Electronic Supplementary Information

for

A red-emitting ratiometric fluorescent probe based on a benzophosphole P-oxide scaffold for the detection of intracellular sodium ion **

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Contents

1. Experimental Details ........................................... S2
   Scheme S1 ..................................................... S3
2. Photophysical Properties ..................................... S10
3. Determination of Dissociation Constant ($K_d$) ................. S10
4. Cell Culture Experiments .................................... S11
5. References .................................................. S11
   Fig. S1 Absorption and emission spectra of NaGY ................. S12
   Fig. S2 Absorption spectral change of NaGY with Na⁺ ............ S12
   Fig. S3 Difference spectra between Na⁺-bound and Na⁺-free forms of NaGY S13
   Fig. S4 Emission spectral change of NaGY with K⁺ ............... S13
   Fig. S5 Emission spectral change with Na⁺ in the presence of K⁺ S14
   Fig. S6 pH dependence of NaGY ................................... S14
   Fig. S7 Trypan blue exclusion tests of cell viability ............. S15
   Fig. S8 Plots of the integrated fluorescence intensity ratio ($I_{565-574}/I_{662-689}$) against [Na⁺] S15
6. NMR Spectra .................................................. S16
1. Experimental Details

General. $^1$H, $^{13}$C{${^1}$H}, and $^{31}$P{${^1}$H} NMR spectra were recorded with a JEOL AL-400 spectrometer in CDCl$_3$, CD$_2$Cl$_2$, DMSO-$d_6$, or THF-$d_8$ (400 MHz for $^1$H, 100 MHz for $^{13}$C, and 162 MHz for $^{31}$P). $^{13}$C{${^1}$H} NMR spectra (150 MHz) of the compounds except 2, 3, 5, and 7 were recorded with a JEOL ECA 600 II spectrometer equipped with an UltraCOOL probe. The chemical shifts in $^1$H NMR spectra are reported in δ ppm using the signals of CHCl$_3$ (7.26 ppm), CH$_2$Cl$_2$ (5.30 ppm), DMSO (2.50 ppm), or THF (1.72 ppm) as an internal standard and those in $^{13}$C NMR spectra are reported using the solvent signals of CDCl$_3$ (77.16 ppm), CD$_2$Cl$_2$ (53.84 ppm) and DMSO-$d_6$ (39.52 ppm) as an internal standard. The chemical shifts in $^{31}$P NMR spectra are reported using H$_3$PO$_4$ (0.00 ppm) as an external standard. Mass spectra were measured with a Bruker micrOTOF Focus spectrometer with the ionization methods of atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI). Thin layer chromatography (TLC) was performed on plates coated with 0.25 mm thickness of silica gel 60F$_{254}$ (Merck). Column chromatography was performed using PSQ100B or PSQ60B (Fuji Silysia Chemicals). Flash chromatography was performed using Isorera (Biotage) equipped with silica gel column (ZIP Sphere cartridge). Recycling preparative HPLC was performed using LC-Forte/R (YMC TECHNOS) equipped with a silica gel column (YMC-Actus SIL SL12S05-2520WX, YMC TECHNOS) or a reversed phase silica gel column (YMC-DispoPack AT ODS, YMC TECHNOS). Recycling preparative gel permeation chromatography (GPC) was performed using LC-918 (Japan Analytical Industry) equipped with polystyrene gel columns (JAIHEL 1H and 2H, Japan Analytical Industry) and CHCl$_3$ as the eluent. Acetonitrile was stored over 3 Å molecular sieves prior to use. Diglycoyl chloride was distilled before use. Anhydrous DMF, THF, and toluene were purchased from Kanto Chemicals and further purified by Glass Contour Solvent Systems. tert-Butyl 2-methoxyphenyl carbamate,\textsuperscript{1} tert-butyl (2,4-dimethoxyphenyl)carbamate,\textsuperscript{2} and 3-bromo-1-phenyl-2-trimethylsilyl benzo[b]phosphole-P-oxide\textsuperscript{3} were prepared according to the literature methods. All other chemicals were purchased from commercial suppliers and used without further purification. All experiments were performed under an argon atmosphere unless otherwise noted.
Synthetic scheme for NaGY and its acetoxymethyl ester (NaGY-AM). **Reagents and conditions:** (a) 1,2-bis(2-chloroethoxy)ethane, NaH, DMF, 2 d, 77%; (b) tert-butyl (2,4-dimethoxyphenyl)carbamate, NaH, KI, DMF, 11 d, 68%; (c) 1:6 TFA/CH₂Cl₂, 1.5 h, 88%; (d) diglycolyl chloride, pyridine, toluene, 100 °C, 3 d, 35%; (e) BF₃·OEt₂, NaBH₄, THF, reflux, 4 h, 94%; (f) NBS, CH₃CN, –30 °C, 16 h, 85%; (g) 4-(tert-butyldimethylsilyloxy)phenylboronic acid, K₃PO₄, Pd(PPh₃)₄, 90 °C, 12 h, 85%; (h) i, NBS, CH₃CN, reflux, 15 h; ii, TBAF, THF, 1.5 h, 70%; (i) ethyl bromoacetate, K₂CO₃, DMF, 50 °C, 16 h, 85%; (j) i, bis(pinacolato)diboron, Pd(dppe)Cl₂, KOAc, 1,4-dioxane, 100 °C, 24 h; ii, 10, Pd(dppe)Cl₂, K₂PO₄, 1,4-dioxane, 100 °C, 17 h 12%; (k) LiOH·H₂O, H₂O, MeOH, 1.5 h, 83%; (l) bromomethyl acetate, Et(Pr)₂N, CH₂Cl₂, 18 h, 9%.

