Electronic Supplementary Information (ESI)

Near-infrared Emitting Lanthanide(III) Complexes as Prototypes of Optical Imaging Agents with Peptide Targeting Ability: a Methodological Approach

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1. General Information

All reactions were carried out under an argon atmosphere unless otherwise stated. Solvents were purified following established protocols. All reagents were used as received from commercial suppliers unless otherwise stated. Peptide synthesis-grade DMF was obtained from Applied Biosystems (Courtaboeuf, France) and used for all solid phase peptide couplings. Anhydrous DMF was obtained by drying the peptide synthesis-grade solvent over activated 4 Å molecular sieves overnight. PyAOP (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate was purchased from AAPTEC (Louisville, KY, USA). Protected amino acids, Wang resin and HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) were purchased from Merck Biosciences (Nottingham, UK). Polypropylene syringes fitted with polypropylene frits and plungers were obtained from Torviq (Niles MI, USA) and Teflon stopcocks from Chromoptic (Courtaboeuf, France). Ultrapure water used for HPLC analysis and purification and copper-catalyzed azide/alkyne cycloaddtions was obtained using a Milli-Q water system from Millipore (Molsheim, France). Reactions were monitored by thin-layer chromatography (TLC) analysis using silica gel (60 F254) plates. Compounds were visualized by UV irradiation and/or spraying with a solution of potassium permanganate, followed by charring at 150 °C. Flash column chromatography was performed on silica gel 60 (230-400 mesh, 0.040-0.063 mm).

^{1}H and ^{13}C NMR spectra were recorded on an Avance II Bruker spectrometer at 250 MHz (^{13}C, 62.9 MHz) or on an Avance III HD NanoBay Bruker at 400 MHz (^{13}C, 101 MHz). Chemical shifts (δ) are given in parts per million from tetramethylsilane (TMS) as internal standard. The following abbreviations are used for the proton spectra multiplicities: s: singlet, d: doublet, t: triplet, m: multiplet, br: broad. Coupling constants (J) are reported in Hertz (Hz). High-resolution accurate mass measurements (HRAM) were performed in positive mode with an ESI source on a Q-TOF mass spectrometer with an accuracy tolerance of 2 ppm by the "Fédération de Recherche" ICOA/CBM (FR2708) platform.

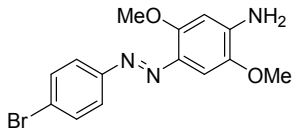
Peptides and conjugates were analyzed by HPLC and MALDI-TOF mass spectrometry. HPLC analyses were carried out on a LaChrom Elite system equipped with a Hitachi L-2130 pump and Hitachi L-2455 diode array detector with autosampler Hitachi L-2200. The machine was equipped with C18 reversed-phase columns, Nucleosil, 300 Å, 5 µm, 250 × 4.6 mm for analysis (unless stated otherwise) and Nucleosil, 300 Å, 5 µm, 250 × 10 mm for purification. Solvents A and B were 0.1% TFA in H_2O and 0.1% TFA in MeCN, respectively. Gradient elution from 5% to 50% B over 30 min, at a flow rate of 1ml/min for analysis (unless stated otherwise) and 3 ml/min for purification was used. MS analyses were performed on an Autoflex MALDI-TOF instrument (Bruker Daltonics, Bremen, Germany) equipped with a 337 nm nitrogen laser and a gridless delayed extraction ion source. The instrument was used in reflector positive ion mode with a 150 ns delay and an accelerating voltage of 19 kV. Instrument control and external calibration were accomplished using Flex-Control software (Bruker). The observed *m*/*z* correspond to the monoisotopic ions. The sample was co-crystallized with a solution of α-cyano-4-hydroxy-cinnamic acid (HCCA) as a matrix, using the dry droplet method.

2. Experimental Procedures

2.1. General procedure (A) for diazo coupling reaction (2a-d)

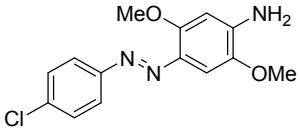
Para-substituted aniline **1a-d** (1.00 equiv.) was added to a solution of $NaNO_2$ (1.00 equiv.) in an ice-water mixture (c=0.5M) which was poured into concentrated aqueous HCl (2.3 mL) cooled in an ice bath. This diazonium salt solution was stirred vigorously for 30 min at 0°C and was then poured into a suspension of 2,5-dimethoxyaniline (1.05 equiv.) in 100 mL of acetate buffer solution (1.2 M, pH = 6) at 0 °C. A red-brown precipitate appeared and the reaction was further stirred for 2h. The coloured precipitate was filtered on Millipore membrane, washed with distilled water and dried under vacuum. The product **2a-d** was purified by flash column chromatography on SiO_2.

a. (E)-4-((4-bromophenyl)diazenyl)-2,5-dimethoxyaniline (2a)



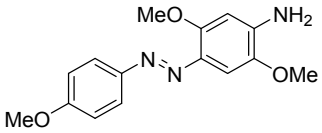
According to the general procedure **A,** and using 4-bromoaniline **1a** (1.00 g, 5.81 mmol) as starting material, after purification by flash column chromatography (PE/EtOAc +0.5% TEA: 7/3 to 5/5), the diazenyl aniline compound **2a** was isolated as a red solid (1.10 g, 57%). R_f = 0.43 (PE/EtOAc: 6/4); Mp = 125 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.70 (d, *J* = 8 Hz, 2H), 7.57 (d, *J* =8 Hz, 2H), 7. 38 (s, 1H), 6.37 (s, 1H), 4.39 (brs, 2H), 3.97 (s, 3H), 3.89 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 55.9, 57.0, 98.2, 98.3, 123.1, 123.9, 127.6, 132.1, 142.0, 142.6, 152.4, 154.9; IR (ATR, neat) ν = 3479, 3369, 2924, 1604, 1505, 1290. HRMS (ESI) *m/z* calcd for $C_{14}H_{15}BrN_3O_2$ $[M+H]^+$ 336.0347, found 336.0346.

b. (E)-4-((4-chlorophenyl)diazenyl)-2,5-dimethoxyaniline (2b)



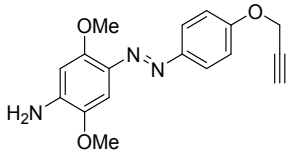
According to the general procedure **A** and starting from 4-chloroaniline **1b** (250 mg, 1.96 mmol), after purification by flash column chromatography (PE/EtOAc: 8/2 to 6/4), the diazenyl aniline product **2b** was isolated as a red solid (150 mg, 26%). R_f = 0.23 (PE/EtOAc: 8/2); Mp = 124 °C.; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.77 (d, *J* = 8 Hz, 2H), 7.42 (d, *J* = 8 Hz, 2H), 7.39 (s, 1H), 6.37 (s, 1H), 4.41 (brs, 2H), 3.96 (s, 3H), 3.89 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 55.9, 57.0, 98.2, 98.3, 123.6, 129.1, 133.6, 134.8, 141.9, 142.6, 152.0, 154.8; IR (ATR, neat) ν = 3477, 3354, 2966, 16171, 1509, 1301. HRMS (ESI) calcd for *m/z* $C_{14}H_{15}ClN_3O_2$ $[M+H]^+$ 292.0852, found 292.0853.

c. (E)-2,5-dimethoxy-4-((4-methoxyphenyl)diazenyl)aniline (2c)



According to the general procedure **A** and starting from *p*-anisidine **1c** (163 mg, 1.24 mmol), after purification by flash column chromatography (PE/EtOAc: 7/3), the diazenyl aniline product **2c** was isolated as a red-orange solid (300 mg, 84%). R_f = 0.81 (PE/EtOAc: 4/6); Mp = 164 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.84 (d, *J* = 8.0 Hz, 2H), 7.38 (s, 1H), 6.96 (d, *J* = 8.0 Hz, 2H), 6.38 (s, 1H), 4.28 (brs, 2H), 3.95 (s, 3H), 3.88 (s, 3H), 3.86 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 55.6, 55.9, 57.2, 98.4, 98.9, 114.1, 124.1, 133.8, 141.3, 141.9, 147.9, 153.9, 160.8; IR (ATR, neat) ν = 3431, 3344, 3222.0, 3004.0, 2924, 2836, 1633, 1578, 1301, 1035. HRMS (ESI) *m/z* calcd for $C_{15}H_{18}N_3O_3$ $[M+H]^+$ 288.1348, found 288.1346.

d. (E)-4-((4-(prop-2-ynyloxy)phenyl)diazenyl)aniline (2d)



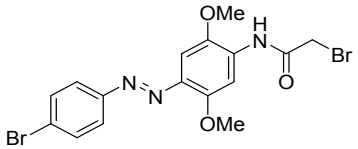
According to the general procedure **A** and starting from 4-prop-2-ynyloxy-phenylamine **1d** (57 mg, 0.39 mmol), after purification by flash column chromatography (PE/EtOAc: 6/4), the diazenyl aniline product **2d** was isolated as a red solid (56 mg, 46%). R_f = 0.33 (PE/EtOAc: 6/4); Mp = 163°C; ^{1}H NMR (250 MHz, $CDCl_3$) δ: 7.84 (d, *J* = 10.0 Hz, 2H), 7.38 (s, 1H), 7.03(d, *J* = 10.0 Hz, 2H), 6.39 (s,

1H), 4.74 (d, *J* = 2.5 Hz, 2H), 4.29 (brs, 2H), 3.96 (s, 3H), 3.89 (s, 3H), 2.54 (t, *J* = 2.5 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 56.0, 56.2, 57.2, 75.9, 78.5, 98.4, 98.9, 115.2, 124.1, 133.9, 141.5, 142.0, 148.5, 154.1, 158.7; IR (ATR, neat) ν= 3453, 3338, 3285, 2920, 2128, 1623, 1508, 1299, 1018. HRMS (ESI) *m/z* calcd for $C_{17}H_{18}N_3O_3$ $[M+H]^+$ 312.1348, found 312.1349.

2.2. General procedure (B) for bromoacetamide formation (3a-d)

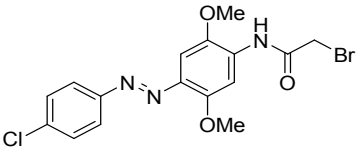
The starting amine **2a-d** (1.00 equiv.) was dissolved in dry THF (20 mL), and the solution was placed in an ice-water bath, then pyridine (1.25 equiv.) was introduced. A solution of bromoacetic bromide (1.00 equiv.) in dry THF (3 mL) was prepared and introduced dropwise to the mixture. The reaction was vigorous stirred for 30 min at 0 °C. After TLC control, the reaction was hydrolyzed at 0°C. The residue was extracted with ethyl acetate, washed twice with water and dried over magnesium sulfate. After filtration, the residue was concentrated and purified by flash column chromatography on SiO_2.

a. (E)-2-bromo-N-(4-((4-bromophenyl)diazenyl)-2,5-dimethoxyphenyl)acetamide (3a)



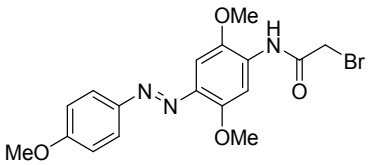
On the basis of the general procedure **B** and starting from the amine **2a** (200 mg, 0.60 mmol), the amide **3a** was isolated after purification by flash column chromatography (PE/EtOAc: 6/4) as a dark orange solid (258 mg, 95%). R_f = 0.59 (PE/EtOAc: 6/4); Mp = 176 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ: 8.98 (brs, 1H), 8.33 (s, 1H), 7.79 (d, *J* = 10 Hz, 2H), 7.60 (d, *J* = 10 Hz, 2H), 7.38 (s, 1H), 4.06 (s, 2H), 4.03 (s, 3H), 3.95 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) δ: 29.7, 56.4, 57.1, 98.0, 104.3, 124.4, 124.8, 131.6, 132.3, 137.2, 142.8, 151.9, 153.2, 163.7; IR (ATR, neat): ν = 3391, 3354, 2970, 1690, 1523, 1477, 830. HRMS (ESI) *m/z* calcd for $C_{16}H_{16}Br_2N_3O_3$ $[M+H]^+$ 455.9558, found 455.9556.

b. (E)-2-bromo-N-(4-((4-chlorophenyl)diazenyl)-2,5-dimethoxyphenyl)acetamide (3b)



On the basis of the general procedure **B** and starting from the amine **2b** (100 mg, 0.34 mmol), the amide **3b** was isolated after purification by flash column chromatography (PE/EtOAc: 6/4) as a dark orange solid (133 mg, 94%). R_f = 0.78 (PE/EtOAc: 6/4); Mp = 170 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ: 8.98 (brs, 1H), 8.34 (s, 1H), 7.84 (dd, *J* = 7.5 Hz and 2.5 Hz, 2H), 7.46 (dd, *J* = 7.5 Hz and 2.5 Hz, 2H), 7.39 (s, 1H), 4.06 (s, 2H), 4.03 (s, 3H), 3.93 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) δ: 29.7, 56.4, 57.1, 98.0, 104.3, 124.2, 129.4, 131.5, 136.4, 137.2, 142.8, 151.6, 153.1, 163.7; IR (ATR, neat): ν = 3371, 2942, 1691, 1595, 1468, 1035. HRMS (ESI) *m/z* calcd for $C_{16}H_{16}BrClN_3O_3$ $[M+H]^+$ 412.0063, found 412.0064.

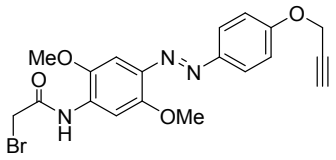
c. (E)-2-bromo-N-(2,5-dimethoxy-4-((4-methoxyphenyl)diazenyl)phenyl)acetamide (3c)



According to the general procedure **B** and starting from the amine **2c** (150 mg, 0.52 mmol), the product **3c** was isolated after purification by flash column chromatography (PE/EtOAc: 7/3) as a red

solid (207 mg, 97%). R_f = 0.82 (PE/EtOAc: 6/4); Mp = 152 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ: 8.94 (brs, 1H), 8.29 (s, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.37 (s, 1H), 6.98 (d, *J* = 7.5 Hz, 2H), 4.05 (s, 2H), 4.01 (s, 3H), 3.93 (s, 3H), 3.86 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) δ: 29.7, 55.6, 56.4, 57.1, 98.2, 104.5, 114.2, 124.8, 130.3, 137.5, 142.8, 147.5, 152.2, 161.8, 163.7; IR (ATR, neat): ν = 3371, 2942, 1691, 1595, 1468, 1035. HRMS (ESI) *m/z* calcd for $C_{17}H_{19}BrN_3O_4$ $[M+H]^+$ 408.0558, found 408.0557.

d. (E)-2-bromo-N-(2,5-dimethoxy-4-((4-(prop-2-ynyloxy)phenyl)diazenyl)phenyl)acetamide (3d)



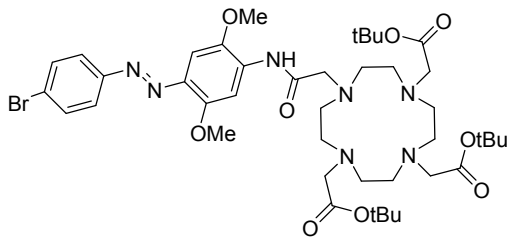
According to the general procedure **B** and starting from the amine **2d** (30 mg, 0.10 mmol), the amide **3d** was isolated after purification by flash column chromatography (PE/EtOAc: 4/6) as a dark orange solid (33 mg, 81%). R_f = 0.83 (PE/EtOAc: 4/6); Mp = 140 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ: 8.94 (brs, 1H), 8.31 (s, 1H), 7.89 (d, *J* = 9.1 Hz, 2H), 7.37 (s, 1H), 7.06 (d, *J* = 9.1 Hz, 2H), 4.75 (d, *J* = 2.4 Hz, 2H), 4.05 (s, 2H), 4.02 (s, 3H), 3.94 (s, 3H), 2.56 (t, *J* = 2.4 Hz, 1H); ^{13}C NMR (63 MHz, $CDCl_3$) δ: 29.7, 56.1, 56.4, 57.1, 76.0, 78.2, 98.1, 104.4, 115.2, 124.6, 130.6, 137.5, 142.7, 148.0, 152.4, 159.6, 163.6; IR (ATR, neat) ν= 3369, 3237, 2924, 2125, 1689, 1596, 1480, 1204, 1030, 833. HRMS (ESI) *m/z* calcd for $C_{19}H_{19}BrN_3O_4$ $[M+H]^+$ 432.0558, found 432.0556.

2.3. General procedure (C) for the synthesis of DO3A derivatives (4a-d)

A solution of triester cyclen[1] (1.00 equiv.) and Na_2CO_3 (3.00 equiv.) in anhydrous THF (5 mL) was stirred for 30 min. at room temperature under argon. A solution of bromoacetamide **3a-d** (1.00 equiv.) in anhydrous THF (2 mL) was added dropwise, and the reaction was refluxed for 8h. After cooling and filtration, the solid residue was washed with dichloromethane and methanol. The filtrate was concentrated and purified by column chromatography on SiO_2.

