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ABSTRACT

THE APPLICATION OF STEREOLITHOGRAPHY TO
THE CREATION OF MOLECULAR MODELS OF AMILORIDE
AND ITS ANALOGUES

by
Thomas John Busanic

Stereolithography, a process by which a computer-guided Ultraviolet (UV) laser is applied onto a liquid monomer resin to form a solid polymer, was used to create solid models of amiloride and two of its analogues. The Cartesian coordinates used as input for the orientation of each atom in a molecule were calculated using *ab initio* molecular orbital theory. Since the structures of the molecules are calculated in this fashion, they represent a more accurate representation of the molecules than that provided by standard molecular modeling kits.

The original purpose of this work was to assist a blind chemist in appreciating the structure of amiloride and two of its analogues. The models not only serve blind individuals, but also serve as a communication link between blind and sighted individuals. The models also have the potential to be used as learning aides to blind and sighted students at the high school and college level.

THE APPLICATION OF STEREOLITHOGRAPHY TO THE CREATION OF MOLECULAR MODELS OF AMILORIDE AND ITS ANALOGUES

by
Thomas John Busanic

A Thesis
Submitted to the Faculty of
New Jersey Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Applied Chemistry

Department of Chemical Engineering,
Chemistry, and Environmental Science

May 1994

APPROVAL PAGE

THE APPLICATION OF STEREOLITHOGRAPHY TO THE CREATION OF MOLECULAR MODELS OF AMILORIDE AND ITS ANALOGUES

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This thesis is dedicated to
John and Maria Busanic

ACKNOWLEDGMENT

The author wishes to express his sincere gratitude to his advisor, Professor Carol A. Venanzi for her guidance and advice throughout this research.

Special thanks to Professors James Grow and Tamara Gund for serving as members of the committee.

The author is grateful to the National Science Foundation for providing further and continued funding for this project.

The author appreciates the help and suggestions from Postdoctoral Research Associates William J. Skawinski and Ronald A. Buono.

Finally, the author thanks Seymore Drazien and coworkers from the Manufacturing Engineering Department of NJIT and George Gonzalez and coworkers from the Academy for the Advancement of Science and Technology for the use of their facilities and for their help.

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CHAPTER 1

INTRODUCTION

1.1 The History of Stereolithography

Stereolithography is a technology that links computer graphics to the rapid fabrication of a solid object. Developed and patented by physicist Charles W. Hull(1), stereolithography converts model files created using computer aided design (CAD) programs into a three-dimensional solid part. The part is constructed via polymerization from a photosensitive monomer (resin). A schematic drawing of an SLA can be seen below in Figure 1.

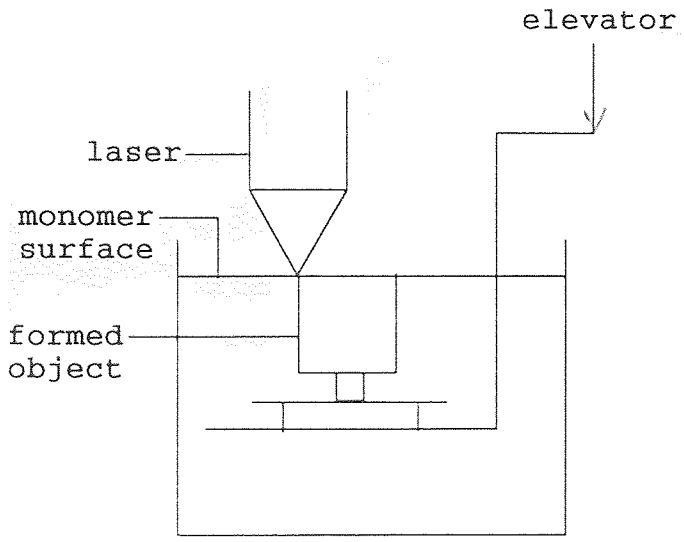
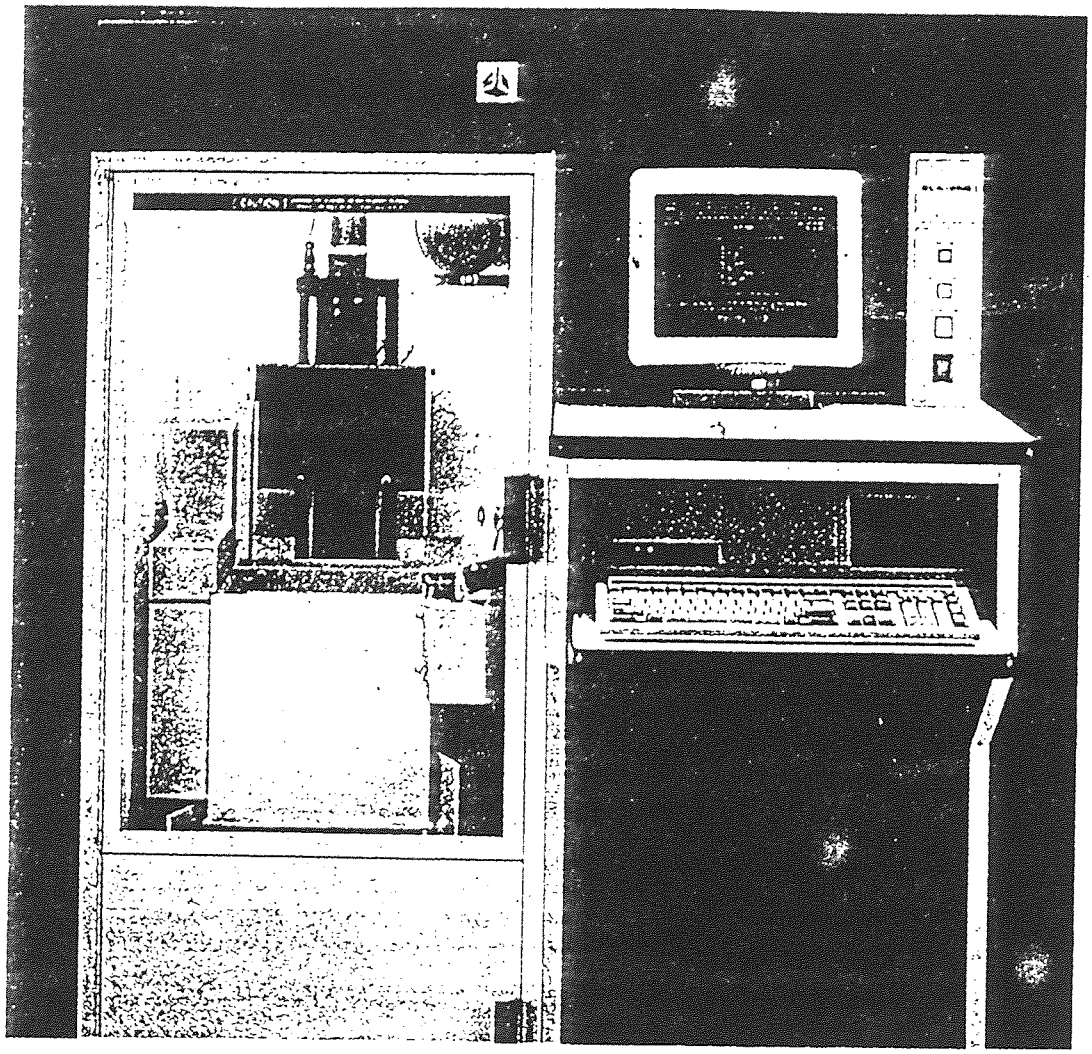


Figure 1 The basic stereolithography apparatus (SLA)(1).

