

Article

Phosphate Monoester Hydrolysis by Supramolecular Phosphatases Functionalized with Lewis Acidic Moieties in Two-Phase Solvent Systems

Hirokazu Okamoto¹, Ayane Nomoto¹, Dahiru Umar Liman¹, Akib Bin Rahman¹, Toshifumi Tojo^{1,2} and Shin Aoki^{1,2,3,*} 

¹ Faculty of Pharmaceutical Science, Tokyo University of Science, 6-3-1 Niijuku, Katsushika-ku 125-8585, Tokyo, Japan

² Research Institute for Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda 278-8510, Chiba, Japan

³ Research Institute for Biomedical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda 278-8510, Chiba, Japan

* Correspondence: shinaoki@rs.tus.ac.jp

Abstract

Protein phosphorylation and dephosphorylation reactions of intracellular molecules catalyzed by enzymes such as kinases and phosphatases are essential reactions in a lot of cellular functions such as intracellular signal transduction in living systems. The design and synthesis of artificial enzyme mimics are important research topics in bioorganic and bioinorganic chemistry. In this paper, we report on the construction of artificial phosphatases via the supramolecular self-assembly of compounds such as an amphiphilic bis(Zn^{2+} -cyclen) (cyclen = 1,4,7,10-tetraazacyclododecane) complex, barbitol derivatives modified with benzocrown ethers and boronophenyl groups, and a copper(II) ion in a two-phase solvent system. We have developed a hypothesis whereby a mono(4-nitrophenyl)phosphate (MNP) substrate coordinates to the $\text{Cu}_2(\mu\text{-OH})_2$ core in supramolecular complexes and is activated either by Lewis acidic units such as alkali metal (Li^+ , Na^+ and K^+)-benzocrown ether complexes or by boronophenyl moieties. The findings suggest that supramolecular phosphatase functionalized with a benzo-12-crown-4- Li^+ complex shows a higher level of activity in the MNP hydrolysis of a two-phase solvent system compared with that of our previous supramolecular phosphatases in terms of hydrolysis activity and catalytic turnover.

Keywords: metallosupramolecular complexes; self-assembly; artificial phosphatases; Lewis acid; hydrolysis



Academic Editor: Giuseppe Gattuso

Received: 15 December 2025

Revised: 17 January 2026

Accepted: 31 January 2026

Published: 13 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

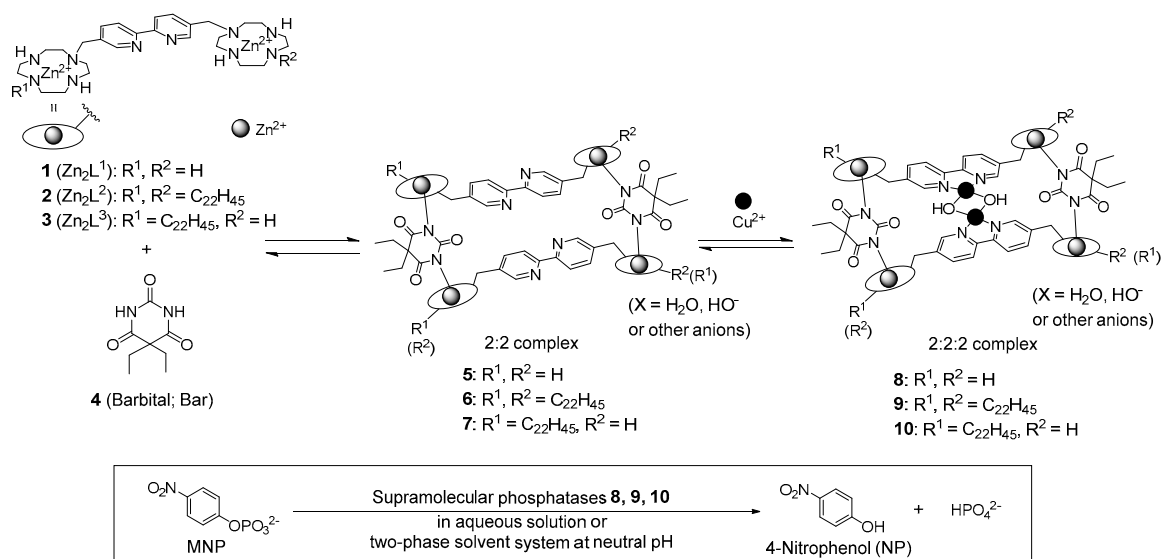
1. Introduction

Phosphorylation and dephosphorylation reactions in living bodies play important roles in intracellular signal transduction and these reactions are catalyzed by natural enzymes such as protein kinases and phosphatases. Alkaline phosphatases (APs) are a group of enzymes that accelerate the hydrolysis of phosphate monoesters and hence play important roles in various tissues in the human body such as the liver, bones, and placenta. APs have been described as potential therapeutic targets for the treatment of cancer and other diseases. AP mimics are small molecules that mimic the activity of AP enzymes, which allow them to provide new methods for the development of drugs to target AP-related diseases. Although many artificial AP models have already been

developed [1–19], only a few have the ability to hydrolyze phosphate monoesters such as mono(4-nitrophenyl)phosphate (MNP) in either a non-catalytic or catalytic manner [20–26]. Recent examples include lanthanum-based materials [27], Cu^{2+} complexes [28,29], the dinuclear Zn^{2+} complex of a macrocyclic Schiff-base-type ligand [30], $\text{MoO}_2(\text{CH}_3)_2\text{L}_2$ complex ($\text{L} = \text{bpy}$ and so on) [31], and Co, Ni, Cu, and Zn complexes of the N-donor bidentate ligand [32].

Generally, it requires tedious tasks to improve the catalytic activity of artificial enzymes via precise structural modification, because most of the previous model compounds are constructed by covalent bonds and their modifications require long synthetic routes and tedious purification processes (sometimes, in low chemical yields). On the other hand, if the construction of artificial supramolecular catalysts was based on the efficient self-assembly of exchangeable molecular units via the use of metal-ligand coordination bonds, it would be a versatile method to develop more efficient enzyme mimetics [33].

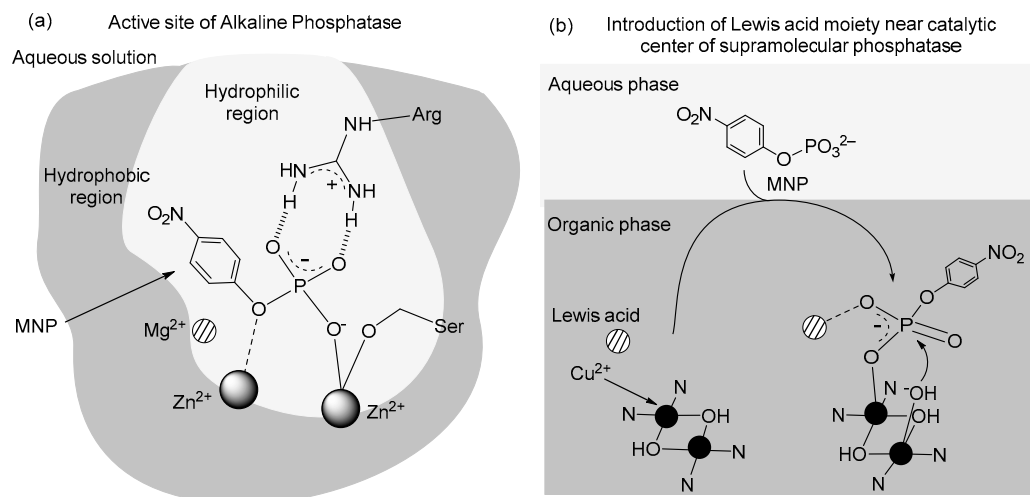
Our previous efforts have been devoted to the construction of supramolecular phosphatase by the self-assembly of dimeric Zn^{2+} -cyclen complexes (cyclen = 1,4,7,10-tetraazacyclododecane), barbitol derivatives, and Cu^{2+} cations (Scheme 1) [34,35]. A bis(Zn^{2+} -cyclen) complex that contains the bipyridyl (bpy) linker **1** (Zn_2L^1) is known to form a 2:2 complex **5** with a deprotonated barbitol 4^{2-} (Bar^{2-}) [36]. Complex **5** and Cu^{2+} form the 1:2 complex **8** (actually a 2:2:2 complex of **1**, Bar^{2-} , and Cu^{2+}) that contains a $\text{Cu}_2(\mu\text{-OH})_2$ core. This structure is considered analogous to the catalytic sites of natural dinuclear metalloenzymes such as AP and it is known to accelerate the hydrolysis of phosphate monoesters such as MNP in aqueous solutions at a neutral pH. However, the MNP hydrolysis activity of **8** is lower than that of AP and the catalytic turnover is negligible.



Scheme 1. Construction of the supramolecular complexes **8–10** by the 2:2:2 self-assembly of Zn_2L complexes (**1–3**), 5,5-diethylbarbitol (**4**), and Cu^{2+} in an aqueous solution at neutral pH and in a two-phase solvent system ($\text{CHCl}_3/\text{H}_2\text{O}$ (pH 7.4)).

Scheme 2a is a schematic drawing of the active site of AP. It is generally accepted that a substrate such as MNP is activated via coordination with two Zn^{2+} ions and then attached by the deprotonated hydroxyl group of serine residue. To mimic the active site of AP, hydrophobic supermolecule **9** was constructed via the 2:2:2 self-assembly of a bis(Zn^{2+} -cyclen) complex **2** (Zn_2L^2) with two hydrophobic long alkyl (C_{22}) chains, deprotonated barbitol units, and Cu^{2+} for higher solubility in organic solvents via the 2:2 complex (**6**) of **2** and **4** (Scheme 1) [37]. We expected product inhibition to be reduced by the release of inorganic phosphate, which is a product of the MNP hydrolysis and inhibits

the regeneration of the $\text{Cu}_2(\mu\text{-OH})_2$ site (Scheme 2b). However, the catalytic turnover was negligible—small for MNP hydrolysis by **9** [37,38]. Next, we designed and synthesized a bis(Zn^{2+} -cyclen) complex with one C_{22} chain (**3**, Zn_2L^3) to construct amphiphilic complex **10** via a 2:2 complex (**7**) of **3** and **4** (and its modified derivatives) with Cu^{2+} , which then accelerates MNP hydrolysis in a catalytic manner [39]. That result could possibly be due to a location of **10** near the organic/aqueous interface.



