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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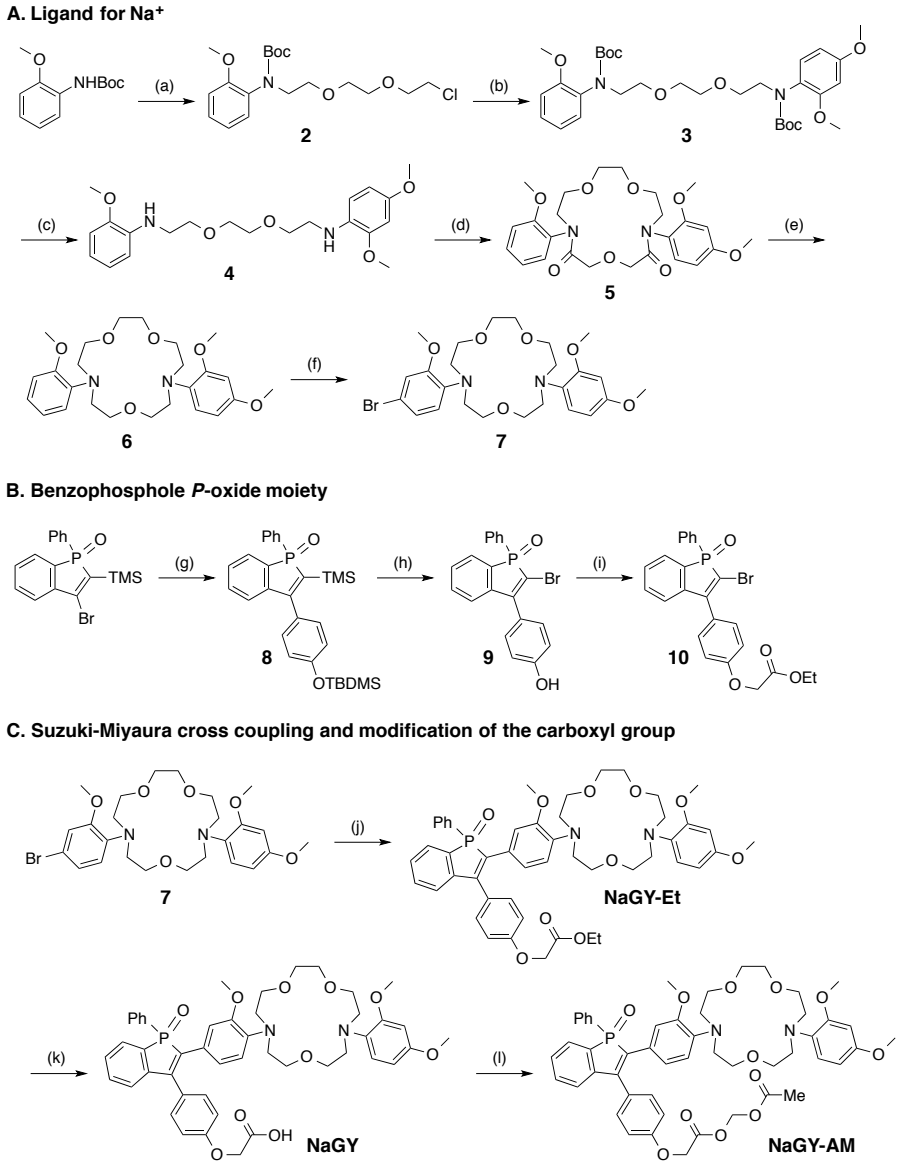
Contents

1. Experimental Details

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_2Cl_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_2Cl_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_2Cl_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_3PO_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 (JAIGEL 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,[1] *tert*-butyl (2,4-dimethoxyphenyl)carbamate,[2] and 3-bromo-1-phenyl-2-trimethylsilyl benzo[*b*]phosphole-*P*-oxide[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.



Scheme. S1 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/CH_2Cl_2, 1.5 h, 88%; (d) diglycolyl chloride, pyridine, toluene, 100 °C, 3 d, 35%; (e) $BF_3{\cdot}OEt_2$, $NaBH_4$, THF, reflux, 4 h, 94%; (f) NBS, CH_3CN, –30 °C, 16 h, 85%; (g) 4-(*tert*-butyldimethylsilyloxy)phenylboronic acid, K_3PO_4, $Pd(PPh_3)_4$, 90 °C, 12 h, 85%; (h) i, NBS, CH_3CN, reflux, 15 h; ii, TBAF, THF, 1.5 h, 70%; (i) ethyl bromoacetate, K_2CO_3, DMF, 50 °C, 16 h, 85%; (j) i, bis(pinacolato)diboron, $Pd(dppf)Cl_2$, KOAc, 1,4-dioxane, 100 ºC, 24 h; ii, **10**, $Pd(dppf)Cl_2$, K_3PO_4, 1,4-dioxane, 100 °C, 17 h 12%; (k) $LiOH{\cdot}H_2O$, H_2O, MeOH, 1.5 h, 83%; (l) bromomethyl acetate, $Et(iPr)_2N$, CH_2Cl_2, 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 $CHCl_3$ three times. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was subjected to silica gel chromatography (PSQ 100B, 3:1 hexane/ethyl acetate; R_f = 0.33) to afford 636 mg (1.71 mmol, 77%) of **2** as colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6, 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). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6, 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 $C_{18}H_{29}O_5NCl$: 374.1734 ([M+H]$^+$); found. 374.1750.

***N*,*N*′-Di(*tert*-butoxycarbonyl)-*N*-(2,4-dimethoxyphenyl)-*N*′-(2-methoxyphenyl)-4,7-dioxa-1,10-diazadecane (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 $CHCl_3$ three times. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, 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; R_f = 0.14) to afford 15.5 g (26.2 mmol, 68%) of **3** as colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6, 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). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6, 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, 55.24, 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 $C_{31}H_{47}O_9N_2$: 591.3282 ([M+H]$^+$); found. 591.3273.

1-(2-Methoxyphenyl)-10-(2,4-dimethoxyphenyl)-4,7-dioxa-1,10-diazadecane (4). To a solution of **3** (9.78 g, 16.5 mmol) in CH_2Cl_2 (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 $NaHCO_3$ solution. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 five times. The combined organic layer was washed with water, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was subjected to flash column chromatography (ZIP Sphere 45 g, 1:3 hexane/ethyl acetate; R_f = 0.32) to afford 5.67 g (14.5 mmol, 88%) of **4** as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.86 (td, 1H, J = 7.8, 1.4 Hz), 6.76 (dd, 1H, J = 7.8, 1.4 Hz), 6.68 (td, 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,

2H, J = 5.4 Hz). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$): δ 152.2, 148.5, 147.2, 138.3, 132.6, 121.3, 116.8, 110.6, 110.1, 109.6, 103.8, 99.3, 70.5, 70.1, 70.0, 55.9, 55.6, 55.5, 44.2, 43.4 (one aliphatic peak is overlapped). HRMS (APCI): m/z calcd. for $C_{21}H_{31}O_5N_2$: 391.2233 ($[M+H]^+$); found. 391.2244.

7-(2-Methoxyphenyl)-13-(2,4-dimethoxyphenyl)-1,4,10-trioxa-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_2Cl_2, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 three times. The combined organic layers were washed twice with water, dried over Na_2SO_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. ^1H NMR (400 MHz, DMSO-d_6, VT 150 °C): δ 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). $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d_6, VT 150 °C): δ 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 $C_{25}H_{33}O_8N_2$: 489.2237 ($[M+H]^+$); found. 489.2218.

7-(2-Methoxyphenyl)-13-(2,4-dimethoxyphenyl)-1,4,10-trioxa-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), $BF_3{\cdot}OEt_2$ (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_2CO_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. ^1H NMR (400 MHz, $CDCl_3$): δ 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), 3.68–3.61 (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); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$): δ 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, 55.53, 53.9, 53.35, 53.27, 52.7 (one aliphatic peak is overlapped). HRMS (APCI): m/z calcd. for $C_{25}H_{37}O_6N_2$: 461.2652 ($[M+H]^+$); found. 461.2666.

7-(4-Bromo-2-methoxyphenyl)-13-(2,4-dimethoxyphenyl)-1,4,10-trioxa-7,13-diazacyclopentadeca

ne (7). To a solution of **6** (800 mg, 1.74 mmol) in anhydrous CH_3CN (10 mL), *N*-bromosuccinimide (325 mg, 1.8 mmol) in anhydrous CH_3CN (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_2CO_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, $CDCl_3$): δ 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, $CDCl_3$): δ 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 $C_{25}H_{36}O_6N_2Br$: 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 μmol), and K_3PO_4 (3.77 g, 17.8 mmol) in a mixture of degassed toluene (32 mL) and degassed H_2O (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_2SO_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): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 44.8. HRMS (APCI): m/z calcd. for $C_{29}H_{38}O_2Si_2P$: 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_3CN (15 mL) was stirred under air with refluxing for 15 h. An aqueous Na_2SO_3 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_2SO_4, filtered, and

concentrated 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 $CHCl_3$ four times. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was subjected to flash column chromatography (ZIP Sphere 30 g, 4:1 $CHCl_3$/ethyl acetate; R_f = 0.28) to afford 917 mg (2.31 mmol, 70%) of **9** as colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): 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). ^{13}C{^{1}H} NMR (150 MHz, DMSO-d_6): 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). ^{31}P{^{1}H} NMR (162 MHz, DMSO-d_6): δ 31.2. HRMS (APCI): *m*/*z* calcd. for $C_{20}H_{15}O_2BrP$: 396.9993 ([*M*+H]$^+$); found. 396.9990.

2-Bromo-3-(4-ethoxycarbonylmethyloxyphenyl)-1-phenylbenzo[*b*]phosphole-*P*-oxide (10). A suspension of ethyl bromoacetate (300 μL, 2.71 mmol), **9** (916 mg, 2.31 mmol), and K_2CO_3 (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 $CHCl_3$. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was subjected to silica gel column chromatography (PSQ100B, 9:1 $CHCl_3$/ethyl acetate; R_f = 0.32) followed by GPC to afford 988 mg (1.96 mmol, 85%) of **10** as colorless oil. ^{1}H NMR (400 MHz, THF-d_8): δ 7.72 (ddd, 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). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): 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, CH_2), 61.7 (s, CH_2), 14.4 (s, CH_3). ^{31}P{^{1}H} NMR (162 MHz, $CDCl_3$): δ 30.1. HRMS (APCI): *m*/*z* calcd. for $C_{24}H_{21}O_4BrP$: 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)Cl_2 (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 K_3PO_4 (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 $CHCl_3$, the organic layer was separated, and the aqueous layer was extracted with $CHCl_3$

five times. The combined organic layer was washed with water, dried over $MgSO_4$, 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/$CHCl_3$/Et_3N to 9:1:1 $CHCl_3$/MeOH/Et_3N). The crude product was further purified by GPC and HPLC (4:6:1 hexane/$CHCl_3$/Et_3N) to afford 103 mg (0.12 mmol, 12%) of **NaGY-Et** as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$): δ 168.8 (s, C), 158.1 (s, C), 156.5 (s, C), 155.2 (s, C), 151.2 (s, C), 146.9 (d, J_{CP} = 22.9 Hz, C), 144.6 (d, J_{CP} = 27.3 Hz, C), 140.5 (s, C), 133.9 (s, C), 133.8 (d, J_{CP} = 96.3 Hz, C), 133.0 (s, CH), 132.2 (d, J_{CP} = 2.8 Hz, CH), 132.0 (d, J_{CP} = 106.3 Hz, C), 131.1 (d, J_{CP} = 10.1 Hz, CH), 131.0 (d, J_{CP} = 99.1 Hz, C), 130.7 (s, CH), 129.0 (d, J_{CP} = 11.5 Hz, CH), 128.7 (d, J_{CP} = 10.1 Hz, CH), 128.5 (d, J_{CP} = 15.7 Hz, C), 125.4 (d, J_{CP} = 10.1 Hz, C), 123.9 (s, CH), 123.6 (d, J_{CP} = 11.4 Hz, CH), 122.4 (d, J_{CP} = 5.7 Hz, CH), 118.6 (s, CH), 115.5 (s, CH), 112.6 (d, J_{CP} = 7.1 Hz, CH), 103.5 (s, CH), 100.1 (s, CH), 71.2 (s, CH_2), 70.9 (s, CH_2), 70.9 (s, CH_2), 69.8 (s, CH_2), 65.5 (s, CH_2), 61.7 (s, CH_2), 55.6 (s, CH_3), 55.5 (s, CH_3), 55.0 (s, CH_3), 53.9 (s, CH_2), 53.2 (s, CH_2), 53.0 (s, CH_2), 52.5(s, CH_2), 14.4 (s, CH_3) (one aromatic peak and two aliphatic are overlapped). ^{31}P{^{1}H} NMR (162 MHz, $CDCl_3$): δ 40.6. HRMS (APCI): m/z calcd. for $C_{49}H_{56}N_2O_{10}P$: 863.3667 ([M+H]$^+$); found. 863.3629.

