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New conjugate of bis(*o*-aminophenoxy)ethane-*N*,*N*,*N'*,*N'*-tetraacetate with naphthalimide as a fluorescent sensor for calcium cations

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CONTENTS

S1. Experimental Section

4-Bromo-1,8-naphthalic anhydride was prepared starting from acenaphthene following the described procedure [S1,S2]. All other reagents were purchased from commercial sources. Acetonitrile used in spectroscopic studies was of HPLC grade.

Melting points were measured on Melt-temp melting point electrothermal apparatus and were uncorrected.

The reaction course and purity of the final products was followed by TLC on silica gel (DC-Alufolien Kieselgel 60 F_{254}, Merck). Column chromatography was conducted over silica gel (Kieselgel 60, particle size 0.063-0.200 mm, Merck).

^{1}H and ^{13}C NMR spectra were recorded on an Avance 300, Avance 400 (Bruker) and Inova 400 (Agilent) spectrometers. The measurements were performed in DMSO-d_6 and D_2O solutions. The chemical shifts (given as δ) were determined with an accuracy of 0.01 ppm relative to the signals corresponding to the residual solvents and recalculated to the internal standard (TMS); the spin-spin coupling constants (J) were measured with an accuracy of 0.1 Hz. The numbering of carbon atoms in the compounds used by us for the description of ^{1}H and ^{13}C NMR spectra below is shown in Scheme S1. ^{1}H NMR spectra of compound **3**, **4** and **BAPTA-NI**, and 2D NMR spectra of **BAPTA-NI** are presented on Figures S2-S7.

LC-ESI-MS analyses were performed on a Finnigan LCQ Advantage mass spectrometer equipped with octopole ion-trap mass-analyzer, MS Surveyor pump, Surveyor auto sampler,

Schmidlin-Lab nitrogen generator (Germany) and Finnigan X-Calibur 1.3 software for data collecting and processing. Isotope patterns were calculated with Molecular Weight Calculator, Version 6.37 (Matthew Monroe). Electron impact (EI) (70 eV) mass spectra were obtained from Finnigan Polaris Q instrument (ion-trap) in standard conditions. Electron impact (EI) (70 eV) mass spectra were obtained from Finnigan Polaris Q instrument (ion-trap) in standard conditions.

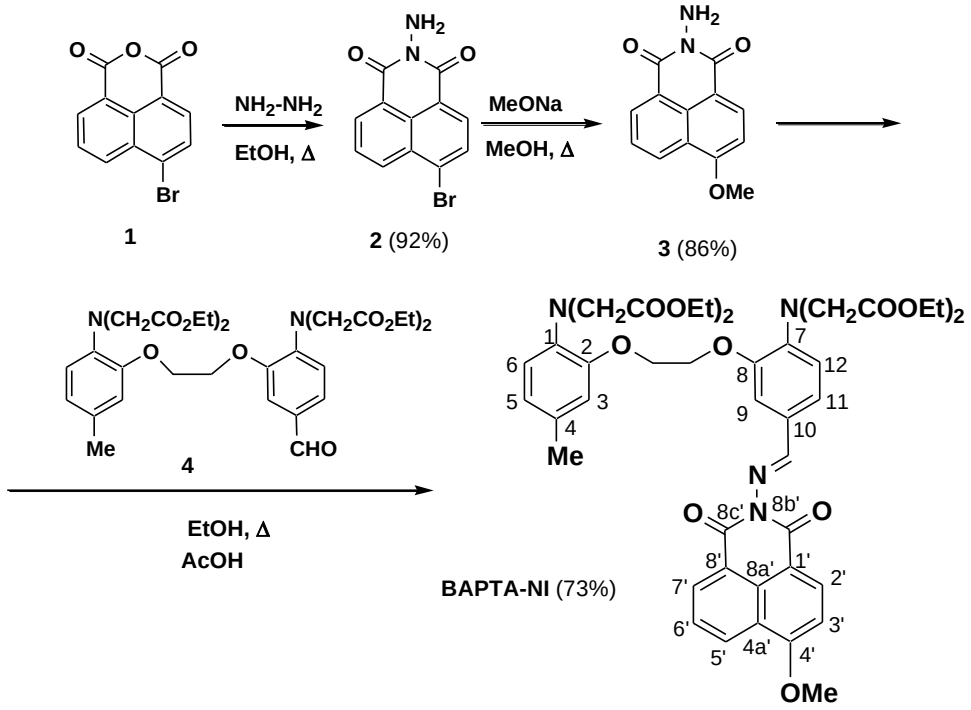
The absorption spectra were taken on a Cary 300 spectrophotometer (Agilent Technologies). The fluorescence quantum yield measurements were performed using a Cary 300 spectrophotometer and a Fluorolog3-221 spectrofluorimeter (Horiba Jobin Yvon). Spectral measurements were carried out in air-saturated acetonitrile solutions at ambient temperature. All measured fluorescence spectra were corrected for the nonuniformity of detector spectral sensitivity. Coumarin 481 in acetonitrile (φ^{fl} = 0.08) [S3] was used as reference for the fluorescence quantum yield measurements. The fluorescence quantum yields were calculated by the Eq. (S1) [S4],

$$\varphi^{fl} = \varphi_R^{fl} \frac{S}{S_R} \cdot \frac{(1 - 10^{-A_R})n^2}{(1 - 10^{-A})n_R^2} \qquad \text{(S1)}$$

wherein φ^{fl} and φ_R^{fl} are the fluorescence quantum yields of the studied solution and the standard compound respectively; A and A_R are the absorptions of the studied solution and the standard respectively; S and S_R are the areas underneath the curves of the fluorescence spectra of the studied solution and the standard respectively; and n and n_R are the refraction indices of the solvents for the substance under study and the standard compound.

Complex formation of compound **BAPTA-NI** with Ca^{2+} was studied by spectrofluorometric and spectrophotometric titration [S5,S6]. The ratio of **BAPTA-NI** to Ca^{2+} was varied by adding aliquots of a solution of calcium perchlorate in acetonitrile of known concentration to a solution of ligand **BAPTA-NI** in the same solvent of known concentration. The fluorescence spectrum of each solution was recorded, and the stability constants of the complexes were determined using the SPECFIT/32 program (Spectrum Software Associates, West Marlborough, MA).

