Molecular Modeling of Bifunctional Chelate Peptide Conjugates. 1. Copper and Indium Parameters for the AMBER Force Field

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In this work we describe the development of parameters for In(III) and Cu(II) for the AMBER* force field as found in the modeling package MacroModel. These parameters were developed using automated procedures from a combination of crystallographic structures and ab initio calculations. The new parameters were added in the form of AMBER* substructures containing specific metal−ligand parameters to the existing force field. These new parameters have produced results in good agreement with experiment without requiring additional changes to the existing AMBER* parameters. These parameters were then utilized to examine the conformational effects caused by the conjugation of InDTPA (DTPA = diethylenetriaminepentaacetic acid) and CuDOTA (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) to the cyclic octapeptide octreotide.

Introduction

Molecular modeling has found extensive use in the design of organic-based pharmaceuticals and has become an important tool for QSAR (quantitative structure activity relationship) and in the development of pharmacophore models, but it has not yet found wide use in the design of radiopharmaceuticals.[1] Molecular modeling can be used successfully for the prediction of radiometal−ligand complex structure, ligand selectivity, coordination number, lipophilicity, and thermodynamic stability. Although not widely applied to date, with the development of suitable molecular mechanics parameters it could also be used for QSAR studies as well as structure-based design of new ligands for metal-based pharmaceuticals targeted toward biological receptors. A common technique in the design of targeted radiopharmaceuticals is the use of bifunctional chelates (BFCs) which are then conjugated to a previously developed substrate for the receptor of interest. Molecular modeling is of great utility in addressing the question of whether this modification to the substrate will have a negative impact on receptor binding.

Molecular mechanics (MM) models of complexes of a variety of metal ions have been developed[2−7] and have been shown to be useful for ligand design. Few reports exist of the use of molecular mechanics in the design and modeling of metal-containing radiopharmaceuticals. Molecular modeling has perhaps been most often used with complexes of the common metal radionuclide ^{99m}Tc.[8−11] In order to fully utilize the techniques of molecular modeling it will first be necessary to develop high-quality parameter sets which allow the modeling of the metal complex.

The AMBER force field as developed by Kollman et al.[12] has found widespread use in the modeling of biological molecules. A recent comparison[13] of several molecular mechanics force fields have found that the AMBER* force field,[14] derived from the original AMBER force field, performs well for modeling small organic molecules as well as biomolecules. The AMBER and AMBER* force fields describe the energy of a molecule with a simple algebraic expression consisting of terms describing bond stretching, angle bending, dihedral rotation, and intermolecular forces, such as electrostatics, van der Waals interactions, and hydrogen bonding. The constants in these equations are obtained from experimental data or ab initio calculations. In designing metal-based radiopharmaceuticals it would seem desirable to take advantage of this widely utilized force field. Use of a well-established biochemically oriented force field with specific metal parameters instead of a specialized force field would allow the interactions of metal complexes and biological molecules to be studied in silico.

The approach that we describe in this work is the addition of AMBER* substructures containing specific metal−ligand parameters to the existing force field. In this way the existing validated force field parameters remain unchanged. Using this

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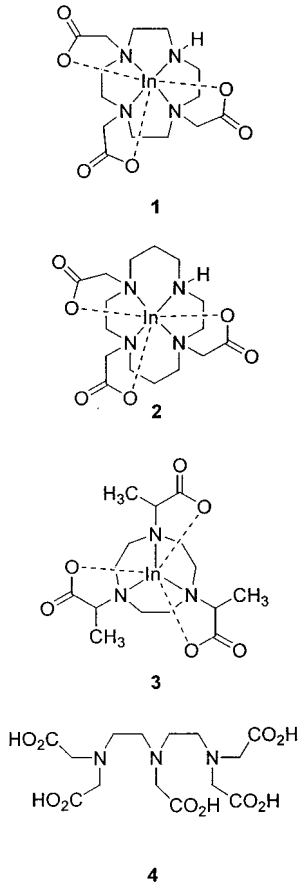

Figure 1. Reference compounds for the In(III) parameters.

approach we have developed copper(II) and indium(III) parameters for the AMBER* force field[14] as implemented within MacroModel.[15] Often coordination to a metal causes changes in the structure of the ligand, typically bond lengthening or shortening.[16] A benefit to our approach is that such changes are easily incorporated. As a demonstration of the utility of this approach we have used the In(III) parameters to examine the conformational effects of conjugation of InDTPA (DTPA = diethylenetriaminepentaacetic acid) to the cyclic octapeptide octreotide, the widely used imaging agent Octreoscan.[17] The Cu(II) parameters were used in a fashion similar to examine the conformational changes caused by the conjugation of CuDOTA (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) to the parent peptide.

