HETEROCYCLES, Vol. 66, 2005, pp. 285 – 297. © The Japan Institute of Heterocyclic Chemistry
Received, 23rd August, 2055, Accepted, 12th October, 2005, Published online, 14th October, 2005. COM-05-S(K)20

ALLOSTERIC BINDING OF ALKALI METAL IONS TO A PSEUDO-CRYPTAND FORMED BY A C-PIVOT TRIPODAL LIGAND CONTAINING 3-HYDROXY-2(1*H*)-PYRIDINONE AND Ga(III) †

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Abstract – A novel *C*-pivot tripodal hexadentate ligand (**3,2-HOPOHL**) composed of 3-hydroxy-2(1*H*)-pyridinone as a bidentate ligand, the ethyleneoxy chain as a spacer, and tris(carboxyric acid) as an anchor was synthesized. **3,2-HOPOHL** recognized only Na^+ ion, suggesting that it pre-organized a cavity due to the electrostatic interaction among the 2(1*H*)-pyridinone rings. UV-VIS spectroscopic analysis indicated that **3,2-HOPOHL** formed a stable intramolecular 1:1 Fe(III) complex in aqueous solution. The stability constant (log *K*) of **3,2-HOPOHL**-Fe(III) complex was estimated to be 27.6 from the competitive reaction with EDTA. ^{1}H-NMR titration of **3,2-HOPOHL**-Ga(III) complex with Na^+ and K^+ ions in $CDCl_3$-CD_3CN indicated the formation of 1:1 complexes. The binding constants of Na^+- and K^+-**3,2-HOPOHL**-Ga(III) complexes were estimated to be 3.3×10^3 and 7.8×10^3 M^{-1}, respectively, the ion selectivity of K^+ toward Na^+ being more than two-fold.

INTRODUCTION

Allostery plays an important role in enzyme regulation. Allosteric regulation is the control of enzyme activity by effectors (ions or molecules) which bind to the enzyme at a site other than the active site, but change the conformation of the active site.[1] An allosteric enzyme is unable to act directly toward a substrate. However, three-dimensional change in enzyme's active site induced by binding of an effector to the allosteric site results in binding of a substrate to its active site. Such phenomenon is called alloste-

† Dedicated to Dr. Kenji Koga, Emeritus Professor of Tokyo University as the memory.

ric effect". Allosteric enzymes exist in the turning point of the metabolic pathway and involve in the metabolic regulation *in vivo*. Metal ions are often seen in allosteric regulation of enzymes.[2] Recently, studies on the reproduction of the allosteric effect by artificial systems have been actively performed in the field of supramolecular chemistry.[3] Nabeshima and co-workers have demonstrated the first example of pseudocrown ethers for allosteric switching of ion regulation.[4] The oligoethyleneglycol-ligating two bipyridine units formed a complex with Cu(I) and as a result the conformation change occurred to form the pseudocrown ether which recognizes alkali metal ions. This concept has been also applied to a pseudocryptands. The recognition of alkali metal ions by pseudocryptands formed by bipyridine-[5] or catechol-armed azacrown ether[6] and metal ions has been reported. The recognition of alkali metal ions by pseudocryptand-like complexes with M_2L_3 coordination mode (M=Fe(III), Ti(IV), and Ga(III); L=β-diketone,[7] catechol,[8] and 8-hydroxyquinoline[9]) has been also reported. However, only a paper[10] on Cs^+ recognition by a pseudocryptand formed by complexation of a *N*-pivot tripodal hexadentate ligand having bipyridine with Fe(II) has been reported.

In our laboratory, synthesis of tripodal hexadentate ligands bearing hydroxyazine-type heterocycles and functional evaluation of their transition metal complexes have been intensively investigated.[11] In the previous paper,[12] we reported for the first time the allosteric binding of alkali metal ions to a pseudocryptand formed by a *C*-pivot tripodal hexadentate ligand (**3,4-HOPOHL**) and Ga(III) as shown in Figure 1.

