFACE AREA (Å ²)		ENCLOSED VOLUME (Å ³)	SURFACE AREA (Å ²)		ENCLOSED VOLUME (Å ³)	Δx(Å)	Δy(Å)	BOX VOLUME (Å ³)	
			Hydro- phobic	Hydro- philic		Hydro- phobic	Hydro- philic					
Methanol	32	36.0	62 = 10 + 52		39.3	166 = 38 + 128		190.0	5.0	4.2	4.5	94.3
Ethanc:	46	53.2	81 = 8 + 73		58.5	201 = 31 + 170		245.9	6.1	4.7	4.5	129.3
Isopropanol	60	70.9	101 = 7 + 94		78.3	229 = 27 + 202		297.9	7.0	6.1	5.2	220.7
n-Butyl alcohol	74	86.5	120 = 8 + 112		97.9	263 = 30 + 233		353.3	8.8	5.0	4.5	197.8
Butyl methyl ether	88	106.2	141 = 5 + 136		116.9	297 = 17 + 280		409.0	10.6	5.0	4.5	238.2
1-Hexanol	102	116.9	159 = 8 + 151		137.9	326 = 30 + 296		461.5	11.4	5.1	4.5	261.1
Pentyl methyl ether	102	118.9	162 = 5 + 157		137.1	330 = 18 + 312		465.5	12.0	5.0	4.5	268.5
Benzyl alcohol	108	110.4	137 = 6 + 131		114.8	288 = 23 + 265		403.5	9.2	7.3	4.5	302.7
Hexyl methyl ether	116	137.5	181 = 5 + 176		156.6	361 = 18 + 343		519.6	13.2	5.1	4.5	305.4
Benzyl methyl ether	122	126.8	160 = 3 + 157		136.7	324 = 11 + 313		460.3	10.8	7.5	4.5	364.1
1-Octanol	130	157.7	196 = 8 + 188		172.0	389 = 30 + 359		572.0	13.9	5.1	4.5	320.1
Benzophenone	182	172.9	206 = 11 + 195		189.2	390 = 39 + 351		590.0	11.6	7.7	6.3	562.9
Diphenylmethanol	184	182.9	208 = 6 + 202		194.6	395 = 22 + 373		603.0	12.0	8.0	6.2	601.2
Benzyl hexyl ether	192	209.9	261 = 2 + 259		236.8	485 = 8 + 477		734.1	16.9	7.4	6.0	746.4
Triphenylmethanol	260	249.8	271 = 4 + 267		274.1	486 = 16 + 470		787.4	11.7	11.8	7.0	961.0
N-isopropyl-p-iodo- amphetamine	303	217.2	252 = 1 + 251		241.6	464 = 3 + 461		728.7	14.6	7.7	7.0	781.3
4-Iodoantipyrine	314	206.9	226 = 10 + 216		220.1	423 = 35 + 388		665.4	12.2	8.5	6.0	619.5
HIPDM	362	273.4	314 = 7 + 307		304.9	559 = 27 + 533		897.4	16.6	10.0	6.7	1110.2

Table 1. (continued)

COMPOUND	M.W.	van der WAALS VOLUME (Å ³)	Molecular Surface			Solvent Accessible Surface			Minimum Box Dimensions			
			SURFACE AREA (Å ²)			SURFACE AREA (Å ²)			ENCLOSED			
			Hydro- philic	Hydro- phobic	Total	Hydro- philic	Hydro- phobic	Total	ENCLOSED VOLUME (Å ³)	Δx(Å)	Δy(Å)	Δz(Å)
^t Cu(PTSM)	308	209.2	236 = 12	+ 224	214.7	446 = 42	+ 404	687.9	14.6	8.5	4.7	583.3
^t Cu(KTSM)	338	213.4	254 = 28	+ 226	236.4	469 = 102	+ 367	736.2	11.9	9.5	8.5	960.9
^t Tc(OHMPAO)												
d,l-boat	383	268.5	285 = 23	+ 262	291.1	499 = 84	+ 415	845.7	12.1	10.9	7.3	962.8
meso-boat	383	268.7	288 = 23	+ 265	304.5	500 = 83	+ 417	848.8	12.0	10.8	7.4	959.0
meso-chair	383	270.3	286 = 25	+ 261	311.6	500 = 93	+ 407	847.2	11.9	10.7	7.9	1005.7
^t SQ31380	505	333.8	339 = 20	+ 319	367.9	586 = 75	+ 511	1008.1	12.4	11.4	10.5	1484.3
^t SQ32014	585	401.8	385 = 30	+ 355	427.1	662 = 111	+ 551	1161.8	14.9	12.9	10.2	1960.5
^t Ga(5-McOsal) ₃ tame	586	460.9	462 = 18	+ 444	495.4	792 = 68	+ 724	1357.6	17.4	16.5	11.0	3158.1
^t Ga(oxine) ₃	548	388.1	372 = 6	+ 366	397.0	656 = 23	+ 633	1139.9	13.8	12.4	9.4	1608.5

†The hydrophilic/hydrophobic area ratios quoted are slightly too low, because the CHEMNOTE facility of the QUANTA package used to compute these areas does not place a charge on the metal ion. This problem is being addressed.

†The CHEMNNOTE facility of the QUANTA package placed the hydrogen atoms in incorrect positions on the ligand. The volume and surface area calculations for the molecule may therefore be slightly incorrect.

Figure 1. Diagram illustrating various models of the molecular surface. A) van der Waals Surface. B) Solvent Accessible Surface. C) Molecular Surface.

derived set of potential functions describing the deformation of specific elements within the molecule, i.e. bond length, bond angles, torsion angles, and steric interaction of non-bonded atoms. The set of potential functions is called the force field, and contains adjustable parameters optimized to obtain the best fit between calculated and empirical properties (*e.g.* geometries, relative conformational energies, and heats of formation). The structures corresponding to minima in the potential functions are conformers of the molecule, and the lowest energy conformers are assumed to be the most prevalent at physiological temperatures. Molecular mechanics calculations have previously been performed on molecules of interest to medicinal chemists, such as proteins (Blaney *et al.*, 1982), metalloproteins (Vedani *et al.*, 1986), nucleic acids (Kollman *et al.*, 1981, Keepers *et al.*, 1982) and enzymes such as Native Human carbonic anhydrase (Vedani and Dunitz, 1985) and triose phosphate isomerase (Alagona *et al.*, 1989).