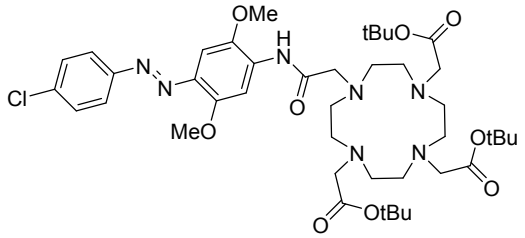
1. a) Triester cyclen : 1,4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane HBr salt. b) S. Mizukami, K. Tonai, M. Kaneko, K. Kikuchi, *J Am. Chem. Soc.* **2008**, *130*, 14376-14377. c) C. Tua, E. A. Osborneb, A. Y. Louie *Tetrahedron*, **2009**, *65*, 1241-1246. d) A. Dadabhoy, S. Faulkner, P. G. Sammes, *J. Chem. Soc., Perkin Trans. 2* **2002**, 348-357.

a. (E)-tert-butyl 2,2',2''-(10-(2-(4-((4-bromophenyl)diazenyl)-2,5-dimethoxyphenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (4a)



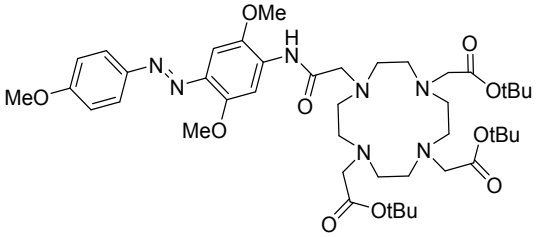
According to the general procedure **C**, the product **4a** was isolated after purification by column chromatography (CH_2Cl_2/MeOH: 95/5) as a red solid (95 mg, 98%). R_f = 0.02 (CH_2Cl_2/MeOH: 95/5); Mp = 146 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.60 (s, 1H), 8.06 (s, 1H), 7.73 (dd, *J* = 4 Hz and 8 Hz, 2H), 7.59 (d, *J* = 4 Hz and 8 Hz, 2H), 7.32 (s, 1H), 3.90 (s, 3H), 3.84 (s, 3H), 3.68-2.15 (m, 24H), 1.40 (m, 27H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 27.9, 27.9, 55.5, 56.1, 56.2, 57.5, 57.6, 81.9, 82.0, 97.8, 106.9, 124.3, 124.6, 132.2, 132.3, 137.5, 143.9, 151.9, 153.0, 171.5, 172.7; IR (ATR, neat): ν = 3350, 2973, 2821, 1724, 1525, 1261, 1223. HRMS (ESI) *m/z* calcd for $C_{41}H_{63}BrN_7O_9$ $[M+H]^+$ 876.3870, found 876.3871.

b. (E)-tert-butyl 2,2',2''-(10-(2-(4-((4-chlorophenyl)diazenyl)-2,5-dimethoxyphenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (4b)



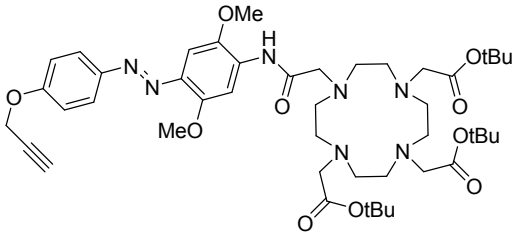
According to the general procedure **C**, the product **4b** was isolated after purification by column chromatography (CH_2Cl_2/MeOH: 95/5) as a red solid (95 mg, 98%). R_f = 0.42 (CH_2Cl_2/MeOH: 95/5); Mp = 134 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ: 8.57 (s, 1H), 8.07 (s, 1H), 7.81 (d, *J* = 10 Hz, 2H), 7.42 (d, *J* = 10 Hz, 2H), 7.32 (s, 1H), 3.90 (s, 3H), 3.84 (s, 3H), 3.50-2.00 (m, 24H), 1.44-1.37 (m, 27H); ^{13}C NMR (63 MHz, $CDCl_3$) δ: 27.9, 55.5, 56.1, 56.2, 57.5, 81.9, 97.8, 106.8, 124.0, 129.3, 132.3, 136.1, 137.4, 143.9, 151.5, 153.0, 171.5, 172.7; IR (ATR, neat): ν = 3384, 2974, 2828, 1724, 1527, 1223, 1105, 1008. HRMS (ESI) *m/z* calcd for $C_{41}H_{63}ClN_7O_9$ $[M+H]^+$ 832.4375, found 832.4376.

c. (E)-tert-butyl 2,2',2''-(10-(2-(2,5-dimethoxy-4-((4-methoxyphenyl)diazenyl)phenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (4c)



According to the general procedure **C**, the product **4c** was isolated after purification by column chromatography (PE/EtOAc: 4/6) as a red-orange solid (219 mg, 57%). R_f = 0.05 (PE/EtOAc: 4/6); Mp = 168 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.33 (s, 1H), 8.07 (s, 1H), 7.87 (dd, *J* = 4 Hz and 8 Hz, 2H), 7.33 (s, 1H), 6.97 (dd, *J* = 4 Hz and 8 Hz, 2H), 3.91 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.45-2.25 (m, 24 H), 1.45-1.39 (m, 27H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 28.0, 55.6, 55.6, 56.2, 57.6, 57.8, 82.0, 98.1, 107.1, 114.2, 124.7, 130.9, 137.9, 143.7, 147.5, 152.2, 161.8, 171.1, 172.8; IR (ATR, neat): ν = 3406, 2980, 2831, 1725, 1594, 1225, 1157, 1104. HRMS (ESI) *m/z* calcd for $C_{42}H_{66}N_7O_{10}$ $[M+H]^+$ 828.4871, found 828.4870.

d. (E)-tert-butyl 2,2',2''-(10-(2-(2,5-dimethoxy-4-((4-(prop-2-ynyloxy)phenyl)diazenyl) phenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (4d)

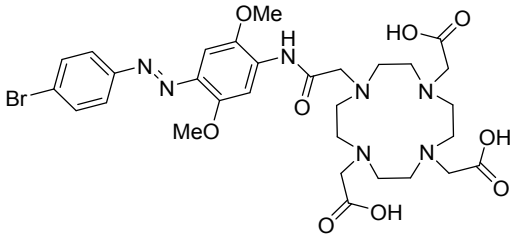


According to the general procedure **C**, the product **4d** was isolated after purification by column chromatography (CH_2Cl_2/MeOH: 9/1) as a red solid (63 mg, 95%). R_f = 0.45 (CH_2Cl_2/MeOH: 9/1); Mp = 143 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ: 8.43 (s, 1H), 8.03 (s, 1H), 7.86 (d, *J* = 8 Hz, 2H), 7.31 (s, 1H), 7.03 (d, *J* = 8 Hz, 2H), 4.72 (d, *J*=2.4 Hz, 2H), 3.88 (s, 3H), 3.82 (s, 3H), 3.29-2.50 (m, 24H), 2.53 (t, *J*=2.4 Hz, 1H), 1.43-1.33 (m, 27H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 27.8, 27.9, 55.4, 56.0, 56.0, 56.1, 57.4, 57.7, 75.9, 78.0, 81.92, 81.9, 97.9, 107.1, 115.1, 124.5, 131.1, 137.7, 143.7, 147.9, 152.2, 159.4, 171.1, 172.7.; IR (ATR, neat) ν = 3360, 3291, 2974, 2834, 2107, 1723, 1673, 1593, 1250, 1023, 840. HRMS (ESI) *m/z* calcd for $C_{45}H_{68}N_7O_{10}$ $[M+H]^+$ 866.5027, found 866.5026.

2.4. General procedure (D) for deprotecting *tert*-butyl esters: (5a-d)

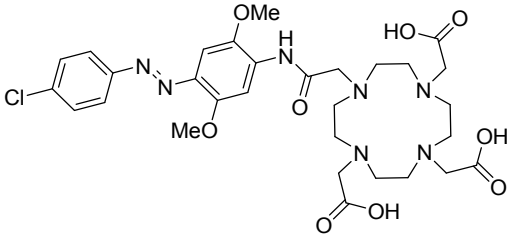
A solution of 2% of triisopropylsilane in trifluoroacetic acid (2mL) was added to the starting ester **4a-d** (0.05 mmol). The deprotection reaction was carried out at room temperature for 60 min. Volatile compounds were evaporated and the product was precipitated with diethyl ether (5mL). After filtration through a Millipore filter and washings with diethyl ether, the residue was dried under vacuum to provide **5a-d** as red gum.

a. (E)-2,2',2''-(10-(2-(4-((4-bromophenyl)diazenyl)-2,5-dimethoxyphenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (5a)



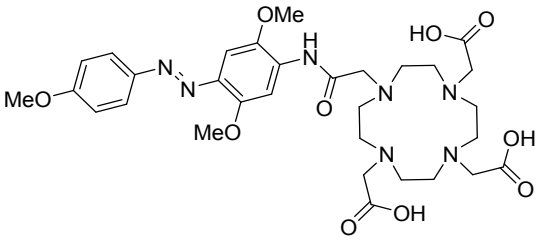
According to the general procedure **D**, the deprotected product **5a** (38 mg) was isolated in quantitative yield as a red gum.^{1}H NMR (250 MHz, MeOD) *δ:* 8.17 (m, 1H), 7.79 (m, 2H), 7.69 (m, 2H), 7.39 (m, 1H), 3.99 (s, 3H), 3.90 (s, 3H), 3.91 (m, 4H), 3.50-2.00 (m, 20H); ^{13}C NMR (101 MHz, MeOD) δ: 211.3, 183.8, 156.3, 133.4, 133.4, 128.8, 125.4, 125.3, 73.9, 57.8, 56.6, 28.4, 28.3, 18.9; IR (ATR, neat): *ν* = 3375, 2974, 2852, 1679, 1593, 1534, 1454, 1394, 1125, 832, 720. HRMS (ESI) *m/z* calcd for $C_{30}H_{41}BrN_7O_9$ $[M+H]^+$ 722.2149, found 722.2148.

b. (E)-2,2',2''-(10-(2-(4-((4-chlorophenyl)diazenyl)-2,5-dimethoxyphenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (5b)



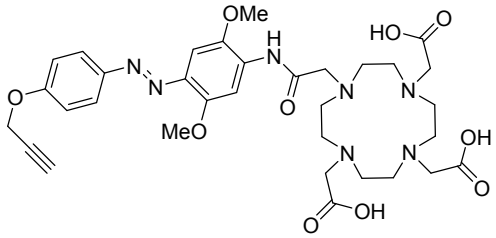
According to the general procedure **D**, the product **5b** (30 mg) was isolated in quantitative yield as a red gum. ^{1}H NMR (250 MHz, MeOD) *δ:* 8.20-8.32 (m, 1H), 7.88 (m, 2H), 7.54 (m, 2H), 7.37 (m, 1H), 4.00 (s, 3H), 3.97 (s, 3H), 3.96-2.00 (m, 24H); ^{13}C NMR (101 MHz, MeOD) *δ:* 18.9, 28.4, 46.4, 49.2, 56.6, 73.9, 125.1, 125.2, 128.8, 130.3, 130.4, 154.8, 156.3, 183.8, 211.3; IR (ATR, neat): *ν* = 3395, 2971, 2854, 1675, 1593, 1183, 721. HRMS (ESI) *m/z* calcd for $C_{30}H_{41}ClN_7O_9$ $[M+H]^+$ 678.2654, found 678.2655.

c. (E)-2,2',2''-(10-(2-(2,5-dimethoxy-4-((4-methoxyphenyl)diazenyl)phenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (5c)



According to the general procedure **D**, the product **5c** (35 mg) was isolated in 88% yield as a dark violet gum. ^{1}H NMR (250 MHz, MeOD) δ: 8.32-8.11 (m, 1H), 7.91 (m, 2H), 7.39 (s, 1H), 7.10-7.06 (m, 1H), 4.01 (s, 3H), 3.91 (brs, 6H), 4.01-2.15 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ: 18.9, 46.3, 49.2, 49.8, 56.1, 56.7, 57.8, 73.8, 115.3, 119.5, 125.7, 128.8, 156.3, 162.8, 163.1, 183.8, 211.3; IR (ATR, neat): *ν* = 3548, 3468, 3408, 3094, 2956, 2837, 1682, 1594, 1251, 1204, 1012, 842. HRMS (ESI) *m/z* calcd for $C_{31}H_{44}N_7O_{10}$ $[M+H]^+$ 674.3149, found 674.3148.

d. (E)-2,2',2''-(10-(2-(2,5-dimethoxy-4-((4-(prop-2-ynyloxy)phenyl)diazenyl)phenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (5d)

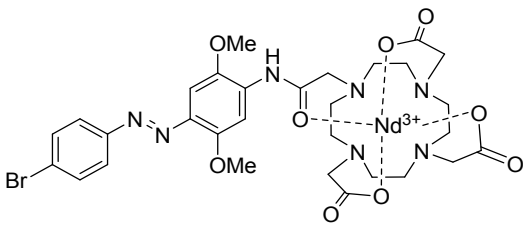


According to the general procedure **D**, **5d** (40 mg) was isolated in 87% yield as a red-violet gum. ^{1}H NMR (250 MHz, MeOD) δ: 8.09-8.31 (m, 1H), 8.09 (s, 1H), 7.89 (m, 2H), 7.38 (brs, 1H), 7.12 (m, 2H), 4.82 (brs, 2H), 3.95 (s, 3H), 3.88 (s, 3H), 4.00-2.00 (m, 26H), 3.01 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 28.3, 44.9, 56.9, 57.8, 58.4, 77.2, 99.3, 110.1, 116.3, 116.4, 119.3, 125.6, 149.0, 152.9, 162.7, 162.8, 163.1, 210.6; IR (ATR, neat): *ν* = 3387, 3285, 2923, 2848, 1674, 1591, 1236, 1017, 720. HRMS (ESI) *m/z* calcd for $C_{33}H_{44}N_7O_{10}$ $[M+H]^+$ 698.3149, found 698.3148.

2.5. General procedure of complexation of triacids with lanthanide salts

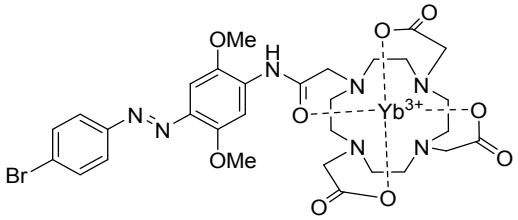
To a solution of the triacid **5a-d** (1.00 equiv.) in methanol (2 mL), $Ln(OTf)_3$ (1.00 equiv.) was added as one single portion. The reaction was carried out for 24 h at 50 °C. Residues were taken up into methanol. Then the volume of solvent was reduced under vacuum and a product was precipitated with diethyl ether (5mL). After filtration through a Millipore filter and washing with diethyl ether, the residue was dried under vacuum to provide complexes as red-violet gums.

a. (E)-2,2',2''-(10-(2-(4-((4-bromophenyl)diazenyl)-2,5-dimethoxyphenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate neodymium(III) complex (5a-Nd)



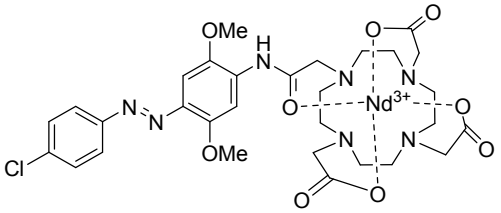
Yield: 92%. HRMS (ESI) *m/z* calcd for $C_{30}H_{38}BrN_7NdO_9$ $[M+H]^+$ 861.0991, found 861.0987.

b. (E)-2,2',2''-(10-(2-(4-((4-bromophenyl)diazenyl)-2,5-dimethoxyphenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate ytterbium(III) complex (5a-Yb)



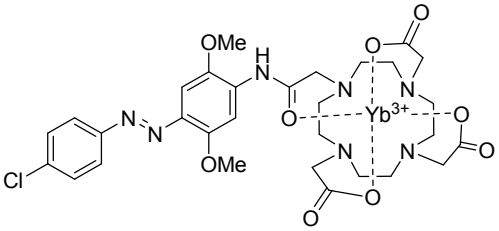
Yield 73%. HRMS (ESI) *m/z* calcd for $C_{30}H_{38}BrN_7O_9Yb$ $[M+H]^+$ 893.1303, found 893.1300.

c. (E)-2,2',2''-(10-(2-(4-((4-chlorophenyl)diazenyl)-2,5-dimethoxyphenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate neodymium(III) complex (5b-Nd)



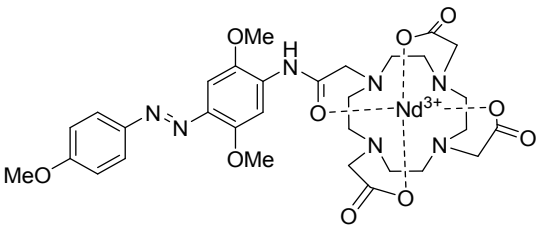
Yield 75%. HRMS (ESI) *m/z* calcd for $C_{30}H_{38}ClN_7NdO_9$ $[M+H]^+$ 817.1496, found 817.1492.

d. (E)-2,2',2''-(10-(2-(4-((4-chlorophenyl)diazenyl)-2,5-dimethoxyphenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate ytterbium(III) complex (5b-Yb)



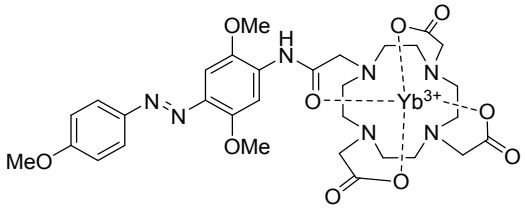
Yield 85%. HRMS (ESI) *m/z* calcd for $C_{30}H_{38}ClN_7O_9Yb$ $[M+H]^+$ 849.1808, found 849.1804.

e. (E)-2,2',2''-(10-(2-(2,5-dimethoxy-4-((4-methoxyphenyl)diazenyl)phenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate neodymium(III) complex (5c-Nd)



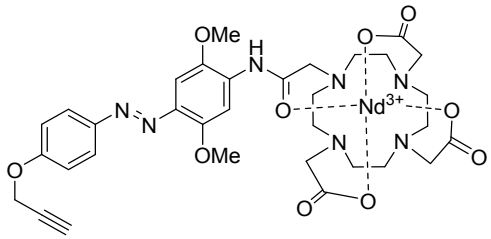
Yield 90%. HRMS (ESI) *m/z* calcd for $C_{31}H_{41}N_7NdO_{10}$ $[M+H]^+$ 813.1992, found 813.1990.

f. (E)-2,2',2''-(10-(2-(2,5-dimethoxy-4-((4-methoxyphenyl)diazenyl)phenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate ytterbium(III) complex (5c-Yb)



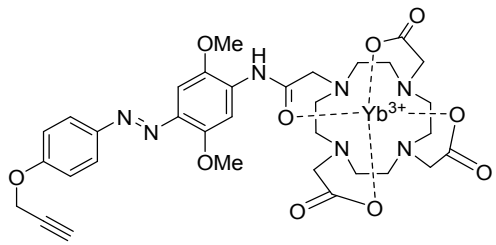
Yield 75%. HRMS (ESI) *m/z* calcd for $C_{31}H_{41}N_7O_{10}Yb$ $[M+H]^+$ 845.2303, found 845.2307.

g. (E)-2,2',2''-(10-(2-(2,5-dimethoxy-4-((4-(prop-2-ynyloxy)phenyl)diazenyl)phenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate neodymium(III) complex (5d-Nd)



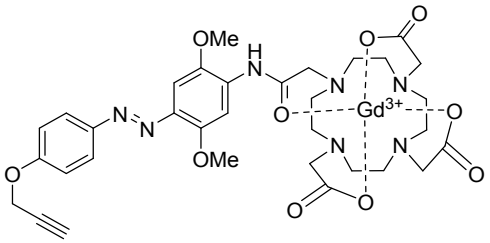
Yield 83%. HRMS (ESI) *m/z* calcd for $C_{33}H_{41}N_7NdO_{10}$ $[M+H]^+$ 837.1992, found 837.1985.

h. (E)-2,2',2''-(10-(2-(2,5-dimethoxy-4-((4-(prop-2-ynyloxy)phenyl)diazenyl)phenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate ytterbium(III) complex (5d-Yb)



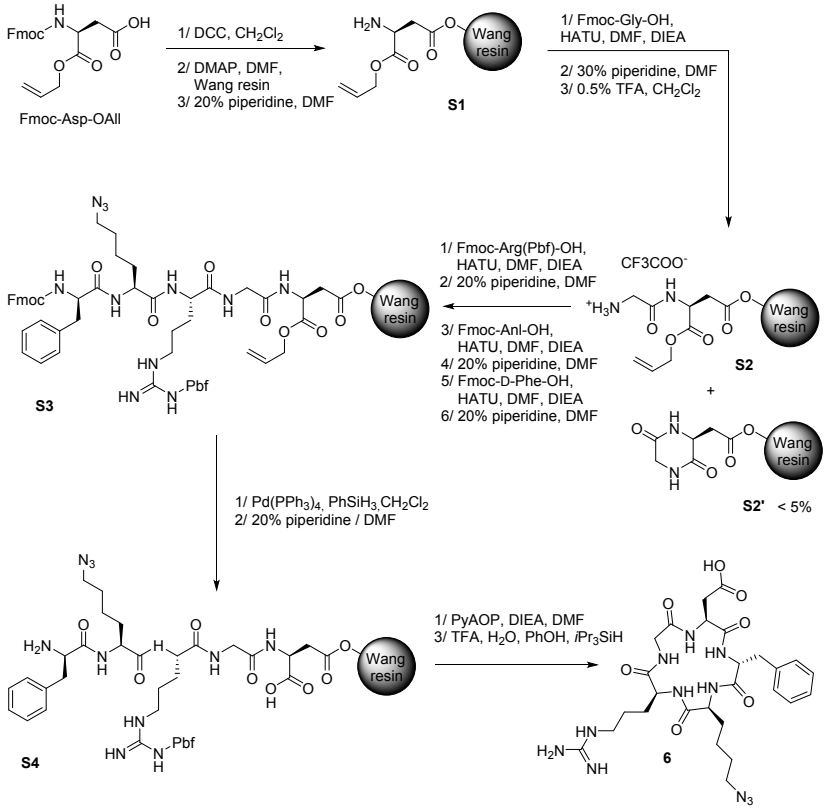
Yield 81%. HRMS (ESI) *m/z* calcd for $C_{33}H_{41}N_7O_{10}Yb$ $[M+H]^+$ 869.2303, found 869.2304.

i. (E)-2,2',2''-(10-(2-(2,5-dimethoxy-4-((4-(prop-2-ynyloxy)phenyl)diazenyl)phenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate gadolinium(III) complex (5d-Gd)



Yield 79%. HRMS (ESI) *m/z* calcd for $C_{33}H_{41}GdN_7O_{10}$ $[M+H]^+$ 853.2156, found 853.2158.