Experimental Section

Both indium and copper ligand complexes were implemented as substructures in Amber* using MacroModel 6.0, running on an SGI Indigo[2] workstation. An all-atom scheme with explicit treatment of hydrogens was used for all calculations. The reference data for the In(III) parameters consisted of a total of seven indium-containing structures, Figure 1, from the Cambridge database[18] with a mean *R* factor of 0.0399. There were three macrocyclic complexes, compounds **1**−**3** (refcodes KAJDEE, KAJDII, KUDCUH) and four complexes of

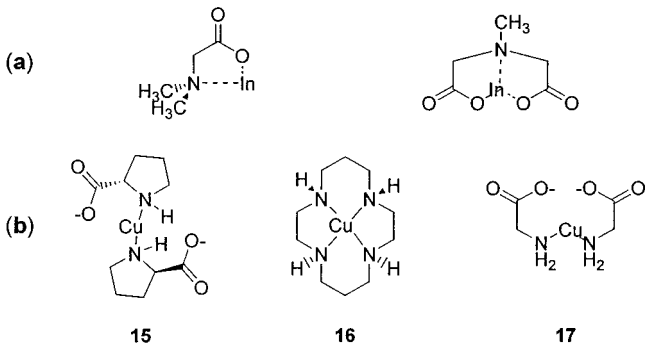

Figure 2. (a) Reference ab initio complexes for the In(III) parameters. The geometries were optimized and frequencies calculated using Gaussian 94 (B3LYP/LANL2DZ). (b) Reference ab initio complexes for the Cu(II) parameters. The geometries were optimized and frequencies calculated using Jaguar (B3LYP/LACVP**).

DTPA, compound **4** (refcodes ZIJTOB, ZIJTUH, ZIJVAP, ZIJUET). In addition to the X-ray structures, two indium aminocarboxylate complexes were optimized and frequencies calculated with density functional calculations (B3LYP/LANL2DZ) within Gaussian94.[19] The structures of these two molecules are shown in Figure 2a.

The reference data for the Cu(II) parameters involved a total of 10 copper-containing crystal structures, shown in Figure 3, from the Cambridge database with a mean *R* factor of 0.0541 (refcodes CUCJOZ01 (**5**), SUKGUA (**6**), DIRJET (**7**), FEKVAS (**8**), VOPSAU (**9**), DEFJIH (**10**), HAFTAJ (**11**), JUMMUZ (**12**), ZEBJIZ (**13**), LEPNOJ (**14**)). These were predominately azamacrocycle complexes as these are the most relevant for use as a radiopharmaceutical. In addition a total of four Cu(II) complexes, complexes **14** (Figure 3) and **15**−**17** (Figure 2b), had their geometries optimized and frequencies calculated (B3LYP/LACVP**) with Jaguar 3.5.[20]

The high and variable coordination number around In could only be reproduced by a rather unusual approach; this allowed investigation of the systems of interest to us, but severely limits transferability to other programs. The MacroModel file format only allows six bonds to a given atom in the input file; in the case of In(III) the indium is typically coordinated to more than six ligating groups. Our approach was to bond only amine ligands to the indium in the input files; the carboxylates were treated as free anions. However, the substructure feature in MacroModel allows identification of In−N−C−COO^- moieties; the substructures developed for In(III) are shown in Figure 4. As these substructures were recognized, a bond was added from the indium atom to each proximate oxyanion. Such added bonds are not limited in number by the program. This procedure also allowed charge flux, lowering the charge on In for each coordinated carboxylate. It should be noted that the coordination environment is determined only at startup; it is not variable in a dynamics run, but only depends on the starting geometry. This alleviates possible problems with discontinuities on bond elongation.

A similar approach was taken in developing parameters for Cu(II) in that a series of substructures, Figure 4, were developed capable of modeling the limited set of complex types we are interested in studying. In those complexes involving carboxylates they were treated as free oxyanions as with the In(III); as the substructure is recognized, a bond between the copper atom and the oxygen is then formed.

The parameters were optimized to fit the selected reference data using an automated parametrization method.[21,22] First, a penalty function

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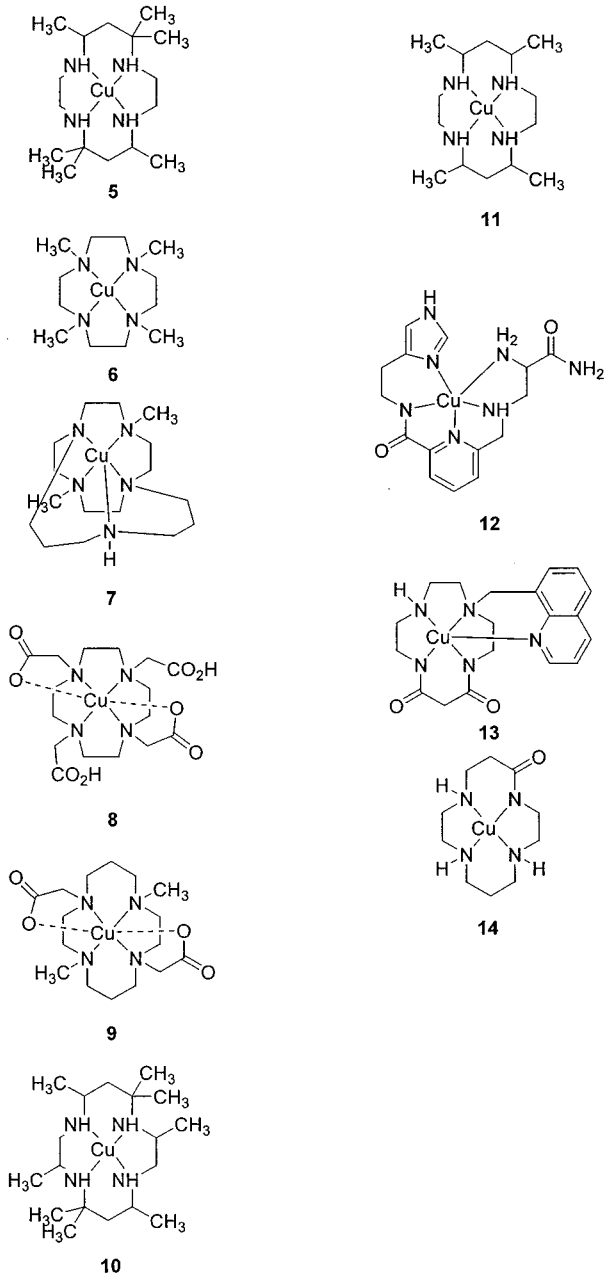

