

**M A S A R Y K O V A
U N I V E R Z I T A**

**FACULTY OF SCIENCE
DEPARTMENT OF CHEMISTRY**

**Unsymmetric Chiral
Ligands in Metallo-
Supramolecular
Chemistry**

Doctoral Thesis

SUBHASIS CHATTOPADHYAY

BRNO 2025

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Supervisors: Prof. RNDr. Radek Marek, Ph.D. & Ing.
Ondřej Jurček, Ph.D. et Ph.D.

Ústav chemie
Program Chemistry

Brno 2025

M U N I
S C I

Bibliografický záznam

Autor:	Subhasis Chattopadhyay, MSc. Masarykova univerzita Přírodovědecká fakulta Ústav chemie
Název práce:	Unsymmetric Chiral Ligands in Metallo-Supramolecular Chemistry
Studijní program:	Chemistry
Vedoucí práce:	Prof. RNDr. Radek Marek, Ph.D. & Ing. Ondřej Jurček, Ph.D. et Ph.D.
Rok:	2025
Počet stran:	97
Klíčová slova:	Žlučová kyselina, supramolekulární koordinanční komplex, asymetrie, flexibilita, samoskládání, orientační samotřídění, chiralita, bioaktivita

Bibliographic record

Author: Subhasis Chattopadhyay, MSc.
Masaryk University
Faculty of Science
Department of Chemistry

Title of Thesis: Unsymmetric Chiral Ligands in Metallo-Supramolecular Chemistry

Degree Programme: Chemistry

Supervisors: Prof. RNDr. Radek Marek, Ph.D. & Ing. Ondřej Jurček, Ph.D. et Ph.D.

Year: 2025

Number of Pages: 97

Keywords: Bile acid, supramolecular coordination complex, unsymmetry, flexibility, self-assembly, orientational self-sorting, chirality, bioactivity

Abstrakt

Většina organických ligandů používaných v metalosupramolekulární chemii je symetrická, rigidní a achirální, na rozdíl od přirozených supramolekulárních sestav. Návrh a samouspořádání nesymetrických, flexibilních a chirálních ligandů do komplexní supramolekulární architektury představuje významnou výzvu v oblasti koordinační chemie. V této studii je zkoumáno koordinačně řízené samouspořádání ligandů odvozených od žlučových kyselin, které rozšiřuje známou škálu chirálních, nesymetrických konstrukcí využívajících derivátů kyseliny ursodeoxycholové (UDCA) a chenodeoxycholové (CDCA, epimer UDCA) nesoucích *bis*- a *tris*-pyridylová koordinační centra. Tyto ligandy dále vykazují pozoruhodnou flexibilitu a objemnost, což vede k charakteristickým strukturám po jejich komplexaci se čtvercově planárním kovovým kationtem Pd²⁺. *Bis*-pyridylový ligand odvozený od UDCA se selektivně samouspořádává a samotřídí do enantiomerně čistého korunovitého supramolekulárního koordinačního komplexu (SCC) Pd₃L₆. Naproti tomu epimerní *bis*-pyridylový ligand odvozený od CDCA, který má relativně nižší flexibilitu, poskytuje směs SCC s obecným vzorcem Pd_nL_{2n} (Pd₂L₄, Pd₃L₆, Pd₄L₈, Pd₅L₁₀ a Pd₆L₁₂). Souhra mezi flexibilitou ligandu a objemností steroidní kostry poskytuje užitečný rámec pro zkoumání supramolekulárního designu a samoskládání s ligandy odvozenými od přírodních molekul. V podobné studii, *tris*-pyridylový ligand odvozený od UDCA prokázal selektivní a přepínatelné sestavování do chirálních nanoklece Pd₆L₈ a Pd₁₂L₁₆, přičemž Pd₁₂L₁₆ představuje vůbec první kuboktahedrálnej samoskladnou sestavu z tritopického ligandu syntetizovanou metodou zaměřenou na stěnu (z anglického face-directed). Tato selektivita se připisuje adaptabilitě a flexibilitě pyridylových skupin ligandu v reakci na různé reakční podmínky, což umožňuje tvorbu homochirálních (ΔΔΔΔ a ΔΔΔΔΔΔ) helikálních izomerů. Použití hmotnostní spektrometrie iontové mobility (IM-MS), ¹H-¹H nukleární Overhauserovy spektroskopie (NOESY), strukturní transformace, ¹H-DOSY NMR spektroskopie, spektroskopie cirkulárního dichroismu (CD) a modelování použitím teorie funkcionálu hustoty (DFT) umožnilo komplexní strukturní analýzu, a tím poskytlo vhled do strukturních konformací zkoumaných SCC. Tato sekvenční studie navíc představuje kombinaci CD spektroskopie a DFT modelování jako potenciální alternativu k rentgenové difrakci monokrystalů (SC-XRD) pro objasnění molekulárních struktur helikálních SCC. Supramolekulární klece Pd₆L₈ a Pd₁₂L₁₆ pocházející z přirozeně se vyskytujících amfifilních transportních molekul, navíc vykazovaly zvýšenou buněčnou absorpci a vyšší protinádorovou účinnost palladiových kationtů v lidských jaterních sféroidech HepG2 než Pd(NO₃)₂. Celkově tato práce podtrhuje užitečnost přirozených nesymetrických stavebních jednotek při konstrukci komplexních chirálních nanostruktur a ukazuje jejich potenciál pro biomedicínské aplikace.

Abstract

Most of the organic ligands employed in metallosupramolecular chemistry are symmetric, rigid, and achiral, in contrast to the natural supramolecular assemblies. The design and self-assembly of unsymmetric, flexible, and chiral ligands into a complex supramolecular architecture represents a significant challenge in the field of coordination chemistry. In this study, the coordination-driven self-assembly of bile-acid-derived ligands is explored, extending the known range of chiral, unsymmetric scaffolds by employing ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA, epimer of UDCA) derivatives bearing *bis*- and *tris*-pyridyl coordination sites. These ligands further exhibit notable flexibility and bulkiness, which result in distinctive structural outcomes upon complexation with the square planar Pd²⁺ metal cation. The UDCA-derived *bis*-pyridyl ligand selectively self-assembles and self-sorts into an enantiopure crown-like Pd₃L₆ supramolecular coordination complex (SCC). In contrast, the epimeric CDCA-derived *bis*-pyridyl ligand, which has relatively lower flexibility, yields a mixture of SCCs with the general formula Pd_nL_{2n} (Pd₂L₄, Pd₃L₆, Pd₄L₈, Pd₅L₁₀, and Pd₆L₁₂). The interplay between the ligand flexibility and the bulkiness of the steroidal backbone provides a useful framework for exploring supramolecular design and self-assembly with natural molecule-derived ligands. In parallel, the UDCA-derived *tris*-pyridyl ligand demonstrated selective and switchable assembly into stellated Pd₆L₈ and Pd₁₂L₁₆ chiral nanocages, the latter representing the first ever face-directed cuboctahedral self-assembly from a tritopic ligand. This selectivity is attributed to the adaptability and flexibility of the ligand's pyridyl groups in response to the varied reaction conditions, which enables the formation of homochiral (ΔΔΔΔ and ΔΔΔΔΔΔΔΔ) helical isomers. The use of ion-mobility mass spectrometry (IM-MS), ¹H-¹H nuclear Overhauser effect spectroscopy (NOESY), structural transformation, ¹H-DOSY NMR spectroscopy, circular dichroism (CD) spectroscopy, and density functional theory (DFT) modeling enabled a comprehensive structural analysis, thereby providing insight to the structural conformations of SCCs under investigation. Moreover, this sequential study presents a combination of CD spectroscopy and DFT modeling as a potential alternative to single-crystal X-ray diffraction (SC-XRD) for elucidating molecular structures of helical SCCs. Furthermore, Pd₆L₈ and Pd₁₂L₁₆ supramolecular cages, originating from naturally occurring amphiphilic transport molecules, showed enhanced cellular uptake and exhibited higher anticancer efficacy of palladium cations in human hepatic HepG2 spheroids than Pd(NO₃)₂. Overall, this work underscores the utility of natural unsymmetric building units in constructing complex chiral nanostructures and highlights their potential for biomedical applications.

Declaration

I declare that I have prepared my thesis independently under the mentorship of the thesis supervisors and using the information sources provided in the thesis. I also acknowledge the assistance of ChatGPT in refining the writing and editing process of this thesis.

Brno 2025


.....
Subhasis Chattopadhyay

Acknowledgements

To the mere chance of existence!

Not to a higher purpose, fate, or reason—but to the randomness.

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Abbreviations

SCC	– Supramolecular coordination complexes
BA	– Bile acid
UDCA	– Ursodeoxycholic acid
CDCA	– Chenodeoxycholic acid
NMR	– Nuclear magnetic resonance
VT NMR	– Variable Temperature Nuclear magnetic resonance
DOSY	– Diffusion-ordered spectroscopy
<i>D</i>	– Diffusion coefficient
<i>D_h</i>	– Hydrodynamic diameter
NOESY	– Nuclear Overhauser effect spectroscopy
IM-MS	– Ion-mobility mass spectrometry
ICP-MS	– Inductively coupled plasma mass spectrometry
CCS	– Collision cross section
CD	– Circular dichroism
SC-XRD	– Single-crystal X-ray diffraction
M:L	– Metal-to-ligand ratio
DFT	– Density functional theory
EPR	– Enhanced permeability and retention
IC ₅₀	– Half-maximal inhibitory concentration
IC:EC	– Intraspheeroid to extracellular concentration
TBACl	– Tetrabutylammonium chloride
TBANO ₃	– Tetrabutylammonium nitrate
ACN	– Acetonitrile
ACN- <i>d</i> ₃	– Trideuteroacetonitrile
DMSO- <i>d</i> ₆	– Hexadeuterodimethyl sulfoxide
RM	– Reaction mixture

1 Introduction

The supramolecular chemistry deals with building complex structures through molecular teamwork,¹ which underpins the design and construction of cavity-containing structures known as cavitands.² The intermolecular interactions are pivotal for creating supramolecular "host-guest" complexes³ resulting in complex architectures⁴ and functional assemblies,⁵ driving advancements in fields ranging from molecular recognition⁶ to materials science, such as sensing,⁷ catalysis,⁸ or drug delivery.⁹ "Host-guest" chemistry, inspired by enzyme-substrate interactions, laid the groundwork for synthetic cavity-containing structures like crown ethers,¹⁰ spherands,¹¹ and cryptands¹² for the selective binding of guest cations, anions, or neutral molecules. With a deeper understanding of intermolecular interactions in natural systems, more complex molecular cavitands, including cucurbiturils,¹³ calixarenes,¹⁴ and cryptophanes,¹⁵ were developed to achieve enhanced selectivity and binding capabilities. Later, in the 1990s, the utilization of "host-guest" interactions led to the development of more sophisticated structures, such as rotaxanes,¹⁶ catenanes,¹⁷ and molecular knots.¹⁸ These advances propelled supramolecular chemistry forward, ultimately contributing to the creation of artificial molecular machines.¹⁹ However, the ever-increasing complexity of the supramolecular structures, with the rapid advancement of the field, often makes the synthesis of covalent architectures synthetically demanding and low-yielding. Therefore, the development of supramolecular coordination complexes (SCCs) offers a suitable alternative for constructing cavity-containing structures.^{20, 21} Additionally, metal centers can provide synergistic therapeutic properties, making them valuable for biological applications.²² Construction of these metallo-supramolecular structures relies on coordination bonds and self-assembly process, referred to as coordination-driven self-assembly. Synthesis of the coordination ligands is often relatively simple, and the self-assembly process generally results in quantitative yield, making the process efficient by avoiding the traditional stepwise approach. Over the past few decades, many artificial coordinating ligands and their SCCs were synthesized, which led to definition of fundamental principles for the self-assembly processes.²⁰ However, unlike natural cavitands, most of these structures are symmetric and achiral. Only a few supramolecular coordination complexes (SCCs) utilize natural molecules as building blocks,²³ whereas many covalent structures are designed with them.²⁴ Exponential growth in the interdisciplinary fields, and emerging boundaries of biology and chemistry, two fundamental sciences of life, clearly emphasize the growing requirement for the natural molecule-based "next-generation" SCCs. Therefore, this thesis primarily focuses on the development of such structures providing a new dimension to the underlying fundamental principles of their formation. Natural molecules (bile acids (BAs) in this case) with their unsymmetry, chirality, flexibility, amphiphilicity, and bulkiness can offer supramolecules with new structural features and enhanced selectivity for

“host-guest” complexes. SCCs are generally divided into two principal categories: (1) finite, or discrete,²⁰ SCCs and (2) infinite SCCs.^{24, 25} This dissertation focuses exclusively on the investigation of finite SCCs, which are self-contained assemblies with defined structural boundaries, while infinite SCCs, which form continuous polymeric networks, are beyond the scope of this study and will not be addressed.

1.1 Metallo-Supramolecular Macrocycles and Cages

1.1.1 General Introduction

Finite, or discrete, SCCs can be broadly classified into two major categories: 1) macrocycles, which may adopt two-dimensional (2D) or three-dimensional (3D) structures, and 2) cages or polyhedral structures, which are inherently three-dimensional (3D) in nature (Figure 1). In 1988, while intending to synthesize allenes, Saalfrank et. al. chanced upon a highly symmetric tetrahedral magnesium-malonate intermediate, laying the foundation of discrete SCCs.²⁷ Understanding this structure led to the synthesis of an ever-increasing number of sophisticated structures with different coordination ligands and metal coordinates.^{20, 28, 29, 30} This has developed a basic understanding of the coordination-driven self-assembly process and established the general guidelines for designing the ligand. The most important properties are encoded in the structure of the coordination ligand, *i.e.*, the type and number of coordination sites, bend angle, steric constraints, and rigidity/flexibility. The type and number of coordination sites available in the ligand, upon recognition by the coordination geometry (*i.e.*, square planar, tetrahedral, octahedral) of the metal coordinates, self-assembles into symmetric polygons and polyhedra, resulting in the discrete SCCs.

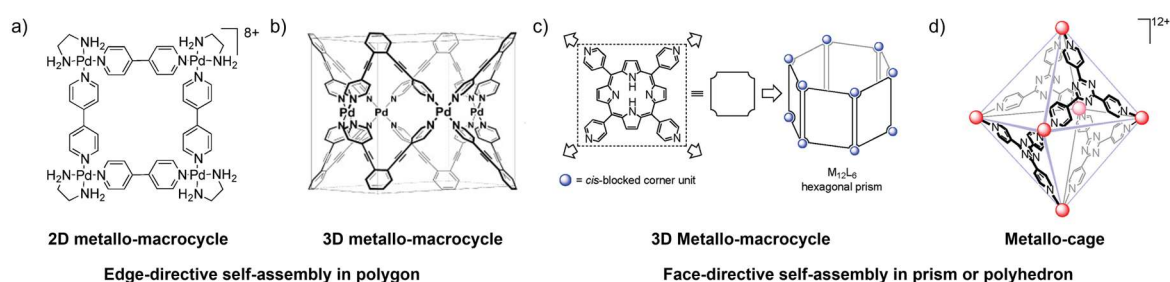


Figure 1. 2D and 3D metallosupramolecular structures. [Adapted (a, d, c) with permission from Ref. 20. Copyright © 2011 American Chemical Society, and adapted (b) from Ref. 31. CC BY-NC 4.0 license. Copyright © 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim]

There are two ways to construct such geometries, where the coordination ligand is placed either on the edges or faces of the polyhedron. So, they are named edge-directed self-assembly and face-directed self-assembly or molecular paneling, respectively (Figure 1). The metal cations reside in each corner of the polygons or prisms, resulting in

macrocycles. Now, depending on the metal coordination geometry (*e.g.*, *cis*-blocked Pd²⁺ (Figure 1a, 1c, and Figure 1d), square planar Pd²⁺ (Figure 1b)) and the type of ligand (edge or face-directed self-assembly), these macrocycles could be 2D or 3D. On the other hand, metallo-cages are structures where the metal cations occupy the vertices of a polyhedron. Therefore, regardless of whether they are formed *via* edge or face-directed self-assembly, they adopt 3D structures.

The self-assembly processes are further sub-categorized by design guidelines such as directional bonding and symmetry interaction.²⁰ Most of the SCCs are made using square planar Pd²⁺ or Pt²⁺ with pyridyl-based ligands, which rely on the directional bonding approach, whereas, metallocycles or cages composed of Fe²⁺, Co²⁺, Ni²⁺, Zn²⁺, Cd²⁺, Ga³⁺ or Ru²⁺ metal cations and tetrazole, imidazole, carboxylate, malonate or 1-(2-pyridinyl)methanimine type of complimentary coordinating units, rely on the symmetry interaction method. This thesis, however, focuses on fundamental design principles specific to pyridyl ligands and square planar Pd²⁺ cations; thus, strategies involving symmetric bonding approaches are not discussed.

1.1.2 Metal-to-ligand Ratio

The first crucial assumption for constructing discrete SCCs is to select the appropriate metal-to-ligand ratio (M:L). To determine this, one must know the coordination number of the metal coordinate, *e.g.*, the coordination number of square planar Pd²⁺ is four; therefore, it can coordinate with four pyridyl coordination sites. Secondly, one must know the topicity or denticity of the ligand, *i.e.*, how many coordination sites are available in a ligand. Therefore one ditopic or *bis*-pyridyl ligand that can bind to **two** metal cations having coordination number **four** would maintain an M:L of **2:4**, which is M:L of 1:2. This metal to ligand ratio, the most studied one, can generate a series of discrete SCCs (macrocycles or cages) with a general formula of Pd_nL_{2n} (n= 2, 3 and so on), resulting in Pd₂L₄, Pd₃L₆, Pd₄L₈, Pd₅L₁₀, Pd₆L₁₂, Pd₇L₁₄, Pd₁₂L₂₄, Pd₂₄L₄₈, Pd₃₀L₆₀ and Pd₄₈L₉₆ (the largest metallocage reported so far).³² Similarly, a *tris*-pyridyl ligand with square planar Pd²⁺ cation will maintain an M:L of 3:4. This results in a series of Pd₃L_{4n} complexes, with so far only four types of SCCs (cages), *i.e.*, Pd₃L₄,^{33–35} Pd₉L₁₂ (one),³⁶ Pd₁₈L₂₄ (one)³⁷ via the edge-directed self-assembly and Pd₆L₈ (the most common type)³⁸ via the face-directed self-assembly, which, relative to the ditopic ligands, makes tritopic ligands less studied. Apart from the above two types of ligands, there are a few tetratopic pyridyl ligands, which are combined with *cis*-blocked Pd²⁺ (Coordination number 2, which makes M:L of 2:1) resulting in mostly open prism-shaped macrocycles or regular polyhedron in few cases.³⁹

The obvious question here is how certainly someone can predict the nuclearity (the number of metal centers) of the SCCs just by looking at the structure of the ligand.

Another key question arises as to whether it is possible to predict the type of self-assembly outcome, specifically, whether a given system will form a polygonal structure or a polyhedral geometry. Therefore, I would like to use this opportunity to point out the incompleteness of the fundamental concepts that must be fulfilled in metallo-supramolecular chemistry. Being a supramolecular chemist, if someone looks at well-ripened organic chemistry, a higher degree of predictability can be observed over the outcome of some synthesis, which is lacking in this young branch of chemistry. A complete understanding of the underlying principles for precise prediction about the outcome is very crucial and the need of the hour to design a new type of ligand which would help to expand the present dataset. I will discuss these gaps one by one in the introduction part, followed by the contributions provided in the results and discussion section of my thesis.

1.1.3 Bend Angle and Flexibility

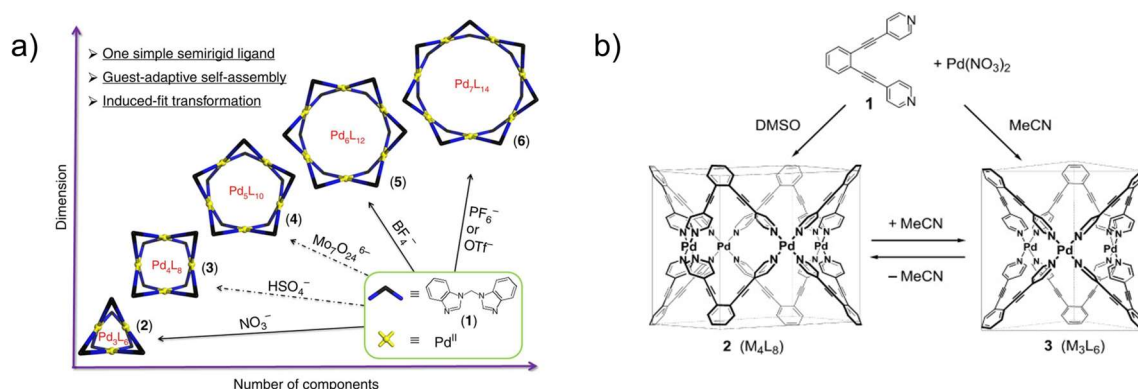


Figure 2. Guest-adaptive self-assembly of rigid, planar, and symmetric ligands. [Adapted (a) from Ref. 40. CC BY-NC 4.0 license. Copyright © 2017 The Authors. Nature Communications published by Springer Nature Limited and (b) from Ref. 31. CC BY-NC 4.0 license. Copyright © 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim]

The question regarding the nuclearity of the SCCs highlights the ligand bend angle, a critical parameter. However, accounting for flexibility, this parameter becomes complex, making it less thoroughly explored and understood. Most ligands used so far are rigid and therefore have a predefined bend angle. The general trend is that the larger the bend angle of the ligands, the higher the nuclearity (size) of the self-assembly.³⁸ This remains true for metallo-cages or polyhedra,^{41,42} however, in the case of metallo-cycles, this is not very straightforward. A recent publication reports the selective formation of interconvertible Pd_nL_{2n} ($n=3, 4, 5, 6, 7$) metallo-macrocycles using semirigid planar pyridinyl ligands having small conformational flexibility (Figure 2a).⁴⁰ The higher nuclearity was achieved through the templating effect of the counteranion of

the metal salt used for the reaction. This phenomenon observed is similar to the natural induced-fit guest-binding mechanism, demonstrating the field's potential to mimic natural processes. Another example is the templating effect provided by the solvent to result in interconvertible Pd_nL_{2n} ($n = 3, 4$) which was revealed by the solid-state crystal structure of these macrocycles (Figure 2b).³¹ Two DMSO molecules together with two nitrate anions template the formation of Pd_4L_8 . When triflate anion was used as a counter anion instead of nitrate or the DMSO solution of Pd_4L_8 was diluted using any other solvent (*e.g.*, methanol, ethanol, 1,4-dioxane, or chloroform), a mixture of Pd_3L_6 and Pd_4L_8 was formed. This demonstrates how sensitive these self-assembly processes are toward counteranions or even solvent molecules. Moreover, it shows that a rigid ligand with a predefined bend angle can form SCCs of varying sizes. Another study utilizing systematic angle variation of rigid, symmetric ditopic ligands suggested that wider bend angles could achieve macrocycles with higher nuclearity (Figure 3), although conformational strain plays the decisive role.⁴³

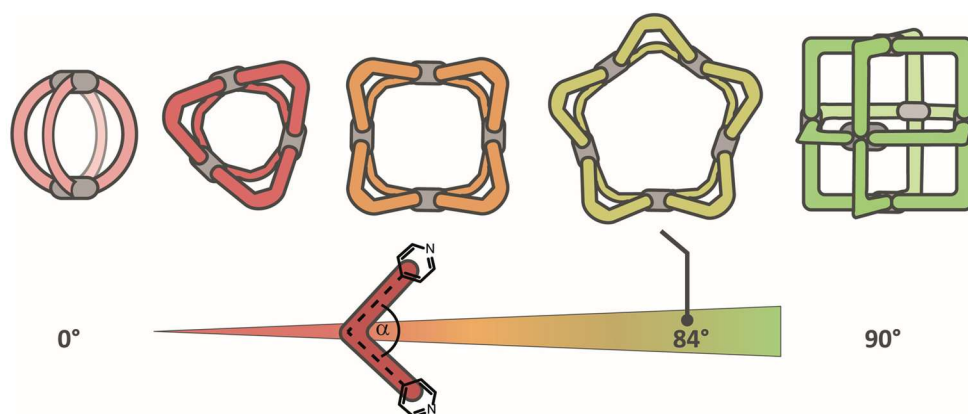


Figure 3. Effect of increasing bend angle and ring strain on macrocyclic self-assemblies of rigid, planar, and symmetric ligands. The red shift in color denotes increasing ring strain. [reprinted from Ref. 43. CC BY-NC-ND 4.0 license. Copyright © 2024 The Authors. Chemistry - A European Journal published by Wiley-VCH GmbH]

While this assessment may hold for planar, rigid, and symmetric ligands, our recent findings indicate that it cannot be universally applied, particularly in designing SCCs incorporating flexible ligands. Notably, DFT calculations of the reported macrocycles reveal that ring strain plays a pivotal role in determining structural outcomes, aligning closely with our observations on macrocycles developed using flexible and unsymmetric ligands, which are described in this thesis.

Now, to claim a general rule of thumb based on only the bend angle of the ligand, one first needs a better understanding of relative rigidity or flexibility to define the bend angle. During the initial development of the field, rigidity coiled with

directionality was given prime importance, providing predefined bend angles. However, if we look at natural supramolecular systems, there are ample examples of highly flexible ligands, and they can adopt specific structures based on the surrounding environment. To mimic the natural systems, understanding the effect of flexibility on the final self-assembly is important. The state of our current understanding is reflected in the $\text{Pd}_{24}\text{L}_{48}$ to $\text{Pd}_{12}\text{L}_{24}$ transition, caused by a change of only 3° in the bend angle of a small rigid ligand.⁴¹ Mathematical calculations show ideal bend angles for coordination polyhedra are $\approx 90^\circ$ for Pd_6L_4 , $\approx 120^\circ$ for $\text{Pd}_{12}\text{L}_{24}$, and $\approx 135^\circ$ for $\text{Pd}_{24}\text{L}_{48}$ for $n \leq 24$. Although, a rigid *bis*-pyridyl ligand with a 149° bend angle, which is close to the ideal value (150°) for $\text{Pd}_{30}\text{L}_{60}$, self-assembles into $\text{Pd}_{24}\text{L}_{48}$ upon heating for an hour. However, prolonged heating results in a mixture of $\text{Pd}_{24}\text{L}_{48}$ and $\text{Pd}_{30}\text{L}_{60}$ (Figure 4a).⁴⁴

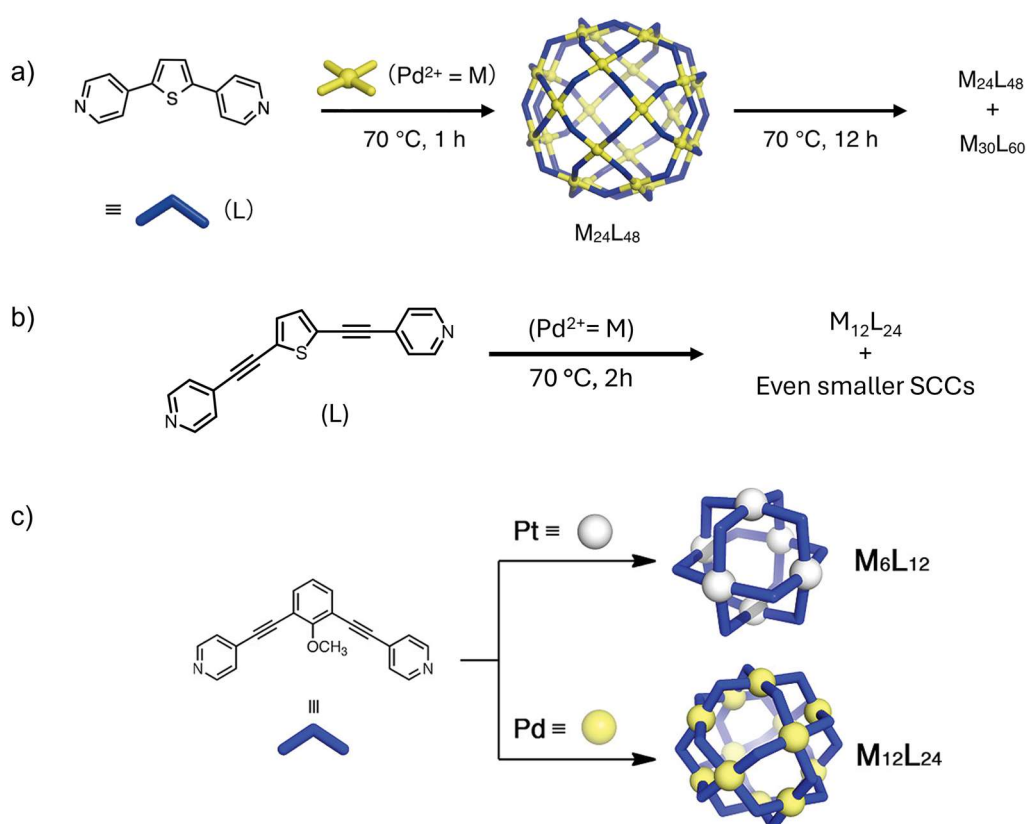


Figure 4. Influence of flexibility and reaction kinetics over self-assembly processes. [Adapted (a, b) with permission from Ref. 44. Copyright © 2016 Elsevier Inc. and (c) with permission from Ref. 45. Copyright © 2011 American Chemical Society]

It suggests self-assembly into a larger structure ($n \geq 24$) undergoes natural kinetic trapping, therefore resulting in kinetically trapped $\text{Pd}_{24}\text{L}_{48}$, and only prolonged heating partially converts it to the thermodynamic $\text{Pd}_{30}\text{L}_{60}$. When the rigidity is slightly compromised by placing an ethynylene spacer group between the pyridyl sites and

thiophene core of the ligand, a mixture of kinetically trapped species, $\text{Pd}_{12}\text{L}_{24}$, and smaller self-assemblies was observed (Figure 4b). A similar *bis*-pyridyl ligand with an ethynylene spacer, upon complexation with Pd^{2+} , results in $\text{Pd}_{12}\text{L}_{24}$ but with relatively inert Pt^{2+} forms kinetically trapped Pt_6L_{12} (Figure 4c).⁴⁵

This suggests that, when provided with a degree of flexibility, the ligands can determine their preferred structural outcome by adjusting their structure. Although being an important parameter, flexibility is poorly defined to generate quantitative numbers. To define and systematically compare the flexibility, which mostly originates by conformational changes of ligands, one approach could be the number of rotatable bonds present in the ligand. However, a better definition would be relating flexibility to the standard deviation in the bend angles of the ligand. Therefore, the key to developing such relations is to design and investigate natural molecule-based flexible ligands and their self-assembly. Nevertheless, the kinetic trapping of the self-assemblies suggests that the self-assembly process is closely related to the natural protein folding process.⁴⁶ Therefore, supramolecular structures are not far from closely mimicking natural systems.

1.1.4 Chirality or Unsymmetry

Apart from rigidity/flexibility, another essential ingredient in natural systems is chirality or unsymmetry, which has not been explored much so far. Recent advancements in the field specifically target the synthesis of unsymmetric, asymmetric, or low-symmetry SCCs. So far, three strategies have been employed.

a) Orientational Self-Sorting

The first one is to develop unsymmetric ligands. Unsymmetric ligands are designed based on two basic strategies: an unsymmetric backbone with the same binding sites and a symmetric/unsymmetric backbone with different binding sites (Figure 5a).⁴⁷ This classification divides these ligands into three categories: type A (unsymmetric backbone with identical binding moieties), type B (unsymmetric backbone with distinct binding moieties), and type C (symmetric backbone with two distinct binding moieties). As the topicity of the ligand increases, the number of possible permutations and combinations of these categories increases with it, making the system more complex.

The introduction of unsymmetric ligands provides a new aspect to the field, known as orientational self-sorting.^{48,49} This process refers to molecules spontaneously and selectively assembling into distinct, well-defined constitutional isomers, typically favoring one over others in a mixture (Figure 5b). For example, Pd_2L_4 can be present in the form of 4 isomers, Pd_3L_6 as 9, Pd_4L_8 as 35, Pd_6L_{12} as 112, and $\text{Pd}_{12}\text{L}_{24}$ even

as 350696 constitutional isomers (excluding their enantiomers). This type of self-sorting provides a particular shape to the nanocavities, unleashing the potential for delivering a chiral environment for enantioselective reactions or molecular recognition with high sensitivity and specificity.

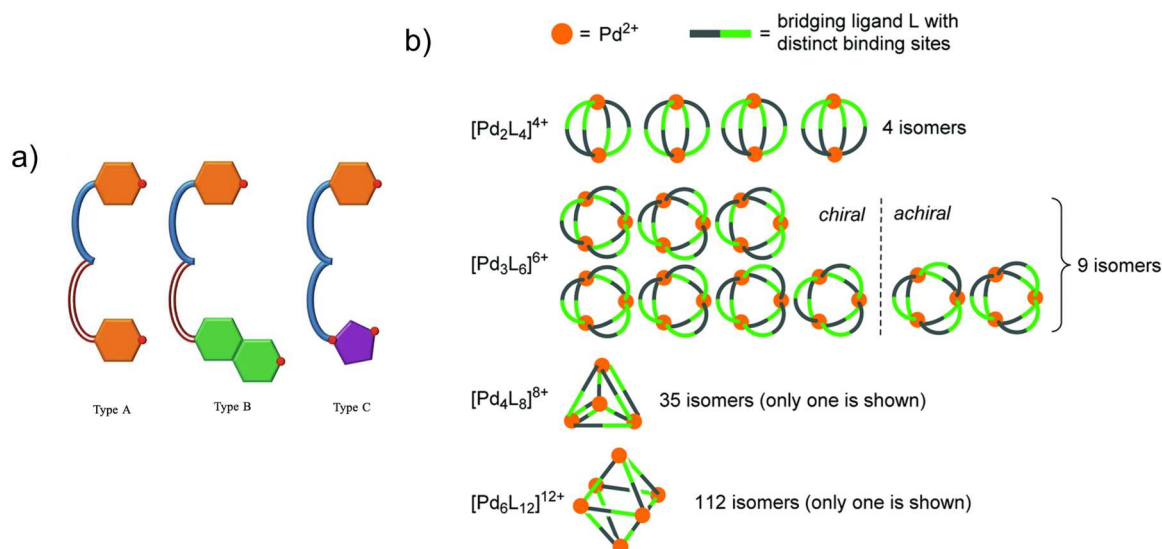


Figure 5. Different types of unsymmetric ligands result in low-symmetry constitutional isomerism in SCCs. [Reprinted (a) with permission from Ref. 47. Copyright © 2021 Elsevier B.V. All rights reserved. and (b) with permission from Ref. 48. Copyright © 2021 Royal Society of Chemistry]

b) Heterolepticity

The second approach to developing unsymmetric SCCs involves a combination of two or more different ligands to construct the SCCs, known as heteroleptic complexes (Figure 6).^{50–55} This process is more complicated than the earlier one, as the presence of multiple ligands can result in a mixture of different homoleptic and heteroleptic SCCs. However, recent advancements provided more control and understanding, resulting in the selective formation of non-statistical heteroleptic SCCs (Figure 6).⁵³ The advantages of such complexes over homoleptic SCCs are multifunctionality, tunable properties, and synergistic effect. However, all the unsymmetric ligands and the majority of the heteroleptic cages are developed using symmetric ditopic pyridyl ligands, which makes the unsymmetric tritopic ligands unexplored.

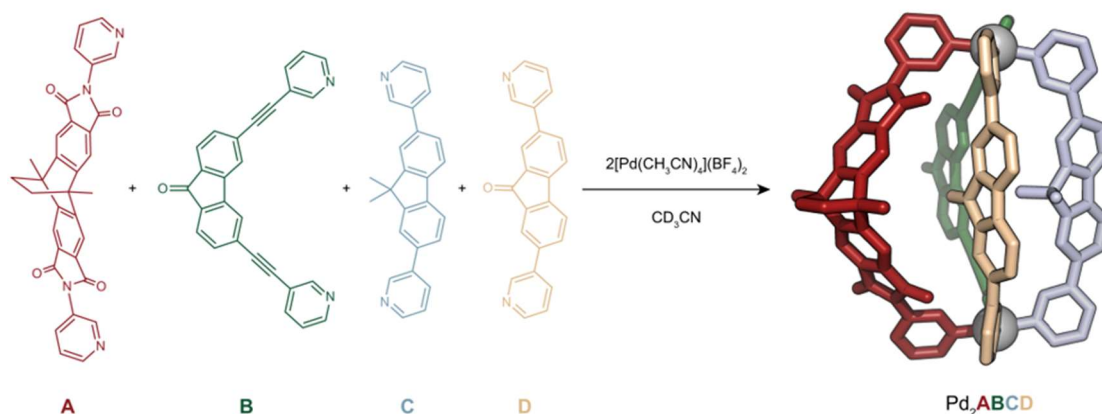


Figure 6. Development of low-symmetry SCCs using heterolepticity. [Reprinted with permission from Ref. 53. Copyright © 2024 The Authors. *Nature Chemistry* published by Springer Nature Limited]

c) Helical Chirality

The third approach targets the development of asymmetric or chiral architecture from symmetric ligands (Figure 7).^{56–59} The basis of the ligand design is the symmetric interaction approach. Initially, these structures are made of first-row transition metal cations (*e.g.*, Fe²⁺, Co²⁺, Cu²⁺, and Ni²⁺) and bipyridine or 1-(2-pyridinyl)methanimine coordinating ligands.^{6,60–62} Asymmetry or chirality in the cages originates from the non-planar asymmetric coordination environment of the metal centers. The ligand in such SCCs, depending on the ligand backbone's length or flexibility, can adopt helicity, resulting in a separate class of SCCs, known as helicates. Therefore, symmetric square-planar coordination geometry (*e.g.*, Pd²⁺ or Pt²⁺) with pyridine coordinating sites was not preferred for constructing these SCCs. Despite this typical behavior, asymmetric or chiral SCCs were recently reported using pyridine coordinating ligands.⁶³ However, their synthesis without using enantiopure building blocks remains challenging; asymmetric cages developed using symmetric ligands result in a racemic mixture (Figure 7a). The chiral resolution was generally achieved using chiral guest molecules (Figure 7b), in rare cases, it is achieved through crystallization.⁵⁷ Nevertheless, constructing enantiopure asymmetric cages using pyridine coordinating ligands with symmetric square-planar coordination spheres of metals is rare.

These previous examples consider the formation of low-symmetry or asymmetric cages, and in some cases, these SCCs have helical chirality. However, the ligands used are symmetric, and the design of the ligands, unlike natural systems, rarely has any chiral centers.²³ There are a few reports where chiral units are appended to the coordinating ligands, *e.g.*, peptide,^{64,65} pentasaccharide,⁶⁶ or short alkyl chains.⁶⁷ This could be considered a surface modification of the ligands. Therefore, the discrete SCCs contain these chiral centers in their peripheral areas, resulting in achiral nanocavities.

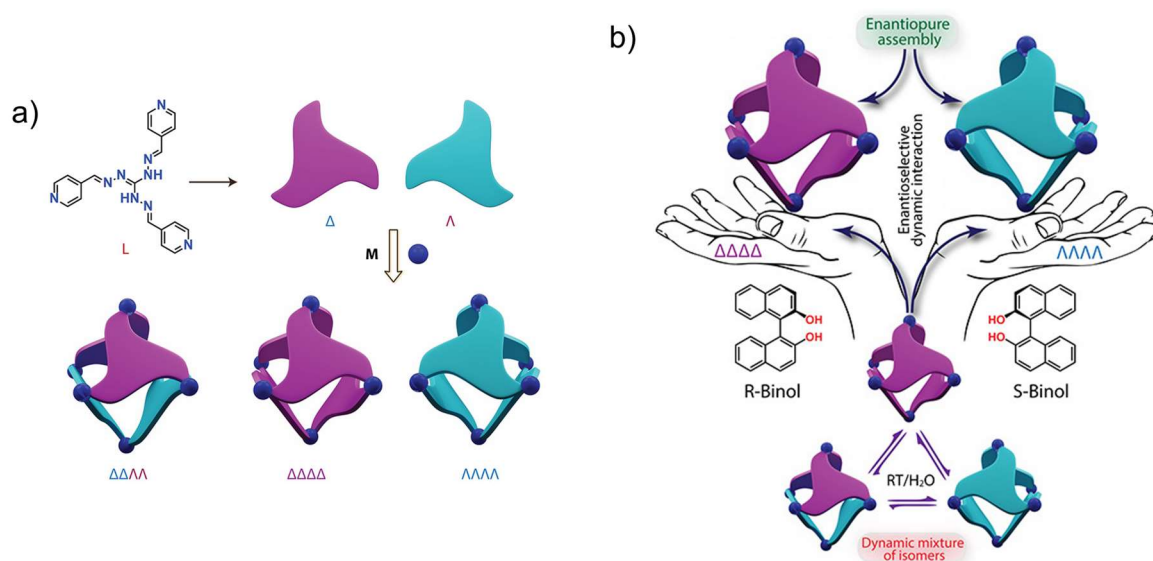


Figure 7. Synthesis and chiral resolution of low-symmetry SCCs from symmetric ligands.

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1.1.5 Natural Molecule-based SCCs

A recent publication describes all the abovementioned factors, reporting the selective formation of natural bile-acid-based enantiopure Pd_3L_6 metallo-macrocycle with a cavity of the complex decorated with chiral centers (Figure 8a).⁶⁸ Furthermore, a similar Pd_3L_6 was reported to form hexagonal microparticles *via* hierarchical self-organization.⁶⁹ Later, the selective formation of small lantern-shaped Pd_2L_4 metallogages was reported using BAs with a standard deviation of $\Delta 14^\circ$ in their bend angles (Figure 8b).⁷⁰ Interestingly, regardless of having such flexibility in their bend angle, these bile-acid-based *bis*-pyridyl ligands selectively form one SCC. Therefore, it seems contrary to the small flexibility of rigid ligands, resulting in a mixture of kinetically trapped self-assemblies; even high flexibility allows the ligands to form a single self-assembly, as observed in natural systems. However, apart from these, there are only a handful of reports on SCCs, *e.g.*, macrocycles or catenanes, made of peptide-based *bis*-pyridyl ligands (Figure 8c-Figure 8d).⁷¹ This highlights the need to develop natural molecule-based unsymmetric and flexible ligands for advanced understanding of the self-assembly process. Moreover, it leaves natural molecule-based tritopic ligands unexplored.

Accordingly, as a continuation of this story and a part of my PhD work, self-assembly process of chenodeoxycholic acid (CDCA)-based *bis*-pyridyl ligand was studied and compared with the analogous UDCA ligand. Being an epimer of UDCA, CDCA provides a fair correlation between ligand bend angle, its effect on flexibility, and the final self-assembly, providing us with valuable insights into the self-assembly process for

unsymmetric and flexible ligands. Furthermore, there is no report of unsymmetric and natural-molecule-based tritopic ligands. Thus, in the second project, extending the series of BA-based ligands, UDCA was converted into a *tris*-pyridyl ligand by appending a coordinating site to both hydroxyls and its alkyl side chain, which brings additional flexibility compared to its *bis*-pyridyl derivatives. This has led to “*flexibility-aided orientational self-sorting*”, resulting in the selective and switchable formation of two distinct metallo-cages guided by the reaction environment.