2.6. Solid phase synthesis of azide-functionalized cyclopeptide (6)



a) H-Asp(O-Wang resin)-OAll (S1)

High loading Wang resin (750 mg, 1.2 mmol/g, 0.9 mmol) was introduced in a polypropylene syringe fitted with a polypropylene frit and was subsequently swollen in anhydrous DMF (10 mL for 30 min) under a dry argon atmosphere. The solvent was then removed under argon and the swelling step was repeated once for 5 min. The syringe was then fitted with a polypropylene plunger and a Teflon stopcock. Fmoc-Asp-OAll (712 mg, 2.0 equiv.) was dissolved in 10 mL anhydrous CH_2Cl_2 under argon and the resulting solution was cooled in an ice bath. DCC (185 mg, 1.0 equiv.) was added and the mixture stirred for 20 min at 0°C. The white dicyclohexylurea precipitate was filtered off under argon, *via* a cannula and through a sintered glass funnel. Solvents were removed *in vacuo* and the white residue was dissolved in 6 mL anhydrous DMF. This solution was transferred under argon to the resin by suction, followed by DMAP (7 mg, 0.1 equiv.) dissolved in 1 mL anhydrous DMF. The resulting suspension was stirred by rotation for 4 h at RT. The resin was extensively washed with DMF then CH_2Cl_2. Capping of the residual hydroxyl groups from the Wang resin was achieved by treatment with Ac_2O (2.5 mL, 29 equiv.) and *N*-methyl-morpholine (2.5 mL, 30 equiv.) in CH_2Cl_2 (10 mL) for 2 h at RT, followed by extensive washes with CH_2Cl_2 then DMF. The Fmoc group was deprotected using 20% piperidine in DMF (3 × 3 min), followed by extensive washes with DMF. The esterification yield (87%, 0.783 mmol) was determined by UV titration of the fluorenylmethylpiperidine co-product (ε = 7800 L.mol^{-1}.cm^{-1} at λ = 301 nm).

b) H-Gly-Asp(O-Wang resin)-OAll·TFA (S2)

In a syringe fitted with a frit, H-Asp(O-Wang resin)-OAll (**S1**, 0.783 mmol) was swollen in DMF (10 mL, 2 × 1 min). The solvent was drained off thoroughly and the syringe was fitted with a plunger and a Teflon stopcock. Fmoc-Gly-OH (477 mg, 2.05 equiv.) dissolved in 3 mL DMF was transferred to the resin by suction, followed by HATU (595 mg, 2.0 equiv.) in 2 mL DMF then DIEA (546 µL, 4.0 equiv.). The resulting suspension was stirred by rotation for 1h at RT, followed by extensive washes with DMF then CH_2Cl_2. A Kaiser test[2] performed on a few beads of resin indicated the absence of any unreacted amine.

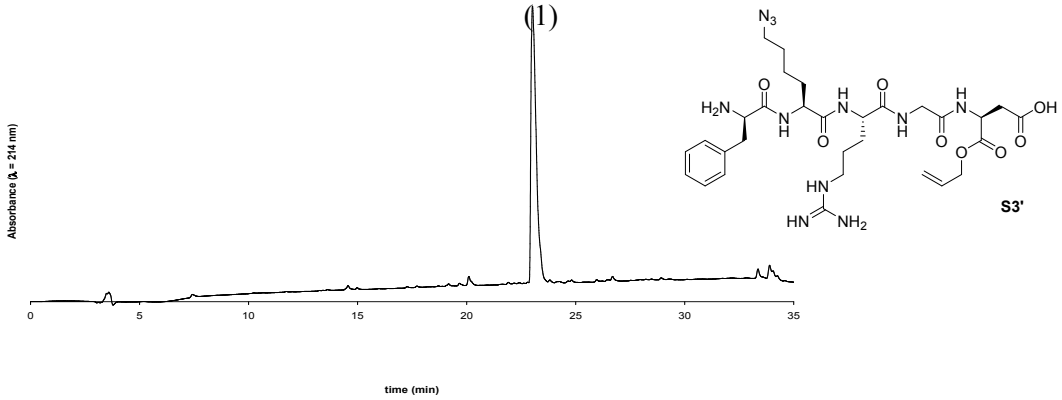
At this stage, we anticipated a side reaction if a standard protocol would be used for the Fmoc deprotection, *e.g.* the formation of the diketopiperazine (DKP) cyclo(Gly-Asp(Wang resin) (**S2'**) arising from the attack of the liberated amine on the allyl ester. Related DKP formation is indeed a common problem encountered during and after treatment with piperidine of Fmoc-Xaa-Yaa esters of Wang or related resin, when Xaa and/or Yaa are Gly or Pro residues.[3] Confirming this, severe yield loss were observed when using a standard Fmoc deprotection protocol (3 × 3 min treatments with 20% piperidine in DMF) followed by storage of the resulting peptidyl resin at RT for a few hours. This could be judged by subsequent coupling of Fmoc-Arg(Pbf)-OH followed by Fmoc deprotection and UV titration of the fluorenylmethylpiperidine co-product. In consequence, a slightly modified Fmoc deprotection protocol was optimized to solve this issue. The resin was treated with 30% piperidine DMF (10 mL, 6 × 10 s) then immediately with CH_2Cl_2 (10 s flow wash) then immediately with 0.5% TFA in CH_2Cl_2 (10 mL, 4 × 2 s) in order to switch off the nucleophilicity of the terminal amine by protonation. The resin was then immediately extensively washed with CH_2Cl_2. UV titration of the fluorenylmethylpiperidine co-product confirmed a quantitative Fmoc deprotection.

c) Fmoc-D-Phe-Anl-Arg(Pbf)-Gly-Asp(O-Wang resin)-OAll (S3)

In a syringe fitted with a frit, H-Gly-Asp(O-Wang resin)-OAll, TFA (assumed 0.783 mmol) was swollen in DMF (2 × 10 mL for 1 min). The solvent was drained off thoroughly and the syringe was fitted with a plunger and a Teflon stopcock. Fmoc-Arg(Pbf)-OH (1.04 g, 2.05 equiv.) dissolved in 3 mL DMF was transferred to the resin by suction, followed by HATU (595 mg, 2.0 equiv.) in 2 mL DMF then DIEA (546 µL, 4.0 equiv.). The resulting suspension was stirred by rotation for 1 h at RT, followed by extensive washes with DMF then CH_2Cl_2. A Kaiser test performed on a few beads of resin indicated the absence of any unreacted amine. The Fmoc group was deprotected using 20% piperidine in DMF (3 × 3 min), followed by extensive washes with DMF. UV titration of the fluorenylmethylpiperidine co-product (0.744 mmol) allowed to indirectly quantifying the amount of diketopiperazine formed during our specific Fmoc deprotection protocol and the subsequent coupling (5%).

Fmoc-ω-azido-norleucine-OH (Fmoc-Anl-OH, 633 mg) then Fmoc-D-Phe-OH (622 mg) were then coupled using the same conditions as for Fmoc-Arg(Pbf)-OH followed by a standard Fmoc deprotection protocol, to give the peptidyl resin Fmoc-D-Phe-Anl-Arg(Pbf)-Gly-Asp(O-Wang resin)-OAll (**S3**). An aliquot (~ 0.5%) of the resin was treated with 20% piperidine in DMF (3 × 3min) then cleaved by treatment with a 1 mL of a TFA/phenol/H_2O/*i*Pr_3SiH cocktail (87.5:5:5:2.5) for 2 h, after Fmoc protection under standard conditions. After filtration of the resin, the solution was poured into ice-cooled Et_2O (10 mL). The white precipitate was isolated by centrifugation, then washed twice with Et_2O (10 mL, followed by centrifugation) and dried under reduced pressure to give a white solid. HPLC analysis indicated an excellent purity of the crude resulting pentapeptide H-D-Phe-Anl-Arg-Gly-Asp-OAll (**S3'**).

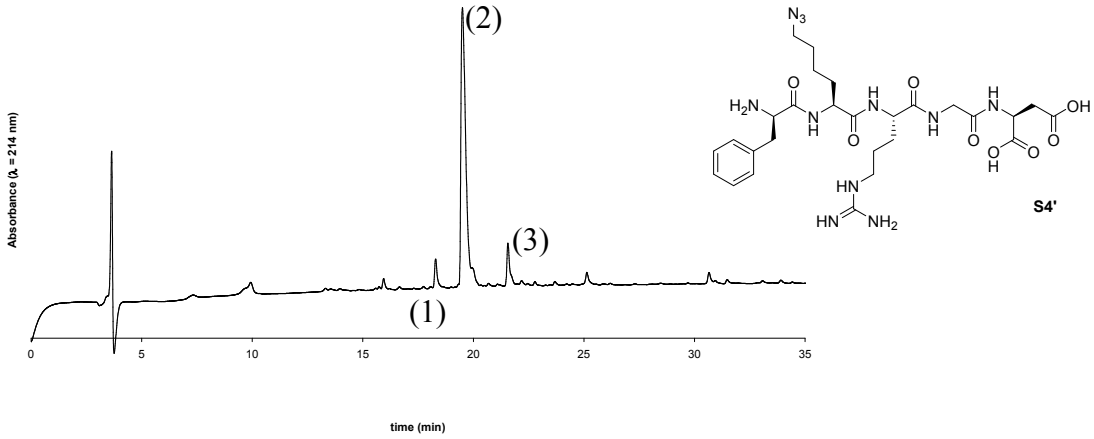
2. E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, *Anal. Biochem.*, 1970, **34**, 595.
3. E. Pedroso, A. Grandas, F. X. C. de las Heras, E. Giralt, *Tetrahedron Lett.*, 1986, **27**, 743.



Peak #	t_R (min)	Observed *m/z*	Attributed to	Calculated *m/z*
1	23.02	688.36	**S3'**	688.35 ($C_{30}H_{46}N_{11}O_8$)

d) Fmoc-D-Phe-Abg-Arg(Pbf)-Gly-Asp(O-Wang resin)-OH (S4)

Under an argon atmosphere, in a syringe fitted with a frit, a plunger and a Teflon stopcock, Fmoc-D-Phe-Anl-Arg(Pbf)-Gly-Asp(O-Wang resin)-OAll (**S3**, assumed 0.740 mmol) was swollen in dry CH_2Cl_2 (10 mL, 2 × 1 min). The solvent was drained off then *tetrakis*(triphenylphosphine)palladium(0) (427 mg, 0.50 equiv.) dissolved in anhydrous CH_2Cl_2 (15 mL) was transferred to the resin by suction, immediately followed by phenylsilane (0.91 mL, 10.0 equiv.). The resulting suspension was stirred by rotation for 2h at RT followed by extensive washes with CH_2Cl_2 then DMF. The dark-coloured peptidyl resin was subsequently washed with a 0.1 M sodium diethyldithiocarbamate solution in NMP then a 1 M pyridinium chloride solution in CH_2Cl_2/MeOH (95:5), these two steps being repeated until the resin recovered its initial yellowish colour- (ca 3-4 times). An aliquot (~ 0.5%) of the resin was then cleaved using the same protocol as for **S3**. HPLC analysis of the resulting white solid indicated that the allyl ester deprotection was complete, as no trace of **S3'** could be detected.

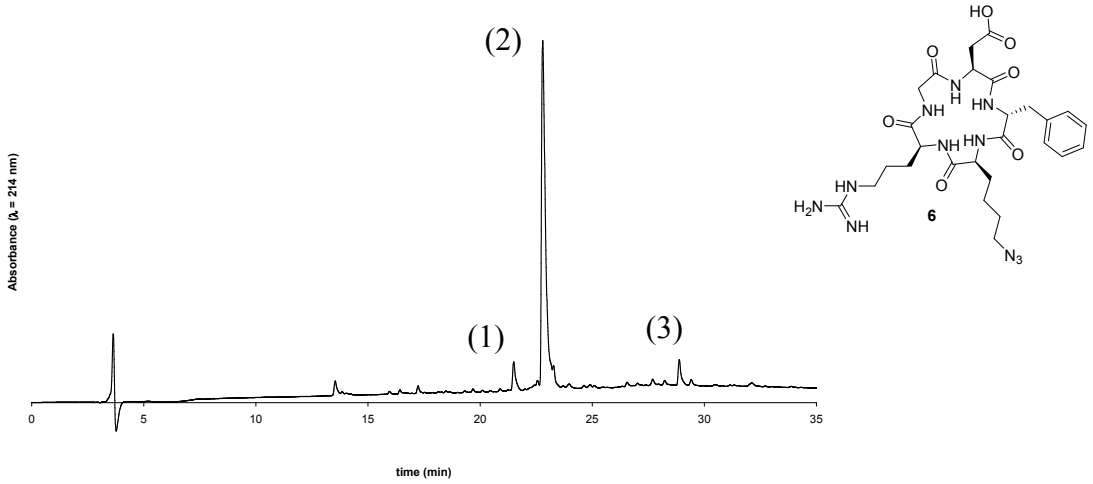


Peak #	t_R (min)	Area %	Observed *m/z*	Attributed to	Calculated *m/z*
1	18.28	4.5	605.31	**S4'** - 43 Da	No structure attributed
2	19.5	87.8	648.32	**S4'**	648.31 ($C_{27}H_{42}N_{11}O_8$)
3	21.56	7.7	-[1]	-	Non-peptidic compound

[1]: no MALDI signal.

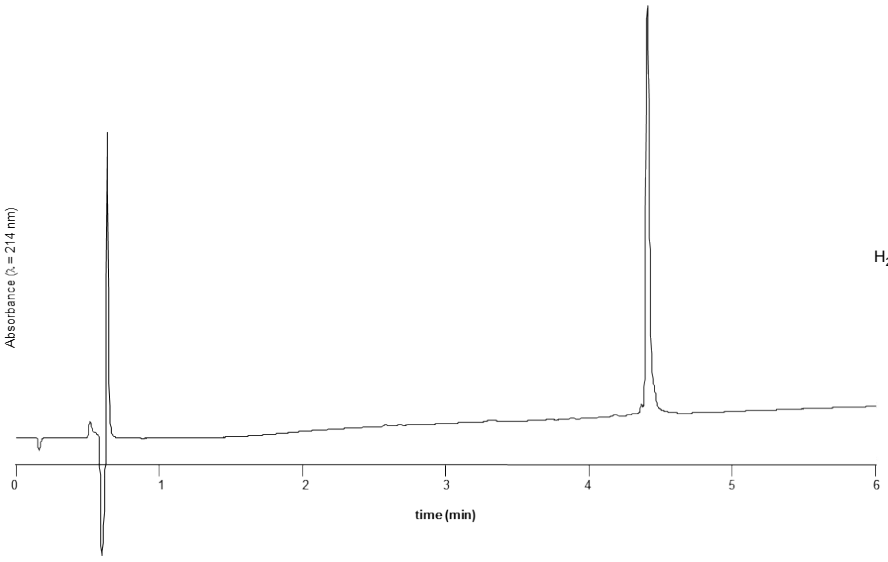
e) Cyclo-($_D$-Phe-Anl-Arg-Gly-Asp) (6)

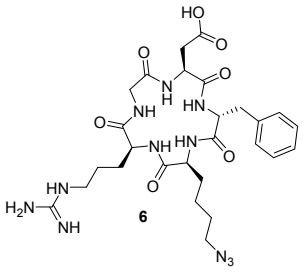
In a polypropylene syringe fitted with a polypropylene frit, Fmoc-D-Phe-Anl-Arg(Pbf)-Gly-Asp(O-Wang resin)-OH (**S4**, assumed 0.736 mmol) was swollen in DMF (10 mL, 2 × 1 min). The Fmoc group was deprotected using 20% piperidine in DMF (3 × 3 min), followed by extensive washes with DMF then 0.5% TFA in -CH_2CL_2 (100 mL, 9.15 equiv., flow wash) then immediately with CH_2Cl_2. The resin was swollen in DMF (2 × 10 mL for 1 min). The solvent was drained off thoroughly and the syringe was fitted with a plunger and a Teflon stopcock. PyAOP (3.84 g, 10 equiv.) dissolved in 10 mL DMF was transferred to the resin by suction, followed by DIEA (2.56 mL, 20 equiv.). The resulting suspension was stirred by rotation for 1.5 h at RT, followed by extensive washes with DMF then CH_2Cl_2. A Kaiser test performed on a few beads of resin indicated the absence of any unreacted amine.
The resin was then cleaved by treatment with a TFA/phenol/H_2O/TIS mixture (87.5:5:5:2.5, 15 mL) for 2h. The resin was filtered off then washed with TFA (3 x 3 mL). The combined supernatants were poured into ice-cold Et_2O (300 mL). The white precipitate was isolated by filtration over a sintered glass funnel, then washed with Et_2O (3 × 100 mL) and dried under reduced pressure to give a white solid.



