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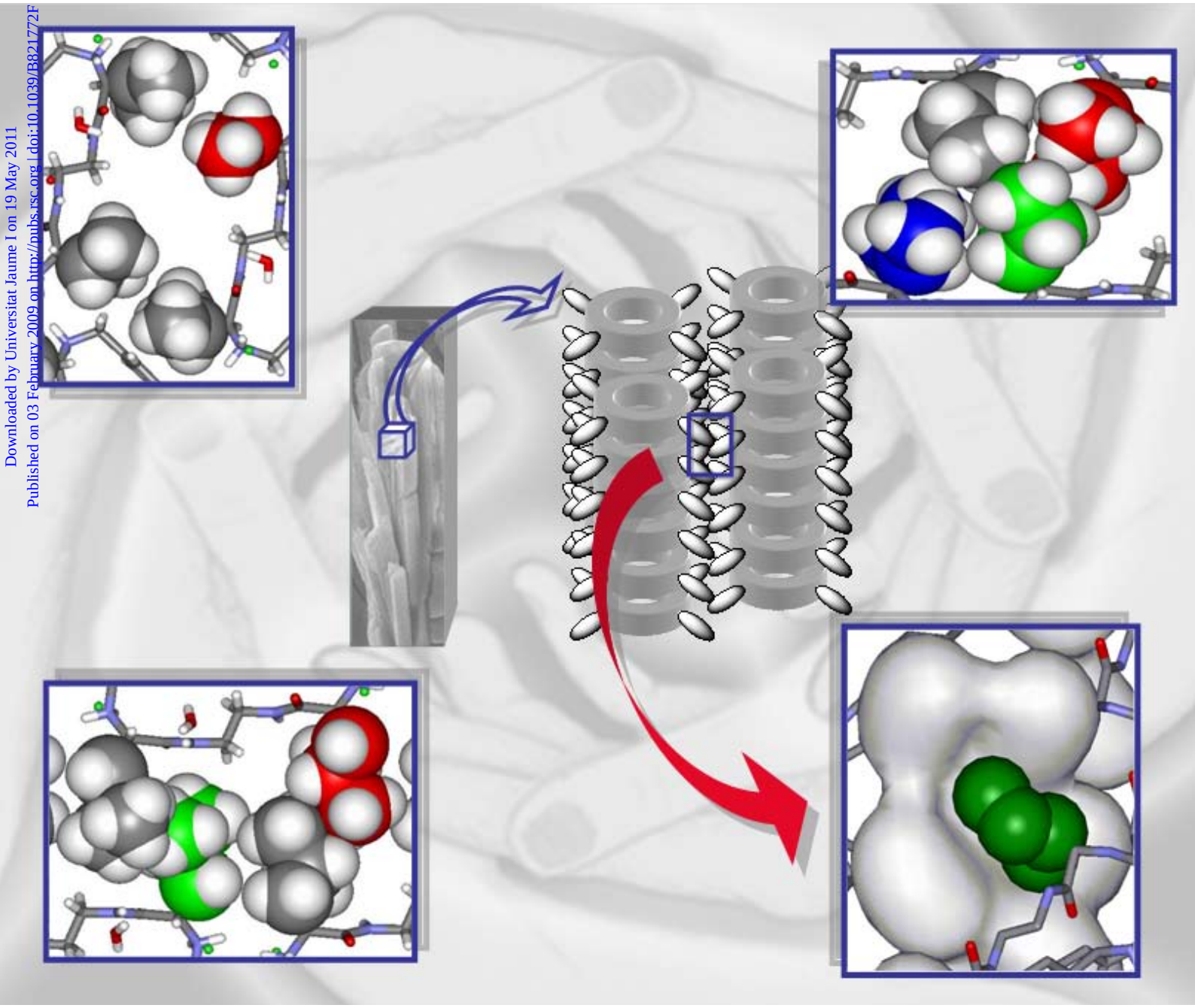

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Crystal structures of the HCl salts of pseudopeptidic macrocycles display "knobs into holes" hydrophobic interactions between aliphatic side chains†