Quantum chemical calculations were carried out by the MOPAC 2016 program package using the PM6 semiempirical method [S7]. CI calculations were performed at optimized geometries, which reached gradient variations less than 0.01 kcal mol^{-1}. The solvent effect was included in geometry optimizations following the "COnductorlike Screening MOdel" (COSMO) implemented in MOPAC 2009. A dielectric constant of ε = 38 and a refraction index of solvent (n) such that n^2 = 2 were used. The CI included eight occupied and eight unoccupied MOs.



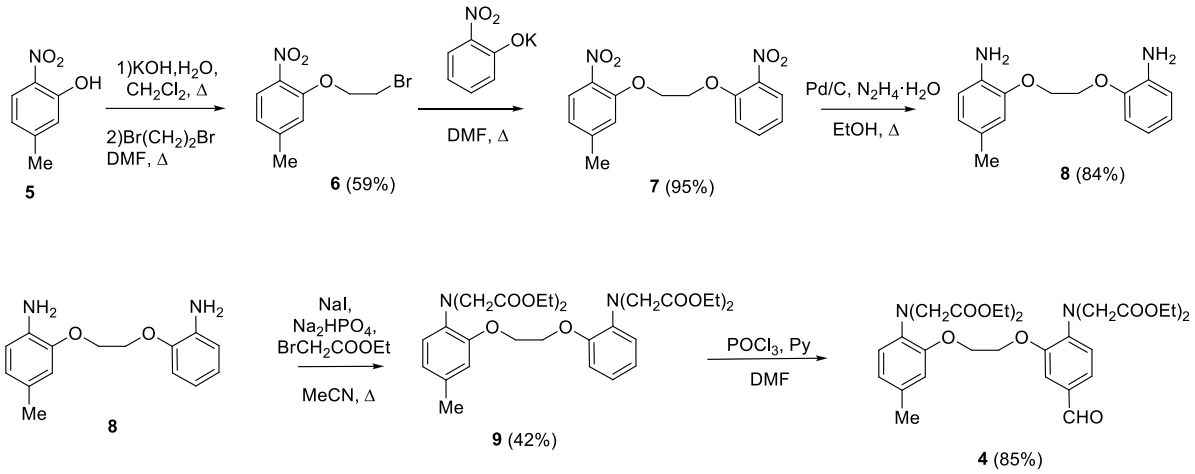
Scheme S1. Synthetic procedure for compound **3** and **BAPTA-NI**. Atom numbering differs from systematic and is given for NMR assignment.

Compounds **2**, **4** and **6-9** (Schemes S1 and S2) were synthesized according previously described procedures [S8-S10].

2-Amino-6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione 2. To a stirred suspension of 4-bromo-1,8-naphthalic anhydride **1** (0.3 g, 1.08 mol) in ethanol (15 ml) at 80ºC in argon atmosphere a solution of hydrazine hydrate (64% w/w, 0.015 ml, 1.08 mmol) in ethanol (5 ml) was added for 40 min. The mixture was stirred at 80 ºC for 3 hours and then cooled to room temperature. The yellow precipitate was filtered off, washed with cold ethanol and dried in air. Yield of **2** was 0.29 g (92%). M.p. 215 – 218 ºC ([S8]: m. p. 215 – 218°C). ^{1}H NMR (400.13 MHz, DMSO-d_6, 27 ºC): δ = 5.81 (bs, 2H, NH_2), 7.97 – 8.05 (m, 1H, H(6′), 8.24 (д, 1H, H(3′), J = 7.8), 8.37(д, 1H, H(2′), J = 7.8), 8.57 (d, 1H, H(5′), J = 8.6), 8.60 (d, 1H, H(7′), J = 7.4).

2-Amino-6-methoxy-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione 3. A mixture of compound **2** (0.282 g, 0.969 mmol) and MeONa (1.0 g, 0.02 mol) in MeOH (25 ml) was refluxed for 5.5 hours. The mixture was cooled to room temperature and the formed precipitate was filtered off, washed with cold ethanol and dried in air to give 0.2 g of product **3** (yield 86%). M. p. 243 – 245 ºC. ^{1}H NMR (300.13 MHz, DMSO-d_6, 22 ºC): δ = 4.13 (s, 3H, OCH_3), 5.77 (s, 2H, NH_2), 7.34 (d, 1H, H(3′), J = 8.3), 7.79 – 7.87 (m, 1H, H(6′)), 8.48 (d, 1H, H(2′), J = 8.3), 8.52 (d, 1H, H(7′), J = 7.3), 855 (d, 1H, H(5′), J = 8.6). ^{13}C NMR (150.93 MHz, DMSO-d_6, 21 ºC): δ = 56.73, 106.47, 113.88, 121.58, 122.85, 126.56, 127.24, 128.43, 131.11, 131.50, 133.47, 160.17, 160.59.

EI-mass m/z: calculated: 242.07, found: 243 (13), 242 (81) ([M^+]), 227 (51), 213 (100), 199 (18), 198 (35), 182 (25), 156 (13), 114 (22), 113 (18).



Scheme S2. Synthetic procedure for compound **4**.

2-(2-Bromoethoxy)-4-methyl-1-nitrobenzene 6. A KOH solution (3.66 g, 65 mmol) in water (20 ml) was added dropwise to a stirred solution of 5-methyl-2-nitrophenol **5** (10.0 g, 65.4 mmol) in CH_2Cl_2 (30 ml) at room temperature. After stirring at 40ºC for 2 hours, the mixture was cooled to room temperature, the solvents were evaporated and the resulting orange powder of potassium 5-methyl-2-nitrophenolate was used in the next synthesis without purification. A mixture of 5-methyl-2-nitrophenolate (11.4 g, 60 mmol) and 1,2-dibromoethane (17.0 ml, 0.2 mol) in DMF (32 ml) was stirred at 120 ºC for 3 hours (until the orange color of the potassium salt disappeared), then cooled to room temperature and diluted with water. The mixture was extracted with dichloromethane (3×50 ml) and the combined organic phases were washed with brine (2×50 ml). After drying over Mg_2SO_4 and evaporating of the solvent, the residue was recrystallized from methanol to give 9.1 g of compound **6** (yield 59%). M. p. 44 – 45 ºC ([S9]: m. p. 44 – 45°C). ^{1}H NMR (400.13 MHz, DMSO-d_6, 20 ºC): δ = 2.38 (s, 3H, CH_3), 3.80 (t, 2H, $\underline{CH_2}CH_2Br$, J = 5.4), 4.48 (t, 2H, $CH_2\underline{CH_2}Br$, J = 5.4), 6.95 (d, 1H, H(5), J = 8.3), 7.22 (s, 1H, H(3)), 7.79 (d, 1H, H(6), J = 8.3).

