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Constructing Sequence-Controlled Heterolayered Dendritic Lanthanide Chelates via a One-Pot Strategy using Orthogonal Chemistry

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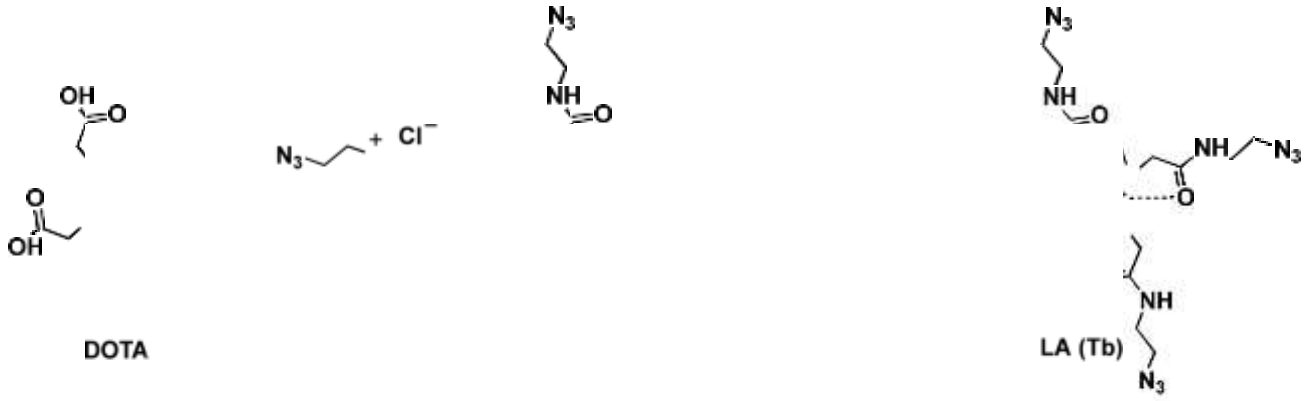
Materials

All chemicals purchased from commercial sources were used without further purification. Anhydrous gadolinium (III) chloride ($GdCl_3$, 99.9%), europium (III) chloride ($EuCl_3$, 99.9%), terbium (III) chloride ($TbCl_3$, 99.9%), dysprosium (III) chloride ($DyCl_3$, 99.9%), *N*,*N*-diisopropylethylamine (DIPEA, 99.5%), trifluoroacetic acid (TFA, 99.9%), triethylamine (TEA) (99%), aqueous ammonia were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).Phosphate buffer saline (PBS, pH 7.4) was purchased from Sangon Biotech (Shanghai, China). Bromoacetyl bromide (98%), hydroxybenzotriazole (HOBt), propargylamine (98%), *tert*-butyl bromoacetate (99%), 2,2,2-trichloroethylchloroformate, tetrakis(acetonitrile)copper(I)hexafluoro-phosphate ($[Cu(CH_3CN)_4]PF_6$), sodium nitrite and *N*-Boc-1,3-propanediamine (99%), 1,4,7,10-tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid (DOTA) were purchased from Inno-Chem (China). 5-Norbornene-2-methylamine (a mixture of isomers), *N*-Boc-1,3-propanediamine (98%), benzotriazol-1-yloxytripyrrolidino-phosphonium hexafluorophosphate (PyBOP), chloroacetyl chloride (99%), 2,2,2-trichloroethyl chloroformate and 2-cyanopyridine were purchased from Energy Chemical. 5-Amino-2-pyridinecarbonitrile was purchased from ChemCruz. 1,4,7,10-Tetraazacyclododecane (cyclen, 98%) was purchased from Strem Chemicals Inc. (Bischheim, France). *N*-Boc-2-azidoethylamine (99%) was purchased from Aikonchem (Jiangsu, China).

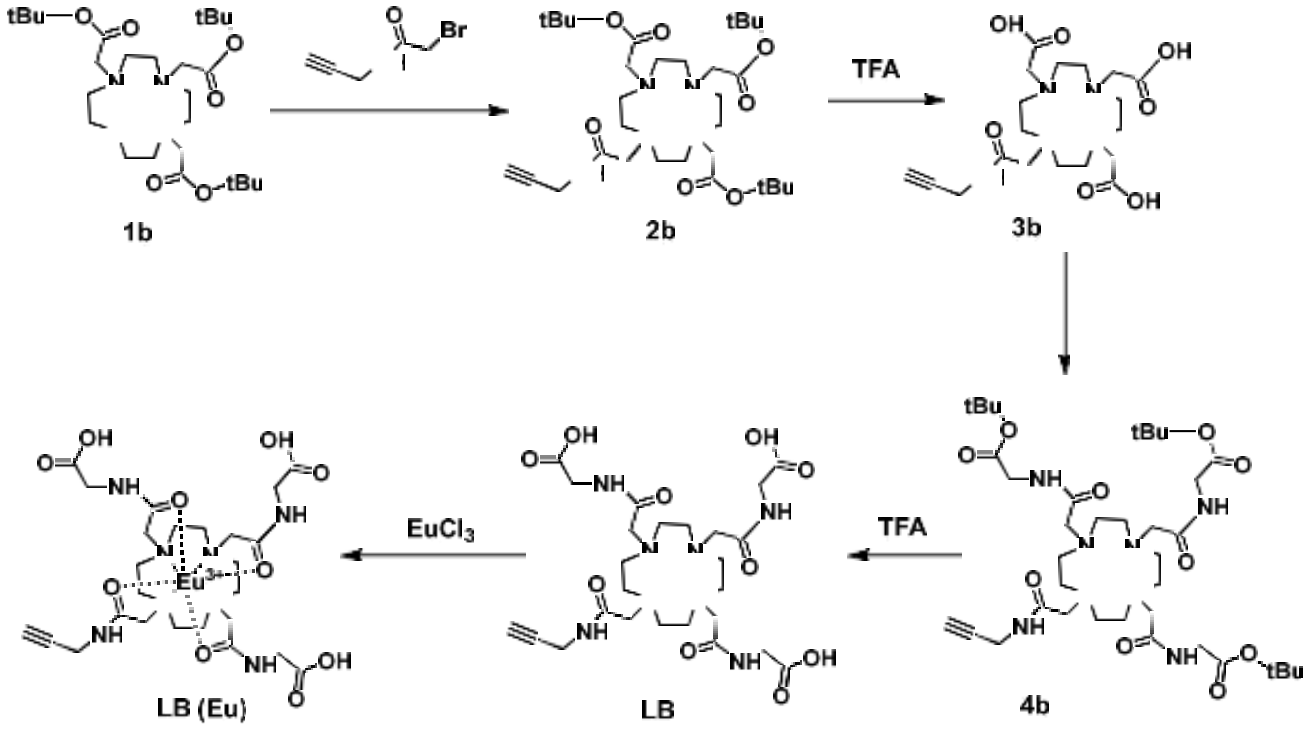
Instruments

Electrospray ionization mass spectrometry (ESI-MS) was performed on an Agilent Technologies ESI-TOF-MS 6224A. Matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was performed on an autoflex maX MALDI-TOF MS (Bruker). ^{1}H NMR and ^{13}C NMR spectra were acquired on a Bruker Plus 500 MHz NMR Spectrometer. Luminescence emission spectra was acquired on a FLS980 spectrometer. Gel filtration chromatography (GFC) was performed on a Dionex UltiMate 3000 system. Dynamic light scattering (DLS) was performed on a Malvern Zetasizer nano ZS instrument.



Scheme S1. Synthesis of Building Block **LA (Tb)**.

Building Block LA (Tb) was synthesized as previously reported.[1]



Scheme S2. Synthesis of Building Block **LB (Eu)**.

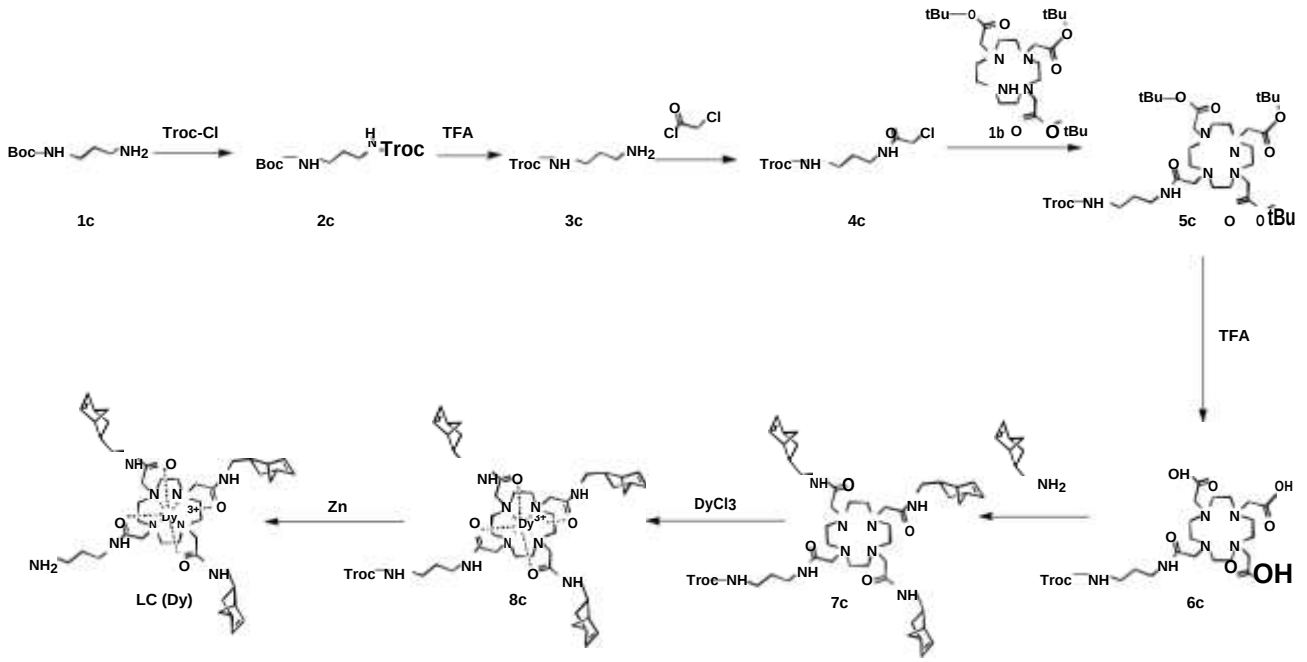
Tri-*tert*-butyl 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (**1b**), **2-bromo-*N*-propargylacetamide**, **tri-*tert*-butyl 2,2',2''-(10-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (2b)**, **2,2',2''-(10-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)-triacetic acid (3b)** were synthesized as previously reported.[1-4]

Tri-*tert*-Butyl 2,2',2''-((2,2',2''-(10-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)tris(acetyl))tris(azanediyl))triacetate (4b). 3b (2.00 g, 4.5 mmol), tert-butyl glycinate (2.37 g, 18.2 mmol), HOBt (2.45 g, 18.2 mmol), and EDC HCl (3.49 g, 18.2 mmol) were dissolved in 100 mL *N*,*N*-dimethylformamide (DMF). The reaction mixture was stirred at RT overnight and concentrated *in vacuo* before 100 mL water was added. The resulting mixture was extracted with DCM (100 mL 3). The combined organic layers were dried with anhydrous sodium sulphate and concentrated *in vacuo* to give crude **4a** (3.15 g, 89%) as a foamy solid, which was directly used in the next step without further purification. ESI-MS calcd. for $C_{37}H_{64}N_8O_{10}Na$ ($[M+Na]^+$) 803.5, found 803.5.

2,2',2''-((2,2',2''-(10-(2-Oxo-2-(prop-2-yn-1-ylamino)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)tris(acetyl))tris(azanediyl))triacetic acid (LB). 4b (2.00 g, 2.6 mmol) was dissolved in

50 mL TFA and stirred overnight. The resulting solution was concentrated *in vacuo* to give the crude product, which was purified by HPLC to give **LB** (1.02 g, 65%) as a foamy solid: ^{1}H NMR (500 MHz, DMSO-d6): δ 12.67 (s, 2H), 8.88-8.10 (m, 4H), 4.01-3.74 (m, 8H), 3.62-2.76 (m, 25H); ^{13}C NMR of LB (151 MHz, DMSO-d6): δ 171.48, 169.65, 158.32, 158.12, 81.15, 73.66, 56.47, 51.16, 41.09, 40.52, 28.37; ESI-MS calcd. for $C_{25}H_{41}N_8O_{10}$ ($[M+H]^+$) 613.3, found 613.4.

Building Block LB (Eu). LB (1.00 g, 1.6 mmol) and $EuCl_3$ (1.27 g, 4.9 mmol) were dissolved in 50 mL water. The pH of the solution was adjusted to 6.5, the resulting solution was stirred at RT overnight and concentrated *in vacuo*. The residue was purified by HPLC to give **LB (Eu)** (1.1 g, 88%) as a white foamy solid: ^{1}H NMR (600 MHz, D_2O): δ 3.50-2.18(m, 20H), 1.89-1.27 (m, 4H), (2.38)-(3.17) (m, 9H); ESI-MS calcd. for $C_{25}H_{38}EuN_8O_{10}$ ($[M-2H]^+$) 763.1923, found 763.1935.



Scheme S3. Synthesis of Building Block **LC (Dy)**.