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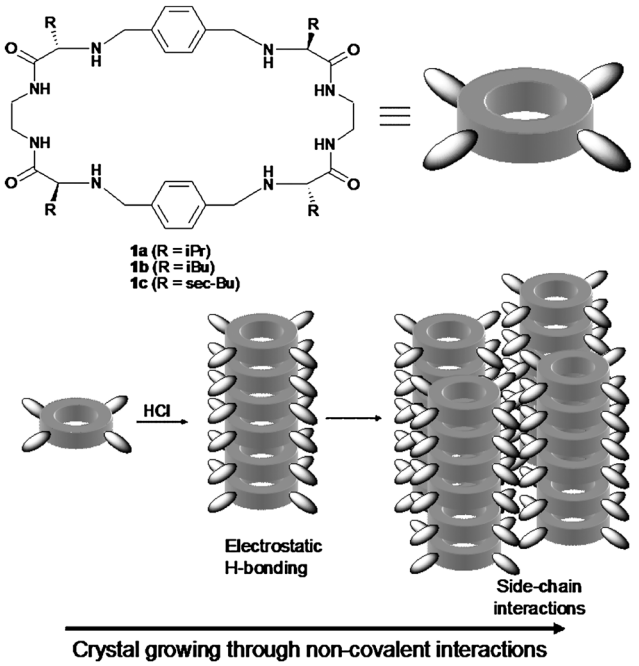
The crystal structures of three HCl salts of pseudopeptidic macrocycles show a H-bonding tubular stack of the rings, where the amino acidic side chains (Val, Leu, Ile) display an inter-columnar "knobs into holes" hydrophobic interaction pattern.

Molecular assembly through non-covalent interactions is a paradigm in modern chemistry.[1] The cooperative action of weak bonds leads to stable complexes with enormous importance in biological chemistry.[2] However, the deep experimental study of those interactions in real systems still remains difficult. The preparation of simplified models often allows a better understanding of the processes at the fundamental physico-chemical level.[3] Thus, specific contributions of the different forces can be visualized and characterized properly. In aqueous solution, hydrophobic interactions are especially important for the structural stability of biomolecules such as functional proteins and nucleic acids.[4] In this context, some of the most interesting interactions are those found in the hydrophobic core of the coiled coil peptide motifs.[5] Those originate from the formation of a water exclusion surface, but crystallographic[6] and thermodynamic[7] data revealed some structural specificity, due to the so called "knobs into holes" complementarity.

Recently, we have prepared and studied new pseudopeptidic macrocycles with a high potential for self-assembling into columnar structures.[8] Although appropriate crystals could not be obtained for the neutral compounds, upon protonation, they can form crystals suitable for the X ray diffraction analysis.[8a,b] Preliminary data showed that the molecules tend to pack into infinite columnar assemblies of nanometrical section. For such a packing to occur (see Scheme 1) two different levels of hierarchical organisation must occur. First, the self-assembling toward the individual columnar structure using the functional groups of the macrocycle (*i.e.* through H-bonding and aromatic interactions). The second level requires the assembling between the individual tubular structures involving side-chain interactions. In order to study such interactions, compounds **1** are very well suited, as they can be easily modified in a modular way. We thus attempted growing crystals[9] for a series of derivatives bearing the residues more often found in the coiled-coil hydrophobic core.[10]



Scheme 1 Schematic representation of the crystal engineering through non-covalent interactions with pseudopeptidic macrocycles **1a–c**

Accordingly, we prepared the corresponding derivatives (**1a–c**) bearing iPr (Val, **1a**), iBu (Leu, **1b**) and *sec*-Bu (Ile, **1c**) side chains but having exactly the same macrocyclic frame.[8b] With this design, protonation would lead to the formation of similar tubular assemblies in the solid state, decorated on the outer surface by the hydrophobic groups of these amino acids.

We prepared the three HCl salts by dissolving the corresponding free amine macrocycle (5 mg) in MeOH (1 ml) and adding a slight excess of concentrated aqueous HCl. The very slow evaporation of the solution yielded crystals suitable for X-ray diffraction analysis.‡ Interestingly, we observed a very different crystallization behaviour among the compounds of the series. Crystals of the Val derivative (**1a**) appeared several months after the preparation of the sample. However, the Leu counterpart (**1b**) required several weeks, while the Ile (**1c**) only needed a couple of days for the suitable crystallization. These preliminary observations suggested important differences in the intermolecular interactions responsible for the crystal packing of the three systems.

