Discoveries from a Phenanthroline-based Dynamic Combinatorial Library: Catenane from Copper(I) or Copper(II) Template?

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We report here a DCL study of a phenanthroline-based building block focusing on catenane formation with copper templates. Two [2]catenanes have been amplified by using Cu⁺ as template from the DCLs that contains no interlocked compound in the absence of copper. In addition, an unexpected Cu²⁺ template effect on the [2]catenane formation was discovered. The observed Cu²⁺ template effect was found to be originated from the in situ reduction of the divalent metal to Cu⁺.

Introduction

Dynamic combinatorial chemistry (DCC),¹ a selection approach of assembling molecular building blocks under thermodynamic control through reversible linkages, has been demonstrated as an efficient strategy for the discovery of unexpected yet successful molecular receptors,² biological ligands/inhibitors³ or complex molecular topologies,⁴ whose structures and syntheses could be unpredictable and/or unattainable. For example, Sanders and co-workers have recently reported a series of elegant examples of complex interlocked architectures including catenanes,⁵ Solomon link⁶ and trefoil knots,⁷ that are otherwise unpredicted and extremely challenging to be synthesized, from aqueous disulfide dynamic combinatorial libraries (DCLs) using simple linear building blocks that can engage in aromatic donor-acceptor and hydrophobic interactions. If molecules having structures that are predesigned to favour catenane formation are used as building blocks in DCL, complex catenanes and other unexpected but potentially highly complicated molecular topologies could be obtained from this self-directing and self-selecting assembly approach. Since the orthogonal ligand arrangement from the tetrahedral phenanthroline-Cu¹ coordination is one of the most successful preorganization strategies for catenane assembly,⁸ we propose the use of building blocks derived from the phenanthroline ligand will efficiently generate DCLs of interlocked structures plus other complex topologies when templated by Cu⁺, allowing the study of complex molecular topologies under thermodynamically-controlled conditions. We report here our initial study on the use of a phenanthroline-based dialdehyde building block for the generation of dynamic combinatorial libraries of catenanes⁹ using hydrazine as the exchange reaction. The DCL behaviour, amplification of two [2]catenanes by Cu⁺ template and their characterisation are described. We also discovered an unexpected Cu²⁺ template effect on the assembly of the [2]catenanes. Further experiments on the coordination chemistry of model systems revealed that the observed template effect from Cu²⁺ is originated from the Cu⁺ generated by the in situ reduction of the divalent metal ion. This unexpected template effect of Cu²⁺ was also applied in the assembly of catenanes under non-DCL conditions. It is believed that the studies of the Cu⁺-phenanthroline coordination motif under DCL conditions could offer an unique opportunity for studying the assembly of catenane and other possible, but not necessarily predictable or imaginable, complex molecular topologies under thermodynamic control, and eventually unveil the rules governing the self-assembly process of these complicated molecular entities.

Results and discussion

Building blocks designs and synthesis

The structure and synthesis of the phenanthroline-based building block 1 is depicted in Scheme 1. The bidentate phenanthroline 1 is designed to coordinate to Cu⁺ in a tetrahedral geometry and that the terminal aldehydes are for hydrazide exchange with hydrazide building blocks. Two dihydrazide building blocks were synthesized and studied. Dihydrazide 2 has a flexible tetraethyleneglycol linker, whereas 3 contains the π-rich dioxynaphthalene unit for possible recognition with complementary π-deficient aromatics.

DCL studies and Cu⁺-templated catenane formation

The first hydrazone DCL was prepared by dissolving 1 mM each of the building blocks 1 and 2 in CHCl₃/MeOH/CF₃COOH (10:10:1). The library solution was analysed by LCMS after 3 days. The HPLC chromatogram showed that the [1+1]macrocycle M1 is the dominant species in the DCL, and it represents 96% of the library materials with the remaining 4% as the [2+2]macrocycle M2 (Fig. 1)
1). Further analysis of the DCL at later time points (i.e. 5 and 7 days) revealed the same library composition, suggesting that the library has reached thermodynamic equilibrium.