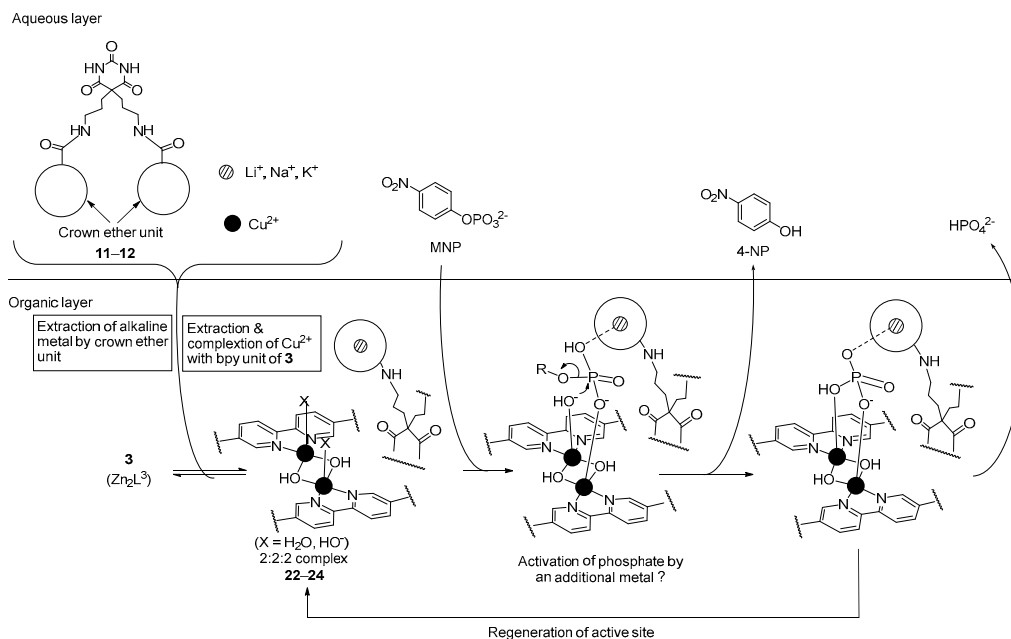
Scheme 2. (a) Schematic diagram of the active center of AP, which contains two Zn^{2+} ions and one Mg^{2+} ion. (b) Concept for activation of the phosphate monoester MNP by Lewis acids placed in close proximity to the $\text{Cu}_2(\mu\text{-OH})_2$ core in a two-phase solvent system.

One of the important findings from our previous studies is that MNP hydrolysis promoted by our metallosupramolecular complexes in two-phase solvent systems obeys the rule of Michaelis–Menten kinetics [37–39]. Therefore, we considered our supramolecular phosphatases including $\text{CHCl}_3/\text{H}_2\text{O}$ solvent systems to be appropriate models of phosphatase reactions.

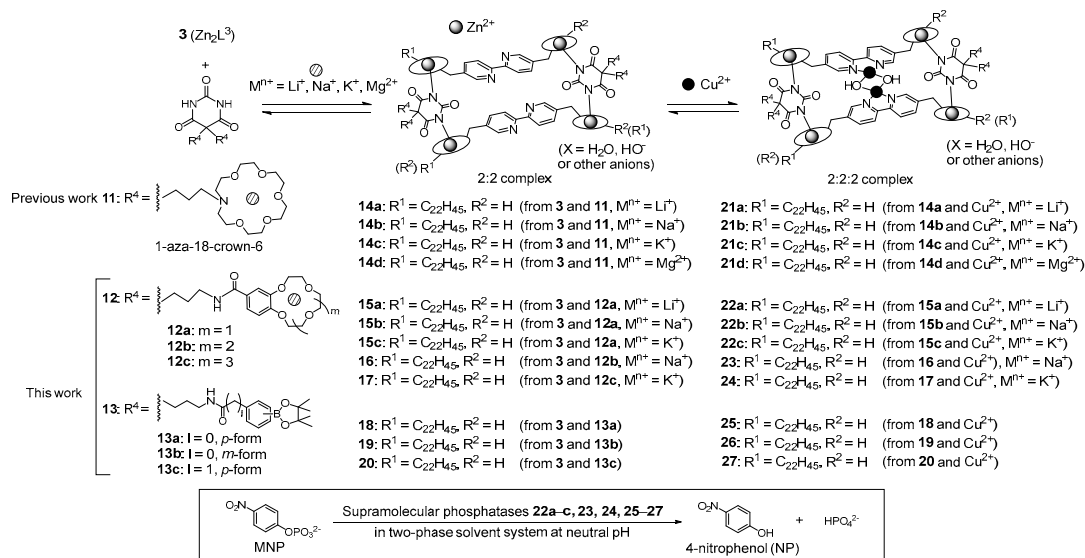
Scheme 2a shows that Mg^{2+} exists as a Lewis acidic site to activate MNP at the active site of AP [40–43]. In order to mimic the situation, we installed the crown-ether-metal (Li^+ , Na^+ , and K^+) complexes near the $\text{Cu}_2(\text{OH})_2$ sites in the metallosupramolecular complexes either to activate MNP or to facilitate the deprotonation of Cu^{2+} -bound H_2O to generate HO^- species near the substrate (Scheme 3), as demonstrated in our previous study [44]. Based on this assumption, we designed and synthesized supramolecular complexes functionalized with a 1-aza-18-crown-6 unit (**21a–d**) via the self-assembly of **3** (Zn_2L^3) with a barbitol building block that possesses 1-aza-18-crown-6 unit **11** and Cu^{2+} via the 2:2 complexes of **3** and **11** (**14a–d**) (Scheme 4) [44]. The results of the MNP hydrolysis by **21** complexed with Li^+ , Na^+ , K^+ , and Mg^{2+} imply that **21a** accelerates the MNP hydrolysis more efficiently than other supramolecular phosphatases, possibly due to the formation of a Li^+ -azacrown ether complex near the $\text{Cu}_2(\mu\text{-OH})_2$ core, as predicted in Scheme 3.

In this study, we design and synthesize barbitol units functionalized with 12, 15, and 18-membered benzocrown ethers (**12a–c**, $m = 1\sim 3$) and boronophenyl groups (**13a–c**, $l = 0, 1$) for the construction of new supramolecular complexes **22–27** via self-assembly with **3** (Zn_2L^3) and Cu^{2+} for more efficient MNP hydrolysis in a two-phase solvent system ($\text{CHCl}_3/\text{H}_2\text{O}$) (Scheme 4). The azacrown ether-unit supramolecular complexes of **21a–d** were changed to benzocrown ether units in **22–24**, because we suspected that the complexation of the azacrown ether units of **21a–d** with metal cations might be hampered by the protonation of the amino groups in the azacrown ether units at a neutral pH. The boronophenyl groups were introduced in **13a–c** and eventually in **25–26** to put Lewis acidic boronyl

groups near the $\text{Cu}_2(\mu\text{-OH})_2$ core of the supramolecular phosphatases. These results are presented below.



Scheme 3. Hypothesis for the hydrolysis of MNP by supramolecular phosphatases (**22–24** shown in Scheme 4) with benzocrown ether-alkaline (or alkaline earth) metal complexes formed from **3** (Zn_2L^3), **12**, Cu^{2+} , and additional metal ions (Li^+ , Na^+ , or K^+) for the MNP hydrolysis in a two-phase solvent system.



Scheme 4. Scheme for the formation of the 2:2:2 supramolecular complexes **21–27** in a two-phase solvent system ($\text{CHCl}_3/\text{H}_2\text{O}$) by the self-assembly of **3** (Zn_2L^3). Bar derivatives were functionalized with a monoaza-18-crown-6 (**11**) (previous work), benzocrown ethers (**12a–c**), boronophenyl units (**13a–c**), and Cu^{2+} .

2. Materials and Methods

All reagents and solvents were of the highest commercial quality and were used with no further purification, unless otherwise noted. MNP was purchased from Nacalai Tesque (Kyoto, Japan) and used without purification. *N,N*-Dimethylformamide (DMF) was distilled from calcium hydride. All aqueous solutions were prepared using deionized

and distilled water [37–39,44]. The Good's buffer reagent was obtained from Dojindo (Kumamoto, Japan) (HEPES (2-(4-(2-hydroxyethyl)-1-piperazinyl)ethanesulfonic acid, $pK_a = 7.6$ at 20 °C). HPLC experiments were carried out using a system consisting of a LC-NetII/ADC HPLC pump, a UV-970 intelligent UV–visible detector, a Rheodine injector (model no. 7125), and a ChromeNAV Lite (JASCO, Japan). For analytical HPLC, a Senshu-Pak Pegasil ODS column (Senshu Scientific Co., Ltd., Tokyo, Japan) (4.6 ϕ $\times$ 250 mm, no. 2012141S) was used [38,39]. For measurement of the UV/Vis spectra, $CHCl_3$ and DMSO were purchased from Nacalai Tesque (Kyoto, Japan). UV/Vis spectra were recorded using either a JASCO (Tokyo, Japan) V-550 or a JASCO V-630 spectrophotometer with quartz cuvettes (path length: 10 mm). IR spectra were recorded on a Perkin-Elmer attenuated total reflectance (ATR)-IR spectrometer 100 (PerkinElmer, Waltham, MA, USA) at room temperature. ^1H -(300 MHz) and ^{13}C -(75 MHz) NMR spectra at 25 ± 0.1 °C were recorded on a JEOL Always 300 spectrometer (JEOL, Tokyo, Japan). Tetramethylsilane (TMS) was used as the internal reference for ^1H - and ^{13}C -NMR measurements in $CDCl_3$ and CD_3OD . Mass spectra were recorded on a Sciex X500R OTOF CAB SCIEX, Framingham, MA, USA) spectrometer and JEOL JMS-700 spectrometer (JEOL, Tokyo, Japan). Thin-layer chromatography (TLC) and alumina gel column chromatography were conducted using a Merck Silica gel 60 F254 plate (Merck KGaA, Darmstadt, Germany) and Wako pure chemical Alumina Gel (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for chromatography, respectively.