Figure 3. Reference compounds for the Cu(II) parameters.

was defined as a weighted sum of squares of differences between values calculated by the force field and the selected reference data. Weight factors were selected as described previously,[21] with slightly lower weight for some less well determined X-ray structures. The penalty function was then minimized by alternating application of Newton−Raphson and Simplex methodologies.[22] The optimization was terminated when no further improvement could be obtained. For the final set, a range was calculated for each parameter, defined as the maximum modification that changes the penalty function by less than 0.1%.[23] Note that this is not a true confidence interval for the parameter, as it does not account for possible errors in the data or linear dependencies between parameters.[22]

Parameter Testing. As a test of the developed parameters, new sets of In(III) aminoacetate structures and Cu(II) azamacrocyclic structures were chosen from the Cambridge database. For indium a total of five structures (Figure 5) with an average *R* factor of 0.03 were

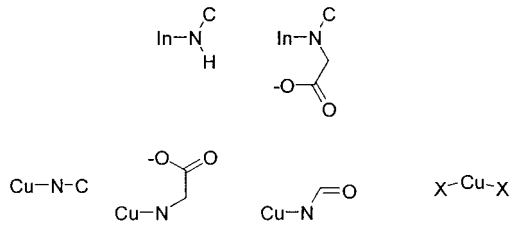

Figure 4. In(III) and Cu(II) substructures implemented within AMBER*.

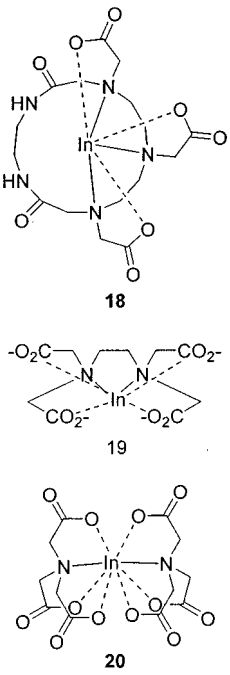

Figure 5. Validation complexes for the In(III) parameters.

chosen: one macrocycle **18** (SIQZIB), two EDTA **19** complexes (ZIJPUD and ZIJQEO), and two dimeric complexes **20** (ZUFXAZ, ZUFXED). These structures were then imported into MacroModel and minimized with the developed parameters. It is important to note that these structures serve as an independent test of the parameters as they were not utilized in developing the parameters.

In a similar manner a total of eight Cu(II) crystal structures (Figure 6) were chosen with an average *R* factor of 0.037: **21** (CEVMAR, CEVMEV), **22** (LEWCOF), **23** (NUJVUJ), **24** (PIMFEW), **25** (POTPUJ), **26** (VALVUZ), and **27 (**ZALFUN). These independent structures were minimized with the developed parameter set and compared to the initial crystal structure as an accuracy test.

Peptide Conformational Searches. The conformational space of the parent peptide octreotide, the InDTPA bifunctional chelate conjugate, and the CuDOTA conjugate, Figure 7, were searched using a two-step process. The initial starting structure of octreotide was that determined by Melacini[24] as obtained from the Protein Data Bank[25] (PDB ID: 1SOC). The structure was then subjected to 5000 steps of a systematic pseudo Monte Carlo search[26] using the AMBER* force field and GB/SA aqueous solvation model and a 50 kJ/mol energy window. After minimization and elimination of duplicate structures the search had produced 250 unique conformations. These were then used as initial starting structures for a low-mode conformational search (LMCS).[27] This search was set to perform 5000 trials and had an energy window of 25 kJ/mol from the lowest energy conformation found.

