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

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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 coronocarbenes (**L1-L3**) containing bipyridine or bithiazole groups. Flexible hemi-macrocycles with potential as clipcarbenes (**L1″-L3″**) are also described. The **c**rystal structures of ligands **L1** and **L3** are detailed and the preliminary biological evaluation of **L1-L3** is presented.

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 into 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 noticeable 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 macrocyclic imidazolium salt family 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 compounds, *N*-*bis*-imidazole-*bis*-heterocyclic (**L1′-L3′**) and *N*-*bis*-imidazolium-*bis*-heterocyclic (**L1″-L3″**) ligands, obtained during the preparation of

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the above-mentioned macrocycles. 1D and 2D NMR spectroscopy and mass spectrometry characterization of each new compound are provided. In addition, the molecular structures of two of the macrocyclic salts, the *N-bis*-imidazolium-*bis*-bipyridine **L1** and the *N-bis*-imidazolium-*bis*-bithiazole **L3**, obtained by single-crystal X-ray diffraction are described.

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

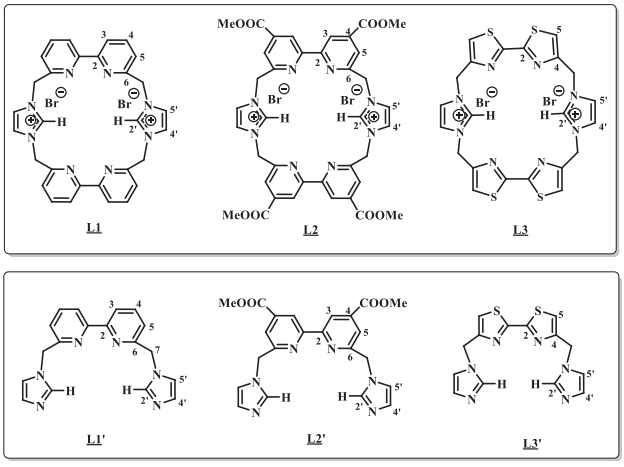
Results and discussion

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).

The first synthetic pathway (pathway A) is a one-pot cyclization. This method uses a stoichiometric mixture of imidazole and dibromide derivative, in dimethylformamide (DMF) as solvent. The reaction is performed in a Schlenk type reactor over a period of 24 to 48 h with solvent 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 an excess of imidazole (10 equivalents) for 1 equivalent of the dibromide derivative in a minimum of solvent (anhydrous DMF) and in the presence of 4 equivalents of (K_2CO_3) at 95 °C for 24 h. Simple extractions permit to isolate the hemi-heterocycles (**L1′-L3′**) with good yields (Scheme 2). The second step consists in the cyclization process and is carried out in high dilution condition in order to favour