The X-ray analysis of the three crystals showed several structural similarities, as well as key fundamental differences. As commonly found for HCl salts, the three compounds crystallized with a different number of water molecules. The macrocycles are tetraprotonated in

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† CCDC reference numbers 665568, 706282 and 706283. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b821772f

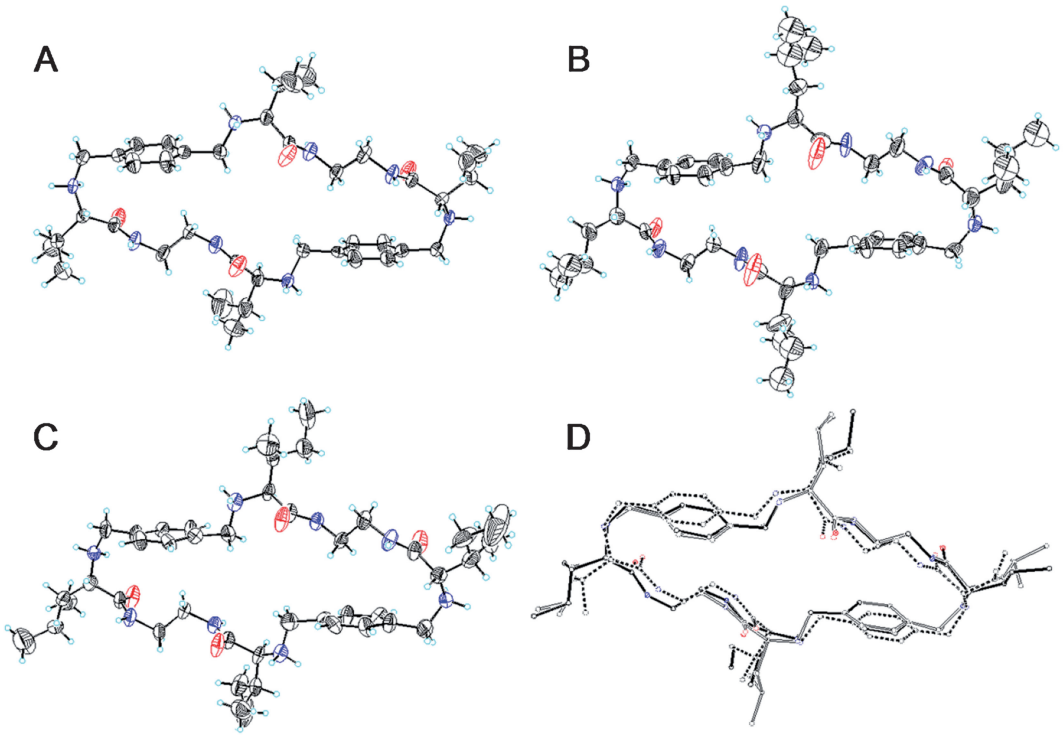

Fig. 1 Molecular structures of the macrocyclic tetracations for: (A) **1a**·4HCl, (B) **1b**·4HCl, (C) **1c**·4HCl, (D) Superimposition of **1a–c**·4HCl.

the crystals. The corresponding structures are shown in Fig. 1. They present an oval shape to minimize electrostatic repulsions between protonated ammonium groups. The elliptically shaped cavities showed very similar dimensions. Thus, measuring the distance (Å) between alternated ammonium nitrogens, we obtain 16.35 (**1a**), 16.04 (**1b**) and 16.09 (**1c**) for the long axis, and 7.46 (**1a**), 6.90 (**1b**) and 6.86 (**1c**) for the short axis. The aromatic *p*-phenylene groups are perpendicular to the macrocyclic main plane. The bis(amide) moieties are in *anti* disposition (with a N–CH_2–CH_2–N torsion angle of 178–180°), setting the amide bond plane also perpendicular to the macrocyclic main plane. This conformation displays H-bonding donor and acceptor sites at both faces of the macrocyclic rings. In all the cases, the side-chains point to the outer part of the ring in a pseudoequatorial position (as initially designed). Resulting from these geometrical similarities, the three different tetracations can be efficiently superimposed (Fig. 1D).

