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Towards the design, synthesis and preliminary biological evaluation of potential corono and clipcarbenes: novel *bis*-imidazolium-*bis*-heterocycle macrocyclic ligands

Hiba Hussein^a, Mihayl Varbanov^a, Bertrand Fournier^{b,c} and Florence Dumarçay-Charbonnier^{a,*}^aUniv Lorraine, CNRS, L2CM, F-54000 Nancy, France^bInstitut Galien Paris-Saclay, CNRS UMR 8612, Université Paris-Saclay, 92296 Châtenay-Malabry, France.^cLaboratoire Structures, Propriétés et Modélisation des Solides, CentraleSupélec, CNRS UMR 8580, Université Paris-Saclay, 91190 Gif-sur-Yvette, France.

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ABSTRACT

The synthesis of novel macrocyclic *N*-*bis*-imidazolium-*bis*-heterocyclic carbenes (NHCs) with rigid and constrained structures to improve the stability of their transition metal coordination complexes in order to obtain efficient photosensitizers is reported. Two pathways have been developed for the preparation of coronacarbenes (**L1**-**L3**) containing bipyridine or bithiazole groups. Flexible hemi-macrocycles with potential as clipcarbenes (**L1'**-**L3'**) are also described. The crystal structures of ligands **L1** and **L3** are detailed and the preliminary biological evaluation of **L1**-**L3** is presented.

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Introduction

Since the first crystal structure reported by Arduengo and co-workers in 1991 [1], *N*-heterocyclic carbenes (NHCs) and their metal complexes have garnered extensive research attention with subsequent broadening of their applications [2]. Most of these supramolecular architectures have been developed for their photoactivable properties as potential photosensitizers (PS) in several fields such as dynamic phototherapy [3], dye-sensitized photovoltaic cells [4], [5] and catalysis [6]. However, only a few studies have focused on the preparation of structures combining both a macrocycle and an imidazolium, and on their metal coordination complexes. Among them, macrocyclic tetra-NHC ligands have been reported with structures similar to porphyrins, showing better electronic transfers to the transition metal due to the presence of the NHC units [7], [8]. Smaller macrocycles containing azolium rings have also been described. In this case, the NHCs embedded in the macrocyclic ring allow synergistic functions and have notably been applied in organometallic catalysis [9].

Moreover, *bis*-heterocyclic ligands have great importance in coordination chemistry, as illustrated by the various symmetric and asymmetric *bis*-heterocyclic cryptands and coronands described in the literature [10]. These compounds and their

complexes display notable photochemical properties (e.g. luminescence, antenna effect) due to their strong chelating donor heteroatom coupled with a delocalized π electron system [11].

Our aim was therefore to combine NHCs and *bis*-heterocyclic entities, and thus their associated photophysical properties, into new organized supramolecular structures: *N*-*bis*-heterocyclic carbene macrocycles.

Hence, we describe two pathways for the synthesis of a novel family of macrocyclic imidazolium salts covalently bearing two imidazolium moieties - as NHC precursors - and two *bis*-heterocyclic units (bipyridine or bithiazole). Our expectation for such structures is to obtain good photophysical properties due to the σ -donor capacity of the imidazolylidene units and the π -conjugated system represented by the *bis*-heterocycle which could absorb light through its π - π^* transitions. These macrocyclic carbenes should be able to ensure the absorption of photons in the visible range. This way, they will have a wider spectral absorbance window, leading to a better photoactivation efficiency as expected from high-performing photosensitizers [12].

We also report the synthesis of new flexible *N*-*bis*-imidazole-*bis*-heterocyclic (**L1'**-**L3'**) and *N*-*bis*-imidazolium-*bis*-heterocyclic (**L1''**-**L3''**) ligands, obtained during the preparation of the above-mentioned macrocycles. 1D and 2D NMR spectroscopy and mass