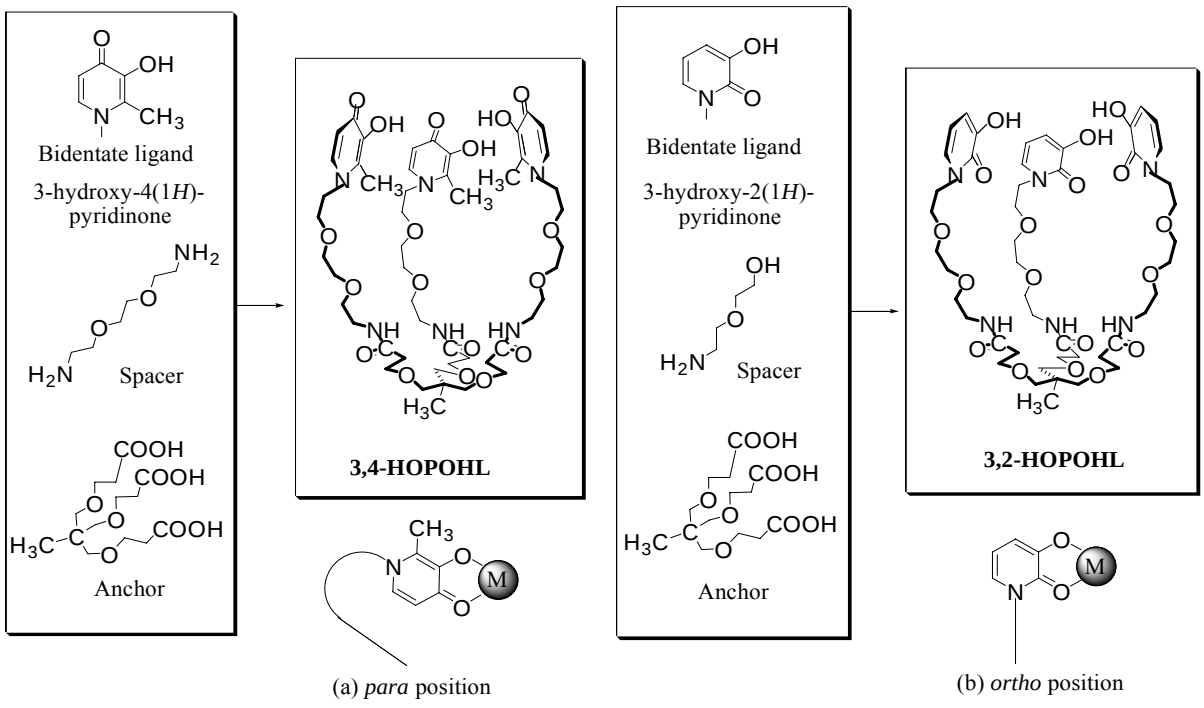


Figure 1 Molecular design of *C*-pivot tripodal hexadentate ligands

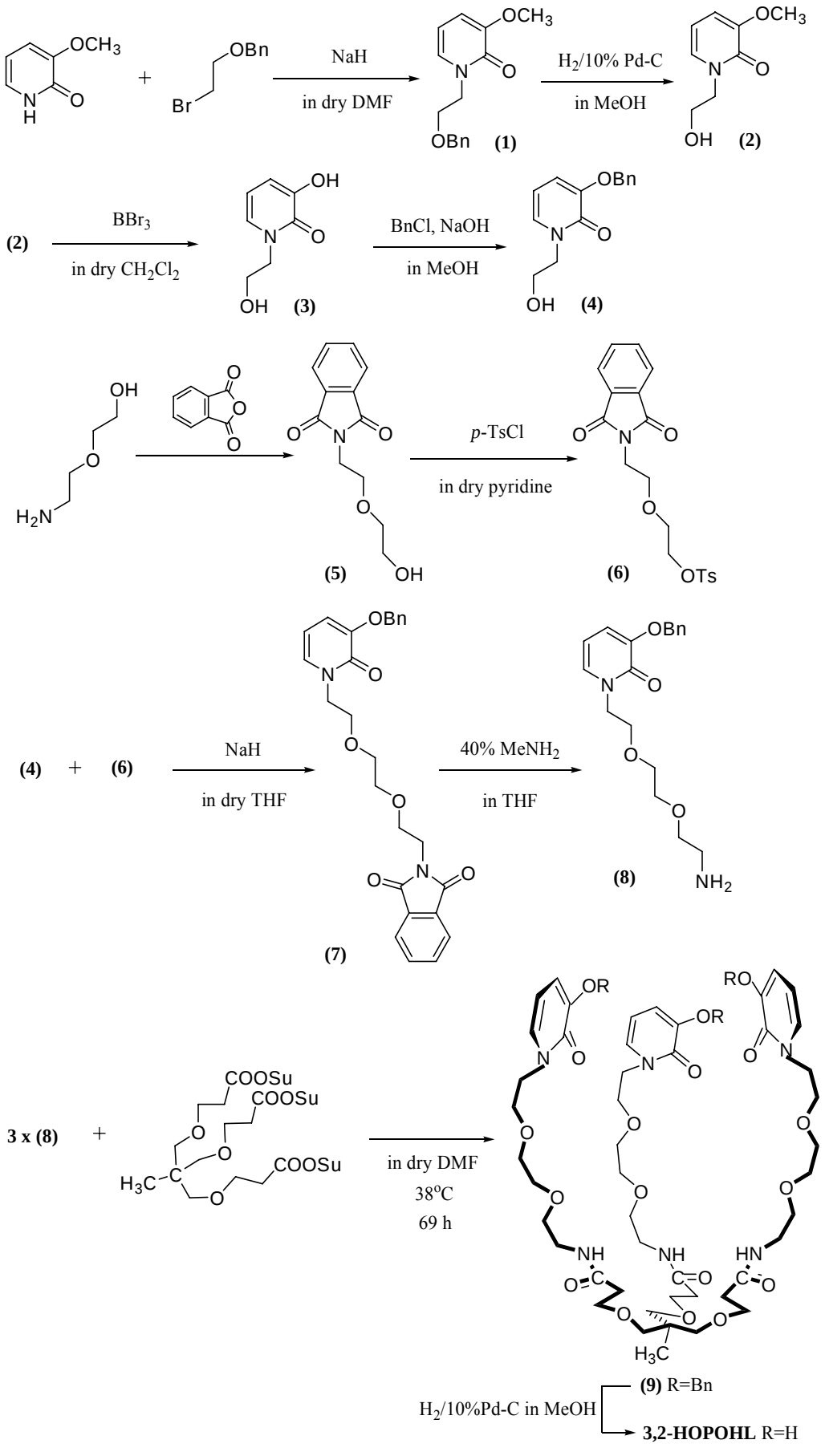
The ion selectivity and binding constant, however, are poor and far below compared to a [2.2.2]cryptand.

It may be attributable that **3,4-HOPOHL**-Ga(III) complex can not construct an ideal three-dimensional cavity like a cryptand, because the 4(1*H*)-pyridinone derivative is linked to the spacer group at the *para* position toward the carbonyl group with a divergent mode as shown in Figure 1-(a).

In search for new compound showing higher ion selectivity and binding constant compared to **3,4-HOPOHL**, we wish to report here synthesis of a novel *C*-pivot tripodal hexadentate ligand (**3,2-HOPOHL**) and the allosteric binding of alkali metal ions to a pseudocryptand formed by complexation of it with Ga(III) as shown in Figure 1. The 2(1*H*)-pyridinone derivative has the spacer group at the *ortho* position toward the carbonyl group as shown in Figure 1-(b), and thus it would be expected to construct an ideal three-dimensional cavity like a cryptand. The difference of **3,4-HOPOHL** and **3,2-HOPOHL** is also discussed.