The most interesting features of the crystal structures emerged from the comparison of the corresponding packing within the lattice. In the three compounds, the macrocyclic rings are stacked forming long channels along either the *c* (Val, **1a**) or the *b* (Leu and Ile, **1b–c**) crystallographic axis (Fig. 2a for Val). Those self-assembled structures are stabilized by a complicated intermolecular hydrogen bond pattern. Within this pattern, ammonium groups are H-bound to chloride anions. Amide groups mainly interact with water molecules. This H-bonding network is very similar for the three compounds. Each ammonium nitrogen atom interacts with two different chlorides, one pseudoaxial and one pseudoequatorial with respect to the macrocycle. These interactions also connect two neighbouring columns. Thus, each chloride occupies a pseudoaxial position for one columnar set, and pseudoequatorial for the neighbouring one. The measured N···Cl distances are within the range for electrostatic H-bonding contacts (3.12–3.21 Å). Amide bond planes are parallel to the column axis. Within the columns, the interactions between amide bonds of stacked macrocycles are mediated by water molecules. The N···Ow distances are 2.8–2.9 Å and the C=O···Ow distances are 2.7–2.8 Å. Overall they lead to a distance between stacked rings of about 7 Å. Thus, the stability of the stacked columnar structures is mainly controlled by the electrostatic and H-bonding interactions provided by the water solvated chloride anions.

The main differences between these compounds arose from the inter-packing of the columnar assemblies (Fig. 2b–d). The relative dispositions between the tubes highly depend on the side chain nature. For Leu (**1b**, Fig. 2C) and Ile (**1c**, Fig. 2D), all the molecules have the same orientation, while for Val (**1a**, Fig. 2B), one half of the molecules are rotated 180° and moved to the middle of the *ab* crystallographic plane. This produces a somehow less efficient crystal packing for Val. Additionally, small differences were observed for the isomeric Leu and Ile derivatives. A lower calculated density of the crystal of **1b** (1.081 g cm^{-3}) compared to **1c** (1.135 g cm^{-3}) suggests a better packing for Ile side chains. Moreover, the crystal for the Leu derivative showed some molecular disorder in the conformation of the side chains.§ Overall, the effectiveness of the packing was in the order Ile > Leu > Val. This trend correlates with the differences observed in the rates of the crystals growing, as well as with the relative hydrophobicity of the corresponding amino acids.[10]

Since those intriguing differences must be related to the intermolecular interactions between side chains, we compared those contacts for the three crystal structures. This is shown in Fig. 3, with the side chain groups highlighted in CPK model. For the Val derivative, each side chain interacts with only one side chain (red in Fig. 3A) of a neighbouring macrocycle. In the case of the Leu derivative, a given side chain interacts with two different side chains (green and red in