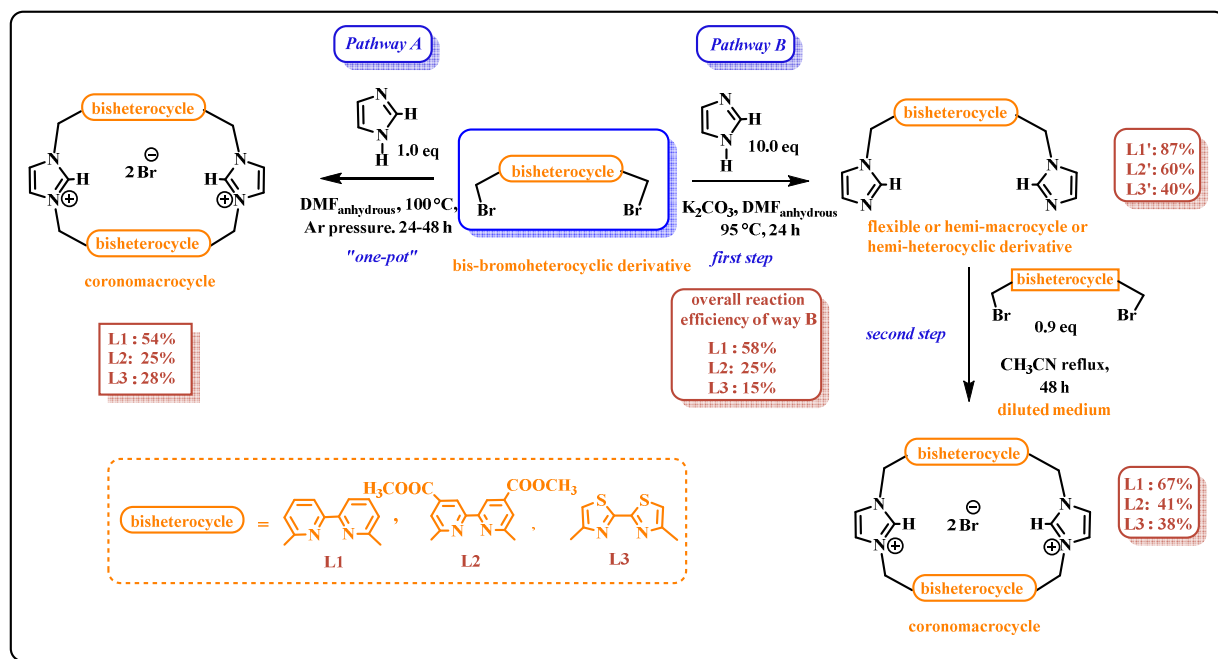
spectrometry characterization of each new compound are provided in the ESI. In addition, the molecular structures of two of the macrocyclic salts, *N*-bis-imidazolium-bis-bipyridine **L1** and *N*-bis-imidazolium-bis-bithiazole **L3**, obtained by single-crystal X-ray diffraction are described.

In parallel, preliminary biological evaluation of macrocycles **L1-L3** was conducted. The results obtained from the cytotoxicity and anti-bacterial studies are presented herein and the biological procedures are provided in the ESI.

Results and Discussion

Synthesis of homoleptic bis-imidazolium-bis-heterocyclic macrocycles

The preparation of bis-imidazolium-bis-heterocycle macrocycles requires the prior synthesis of bis-(bromomethyl)-bis-heterocycles [10], [11], [13] as substrates. Therefore, macrocycles (**L1-L3**) possessing bipyridine, bipyridine bis-ester and bithiazole units were prepared *via* two synthetic pathways for cyclization (Scheme 1).