***tert*-Butyl-(2,2,2-trichloroethyl)propane-1,3-diyldicarbamate (2c).** 2,2,2-Trichloroethylchloro-formate (7.31 g, 34.5 mmol) was added dropwise to a solution of **1c** (5 g, 28.7 mmol) and DIPEA (6.0 mL, 34.5 mmol) in DCM (200 mL) at 0 °C. The resulting solution was stirred at RT for 8 h before extracted with 1 M aqueous hydrochloric acid (HCl, 200 mL). The organic layer was extracted with water (200 mL 2) and dried with anhydrous sodium sulphate. The resulting solution was concentrated *in vacuo* to give crude **2c** as a white solid (7.43 g, 74%):^{1}H NMR (600 MHz, DMSO-d6) δ 7.65 (s, 1H), 6.78 (s, 1H), 4.79 (s, 2H), 3.02 (d, *J* = 6.6 Hz, 2H), 2.93 (d, *J* = 6.6 Hz, 2H), 1.53 (p, *J* = 7.2 Hz, 2H), 1.37 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.56, 154.97, 95.69, 74.46, 38.00, 37.08, 30.44, 28.40.

2,2,2-Trichloroethyl-(3-aminopropyl)carbamate (3c). 2c (5.00 g, 14.4 mmol) was dissolved in a solution (100 mL) of TFA and DCM (1:1 v/v). The resulting mixture was stirred at RT for 10 h and concentrated *in vacuo* to give **3c** as a colourless oil (3.6 g, 99%), which was directly used in the next step without further purification. A small portion of **3c** was purified for characterization (as **3c** TFA): ^{1}H NMR of (500 MHz, DMSO-d6): δ 7.99 (br s, 3H), 7.84-7.80 (m, 1H), 4.79 (s, 2H), 3.11 (q, *J* = 6.4 Hz, 2H), 2.80 (br s, 2H), 1.75-1.71 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d6): δ 158.95, 155.02, 117.49, 96.70, 73.81, 49.01, 38.15, 37.12, 27.89; ESI-MS calcd. for $C_6H_{12}Cl_3N_2O_2$ ($[M+H]^+$) 249.0 , found 249.2 .

2,2,2-Trichloroethyl-(3-(2-chloroacetamido)propyl)carbamate (4c). Chloroacetyl chloride (4.48 g, 40.0 mmol) was added dropwise to a solution of **3c** (5 g, 20.0 mmol) and DIPEA (6.95 mL, 40.0 mmol) in dichloromethane (DCM, 200 mL) at 0 °C. The resulting solution was stirred at RT for 8 h and concentrated *in vacuo*. The residue was purified with flash chromatography (hexanes/ethyl acetate (Hex/EA) = 5:1 to 2:1) to give **4c** (4.2 g, 65%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.07 (br s, 1H), 5.62 (br s, 1H), 4.74 (s, 2H), 4.07 (s, 2H), 3.40 (q, *J* = 6.3 Hz, 2H), 3.29 (q, *J* = 6.2 Hz, 2H), 1.77-1.73 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.71, 155.21, 95.60, 74.53, 42.61, 37.87, 36.43, 29.72.

Tri-*tert*-butyl-2,2',2''-(10-(2-oxo-2-((3-(((2,2,2-trichloroethoxy)carbonyl)amino)propyl)amino)-ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (5c). Tri-*tert*-butyl-2,2',2''-(1,4,7,10-tetraazacyclodo-decane-1,4,7-triyl)triacetate (**1b**) (4.74 g, 9.2 mmol), **4c** (3 g, 9.23 mmol) and DIPEA (1.60 mL, 9.2 mmol) were dissolved in 100 mL acetonitrile. The reaction mixture was stirred at 50 °C overnight and concentrated *in vacuo*. The crude product was purified with flash chromatography (MeOH/DCM = 0:100 to 10:100) to give **5c** (4.07 g, 55%) as a light yellow solid: ^{1}H NMR (600 MHz, C_6D_6): δ 8.60 (br s, 1H), 6.83 (br s, 1H), 4.72 (s, 2H), 3.35-3.31 (m, 2H), 3.30-3.27 (m, 2H), 3.20-3.18 (m, 2H), 3.09-3.07 (m, 4H), 2.91-2.89 (m, 2H), 2.86-2.83 (m, 4H), 2.75-2.73 (m, 4H), 2.47-2.43 (m, 4H), 2.10-2.07 (m, 4H), 1.59-1.56 (m, 2H), 1.35 (s, 27H); ^{13}C NMR (151 MHz, C_6D_6): δ 171.86, 170.15, 154.62, 96.57, 80.05, 79.96, 74.19, 57.78, 56.84, 56.22, 54.95, 53.84, 52.08, 37.93, 35.47, 30.06, 27.84, 27.72; ESI-MS calcd. for $C_{34}H_{62}Cl_3N_6O_9$ ($[M+H]^+$) 803.4, found 803.5.

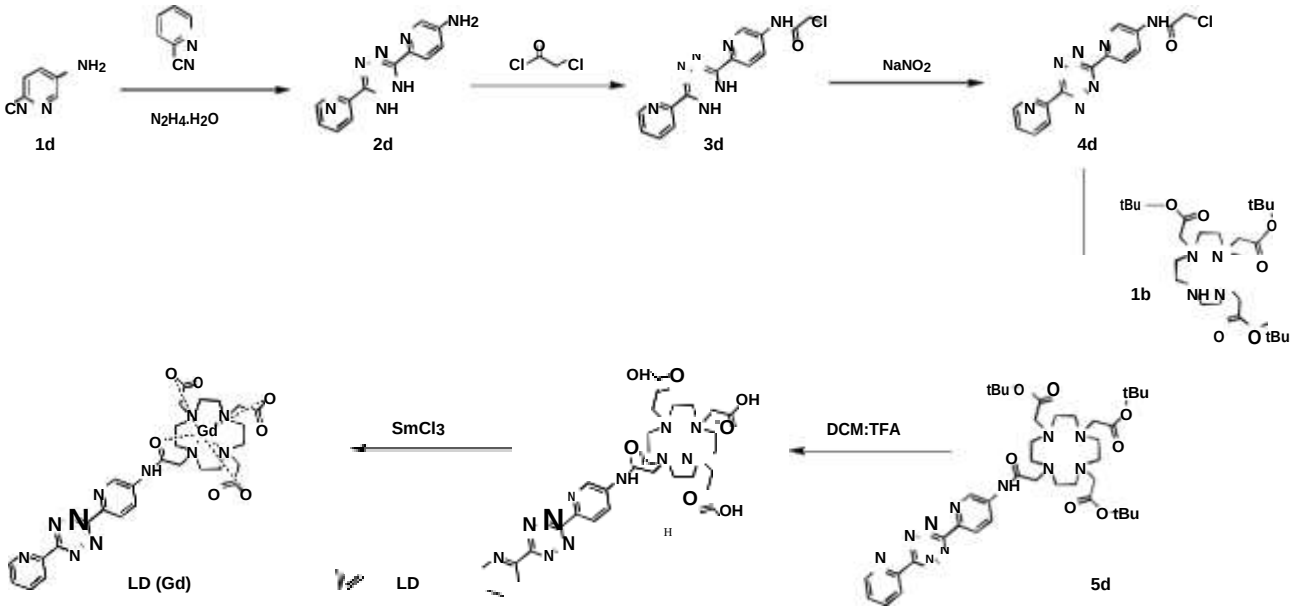
2,2',2''-(10-(2-Oxo-2-((3-(((2,2,2-trichloroethoxy)carbonyl)amino)propyl)amino)ethyl)-1,4,7,10-tetra-azacyclododecane-1,4,7-triyl)triacetic acid (6c). 5c (4.00 g, 4.98 mmol) was dissolved in 50 mL TFA and stirred overnight. The resulting solution was concentrated *in vacuo* to give crude **6c** (3.0 g, 95%) as a golden oil, which was directly used in the next step without further purification. A small portion of **6c** was purified for characterization: ^{1}H NMR (600 MHz, DMSO-d6): δ 8.24 (s, 1H), 8.08 (s, 1H), 4.78 (s, 2H), 3.44-3.42 (m, 7H), 3.16-2.54 (m, 20H), 1.63-1.59 (m, 2H); ^{13}C NMR (151 MHz, DMSO-d6): δ 170.78, 170.60, 154.95, 96.86, 73.72, 59.15, 56.02, 55.16, 51.41, 50.55, 50.40, 49.07, 40.52, 38.43, 36.31, 29.46; ESI-MS calcd. for $C_{22}H_{39}Cl_3N_6O_9$ ($[M+H]^+$) 635.2, found 635.5 .

2,2,2-Trichloroethyl (3-(2-(4-(2-((((1*R*,2*S*,4*R*)-bicyclo[2.2.1]hept-5-en-2-yl)methyl)amino)-2-oxoethyl)-10-(2-((((1*S*,2*R*,4*S*)-bicyclo[2.2.1]hept-5-en-2-yl)methyl)amino)-2-oxoethyl)-7-(2-((((2*R*,4*R*)-bicyclo-[2.2.1]hept-5-en-2-yl)methyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetamido)pro-pyl)carbamate (7c). 6c (3.00 g, 4.73 mmol), 5-norbornene-2-methylamine

(a mixture of isomers) (2.62 g, 21.3 mmol), HOBt (2.88 g, 21.3 mmol), EDC HCl (4.09 g, 21.3 mmol) and DIPEA (3.7 mL, 21.3 mmol) were dissolved in 100 mL DMF. The reaction mixture was stirred at RT overnight and concentrated *in vacuo* before 100 mL DCM was added. The resulting mixture was extracted with water (100 mL 3). The organic layers were dried with anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by HPLC to give **7c** (3.36 g, 75%) as a golden oil: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.10-8.02 (m, 4H), 6.83 (br s, 1H), 6.05–5.98 (m, 6H), 4.84 (s, 2H), 3.52-3.38 (m, 2H), 3.29-3.00 (m, 16H), 2.84-2.16 (m, 22H), 2.16-1.31 (m, 17H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.7, 157.8, 135.9, 95.6, 71.3, 59.5, 55.1, 46.9, 42.9, 39.6, 37.1, 34.0, 30.7, 28.6; ESI-MS calcd. for $C_{46}H_{71}Cl_3N_9O_6$ ($[M+H]^+$) 950.5, found 950.3.

Troc-LC (Dy) (8c). **7c** (3.36 g, 3.5 mmol) and $DyCl_3$ (2.85 g, 10.6 mmol) were dissolved in a solution (100 mL) of acetonitrile and water (1:1 v/v). The pH of the solution was adjusted to 6.5. The resulting solution was stirred at RT overnight and concentrated *in vacuo*. The residue was purified by HPLC to give **8c** (3.3 g, 84%) as a colorless oil: ESI-MS calcd. for $C_{50}H_{70}Cl_3DyF_6N_9O_{10}$ ($[M+2CF_3COO^-]^+$) 1339.4, found 1339.4 .

Building Block LC (Dy). 8c (3.30 g, 2.9 mmol) were dissolved in a solution (100 mL) of 10 M ammonium acetate solution and THF (1:1 v/v). Zinc powder (50 mg) was added and the resulting solution was stirred at RT for 8 h before filtration. The filtrate was concentrated *in vacuo*. The residue was purified by HPLC to give **LC (Dy)** (2.39 g, 86%) as a white solid: ESI-MS calcd. for $C_{45}H_{68}DyF_3N_9O_6$ ($[M+CF_3COO^-H]^+$) 1051.4542, found 1051.4546.



Scheme S4. Synthesis of Building Block **LD (Gd)**

6-(6-(Pyridin-2-yl)-1,2-dihydro-1,2,4,5-tetrazin-3-yl)pyridin-3-amine (2d). 2d was synthesized as previously reported.[5]

2-Chloro-*N*-(6-(6-(pyridin-2-yl)-1,2-dihydro-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)acetamide (3d). Chloro-acetyl chloride (3.13 g, 27.7 mmol) was added dropwise to a solution of **2d** (3.5 g, 13.8 mmol) and TEA (3.84 mL, 27.7 mmol) in tetrahydrofuran (THF, 100 mL) at 0 °C. The resulting solution was stirred at RT and concentrated *in vacuo.* The crude product was purified with flash chromatography (DCM/MeOH = 100:1 to 10:1) to give **3d** (4.1 g, 90%) as a yellow solid: ESI-MS calcd. for $C_{14}H_{13}ClN_7O$ ($[M+H]^+$) 330.1, found 331.0 .

2-Chloro-*N*-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)acetamide (4d). $NaNO_2$ (2.58 g, 37.4 mmol) was added to a solution of **3d** (4.1 g, 12.5 mmol) in acetic acid at RT for 1 h. The resulting solution was concentrated *in vacuo*. The residue was purified with flash chromatography (DCM/MeOH = 100:1 to 10:1) to give **4d** (3.6 g, 89%) as a red solid: ^{1}H NMR (500 MHz, DMSO-d6)
δ 10.95 (s, 1H), 9.06 (d, *J* = 2.4 Hz, 1H), 8.99 – 8.90 (m, 1H), 8.63 (dd, *J* = 29.4, 8.2 Hz, 2H), 8.43 (dd, *J* = 8.6, 2.5 Hz, 1H), 8.16 (td, *J* = 7.7, 1.8 Hz, 1H), 7.73 (ddd, *J* = 7.6, 4.7, 1.2 Hz, 1H), 4.40 (s, 2H); ESI-MS calcd. for $C_{14}H_{11}ClN_7O$ ($[M+H]^+$) 327.7, found 328.4 .