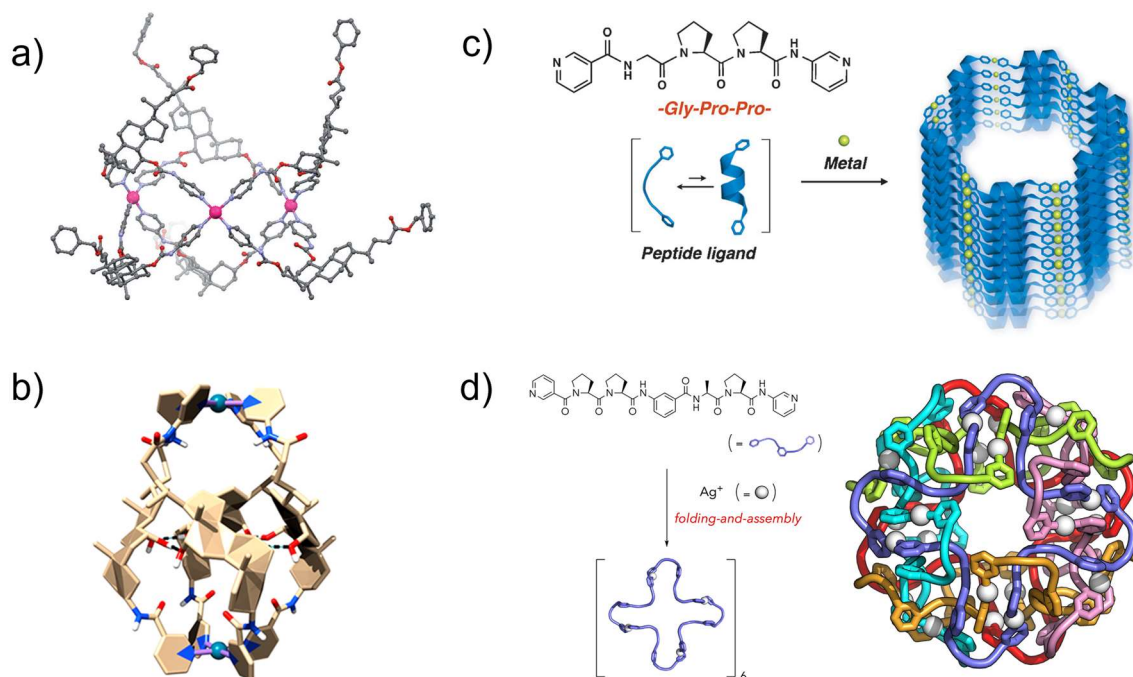


Figure 8. Natural-molecule-based chiral SCCs. [Adapted (a) from Ref. 68. CC BY-NC 4.0 license. Copyright © 2015 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim; (b) with permission from Ref. 70. Copyright © 2015 American Chemical Society; (c) from Ref. 72. CC BY-NC 4.0 license. Copyright © 2014 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim; (d) with permission from Ref. 71. Copyright © 2020 Elsevier Inc.]

1.1.6 Biomedical Applications of SCCs

In addition to the fundamental aspects, these discrete SCCs can provide various interesting applications like recognition, enantioselective catalysis, or separation. However, as the topic revolves around natural molecules abundant in the human body, their investigation instinctively led to their biomedical evaluation. Despite the exponential increase in the number of discrete SCCs since their discovery, biomedical research and applications remain an emergent field of study.⁷³ Recent discoveries have shown their potential to be a promising candidate for modern therapeutics.^{22,74,75} Since most discrete SCCs are composed of bioactive heavy metals like Pd²⁺ or Pt²⁺, they are

primarily studied for their anticancer or antitumor characteristics. Small platinum or palladium-based anticancer drugs lack bioavailability, stability, and solubility, and have deleterious side effects due to insufficient targeting ability. Therefore, these SCCs, particularly macrocycles, cages, or capsules, are developed as drug carriers by forming host-guest complexes with small platinum metallo-drugs. For example, a series of Pd₂L₄ metallo-cages⁷⁶ were exposed to A549, SKOV-3, or HepG2 cancer cell line, where overall the free cages showed lower toxicity compared to the well-known anticancer drug cisplatin. However, the anticancer activity of the host-guest complex with cisplatin (2:1 cisplatin: Pd₂L₄) was investigated in human ovarian carcinoma SKOV-3, and this complex showed 10-fold toxicity compared to cisplatin and the cage alone (Figure 9a). Co-administration of cisplatin, free ligand, and Pd²⁺ precursor to the cell-culture medium did not result in the same increase in their half-maximal inhibitory concentration (IC₅₀) values.

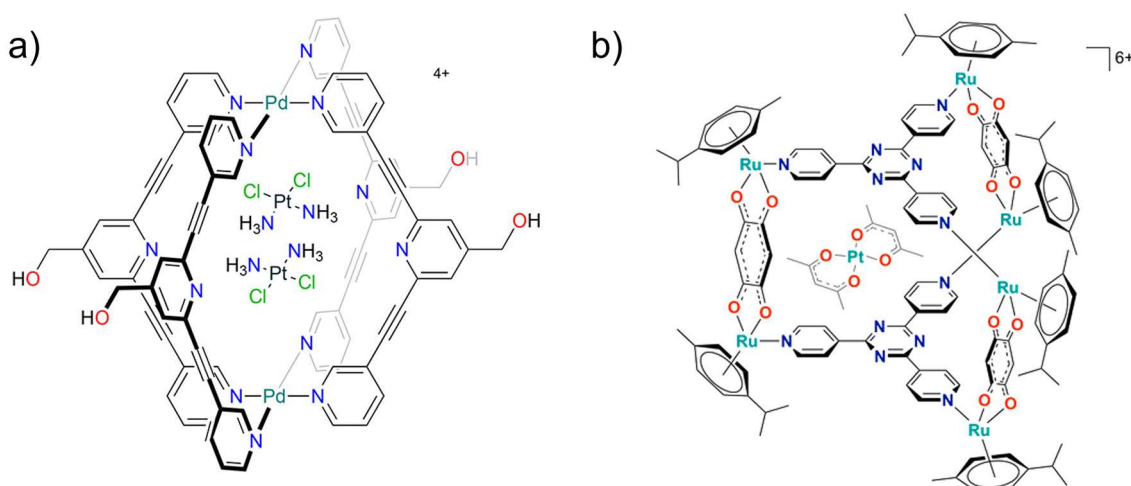


Figure 9. Bioactive host-guest inclusion complexes of SCCs. [Adapted from Ref. 73. Copyright © 2017 American Chemical Society]

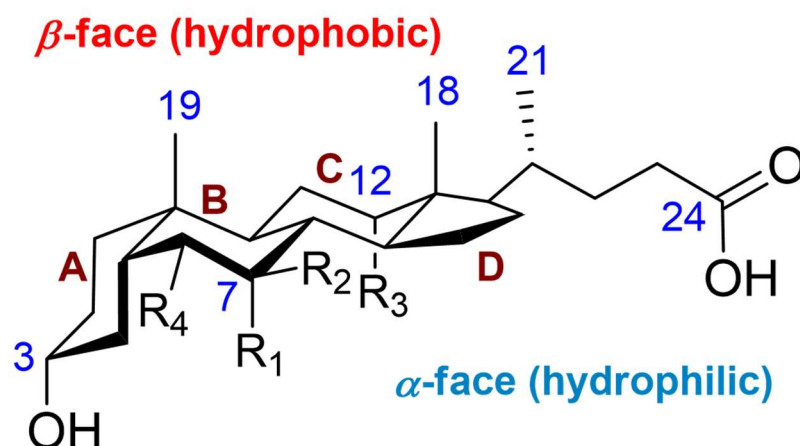
Another interesting SCC, made of Ru²⁺ and tritopic pyridyl ligand, upon encapsulation of [Pt(acac)]²⁺ (acac = acetylacetonato), the 1:1 host-guest complex displays an IC₅₀ value of approximately 1 μM on human ovarian cancer cell line A2780 (Figure 9b). Although the cage itself has an IC₅₀ value of around 23 μM, [Pt(acac)]²⁺ does not show any cytotoxicity.⁷⁷ Therefore, this indicates that nanocavities of SCCs, by providing shelter, can enhance the stability or solubility of the drugs in the biological environment, thus providing more efficient delivery to target tissues or cells. Moreover, these SCCs can enhance the bioavailability as exhibited by bigger⁷⁸ and highly charged complexes⁷⁹, which selectively accumulate in cancer cells, known as the “enhanced permeability and retention effect” (EPR effect). The main aim of chemotherapy is to increase selectivity, reducing the general toxicity and so the side effects. The versatility of SCCs allows surface modifications to tune their selectivity. This approach using synthetic

ligands relies on the trial-and-error method, which is generally demanding. This again emphasizes that natural molecules, which can function as transporters and are specific to a limited number of organs, should be used to develop SCCs. Therefore, bile-acid-based SCCs developed during my studies were exposed to spheroids of hepatoblastoma HepG2 cells. Their toxicity in correlation with their enhanced cellular uptake was investigated, displaying the relation of charge and size of SCCs, synergistically with the inherent transportation ability of BAs.

1.2 Bile Acids (BAs)

1.2.1 Structural Features

BAs are a group of steroid acids found predominantly in the bile of mammals and other vertebrates. Their biosynthesis occurs in the liver through a multi-step process known as the classical (acidic) or neutral pathway, serving as a primary pathway for hepatic cholesterol catabolism. BAs are characterized by a steroid skeleton composed of three cyclohexane rings (A, B, and C) and one cyclopentane ring (D), connected to an alkyl side chain that includes a carboxyl group (Figure 10).



Cholic acid (CA)	$R_1 = \text{OH}, R_2 = \text{H}, R_3 = \text{OH}, R_4 = \text{H}$
Ursodeoxycholid acid (UDCA)	$R_1 = \text{H}, R_2 = \text{OH}, R_3 = \text{H}, R_4 = \text{H}$
Chenodeoxycholic acid (CDCA)	$R_1 = \text{OH}, R_2 = \text{H}, R_3 = \text{H}, R_4 = \text{H}$
Lithocholic acid (LCA)	$R_1 = \text{H}, R_2 = \text{H}, R_3 = \text{H}, R_4 = \text{H}$
Deoxycholic acid (DCA)	$R_1 = \text{H}, R_2 = \text{H}, R_3 = \text{OH}, R_4 = \text{H}$
Hyodeoxycholic acid (HDCA)	$R_1 = \text{H}, R_2 = \text{H}, R_3 = \text{H}, R_4 = \text{OH}$

Figure 10. Chemical structure of bile acids.

The steroid backbone is decorated with hydroxyl and methyl groups and contains 9-11 well-defined chiral centers, which make them enantiopure building units. The amphiphilic nature of BAs arises from these two faces of the steroid skeleton: a hydrophilic concave face (α -face) with hydroxyl groups and a hydrophobic convex face (β -face) with methyl groups. BAs differ in the number, location, and spatial orientation of their hydroxyl groups, which influence their solubility, amphiphilicity, and biological activity. These hydroxyl and carboxylic acid groups can be chemically modified to create desired coordinating or protected sites of ligands as required. The position and orientation of these hydroxyl groups and the linkers used to attach the coordination groups can further determine the flexibility of the resulting ligands, which can be further enhanced by including the alkyl chain into the coordination. The characteristic steroid skeleton of BAs and the methyl groups make them bulky, which can impose steric constraints that can provide control over supramolecular self-assembly products. These qualities enable BAs to generate a range of complementary enantiopure ligands with tunable coordinating sites, bend angles, flexibility, steric constraints, and amphiphilicity. These structural features make BAs highly appealing for developing sophisticated unsymmetric supramolecular building units with high chiral density, suitable for constructing the “next generation” SCCs.

1.2.2 Bio-functional Assessment

BAs regulate the digestion and absorption of dietary lipids and fat-soluble vitamins (A, D, E, and K). This digestive function is linked to the recycling process known as enterohepatic circulation (Figure 11), the most efficient circulation in the human body, reabsorbs approximately 95% of bile acids.⁸⁰ This cycle involves the secretion of bile by the liver into the gallbladder where it is stored, followed by the release in the small intestine in response to food intake (particularly fats), reabsorption in the terminal ileum (mostly mediated by apical sodium-dependent bile acid transporter (ASBT)) into the bloodstream *via* the portal vein, and transporting back to the liver. Moreover, bile acids act as potent signaling molecules. They activate nuclear receptors such as the farnesoid X receptor (FXR) and the G protein-coupled bile acid receptor 1 (GPBAR1, also known as TGR5). These signaling processes control lipid and glucose metabolism, modulate inflammation, and influence energy balance, overall maintaining metabolic homeostasis and supporting liver and intestinal health.

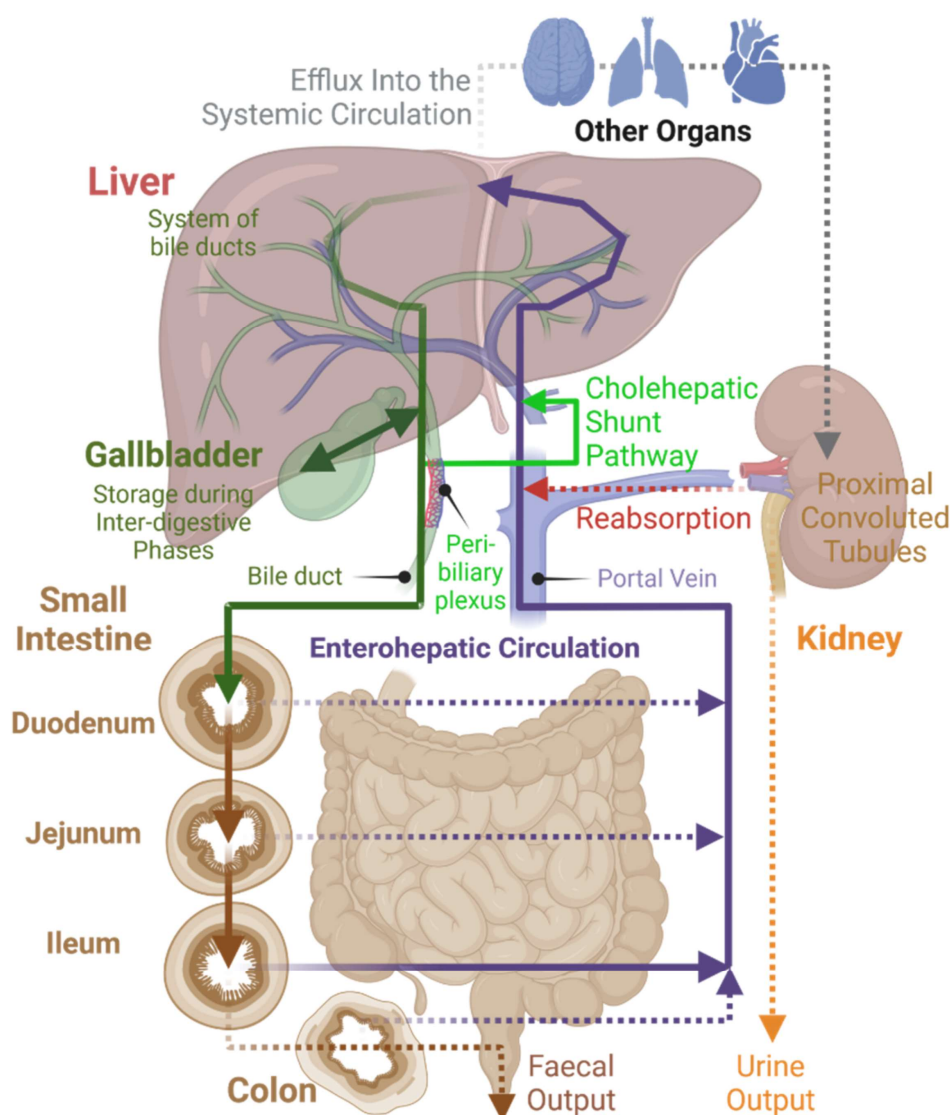


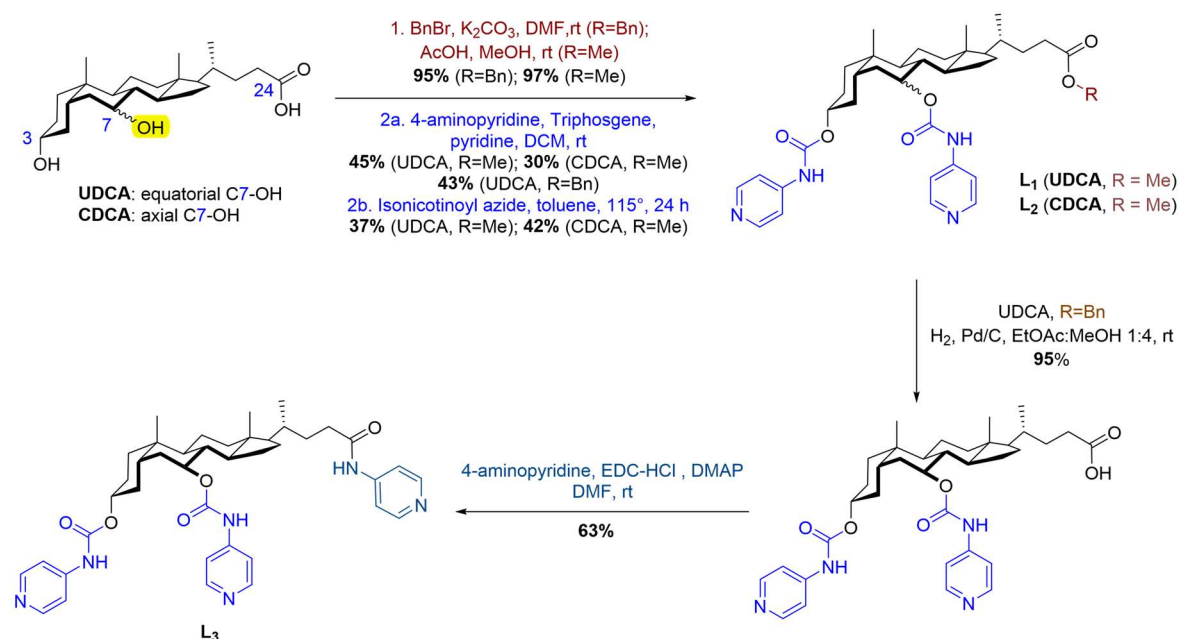
Figure 11. Enterohepatic circulation of bile acids. [Reprinted from Ref. 80. CC BY-NC 4.0 license. Copyright © 2022 The Author(s), Molecules published by MDPI]

Moreover, UDCA is an FDA (the Food and Drug Administration)-approved medication used to treat liver and gallbladder problems, such as primary biliary cholangitis (PBC) and cholesterol gallstones. It promotes bile flow, lowers liver damage in cholestatic liver disorders, and may help liver function in transplant patients by lowering bile acid toxicity. Therefore, in addition to the appealing structural features, BAs hold the potential for biomedical applications targeting the transport and enhanced cellular uptake to the organs involved in the enterohepatic circulation, especially the Pd-SCCs as an anticancer agent.

2 Results and Discussion

2.1 Synthesis of Bile Acid-Based Pyridyl Ligands

A general approach was established for synthesizing unsymmetric chiral building units for metallo-supramolecular complexes using bile acids (Scheme 1). The initial focus was on developing bile-acid-based ditopic pyridyl ligands. Building on the prior work of Jurček et al.,^{68,69} the alkyl carboxylic acid group at C24 was methylated to prevent it from interfering with synthesis and later in coordination processes. Previous studies indicated that a UDCA-based *bis*-pyridyl ligand narcissistically self-assembles into an enantiomerically pure Pd₃L₆ macrocycle upon coordination with Pd²⁺ ions. To deepen the understanding of coordination-driven self-assembly and orientational self-sorting, the bile acid family of coordinating ligand was extended using chenodeoxycholic acid (CDCA) as the ligand's core, an epimer of UDCA. The hydroxyl groups at C3 and C7 were appended by pyridyl coordination sites via carbamate linkages. Two synthetic strategies were employed: 1) the triphosgene pathway, and 2) through isonicotinoyl azide, leading to **L**₁ (UDCA ligand, 45% vs. 37%, respectively) and **L**₂ (CDCA ligand, 30% vs. 42%, respectively) (Scheme 1).



Scheme 1. Sequential synthesis of *bis*- and *tris*-pyridyl ligands from UDCA and CDCA.

To further explore the influence of flexibility, topicity, and BAs as molecular panels on self-assembly, a *tris*-pyridyl ligand (**L**₃) was synthesized by converting the carboxylic acid on the flexible alkyl chain of UDCA into a pyridyl group through amide

bond formation using 4-aminopyridine. The approach for modifying C3 and C7 hydroxyls remained consistent with that of the *bis*-pyridyl ligand **L1**. The alkyl carboxylic acid was first protected as a benzyl ester. Following the incorporation of pyridyl coordinating sites at the C3 and C7 hydroxyls, the benzyl group was selectively removed by hydrogenation, which subsequently allowed for amide bond formation at C24. This synthetic strategy allowed for the precise conversion of the head, tail, and core (belly) regions of bile acids into coordination sites as needed, covering edge-directive and face-directive self-assembly mechanisms.

This approach facilitates a systematic comparison of interconnected parameters inherent to natural molecules, such as bend angle, flexibility, and steric influence, while integrating a chiral steroidal scaffold and unsymmetry into the resulting coordination systems. The ditopic ligands primarily formed metallo-macrocycles, whereas the tri-topic ligands yielded metallo-cages, each of which is discussed in detail below. Overall, this synthetic framework provides a platform to explore supramolecular coordination chemistry with unsymmetric, chiral, and flexible building blocks derived from natural products.

2.2 Selectivity of Orientational Self-Sorting of Metallo-Supramolecular Macrocycles

2.2.1 Synthesis and Characterization

The complexation reaction was performed with a 10 mM solution of UDCA and CDCA-based *bis*-pyridyl ligands (**L1** and **L2**) with Pd(NO₃)₂·2H₂O in DMSO-*d*₆ at M:L 1:2, 55 °C for 1 hour. ¹H-NMR spectroscopic analysis showed significant chemical shift changes for carbamate -NH- and aromatic protons, indicating the quantitative formation of the metallo-complexes (Figure 12). The complexation of **L1** shows sharp signals and an identical number of proton signals to that of the free ligand, which suggests the formation of a single symmetric species. In contrast, the signals for the complexation of **L2** became broad and unsymmetric, which might suggest the formation of a mixture of species or conformational isomers.

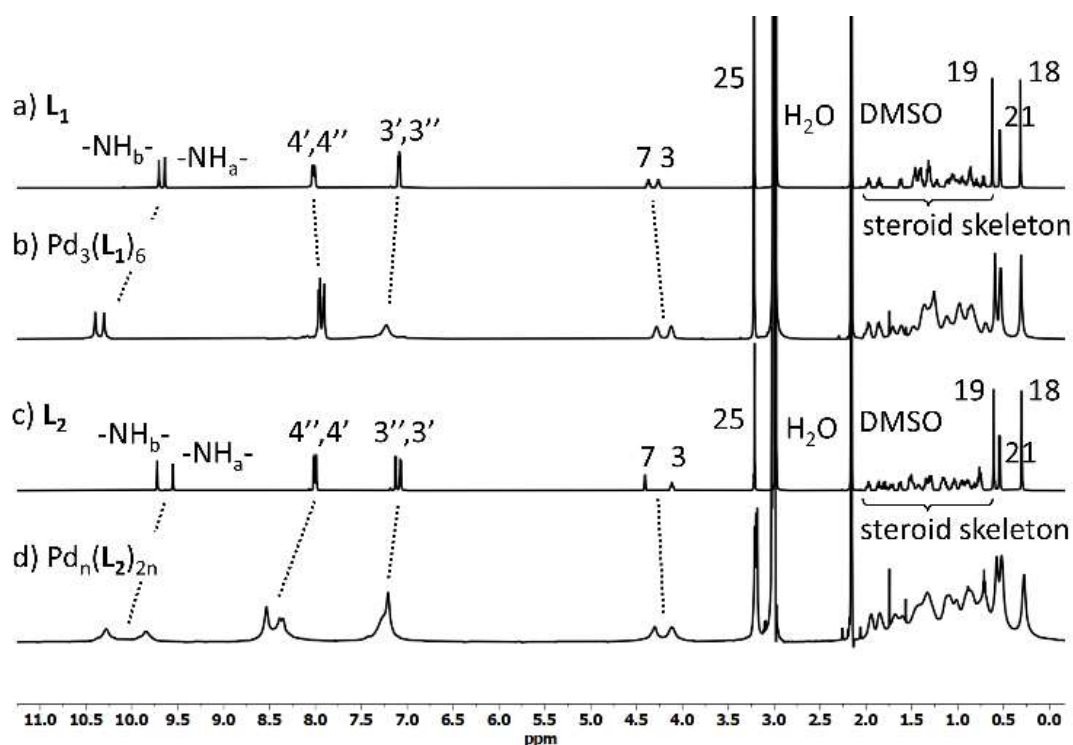


Figure 12. ^1H -NMR spectra of L_1 , L_2 , and their complexation reaction with $\text{Pd}(\text{NO}_3)_2$ (700 MHz, 298.2 K, $\text{DMSO-}d_6$). [Reprinted from Ref. 81. CC BY-NC 4.0 license. Copyright © 2024 The Authors. *Angewandte Chemie International Edition* published by Wiley-VCH GmbH]

These solutions were further analyzed using IM-MS and ^1H -DOSY NMR spectroscopy (Figure 13), which supports the assumptions made based on the ^1H -NMR spectroscopy. Complexation of L_1 results in the selective formation of $\text{Pd}_3(\text{L}_1)_6$, whereas that of L_2 produces a mixture of species having a general formula $\text{Pd}_n(\text{L}_2)_{2n}$, ranging from $\text{Pd}_2(\text{L}_2)_4$ to the largest $\text{Pd}_6(\text{L}_2)_{12}$.

To control the distribution of the species, preferably limited to one system, various complexation reactions were performed with L_2 , such as using various solvents or their mixtures (e.g., acetone- d_6 , or solvent mixtures of $\text{D}_2\text{O}:\text{DMSO-}d_6$ 1:5 and acetonitrile- $d_3:\text{DMSO-}d_6$ 9:1), palladium salts (e.g., $\text{Pd}(\text{CH}_3\text{CN})_4(\text{BF}_4)_2$), temperature (e.g., 70 °C and 120 °C), and varying time (e.g., 2, 5, and 24 hours), which led to similar or more complex mixtures (as observed in ^1H -NMR spectra (Figure S1) or insoluble precipitates. To further investigate the complexation process, the reaction pathway was modified. Complexation was first carried out with 10 mM L_2 in $\text{DMSO-}d_6$ using PdCl_2 at M:L 1:1 at 55 °C for 1 hour, yielding a narrow distribution of species with the general formula $\text{Pd}_n(\text{L}_2)_n\text{Cl}_{2n}$ (Figure S2 and Figure S3). When AgBF_4 was subsequently added, a similar distribution of $\text{Pd}_n(\text{L}_2)_{2n}$ species was observed (Figure S2 and Figure S4). Thus, it appears that upon complexation, L_2 tends to form a range of kinetically trapped species, likely due to comparable energy profiles associated with their formation.

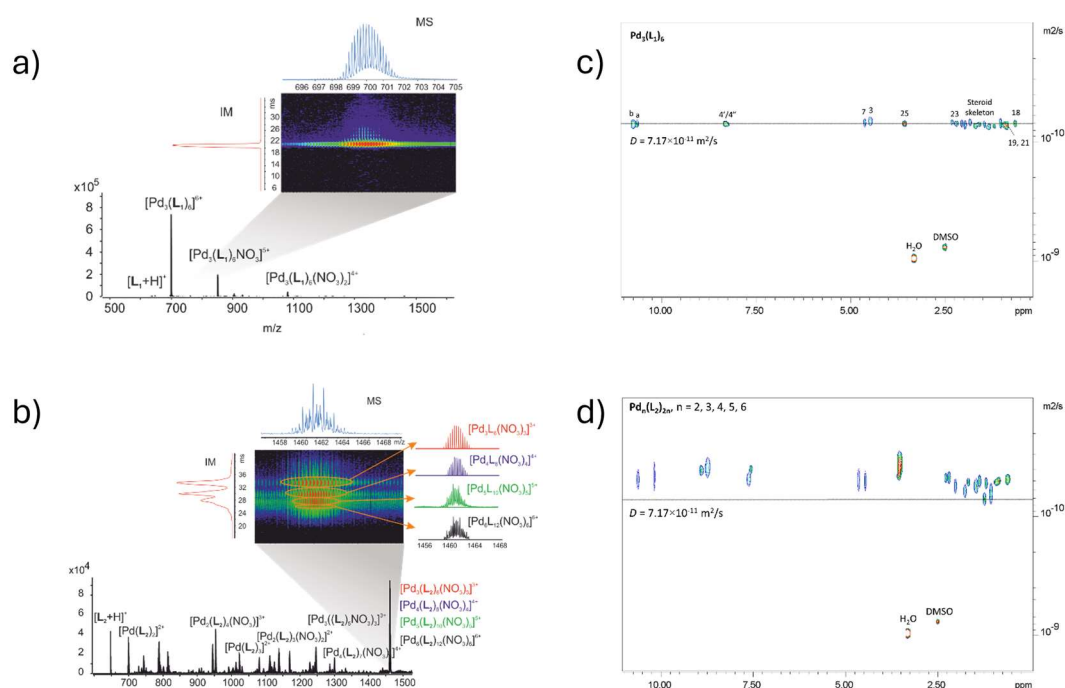


Figure 13. IM-MS and ^1H -DOSY NMR (700 MHz, 303.2 K, $\text{DMSO}-d_6$) spectra of the complexation mixtures of L_1 and L_2 . [Adapted from Ref. 81. CC BY-NC 4.0 license. Copyright © 2024 The Authors. *Angewandte Chemie International Edition* published by Wiley-VCH GmbH]

2.2.2 Computational Modeling

While SC-XRD is widely regarded as the most accurate method for structural determination, obtaining high-quality single crystals can be challenging for many systems. There is a growing need for alternative, reliable, and straightforward methods, particularly for complexes involving unsymmetric and flexible ligands. Therefore, to elucidate the structures of the coordination complexes, a combination of techniques was employed, including IM-MS, DFT modeling, ^1H -DOSY NMR, ^1H - ^1H NOESY NMR, CD spectroscopy, and structure-transformation analysis.

For the $\text{Pd}_3(\text{L}_1)_6$ complex, the unsymmetry of the ligand theoretically allows for 9 possible conformational isomers. However, many of these structures are not energetically, sterically, or geometrically accessible under the reaction conditions. Additionally, the identical number of ^1H -NMR chemical shifts and splitting pattern comparable with the free ligand suggests the formation of a symmetric self-assembly, indicating the presence of C_3 symmetry in $\text{Pd}_3(\text{L}_1)_6$. Given these considerations, only two conformational isomers are feasible: a crown-like structure and a barrel-like structure, both of which were further examined through energy-optimized DFT models (Figure 14). The $\text{Pd}_3(\text{L}_1)_6$ complex can be conceptually divided into a top and bottom section by an imaginary plane passing through the Pd^{2+} centers. Each section consists of three ligands

linked into a cyclic arrangement with a (C7-pyridyl)-Pd²⁺-(C3-pyridyl) connectivity, described as 7-Pd-3. The distinction between the crown and barrel forms arises from the relative orientations of these cycles: in the crown form, both cycles align in the same direction, creating a 3,3-Pd-7,7 coordination sphere (Figure 14a). Conversely, in the barrel form, the cycles are oriented in opposite directions, resulting in a 3,7-Pd-3,7 coordination sphere (Figure 14b).

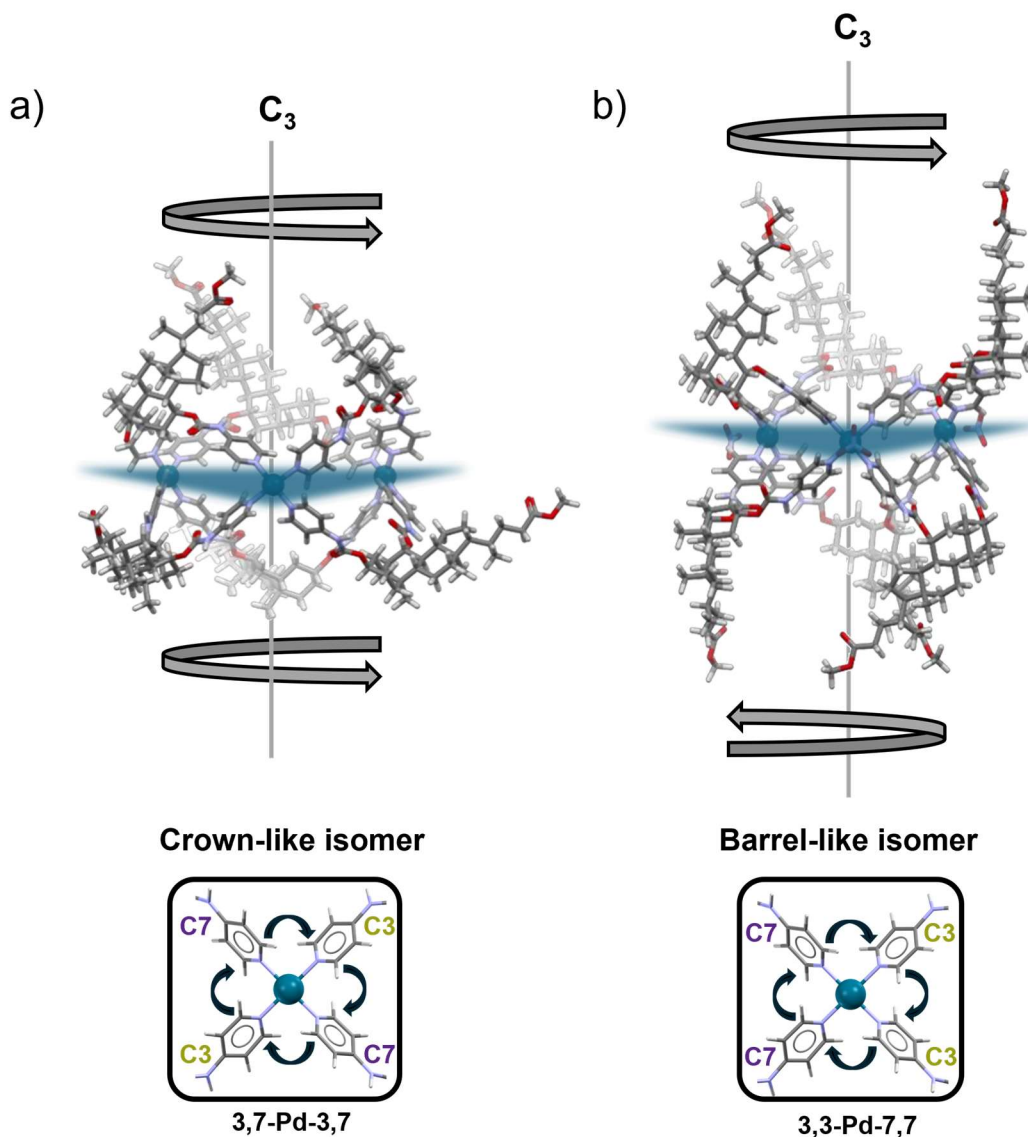


Figure 14. DFT-optimized constitutional isomers of $\text{Pd}_3(\text{L}_1)_6$ having C_3 symmetry. [Adapted from Ref. 81. CC BY-NC 4.0 license. Copyright © 2024 The Authors. *Angewandte Chemie International Edition* published by Wiley-VCH GmbH]

A comparison of the ground state energies of the optimized molecular models of $\text{Pd}_3(\text{L}_1)_6$ revealed a difference of 10 kcal/mol, favoring the crown-like model over the barrel-like isomer. Given the amphiphilic nature and inherent tendency to aggregate

in polar environments, bile acids are likely to favor the self-assembly of the crown-like isomer. To further support the computational analysis, the diameter of the DFT-optimized models is compared with the experimental diameter obtained using IM-MS and ^1H -DOSY NMR spectra. IM-MS provides a collision cross section (CCS) value of $973.7 \text{ \AA}^2$ from which the diameter of the complex was found to be 3.5 nm, and the diffusion coefficient was $D = 7.17 \times 10^{-11} \text{ m}^2/\text{s}$ accounting for the hydrodynamic diameter (D_h) of 3.4 nm (applying Stokes–Einstein–Sutherland equation). These experimental values are in good agreement with the theoretical crown-like model (3.3 nm).

2.2.3 ^1H - ^1H NOESY NMR

^1H - ^1H NOESY NMR analysis of the $\text{Pd}_3(\text{L}_1)_6$ complex revealed strong dipolar nuclear spin-spin interactions between the C25-methyl group on the flexible alkyl chain and several protons of the steroid nucleus, consistent with a crown-like self-assembly (Figure 15). This arrangement places the C25-methyl group in proximity to adjacent ligands. In contrast, a barrel-like assembly would direct the C25-methyl outward, preventing such interactions.

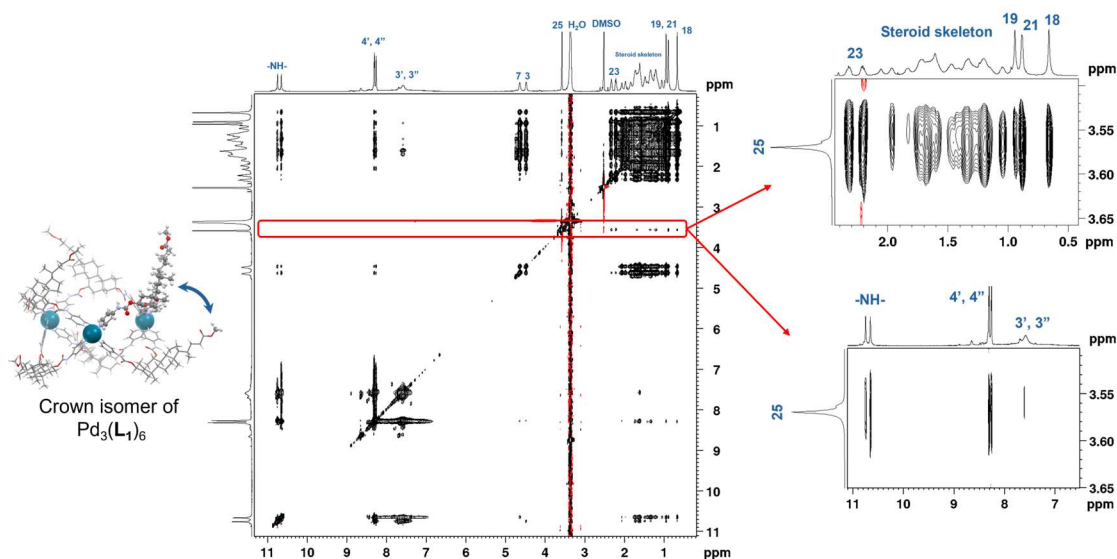


Figure 15. ^1H - ^1H NOESY NMR spectra (700 MHz, 298.2 K, $\text{DMSO}-d_6$) of $\text{Pd}_3(\text{L}_1)_6$. [Adapted from Ref. 81. CC BY-NC 4.0 license. Copyright © 2024 The Authors. *Angewandte Chemie International Edition* published by Wiley-VCH GmbH]

2.2.4 Structure-transformation Analysis

To validate the structure, a structure-transformation analysis using tetrabutylammonium chloride (TBACl) was performed on the crown-like $\text{Pd}_3(\text{L}_1)_6$ complex (Figure 16). ^1H -NMR analysis showed a single new set of signals for coordinated L_1

alongside signals for free **L1**, indicating the formation of either *cis* or *trans* isomer of the $\text{Pd}_3(\text{L}_1)_3\text{Cl}_6$ (Figure S5). ^1H -DOSY NMR measured a diffusion coefficient of $7.83 \times 10^{-11} \text{ m}^2/\text{s}$ (Figure S6), corresponding to a hydrodynamic diameter (D_h) of 3.1 nm, smaller than the crown-like $\text{Pd}_3(\text{L}_1)_6$ ($D = 7.17 \times 10^{-11} \text{ m}^2/\text{s}$; $D_h = 3.4 \text{ nm}$, DMSO- d_6 , 303.2 K, Figure 13c).

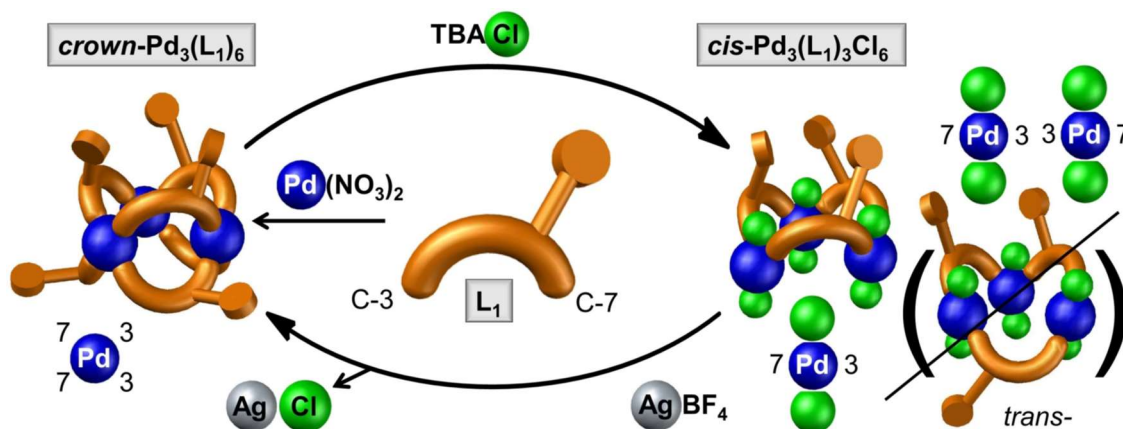


Figure 16. Structure-transformation analysis of $\text{Pd}_3(\text{L}_1)_6$. [Adapted from Ref. 81. CC BY-NC 4.0 license. Copyright © 2024 The Authors. Angewandte Chemie International Edition published by Wiley-VCH GmbH]

Moreover, the addition of AgBF_4 to the solution reproduces the same $\text{Pd}_3(\text{L}_1)_6$ metallo-cycle (Figure S5). Molecular modeling also confirmed that this matches the *cis*-isomer (Figure S7), indicating its selective formation through the deconstruction of the crown-like structure. The barrel-like $\text{Pd}_3(\text{L}_1)_6$ would produce both *cis*- and *trans*-forms, further supporting the crown-like structural assignment.

2.2.5 CD Spectroscopy

The CD spectroscopic analysis of the $\text{Pd}_3(\text{L}_1)_6$ complex displays a strong negative band at approximately 275 nm and a strong positive band around 250 nm, resulting in a (- +) CD couplet. In contrast, the free ligand exhibits weak CD signals, likely due to its inherent chiral centers. This CD spectral pattern confirms the self-assembly of **L1**, introducing helical or super chirality. Furthermore, the CD absorption profile offers insights into the formation of either a 3,3-Pd-7,7 or 3,7-Pd-3,7 coordination sphere. For further clarity, this is elaborated in the section 2.3; however, the observed (- +) CD couplet serves, for now, as indicative evidence supporting the proposed crown-like self-assembly (Figure S8).