When the DCL was prepared in the presence of 0.5 mM Cu\textsuperscript{+}, a library of a different composition was obtained. Addition of Cu\textsuperscript{+} to the DCL templated the formation of a new species (C1-Cu\textsuperscript{+}), which was not detectable in the metal-free DCL, to 71% at the expense of other library members. Pure sample of C1-Cu\textsuperscript{+} can be obtained by preparative HPLC or flash column chromatography for further analysis (see ESI for details). Results from ESI-MS, HRMS and MS/MS studies on C1-Cu\textsuperscript{+} are consistent with an interlocked structure (Fig. 2). The mass spectrum of C1-Cu\textsuperscript{+} shows peaks at m/z = 571.4 [C1+Cu+3H]\textsuperscript{4+}, 761.5 [C1+Cu+2H]\textsuperscript{3+} and 1141.3 [C1+Cu+H]\textsuperscript{2+} that correspond to the tetra-, tri- and di-cation of C1-Cu\textsuperscript{+} respectively. Fragmentation of the ion at m/z = 1141.3 [C1+Cu+H]\textsuperscript{2+} directly resulted in daughter ions at m/z = 1109.3 and 1171.3 that correspond to [M1+H]\textsuperscript{+} and [M1+Cu]\textsuperscript{+}. The observation that a [2+2] species directly fragment to a [1+1] and other smaller species without any other fragments with m/z larger than that of the [1+1]macrocycle is typical for a catenane, as other topologies such as a [2+2]macrocyle or a molecular figure-of-eight tied by Cu\textsuperscript{+} coordination should produce larger fragments such as those derived from the [2+2] or [2+1] building block combination in the MS/MS experiments.\textsuperscript{4a-c,5,10} Furthermore, to further confirm the catenane topology, the metal-free C1 obtained from treatment of C1-Cu\textsuperscript{+} with NaCN was also analysed by MS and MS/MS. The mass spectrum of the sample showed a peak at m/z = 1132.3 [C1+2Na]\textsuperscript{+} that corresponds to the expected copper free C1. MS/MS analysis of C1 again resulted in only M1 (m/z = 1131.4 [M1+Na]\textsuperscript{+}) and its fragments (m/z = 1088.3, 1040.4, 1013.3 and 987.3) as the only species observed in the MS/MS experiment (Fig. 2). No larger species than that derived from M1 was observed in the tandem mass spectrum, further confirming the catenane topology of C1 and that a [2]catenane was successfully amplified by the Cu\textsuperscript{+} template from the dynamic library upon the anticipated phenanthroline-Cu\textsuperscript{+} coordination. H NMR studies on C1-Cu\textsuperscript{+} revealed multiple sets of overlapped resonances in the aromatic region, suggesting the presence of different inequivalent conformations of the catenane.

Scheme 1. Synthesis of the dialdehyde building block 1, the dihydrazides 2 and 3, and the phenanthroline derivatives 4 and 5.

Scheme 2. Generation of hydrazine DCL from dialdehyde 1 and dihydrazide 2. The [2]catenane C1-Cu\textsuperscript{+} was amplified by Cu\textsuperscript{+} template.
Indeed, the $^1$H NMR spectrum of M1 does show at least six different sets of aromatic signals that are identified as the phenylene protons from the corresponding COSY spectrum (Fig. S19, S20), suggesting that both the syn and anti isomers of the hydrazone are present in M1 (and also most likely in C1-Cu$^+$) that lead to the inequivalent aromatic chemical environments.

**Fig. 1** HPLC analysis of a 1 mM DCLs of 1 and 2 (a) in the absence of template and in the presence of 0.5 mM of (b) Cu$^+$ and (c) Cu$^{2+}$. Absorbance was monitored at 285 nm.

The dynamic nature of the catenane assembly was studied by a series of equilibration experiments. First, instead of adding the Cu$^+$ template at the beginning of the DCL preparation, the metal ion was added to a DCL that has been equilibrated for 3 days. Concentration of C1-Cu$^+$ in the DCL gradually increased from undetectable to ca. 70% with the remaining 30% as M1, which is almost the same as that of the DCL prepared when Cu$^+$ was introduced at the beginning of the DCL evolution (Fig. S28). The observation that the same equilibrium position could be reached from two different starting points suggests that the library is dynamic and is under thermodynamic control. Second, the effect of the building block concentration on the equilibrium position and yield of C1-Cu$^+$ was studied. DCLs with different concentrations of 1 and 2 were setup and their equilibrium compositions were compared. In accord with the Le Châtelier’s principle, an increase in the yield of C1-Cu$^+$ from 36% to 71% to 93% when the building block concentrations were increased from 0.1 mM to 1 mM to 5 mM at a building block/Cu$^+$ ratio of 2:1 (Fig. S26). Third, effect of the Cu$^+$ template to building block ratio was studied. DCLs with 0.1, 0.5, 1 and 2 eq. of Cu$^+$ relative to 1 were prepared. From 0.1 eq. to 0.5 eq. of Cu$^+$, an increase in the yield of C1-Cu$^+$ from 17% to 71% was observed. Further increasing the template concentration to 1 eq. and 2 eq. of Cu$^+$ relative to 1, on the other hand, lead to a decrease of the yield of C1-Cu$^+$ to 68% to 48% respectively (Fig. S27). Presumably, at Cu$^+$ concentration below 0.5 eq., increase in the concentration of Cu$^+$ templates the formation of the 2:1 phenanthroline-Cu$^+$ complex and lead to a higher yield of C1-Cu$^+$. However, further increase in Cu$^+$ concentration could shift the equilibrium towards the 1:1 phenanthroline-Cu$^+$ complex which favors the formation of the macrocycle instead of the [2]catenane.

