Supporting Information "**Two-photon probes for Golgi apparatus: Detection of Golgi apparatus in live tissue by two-photon microscopy**"

Ji-Woo Choi[†], Seung Taek Hong[†], Mun Seok Kim[‡], Kyu Cheol Paik[‡], Man So Han[‡], and Bong Rae Cho*[,‡,§]

[†]*KU-KIST Graduate School of Converging Science and Technology, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul, 02841, Republic of Korea*

[‡] *Department of Chemistry, Daejin University, 1007 Hoguk-ro, Pocheon-si, Gyeonggi-do, 11159, Republic of Korea*

[§] *Department of Chemistry, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul, 02841, Republic of Korea*

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Synthesis

A and **Pyr-SIM** were prepared in our earlier works.[1,2] The synthetic scheme for BGolgi-blue is shown below.

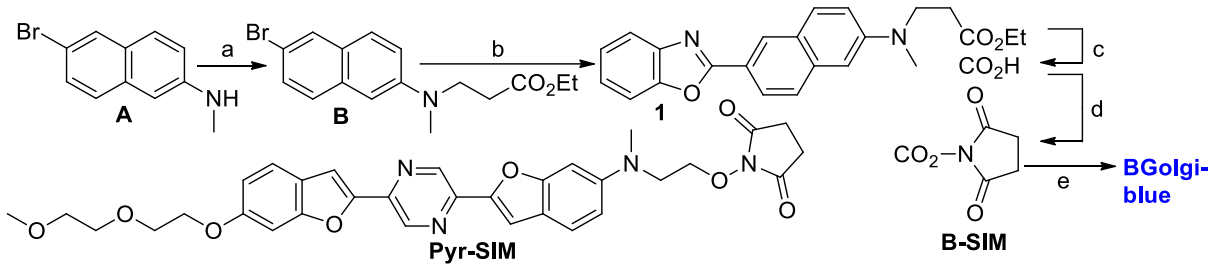

Figure S1. Structures of B-SIM and Pyr-SIM, and synthesis of BGolgi-blue. (a) CH_2=$CHCO_2Et$, $(CF_3)_2CHOH$, 60 °C. (b) Benzoxazole, $Pd(OAc)_2$, CuI, Cs_2CO_3, dimethylformamide (DMF), 90 °C. (c) KOH, EtOH, rt. (d) *N*-hydroxysuccinimide, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide·HCl (EDCl), 4-dimethylaminopyridine (DMAP), CH_2Cl_2, rt. (e) SDYQRL, Et_3N, dimethylsulfoxide (DMSO), rt.

Compound B

A solution of **A** (0.5 g, 2.1 mmol) and ethyl acrylate (0.64 g, 6.4 mmol) in $(CF_3)_2CHOH$ (2 mL) was stirred at 60 °C for 2 h. The solvent was evaporated and the product was purified by silica gel column chromatography using hexane/EtOAc (3/1) as the eluent. The product was obtained as a yellow oil. Yield: 0.68 g (96%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.82 (1 H, d, J = 2.0 Hz), 7.59 (1 H, d, J = 8.9 Hz), 7.50 (1 H, d, J = 8.9 Hz), 7.42 (1 H, J = 8.9, 2.0 Hz), 7.16 (1 H, dd, J = 8.9, 2.7 Hz), 6.86 (1 H, m), 4.14 (2 H, q, J = 7.1 Hz), 3.78 (2 H, t, J = 7.2 Hz), 3.02 (3 H, s), 2.61 (2 H, t, J = 7.2 Hz), 1.26 (3 H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.4, 147.1, 133.7, 129.7, 129.6, 128.3, 128.1, 128.0, 117.0, 115.4, 106.5, 61.0, 48.9, 38.6, 32.3, 14.5.

Compound 1

A flame-dried round-bottom flask with a magnetic stirring bar was charged with CuI (0.04g, 0.21 mmol), $Pd(OAc)_2$ (0.01g, 0.05 mmol), PPh_3 (0.03 g, 0.11 mmol), Cs_2CO_3 (0.45 g, 1.3 mmol), Benzoxazole (0.26 g, 2.1 mmol), and **B** (0.36 g, 1.1 mmol). Dimethyl formamide (DMF, 3 mL) was then added to the mixture using a syringe under Ar. The reaction mixture was heated to 90°C for 30 min, cooled to rt, and filtered through a small pad of celite. The solid residue

was washed with CH_2Cl_2 (10 mL) and the combined organic layers were evaporated. The product was purified by silica gel column chromatography using 9% EtOAc in *n*-hexane as the eluent. Yield: 0.27 g (67%); ^{1}H NMR (700 MHz, DMSO-d_6): δ 8.61 (1 H, d, J = 1.7 Hz), 8.09 (1 H, dd, J = 8.5, 1.7 Hz), 7.97 (1 H, d, J = 9.0 Hz), 7.82 (1 H, d, J = 8.5 Hz), 7.80–7.77 (2 H, m), 7.42–7.39 (2 H, m), 7.32 (1 H, dd, J = 9.4, 2.6 Hz), 7.02 (1 H, d, J = 2.1 Hz), 4.05 (2 H, q, J = 7.1 Hz), 3.79 (2 H, t, J = 7.1 Hz), 3.04 (3 H, s), 2.61 (2 H, t, J = 7.1 Hz), 1.16 (3 H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 164.1, 151.0, 148.2, 142.6, 137.0, 130.5, 128.2, 127.0, 126.1, 124.8, 124.7, 124.6, 120.4, 119.8, 116.6, 110.6, 106.1, 61.0, 48.7, 38.7, 32.3, 14.4.

B-SIM

KOH (1 N, 3 mL) was added to a solution of **1** (0.17 g, 0.45 mmol) in ethanol (1 mL) and the solution was stirred for 3 h. The solvent was evaporated, and the product mixture was dissolved in water and acidified with 1 N HCl. The product was extracted with ethyl acetate, and the combined organic layer was washed with brine, dried over $MgSO_4$, and the solvent was evaporated. A mixture of the crude product (0.15 g, 0.44 mmol), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide·HCl (EDCl, 0.09 g, 0.48 mmol), and 4-(dimethylamino)pyridine (DMAP, 0.005 g, 0.04 mmol) in CH_2Cl_2 (5 mL) was stirred for 30 min. *N*-Hydroxysuccinimide (0.06 g, 0.48 mmol) was then added to this mixture, and the mixture was stirred at rt for 12 h under Ar. The mixture was extracted with CH_2Cl_2 and the organic layer was dried over anhydrous $MgSO_4$; then, filtration was carried out to obtain a light-yellow solution. After concentration, the solution was precipitated with ether to give a light yellow solid. The solid was collected and dried in vacuo for 8 h. The product was used for the next step without further purification.

BGolgi-blue

A mixture of crude B-SIM (0.050 g, 0.11 mmol), Et_3N (0.020 g, 0.17 mmol), and trans-Golgi-network peptide (SDYQRL, InterPharm, Gyeonggi-do, Korea) (0.090 g, 0.11 mmol) in dimethylsulfoxide (DMSO) was stirred at rt for 2 h. The precipitates produced upon the addition of ether were collected and dissolved in DMSO. The product was purified by semi-preparative reverse-phase high-performance liquid chromatography (RP-HPLC, SunFire Preparative Column C18 OBD, 5 μm, 19 × 50 mm) on a WATERS HPLC system (Waters Corporation, Milford, MA) using step gradient elution with 30–90% MeOH/H_2O at flow rate

25 mL/min over 10 min. Yield: 0.07 g (56%); ^{1}H NMR (700 MHz, DMSO-d_6): δ 9.17 (1 H, s), 8.60 (1 H, d, *J* = 1.7 Hz), 8.40 (1 H, d, *J* = 6.9 Hz), 8.21 (1 H, d, *J* = 7.3 Hz), 8.10 (1 H, d, *J* = 7.7 Hz), 8.07 (1 H, dd, *J* = 8.5, 1.7 Hz), 7.96 (1 H, d, *J* = 9.5 Hz), 7.95 (1 H, d, *J* = 7.5 Hz), 7.91 (1 H, d, *J* = 7.3 Hz), 7.85–7.82 (1 H, m), 7.80 (1 H, d, *J* = 8.5 Hz), 7.79–7.77 (2 H, m), 7.49 (1 H, br s), 7.41–7.39 (2 H, m), 7.31 (1 H, dd, *J* = 9.0, 2.6 Hz), 7.24 (1 H, br s), 7.00 (2 H, d, *J* = 8.5 Hz), 6.99 (1 H, d, *J* = 3.4 Hz), 6.79 (1 H, br s), 6.64 (2 H, d, *J* = 8.5 Hz), 5.19 (1 H, br s), 4.52–4.50 (1 H, m), 4.38–4.30 (3 H, m), 4.24–4.19 (2 H, m), 3.78–3.70 (2 H, m), 3.61–3.58 (1 H, m), 3.57–3.54 (1 H, m), 3.11–3.08 (2 H, m), 3.02 (3 H, s), 2.94–2.92 (1 H, m), 2.68–2.59 (2 H, m), 2.45–2.41 (1 H, m), 2.14–2.06 (2 H, m), 1.91–1.86 (1 H, m), 1.78–1.74 (1 H, m), 1.71–1.68 (1 H, m), 1.65–1.60 (1 H, m), 1.55–1.47 (5 H, m), 1.28–1.22 (1 H, m), 0.87 (3 H, d, *J* = 6.9 Hz), 0.81 (3 H, d, *J* = 6.4 Hz). HRMS (ESI): *m/z* calcd. for [$C_{54}H_{68}N_{12}O_{14}$+H^+]: 1109.5051, found: 1109.5056.