2.2.6 Complexation of the Epimer (**L**₂)

The complexation of **L**₂ has yielded a mixture of species having the general formula Pd_n(**L**₂)_{2n}. Some of the complexes can exist in multiple structures, whereas others form only one. For example, Pd₂(**L**₂)₄ forms only a lantern-shaped cage, whereas Pd₃(**L**₂)₆, and Pd₅(**L**₂)₁₀, exist solely as polygonal macrocyclic structure. Now, Pd₄(**L**₂)₈ complex can adopt a polygonal macrocyclic, tetrahedral, or mechanically interlocked structure. Similarly, the Pd₆(**L**₂)₁₂ complex also has the possibility of forming polygonal macrocyclic, cuboid, or mechanically interlocked structures. Accordingly, the DFT models were constructed (Figure S9), and the theoretical CCS values were compared with the CCS values obtained from the IM-MS. The small differences between the CCS values of these constitutional isomers make precise structural determination challenging. However, ¹H-¹H NOESY NMR spectra show similar cross-peaks as observed for the Pd₃(**L**₁)₆ macrocycle (Figure S10). Also, the CD spectra of the mixture show a (- +) CD couplet (Figure S8), although it has a relatively lower signal intensity. This suggests a significant population of species existing in a crown-like macrocyclic assembly, and a small amount of the complexes may deviate from the crown-like structure, accounting for the intensity drop observed in the CD spectra. Moreover, the theoretical calculation of the ground state energy optimized structures of Pd₃(**L**₂)₆ macrocycle has a 10 kcal/mol preference for the crown-like structure over the barrel-like assembly.

2.2.7 Structural Comparison of Eimers: Bend Angle and Ring Strain

The energy-optimized DFT model shows a bend angle ($\angle \text{N}_{\text{C3-pyridyl}}-\text{C5}-\text{N}_{\text{C7-pyridyl}}$) of 117° for **L**₁ in the free form and a range of 89 to 105° in the Pd₃(**L**₁)₆ macrocycle. In contrast, **L**₂ has a bend angle of 72° in its free form, and the bend angles in the coordination complexes range from 70–91° ($\Delta 20^\circ$) (Figure 17), excluding the less abundant and energetically less probable Pd₂(**L**₂)₄ (as observed from IM-MS and ¹H-DOSY NMR analysis). Therefore, **L**₁ maintains a larger bend angle and displays a higher deviation of $\Delta 28^\circ$, compared to **L**₂ with $\Delta 20^\circ$ deviation. Overall, a larger bend angle results in a smaller Pd₃L₆ macrocycle, while smaller bend angles favor the formation of larger macrocycles, such as Pd₄L₈, Pd₅L₁₀, and Pd₆L₁₂. The assumption that larger bend angles always produce bigger coordination complexes does not apply to non-planar (rather bulky), flexible, and unsymmetric ligands and their metallo-cycles.

For **L**₂, the smaller bend angle or lower flexibility induces considerable geometric strain, which is further amplified by the bulky peripheral parts (C and D rings (Figure S11), alkyl chain) of the steroid moiety. Consequently, the thermodynamically favored Pd₃L₆ complex for **L**₁ is not the most stable for **L**₂. To relieve these strains, a distribution of kinetic species is observed, with a preference for larger macrocyclic structures. The reduced flexibility of **L**₂ amplifies its bulkiness and therefore guides the formation of double-walled polygonal structures over the polyhedral structures, making the

formation of tetrahedral (Pd_4L_8), cuboid (Pd_6L_{12}), or mechanically interlocked conformational isomers unlikely. In summary, it seems that increased flexibility of the ligand enhances its tendency for orientational self-sorting. This is a summary of the work from **Publication 1**, and please follow the original article in Appendix A for more information.

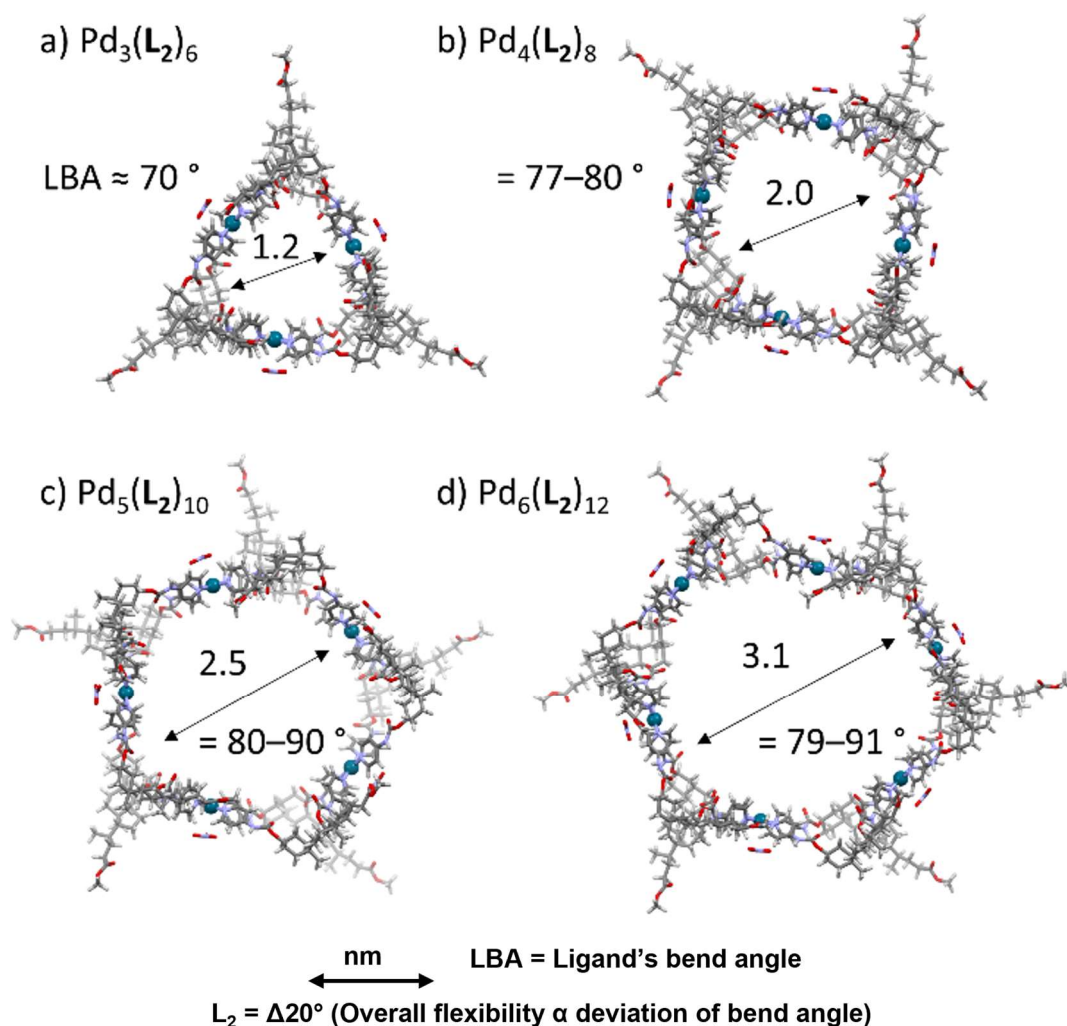


Figure 17. DFT-optimized crown-like $\text{Pd}_n(\text{L}_2)_{2n}$ macrocycles and nomenclature of rings of steroid nucleus. [Adapted from Ref. 81. CC BY-NC 4.0 license. Copyright © 2024 The Authors. *Angewandte Chemie International Edition* published by Wiley-VCH GmbH]

2.3 Flexibility-aided Orientational Self-Sorting and Transformation of Homochiral, and Bioactive Metallo-supramolecular Cages

2.3.1 Synthesis and Characterisation

To further investigate the effect of increased flexibility, topicity, and role of bile acid as a molecular panel in self-assembly, a UDCA-based *tris*-pyridyl ligand (**L**₃) was synthesized and the initial complexation reaction of **L**₃ (10 mM in DMSO-*d*₆) with [Pd(CH₃CN)₄](BF₄)₂ (M:L 3:4, 70 °C, 1 h) shows the formation of a mixture of Pd₆(**L**₃)₈ and Pd₁₂(**L**₃)₁₆ SCCs (reaction mixture 1 (**RM** 1), Figure 18a (green), Figure 18b and Figure S12).

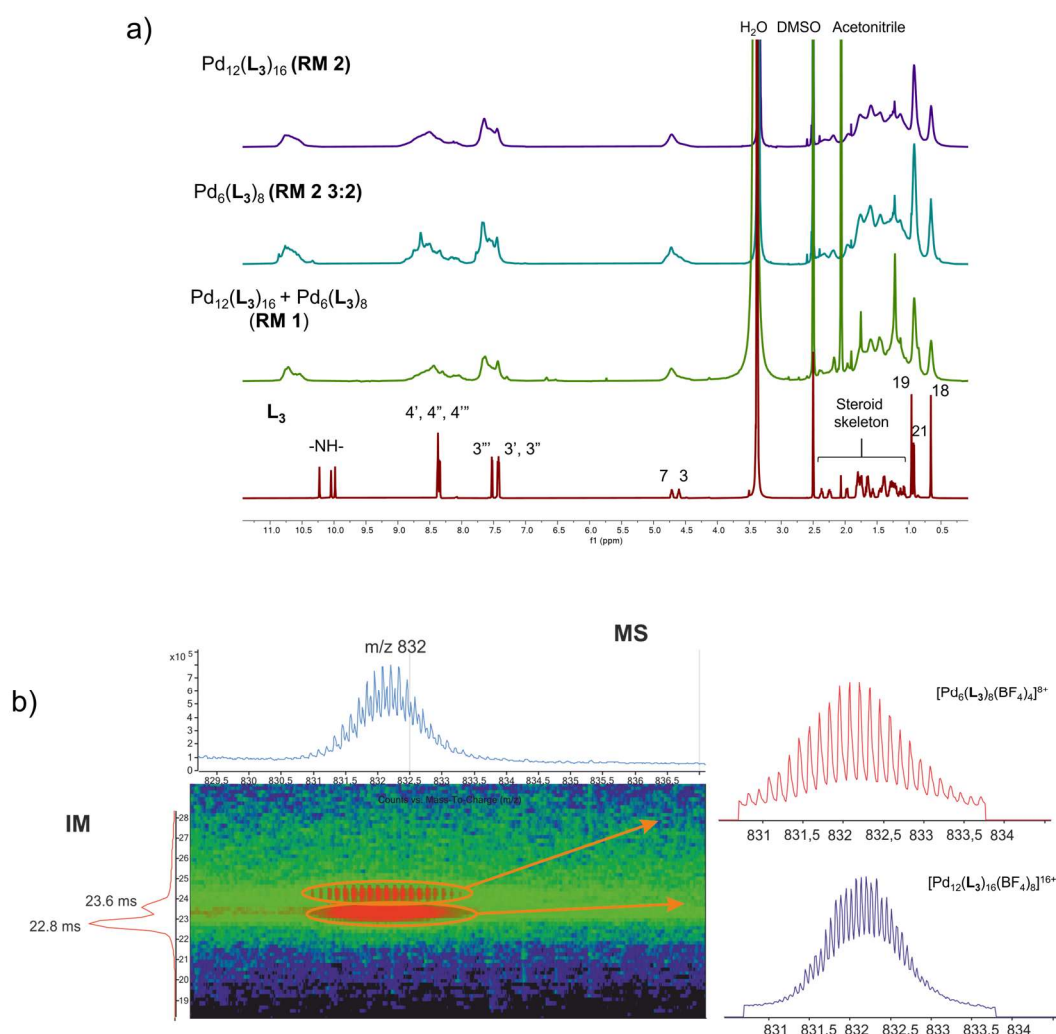


Figure 18. ¹H-NMR (700 MHz, 298.2 K, DMSO-*d*₆) and IM-MS spectra of Pd₁₂(**L**₃)₁₆ and Pd₆(**L**₃)₈. [Adapted from Ref. 82. CC BY-NC 4.0 license. Copyright © 2025 The Authors. *Angewandte Chemie International Edition* published by Wiley-VCH GmbH]

The selective formation of coordination cages using ligand **L3** (10 mM) was achieved with $\text{Pd}(\text{NO}_3)_2$ (70 °C, 1h) under three different reaction conditions:

1. M:L of **3:4** in **DMSO-*d*₆**, yielding $\text{Pd}_{12}(\text{L}_3)_{16}$ (**RM 2**).
2. M:L of **3:2** in **DMSO-*d*₆**, yielding $\text{Pd}_6(\text{L}_3)_8$ (**RM 2 3:2**; Figure 18 and Figure 19).
3. M:L of **3:4**, but in a solvent mixture of **95:5 ACN-*d*₃:DMSO-*d*₆**, yielding $\text{Pd}_6(\text{L}_3)_8$ (**RM 3**, Figure S13 and Figure S14).

Hydrodynamic diameters of 5.8 nm ($D = 4.98 \times 10^{-11} \text{ m}^2/\text{s}$, **RM2**, $\text{Pd}_{12}(\text{L}_3)_{16}$) and 4.8 nm ($D = 5.98 \times 10^{-11} \text{ m}^2/\text{s}$, **RM2 3:2**, $\text{Pd}_6(\text{L}_3)_8$) were determined from diffusion coefficients using the Stokes-Einstein equation. These values show good to moderate agreement with diameters estimated from CCS data *via* IM-MS, confirming the formation of $\text{Pd}_6(\text{L}_3)_8$ and $\text{Pd}_{12}(\text{L}_3)_{16}$. Moreover, IM-MS analysis of this sample confirms that $\text{Pd}_{12}(\text{L}_3)_{16}$ exists as a monomer rather than a dimer of $\text{Pd}_6(\text{L}_3)_8$, based on factors such as specific intensity abundance, concentration independence, collision cross-section, and the presence of odd-charge states in the ions.

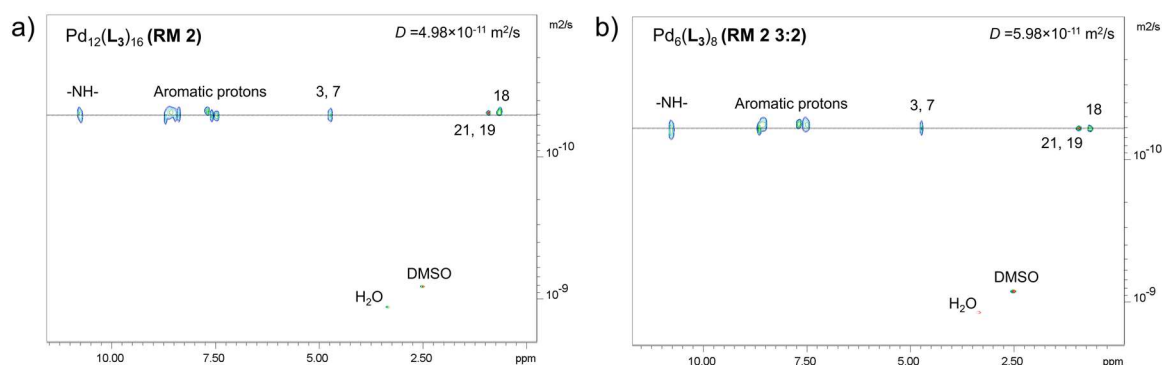


Figure 19. ^1H -DOSY NMR (700 MHz, 303.2 K, **DMSO-*d*₆**) spectra of $\text{Pd}_{12}(\text{L}_3)_{16}$ and $\text{Pd}_6(\text{L}_3)_8$.

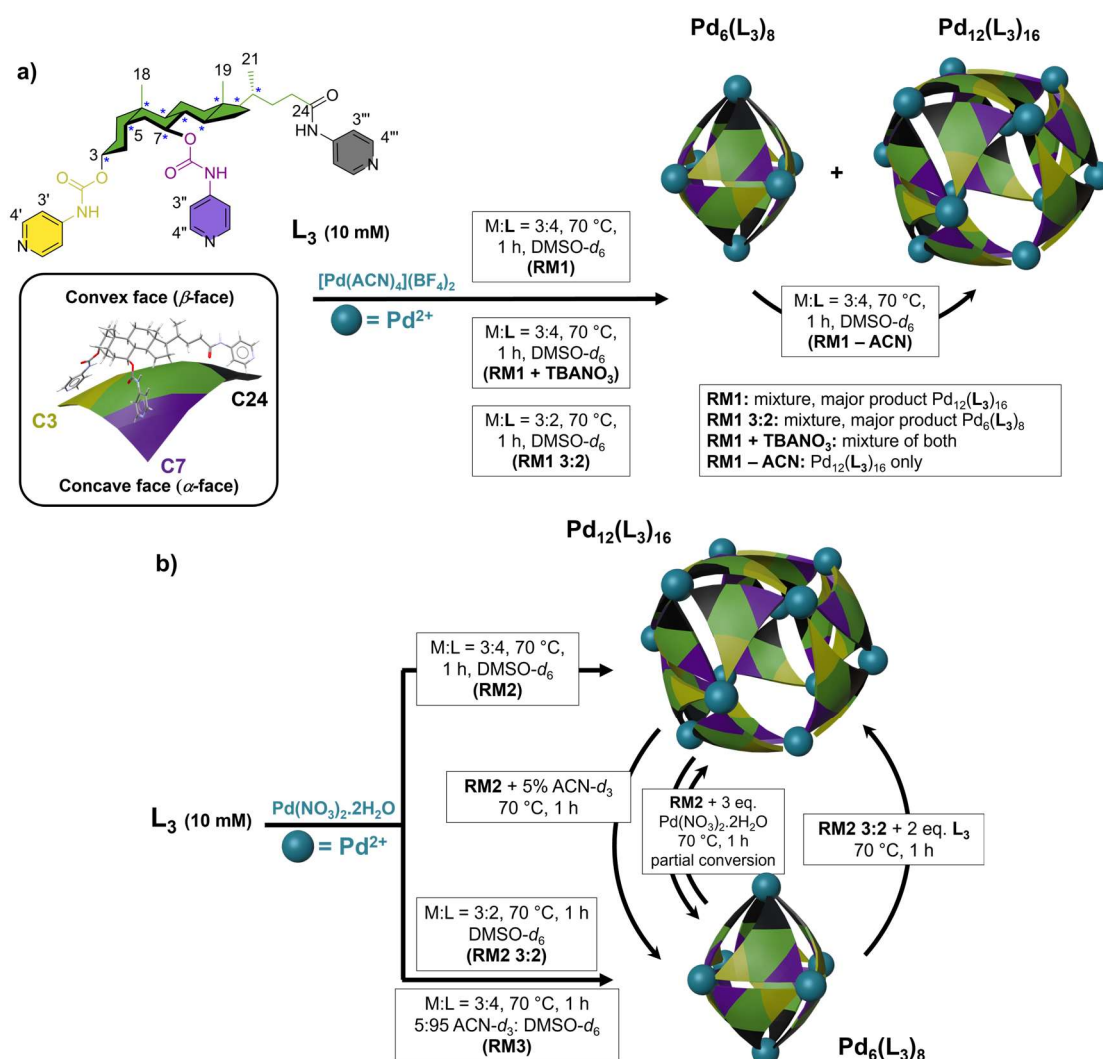
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2.3.2 Interspecific Transformation

To investigate the potential for interspecies transformations and the reaction kinetics of the coordination assemblies, a series of reactions was carried out (described in Scheme 2) and monitored using ^1H NMR spectroscopy, ^1H - ^1H DOSY NMR, and IM-MS. Complexation of ligand **L3** under standard **RM1** conditions predominantly yielded the $\text{Pd}_{12}(\text{L}_3)_{16}$ species, comprising approximately 75% of the total product distribution relative to $\text{Pd}_6(\text{L}_3)_8$. In contrast, repeating the **RM1** procedure with an excess of metal salt (metal-to-ligand ratio of 3:2, **RM1 3:2**) favored the formation of $\text{Pd}_6(\text{L}_3)_8$ as the major species. Additionally, subjecting the **RM1** reaction mixture to prolonged heating (70 °C, 24 hours) shifted the equilibrium further toward $\text{Pd}_6(\text{L}_3)_8$. These relative

product distributions were determined by comparing the intensities of the drift peaks in the IM-MS spectra.

Interestingly, when analogous reaction conditions were employed using $\text{Pd}(\text{NO}_3)_2$ as the metal source, *i.e.*, **RM2** and **RM2 3:2**, selective formation of $\text{Pd}_{12}(\text{L}_3)_{16}$ and $\text{Pd}_6(\text{L}_3)_8$ was achieved, respectively. These observations suggest that variables such as the presence of excess metal ions, the nature of the counter anions, and solvent effects (*e.g.*, the coordinating ability of acetonitrile) may significantly influence this dynamic coordination chemistry and related interspecies transformations. Each of these factors was therefore investigated independently in subsequent experiments.



Scheme 2. Schematic representation of coordination-driven self-assemblies of L_3 and their interconversion into homochiral $\text{Pd}_{12}(\text{L}_3)_{16}$ cuboctahedron and $\text{Pd}_6(\text{L}_3)_8$ octahedron. [Adapted from Ref. 82. CC BY-NC 4.0 license. Copyright © 2025 The Authors. Angewandte Chemie International Edition published by Wiley-VCH GmbH]

• Solvent-controlled experiments

To evaluate the influence of acetonitrile on the formation and interconversion of coordination species, solvent-controlled experiments were systematically conducted. In the first experiment, the **RM1** solution was evaporated overnight under vacuum at 40 °C to remove residual acetonitrile. The resulting solid was then redissolved in DMSO-*d*₆ and heated at 70 °C for 1 hour (denoted as **RM1-ACN**). Under these conditions, complete conversion to Pd₁₂(L₃)₁₆ was observed. ¹H NMR spectroscopy confirmed the near-complete removal of acetonitrile, with the Pd:ACN molar ratio decreasing from 1:4 in the original **RM1** mixture to approximately 1:0.2.

To further probe the effect of acetonitrile, a parallel reaction was conducted under **RM2** conditions but using a mixed solvent system of 5% (v/v) ACN-*d*₃ in DMSO-*d*₆ (denoted as **RM3**). Due to the poor solubility of L₃ in neat ACN-*d*₃, this solvent mixture was chosen to maintain solubility. The resulting Pd:ACN ratio in **RM3** was approximately 1:128, in contrast to the 1:4 ratio in **RM1**. Under these conditions, selective formation of Pd₆(L₃)₈ was observed.

Moreover, when 5% (v/v) ACN-*d*₃ was introduced post-synthetically to the **RM2** reaction mixture (denoted as **RM2 + 5% ACN-*d*₃**), followed by heating at 70 °C for 1 hour, a complete transformation to Pd₆(L₃)₈ was again observed. These experiments collectively demonstrate a strong dependence of product distribution on the concentration of acetonitrile. Specifically, higher concentrations of acetonitrile favor the formation of Pd₆(L₃)₈, while lower concentrations shift the equilibrium toward Pd₁₂(L₃)₁₆.

• Anion exchange

Now, the comparison between **RM1-ACN** and **RM2**, where almost an identical environment is established except for the counter anions, *i.e.*, BF₄⁻ or NO₃⁻, demonstrated that these counter anions do not strongly influence the reaction kinetics, as both reactions led to the formation of Pd₁₂(L₃)₁₆. To further investigate this matter, 1.5 eq. (M:L:NO₃⁻ 3:4:6) and 3 eq. (M:L:NO₃⁻ 3:4:12) of TBANO₃ with respect to L₃ were subsequently added to **RM1** (post-synthesis), and the reaction mixture was heated at 70 °C for 1 h after every addition and even continued for 24 hours (denoted as **RM1+TBANO₃**). Separately, the same amounts of the TBANO₃ were introduced with the precursors of **RM1** as well, accounting for the presence of excess nitrate anion from the beginning, but in all these abovementioned cases, the solution is composed of the mixtures of SCCs. These additional experiments reassure that the nitrate anion does not strongly influence the equilibrium between the SCCs.

- **Metal-ligand ratio**

This reflects clarity regarding **RM2 3:2** that it is the presence of excess metal cations responsible for the formation of $\text{Pd}_6(\text{L}_3)_8$. Furthermore, when two equivalents of free L_3 were introduced to **RM2 3:2**, followed by heating at 70 °C for 1 hour (denoted as **RM2 3:2+2 eq. L₃**), shifting the M:L 3:2 to M:L 3:4, results in transformation into $\text{Pd}_{12}(\text{L}_3)_{16}$. Similarly, when 3 equivalents of $\text{Pd}(\text{NO}_3)_2$ were introduced to a solution of **RM2** (denoted as **RM2+3 eq. Pd(NO₃)₂**) resulted in the partial conversion of $\text{Pd}_{12}(\text{L}_3)_{16}$ to $\text{Pd}_6(\text{L}_3)_8$. These experiments further support the hypothesis on the influence of excess metal cations on the reaction dynamics. ^1H - ^1H DOSY NMR spectroscopic analysis was summarized in Table S1, and for further experimental and analytical details, please follow Error! Reference source not found..

- **Solvent/metal ion effect on dynamics and equilibrium**

The formation of two SCCs in the reaction mixture suggests the formation of thermodynamic and kinetically trapped species. Hiraoka *et al.* have shown that ACN accelerates ligand exchange *via* a solvent-assisted self-assembly process, which prevents the formation of primitive intermediates leading to kinetic products, thereby leading to thermodynamic products.⁸³ In this system, the presence of excess ACN favors the $\text{Pd}_6(\text{L}_3)_8$ complex, making it the thermodynamic product, whereas its absence in DMSO leads to the kinetically favored $\text{Pd}_{12}(\text{L}_3)_{16}$.

This can be attributed to ACN increasing the reversibility of Pd(II)-ligand coordination bonds, enhancing the dynamic nature of the assembly. During self-assembly, ligands and metals initially form oligomers of varying sizes before final cage formation.⁸⁴ Under excess ACN, faster ligand exchange results in smaller oligomers, directing the system toward $\text{Pd}_6(\text{L}_3)_8$. A similar effect is observed with excess $\text{Pd}(\text{NO}_3)_2$, where abundant Pd^{2+} ions favor the formation of smaller oligomers and thus smaller final assemblies. In contrast, slower exchange in the absence of ACN allows oligomers to grow larger and become trapped, ultimately yielding the larger $\text{Pd}_{12}(\text{L}_3)_{16}$ species. Moreover, the interface flexibility of the ligand itself participates in the nucleation and growth of these supramolecular networks.⁸⁵

2.3.3 ^1H NMR Broadening

As for the broadening of the ^1H NMR signals, the larger $\text{Pd}_{12}(\text{L}_3)_{16}$ (**RM2**) cage has a slightly broader NMR lines than $\text{Pd}_6(\text{L}_3)_8$ (**RM2 3:2**) (Figure 18a). A similar pattern of chemical shift is also suggestive of both SCCs being composed of the same subunits. The broadening of the resonance lines, keeping the ^1H - ^1H DOSY NMR spectra in mind, could be associated with large conformational changes in the near vicinity of pyridyls, *i.e.*, on the C3-O-CO-NH-pyridine, C7-O-CO-NH-pyridine, and C24-CO-NH-pyridine

axes, being manifested in solution along with the unsymmetry present in the coordination sites. Variable-temperature ^1H NMR studies of **RM2** (Figure S15) revealed signal sharpening and the appearance of a second set of resonances at elevated temperatures. Moreover, these structural changes affect only a minor fraction of the species and are transient, as they are induced by elevated temperature and revert to the original form upon cooling (Table S1) with slightly resolved NMR signals. The possible explanation could be that during direct synthesis at 70 °C for 1 hour (**RM1**), some ligands appear to become trapped in conformations with local energy minima. In contrast, heating to higher temperatures facilitates access to global minimum conformations that better satisfy the spatial and geometric requirements of the assembled complex, and these conformations are retained upon cooling. This suggests the formation of a temporary constitutional isomer (through rotation of the ligand, altering the connectivity in the SCC) rather than a conformational isomer (through conformational changes within the ligand without altering its connectivity in the SCC), as one would assume that the spectral changes would be more subtle in such a case. However, the formation of a completely new species, although less probable, cannot be ignored, but its short life span limits its characterization.

2.3.4 Computational Modelling

To elucidate the structure of the species, numerous crystallization setups were employed. However, high-quality single crystals could not be obtained due to the inherent flexibility of the species. Thus, as an alternative approach, the focus was set on DFT modeling and CD spectroscopic analysis.

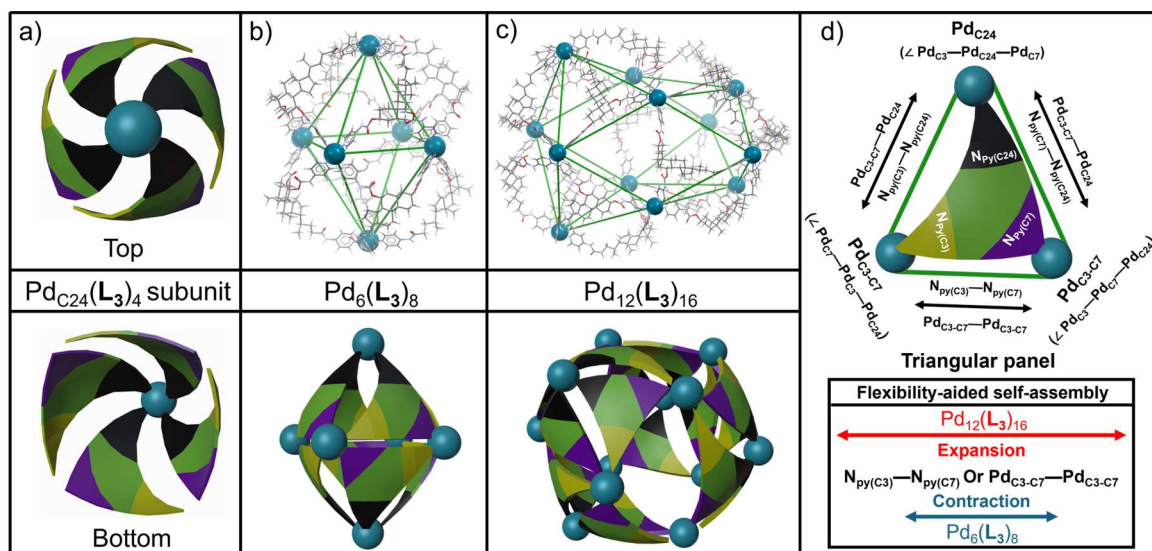


Figure 20. a)-c) Cartoon and DFT-optimized models of SCCs derived from L_3 and d) the nomenclature used for the molecular panel. [Adapted from Ref. 82. CC BY-NC 4.0 license. Copyright © 2025 The Authors. Angewandte Chemie International Edition published by Wiley-VCH GmbH]

Theoretically, there are many conformational isomers possible for the $\text{Pd}_6(\text{L}_3)_8$ and $\text{Pd}_{12}(\text{L}_3)_{16}$ metallo-cages, however, the structure of L_3 limits the formation of many isomers. L_3 can be observed as an elongated (towards C24) triangular panel with two faces, a concave (α) face containing pyridyls (Py) and a convex (β) face featuring methyl groups (C18, C19, and C21). Considering the shape and related steric strains of the self-assembly, L_3 should always point the convex face outside of the complex. As for the elongated shape, the panels will be oriented to maintain high symmetry, thereby lowering the geometric strains. Considering these points, a structural subunit was designed (Figure 20a), where four ligands are coordinated to Pd^{2+} ions through their Py_{C24} (denoted as Pd_{C24}) with their β -faces oriented outward - $\text{Pd}_{\text{C24}}(\text{L}_3)_4$. This model aligns with the CD spectroscopic study discussed in detail later. Two of these subunits were then connected through four additional Pd^{2+} ions using the remaining pyridyls at C3 and C7 (denoted as $\text{Pd}_{\text{C3-C7}}$), forming $\text{Pd}_6(\text{L}_3)_8$ (Figure 20b). The resulting optimized structure of $\text{Pd}_6(\text{L}_3)_8$ is a symmetric, axially elongated stellated octahedron. Similarly, four $\text{Pd}_{\text{C24}}(\text{L}_3)_4$ subunits were connected, two $\text{Pd}_{\text{C24}}(\text{L}_3)_4$ connected sideways with two $\text{Pd}_{\text{C3-C7}}$, resulting in a dimer, which was further connected, resembling the structure of a tennis ball, resulting in a cuboctahedral $\text{Pd}_{12}(\text{L}_3)_{16}$ structure (Figure 20c). The optimized structure represents an unprecedented formation of a low-symmetry stellated cuboctahedron *via* face-directive self-assembly of a tritopic ligand.

2.3.5 Flexibility-Aided Orientational Self-Sorting

The complexes were compared by measuring the edges and the angles of the triangular panel (represented by the $\text{Pd}-\text{Pd}$ distances and $\angle\text{Pd}-\text{Pd}-\text{Pd}$ angles) and the $\text{N}_{\text{Py}}-\text{N}_{\text{Py}}$ distances in the ligand (Figure 20d). The increase in the average $\text{Pd}_{\text{C24}}-\text{Pd}_{\text{C3-C7}}$ and $\text{Pd}_{\text{C3-C7}}-\text{Pd}_{\text{C3-C7}}$ distances from $\text{Pd}_6(\text{L}_3)_8$ to $\text{Pd}_{12}(\text{L}_3)_{16}$ are 1.1 Å and 4.3 Å, respectively. The average angles of the panel show the transformation of the triangular panel from equilateral ($\text{Pd}_6(\text{L}_3)_8$) to isosceles triangle ($\text{Pd}_{12}(\text{L}_3)_{16}$).

The increase in $\text{Pd}_{\text{C3-C7}}-\text{Pd}_{\text{C3-C7}}$ distance is reflected by the increase in $\angle\text{N}_{\text{Py}(\text{C3})}-\text{C5}-\text{N}_{\text{Py}(\text{C7})}$ bend angle from 90-93° to 105-127° in $\text{Pd}_6(\text{L}_3)_8$ to $\text{Pd}_{12}(\text{L}_3)_{16}$, respectively. Therefore, the regular consideration that a higher bend angle results in larger species remains valid for the self-assembly into polyhedral structures. This in comparison with $\angle\text{N}_{\text{Py}(\text{C3})}-\text{C5}-\text{N}_{\text{Py}(\text{C7})}$ bend angle of $\text{Pd}_3(\text{L}_1)_6$ metallo-cycle, which ranges from 89-105°, demonstrates the magnitude of flexibility in the UDCA-based pyridyl ligands.

In turn, the increasing length of $\text{Pd}_{\text{C24}}-\text{Pd}_{\text{C3-C7}}$ represents the decrease in the convexity and further elongation of the ligand towards C24. Comparison with the DFT optimized free L_3 shows significant stretching in $\text{N}_{\text{Py}(\text{C3})}-\text{N}_{\text{Py}(\text{C24})}$ direction, 5 Å and 4 Å for $\text{Pd}_6(\text{L}_3)_8$ and $\text{Pd}_{12}(\text{L}_3)_{16}$, respectively, being dominantly contributing to the elongation. Whereas $\text{N}_{\text{Py}(\text{C7})}-\text{N}_{\text{Py}(\text{C24})}$ distance remains similar to that of $\text{Pd}_6(\text{L}_3)_8$ and 0.8 Å

longer than in $\text{Pd}_{12}(\text{L}_3)_{16}$, $\text{N}_{\text{Py}(\text{C}3)}\text{—N}_{\text{Py}(\text{C}7)}$ distance stays median compared to both species.

This highlights that the ligand, by contracting and expanding, can selectively adopt either octahedral or cuboctahedral structures. Thereby, it again validates that increased flexibility of the ligand enhances its tendency for orientational self-sorting. This adaptivity of the structure originates from the inherent flexibility present in the structure of the ligand, which allows the selective formation of species that are non-templated,^{31,40} not assisted by changing coordination sites⁵⁸ or metal cations,⁴⁵ and by triggering chemical changes in the ligand structure.⁸⁶ Therefore, it represents a novel ligand design to gain control over selectivity referred to as flexibility-aided orientational self-sorting.

Additionally, rotating one ligand either clockwise or counterclockwise in $\text{Pd}_6(\text{L}_3)_8$ generates less stable conformational isomers, as reflected by the increased energies of these DFT-optimized structures. This instability arises from the geometric strain caused by the reduction of the symmetry in the complex, suggesting a preference for the formation of homochiral species.

2.3.6 CD Spectroscopy

The DFT models of these metallo-cages reveal that the complexation process induces helicity within the ligand structures. To gain a deeper understanding of these fine structural details, CD spectroscopic analysis was conducted and compared with both the previously developed Pd_3L_6 metallo-macrocycle and simulated CD spectra (Figure 21a). The CD spectra for the $\text{Pd}_6(\text{L}_3)_8$ to $\text{Pd}_{12}(\text{L}_3)_{16}$ cages display similar absorption patterns, featuring a strong positive band at 275 nm and a strong negative band at 260 nm, forming a (+ -) CD couplet. In contrast, the CD spectra for the previously reported crown-like Pd_3L_6 metallo-macrocycle, synthesized from a comparable UDCA-based *bis*-pyridyl ligand (**Ld**), exhibit an almost inverse (- +) CD couplet.

A detailed examination of the coordination sites around the $\text{Pd}_{\text{C}3\text{-C}7}$ centers reveals their role in determining the opposite helicities. In case of $\text{Pd}_6(\text{L}_3)_8$ or $\text{Pd}_{12}(\text{L}_3)_{16}$ the coordination sites around $\text{Pd}_{\text{C}3\text{-C}7}$ follows a clockwise C3-C7-C3-C7 (3,7- $\text{Pd}_{\text{C}3\text{-C}7}$ -3,7) connectivity (Figure 21b). This connectivity makes the $\text{Pd}_6(\text{L}_3)_8$ or $\text{Pd}_{12}(\text{L}_3)_{16}$ an ensemble of four or eight right-handed helices, as observed following two diagonally interconnected ligands through the C24-C3-Pd-C3-C24 backbone. Consequently, the CD spectra confirm orientational self-sorting into homochiral quadrupole ($\Delta\Delta\Delta\Delta$) or octupole ($\Delta\Delta\Delta\Delta\Delta\Delta\Delta\Delta$) helical structures.

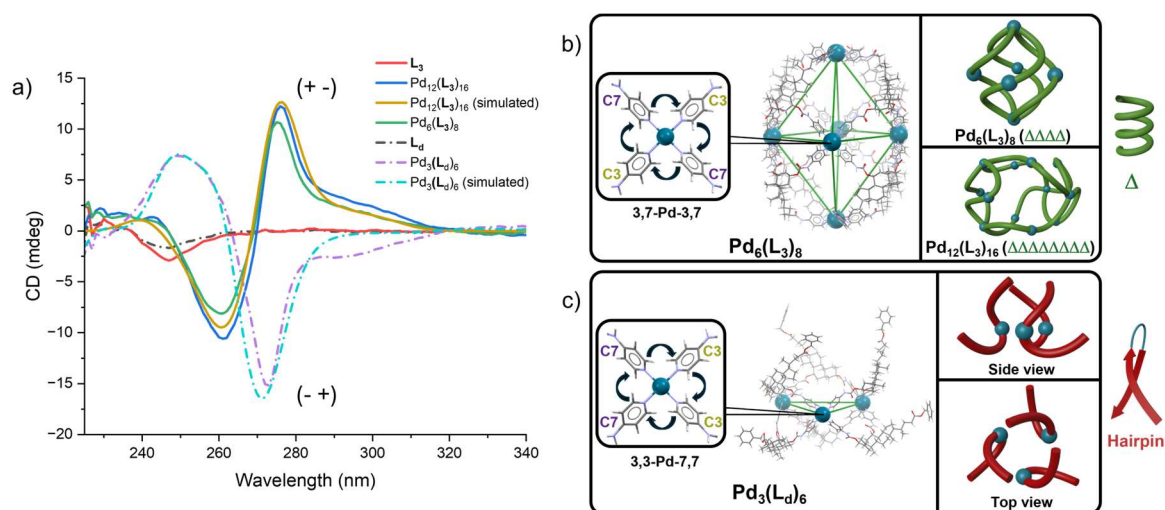


Figure 21. CD spectroscopic analysis (MeOH:DMSO-*d*₆ 1000:1, 25 °C) to elucidate the structure of UDCA-based supramolecular assemblies. [Adapted from Ref. 82. CC BY-NC 4.0 license. Copyright © 2025 The Authors. *Angewandte Chemie International Edition* published by Wiley-VCH GmbH]

In contrast, the $\text{Pd}_3(\text{L}_4)_6$ complex exhibits a different coordination pattern, with the $\text{Pd}_{\text{C3-C7}}$ centers arranged in a clockwise C3-C3-C7-C7 (3,3-Pd_{C3-C7}-3,3) connectivity (Figure 21c). This connectivity represents the structure being a collection of three hairpin loops, similarly following the C24-C3-Pd-C3-C24 backbone. The barrel-like structure of $\text{Pd}_3(\text{L}_4)_6$, characterized by 3,7-Pd_{C3-C7}-3,7 connectivity, should ideally produce a (+ -) CD couplet. This observation further confirms the formation of homochiral crown-like assemblies in $\text{Pd}_3(\text{L}_1)_6$ and $\text{Pd}_n(\text{L}_2)_{2n}$ ($n = 2-6$) macrocycles. Additionally, the strong correlation between the simulated and experimental CD spectra lends further validation to these conclusions.

2.3.7 Biological activity

CD spectroscopy can also effectively monitor the aqueous stability of $\text{Pd}_6(\text{L}_3)_8$ and $\text{Pd}_{12}(\text{L}_3)_{16}$ at much lower biologically relevant concentrations, *e.g.*, 625 nm and 1250 nm (Figure S16). Given the transport capability of bile acids in enterohepatic circulation and Pd^{2+} 's biological activity, hepatospheroids of HepG2 cells were used to evaluate toxicity. While the ligand exhibited minimal toxicity after 8 days, both inorganic $\text{Pd}(\text{NO}_3)_2$ and metallo-cages reduced spheroid viability as Pd^{2+} concentrations increased. At equimolar Pd^{2+} concentrations (24 μM), spheroid viability dropped to 90% for $\text{Pd}(\text{NO}_3)_2$, 78% for $\text{Pd}_6(\text{L}_3)_8$, and 65% for $\text{Pd}_{12}(\text{L}_3)_{16}$, as measured by ATP assay (Figure 22a) and spheroid size monitoring. Inductively coupled plasma mass spectrometry (ICP-MS) analysis revealed palladium uptake correlated negatively with spheroid viability (Figure 22b). Spheroids treated with 24 μM $\text{Pd}(\text{NO}_3)_2$ contained 0.15 ng palladium per spheroid, whereas 4 μM $\text{Pd}_6(\text{L}_3)_8$ and 2 μM $\text{Pd}_{12}(\text{L}_3)_{16}$ delivered 1.5 ng and 4.26 ng, respectively, indicating higher uptake efficiencies of 0.2% and 0.7%

compared to 0.023% for $\text{Pd}(\text{NO}_3)_2$. The intraspheroid to extracellular concentration ratios (IC:EC) were 249 for $\text{Pd}_{12}(\text{L}_3)_{16}$ and 51 for $\text{Pd}_6(\text{L}_3)_8$, indicating significantly greater uptake compared to that of $\text{Pd}(\text{NO}_3)_2$ which was 4.4. This highlights that larger, highly charged complexes are more readily absorbed by hepatospheroids, resulting in enhanced toxic effects.