Peak #	t_R (min)	Area %	Observed *m/z*	Attributed to	Calculated *m/z*
1	21.48	4.3	587.30	**6** - 43 Da	No structure attributed
2	22.79	90.1	630.30	**6**	630.30 ($C_{27}H_{40}N_{11}O_7$)
3	28.87	4.6	1259.60	cyclic dimer	1259.61 ($C_{54}H_{79}N_{22}O_{14}$)

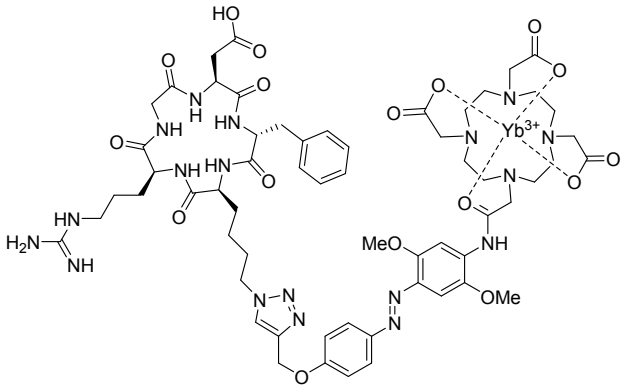
The crude mixture was purified by RP-HPLC to give the desired azido-cyclopeptide **6** as a white powder (288 mg, 43 % over 13 steps, based on initial resin loading, and taking into account a trifluoroacetate counter ion but not any eventual residual water).
HPLC trace of the pure product **6** (Chromolith column HR RP-18e, 150 Å, 10 × 4.6 mm, 3 mL/min flow rate, gradient 5 to 50% B over 5 min):





2.7. Click reaction with azido-containing cRGD derivative : 7

a) Copper catalyzed azide/alkyne cycloaddition providing the 2,2',2''-(10-(2-(4-((E)-(4-((1-(4-((2S,5S,11S,14R)-14-benzyl-11-(carboxymethyl)-5-(3-guanidinopropyl)-3,6,9,12,15-pentaoxo-1,4,7,10,13-pentaazacyclopentadecan-2-yl)butyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)diazenyl)-2,5-dimethoxyphenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate ytterbium(III) complex (7-Yb)

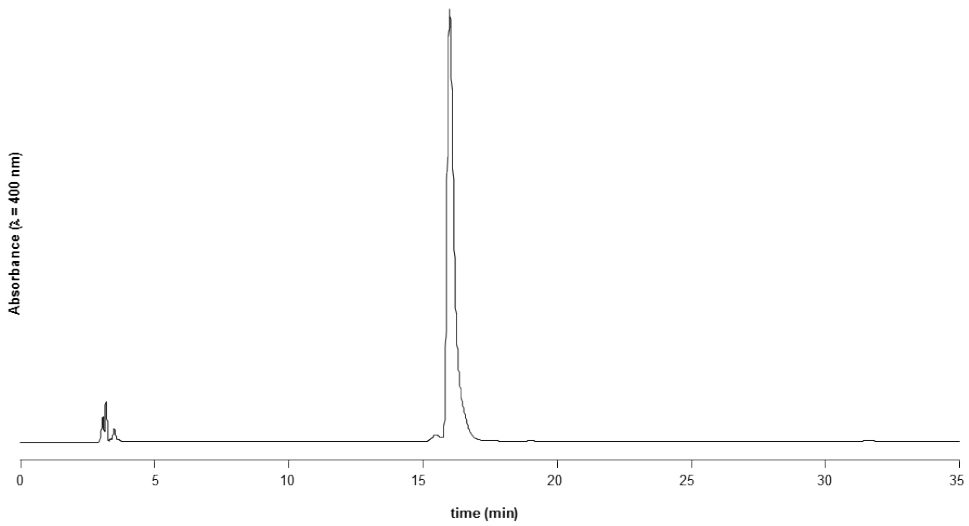


Solution **A**: Under an argon atmosphere, a solution of complex **5d-Yb** (15.0 mg, 17.90 µmol, 1.00 equiv.) in H_2O (500 µL) was mixed with a solution of azido-cyclopeptide **6** (16.9 mg, 26.85 µmol, 1.50 equiv.) in HEPES buffer (500 µL, 400 mM, pH=7.5); this solution **A** was diluted with 500 µL H_2O, followed by one drop of DMF to solubilise the peptide.

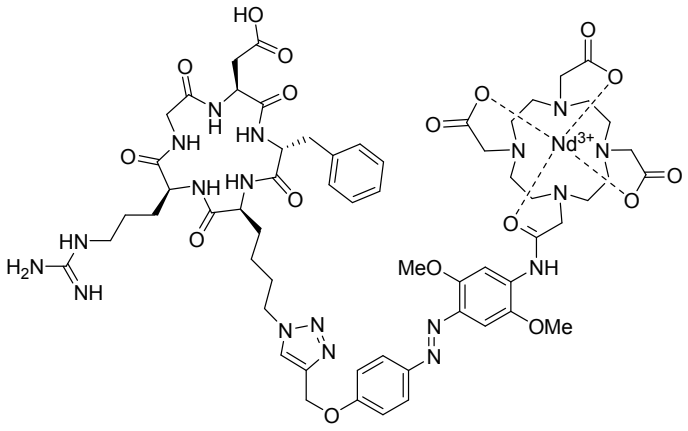
Separately, in a 1.5 mL centrifuge tube, a catalyst solution **B** was prepared: ligand THPTA (tris(3-hydroxypropyltriazolylmethyl)amine) (41.27 mg, 94.98 µmol, 5.30 equiv.) and aminoguanidine (29.7 mg, 268.5 µmol, 15.0 equiv.) were dissolved in 500 µL H_2O. A solution of $CuSO_4 \cdot 5H_2O$ (17.88 mg, 71.6 µmol, 4 equiv.) in 200 µL H_2O was then added (apparition of an intensive blue colour), followed by a solution of sodium ascorbate (27.94 mg, 143.2 µmol, 8 equiv.) in 250 µL H_2O (the solution became slightly yellow). The mixture **B** was immediately added to solution **A**.

The reaction (**A**+**B**) was vigorously stirred for 30 min at RT, followed by analytical HPLC to confirm the completion of the cycloaddition reaction, then quenched by addition of 1 mL of a 5% aqueous TFA solution. The content of the flask was centrifuged to remove the precipitate and the supernatant was purified by semi-preparative HPLC (gradient 22/78 to 37/63 A/B, for 30 min., t_R = 17.2 min.), leading to the expected complex 7-Yb (11.5 mg, 40%, taking into account one trifluoroacetate counteranion but no eventual water content). HRMS (ESI) *m*/*z*: $[M+2H]^{2+}$ calculated for $C_{60}H_{81}N_{18}O_{17}Yb$ 749.7708, found 749.7704.

HPLC trace of the pure product (gradient 20 to 70% B over 30 min):



b) Copper catalyzed azide/alkyne cycloaddition providing the 2,2',2''-(10-(2-(4-((E)-(4-((1-(4-((2S,5S,11S,14R)-14-benzyl-11-(carboxymethyl)-5-(3-guanidinopropyl)-3,6,9,12,15-pentaoxo-1,4,7,10,13-pentaazacyclopentadecan-2-yl)butyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)diazenyl)-2,5-dimethoxyphenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate neodymium(III) complex (7-Nd)

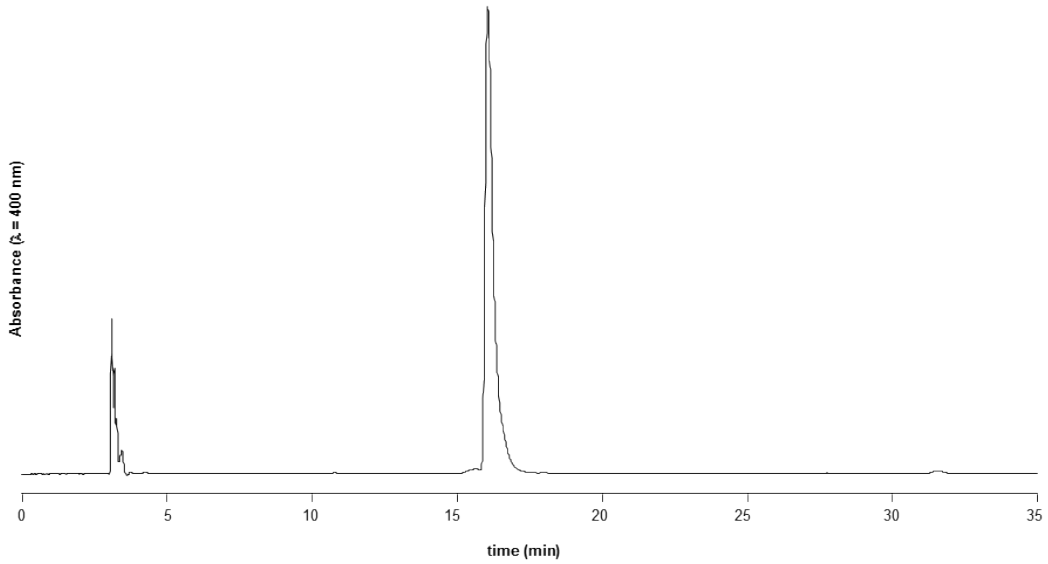


Solution **A**: Under an argon atmosphere, a solution of complex **5d-Nd** (15.0 mg, 17.29 µmol, 1.00 equiv.) in H_2O (500µL) was mixed with a solution of azido-cyclopeptide **6** (16.3 mg, 25.94 µmol, 1.50 equiv.) in HEPES buffer (500µL, 100 mM, pH=7.5).

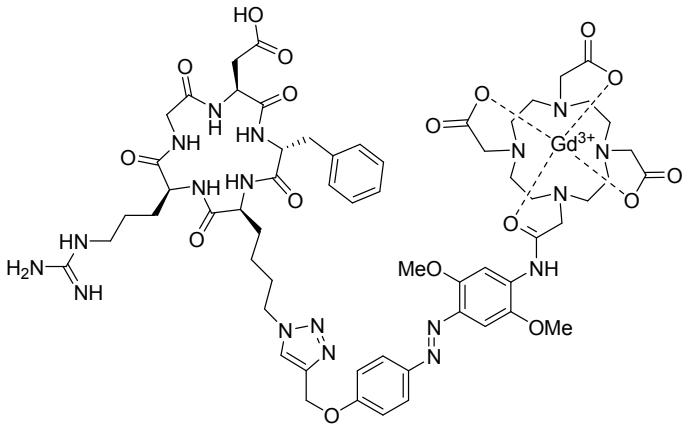
Separately in a 1.5 mL centrifuge tube, a catalyst solution **B** was prepared: ligand THPTA (37.3 mg, 85.85 µmol, 5.00 equiv.) and aminoguanidine (28.7 mg, 259.4 µmol, 15.00 equiv.) were dissolved in 500 µL of H_2O. A solution of $CuSO_4.5H_2O$ (17.3 mg, 69.16 µmol, 4.00 equiv.) in 200 µL H_2O was then added (apparition of an intense blue colour), followed by a solution of sodium ascorbate (27.4 mg, 138.32 µmol, 8.00 equiv.) in 250 µL H_2O (the solution became slightly yellow). The mixture **B** was immediately added to the solution **A**.

The reaction (**A**+**B**) was vigorously stirred for 30 minutes at RT, followed by analytical HPLC then quenched by addition of 1mL of a 5% aqueous TFA solution. The content of the flask was centrifuged to remove the precipitate and the supernatant was purified by semi-preparative HPLC (gradient CH_3CN/H_2O (+0.1% TFA) 22/78 to 37/63, 3mL/min., 30 min., t_R=17.2 min.) leading to the expected complex **7-Nd** (40% yield, taking into account one trifluoroacetate counter anion but no eventual water content). HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{60}H_{81}N_{18}NdO_{17}$ 733.7547, found 733.7550.

HPLC trace of the pure product (gradient 20 to 70% B over 30 min):



c) Copper catalyzed azide/alkyne cycloaddition providing the 2,2',2''-(10-(2-(4-((E)-(4-((1-(4-((2S,5S,11S,14R)-14-benzyl-11-(carboxymethyl)-5-(3-guanidinopropyl)-3,6,9,12,15-pentaoxo-1,4,7,10,13-pentaazacyclopentadecan-2-yl)butyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)diazenyl)-2,5-dimethoxyphenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate gadolinium(III) complex (7-Gd)



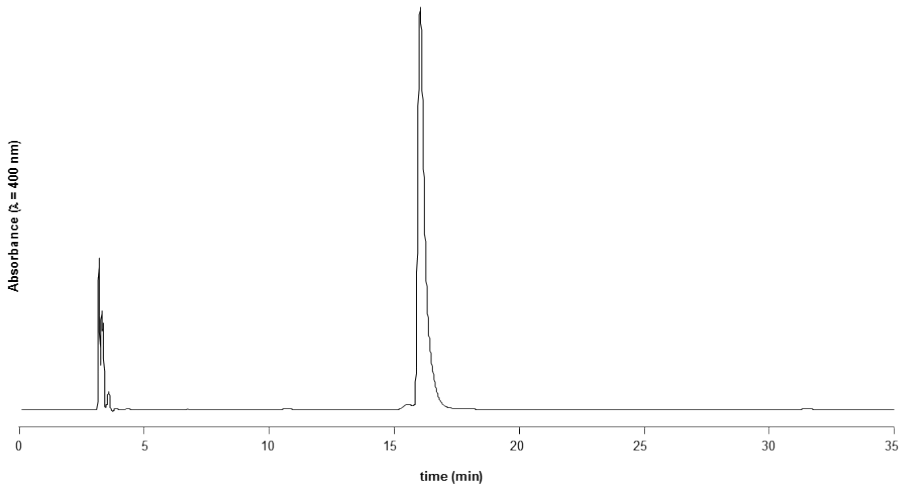
Solution **A**: A solution of complex **5d-Gd** (18.0 mg, 21.55 µmol, 1.00 equiv.) in H_2O (500µL) was mixed with a solution of azido-cyclopeptide **6** (20.3 mg, 32.32 µmol, 1.50 equiv.) in HEPES buffer (500µL, 100 mM, pH=7.5).

Separately in a 1,5 mL centrifuge tube, a catalyst solution **B** was prepared: ligand THPTA (46.8 mg, 107.75 µmol, 5.00 equiv.) and aminoguanidine (35.7 mg, 323.25 µmol, 15.00 equiv.) were dissolved in 500 µL of H_2O. A solution of $CuSO_4.5H_2O$ (21.5 mg, 86.2 µmol, 4.00 equiv.) in 200 µL of H_2O was then added (apparition of an intensive blue colour), followed by a solution of sodium ascorbate (34.2 mg, 172.4 µmol, 8.00 equiv.) in 250 µL of H_2O (the solution became slightly yellow). The mixture **B** was immediately added to the solution **A**.

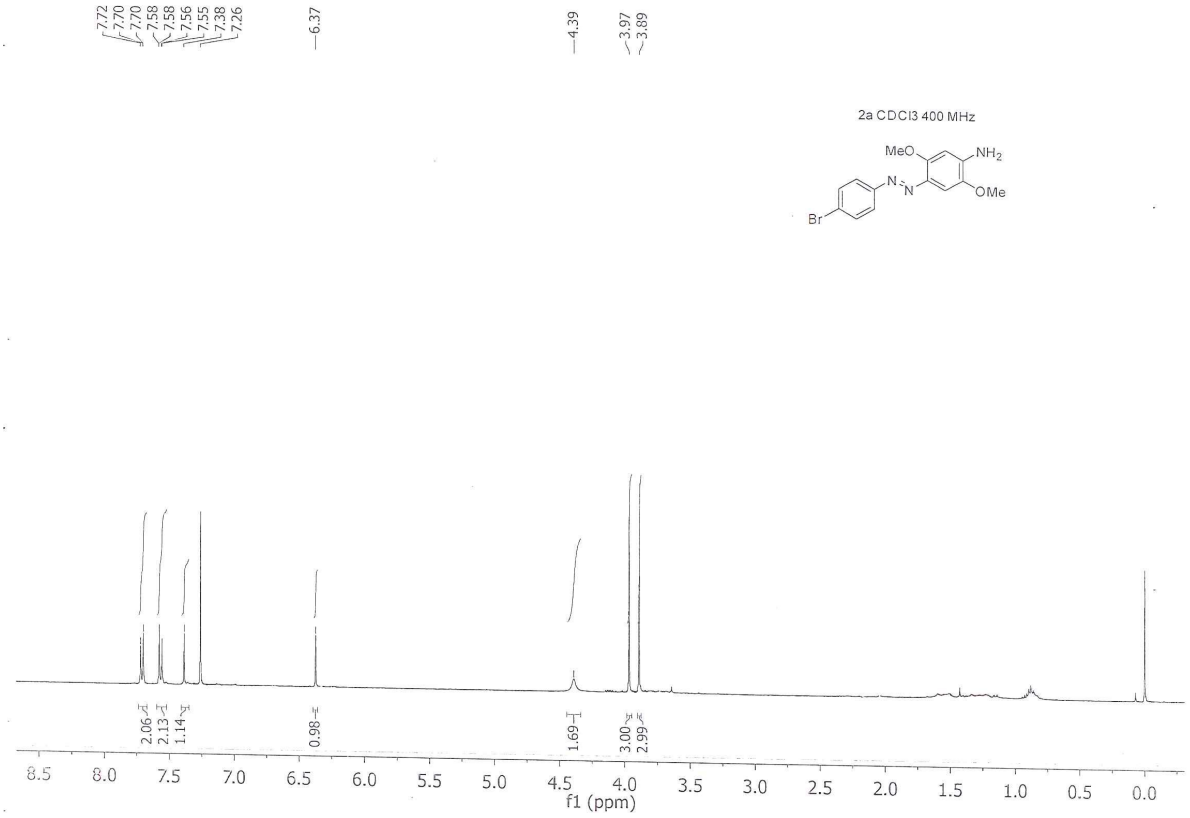
The reaction (**A**+**B**) was vigorously stirred for 30 minutes at RT, followed by analytical HPLC then quenched by addition of 1mL of an aqueous 5% TFA. The content of the flask was centrifuged to remove the precipitate and the supernatant was purified by semi-preparative HPLC (Nucleosil, RP-C18, gradient CH_3CN/H_2O (+0.1% TFA) 22/78 to 37/63, 3mL/min., 30 min., t_R = 15.8 min.) leading to the expected complex **7-Gd** (36% yield, taking into account one trifluoroacetate counteranion but

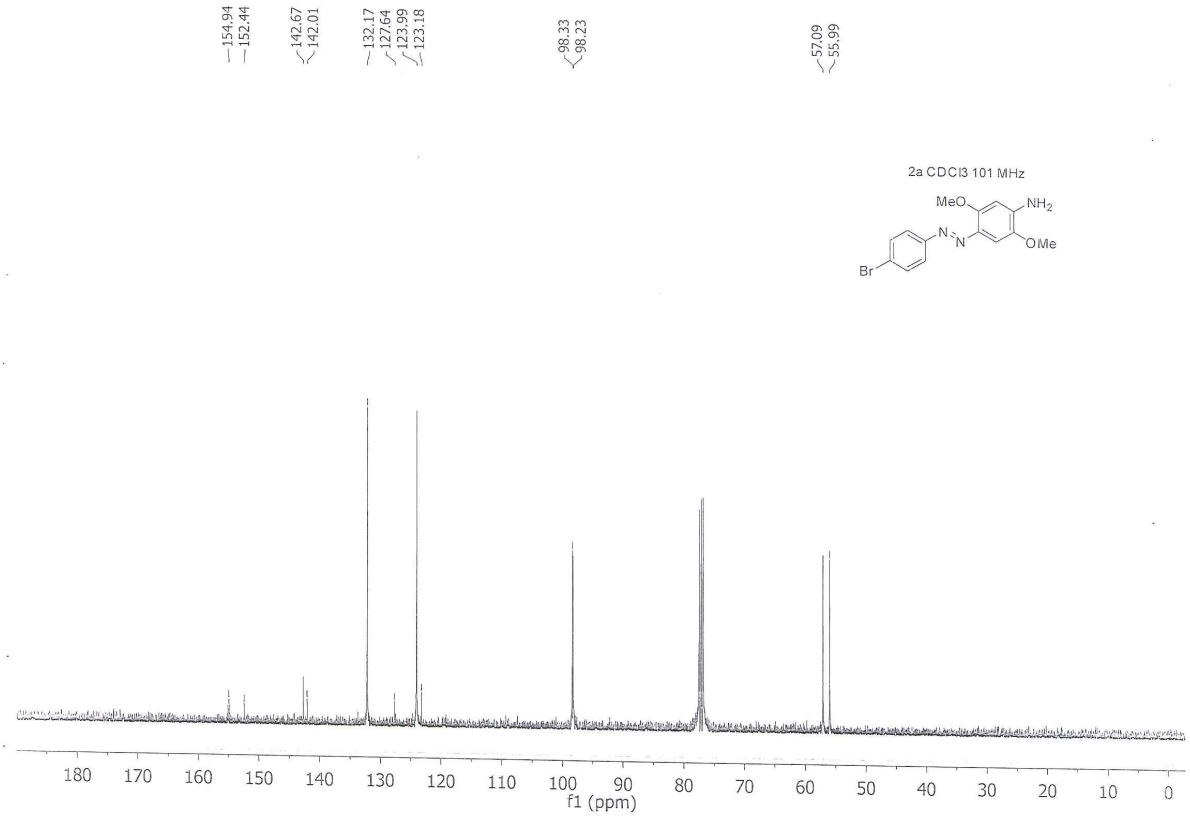
no eventual water content). HRMS (ESI) m/z: $[M]^+$ calculated for $C_{60}H_{80}GdN_{18}O_{17}$ 1482.5201, found 1482.5175.