There are a number of molecular mechanics programs in current use, all of which employ different potential functions. Thus, the computational chemist must decide on which program to use for the application at hand. A primary objective of our program is the testing of the accuracy of structure reproduction of selected force fields and of surface area/volume calculation programs. Since we ultimately also wish to model the interactions of the metal complexes with large molecules such as proteins and lipids, it was decided to test the structure reproduction capabilities of programs designed to model small molecules and those designed for large molecules. The structures input into the programs were complexes of low spin nickel (II) with saturated amine donor ligands. Square-planar nickel complexes were chosen as examples because: (i) a number of crystal structures of these complexes exist; (ii) the geometric constraints of fitting a ligand around a metal atom with vacant axial sites are more stringent than around an octahedral metal ion, where errors in the force field can be masked by compensating non-bonding effects; and (iii) an adequate force field modeling these compounds exists (Hancock *et al.*, 1988), providing a template for the modification of force fields that were not initially designed to handle metal ions.

GENERAL FEATURES OF PROGRAMS AND FORCE FIELDS

Minimizers Used in the Force Fields

The different molecular mechanics programs not only employ different force fields, but they differ also in methods of locating potential energy minima. All minimization routines operate by "descent series methods", in which coordinates are modified from one iteration to the next in order to decrease the energy. The methods are uniformly unable to locate global minima in the potential energy surface (Cohen *et al.*, 1990).

Simplex. The simplex method is a non-derivative method used on an atom by atom basis until the maximum force on any single atom is less than a specified value. In highly distorted structures the potential energy surface and its derivatives are often discontinuous. The simplex method can handle these surfaces, whereas derivative based procedures cannot.

Steepest Descent. In the steepest descent method coordinates are modified by increments proportional to the first derivative of the potential energy function at the current position. No information from previous iterations is used. It works well for very distorted systems where the direction of the maximum change will vary greatly from one iteration to the next. The method exhibits poor convergence. The required computer storage space is proportional to the number of variables.

Conjugate Gradient. The conjugate gradient method accumulates information about the potential energy function from one iteration to the next. The convergence is superior to that of the steepest descent. Storage requirements are greater than those of the steepest descent algorithm, but are still linear with respect to the number of variables.

Newton-Raphson. The Newton-Raphson method employs a second derivative matrix which is analytically calculated or numerically approximated. Storage requirements are therefore proportional to the square of the number of variables. The matrix is inverted at each iteration; consequently the time of run is proportional to the cube of the number of variables.

Line Searches. Line searches operate as follows: from the current position a direction in a space is chosen, the dimensionality of which is equal to the number of adjustable parameters. The parameters (coordinates and rotational parameters) are modified along that line until a minimum in the function is bracketed.

Force Field Programs Considered

All molecular mechanics programs minimize the internal energy of the molecule of interest. The energy function has the following general form:

$$E_{\text{(total)}} = E_{\text{(stretch)}} + E_{\text{(bend)}} + E_{\text{(dihedral)}} + E_{\text{(Van der Waals)}} + E_{\text{(electrostatic)}} + E_{\text{(H-bond)}}.$$

Electrostatic energy and hydrogen-bonding were omitted in all the calculations described below. The functional forms assumed by the individual energy terms differ from one program to the next. The references cited in the description of specific programs (below) may be consulted for information concerning the individual terms.

MM2 (Allinger and Yuh, 1980), uses the best known small-molecule force field (Seibel and Kollman, 1990). This force field is regarded in general to be a poor choice for calculations involving macromolecules (Cohen *et al*, 1990). The program possesses no molecular graphics interface, and molecule building is restricted to the ability to affix hydrogen atoms to molecular skeletons. MM2 uses the Newton Raphson minimization technique. The force field was originally developed for hydrocarbons, but has since been parameterized for a number of organic functionalities. The version used was modified by Drew (Drew *et al*, 1985) to incorporate atoms with a coordination number of more than four, and which exhibited periodicity of bonds about the metal center, thus obviating explicit differentiation between *trans* and *cis* angles. Drew does not use the version of MM2 developed for conjugated

systems (MMP2) in his work, rather employing a classical force field approach. Drew (Drew *et al.*, 1985) estimates organic parameters for unsaturated molecules from Allinger's original MM2 (1980) parameters.

MM2 only defines out-of-plane bending parameters for certain atom types, of which the metal is not one, so one can not enforce planarity of the square-planar metal ion with out-of-plane angle constraints. MM2 also does not differentiate between lengths of bonds between unsaturated carbon atoms joined by single and double bonds. Bonding potentials exhibit a minimum at short interatomic distances and a steep quadratic fall-off at high bond lengths. Consequently, if the bond length gets too big, as is often the case in severely distorted structures, it stays too big. Drew does not differentiate between the force constants of *cis* and *trans* N-M-N angles, and in separate publications uses two different $k_0(N-M-N)$ values. MM2 runs into overflow errors when calculating torsion angles of collinear bonds, e.g. those around metal centers. If these errors are encountered before the first cycle of refinement, the program terminates without error messages giving the incorrect impression that all is well.

BOYD (Boyd *et al.*, 1973) uses the MHB force field (McDougall, Hancock and Boeyens, 1978; Hancock *et al.*, 1988), which has been extensively parametrized for nickel complexes. The program employs no molecular graphics interface. BOYD uses the Newton Raphson minimization technique. Buckingham potentials (Buckingham, 1938) are used for nonbonded interactions, that can fail at low interatomic distances (Hirshfelder *et al.*, 1954). Energy minimization is compromised if the first three atoms in the input data set are bonded or if zero potentials are included in the minimization.