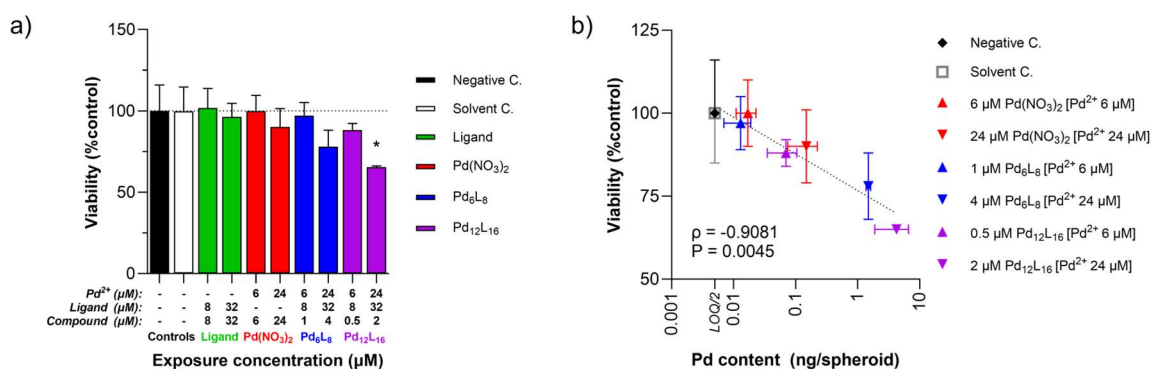


Figure 22. Biological studies to investigate the toxicity and the cellular uptake of $\text{Pd}_6(\text{L}_3)_8$ and $\text{Pd}_{12}(\text{L}_3)_{16}$ coordination cages. [Adapted from Ref. 82. CC BY-NC 4.0 license. Copyright © 2025 The Authors. *Angewandte Chemie International Edition* published by Wiley-VCH GmbH]

3 Conclusion

This thesis provides a concept to synthesize supramolecular building units from natural bile acids, resulting in the “next generation” supramolecular coordination complexes. This takes advantage of the inherent unsymmetry, chirality, flexibility, amphiphilicity, and bulky steroid skeleton of the bile acids. Chemical modification of the hydroxyl groups of different bile acids into coordination sites provides varying bend angles with different amounts of flexibility, allowing fair comparison of the systems for deeper insights regarding the self-assembly process. Moreover, the inclusion of the flexible alkyl chain of bile acid in the coordination process induces a higher magnitude of overall flexibility, ultimately leading to stimuli-responsive interconvertible coordination cages. This approach, in a symmetric, achiral, and rigid ligand-dominated metallosupramolecular field, is novel and extends the library of metallosupramolecular species, bringing them closer to natural mimetics. Sequential study of pyridyl ligands derived from UDCA and CDCA provided an advanced understanding of flexibility and bulkiness over selectivity and orientational self-sorting.

UDCA (**L**₁) and CDCA (**L**₂)-based *bis*-pyridyl ligand upon square-planar complexation with Pd²⁺ shows the selective formation of Pd₃(**L**₁)₆ macrocycle and a mixture of macrocycles from Pd₂(**L**₂)₄ to Pd₆(**L**₂)₁₂, respectively. A comparison between the epimeric pair reveals that the deviation in bend angle of **L**₁ and **L**₂ is Δ28° (89-117°) and Δ20° (70-90°) in their respective supramolecular complexes. This observation is contrary to the well-accepted general rule that a higher bend angle results in a larger coordination species. A higher standard deviation of bend angle, which means higher flexibility, can reduce the steric constraints imposed by the bulky steroid rings, resulting in the selectivity of the orientational self-sorting into Pd₃(**L**₁)₆. When flexibility is compromised, the preference for **L**₂ shifts towards looser and larger macrocyclic species to compensate for the structural rigidity and increased tension. IM-MS, ¹H- DOSY NMR, ¹H-¹H NOESY NMR, DFT modeling, and structure-transformation analysis, in agreement with each other, helped to establish the structural conformation of these macrocycles. These observations represent valuable progress in understanding and mimicking natural systems made of non-planar, bulky, unsymmetric, and chiral molecules.

As a continuation of the study, UDCA-based unsymmetric *tris*-pyridyl ligand (**L**₃), where an alkyl chain was incorporated as a pyridyl coordinating site, resulted in the selective formation of homochiral octahedra cage Pd₆(**L**₃)₈ and introduced a new type cuboctahedral cage Pd₁₂(**L**₃)₁₆ that are interconvertible. The flexibility of the alkyl chain pyridyl group (C24), in combination with the flexibility of the C3 and C7 pyridyl sites, allows expansion or contraction of the ligand to adopt cuboctahedral or

octahedral geometry in response to the reaction conditions, such as reaction stoichiometry or catalytic amount of coordinating solvent. The inherent adaptivity of the ligand, without directly influencing the ligand's shape by photochemical or templating methods, is often observed in large natural systems like enzymes or proteins. Therefore, advanced control over the formation and interconversion of supramolecular coordination complexes engaged in thermodynamic equilibrium is referred to as "flexibility-aided orientational self-sorting".

Another interesting feature of the supramolecular complexes is that they adopt helical structures. Comparison of the CD spectra of the macrocycle $\text{Pd}_3(\text{L}_1)_6$ and the cages $\text{Pd}_6(\text{L}_3)_8$ and $\text{Pd}_{12}(\text{L}_3)_{16}$ showed almost opposite CD bands. This has helped to establish the connectivity of the ligand around the coordination center, *e.g.*, 3,3-Pd-7,7 or 3,7-Pd-3,7, and is further supported by DFT modeling. This has confirmed orientational self-sorting and structural isomerism of the coordination cages into right-handed quadruple ($\Delta\Delta\Delta\Delta$) or octuple ($\Delta\Delta\Delta\Delta\Delta\Delta\Delta\Delta$) helices and that of the macrocycles to be an ensemble of beta-hairpin loops altering their handedness. CD spectroscopy is a well-established, sensitive, fast, and reliable tool for large chiral systems such as DNAs, proteins, or enzymes. Therefore, this sequential development of these artificial systems and their comparison presents CD spectroscopy as a potential alternative to determine the structure of chiral supramolecular complexes comparable to often challenging single-crystal X-ray diffraction.

The biological investigation of the cages showed enhanced cellular uptake and increased cytotoxicity of Pd^{2+} cations in HepG2 spheroids, with the anticancer effect increasing with size and charge. The ICP-MS analysis demonstrated that the Pd^{2+} uptake was 12-fold ($\text{Pd}_6(\text{L}_3)_8$) and 56-fold ($\text{Pd}_{12}(\text{L}_3)_{16}$) higher than that of inorganic $\text{Pd}(\text{NO}_3)_2$. This behavior emphasizes the value of tailored bile-acid-based ligands and the therapeutic promise of bile-acid-based complexes for advanced biomedical applications.

These supramolecular complexes also represent water-soluble hydrophobic nano-pockets with an impressive chiral density not reported so far. For example, $\text{Pd}_3(\text{L}_1)_6$, $\text{Pd}_6(\text{L}_3)_8$, and $\text{Pd}_{12}(\text{L}_3)_{16}$ have 60, 80, and 160 chiral centers embedded in their structures. Therefore, these complexes are also promising candidates in molecular recognition, enantioselective catalysis, and materials science.

Overall, one of the future directions of metallo-supramolecular chemistry lies in the design and control of unsymmetric supramolecular coordination complexes containing chiral nanocavities. By modulating the connectivity between the ligand and metal node, such as introducing additional denticity, control over the handedness of supramolecular coordination complexes is an interesting discovery. The *flexibility-aided orientational self-sorting* approach introduced here also represents a novel

strategy for supramolecular self-assembly, which led to stimuli-responsive interconversion of cavity-containing structures. Further advancements in this field will require a comprehensive understanding, eventually resulting in substrate-selective recognition or reversible dynamic structural changes that facilitate efficient substrate transformation as observed in natural molecular machines.

In my opinion, the aim of supramolecular chemistry revolves around mimicking natural systems and functionalities; therefore, bridging synthetic and biological domains offers fascinating opportunities for future research and development.

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Appendix A [Publications]

- **Publication 1**

Jurček, O.; **Chattopadhyay, S.**; Kalenius, E.; Linnanto, J. M.; Kiesilä, A.; Jurček, P.; Radiměřský, P.; Marek, R. Unsymmetric Chiral Ligands for Large Metallo-Macrocycles: Selectivity of Orientational Self-Sorting. *Angew. Chem. Int. Ed.* **2024**, 63, e202409134.

<https://doi.org/10.1002/anie.202409134>

- **Publication 2**

Chattopadhyay, S.; Durník, R.; Kiesilä, A.; Kalenius, E.; Linnanto, J. M.; Babica, P.; Kuta, J.; Marek, R.; Jurček, O. Flexibility-Aided Orientational Self-sorting and Transformations of Bioactive Homochiral Cuboctahedron Pd₁₂L₁₆. *Angew. Chem. Int. Ed.* **2025**, 64, e202513902.

<https://doi.org/10.1002/anie.202513902>

- **Publication 3**

Marzabad, M. A.; **Chattopadhyay, S.**; Hietala, S.; Nonappa, N.; Marek, R.; Jurček, O. Robust Self-Healing Metallo-Supergels of Folic Acid: Potential Sustainable Gelator for Oilfield Applications. *Chem. Eur. J.* **2025**, 31, e202500748.

<https://doi.org/10.1002/chem.202500748>

(Publication 3 is not part of my thesis, but it represents a collaborative work performed within the research group during my doctoral studies)

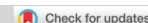
- **Manuscript Preparation and Pending Patent**

Chattopadhyay, S. et al., Self-assembly of Bile Acid-based Nanoparticles for Efficient Drug Targeting

Publication 1



Research Article



Supramolecular Chemistry

How to cite: *Angew. Chem. Int. Ed.* **2024**, *63*, e202409134
doi.org/10.1002/anie.202409134

Unsymmetric Chiral Ligands for Large Metallo-Macrocycles: Selectivity of Orientational Self-Sorting

Ondřej Jurček,* Subhasis Chattopadhyay, Elina Kalenius, Juha M. Linnanto, Anniina Kiesilä, Pia Jurček, Petr Radiměřský, and Radek Marek

In memory of professor Erkki Kolehmainen, a great person and one of the pioneers of supramolecular chemistry of bile acids.

Abstract: Nature uses various chiral and unsymmetric building blocks to form substantial and complex supramolecular assemblies. In contrast, the majority of organic ligands used in metallosupramolecular chemistry are symmetric and achiral. Here we extend the group of unsymmetric chiral bile acids used as a scaffold for organic bispyridyl ligands by employing chenodeoxycholic acid (CDCA), an epimer of the previously used ursodeoxycholic acid (UDCA). The epimerism, flexibility, and bulkiness of the ligands leads to large structural differences in coordination products upon reaction with $\text{Pd}(\text{NO}_3)_2$. The UDCA-bispyridyl ligand self-assembles quantitatively into a single crown-like Pd_3L_6 complex, whereas the CDCA ligand provides a mixture of coordination complexes of general formula Pd_nL_{2n} , i.e., Pd_2L_4 , Pd_3L_6 , Pd_4L_8 , Pd_5L_{10} , and even Pd_6L_{12} containing an impressive 120 chiral centers. The coordination products were studied by a combination of analytical methods, with ion-mobility mass spectrometry (IM-MS) providing valuable details on their structure and allowed an effective separation of m/z 1461 to individual signals according to the arrival time distribution, thereby revealing four different ions of $[\text{Pd}_3\text{L}_6(\text{NO}_3)_3]^{3+}$, $[\text{Pd}_4\text{L}_8(\text{NO}_3)_4]^{4+}$, $[\text{Pd}_5\text{L}_{10}(\text{NO}_3)_5]^{5+}$, and $[\text{Pd}_6\text{L}_{12}(\text{NO}_3)_6]^{6+}$. The structures of all the complexes were modelled using DFT calculations. Finally, the challenges and conclusions in determining the specific structural identity of these unsymmetric species are discussed.

Introduction

Nature creates various substantial and complex supramolecular self-assemblies using chiral and unsymmetric components. Even for the obvious importance of understanding these processes, the area of using natural compounds as building blocks in supramolecular chemistry is not extensively explored. There are just a few known synthetic coordination assemblies built of ligands containing small terpenoids,^[1] peptides,^[2] cyclodextrins,^[3–5] or quite recently using bile acids.^[6–8]

The self-assembly of organic ligands and metal centers—of which transition-metal cations are frequently used—leads to distinct metallosupramolecular structures such as dimeric or multimeric capsules, molecular polyhedra, metallacycles, interlocked structures, or metallopolymer such as metal-organic frameworks.^[9–17] The self-assemblies derived from $\text{Pd}(\text{II})$ salts have rich history and their development is continuously progressing.^[9–11,15] However, most of metallosupramolecular architectures are highly symmetric as being built of symmetric ligands. The field of low-symmetry supramolecular species is still in its infancy but it has gained more and more attention lately.^[18]

The biggest challenge in the synthesis of complexes from unsymmetric coordination ligands is the limited control over the ligand's relative orientation in the self-assembly which can lead to a mixture of many constitutional isomers of the assembly, e.g., Pd_2L_4 can be present in the form of 4 isomers, Pd_3L_6 as 9, Pd_4L_8 as 35, and Pd_6L_{12} even as 112 isomers (excluding their enantiomers). The process of unsymmetric ligand self-assembly into a limited number of isomers,

[*] Dr. O. Jurček
Department of Natural Drugs, Faculty of Pharmacy, Masaryk University
Palackého 1946/1, CZ-61200 Brno, Czechia
E-mail: 239999@mail.muni.cz
Dr. O. Jurček, MSc. S. Chattopadhyay, MSc. P. Radiměřský,
Prof. Dr. R. Marek
Department of Chemistry, Faculty of Science, Masaryk University
Kamenice 5, CZ-62500 Brno, Czechia
Dr. O. Jurček, MSc. S. Chattopadhyay, Dr. P. Jurček,
Prof. Dr. R. Marek
CEITEC—Central European Institute of Technology, Masaryk University
Kamenice 5, CZ-62500 Brno, Czechia

Dr. E. Kalenius, Dr. A. Kiesilä
Department of Chemistry, University of Jyväskylä
P. O. Box 35, FI-40014 Jyväskylä, Finland

Dr. J. M. Linnanto
Institute of Physics, University of Tartu
W. Ostwald Street 1, 50411, Tartu, Estonia

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preferably a single one, is described as “orientational self-sorting”.^[19] Other challenge in this area is the accurate determination of molecular structure of these coordination products which can be done either by a combination of analytical methods such as NMR spectroscopy, mass spectrometry (MS), and molecular modelling,^[6] or the most reliably by X-ray crystallography.^[19,20] Ion mobility mass spectrometry (IM-MS) can further provide information on conformational dynamics and structural and/or conformational monodispersity. In some cases, IM-MS can reveal the existence of higher nuclearity complexes that are not possible to be observed in mass spectra owing to their low abundance and overlapping m/z values.^[21,22]

Smallest Pd_3L_4 low-symmetric assemblies are the most studied species so far.^[7,23–28] Larger Pd_3L_6 complexes (often referred to as double-walled triangles) prepared of unsymmetric ligands were introduced by Jurček et al. in 2015,^[6] since then there are two other examples.^[8,29] So far, there are two different structural organizations described for Pd_3L_8 , tetrahedral^[19] or double-walled square^[29,30] built of unsymmetric ligands. There are Pd_3L_{10} complexes built of a symmetric ligand either via guest-adaptive self-assembly^[31] or recently without the use of a template.^[32] There is no Pd_3L_{10} built of unsymmetric ligand. Finally, there is a number of examples of large coordination Pd_6L_{12} species built of ditopic symmetric ligands forming mostly octahedral,^[20,33–44] but also crown-like macrocyclic^[31,45] assemblies. Out of these, there is only one example of octahedral Pd_6L_{12} complex assembled of low-symmetry achiral ligand (Figure 1a) by Severin et al.^[19]

The bile acids (BAs) are amphiphilic triterpenoids with rigid lipophilic core decorated by variable number of hydroxyl groups on their concave hydrophilic α -side and a flexible alkyl chain bearing carboxylic acid. Their main physiological function is in mediating absorption and transport of lipids and lipid soluble nutrients from digestive tract, but there is an increasing evidence that their physiological importance goes further than just that.^[46] BAs found their applications in supramolecular chemistry because of their structural features, reactivity, and physicochemical properties,^[47–50] but it was not until recently, that they were introduced to coordination supramolecular chemistry.^[6] We

demonstrated that ditopic pyridyl ligands derived from ursodeoxycholic acid (UDCA) can form in a narcissistic way large, chiral, concave, supramolecular Pd_3L_6 macrotetra-cycles with $\text{Pd}(\text{II})$ of square planar coordination geometry.^[6,8] We have further shown that by using an amphiphilic UDCA-bispyridyl-carboxylic tritopic ligand we can induce self-organization of Pd_3L_6 into microscopic hexagonal particles upon addition of water to its DMSO solution.^[8] In this article we introduce a new epimeric BA as a building block in coordination supramolecular chemistry—chenodeoxycholic acid (CDCA). The only structural difference between both 3,7-dihydroxy BAs, the UDCA and the CDCA (ligands derived shown in Figure 1b), is in spatial orientation of the hydroxyl at position C-7, equatorial or axial, respectively. This leads to a difference in bend angle enclosed between $\text{OH}@C3\text{--}C5\text{--OH}@C7$, i.e., 110° (UDCA) or 90° (CDCA). Therefore, the CDCA-bispyridyl ligand provides new geometric features which can lead to different complexes than known Pd_3L_6 . This was indeed confirmed in our experiments revealing a mixture of larger complexes including the superchiral Pd_6L_{12} containing 120 chiral centers. Such chiral density has never been contained in a single artificial metallosupramolecular species. The ligand flexibility and with that connected structural variability of self-assemblies derived from UDCA- and CDCA-bispyridyl ligands is discussed. The study also opens an important chapter of structural predictability of complexes made of flexible, bulky, unsymmetric, and chiral ligands, and further provides an insight into a mechanism of selectivity of orientational self-sorting.

Results and Discussion

The bispyridyl ligands derived from ursodeoxycholic (L_1) and chenodeoxycholic (L_2) bile acid (Figure 1b) were prepared in two steps. The carboxylic acid group of BAs was converted into methyl ester followed by transformation of hydroxyl groups into carbamoyl pyridines. The carbamates were formed either via triphosgene reaction pathway or via isonicotinoyl azide pathway (for details, see Supporting Information). The triphosgene pathway, reported previously,^[6,8] provided higher yields for L_1 (45 % vs. 37 %), whereas isonicotinoyl azide pathway provided higher yields for L_2 (30 % vs. 42 %).

In general, a ligand (L_1 or L_2) was dissolved in $\text{DMSO-}d_6$ and the resulting 10 mM solution was used to dissolve $\text{Pd}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ ($\text{L}:\text{M}$ ratio 2:1). The mixture was heated for 1 h at 55°C . The course of the complexation reaction was followed using ^1H NMR spectroscopy (Figure 2). The complexation of L_1 leads to a deshielding and broadening of the carbamate $-\text{NH}-$ and pyridyl $\text{H}3'$, $\text{H}3''$ atoms and a slight shielding of pyridyl $\text{H}4'$, $\text{H}4''$ atoms, which was attributed to a formation of $\text{Pd}_3(\text{L}_1)_6$ complex (Figure 2a, 2b). The complex and L_1 have the same number of ^1H NMR signals, indicating that the supramolecular assembly is formed spontaneously in symmetric and precise manner.

In theory, there can be 9 possible constitutional isomers of Pd_3L_6 , but often only the thermodynamically most stable

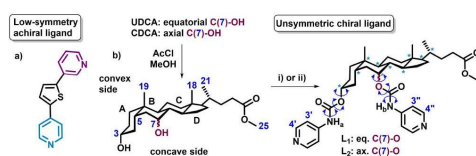


Figure 1. a) Structure of low-symmetry ligand by Severin et al.^[19] b) Reaction Scheme of the preparation of the ligands by two pathways: i) triphosgene, 4-aminopyridine, pyridine, DCM; ii) isonicotinoyl azide, toluene. The chemical formulae and partial proton numbering of unsymmetric ligands L_1 (UDCA-based) and L_2 (CDCA-based) are shown. Blue asterisks mark stereocenters which are similar in both BAs (10 in total). Blue arrows indicate rotational flexibility of the ligands. Letters A–D denote rings of the steroid skeleton.

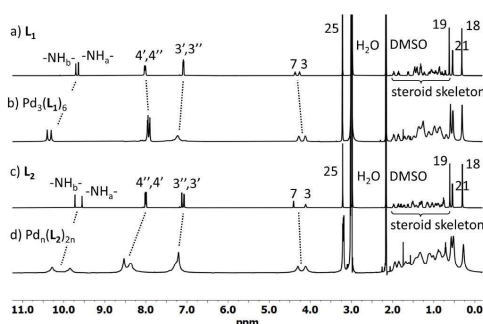


Figure 2. ^1H NMR spectra of a) ligand L_1 , b) $\text{Pd}_3(\text{L}_1)_6$, c) ligand L_2 , and d) mixture of Pd^{2+} - L_2 complexes.

one is formed via the orientational self-sorting and can be detected in the reaction mixture.^[19] As we elaborately discussed and studied previously using structurally similar ligands derived from the UDCA (Figure S15, C-24 was either benzyl ester or carboxylic acid, while in here it is the methyl ester),^[6,8] based on the ^1H NMR analysis of the L_1 complex we can assume that the final self-assembly is symmetric. The stoichiometry of the complex was determined by ESI-Q TOF MS as $\text{Pd}_3(\text{L}_1)_6$ (Figure 3), further suggesting C_3 rotational symmetry.

Such complex can be represented by two possible constitutional isomers—crown- (Figure 4, previously called “flower”^[(6,8)]) or barrel-like complex, where the six ligands are interconnected by three square-planar Pd(II) centers (PdN₂ coordination sphere). The $\text{Pd}_3(\text{L}_1)_6$ complex can be divided into a top and a bottom part by an imaginary plane going through the Pd(II) centers. Each part contains three ligands interconnected into a cycle with connectivity (C7-pyridyl)-Pd(II)-(C3-pyridyl), i.e., 7-Pd-3. This top and bottom cycles differ in their orientation to each other in the crown- and the barrel-form, i.e., both cycles travel in the same direction (crown, 7,7-Pd-3,3 coordination sphere) (Figure 4, Scheme 1) or in the opposite direction (barrel, 7,3-Pd-3,7 coordination sphere) (Figure S32).

This was further confirmed by performing a logical structure-transformation analysis (Scheme 1). The principle of the analysis consists in the preparation of trimeric macrocycles by addition of tetrabutylammonium chloride (TBACl) and analysis of the products. The hypothesis is that direct deconstruction of the crown-like $\text{Pd}_3(\text{L}_1)_6$ using TBACl leads to a single constitutional crown-like isomer $\text{Pd}_3(\text{L}_1)_3\text{Cl}_3$ (also referred to as *cis*), while the same deconstruction of the barrel-like $\text{Pd}_3(\text{L}_1)_6$ complex leads to two constitutional isomers, the crown-like and an upturned one (also referred to as *trans*) (Scheme 1), which shall be possible to follow using ^1H - and ^1H DOSY NMR.

As expected, the ^1H NMR spectrum of the TBACl reaction shows a new single set of signals of coordinated L_1 , next to a signal of free ligand L_1 . The chemical shifts are different to those of $\text{Pd}_3(\text{L}_1)_6$ confirming quantitative

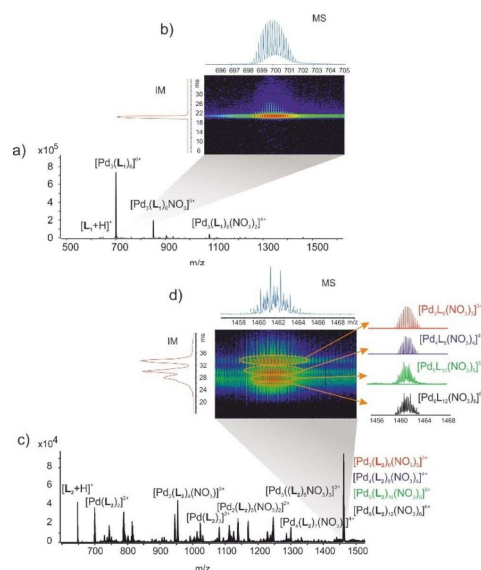


Figure 3. Comparison of the ESI-MS spectra and ion-mobility arrival time distributions for spectra measured from L_1 -Pd(II) and L_2 -Pd(II) complexation reactions. a) ESI-MS spectrum of L_1 -Pd(II) complexation showing exclusive formation of $\text{Pd}_3(\text{L}_1)_6$, b) ion-mobility arrival time distribution and heat plot for the $[\text{Pd}_3(\text{L}_1)_6]^{2+}$ ion, c) ESI-MS spectrum of L_2 -Pd(II) complexation showing the formation of a variety of Pd(II) complexes, and d) ion-mobility arrival time distribution and heat plot for the ion at m/z 146, which is shown to consist of a mixture of $[\text{Pd}_3(\text{L}_2)_6(\text{NO}_3)_3]^{3+}$, $[\text{Pd}_4(\text{L}_2)_8(\text{NO}_3)_4]^{4+}$, $[\text{Pd}_5(\text{L}_2)_{10}(\text{NO}_3)_5]^{5+}$, and $[\text{Pd}_6(\text{L}_2)_{12}(\text{NO}_3)_6]^{6+}$.

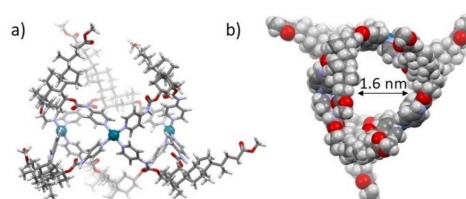
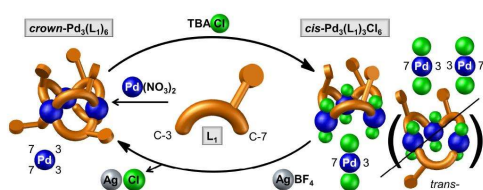


Figure 4. a) Optimized (CAM-B3LYP with SCRF methanol model and LANL2DZ + 6-311G(d,p) method) molecular model of the crown-like $\text{Pd}_3(\text{L}_1)_6$ complex (side view) and b) top view of complex shown with a CPK model.

formation of a new single coordination product $\text{Pd}_3(\text{L}_1)_3\text{Cl}_3$ (Figure S35). The ESI-MS analysis of the experiment shows presence of $[(\text{L}_1)_3\text{Pd}_3\text{Cl}_3\text{Na}]^+$ or $[(\text{L}_1)_3\text{Pd}_3\text{Cl}_3]^+$ ions confirming the expected progress of the reaction towards the formation of a zero net charge $\text{Pd}_3(\text{L}_1)_3\text{Cl}_3$ complex (thus not directly observable by MS). These results confirm that treatment of the C_3 -symmetric crown-like complex $\text{Pd}_3(\text{L}_1)_6$



Scheme 1. Structure-transformation analysis: reversible conversion reaction between $\text{Pd}_3(\text{L}_1)_6$ and trimeric $\text{Pd}_3(\text{L}_1)_3\text{Cl}_6$ species suggesting the existence of a crown-like architecture.

leads to another C_3 -symmetric crown-like complex $\text{Pd}_3(\text{L}_1)_3\text{Cl}_6$. Moreover, the original crown-like $\text{Pd}_3(\text{L}_1)_6$ can be fully recovered from this mixture by AgCl treatment and heating at 55°C , as confirmed by ^1H NMR spectroscopy and MS.

In the following step, all reaction products were studied by ^1H DOSY NMR spectroscopy. The diffusion coefficient for the single hexameric species $\text{Pd}_3(\text{L}_1)_6$ is $D = 7.17 \times 10^{-11} \text{ m}^2/\text{s}$ (Figure 5a, considering its roughly spherical

shape corresponding to 3.4 nm hydrodynamic radius). The *cis*-form of $\text{Pd}_3(\text{L}_1)_3\text{Cl}_6$ provided $D_{\text{cis}} = 7.83 \times 10^{-11} \text{ m}^2/\text{s}$ (Figure S36, corresponding roughly to 3.1 nm radius). This is consistent with the *cis*- rather than the *trans* isomer, as it was also confirmed by comparison with measurements of their molecular models, which show a diameter 3.6 and 4.4 nm, respectively (Figure S34).

Another evidence of the presence of the crown-like self-assembly was obtained by ^1H - ^1H NOESY NMR analysis of the $\text{Pd}_3(\text{L}_1)_6$ solution (Figure S33). The analysis of the spectrum reveals strong interaction of C25-methyl group of ester on the flexible alkyl side chain with multiple protons of the steroid nucleus (including the interaction with methyls C18, C19, and C21) and a weak interaction with pyridylcarbamates. This can be seen as “tapping” of the C25-methyl on the convex face of the above lying neighboring steroid ligand which can only be possible in the crown-like assembly. In contrast, the C25-methyls in the barrel-like assembly point outwards the central Pd-plane of the $\text{Pd}_3(\text{L}_1)_6$ complex and thus have no possibility for measurable ^1H - ^1H dipolar (NOE) interaction with other neighboring ligands.

The comparison of the collision cross-section (CCS) of the crown-like $\text{Pd}_3(\text{L}_1)_6$ with the previously published similar Pd_3L_6 self-assemblies further confirms the presence of the crown-like structure. The crown-like complexes of the general formula $[\text{Pd}_3(\text{L}_n)_6]^{6+}$ are represented by $^{DT}\text{CCS}_{\text{N}_2}$ 1106–1191 $\text{\AA}^2$ for the largest UDCA-based “waving” ligand (Figure S15a),^[6] 919.4 $\text{\AA}^2$ for the smallest ligand (Figure S15b),^[8] and 973.7 $\text{\AA}^2$ using the current medium-sized ligand L_1 .

Finally, the comparison of the ground state energies of optimized molecular models of $[\text{Pd}_3(\text{L}_1)_6(\text{NO}_3)_3]^{3+}$ shows an energy difference of 10 $\text{kcal}\cdot\text{mol}^{-1}$ in favor of the crown-like model (yet the energy difference can slightly differ in relation to the position of nitrate anions surrounding the Pd^{2+} center). The reasons for the crown-like architecture are likely encompassed in the ligand’s amphiphilic character and natural tendency of BAs to undergo aggregation in polar environment, which together with the relatively flexible binding sites provide conditions for orientational self-sorting into a single constitutional complex crown- $\text{Pd}_3(\text{L}_1)_6$.

In the case of L_2 complexation, the ^1H NMR spectrum of the resulting reaction mixture shows complete coordination of the ligand as seen by signal broadening, but this reaction results in a mixture of coordination complexes (Figure 2d). At least two different sets of overlapping pyridyl signals can be recognized in the spectrum, suggesting the presence of at least two different coordination species or constitutional isomers (similar observation where constitutional isomers could be distinguished based on ^1H NMR spectra was previously shown^[6]). The ^1H resonances of the carbamate $-\text{NH}-$ groups and pyridyl H3', H3'' atoms are shifted to higher frequencies. It is also noteworthy that H4', H4'' atoms are deshielded in contrast to those in L_1 . To induce orientational self-sorting process and selectivity of the reaction of $\text{Pd}(\text{II})$ with L_2 we screened different conditions (varying solvent system (Figure S37), temperature) and used different palladium salts (Figure S38), but the reactions generally led

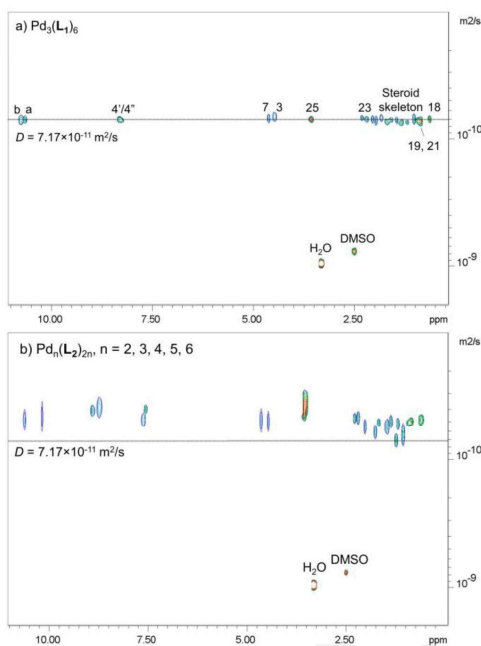


Figure 5. ^1H DOSY NMR spectrum (700 MHz, $\text{DMSO}-d_6$, 303 K) of a) $\text{Pd}_3(\text{L}_1)_6$ ($D = 7.17 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$), b) $\text{Pd}_3(\text{L}_2)_{2m}$ where $n = 3, 4, 5$, or 6 , showing multiple species in the reaction mixture (the reference line is set for comparison at $D = 7.17 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$, which corresponds to $\text{Pd}_3(\text{L}_1)_6$).

to even more complex mixtures and insoluble precipitates. Moreover, we also tested another approach, where, in the first step, a reaction of **L**₂ with PdCl₂ led to a mixture of fewer coordination products of a general formula Pd_n-(**L**₂)_nCl_{2n}, and in the second step reaction with AgBF₄ was expected to lead to a simpler mixture of products with general formula Pd_n(**L**₂)_{2n}, but, again, this was not the case (Figure S38d,e, Figure S39).

The mixture from the reaction with Pd(NO₃)₂ was analyzed by ESI-MS followed by IM-MS (Figure 3c). The ESI-MS analysis of the **L**₂-Pd(II) complexation reaction confirmed a mixture of several products. Ions of various stoichiometry were assigned to, e.g., [Pd₃(**L**₂)₄(NO₃)₃]³⁺, [Pd₂(**L**₂)₃(NO₃)₂]²⁺, [Pd₃(**L**₂)₅(NO₃)₃]³⁺, [Pd₄(**L**₂)₇(NO₃)₄]⁴⁺, or a signal with *m/z* 1461 corresponding to [Pd₃(**L**₂)₆(NO₃)₃]³⁺, but possibly also to larger overlapping species like [Pd₄(**L**₂)₈(NO₃)₄]⁴⁺, [Pd₅(**L**₂)₁₀(NO₃)₅]⁵⁺, or even [Pd₆(**L**₂)₁₂(NO₃)₆]⁶⁺ (Table S1). Arrival time distribution (ATD) separation at *m/z* 1461, indeed, reveals four different ions of [Pd₃(**L**₂)₆(NO₃)₃]³⁺, [Pd₄(**L**₂)₈(NO₃)₄]⁴⁺, [Pd₅(**L**₂)₁₀(NO₃)₅]⁵⁺, and [Pd₆(**L**₂)₁₂(NO₃)₆]⁶⁺ (Figure 3d).

The size and number of supramolecular self-assemblies of **L**₂ were also followed using ¹H DOSY NMR spectroscopy. The spectrum (Figure 5b) shows a mixture of complexes which are similar or larger than the Pd₃(**L**₁)₆ self-assembly.

Despite many attempts to obtain single crystals of **L**₁ or **L**₂ complexes for X-ray diffraction structural analysis, we were not successful. Therefore, the structures of **L**₂ complexes were also modelled and optimized by DFT calculations. In theory, these complexes can undertake shapes of macrocycles, but in some cases, based on their stoichiometry, they could also form distinct cages, or even interlocked structures. Pd₃(**L**₂)₆ (Figure 6a, S41a) can contain a crown-like macrocycle similar to that of Pd₃(**L**₁)₆. This was also suggested by the comparison of ground state energies of their optimized molecular models of [Pd₃(**L**₂)₆(NO₃)₃]³⁺, where the difference 10 kcal.mol⁻¹ was similarly in favor of the crown-like model compared to the barrel-like isomer. On the other hand, the Pd₄(**L**₂)₈ complex can form either a macrocycle (Figure 6b) or a tetrahedral self-assembly (Figure S42). The Pd₅(**L**₂)₁₀ complex is contained into only possible macrocyclic structure (Figure 6c). And the largest of them all, Pd₆(**L**₂)₁₂, can form a macrocycle (Figure 6d), a cuboid structure (Figure S42), or even mechanically interlocked structure made of two Pd₃(**L**₂)₆ macrocycles.

In the next step, based on the models we calculated theoretical values of CCS and compared them with the experimental ones. IM-MS experiments provide ^{DT}CCS_{He} values of 905.1 Å² and 1206.7 Å² for ions [Pd₃(**L**₂)₆(NO₃)₃]³⁺ and [Pd₄(**L**₂)₈(NO₃)₄]⁴⁺, respectively. These have a good correlation to theoretical ^{DTM}CCS_{He} values for [Pd₃(**L**₂)₆(NO₃)₃]³⁺ either 924.7 Å² (barrel, Figure S41d) or 949.9 Å² (crown isomer, Figure S41a) and for [Pd₄(**L**₂)₈(NO₃)₄]⁴⁺ either 1206.8 (tetrahedron) or 1237.7 Å² (crown isomer) (Figure S42). The differences between the constitutional isomers are relatively small, which somehow limits the ultimate structural determination (see Table S2 in SI). Yet, the tetrahedral, cuboid, and mechanically interlocked struc-

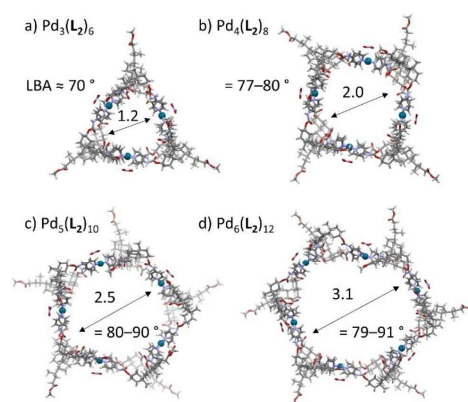


Figure 6. Top view of optimized (CAM-B3LYP with SCRF methanol model and LANL2DZ + 6-311G(d,p) method) molecular models of macrocyclic crown-like complexes: a) triangular [Pd₃(**L**₂)₆(NO₃)₃]³⁺, b) square [Pd₄(**L**₂)₈(NO₃)₄]⁴⁺, c) pentagonal [Pd₅(**L**₂)₁₀(NO₃)₅]⁵⁺, and d) hexagonal [Pd₆(**L**₂)₁₂(NO₃)₆]⁶⁺ (the cavity diameter is shown in nm, LBA stands for ligand bend angle).

tures are likely not plausible because of the bulkiness of the peripheral areas of the ligand **L**₂ (ring C and D bearing the alkyl side chain), its flexibility, and conclusions made based on analytical data collected.

The ¹H-¹H NOESY NMR analysis of the mixture showed similar interactions as in the case of Pd₃(**L**₁)₆ of the C25-methyl group of ester and protons of the steroid nucleus and pyridylcarbamates confirming the crown-like architecture for the complexes (Figure S40).

Finally, the chirality of the ligands **L**₁, **L**₂, and their complexes was studied by electronic circular dichroism (CD) (Figure 7). In general, the change of the CD signals of complexes compared to their ligands suggests that the dichroism is mainly related to structural organization of the complexes mediated through metal to ligand coordination. The experimental results obtained correspond to those previously published for the crown-like Pd₃**L**₆ complexes^[6,8] confirming similar crown-like isomerism. Nevertheless, this seems to be more relevant for the spectrum of Pd₃(**L**₁)₆ where, at the same concentration of the ligand complexed, the signal intensity is higher than the one in the case of complexes made of **L**₂. The lower signal intensity of the mixture of Pd_n(**L**₂)_{2n} complexes could further suggest that slight differences in structural organization are present within the mixture. We presume that while the organization of the ligands in Pd₃(**L**₁)₆ follows ideally the 7-Pd-3 directionality of the crown-like self-assembly (7,7-Pd-3,3 coordination sphere), the Pd_n(**L**₂)_{2n} complexes might contain structural discrepancies from the crown-like architecture and thus provide less optically active species. These structural aberrations could easily be possible for the smaller macrocycles, e.g., Pd₃(**L**₂)₆, where the steric hindrance plays

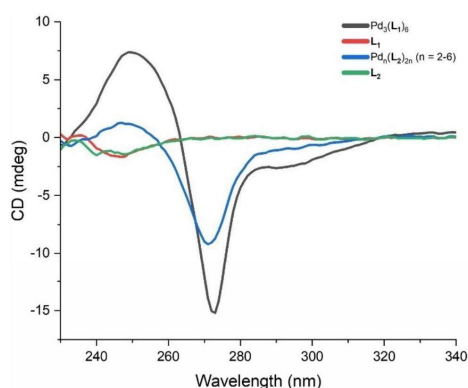


Figure 7. Comparison of electronic circular dichroism spectra of ligand L_1 , L_2 , $Pd_3(L_1)_6$, and $Pd_3(L_2)_{2n}$ (where $n=2-6$) ($c=10 \mu M$ with respect to ligand, $t=25^\circ C$, mixture of methanol and DMSO- d_6 in 1000:1 ratio).

larger role in orientational self-sorting as the intramolecular distances between ligands are smaller (Figure S41).

In general, the exact structural determination of coordination products with unsymmetric and flexible ligands is challenging (if not being supported by a crystal structure), especially, if the subject of the analyses is an unresolvable mixture of products as in our case. Therefore, new analytical strategies need be developed. Over the years, various strategies have been described in design and analyses of 2D and 3D metallocupramolecular architectures using symmetric ligands, where directional bonding, symmetry interaction, or molecular paneling approaches are the most extensively used and adopted.^[51,52] The key factors to be considered in the coordination self-assembly processes are ligand's geometry, its bend angle, structural rigidity/flexibility, and coordination sphere of the metal center.

It is noteworthy, that while in the metallocupramolecular chemistry of symmetric and rigid ligands a general rule applies where the larger is the bend angle the larger is the resulting self-assembly,^[52-55] herein it follows rather opposite trend. Nevertheless, this rule seems to be mainly applicable in the construction of coordination cages or polyhedra and might not hold strong for the formation of metalla-cycles.

Introducing the unsymmetric, flexible, but also bulky bile-acid-based ligands brings other structural features. Herein, we investigate and compare them for the ligands L_1 and L_2 . DFT calculation of an equilibrium geometry of L_1 (ground state in gas phase using B3LYP/6-31G*) provided a model enclosing 117° bend angle between pyridyl N@C3–C5-pyridyl N@C7 atoms (Figure S1c). Moreover, previously, we could also observe other conformational isomers found in a crystal structure of similar UDCA-based ligand, where four ligand molecules of an asymmetric unit enclose 112 , 115 , 137 , and 145° bend angle (Figure S14, this shall be taken carefully considering crystal constraints).^[8]

Furthermore, the bend angle of L_1 was measured in the optimized molecular model of $Pd_3(L_1)_6$ where it ranges from 89 to 105° . Overall, this represents quite a large flexibility of L_1 bend angle between 89 – 145° ($\Delta 56^\circ$), and if excluding the bend angles observed in the solid state, 89 – 117° ($\Delta 28^\circ$).

On the other side, the CDCA-based ligand L_2 possesses 72° bend angle in its equilibrium geometry (Figure S16c). The ligand's bend angle was further measured in molecular models of all coordination species, for $Pd_3(L_2)_4$ ranging 42 – 44° , macrocyclic $Pd_3(L_2)_6$ around 70° , macrocyclic $Pd_4(L_2)_8$ 77 – 80° , tetrahedral $Pd_4(L_2)_8$ 73 – 86° , macrocyclic $Pd_5(L_2)_{10}$ 80 – 90° , macrocyclic $Pd_6(L_2)_{12}$ 79 – 91° , and cuboid $Pd_6(L_2)_{12}$ 92 – 105° . Overall, ranging between 42 – 90° ($\Delta 48^\circ$), or when excluding the less abundant or less probable cases of $Pd_2(L_2)_4$ and cuboid $Pd_6(L_2)_{12}$, 70 – 90° ($\Delta 20^\circ$). Comparing the overall bend angles of both ligands, the L_1 roughly covers the range 90 – 120° (30°) while the L_2 covers the range 70 – 90° (20°), making them geometrically complementary building blocks. It appears that the higher is the flexibility of the unsymmetric ligand the higher is the probability of its orientational self-sorting into a single product.

