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# Efficient syntheses of artificial nucleases containing mono-, di- and tri-[12]aneN$_3$ ligating units through click chemistry

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ABSTRACT

Mono-, di-, and tri-nucleating [12]aneN$_3$ ligands **3**–**5** have been efficiently prepared through the copper-mediated click reactions between propargyl modified [12]aneN$_3$ **2** and mono-, bis-, and tri-azido compounds. Their corresponding zinc(II) and copper(II) complexes showed excellent catalytic activity and strong synergetic effects in the cleavage of RNA model phosphate diester.



The development of artificial nucleases capable of mimicking the action of nature enzymes is a long-standing goal in bioorganic chemistry [1–6]. These studies will offer many applications, such as in understanding the chemistry of the natural nucleases, providing valuable tools in the manipulation of DNA, and developing the drugs for gene diagnosis and treatments. According to the active structures of the natural enzymes, much attention has been paid to the design and preparation of synthetic metallonucleases where metal ions complexed to suitable organic ligands perform their catalytic function in the cleavage of phosphodiester bonds [7–12]. Ligands capable of coordinating two or more metal ions play important roles in the design of artificial nucleases due to the following advantages of multinuclear complexes: 1) by double Lewis acid activation of a phosphate; 2) through bifunctional catalysis in which the metal ions activate the phosphate and supply a metal-coordinated hydroxide acted as nucleophile or base; 3) as an electrostatic reservoir of positive charge to interact with the anionic phosphate to stabilize the transition state for the phosphoryl transfer reaction; 4) by assisting the departure of the phosphate's leaving group through coordination. However, syntheses of these ligands are often expensive and time-consuming. To develop an efficient method in the preparation of multi-nucleating ligands is always desirable.

As a modular synthetic approach, click chemistry has been explosively applied in all areas of modern chemistry from drug discovery to material science in recent years [13–15]. This method refers to a Cu(I) catalyzed Huisgen 1,3-dipolar cycloaddition of azides and alkynes, which provides 1,4-disubstituted 1,2,3-triazole ring in high yield without the need for further purification, without generating side reactions and proceeding under friendly conditions, in aqueous media at room temperature. On the other hand, [12]aneN$_3$ unit is a very useful building block in the design and construction of artificial nucleases due to its facial tridentate coordinating ability. The systems containing one or more [12]aneN$_3$ units have shown prominent results in the detailed mechanism studies and excellent catalytic performance [16–20]. Recently we found that the dinuclear zinc(II) complex of 1,3-*bis*-[12]aneN$_3$-propane accelerates the cleavage of model phosphate diesters by a remarkable factor of $10^{12}$-fold, when compared to the background reactions [21,22].

With the above consideration, we report here on the syntheses and characterization of artificial nucleases containing mono-, di- and tri-nucleating [12]aneN$_3$ ligating units through click reactions between propargyl modified [12]aneN$_3$ and various azides.

Our syntheses started from tri-*tert*-butyl-3-hydroxy-1,5,9-triazacyclododecane-1,5,9-tricarboxylate **1**, which was prepared according to the literature reported by Lönnberg [16]. The synthetic route of the propargyl modified [12]aneN$_3$ compound, tri-*tert*-butyl-3-(prop-2-ynyloxy)-1,5,9-triazacyclododecane-1,5,9-tricarboxylate **2** is outlined in Scheme 1. The reaction of **1** with propargyl bromide in freshly distilled anhydrous DMF smoothly afforded **2** in 96% yield in the presence of sodium hydride at room temperature [23].

The mono-, bis-, and tri-azido compounds used for the following click reactions were prepared from the corresponding bromides following literature methods [24–26]. Among them, ethyl azide was not isolated but directly used in the click reaction.