QUANTA (Polygen, 1990), uses the force field CHARMM (Brooks *et al.*, 1983), developed exclusively for use with macromolecules, such as proteins (Simon and Nugiel, 1990). It supposedly yields only qualitative results with small molecules (Cohen *et al.*, 1990). QUANTA possesses a molecular graphics interface. The program uses both conjugate gradient and Newton Raphson minimization algorithms. Torsions within aromatic rings are defined exclusively by out-of-plane angle constraints. Periodicity of angles about metal atoms is not included in the program, so the user has to distinguish between *cis* and *trans* atom types explicitly in the force field.

SYBYL (Tripos, 1988), uses a force field designed to be intermediate between those designed for use with large and small molecules (Clark *et al.*, 1989). SYBYL uses line searches, in conjunction with conjugate gradient and Newton-Raphson minimization techniques. It possesses a molecular graphics interface, with a utility to affix hydrogen atoms to molecular skeletons. The user has to explicitly distinguish between *trans* and *cis* atom types, as in QUANTA above.

An ionic bonding model was initially attempted, using QUANTA, with [niamhp] (the structure with least strained metal ion (Wade, 1989)) in order to obviate the need to define separate *cis* and *trans* donor atoms (see below). The nickel ion migrated during refinement, resulting in bad N-Ni-N angles. The size of the nickel ion was increased to an unrealistic radius, without the structural buckling being alleviated; excessively large nickel would simply leave the cavity of the macrocycle. Thus, the ionic bonding model has been rejected in favor of covalent metal to ligand bonding.

Modifications to Force Fields

MM2DREW: Drew does not differentiate between the force constants of *cis* and *trans* N-M-N angles, and in separate publications uses two different N-M-N angle stiffnesses, of 0.30 (Drew *et al*, 1987) and 0.03 (Drew *et al*, 1986) mdyn/angstrom. The former angle stiffness was used in the present work due to its similarity with those in the MHB force field (McDougall, Hancock and Boeyens, 1978; Hancock *et al*, 1988), which was explicitly developed to model low-spin Ni(II). The force field contained no non-bonded parameters for nickel. However, in force field reproductions of [galzuo], which has strong ligand to metal non-bonding interactions (Wade, 1989), it was discovered that the choice of Van der Waals repulsions due to the metal was not crucial. The non-bonded parameters ultimately used in the present work were interpolations of other metallic non-bonded parameters by the method of Allinger (1976).

BOYD: No modifications were made to the MHB force field, since it was specifically parametrized for low spin Ni(II).

CHARMM: A force field involving electrostatic metal to ligand bonding was initially tested and rejected because unrealistic coordination geometries were predicted. The original force field was not set up for calculations involving metal complexes, so it was augmented in the present work with the low-spin Ni(II) parameters in the MHB force field (McDougall, Hancock and Boeyens, 1978; Hancock *et al*, 1988). A further modification made to the force field was the identification of the stiffness of the *cis* and *trans* N-M-N angles with the average of those in the MHB force field.

TRIPOS 5.2: The original force field was not parametrized for metals, so the MHB parameters for low spin Ni(II) were appended to the force field as with QUANTA above.

Randomization of Coordinates

A general problem with potential energy minimization algorithms is that of locating the global energy minimum. There is no guarantee of locating this minimum and all algorithms to date are capable of falling into local energy minima. Since in most cases the structures to be minimized are built with a molecular graphics package, the onus is on the computational chemist to construct an initial structure close to the global energy minimum. This requires a lot of experience with conformational structural chemistry, which is sometimes not available. It is desirable to test, therefore, the propensity of the molecular mechanics packages for entrapment by local minima in the potential energy surface.

Randomization of coordinates and subsequent re-refinement has been proposed by Clark *et al* (1989) to determine sensitivity of refined to initial coordinate position (*i.e.* the propensity of the force field or molecular mechanics program to locate a local vs. a global minimum in the potential energy surface). Clark *et al* (1989) in their validation of the SYBYL program (Tripos, 1988), truncate all atomic coordinates to one decimal place, and

minimize the resulting structures. This method was tested by Ferguson and Raber (1989) in their search for different conformations of alkanes. They found that truncation effectively translates all atoms in the direction of the origin of the coordinate system. The motions of the atoms during the attempted randomization are, therefore, correlated.

This truncation of coordinates is attractive in terms of ease of computation. Rounding of coordinates also features ease of computation and it randomizes coordinates more than does truncation, and it was thus considered as a randomization strategy. It was found, however, that in the case of symmetrical molecules with symmetry elements aligned parallel to the axes of the coordinate system, correlated (*i.e.* non-random) distortions of the molecular structures were effected. Also, truncation of coordinates to one decimal place moves atoms by at most the square root of 3, or 1.73 Å, whereas rounding coordinates changes each one by at most 0.5 Å, displacing atoms by at most 0.87 Å (one-half the square root of 3). This randomization strategy was abandoned.

Ferguson and Raber (1989) add random increments to coordinates and re-refine in order to locate global minima in the potential energy surface. Following their example, random increments were added to all atomic coordinates and the resulting distorted molecules were energy minimized.

Calculations

The structures of 38 Ni(II) complexes were examined (Figure 2). The Cambridge Crystallographic Database (CCDF) was searched for square planar Ni(II) complexes with N-donor atoms, and 35 structures were retrieved. Two additional unpublished structures were added that have unusual features, unstrained Ni-N bonds ([niamhp] (Wade, 1989)), and highly distorted N-Ni-N and Ni-N-C angles ([nibaph]). One further structure, [nibaeh], was added for comparison to [fitxel], which has similar R-factor and structure. Those structures retrieved from the database will be referred to by their Cambridge Crystallographic Database refcode. Structures not yet included in the database will be referred to by a 6-letter mnemonic. All structures were randomized and then minimized as described above. The calculated and crystallographic structures were then compared to see if the metal geometry and the ligand structures were adequately reproduced.