4-Methyl-1-nitro-2-[2-(2-nitrophenoxy)ethoxy]benzene 7. A mixture of compound **6** (4.64 g, 178.5 mmol) and potassium 2-nitrophenolate (3.47 g, 196 mmol) in DMF (9.0 ml) was stirred at 130ºC for 2 hours. After cooling to room temperature, the precipitate formed was filtered off, washed with water and dried in vacuum to give 5.4 g of product **7**, yield 95%. M.p. 150 – 151 ºC ([S9]: m. p. 150 – 152 °C). ^{1}H NMR (400.13 MHz, DMSO-d_6, 21 ºC): δ = 2.39 (s, 3H, CH_3), 4.49 – 4.55 (m, 4H, $C\underline{H_2}C\underline{H_2}$), 6.94 (d, 1H, H(5), J = 8.2), 7.14 (dt, 1H, H(10), J_1 = 7.8, J_2 = 0.9), 7.26 (s, 1H, H(3)), 7.44 (dd, 1H, H(9), J_1 = 8.5, J_2 = 0.9), 7.65 (dt, 1H, H(11), J_1 = 8.0, J_2 = 1.7), 7.77 (d, 1H, H(6), J = 8.2), 7.85 (dd, 1H, H(12), J_1 = 8.0, J_2 = 1.7).

2-[2-(2-Aminophenoxy)ethoxy]-4-methylaniline 8. To a stirred suspension of compound **7** (5.4 g, 169 mmol) and 10% Pd/C (0.437 g) in EtOH (70 ml) at 78 ºC in argon atmosphere, hydrazine hydrate (37 ml) was added dropwise. The mixture was refluxed for 2 hours, then cooled to room temperature and filtered. The filtrate was evaporated to leave a brown oil, which was crystallized from ethanol to give 3.3 g of product **8**, yield 75%. M. p. 117 – 118 ºC ([S9]: m. p. 118 – 119 °C). ^{1}H NMR (400.13 MHz, DMSO-d_6, 21 ºC): δ = 2.16 (s, 3H, CH_3), 4.26 (bs, 4H, $C\underline{H_2}C\underline{H_2}$), 4.47 (bs, 2H, NH_2), 4.68 (bs, 2H, NH_2), 6.46 – 6.56 (m, 3H, H(5), H(6), H(12)), 6.60 – 6.75 (m, 3H, H(10), H(11), H(3)), 6.85 (d, 1H, H(9), J = 7.9).

2-(2-{2-[Bis(2-ethoxy-2-oxoethyl)amino]phenoxy}ethoxy)-*N*,*N*-bis(2-ethoxy-2-oxoethyl)-4-methylaniline 9. A mixture of compound **8** (3.65 g, 14 mmol), ethyl 2-bromoacetate (7.8 ml, 70 mmol), Na_2HPO_4 (9.7 g) and NaI (0.86 g) in MeCN (53 ml) was refluxed in argon atmosphere for 18 hours and then cooled to room temperature. The mixture was poured into water (100 ml), and the product was extracted with dichloromethane. The combined organic phases were washed with water. Then, the solvent was removed in vacuum and the residue was recrystallized from ethanol to give 3.57 g (yield 42%) of compound **9**. M. p. 109 – 110 ºC. ^{1}H NMR (400.13 MHz, DMSO-d_6, 21 ºC): δ: 1.00 – 1.10 (m, 12H, 4×$CH_2\underline{CH_3}$), 2.21 (s, 3H, CH_3), 3.86 – 4.00 (m, 8H, 4×$\underline{CH_2}CH_3$), 4.04 (bs, 4H, 2×$C\underline{H_2}$COOEt), 4.08 (bs, 4H, 2×$C\underline{H_2}$COOEt), 4.17 (bs, 4H, $C\underline{H_2}C\underline{H_2}$), 6.57 – 6.66 (m, 2H, H(5), H(6)), 6.67 – 6.73 (m, 1H, H(12)), 6.76 (s, 1H, H(3)), 6.79 – 6.89 (m, 2H, H(10), H(11)), 6.90 – 6.98 (m, 1H, H(9)).

4-[Bis(2-ethoxy-2-oxoethyl)amino]-3-(2-{2-[bis(2-ethoxy-2-oxoethyl)amino]-5-methyl-phenoxy}ethoxy)benzaldehyde 4. Compound **9** (0.4 g, 0.7 mmol) and pyridine (75 μl) were dissolved in of DMF (1 ml), cooled to 0ºC, and $POCl_3$ (0.55 ml) was added dropwise. After stirring at room temperature for 30 min, the mixture was heated to 60ºC and stirred for 5 hours. The mixture was cooled to room temperature and poured into ice water (50 ml), and the product was extracted with dichloromethane. The combined organic phases were washed with water, the solvent was removed in vacuum to leave 0.35 g (yield 85%) of compound **4**. M. p. 99 – 100 ºC. ^{1}H NMR (400.13 MHz, DMSO-d_6, 20 ºC): δ = 0.98 – 1.14 (m, 12H, 4×$CH_2\underline{CH_3}$), 2.22 (s, 3H, CH_3), 3.87 – 4.10 (m, 12H, 6×CH_2), 4.13 – 4.28 (m, 8H, 4×CH_2), 6.57 – 6.68 (m, 2H, H(5), H(6)), 6.72 (d, 1H, H(12), J = 8.3), 6.76 (s, 1H, H(3)), 7.38 (s, 1H, H(9)), 7.43 (d, 1H, H(11), J = 8.3), 9.76 (s, 1H, CHO). ^{13}C NMR (100.60 MHz, DMSO-d_6, 18 ºC): 13.80 (2×$CH_2\underline{CH_3}$), 13.87 (2×$CH_2\underline{CH_3}$), 53.17 (2×NCH_2), 53.53 (2×NCH_2), 60.04 (2×$C\underline{H_2}CH_3$), 60.46 (2×$C\underline{H_2}CH_3$), 66.66 ($\underline{C}H_2CH_2$), 67.40 ($CH_2\underline{C}H_2$), 111.53 (C(9)), 114.09 (C(3), 116.05 (C(12)), 118.23 (C(5)), 125.60 (C(11)), 128.78 (C(10), 130.59 (C(4)), 136.38 (C(1)), 144.59 (C(7)), 148.66 (C(8)), 149.55 (C(2)), 170.25 (2×C=O), 170.85 (2×C=O), 190.69 (CHO). EI-mass m/z: calculated: 630.28,

found: 630 (4), 585 (16), 584 (56), 557 (93), 529 (31), 336 (69), 249 (60), 234 (50), 220 (36), 176 (100), 148 (83), 162 (72).