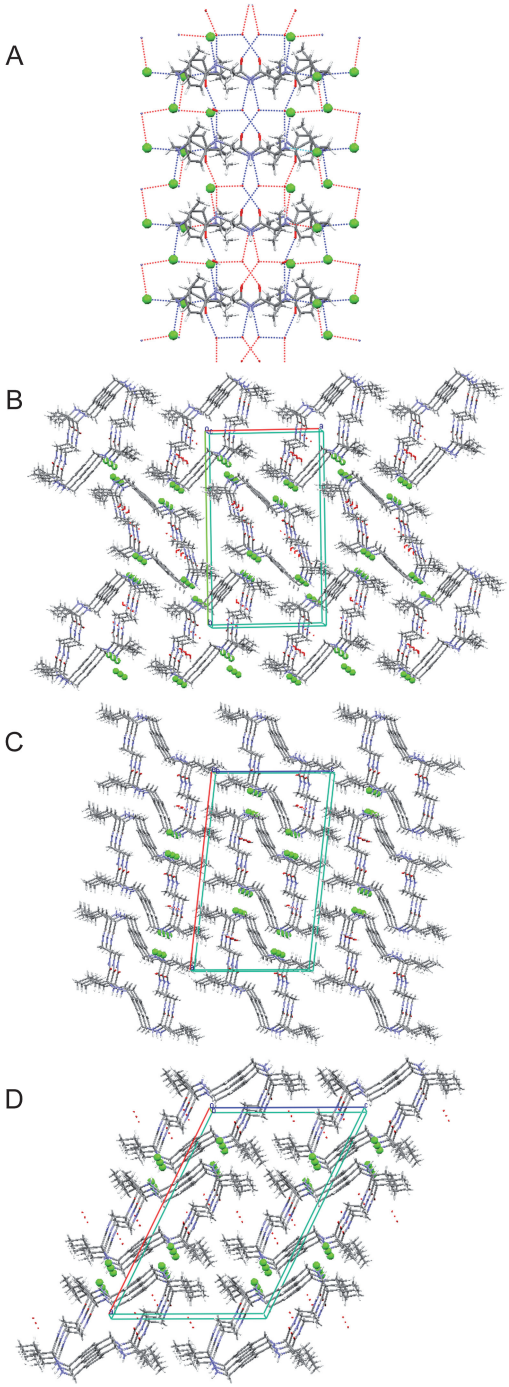

Fig. 2 (A) Columnar packing (side view) for **1a**·4HCl, where H-bonds are shown as dashed lines. (B) Upper view of the columnar packing of **1a**·4HCl, as well as those for (C) **1b**·4HCl and for (D) **1c**·4HCl. Chloride anions are represented as green spheres.

Fig. 3B) of close rings. Finally, the Ile counterpart presents the best packing, as every *sec*-Bu group interacts with three neighbouring side chains (blue, green and red in Fig. 3C).

We further analysed the hydrophobic core for the Ile derivative (**1c**), since this is the one showing the most efficient interactions. The Ile amino acids defining the core set in antiparallel disposition (Fig. 4A). The *sec*-Bu groups are perpendicular with respect to the columns, directing their Cα–Cβ vector towards the centre of the core.

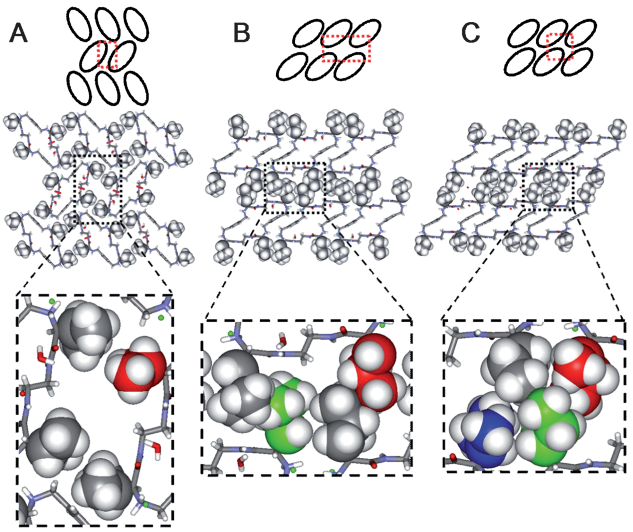

Fig. 3 Schematic (up) and stick (middle) representation of the alignment between columns in (A) **1a**, (B) **1b** and (C) **1c**. In the insets, a magnification of the side chain-side chain contacts is shown (CPK).

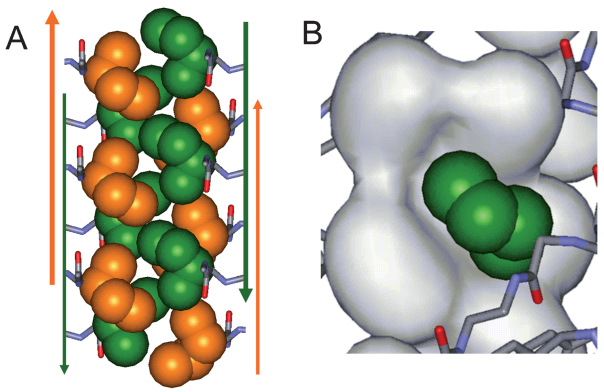

Fig. 4 (A) Representation of the hydrophobic core (CPK, no H-atoms) in compound **1c**·4HCl. The columns have been coloured in green or orange attending to the direction (arrows) of the corresponding Ile amino acid carbonyls. (B) Representation of the "knobs into holes" contacts.