The utility in SYBYL to generate coordinates for hydrogen atoms was used where these were not included in published crystal structures. FORTRAN programs were written to (a) randomize coordinates of a crystal structure, (b) prepare input file for the differing molecular mechanics programs, and (c) compare and compile statistics on calculated and observed structures. In the randomization of coordinates, increments added to coordinates were determined by random number generator (Hewlett Packard, 1975) and scaled to be between -0.5 and +0.5, resulting in an overall maximum displacement of atoms of 1.73 Å. All structures were submitted to BOYD, DREW, SYBYL, and QUANTA force field programs. All resulting structural features were compared to those in the crystal structure, the exception being undefined torsion angles about the metal atoms where bonds are collinear. All calculations were performed on a Silicon Graphics Iris 4D workstation.

Figure 2 (13 pages). Stereoscopic stick drawings illustrating the molecular structure of each of the 38 nickel(II) complexes considered. In each case the square-planar Ni(II) ion is bonded to four nitrogen donor atoms that are part of saturated aliphatic amine or polyamine ligands.

NIBAPH NIBAЕН

TMTZNI TRIENP ZEGCAP

FANRAG OCMFNT TMENIC

FIIC.MOF GALZUO MTAZNI

FTSKFX FITNEB FITXEL

VFMT7NI FAGWAL FIBHED

ΠΟΚΤΑΥ ΠΟΝΣΑΚ ΠΙΚΡΟΥ

nm7c:nt docduw dogcor

CUKXUB DACONI DITMUO

ROPJOF BOZZOF BUWSOB

RIJFIJ BISKAP BIXWIO

NIT ΔMHP ΔM71INT RADSAΑ

Comparison of Observed and Calculated Structures

Most publications detailing a new force field or molecular mechanics program are accompanied by a few examples demonstrating their quality of structure reproduction. This efficiency is usually demonstrated as a comparison of the calculated and observed (*i.e.* crystallographically determined) structures. The literature is lacking in methods for general statistical comparison between molecules (Clark *et al*, 1989). There are also problems encountered in the choice of experimental structures to which to compare calculations, since there is considerable latitude in the quality of crystal structures, even those with low R-factors (Jones, 1984).

The root mean square deviation of atomic positions between calculated and crystal structures are often used as a measure of distortion of overall shape due to the force field. However, this procedure can be misleading, as a small movement at the base of a chain can result in large deviations in the coordinates of atoms at the end of the chain. Clark, *et al* (1989) propose a statistical analysis of deviations between structures involving more meaningful chemical entities such as bond lengths, bond angles, and torsion angles. This method is employed in the current work.

The statistical analysis of the programs included calculations of the root mean square deviation (R.M.S.D.), the maximum deviation, the mean and the standard deviation of bond lengths, bond angles, dihedral angles and coordinates of atoms. The statistical data pertaining to ligands were compared with those derived by Clark *et al* (1989). The structural features of the metal complexes have been divided into contributions from the ligand and the metal force fields. The mean of the deviation of structural features shows the net deviation of the parameter from the crystal structure, *i.e.* the direction in which the force field parameters governing the feature must be adjusted to improve the structural reproduction. The compound exhibiting the greatest discrepancy between calculated and observed structure is identified in order to see if the discrepancy is due to an unreliable crystal structure, or if it is due to an incorrectly parametrized force field. The statistics on the displacements of non-hydrogen atoms were included in the following tables for completeness.

A number of structures retrieved from the database were of the same complex, though R-factors differed: [cafhum] (R=5.5), [fagwal] (R=6.2) and [fiskex] (R=4.7) are identical, [cikdop] (R=13.0) and [daconi] (R=8.7) are identical, and [nibaeh] (R=5.9) and [fixel] (R=5.5) are identical. It was decided to repeat the statistical test with unique structures, also eliminating those structures with an R-factor greater than 9.0.

Comparison of Accuracy of Structure Prediction Programs

Initially, crystallographic coordinates were used as a starting point for molecular mechanics minimizations with the four programs under consideration. Tables I-IV present statistical data describing the deviation between calculated and observed structures for the 35 structures retrieved from the crystallographic database and those added. Table V compares the force field programs. The quantities in the Tables have all been rounded off to two decimal places, so while the standard deviation of a quantity might appear to be zero, it is a non-zero number less in

Table I: Statistics of deviations of crystal structures from those calculated by QUANTA (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	132	0.02	0.07	0.01	0.01	amzuni
Bond (Lig)	536	0.03	0.13	-0.01	0.01	amzuni
Angl (N-M-N)	198	1.92	-7.84	-0.12	0.29	clkdop
Angl (M-N-C)	265	2.75	9.15	0.81	0.82	ocmeni
Angl (Lig)	657	2.28	-9.84	0.34	0.38	emtzni
Tors (M-N)	534	6.21	24.50	-0.16	0.36	zegcap
Tors (Lig)	980	6.83	27.74	0.06	0.49	mtazni
Atom. Displ.	575	0.18	0.97	0.13	0.14	zegcap

Table II: Statistics of deviations of crystal structures from those calculated by SYBYL (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	132	0.03	-0.09	-0.02	0.02	clkdop
Bond (Lig)	536	0.03	0.13	-0.01	0.01	amzuni
Angl (N-M-N)	198	1.68	6.84	0.00	0.00	ocmeni
Angl (M-N-C)	265	2.70	7.98	1.13	1.14	dogcor
Angl (Lig)	657	2.41	8.13	0.01	0.16	clkdop
Tors (M-N)	538	4.65	-16.75	-0.08	0.08	doktay
Tors (Lig)	980	4.16	-19.61	0.06	0.05	dogcor
Atom. Displ.	575	0.32	3.01	0.11	0.11	nibaeh

Table III: Statistics of deviations of crystal structures from those calculated by MM2DREW (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	132	0.05	-0.05	-0.03	0.03	dukpuu
Bond (Lig)	536	0.03	0.13	0.00	0.00	amzuni
Angl (N-M-N)	198	1.92	12.87	0.28	0.28	nibaph
Angl (M-N-C)	265	2.17	15.78	0.59	0.58	dukpuu
Angl (Lig)	657	2.16	-10.94	-0.44	0.44	dukpuu
Tors (M-N)	538	3.60	-27.24	-0.19	0.18	nibaph
Tors (Lig)	980	4.54	-22.66	0.15	0.14	dukpuu
Atom. Displ.	575	0.11	0.49	0.09	0.08	dukpuu