2-{4-[Bis(2-ethoxy-2-oxoethyl)amino]-3-(2-{2-[bis(2-ethoxy-2-oxoethyl)amino]-5-methyl-phenoxy}ethoxy)phenyl]methylideneamino}-6-methoxy-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione, BAPTA-NI. To a mixture of compound **4** (0.05 g, 0.08 mmol) and compound **3** (0.02 g, 0.08 mmol) in EtOH (2.0 ml), AcOH (25 μl) was added. The mixture was refluxed for 12 hours and then cooled to room temperature. The precipitate was filtered off, washed with cold ethanol and dried in vacuum. Yield of **BAPTA-NI** was 49 mg (73%). M. p. 161 – 162 ºC. ^{1}H NMR (400.13 MHz, DMSO-d_6, 20 ºC): δ = 0.97 – 1.14 (m, 12H, 4×$CH_2\underline{CH_3}$), 2.22 (s, 3H, CH_3), 3.87 – 4.31 (m, 23H, $\underline{CH_2}C\underline{H_2}$, 4×N$\underline{CH_2}$, 4×$\underline{CH_2}CH_3$, OCH_3), 6.60 (d, 1H, H(6), J = 8.0), 6.64 (d, 1H, H(5), J = 8.0), 6.73 – 6.80 (m, 2H, H(3), H(12)), 7.33 – 7.41 (m, 2H, H(3′), H(11), 7.54 (s, 1H, H(9)), 7.83 – 7.91 (m, 1H, H(6′)), 8.47 – 8.57 (m, 3H, H(5′), H(7′), CH=N), 8.61 (d, 1H, H(2′), J = 8.3). ^{13}C NMR (150.93 MHz, DMSO-d_6, 19 ºC): δ = 13.85 (2×$CH_2\underline{C}H_3$), 13.90 (2×$CH_2\underline{C}H_3$), 20.52 (CH_3), 53.16 (2×N$\underline{CH_2}$), 53.40 (2×N$\underline{CH_2}$), 56.77 (OCH_3), 60.09 (2×$\underline{C}H_2CH_3$), 60.40 (2×$\underline{C}H_2CH_3$), 66.73 ($\underline{C}H_2CH_2$), 67.37 ($CH_2\underline{C}H_2$), 106.54 (C(3′)), 110.58 (C(4′)), 111.20 (C(9)), 114.03 (C(3)), 114.53 (C(1′)), 116.63 (C(12)), 118.10 (C(6)), 121.73 (C(5)), 122.74 (C(8′)), 123.36 (C(4a′)), 124.41 (C(10) or C(8)), 124.86 (C(11)), 127.04 (C(6′)), 128.45 (C(2′) or C(7′)), 128.92 (C(8a′)), 130.92 (C(4)), 131.79 (C(2′) or C(7′)), 134.07 (C(5′)), 136.79 (C(1)), 143.29 (C(7)), 149.35 (C(10) or C(8)), 149.83 (C(2)), 160.53 (C(8b′) or C(8c′)), 160.99 (C(8b′) or C(8c′)), 170.85 (4×$\underline{C}OOEt$), 171.30 (CH=N). ESI-mass m/z: calculated: 854.34; found: 877.8 (100) ($[M+Na]^+$), 856.5 (19) ($[M+H]^+$).

S2 Figures S1–S8

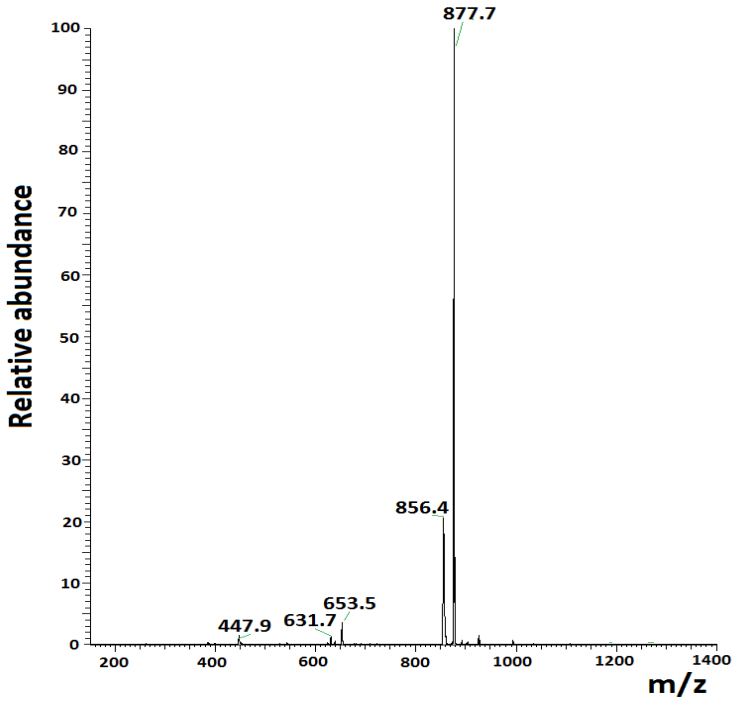


Figure S1. ESI MS spectrum of solution of compound **BAPTA-NI** in H_2O containing 1 equiv. of $Ca(ClO_4)_2$.