Tri-*tert*-butyl 2,2',2''-(10-(2-oxo-2-((6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)amino)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (5d). 4d (3.6 g, 11.0 mmol), **1b**

(5.66 g, 11.0 mmol), DIPEA (1.9 mL, 11.0 mmol), and 50 mL acetonitrile were added to a 100 mL flask. The reaction mixture was stirred at 50 °C overnight and concentrated *in vacuo*. The crude product was purified with flash chromatography (MeOH/DCM = 1:100 to 10:100) to give **5d** (5.5 g, 62%) as a red solid: ESI-MS calcd. for $C_{40}H_{60}N_{11}O_7$ ($[M+H]^+$) 806.5, found 806.6 .

2,2',2''-(10-(2-Oxo-2-((6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)amino)ethyl)-1,4,7,10-tetra-azacyclododecane-1,4,7-triyl)triacetic acid (6d). 5d (5.5 g, 6.8 mmol) was dissolved in 50 mL TFA and stirred overnight. The resulting solution was concentrated *in vacuo* to give crude **6d** (4.2 g, 96%) as a red solid, which was directly used in the next step without further purification. ESI-MS calcd. for $C_{28}H_{36}N_{11}O_7$ ($[M+H]^+$) 638.3, found 638.5 .

Building Block LD (Gd). 6d (4.2 g, 6.6 mmol) and $GdCl_3$ (5.08 g, 19.8 mmol) were dissolved in a solution (100 mL) of 10 M ammonium acetate solution. The pH of the solution was adjusted to 7.0. The resulting solution was stirred at RT overnight and concentrated *in vacuo.* The residue was purified by HPLC to give **7d** (4.3 g, 84%) as a red solid: ESI-MS calcd. for $C_{28}H_{34}N_{11}O_7Gd$ ($[M+2H]^{2+}$) 398.0936, found 398.0870.

SHELLs constructed through the sequential approach

Synthesis of OPG1 (Tb, Eu). To a flask containing **LA (Tb)** (100 mg, 0.12 mmol), **LB (Eu)** (1.44 mmol), 4 mL CH_3CN/H_2O (1:1 v/v) was added. The resulting solution was stirred under N_2 for 15 min before $[Cu(CH_3CN)_4]PF_6$ (44 mg, 0.12 mmol) in 1 mL water was added quickly. The resulting mixture was stirred at 35 °C for 12 h before 15 mL water was added. The resulting solution was purified by RP-HPLC to give **OPG1 (Tb, Eu)** (304 mg, about 0.08 mmol, 80%) as a white solid.

Synthesis of OPG2 (Tb, Eu, Dy). To a flask was added **OPG1 (Tb, Eu)** (200 mg, 0.05 mmol), PyBOP (640 mg, 1.2 mmol), DIPEA (160 mg, 1.2 mmol), and 5 mL DMF. The resulting solution was stirred at 0 °C for 15 min before **LC (Dy)** (2.1 g, about 1.8 mmol) in 5 mL DMF was added quickly. The resulting mixture was stirred at 35 °C for 12 h before 15 mL methanol was added. The resulting solution was transferred to an MWCO 3 kDa dialysis bag and dialyzed against CH_3OH/H_2O (1:1 v/v). The dialysate was changed every 8 h for three times. Then the solution in the dialysis bag was concentrated *in vacuo* to give **OPG2 (Tb, Eu, Dy)** (720 mg, about 0.04 mmol, 80%) as a yellowish solid.

Synthesis of OPG3 (Tb, Eu, Dy, Gd). To a flask was added **OPG2 (Tb, Eu, Dy)** (100 mg, 0.006 mmol), **LD (Gd)** (510 mg, 0.65 mmol), and 3 mL DMSO. The resulting solution was stirred at 30 °C in dark for 12 h before 12 mL H_2O was added. Then the solution was subjected to ultrafiltration (Ultracel®-30K), which was repeated for 3 times. The remaining solution was concentrated *in vacuo* to give **OPG3 (Tb, Eu, Dy, Gd)** (130 mg, 0.003 mmol, 54%) as a brown solid.

SHELLs constructed through the one-pot approach

Synthesis of OPG3 (Tb, Eu, Dy, Gd). To a flask containing **LA (Tb)** (5 mg, 0.0047 mmol), **LB (Eu)** (18 mg, 0.023 mmol, 1.2 eq to the relevant reacting group), $[Cu(CH_3CN)_4]PF_6$ (2 mg, 0.005 mmol), 0.5 mL DMSO was added. The resulting solution was stirred at 50 °C under nitrogen atmosphere. After the reaction was complete (about 2 h, monitored by MALDI-TOF MS), 1 mL DMSO containing **LC (Dy)** (157 mg, 0.135 mmol, 2 eq to the relevant reacting group), PyBOP (70 mg, 0.135 mmol) and DIPEA (17 mg, 0.135 mmol) was added. The resulting solution was stirred at 40 °C before the reaction was complete (about 4 h, monitored by GFC). To this solution 1 mL DMSO containing **LD (Gd)** (0.95 g, 0.40 mmol, 3 eq to the relevant reacting group) was added. The resulting solution was stirred at RT for 30 min before subjected to ultrafiltration (Ultracel®-30K). The purification process was repeated for three times to ensure the impurities were completely removed.

Large-scale synthesis of OPG3 (Tb, Eu, Dy, Gd). To a flask containing **LA (Tb)** (80 mg, 0.075 mmol), **LB (Eu)** (290 mg, 0.37 mmol, 1.2 eq to the relevant reacting group), [Cu $(CH_3CN)_4$]PF_6 (32 mg, 0.08 mmol), 8.0 mL DMSO was added. The resulting solution was stirred at 50 °C under nitrogen atmosphere. After the reaction was complete (about 3 h, monitored by MALDI-TOF MS), 16 mL DMSO containing **LC (Dy)** (2.51 g, 2.16 mmol, 2 eq to the relevant reacting group), PyBOP (1.12 g, 2.16 mmol) and DIPEA (270 mg, 2.16 mmol) was added. The resulting solution was stirred at 40 °C before the reaction was complete (about 5 h, monitored by GFC). To this solution 16 mL DMSO containing **LD (Gd)** (15.20 g, 6.40 mmol, 3 eq to the relevant reacting group) was added. The resulting solution was stirred at RT for 1 h before 120 mL H_2O was added. The resulting solution was divided into several portions, which were subjected to ultrafiltration (Ultracel®-30K), respectively. Then the remaining solutions were combined and concentrated *in vacuo* to give **OPG3 (Tb, Eu, Dy, Gd)** (0.74 g, 0.017 mmol, 23% from **LA (Tb)**) as a brown solid, which was characterized by GFC (Figure 8d).

Sample preparation for MALDI-TOF and ESI-MS *via* ion exchange

1 mg sample in 1 mL H_2O was mixed with excess trifluoroacetic acid (TFA, 10 L). The resulting mixture was stirred for 10 min at RT before 10 mL deionized water was added. The resulting solution was transferred to an MWCO 3 kDa Millipore Filter for ultrafiltration. The remaining solution was diluted with 1 mL 0.1% TFA in deionized water for MALDI-TOF and ESI-MS analysis. MALDI-TOF was performed using 2,5-dihydroxybenzoic acid (DHB) as the matrix.

Sample preparation for gel filtration chromatography (GFC)

All samples were prepared at 1 mg/mL with 10 PBS. GFC analysis was carried out on a Superose™6 10/300 GL column (GE Healthcare) with 10 PBS as the eluent and the flow rate fixed at 0.50 mL/min for all samples. The detection wavelengths for GFC were set at both 210 and 254 nm. 20 μL of the sample solution was injected for each analysis.

Sample preparation for dynamic light scattering (DLS)

OPG1, **OPG3** were prepared at about 10 mg/mL with 1 PBS solution (pH 7.4), while **OPG2** were prepared at about 5 mg/mL with 1 PBS solution (pH 7.4) containing 10% methanol. All samples were filtered through filters (pore size: 0.22 μm) for three times before analysis. Dynamic light scattering (DLS) was performed at 25 ˚C with a scattering angle of 173˚. The refractive index and absorption of sample materials were set as 1.450 and 0.001, respectively. The dispersant was set as water.

Inductively coupled plasma-optical emission spectrometry (ICP-OES)

The ratios of Tb: Eu in **OPG1 (Tb, Eu)**, Tb: Eu: Dy in **OPG2 (Tb, Eu, Dy)**, and Tb: Eu: Dy: Gd in **OPG3 (Tb, Eu, Dy, Gd)** were measured to be 1: 4.0; 1: 4.0: 12.0, and 1: 4.0: 12.0: 31.7, indicating the successful construction of **OPG1 (Tb, Eu)** and **OPG2 (Tb, Eu, Dy)** and the presence of structural imperfection in **OPG3 (Tb, Eu, Dy, Gd)**.

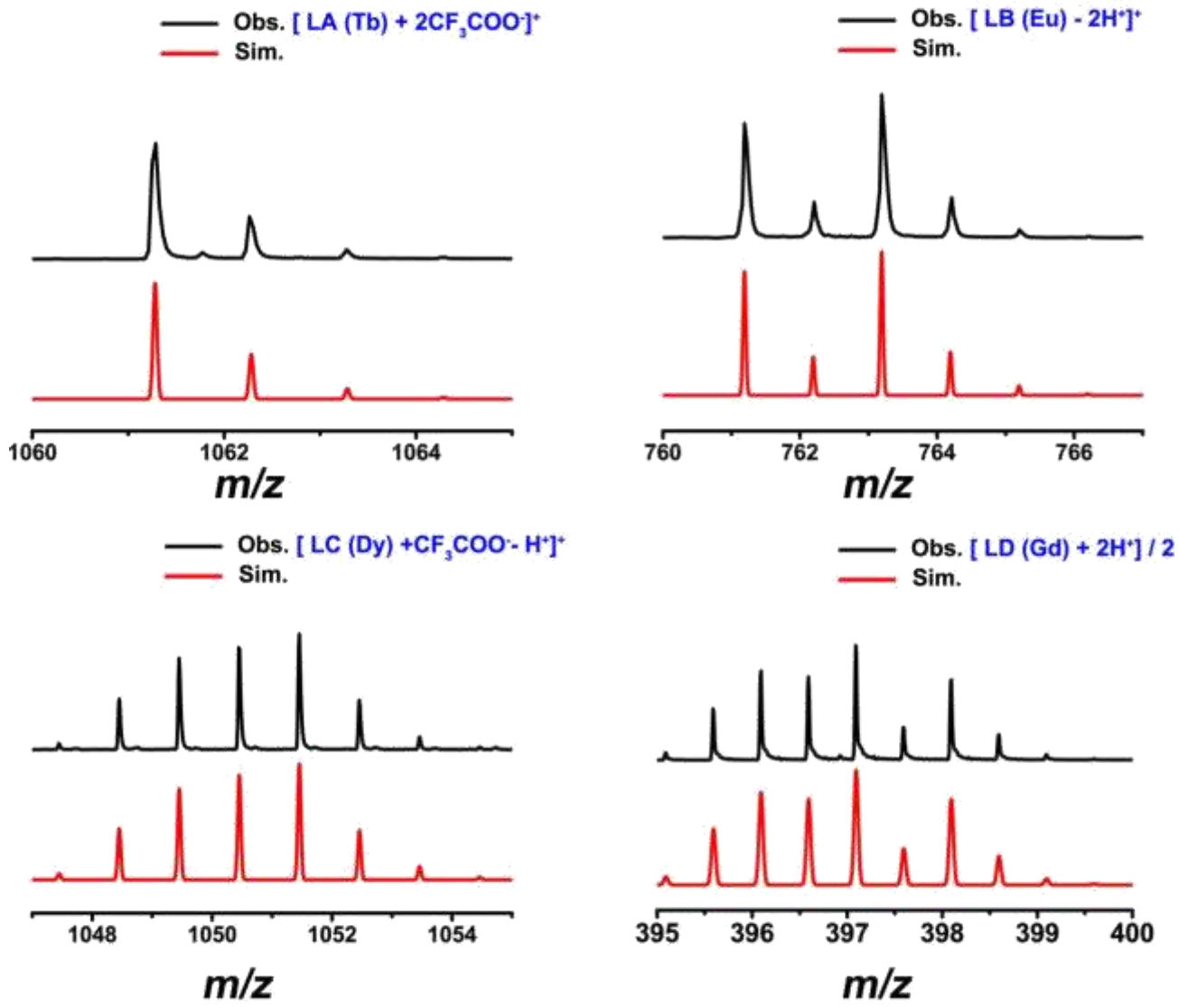


Figure S1. HR-MS spectra of Ligand **LA (Tb)**, **LB (Eu)**, **LC (Dy)**, and **LD (Gd)**.