The ^{1}H NMR spectrum of SDYQRL in DMSO has 12 peaks (1–8 and *) in the region δ 9.3–5.0 (Figure S2), of which all but two doublets at δ 7.03 (2 H, d, *J* = 8.5 Hz) and 6.64 (2 H, d, *J* = 8.5 Hz) disappear in D_2O (Figure S1d). Hence, the two peaks can be assigned to the aromatic protons of tyrosine (5 and 6). In addition, the singlets at δ 9.17 (1 H, s), 8.13 (3 H, br s), 7.24 (1 H, s), 6.82 (1 H, s), and 5.19 (1 H, br s) can be assigned to the phenolic OH of tyrosine (4), -C(NH_2)=NH protons of arginine (3), amide protons of glutamine (8), and OH proton of serine (7), respectively. In addition, the triplet at δ 7.56 (1 H, t, *J* = 1.7 Hz) can be assigned to the N-H proton of arginine (2), and the doublets at δ 8.70, 8.17, 8.08, 8.01, and 7.94 (*J* = 6.9–7.7 Hz) can be assigned to the N-H protons (*) next to the C=O groups, respectively (Figure S1c). The 10 peaks at δ 9.3–5.0 in the ^{1}H NMR of compound **1** are assigned as a–j in Figure S1b. The ^{1}H NMR of BGolgi-blue is almost the same as the sum of those of SDYQRL and **1**, except for the changes in the chemical shifts of the N-H protons (2 and *), which can be attributed to the change in the magnetic environment around the C-H and N-H bonds upon conjugation, and the disappearance of the -C(NH_2)=NH protons of arginine (3) that may have been broadened. The relative areas are consistent with the proposed structure.

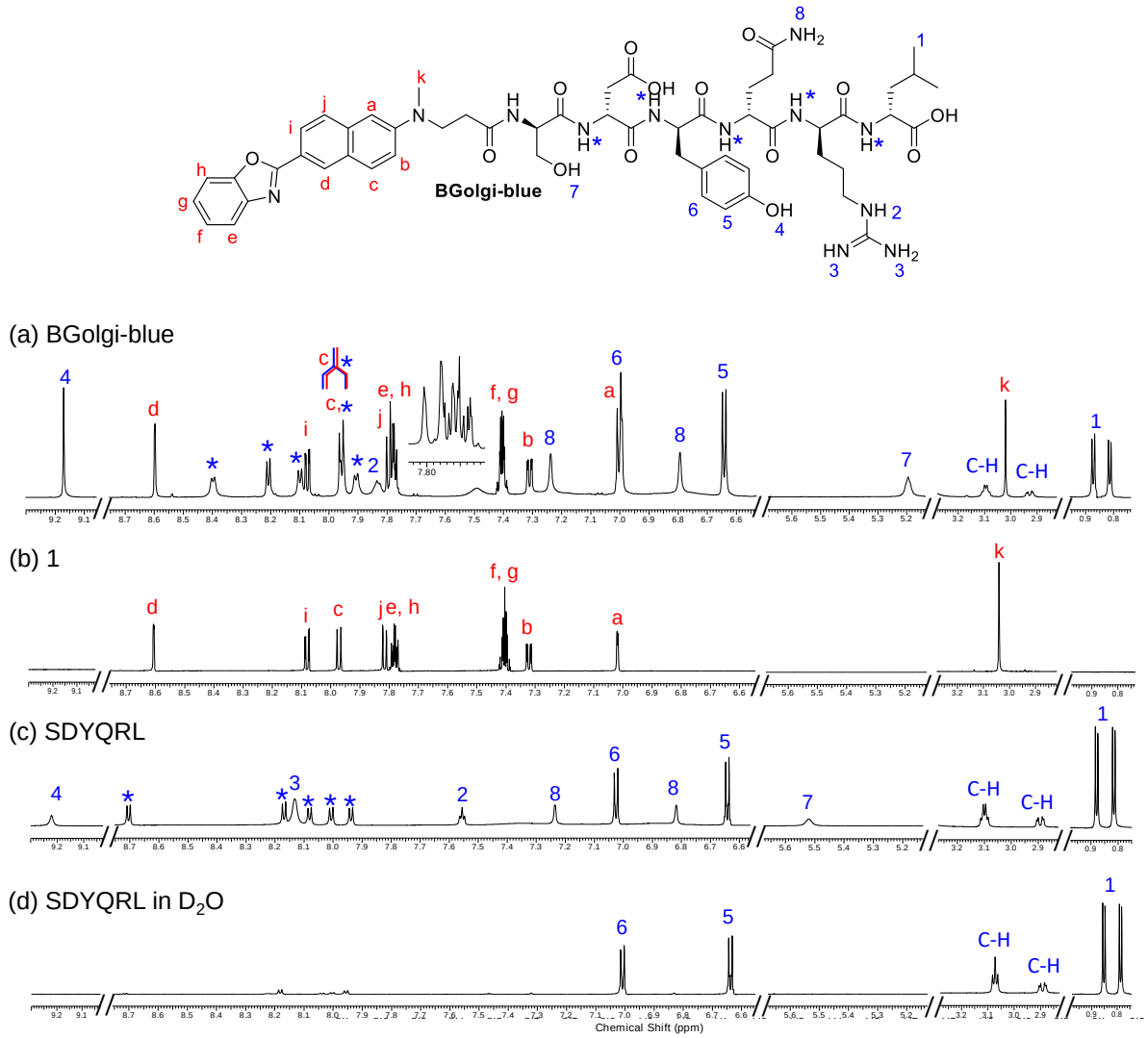

Figure S2. (a) ^{1}H NMR spectrum of BGolgi-blue in DMSO. (b,c) ^{1}H NMR spectrum of compound **1** (b) and SDYQRL (c) in DMSO and (d) of SDYQRL in D_2O. The protons in the fluorophore and SDYQRL moieties are indicated as a–l and 1–8, respectively. The N-H bonds next to the C=O groups in the SDYQRL moiety are denoted by asterisks (*). Most of the aliphatic C-H peaks are excluded for simplicity.

PGolgi-yellow

PGolgi-yellow was synthesized from Pyr-SIM and SDYQRL as above. Yield: 0.050 g (40%); ^{1}H NMR (700 MHz, DMSO-d_6): δ 9.17 (1 H, s), 9.11 (1 H, d, J = 1.3 Hz), 9.09 (1 H, d, J = 1.3 Hz), 8.38 (1 H, br s), 8.20 (1 H, d, J = 7.3 Hz), 8.08 (1 H, d, J = 7.7 Hz), 7.95−7.92 (2 H, m), 7.88 (1 H, br s), 7.66 (1 H, s), 7.63 (1 H, d, J = 8.5 Hz), 7.61 (1 H, s), 7.52 (1 H, d, J = 9.0 Hz), 7.34 (1 H, d, J = 1.7 Hz), 7.25 (1 H, s), 7.00 (2 H, d, J = 8.5 Hz), 6.98 (1 H, dd, J = 8.5, 1.7 Hz), 6.94 (1 H, d, J = 1.9 Hz), 6.84 (1 H, dd, J = 9.0, 1.9 Hz), 6.79 (1 H, s), 6.64 (2 H, d, J = 8.5 Hz), 5.19 (1 H, br s), 4.53–4.50 (1 H, m), 4.38−4.28 (3 H, m), 4.23−4.18 (4 H, m), 3.79−3.78 (2 H, m), 3.68−3.64 (2 H, m), 3.62−3.61 (3 H, m), 3.56−3.53 (1 H, m), 3.49−3.47 (2 H, m), 3.26 (3 H, s), 3.17 (1 H, s), 3.10−3.08 (2 H, m), 2.95 (3 H, s), 2.94−2.92 (1 H, m), 2.67−2.61 (1 H, m), 2.60−2.58 (1 H, m), 2.56−2.45 (1 H, m), 2.44−2.40 (1 H, m), 2.15−2.06 (2 H, m), 1.91−1.86 (1 H, m), 1.79−1.74 (1 H, m), 1.72−1.68 (1 H, m), 1.66−1.60 (1 H, m), 1.55−1.47 (5 H, m), 0.87 (3 H, d, J = 6.8 Hz), 0.81 ppm (3 H, d, J = 6.4 Hz). HRMS (ESI): *m/z* calcd. for [$C_{62}H_{80}N_{13}O_{18}$+H^+]: 1294.5739, found: 1294.5731.