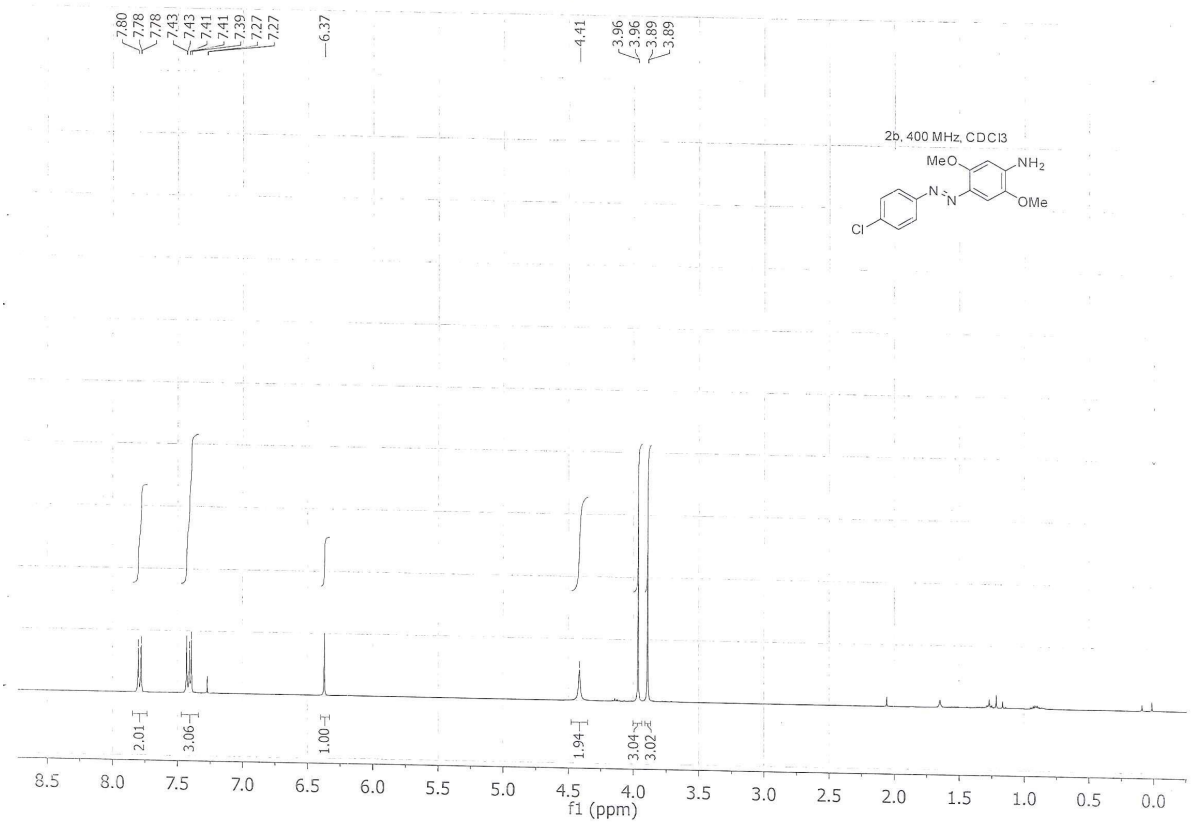
HPLC trace of the pure product (gradient 20 to 70% B over 30 min):

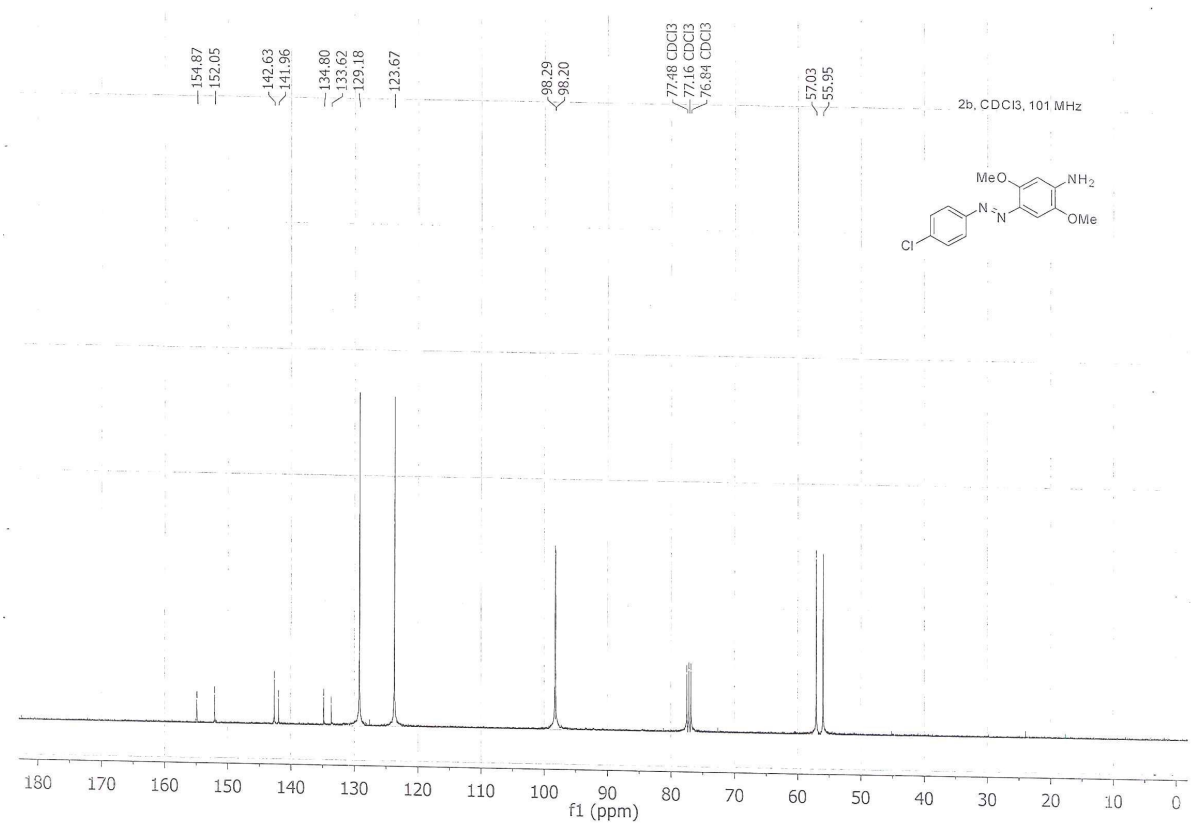


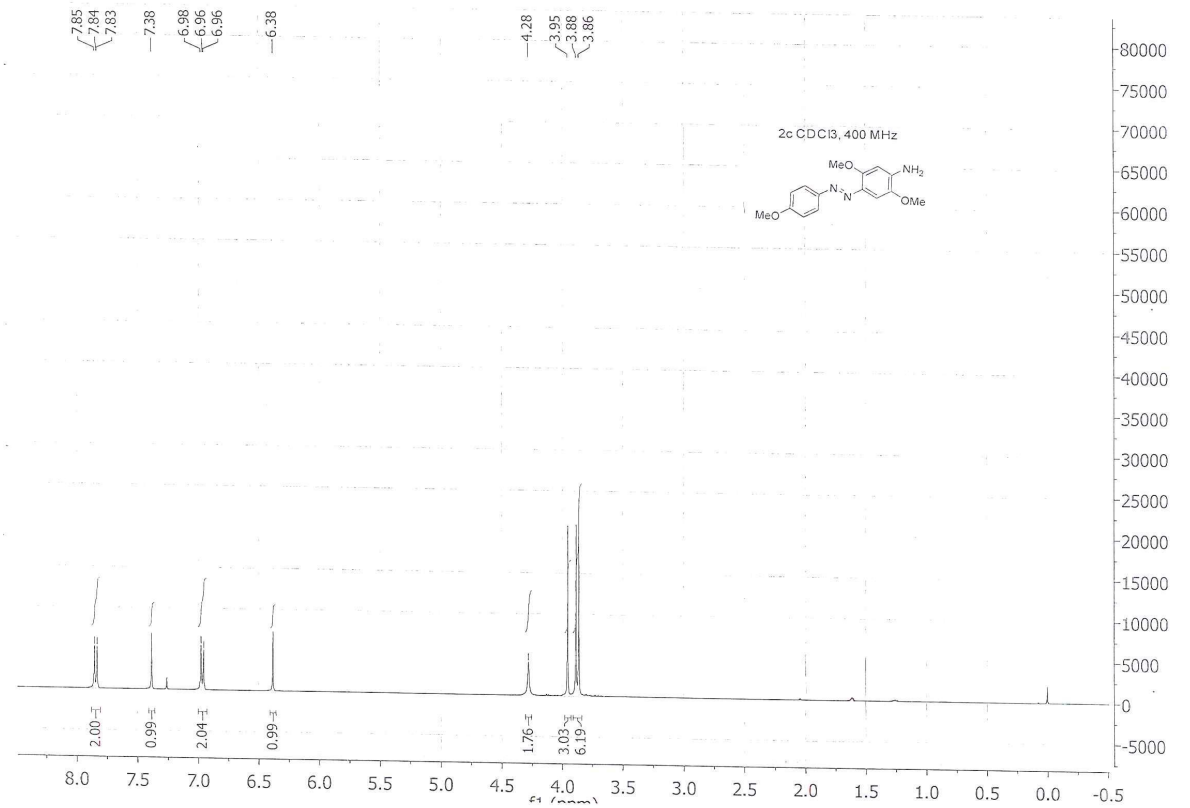
3. NMR Spectra

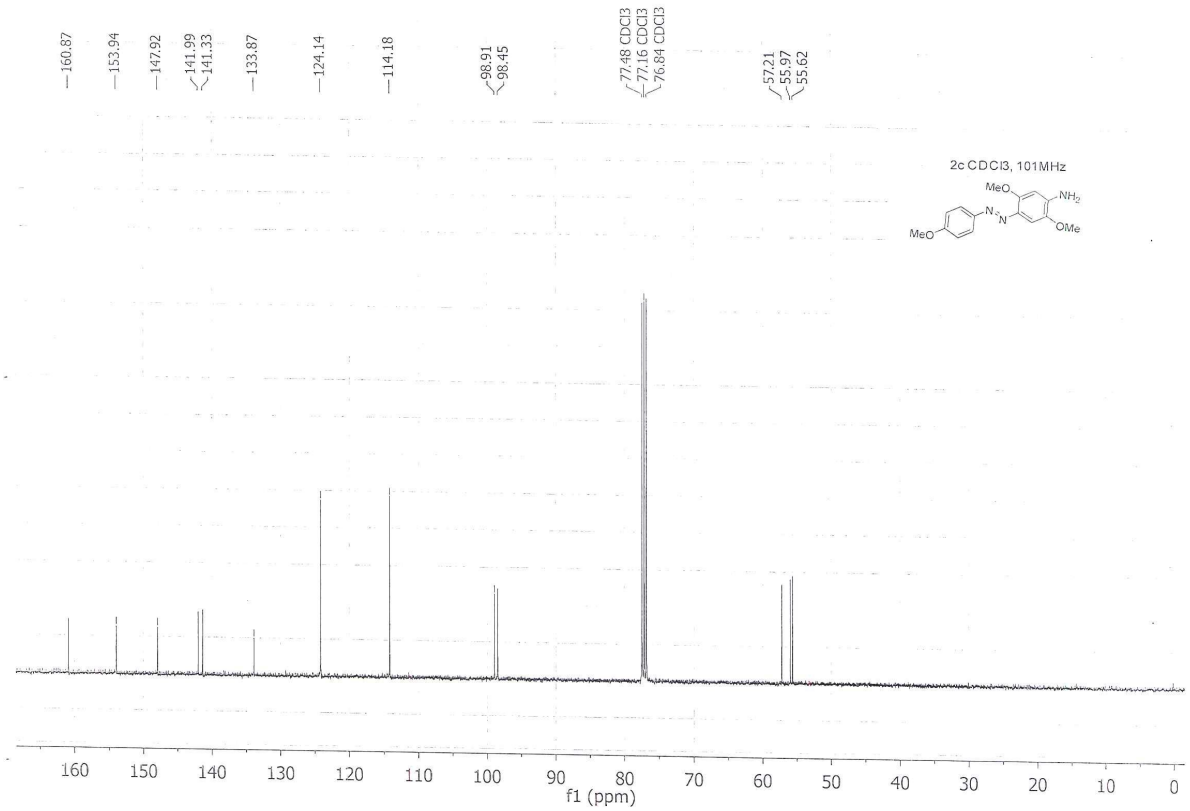


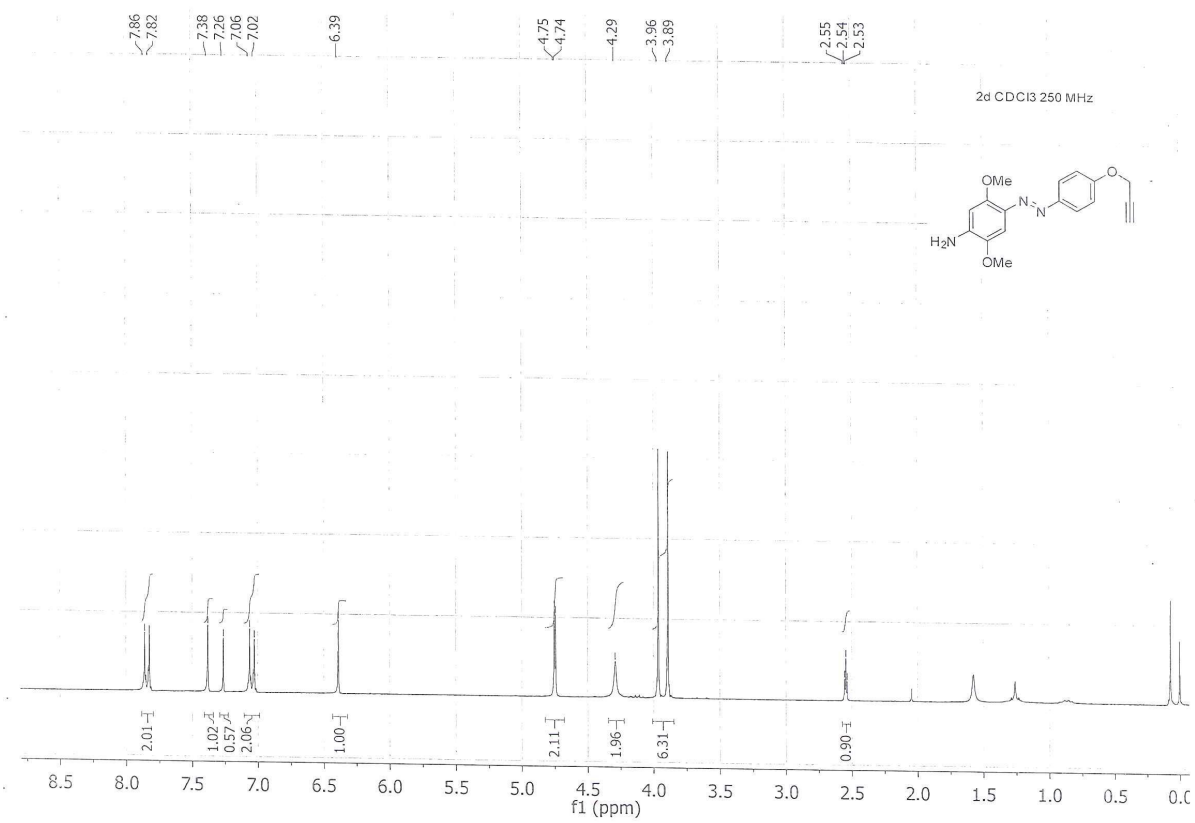


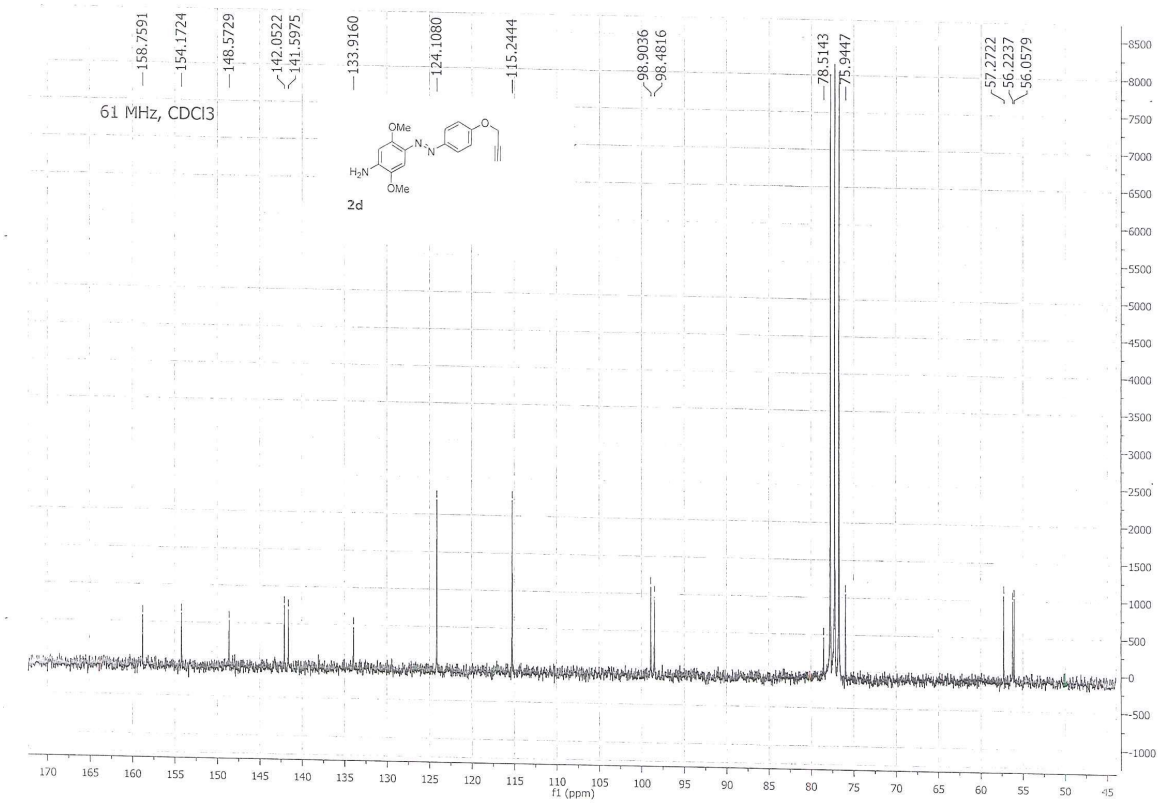

7.80
7.78
7.78
7.43
7.43
7.41
7.41
7.39
7.27
7.27
6.37
4.41
3.96
3.96
3.89
3.89
2b, 400 MHz, CDCl3
MeO
NH2
N
N
OMe
Cl
2.01
3.06
1.00
1.94
3.04
3.02
f1 (ppm)