The molecular modelling has further shown that not only bending of flexible ligands' moieties is responsible for their flexibility, but also the rotation around the C3–CO–NH–pyridine axis plays an important role. Especially for L_2 , the rotational freedom around the axis supplies to some extent the ligands' structural limitations given by a narrower bend angle. In connection to this, small energetical variability was also observed when comparing various conformational species of the crown-like $Pd_3(L_2)_6$ complexes. The differences in carbamate-pyridyl orientations additionally vary the overall size of the whole $Pd_3(L_2)_6$ complex, i.e., when all protons of –NH– group point away from the C3 axis the Pd–Pd distance in the complex is about 0.5 – $1 \text{ \AA}$ larger than that of the complex where one of the hydrogen –NH– atom points towards the C3 axis (or towards the C7). This size variability can also be somehow supported by a slightly broader ATD for $[Pd_3(L_2)_6(NO_3)_3]^{3+}$ in IM-MS (which can also be seen as another manifestation of “tapping” C25 methyl as observed by NOESY NMR).

Moreover, if we only consider the comparable species Pd_3L_6 , the bend angle of L_1 in $Pd_3(L_1)_6$ complex ranges between 89 – 105° , while the bend angle of L_2 in $Pd_3(L_2)_6$ is limited to about 70° suggesting structural rigidity and increased tension in the latter one. This is making it energetically less favorable product, which ultimately can be the reason for structural preferences of L_2 towards rather looser and larger macrocyclic species, where the bend angle can slightly vary. It also appears that L_1 feels lesser steric hindrance when being assembled in the complex because of different orientation of the side chain with C and D steroid rings. Indeed, the difference in ligands' bulkiness can be observed from the comparison of the molecular models of L_1 and L_2 (Figure S44). In case of L_2 , the volume of C+D moiety extends in one direction over the length of steroidal A and B rings bearing the pyridyl binding sites. This structural organization could be sterically limiting the orientational self-sorting and self-assembly into regular crown-like architecture and thus causing suggested structural



aberrations (some possible constitutional isomers of $\text{Pd}_3(\text{L}_2)_6$ are shown in Figure S41).

Conclusion

Herein, we introduced chenodeoxycholic acid (CDCA) as a core unit of the bispyridyl ligand to be used in metallosupramolecular chemistry. A comparison to the known framework of ursodeoxycholic acid (UDCA) is presented with a focus on differences in their molecular structure and resulting $\text{Pd}(\text{II})$ macrocycles. In general, both ligands provide macrocyclic structures. While the UDCA ligand leads selectively via orientational self-sorting to a single constitutional isomer of Pd_3L_6 , the CDCA ligand results in a mixture of mostly larger macrocyclic complexes Pd_nL_{2n} , starting from Pd_3L_6 all the way up to a giant Pd_6L_{12} .

The lack of symmetry, bulkiness, and partial flexibility of the ligands might, at first, rather complicate the prediction of their final self-assemblies, yet once some systematic structural code is unveiled, it leads to a general better understanding of the important self-assembly behavior of unsymmetric, chiral and flexible building blocks with a relevance to natural processes (yet still much more complex). The elemental rules of ligand bend angle, rigidity, and metal geometry can be a good starting point when working with ligands slightly diverging from the symmetry. Nevertheless, several other features need to be considered when explaining the orientational self-sorting of truly unsymmetric ligands like ours, such as flexibility of bend angle, directionality, and steric requirements, together with natural propensity of BAs to aggregate because of their amphiphilic character. The logical structure-transformation analysis as we introduced serves well as a method for structural confirmation. Also, CD spectroscopy might represent an important new approach for structural characterization of supramolecular self-assemblies composed of chiral unsymmetric coordination ligands and it can also serve well in the description of their regularity.

There is an intriguing orientational self-sorting when using L_1 , a feature which can now be related to the ligand's optimal bend angle, flexibility, and distribution of bulkiness. On the other hand, there is a general specificity mounted in the bile acid skeleton, as also shown when using L_2 , steering the course of coordination self-assembly preferably towards the crown-like macrocyclic complexes, yet in this case of varying size.

Moreover, the spatial orientation of hydroxyl groups at C-3 and C-7 of CDCA are the same as in the molecule of cholic acid—the least toxic and the most water-soluble bile acid commercially available, which predestines its similar utilization in coordination supramolecular chemistry. High level of chiral density of these macrocyclic complexes is another interesting feature with possible future applications, e.g., in enantioselective catalysis, or in mediation of chiral recognition and enantioselective separations.

Supporting Information

The authors have cited additional references within the Supporting Information.^[54–59]

Acknowledgements

We thank Dr. Miroslava Bittová and Dr. Antonín Bednář for measuring some of the MS spectra. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie and is co-financed by the South Moravian Region under grant agreement No. 665860 (OJ). This publication reflects only the authors' view and the EU is not responsible for any use that may be made of the information it contains. We acknowledge the Czech Science Foundation (Grant No. 24-10760S to R.M.) and the Core Facilities Josef Dadok National NMR Center and Biomolecular Interactions and Crystallography of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2023042) and the European Regional Development Fund-Project "UP CIISB" (No. CZ.02.1.01/0.0/0.0/18_046/0015974). JML thanks Estonian Research Council (Grant PSG264). Open Access publishing facilitated by Masarykova univerzita, as part of the Wiley - CzechELib agreement.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: self-assembly · epimerism · chirality · cage compounds · supramolecular chemistry

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Manuscript received: May 14, 2024

Accepted manuscript online: June 6, 2024

Version of record online: August 1, 2024

Publication 2



Research Article



How to cite: *Angew. Chem. Int. Ed.* **2025**, e202513902
doi.org/10.1002/anie.202513902

Coordination Cages

Flexibility-Aided Orientational Self-Sorting and Transformations of Bioactive Homochiral Cuboctahedron Pd₁₂L₁₆

Subhasis Chattopadhyay, Robin Durník, Anniina Kiesilä, Elina Kalenius, Juha M. Linnanto, Pavel Babica, Jan Kuta, Radek Marek, and Ondřej Jurček*

This work is dedicated to Professor Michaela Vorlíčková, a leading expert in CD spectroscopy of nucleic acids, on the occasion of her 80th birthday

Abstract: The rational design and selective self-assembly of flexible and unsymmetric ligands into large coordination complexes is an eminent challenge in supramolecular coordination chemistry. Here, we present the coordination-driven self-assembly of natural ursodeoxycholic-bile-acid-derived unsymmetric *tris*-pyridyl ligand (**L**) resulting in the selective and switchable formation of chiral stellated Pd₆L₈ and Pd₁₂L₁₆ cages. The selectivity of the cage originates in the adaptivity and flexibility of the arms of the ligand bearing pyridyl moieties. The interspecific transformations can be controlled by changes in the reaction conditions. The orientational self-sorting of **L** into a single constitutional isomer of each cage, i.e., homochiral quadruple and octuple right-handed helical species, was confirmed by a combination of molecular modelling and circular dichroism. The cages, derived from natural amphiphilic transport molecules, mediate the higher cellular uptake and increase the anticancer activity of bioactive palladium cations as determined in studies using in vitro 3D spheroids of the human hepatic cells HepG2.

Introduction

Natural chiral hydrophobic cavities are important for many biological functions, e.g., for recognition as parts of transport proteins or for substrate-specific transformations as parts of enzymes. To understand and mimic these natural systems and their (supra)molecular mechanisms of action, the development of their artificial counterparts (e.g., cages, macrocycles) from chiral molecules is desirable. Easing such efforts, nature readily offers convenient chiral building blocks (terpenoids, amino acids, or carbohydrates) which can be utilized. Intermolecular-interaction-mediated self-assembly together with metal coordination are essential natural processes to construct such higher-order structures and can easily be adapted in the development of artificial systems.

The discovery of supramolecular coordination cages (SCCs) via coordination-driven self-assembly by Saalfrank et al.^[1] led to the rapid development of a large group of metallo-cycles and metallo-cages mainly using rigid and symmetric *bis/tris*-pyridyl coordinating ligands and tetravalent square-planar Pd²⁺.^[2] The edge-directed self-assembly of different *bis*-pyridyl ligands resulted in the formation of SCCs and macrocycles with the general formula Pd_nL_{2n}, e.g., Pd₂L₄, Pd₃L₆, Pd₄L₈, Pd₆L₁₂, Pd₁₂L₂₄, Pd₂₄L₄₈, Pd₃₀L₆₀, or Pd₄₈L₉₆—the largest SCC described so far.^[3] Whereas *tris*-pyridyl ligands led to Pd_{3n}L_{4n} SCCs, with only four types so far, i.e., Pd₃L₄,^[4–6] Pd₉L₁₂ (only one),^[7] Pd₁₈L₂₄^[8] (one) via edge-directed self-assembly, and the most common Pd₆L₈^[9] via face-directed self-assembly.

[*] S. Chattopadhyay, Prof. Dr. R. Marek, Dr. O. Jurček
Department of Chemistry, Faculty of Science, Masaryk University,
Kamenice 5, Brno CZ-62500, Czechia
E-mail: jurceko@pharm.muni.cz

S. Chattopadhyay, R. Durník, Dr. O. Jurček
Department of Natural Drugs, Faculty of Pharmacy, Masaryk
University, Palackého 1946/1, Brno CZ-61200, Czechia

S. Chattopadhyay, Prof. Dr. R. Marek, Dr. O. Jurček
CEITEC—Central European Institute of Technology, Masaryk
University, Kamenice 5, Brno CZ-62500, Czechia

R. Durník
Department of Biochemistry, Faculty of Science, Masaryk University,
Kamenice 5, Brno CZ-62500, Czechia

R. Durník, Assoc. Prof. P. Babica, Dr. J. Kuta
RECETOX, Faculty of Science, Masaryk University, Kotlarska 2, Brno
CZ-61137, Czechia

Dr. A. Kiesilä, Dr. E. Kalenius
Department of Chemistry, University of Jyväskylä, P. O. Box 35,
Jyväskylä FI-40014, Finland

Dr. J. M. Linnanto
Institute of Physics, University of Tartu, W. Ostwald Street 1, Tartu
50411, Estonia

Prof. Dr. R. Marek
National Center for Biomolecular Research, Faculty of Science,
Masaryk University, Kamenice 5, Brno CZ-62500, Czechia

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More than three decades after the first discovery, most of these metallo-supramolecular complexes are achiral and symmetric. Several approaches have been employed to construct low-symmetry, unsymmetric, or chiral coordination complexes using unsymmetric bidentate ligands,^[10] a combination of multiple symmetric ligands (heteroleptic complexes),^[11,12] or even single-type symmetric ligands.^[13–16] However, unlike natural systems, the presence of stereogenic carbons in the structure of ligands is rare, mostly limited to the peripheral areas of ligands and their resulting complexes,^[17] e.g., using peptides,^[18,19] pentasaccharide,^[20] or short alkyl chains.^[21]

To mimic the natural systems more closely and to develop the “next generation” SCCs, where the chiral centres will line their inner cavities requires careful design of ligands from inherently chiral natural compounds. Following this concept, Jurček et al. introduced the metallo-supramolecular macrocycle Pd₃L₆ containing 60 stereogenic carbons using the natural bile acid (BA) ursodeoxycholic acid (UDCA) as a core for *bis*-pyridyl ligands.^[22,23] A comparable study reports Pd₂L₄ SCCs using cholic-, deoxycholic-, or lithocholic-acid-based *bis*-pyridyl ligands.^[24] Recently, it was shown that ligands derived from chenodeoxycholic acid, an epimer of UDCA, can form even larger complexes, namely, Pd₂L₄, Pd₃L₆, Pd₄L₈, Pd₅L₁₀, and Pd₆L₁₂, having 120 stereogenic carbons.^[25] Other than this, intriguing coordination complexes have been reported using peptide-based *bis*-pyridyl ligands in the process of folding and assembly,^[26] but leaving triptic natural-molecule-based ligands unstudied.

Moreover, most ligands used to build coordination complexes are rather small and rigid. The directionality of the binding sites together with the rigidity of the ligand predefine their bend angles. These characteristics are crucial to control the self-assembly processes of symmetric and rigid ligands. Small differences in the bend angle of the ligand lead to significant changes in the final self-assembly products, e.g., a difference of 3° in the bend angle leads to the Pd₂₄L₄₈ → Pd₄₈L₉₆ or Pd₁₂L₂₄ → Pd₂₄L₄₈ transformation.^[27–29] However, the use of small, and to some extent flexible, ligands typically results in a mixture of kinetically trapped coordination complexes.^[30] In contrast, the large, flexible, and unsymmetric UDCA-based *bis*-pyridyl ligands, with a deviation of their bend angle up to 30° (90°–120°), showed the selective formation of a single constitutional isomer of Pd₃L₆.^[22,23,25] In comparison, the epimeric flexible chenodeoxycholic-acid-based ligand spans a bend angle range of 70°–90° (Δ 20°) resulting in a mixture of macrocyclic complexes (Pd₂L₄, Pd₃L₆, Pd₄L₈, Pd₅L₁₀, and Pd₆L₁₂). It has been proposed that the greater flexibility of the unsymmetric ligand together with certain steric restrictions increase the probability of forming a single species via orientational self-sorting.^[25] Still, a better understanding of the effect of the structural behaviour of the ligand on the final self-assembly is required.

Even after a rapid increase in the number of architectonically appealing SCCs, studies of their biomedical aspects and applications are limited.^[31] However, recent contributions demonstrate their drug-loading ability and promising anticancer therapeutical potential.^[32,33] From this perspective, the utilization of natural molecules in the construction of

biocompatible SCCs is appealing. Out of the vast library of natural compounds, BAs meet well the requirements for the design of chiral, unsymmetric, flexible, and biocompatible ligands. The BAs are biosynthesized in the human body, where they play a key role in the digestion and transport of lipids and lipid-soluble nutrients within the enterohepatic circulation using various passive and active transport processes.^[34] BAs are commercially easily available, enantiomerically pure (containing 9–11 chiral centres), and possess a conformationally defined rigid steroid skeleton decorated with hydroxyl groups and a flexible alkyl side chain bearing a carboxylic acid group, that can be easily synthetically transformed into pyridyl coordination sites.^[22–25] However, preparation of the *tris*-pyridyl ligand from a BA, its coordination-driven self-assembly with square-planar tetravalent Pd²⁺, demonstration of the potential of the ligand for orientational self-sorting and flexibility-permitted selective self-assembly, together with evaluation of its biomedical potential have been missing until now.

Results and Discussion

Natural UDCA was decorated in four synthetic steps with three 4-aminopyridine groups, attached either through carbamate bonds to C3 and C7 hydroxyls or by an amide bond to the C24 carboxylic acid, resulting in a tridentate ligand (**L**) (Figure 1, Supporting Information section 2.1). A molecular model of **L** can be visualized as an elongated triangular panel (towards C24) having two faces, concave (α) and convex (β), containing either pyridyls (Py) or methyls (C18, C19, and C21), respectively (Figure 1).

The initial complexation of **L** (10 mM) with [Pd(ACN)₄](BF₄)₂ (metal to ligand ratio M:L 3:4) in [D₆]-DMSO (70 °C, 1 h), marked as reaction mixture 1 (RM1) (Figure 1a), led to a quantitative (i.e., the spectrum lacks signals corresponding to the non-coordinated free ligand) formation of coordination species as confirmed by ¹H NMR spectroscopy (Figure 2a, green, Figure S13). The ¹H NMR signals showed a high-frequency coordination shift. The significant broadening of the ¹H signals and the presence of multiple aromatic signals give rise to a few possibilities considering the unsymmetry and flexibility of **L**, the formation of: 1) a mixture of coordination complexes with varying molecular formula (size), 2) multiple architectures of a complex having the same molecular formula, 3) multiple constitutional isomers of a single architecture (varying C3-C7-C24-pyridyl-Pd²⁺ connectivity-rotation of triangular panel), 4) multiple conformational isomers of a coordination complex, or 5) a combination of the abovementioned possibilities.

In the next step, the reaction mixture was analysed by electrospray ionization (ESI-MS) and ion mobility mass spectrometry (IM-MS) (Figure S14) revealing charge state distributions for ions [Pd₆L₈(BF₄)_n]⁽¹²⁻ⁿ⁾⁺ (*n* = 1–8) and [Pd₁₂L₁₆(BF₄)_n]⁽²⁴⁻ⁿ⁾⁺ (*n* = 6–16) (Table S1, being in the size range of small proteins, ca 7 and 14 kDa, respectively). The drift tube collision cross sections in nitrogen (^DTCCS_{N₂}) of [Pd₁₂L₁₆(BF₄)₁₄]¹⁰⁺ and [Pd₆L₈(BF₄)₂]¹⁰⁺ were 1976 and 1420 Å², corresponding roughly to diameters of 5.0 and

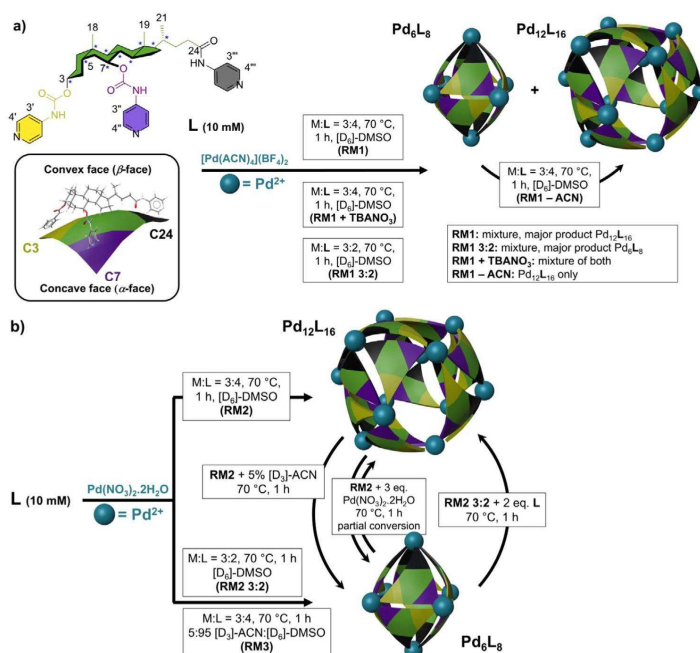


Figure 1. Coordination-driven self-assembly of **L** into stellated helical octahedral Pd_6L_8 and cuboctahedral $\text{Pd}_{12}\text{L}_{16}$ SCCs and their transformation reactions: a) using $[\text{Pd}(\text{ACN})_4](\text{BF}_4)_2$, b) using $\text{Pd}(\text{NO}_3)_2$. The blue asterisk denotes chiral centres of the steroid skeleton.

4.3 nm, respectively. According to the IM-MS, the formation of the larger cage $\text{Pd}_{12}\text{L}_{16}$ was more abundant under these conditions (~75% of $\text{Pd}_{12}\text{L}_{16}$ compared to Pd_6L_8). Furthermore, the number of species and their diffusion coefficients (*D*) were determined by ^1H DOSY NMR (Figure S15).

The presence of two species was confirmed, and hydrodynamic diameters were calculated from the diffusion coefficients using the Stokes–Einstein equation, 4.8 nm (Pd_6L_8) and 5.8 nm ($\text{Pd}_{12}\text{L}_{16}$). This provided a moderate fit with the sizes observed using the IM-MS data (Table S1) and confirmed the formation of Pd_6L_8 and $\text{Pd}_{12}\text{L}_{16}$ SCCs. Additionally, MS analysis confirmed that $\text{Pd}_{12}\text{L}_{16}$ is a monomer rather than a dimer of Pd_6L_8 based on the specific intensity abundance, concentration independence, collision cross section, and the presence of odd charge states of the ions (Supporting Information Section 2.2.1).

The selective formation of the $\text{Pd}_{12}\text{L}_{16}$ cage was achieved by dissolving **L** in $[\text{D}_6]$ -DMSO (10 mM) and using a different metal salt $\text{Pd}(\text{NO}_3)_2$ at M:L 3:4 (70 °C, 1 h) (RM2; Figures 1b,2a,b, Supporting Information Section 2.2.3). The selective formation of the Pd_6L_8 cage was achieved under two different reaction conditions, where **L** (10 mM) reacts with: 1) $\text{Pd}(\text{NO}_3)_2$ at M:L 3:2 ratio in $[\text{D}_6]$ -DMSO (70 °C, 1 h) (RM2 3:2; Figures 1b,2a,c, Supporting Information Section 2.2.3); and 2) $\text{Pd}(\text{NO}_3)_2$ at M:L 3:4 in the solvent

mixture $[\text{D}_3]$ -ACN:[D_6]-DMSO 5:95 (70 °C, 1 h) (RM3; Supporting Information Section 2.2.5). The solvent system was adjusted according to the low solubility of **L** in neat $[\text{D}_3]$ -ACN. The ^1H NMR spectra of both cages in $[\text{D}_6]$ -DMSO were distinguished by a sharp singlet signal at about 8.7 ppm indicating the presence of Pd_6L_8 (Figure 2a). The ^1H DOSY NMR experiments then reliably confirmed the selective formation of $\text{Pd}_{12}\text{L}_{16}$ or Pd_6L_8 in both solvent systems (Table S3). The values of the diffusion coefficient (*D*) for products in RM2, RM2 3:2, and RM3 were $4.98 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$ (4.9 nm, Figure 2b), $5.98 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$ (4 nm, Figure 2c), and $6.24 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$ (the presence of $[\text{D}_3]$ -ACN slightly increases *D*, Figure S33), respectively.

In the next step, we investigated a possible interspecific transformation of the coordination species followed by ^1H NMR and ^1H DOSY NMR techniques. Post-synthetic addition of 5% (v/v) $[\text{D}_3]$ -ACN to the $[\text{D}_6]$ -DMSO solution of $\text{Pd}_{12}\text{L}_{16}$ (RM2) (heated at 70 °C, 1 h) led to complete conversion to Pd_6L_8 (Supporting Information Section 2.2.6.1). In another experiment, adding two equivalents of **L** to the $[\text{D}_6]$ -DMSO solution of Pd_6L_8 (RM2 3:2), changing the M:L ratio from 3:2 to 3:4 (heated at 70 °C, 1 h), led to complete conversion to $\text{Pd}_{12}\text{L}_{16}$ (Supporting Information Section 2.2.6.2). Conversely, three equivalents of $\text{Pd}(\text{NO}_3)_2$ were added to the $\text{Pd}_{12}\text{L}_{16}$ solution (RM2), changing the ratio M:L from

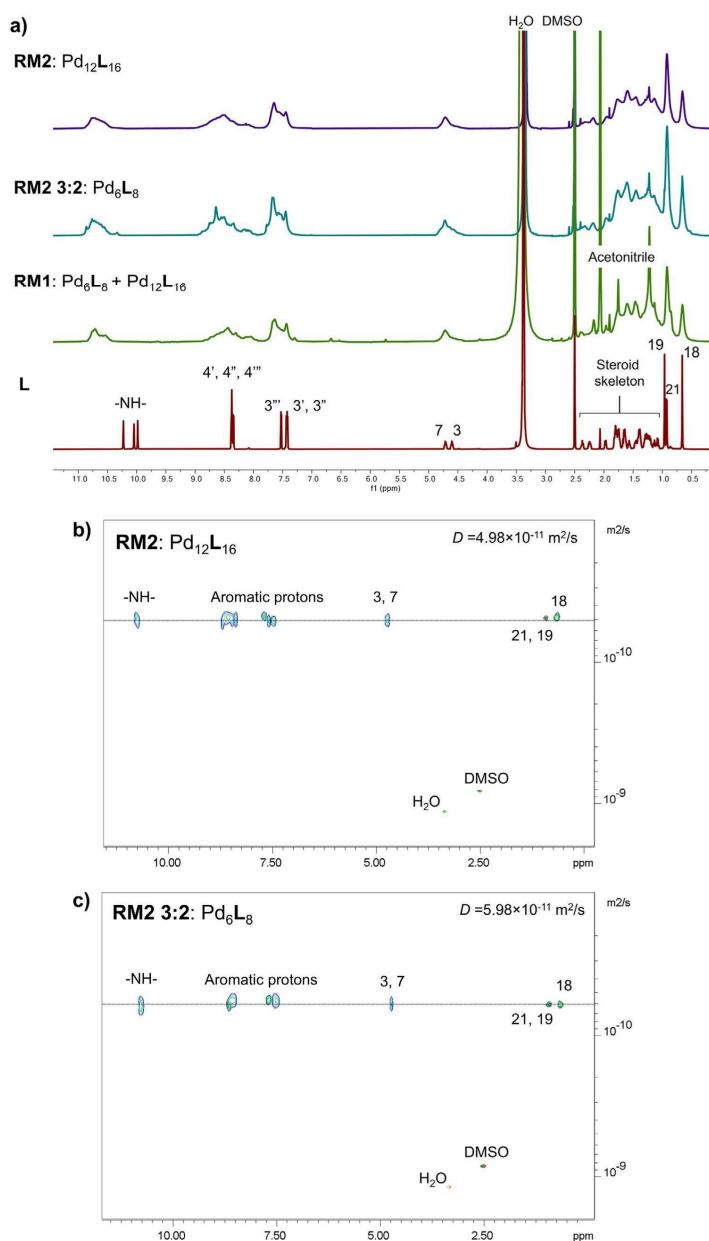


Figure 2. NMR characterisation of Pd_6L_8 and $\text{Pd}_{12}\text{L}_{16}$. a) ^1H NMR spectra of **L**, mixture of Pd_6L_8 and $\text{Pd}_{12}\text{L}_{16}$ (**RM1**), Pd_6L_8 (**RM2 3:2**), and $\text{Pd}_{12}\text{L}_{16}$ (**RM2**) in $[\text{D}_6]\text{-DMSO}$ at 298.2 K and 700 MHz. ^1H DOSY NMR spectra of b) $\text{Pd}_{12}\text{L}_{16}$ (**RM2**) and c) Pd_6L_8 (**RM2 3:2**) ($[\text{D}_6]\text{-DMSO}$, 303.2 K and 700 MHz).

3:4 to 3:2 (heated at 70 °C, 1 h). Spectroscopic analyses showed partial conversion of $\text{Pd}_{12}\text{L}_{16}$ to Pd_6L_8 (Supporting Information Section 2.2.6.3), even after prolonged heating. A comparison of the diffusion coefficients for all reaction products is shown in Table S3.

Considering these observations and the related literature,^[35,36] we suggest a hypothesis for the reaction mechanism of self-assembly processes. The coordination effect of the coordinating species participating in the reaction mixture decreases in the sequence: pyridyl >> DMSO > NO_3^- > acetonitrile >> BF_4^- .^[37] At the same time, their coordination ability should also be considered through their concentration factors, thus the effect of $[\text{D}_6]$ -DMSO, present in all experiments in very high excess, can be considered as comparable. Therefore, we hypothesize that the L -pyridyls $\leftrightarrow \text{NO}_3^- \leftrightarrow$ acetonitrile exchange affects the self-assembly processes the most significantly (ignoring the effect of the weak coordination of BF_4^-). A reaction mixture may contain a Pd_6L_8 self-assembly as a minor product in a mixture with $\text{Pd}_{12}\text{L}_{16}$ if $[\text{Pd}(\text{ACN})_4](\text{BF}_4)_2$ (3:4 M:L, Pd:ACN ratio 1:4) was used or as neat single product when using $\text{Pd}(\text{NO}_3)_2$ with 5% (v/v) $[\text{D}_3]$ -ACN in $[\text{D}_6]$ -DMSO as the solvent system (Pd:ACN ratio 1:128). In the case of $[\text{Pd}(\text{ACN})_4](\text{BF}_4)_2$, Pd_6L_8 is present as a minor product in a mixture with $\text{Pd}_{12}\text{L}_{16}$ (as determined by comparison of the heights of the drift peaks by IM-MS, Table S1), whereas in a large excess of ACN, Pd_6L_8 is the only species. Also, prolonged heating of the $[\text{Pd}(\text{ACN})_4](\text{BF}_4)_2$ reaction leads to an increase in the concentration of Pd_6L_8 . In addition, the reaction of L (10 mM) with $[\text{Pd}(\text{ACN})_4](\text{BF}_4)_2$ at M:L 3:2 in $[\text{D}_6]$ -DMSO (70 °C, 1 h) was carried out, where the Pd_6L_8 species was observed as the major product in a mixture with $\text{Pd}_{12}\text{L}_{16}$ (Table S2, Figure 1a). In general, the higher the concentration of ACN the higher the ratio of Pd_6L_8 to $\text{Pd}_{12}\text{L}_{16}$. Finally, we also performed an experiment where the product was prepared via RM1 and the reaction mixture was subsequently evaporated to dryness under vacuum to remove/reduce the ACN content. The solid residue was re-dissolved in $[\text{D}_6]$ -DMSO at 70 °C (RM1-ACN). ^1H NMR spectroscopy showed only a trace amount of ACN remaining (the ratio between Pd:ACN was reduced from 1:4 to 1:0.2, Figure S16). The ^1H -DOSY NMR spectrum showed complete conversion of the $\text{Pd}_{12}\text{L}_{16}$ and Pd_6L_8 mixture into $\text{Pd}_{12}\text{L}_{16}$ only (Figure S17), which further confirms that it is the elevated amount of ACN that directs the reaction towards Pd_6L_8 . In this experiment, we could also compare the effect of a palladium counter-anion, having the reaction mixture containing either BF_4^- or NO_3^- anion in $[\text{D}_6]$ -DMSO in $[\text{M}]:[\text{L}] = 3:4$ ratio. Both reaction mixtures provided the $\text{Pd}_{12}\text{L}_{16}$ complex, suggesting that the anion effect on the final self-assembly is similar or negligible, unlike the eminent effect of ACN.

A similar situation can also be observed in the case of varying $\text{Pd}(\text{NO}_3)_2$ concentration, i.e., a higher concentration of $\text{Pd}(\text{NO}_3)_2$ in RM2 3:2 leads to selective formation of Pd_6L_8 in comparison to RM2, or the addition of an excessive amount of $\text{Pd}(\text{NO}_3)_2$ to $\text{Pd}_{12}\text{L}_{16}$ (prepared under conditions of RM2), shifting the molar ratio M:L from 3:4 to 3:2 (resulting in three equivalents of $\text{Pd}(\text{NO}_3)_2$ present as a free salt in

the reaction mixture), leads to a partial transformation to Pd_6L_8 . In contrast, the addition of L to RM2 3:2, shifting the M:L from 3:2 to 3:4 (decreasing the concentration of free $\text{Pd}(\text{NO}_3)_2$), promotes the formation of $\text{Pd}_{12}\text{L}_{16}$.

To further distinguish the effect of nitrate or palladium, we have carried out additions of TBANO_3 to RM1 (Supporting Information Section 2.2.2.2). 1.5 eq. (M:L: NO_3^- 3:4:6) and 3 eq. (M:L: NO_3^- 3:4:12) of TBANO_3 with respect to L were subsequently added and the reaction mixture was heated at 70 °C for 1 h upon every addition (Figure S18a,b). ^1H DOSY NMR analysis showed that the solutions contained mixtures of SCCs (even after continued heating for 24 h) (Figure S18c). These experiments indicate that the addition of nitrate does not influence strongly the equilibrium between the SCCs and it is rather the palladium concentration or the ACN content that control the interspatial transformation and direct the equilibrium towards the Pd_6L_8 product.

The presence of two different species in the reaction mixture suggests the formation of thermodynamic and kinetically trapped species. A previous study by Hiraoka et al. showed that the use of ACN as solvent accelerates the ligand exchange rate (solvent-assisted exchange self-assembly process) which prevents the formation of primitive intermediates leading to kinetic products, thus it is promoting the formation of a thermodynamic product.^[36] The participation of ACN in the self-assembly process results in preference for the Pd_6L_8 complex which can thus be seen as a thermodynamic product, whereas its absence in DMSO shifts the equilibrium towards the kinetic product $\text{Pd}_{12}\text{L}_{16}$.

If we consider that ACN allows greater coordination bond reversibility and increases the dynamic character of the Pd(II)-ligand coordination bond, a mechanism of reaction selectivity could be deduced. In the process of self-assembly, ligands form variously sized oligomers with metals before the final transformation into a cage (cyclization).^[38] In the presence of an increased concentration of ACN, the more dynamic ligand exchange can be responsible for the formation of a greater number of smaller oligomers, ultimately leading to a smaller Pd_6L_8 complex. This can also be true with an elevated concentration of $\text{Pd}(\text{NO}_3)_2$ where the increased concentration of palladium cations plays a key role in the formation of the oligomer (the higher the Pd^{2+} concentration, the smaller the oligomer and the smaller the final complex). In contrast, in the absence of ACN, the oligomers grow larger as the ligand exchange is slower, ultimately leading to large kinetically trapped oligomers which eventually transform into $\text{Pd}_{12}\text{L}_{16}$.

Nuclear shielding in diamagnetic systems is highly local in nature, therefore the measured ^1H NMR shifts for ligands in Pd_6L_8 and $\text{Pd}_{12}\text{L}_{16}$ are very similar, also being composed of similarly built subunits (Figure 3a). However, the larger $\text{Pd}_{12}\text{L}_{16}$ cage has a slightly longer correlation time (shorter relaxation time) and therefore somewhat broader NMR lines (Figure 2a). Additionally, we assume that the line width and apparent presence of multiple sets for the proton NMR signals of pyridyls and the -NH- groups indicate the representation of multiple stable conformational isomers of L in the complexes. In this regard, a variable temperature ^1H NMR study of RM2 was conducted over the temperature

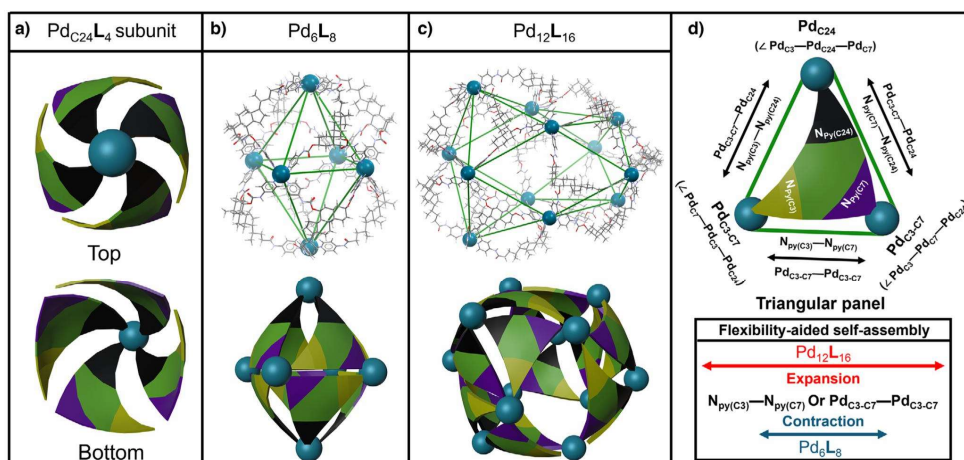


Figure 3. Computational models and cartoon representations. a) $\text{Pd}_{\text{C}24}\text{L}_4$ building subunit, b) Pd_6L_8 , c) $\text{Pd}_{12}\text{L}_{16}$, and d) nomenclatures used for the triangular panel.

range 298.2–398.2 K in increments of 10 K (Figure S27, Supporting Information Section 2.2.4). The study shows sharpening of the proton signals with increasing temperature, but also the eventual appearance of a second set of signals to the detriment of the original set corresponding to $\text{Pd}_{12}\text{L}_{16}$. This can represent either formation of conformational or constitutional isomers, or the formation of a new coordination product (these could not be confirmed by MS). At the end of the experiment, the sample was cooled back down to 298.2 K and the ^1H - and ^1H DOSY NMR spectra that were recorded showed the presence of a single coordination species $\text{Pd}_{12}\text{L}_{16}$ but represented by somehow sharper and better distinguished ^1H NMR signals (Figures S27 and S28). For comparison, the reaction RM2 was carried out (M:L 3:4, 10 mM L , $\text{Pd}(\text{NO}_3)_2$, $[\text{d}_6]\text{-DMSO}$) at 100 °C, heated for 1 h, and ^1H - and ^1H DOSY NMR spectra were recorded showing the formation of $\text{Pd}_{12}\text{L}_{16}$ similar to the previous case of incremental heating to 125 °C (Figure S29).

To our understanding,^[39] the appearance of a complex second set of multiplets with a significant chemical shift refers to the formation of a new coordination self-assembly, most likely a new constitutional isomer (caused by rotation of the ligand(s)). This is more probable than the formation of new conformational isomers, as we assume that the spectral changes would be more subtle in such a case. Moreover, the structural changes are represented in a smaller fraction of the species and are temporary as they are connected to the elevated temperature and the original form is restored upon cooling. In contrast, the signal sharpening which remains even upon cooling could be related to conformational changes, i.e., in direct synthesis at 70 °C for 1 h, some ligands are trapped in local minima, whereas heating to higher temperatures helps them reach global minima conformations corresponding better to

complex spatial requirements (where they also remain upon cooling).

As we previously foretold, comparing the self-assemblies of epimeric *bis*-pyridyl ligands derived from UDCA and chenodeoxycholic acid^[25] the structural selectivity for the self-assembly lies in the ligand's geometry and the flexibility provided by the bending of the ligand molecule but also by a conformational change through rotation on the C3-O-CO-NH-pyridine axis.

Concerning the present ligand **L**, we believe that even larger conformational changes in the near vicinity of pyridyls, i.e., on the C3-O-CO-NH-pyridine, C7-O-CO-NH-pyridine, and C24-CO-NH-pyridine axes, are manifested in solution. The conformational flexibility was further demonstrated by experiments using the *mono*-pyridyl ligands $\text{L}_{\text{M}3}$ and $\text{L}_{\text{M}24}$, where the binding moiety is at either position C3 or C24, respectively. For more details, please see Supporting Information Section 4.

Finally, having both neat species in hand, many attempts were made to grow a single crystal, but none of the crystals obtained showed X-ray diffraction. Therefore, a combination of circular dichroism (CD) and molecular modelling was employed as an alternative for the structural analysis at the molecular level. Computational models of Pd_6L_8 and $\text{Pd}_{12}\text{L}_{16}$ were prepared and their energy was optimized using the DFT approach (Figure 3). An important steric restraint, resulting from the presence of two faces of **L**, is that the convex face should always point outside of the complex. Also, the elongated shape of **L** towards C24 further restricts its orientation in the final self-assembly. This implies that the structure of **L** prohibits the formation of many theoretically possible constitutional isomers of SCCs. Following this, the structural subunit $\text{Pd}_{\text{C}24}\text{L}_4$ was constructed, where four ligands are connected to Pd^{2+} by their C24-pyridyls (marked

as $\text{Pd}_{\text{C}24}$) with the β -faces pointing outwards (Figure 3a, the model subunit was designed in correlation with a CD spectroscopic study which is described in detail later). Two of these subunits were interconnected through four Pd^{2+} ions with the remaining pyridyls at C3 and C7 (marked as $\text{Pd}_{\text{C}3-\text{C}7}$), leading to Pd_6L_8 . The resulting optimized structure of Pd_6L_8 represents an axially elongated, symmetric, and stellated octahedron (Figure 3b). To further support such structural organization, we performed semiempirical calculations on the Pd_6L_8 complex using the PM6 method (palladium charge balanced by two nitrates) to study the effect of the rotation of **L** on the structure (constitutional isomerism). Initially, we found that the rotation of **L** is geometrically preferred in the counterclockwise direction, whereas rotation in the clockwise direction leads to large distances between the pyridyls and the *tris*-pyridyl-coordinated Pd cation that significantly change the overall shape of the complex and increase its total energy by a few tens of kcal per mole. In contrast, the complex with the ligand counterclockwise-rotated retains its original shape overall but leads to an energy increase of about 7 kcal mol⁻¹ in comparison to the original symmetric complex suggested. If all the ligands in the complex were similarly rotated, the total energy would increase by about 40 kcal mol⁻¹ (approximately 5 kcal mol⁻¹ per ligand rotation). Finally, we compared the total energies of the ligands taken out of both optimized Pd_6L_8 complexes (original and rotated). The differences in total energy between these two ligand conformations result similarly in about 4–6 kcal mol⁻¹, suggesting that the energy difference between the complexes originates mostly in the conformational change of the ligand, while the energy of the coordination interactions between Pd(II) and the pyridyls of the ligand seem to be quite similar for both complexes.

The structure of $\text{Pd}_{12}\text{L}_{16}$ was similarly constructed using four $\text{Pd}_{\text{C}24}\text{L}_4$ subunits. Two $\text{Pd}_{\text{C}24}\text{L}_4$ subunits were placed sideways to each other and connected by two $\text{Pd}_{\text{C}3-\text{C}7}$. Two of these dimeric units were then joined together with the remaining $\text{Pd}_{\text{C}3-\text{C}7}$ (resembling the structure of a tennis ball) and fulfilling the square planar coordination sphere by pyridyls forming the cuboctahedral $\text{Pd}_{12}\text{L}_{16}$ species. The geometry-optimized structure of $\text{Pd}_{12}\text{L}_{16}$ illustrates a low-symmetry stellated cuboctahedron, where each triangular face contains one **L**, two opposite square faces have two **L**s each, and each remaining square face has one **L** in an alternating up and down (zig-zag) fashion (Figure 3c). This supramolecular cuboctahedron, cultivating the face-directive self-assembly of a tritopic ligand, is unprecedented.

The structural behaviour of **L** in Pd_6L_8 and $\text{Pd}_{12}\text{L}_{16}$ can be easily described and followed through the Pd–Pd distances. The average $\text{Pd}_{\text{C}24}-\text{Pd}_{\text{C}3-\text{C}7}$ and $\text{Pd}_{\text{C}3-\text{C}7}-\text{Pd}_{\text{C}3-\text{C}7}$ distances for Pd_6L_8 are 18.4 and 15.6 Å, and those of $\text{Pd}_{12}\text{L}_{16}$ are 19.5 and 19.9 Å, respectively. The average angles of the triangular faces for Pd_6L_8 are 50° (for $\angle\text{Pd}_{\text{C}3}-\text{Pd}_{\text{C}24}-\text{Pd}_{\text{C}7}$) and 65° (for $\angle\text{Pd}_{\text{C}7}-\text{Pd}_{\text{C}3}-\text{Pd}_{\text{C}24}$ and $\angle\text{Pd}_{\text{C}3}-\text{Pd}_{\text{C}7}-\text{Pd}_{\text{C}24}$), whereas those of $\text{Pd}_{12}\text{L}_{16}$ contain 60° in both cases (Figure 3d, Tables S4–S7). Thus, the octahedron is made of isosceles triangles, while the cuboctahedron is composed of nearly equilateral triangular panels. The $\text{Pd}_{3-7}-\text{Pd}_{3-7}$ distances correlate to the $\text{N}_{\text{Py}(\text{C}3)}-\text{N}_{\text{Py}(\text{C}7)}$ distance corresponding to a change of $\angle\text{N}_{\text{Py}(\text{C}3)}-\text{C}5-\text{N}_{\text{Py}(\text{C}7)}$ bend angle of **L**. The $\angle\text{N}_{\text{Py}(\text{C}3)}$ —

$\text{C}5-\text{N}_{\text{Py}(\text{C}7)}$ bend angle range is 90°–93° for Pd_6L_8 and 105°–127° for $\text{Pd}_{12}\text{L}_{16}$ (Figure S40). At the same time, a small increase in the $\text{Pd}_{\text{C}3-\text{C}7}-\text{Pd}_{\text{C}24}$ distance for $\text{Pd}_{12}\text{L}_{16}$ demonstrates the decrease in the convexity of **L**. In summary, although the ligand is an unsymmetric building block in its nature, inside the complexes it can effectively adapt because of its flexibility and it is contained as a regular symmetric panel according to the steric and geometric requirements of the given self-assembly (resembling the multicomponent self-assembly process of viral capsids). In particular, **L** adapts to the structure of a stellated octahedron or cuboctahedron in directions $\text{N}_{\text{Py}(\text{C}3)}-\text{N}_{\text{Py}(\text{C}7)}$, $\text{N}_{\text{Py}(\text{C}3)}-\text{N}_{\text{Py}(\text{C}24)}$, $\text{N}_{\text{Py}(\text{C}7)}-\text{N}_{\text{Py}(\text{C}24)}$ differing by 3.1, 1.0, and 0.8 Å, with respect to each other, with higher standard deviations observed for $\text{Pd}_{12}\text{L}_{16}$ (Figure 3d, Table S8). In comparison, the DFT-optimized model of **L** (ground state in polar solvent, B3LYP/6–31G*) has a medial $\text{N}_{\text{Py}(\text{C}3)}-\text{N}_{\text{Py}(\text{C}7)}$ distance to both species, an $\text{N}_{\text{Py}(\text{C}7)}-\text{N}_{\text{Py}(\text{C}24)}$ distance similar to that of Pd_6L_8 , and is 0.8 Å longer than in $\text{Pd}_{12}\text{L}_{16}$, but it is significantly stretched in the direction $\text{N}_{\text{Py}(\text{C}3)}-\text{N}_{\text{Py}(\text{C}24)}$ by 5.0 and 4.0 Å as compared to Pd_6L_8 and $\text{Pd}_{12}\text{L}_{16}$, respectively (Table S8).