RESULTS AND DISCUSSION

Synthesis of a *C*-pivot tripodal hexadentate ligand: The synthetic procedure for a *C*-pivot tripodal hexadentate ligand (**3,2-HOPOHL**) was depicted in Scheme 1. A commercially available 3-methoxy-2(1*H*)-pyridinone was allowed to react with benzyl 2-bromoethyl ether in dry DMF to give compound (**1**) in 65% yield. The debenzylation of compound (**1**) by the catalytic hydrogenation afforded compound (**2**) in 77% yield. Compound (**2**) was subjected to the demethylation with 1M BBr_3 in dry CH_2Cl_2 to give compound (**3**) in 89% yield. Treatment of compound (**3**) with benzyl chloride in the presence of NaOH under reflux gave compound (**4**) in 57% yield. The condensation of 2-(2-aminoethoxy)ethanol and phthalic anhydride gave compound (**5**) in 46% yield. Compound (**5**) was converted into the corresponding *O*-tosyl derivative (**6**) in 55% yield. The reaction of compound (**4**) with (**6**) afforded compound (**7**) in 62% yield. Subsequently the deprotection of the phthaloyl group with methylamine gave compound (**8**) in 73% yield. The coupling of three equimolar amount of compound (**8**) with 1,1,1-tris(succinimideoxycarbonylethoxymethyl)- ethane[13] in dry DMF at 38 °C for 69 h[14] gave the *O*-benzyl-protecting tripodal compound (**9**) in 67% yield, which was purified by gel chromatography on Toyopearl HW-40 with MeOH as an eluent. Finally, the debenzylation of compound (**9**) by the catalytic hydrogenation smoothly proceeded to give the desired *C*-pivot tripodal hexadentate ligand (**3,2-HOPOHL**) in 82% yield as a colorless amorphous solid. The structural assignment of **3,2-HOPOHL** was carried out by means of ^{1}H-NMR and IR spectral analyses. Three characteristic olefinic protons at C-4, C-5 and C-6 positions of the pyridinone ring were observed at δ 6.82, 6.14, and 6.93 ppm, respectively. The ethyleneoxy and the methyl protons of the anchor moiety were observed at δ 2.43-4.17 and 0.87 ppm, respectively. The absorption bands due to -OH, C=O, and C-O-C stretching vibrations were observed at 3415, 1646, and 1102 cm^{-1}, respectively. Further, **3,2-HOPOHL** showed the hydroxamic acid test (+).



Scheme 1 Synthetic procedure for *C*-pivot tripodal hexadentate ligand (**3,2-HOPOHL**)

Properties of 3,2-HOPOHL

Conformation in solution: ^{1}H-NMR spectrum of **3,2-HOPOHL** in DMSO-d_6 solution at 20 °C exhibited one set of signals, indicating that it possesses the pseudo-C_3-symmetrical structure. The temperature dependence of the amide proton chemical shift was measured at various temperatures from 20 to 80 °C, and plots of chemical shifts vs. temperatures gave a straight line. (not shown) The temperature dependence coefficient for a strong intramolecular hydrogen bond has been reported to be usually less than -3×10^{-3} ppm deg^{-1}.[15] A large temperature dependence coefficient (-6.0×10^{-3} ppm deg^{-1}) indicated that no strong intramolecular hydrogen bond exists in DMSO-d_6 solution.

Recognition of Na^+ ion by the pre-organization

In the case of **3,4-HOPOHL**, any change in the chemical shift was not observed upon adding an equimolar amount of alkali metal ions in $CDCl_3$-CD_3CN solution. On the other hand, in the case of **3,2-HOPOHL**, the apparent lower magnetic field shift was observed when an equimolar amount of $NaClO_4$ was added to the solution. Plots of $\Delta\delta$ vs. [Na^+]/[**3,2-HOPOHL**] showed a curvature as shown in Figure 2, and the intersection point of the extrapolation of two lines was 1.06, indicating the formation of a 1:1 Na^+ complex. The binding constant K was determined by the ^{1}H-NMR titration of host (**3,2-HOPOHL**) with guest (Na^+ ion) in $CDCl_3$-CD_3CN solution. Under the Benesi-Hildebrand conditions,[16] the following equation is used;

$$1/\Delta\delta_{obs}H=1/\Delta\delta_{comp}H + 1/\Delta\delta_{comp}H\times1/K\times1/[guest]$$

Where $\Delta\delta_{obs}H$ is the observed chemical shift change, and [guest] is the total concentration of alkali metal ion. A straight line was obtained when 1/[guest] was plotted against $1/\Delta\delta_{obs}H$ as shown in Figure 3. The binding constant K was calculated to be 160 M^{-1} from the slope and intercept.

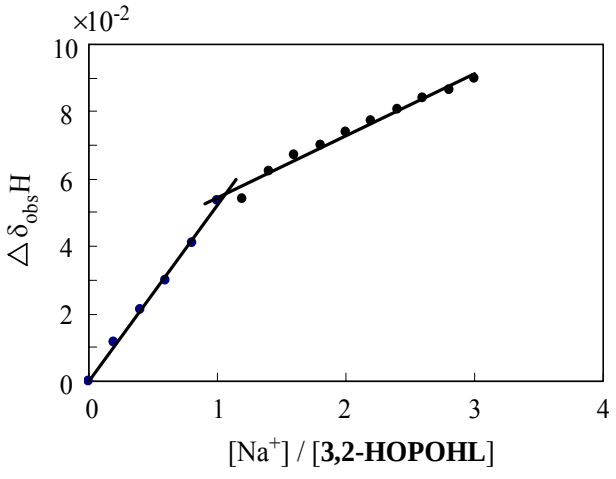


Figure 2 Plots of $\Delta\delta_{obs}H$ vs. the molar ratio of Na^+ ion to **3,2-HOPOHL**

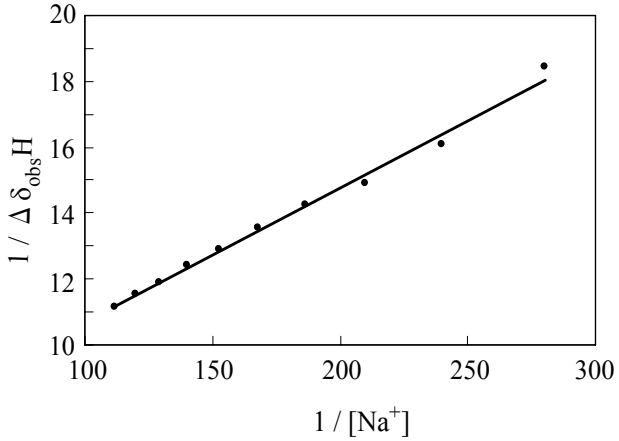


Figure 3 The Benesi-Hildebrand plot of Na^+-**3,2-HOPOHL**

It is noteworthy that the three ring protons of 2(1*H*)-pyridinone of **3,2-HOPOHL** largely shifted to the lower magnetic field upon complexation with Na^+ ion. Only difference between two *C*-pivot tripodal hexadentate ligands is the structure of heterocyclic bidentate ligands, that is, 3-hydroxy-4(1*H*)-pyridinone in **3,4-HOPOHL** vs. 3-hydroxy-2(1*H*)-pyridinone in **3,2-HOPOHL**. This striking difference in the binding property toward Na^+ ion may be explained by the pre-organization of **3,2-HOPOHL** suitable for Na^+ accommodation, although the supporting spectral data could not be obtained.