The 10 peaks (a–j) at δ 9.3–6.5 in the ^{1}H NMR spectrum of **Pyr-SIM** in DMSO are assigned in Figure S3b. The ^{1}H NMR spectrum of PGolgi-yellow is almost the same as the sum of those of SDYQRL and **Pyr-SIM**, except for the changes in the chemical shifts of the N-H protons (2 and *) that can be attributed to the changes in the magnetic environment around the N-H bonds upon conjugation, and the disappearance of the N-H proton (*) and -C(NH_2)=NH protons of arginine (3) that may have been broadened (Figure S3a,c). The relative area of peaks 4, 7, and a–j is 1H, while those of 5, 6, 8, k, l, and 1 are 2H, 2H, 2H, 3H, 3H, and 6H (Figure S3a), respectively, as expected for the proposed structure. These results confirm that **2** is conjugated to SDYQRL in a 1:1 ratio.

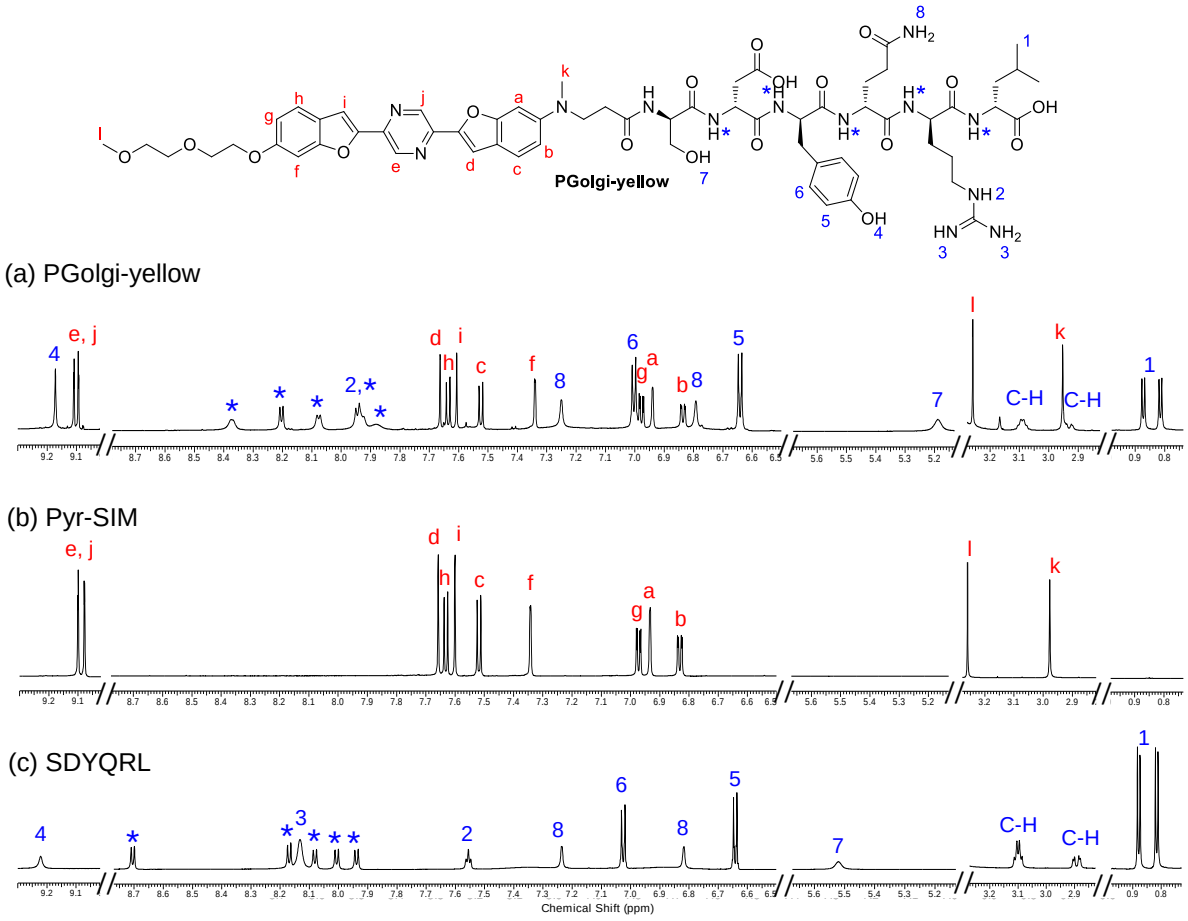

Figure S3. (a) ^{1}H NMR spectrum of PGolgi-yellow in DMSO. (b,c) ^{1}H NMR spectrum of **Pyr-SIM** (b) and SDYQRL (c) in DMSO. The protons in the fluorophore and SDYQRL moieties are indicated as a–l and 1–8, respectively. The N-H bonds next to the C=O groups in the SDYQRL moiety are denoted by asterisks (*). Most of the aliphatic C-H peaks are excluded for simplicity.

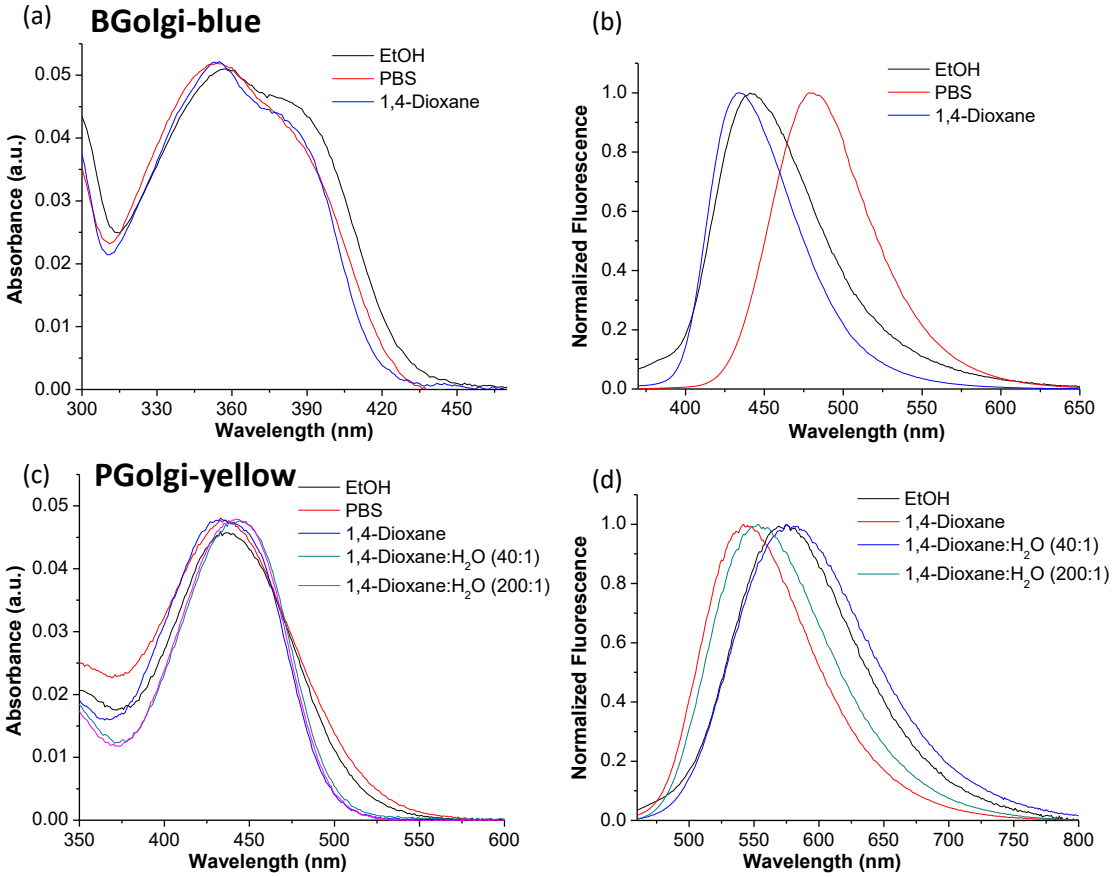

Figure S4. (a, c) Absorption and (b, d) Normalized emission spectra of (a, b) BGolgi-blue and (c, d) PGolgi-yellow in various solvents.