The selective formation of different complexes using a single usually small and relatively rigid ligand were previously achieved by: 1) a templating effect of the solvent^[40] or counter anion,^[41,42] 2) using different metal nodes (i.e., Pt^{2+} and Pd^{2+}),^[43] 3) using different binding sites of the same ligand,^[44] or 4) using photoactive ligands via changing their conformation.^[45] Fine balancing and prediction of the structural flexibility in the design of a ligand is a challenging task (often resulting in a mixture of kinetically trapped species with limited flexibility)^[30,43] and the construction of larger species with flexible ligands rather relies on the method of trial-and-error. Therefore, the selective formation of coordination complexes utilizing the inherent flexibility of the ligand represents a novel approach, which we refer to as *flexibility-aided self-assembly*, moreover, considering the unsymmetry of the ligand, we propose to term this phenomenon *flexibility-aided orientational self-sorting*.

Finally, the CD spectra of **L**, Pd_6L_8 , and $\text{Pd}_{12}\text{L}_{16}$ were recorded to investigate the chirality of the SCCs (Figure 4a). **L** gives a single negative CD band at 247 nm in the spectral region studied, whereas Pd_6L_8 and $\text{Pd}_{12}\text{L}_{16}$ display a strong positive band at 275 nm and strong negative band at 260 nm, resulting in a (+ –) CD couplet. The coordination sphere of $\text{Pd}_{\text{C}3-\text{C}7}$ in models of Pd_6L_8 and $\text{Pd}_{12}\text{L}_{16}$ shows a clockwise C3–C7–C3–C7 connectivity (3,7- $\text{Pd}_{\text{C}3-\text{C}7}$ -3,7) which induces right-handed helicity (Δ), as observed on following two diagonally interconnected ligands through the C24–C3–Pd–C3–C24 backbone (Figures 4b and S41). This helicity is present in both cages, leading overall to a right-handed quadruple ($\Delta\Delta\Delta\Delta$) for Pd_6L_8 or octuple helices ($\Delta\Delta\Delta\Delta\Delta\Delta\Delta\Delta$) for $\text{Pd}_{12}\text{L}_{16}$. In general, the structural organization of natural polymers into a right-handed double helix is quite abundant, e.g., the most common form of DNA, canonical B-DNA, is represented by a similar (+ –) CD couplet.^[46] The CD spectra of the SCCs were further compared with the previously reported *bis*-pyridyl UDCA-based ligand **L**_d and its macrocyclic complex $\text{Pd}_3(\text{L}_d)_6$ ^[22] (Figure 4c) where, interestingly, almost the opposite (– +) CD couplet was

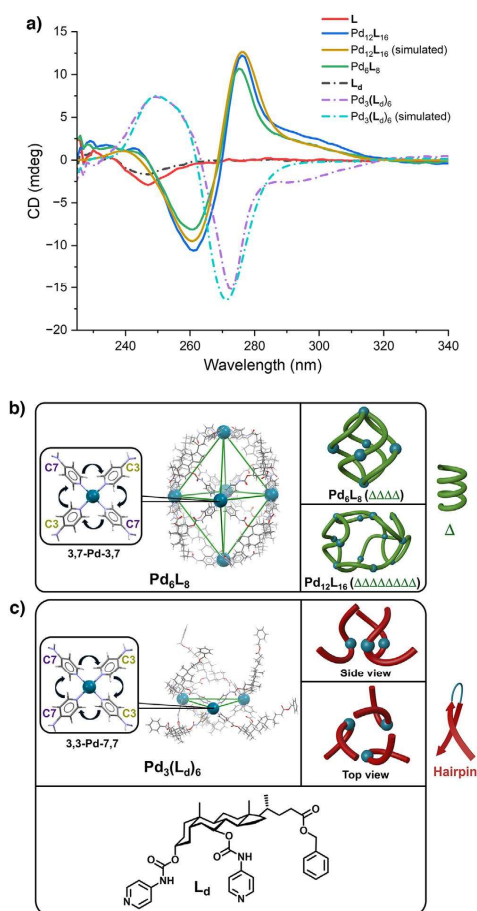


Figure 4. Structural analysis of supramolecular coordination complexes using CD spectroscopy. a) CD spectra of ligands and their coordination complexes in methanol at 25 °C. Interpretation of helical structures of b) Pd₆L₈ or Pd₁₂L₁₆, and c) Pd₃(L_d)₆, following the C24-C3-Pd-C3-C24 backbone.

observed (Figure 4a). It was demonstrated that a single crown-like constitutional isomer of Pd₃(L_d)₆ was formed where the Pd²⁺ centres have a different clockwise connectivity C3-C3-C7-C7 (3,3-Pd-7,7) (Figure 4c). If, similarly, the curvature of the C24-C3-Pd-C3-C24 backbone is followed, a hairpin structure is observed. This makes Pd₃(L_d)₆ an ensemble of three hairpin subunits accounting for the opposite CD couplet. Interestingly, a similar inverse CD couplet can also be observed in nature representing the duplex-to-hairpin transformation of B-DNA.^[46]

To further support the structural assignment, we carried out a computational study at the time-dependent DFT level to

calculate the CD spectrum of Pd₁₂L₁₆. Even though the large size of the Pd₁₂L₁₆ complex and the computational demands connected with that led us to make certain approximations in the DFT calculations, the resulting spectrum using a fragment-based approach is in very good agreement with the experimental one (Figure 4a, Supporting Information Section S.1). The calculations also describe well the spectrum of Pd₃(L_d)₆ (Figure 4a).

Therefore, a different structural organization of the same steroidal backbone in a supramolecular complex can lead to a different CD spectrum. Finding such a relationship between the CD signal and the structure represents a very useful tool in elucidating the connectivity of the ligands, i.e., 3,7-Pd_{C3-C7}-3,7 and the 24,24-Pd_{C24-24} corresponding to it, confirming the structural isomerism in Pd₆L₈ and Pd₁₂L₁₆. On that account, revealing the origin of the CD signals in these SCCs and comparing them with known supramolecular species (Supporting Information Section S.1) and natural products, the CD method provides a potent alternative for the structural determination of chiral supramolecular species comparable to the often challenging single-crystal X-ray diffraction.

Moreover, CD spectroscopy may serve as a reliable and sensitive method to study the aqueous stability of Pd₆L₈ and Pd₁₂L₁₆ at the low concentrations relevant for biological studies (Figure S42). Consequently, considering the inherent transport ability of BAs within the enterohepatic circulation and the biological activity of Pd²⁺, we used spheroids of the human hepatoblastoma cell line HepG2 as a 3D in vitro model of the target liver tissue. The hepatospheroids were exposed to samples of Pd(NO₃)₂, L, Pd₆L₈, and Pd₁₂L₁₆. While L has shown negligible toxicity after an 8-d exposure, the spheroid viability in response to an inorganic salt or SCCs decreased with increasing concentrations of Pd²⁺ ions. A comparison of equimolar Pd²⁺ concentrations (24 μM) of Pd(NO₃)₂, Pd₆L₈, and Pd₁₂L₁₆ shows spheroid viability reduced to 90%, 78%, and 65% of the control, respectively, as determined by the adenosine triphosphate (ATP) assay (Figure 5a) or by following on the spheroid size (Figure S57a, Table S9). At the end of the experiments, the spheroids were isolated, carefully washed, and submitted for studies by inductively coupled plasma mass spectrometry (ICP-MS) to determine the palladium content. The palladium content in the spheroids displays a significant negative correlation with their viability (Figure 5b) and size (Figure S57b). The spheroids treated with 24 μM Pd(NO₃)₂ contained a low palladium content of 0.15 ng per spheroid, whereas 4 μM Pd₆L₈ and 2 μM Pd₁₂L₁₆ delivered 1.5 and 4.26 ng of bioactive palladium per spheroid, respectively (Figure 5b, Table S8). This corresponds to 0.023% cellular absorption efficiency at 24 μM Pd(NO₃)₂ (in total 638.5 ng of Pd(II) was applied), while the Pd₆L₈ and the Pd₁₂L₁₆ showed 0.2% and 0.7% uptake efficiency, respectively, at the equivalent palladium dose. The intraspheroid to extracellular concentration (IC:EC) ratios calculated across different species exhibited even more pronounced differences due to the reduced spheroid size in response to SCCs. At the high palladium dose, the IC:EC ratio was 248 for Pd₁₂L₁₆ and 51 for Pd₆L₈, representing a 56-fold and 12-fold increase, respectively, compared to the IC:EC ratio of 4.4 observed for

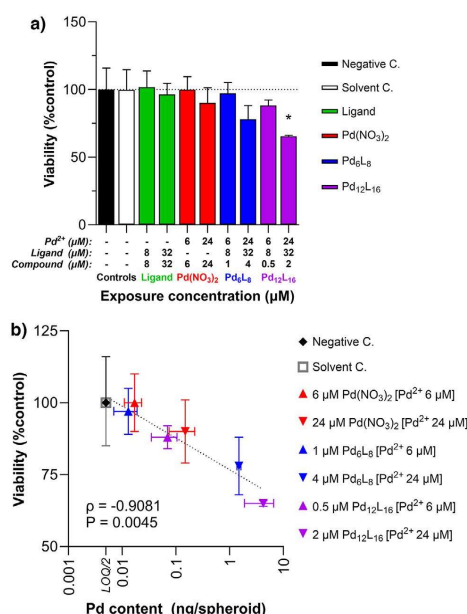


Figure 5. Toxicological studies of the SCCs. a) Concentration-response of HepG2 spheroid viability (ATP content) after 8 days of exposure to Pd(NO₃)₂, L, Pd₆L₈, and Pd₁₂L₁₆. The asterisk (*) indicates a statistically significant difference from the solvent control. b) Relation of spheroid viability to palladium content measured in spheroids. ρ represents Spearman's rank correlation coefficient with a P value.

Pd(NO₃)₂ (Table S9, Figure S57c). These results show that with larger and more charged SCCs, the affinity for uptake by the hepatospheroids, and consequently their toxic effects, increased (Figures 5b and S57b).

Conclusion

The majority of the artificial metallo-supramolecular complexes, unlike natural systems, are made of achiral, symmetric, and rigid ligands. In contrast, nature is ruled by the handedness of molecules, whereas the amino acids build and regulate living organisms through their left-handedness, evolution assigned carbohydrates with right-handed features. Control over the handedness of supramolecular coordination complexes (SCCs), by simply changing the connectivity of the ligand to a metal node, e.g., by introducing additional denticity to the ligand, is a very interesting feature which can lead to stimuli-responsive interconversion of such cavity containing structures.

In this study, we provide a concept to design chiral, unsymmetric, flexible ligands employing natural bile acids in the construction of the “next generation” supramolecular coordination cages (SCCs). Implementing this concept we

have introduced the first natural-molecule-based unsymmetric tris-pyridyl ligand (L) derived from ursodeoxycholic bile acid. Because of the inherent flexibility, L can structurally adjust by expanding or contracting in coordination self-assemblies with square-planar Pd(II) into a Pd₆L₈ octahedron or a giant Pd₁₂L₁₆ cuboctahedron (5 nm in diameter). We refer to this controllable self-assembly process as “flexibility-aided orientational self-sorting”. At the same time, Pd₁₂L₁₆ represents the very first face-directed self-assembly of a tridentate ligand into the cuboctahedron, introducing a new group of M₁₂L₁₆ species.

So far, product mixtures resulting from rigid and symmetric ligands usually contain smaller species as the kinetically trapped ones,^[30,43] whereas, in our case, it was found that using the unsymmetric and flexible ligand, the larger Pd₁₂L₁₆ species represents the metastable form. Similarly in nature, while some large kinetic self-assemblies might be less stable because of increased complexity and the potential for structural defects, others can be exceptionally stable because of robust intermolecular interactions and intricate design. Our example seems to be reaching the natural complexity.

The course of the reaction and the resulting product can be controlled by the choice of reaction conditions or later via interspecific transformations between Pd₆L₈ and Pd₁₂L₁₆. The process of structural switching has a special significance in view of the system adaptation to the environment representing a step forward towards artificial adaptive materials derived from natural products.

As for the structural elucidation, single-crystal X-ray diffraction is of paramount importance for SCCs. However, getting a quality single crystal is a challenging task in many cases, especially for flexible SCCs like ours. Accordingly, we employed various analytical techniques, with importance laid on CD spectroscopy in combination with molecular modelling, which confirm the orientational self-sorting and structural isomerism of the SCCs into right-handed quadruple (ΔΔΔΔ) or octuple (ΔΔΔΔΔΔΔΔ) helices. We envisage that CD spectroscopy, being a well-established, sensitive, fast, and reliable tool for large chiral systems (e.g., DNA, proteins), can also be used to predict the structural organization of chiral metallo-supramolecular complexes.

Because of their tailored composition, these water-soluble SCCs show the enhanced cellular absorption and increased toxicity effect of palladium(II) cations when studied with spheroids of hepatoblastoma HepG2 cells. This anticancer effect of Pd²⁺ amplifies with increasing charge and size of the SCC. ICP-MS analysis of hepatospheroids reveals 12-fold (Pd₆L₈) or 56-fold (Pd₁₂L₁₆) higher uptake of toxic Pd²⁺ than that of inorganic Pd(NO₃)₂. This interesting toxicological behaviour of bile-acid-based SCCs highlights the importance of developing natural molecule-based coordination ligands and their supramolecular complexes for modern therapeutics.

The steroidal SCCs developed mimic natural hydrophobic cavities containing 80 (Pd₆L₈) or a record 160 chiral centres (Pd₁₂L₁₆) in their structures. Therefore, these SCCs, along with possible biomedical applications, are equally attractive for molecular recognition, enantioselective catalysis, and material science. We believe that the future of metallo-supramolecular chemistry lies in the development and control



of SCCs containing chiral nanocavities. Advancement in the field requires crucial understanding of their structural design and of their additional features such as substrate selective recognition, concerted interplay of reactive functionalities, or reversible dynamic structural changes for efficient substrate-product turnover. The *flexibility-aided orientational self-sorting* we have introduced herein represents a novel approach leading to such supramolecular self-assemblies at the interface of synthetic and biological fields. It will certainly be an interesting endeavour to continue in the future.

Supporting Information

The authors have cited additional references within the Supporting Information.^[47–54]

Acknowledgements

The authors thank Dr. Miroslava Bittová for measuring some of the MS spectra. This work has received support from the Czech Science Foundation (Grant No. 24–10760S to R.M.) and the Grant Agency of Masaryk University (MUNI/A/1575/2023 and MUNI/C/0123/2023). The authors acknowledge the Core Facility NMR of CIISB, Instruct-CZ Center, supported by MEYS CR (LM2023042) and the European Regional Development Fund Project “UP CIISB” (No. CZ.02.1.01/0.0/0.0/18_046/0015974). This work has been supported by the RECETOX Research Infrastructure (LM2023069) financed by MEYS CR. This work was also supported by the European Union’s Horizon 2020 research and innovation program under grant agreement No 857560 (CETOCOEN Excellence). This publication reflects only the author’s view, and the European Commission is not responsible for any use that may be made of the information it contains.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Biological activity • Chirality • Self-assembly • Structural transformation • Supramolecular chemistry

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Manuscript received: June 25, 2025

Revised manuscript received: July 23, 2025

Accepted manuscript online: July 23, 2025

Version of record online: ■■■■■

Publication 3



Chemistry – A European Journal

Research Article
doi.org/10.1002/chem.202500748

www.chemeurj.org

Robust Self-Healing Metallo-Supergels of Folic Acid: Potential Sustainable Gelator for Oilfield Applications

Mahya Asgharian Marzabad,^[a, b, c] Subhasis Chattopadhyay,^[a, b, c] Sami Hietala,^[d] Nonappa Nonappa,^{*[e]} Radek Marek,^[a, c] and Ondřej Jurček^{*[a, b, c]}

The majority of known metallosupramolecular gels are based on carefully designed ligands using extensive chemical synthesis. Their gelation is often limited to a certain specific metal salt. We demonstrate that in the presence of a wide group of metal salts natural and readily available folic acid (FA) can act as a supergelator. We report a systematic investigation of 17 mechanically robust FA-based metallogels at extremely low concentrations (<0.2 wt%). Using oscillatory rheological measurements, we further show that these metallogels undergo rapid recovery and self-healing, recovering up to 95% of their original stiffness within 1 min. Among the metallogels studied, FA-chromium(III)

acetate gel (0.4 wt%) displayed the highest stiffness with a storage modulus of 4 kPa. More importantly, the stiffness, recovery, and sol $\leftrightarrow$ gel transitions can be readily tuned by changing either the metal salt or the concentration. Using a combination of various analytical methods, we also suggest a structure of self-assembly in the metallogel network. This study defines non-toxic FA as a robust and sustainable building block for metallogels—mechanically tunable, multi-responsive soft materials. Finally, as a proof-of-concept experiment, we demonstrate that the FA-chromium(III) acetate gel can be considered as a potent sustainable gellator for enhanced oil recovery applications.

1. Introduction

Molecular gels are formed by self-assembly of low molecular weight gelators in the presence of solvent molecules. As a branch of supramolecular chemistry, molecular gels constitute an attractive research topic in the development of functional smart soft materials. Their self-assembly is driven by either one or a combination of several supramolecular interactions such as H-bonding, π - π stacking, van der Waals forces, charge-

transfer interactions, or halogen bonding.^[1–3] Because of the weak nature of these non-covalent interactions, molecular gels often provide facile reversibility when subjected to mechanical or other external stimuli. Therefore, they find potential applications in catalysis,^[4,5] sensing,^[6] biological applications,^[7,8] controlled release,^[9] and removing pollutants from water.^[10]

Organic molecules with metal coordination sites allow metal-ion-induced gelation, commonly referred to as metallo-supramolecular gels or simply metallogels. Metallogels combine the topological multiplicity of the metal–ligand interaction with multilevel hierarchical superstructures. The metal–ligand interaction can lead to a primary assembly forming a one-dimensional scaffold or can cross-link the available one-dimensional molecular assembly into a two-dimensional or three-dimensional complex network. Importantly, a metal cation can interact with an organic ligand through a coordinate covalent bond or a non-covalent ion–dipole interaction. Metallogels offer multiresponsive behavior by readily undergoing a gel $\leftrightarrow$ sol transition. This can result in tunable stiffness, adaptivity, and orderliness of the gels.^[11–17] A wide range of stimuli such as temperature, oxidation–reduction, pH, and electric and magnetic fields have been used to exploit the properties of coordinated metal ions.^[16,18–20]

In general, the ability to accept electrons and a wide range of coordination geometries of metal cations have led to the incorporation of transition metals and lanthanides into supramolecular metallogel networks.^[21] For example, it has been shown that pyridyl-appended bis-urea derivatives form metallogels in the presence of silver and copper and also allow in situ formation of nanoparticles.^[22] Functionalized derivatives of pyridine, bipyridine, and terpyridine have also been studied for anion/cation selective gelation, self-healing properties, the formation of size- and shape-selective nanoparticles in situ, and gelation-induced

[a] M. A. Marzabad, S. Chattopadhyay, R. Marek, O. Jurček
Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czechia
E-mail: jurcekondrej@mail.muni.cz

[b] M. A. Marzabad, S. Chattopadhyay, O. Jurček
Department of Natural Drugs, Faculty of Pharmacy, Masaryk University, Brno, Czechia

[c] M. A. Marzabad, S. Chattopadhyay, R. Marek, O. Jurček
CEITEC – Central European Institute of Technology, Masaryk University, Brno, Czechia

[d] S. Hietala
Department of Chemistry, University of Helsinki, Helsinki, Finland

[e] N. Nonappa
Faculty of Engineering and Natural Sciences, Tampere University, Tampere, Finland
E-mail: nonappa@tuni.fi

CEITEC @CEITEC_Brno; Masaryk University @MasarykUni; University of Helsinki @helsinkiuni; Tampere University @TampereUni

Supporting information for this article is available on the WWW under <https://doi.org/10.1002/chem.202500748>

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luminescence. However, existing metallogels are usually specific to a particular metal ion or a counter anion.^[23]

In the form of folate, folic acid (FA or vitamin B₉) belongs to a class of water-soluble B vitamins. FA is also a widely studied small-molecular vector for drug delivery targeted at cancer cells.^[24] Despite extensive studies of the biological significance of FA, metal-to-FA complexation and related research on soft functional materials have been limited.^[25–28] The presence of carboxylic acid groups and primary, secondary, and tertiary amines in the FA molecule make it quite a complex molecule capable of a variety of types of hydrogen bonding (as observed in its crystal structures)^[29–31] and metal-coordination. Fazary et al. studied the complexation equilibria of FA with Fe(III), Fe(II), Ca(II), and Zn(II) in aqueous solutions. It was suggested that the metal ions coordinate with the carboxylates of the FA, making binary complexes. The stability constants of these complexes decreased in the order Fe(III) > Fe(II) > Zn(II) > Ca(II).^[25] Metal-folate complexes can display high structural diversity, e.g., carboxylates of FA can function like a monodentate or a bidentate ligand binding to a single metal, or like a bridging bidentate ligand coordinating to two metals.^[27,28]

Although neat FA was studied for its gelation properties in a DMSO/water system,^[32] the resulting gel was mechanically weak, limiting its application. Therefore, several research attempts have been made to improve the mechanical properties of FA-based gels. Song et al. prepared hybrid injectable gels by adding different amounts of agar to FA in a DMSO/water mixture. It was shown that agar molecules are involved in the process of self-assembly via hydrogen bonds forming a denser 3D network, which improved the strength of the hybrid gel.^[33] In another study, the FA-based supramolecular hydrogel was formed using Zn(NO₃)₂ and studied as a material for 3D printing.^[34] Embedding various metal salts inside molecular and polymeric gels can further fine-tune their properties increasing the scope of diverse applications.

The structural organization of FA molecules in aqueous environments in the form of liquid crystals or organo-/hydrogels has been previously studied.^[34–38] The FA or folate was found to form tetrameric structures through hydrogen bond interactions among pterin heterocycles (*Hoogsteen*-bonded tetrad), assemblies analogous to those of the G-tetrads (model comparison in Figure S1). These disk-like arrays were further self-assembled through π - π -stacking to form tertiary columnar structures (similar to the well-known G-quadruplexes) where they are continuously rotated to each other, generating chiral columns. These columns further aggregate into fibrils, which entangle into a 3D fibrillar network of gels.^[39,40]

Despite the potential to create a diverse library of metallogels using FA, there exists no such systematic study to fully understand and eventually exploit this possibility. Herein, we provide an extensive investigation of FA-metal salt gelation using a total of 33 metal salts complemented by a systematic evaluation of their mechanical properties. We show that FA acts as a supragelator that results in 17 metallogels at concentrations $\leq 0.2\%$ (all % refers to w/v%, unless stated otherwise). More importantly, strong metallogels were obtained even at 0.07%

in the presence of chromium(III) acetate. We show that the FA metallogels undergo rapid recovery, self-healing, and thixotropy under shear rheology. We also demonstrate the macroscopic self-healing of FA metallogels. Electron microscopy imaging revealed that all gels are composed of highly entangled fibrillar networks. Further insight into structure and morphology is provided using Fourier transformed infra-red spectroscopy (FT-IR), UV-Vis spectroscopy, circular dichroism (CD), nuclear magnetic resonance spectroscopy (NMR), powder X-ray diffraction (PXRD), and differential scanning calorimetry (DSC), suggesting new behavior of FA and a mechanism of self-assembly in the presence of Cr(III)-acetate.

The utilization of chromium-based gels for enhanced oil recovery (EOR) represents a promising cost-effective approach to increase the efficiency of oil mining. Chromium-based polymeric gels are especially potent in enhancing oil recovery from unconventional reservoirs (e.g., with fractured formations). The gels act as conformance control agents that decrease water cuts in the oil fractions harvested and/or provide gas shutoffs. The process of employing chromium-based gels is generally simple; gel precursors are prepared in solution and injected into the reservoir to modify the flow of fluid and to increase the displacement of oil.^[41] To optimize the gelation processes and to ensure optimal oil recovery operations, a choice of gel components, their concentrations, operating temperature, and environmental and geological conditions must be considered carefully. Alongside these, there are also concerns about environmental safety, acute to chronic toxicity, sustainability, and naturally the cost. A number of promising, mostly polymer-based, metallogels have been studied with positive outputs in field tests.^[42–47] For example, many oilfields in China have already undergone conformance control using chromium gels, e.g., the Huoshaoshan block in the Xinjiang oilfield. This block was developed by water injection in its early stage, but the poor physical properties of the original heterogeneous formation led to the development of fractures resulting in low oil recovery. Field experiments with organic chromium gels were done in five groups of water injection wells, where the daily oil production of the 10 corresponding oil wells increased from 20.2 to 35.9 t, and the water content of the fluid produced decreased from 59.5% to 46.2%.^[48]

In general polyacrylamide-based chromium(III) gels are the most studied group of conformance control agents.^[42–47,49–51] However, drawbacks in their use are usually their low thermal stability and their sensitivity to high salinity.^[41] For these reasons, there is an ongoing search for advanced gel materials which can provide a broader range of applications in oil mining processes, while, maintaining overall safety and low cost. The initial assessment of the FA-Cr(III)-acetate metallogel encompasses the evaluation of gel formation in simulated seawater conditions, its aging, and scrutiny of thermal stability profiles. The FA-Cr(III)-acetate metallogel represents a promising candidate for EOR and shows good resistance to external stress. The use of sustainable and cheap FA provides metallogel stability, but also healable, thixotropic, and time-controllable properties that can offer a new direction in EOR.

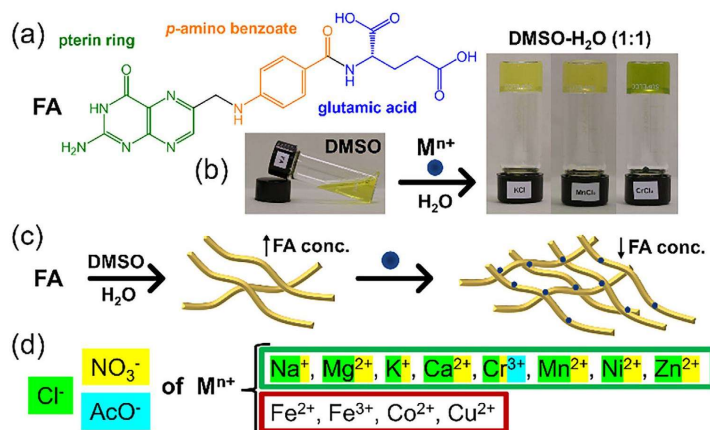


Figure 1. (a) Chemical structure of folic acid (FA). (b) Addition of aqueous solution of a metal salt to DMSO solution of FA promotes gelation as indicated by photographs showing resistance to flow upon inversion of the vial. (c) Self-assembly of FA at high concentration and in the presence of sea-water having excessive amounts of other metal salts, especially of Ca^{2+} or Mg^{2+} , which can lead to unwanted partial precipitation of the gelator and thus decrease the effective gelator concentration resulting in gels with low mechanical strength and poor homogeneity.^[41] In addition, K^+ and Na^+ significantly affect the FA coordination self-assembly (showing a preference for Na^+ ^[35–37]). (d) Salts studied in this work: cations in the green frame promoted gelation, no gelation was observed using cations in the red frame. Green, yellow, and blue highlights refer to counter anions used for successful gelation.

2. Results and Discussion

2.1. FA-Based Metallogels

We have tested the gelation of FA in a 1:1 (v/v) DMSO:H₂O solvent system in the presence of monovalent, divalent, and trivalent metal salts. We have used 11 different metals as their chlorides, nitrates, and acetates, resulting in 33 different combinations (Table S1). Interestingly, we observed metallogelation in 17 systems leading to transparent yellowish or greenish-yellow metallogels, depending on the metal ion under investigation (Figure 1 and Figure S2). All gels were formed at a concentration of 0.2%. Therefore, FA, together with metal salts, is referred to as a supergelator. Notably, the chromium(III) acetate gel formed at the extremely low concentration of 0.07%. Monovalent, divalent, and trivalent metals resulted in four (out of 6 tested), ten (of 13 tested), and three gels (of 6 tested), respectively. Of the 11 metal cations tested, eight metallogels were obtained with chlorides, and eight with nitrates, but only one metallogel was formed using an acetate salt (Figure 1).

The experiments were also carried out with representative salts of each valence group, NaCl, ZnCl₂, CrCl₃, and Cr(III)-acetate in solvents with different H₂O:DMSO ratios (0.4% of FA), 6:4, 7:3, 8:2, or 9:1. In all cases, weaker gels were formed in the 6:4 solvent system than in the case of 5:5. In the case of Cr(III)-acetate, the gels were formed all the way up to the 9:1 ratio, and the time required for the gel to form was prolonged up to a week, but the strength of the gels decreased. In comparison, the Cr(III)-acetate metallogel (0.2%) in H₂O:DMSO 1:1 ratio formed a strong gel within 4–5 days (Figure S23). In general, the H₂O:DMSO 1:1 (v/v) ratio was optimal, providing metallogels with the highest mechanical strengths as determined by the vial inversion test.

Moreover, we avoided using alkaline metal bases, e.g., KOH or NaOH to facilitate the formation of carboxylates.^[25,34] By this approach, we aim to suppress the formation of strong coordination bonds between metal salts and carboxylates. It can play an important role when a mixture of salts is used, e.g., in advanced levels of oil mining, gelation is performed in the presence of sea-water having excessive amounts of other metal salts, especially of Ca^{2+} or Mg^{2+} , which can lead to unwanted partial precipitation of the gelator and thus decrease the effective gelator concentration resulting in gels with low mechanical strength and poor homogeneity.^[41] In addition, K^+ and Na^+ significantly affect the FA coordination self-assembly (showing a preference for Na^+ ^[35–37]).

2.2. Rheological Properties of Metallogels

In general, metallogels have been studied for their unique rheological properties such as self-healing and thixotropy.^[16,52–56] We have used oscillatory shear rheological measurements to determine the mechanical properties of the FA metallogels. Accordingly, frequency-sweep, time-sweep, strain-sweep, temperature-sweep, and step-strain experiments were performed. For rheological studies, 0.4% of premade gels were used.

The time-sweep experiment provides insights into the kinetics of gelation. The gelation behavior of the samples was monitored for up to 60 min after loading. Figure 2a,b show a comparison of the representative time-sweep experiments of the 0.4% metallogels derived from chloride and nitrate salts of K^+ , Mn^{2+} , and Cr^{3+} . The elastic modulus G' was found to be 0.1 kPa, 0.4 kPa, and 0.8 kPa, for FA-KCl, FA-MnCl₂, and FA-CrCl₃, respectively (Figure 2a, b). Thus, in the case of chlorides, the gel stiffness follows the order: monovalent < divalent < trivalent

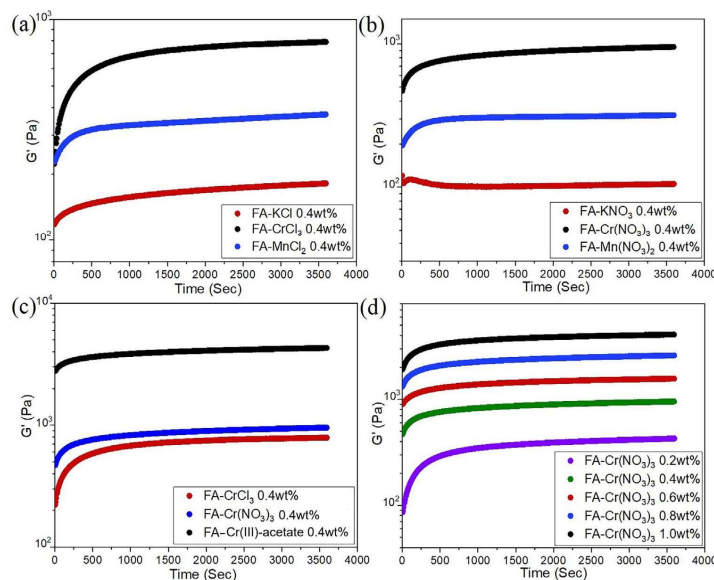


Figure 2. Time-sweep experiments for 0.4% FA metallogels in DMSO:water (1:1) (a) comparison of chlorides of mono-, di-, and trivalent cations, (b) comparison of nitrates of mono-, di-, and trivalent cations, c) comparison of the effect of the anion for Cr^{3+} , and d) the effect of the FA concentration in $\text{Cr}(\text{NO}_3)_3$ gel samples.

cation. The trend was similar when the nitrate salts were used. Metallogels containing various divalent cations seem to have similar properties based on the comparison of their G' values (Figure S3). Figure 2c,d show a comparison of the time-sweep experiments for different anions of Cr^{3+} and variable concentration $\text{FA-Cr}(\text{NO}_3)_3$ experiments, respectively. In general, the higher the concentration of components, the stiffer the metallogel. For a comparison of samples containing metal chlorides or nitrates, see Figure S3 in Supporting Information. Neat FA (without a metal salt) in DMSO:water (0.4%) could not be used for comparison with the metallogels as the sample does not form a gel (up to about 1.0%).

Figure S4 and Table S2 show results from frequency-sweep experiments of selected monovalent, divalent, and trivalent chlorides, nitrates, and acetates. Typically, the storage modulus G' is greater than the loss modulus G'' over an entire range of frequencies in the linear viscoelastic region (LVR).^[11,57] In our experiments, G' values are an order of magnitude higher than those of G'' , suggesting that the materials under study are viscoelastic solids. For all of the metallogels studied, the frequency-sweep results show that G' and G'' are independent of the frequency. The storage modulus G' decreased in the order of $\text{Cr}^{3+} > \text{Mn}^{2+} > \text{K}^+$ for chlorides and nitrates. When the anions were varied in the case of the Cr^{3+} salt, the G' followed the order $\text{FA-Cr(III)-acetate} > \text{FA-Cr}(\text{NO}_3)_3 > \text{FA-CrCl}_3$ (Figure S4e). Notably, among all the metal salts tested, the Cr(III)-acetate forms the strongest gels. The G' of $\text{FA-Cr(III)-acetate}$ metallogel (4 kPa) was

~4 times higher than that of samples with Cr^{3+} nitrate or chloride salt. In another example comparing Cr(III)-acetate with KCl metallogels, the G' of Cr(III)-acetate metallogel is even 40 times higher. The G' values increase further with increasing concentration of the $\text{FA-Cr}(\text{NO}_3)_3$ metallogel (Figure S4f), indicating the impact of an increased density of cross-linkers on the mechanical properties of the metallogel. A reliable concentration study could not be performed with the $\text{FA-Cr(III)-acetate}$ metallogels as they were found to be extremely strong and the samples slipped off the plates of the rheometer. Overall, the results suggest that the valency of the metal cations plays an important role in the stability and stiffness of the gel.

2.3. Self-Healing and Recovery Studies

Step-strain experiments were performed to investigate the gel $\leftrightarrow$ sol transition and self-healing as the gel strength recovery (Figure 3). The gels showed a rapid decrease in G' in response to an increased strain by turning into viscoelastic liquids. The application of increased strain over 60 s further breaks the structure as shown by the gradual decrease in the G' values. Upon switching to a lower strain, the gels recover most of their original mechanical strength almost instantly, which reflects the rapid self-healing properties of FA-metallogels. Notably, in the step-strain experiments, the recovery in the first cycle is not 100% of the original value. However, in the following cycles,

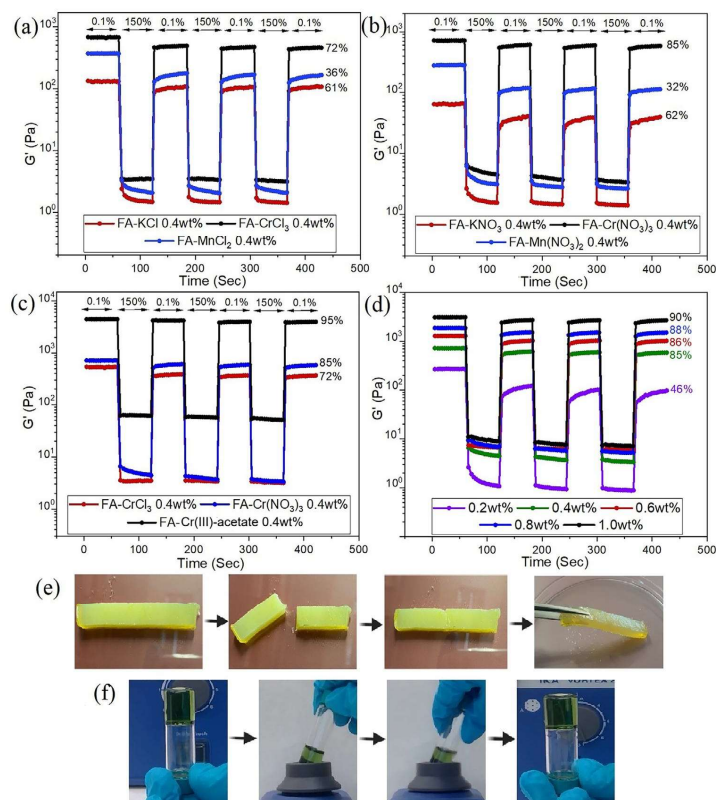


Figure 3. Step-strain experiments showing the self-healing properties of 0.4% DMSO:water (1:1) FA-metallogels: (a) comparison of the self-recovery properties of FA-metallogels derived from chloride salts of mono-, di-, and trivalent cations, (b) comparison of nitrates of mono-, di-, and trivalent cations, (c) comparison of the self-recovery properties of FA-metallogels with different counter anions of Cr^{3+} , (d) the effect of concentration on the self-recovery of FA-Cr(NO₃)₃ metallogel. (e) Photographs showing the macroscopic self-healing of 1.0% DMSO:water (1:1) FA-NaCl metallogel, and (f) photographs showing snapshots from Video S2 demonstrating the gel-sol transition by the vortexing of the 0.2% DMSO:water (1:1) FA-Cr(III)-acetate metallogel.

they fully recover to the second G' value. This is presumably due to a rearrangement of the gel structure in the first cycle and is typically also observed for other gels.^[52,55] The process can be repeated for several cycles (Figure 3, herein for three cycles). Figure 3a shows the step-strain rheological data for FA-metallogels of KCl, MnCl₂, and CrCl₃. For FA-metallogels with KCl and MnCl₂, the equilibrium point was not reached compared to that of CrCl₃. This suggests that the gels require a longer time (rest time) to recover completely (the recovery percentage for the first cycle is shown for each plot). Similar recovery behavior was also found for nitrates (Figure 3b). When three different salts of chromium were compared, the metallogel made of FA-Cr(III)-acetate showed the highest recovery of 95% in the first cycle (Figure 3c). In general, all of the FA-metallogels with Cr^{3+} showed high recovery in their first cycle compared to other metal ions. The percentage recovery in the first cycle

increased from 50% to 90% as the concentration was increased from 0.2% to 1.0% of FA-Cr(NO₃)₃ metallogels (Figure 3d). Therefore, the results suggest that the extent of recovery depends positively on the strength of the gel. The strength of the gel depends on the metal cations and the concentration, and lesser on the counter anion used. Self-healing was also observed with macroscopic gel samples. For these experiments, we prepared a free-standing 1.0% of FA-NaCl gel. The gel was cut into pieces which were subsequently re-joined together. After 24 h, a uniform gel piece that could be lifted without any breakage was re-formed (Figure 3e, Video S1). Furthermore, the immediate transition from sol to gel was observed when the FA-Cr(III)-acetate metallogel (0.2%) sample was subjected to intense mechanical perturbation using vortexing. The sol returned to the gel state immediately when vortexing was stopped (see Video S2). For more step-strain experiments comparing metal chlorides and

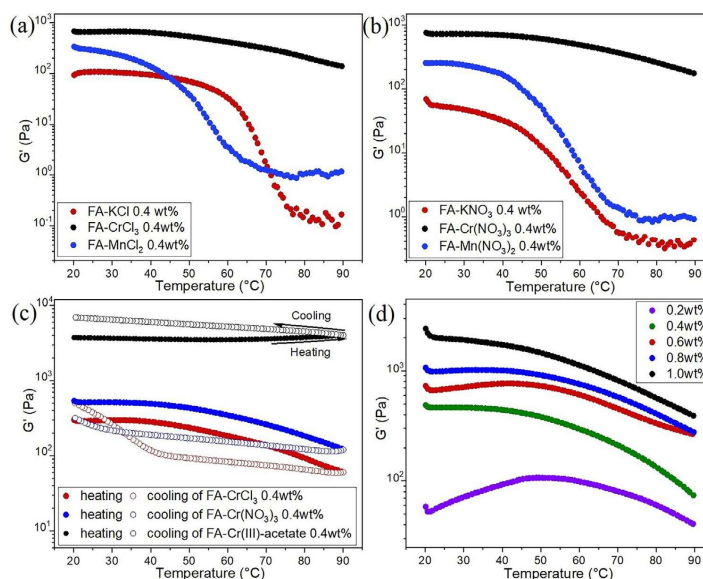


Figure 4. Temperature sweep rheological experiments of 0.4% DMSO:water (1:1) FA-metallogels (a) comparison of the self-recovery properties of FA-metallogels derived from chlorides of mono-, di-, and trivalent cations, (b) comparison of the self-recovery properties of FA-metallogels derived from nitrates of mono-, di-, and trivalent cations, (c) effect of an anion on the thermal properties of FA-Cr³⁺ metallogels, and (d) effect of concentration on gel melting properties of FA-Cr(NO₃)₃ metallogels.

nitrate, see Figure S5 and Table S3 in Supporting Information. The results suggest that the gels are particularly mechanically robust, self-healing, and thixotropic.