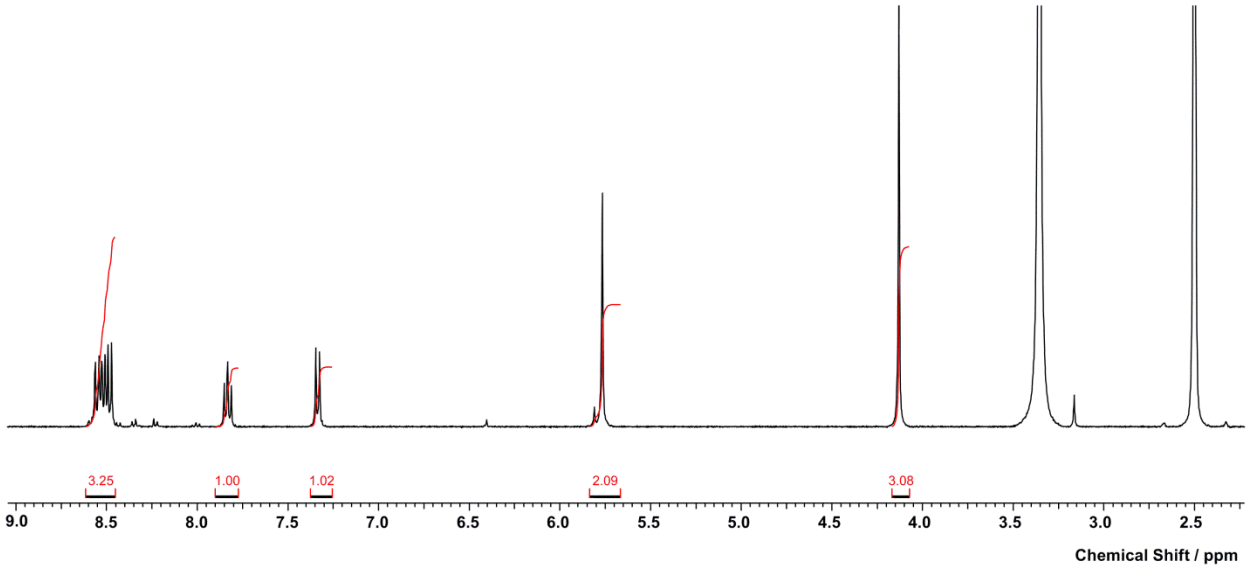


Figure S2. ^{1}H NMR spectrum of compound **3** in DMSO-d_6.

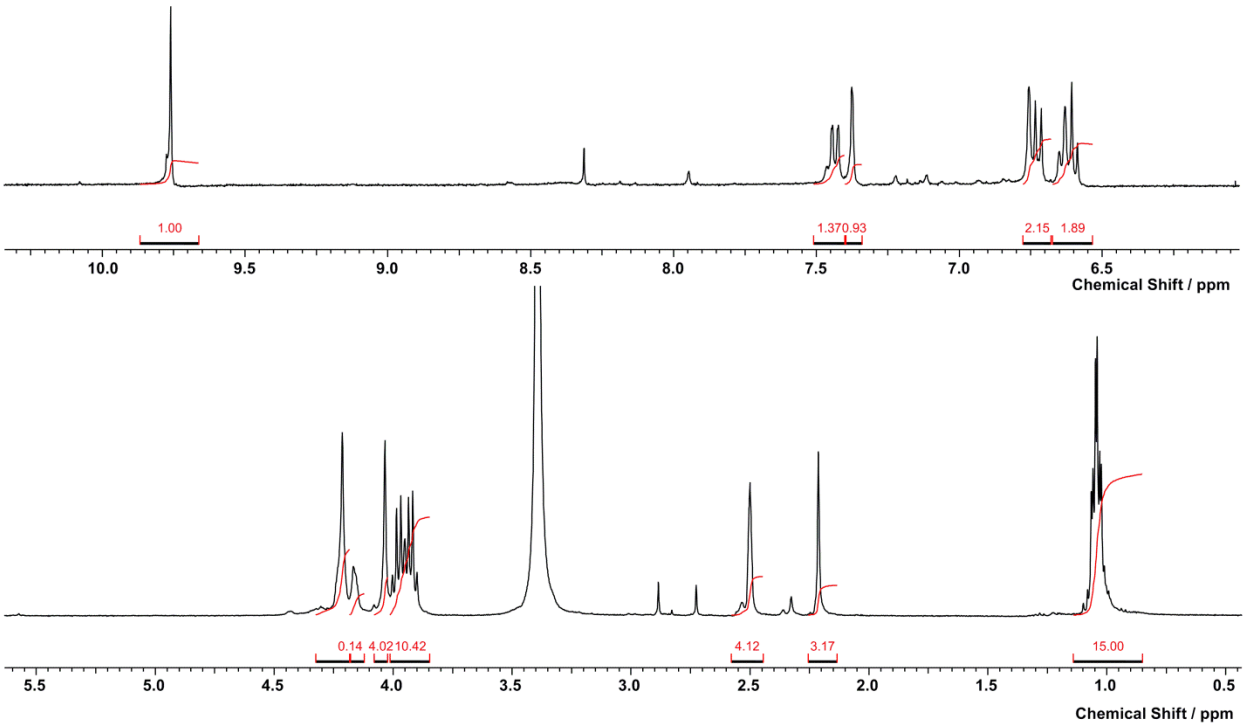


Figure S3. ^{1}H NMR spectrum of compound **4** in DMSO-d_6.