The stereolithography apparatus used to build the molecular models was the SLA-250 from 3D Systems(2) of

facing 2



3D Systems

Figure 2 Photograph of the SLA-250(2).

Valencia, California. A picture of the SLA-250 can be seen in Figure 2 on the facing page. This SLA consists of a 7.8 gallon vat equipped with an elevator table(3). The vat contains the resin used to create the model. The SLA also consists of a helium/cadmium UV laser.

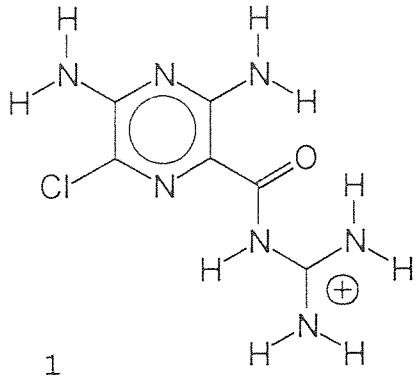
The SLA builds models through photopolymerization. When the laser beam is applied to the monomeric resin (which is a liquid), the resin polymerizes. Once the resin polymerizes, it solidifies. The laser beam cures the resin along the xy-plane of the model. After each xy-plane is cured by the laser, the laser is directed along the z-axis to begin curing the next xy-plane (or slice). The elevator is then lowered to expose subsequent layers of resin to the laser.

Since parts made using stereolithography are created layer by layer, temporary supports are needed to ensure that the part does not collapse during the fabrication process. This support can also be created via CAD programs.

The fumes generated by the liquid resin are carcinogenic. Because of this health hazard, the SLA must have sufficient ventilation. Once the SLA has completed building the entire part, isopropyl alcohol is used to clean off the resin of the support of the part. The part is then placed in an ultraviolet oven to further cure the resin to ensure that the model contains no liquid resin. Once the photopolymer is solidified, it cannot be converted back to liquid form.

1.2 The Importance of Amiloride and Amiloride Analogues

Amiloride, 1, is the generic name of 3,5-diamino-6-chloro-*N*-(diaminomethylene)pyrazine carboxamide(4). In biological systems, amiloride is protonated. The site of protonation has been determined to be at the imino nitrogen(5), as shown below.



1

Prior to the discovery of amiloride and other pyrazinoylguanidines, diuretics caused an increase in potassium ion secretion(6). This effect causes hypokalemia, which can result in a variety of adverse clinical manifestations including cardiac disturbances, anorexia, muscle weakness, and lethargy(7). This deficiency prompted the search for potassium ion-sparing diuretics to use as companion drugs with the potassium ion-losing agents.

Pyrazinoylguanidines were chemically novel being: 1) a member of an uncommon heterocyclic class, pyrazine; 2) a rare, stable acylquanidine (guanidines are usually labile); and 3) a base (most diuretics are acidic or neutral

compounds)(7). Amiloride emerged as the optimal member of the pyrazinoylguanidine series, having a nearly ideal balance of diuretic, saluretic, and antikaliuretic properties.

Amiloride and amiloride analogues possess a broad range of unique activity in biological systems by acting in the blocking of Na^+/H^+ exchangers, Na^+/Ca^{2+} exchangers, and Na^+ channels.

The Na^+/H^+ exchanger contributes in a major way to the total economy of the cell through intracellular pH homeostasis, cell volume regulation, and solute uptake(8,9). The activity of a large number of pyrazinoylguanidine analogues has been systematically evaluated in several cell types. Although absolute values for inhibition constants vary from cell to cell, the rank order of potency of most analogues relative to amiloride is remarkably constant(7). From the studies conducted by Simchowitz and coworkers(7) on amiloride and its analogues, several key points were made:

1) The unsubstituted guanidino moiety is required to preserve activity.

2) Substitution of the 6-chloro group by bromo or iodo groups result in increases in potency. However, substitution of the 6-chloro group by a fluoro group or hydrogen leads to a tenfold decrease in potency. Iodo groups are larger and more lipophilic than the chloro group, attributes which help to anchor the drug more firmly to the hydrophobic residues of the transport protein.

3) Substitution at the 5-amino nitrogen atom produce the greatest enhancement of activity. Replacement of one of the 5-amino hydrogens by ethyl, butyl, hexyl, or phenyl groups results in increases in drug potency. Replacement of both hydrogen atoms by alkyl groups results in even greater drug potency. This effect reaches a maximum when the number of carbons substituted on the 5-amino nitrogen reaches six carbons.

Most cells possess a Na^+/Ca^{2+} exchange mechanism that, in excitable tissues such as nerve and muscle, helps maintain a low cytosolic Ca^{2+} level under steady-state conditions(10,11). This exchange system also influences cell growth and function in cell types such as murine erythroleukemia cells(12) and human neutrophils(13,14). Unlike the Na^+/H^+ exchange mechanism, substitution at the guanidino group enhances the activity of Na^+/Ca^{2+} exchange.

Amiloride has been shown to be a potent inhibitor of Na^+ transport in a variety of cellular and epithelial transport systems(15-18). Analysis of the activities of Na^+ channel blocking of various amiloride analogues done by Cuthbert and Fanelli(19) reveal three major regions of interest: First, as was the case with Na^+/Ca^{2+} exchange, substitution of large hydrophobic groups on one of the terminal guanidino nitrogen atoms dramatically enhances the activity of the blocking of Na^+ channels. Second, in contrast to Na^+/H^+ exchange, an unsubstituted 5-amino group is essential for inhibition of the Na^+ channel(18-21).

Finally, optimal activity is observed when a chlorine atom occupies the 6-position of the pyrazine ring(18-19,21).

Analysis of the activities of Na^+ channel blocking in frog epithelia were conducted by Li et al.(21,22). These studies included both ring substitution and side-chain modifications. Results of these studies are shown below in Table 1.

Table 1 Structure-activity relationships for amiloride and various analogues, calculated by Li et al(21,22).

analogue	substituent on pyrazine ring position 5	position 6	k_{on} ($s^{-1}\mu M$)	k_{off} (s^{-1})	block time (ms)
1	NH_2	Cl	13.17±0.25	3.93±0.19	255
2	NH_2	Br	14.19±1.09	5.58±0.92	179
3	NH_2	I	11.43±0.90	17.41±0.40	57
4	NH_2	F	13.54±0.65	32.20±1.57	31
5	NH_2	H	14.47±0.68	176.25±17.73	6
6	H	Cl	3.32±0.44	10.89±1.35	92
7	Cl	Cl	5.16±0.46	151.10±16.48	7
	extended side-chain				
18	-O-		1.22±0.07	20.67±3.72	48
19	-NH-		2.16±0.11	3.41±0.55	293

As can be seen from Table 1, substitution of the position-6 chlorine by either bromine, **2**, fluorine, **4**, or hydrogen, **5**, raises the value of the on-rate constant, k_{on}. However, the off-rate constant, k_{off}, for analogues **2**, **4**, and **5** are also greater than that of amiloride, **1**. Since the

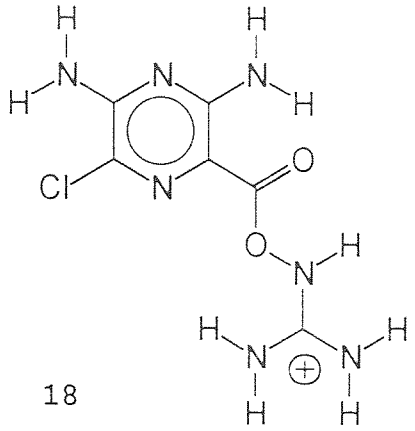
block time is simply the inverse of k_{off}, a lower k_{off} value corresponds to a greater block time. Substitution of the position-6 chlorine by iodine, **3**, has the effect of decreasing the k_{on} and increasing the k_{off}, thus making it a weaker Na^+ channel blocker than amiloride.