2.1. Synthesis

***N,N'*-((2,4,6-Trioxohexahydropyrimidine-5,5-diyl)bis(propane-3,1-diyl))bis(2,3,5,6,8,9-hexahydrobenzo[*b*][1,4,7,10]tetraoxacyclododecine-12-carboxamide) (12a)**

To a solution of compound **36a** [45,46] (0.052 g, 0.18 mmol) and **35** [38] (0.022 g, 0.071 mmol) in distilled CH_2Cl_2 (1.5 mL), *N,N*-diisopropylethylamine (0.082 mL, 0.48 mmol) was added at room temperature. The reaction mixture was stirred at room temperature for 12 h under an argon atmosphere. After concentration of the reaction mixture under reduced pressure, the resultant residue was purified by preparative reversed-phase HPLC (RP-HPLC) [H_2O (0.1% TFA)/ CH_3CN (0.1% TFA), 80:20 to 35:65 (0 to 30 min), $t_r = 17$ min] and lyophilization to give **12a** as a pale yellow solid (18 mg, 35%). M.p. 136.2–137.0 °C. IR (ATR) cm^{-1} : 2924, 1685, 1629, 1575, 1502, 1449, 1308, 1273, 1198, 1125, 1074, 1027, 899, 834, 798, 761, 719, 497, 445, 436, 418, 497. ^1H NMR (300 MHz, CD_3OD /TMS) δ : 7.50 (d, 2H, $J = 2.4$ Hz), 7.47 (dd, 2H, $J = 8.3$, 2.0 Hz), 7.065 (d, 2H, $J = 8.4$ Hz), 4.21–4.19 (m, 8H), 3.85–3.78 (m, 8H), 3.735 (brs, 8H), 2.03–1.97 (m, 4H), 1.56–1.46 (m, 4H). ^{13}C -NMR (75 MHz, CD_3OD /TMS) δ : 148.5, 143.3, 128.6, 124.9, 103.5, 97.5, 92.8, 91.6, 46.9, 45.5, 45.3, 45.0, 44.1, 43.9, 30.4, 14.3, 11.0 ppm. HRMS (FAB): calcd. for $C_{36}H_{47}N_4O_{13}$ [$M+H$] $^+$ 743.3140; found 743.3167. The ^1H - and ^{13}C -NMR spectra of **12a** are shown in Figure S1 of the Supplementary Materials.

***N,N'*-((2,4,6-Trioxohexahydropyrimidine-5,5-diyl)bis(propane-3,1-diyl))bis(2,3,5,6,8,9,11,12-octahydrobenzo[*b*][1,4,7,10,13]pentaoxacyclopentadecine-15-carboxamide) (12b)**

To a solution of **36b** [45,46] (0.048 g, 0.144 mmol) and **35** [38] (0.016 g, 0.052 mmol) in distilled CH_2Cl_2 (0.5 mL), *N,N*-diisopropylethylamine (0.065 mL, 0.38 mmol) was added at room temperature. The reaction mixture was stirred at room temperature for 17 h under an argon atmosphere. Following concentration of the reaction mixture under reduced pressure, the resultant residue was purified using preparative RP-HPLC [H_2O (0.1% TFA)/ CH_3CN (0.1% TFA), 80:20 to 35:65 (0 to 30 min), $t_r = 17$ min] and lyophilization to give **12b** as a pale yellow solid (17 mg, 38%). M.p. 116–117 °C (dec.). IR (ATR) cm^{-1} : 3252, 2924, 1682, 1633, 1580, 1506, 1452, 1317, 1269, 1201, 1123, 1044, 937, 832, 798, 759, 719, 516, 496, 434, 418. ^1H NMR (300 MHz, CD_3OD /TMS) δ : 7.41 (d, 2H, $J = 1.8$ Hz), 7.39–7.37 (m, 2H), 6.94 (d, 2H, $J = 7.8$ Hz), 4.13–4.11 (m, 8H), 3.87–3.83 (m, 8H), 3.74–3.69 (m, 16H), 2.03–1.98

(m, 4H), 1.54–1.49 (m, 4H). ^{13}C -NMR (75 MHz, $\text{CD}_3\text{OD}/\text{TMS}$) δ : 174.7, 169.8, 153.1, 149.7, 128.4, 122.3, 114.0, 113.7, 71.6, 71.0, 70.9, 70.1, 69.9, 69.6, 56.6, 40.4, 37.2, 26.2 ppm. HRMS (FAB): calcd. for $\text{C}_{40}\text{H}_{54}\text{KN}_4\text{O}_{15}$ $[\text{M} + \text{K}]^+$ 869.3217; found 869.3217. The ^1H - and ^{13}C -NMR spectra of **12b** are shown in Figure S2 of the Supplementary Materials.

***N,N'*-((2,4,6-Trioxohexahydropyrimidine-5,5-diyl)bis(propane-3,1-diyl))bis(2,3,5,6,8,9,11,12,14,15 decahydrobenzo[*b*][1,4,7,10,13,16]hexaoxacyclooctadecine-18-carboxamide) (12c)**

To a solution of **36c** [45,46] (0.037 g, 0.100 mmol) and **35** [38] (0.013 g, 0.042 mmol) in distilled CH_2Cl_2 (0.4 mL), *N,N*-diisopropylethylamine (0.043 mL, 0.25 mmol) was added at room temperature. The reaction mixture was stirred at room temperature for 17 h under an argon atmosphere. After evaporation of the reaction mixture under reduced pressure, the resultant residue was purified using preparative RP-HPLC [H_2O (0.1% TFA)/ CH_3CN (0.1% TFA), 80:20 to 35:65 (0 to 30 min), t_r = 19 min] and was lyophilized to give **12c** as a pale yellow solid (14 mg, 37%). M.p. 96–96.5 °C. IR (ATR) cm^{-1} : 3400, 2866, 1724, 1702, 1629, 1579, 1543, 1504, 1449, 1318, 1269, 1220, 1122, 1051, 942, 845, 789, 762, 494, 442. ^1H NMR (300 MHz, $\text{CD}_3\text{OD}/\text{TMS}$) δ : 7.41 (d, 2H, J = 1.8 Hz), 7.39–7.37 (m, 2H), 6.94 (d, 2H, J = 7.8 Hz), 4.13–4.11 (m, 8H), 3.87–3.83 (m, 8H), 3.74–3.69 (m, 16H), 2.03–1.98 (m, 4H), 1.54–1.49 (m, 4H). ^{13}C -NMR (75 MHz, DMSO/TMS) δ : 172.8, 165.3, 150.4, 149.7, 147.3, 126.6, 120.3, 111.7, 111.5, 69.8, 69.7, 68.4, 67.9, 54.4, 35.7, 25.0 ppm. HRMS (FAB): calcd. for $\text{C}_{40}\text{H}_{54}\text{KN}_4\text{O}_{15}$ $[\text{M} + \text{K}]^+$ 869.3217; found 869.3217. The ^1H - and ^{13}C -NMR spectra of **12c** are shown in Figure S3 of the Supplementary Materials.

***N,N'*-((2,4,6-Trioxohexahydropyrimidine-5,5-diyl)bis(propane-3,1-diyl))bis(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzamide) (13a)**

To a solution of **35** [38] (0.020 g, 0.063 mmol), *N,N*-diisopropylethylamine (0.049 mL, 0.28 mmol), and HATU (54 mg, 0.14 mmol) in distilled DMF (0.5 mL), **37a** (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid, Tokyo Chemical Industry (TCI) Co., Ltd., Tokyo, Japan) (0.035 g, 0.14 mmol) was added at room temperature. The reaction mixture was stirred for 4 h under an argon atmosphere. After evaporation of the reaction mixture under reduced pressure, the resultant residue was dissolved in AcOEt, washed with sat. NH_4Cl aq. (10 mL $\times$ 3) and brine, dried over Na_2SO_4 , and filtered. The filtrate was evaporated and the crude compound was recrystallized from hexanes/AcOEt to give **13a** as a colorless solid (39 mg, 88%). M.p. 167–168 °C. IR (ATR) cm^{-1} : 3236, 2979, 1727, 1704, 1636, 1538, 1397, 1358, 1317, 1269, 1205, 1141, 1088, 1019, 962, 854, 769, 690, 653, 578, 558, 492, 454. ^1H -NMR (300 MHz, $\text{CD}_3\text{OD}/\text{TMS}$) δ : 7.82–7.74 (m, 8H), 2.04–2.02 (m, 4H), 1.54–1.35 (m, 4H), 1.35 (brs, 28H). ^{13}C -NMR (75 MHz, $\text{CD}_3\text{OD}/\text{TMS}$) δ : 174.6, 170.3, 138.2, 135.7, 127.5, 85.5, 56.6, 40.5, 37.3, 26.2, 25.1. ^{11}B -NMR (128 MHz, CD_3OD , $\text{BF}_3 \cdot \text{OEt}_2$) δ : 31.3 (brs). HRMS (ESI-MS) m/z : 701.3754 (calcd. for $\text{C}_{36}\text{H}_{49}^{10}\text{B}_2\text{N}_2\text{O}_9$ $[\text{M} + \text{H}]^+$: 701.3753). The ^1H - and ^{13}C -NMR spectra of **13a** are shown in Figure S4 of the Supplementary Materials.

***N,N'*-((2,4,6-Trioxohexahydropyrimidine-5,5-diyl)bis(propane-3,1-diyl))bis(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzamide) (13b)**

To a solution of **35** (0.021 g, 0.066 mmol), *N,N*-diisopropylethylamine (0.050 mL, 0.28 mmol), and HATU (58 mg, 0.16 mmol) in distilled DMF (0.5 mL), **37b** (3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid, TCI, Japan) (0.039 g, 0.16 mmol) was added at room temperature. The reaction mixture was stirred for 2 h under an argon atmosphere. After evaporating the reaction mixture, the resultant residue was dissolved in AcOEt, washed with sat. NH_4Cl aq. (10 mL $\times$ 3) and brine, dried over Na_2SO_4 and then filtered. The filtrate was evaporated under reduced pressure and the crude compound was recrystallized from hexanes/AcOEt to give **13b** as a colorless solid (39 mg, 84%). M.p. 158–159 °C. IR (ATR) cm^{-1} : 2977, 1761, 1724, 1687, 1626, 1598, 1579, 1485, 1413, 1360, 1320, 1300, 1270, 1216, 1141, 1083, 963, 856, 754, 705, 652, 558, 496, 456, 429. ^1H -

NMR(300 MHz, CD₃OD/TMS) δ : 8.16 (s, 2H), 7.88–7.85 (m, 4H), 7.44 (t, 2H), 2.02–1.99 (m, 4H), 1.59–1.48 (m, 4H), 1.35 (brs, 28H). ¹³C-NMR (75 MHz, CD₃OD/TMS) δ : 174.7, 170.3, 151.2, 138.7, 135.2, 134.4, 131.2, 129.0, 85.4, 56.6, 40.7, 37.3, 26.2, 25.3. ¹¹B-NMR (128 MHz, CD₃OD, BF₃·OEt₂) δ : 32.9 ppm. HRMS (ESI-MS) m/z : 701.3753 (calcd. for C₃₆H₄₉¹⁰B₂N₂O₉ [M+H]⁺: 701.3753). The ¹H- and ¹³C-NMR spectra of **13b** are shown in Figure S5 of the Supplementary Materials.

