Design and Synthesis of Novel Peptide-Metal Organic Frameworks

Moustafa Mahmoud Abdel Wahid Hamaad

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By

Moustafa Mahmoud Abdel Wahid Hamaad

Rudolf Wehmschulte, Dr. rer. nat.
Professor
Biomedical and Chemical Engineering and Sciences
Major Advisor

Yi Liao, Ph.D.
Professor
Biomedical and Chemical Engineering and Sciences

Alan B. Brown, Ph.D.
Professor
Biomedical and Chemical Engineering and Sciences

Robert Usselman, Ph.D.
Assistant Professor
Biomedical and Chemical Engineering and Sciences

Maria E. Pozo de Fernandez, Ph.D.
Assistant Professor
Biomedical and Chemical Engineering and Sciences

Andrew D. Knight, Ph.D.
Professor and Department Head
Biomedical and Chemical Engineering and Science
Abstract

Design and Synthesis of Novel Peptide-Metal Organic Frameworks

Author: Moustafa Mahmoud Abdel Wahid Hamaad

Advisor: Rudolf Wehmschulte, Dr. rer. nat.

Metal-organic frameworks (MOFs) are a class of crystalline porous materials with extended network structures formed through the reticular assembly of inorganic and organic molecular building blocks through strong coordination bonds. Compared to other porous materials, MOFs offer a high degree of structural diversity as their shape and pore size as well as functionality can be tuned by the precise selection and design of their metal centers and organic struts. Due to their high surface areas, pore volumes, and the possibility of introduction of a myriad of chemical functionalities in their structures, MOFs have gained popularity for applications in many fields such as gas storage, catalysis and separation.

Peptides are attractive biomolecules to serve as linkers in the construction of MOFs since they are chiral and offer the possibility of introduction of various functional groups in the MOF structure. Natural peptide assemblies play a central role in function and construction of living systems and there has been a substantial interest over the past decade in the design and construction of artificial peptide-based assemblies with targeted structural, physical and chemical properties, with the goal of mimicking natural biological functions. Despite the great advances in the design and synthesis of metal organic frameworks (MOFs), the synthesis of frameworks using peptide linkers is less
well explored. Due to the flexible nature of peptides, obtaining a crystalline peptide MOF is highly challenging. Also, many of the current strategies for synthesizing metal peptide frameworks are based on coordination of the C-terminus and N-terminus as well side chains of peptide linkers with single metal ions. These strategies lead to compact, low dimensional and random frameworks that can not be made by the rational design principles of reticular chemistry.

In this dissertation, we describe the design and synthesis of novel peptide-based linkers for the construction of peptide-metal organic frameworks. Our strategy is based on synthesizing peptide linkers where both the C-terminus and the N-terminus ends of the peptides have ditopic 1,3-benzene dicarboxylate groups attached to them, thus converting them into tetracarboxylate-based linkers. We describe our work for the synthesis of novel 3D metal-peptide frameworks using our tetracarboxylate peptide linkers. The chelating ability of the carboxylate groups of our tetracarboxylate peptide linkers provides more structural rigidity and stability, and favor the formation of polynuclear clusters with fixed overall coordination geometry and connectivity. The strong bonding between our carboxylate-based peptide linkers and the metal centers of the secondary building units (SBUs) is expected to yield flexible 3D porous peptide MOFs with robust stability, thus opening the door for the development of new biomimetic 3D porous materials for application in various fields such as enzyme-mimicking catalysis, biomolecular recognition and chiral separations.
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<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Et$_2$O</td>
<td>Diethyl ether</td>
</tr>
<tr>
<td>EDC·HCl</td>
<td>1-ethyl-3-(3-dimethylaminopropyl)carbodiimide Hydrochloride</td>
</tr>
<tr>
<td>CDCl$_3$</td>
<td>Deuterated chloroform</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>DMSO-d$_6$</td>
<td>Deuterated DMSO</td>
</tr>
<tr>
<td>D$_2$O</td>
<td>Heavy water (deuterium oxide)</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMA</td>
<td>Dimethylacetamide</td>
</tr>
<tr>
<td>DIEA</td>
<td>N,N-Diisopropylethylamine</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MBB</td>
<td>Molecular Building Block</td>
</tr>
<tr>
<td>MOF</td>
<td>Metal-Organic Framework</td>
</tr>
<tr>
<td>MOP</td>
<td>Metal-Organic Polyhedron</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution Mass spectrometry</td>
</tr>
<tr>
<td>MOMs</td>
<td>Metal-organic materials</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>tbo</td>
<td>twisted boracite</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>Na$_2$SO$_4$</td>
<td>Sodium sulfate</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared spectroscopy</td>
</tr>
<tr>
<td>SBB</td>
<td>Supermolecular Building Block</td>
</tr>
<tr>
<td>SBL</td>
<td>Supermolecular Building Layer</td>
</tr>
<tr>
<td>SBU</td>
<td>Secondary building unit</td>
</tr>
<tr>
<td>sql</td>
<td>square lattice</td>
</tr>
<tr>
<td>PXRD</td>
<td>Powder X-Ray Diffraction</td>
</tr>
<tr>
<td>HBTU</td>
<td>2-(1H-Benzotriazolo-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate</td>
</tr>
<tr>
<td>HOBT</td>
<td>1-hydroxybenzotriazole hydrate</td>
</tr>
<tr>
<td>kgm</td>
<td>Kagomé lattice</td>
</tr>
<tr>
<td>kag</td>
<td>Kagomé net</td>
</tr>
<tr>
<td>pcu</td>
<td>primitive cubic</td>
</tr>
</tbody>
</table>
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Chapter 1: Introduction

1.1. Metal Organic Materials (MOMs)

Over the past few decades, much progress has been made in the development of porous functional materials which became an important class of solid state materials due to their versatile properties and wide potential applications. Through the synthesis and design principles of coordination and supramolecular chemistry, much effort has been made in the development of organic-inorganic hybrid materials namely metal-organic materials (MOMs)\(^1\)\(^-\)\(^6\) such as metal-organic polyhedra (MOPs) and metal-organic frameworks (MOFs) for multiple targeted applications.

MOMs are readily modular and functional materials with large pores and cavities with structures that range from periodic 0D polyhedra to 1D, 2D, 3D extended frameworks.\(^7\) The precise design of the organic ligand allows for the control of various tunable properties such as those based on the ligand shape\(^8,9\) (coordination angle), size\(^10,11\) (ligand expansion) and introduction of a wide group of organic functional groups into the pores of MOMs through functionalization of the ligand\(^12\)\(^-\)\(^15\). The inorganic element or metal ions provide different coordination geometries which allows them serve as appropriate molecular building blocks.\(^11,16\)

Metal-organic frameworks are a class of crystalline porous materials with extended structures composed of metal centers or inorganic clusters bridged by organic linkers through metal-linker coordination bonds.\(^17,18\) Compared to other porous materials, MOFs offer a high degree of structural diversity as their shape and pore size as well as functionality can tuned by the precise selection of metal centers, organic linkers and the reticular chemistry synthesis conditions.\(^17,19\)\(^-\)\(^21\) An attractive feature of MOFs is the potential to target structures of particular nets with targeted properties by implementation of the molecular building block (MBB) approach. This building
block strategy for targeting structures relies on the practice of reticular chemistry for the rational construction of solid materials where the process depends on pre-selecting or pre-designing building blocks such as metal ions, coordination metal clusters, and organic ligands to contain specific geometric and structural properties such that during the \textit{in situ} assembly or coordination process these building blocks would code for a specific net.\textsuperscript{16,18, 22-25} In other words, the MBB approach calls for the construction of made-to-order functional materials for targeted applications based on the pre-designed structural and chemical properties of the building blocks.

### 1.2 Metal-cluster building blocks and carboxylate-based Metal Organic Frameworks.

Synthetic reticular MOF chemistry typically employs metal clusters rather than single metal ions\textsuperscript{18}. Single metal ions can be challenging to use in MOF chemistry as they can adopt different coordination geometries giving rise to too much structural diversity in addition to lack of orientation or directionality making the rational design of MOFs based on single metal ions challenging as evidenced by frameworks based on Cu(I) ions and linear N-heterocyclic bridging ligands such as bipyridine and other related links.\textsuperscript{11,26,27} Single metal ions offer a limited number of geometries as there connectivity is generally low (<8) while metal-clusters can afford highly connected building blocks with diverse shapes and geometries.\textsuperscript{16} The points of extension of a metal-cluster MBB define the connectivity and the geometry of the resultant secondary building unit (SBU); in this context the SBU can be defined as an aggregate of metal ions that are attached together by multidentate functional groups (e.g., carboxylates) into clusters.\textsuperscript{17,18} Figure 1.1 shows examples of some rigid metal-carboxylate clusters that have been used in reticular MOF synthesis and the resulting building block units as well as the geometries associated with the connectivity of these building blocks. It is worthy to mention that for a carboxylate-based cluster SBU for MOF
synthesis, the geometry of such a SBU is determined by the carboxylate carbons of the ligand, which are considered the points from which the MOF will extend. \textsuperscript{11,17,18,29,8}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Some common metal-carboxylate MBB clusters used in MOF synthesis: (a) a dinuclear paddlewheel cluster with four bridging carboxylates and two axial ligands, M\textsubscript{2}(RCO\textsubscript{2})\textsubscript{4}L\textsubscript{2}. The paddlewheel can act as a square or octahedral building unit. (b) a trinuclear-oxo-hexacarboxylate cluster with six bridging carboxylates and three terminal ligands, M\textsubscript{3}O(RCO\textsubscript{2})\textsubscript{6}L\textsubscript{3}, an example is the basic chromium acetate cluster. This metal-carboxylate cluster can act as a trigonal prism building block. (c) a tetranuclear cluster with six bridging carboxylates, an example of this cluster is the basic zinc acetate cluster. This metal cluster can serve as an octahedral building unit. Reprinted with permission from ref \textsuperscript{11}, copyright: 2012 American Chemical Society, and from ref \textsuperscript{123}, copyright: 2010 John Wiley & Sons: Hoboken, NJ.}
\end{figure}
When multiple metal ions are stitched together or arranged as a cluster by carboxylate linkers, the outcome is the formation of rigid well-defined SBUs with intrinsic geometric properties, facilitating the rational design of MOFs and avoiding lack of directionality associated with single metal ions. An advantage of using chelating ligands in formation of metal clusters such as carboxylate based ligands is the structural stability of the framework produced which is an important criteria based on an application perspective in which permeant pores of the framework are desired. Metal-cluster MBBs are formed in situ during the self-assembly process and are mainly not isolable as individual clusters.

One of the most widely employed MBBs in MOF synthesis chemistry is the dimetallic paddlewheel cluster (Figure 1.1), their structure is composed of two metal centers that are bridged by four ligands which occupy the equatorial coordination positions while axial sites (falling along the metal-metal axis) are occupied by terminal ligands that cap these sites. There are a variety of different structures that can be produced when employing a paddlewheel unit depending on the ligands that occupy the six sites of the equatorial and axial positions and how those ligands are used; either as bridging ligands to other neighboring paddlewheel sites or as capping ligands preventing propagation over the sites they occupy. As shown in Figure 1.2, when both equatorial sites are occupied with bridging carboxylate ligands while the axial sites are capped by a terminal ligand (e.g. pyridine, water/solvent ligand) preventing propagation along the axial sites, a square building unit is obtained where the four bridging carboxylate groups are the points of extension occupying the four vertices of this square cluster (a 4-c MBB). Square paddlewheels are used to obtain 2D square grid MOFs structures (Figure 1.2).
Figure 1.2. Dimetallic paddlewheel building blocks can be used to construct 2D MOFs (a) or 3D MOFs (b) based on how the six sites of the equatorial and axial ligands are used; bridging ligands bridge neighboring dinuclear sites while capping ligands prevent propagation along the sites they occupy. Reprinted with permission from ref 11, copyright: 2012 American Chemical Society.

The open metal sites of the axial positions of the square paddlewheel cluster provide extra points of extension which can be occupied by two other linkers perpendicular to the square plane. In the case that all sites of the paddlewheel are used by bridging linkers, the resulting building units acts as a 6-c octahedral building unit and can be used to construct 3D cubic grid MOF structures11 (Figure 1.2). This octahedral building unit has been employed for obtaining pillared MOFs where paddlewheels from 2D layers are linked through their axial sites by ditopic linkers such as bipyridines.33

Yaghi and coworkers reported the synthesis of MOF-2, a permanently porous MOF with formula Zn(BDC)(H2O), based on the linear ditopic 1,4-benzenedicarboxylate (BDC) ligand.34 The structure of MOF-2 shows the carboxylates of the BDC ligand coordinated in a bis-
monodentate mode to two square pyramidal Zn$^{+2}$ ions to form the Zn$_2$(−COO)$_4$(H$_2$O) dinuclear paddlewheel metal-carboxylate cluster with two water molecules occupying the axial positions of the paddlewheel (Figure 1.3). The BDC ligand links the Zn$_2$(−COO)$_4$(H$_2$O) paddlewheel units resulting in the formation of two-periodic 2D square-grid (sq1) network layer structures in which the paddlewheel serves a 4-c square building unit.

Figure 1.3. Structure of MOF-2. Color code: black, C; red, O; blue polyhedra, Zn. Reprinted with permission from ref$^{37}$, copyright: 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, and from ref$^{17}$ with permission from Springer Nature.

HKUST-1, a 3D microporous MOF reported by Williams and co-workers,$^{35}$ was constructed from 4-c square Cu paddlewheel clusters (generated in situ), Cu$_2$(−COO)$_4$L$_2$, linked by the 3-c tritopic linker benzene-1,3,5-tricarboxylate (BTC) to afford a 3,4-c tbo net with the formula Cu$_3$(BTC)$_2$(H$_2$O)$_3$ (Figure 1.4). The axial sites in the Cu paddlewheel clusters of HKUST-1 are occupied by terminal water ligands and can be removed by heating to provide the anhydrous form, Cu$_3$(BTC)$_2$, with open-metal sites while maintaining the stability of the framework.$^{35}$
Figure 1.4. The structure of HKUST-1 formed by the combination of square Cu paddlewheels and BTC ligands. The topology of the framework is a 3,4-c net topology. The axial water terminal ligands of the square paddlewheels are omitted for clarity. Color code: black, C; red, O; and blue, Cu. The orange and yellow balls are at the center of the associated cavities and represent the empty spaces in the framework. Reprinted with permission from ref 37, copyright: 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

The structure of MOF-5, reported by Yaghi and co-workers,36 shows this MOF to be constructed of tetranuclear Zn cluster units Zn₄O(CO₂)₆, formed in situ under the reaction conditions, and linear ditopic 1,4-benzenedicarboxylate (BDC) linkers (Figure 1.5). The Zn atoms in this tetranuclear cluster are bridged by a μ₄-O group defining a tetrahedral central oxide that is surrounded by four tetrahedrally coordinated Zn²⁺ ions (Zn₄O). The four Zn atoms of this central oxide unit are bound to six bridging carboxylate groups of the BDC linker which span the edges of the Zn₄O central tetrahedron in an octahedral fashion with the carbon atoms of the carboxylate moieties serving as points of extension of this octahedral cluster. The resulting MOF-5 is a 3D MOF with open-cubic like network topology, pcu topology, in which the vertices are the octahedral units and the edges are the benzene struts.
Figure 1.5. The structure of MOF-5. Color code: black, C; red, O; and blue, Cu. The orange and yellow balls are at the center of the associated cavities and represent the empty spaces in the framework. Reprinted with permission from ref 37, copyright: 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

The desolvated crystals of MOF-5 obtained after removal of the guest molecules from the pores retained their integrity demonstrating the stability of these carboxylate based MOF structures. MOF-5 was demonstrated to be a modular structure, and a series of isoreticular (pcu type net) frameworks (IRMOF) based on MOF-5 were synthesized by variation of the length as well as the functionality of the linker(Figure 1.6). This allowed the synthesis of frameworks with larger pore size by expansion of the linker, and introduction of different functional groups in the pores such as –Br, and –NH$_2$ leading to control over the pore chemistry. These materials demonstrated a high methane storage capacity at room temperature.
1.3. The Supramolecular Building Blocks (SBBs) Approach.

Obtaining molecular building blocks (MBBs) of high connectivity is a desirable target in MOF crystal chemistry but this is challenged by the difficulty in isolating reaction conditions that allow the formation of MBBs with high connectivity (i.e., $\geq 12$).\textsuperscript{39-45} The need to design highly connected and more complex MBBs has led to the development of the Supramolecular Building Blocks (SBBs) approach.

Simple MBBs having a connectivity of 8 or greater can be challenging to be obtained systematically by means of simple organic ligands or polynuclear clusters.\textsuperscript{22} Designing more complex and more elaborate building blocks through the assembly of relatively simple 3- or 4-connected MBBs leading to larger supramolecular building blocks is the basis of the SBBs approach.\textsuperscript{22,46} In other words, the SBBs approach relies on the utilization of 0D Molecular Organic Polyhedra (MOPs) as SBB units with larger dimensions and more connectivity in the construction.
of MOFs, an approach with potential to target novel MOFs and control over the target framework and specific applications.47-49

In most of the reported MOPs, the assembly of single metal ions, inorganic MBBs or oxide-based metal clusters with bent ligands is used in the construction process of these MOPs.50-52

We will here discuss MOP-1,53 which was reported in 2001, and structures derived using this MOP as a MBB. MOP-1 is constructed using the metal oxide clusters as the MBB (Figure 1.7), more specifically it is constructed from dicopper paddle wheel SBUs (Cu$_2$(-COO)$_4$) SBUs that are linked by H$_2$mBDC linkers that have a bent angle between the coplanar carboxylic binding groups of approximately 120° resulting in the formation of a 0D polyhedron composed of 12 Cu-paddle wheel units connected with 24 mBDC linkers that is considered as a truncated cuboctahedron (cuo) offering 12 points of extension on the activated metal sites of the outer copper paddle wheel moieties rendering them into a 12-c cuo building block where the functionalizable apical positions of the di copper paddle wheel (square MBB) are considered as the vertices of the polyhedron while the mBDC linkers (2-c units) are considered the links of the polyhedron giving rise to an augmented cuboctahedron (i.e., truncated cuboctahedron).

**Figure 1.7.** Construction of MOP-1 from 12 Cu-paddlewheel units and 24 m-H$_2$BDC linkers producing a truncated cuboctahedron that could serve as a 12-c cuo node through the functionalizable open metal sites of the apical positions of the paddlewheels. The terminal axial ligands of the Cu-paddlewheel are omitted for clarity. Reprinted with permission from ref 56, copyright: 2019 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.
To illustrate the use of this type of cuboctahedron as a 12-c SBBs for the construction of MOFs based on the assembly of polyhedral, Furukawa et al reported the synthesis of MOP-15 which is an analogue of MOP-1 built using 5-NH$_2$-mH$_2$BDC linkers with 12 Cu-paddle wheel units connected with 24 5-NH$_2$-mBDC linkers generating a truncated cuboctahedron structure (Figure 1.8). Both axial sites in the paddlewheel units of MOP-15 are occupied by two water molecules. MOP-15 was subsequently reacted with the ditopic linear bipyridine ligand in a solvent mixture of N,N'-dimethylacetamide (DMA) and ethanol where the labile terminal ligands (water) of the twelve outer axial sites of the copper paddlewheel units are substituted by the linear ditopic bipyridine linker to afford a 12-c fcu polyhedral MOF (Figure 1.8) where each MOP-15 is connected to 12 other neighboring cuboctahedral MOP-15 via 12 ditopic bipyridine linkers in a bridging fashion.

![Diagram](image.png)

**Figure 1.8.** Construction of MOP-15 and view of the 12-c polyhedral MOF structure. Each MOP-15 is linearly bridged to 12 others by the apical positions of the 12 paddlewheels by using the ditopic 4,4'-bipyridine linker. Adapted from ref 22 with permission, copyright: 2014 The Royal Society of Chemistry.

We can describe this example of MOP to MOF conversion as follows: a discrete closed MOP is formed and in case of MOP-15 the structure is “capped” by water molecules occupying the axial positions of the Cu-paddlewheels. By replacing the terminal capping ligands with a suitable bridging ligand, extended MOF structures comprised of the MOP can be generated.
1.4. **Supramolecular Building Layers (SBLs) Approach for construction of MOFs.**

The molecular building blocks approach has allowed crystal engineers to gain better control regarding the design and construction of MOFs opening new horizons to establish new approaches for the construction of MOFs with new topologies and specific targeted applications. Eddaoudi *et al.* developed a powerful strategy for the construction of MOFs based on pillaring of 2D layers. This strategy named as the Supramolecular Building Layers approach allowed for the construction of 3-periodic MOFs through pillaring layers of 2-periodic sheets. This approach has opened horizons for the design of pillared 3-D MOFs with higher degree of complexity.

The pillaring of already known and well established 2D nets can facilitate the rational design of MOFs as there are only five edge transitive nets that are known to exist (Figure 1.9), *sql* (square lattice), *kgm* (Kagomé), *hcb* (honey comb), *kgd* (Kagomé dual) and *hxl* (hexagonal lattice) which can be targeted for pillaring into predicted 3-periodic MOFs.

![Figure 1.9](image.png)

*Figure 1.9.* Illustration of the five edge transitive layers (top) and their augmented forms (bottom). Reproduced from ref 22 with permission, copyright: 2014 The Royal Society of Chemistry. Reprinted from ref 22 with permission, copyright: 2014 The Royal Society of Chemistry.
The SBLs approach relies on the principle of using readily targeted 2D metal-organic MOF layers as building units to construct 3D functional porous MOFs. The chemical cross-linking (pillaring) of the layers through possible pillaring or bridging sites such as open metal sites or through a functionalizable position of the organic ligand is the process that leads to the construction of these pillared 3-periodic MOFs.\textsuperscript{22,57}

It is worth mentioning that by a careful look at the augmented layer forms of the five edge-transitive nets, it could be realized that out of these five edge-transitive nets there are two nets, the sql and the kgm, that can be considered as the assembly of squares as these nets are composed solely of 4-c vertices.\textsuperscript{22,32,57-59} The sql and the kgm can thus be targeted by using metals that are able to form the 4-c paddlewheel such as Cu and Zn.

The SBLs strategy calls for the precise selection of metals for the construction of the targeted layers as well as the precise selection of organic ligands that would pillar the layers. Based on the pre-targeted layer and the choice of the pillars the material designer could construct and functionalize a myriad of 3D MOFs for targeted specific applications. Expansion of the pillaring component leads to construction of isoreticular 3D MOFs with expansion of the confined space while the overall framework and network topology does not change.\textsuperscript{22} One advantage of this approach is that if the layers remain intact, meaning that if there is no expansion along the layers and expansion is along the pillars only, then pores or the windows of the layers will remain intact or unexpanded which precludes the possibility framework interpenetration which is a concern of many expanded MOFs and leads to drastic reduction in the porosity of the framework.\textsuperscript{22,65,66}

There are two common strategies through which pillaring of 2D layers could be achieved; Axial-to-Axial pillaring (A-A)\textsuperscript{22,57,67-71} and Ligand-to-Ligand pillaring (L-L)\textsuperscript{22,57,72-74} depending
on the method of coordination of the organic pillaring linker and the resulting assembly or pillaring of the layers.

1.4.1. Axial-to-Axial pillaring Strategy.

The Axial-to-Axial pillaring method is apparently the most obvious and simplest design method for pillaring of MOF layers and is accomplished through the axial position of the metal clusters used in the construction of the 2D MOF layers.\textsuperscript{22,57,67-71} In these 2D layers, the axial position of the metal cluster is typically occupied by terminal ligands (water, DMF, pyridine) and are not considered part of the framework topology. Exchange of these terminal ligands with pillaring ligands of choice results in crosslinking of the separate 2D layers into periodic 3D MOF structures. Figure 1.10 shows some examples of N-heterocyclic ditopic ligand that have been used as pillaring ligands.\textsuperscript{22}

![Figure 1.10](image.png)

Figure 1.10. Examples of the structures of some pillaring ligands used in the Axial-to-Axial approach.

MOF 2D layers constructed based on the sql and kgm topology provide some of the most common examples of MOFs constructed using this strategy. The sql and kgm 2D layers are composed of 4-vertices and as such are easily build by the assembly of square MBBs.
(M₂(OOCR)₄(A)₂; M = metal (e.g. Cu⁺², Zn⁺², Co⁺²), A = Axial ligand) such as the (4-c) square paddlewheel dinuclear MBBs that are bridged by organic ditopic ligands (e.g., benzenedicarboxylates).⁸⁻²⁹,³²,³⁴,⁵⁸⁻⁷⁰ An example of a MOF displaying a 2D sql layer structure is MOF-2, Zn(BDC)(H₂O) (Figure 1.11), which is constructed from linear benzene-1,4-dicarboxylic acid (BDC) ditopic linkers and Zn₂(-COO₄) paddlewheel building units providing a 2D square grid net (sql net).³⁴

**Figure 1.11.** (a) Structure of MOF-2 constructed from linear H₂BDC linkers and Zn₂(-COO)₄(H₂O)₂ paddlewheel SBU. (b) 2D square grid (sql-a) topological representation of the structure of MOF-2. (c) Representation of the square paddlewheel as a square 4-c vertex figure. The capping terminal ligands of the paddlewheel are omitted for clarity. Color code: black, C; red, O; blue, Zn. Hydrogen atoms are omitted for clarity. Part (a): Reprinted with permission from ref ³⁷, copyright: 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

Considering 2D MOFs based with sql or kgm topology and based on the 4-c M₂(-COO₂)₄ (A)₂ (M = metal, A = axial ligand) square paddlewheel MBBs, the axial positions of the square paddlewheel are positioned outward and orienting at both the upper and lower surfaces of the 2D MOF layer. Using a linear ditopic or bifunctional organic pillaring ligand (i.e., bipyridine,⁶⁷,⁷⁸,⁸¹⁻⁸⁴ functionalized bipyridine,⁸⁵,⁸⁶ DABCO⁸⁷⁻⁹⁰ (1,4-diazabicyclo[2.2.2]octane)) would replace the terminal ligands (such as water or pyridine) occupying the axial sites of the paddlewheel allowing
the coordination of the linear pillaring ligand through these axial sites, resulting in crosslinking of
the separate two-periodic MOF layers (Figure 1.12). It is worthy to mention that by this pillaring
by the linear ditopic linker, the dinuclear paddlewheel becomes a 6-c octahedral MBB or node.

**Figure 1.12.** (a) Schematic representation of the A-A pillaring of a 2D sql net, made from linear
ditopic linkers and paddlewheel (M₂(O₂CR)₄A₂) MBB, to form a 3D pillared paddlewheel metal
organic framework with pcu topology. (b) Schematic representation of the A-A pillaring of a 2D
gkm net, made from linear ditopic linkers and paddlewheel (M₂(O₂CR)₄A₂) MBB, to form a 3D
pillared paddlewheel metal organic framework with kag topology. Adapted from ref 22 with
permission from the Royal Society of Chemistry.

As illustrated in Figure 1.12a, employing sql-MOFs as SBLs constructed from ditopic
carboxylate linkers and square paddlewheels, and applying the Axial-to-Axial pillaring method
using linear organic ligands to bridge the layers through 6-c octahedral nodes has been used to
synthesize 3D MOFs having pcu topology.¹³,²²,⁸⁴,⁸⁷,⁸⁸,⁹¹-⁹⁵ Employing gkm-MOFs as SBLs,
constructed from ditopic carboxylate linkers and square paddlewheels, and applying the Axial-to-Axial pillaring method using linear organic ligands to bridge the layers has been used to synthesize 3D MOFs (Figure 1.12b) having the kag topology.\textsuperscript{22,78-99}

Gadzikwa reported the assembly of a MOF, KSU-100, with pecu topology using 2,2′-diamino-[1,1′-biphenyl]-4,4′-dicarboxylic acid (BPDC-(NH\textsubscript{2})\textsubscript{2}) linkers and dipyridyl glycol (DPG) pillars.\textsuperscript{91} This MOF, KSU-100, has the ditopic BPDC-(NH\textsubscript{2})\textsubscript{2} linkers connected through Zn paddlewheel clusters forming the 2D sql layer, the sql layers are then pillared together by the dipyridyl glycol (DPG) forming the 3D pecu framework (Figure 1.13). It could be noticed that the BPDC-(NH\textsubscript{2})\textsubscript{2} linker has two amine functional groups and the pillaring dipyridyl glycol (DPG) pillaring linker has two hydroxide groups. The resulting pillared 3D pecu MOF has these amine and hydroxide functional groups exposed in the pores of the MOF. This demonstrates the modularity of this method as it allows for the introduction of different functionalities in the MOF structure to target specific applications or for further MOF post-synthesis covalent functionalization.

\textbf{Figure 1.13.} (a) Linkers employed for the construction of the pillared MOF. (b) Network structure of the 3D pillared MOF (KSU-100) with pecu topology. (c) Network unit of the MOF (KSU-100). Reprinted from ref \textsuperscript{91} with permission.
A \textit{kag} MOF, KSU-1, was synthesized by Gadzikwa using 2-amino-1,4-benzenedicarboxylic acid (BDC-NH$_2$) linkers and dipyridyl glycol (DPG) as pillars under low-temperature nucleation conditions.\textsuperscript{96} The MOF structure had 2D \textit{kag}m type layers formed by BDC-NH$_2$ linkers that connected by Zn paddlewheel clusters, these layers are pillared together by the DPG pillaring linkers forming the \textit{kag} type MOF (Figure 1.14).

\textbf{Figure 1.14.} (a) Linkers employed for the construction of the pillared MOF. (b) Network structure of the 3D pillared MOF (KSU-1) with \textit{kag} topology. (c) Network unit of the MOF (KSU-1) and its schematic representation. Reprinted from ref\textsuperscript{96} with permission, copyright: 2019 American Chemical Society.

\section*{1.4.2 Ligand-to-Ligand Pillaring Strategy.}

The Ligand-to-Ligand pillaring method relies on the precise design and selection of polytropic ligands capable of achieving this type of pillaring through formation of the 2D SBLs and bridging the layers.\textsuperscript{22,57,72-74} We will here discuss the pillaring strategies based on di-isophthalate ligands having “l”-shaped pillaring cores (Figure 1.15) and the targeted topologies when employing metal square MBBs.
Figure 1.15. Structure of “I”-shaped di-isophthalate ligand employed in the ligand-to-ligand pillaring technique for construction of 3D pillared MOFs.

Targeting the sql and kgm edge-transitive layers can be accomplished through the assembly of 4-c square paddlewheels with 2-c organic ligands (e.g. isophthalates). The angular isophthalate based carboxylate ligand termed 1,3-benzenedicarboxylic acid (also written as 1,3-BDC or H₂mBDC) (Figure 1.16) is a versatile organic ligand; it leads to a large structural diversity when linked with the paddlewheel unit (M₂(-COO₄)); 0D discrete metal-organic polyhedra (also known as nanoballs) \(^{53}\), 2D kgm nets, \(^{32,77,78}\) and 2D sql nets, \(^{29,58,79,80,100}\) are examples of the structures formed from 1,3-benzenedicarboxylic acid linked paddlewheels.

Figure 1.16. Structure of 1,3-benzenedicarboxylic acid.

1,3-benzenedicarboxylic acid is suitable for the linking of square SBUs at 120°, example of square SBU in this context is the metal square paddlewheels M₂(O₂CR)₄(A)₂ [M = metal, A = axial ligand].\(^{32}\) As illustrated in Figure 1.17, two nSBUs (nanoscale secondary building units) can be formed if the molecular squares are linked by 1,3-benzenedicarboxylate at 120° angle at their vertices: a square nSBU (formed by a cluster of four square carboxylate-bridged di-metal SBUs) (Figure
1.17a), or a triangular nSBU (formed by a cluster of three square carboxylate-bridged di-metal SBUs) (Figure 1.17b). This arrangement of nSBU controls the overall topology of the resultant 2D structure, the assembly of square nSBUs yields the 2D square grid (sql) lattice with the square paddlewheels positioned at the lattice points and are bridged by the 1,3-benzenedicarboxylate ligand giving rise to an undulating sheet structure due to the 120° angle of the 1,3-benzenedicarboxylate ligand (Figure 1.17c). The assembly of the triangular nSBU yields the 2D kagomé (kgm) hexagonal lattice where the square paddlewheels are positioned at the lattice points and are bridged by the 1,3-benzenedicarboxylate ligand. Due to the 120° angle imparted by the 1,3-benzenedicarboxylate, the kgm layers are undulating as a result of this curvature induced by the ligand (Figure 1.17d).

**Figure 1.17.** (a) schematic representation of linking square MBBs through 1,3-BDC type ligand; square arrangement of four square MBBs at their vertices forming a square nSBU. (b) illustration of linking square MBBs through 1,3-BDC type ligand; triangular arrangement of three square MBBs at their vertices forming a triangular nSBU. (c) 2D sql lattice formed through the arrangement of square of squares (d) 2D kgm lattice formed through the arrangement of triangles of squares. The axial ligands of the paddlewheel are omitted for clarity.
Zaworotko and co-workers reported the synthesis of a 2D kagomé (kgm) hexagonal lattice structure composed of Cu₂(-COO₂) paddlewheels and 1,3-benzenedcarboxylic acid ligand (Figure 1.18a).\textsuperscript{32} Another lattice 2D lattice was synthesized by Zaworotko and coworkers composed of Cu₂(-COO₂) paddlewheels and 1,3-benzenedcarboxylic acid ligand but had the square grid (sql) topology (Figure 1.18b).\textsuperscript{58}

Figure 1.18. (a) Construction of a 2D kgm lattice from Cu₂(-COO)₄ paddlewheel and 1,3-benzenedcarboxylic acid ligand (H₂mBDC) and the associated augmented net (kgm-a). (b) Construction of a 2D sql lattice from Cu₂(-COO)₄ paddlewheel and 1,3-benzenedcarboxylic acid ligand (H₂Mbdc) and the associated augmented net (sql-a). Part (a): Reproduced with permission from ref \textsuperscript{37}, copyright: 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

If we consider SBLs (supramolecular building layers) based on the square paddlewheel and isophthalate moieties as organic bridging linkers, it could be noticed that the 5-position of isophthalate units are oriented outward toward the upper and lower surfaces of the MOF layer.\textsuperscript{22} By the careful selection, design and synthesis of ligands through conventional organic chemistry, these positions (i.e., the 5-position of isophthalate moieties) from two layers can be bridged
chemically resulting in the covalent pillaring of the neighboring layers (Figure 1.19).\textsuperscript{22,57} Considering the design principle of tetracarboxylate ligands with “I”-shaped pillaring cores, the ligand contains two bridging isophthalate moieties that pillar 2D layers along the covalent pillaring core linkage of the tetracarboxylate ligand.\textsuperscript{22,57} Increasing the distance between the layers (expansion of the confined space) can be accomplished by the organic synthesis of a longer ligand than the original ligand.\textsuperscript{72,103} The introduction of functionality into the MOF pores can accomplished through functionalizing the pillaring core of the ligand allowing for the targeting of the MOF for specific applications.\textsuperscript{72,103}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1_19.png}
\caption{(a) Layer segment of a 2D \textit{kgm}-MOF, made from 1,3-BDC ligand and M\textsubscript{2}(OOCR)A\textsubscript{2} paddlewheel MBB, and the corresponding kagomé lattice net representation (overhead view). (b) Layer segment of a 2D \textit{sql}-MOF, made from 1,3-BDC ligand and M\textsubscript{2}(OOCR)A\textsubscript{2} paddlewheel MBB, and the corresponding square lattice net representation (overhead view). (c) side view of the kagomé lattice net and the square lattice net respectively (d) Scheme representing the ligand-to-ligand pillaring technique using an exemplary “I”-shaped ligand and a side view of the resulting pillared MOF. Adapted from ref \textsuperscript{57} with permission, copyright: 2011 American Chemical Society.}
\end{figure}
Summarizing these concepts, in order to construct 3D MOFs through cross linking SBLs based on the sql and kgm nets through the Ligand-to-Ligand pillaring method, the ligand has to contain as part of its structure two isophthalate bridging moieties (i.e., a 4-c ligand) which coordinate to form the 4-c metal square paddlewheel MBBs resulting in the pillaring of adjacent layers through the covalent linkage of the di-isophthalate ligand leading to construction of 3-periodic MOFs based on a (4,4)-c topology.

1.4.2.1 Ligand-to-Ligand pillaring: the nbo/fof-MOF platform

If the assembling of square/rectangle tetracarboxylate ligands with 4-c paddlewheels and the resulting 3-periodic MOF is based on Ligand-to-Ligand pillaring of kgm SBLs then the resulting MOF would be an nbo/fof-type MOF in which the kgm layers are pillared through Ligand-to-Ligand pillaring in a staggered fashion (Figure 1.20). The nbo/fof is the default topology for assembling square/rectangle tetracarboxylate ligands (with two coplanar dicarboxylate groups with a 120° angle) with 4-c paddlewheel units.

Figure 1.20. Schematic of the ligand-to-ligand pillaring of kgm layers to form the nbo/fof net. Adapted with permission from ref 22, copyright: 2014 The Royal Society of Chemistry.
An example of a MOF displaying nbo/fof net topology is NOTT-101 obtained by assembly of a rigid tetracarboxylate ligand having a rectangular-planar geometry (the four carboxylate groups on the two isophthalate moieties lie in the same plane) with dicopper paddlewheels (Figures 1.21 a and b), the vertices of the of the paddlewheels are linked into Kagome’ layers (forming triangular and hexagonal windows) that are pillared by isophthalate linking bridges of the ligand in a staggered fashion (ligand-to-ligand pillaring).\textsuperscript{72,103} The nbo network described here can also be viewed as the packing and combination of two types of metal-ligand cages or cavities.\textsuperscript{72,103} The first cage is comprised of six ligands and twelve dicopper paddlewheel units with a stoichiometry of [Cu\textsubscript{24}(L\textsubscript{6})] for the cage which has an ellipsoidal shape (Figure 1.21c).\textsuperscript{72,103} The [Cu\textsubscript{24}(L\textsubscript{6})] cage has two triangular windows that lie above and below the cage, the triangular window results from triangles of square paddlewheels (i.e. three dicopper paddlewheels bridged by isophthalate moieties of the ligand).\textsuperscript{72,103} The other cage is comprised of six ligands and six dicopper paddlewheel unit with a stoichiometry of [Cu\textsubscript{12}(L\textsubscript{6})] and has a spherical shape (Figure 1.21d).\textsuperscript{72,103} The [Cu\textsubscript{12}(L\textsubscript{6})] cage is connected through isosceles triangular windows for which the length of the ligand defines the length of the two equal sides of the isosceles triangle. The packing and connection of these two types of cages (the large ellipsoidal [Cu\textsubscript{24}(L\textsubscript{6})] cage and the small spherical [Cu\textsubscript{12}(L\textsubscript{6})] cage) results in generating the tilling of the nbo/fof type network (Figures 1.21e and d).\textsuperscript{72,103} It could be noticed that the small spherical [Cu\textsubscript{12}(L\textsubscript{6})] cage spans two layers in the network structure.
The dicopper paddlewheels in NOTT-101 define a square planar 4-c node.\textsuperscript{72,103} The notation -c stands for “coordinated” rather than “connected”.\textsuperscript{104} The tetracarboxylate rigid linker in NOTT-101 serve as square or rectangular planar 4-c nodes (Figure 1.22a).\textsuperscript{72,103} The assembly of these two types of planar 4-c nodes generates the (4,4)-c network of NOTT-101 with \textbf{nbo} topology.\textsuperscript{72,103} Topological deconstruction approaches consider that the tetracarboxylate linker contains two 3-c branch points (nodes) (Figure 1.22b).\textsuperscript{104,105} If the topology is described considering the ligand as two 3-c nodes instead of a single planar 4-c node then the derived net is the (3,4)-c \textbf{fof} net.\textsuperscript{104,105} The \textbf{fof} net is derived from the basic \textbf{nbo} net by replacing the 4-c ligand node by two 3-c nodes.
The \textbf{nbo/fof} topological description of NOTT-101 describes the basic (4,4)-c \textbf{nbo} net with the ligand as a 4-c single node and the derived (3,4)-c \textbf{fof} net considering the ligand as two 3-c nodes.

![Diagram](image)

\textbf{Figure 1.22.} Two possible abstractions of planar tetratopic linker + SBUs: (a) planar tetracarboxylate linker can be regarded as one four coordinated vertex (a single 4-c node), (b) or as two 3-c vertices (two 3-c branch points or nodes). Red points are branch points of the linker, blue spheres represent one kind of metal SBU (the 4-c dicopper paddlewheel in case of NOTT-101).

MOF-505 was constructed using rectangular BPTC linkers (H$_4$BPTC = 3,3′, 5,5′-biphenyltetracarboxylic acid) and dicopper paddlewheel SBUs affording a 3D framework of \textbf{nbo/fof} type topology viewed as staggered \textbf{kgm} layers linked together via ligand-to-ligand cross-linking (Figure 1.23).\textsuperscript{10,22,110} MOF-505 exhibits two types of 4-c nodes, the dicopper paddlewheels and the rectangular tetracarboxylate linker, affording the (4,4)-c \textbf{nbo} type network.\textsuperscript{104,105,110} Topological deconstruction would describe the tetracarboxylate linker as two 3-c nodes instead of a single planar 4-c node rendering the (3,4)-c derived \textbf{fof} net (Figure 1.23).\textsuperscript{104,105} The topology of MOF-505 is described as \textbf{nbo/fof} where the (4,4)-c \textbf{nbo} net is the basic net and the derived net is the (3,4)-c \textbf{fof} net.\textsuperscript{104}
**Figure 1.23.** The structure of MOF-505 formed by assembly of paddlewheel units with the rectangular BPTC ligand affording pillared \( \text{kgm} \) layers generating an \text{nbo/fof} type MOF. In the augmented form of the \text{fof} net, the tetracarboxylate ligand is represented as two triangular nodes in the same plane (two 3-c vertices joined by an extra edge) which together with the square planar paddlewheel units gives rise to the derived (3,4)-c \text{fof} net. Color code: black, C; red, O; and blue polyhedra, Cu. The yellow balls represent the empty spaces in the framework. Reprinted with permission from ref \textsuperscript{37}, copyright: 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

1.4.2.2. Ligand-to-Ligand pillaring: the \text{ssa/sty}-MOF platform

If the geometry of the square/rectangle tetracarboxylate deviates from the linear geometry, then other topologies based on the \text{kgm} SBLs can be produced.\textsuperscript{10,22,104,105} If the central core of the diisophthalic-based tetracarboxylate is bent, then this bent position causes the ligand to not point in a straight fashion up or down the layer but could rather be oriented outside or inside a certain window in the layer.\textsuperscript{10,22,104,105} The bending of the central core of the ligand geometrically prevents the formation of the staggered pillaring mode of \text{kgm} layers that is characteristic of the \text{nbo/fof} net but instead leads to generation of an eclipsed pillaring of the \text{kgm} layers leading to formation of an \text{ssa/sty} type MOF (Figure 1.24).\textsuperscript{10,22,104,105} In other words, if the tetracarboxylate ligands are not linear, this geometric deviation allows the resultant MOF to deviate from the default \text{nbo/fof} topology producing non-default topologies.
Figure 1.24. Schematic of the ligand-to-ligand pillaring of kgm layers to form the ssa/sty net. Reproduced with permission from ref \textsuperscript{22}, copyright: 2014 The Royal Society of Chemistry.