tert-Butyl (8-chloro-3,6-dioxaoctyl)(2-methoxyphenyl)carbamate (2). To a suspension of 1,2-bis(2-chloroethoxy)ethane (1.38 mL, 8.80 mmol) and NaH (55% in mineral oil, 212 mg, 4.84 mmol) in anhydrous DMF (50 mL), tert-butyl 2-methoxyphenylcarbamate (439 mg, 2.20 mmol) in anhydrous DMF (1.0 mL) was added dropwise over 5 min. After stirring for 2 days at ambient temperature, the mixture was quenched with water at 0 °C. The resulting organic layer was separated,
and the aqueous layer was extracted with CHCl₃ three times. The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was subjected to silica gel chromatography (PSQ 100B, 3:1 hexane/ethyl acetate; Rf = 0.33) to afford 636 mg (1.71 mmol, 77%) of 2 as colorless oil. ¹H NMR (400 MHz, DMSO-d₆, VT 80 °C): δ 7.23 (t, 1H, J = 7.8 Hz), 7.15 (d, 1H, J = 7.8 Hz), 7.02 (d, 1H, J = 7.8 Hz), 6.90 (t, 1H, J = 7.8 Hz), 3.80–3.74 (m, 3H), 3.66–3.42 (m, 12H), 1.31 (br, 9H). ¹³C(¹H) NMR (100 MHz, DMSO-d₆, VT 80 °C): δ 154.7, 153.9, 130.8, 129.1, 127.6, 119.8, 111.8, 78.4, 70.3, 69.4, 69.2, 67.8, 55.2, 48.1, 42.9, 27.6. HRMS (ESI): m/z calcd. for C₁₈H₂₅O₉NCl: 374.1734 ([M+H]+); found. 374.1750.

N,N'-Di(tert-butoxycarbonyl)-N-(2,4-dimethoxyphenyl)-N'-(2-methoxyphenyl)-4,7-dioxaoctadecane (3). To a suspension of 2 (15.1 g, 38.9 mmol), NaH (55% in mineral oil, 3.01 g, 69.0 mmol), and KI (1.50 g, 9.05 mmol) in anhydrous DMF (20 mL), tert-butyl (2,4-dimethoxyphenyl)carbamate, (12.8 g, 50.4 mmol) was added dropwise over 20 min. After stirring for 11 days at ambient temperature, the mixture was quenched with water. The resulting organic layer was separated, and the aqueous layer was extracted with CHCl₃ three times. The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude mixture was filtered through a plug of silica gel (PSQ 100B, 2:1 hexane/ethyl acetate), and the volatiles were removed under reduced pressure. The resulting crude product was subjected to flash chromatography (PSQ 60B, 3:1 hexane/ethyl acetate; Rf = 0.14) to afford 15.5 g (26.2 mmol, 68%) of 3 as colorless oil. ¹H NMR (400 MHz, DMSO-d₆, VT 70 °C): δ 7.22 (td, 1H, J = 7.8, 1.6 Hz), 7.12 (dd, 1H, J = 7.8, 1.6 Hz), 7.01 (m, 2H), 6.87 (td, 1H, J = 7.8, 1.6 Hz), 6.56 (d, 1H, J = 2.8 Hz), 6.43 (dd, 1H, J = 7.2, 2.8 Hz), 3.87–3.74 (m, 9H), 3.46–3.36 (m, 8H), 3.11 (s, 4H), 1.30 (br, 18H). ¹³C(¹H) NMR (100 MHz, DMSO-d₆, VT 70 °C): δ 158.9, 155.5, 154.7, 153.9, 130.7, 129.4, 129.2, 127.7, 123.8, 119.8, 111.8, 104.3, 98.9, 78.4, 78.2, 69.2, 67.7, 55.3, 55.2, 55.15, 55.06, 54.98, 48.1, 27.6 (one carbonyl peak and two aliphatic peaks are overlapped). HRMS (APCI): m/z calcd. for C₃₀H₄₇O₉N₂: 591.3282 ([M+H]+); found. 591.3273.

1-(2-Methoxyphenyl)-10-(2,4-dimethoxyphenyl)-4,7-dioxaoctadecane (4). To a solution of 3 (9.78 g, 16.5 mmol) in CH₂Cl₂ (85 mL), trifluoroacetic acid (15.0 mL) was added under air. After stirring for 1.5 h at ambient temperature, the volatiles were removed under reduced pressure. The resulting oil was neutralized with an aqueous NaHCO₃ solution. The organic layer was separated, and the aqueous layer was extracted with CH₂Cl₂ five times. The combined organic layer was washed with water, dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude mixture was subjected to flash column chromatography (ZIP Sphere 45 g, 1:3 hexane/ethyl acetate; Rf = 0.32) to afford 5.67 g (14.5 mmol, 88%) of 4 as colorless oil. ¹H NMR (400 MHz, CDCl₃): δ 6.86 (td, 1H, J = 7.8, 1.4 Hz), 6.76 (dd, 1H, J = 7.8, 1.4 Hz), 6.08 (d, 1H, J = 7.8, 1.4 Hz), 6.62 (dd, 1H, J = 7.8, 1.4 Hz), 6.54 (d, 1H, J = 8.6 Hz), 6.45 (d, 1H, J = 2.4 Hz), 6.40 (dd, 1H, J = 8.6, 2.4 Hz), 4.38 (br, 2H), 3.82 (s, 3H), 3.80 (s, 3H), 3.77–3.72 (m, 7H), 3.67 (s, 4H), 3.33 (t, 2H, J = 5.4 Hz), 3.28 (t, S4
7-(2-Methoxyphenyl)-13-(2,4-dimethoxyphenyl)-1,4,10-trioxo-7,13-diazacyclopentadecane-8,12-dione (5). To a flask containing anhydrous toluene (1 L), a solution of 4 (4.67 g, 12.0 mmol) in a mixture of anhydrous pyridine (20 mL) and anhydrous toluene (80 mL) and a solution of diglycolyl chloride (2.08 g, 12.0 mmol) in anhydrous toluene (100 mL) were dropped simultaneously over 24 h at 100 °C. The solution was further stirred at 100 °C for 2 days, and then all volatiles were removed under reduced pressure. After addition of water and CH$_2$Cl$_2$, the organic layer was separated, and the aqueous layer was extracted with CH$_2$Cl$_2$ three times. The combined organic layers were washed twice with water, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The crude mixture was subjected to silica gel column chromatography (PSQ 100B, 19:1 chloroform/methanol; $R_f = 0.34$) followed by GPC to afford 2.04 g (4.18 mmol, 35%) of 5 as colorless solid. $^1$H NMR (400 MHz, DMSO-$d_6$, VT 150 °C): $\delta$ 7.40–7.21 (m, 3H), 7.10 (d, 1H, $J = 7.6$ Hz), 7.01 (t, 1H, $J = 7.6$ Hz), 6.65 (s, 1H), 6.58 (d, 1H, $J = 8.4$ Hz), 4.08 (br, 3H), 3.85–3.77 (m, 10H), 3.62 (br, 6H), 3.52 (br, 6H). $^{13}$C({$^1$H}) NMR (100 MHz, DMSO-$d_6$, VT 150 °C): $\delta$ 168.3, 168.1, 159.6, 155.6, 154.7, 130.5, 130.1, 128.3, 120.0, 119.9, 112.4, 112.3, 105.1, 99.5, 69.3, 69.2, 66.7, 66.6, 66.1, 55.32, 55.26, 54.92, 54.88, 46.7 (one aliphatic peak is overlapped). HRMS (APCI): $m/z$ calcd. for C$_{25}$H$_{30}$O$_4$N$_2$: 489.2237 ([M+H]$^+$); found. 489.2218.