***N,N'*-(2,4,6-Trioxohexahydropyrimidine-5,5-diyl)bis(propane-3,1-diyl)bis(2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetamide) (13c)**

To a solution of **35** (0.017 g, 0.056 mmol), *N,N*-diisopropylethylamine (0.042 mL, 0.24 mmol), and PyBOP (64 mg, 0.123 mmol) in distilled DMF (0.6 mL), **37c** (2-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]acetic acid, TCI, Japan) (0.032 g, 0.12 mmol) was added at room temperature. The reaction mixture was stirred for 2 h under an argon atmosphere. After the reaction mixture was concentrated under reduced pressure, the resultant residue was dissolved in CHCl₃, washed with sat. NH₄Cl aq. (20 mL × 3), washed with brine, dried over Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure, and the crude product was purified using silica gel column chromatography (hexanes/AcOEt = 4/1 to 1/1) to give **13c** as a colorless solid (24 mg, 61%). M.p. 112.5–113.0 °C. IR (ATR) cm^{−1}: 3369, 2978, 1724, 1696, 1637, 1611, 1548, 1357, 1269, 1214, 1141, 1087, 1021, 962, 856, 654, 494, 463, 441. ¹H-NMR(300 MHz, CD₃OD/TMS) δ : 7.69 (d, 4H, *J* = 8.1 Hz), 7.28 (d, 4H, *J* = 8.4 Hz), 3.49 (s, 4H), 3.12 (t, 4H, *J* = 6.8 Hz), 1.90–1.85 (m, 4H), 1.41–1.29 (m, 28H). ¹³C-NMR (75 MHz, CD₃OD/TMS) δ : 174.5, 173.8, 140.3, 136.1, 129.5, 85.1, 56.5, 44.1, 40.1, 37.1, 26.0, 25.2 ppm. ¹¹B-NMR (128 MHz, CD₃OD, BF₃·OEt₂) δ : 31.1 (brs). HRMS (ESI-MS) m/z : 731.3997 (calcd. for C₃₈H₅₃¹¹B₂N₄O₉ [M+H]⁺: 731.3993). Anal. Calcd. for C₃₈H₅₄B₂N₄O₁₀·H₂O; C, 60.98; H, 7.27; N, 7.49; found: C, 60.83; H, 7.18; N, 7.26. The ¹H- and ¹³C-NMR spectra of **13c** are shown in Figure S6 of the Supplementary Materials

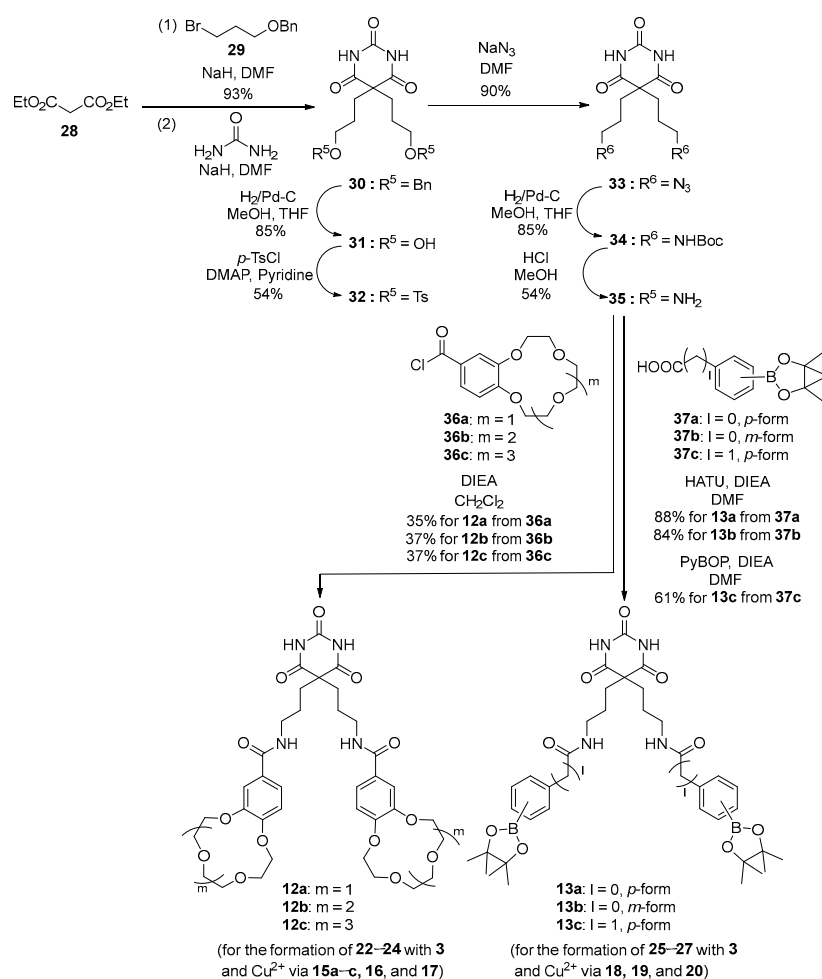
2.2. Hydrolysis of MNP

The hydrolysis of MNP was carried out in CHCl₃/50 mM HEPES (pH 7.4) with *I* = 0.1 (LiNO₃) (2/8) (for **22a**), CHCl₃/50 mM HEPES (pH 7.4) with *I* = 0.1 (NaNO₃) (2/8) (for **22b**, **23** and **25–27**), and in CHCl₃/50 mM HEPES (pH 7.4) with *I* = 0.1 (KNO₃) (2/8) (for **22c** and **24**) at 37 °C. Stock solutions of **12a–c** (0.75 mM in H₂O), **13a–c** (0.75 mM in CHCl₃), and Cu(ClO₄)₂·6H₂O (6 mM in H₂O) were used to prepare sample solutions of **22a–c** and **23–27** in CHCl₃/50 mM HEPES (pH 7.4) with *I* = 0.1 (LiNO₃, NaNO₃ or KNO₃) (2/8) (total volume = 3.0 mL). A stock solution of MNP (20 mM) in water was prepared for the hydrolysis reaction. Prior to the hydrolysis of MNP in the two-phase solvent system, the reaction mixtures of **3**, **12a–b**, **13a–c**, and Cu(ClO₄)₂ in CHCl₃/50 mM HEPES (pH 7.4) with *I* = 0.1 (LiNO₃, NaNO₃ or KNO₃) (2/8) were incubated for 24 h at 37 °C using a controlled incubator (BioShaker BR-23FP, TAITEC, Japan) at a shaking speed of 150 rpm to form **22a–c** and **23–27** in situ, to which an aqueous solution of MNP was added. All of the hydrolysis experiments were performed at a shaking speed of 150 rpm in triplicate. The yields of MNP hydrolysis by the supramolecular complexes were calculated based on the increase in the absorption at 400 nm corresponding to the released 4-nitrophenol (NP) (ϵ_{400} value of NP is $1.35 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ at pH 7.4) ([NP produced in the presence of the supramolecular complex]–[NP produced in the absence of the supramolecular complex]) in the aqueous layer [38,39,44]. The partition ratio of NP (79% in aqueous solution), which was determined in a previous report, was used to calculate the yields in the CHCl₃/H₂O system.

3. Results and Discussion

3.1. Synthesis of the Barbitol Derivatives Functionalized with Crown Ether Units and Boronophenyl Moieties

The synthesis of new barbitol derivatives is described in Scheme 5. The common intermediate **32** was synthesized from diethyl malonate (**28**) and **29** via **30** and **31** according to our previous methods [38,39,44], and a tosylate of **30** (**32**) was reacted with NaN_3 to afford **33**. Reduction of the azido groups of **33** produced diamino compounds, which were converted to bis-Boc protected compound **34** for easy purification. Two Boc groups of **34** were deprotected by HCl, and the resultant amino intermediate **35** [39] was reacted with the chlorinated 12, 15, and 18-membered benzocrown ethers **36a–c**, which were prepared from ethyl 3,4-dihydroxybenzoate to obtain **12a–c**, respectively, following the descriptions in the previous literature [45]. In a similar manner, **35** was reacted with pinacol esters of boron-containing acids **37a–c** to obtain **13a–c**.



Scheme 5. Synthesis of the barbitol units functionalized with crown ethers and boronic acid esters.

3.2. Complexation of **3** (Zn_2L^3) with **12a** and Cu^{2+} as Studied by UV/Vis Titration

The complexation of **3** [39] (40 μM) with barbitol derivatives **12a–c** and **13a–c** and $\text{Cu}(\text{NO}_3)_2$ was checked by UV/Vis titrations in a mixture of DMSO and 50 mM HEPES buffer (pH 7.4 with $I = 0.1$ (LiNO_3)), because the solubility of **3** was low in either organic (CHCl_3 and CH_2Cl_2) and aqueous solvents. The results of typical titrations of **15a** ($[\text{15a}] = 20 \mu\text{M}$ prepared from **3** (40 μM) and **12a** (40 μM)) with $\text{Cu}(\text{NO}_3)_2$ are shown in Figure 1. The absorption of **15a** at 307 nm (λ_{max}) was enhanced at the increased concentrations of Cu^{2+} and reached a plateau with two equations of Cu^{2+} , suggesting an almost

quantitative complexation of **15a** and Cu^{2+} to form **22a**, which is the 2:2:2 complex of **3**, **12a**, and Cu^{2+} . Similar results were obtained in the UV/Vis titration experiments of **3** with **12b,c** and **13a–c**, and then with Cu^{2+} .