Examples of MOFs displaying the ssa/sty topology include PCN-12\textsuperscript{111} and ZJU-25,\textsuperscript{112} in which bending of the central core of the ligands used in their construction prevents the staggered pillaring of their respective kgm SBLs and instead generates eclipsed pillaring of the kgm SBLs leading to non-default ssa/sty type frameworks.\textsuperscript{10,22,104,105,111,112}

PCN-12 was constructed from the bent ligand 5,5'- methylene-di-isophthalate (H\textsubscript{4}mdip) and dicopper paddlewheels to yield a 3D pillared MOF with ssa/sty topology (Figure 1.25).\textsuperscript{111,10,104,105}

Figure 1.25. Structure of PCN-12 constructed from H\textsubscript{4}mdip ligand and dicopper paddlewheel SBUs. Introduction of bending into the ligand leads to eclipsed pillaring of kgm SBLs leading to the ssa/sty topology. Reproduced with permission from ref \textsuperscript{10}, copyright: 2019 American Chemical Society.
ZJU-25 was constructed from the bent ligand (5,5′-(9H-fluorene-2,7-diyl) diisophthalic acid (H$_4$FDDI) and dicopper paddlewheels to yield a 3D pillared MOF with ssa/sty topology (Figure 1.26).$^{112,10,104,105}$

Figure 1.26. (a) The structure of ZJU-25 constructed from H$_4$FDDI ligand and dicopper paddlewheel SBUs viewed along the c axis (b) The framework topology of ZJU-25 in its augmented form (ssa/sty-a) net. , the tetracarboxylate ligand is represented as two triangular nodes in the same plane (two 3-c vertices joined by an extra edge) and the paddlewheel units are represented as square units. Reproduced with permission from ref $^{112}$, copyright: 2013 The Royal Society of Chemistry

If the tetracarboxylate linker in PCN-12 and ZJU-25 is considered a 4-c node and the dicopper paddlewheel in both MOFs is considered a 4-c node then the combination of these two 4-c nodes would lead to the basic (4,4)-c ssa net (Figure 1.27).$^{104,105}$ Topological deconstruction approaches would consider the tetracarboxylate linker as having two 3-c branch points instead of considering
the linker as a single 4-c node, combing the linker as two 3-c nodes with the 4-c node of the dicopper paddlewheel yield the derived (3,4)-c sty net (Figure 1.27).\textsuperscript{104,105} The topology of PCN-12 and ZJU-25 is described as ssa/sty where the (4,4)-c ssa net is the basic net and the derived net is the (3,4)-c sty net.\textsuperscript{104,105} In the augmented form of the sty net (sty-a), the tetracarboxylate ligand is represented as two triangular nodes in the same plane (two 3-c vertices joined by an extra edge) and the paddlewheel units are represented as square units (Figure 1.27e).

\textbf{Figure 1.27.} (a and b) Two possible abstractions of planar tetratopic linker + SBUs: (a) planar tetracarboxylate linker can be regarded as one four coordinated vertex (a single 4-c node), (b) or as two 3-c vertices (two 3-c branch points or nodes). Red points are branch points of the linker, black spheres represent one kind of metal SBU (the 4-c dicopper paddlewheel in case of PCN-12 and ZJU-25. (c) The basic (4,4)-c ssa net. (d) The derived (3,4)-c sty net. (e) The augmented form (sty-a) of the sty net, the tetracarboxylate ligand is represented as two triangular nodes in the same plane (two 3-c vertices joined by an extra edge) and the paddlewheel units are represented as square units. Reprinted with permission from ref \textsuperscript{104} (copyright: 2014 The Royal Society of Chemistry) and ref \textsuperscript{105} (copyright: 2013 American Chemical Society).

1.4.2.3. **Ligand-to-Ligand pillaring: The ssb/stx-MOF and the lvt/lil-MOF platforms**

Considering sql layers obtained through the assembly of isophthalates with square paddlewheel MBBs, there are two distinguishable types of sql layers that can be obtained, the sql-1 and the sql-2, that differ in the arrangement of the isophthalate moieties around the paddlewheels (Figure
The 5-position of the isophthalate moieties points up or down from the sql layer in a distinguishable different fashion when comparing the two types of sql layers (sql-1 and sql-2).

Figure 1.28. Schematic representing two possible types of sql layers that can be obtained with isophthalates and square paddlewheel MBBs, sql-1 and sql-2. Orange lines indicate isophthalates pointing “up”, blue lines indicate isophthalates pointing “down”. Reproduced with permission from ref 22, copyright: 2014 The Royal Society of Chemistry.

In order to systematically distinguish between these two types of sql layers, we discuss the structural chemistry of calixarene compounds, specifically calix[4]arene (Figure 1.29).101,102 The three chemical conformations for calix[4]arene shown in Figure 1.29 differ in the orientation of

Figure 1.29. Three possible different chemical conformations of calix[4]arene. (a) cone, (b) 1,2-alternate, (c) 1,3-alternate.
the arene rings with respect to one another. In the cone conformation (Figure 1.29a), all arene rings point up. The 1,2-alternate conformation (Figure 1.29b) has two adjacent arene groups pointing up and two other adjacent arene groups pointing down while the 1,3-alternate conformation (Figure 1.29c) has opposite arene groups oriented in the same direction.

The square grid lattice (sql) can be obtained by the assembly of square nSBUs (nanoscale secondary building units) where the square nSBU is constructed from four 1,3-dicarboxylate linkers and four paddlewheel units.\textsuperscript{58,100} Considering the structure of the sql-1 type of sql-layers, there are two types of nSBU in the structure of this square lattice layer as illustrated in Figure 1.30(a); a cone nSBU and a 1,3-alternate nSBU.\textsuperscript{100} The cone nSBU exhibits a conformation where all four dicarboxylate ligands orient in the same direction (the 5-position of the isophthalate orient in the same direction) which resembles that of the cone conformation of calix[4]arene, while the 1,3-alternate nSBU has a conformation where opposite dicarboxylate ligands are oriented in the same direction which resembles the conformation of 1,3-alternate calix[4]arene.\textsuperscript{100} The sql-1 square lattice layer here is assembled from alternating cone and 1,3-alternate nSBUs giving rise to the undulating structure of the lattice layer. Regarding the structure of the sql-2 type lattice layer, there is one type of nSBU in this layer which is the 1,2-alternate nSBU as illustrated in Figure 1.30(b).\textsuperscript{100} The 1,2-alternate exhibits a conformation where two adjacent carboxylate ligands orient up and two other adjacent carboxylate ligand orient down which resembles the conformation of 1,2-alternate calix[4]arene.\textsuperscript{100} The sql-2 square lattice layer here is assembled from 1,2-alternate nSBUs.
Taking into account these two possible types of sql layers (sql-1 and sql-2), when it comes to the Ligand-to-Ligand pillaring method, there will exist different Ligand-to-Ligand pillaring possibilities considering that the ligand is functionalized at the 5-position of the isophthalate moieties.\textsuperscript{22} This functionalized position of the pillaring ligand will point up and down from the sql layer pillaring the layers in distinct different fashions or arrangements.

Taking into consideration the conformation of the tetracarboxylate ligand (di-isophthalic based ligands) used, assembling square/rectangle tetracarboxylate ligands (with both isophthalate groups of the ligand having coplanar dicarboxylate groups with a 120° angle) with 4-c paddlewheels would result in a 3-periodic MOF with an ssb/stx topology if the supermolecular building layers (SBLs) are based on the sql-1 layer.\textsuperscript{22} The structure of an MOF with ssb/stx topology can be considered as Ligand-to-Ligand pillaring of sql-1 SBLs in which the layers are pillared in an eclipsed
fashion. If assembling square/rectangle tetracarboxylate ligands with 4-c paddlewheels and the resulting 3-periodic MOF is based on Ligand-to-Ligand pillaring of sql-2 SBLs then the MOF would be an lvt/lil-type MOF.

Examples of MOFs displaying the ssb/stx topology:

An example of a MOF displaying the ssb/stx topology is NOTT-109 reported by Schröder et al which is constructed from 4-c dicopper paddlewheel centers and 4-c tetracarboxylate ligands giving rise to a (4,4)-c network adopting the ssb net topology displaying square windows (Figure 1.31).

![Figure 1.31](image)

**Figure 1.31.** (a) Structure the tetracarboxylate ligand used in construction of NOTT-109. (b) Dicopper paddlewheel SBU (axial terminal ligands omitted for clarity). (c) Structure of the square grid of NOTT-109 formed by isophthalate units and dicopper paddlewheel SBUs. (d) View of the framework of NOTT-109. Reproduced with permission from ref 103, copyright: 2013 American Chemical Society.

As illustrated in Figure 1.32, the network structure of NOTT-109 displays two types of metal-ligand cages, the large cage comprised of eight dicopper paddlewheel units and sixteen ligands with a stoichiometry for the cage of \([\text{Cu}_{16}(L)_{16}]\) (Figure 1.32c) and a smaller cage comprised of eight dicopper paddlewheel units and four ligands with a stoichiometry for the cage of \([\text{Cu}_{16}(L)_{4}]\)
These two types of cages give the overall framework structure of NOTT-109 as displayed in Figure 1.32e. The tetracarboxylate ligand used in the construction of NOTT-109 is considered a square planar ligand since the four carboxylate groups on the isophthalate lie on the same plane with each of the two coplanar dicarboxylate of the isophthalate moieties of the ligand displaying an angle of $120^\circ$ (figure 1.32a). The ligand has a bulky central aromatic naphthalene core in its backbone which induces steric hindrance and as a result precludes the formation of the triangular windows formed by three Cu paddlewheel units bridged by three isophthalate groups of three ligands that form the main cage which is characteristic of the default nbo/fof MOF topology for assembling square/rectangular diisophthalate ligands with 4-c paddlewheels. This steric hindrance induced by the bulky aromatic naphthalene core of the ligand leads instead to expansion of the windows to yield square windows constructed by four Cu-paddlewheels units (as opposed to three in the triangular window) and four isophthalates leading to formation of square grid (sql) SBLs which are pillared in an eclipsed fashion forming an ssb/stx topology type MOF. In other words, introducing steric hindrance in the core of the tetracarboxylate ligand used to construct NOTT-109 creates a structural irregularity defined as a geometry mismatch that leads to a MOF with a non-default topology.

The topology of NOTT-109 is described as an ssb/stx topology, if from a topological standpoint the tetracarboxylate ligand serves as a 4-c node when combined with the 4-c paddlewheels to afford a (4,4)-c pillared MOF then the topology of NOTT-109 is described as ssb. In order
Figure 1.32. (a) Structure of ligand used in construction of NOTT-109, steric hindrance introduced by the bulky naphthalene core. (b) Dicopper paddlewheel SBU (axial terminal ligands omitted for clarity). (c) View of the large [Cu$_{16}$(L)$_{16}$] cage of NOTT-109 (d) View of the small [Cu$_{16}$(L)$_{4}$] cage of NOTT-109. (e) View of the overall framework structure of NOTT-109. Color code: black, C; red, O; blue, Cu. Hydrogen atoms are omitted for clarity. Parts (c), (d) and (e) Reprinted with permission from ref 72, copyright: 2009 American Chemical Society.

to further completely characterize the topology, topological deconstruction suggests that the tetracarboxylate linker contains two 3-c branch points (nodes) (Figure 1.33b). If the topology is described considering the ligand as two 3-c nodes instead of a single planar 4-c node then the derived net is the (3,4)-c stx net (Figure 1.33). In other words, the stx net is derived from the basic ssb net by replacing the 4-c ligand node by two 3-c nodes. The ssb/stx topological description of NOTT-109 describes the basic (4,4)-c ssb net with the ligand as a 4-c single node and the derived (3,4)-c stx net considering the ligand as two 3-c nodes.
Figure 1.33. Two possible abstractions of planar tetratopic linker + SBUs: (a) planar tetracarboxylate linker can be regarded as one four coordinated vertex (a single 4-c node), (b) or as two 3-c vertices (two 3-c branch points or nodes). Red points are branch points of the linker, blue spheres represent one kind of metal SBU (the 4-c dicopper paddlewheel in case of NOTT-109). (c) Representation of the basic ssb net and (d) the derived (3,4)-c stx net. Parts (c) and (d) are reproduced with permission from ref 104, copyright: 2014 The Royal Society of Chemistry.

Another MOF displaying ssb/stx topology and isoreticular with NOTT-109 is the MMPF-1 MOF reported by Wang and co-workers.\textsuperscript{106,104} MMPF-1 was constructed by the assembly of a diisophthalate-derived porphyrin based ligand in which a porphine macrocycle bridges two isophthalate moieties (5,15-bis(3,5-dicarboxyphenyl)porphine) with dicopper paddlewheel SBU (Figure 1.34) to yield a 3D porous pillared framework, MMPF-1.\textsuperscript{106}

Figure 1.34. (a) Structure of the 5,15-bis(3,5-dicarboxyphenyl)porphine ligand and (b) dicopper paddlewheel SBU used in the construction of MMPF-1. Color code: black, C; red, O; blue, Cu. Hydrogen atoms are omitted for clarity.
The structure of MMPF-1 displays a nanoscopic cage composed of sixteen ligands connecting eight dicopper paddlewheel SBUs as illustrated in Figure 1.35a. Since MMPF-1 was prepared by the assembly of the free-base tetracarboxylate ligand (Figure 1.16a) with Cu^{2+} ions, insertion of Cu^{2+} ions into the center of the porphyrin core ring of the ligand occurred in the course of the assembly reaction. The extended structure of MMPF-1 along the ab plane (Figure 1.35b) displays a square grid structure formed by isophthalate units of ligand and dicopper paddlewheel units.

**Figure 1.35.** (a) Nanoscopic cage of MMPF-1 formed by sixteen ligands and eight dicopper paddlewheel SBUs. (b) View of the extended square grid structure of MMPF-1 in the ab plane formed of nanoscopic cages. The yellow balls represent the empty space in the framework. Reprinted with permission from ref 106, copyright: 2011 The American Chemical Society.

The structure of the ligand used to construct MMPF-1 contains a bulky porphyrine macrocycle as part of its core structure which introduces steric hindrance preventing the formation of the triangular window characteristic of the nbo/fot topology and instead adopts an ssb/stx net structure similar to that displayed by NOTT-109 in which a bulky central aromatic naphthalene group in the ligand structure introduced steric hindrance.106,107,103
Mori reported the synthesis of a series 3D MOFs similar to MMPF-1 but instead of using the free-base porphyrin tetracarboxylate ligand used in the synthesis of MMPF-1, metalated analogues of the same ligand were used where the central porphyrin core of the ligand was metalated with different metals (Zn$^{2+}$, Ni$^{2+}$, Pd$^{2+}$, Mn$^{3+}$(NO$_3$), Ru$^{2+}$(CO)) before using them in the construction of the MOFs (Figure 1.36).

**Figure 1.36.** (a) Structure of the metalated 5,15-bis(3,5-dicarboxyphenyl)porphine ligand, MDDCPP (M = Zn$^{2+}$, Ni$^{2+}$, Pd$^{2+}$, Mn$^{3+}$(NO$_3$), Ru$^{2+}$(CO)) (b) dicopper paddlewheel SBU used in the construction of [Cu$_2$(MDDCPP)] 3D MOFs. Color code: black, C; red, O; blue, Cu. Hydrogen atoms are omitted for clarity.

Mori synthesized a series of porphyrin-based metal carboxylate frameworks by the assembly of the diisophthalate-derived metalated porphyrin based ligands with dicopper paddlewheel SBUs to yield a series of 3D porous pillared frameworks with the composition [Cu$_2$(MDDCPP)] (M = Zn$^{2+}$, Ni$^{2+}$, Pd$^{2+}$, Mn$^{3+}$(NO$_3$), Ru$^{2+}$(CO)) (Figures 1.37 and 1.38) that had similar structures as NOTT-109 and MMPF-1.

**Figure 1.37.** (a) A Zn$^{2+}$ metalated ligand moiety with four dicopper paddlewheels. (b) Structure of a cage assembled by the ligand and dicopper paddlewheels. Reprinted with permission from ref 107, copyright: 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.
Figure 1.38. (a) Extended framework structure of a porphyrin-based metal carboxylate framework (b) Extended structure of the framework along the c-axis illustrating the square grid structure of the 3D MOF. Color code: grey, C; red, O; blue, N; green, Cu, purple, Zn. Reprinted with permission from ref\textsuperscript{107}, copyright: 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

Examples of MOFs displaying the lvt/lil topology:

If assembling square/rectangle tetracarboxylate ligands with 4-c paddlewheels and the resulting 3-periodic MOF is based on Ligand-to-Ligand pillaring of sql-2 SBLs then the MOF would be an lvt/lil-type MOF as illustrated in Figure 1.39.\textsuperscript{22}

Figure 1.39. Schematic representation of the sql-2 layer obtained from isophthalates and square paddlewheel MBBs (orange lines indicate isophthalates pointing up and blue lines represent isophthalates pointing down) and the ligand-to-ligand pillaring of the sql-2 SBLs layers to form the lvt/lil net. Adapted with permission from ref \textsuperscript{22}, copyright: 2014 The Royal Society of Chemistry.
Zaworotko reported a 3D MOF with \textbf{lvt/lil} topology constructed from a tetracarboxylic acid imide based ligand $\text{H}_4\text{BIPA-TC}$ (5,5′-(1,3,6,8-tetraoxobenzo[\textit{Imn}][3,8] phenanthroline-2-7-diyl)bis-1,3-benzenedicarboxylic acid) and dicopper paddlewheel ($\text{Cu}_2(-\text{COO})_4$) square MBBs (Figure 1.40).\textsuperscript{108} The vertices of the paddlewheels assemble into squares adopting the 1,2-alternate calixarene-like partial cone conformations rendering square grid 4,4-net layers of $\text{sql}$-2 type.\textsuperscript{108} The layers are pillared or cross-linked through the BIPA-TC ligands (ligand-to-ligand pillaring) in a zigzag like fashion generating a 3D pillared MOF with \textbf{lvt/lil} topology.\textsuperscript{108}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image}
\caption{Construction of a 3D pillared \textbf{lvt/lil} type MOF from $\text{H}_4\text{BIPA-TC}$ and dicopper paddlewheels ($\text{Cu}_2(-\text{COO})_4$). Reproduced with permission from ref\textsuperscript{108}, copyright: 2011 The Royal Society of Chemistry.}
\end{figure}

This 3D pillared MOF is described as having an \textbf{lvt} topology considering that the tetracarboxylate ligand serves as a 4-c node when combined with the 4-c paddlewheels to afford a (4,4)-c pillared MOF, the \textbf{lvt} topology describes the basic net in this case (Figure 1.41a).\textsuperscript{108,105} In order to completely characterize the topology, the tetracarboxylate ligand can be deconstructed topologically into two 3-c nodes instead of a single 4-c node.\textsuperscript{105} Replacing the 4-c ligand node in the \textbf{lvt} net by two 3-c nodes gives the derived \textbf{lii} net (Figure 1.41b).\textsuperscript{105} The \textbf{lvt/lii} topological
description of the 3D MOF describes the basic (4,4)-c \textit{lvt} net with the ligand as a 4-c single node and the derived (3,4)-c \textit{lil} net considering the ligand as two 3-c nodes.

**Figure 1.41.** (a) The \textit{lvt} net shown in the augmented form, the tetracarboxylate ligand is represented as a single square planar node which together with the square planar paddlewheel units gives rise to the basic \textit{lvt} net. (b) The \textit{lil} net shown in the augmented form, the tetracarboxylate ligand is represented as two triangular nodes in the same plane (two 3-c vertices joined by an extra edge) which together with the square planar paddlewheel units gives rise to the derived \textit{lil} net. Reprinted with permission from ref\textsuperscript{105}, copyright: 2013 American Chemical Society.

In summary for the Ligand-to-Ligand pillaring (of supramolecular building layer strategy for construction of 3D pillared MOFs, we have discussed the pillaring strategies based on di-isophthalate ligands having “I”-shaped pillaring cores and the targeted topologies when employing metal square MBBs. Ligand-to-Ligand pillaring of kgm layers can lead to construction of 3D MOFs with \textit{nbo/fof} or \textit{ssa/sty} type nets, while Ligand-to-Ligand pillaring of sql layers can lead to construction of MOFs with \textit{ssb/stx} or \textit{lvt/lil} type nets.
1.5. Metal-Peptide Frameworks

Peptides and amino acids are an attractive class of organic ligands for the construction of extended MOF structures due to their rich coordination chemistry through the carboxylate, amino groups as well as through side chain groups.\textsuperscript{113-115} Peptides are polymeric biomolecules composed of amino acids linked through peptide bonds; a peptide is a linear chain of two or more amino acids linked via amide bonds.\textsuperscript{116} There are 20 naturally occurring amino acids, each amino acid contains an α-carbon to which a carboxyl group, an amine group and a side chain are attached. The variation in the structure and functionality of these chain groups determines the chemical and physical properties of these amino acids.

An amide bond between the nitrogen atom of one amino acid and the acyl carbon atom of another amino acid is referred to as a peptide bond, for example the structure of the dipeptide Ala-Phe shown below (Figure 1.42):

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{peptide.png}
\caption{Chemical structure of the Ala-Phe dipeptide.}
\end{figure}
Peptides can act as organic ligands by coordinating metal ions through both their amino terminus and carboxylic acid terminus groups.\textsuperscript{113-115} The terminal amino group can coordinate to metal ions in a monodentate coordination mode or through a chelation mode via formation of a five membered chelate ring in which the terminal amine group as well as the oxygen atom of the neighboring amide group both coordinate to the same metal forming a five membered chelate ring (O,N-chelation mode (Figure 1.43a)).\textsuperscript{113-115} Coordination of the terminal carboxylate group to the metal ion can occur through any of the well-known modes such as chelation, monodentate, bidentate or tridentate (Figure 1.43b).\textsuperscript{113-115} Side chains of amino acids residues in the peptide sequence can add more metal binding groups to the peptide.\textsuperscript{113-115} It is through these coordinating sites of peptides with metal ions that extended metal ion-peptide framework can be created.

**Figure 1.43.** (a) Coordination modes of the terminal amino group of peptides: monodentate and five –membered chelate mode. (b) Monodentate, bidentate and chelate coordination modes of the terminal carboxylate group of peptides. (c) Structures of some dipeptides and tripeptides used for creating metal-peptide frameworks.
Gastaldo and co-workers reported the synthesis of a metal-peptide polymer by the reaction of a tripeptide ligand (Gly-His-Gly) with Cu(II) where the structure of the resulting metal-peptide polymer was based on coordination of the side chains of the tripeptide with the copper metal (Figures 1.44 a,b,c). In the framework structure, each Cu (II) center adopts a distorted square pyramidal configuration that is based on the tridentate chelation mode of the histidine residue and the monodenate bonding of the carboxyl groups belonging to the terminal glycine residue (Figures 1.44 b,c). The helicoidal Cu-peptide-Cu chains are bridged or interconnected into a 3D open framework through the formation of $\mu_2$-carboxylate bridges of C-terminal Gly. The framework exhibited solvent-accessible nanospace or pores close to 60% of the total volume. This empty space is decorated with chiral binding sites characteristic of the Gly-His-Gly ligand used in construction of the framework including amide, amino, and imidazole groups, pointing into the channels (Figure 1.4c) which contributed towards the capability of the framework for enantioselective separation of chiral drugs such as methamphetamine and ephedrine.
Rosseinksy et al. reported the synthesis of 2D metal-peptide layers by using short flexible peptides such as the dipeptide Gly-Ala where the dipeptide is arranged in a regular array through coordination of the C-terminus carboxylate and amino groups with metal centers.\textsuperscript{118} Using Gly-Ala dipeptide as a linker with Zn (II) afforded 2D-sheet-like metal peptide layers where the zinc ions are tetrahedrally coordinated to four dipeptide ligands, two dipeptide ligands are coordinated by the C-terminal Ala carboxylate groups and two by the N-terminal Gly amine groups (Figure 1.45).\textsuperscript{118} Each ligand is coordinated to two zinc ions resulting in the formations of layers with 2D grid-like structure (Figure 1.45a).\textsuperscript{118} The adjacent layered are stacked or assembled in an eclipsed stacking mode through β-sheet like hydrogen bonding between all the amide groups in adjacent layers leading to 1D square-shaped pores (Figure 1.45b).\textsuperscript{118} Upon desolvation (removal of
adsorbed guest molecules) the pores become narrowed and collapse. In this case, the 2D grid-like structures form 3D frameworks through intermolecular hydrogen bonding.

Figure 1.45. (a) Space filling Representation of a layer of the metal peptide framework constructed from Gly-Ala dipeptide ligands and Zn(II). (b) Stacking of the 2D layers through hydrogen bonding interactions. (c) and (d) Structure of the Gly-Ala dipeptide. Reprinted from ref 118 with permission from the American Association for the Advancement of Science.

Rosseinsky reported a metal-peptide coordination polymer assembled using the dipeptide glycylthreonine (Gly-Thr) and Zn (II) affording 2D metal-peptide layers where each Zn (II) center is six-fold coordinated by four dipeptide molecules leading to a distorted octahedral geometry (Figure 1.46). Two peptides coordinate through the C-terminus carboxylate group of the Thr residue and the other two coordinate to the metal center through a chelate coordination mode by the amine and oxo group belonging to the N-terminus Gly residue forming a five membered chelate structure, each dipeptide acts as a μ2 -linker connecting two metal centers resulting in the formation of 2D grid-like layers. The layers are packed through hydrogen bonding interaction between the N-H and C=O groups of the neighboring layers leading to 1D square-shaped pores or
The presence of the Thr polar side chain adds an additional hydrogen bonding interaction between the N-terminus amino groups and the -OH groups of the threonine side chain which reinforces the structural stability of the 3D framework formed through packing of 2D grid-like metal peptide sheets through intermolecular hydrogen bonding. This extra stability is specific to the polar Thr side chain through formation of additional hydrogen bonding which is not present in frameworks constructed from dipeptides with apolar amino acid residues such as the Ala residue in the Gly-Ala dipeptide.

Figure 1.46. (a) Octahedral coordination of the peptide linkers around the Zn (II) ion. (b) Grid-like space filling representation of metal peptide framework constructed from Gly-Thr dipeptide and Zn (II) (right), and interlayer hydrogen-bonding interactions (represented as dashed lines) (left). Color code Zn dark blue, O red, C gray, N blue, H white. (c) Structure of the Gly-Thr dipeptide linker. (d) Structure of the 2D grid like layer of the metal peptide framework. Part (a) and (b) are reprinted with permission from ref 119 (copyright: 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim). Part (d) reprinted with permission from ref 115 (copyright: 2021 American Chemical Society).
Assembly of the dipeptide Gly-Ser with Zn (II) afforded a metal-peptide polymer that is isostructural to that constructed using the dipeptide Gly-Ala and Zn (II).\textsuperscript{120} Zinc ions are tetrahedrally coordinated to four Gly-Ser dipeptide ligands, two dipeptide ligands are coordinated by the C-terminal Ser monodentate carboxylate groups and two by the N-terminal Gly amine groups (Figure 1.47a).\textsuperscript{120} Each ligand is coordinated two zinc ions resulting into the formation of layers with 2D grid-like structure (Figure 1.47c).\textsuperscript{120} The 2D sheets are stacked through hydrogen bonding interaction involving N-H and C=O groups between peptides in neighboring layers giving rise to 1D pores or channels occupied by MeOH guest molecules (MeOH is the solvent used for synthesis of this MOF) which are hydrogen bonded to –OH groups from the Ser sidechain (Figures 1.47 c and d).\textsuperscript{120}

Figure 1.47. (a) Representation of the tetrahedral coordination environment around the Zn (II) and the peptide to metal connectivity of the metal peptide framework constructed from Gly-Ser linker and Zn (II) ions. (b) Structure of the Gly-Ser dipeptide linker. (c) Representation of a 2D layer of the metal peptide framework showing the square-like grid structure. The pockets are filled with methanol guest molecules in the solvated structure which are hydrogen bonded to the –OH groups from the –CH$_2$OH side chain of the serine residues (c) β-sheet like packing of the layers due to hydrogen bonding interactions (represented by red dashed lines) between N-H and C=O groups from neighboring layers. Color code: Zn, dark blue; O, red; C, grey; N, blue; H, white. Reproduced from ref\textsuperscript{120} with permission from Springer Nature.
The dipeptide Gly-Gly has been used to construct a 2D metal-peptide MOF through coordination with Zn (II) where the terminal amino group of the dipeptide coordinates to the metal through a five membered chelation ring while each Gly-Gly ligand coordinates through the terminal carboxylate group in a monodentate mode bridging two metal ions (Figure 1.48).\textsuperscript{121} As a consequence, each octahedral metal ion is connected to four other metal ions through four bridging Gly-Gly dipeptide ligands leading to 2D framework structures.\textsuperscript{121}

**Figure 1.48.** Structure of the Gly-Gly dipeptide ligand. Representation of the coordination modes of the Gly-Gly ligand with Zn (II) ions. Structural illustration of the metal peptide framework constructed from Gly-Gly ligands and Zn (II) ions. Part (c) is reprinted with permission from ref \textsuperscript{114}, copyright: 2017 Elsevier B.V.

Varying the side chains groups of peptide ligands with metal binding sites allows the construction of 3D metal-peptide frameworks.\textsuperscript{122} The natural peptide carnosine (β-Ala-L-His) consists of L-histidine and β-alanine (Figure 1.49a), the β-alanine residue has an extra CH\textsubscript{2} group while the imidazolate function of the histidine residue provides two additional metal coordination thus giving carnosine two more binding sites in relative to other peptide ligands such Gly-Thr, Gly-Ala and Gly-Ser.\textsuperscript{122} In the structure of the 3D MOF constructed from carnosine and Zn (II) ions, each carnosine molecule links four tetrahedral Zn (II) ions, two of those Zn (II) ions are...
bridged by the deprotonated imidazole side chain ring of the histidine residue (Figure 1.49).\textsuperscript{122}  
This work emphasizes a strategy for construction of a 3D metal peptide framework by introduction of extra metal binding sites (e.g. imidazolate side chain) which in contrast to other peptide-based MOFs such as those constructed from Gly-Ala and Gly-Thr dipeptides, this three-dimensional structure is a result of coordination bonds between the peptide ligand and metal ions without additional inter- or intramolecular hydrogen bonding interactions.

\textbf{Figure 1.49.} (a) Structure of carnosine, β-Ala-L-His. (b) Structural representation of carnosine coordination mode with Zn (II). Structural illustration of the metal peptide framework constructed from carnosine and Zn (II). Part (c) is reprinted with permission from ref \textsuperscript{114}, copyright: 2017 Elsevier B.V.

1.6. Scope and Objective of Research Work

There are considerable drawbacks on the current strategies for synthesis of metal-peptide frameworks that rely on the peptide C-terminus, N-terminus and side chains of amino acid residues as coordination sites with metals. The frameworks produced are by mostly low-dimensional and compact frameworks, with higher dimensions arising from stacking of peptide metal framework layers through hydrogen bonding interaction or through extra metal coordination sites provided by side chains of amino acid residues.\textsuperscript{117-122} One other drawback, is that substitution one of the amino
acid residues in the peptide linker with a different amino acid leads to obtaining different metal-peptide structures because of the different metal-peptide coordination modes or/and the different coordination spheres (number and configuration) of the metal ions which is a consequence of using single metal ions in the construction of these metal peptide frameworks.\textsuperscript{117-122,113-115} With the lack of orientation or directionality, this approach leads to rather random frameworks that cannot be made by design. Moreover, they show no variability and structural diversity, because changing the amino acids would result in different structure outcomes with presumably different properties. The rational design of metal organic frameworks is a fundamental principle of reticular chemistry,\textsuperscript{17} this principle is challenging to achieve with the current strategies for construction of metal peptide organic frameworks.

The scope of our research is to design and synthesize novel peptide linkers with the general structure displayed in Figure 1.50 where both the N-terminus and the C-terminus ends of the peptide are attached to ditopic 1,3-benzene dicarboxylate groups (isophthalate moieties).

![Figure 1.50. Chemical structure of novel tetracarboxylate peptide-based linkers.](image)
These ditopic benzene carboxylate groups would serve as the coordination centers with metals such as Cu(II) forming the dicopper paddlewheel square metal cluster, which is a rigid well-defined SBU (secondary building unit) with intrinsic geometric properties, facilitating the rational design of MOFs and avoiding lack of directionality associated with single metal ions. An advantage of using chelating ligands in formation of metal clusters such as carboxylate based ligands is the structural stability of the framework produced which is an important criteria based on an application perspective in which permeant pores of the framework are desired. This is in contrast to the metal-peptide frameworks reported in the literature where the carboxylate and amine functionalities of the amino acid residues of the peptides where used as coordination sites with single metal ions for construction of peptide MOFs that lacked rigidity and orientation which lead to random frameworks that cannot be made by design.

Our flexible peptide ligands contains two isophthalate moieties serving as “I” shaped tetracarboxylate ligands (4-c ligands) and are used to construct 2-dimensional supramolecular building layers (SBLs) based on the 4-c dicopper square paddlewheel MBBs (sql or kgm 2D-layers) resulting in the pillaring of adjacent layers through the peptide core of the di-isophthalate ligand leading to construction of 3-D MOFs based on a (4,4)-c topology. This is the Ligand-to-Ligand (L-L) pillaring strategy for construction of 3D periodic MOFs and if the pillaring is based on 2D kgm layers then the resultant 3D MOF could be of the nbo/fof or ssa/sty nets, if the pillaring is based on 2D sql layers then the resultant 3D MOF could be of the sbb/stx or lvt/lil nets. It is worth mentioning that the use of flexible diisophthalate linkers in the construction of MOFs can lead to unprecedented architectures with new topologies that cannot be readily obtained using rigid linkers. This is due to the different conformations that can be adopted by flexible ligands during the self-assembly process.
The scope of this research includes an organic synthetic part concerned with the design, synthesis and characterization of novel tetracarboxylate peptide ligands as well as a reticular chemistry part concerned with the synthesis and characterization of novel metal peptide frameworks based on these peptide tetracarboxylate ligands through the Ligand-to-Ligand pillaring of supramolecular building layers strategy for construction of 3D MOFs.

Metal-peptide frameworks are highly sought out materials for their modularity, structural flexibility, biocompatibility, adaptability and structural control opening up exciting opportunities for their use in fields such as, artificial enzyme-mimicking catalysis, chiral separation and bimolecular recognition. These materials have gained interest of the supramolecular chemistry community for their potential to create great opportunities for addressing key challenges facing the field of porous materials. Our strategy for design and synthesis of metal-peptide frameworks introduced in this chapter would lead to construction of more stable, robust and porous 3D metal-peptide frameworks with the potential of addressing key challenges facing development of current biomimetic porous materials.
1.7. References


2.1. Introduction

Metal organic frameworks (MOFs) are a class of supramolecular materials with extended networks formed through the reticular assembly of bi- or multidentate ligands with metal ions via coordination bonds. MOFs have gained popularity for applications in many fields such as gas sorption, chiral recognition, catalysis and separation.

The chemical structures of many of the ligands that are used in MOF construction consist of rigid aromatic systems as part of their core structure, serving as spacers between metal coordination complexes. Following the rules of reticular principles in the design and synthesis of MOFs provides extended structures with a myriad of networks such as tetrahedral, cubic or octahedral where the corners are occupied with metal complexes. Biomolecules are considered an attractive pool for the supramolecular chemist for the construction of new types of MOFs as they provide an attractive platform due to their diverse structures and functionality. Bio-MOFs have attracted attention for applications in medicine and biology due to their biocompatibility and biodegradability. There have been various approaches to decorate known MOFs with biomolecules such as peptides, proteins and nucleic acids, but examples of MOFs that are constructed from biomolecules are rare.

Peptides are attractive biomolecules to serve as ligands in the construction of MOFs since they are chiral and offer the possibility of introduction of various functional groups and building blocks in their structure through conventional organic synthesis. Natural peptide assemblies play a
central role in function and construction of living systems\textsuperscript{16} and there has been a substantial interest over the past decade in the design and construction of artificial peptide-based assemblies with targeted structural, physical and chemical properties, with the goal of mimicking natural biological functions.\textsuperscript{17} Peptide chemistry offers the supramolecular chemist unlimited structural diversity besides the intrinsic chirality and flexibility of peptide molecular structures making peptides prime candidates for the construction of extended metal peptide frameworks (peptidic MOFs).\textsuperscript{17,18} The flexibility of the peptide linker used in construction of peptide metal frameworks allows the pore conformations to adapt to the nature and loading of guest molecules opening a range of routes in terms of controlling functions which are not possible in case of rigid frameworks constructed from rigid linkers that limit the ability of host molecules to rearrange.\textsuperscript{17,9,13,19,20} The ability of MOFs constructed form flexible linkers such as peptides to expand or contract upon uptake and release of guest molecule (breathing) is in contrast to rigid frameworks that are unaffected by molecular binding.\textsuperscript{17,9,13,19,20} This breathing ability of flexible peptide MOFs mimics the ability of natural enzymes which undergo structural transitions upon binding to small molecules.\textsuperscript{17,20} The broad choice of side-chain groups of amino acid residues in peptide linkers can be used by the supramolecular chemist to engineer the pores or pockets of peptide MOF with specific chiral functional groups.\textsuperscript{17,18}

Fascinatingly, the adoption of concepts of flexible frameworks constructed from naturally occurring molecules such as peptides should open the door to the preparation of complex crystalline bio-hybrid frameworks that incorporate functionality as well as flexible porosity. Using peptide linkers in construction of porous materials allows for generating unique biomimetic materials were peptide linkers are embedded in a crystalline lattice separated from each other,\textsuperscript{17} thus generating tunable nanopores or cavities with the ability to undergo conformational changes...
similar to natural protein systems facilitating the ability to undergo adaptable regulations to guest molecules as well as control over the size, shape and functionality of the internal pores.\textsuperscript{17,18}

The construction of networks from peptidic ligands are sought after for their modularity, structural flexibility, biocompatibility, adaptability and structural control, creating great opportunities for addressing key challenges facing the field of porous materials.\textsuperscript{17,18} Despite the great advances in the construction of metal organic frameworks (MOFs), the emergence of frameworks constructed using peptide ligands is less well-explored.\textsuperscript{17} The high conformational flexibility of peptides makes the process of obtaining a crystalline peptide MOF difficult and highly challenging which have hampered the development of crystalline peptide MOFs.\textsuperscript{21,22,5} Also, many of the reported peptide metal organic frameworks are low dimensional and compact frameworks that rely on the peptide C-terminus, N-terminus and side chains of amino acid residues as coordination sites with single metal ions.\textsuperscript{17,18,9,12,14} These current strategies for construction of metal peptide frameworks lead to rather random frameworks that cannot be made by design.

The effective designing of a MOF and its synthesis relies on the precise choice of well-designed organic linkers acting as bridges or building blocks, with metal ions or metal clusters as nodes.\textsuperscript{23-26,1,4} Isophthalate (1,3-benzenedicarboxylate or 1,3-bdc) is a dicarboxylate based linker subtended with a 120° angle by the carboxylate moieties allowing it to act as an angular linker between the vertices of square paddlewheel \([\text{M}_2(\text{-COO})_4]\) molecular building blocks (MBBs) and sustaining square of square leading to construction of 2D undulating square grid (sql) supramolecular isomers\textsuperscript{27-31} or sustaining triangles of squares leading formation of 2D Kagomé lattice (kgm) supramolecular isomers.\textsuperscript{32-34} Extension of the isophthalate moiety at the 5-position with another isophthalate moiety can pillar both 2D square grid or 2D Kagomé lattice supramolecular isomers into 3D frameworks with various topologies.\textsuperscript{23,35} This is the Ligand-to-
Ligand pillaring of supramolecular building layers approach for construction 3D MOFs and if the pillaring is based on 2D Kagomé lattice layers, the resultant 3D MOF could be of the **nbo/fof** or **ssa/sty** nets.\(^{23,35}\) If the pillaring is based on 2D **square grid** layers then the resultant 3D MOF could be of the **ssb/stx** or **lvt/lil** nets.\(^{23,35}\) Thus, rigidly linking two isophthalate (1,3-benzenedicarboxylate) moieties at the 5-position to form diisophthalate ligands can afford 3D MOFs with special topologies.

### 2.2. Scope of research work and strategy

In this chapter we present the design and organic synthesis of three novel linkers (**L1**, **L2** and **L3**) as displayed in Figure 2.1.

![Figure 2.1. Chemical Structure L1, L2 and L3.](image)

**Figure 2.1.** Chemical Structure L1, L2 and L3.
The structure of $L_1$ (Figure 2.1) is based on attaching two ditopic 1,3-benzene dicarboxylate groups (isophthalate moieties) to the N-terminus and C-terminus ends of the amino acid glycine. The structures of $L_2$ and $L_3$ (Figure 2.1) are based on attaching two isophthalate moieties to the N-terminus and C-terminus ends of the Gly-Gly dipeptide and the Gly-Gly-Gly tripeptide respectively. Thus, our strategy is based on designing isophthalate-derived peptide ligands, in which a pair of isophthalates are bridged by a peptide core. These ditopic 1,3-benzene carboxylate groups (isophthalate moieties) would serve as coordination centers with metals such as Cu (II) forming the dicopper paddlewheel square metal cluster, which is a rigid well-defined SBU (secondary building unit) with intrinsic geometric properties. Thus, the assembly of our tetracarboxylate peptide based ligands with dicopper paddlewheels SBU$s$ would lead to construction of 3D porous peptide-based frameworks and facilitate the rational design of peptide-based metal organic frameworks (peptide-MOFs).