7-(2-Methoxyphenyl)-13-(2,4-dimethoxyphenyl)-1,4,10-trioxo-7,13-diazacyclopentadecane (6). To a suspension of 5 (870 mg, 1.78 mmol) and NaBH$_4$ (503 mg, 13 mmol) in anhydrous THF (18 mL), BF$_3$OEt$_2$ (8.4 mL, 67 mmol) was carefully added at room temperature. After stirring with refluxing for 4 h, the mixture was neutralized with an aqueous K$_2$CO$_3$ solution and the aqueous layer was extracted with CHCl$_3$ three times. The combined organic layer was dried over anhydrous MgSO$_4$, filtered, and concentrated under reduced pressure. The crude mixture was subjected to silica gel column chromatography (PSQ 100B, 5:5:1 hexane/ethylacetate/triethylamine; $R_f = 0.49$) to afford 770 mg (1.67 mmol, 94%) of 6 as colorless oil. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.05 (dd, 1H, $J = 7.6, 1.6$ Hz), 7.03 (d, 1H, $J = 8.4$ Hz), 6.94 (td, 1H, $J = 7.6, 1.6$ Hz), 6.88 (td, 1H, $J = 7.6, 1.6$ Hz), 6.84 (dd, 1H, $J = 7.6, 1.6$ Hz), 6.45 (d, 1H, $J = 2.8$ Hz), 6.39 (dd, 1H, $J = 8.4, 2.8$ Hz), 3.82 (s, 3H), 3.80 (s, 3H), 3.78 (s, 3H), 3.72 (t, 2H, $J = 5.4$ Hz), 3.63–3.61 (m, 8H), 3.58 (t, 2H, $J = 6.0$ Hz), 3.47 (m, 4H), 3.38 (t, 2H, $J = 5.6$ Hz), 3.34 (t, 2H, $J = 5.4$ Hz); $^{13}$C({$^1$H}) NMR (150 MHz, CDCl$_3$): $\delta$ 156.5, 155.2, 153.0, 140.6, 133.9, 124.0, 122.3, 121.0, 120.9, 111.9, 103.5, 100.2, 71.22, 71.17, 70.9, 70.0, 69.9, 55.65, 55.57, 55.5, 53.9, 53.35, 53.27, 52.7 (one aliphatic peak is overlapped). HRMS (APCI): $m/z$ calcd. for C$_{25}$H$_{37}$O$_8$N$_2$: 461.2652 ([M+H]$^+$); found. 461.2666.

7-(4-Bromo-2-methoxyphenyl)-13-(2,4-dimethoxyphenyl)-1,4,10-trioxo-7,13-diazacyclopentadeca
ne (7). To a solution of 6 (800 mg, 1.74 mmol) in anhydrous CH$_2$CN (10 mL), N-bromosuccinimide (325 mg, 1.8 mmol) in anhydrous CH$_2$CN (10 mL) was added dropwise at $-30$ °C over 10 min. After stirring at room temperature for 16 h, the mixture was quenched with an aqueous K$_2$CO$_3$ solution. All volatiles were removed under reduced pressure, and the resulting mixture was extracted with CHCl$_3$ five times. The combined organic layer was washed with water, dried over anhydrous MgSO$_4$, filtered, and concentrated under reduced pressure. The crude mixture was subjected to silica gel column chromatography (PSQ 100B, 5:5:1 hexane/ethyl acetate/triethylamine; $R_f = 0.49$) to afford 784 mg (1.45 mmol, 85%) of 7 as colorless oil. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.02–6.98 (m, 2H), 6.92–6.90 (m, 2H), 6.45 (d, 1H, $J = 2.8$ Hz), 6.39 (dd, 1H, $J = 8.8, 2.8$ Hz), 3.81 (s, 3H), 3.80 (s, 3H), 3.78 (s, 3H), 3.69 (t, 2H, $J = 5.8$ Hz), 3.67–3.58 (m, 8H), 3.56 (t, 2H, $J = 6.0$ Hz), 3.46–3.40 (m, 4H), 3.36 (t, 2H, $J = 6.0$ Hz), 3.32 (t, 2H, $J = 5.0$ Hz). $^{13}$C($^1$H) NMR (100 MHz, CDCl$_3$): $\delta$ 156.6, 155.3, 153.6, 139.7, 133.8, 124.2, 123.7, 122.1, 115.2, 114.2, 103.4, 100.1, 71.1, 71.0, 70.9, 69.9, 69.7, 55.8, 55.6, 55.5, 53.9, 53.3, 53.2, 52.7 (one aliphatic peak is overlapped). HRMS (APCI): $m/z$ calcd. for C$_{23}$H$_{38}$O$_5$N$_3$Br: 539.1757 ([M+H]$^+$); found. 539.1738.

