ABSTRACT

INCLUSION COMPOUNDS OF LEUCYL-ALANINE

Abdolreza Yazdani  Advisor:  
University of Guelph, 2011  Professor D. V. Soldatov

The ability of L-leucyl-L-alanine dipeptide (Leu-Ala) to form inclusion compounds with ~20 guest molecules was studied using preparation, thermal analysis (TGA and DSC), X-ray diffraction (single crystal and powder), spectroscopy (FT-IR), and other methods. Inclusion compounds with unsubstituted pyridine, ten pyridine derivatives, quinoline, isoquinoline and guaiacol were prepared for the first time and their composition, thermal stability and dissociation mechanisms were studied. The crystal structures and bulk properties of inclusions with three methylpyridine isomers were investigated. The T/X phase diagrams of the systems Leu-Ala – DMSO and Leu-Ala – guaiacol were determined to reveal the conditions of formation, limits of existence and modes of decomposition of the inclusion phases which form as 1:1 binary compounds in the systems. The irreversible cyclization of the Leu-Ala host molecule when the molecule is a part of various inclusion compounds or a guest-free solid phase of the dipeptide was studied. The inclusion of water in Leu-Ala was studied by the determination of the sorption isotherm at 298 K and crystal phase analysis of the sorption products. This work presents the first comprehensive study on the clathration ability of a layered peptide matrix as a host material.
Acknowledgments

First and foremost, I am heartily thankful to my supervisor, Dr. Dmitriy Soldatov, for giving me the opportunity to work in his laboratory and carry out my master project under his supervision. His encouragement, guidance and support from the beginning to end of my project over the last two years has enabled me to develop an understanding of the subject.

I am also grateful to Dr. James Britten and Dr. Mark Baker for their advice and support during the last two years and Dr. Dan Thomas for reading my thesis.

Thanks to all past and present group members that I have had the pleasure to work with, including Emeka Okeke, Chris Brown, David Ben-Israel, and Julia Crewson. I am especially thankful to Vadim Chirmanov who helped me in phase diagrams determinations.

Last but not the least, I would like to thank my family for all their love and encouragement. I offer my regards and blessings to all of those who supported me in any respect during the completion of the project. Thank you.
# Table of Contents

<table>
<thead>
<tr>
<th>Chapter One: Introduction into Supramolecular Chemistry and Inclusion Compounds</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Supramolecular chemistry</td>
<td>2</td>
</tr>
<tr>
<td>1.2 Host – guest chemistry</td>
<td>2</td>
</tr>
<tr>
<td>1.3 Crystalline inclusion compounds</td>
<td>6</td>
</tr>
<tr>
<td>1.4 Molecular crystals</td>
<td>9</td>
</tr>
<tr>
<td>1.5 References</td>
<td>13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter Two: Using Peptides in the Design of New Host Materials</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Peptides as host materials</td>
<td>16</td>
</tr>
<tr>
<td>2.2 Hosts with tubular cavity space</td>
<td>18</td>
</tr>
<tr>
<td>2.2.1 Urea and thiourea</td>
<td>18</td>
</tr>
<tr>
<td>2.2.2 Zeolites</td>
<td>19</td>
</tr>
<tr>
<td>2.2.3 Organic zeolites</td>
<td>21</td>
</tr>
<tr>
<td>2.2.4 Peptide nanotubes and porous solids</td>
<td>23</td>
</tr>
<tr>
<td>2.3 Layered host solids</td>
<td>30</td>
</tr>
<tr>
<td>2.3.1 Layered solids and intercalates</td>
<td>30</td>
</tr>
<tr>
<td>2.3.2 Graphite</td>
<td>30</td>
</tr>
<tr>
<td>2.3.3 Clays</td>
<td>33</td>
</tr>
<tr>
<td>2.3.4 Organic clays</td>
<td>39</td>
</tr>
<tr>
<td>2.3.5 Bile acids</td>
<td>43</td>
</tr>
<tr>
<td>2.3.6 Layered peptide inclusion compounds</td>
<td>44</td>
</tr>
<tr>
<td>2.4 Research direction</td>
<td>48</td>
</tr>
<tr>
<td>2.5 References</td>
<td>52</td>
</tr>
</tbody>
</table>
Chapter Three: Experimental

3.1 Chemicals.................................................................57

3.2 Preparation and characterization procedures..............................58

3.2.1 Synthesis and characterization of inclusion compounds........58

3.2.1.1 Crystallization and characterization methods..............58

3.2.1.2 Immersion method.............................................60

3.2.1.3 Isopiestic method.............................................61

3.2.2 Determination of phase diagrams.................................64

3.2.2.1 Leu-Ala – DMSO phase diagram..............................70

3.2.2.2 Leu-Ala – guaiacol phase diagram.............................76

3.3 Instrumental methods and equipment..................................77

3.3.1 Thermal analysis (TGA and DSC)..................................77

3.3.2 Visual observations..................................................79

3.3.3 FT/IR spectroscopy..................................................80

3.3.4 X-Ray diffraction analysis...........................................80

3.4 References........................................................................82

Chapter Four: Clathrate Formation in the LA – DMSO and LA – Guaiacol Binary Systems

4.1 Phase diagram of the system LA – DMSO and the properties of LA*DMSO inclusion compound........................................84

4.1.1 Description of the phase diagram...................................84

4.1.2 Irreversible degradation of LA......................................88

4.1.3 Isolation and properties of the LA*DMSO inclusion compound .................................................................90
4.2 Phase diagram of the system LA – guaiacol and the properties of LA*guaiacol inclusion compound.................................98

4.2.1 Description of the phase diagram.................................98

4.2.2 Isolation and properties of the LA*guaiacol inclusion compound.................................................................101

4.3 Conclusion......................................................................103

4.4 References.......................................................................106

Chapter Five: Clathration Ability of LA with Respect to Aromatic Guest Molecules.....108

5.1 Inclusion compounds of Leu-Ala with various guests.................108

5.2 Clathrates of Leu-Ala with methylpyridines........................112

5.2.1 Crystal structures........................................................112

5.2.2 Formation and stability...............................................120

5.3 References.......................................................................126

Chapter Six: The Formation of Tetrahydrate in the System LA – Water ............128

6.1 Properties of the Leu-Ala tetrahydrate................................128

6.2 Sorption isotherm of water by solid LA............................131

6.3 References.......................................................................138

Chapter Seven: Future Directions.............................................140
### List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>Idealized formulas for some selected clays</td>
<td>35</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>Temperatures (°C) and relative magnitudes of thermal effects of phase transitions in the system LA – DMSO registered by DSC</td>
<td>66</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>Temperatures (°C) and relative thermal effects of phase transitions in the system LA – guaiacol registered by DSC</td>
<td>68</td>
</tr>
<tr>
<td>Table 3.3</td>
<td>The compositions of liquid and solid phases (% LA) in the LA – DMSO system equilibrated at three temperatures (three determinations for each value)</td>
<td>75</td>
</tr>
<tr>
<td>Table 3.4</td>
<td>The compositions of liquid and solid phases (% LA) in the LA – guaiacol system equilibrated at three temperatures (three determinations for each value)</td>
<td>76</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Temperatures of incongruent melting of the inclusion compound LA*DMSO registered in DSC experiments with various heating rates</td>
<td>85</td>
</tr>
<tr>
<td>Table 5.1</td>
<td>Inclusion compounds prepared by crystallization and/or immersion methods and their properties from TGA experiments</td>
<td>113</td>
</tr>
<tr>
<td>Table 5.2</td>
<td>Crystal data for Leu-Ala inclusions with methylpyridine isomers</td>
<td>117</td>
</tr>
<tr>
<td>Table 5.3</td>
<td>Interlayer distance in the studied inclusion compounds</td>
<td>119</td>
</tr>
<tr>
<td>Table 6.1</td>
<td>Sorption of water by dry LA powder samples as a function of partial water vapor pressure: the results of isopiestic and powder XRD experiments (298 K)</td>
<td>133</td>
</tr>
</tbody>
</table>
### List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>The guest methanol molecule (CH$_3$OH) in the hydroquinone cage. A fragment of the crystal structure of the 3[C$_6$H$_4$(OH)$_2$]*CH$_3$OH clathrate.</td>
</tr>
<tr>
<td>1.2</td>
<td>Complex of [2.2.2] cryptand with potassium cation, N(CH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$)$_3$N*K$^+$</td>
</tr>
<tr>
<td>2.1</td>
<td>Topologies of zeolites. a. Sodalite type, b. Linde type, c. Faujasite type, d. AlPO$_4$-5, e. ZSM-5. The vertices present the positions of AlO$_4$ and SiO$_4$ tetrahedra.</td>
</tr>
<tr>
<td>2.2</td>
<td>a. Distorted diamond topology in 3D structure of [Ni(NCS)$_2$(4-MePy)$_4$]*(C$_6$H$_6$). Small cavities are represented by rods. b. Hexagonal molecular structure of a macrocycle used in the design of organic zeolites.</td>
</tr>
<tr>
<td>2.3</td>
<td>a. Molecular structure of eight-residue cyclic peptide ring. b. Peptide rings self-assemble to form a tubular configuration. The peptide side chains stand outside of the hollow tubular structure.</td>
</tr>
<tr>
<td>2.4</td>
<td>a. Spiral assembly of AV (left) and VA (right) dipeptide molecules that create a channel. b. The channel shape in AV and VA shown as a set of inscribed disks threaded on a sixfold screw axis.</td>
</tr>
<tr>
<td>2.5</td>
<td>Spiral H-bonding assembly of VV and LS that forms a nanotube (top). Schematic illustration of the packing of peptide nanotubes into the crystal structure.</td>
</tr>
<tr>
<td>2.6</td>
<td>Illustration of pore space in eight hydrophobic dipeptides. The van der Waals outlines for He and Xe are given on the same scale for comparison.</td>
</tr>
<tr>
<td>2.7</td>
<td>Expected crystal structure of graphite.</td>
</tr>
<tr>
<td>2.8</td>
<td>In-plane structure model for the inclusion of graphite with alkali metal atoms (MC$_8$). Circles denote the intercalated atoms (ions) which are superimposed on the graphite hexagonal layer.</td>
</tr>
<tr>
<td>2.9</td>
<td>Structure of 1:1 and 2:1 layers in clays, where X (shaded circles) is usually OH and M can be Al, Mg, Fe, etc.</td>
</tr>
<tr>
<td>2.10</td>
<td>Montmorillonite network with the hydrated cations intercalated between the aluminosilicate layers.</td>
</tr>
<tr>
<td>2.11</td>
<td>The principle structure of the pillared clay.</td>
</tr>
<tr>
<td>2.12</td>
<td>Preparation of a pillared clay.</td>
</tr>
<tr>
<td>2.13</td>
<td>Calixarene (left) and calix[4]arene (right).</td>
</tr>
</tbody>
</table>
Figure 2.14. The bilayer structure of Na₅[calix[4]arene sulfonate]*12H₂O. The lines are least-squares best planes of the aromatic carbon atoms bonded to the -SO₃ groups……39

Figure 2.15. Deprotonation of H₂PzDCA by primary amine (R=H, R’=benzyl) and secondary amine (R=R’=benzyl)………………………………………………………………………………41

Figure 2.16. Hydrogen-bonded sheet in HPzDCA (left). ABA pattern (middle). AB pattern (right)………………………………………………………………………………………………42

Figure 2.17. Molecular structure of two bile acids: deoxycholic acid if R₁=H and R₂=OH; cholic acid if R₁=OH and R₂=OH (left). Deoxycholic acid host framework along the channels (right)………………………………………………………………………………44

Figure 2.18. Typical molecular arrangement of an inclusion compound with Leu-Ala….45

Figure 2.19. Layered structure of inclusion crystals of Leu-Ala with various sulfoxides. The arrows indicate the arrangement of dipeptide molecules (from the C-terminal to the N-terminal). Leu-Ala with isobutyl methyl sulfoxide (top), dimethyl sulfoxide (middle), and benzyl methyl sulfoxide (bottom)………………………………………………………47

Figure 3.1. Water sorption by LA dipeptide under two different partial pressures of water: 88.0% (squares) and 75.1% (triangles)…………………………………………………………63

Figure 3.2. DSC thermograms of DMSO samples from old bottles (top and middle) and a new bottle kept sealed in a dry glove box (bottom). The observed differences in the melting point are due to traces of water absorbed from the atmosphere…………71

Figure 3.3. Apparent decrease in the melting temperature of DMSO when samples of different size are used. The size of the samples varies from 10.63 mg (top) to 2.22 mg (bottom). The decrease is not real and is caused by the massive high-pressure capsule…72

Figure 3.4. SEM images showing the shape and dimensions of crystals of solid Leu-Ala*DMSO present in the mixture with saturated liquor at room temperature……74

Figure 3.5. A typical TGA thermogram of saturated liquor in the system LA – DMSO. The plateau due to the solid LA residue is enlarged and shown in the insert………………75

Figure 4.1. Phase diagram of the system LA – DMSO shown only for the 0-200°C temperature range. Solid circle points are obtained from DSC experiments. Typical error is 0.5-0.8°C. Error bars are shown when the error was larger due to irregular shape of the DSC peak. Open circles: points obtained from the solubility experiments. For the values and errors see Tables 3.1 and 3.3………………………………………………87

Figure 4.2. Selected DSC thermograms of samples with different LA/DMSO ratios…….89

Figure 4.3. Layered structure of inclusion crystal of Leu-Ala with DMSO………………91
Figure 4.4. Crystal shape of LA*DMSO inclusion compound (microscope). Image size is 10 mm by 7.5 mm ..........................................................92

Figure 4.5. Thermal dissociation of the LA*DMSO clathrate. Dashes show the positioning of expected plateaus for the indicated decomposition products……………93

Figure 4.6. 3D FT-IR spectrum of the gaseous products released in the course of the LA*DMSO TG analysis........................................................................95

Figure 4.7. IR spectra of gases evolved in a TGA decomposition of LA*DMSO sample (in grey). The spectra correspond to DMSO (a), H2O (b) and cyclic LA (c). Spectra from a library (in black) are shown for comparison. In case of cyclic LA, the binary system is for solid material and so it shows several extra lines such as broad lines in the 3200-3500 cm⁻¹ range due to stretching vibrations of N-H groups involved in hydrogen bonding….96

Figure 4.8. DSC curve for the LA*DMSO clathrate........................................97

Figure 4.9. Phase diagram of the system LA – guaiacol shown only for the 0-200°C temperature range. Solid circle points are obtained from DSC experiments. Typical error is 0.5-0.8°C. Error bars are shown when the error was larger due to irregular shape of the DSC peak. Open circles: points obtained from the solubility experiments. For the values and errors see Tables 3.2 and 3.4.................................................................99

Figure 4.10. Thermal dissociation of LA*guaiacol. Calculated compositions are shown by dashes...............................................................102

Figure 4.11. DSC curve for LA*guaiacol clathrate........................................103

Figure 5.1. Crystals obtained by crystallization of Leu-Ala from different solvents: top left: DMSO; top right: 2-methylpyridine; bottom left: 3-methylpyridine; bottom right: 4-methylpyridine...............................................................114

Figure 5.2. ORTEP projections of the guest and host molecules in the crystal structures of Leu-Ala*2-methylpyridine (top), Leu-Ala*3-methylpyridine (middle), Leu-Ala*4-methylpyridine (bottom). H-atoms are omitted..........................116

Figure 5.3. A fragment of crystal packing of Leu-Ala*4-MePy along the a-axis. The arrows point C-terminal end to N-terminal end (top). Spacefill projection of Leu-Ala*4-MePy crystal packing (bottom)..............................................118

Figure 5.4. Overlay of TGA thermograms of LA inclusion compounds with methylpyridine isomers. Dashes show the positioning of expected plateaus for the indicated decomposition products........................................122

Figure 5.5. DSC thermograms of LA and its inclusions with pyridine and methylpyridines.................................................................123
Figure 5.6. Sorption of pyridine and methylpyridines by LA as a function of time........125

Figure 6.1. Crystal packing in Leu-Ala*4H₂O along the a axis.................................129

Figure 6.2. Crystals of Leu-Ala*4H₂O under microscope. Image size is 10 mm by 7.5 mm......................................................................................................................130

Figure 6.3. P/X dependence of H₂O:LA molar ratio (X) on the partial water vapor pressure (P/P₀) over solid LA at 298 K. Circular and square points represent types I and II powder diffraction patterns, respectively. Solid points were obtained using sulfuric acid solutions and open circle points were obtained using saturated salt solutions (see Table 6.1). Error bars do not account for a possible systematic error due to surface sorption of water on the LA and LA*4H₂O phases..............................132

Figure 6.4. Powder X-ray diffraction patterns of totally dehydrated Leu-Ala, Pattern I (a); inclusion compound of Leu-Ala with H₂O obtained from sorption experiment, Pattern II (b); powdered crystals of LA*4H₂O (c)..................................................134
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-MePy</td>
<td>2-methylpyridine</td>
</tr>
<tr>
<td>3-MePy</td>
<td>3-methylpyridine</td>
</tr>
<tr>
<td>4-MePy</td>
<td>4-methylpyridine</td>
</tr>
<tr>
<td>AI</td>
<td>Alanyl-isoleucine (L,L isomer)</td>
</tr>
<tr>
<td>Ala-Ile</td>
<td>Alanyl-isoleucine (L,L isomer)</td>
</tr>
<tr>
<td>Ala-Val</td>
<td>Alanyl-valine (L,L isomer)</td>
</tr>
<tr>
<td>AV</td>
<td>Alanyl-valine (L,L isomer)</td>
</tr>
<tr>
<td>Cyclo-Leu-Ala</td>
<td>Cyclic leucyl-alanine (L,L isomer)</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>IA</td>
<td>Isoleucyl-alanine (L,L isomer)</td>
</tr>
<tr>
<td>Ile-Ala</td>
<td>Isoleucyl-alanine (L,L isomer)</td>
</tr>
<tr>
<td>Ile-Val</td>
<td>Isoleucyl-valine (L,L isomer)</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>IV</td>
<td>Isoleucyl-valine (L,L isomer)</td>
</tr>
<tr>
<td>LA</td>
<td>Leucyl-alanine (L,L isomer)</td>
</tr>
<tr>
<td>Leu-Ala</td>
<td>Leucyl-alanine (L,L isomer)</td>
</tr>
<tr>
<td>Leu-Leu</td>
<td>Leucyl-leucine (L,L isomer)</td>
</tr>
<tr>
<td>Leu-Leu-Leu</td>
<td>Leucyl-leucyl-leucine (L,L,L isomer)</td>
</tr>
<tr>
<td>Leu-Ser</td>
<td>Leucyl-serine (L,L isomer)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>LS</td>
<td>Leucyl-serine (L,L isomer)</td>
</tr>
<tr>
<td>MePy</td>
<td>Methylpyridine</td>
</tr>
<tr>
<td>$P_0$</td>
<td>Pressure of water vapor over pure water</td>
</tr>
<tr>
<td>P/X</td>
<td>Pressure/concentration</td>
</tr>
<tr>
<td>PXRD</td>
<td>Powder X-ray diffraction</td>
</tr>
<tr>
<td>Py</td>
<td>Pyridine</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>TG</td>
<td>Thermogravimetry, thermogravimetric</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric analysis</td>
</tr>
<tr>
<td>TO</td>
<td>Tetrahedral-octahedral</td>
</tr>
<tr>
<td>TOT</td>
<td>Tetrahedral-octahedral-tetrahedral</td>
</tr>
<tr>
<td>T/X</td>
<td>Temperature/concentration</td>
</tr>
<tr>
<td>VA</td>
<td>Valyl-alanine (L,L isomer)</td>
</tr>
<tr>
<td>Val-Ala</td>
<td>Valyl-alanine (L,L isomer)</td>
</tr>
<tr>
<td>Val-Ile</td>
<td>Valyl-isoleucine (L,L isomer)</td>
</tr>
<tr>
<td>Val-Val</td>
<td>Valyl-valine (L,L isomer)</td>
</tr>
<tr>
<td>VI</td>
<td>Valyl-isoleucine (L,L isomer)</td>
</tr>
<tr>
<td>VV</td>
<td>Valyl-valine (L,L isomer)</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
</tr>
</tbody>
</table>
Molecular structure of compounds used in this work:

2-ethylpyridine

\[
\text{CH}_3
\]

2,4,6-trimethylpyridine

\[
\text{CH}_3
\]

2-methylpyridine

\[
\text{CH}_3
\]

3,4-dimethylpyridine

\[
\text{CH}_3
\]

3,5-dimethylpyridine

\[
\text{CH}_3
\]

3-methylpyridine

\[
\text{CH}_3
\]

4-benzylpyridine

\[
\text{CH}_3
\]

4-ethylpyridine
4-methylpyridine

4-pyridinepropanol

Cyclic leucyl-alanine

Dimethylsulfoxide (DMSO)

Guaiacol

Isoquinoline

Leucyl-alanine (Leu-Ala)
Leucyl-leucine (Leu-Leu)

Leucyl-leucyl-leucine (Leu-Leu-Leu)

Pyridine

Quinoline
Chapter One

Introduction into

Supramolecular Chemistry and

Inclusion Compounds
1. Introduction into Supramolecular Chemistry and Inclusion Compounds

1.1 Supramolecular chemistry

Supramolecular chemistry has been defined by Jean-Marie Lehn, who won a Nobel Prize for his work in the area in 1987, as the "chemistry of molecular assemblies and of the intermolecular bond". Another of his well-known definitions is "chemistry beyond the molecule". While traditional chemistry focuses on the covalent bond, supramolecular chemistry examines the weaker non-covalent interactions between molecules, such as hydrogen bonds and van der Waals interactions. The study of non-covalent interactions is crucial in understanding various physical, chemical, and biological processes, such as the structure and function of complex polymolecular assemblies in living organisms.