In this chapter we discuss our attempts to synthesize novel 3D peptide MOFs based on our flexible peptide ligands ($L_1$, $L_2$ and $L_3$) which contains two isophthalate moieties serving as “I” shaped tetracarboxylate ligands (4-c ligands) and are used to construct 2-dimensional supramolecular building layers (SBLs) based on the 4-c dicopper square paddlewheel MBBs (sql or kgm 2D-layers) resulting in the pillaring of adjacent layers through the peptide core of the di-isophthalate ligand leading to construction of 3-D MOFs based on a (4,4)-c topology (Figure 2.2). This is the Ligand-to-Ligand (L-L) pillaring strategy for construction of 3D periodic MOFs, and if the pillaring is based on 2D kgm layers, the resultant 3D MOF could be of the nbo/fof or ssa/sty nets. If the pillaring is based on 2D sql layers, the resultant 3D MOF could be of the ssb/stx or lvt/lil nets which are the possible topological platforms that could be obtained following this strategy for construction of 3D pillared MOFs (Figure 2.2). It is worth
mentioning that the use of flexible diisophthalate linkers in the construction of MOFs can lead to unprecedented architectures with new topologies that cannot be readily obtained using rigid linkers. This is due to the different conformations that can be adopted by flexible ligands during the self-assembly process which is also a reason for the difficulty of constructing MOFs using flexible ligands compared to using rigid ligands.

Figure 2.2. (a) Layer segment of a 2D kgm-MOF, made from 1,3-BDC ligand and M\(_2\)(OOCR)A\(_2\) paddlewheel MBB, and the corresponding Kagomé lattice net representation (overhead view). (b) Layer segment of a 2D sql-MOF, made from 1,3-BDC ligand and M\(_2\)(OOCR)A\(_2\) paddlewheel MBB, and the corresponding square lattice net representation (overhead view). (c) side view of the Kagomé lattice net and the square lattice net respectively (d) Scheme representing the ligand-to-ligand pillaring technique using an exemplary “I” shaped tetracarboxylate peptide-based ligand and the possible framework topologies of the resulting pillared MOFs. Adapted from ref \(^{35}\) with permission, copyright: 2011 American Chemical Society, and from ref \(^{23}\) with permission from the Royal Society of Chemistry.
2.3. Results and Discussion

2.3.1. Design and synthesis of ligands.

Initially, key intermediate compound 3 was prepared according to Scheme 2.1. The Basic hydrolysis of the commercially available trimethyl 1,3,5-benzenetricarboxylate (compound 1) using 1 equivalent of aqueous NaOH in MeOH to afford compound 2 (1,3,5-benzenetricarboxylic acid dimethyl ester) was accomplished through a reported procedure with major modifications for the purification of the final product.\textsuperscript{37} Conversion of compound 2 to the corresponding acid chloride (compound 3) was achieved by refluxing in SOCl\textsubscript{2} and adding few drops of DMF as a catalyst. Conversion of compound 2 to the acyl chloride could also be accomplished under milder conditions at room temperature by using oxalyl chloride with few drops of DMF as a catalyst.

Scheme 2.1. Synthesis of key intermediate compound 3 (dimethyl 5-(chlorocarbonyl) isophthalate)
**Synthesis of L1.** The synthesis of the glycine tetracarboxylate linker (L1) was accomplished through Scheme 2.2. The coupling reaction of the commercially available aromatic amine (compound 4) with the N-Boc protected glycine amino acid (compound 5) to afford compound 6 was accomplished through a reported procedure,\(^3\) but with major modifications to the synthesis conditions where we have employed a different peptide coupling reagent HBTU (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) to accomplish the coupling of the aromatic amine 4 with compound 5. We also have also improved the purification conditions of compound 6 which we purified through recrystallization from hot EtOAc thus avoiding using column chromatography for purification which was the purification method used by the reported procedure. Our modifications allowed for the synthesis of key compound 6 through a reproducible and convenient route suitable for multigram production and with easier purification as well as higher yields compared to the reported procedure. Removal of the Boc protecting group of compound 6 was performed under acidolytic conditions using trifluoroacetic acid (TFA) affording the corresponding trifluoroacetate salt (compound 7). The acylation of compound 7 with the synthesized intermediate compound 3 (dimethyl 5-(chlorocarbonyl)isophthalate) in dry CH\(_2\)Cl\(_2\) and in the presence of triethylamine afforded the tetraester compound 8. Subsequent ester hydrolysis of compound 8 using 1N LiOH afforded the glycine tetracarboxylate linker, L1, (compound 9). The overall yield is 24%.
Scheme 2.2. Synthesis of L1, a glycine tetracarboxylate ligand

\[
\text{H}_2\text{COOC}_3^+ + \text{CH}_2\text{Cl}_2 / \text{MeOH (9:1)} \\
0 ^\circ \text{C} \text{ to rt, 4h} \\
98% \\
\rightarrow \text{TFA-H}_2\text{N}^+ - \text{O} \rightarrow \text{N} \rightarrow \text{P} \rightarrow \text{COOCH}_3^-
\]

\[
\text{H}_3\text{COOC}_3^+ + \text{CH}_2\text{Cl}_2 / \text{TEA} \\
0 ^\circ \text{C} \text{ to rt, 16h} \\
54% \\
\rightarrow \text{H}_3\text{COOC}-\text{COOCH}_3^-
\]

\[
\text{H}_3\text{COOC}_3^+ + \text{MeOH/THF/H}_2\text{O (1:1:1)} \\
0 ^\circ \text{C} \text{ to rt, 24h} \\
67% \\
\rightarrow \text{HOOC-} \rightarrow \text{COOH}
\]
**Synthesis of L2.** The synthesis of the **gly-gly tetracarboxylate linker (L2)** was accomplished through Scheme 2.3. Coupling of the aromatic amine (compound 4) with Boc-Gly-OH (compound 5) using the peptide coupling reagent HBTU afforded compound 6. Deprotection of the Boc group of compound 6 under acidolytic conditions using trifluoroacetic acid (TFA) afforded the corresponding trifluoroacetate salt compound 7. The trifluoroacetate salt (compound 7) was coupled with Boc-Gly-OH (compound 5) using the coupling reagent HBTU in DMF and DIEA to afford the dipeptide compound 10. The N-Boc protecting group of compound 10 was removed under acidolytic conditions using trifluoroacetic acid (TFA) to afford corresponding trifluoroacetate salt (compound 11). The acylation reaction of compound 11 with dimethyl 5-(chlorocarbonyl)isophthalate (compound 3) in dry CH$_2$Cl$_2$ and in the presence of triethylamine afforded the tetraester compound 12. Even though this reaction involved a simple workup for purification of compound 12, yields of compound 12 ranged between 29-58% in different batches following this reaction which could be attributed to the low solubility of the free amine of compound 11 in CH$_2$Cl$_2$. In order to improve the yield of compound 12, we elected to perform the coupling of compound 11 with 1,3,5-benzenetricarboxylic acid dimethyl ester (compound 2) using the peptide coupling reagent HBTU in DMF and in the presence of TEA which afforded compound 12 in 60 % yield. Basic hydrolysis of compound 12 using 1N LiOH afforded the Gly-Gly dipeptide tetracarboxylate linker (compound 13). The overall yield is 17%.
Scheme 2.3. Synthesis of L2, a Gly-Gly dipeptide tetracarboxylate ligand
Synthesis of L3 (Gly-Gly-Gly tripeptide tetracarboxylate ligand). Initially key intermediate compound 15 was prepared according to Scheme 2.4. Basic hydrolysis of compound 1 using 1 eq aq NaOH afforded compound 2 which was converted to the acyl chloride (compound 3) using thionyl chloride (SOCl₂) or oxalyl chloride ((COCl)₂) with a few drops of DMF as a catalyst. The condensation of dimethyl 5-(chlorocarbonyl)isophthalate (compound 3) with glycine (compound 14) and using aq NaHCO₃ as a base affording compound 15 was performed according to a reported procedure for synthesis of similar compounds.⁴⁹ A major drawback of this procedure is the low yield of the target compound 15 (17%). This could be attributed to the hydrolysis of the acyl chloride compound 3 to the carboxylic acid derivative under the aqueous conditions of the reaction leading to low yields of the desired product.

Scheme 2.4. Synthesis of intermediate compound 15

\[
\begin{align*}
\text{H}_3\text{COOC} & \text{COCOCH}_3 \\
\text{COOCH}_3 & \\
\text{NaOH}_{aq} & 1 \text{ M} \\
\text{MeOH} & \\
\text{SOCl}_2 & \text{DMF} (\text{cat}) \\
\text{Reflux, 16h} & 91\% \\
\text{Or:} & \\
\text{(COCl)}_2 & \text{CH}_2\text{Cl}_2, \text{DMF} (\text{cat}) \\
0 \text{ °C to rt, 16h} & 95\% \\
\end{align*}
\]
Due to the low yields of target compound 15 obtained through applying Scheme 2.4, we elected to follow a different path for the synthesis of compound 15 (Scheme 2.5) based on reported procedures for the synthesis of similar compounds. We elected to use the commercially available glycine tert-butyl ester hydrochloride (compound 16) which allowed us to perform the reaction of dimethyl 5-(chlorocarbonyl)isophthalate (compound 3) with glycine tert-butyl ester hydrochloride (compound 16) using an organic solvent (dry CH₂Cl₂) and in the presence of triethylamine affording the Boc protected compound 17 with an 87% yield after column chromatography purification. Deprotection of the tert-butyl group of compound 17 under acidolytic conditions using trifluoroacetic (TFA) afforded target compound 15 with a 76% yield.

Scheme 2.5. Synthesis of intermediate compound 15
The synthesis of the Gly-Gly-Gly tripeptide tetracarboxylate ligand (L3) was executed as shown in Scheme 2.6. Coupling of the aromatic amine (compound 4) with Boc-Gly-OH (compound 5) using the peptide coupling reagent HBTU afforded compound 6. Deprotection of the Boc group of compound 6 under acidolytic conditions using trifluoroacetic acid (TFA) afforded the corresponding trifluoroacetate salt compound 7. The trifluoroacetate salt (compound 7) was coupled with Boc-Gly-OH (compound 5) using the coupling reagent HBTU in DMF and DIEA to afford the dipeptide compound 10. The N-Boc protecting group of compound 10 was removed under acidolytic conditions using trifluoroacetic acid (TFA) to afford corresponding trifluoroacetate salt (compound 11). The coupling of compound 11 with the synthesized key intermediate compound 15 using the water soluble peptide coupling reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) in the presence of 1-hydroxybenzotriazole (HOBt) afforded the tripeptide tetramethyl ester compound 18. Basic hydrolysis of compound 18 using 1N LiOH afforded the Gly-Gly-Gly tripeptide tetracarboxylate linker L3 (compound 19). The overall yield is 20%.
Scheme 2.6. Synthesis of L3, a Gly-Gly-Gly tripeptide tetracarboxylate ligand

\[
\text{H}_3\text{COOC} + \text{COOCH}_3 \quad \text{4} \quad \text{HBTU} \quad \text{DIEA} \quad \text{DMF} \quad \text{RT, 20h} \quad 69\%
\]

\[
\text{TFA} \quad \text{CH}_2\text{Cl}_2 / \text{MeOH (9:1)} \quad 0 \degree \text{C to rt, 4h} \quad 98\%
\]

\[
\text{TFA-H}_2\text{N} \quad \text{5} \quad \text{COOCH}_3 \quad \text{7}
\]

\[
\text{TFA-H}_2\text{N} \quad \text{COOCH}_3 \quad \text{10} \quad \text{TFA} \quad \text{CH}_2\text{Cl}_2 / \text{MeOH (9:1)} \quad 0 \degree \text{C to rt, 4h} \quad 98\%
\]

\[
\text{TFA-H}_2\text{N} \quad \text{COOCH}_3 \quad \text{11}
\]

\[
\text{H}_3\text{COOC} \quad \text{COOCH}_3 \quad \text{15} \quad \text{EDC-HCl} \quad \text{HOBt} \quad \text{TEA} \quad 0 \degree \text{C to rt, 16h} \quad 71\%
\]

\[
\text{H}_3\text{COOC} \quad \text{COOCH}_3 \quad \text{18}
\]

\[
\text{1N LiOH} \quad \text{MeOH/THF/H}_2\text{O (1:1:1)} \quad 0 \degree \text{C to rt, 24h} \quad 58\%
\]

\[
\text{COOH} \quad \text{L3}
\]
2.3.2. Synthesis of The Metal Organic Framework Complexes

Synthesis of Metal complex using L1 and Cu(NO$_3$)$_2$ · 2.5H$_2$O. In order to synthesize single crystals of the MOF (MH-1-Gly), Cu(NO$_3$)$_2$·2.5H$_2$O and L1 were mixed combinatorially under a variety of solvothermal conditions (Scheme 2.7).

Scheme 2.7. Synthesis of MH-1-Gly.

Due to the high flexibility of the ligand (L1) the synthesis of crystalline MOF samples of MH-1-Gly proved challenging. We have performed dozens of solvothermal experiments under different conditions that included variations in temperature, solvent systems and modulators in order to grow single MOF crystals suitable for single crystal X-ray diffraction analysis. We here report the best solvothermal reaction conditions for obtaining single MOF crystals.

Solvothermal reaction conditions 1: To a solution of the linker L1 (21.5 mg, 0.05 mmol) in 1.4 mL solvent mixture of DMF/DMA/EtOH/H$_2$O (5:5:1:1 v/v/v/v) in a 5 mL scintillation vial was added 23.3 mg Cu(NO$_3$)$_2$·2.5H$_2$O (0.1 mmol). Acetic acid (0.2 mL) was then added followed by 0.1 mL pyridine. The vial was capped, and the clear solution was heated in an oven for 48 h at 80 °C. This solvothermal reaction condition afforded green block shaped single crystals (Figure 2.3).
Figure 2.3. Microscopic image of the green colored single crystals of as-synthesized MH-1-Gly obtained under solvothermal conditions 1.

X-ray diffraction experiments of the single crystals of the MOF MH-1-Gly obtained under solvothermal conditions 1 were performed at the chemical crystallography laboratory of University of Oklahoma and the X-ray data modeling showed the coordination environment of Cu(II) around the isophthalate moieties (1,3-benzenedicarboxylate moieties) of the ligand where two Cu$^{2+}$ cations are bridged by four carboxylate groups forming the dicopper paddlewheel [Cu$_2$(-COO)$_4$] molecular building block with two water molecules occupying the axial positions. A high degree of disorder has been found for the core glycine structure between the two isophthalate moieties of the ligand, which can be ascribed to the conformational flexibility of this part of the linker and the high disorder of the linker within the framework. The glycine core structure of the ligand could not be resolved and modelled from the x-ray data, and the general structure of the MOF could not be obtained. In order to obtain the complete structure of the MOF, X-ray diffraction data collected using synchrotron radiation would be a choice to be considered as exemplified by structures of several MOFs determined using X-ray data collected using synchrotron radiation.$^{41,44}$ The identity of the core Gly structure of the ligand was confirmed through $^1$H-NMR experiments where $^1$H-NMR of digested MOF sample crystals of MH-1-Gly in d$_6$-DMSO / DCI (20 wt% in D$_2$O) mixture allowed re-isolation of the ligand which could then be analyzed by $^1$H-NMR experiments (Figure 2.4).
Figure 2.4. $^1$H-NMR spectrum of digested MH-1-Gly in d$_6$-DMSO / DCI (20 wt% in D$_2$O) mixture.

We carried out powder X-ray diffraction (PXRD) experiments to examine the effect of solvent exchange on the framework structure of MH-1-Gly (Figure 2.5). Solvent exchange of the nonvolatile solvents (DMF, DMA, H$_2$O) included in the cavities of the framework with acetone was performed and the sample was left immersed in acetone for several days. The PXRD pattern of the sample immersed in acetone appeared to be broadened slightly but was very similar to that of the as-synthesized MH-1-Gly sample (Figure 2.5). This indicates that the nonvolatile solvents included in the cavities of the framework could be exchanged with acetone without collapse or alteration of the framework structure. Acetone would be an activating solvent of choice prior to gas adsorption measurements experiments.
Figure 2.5. Experimental PXRD patterns for as-synthesized MH-1-Gly and after immersion in acetone. Patterns provided here are for as-synthesized samples, thus the hump over a wide range of two-theta is due to the inclusion of large amount of disordered solvent molecules.

In order to evaluate the thermal stability of MOF MH-1-Gly obtained under solvothermal conditions 1, thermogravimetric analysis (TGA) was carried out in an N₂ environment. TGA analysis revealed a nearly 38% weight loss from 31 to ~ 185 °C which can be attributed to removal of guest solvent molecules from the surface and the channels (Figure 2.6). A near steady plateau follows from ~ 185 °C to ~ 270 °C with a nearly 10 % mass loss between 185 and 270 °C which can be attributed to removal of coordinated water molecules. The mass loss beyond 270 °C is due to decomposition of the complex framework. Thus MH-1-Gly can be stable to a temperature of about 270 °C.
The following solvothermal conditions have also resulted in crystalline products:

Solvothermal reaction conditions 2: To a solution of the linker L1 (10.8 mg, 0.025 mmol) in 0.85 mL solvent mixture of DMF/DMSO/EtOH/Acetonitrile (2:2:1:1 v/v/v/v) in a 5 mL scintillation vial was added Cu(NO₃)₂ • 2.5H₂O (11.7 mg, 0.05 mmol). Acetic acid (0.1 mL) was then added followed by 0.05 mL pyridine. The vial was capped, and the clear solution was heated in an oven for 48 h at 80 °C to afford green-block crystals (Figure 2.7).

**Figure 2.6.** TGA plot of MH-1-Gly.

**Figure 2.7.** Microscopic image of the green colored crystals of MH-1-Gly obtained under solvothermal conditions 2.
The powder X-ray diffraction (PXRD) patterns of the as-synthesized crystal sample obtained under conditions 2 closely matched the patterns of the as-synthesized MH-1-Gly sample obtained under conditions 1 indicating that they are isostructural (Figure 2.8).

![Figure 2.8](image)

**Figure 2.8.** Experimental PXRD patterns for MH-1-Gly obtained under solvothermal conditions 1 and 2. Patterns provided here are for as-synthesized samples, thus the hump over a wide range of two-theta is due to the inclusion of large amount of disordered solvent molecules.

Solvothermal reaction conditions 3: To a solution of the linker **L1** (10.8 mg, 0.025 mmol) in 0.85 mL solvent mixture of DMF/DMSO/EtOH/Acetonitrile/H₂O (2:2:1:1:1 v/v/v/v) in a 5 mL scintillation vial was added Cu(NO₃)₂·2.5H₂O (11.7 mg, 0.05 mmol). Acetic acid (0.1 mL) was then added followed by 0.05 mL pyridine. The vial was capped, and the clear solution was heated in an oven for 72 h at 80 °C to afford green block crystals. The yield of the crystals obtained under these conditions was too low for PXRD experimentation.
Synthesis of Metal complex using L2 and Cu(NO\textsubscript{3})\textsubscript{2} \cdot 2.5H\textsubscript{2}O. In order to synthesize the metal peptide framework MH-2-Gly-Gly by the solvothermal reaction of Cu(NO\textsubscript{3})\textsubscript{2} \cdot 2.5H\textsubscript{2}O with the Gly-Gly tetracarboxylic acid ligand L2 (Scheme 2.8) we have performed a great number of years long series of solvothermal reactions under various conditions that included different temperature, solvent systems, modulators and molar ratios of ligand and metal source in order to obtain single crystals of the MOF suitable for single X-ray diffraction experiments.


The synthesis of peptide MOF single crystals (MH-2-Gly-Gly) using L2 is highly challenging due the conformational flexibility imparted by the Gly-Gly dipeptide core structure of the linker. We here discuss the best synthetic conditions for the synthesis of the MOF (MH-2-Gly-Gly).

Solvothermal reaction conditions 1: L2 (24.4 mg, 0.05 mmol) and Cu(NO\textsubscript{3})\textsubscript{2} \cdot 2.5H\textsubscript{2}O (23.3 mg, 0.1 mmol) were dissolved in 1.7 ml of a solvent mixture of DMF/EtOH/H\textsubscript{2}O (5:1:1 v/v/v) in a 5 mL scintillation vial and 0.1 mL pyridine was added. The vial was capped, and the clear solution was heated at 75 °C for 48 h to yield green block crystals. The crystals obtained under these conditions were not single crystals.

Solvothermal reaction conditions 2: L2 (24.4 mg, 0.05 mmol) and Cu(NO\textsubscript{3})\textsubscript{2} \cdot 2.5H\textsubscript{2}O (23.3 mg, 0.1 mmol) were dissolved in 1.7 ml of a solvent mixture of DMF/DMA/EtOH/H\textsubscript{2}O (5:1:1:1 v/v/v/v)
v/v/v/v) in a 5 mL scintillation vial and 0.1 mL pyridine was added. The vial was capped, and the clear solution was heated at 75 °C for 48 h to yield green block crystals. The crystals obtained under these conditions were not single crystals.

Solvothermal reaction conditions 3: \textbf{L2} (24.4 mg, 0.05 mmol) and Cu(NO$_3$)$_2$ · 2.5H$_2$O (23.3 mg, 0.1 mmol) were dissolved in 1.7 ml of a solvent mixture of DMF/DMA/EtOH/H$_2$O (5:5:1:1 v/v/v/v) in a 5 mL scintillation vial and 0.2 mL acetic acid was added followed by addition of 0.1 mL pyridine. The vial was capped, and the clear solution was heated at 80 °C for 48 h to yield green square crystals (Figure 2.9). The crystals obtained under these conditions were single crystals suitable for X-ray single crystal analysis.

![Microscopic image of the single crystals of the MOF obtained under solvothermal conditions 3.](image)

**Figure 2.9.** Microscopic image of the single crystals of the MOF obtained under solvothermal conditions 3.

X-ray diffraction experiments of the peptide MOF (MH-2-Gly-Gly) samples obtained under solvothermal conditions 3 were performed at the small molecule X-ray chemical crystallography laboratory of the University of Oklahoma. The coordination environment of Cu(II) around the isophthalate moieties of the ligand were fully resolved in the crystal data of MH-2-Gly-Gly and could be modeled but the core Gly-Gly dipeptide structure between the two isophthalate moieties of the linker was not fully resolved in the electron density maps of the crystal structure due to high
disorder of this part of the ligand and could not be modeled. This can be ascribed to the conformational flexibility of this part of the linker and the high disorder of the linker within the framework. However, the identity of the core Gly-Gly dipeptide structure of the ligand was confirmed through $^1$H-NMR experiments where $^1$H-NMR of digested MOF sample crystals of MH-2-Gly-Gly in $d_6$-DMSO / DC1 (20 wt% in D$_2$O) mixture allowed re-isolation of the ligand which could then be examined by $^1$H-NMR experiments which verified the structure and integrity of the ligand (Figure 2.10).

![Figure 2.10](image)

Figure 2.10. $^1$H-NMR spectrum of digested MH-2-Gly-Gly in a $d_6$-DMSO / DC1 (20 wt% in D$_2$O) mixture.

The coordination environment of Cu(II) around the isophthalate moieties (1,3-benzenedicarboxylate moieties) of the ligand were fully resolved in the crystal data of the MOF (MH-2-Gly-Gly) of the single crystal X-ray experiments performed at the University of Oklahoma and could be modelled. Based on these data we were able to analyze the overall structure of the
peptide MOF MH-2-Gly-Gly. Single-crystal X-ray structure determination of MH-2-Gly-Gly reveals that it is sustained by $[\text{Cu}_2(-\text{COO})_4(\text{H}_2\text{O})_2]$ paddlewheel molecular building blocks MBB in which two Cu$^{2+}$ cations are bridged by four carboxylate groups (Cu–Cu separation $d = 2.653$ (11) Å) that are distributed around the Cu–Cu axis defining the paddlewheel as a square-planar 4-c node (Figure 2.11 b). The Cu$^{2+}$ cations adopt a square pyramidal geometry, since they each coordinate to four oxygen atoms from carboxylate groups (the equatorial Cu–O distances span the range of 1.8390 (3) - 2.0139 (3) Å) and H$_2$O molecules occupy the axial sites of the paddlewheels. The axially bound water molecules show the Cu1–O8 and Cu2–O9 distances to be 2.2722 (3) and 2.2713(3) Å, respectively. Each ligand is coordinated to four paddlewheel units where each isophthalate moiety (1,3-benzenedicarboxylate moiety) of the ligand links two paddlewheel $[\text{Cu}_2(-\text{COO})_4]$ units. The vertices of the $[\text{Cu}_2(\text{carboxylate})_4]$ paddlewheel square MBB are linked by the 1,3 carboxylate groups of the diisophthalate moiety at both ends of the linker into squares of squares adopting alternating cone calixarene-like conformations and 1,3-alternate calix[4]arene-like partial cone conformations rendering 2D square grid layers (4,4-nets) of the sql-1 type$^{30,27,23}$ (Figure 2.12) that are in turn pillared via ligand-to-ligand cross-linking in an eclipsed fashion into a 3D MOF with a ssb/stx type topology$^{23,35}$ (Figure 2.13). Due to the 120° angle subtended by the two carboxyls of the two isophthalate moieties of the ligand (1,3-benzenedicarboxylate moieties), the pillared sql-1 layers in the MOF exhibit an undulating or zigzagged layered structure as a result of this curvature induced by the isophthalate moieties.
Figure 2.11. (a) Gly-Gly tetracarboxylate ligand (L2). (b) Dicopper paddlewheel SBU, Color code: black, C; red, O; blue, Cu. Hydrogen atoms are omitted for clarity.

<table>
<thead>
<tr>
<th>Bond</th>
<th>Distance (Å)</th>
<th>Angle (deg)</th>
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<tbody>
<tr>
<td>Cu1-Cu2</td>
<td>2.6525(11)</td>
<td></td>
</tr>
<tr>
<td>Cu1-O8</td>
<td>2.2722(329)</td>
<td></td>
</tr>
<tr>
<td>Cu1-O1B</td>
<td>1.8538(310)</td>
<td>90.6(14)</td>
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<tr>
<td>Cu1-O1A</td>
<td>1.9549(276)</td>
<td>95.2(14)</td>
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<tr>
<td>Cu1-O4B</td>
<td>1.8425(357)</td>
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</tr>
<tr>
<td>Cu1-O4A</td>
<td>1.8732(289)</td>
<td>87.7(13)</td>
</tr>
<tr>
<td>Cu2-O9</td>
<td>2.2713(345)</td>
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<tr>
<td>Cu2-O3A</td>
<td>1.8390(287)</td>
<td>92.2(13)</td>
</tr>
<tr>
<td>Cu2-O2A</td>
<td>2.0139(296)</td>
<td>98.2(12)</td>
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<tr>
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<td>1.9697(299)</td>
<td>166.2(14)</td>
</tr>
<tr>
<td>Cu2-O3B</td>
<td>1.9832(302)</td>
<td>164.7(14)</td>
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<tr>
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<tr>
<td>O4A-Cu1-O8</td>
<td>91.1(14)</td>
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</tr>
</tbody>
</table>

Table 1. Selected bond distances (Å) and angles (deg) of MH-2-Gly-Gly.
Figure 2.12. (a) View of the extended crystal structure of MH-2-Gly-Gly displaying the square grid structure formed by isophthalate units of the ligand and dicopper paddlewheel units $[\text{Cu}_2(\text{COO})_4]$ generating square shaped windows. (b) The connections of the isophthalate units and the square paddlewheel units forming the square grid structure display alternating cone and 1,3-alternate calix[4]arene like conformations rendering the sq1-1 type square grid structure. (c) Side view of a one layer structure of MH-2-Gly-Gly showing the undulating nature of the layer. Color code: black, C; red, O; blue, Cu. Hydrogen atoms are omitted for clarity.
Figure 2.13. (a) View of the extended crystal structure of MH-2-Gly-Gly displaying the ligand-to-ligand pillaring of two sql-1 layers of MH-2-Gly-Gly. The core Gly-Gly dipeptide structure of the ligand was not resolved in the single X-ray experiment due to high disorder ascribed to the conformational flexibility of this part of ligand. However, the structure and integrity of the ligand was confirmed through $^1$H-NMR experiments of digested samples of MH-2-Gly-Gly. (b) The dicopper paddlewheel SBU and the L2 ligand used for construction of MH-2-Gly-Gly. (c) Each L2 linker coordinates to four dicopper paddlewheels SBUs. Color code: black, C; red, O; blue, Cu. Hydrogen atoms are omitted for clarity.
The structure of our peptide MOF, MH-2-Gly-Gly, is similar to that of NOTT-109 reported by Schröder et al. NOTT-109 is constructed from the assembly of a polyphenyltetracarboxylate ligand with dicopper paddlewheel units leading to the formation of sq\text{1} supramolecular building layers (SBLs), which are pillared via ligand-to-ligand pillaring in an eclipsed fashion forming a 3D MOF with sq\text{b}/stx topology (Figure 2.14).\textsuperscript{41-43,4} The bulky central naphthalene group in the ligand used for construction of NOTT-109 precludes formation of the triangular windows characteristic of the nbo/fof MOFs and instead leads to formation of sq\text{1} supramolecular building layers which are pillared forming an sq\text{b}/stx MOF.\textsuperscript{41-43,4} Other reported MOFs that display sq\text{b}/stx topology is the MMPF-1 MOF reported by Wang et al\textsuperscript{44,43} (discussed in Chapter 1).

**Figure 2.14.** (a) and (b) Structure of the tetracarboxylate ligand and dicopper paddlewheel SBU used for construction of NOTT-109, respectively. (c) Extended structure of the overall framework of NOTT-109. (d) and (e) Structure of tetracarboxylate the ligand and dicopper paddlewheel SBU used for construction of MH-2-Gly-Gly, respectively. (f) Extended structure of the overall framework of MH-2-Gly-Gly.
If we view the paddlewheels in our peptide MOF, MH-2-Gly-Gly, as square planar 4-c nodes and the linker as a 4-c node having rectangular geometry, then the connection of these two types of planar 4-c nodes generates a (4,4)-c network adopting the ssb net topology (Figure 2.15).\textsuperscript{43,45} Topological deconstruction approaches suggest that the tetracarboxylate linker contains two 3-c branch points (nodes).\textsuperscript{43,45} If the topology is described considering the ligand as two 3-c nodes instead of a single planar 4-c node then the derived net is the (3,4)-c stx net (Figure 2.15).\textsuperscript{43,45} In other words, the stx net is derived from the basic ssb net by replacing the 4-c ligand node by two 3-c nodes. The ssb/stx topological description of our peptide MOF MH-2-Gly-Gly describes the basic (4,4)-c ssb net with the ligand as a 4-c single node and the derived (3,4)-c stx net with the ligand considered as two 3-c nodes.

**Figure 2.15.** Two possible abstractions of the Gly-Gly tetratopic linker (L2) + SBUs: (a) The tetracarboxylate linker can be regarded as one four coordinated vertex (a single 4-c node), (b) or as two 3-c vertices (two 3-c branch points or nodes). Red points are branch points of the linker, blue spheres represent one kind of metal SBU (the 4-c dicopper paddlewheel in case of MH-2-Gly-Gly). (c) Representation of the basic ssb net and (d) the derived (3,4)-c stx net. Part (c) and (d) are adapted from ref\textsuperscript{43} with permission from the Royal Society of Chemistry.
The structure of the peptide MOF M-2-Gly-Gly may also be regarded as an assembly of nanoscopic cage structures. Sixteen peptide ligands connect eight paddlewheel SBUs to form a nanoscopic cage, eight of the sixteen peptide ligands are face-on peptide ligands while the other eight only provide isophthalate units (Figure 2.16 a). Four dicopper paddlewheel SBUs are bridged by four isophthalate moieties of four different peptide ligands to form the top of the cage; they are pillared to four dicopper paddlewheel SBUs at the bottom of the cage through eight different peptide ligands (Figure 2.16 a). The cage has an approximate spherical cavity diameter of 20 Å with a stoichiometry of the cage of \([\text{Cu}_{16}(\text{L})_{16}]\). This cage structure displayed in the framework of MH-2-Gly-Gly is similar to the cage structure displayed in the framework of NOTT-109 (Figure 2.16 b).\(^{41}\)

**Figure 2.16.** (a) View of the structure of the nanoscopic cage of MH-2-Gly-Gly consisting of sixteen L2 ligands (eight are face-on peptide ligands, and the other eight only provide isophthalate units). (b) View of the structure of the cage of NOTT-109. Part (b) is adapted from ref\(^{41}\) with permission, copyright: 2009 American Chemical Society.
We carried out powder X-ray diffraction (PXRD) experiments to examine the effect of solvent exchange on the framework structure of MH-2-Gly-Gly. Solvent exchange of the nonvolatile solvents (DMF, DMA, H2O) included in the cavities of the framework with MeOH was performed and the sample was left immersed in MeOH for several days. The PXRD patterns of the sample immersed in MeOH appeared to be different from that of the as-synthesized sample (Figure 2.17). In contrast, the PXRD patterns of samples for which solvent exchange was performed with acetone were similar to that of the as-synthesized sample (Figure 2.18). This indicates that the nonvolatile solvents included in the cavities of the framework could be exchanged with acetone without collapse or alteration of the framework structure. Acetone would be an activating solvent of choice prior to gas adsorption measurements. Samples of MH-2-Gly-Gly that were solvent exchanged with acetone were digested in d6-DMSO / DCl (20 wt% in D2O) mixture, and 1H-NMR analysis of the digested samples showed no detectable resonance peaks for DMF or DMA (Figure 2.10), which indicates that those nonvolatile solvents were almost completely exchanged with acetone.
Figure 2.17. Experimental PXRD patterns for as-synthesized MH-2-Gly-Gly and after immersion in MeOH. Patterns provided here are for as-synthesized samples, thus the hump over a wide range of two-theta is due to the inclusion of large amount of disordered solvent molecules.

Figure 2.18. Experimental PXRD patterns for as-synthesized MH-2-Gly-Gly and after immersion in acetone. Patterns provided here are for as-synthesized samples, thus the hump over a wide range of two-theta is due to the inclusion of large amount of disordered solvent molecules.
In order to evaluate the thermal stability of the peptide MOF MH-2-Gly-Gly, thermogravimetric analysis (TGA) was carried out in an N\textsubscript{2} environment. TGA analysis revealed a nearly 38\% weight loss from 27 to \~ 170 °C which can be attributed to removal of guest solvent molecules from the surface and the channels and removal of the two terminal H\textsubscript{2}O ligands liberated from the copper paddlewheel SBUs (Figure 2.19). A steady plateau follows from \~ 170 °C to \~ 260 °C, and the mass loss beyond 260 °C is due to decomposition of the complex framework. Thus MH-2-Gly-Gly can be stable to a temperature of about 260 °C.

![Figure 2.19. TGA plot of MH-2-Gly-Gly.](image)

**Synthesis of Metal complex using L\textsubscript{3} and Cu(NO\textsubscript{3})\textsubscript{2} \cdot 2.5H\textsubscript{2}O.** In order to synthesize the metal peptide framework MH-3-Gly-Gly-Gly-Gly by the solvothermal reaction of Cu(NO\textsubscript{3})\textsubscript{2} \cdot 2.5H\textsubscript{2}O with the Gly-Gly-Gly tetracarboxylic acid ligand L\textsubscript{3} (Scheme 2.9) we have performed a long series of solvothermal reactions under various conditions that included different temperature, solvent systems, modulators and molar ratios of ligand and metal source. The L\textsubscript{3} linker has a Gly-Gly-Gly tripeptide core structure between two isophthalate units imparting a higher degree of
conformational flexibility compared to $\text{L1}$ and $\text{L2}$, and we here report the best solvothermal conditions for our attempts to synthesize the MH-3-Gly-Gly-Gly metal peptide framework.

**Scheme 2.9. Synthesis of MH-3-Gly-Gly-Gly.**

Solvothermal reaction conditions 1: To a solution of the linker $\text{L3}$ (13.6 mg, 0.025 mmol) in 0.95 mL solvent mixture of DMF/DMSO/EtOH/Acetonitrile (2:2:1:1 v/v/v/v) in a 5 mL scintillation vial was added Cu(NO$_3$)$_2$‧2.5H$_2$O (11.7 mg, 0.05 mmol). Acetic acid (0.05 mL) was then added followed by 0.05 mL pyridine. The vial was capped, and the clear solution was heated in an oven for 48 h at 80 °C to afford hexagonal like crystals. (Figure 2.20).

**Figure 2.20.** Microscopic image of the hexagonal like crystals of MH-3-Gly-Gly-Gly.

$^1$H-NMR experiments of digested samples of these hexagonal like crystals of MH-3-Gly-Gly-Gly in d$_6$-DMSO / DCl (20 wt% in D$_2$O) mixture confirmed the inclusion of the Gly-Gly-Gly tetracarboxylate ligand ($\text{L3}$) in the crystal structure of the MH-3-Gly-Gly-Gly complex (Figure 2.21), and PXRD confirmed that the material was crystalline (Figure 2.22).
**Figure 2.21.** $^1$H-NMR spectrum of digested MH-3-Gly-Gly-Gly in a d$_6$-DMSO / DCl (20 wt% in D$_2$O) mixture.

**Figure 2.22.** Experimental PXRD pattern for as-synthesized MH-3-Gly-Gly-Gly. Patterns provided here are for as-synthesized samples, thus the hump over a wide range of two-theta is due to the inclusion of large amount of disordered solvent molecules.
In order to evaluate the thermal stability of MH-3-Gly-Gly-Gly obtained under solvothermal conditions 1, thermogravimetric analysis (TGA) was carried out in an N₂ environment. TGA analysis revealed a nearly 40% weight loss from 31 to ~ 205 °C which can be attributed to removal of guest solvent molecules from the surface and the channels (Figure 2.23). A near steady plateau follows from ~ 205 °C to ~ 270 °C with a nearly 6 % mass loss between 205 and 270 °C which can be attributed to removal of possible coordinated pyridine molecules. The mass loss beyond 270 °C is due to decomposition of the complex framework. Thus MH-3-Gly-Gly-Gly can be stable to a temperature of about 270 °C.

Figure 2.23. TGA plot of MH-3-Gly-Gly-Gly.
2.4. Conclusion.

We have accomplished the design and synthesis of a novel glycine based tetracarboxylic acid linker and two novel peptide-based tetracarboxylic acid linkers based on the dipeptide Gly-Gly and the tripeptide Gly-Gly-Gly. We were able to obtain single crystals of the peptide metal organic framework MH-2-Gly-Gly using our Gly-Gly dipeptide tetracarboxylate linker and were able to determine the framework structure despite the high disorder in the dipeptide core structure of the ligand. MH-2-Gly-Gly appeared to be a 3D pillared MOF with an ssb/stx topology. These results proved the success of our strategy to construct 3D peptide MOFs based on the Ligand-to-Ligand pillaring of supramolecular building layers (SBLs) and to the best of our knowledge represent first 3D peptide-based MOFs based on the Ligand-to-Ligand pillaring strategy for construction of MOFs. We have successfully synthesized single crystals of MH-1-Gly using our single amino acid Gly-based tetracarboxylate linker for which single X-ray analysis showed that MH-1-Gly was sustained by dicopperpaddlewheel SBUs but the overall structure of the MOF could not be determined due to the high disorder in the core Gly structure of the ligand. We have synthesized crystalline material of MH-3-Gly-Gly-Gly through the solvothermal reaction of our Gly-Gly-Gly tripeptide tetracarboxylate linker with Cu(NO₃)₂ · 2.5 H₂O, and NMR experiments of digested samples of MH-3-Gly-Gly-Gly confirmed the inclusion of the linker in the crystal structure of the MH-3-Gly-Gly-Gly complex.
2.5. Experimental procedures.

2.5.1. General procedures. Chemicals and solvents were obtained from commercial sources and were used without further purification unless otherwise specified. All organic reactions were carried out in oven dried glassware with dry solvents under an atmosphere of dry nitrogen unless otherwise specified. Analytical TLC was performed on Merck 60 F254 silica gel plates with a fluorescent indicator with a 254 nm excitation wavelength. Compounds were visualized under UV light at 254 nm wavelength. Column chromatography was carried out on silica gel 60 (particle size 240-400 mesh). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Compound lyophilization was performed using a Labconco freezone 4.5 liter -84C benchtop freeze drier apparatus. NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer or an Oxford AS400 Spectrometer ($^1$H 400 MHz, $^{13}$C 100 MHz) at 25 °C. $^1$H NMR chemical shift values were determined relative to residual protonated solvent signals as internal standard ((CD$_3$)(CD$_2$H)SO in (CD$_3$)$_2$SO, δ 2.50 ppm), (CHCl$_3$ in CDCl$_3$, δ 7.26 ppm). The chemical shifts for $^1$H NMR are expressed in ppm, followed by the multiplicity (br, broad; s, singlet; d, doublet; t, triplet; q, quadruplet, qt, quintet; m, multiplet), coupling constants (J, in Hertz, Hz), and integration. $^{13}$C NMR spectra were referenced to the solvent signal (δ 39.52 ppm for (CD$_3$)$_2$SO, δ 77.00 ppm for CDCl$_3$ and δ 49.00 ppm for CD$_3$OD). All $^{13}$C NMR spectra were recorded with complete proton decoupling. NMR signals of spectra were assigned using gradient COSY (correlation spectroscopy), HSQC (heteronuclear single quantum coherence) and HMBC (heteronuclear multiple bond correlation). High-resolution mass spectra were acquired on an Agilent 6560 IM-QTOF mass spectrometer.
2.5.2. Solution $^1$H-NMR spectra of digested MOF samples.

Typically, for the digestion of the MOFs, the as-synthesized MOF sample was solvent exchanged with DMF twice daily for 2-3 days followed by acetone exchange twice daily for 2-3 days. The sample was then left to dry in air for 12 h. DMSO-$d_6$ (0.5 mL) and DCl (20 µL, 20 wt. % in D$_2$O), were added to a 4 mL vial containing the dried MOF (~10 mg), and the resulting suspension was left to rest at room temperature for 5-10 min until clear solution was obtained. The $^1$H NMR spectra were recorded immediately after (~10 min). $^1$H NMR spectra on digested solutions of MOFs were acquired on a Bruker Avance 400 MHz spectrometer, with chemical shifts of linkers identified by comparing with spectra of the pure linker.

2.5.3. Powder X-ray diffraction (PXRD)

Powder patterns were recorded on a Bruker AXS D8 Advance Phaser diffractometer with Cu Kα-1 radiation ($\lambda = 1.5406$ Å, operating at 30 kV and 10 mA) over a range of $5^\circ < 2\theta < 30^\circ$, with a step size of 0.02° steps and a 1.0 s counting time per step. The supernatant of fresh as-synthesized was exchanged with DMF, and the samples were filtered through a 0.45 µm nylon membrane filter. Collected sample was spread on a Si-Einkristalle plate immediately before PXRD measurements.