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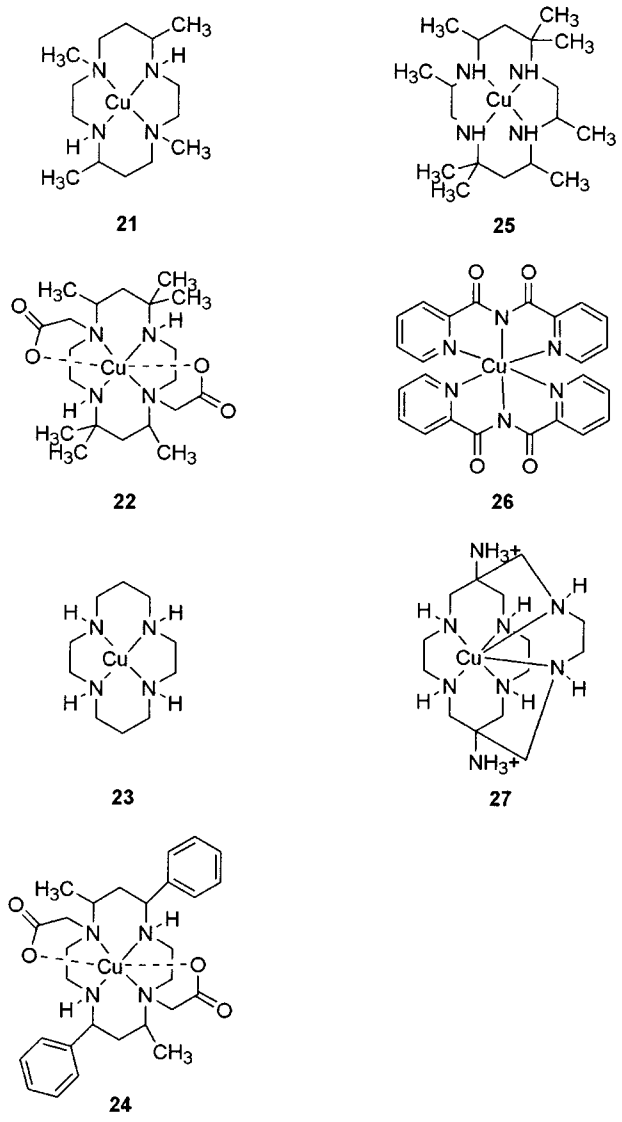

Figure 6. Validation complexes for the Cu(II) parameters.

In a similar procedure the conformational space of InDTPA−octreotide was explored as well. The initial structure was built from the crystal structures of InDTPA (ZIJTOB) and the same structure of octreotide as used above. The initial Monte Carlo search was run for 1000 trials and used a 50 kJ/mol energy window. After minimization and elimination of duplicate conformations the search had produced 382 unique conformations. These were then used as starting structures for a LMCS search using 5000 trials and an energy window of 25 kJ/mol. This search produced a total of 179 conformations of which only 61 structures had fully converged. The GB/SA solvation model has been found to have problems reaching convergence on highly charged groups such as InDTPA. The structures from this search were then fully minimized without solvation, resulting in 98 unique conformations.

The initial structure of CuDOTA−octreotide was built from the crystal structures of CuDOTA (FEKVAS) and the structure of octreotide used previously. Rather than using an initial Monte Carlo search, sequential LMCS searches were utilized to explore conformational space. As with the InDTPA conjugate, the GB/SA solvation model had difficulties in reaching convergence with the CuDOTA structure; the conformational searches were than performed without this model. An additional modification was made to the initial starting structure in order to circumvent a problem with the LMCS procedure. The LMCS procedure performs a chirality check on all four-coordinate atoms; a four-coordinate copper fails this test. When one of the carboxylates was directly bound to the copper atom, making the copper five-coordinate, the LMCS procedure performed normally.

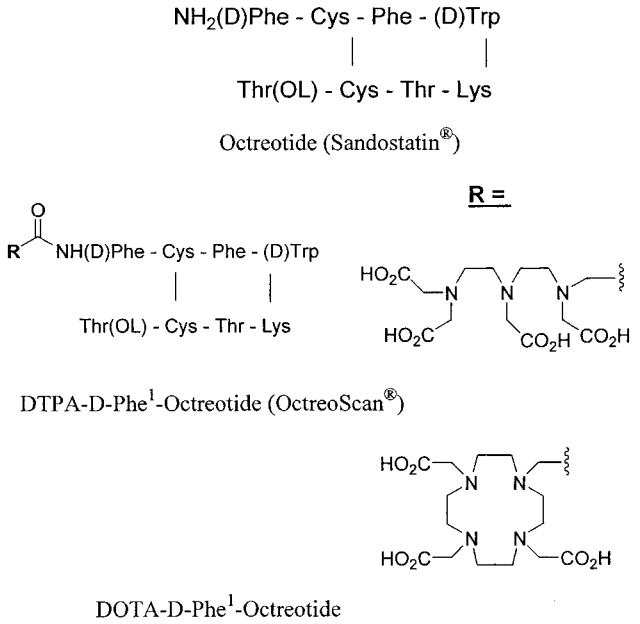

Figure 7. Structure of the somatostatin analogue octreotide and the DTPA and DOTA conjugates.

The initial LMCS search used 5000 trials and an energy window of 25 kJ/mol. This produced a total of 396 conformations, which were not subjected to a full minimization. These structures were then used as starting conformations for a subsequent LMCS search of 5000 trials and a 25 kJ/mol energy window. After full minimization a total of 175 unique conformations remained; these were used for a final LMCS search of 5000 trials with the same energy requirements. This search after full minimization produced a total of 250 unique conformations with the same global minimum as found previously.

Results and Discussion

The selection of force field parameters for organic molecules has been extensively addressed.[28,29] A key feature of a molecular mechanics parameter set is transferability; a parameter developed for a specific interaction is applicable regardless of the environment in which it occurs; thus relatively small parameter sets can model a variety of molecules. With coordination compounds the derived parameters are not as transferable as those for simple organic molecules primarily due to the different geometries which can occur at the metal center and thus have to be accounted for. Most commercial modeling packages have parameters which cover the majority of organic molecules and common functional groups. However, little attention has been spent developing parameters for metals of interest as potential radiopharmaceuticals or other diagnostic imaging agents.