Substitution of the position-5 amino group by hydrogen, **6**, or chlorine, **7**, reduces the k_{on} and increases the k_{off} relative to amiloride, making both analogues weaker Na^+ channel blockers than amiloride. This seems unusual in the case of analogue **7** since there are electronegative substituents at positions 5 and 6 of the pyrazine ring. Li et al.(21) have suggested that the electron-donating amino group at position 5 of amiloride stabilizes the complex by increasing the electron density of the ligand with respect to analogue **7**.

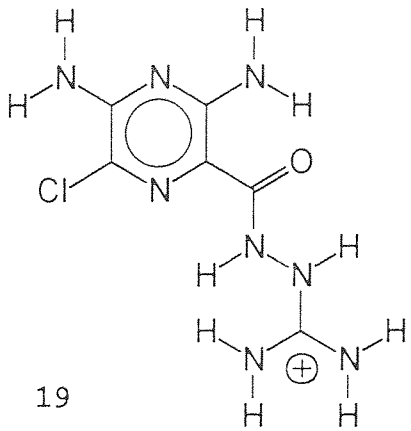
Extending the side chain of amiloride by inserting an oxygen, **18**, or secondary amino group, **19**, between the carbonyl carbon and the imino nitrogen has the effect of reducing the k_{on} with respect to amiloride. The k_{off} for analogue **18** is greater than that of amiloride, making it a weaker Na^+ channel blocker than amiloride. The k_{off} for analogue **19** is smaller than that of amiloride, which corresponds to a longer block time.

C.A. Venanzi and W.J. Skawinski, in unpublished results, determined that analogues **18** and **19** are non-planar molecules, unlike analogues **1-7**. Due to the fact that analogues **18** and **19** are non-planar molecules, these two

analogues are of interest. The protonated forms of analogues **18** and **19** can be seen below.



18



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1.3 How SLA Can Assist Both Blind Chemists and Sighted Chemists

Creating models using stereolithography is a novel approach to the building of molecular models. These models may assist both blind and sighted chemists. These models were made to represent molecules with calculated, rather than average, bond lengths, bond angles, and atom size. Additional work is being done to encode properties of atoms such as atom type or electrostatic potential onto the surface of the model by extruding letters or dots from the surface of the model.

SLA models have the potential to allow blind scientists and blind students to gain access to complex visual information through tactile sense. Thus, SLA models serve blind individuals in two ways. First, SLA models can provide blind scientists with a useful tool that enables them to

obtain more information about a given molecule. Second, SLA models can be used as instructional aids to provide blind students with a better understanding of chemical structure.

SLA models can assist sighted scientists. These models have the potential to allow sighted scientist to communicate with blind colleagues more easily. SLA models can also give the sighted scientist a more realistic chemical model than that of a molecular model set. Such molecular model sets come with standard bond lengths, bond angles, and atom sizes, which may not be appropriate for molecules such as amiloride. The standard hand-held molecular models do not incorporate realistic torsional barriers and cannot be used to identify the low energy conformers of complicated structures, such as analogues **18** and **19**. In addition, the modeling kits cannot accurately represent structures such as transition states of reactions. The advantage of stereolithography is that it can be used to produce accurate solid models of molecular structures calculated from *ab initio* quantum chemistry and other techniques.

Molecular models created using stereolithography can also provide blind and sighted scientists with additional information about a given molecule. Properties encoded on the surface of a model can give a three-dimensional perspective of a given property. In addition, complementary shapes of molecules can be made. This complementary shape can represent the steric and electrostatic aspects of the binding site of a given molecule. In the case of amiloride,

where the molecular structure of the ion channel is unknown, it is important to use the properties of the analogues to input information about the complementary binding site.

SLA models of molecules can be used as instructional tools for high school and college chemistry students. These models can provide the student with a view of molecular structure that is more realistic than that of a standard molecular modeling kit.

CHAPTER 2

ELECTROSTATIC POTENTIAL MAPS OF AMILORIDE AND ITS ANALOGUES

2.1 Minimum Energy Conformation Calculations of Amiloride and its Analogues

The data used as input to the computer-aided design program were calculated by thesis advisor Carol A. Venanzi and postdoctoral research associate William J. Skawinski. The calculations were done via *ab initio* quantum chemistry, a quantum mechanics method used to determine the structure, energy, and other physical properties of a molecule(23). Two types of basis sets, comprised of Gaussian functions, were used in the calculation of amiloride and analogues **18** and **19**.

For computational facility, the geometry of atomic orbitals are defined by linear combinations of primitive Gaussian functions. These linear combinations constitute a basis set. The first basis set used to calculate the optimal geometry of amiloride and analogues **18** and **19** was the 3-21G* basis set, in which a complete set of six second-order Gaussian primitives was added to two basis functions allocated to describe each valence electron orbital. The second basis set used was the 6-31G*. This basis set is constructed by the addition of a set of six second-order (d-type) Gaussian primitives to the 6-31G basis set, which comprises inner shell functions each written in terms of six

Gaussian functions and two valence shell functions represented by three and one primitive Gaussians, respectively(23).

The minimum energy conformers of analogues **18** and **19** were obtained by optimizing the geometry of the molecule for various increments in two torsional angles: HNOC and NOCO for analogue **18**, HNNC and NNCO for analogue **19**.

2.2 Electrostatic Potential Calculations of Amiloride and Analogues 18 and 19

The molecular electrostatic potential (MEP) represents the energy of interaction between a positive point charge and the nuclei and electrons of a molecule. The electrostatic potential at a point $\mathbf{r}^{\rightarrow}$ is given, in atomic units, by

$$V^{ES}(\mathbf{r}^{\rightarrow}) = \sum_{A} \frac{Z_A}{|\mathbf{R}^{\rightarrow}_A - \mathbf{r}^{\rightarrow}|} - \int \frac{\rho(\mathbf{r}'^{\rightarrow})d\mathbf{r}'^{\rightarrow}}{|\mathbf{r}'^{\rightarrow} - \mathbf{r}^{\rightarrow}|} \qquad (1)$$

where Z_A is the charge on nucleus A

$\mathbf{R}^{\rightarrow}_A$ is the location of nucleus A

$\rho(\mathbf{r}^{\rightarrow})$ is the electronic density function

The two terms on the right of Equation 1 correspond, respectively, to the nuclear and electronic contributions to the potential(24).