2.5.4. Thermogravimetric analysis (TGA)

TGA was performed on a Perkin Elmer TGA 4000 instrument under nitrogen atmosphere. Samples were heated at a rate of 5 °C/min. All samples were extensively solvent exchanged with fresh DMF prior to analysis.
2.5.5. Single X-ray Diffraction

As-synthesized MOF samples of MH-1-Gly and MH-2-Gly-Gly were shipped to the Chemical Crystallography laboratory at the University of Oklahoma. Single-crystal X-ray diffraction data were collected using a D8 Quest κ-geometry diffractometer with a Bruker Photon II cmos area detector and an Incoatec Iμs microfocus Mo Kα source (λ = 0.71073 Å).

2.5.6. Experimental section for synthesis of organic compounds and characterization data

1,3,5-benzenetricarboxylic acid dimethyl ester (2).\(^{37}\) Compound 1 (9.0 g, 35.7 mmol) was charged into a 1000 mL round bottom flask and 810 mL MeOH was added to produce a suspension. 1 N aq NaOH (32.1 ml, 32.1 mmol) was added dropwise to the reaction mixture and was stirred vigorously at rt for 18-22 h (the solid material completely disappeared after 8 h). The solvents were removed under reduced pressure and 600 mL CH\(_2\)Cl\(_2\) was added to the residue and stirred for 1 h after which the mixture was filtered and the collected solid was transferred to a separating funnel and was extracted with 600 mL sat NaHCO\(_3\) solution. The basic extracts were then filtered, and the filtrate was acidified by the addition of concentrated aq HCl (pH~2) to yield a milky white solid which was extracted with EtOAc (3 × 100 ml) and dried over Na\(_2\)SO\(_4\). The solvents were removed under reduced pressure and the solid was dried under high vacuum after which 400-500 mL CH\(_2\)Cl\(_2\) was added to the solid and the mixture was stirred for 1 h followed by filtration. The solid material on the filter paper was discarded while the filtrate was collected and dried over Na\(_2\)SO\(_4\). The solvents were then removed under reduced pressure to afford compound 2 as a white solid. (4.46 g, 18.7 mmol, 52%).

![Structure of compound 2](image_url)
\(^1\)H NMR (400 MHz, DMSO) \(\delta\) 13.71 (b, 1H, -COOH), 8.63 (d, \(J = 1.6\) Hz, 2H, Ar-H), 8.60 (t, \(J = 1.6\) Hz, 1H, Ar-H), 3.92 (s, 6H, -COOCH\(_3\) \(\times 2\)).

\(^{13}\)C\(^{1}\)H NMR (101 MHz, DMSO) \(\delta\) 165.07 (1C, C1), 164.20 (2C, C6), 133.16 (2C, C3), 132.61 (1C, C5), 131.73 (1C, C2), 130.34 (2C, C4), 52.26 (2C, C7).

**Dimethyl-5-(chlorocarbonyl) isophthalate (3).** Thionyl chloride procedure: Compound 2 (5.0 g, 20.9 mmol) was charged into a 250 mL flask and 84 mL SOCl\(_2\) was added followed by 5 drops anhydrous DMF (cat) and the reaction mixture was refluxed for 16 h under nitrogen atmosphere after which SOCl\(_2\) was removed by distillation and the residue was dried under high vacuum to obtain compound 3 as an off white solid that was used without further purification (4.91 g, 19.1 mmol, 91%).

Oxalyl chloride procedure: Compound 2 (5.0 g, 20.9 mmol) was charged into a 250 mL flask and 50 mL anhydrous CH\(_2\)Cl\(_2\) was added. The reaction mixture was cooled to 0 °C and 3.6 ml (41.9 mmol) oxalyl chloride was added followed by 5 drops anhydrous DMF (cat) and left to stir at 0 °C for 20 min. The reaction mixture was then warmed to rt and left to stir under nitrogen atmosphere overnight for 16h. The solvents were then removed under reduced pressure and the residue was co-evaporated with anhydrous CH\(_2\)Cl\(_2\) (20 mL \(\times 3\)) under reduced pressure after which the residue was dried under high vacuum to afford compound 3 as an off white solid that was used without further purification (5.07 g, 19.8 mmol, 95 %).
5-(2-((Tert-butoxycarbonyl)amino)-acetamido)-isophthalic acid dimethyl ester (6). Boc-Gly-OH (Compound 5) (4.78 g, 27.3 mmol, 1.1 eq) was charged into an oven dried 100 mL flask and 20 mL anhydrous DMF was added followed by 9.5 mL DIEA (54.5 mmol, 2.2 eq). To this stirred reaction mixture was added HBTU (2-(1H-Benzotriazlo-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) (12.41 g, 32.7 mmol, 1.3 eq) and was left to stir for 15 min after which a suspension of dimethyl-5-aminoisophthalate (compound 4) (5.19 g, 24.8 mmol, 1eq) in 16 mL anhydrous DMF was added. The reaction mixture was left to stir under nitrogen atmosphere for 20 h at rt. On completion, the solvents were concentrated under reduced pressure to about 10 mL and the residue was diluted with 400 mL EtOAc and the organic layer was washed with DI H2O (150 mL × 4), sat NaHCO3 (100 mL × 3), 10 % citric acid (100 mL × 2), DI H2O (150 mL × 1) and brine (150 mL × 1). The organic layer was dried over anhydrous Na2SO4. The solvents were then removed under reduced pressure and the residue was triturated with EtOAc followed by filtration. The collected solid was then recrystallized from hot EtOAc to afford compound 6 as a white solid (6.29 g, 17.2 mmol, 69 %).

1H NMR (400 MHz, DMSO) δ 10.40 (s, 1H, Ar-NH), 8.47 (s, 2H, Ar-H), 8.14 (s, 1H, Ar-H), 7.13 (t, J = 6.0 Hz, 1H, Gly-NH), 3.88 (s, 6H, -COOCH3 × 2), 3.74 (d, J = 6.0 Hz, 2H, Gly-α), 1.39 (s, 9H, Boc).
13C$^1$H NMR (101 MHz, DMSO) δ 168.99 (1C, C7), 165.27 (2C, C5), 155.94 (1C, C9), 139.89 (1C, C4), 130.69 (2C, C2), 123.96 (1C, C1), 123.49 (2C, C3), 78.15 (1C, C10), 52.54 (2C, C6), 43.86 (1C, C8), 28.20 (3C, C11).

HRMS (ESI) (m/z): Exact mass calculated for C$_{17}$H$_{22}$N$_2$NaO$_7$ [M+Na]$^+$: 389.1325. Found: 389.1325.

5-(2-Aminoacetamido)-isophthalic acid dimethyl ester trifluoroacetate (7).$^{38}$ Compound 6 (6.26 g, 17.1 mmol) was charged into an oven dried 250 mL flask and 40 mL anhydrous CH$_2$Cl$_2$ was added. Anhydrous MeOH (8.9 mL) was then added to improve the solubility of compound 6 (DCM:MeOH 9:1). The reaction mixture was cooled to 0 °C and a solution of TFA (79 mL) in 40 mL anhydrous CH$_2$Cl$_2$ was added dropwise via an addition funnel and the reaction mixture was left to stir at 0 °C for 30 min then warmed to rt and stirred for 4 h under nitrogen atmosphere. Upon completion of the reaction, as monitored by TLC, solvents were then removed under reduced pressure and the residue was co-evaporated with Et$_2$O (20 mL × 10) in order to remove residual TFA. The resulting white solid was washed (stirred) with 400 mL Et$_2$O followed by filtration. The collected solid was dried under high vacuum to afford compound 7 as a white solid (6.39 g, 16.8 mmol, 98%).
\[ \text{N-Dimethyl-5-carbonylisophthalate-Gly-O(dimethyl-5-aminoisophthalate)} \ (8) \ (\text{new compound}) \]

Compound 7 (1.72 g, 4.5 mmol, 1 eq) was charged into a two neck 250 mL flask and 60 mL anhydrous CH\(_2\)Cl\(_2\) was added followed by 1.4 mL TEA (9.9 mmol, 2.2 eq). The reaction mixture was cooled to 0 °C and compound 3 (1.28 g, 5.0 mmol, 1.1 eq) in 15 mL anhydrous CH\(_2\)Cl\(_2\) was added dropwise and left to stir at 0 °C for 20 min (the reaction mixture is a clear solution) then warmed to room temperature and stirred for 16 h under nitrogen atmosphere. On completion, the reaction mixture showed a gel like precipitate which was filtered. The collected solid after filtration was washed with a few mL of CH\(_2\)Cl\(_2\), washed with DI H\(_2\)O, sat NaHCO\(_3\) and H\(_2\)O and dried under high vacuum. The solid was then triturated with hot EtOAc, left to cool then filtered and the
collected solid was dried under high vacuum to afford compound 8 as a white solid (1.2 g, 2.5 mmol, 54%).

![Chemical structure of compound 8]

$^1$H NMR (400 MHz, DMSO) $\delta$ 10.54 (s, 1H, Ar-NH), 9.41 (t, $J = 5.6$ Hz, 1H, Gly-NH), 8.73 (d, $J = 1.1$ Hz, 2H, Ar-H), 8.60 (s, 1H, Ar-H), 8.50 (d, $J = 1.0$ Hz, 2H, Ar-H), 8.15 (s, 1H, Ar-H), 4.12 (d, $J = 5.6$ Hz, 2H, Gly-$\alpha$), 3.93 (s, 6H, -COOCH$_3$ $\times$ 2), 3.88 (s, 6H, -COOCH$_3$ $\times$ 2).

$^{13}$C ($^1$H) NMR (101 MHz, DMSO) $\delta$ 168.27 (1C, C9), 165.25 (2C, C5), 164.98 (2C, C14), 164.87 (1C, C7), 139.81 (1C, C10), 135.07 (1C, C4), 132.26 (2C, C3), 132.04 (1C, C1), 130.71 (2C, C2), 130.60 (2C, C12), 124.13 (1C, C13), 123.68 (2C, C11), 52.74 (2C, C6), 52.57 (2C, C15), 43.58 (1C, C8).

HRMS (ESI) (m/z): Exact mass calculated for C$_{23}$H$_{22}$N$_2$NaO$_{10}$ [M+Na]$^+$: 509.1172. Found: 509.1172.

**N-5-Carboxylisophthalate-Gly-O(5-aminoisophthalate) (L1, compound 9)(new compound).**

Compound 8 (0.96 gm, 2.0 mmol, 1eq) was charged into a 100 mL flask and 40 mL MeOH/THF (1:1) was added. The resulting suspension was cooled to 0 °C followed by addition of 19.8 mL 1N LiOH (19.8 mmol, 10 eq) dropwise. The reaction was stirred at 0 °C for 3-4h then warmed to rt
and left to stir for 24 h. Upon completion of the reaction (TLC monitoring), the reaction mixture was transferred to a separating funnel and EtOAc was added till two layers appeared, the aqueous layer was separated and cooled to 0 °C followed by acidification to pH~2 with 1 M HCl to obtain a white solid which was filtered through a Buchner funnel with a sealed-in glass fritted disc of fine frit, and washed with DI H₂O (100 mL × 3). The solid was collected and lyophilized to obtain L1 (compound 9) as a white solid (0.57 g, 1.3 mmol, 67%).

![L1](image.png)

**1H NMR (400 MHz, DMSO) δ**: 13.38 (b, 4H, COOH), 10.45 (s, 1H, Ar-NH), 9.35 (t, J = 5.7 Hz, 1H, Gly-NH), 8.70 (d, J = 1.2 Hz, 2H, Ar-H), 8.60 (s, 1H, Ar-H), 8.46 (d, J = 1.0 Hz, 2H, Ar-H), 8.16 (s, 1H, Ar-H), 4.11 (d, J = 5.6 Hz, 2H, Gly-α).

![L1](image.png)

**13C{1H} NMR (101 MHz, DMSO) δ**: 168.23 (1C, C8), 166.48 (2C, C5), 166.19 (2C, C12), 165.21 (1C, C6), 139.53 (1C, C9), 134.86 (1C, C4), 132.45 (1C, C1), 132.17 (2C, C3), 131.78 (2C, C2), 131.68 (2C, C11), 124.69 (1C, C13), 123.73 (2C, C10), 43.58 (1C, C7).

Boc-Gly-Gly-O(Dimethyl-5-aminoisophthalate) (10) (new compound). Boc-Gly-OH (compound 5) (1.48 g, 8.5 mmol, 1.2 eq) was charged into a 100 mL oven dried round bottomed flask and 20 mL anhydrous DMF was added followed by 1.6 mL DIEA (8.9 mmol, 1.25 eq). HBTU (3.22 g, 8.5 mmol, 1.2 eq) was then added and the reaction mixture was left to stir at rt for 20 min. A solution of compound 7 (2.69 g, 7.1 mmol, 1 eq) and DIEA (1.6 mL, 8.9 mmol, 1.25 eq) in 20 mL anhydrous DMF was then added to the reaction mixture and was left to stir at rt under nitrogen atmosphere for 18-20 h. On completion, the reaction mixture was concentrated under reduced pressure (to a volume of about 5-10 mL) and the residue was diluted with 400-450 mL EtOAc, washed with DI H2O (200 mL \( \times \) 3), sat NaHCO3 (100 mL \( \times \) 3), 10 % citric acid (100 mL \( \times \) 2), DI water (100 mL \( \times \) 1) and brine (150 mL\( \times \) 1) followed by drying the organic layers under Na2SO4. The organic solvents were then removed under reduced pressure and the solid was triturated with EtOAc and filtered. The collected solid was recrystallized from hot EtOAc to afford compound 10 as a white solid (2.24 g, 5.3 mmol, 75 %).

\[
\begin{align*}
\text{NH} & \quad \text{COOCH}_3 \\
\text{C}_6\text{H}_5 & \quad \text{COOCH}_3
\end{align*}
\]

\( ^1H \) NMR (400 MHz, DMSO) \( \delta \) 10.26 (s, 1H, Ar-NH (d)), 8.50 (d, \( J = 0.9 \) Hz, 2H, Ar-H (c)), 8.25 (t, \( J = 5.6 \) Hz, 1H, Gly-NH (f)), 8.16 (s, 1H, Ar-H (b)), 7.13 (t, \( J = 5.9 \) Hz, 1H, Gly-NH (h)), 3.91 (d, \( J = 5.8 \) Hz, 2H, Gly-\( \alpha \) (e)), 3.88 (s, 6H, -COOCH\( _3 \) \( \times \) 2 (a)), 3.61 (d, \( J = 5.9 \) Hz, 2H, Gly-\( \alpha \) (g)), 1.38 (s, 9H, Boc (i)).
$^{13}$C\text{\text{\text{}^{1}H}}$ NMR (101 MHz, DMSO) δ 169.96 (1C, C9), 168.36 (1C, C7), 165.19 (2C, C5), 155.99 (1C, C11), 139.62 (1C, C4), 130.68 (2C, C2), 124.12 (1C, C1), 123.60 (2C, C3), 78.22 (1C, C12), 52.47 (2C, C6), 43.37 (1C, C10), 42.78 (1C, C8), 28.13 (3C, C13).

HRMS (ESI) (m/z): Exact mass calculated for C$_{19}$H$_{25}$N$_{3}$NaO$_{8}$ [M+Na]$^+$: 446.1539. Found: 446.1536.

H-Gly-Gly-O(Dimethyl-5-aminoisophthalate)-TFA (11) (new compound) Compound 10 (2.17 g, 5.1 mmol) was charged into an oven dried 250 mL flask followed by addition of 40 mL anhydrous CH$_2$Cl$_2$. MeOH (8 mL) was then added to improve the solubility of compound 10 (CH$_2$Cl$_2$ / MeOH 9:1). The reaction mixture was cooled to 0 °C followed by addition of a solution of 25 mL TFA in 40 mL anhydrous CH$_2$Cl$_2$ dropwise via an addition funnel. The reaction was stirred at 0 °C for 30 min then warmed to rt and left to stir for 4 h. Upon completion of the reaction (TLC monitoring), the solvents were then removed under reduced pressure and the residue was co-evaporated with Et$_2$O (20 mL × 10) till a white solid appeared (could also be co-evaporated with EtOAc instead of Et$_2$O). The solid was washed (stirred) with 300 mL Et$_2$O followed by filtration. The collected solid was dried under high vacuum to afford compound 11 as a white solid (2.20 g, 5.0 mmol, 98%).
H NMR (400 MHz, DMSO) δ 10.57 (s, 1H, Ar-NH (d)), 8.83 (t, J = 5.7 Hz, 1H, Gly-NH (f)), 8.49 (d, J = 1.4 Hz, 2H, Ar-H (c)), 8.17 (s, 1H, Ar-H (b)), 8.12 (s, 3H, Gly-NH$_3^+$ (h)), 4.03 (d, J = 5.7 Hz, 2H, Gly-α (e)), 3.90 (s, 6H, -COOCH$_3$ × 2), 3.68 (s, 2H, Gly-α (g)).

$^{13}$C($^1$H) NMR (101 MHz, MeOD) δ 169.69 (1C, C9), 168.11 (1C, C7), 167.24 (2C, C5), 163.25-162.91 (TFA), 140.44 (1C, C4), 132.60 (2C, C2), 126.69 (1C, C1), 125.78 (2C, C3), 53.00 (2C, C6), 43.90 (1C, C10), 41.54 (1C, C8).

N-Dimethyl-5-carbonylisophthalate-Gly-Gly-O(dimethyl-5-aminoisophthalate) (12) (new compound). Compound 12 was synthesized according to procedure 1 or procedure 2.

Procedure 1: Compound 11 (2.02 g, 4.6 mmol, 1 eq) was charged into a two neck 250 mL oven dried flask and 90 mL anhydrous CH$_2$Cl$_2$ was added followed by 1.8 mL DIEA (10.1 mmol, 2.2 eq). The reaction mixture was a white suspension and was cooled to 0 °C. Dimethyl 5-(chlorocarbonyl) isophthalate (Compound 3) (1.30 g, 5.1 mmol, 1.1 eq) was dissolved in 15 mL anhydrous CH$_2$Cl$_2$ and was added to the reaction mixture dropwise via a syringe, the reaction was
left to stir at 0 °C for 30 min then warmed to room temperature and stirred for 18 h under nitrogen atmosphere. On completion, the reaction mixture had a gel like form and was filtered, the solid was washed with DCM (80-100 mL), EtOAc (80-100 mL) and dried under vacuum. The collected solid was then washed with DI H₂O (80-100mL), sat NaHCO₃ (80-100 mL) and DI H₂O (80-100 mL) after which it was dried under high vacuum. The solid was then washed with EtOAc (80-100 mL) and dried under high vacuum to afford compound 12 as a white solid (1.45 g, 2.7 mmol, 58%).

Procedure 2: 1,3,5-benzenetricarboxylic acid dimethyl ester (Compound 2) (1.31 g, 5.5 mmol, 1.1 eq) was charged into a 250 mL oven dried round bottomed flask followed by addition of 30 mL anhydrous DMF. Triethylamine (TEA) (0.92 mL, 6.6 mmol, 1.3 eq) was then added and the reaction mixture was left to stir for 5-10 min after which was added HBTU (2.50 g, 6.6 mmol, 1.3 eq) and the reaction mixture was left to stir for an additional 20-30 min at rt under nitrogen atmosphere. A solution mixture compound 11 (2.18 g, 5.0 mmol, 1 eq) and TEA (0.84 mL, 6.0 mmol, 1.2 eq) in 95 mL anhydrous DMF was then added to the reaction mixture and left to stir for 20 h at rt under nitrogen atmosphere. On completion, the reaction mixture was concentrated under reduced pressure (to an approximate volume of 20-30 mL) and EtOAc (150-200 ml) was added to the residue and stirred for 1 h followed by filtration and the collected solid was dried under vacuum. The solid was then washed with DI H₂O (100 mL), sat NaHCO₃ (100-150 mL) and DI H₂O (100 mL) after which it was dried under high vacuum. The solid was then washed with 80 mL MeOH (stirred) and dried, the collected solid was triturated with hot EtOAc and left to cool to rt after which it was filtered and dried under high vacuum to afford compound 12 as a white solid (1.63 g, 3.0 mmol, 60%).
$^1\text{H NMR}$ (400 MHz, DMSO) $\delta$ 10.31 (s, 1H, Ar-NH), 9.42 (t, $J = 5.7$ Hz, 1H, Gly-NH (d)), 8.74 (d, $J = 1.5$ Hz, 2H, Ar-H (c)), 8.60 (t, $J = 1.5$ Hz, 1H, Ar-H (b)), 8.54 (d, $J = 1.4$ Hz, 2H, Ar-H (i)), 8.49 (t, $J = 5.8$ Hz, 1H, Gly-NH (f)), 8.17 (t, $J = 1.4$ Hz, 1H, Ar-H (j)), 3.99 (d, $J = 5.6$ Hz, 2H, Gly-\(\alpha\), (e)), 3.95-3.93 (m, 8H, Gly-\(\alpha\), (g, 2H), and –COOCH$_3$ $\times$ 2 (a, 6H), overlapped), 3.88 (s, 6H, COOCH$_3$ $\times$ 2 (k)).

$^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, DMSO) $\delta$ 169.28 (1C, C9), 168.34 (1C, C11), 165.20 (2C, C2), 164.96 (1C, C7), 164.95 (2C, C16), 139.67 (1C, C12), 135.15 (1C, C6), 132.30 (2C, C5), 131.93 (1C, C3), 130.72 (2C, C4), 130.51 (2C, C14), 124.12 (1C, C15), 123.61 (2C, C13), 52.64 (2C, C1), 52.48 (2C, C17), 42.96 (1C, C8), 42.83 (1C, C10).

**HRMS (ESI) (m/z):** Exact mass calculated for C$_{25}$H$_{25}$N$_{3}$NaO$_{11}$ [M+Na]$^+$: 566.1387. Found: 566.1387.

**N-5-Carbonylisophthalate-Gly-Gly-O(5-aminoisophthalate) (L2, compound 13) (new compound).** Compound 12 (1.31 g, 2.4 mmol) was charged into a 250 mL round bottomed flask and 50 mL of a MeOH/THF (1:1) solvent mixture was added. The reaction mixture was cooled to 0 °C followed by addition of 24.2 mL 1 N LiOH (16.0 mmol, 10 eq) dropwise. The reaction
mixture was stirred for 3-4h at 0 °C then warmed to r.t and left to stir for 24 h. Upon completion of the reaction (TLC monitoring), the reaction mixture was then transferred into a separating funnel and EtOAc was added till two layers appeared, the aqueous layer was separated and cooled to 0 °C followed by acidification to pH 2 using 1 N HCl to obtain a white solid which was filtered and washed with DI H₂O (100 mL × 3). The solid was collected and lyophilized to obtain L₂ (compound 13) as a white solid (0.68 g, 1.4 mmol, 58%).

\[ \text{L}_2 \]

\[^1\text{H} \text{NMR} (400 \text{ MHz, DMSO}) \delta 13.43 (\text{b, 4H, -COOH}), 9.32 (\text{t, } J = 5.8 \text{ Hz, 1H, Gly-NH (d)}), 8.69 (\text{d, } J = 1.6 \text{ Hz, 2H, Ar-H (b)}), 8.59 (\text{t, } J = 1.6 \text{ Hz, 1H, Ar-H (a)}), 8.47 (\text{d, } J = 1.5 \text{ Hz, 2H, Ar-H (i)}), 8.44 (\text{t, } J = 5.8 \text{ Hz, 1H, Gly-NH (f)}), 8.16 (\text{t, } J = 1.5 \text{ Hz, 1H, Ar-H (j)}), 3.97 (\text{d, } J = 5.7 \text{ Hz, 2H, Gly-\(\alpha\) (e)}), 3.93 (\text{d, } J = 5.7 \text{ Hz, 2H, Gly-\(\alpha\) (g)}).

\[ \text{L}_2 \]

\[^{13}\text{C} \{^1\text{H} \text{NMR} (101 \text{ MHz, DMSO}) \delta 169.42 (1\text{C, C10}), 168.29 (1\text{C, C8}), 166.47 (2\text{C, C5}), 166.22 (2\text{C, C15}), 165.27 (1\text{C, C6}), 139.44 (1\text{C, C11}), 134.92 (1\text{C, C4}), 132.40 (1\text{C, C1}), 132.23 (2\text{C, C3}), 131.84 (2\text{C, C2}), 131.66 (2\text{C, C13}), 124.71 (1\text{C, C14}), 123.63 (2\text{C, C12}), 42.91 (1\text{C, C7}), 42.87 (1\text{C, C9}).\]
**HRMS (ESI) (m/z):** Exact mass calculated for C$_{21}$H$_{17}$N$_3$NaO$_{11}$ [M+Na]$^+$: 510.0761. Found: 510.0727.

**5-(Tert-butoxycarbonylmethyl-carbamoyl)-isophthalic acid dimethyl ester (17) (new compound).** To an oven dried two neck flask with a stir bar was charged glycine tert-butyl ester hydrochloride (compound 16) (1.0 g, 6.0 mmol, 1eq) and 30 mL anhydrous CH$_2$Cl$_2$ was added followed by 1.8 mL TEA (13.1 mmol, 2.2 eq), and the reaction mixture was stirred for 5-10 min then cooled to 0 °C. A solution of dimethyl 5-(chlorocarbonyl) isophthalate (compound 3) (1.68 g, 6.6 mmol, 1.1 eq) in 7 mL anhydrous CH$_2$Cl$_2$ was then added dropwise via a syringe and the reaction mixture was stirred at 0 °C for 30 min then warmed to r.t and stirred for 16 h under nitrogen atmosphere. On completion, the solvents were removed under reduced pressure and the crude residue was purified via column chromatography (30:70 EtOAc/hexanes then 50:50 EtOAc/hexanes) to afford compound 17 as a white solid (1.81 g, 5.2 mmol, 87%).

![Chemical Structure of 17](image)

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.76 (s, Ar-H, 1H), 8.62 (s, 2H, Ar-H), 6.98 (t, $J = 4.1$ Hz, 1H, Gly-NH), 4.15 (d, $J = 5.0$ Hz, 2H, Gly-α), 3.94 (s, 6H, -COOCH$_3$ × 2), 1.49 (s, 9H, Boc).
$^{13}$C($^1$H) NMR (101 MHz, CDCl$_3$) $\delta$ 168.96 (1C, C9), 165.44 (2C, C2), 165.32 (1C, C7), 134.73 (1C, C6), 133.48 (1C, C3), 132.16 (2C, C5), 131.25 (2C, C4), 82.81 (1C, C10), 52.63 (2C, C1), 42.64 (1C, C8), 28.07 (3C, C11).

HRMS (ESI) (m/z): Exact mass calculated for C$_{17}$H$_{21}$NaO$_7$ [M+Na]$^+$: 374.1216. Found: 374.1216.

5-(Carboxymethyl-carbamoyl)-isophthalic acid dimethyl ester (15) (new compound).

Synthesis of compound 15 was accomplished via Scheme 2.4 or Scheme 2.5.

Synthesis via Scheme 2.4: To a stirred mixture of Glycine (compound 14) (0.59 g, 8.0 mmol) and NaHCO$_3$ (2.0 g, 24.1 mmol) in 60 mL water was added dimethyl 5-(chlorocarbonyl) isophthalate (compound 3) (2.0 g, 8.4 mmol) in several portions at r.t. The reaction mixture was left to stir for 16 h. The reaction mixture was then filtered, and the filtrate was cooled to 0 °C followed by acidification to pH~2 using 1N HCl. The acidified filtrate was left overnight in the fridge for 16 h after which a white solid appeared which was filtered and dried under high vacuum. The solid crude was then recrystallized from a hot EtOAc/hexane solution to afford compound 15 as a white solid (0.4 g, 1.4 mmol, 17%).

Synthesis via Scheme 2.5 from synthesized compound 17: A solution of compound 17 (1.77 g, 5.0 mmol) in 20 mL anhydrous CH$_2$Cl$_2$ was cooled to 0 °C followed by addition of 20 mL of TFA dropwise via an addition funnel. The reaction mixture was left to stir for 30 min at 0 °C, warmed to r.t and stirred for 16 h. Upon completion of the reaction, as monitored by TLC, the solvents
were removed under reduced pressure and the residue was co-evaporated with Et₂O (10 mL × 8) till a white solid appeared. The solid was then stirred (washed) with 200 mL Et₂O for 4 h followed by filtration. The collected solid was dried under high vacuum to afford compound 15 as a white solid (1.12 g, 3.8 mmol, 76%).

![Structure of compound 15]

**¹H NMR (400 MHz, DMSO)** δ 12.70 (s, -COOH), 9.35 (t, J = 5.6 Hz, 1H, Gly-NH), 8.70 (s, 2H, Ar-H), 8.61 (s, 1H, Ar-H), 3.96 (d, J = 5.7 Hz, 2H, Gly-α), 3.93 (s, 6H, -COOCH₃ × 2).

![Structure of compound 15]

**¹³C {¹H} NMR (101 MHz, DMSO)** δ 170.87 (1C, C9), 164.88 (2C, C2), 164.55 (1C, C7), 134.98 (1C, C6), 132.04 (2C, C5), 131.94 (1C, C3), 130.57 (2C, C4), 52.61 (2C, C1), 41.29 (1C, C8).

**HRMS (ESI) (m/z):** Exact mass calculated for C₁₃H₁₃NNaO₇ [M+Na]⁺: 318.0590. Found: 318.0577.

**N-Dimethyl-5-carbonylisophthalate-Gly-Gly-Gly-O(dimethyl-5-aminoisophthalate) (18) (new compound).** Compound 15 (0.68 g, 2.3 mmol, 1eq) was charged into a 50 mL oven dried round bottomed flask followed by addition of 10 mL anhydrous DMF and 0.4 mL TEA (2.9 mmol, 1.25 eq), and the reaction mixture was cooled to 0 °C and HOBt (0.42 g, 2.7 mmol, 1.2 eq),
EDC·HCl (0.53 g, 2.7 mmol, 1.2 eq) were added. The reaction mixture was left to stir for 15 min after which a solution of compound 11 (1.0 g, 2.3 mmol, 1 eq) and 0.4 ml TEA (2.9 mmol, 1.25 eq) in 15 mL anhydrous DMF were added, and the reaction mixture was stirred at 0 °C for 30 min then warmed to r.t and stirred for 16 h. On completion, the reaction mixture was concentrated under reduced pressure (to about 5 mL) and 200 mL Et₂O was added to induce precipitation which afforded a sticky residue which was left suspended in the Et₂O layer for 6 h. After filtration, the collected solid was washed with DI H₂O, sat NaHCO₃, 10 % citric acid and DI H₂O. The solid was dried under high vacuum after which it was washed with EtOAc (60-80 mL) then washed (stirred) with 80 mL MeOH. The washing of the solid with MeOH was repeated several times then was left suspended in 80 mL MeOH for 16 h (purity was monitored through TLC (EtOAc/hexanes 9:1)). The solid was filtered, collected and dried under high vacuum to afford compound 18 as a white solid (0.98 g, 1.6 mmol, 71%).

\[ \text{H NMR (400 MHz, DMSO)} \delta 10.10 (s, 1H, Ar-NH (j)), 9.32 (t, J = 5.7 Hz, 1H, Gly-NH (d)), 8.65 (d, J = 1.5 Hz, 2H, Ar-H (c)), 8.54 (t, J = 1.5 Hz, 1H, Ar-H (b)), 8.50 (t, J = 5.7 Hz, 1H, Gly-NH, (h)), 8.45 (d, J = 1.4 Hz, 2H, Ar-H (k)), 8.28 (t, J = 5.9 Hz, 1H, Gly-NH (f)), 8.10 (t, J = 1.4 Hz, 1H, Ar-H (n)), 3.98 (d, J = 5.6 Hz, 2H, Gly-\(\alpha\) (e)), 3.94 (d, J = 5.9 Hz, 2H, Gly-\(\alpha\) (i)), 3.91 (s, 6H, -COOCH₃ × 2 (a)), 3.86 (s, 6H COOCH₃ × 2 (m)), 3.81 (d, J = 5.6 Hz, 2H, Gly-\(\alpha\) (g)).

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\[ \text{**\text{\textsuperscript{13}C\text{\textsuperscript{1}H}}} \text{ NMR (101 MHz, DMSO)} \delta 169.64 (1C, C11), 169.39 (1C, C9), 168.21 (1C, C7), 165.12 (2C, C18), 164.86 (2C, C5), 164.77 (1C, C13), 139.52 (1C, C4), 134.98 (1C, C14), 132.16 (2C, C15), 131.84 (1C, C17), 130.53 (2C, C16), 130.37 (2C, C2), 124.08 (1C, C1), 123.63 (2C, C3), 52.57 (2C, C19), 52.42 (2C, C6), 42.91 (1C, C10), 42.72 (1C, C8), 42.37 (1C, C12). \]

**HRMS (ESI) (m/z):** Exact mass calculated for C\textsubscript{27}H\textsubscript{28}N\textsubscript{4}NaO\textsubscript{12} [M+Na]\textsuperscript{+}: 623.1601. Found: 623.1601.

**N-5-Carboxylisophthalate-Gly-Gly-Gly-O(5-aminoisophthalate) (L3, 19) (new compound).**

Compound 18 (0.93 g, 1.6 mmol, 1eq) was charged into a 100 mL round bottomed flask and 40 mL of MeOH/THF (1:1) solvent mixture was added. The reaction mixture was cooled to 0 °C followed by addition of 16 mL 1 N LiOH (16.0 mmol, 10 eq) dropwise. The reaction mixture was stirred for 1-2 h at 0 °C then warmed to r.t and left to stir for 24 h. Upon completion of the reaction (TLC monitoring), the reaction mixture was then transferred into a separating funnel and EtOAc was added till two layers appeared, the aqueous layer was separated and cooled to 0 °C followed by acidification to pH 2 with 1 N HCl, the acidified aqueous layer was left to stand for 3-4 h till a white precipitate formed which was filtered and lyophilized to afford L3 (compound 19) as a white solid (0.36 g, 0.66 mmol, 43%).
$^1$H NMR (400 MHz, DMSO) $\delta$ 13.37 (b, 4H, COOH), 10.22 (s, 1H, Ar-H (j)), 9.24 (t, $J = 5.8$ Hz, 1H, Gly-NH (d)), 8.68 (d, $J = 1.6$ Hz, 2H, Ar-H (c)), 8.59 (t, $J = 1.5$ Hz, 1H, Ar-H (b)), 8.44 (d, $J = 1.5$ Hz, 2H, Ar-H (k)), 8.38 (t, $J = 5.7$ Hz, 1H, Gly-NH (h)), 8.30 (t, $J = 5.8$ Hz, 1H, Gly-NH (f)), 8.16 (t, $J = 1.5$ Hz, 1H, Ar-H (n)), 3.98 (d, $J = 5.7$ Hz, 2H, Gly-α (e)), 3.94 (d, $J = 5.8$ Hz, 2H, Gly-α (i)), 3.82 (d, $J = 5.7$ Hz, 2H, Gly-α (g)).

$^{13}$C ($^1$H) NMR (101 MHz, DMSO) $\delta$ 169.39 (1C, C8 or C10), 169.38 (1C, C8 or C10), 168.10 (1C, 12), 166.38 (1C, C1), 166.11 (1C, C17), 165.07 (1C, C6), 139.31 (1C, C13), 134.88 (1C, 5), 132.29 (1C, C3), 132.10 (2C, C4), 131.72 (2C, C2), 131.55 (2C, C15), 124.66 (1C, C16), 123.64 (2C, C14), 42.86 (1C, C9), 42.77 1C, C11), 42.15 (1C, C7).

2.6. NMR Characterization data of synthesized Organic Compounds

Figure 2.24. $^1$H-NMR spectrum of compound 6 in DMSO-d$_6$.

Figure 2.25. $^{13}$C-NMR spectrum of compound 6 in DMSO-d$_6$.
Figure 2.26. $^1$H-NMR spectrum of compound 8 in DMSO-$d_6$.

Figure 2.27. $^{13}$C-NMR spectrum of compound 8 in DMSO-$d_6$. 
Figure 2.28. $^1$H-NMR spectrum of compound 9 in DMSO-d$_6$.

Figure 2.29. $^{13}$C-NMR spectrum of compound 9 in DMSO-d$_6$. 
Figure 2.30. $^1$H-NMR spectrum of compound 10 in DMSO-d$_6$.

Figure 2.31. $^{13}$C-NMR spectrum of compound 10 in DMSO-d$_6$. 
Figure 2.32. COSY spectrum of compound 10 in DMSO-d₆.

Figure 2.33. HSQC spectrum of compound 10 in DMSO-d₆.
Figure 2.34. HMBC spectrum of compound 10 in DMSO-d$_6$. 
Figure 2.35. $^1$H-NMR spectrum of compound 11 in DMSO-d$_6$.

Figure 2.36. $^{13}$C-NMR spectrum of compound 11 in Methanol-d$_4$. 
Figure 2.37. $^1$H-NMR spectrum of compound 12 in DMSO-d$_6$.

Figure 2.38. $^{13}$C-NMR spectrum of compound 12 in DMSO-d$_6$. 
Figure 2.39. COSY spectrum of compound 12 in DMSO-d$_6$.

Figure 2.40. HSQC spectrum of compound 12 in DMSO-d$_6$. 
Figure 2.41. HMBC spectrum of compound 12 in DMSO-d$_6$. 
Figure 2.42. $^1$H-NMR spectrum of compound 13 in DMSO-$d_6$.

Figure 2.43. $^{13}$C-NMR spectrum of compound 13 in DMSO-$d_6$. 

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Figure 2.44. $^1$H-NMR spectrum of compound 17 in CDCl₃.

Figure 2.45. $^{13}$C-NMR spectrum of compound 17 in CDCl₃.
Figure 2.46. $^1$H-NMR spectrum of compound 15 in DMSO-d$_6$.

Figure 2.47. $^{13}$C-NMR spectrum of compound 15 in DMSO-d$_6$. 
Figure 2.48. $^1$H-NMR spectrum of compound 18 in DMSO-d$_6$.

Figure 2.49. $^{13}$C-NMR spectrum of compound 18 in DMSO-d$_6$. 
Figure 2.50. COSY spectrum of compound 18 in DMSO-d$_6$.

Figure 2.51. HSQC spectrum of compound 18 in DMSO-d$_6$. 
Figure 2.52. HMBC spectrum of compound 18 in DMSO-d₆.
Figure 2.53. $^1$H-NMR spectrum of compound 19 in DMSO-d$_6$.

Figure 2.54. $^{13}$C-NMR spectrum of compound 19 in DMSO-d$_6$. 
Figure 2.55. COSY spectrum of compound 19 in DMSO-d$_6$.

Figure 2.56. HSQC spectrum of compound 19 in DMSO-d$_6$. 
Figure 2.57. HMBC spectrum of compound 19 in DMSO-d₆.
2.7. HRMS characterization data for synthesized organic compounds.

**Figure 2.58.** HRMS of compound 6.

**Figure 2.59.** HRMS of compound 8.
Figure 2.60. HRMS of compound 9.

Figure 2.61. HRMS of compound 10.
Figure 2.62. HRMS of compound 12.

Figure 2.63. HRMS of compound 13.
Figure 2.64. HRMS of compound 17.

Figure 2.65. HRMS of compound 15.
Figure 2.66. HRMS of compound 18.

Figure 2.67. HRMS of compound 19.
2.8. References


3.1. Introduction

The development of chiral crystalline porous materials has gained substantial interest in chemistry and materials science over the past decade. These homochiral metal-organic frameworks are constructed from a combination of metal ions or clusters with organic linkers forming porous materials providing nanocavities or pore structures with a chiral environment which has made them useful in various stereoselective applications such as chiral recognition and separation, asymmetric catalysis, and chemical sensing.

Most ligands used in the construction of metal-organic frameworks (MOFs) as well as those used in the constructions of homochiral MOFs are composed of rigid aromatic components, resulting in the generation of fixed rigid internal cavities. The rigid character of current homochiral MOFs, however, is not desirable; as adjustable pore shape with adaptability are critical factors that govern the regulation of the guest-to-frameworks interactions. Among the challenges of current chiral porous materials, is the lack of remarkable enantioselectivity and specific tight-affinity binding toward important biomolecules such as carbohydrates, nucleotides, amino acids, peptides, etc. The current chiral porous materials are usually considered highly hydrophobic, resulting in a poor solution processability as well as poor compatibility, thus severely restricting practical applications of these materials in chemical engineering and biological technology.
The design and development of chiral porous materials possessing excellent enantioselectivity, adaptability, flexible host-framework interactions, and biocompatibility, has become an emerging challenge that has attracted the interest of the supramolecular chemistry community. In order to address key issues with current chiral porous materials, several criteria should be considered when designing a perspective novel chiral ligand: (i) the chiral ligand should have chiral sites as part of its structure and be synthetically feasible; (ii) The chiral ligand should be flexible, thus imposing flexibility to the resulting framework with capability of adaptive guest-framework interactions; (iii) the ability to tune the ligand’s structure hydrophobic-hydrophilic properties. In this perspective, the use of peptide-derived ligands to construct chiral artificial metal-peptide assemblies through the assembly of peptides with metals affording porous chiral materials is a new approach that could overcome the drawbacks and challenges of current chiral porous materials.

Natural peptide assemblies, such as proteins and enzymes, are central to the functions of living systems and are capable of performing adaptive functions with a high degree selectivity and efficiency. With the goal of mimicking biological systems and functions of natural peptide assemblies, artificial metal-peptide assemblies with tailored structural, chemical and physical properties have gained substantial interest over the past decade. The internal pores or cavities of chiral porous artificial metal-peptide assemblies constructed using flexible peptide-derived ligands would possess conformational adaptability due to the inherent flexibility imposed in their structure, thus having the potential to undergo a myriad of conformational variations that are reminiscent to natural biological systems such as enzymes. Furthermore, these cavities or pores contain chiral binding sites stemming from the side-chain groups of the amino acid residues of the peptide-derived ligands used in the construction of these artificial-metal peptide assemblies, thus offering wide structural variability and functionality as well as introducing highly specific
biomolecular chiral recognition sites with exceptional enantioselectivities in the cavities of these materials which are difficult to accomplish in current chiral MOFs.\textsuperscript{25} The tuning of the hydrophobic-hydrophilic property of these chiral porous materials could be achieved through varying the side-chain groups of the peptide-derived linkers, thus improving the materials’ solution processability and compatibility for practical applications.\textsuperscript{25}

Highly specific and stereoselective recognition of important biomolecules such as carbohydrates and amino acids is an emerging challenge in the biological and medical fields which has proved difficult to accomplish in current chiral and synthetic systems; as the chiral binding sites and the cavity adaptability are important factors that significantly influence targeted bimolecular recognition.\textsuperscript{25} Artificial metal-peptide assemblies with biomimetic cavities, structural flexibility and adaptability could offer promising materials that would facilitate specific bimolecular recognition with high selectivity and affinity for biological and medical applications.\textsuperscript{34,38}

Artificial metal-peptide assemblies have great potential to act as materials for enantiomer separation which remains a great challenge in the pharmaceutical industry.\textsuperscript{39-40} The chiral side-chain functional groups of the amino acids used in the construction of the peptide-derived ligands can offer advantage in terms of enantiospecific guest-to-host interactions, thus homochiral peptide assemblies can be excellent candidates to act as solid support materials capable of highly specific enantioselectivities.\textsuperscript{9,25,41}

3.1.1. Enzyme-Mimicking Catalysis.

Enzymes are considered the fastest and most selective catalysts from nature and can catalyze reactions with rate enhancements of $10^{17}$-fold and with selectivities of $>99\%$ enantiomeric
Enzymes fold into three-dimensional structures, which is fundamental for their exceptional catalytic properties. In biological systems, enzymes are constructed by the 20 genetically encoded amino acids with unique specific sequences which underlies their ability to fold into specific structures with defined conformations allowing for tuning the dynamics and positions of the functional groups required for binding and catalysis. Designing and developing more efficient and selective synthetic catalysts with the ability to mimic the mode of action of natural enzymes has gained enormous attention of in supramolecular chemistry community due to its practical applications.