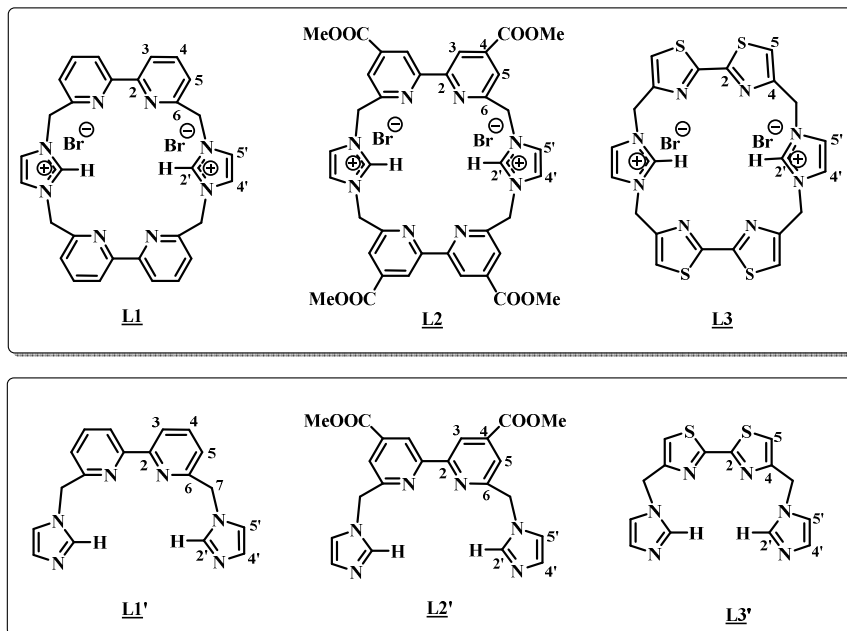


Scheme 1. Synthetic pathways towards hemi- and macro-bis-heterocycles.

The first synthetic pathway (pathway A) is a one-pot cyclization using a stoichiometric mixture of imidazole and the dibromide derivative, in dimethylformamide (DMF) as solvent. The reaction is performed in a Schlenk type reactor over a period of 24 to 48 hours at 100 °C under argon pressure. The bis-imidazolium-bis-heterocyclic-bromide macrocycles **L1-L3** are obtained with reasonable yields.

The second synthetic pathway (pathway B) is a two-step macrocyclization procedure. The first step consists of synthesizing the hemi-heterocyclic derivatives (**L1'-L3'**) using the method described by Benoît and co-workers [14] with a 10 equivalent excess of imidazole for 1 equivalent of the dibromide derivative in a minimum of solvent (anhydrous DMF) in the

presence of 4 equivalents of K₂CO₃ at 95 °C for 24 hours. Simple extraction allows the hemi-heterocycles (**L1'-L3'**) to be isolated with good yields. The second step consists of the cyclization process and is carried out under high dilution conditions in order to favour the intramolecular reaction. The reaction is performed using 1 equivalent of the hemi-heterocyclic derivative (**L1'-L3'**) to 0.9 equivalents of the corresponding bis-bromoheterocyclic derivative. Simple extraction with water and dichloromethane allows removal of the unreacted dibromide derivative. The expected bis-imidazolium-bis-heterocycle bromide salt **L1-L3** is then dissolved in the aqueous phase and freeze-dried. **L1-L3** are obtained pure without any further purification with good yields.



Scheme 2. Representation of the homoleptic *bis*-imidazolium-*bis*-heterocyclic macrocycles **L1**- **L3** (top) and flexible *bis*-imidazole-*bis*-heterocycles **L1'**, **L2'** and **L3'** (bottom).

The NMR 1D (¹H and ¹³C) and 2D (HMBC, HSQC) spectroscopy and mass spectrometry of **L1-L3** and **L1'-L3'** are detailed in the ESI.

The two pathways A and B described in Scheme 1, show the successful synthesis of three homoleptic bis-heterocyclic macrocycles **L1-L3**. Pathway A affords the desired macrocycles in only one step under pressure and high temperature. The reaction time is faster (between 24 to 48 hours) compared to pathway B (24 hours for the first step and 48 hours for the second step). The main difference between the two pathways lies in the purification step. In pathway A, purification on silica chromatography is necessary. Due to their high polarity, purification of the *bis*-imidazolium-*bis*-heterocycle cationic macrocycle requires a polar acetone/water/KNO₃ (saturated) eluent. However, only a fraction of the macrocycle is recovered and the rest remains attached to the column even if the eluent is very polar. Reverse phase column purification did not result in any improvement.

In contrast, in the first step of pathway B, extraction using a mixture of dichloromethane/water is sufficient to recover the hemi-macrocycle (**L1'-L3'**) from the organic phase. The excess imidazole remains in the aqueous phase. During the second step

of pathway B, extraction with the same solvents (dichloromethane/water) is also sufficient to recover the ligand (**L1-L3**) from the aqueous phase without the need for further purification, which simplifies the procedure.

X-Ray crystallography

Pure crystals of ligands **L1** and **L3**, obtained by slow evaporation in a CH₂Cl₂/MeOH solvent mixture, were analysed by single-crystal X-ray diffraction. Crystallization attempts by slow evaporation were also made with ligand **L2** but failed to provide suitable crystals. Details regarding data collection and processing are provided in the ESI.