NaGY. A solution of **NaGY-Et** (13 mg, 15 μmol) and $LiOH{\cdot}H_2O$ (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 $CHCl_3$, the organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, 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 H_2O/CH_3CN with 0.1% TFA to 1:1 H_2O/CH_3CN with 0.1% TFA). Lyophilization of the obtained product gave 10 mg (1.2 mmol, 83%) of **NaGY** as yellow solid. ^{1}H NMR (400 MHz, CD_2Cl_2): 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). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$): δ 170.7 (s, C), 162.2 (s, C), 158.8 (s, C), 153.1 (s, C), 152.0 (s, C), 150.2 (d, J_{CP} = 17.3 Hz, C), 143.9 (d, J_{CP} = 25.8 Hz, C), 133.4 (s, CH), 133.9 (d, J_{CP} = 96.5 Hz, C), 132.6 (s, CH), 131.8 (d, J_{CP} = 106.3 Hz, C), 131.1 (d, J_{CP} = 10.1 Hz, CH), 130.5 (s, CH), 130.0 (d, J_{CP} = 99.1 Hz, C), 129.5 (d, J_{CP} = 10.1 Hz, CH), 129.2 (d, J_{CP} = 11.4 Hz, CH), 127.0 (d, J_{CP} = 14.3 Hz, CH), 125.4 (s, CH), 124.3 (d, J_{CP} = 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, J_{CP} = 5.7 Hz, CH), 106.3 (s, CH), 100.1 (s, CH), 70.2 (s, CH_2), 70.1 (s, CH_2), 68.8 (s, CH_2), 68.2 (s,

CH_2), 65.3 (s, CH_2), 64.4 (s, CH_2), 64.3 (s, CH_2), 57.4 (s, CH_2), 57.3 (s, CH_2), 56.6 (s, CH_3), 56.0 (s, CH_3), 55.7 (s, CH_3), 55.2 (s, CH_2), 53.4 (s, CH_2) (one aliphatic peak is overlapped). $^{31}P\{^1H\}$ NMR (162 MHz, CD_2Cl_2): δ 40.7. HRMS (APCI): *m/z* calcd. for $C_{47}H_{52}N_2O_{10}P$: 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 CH_2Cl_2 (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 H_2O/CH_3CN with 5 mM ammonium formate to CH_3CN 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 ^{13}C NMR spectrum. ^1H NMR (400 MHz, acetone-d_6) 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). $^{31}P\{^1H\}$ NMR (162 MHz, CD_2Cl_2): δ 40.6. HRMS (ESI): *m/z* calcd. for $C_{50}H_{56}N_2O_{12}P$: 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 Ca^{2+} and Mg^{2+}, or 0.1 mM of Mn^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}. 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 Ca^{2+} and Mg^{2+}, 1 M stock solutions in 50 mM HEPES buffer (pH 7.4) were prepared. For Mn^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}, 10 mM stock solutions of the metal salt in 50 mM HEPES buffer (pH 7.4) were prepared. For Fe^{3+}, 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 $Me_4NOH{\cdot}5H_2O$.

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):

$$A_{410} = \frac{A_0 K_d + A_\infty [\mathrm{Na}^+]}{K_d + [\mathrm{Na}^+]} \qquad \text{(eq. 1)}$$

where A_0 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):

$$I_{575}/I_{700} = \frac{R_{\max}K_d'[\mathrm{Na}^+]+R_{\min}}{1+K_d'[\mathrm{Na}^+]}, \quad K_d' = K_d\left(\frac{S_{f2}}{S_{b2}}\right) \qquad \text{(eq. 2)}$$

where R_{min} and R_{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 (5×10^4) 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 μM **NaGY-AM** in DMEM for 30 min at 37 °C and rinsed three times with DMEM. After the plate was filled with 200 μL 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 staring acquisition, and then the K^+-free medium containing 140 mM Na^+ in PBS (pH 7.4) was added to the cells.

5. References

1. I. Nakamura, U. Yamagishi, D. Song, S. Konta, Y. Yamamoto, *Angew. Chem. Int. Ed.,* 2007, **46**, 2284–2287.
2. T. Hashimoto, H. Nakatsu, Y. Takiguchi, K. Maruoka, *J. Am. Chem. Soc.,* 2013, **135**, 16010–16013.
3. A. Fukazawa, Y. Ichihashi, Y. Kosaka, S. Yamaguchi, *Chem. Asian. J.,* 2009, **4**, 1729–1740.

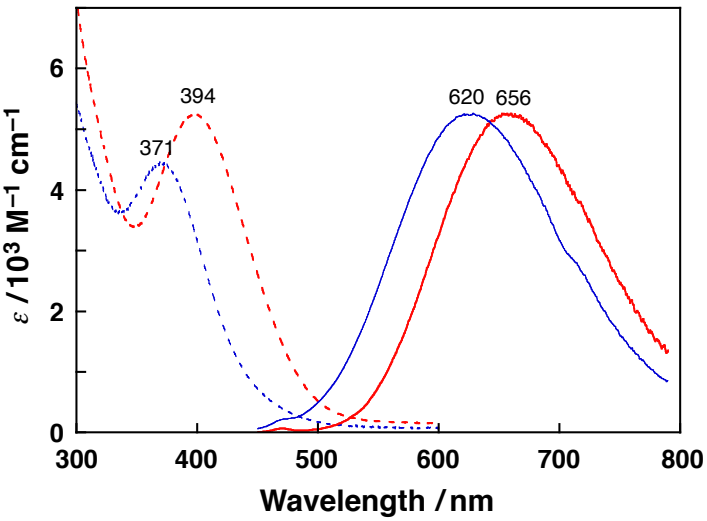


Fig. S1 Absorption (dashed line) and emission (solid line) spectra of **NaGY** in 50 mM HEPES buffer (pH 7.4) containing 1% DMSO. Red and blue lines represent before and after addition of 200 mM Na^+, respectively. The emission spectra were measured with the excitation wavelength at 405 nm.

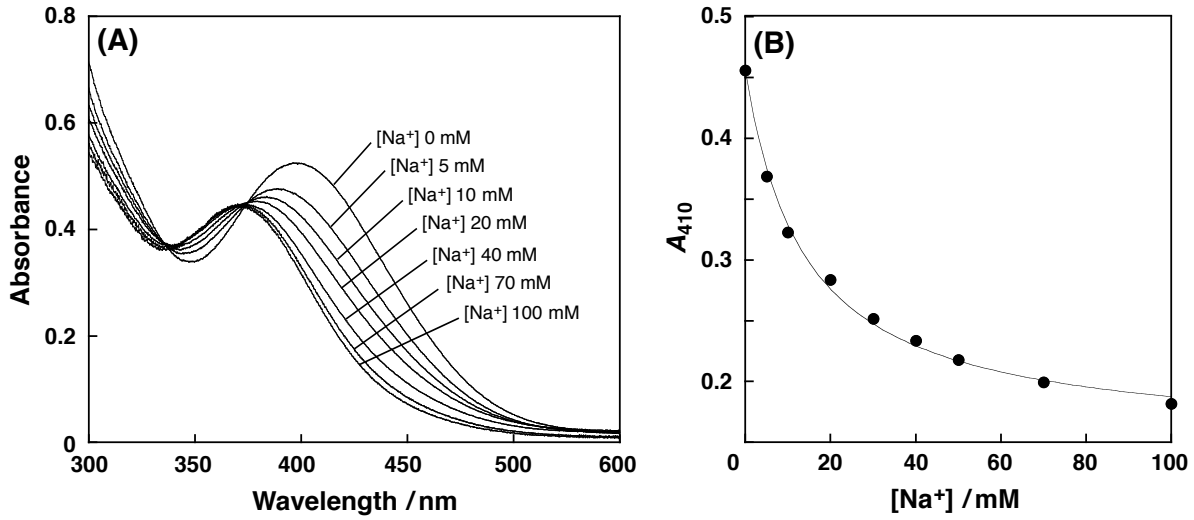


Fig. S2 (A) Absorption spectral change of **NaGY** upon addition of various concentration of Na^+ in an aqueous buffered solution. (B) Plots of the absorbance at 410 nm (A_{410}) as a function of [Na^+] with best-fit curves for the dissociation constant 14.0 ± 0.1 mM.

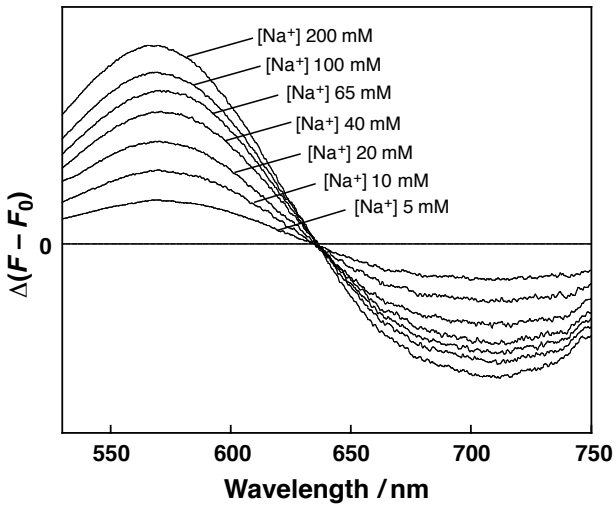


Fig. S3 Difference spectra between Na^+-bound and Na^+-free forms during the titration of **NaGY** (25 μM) with Na^+ (0, 5, 10, 20, 40, 65, 100, and 200 mM) in 50 mM HEPES (pH 7.4) with the excitation at 405 nm.

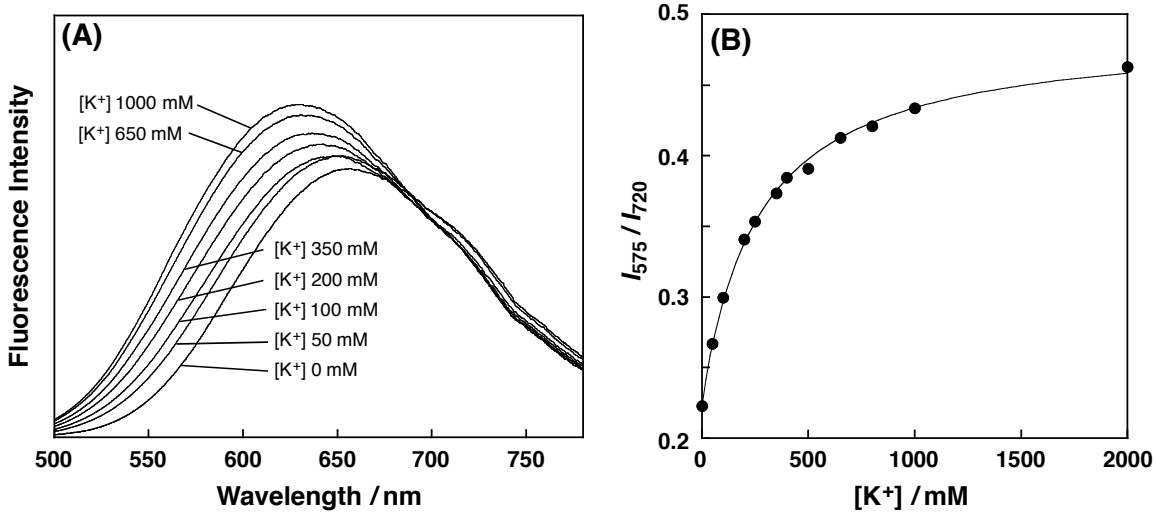


Fig. S4 (A) Emission spectral change of **NaGY** (25 μM) upon addition of KCl (0, 50, 100, 200, 350, 650, and 1000 mM) in 50 mM HEPES (pH 7.4) with the excitation at 405 nm. (B) Plots of the fluorescence intensity ratio between 575 and 720 nm (I_{575}/I_{720}) with a best curve fitting for a dissociation constant of 223 ± 7 mM.

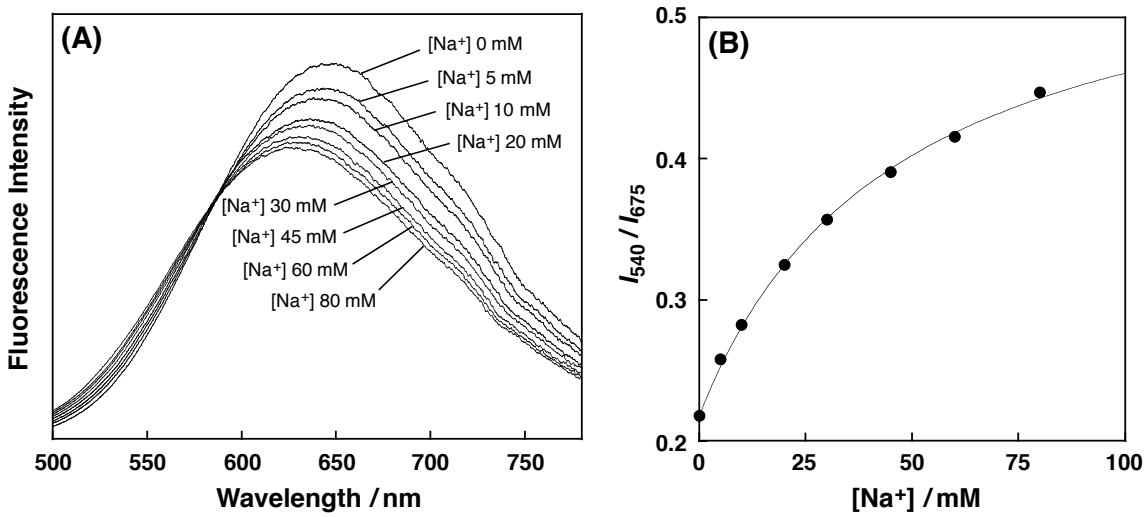


Fig. S5 (A) Emission spectral change of **NaGY** (25 μM) upon addition of NaCl (0, 5, 10, 20, 30, 45, 60, and 80 mM) in 50 mM HEPES (pH 7.4) containing 150 mM of K^+ with the excitation at 405 nm. (B) Plots of the fluorescence intensity ratio between 540 and 675 nm (I_{540}/I_{675}) with a best curve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

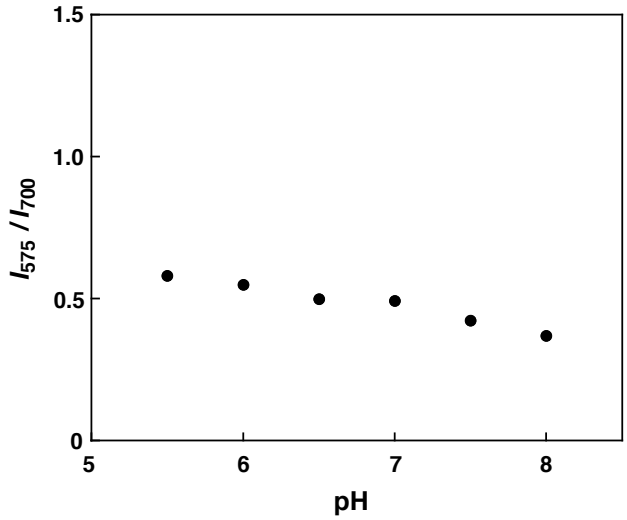


Fig. S6 Plots of the fluorescence intensity ratio between 575 and 700 nm as a function of pH value for 25 μM **NaGY**.