In order to explain the remarkable reactivity and selectivity of enzymes, it is important to mention the unique structural features involved in natural enzymatic systems: (i) the confinement of the active site of the enzyme containing the key catalytic groups; (ii) the presence of different functional groups within the active site of the enzyme, and (iii) flexibility of the active site.

The catalytic sites of enzymes are confined within cavities or pockets that have specific shape and size which ensures that a substrate with specific shape and size fits nicely, and confinement increases the concentration of the substrate around its reactive center leading to both selectivity and reaction rate enhancement. The multiple functional groups within the catalytic site permit the binding, activation and stabilization of the substrates and intermediates through non-covalent interactions. Flexibility of the catalytic cavity or pocket allows it to undergo induced-fit changes during catalytic reactions to ensure favorable substrate interactions, thus providing extra stabilization for the substrate interactions and the transition states. In much the same way that a unique and specific enzyme-substrate interaction require that the enzyme undergoes a conformational change in order to accommodate and conform to the structure of its substrate and transitions states, so too should synthetic supramolecular systems have the ability to
conform to accommodate their guest molecules for optimal binding. The remarkable catalytic properties and selectivities of natural enzymes are due to contribution of all these three structural key properties.

Inspired by natural enzymes, supramolecular chemists have spent decades attempting to synthesize supramolecular catalyst systems that copy different functions and properties of natural enzyme with the goal of developing catalysts that mimic natural enzymes, thus developing better innovative approaches toward the discovery of catalyst systems of unprecedented reactivity.

In order to achieve confinement, supramolecular chemists have designed and assembled rigid molecular capsules or cages with definite shapes and sizes. The work of Raymond, Bergman and Toste demonstrate the importance of localization or encapsulation of active catalytic sites into confined spaces. Tefler and co-workers have designed and synthesized unique four-component MOFs with confined-spaces and multiple functionality decorating these spaces. A notable multicomponent MOF synthesized by Tefler and co-workers with the goal of targeting enzyme-like complexity is MUF-77 which was constructed using an organocatalyst linker with a prolinyl group and two different modulating linkers that tuned the spatial environment around the catalytic group in the cavities of MUF-77 which showed an increase in reaction rate and the enantiomeric excess of a series of aldol reactions. These models where designed following the idea that increasing the concentration of a substrate and its binding in close proximity to a catalytic or reactive site within a confined space shall lead to reaction rate enhancement.

To demonstrate the importance of flexibility of the confined catalytic cavity or pocket, Sanders and co-workers developed and studied a series of cyclic metalloporphyrin oligomers as host systems for acyl-transfer reactions. Pyridyl and imidazolyl derivatives were used as substrates which both bind simultaneously to the zinc-porphyrin within the cavity of the host system adopting
a specific orientation resembling the transition state of the reaction. The host system presented some flexibility and significant distortion in order to accommodate the product.\textsuperscript{55}

While natural enzymatic properties have had some success when applied separately in different supramolecular enzyme mimic systems, combining all three properties of natural enzymes in one supramolecular structure has been difficult.\textsuperscript{46,47} Molecular capsules or cages for example, are mostly rigid structures and this rigidity can lead to weak guest or substrate binding and poor stabilization of the guest as well as the transition state interactions thus hindering the catalytic activity.\textsuperscript{56} It is crucial that an adequate enzyme mimic system should have all three features of natural enzymes. Indeed, it is a highly challenging process to design and synthesize an artificial enzyme-mimic with an active site that combines all three properties of natural enzymes: i) that possess a specific shape and size, ii) surrounded by reactive chemical functionalities that allow for substrate binding, activation and stabilization, iii) that are flexible with ability to adapt to substrate as well as transition state interactions.

From a structural and chemical perspective, the self-assembling of 3D metal-peptide frameworks as scaffolds to generate adequate enzyme-mimics offers great opportunities to mimic the exceptional catalytic properties of natural enzymes,\textsuperscript{25} and offers the possibility to affect reactions only known for natural enzymes.\textsuperscript{57} The tailored and targeted assembly of flexible peptide-derived ligands and metals could generate conformationally flexible peptide-metal organic frameworks able to adapt to substrate and transition state interactions.\textsuperscript{25} The chemical environment of the pores or cavities of the these 3D porous metal peptide frameworks would be surrounded by natural chiral binding functionalities with controlled size and possessing substrate-adaptive features, thus bearing similar chemical environments with the chiral pockets of natural enzymes, allowing for substrate binding, activation and stabilization of both substrate and transition states.
Indeed, 3D metal-peptide frameworks could be excellent candidates as artificial enzyme-mimicking systems due to their ability to bear all three structural features responsible for the remarkable catalytic performance of natural enzymes (Figure 3.1).

![Figure 3.1. Schematic representation showing the natural enzymatic features and how they are introduced into 3D metal-peptide frameworks. Part (a) adapted with permission from ref 83, copyright: 2019 American Chemical Society.](image)

Artificial metal-peptide assemblies are expected to have an extraordinary impact in the field of materials science as next-generation versatile biomimetic materials with profound potential in enhancing current conventional chiral porous materials.

Metal-organic frameworks can be considered more than just robust extended framework structures with high porosity, as their pores can be further decorated with unique chemical functionalities thus tailoring MOFs for targeted applications. One of the ways of introducing functionality into MOFs is through the initial solvothermal synthesis where the linking ligand is “tagged” with an additional desired functional group offering the opportunity to form MOF structures where this group projects into the pores of the MOF network. Another way is through post-synthetic modifications (PSM) where preformed MOF precursors with “tagged” functional groups can be transformed into other functional groups via a variety of organic transformations using simple reagents. Cohen and co-workers have applied the post-synthetic modification approach to introduce a wide variety of functional groups into the lattice structure of IRMOF-3 using anhydrides and isocyanates as chemical reagents. Yaghi and co-workers employed the post-synthetic method to introduce an enzyme-like structure into the pores of a multivariate MOF (MTV-IRMOF-74-III) through seven post-synthetic covalent functionalization reactions. Introduction of such myriad of functionality into MOF pores through post assembly modifications further increases the structural and chemical complexity of MOF pores.

A multi-functionalized type of MOF which involves employing linkers with similar parent structure but with different functionalities is known as Multivariate Metal-Organic Framework (MTV-MOF). Yaghi and co-workers reported mixed-dicarboxylate MOFs based on the IRMOF platform system where they synthesized a series of MTV-MOFs combining the BDC (1,4-benzenedicarboxylate) linker and one or more of the functionalized bdc linkers, and different functionalities were successfully incorporated in a single framework (Figure 3.2).
Figure 3.2. Multivariate metal-organic frameworks (MTV-MOFs) dicarboxylate linkers with different functionalities. Adapted from Ref 65 with permission from American Association for the Advancement of Science.

Cohen and co-workers synthesized a multivariate mixed-linker bifunctional MOF (UiO-66-(Br)(NH₂) using 1,4-dicarboxylate linkers (BDC) with –Br and –NH₂ functionalities (BDC-NH₂ and BDC-Br), the resultant multifunctional MOF had the two functional groups (–Br and –NH₂) distributed through the MOF. Yaghi and co-workers prepared a series of mixed linker multivariate MOFs (MTV-IRMOF-74-III) using two types of dicarboxylate organic linkers, H₄L-CH₃ (functionalized with methyl groups) and H₄L-CH₂NHBoc (functionalized with Boc-protected primary amines) (Figure 3.3). The MTV-IRMOF-74-III series reported by Yaghi and co-workers were synthesized using various ratios of the H₄L-CH₃ and H₄L-CH₂NHBoc linkers, hereafter, MTV-IRMOF-74-III-(CH₃)ₓ(CH₂NHBoc)₁₋ₓ, x = 0.2, 0.4, 0.6 and 0.8.
Figure 3.3. Multivariate metal organic framework IRMOF-74-III series (MTV-IRMOF-74-III-(CH$_3$)$_{1-x}$(CH$_2$NHBoc)$_x$) and the organic linkers employed in the synthesis of MTV-IRMOF-74-III-(CH$_3$)$_{1-x}$(CH$_2$NHBoc)$_x$. Adapted from Ref 57 with permission, copyright: 2016, American Chemical Society.

Using more than one type of functionalized linker with the same parent structure in the synthesis of multivariate-MOFs could lead to generation of MOF products where the linkers are incorporated into the MOF structure in random positions, meaning that the linkers could be non-uniformly distributed throughout the MOF and as a result, the pores of the multivariate MOF could be non-uniformly functionalized. Despite the non-uniform distribution of functionality in mixed linker multivariate-MOFs, an advantage of multivariate mixed linker multivariate-MOFs is the more accessible pore volume compared to fully functionalized MOFs constructed using a single functionalized linker. This is an important factor when the MOF is used for further applications or reactions (such as post-synthetic reactions) where multiple post-synthetic reactions can be carried out in tandem on the MOF without the challenge of low yields due to diminished porosity and accessible pore volume.
3.2. **Scope of research work and strategy.**

In this chapter we present the design and organic synthesis of a series of novel flexible chiral peptide-derived linkers as displayed in Figure 3.4.

![Chemical structures of novel tetracarboxylate peptide-derived chiral linkers with various functional groups.](image)

**Figure 3.4.** Chemical structures of novel tetracarboxylate peptide-derived chiral linkers with various functional groups. The Phe-Gly tetracarboxylate linker has a hydrophobic phenyl side chain group, the Ser-Gly tetracarboxylate linker has a polar hydroxyl side chain group, the Tryp-Gly tetracarboxylate linker has a hydrophobic aromatic indole side chain group, the Ala-Gly tetracarboxylate linker has a hydrophobic methyl side chain group.
The N-terminus and the C-terminus of the peptides displayed in Figure 3.4 have been chemically modified to bear two ditopic 1,3-benzene dicarboxylate groups (isophthalate units), thus converting the peptides into tetratopic carboxylate linkers with chiral side chain groups stemming from the amino acid residues used in construction of the peptide linkers. The dipeptide-based tetracarboxylate linkers have a Phe-Gly, Ser-Gly, Tryp-Gly and Ala-Gly dipeptide cores between the isophthalate moieties (Figure 3.4). The ditopic 1,3-benzene carboxylate groups (isophthalate groups) of these linkers would serve as coordination sites with metals such as Cu (II) forming the dicopper paddlewheel metal cluster, which is considered a rigid well-defined SBU with unique intrinsic geometric properties affording stable, porous, and robust metal-organic carboxylate framework structures. The assembly of our tetratopic carboxylate peptide-derived chiral linkers with dicopper paddlewheel would lead to construction of novel homochiral 3D peptide metal-organic frameworks. The design strategy of our homochiral peptide metal-organic frameworks is based on the Ligand-to-Ligand (L-L) pillaring of supramolecular building layers strategy for construction of stable and robust 3D periodic porous metal organic frameworks. Ligand-to-Ligand pillaring of sql 2D supramolecular building layers based on the 4-c dicopper paddlewheel molecular building blocks would lead to 3D MOFs with the ssb/stx or lvt/lil topological platforms, while Ligand-to-Ligand pillaring of kgm 2D supramolecular building layers based on the dicopper paddlewheel molecular building blocks would lead to 3D MOFs with the nbo/fof or ssa/sty platforms. Details of the Ligand-to-Ligand pillaring strategy is discussed in the introduction section of chapter 2. It is worth mentioning that due to the different conformations that could be adopted by flexible diisophthalate linkers during the assembly process of the MOFs, new topologies associated with resulting MOFs with unprecedented architectures could be obtained which could open the door to access new framework topologies beyond those
associated with rigid linkers. Our design strategy for construction of robust 3D metal-peptide frameworks has great potential to overcome the current challenges in construction of metal-peptide frameworks that rely on the peptide C-terminus, N-terminus and side chains of amino acid residues as coordination sites with single metal ions leading to low dimensional and compact frameworks.

Using our tetracarboxylate chiral peptide-derived linkers for construction of 3D porous peptide-metal organic framework is expected to yield biomimetic 3D porous framework structures where the backbone is a flexible peptide core moiety and the corners are dicopper paddlewheel secondary building units (metal clusters) associated with the isophthalate units of the linker (Figure 3.5). From a chemical and structural perspective, our metal-peptide frameworks have the potential to overcome the significant key challenges facing the development of chiral biomimetic materials. The backbone of our peptide-organic frameworks would be of a flexible peptide structure, thus imparting flexibility and adaptability within the cavities or pores of the framework and allowing the ability to undergo structural conformational variations to accommodate guest interactions. The cavities would contain numerous natural chiral side-chain binding units that can be used to tune the chiral pores for specific functions, and enabling enantioselective recognition as well as secondary interactions with guest molecules. The tuning of the hydrophobic-hydrophilic properties of the cavities of the framework structure could be achieved through varying the side-chain groups of the peptide linkers with a myriad of choice among polar and apolar natural side chain-groups. From a design perspective, our chiral peptide-metal organic frameworks are expected to be 3D porous frameworks that combine all features of natural protein systems such as enzymes; flexibility, adaptability, confinement, and pores decorated with multiple chiral functionalities, thus having the potential to serve as platforms for developing more efficient bioinspired enzyme-
mimicking catalysts. Another potential application is to act as more efficient and stable 3D chiral porous structures for biomolecular recognition and chiral separations due to the presence of natural chiral side-chain functional groups within the pores or cavities capable of specific enantioselective binding with guest molecules.

**Figure 3.5.** Schematic displaying the construction of novel bioinspired 3D homochiral metal-peptide frameworks mimicking natural protein systems such as enzymes.

In chapter 2, we reported the synthesis of a novel tetracarboxylate Gly-Gly dipeptide-derived linker and optimization of the solvothermal conditions for the synthesis of our Cu-based peptide MOF (MH-2-Gly-Gly) using this linker (Scheme 3.1). The peptide based MOF, MH-2-Gly-Gly, was obtained by the solvothermal reaction between the Gly-Gly tetratopic carboxylate linker and Cu(NO₃)₂ · 2.5H₂O (linker : metal molar ratio = 1 : 2) in a mixture solvent of DMF/DMA/EtOH/H₂O (v/v/v/v = 5 : 5 : 1 : 1) in the presence of acetic acid as a modulator and pyridine. These conditions yielded single crystals of the peptide MOF MH-2-Gly-Gly for which single crystal X-ray analysis experiments performed at the University of Oklahoma revealed that
this peptide MOF was sustained by square dicopper paddlewheel \([\text{Cu}_2(\text{COO})_4]\) secondary building units, the vertices of which are linked into squares of squares rendering square grid layers (sq1-1) that are in turn pillared via ligand-to-ligand cross linking into a 3D MOF that exhibited \text{ssb/stx}\ topology. Details of the synthesis and analysis of the structure of the MH-2-Gly-Gly peptide MOF are discussed in chapter 2.

**Scheme 3.1. Solvothermal synthesis of MH-2-Gly-Gly**

In the work presented in this chapter, we investigate the synthesis of novel peptide-based metal organic frameworks using our chiral peptide-derived flexible linkers. We discuss our attempts for the solvothermal synthesis of our homochiral peptide-metal organic frameworks through the single-linker approach where only one type of functionalized tetracarboxylate chiral peptide-derived linker is used to obtain a homochiral single-linker metal organic framework (Figure 3.6 (a)). We also discuss our attempts for the synthesis of Multivariance metal-organic frameworks (MTV-MOFs) via the mixed-linker approach where we used a mixture of the un-functionalized tetracarboxylate Gly-Gly dipeptide linker and one of the functionalized tetracarboxylate dipeptide linkers to obtain a series of functionalized multivariate peptide-metal organic frameworks, hereafter, MH-MTV-(Gly-Gly)$_n$-(AA-Gly)$_k$; AA stands for the amino acid in the peptide sequence of the linker (Figure 3.6 (b)).
**Figure 3.6.** (a) Schematic representing the single-linker approach for the synthesis of single-linker fully functionalized homochiral metal-peptide framework. (b) Schematic representing the mixed-linker approach for the synthesis functionalized multivariate metal-peptide frameworks.
3.3. Results and Discussion

3.3.1. Design and synthesis of linkers.

The synthesis of the Phe-Gly tetracarboxylate linker (compound 10) was accomplished according to Scheme 3.2. Coupling of the commercially available aromatic amine (compound 1) with Boc-Gly-OH (compound 2) using the peptide coupling reagent HBTU (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) in the presence of DIEA (N,N-diisopropylethyl amine) afforded the amide compound 3. Deprotection of the Boc group of compound 3 under acidolytic conditions using trifluoroacetic acid (TFA) afforded the corresponding trifluoroacetate salt compound 4. Coupling of trifluoroacetate salt (compound 4) with Boc-Phe-OH (compounds 5) using the peptide coupling reagent HBTU in the presence of 1-hydroxybenzotriazole hydrate (HOBt) and DIEA in DMF afforded the dipeptide compound 6. The N-Boc protecting group of compound 6 was removed under acidolytic conditions using trifluoroacetic acid (TFA) to afford the corresponding trifluoroacetate salt compound 7. The acylation reaction of compound 7 with dimethyl 5-(chlorocarbonyl)isophthalate (compound 8) in dry CH₂Cl₂ and in the presence of Et₃N afforded the tetraester compound 9. Basic hydrolysis of the methylester groups of compound 9 1N LiOH afforded the Phe-Gly dipeptide tetracarboxylate linker (compounds 10). The overall yield is 19%.

The synthesis of key intermediate compound 8 that was employed in Scheme 3.2 was discussed in Chapter 2 (Scheme 2.1 for synthesis of compound 3 in Chapter 2)
Scheme 3.2. The design and synthesis of the Phe-Gly dipeptide tetracarboxylate linker (compound 10).

Reagents and conditions: (a) HBTU, DIEA, DMF, rt, 20 h, 69%; (b) TFA, CH₂Cl₂, or CH₂Cl₂/MeOH 9:1 (v:v), 0 °C to rt, 4 h, 98%; (c) HBTU, DIEA, DMF, rt, 18-20 h, 62%; (d) TFA, CH₂Cl₂/MeOH 9:1 (v:v), 0 °C to rt, 2-3 h, 95%; (e) CH₂Cl₂, Et₃N, 0 °C to rt, 18 h, 68%; (f) LiOH·H₂O, MeOH/THF/H₂O 1:1:1 (v:v:v), 0 °C to rt, 24 h, 70%.
The synthesis of the Ala-Gly tetracarboxylate linker (compound 13) was accomplished according to Scheme 3.3. Coupling of the commercially available aromatic amine (compound 1) with Boc-Gly-OH (compound 2) using the peptide coupling reagent HBTU (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) in the presence of DIEA (N,N-diisopropylethyl amine) afforded the amide compound 3. Deprotection of the Boc group of compound 3 under acidolytic conditions using trifluoroacetic acid (TFA) afforded the corresponding trifluoroacetate salt compound 4. The coupling of compound 4 with the synthesized key intermediate compound 11 using the water soluble peptide coupling reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC-HCl) in the presence of 1-hydroxybenzotriazole (HOBt) afforded the tripeptide tetramethyl ester compound 12. Basic hydrolysis of the methylester groups of compound 12 1N LiOH afforded the Ala-Gly dipeptide tetracarboxylate linker (compounds 13). The overall yield is 17%. 

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Scheme 3.3. The design and synthesis of the Ala-Gly dipeptide tetracarboxylate linker (compound 13).

Reagents and conditions: (a) HBTU, DIEA, DMF, rt, 20 h, 69%; (b) TFA, CH₂Cl₂, or CH₂Cl₂/MeOH 9:1 (v:v), 0 °C to rt, 4 h, 98%; (c) EDC·HCl, HOBt, Et₃N, DMF, 0 °C to rt, 16 h, 40%; (d) LiOH·H₂O, MeOH/THF/H₂O 1:1:1 (v:v:v), 0 °C to rt, 24 h, 62%.
The synthesis of key intermediate compound 11 that was employed in Scheme 3.3 for the synthesis of the Ala-Gly tetracarboxylate linker was accomplished according Scheme 3.4 below:

**Scheme 3.4. The design and synthesis of intermediate compound 11.**

Reagents and conditions: (a) NaOH$_{aq}$ 1M, MeOH, rt, 18-20 h, 52%; (b) SOCl$_2$, DMF (cat), reflux, 16 h, 91% or (COCl)$_2$, CH$_2$Cl$_2$, DMF (cat), 0 °C to rt, 16 h, 95%; (c) Et$_3$N, CH$_2$Cl$_2$, 0 °C to rt, 16 h, 84%; (d) TFA, CH$_2$Cl$_2$, 0 °C to rt, 16 h, 81%.

Basic hydrolysis of compound 14 using 1 eq aq NaOH afforded compound 15 which was converted to the acyl chloride (compound 8) using thionyl chloride (SOCl$_2$) or oxalyl chloride ((COCl)$_2$) with a few drops of DMF as a catalyst. The acylation reaction of dimethyl 5-(chlorocarbonyl)isophthalate (compound 8) with the commercially available alanine tert-butyl ester hydrochloride (compound 16) in anhydrous CH$_2$Cl$_2$ and in the presence of triethylamine affording the Boc protected compound 17 was performed according to reported procedures for the synthesis of similar compounds.$^{79}$ Deprotection of the tert-butyl group of compound 17 under acidolytic conditions using trifluoroacetic acid (TFA) afforded target compound 11 with an 81 % yield.
The synthesis of the Ser-Gly and Tryp-Gly tetracarboxylate linkers (compounds 26 and 27 respectively) were accomplished according to the general Scheme 3.5. Coupling of the commercially available aromatic amine (compound 1) with Boc-Gly-OH (compound 2) using the peptide coupling reagent HBTU (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) in the presence of DIEA (N,N-diisopropylethyl amine) afforded the amide compound 3. Deprotection of the Boc group of compound 3 under acidolytic conditions using trifluoroacetic acid (TFA) afforded the corresponding trifluoroacetate salt compound 4. Coupling of trifluoroacetate salt (compound 4) with different N-Boc protected amino acids (compounds 18, and 19) using the water soluble carbodiimide coupling reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) in the presence of 1-hydroxybenzotriazole hydrate (HOBt) and Et₃N in DMF afforded the respective dipeptides 20 and 21. The N-Boc protecting group of compound 20 and 21 was removed under acidolytic conditions using trifluoroacetic acid (TFA) in the presence of anisole to afford a series of the corresponding trifluoroacetate salts compounds 22 and 23. Coupling of compound 22 and 23 with 1,3,5-benzenetricarboxylic acid dimethyl ester (compound 15) using the water soluble carbodiimide coupling reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) in the presence of 1-hydroxybenzotriazole hydrate (HOBt) and Et₃N in DMF afforded the respective tetramethylester compounds 24 and 25. Basic hydrolysis of the methylester groups of compounds 24 and 25 using 1N LiOH afforded the Ser-Gly and Tryp-Gly dipeptide tetracarboxylate linkers (compounds 26 and 27 respectively).
Scheme 3.5. The design and synthesis of the Ser-Gly and Tryp-Gly dipeptide tetracarboxylate linkers (Compounds 26 and 27 respectively).

Reagents and conditions: (a) HBTU, DIEA, DMF, rt, 20 h, 69%; (b) TFA, CH₂Cl₂, or CH₂Cl₂/MeOH 9:1 (v:v), 0 °C to rt, 4 h, 98%; (c) EDC·HCl, HOBt, Et₃N, DMF, 0 °C to rt, 18-20 h, 68-87%; (d) TFA, CH₂Cl₂/MeOH 9:1 (v:v), anisole, 0 °C to rt, 2-3 h, 91-93%; (e) EDC·HCl, HOBt, Et₃N, DMF, rt, 18-20 h, 41-38%; (f) LiOH·H₂O, MeOH/THF/H₂O 1:1:1 (v:v:v), 0 °C to rt, 24 h, 46-75%.

The use of functional linkers for the synthesis of metal organic frameworks for novel applications is highly sought after. However, synthesis of metal organic frameworks using functional linkers is challenging; the incorporation of functional groups on the linking ligands can introduce steric and solubility characteristics that could interfere with MOF formation through conventional solvothermal synthesis.\textsuperscript{58,80,81} Using our novel chiral functionalized tetracarboxylate dipeptide linkers for the synthesis of metal-peptide frameworks through conventional solvothermal synthesis can be challenging due to the high flexibility of these peptide derived linkers as well as due to the steric and structural effects imparted by the chiral functional groups of the linkers. In this chapter we report the best solvothermal conditions for the synthesis of the metal-peptide frameworks from a long series of solvothermal experiments that were performed under different conditions that included different temperature, solvent systems, modulators and molar ratios of linker and metal source.


Synthesis of Metal complex using the Ala-Gly dipeptide tetracarboxylate linker (compound 13) and Cu(NO\textsubscript{3})\textsubscript{2} \cdot 2.5H\textsubscript{2}O. In order to synthesize the metal-peptide organic framework MH-4-Ala-Gly, the Ala-Gly dipeptide tetracarboxylate linker and Cu(NO\textsubscript{3})\textsubscript{2} \cdot 2.5H\textsubscript{2}O were mixed combinatorially under a variety of solvothermal conditions (Scheme 3.6).

![Scheme 3.6. Synthesis of MH-4-Ala-Gly.](image)

Solvothermal reaction conditions: To a solution of the linker (compound 13) (10.8 mg, 0.022 mmol) in 0.85 mL solvent mixture of DMF/DMSO/EtOH/Acetonitrile (2:2:1:1 v/v/v) in a 5 mL scintillation vial was added 11.7 mg Cu(NO$_3$)$_2$ · 2.5H$_2$O (0.05 mmol). Acetic acid (0.1 mL) was then added followed by 0.05 mL pyridine. The vial was capped, and the clear solution was heated in an oven for 48 h at 80 ºC to afford green square-like crystals (Figure 3.7).

![Microscopic image of the square-like crystal structures of MH-4-Ala-Gly.](image)

Figure 3.7. Microscopic image of the square-like crystal structures of MH-4-Ala-Gly.

$^1$H-NMR experiments on digested samples of these square-like crystals of MH-4-Ala-Gly in d$_6$-DMSO / DCl (20 wt% in D$_2$O) mixture allowed re-isolation of the Ala-Gly tetracarboxylate linker, and $^1$H-NMR analysis confirmed the inclusion of the linker in MH-4-Ala-Gly crystals (Figure 3.8).
Figure 3.8. $^1$H-NMR spectrum of digested MH-4-Ala-Gly in $d_6$-DMSO / DCl (20 wt% in $D_2O$) mixture.

In chapter 2, we reported the synthesis of the Cu-based 3D peptide-metal organic framework MH-2-Gly-Gly using our Gly-Gly dipeptide tetracarboxylate linker (Figure 3.9). Our analysis of the structure of MH-2-Gly-Gly revealed that the framework displayed the ssb/stx topology (details of the synthesis, characterization and analysis of the framework structure of MH-2-Gly-Gly is discussed in chapter 2).
Figure 3.9. Solvothermal synthesis of MH-2-Gly-Gly.

The powder X-ray diffraction patterns of the as-synthesized crystal sample of MH-4-Ala-Gly are similar to those of MH-2-Gly-Gly (Figure 3.10). This indicates that MH-4-Ala-Gly is isostructural with MH-2-Gly-Gly which displayed an ssb/stx type network.

Figure 3.10. Experimental PXRD patterns of MH-2-Gly-Gly and MH-4-Ala-Gly. Patterns provided here are for as-synthesized samples, thus the hump over a wide range of two-theta is due to the inclusion of large amount of disordered solvent molecules.
Synthesis of Metal complex using the Ser-Gly dipeptide tetracarboxylate linker (compound 26) and Cu(NO₃)₂ · 2.5H₂O. In order to synthesize the metal-peptide organic framework MH-5-Ser-Gly, the Ser-Gly dipeptide tetracarboxylate linker and Cu(NO₃)₂ · 2.5H₂O were mixed combinatorially under a variety of solvothermal conditions (Scheme 3.7).

**Scheme 3.7. Synthesis of MH-5-Ser-Gly.**

Solvothermal reaction conditions: To a suspension of the linker (compound 26) (13.0 mg, 0.025 mmol) in 0.85 mL solvent mixture of DMF/DMSO/EtOH/Acetonitrile/H₂O (2:2:1:1 v/v/v/v) in a 5 mL scintillation vial was added 11.7 mg Cu(NO₃)₂ · 2.5H₂O (0.05 mmol). Acetic acid (0.1 mL) was then added followed by 0.05 mL pyridine, and 400 mL solvent mixture of DMF/DMSO/EtOH/Acetonitrile (2:2:1:1 v/v/v) was added and the reaction mixture became a clear solution. The vial was capped, and the clear solution was heated in an oven for 48 h at 80 °C to afford green square-like crystals (Figure 3.11).

**Figure 3.11.** Microscopic image of the square-like crystal structures of MH-5-Ser-Gly.
$^1$H-NMR experiments of digested samples of these square-like crystals of MH-5-Ser-Gly in d$_6$-DMSO / DCI (20 wt% in D$_2$O) mixture allowed re-isolation of the Ser-Gly tetracarboxylate linker, and $^1$H-NMR analysis confirmed the inclusion of the linker in MH-5-Ser-Gly crystals (Figure 3.12).

![Diagram of chemical structure]

**Figure 3.12.** $^1$H-NMR spectrum of digested MH-5-Ser-Gly in d$_6$-DMSO / DCI (20 wt% in D$_2$O) mixture.

The powder X-ray diffraction patterns of the as-synthesized crystal sample of MH-5-Ser-Gly are similar to those of MH-2-Gly-Gly (Figure 3.13). This indicates that MH-5-Ser-Gly is isostructural with MH-2-Gly-Gly which displayed an ssb/stx type network.
Figure 3.13. Experimental PXRD patterns of MH-2-Gly-Gly and MH-5-Ser-Gly. Patterns provided here are for as-synthesized samples, thus the hump over a wide range of two-theta is due to the inclusion of large amount of disordered solvent molecules.

Synthesis of Metal complex using the Tryp-Gly dipeptide tetracarboxylate linker (compound 27) and Cu(NO$_3$)$_2$ 2.5H$_2$O. In order to synthesize the metal-peptide organic framework MH-6-Tryp-Gly, the Tryp-Gly dipeptide tetracarboxylate linker and Cu(NO$_3$)$_2$ 2.5H$_2$O were mixed combinatorially under a variety of solvothermal conditions (Scheme 3.8).

Solvothermal reaction conditions: To a solution of the linker (compound 27) (15.5 mg, 0.025 mmol) in 0.85 mL solvent mixture of DMF/DMSO/EtOH/Acetonitrile (2:2:1:1 v/v/v) in a 5 mL scintillation vial was added 0.4 mL solvent mixture of DMF/DMSO/EtOH/Acetonitrile/H₂O (2:2:1:1:1 v/v/v/v) followed by addition of 11.7 mg Cu(NO₃)₂·2.5H₂O (0.05 mmol). Acetic acid (0.1 mL) was then added followed by 0.05 mL pyridine. The vial was capped, and the clear solution was heated in an oven for 72 h at 80 °C to afford green hexagonal-like crystals (Figure 3.14).

![Microscopic image of the hexagonal-like crystal structures of MH-6-Trypt-Gly.](image)

**Figure 3.14.** Microscopic image of the hexagonal-like crystal structures of MH-6-Trypt-Gly.

¹H-NMR experiments of digested samples of these square-like crystals of MH-6-Trypt-Gly in d₆-DMSO / DCl (20 wt% in D₂O) mixture allowed re-isolation of the Trypt-Gly tetracarboxylate linker, and ¹H-NMR analysis confirmed the inclusion of the linker in MH-6-Trypt-Gly crystals (Figure 3.15), and PXRD confirmed that the material was crystalline (Figure 3.16).
Figure 3.15. $^1$H-NMR spectrum of digested MH-6-Trypt-Gly in $d_6$-DMSO / DCl (20 wt% in D$_2$O) mixture.

Figure 3.16. Experimental PXRD patterns of MH-6-Trypt-Gly. Patterns provided here are for as-synthesized samples, thus the hump over a wide range of two-theta is due to the inclusion of large amount of disordered solvent molecules.
Synthesis of Metal-Peptide Frameworks using the Mixed-Ligand approach.

In order to synthesize the multivariate mixed-linker MOF MH-MTV-(Gly-Gly)(Tryp-Gly) through the solvothermal reaction of Cu(NO$_3$)$_2$ · 2.5H$_2$O with a mixture of the Gly-Gly tetracarboxylate linker and the Tryp-Gly tetracarboxylate linker (Scheme 3.9) we employed the same conditions that we previously used for the synthesis the single linker peptide MOF MH-2-Gly-Gly using the Gly-Gly tetracarboxylate linker (discussed in chapter 2).


Solvothermal reaction conditions: Gly-Gly tetracarboxylate linker (0.0195 g, 0.04 mmol, 80 % of total linker mixture), Tryp-Gly tetracarboxylate linker (0.0062 g, 0.01 mmol, 20 % of total linker mixture).
mixture) and Cu(NO$_3$) ∙ 2.5H$_2$O (0.0233 gm, 0.1 mmol) were dissolved in 1.7 mL of a solvent mixture of (DMF:DMA:EtOH:H$_2$O 5:5:1:1 v/v/v/v) in a 5 mL scintillation vial and 0.2 mL acetic acid (as a modulator) was added followed by addition of 0.1 mL pyridine. The vial was capped, and the clear solution was heated at 80 °C for 48 h in an oven to yield green square block crystals. Figure 3.17 shows the powder X-ray diffraction (PXRD) patterns of the as-synthesized crystal sample of the multivariate MOF MH-MTV-(Gly-Gly)$_{0.8}$(Tryp-Gly)$_{0.2}$ closely matched the patterns of the as-synthesized peptide MOF MH-2-Gly-Gly which was constructed using the Gly-Gly tetracarboxylate single linker and displayed an ssb/stx type network (Chapter 2). This indicates that MH-MTV-(Gly-Gly)$_{0.8}$(Asp-Gly)$_{0.2}$ is isostructural with MH-2-Gly-Gly.

Figure 3.17. Experimental PXRD patterns of MH-2-Gly-Gly and MH-MTV-(Gly-Gly)$_{0.8}$(Tryp-Gly)$_{0.2}$. Patterns provided here are for as-synthesized samples, thus the hump over a wide range of two-theta is due to the inclusion of large amount of disordered solvent molecules.
To determine the inclusion of the Gly-Gly tetracarboxylate linker and the Tryp-Gly tetracarboxylate linker in MH-MTV-(Gly-Gly)_{0.8}(Tryp-Gly)_{0.2} we have performed $^1$H-NMR of digested sample crystals of MH-MTV-(Gly-Gly)_{0.8}(Tryp-Gly)_{0.2} in d$_6$-DMSO / DCl (20 wt% in D$_2$O) mixture (Figure 3.18). The resonance peaks at 3.19-3.23 ppm (m) and at 3.31-3.28 ppm (m) correspond to the Tryp-β hydrogens of the Tryp-Gly tetracarboxylate linker. The resonance peak at 3.92 ppm and at 3.95 ppm correspond to the Gly-α protons of the two Gly residues of the Gly-Gly tetracarboxylate linker and are overlapped with the resonance peak corresponding to the Gly-α hydrogens of the Gly residue of the Tryp-Gly linker. The resonance peak at 4.79-4.75 ppm (m) corresponds to the Tryp-α hydrogen of the Tryp-Gly linker. The resonance peaks at 6.94 ppm (t), 7.01 ppm (t), 7.18 ppm (s) 7.28 ppm (d) and 7.67 ppm (d) correspond to the indole aromatic hydrogens of the Tryp residue of the Tryp-Gly linker.

Resonance peaks at 8.13 ppm, 8.46 ppm, 8.52 ppm, 8.56 ppm, 8.60 ppm and 8.66 ppm correspond to aromatic protons of the isophthalate moieties of both the Gly-Gly and the Tryp-Gly linkers as indicated in Figure 3.18.
**Figure 3.18.** $^1$H-NMR spectrum of digested MH-MTV-(Gly-Gly)$_{0.8}$(Tryp-Gly)$_{0.2}$ in $d_6$-DMSO / DCl (20 wt% in D$_2$O) mixture. Inset, chemical structures of the Tryp-Gly and the Gly-Gly tetracarboxylate linkers.

In order to synthesize the multivariate mixed-linker MOF MH-MTV-(Gly-Gly)(Phe-Gly) through the solvothermal reaction of Cu(NO$_3$)$_2$ · 2H$_2$O with a mixture of the Gly-Gly tetracarboxylate linker and the Phe-Gly tetracarboxylate linker (Scheme 3.10) we employed the same conditions that we previously used for the synthesis the single linker peptide MOF MH-2-Gly-Gly using the Gly-Gly tetracarboxylate linker (discussed in chapter 2).

Solvothermal reaction conditions: Gly-Gly tetracarboxylate linker (0.0195 g, 0.04 mmol, 80 % of total linker mixture), Phe-Gly tetracarboxylate linker (0.0058 g, 0.01 mmol, 20 % of total linker mixture) and Cu(NO₃)₂ · 2.5H₂O (0.0233 gm, 0.1 mmol) were dissolved in 1.7 mL of a solvent mixture of (DMF:DMA:EtOH:H₂O 5:5:1:1 v/v/v/v) in a 5 mL scintillation vial and 0.2 mL acetic acid (as a modulator) was added followed by addition of 0.1 mL pyridine. The vial was capped, and the clear solution was heated at 80 °C for 48 h in an oven to yield green square block crystals. Figure 3.19 shows the powder X-ray diffraction (PXRD) patterns of the as-synthesized crystal sample of the multivariate MOF MH-MTV-(Gly-Gly)₀.₈(Phe-Gly)₀.₂ resembled the patterns of the as-synthesized peptide MOF MH-2-Gly-Gly which was constructed using the Gly-Gly tetracarboxylate single linker and displayed an ssb/stx type network (Chapter 2). This indicates that MH-MTV-(Gly-Gly)₀.₈(Phe-Gly)₀.₂ is isostructural with MH-2-Gly-Gly.
Figure 3.19. Experimental PXRD patterns of MH-2-Gly-Gly and MH-MTV-(Gly-Gly)_{0.8}(Phe-Gly)_{0.2}. Patterns provided here are for as-synthesized samples, thus the hump over a wide range of two-theta is due to the inclusion of large amount of disordered solvent molecules.

To determine the inclusion of the Gly-Gly tetracarboxylate linker and the Phe-Gly tetracarboxylate linker in MH-MTV-(Gly-Gly)_{0.8}(Phe-Gly)_{0.2} we have performed $^1$H-NMR of digested sample crystals of MH-MTV-(Gly-Gly)_{0.8}(Phe-Gly)_{0.2} in d$_6$-DMSO / DCl (20 wt% in D$_2$O) mixture (Figure 3.20). The resonance peaks at 3.07-3.00 ppm (m) and at 3.23-3.18 ppm (m) correspond to the Phe-β hydrogens of the Phe-Gly tetracarboxylate linker. The resonance peak at 3.92 ppm and at 3.95 ppm correspond to the Gly-α protons of the two Gly residues of the Gly-Gly tetracarboxylate linker and are overlapped with the resonance peak corresponding to the Gly-α hydrogens of the Gly residue of the Phe-Gly linker. The resonance peak at 4.79-4.75 ppm (m) corresponds to the Phe-α hydrogen of the Phe-Gly linker. The resonance peaks at 7.13 ppm (t),
7.21 ppm (t) and 7.33 ppm (d) correspond to the phenyl aromatic hydrogens of the Phe residue of the Phe-Gly linker.

Resonance peaks at 8.12 ppm, 8.46 ppm, 8.51 ppm, 8.56 ppm and 8.66 ppm correspond to aromatic protons of the isophthalate moieties of both the Gly-Gly and the Phe-Gly linkers as indicated in Figure 3.20.

**Figure 3.20.** $^1$H-NMR spectrum of digested MH-MTV-(Gly-Gly)$_{0.8}$(Phe-Gly)$_{0.2}$ in d$_6$-DMSO / DCl (20 wt% in D$_2$O) mixture. Inset, chemical structures of the Phe-Gly and the Gly-Gly tetracarboxylate linkers.
3.4. Conclusion.