154.87
152.05
142.63
141.96
134.80
133.62
129.18
123.67
98.29
98.20
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
57.03
55.95
2b, CDCl3, 101 MHz
MeO
NH2
N
N
OMe
Cl
f1 (ppm)


7.85
7.84
7.83
7.38
6.98
6.96
6.96
6.38
4.28
3.95
3.88
3.86
2c CDCl3, 400 MHz
MeO
NH2
OMe
MeO
2.00
0.99
2.04
0.99
1.76
3.03
6.19
f1 (ppm)


160.87
153.94
147.92
141.99
141.33
133.87
124.14
114.18
98.91
98.45
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
57.21
55.97
55.62
2c CDCl3, 101MHz
MeO
NH2
OMe
MeO
f1 (ppm)


7.86
7.82
7.38
7.26
7.06
7.02
6.39
4.75
4.74
4.29
3.96
3.89
2.55
2.54
2.53
2d CDCl3 250 MHz
OMe
N
N
O
H2N
OMe
2.01
1.02
0.57
2.06
1.00
2.11
1.96
6.31
0.90
f1 (ppm)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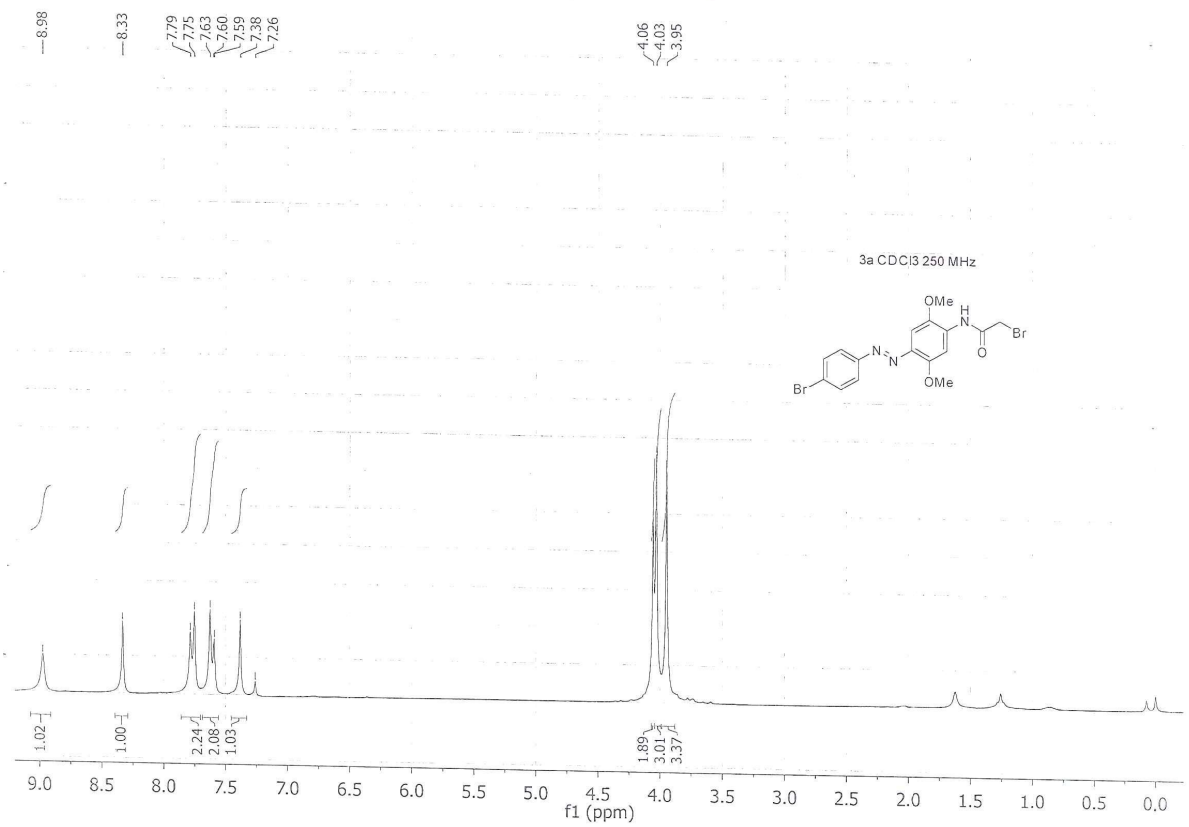
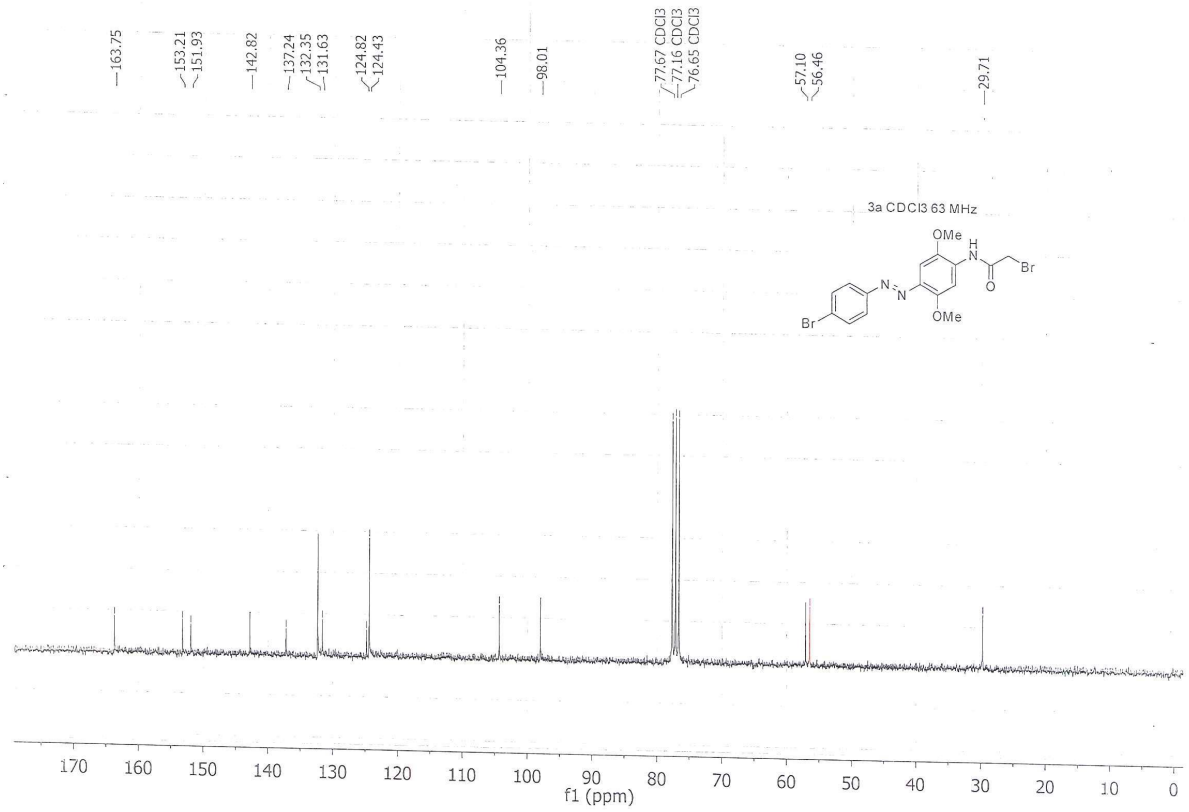
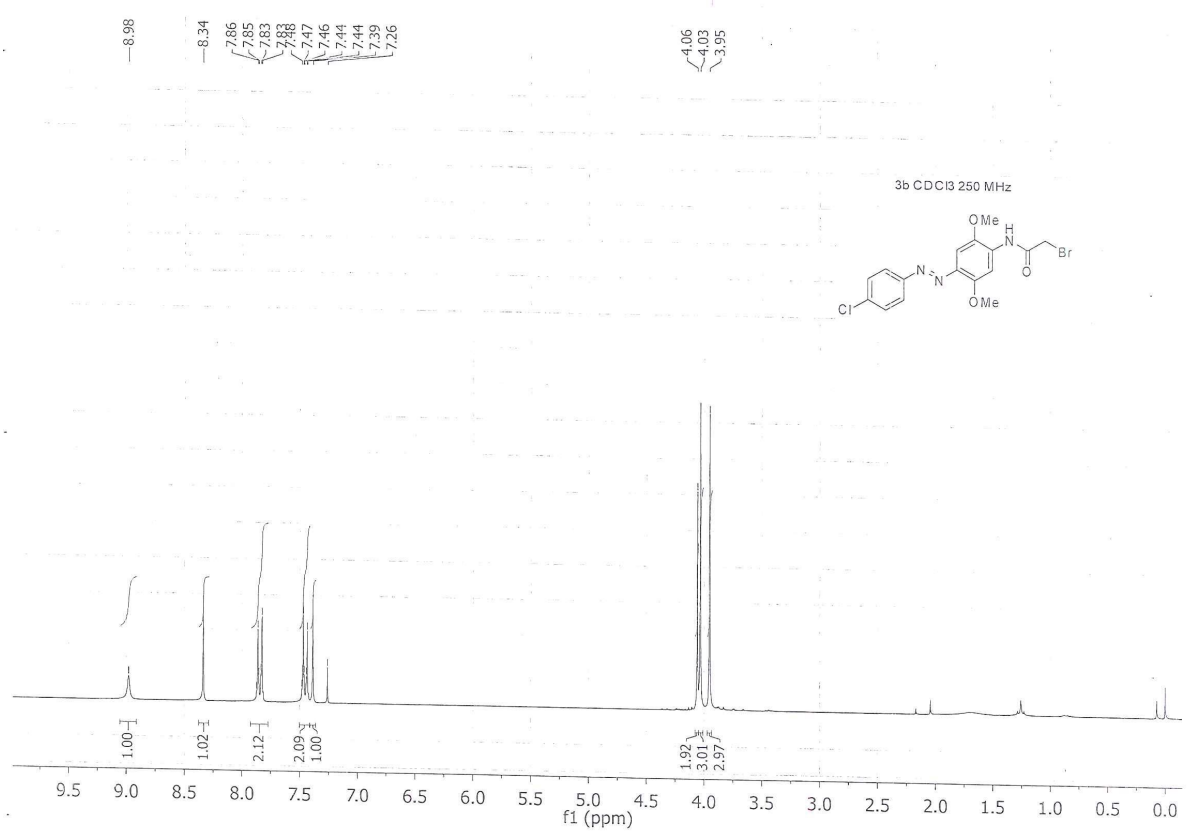
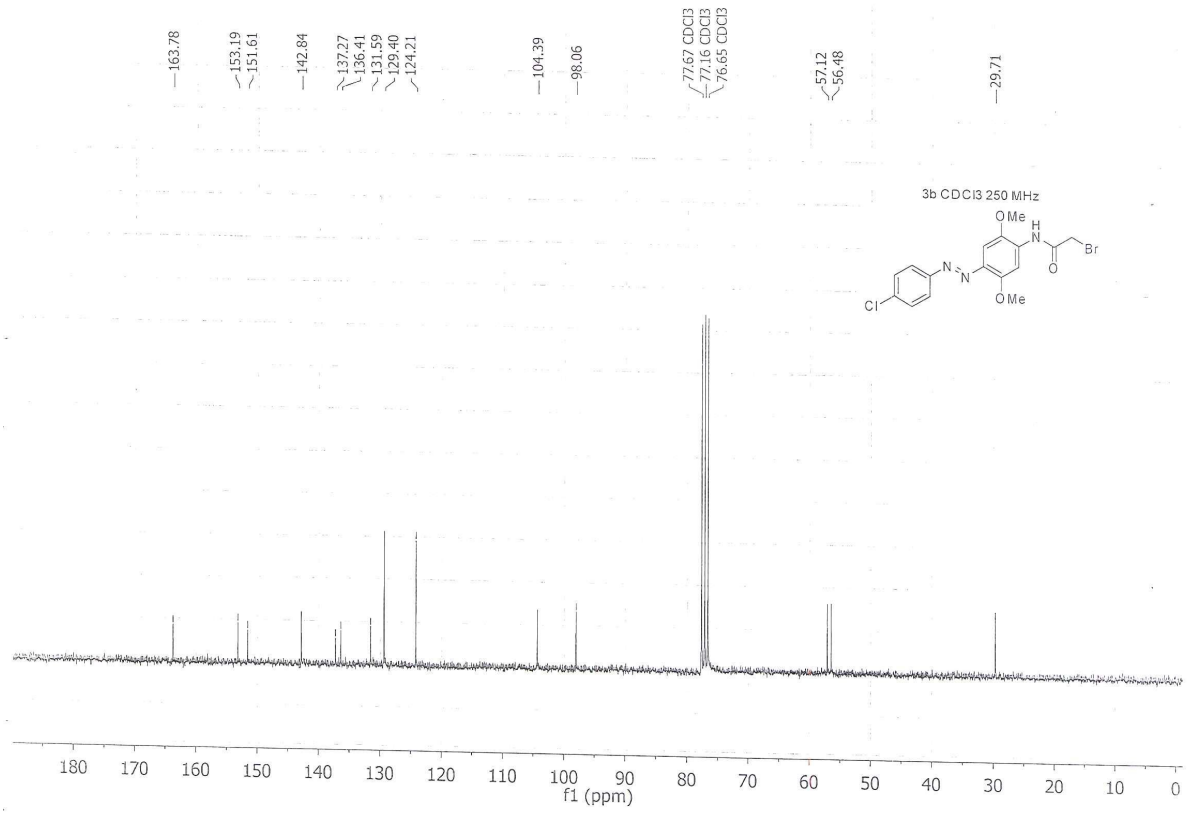
154.1724
148.5729
142.0522
141.5975
133.9160
124.1080
115.2444
98.9036
98.4816
78.5143
75.9447
57.2722
56.2237
56.0579
61 MHz, CDCl3
OMe
N
N
O
H2N
OMe
2d
f1 (ppm)


8.98
8.33
7.79
7.75
7.63
7.60
7.59
7.38
7.26
4.06
4.03
3.95
3a CDCl3 250 MHz
OMe
N
H
Br
N
N
O
Br
OMe
1.02
1.00
2.24
2.08
1.03
1.89
3.01
3.37
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


163.75
153.21
151.93
142.82
137.24
132.35
131.63
124.82
124.43
104.36
98.01
77.67 CDCl3
77.16 CDCl3
76.65 CDCl3
57.10
56.46
29.71
3a CDCl3 63 MHz
OMe
N
H
Br
N
N
O
Br
OMe
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