2-Trimethylsilyl-3-(4-tert-butyldimethylsiloxyphenyl)-1-phenylbenzo[b]phosphole-P-oxide (8). A solution of 3-bromo-1-phenyl-2-trimethylsilylbenzo[b]phosphole-P-oxide (1.48 g, 3.94 mmol), 4-(tert-butyldimethylsiloxy)phenylboronic acid (1.30 g, 5.16 mmol), Pd(PPh$_3$)$_4$ (89 mg, 77 $\mu$mol), and K$_2$PO$_4$ (3.77 g, 17.8 mmol) in a mixture of degassed toluene (32 mL) and degassed H$_2$O (8 mL) was stirred at 90 °C for 12 h. Then, the organic layer was separated, and the aqueous layer was extracted with ethyl acetate three times. The combined organic layer was washed with water, dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (PSQ 100B, 5:1 CHCl$_3$/ethyl acetate) followed by GPC to afford 1.78 g (3.43 mmol, 85%) of 8 as colorless oil (9:1 CHCl$_3$/ethyl acetate; $R_f = 0.25$). $^1$H NMR (400 MHz, THF-d$_8$): $\delta$ 7.67 (dd, 2H, $J = 11.6, 8.4$ Hz), 7.50–7.38 (m, 2H), 7.38–7.32 (m, 3H), 7.32–7.15 (m, 3H), 7.00–6.91 (m, 3H), 0.99 (s, 9H), 0.22 (s, 6H), $-0.20$ (s, 9H). $^{13}$C($^1$H) NMR (150 MHz, CDCl$_3$): $\delta$ 165.3 (d, $J_{CP} = 10.5$ Hz, C), 156.3 (s, C), 145.4 (d, $J_{CP} = 34.5$ Hz, C), 135.7 (d, $J_{CP} = 57.5$ Hz, C), 135.4 (d, $J_{CP} = 99.2$ Hz, C), 132.6 (s, CH), 131.9 (s, CH), 131.2 (d, $J_{CP} = 10.5$ Hz, CH), 131.1 (d, $J_{CP} = 94.8$ Hz, C), 129.9 (d, $J_{CP} = 20.1$ Hz, C), 129.7 (br, CH), 129.4 (d, $J_{CP} = 10.5$ Hz, CH), 128.9 (d, $J_{CP} = 12.9$ Hz, CH), 128.6 (d, $J_{CP} = 10.5$ Hz, CH), 123.9 (d, $J_{CP} = 12.9$ Hz, C), 120.4 (s, CH), 25.9 (s, CH$_3$), 18.5 (s, C), 0.2 (s, CH$_3$), $-4.2$ (s, CH$_3$). $^{31}$P($^1$H) NMR (162 MHz, CDCl$_3$): $\delta$ 44.8. HRMS (APCI): $m/z$ calcd. for C$_{29}$H$_{36}$O$_5$Si$_3$P: 505.2142 ([M+H]$^+$); found. 505.2153.

2-Bromo-3-(4-hydroxyphenyl)-1-phenylbenzo[b]phosphole-P-oxide (9). A solution of N-bromosuccinimide (854 mg, 4.80 mmol) and 8 (1.69 g, 3.35 mmol) in CH$_2$CN (15 mL) was stirred under air with refluxing for 15 h. An aqueous Na$_2$SO$_4$ solution was added, and then all the volatiles were removed under reduced pressure. The resulting mixture was extracted with CHCl$_3$ four times. The combined organic layer was washed with brine, dried over anhydrous Na$_2$SO$_4$, filtered, and...
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trated under reduced pressure. Tetra(n-butyl)ammonium fluoride (952 mg, 3.64 mmol) in THF (15 mL) was then added and the mixture was stirred under air at ambient temperature for 1.5 h. After the solvent was removed under reduced pressure, water was added and the mixture was extracted with CHCl₃ four times. The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude mixture was subjected to flash column chromatography (ZIP Sphere 30 g, 4:1 CHCl₃/ethyl acetate; R_f = 0.28) to afford 917 mg (2.31 mmol, 70%) of 9 as colorless solid. ¹H NMR (400 MHz, DMSO-d₆): δ 9.97 (s, 1H), 7.76–7.63 (m, 4H), 7.62–7.53 (m, 3H), 7.48 (td, 1H, J = 7.2, 3.2 Hz), 7.39 (d, 2H, J = 8.8 Hz), 7.28 (dd, 1H, J = 7.2, 3.2 Hz), 6.97 (d, 2H, J = 8.8 Hz). ¹³C{¹H} NMR (150 MHz, DMSO-d₆): δ 158.2 (s, C), 152.2 (d, J_CP = 22.9 Hz, C), 142.0 (d, J_CP = 22.9 Hz, C), 132.9 (s, CH), 132.3 (s, CH), 131.0 (d, J_CP = 104.9 Hz, C), 130.4 (d, J_CP = 11.5 Hz, CH), 129.5 (s, CH), 128.8 (d, J_CP = 11.6 Hz, CH), 128.74 (d, J_CP = 12.9 Hz, CH), 128.66 (d, J_CP = 12.9 Hz, CH), 128.0 (d, J_CP = 104.9 Hz, C), 123.3 (d, J_CP = 10.1 Hz, CH), 122.1 (d, J_CP = 11.4 Hz, C), 116.7 (d, J_CP = 104.1 Hz, C), 115.3 (s, CH). ³¹P{¹H} NMR (162 MHz, DMSO-d₆): δ 31.2. HRMS (APCI): m/z calcd. for C₆H₅OBrP: 396.9993 ([M+H]+); found. 396.9990.