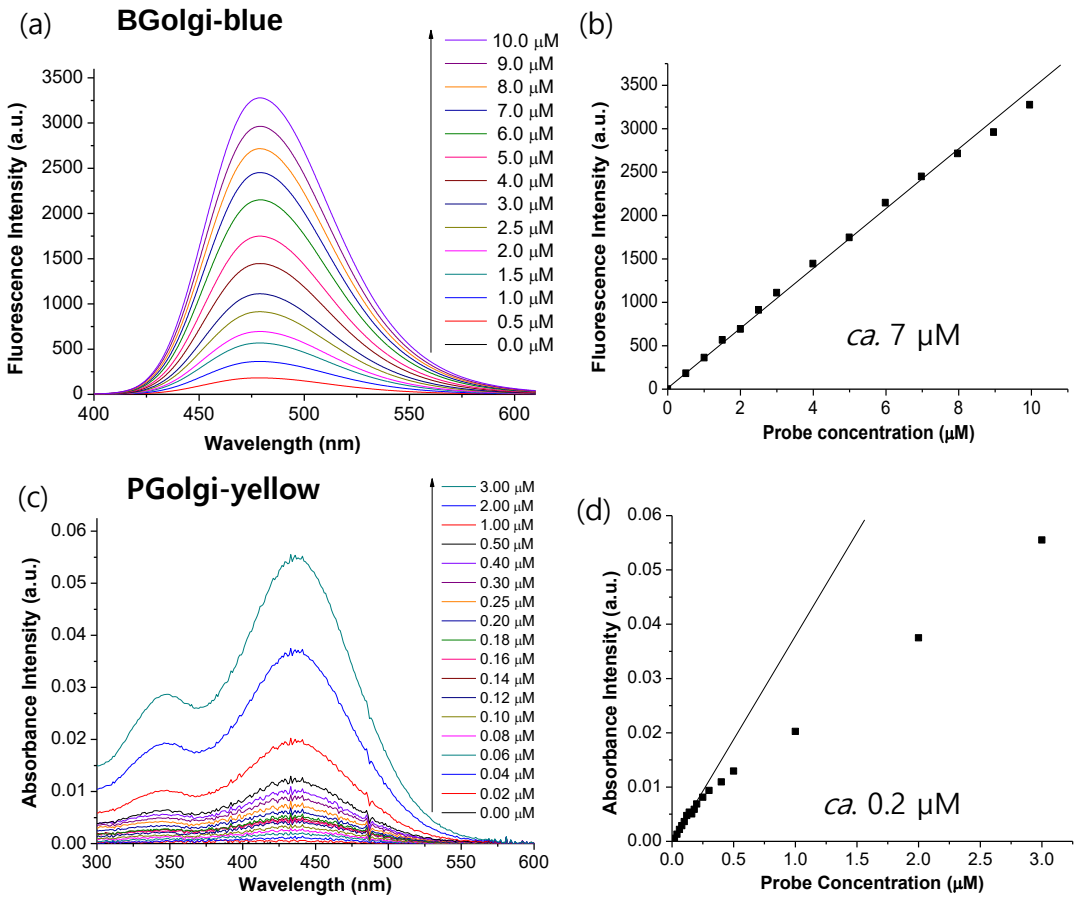

Figure S5. (a) Fluorescence and (c) absorption spectra, and plots of (b) the fluorescence intensity and (d) absorbance against probe concentration for (a, b) BGolgi-blue and (c, d) PGolgi-yellow in PBS buffer. The excitation wavelength was (a) 357 nm.

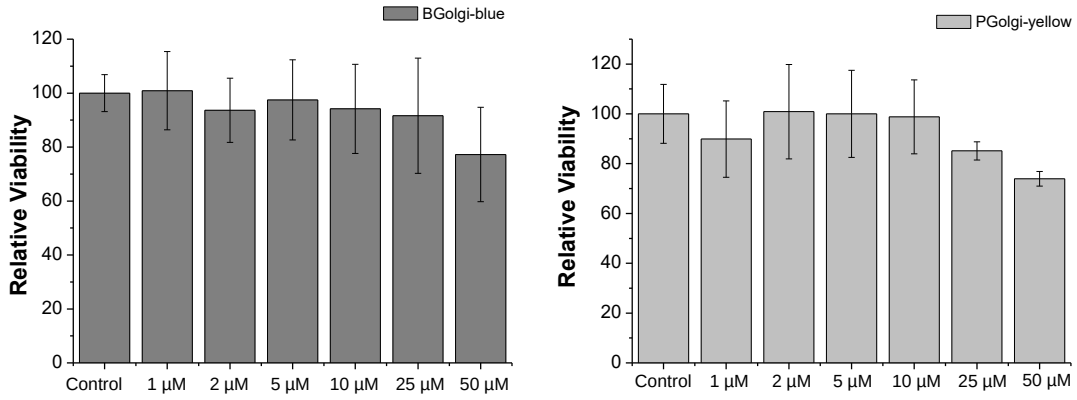

Figure S6. Viability of HeLa cells in the presence of BGolgi-blue and PGolgi-yellow probes, measured using a CCK-8 kit. The cells were incubated with the probes (0–50 μM) for 16 h.

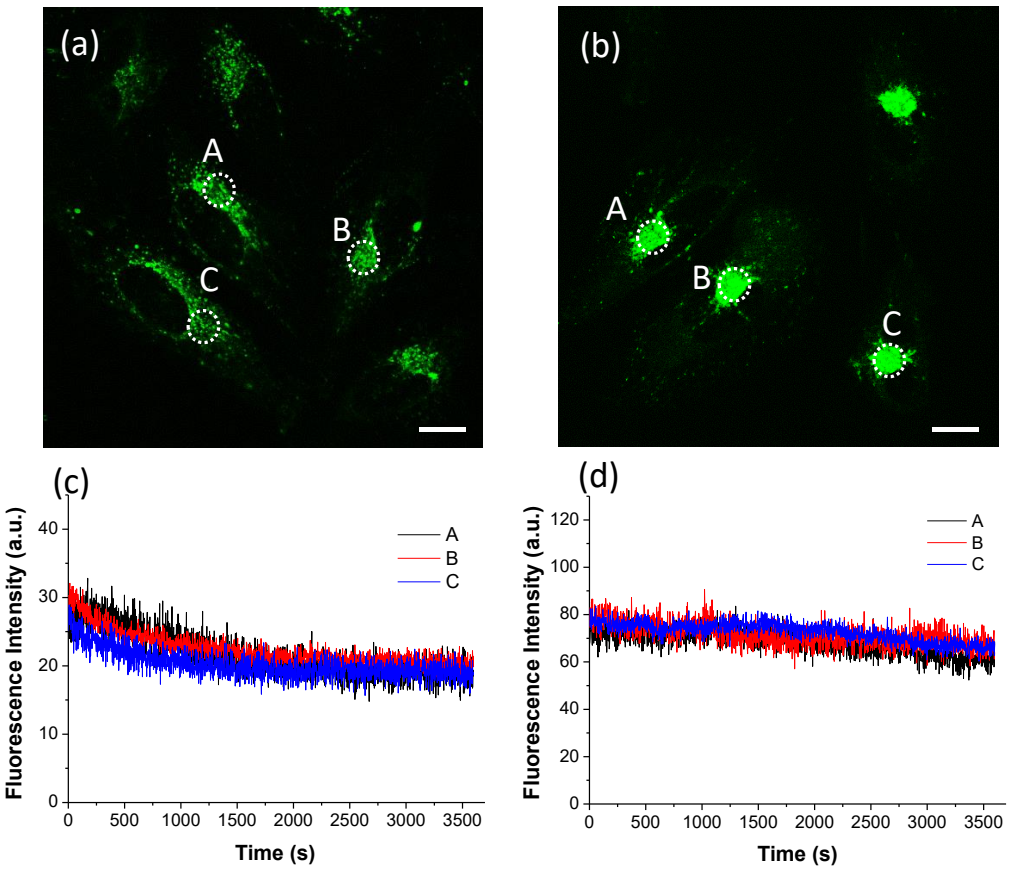

Figure S7. (a, b) Two-photon microscopy (TPM) images of HeLa cells labeled with BGolgi-blue (a) and PGolgi-yellow (b) collected at 400–450 nm and 550–650 nm, respectively. (c, d) The relative two-photon excited fluorescence (TPEF) intensity as a function of time. The digitized intensity was recorded with 1.63-s intervals for a duration of 1 h using *xyt* mode. The TPEF intensities at positions A–C were collected upon excitation at 750 nm with femtosecond pulses. Scale bar: 30 μm.

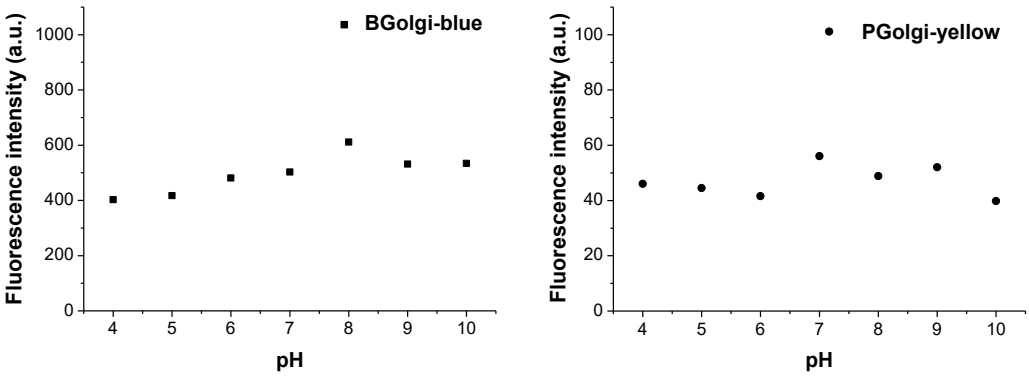

Figure S8. Effect of the pH (4.0–10.0) on the one-photon fluorescence intensity of 2 μM BGolgi-blue and PGolgi-yellow in universal buffer (0.1 M citric acid, 0.1 M KH_2PO_4, 0.1 M $Na_2B_4O_7$, 0.1 M Tris, and 0.1 M KCl) and 30% 1,4-dioxane/universal buffer solution.