Supramolecular chemistry has opened new opportunities for materials science by introducing novel principles on how chemical compounds can be generated. One of these principles is the inclusion of chemical species inside cavities formed by other species, as exploited in host-guest chemistry.

1.2 Host – guest chemistry

Historically the emergence of supramolecular chemistry was preceded by the development of host-guest chemistry which currently remains one of its major subdisciplines.
Host-guest chemistry studies inclusion compounds, where chemical species of two types, the "host" and "guest", associate with each other by means of non-covalent interactions. The nature of inclusion compounds remained a puzzle for a long time until crystal structure studies by X-ray diffraction methods became possible.

In 1947-1948 Herbert Powell reported the crystal structure of inclusion compounds of hydroquinone with SO₂ and other gases. In a major work published in 1948, Powell introduced the term "clathrate" and formulated a new principle according to which the formation of compounds between two components may occur due to their spatial complementarity rather than at the expense of chemical bonds.² The term "clathrate" is derived from the Latin word "clathratus" which means "enclosed or protected by the cross bars of a grating". Powell discussed examples in which either two or more identical molecules interpenetrate and enclose each other, or when molecules of one kind create a cage structure of suitable form that imprisons molecules of a second kind to give compounds. He suggested the word "clathrate" to indicate the situation where the molecule is completely enclosed by the host and cannot escape from its surroundings.

The cage structure of hydroquinone clathrate studied by Powell and coworkers is illustrated in Figure 1.1.³ ⁴ The host framework comprises two interpenetrating giant “molecules” of hydrogen-bonded quinol units. The methanol molecules are imprisoned in the cavities existing between these two giant “molecules”.

³

³
Although many inclusion compounds have been prepared and studied since the first reports by A. Cronstedt (described the first zeolite mineral in 1756) and J. Priestly (reported the first clathrate hydrate in 1777), Powell’s work explained the nature of these compounds, triggered intense experimental research by many groups in the world, and contributed to the development of supramolecular chemistry. A good insight into the scope of experimental work conducted in the second part of the twentieth century is given in reviews on inclusion compounds of graphite, zeolites, hydroquinone, urea and thiourea, cholic acid, cyclodextrins, metal complexes, and reviews on general concepts such as nature, structure, stoichiometry, and systematization of inclusion compounds.

In 1967, Charles Pedersen isolated and reported the first molecular receptor, called “crown-ether.” The crown-ethers molecules became the first ligands to form coordination compounds with the cations of alkali metals. The structure and stability of such complexes is
governed by both chemical interaction and spatial complementarity between the host (ligand) and guest (metal cation). Jean-Marie Lehn and Donald Cram introduced other molecular receptors: cryptands, molecules surrounding their cavity from three sides (Figure 1.2), and spherands, molecules with a rigid geometry of coordination cavity. The importance of these discoveries was recognized by awarding Cram, Lehn and Pedersen the Nobel Prize in Chemistry in 1987 “for their development and use of molecules with structure-specific interactions of high selectivity”. It should be noted that the idea of molecular receptors and their ability to recognize particular species was known before these discoveries. By 1906, Paul Ehrlich devised the concept of molecular receptors by stating that any molecule can only have an effect on the human body if it is bound. In 1894, Emil Fischer proposed a molecular recognition concept which is known as the 'lock and key' principle.

![Figure 1.2. Complex of [2.2.2] cryptand with potassium cation,](image)

\[ \text{N(CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{)}_3\text{N}\ast\text{K}^+ \]
1.3 Crystalline inclusion compounds

Inclusion compounds form as a result of the inclusion of the molecules of one kind (guest) in the cavities of molecules of another kind (host), or in the cavities of a host crystal framework. When the inclusion occurs in the cavity formed by a single molecule (or several molecules), the compound is called a molecular clathrate (discrete system). When the inclusion occurs in the cavities of a crystal framework, the compound is called a cage clathrate (infinite system). This thesis is devoted to cage clathrates (crystalline inclusion compounds) and therefore they will be discussed in more detail.

The formation of an inclusion compound is driven by thermodynamics. For the simplest reaction between a solid host (H) and gaseous guest (G):

$$H_{\text{solid}} + G_{\text{gas}} = [H*G]_{\text{solid}}$$  \[1.1\]

the equilibrium constant (K) is given by:

$$K = [H*G] / [H] [G] = 1/P_G$$  \[1.2\]

That is, the constant is inversely proportional to the partial pressure of the guest over the solid host – solid clathrate mixture.

Free energy of the process ($\Delta G$) is given by:

$$\Delta G = \Delta H - T \Delta S$$  \[1.3\]

The formation of any inclusion compound, a highly organized molecular system, leads to the decrease in entropy, so $\Delta S < 0$. Therefore, in order for the process to occur, $\Delta H$ should be $< 0$ to give an overall negative $\Delta G$. In other words, the clathrate formation process is driven by the enthalpy change and against entropy.
In cage clathrates, host molecules dominate: they form a crystal framework with some cavity space where guest molecules reside. Thus the guest molecule is 'included' in the cavities and is bound to the host by non-covalent interactions; this host-guest interaction requires that the guest molecule is complementary to the cavity space. At the same time, very often the guest species are active participants in the formation of inclusion architectures, and may contribute to a greater or lesser extent to the formation of a particular architecture or may facilitate different crystal structures.\textsuperscript{13}

Classification of inclusion compounds by the topology of the cavity space of the host framework distinguishes three major types: "layer-type" (two dimensionally open), "channel-type" (one-dimensionally open), and "cage-type" (totally enclosed). These three types are also known as "intercalates", "tubulates", and "cryptates" respectively.\textsuperscript{14}

Crystalline inclusion compounds have been prepared with a wide variety of host molecules, from inorganic to metal-organic and purely organic. Among many examples, zeolites, Hoffman and Werner clathrates, urea clathrates, choleic acids, and cyclodextrin inclusion complexes are briefly mentioned here\textsuperscript{7}. Zeolites are robust aluminosilicate microporous solids which have been used as ‘molecular sieves’. The inclusion chemistry of zeolites depends very much on the pore size and other properties of accessible cavities. The MFI class of zeolites is of enormous importance in the petrochemical industry for diffusion shape selectivity in xylene isomerisation. Hoffman- and Werner-type inclusion compounds are polymeric or molecular crystalline solids where crystal lattice voids result from the assembly of metal complexes. Classical Hoffman inclusion compounds have the general
formula M(NH$_3$)$_2$M'(CN)$_4$*2G, where M is an octahedrally coordinated metal cation such as Mn(II)-Zn(II) or Cd(II), M' is square-planar coordinated metal cation such as Ni(II), Pd(II) or Pt(II), and G is a small aromatic molecule. Werner clathrates have a general formula of trans-MX$_2$A$_4$*xG, where M is an octahedrally coordinated metal(II) cation such as Mn(II)-Zn(II), Cd(II), Hg(II), X is a singly charged anionic ligand such as NCS$^-$, NCO$^-$, CN$^-$, NO$_3^-$, NO$_2^-$, Cl$^-$, Br$^-$ or I$^-$, A is a neutral, substituted pyridine, and G is a small guest molecule. Werner clathrates have been used to separate o-, m- and p-isomers of disubstituted benzenes by chromatographic methods. Urea is an example of purely organic host molecule; it forms clathrates with long-chain hydrocarbons. Urea molecules self-assemble via intermolecular hydrogen bonds between the acidic protons of NH$_2$ groups and the oxygen atoms of the adjacent molecules to form hollow tubes in which guests of small cross-section can fit. Bile acids and their derivatives are intermediate between small organic molecules and biopolymers. They form inclusion compounds with a chiral hydrogen-bonded host framework. For example, deoxycholic acid forms channel-type inclusions with a wide variety of guest molecules; these crystalline inclusions are known as “choleic acids”. Versatile host molecules have been employed to mimic the structure and function of biological enzymes. Enzyme mimics require a host which can bond to the guest quickly, selectively, and reversibly to catalyze chemical reactions. Cyclodextrins are among host molecules used for the construction of small molecule enzyme mimics. It should be noted that only few studies are available with peptides as prospective host materials. Peptide solids as host materials are reviewed in Chapter Two.
1.4 Molecular crystals

When the formation of crystalline solids is governed by non-covalent intermolecular interactions, such as in all new materials reported in this thesis, the resulting solids are molecular crystals. The components of a molecular crystal retain their chemical identity although the crystalline solid exhibits a number of new properties not inherent in the initial components. For example, it is possible to stabilize reactive molecules or unusual species in the crystals of inclusion compounds due to so-called supramolecular stabilization, which is kinetic or thermodynamic stabilization in a particular supramolecular environment.\textsuperscript{15} Pharmaceutical products need to be chemically stable at least for two years for marketing. Since the active components of drugs might decompose over time, we can utilize kinetic stabilization to increase lifetime of drugs. In 1988, Tomono and coworkers improved stability of some photosensitive drugs by inclusion in cyclodextrins.\textsuperscript{16} Although weaker interactions such as hydrogen bonds or van der Waals forces are individually insignificant, the cumulative contribution of multiple intermolecular interactions can stabilize unusual or unexpected chemical species; this phenomenon is known as thermodynamic stabilization. The best-known example is the so-called “blue reaction”, the appearance of blue color upon contact of iodine with starch. The structural studies indicate that the guest iodine molecules are included and polymerized in a polyiodide chain inside the helix form of the amylose host (amylose is a component of starch).\textsuperscript{17}

The formation of molecular crystals occurs through the process of molecular self-assembly. Molecular self-assembly is the process by which a group of molecules adopts a
defined well-organized arrangement driven by non-covalent interactions without guidance or management from an outside source. They are usually referred to as "non-covalent" or "weak interactions", as opposed to the thermodynamically and kinetically stable covalent bonds. The forces that can control the process range from the coordination bond to van der Waals interactions. Self-assembly of molecules is usually a spontaneous and reversible process which can be facilitated or reversed by a solvent, pH, or external parameters such as temperature. DNA is an example of a self-assembled supermolecule that is made up of two complementary molecular strands held together by π-π stacking and hydrogen bonding.

The process of self-assembly obeys certain rules dictated by the necessity to minimize the energy of a molecular system, or by symmetry and the periodic nature of crystals. For example, chiral molecules will form a crystal in a chiral space group and the insertion of an asymmetric guest in a symmetric cavity will necessarily result in its disordering. One more example is Etter’s rules for preferential hydrogen-bond patterns in crystals described further in the text.

The term "non-covalent interactions" combines several types of attractive and repulsive forces, some of which are listed below: Coordination bond (typical energy from 200 to 500 kJmol⁻¹), Hydrogen bond (4 to 120 kJmol⁻¹), π-π Interactions (50 kJmol⁻¹) and Van der Waals interactions (10 kJmol⁻¹). Since Hydrogen bond and Van der Waals interactions play an important role for self-assembly of the dipeptide molecules studied in our work, they will be discussed in a greater degree of detail.
Hydrogen bonding is perhaps the most widely utilized intermolecular interaction in supramolecular systems because of its relative strength and directional nature. In this interaction, the hydrogen atom attached to an electronegative atom or electron-withdrawing group (donor) is attracted to another electronegative atom or group (acceptor) of an adjacent molecule. The strength of the bond depends on the nature of 'donor' and 'acceptor'. An excellent example is the formation of carboxylic acid dimers which results in the shift of the \( \nu(\text{OH}) \) infrared stretching frequency from about 3400 cm\(^{-1}\) to about 2500 cm\(^{-1}\), accompanied by a significant broadening and intensifying of the absorption. A strategy for increasing the strength of non-covalent forces is the use of Charge-Assisted Hydrogen Bonds for complementary interactions between moieties. Since the hydrogen bond is mostly electrostatic, an increase in charge on components can increase Coulombic stabilization. A charge-assisted hydrogen bond is an exceptionally strong interaction between the oppositely charged participants. The strength and directionality of hydrogen bonds are two important reasons for their utilization in the reliable design of new molecular crystals in crystal engineering. Etter and coworkers proposed a set of rules to predict a resulting structure pattern in reasonably strongly hydrogen-bonded systems after extensive studies of preferential hydrogen-bond patterns in organic crystals. Etter's rules concerning hydrogen bonded organic compounds have the following general main statements:

1. All good proton donors and acceptors are used in hydrogen bonding.
2. Six-membered-ring intramolecular hydrogen bonds form in preference to intermolecular hydrogen bonds.
3. The best proton donors and acceptors remaining after intramolecular hydrogen bond formation make intermolecular hydrogen bonds to one another.
Van der Waals interactions arise from the polarization of an electron cloud by the proximity of an adjacent atom, resulting in a weak electrostatic interaction. Very often the interactions between permanent dipoles are also regarded as van der Waals. Although the utilization of van der Waals interactions in supramolecular design is less evident, they are very important in host-guest chemistry. Many host materials require assistance of other molecules, guests, to fill their cavities and achieve 'close packing' arrangement. Guest molecules in classical clathrates are held only by van der Waals interactions. The importance of van der Waals interactions arises from two facts: 1) numerous van der Waals interactions form when a large number of atoms are in van der Waals contact and when the effect is summed over many atom pairs it can be substantial; 2) van der Waals interactions are always present and their role is much more significant in the absence of other, stronger interactions. Close packing maximizes the total energy of van der Waals interactions between the atoms and minimizes the free energy of the crystal. In fact, close packing in solid state is a significant driving force in the formation of crystalline solids with a particular structure. According to the Close Packing Principle of Kitaigorodsky, molecules undergo a shape simplification as they progress towards dimers, trimers, higher oligomers, and ultimately crystals to maximize favorable isotropic van der Waals interactions.20
1.5 References


Chapter Two

Using Peptides in the Design of
New Host Materials
2. Using Peptides in the Design of New Host Materials

2.1 Peptides as host materials

Peptides are very attractive as host materials. One reason for that is their biological nature and thus potential utilization in various applications where non-toxic, biocompatible and degradable host materials are required. Examples of such applications are the storage, transport, and stabilization of drugs in the host peptide matrices, storage and slow release of flavors, odors and signaling chemicals (pheromones, allelochemicals), and the modeling of biological structures and functions such as transport through membrane pores and ion channels.

Another feature that makes peptides a promising class of host molecules is that the crystal frameworks they build are based on hydrogen bonds. These hydrogen bonds, some of which are charge-assisted, are numerous and strong. Due to their great number and strength, as well as directionality, the hydrogen bonds can create relatively robust and stable porous architectures mimicking host matrices based on covalent bonds. At the same time, the hydrogen bonded solids are “soft” materials: they can easily dissociate and reassemble, or change their structure, upon mild changes in external conditions (pH, temperature, solvents) induced by an operator in a well-controlled manner. Materials of this kind are of special interest in modern materials science.1,2,3
A third reason that makes peptides attractive is the almost unlimited number of potential host molecules that can be created from a limited number of amino acids. In other words, the design of a new host material with specific desired properties could be accomplished as “engineering” on the molecular level by choosing a suitable sequence of amino acid residues. However, it is not clear how to program and tune the host properties of peptide materials on molecular level. Screening all possible peptides for inclusion ability would be impossible. Nevertheless, extensive studies are necessary in order to understand the relationship between the molecular and crystal structure of peptides and to develop reliable strategies in the design of new peptide hosts. High cost and time requirements explain why very few studies on peptide host materials have been conducted so far and the field remains virtually undeveloped.

Historically most host materials that were discovered early, studied well, and subjected to further modifications, were based on very simple molecular building units: zeolites, urea and thiourea, clathrate hydrates, graphite and its modifications, and clays. Therefore, present-day attempts to design peptide-based host materials are often based on analogy to these host types that are already known and mimicking their structure or properties.

In this chapter several design strategies based on such analogy are reviewed including a short description of the well-known host types and their use in creating novel host materials based on advanced organic molecules and peptides.
2.2 Hosts with tubular cavity space

2.2.1 Urea and thiourea

Urea and thiourea are known to form solid clathrates with linear organic compounds such as $n$-alkanes.$^4,5,6$ Urea molecules form an extensive hydrogen-bonded network through H-bonds between the acidic protons of the NH$_2$ groups and the O-atoms of adjacent molecules, maximizing intermolecular interactions. The result is the formation of chiral, helical hollow tubes which are almost ideal linear, parallel cylinders with inner van der Waals diameter of 5.5-5.8 Å. $^7$ This host tunnel structure forms only if appropriate guest molecules are included in the channels. Removal of the guest results in the collapse of the channel structure. In this case, a different structure forms which does not contain empty tunnels. Thiourea also forms a tubular structure in the presence of an appropriate guest species. However, the channel in thiourea includes wide and narrow parts and has a larger cross-section than in urea clathrates. Since its channels are larger, thiourea can form inclusion compounds with guest molecules such as branched hydrocarbons, cyclohexane, and ferrocene, all of which cannot be included in the structure of urea. $^7$ The affinity of urea towards some guests has been used for various applications. For example, Hayes et al. have reported the use of urea for separation of a saturated free fatty acid from a mixture derived from low erucic acid rapeseed oil.$^8$
2.2.2 Zeolites

Zeolites are crystalline aluminosilicates with a porous anionic framework formed by linked AlO$_4$ and SiO$_4$ polyhedra. Although silicon is the key element in zeolite, in many cases AlO$_4^-$ can be easily substituted within the neutral SiO$_4$ sites. Introduction of alumina makes the neutral framework negatively charged, which requires the presence of metal cations to balance it. These cations are usually located within the solid cavities and channels, but do not clog them. Zeotypes are another type of microporous materials in which a wide range of TO$_4$ species (T = tetrahedral center such as Ge, Ga, P, As etc.) have been used instead of AlO$_4$ and SiO$_4$ sites. So far, more than 50 natural zeolites have been isolated, while hundreds of new zeolites and zeotypes have been synthesized.

The importance of zeolites arises from the presence of the channels and cavities in their structure which might accommodate metal cations, water and a vast range of other guest species. The most important property of the zeolite structure is the aluminosilicate framework which is robust enough that the guest species can enter and leave the cavities and channels with no destructive effect on the host structure. In other words, zeolites are able to absorb guest species reversibly. Consequently, zeolites have been used as “molecular sieves”, separating guest species based on their channel and cavity pore size. Each framework topology has a unique system of cavity space (Figure 2.1). Sodalite, the simplest zeolite, has only cavities built by four- and six-membered rings which are not large enough to accommodate many guest species. Linde topology is based on sodalite cages, but contains an additional four-membered ring. This makes the cavity accessible through eight-membered
rings. Faujasite type, an extended Linde structure, incorporates a three-dimensional 12-ring channel system. In contrast, ZSM-5 is not based on the sodalite motif. ZSM-5 has a complex structure made up of 10-ring channels.\textsuperscript{11}

Most zeolite applications are based on their basic qualities: sorption, ion exchange and catalysis. They have a wide range of industrial applications in catalysis and separation science, particularly in the petrochemical industry for separation of hydrocarbons and purification of gases and liquids. The most interesting use of natural zeolites has been reported in radioactive waste encapsulation.\textsuperscript{12} The application of zeolites as catalysts for the production of petrochemicals has stimulated interest in the synthesis of zeolite materials.

Figure 2.1. Topologies of zeolites. a. Sodalite type, b. Linde type, c. Faujasite type, d. AlPO\textsubscript{4}-5, e. ZSM-5. The vertices present the positions of AlO\textsubscript{4} and SiO\textsubscript{4} tetrahedra\textsuperscript{11}
2.2.3 Organic zeolites

The term “organic zeolite” was used for the first time by Barrer and coworkers\textsuperscript{13} to define any solid able to reversibly and selectively absorb large amounts of hydrophobic (organic) species while having a poor tendency toward sorption of inorganic compounds. In fact, the “zeolite” label was used to show the porosity of the material, while the “organic” label was used to demonstrate the hydrophobic nature of the pore surface. In other words, organic zeolites mimic zeolites’ properties by having permanent porosity even though they have a different chemical nature. Currently, there is a great interest in the design of organic and metal-organic materials mimicking zeolites’ behavior. The most important characteristic feature of these permanently porous materials is to remain intact upon guest release, as zeolites do. The host framework exists independent of the guest solvent, even though it is usually not stable thermodynamically and exists for kinetic reasons.\textsuperscript{14} [Ni(NCS)\textsubscript{2}(4-MePy)\textsubscript{4}] forms inclusion compounds with benzene molecules, located in zigzag channels running in three directions. The system of channels, which has a distorted diamondoid topology, contains small and large cavities (Figure 2.2 a). Nevertheless, the guest molecules usually occupy the small cavities only.\textsuperscript{15} Macrocycles also have been found to be interesting building blocks from which to make microporous structures. Some rigid organic macrocycles are hydrogen bonded to each other to form layers which could be aligned in a tubular structure (Figure 2.2 b).\textsuperscript{16}
The design of organic zeolites requires solving two general problems. The first problem is how to make materials with the desired volume and geometry of the cavity space. Making organic zeolites with the desired cavity space is very much dependent on the building units that may be utilized. Properties of the final structure usually are determined by the properties of these molecular building blocks. The geometry of the void space is a primary characteristic of the microporous material that can be used to understand total capacity, selectivity towards certain guests, kinetics of inclusion and other important properties. The second problem is to make these materials stable enough to operate in a desired range of experimental conditions. Real zeolites are built upon strong covalent bonds. Organic zeolites, on the other hand, are based on weaker interactions. This means that they
have a greater tendency towards dissociation or collapse to a more stable, dense form. Microporosity requires robustness and overall stability, while weaker interactions in the organic zeolites facilitate flexibility of the crystal structure. A proper balance of strong and weak interactions within a desired architecture could make it robust and flexible at the same time. The best molecules for making such microporous materials are those that avoid close packing or molecules which already have a void space, especially those which cannot create close packing by filling the internal space with the fragments of other molecules in the host matrix.