8.98
8.34
7.86
7.85
7.83
7.48
7.47
7.46
7.44
7.44
7.39
7.26
4.06
4.03
3.95
3b CDCl3 250 MHz
OMe
H
N
Br
N
N
O
Cl
OMe
1.00
1.02
2.12
2.09
1.00
1.92
3.01
2.97
f1 (ppm)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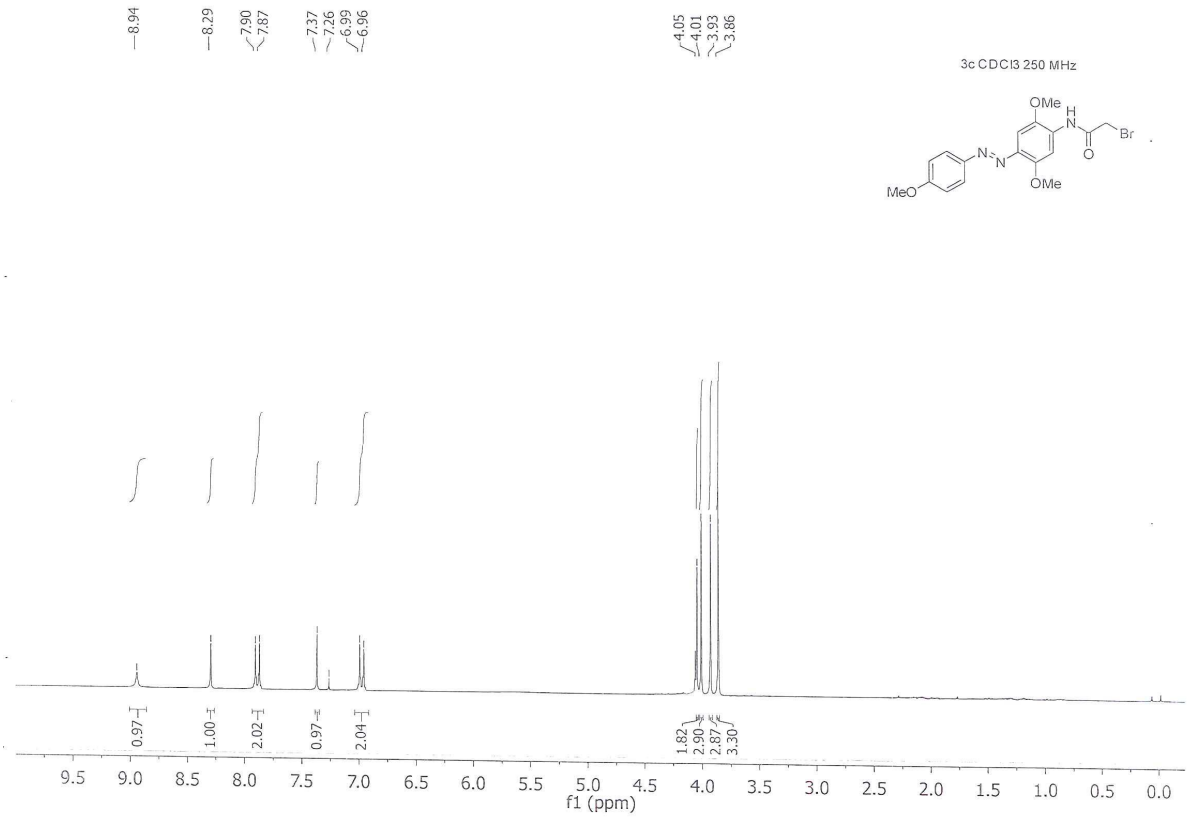
153.19
151.61
142.84
137.27
136.41
131.59
129.40
124.21
104.39
98.06
77.67 CDCl3
77.16 CDCl3
76.65 CDCl3
57.12
56.48
29.71
3b CDCl3 250 MHz
OMe
H
N
Br
N
N
O
Cl
OMe
f1 (ppm)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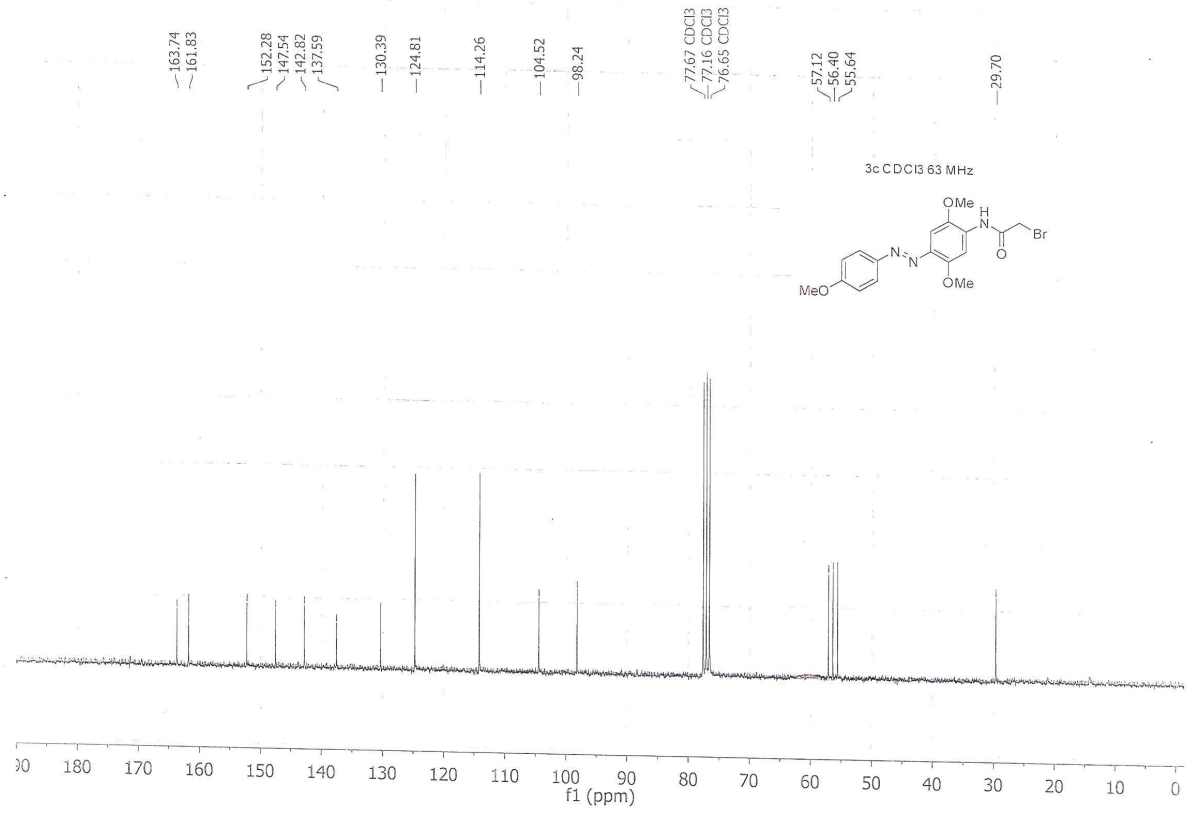
8.29
7.90
7.87
7.37
7.26
6.99
6.96
4.05
4.01
3.93
3.86
3c CDCl3 250 MHz
OMe
H
N
Br
O
N
N
MeO
OMe
0.97
1.00
2.02
0.97
2.04
1.82
2.90
2.87
3.30
f1 (ppm)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147.54
142.82
137.59
130.39
124.81
114.26
104.52
98.24
77.67 CDCl3
77.16 CDCl3
76.65 CDCl3
57.12
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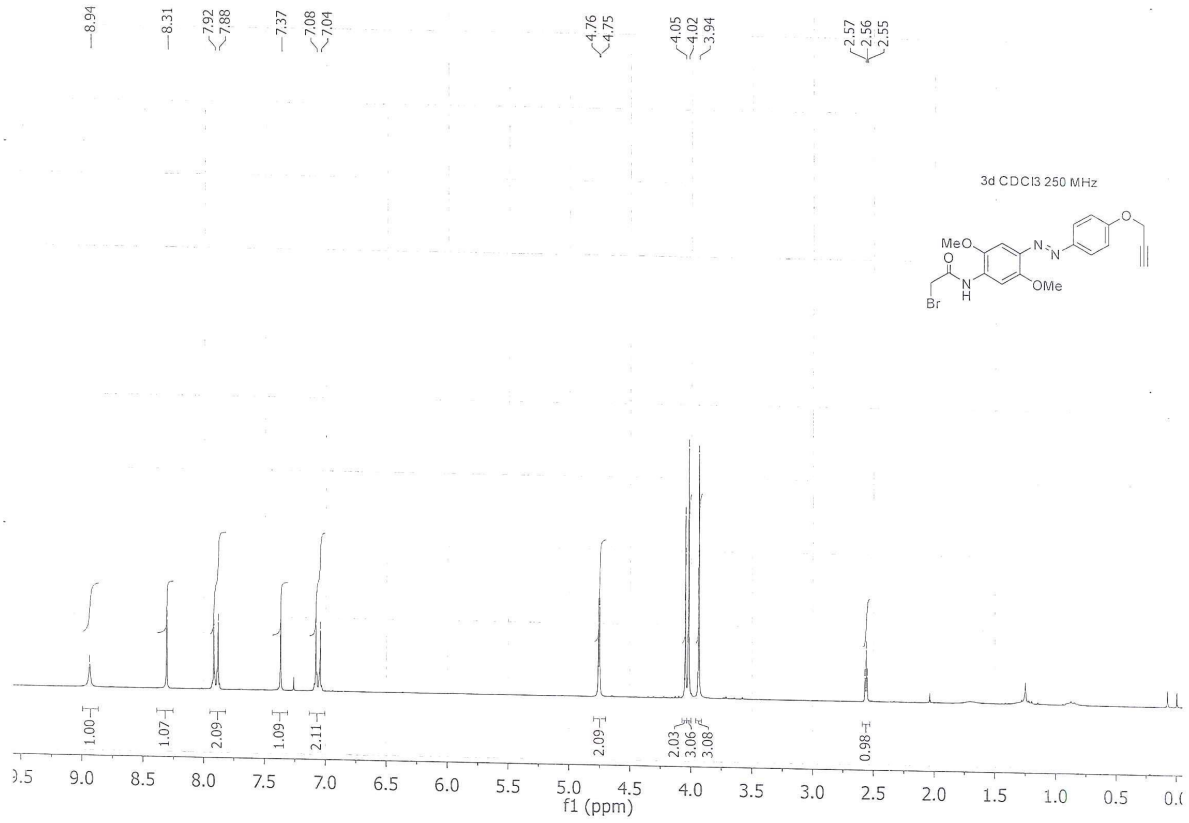
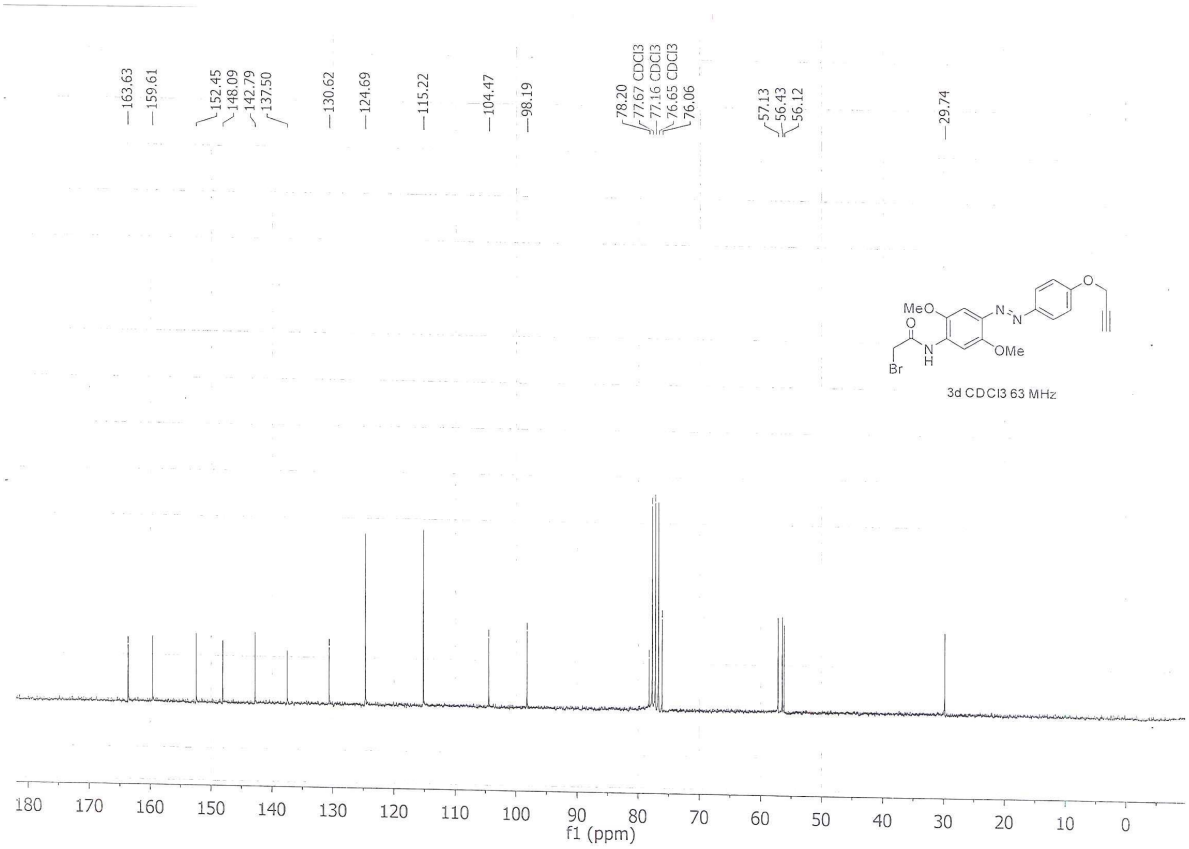
N
Br
O
N
N
MeO
OMe
f1 (ppm)