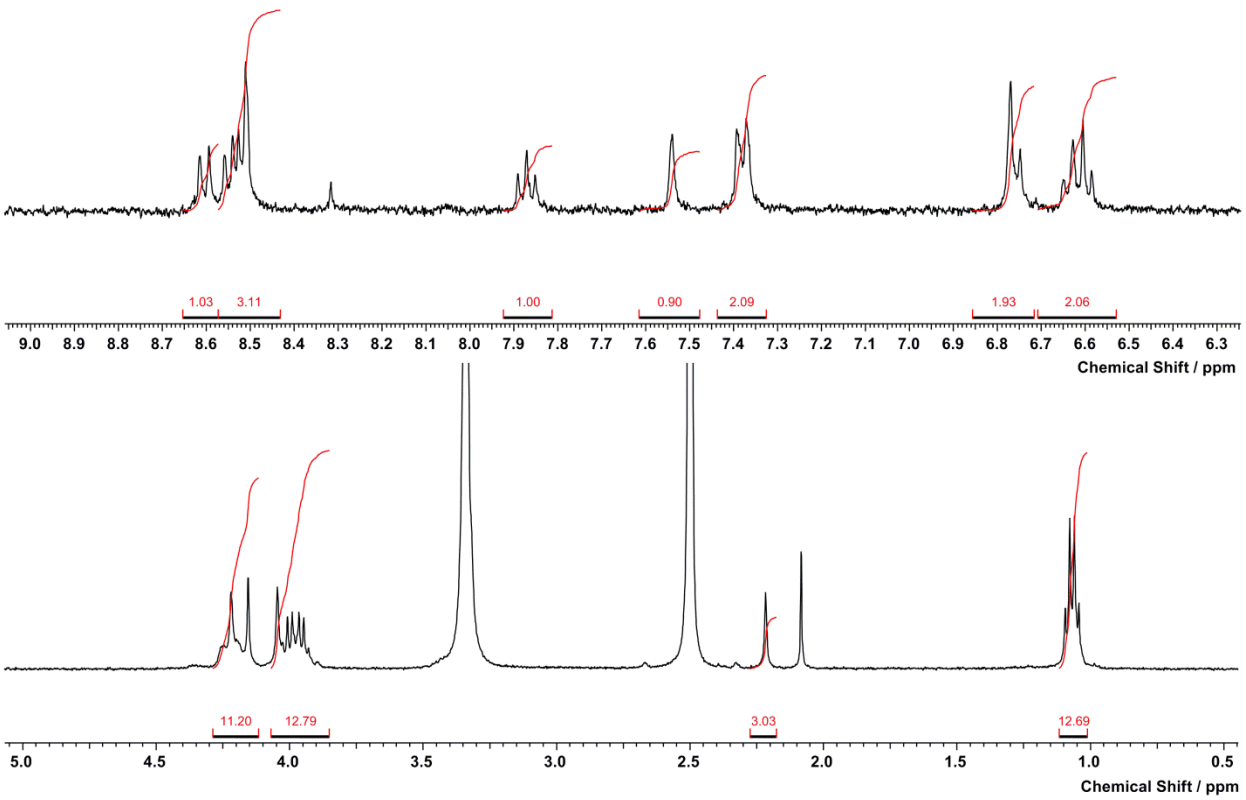


Figure S4. ^{1}H NMR spectrum of compound **BAPTA-NI** in DMSO-d_6.

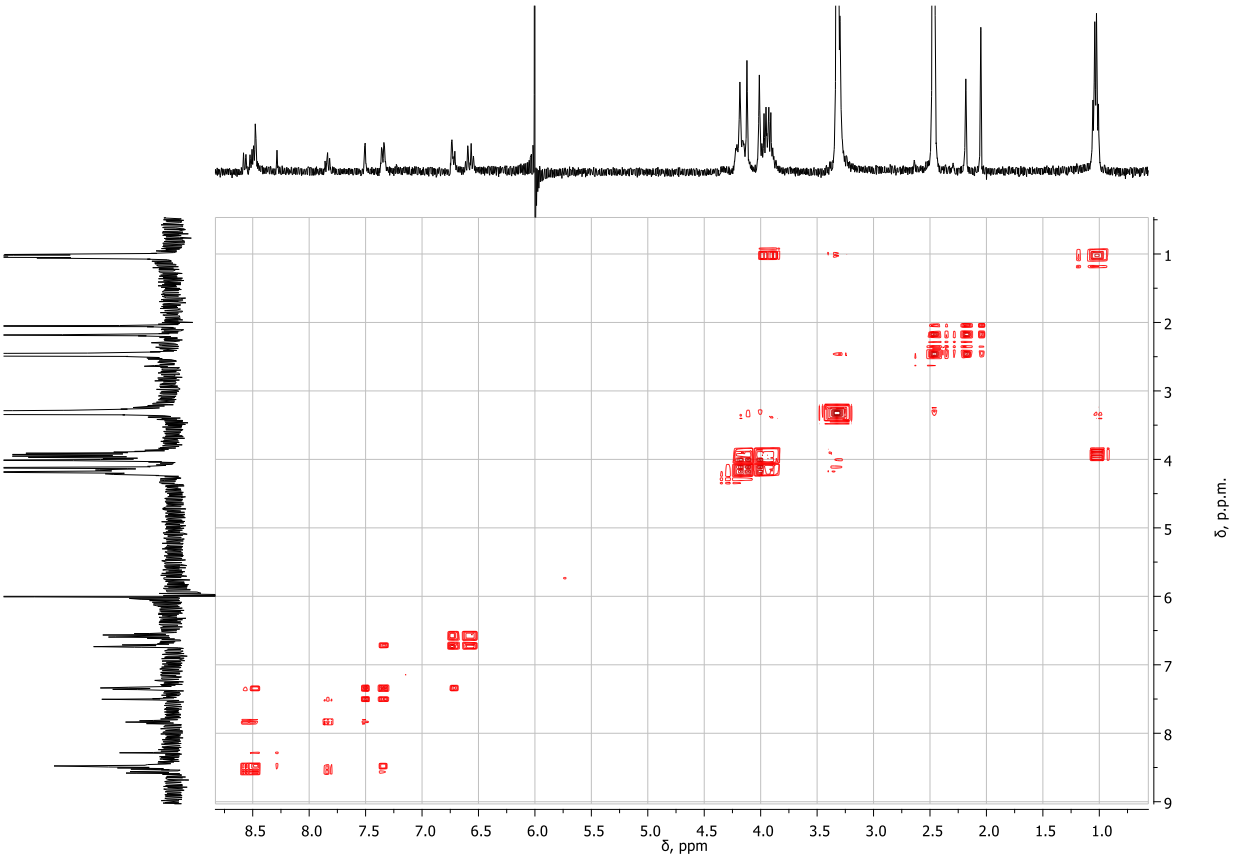


Figure S5. ^{1}H-^{1}H COSY spectrum of compound **BAPTA-NI** in DMSO-d_6.