The torsion angles for the side chains are: CO–Cα–Cβ–Cγ ≈ 70° and Cα–Cβ–Cγ–Cδ ≈ 160°. This geometrical relationship produces a perfect interdigitation of the alkyl groups. Thus, every *sec*-Bu side chain displays two "knobs" (corresponding to Cδ and the methyl at Cβ, respectively) which nicely fit into the "holes" formed by the surrounding side chains within the core (Fig. 4B). Following our initial analogy with coiled coil peptides, this hydrophobic core would mimic an antiparallel tetramer with a perpendicular packing mode.[11] As far as we know, this configuration is unprecedented for the crystal packing of a fully synthetic pseudopeptidic structure.

In summary, here we report on the crystal structures of the tetra-HCl salts of three pseudopeptidic macrocycles. The macrocycles pack through an intricate hydrogen bonding pattern establishing a columnar assembly of the rings. Side chain–side chain contacts provide an essential element for the interaction between columns. As a result, the observed packing of the columns correlates with the hydrophobicity of the side chains. Thus, our systems have served to visualize the hydrophobic contacts between side chains, mimicking

the "knobs into holes" interactions present in many natural protein motifs. We hope that our crystal engineering approach could serve as a scaffold to study other non-covalent weak interactions in an easy and straightforward manner.

Acknowledgements

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Notes and references

‡ Crystallographic data for compound **1a** (CCDC-665568): $C_{40}H_{68}N_8O_4^{4+}\cdot 4Cl^-\cdot 4H_2O$, $M = 938.89$, orthorhombic, $a = 15.521(3)$ Å, $b = 26.412(5)$ Å, $c = 6.9655(14)$ Å, $V = 2855.5(10)$ Å^3, $T = 298$ K, space group $P2_12_12$ (No. 18), $Z = 2$, 23532 reflections measured, 8450 unique (Rint = 0.097) which were used in all calculations. Final $wR2 = 0.257$ and $R1 = 0.100$ for data with $I>2\sigma(I)$. Flack-x-parameter −0.02(14).
Compound **1b** (CCDC-706283): $C_{44}H_{76}N_8O_4^{4+}\cdot 4Cl^-\cdot 2H_2O$, $M = 958.96$, monoclinic, $a = 26.413(5)$ Å, $b = 7.1135(10)$ Å, $c = 15.775(3)$ Å, $\beta = 96.299(16)°$, $V = 2946.1(9)$ Å^3. $T = 173$ K, space group $C2$ (No. 5), $Z = 2$, 14292 reflections measured, 5225 unique (Rint = 0.233) which were used in all calculations. Final $wR2 = 0.313$ and $R1 = 0.142$ for data with $I>2\sigma(I)$. Flack-x-parameter −0.3(3).
Compound **1c** (CCDC-706282): $C_{44}H_{76}N_8O_4^{4+}\cdot 4Cl^-\cdot 7H_2O$, $M = 1049.04$, monoclinic, $a = 26.700(5)$ Å, $b = 7.0199(14)$ Å, $c = 17.975(4)$ Å, $\beta = 115.95(3)°$, $V = 3029.4(10)$ Å^3. $T = 173$ K, space group $C2$ (No. 5), $Z = 2$, 9170 reflections measured, 4906 unique (Rint = 0.151) which were used in all calculations. Final $wR2 = 0.310$ and $R1 = 0.113$ for data with $I>2\sigma(I)$. Flack-x-parameter 0.0(2).

§ The terminal atoms of the isobutyl side chains in **1b** are disordered over two positions with site occupation factors of 0.51(1) and 0.63(3) for the major component. The C–C bond lengths had been restrained to 1.54(2) Å and 1–3 distances to 2.5(5) Å.

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