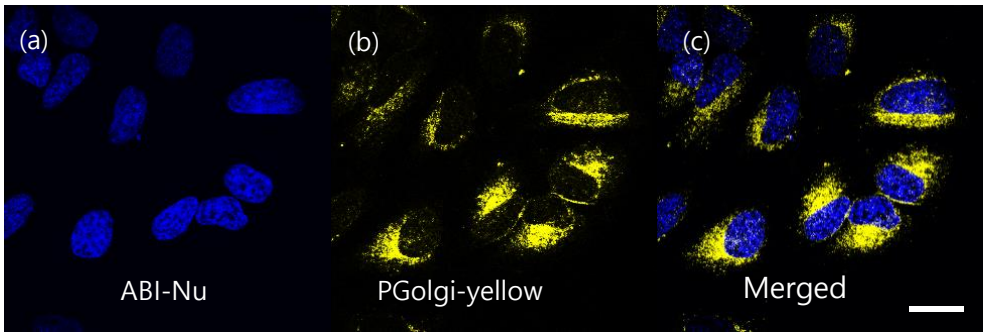

Figure S9. (a, b) Dual-color TPM images of HeLa cells co-labeled with ABI-Nu and PGolgi-yellow. (c) Merged image. The images were collected in Ch1 (b) and Ch2 (c), respectively, upon excitation at 750 nm. Scale bar: 30 μm.

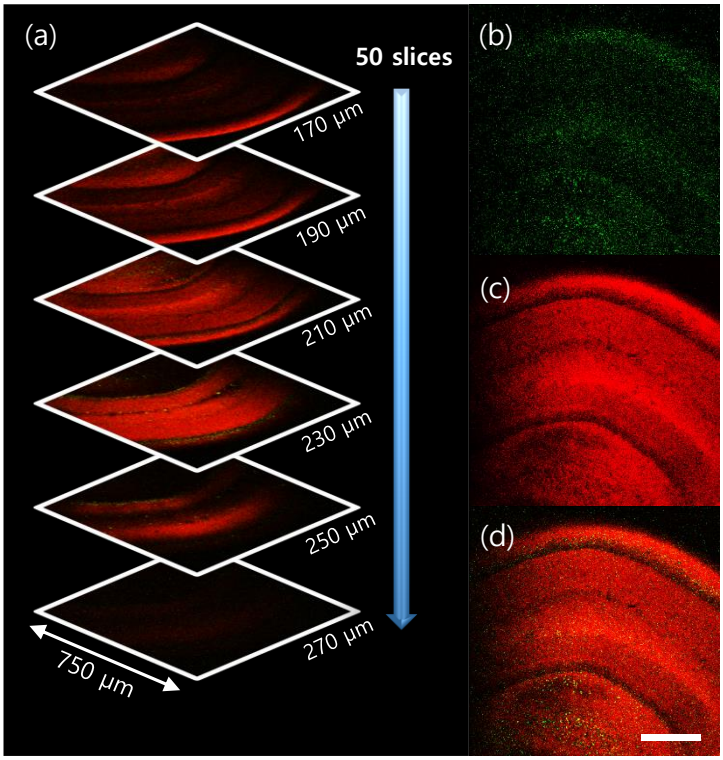

Figure S10. (a) Sectional TPM images of a brain tissue co-labeled with PGolgi-yellow and ABI-Nu at depths of 170–270 µm at 10× magnification. The images were collected at Ch1 (ABI-Nu) and Ch2 (PGolgi-yellow) upon excitation at 750 nm. (b-d) TPM images collected at Ch1 (b) and Ch2 (c) and a merged image (d) at a depth of 220 µm. Scale bar: 100 µm.