2.2.4 Peptide nanotubes and porous solids

Macrocycles are found to be useful building blocks to create micro/nanoporous tubular structures. Peptides are naturally occurring molecules that can be used to create these porous materials. Polypeptides that can build tubular or helical structures, also referred to as nanotubes, have attracted great attention. Recent studies indicate that certain oligopeptides self-assemble to create a porous structure in the solid state. These cyclic oligomers form H-bonded tubular structures with a channel inside the tube.17

One such class of organic nanotubes based on cyclic polypeptides was studied by Ghadiri and co-authors.18,19 Upon crystallization, these compounds produce a tubular structure hundred of nanometers long with an internal diameter of 7-8 Å. In this nanotube structure, a great number of peptide units interact through a giant network of hydrogen
bonds. The rings of eight-residue cyclic peptides connect through H-bonds to build a rigid hollow tube. The residues have a flat conformation in which all backbone amide functionalities stand perpendicular to the plane of the structure. The peptide side chains lie outside of the hollow tubular structure (Figure 2.3). In the structure the holes of the macrocycles are combined in a channel that falls into close packing structure upon inclusion of the guest species into the channel space. Peptide hollow tubes exist both in crystal form and the solution, and may encapsulate the molecules of solvent. Pavon and coworkers reported crystalline inclusion of two cyclo-hexapeptides. In the structure the peptide molecules keep a ring shape, but they do not combine into tubes. Guest species reside either inside or outside the rings. Big cyclic peptides do not usually form ring shaped structures or nanotubes.

Figure 2.3. a. Molecular structure of eight-residue cyclic peptide ring. b. Peptide rings self-assemble to form a tubular configuration. The peptide side chains stand outside of the hollow tubular structure

18
Hydrophobic dipeptides, such as L-alanyl-L-valine (AV) and L-valyl-L-alanine (VA), produce useful microporous materials with the ability to host small organic molecules. The AV and VA porous crystals are hexagonal with $P6_1$ space group and nearly identical lattice parameters. The molecules self-assemble through hydrogen bonds as a $6_1$ spiral to form a channel. The channel shape in both dipeptides can be represented by disks threaded on a sixfold screw axis (Figure 2.4). Hence the channels are chiral and could be used for separation of optical isomers. The channels have a smooth, constant average diameter of 5.13Å and 4.90Å, for AV and VA respectively. Another feature of these dipeptides is the stability of their porous framework. Most porous materials collapse and become denser once the guest species are removed. However, these two reported dipeptides exist in a porous form even when crystallized in the absence of a guest template. Based on this observation, the dipeptide materials were classified as organic zeolites or “biozeolites.”
Figure 2.4. a. Spiral assembly of AV (left) and VA (right) dipeptide molecules that create a channel. b. The channel shape in AV and VA shown as a set of inscribed disks threaded on a sixfold screw axis $^{21}$
The studies of these two dipeptides, Ala-Val (AV) and Val-Ala (VA), have been extended to other dipeptides including Ile-Ala (IA), Ala-Ile (AI), Ile-Val (IV), Val-Ile (VI), Val-Val (VV) and Leu-Ser (LS) by Soldatov and co-workers. All crystals are similar with a spiral self-assembly of dipeptide molecules producing a nanotube along the hexagonal axis of the structure (Figure 2.5). In order to build the helix structure, the dipeptides adopt a trans-conformation in which the two side groups are on the opposite sides of the dipeptide backbone. As shown in Figure 2.5, each of the nanotubes H-bonds to and slightly overlaps with the six parallel adjacent nanotubes. The resulting one-dimensional channels are isolated from each other. The inner walls of the channels are formed by hydrocarbon fragments and are essentially hydrophobic, and the inner diameter of the nanotube varies within 5.4-3.0 Å. Leu-Ser forms a unique structure with a similar H-bonding spiral assembly. The nanotube inner diameter is 4.3 Å (Figure 2.5). In contrast to other dipeptides which form right handed channels, the channel in Leu-Ser is left handed.
By definition, the total porosity of a material is the fraction of its volume which is freely accessible for guest species. This means that the porosity is just a part of total void space in the material. Direct sorption experiments conducted for all the dipeptides studied indicated that 4 to 13% of the materials’ volume was reversibly accessible to He and Xe gases. As illustrated in Figure 2.6, each biozeolite has a unique geometry of its void space and may be selective to a particular guest molecule. For example, VA exhibits four times greater tendency towards sorption of Xe as compared to AV.
The helical type of assembly was observed in a number of dipeptides,\textsuperscript{23,24} and may become a common principle in the design of porous solids. Not all peptide nanotubes are hydrophobic pores. In a series of dipeptides such as phenylalanyl-phenylalanine (FF) each molecule is H-bonded to two neighbors on one side and two neighbors on the other side. In order to build such a tubular structure, the dipeptides adopt a \textit{cis}-conformation in which two side groups are on the same side of the dipeptide backbone. As the hydrophobic fragment inhabits the outer surface of the nanotube, the inner surface is hydrophilic.\textsuperscript{17}
2.3 Layered host solids

2.3.1 Layered solids and intercalates

Layered solids are characterized by a two-dimensional sheet arrangement in which the components of the sheet interact by stronger bonds, such as covalent bonds, while the adjacent sheets link to each other by weaker interactions, such as van der Waals forces. Various materials are considered to be layered solids, such as graphite, metal phosphates and phosphonates, clay minerals, organic clays and a range of other inorganic, organic and coordination compounds. Some layered solids have been known and used since ancient times. For example, the property of clays to absorb water and become a plastic mass is used in the production of porcelain which is generally believed to have originated in China during the Shang Dynasty in 1600 BC.

Layered solids are important as potential host materials because in many cases, molecular guest species may be inserted between the layers, causing them to expand. This kind of inclusion is known as intercalation.

2.3.2 Graphite

The chemistry of layered intercalates began in 1840 with the report that graphite was able to intercalate sulfuric acid between consecutive layers of its 'chicken wire' mesh. The
structure of graphite is an infinite sheet comprising of six-membered rings with $sp^2$ hybridized carbon atoms. The layers aggregate at a distance of about 3.35 Å, which maximizes $\pi-\pi$ stacking interactions (Figure 2.7).

![Figure 2.7. Expected crystal structure of graphite](image)

The Pauling electronegativity of carbon is 2.5, suggesting that possible loss or gain of electrons by guest species that can fit between the layers may occur, depending on the electron donor or acceptor nature of the guest. In fact, graphite forms intercalates with both metal atoms, in which metal reduces the graphite layers, and halogen atoms, in which halogen oxidizes them. Typical metal complexes include LiC$_6$ (used as the negative electrode in Li-ion batteries) and MC$_8$, where M = K, Rb, Cs, Ca, Sr, Ba, Sm, Eu, and Yb (Figure 2.8). Since these atoms are relatively large in comparison with carbon atoms (M in MC$_8$...
complexes), the graphite layers slip on each other giving enough space to the intercalated guest atoms.\textsuperscript{26}

Figure 2.8. In-plane structure model for the inclusion of graphite with alkali metal atoms (MC\textsubscript{8}). Circles denote the intercalated atoms (ions) which are superimposed on the graphite hexagonal layer

Graphite can be used as a dry, low temperature lubricant because the carbon layers easily slip on one other (one might remember the slippery feel of a soft pencil lead). Interestingly, the lubricant properties of graphite depend crucially on the presence of intercalated oxygen. In the absence of oxygen which behaves as a sort of molecular ball bearing, graphite becomes much less slippery. This proved to be a particular problem for the use of graphite lubricants in the space program.\textsuperscript{27}
2.3.3 Clays

The term clay usually refers to sheet aluminosilicates (minerals composed of aluminum, silicone, and oxygen) that have an appreciable amount of cation-exchange capacity. The main standard units, SiO$_4$ tetrahedra and AlO$_6$ octahedra, may form a large number of structural types and in all crystal structures, they are linked together so that every oxygen atom is common to two tetrahedra. The current definition of a clay describes it in structural terms as a phyllosilicate (parallel sheets of silicate tetrahedra) consisting of two-dimensional tetrahedral sheets fused to octahedral sheets and, if necessary for charge neutrality, coordinated to charge-balancing cations. Two types of building blocks which are present in phyllosilicates are tetrahedra where each silicon (Si) is surrounded with four oxygen atoms, and octahedra where each aluminum (or magnesium) atom is surrounded by six oxygen atoms. Clay minerals are composed of two distinct types of connected layers, one consisting of corner-linked Si-tetrahedra, the other edge-linked Al-octahedra or, in some cases, Mg-octahedra. Different combinations of the layers make two possible clay layered structures:

a) Clays with TOT-type layer structure

In these clays, each layer is composed of three sub-layers, tetrahedral-octahedral-tetrahedral. This is a 2:1 phyllosilicate mineral with one Al-octahedral (or Mg-octahedral) sheet sandwiched between two Si-tetrahedral sheets. Separate TOT layers are loosely bonded by weak van der Waals forces. Montmorillonite is one example of a TOT-type clay which is well known due to its primary catalytic interest. Montmorillonite is characterized by pronounced swelling when is wet and shrinking when is dried.
b) Clays with TO-type layer structure

In TO-type clays, each layer is composed of two sub-layers, tetrahedral-octahedral. This is a 1:1 phyllosilicate mineral with one Al-octahedral sheet bonded to one Si-tetrahedral sheet by shared (apical) oxygen atoms of the tetrahedral sheet. Two adjacent layers are held together by hydrogen bonding. Consequently, the structure is fixed and no expansion occurs between layers when the clay is wetted. Kaolinite is one of the best known examples of this type.\(^\text{29}\)

Figure 2.9 illustrates the 1:1 and 2:1 structures and Table 2.1 shows idealized formulas for some selected clays.

Figure 2.9. Structure of 1:1 and 2:1 layers in clays, where X (shaded circles) is usually OH and M can be Al, Mg, Fe, etc.\(^\text{29}\)

There has been rapid growth in the studies carried out on sheet aluminosilicates. In particular, layered aluminosilicates are very versatile hosts for organic molecules. The nature of the clay-organic interaction can be altered by changing the Si:Al ratio in the host matrix.
Also, the host can be modified by introducing a heteroatom in the lattice. Moreover, the size and geometry of the interlayer space can be altered by pillaring. The intercalation of a guest can also change the structure. For example, while most of aluminosilicate hosts are centrosymmetric, the intercalation of organic guests may result in a noncentrosymmetric structure.27

<table>
<thead>
<tr>
<th>Clay</th>
<th>Idealized formula</th>
<th>Layer type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrophyllite</td>
<td>Al$_4$Si$<em>8$O$</em>{20}$(OH)$_4$</td>
<td>TOT</td>
</tr>
<tr>
<td>Talc</td>
<td>Mg$_6$Si$<em>8$O$</em>{20}$(OH)$_4$</td>
<td>TOT</td>
</tr>
<tr>
<td>Muscovite</td>
<td>K$_2$Al$_4$Si$_6$Al$<em>2$O$</em>{20}$(OH)$_4$</td>
<td>TOT</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>M$_{x/n}$$^{n+}$aH$<em>2$O, Al$</em>{4-x}$Mg$_x$Si$<em>8$O$</em>{20}$(OH)$_4$</td>
<td>TOT</td>
</tr>
<tr>
<td>Hectorite</td>
<td>M$_{x/n}$$^{n+}$aH$<em>2$O, Al$</em>{6-x}$Li$_x$Si$<em>8$O$</em>{20}$(OH)$_4$</td>
<td>TOT</td>
</tr>
<tr>
<td>Beidellite</td>
<td>M$<em>{x/n}$$^{n+}$aH$<em>2$O, Al$</em>{4}$Si$</em>{8-x}$Al$<em>x$O$</em>{20}$(OH)$_4$</td>
<td>TOT</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>Al$_{2}$Si$_2$O$_5$(OH)$_4$</td>
<td>TO</td>
</tr>
<tr>
<td>Serpentine</td>
<td>Mg$_3$Si$_2$O$_5$(OH)$_4$</td>
<td>TO</td>
</tr>
</tbody>
</table>

Intercalation is a very important property of clays and the best known example of it is the swelling of clay in water. This swelling is caused by the insertion of guest species (water in this case) between adjacent layers in the clay structure. The layers in montmorillonite crystals are loosely held together by weak oxygen-to-oxygen and cation-to-oxygen
interactions. Hence, water molecules and various ions are attracted between the layers (into the interlayer space), causing expansion of the crystal. Montmorillonite is characterized by pronounced expansion of the structure upon intercalation (Figure 2.10). A great number of polar organic molecules can be intercalated in montmorillonite clay.

![Figure 2.10. Montmorillonite network with the hydrated cations intercalated between the aluminosilicate layers](image)

Numerous studies have been conducted to investigate clay absorption properties.\textsuperscript{30,31} Pure clay minerals (kaolinite, illite, and montmorillonite) have shown high absorption of volatile organic compounds such as acetone, benzene, and toluene in the vapor phase.\textsuperscript{32} Clays can also potentially be used as carriers for controlled release of pesticides as reported by Celis et al.\textsuperscript{33}
Pillared clays are modified clay materials with enhanced host properties. Many types of clay experience physical collapse at elevated temperatures. At these temperatures, the interlamellar (that is intercalated) solvent species, either water or organic molecules, are ejected and the layers aggregate and stick. However this collapse can be prevented by inserting pillars which keep individual layers apart (Figure 2.11). Tsiao et al. reported the effective interlamellar spacing of montmorillonite increased from 5.4 to 8.0 Å after pillaring with aluminium polyoxohydroxy Keggin cations. In order to prepare the alumina pillared montmorillonite (Al-PILC), the exchangable Na⁺ cations between the layers in natural montmorillonite are replaced by the Keggin ion \([\text{Al}_{13}\text{O}_4\text{(OH)}_{24}\text{(H}_2\text{O})_{12}]^{7+}\) (Figure 2.12).
Another advantage of the pillared clays is the possibility that a new type of pores can be introduced. Pillaring is driven by ion exchange, and pillars attach themselves to the ion-exchange sites of the host layer. So, by altering the layer composition and thereby the cation exchange capacity, the number of pillars can be varied. Also, the pore size can be customized by changing the size of the pillars in use. Molinard et al. studied sorption of some organic and inorganic gases in various pillared clays. It became clear that the type of a pillar that was used to connect the clay layers has a great influence on the sorption properties of the pillared clays.
2.3.4 Organic clays

Organically-based materials that mimic the layered organization and host properties of clays are called "organic clays". This term was used by Atwood et al.\textsuperscript{36} in 1989 for layered structures of the calix[4]arene sulfonate of alkali metals. Calixarene is a macrocycle formed from condensation of a substituted phenol with an aldehyde (Figure 2.13). The bilayer structure found in Na\textsubscript{5}[calix[4]arene sulfonate]\textsuperscript{*12H\textsubscript{2}O, for example, resembles a 2:1 layer type of clay, if one considers -SO\textsubscript{3} groups as a part of the hydrated layer (Figure 2.14).

![Figure 2.13. Calixarene (left) and calix[4]arene (right)](image)

![Figure 2.14. The bilayer structure of Na\textsubscript{5}[calix[4]arene sulfonate]\textsuperscript{*12H\textsubscript{2}O. The lines are least-squares best planes of the aromatic carbon atoms bonded to the -SO\textsubscript{3} groups\textsuperscript{36}](image)
Figure 2.14 illustrates the bilayer arrangement of the calix[4]arene anions which form an organic layer. The inorganic layer is formed by water molecules, sodium ions, and the $-\text{SO}_3^-$ groups. This general structure is similar throughout the studied series and resembles that of clay minerals. Some of these similarities are as follows:

a) The repeat distance in the sodium salt of the calixarene close to smectite or vermiculite.
b) The hydrated layer thickness in vermiculite close to the inorganic layer in Na$_5$[calix[4]arene sulfonate]*12H$_2$O.
c) The presence of cation-exchange capabilities.

Clays which can intercalate guest species between 2D layers are of considerable commercial interest because of their applications in separation and catalysis.$^{37}$ Therefore, another interesting direction is the design and synthesis of lamellar materials that can mimic the structure and properties of naturally occurring clays.$^{38}$ The ultimate goal is to use these new layered solids in host-guest chemistry for such applications as storage, separation of chemicals, chemical sensing, and catalysis.$^{39}$ These materials differ from naturally occurring clays and inorganic clay-like minerals, as strong bonds like covalent bonds found in clays have been replaced by weaker interactions like hydrogen bonds. On the other hand, while many types of clay have high affinity towards the inclusion of water or other hydrophilic components, the organic clay mimics tend to intercalate hydrophobic organic species.

There are advantages in using hydrogen bonds as the 'supramolecular glue' in creating clay-like materials. First of all, the hydrogen-bonded materials can be easily created using a self-assembly process and disassembled if necessary, the process being totally reversible and
reproducible. By changing the building blocks, one can design various systems with the specific size and shape of the interlayer cavity space. While these solids are not always as robust as their inorganic counterparts (as the hydrogen bonds are weaker), the hydrogen-bonded layered materials may be strong enough to remain undamaged during intercalation. The strength of hydrogen-bonded layered solids can be increased by using charge-assisted hydrogen bonds which are directional, reliable, and up to four times stronger than regular strong hydrogen bonds.\textsuperscript{40}

Carboxylic anions and ammonium cations have been used as building units to design ionic organic materials with a supramolecular framework. This is mainly due to the complementary nature of the strong hydrogen bond donors of the cations and the strong hydrogen bond acceptors of the anions.\textsuperscript{41,42} Beatty et al.\textsuperscript{43} showed that if 3,5-pyrazoledicarboxylic acid (H\textsubscript{2}PzDCA) is allowed to react with primary and secondary amines (Figure 2.15), the monoanions (HPzDCA\textsuperscript{−}) can connect in two dimensions and form a stable 2D layered structure.

![Figure 2.15. Deprotonation of H\textsubscript{2}PzDCA by primary amine (R=H, R´=benzyl) and secondary amine (R=R´=benzyl)](image-url)
The complexes in both cases display a lamellar structure in the solid state with similar anionic sheet: carboxylic acid – carboxylate OH···O hydrogen bonds connect the monoanions into parallel linear chains cross-linked by NH···O hydrogen bonds. For primary amines, two ammonium carboxylate and one ammonium pyrazolate hydrogen bonds attach the ammonium cation to the anionic layers and eventually the overall motif exhibits bilayer arrangement, ABA, mimicking the TOT pattern in naturally occurring clays. However, secondary amines participate in one NH···O and one NH···N hydrogen bond and the benzyl groups of each cation lie above and below the anions, resulting in an AB structural pattern which mimics the TO pattern in naturally occurring clays (Figure 2.16). However, it has not been determined if these materials also mimic the physico-chemical properties of clays as sorbents.

Beatty et al. also synthesized the pillared clay mimics from dicarboxylic acids and flexible diamines. Recently, a metal-containing dicarboxylic acid was used to make a 2D layered structure with a metal-organic framework.

Figure 2.16. Hydrogen-bonded sheet in HPzDCA (left). ABA pattern (middle). AB pattern (right)
2.3.5 Bile acids

Bile acids are steroid carboxylic acids derived from cholesterol (Figure 2.17). The structure of this large family of molecules consists of four rings with a side-chain terminating in a carboxylic acid. IUPAC recommends labeling these four rings from left to right. Based on IUPAC nomenclature, bile acids have a hydroxyl group on position 3. By changing the R substituents, many derivatives can be formed.

