

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 tritopic 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 architecturally 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 (^{DT}CCS_{N₂}) of [Pd₁₂L₁₆(BF₄)₁₄]¹⁰⁺ and [Pd₆L₈(BF₄)₂]¹⁰⁺ were 1976 and 1420 Å², corresponding roughly to diameters of 5.0 and