MEP calculations of various amiloride analogues with pyrazine ring modifications previously were done by Venanzi et al.(5,25,26). Analysis of the MEP maps of the model encounter complexes of these analogues with the formate ion

indicated four points. First, a stable Na^+ channel blocking complex is formed with analogues that have a localized minimum off the 6-position of the pyrazine ring. Second, the stability of the blocking complex is directly related to the depth of the minimum. Third, substitution at position 5 of the pyrazine ring affects not only the depth but also the location of the minimum off position 6. Finally, steric factors may influence the optimal binding of the 6-position ligand to the ion channel. From these studies, a pharmocophore was defined for amiloride(26,27) in which the distance between the proton donors of the guanidinium group and the distinctive MEP minimum off the 6-position of the pyrazine ring was considered to be an important feature. Subsequent solvation studies of amiloride(28,29) supported the pharmocophore hypothesis.

Once the minimum energy conformation calculations for amiloride and analogues **18** and **19** were completed, the single point energy and the electrostatic potentials of each analogue were calculated. The basis set used for these calculations was the STO-3G basis set(27). As in previous work, a formate ion was used to mimic an acidic amino acid that forms an encounter complex with each of the analogues. This encounter complex represents the complex formed when the analogue interacts with a negative ion channel. The formate ion was chosen because it is simple in structure and the exact geometry of the encounter complex is still unknown. The formate ion was positioned to be coplanar with

the guanidino group of each analogue. Each of the two oxygens of the formate ion were positioned equidistant from the each of the two hydrogens of the positive ion channel of the guanidino group. The MEP maps of amiloride and analogues **18** and **19** were calculated using the molecular modeling program SPARTAN(30), created by Wavefunction, Inc., Irvine, CA. The Cartesian coordinates of amiloride and analogues **18** and **19** were imported into the SPARTAN program. Single point energy calculations were done on each analogue. Next, the electrostatic potential was calculated for each analogue. The MEPs were displayed as slices taken from the volume of the electron density of the molecules. The molecular electrostatic potential maps of amiloride and analogues **18** and **19** can be seen in Figures 3-5 in Appendix A. Each MEP map is coplanar with the pyrazine ring. The units of the electrostatic potentials in Figures 3-5 are kcal/mol. The contour lines in Figures 3-5 are separated by 2 kcal/mol. As can be seen in Figures 4 and 5, analogues **18** and **19** both have localized minima off the 6-position of the pyrazine ring. With regards to the MEP maps of analogues **18** and **19** two observations can be made. First, extending the side chain with the insertion of an amino group (**19**) or an oxygen atom (**18**) increases the size of the local minima off the 6- and the 1-position of the pyrazine ring. Also, the values of the minima decrease in the order **1**>**19**>**18**. This may be due to the fact that the extended chain of **18** and **19** have,

respectively, two and one pair of nonbonding electrons which may interact with the pyrazine ring.

In future work, the molecular electrostatic potentials of analogues **18** and **19** will be encoded onto molecular models created using stereolithography. This can be done by extruding numbers to mark the electrostatic potential at a specific point.

CHAPTER 3

METHODOLOGY OF STEREOLITHOGRAPHY

3.1 The Use of the Computer-Aided Design Program I-DEAS to Create STL Model Files

3.1.1 Creating the STL Files for the Molecular Models

The first step in the generation of a three dimensional model using stereolithography is creating model files using computer aided design (CAD) programs. The CAD program used by the author was I-DEAS(31), created by Structural Dynamics Research Corporation of Milford, OH.

Using the data calculated by thesis advisor Carol A. Venanzi and postdoctoral research associate William J. Skawinski, coordinates of atoms of the protonated forms of amiloride and analogues **18** and **19** were used as input for the CAD program. The data used is shown in Tables 3-7 in Appendix B. It should be noted that although the data in Tables 3-7 are in Angstroms, the units used in the I-DEAS program were inches.

Each atom was represented by a sphere. The size of the sphere depends on the atom's van der Waal's radius. Spheres sharing space in common represent bonds. Since the CAD program recognizes two spheres sharing common space as two separate objects, it was necessary to combine bonded spheres to be represented as one object. This was done using the

'JOIN' command in I-DEAS. Once a sphere was joined to another, the objects become inseparable.

Once all of the atoms of a model were joined, the model was moved into positive space by using the 'TRANSLATE' command. The model was then scaled down by a factor of 0.25 in order for it to fit within the dimensions of the SLA vat. Next, the 'TRIANGULATE' command was used to define all points of each model.

Once the object created in the CAD program was triangulated, it was converted to an STL file, which is a file containing several triangular surface facets the that define the solid object(32).

3.1.2 Creating the STL Files for the Complementary Shapes of Amiloride

The complementary shapes of amiloride were of interest in order to represent a model of the binding site of amiloride. The two complimentary shapes of amiloride were generated in the I-DEAS program in two steps. First, the 'CREATE' command was used to create a block. The dimensions of the block in the xy plane were made larger than that of amiloride in order for the complementary shape to contain the entire molecular model. The top of the block was set at the midpoint of the plane of the molecule. The bottom of the block was set at 0.2 inches below the lowest point of the model (after scaling). Next, the I-DEAS command 'CUT' was used to carve out of the block any space contained by the model of amiloride. In order for the models to fit into the

complementary shapes of amiloride, the amiloride model was scaled up by a factor of 1.1 before it was carved into the block.

The second complementary shape of amiloride was made in the same fashion, with one exception. The z-dimensions of the second complementary shape differed from that of the first in that the top of the block was set at 0.2 inches above the highest point of the model, and the bottom of the block was set at the midpoint of the plane of the amiloride model. The 'ROTATE' command was then used to flip the second complementary shape so that the open end faced upward. Due to the planarity of amiloride, the complementary shapes created are mirror images of each other. The complementary shapes were then 'TRANSLATED' into positive space and converted to STL files in the same way as the molecular models.

3.1.3 Creating the STL Files for the Supports

The support of the molecular models and the complementary shapes of amiloride were also created in the I-DEAS program. Supports were necessary to prevent layer of the model from collapsing during the building process. The supports were made by creating blocks with the dimensions in the xy-plane equal to that of the model it was to support. This was done by creating a cylinder with the same facet size and radius for each sphere. The upper face of each cylinder was set at the radius of the sphere; the lower face of all of the

cylinders was set at 0.2 inches below the lowest point of the model (after scaling down). The supports were then triangulated and converted to STL file format the same way as the models.

3.2 Slicing and Merging the STL Files

3.2.1 Slicing the STL Files

Once the STL files were created, it was necessary to use the SLA slice computer from 3D Systems to 'SLICE' the STL files. This procedure converts the STL file format into a series of slices in the xy-plane of the model. The 'SLICE' command consists of several key parameters, as shown below in Table 2.

Table 2 Values used for the slicing parameters of model and support files.