Figure 2.17 illustrates the molecular structure of two bile acids: deoxycholic acid and cholic acid. Deoxycholic acid has been known as a host matrix for a long time. Since the molecule is chiral, the produced inclusion crystals are also chiral. The inclusion compounds exist as molecular crystals with an extensive system of hydrogen bonds and are often referred to as “choleic acids”. Cholic acid has also been recently reported to form channel-type inclusion compounds with a variety of organic guest molecules. The two acids are similar in molecular structure but the crystal structure of their inclusions vary notably.45,46

Chemical modification of bile acids brings a versatile type of host materials which can form a wide range of inclusion compounds. Over three hundred crystal structures of inclusion compounds of bile acids and their derivatives have been reported. As a rule, they self-assemble to form a biocompatible host framework with channel-type cavities. The channels are chiral and therefore can recognize guest species not only by size and shape, but also by chirality.48
2.3.6 Layered peptide inclusion compounds

Few inclusion compounds based on a layered peptide matrix have been reported. Supramolecular self assembly of peptides is driven by hydrogen-bond formation in the structure and the segregation of hydrophilic and hydrophobic parts. The crystal structures of many dipeptides offer distinct hydrophobic and hydrophilic layers. The hydrophilic layers typically incorporate a hydrogen-bonding motif with two $\text{NH}_3^+$-$\text{OOC}$ head-to-tail links, with the third amino H atom, which cannot find a peptide main chain acceptor, pointing straight into the adjacent hydrophobic layer (Figure 2.18).
Leucyl-alanine has been known to form a crystalline solvate with DMSO.\textsuperscript{53} Akazome et al. reported inclusion compounds of some dipeptides with sulfoxides.\textsuperscript{54-56} Their attention was first focused on dipeptides with aliphatic amino acids in the backbone. The layer structure is built by hydrogen bonds between terminal amino and carboxyl groups, while the cavities are produced by the alkyl side chains of the dipeptide which stand perpendicular to the layer. In Figure 2.19, the crystal structures of inclusion compounds of Leu-Ala with isobutyl methyl sulfoxide, dimethyl sulfoxide, and benzyl methyl sulfoxide are illustrated. Interestingly, the interlayer distance expands from 9.4 Å to 11.1 Å when those different guests are included. In other words, the interlayer distance varies in response to the size of a specific guest. The dipeptide molecules in the inclusion compounds of isobutyl methyl sulfoxide and dimethyl sulfoxide pile up in the anti-parallel direction, while in the inclusion compound with benzyl methyl sulfoxide they stack in a parallel direction. The chiral molecule yields a chiral space group as expected. In the inclusions of phenylglycyl-phenylglycine (D,D- and L,D- isomers), the dipeptide molecules are arranged in parallel to
construct a wavy sheet and the sulfoxide guest molecules are accommodated in a channel generated between the layers.\textsuperscript{54,55}

A series of layered inclusion compounds was also reported for leucyl-leucyl-leucine tripeptide, with various guest species such as methanol/water,\textsuperscript{57} pyridine and methylpyridines.\textsuperscript{58}
Figure 2.19. Layered structure of inclusion crystals of Leu-Ala with various sulfoxides. The arrows indicate the arrangement of dipeptide molecules (from the C-terminal to the N-terminal). Leu-Ala with isobutyl methyl sulfoxide (top), dimethyl sulfoxide (middle), and benzyl methyl sulfoxide (bottom)\textsuperscript{56}
2.4 Research direction

Over the last 60 years, host-guest chemistry has introduced numerous new materials and contributed to the development of supramolecular chemistry as a new discipline with a high impact on modern materials science. Many host materials have found industrial applications worldwide, such as zeolites in petrochemistry. There is a great demand for new host materials that would either replace those utilized currently or enable new technologies and applications. A special interest exists in biocompatible, environment-friendly host materials whose structure could be extensively modified on a molecular level to tune the materials properties.

Lower peptides are versatile, naturally occurring molecules that could satisfy this demand. The host-guest chemistry of peptides remains virtually unexplored and the potential of peptide host materials cannot be reliably evaluated due to the lack of experimental data.

A general purpose of this project was to conduct comprehensive studies on one dipeptide. Previous studies never focused on the clathration ability of peptides. Our purpose was to use a combination of methods to elucidate the clathration ability of a dipeptide with respect to a range of potential guests. In particular, the following questions had to be addressed:

1. What variety of inclusion compounds can the host dipeptide form with organic molecules? This question necessitates screening a range of potential guest candidates. We were especially interested in aromatic guests since no dipeptide inclusions with aromatic guests
have been reported. We also wanted to study at least one crystal structure with an aromatic
guest down to atomic resolution.

2. Which molecules would be good guest candidates and which would not? In other words,
what should be the ideal guest molecule for a chosen dipeptide.

3. What are the typical compositions of the inclusions? How does the host-guest
stoichiometry depend on the guest?

4. What are the parameters of formation and dissociation of the inclusion compounds in
various conditions? Are the inclusion compounds stable thermodynamically or kinetically
and what are the limits of their existence? How do the stability and other properties depend
on the guest molecular structure and the crystal structure of the clathrate?

5. What are the conditions of formation of the inclusion compounds and what experimental
procedures can be used to synthesize and handle the new materials? This included the
development or adaptation of synthetic and characterization methods that could be applied in
future studies as well.

The leucyl-alanine dipeptide has been chosen as the host molecule of this study.
Although the inclusion properties of Leu-Ala have not been comprehensively studied, a few
inclusion compounds of the dipeptide have been isolated. Some of them were studied
structurally, while the crystal structure of a guest-free form has never been reported. These
facts were regarded as indications of versatile clathration ability of the dipeptide, making it a
good candidate for our studies.
It has been seen that Leu-Ala forms inclusion compounds with a layered structure, with guest species residing in the interlayer space. The layered organization is common in peptides and has a structural analogy to clays. As discussed in this chapter, the structural analogy strategy proved to be very useful in the design of new host materials. This strategy led to the development of zeolite mimics based upon various organically based molecules and some peptides. Therefore, the development of clay-like materials based on peptides seemed to be a useful and promising strategy.

Prior to this work, only a few structures have been reported and no studies on physico-chemical properties of layered peptide materials had been conducted. Therefore, one specific purpose of this project was to acquire physico-chemical data for a number of inclusions and to study in detail the formation and properties of inclusion compounds in three host – guest binary systems.

A set of experimental methods was used as described in Chapter 3 including preparation and characterization techniques. These methods, previously used successfully to isolate and study inclusion compounds of other hosts, had to be modified for peptide materials. The adaptation of these methods and development of new approaches to study peptide inclusion materials was a part of this project. For example, in this work we applied the determination of host-guest phase diagrams as a method to study inclusion properties of peptide materials for the first time. We also extensively used the methods of thermal analysis and sorption techniques.
In summary, the purpose of this project was to comprehensively study the clathration ability of the Leu-Ala dipeptide as an example of a layer-type peptide host material. Deep understanding of the inclusion chemistry of Leu-Ala would help to evaluate the potential of lower peptides as a new family of host materials. Finally, this project was planned in a way to facilitate future studies in the field.
2.5 References


Chapter Three

Experimental
3. Experimental

3.1 Chemicals

Dimethylsulfoxide (DMSO) was obtained from Sigma-Aldrich (99.9+, water content <0.005%). For phase diagram determinations, a new bottle of the solvent was used; it was kept sealed and used in a dry glovebox. The melting point, determined in a DSC experiment, was 18.1(8)°C, which compares well with the literature value of 18.8(3)°C.\(^1\) Guaiacol was from Sigma (reagent grade). The chemical was protected from light and kept in a dry glovebox. Its melting point (DSC) was 26.3(8)°C (lit. 28.0(4)°C).\(^2\) From the freezing point depression of 1.7(8)°C and the freezing point lowering of 5.4°C per mole of water per liter of solvent reported previously,\(^3\) the amount of impurities including water in the guaiacol used were less than 0.5(3)%. Other solvents used in this work were of reagent grade or better with the content of the main substance of 98% or higher. Leu-Ala (L,L-isomer) dipeptide was from Chem-Impex International. The chemical from different bottles varied in water content (6 to 23%). When a dry sample was required, it was dehydrated as described in section 3.2.1.2. A TGA determination was conducted for all as-received and dehydrated samples before each use of the chemical to determine precise water content or to confirm the absence of water after its dehydration.
3.2 Preparation and characterization procedures

3.2.1 Synthesis and characterization of inclusion compounds

3.2.1.1 Crystallization and characterization methods

Inclusion compounds of Leu-Ala were prepared by crystallization of the dipeptide from each corresponding guest solvent. A small amount of water was added to increase the solubility of Leu-Ala. Two variations of this method were used. (1) Preparation of the hot solutions followed by slow cooling to room temperature. This method was used in most cases. (2) Isothermal evaporation of solutions at room temperature. Typically, a saturated solution was prepared, separated by filtration from excess solid, and left to evaporate slowly. This method was used for highly volatile guest solvents or as a second step when method (1) did not produce a solid product.

In a typical crystallization experiment, Leu-Ala (~100 mg, 0.5 mmol), taken directly from the bottle, was covered by a guest solvent. The mixture was heated for ~20 seconds to ~100°C (or close to boiling point for volatile solvents), with stirring and a minimal amount of water added dropwise when necessary until all of the solid dissolved. The resulting transparent solution was left to cool slowly to room temperature. Depending on the solvent used, crystals were recovered on the next day or after several days. In some cases, in order to induce the crystal growth, the vial cap was slightly opened and the solvent was allowed to evaporate.
In order to prepare the crystals of Leu-Ala*DMSO, evaporation of aqueous solution was also used. Leu-Ala (~220 mg, 1 mmol), taken directly from the bottle was dissolved in a solution of DMSO (0.15 ml) in water (5 ml). The solution was separated by filtration and left to evaporate slowly at room temperature. Needle-shaped crystals were recovered after 2 weeks.

All isolated crystals were inspected visually (microscope) and tested for their stability in air. The decomposition rate of inclusion compounds due to guest release varied. For instance, the inclusion crystals with 4-methylpyridine were stable for several days and the inclusion with DMSO did not reveal any decomposition, at least over several weeks. To preserve the crystals obtained from guest loss, they were typically stored under mother liquor and taken and dried with filter paper when needed. All crystalline products were colorless, but the crystals varied in shape. Inclusion compounds of Leu-Ala with 3-methylpyridine and 2,4,6-trimethylpyridine were prismatic; those with 4-methylpyridine were obtained as plates. Other crystals had a needle shape.

The stoichiometry and thermal stability of inclusion compounds were studied in TGA experiments. In all cases complete mass loss was observed by 300°C, and occurred in three steps: guest release, water release in a cyclization reaction of Leu-Ala, and complete evaporation of cyclo-Leu-Ala. The TGA results confirmed a 1:1 host to guest molar ratio for most inclusions (see Table 5.1 in Chapter 5). For some inclusions an initial step due to the presence of a small amount of solvent in the sample was observed. For example, the crystals of Leu-Ala*DMSO subjected to TGA analysis revealed some amount of liquid DMSO. The
release of this excess of solvent occurred at low temperature and was distinguishable from the release of guest solvent occurring at higher temperatures. In some cases, FT-IR coupled to TGA was used for determination of the components of the evolved gas. The temperature limit of existence of some inclusion compounds was studied by DSC technique using samples hermetically sealed in high-pressure capsules. The character of phase transitions observed in a DSC experiment was determined visually by heating samples in sealed capillary tubes on a melting temperature apparatus. Inclusion crystals with methylpyridines were characterized structurally on a single-crystal X-ray diffractometer, at 100 K to improve the X-ray diffraction data and protect inclusion crystals from decomposition. The 1:1 stoichiometry obtained from the single-crystal X-ray diffraction studies corresponded well to the composition determined from the TGA experiments.

3.2.1.2 Immersion method

Another technique which was used to prepare inclusion compounds is known as the immersion method.\textsuperscript{4} A suspension of dry Leu-Ala (~100 mg, 0.5 mmol) in a neutral solvent (~5 g) was stirred together with an excess of guest solvent (~5 mmol) at ambient temperature at least for one day. Benzene, hexane or heptane was used as the neutral solvent. The powder-like inclusion compound was separated by filtration and washed with ethyl ether (several drops). The product was studied by TGA. The results for the powder-like inclusion compounds matched the results for crystalline inclusion compounds.
Chem-Impex International does not include information about the water content of Leu-Ala dipeptides. Since the as-received chemicals contained anywhere from 6 to 23% of water by mass and could absorb moisture from air (Leu-Ala can accommodate up to 4 moles of water in its cavity space)\(^5\), we developed a special procedure to prepare water-free Leu-Ala: A sample of Leu-Ala of ~200 mg was ground in an agate mortar thoroughly and dried in a ventilation oven at 65°C repeatedly, each time for 15 minutes, until no mass change was observed. Then the vial was sealed and kept in a dry box under dry nitrogen atmosphere. Further manipulations were done inside the glove box.

### 3.2.1.3 Isopiestic method

A Sorption (isopiestic) experiment in general is a method involving the uptake of a sorbate vapor (different guest species) by a solid sorbent (Leu-Ala host matrix). In this work, the isopiestic experiments were used as both a method of preparation of an inclusion compound and a method to determine the guest to host molar ratio in the final product. Previously reported procedures\(^6,7,8,9\) were used with some modifications.

A thorough control of the Leu-Ala sorbent is necessary during the preparation of the sorption experiment since water may compete with the main guest. The same procedure of Leu-Ala dry powder preparation used for the immersion method (see previous section) was also utilized for isopiestic experiment. However, considering the importance of having a thoroughly dehydrated homogenously ground host material, an additional step was applied.
For this additional step, Leu-Ala (which was already ground and dried) was ground again in an agate mortar, placed in a weighed vial, dried in a ventilation oven at 65°C for 15 min, and the vial was weighed again and used directly in the isopiestic experiment.

Dehydrated Leu-Ala powder prepared as described above (60-90 mg, 0.3-0.45 mmol) in a vial and pure guest solvent in a separate vial were both placed in a hermetically closed flask to equilibrate. The samples of Leu-Ala exposed to the vapors of a desired guest showed a mass increase that was monitored periodically using an analytical balance. An empty reference vial was also placed in the same flask and the mass increase due to the guest solvent vapor was measured to account for the weight of the guest in the gaseous phase inside the vial with the sample. The Leu-Ala sample was weighed several times during the first day and every day afterward to monitor the guest uptake by the host until an equilibrium was established. The mass increase (by molar ratio) over time was plotted and the experiment continued until a plateau was observed. It usually took less than two weeks to reach the equilibrium, but we continued monitoring mass increase for a longer time to make sure no further sorption occurred.

In the Leu-Ala – water isotherm sorption experiments, Leu-Ala samples (~100 mg), dehydrated as described above, were exposed to various relative pressures \( \frac{P}{P_0} \) of water vapor. For this purpose, a series of water-sulfuric acid solutions with a given concentration was prepared and used as a source of water vapor of a known pressure over solution. Saturated solutions of NaCl, NH₄Cl and Ca(NO₃)₂ were also used for the same purpose. The experiments were performed at room temperature (298 K). The mass increase (by molar
ratio) versus vapor pressure of water was plotted to give the dependence of the sample composition on the water vapor pressure. The time needed to establish equilibrium varied from 24 hours to 21 days (Figure 3.1). The values of vapor pressure of water over sulfuric acid and saturated salt solutions, as well as the density – concentration dependence for sulfuric acid, were taken from the reference literature.\textsuperscript{10,11} The numerical results are listed in Table 6.1 in Chapter 6.

![Graph showing water sorption by LA dipeptide under two different partial pressures of water: 88.0% (squares) and 75.1% (triangles).](image)

Figure 3.1. Water sorption by LA dipeptide under two different partial pressures of water:

88.0% (squares) and 75.1% (triangles)
3.2.2 Determination of phase diagrams

The phase diagrams of two host – guest binary systems were determined in this work: Leu-Ala – DMSO and Leu-Ala – guaiacol. The diagrams were studied in a full concentration range and from -80°C to ~200°C (no solid phases exist above 200°C in the systems). Since water can act as a third component, considerably changing the temperature and character of the phase transitions, special care was taken to have all samples free of water. The Leu-Ala dipeptide was dehydrated using the same procedure as in the immersion method of synthesis (section 3.2.1.2). The dry Leu-Ala, DMSO and guaiacol were kept in a dry glovebox (over P₂O₅) filled with nitrogen. All samples were prepared in the glovebox and used in a way excluding any possibility of their contact with the atmosphere.

Two methods were used to obtain experimental points on the phase diagrams. (1) The samples (~10 mg) with various Leu-Ala:guest ratios were prepared using the dipeptide, guest (DMSO or guaiacol), or the corresponding inclusion compound. The samples were hermetically sealed in high-pressure DSC capsules (glovebox). The samples were studied on a DSC analyzer to obtain the temperatures of phase transformations. The DSC thermograms were recorded typically from -80°C to +200°C at the rate of 1°C/min. The slow heating rate was used to avoid overheating of samples containing inclusion compounds above the temperature of their incongruent melting. The temperatures of observed endothermal effects were plotted on the phase diagram. Over 50 DSC experiments were done for each phase diagram in order to locate solidus and liquidus curves. DSC results used to construct the phase diagram for LA – DMSO system are shown in Table 3.1 and DSC results used for the
system LA – guaiacol are shown in Table 3.2. The phase diagrams are shown in Figures 4.1 and 4.9 in Chapter 4.
Table 3.1. Temperatures (°C) and relative magnitudes of thermal effects of phase transitions in the system LA – DMSO registered by DSC

<table>
<thead>
<tr>
<th>N</th>
<th>Sample</th>
<th>% LA</th>
<th>m, mg</th>
<th>Eut. T</th>
<th>Eut. TE</th>
<th>Per. T (range)</th>
<th>Per./L TE</th>
<th>Liq. T (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R19</td>
<td>0</td>
<td>10.63</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>18.1(8)</td>
</tr>
<tr>
<td>2</td>
<td>R25</td>
<td>4.9</td>
<td>14.81</td>
<td>17.3*</td>
<td>100</td>
<td>-</td>
<td>1.2*</td>
<td>113 (104-122)</td>
</tr>
<tr>
<td>3</td>
<td>R23</td>
<td>10.8</td>
<td>19.29</td>
<td>16.3*</td>
<td>87.3</td>
<td>-</td>
<td>2.0*</td>
<td>121 (113-129)</td>
</tr>
<tr>
<td>4</td>
<td>R26</td>
<td>15.2</td>
<td>10.05</td>
<td>16.3*</td>
<td>74.2</td>
<td>-</td>
<td>7.1*</td>
<td>125 (119-131)</td>
</tr>
<tr>
<td>5</td>
<td>R27</td>
<td>20.4</td>
<td>10.07</td>
<td>15.8*</td>
<td>74.8</td>
<td>-</td>
<td>4.0*</td>
<td>134 (131-137)</td>
</tr>
<tr>
<td>6</td>
<td>R20</td>
<td>25.1</td>
<td>12.07</td>
<td>15.6*</td>
<td>61.7</td>
<td>-</td>
<td>8.4*</td>
<td>138 (136-140)</td>
</tr>
<tr>
<td>7</td>
<td>R28</td>
<td>29.8</td>
<td>11.19</td>
<td>15.9*</td>
<td>55.6</td>
<td>-</td>
<td>14.5*</td>
<td>141.5 (140-143)</td>
</tr>
<tr>
<td>8</td>
<td>R29</td>
<td>34.8</td>
<td>9.76</td>
<td>17.3*</td>
<td>55.8</td>
<td>-</td>
<td>20.8*</td>
<td>144 (143-145)</td>
</tr>
<tr>
<td>9</td>
<td>R46</td>
<td>37.0</td>
<td>9.76</td>
<td>16.3*</td>
<td>48.4</td>
<td>144.6*</td>
<td>23.0*</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>R22</td>
<td>41.8</td>
<td>11.06</td>
<td>14.2*</td>
<td>39.6</td>
<td>143.7*</td>
<td>27.7*</td>
<td>154 (151-157)</td>
</tr>
<tr>
<td>11</td>
<td>R30</td>
<td>44.1</td>
<td>10.29</td>
<td>16.9*</td>
<td>40.7</td>
<td>144.1*</td>
<td>29.8*</td>
<td>156 (152-160)</td>
</tr>
<tr>
<td>12</td>
<td>R21</td>
<td>49.2</td>
<td>14.39</td>
<td>12.6</td>
<td>30.0</td>
<td>143.7*</td>
<td>25.5 b</td>
<td>162 (155-169)</td>
</tr>
<tr>
<td>13</td>
<td>R31</td>
<td>56.2</td>
<td>9.96</td>
<td>16.9*</td>
<td>24.6</td>
<td>143.6*</td>
<td>34.9 b</td>
<td>168.5 (156-181)</td>
</tr>
<tr>
<td>14</td>
<td>R32</td>
<td>58.5</td>
<td>7.75</td>
<td>15.6*</td>
<td>17.4</td>
<td>145.3*</td>
<td>52.6</td>
<td>171 (157-185)</td>
</tr>
<tr>
<td>15</td>
<td>R43</td>
<td>60.7</td>
<td>9.08</td>
<td>13.7</td>
<td>13.8</td>
<td>144.5*</td>
<td>49.4</td>
<td>h</td>
</tr>
<tr>
<td>16</td>
<td>R40</td>
<td>63.1</td>
<td>9.09</td>
<td>8.2</td>
<td>7.9</td>
<td>146.0*</td>
<td>65.1</td>
<td>h</td>
</tr>
<tr>
<td>17</td>
<td>R33</td>
<td>66.4</td>
<td>10.04</td>
<td>11.7</td>
<td>6.8</td>
<td>146.8*</td>
<td>63.1</td>
<td>h</td>
</tr>
<tr>
<td>18</td>
<td>R34</td>
<td>69.6</td>
<td>9.29</td>
<td>7.0</td>
<td>1.3</td>
<td>147.1*</td>
<td>96.5</td>
<td>n</td>
</tr>
<tr>
<td>19</td>
<td>R48</td>
<td>72.1</td>
<td>9.08</td>
<td>-</td>
<td>-</td>
<td>143.0*</td>
<td>44.9 j</td>
<td>h</td>
</tr>
<tr>
<td>20</td>
<td>R18</td>
<td>72.1</td>
<td>10.25</td>
<td>-</td>
<td>-</td>
<td>147.7*</td>
<td>87.5</td>
<td>h</td>
</tr>
<tr>
<td>21</td>
<td>V28-31</td>
<td>72.1</td>
<td>10.41</td>
<td>-</td>
<td>-</td>
<td>146.6*</td>
<td>49.0 j</td>
<td>n</td>
</tr>
<tr>
<td>22</td>
<td>R45</td>
<td>74.1</td>
<td>8.78</td>
<td>-</td>
<td>-</td>
<td>149.2*</td>
<td>100</td>
<td>n</td>
</tr>
<tr>
<td>23</td>
<td>R35</td>
<td>76.3</td>
<td>9.46</td>
<td>-</td>
<td>-</td>
<td>144.5*</td>
<td>53.8*</td>
<td>h</td>
</tr>
<tr>
<td>24</td>
<td>R39</td>
<td>80.6</td>
<td>9.55</td>
<td>-</td>
<td>-</td>
<td>144 (140-148) j</td>
<td>30.0</td>
<td>n</td>
</tr>
<tr>
<td>25</td>
<td>R44</td>
<td>82.6</td>
<td>9.55</td>
<td>-</td>
<td>-</td>
<td>143 (138-148) i</td>
<td>25.7</td>
<td>n</td>
</tr>
<tr>
<td>26</td>
<td>R36</td>
<td>85.8</td>
<td>9.51</td>
<td>-</td>
<td>-</td>
<td>143.5 (139-148) j</td>
<td>17.1</td>
<td>h</td>
</tr>
<tr>
<td>27</td>
<td>R42</td>
<td>89.0</td>
<td>9.46</td>
<td>-</td>
<td>-</td>
<td>141 (138-144) i</td>
<td>17.1</td>
<td>h</td>
</tr>
<tr>
<td>28</td>
<td>R38</td>
<td>91.4</td>
<td>10.49</td>
<td>-</td>
<td>-</td>
<td>140.5 (137-144) j</td>
<td>11.6</td>
<td>h</td>
</tr>
<tr>
<td>29</td>
<td>R37</td>
<td>94.9</td>
<td>10.54</td>
<td>-</td>
<td>-</td>
<td>m</td>
<td>m</td>
<td>n</td>
</tr>
<tr>
<td>30</td>
<td>R41</td>
<td>100</td>
<td>9.79</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>n</td>
</tr>
</tbody>
</table>