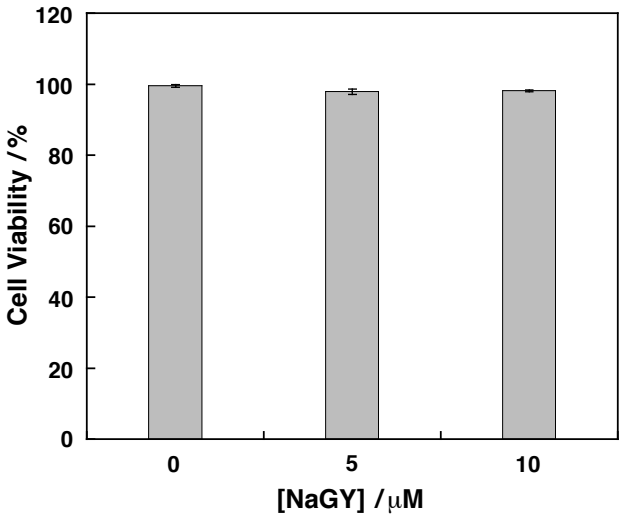


Fig. S7 Trypan blue exclusion tests of cell viability. Cells were incubated without or with **NaGY** (5 and 10 μM) at 37 °C for 4 h. Error bar = S. D. (N = 6)

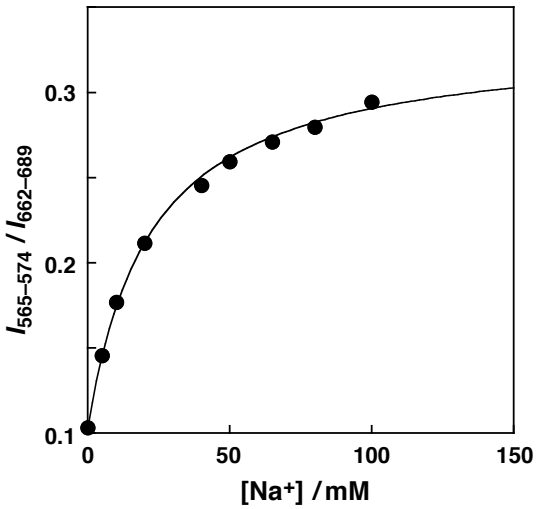


Fig. S8 Plots of the integrated fluorescence intensity ratio between 565-574 nm ($I_{565\text{-}574}$) and 662-689 nm ($I_{662\text{-}689}$) observed in **Fig. 3** with a best curve fitting for a dissociation constant of 16.8 ± 1.0 mM.

6. NMR spectra

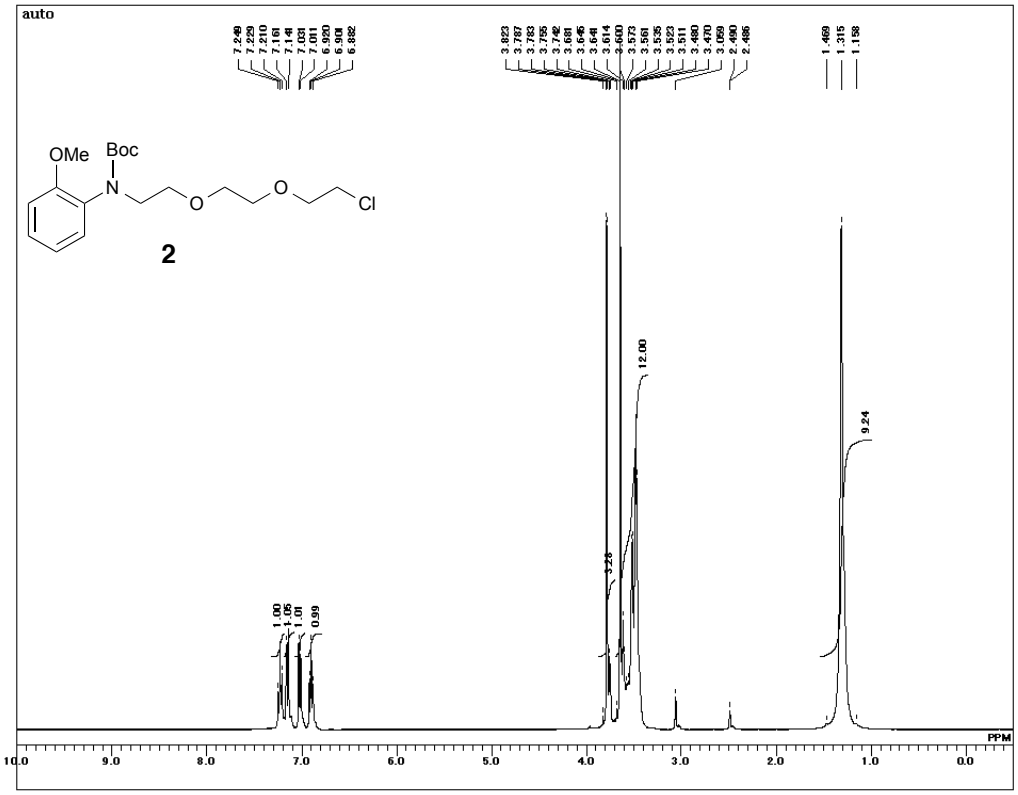


Fig. S6 ^{1}H NMR spectrum (400 MHz) of **2** in DMSO-d_6 at 80 ºC.

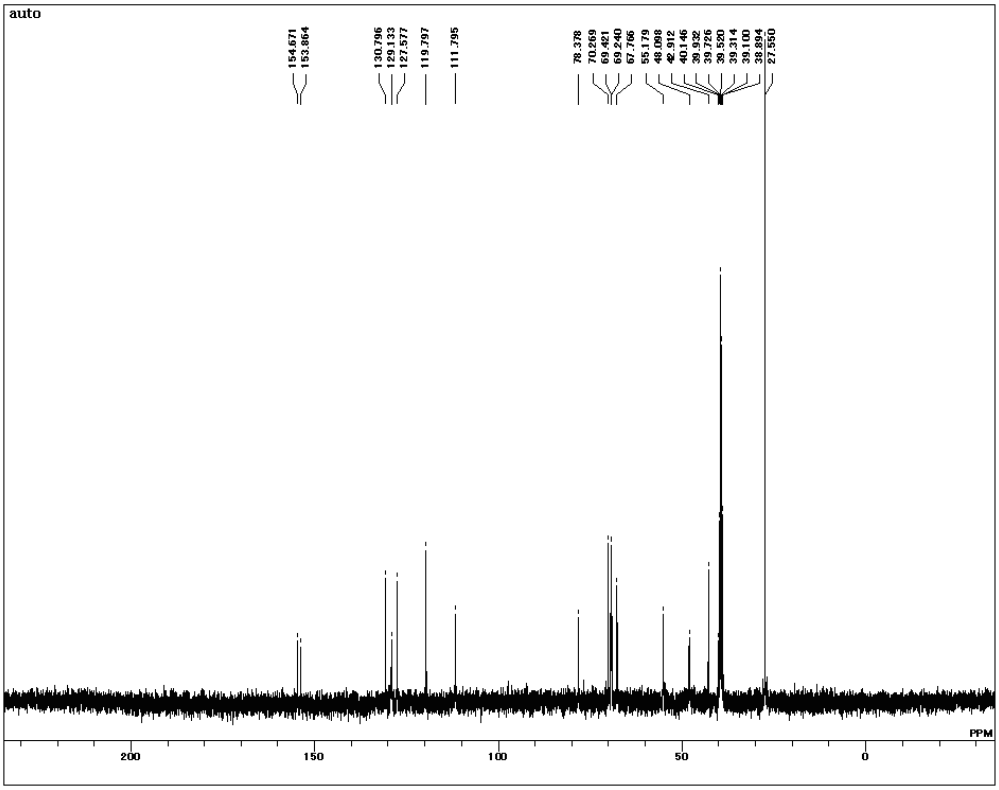


Fig. S7 ^{13}C NMR spectrum (100 MHz) of **2** in DMSO-d_6 at 80 °C.

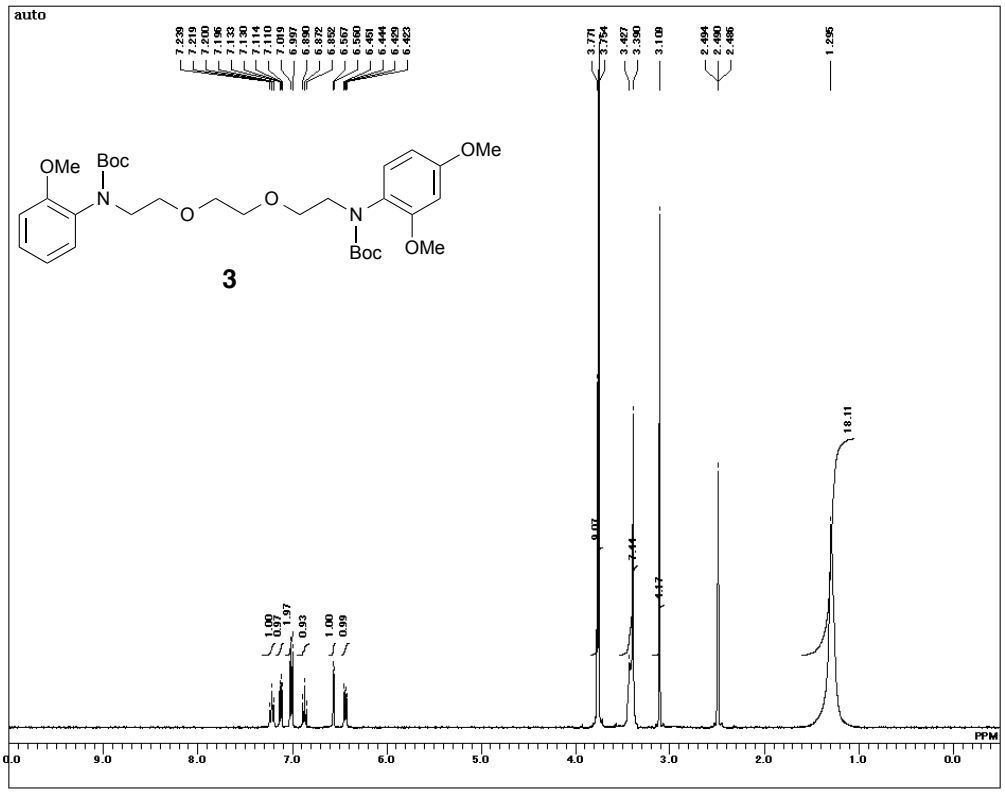


Fig. S8 ^{1}H NMR spectrum (400 MHz) of **3** in DMSO-d_6 at 70 °C.