2.4. Temperature-Sweep Experiment

The gel→sol transition (melting profile) of metallogels was studied by temperature-sweep rheological experiments (Figure 4a–d). The premade gels were heated (20°C–90°C) and cooled (90°C–20°C) and the moduli were followed. The gels showed rapid changes in their elastic moduli upon gel→sol transition, a typical characteristic of supramolecular gels.^[52] The elastic moduli of gels decreased by two orders of magnitude upon melting. Most of the gels showed the initiation of network melting (T_{onset}) between 45°C and 55°C. Minima were recorded when the melting was completed (gel melting temperature T_{gel}), which was between 55°C and 60°C, depending on the gel under study. Surprisingly, unlike other gels, no gel→sol transition was observed for any chromium gel (Figure 4c,d) in the temperature range of the sweep experiments. This implies that chromium metallogels only soften during heating in this temperature range and do not melt. In the cooling cycle, the gels with lower G' values eventually reach the original G' values (loop). Significantly, for the FA-Cr(III)-acetate sample, the G' value decreased slightly as the temperature increased up to 60°C and then slightly increased (Figure 4c). Cooling the sample led to a

further increase in the storage modulus. Between the beginning and the end of the experiment, $\Delta G'$ was almost 2 kPa. This might imply that the gel network undergoes structural reorganization under heating leading to a stronger gel (but it could also correspond to a possible partial evaporation of solvent). For additional results comparing the effect of metal chlorides and nitrates on FA-metallogels, see Figure S6 and Table S4 in Supporting Information.

2.5. Critical Strain and Yield Point

Beyond the linear-viscosity region, G' falls below G'' and the gel transforms into a liquid-like form (sol). The rheological properties of soft materials display nonlinear behavior with a sudden decrease in their storage modulus above a certain strain level. This region is known as critical strain. The gel structure remains intact below the critical strain, but it is disrupted when the level of strain applied reaches above it. Thus, under high oscillatory stress, the solid-like gel begins to flow. The term “yield stress” or “yield point” is the value of the crossover of G'' and G' , representing the stiffness.^[58] Yield stress and critical strain values are useful for understanding the linear viscoelastic regime of soft materials. For FA-metallogels, the yield point values varied from 61% to 97%, depending on the cation and counter anion (Figure 5a–c). For the Cr(NO₃)₃ metallogel, the yield point values increased from 49% (for 0.2% gel) to 98% (for 1.0% gel), Figure 5d.

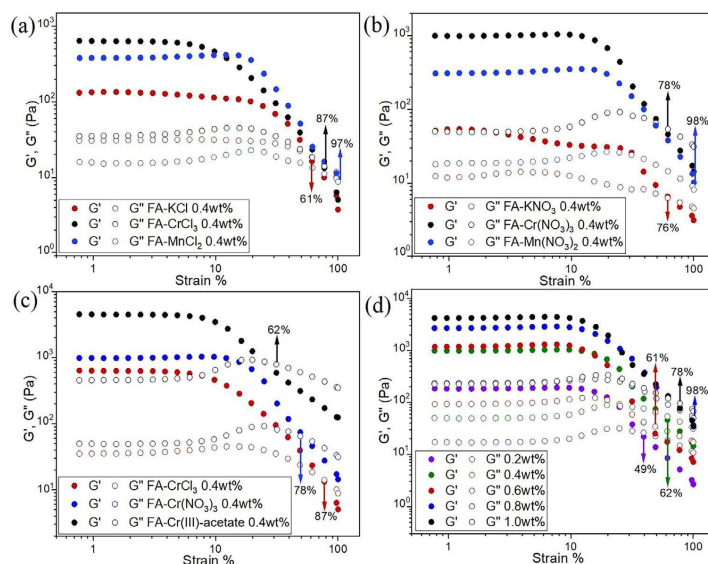


Figure 5. Strain-sweep experiments for 0.4% DMSO:water (1:1) FA-metallogels: (a) comparison of the critical strain of FA-metallogels derived from chlorides of mono-, di-, and trivalent cations, (b) comparison of the critical strain of FA-metallogels derived from nitrates of mono-, di-, and trivalent cations, (c) effect of an anion on the critical strain of Cr^{3+} , and (d) effect of concentration on the critical strain of FA- $\text{Cr}(\text{NO}_3)_3$ metallogels.

For additional results comparing the effect of metal chlorides and nitrates on FA-metallogels, see Figure S7 and Table S5 in Supporting Information.

Systematic rheological experiments were performed for all gels of the study (see Supporting Information, in Figure S8 we plot G' at 1.194 rad/s vs. various metal salts). As could also be observed from their G' values, the stability of the gels increases as their valence increases. The metallogels obtained with Na^+ , K^+ , Ni^{2+} , and Mg^{2+} salts degraded after 3–4 weeks at room temperature, other gels were stable for months to a year. Interestingly, the FA- $\text{Cr}(\text{III})$ -acetate gel remained unchanged for almost three years.

2.6. Gelation Mechanism and Interactions

2.6.1. Fourier Transform Infrared (FT-IR) Spectroscopy

The FT-IR spectra of samples of FA powder, $\text{Cr}(\text{III})$ -acetate, FA gel (DMSO:water 1:1), and FA- $\text{Cr}(\text{III})$ -acetate metallogel (DMSO:water 1:1) were compared (Figure S9a). The FT-IR spectrum of FA powder in the range of 3600–3000 cm^{-1} shows several vibrational bands: 3539 cm^{-1} , represents the stretching vibration band of $\text{O}-\text{H}$ in $-\text{COOH}$ groups; 3412 and 3319 cm^{-1} , the antisymmetric and symmetric stretching vibrations (ν_{as} , ν_{s}) of $-\text{N}-\text{H}$ in $-\text{NH}_2$ groups; and ν_{as} , ν_{s} of $\text{C}-\text{H}$ at 2926, 2843 cm^{-1} . In comparison, the broad shifted bands at 3380 and 3284 cm^{-1} , present in both gel samples, suggest hydrogen bonding between FA molecules resulting in the formation of known pterin-based tetrads.^[38] In

comparison, the spectrum of the metallogel shows an additional vibration at 3660 cm^{-1} reflecting the stretching vibration of a free enolic $-\text{OH}$ group (Figure S9c)^[59] and suggesting the presence of a lactim tautomer (Scheme S1). The metallogel also shows very strong bands at 2987, 2972, and 2900 cm^{-1} corresponding to the $\text{O}-\text{H}$ vibrations of carboxyls and $\text{C}-\text{H}$, and $\text{N}-\text{H}$ stretching. It has been shown, that these signals can also provide information on the structural organization mediated through a complex system of hydrogen bonding, while the peaks around 3012 cm^{-1} are attributed to the organization in disc-like arrays (signals at 3150 cm^{-1} would correspond to a ribbon-like pattern, but are missing in our case).^[41,60,61] At the lower wavenumbers, the absorption bands of $\text{C}=\text{O}$ stretching of the $-\text{COOH}$ or $-\text{CONH}-$ groups of FA at 1690 and 1620 cm^{-1} , respectively, and the $-\text{NH}-$ of $-\text{CONH}-$ at 1602 cm^{-1} merged into a wide absorption band at 1647 cm^{-1} in both gel samples indicating the involvement of these groups in hydrogen bonding. Comparing the spectra of both gels, the common strong broad band at 1647 cm^{-1} belongs to the $\text{C}=\text{O}$ stretching of CONH , COOH , or a conjugated carboxyl indicating the involvement of these groups in hydrogen bonding or adjacent metal coordination. The characteristic absorption band of the phenyl ring at 1480 cm^{-1} in FA powder, shifts to 1400 cm^{-1} in gels. There are also several differences between the gels: the $\text{O}-\text{H}$ bending band is larger for the metallogel sample, and the metallogel shows an additional aromatic amine $\text{C}-\text{N}$ vibration at 1250 cm^{-1} and an alcohol $\text{C}-\text{O}$ stretching vibration at 1050 cm^{-1} . In both gels, we can observe the strong $\text{S}=\text{O}$ stretching vibrations at 1012 and 951 cm^{-1} of the DMSO molecules.

Based on the differences observed between the FA-gel and metallogel we speculate that FA undergoes a partial chemical transformation in the presence of chromium salt which subsequently directs the 3D self-assembly into the complex network of metallogel. The pH of FA-Cr(III)-acetate gel is 6, which suggests that the FA is mostly present in the form of folate. Previous studies have also shown tautomerism of folate manifested in aqueous solution in the form of an equilibrium mixture of N3 and N1-lactams (keto-tautomers) rather than as a lactim (enol-tautomer) (Scheme S1,^[62] electrostatic potential map of N3-lactam in Figure S21). The addition of Cr(III)-acetate can lead subsequently, to establishing a new equilibrium among various forms, e.g., the presence of acetate can stabilize the hydrogen bonding of lactam but also lactim forms,^[63] and the lactam tetrads can bind the Cr(III) ion through ion-dipole interactions (Scheme S1). Overall, several chemical forms of FA seem to be present in the complex structure of the FA-Cr(III)-acetate metallogel at the same time, which makes it rather difficult to describe in detail using IR spectroscopy. Nevertheless, this method provides us with valuable initial information on the gel structure that can be further evaluated by a combination of other analytical techniques.

Not only the structure of FA but also the form of chromium(III) acetate plays an important role in the gelation process. Chromium(III) acetate basic is a trimeric cluster, $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_3)_6(\text{OH}_2)_3](\text{O}_2\text{CCH}_3)_3$, with a well-defined Cr_3O core stabilized by acetate ligands and a central μ_3 -oxo bridge. Previous reports indicate that the Cr_3O cluster exhibits stability in an aqueous environment, although ligand exchange can occur depending on the coordination environment and pH conditions.^[64,65] In the IR spectrum of neat chromium(III) acetate basic, distinct vibrational modes are observed in the region of $500\text{--}700\text{ cm}^{-1}$ corresponding to the Cr—O—Cr core (Cr_3O): band at 544 cm^{-1} attributed to Cr—O—Cr asymmetric stretching, 620 cm^{-1} band assigned to Cr—O—Cr bending vibrations, and band at 654 cm^{-1} referring to Cr—O stretching modes (Figure S9d).^[66] The gel sample exhibited a broad band in this spectral region with one sharper band at about 708 cm^{-1} consistent with vibrational modes of the Cr_3O core (Figure S9d). The broadening is caused by non-covalent and coordination interactions of the Cr_3O core with FA functionalities assuming that under the gelation conditions, the acetates are to some extent replaced by FA carboxylates.^[65] At the same time, having a complex equilibrium reaction going on here, we consider that some amount of *tris*-chromium(III) cluster decomposes into *mono*-chromium(III) cations which can be incorporated within pterin tetrads through ion-dipole interactions, stabilizing overall the metallogel network. Although the ion-dipole interaction is a weaker non-covalent force, in its cooperativity it is exceptionally robust and supports the formation of strong gels even in the presence of a multi-component mixture such as seawater (experiments shown below).

2.6.2. CD Spectroscopy

The CD spectra of FA in DMSO with and without metal salts show no chiral self-assemblies at the given concentrations of

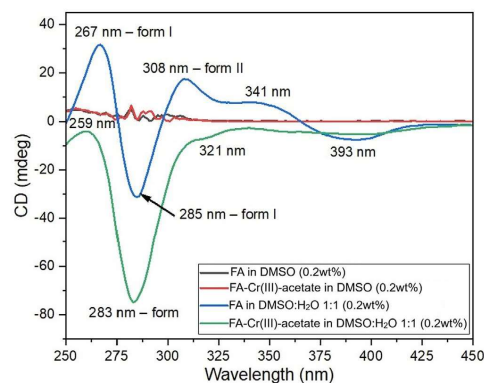


Figure 6. Circular dichroism spectra of FA and FA and Cr(III)-acetate in DMSO or DMSO:H₂O mixture showing the presence of two forms of supramolecular aggregates.

the components; any molecular chirality is silent in the CD spectra. On the other hand, the addition of water to the DMSO solution induces significant changes in the spectra because a supramolecular chiral self-assembly is formed (Figure 6). In the case of neat FA, the spectral shape is characterized by a broad positive band with a maximum at 267 nm, followed by a broad negative band at 285 nm, which further continues to a positive band at 308 nm, complemented by a lower very broad positive band at 341 nm and a similar but negative band at 393 nm. The addition of chromium(III) acetate leads to significant changes in the spectrum and the disappearance of several of the bands characteristic of neat FA. The intensity of the negative band at 283 nm significantly increases and is complemented by a weak band at 321 nm, after which the spectrum approximates to zero, but still has a very broad negative band at around 390 nm.

These results can be compared with those previously observed and elucidated by Gottarelli et al.,^[37] where the folate has been found to form two different hexagonal columnar mesophases, I and II, in the presence of Na^+ or K^+ . This was related to the formation of *Hoogsteen*-bonded tetramers and their pterin-mediated π – π stacking into larger columnar helical aggregates. Form I is characterized by a positive band at about 270 nm and a negative one at 290 nm, whereas form II has a positive band at about 310 nm. The difference between the two phases is minimal, originating mainly in the tightness of stacking. Form I shows a greater aromatic stacking distance than form II, which is tighter. The attractive interaction between quadruplexes was also found to be significantly lower in pure water and increased with increasing concentrations of salts (the effect of Na^+ is greater than that of K^+). Both forms show that the glutamate side-chain is rather mobile in the structure, not directly affecting the chiral aggregation.^[37]

Similarly, in our samples, we can also observe the presence of both forms, I and II. The FA gel sample shows the presence of form I, having positive bands at 267 and 285 nm, and form II with a band at 308 nm. In contrast, the addition of a Cr^{3+} salt

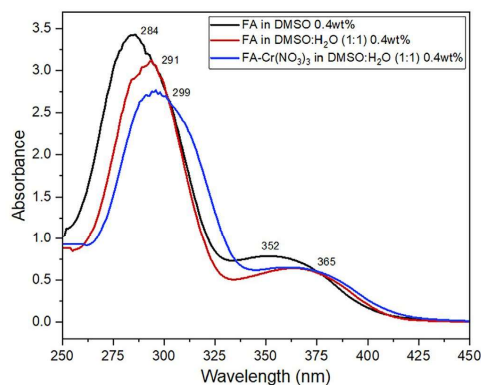


Figure 7. UV-Vis spectra of FA powder in DMSO, FA gel, and FA-Cr(NO₃)₃ gel in DMSO:water (1:1) (all experiments were measured at 0.4 wt% and room temperature).

leads to the appearance of a very strong signal at 283 nm, which can be explained by the presence of only form I. Interestingly, the measurements were also carried out at various time periods. Whereas at 15 min the FA gel sample shows the presence of only form I, at 45 min form II also appears (after this period the composition of the sample remains the same) (Figure S10). In contrast, the spectrum of the FA-Cr(NO₃)₃-metallogel sample does not change over time after the first measurement at 15 min. These findings can be referred to the previously observed trend where the tightness and length of stacked quadruplexes decreased with increasing size of the cation, Na⁺ versus K⁺,^[37] where Cr³⁺, being an even larger cation, can lead selectively to the formation of the looser form I. Nevertheless, the tightness of the interaction among quadruplexes does not correlate directly with the strength of metallogels, and other structural and physicochemical factors will also be considered. Another interpretation of the presence of form I is correlated with the presence of tris-chromium(III) acetate cluster. Being a metal node too large to be effectively intercalated within the tetrads, the cluster preferably coordinates with carboxylates of the side chain. This can similarly disturb the tight packing of pterin tetrads by “pulling” the FA molecules apart. These together with the hydrogen-bonded and/or coordinated molecules of solvents contribute to tetrad’s distortion and increased distancing.

2.6.3. UV-Visible Spectroscopy

UV-Vis spectra of FA in DMSO, and FA-gel and FA-Cr(NO₃)₃-metallogel in DMSO:H₂O (1:1) were measured at 0.4 wt% and at room temperature (Figure 7). The absorption bands of the pterin ring in the FA-DMSO solution appear at 284 and 352 nm and are attributed to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively. The corresponding absorption bands of the pterin ring in the FA gel show the bathochromic shift of 7 and 10 nm, respectively, which indicates that FA molecules self-assemble in the DMSO:H₂O solvent mixture through π - π stacking interactions in

a J-type aggregate. J-aggregates are represented by an “angle of slippage” larger than 32° (the angle between the line of centers of a column of molecules and the long axis of any one of the parallel molecules). Thus, the heterocycles stacked on top of each other in tetrads are rotated to each other forming a helical supramolecular structure. This can be further supported by decreasing the absorption intensity of the peak at 291 nm. In the case of FA-Cr(NO₃)₃-metallogel, the absorption bands of pterin show an additional 8 nm bathochromic shift as well as an additional lowering of the absorbance when compared to FA gel, which is connected to the effect of electrostatic ion-dipole interactions of the metal to the carbonyls of the pterin tetrad and additional aggregation in this complex gel network.

2.6.4. ¹H-NMR Spectroscopy and Variable Temperature NMR

NMR spectroscopy experiments have been used to reveal the non-covalent interactions involved in gel formation and its dynamics. However, the NMR spectroscopic characterization of certain metal complexes is a challenging task because some of them are paramagnetic.^[67,68] Therefore, out of all of the samples studied, the FA-KCl, FA-NaCl, FA-ZnCl₂, and FA-Zn(NO₃)₂ gel samples were selected for the NMR studies as diamagnetic species, and one representative of the paramagnetic group of metals, Cr(III)-acetate, was included.

Initially, we measured the ¹H NMR spectrum of the FA sample in DMSO-*d*₆ and in the mixture DMSO-*d*₆:D₂O 1:1. Whereas the FA in DMSO-*d*₆ provides a single set of ¹H NMR signals (Figure S11), the FA solution in the mixture DMSO-*d*₆:D₂O 1:1 provides two sets of broader ¹H signals, suggesting the involvement of water molecules in the FA self-assembly, possibly via hydrogen bonding (Figure 8). The very broad ¹H signals for carboxylic acid groups and -NH₂ can also suggest the formation of intermolecular H-bonding. Moreover, the absence of a signal for hydroxyl protons in DMSO-*d*₆ suggests that the lactim tautomer is not significantly represented in the liquid sample. The overlapping sets of signals, a major and a minor set in approximately 4:1 ratio have a chemical shift difference $\Delta\delta = 0.08$ ppm, where the minor set is at lower frequencies. Their presence can have several explanations, related to the previous structural studies. The major signal set can represent the formation of hydrogen-bonded tetrads with the minor set representing their short oligomeric π - π -stacked aggregates or even a ribbon-like assembly in solution.^[38,60,61] When analyzing the NMR data we should also keep in mind that large(r) polymeric aggregates are not visible in a regular solution-state ¹H NMR spectrum. Thus, we cannot see the pivotal gel polymeric framework but only small fragments of its possible building blocks. Figure 8 further shows a comparison of the spectrum of neat FA with spectra of various FA metallogels at 0.4 wt% in DMSO-*d*₆:D₂O 1:1 mixture. Several differences can be observed in the ¹H NMR spectra. The minor second set of signals observed in the neat sample of FA disappears in all of the metallogels studied, likely because of metal-induced aggregation into larger aggregates. In the Zn(NO₃)₂ sample, next to the familiar major signal set, a new second set of signals appears at higher frequencies with $\Delta\delta = 0.20$ ppm. This may represent tetrads carrying Zn²⁺. ZnCl₂, NaCl,

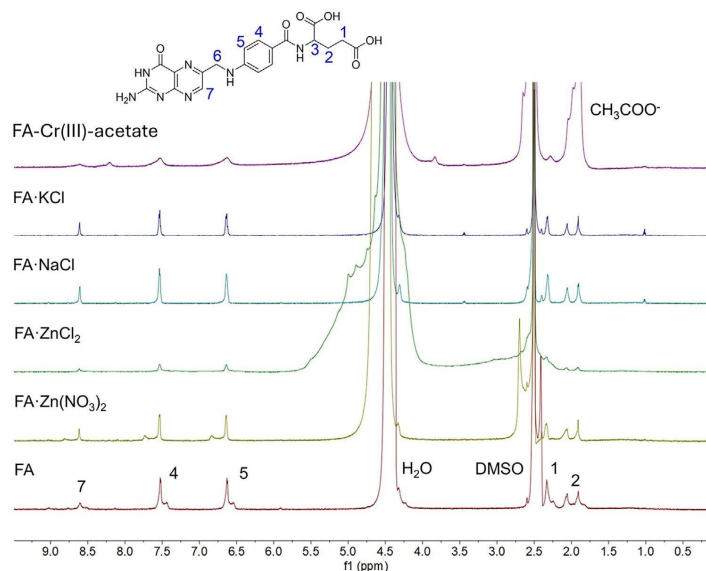


Figure 8. ^1H NMR of folic acid (FA) and its metallogels (0.4 wt% of FA and metal salt:FA 1:1) in $\text{DMSO}-d_6$: D_2O 1:1 (298.2 K, 700 MHz).

and KCl samples provide only a single set of signals similar to the major signals of neat FA representing free tetramers. In general, chlorides form stronger gels than nitrates, which could be translated here into a more effective polymerization of gel fibers using chlorides rather than nitrate. Finally, the addition of paramagnetic Cr(III)-acetate leads to a significant broadening of all FA signals and the appearance of a new signal at 8.21 ppm (this may originate from a signal of coordinated species—a paramagnetic shift of some proton signal).

In order to suppress the effect of water on the gelation of FA and to see any possible changes in the chemical shifts of FA induced by interactions with metal salts, we performed an ^1H NMR titration experiment of FA $\text{DMSO}-d_6$ solution with $\text{Zn}(\text{NO}_3)_2$ (Figure S12). The FA $\text{DMSO}-d_6$ solution gives only one set of NMR signals and there are no changes in the spectra upon the addition of metal salt, except for a broadening of the $-\text{NH}_2$ -signal. Finally, variable temperature (VT, from 298.2 K to 348.2 K) ^1H NMR experiments were carried out to follow on sol $\leftrightarrow$ gel transitions of FA gel (0.2 wt%) and FA metallogels (0.8 wt% of FA; using $\text{Zn}(\text{NO}_3)_2$, ZnCl_2 , NaCl, KCl, and Cr(III)-acetate in 1:1 ratio with FA) prepared in a $\text{DMSO}-d_6$: D_2O 1:1 mixture (Figure S13–18). In all cases a significant increase in the signal intensity was observed with increasing temperature of the sample, suggesting a gel $\rightarrow$ sol transition (melting of the gel leading to an increased concentration of small assemblies of FA). As in the temperature-sweep rheologic experiments, gradually cooling the samples inside the probe to 298.2 K led to a significant decrease in the intensity of the NMR signals representing the sol $\rightarrow$ gel transition (gel recovery).

Even though we could not observe significant changes in the ^1H NMR spectra suggesting effective metal-FA interaction, we could follow the presence of smaller FA-aggregates. We can assume that the presence of metal cations leads to the swift formation of larger polymeric aggregates which are not directly visible using liquid-state NMR.

2.6.5. Powder X-Ray Diffraction (PXRD)

To gain further insight into the solid-state properties of the metallogels, PXRD measurements were performed on dried samples. In general, the samples do not show any signs of crystallinity from the PXRD pattern (e.g., FA-Cr(III)-acetate in Figure S19a), suggesting that the self-assembled aggregates in metallogels are too small (the reported size at the same concentration of FA in the presence of K^+ or Na^+ is 9 stacked tetramers)^[37] or too few to be detected by PXRD (but aggregates are apparently present as shown by the more sensitive CD spectroscopy). Also, it is well documented in the literature that thixotropic gels display an amorphous nature in their XRD pattern.^[69]

2.6.6. Differential Scanning Calorimetry (DSC)

For DSC studies, dry or wet samples were weighed into a pan and measured. In general, the thermal responsiveness of the gels is influenced by their structural features, e.g., nanofiber structure, and/or their crosslinking in the gels. The supramolecular gel network is therefore quite sensitive to temperature, particularly near the critical gelation temperature. For example, the DSC curve of

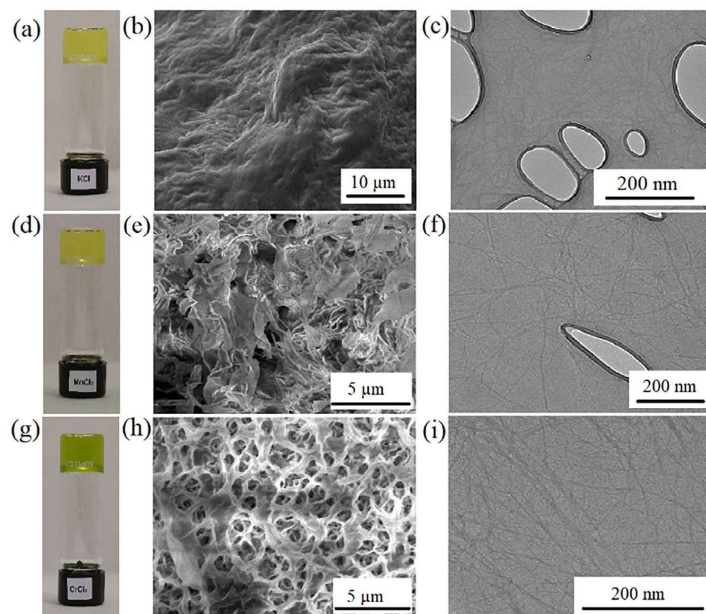


Figure 9. (a) Photograph of 0.2 wt% FA-KCl metallogel in DMSO:water mixture (1:1), (b) SEM image of freeze dried FA-KCl metallogel, (c) TEM image of FA-KCl metallogel. (d) Photograph of 0.2 wt% FA-MnCl₂ metallogel in DMSO:water mixture (1:1), (e) SEM image of freeze dried FA-MnCl₂ metallogel, (f) TEM image of FA-MnCl₂ metallogel. (g) Photograph of 0.2 wt% FA-CrCl₃ metallogel in DMSO:water mixture (1:1), (h) SEM image of freeze dried FA-CrCl₃ metallogel, (i) TEM image of FA-CrCl₃ metallogel.

the FA-Mg(NO₃)₂ sample (Figure S19b) shows endothermic peaks between 45°C and 60°C that account for the gel-solution phase transitions. On the other hand, the FA-Cr(NO₃)₃ (Figure S12b) and the FA-Cr(III)-acetate (Figure S12c) gel samples do not follow the same trend as they show only negligible decrease in heat flow over the temperature range. The gels do not melt (only soften), which is in agreement with the results of rheological temperature-sweep experiments and can also be observed from the VT ¹H NMR spectrum.

2.6.7. Electron Microscopy (EM)

To investigate the structure of the supramolecular assemblies, we performed EM imaging of all of the gels studied. The TEM images of all 17 metallogels revealed the presence of highly entangled fibrillar networks. The diameter of the fiber varied from 5 to 50 nm. The type and size of the fiber were similar for all of the gels studied. However, in some cases, a strong film formed as the gels dried and prevented the extraction of finer details. For SEM imaging, the gels were freeze-dried before sputtering and imaging. The SEM images of freeze-dried gels also displayed highly entangled networks. Figure 9 shows representative TEM and SEM images of the FA-KCl, FA-MnCl₂, and FA-CrCl₃ metallogels. Figure S20 shows the remaining TEM and SEM images of the metallogels obtained.

2.6.8. Enhanced Oil Recovery Using Metallogels

To explore the potential applications of the FA-Cr(III)-acetate gel (0.2%), we carried out proof-of-concept experiments for oil recovery. Accordingly, we carried out experiments on the effect of simulated seawater (a mixture of NaCl, MgSO₄, CaCl₂, and KCl in distilled water (with overall salt concentration 34 g/L)) (Table S7 and S8) or saturated brine solution (335 g/L) on gel formation and stability, changing reagents' concentration and ratio (simulating possible dilution in oil reservoir) and following the rate of gel formation and its strength at ambient temperature or 150°C (mimicking conditions of high-temperature oil reservoirs) (Table S6), following on the effect of thermal stress (heating the samples for 12 h at 100°C, 120°C, 150°C, 200°C, and 250°C), or aging of the samples for one year (Figure 10 and S22, Table S7).

The gels can be formed at ambient temperature at various concentrations down to 0.07%. The gels are also formed at various FA to Cr(III)-acetate ratios, i.e., from 1:1 down to 100:1 ratio. At 150°C, strong gels are slowly formed at 0.4% concentration (FA:Cr(III)-acetate 1:1) within 24 h and are further stiffening for up to 7 days of the study (Table S6).

Moreover, the effect of thermal stress (heating the 0.2% FA:Cr(III)-acetate 1:1 samples for 12 h at 100°C, 120°C, 150°C, 200°C, and 250°C) and aging the samples for one year (0.2% FA FA:Cr(III)-acetate 1:1 in water:DMSO or seawater:DMSO 1:1) were

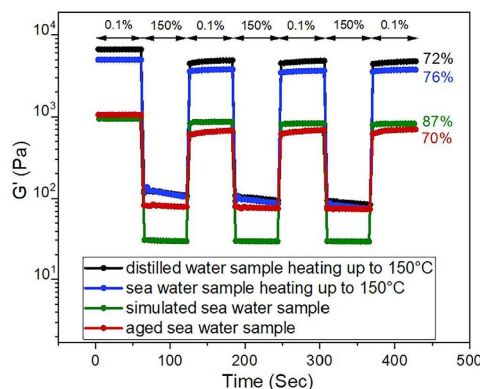


Figure 10. Step-strain experiments showing the self-healing properties of FA-Cr(III)-acetate metallo gels (0.2 wt%) under various conditions mimicking those in field tests, i.e., thermal stress at 150°C, seawater environment, and aging effect.

followed by rheological measurements. Frequency-sweep rheological experiments on these samples showed that the materials remained viscoelastic solids upon aging or thermal treatment up to 200°C (Figure S22f,g). Time-sweep experiments showed that the gel strength of the samples remained constant as follows for 60 min (Figure S22b,c). Temperature-sweep experiments further confirmed the good thermal stability of the gels within the 20°C–80°C range (Figure S22d,e). The sudden drop in gel strength at 250°C is likely caused by the thermal decomposition of FA.^[70–72] whereas at the lower temperatures studied, i.e., 100°C, 120°C, 150°C, and 200°C, the gels increased in strength as followed by G' values (Table S7). Considering that gelation is a dynamic process driven by temperature-dependent kinetic factors, the higher the temperature, the faster the rearrangement towards thermodynamically favored rearrangement of the gel structure resulting in a stronger gel network. Step-strain and strain-sweep experiments using the thermally treated samples showed critical strain values around 80% after one cycle, even in the presence of seawater (Figure 10, S22a,h,i,j).

The effect of simulated seawater on gel formation was studied at ambient temperature and at 150°C (Table S8). Rigid gel (0.4 wt% FA in seawater:DMSO mixture 1:1) can be formed at ambient conditions after 7 days. The flowing gel can be formed at 150°C under the same conditions and time. Excellent stability of FA:Cr(III)-acetate metallo gel (0.4 wt%, 1:1 ratio in water:DMSO 1:1) was further confirmed in the presence of saturated brine solution at room temperature but also under heating for one week at 150°C.

Overall, the results show that the FA-Cr(III)-acetate gels are mechanically robust, thermally stable, and maintain their self-healing and thixotropic properties. Moreover, the stability of the gels increases with an increasing temperature up to 200°C. The gels are not sensitive to the presence of other cations/anions in the solvent system, but quite the opposite, their mechanical

strength increases in the presence of seawater (and further upon heating) (Table S7).

3. Conclusions

In this work, we show that the incorporation of quite a large group of various metal salts via the hierarchical self-assembly of natural FA results in supramolecular metallo gels with a robust mechanical elastic modulus at concentrations below 0.2%. Combinations of FA with chlorides, nitrates, and acetates of Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Fe^{3+} , and Cr^{3+} were investigated, out of which 17 supragels were prepared and thoroughly studied for their rheological behavior, morphology, and structure using SEM, TEM, $^1\text{H-NMR}$, FTIR, CD, UV-Vis, PXRD, and DSC. Rheological studies proved that the addition of a metal ion to FA significantly increases the strength of the gels. Comparing their storage modulus values G' , the strength of the gels decreases in a row for chlorides and nitrates in the same way: $\text{Cr}^{3+} > \text{Ca}^{2+} > \text{Zn}^{2+} > \text{Mn}^{2+} > \text{Mg}^{2+} > \text{Ni}^{2+} > \text{Na}^+ > \text{K}^+$. The FA-based metallo gels studied show that their strengths are also influenced by the counter anion, chlorides mostly resulting in stronger gels than nitrates (with the exception of Cr^{3+}). In the case of chromium(III)-based systems it seems that the effect of the cation represents a dominant factor in the strength of the gels with, exceptionally, the strongest gel formed using Cr(III)-acetate (it being also the only acetate-based metallo gel found in this work). The robustness of FA gelation with metals is outstanding in its non-selective character, as the FA can form strong gels with 17 metal salts in a relatively broad range of concentrations and conditions.

Given the large group of variable metal salts tested, monovalent, divalent, or trivalent alkaline, alkaline earth, or transition metal salts, their morphology varies (as seen by SEM and TEM) and their (supra)molecular structure can also vary along with various mechanical properties, as established by rheological studies. Of the group of metallo gels, we have closely investigated the molecular structure of the strongest, the most stable, and for us currently, the most interesting metallo gel derived from Cr(III)-acetate. Using a combination of analytical techniques, we found that FA follows a general trend of self-assembly into tetrad and their stacking into small columnar aggregates. The Cr^{3+} can be partly positioned in the center of these tetrads but can also decorate the outside of the aggregates that arise and lead to their additional interlinking. We hypothesize that the pivotal network forming π - π and Cr(III)-mediated columnar stacks of tetrads are greatly supplemented by coordination between various chromium(III) nodes and glutamate carboxylates, complemented by numerous hydrogen bonds among the folates, solvent molecules (DMSO, water), and acetates (Figure 11, Scheme S1).

We show that the FA-Cr(III)-acetate gel is a potential candidate for oil recovery applications based on our proof-of-concept experiments as it overcomes the currently known limitations of various polyacrylamide-based polymeric gels. The folic acid gelator and the FA-Cr(III)-acetate-based gels in particular show multiple beneficial features in a single system, which were not

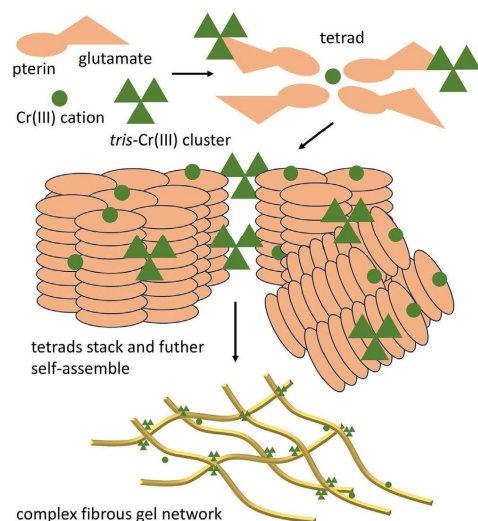


Figure 11. Mode of self-assembly of FA in the presence of Cr(III)-acetate in DMSO:water mixture.

found together in any other gelation system for EOR. Results of the laboratory study suggest that this novel gel technology should be operational and environmentally attractive for field tests. The non-toxic and natural FA is a broad spectral gelator with various metal salts, forming saline- and thermo-resistant adjustable gels. The FA can be a gelator for high and low permeability zones depending on gel formulation and application. The gels introduced in our technology represent a single-fluid system, i.e., the simple injection of a single concentrated DMSO solution to a seawater phase in situ leads to gelation, where the strength of the gel (ranging from highly flowing to rigid, rubbery gels) can be controlled by the choice of metal salt or by varying the concentrations or the component ratio, which ultimately makes them applicable to a wide range of oilfield requirements and environmental and geological conditions. As stable gels can be achieved under various conditions and over a substantial range of FA to metal salts ratios, suggesting that precise formulations, which might be generally difficult to achieve during fieldwork, are not necessary prerequisites for practical and successful applications of the gels.

The chromium(III) acetate gels show beneficial thermal properties, high stability (up to 200°C), and slow formation rate at 150°C which provides a reasonable delay of gelation necessary for thorough penetration of the gelating mixture in the reservoir matrix. The gels show undisturbed (or even elevated) ability of gelation in seawater (also at an elevated temperature of 150°C) and long-term stability in (sea)water or a highly saline saturated environment. Moreover, their thixotropic behavior could provide benefits in certain oil mining applications where injection of pre-formed gel is desirable, i.e., the gel technology can also be used in high permeability zones, and the quality of the gel does not

change by shearing during pumping or flowing through pore throats, being it also capable to perform under dynamic reservoir conditions.

DMSO as a co-solvent does not pose any significant environmental risk^[73–75] and can have some additional benefits, e.g., it helps to improve the thermal stability of gels, decreases the total volume needed for in situ injection, and shows the higher viscosity and good miscibility with an aqueous solution that is related to the slow diffusion throughout the aqueous volume that supports the formation of a homogenous and stable gel.

Finally, this study shows FA in combination with various metal salts to be a powerful gelator and predestines it to become an interesting, biocompatible, and sustainable building block for the materials chemistry of the future. We believe that because of their biocompatibility, these materials could also find uses in other laboratory and industrial processes, especially in the pharmaceutical and biomedical sciences and technologies.

Acknowledgments

This work has received support from the Czech Science Foundation (Grant No. 24–10199S to R.M.) and the Grant Agency of Masaryk University (MUNI/A/1575/2023). We acknowledge the CEITEC Core Facilities Josef Dadok National NMR Center, Cryo-Electron Microscopy and Tomography, Biomolecular Interactions and Crystallography, and BIC of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2018127, LM2023042) and the European Regional Development Fund-Project “UP CIISB” (No. CZ.02.1.01/0.0/0.0/0.0/18_046/0015974). CzechNanoLab project LM2023051 funded by MEYS CR is gratefully acknowledged for the financial support of the measurements at CEITEC Nano Research Infrastructure. We thank the Research Council of Finland for project funding (No. 352900) and Photonics Research and Innovation (PREIN) flagship. This work utilized the Tampere Microscopy Centre for imaging facilities.

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Conflict of Interests

M.A.M., R.M., and O.J. have filed a patent application regarding this work. The authors declare no other competing interests.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: folic acid · gel · metallogel · oil mining · rheology · supramolecular chemistry

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Manuscript received: February 26, 2025

Revised manuscript received: March 12, 2025

Version of record online: April 9, 2025

Appendix B [Supporting Information]

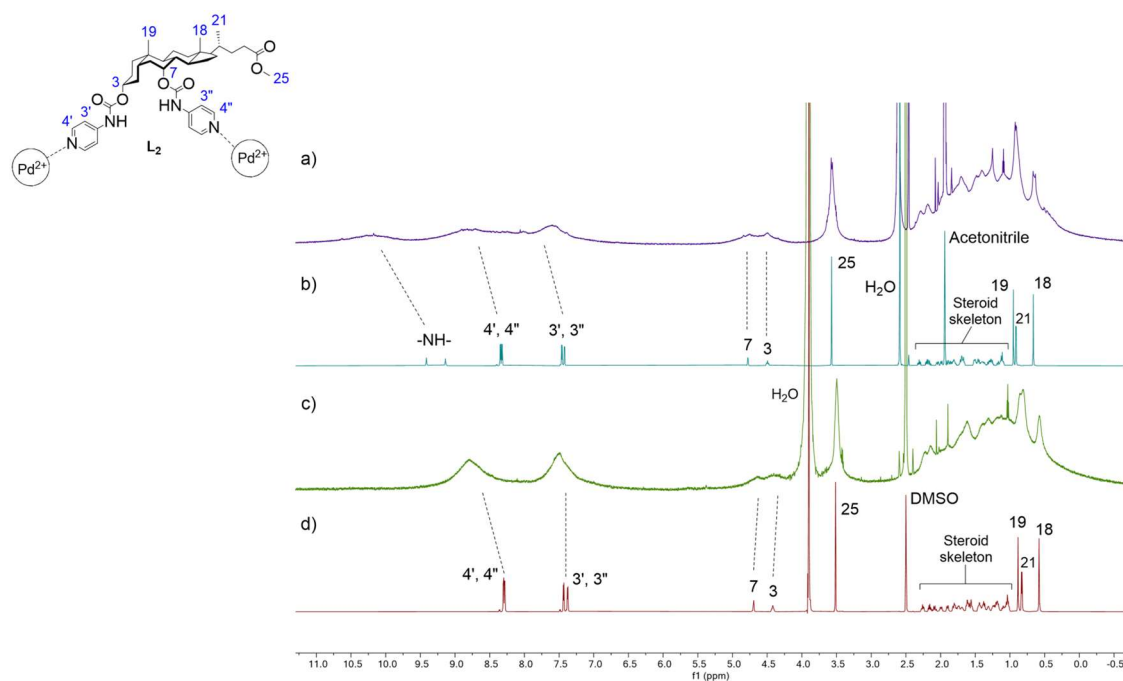


Figure S1. ^1H -NMR spectra of the complexation reaction of L_3 with $\text{Pd}(\text{NO}_3)_2$, M:L 1:2, 70 °C, 1 h in acetonitrile- d_3 : $\text{DMSO}-d_6$ 9:1 and D_2O : $\text{DMSO}-d_6$ 1:5 (700 MHz, 298.2 K).

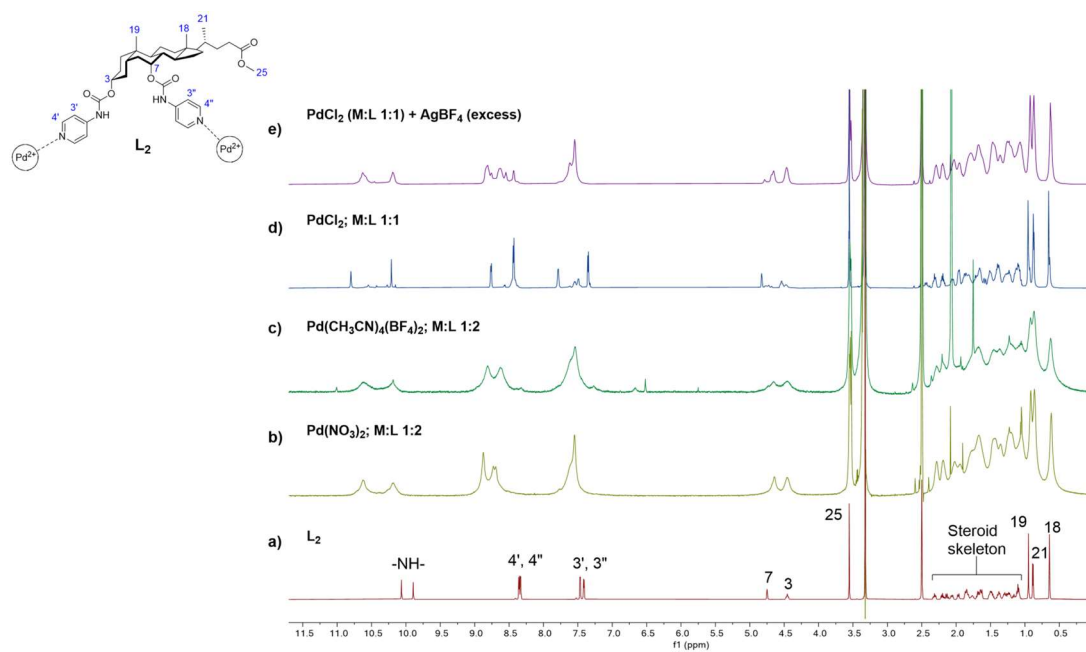


Figure S2. ^1H -NMR spectra (700 MHz, 298.2 K, $\text{DMSO}-d_6$) of the complexation reaction of L_3 with different metal salts.