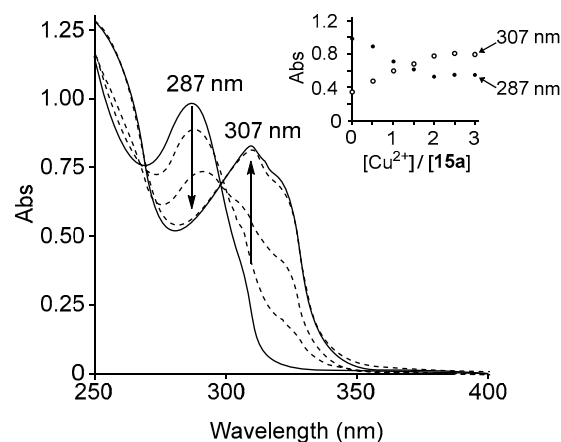


Figure 1. UV/Vis titrations of **15a** (from **3** + **12a**) (20 μM) at the increasing concentrations of $\text{Cu}(\text{NO}_3)_2$ in DMSO/50 mM HEPES buffer (pH 7.4 with $I = 0.1$ (LiNO_3)) (4/96) at 37 $^\circ\text{C}$. Solid curves indicate UV/Vis absorption spectra of **15a** before and after addition of Cu^{2+} . Dashed curves are representative UV/Vis absorption spectra of **15a** in the middle of UV/Vis titrations and two arrows indicate change of absorption at 287 nm and 307 nm upon addition of Cu^{2+} .

3.3. Hydrolysis of MNP by 2:2:2 Complexes in a Two-Phase Solvent System

The hydrolysis reactions of MNP (100 μM in total solution) by **21c**, **22a–c**, and **23–27** (20 μM in the total amount of solvent) were performed in CHCl_3 /50 mM HEPES buffer containing Li^+ (50 mM), Na^+ (50 mM), and K^+ (50 mM) (pH 7.4 with $I = 0.1$ (LiNO_3 or KNO_3) (2/8) at 37 $^\circ\text{C}$, because we expected MNP to be activated by the complexes of the benzocrown ether units with these metal cations in the CHCl_3 phase, as predicted in Scheme 3. Figure 2a shows the results of the hydrolysis of MNP (1000 μM in total solution) in the presence of **21c**, **22a**, and **23–27** (20 μM) for comparison with **10**, which had no Lewis acidic moiety. Supramolecular complexes **25–27** containing boronophenyl units showed a lower level of hydrolysis activity compared with that of the other complexes shown in Figure 2a.

As is shown in Figure 2b, the hydrolysis of MNP (100, 200, 500, 800, and 1000 μM) by **22a** (20 μM) afforded more than 20 μM of *p*-nitrophenol (NP), which is a product of MNP hydrolysis, indicating that **22a** functions as a catalyst (100% hydrolysis yield based on the used **22a** is depicted by the dashed line in Figure 2a–c).

The reactivities of metallosupramolecular complexes functionalized with a benzo-12-crown-4 unit in the presence of Li^+ , Na^+ , and K^+ (**22a–c**) are compared in Figure 2c, which reveals that the highest level of hydrolysis activity was achieved by **22a** among these complexes. That result was possibly due to the complexation of its 12-crown-4 units with Li^+ in the organic phase [47–50]. The hydrolysis yields of MNP by metallosupramolecular phosphatases described above along with their catalytic turnover numbers (CTNs) when $[\text{MNP}] = 1000 \mu\text{M}$ and $[\text{supramolecular complex}] = 20 \mu\text{M}$ are presented in Figure 3, in which **22a** exhibits higher level of MNP hydrolysis yield and CTN than other supramolecular complexes. Unfortunately, the CTN values of **23** and **24**, in which 15-crown-5 and 18-crown-6 units are expected to form 1:1 complexes with Na^+ and K^+ , were smaller than that of **22a**. It was also turned out that supramolecular complexes functionalized with boronophenyl units (**25–27**) exhibited lower levels of hydrolysis activity (particularly **25** and **26**) and CTN values than those of **22–24** functionalized with benzocrown ether units.

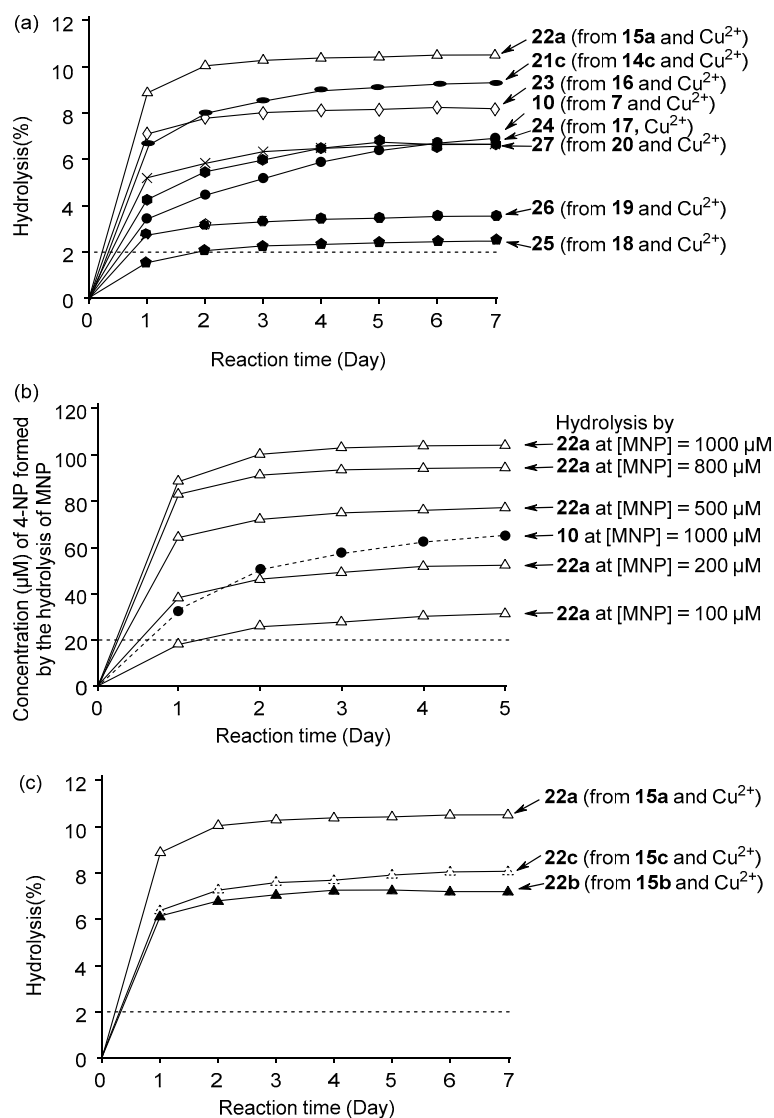


Figure 2. (a) The hydrolysis yields (%) of MNP (1000 μM in total solution) by **22a** (20 μM) in CHCl₃/50 mM HEPES (pH 7.4 with $I = 0.1$ (LiNO₃)), **23** and **25–27** (20 μM) in CHCl₃/50 mM HEPES (pH 7.4 with $I = 0.1$ (NaNO₃)), as well as **24** in CHCl₃/50 mM HEPES (pH 7.4 with $I = 0.1$ (KNO₃)) and **10** (20 μM) in CHCl₃/50 μM HEPES (pH 7.4 with $I = 0.1$ (LiNO₃)). (b) Time-dependent change in the concentrations of 4-nitrophenol (4-NP) formed by the MNP hydrolysis in the presence of **22a** (20 μM) at [MNP] = 100, 200, 500, 800, and 1000 μM (solid lines with open triangles) and change in the concentration of 4-NP by the MNP hydrolysis ([MNP] = 1000 μM) in the presence of **10** (dashed line with filled circles) in CHCl₃/50 mM HEPES (pH 7.4 with $I = 0.1$ (LiNO₃)). (c) The results of hydrolysis (%) of MNP (1000 μM in total solution) by **22a** (20 μM in total solution) in CHCl₃/50 mM HEPES (pH 7.4 with $I = 0.1$ (LiNO₃)), **22b** (20 μM) in CHCl₃/50 mM HEPES (pH 7.4 with $I = 0.1$ (NaNO₃)), and **22c** (20 μM) in CHCl₃/50 mM HEPES (pH 7.4 with $I = 0.1$ (KNO₃)). The dashed lines in Figure 2a–c indicate one equivalent of supramolecular complexes, which corresponds to 100% hydrolysis based on the supramolecular complexes used in each experiments).

The results of the hydrolysis reactions of MNP (100, 200, 500, 800, and 1000 μM) were analyzed using Lineweaver–Burk plots with the reciprocal of the MNP concentration on the horizontal axis (X-axis) and the reciprocal of the hydrolysis rate on the vertical axis (Y-axis), as shown in Figure 4. Plain curves were obtained for **22a–c**, **23**, **24**, and **27** in the two-phase solvent system, which suggests that these MNP hydrolysis reactions follow Michaelis–Menten’s kinetics like a natural enzyme, as previously reported by us [37–39,44]. The slopes of Lineweaver–Burk plots for **22a–c**, **23**, and **24** are smaller than that of **27**,

indicating greater reactivity of **22a–c**, **23**, and **24** than of **27**. Note that the data of **25** and **26** are not included in Figure 4, because their Y-axis intercept values were close to or below zero and thus we could not determine their $V_{\max}$ values.

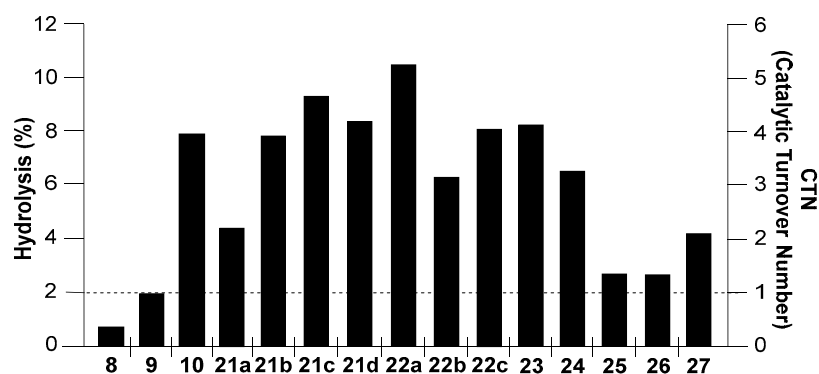


Figure 3. Comparison of MNP hydrolysis yields (left Y-axis) and catalytic turnover numbers (CTNs) (right Y-axis) of **8**, **9**, **10**, **21**, **22**, and **23–27** (20 μM in total solution) at $[\text{MNP}] = 1000 \mu\text{M}$ in $\text{CHCl}_3/50 \text{ mM HEPES}$ buffer (pH 7.4) with $I = 0.1$ (LiNO_3 or NaNO_3 or KNO_3) (2/8) at 37°C .