Ave. | 16.2(6) o | 145.4(7)
Samples sealed in high pressure pans; heating rate 1°C/min. The thermal effect for each type of phase transition is expressed as percentage of the strongest effect. Temperature values used in the calculations of average values are marked with an asterisk. Abbreviation used: %LA, content of LA in the sample; m, mass of the sample; Eut. T, the temperature of eutectics; Eut. TE, the relative magnitude of the endotherm of the eutectic transition; Per. T, the temperature of peritectics; Per./L TE, the relative magnitude of the endotherm of the peritectic and liquidus transition; Liq. T, the temperature of liquidus

- Peritectics + liquidus. Thermal effect from peak heights (rather than integral intensities)
- From bottle, Sigma-Aldrich, 99.9+%, water content <0.005%
- Lit. m.p. for DMSO is 18.8(3)°C
- Liquidus only
- Peritectic point: 37(2)% LA; 145.4(7) °C
- Higher values due to irregular shape of the peak
- Liquidus is not reached because of irreversible decomposition of the sample
- Inclusion compound prepared by crystallization
- Wide peak
- Inclusion compound prepared by immersion method
- Eutectic point: 0.37(4) %LA; 16.2(6) °C

* Effected by decomposing LA
  - Effects are not resolved due to irreversible decomposition of LA
  - Irreversible effect of complex shape starting from an exotherm at ~115°C
  - Eutectic point: 0.37(4) %LA; 16.2(6) °C
Table 3.2. Temperatures (°C) and relative thermal effects of phase transitions in the system LA – guaiacol registered by DSC

<table>
<thead>
<tr>
<th>N</th>
<th>Sample</th>
<th>% LA m, mg</th>
<th>Eut. T</th>
<th>Eut. TE</th>
<th>Per. T (range)</th>
<th>Per./L TE</th>
<th>Liq. T (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R55</td>
<td>0</td>
<td>10.65</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>27.0(3)</td>
</tr>
<tr>
<td>2</td>
<td>R61</td>
<td>4.8</td>
<td>15.4</td>
<td>25.3</td>
<td>100</td>
<td>-</td>
<td>7.2</td>
</tr>
<tr>
<td>3</td>
<td>R62</td>
<td>10.2</td>
<td>9.85</td>
<td>25.6</td>
<td>87.6</td>
<td>-</td>
<td>19.7</td>
</tr>
<tr>
<td>4</td>
<td>R60</td>
<td>15.7</td>
<td>12.45</td>
<td>25.5</td>
<td>90.9</td>
<td>122.4</td>
<td>33.4</td>
</tr>
<tr>
<td>5</td>
<td>R66</td>
<td>17.1</td>
<td>9.68</td>
<td>25.1</td>
<td>79.5</td>
<td>125.2</td>
<td>29.0</td>
</tr>
<tr>
<td>6</td>
<td>R57</td>
<td>19.2</td>
<td>14.02</td>
<td>24.8</td>
<td>70.3</td>
<td>122.3</td>
<td>30.2</td>
</tr>
<tr>
<td>7</td>
<td>R74</td>
<td>20</td>
<td>10.72</td>
<td>26.2</td>
<td>77.1</td>
<td>125.2</td>
<td>28.0</td>
</tr>
<tr>
<td>8</td>
<td>R64</td>
<td>24.3</td>
<td>9.84</td>
<td>24.7</td>
<td>61.5</td>
<td>122.3</td>
<td>41.3</td>
</tr>
<tr>
<td>9</td>
<td>R63</td>
<td>31.0</td>
<td>10.40</td>
<td>23.5</td>
<td>47.5</td>
<td>123.1</td>
<td>52.9</td>
</tr>
<tr>
<td>10</td>
<td>R75</td>
<td>37.0</td>
<td>9.52</td>
<td>24.4</td>
<td>46.8</td>
<td>123.1</td>
<td>73.1</td>
</tr>
<tr>
<td>11</td>
<td>R58</td>
<td>37.9</td>
<td>18.94</td>
<td>23.0</td>
<td>38.0</td>
<td>122.5</td>
<td>62.0</td>
</tr>
<tr>
<td>12</td>
<td>R76</td>
<td>40.0</td>
<td>9.41</td>
<td>23.6</td>
<td>36.9</td>
<td>123.6</td>
<td>75.3</td>
</tr>
<tr>
<td>13</td>
<td>R77</td>
<td>42.8</td>
<td>12.46</td>
<td>23.5</td>
<td>31.8</td>
<td>124.0</td>
<td>75.0</td>
</tr>
<tr>
<td>14</td>
<td>R66</td>
<td>45.9</td>
<td>10.28</td>
<td>23.1</td>
<td>14.7</td>
<td>122.3</td>
<td>74.5</td>
</tr>
<tr>
<td>15</td>
<td>R83</td>
<td>49.3</td>
<td>9.69</td>
<td>23.2</td>
<td>21.0</td>
<td>123.6</td>
<td>78.1</td>
</tr>
<tr>
<td>16</td>
<td>R78</td>
<td>53.7</td>
<td>9.24</td>
<td>23.0</td>
<td>8.9</td>
<td>124.7</td>
<td>80.0</td>
</tr>
<tr>
<td>17</td>
<td>R68</td>
<td>55.5</td>
<td>10.84</td>
<td>-</td>
<td>-</td>
<td>120.3</td>
<td>82.1</td>
</tr>
<tr>
<td>18</td>
<td>R80</td>
<td>58.6</td>
<td>9.10</td>
<td>-</td>
<td>-</td>
<td>121.7</td>
<td>80.9</td>
</tr>
<tr>
<td>19</td>
<td>R59</td>
<td>59.7</td>
<td>7.07</td>
<td>-</td>
<td>-</td>
<td>120.1</td>
<td>78.3</td>
</tr>
<tr>
<td>20</td>
<td>R73</td>
<td>62.0</td>
<td>8.62</td>
<td>-</td>
<td>-</td>
<td>123.9</td>
<td>100</td>
</tr>
<tr>
<td>21</td>
<td>R70</td>
<td>64.2</td>
<td>11.48</td>
<td>-</td>
<td>-</td>
<td>123.9</td>
<td>86.2</td>
</tr>
<tr>
<td>22</td>
<td>R71</td>
<td>69.9</td>
<td>11.77</td>
<td>-</td>
<td>-</td>
<td>119.8</td>
<td>79.3</td>
</tr>
<tr>
<td>23</td>
<td>R81</td>
<td>73.2</td>
<td>9.96</td>
<td>-</td>
<td>-</td>
<td>121.8</td>
<td>69.8</td>
</tr>
<tr>
<td>24</td>
<td>R79</td>
<td>82.5</td>
<td>10.45</td>
<td>-</td>
<td>-</td>
<td>119 (117-121)</td>
<td>48.3</td>
</tr>
<tr>
<td>25</td>
<td>R84</td>
<td>88.1</td>
<td>11.75</td>
<td>-</td>
<td>-</td>
<td>118 (114-122)</td>
<td>25.9</td>
</tr>
<tr>
<td>Ave.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24.3(5)</td>
<td>122.4(6)</td>
</tr>
</tbody>
</table>

\(^{a}\) See footnote \(^{a}\) under Table 3.1

\(^{b}\) Peritectics + liquidus. Thermal effect from peak heights (rather than integral intensities)

\(^{c}\) From bottle, Sigma

\(^{d}\) Lit. m.p. for guaiacol is 28.0(4)°C

\(^{e}\) Liquidus only

\(^{f}\) Peritectic point: 15(2)% LA; 122.4(6)°C

\(^{g}\) Liquidus is not reached because of irreversible decomposition of the sample

\(^{h}\) Inclusion compound prepared by immersion method

\(^{i}\) Effected by decomposing LA

\(^{j}\) Eutectic point: 0.05(2) %LA; 24.3(5)°C

---

68
(2) The second experimental method used to study the phase diagrams was based on the analysis of the liquid and solid phases equilibrated at a certain temperature (solubility method). The mixtures of Leu-Ala and guest solvent containing both liquid and solid phases were equilibrated (stirring) in a thermostatted cell, protected from the atmosphere. The sample of liquor was taken at different time intervals, including 15 min, 30 min, 45 min and 60 min, to make sure the equilibrium had been reached. For all samples, the equilibrium was reached within 15 min. In all cases a portion of solid-free liquor was taken from the mixture using a pre-heated pipette equipped with a Millipore filter (0.45 µm). The liquid sample was examined in a TGA experiment at 1°C/min heating rate to measure the amount of free DMSO in the liquor and thus determine the solubility of the solid phase (Leu-Ala*DMSO) at this temperature. The points, each obtained as an average of several determinations, were plotted on the phase diagram to identify the liquidus curve. The solid phase was also recovered, separated from liquor and studied by TGA to determine and plot on the diagram its composition.

The solidus and liquidus curves on the phase diagrams and the fields of co-existing phases were located taking into account the experimental points, the character and magnitude of observed DSC effects, visual observations and other information available, as well as general knowledge on phase diagrams and inclusion compounds formation. Further experimental details of this work are given in the two following sections.
3.2.2.1 Leu-Ala – DMSO phase diagram

Pure DMSO not containing any water has a melting point of 18.8(3)°C. However, the melting point of DMSO is highly affected by the presence of water. DMSO-water mixtures exhibit abnormal behavior with respect to melting temperature and addition of water to DMSO causes a large decrease in the numerical value of the melting point. For example DMSO containing 10% water by weight melts at -4°C and DMSO containing 20% water by weight melts at -33°C. We observed that the DMSO melting point sensitivity to traces of water leads to an apparent decrease in the melting temperature of the eutectic mixture (Figure 3.2). In fact, the decrease is caused, to some extent, by small amounts of water available in the samples. Since DMSO is water sensitive, a special sealed DMSO bottle was ordered which was kept inside the glove box, and all manipulations were done in the glove box under a dry nitrogen atmosphere. A DSC experiment was done on a neat DMSO sample to ensure its purity. The result confirms a 18.1(8)°C melting point for DMSO, which is within acceptable range of literature value (18.8(3)°C).

In our DSC experiments, in order to protect the samples from moisture and the loss of volatile components, we used hermetically sealed high-pressure capsules. However, the accuracy of DSC measurements on the samples sealed in these massive capsules is lower than in regular DSC experiments. In particular, for the samples of small size the DSC peaks shifted to lower temperatures. This shift is not real and would not happen if the calibration parameters did not depend on the sample size. Apparently for the high-pressure capsules the calibration parameters obtained for large samples are not adequate for samples of smaller
size. Another error arises from irregular shape of the peaks for some of our samples. Broader peaks give lower onset temperatures than they should. These experimental problems are illustrated in Figure 3.3.

![Figure 3.2](image_url)

Figure 3.2. DSC thermograms of DMSO samples from old bottles (top and middle) and a new bottle kept sealed in a dry glove box (bottom). The observed differences in the melting point are due to traces of water absorbed from the atmosphere.

Due to the experimental problems described above, points which define the eutectic line on the phase diagram are observed at lower temperature for the samples with small amounts of eutectic mixture (which is almost pure DMSO in this case). Therefore, those
points were excluded from the calculation of the average temperature of the eutectic line on the diagram.

Figure 3.3. Apparent decrease in the melting temperature of DMSO when samples of different size are used. The size of the samples varies from 10.63 mg (top) to 2.22 mg (bottom). The decrease is not real and is caused by the massive high-pressure capsule

In the high-temperature region of the phase diagram, the system is complicated by irreversible degradation of the Leu-Ala dipeptide (see section 4.1.2). This process is greatly accelerated by the presence of a liquid phase. The degradation of guest-free LA occurs above ~115°C, while the peritectic line on the diagram is at 145.4(7)°C. This leads to two
consequences. First, the samples containing the guest-free phase of LA and the LA*DMSO inclusion phase show complex DSC thermograms in this region due to the overlap of irreversible degradation of LA and incongruent melting of LA*DMSO. Therefore, the temperature of the peritectic line in this region cannot be determined reliably. These experimental points are shown with vertical bars (estimated experimental error) on the phase diagram and they were not used in the calculation of the average peritectic temperature of 145.4(7)°C.

The second consequence is the rapid accumulation of LA degradation products in the liquor above the peritectic line which makes it impossible to reliably locate the liquidus curve. Therefore, the corresponding experimental points are shown with vertical bars and approximate positioning of the liquidus curve is shown by dashes.

Another experimental problem was the formation of gel-like phase when large amounts of LA were equilibrated with DMSO (solubility experiment). This complicated the selection of liquid and solid phases from the mixture for analysis. In order to confirm or exclude the formation of gel, the gel-like phase was studied using scanning electron microscopy (SEM). The resulting images (Figure 3.4) show the presence of very small crystals in the sample. The crystals had the shape of bundles 10µm in length, 1µm wide and ~0.2µm thick. First, this observation excluded the formation of gel in the system. Second, it suggested the use of very fine filter to isolate liquid phase for analysis. Thus, the Millipore 0.45 µm filters were used for this purpose.
Figure 3.4. SEM images showing the shape and dimensions of crystals of solid Leu-Ala*DMSO present in the mixture with saturated liquor at room temperature

A typical TGA thermogram of liquor equilibrated with solid at room temperature and separated using the Millipore filter is shown in Figure 3.5. The thermogram reveals two steps: the first mass loss corresponds to the evaporation of free DMSO solvent, and the resulting plateau yields the amount of LA in the sample. The compositions of the liquor determined by this method for different equilibration temperatures are given in Table 3.4. The composition of the solid phase in equilibrium at room temperature was also determined to confirm the stoichiometry of the inclusion compound phase (Table 3.4). The result is in excellent agreement with the 1:1 host to guest molar ratio. That gives us an extra experimental point which is shown on the phase diagram.
Figure 3.5. A typical TGA thermogram of saturated liquor in the system LA – DMSO. The plateau due to the solid LA residue is enlarged and shown in the insert.

Table 3.3. The compositions of liquid and solid phases (% LA) in the LA – DMSO system equilibrated at three temperatures (three determinations for each value)

<table>
<thead>
<tr>
<th>T, °C</th>
<th>25</th>
<th>50</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquor</td>
<td>0.37(4)</td>
<td>0.40(4)</td>
<td>0.52(4)</td>
</tr>
<tr>
<td>Solid phase&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.0(5)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Calculated % LA for LA*DMSO is 72.1
3.2.2.2 Leu-Ala – guaiacol phase diagram

Guaiacol is air and light sensitive. Samples darken upon exposure to air and light, so the guaiacol bottle used in our experiments was kept sealed in a dark place. Hermetically sealed high-pressure capsules were used for DSC experiments in order to protect the samples from air and moisture. As in the case of the LA – DMSO system, the LA – guaiacol system was complicated by irreversible degradation of LA. Since the LA*guaiacol melting temperature (122.4(6)°C) is in the same range as the degradation of guest-free LA (above 115°C), the determination of peritectic temperature for samples containing considerable amounts of guest-free LA was impossible. These points (shown in Figure 4.9 with vertical bars) were excluded from the average peritectic temperature calculation. The positioning of the liquidus curve above 130°C was also determined only approximately. The solubility experiments were also conducted for this system. The compositions of liquid and solid phases determined by this method for different equilibrium temperatures are given in Table 3.4.

Table 3.4. The compositions of liquid and solid phases (% LA) in the LA – guaiacol system equilibrated at three temperatures (three determinations for each value)

<table>
<thead>
<tr>
<th>T, °C</th>
<th>25</th>
<th>50</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquor</td>
<td>0.05(1)</td>
<td>0.05(1)</td>
<td>0.08(2)</td>
</tr>
<tr>
<td>Solid phaseᵃ</td>
<td>62.3(5)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

ᵃ Calculated % LA for LA*guaiacol is 62.0
The studied system is complicated in the low-temperature (subsolidus) region due to polymorphism of guaiacol. It has been reported that guaiacol forms at least two crystalline phases, called α and β.\textsuperscript{14} For slowly cooled samples, reported endotherm was assigned to a crystal-crystal transition in the temperature range of -23°C to -13°C. Rapid cooling of guaiacol, however, causes vitrification and induces a phase transition from glassy to an amorphous state at -68°C and crystallization at -43°C upon heating.\textsuperscript{15} We observed random thermal effects in the temperature range of -10°C to -60°C which corresponded to the previously reported phase transitions. Further studies are necessary to elucidate this polymorphism but they are beyond the scope of this research.

### 3.3 Instrumental methods and equipment

#### 3.3.1 Thermal analysis (TGA and DSC)

Thermal analysis is a useful tool for the characterization of materials including inclusion compounds.\textsuperscript{16,17} Thermal Gravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) are two methods commonly used for quantitative studies on stoichiometry, thermal stability and dissociation thermodynamics of inclusion compounds.

The TGA technique determines changes in weight as a response to change in temperature and therefore the mass of the substance is monitored as a function of temperature. TGA is a very useful method to determine accurate host:guest ratios when the
guest is relatively volatile. Release of the volatile guest occurs as the temperature increases. Eventually on TG curve, the guest component escapes from the system to yield a plateau corresponding to a guest-free form of the host material.

A Q5000-IR TGA analyzer from TA Instruments was used. Crystals taken from under their mother solutions were dried on a filter paper. Powder samples were analyzed as such. Approximately 10 mg of sample in a 100 µl platinum pan was used with a linear heating rate of either 5°C/min or 1°C/min, up to 300°C under a purge of dry nitrogen gas (25 ml/min). TGA curves were recorded as a plot of percent weight against temperature. The experiments were controlled and analyzed with Q Advantage and Universal Analysis software supplied with the instrument.

DSC is another thermoanalytical technique which determines the difference in the amount of heat which is required to increase the temperature of the sample and the reference measured as a function of temperature. Both the sample and reference are maintained at the same temperature throughout the experiment. The DSC method is a very important tool for characterization of new materials. It has been used to determine the temperature of melting or other physical transformations based on the principle that more or less heat will need to flow to the sample than to the reference to maintain both at the same temperature. In this research, DSC measurements were used to determine the temperatures of various phase transitions occurring in the systems studied.
A Q2000 DSC analyzer from TA Instruments was used for samples of both pure compounds (pure DMSO, guaiacol, Leu-Ala and inclusion compounds) and their mixtures. The instrument was calibrated using indium metal. Several sets of calibration parameters were acquired for experiments with heating rates of 1°C/min, 5°C/min and 10°C/min. Usually about 10 mg sample was analyzed in a high-pressure capsule under a flow of nitrogen gas (50 ml/min). The samples were heated from -80°C to 300°C using a linear heating rate of 1°C/min, 5°C/min or 10°C/min. The experiments were controlled and analyzed with Q Advantage and Universal Analysis software supplied with the instrument.

3.3.2 Visual observations

The stability of inclusion crystals was monitored visually using a microscope. For this purpose, crystals were separated from the mother solution and kept on a glass slide in air at ambient conditions. The temperatures and the characters of melting of some of the inclusion compounds were determined using Mel-Temp 1101D apparatus from Barnstead International. The samples were sealed in capillary tubes and heated at a rate of ~5°/min. The physical changes of the samples were monitored visually.
3.3.3 FT/IR spectroscopy

Fourier Transform Infrared Spectroscopy is used to obtain an infra-red spectrum of samples. A Nicolet iS10 spectrometer was used to study both solid samples and gases evolved during the TGA experiments. FT/IR was used for identification of the components of the evolved gas. The TGA/FT-IR module allows evolved gases to be introduced to a gas cell of the spectrometer through a transfer line which is heated up to 220°C to prevent the condensation of the evolved gas. The infra-red spectra were collected at a 0.483 cm\(^{-1}\) spectral resolution over a spectral range of 4000 to 500 cm\(^{-1}\) with a sampling interval of 60 seconds (46 scans per spectrum) for 60 min. The experiments were controlled and analyzed with OMNIC Series Software supplied with the instrument.