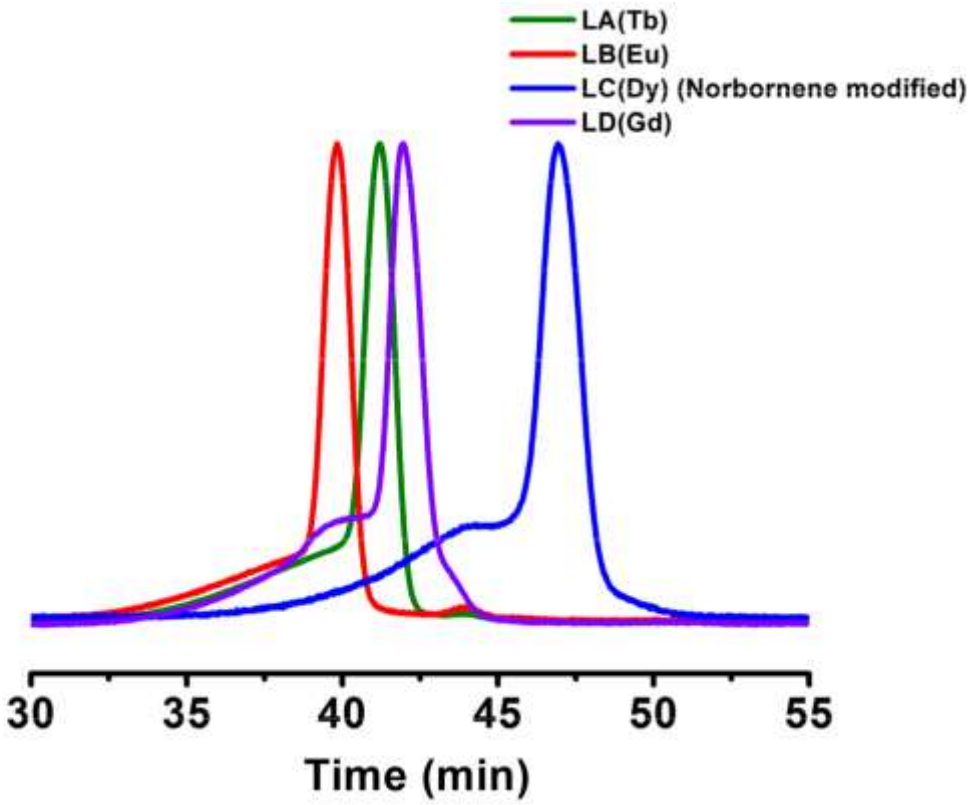


Figure S2. Gel filtration chromatography (GFC) traces of **LA (Tb)**, **LB (Eu)**, **LC (Dy)**, and **LD (Gd)**.

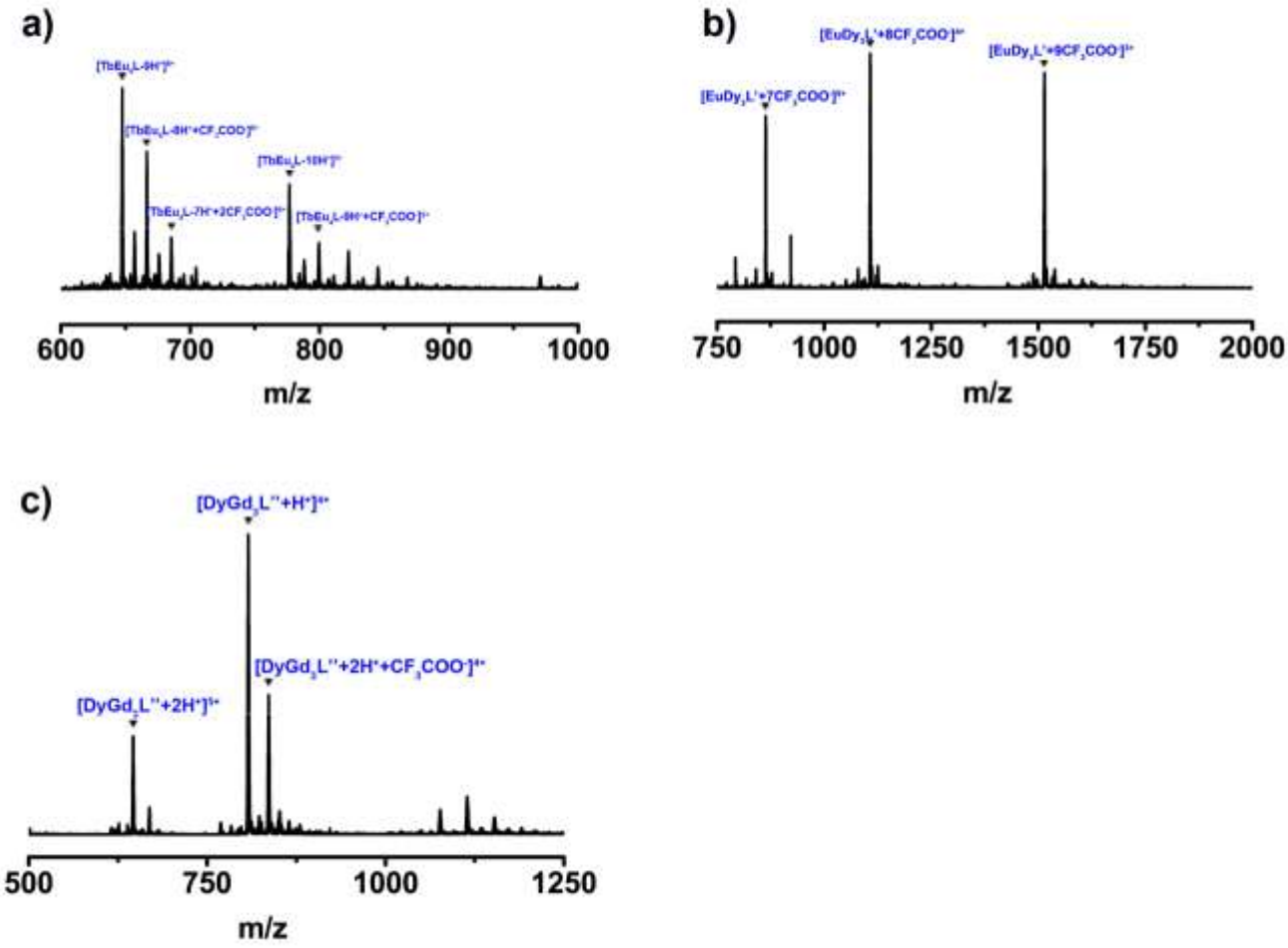


Figure S3. HR-MS spectra of (a) **LA (Tb)-4LB (Eu)** (**TbEu$_4$L**), (b) **LB (Eu)-3LC (Dy)** (**EuDy$_3$L'**), and (c) **LC (Dy)-3LD (Gd)** (**DyGd$_3$L''**).

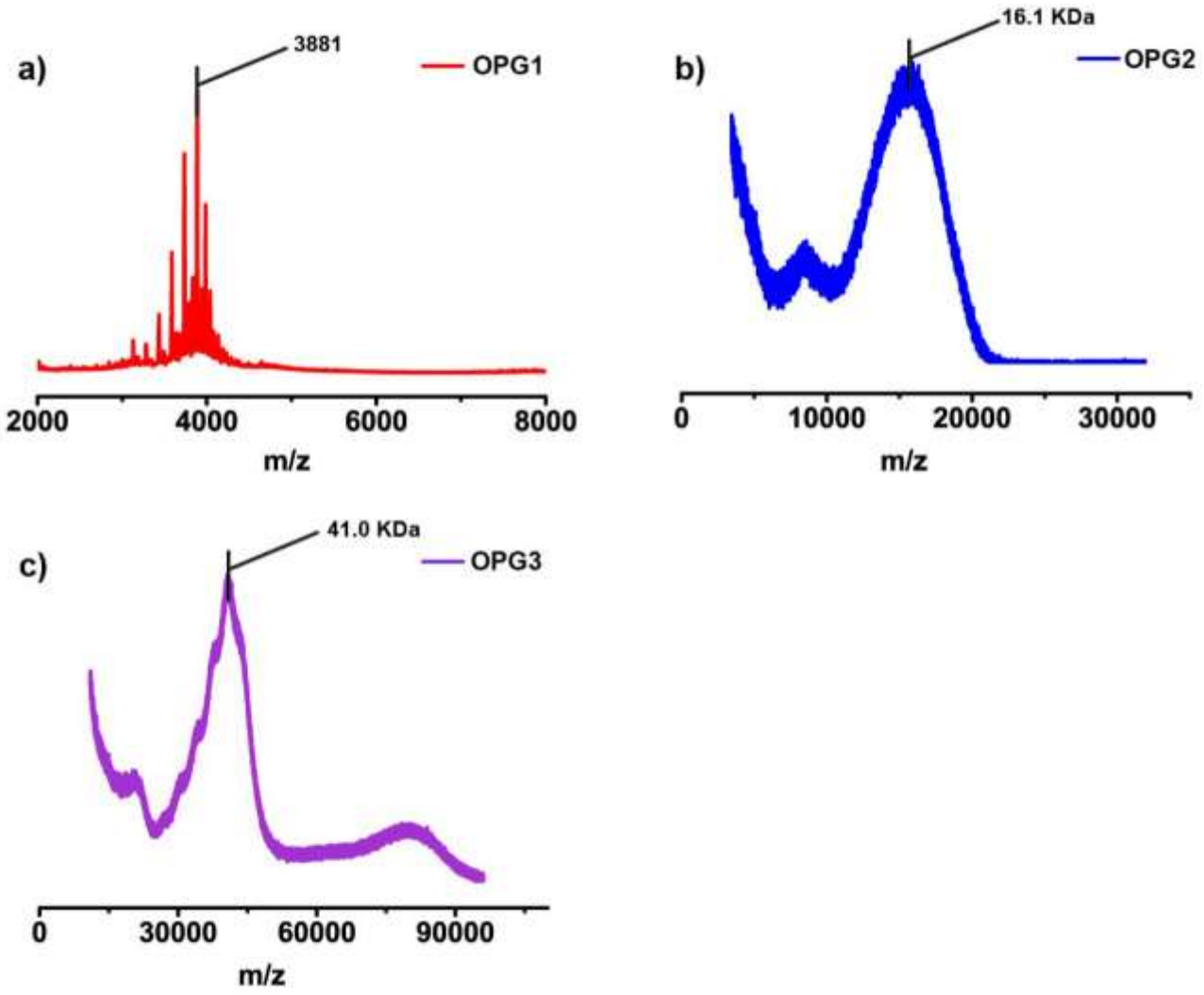


Figure S4. MALDI-TOF MS spectra of (a) **OPG1 (Tb, Eu)**, (b) **OPG2 (Tb, Eu, Dy)**, and (c) **OPG3 (Tb, Eu, Dy, Gd)**.

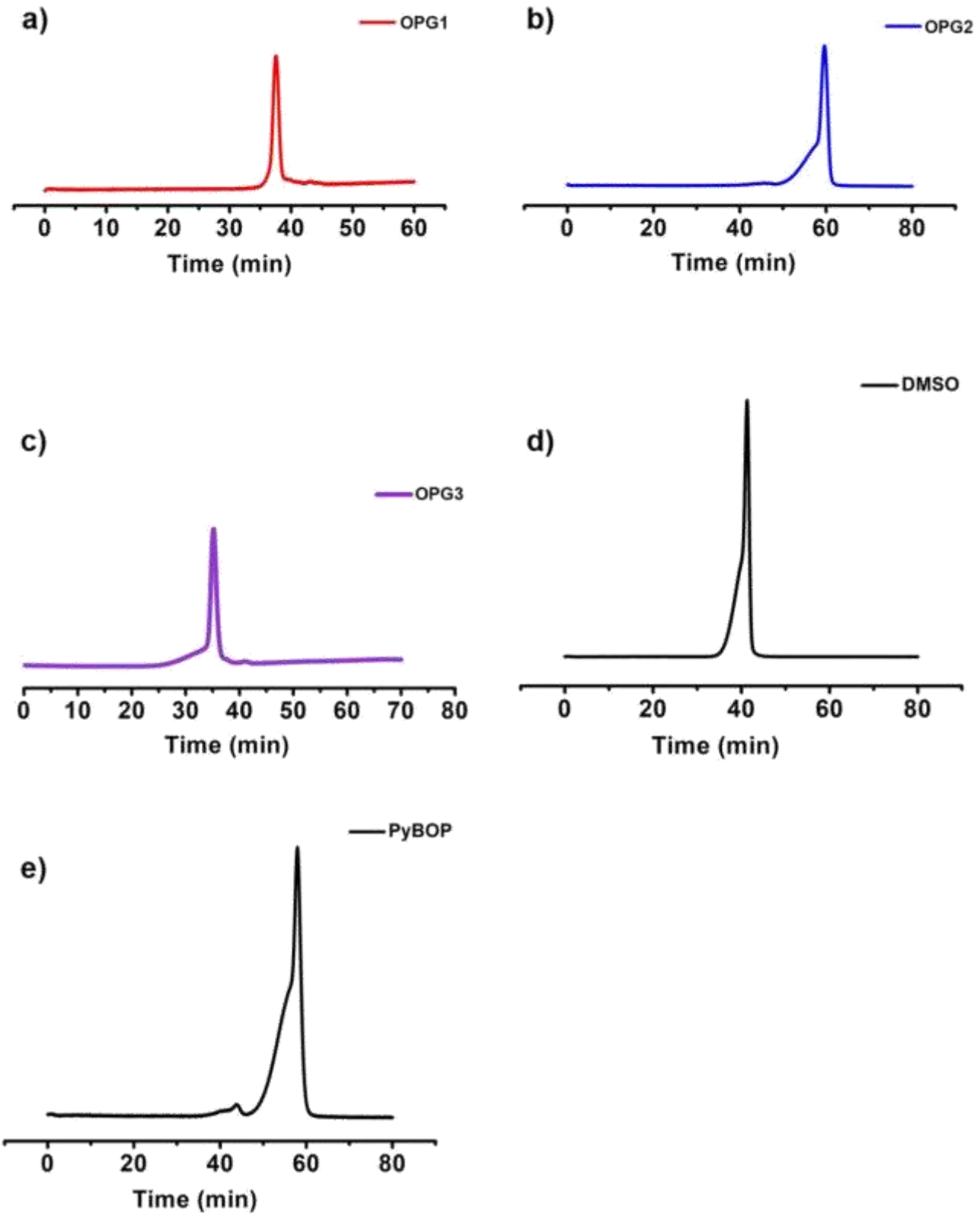


Figure S5. The GFC trace of (a) **OPG1 (Tb, Eu)**, (b) **OPG2 (Tb, Eu, Dy)**, (c) **OPG3 (Tb, Eu, Dy, Gd),** (d) **DMSO**, and (e) **PyBOP**.