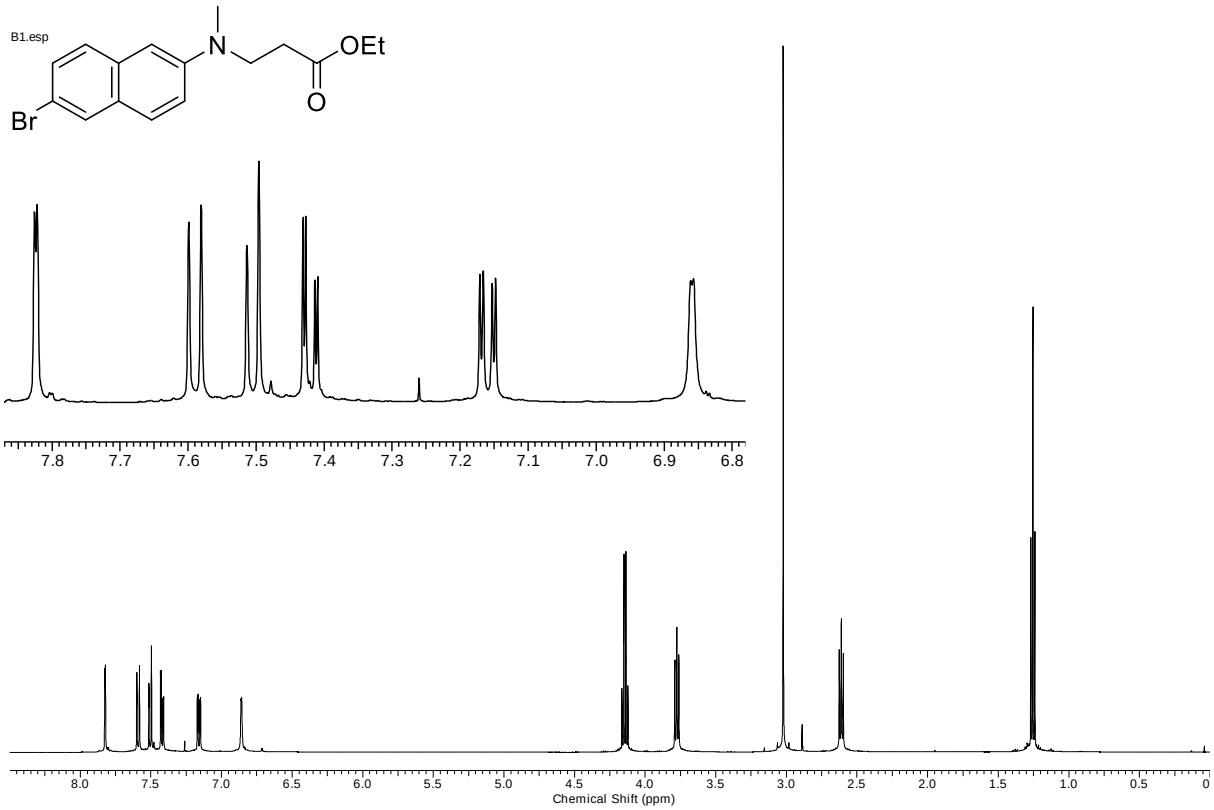

Figure S11. ^{1}H NMR spectrum (500 MHz) of ***Compound B*** in $CDCl_3$**.**

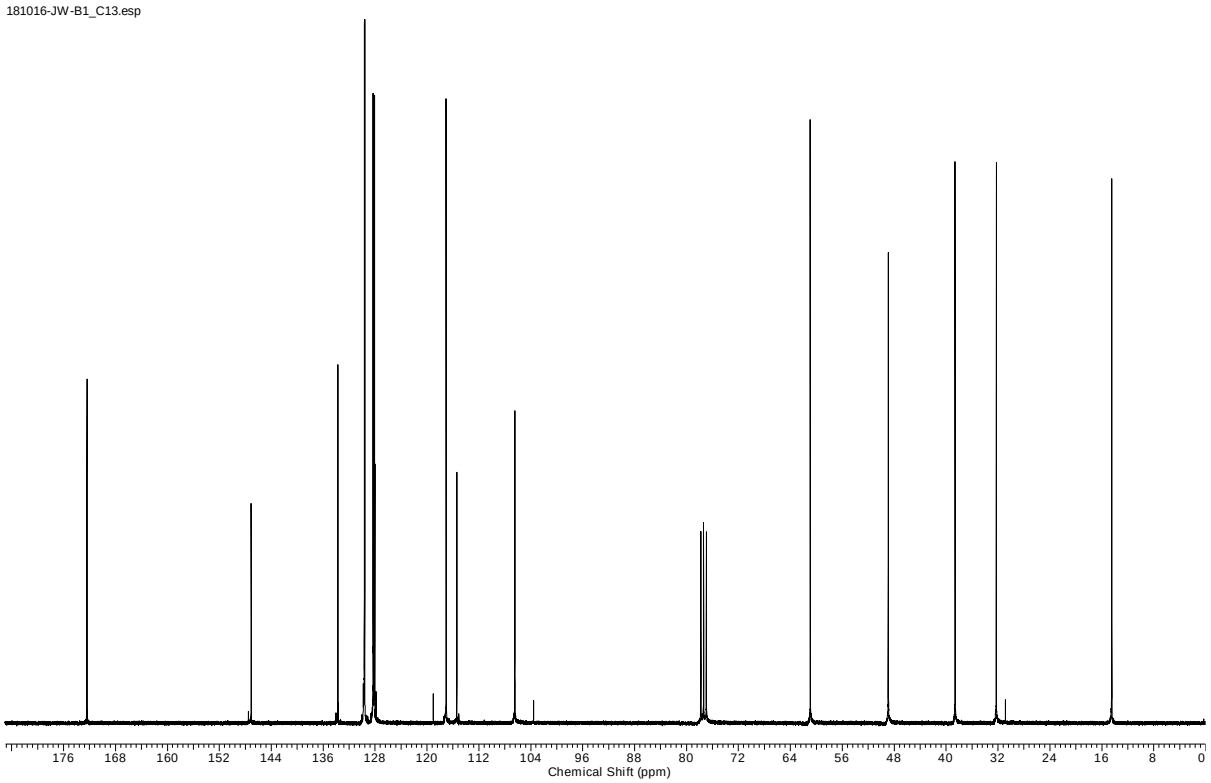

Figure S12. ^{13}C NMR spectrum (100 MHz) of ***Compound B*** in $CDCl_3$**.**

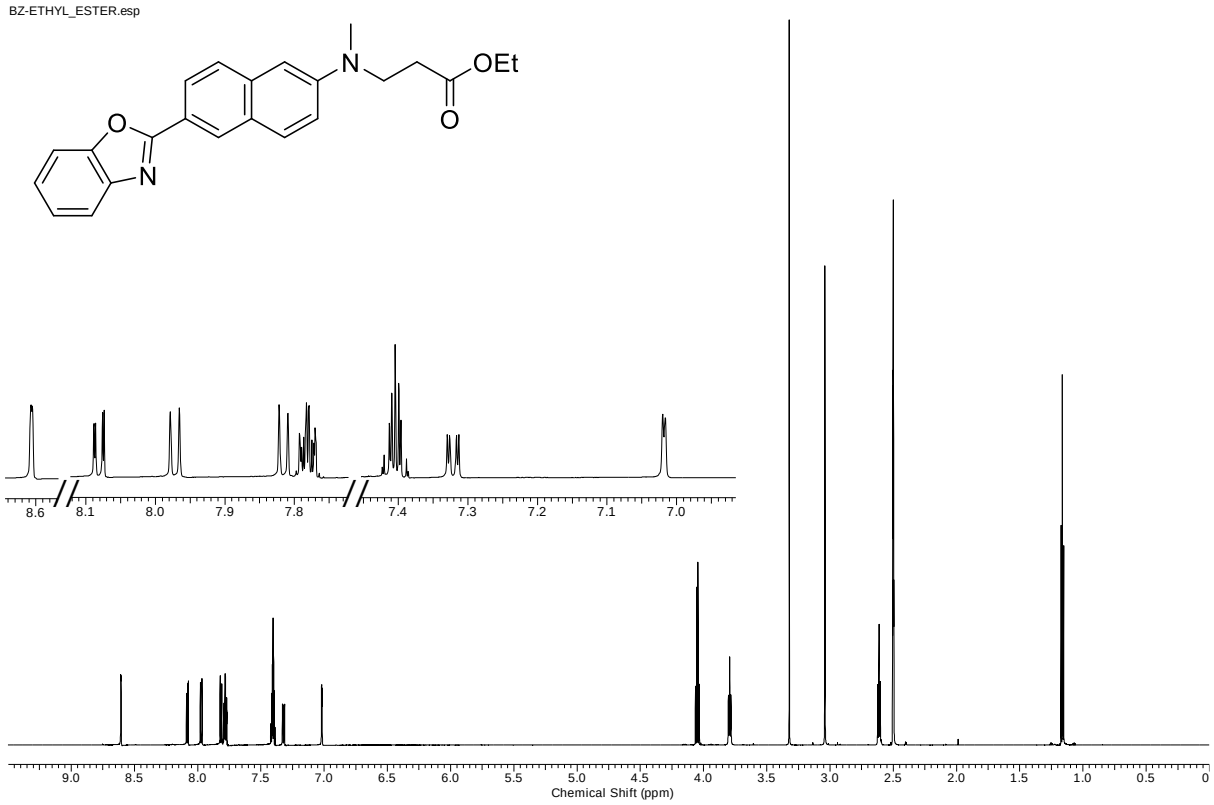

Figure S13. ^{1}H NMR spectrum (700 MHz) of ***Compound 1*** in DMSO-d_6.

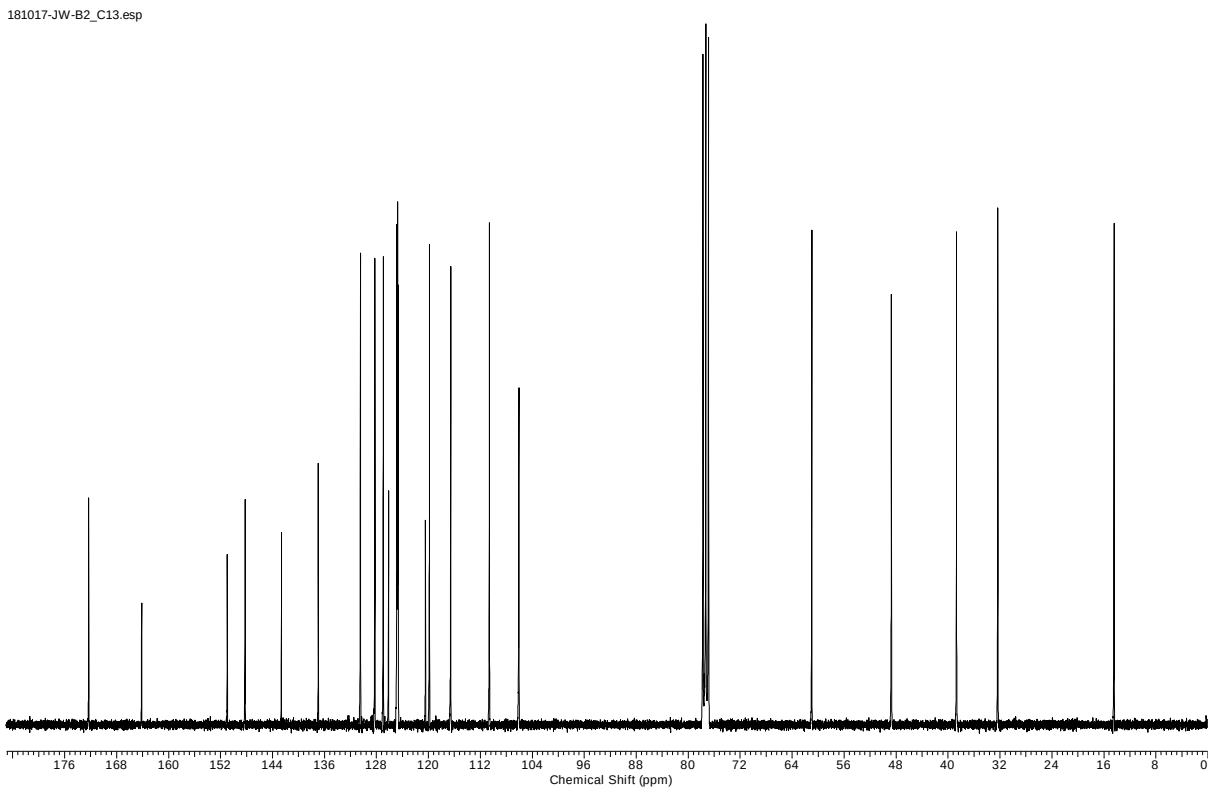

Figure S14. ^{13}C NMR spectrum (100 MHz) of ***Compound 1*** in $CDCl_3$.