3.3.4 X-Ray diffraction analysis

X-Ray diffraction is a crucial tool for the characterization of new crystalline compounds. Single-crystal X-ray diffraction analysis makes it possible to determine the crystal structure of a sample down to atomic resolution. Therefore, it reveals the nature, composition and structural details of the compound. Powder X-ray diffraction is another technique in materials chemistry which is widely used for characterization of crystalline solids and identification of crystal phases. In this work, the X-ray diffraction experiments of single crystals and powder samples were conducted to confirm the nature or presence of certain crystalline phases. Full details of these studies are beyond the scope of this thesis.
Single-crystal X-ray diffraction experiments were conducted at 100(2) K to prevent
the crystals from decomposition and to reduce thermal motion of the atoms. The crystals
were taken directly from their mother liquor, immediately frozen and studied on a single-
crystal X-ray diffractometer with a Rigaku CuK\(_\alpha\) (\(\lambda = 1.54184\) Å) radiation source (rotating
anode) and a Bruker SMART6000 CCD detector (X-ray Facility at the Department of
Chemistry, McMaster University). The crystal structures were solved using the WinGX suite
of programs\(^{18}\) including SUPERFLIP and SHELXL software for crystal structure solution
and refinement. The analysis of crystal packing and structure visualization were done using
the XP and Mercury programs.

Powder X-ray diffraction experiments were conducted at room temperature on a
SuperNova single-crystal diffractometer equipped with a CuK\(_\alpha\) radiation microsource and
Atlas CCD detector. The powder patterns were generated and analyzed using CrysAlisPro
software (version 171.34.36) supplied with the instrument. The powder samples obtained in
the Leu-Ala – water isopiestic experiments were sealed in thin-glass capillaries which were
mounted on a goniometer head.
3.4 References


Chapter Four

Clathrate Formation in the LA – DMSO and LA – Guaiacol Binary Systems

4.1 Phase diagram of the system LA – DMSO and the properties of LA*DMSO inclusion compound

4.1.1 Description of the phase diagram

The determined X/T phase diagram of the LA – DMSO system is shown in Figure 4.1. The corresponding coordinates of the experimental points are listed in Tables 3.1 and 3.3 in Chapter 3. In the studied temperature range (-80°C to +200°C) only one binary compound forms with the 1:1 stoichiometry, LA*DMSO. According to the phase diagram study, the compound exists and is thermodynamically stable in the following concentration-temperature ranges. No low temperature limit for the compound existence has been detected in the studied range of -80°C to +200°C. The high temperature limit is defined by the temperature of its incongruent melting at 145.4(7) °C (peritectic equilibrium):

\[ \text{LA*DMSO}_{\text{solid}} = \text{LA}_{\text{solid}} + \text{liquor} \]  [4.1]

It should be noted that the LA*DMSO compound can exist as a metastable solid phase at higher temperatures. This is possible because the process [4.1] occurs with the formation of a pure LA solid which presumably has a different crystal structure. Therefore, the process depends on the nucleation and growth of this new phase. It was noted that when the samples containing LA*DMSO were heated faster, the incongruent melting was observed at a much higher temperature (Table 4.1). This effect was especially pronounced for the samples corresponding to either pure LA*DMSO or LA*DMSO mixed with liquor. The
seeds of pure LA solid phase are absent in such samples and hence the process is kinetically delayed.

Table 4.1. Temperatures of incongruent melting of the inclusion compound

<table>
<thead>
<tr>
<th>N</th>
<th>Sample</th>
<th>% LA</th>
<th>m, mg</th>
<th>Heating rate</th>
<th>Per. T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R18 a</td>
<td>72.1</td>
<td>10.25</td>
<td>1°C/min</td>
<td>148°C</td>
</tr>
<tr>
<td>2</td>
<td>R6 a</td>
<td>72.1</td>
<td>9.28</td>
<td>5°C/min</td>
<td>168°C</td>
</tr>
<tr>
<td>3</td>
<td>R53 a</td>
<td>72.1</td>
<td>9.87</td>
<td>10°C/min</td>
<td>172°C</td>
</tr>
</tbody>
</table>

a Inclusion compound prepared by immersion method

In the 0 to 72.1% LA concentration range the LA*DMSO compound exists as a mixture with solid DMSO below 16.2(6)°C. At this temperature the eutectic mixture of solid DMSO and LA*DMSO melts. The eutectic mixture is almost degenerate and its temperature is very close to the melting point of DMSO, which is 18.1(8)°C as detected in our DSC experiment (literature data is 18.8(3)°C). In other words, the eutectic mixture is almost pure DMSO. This fact has been also confirmed in the solubility measurements; the amount of LA in the mixture at 25°C is 0.37(4)%.

Above the eutectic line, LA*DMSO exists as a stable phase mixed with liquor. This X/T area, also called the crystallization field, is defined by the concentration of saturated liquor (liquidus curve), the composition line of LA*DMSO, and the eutectic and peritectic temperatures of 16.2(6)°C and 145.4(7)°C, respectively. It is interesting that the solubility of LA*DMSO is very low at temperatures below ~80°C but sharply increases above. That
means the crystallization of LA*DMSO by cooling hot solutions of LA in DMSO should be a very efficient method to prepare the compound, with nearly quantitative yield expected.

In the 72.1% to 100% LA concentration range the LA*DMSO compound exists as a mixture with the solid phase of pure LA, up to the peritectic line at 145.4(7)°C.

Above the peritectic line, the crystallization field of LA exists (LA + liquor); however, the preparation of crystalline LA would be very problematic due to its irreversible degradation at these temperatures as described in the next section.

The 1:1 stoichiometry of LA*DMSO was confirmed by DSC experiments since the samples with exact 1:1 composition revealed no effect at the eutectic temperature but showed maximal thermal effect at the temperature of the peritectic line (Figure 4.2). This composition was also confirmed by the TG analysis of the solid phase equilibrated with DMSO liquor at room temperature (shown as an open circle on the phase diagram), as well as analysis of the LA*DMSO compound obtained by crystallization and immersion methods (see section 4.1.3). However, taking into account the clathrate nature of the compound, we cannot exclude the existence of the same phase with lower DMSO content (that is where some molecules of guest DMSO are missing and the corresponding cavities in the crystal structure are vacant). Therefore, the solid phase of the inclusion compound can be, in fact, a phase of variable composition ("solid solution"), with a possible lower DMSO concentration limit as shown by a dotted line on the phase diagram to the right of the LA*DMSO line.
Figure 4.1. Phase diagram of the system LA – DMSO shown only for the 0-200°C temperature range. Solid circle points are obtained from DSC experiments. Typical error is 0.5-0.8°C. Error bars are shown when the error was larger due to irregular shape of the DSC peak. Open circles: points obtained from the solubility experiments. For the values and errors see Tables 3.1 and 3.3
4.1.2 Irreversible degradation of LA

The studied system was complicated by an irreversible process of LA degradation. The DSC thermogram of LA indicates a complex shape starting with an exotherm at \( \sim 115^\circ \text{C} \) (Figure 4.2). The TGA of LA shows a one step process with a mass loss corresponding to one mole of water. From the exothermic effect on the DSC curve, which is an indication of an irreversible process, and from the mass loss of one mole of water in TGA, we supposed that the process is an intramolecular condensation (cyclization) reaction leading to the cyclic form of LA:

\[
\begin{array}{c}
\text{H}_2\text{N}\quad \text{O} \\
\text{CH}_3 \\
\text{H}_3\text{C} \\
\text{CH}_3
\end{array} \quad \Delta T \quad -\text{H}_2\text{O} \quad \begin{array}{c}
\text{CH}_3 \\
\text{H}_3\text{C} \\
\text{H}_3\text{C} \\
\text{H}_3\text{C}
\end{array} \\
\text{[4.2]}
\]

It was demonstrated previously that the intramolecular cyclization is a common fragmentation mechanism in mass-spectrometry experiments on dipeptides. A significant number of studied dipeptides exhibited this transformation as a thermally-induced intramolecular condensation reaction.\textsuperscript{2,3,4}
Figure 4.2. Selected DSC thermograms of samples with different LA/DMSO ratios

Figure 4.2. Selected DSC thermograms of samples with different LA/DMSO ratios
A peritectic effect for samples with high LA content appears at lower temperatures, because it is superimposed by the effect of irreversible cyclization of LA. Since LA cyclization occurs at lower temperature than clathrate incongruent melting, it shifts the peritectic effect to lower temperatures. In particular, the reaction produces H\textsubscript{2}O, and a third component would reduce the melting point. Due to the cyclization reaction, the LA crystallization field over the peritectic line cannot be accurately outlined and the liquidus is plotted as short dashes on the phase diagram. The cyclization reaction was confirmed in TGA experiments with the inclusion compound (section 4.1.3).

4.1.3 Isolation and properties of the LA*DMSO inclusion compound

Leucyl-alanine has been known to form an inclusion compound with DMSO\textsuperscript{5,6}. In these two papers, only the crystal structure has been described, while neither a detailed synthesis nor properties of the compound have been reported.

The LA*DMSO compound crystallizes in the orthorhombic space group \textit{P}2\textsubscript{1}2\textsubscript{1}2\textsubscript{1}. The molecules of the Leu-Ala dipeptide build a 2D network through hydrogen bond formation between the terminal amino and carboxyl groups with an N\textsuperscript{\text┄}O distance of 2.79Å. The hydrogen-bonded layers are stacked at a distance of 9.4Å and interact with each other only by van der Waals forces. The alkyl side chains, which stand perpendicular to the layer, produce cavities which are occupied by the DMSO molecules. The DMSO molecules are hydrogen-bonded to the Leu-Ala backbone. The dipeptide molecules in the LA*DMSO
compound pile up in an anti-parallel arrangement in the C-terminal to N-terminal direction (Figure 4.3). From the reported crystal structure studies, LA*DMSO is an inclusion compound with the DMSO molecules included as guest species in the host matrix of the LA dipeptide.

![Layered structure of inclusion crystal of Leu-Ala with DMSO](image)

Figure 4.3. Layered structure of inclusion crystal of Leu-Ala with DMSO

In our study the LA*DMSO inclusion compound was isolated by three methods, including crystallization from an aqueous solution (similar to the method by Mitra\(^5\)) and the immersion method (according to the procedure by Akazome et al.\(^6\)). Based on our phase diagram study, we also introduced a third method of direct crystallization from hot DMSO solution. The crystallization products revealed colorless prismatic crystals (Figure 4.4). In air, the crystals do not show any sign of decomposition for at least several weeks. The product of the immersion method of synthesis was a fine-crystalline, powder-like material. All of the products were nearly identical in composition and properties.
Figure 4.4. Crystal shape of LA*DMSO inclusion compound (microscope).

Image size is 10 mm by 7.5 mm

The TGA results confirm the formation of the LA*DMSO inclusion compound with a 1:1 host to guest molar ratio (Figure 4.5). The observed mass loss percent of 34.0% is in a good agreement with the calculated mass loss percent (34.3%) for the loss of guest DMSO followed by the loss of one mole of water due to the cyclization reaction:

\[ \text{LA*DMSO} \rightarrow \text{LA} + \text{DMSO} \uparrow \]  \[4.3\]
LA → Cyclo-LA + H₂O ↑ \[4.4\]

Figure 4.5. Thermal dissociation of the LA*DMSO clathrate. Dashes show the positioning of expected plateaus for the indicated decomposition products.

The dashed lines in Figure 4.5 correspond to the calculated compositions of the LA*DMSO, pure LA and cyclic LA compounds. The guest release and product sublimation start at onset temperatures of 144°C and 240°C, respectively. The steps due to the guest and
water release are not well separated. It should be noted that the cyclization of pure, guest-free LA occurs at a much lower temperature of ~115°C (see the previous section). Based on this fact, we proposed that the release of a guest triggers the cyclization reaction which does not occur otherwise due to the stabilization of LA molecules in the clathrate phase.

In order to verify this hypothesis and to confirm the cyclization reaction, the gases evolved during the TGA experiment were studied with an FT-IR spectrometer. The TGA instrument was connected to the spectrometer with a gas cell through a heated transfer line and IR spectra were recorded simultaneously with the TGA results. The evolution of IR spectra over time is shown in Figure 4.6. The total 3D spectrum can be decomposed into the spectra of three individual substances (Figure 4.7). They correspond to DMSO, water and cyclic LA. In other words, the spectral study confirms the occurrence of processes [4.3] and [4.4]. It also confirms that the release of water occurs simultaneously with the release of the guest DMSO, or that process [4.3] induces process [4.4] and therefore the two steps in the TGA experiment cannot be separated.
Figure 4.6. 3D FT-IR spectrum of the gaseous products released in the course of the
LA*DMSO TG analysis
Figure 4.7. IR spectra of gases evolved in a TGA decomposition of LA*DMSO sample (in grey). The spectra correspond to DMSO (a), H2O (b) and cyclic LA (c). Spectra from a library (in black) are shown for comparison. In case of cyclic LA, the binary system is for solid material and so it shows several extra lines such as broad lines in the 3200-3500 cm\(^{-1}\) range due to stretching vibrations of N-H groups involved in hydrogen bonding.
The melting of LA\*DMSO inclusion crystals was visually observed at 152°C using a melting temperature apparatus. At this temperature, the compound turned to another solid plus liquor. DSC experiments conducted for the samples of the LA\*DMSO inclusion compound heated in a close volume show a strong endotherm followed by another, smaller endotherm (Figure 4.8). The first peak with onset temperature of 147.5°C corresponds to the incongruent melting of LA\*DMSO clathrate into solid LA and liquor. The second endotherm marks the liquidus curve. Apparently, the cyclization reaction starts as soon as the first amounts of liquor form. In other words, the system is not truly binary above the melting point and the registered temperature of the liquidus is only approximate. Further heating reveals a strong exothermal effect above 200°C due to further irreversible degradation of the peptide.

Figure 4.8. DSC curve for the LA\*DMSO clathrate
4.2 Phase diagram of the system LA – guaiacol and the properties of LA\(^*\)guaiacol inclusion compound

4.2.1 Description of the phase diagram

The determined X/T phase diagram of the LA – guaiacol system is shown in Figure 4.9. The corresponding coordinates of the experimental points are listed in Tables 3.2 and 3.4. The phase diagram shows similarities to the phase diagram of the LA – DMSO system described in section 4.1. Only one compound was observed in the studied temperature range of -80°C to +200°C, LA\(^*\)guaiacol. The compound is thermodynamically stable up to 122.4(6)°C where it melts incongruently into solid LA and liquor (peritectic equilibrium):

\[
\text{LA}^{*}\text{guaiacol}_{\text{solid}} = \text{LA}_{\text{solid}} + \text{liquor} \quad [4.5]
\]

Process [4.5] occurs with the formation of a new solid phase, LA. Since the process depends on the nucleation of this solid phase, the LA\(^*\)guaiacol compound can be overheated above its melting point.

The eutectic mixture of LA\(^*\)guaiacol with solid guaiacol melts at 24.3(5)°C (0-62.0% LA concentration). The eutectic is almost degenerate as its temperature is close to the melting point of pure guaiacol (26.3(8)°C as determined in our DSC experiment; literature value is 28.0(4)°C\(^7\)). The fact that the eutectic mixture is nearly pure guaiacol is also confirmed by the solubility experiment. The concentration of LA in the mixture at 25°C is 0.05(2)% which is even lower than for LA*DMSO solubility in DMSO.
Figure 4.9. Phase diagram of the system LA – guaiacol shown only for the 0-200°C temperature range. Solid circle points are obtained from DSC experiments. Typical error is 0.5-0.8°C. Error bars are shown when the error was larger due to irregular shape of the DSC peak. Open circles: points obtained from the solubility experiments. For the values and errors see Tables 3.2 and 3.4
The crystallization field of the compound is defined by the eutectic line, composition line of LA\textsuperscript{*}guaiacol, liquidus curve and peritectic temperature of 122.4(6)°C. In the 62.0-100% LA concentration range, the LA\textsuperscript{*}guaiacol compound exists as a mixture with the solid phase of pure LA up to the peritectic line. The peritectic temperature is considerably lower than in LA\textsuperscript{*}DMSO system. Nevertheless, it is still higher than the decomposition temperature of pure LA. Since the irreversible cyclization of LA starts at ~115°C as an exothermic effect, it makes it impossible to properly define the crystallization field of LA; the approximate location of liquidus is shown by dashes above the peritectic line. In fact, due to the formation of cyclic LA in this X/T area, the system is not binary anymore.

The 1:1 stoichiometry of LA\textsuperscript{*}guaiacol was observed in various independent experiments, similar to LA\textsuperscript{*}DMSO. Samples with an exact 1:1 composition show no effect of the eutectic melting, but maximal thermal effect of the peritectics. The composition of the LA\textsuperscript{*}guaiacol compound was also confirmed by TG analysis of solid phase equilibrated with guaiacol liquor at room temperature which is shown as an open circle on the phase diagram. TG analysis of the isolated product, obtained by immersion method, provides another confirmation of the 1:1 stoichiometry of the clathrate which is discussed in section 4.2.2. Since the clathrate might exist with lower guaiacol content where some cavities in the crystal structure are vacant, the solid phase of the inclusion compound can be a phase of variable composition with higher LA content. The corresponding possible concentration limit is shown by a dotted line on the right side of the LA\textsuperscript{*}guaiacol line on the phase diagram.
4.2.2 Isolation and properties of the LA*guaiacol inclusion compound

By analogy with other inclusion compounds of LA and by its properties, LA*guaiacol is an inclusion compound with guest guaiacol accommodated between H-bonded layers of LA. The LA*guaiacol clathrate was obtained by the immersion method in a fine-crystalline, powder-like form. The product was used for TGA and DSC experiments.

TG analysis of LA*guaiacol confirms formation of the inclusion compound with 1:1 stoichiometry (Figure 4.10). The TGA curve clearly reveals a plateau corresponding to the loss of 1 mole of guaiacol. The observed mass loss of 37.3% is in a good agreement with the calculated mass loss of 38.0% for process [4.6]. The second mass loss step, corresponding to the release of 1 mole of H\textsubscript{2}O in the cyclization reaction [4.7], is not resolved well and does not fit the calculated positioning for cyclic LA.

\[
\text{LA*guaiacol} \rightarrow \text{LA} + \text{guaiacol} \uparrow \quad [4.6]
\]

\[
\text{LA} \rightarrow \text{Cyclo-LA} + \text{H}_2\text{O} \uparrow \quad [4.7]
\]

The dashes in Figure 4.10 correspond to the initial LA*guaiacol and calculated mass losses of 1 mole of guaiacol and 1 mole of H\textsubscript{2}O. The guest release and product sublimation occur at onset temperatures of 111\,°C and 229\,°C, respectively. The guest release and H\textsubscript{2}O release steps are well separated. In contrast, the H\textsubscript{2}O mass loss exceeds the expected range. This implies that the cyclization process overlaps with the sublimation of cyclic LA.
A DSC experiment was conducted on the LA*guaiacol inclusion compound to determine its thermal stability and the highest temperature at which it is still thermodynamically stable (Figure 4.11). Upon heating in a closed volume, the compound experiences incongruent melting with a strong endotherm at 123.8°C, followed by liqudus superimposed by LA cyclization effect. Apparently, LA molecules released after the clathrate melts undergo an immediate cyclization reaction.
4.3 Conclusion

The two binary systems of this chapter present the first detailed physico-chemical study on clathrate formation of layered inclusion compounds of dipeptides. Two phase diagrams of this study reveal the stability characteristics of the LA*DMSO and LA*guaiacol inclusion compounds and phase relations between initial compounds and an inclusion compound they form. The two phase diagrams are similar in many aspects. Both indicate the formation of an inclusion compound as a thermodynamically stable solid phase with 1:1 host
to guest stoichiometry. In both cases, the inclusion compound melts incongruently into a
guest-free form of LA and liquor (equations 4.1 and 4.5).

When the melting points of pure host and guest are comparable, the inclusion
compound they form usually melts congruently.\textsuperscript{8,9} However, when the melting point of the
host is considerably higher, the inclusion compound usually melts incongruently, to produce
a guest-rich liquor and a guest-free solid phase of host.\textsuperscript{10,11,12,13} This is the case for the two
systems studied in this work.

The solubility of the clathrate in the guest liquid is very low in both systems, but it
increases significantly as temperature rises. That suggests that crystallization by cooling hot
solutions of LA in the guest solvent is a good method to prepare the inclusion compound.
Many crystals isolated in this work and described in the next chapter were obtained by this
method.