In previous studies we and our collaborators have developed molecular mechanics (MM) parameters for the TAFF force field, found in the commercial molecular modeling package SYBYL.[30] These were used for modeling Ga(III) and In(III) octahedral complexes,[31,32] technetium(V) mono-oxo complexes,[33] and

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Gd(III)-based MRI contrast agents.[34] The parameters were determined in an iterative fashion through comparison with selected crystal structures. The most critical parameter found to affect the metal complex structures was the metal−ligand bond length as well as the force constant for this motion. In all cases, the effects of electrostatics were ignored so that the metal complex structure is determined solely by steric effects.

To date our radiometal force fields have been relatively simple with only a covalent description of the environment about the metal center. Even with these relatively simple force fields, they have proven useful in predicting coordination number and stereochemical preferences. In order to develop more accurate tools for QSAR studies, more sophisticated force fields are required. Developing such force fields presents a 2-fold problem. The first is choosing and evaluating relevant data, the second is fitting of the developed parameters to this data.

In recent years quantum mechanical calculations have been used to extend the available data set for parameter development. Ab initio calculations have most commonly been employed to determine properties corresponding to experimental observables (e.g., structures or rotational barriers), which have been used in lieu of observed data. The CFF force field developed by Hagler et al has made extensive use of ab initio calculations at the HF/6-31G* level, with subsequent scaling to reproduce the available experimental data.[35,36] This method was then used in the development of the Merck force field, MMFF, by Halgren et al.[37−42] Instead of relying on parameter scaling to fit experimentally derived data, the Merck group used increasing levels of theory for different types of quantum mechanical predictions.

The force fields developed by Hagler et al.[35,36] and Halgren[37−42] have been shown to be among the most reliable currently available.[13] Norrby and co-workers have extended upon this work, developing a method for adding parameters to existing force fields.[43] Their work has resulted in a nonproprietary set of procedures for automation of molecular mechanics parametrization. These procedures have been successfully applied by Brandt et al.[23] in order to develop ruthenium(II) parameters for the MM3*[44] force field as implemented in the commercial package MacroModel.[15]

The development of these force field parameters consists of several distinct steps:

(i) Collection of reference data and definition of a penalty function. Usually the penalty function is a weighted sum of squares of deviations between reference data points and the corresponding calculated force field values.

(ii) Definition of new functional groups. Even within the context of an existing force field, parametrization of new functional groups commonly requires definition of what atom types and bond types to use.

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Table 1. AMBER* Parameters for In(III)[a]

group	bond length (Å)	stretching constant (kcal/mol Å^2)	bond moment (D)
In−N	2.3439 ± 0.06	52.4947 ± 3.4338	−1.1094 ± 0.0196
N−C	1.4718 ± 0.0039	465.4204 ± 7.1051	0.1896 ± 0.0149
N−H	1.0080 ± 0.02	495.2737 ± 9.2091	−1.4347 ± 0.0151
C═O	1.2324 ± 0.0049	753.3836 ± 4.9755	−0.1906 ± 0.0102
C−(O−)	1.2717 ± 0.0054	389.6917 ± 7.7339	3.9450 ± 0.0097
In−(O−)	2.2093 ± 0.0057	231.8012 ± 4.9359	5.0425 ± 0.0111

group	angle (deg)	bending constant (kcal/mol)
In−N−C	109.6611 ± 0.5062	27.6302 ± 1.0327
In−N−H	106.7439 ± 0.895	23.9144 ± 1.1036
N−In−N	125.6148 ± 0.9402	12.8810 ± 0.4788

[a] The range indicated for each parameter represents the change in that parameter which causes a 0.1% increase in the penalty function.

(iii) Choice of initial parameter values.

(iv) Refinement of parameters (optimization of the penalty function).

(v) Testing and validation of the final parameter set.

The reference data utilized in this work consists of crystallographic data obtained from the Cambridge Structural Database,[18] and density functional (DFT) calculations utilizing the B3LYP functional. In determining what functional groups are required for the parametrization it is important to consider the type of coordination complexes to be studied. The use of metal coordination complexes for use in vivo imposes many limitations, which affect the choice of suitable ligands. In the case of In(III) commonly utilized ligands are polyaminocarboxylates, hydroxyaromatics, and azamacrocycles. Examples of some commonly utilized polyaminocarboxylate ligands are the ligands EDTA (**19**) and DTPA (**4**). In addition, certain restraints are imposed by the MacroModel program itself. The structural file format used prohibits the specification of more than six bonds to a given atom.

In all the complexes a similar coordination scheme is observed: the amines are bound to the indium, and the complexes have either four or five carboxylates coordinated to the metal. It was decided to model the complexes with explicit In−N bonds; a bond between In and O^- is added if certain geometrical constraints are met. With this approach it was possible to have coordination numbers greater than six about the metal center. In order to minimize the number of new AMBER* parameters to be developed, the two new functional groups shown in Figure 4 were defined. The refinement procedure was successful, with the penalty function reaching a minimum value. The resultant AMBER* parameters are shown in Table 1.