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Fe(III) complex formation: UV-VIS spectra of a 1:1 molar mixture of **3,2-HOPOHL** and Fe(III) in aqueous solution were measured in the range from pH 2.9 to 8.4 as shown in Figure 4. The absorption maxima due to the ligand-to-metal charge transfer were observed at 500-515 and 410-420 nm. The absorption maxima did not change even at increasing pH, although the absorbance decreased with an increase of pH, indicating that **3,2-HOPOHL** makes a very stable Fe(III) complex even at the acidic region. λ_{max} and ε values of the complex at pH 2.9 were 515 (4300 $dm^3mol^{-1}cm^{-1}$) and 420 nm (3300 $dm^3mol^{-1}cm^{-1}$). These values are comparable to the reported values[17,18] of a **3,2-HOPO**-Fe(III) (3:1) complex, suggesting that **3,2-HOPOHL** forms a stable intramolecular 1:1 Fe(III) complex. The 1:1 stoichiometry was also supported by the result of the molar ratio method as shown in Figure 5, in which an intersection point was 1.03.

The stability constant of Fe(III) complex: The relative stability constant ($K_{Fe(L)}$) of a hexadentate ligand with Fe(III) is defined by the following equation.

$$Fe^{3+} + L^{3-} \rightleftharpoons Fe(L) \qquad K_{Fe(L)} = [Fe(L)]/[Fe^{3+}][L^{3-}]$$

The competitive Fe(III) exchange reaction[19] between EDTA and **3,2-HOPOHL** was carried out in order to obtain the stability constant of Fe(III) complex. Three p*Ka* values of the ligand are necessary for calculation. These values, however, were approximated by the p*Ka* value (8.66)[18] of the model bidentate ligand, 3-hydroxy-2(1*H*)-pyridinone, owing to the limitation of the solubility. Absorbance at 515 nm was monitored in order to determine the equilibrium point. (not shown) The relative stability constant was calculated from p*Ka* values[19] of EDTA, p*Ka* of 3-hydroxy-2(1*H*)-pyridinone, the stability constant of Fe(EDTA) (log*K*=25.1[20]), and the equilibrium constant. The relative stability constant was calculated to be 27.6 in log*K*, being smaller than natural ferrioxamine B (log*K*=30.5[20]).

Ga(III) complex formation: ^{1}H-NMR spectrum of Fe(III) complex could not be measured due to the paramagnetic character of Fe(III). Ga(III) ion is diamagnetic and its ion radius is very close to Fe(III). Ga(III) complex, therefore, was prepared by mixing an equimolar amount of **3,2-HOPOHL** and $Ga(acac)_3$ in $CDCl_3$ solution.[12] On ^{1}H-NMR spectrum of the Ga(III) complex, two signals due to an olefinic proton and methylene protons adjacent to *N*-1 of the 2(1*H*)-pyridinone ring apparently shifted to the down field compared to those of free ligand; Δδ 0.25 for 5-H, 0.04 ppm for -CH_2-N-. On the other hand, two signals due to olefinic protons of the 2(1*H*)-pyridinone ring shifted to the upper field

compared to those of free ligand; Δδ 0.11 for 4-H, 0.17 ppm for 6-H. Further, the broadening of all signals of the Ga(III) complex was observed owing to a decrease of flexibility upon complexation.

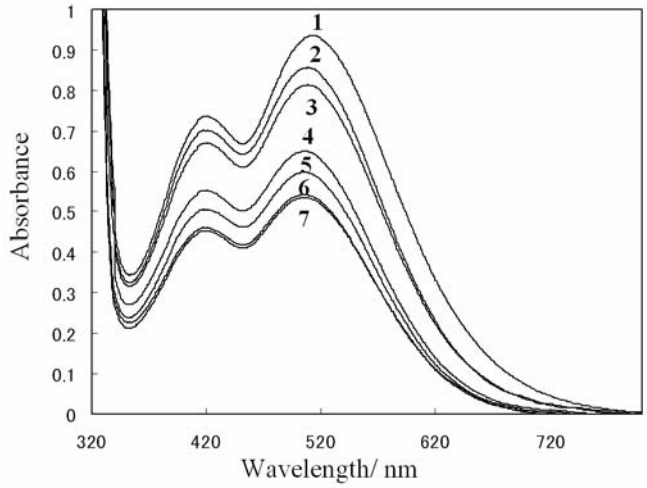

Figure 4 Spectral change of **3,2-HOPOHL**-Fe(III) complex in aqueous solution; **1**(pH 2.9), **2**(3.3), **3**(3.9) **4**(5.9), **5**(7.1), **6**(8.0), and **7**(8.4)

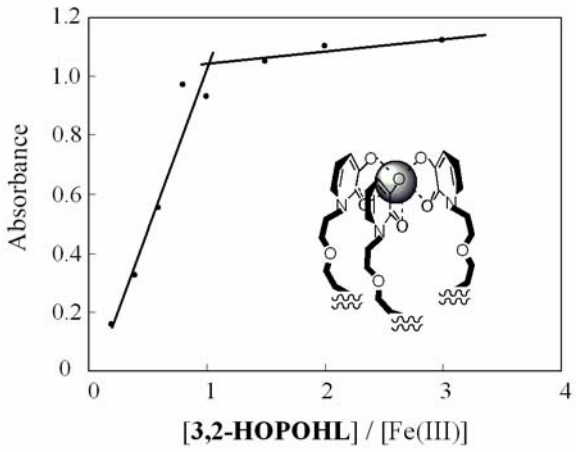

Figure 5 Plots of absorbance at 515 nm vs. the molar ratio of Fe(III) to **3,2-HOPOHL** at pH 2.9