The condensation reaction complicates the two systems. In previous studies\textsuperscript{2,3,4},
cyclization of dipeptides was observed in the gaseous phase, while in our work it occurs as a
solid-state process. Remarkably, the temperature at which this intramolecular reaction occurs
depends on the phase which was subjected to heating. For LA*DMSO, LA*guaiacol and
guest-free LA, it occurs mostly above \(\sim 170^\circ\text{C}\), \(\sim 130^\circ\text{C}\) and \(\sim 115^\circ\text{C}\), respectively. LA
decomposes before melting. The two studied clathrates show different thermal stability. In
both cases however, the observed peritectic temperature is higher than the temperature of LA
cyclization. No exotherm is observed at \(\sim 115^\circ\text{C}\) when a solid clathrate phase is heated,
therefore LA cyclization does not happen when LA molecules reside in the clathrates. Thus, the guest stabilizes the host framework and prevents the host molecules from decomposition. The stabilization of host molecules by guest molecules, facilitated by van der Waals interactions, is called contact stabilization. The contact stabilization phenomena usually occur when the stability of a molecule is comparable to the stability of its decomposition products, and non-covalent interactions play a decisive role for favoring the formation of the molecule.\textsuperscript{14}

In general, the significance of these results is that they form a basis for the physico-chemical understanding of the formation of layered inclusion compounds between peptides and liquid organic guests. In particular, this understanding has facilitated progress on screening of aromatic guest candidates as described in the next chapter.
4.4 References


Chapter Five

Clathration Ability of LA with Respect to Aromatic Guest Molecules
5. Clathration Ability of LA with Respect to Aromatic Guest Molecules

5.1 Inclusion compounds of Leu-Ala with various guests

Prior to our study, few inclusion compounds of Leu-Ala as host had been reported. These were LA*DMSO\(^1\), a series of inclusions with sulfoxides LA*Guest\(^2\), and the tetrahydrate LA*4H\(_2\)O\(^3\) which also can be regarded as an inclusion compound. One goal of our study was to define other molecules that could act as guests to yield layered inclusions with LA. We were especially interested in aromatic molecules such as pyridine and its derivatives. Pyridine is an aromatic molecule with the ability to form a hydrogen bond to peptides both in solutions and in the crystal. The pyridyl ring is a frequent fragment in many biologically active molecules, such as nicotine and nicotinamide (vitamin B\(_3\)). No inclusions of LA with aromatic guest molecules have been reported in the literature. Therefore, we attempted to study a range of potential guest solvents including pyridine and its derivatives. Quinoline and isoquinoline were added to this list as examples of condensed aromatic molecules which occur naturally and can act as flavors or exhibit other bio-related properties. We also used guaiacol as another naturally occurring molecule as many of its derivatives are important components of various natural flavors (such as vanillin). Finally, benzene was used as an example of aromatic molecule that cannot form strong hydrogen bonds.

The results of the crystallization experiments are listed in Table 5.1. Crystalline products of crystallization from the corresponding guest solvent or powder-like products obtained by immersion method were examined by TGA to determine their composition and
relative thermal stability. Data for all new inclusion compounds of this study are listed in the Table, as well as new results for two compounds previously reported.

No inclusion compound was obtained with benzene. With all other attempted guests, inclusion compounds with a 1:1 host to guest stoichiometry were isolated. These guests were pyridine and its ten mono-, di-, or tri-substituted derivatives, quinoline, isoquinoline and guaiacol. Among pyridine derivatives, 4-benzylpyridine contains two separated aromatic rings in the same molecule.

For each inclusion compound in Table 5.1, three onset temperatures are listed which mark the start of three processes in TGA: the dissociation of the clathrate into solid LA and gaseous guest [5.1], cyclization of LA with release of water [5.2] and sublimation of cyclo-LA [5.3].

\[
\begin{align*}
\text{LA*Guest} & \rightarrow \text{LA} + \text{Guest} \uparrow & [5.1] \\
\text{LA} & \rightarrow \text{cyclo-LA} + \text{H}_2\text{O} \uparrow & [5.2] \\
\text{cyclo-LA} & \rightarrow \text{cyclo-LA} \uparrow & [5.3]
\end{align*}
\]

The total of [5.1], [5.2] and [5.3] was 100% in all cases. The calculated mass loss for [5.1] and [5.2] was in excellent agreement with experimentally observed values in most cases. For some inclusions, the processes [5.1] and [5.2] overlap and the total mass loss due to both guest and water release should be used instead. One can see from Table 5.1 that an overlap as well as deviations from calculated values are observed for inclusions which start
to dissociate at higher temperatures or for guest solvents with higher melting point. Possible causes of these deviations are the overlap of [5.1] and [5.2] with the sublimation [5.3], and partial melting of the samples that can occur at high temperatures.

The thermal stability of the studied inclusion compounds ranges considerably (the onset temperature of [5.1] is used here as a quantitative measure of thermal stability). There are two factors that will affect the onset temperature of [5.1]. One is the volatility of the guest: inclusions with guests which have lower boiling point are generally expected to be less stable. Another is the strength of host – guest interactions in the inclusion phase, i.e. the complementarity between host cavity and the guest molecule. Therefore, one should compare the order of thermal stability with the volatility of the corresponding neat guest solvents.

Guest solvents in Table 5.1 are listed in order of increasing boiling point. One can easily see that the thermal stability of inclusion phases does not follow this order. Therefore, we should conclude that the host – guest complementarity plays a crucial role in the stability of LA inclusion compounds.

Taking into account the volatility of guests, inclusions with pyridine and 4-methylpyridine are among the most stable, while inclusions with 2-ethylpyridine and 2,4,6-trimethylpyridine are the least stable ones. Evidently, a substituent in the 2-position on the pyridine ring makes the inclusion of the guest in the structure more difficult. This could be because the 2-substituent points out towards the dipeptide backbone and may weaken hydrogen bonding between the N-atom of the guest and the amino group of the dipeptide.
Other guests which do not appear to fit well in the host matrix are quinoline, isoquinoline and guaiacol.

The results of this study can be summarized by the following conclusions:

1. LA readily forms inclusion compounds with a 1:1 host to guest ratio with a wide range of pyridines.
2. LA can form inclusion compounds with molecules that vary in nature: water, aliphatic organics, and aromatics. The guest molecules may vary in size and shape, and possess different functional groups. Further studies are necessary to define the range of molecules that can be included as guests in the LA matrix.
3. The ideal guest molecule should both have a hydrophobic part and be able to form a hydrogen bond. Thus, pyridine derivatives are very suitable guests. The water molecule can form hydrogen bonds but does not have a hydrophobic fragment; it does form an inclusion with LA but its stability is low. On the other hand, organic molecules that cannot form hydrogen bonds do not form inclusions at all. One example is benzene; others are hexane and heptane which do not react and can be used as neutral solvents in the immersion method (see section 3.2.1.2).
4. Thermal stability of LA inclusions ranges considerably. Host – guest complementarity seems to have the greatest impact on the thermal stability.
5. The atom on guest molecule taking part in the hydrogen bond to the host matrix should not be obscured by other groups. For example, 2-substituents on the pyridine ring lower the stability of the inclusion compounds formed.
5.2 Clathrates of Leu-Ala with methylpyridines

5.2.1 Crystal structures

Akazome et al. have reported inclusion compounds of some dipeptides with sulfoxides\textsuperscript{2,4,5}. Their attention was primarily focused on dipeptides with aliphatic amino acids as their backbone. All of their reported inclusion compounds form layered structures built through hydrogen bonding between terminal amino and carboxyl groups of the dipeptide molecules, with the cavities being produced by the alkyl side chains of the dipeptide which stand perpendicular to the layer. The guest molecules are included between the layers, in the cavities provided by the alkyl side chains.

No inclusion compounds of Leu-Ala with an aromatic guest species has been reported before. As reported in 5.1, we isolated a number of Leu-Ala inclusions with aromatic guests. Fortunately, we obtained X-ray diffraction analysis quality single crystals of the inclusion compounds of 2-methylpyridine, 3-methylpyridine and 4-methylpyridine. The shape and size of the crystals are shown in Figure 5.1. The crystals obtained from different solvents had a different shape which indicated that they might have different structures.
Table 5.1. Inclusion compounds prepared by crystallization and/or immersion methods and their properties from TGA experiments

<table>
<thead>
<tr>
<th>Compound</th>
<th>b.p. (guest)°C</th>
<th>$T_{\text{onset}}$°C (guest release)</th>
<th>$T_{\text{onset}}$°C (water release)</th>
<th>$T_{\text{onset}}$°C (the rest)</th>
<th>Mass loss/% a</th>
<th>Calc. mass loss/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu-Ala*4H₂O</td>
<td>100</td>
<td>57</td>
<td>149</td>
<td>220</td>
<td>25.7; 6.8</td>
<td>26.3, 6.6</td>
</tr>
<tr>
<td>Leu-Ala*pyridine</td>
<td>115</td>
<td>137</td>
<td>177</td>
<td>227</td>
<td>28.3; 6.2</td>
<td>28.1, 6.4</td>
</tr>
<tr>
<td>Leu-Ala*2-methylpyridine</td>
<td>129</td>
<td>131</td>
<td>153</td>
<td>235</td>
<td>31.4; 5.1</td>
<td>31.5, 6.1</td>
</tr>
<tr>
<td>Leu-Ala*3-methylpyridine</td>
<td>144</td>
<td>136</td>
<td>152</td>
<td>230</td>
<td>31.9; 5.1</td>
<td>31.5, 6.1</td>
</tr>
<tr>
<td>Leu-Ala*4-methylpyridine</td>
<td>145</td>
<td>152 c</td>
<td>-</td>
<td>226</td>
<td>37.7</td>
<td>37.6</td>
</tr>
<tr>
<td>Leu-Ala*2-ethylpyridine</td>
<td>149</td>
<td>110</td>
<td>177</td>
<td>223</td>
<td>34.8; 5.5</td>
<td>34.6; 5.8</td>
</tr>
<tr>
<td>Leu-Ala*4-ethylpyridine</td>
<td>168</td>
<td>143 c</td>
<td>-</td>
<td>226</td>
<td>40.3</td>
<td>40.4</td>
</tr>
<tr>
<td>Leu-Ala*2,4,6-trimethylpyridine</td>
<td>171</td>
<td>111</td>
<td>182</td>
<td>229</td>
<td>37.2; 7.2</td>
<td>37.5, 5.6</td>
</tr>
<tr>
<td>Leu-Ala*3,5-dimethylpyridine</td>
<td>172</td>
<td>99; 127 d</td>
<td>183</td>
<td>223</td>
<td>34.5; 6.0</td>
<td>34.7, 5.8</td>
</tr>
<tr>
<td>Leu-Ala*3,4-dimethylpyridine</td>
<td>179</td>
<td>140</td>
<td>156</td>
<td>219</td>
<td>35.9; 5.1</td>
<td>34.7, 5.8</td>
</tr>
<tr>
<td>Leu-Ala*DMSO</td>
<td>189</td>
<td>144 c</td>
<td>-</td>
<td>228</td>
<td>34.0</td>
<td>34.3</td>
</tr>
<tr>
<td>Leu-Ala*guaiacol e</td>
<td>205</td>
<td>107</td>
<td>164</td>
<td>210</td>
<td>37.2; 9.2</td>
<td>38.0; 5.5</td>
</tr>
<tr>
<td>Leu-Ala*quinoline</td>
<td>237</td>
<td>118</td>
<td>184</td>
<td>223</td>
<td>40.5; 5.9</td>
<td>40.0, 5.4</td>
</tr>
<tr>
<td>Leu-Ala*isoquinoline</td>
<td>243</td>
<td>144</td>
<td>168</td>
<td>219</td>
<td>38.1; 6.5</td>
<td>40.0, 5.4</td>
</tr>
<tr>
<td>Leu-Ala*4-benzylpyridine e</td>
<td>288</td>
<td>132 c</td>
<td>-</td>
<td>217</td>
<td>45.3</td>
<td>50.4</td>
</tr>
<tr>
<td>Leu-Ala*4-pyridinepropanol e</td>
<td>289</td>
<td>132 c</td>
<td>-</td>
<td>212</td>
<td>52.5</td>
<td>45.8</td>
</tr>
</tbody>
</table>

a Literature value of boiling point of the corresponding guest solvent
b Mass loss due to guest and water release respectively. The residue after the last step was close to zero
c The guest release occurred simultaneously or overlapped with the release of water
d The thermogram is complicated indicating a two step process for the guest release
e Inclusion compounds which were obtained by immersion method. All others were prepared by crystallization method
Figure 5.1. Crystals obtained by crystallization of Leu-Ala from different solvents: top left: DMSO; top right: 2-methylpyridine; bottom left: 3-methylpyridine; bottom right: 4-methylpyridine

A summary of preliminary X-ray diffraction analysis results on the studied crystals is given in Table 5.2. The inclusions of all three methylpyridine isomers crystallize in the orthorhombic space group $P2_12_12_1$ with similar unit cell dimensions and can be regarded as isostructural. ORTEP projections of the guest and host molecules found in the crystal structures are shown in Figure 5.2.
All three inclusions have a layered structure similar to the structure of LA*DMSO. The molecules of methylpyridine reside in the interlayer space and form a hydrogen bond to the amino group of LA: N\textsubscript{LA} – H···N\textsubscript{MePy}. The ideal host to guest molar ratio is 1:1 in all the structures. The crystal packing in LA*4-methylpyridine along the \textit{a}-axis is shown in Figure 5.3, top. The host Leu-Ala molecules self-assemble to form a layer structure through intermolecular hydrogen bonding between their terminal amino and carboxyl groups. In the cavity space between the layers, the 4-methylpyridine guest molecules are included. They form a hydrogen bond from their nitrogen atom to the amino proton of the host Leu-Ala backbone, with an N···N distance of 2.89Å. The sheets pile up in an anti-parallel direction along the C-terminal to N-terminal. The layers of the Leu-Ala backbone resemble the \(\beta\)-sheet of a protein. In the spacefill projection (Figure 5.3, bottom), the \(\beta\)-sheets which form the dipeptide backbone are colored black, the guest 4-methylpyridine is gray and the alkyl groups (alkyl = methyl and isobutyl) of Leu-Ala are white. This space-filling model reveals how the guest molecules are arranged between the host layers and are intercalated in the cavities produced by the alkyl side chains of the dipeptide which stand perpendicular to the layer. LA*2-methylpyridine and LA*3-methylpyridine have similar structures.
Figure 5.2. ORTEP projections of the guest and host molecules in the crystal structures of Leu-Ala*2-methylpyridine (top), Leu-Ala*3-methylpyridine (middle), Leu-Ala*4-methylpyridine (bottom). H-atoms are omitted.
Table 5.2. Crystal data for Leu-Ala inclusions with methylpyridine isomers

<table>
<thead>
<tr>
<th>Compound</th>
<th>LA(^{*})2-MePy</th>
<th>LA(^{*})3-MePy</th>
<th>LA(^{*})4-MePy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature / K</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Crystal shape</td>
<td>plate</td>
<td>plate</td>
<td>block</td>
</tr>
<tr>
<td>Crystal size / mm</td>
<td>0.45x0.22x0.20</td>
<td>0.32x0.30x0.07</td>
<td>0.50x0.50x0.50</td>
</tr>
<tr>
<td>Crystal system</td>
<td>orthorhombic</td>
<td>orthorhombic</td>
<td>orthorhombic</td>
</tr>
<tr>
<td>Space group</td>
<td>(P2_12_12_1)</td>
<td>(P2_12_12_1)</td>
<td>(P2_12_12_1)</td>
</tr>
<tr>
<td>(a) / Å</td>
<td>5.459(1)</td>
<td>5.338(1)</td>
<td>5.191(1)</td>
</tr>
<tr>
<td>(b) / Å</td>
<td>15.720(2)</td>
<td>15.936(4)</td>
<td>15.968(4)</td>
</tr>
<tr>
<td>(c) / Å</td>
<td>19.415(2)</td>
<td>19.894(5)</td>
<td>20.013(5)</td>
</tr>
<tr>
<td>(V) / Å(^3)</td>
<td>1666.1(4)</td>
<td>1692.3(7)</td>
<td>1658.9(7)</td>
</tr>
<tr>
<td>(Z)</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>(D_{calc.}) / g cm(^{-3})</td>
<td>1.178</td>
<td>1.159</td>
<td>1.183</td>
</tr>
<tr>
<td>Total reflections</td>
<td>11022</td>
<td>11241</td>
<td>10919</td>
</tr>
<tr>
<td>Unique ((I&gt;2\sigma(I)))</td>
<td>2789</td>
<td>2647</td>
<td>2784</td>
</tr>
<tr>
<td>(R) (reflections with ((I&gt;2\sigma(I))))</td>
<td>0.030</td>
<td>0.049</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Although all inclusion compounds with Leu-Ala that we have studied have similar structures, they vary in their interlayer distance. The half-length of the \(c\)-axis, corresponding to the interlayer distance, expands from 9.707(1) Å to 10.007(2) Å when different isomer guests are included. In other words, the interlayer distance in the inclusion crystal is variable in response to the size and shape of a specific guest. Table 5.3 provides a comparison of interlayer distances as an important structural characteristic of the studied crystal structures.
Figure 5.3. A fragment of crystal packing of Leu-Ala*4-MePy along the $\alpha$-axis. The arrows point C-terminal end to N-terminal end (top). Spacefill projection of Leu-Ala*4-MePy crystal packing (bottom)
Table 5.3. Interlayer distance in the studied inclusion compounds

<table>
<thead>
<tr>
<th>Inclusion compound</th>
<th>Interlayer distance / Å</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA*DMSO (^a)</td>
<td>9.353(1)</td>
<td>Akazome et al. (^2)</td>
</tr>
<tr>
<td>LA*2-MePy</td>
<td>9.707(1)</td>
<td>This work</td>
</tr>
<tr>
<td>LA*3-MePy</td>
<td>9.947(2)</td>
<td>This work</td>
</tr>
<tr>
<td>LA*4-MePy</td>
<td>10.007(2)</td>
<td>This work</td>
</tr>
</tbody>
</table>

\(^a\) LA*DMSO was studied at room temperature, while the inclusions with methylpyridines were studied at 100 K.

Another characteristic is packing efficiency, which is a very important factor of stability in molecular crystals.\(^6,7\) Due to the nearly identical molecular volume of the methylpyridine isomers, the densities of the studied inclusion crystals (Table 5.2) must directly relate to the packing coefficients of the crystal structures. Therefore, the inclusion with 4-MePy has the highest packing coefficient and the inclusion with 3-MePy the lowest: 4-MePy > 2-MePy > 3-MePy. One can see that LA*4-MePy has the highest efficiency in spite of having the greatest interlayer distance in the clathrate. This is possibly due to the high flexibility of the peptide \(\beta\)-sheet layer based on hydrogen bonds. The host layer structure easily changes to accommodate different guest molecules. This property of the layered peptide matrix could be used to incorporate larger molecules of biological importance, although the limits of this flexibility remain unknown.

Layered organization is very common in the crystals of hydrophobic dipeptides.\(^8\) The three clathrates structurally characterized in this work and the previously reported
LA*DMSO\(^2\) have a layered structure. A similar structural motif was observed in the clathrate of another dipeptide with DMSO, Leu-Leu*DMSO (monoclinic, \(P2_1\)), with the interlayer distance defined as \(c \cdot \sin \beta = 11.09 \text{ Å}\).\(^9\) Layered organization was also observed in a series of inclusions of Leu-Leu-Leu with pyridine and the three methylpyridine isomers, with the interlayer distance ranging between 11.5 and 11.8 Å.\(^10\) We can expect layered organization for all inclusions isolated in our studies. Structural analysis of these inclusions currently underway confirms this conclusion but the results are beyond the scope of this thesis.

5.2.2 Formation and stability

It was interesting to compare the stability of the inclusion compounds of the three methylpyridine isomers as guests, as well as the parameters of the host – guest reaction. First, we wanted to see how the differences in the methyl substituent position and the resulting crystal structure affect the properties of the bulk inclusion materials. Second, the separation of isomers is one of the important uses of inclusion compounds (see Chapter 2). The inclusion with unsubstituted pyridine was added to these studies for comparison.

As one can see from Table 5.1, the thermal stability of the inclusions with the three methylpyridines correlates with the boiling point of the guest. However, for the 2- and 4-isomers, the dissociation of the clathrates in the TGA experiment starts at a higher temperature, unlike for the 3-methylpyridine isomer.
The TGA curves of the three inclusions are compared in Figure 5.4. It was a reproducible result that the clathrate with the 4-isomer dissociates at a considerably higher temperature than the clathrate with the 3-isomer, in spite of the two methylpyridines having nearly the same boiling temperature. In other words, 4-methylpyridine is held more tightly in the host matrix.

This result is in excellent agreement with higher density of the 4-methylpyridine clathrate that implies higher packing efficiency of molecules in the crystal. The order of packing efficiency discussed in the previous section 4-MePy > 2-MePy > 3-MePy correlates very well with the order of thermal stability of the clathrates if we compare the differences between the guest release onset temperature and the boiling point of the corresponding methylpyridine.

The differences in the thermal stability of the inclusion phases also cause different pathways of their thermal degradation. The TG curve for the 2-methylpyridine clathrate reveals distinct steps corresponding to the release of guest and water. In the case of the clathrate with 4-methylpyridine, the whole process occurs as a single step.