Parameter	Model	Support
Slice Output Scale	1.000	1.000
Resolution	5000	5000
Layer Thickness (inches)	0.005	0.010
X-Hatch Spacing (inches)	0.01	0.2
Y-Hatch Spacing (inches)	0.0	0.0
60/120 Degree Hatch Spacing (inches)	0.01	0.2
X-Skin Fill Spacing (inches)	0.004	0.0
Y-Skin Fill Spacing (inches)	0.0	0.0

'LAYER THICKNESS' determines the thickness of the slices generated. For example, if the layer thickness for

the model was set to 0.010, the solid model generated will not be defined as well as the model in the CAD program. On the other hand, if the layer thickness is to 0.001, the 'SLICE' procedure, and the SLA building process, will take much more time than normally required (20 minutes and up to 24 hours, respectively). The layer thickness of the support was set to 0.010, since the support would later be discarded.

A second key parameter within the 'SLICE' procedure is the 'HATCH FILL SPACING'. During the building of the solid model, the laser is directed digitally along the x-, y-, and z-axis. The 'HATCH FILL SPACING' parameter determines how closely each row created by the laser is exposed to the resin along the x-, y-, and z-axis. This parameter is especially important since it determines whether or not enough of the resin will be cured to generate solid models. For example, the hatch fill spacing of the supports were set at relatively high values, which served several purposes. First, since less resin was being cured for the support, a minimal amount of resin was used to create the supports. Also, it was easier to remove the supports from the models after the building process since the supports were not held together as well as the models. A third purpose was time conservation. Since the laser traced over less surface area for the supports, it took less time to build the supports. Finally, the laser itself was conserved, since it was not used unnecessarily for the building of the support.

3.2.2 Merging the SLI Files

The 'SLICE' procedure converts the STL files into SLI file format. These SLI files were then combined into a file format used by the SLA computer. This was done through the use of the 'MERGE' procedure. The 'MERGE' procedure combines the SLI files of the models and supports and generates four files: V, R, L, and PRM files. Merging the SLI files allows simultaneous building of the parts and supports while maintaining individual building parameters assigned in the 'SLICE' procedure.

Since two models were built at one time (to conserve time and resin), the SLI files of the models and their supports were 'MERGED' together. The V file generated by the 'MERGE' procedure consisted of the information necessary for the SLA to build the models. The L and R files consisted of layer and range information, respectively. The PRM file consisted of various parameters also used in the building of the models.

3.2.3 Creating the Solid Model in the SLA

Due to the health hazards associated with the liquid monomer resin and the fumes it generates, certain safety precautions were necessary. The SLA is kept in an isolated room with sufficient ventilation. The fumes generated from the resin accumulate in vat chamber during the photopolymerization process. To protect the SLA operator during the building process, the chamber door was shut at all times with the

exception of loading the resin and removing the parts. Filter masks and goggles were worn by the operator when working in the SLA chamber. When handling the resin and the solid parts, rubber gloves were worn. Disposal of any wasted resin was done only after the resin was cured completely.

Once the SLI files were merged, the V, L, R, and PRM files were transferred to the SLA computer that controls the laser and the elevator of the SLA. Once the building of the model was initiated, the computer-directed laser began to polymerize the liquid resin with which it came into contact. After each slice in the xy-plane was exposed to the laser beam, any liquid resin above the slice was removed by a blade within the vat that swept over the top of the layer.

The elevator within the vat of the SLA was also controlled by the SLA computer. After the top of the layer was swept by the blade, the elevator was lowered along the z-axis. The amount the elevator was lowered depended on the value of the layer thickness assigned in the 'SLICE' procedure. This procedure was repeated for each slice of the model.

Once the SLA had completed the building of the model, the elevator was directed up to remove the solid model from the liquid resin. The models were removed from the SLA chamber and cleaned with isopropyl alcohol, which removed the support from the model.

At this point, the models still contained some liquid resin. To complete the curing process, the models were

placed in a UV oven for two hours. This process, called post curing, cures any liquid resin remaining. After the post curing process, it was not necessary to use gloves in the handling of the models.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Solid Models Used to Enable a Blind Chemist to Visualize Molecular Structure

The original purpose of creating solid molecular models from calculated geometries was to assist a blind chemist, William J. Skawinski, in acquiring spatial information of the structures of amiloride and analogues **18** and **19**. Upon tactile inspection of the SLA models, Dr. Skawinski was able to distinguish between atoms of differing atomic radii. Since some atoms have very similar atomic radii, such as aromatic carbon and chlorine (1.85 and 1.8 angstroms, respectively), different facet sizes were assigned to these atoms in the I-DEAS CAD program. Due to the fact that atoms of similar size were assigned different facet values, Dr. Skawinski was also able to distinguish between aromatic carbon and chlorine atoms.

The models created using the SLA also presented Dr. Skawinski with an improved spatial appreciation of the structures of amiloride and analogues **18** and **19**. Previously, the primary means by which Dr. Skawinski was able to inspect the molecular structure of these analogues was to inspect standard molecular models with standard bond lengths, bond angles, and atomic radii. Thus, the models created using the SLA are an improvement of standard modeling kits for two

reasons. First, different atom types can be easily discerned, which is difficult when inspecting standard models. Second, the SLA molecular models represent accurate structures of molecules, since the structures are calculated using quantum mechanics.

4.2 SLA Models Used as a Tool in Understanding the Relationship Between Structure and Function

The models of amiloride and analogues **18** and **19** also have the potential to aid sighted researchers in understanding the relationship between the structure and function of a given molecule. Upon inspection of the minimum energy conformation models of amiloride and analogues **18** and **19**, the author noted that although the side chains of **18** and **19** are longer than that of amiloride, the relative positions of the guanidinium hydrogens and the chlorine bonded to the pyrazine ring are very similar, and fit the spatial requirements of the amiloride pharmacophore.

The SLA models of the complimentary sites of amiloride also provide qualitative information on the structure of the amiloride binding site. Although neither the models of **18** nor **19** fit entirely into the model of the complimentary site of amiloride, the guanidinium hydrogens and the chlorine atom fit into the complimentary site of amiloride. This supports the theory that the guanidinium hydrogens and the 6-position substituent, chlorine, are essential to the blocking of the Na^+ channel.

Superposition of the two guanidinium hydrogens (H28 and H29 in Figures 4 and 5) and the chlorine (Cl19 in Figures 4 and 5) of analogues **18** and **19** onto the corresponding atoms of amiloride (H20, H23, and Cl15 in Figure 3) using SPARTAN shows that the MEP minimum off the chlorine in analogues **18** and **19** are located in the same region of space as that of amiloride. This further supports the theory that molecules that are active Na^+ channel blockers possess the same molecular features of the amiloride pharmacophore.

CHAPTER 5

CONCLUSIONS AND SUGGESTIONS

5.1 Encoding The SLA Models to Further Assist Blind Individuals

Work is currently underway to encode various properties onto the surface of the spheres of the SLA molecular models. Such properties can be encoded by extruding dots or other characters within the CAD program I-DEAS. For example, the atomic symbol can be encoded onto each sphere. Another example is encoding the ring position onto a cyclic molecular model. A third possibility of encoding information onto the surface of molecular models created using SLA technology is to encode molecular properties such as electron density or electrostatic potential.