The click reactions between **2** and the mono-, di- and tri-azide were processed smoothly and efficiently in DMF–$H_2O$–THF mixture

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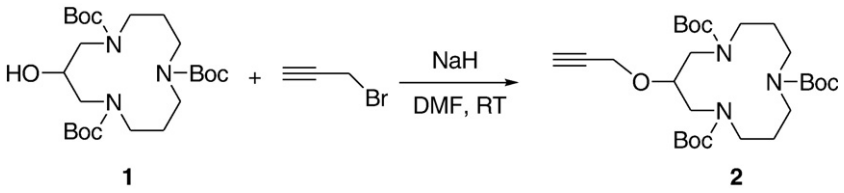
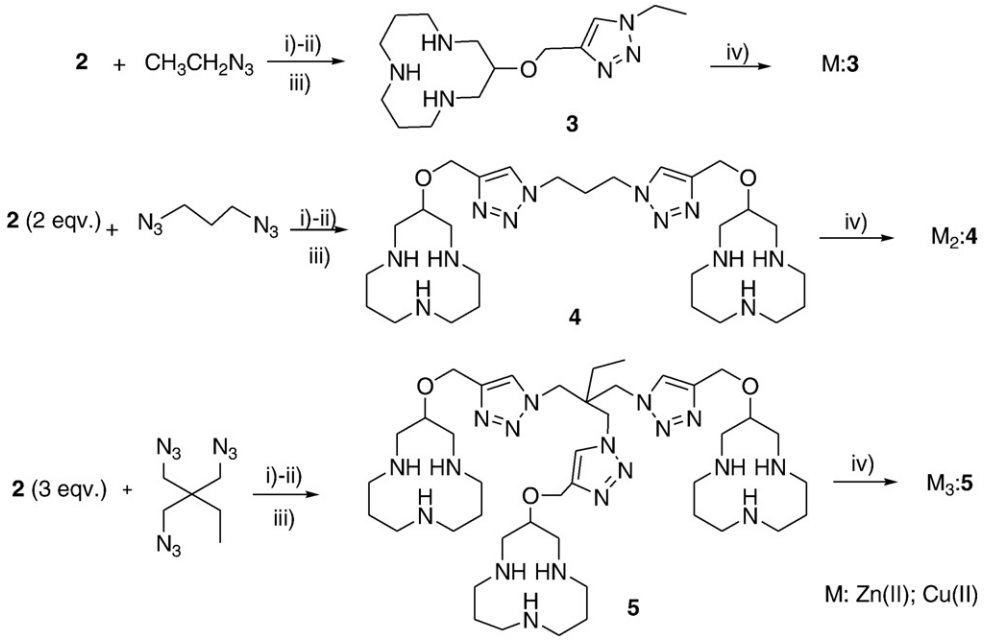



**Scheme 1.** Preparation of the propargyl modified [12]aneN$_3$.

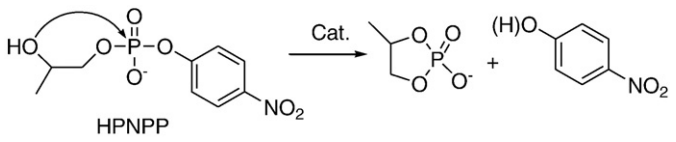



**Scheme 2.** Reagents and conditions: i) VcNa, $CuSO_4 \cdot 5H_2O$, DMF–$H_2O$–THF, RT, 3–4 h; ii) MeOH, HCl(aq.), reflux, 6 h; iii) NaOH (10 M), 0.5 h; iv) $Zn(OTf)_2$ or $Cu(OTf)_2$.

(volume ratio 4:3:3) in the presence of hydrated copper(II) sulfate and sodium ascorbate at room temperature under $N_2$ (Scheme 2) [27]. The reactions were monitored by TLC and stopped when the starting material was found disappearing. After the treatment with saturated $NH_4Cl$ aqueous solution, the resulted Boc-protected triazole compounds were isolated through flash chromatography on silica gel with the yields of 80–86%, they were not completely dried but directly used in the next step. The removal of Boc protecting groups was processed in refluxing methanol in the presence of concentrated HCl for 6 h. The resulted hydrochloride salts were then neutralized with sodium hydroxide (10 M) to afford the free ligands **3**–**5**.