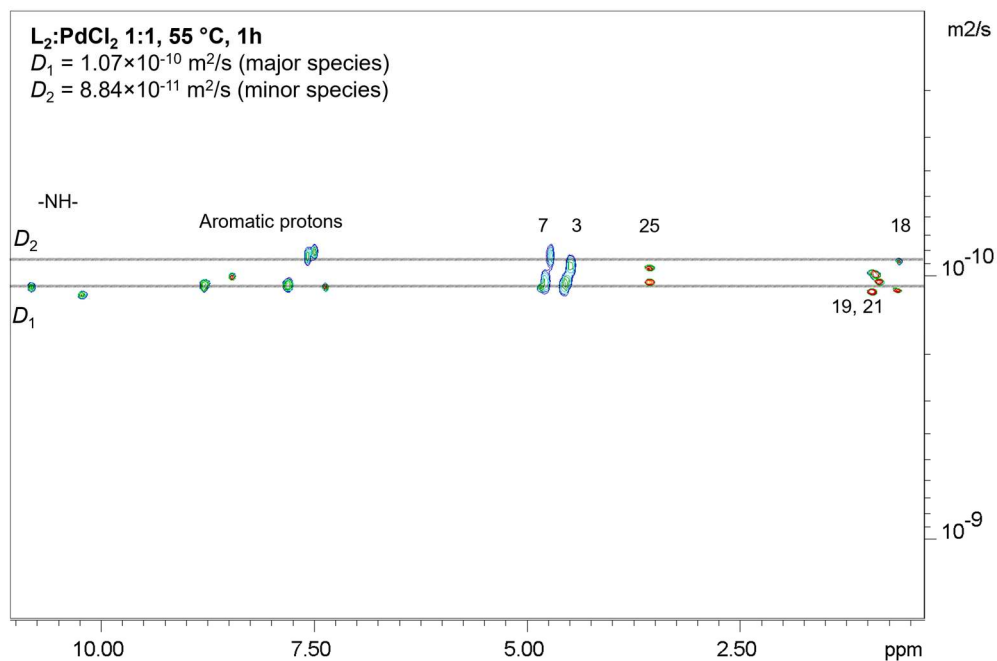


Figure S3. ¹H-DOSY NMR (700 MHz, 303.2 K, DMSO-*d*₆) spectra of the complexation reaction of L₂ with PdCl₂.

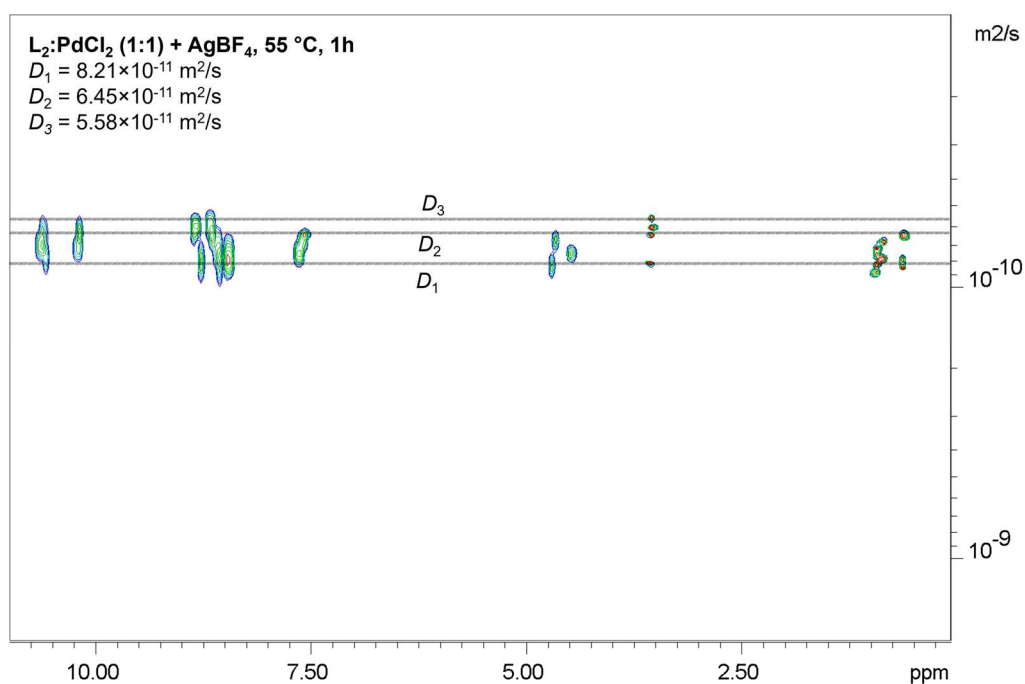


Figure S4. ¹H-DOSY NMR (700 MHz, 303.2 K, DMSO-*d*₆) spectra of the complexation reaction of L₂ with PdCl₂, followed by the addition of AgBF₄ and heating at 55 °C for 1h.

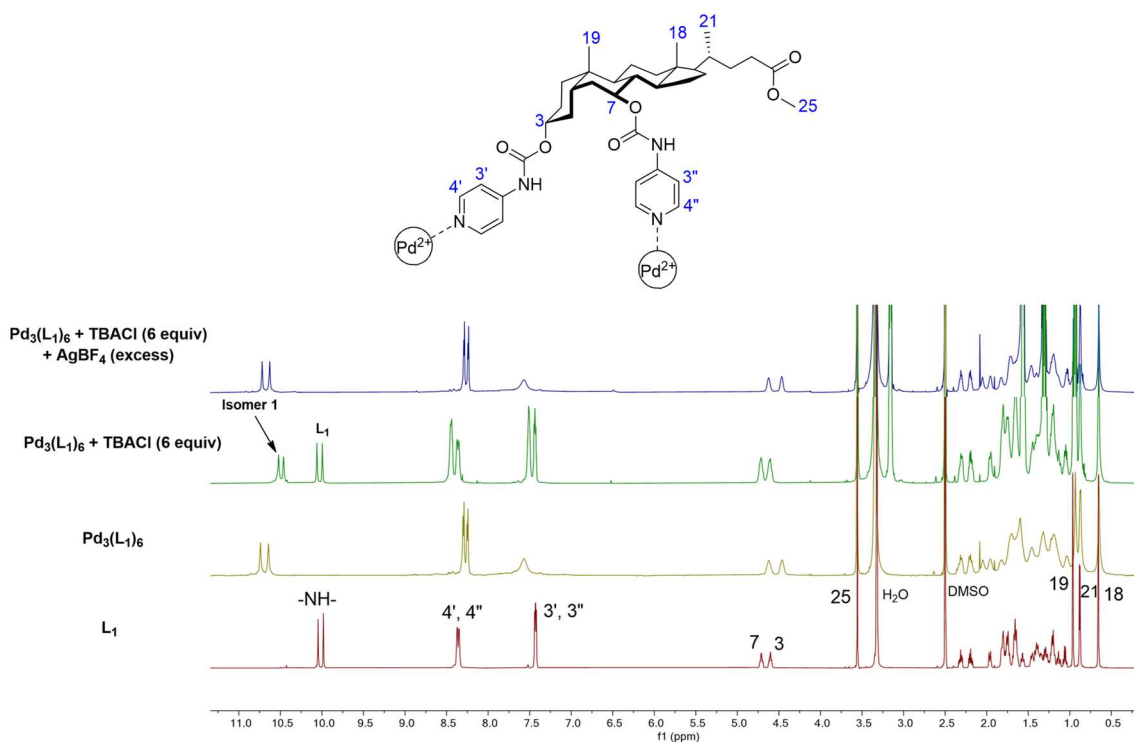


Figure S5. ^1H -NMR spectroscopic analysis (700 MHz, 298.2 K, $\text{DMSO-}d_6$) of structure-transformation study of $\text{Pd}_3(\text{L}_1)_6$.

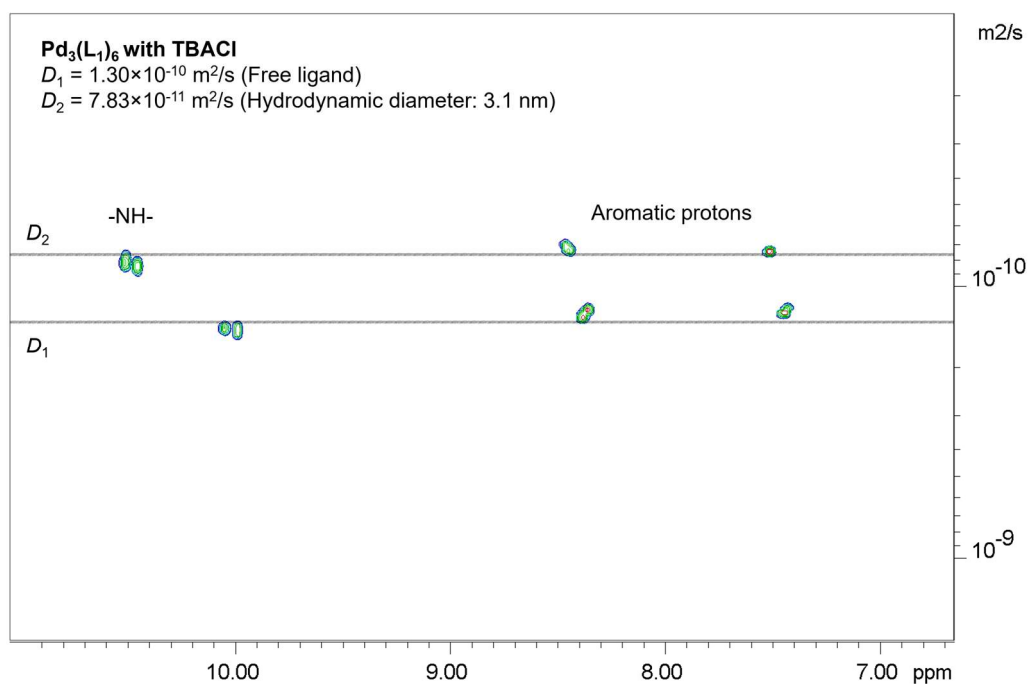


Figure S6. ^1H -DOSY NMR (700 MHz, 303.2 K, $\text{DMSO-}d_6$) spectra of $\text{Pd}_3(\text{L}_1)_6$ after the addition of TBACl and heating at 55 $^\circ\text{C}$ for 1h.

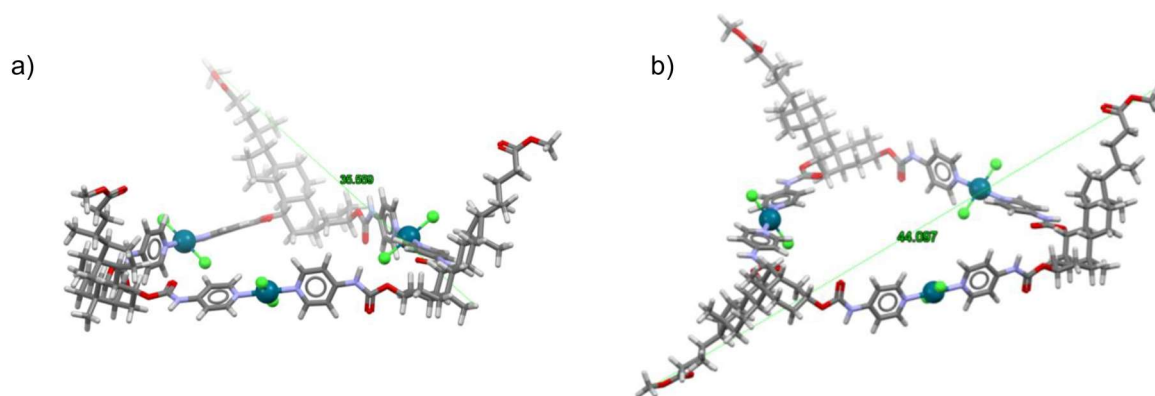


Figure S7. DFT-optimized models of *cis*- and *trans*- $\text{Pd}_3(\text{L}_1)_3\text{Cl}_6$.

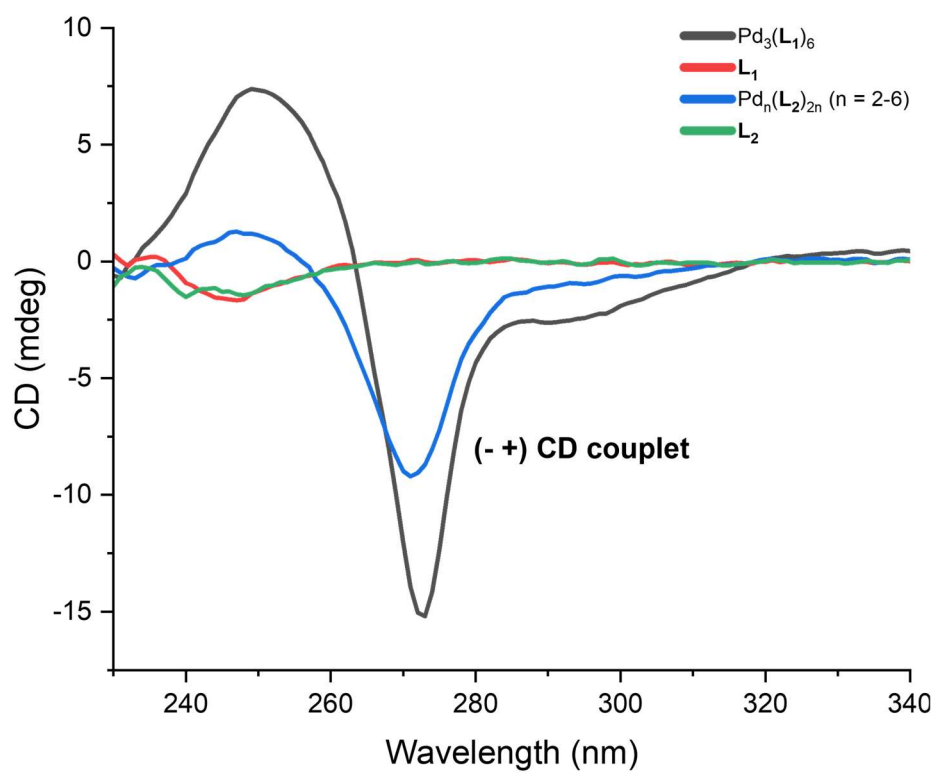


Figure S8. CD spectra (MeOH:DMSO- d_6 1000:1, 25 °C) of L_1 , L_2 , and their complexation reactions with $\text{Pd}(\text{NO}_3)_2$.

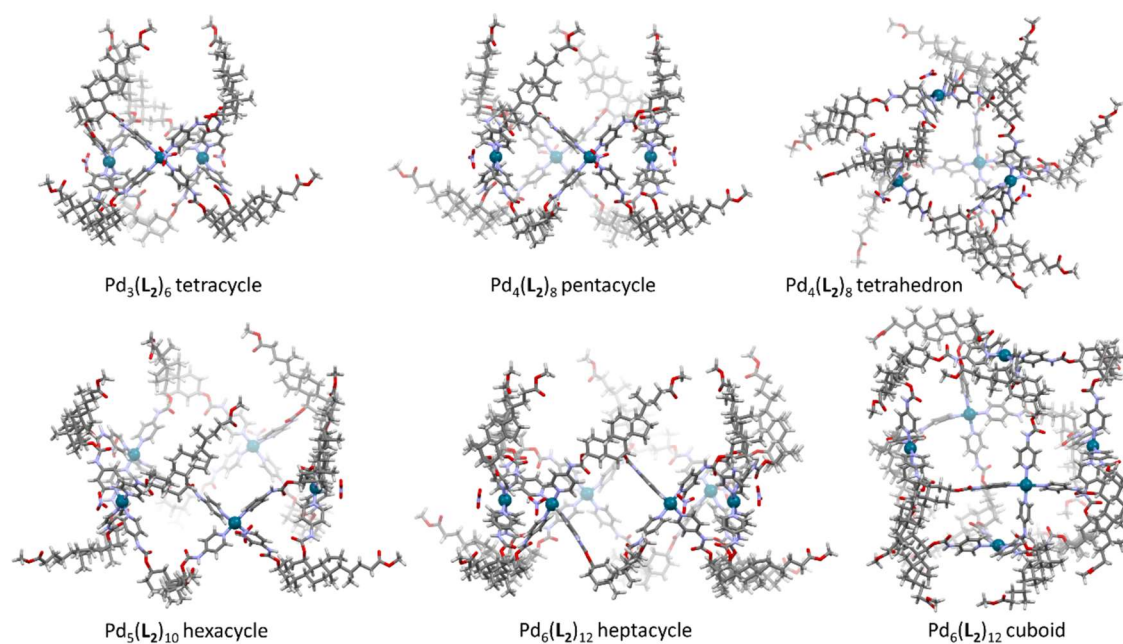


Figure S9. DFT-optimized models of the possible constitutional isomers of $\text{Pd}_n(\text{L}_2)_{2n}$ mixture.

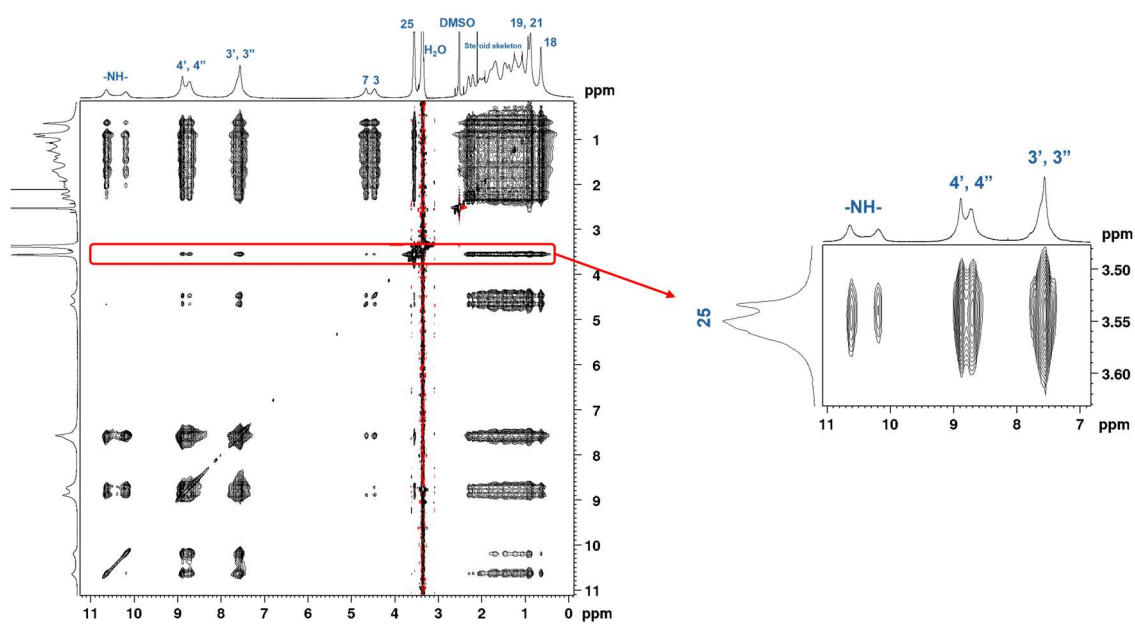


Figure S10. ^1H - ^1H NOESY NMR spectra (700 MHz, 298.2 K, $\text{DMSO}-d_6$) of $\text{Pd}_n(\text{L}_2)_{2n}$ mixture.

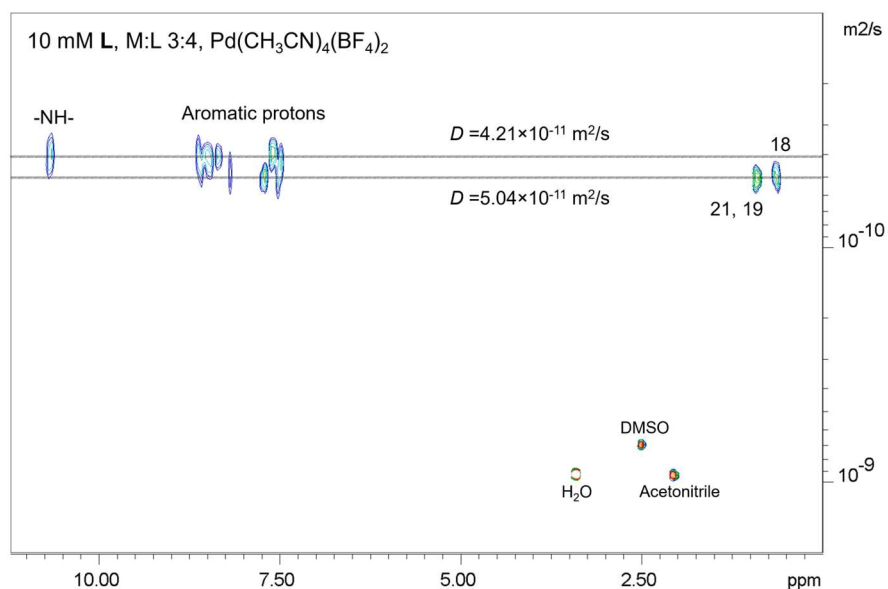
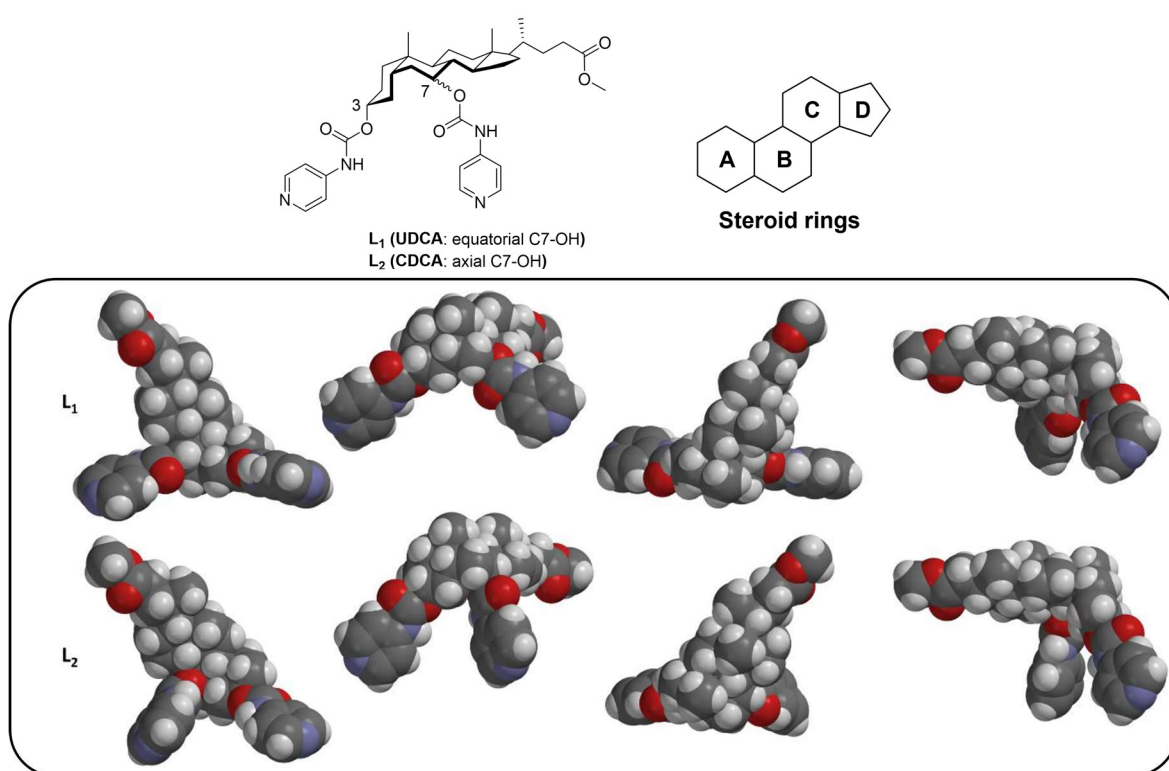


Figure S12. ^1H -DOSY NMR spectra (700 MHz, 303.2 K, $\text{DMSO}-d_6$) of $\text{Pd}_{12}(\text{L}_3)_{16}$ and $\text{Pd}_6(\text{L}_3)_8$ mixture.

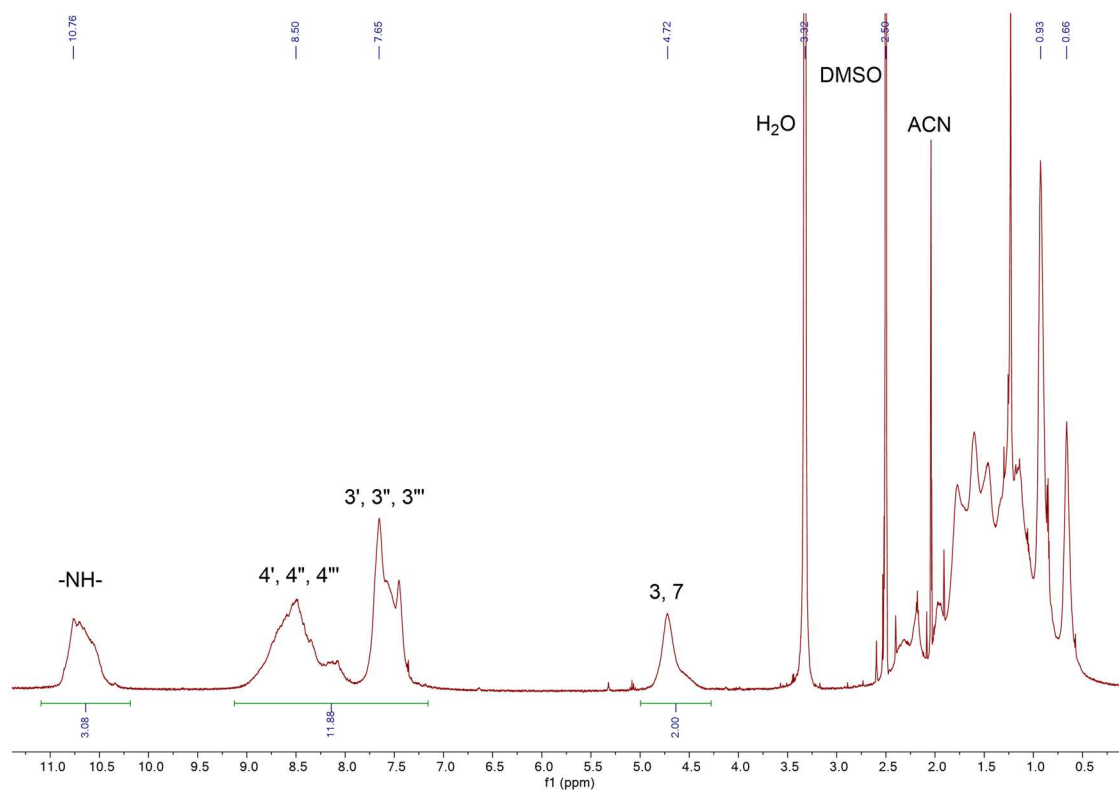


Figure S13. ¹H-NMR spectra (700 MHz, 298.2 K, DMSO-*d*₆) of RM 3.

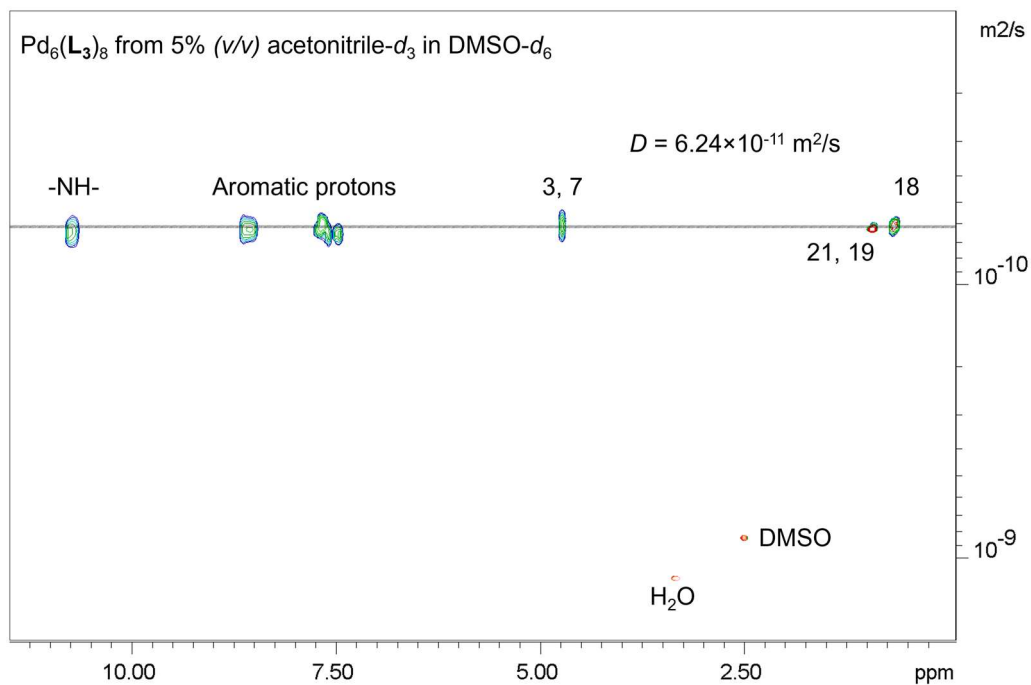


Figure S14. ¹H-DOSY NMR spectra (700 MHz, 303.2 K, DMSO-*d*₆) of RM 3.

Table S1. Comparison of diffusion coefficients (D) of products as obtained using ^1H -DOSY NMR spectroscopy (700 MHz, 303.2 K).

Experiment	Reaction conditions	Product	D_{product} (m^2/s)
RM1	$\text{Pd}(\text{CH}_3\text{CN})_4(\text{BF}_4)_2:\text{L}_3 = 3:4$ in $[\text{D}_6]$ -DMSO	$\text{Pd}_6(\text{L}_3)_8 + \text{Pd}_{12}(\text{L}_3)_{16}$	4.21×10^{-11} 5.04×10^{-11}
RM1 – ACN	$\text{Pd}(\text{CH}_3\text{CN})_4(\text{BF}_4)_2:\text{L}_3 = 3:4$ in $[\text{D}_6]$ -DMSO (evaporated and redissolved)	$\text{Pd}_{12}(\text{L}_3)_{16}$	4.96×10^{-11}
RM1 + TBANO₃	RM1 + 2×1.5 equiv. (1+1 h heating)	$\text{Pd}_6(\text{L}_3)_8 + \text{Pd}_{12}(\text{L}_3)_{16}$	5.10×10^{-11} 5.58×10^{-11}
RM2	$\text{Pd}(\text{NO}_3)_2:\text{L}_3 = 3:4$ in $[\text{D}_6]$ -DMSO	$\text{Pd}_{12}(\text{L}_3)_{16}$	4.98×10^{-11}
RM3	$\text{Pd}(\text{NO}_3)_2:\text{L}_3 = 3:4$ in 5% (v/v) $[\text{D}_3]$ -ACN in $[\text{D}_6]$ -DMSO	$\text{Pd}_6(\text{L}_3)_8$	6.24×10^{-11}
RM2 + $\text{Pd}(\text{NO}_3)_2$	RM2 + 3 equiv. $\text{Pd}(\text{NO}_3)_2$	$\text{Pd}_6(\text{L}_3)_8 + \text{Pd}_{12}(\text{L}_3)_{16}$	4.29×10^{-11} 5.62×10^{-11}
RM2 + $[\text{D}_3]$-ACN	RM2 + 5% $[\text{D}_3]$ -CAN	$\text{Pd}_6(\text{L}_3)_8$	5.58×10^{-11}
RM2 (VT-NMR)	$\text{Pd}(\text{NO}_3)_2:\text{L}_3 = 3:4$ in $[\text{D}_6]$ -DMSO	$\text{Pd}_{12}(\text{L}_3)_{16}$	4.96×10^{-11} (up to 125 °C) 5.10×10^{-11} (at 100 °C)
RM2 3:2	$\text{Pd}(\text{NO}_3)_2:\text{L}_3 = 3:2$ in $[\text{D}_6]$ -DMSO	$\text{Pd}_6(\text{L}_3)_8$	5.98×10^{-11}
RM2 3:2 + L_3	RM2 3:2 + 2 eq. L_3	$\text{Pd}_{12}(\text{L}_3)_{16}$	4.29×10^{-11}
Neat sample of L_3	L_3 in $[\text{D}_6]$ -DMSO	L_3	1.57×10^{-10}

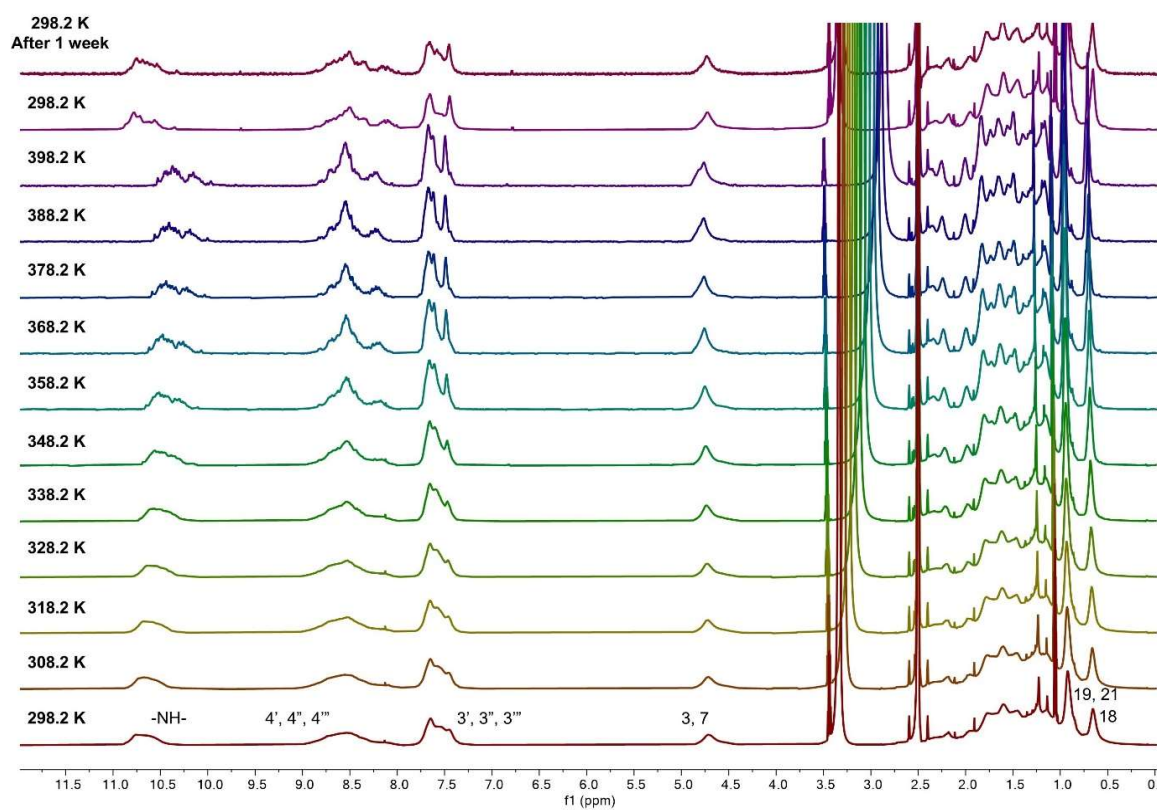


Figure S15. Variable temperature ¹H-NMR (700 MHz, DMSO-*d*₆) study of Pd₁₂(L₃)₁₆ (RM2).

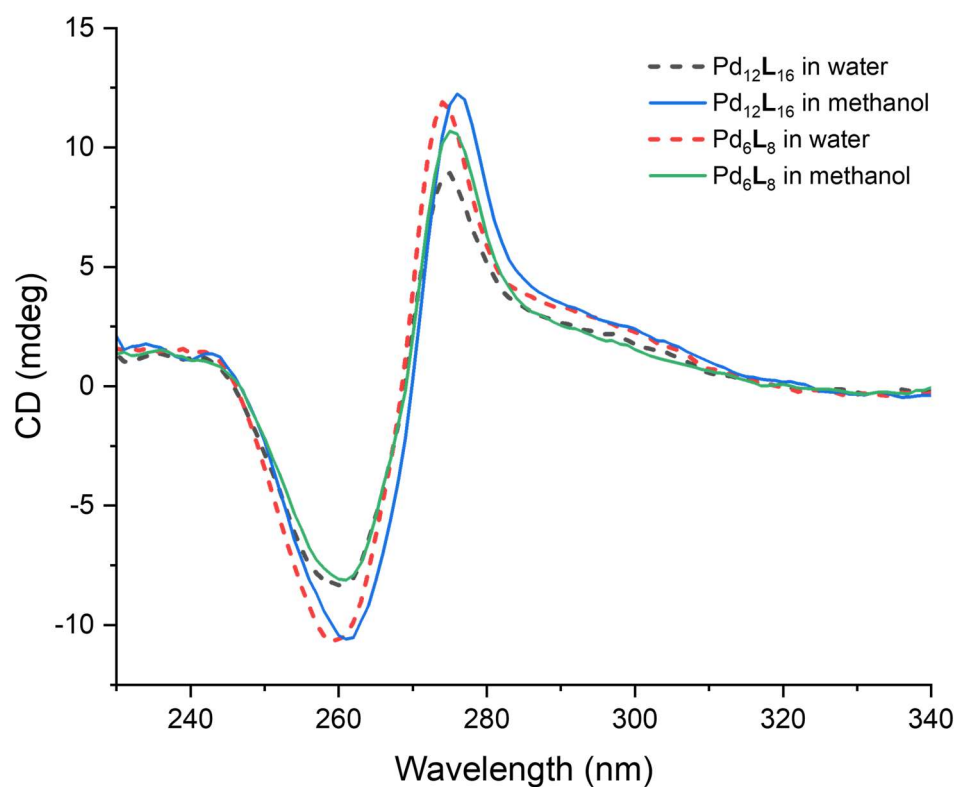


Figure S16. Comparison of CD spectra (25 °C) of $\text{Pd}_6(\text{L}_3)_8$ and $\text{Pd}_{12}(\text{L}_3)_{16}$ in $\text{MeOH}:\text{DMSO-}d_6$ 1000:1 and $\text{H}_2\text{O}:\text{DMSO-}d_6$ 1000:1; accounting for 625 nm and 1250 nm concentration of $\text{Pd}_{12}(\text{L}_3)_{16}$ and $\text{Pd}_6(\text{L}_3)_8$ respectively.

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Appendix C [Conference Contributions]

1. **36th Central European NMR Meeting (*NMR Valtice-2023*)**
 April 2 – 5, 2023 | Valtice, Czechia
Oral Presentation: ^1H DOSY NMR spectroscopy of bile acid-based metallosupramolecular structures

2. **17th International Symposium on Macrocyclic and Supramolecular Chemistry (*ISMSC-2023*)**
 April 25 – 29, 2023 | Reykjavík, Iceland
Poster Presentation: Self-Assembly of Bile Acid Derivatives into Metallosupramolecular Cages

3. **Phytochemical Society of Europe Trends in Natural Products 2024 Young Scientists' Meeting (*PSE-2024*)**
 May 21 – 24, 2024 | Brno, Czechia
Poster Presentation: Bioactive 3D supramolecular coordination architectures of bile acids

Appendix D [Curriculum Vitae]

CURRICULUM VITAE

PERSONAL INFORMATION

Subhasis Chattopadhyay

+91- 7319120548 / +420 774 115 853

subhasischattopadyay101@gmail.com

Date of birth 09.03.1995 | Nationality Indian

AREA OF INTERESTS

- Synthesis and medicinal applications of natural products and derivatives
- Supramolecular coordination complexes
- Self-assembly of supramolecules into porous nanoparticles

PROFESSIONAL EXPERIENCE

April – June
2022

Development of Porous Steroidal Nanoparticles, *Erasmus+ Traineeship (3 months)*

Adj. Prof. Manu Lahtinen, Department of Chemistry
University of Jyväskylä, Finland

August 2017 –
July 2018

Towards Biomimetic Synthetic Approach for Carpatamides to Daryamides Conversions, *M.Sc. 1-year Research Project*

Asst. Prof. Gokarneswar Sahoo, Department of Chemistry
NIT Rourkela, India

May – July
2017

Paraquinols and Epoxy-paraquinols as Key Skeletons in Natural Products: A Synthetic effort, *Summer Internship*

Asst. Prof. Gokarneswar Sahoo, Department of Chemistry,
NIT Rourkela, India

ACADEMIC
PROFILE

Post-doctoral Research: Dec 2024 - present	Photoswitchable Metallosupramolecular Cages Dr. Pia Jurček Department of Chemistry, Masaryk University, Czechia
Ph.D. 2019 - present	Unsymmetric chiral ligands in metallo-supramolecular chemistry Prof. Radek Marek, Asst. Prof. Ondřej Jurček Department of Chemistry, Masaryk University, Czechia
M. Sc. (Master of Science) 2016 - 2018	Chemistry: Heterocyclic Chemistry, Molecular Spectroscopy, Organometallic Chemistry (Professional Electives), CGPA: 9.27 (Placed 1st position) Department of Chemistry, NIT Rourkela, India
B. Sc. (Bachelor of Science) 2012 - 2016	Chemistry (Honors) with Physics, Mathematics (Electives) 69.12% (Top 20 students at the university) Krishnagar Government College, University of Kalyani, West Bengal, India
Higher Secondary 2010 - 2012	Science with English, Bengali (Compulsory Languages), Chem- istry, Physics, Mathematics, Biological Science (Elective), Envi- ronmental Science 81.40% , Nabadwip Bakultala High School, Nabadwip, West Bengal, India

ADDITIONAL
INFORMATIONAwards &
Achievements

- **2016: All India Rank 647** in JAM (Joint Admission Test for M.Sc.) in Chemistry (India).
- **2017: Lectureship, All India Rank 28** in CSIR-UGC NET (June) in Chemistry (India).
- **2017: Certificate of Merit** for securing 1st position in 1st year M.Sc., **Chemistry** in the academic session 2016-2017 (NIT Rourkela, India).

- **2018: All India Rank 1258** in GATE (Graduate Aptitude Test in Engineering) in Chemistry (India).
- **2019: Institute Medal for Branch Topper** for holding **1st position** in **M.Sc. in Chemistry**, NIT Rourkela (India) for the year 2018.
- **2024: Award of the Head of the Department of Chemistry**, Masaryk University, Czechia.
- **2025: Petr Sadmera Prize 2025** to the group of authors for the publication “*Unsymmetric Chiral Ligands for Large Metallo-Macrocycles: Selectivity of Orientational Self-Sorting* (*Angew. Chem. Int. Ed.* **2024**, *63*, e202409134; <https://doi.org/10.1002/anie.202409134>)” in the field of **Nuclear Magnetic Resonance**.

Professional Skills

- Advanced knowledge of NMR techniques (^1H , ^{13}C , DEPT, COSY, HSQC, HMBC, DOSY) and CD spectroscopy for supramolecular structure elucidation
- Competent in air- and moisture-sensitive chemistry (e.g., LiAlH_4 , Pd/C hydrogenation, cross-coupling reactions)
- Well-versed in purification techniques such as column chromatography, distillation, preparative TLC, and crystallization
- Proficient in FT/ATR-IR and mass spectrometry for molecular characterization
- Skilled in scanning electron microscopy (SEM), including meticulous sample preparation
- Conversant in scientific and analytical software: ChemDraw, MestReNova, Bruker TopSpin, Origin, Mercury, Avogadro, Blender, and MS Office

Teaching Assistance & Supervision

- Supervised and mentored undergraduate and high school students (Bc. Eliška Šranková, Mgr. Petr Radiměšský, Mgr. Sára Kollárová, Mgr. Robin Durník) in laboratory research
- Provided hands-on training in experimental techniques, data analysis, and scientific methodology, fostering independent research skills