5.2 Using SLA to Model Electrostatic Potential

Due to the importance of the relationship between electrostatic potential and function, work is underway to create solid models depicting the electrostatic potential. This can be done two ways. One option is to use the I-DEAS program to extrude dots or numbers from the surface of a given model to represent the electrostatic potential at a given point. A second method involves utilizing the program MATHEMATICA(33), created by Wolfram Research Inc., Champagne, IL. MATHEMATICA is a program that converts a

three-dimensional mathematical function into CAD format. Once a given function is converted to CAD format, it could be converted to an STL file and, eventually, to a solid model.

Since extruded dots or numbers on the surface of the solid models may be used by blind researchers and students to differentiate atom types, the second option seems more viable. This procedure can be done once the MEP calculated using SPARTAN can be converted into a mathematical function.

5.3 The Possibility of Building Models of Reaction Intermediates

Using stereolithography to build molecular models has the potential to create models of chemical species that are either difficult or impossible to isolate, for example, a reaction intermediate. The structure of such a species can be calculated using quantum mechanics methods. The results of the quantum mechanics calculations can then be used as input for a CAD program, and in turn, an SLA can be used to build such a model.

5.4 Using SLA Molecular Models as Learning Aides

Molecular models generated from an SLA have the potential to be used as instructional aids. SLA molecular models can be used in high school or college chemistry classes to more accurately depict the structures of molecules. Both blind and sighted students can benefit from inspecting SLA

molecular models. Sighted students can use these models to better understand topics such as stereochemistry and chemical bonding. Blind students can use SLA models to introduce themselves to chemistry and to better understand molecular structure and properties.

5.5 Conclusion

Molecular models created using stereolithography technology have been shown to serve many purposes. These models present a novel use for the SLA, which is normally used in the field of manufacturing and engineering. SLA-created molecular models can help to bridge the communication gap between blind and sighted individuals. Finally, these models also provide an accurate representations of molecules and molecular properties.

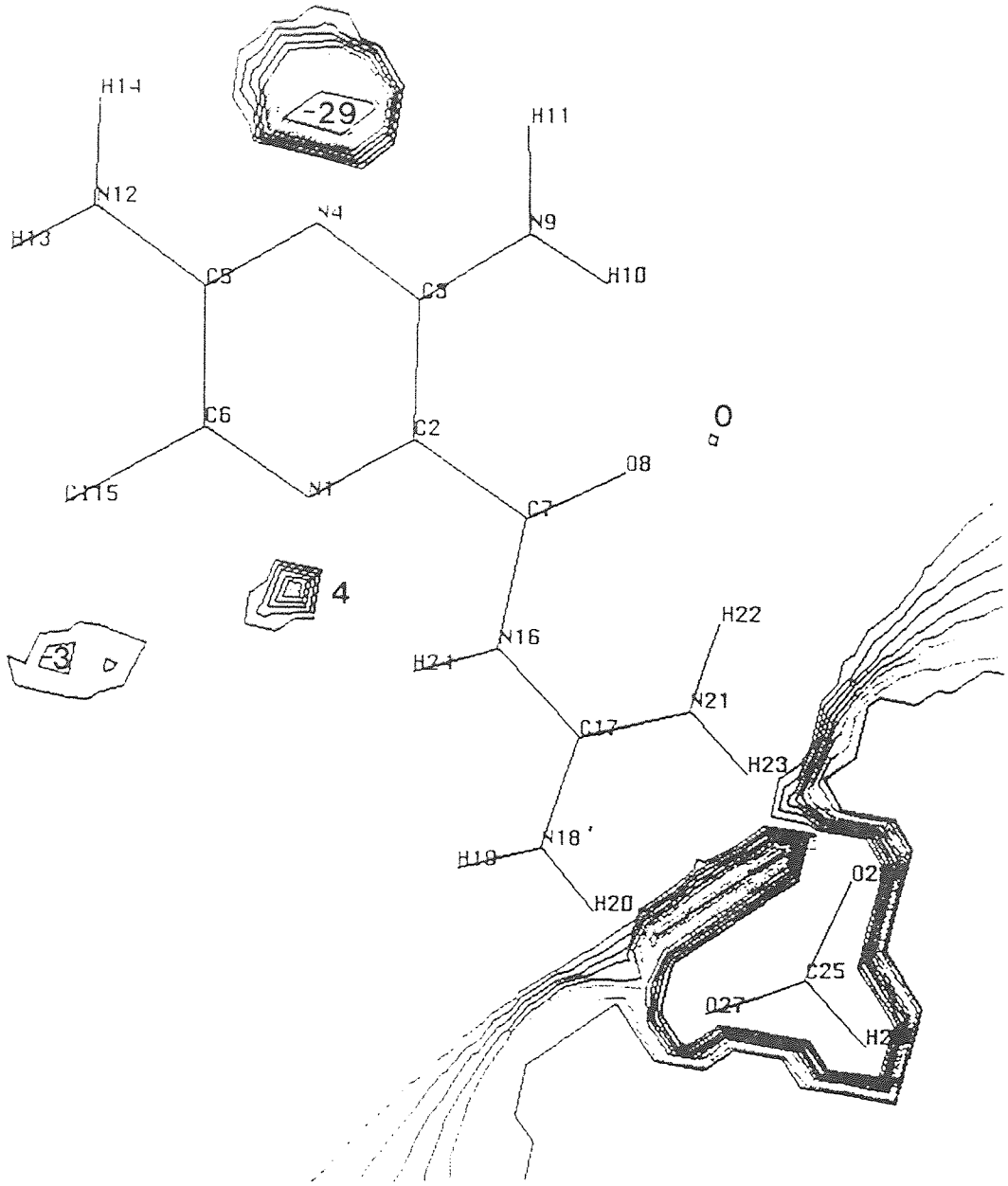

Figure 3 Molecular Electrostatic Potential (MEP) map of Amiloride, 1.