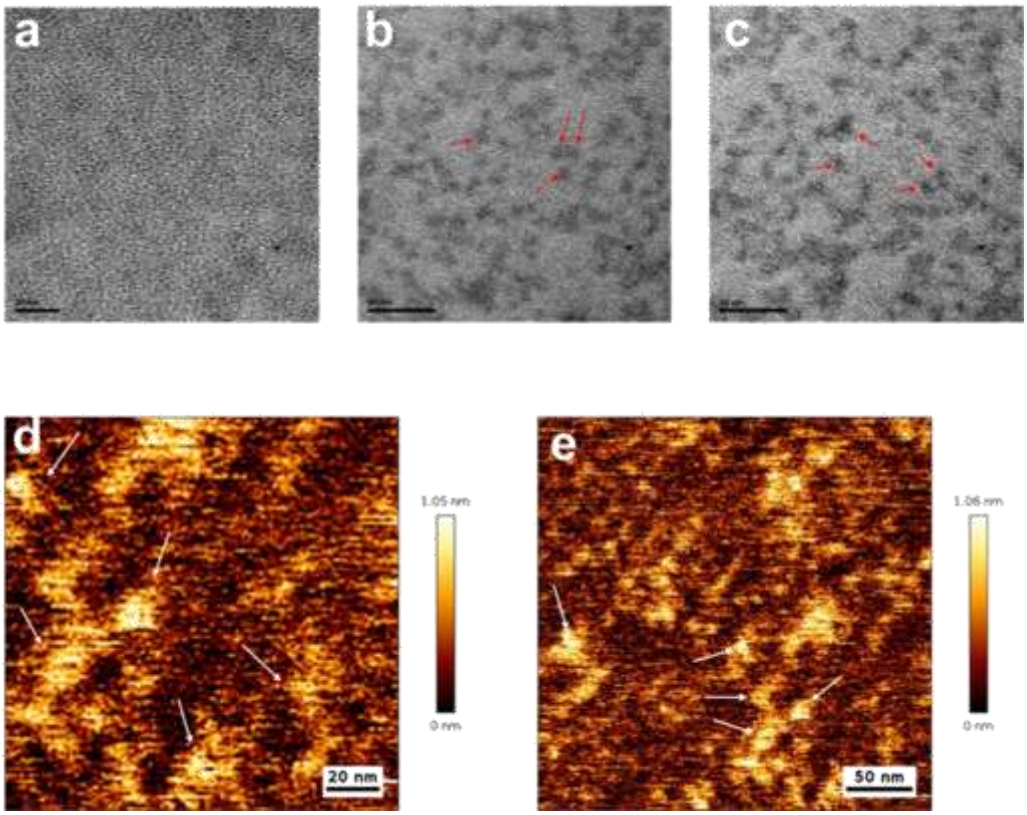


Figure S6. TEM images of **OPG1 (Tb, Eu)** (a), **OPG2 (Tb, Eu, Dy)** (b), and **OPG3 (Tb, Eu, Dy, Gd)** (c), and AFM images of **OPG2 (Tb, Eu, Dy)** (d) and **OPG3 (Tb, Eu, Dy, Gd)** (e).

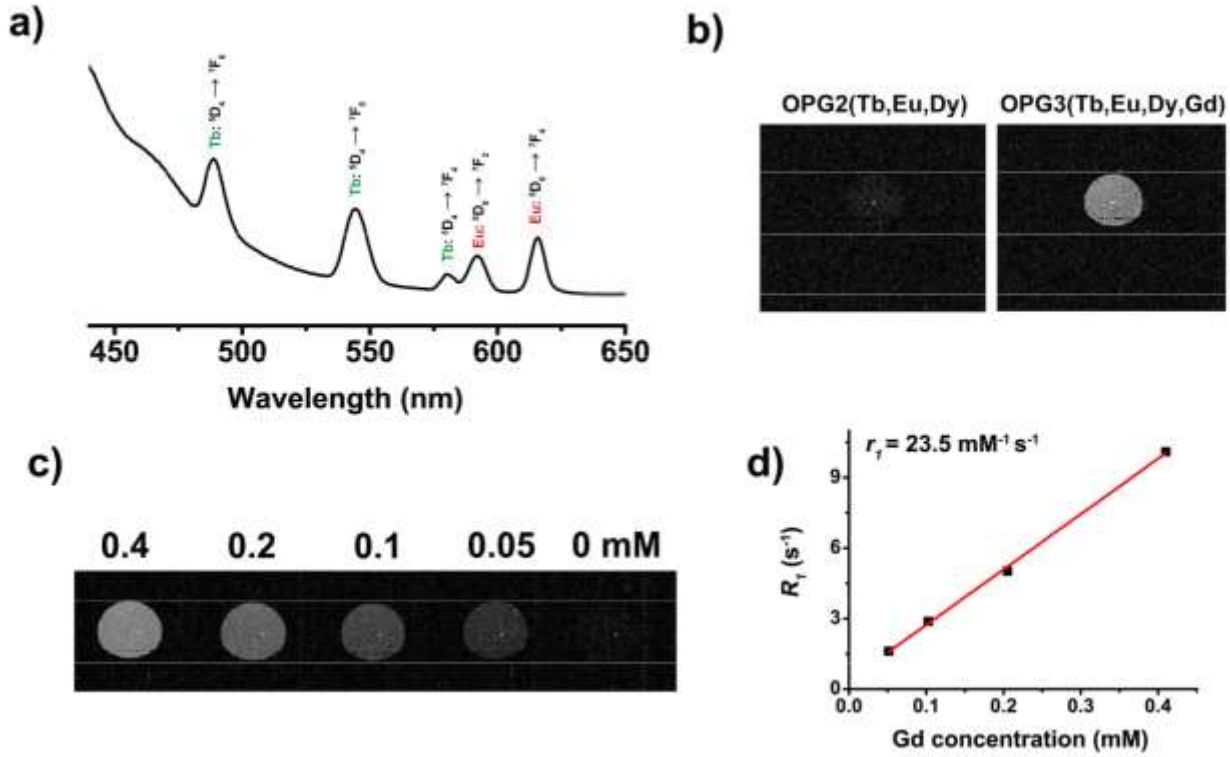


Figure S7. (a) Luminescent emission spectra of **OPG1 (Tb, Eu)** with an excitation wavelength at 380 nm. (b) T_1-weighted MR images of **OPG2 (Tb, Eu, Dy)** and **OPG3 (Tb, Eu, Dy, Gd)** phantoms. The molecular concentration (Tb concentration) was 1 μM for both samples. (c) T_1-weighted MR images of **OPG3 (Tb, Eu, Dy, Gd)** phantoms at indicated Gd concentrations. (d) T_1 relaxivity measurement of **OPG3 (Tb, Eu, Dy, Gd)**.

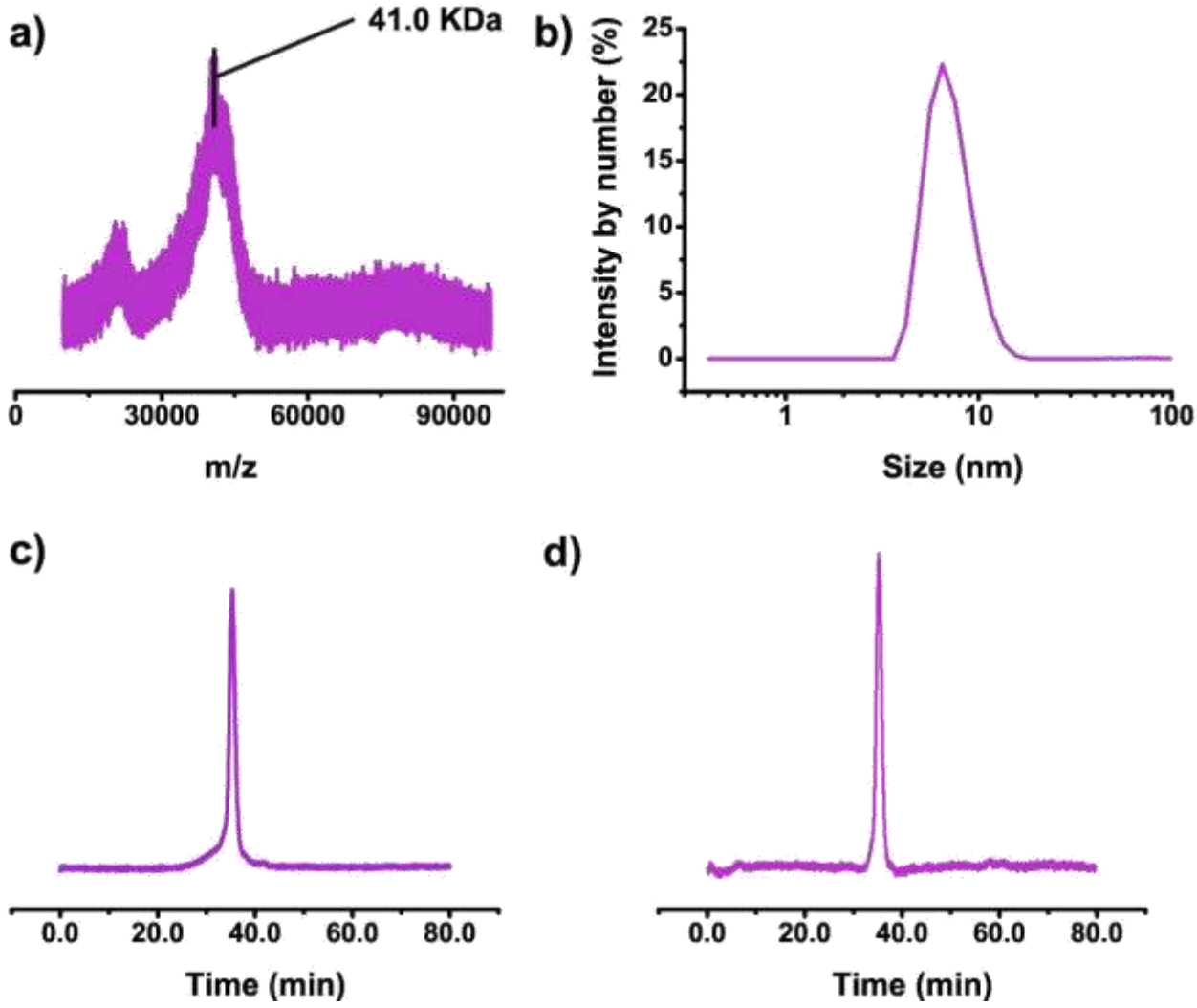


Figure S8. (a) MALDI-TOF MS spectra, (b) DLS analysis, and the GFC traces of **OPG3 (Tb, Eu, Dy, Gd)** synthesized by the one-pot approach in small scale (c) and large scale (d).