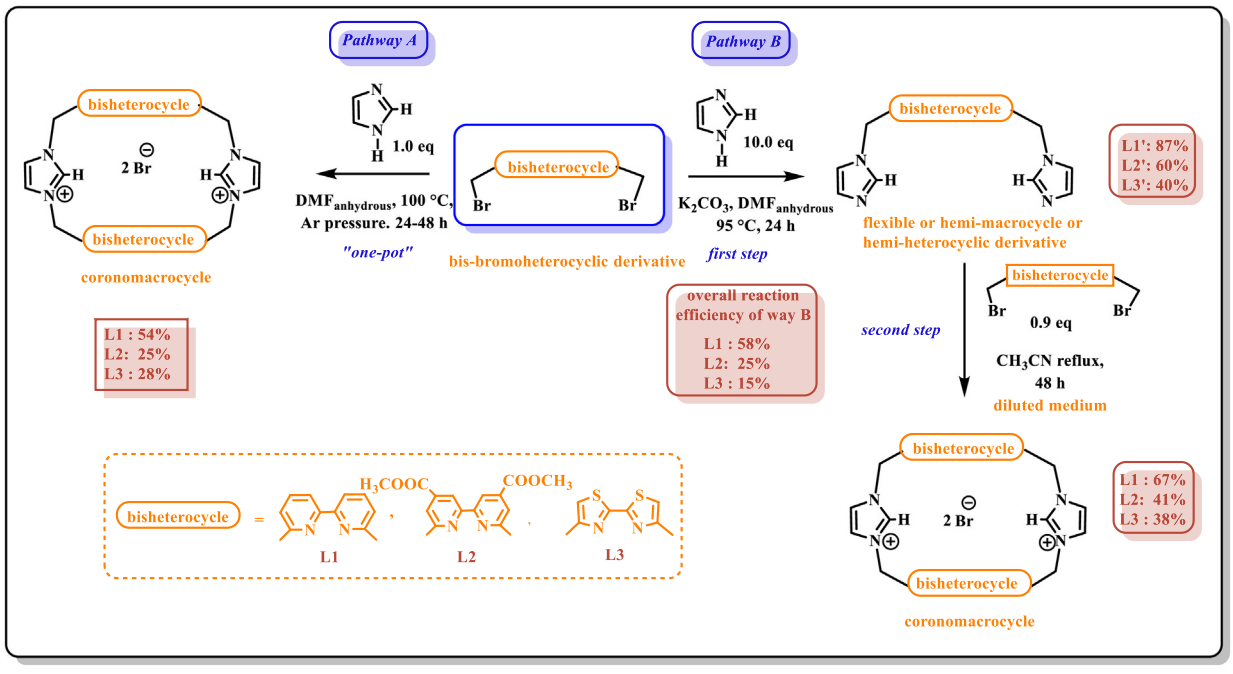


Scheme 2. Representation of the homoleptic *bis*-imidazolium-*bis*-heterocyclic macrocycles **L1**- **L3** (top) and flexible *bis*-imidazole-*bis*-heterocycles **L1′**, **L2′** and **L3′** (bottom).

the intramolecular reaction. The stoichiometry is then 1 equivalent of the hemi-heterocyclic derivative (**L1′-L3′**) to 0.9 equivalent of the homoleptic corresponding *bis*-bromoheterocyclic derivative (second step pathway B, Scheme 1). Simple extractions with water and dichloromethane permit to remove the unreacted dibromide derivative. The expected *bis*-imidazolium-*bis*-heterocycle bromide salt **L1-L3** is then dissolved in the aqueous phase and is finally freeze-dried. **L1-L3** are obtained pure without any further purification with good yields.

The NMR 1D (^{1}H and ^{13}C) 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 on Scheme 1, show the successful synthesis of three homoleptic *bis*–heterocyclic macrocycle **L1-L3**. The pathway A is an original way and give, in only one step, the desired macrocycle under pressure and high temperature. The reaction time is faster (between 24 and 48 h) compared to



Scheme 1. Synthetic pathways to hemi- and macro-*bis*-heterocycles.

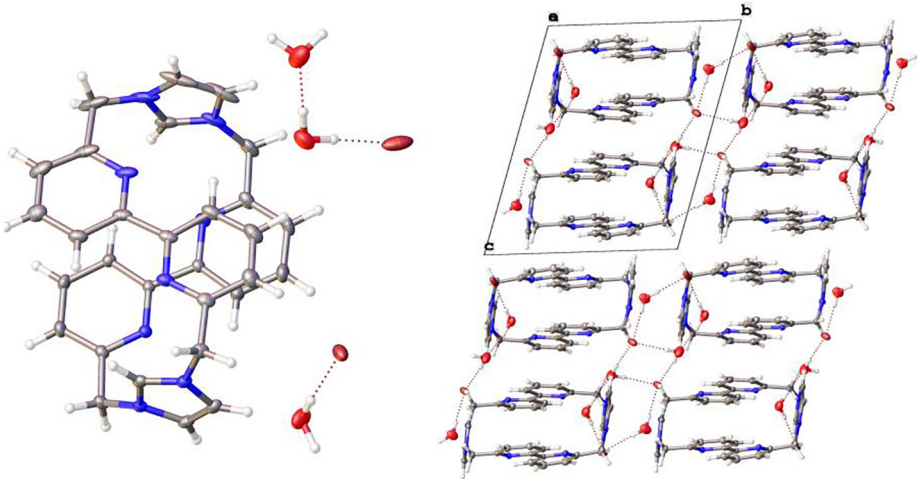


Figure 1. X-Ray crystallographic structure of ligand **L1**.