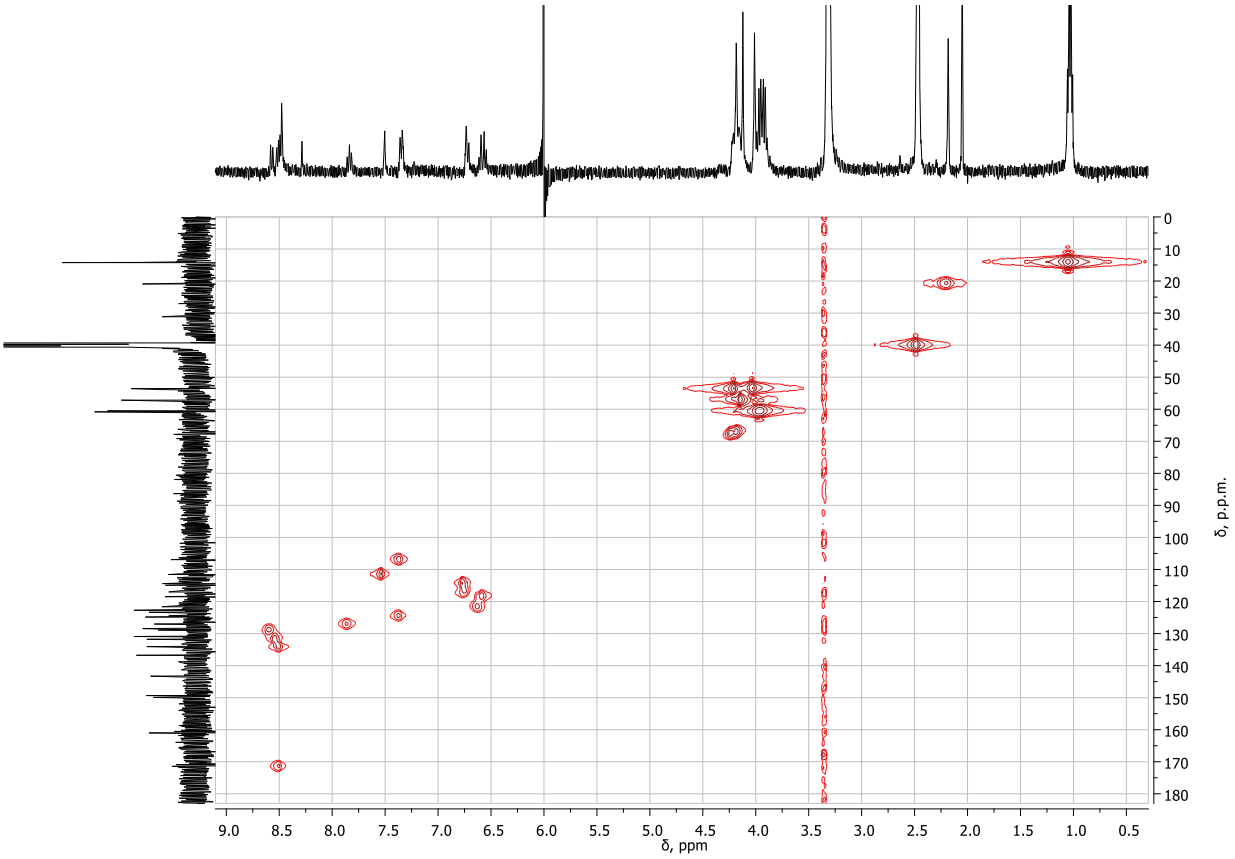


Figure S6. HSQC spectrum of compound **BAPTA-NI** in DMSO-d_6.

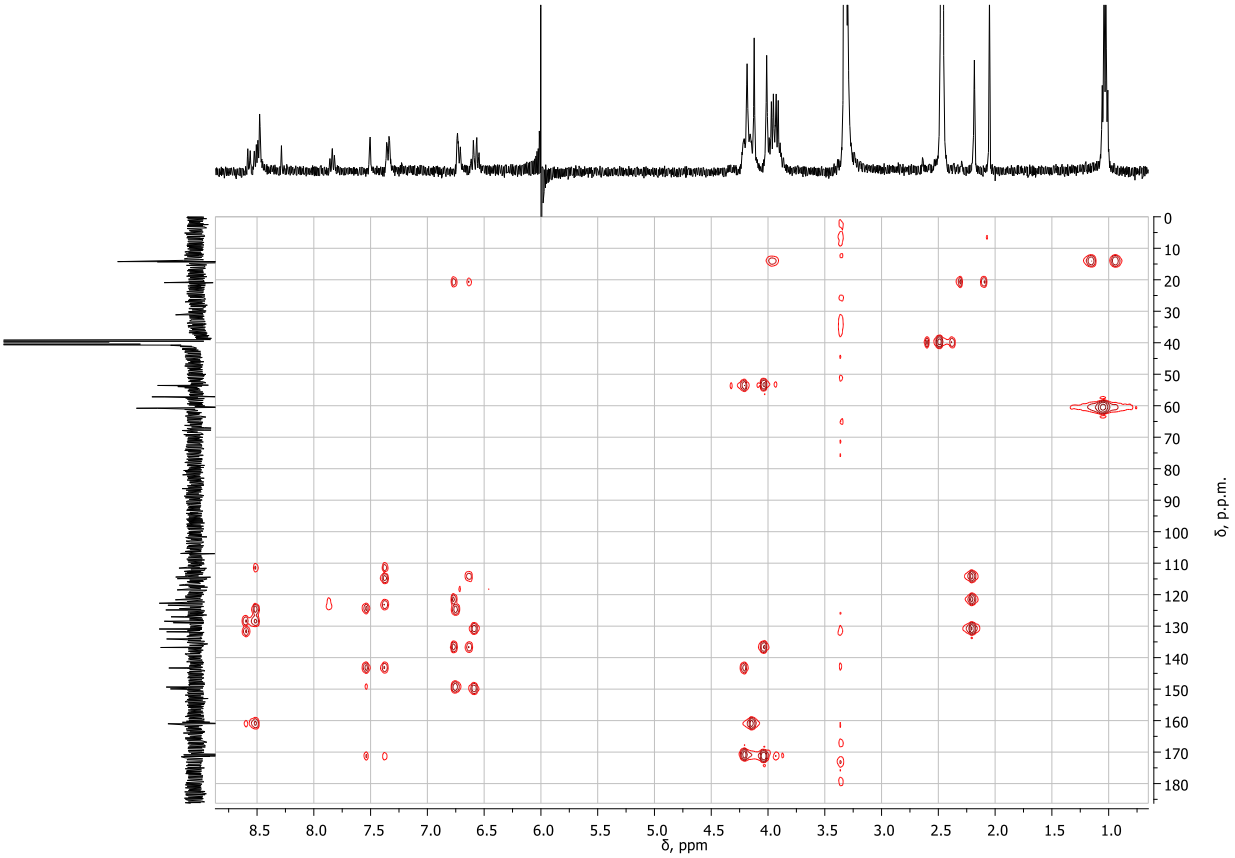


Figure S7. HMBC spectrum of compound **BAPTA-NI** in DMSO-d_6.