**2-Bromo-3-(4-ethoxycarbonylmethoxyphenyl)-1-phenylenbenzophosphole-P-oxide (10).** A suspension of ethyl bromoacetate (300 µL, 2.71 mmol), 9 (916 mg, 2.31 mmol), and K₂CO₃ (749 mg, 5.42 mmol) in DMF (15 mL) was stirred under air at 50 °C for 16 h. After addition of water, the aqueous layer was extracted with CHCl₃. The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude mixture was subjected to silica gel column chromatography (PSQ100B, 9:1 CHCl₃/ethyl acetate; R_f = 0.32) followed by GPC to afford 988 mg (1.96 mmol, 85%) of 10 as colorless oil. ¹H NMR (400 MHz, THF-d₇): δ 7.72 (dd, 2H, J = 12.8, 8.3, 1.2 Hz), 7.63 (dd, 1H, J = 9.2, 7.6 Hz), 7.57–7.51 (m, 1H), 7.51–7.42 (m, 5H), 7.42–7.35 (m, 1H), 7.26 (dd, 1H, J = 7.6, 3.2 Hz), 7.09 (d, 2H, J = 9.2 Hz), 4.74 (s, 2H), 4.21 (q, 2H, J = 7.2 Hz), 1.26 (t, 3H, J = 7.2 Hz). ¹³C{¹H} NMR (100 MHz, CDCl₃): δ 168.8 (s, C), 158.8 (s, C), 152.4 (d, J_CP = 23.1 Hz, C), 143.3 (d, J_CP = 23.0 Hz, C), 133.4 (d, J_CP = 1.7 Hz, CH), 133.1 (d, J_CP = 2.5 Hz, CH), 131.5 (d, J_CP = 11.6 Hz, CH), 131.3 (d, J_CP = 104.9 Hz, C), 130.4 (s, CH), 130.0 (d, J_CP = 8.6 Hz, CH), 129.3 (d, J_CP = 11.6 Hz, CH), 129.2 (d, J_CP = 13.2 Hz, CH), 128.7 (d, J_CP = 107.7 Hz, C), 125.8 (d, J_CP = 17.5 Hz, C), 124.0 (d, J_CP = 10.1 Hz, CH), 118.3 (d, J_CP = 104.9 Hz, C), 115.1 (s, CH), 65.5 (s, CH₂), 61.7 (s, CH₃), 14.4 (s, CH₃). ³¹P{¹H} NMR (162 MHz, CDCl₃): δ 30.1. HRMS (APCI): m/z calcd. for C₂₅H₂₀OBrP: 483.0361 ([M+H]+); found. 483.0349.

**NaGY-Et.** A suspension of 7 (539 mg, 1.00 mmol), bis(pinacolato)diboron (263 mg, 1.00 mmol), Pd(dppf)Cl₂ (73.4 mg, 0.10 mmol), and KOAc (206 mg, 2.12 mmol) in 1,4-dioxane (5.0 mL) was heated to 100 °C for 24 h. After complete consumption of 7, a mixture of 10 (520 mg, 1.1 mmol) and K₃PO₄ (1.12 g, 5.35 mmol) in degassed 1,4-dioxane (3.0 mL) and water (1.0 mL) was added. After stirring at 100 °C for 17 h, all the volatiles were removed under reduced pressure. After addition of water and CHCl₃, the organic layer was separated, and the aqueous layer was extracted with CHCl₃.
five times. The combined organic layer was washed with water, dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude mixture was subjected to silica gel column chromatography (PSQ 100B, the eluent was gradually changed from 4:6:1 hexane/CHCl₃/Et₂N to 9:1:1 CHCl₃/MeOH/Et₂N). The crude product was further purified by GPC and HPLC (4:6:1 hexane/CHCl₃/Et₂N) to afford 103 mg (0.12 mmol, 12%) of NaGY-Et as yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 7.80 (dd, 2H, J = 12.4, 8.0 Hz), 7.64 (dd, 1H, J =9.2, 7.6 Hz), 7.53–7.38 (m, 4H), 7.35–7.28 (m, 3H), 7.13 (dd, 1H, J = 8.4, 1.6 Hz), 7.01 (d, 2H, J = 8.8 Hz), 6.98 (d, 1H, J = 8.8 Hz), 6.86 (dd, 1H, J =8.4, 1.6 Hz), 6.65 (d, 1H, J = 8.4 Hz), 6.62 (s, 1H), 6.44 (d, 1H, 2.4 Hz), 6.37 (dd, 1H, J = 8.8, 2.4 Hz), 4.67 (s, 2H), 4.29 (q, 2H, J = 7.2 Hz), 3.78 (s, 3H), 3.76 (s, 3H), 3.65 (t, 2H, J = 5.2 Hz), 3.62–3.56 (m, 8H), 3.52 (t, 2H, J = 6.0 Hz), 3.46–3.35 (m, 7H), 3.33 (t, 2H, J = 6.0 Hz), 3.28 (t, 2H, J = 5.2 Hz), 1.32 (t, 3H, J = 7.2 Hz). ¹³C{¹H} NMR (150 MHz, CDCl₃): δ 168.8 (s, C), 158.1 (s, C), 156.5 (s, C), 155.2 (s, C), 151.2 (s, C), 146.9 (d, JCP = 22.9 Hz, C), 144.6 (d, JCP = 27.3 Hz, C), 140.5 (s, C), 133.9 (s, C), 133.8 (d, JCP = 96.3 Hz, C), 133.0 (s, CH), 132.2 (d, JCP = 2.8 Hz, CH), 132.0 (d, JCP = 106.3 Hz, C), 131.1 (d, JCP = 10.1 Hz, CH), 131.0 (d, JCP = 99.1 Hz, C), 130.7 (s, CH), 129.0 (d, JCP = 11.5 Hz, CH), 128.7 (d, JCP = 10.1 Hz, CH), 128.5 (d, JCP = 15.7 Hz, C), 125.4 (d, JCP = 10.1 Hz, C), 123.9 (s, CH), 123.6 (d, JCP = 11.4 Hz, CH), 122.4 (d, JCP = 5.7 Hz, CH), 118.6 (s, CH), 115.5 (s, CH), 112.6 (d, JCP = 7.1 Hz, CH), 103.5 (s, CH), 100.1 (s, CH), 71.2 (s, CH₂), 70.9 (s, CH₂), 69.8 (s, CH₂), 65.5 (s, CH₂), 61.7 (s, CH₂), 55.6 (s, CH₂), 55.5 (s, CH₂), 55.0 (s, CH₂), 53.9 (s, CH₂), 53.2 (s, CH₂), 53.0 (s, CH₂), 52.5(s, CH₂), 14.4 (s, CH₂) (one aromatic peak and two aliphatic are overlapped). ³¹P{¹H} NMR (162 MHz, CDCl₃): δ 40.6. HRMS (APCI): m/z calcd. for C₉₀H₆₈N₄O₁₀P: 863.3667 ([M+H]+); found: 863.3629.