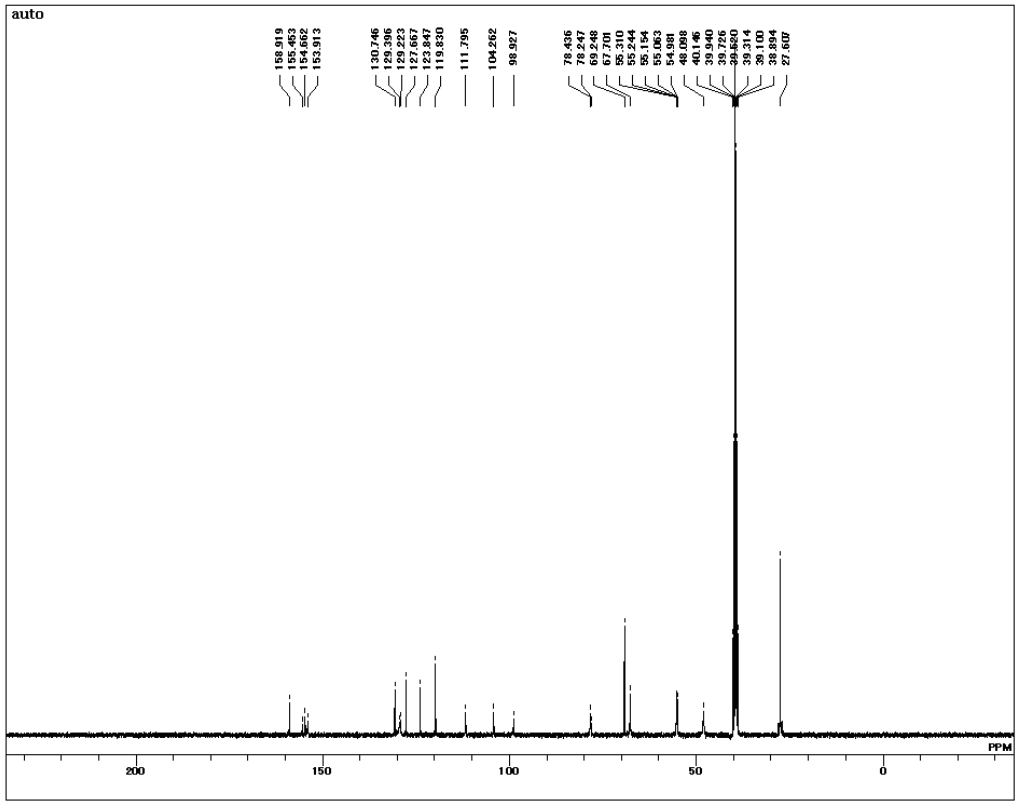


Fig. S9 ^{13}C NMR spectrum (100 MHz) of **3** in DMSO-d_6 at 70 °C.

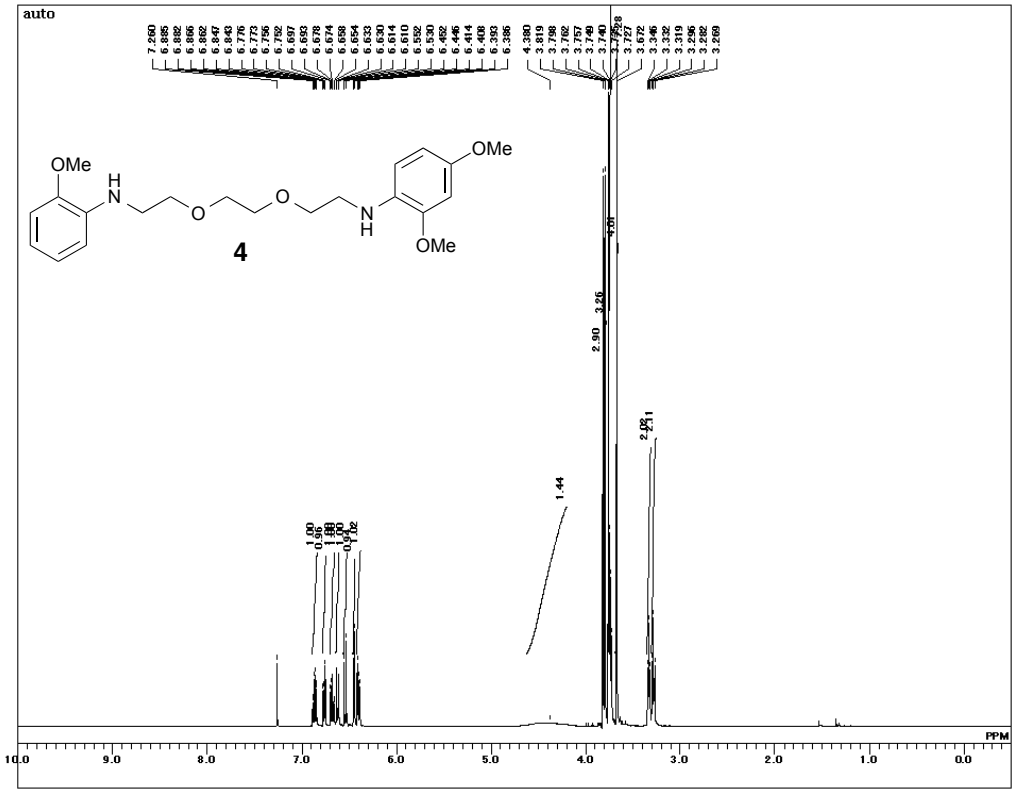


Fig. S10 ^{1}H NMR spectrum (400 MHz) of **4** in $CDCl_3$.

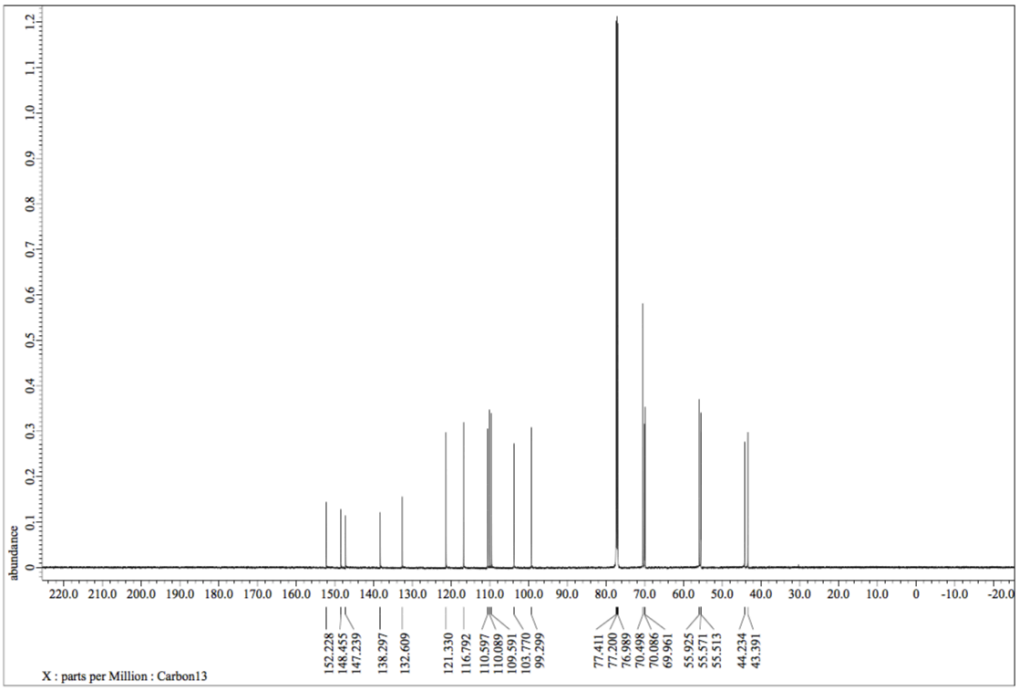


Fig. S11 ^{13}C NMR spectrum (150 MHz) of **4** in $CDCl_3$.

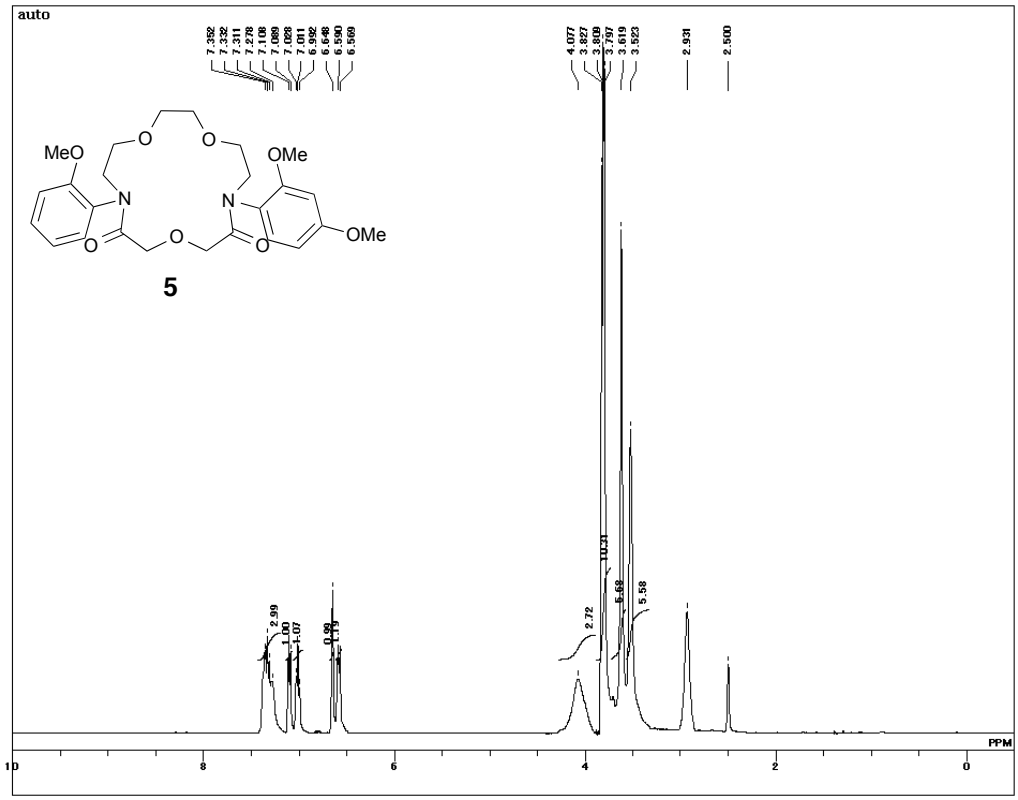


Fig. S12 ^{1}H NMR spectrum (400 MHz) of **5** in DMSO-d_6 at 150 °C.

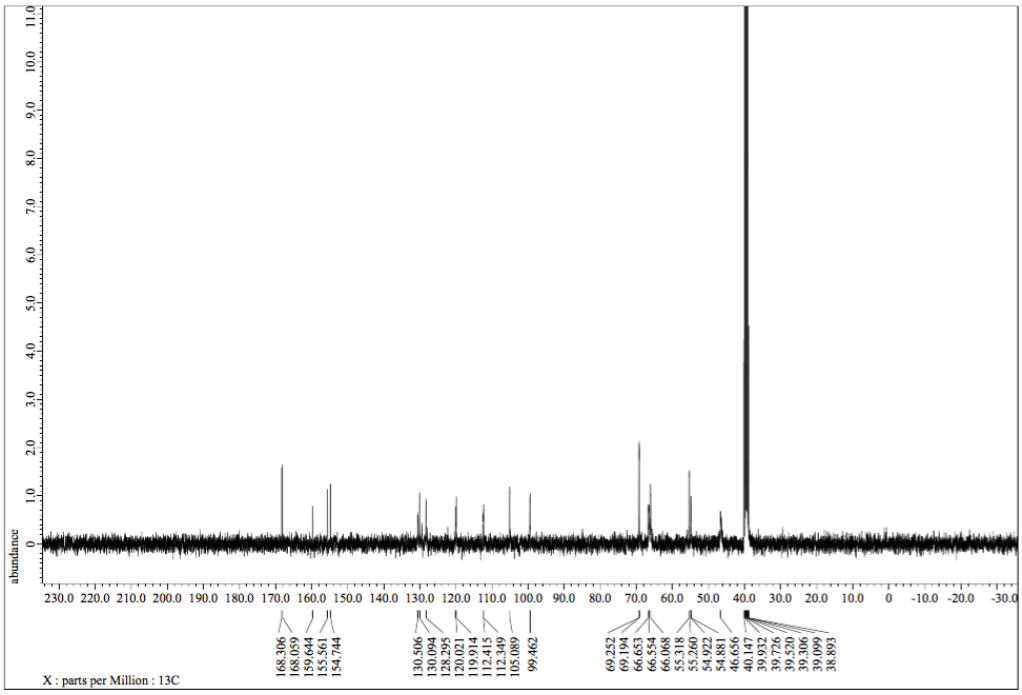


Fig. S13 ^{13}C NMR spectrum (100 MHz) of **5** in DMSO-d_6 at 150 °C.

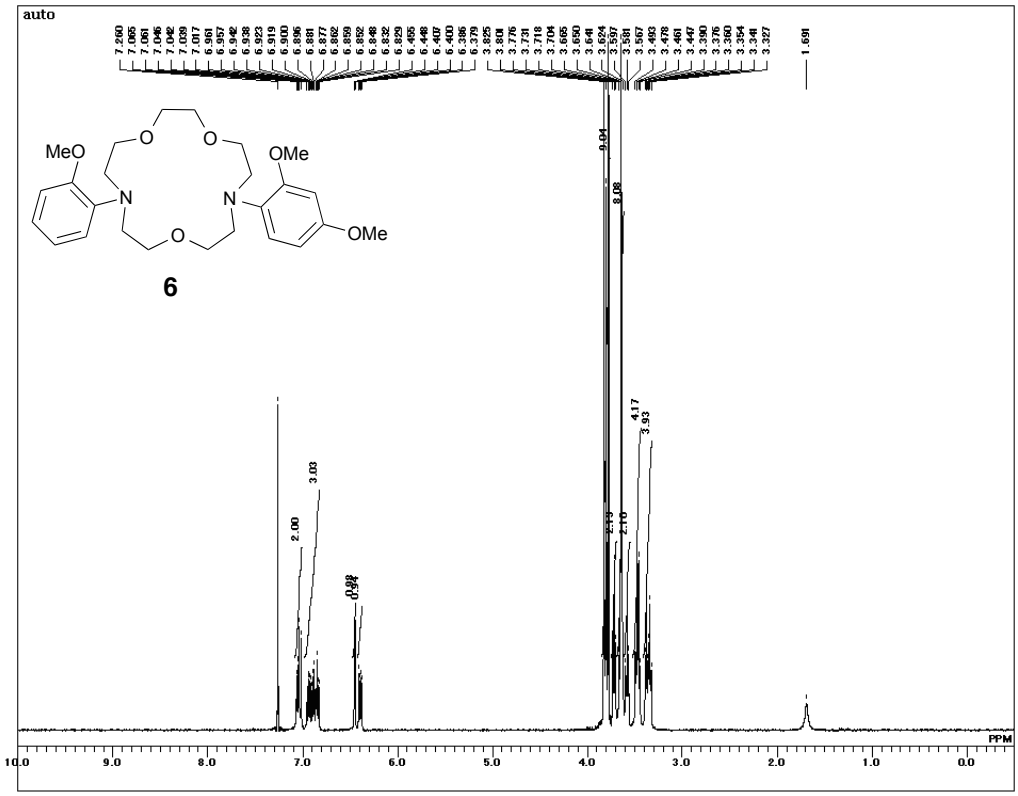


Fig. S14 ^{1}H NMR spectrum (400 MHz) of **6** in $CDCl_3$.