**Fig. 2** ESI-MS analysis of C1-Cu$^+$ and C1. (a) The MS and (b) MS/MS spectra of C1-Cu$^+$. The MS/MS spectrum was obtained from fragmenting the peak at m/z = 1141.3. (c) The MS of spectrum C1; and (d) the MS/MS spectrum from fragmentation of the peak at m/z = 1132.3. A similar hydrazone DCL was also prepared from 1 and 3 under the same conditions (Scheme S4). The library mixture was analysed by LCMS which showed the [1+1]macrocycle M3 as the only detectable library member. No [2+2]macrocycle similar to M2 was found. Similar to the DCL of 1 and 2, the presence of 0.5 mM Cu$^+$ templates the formation of the [2]catenane C2-Cu$^+$ to 90% (Fig. S26). The interlocked structure of C2-Cu$^+$ and its metal-free form C2 were also characterised by ESI-MS and MS/MS. Fragmentation behaviour...
similar to that of C1-Cu+ and C1 was observed, confirming the catenate topology of C2-Cu2+ (Fig. S34, S35). Since building block 3 is designed with the π-rich dioxynaphthalene core for further molecular recognition with complementary π-deficient unit, we tested if the use of the π-deficient cycloisoparquat(p-phenylene) (CBPQT4+) macrocycle could amplify other interlocked compounds from the DCL. Due to the insolubility of CBPQT4+ under the DCL condition, attempts to use the π-deficient macrocycle as template, in the form of a concentrated MeCN solution, were unsuccessful. LCMS analysis of the filtered library solution revealed the presence of only C2-Cu+ and M3, in a distribution as if the macrocyclic template was not added (Fig. S29). No incorporation of the π-deficient unit to the π-rich library members was observed.

Cu+ templates catenate formation in DCL

In addition to Cu+, other metal ions including Li+, Na+, K+, Mg2+, Ca2+, Al3+, Mn2+, Fe2+, Fe3+, Co2+, Ni2+, Cd2+, Ce3+ and Er3+ were also tested as templates for the DCL of 1 and 2. While these metal ions did not perturb the equilibrium position and the same library compositions as the metal-free DCL were observed (Fig. S25), C1-Cu+ was amplified by addition of Cu2+. While the redox switching of Cu2+/+ has been demonstrated to induce the circumrotation and conformation change of catenanes and other complex topologies with variable coordination geometry and coordination number,13 templating [2]catenate assembly from two bidentate phenanthroline precursors by Cu2+ is not known as far as we are concerned. Compared with the Cu+ template, Cu2+ only amplifies C1-Cu+ to 44% of the library materials. The lower efficiency of Cu2+ to template C1 in the DCL may suggest that (1) Cu2+ coordination to the [2]catenate is thermodynamically less stable than that of Cu+ or (2) the mechanism of Cu2+ template involves not only Cu2+-phenanthroline coordination.

In situ reduction of CuII to CuI

To elucidate the origin of Cu2+ template effect on the [2]catenate assembly, the coordination chemistry of the phenanthroline derivative 4 and 5 to Cu2+ was studied. Complexation of 4 to CuCl2 in 2:1 ratio in CH2Cl2/MeOH at room temperature initially resulted in a yellow solution that gradually turned red. UV-Vis analysis showed that the red species has an absorption maximum at 445 nm which is consistent with the reported MLCT band of CuI-phenanthroline (Fig. 3a).14 The absorption spectrum also resembles to that of the independently synthesized [Cu(4)4][PF6]4 indicating a reduction of Cu2+ to Cu+ upon coordination of 4 under the experimental conditions. Similar CuI to Cu+ reduction has also been observed in cross-coupling reactions involving copper as the catalysts,15 but such effect in molecular self-assembly involving the metal is not known. By comparing the relative absorbance, a ca. 60% conversion of CuII to Cu+ has been estimated, which correlates very well with the observed relative yields (71% vs. 44%) of C1-Cu+ in the corresponding DCLs. The observed in situ reduction is also supported by 1H NMR studies. The red solution obtained from a 2:1 mixture of 4 and CuCl2 in CD3CN showed no paramagnetically shifted resonances due to the d6 CuII, and that the 1H spectrum is consistent with the formation of [Cu(4)4]4+ (Fig. S24).