Table IV: Statistics of deviations of crystal structures from those calculated by BOYD (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	132	0.02	-0.07	-0.01	0.01	mtazni
Bond (Lig)	536	0.03	0.13	-0.01	0.01	amzuni
Angl (N-M-N)	198	1.95	-8.00	0.11	0.15	cikdop
Angl (M-N-C)	265	2.80	9.93	1.09	1.08	ocmeni
Angl (Lig)	657	1.99	7.24	0.44	0.47	amzuni
Tors (M-N)	538	7.05	23.31	-0.16	0.16	fibhed
Tors (Lig)	980	5.86	-23.23	0.13	0.12	fibhed
Atom. Displ.	575	0.36	1.10	0.26	0.26	doktay

magnitude than 0.005. All bond lengths are overestimated on average. If not corrected for rigid body motions, bond lengths in crystal structures can be underestimated by 0.005 to 0.015 angstroms (Clark *et al.*, 1989).

Among the least well reproduced structures in these comparisons were the compounds [amzuni], [cikdop], [ocmeni], [emtzni], [zegcap], [mtazni], [dogcor], [doklay] and [nibaeh]. The bad reproductions of [cikdop] and [mtazni] could be due to bad crystal structures, their R-factors being 13.0 and 14.7 respectively. The same could apply to [amzuni] and [emtzni]. Although the latter have relatively low crystallographic R-factors (8.5 and 4.2 respectively), they also have a number of very long C-C bonds (1.60 Å to 1.65 Å), that would not be reproduced by force field programs. [Amzuni] consistently has the ligand bonds worst reproduced by all programs. The crystallographic R-factors of all the other complexes are low, and the structures are plausible, so failure to reproduce these structures must reflect an inadequacy of the programs.

The programs that perform best at reproducing the structures of the square-planar nickel complexes are BOYD and MM2DREW when crystallographic coordinates are used as the starting point for minimization. QUANTA does not feature among the programs best reproducing structural elements. The best RMSD's of the programs BOYD, MM2DREW and SYBYL are, however, comparable.

Tables VI to IX present statistical data describing the deviation between calculated and observed structures for the subset of structures retrieved from the crystallographic database with low R-factors. Table X compares the force field programs. Comparison of Tables V and X show that there is no effect on the order of the programs' capability to reproduce the structures, upon paring the structures with high R-factor. There is, in fact, an increase in the best RMSD of differences in the M-N-C angles, and in the ligand torsion angles upon removal of structures of high R-factor.

The complex [nibaph] exhibits an unusually large Ni-N-C angle due to steric strain in the ligand. The Ni-N-C angle in this complex is stretched out to 119.4°, the greatest such distortion known. Bond angles (Ni-N-C) of 116.0°, 114.6°, 114.4° and 116.5° were calculated by the programs BOYD, DREW, SYBYL, and QUANTA, respectively. Thus, QUANTA best reproduces the distortion in the Ni-N-C angle, although only slightly better than BOYD.

Structure Reproduction After Coordinate Randomization

As a test of minimization strategies structure reproductions were also investigated starting from randomized crystallographic coordinates. All the structures retrieved from the crystallographic database had their coordinates randomized as described above and were then minimized with each of the four programs. Tables XI to XIV present statistical data describing the deviation between calculated and observed structures. BOYD and MM2DREW uniformly converged on local energy minima, yielding structure reproductions so bad as to be useless to the computational chemist. Table XV summarizes the results and compares the remaining two programs, QUANTA and SYBYL. SYBYL best reproduces the most structural elements, while QUANTA calculations lead to the worst RMSD values

Table V: Summary of statistics and comparison of structure reproduction abilities of programs.

Structural Feature	Tripos RMSD	Best Reproduction		Worst Reproduction			R-F
		RMSD	Program	Max. Dev.	Struct.	Program	
Bond (M-N)		0.02	BOYD	-0.09	ditmuo	MM2DREW	5.1
Bond (Lig)	0.0	0.03	BOYD	0.14	amzuni	MM2DREW	8.5
Angl (N-M-N)		1.68	SYBYL	12.87	nibaph	MM2DREW	6.0
Angl (M-N-C)		2.17	MM2DREW	15.78	dukpuu	MM2DREW	4.2
Angl (Lig)	2.50	1.99	BOYD	-10.94	dukpuu	MM2DREW	4.2
Tors (M-N)		3.61	MM2DREW	-27.24	nibaph	MM2DREW	6.0
Tors (Lig)	9.50	4.16	SYBYL	27.74	mtazni	QUANTA	14.7
Atom. Displ.	0.25	0.11	MM2DREW	3.01	nibaeh	SYBYL	5.9

- (a) The figures in the column labeled "Tripos RMSD" refer to those determined by Clark *et al* (1989) for 76 organic molecules as a validation of the Tripos force field.
- (b) R-F is the crystallographic R-factor for the structure least well reproduced by the force field programs.

Table VI: Statistics of deviations of unique crystal structures, R-factors less than 9.0, from those calculated by QUANTA (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	112	0.02	0.07	0.01	0.01	amzuni
Bond (Lig)	469	0.03	0.13	-0.01	0.01	amzuni
Angl (N-M-N)	168	1.88	6.32	-0.07	0.29	nibaph
Angl (M-N-C)	225	2.82	9.15	0.81	0.82	ocmeri
Angl (Lig)	583	2.25	-9.84	0.37	0.41	emtzni
Tors (M-N)	454	6.38	24.50	-0.02	0.35	zegcap
Tors (Lig)	876	6.90	-27.28	-0.03	0.52	zegcap
Atom. Displ.	499	0.19	0.97	0.13	0.14	zegcap

Table VII: Statistics of deviations of unique crystal structures, R-factors less than 9.0, from those calculated by SYBYL (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	112	0.03	-0.05	-0.02	0.02	fibhed
Bond (Lig)	469	0.03	0.13	-0.01	0.02	amzuni
Angl (N-M-N)	168	1.64	6.84	0.05	0.04	ocmeni
Angl (M-N-C)	225	2.79	7.98	1.15	1.17	dogcor
Angl (Lig)	583	2.33	-6.61	0.07	0.19	emtzni
Tors (M-N)	454	4.94	-16.75	-0.02	0.02	doktay
Tors (Lig)	876	4.22	-19.61	-0.01	0.01	dogcor
Atom. Displ.	499	0.09	0.46	0.07	0.07	doktay