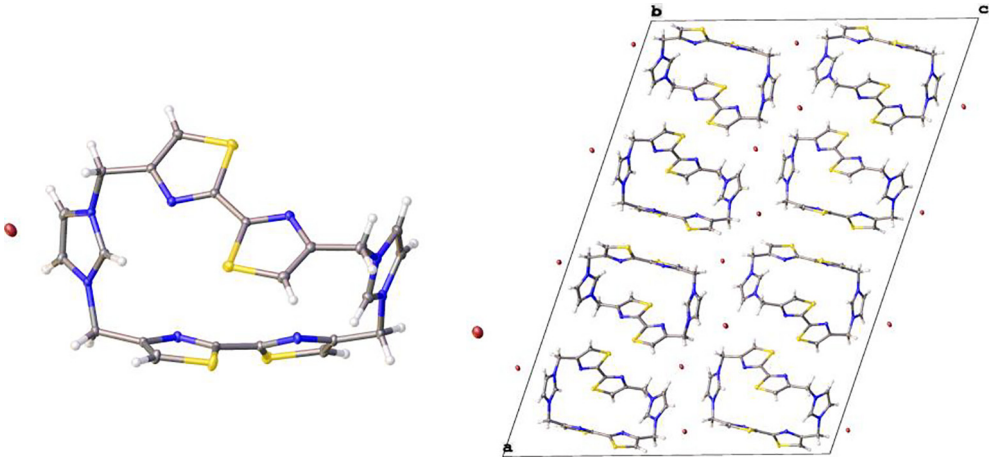
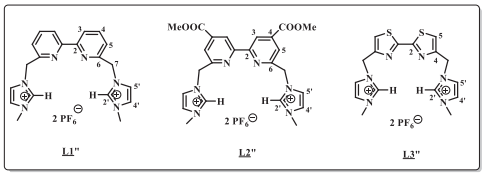


Figure 2. X-Ray crystallographic structure of ligand **L3.**



Scheme 3. Structures of the methyl *bis*-imidazolium-*bis*-heterocycles hexafluorophosphate **L1″-L3″**.

pathway B (24 h for the first step and 48 h 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. Indeed, due to their high polarity, purification of the *bis*-imidazolium-*bis*-heterocycle cationic macrocycle requires a polar acetone/water/KNO_3 (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. A reverse phase column purification did not result in any improvement.

In contrast, in pathway B, extraction in a mixture of dichloromethane/water is sufficient to recover during the first step, the hemi-macrocycle (**L1′-L3′**) in organic phase. The excess of imidazole is eliminated in aqueous phase. During the second step of the pathway B, extraction with the same solvents (dichloromethane/water) is also sufficient to recover the pure ligand (**L1-L3**) in the aqueous phase without further purification, which simplifies the procedure.

X-Ray crystallography

Salt crystals of ligands **L1** and **L3**, obtained by slow evaporation in a CH_2Cl_2/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 [15–17] (Figs. 1 and 2).

Synthesis of methyl-bis-imidazolium-bis-heterocycle

In order to obtain new NHC pincer ligands (or flexible compounds) from *bis*-imidazole-*bis*-heterocycles **L1′-L3′**, it is necessary to quarternarize 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 KPF_6 solution. The obtained yields are good and range from 83 to 90%. Their structures are represented in Scheme 3. Kuhn and co-workers [18] also

Table 1
Antimicrobial activity (minimum inhibitory concentration, MIC) and selectivity index of **L1**, **L2**, **L3** on 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 greater than 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.

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-methyl-imidazole.

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 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). The macrocycle **L1** is the most cytotoxic compound, followed by **L2**, whereas **L3** is the least toxic 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 another Gram-negative pathogen, *Escherichia coli* (MIC = 128 μg/ mL), yet remaining in the same range of antibacterial activity. 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).

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 bioactivity (cytotoxicity and antibacterial activity were tested). 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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Appendix A. Supplementary data

Crystallographic data for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC: 2,062,288 for Ligand **L1** and 2,062,252 for Ligand **L3.** Supplementary data (experimental procedures and characterization data for new compounds) to this article can be found online at https://doi.org/10.1016/j.tetlet.2021.153288.

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