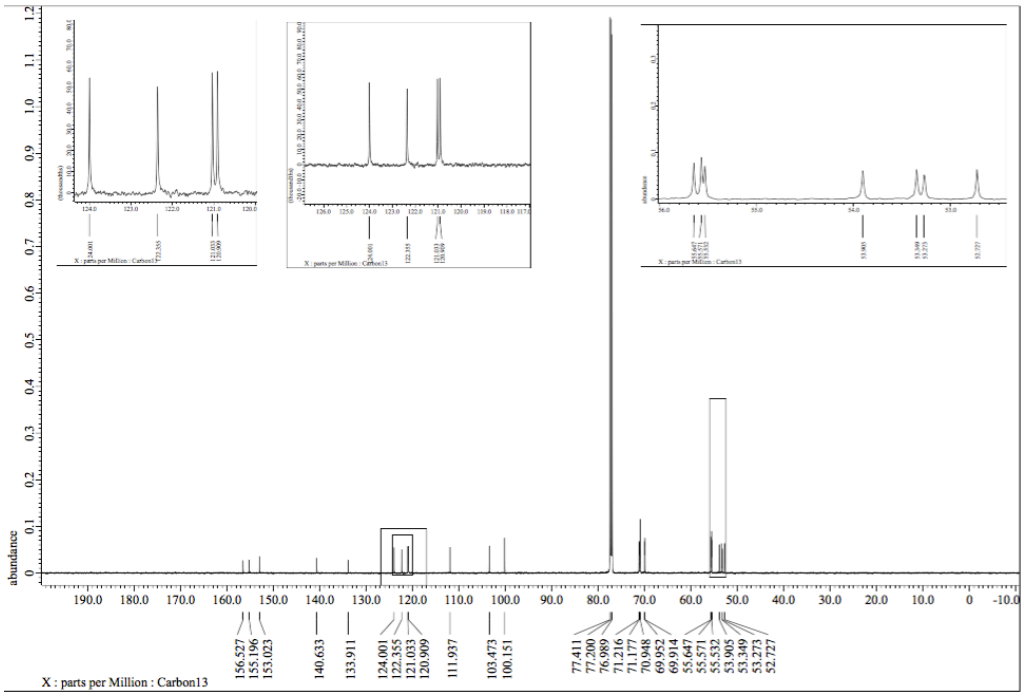


Fig. S15 ^{13}C NMR spectrum (150 MHz) of **6** in $CDCl_3$.

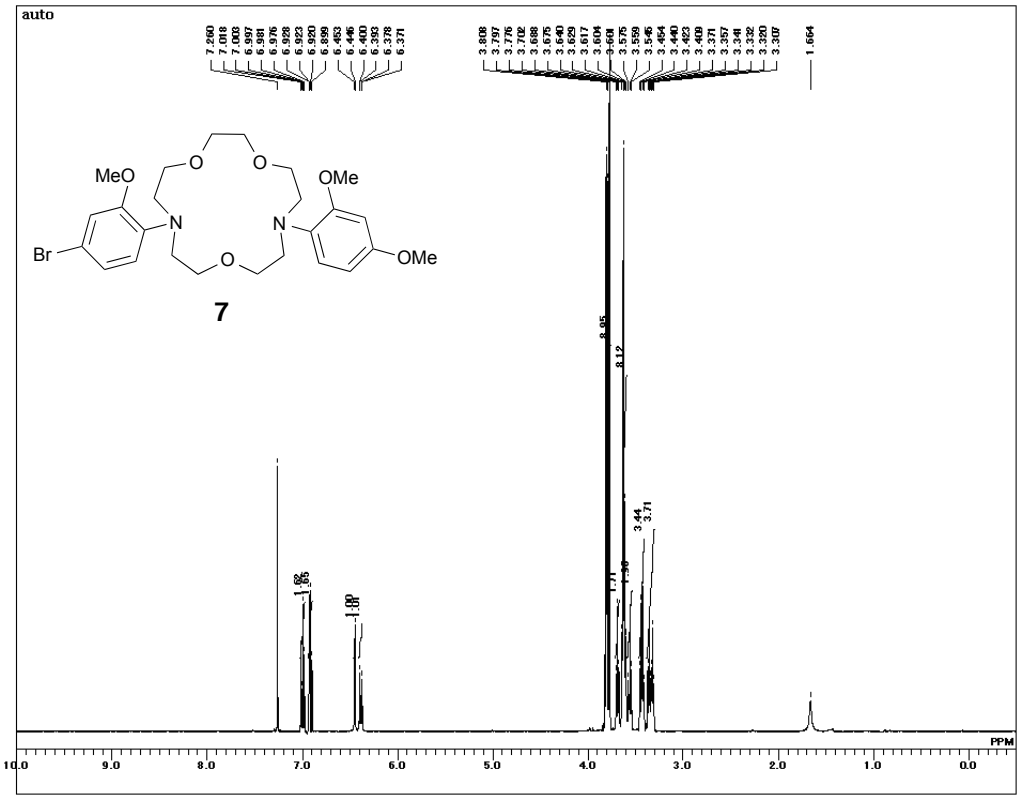


Fig. S16 ^{1}H NMR spectrum (400 MHz) of **7** in $CDCl_3$.

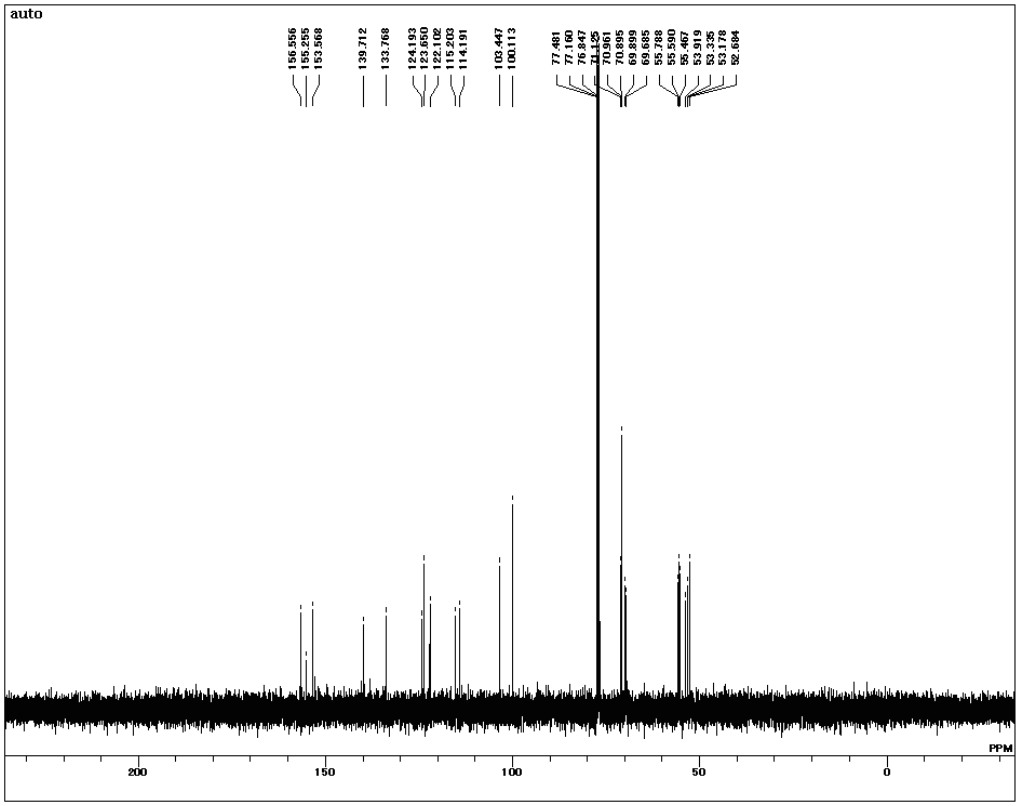


Fig. S17 ^{13}C NMR spectrum (100 MHz) of **7** in $CDCl_3$.

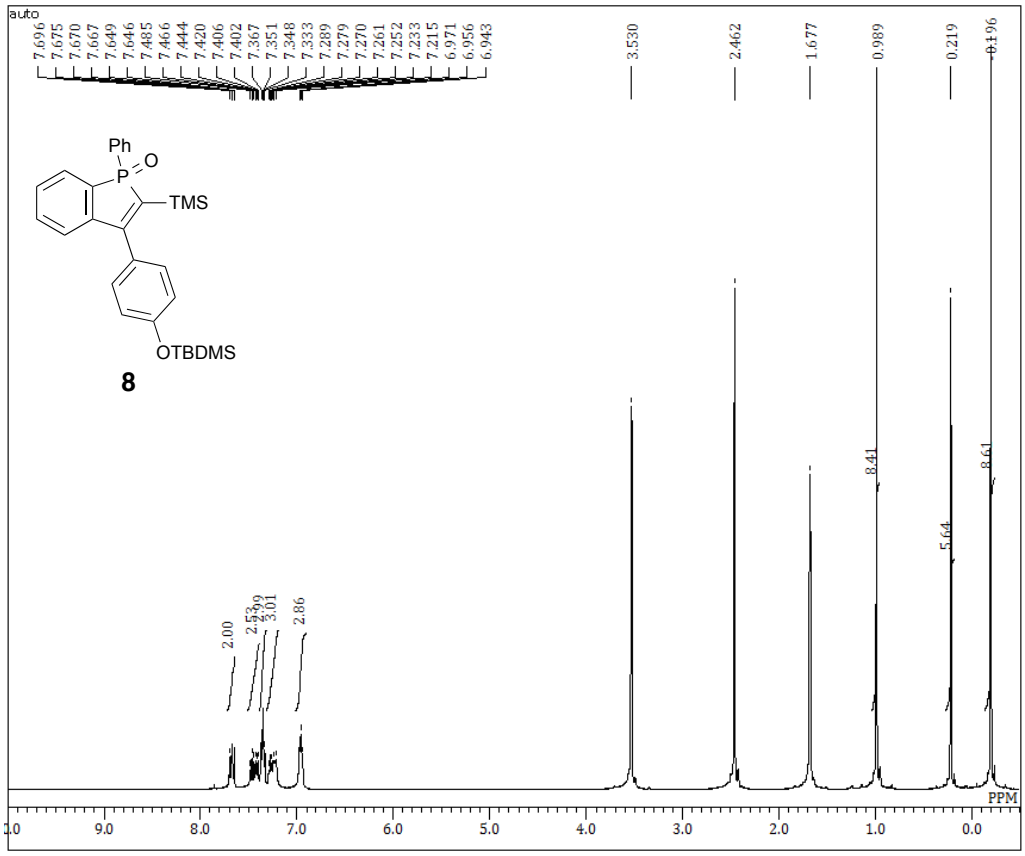


Fig. S18 ^{1}H NMR spectrum (400 MHz) of **8** in THF-d_8.

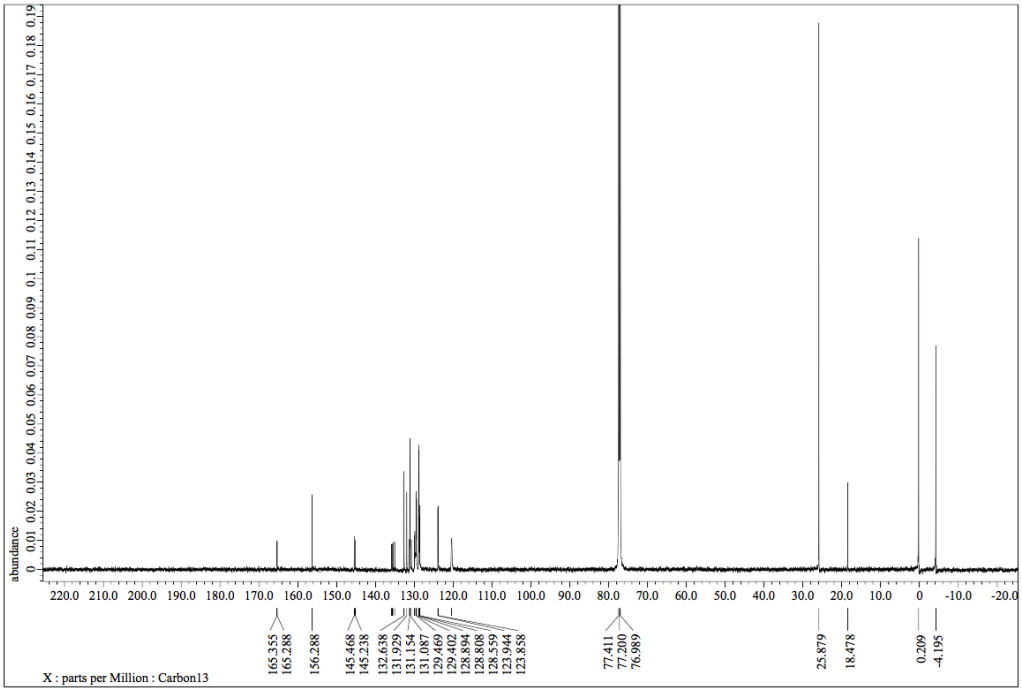


Fig. S19 ^{13}C NMR spectrum (150 MHz) of **8** in $CDCl_3$.