We have successfully accomplished the design and synthesis of novel tetracarboxylate peptide-derived chiral linkers with various functional groups based on the dipeptide Phe-Gly, the dipeptide Ser-Gly, the dipeptide Tryp-Gly and the dipeptide Ala-Gly. We have investigated the solvothermal synthesis of novel homochiral metal-peptide frameworks using our tetracarboxylate peptide-derived chiral linkers and copper (II) nitrate under various conditions. We have identified solvothermal synthesis reaction conditions for the synthesis of the homochiral metal-peptide framework MH-4-Ala-Gly using our Ala-Gly dipeptide tetracarboxylate linker and copper (II) nitrate, and PXRD experiments showed that the crystalline product was isostructural with our copper paddlewheel-based metal-peptide framework MH-2-Gly-Gly which displayed ssb/stx type network (Synthesis and characterization of MH-2-Gly-Gly is described in Chapter 2). We have also identified solvothermal conditions for the synthesis of the copper-based metal-peptide framework MH-5-Ser-Gly using our Ser-Gly dipeptide tetracarboxylate. PXRD experiments showed that MH-5-Ser-Gly was isostructural with our metal-peptide framework MH-2-Gly-Gly which displayed an ssb/stx topology. We have synthesized crystalline material of MH-6-Tryp-Gly using our Tryp-Gly tripeptide tetracarboxylate linker and copper (II) nitrate, and NMR experiments of digested samples conformed inclusion of the linker in the MH-6-Trp-Gly complex. We have synthesized and characterized the multivariate MOF MH-MTV-(Gly-Gly)_{0.8}(Tryp-Gly)_{0.2} and the multivariate MOF MH-MTV-(Gly-Gly)_{0.8}(Phe-Gly)_{0.2} were we used a mixture of the un-functionalized tetracarboxylate Gly-Gly dipeptide linker and one of the functionalized tetracarboxylate dipeptide linkers. We hope that this work will open opportunities for the use homochiral metal-peptide frameworks as, for example, artificial enzyme-mimicking catalysis, chiral separations and biomolecular recognition.
3.5. **Experimental procedures.**

3.5.1. **General procedures.** Chemicals and solvents were obtained from commercial sources and were used without further purification unless otherwise specified. All organic reactions were carried out in oven dried glassware with dry solvents under an atmosphere of dry nitrogen unless otherwise specified. Analytical TLC was performed on Merck 60 F254 silica gel plates with a fluorescent indicator with a 254 nm excitation wavelength. Compounds were visualized under UV light at 254 nm wavelength. Column chromatography was carried out on silica gel 60 (particle size 240-400 mesh). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Compound lyophilization was performed using a Labconco freezone 4.5 liter -84 °C benchtop freeze dryer apparatus. NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer (\(^1\)H 400 MHz, \(^{13}\)C 100 MHz) at 25 °C. \(^1\)H NMR chemical shift values were determined relative to residual protonated solvent signals as internal standard ((CD\(_3\))(CD\(_2\)H)SO in (CD\(_3\))\(_2\)SO, δ 2.50 ppm). The chemical shifts for \(^1\)H NMR are expressed in ppm, followed by the multiplicity (br, broad; s, singlet; d, doublet; t, triplet; q, quadruplet, qt, quintet; m, multiplet), coupling constants (J, in Hertz, Hz), and integration. \(^{13}\)C NMR spectra were referenced to the solvent signal (δ 39.52 ppm for (CD\(_3\))\(_2\)SO. All \(^{13}\)C NMR spectra were recorded with complete proton decoupling. NMR signals of spectra were assigned using gradient COSY (correlation spectroscopy), HSQC (heteronuclear single quantum coherence) and HMBC (heteronuclear multiple bond correlation). High-resolution mass spectra were acquired on an Agilent 6560 IM-QTOF mass spectrometer.

3.5.2. **Solution \(^1\)H NMR spectra of digested MOF samples.**

Typically, for the digestion of the MOFs, the as-synthesized MOF sample was solvent exchanged with DMF twice daily for 2-3 days followed by acetone exchange twice daily for 2-3 days. The
sample was then left to dry in air for 12 h. DMSO-d$_6$ (0.5 mL) and DCl (20 µL, 20 wt. % in D$_2$O), were added to a 5 mL vial containing the dried MOF (~10 mg), and the resulting suspension was left to rest at room temperature for 5-10 min until clear solution was obtained. The $^1$H NMR spectra were recorded immediately after (~10 min). $^1$H NMR spectra on digested solutions of MOFs were acquired on a Bruker Avance 400 MHz spectrometer, with chemical shifts of linkers identified by comparing with spectra of each pure linker.

3.5.3. Powder X-ray diffraction (PXRD)

Powder patterns were recorded on a Bruker AXS D8 Advance Phaser diffractometer with Cu Kα radiation ($\lambda = 1.5406$ Å, operating at 30 kV and 10 mA) over a range of $5^\circ < 2\theta < 30^\circ$, with a step size of 0.02° steps and a 1.0 s counting time per step. The supernatant of fresh as-synthesized was exchanged with DMF, and the samples were filtered through a 0.45 µm nylon membrane filter. Collected sample was spread on a Si-Einkristalle plate immediately before PXRD measurements.

3.5.4. General procedure for the deprotection of the Boc-group.

The respective Boc protected compound was dissolved in anhydrous CH$_2$Cl$_2$ or CH$_2$Cl$_2$:CH$_3$OH 9:1 (depending on solubility), and anisole was added (unless otherwise stated). The reaction mixture was cooled to 0 °C and a solution of trifluoroacetic acid (equal amount as the total solvent amount) in anhydrous CH$_2$Cl$_2$ was added dropwise via an addition funnel. The reaction was stirred at 0 ºC for 30 min then warmed to rt and left to stir for 2-3 h. Upon completion of the reaction (TLC monitoring), the solvents were removed under reduced pressure and the residue was co-evaporated with Et$_2$O (30 mL × 10) till a solid appeared (could also be co-evaporated with EtOAc instead of Et$_2$O). The solid residue was dried under high vacuum, after which it was washed (stirred) with 300 mL Et$_2$O, and left soaked in Et$_2$O for 5-6 h. The solid was then filtered and the
collected solid was dried under high vacuum to afford the respective products as trifluoroacetate salts that were used in next steps without further purification.

3.5.5. General procedure for the alkaline hydrolysis of the methyl ester.

To a mixture of the respective methyl ester compound (1 eq) in a MeOH/THF (1:1) solvent mixture was added 1 N LiOH·H₂O(aq) (10 eq) at 0 °C. The reaction mixture was stirred for 3-4 h at 0 °C then warmed to room temperature and left to stir for 24 h. Upon completion of the reaction (TLC monitoring), the reaction mixture was transferred into a separating funnel, and EtOAc was added till two layers appeared, the aqueous layer was separated and cooled to 0 °C followed by acidification to pH 2 using 1 N HCl to obtain a white solid which was filtered and washed with DI H₂O (100 mL × 3). The solid was collected and lyophilized to obtain the respective products as white solids.

3.5.6. Experimental section for synthesis of organic compounds and characterization data

5-(2-((Tert-butoxycarbonyl)amino)-acetamido)-isophthalic acid dimethyl ester (3). Synthesis of compound 3 was performed according to a procedure described in chapter 2 (chapter 2, section 2.5.6, synthesis of compound 6).

5-(2-Aminoacetamido)-isophthalic acid dimethyl ester trifluoroacetate (4). Synthesis of compound 3 was performed according to a procedure described in chapter 2 (chapter 2, section 2.5.6, synthesis of compound 7).
**Boc-Phe-Gly-O(dimethyl-5-aminoisophthalate) (6) (new compound).** The commercially available Boc-Phe-OH (compound 5) (1.94 g, 7.3 mmol, 1.0 eq) was charged into a 100 mL oven dried round bottomed flask and 20 mL anhydrous DMF was added followed by 1.6 ml DIEA (9.1 mmol, 1.25 eq), HBTU (3.33 g, 8.8 mmol, 1.2 eq) was then added followed by HOBt (1.34 g, 8.8 mmol, 1.2 eq), and the reaction mixture was left to stir at rt for 20 min. A solution of compound 4 (2.78 g, 7.3 mmol, 1 eq) and DIEA (1.6 mL, 9.1 mmol, 1.25 eq) in 20 mL anhydrous DMF was then added to the reaction mixture via a syringe, and the reaction mixture was left to stir at rt under nitrogen atmosphere for 18-20h. On completion, the reaction mixture was concentrated under reduced pressure (to a volume of about 5-10 mL) and the residue was diluted with 400-450 mL EtOAc, washed with DI H2O (200 mL × 3), sat NaHCO3 (150 mL × 3), 10 % citric acid (100 mL × 1), DI water (100 mL × 1) and brine (150 mL× 1) followed by drying the organic layers over Na2SO4. The organic solvents were then removed under reduced pressure and the solid was triturated with EtOAc and filtered. The collected solid was dissolved in a hot EtOAc/hexanes mixture and left to cool to room temperature and then overnight in the fridge. A white gel-like solid precipitated out, the solid was filtered and collected while the filtrate was concentrated under reduced pressure and left overnight in the fridge to yield a second crop of solid which was filtered and collected. The collected solids were dried under high vacuum to yield compound 6 as a white solid (2.30 g, 62%).
\(^1\)H NMR (400 MHz, DMSO) \(\delta\) 10.28 (s, 1H, Ar-NH (f)), 8.51 (s, 2H, Ar-H (g)), 8.41 (t, \(J = 5.6\) Hz, 1H, Gly-NH (d)), 8.18 (s, 1H, Ar-H (h)), 7.29 – 7.18 (m, 5H, Ar-H (Phe)), 7.07 (d, \(J = 8.1\) Hz, 1H, Phe-NH (b)), 4.25 – 4.20 (m, 1H, Phe-\(\alpha\) (c)), 3.94 (d, \(J = 5.6\) Hz, 2H, Gly-\(\alpha\) (e)), 3.90 (s, 6H, -COOCH\(_3\) (j)), 3.05 (dd, \(J = 13.7, 4.0\) Hz, 1H, Phe-\(\beta\) (l)), 2.79 (dd, \(J = 13.6, 10.6\) Hz, 1H, Phe-\(\beta\) (l)), 1.30 (s, 9H, Boc (a)).

\(^{13}\)C\({\^{1}}\)H NMR (101 MHz, DMSO) \(\delta\) 172.24 (1C, C5), 168.30 (1C, C7), 165.20 (2C, C12), 155.46 (1C, C3), 139.63 (1C, C8), 138.15 (1C, C15), 130.73 (2C, C10), 129.16 (2C, C16 or C17), 127.98 (2C, C16 or C17), 126.14 (1C, C18), 124.14 (1C, C11), 123.58 (2C, C9), 78.18 (1C, C2), 55.78 (1C, C4), 52.50 (2C, C13), 42.84 (1C, C6), 37.23 (1C, C14), 28.07 (3C, C1).

**HRMS (ESI)** Exact mass calculated for \(C_{26}H_{31}N_{3}NaO_{8}\) [M + Na]\(^+\), 536.2009. Found 536.2009.

**H-Phe-Gly-O(dimethyl-5-aminoisophthalate)-TFA (7) (new compound).** Compound 6 (2.26 g, 4.4 mmol) was reacted following the general procedure for the deprotection of the Boc-group. Anhydrous CH\(_2\)Cl\(_2\) (24 mL), MeOH (5.3 mL), TFA (a solution of 45 mL TFA in 24 mL anhydrous CH\(_2\)Cl\(_2\)). The product compound 7 was obtained as a white solid (2.22 g, 95%).

![Diagram of compound 6](image-url)
$^1$H NMR (400 MHz, DMSO) δ 10.61 (s, 1H, Ar-NH (e)), 8.99 (t, $J = 5.5$ Hz, 1H, Gly-NH (c)), 8.49 (s, 2H, Ar-NH (f)), 8.22 (s, 3H, -NH$_3^+$), 8.18 (s, 1H, Ar-H (g)), 7.37 – 7.27 (m, 5H, Phe), 4.15 (b, 1H, Phe-α (b)), 4.03 (d, $J = 5.6$ Hz, 2H, Gly-α (d)), 3.90 (s, 6H, -COOCH$_3$ (h)), 3.18 (dd, $J = 14.0, 5.2$ Hz, 1H, Phe-β (j)), 3.00 (dd, $J = 14.0, 8.1$ Hz, 1H, Phe-β (j)).

$^{13}$C NMR (101 MHz, DMSO) δ 168.62 (1C, C2), 167.64 (1C, C4), 165.19 (2C, C9), 158.25-157.95 (TFA), 139.63 (1C, C5), 134.86 (1C, C12), 130.74 (2C, C7), 129.51 (2C, 13), 128.49 (2C, C14), 127.13 (1C, C15), 124.15 (1C, C8), 123.62 (2C, C6), 53.39 (1C, C1), 52.51 (2C, C10), 42.77 (1C, C3), 37.01 (1C, C11).

**Dimethyl 5-(chlorocarbonyl) isophthalate (8).** Synthesis of compound 8 was performed according to a procedure described in chapter 2 (chapter 2, section 2.5.6, synthesis of compound 3).

**N-Dimethyl-5-carbonylisophthalate-Phe-Gly-O(dimethyl-5-aminoisophthalate) (9) (new compound).** Compound 7 (2.15 g, 4.1 mmol, 1 eq) was charged into a two neck 250 mL oven dried flask and 40 mL anhydrous CH$_2$Cl$_2$ was added followed by 1.3 mL Et$_3$N (9.0 mmol, 2.2 eq), and the reaction mixture was a clear solution and was cooled to 0 °C. Dimethyl 5-(chlorocarbonyl) isophthalate (Compound 8) (1.15 g, 4.5 mmol, 1.1 eq) was dissolved in 15 mL anhydrous CH$_2$Cl$_2$
and was added to the reaction mixture dropwise via a syringe. The reaction was left to stir at 0 °C for 30 min then warmed to room temperature and stirred for 18 h under nitrogen atmosphere. On completion, the reaction mixture was a white suspension which was filtered, and the collected solid was washed with DI H₂O followed by EtOAc, and dried under vacuum to yield a fraction of the product (compound 9). The filtrate was diluted with 60 mL CH₂Cl₂ and washed with DI H₂O (80 mL), sat NaHCO₃ (80 × 2), 10% citric acid (80 × 2), DI H₂O (80-100 mL) and dried over Na₂SO₄. The organic solvents were then removed under reduced pressure and the solid was triturated with EtOAc, and filtered. The collected solid was triturated with MeOH, filtered and dried under high vacuum to afford another fraction of the product compound 9 which was combined with the other fraction. (white solid, 1.77 g, 68%).

\[ \text{1H NMR (400 MHz, DMSO)} \delta 10.38 \text{ (s, 1H, Ar-NH(h))}, 9.28 \text{ (d, } J = 8.1 \text{ Hz, 1H, Phe-NH (d))}, 8.64 \text{ (s, 3H, Ar-H (c) and Gly-NH (f) overlapped)}, 8.57 \text{ (s, 1H, Ar-H (b))}, 8.53 \text{ (s, 2H, Ar-H (i))}, 8.18 \text{ (s, 1H, Ar-H (j))}, 7.36 \text{ (d, } J = 7.4 \text{ Hz, 2H, Phe (m))}, 7.26 \text{ (t, } J = 7.4 \text{ Hz, 2H, (Phe (n))}, 7.17 \text{ (t, } J = 7.2 \text{ Hz, 1H, (Phe (o))}, 4.84 - 4.79 \text{ (m, 1H, Phe-α (e))}, 4.98 - 3.96 \text{ (m, 2H, Gly-α (g))}, 3.92 \text{ (s, 6H, -CCOCH₃ (a))}, 3.89 \text{ (s, 6H, -COOCH₃ (k))}, 3.24 \text{ (dd, } J = 13.6, 3.7 \text{ Hz, 1H, Phe-β (l))}, 3.11 - 3.04 \text{ (m, 1H, Phe-β (l))}.\]
\(^{13}\text{C}\{^1\text{H}\} \text{ NMR} (101 \text{ MHz, DMSO}) \delta\) 171.65 (1C, C12), 168.26 (1C, C14), 165.20 (2C, C21), 164.93 (2C, C19), 164.77 (1C, C5), 139.71 (1C, C15), 138.24 (1C, C8), 135.24 (1C, C4), 132.36 (2C, C3), 131.87 (1C, C1), 130.74 (2C, C2), 130.41 (2C, C17), 129.07 (2C, C9), 128.06 (2C, C10), 126.24 (1C, C11), 124.08 (1C, C18), 123.53 (2C, C16), 55.16 (1C, C6), 52.63 (2C, C22), 52.49 (2C, C20), 42.90 (1C, C13), 36.79 (1C, C7).

**HRMS (ESI)** Exact mass calculated for C\(_{32}\)H\(_{31}\)N\(_{3}\)NaO\(_{11}\)\([\text{M} + \text{Na}]^+\), 656.18563. Found 656.1853.

**N-5-Carboxylisophthalate-Phe-Gly-O(5-aminoisophthalate) (compound 10) (new compound).** The title compound was prepared via general procedure for the alkaline hydrolysis of the methyl ester using compound 9 (1.8 g, 2.8 mmol, 1eq), MeOH/THF (1:1) 40 mL, 1 N LiOH·H\(_2\)O\(_{(aq)}\) (20.9 mL, 20.9 mmol, 10 eq). Compound 10 was obtained as a white solid after lyophilization (1.2 g, 70%).
\(^1\)H NMR (400 MHz, DMSO) \(\delta\) 13.34 (b, 4H, -COOH), 10.31 (s, 1H, Ar-NH (h)), 9.17 (d, \(J = 8.2\) Hz, 1H, Phe-NH (d)), 8.60 (d, \(J = 1.6\) Hz, 2H, Ar-H (c)), 8.58 (t, \(J = 3.8\) Hz, 1H, Gly-NH (f)), 8.54 (t, \(J = 1.6\) Hz, 1H, Ar-H (b)), 8.45 (d, \(J = 1.5\) Hz, 2H, Ar-H (i)), 8.15 (t, \(J = 1.5\) Hz, 1H, Ar-H (j)), 7.33 (d, \(J = 7.2\) Hz, 2H, Ar-H, (Phe (m))), 7.23 (t, \(J = 7.5\) Hz, 2H, Ar-H, (Phe (n))), 7.14 (t, \(J = 7.3\) Hz, 1H, Ar-H, (Phe (o))), 4.83 – 4.77 (m, 1H, Phe-\(\alpha\) (e)), 3.95 (d, \(J = 5.7\) Hz, 2H, Gly-\(\alpha\) (g)), 3.21 (dd, \(J = 13.8, 3.8\) Hz, 1H, Phe-\(\beta\) (l)), 3.06 (dd, \(J = 13.6, 11.1\) Hz, 1H, Phe-\(\beta\) (l)).

\(^{13}\)C\({^1}\)H NMR (101 MHz, DMSO) \(\delta\) 171.72 (1C, C12), 168.11 (1C, C14), 166.39 (2C, C20), 166.13 (2C, 19), 164.99 (1C, C5), 139.39 (1C, C15), 138.37 (1C, C8), 134.92 (1C, C4), 132.27 (1C, C1), 132.18 (2C, C3), 131.83 (2C, C2), 131.54 (2C, C17), 129.06 (2C, C9), 128.03 (2C, C10), 126.20 (1C, C11), 124.61 (1C, C18), 123.50 (2C, C16), 55.05 (1C, C6), 42.87 (1C, C13), 36.85 (1C, C7).

**HRMS (ESI)** Exact mass calculated for C\(_{28}\)H\(_{23}\)N\(_3\)NaO\(_{11}\) [M + Na]\(^+\), 600.1230. Found 600.1322.

**N-Dimethyl-5-carbonylisophthalate-Ala-Gly-O(dimethyl-5-aminoisophthalate) (12) (new compound).** The carboxylic acid compound 11 (1.20 g, 3.9 mmol, 1.1 eq) was charged into a 100 mL oven dried round bottomed flask followed by addition of 20 mL anhydrous DMF and 0.7 mL TEA (4.6 mmol, 1.3 eq). The reaction mixture was cooled to 0 °C and HOBt (0.71 g, 4.6 mmol, 1.3 eq) and, EDC·HCl (0.90 g, 4.6 mmol, 1.3 eq) were added, and the mixture was left to stir for 15 min after which a solution of compound 4 (1.34 g, 3.5 mmol, 1 eq) and 0.6 ml TEA (4.2 mmol,
1.2 eq) in 15 mL anhydrous DMF was added to the reaction mixture via a syringe, and left to stir at 0 °C for 30 min then warmed to r.t and stirred for 16h. On completion, the reaction mixture was concentrated under reduced pressure (to about 5 mL), and 200 mL Et₂O was added to induce precipitation for which a sticky residue was formed and was left suspended in the Et₂O layer for 4-5 h after which the Et₂O layer was decanted. DI H₂O (400 mL) was added to the residue and was left to stir for 3-4 h after which the solid was filtered, and the collected solid was washed with sat NaHCO₃ then filtered, washed with 10 % citric acid then filtered, and washed with DI H₂O and then filtered. The solid was dried under high vacuum after which it was washed with EtOAc (60-80 mL) and left soaking in EtOAc for 4-5 h after which it was filtered, then washed (stirred) with 80 mL MeOH and left soaking in MeOH overnight. The solid was filtered, dried, and recrystallized from hot EtOH (150-200 mL) to afford title compound 12 as a white solid (0.77 g, 40%).

![Chemical Structure](image)

1H NMR (400 MHz, DMSO) δ 10.24 (s, 1H, Ar-NH (h)), 9.27 (d, J = 6.0 Hz, 1H, Ala-NH (d)), 8.76 (s, 2H, Ar-H (c)), 8.60 (s, 1H, Ar-H (b)), 8.57 (s, 2H, Ar-H (i)), 8.52 (t, J = 5.0 Hz, 1H, Gly-NH (f)), 8.18 (s, 1H, Ar-H (j)), 4.55-4.50 (m, 1H, Ala-α(e)), 3.93 (s, 8H, -COOCH₃ (a) and Gly-α (g) overlapped), 3.88 (s, 6H, -COOCH₃ (k)), 1.43 (d, J = 6.9 Hz, 3H, Ala-CH₃ (m)).
\(^{13}\)C\(^{1}\)H NMR (101 MHz, DMSO) δ 172.73 (1C, C9), 168.38 (1C, C11), 165.19 (2C, C5), 164.98 (2C, C16), 164.83 (1C, C7), 139.67 (1C, C12), 135.15 (1C, C4), 132.55 (2C, C3), 131.94 (1C, C1), 130.73 (2C, C2), 130.41 (2C, C14), 124.12 (1C, C15), 123.54 (2C, C13), 52.62 (2C, C6), 52.47 (2C, C17), 49.73 (1C, C8), 42.86 (1C, C10), 17.34 (1C, C18).

HRMS (ESI) Exact mass calculated for C\(_{26}\)H\(_{27}\)N\(_{3}\)NaO\(_{11}\) [M + Na]\(^+\), 580.1543. Found 580.1548.

N-5-Carboxylisophthalate-Ala-Gly-O(5-aminoisophthalate) (compound 13) (new compound). The title compound was prepared via the general procedure for the alkaline hydrolysis of the methyl ester using compound 12 (0.74 g, 1.3 mmol, 1eq), MeOH/THF (1:1) 26 mL, 1 N LiOH\(\cdot\)H\(_{2}\)O\(_{aq}\) (13.2 mL, 13.2 mmol, 10eq). Compound 13 was obtained as a white solid after lyophilization (0.4 g, 62%).
\( ^1H \text{NMR} (400 \text{ MHz, DMSO}) \delta 13.37 (b, 4H, -\text{COOH}), 10.22 (s, 1H, Ar-\text{NH} (h)), 9.17 (d, J = 6.8 \text{ Hz}, 1H, \text{Ala}-\text{NH} (d)), 8.72 (d, J = 1.5 \text{ Hz}, 2H, \text{Ar-H} (c)), 8.59 (t, J = 1.5 \text{ Hz}, 1H, \text{Ar-H} (b)), 8.49 (d, J = 1.4 \text{ Hz}, 2H, \text{Ar-H} (i)), 8.44 (t, J = 5.8 \text{ Hz}, 1H, \text{Gly-NH} (e)), 8.17 (t, J = 1.4 \text{ Hz}, 1H, \text{Ar-H} (k)), 4.58-4.51 (m, J = 7.1 \text{ Hz}, 1H, \text{Ala-}\alpha (f)), 3.98 – 3.87 (m, 2H, \text{Gly-}\alpha (g)), 1.42 (d, J = 7.2 \text{ Hz}, 3H, \text{Ala-CH}_3 (l)). \)

\( ^{13}C\{^1H\} \text{NMR} (101 \text{ MHz, DMSO}) \delta 172.75 (1C, C8), 168.22 (1C, C10), 166.37 (1C, C5), 166.15 (1C, C15), 165.02 (1C, C6), 139.37 (1C, C11), 134.93 (1C, C4), 132.39 (2C, C3), 132.29 (1C, C1), 131.79 (2C, C2), 131.47 (2C, C13), 124.63 (1C, C14), 123.53 (2C, C12), 49.52 (1C, C7), 42.83 (1C, C9), 17.46 (1C, C16). \)

**HRMS (ESI)** Exact mass calculated for \( C_{22}H_{19}N_3O_11 [M + Na]^+ \), 524.0917. Found 524.0917.

**1,3,5-Benzene tri-carboxylic acid dimethyl ester (15).** Synthesis of compound 15 was performed according to a procedure described in chapter 2 (chapter 2, section 2.5.6, synthesis of compound 2).

**5-(1-Tert-butoxycarbonyl-ethylcarbamoyl)-isophthalic acid dimethyl ester (17) (new compound).** To an oven dried two neck 100 mL flask with a stir bar was charged alanine tert-
butyl ester hydrochloride (compound 16) (1.28 g, 7.0 mmol, 1eq) and 30 mL anhydrous CH₂Cl₂ was added followed by 2.2 mL TEA (15.8 mmol, 2.25 eq). The reaction mixture was stirred for 5-10 min then cooled to 0 °C. A solution of dimethyl 5-(chlorocarbonyl) isophthalate (compound 8) (1.99 g, 7.7 mmol, 1.1 eq) in 10 mL anhydrous CH₂Cl₂ was then added dropwise via a syringe and the reaction mixture was stirred at 0 °C for 30 min then warmed to r.t and stirred for 16 h under nitrogen atmosphere. On completion, 40 mL DI H₂O was added to the reaction mixture and was left to stir for 15 min, after which the reaction mixture was transferred to a separating funnel and the aqueous layer was discarded. The organic layer was washed with brine (50 mL × 1), and dried over Na₂SO₄. The organic layer was evaporated under reduced pressure to obtain an oily residue which was purified via column chromatography (30:70 EtOAc/Hexanes) to afford the title compound 17 as a white solid (2.15 g, 5.2 mmol, 84%).

![Chemical Structure](image)

\(^1\)H NMR (400 MHz, DMSO) δ 9.19 (d, J = 6.8 Hz, 1H, Gly-NH (c)), 8.70 (d, J = 0.7 Hz, 2H, Ar-H (d)), 8.58 (s, 1H, Ar-H (e)), 4.43-4.35 (m, 1H, Ala- α (b)), 3.94 (s, 6H, -COOCH₃ (f)), 1.42 (s, 9H, Boc (a)).
$^{13}$C{$^1$H} NMR (101 MHz, DMSO) $\delta$ 171.59 (1C, C3), 164.92 (2C, C10), 164.35 (1C, C5), 135.11 (1C, C6), 132.24 (2C, C7), 131.92 (1C, C9), 130.51 (2C, C8), 80.44 (1C, C2), 52.62 (2C, C11), 49.15 (1C, C4), 27.60 (3C, C1), 16.60 (1C, C12).

**HRMS (ESI)** Exact mass calculated for C$_{18}$H$_{23}$NNaO$_7$ [M + Na]$^+$, 388.1372. Found 388.1372.

5-(1-Carboxy-ethylcarbamoyl)-isophthalic acid dimethyl ester (11) (new compound). A solution of compound 17 (1.76 g, 4.8 mmol) in 10 mL anhydrous CH$_2$Cl$_2$ was cooled to 0 °C followed by addition of a solution of TFA (10 mL) in anhydrous CH$_2$Cl$_2$ (10 mL) dropwise via an addition funnel. The reaction mixture was left to stir for 30 min at 0 °C, then warmed to r.t and stirred for 16 h. Upon completion of the reaction, as monitored by TLC, the solvents were removed under reduced pressure and the residue was co-evaporated with EtOAc (15 mL $\times$ 8) till a white solid appeared. The solid was then stirred (washed) with 100 mL Et$_2$O for 4 h, and left soaking in the Et$_2$O layer overnight. The solid was filtered, and the collected solid was dried under high vacuum to afford compound 15 as a white solid that was used without further purification (1.2 g, 81%).
$^1$H NMR (400 MHz, DMSO) $\delta$ 12.65 (s, 1H, -COOH (a)), 9.20 (d, $J = 7.0$ Hz, 1H, Gly-NH (c)), 8.72 (s, 2H, Ar-H (d)), 8.59 (s, 1H, Ar-H (e)), 4.51-4.44 (m, 1H, Ala-$\alpha$ (b)), 3.94 (s, 6H, -COOCH$_3$ (f)), 1.43 (d, $J = 7.2$ Hz, 3H, Ala-CH$_3$ (g)).

$^{13}$C($^1$H) NMR (101 MHz, DMSO) $\delta$ 173.86 (1C, C1), 164.95 (2C, C8), 164.21 (1C, C3), 135.13 (1C, C4), 132.26 (2C, C5), 131.92 (1C, C7), 130.51 (2C, C6), 52.63 (2C, C9), 48.36 (1C, C2), 16.73 (1C, C10).

HRMS (ESI) Exact mass calculated for C$_{14}$H$_{15}$NNaO$_7$ [M + Na]$^+$, 332.0746. Found 332.0746.

**Boc-Ser-Gly-O(dimethyl-5-aminoisophthalate) (20) (new compound).** Compound 4 (2.61 g, 6.9 mmol, 1 eq) was charged in a two neck 100 mL flask, anhydrous DMF (30 mL) was added followed by Et$_3$N (1.2 mL, 8.6 mmol, 1.25 eq). The reaction mixture was cooled to 0 °C, and EDC·HCl (1.58 g, 8.3 mmol, 1.2 eq) was added followed by HOBt (1.26 g, 8.3 mmol, 1.2 eq). A solution of Boc-Ser-OH (compound 18) (1.41 g, 6.9 mmol, 1 eq) and Et$_3$N (1.2 mL, 8.6 mmol, 1.25 eq) in 25 mL anhydrous DMF was then added to the reaction mixture dropwise via a syringe, and the reaction mixture was left to stir at 0 °C for 15-20 min then warmed to room temperature.
and left to stir overnight for 16 hr. On completion, the reaction mixture was concentrated under reduced pressure (to a volume of about 5-10 mL) and the residue was diluted with 400-450 mL EtOAc, washed with DI H₂O (200 mL × 3), sat NaHCO₃ (100 mL × 3), 10% citric acid (100 mL × 2), DI water (100 mL × 1) and brine (150 mL × 1) followed by drying the organic layers under Na₂SO₄. The organic solvents were removed under reduced pressure to afford an off-white solid residue, the solid was dissolved in EtOAc and hexanes were added portion-wise till a white solid precipitated out of the solution. The solid was filtered and collected while the filtrate was concentrated under reduced pressure and hexanes were added portion-wise till a white solid precipitated out which was filtered, and the solids were combined. The solid was dissolved in a hot EtOAc/hexanes mixture and left to cool to room temperature, then left overnight in the fridge after which a white solid precipitated out. The purity was monitored by TLC (EtOAc/hexanes 7:3), and purification of the solid through precipitation from a hot EtOAc/hexanes mixture could be repeated if necessary. The solid was filtered, and dried under high vacuum to afford compound 20 was a white solid (2.11 g, 68%).

\[ \text{HNMR (400 MHz, DMSO)} \delta \text{10.14 (s, 1H, Ar-NH (f)), 8.50 (s, 2H, Ar-H (g)), 8.35 (t, } J = 5.6 \text{ Hz, 1H, Gly-NH (d)), 8.18 (s, 1H, Ar-H (h)), 6.87 (d, } J = 7.2 \text{ Hz, 1H, Ser-NH (b)), 4.97 (t, } J = 5.8 \text{ Hz, 1H, Ser-CH}_2\text{OH (m), 4.05-4.00 (m, 1H, Ser- } \alpha \text{ (c)), 3.93 (dd, } J = 5.4, 2.9 \text{ Hz, 2H, Gly- } \alpha \text{ (e), 3.90 (s, 6H, } \text{-COOCH}_3\text{(j)), 3.64 – 3.62 (m, 2H, Ser-CH}_2\text{OH (l))}. \]
$^{13}$C($^1$H) NMR (101 MHz, DMSO) $\delta$ 171.02 (1C, C5), 168.35 (1C, C7), 165.18 (2C, C12), 155.51 (1C, C3), 139.52 (1C, C8), 130.74 (2C, C10), 124.22 (1C, C11), 123.59 (2C, C9), 78.45 (1C, C2), 61.68 (1C, C14), 56.99 (1C, C4), 52.50 (2C, C13), 42.92 (1C, C6), 28.11 (3C, C1).

HRMS (ESI) Exact mass calculated for C$_{20}$H$_{27}$N$_{3}$NaO$_{9}$ [M + Na]$^+$, 476.16450. Found 476.1637.

H-Ser-Gly-O(dimethyl-5-aminoisophthalate)-TFA (22) (new compound). Compound 20 (1.96 g, 4.3 mmol) was reacted following the general procedure for the deprotection of the Boc-group. Anhydrous CH$_2$Cl$_2$ (24 mL), anhydrous MeOH (5.3 mL), anisole (940 µL, 8.6 mmol, 2 eq), TFA (a solution of 45 mL TFA in 24 mL anhydrous CH$_2$Cl$_2$). The product compound 22 was obtained as a white solid (1.82 g, 91%).

$^1$H NMR (400 MHz, DMSO) $\delta$ 10.57 (s, 1H, Ar-NH (e)), 8.86 (s, 1H, Gly-NH (c)), 8.48 (s, 2H, Ar-H (f)), 8.16 (d, $J = 11.8$ Hz, 4H, Ar-H (g) and -NH$_3^+$ (a) overlapped), 5.59 (s, 1H, -CH$_2$OH (l)), 4.07-4.03 (m, 2H, Gly-α), 3.90 – 3.76 (m, 9H, -COOCH$_3$ × 2 (h), Ser-α (b) and Ser-CH$_2$OH (j) overlapped).
\[^{13}\text{C}\{^1\text{H}\} \text{NMR (101 MHz, DMSO)} \delta 167.78 (1\text{C}, \text{C}2), 167.49 (1\text{C}, \text{C}4), 165.19 (1\text{C}, \text{C}9), 158.41-157.53 (\text{m, TFA}), 139.62 (1\text{C}, \text{C}5), 130.75 (2\text{C}, \text{C}7), 124.15 (1\text{C}, \text{C}8), 123.58 (2\text{C}, \text{C}6), 118.78-115.79 (\text{TFA}), 60.35 (1\text{C}, \text{C}11), 54.34 (1\text{C}, \text{C}1), 52.52 (2\text{C}, \text{C}10), 42.83 (1\text{C}, \text{C}3).\]

\text{Boc-Trypt-Gly-O(dimethyl-5-aminoisophthalate) (21) (new compound).} Compound 4 (1.50 g, 3.9 mmol, 1 eq) was charged in a two neck 100 mL flask, anhydrous DMF (20 mL) was added followed by Et\textsubscript{3}N (1.4 mL, 9.8 mmol, 2.5 eq). The reaction mixture was cooled to 0 °C, and EDC\cdot\text{HCl} (0.91 g, 4.7 mmol, 1.2 eq) was added followed by HOBt (0.72 g, 4.7 mmol, 1.2 eq). A solution of Boc-Trypt-OH (compound 19) (1.20 g, 3.9 mmol, 1 eq) in 10 mL anhydrous DMF was then added to the reaction mixture dropwise via a syringe, and the reaction mixture was left to stir at 0 °C for 15-20 mins then warmed to room temperature and left to stir overnight for 16 hrs. On completion, the reaction mixture was concentrated under reduced pressure (to a volume of about 5-10 mL) and the residue was diluted with 400-450 mL EtOAc, washed with DI H\textsubscript{2}O (200 mL × 3), sat NaHCO\textsubscript{3} (100 mL × 3), 10 % citric acid (100 mL × 2), DI water (100 mL × 1) and brine (150 mL× 1). It was noticed that after washing with brine, a white solid started to crash out of the EtOAc layer, which increased over time. The solid was filtered and collected, and the filtrate was evaporated under reduced pressure to obtain a yellowish white solid. The solids were combined and dried under high vacuum followed by tituration with hot EtOAc (purity was monitored by TLC) and left to cool to rt. The solid was filtered, and dried under high vacuum to obtain title compound 21 as a white light fluffy solid (1.87 g, 87%).
\[ \text{H NMR (400 MHz, DMSO)} \delta 10.91 \text{ (s, 1H, indole-NH (f))}, 10.47 \text{ (s, 1H, Ar-NH (m))}, 8.55 \text{ (d, } J = 1.0 \text{ Hz, 2H, Ar-H (n))}, 8.48 \text{ (t, } J = 5.6 \text{ Hz, 1H, Gly-NH (k))}, 8.18 \text{ (s, 1H, Ar-H (o))}, 7.62 \text{ (d, } J = 7.8 \text{ Hz, 1H, indole-H (b))}, 7.33 \text{ (d, } J = 8.0 \text{ Hz, 1H, indole-H (a))}, 7.19 \text{ (d, } J = 2.0 \text{ Hz, 1H, indole-H (e))}, 7.06 \text{ (t, } J = 7.2 \text{ Hz, 1H, indole-H (d))}, 7.00-6.94 \text{ (m, 2H, indole-H (c) and Trp-NH (j) overlapped)}, 4.27-4.22 \text{ (m, 1H, Trp-}\alpha \text{ (r))}, 3.95 \text{ (d, } J = 3.8 \text{ Hz, 2H, Gly-}\alpha \text{ (l))}, 3.89 \text{ (s, 6H, -COOCH}_3 \text{ (p))}, 3.15 \text{ (dd, } J = 14.6, 4.2 \text{ Hz, 1H, Trp-}\beta \text{ (q))}, 2.97 \text{ (dd, } J = 14.5, 9.5 \text{ Hz, 1H, Trp-}\beta \text{ (q))}, 1.31 \text{ (s, 9H, Boc (g))}.

\[ \text{\textsuperscript{13}C\text{\{H\} NMR (101 MHz, DMSO)} \delta 172.56 \text{ (1C, C5)}, 168.44 \text{ (1C, C7)}, 165.23 \text{ (2C, C12)}, 155.47 \text{ (1C, C3)}, 139.71 \text{ (1C, C8)}, 136.06 \text{ (1C, C16)}, 130.69 \text{ (2C, C10)}, 127.34 \text{ (1C, C15)}, 124.13 \text{ (1C, C11)}, 123.71 \text{ (1C, C22)}, 123.64 \text{ (2C, C9)}, 120.73 \text{ (1C, C19)}, 118.41 \text{ (1C, C17)}, 118.11 \text{ (1C, C18)}, 111.25 \text{ (1C, C20)}, 110.11 \text{ (1C, C21)}, 78.19 \text{ (1C, C2)}, 55.32 \text{ (1C, C4)}, 52.49 \text{ (2C, C13)}, 42.92 \text{ (1C, C6)}, 28.08 \text{ (3C, C1)}, 27.48 \text{ (1C, C14)}.

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HRMS (ESI) Exact mass calculated for C_{28}H_{32}N_{3}NaO_{8} [M + Na]^+ 575.21178. Found 575.2128.

H-Trypt-Gly-O(dimethyl-5-aminoisophthalate)-TFA (23) (new compound). Compound 21 (1.0 g, 1.8 mmol) was reacted following the general procedure for the deprotection of the Boc-group. Anhydrous CH₂Cl₂ (15 mL), anhydrous MeOH (3.3 mL), anisole (585 µL, 5.4 mmol, 3 eq), TFA (a solution of 15 mL TFA in 15 mL anhydrous CH₂Cl₂). The product compound 23 was obtained as a white solid (0.95 g, 93%).

![Diagram of compound 23]

¹H NMR (400 MHz, DMSO) δ 11.07 (s, 1H, 1H, indole-NH (f)), 10.62 (s, 1H, s, 1H, Ar-NH (m)), 9.10 (t, J = 5.5 Hz, 1H, Gly-NH (k)), 8.50 (s, 2H, Ar-H (n)), 8.19 (s, 1H, Ar-H (o)), 8.12 (b, 3H, -NH₃⁺), 7.73 (d, J = 7.8 Hz, 1H, indole-H (b)), 7.38 (d, J = 8.0 Hz, 1H, indole-H (a)), 7.27 (d, J = 1.8 Hz, 1H, indole-H (e)), 7.11 (t, J = 7.5 Hz, 1H, indole-H (d)), 7.02 (t, J = 7.4 Hz, 1H, indole-H (c)), 4.14-4.11 (m, 1H, Trp-α (r)), 4.06 – 4.04 (m, 2H, Gly-α (l)), 3.90 (s, 6H, -COOCH₃ (p)), 3.32 (dd, J = 15.0, 5.0 Hz, 1H, Trp-β (q)), 3.14 (dd, J = 14.7, 8.4 Hz, 1H Trp-β (q)).
\[^{13}\text{C}\{^1\text{H}\}\text{ NMR (101 MHz, DMSO)}\, \delta\, 169.11 (1\text{C}, \text{C}2), 167.75 (1\text{C}, \text{C}4), 165.20 (2\text{C}, \text{C}9), 158.06-157.75 (\text{TFA}), 139.66 (1\text{C}, \text{C}5), 136.32 (1\text{C}, \text{C}13), 130.76 (2\text{C}, \text{C}7), 127.05 (1\text{C}, \text{C}12), 125.02 (1\text{C}, \text{C}15), 124.15 (1\text{C}, \text{C}8), 123.61 (3\text{C}, \text{C}6 \text{and C}19 \text{overlapped}), 121.12 (1\text{C}, \text{C}16), 118.41 (1\text{C}, \text{C}14), 111.46 (1\text{C}, \text{C}17), 106.74 (1\text{C}, \text{C}18), 52.63 (1\text{C}, \text{C}1), 52.53 (2\text{C}, \text{C}10), 42.84 (1\text{C}, \text{C}3), 27.44 (1\text{C}, \text{C}11).

\text{N-Dimethyl-5-carbonylisophthalate-Sep-Gly-O(dimethyl-5-aminoisophthalate) (24) (new compound).} \] = 1,3,5-Benzene-teretricarboxylic acid dimethyl ester (Compound 15) (0.57 g, 2.4 mmol, 1.1 eq) was charged into a two neck 100 mL oven dried round bottomed flask followed by addition of 14 mL anhydrous DMF. Triethylamine (TEA) (0.4 mL, 2.9 mmol, 1.3 eq) was added, and the reaction mixture was cooled to 0 °C and left to stir for 5-10 min after which time EDC·HCl (0.55 g, 2.9 mmol, 1.3 eq) was added followed by HOBT (0.72 g, 2.9 mmol, 1.3 eq), and the reaction mixture was left to stir at 0 °C for 15 mins. A solution of compound 22 (1.1 g, 2.2 mmol, 1 eq) and TEA (0.36 mL, 2.6 mmol, 1.2 eq) in 15 mL anhydrous DMF was then added to the reaction mixture dropwise via a syringe, and the reaction mixture was left to stir for at 0 °C for 15-20 mins then warmed to rt and left to stir for 18 h under nitrogen atmosphere. On completion, the reaction mixture was concentrated under reduced pressure (to an approximate volume of 10 mL), and Et\(_2\)O was added to induce precipitation. A sticky residue was obtained and, the Et\(_2\)O layer was decanted, and the residue was dried under vacuum after which time DI H\(_2\)O (400-500 mL) was added and
the mixture was left to stand for 3 h then stirred for an additional hour. The solid was filtered, and washed with DI H$_2$O (100 mL), sat NaHCO$_3$ (100-150 mL), 10 % citric acid (100 mL), and DI H$_2$O (100 mL) after which it was dried under high vacuum. The solid was washed with 80-100 mL EtOAc (stirred), filtered, then dried, and the collected solid was washed (stirred) with 80-100 mL MeOH, filtered, then dried. The solid was dissolved in hot EtOH and left to cool to rt and left in the fridge overnight after which time a white solid precipitated out which was filtered and collected. The filtrate was concentrated under reduced pressure and left to cool in the fridge to obtain second crop of a white solid precipitate. The solids where combined and dried under high vacuum to afford title compound 24 (0.53 g, 41%).