NaGY. A solution of NaGY-Et (13 mg, 15 µmol) and LiOH·H₂O (1.3 mg, 30 µmol) in MeOH (1.0 mL) and water (0.5 mL) was stirred under air at ambient temperature for 90 min. Then all the volatiles were removed under reduced pressure. After addition of water and CHCl₃, the organic layer was separated, and the aqueous layer was extracted with CHCl₃ three times. The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude mixture was subjected to reversed phase silica gel column chromatography and reversed-phase HPLC (the eluent was gradually changed from 4:1 H₂O/CH₂CN with 0.1% TFA to 1:1 H₂O/CH₂CN with 0.1% TFA). Lyophilization of the obtained product gave 10 mg (1.2 mmol, 83%) of NaGY as yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 7.97–7.21 (m, 12H), 7.16–6.89 (br, 4H), 7.85–6.56 (m, 2H), 6.43 (br, 1H), 4.69 (s, 2H), 4.13–2.92 (m, 29H). ¹³C{¹H} NMR (150 MHz, CDCl₃): δ 170.7 (s, C), 162.2 (s, C), 158.8 (s, C), 153.1 (s, C), 152.0 (s, C), 150.2 (d, JCP = 17.3 Hz, C), 143.9 (d, JCP = 25.8 Hz, C), 133.4 (s, CH), 133.9 (d, JCP = 96.5 Hz, C), 132.6 (s, CH), 131.8 (d, JCP = 106.3 Hz, C), 131.1 (d, JCP = 10.1 Hz, CH), 130.5 (s, CH), 130.0 (d, JCP = 99.1 Hz, C), 129.5 (d, JCP = 10.1 Hz, CH), 129.2 (d, JCP = 11.4 Hz, CH), 127.0 (d, JCP = 14.3 Hz, CH), 125.4 (s, CH), 124.3 (d, JCP = 11.5 Hz, CH), 122.5 (s, CH), 121.3 (s, CH), 118.7 (s, C), 117.7 (s, C), 115.8 (s, C), 115.5 (s, CH), 113.2 (d, JCP = 5.7 Hz, CH), 106.3 (s, CH), 100.1 (s, CH), 70.2 (s, CH₂), 70.1 (s, CH₂), 68.8 (s, CH₂), 68.2 (s,
CH₂), 65.3 (s, CH₂), 64.4 (s, CH₂), 64.3 (s, CH₂), 57.4 (s, CH₂), 57.3 (s, CH₂), 56.6 (s, CH₃), 56.0 (s, CH₃), 55.7 (s, CH₃), 55.2 (s, CH₂), 53.4 (s, CH₂) (one aliphatic peak is overlapped). ³¹P{¹H} NMR (162 MHz, CD₂Cl₂): δ 40.7. HRMS (APCI): m/z calcd. for C₄₇H₅₂N₂O₁₀P: 835.3354 ([M+H]+); found. 835.3360.

NaGY-AM. A solution of NaGY (49.8 mg, 0.06 mmol), bromomethyl acetate (70 µL, 0.71 mmol), and ethyldi(isopropyl)amine (30 µL, 0.18 mmol) in CH₂Cl₂ (2.5 mL) was stirred at room temperature for 18 h. After all the volatiles were removed under reduced pressure, the mixture was washed with hexane three times and purified by reversed-phase HPLC (the eluent was gradually changed from 1:1 H₂O/CH₃CN with 5 mM ammonium formate to CH₃CN with 5 mM ammonium formate). Lyophilization of the obtained product gave NaGY-AM as an a yellow solid (5.1 mg, 9%); however, the quantifies were not sufficient to measure the ¹³C NMR spectrum. ¹H NMR (400 MHz, acetone-d₆) δ 7.84 (d, 2H, J = 7.2 Hz, 12.0 Hz), 7.66-7.36 (m, 9H), 7.20-7.13 (m, 3H), 7.10-6.93 (m, 1H), 6.74 (br, 2H), 6.56-6.35 (m, 2H), 5.84 (s, 2H), 4.92 (s, 2H), 3.77 (s, 3H), 3.75 (s, 3H), 3.68-3.49 (m, 10H), 3.48-3.30 (m, 9H), 3.24 (t, 2H, J = 6.0 Hz), 3.18 (t, 2H, J = 4.8 Hz), 2.07 (s, 3H). ³¹P{¹H} NMR (162 MHz, CD₂Cl₂): δ 40.6. HRMS (ESI): m/z calcd. for C₅₀H₅₆N₂O₁₂P: 907.3571 ([M+H]+); found. 907.3587.
2. Photophysical properties

Photophysical measurements. All spectroscopic measurements of NaGY were performed in 50 mM HEPES buffer (pH 7.4) containing 1% DMSO. UV-vis absorption spectra of NaGY were recorded on a Shimadzu UV-3150 spectrometer with a resolution of 0.2 nm using a 100 µM solution in a 1 cm square quartz cuvette. Emission spectra (25 µM of NaGY) were measured using a Hitachi F-4500 spectrometer with a slit width of 10 nm. The photomultiplier voltage was 700 V. Absolute fluorescence quantum yields were determined with a Hamamatsu photonics PMA-11 calibrated integrating sphere system.