Presumably, the tetrahedral coordination geometry enforced by the two phenanthrolines modulates the reduction potential of CuII such that Cu2+ could be reduced to Cu+, possibly by protic reagents, upon coordination to the bidentate ligand under the experimental conditions.15,16 X-ray structure of [Cu(4)4][PF6]4 revealed the expected tetrahedral coordination geometry around the metal (Fig. 3a). In addition, the observed π-stacking interaction between the phenanthroline and the aromatic substituents may also reinforce the stability of the tetrahedral coordination motif and facilitate the in situ reduction. Indeed, the complexation reaction of 5 to CuII resulted in a much less efficient reduction to Cu+ as evident by both UV-Vis and 1H NMR studies (Fig. 3b and S24). X-ray diffraction study of the crystalline product obtained from a 2:1 mixture of 5 and CuCl2 showed only the 5-coordinated [Cu(5)Cl3] with a distorted square pyramidal structure (Fig. 3b). The formation of such 1:1 complex suggests that Cu2+phenanthroline coordination alone is not sufficient to explain the observed amplification of the [2]catenate in the DCL because the required preorganized ML2 complex could not be formed. Together with the UV-Vis and NMR data, it is believed that the reduction of CuII to Cu+, and the subsequent formation of the tetrahedral ML2 complex constitute the major part of the observed template effect.

Fig. 3 UV-Vis spectra of a 2:1 complex mixture of the phenanthroline ligands (a) 4 and (b) 5 with Cu+ (red) and Cu2+ (blue). The absorption band at 445 nm is assigned as the CuI-phenanthroline MLCT.15

Fig. 4 Molecular structures of (a) [Cu(4)4]4+ (the PF6 anion is omitted for clarity) and (b) [CuI(5)Cl3]. Selected bond lengths (Å) and angles (deg) for [Cu(4)4]4+: Cu1–N1 2.029(2), Cu1–N2 2.038(3), Cu1–N3 2.048(3), Cu1–N4 2.032(3), N1–Cu1–N2 82.1(8), N3–Cu1–N4 82.2(7), N1–Cu1–N3 123.5(7), N2–Cu1–N4 132.8(8); for [CuI(5)Cl3]: Cu1–N1 2.048(3), Cu1–N2 2.038(3), Cu1–N3 2.048(3), Cu1–N4 2.032(3), N1–Cu1–N2 82.1(8), N3–Cu1–N4 82.2(7), N1–Cu1–N3 123.5(7), N2–Cu1–N4 132.8(8); for [CuI(5)Cl3]: Cu1–N1 2.048(3), Cu1–N2 2.038(3), Cu1–N3 2.048(3), Cu1–N4 2.032(3), N1–Cu1–N2 82.1(8), N3–Cu1–N4 82.2(7), N1–Cu1–N3 123.5(7), N2–Cu1–N4 132.8(8); for [CuI(5)Cl3]: Cu1–N1 2.048(3), Cu1–N2 2.038(3), Cu1–N3 2.048(3), Cu1–N4 2.032(3), N1–Cu1–N2 82.1(8), N3–Cu1–N4 82.2(7), N1–Cu1–N3 123.5(7), N2–Cu1–N4 132.8(8)

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Two [2]catenanes have been amplified in high yields by using Cu⁺ as the template (58%), a [2]catenane under construction by increasing the library complexity are currently alternative templates for a n assembly event. Further studies of the secondary amine by NaBH₄. Albeit in a lower yield than if Cu⁺ is a aid analysis, the dynamic imine was reduced to the corresponding with phenanthroline-based building blocks. The interlocked structure of C₃-Cu⁺ has been confirmed by NMR, ESI-MS and MS/MS (Fig. S22, S23, S36, S37).

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**Notes and references**


11 Attempt to reverse the library composition from the catenane to the macrocycle by removal of the Cu⁺ template was not tested because of the incompatibility of common demetallating reagents such as CN⁻ with the acidic DCL condition.