Table VIII: Statistics of deviations of unique crystal structures, R-factors less than 9.0, from those calculated by MM2DREW (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	112	0.06	-0.49	-0.02	0.02	dukpuu
Bond (Lig)	469	0.03	0.14	0.00	0.00	amzuni
Angl (N-M-N)	168	1.82	12.87	0.31	0.31	nibaph
Angl (M-N-C)	225	2.21	15.78	0.63	0.63	dukpuu
Angl (Lig)	583	2.12	-10.94	-0.40	0.40	dukpuu
Tors (M-N)	454	3.60	-27.24	-0.06	0.06	nibaph
Tors (Lig)	876	4.45	-22.66	0.07	0.06	dukpuu
Atom. Displ.	499	0.12	0.49	0.08	0.08	dukpuu

Table IX: Statistics of deviations of unique crystal structures, R-factors less than 9.0, from those calculated by BOYD (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	112	0.02	0.05	-0.00	0.00	amzuni
Bond (Lig)	469	0.03	0.13	-0.01	0.00	amzuni
Angl (N-M-N)	168	1.96	5.47	0.19	0.22	nibaph
Angl (M-N-C)	225	2.91	9.93	1.16	1.15	ocmeni
Angl (Lig)	583	1.93	7.24	0.47	0.50	amzuni
Tors (M-N)	454	7.49	23.31	-0.14	0.14	fibhed
Tors (Lig)	876	5.95	-23.23	0.16	0.15	fibhed
Atom. Displ.	499	0.37	1.10	0.27	0.26	doktay

Table X: Summary of statistics and comparison of structure reproduction abilities of programs with unique crystal structures, R-factor less than 9.0.

Structural Feature	Tripos RMSD	Best Reproduction		Max. Dev.	Worst Reproduction		R-F
		RMSD	Program		Struct.	Program	
Bond (M-N)		0.02	BOYD	0.07	amzuni	QUANTA	8.5
Bond (Lig)	0.0	0.03	BOYD	0.14	amzuni	MM2DREW	8.5
Angl (N-M-N)		1.64	SYBYL	12.87	nibaph	MM2DREW	6.0
Angl (M-N-C)		2.21	MM2DREW	15.78	dukpuu	MM2DREW	4.2
Angl (Lig)	2.50	1.93	BOYD	-10.94	dukpuu	MM2DREW	4.2
Tors (M-N)		3.60	MM2DREW	-27.24	nibaph	MM2DREW	6.0
Tors (Lig)	9.50	4.22	SYBYL	-27.28	zegcap	QUANTA	13.6
Atom. Displ.	0.25	0.11	MM2DREW	3.01	nibaeh	SYBYL	5.9

- (a) The figures in the column labeled "Tripos RMSD" refer to those determined by Clark *et al* (1989) for 76 organic molecules as a validation of the Tripos force field.
- (b) R-F is the crystallographic R-factor for the structure least well reproduced by the force field programs.

Table XI: Statistics of deviations of crystal structures from those calculated by QUANTA using randomized coordinates (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	132	0.02	0.07	0.01	0.01	amzuni
Bond (Lig)	536	0.03	0.13	-0.01	0.01	amzuni
Angl (N-M-N)	198	2.40	-8.77	0.10	0.28	badssa
Angl (M-N-C)	265	2.97	9.48	0.93	0.93	badssa
Angl (Lig)	657	2.26	-8.56	0.29	0.34	emtzn
Tors (M-N)	538	8.91	30.12	-0.02	0.32	badssa
Tors (Lig)	980	7.75	-32.79	-0.11	0.50	badssa
Atom. Displ.	575	0.26	1.29	0.19	0.20	badssa

Table XII: Statistics of deviations of crystal structures from those calculated by SYBYL, using randomized coordinates (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	132	0.03	-0.09	-0.02	0.02	clkdop
Bond (Lig)	536	0.03	0.13	-0.01	0.01	amzuni
Angl (N-M-N)	198	1.91	-7.40	0.17	0.17	clkdop
Angl (M-N-C)	265	2.79	8.19	1.16	1.17	dogcor
Angl (Lig)	657	2.42	7.74	0.02	0.17	clkdop
Tors (M-N)	538	5.02	15.76	-0.19	0.20	doktay
Tors (Lig)	980	4.65	-19.66	0.06	0.12	dogcor
Atom. Displ.	575	0.14	0.45	0.13	0.13	doktay

Table XIII: Statistics of deviations of crystal structures from those calculated by MM2DREW, using randomized coordinates (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	132	0.10	-0.55	-0.04	0.04	daconi
Bond (Lig)	536	0.04	-1.58	-0.00	0.00	fibhed
Angl (N-M-N)	198	15.69	83.01	5.12	5.04	cukxub
Angl (M-N-C)	265	5.07	20.09	0.29	0.29	tmtzni
Angl (Lig)	657	5.07	29.93	-0.22	0.22	fibhed
Tors (M-N)	538	19.57	-260.73	-0.45	0.44	tmenic
Tors (Lig)	980	21.82	-248.41	-0.17	1.28	zegcap
Atom. Displ.	575	0.44	2.52	0.33	0.33	fucmof

Table XIV: Statistics of deviations of crystal structures from those calculated by BOYD, using randomized coordinates (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	132	5.06	-22.07	-2.13	2.11	amzuni
Bond (Lig)	536	10.39	-61.71	-5.40	5.45	bopjof
Angl (N-M-N)	198	41.27	124.50	17.21	18.58	cafhum
Angl (M-N-C)	265	36.49	102.88	11.39	11.99	amzuni
Angl (Lig)	657	40.49	107.11	19.57	19.51	tmenic
Tors (M-N)	538	101.39	268.11	1.92	3.83	daconi
Tors (Lig)	980	98.59	-269.18	0.32	2.75	zegcap
Atom. Displ.	575	10.26	51.03	6.79	6.78	bopjof

Table XV: Summary of statistics and comparison of structure reproduction abilities of programs from randomized coordinates.