Recognition of alkali metal ions by a pseudocrypyand formed by 3,2-HOPOHL and Ga(III): A change in the chemical shift of each signal was observed when **3,2-HOPOHL**-Ga(III) complex was mixed with alkali metal ions, suggesting the cooperative recognition of alkali metal ions with the Ga(III) complex. In the case of Na^+, the formation of 1:1 complex was confirmed by the molar ratio plot; plots of $\Delta\delta_{obs}H$ vs. the molar ratio [Na^+]/[**3,2-HOPOHL**-Ga(III) complex] gave an intersection point at 0.99. The binding constant *K* was determined by ^{1}H-NMR titration of **3,2-HOPOHL**-Ga(III) complex with Na^+ ion in $CDCl_3$-CD_3CN solution.

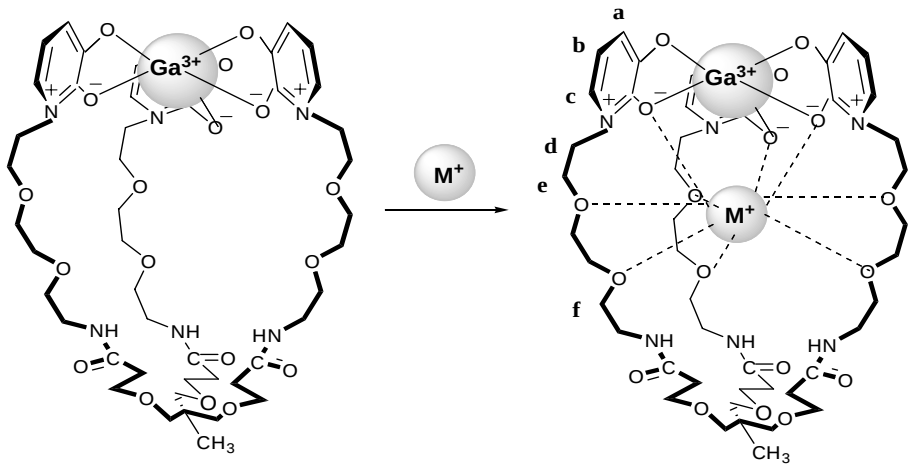

Figure 6 A possible structure of the allosteric binding of alkali metal ions by pseudocryptand formed by **3,2-HOPOHL**-Ga(III) complex

Under the Benesi-Hildebrand conditions,[16] the binding constant *K* of the host with Na^+ ion was calculated to be 3.3×10^3 M^{-1} from the slope and intercept. In the case of K^+ ion, the formation of 1:1 complex was also confirmed by the molar ratio plot; an intersection point at 1.02. The binding constant *K* was calculated to be 7.8×10^3 M^{-1}. The binding constants toward Na^+ and K^+ ions of **3,2-HOPOHL**-Ga(III) complex are far larger than those[12] (*K*=5.9x10^2 for Na^+ and 5.8x10^2 M^{-1} for K^+) of **3,4-HOPOHL**-Ga(III) complex. It may be attributable that **3,2-HOPOHL**-Ga(III) complex can construct a suitable cavity like a cryptand compared to **3,4-HOPOHL**-Ga(III) complex. The spacer group at the *ortho* position toward the C=O group would be favorable to form a cryptand-like cavity. Further, large downfield shifts are observed toward protons of the 3-hydroxy-2(1*H*)-pyridinone ring and of the ethyleneoxy moiety (**a-f** in Figure 6), indicating that Na^+ and K^+ ions locate at nearly central position upon complexation.

EXPERIMENTAL

1-(2-Benzyloxyethyl)-3-methoxypyridin-2(1*H*)-one (1)

To a suspension of NaH (60% in an oil, 1.05 g, 23.9 mmol), which was washed with hexane, was slowly added a solution of 3-methoxy-2(1*H*)-pyridinone (3.0 g, 23.9 mmol) in dry DMF (7 mL), and then the mixture was stirred for 1 h on an ice bath. To the mixture was added a solution of benzyl 2-bromoethyl ether (5.15 g, 23.9 mmol) in dry DMF (5 mL), and then the reaction mixture was stirred for 24 h at rt. After evaporation of the solvent, the residue was dissolved in H_2O (10 mL), and then extracted with AcOEt (50 mLx3). The combined organic layers were dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residual oil (6.75 g) was purified by column chromatography on silica gel 60 (60-200 μm) with $CHCl_3$:acetone:EtOH (100:5:1) mixture to give the product (**1**) (4.04 g, 65%) as a yellow oil; IR(neat): 3062, 2940, 1654, 1101, 1052, and 740 cm^{-1}; ^{1}H-NMR(δ, $CDCl_3$, 400 MHz): 3.79 (2H, t, *J*=5.0 Hz, N- CH_2), 3.82 (3H, s, O-Me), 4.19 (2H, t, *J*=5.0 Hz, BnO-CH_2), 4.47 (2H, s, Ph-CH_2), 6.09 (1H, t, *J*= 7.3 Hz, 5-H), 6.63 (1H, dd, *J*=1.7 and 7.3 Hz, 4-H), 7.02 (1H, dd, *J*=1.7 and 7.3 Hz, 6-H), and 7.23-7.32 ppm (5H, m, Ph). *Anal*. Calcd for $C_{15}H_{17}NO_3 \cdot 0.5H_2O$: C, 67.15; H, 6.76; N, 5.22. Found: C, 67.01; H, 6.58; N, 5.38.