The three new ligands were characterized with $^1$H NMR, $^{13}$C NMR, and high resolution mass spectra [28]. It can be seen that the protons on the triazole moieties generally appear at 7.50–7.60 ppm in singlet, the protons from CH—O—$CH_2$ moieties between triazole and [12]aneN$_3$ units appear at 3.66 (multiplet) and 4.70 (singlet) ppm, respectively. The protons from methylene groups of [12]aneN$_3$ generally appear around 1.60, 2.50, and 2.80 ppm as multiplet, which are consistent with those reported in the literature [16].

The corresponding mono-, di-, and tri-nuclear zinc(II) and copper (II) complexes were prepared in situ following the literature method, i.e. by mixing ligand, base, and metal salts in appropriate ratio [21,22]. The catalytic activities of zinc(II) and copper complexes on the methanolysis of phosphate diester, 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNPP), which is often used as model compound of RNA (Scheme 3), were tested [29]. The results are summarized in Table 1.




**Scheme 3.** Methanolysis of phosphate HPNPP.

It can be seen that relative to the methoxide promoted background reaction, the mono-nuclear, di-nuclear, and tri-nuclear zinc(II) complexes at $1.0\times10^{-4}$ mol dm$^{-3}$ could accelerate the reactions by $6.5\times10^5$, $4.4\times10^7$, and $1.5\times10^9$ folds, respectively. $k_{obs}$ of the di-nuclear and tri-nuclear zinc(II) complexes are 56 and 210-fold larger, respectively, than that of the mononuclear complex Zn:**3**. For the corresponding copper complexes, excellent catalytic activities were also observed. At the concentration of $1.0\times10^{-4}$ mol dm$^{-3}$, the mono-nuclear, di-nuclear, and tri-nuclear copper(II) complexes could accelerate the reactions by $4.9\times10^5$, $4.7\times10^7$, and $7.3\times10^8$ folds, respectively. The di-nuclear and tri-nuclear complexes also showed good synergistic effects, $k_{obs}$ of the di-nuclear and tri-nuclear copper

**Table 1**
Kinetic data for the cleavage of HPNPP (0.04 mM) by zinc(II) and copper(II) complexes (0.1 mM) with ligands **3**, **4**, and **5** at 25.0 ± 0.1 °C.

| Catalyst | $k_{obs}$ s$^{-1}$ | $^s$pH[a] | $k_{obs}^{MeO-}$s$^{-1}$ [b] | Acceleration | Relative $k$[c] |
|---|---|---|---|---|---|
| Zn:**3** | $3.1\times10^{-4}$ | 10.04 | $4.77\times10^{-10}$ | $6.5\times10^5$ | |
| $Zn_2$:**4** | $1.74\times10^{-2}$ | 9.96 | $3.96\times10^{-10}$ | $4.4\times10^7$ | 56 |
| $Zn_3$:**5** | $6.51\times10^{-2}$ | 9.35 | $9.73\times10^{-11}$ | $1.5\times10^9$ | 210 |
| Cu:**3** | $6.2\times10^{-5}$ | 9.40 | $1.26\times10^{-10}$ | $4.9\times10^5$ | |
| $Cu_2$:**4** | $5.5\times10^{-3}$ | 9.40 | $1.17\times10^{-10}$ | $4.7\times10^7$ | 89 |
| $Cu_3$:**5** | $1.2\times10^{-2}$ | 8.70 | $1.65\times10^{-11}$ | $7.3\times10^8$ | 194 |

[a] The reaction $^s$pH for the self-buffered metal complex solutions [30].
[b] Data for the methoxide promoted background reaction. Second-order rate constants for the cleavage of HPNPP by methoxide are $2.56\times10^{-3}$ M$^{-1}$ s$^{-1}$ [31].
[c] $k_{obs}$(M$_2$:**4**)/$k_{obs}$(M:**3**) or $k_{obs}$(M$_3$:**5**)/$k_{obs}$(M:**3**).