Metal ion selectivity. The fluorescence spectra of NaGY was measured in the presence of 150 mM Na⁺ and K⁺, 10 mM Cu²⁺ and Mg²⁺, or 0.1 mM of Mn²⁺, Fe³⁺, Ni²⁺, Cu²⁺, and Zn²⁺. All metal sources used here were chloride salt. For Na⁺ and K⁺, each chloride salt was directly added to the solution of 25 µM NaGY (2.0 mL) in a cuvette. For Ca²⁺ and Mg²⁺, 1 M stock solutions in 50 mM HEPES buffer (pH 7.4) were prepared. For Mn²⁺, Ni²⁺, Cu²⁺, and Zn²⁺, 10 mM stock solutions of the metal salt in 50 mM HEPES buffer (pH 7.4) were prepared. For Fe³⁺, a 10 mM stock solution in 1 mM HCl aq (pH ~3) was prepared. Subsequently, a 20 µL of the stock solution was added to the 2.0 mL of sample. To the each solution containing the indicated metal ion, NaCl (17.53 mg, 0.3 mmol) was directly added. The samples were stirred for 5 min and the emission spectra were measured. The fluorescence intensity ratios between 575 and 700 nm (I_575/I_700) were calculated.

pH Dependence. The fluorescence spectra of NaGY (25 µM) were measured in a buffered solution at various pH values. Each pH-buffered solution was prepared with MES for pH 5.5–7 and HEPES for pH 7.5 and 8, where pH values were adjusted by using Me₄NOH·5H₂O.

3. Determination of dissociation constant (K_d)

Absorption spectroscopic titration of NaGY with Na⁺ was performed in 50 mM HEPES buffer (pH 7.4) containing 1% DMSO. An initial absorption spectrum of NaGY (100 µM) was measured and an appropriate amount of NaCl was directly added to the solution. The absorbance at 410 nm (A_{410}) was plotted against total [Na⁺], and the experimental data were analyzed by non-linear least square curve fitting using the following equation (eq. 1):

\[ A_{410} = \frac{A_0K_d + A_\infty [Na^+]}{K_d + [Na^+]} \]  (eq. 1)

where A₀ and A_∞ represent the initial and final absorbance values at 410 nm, respectively.

Ratiometric titration of NaGY with Na⁺ in fluorescence was conducted according to the experimental procedure described above, except the concentration of NaGY used (25 µM). The ratio of the fluorescence intensities at 575 and 700 nm (I_575/I_700) was plotted against total [Na⁺]. The data was analyzed by curve fitting using the following equation (eq. 2):

\[ A_{410} = \frac{A_0K_d + A_\infty [Na^+]}{K_d + [Na^+]} \]  (eq. 2)
\[ \frac{I_{575}}{I_{700}} = \frac{R_{\text{max}} K_d [\text{Na}^+] + R_{\text{min}}}{1 + K'_d [\text{Na}^+]}, \quad K'_d = K_d \left( \frac{S_{f2}}{S_{b2}} \right) \]  

(eq. 2)

where \( R_{\text{min}} \) and \( R_{\text{max}} \) are the minimum and maximum ratio values, respectively. The expression \( S_{f2} \) and \( S_{b2} \) are the emission intensities of the Na\(^+\)-free and Na\(^+\)-bound forms at 700 nm. The determination of \( K_d \) values for K\(^+\) as well as Na\(^+\) in the presence of 150 mM K\(^+\) were performed in the same manner described above, except for the selected wavelengths for the calculations (\( I_{575}/I_{720} \) for K\(^+\) and \( I_{540}/I_{675} \) for Na\(^+\) in the presence of 150 mM K\(^+\), respectively).

4. Cell culture experiments

HeLa cells (RIKEN Cell Bank, Japan) were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Sigma) containing 10% fetal bovine serum (FBS, Gibco) and 1% Antibiotic-Antimycotic (AA, Sigma) at 37 °C in a 5% CO\(_2\)/95% air incubator. Cells (5 \( \times \) 10\(^4\)) were seeded in glass-bottom 8-well plates three days before imaging. For ratiometric imaging of intracellular Na\(^+\), the incubation medium was removed from the cells, and then the cells were incubated with 10 \( \mu \)M NaGY-AM in DMEM for 30 min at 37 °C and rinsed three times with DMEM. After the plate was filled with 200 \( \mu \)L of DMEM, the fluorescence images were recorded with a 405 nm laser using LSM 780 confocal laser-scanning microscope (Zeiss) equipped with a GaAsP multi-channel spectral detector. For the ratiometric imaging of intracellular Na\(^+\), the integrated emission intensities in the range of 565–574 nm (\( I_{565-574} \)) and 662–689 nm (\( I_{662-689} \)), corresponding to the Na\(^+\)-bound and Na\(^+\)-free forms, respectively, were collected using a ImageJ software and the ratio images (\( I_{565-574} \) and \( I_{662-689} \)) were obtained on a pixel-by-pixel basis.

Imaging of Na\(^+\) dynamics in living cells was performed as follows: the incubation culture was removed from the cells 10 min after starting acquisition, and then the K\(^+\)-free medium containing 140 mM Na\(^+\) in PBS (pH 7.4) was added to the cells.