$^1$H NMR (400 MHz, DMSO) δ 10.20 (s, 1H, Ar-H (h)), 9.10 (d, $J = 6.5$ Hz, 1H, Ser-NH (d)), 8.77 (d, $J = 1.5$ Hz, 2H, Ar-H (c)), 8.60 (t, $J = 1.4$ Hz, 1H, Ar-H (b)), 8.60 – 8.54 (m, 3H, Ar-H× 2 (i) and Gly-NH (f) overlapped ), 8.17 (t, $J = 1.3$ Hz, 1H, Ar-H (j)), 5.14 (b, 1H, Ser-CH$_2$OH (l)), 4.57 – 4.54 (m, 1H, Ser-α (e)), 3.95 (d, $J = 5.1$ Hz, 2H, Gly-α (g)), 3.93 (s, 6H, -COOCH$_3$ (a)), 3.88 (s, 6H, -COOCH$_3$ (k)), 3.85-3.84 (m, 2H, Ser-CH$_2$OH (m)).
$^{13}\text{C}^{\{\text{H}\}}\text{ NMR (101 MHz, DMSO)} \delta 170.42 (1\text{C}, \text{C9}), 168.31 (1\text{C}, \text{C11}), 165.17 (2\text{C}, \text{C5}), 165.10 (2\text{C}, \text{C16}), 164.97 (1\text{C}, \text{C7}), 139.58 (1\text{C}, \text{C12}), 135.18 (1\text{C}, \text{C4}), 132.53 (2\text{C}, \text{C3}), 131.98 (1\text{C}, \text{C1}), 130.73 (2\text{C}, \text{C2}), 130.41 (2\text{C}, \text{C14}), 124.16 (1\text{C}, \text{C15}), 123.54 (2\text{C}, \text{C13}), 61.22 (1\text{C}, \text{C18}), 56.92 (1\text{C}, \text{C8}), 52.63 (2\text{C}, \text{C6}), 52.48 (2\text{C}, \text{C17}), 42.94 (1\text{C}, \text{C10}).$

HRMS (ESI) Exact mass calculated for C$_{26}$H$_{27}$N$_3$NaO$_{12}$ [M + Na]$^+$, 596.14924. Found 596.1492.

N-Dimethyl-5-carbonylisophthalate-Tryptophan-Glycine-O(dimethyl-5-aminoisophthalate) (25) (new compound). 1,3,5-Benzene tricarboxylic acid dimethyl ester (compound 15) (0.42 g, 1.8 mmol, 1.1 eq) was charged into a two neck 100 mL oven dried round bottomed flask followed by addition of 15 mL anhydrous DMF. Triethylamine (TEA) (0.3 mL, 2.1 mmol, 1.3 eq) was added, the reaction mixture was cooled to 0 °C and left to stir for 5-10 min after which time EDC·HCl (0.41 g, 2.1 mmol, 1.3 eq) was added followed by HOBt (0.32 g, 2.1 mmol, 1.3 eq), and the reaction mixture was left to stir at 0 °C for 15 min. A solution mixture of compound 23 (0.91 g, 1.6 mmol, 1 eq) and TEA (0.27 mL, 1.9 mmol, 1.2 eq) in 15 mL anhydrous DMF was then added to the reaction mixture dropwise via a syringe, and the reaction mixture was left to stir for at 0 °C for 15-20 min, warmed to rt and left to stir for 18 h under nitrogen atmosphere. On completion, the reaction mixture was concentrated under reduced pressure (to an approximate volume of 10 mL),
and Et₂O was added to induce precipitation. A sticky residue was obtained and, the Et₂O layer was decanted followed and the residue was dried under vacuum after which DI H₂O (400-500 mL) was added and the mixture was left to stand for 3-5 h then stirred for an additional hour. The solid was filtered, and washed with DI H₂O (100 mL), sat NaHCO₃ (100-150 mL), 10 % citric acid (100 mL), and DI H₂O (100 mL) after which it was dried under high vacuum. The solid was then washed with 80-100 mL EtOAc (stirred), filtered and dried, the collected solid washed (stirred) with 80-100 mL MeOH, and filtered then dried. The solid was dissolved in hot EtOH, and a hot filtration was done to remove undissolved particulate matter. The hot filtrate was left to cool to rt and left in the fridge overnight after which time a white solid precipitated out which was filtered and collected, and the filtrate was concentrated under reduced pressure and left to cool in the fridge to obtain second crop of a white solid precipitate. The solids were combined and dried under high vacuum to afford title compound 25 as an off-white solid (0.37 g, 38%).

\(^1\)H NMR (400 MHz, DMSO) \(\delta\) 10.81 (s, 1H, indole-NH (f)), 10.34 (s, 1H, Ar-NH (m)), 9.27 (d, \(J = 7.6\) Hz, 1H, Trp-NH (j)), 8.67-8.66 (m, 3H, Ar-H × 2 (i) and Gly-NH (k) overlapped), 8.56 (t, \(J = 1.4\) Hz, 1H, Ar-H, (h)), 8.55 (d, \(J = 1.3\) Hz, 2H, Ar-H, (n)), 8.18 (s, 1H, Ar-H (o)), 7.71 (d, \(J = 7.8\) Hz, 1H, indole-H (b)), 7.32 (d, \(J = 8.0\) Hz, 1H, indole-H (a)), 7.23 (d, \(J = 2.0\) Hz, 1H, indole-H (e)), 7.06 (t, \(J = 7.3\) Hz, 1H, indole-H (d)), 6.99 (t, \(J = 7.4\) Hz, 1H, indole-H (c)), 4.85-4.80 (m,
1H, Trp-α (r)), 4.01 – 3.96 (m, 2H, Gly-α (l)), 3.92 (s, 6H, -COOCH₃ (g)), 3.88 (s, 6H, -COOCH₃ (p)), 3.33 (d, J = 4.2 Hz, 1H, Trp-β (q)), 3.24 (dd, J = 14.6, 10.2 Hz, 1H, Trp-β (q)).

13C ¹H NMR (101 MHz, DMSO) δ 172.05 (1C, C9), 168.34 (1C, C11), 165.20 (2C, C5), 164.93 (2C, C16), 164.82 (1C, C7), 139.70 (1C, C12), 136.05 (1C, C26), 135.21 (1C, C4), 132.44 (2C, C3), 131.88 (1C, C1), 130.73 (2C, C2), 130.38 (2C, C14), 127.23 (1C, C19), 124.10 (1C, C15), 123.54 (3C, C13 and C20 overlapped), 120.86 (1C, C23), 118.41 (1C, C25), 118.22 (1C, C24), 111.29 (1C, C22), 110.43 (1C, C21), 54.82 (1C, C8), 52.61 (2C, C6), 52.48 (2C, C17), 42.96 (1C, C10), 27.06 (1C, C18).


N-5-Carboxylisophthalate-Ser-Gly-O(5-aminoisophthalate) (compound 26) (new compound). The title compound was prepared via the general procedure for the alkaline hydrolysis of the methyl ester using compound 24 (0.72 g, 1.3 mmol, 1 eq), MeOH/THF (1:1) 25 mL, 1 N LiOH·H₂Oₐq) (13 mL, 13.0 mmol, 10 eq). On reaction completion, the reaction mixture was transferred into a separating funnel and EtOAc was added till two layers appeared, and the aqueous layer was separated and cooled to 0 °C. Acetone (10 mL) was added to the cooled aqueous layer followed by acidification to pH 2 using 1 N HCl. The acidified aqueous layer was left in the
fridge till a white solid precipitated out (could be left overnight in the fridge to induce precipitation if required). The white solid was filtered, and washed with DI H$_2$O (100 mL × 3). The solid was collected and lyophilized to obtain the desired product (compound 26) as white solid (0.3 g, 46%).

![Chemical structure of compound 26](image)

$^1$H NMR (400 MHz, DMSO) δ 13.39 (b, 4H, -COOH), 10.16 (s, 1H, Ar-NH (h)), 8.97 (d, $J = 7.2$ Hz, 1H, Ser-NH (d)), 8.73 (d, $J = 1.5$ Hz, 2H, Ar-H (c)), 8.61 (t, $J = 1.5$ Hz, 1H, Ar-NH (b)), 8.51 (t, $J = 5.8$ Hz, 1H, Gly-NH (f)), 8.47 (d, $J = 1.4$ Hz, 2H, Ar-H (i)), 8.18 (t, $J = 1.4$ Hz, 1H, Ar-H (j)) 5.16 (b, 1H, Ser-CH$_2$OH (m)), 4.57 (dd, $J = 12.3$, 6.5 Hz, 1H, Ser-α (e)), 3.95 (d, $J = 5.7$ Hz, 2H, Gly-α (g)), 3.87–3.79 (m, 2H, Ser-CH$_2$OH (l)).

$^{13}$C-$^1$H NMR (101 MHz, DMSO) δ 170.47 (1C, C8), 168.17 (1C, C10), 166.36 (2C, C5), 166.15 (1C, C15), 165.28 (1C, C4), 139.26 (1C, C11), 134.92 (1C, C4), 132.36 (3C, C1 and C3 overlapped, assigned through HSQC), 131.80 (2C, C2), 131.50 (2C, C13), 124.72 (1C, C14), 123.58 (2C, C12), 61.42 (1C, C16), 56.76 (1C, C7), 42.94 (1C, C9).

HRMS (ESI) Exact mass calculated for C$_{22}$H$_{19}$N$_3$NaO$_{12}$ [M + Na]$^+$, 540.0866. Found 540.0866.
N-5-Carbonylisophthalate-Trypt-Gly-O(5-aminoisophthalate) (compound 27) (new compound). The title compound was prepared via the general procedure for the alkaline hydrolysis of the methyl ester using compound 25 (0.27 g, 0.41 mmol, 1eq), MeOH/THF (1:1) 8 mL, 1 N LiOH$\cdot$H$_2$O$_{aq}$ (4.1 mL, 4.1 mmol, 10 eq). Compound 27 was obtained as a white solid after lyophilization (0.16 g, 75%).

$^1$H NMR (400 MHz, DMSO) δ 13.28 (b, 4H, COOH), 10.70 (s, 1H, indole-NH (q)), 10.22 (s, 1H, Ar-NH (h)), 9.09 (d, $J = 7.8$ Hz, 1H, Trp-NH (d)), 8.57 (d, $J = 1.5$ Hz, 2H, Ar-H (c)), 8.53 (t, $J = 5.7$ Hz, 1H, Gly-NH (f)), 8.48 (t, $J = 1.5$ Hz, 1H, Ar-H (b)) 8.40 (d, $J = 1.4$ Hz, 2H, Ar-H (i)), 8.09 (t, $J = 1.4$ Hz, 1H, Ar-H (j)), 7.62 (d, $J = 7.8$ Hz, 1H, Indole-H(m)), 7.22 (d, $J = 8.0$ Hz, 1H, indole-H(p)), 7.13 (d, $J = 2.1$ Hz, 1H, indole-H(r)), 6.96 (t, $J = 7.1$ Hz, 1H, indole-H(o)), 6.89 (t, $J = 7.1$ Hz, 1H, indole-H(n)), 4.77 – 4.71 (m, 1H, Trp-α (e)), 3.88 (d, $J = 6.2$ Hz, 2H, Gly-α (g)), 3.25 (dd, $J = 14.6$, 4.0 Hz, 1H, Trp-β (l), overlapped with H$_2$O impurity peak), 3.15 (dd, $J = 14.6$, 10.3 Hz, 1H, Trp-β (l), overlapped with H$_2$O impurity peak).
$^{13}$C-$^1$H NMR (101 MHz, DMSO) δ 172.0 (1C, C8), 168.17 (1C, C10), 166.38 (2C, C5), 166.12 (2C, C15), 165.00 (1C, C6)), 139.40 (1C, C11)), 136.03 (1C, C24), 134.92 (1C, C4), 132.26 (3C, C1 and C3 overlapped, assigned by HSQC), 131.81 (2C, C2), 131.47 (2C, C13), 127.25 (1C, C17), 124.63 (1C, C14), 123.53 (2C, C12), 123.47 (1C, C18), 120.83 (1C, C21), 118.41 (1C, C23), 118.20 (1C, C22), 111.27 (1C, C20), 110.57 (1C, C19), 54.68 (1C, C7), 42.93 (1C, C9), 27.14 (1C, C16).

**HRMS (ESI)** Exact mass calculated for C$_{30}$H$_{24}$N$_4$NaO$_{11}$ [M + Na]$^+$, 639.1339. Found 639.1339.
3.6. NMR Characterization data of synthesized Organic Compounds

Figure 3.21. $^1$H-NMR spectrum of compound 6 in DMSO-$d_6$.

Figure 3.22. $^{13}$C-NMR spectrum of compound 6 in DMSO-$d_6$. 
Figure 3.23. COSY spectrum of compound 6 in DMSO-d$_6$.

Figure 3.24. HSQC spectrum of compound 6 in DMSO-d$_6$. 
Figure 3.25. $^1$H-NMR spectrum of compound 7 in DMSO-$d_6$.

Figure 3.26. $^{13}$C-NMR spectrum of compound 7 in DMSO-$d_6$. 
Figure 3.27. $^1$H-NMR spectrum of compound 9 in DMSO-$d_6$.

Figure 3.28. $^{13}$C-NMR spectrum of compound 9 in DMSO-$d_6$. 
Figure 3.29. COSY spectrum of compound 9 in DMSO-d$_6$. 
Figure 3.30. \(^1\)H-NMR spectrum of compound 10 in DMSO-d\(_6\).

Figure 3.31. \(^{13}\)C-NMR spectrum of compound 10 in DMSO-d\(_6\).
Figure 3.32. COSY spectrum of compound 10 in DMSO-$d_6$.

Figure 3.33. HSQC spectrum of compound 10 in DMSO-$d_6$. 
Figure 3.34. HMBC spectrum of compound 10 in DMSO-d$_6$. 
Figure 3.35. $^1$H-NMR spectrum of compound 12 in DMSO-$d_6$.

Figure 3.36. $^{13}$C-NMR spectrum of compound 12 in DMSO-$d_6$. 
Figure 3.37. COSY spectrum of compound 12 in DMSO-d$_6$. 
Figure 3.38. $^1$H-NMR spectrum of compound 13 in DMSO-$d_6$.

Figure 3.39. $^{13}$C-NMR spectrum of compound 13 in DMSO-$d_6$. 

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Figure 3.40. COSY spectrum of compound 13 in DMSO-d$_6$.

Figure 3.41. HSQC spectrum of compound 13 in DMSO-d$_6$. 
Figure 3.42. HMBC spectrum of compound 13 in DMSO-d$_6$. 
Figure 3.43. $^1$H-NMR spectrum of compound 17 in DMSO-$d_6$.

Figure 3.44. $^{13}$C-NMR spectrum of compound 17 in DMSO-$d_6$. 
Figure 3.45. $^1$H-NMR spectrum of compound 11 in DMSO-d$_6$.

Figure 3.46. $^{13}$C-NMR spectrum of compound 11 in DMSO-d$_6$. 

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Figure 3.47. $^1$H-NMR spectrum of compound 20 in DMSO-d$_6$.

Figure 3.48. $^{13}$C-NMR spectrum of compound 20 in DMSO-d$_6$. 
Figure 3.49. COSY spectrum of compound 20 in DMSO-d₆.

Figure 3.50. HSQC spectrum of compound 20 in DMSO-d₆.
Figure 3.51. HMBC spectrum of compound 20 in DMSO-d$_6$. 

![HMBC Spectrum](image-url)
Figure 3.52. $^1$H-NMR spectrum of compound 22 in DMSO-d$_6$.

Figure 3.53. $^{13}$C-NMR spectrum of compound 22 in DMSO-d$_6$. 
Figure 3.54. $^1$H-NMR spectrum of compound 21 in DMSO-d$_6$.

Figure 3.55. $^{13}$C-NMR spectrum of compound 21 in DMSO-d$_6$. 

250
Figure 3.56. COSY spectrum of compound 21 in DMSO-d$_6$.

Figure 3.57. HSQC spectrum of compound 21 in DMSO-d$_6$. 
Figure 3.58. HMBC spectrum of compound 21 in DMSO-d$_6$. 
Figure 3.59. $^1$H-NMR spectrum of compound 23 in DMSO-$d_6$.

Figure 3.60. $^{13}$C-NMR spectrum of compound 23 in DMSO-$d_6$. 

253
Figure 3.61. $^1$H-NMR spectrum of compound 24 in DMSO-$d_6$.

Figure 3.62. $^{13}$C-NMR spectrum of compound 24 in DMSO-$d_6$. 
Figure 3.63. $^1\text{H}-\text{NMR}$ spectrum of compound 25 in DMSO-d$_6$.

Figure 3.64. $^{13}\text{C}-\text{NMR}$ spectrum of compound 25 in DMSO-d$_6$. 
Figure 3.65. $^1$H-NMR spectrum of compound 26 in DMSO-$d_6$.

Figure 3.66. $^{13}$C-NMR spectrum of compound 26 in DMSO-$d_6$. 

256
Figure 3.67. COSY spectrum of compound 26 in DMSO-d$_6$.

Figure 3.68. HSQC spectrum of compound 26 in DMSO-d$_6$. 
Figure 3.69. HMBC spectrum of compound 26 in DMSO-d$_6$. 
Figure 3.70. $^1$H-NMR spectrum of compound 27 in DMSO-$d_6$.

Figure 3.71. $^{13}$C-NMR spectrum of compound 27 in DMSO-$d_6$. 
**Figure 3.72.** COSY spectrum of compound 27 in DMSO-d$_6$.

**Figure 3.73.** HSQC spectrum of compound 27 in DMSO-d$_6$. 
Figure 3.74. HMBC spectrum of compound 27 in DMSO-d$_6$. 

![HMBC spectrum of compound 27 in DMSO-d$_6$.]
3.7. HRMS characterization data for synthesized organic compounds.

Figure 3.75. HRMS of compound 6.

Figure 3.76. HRMS of compound 9.
Figure 3.77. HRMS of compound 10.

Figure 3.78. HRMS of compound 12.
Figure 3.79. HRMS of compound 13.

Figure 3.80. HRMS of compound 17.
Figure 3.81. HRMS of compound 11.

Figure 3.82. HRMS of compound 20.
Figure 3.83. HRMS of compound 21.

Figure 3.84. HRMS of compound 24.

Figure 3.85. HRMS of compound 25.
**Figure 3.86.** HRMS of compound 26.

**Figure 3.87.** HRMS of compound 27.
3.8. References


Chapter 4: Design and Synthesis of a novel tetracarboxylate linker based on the Asp-Gly dipeptide and investigation of the synthesis of a peptide based mixed linker multivariate Metal Organic Framework with free carboxylate groups (MOF-COOH).

4.1. Introduction

Metal organic frameworks (MOFs) are a new class of advanced porous materials formed by the coordination metal ions or clusters with ligands\(^1\)-\(^3\). MOFs have gained tremendous attention over the past years, and this stems from the porous characters of these materials, the large surface areas, tunable pore sizes and the structural as well as the topological tunability\(^1\)-\(^3\). MOFs have promising applications in diverse fields driving the interest in this class of compounds, including separation\(^4\)-\(^6\), adsorption with emphasis on gas storage, sensing\(^7\), and heterogeneous catalysis\(^8\)-\(^9\).

The wide and diverse applicability of MOFs stems from properties of the MOF and the ability to introduce functional groups in the pores of the MOFs. Designing and synthesizing a MOF with a desired functionality allows the opportunity to target the MOF for a specific application\(^1\),\(^4\),\(^10\),\(^11\). Examples of functionalities introduced on MOFs include \(-\text{NH}_2\), \(-\text{SO}_3\text{H}\), \(-\text{X}(\text{Cl, Br, F, I})\), \(-\text{CF}_3\), \(-\text{COOH}\)^\(^10\),\(^11\). The carboxylate group \((-\text{COOH})\) has gained interest and many MOFs with free \(-\text{COOH}\) groups have been prepared\(^12\)-\(^16\),\(^10\). Free \(-\text{COOH}\) groups on MOFs import various properties into the MOF material due to their Bronsted acidity, binding ability with different compounds and gas molecules, and polarity\(^10\). The MOF-COOHs have been identified as promising materials for diverse applications that are based on the free \(-\text{COOH}\) functional group such as adsorption and separation\(^10\),\(^12\)-\(^15\), proton conductivity\(^10\),\(^16\)-\(^17\) and heterogeneous catalysis\(^10\),\(^18\).
Several strategies have been employed to introduce free –COOH functionality into the pores of MOF surfaces;\textsuperscript{10} of these methods, two of them have been mainly used, the direct synthesis method (DS) and the post-synthetic modification (PSM).\textsuperscript{10} In the direct synthesis approach (DS), a single organic ligand (single-ligand approach) having a sufficient number of –COOH groups is used to directly synthesize MOFs bearing free –COOH groups where one or more uncoordinated or extra –COOH groups is apparent in the MOF structure.\textsuperscript{10,16,12,14} If a mixture of ligands is used in the construction of the MOF-COOHs, then this is the direct synthesis via the mixed-ligand approach.\textsuperscript{10,13,15} Metal organic frameworks with free uncoordinated –COOH functionalities in the framework can be constructed when extra uncoordinated –COOH exist due several factors including restricted deprotonation of extra –COOH groups of the ligand under the synthesis conditions, the solvent, steric factors, the use of modulators, the metal salt, the use of secondary ligand and the pH of the synthesis conditions.\textsuperscript{10}

4.2. Scope of research work and strategy

Of the strategies of preparing novel MOF-COOHs with free uncoordinated –COOH groups in the framework is the design and synthesis of new ligands that would open new pathways for the study and application in many fields. In this work we report the design and synthesis of a novel tetracarboxylate Aspartic-Glycine dipeptide linker where both, the N-terminus and the C-terminus of the dipeptide have been chemically modified to bear two isophthalate units converting the dipeptide into a tetratopic carboxylate linker with a chiral extra carboxylate group stemming from the side chain of the aspartic acid residue (Figure. 4.1)
In our previous work (chapter 2), we have accomplished the design and synthesis of a novel tetratopic carboxylate Gly-Gly dipeptide linker (Figure 4.2) and we have optimized the conditions for the solvothermal synthesis of our Cu-based peptide MOF (MH-2-Gly-Gly) using this linker. Our peptide based MOF, MH-2-Gly-Gly, was obtained by the solvothermal reaction between the Gly-Gly tetratopic carboxylate linker and Cu(NO$_3$)$_2$·2.5H$_2$O (linker : metal molar ratio = 1 : 2) in a mixture solvent of DMF : DMA : EtOH : H$_2$O (v/v/v/v = 5 : 5 : 1 : 1) in the presence of acetic acid as a modulator and pyridine. These conditions yielded single crystals of the peptide MOF MH-2-Gly-Gly for which single crystal X-ray analysis experiments performed at the University of Oklahoma revealed that this peptide MOF was sustained by square dicopper paddlewheel [Cu$_2$(COO)$_4$] secondary building units, the vertices of which are linked into squares of squares rendering square grid layers (sql-1) that are in turn pillared via ligand-to-ligand cross linking into a 3D MOF that exhibited ssb/stx topology. Details of the synthesis and analysis of the structure of the MH-2-Gly-Gly peptide MOF are discussed in chapter 2.
Homochiral peptide-based MOFs have potential applications in many fields that would benefit from the chiral property of these peptide-based MOF. For example, homochiral peptide MOFs are promising candidates as solid-supported chiral adsorbents for separation of racemic mixtures\textsuperscript{21}, the chiral pockets in their structure can be used for chiral recognition of substrates leading to preferential interaction of one of the enantiomers and hence to separation of the racemic mixture\textsuperscript{21}. During the synthesis of many drug compounds, many end as racemic mixtures. Drug compounds that exist as enantiomers generally display different pharmacological action with one enantiomeric form showing more biological effect while the other form could display low or no biological activity\textsuperscript{20}. Enantiomeric drug compounds could also result in toxicological effects leading to undesired side reactions\textsuperscript{20,21}. Discovery of chiral adsorbents that can be used in separation of individual enantiomers is of primary importance in the field of drug research and the pharmaceutical industry.

In the work presented in this chapter, we investigate the synthesis of a novel peptide based MOF-COOHs with free –COOH in the pores of the MOF via the mixed-ligand direct synthesis approach where we used a mixture of the achiral Gly-Gly tetracarboxylate linker and the chiral Asp-Gly tetratcarboxylate linker (Figure 4.3). Using this mixed linker approach, we anticipated that our tertratopic carboxylate Asp-Gly linker would introduce chirality into this mixed linker.
MOF leading to our approach to the synthesis of homochiral MOFs where the linker is a peptide based tetracarboxylate linker with chirality of the linker stemming from the chirality of the amino acids used in synthesizing the linker. This peptide based homochiral MOF would have potential applications in many fields that would benefit from the chiral property of this peptide-based MOF.

**Figure 4.3.** Schematic illustration of the mixed-linker strategy for construction of homochiral peptide-based MOF with free chiral –COOH groups through combination of chiral and achiral organic linkers.

We investigate the employment of the same solvothermal conditions that we previously used to obtain single crystals of the peptide-based MOF MH-2-Gly-Gly using the Gly-Gly linker in the construction of our homochiral mixed-linker MOF. We also investigate the effect of changing the amount of acetic acid (as a modulator) in the synthesis of our mixed linker MOF.
4.3. Results and Discussion

4.3.1. Design and synthesis of the Asp-Gly tetracarboxylate linker.

The design and synthesis Asp-Gly tetracarboxylate linker (compound 10) was executed as shown in Scheme 4.1.

Coupling of the commercially available aromatic amine dibenzyl-5-aminoisophthalate (1) with Boc-Gly-OH (2) using the peptide coupling reagent HBTU (2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) in DMF in the presence of DIEA (N,N-diisopropylethyl amine) afforded the amide compound 3. The Boc-protecting group of compound 3 was then removed under acidolytic conditions using trifluoroacetic acid (TFA) giving the corresponding trifluoroacetate salt (compound 4). The coupling of compound 4 with Boc-Asp(Obzl)-OH (compound 5) using the water soluble carbodiimide coupling reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC-HCl) in the presence of 1-hydroxybenzotriazole hydrate (HOBr) and DIEA in DCM afforded the dipeptide compound 6.
Scheme 4.1. The design and synthesis of the Asp-Gly tetracarboxylate linker (compound 10).
Having accomplished the synthesis of compound 6 which has an N-Boc protected Asp-Gly dipeptide unit as part of its structure, the next step would be the deprotection of the N-Boc of 6 to get the free amine compound 7 but this deprotection step deemed challenging if done under commonly used acidolytic conditions employing strong acids such as trifluoroacetic acid (TFA) which is reported to cause formation of aspartimide products as a side reaction in peptides with aspartic acid residues thus significantly lowering the yield of the desired target compound.\textsuperscript{24,23,26}

The main side reaction in the synthesis of Asp-containing peptides is aspartimide formation and this side reaction can occur under both acid and base conditions and remains one of the most formidable obstacles in peptide synthesis.\textsuperscript{22-26} The undesired aspartimide side reaction results in poor yields of target compounds and inaccessible peptide sequences.\textsuperscript{22-26} Costly and time-consuming purification steps are a frequent problem encountered as a result of the aspartimide side reaction.\textsuperscript{30,26} Acid-catalysed aspartimide formation has been reported to occur in solution or solid-phase peptide synthesis and is common in Boc/Bzl protection strategy during removal of the temporary protecting Boc group using acids such as TFA as well as during the final cleavage step of the peptide chain form the solid support in solid-phase peptide synthesis using strong acids such as hydrofluoric acid (HF) or trifluoromethanesulfonic acid (TFMSA).\textsuperscript{24,23,26} TFA-based cleavage of Boc side-chain protecting groups has been reported to cause formation of aspartimide products in synthesis of the cyclic peptide argifin.\textsuperscript{27} Base-catalyzed aspartimide side reaction has been reported in Fmoc/t-Bu protection schemes in solid phase peptide synthesis, particularly with the use of piperidine, which is the standard secondary amine applied in the cleavage of the Fmoc (9-fluorenylmethoxycarbonyl) group during the synthetic cycles in Fmoc/t-Bu solid phase peptide synthesis.\textsuperscript{25,23,28} The aspartimide side reaction involves nucleophilic attack of the amide nitrogen of the amino acid following the aspartyl residue in the peptide sequence to the $\beta$-protected carboxyl
moiety of the aspartyl residue (Figure 4.4). Given the need for orthogonal protection schemes in peptide synthesis, the β-functional group in the side chain of Asp is commonly protected as ester, thereby acting as leaving groups in the aspartimide side reaction. The aspartimide formation reaction is highly sequence dependent with the Asp-Gly sequence considered the most prone to this aspartimide side reaction due to Gly being the least sterically hindered amino acid thus facilitating the attack of the amide nitrogen to the β-protected carboxyl moiety of the aspartyl residue leading to formation of the cyclic imide ring of the aspartimide side product. The aspartimide side reaction is an intramolecular displacement reaction of the β-carboxyl ester protecting group of the aspartyl residue leading to the concomitant formation of amino-succinimide structures.

![Figure 4.4. Mechanism of aspartimide side reaction formation and the products from its reaction with nucleophiles.](image-url)
A major concern regarding formation of the aspartimide side reaction is the susceptibility of the two carbonyl carbons of the imide ring to attack by nucleophiles such as piperidine, water or hydroxide ion either in the synthesis or purification steps, leading to opening of the imide ring and formation of additional byproducts derived from this nucleophilic opening of the amino-succinyl moiety. The hydrolysis of the succinimide moiety through attack by nucleophiles (H₂O or OH⁻) to the β-carboxyl of Asp leads to formation of the α-aspartyl peptide having a natural backbone. However, the hydrolysis of the succinimide moiety through attack by nucleophiles (H₂O or OH⁻) to the α-carboxyl leads to formation β-isoaspartyl peptide with an unnatural backbone which is reported to be the main product. The aspartimide ring is prone to epimerization at the α-carbon ultimately leading to the formation of several aspartyl byproducts. Of the main problems associated with the aspartimide side reaction are the reduced yields of the target peptide and the difficulty in separation and purification of the byproducts.

Aqueous phosphoric acid (85 wt% H₃PO₄) has been reported as a mild reagent for the deprotection of the N-Boc group of N-Boc amino acids. The mildness of the reactions conditions when using aqueous phosphoric acid (85 wt%) as a deprotecting reagent has been reported to preserve the stereochemical integrity of the N-Boc amino acids. We elected to use aqueous phosphoric acid (85 wt% H₃PO₄) as a mild reagent for the deprotection of the N-Boc group of compound 6, thus avoiding the use strong acids in this step such as trifluoroacetic acid (TFA); phosphoric acid (pKₐ 2.15) is considered a much weaker acid than trifluoroacetic acid (pKₐ 0.3). To the best of our knowledge, this is the first use of aqueous phosphoric acid (85 wt% H₃PO₄) as a mild deprotecting reagent for the Boc group in an Asp-Gly peptide compound.
In a typical reported method using aqueous phosphoric acid as a deprotecting reagent for the Boc group, the reaction is executed by adding 85 wt % aqueous phosphoric acid to a solution of the substrate in a suitable organic solvent of good solubility for the substrate (THF, methylene chloride, acetonitrile or toluene). The reaction mixture is vigorously stirred at room temperature until reaction is complete (3-14h), effective mixing is a critical factor in driving the reaction to completion within reasonable time frames. The reaction is worked up by dilution with water, and sodium hydroxide solution is added to adjust the pH to 7-8. After extractive workup with a suitable organic solvent and removal of the solvent, the product is obtained in high purity without further purification. The reaction is to be conducted at high concentration, typically with 1mL of solvent per gram of substrate and 2.5-5 equivalents of 85 wt % aqueous phosphoric acid. The reaction slows down and proceeds sluggishly if larger amount of organic solvent is used (>5 mL/g substrate), and this was attributed to formation of a biphasic reaction mixture that slows down the reaction significantly. In case of milligram scale reactions, the substrate is dissolved in 0.5 mL of a suitable organic solvent, and 0.5 mL of aqueous 85 wt% phosphoric were used. We attempted to perform the Boc deprotection of compound 6 using the reported procedure; the dipeptide 6 (1.00 g, 1.4 mmol) was dissolved in 1mL CH2Cl2 and to this solution mixture was added aqueous phosphoric acid (85%, 0.23 mL, 3.3 mmol). The reaction was left to stir vigorously. After 30 min of stirring it was noticed that the reaction became a sticky gummy material and stirring stopped. After reaction workup by pH adjustment using 50% aq NaOH followed by DCM extractions, NMR analysis showed incomplete reaction and a mixture of the Boc protected starting material (compound 6) and the desired product (compound 7) were present. The incomplete reaction could be attributed to the formation of a large amount of gummy material (the phosphate salt of compound 7) after 30 min from the reaction start, the gummy material would sequester the starting
material (compound 6) and prevent it from reaction. Also, this gummy material has hampered the stirring of the reaction leading to complete stop of stirring, this prevented effective mixing which is a critical factor in driving the reaction to completion.

It was elected to repeat the reaction but with some modification by redesigning the reaction where a solution of the substrate compound 6 (1.0 g, 1.4 mmol) in DCM (1 mL) was added dropwise to a vigorously stirred aqueous 85 wt% phosphoric acid (0.23 mL, 3.3 mmol). We anticipated that this could prevent the formation of the gummy material but after 30 min stirring at room temperature a gummy material did form. The reaction was worked up by pH adjustment using 50% aq NaOH followed by DCM extraction and NMR analysis showed incomplete deprotection reaction.

The reaction was repeated with another modification. Compound 6 (0.5 g) was dissolved in 0.5 mL DCM and 0.12 mL aqueous 85 wt% phosphoric acid was added dropwise and the reaction was stirred vigorously at room temperature. After 30 min a sticky gummy material appeared, and the reaction stopped stirring, 0.38 mL aqueous 85 wt% phosphoric acid was then added to reaction mixture (at this point the total amount of aqueous 85 wt% phosphoric acid in the reaction mixture would be 0.5 mL, which is the amount used in the reported procedure for milligram scale reactions), and 2 mL DCM was added to the reaction mixture. This dilution step through addition of more amounts of the organic solvent resulted in breakage of the gummy material and allowed vigorous stirring of the reaction mixture. Even though, dilution of the reaction mixture through addition of more amounts of the organic solvent (>5 mL/g substrate) results in generation of a biphasic reaction mixture thus slowing down the reaction significantly, but this was compensated by vigorously stirring the reaction for longer times (4-5 h) at room temperature. After reaction workup by pH adjustment using 50% aq NaOH followed by DCM extractions, NMR analysis
shows complete reaction and formation of the desired product 7. Compound 7 was obtained in 82% yield with both 1H NMR, 13C NMR and HRMS confirming the structure of compound 7 and showing the benzyl protecting group of the asp residue to be intact and indicating no detectable aspartimide side product. The high yield obtained of compound 7 and its structure confirmation through NMR analysis indicates the success of using aqueous phosphoric acid (H₃PO₄, 85% wt) as a mild reagent for the deprotection of the Boc group of compound 6 with prevention or minimization of aspartimide side product formation. To the best of our knowledge, this is the first use of aqueous phosphoric acid (85% H₃PO₄) for the prevention of the aspartimide side reaction in an Asp-containing peptide.

The acylation reaction of compound 7 with dibenzyl-5-(chlorocarbonyl)isophthalate (compound 8) in anhydrous DCM and in the presence of TEA afforded the tetrabenzyl ester dipeptide compound 9.

The final reaction in Scheme 4.1 is the deprotection of the benzyl ester groups of compound 9 to afford the final compound 10. Although numerous methods are known for the removal of benzyl ester protecting groups, we elected to use the mild and efficient catalytic hydrogenation method. The direct basic hydrolysis of the benzyl esters of compound 9 was avoided in order to prevent any racemization. The catalytic hydrogenation reaction to deprotect the benzyl ester groups of compound 9 was complicated by the low solubility of 9 in most organic solvents. Hydrogenolysis of the benzyl groups of compound 9 was performed using 10% Pd/C in MeOH : DMA (v/v = 1:1) to afford the tetracarboxylic acid Gly-Asp dipeptide linker (compound 10). We elected to use a mixture solvent of MeOH and DMA in a 1:1 ratio to accomplish this reaction; compound 9 had low solubility in MeOH but was soluble in a 1:1 solvent mixture of MeOH/DMA. After the reaction filtration to remove the catalyst and workup, NMR analysis showed some DMA remained.
in the product, washing the product several times with DI H₂O helps in removing this residual DMA.

The synthesis of key intermediate compounds 5 and 8 that were employed in Scheme 4.1 were performed as follows:

The synthesis of key intermediate compound 5 was executed using a reported method as shown in Scheme 4.2. The amine group of the commercially available L-aspartic acid-4-benzyl ester (compound 11) was protected by introducing a Boc group following the reaction of L-aspartic acid-4-benzyl ester with di-tert-butyl dicarbonate in dioxane/water in the presence of triethylamine to yield the Boc protected compound N-Boc-L-aspartic acid-4-benzyl ester (compound 5).

Scheme 4.2. Conversion of L-aspartic acid-4-benzyl ester (11) to the N-Boc protected amino acid derivative (5).

The synthesis of key intermediate compound (8) was executed as shown in Scheme 4.3. Initially, trimesic acid (12) was converted to trimesic acid chloride (13) following a reported procedure; trimesic acid (12) was reacted with oxalyl chloride in anhydrous DCM with a few drops of DMF (as a catalyst) to afford trimesic acid chloride (13). The careful reaction of 2.0 equiv of benzyl alcohol (14) with trimesic acid chloride (13) in the presence of triethylamine followed by a mild basic hydrolysis produced the corresponding 1,3,5-benzenetricarboxylic acid dibenzyl ester (15).
Reaction of compound 15 with oxalyl chloride in anhydrous DCM in the presence of few drops of DMF as a catalyst afforded the acid chloride compound 8.

**Scheme 4.3. Design and Synthesis of dibenzyl-5-(chlorocarbonyl)isophthalate (8)**

4.3.2 **Metal Organic Framework synthesis and screening**

In order to synthesize the multivariate mixed-linker MOF MH-MTV-(Gly-Gly)(Asp-Gly) through the solvothermal reaction of Cu(NO$_3$)$_2$ · 2.5H$_2$O with a mixture of the Gly-Gly tetracarboxylate linker and the Asp-Gly tetracarboxylate linker (Scheme 4.4) we employed the same conditions that we previously used for the synthesis the single linker peptide MOF MH-2-Gly-Gly using the Gly-Gly tetracarboxylate linker (discussed in chapter 2) and we have performed several solvothermal experiments under these conditions were we varied the amount of acetic acid (a modulator) added.

Solvothermal reaction conditions 1: Gly-Gly tetracarboxylate linker (0.0195 g, 0.04 mmol, 80% of total linker mixture), Asp-Gly tetracarboxylate linker (0.0055 g, 0.01 mmol, 20% of total linker mixture) and Cu(NO$_3$)$_2$ · 2.5H$_2$O (0.0233 gm, 0.1 mmol) were dissolved in 1.7 mL of a solvent mixture of (DMF:DMA:EtOH:H$_2$O 5:5:1:1 v/v/v/v) in a 5 mL scintillation vial and 0.2 mL acetic acid (as a modulator) was added followed by addition of 0.1 mL pyridine. The vial was capped, and the clear solution was heated at 80 °C for 72 h in an oven.

Under solvothermal conditions 1, we used a mixture of ligands were the Gly-Gly tetracarboxylate linker composed 80% of the linker mixture while the Asp-Gly tetracarboxylate linker composed 20% of the mixture. These conditions yielded very low yield of square shaped crystals. It is
possible that the acidity of the medium prevented the deprotonation of the ligands and crystal growth, this extra acidity could possibly be due to the extra carboxylate group of the aspartic acid residue side chain in the Asp-Gly linker.

We decided to change the amount of acetic acid and monitor the effect on crystal growth, the following trials were performed with varying amounts of acetic acid:

Solvothermal conditions 2: Gly-Gly tetracarboxylate linker (0.0195 g, 0.04 mmol, 80 % of total linker mixture), Asp-Gly tetracarboxylate linker (0.0055 g, 0.01 mmol, 20 % of total linker mixture) and Cu(NO₃) ∙ 2.5H₂O (0.0233 gm, 0.1 mmol) were dissolved in 1.7 mL of a solvent mixture of (DMF:DMA:EtOH:H₂O 5:5:1:1 v/v/v/v) in a 5 mL scintillation vial and 0.1 mL acetic acid (as a modulator) was added followed by addition of 0.1 mL pyridine. The vial was capped, and the clear solution was heated at 80 °C in an oven.

Solvothermal reaction conditions 2 yielded crystals after 24h but the quality of the crystals was not good.

Solvothermal reaction conditions 3: Gly-Gly tetracarboxylate linker (0.0195 g, 0.04 mmol, 80 % of total linker mixture), Asp-Gly tetracarboxylate linker (0.0055 g, 0.01 mmol, 20 % of total linker mixture) and Cu(NO₃) ∙ 2.5H₂O (0.0233 gm, 0.1 mmol) were dissolved in 1.7 mL of a solvent mixture of (DMF:DMA:EtOH:H₂O 5:5:1:1 v/v/v/v) in a 5 mL scintillation vial and 0.15 mL acetic acid (as a modulator) was added followed by addition of 0.1 mL pyridine. The vial was capped, and the clear solution was heated at 80 °C for 48 h in an oven.

Solvothermal reaction conditions 4: Gly-Gly tetracarboxylate linker (0.0195 g, 0.04 mmol, 80 % of total linker mixture), Asp-Gly tetracarboxylate linker (0.0055 g, 0.01 mmol, 20 % of total linker mixture) and Cu(NO₃) ∙ 2.5H₂O (0.0233 gm, 0.1 mmol) were dissolved in 1.7 mL of a solvent
mixture of (DMF:DMA:EtOH:H\textsubscript{2}O 5:5:1:1 v/v/v/v) in a 5 mL scintillation vial and 0.17 mL acetic acid (as a modulator) was added followed by addition of 0.1 mL pyridine. The vial was capped, and the clear solution was heated at 80 °C for 48 h in an oven.