1-(2-Hydroxyethyl)-3-methoxypyridin-2(1*H*)-one (2)

A suspension of 10% Pd-C (80 mg) in MeOH (15 mL) was prehydrogenated with H_2 for 30 min. To the suspension was added a solution of **1** (800 mg, 3.08 mmol) in MeOH (35 mL). After hydrogenation with H_2 under atmospheric pressure for 3 h at rt, the catalyst was removed by filtration. The filtrate was evaporated to give the residue, which was purified by column chromatography on silica gel 60 with $CHCl_3$:MeOH (9:1) mixture to give the product (**2**) (405 mg, 77%) as a colorless solid; mp: 109-112 °C; IR(KBr): 3322, 2954, 1652, 1191, 1072, and 740 cm^{-1}; ^{1}H-NMR(δ, $CDCl_3$, 400 MHz): 3.83 (3H, s, O-Me), 3.96 (2H, t, *J*=5.0 Hz, N-CH_2), 4.18 (2H, t, *J*=5.0 Hz, CH_2-OH), 6.17 (1H, t, *J*=7.3 Hz, 5-H), 6.67 (1H, dd,

J=1.4 and 7.3 Hz, 4-H), and 6.95 ppm (1H, dd, *J*=1.4 and 7.3 Hz, 6-H). *Anal*. Calcd for $C_8H_{11}NO_3$: C, 56.80; H, 6.55; N, 8.28. Found: C, 56.49; H, 6.38; N, 8.21.

3-Benzyloxy-1-(2-hydroxyethyl)pyridin-2(1*H*)-one (4)

To a solution of **2** (2 g, 11.8 mmol) in dry CH_2Cl_2 (20 mL) was added a solution of 1M BBr_3 in dry CH_2Cl_2 (11.8 mL) at -30 °C. The reaction mixture was stirred for 24 h at rt, and then MeOH was added to the mixture at -30 °C. After evaporation of the solvent, the residue was dissolved in H_2O and neutralized with 1M NaOH. After removal of the solvent, the residue was recrystallized from EtOH to give 3-hydroxy-1-(2-hydroxyethyl)pyridine-2(1*H*)-one (**3**) (1.60 g, 89%) as a colorless solid; hydroxamic acid test: positive; mp: 140-142 °C; IR(KBr): 3394, 2979, 2888, 1647, 1553, and 1471 cm^{-1}; ^{1}H-NMR(δ, CD_3OD, 400 MHz): 3.82 (2H, t, *J*=5.1 Hz, -CH_2-OH), 4.10 (2H, t, *J*=5.1 Hz, N-CH_2), 6.21 (1H, t, *J*=7.0 Hz, 5-H), 6.82 (1H, dd, *J*=1.6 and 5.6 Hz, 4-H), and 7.08 ppm (1H, dd, *J*= 1.6 and 5.6 Hz, 6-H).

To a solution of **3** (1.6 g, 10.3 mmol) in MeOH (20 mL) was added 4M NaOH (3 mL) and benzyl chloride (5 mL), and then the reaction mixture was refluxed for 24 h. After evaporation of the solvent, the residue was dissolved in H_2O (50 mL), and extracted with CH_2Cl_2 (50 mLx3). The combined organic layers were washed with saturated NaCl solution (30 mL), and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residual oil was purified by column chromatography on silica gel 60 with $CHCl_3$:MeOH (9:1) mixture to give the product (**4**) (1.41 g, 57%) as a colorless solid; mp: 89-90 °C; IR(neat): 3370, 2948, 2875, 1648, 1593, 1554, 1497, 1454, 1231, 744, and 698 cm^{-1}; ^{1}H-NMR(δ, $CDCl_3$, 400 MHz): 3.96 (2H, t, *J*=4.4 Hz, N-CH_2), 4.17 (2H, t, *J*=4.4 Hz, -CH_2-OH), 5.11 (2H, s, -CH_2-), 6.08 (1H, t, *J*=7.0 Hz, 5-H), 6.69 (1H, dd, *J*=1.4 and 7.0 Hz, 4-H), 6.94 (1H, dd, *J*=1.4 and 7.0 Hz, 6-H), and 7.30-7.44 ppm (5H, m, Ph). *Anal*. Calcd for $C_{14}H_{15}NO_3 \cdot 0.1H_2O$: C, 68.06; H, 6.20; N, 5.67. Found: C, 68.14; H, 6.15; N, 5.82.

2-(2-(2-Hydroxyethoxy)ethyl)isoindoline-1,3-dione (5)

A mixture of 2-(2-aminoethoxy)ethanol (5.00 g, 47.5 mmol) and phthalic anhydride (7.04 g, 47.5 mmol) was heated at 180 °C on an oil bath. The residue was recrystallized from H_2O to give the product (**5**) (5.21 g, 46%) as a colorless solid; mp: 61.5-63.0 °C; IR(KBr): 3417, 2940, 2873, 1641, 1120, and 794 cm^{-1}; ^{1}H-NMR(δ, $CDCl_3$, 400 MHz): 3.60 (2H, t, *J*=5.3 Hz, CH_2-O), 3.68 (2H, t, *J*=5.3 Hz, O-CH_2), 3.75 (2H, t, *J*=5.3 Hz, N-CH_2), 3.91 (2H, t, *J*=5.3 Hz, CH_2-OH), 7.72 (2H, q, *J*=3.1 Hz, 5-H and 6-H isoindoline-1,3-dione), and 7.85 ppm (2H, q, *J*=3.1 Hz, 4-H and 7-H isoindoline-1,3-dione). *Anal*. Calcd for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.95. Found: C, 60.97; H, 5.32; N, 5.87.

2-(2-(2-*O*-*p*-Toluenesulfonylethoxy)ethyl)isoindoline-1,3-dione (6)

To a solution of *p*-toluenesulfonyl chloride (4.05 g, 21.2 mmol) in dry pyridine (40 mL) was slowly added **5** (5 g, 21.2 mmol) on an ice bath, and then the reaction mixture was stirred for 6 h. To the mixture was added ice water, and then the aqueous solution was extracted with CH_2Cl_2 (25 mLx4). The

combined organic layers were washed with cold 6M HCl (30 mLx7), saturated NaCl solution (30 mL), and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was recrystallized from AcOEt to give the product (**6**) (4.57 g, 55%) as a colorless solid; mp: 81.0-83.0 °C; IR(KBr): 2913, 2871, 1715, 1351, 1178, 1116, 813, and 777 cm^{-1}; ^{1}H-NMR(δ, $CDCl_3$, 400 MHz): 2.43 (3H, s, CH_3), 3.66 (4H, m, CH_2-O-CH_2), 3.83 (2H, t, *J*=5.6 Hz, N-CH_2), 4.11 (2H, t, *J*=5.6 Hz, O-CH_2), 7.32 (2H, d, *J*=8.0 Hz, 3-H and 5-H *p*-tosyl), 7.72 (2H, q, *J*=3.1 Hz, 5-H and 6-H indoline-1,3-dione), 7.75 (2H, d, *J*=8.0 Hz, 2-H and 6-H *p*-tosyl), and 7.84 ppm (2H, q, *J*=3.1 Hz, 4-H and 7-H indoline-1,3-dione). *Anal*. Calcd for $C_{19}H_{19}NO_6S$: C, 58.60; H, 4.92; N, 3.60. Found: C, 58.38; H, 4.72; N, 3.74.