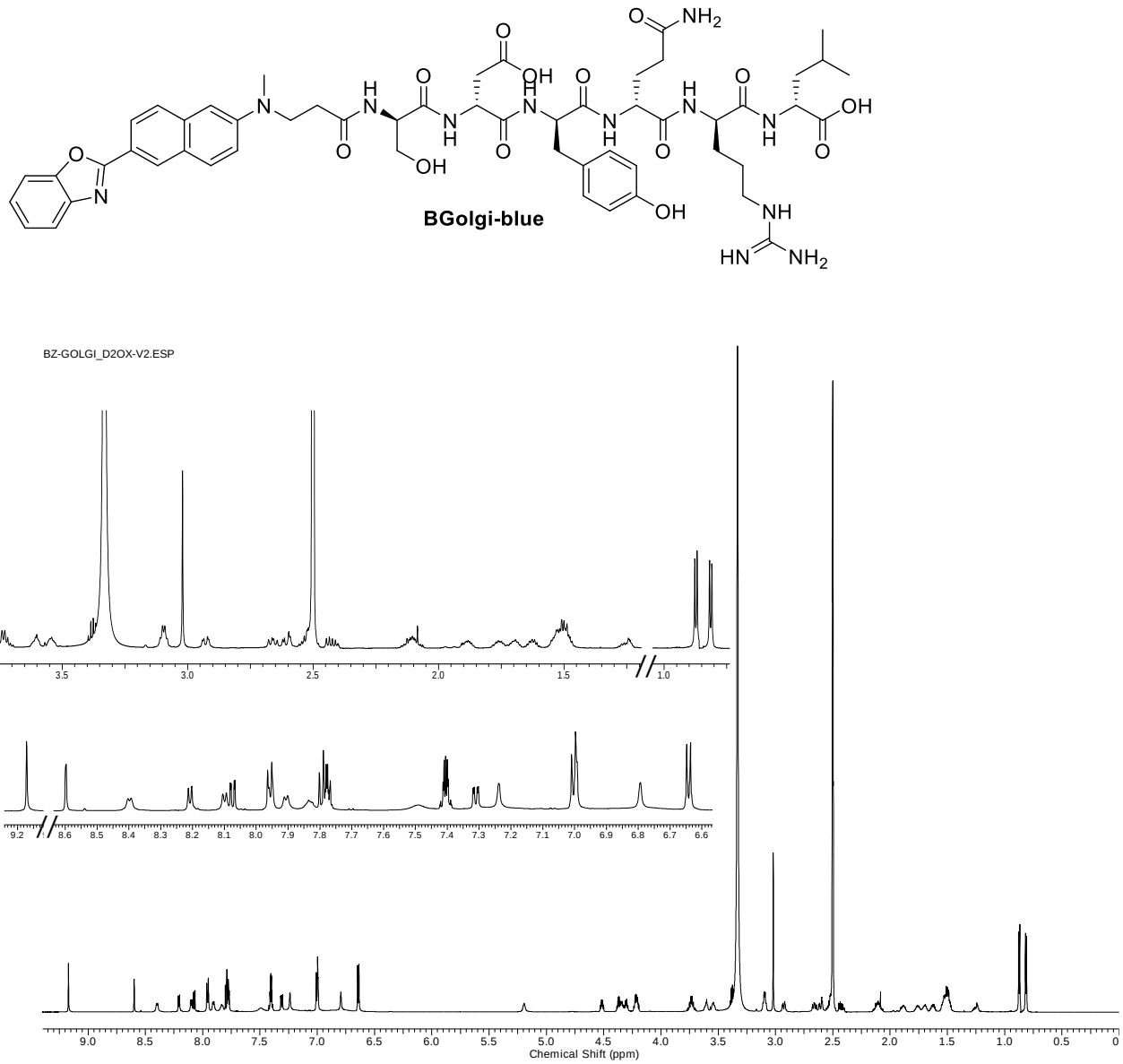

Figure S15. ^{1}H NMR spectrum (700 MHz) of ***BGolgi-blue*** in DMSO-d_6.

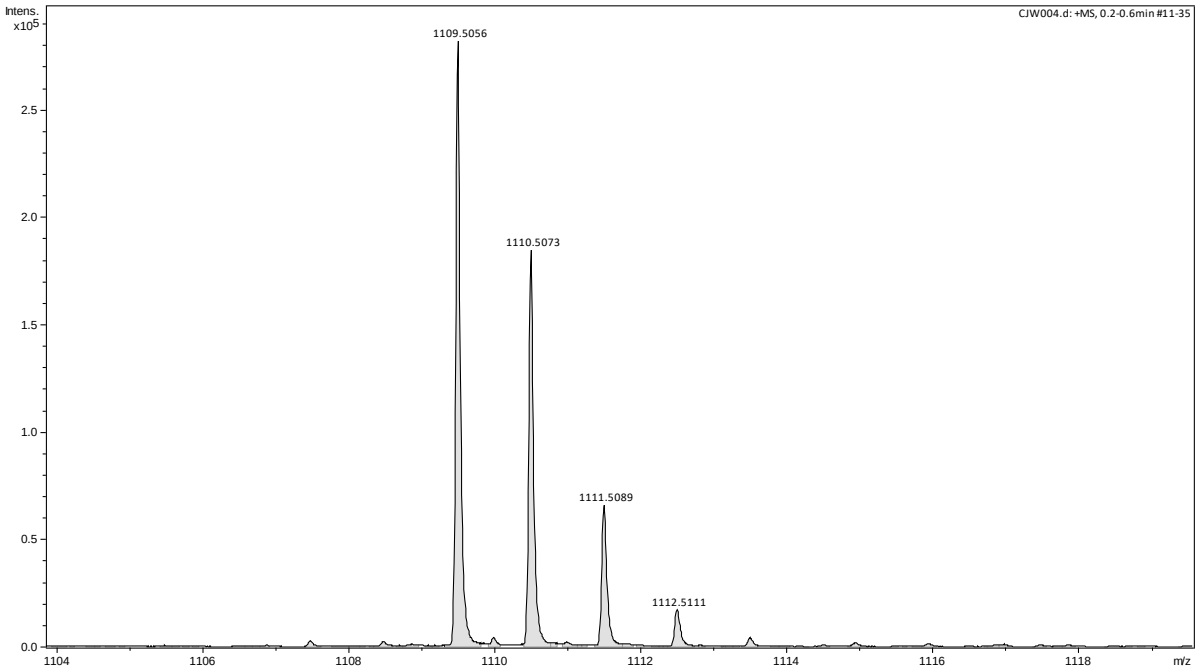

Figure S16. HRMS spectrum of ***BGolgi-blue.***

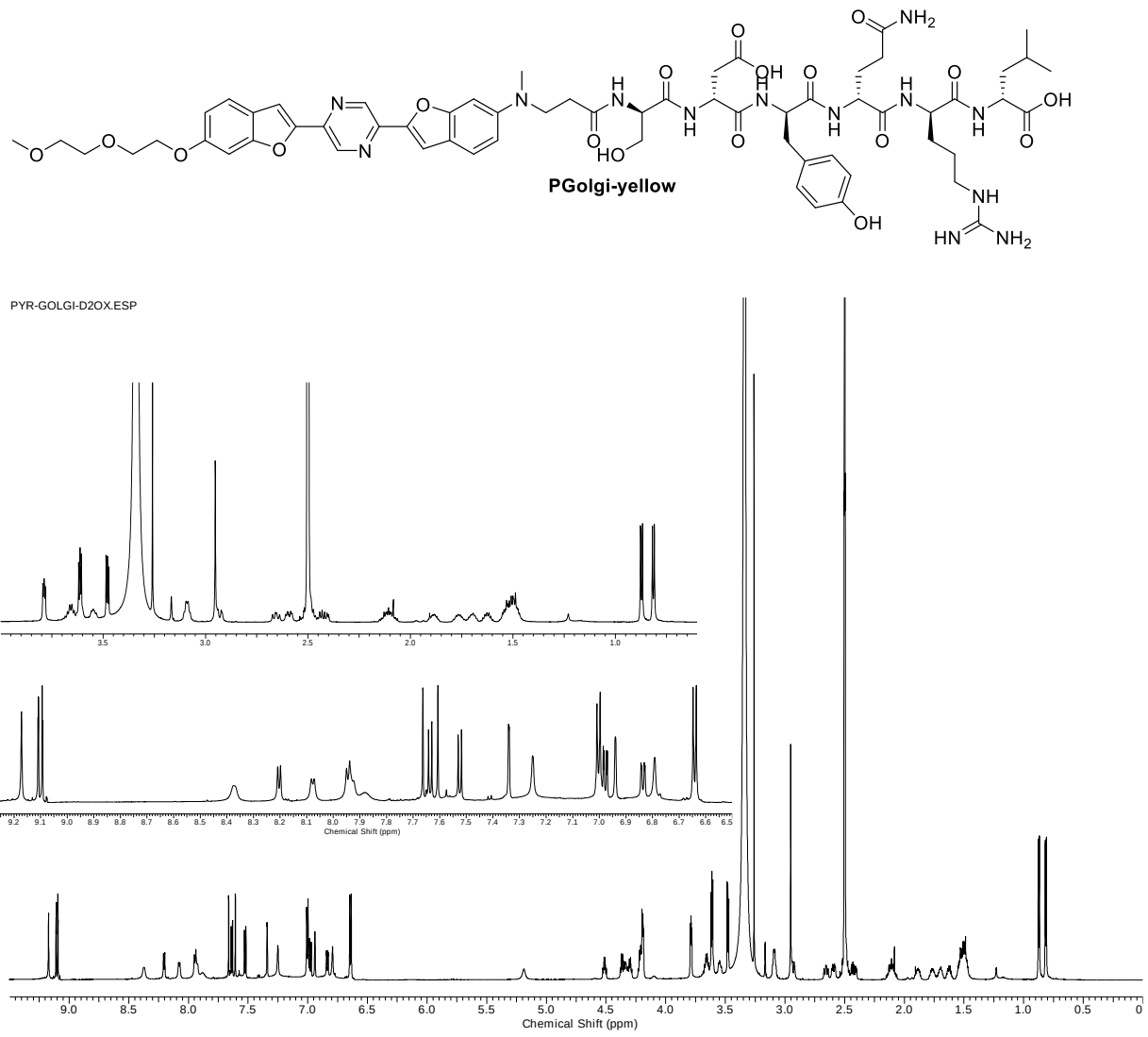

Figure S17. ^{1}H NMR spectrum (700 MHz) of ***PGolgi-yellow*** in DMSO-d_6.