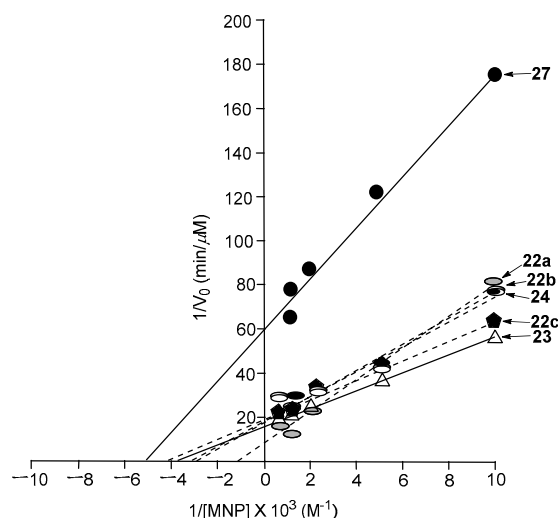


Figure 4. Lineweaver–Burk plots for the hydrolysis of MNP promoted by **22a–c**, **23**, **24**, and **27** (20 μM in total solution) at $[\text{MNP}] = 100, 200, 500$, and $1000 \mu\text{M}$ in $\text{CHCl}_3/50 \text{ mM HEPES}$ buffer (pH 7.4) with $I = 0.1$ (LiNO_3 , NaNO_3 or KNO_3) (2/8) at 37°C .

The K_m (the concentration of the substrate giving half of the maximum velocity), $V_{\max}$ (the maximum reaction velocity), and k_{cat} (rate constants for the catalytic conversion of the substrate into the product) values of the MNP hydrolysis were calculated from the Lineweaver–Burk plots of **22a–c** and **23–27** in Figure 4 and are listed in Table 1. As is described above, **22a** exhibited the highest values for CTN, $V_{\max}$, and k_{cat} among all supramolecular complexes with the exception of **25** (Entry 9), which showed a higher $V_{\max}$ compared with that of all other supramolecular complexes. The $V_{\max}$ and k_{cat} values of **22a** that were higher than those of the other supramolecular complexes could be explained by the formation of a $\text{Li}^+\text{-OH}^-$ moiety on the benzo-12-crown-4- Li^+ complex unit and/or by the direct activation of MNP via the coordination of MNP with the benzo-12-crown-4- Li^+ complex. The $V_{\max}$ and k_{cat} values of **22a** (with Li^+) are greater than those of **21a** (with Li^+), possibly due to the more efficient complexation of benzocrown ether units in **22a** with Li^+ than that of azacrown ether units in **21a**, nitrogen atoms in which are considerably protonated at a neutral pH. It should be noted that **27** possessing a boronophenyl group on

the (3-phenyl)propionyl side chain ($l = 1$ in Scheme 4) exhibits moderate reactivity with a CTN value of ca. 2.1, which is slightly greater than those of **25** and **26**, having boron atoms on the benzoyl side chains ($l = 0$ in Scheme 4). These data may suggest that the distance between the $\text{Cu}_2(\mu\text{-OH})_2$ active site and boronophenyl groups on the side chain of barbitol units affects the reaction mode (e.g., interaction with the substrate and products) and catalytic activity of these metallosupramolecular phosphatases (their K_m values are higher than those of other supramolecular complexes, although we could not calculate exact values).

Table 1. Typical kinetic (Michaelis–Menten kinetics) parameters for the hydrolysis of MNP by **8** and **AP** in a single-phase solvent system (10 mM HEPES buffer (pH 7.4) with $I = 0.1$ (NaNO_3)) at 37°C , and by **8**, **10**, **21a–d**, **22a–c**, and **23–27** in a two-phase solvent system (CHCl_3 /50 mM HEPES buffer (pH 7.4) with $I = 0.1$ (LiNO_3 , NaNO_3 or KNO_3) (2/8)) at 37°C .

Entry	Cat. ^[a]	$\text{M}^{\text{n+}}$	V_{max} ($\mu\text{M}\cdot\text{min}^{-1}$)	K_m (μM)	k_{cat} (min^{-1}) ^[b]	K_i (μM)	K_m/K_i	CTN ^[c]
1	AP ^[d]	-	1.3 ± 0.1 ^[e]	7 ± 4 ^[e]	$(2.4 \pm 0.2) \times 10^3$ ^[e]	3 ± 1 (competitive)	ca. 2.3	$>10^3$ ^[f]
2	8 ^[d]	-	$(8.9 \pm 0.2) \times 10^{-2}$ ^[d]	$(4.1 \pm 0.3) \times 10^2$ ^[d]	$(8.9 \pm 0.2) \times 10^{-4}$ ^[d]	ca. 15 (mixed-type)	ca. 27	0.4
3	9 ^[g]	-	$(1.4 \pm 0.4) \times 10^{-2}$ ^[g]	$(5.4 \pm 0.5) \times 10^2$ ^[g]	$(7.0 \pm 2.0) \times 10^{-4}$ ^[g]	ca. 15 (competitive)	ca. 36	1.0
4	10 ^[h]	-	$(6.8 \pm 0.3) \times 10^{-2}$ ^[h]	$(3.8 \pm 0.2) \times 10^2$ ^[h]	$(3.4 \pm 0.2) \times 10^{-3}$ ^[h]	ca. 80 ^[h] (mixed-type)	ca. 4.8 ^[h]	~4
5	21a	Li^+	$(2.2 \pm 0.3) \times 10^{-2}$	90 ± 6	$(1.1 \pm 0.2) \times 10^{-3}$	n.d.	n.d.	2.4
6	21b	Na^+	$(4.4 \pm 0.2) \times 10^{-2}$	$(2.3 \pm 0.3) \times 10^2$	$(2.2 \pm 0.1) \times 10^{-3}$	ca. 40 (mixed-type)	ca. 4.9	3.9
7	21c ^[i]	K^+	$(7.9 \pm 0.4) \times 10^{-2}$	$(5.9 \pm 0.3) \times 10^2$	$(4.0 \pm 0.2) \times 10^{-3}$	ca. 97 (mixed-type)	ca. 5.2	4.7
8	21d	Mg^{2+}	$(3.3 \pm 0.4) \times 10^{-2}$	51 ± 3	$(1.7 \pm 0.2) \times 10^{-3}$	n.d.	n.d.	4.3
9	22a ^[j]	Li^+	$(9.9 \pm 4.0) \times 10^{-2}$	$(6.2 \pm 3.5) \times 10^2$	$(5.0 \pm 2.0) \times 10^{-3}$	ca. 22	ca. 48	5.3
10	22b ^[k]	Na^+	$(6.0 \pm 1.3) \times 10^{-2}$	$(3.5 \pm 0.8) \times 10^2$	$(3.0 \pm 0.6) \times 10^{-3}$	n.d. ^[m]	n.d. ^[m]	3.6
11	22c ^[l]	K^+	$(7.2 \pm 3.2) \times 10^{-2}$	$(3.6 \pm 2.0) \times 10^2$	$(3.6 \pm 1.6) \times 10^{-3}$	n.d. ^[m]	n.d. ^[m]	4.1
12	23 ^[k]	Na^+	$(6.7 \pm 1.2) \times 10^{-2}$	94 ± 2	$(3.3 \pm 0.6) \times 10^{-3}$	n.d. ^[m]	n.d. ^[m]	4.1
13	24 ^[j]	K^+	$(5.7 \pm 0.4) \times 10^{-2}$	51 ± 3	$(2.8 \pm 0.2) \times 10^{-3}$	n.d. ^[m]	n.d. ^[m]	3.3
14	25 ^[k]	-	n.d. ^[m]	n.d. ^[m]	n.d. ^[m]	n.d. ^[m]	n.d. ^[m]	1.3
15	26 ^[k]	-	n.d. ^[m]	n.d. ^[m]	n.d. ^[m]	n.d. ^[m]	n.d. ^[m]	1.3
16	27 ^[k]	-	$(1.6 \pm 0.1) \times 10^{-2}$	$(1.7 \pm 0.3) \times 10^2$	$(8.0 \pm 0.4) \times 10^{-4}$	n.d. ^[m]	n.d. ^[m]	2.1

^[a] Cat = catalyst. ^[b] Calculated by $V_{\text{max}}/[\text{Catalyst}]$. ^[c] Catalytic turnover numbers determined at $[\text{MNP}] = 1000 \mu\text{M}$. ^[d] From Reference [36] (in a single aqueous solution (10 mM HEPES, pH 7.4 with $I = 0.1$ (NaNO_3))). ^[e] From Reference [51] (in a single aqueous solution (10 mM HEPES (pH 7.4 with $I = 0.1$ (NaNO_3))). ^[f] Calculated from data in Reference [51]. ^[g] From Reference [37]. ^[h] From Reference [39]. ^[i] From Reference [44]. ^[j] Determined in CHCl_3 /50 mM HEPES (pH 7.4 with $I = 0.1$ (LiNO_3))) (2/8). ^[k] The MNP hydrolysis was carried out in CHCl_3 /50 mM HEPES (pH 7.4 with $I = 0.1$ (NaNO_3))) (2/8). ^[l] Determined in CHCl_3 /50 mM HEPES (pH 7.4 with $I = 0.1$ (KNO_3))) (2/8). ^[m] Not determined.