1-(2-(2-(2-Phthalimidoethoxy)ethoxy)ethyl)-3-benzyloxypyridine-2(1*H*)-one (7)

To a suspension of NaH (60% in an oil, 179 mg, 4.48 mmol), which was washed with hexane, was slowly added a solution of **4** (1.00 g, 4.07 mmol) in dry THF (10 mL). The mixture was stirred for 5 h at -10 °C, and then a solution of **6** (1.58 g, 4.07 mmol) in dry THF (10 mL) was added to the mixture at -10 °C. The reaction mixture was heated to reflux for 18 h. After evaporation of the solvent, the residue was dissolved in H_2O (10 mL), extracted with AcOEt (50 mLx3). The combined organic layers were washed with saturated NaCl solution (30 mL), and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residual oil (2.16 g) was purified by column chromatography on silica gel 60 with AcOEt:hexane (5:1) mixture to give the product (**7**) (1.18 g, 62%) as a yellow oil; IR(neat): 2953, 2919, 2865, 1711, 1643, 1601, 1493, 1431, 1278, 1189, 1114, 777, 746, and 723 cm^{-1}; ^{1}H-NMR(δ, $CDCl_3$, 400 MHz): 3.51 (2H, t, *J*=3.4 Hz, CH_2O), 3.57 (2H, t, *J*=3.4 Hz, OCH_2), 3.69 (2H, t, *J*=5.8 Hz, CH_2O), 3.72 (2H, t, *J*=5.8 Hz, N-CH_2), 3.87 (2H, t, *J*=5.8 Hz, CH_2-N(C=O)$_2$), 4.10 (2H, t, *J*=5.8 Hz, OCH_2), 5.98 (1H, t, *J*=7.0 Hz, 5-H), 6.63 (1H, dd, *J*=1.4 and 5.8 Hz), 6.99 (1H, dd, *J*=1.4 and 5.8 Hz), 7.29-7.44 (5H, m, Ph), 7.71 (2H, q, *J*=2.4 Hz, 5-H and 6-H isoindoline-1,3-dione), and 7.84 (2H, q, *J*=2.4 Hz, 4-H and 7-H isoindoline-1,3-dione). *Anal*. Calcd for $C_{26}H_{26}N_2O_6 \cdot 0.5H_2O$: C, 66.23; H, 5.77; N, 5.94. Found: C, 66.39; H, 5.76; N, 5.69.

1-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)-3-benzyloxypyridine-2(1*H*)-one (8)

To a solution of **7** (850 mg. 1.83 mmol) in THF (10 mL) was added 40% aqueous $MeNH_2$ solution (200 mL), and then the reaction mixture was heated for 18 h at 60 °C. After evaporation of the solvent, the residual oil was purified by column chromatography on aluminum oxide (63-200 μm) with $CHCl_3$:MeOH (10:1) mixture to give the product (**8**) (450 mg, 73%) as a yellow oil; IR(neat): 3428, 2925, 1647, 1233, 1114, and 755 cm^{-1}; ^{1}H-NMR(δ, $CDCl_3$, 400 MHz): 2.82 (2H, t, *J*=5.3 Hz, CH_2NH_2), 3.45 (2H, t, *J*=5.3 Hz, OCH_2), 3.57 (4H, m, O-CH_2-CH_2-O), 3.80 (2H, t, *J*=5.1 Hz, CH_2O), 4.17 (2H, t, *J*=5.1 Hz, N-CH_2), 5.99 (1H, t, *J*=7.0 Hz, 5-H), 6.65 (1H, dd, *J*=1.6 and 5.8 Hz, 4-H), 7.02 (1H, dd, *J*=1.6 and 5.8 Hz, 6-H), and 7.30-7.44 ppm (5H, m, Ph). *Anal*. Calcd for $C_{18}H_{24}N_2O_4 \cdot H_2O$: C, 61.70; H, 7.48; N, 7.99. Found: C, 61.68; H, 7.19; N, 7.92.

1,1,1-Tris{carbonylethoxymethyl-7-amino-10,13-dioxaoctyl-16-(1',2'-dihydro-3'-hydroxy-2'-oxo-pyridin-1'-yl)}ethane (3,2-HOPOHL)

To a solution of compound (**8**) (232 mg, 0.698 mmol) in dry DMF (5 mL) was added a solution of 1,1,1-tris(succinimideoxycarbonylethoxymethyl)ethane[13] (146 mg, 0.233 mmol) in dry DMF (5 mL), and then the reaction mixture was stirred for 69 h at 38 °C. After evaporation of the solvent under reduced pressure, H_2O (50 mL) was added to the residue, and the aqueous solution was extracted with $CHCl_3$ (50 mLx3). The combined organic layers were successively washed with H_2O (25 mL), 5% $NaHCO_3$ (50 mLx2), 5% citric acid (50 mLx2), saturated NaCl solution (50 mL), and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by gel chromatography on Toyopearl HW-40 with MeOH as an eluent to give 1,1,1-tris{carbonylethoxymethyl-7-amino-10,13-dioxaoctyl-16-(3'-benzyloxy-1',2'-dihydro-2'-oxo-pyridin-1'-yl)}ethane (**9**) (200 mg, 67%) as a yellow oil; IR(neat): 3300, 1648, 1109, 751, and 699 cm^{-1}; ^{1}H-NMR (δ, $CDCl_3$, 400 MH_Z): 0.87 (3H, s, -C-CH_3), 2.41 (6H, t, *J*=6.1 Hz, -CH_2CO), 3.23 (6H, s, O-CH_2-C), 3.39 (6H, t, *J*=5.4 Hz, CH_2-NH), 3.47 (6H, t, *J*=5.4 H_Z, O-CH_2-), 3.53 (12H, m, O-CH_2-CH_2-O), 3.62 (6H, t, *J*=6.1 H_Z, CH_2-O), 3.79 (6H, t, *J*=5.1 Hz, -N-CH_2-), 4.14 (6H, t, *J*=5.1 H_Z, O-CH_2-), 5.09 (6H, s, CH_2-Ph), 6.01 (1H, t, *J*=7.0 Hz, 5-H), 6.66 (1H, dd, *J*=1.4 and 5.8 Hz, 4-H), 6.99 (1H, dd, *J*=1.4 and 5.8 Hz, 6-H), and 7.27-7.43 ppm (5H, m, Ph).