References

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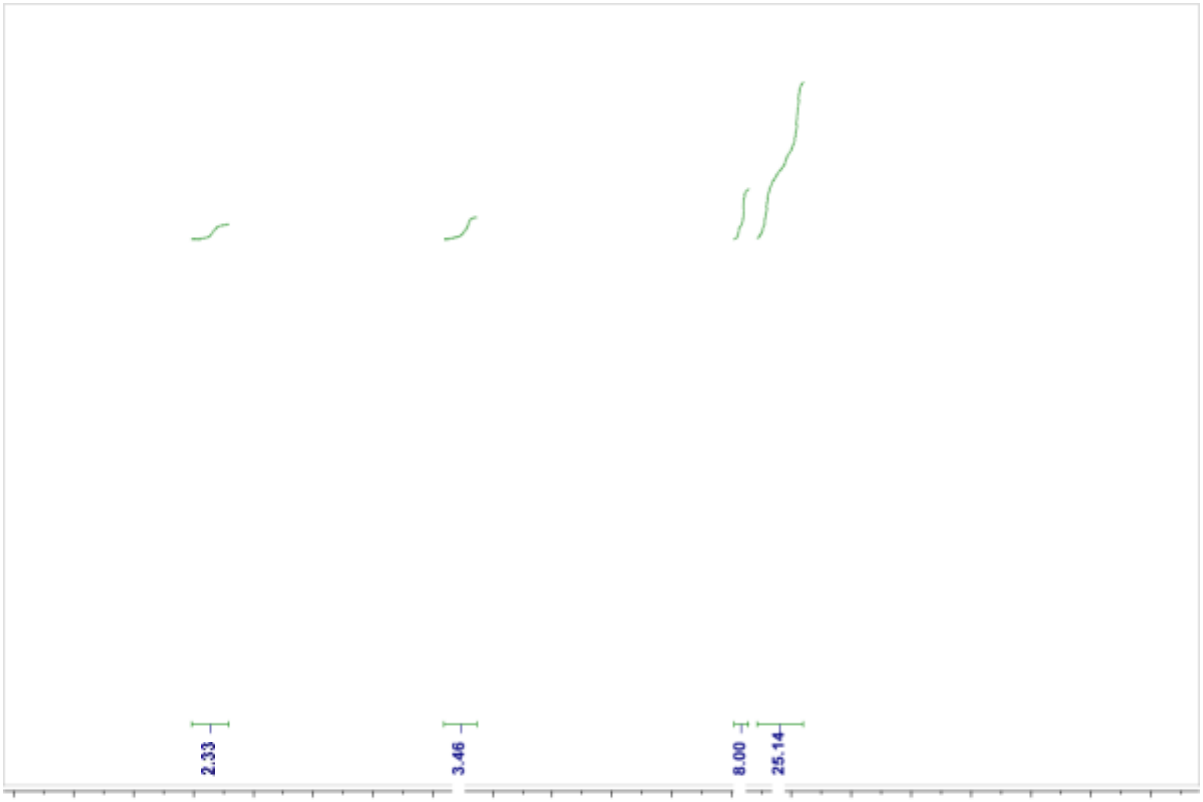
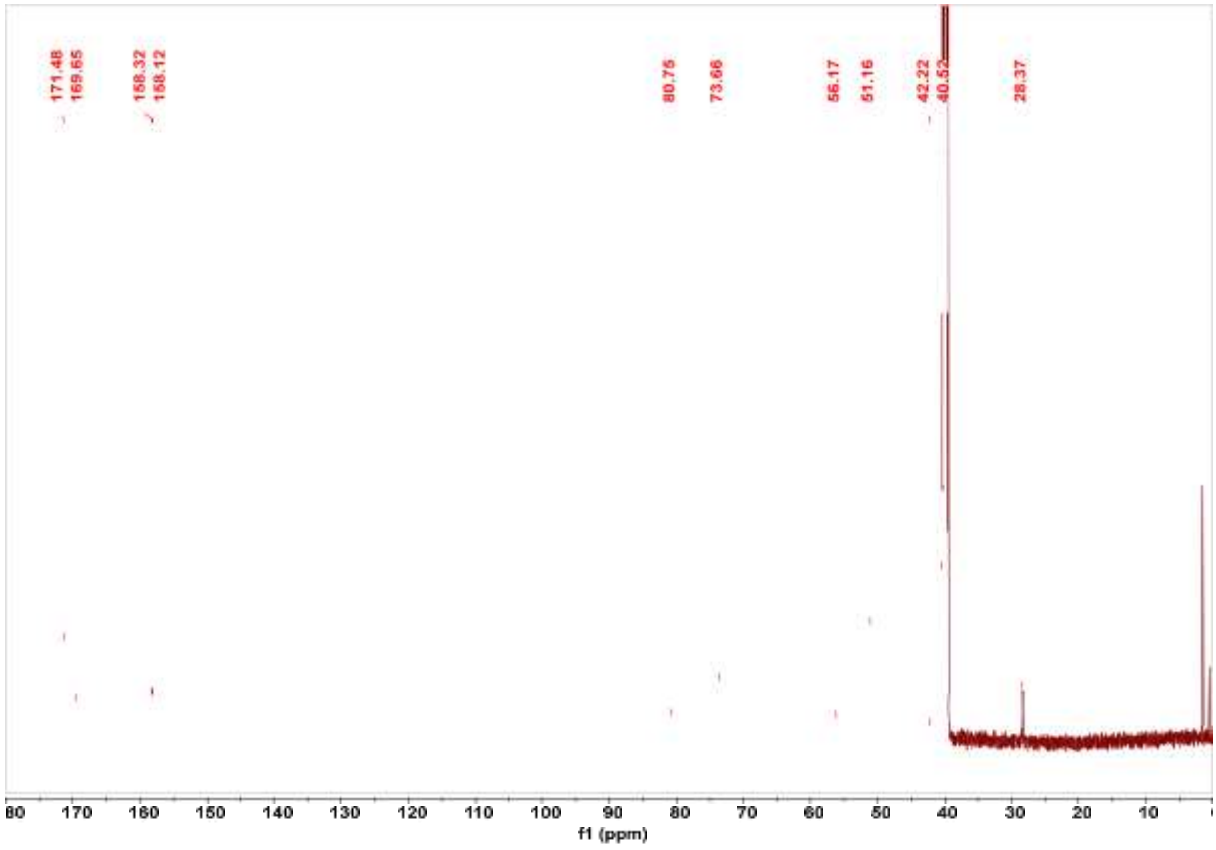
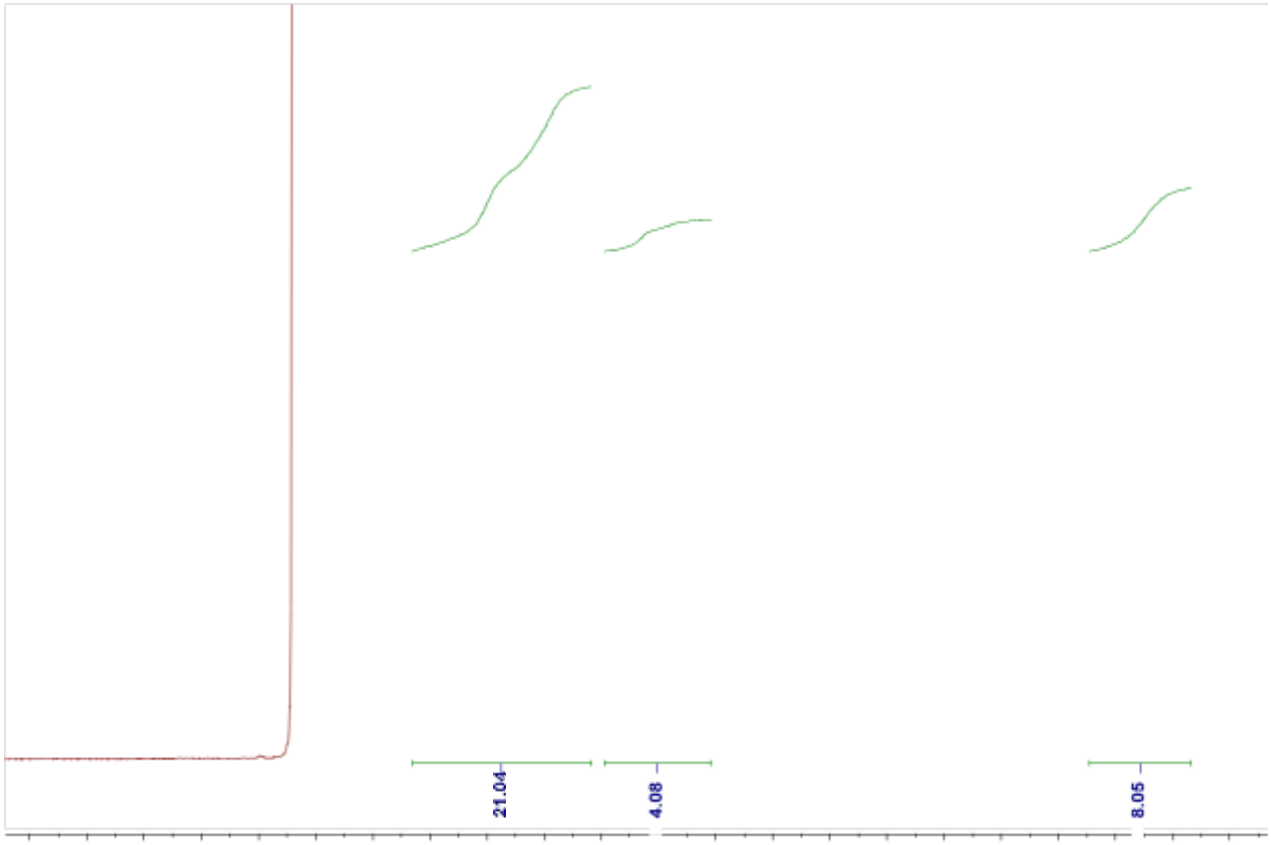