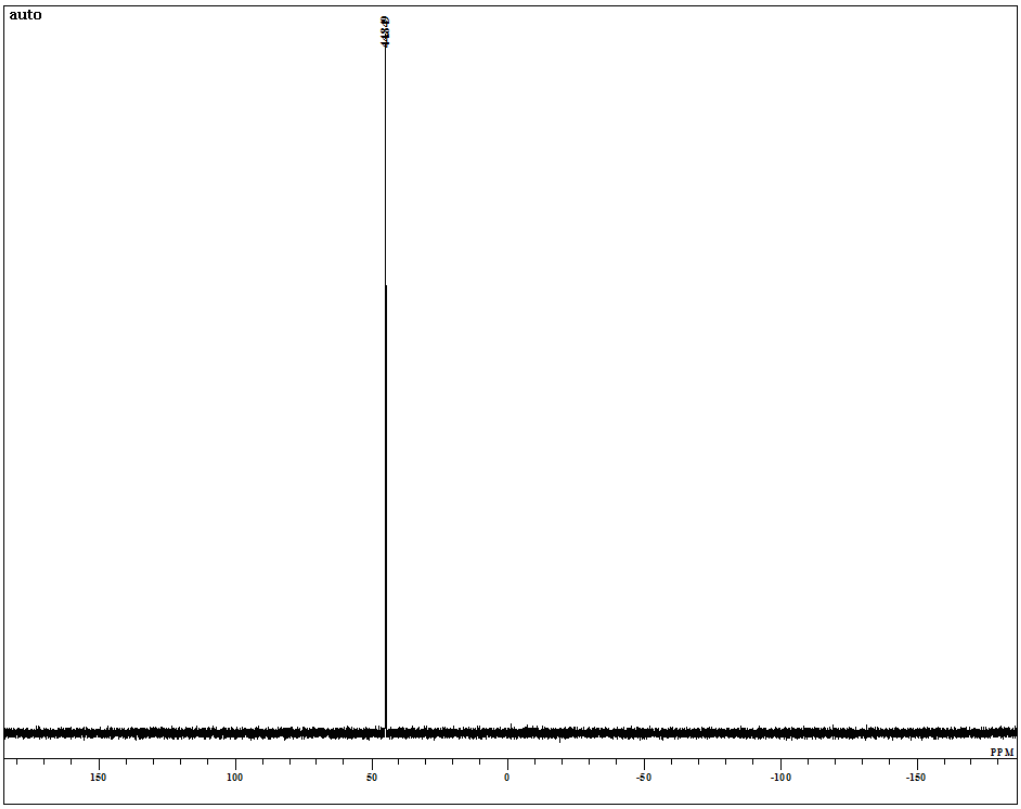


Fig. S20 ^{31}P NMR spectrum (162 MHz) of **8** in $CDCl_3$.

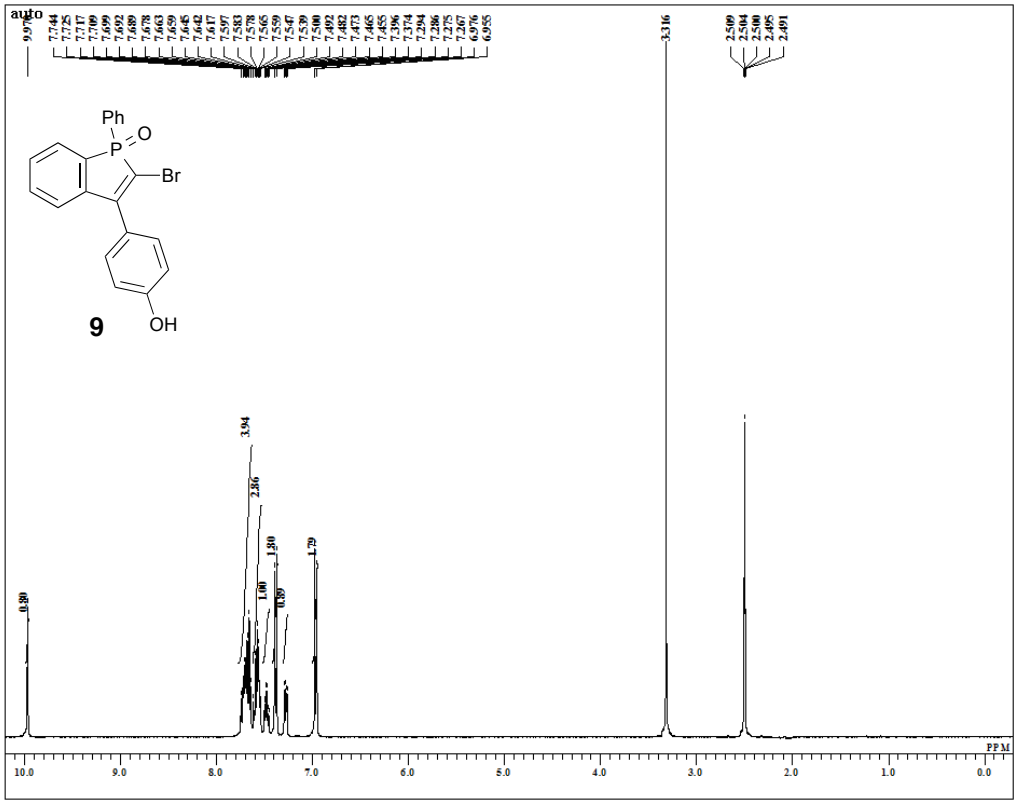


Fig. S21 ^{1}H NMR spectrum (400 MHz) of **9** in DMSO-d_6.

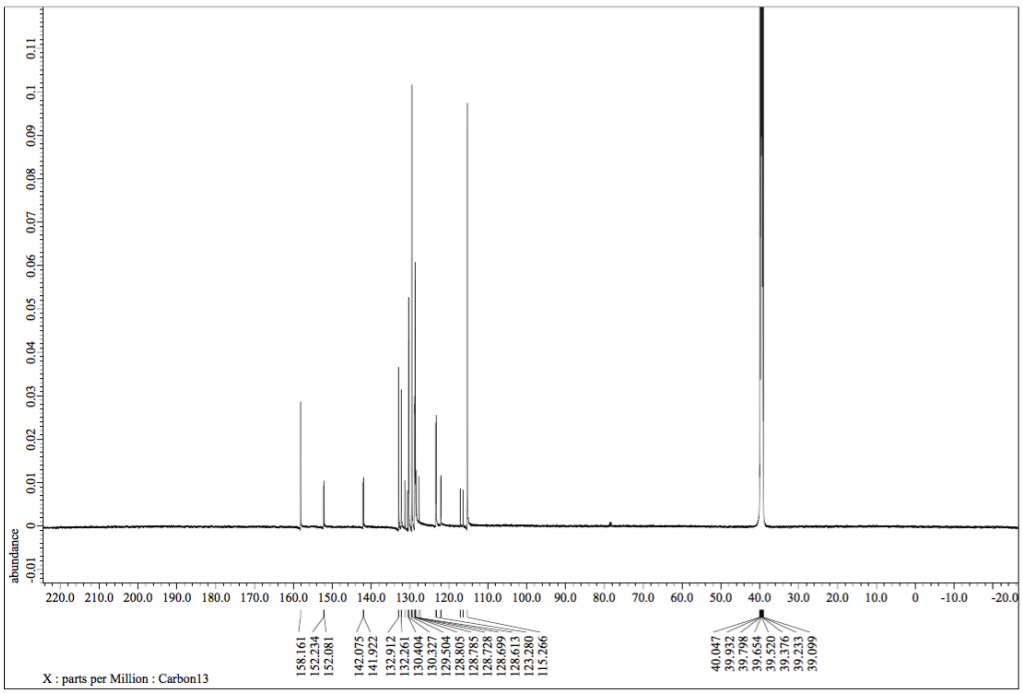


Fig. S22 ^{13}C NMR spectrum (150 MHz) of **9** in DMSO-d_6.

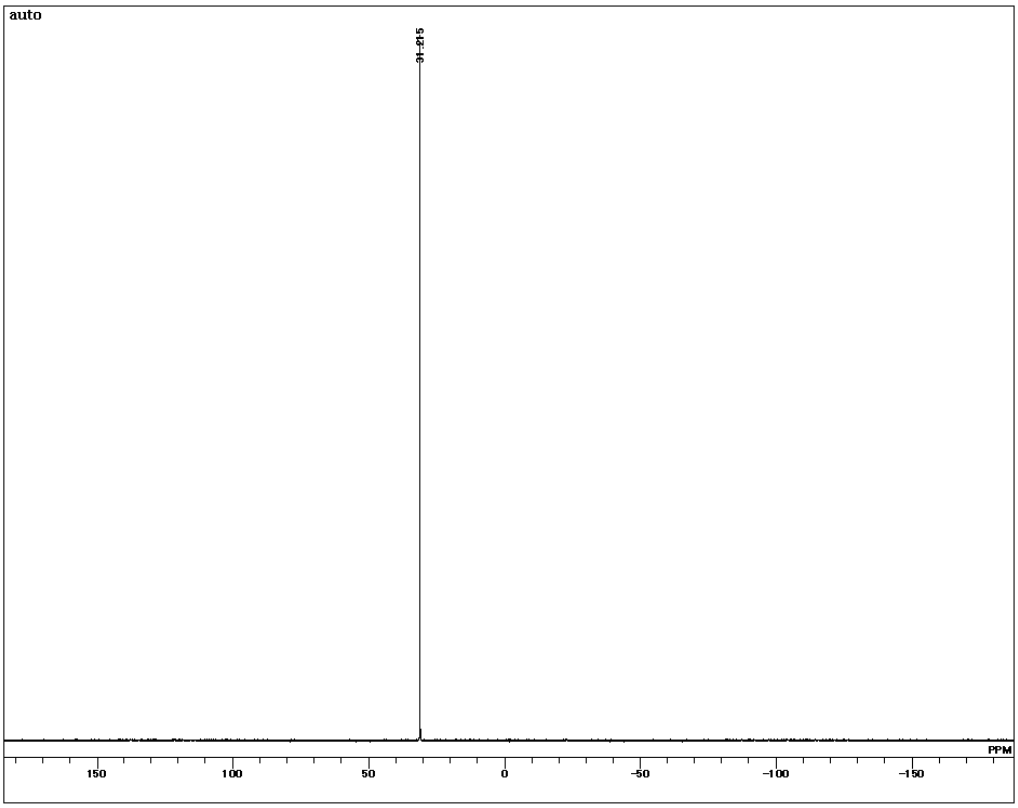


Fig. S23 ^{31}P NMR spectrum (162 MHz) of **9** in DMSO-d_6.

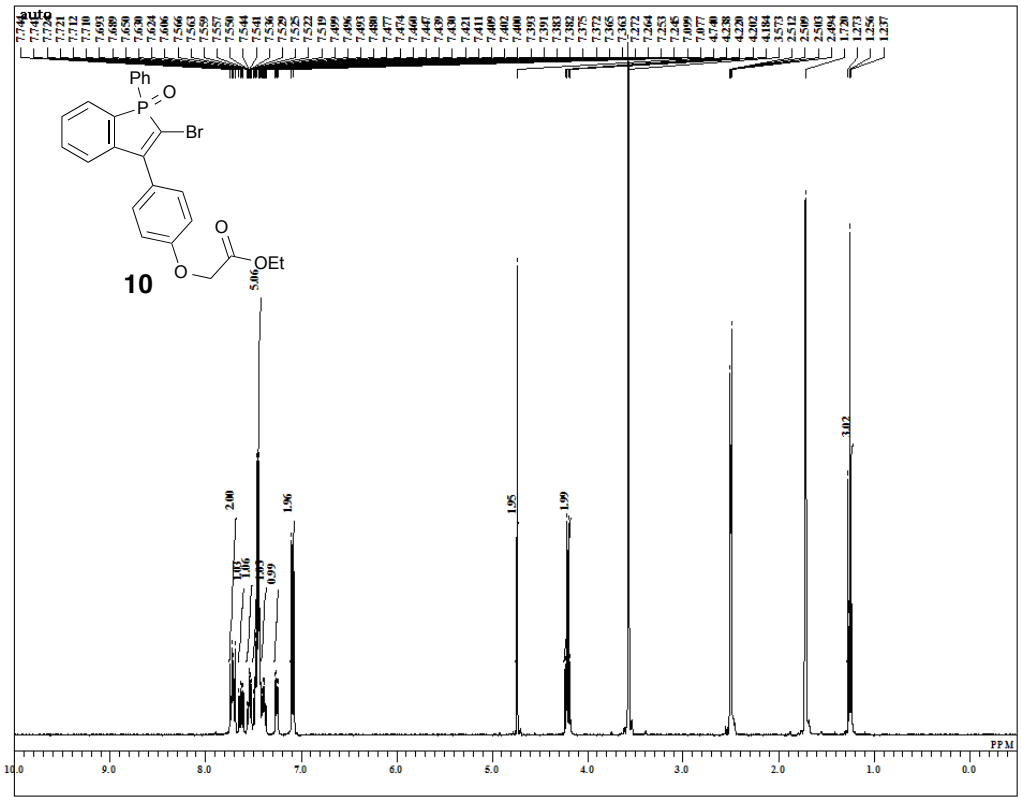


Fig. S24 ^{1}H NMR spectrum (400 MHz) of **10** in THF-d_8.