Structural Feature	Tripos RMSD	Best Reproduction		Max. Dev.	Worst Reproduction		R-F
		RMSD	Program		Struct.	Program	
Bond (M-N)		0.02	QUANTA	0.09	clkdop	SYBYL	13.0
Bond (Lig)	0.03	0.03	QUANTA	0.13	amzuni	SYBYL	8.5
Angl (N-M-N)		1.91	SYBYL	-8.77	badssa	QUANTA	9.0
Angl (M-N-C)		2.79	SYBYL	9.48	badssa	QUANTA	9.0
Angl (Lig)	2.50	2.26	QUANTA	-8.56	emtzni	QUANTA	4.2
Tors (M-N)		5.02	SYBYL	30.13	badssa	QUANTA	7.0
Tors (Lig)	9.65	4.65	SYBYL	-32.79	badssa	QUANTA	7.0
Atom. Displ.	0.25	0.14	SYBYL	1.29	badssa	QUANTA	7.0

- (a) The figures in the column labeled "Tripos RMSD" refer to those determined by Clark *et al* (1989) for 76 organic molecules as a validation of the Tripos force field.
- (b) R-F is the crystallographic R-factor for the structure least well reproduced by the force field programs.

Table XVI: Statistics of deviations of unique crystal structures, R-factors less than 9.0, from those calculated by QUANTA, using randomized coordinates (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	112	0.02	0.07	0.01	0.01	amzuni
Bond (Lig)	469	0.03	0.13	-0.01	0.01	amzuni
Angl (N-M-N)	168	2.45	-8.77	0.18	0.37	badssa
Angl (M-N-C)	225	3.08	9.48	0.95	0.95	badssa
Angl (Lig)	583	2.23	-8.56	0.31	0.37	emtzni
Tors (M-N)	454	9.42	30.13	0.13	0.37	badssa
Tors (Lig)	876	7.92	-32.79	-0.23	0.56	badssa
Atom. Displ.	499	0.27	1.29	0.20	0.21	badssa

Tables XVI to XIX present the statistical data describing the deviation between observed structures and structures calculated from randomized coordinates, for the subset of crystallographic structures which are unique and have R factors less than 9.0. The BOYD and DREW programs uniformly converged on false minima far from the observed structure. Table XX summarizes the results and compares the SYBYL and QUANTA force field programs. Inspection of the Tables shows that again there is very little improvement upon paring the dataset to unique structures of low R-factor. As with the minimizations in Table XV, the program of choice seems to be SYBYL.

The overall force field reproductions of structures are best effected by the programs DREW and BOYD, although QUANTA was best able to reproduce the large Ni-N-C bond angle in [nibaeh]. DREW and BOYD performed very poorly in reproducing structures that simulated bad guesses (i.e. those relatively far from the global energy minimum), while SYBYL performed well. Omission of duplicate structures, and those with high R-factor, had uniformly little effect on the statistics describing the structure reproduction power of the programs. Thus, it seems that BOYD and DREW should be used when close to the global minimum, and SYBYL should be used when far away. The difficulty with this reasoning is that one is not always aware of how bad a guess has been input into the molecular mechanics program, and thus what minimization routine to use. Inspection of the relative success each program has at reproducing structures indicates that the force field programs BOYD, MM2DREW and SYBYL are comparable in structure reproductive power. Since SYBYL's force field was developed to be intermediate between those for small and large molecules, it can be used to model interactions of metal complexes with proteins. Thus, it might be concluded that of the four molecular mechanics programs tested, SYBYL is the one of choice for structure reproduction.

We have also applied these programs in an attempt to evaluate the energetics of conformational isomerism in the *meso*- and *d,l*-isomers of the radiopharmaceutical Tc-HMPAO. As described in our year 1 progress report, analysis of published crystallographic data for these compounds suggested that the disparate cerebral pharmacokinetics of these tracers might be explained by structural differences that affect the energetics of TcN₂ C₃-ring conformational isomerism. The independent crystallographic data for the *meso* and *d,l*-TcHMPAO complexes show that, in the case of the *meso*-isomer, alkyl substituents on the ligand backbone might block access of sulfhydryl reductants to the metal coordination sphere, thereby slowing the kinetics of Tc-99m trapping.

Molecular mechanics calculations were conducted to determine whether the unique solid-state conformation of the *meso*-Tc-HMPAO complex reflects a substantial energetic preference for a configuration that blocks access to the vacant axial metal coordination site. These calculations suggest that chair-boat conformational isomerism does not involve substantial energetic differences for either the *meso* or *d,l*-isomers. Thus, this would not appear to support our hypothesis of a structural explanation for differences in the rates at which these molecules react with glutathione. However, with the current modeling software, the energetics of distortions in Tc-N bonds have been neglected. This appears to be a valid approach, as the positions of the Tc-N bonds do not change substantially between the chair and boat conformers of *meso*-Tc-HMPAO that are characterized. Nevertheless, final resolution of this problem must await our development of adequate force-fields to model Tc=N double bonds.