4. Conclusions

In this paper, we report on the design and synthesis of novel metallosupramolecular phosphatases (**22–27**) from bis(Zn^{2+} -cyclen) complex **3** as well as various barbitol analogs functionalized with Lewis acidic moieties and Cu^{2+} ions to improve MNP hydrolysis activity. The supramolecular complex **22a** that contains benzo-12-crown-4- Li^+ complex achieved the highest level of catalytic activity among our metallosupramolecular phosphatases in terms of reaction rates, possibly due to the complexation of the benzo-12-crown-4 units with Li^+ near the active site of the supermolecule that activates MNP. The reactivity of supramolecular phosphatases with boronophenyl groups is dependent on linker length between boronyl site and barbitol moieties. These findings should help with the establishment of more efficient processes for both enzyme-model reactions and phase-transfer catalysis.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/org7010009/s1>. Figure S1: (a) ^1H NMR (300 MHz) and (b) ^{13}C NMR (75 MHz) spectra of **12a** (in $\text{CD}_3\text{OD}/\text{TMS}$). Figure S2: (a) ^1H NMR (300 MHz) and (b) ^{13}C NMR (75 MHz) spectra of **12b** (in $\text{CD}_3\text{OD}/\text{TMS}$). Figure S3: (a) ^1H NMR (300 MHz) and (b) ^{13}C NMR (75 MHz) spectra of **12c** (in $\text{CD}_3\text{OD}/\text{TMS}$). Figure S4: (a) ^1H NMR (300 MHz) and (b) ^{13}C NMR (75 MHz) spectra of **13a** (in $\text{CD}_3\text{OD}/\text{TMS}$). Figure S5: (a) ^1H NMR (300 MHz) and (b) ^{13}C NMR (75 MHz) spectra of **13b** (in $\text{CD}_3\text{OD}/\text{TMS}$). Figure S6: (a) ^1H NMR (300 MHz) and (b) ^{13}C NMR (75 MHz) spectra of **13c** (in $\text{CD}_3\text{OD}/\text{TMS}$).

Author Contributions: H.O. carried out the synthesis of the dizinc complexes and barbitol derivatives, UV/Vis titrations of supramolecular complexes, and hydrolysis of MNP by supramolecular complexes. A.N., D.U.L. and A.B.R. carried out some of the measurements for the hydrolysis of MNP by the supramolecular complexes. T.T. and S.A. supervised all experiments and the preparation of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grants-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan (Nos. 16K10396, and 17K08225 for S.A., and “Academic Frontiers” project for private universities: matching funds from MEXT, and the TUS (Tokyo University of Science) fund for strategic research areas.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Acknowledgments: We wish to express our appreciation for the assistance of Yayoi Yoshimura (Faculty of Pharmaceutical Sciences, Tokyo University of Science) for obtaining the mass spectra, Motoo Iida for the NMR measurements, and Hitomi Isoda (Research Institute for Science and Technology, Tokyo University of Science) for the elemental analyses.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Chakraborty, T.; Mukherjee, S.; Parveen, R.; Chandra, A.; Samanta, D.; Das, D. A Combined Experimental and Theoretical Rationalization of an Unusual Zinc(II)-Mediated Conversion of 18-membered Schiff-base Macrocycles to 18-Membered Imine-Amine Macrocycles with Imidazolidine Side Rings: An Investigation of their Bio-Relevant Catalytic Activities. *New J. Chem.* **2021**, *45*, 2550–2562. [[CrossRef](#)]
2. Koike, T.; Inoue, M.; Kimura, E.; Shiro, M. Novel Properties of Cooperative Dinuclear Zinc(II) Ions: The Selective Recognition of Phosphomonoesters and their P-O Ester Bond Cleavage by a New Dinuclear Zinc(II) Cryptate. *J. Am. Chem. Soc.* **1996**, *118*, 3091–3099. [[CrossRef](#)]
3. Vance, D.H.; Czarnik, A.W. Functional Group Convergency in a Binuclear Dephosphorylation Reagent. *J. Am. Chem. Soc.* **1993**, *115*, 12165–12166. [[CrossRef](#)]
4. Seo, J.S.; Sung, N.-D.; Hynes, R.C.; Chin, J. Structure and Reactivity of a Dinuclear Cobalt(III) Complex with a Bridging Phosphate Monoester. *Inorg. Chem.* **1996**, *35*, 7472–7473. [[CrossRef](#)]

5. Williams, N.H.; Lebuis, A.-M.; Chin, J. A Structural and Functional Model of Dinuclear Metallophosphatases. *J. Am. Chem. Soc.* **1999**, *121*, 3341–3348. [\[CrossRef\]](#)
6. Pecina, A.; Rosa-Gastaldo, D.; Riccardi, L.; Franco-Ulloa, S.; Milan, E.; Scrimin, P.; Mancin, F.; De Vivo, M. On the Metal-Aided Catalytic Mechanism for Phosphodiester Bond Cleavage Performed by Nanozymes. *ACS Catal.* **2021**, *11*, 8736–8748. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Jeon, H.; Vazquez-Lima, H.; Jeong, H.; Cho, K.-B.; Hong, S. Mono- and Dinuclear zinc Complexes Bearing Identical Bis(thiosemicarbazone) Ligand that Exhibit Alkaline Phosphatase-like Catalytic Reactivity. *JBIC J. Biol. Inorg. Chem.* **2022**, *27*, 37–47. [\[CrossRef\]](#)
8. Koike, T.; Kimura, E. Roles of Zinc(II) Ion in Phosphatases. A Model Study with Zinc(II)-Macrocyclic Polyamine Complexes. *J. Am. Chem. Soc.* **1991**, *113*, 8935–8941. [\[CrossRef\]](#)
9. Kimura, E.; Kodama, Y.; Koike, T.; Shiro, M. Phosphodiester Hydrolysis by a New Zinc(II) Macrocyclic Tetraamine Complex with an Alcohol Pendant: Elucidation of the Roles of Ser-102 and Zinc(II) in Alkaline Phosphatase. *J. Am. Chem. Soc.* **1995**, *117*, 8304–8311. [\[CrossRef\]](#)
10. Kimura, E.; Hashimoto, H.; Koike, T. Hydrolysis of Lipophilic Esters Catalyzed by a Zinc(II) Complex of a Long Alkyl-Pendant Macrocyclic Tetraamine in Micellar Solution. *J. Am. Chem. Soc.* **1996**, *118*, 10693–10970. [\[CrossRef\]](#)
11. Kamiskaia, N.V.; He, C.; Lipperd, S.J. Reactivity of μ -Hydroxodizinc(II) Centers in Enzymatic Catalysis Through Model Studies. *Inorg. Chem.* **2000**, *39*, 3365–3373. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Knight, D.A.; Delenhanty, J.B.; Goldman, E.R.; Bongard, J.; Sterich, F.; Edwards, L.W.; Chang, E.L. Carboxylic Acid Functionalized Cobalt(III) Cyclen Complexes for Catalytic Hydrolysis of Phosphodiester Bonds. *Dalton Trans.* **2004**, 2006–2011. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Belousoff, M.J.; Duriska, M.B.; Graham, B.; Batten, S.R.; Moubaraki, B.; Murray, K.S.; Spiccia, L. Synthesis, X-ray Crystal Structures, Magnetism, and Phosphate Ester Cleavage Properties of Copper(II) Complexes of N-Substituted Derivatives of 1,4,7-Triazacyclononane. *Inorg. Chem.* **2006**, *45*, 3746–3755. [\[CrossRef\]](#)
14. Subat, M.; Woinaroschy, K.; Garstl, C.; Sarkar, B.; Kaim, W.; König, B. 1,4,7,10-Tetraazacyclododecane Metal Complexes as Potent Promoters of Phosphodiester Hydrolysis under Physiological Conditions. *Inorg. Chem.* **2008**, *47*, 4661–4668. [\[CrossRef\]](#)
15. Dutta, N.; Halder, S.; Vijaykumar, G.; Paul, S.; Chattopadhyay, A.P.; Carrella, L.; Bera, M. Phosphatase-Like Activity of Tetranuclear Iron(III) and Zinc(II) Complexes. *Inorg. Chem.* **2018**, *57*, 10802–10820. [\[CrossRef\]](#)
16. Emmanuel, Y.T.; Williams, N.H. Enhancing Phosphate Diester Cleavage by a Zinc Complex Through Controlling Nucleophile Coordination. *Chem.-A Eur. J.* **2015**, *21*, 7053–7056. [\[CrossRef\]](#)
17. Chapman, W.H.; Breslow, R. Selective Hydrolysis of Phosphate Esters, Nitrophenyl Phosphates and UpU, by Dimeric Zinc Complexes Depends on the Spacer Length. *J. Am. Chem. Soc.* **1995**, *117*, 10693–10970. [\[CrossRef\]](#)
18. Jiang, F.; Huang, L.; Meng, X.; Du, J.; Yu, X.; Zhao, Y.; Zeng, X. Metallomicellar Catalysis: Hydrolysis of Phosphate Monoester and Phosphodiester by Cu(II), Zn(II) Complexes of Pyridyl Ligands in CTAB Micellar Solution. *J. Colloid Interface Sci.* **2006**, *303*, 236–242. [\[CrossRef\]](#)
19. Williams, N.H.; Takasaki, B.; Wall, M.; Chin, J. Structure and Nuclease Activity of Simple Dinuclear Metal Complexes: Quantitative Dissection of the Role of Metal Ions. *Acc. Chem. Res.* **1999**, *32*, 485–493. [\[CrossRef\]](#)
20. Kimura, E. Dimetallic Hydrolases and their Model. *Curr. Opin. Chem. Biol.* **2000**, *4*, 207–213. [\[CrossRef\]](#)
21. Hettich, R.; Schneider, H.J. Cobalt(III) Polyamine Complexes as Catalysts for the Hydrolysis of Phosphate Esters and of DNA. A Measurable 10 Million-Fold Rate Increase. *J. Am. Chem. Soc.* **1997**, *119*, 5638–5647. [\[CrossRef\]](#)
22. Der, B.S.; Edwards, D.R.; Kuhlman, B. Catalysis by a De Novo Zinc-Mediated Protein Interface: Implications for Natural Enzyme Evolution and Rational Enzyme Engineering. *Biochemistry* **2012**, *51*, 3933–3940. [\[CrossRef\]](#)
23. Zhang, X.; Zhu, Y.; Zheng, X.; Phillips, D.L.; Zhao, C. Mechanistic Investigation on the Cleavage of Phosphate Monoester Catalyzed by Unsymmetrical Macrocyclic Dinuclear Complexes: The Selection of Metal Centers and the Intrinsic Flexibility of the Ligand. *Inorg. Chem.* **2014**, *53*, 3354–3361. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Xue, S.S.; Zhao, M.; Ke, Z.F.; Cheng, B.C.; Su, H.; Cao, Q.; Cao, Z.K.; Wang, J.; Ji, L.N.; Mao, Z.W. Enantioselective Hydrolysis of Amino Acid Esters Promoted by Bis(β -cyclodextrin) Copper Complexes. *Sci. Rep.* **2016**, *6*, 22080. [\[CrossRef\]](#)
25. Naik, K.; Nevrekar, A.; Korera, D.G.; Kotian, A.; Kamat, K.V.; Revankar, V.K. Pyrazole Bridged Dinuclear Cu(II) and Zn(II) Complexes as Phosphatase Models: Synthesis and Activity. *J. Mol. Struct.* **2016**, *1125*, 671–679. [\[CrossRef\]](#)
26. Bahia, S.B.B.B.; Vargas, C.A.A.; Denadai, A.M.L.; Araujo, M.H.; Moraes, A.H.; Brandao, T.A.S. Efficient Catalysis of Phosphate Ester Hydrolysis by Bare Silica. *J. Phys. Chem.* **2020**, *124*, 17111–17120. [\[CrossRef\]](#)
27. Yan, X.; Wu, B.; Zhang, Y.; Yang, Z.; Zhou, M.; Westerhoff, P.; Pan, B. Lanthanum Materials Enable Catalytic Hydrolysis of Phosphate Esters: Implications for Water Treatment and Eutrophication Control. *Environ. Sci. Technol. Lett.* **2024**, *11*, 878–885. [\[CrossRef\]](#)