A suspension of 10% Pd-C (20 mg) in MeOH (20 mL) was prehydrogenated with H_2 for 30 min. To the suspension was added a solution of **9** (200 mg, 0.156 mmol) in MeOH (10 mL). After hydrogenation with H_2 under atmospheric pressure for 2 h at rt, the catalyst was removed by filtration. The filtrate was evaporated to give the crude product, which was purified by gel chromatography on Toyopearl HW-40 with MeOH as an eluent to afford the desired product (**3,2-HOPOHL**) (131 mg, 82%) as a colorless amorphous solid; hydroxamic acid test: positive; IR (KBr): 3415, 1646, 1595, 1264, and 1102 cm^{-1}; ^{1}H-NMR (δ, $CDCl_3$, 400 MH_Z): 0.87 (3H, s, -C-CH_3), 2.43 (6H, t, *J*=5.8 Hz, -CH_2CO), 3.25 (6H, s, O-CH_2-C), 3.41 (6H, t, *J*=5.1 Hz, CH_2-NH), 3.49 (6H, t, *J*=5.1 H_Z, O-CH_2-), 3.55 (12H, m, O-CH_2-CH_2-O), 3.64 (6H, t, *J*=5.8 H_Z, CH_2-O), 3.81 (6H, t, *J*=5.3 Hz, -N-CH_2-), 4.17 (6H, t, *J*=5.3 H_Z, O-CH_2-), 6.14 (1H, t, *J*=7.0 Hz, 5-H), 6.82 (1H, dd, *J*=1.4 and 5.6 Hz, 4-H), and 6.93 ppm (1H, dd, *J*=1.4 and 5.6 Hz, 6-H). *Anal*. Calcd for $C_{47}H_{72}N_6O_{18}\cdot 2.5H_2O$: C, 53.55; H, 7.36; N, 7.97. Found: C, 53.60; H, 7.04; N, 7.67.

Measurement of UV-VIS spectra of Fe(III) complex: 3,2-HOPOHL (109 mg, 0.108 mmol) was dissolved in deionized H_2O (50 mL). A 1.0 mL volume of the sample was mixed with an equimolar amount of the standard $Fe(NO_3)_3$ solution (2.17 mM) and diluted to 10.0 mL ($2.17x10^{-4}$ M); $\lambda_{max}(\varepsilon)$= 515 (4300 $dm^3mol^{-1}cm^{-1}$) and 420 nm (3300 $dm^3mol^{-1}cm^{-1}$).

The molar ratio plot: 3,2-HOPOHL (109 mg, 0.108 mmol) was dissolved in deionized H_2O (50 mL). A 0.5 mL volume of the standard aqueous $Fe(NO_3)_3$ solution (2.17 mM) was mixed with an appropriate

amount of the sample solution. Visible spectra of the mixture were measured. Absorbance of each solution at 515 nm was plotted against the molar ratio of [**3,2-HOPOHL**]/[Fe(III)]; intersection point=1.03.

Fe(III) exchange reaction: A buffer solution (5 mL phosphate buffer (pH=6.5, and [KNO_3]=0.23 M) containing 4.34×10^{-6} mol of Fe(III) complex was prepared. An EDTA solution was prepared by dissolving EDTA・2Na (2.91 mg, 8.68×10^{-6} mol) in phosphate buffer, and diluting to 10.0 mL. The Fe(III) exchange reaction was initiated by mixing of the complex solution (2.0 mL) with the EDTA solution (2.0 mL), and followed by monitoring a decrease of absorbance at 515 nm.

Measurement of the binding constant of Na^+ ion to 3,2-HOPOHL: **3,2-HOPOHL** (2.354 mg, 2.33 μmol) was dissolved in $CDCl_3$:CD_3CN (1:1) mixture (0.78 mL), and then ^{1}H-NMR spectrum was measured. 4μL portions of stock $NaClO_4$ solution (2.859 mg, 2.33×10^{-5} mol/200 μL) in $CDCl_3$:CD_3CN (1:1) mixture was directly added to the solution, and then ^{1}H-NMR spectrum of the resulting solution was again measured. $\Delta\delta_{obs}$H vs. molar ratio of [Na^+]/[**3,2-HOPOHL**] was plotted. The binding constant was calculated according to the Benesi-Hildebrand equation; *K*=160.

Measurement of the binding constant of Na^+ and K^+ ions to 3,2-HOPOHL-Ga(III) complex: **3,2-HOPOHL** (2.354 mg, 2.33 μmol) and $Ga(acac)_3$ (0.832 mg, 2.33 μmol) were dissolved in $CDCl_3$:CD_3CN (1:1) mixture (0.78 mL), and then ^{1}H-NMR spectrum was measured. 4 μL portions of the stock $NaClO_4$ solution (2.859 mg, 2.33×10^{-5} mol, 200 μL) or 9 μL portions of the stock $KClO_4$ solution (1.436 mg, 1.04×10^{-5} mol, 700 μL) in $CDCl_3$:CD_3CN (1:1) mixture was directly added to the **3,2-HOPOHL**-Ga(III) complex solution, and then ^{1}H-NMR spectrum of the resulting solution was again measured. $\Delta\delta_{obs}$H vs. molar ratio of [K^+ or Na^+]/[**3,2-HOPOHL**-Ga(III) complex] was plotted. The binding constant was calculated according to the Benesi-Hildebrand equation.

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