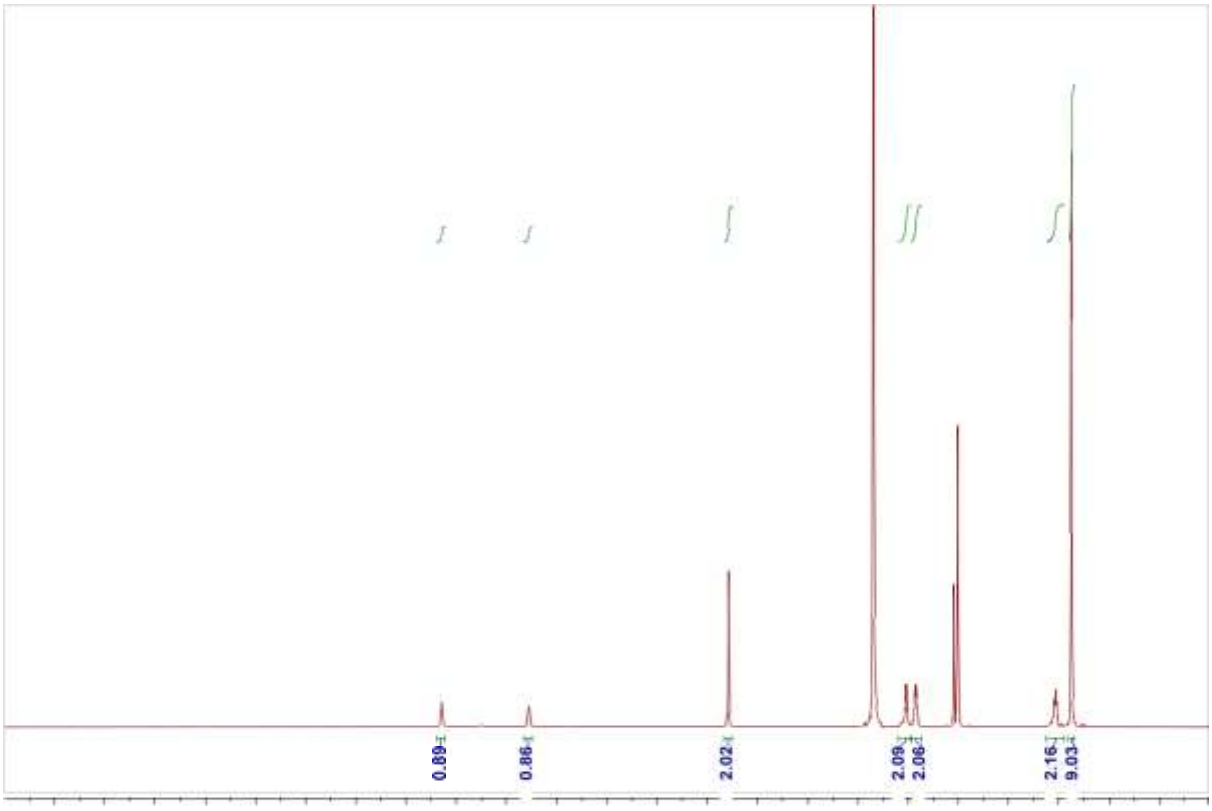
^{1}H NMR of **LB**



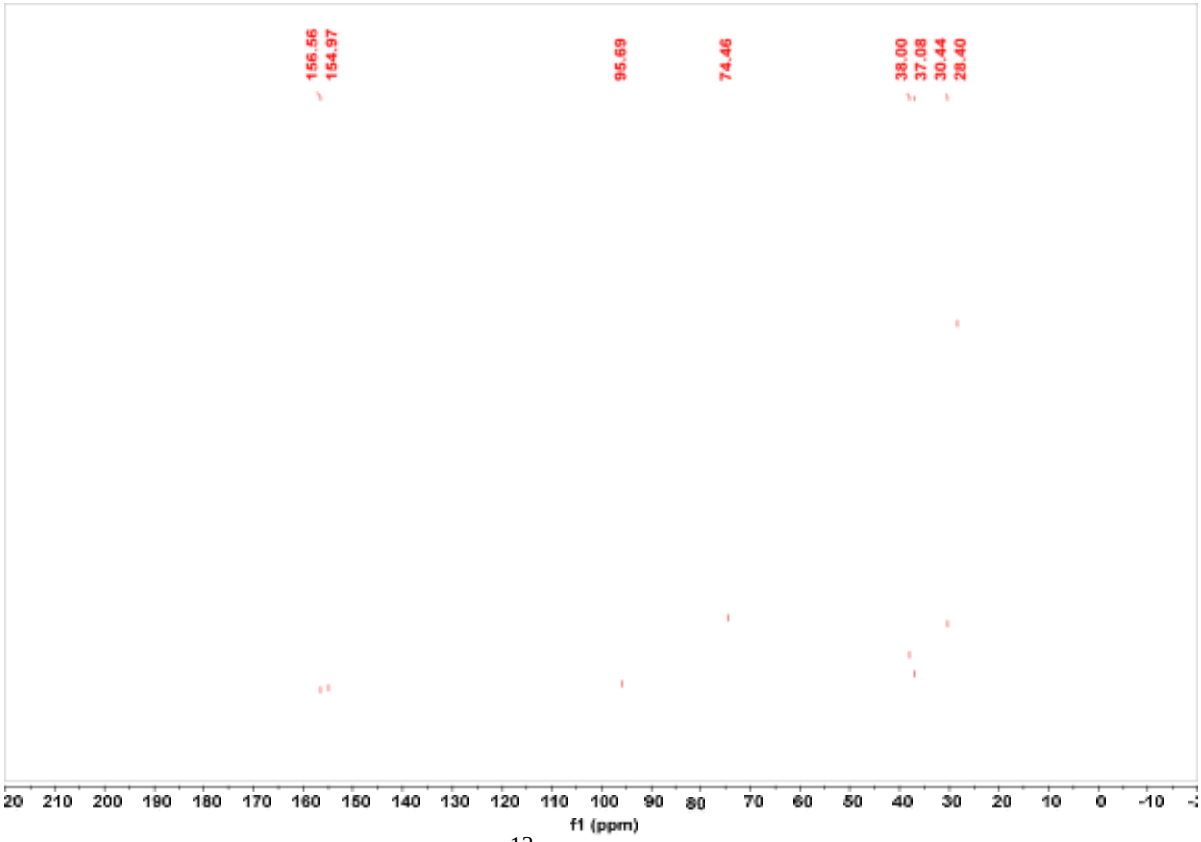
^{13}C NMR of **LB**



^{1}H NMR of **LB (Eu)**



$_1$H NMR of **2c**



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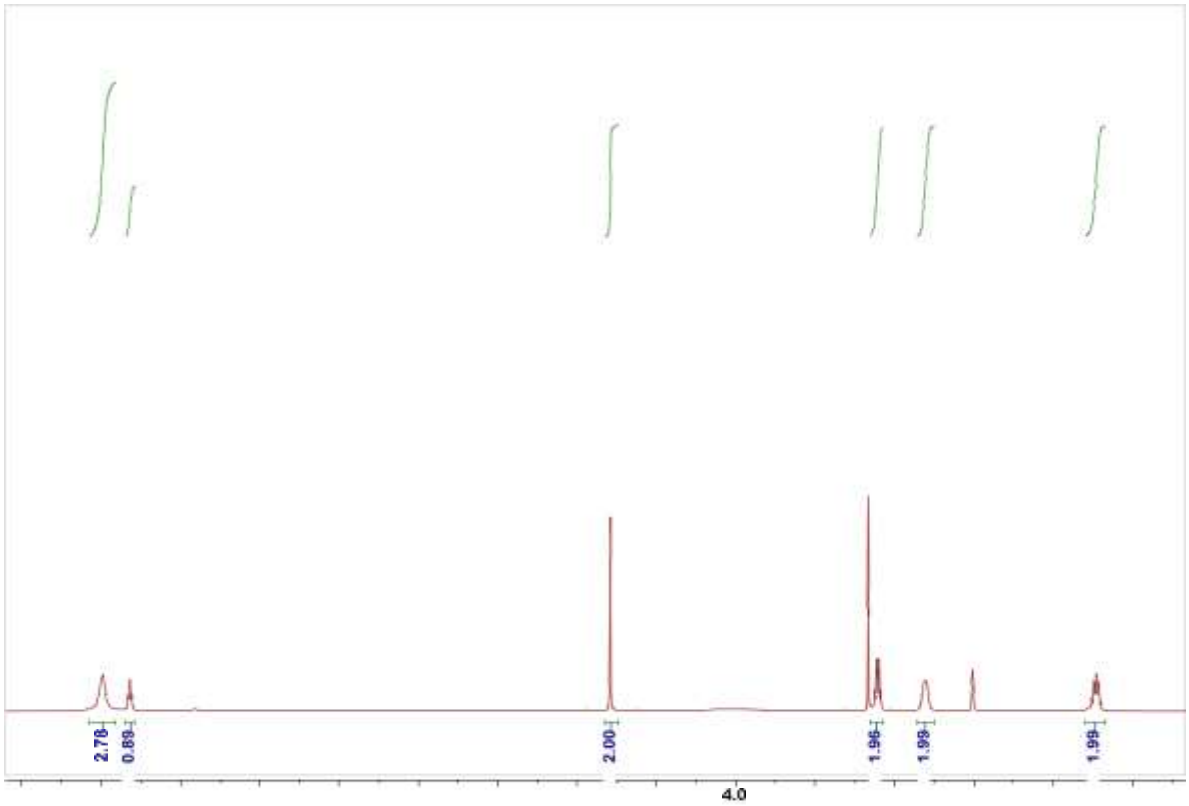
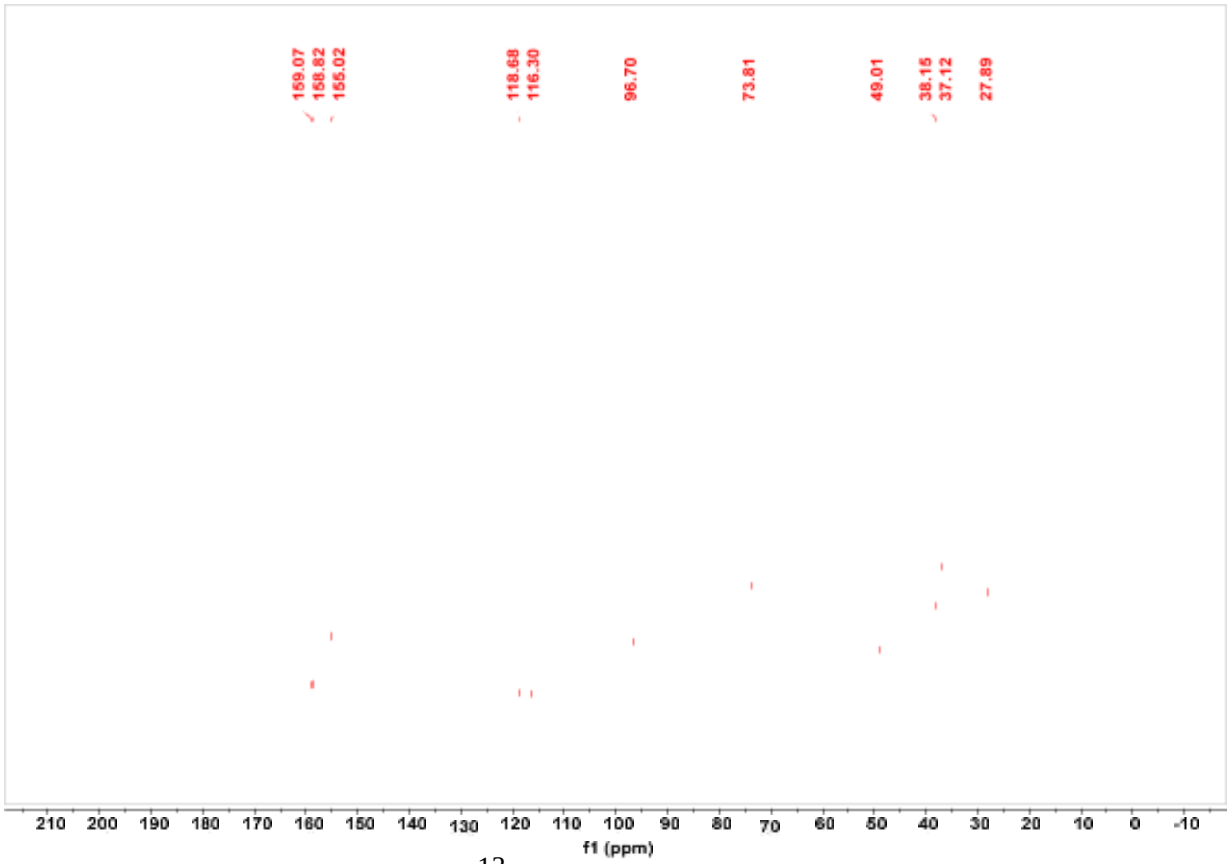
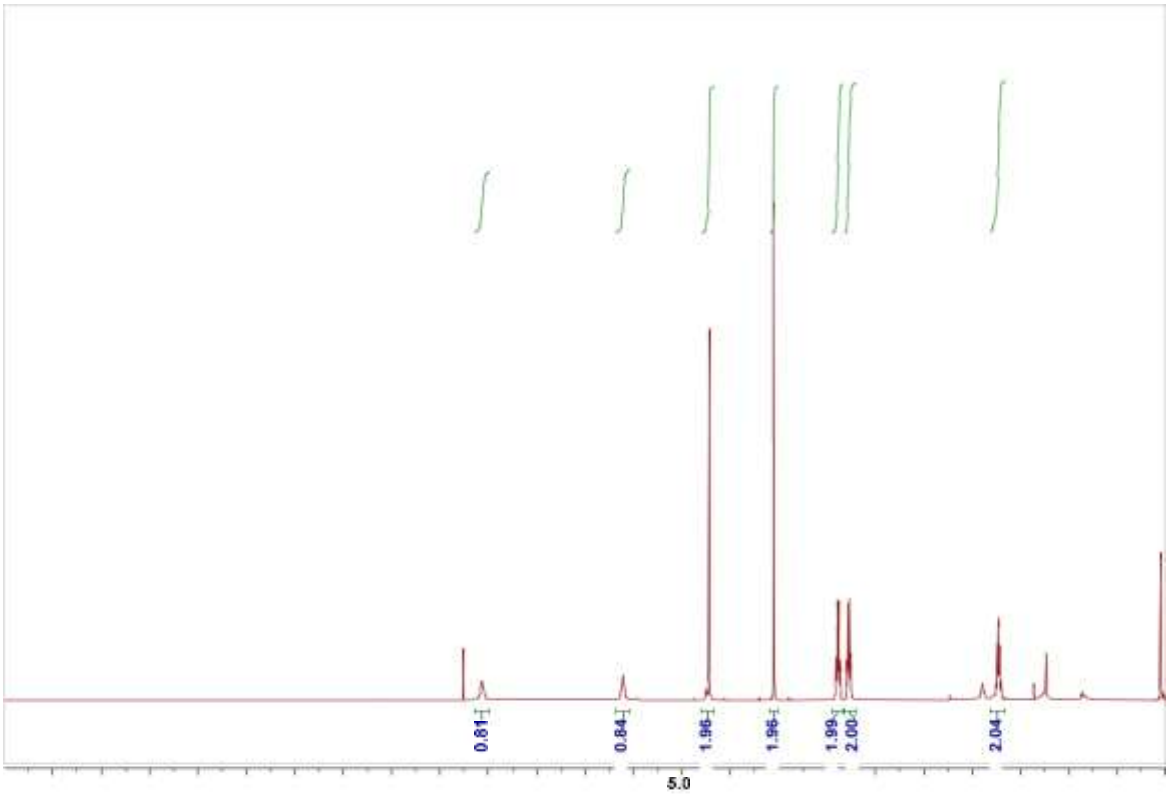