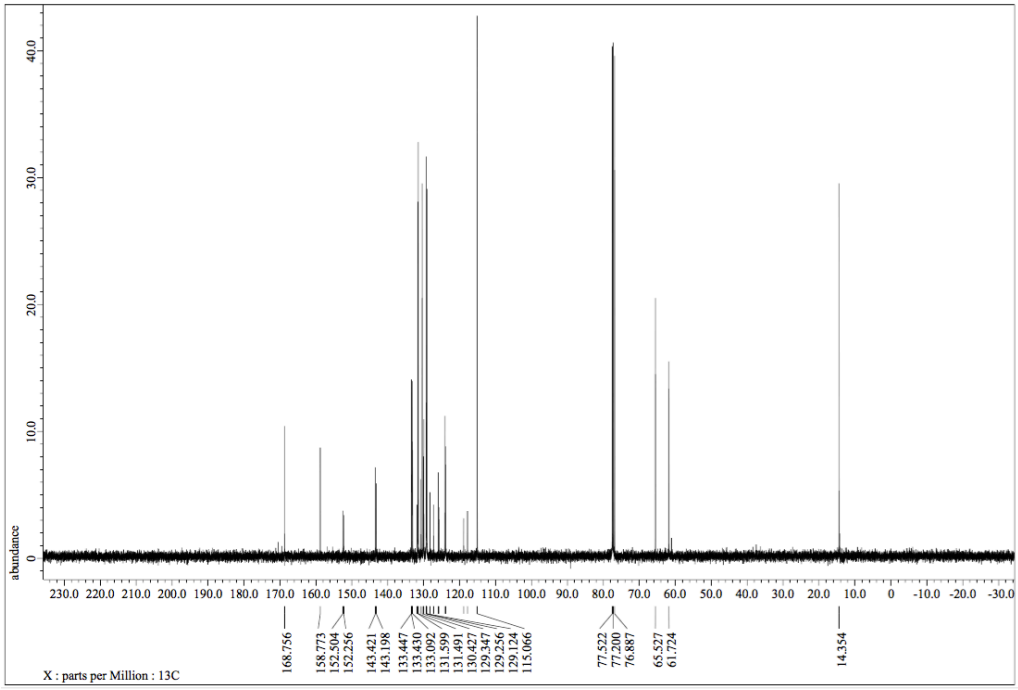


Fig. S25 ^{13}C NMR spectrum (100 MHz) of **10** in $CDCl_3$.

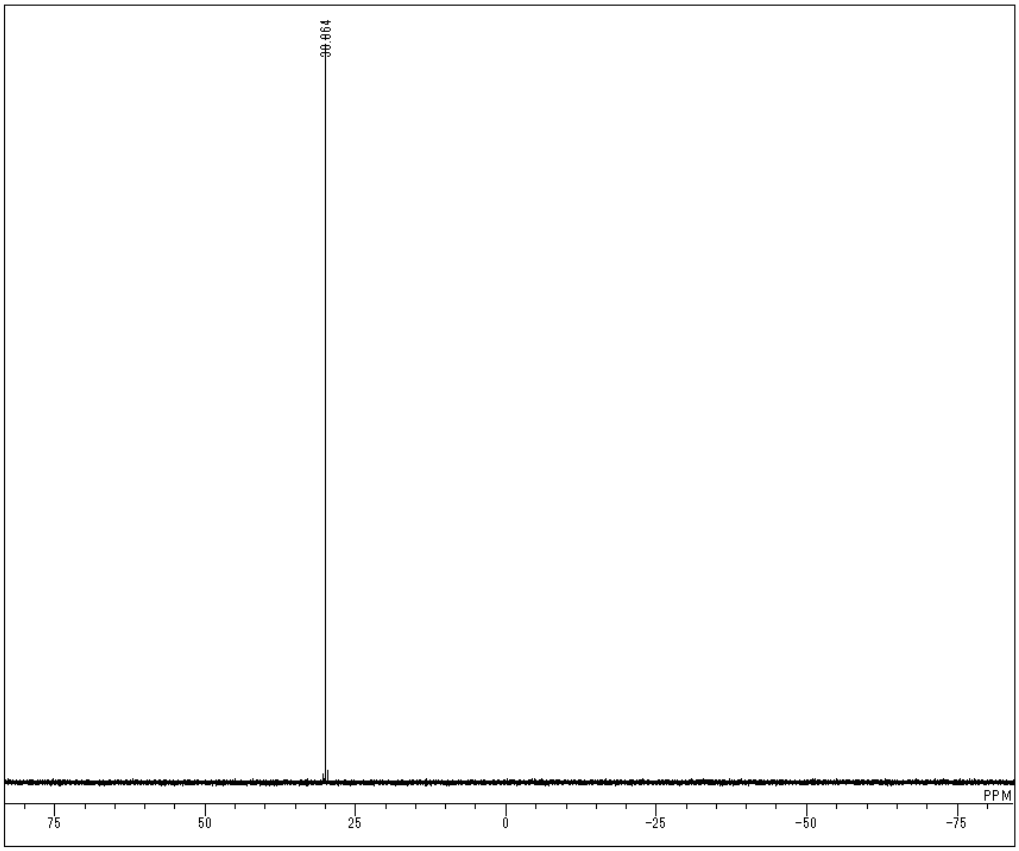


Fig. S26 ^{31}P NMR spectrum (162 MHz) of **10** in $CDCl_3$.

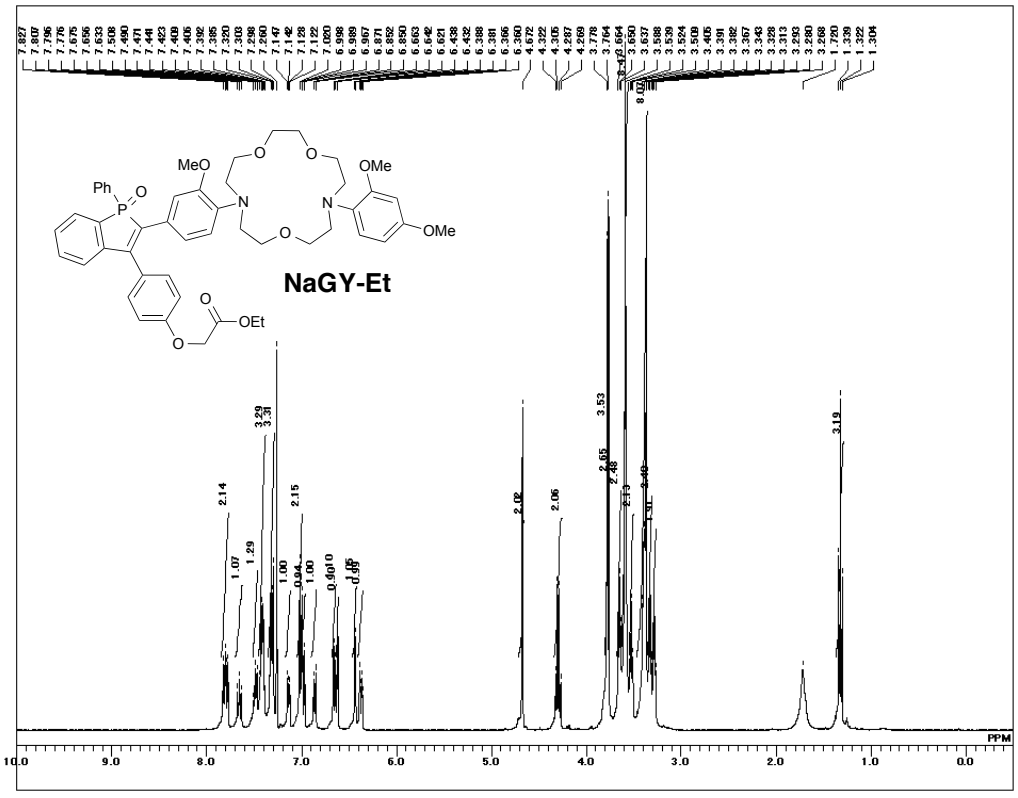


Fig. S27 ^{1}H NMR spectrum (400 MHz) of **NaGY-Et** in $CDCl_3$.

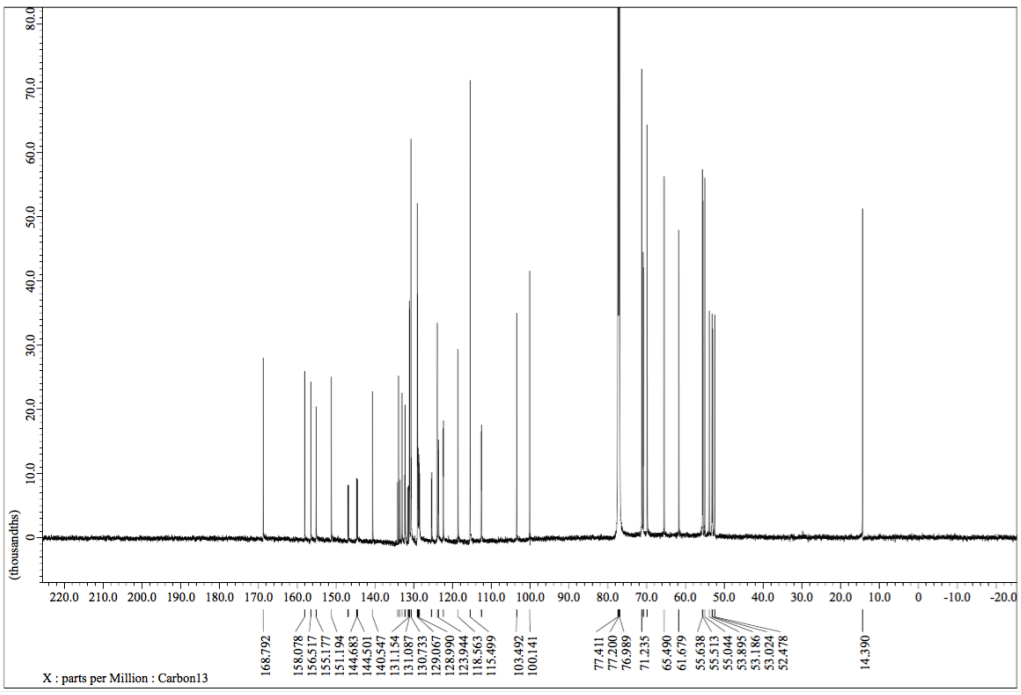


Fig. S28 ^{13}C NMR spectrum (150 MHz) of **NaGY-Et** in $CDCl_3$.

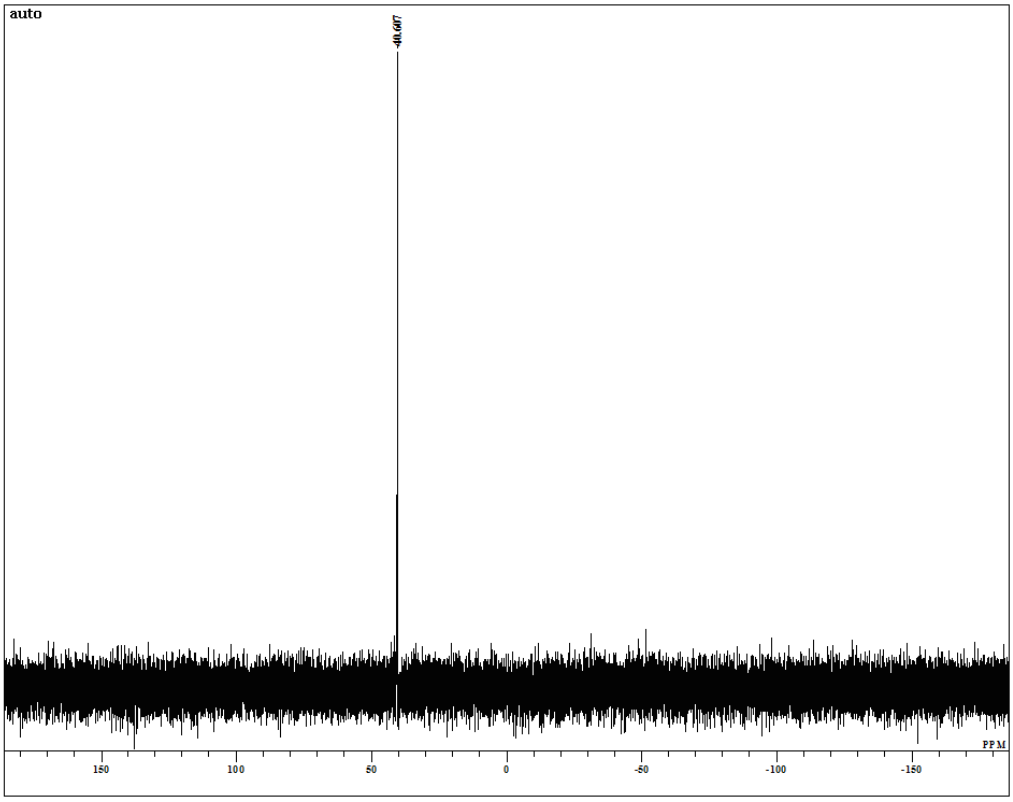


Fig. S29 ^{31}P NMR spectrum (162 MHz) of **NaGY-Et** in $CDCl_3$.