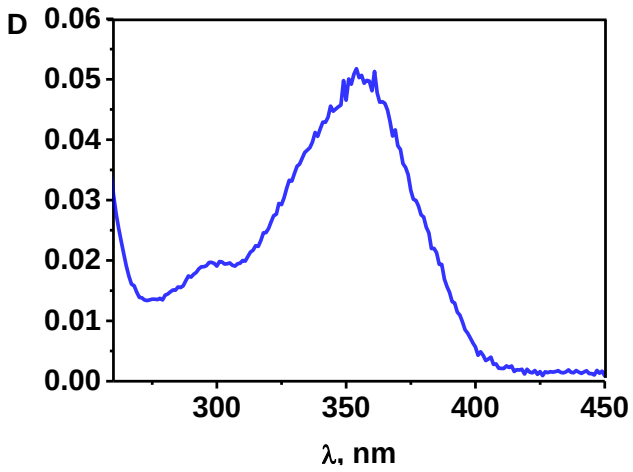


Figure S8. Absorption spectra of **BAPTA-NI** in acetonitrile ($4 \cdot 1^{-6}$mol dm^{-3}).

a *b*

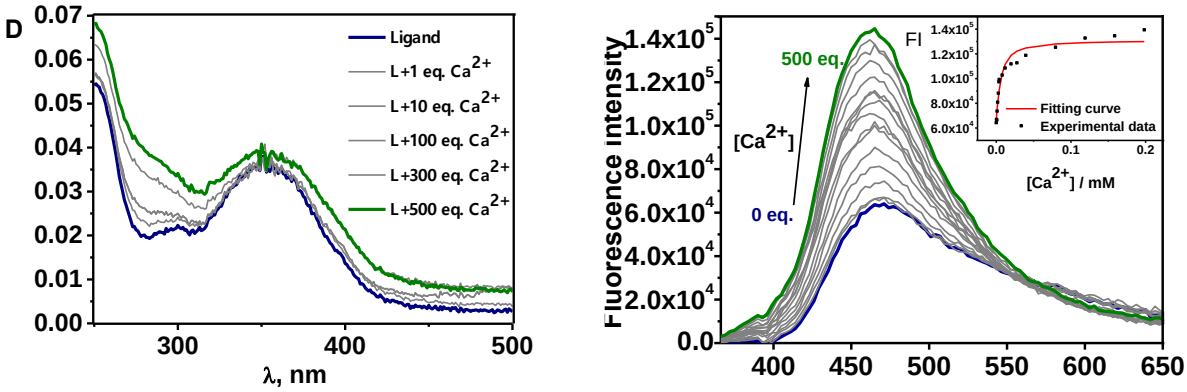


Figure S9. Changes in absorption spectra (*a*) and emission spectra (*b*) of compound **BAPTA-NI** in water ($4 \cdot 10^{-6}$ mol dm^{-3}) in the absence and presence of $Ca(ClO_4)_2$, excitation wavelength 350 nm. The upper insets show the dependence of fluorescence intensity (FI) at 471 nm on the concentration of Ca^{2+}.

Table S1. Spectral characteristics of **BAPTA-NI** in water.

	λ_{max}^{abs}/nm	λ_{max}^{fl} /nm	φ^{fl}	lgK[(L)·Ca^{2+}]
BAPTA-NI	350	471 (λ_{ex}=350)	0.0022	5.4 ± 0.1

S3. Determination of detection limit

The detection limit was determined from the fluorescence titration data based on a reported method [S11]. The detection limit was calculated with the following equation:

$$\text{Detection limit} = 3\sigma/K$$

, where σ is the standard deviation of blank measurement, and K is the slop between the fluorescence versus Ca^{2+} concentration. There was a good linearity at micro molar concentration levels between fluorescent intensity data at 471 nm and concentrations of Ca^{2+} in water to $3.6 \cdot 10^{-6}$ M. The linear equation was found to be $y = 9.45 \cdot 10^{9} \cdot x + 62312$ ($r = 0.990$), where y is the fluorescent intensity data at 471 nm measured at a given Ca^{2+} concentration and x represents the concentration of Ca^{2+} added. So, the detection limit for Ca^{2+} was calculated to be $4 \cdot 10^{-7}$ M (Detection limit=3σ/K=3·1155/9.45×10^{-9} M ≈ $4 \cdot 10^{-7}$ M).

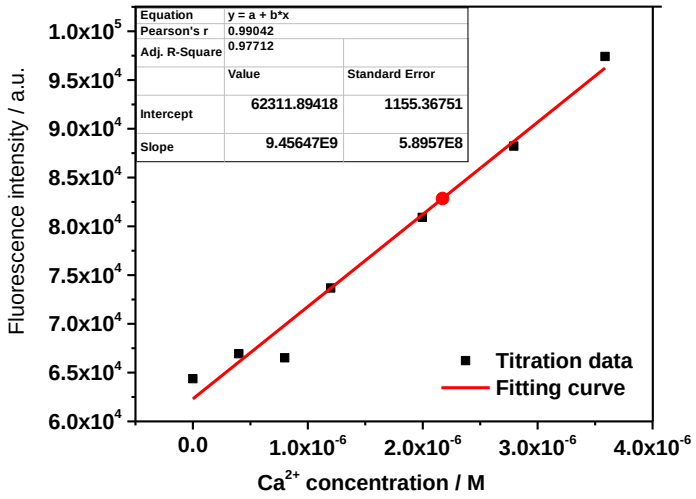


Figure S10. Normalized response of fluorescence signal of **BAPTA-NI** in H_2O ($4 \cdot 10^{-6}$ M) in the presence of increasing amount of Ca^{2+} (from blank solution to 3.6×10^{-6} M). λ_{ex} = 350 nm; λ_{em} = 471 nm.

For acetonitrile solution there was a good linearity between fluorescent intensity data at 437 nm and concentrations of Ca^{2+} to $8.0 \cdot 10^{-6}$ M. The linear equation was found to be $y = 5.04 \cdot 10^9 \cdot x + 122068$ (r = 0.999), the detection limit for Ca^{2+} was calculated to be $7 \cdot 10^{-7}$ M (Detection limit=3σ/K=$3 \cdot 1212/5.04 \times 10^{-9}$ M ≈ $7 \cdot 10^{-7}$ M).

S4 References

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