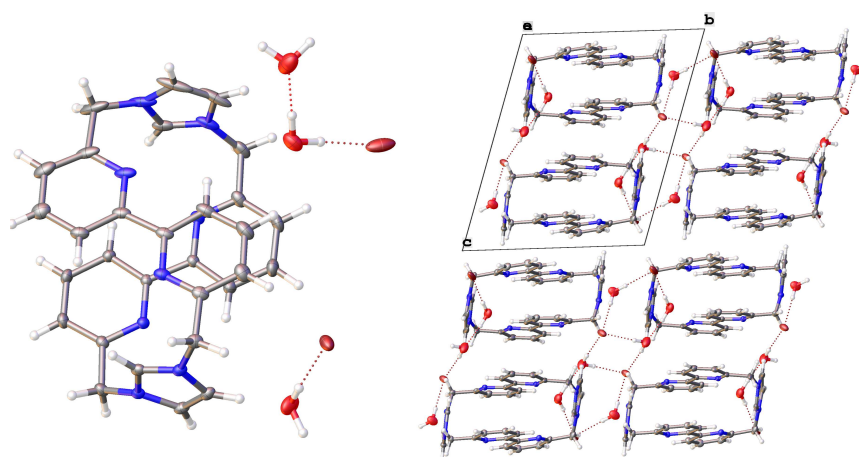


Figure 1. X-Ray crystallographic structure of ligand **L1**.

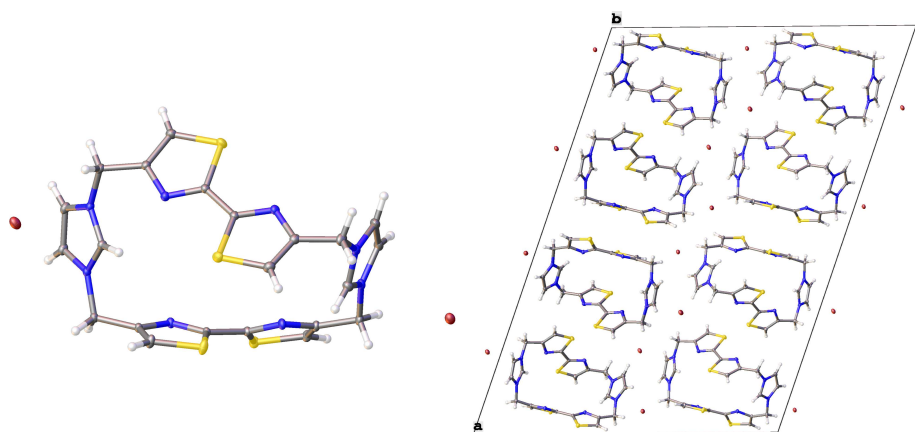
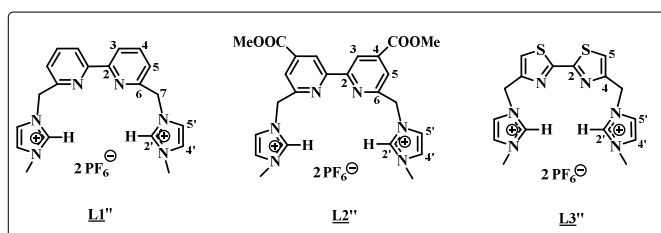


Figure 2. X-Ray crystallographic structure of ligand **L3**.

Synthesis of methyl-*bis*-imidazolium-*bis*-heterocycles

In order to obtain new NHC pincer ligands (or flexible compounds) from *bis*-imidazole-*bis*-heterocycles **L1'**-**L3'**, it is necessary to quarternize the nitrogen of the imidazole ring. The preparation of methyl *bis*-imidazolium-*bis*-heterocycle hexafluorophosphates **L1''**-**L3''** consisted of a conventional methylation reaction using methyl iodide in DMF at 90 °C overnight. After cooling to room temperature, the reaction mixture is precipitated as a hexafluorophosphate salt using a saturated KPF₆ solution. The obtained yields range from 83-90% and their structures are represented in Scheme 4. Kuhn and co-workers [18] also described the synthesis of the **L1''** hexafluorophosphate ligand in 80% yield by a direct synthesis route using 1 equivalent of 6,6'-*bis*-(bromomethyl)-2,2'-bipyridine in the presence of 5 equivalents of 1-methylimidazole.