Table XVII: Statistics of deviations of unique crystal structures, R-factors less than 9.0, from those calculated by SYBYL, using randomized coordinates (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	112	0.03	-0.05	-0.02	0.02	fibhed
Bond (Lig)	469	0.03	0.13	-0.02	0.02	amzuni
Angl (N-M-N)	168	1.85	6.71	0.21	0.22	ocmeni
Angl (M-N-C)	225	2.87	8.19	1.18	1.19	dogcor
Angl (Lig)	583	2.33	6.65	0.08	0.20	amzuni
Tors (M-N)	454	5.20	15.76	-0.11	0.13	doklay
Tors (Lig)	876	4.60	-19.67	-0.01	0.11	dogcor
Atom. Displ.	499	0.14	0.45	0.13	0.13	doklay

Table XVIII: Statistics of deviations of unique crystal structures, R-factors less than 9.0, from those calculated by MM2DREW, using randomized coordinates (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	112	0.10	-0.55	-0.04	0.04	daconi
Bond (Lig)	469	0.05	-0.58	-0.00	0.00	fibhed
Angl (N-M-N)	168	15.25	83.02	5.17	5.08	cukxub
Angl (M-N-C)	225	5.01	20.06	0.45	0.44	tmtzni
Angl (Lig)	583	5.08	29.93	-0.20	0.20	fibhed
Tors (M-N)	454	20.62	-260.73	-0.13	0.13	tmenic
Tors (Lig)	876	22.14	-248.41	-0.39	1.40	zagcap
Atom. Displ.	499	0.45	2.52	0.33	0.33	fucmof

Table XIX: Statistics of deviations of unique crystal structures, R-factors less than 9.0, from those calculated by BOYD, using randomized coordinates (angstroms and degrees).

Structural Feature	No.	R.M.S.	Max. Dev.	Mean	Std. Dev.	Structure with greatest deviation
Bond (M-N)	112	4.67	-22.07	-1.86	1.84	amzuni
Bond (Lig)	469	10.38	-61.71	-5.41	5.46	bopjof
Angl (N-M-N)	168	39.12	120.92	15.39	17.23	amzuni
Angl (M-N-C)	225	36.48	102.88	12.15	12.79	amzuni
Angl (Lig)	583	40.61	107.11	19.83	19.75	tmenic
Tors (M-N)	454	104.10	268.11	2.71	4.54	daconi
Tors (Lig)	876	101.05	-269.18	-0.72	2.98	segcap
Atom. Displ.	499	10.10	51.03	6.65	6.65	bopjof

Table XX: Summary of statistics and comparison of structure reproduction abilities of programs with unique crystal structures, R-factor less than 9.0 from randomized coordinates.

Structural Feature	Tripos RMSD	Best Reproduction		Max. Dev.	Worst Reproduction		R-F
		RMSD	Program		Struct.	Program	
Bond (M-N)		0.02	QUANTA	0.07	amzuni	SYBYL	8.5
Bond (Lig)	0.03	0.03	QUANTA	0.13	amzuni	SYBYL	8.5
Angl (N-M-N)		1.85	SYBYL	-8.77	badaaa	QUANTA	9.0
Angl (M-N-C)		2.87	SYBYL	9.48	badaaa	QUANTA	9.0
Angl (Lig)	2.50	2.23	QUANTA	-8.56	emtzni	QUANTA	4.2
Tors (M-N)		5.20	SYBYL	30.13	badaaa	QUANTA	7.0
Tors (Lig)	9.65	4.60	SYBYL	-32.79	badaaa	QUANTA	7.0
Atom. Displ.	0.25	0.14	SYBYL	1.29	badaaa	QUANTA	7.0

- (a) The figures in the column labeled "Tripos RMSD" refer to those determined by Clark *et al* (1989) for 76 organic molecules as a validation of the Tripos force field.
- (b) R-F is the crystallographic R-factor for the structure least well reproduced by the force field programs.

Gallium-68 Radiopharmaceuticals for Imaging the Heart

A number of ^{68}Ga -radiopharmaceuticals have been described that can be employed for imaging the heart with PET. However, despite high heart uptake and excellent heart/blood ratios, all previously reported Ga-tracers are cleared fairly rapidly from the myocardium. Thus, the pharmacokinetics of these tracers prevents complete exploitation of the 68 minute half-life of the gallium-68 radiolabel (e.g. by allowing long image acquisition periods and/or tracer administration at a site remote from the camera). We have now synthesized and evaluated a series of cationic ^{67}Ga -Schiff base complexes that exhibit myocardial uptake upon intravenous injection into rats followed by prolonged retention of the gallium radiolabel in the heart.

Gallium-67 complexes of the hexadentate N,N' -bis(3-aminopropyl)ethylenediamine bis(4,6-dimethoxysalicylaldehyde) (I) and bis(4-diethylaminosalicylaldehyde) (II) ligands were prepared by reaction of no-carrier-added (^{67}Ga)-gallium(III) acetylacetonate with the corresponding tris(salicylaldehydes) in hot ethanol. The radiochemical purity of these ^{67}Ga -radiotracers exceeded 99% based on thin layer chromatography using a C18-silica gel plate eluted with methanol. Cellulose acetate electrophoresis studies demonstrate that these ^{67}Ga -tracers are cationic, as would be expected for Ga^{3+} complexes with $\text{N}_4\text{O}_2^{2-}$ ligands. Despite their cationic nature, the ^{67}Ga complexes of I and II are lipophilic with $\log P$ values of 1.7 and 2.5, respectively (where P is the octanol/water partition coefficient).

The biodistribution of each of these radiotracers was determined following intravenous injection into ether anesthetized rats. Both radiopharmaceuticals afford significant myocardial uptake of activity and in both cases the ^{67}Ga -activity is completely retained in the heart from 1 minute to 2 hours post-injection. The ^{67}Ga complex of I exhibits the best myocardial uptake with 1% of the injected dose in the heart/blood ratios beyond 5 minutes post-injection. Additional compounds of this class remain under investigation.

Publications

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P.W. Wade, A.T. McKenzie, J.G. Stowell, and M.A. Green. "Evaluation of Structural Features in Metal-Based Tracers for Imaging the Brain," Scientific Exhibit, Society of Nuclear Medicine 38th Annual Meeting, Cincinnati, Ohio, June, 1991.

B.W. Tsang, C.J. Mathias, M.A. Green. "A Gallium-68 Radiopharmaceutical that is Retained in Myocardium: $^{68}\text{Ga}[(4,6\text{-MeO}_2\text{sal}_2\text{BAPEN})^+]$." *J. Nucl. Med.* **34**, 1127-1131, 1993.

PATENT APPLICATIONS

"Radiopharmaceuticals for Imaging the Heart," M.A. Green and B.W. Tsang, U.S. Patent Application Serial No. 07/861,778. Filed April 2, 1992. (For gallium-68 radiopharmaceuticals that are retained in myocardium.)

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