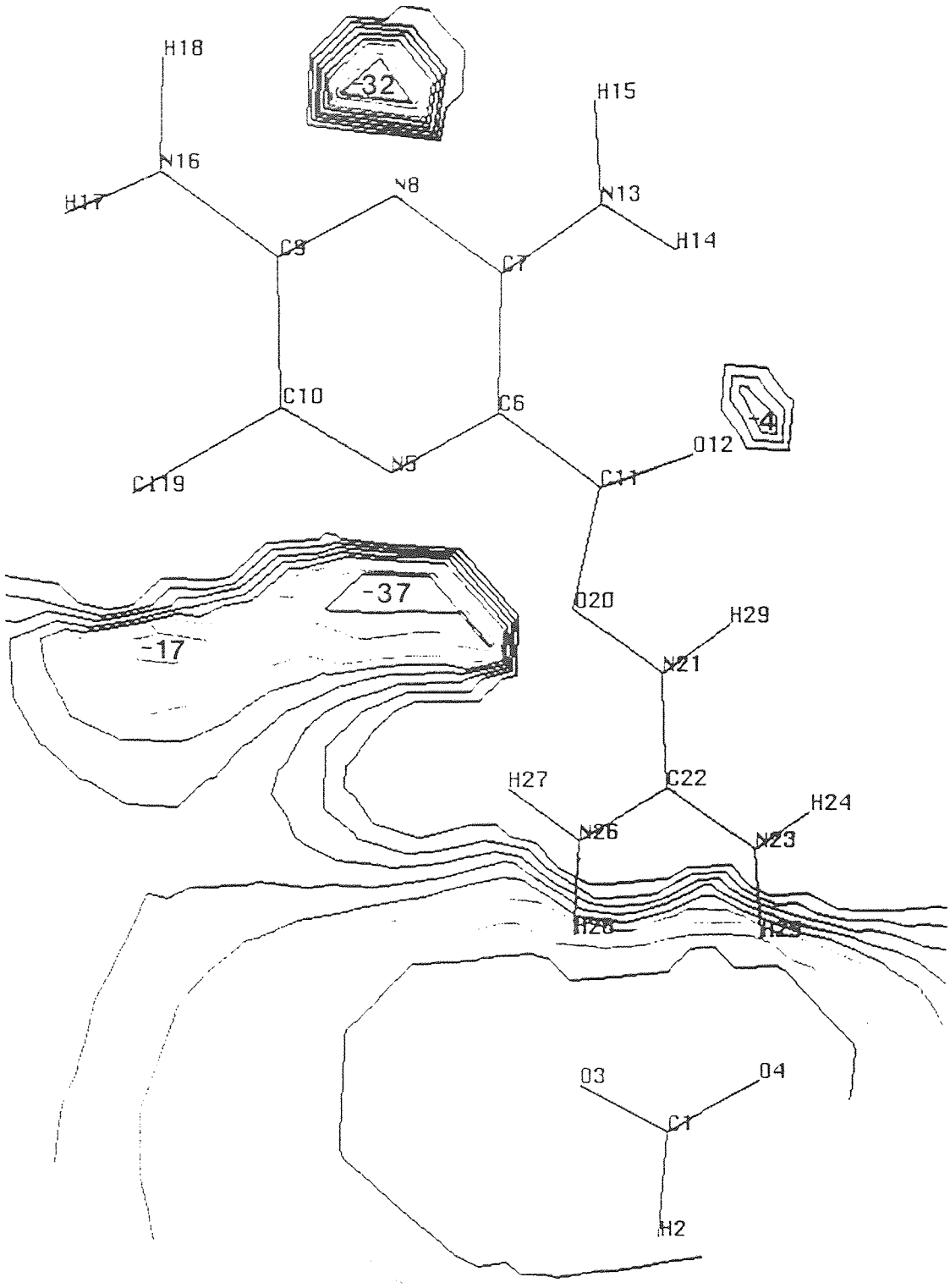

Figure 4 Molecular Electrostatic Potential (MEP) map of Analogue 18.

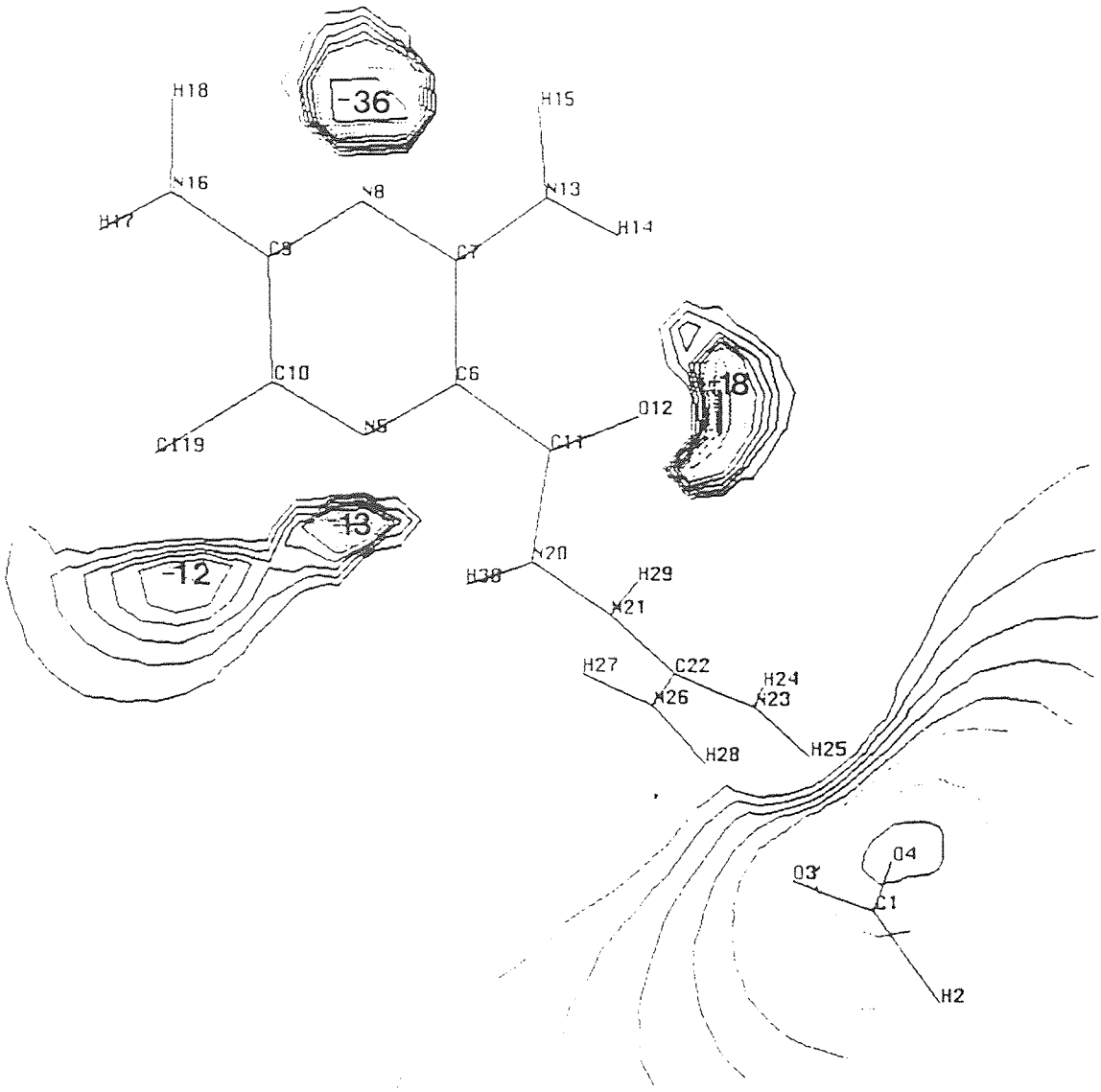

Figure 5 Molecular Electrostatic Potential (MEP) map of Analogue 19.