Scheme 4. Structures of the methyl *bis*-imidazolium-*bis*-heterocycles hexafluorophosphate **L1''**-**L3''**.

NMR (1H and 2D) spectroscopy and mass spectrometry were used to determine the structures of the new pincer ligands **L1''**, **L2''** and **L3''** and can be viewed in the ESI.

Biological evaluation

Preliminary biological evaluation of the macrocycles (**L1**-**L3**) showed encouraging results. All three macrocycles display varying degrees of cytotoxicity when tested at 100 µg/mL on MRC-5 cells, expressed as the cytotoxic concentration (CC). Macrocycle **L1** is the most cytotoxic compound, followed by **L2**, whereas **L3** is the least cytotoxic against the MRC-5 cells, with cell viabilities of 89%, 95% and 98%, respectively. Macrocycles **L1**-**L3** display varying antimicrobial activity as measured by their minimal inhibitory concentrations (MIC) (Table 1) [19]. Only **L1** shows noteworthy antibacterial activity (MIC ≤ 256 µg/mL) and is the most active against Gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis* (MIC = 64 µg/mL). Furthermore, **L1** shows antibacterial activity against the Gram-negative bacteria *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, where both pathogens have the same degree of susceptibility (MIC = 256 µg/mL). In contrast, **L1** is slightly more active against the Gram-negative pathogen, *Escherichia coli* (MIC = 128 µg/mL). This observation is compelling, in terms of the resistance of Gram-negative bacteria, due to the presence of lipopolysaccharides in their outer membrane. Our results show that **L1** has a selectivity index (SI) value (SI = CC/MIC) greater than 1 for two of the screened pathogens *Staphylococcus aureus* and *Enterococcus faecalis* (Table 1).

Table 1. Antimicrobial activity (minimum inhibitory concentration, MIC) and selectivity index of **L1**, **L2**, **L3** for five pathogenic bacteria. The cytotoxic concentration (CC) used for calculation of the SI is 100 µg/mL, allowing the survival of more than 50% of the host cells. The values in bold are considered as noteworthy (MIC ≤ 256 µg/mL, SI > 1). Sa = *Staphylococcus aureus*, Ef = *Enterococcus faecalis*, Pa = *Pseudomonas aeruginosa*, Ec = *Enterobacter cloacae*; Ec = *Escherichia coli*; Kp = *Klebsiella pneumoniae*.

Compound	MIC (µg/mL)					SI (CC/MIC)				
	Sa	Ef	Pa	Ec	Kp	Sa	Ef	Pa	Ec	Kp
L1	64	64	256	128	256	1.56	1.56	0.39	0.78	0.39
L2	≥ 256	≥ 256	≥ 256	≥ 256	≥ 256	≤ 0.39	≤ 0.39	≤ 0.39	≤ 0.39	≤ 0.39
L3	≥ 256	≥ 256	≥ 256	≥ 256	≥ 256	≤ 0.39	≤ 0.39	≤ 0.39	≤ 0.39	≤ 0.39

A selectivity index value greater than 1 indicates that the compound is more toxic to the pathogenic bacteria than to human cells. The greater the selectivity index value, the safer the compound is for the host.

Conclusion

We have described the synthesis of novel homoleptic *bis*-imidazolium-*bis*-heterocycle macrocycles with bipyridine or bithiazole units (**L1-L3**), as well as interesting reaction intermediates: *bis*-imidazole-*bis*-heterocyclic compounds (**L1'-L3'**), and methyl-*bis*-imidazolium heterocyclic derivatives (**L1''-L3''**). The targeted applications of these compounds would be in biomedical research, most notably for antimicrobial photodynamic therapy (aPDT). It is also important to note that these new macrocycles are soluble in water. They have also shown promising bioactivities (cytotoxicity and anti-bacterial activity). Complexation studies *via* the formation of their carbenes in the cases of ligand **L** and **L''** with transition metals is currently under way.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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This manuscript is dedicated to Pr. Alain Marsura

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Graphical Abstract

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Towards the design, synthesis and preliminary biological evaluation of potential coronoids and clipcarbenes: novel *bis*-imidazolium-*bis*-heterocycle macrocyclic ligands

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