5. References

**Fig. S1** Absorption (dashed line) and emission (solid line) spectra of NaGY in 50 mM HEPES buffer (pH 7.4) containing 1% DMSO. Red and blue lines represent before and after addition of 200 mM Na\(^+\), respectively. The emission spectra were measured with the excitation wavelength at 405 nm.

**Fig. S2** (A) Absorption spectral change of NaGY upon addition of various concentration of Na\(^+\) in an aqueous buffered solution. (B) Plots of the absorbance at 410 nm ($A_{410}$) as a function of [Na\(^+\)] with best-fit curves for the dissociation constant 14.0 ± 0.1 mM.
**Fig. S3** Difference spectra between Na⁺-bound and Na⁺-free forms during the titration of NaGY (25 µM) with Na⁺ (0, 5, 10, 20, 40, 65, 100, and 200 mM) in 50 mM HEPES (pH 7.4) with the excitation at 405 nm.

**Fig. S4** (A) Emission spectral change of NaGY (25 µM) upon addition of KCl (0, 50, 100, 200, 350, 650, and 1000 mM) in 50 mM HEPES (pH 7.4) with the excitation at 405 nm. (B) Plots of the fluorescence intensity ratio between 575 and 720 nm ($I_{575}/I_{720}$) with a best curve fitting for a dissociation constant of 223 ± 7 mM.
Fig. S5 (A) Emission spectral change of NaGY (25 μM) upon addition of NaCl (0, 5, 10, 20, 30, 45, 60, and 80 mM) in 50 mM HEPES (pH 7.4) containing 150 mM of K+ with the excitation at 405 nm. (B) Plots of the fluorescence intensity ratio between 540 and 675 nm ($I_{540}/I_{675}$) with a best curve fitting for a dissociation constant of 32 ± 2.1 mM.

Fig. S6 Plots of the fluorescence intensity ratio between 575 and 700 nm as a function of pH value for 25 μM NaGY.
**Fig. S7** Trypan blue exclusion tests of cell viability. Cells were incubated without or with NaGY (5 and 10 μM) at 37 °C for 4 h. Error bar = S. D. (N = 6)

**Fig. S8** Plots of the integrated fluorescence intensity ratio between 565-574 nm ($I_{565-574}$) and 662-689 nm ($I_{662-689}$) observed in Fig. 3 with a best curve fitting for a dissociation constant of 16.8 ± 1.0 mM.
6. NMR spectra

Fig. S6 $^1$H NMR spectrum (400 MHz) of 2 in DMSO-$d_6$ at 80 °C.

Fig. S7 $^{13}$C NMR spectrum (100 MHz) of 2 in DMSO-$d_6$ at 80 °C.
**Fig. S8** $^1$H NMR spectrum (400 MHz) of 3 in DMSO-$d_6$ at 70 °C.

**Fig. S9** $^{13}$C NMR spectrum (100 MHz) of 3 in DMSO-$d_6$ at 70 °C.
Fig. S10 $^1$H NMR spectrum (400 MHz) of 4 in CDCl$_3$.

Fig. S11 $^{13}$C NMR spectrum (150 MHz) of 4 in CDCl$_3$. 
Fig. S12 $^1$H NMR spectrum (400 MHz) of 5 in DMSO-$d_6$ at 150 °C.

Fig. S13 $^{13}$C NMR spectrum (100 MHz) of 5 in DMSO-$d_6$ at 150 °C.
Fig. S14 $^1$H NMR spectrum (400 MHz) of 6 in CDCl$_3$.

Fig. S15 $^{13}$C NMR spectrum (150 MHz) of 6 in CDCl$_3$. 
Fig. S16 \(^1\)H NMR spectrum (400 MHz) of 7 in CDCl\(_3\).

Fig. S17 \(^{13}\)C NMR spectrum (100 MHz) of 7 in CDCl\(_3\).
Fig. S18 $^1$H NMR spectrum (400 MHz) of 8 in THF-$d_8$.

Fig. S19 $^{13}$C NMR spectrum (150 MHz) of 8 in CDCl$_3$. 
Fig. S20 $^{31}$P NMR spectrum (162 MHz) of 8 in CDCl$_3$.

Fig. S21 $^1$H NMR spectrum (400 MHz) of 9 in DMSO-$d_6$. 

S23
**Fig. S22** $^{13}$C NMR spectrum (150 MHz) of 9 in DMSO-$d_6$.

**Fig. S23** $^{31}$P NMR spectrum (162 MHz) of 9 in DMSO-$d_6$. 
Fig. S24 $^1$H NMR spectrum (400 MHz) of 10 in THF-$d_8$.

Fig. S25 $^{13}$C NMR spectrum (100 MHz) of 10 in CDCl$_3$. 
Fig. S26 $^{31}$P NMR spectrum (162 MHz) of 10 in CDCl$_3$.

Fig. S27 $^1$H NMR spectrum (400 MHz) of NaGY-Et in CDCl$_3$. 
Fig. S28 $^{13}$C NMR spectrum (150 MHz) of NaGY-Et in CDCl$_3$.

Fig. S29 $^{31}$P NMR spectrum (162 MHz) of NaGY-Et in CDCl$_3$. 
Fig. S30 $^1$H NMR spectrum (400 MHz) of NaGY in CD$_2$Cl$_2$.

Fig. S31 $^{13}$C NMR spectrum (150 MHz) of NaGY in CD$_2$Cl$_2$. 

S28
Fig. S32 $^{31}$P NMR spectrum (162 MHz) of NaGY in CD$_2$Cl$_2$.

Fig. S33 $^1$H NMR spectrum (400 MHz) of NaGY-AM in acetone-$d_6$. 
Fig. S33 $^{31}$P NMR spectrum (162 MHz) of NaGY-AM in CDCl$_3$. 