28. Meza-González, B.M.; Guzmán, F.C. Hydrolysis of Ester Phosphates Mediated by a Copper Complex. *Phys. Chem. Chem. Phys.* **2023**, *25*, 18652–18658. [\[CrossRef\]](#)
29. Dasgupta, S.; Aullón, G.; Zangrando, E.; Das, D. Mapping the Working Route of Phosphate Monoester Hydrolysis Catalyzed by Copper Based Models with Special Emphasis on the Role of Oxoanions by Experimental and Theoretical Studies. *New J. Chem.* **2019**, *43*, 2501–2512. [\[CrossRef\]](#)
30. Kou, H.; Wang, Y.; Ding, P.; Cheng, X.; Zhou, G. Synthesis, Crystal Structure, Phosphate Hydrolysis Activity and Antibacterial Activity of Macrocyclic Dinuclear Zn(II) Complex with Benzyl Pendant-Arms. *J. Mol. Struct.* **2020**, *1216*, 128299. [\[CrossRef\]](#)
31. Lanuza, J.; Gonzalez, A.S.; Bandeira, N.A.G.; Lopez, X.; Gil, A. Mechanistic Insights into Promoted Hydrolysis of Phosphoester Bonds by MoO₂Cl₂(DMF)₂. *Inorg. Chem.* **2021**, *60*, 11177–11191. [\[CrossRef\]](#)
32. Pattanaik, K.; Sabiah, S. Synthesis, Structure, Hirshfeld Surface Analysis, and Phosphoester Hydrolysis of Mononuclear Metal(II) Chloride Complexes with Piperidine-Based Ligand. *J. Mol. Struct.* **2025**, *1331*, 141542. [\[CrossRef\]](#)
33. Mahmudov, K.T.; Kopylovich, M.N.; Maharramov, A.M.; Kurbanova, M.M.; Gurbanov, A.V.; Pombeiro, A.J.L. Barbituric Acids as a Useful Tool for the Construction of Coordination and Supramolecular Compounds. *Coord. Chem. Rev.* **2014**, *265*, 1–37. [\[CrossRef\]](#)
34. Aoki, S.; Zulkefeli, M.; Hisamatsu, Y.; Kitamura, M. *Synergy in Supramolecular Chemistry*; Nabeshima, T., Ed.; CRC Press, Taylor & Francis Group: Boca Raton, FL, USA, 2015; pp. 33–56.
35. Aoki, S.; Bin Rahman, A.; Hisamatsu, Y.; Miyazawa, Y.; Zulkefeli, M.; Saga, Y.; Tanaka, T. Development of Metallosupramolecular Phosphatases Based on the Combinatorial Self-Assembly of Metal Complexes and Organic Building Blocks for the Catalytic Hydrolysis of Phosphate Monoesters. *Results Chem.* **2021**, *3*, 100133. [\[CrossRef\]](#)
36. Zulkefeli, M.; Hisamatsu, Y.; Suzuki, A.; Miyazawa, Y.; Shiro, M.; Aoki, S. Supramolecular Phosphatases Formed by the Self-Assembly of the Bis(Zn²⁺-Cyclen) Complex, Copper(II), and Barbitol Derivatives in Water. *Chem.-Asian J.* **2014**, *9*, 2831–2841. [\[CrossRef\]](#)
37. Hisamatsu, Y.; Miyazawa, Y.; Yoneda, K.; Miyauchi, M.; Zulkefeli, M.; Aoki, S. Supramolecular Complexes Formed by the Self-Assembly of Hydrophobic Bis(Zn²⁺-Cyclen) Complexes, Copper, and Di- or Triimide Units for the Hydrolysis of Phosphate Mono- and Diesters in Two-phase Solvent Systems (Cyclen = 1,4,7,10-tetraazacyclododecane). *Chem. Pharm. Bull.* **2016**, *64*, 451–464. [\[CrossRef\]](#)
38. Miyazawa, Y.; Rahman, A.B.; Saga, Y.; Imafuku, H.; Hisamatsu, Y.; Aoki, S. Catalytic Hydrolysis of Phosphate Monoester by Supramolecular Complexes Formed by the Self-Assembly of a Hydrophobic Bis(Zn²⁺-cyclen) Complex, Copper, and Barbitol Units that are Functionalized with Amino Acids in a Two-Phase Solvent System. *Micromachines* **2019**, *10*, 452. [\[CrossRef\]](#) [\[PubMed\]](#)
39. Rahman, A.B.; Imafuku, H.; Miyazawa, Y.; Kafle, A.; Sakai, H.; Saga, Y.; Aoki, S. Catalytic Hydrolysis of Phosphate Monoester by Supramolecular Phosphatases Formed from a Monoalkylated Dizinc(II) Complex, Cyclic Diimide Units, and Copper(II) in Two-Phase Solvent System. *Inorg. Chem.* **2019**, *58*, 5603–5616. [\[CrossRef\]](#)
40. Kim, E.E.; Wyckoff, H.W. Reaction Mechanism of Alkaline Phosphatase Based on Crystal Structures. Two-Metal Ion Catalysis. *J. Mol. Biol.* **1991**, *218*, 449–464. [\[CrossRef\]](#)
41. Wang, J.; Stieglitz, K.A.; Kantrowitz, E.R. Metal Specificity Is Correlated with Two Crucial Active Site Residues in *Escherichia coli* Alkaline Phosphatase. *Biochemistry* **2005**, *44*, 8378–8386. [\[CrossRef\]](#)
42. Stec, B.; Holtz, K.M.; Kantrowitz, E.R. A Revised Mechanism for the Alkaline Phosphatase Reaction Involving Three Metal Ions. *J. Mol. Biol.* **2000**, *299*, 1303–1311. [\[CrossRef\]](#)
43. Anderson, R.A.; Bosron, W.F.; Kennedy, F.S.; Valle, B.L. Role of Magnesium in *Escherichia coli* Alkaline Phosphatase. *Proc. Nat. Acad. Sci. USA* **1975**, *72*, 2989–2993. [\[CrossRef\]](#)
44. Rahman, A.B.; Okamoto, H.; Miyazawa, Y.; Aoki, S. Design and Synthesis of Supramolecular Phosphatases Formed from a Bis(Zn²⁺-Cyclen) Complex, Barbitol-Crown-K⁺ Conjugate and Cu²⁺ for the Catalytic Hydrolysis of Phosphate Monoester. *Eur. J. Inorg. Chem.* **2021**, *2021*, 1213–1223. [\[CrossRef\]](#)
45. Calderon, V.; Garcia, F.C.; De La Pena, J.L.; Maya, E.M.; Garcia, J.M. Synthesis and Characterization of New Aromatic Polyamides Bearing Crown Ethers or their Dipodal Counterparts in the Pendant Structure. I. Benzo-12-crown-4 and *ortho*-bis(2-ethoxyethoxy)benzene. *J. Polym. Sci. Part A Polym. Chem.* **2006**, *44*, 2270–2281. [\[CrossRef\]](#)
46. Calderon, V.; Schwarz, G.; Garcia, F.; Tapia, M.J.; Valente, A.J.M.; Burrows, H.D.; Garcia, J.M. Synthesis and Characterization of New Aromatic Polyamides Bearing Crown Ethers and Acyclic Ethylene Oxide Units in the Pendant Structure. III. Benzo-18-crown-6 Systems and their Open-Chain Counterparts. *J. Polym. Sci. Part A Polym. Chem.* **2006**, *44*, 6252–6269. [\[CrossRef\]](#)
47. Miyazaki, T.; Yanagida, S.; Itoh, A.; Okahara, M. Synthesis and Alkali-Cation Complexing Properties of 12-crown-4 Derivatives. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2005–2009. [\[CrossRef\]](#)
48. Pedersen, C.J. Cyclic Polyethers and their Complexes with Metal Salts. *J. Am. Chem. Soc.* **1967**, *89*, 7017–7036. [\[CrossRef\]](#)
49. Pedersen, C.J. The Discovery of Crown Ethers (Noble Lecture). *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1021–1027. [\[CrossRef\]](#)

50. Gokel, G.W.; Leevy, W.M.; Weber, M.E. Crown Ethers: Sensors for Ions and Molecular Scaffolds for Materials and Biological Models. *Chem. Rev.* **2004**, *104*, 2723–2750. [[CrossRef](#)] [[PubMed](#)]
51. Zulkefeli, M.; Suzuki, A.; Shiro, M.; Hisamatsu, Y.; Kimura, E.; Aoki, S. Selective Hydrolysis of Phosphate Monoester by a Supramolecular Phosphatase Formed by the Self-Assembly of a Bis(Zn²⁺-cyclen) Complex, Cyanuric Acid, and Copper in an Aqueous Solution (Cyclen = 1,4,7,10-Tetraazacyclododecane). *Inorg. Chem.* **2011**, *50*, 10113–10123. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.