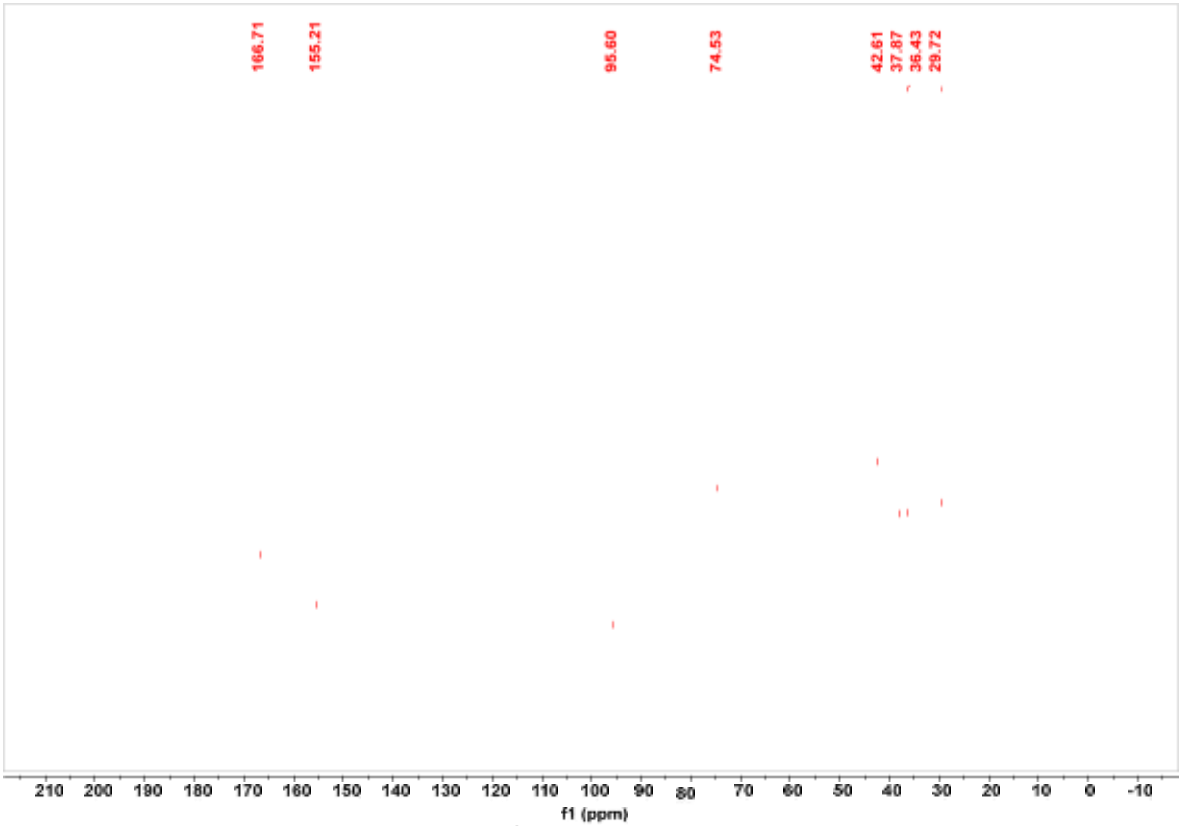
$_1$H NMR of **3c TFA**



^{13}C NMR of **3c TFA**



$_1$H NMR of **4c**



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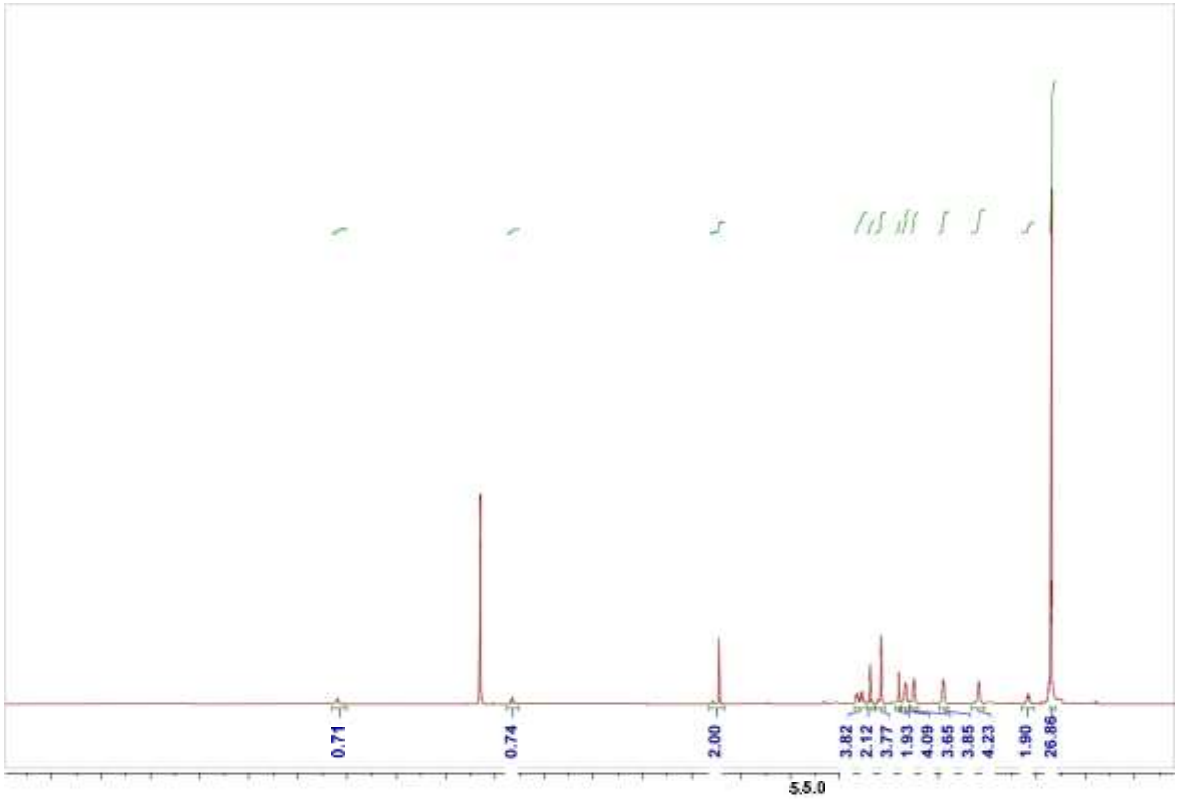
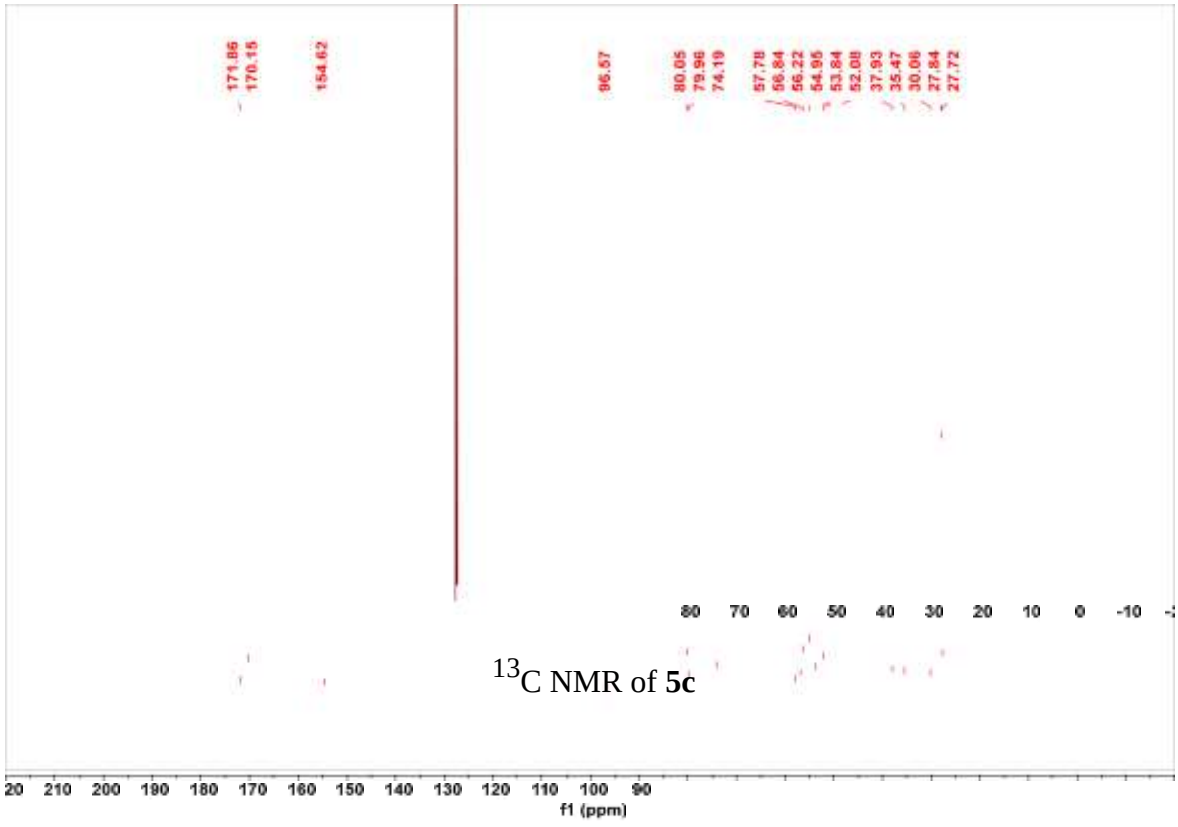


$_1$H NMR of



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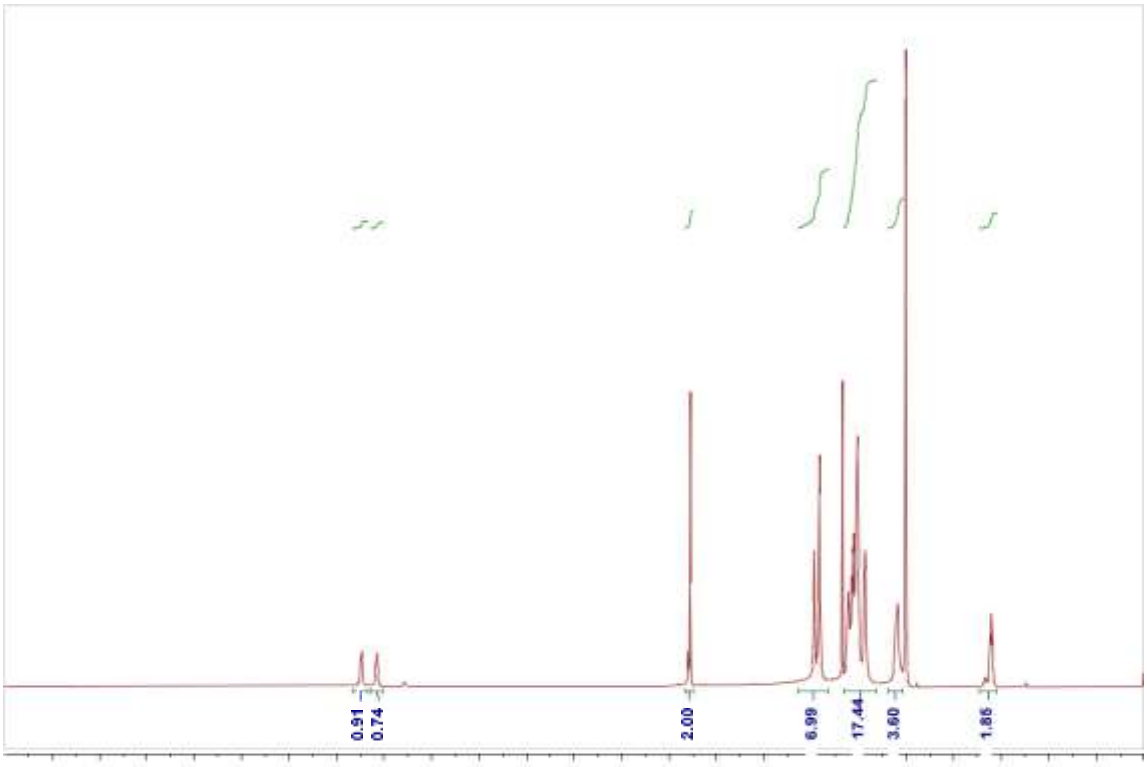
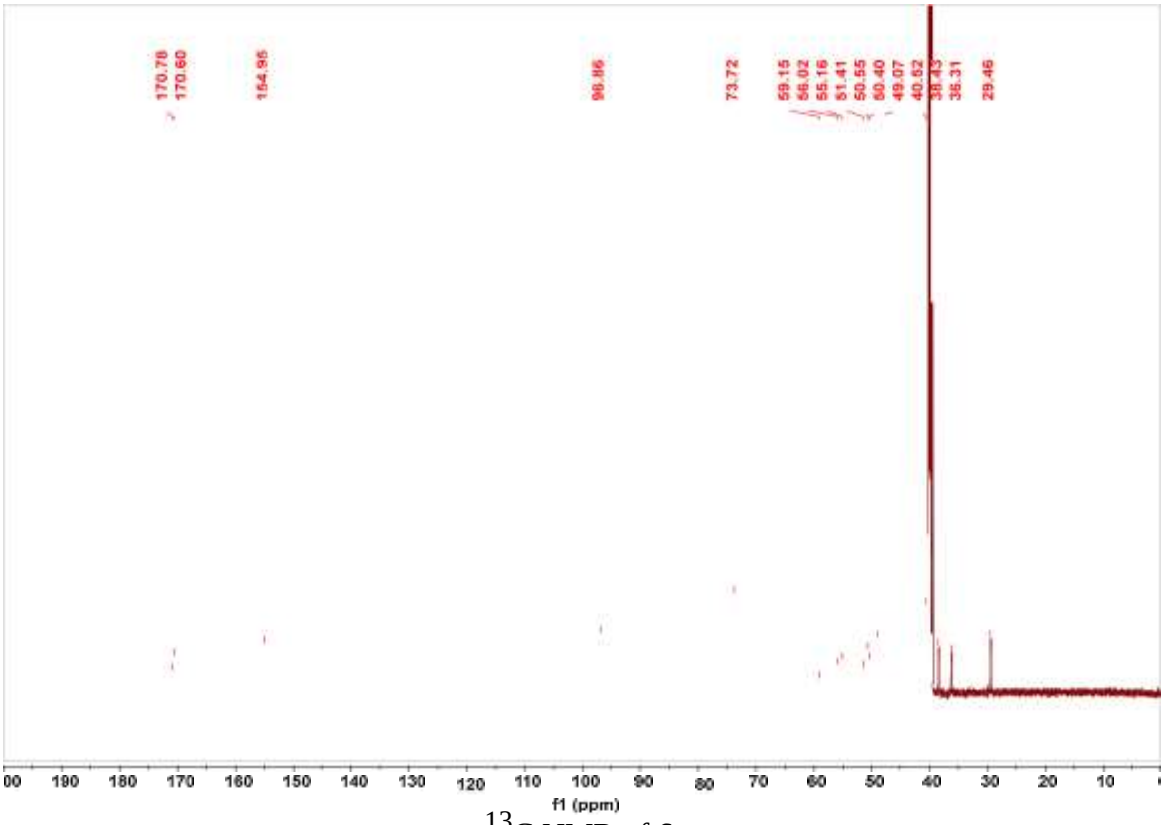


^{1}H NMR of **6c**



^{13}C NMR of **6c**