APPENDIX B

Table 3 3-21G* calculation results for the structure of amiloride, **1**, calculated by Carol A. Venanzi

Atom Symbol	Radius (Angstroms)	Cartesian Coordinates (Angstroms)		
N	1.5	-0.558990	-0.596026	0.000000
C	1.85	0.000000	0.620711	0.000000
C	1.85	-0.836512	1.748303	0.000000
N	1.5	-2.165850	1.587738	0.000000
C	1.85	-2.696165	0.378951	0.000000
C	1.85	-1.834849	-0.753773	0.000000
C	1.85	1.427322	0.698081	0.000000
O	1.4	2.095830	1.726515	0.000000
N	1.5	-0.382405	2.994140	0.000000
H	1.2	0.596731	3.188547	0.000000
H	1.2	-1.048218	3.738314	0.000000
N	1.5	-4.015235	0.265634	0.000000
H	1.2	-4.483780	-0.614173	0.000000
H	1.2	-4.559027	1.103909	0.000000
Cl	1.8	-2.510845	-2.360346	0.000000
N	1.5	2.044884	-0.565695	0.000000
C	1.85	3.369594	-0.797105	0.000000
N	1.5	3.780127	-2.057479	0.000000
H	1.2	3.134449	-2.820115	0.000000
H	1.2	4.751420	-2.292090	0.000000
N	1.5	4.224112	0.197964	0.000000
H	1.2	3.859686	1.138590	0.000000
H	1.2	5.211500	0.047375	0.000000
H	1.2	1.392126	-1.331544	0.000000

Table 4 3-21G* calculation results for the structure of analogue **18**, calculated by William J. Skawinski

Atom Symbol	Radius (Angstroms)	Cartesian Coordinates (Angstroms)		
N	1.5	-0.794420	-0.528839	-0.000035
C	1.85	-0.453204	0.765955	-0.000034
C	1.85	-1.471319	1.732733	0.000011
N	1.5	-2.753179	1.345751	0.000052
C	1.85	-3.067516	0.063725	0.000051
C	1.85	-2.024085	-0.903839	0.000005
C	1.85	0.918814	1.125315	-0.000090
O	1.4	1.436791	2.219506	-0.000099
N	1.5	-1.243073	3.040464	0.000016
H	1.2	-0.316198	3.410203	-0.000013
H	1.2	-2.030946	3.653862	0.000049
N	1.5	-4.347797	-0.272919	0.000092
H	1.2	-4.656545	-1.220698	0.000094
H	1.2	-5.027397	0.459300	0.000123
Cl	1.8	-2.413460	-2.602790	0.000002
O	1.4	1.755678	-0.005750	-0.000142
N	1.5	3.125298	0.368758	-0.000199
C	1.85	3.955309	-0.657378	0.000031
N	1.5	5.264544	-0.432547	0.000294
H	1.2	5.640047	0.493411	0.000105
H	1.2	5.920815	-1.185615	0.000562
N	1.5	3.463186	-1.878330	-0.000116
H	1.2	2.467819	-1.999376	-0.000186
H	1.2	4.046584	-2.688052	-0.000262
H	1.2	3.294978	1.358924	0.000855

Table 5 3-21G* calculation results for the structure of analogue **19**, as calculated by William J. Skawinski

Atom Symbol	Radius (Angstroms)	Cartesian Coordinates (Angstroms)		
N	1.5	-0.953274	-0.630026	-0.158261
C	1.85	-0.373837	0.576767	-0.129558
C	1.85	-1.184473	1.711537	0.032868
N	1.5	-2.510089	1.567480	0.154983
C	1.85	-3.060845	0.368270	0.123958
C	1.85	-2.225630	-0.771918	-0.041313
C	1.85	1.055105	0.645549	-0.263596
O	1.4	1.735542	1.659198	-0.258317
N	1.5	-0.703452	2.949571	0.073647
H	1.2	0.277136	3.117982	-0.012246
H	1.2	-1.346517	3.703351	0.191990
N	1.5	-4.378598	0.272956	0.248785
H	1.2	-4.860414	-0.599034	0.234353
H	1.2	-4.901847	1.116010	0.361563
Cl	1.8	-2.928943	-2.366112	-0.085438
N	1.5	1.674555	-0.604598	-0.380830
N	1.5	3.009217	-0.600238	-0.776078
C	1.85	3.960275	-0.595120	0.160188
N	1.5	5.222330	-0.423888	-0.203451
H	1.2	5.477587	-0.258352	-1.155211
H	1.2	5.966442	-0.436628	0.462824
N	1.5	3.631503	-0.791822	1.416998
H	1.2	2.666233	-0.909998	1.652431
H	1.2	4.306585	-0.801180	2.152241
H	1.2	3.219094	-0.343480	-1.720000
H	1.2	1.134478	-1.424914	-0.574784

Table 6 6-31G* calculation results for the structure of analogue **18**, calculated by William J. Skawinski

Atom Symbol	Radius (Angstroms)	Cartesian Coordinates (Angstroms)		
N	1.5	-0.786356	-0.525299	-0.137047
C	1.85	-0.415595	0.759229	-0.063253
C	1.85	-1.406530	1.739705	0.103876
N	1.5	-2.692475	1.375595	0.185665
C	1.85	-3.036160	0.103339	0.110529
C	1.85	-2.020029	-0.878345	-0.058892
C	1.85	0.982717	1.075131	-0.181025
O	1.4	1.507480	2.147068	-0.141838
N	1.5	-1.156777	3.043625	0.189796
H	1.2	-0.231649	3.403019	0.139000
H	1.2	-1.926051	3.663457	0.307874
N	1.5	-4.321988	-0.210852	0.196428
H	1.2	-4.643741	-1.149544	0.146922
H	1.2	-4.984510	0.522868	0.314623
Cl	1.8	-2.448406	-2.564804	-0.160855
O	1.4	1.752200	-0.040266	-0.388743
N	1.5	3.086745	0.238689	-0.451942
C	1.85	3.869081	-0.682761	0.103924
N	1.5	5.165145	-0.432424	0.200354
H	1.2	5.563068	0.391050	-0.196462
H	1.2	5.798834	-1.114666	0.554822
N	1.5	3.354149	-1.823148	0.501810
H	1.2	2.370407	-1.978517	0.423242
H	1.2	3.928148	-2.571388	0.822683
H	1.2	3.290955	1.219879	-0.419908

Table 7 6-31G* calculation results for the structure of analogue **19**, calculated by William J. Skawinski

Atom Symbol	Radius (Angstroms)	Cartesian Coordinates (Angstroms)		
N	1.5	-0.963435	-0.628789	-0.150418
C	1.85	-0.382394	0.577042	-0.114562
C	1.85	-1.192538	1.712709	0.044004
N	1.5	-2.519285	1.570438	0.155452
C	1.85	-3.071616	0.372152	0.117515
C	1.85	-2.236880	-0.768966	-0.043716
C	1.85	1.061949	0.626127	-0.231759
O	1.4	1.739414	1.619935	-0.218794
N	1.5	-0.720406	2.956251	0.092665
H	1.2	0.253936	3.138663	0.016590
H	1.2	-1.366475	3.703746	0.207407
N	1.5	-4.391846	0.278981	0.231999
H	1.2	-4.866546	-0.593323	0.211059
H	1.2	-4.919613	1.115656	0.342467
Cl	1.8	-2.942264	-2.361971	-0.096805
N	1.5	1.694113	-0.621127	-0.330140
N	1.5	2.994415	-0.593707	-0.769246
C	1.85	3.977250	-0.588309	0.139601
N	1.5	5.209813	-0.329424	-0.258951
H	1.2	5.416056	-0.123598	-1.211784
H	1.2	5.976960	-0.339911	0.376275
N	1.5	3.717250	-0.891456	1.389315
H	1.2	2.772182	-1.047334	1.667858
H	1.2	4.433238	-0.937182	2.079810
H	1.2	3.165106	-0.099873	-1.621543
H	1.2	1.159365	-1.395510	-0.663341

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