(II) complexes are 89 and 194-fold larger than that of the mononuclear copper(II) complex.

In conclusion, we have successfully applied the copper-mediated click reactions for the preparation of mono-, di-, and tri-nucleating [12]aneN$_3$ ligands in higher yields. The synthetic route is efficient and versatile. Compound **2** is a useful building block in the construction of the functional bio-molecules containing [12]aneN$_3$, such as modified sugar and peptides. The preliminary work has shown that the zinc(II) and copper complexes of those novel [12]aneN$_3$ ligands are very efficient in the cleavage of RNA model compound HPNPP. Further kinetic works in methanol and aqueous solution are being undertaken in our group.

## Acknowledgements

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[22] Z.-L. Lu, C.T. Liu, A.A. Neverov, R.S. Brown, J. Am. Chem. Soc. 129 (2007) 11642.
[23] The procedure for the preparation of **2**: to a solution of tri-tert-butyl-3-hydroxy-1,5,9-triazacyclododecane-1,5,9-tricarboxylate (0.51 mmol 0.25 g) in 4 ml dry DMF was added propargyl bromide (0.62 mmol 0.095 g) and NaH (1.3 mmol 0.060 g). The mixture was stirred for 3 h at room temperature. Then 2 ml MeOH was added dropwise to quench the excess NaH, and the solvent was removed in vacuo. To the residue 10 ml of saturated sodium chloride solution was added, the aqueous phase was extracted with ethyl acetate (4×15 ml). The combined organic phase was dried over $Na_2SO_4$, filtered, and concentrated in vacuo. The crude material was purified by flash chromatography on silica gel (EtOAc/Pet. Spirit 1:8) to give 2 as a colorless oil (0.26 g, 96% yield): $^1$H NMR (400 MHz, $CDCl_3$) δ 4.24 (d, J = 2.0, 2H), 4.03–3.93 (m, 1H), 3.60–3.33 (m, 8H), 3.25–3.07 (m, 4H), 2.39 (t, J = 2.1, 1H), 2.10–1.93 (m, 2H), 1.86–1.71 (m, 2H), 1.45 (s, 18H), 1.44 (s, 9H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 156.44, 156.16, 80.14, 79.80, 77.36, 75.09, 74.49, 57.45, 49.52, 46.85, 45.15, 29.46, 28.60, 28.55. HR-MS cacld. for $C_{27}H_{48}N_3O_7$ $(M+H)^+$: 526.3492. Found: 526.3487.
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[27] Caution: due to their potentially explosive character, all reactions involving mono-, bis-, and tri-azido compounds were carried out with the appropriate protection under a well-ventilated hood. General procedure for the preparation of ligands **3**–**5**: i) preparation of triazole compounds through click reactions: to a solution of 2 (1.8 mmol 0.945 g) in 10 ml of DMF–$H_2O$–THF mixture (volume ratio 4:3:3) were added sodium ascorbate (10 mol%, 35, 5 mg 0.18 mmol), $CuSO_4 \cdot 5H_2O$ (5 mol%, 14.4 mg 0.09 mmol), and the respective azides to make sure that the ratio of alkyne and azido groups is 1:1. The mixture was stirred at room temperature for 4 h, then poured into 10 ml of saturated $NH_4Cl$ aqueous solution. THF was removed under reduced pressure, and the aqueous solution was extracted with DCM (5×15 ml). The combined organic phases were washed with water (3×50 ml), dried over $Na_2SO_4$, and concentrated in vacuo. The crude material was purified by flash chromatography on silica gel (EtOAc/Pet. Spirit 5:1) to give Boc-protected compounds of **3**–**5** as a white solid with yield of 80–86%. All these compounds were used without identification for the next step. ii) Removal