Figure 5.5 compares the DSC thermograms of LA and its inclusions with pyridine and methylpyridines. The DSC experiments reveal the stability of the inclusion compounds in a closed volume, that is with high partial pressure of a released component. It should be noted that in our DSC experiments, guest release was not possible since the samples were sealed in high-pressure capsules. The beginning of the first thermal effect on the thermogram
(which could be endo or exo) marks the highest temperature limit of existence for the corresponding solid phase. Upon heating in a closed volume, all inclusion compounds experience a strong endotherm on the DSC thermogram which corresponds to the incongruent melting of the inclusion compound followed by another endotherm which corresponds to liquidus overlapped with LA cyclization:

\[
\text{LA}^*\text{MePy}_{\text{solid}} = \text{LA}_{\text{solid}} + \text{liquor} \quad [5.4]
\]

Figure 5.4. Overlay of TGA thermograms of LA inclusion compounds with methylpyridine isomers. Dashes show the positioning of expected plateaus for the indicated decomposition products.
Cyclization of guest-free LA starts at \( \sim 115^\circ\text{C} \) with an exotherm and the thermogram has a complex shape. The inclusion compounds indicate different thermal stability, but they all can exist at a higher temperature than the guest-free phase of LA. An interesting result is that the inclusion compound with pyridine is the most stable in spite of being the most volatile guest in this series. Therefore, the inclusion of a guest in LA stabilizes the molecule of dipeptide, and different guests provide different degree of this stabilization.

Figure 5.5. DSC thermograms of LA and its inclusions with pyridine and methylpyridines
The TGA and DSC experiments clearly indicate that the geometry of guest species has important consequences on the stability of the resulting inclusions, the overall mechanism of their decomposition and the temperature at which the degradation of the host LA molecule occurs.

Sorption experiments conducted with pyridine and its methyl derivatives revealed that the dissociation reaction as studied by TGA is reversible. Guest-free Leu-Ala readily reacts with vapors of these solvents as judged from significant mass increase of the dipeptide samples in the atmosphere of these guests (up to 40% mass uptake). The process occurs faster with pyridine (equation 5.5) and slower with methylpyridines. As shown in Figure 5.6, the reaction with pyridine is complete in four days and corresponds approximately to a 1:1 host to guest stoichiometry of the final product. The reaction with methylpyridines was much slower, indicating kinetic problems. The first uptake of about half a mole of methylpyridine was followed by a very slow uptake that was not complete over one month. The slow step can be attributed to a slow diffusion of guest to the empty cavities inside the particles of the dipeptide. It should be noted that the rate of sorption depended strongly on the history of sample preparation and varied considerably even for pyridine. Additional studies are required in order to elucidate what factors affect sorption and to evaluate the dipeptide as a potential sorbent material.

\[ \text{LA}_{\text{solid}} + \text{Py}_{\text{gas}} = \text{LA*Py}_{\text{solid}} \]  

[5.5]
Figure 5.6. Sorption of pyridine and methylpyridines by LA as a function of time
5.3 References


Chapter Six

The Formation of Tetrahydrate in the System LA – Water
6. The Formation of Tetrahydrate in the System LA – Water

6.1 Properties of the Leu-Ala tetrahydrate

In the two previous chapters, we described a number of inclusion compounds with the Leu-Ala dipeptide as host. Several crystal structures were reported in this thesis and by three other research groups. However, the isolation of crystalline guest-free Leu-Ala has never been reported. Görbitz studied the crystals of Leu-Ala dipeptide as purchased from Sigma which were larger than required (several mm).\(^1\) His studies revealed that the crystals contain four moles of water in a layered structure (interlayer distance 9.941(1) Å). The water molecules, acting as guests, are segregated in columns in the crystal, as are the hydrophobic side chains of the dipeptide. The layers incorporate two head-to-tail hydrogen bonds as in other inclusion crystals of LA, but unlike other structures, one of the bonds is interrupted by a bridging water molecule (Figure 6.1). The structural stoichiometry of the compound is Leu-Ala*4H\(_2\)O.

Since the water molecule is too small to fill the whole cavity in the Leu-Ala layered structure, four H\(_2\)O molecules fill the cavity space and the \(\beta\)-sheets bend in wave-like nets. In the case of other studied clathrates when the guest molecule is larger, it fills the interlayer cavity space well and, as a result, only one molecule of the guest is included. The water molecules form an extensive system of hydrogen bonds which connect the \(\beta\)-sheets. Therefore, unlike other inclusions of LA, the tetrahydrate has a 3D H-bonded network.
Samples of the chemical from different bottles (even from the same company; see Chapter 3) considerably varied in the amount of water that they contained. Therefore, we crystallized the chemical from water. Although Görbitz stated that the compound does not crystallize as elongated prisms,¹ our crystals had exactly this shape (Figure 6.2). TGA confirmed the composition LA*4H₂O of the bulk crystalline product (Table 5.1). The DSC thermogram of the tetrahydrate (sealed capsule) shows a sharp endotherm with T_{onset} = 52.3°C. Visual observation of heated crystals sealed in a capillary tube confirms the incongruent-type melting of the compound (~57°C). Further heating results in a complex series of endo- and exothermal effects on the DSC thermogram above 100°C. Visual observations indicate disappearance of the solid phase at ~132°C. These results reveal similarities of the system LA – water with the two other systems studied and described in Chapter 4.
The terahydrate is the only compound in the studied series with a 1:4 host to guest molar ratio, while all the other inclusion compounds have a 1:1 stoichiometry. The interesting unusual stoichiometry of the LA*4H₂O inclusion compound made us think of other possible compounds which might form in this system. Since the vapor pressure of water can be easily controlled using salt or sulfuric acid solutions, we used an isopiestic technique to study the dependence of equilibrium water pressure over solid phase as a function of X in the system LA – water.
6.2 Sorption isotherm of water by solid LA

The sorption isotherm of water by solid Leu-Ala at 298 K is shown in Figure 6.3. Numerical data and some details of the isopiestic experiment are listed in Table 6.1. Increasing water vapor pressure from zero to \( \sim 0.86 \ P/P_0 \) results in the gradual uptake of \( \sim 1 \) mole of water. At a pressure of \( 0.86 \pm 0.05 \ P/P_0 \), the uptake rises stepwise to \( \sim 4 \) moles of water per 1 mole of the dipeptide. The water uptake is reversible as was demonstrated for several samples. The equilibrium is attainable in 1 to 20 days for the direct (sorption) process.

After the equilibrium in the samples was reached, they were hermetically sealed in thin-wall glass capillary tubes and studied by the powder XRD. Only two different powder patterns were observed as shown in Figure 6.4 and in Table 6.1. Pattern I was observed for totally a dehydrated Leu-Ala dipeptide and for all samples equilibrated under water vapor pressure \( < 0.86 \ P/P_0 \). No shift of diffraction peaks was observed indicating no change in crystal structure or unit cell dimensions. Pattern II was observed for samples equilibrated under water vapor pressure \( > 0.86 \ P/P_0 \). It was identical to the powder pattern of the crystals of LA tetrahydrate, LA\*4H_2O, and corresponded well to the pattern calculated from the crystal structure data. Again, no shift of the diffraction peaks was observed for the different samples.
Figure 6.3. P/X dependence of H$_2$O:LA molar ratio (X) on the partial water vapor pressure ($P/P_0$) over solid LA at 298 K. Circular and square points represent types I and II powder diffraction patterns, respectively. Solid points were obtained using sulfuric acid solutions and open circle points were obtained using saturated salt solutions (see Table 6.1). Error bars do not account for a possible systematic error due to surface sorption of water on the LA and LA*4H$_2$O phases.
Table 6.1. Sorption of water by dry LA powder samples as a function of partial water vapor pressure: the results of isopiestic and powder XRD experiments (298 K)

<table>
<thead>
<tr>
<th>N</th>
<th>Sample</th>
<th>Solution used</th>
<th>Water pressure $(P/P_0)$</th>
<th>Molar ratio H₂O:LA (X)</th>
<th>Estimated error for X</th>
<th>Time required (days)</th>
<th>Structural type a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R107</td>
<td>10.0% H₂O in H₂SO₄</td>
<td>0.0003</td>
<td>0.02</td>
<td>0.04</td>
<td>1</td>
<td>Type I</td>
</tr>
<tr>
<td>2</td>
<td>R106</td>
<td>20.0% H₂O in H₂SO₄</td>
<td>0.006</td>
<td>0.11</td>
<td>0.06</td>
<td>1</td>
<td>Type I</td>
</tr>
<tr>
<td>3</td>
<td>R105</td>
<td>30.0% H₂O in H₂SO₄</td>
<td>0.052</td>
<td>0.15</td>
<td>0.07</td>
<td>1</td>
<td>Type I</td>
</tr>
<tr>
<td>4</td>
<td>R104</td>
<td>40.0% H₂O in H₂SO₄</td>
<td>0.165</td>
<td>0.37</td>
<td>0.08</td>
<td>1</td>
<td>Type I</td>
</tr>
<tr>
<td>5</td>
<td>R115</td>
<td>48.1% H₂O in H₂SO₄</td>
<td>0.312</td>
<td>0.41</td>
<td>0.13</td>
<td>2</td>
<td>Type I</td>
</tr>
<tr>
<td>6</td>
<td>R103</td>
<td>50.0% H₂O in H₂SO₄</td>
<td>0.353</td>
<td>0.53</td>
<td>0.11</td>
<td>2</td>
<td>Type I</td>
</tr>
<tr>
<td>7</td>
<td>R116</td>
<td>51.8% H₂O in H₂SO₄</td>
<td>0.389</td>
<td>0.40</td>
<td>0.13</td>
<td>2</td>
<td>Type I</td>
</tr>
<tr>
<td>8</td>
<td>R117</td>
<td>56.0% H₂O in H₂SO₄</td>
<td>0.481</td>
<td>0.57</td>
<td>0.13</td>
<td>2</td>
<td>Type I</td>
</tr>
<tr>
<td>9</td>
<td>R118</td>
<td>57.6% H₂O in H₂SO₄</td>
<td>0.515</td>
<td>0.66</td>
<td>0.14</td>
<td>2</td>
<td>Type I</td>
</tr>
<tr>
<td>10</td>
<td>R109</td>
<td>saturated Ca(NO₃)₂</td>
<td>0.559</td>
<td>0.74</td>
<td>0.10</td>
<td>2</td>
<td>Type I</td>
</tr>
<tr>
<td>11</td>
<td>R120</td>
<td>60.0% H₂O in H₂SO₄</td>
<td>0.565</td>
<td>0.70</td>
<td>0.13</td>
<td>2</td>
<td>Type I</td>
</tr>
<tr>
<td>12</td>
<td>R102</td>
<td>60.0% H₂O in H₂SO₄</td>
<td>0.565</td>
<td>0.67</td>
<td>0.17</td>
<td>2</td>
<td>Type I</td>
</tr>
<tr>
<td>13</td>
<td>R77</td>
<td>saturated NH₄Cl</td>
<td>0.568</td>
<td>0.83</td>
<td>0.09</td>
<td>2</td>
<td>Type I</td>
</tr>
<tr>
<td>14</td>
<td>R119</td>
<td>62.9% H₂O in H₂SO₄</td>
<td>0.624</td>
<td>0.78</td>
<td>0.14</td>
<td>2</td>
<td>Type I</td>
</tr>
<tr>
<td>15</td>
<td>R121</td>
<td>67.1% H₂O in H₂SO₄</td>
<td>0.702</td>
<td>0.86</td>
<td>0.15</td>
<td>2</td>
<td>Type I</td>
</tr>
<tr>
<td>16</td>
<td>R101</td>
<td>70.0% H₂O in H₂SO₄</td>
<td>0.751</td>
<td>0.94</td>
<td>0.15</td>
<td>2</td>
<td>Type I</td>
</tr>
<tr>
<td>17</td>
<td>R108</td>
<td>saturated NaCl</td>
<td>0.758</td>
<td>0.94</td>
<td>0.10</td>
<td>2</td>
<td>Type I</td>
</tr>
<tr>
<td>18</td>
<td>R122</td>
<td>71.8% H₂O in H₂SO₄</td>
<td>0.780</td>
<td>1.08</td>
<td>0.13</td>
<td>2</td>
<td>Type I</td>
</tr>
<tr>
<td>19</td>
<td>R123</td>
<td>74.4% H₂O in H₂SO₄</td>
<td>0.817</td>
<td>1.23</td>
<td>0.14</td>
<td>2</td>
<td>Type I</td>
</tr>
<tr>
<td>20</td>
<td>R124</td>
<td>77.3% H₂O in H₂SO₄</td>
<td>0.852</td>
<td>1.54</td>
<td>0.12</td>
<td>7</td>
<td>Type I</td>
</tr>
<tr>
<td>21</td>
<td>R100</td>
<td>80.0% H₂O in H₂SO₄</td>
<td>0.880</td>
<td>3.80</td>
<td>0.20</td>
<td>15</td>
<td>Type II</td>
</tr>
<tr>
<td>22</td>
<td>R125</td>
<td>83.6% H₂O in H₂SO₄</td>
<td>0.913</td>
<td>3.85</td>
<td>0.20</td>
<td>21</td>
<td>Type II</td>
</tr>
<tr>
<td>23</td>
<td>R99</td>
<td>90.0% H₂O in H₂SO₄</td>
<td>0.955</td>
<td>4.16</td>
<td>0.22</td>
<td>10</td>
<td>Type II</td>
</tr>
<tr>
<td>24</td>
<td>R74</td>
<td>100.0% H₂O in H₂SO₄</td>
<td>1</td>
<td>4.39</td>
<td>0.30</td>
<td>12</td>
<td>Type II</td>
</tr>
</tbody>
</table>

*a From powder X-ray diffraction analyses*
From the results obtained, only two thermodynamically stable solid phases exist in the system LA – H₂O. One phase is observed when the water vapor pressure is low. This phase has the same crystal structure as totally dehydrated LA and exhibits a powder
diffraction pattern of type I (Figure 6.4). It is not clear at the moment if this phase can accommodate water molecules in its structure. The isotherm indicates an uptake of up to 1 mole of water (~9% by mass). At the same time, the lack of shifting in the diffraction peaks proves the structure does not expand; some expansion might be expected taking into account the considerable amount of water in the sample and flexibility of peptide crystals in general. On the other hand, the water uptake may occur due to adsorption on the developed surface of the peptide material. As one can see from Figure 6.4, the diffraction peaks of Pattern I are very broad, implying the crystallites are of small size. It is also not clear what fraction of the sample is crystalline and if a significant amount of amorphous phase is present. Therefore, we can tentatively assume the solid phase of Pattern I is the guest-free LA.

It appears that the guest-free form of LA does not form crystals easily. Heating of the dehydrated LA in nitromethane does not change the diffraction pattern, with the peaks remaining broad. The difficulty of forming its own densely packed crystal structure may be the reason why LA is a versatile host material. The subject was discussed by Kitaigorodsky from the viewpoint of the Principle of Close Packing. The molecules of irregular shape that cannot create efficient packing without other molecules have been proved to be excellent hosts. The first two strong peaks on Pattern I correspond to $d$ distances of ~12.5 and ~7.05 Å. It is not likely the solid has a layered structure with the interlayer distance of 12.5 Å (the interlayer distance in four inclusions listed in Table 5.3 varies from 9.353 to 10.007 Å). It is worth mentioning that another dipeptide, Leu-Leu, forms similar layered inclusions with DMSO, and several alcohols, while with water it forms a totally different structure with channels partially filled with water.
At water pressures > 0.86 \( P/P_0 \), the tetrahydrate phase \( \text{LA}^*4\text{H}_2\text{O} \) forms and is seen in diffraction Pattern II:

\[
\text{LA}_{\text{solid}} + 4 \text{H}_2\text{O}_{\text{gas}} = \text{LA}^*4\text{H}_2\text{O}_{\text{solid}} \quad [6.1]
\]

The value \( P/P_0 = 0.86 \pm 0.05 \) is the equilibrium water vapor pressure over the tetrahydrate. This is also the minimal pressure at which the tetrahydrate may form in the process [6.1] and therefore can be referred to as the “gate pressure”. As one can see, the equilibrium pressure over the tetrahydrate is only slightly lower than over pure water. In other words, the thermodynamic stability of the tetrahydrate is very low. For comparison, the equilibrium vapor pressure of acetone over several inclusion compounds with this guest ranges from \( P/P_0 = 0.39 \) to 0.75 \(^8\) and all those compounds easily dissociate. Since our data were obtained at 298 K, it is possible to estimate the standard free energy of the process [6.1]:

\[
\Delta G^{\circ}_{298} = RT \ln(P/P_0) = 8.31 \times 298 \ln(0.86 \pm 0.05) = 373 \pm 144 \text{ J/(mole of } \text{H}_2\text{O})
\]
or

\[
\Delta G^{\circ}_{298} = 1.5 \pm 0.6 \text{ kJ/mol}
\]

for the reaction [6.1]. The low free energy can be partially explained by strong contribution of the entropy term as four moles of guest transfer from gaseous phase to the solid.

It would be interesting to isolate and study single crystals of the guest-free form of LA. Taking into account the low stability of the tetrahydrate, several methods can be suggested. One would involve the crystallization of LA from an aqueous solution above the temperature of incongruent melting of the tetrahydrate of 52°C. Another method would
involve crystallization from mixed solvents containing some amount of water and an organic solvent that cannot act as a guest. These studies are currently underway.
6.3 References


Chapter Seven

Future Directions
This work is the first comprehensive study on clathration ability and inclusion properties of a layer-type peptide material. Fourteen new and two previously known inclusion compounds of the Leu-Ala dipeptide have been isolated and studied to various degree of detail. The formation of three inclusion compounds was studied by investigation of heterogeneous phase equilibria in the corresponding host – guest binary systems. This study is the first step in evaluating the potential of lower peptides as layer-type host materials. The results of this study, as well as the use of experimental approaches and procedures, will form a basis for future studies in the area. Some possible future directions of this work are briefly mentioned below.

This study outlined what types of molecules are the best guest candidates for inclusion in the peptide matrix of LA. Molecules that possess both a hydrophobic moiety and are able to form a strong H-bond to the host layer appear to be the most suitable. Pyridine and its derivatives illustrate this concept. It is not clear, however, to what extent this conclusion can be extended to other types of organic molecules. For example, guaiacol satisfies the requirements and does form an inclusion but its stability is noticeably lower. Further studies with solvents representing other classes of organic compounds are required. It would be also interesting to see how the stability of the inclusions changes as we use other dipeptide or tripeptide matrices, as there may be differences due to the size and shape of the peptide side groups.
The evaluation of sorption properties of layered peptides is an interesting direction of practical importance. The gate pressure is a quantitative characteristic of the inclusion formation reaction and the stability of the inclusion phase (Chapter 6). The determination of gate pressures for a range of guests would be useful to provide a comparison of stability of the inclusions in relation to their structure and the nature of guest. Sorption kinetics is another aspect that requires additional studies, as observed for the reaction of LA with methylpyridines (Chapter 5).

This work demonstrated the flexibility of the peptide matrix, which easily adapts to guest molecules of a various geometry and chemical nature. However, it was also seen that this adaptation occurs at the expense of overall stability of the inclusion solid formed. Therefore, the limits of this flexibility should be investigated. It is interesting to see how far the interlayer distance could be extended to incorporate larger molecules such as vitamins and pharmaceuticals. It would be important to examine if the choice of peptide could substantially increase the stability for a given guest or if this effect is not significant. The introduction of pillars in the layered structure as illustrated in Chapter 2 for clays may be a promising strategy of increasing the overall stability of the peptide matrix and utilizing it for bulkier guest molecules.

Another evident direction of future studies would be the screening of guests with practical importance as flavors as well as other light bioactive molecules. Guaiacol derivatives form a large class of flavor components and could be investigated in greater detail. Other such classes are the derivatives of pyrazines or various esters. The testing of
guests which are solid under ambient conditions would require methods different from those used in our work. A solid-solid mechanochemical reaction or solvent-assisted grinding may be considered. The latter possibility would be dependent on finding an appropriate neutral solvent which would both facilitate the reaction and not act as guest at the same time.

Further research is necessary to evaluate peptide layered solids as materials for separation or molecular recognition of guest chemicals. It was clearly seen that the thermal stability of studied inclusions (Chapter 5) is defined by the host-guest complementarity rather than just by the volatility of the guest solvent. It is not clear, however, how selective the peptide matrix could be for a given guest. Direct competition experiments are necessary because the methods used in this work would hardly provide this kind of quantitative information.

The cyclization of the LA dipeptide was rather unexpected but a very interesting result of this study. As was demonstrated in Chapters 4 and 5, the parameters of this reaction strongly depend on the solid phase where the reagent molecules reside. Further studies would be of interest to both organic chemists, as a controlled, solvent-free method of synthesis of cyclic dipeptides, and to supramolecular chemists, as an example of supramolecular stabilization of dipeptides in inclusion compounds. The range of dipeptides that can be cyclized as a solid-state process starting from their inclusion compounds is of particular interest.
Although we introduced some new methods and approaches in the studies of this work, they need further verification and improvement. Numerous problems were discussed in Chapter 3 that we faced while studying the peptide – guest phase diagrams. Although the phase diagram provides a great amount of useful information, it is still a time and labor consuming method. We were able to solve many technical problems and acquired certain expertise but more work is necessary and new technical solutions would be pertinent.

In general, although this study added new data and overall understanding on the inclusion properties of layered peptides, the experimental material in the field remains scarce and more experimental studies would help. New data on stoichiometry, crystal structure, stability and other properties are highly required. For example, it is not clear if the host:guest ratio is variable in a particular inclusion phase, how diverse the inclusions are in their crystal structures, and what happens if a mixed guest is used. We hope further studies will answer many of the questions listed above.