The use of molecular mechanics to study Cu(II) complexes presents a challenge due to the variety of coordination numbers and geometries adopted by copper. Copper in the +2 oxidation state accommodates anywhere from four to six donor atoms, resulting in many coordination geometries. Complex geometries such as distorted octahedral, trigonal prismatic, square pyramidal, trigonal bipyramidal, tetrahedral, and square planar have all been found experimentally. This presents a major hurdle in developing force field parameters, with each coordination geometry requiring a unique set of parameters. The task is simplified somewhat in that we will focus primarily on azamacrocyclic ligands, limiting the possible coordination geometries. Ligands which have been utilized as copper-based radiopharmaceuticals have been polyaminocarboxylates, aza-

Table 2. AMBER* Parameters for Cu(II)[a]

group	bond length (Å)	stretching constant (kcal/mol Å^2)	bond moment (D)
Cu−N−C			
Cu−N	2.092 ± 0.0059	204.358 ± 0.1873	0.3324 ± 0.0034
N−C	1.5159 ± 0.0005	132.413 ± 1.9588	−0.8436 ± 0.0033
Cu−N−C−(C═O)−O−			
Cu−N	2.1655 ± 0.0084	242.2446 ± 5.1558	0.3518 ± 0.0086
C═O	1.2559 ± 0.0039	500.5697 ± 2.4614	−0.1884 ± 0.0098
Cu−O−	1.4363 ± 0.0092	250.7881 ± 4.1856	0.6018 ± 0.0022
Cu−N−C═O			
Cu−N	1.9275[b]	201.2229 ± 0.9526	3.1986[b]

group	angle (deg)	bending constant (kcal/mol)
?−Cu−?	180 ± 0.0059	2.9052 ± 0.0034
?−Cu−?	69.715 ± 0.0008	4.0428 ± 0.0009

[a] The range indicated for each parameter represents the change in that parameter which causes a 0.1% increase in the penalty function. [b] A range for this parameter was unable to be calculated.

Table 3. Calculated Structure to X-ray Fitting Results

structure	rms fit all atoms	rms fit only In and N
1	0.202	0.035
2	0.357	0.095
3	0.163	0.021
4 (ZIJTOB)	0.527	0.045
4 (ZIJTUH)	0.569	0.091
4 (ZIJVAP)	0.465	0.063
4 (ZIJVET)	0.469	0.063
av	0.393	0.068

macrocycles, and macrocyclic polyaminocarboxylates.[45] Only the last two classes have been found to possess stabilities high enough for use in vivo. A procedure identical to that used for indium was followed for the development of a copper force field. The resulting parameters are found in Table 2.

Performance of the Parameters. The seven In(III) reference structures (Figure 1) were then modeled using these parameters and compared to the experimentally determined crystal structures in order to validate the parameter set. The results are found in Table 3; overall the root-mean-square (rms) fits are good. The major errors are found in the carboxylate positions as might be expected from a primarily electrostatic interaction. A closer examination of the performance of the developed parameters finds that the average difference in In−L bond lengths between the X-ray structures and the modeled structure is 0.0048 ± 0.042 Å. The average difference in the L−In−L angles was found to be 0.39° ± 2.25°.

When these parameters were used to model the independent set of structures (Figure 5), quite similar results were obtained. The average difference in In−L bond lengths was found to be 0.0046 ± 0.056 Å. The average difference in the L−In−L angles was found to be 0.32° ± 2.67°. With such good agreement between the two sets of structures it appears that these parameters can adequately model aminocarboxylate complexes of In(III).

The Cu(II) reference structures (Figure 3) were then compared to the crystal structures; the results are shown in Table 4. A comparison of the rms fits finds lower agreement than that achieved with In(III). The average Cu−L bond difference is 0.031 ± 0.17 Å, while the average L−Cu−L angle difference is 1.22° ± 15.87°. These lower agreements are most likely due to the much wider variety of structures found in the Cu(II) reference structures when compared to the In(III) reference structures.

Table 4. Comparison of Calculated Structure to X-ray for Cu(II) Complexes

structure	rms fit all atoms	rms fit only Cu and N
5	0.386	0.408
6	0.267	0.110
7	0.155	0.075
8	0.329	0.148
9	0.313	0.220
10	0.475	0.413
11	0.359	0.210
12	1.640	0.868
13	0.296	0.125
14	0.275	0.172
av	0.350	0.275

Indeed when the more homogeneous set of structures in the independent test set (Figure 6) was modeled with these parameters, slightly better results were obtained. The average Cu−L bond difference is 0.023 ± 0.25 Å, while the average L−Cu−L angle difference is 0.070° ± 7.96°. While we see that the differences in bond lengths between the two sets remain approximately the same, we see an improvement in the angles with the more homogeneous test set.