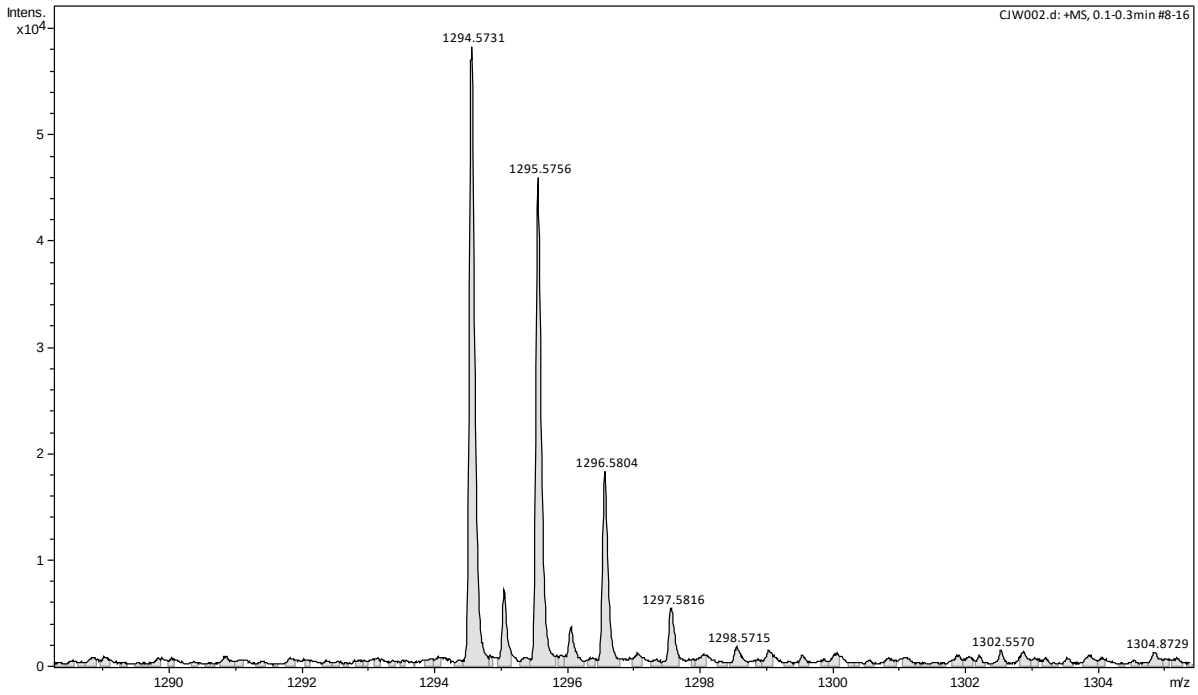

Figure S18. HRMS spectrum of ***PGolgi-yellow.***

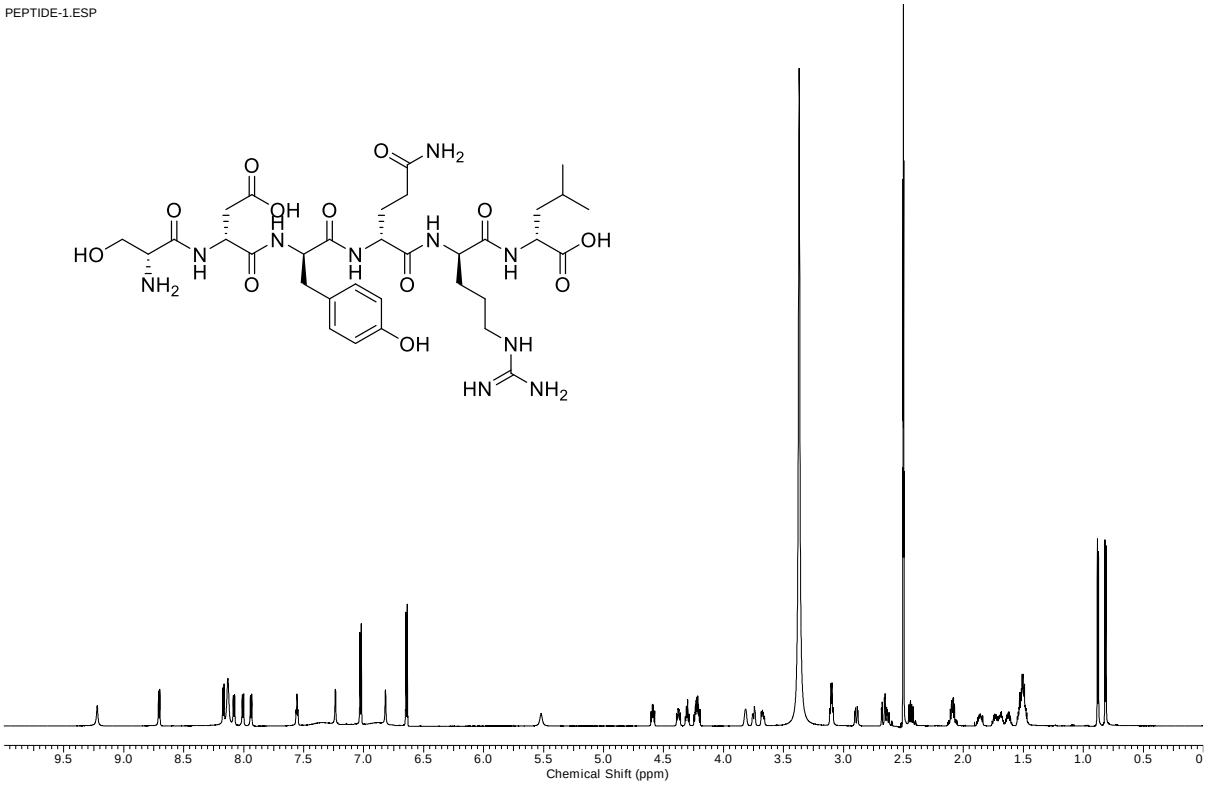

Figure S19. ^{1}H NMR spectrum (700 MHz) of ***SDYQRL*** in DMSO-d_6.

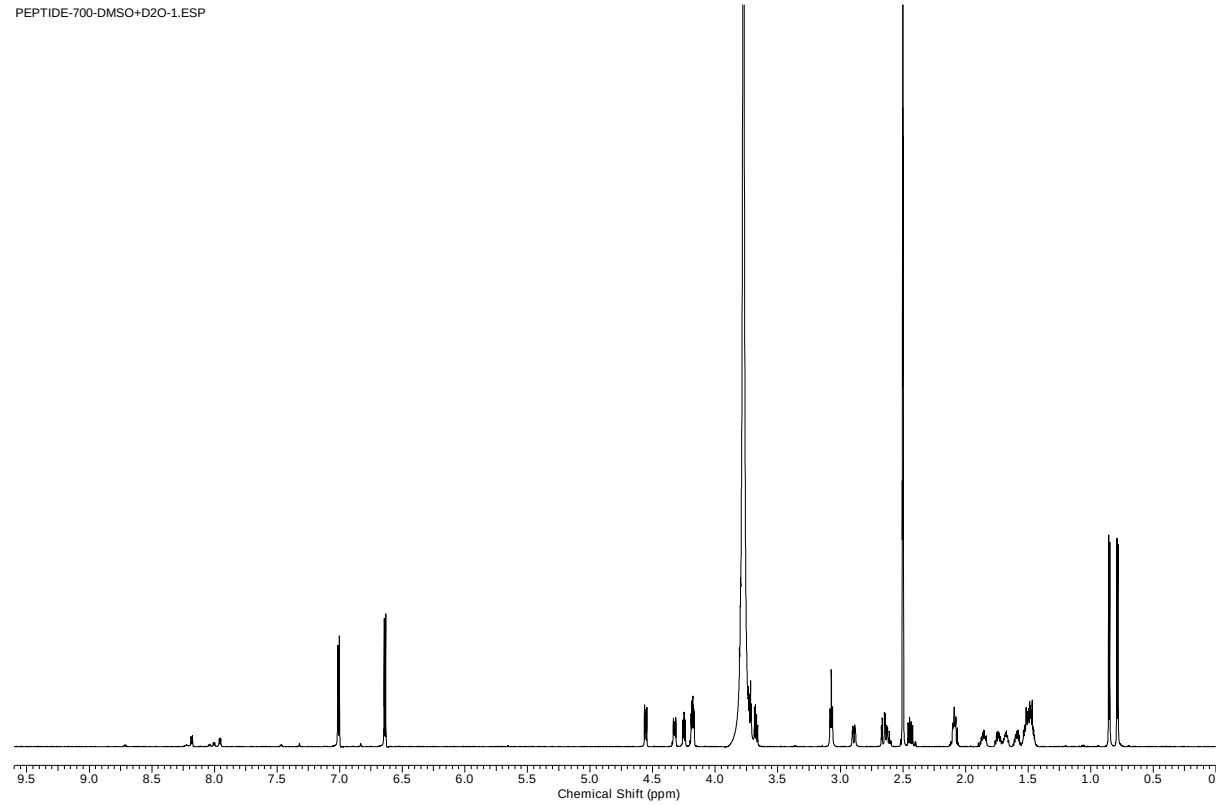

Figure S20. ^{1}H NMR spectrum (700 MHz) of ***SDYQRL*** in DMSO-d_6 upon addition of D_2O.

Reference.

1. X. Gao, Y. Zhang and B. Wang, *Tetrahedron.*, 2005, 61, **38**, 9111.

2. J. W. Choi, S. T. Hong, D. E. Kang, K. C. Paik, M. S. Han, C. S. Lim and B. R. Cho, *Anal Chem*., 2016, 88, **19**, 9412.