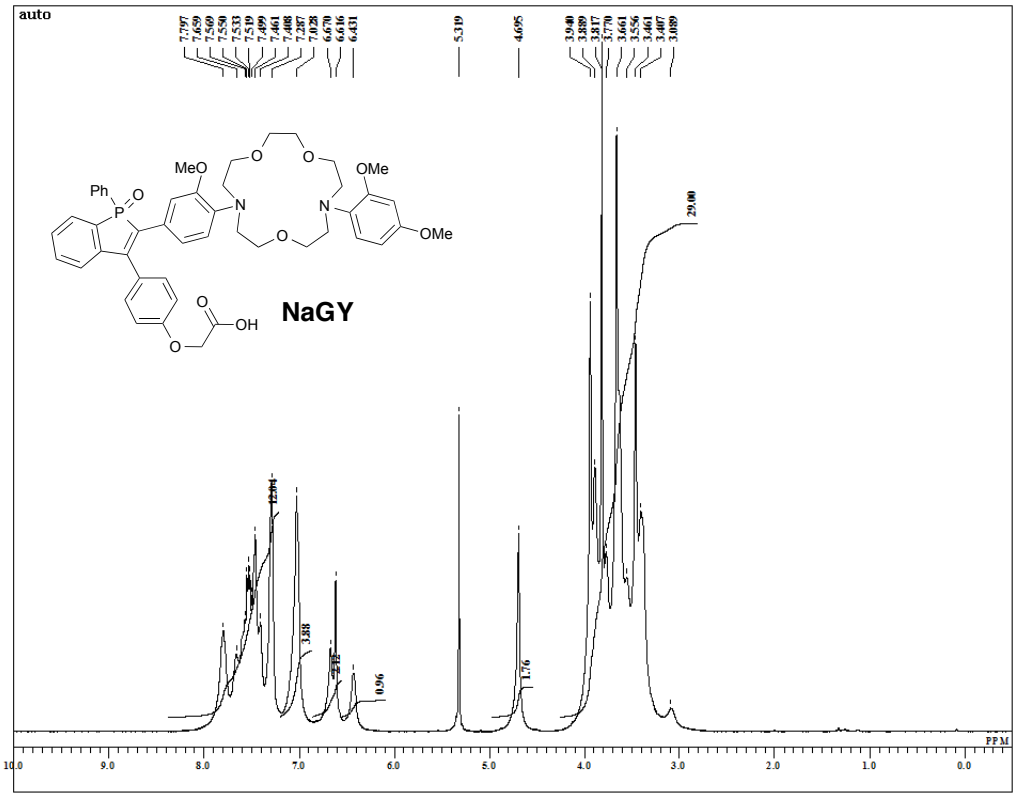


Fig. S30 ^{1}H NMR spectrum (400 MHz) of **NaGY** in CD_2Cl_2.

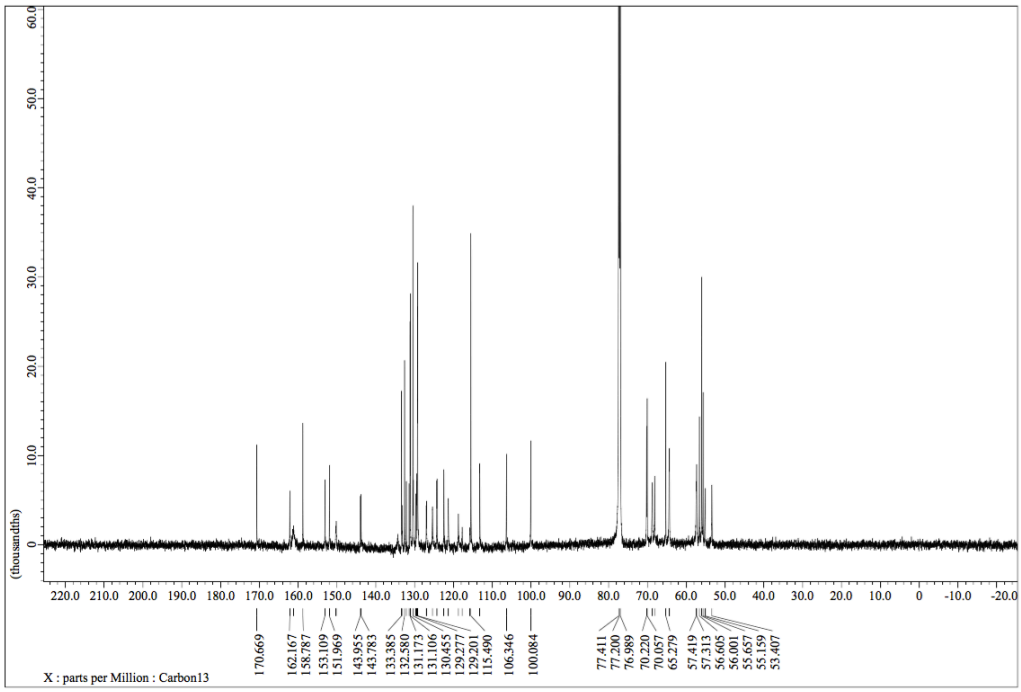


Fig. S31 ^{13}C NMR spectrum (150 MHz) of **NaGY** in CD_2Cl_2.

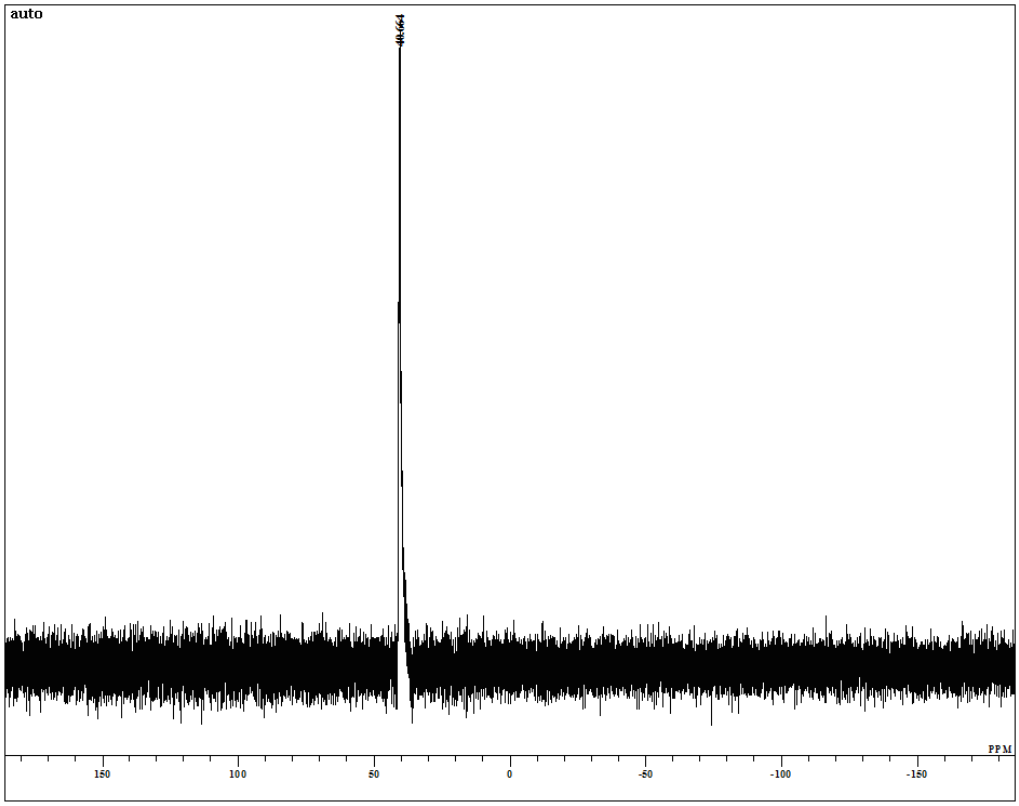


Fig. S32 ^{31}P NMR spectrum (162 MHz) of **NaGY** in CD_2Cl_2.

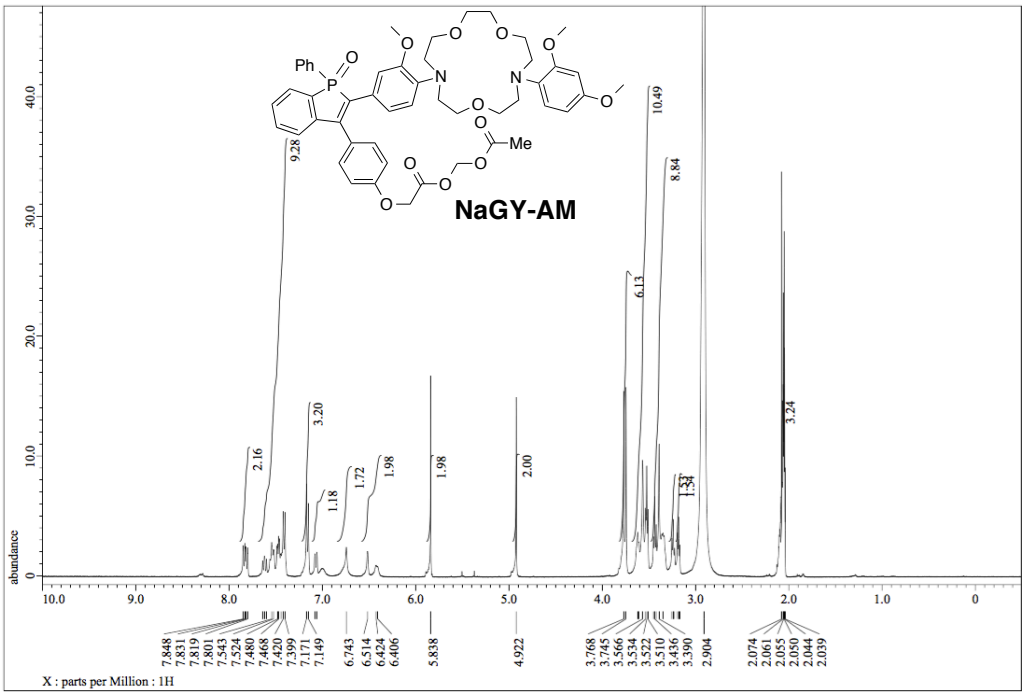


Fig. S33 ^{1}H NMR spectrum (400 MHz) of **NaGY-AM** in acetone-d_6.

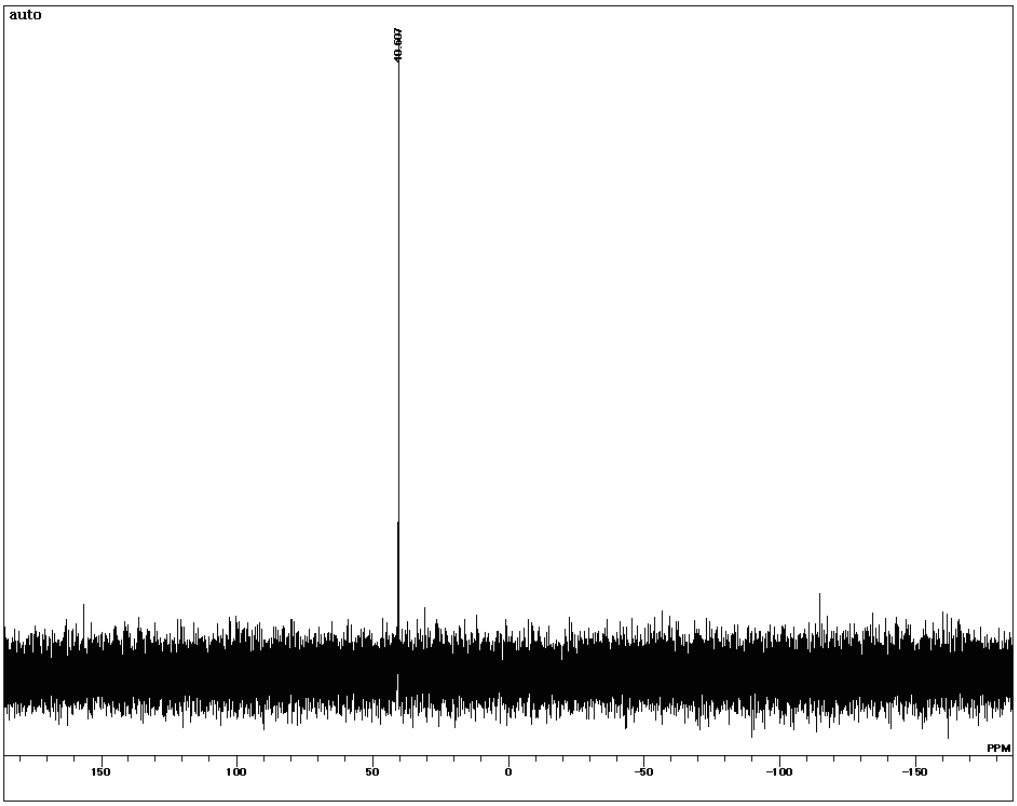


Fig. S33 ^{31}P NMR spectrum (162 MHz) of **NaGY-AM** in $CDCl_3$.