Modeling of Radiometal−Bifunctional Chelate−Peptide Conjugates. In order to show the utility of this type of parametrization we have used our parameters to examine the eight amino acid somatostatin analogue octreotide,[46] In^{III}DTPA−octreotide,[17] and Cu^{II}DOTA−octreotide (Figure 7). The conformational preference of octreotide has been studied both in solution by NMR and in the solid state through X-ray crystallography.[24,47] Melacini et al.[24] determined that in solution the behavior of the cyclic peptide was best described with a multiconformational model, the commonly proposed antiparallel β-sheet, and a 3_{10} helix-like fold. These results were arrived at through molecular modeling of the peptide using the CVFF force field found in the commercial package DISCOVER, with distance geometry criteria provided by two-dimensional NMR data. Thus the parent peptide provides a well-studied test for our modeling methods.

The human somatostatin receptor has been found to have five subtypes, sst1, sst2, sst3, sst4, and sst5.[48] All five of these subtypes have been found to bind somatostatin with high affinity; however, octreotide and various analogues have all been found to possess different affinities for the various subtypes. Of particular importance to the design of metal-containing diagnostic and therapeutic agents was that changes in the metal and bifunctional chelate utilized affected the binding affinities to the various receptor subtypes.[49] Molecular modeling of such peptides conjugated to various bifunctional chelates and various metals could lend insight into the factors affecting binding to the somatostatin receptor subtypes.

As a first step in such a study we have modeled the parent peptide, the InDTPA conjugate, and the CuDOTA conjugate with our modified AMBER* force field using the GB/SA

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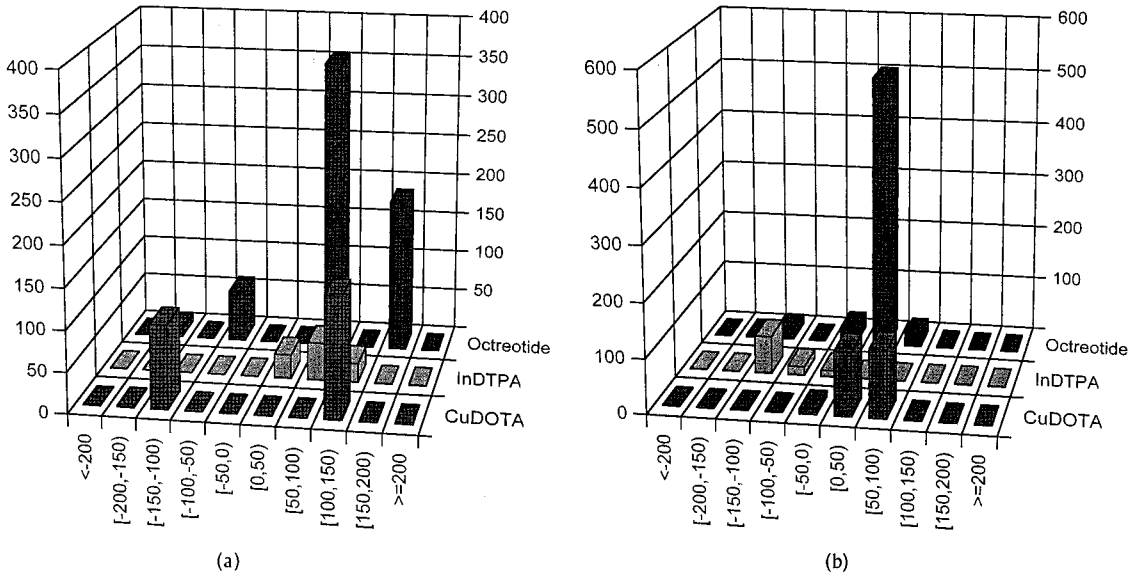

Figure 8. Histograms of the ϕ (a) and ψ (b) values for position 1 ((D)Phe) of octreotide, the InDTPA conjugate, and the CuDOTA conjugate. The height of the columns represents the number of times torsions of the indicated range were found in the search results.

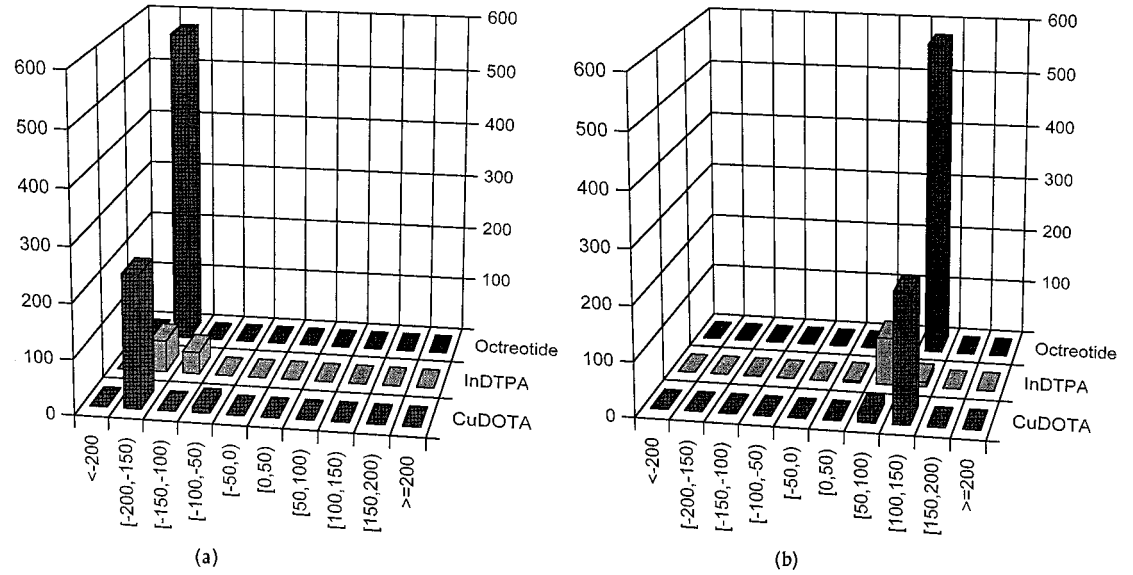

Figure 9. Histograms of the ϕ (a) and ψ (b) values for position 6 (Thr) of octreotide, the InDTPA conjugate, and the CuDOTA conjugate. The height of the columns represents the number of times torsions of the indicated range were found in the search results.