of Boc protecting groups through hydrolysis: The above prepared compounds were dissolved in MeOH (20 ml), and concentrated HCl (1.0 ml) was added to the above solution. The mixture was stirring under reflux for 5 h. After cooling the reaction mixture to 0 °C, the hydrochlorides salts of **3**–**5** were precipitated as white solid. iii) Preparation of free ligands by neutralization: to the solution of the above hydrochlorides salts in $H_2O$ (20 ml) was added 10 ml of 10 M NaOH. The mixture was stirred for 0.5 h at room temperature, then extracted with DCM (7×15 ml). The combined organic phase was dried over $Na_2SO_4$ and solid NaOH, filtered, and concentrated under reduce pressure to get the free ligands **3**–**5** as yellow oil.
[28] Analytical data for the ligands: **3** (70% yield); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1H), 4.65 (s, 2H), 4.37 (q, J = 7.2, 2H), 3.70–3.56 (m, 1H), 2.97–2.62 (m, 12H), 2.36 (br, 2H), 1.69–1.46 (m, 7H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 145.72, 121.76, 77.36, 62.84, 50.78, 49.51, 49.12, 45.33, 27.26, 15.53. HR-MS cacld. for $C_{14}H_{29}N_6O$, $(M+H)^+$: 297.2403. Found: 297.2413. 4 (85% yield).$^1$H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 2H), 4.61 (s, 4H), 4.30 (t, J = 6.2, 4H), 3.66–3.50 (m, 2H), 2.87–2.61 (m, 24H), 2.47 (t, J = 6.2, 2H), 2.24 (br, 4H), 1.64–1.45 (m, 8H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 145.85, 123.14, 77.36, 62.59, 50.65, 49.38, 48.96, 46.73, 30.58, 27.21. HR-MS cacld. for $C_{27}H_{53}N_{12}O_2$ $(M+H)^+$: 577.4414. Found: 577.4426. 5 (80% yield); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 3H), 4.63 (s, 6H), 4.30 (s, 6H), 3.65–3.54 (m, 3H), 2.90–2.60 (m, 36H), 2.22 (br, 7H), 1.66–1.41 (m, 12H), 1.11–0.97 (m, 5H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 145.38, 125.56, 77.36, 62.50, 50.94, 50.66, 49.39, 48.97, 44.42, 27.15, 23.63, 7.45. HR–MS cacld. for $C_{42}H_{81}N_{18}O_3$ $(M+H)^+$: 885.6739. Found: 885.6743.
[29] The kinetics of methanolysis of HPNPP promoted by zinc(II) and copper complexes were performed following the procedure in literature [21–22] at the self-buffered condition with HPNPP at $4.0\times10^{-5}$ mol dm$^{-3}$ and temperature at 25 ± 0.1 °C. The stock solutions of the complexes at $1\times10^{-3}$ mol dm$^{-3}$ were prepared in pure methanol by sequential addition of aliquots of stock solutions of sodium methoxide, ligand and $Zn(CF_3SO_3)2$ such that the relative amounts were 0.5:1.0:1.0, 1.0:1.0:2.0, and 1.5:1.0:3.0 for mononuclear, dinuclear, and trinuclear complexes, respectively.
[30] For the designation of pH in non-aqueous solvents we use the forms recommended by the IUPAC, Compendium of Analytical Nomenclature. Definitive Rules 1997 3rd ed., Blackwell, Oxford, U. K. 1998. If one calibrates the measuring electrode with aqueous buffers and then measures the pH of an aqueous buffer solution, the term $^{w}_{w}$pH is used; if the electrode is calibrated in water and the 'pH' of the neat buffered methanol solution then measured, the term $^{s}_{w}$pH is used; and if the electrode is calibrated in the same solvent and the 'pH' reading is made, then the term $^{s}_{s}$pH is used. Since the autoprotolysis constant of methanol is $10^{-16.77}$, neutral $^{s}_{s}$pH is 8.4.
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