Solvothermal reactions conditions 3 and 4 both yielded square shaped block crystals with solvothermal reaction condition 4 yielding better quality crystals. Figure 4.5 shows the powder X-ray diffraction (PXRD) patterns of the as-synthesized crystal sample of the multivariate MOF MH-MTV-(Gly-Gly)\textsubscript{0.8}(Asp-Gly)\textsubscript{0.2} obtained under solvothermal reaction conditions 4 closely matched the patterns of the as-synthesized peptide MOF MH-2-Gly-Gly which was constructed using the Gly-Gly tetracarboxylate single linker and displayed an ssb/stx type network (Chapter 2). This indicates that MH-MTV-(Gly-Gly)\textsubscript{0.8}(Asp-Gly)\textsubscript{0.2} is isostructural with MH-2-Gly-Gly.

![PXRD patterns](image)

**Figure 4.5.** Experimental PXRD patterns of MH-2-Gly-Gly and MH-MTV-(Gly-Gly)\textsubscript{0.8}(Asp-Gly)\textsubscript{0.2}. Patterns provided here are for as-synthesized samples, thus the hump over a wide range of two-theta is due to the inclusion of large amount of disordered solvent molecules.
To determine the inclusion of the Gly-Gly tetracarboxylate linker and the Asp-Gly tetracarboxylate linker in MH-MTV-(Gly-Gly)_{0.8}(As-Gly)_{0.2} we have performed $^1$H-NMR of digested sample crystals of MH-MTV-(Gly-Gly)_{0.8}(Asp-Gly)_{0.2} obtained under solvothermal reaction conditions in $d_6$-DMSO / DCl (20 wt% in D$_2$O) mixture (Figure 4.6). The resonance peaks at 2.84-2.81 ppm (m, 1H) and at 2.74-2.68 ppm (m, 1H) correspond to the Asp-β hydrogens of the Asp-Gly tetracarboxylate linker. The resonance peak at 4.74 ppm (1H) corresponds to the Asp-α hydrogen of the Asp-Gly linker. These resonance peaks are assigned to protons corresponding to the Asp-Gly tetracarboxylate linker indicating the presence of this linker in the framework. Their integration values are based on the Asp-Gly linker.

The resonance peak at 3.88 ppm and at 3.91 ppm correspond to Gly-α protons of the two Gly residues of the Gly-Gly tetracarboxylate linker and are overlapped with the resonance peak corresponding to the Gly-α hydrogens of the Gly residue of the Asp-Gly linker. Resonance peaks at 8.07 ppm, 8.40 ppm, 8.50 ppm and 8.59 ppm correspond to aromatic protons of both the Gly-Gly and the Asp-Gly linkers.
**Figure 4.6.** $^1$H-NMR spectrum of digested MH-MTV-(Gly-Gly)$_{0.8}$(Asp-Gly)$_{0.2}$ in d$_6$-DMSO / DCl (20 wt% in D$_2$O) mixture. Inset, chemical structure of Asp-Gly tetracarboxylate and Gly-Gly tetracarboxylate linkers.

**Fourier-transform infrared spectroscopy (FTIR) Characterization.**

Fourier-transform infrared spectroscopy (FTIR) is particularly a useful technique for the characterization of compounds comprising functional groups such as –COOH. We have performed AT-FTIR analysis of our multivariate MOF MH-MTV-(Gly-Gly)$_{0.8}$(Asp-Gly)$_{0.2}$ in order to assess the presence of free uncoordinated –COOH groups in the MOF. We have also performed AT-FTIR analysis of the Gly-Gly tetracarboxylate linker, the Asp-Gly tetracarboxylate linker and our peptide MOF MH-2-Gly-Gly (constructed using the Gly-Gly tetracarboxylate single linker) for comparison and assessment.
The IR spectrum of the Asp-Gly linker (Figure 4.7) displays a band at 1708 cm\(^{-1}\) which corresponds to the stretching vibration of the C=O group of the side chain carboxylic acid group of the Asp residue.\(^{42}\) The lower absorption frequency compared to that of a free carboxylic acid of a monomer suggest that this carboxylic acid group participates in hydrogen bonding with other carboxylic acid groups in the ligand resulting in the formation of carboxylic acid dimers.\(^{39}\) The band at 1673 cm\(^{-1}\) corresponds to the stretching vibration of the C=O group of the COOH groups attached to the aromatic ring; they are at a lower frequency due to conjugation with the phenyl ring as well as due to formation of carboxylic acid dimers.\(^{16,39}\) The broad band 2519 cm\(^{-1}\) originates from the strong hydrogen bonds between facing carboxylates groups which confirms the formation of --COOH dimers.\(^{16,39}\) The band at 1639 cm\(^{-1}\) corresponds to the amide I band which is the carbonyl absorption band resulting from the C=O stretching vibration of the amide bonds.\(^{43}\) The band at 1596 cm\(^{-1}\) are due to the C=C aromatic ring stretch.\(^{39}\) The band at 1541 cm\(^{-1}\) corresponds to the amide II band which results from the coupling of the N-H bending and C-N stretching vibrations.\(^{43}\)

![Figure 4.7. AT-FTIR spectrum of the Asp-Gly tetracarboxylate linker.](image)
The IR spectrum of the Gly-Gly tetracarboxylae linker (Figure 4.8) shows a band at 1690 cm\(^{-1}\) corresponding to the stretching vibration of the C=O group of the COOH groups attached to the aromatic ring, they are at a lower frequency due to conjugation with the aromatic ring as well as due to formation of carboxylic acid dimers.\(^{16,39}\) The band at 1640 cm\(^{-1}\) corresponds to the amide I band which is the carbonyl absorption band resulting from the C=O stretching vibration of the amide bonds.\(^{43}\) The band at 1607 cm\(^{-1}\) corresponds to the C=C aromatic ring stretch. The band displayed at 1547 cm\(^{-1}\) corresponds to the amide II band resulting from the coupling of the N-H bending and C-N stretching vibrations.\(^{43}\)

![Figure 4.8. AT-FTIR spectrum of the Gly-Gly tetracarboxylate linker.](image)

In the IR spectrum of the multivariate MOF MH-MTV-(Gly-Gly)\(_{0.8}\)(Asp-Gly)\(_{0.2}\) (Figure 4.9) it is noticed the disappearance of the sharp peaks at 1690 cm\(^{-1}\) for the Gly-Gly linker and at 1672 cm\(^{-1}\) for the Asp-Gly linker that were assigned to the stretching vibration of the C=O group of the COOH groups attached to the phenyl rings of the linkers, these peaks disappear after the
coordination of four carboxylate groups of the linker with copper and is accompanied by the appearance of two peaks at 1627 cm\(^{-1}\) and 1367 cm\(^{-1}\) in the spectrum of the multivariate MOF which are assignable to the asymmetric and symmetric carboxylate (-CO\(_2\)) vibrations following coordination of four carboxylic groups of the linker with the copper.\textsuperscript{14,40} It is notable that a peak is observed at 1721 cm\(^{-1}\) which can be ascribed to the C=O stretching vibration of the side chain –COOH group of the aspartic acid residue which suggests that these –COOH groups are uncoordinated and is free inside the pores of the multivariate MOF MH-MTV-(Gly-Gly)\(_{0.8}\)(Asp-Gly)\(_{0.2}\).\textsuperscript{42,41}

![AT-FTIR spectrum of the multivariate MOF MH-MTV-(Gly-Gly)\(_{0.8}\)(Asp-Gly)\(_{0.2}\).](image)

**Figure 4.9.** AT-FTIR spectrum of the multivariate MOF MH-MTV-(Gly-Gly)\(_{0.8}\)(Asp-Gly)\(_{0.2}\).

Figure 4.10 shows a comparison between the AT-FTIR spectrum of the peptide MOF MH-2-Gly-Gly which is constructed using the Gly-Gly tetracarboxylate as a single linker (chapter 2) and the AT-FTIR multivariate MOF MH-MTV-(Gly-Gly)\(_{0.8}\)(Asp-Gly)\(_{0.2}\). It could be noticed the resemblance between the two spectra except for the appearance of a peak at 1721 cm\(^{-1}\) in the
spectrum of the multivariate MOF MH-MTV-(Gly-Gly)_{0.8}(Asp-Gly)_{0.2} which is not present in the spectrum of MH-2-Gly-Gly and could be attributed to presence free uncoordinated carboxylic acid groups of the side chain of the aspartic acid residue in the pores of the multivariate MOF MH-MTV-(Gly-Gly)_{0.8}(Asp-Gly)_{0.2}.

**Figure 4.10.** Overlay of the AT-FTIR spectra of MH-2-Gly-Gly and MH-MTV-(Gly-Gly)_{0.8}(Asp-Gly)_{0.2}.
4.4. Conclusion

We have successfully accomplished the design and synthesis of a novel Asp-Gly dipeptide tetracarboxylate linker. We have investigated various solvothermal conditions for the synthesis of a novel homochiral multivariate metal-peptide framework MH-MTV-(Gly-Gly)$_{0.8}$(Asp-Gly)$_{0.2}$ were we used a mixture of the un-functionalized tetracarboxylate Gly-Gly dipeptide linker and the functionalized chiral Asp-Gly tetracarboxylate dipeptide linker, and copper (II) nitrate under various solvothermal reaction conditions. We have identified solvothermal synthesis reaction conditions for the synthesis of the multivariate metal-peptide framework MH-MTV-(Gly-Gly)$_{0.8}$(Asp-Gly)$_{0.2}$, and PXRD experiments showed that the crystalline product was isostructural with our copper paddlewheel-based metal-peptide framework MH-2-Gly-Gly which displayed ssb/stx type network (Synthesis and characterization of MH-2-Gly-Gly is described in Chapter 2). Fourier-transform infrared spectroscopy (FTIR) analysis showed the presence of free carboxylate groups in the pores of MH-MTV-(Gly-Gly)$_{0.8}$(Asp-Gly)$_{0.2}$. We hope that this work would open opportunities for the use homochiral metal-peptide frameworks with free carboxylate groups in various fields, for example, chiral separations, proton conductivity and catalysis.
4.5. Experimental procedures.

4.5.1. General procedures. Chemicals and solvents were obtained from commercial sources and were used without further purification unless otherwise specified. All organic reactions were carried out in oven dried glassware with dry solvents under an atmosphere of dry nitrogen unless otherwise specified. Analytical TLC was performed on Merck 60 F254 silica gel plates with a fluorescent indicator with a 254 nm excitation wavelength. Compounds were visualized under UV light at 254 nm wavelength. Column chromatography was carried out on silica gel 60 (particle size 240-400 mesh). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Compound lyophilization was performed using a Labconco freezone 4.5 liter -84 °C benchtop freeze drier apparatus. NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer ($^1$H 400 MHz, $^{13}$C 100 MHz) at 25 °C. $^1$H NMR chemical shift values were determined relative to residual protonated solvent signals as internal standard ((CD$_3$)(CD$_2$H)SO in (CD$_3$)$_2$SO, δ 2.50 ppm). The chemical shifts for $^1$H NMR are expressed in ppm, followed by the multiplicity (br, broad; s, singlet; d, doublet; t, triplet; q, quadruplet, qt, quintet; m, multiplet), coupling constants (J, in Hertz, Hz), and integration. $^{13}$C NMR spectra were referenced to the solvent signal (δ 39.52 ppm for (CD$_3$)$_2$SO and δ 49.00 ppm for CD$_3$OD). All $^{13}$C NMR spectra were recorded with complete proton decoupling. NMR signals of spectra were assigned using gradient COSY (correlation spectroscopy), HSQC (heteronuclear single quantum coherence) and HMBC (heteronuclear multiple bond correlation). High-resolution mass spectra were acquired on an Agilent 6560 IM-QTOF mass spectrometer.

4.5.2. Solution $^1$H NMR of digested MOF samples.

Typically, for the digestion of the MOFs, the as-synthesized MOF sample was solvent exchanged with DMF twice daily for 2-3 days followed by acetone exchange twice daily for 2-3 days. The
sample was then left to dry in air for 12 h. DMSO-d$_6$ (0.5 mL) and DCl (20 μL, 20 wt. % in D$_2$O), were added to a 5 mL vial containing the dried MOF (~10 mg), and the resulting suspension was left to rest at room temperature for 5-10 min until clear solution was obtained. The $^1$H NMR spectra were recorded immediately after (~10 min). The $^1$H NMR spectra were recorded immediately after (~10 min). $^1$H NMR spectra on digested solutions of MOFs were acquired on a Bruker Avance 400 MHz spectrometer, with chemical shifts of linkers identified by comparing with spectra of each pure linker.

4.5.3. Powder X-ray diffraction (PXRD)

Powder patterns were recorded on a Bruker AXS D8 Advance Phaser diffractometer with Cu Kα-1 radiation (λ = 1.5406 Å, operating at 30 kV and 10 mA) over a range of 5° < 2θ < 30°, with a step size of 0.02° steps and a 1.0 s counting time per step. The supernatant of fresh as-synthesized was exchanged with DMF, and the samples were filtered through a 0.45 µm nylon membrane filter. Collected sample was spread on a Si-Einkristalle plate immediately before PXRD measurements.

4.5.4. Fourier-transform infrared spectroscopy (FTIR)

IR spectra were recorded on a Nicolet iS5 FTIR Spectrometer with an ATR set (attenuated total reflection). Prior to analysis, the supernatant of fresh as-synthesized MOF crystal samples was exchanged with fresh DMF and the process was repeated three to two times per day for 3 days to obtain a MOF sample with washed interior which was filtered through a 0.45 μm nylon membrane filter.
4.5.5. Experimental section for synthesis of Organic compounds and characterization data

2-(Tert-butoxyarbonyl)amino)acetamindo-isopthalic acid dibenzylester (3) (new compound). Boc-Gly-OH (2) (1.33 g, 7.6 mmol, 1.1 eq) was charged into a 100 mL flask, 15 mL of anhydrous DMF was added followed by DIEA (2.6 mL, 15.2 mmol, 2 eq) and the reaction mixture was left to stir under nitrogen atmosphere for 10 min. To this reaction mixture was then added HBTU (2-(1H-Benzotriazlo-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) (3.45 gm, 9.1 mmol, 1.2 eq) and left to stir at room temp for 15 min. A solution of the commercially available dibenzyl-aminoisophthalate (compound 1) (2.49 gm, 6.9 mmol) in 15 mL anhydrous DMF was added to the reaction mixture dropwise via a syringe, and the reaction mixture was left to stir under nitrogen atmosphere for 20 h at room temperature. On completion, the reaction mixture was then concentrated under reduced pressure (not all the DMF was evaporated, about 5 mL was left) and the residue was diluted with 400 mL EtOAc, the organic layers were washed with DI H₂O (200 mL × 3) and the H₂O extracts were discarded. The organic layer was then washed with sat NaHCO₃ (2×150 mL) followed by a wash with 10% citric acid (1×100 mL) then a wash with DI H₂O (1×100 mL) followed by a wash with brine (1×100 mL). The organic layer was dried over Na₂SO₄. The organic solvent then was evaporated under reduced pressure and a sticky oily residue was obtained which was co-evaporated under reduced pressure several times with an EtOAc/hexanes mixture (1:1) till a solid residue appeared. The solid was purified by recrystallization from hot EtOAc/hexanes, the hot EtOAc/hexanes solution was left to cool to room temperature, then placed in the fridge overnight leading to formation yellowish brown crystals. The crystals were filtered, and the mother liquor filtrate was concentrated and placed in the fridge to get a second crop of crystals. The crystals were combined and washed with cold EtOAc/hexanes. The crystals were then dissolved in hot MeOH and left to cool in the fridge
overnight; a white solid precipitated from the clear MeOH solution. This precipitation process from hot MeOH was repeated three times and purity was monitored by TLC (EtOAc/Hexanes 1:1). Recovered 1.65 g (46%) of compound 3 as a white solid.

\[ \text{3} \]

$^1$H NMR (400 MHz, DMSO) $\delta$ 10.42 (s, Ar-NH (d), 1H), 8.52 (d, $J = 1.4$ Hz, Ar-H (e), 2H), 8.21 (t, $J = 1.3$ Hz, Ar-H (f), 1H), 7.49 – 7.36 (m, Bn $\times$ 2, 10H), 7.11 (t, $J = 6.0$ Hz, Glyc-NH (b), 1H), 5.39 (s, PhCH$_2$O $\times$ 2 (g), 4H), 3.74 (d, $J = 6.1$ Hz, Gly-$\alpha$ (c), 2H), 1.40 (s, Boc (a), 9H).

$^{13}$C ($^1$H) NMR (101 MHz, DMSO) $\delta$ 168.94 (1C, C11), 164.61 (2C, C6), 155.88 (1C, C13), 139.93 (1C, C10), 135.79 (2C, C4), 130.73 (2C, C8), 128.54 (4C, C3 or C2), 128.23 (2C, C1), 128.09 (4C, C3 or C2), 124.10 (1C, C7), 123.66 (2C, C9), 78.11 (1C, C14), 66.67 (2C, C5), 43.85 (1C, C12), 28.17 (3C, C15).

**HRMS (ESI)** Exact mass calculated for C$_{29}$H$_{30}$N$_2$NaO$_7$ [M + Na]$^+$, 541.1951. Found 541.1951.
2-Aminoacetamindo isophthalic acid dibenzylester·TFA (4) (new compound). Compound 3 (1.65 g, 3.2 mmol) was charged into a 100 mL round bottomed flask and 15 mL anhydrous DCM was added. The reaction mixture was cooled to 0 °C and 15.4 mL TFA in 10 mL anhydrous DCM was added dropwise via an addition funnel, the reaction mixture was stirred at 0 °C for 30 min then warmed to room temperature and left to stir for 2 h under nitrogen atmosphere. The solvents were then removed under reduced pressure, and the residue was co-evaporated with EtOAc under reduced pressure and this co-evaporation process was repeated eight times (80 mL × 8) in order to remove any residual TFA left. A final co-evaporation with Et₂O (50 mL × 2) was performed to obtain a white solid. The solid was dried under high vacuum for 1-2 h then washed (stirred for 4-5 h) with Et₂O and left soaking in Et₂O overnight. The solid was filtered and dried under high to afford compound 4 as a white solid (1.43 g, 84%).

\[
\text{H NMR (400 MHz, DMSO) } \delta 10.96 (s, 1H, Ar-NH (c)), 8.50 (d, J = 1.3 Hz, 2H, Ar-H (d)), 8.27 (s, 1H, Ar-H (e)), 8.20 (s, 2H, NH₂ (a)), 7.49 – 7.34 (m, 10H, Bn × 2), 5.39 (s, 4H, PhCH₂O × 2 (f)), 3.82 (s, 2H, Gly-α (b))
\]
$^{13}$C\{$^1$H\} NMR (101 MHz, DMSO) δ 165.53 (1C, C11), 164.43 (2C, C6), 157.85 (TFA), 139.16 (1C, C10), 135.72 (2C, C4), 130.99 (2C, C8), 128.55 (4C, C3 or C2), 128.28 (2C, C1), 128.15 (4C, C3 or C2), 124.72 (1C, C7), 123.71 (2C, C9), 66.80 (2C, C5), 41.10 (1C, C12).

N-Boc-L-Aspartic acid-4-benzyl ester (5)\textsuperscript{36}. The commercially available L-Aspartic acid-4-benzyl ester (compound 11) (1.0 g, 4.5 mmol) was charged into a 100 mL round bottomed flask and 10 mL of H$_2$O/Dioxane (1:1) was added followed by Et$_3$N (0.75 mL, 5.3 mmol) then DI H$_2$O (5 mL) was added. The reaction mixture was cooled to 0 °C and a solution of di-tert-butyl decarbonate (Boc$_2$O, 1.08 g, 5.0 mmol) in dioxane (5 mL) was added dropwise using an addition funnel. The reaction mixture was left to stir at 0 °C for 20 min then warmed to room temperature and left to stir for 16 h. The dioxane solvent was then evaporated under reduced pressure, and the aqueous layer residue was cooled to 0 °C and acidified to pH ~ 1 using 1.0 M HCl. The aqueous layer was then extracted with EtAOc (50 mL × 3), the combined organic layers were washed with brine and dried over NaSO$_4$. The solvents were removed under reduced pressure and the residue was dried under high vacuum to afford compound 5 (1.33 g, 92%) as an off white solid.

![N-Boc-L-Aspartic acid-4-benzyl ester (5)](image)

$^1$H NMR (400 MHz, DMSO) δ 12.78 (s, 1H, -COOH), 7.37-7.32 (m, 5H, Bn), 7.19 (d, $J = 8.5$ Hz, 1H, -NH), 5.11 (s, 2H, PhCH$_2$O), 4.37-4.32 (m, 1H, Asp-α), 2.84 (dd, $J = 16.1$, 5.6 Hz, 1H, Asp-β), 2.69 (dd, $J = 16.1$, 8.1 Hz, 1H, Asp-β), 1.38 (s, 9H, Boc-H).
Boc-Asp(OBn)-Gly-O(dibenzy1-5-aminoisophthalate) (6) (new compound). Compound 5 (Boc-Asp(OBzl)-OH) (1.58 g, 4.9 mmol, 1 eq) was charged into a two neck 250 mL flask, 34 mL anhydrous DCM was added and the mixture was cooled to 0 °C. To the reaction mixture was added a solution mixture of compound 4 (2.60 g, 4.9 mmol, 1 eq) and DIEA (0.94 mL, 5.4 mmol, 1.1 eq) in 34 mL anhydrous DCM. Hydroxybenzotriazole hydrate (HOBt hydrate) (0.90 g, 5.9 mmol, 1.2 eq) was added, and the resulting mixture was stirred at 0 °C for 10 min. To this reaction mixture was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC hydrochloride, 1.1242 g, 5.8646 mmol, 1.2 eq) and was stirred at 0 °C for 15 min, then warmed to room temperature and left to stir for 16 h under nitrogen atmosphere. On completion, the reaction mixture was diluted with EtOAc (200 mL) and the organic layer was washed with sat NaHCO₃ (3×140 mL), washed with 10% citric acid (1×100 mL), washed with DI H₂O (1×100 mL), washed with brine (1×100 mL) and dried over Na₂SO₄. The organic solvent was then removed under reduced pressure to afford a sticky residue. The residue was dissolved in a hot EtOAc/hexanes mixture and left to cool to room temperature then left overnight in the fridge. A white solid precipitated out, the solid was filtered and purified by column chromatography (SiO₂, 50 % EtOAc/hexanes then 70% EtOAc/hexanes). The product fractions were collected, and solvents
removed under reduced pressure. The product was further purified by precipitation from hot EtOAc/hexanes mixture to afford the product (compound 6) as a white solid (2.3689 g, 67%).

![Chemical Structure](image)

$^1$H NMR (400 MHz, DMSO) $\delta$ 10.66 (s, 1H, Ar-NH (f)), 8.48 (d, $J = 1.3$ Hz, 2H, Ar-H (g)), 8.24 (s, 1H, Ar-H (h)), 7.59 (d, $J = 8.2$ Hz, 1H, Asp-NH (b)), 7.49 – 7.21 (m, 15H, Bn × 3), 5.39 (s, 4H, PhCH$_2$O × 2), 5.16 (t, $J = 5.7$ Hz, 1H, Gly-NH (d)), 4.54-4.49 (m, 3H, Gly-$\alpha$ (e)) and Asp-$\alpha$ (c overlapped), 4.32-4.21 (m, 2H, PhCH$_2$O (Asp)), 3.10 (dd, $J = 17.6$, 9.3 Hz, 1H, Asp-$\beta$ (m)), 2.63 (dd, $J = 17.7$, 5.6 Hz, 1H, Asp-$\beta$ (m)), 1.39 (s, 9H, Boc-H (a)).

![Chemical Structure](image)

$^{13}$C$^{1}$H NMR (101 MHz, DMSO) $\delta$ 171.22 (1C, C19), 170.22 (1C, C13), 168.17 (1C, C11), 164.56 (2C, C6), 155.35 (1C, C15), 139.69 (1C, C10), 136.01 (1C, C21), 135.78 (2C, C4 ), 130.76 (2C, C8), 128.53 (4C, C3 or C2), 128.32 (2C, C22 or C23), 128.22 (2C, C1), 128.09 (4C, C3 or C2), 127.89 (1C, C24), 127.74 (2C, C22 or C23), 124.29 (1C, C7), 123.72 (2C, C9), 78.54 (1C, C16), 66.67 (2C, C5), 65.63 (1C, C14), 50.96 (1C, C12), 42.94 (1C, C20), 36.20 (1C, C18), 28.10 (3C, C17).
HRMS (ESI) Exact mass calculated for C$_{40}$H$_{41}$N$_3$NaO$_{10}$ [M + Na]$^+$, 746.2690. Found 746.2690.

**Boc deprotection of compound 6: Synthesis of H-Asp(OBn)-Gly-O(dibenzyl-5-aminoisophthalate) (7) (new compound).** Following a reported procedure for the deprotection of the Boc using 85 wt % aqueous phosphoric acid (85 wt% H$_3$PO$_4$), the N-Boc dipeptide 6 (1.00 g, 1.4 mmol, 1eq) was charged in a 10 mL round-bottomed flask and 1mL DCM was added. To this solution mixture was then added 0.23 mL of 85 wt % aqueous phosphoric acid at room temperature, and the reaction was left to stir vigorously. After 30 min of stirring it was noticed that the reaction became a sticky gummy material that could not be stirred further thus hampering the completion of the reaction. DI H$_2$O (5 mL) was added and the reaction mixture was cooled to 0 °C. A 50 wt % solution of NaOH was added dropwise slowly to adjust the pH to 8. The mixture was then extracted with DCM (3×60 mL) and the combined organic phases were dried under Na$_2$SO$_4$. The organic solvent was evaporated under reduced pressure to get a white solid (0.86 g). NMR analysis showed incomplete deprotection, a mixture of the N-Boc protected starting material (compound 6) and the desired product (compound 7) are present.

It was elected to repeat the reaction but with some modifications that included a reverse in the order of addition of the substrate and 85 wt% phosphoric acid. A solution of the N-Boc dipeptide compound 6 (1.0 gm in 1 mL DCM) was added dropwise to a vigorously stirred aqueous 85 wt% phosphoric acid (0.23 mL). We anticipated that this could prevent the formation of the gummy material but after 30 min stirring at room temperature a gummy material did form which prevented further stirring of the reaction. The reaction was worked up by pH adjustment followed by DCM extraction, NMR analysis showed incomplete deprotection of N-Boc group of compound 6.
The reaction was repeated with another modification. Compound 6 (0.5 g, 0.69 mmol, 1 eq) was dissolved in 0.5 mL DCM and 0.12 mL aqueous 85 wt% phosphoric acid was added dropwise and the reaction was left to stir vigorously at room temperature. After 30 min a sticky gummy material appeared, and the reaction stopped stirring. 0.38 mL aqueous 85 wt% phosphoric acid were added to reaction mixture (at this point the total amount of aqueous 85 wt% phosphoric acid in the reaction mixture would be 0.5 mL, which is the amount used in the reported procedure for milligram scale reactions\textsuperscript{32,33}), and an additional 2 mL DCM was added to the reaction mixture, the reaction mixture became stirrable and was stirred vigorously at room temperature for 3-4 hours. On completion, 5 mL of DI H\textsubscript{2}O was added, and the reaction mixture was cooled to 0 °C. A 50 wt % solution of NaOH was then added dropwise slowly to adjust the pH to 8. The mixture was then extracted with DCM (3×60 mL) and the combined organic phases were dried under Na\textsubscript{2}SO\textsubscript{4}. The organic solvent was evaporated under reduced pressure to afford the desired product (compound 7) as an off white solid (0.352 g, 82%). NMR analysis showed the reaction to be complete.

\textbf{1}H NMR (400 MHz, DMSO) δ 10.75 (s, 1H, Ar-NH (e)), 8.48 (d, J = 1.5 Hz, 2H, Ar-H (f)), 8.24 (t, J = 1.5 Hz, 1H, Ar-H (g)), 7.49 – 7.21 (m, 15H, Bn × 3), 5.38 (s, 4H, PhCH\textsubscript{2}O × 2), 5.19 (t, J = 5.2 Hz, 1H, Gly-NH (c)), 4.50 (d, J = 4.7 Hz, 2H, Gly-α (d)), 4.24 (s, 2H, PhCH\textsubscript{2}O (Asp)), 3.85 (dd, J = 8.7, 5.2 Hz, 1H, Asp-α (b)), 3.04 (dd, J = 17.8, 8.7 Hz, 1H, Asp-β (l)), 2.43 (dd, J = 17.8, 5.2 Hz, 1H, Asp-β (l)), 2.14 (s, 2H, NH\textsubscript{2} (a)).
\[^{13}\text{C}\{^{1}\text{H}}\] NMR (101 MHz, DMSO) δ 179.73 (1C, C16), 175.12 (1C, C13), 165.06 (1C, C11), 164.51 (2C, C6), 142.49 (1C, C10), 139.51 (1C, C18), 135.76 (2C, C4), 130.82 (2C, C8), 128.55 (4C, C3 or C2), 128.26 (2C, C19 or C20), 128.15 (4C, C3 or C2), 127.98 (2C, C1), 126.56 (1C, C21), 126.37 (2C, C19 or C20), 124.49 (1C, C7), 123.62 (2C, C9), 66.72 (2C, C5), 62.87 (1C, C14), 50.55 (1C, C12), 40.99 (1C, C17), 37.59 (1C, C15).

**HRMS (ESI)** Exact mass calculated for C\(_{35}\)H\(_{33}\)N\(_3\)NaO\(_8\) [M + Na]\(^+\), 646.2165. Found 646.2165.

N-Dibenzy1-5-carbonylisophthalate-Asp(OBn)-Gly-O(dibenzy1-5-aminoisophthalate)  (9)  
(new compound). Compound 7 (0.59 g, 0.94 mmol, 1 eq) was charged into a 100 mL round bottom flask and 30 mL dry DCM was added. Triethyl amine (0.16 mL, 1.1 mmol) was then added, and the reaction mixture was cooled to 0 °C. A solution of compound 8 (1.0 mmol, 1.1 eq) in 5 mL dry DCM was then added to the reaction mixture dropwise via a syringe, the reaction was left to stir at 0 °C for 20 min, then stirred for 16 h at room temperature under nitrogen atmosphere. On completion, the solvents were removed under reduced pressure and 40-50 mL saturated aq NaHCO\(_3\) was added to the residue followed by 100 mL DCM. The mixture was then transferred to a separating funnel, and the basic aqueous layer was discarded, the organic layer was washed with another 50 mL saturated aq NaHCO\(_3\), washed with 10 % citric acid (1×50 ml), and washed
with DI H₂O, and the organic layer was dried over Na₂SO₄. The solvents were removed under reduced pressure, and the sticky residue was co-evaporated with EtOAc/hexanes and dried under high vacuum. The solid was titurated with EtOAc and filtered. The solid was then purified by precipitation form a hot clear EtOAc solution to afford the product 9 as a white solid (0.46 g, 49%).

![Chemical Structure](image)

^1H NMR (400 MHz, DMSO) δ 10.30 (s, 1H, Ar-NH (k)), 9.40 (d, J = 7.3 Hz, 1H, Asp-NH (g)), 8.72 (s, 2H, Ar-H (f)), 8.64 (s, 1H, Ar-H (e)), 8.56 (s, 2H, Ar-H (l)), 8.51 (t, J = 5.5 Hz, 1H, Gly-NH (i)), 8.21 (s, 1H, Ar-H (m)), 7.48 – 7.23 (m, 25H, Bn × 5), 5.42 (s, 4H, PhCH₂O × 2 (d)), 5.37 (s, 4H, PhCH₂O × 2 (n)), 5.13 – 5.06 (m, 2H, PhCH₂O (Asp)), 4.94 (dd, J = 13.6, 7.5 Hz, 1H, Asp-α (h)), 3.90 (d, J = 5.7 Hz, 2H, Gly-α (j)), 3.04 (dd, J = 16.8, 5.2 Hz, 1H, Asp-β (r)), 2.89 (dd, J = 16.3, 9.2 Hz, 1H, Asp-β (r)).

![Chemical Structure](image)
\(^{13}\)C\(^{1}\)H NMR (101 MHz, DMSO) \(\delta\) 175.43 (1C, C27), 174.40 (1C, C13), 164.89 (1C, C11), 164.56 (1C, C15), 164.50 (2C, C20), 164.22 (2C, C6), 139.44 (1C, C10), 135.75 (2C, C22), 135.68 (3C, C4 and C29 overlapped), 134.30 (1C, C16), 132.48 (1C, C19), 132.27 (2C, C17), 130.85 (2C, C18), 130.75 (2C, C8), 128.53 (4C, C23 or C24), 128.37 (2C, C25), 128.25 (4C, C23 or C24), 128.23 (4C, C3 or C2), 128.11 (4C, C3 or C2), 127.97 (2C, C1), 127.80 (2C, C30 or C31), 126.56 (1C, C32), 126.37 (2C, C30 or C31), 124.60 (1C, C7), 123.68 (2C, C9), 66.90 (2C, C21), 66.71 (2C, C5), 62.89 (1C, C14), 49.03 (1C, C12), 41.32 (1C, C28), 34.74 (1C, C26).

N-5-Carboxylisophthalic acid-Asp(OH)-Gly-O(5-aminoisophthalic acid) (10)(new compound). To a stirred solution of compound 9 (0.059 g, 0.06 mmol, 1 eq) in 4 mL anhydrous MeOH : DMA (1:1) solvent mixture was added 10% palladium on carbon (0.006 g, 10% by wt of substrate) at room temperature under nitrogen atmosphere. The nitrogen was exchanged with a H\(_2\) atmosphere (1 atm H\(_2\)) and the reaction was stirred for 8 h. The reaction mixture was filtered through Celite, and the Celite layer filter cake was washed with few a mL of MeOH. The filtrate was concentrated under reduced pressure, and the residue was diluted with EtOAc and transferred to a separating funnel where the organic layer was extracted with saturated sodium bicarbonate solution, the aqueous layer was separated and cooled to 0 °C followed by acidification to pH = 2 using 1 N HCl, and was left to stand till a gel-like precipitate appeared that was filtered through a Buchner funnel with fine fritted disc. The precipitate was washed several times with DI H\(_2\)O, collected and lyophilized to afford compound 10 as a white solid (0.0078 g, 24%).

In an alternate workup, after filtration of the reaction mixture through Celite and concentration of the filtrate under pressure, water was added to induce precipitation of the product. Filtration followed by lyophilization affords compound 10 as a white solid.
\(^1\)H NMR (400 MHz, DMSO) \(\delta\) 13.27 (b, 5H, -COOH), 10.29 (s, 1H, Ar-NH (h)), 9.17 (d, \(J = 7.6\) Hz, 1H, Asp-NH (d)), 8.64 (s, 2H, Ar-H (c)), 8.59 (s, 1H, Ar-H (b)), 8.42 – 8.39 (m, 3H, Ar-H (2H, (i)) overlapped Gly-NH (1H. (f))), 8.16 (s, 1H, Ar-H (j)), 4.83 (dd, \(J = 13.6, 7.5\) Hz, 1H, Asp-\(\alpha\) (e)), 3.93 (qd, \(J = 16.7, 5.6\) Hz, 2H, Gly-\(\alpha\) (g)), 2.88 (dd, \(J = 15.4, 5.6\) Hz, 1H, Asp-\(\beta\) (k)), 2.76 (dd, \(J = 15.7, 7.9\) Hz, 1H, Asp-\(\beta\) (k)).

\(^{13}\)C\({\(^1\)H}\) NMR (101 MHz, DMSO) \(\delta\) 172.58 (1C, C17), 169.87 (1C, C8), 168.19 (1C, C6), 166.38 (2C, C15), 166.08 (2C, C1), 164.57 (1C, C10), 139.33 (1C, C5), 134.83 (1C, C11), 132.35 (1C, C14), 131.98 (2C, C12), 131.75 (2C, C13), 131.65 (2C, C2), 124.63 (1C, C3), 123.66 (2C, C4), 49.71 (1C, C9), 42.94 (1C, C7), 36.51 (1C, C16).

**HRMS (ESI)** Exact mass calculated for \(C_{23}H_{10}N_{3}NaO_{13}[M+Na]^+\), 568.0816. Found 568.0816.

**Benzene-1.3.5-tricarbonyl trichloride (13).**\(^{37}\) Trimesic acid (12) (5 g, 23.8 mmol) was charged into a 250 mL round-bottomed flask, and anhydrous DCM (100 mL) was added. The reaction mixture was cooled to 0 °C and oxalyl chloride (9.07 mL, 107.2 mmol, 4.5 eq) was added dropwise
over a period of 15 min under nitrogen atmosphere followed by addition of 0.3 mL anhydrous DMF to the reaction mixture. The reaction mixture was stirred for 30 min at 0 °C, then was stirred for 4 h at room temperature after which the reaction mixture was stirred in an oil bath under reflux at a temperature of 50 °C for another 16 h. On completion, the solvents were removed under reduced pressure and the residue was co-evaporated with anhydrous DCM (5×30 mL) to remove excess oxalyl chloride. The residue was dried under high vacuum to afford the trimesic acid chloride (13) as a pale-yellow orange semisolid that was used without further purification.

1,3,5-Benzenetricarboxylic acid dibenzyl ester (15).38 To the crude 1,3,5-benzenetricarbonyl trichloride (13) (6.32 g, 23.8 mmol) was added 214 mL solvent mixture of anhydrous dichloromethane/THF (8:1), and the solution was cooled to -20 °C. To this cooled solution were added slowly over a course of 1.5 h via an addition funnel a solution mixture of benzyl alcohol (14) (4.92 mL, 47.6 mmol, 2 eq) and triethylamine (9.9 mL, 71.4 mmol, 3 eq) in 40 mL anhydrous dichloromethane/THF (8:1). The reaction mixture was left to stir for an additional 1 h at -20 °C, then stirred for another 1 h at rt. To the reaction mixture was then added 2 M aqueous sodium bicarbonate solution (36 mL), and left to stir for 30 min at rt. The organic solvents were evaporated under reduced pressure, then 240 mL of H₂O/MeOH mixture (1:1) was added to the residue and the pH was adjusted to ~ 10 using 1 M NaOH. The mixture was then extracted with hexanes (2×48 mL) and the hexane extracts were discarded. The organic volatiles were removed again under
reduced pressure and the pH was adjusted using 0.1 M HCl to ~ 8.5, and the mixture was then extracted with EtOAc (4×70 mL). The organic solvents were removed under reduced pressure to afford an oily residue which was dissolved in CH₂Cl₂ and hexanes were added slowly till a solid precipitate appeared which was filtered and dried under vacuum. The solid was dissolved in minimal EtOAc and was purified by column chromatography using CH₂Cl₂/acetone (30:1) as an eluent to provide compound 15 as a white solid, which was further purified through recrystallization from a hot CH₂Cl₂/hexanes mixture to obtain a white crystalline product of 15 (0.97 g, 10%).

$$\text{1H NMR (400 MHz, DMSO) } \delta 13.75 \text{ (s, COOH, 1H), 8.67 \text{ (s, 3H, Ar-H), 7.36-7.50 \text{ (m, (PhCH₂O} \times 2)), 10H), 5.41 \text{ (s, 4H, (PhCH₂O} \times 2)).}$$

$$\text{13C\{^1H\} NMR (101 MHz, DMSO) } \delta 165.51 \text{ (1C, C11), 164.05 \text{ (2C, C6), 135.64 \text{ (2C, C4), 133.77 \text{ (2C, C9), 133.25 \text{ (1C, C8), 132.33 \text{ (1C, C10), 130.87 \text{ (2C, C7), 128.55 (4C, C2 or C3), 128.30 \text{ (2C, C1), 128.18 (4C, C2 or C3), 66.94 (2C, C5).}}$$
Dibenzyl-5-(chlorocarbonyl)isophthalate (8). Compound 15 (0.41 gm, 1.0 mmol, 1 eq) was dissolved in 15 mL anhydrous DCM, and two drops of DMF were added. The reaction mixture was cooled to 0 °C and oxalyl chloride (0.22 mL, 2.6 mmol, 2.5 eq) was added dropwise. The reaction mixture was stirred at 0 °C for 20 min and stirred at room temperature for 8 h under nitrogen atmosphere. The solvents were then removed under reduced pressure and the residue was co-evaporated with dry DCM (5×20 ml) under reduced pressure, and the solid residue was dried under high vacuum to afford the acid chloride compound 8 that was then used without further purification.
4.6. NMR Characterization data of synthesized Organic Compounds

Figure 4.11. $^1$H-NMR spectrum of compound 3 in DMSO-d$_6$.

Figure 4.12. $^{13}$C-NMR spectrum of compound 3 in DMSO-d$_6$. 

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Figure 4.13. HSQC spectrum of compound 3 in DMSO-d$_6$.

Figure 4.14. HMBC spectrum of compound 3 in DMSO-d$_6$. 
Figure 4.15. $^1$H-NMR spectrum of compound 4 in DMSO-d$_6$.

Figure 4.16. $^{13}$C-NMR spectrum of compound 4 in DMSO-d$_6$. 
Figure 4.17. $^1$H-NMR spectrum of compound 6 in DMSO-$d_6$. 

Figure 4.18. $^{13}$C-NMR spectrum of compound 6 in DMSO-$d_6$. 

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Figure 4.19. COSY spectrum of compound 6 in DMSO-d$_6$.

Figure 4.20. HSQC spectrum of compound 6 in DMSO-d$_6$. 
Figure 4.21. HMBC spectrum of compound 6 in DMSO-d$_6$. 
Figure 4.22. $^1$H-NMR spectrum of compound 7 in DMSO-d$_6$.

Figure 4.23. $^{13}$C-NMR spectrum of compound 7 in DMSO-d$_6$. 

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Figure 4.24. $^1$H-NMR spectrum of compound 9 in DMSO-$d_6$.

Figure 4.25. $^{13}$C-NMR spectrum of compound 9 in DMSO-$d_6$. 
Figure 4.26. HSQC spectrum of compound 9 in DMSO-d$_6$.

Figure 4.27. HMBC spectrum of compound 9 in DMSO-d$_6$. 
Figure 4.28. $^1$H-NMR spectrum of compound 10 in DMSO-$d_6$.

Figure 4.29. $^{13}$C-NMR spectrum of compound 10 in DMSO-$d_6$. 
Figure 4.30. COSY spectrum of compound 10 in DMSO-d$_6$. 

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4.7. HRMS characterization data for synthesized organic compounds.

**Figure 4.31.** HRMS of compound 3.

**Figure 4.32.** HRMS of compound 6.
Figure 4.33. HRMS of compound 7.

Figure 4.34. HRMS of compound 10.
4.8. References