solvation model[50] found in MacroModel. The lowest energy conformation found in our conformational searches compares favorably to that reported by Melacini. An rms fit of the α carbons between the lowest energy conformation found with our search procedure and the structure reported by Melacini was found to be 0.321 Å.

Rather than comparing individual structures, we analyzed the populations of low-energy conformations produced by our searches. Each population of conformations was analyzed in terms of their ϕ and ψ values, and histograms of the frequency at which these torsions were found were plotted. As might be expected, the largest differences were found in position 1 (D)Phe to which the metal−BFC is directly bound, Figure 8. The threonine in position 6 also was found to have different torsional preferences when a metal−BFC moiety was present, Figure 9. In Figure 10, the terminal threonol position 8, we see small differences in the torsional profiles between the parent peptide and the conjugates. The exact cause of these differences is currently under investigation but appears to be the result of steric interactions and the formation of additional hydrogen bonds between the bifunctional chelate and the peptide.

Conclusions

This work illustrates the utility of Norrby's automated parametrization method for developing specific metal ligand parameters for the AMBER* force field. This method provides an important tool for the fields of bioinorganic chemistry and nuclear medicine, in that it is relatively simple to develop metal parameters for a well-accepted force field widely used in modeling peptides and proteins. An initial application of this methodology has been to examine the influence two commonly utilized bifunctional chelates, DTPA and DOTA, have on a peptide's conformational preferences.

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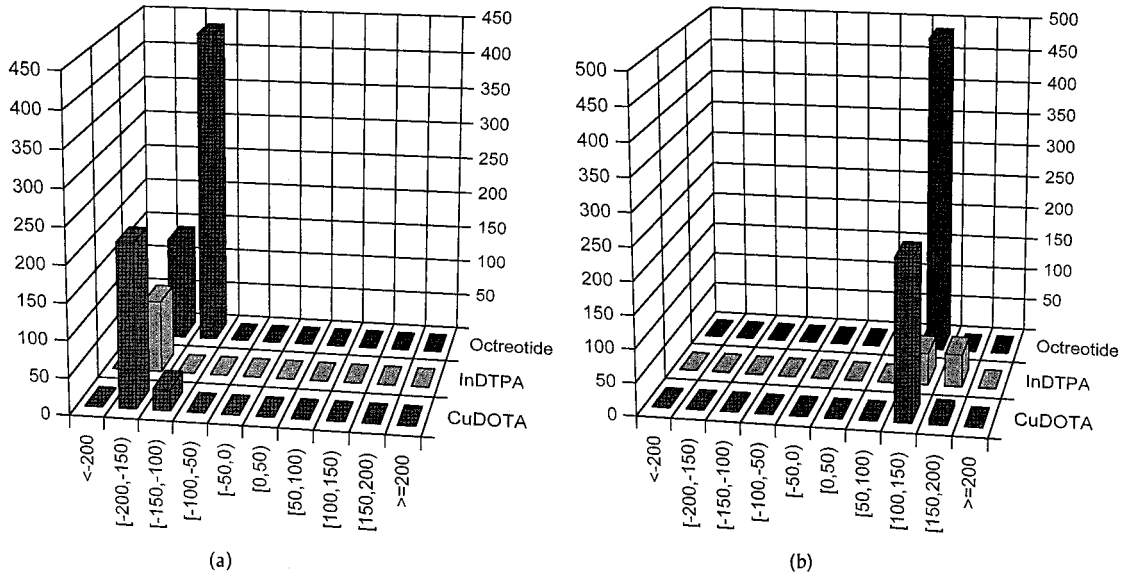

Figure 10. Histograms of the ϕ (a) and ψ (b) values for position 8 (ThrOL) of octreotide, the InDTPA conjugate, and the CuDOTA conjugate. The height of the columns represents the number of times torsions of the indicated range were found in the search results.

The cyclic peptide octreotide has been studied though Monte Carlo conformational search procedures and the GB/SA solvation model in order to find the low-energy conformations. In a similar fashion the same peptide conjugated to InDTPA and CuDOTA was studied. The presence of these metal−BFC groups was found to have a significant effect on the conformational preference of the peptide, possibly explaining the changes in binding affinity to the various somatostatin receptor subtypes. It must be noted that these are only preliminary studies; studies currently underway involve other BFC−peptide conjugates and several analogues of octreotide.

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Supporting Information Available: A complete set of the AMBER* parameters described in this work. This material is available free of charge via the Internet at http://pubs.acs.org.

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