8.94
8.31
7.92
7.88
7.37
7.08
7.04
4.76
4.75
4.05
4.02
3.94
2.57
2.56
2.55
3d CDCl3 250 MHz
MeO
O
N
N
O
N
H
OMe
Br
1.00
1.07
2.09
1.09
2.11
2.09
2.03
3.06
3.08
0.98
f1 (ppm)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148.09
142.79
137.50
130.62
124.69
115.22
104.47
98.19
78.20
77.67 CDCl3
77.16 CDCl3
76.65 CDCl3
76.06
57.13
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56.12
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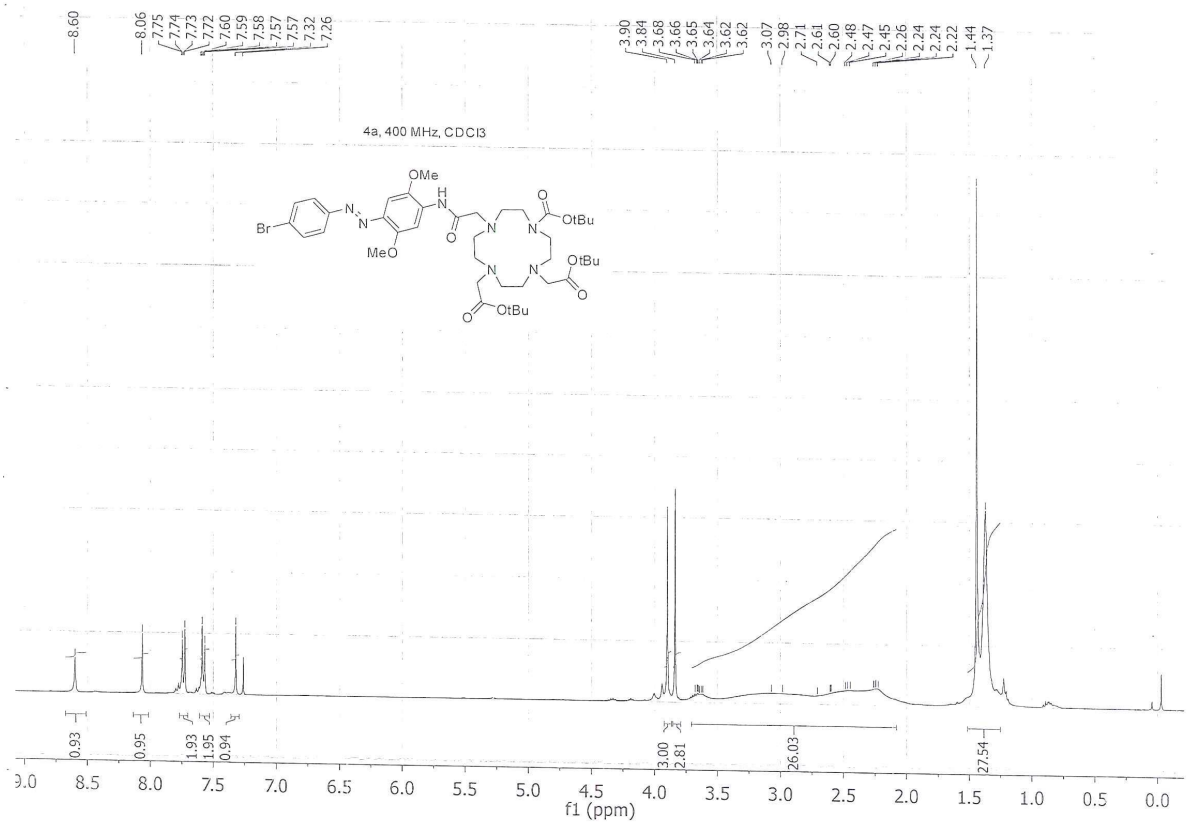
N
O
N
H
OMe
Br
3d CDCl3 63 MHz
f1 (ppm)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3.68
3.66
3.65
3.64
3.62
3.62
3.07
2.98
2.71
2.61
2.60
2.48
2.47
2.45
2.26
2.24
2.24
2.22
1.44
1.37
4a, 400 MHz, CDCl3
OMe
H
N
Br
N
N
MeO
O
OtBu
OtBu
OtBu
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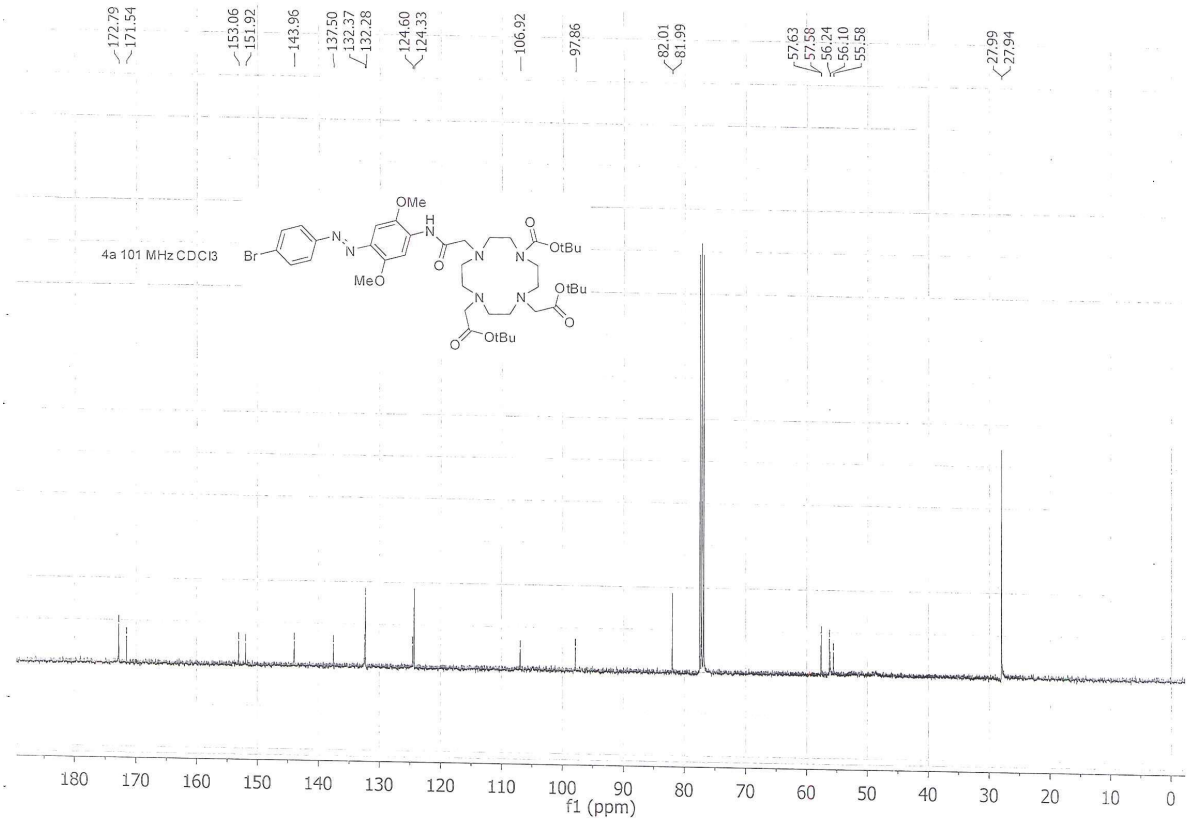
f1 (ppm)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81.99
57.63
57.58
56.24
56.10
55.58
27.99
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4a 101 MHz CDCl3
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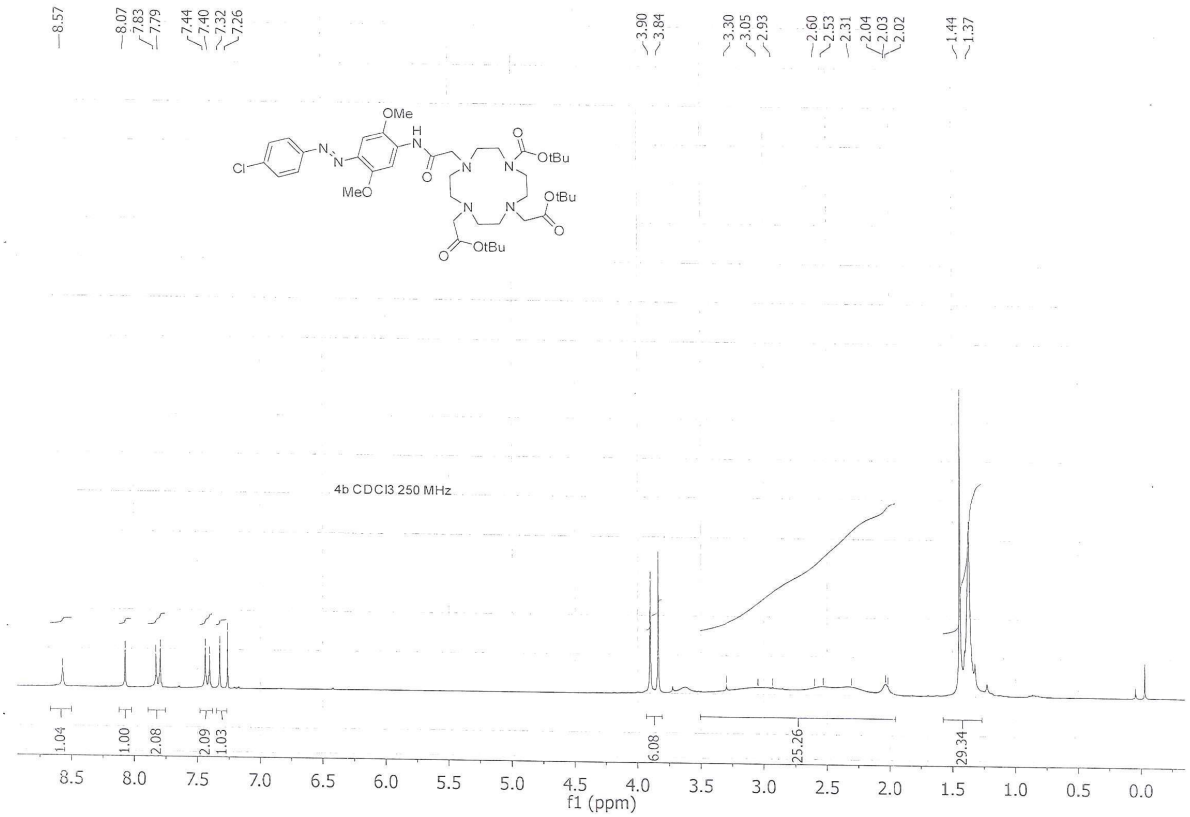
H
N
Br
N
N
MeO
O
OtBu
OtBu
OtBu
f1 (ppm)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2.60
2.53
2.31
2.04
2.03
2.02
1.44
1.37
OMe
H
N
Cl
N
N
MeO
O
N
N
N
N
OtBu
OtBu
OtBu
O
O
O
4b CDCl3 250 MHz
1.04
1.00
2.08
2.09
1.03
6.08
25.26
29.34
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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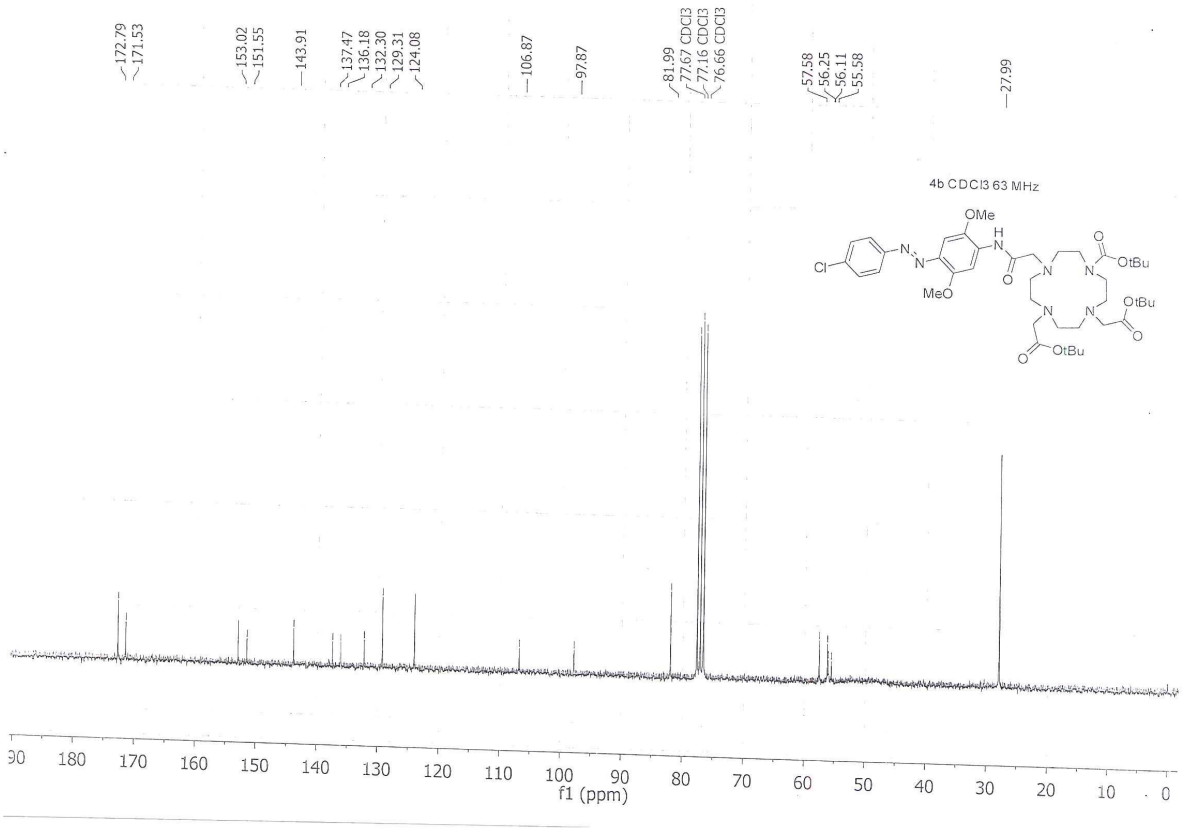
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97.87
81.99
77.67 CDCl3
77.16 CDCl3
76.66 CDCl3
57.58
56.25
56.11
55.58
27.99
4b CDCl3 63 MHz
OMe
H
N
Cl
N
N
MeO
O
N
N
N
N
OtBu
OtBu
OtBu
O
O
O
90
180
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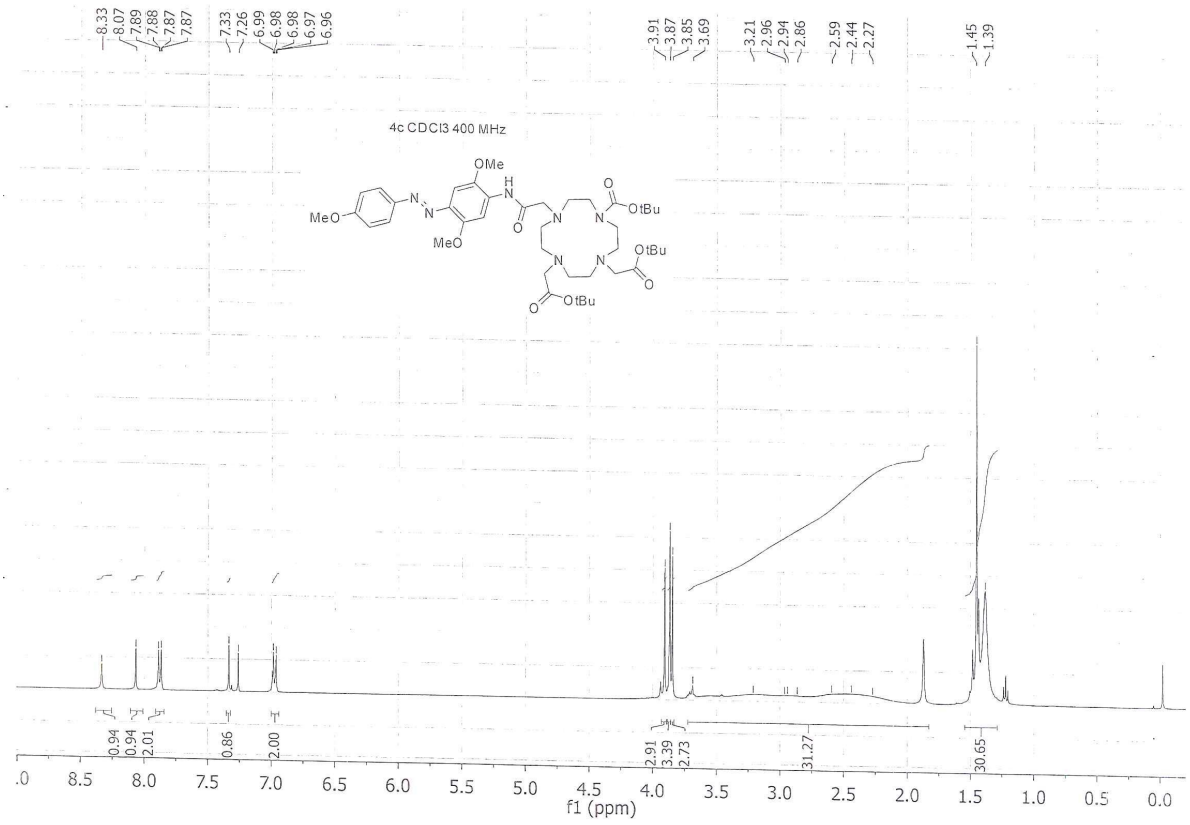
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3.91
3.87
3.85
3.69
3.21
2.96
2.94
2.86
2.59
2.44
2.27
1.45
1.39
4c CDCl3 400 MHz
OMe
MeO
MeO
OtBu
OtBu
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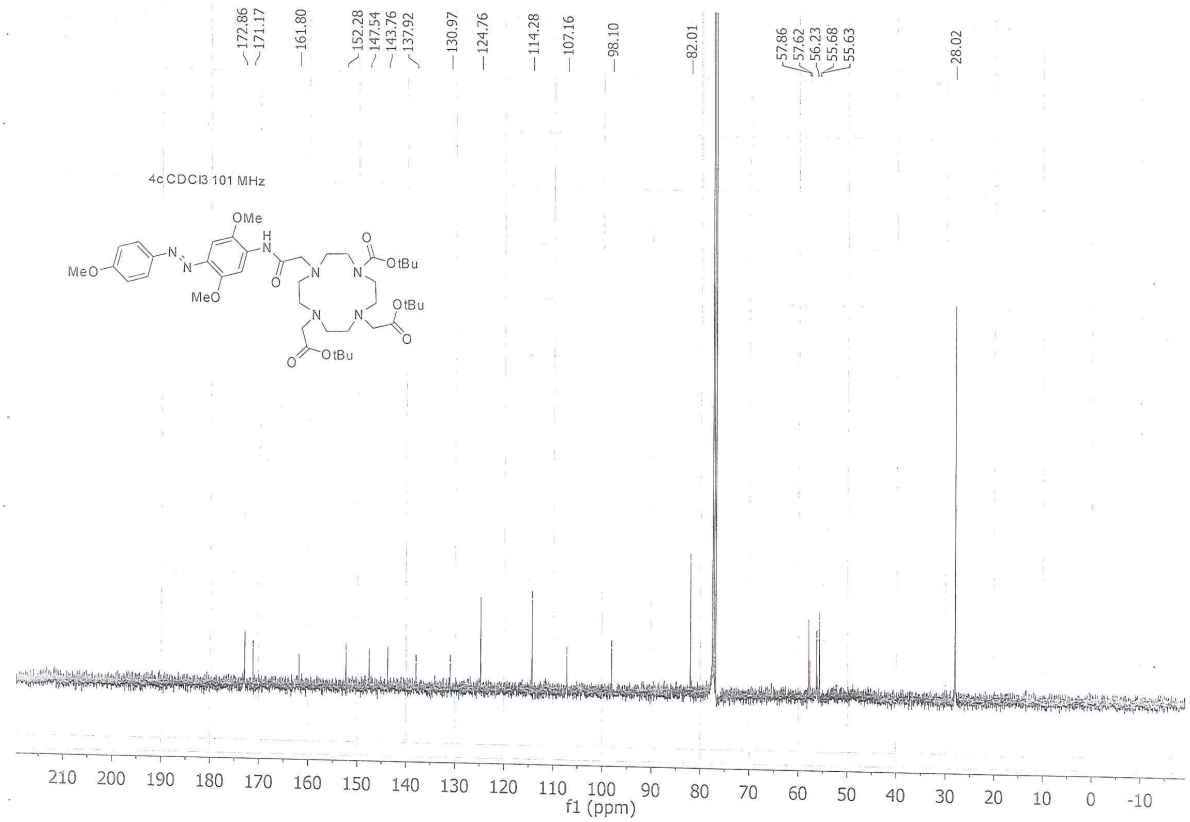
2.73
31.27
30.65
f1 (ppm)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82.01
57.86
57.62
56.23
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55.63
28.02
4c CDCl3 101 MHz
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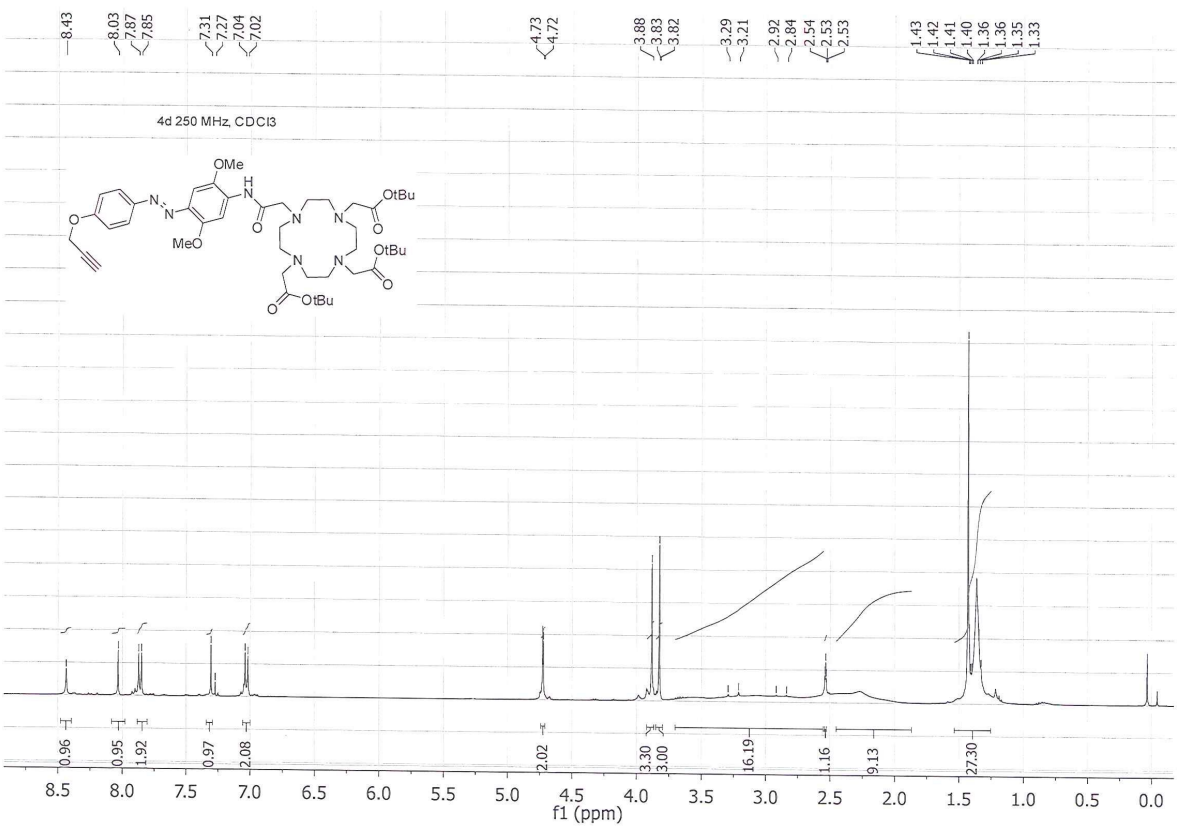
MeO
MeO
OtBu
OtBu
OtBu
f1 (ppm)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3.82
3.29
3.21
2.92
2.84
2.54
2.53
2.53
1.43
1.42
1.41
1.40
1.36
1.36
1.35
1.33
4d 250 MHz, CDCl3
OMe
MeO
OtBu
OtBu
OtBu
0.96
0.95
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2.08
2.02
3.30
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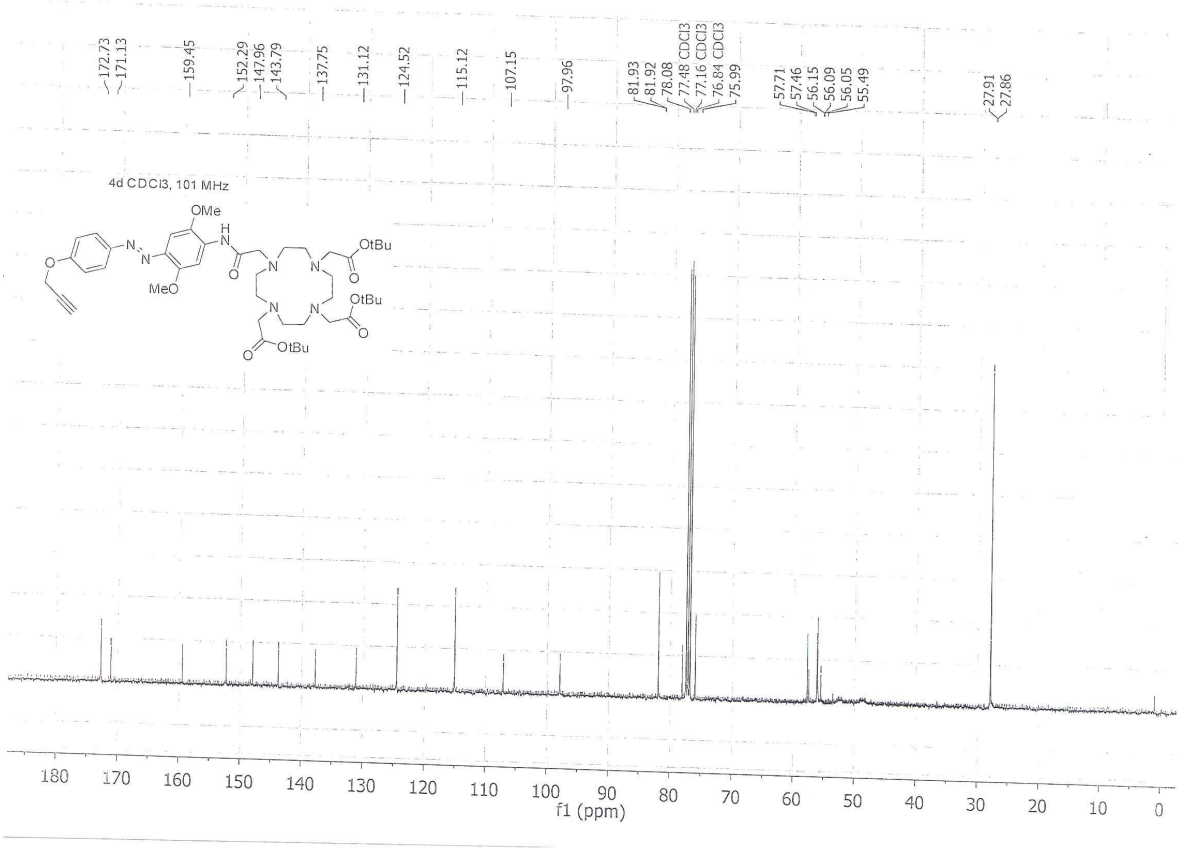
f1 (ppm)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107.15
97.96
81.93
81.92
78.08
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
75.99
57.71
57.46
56.15
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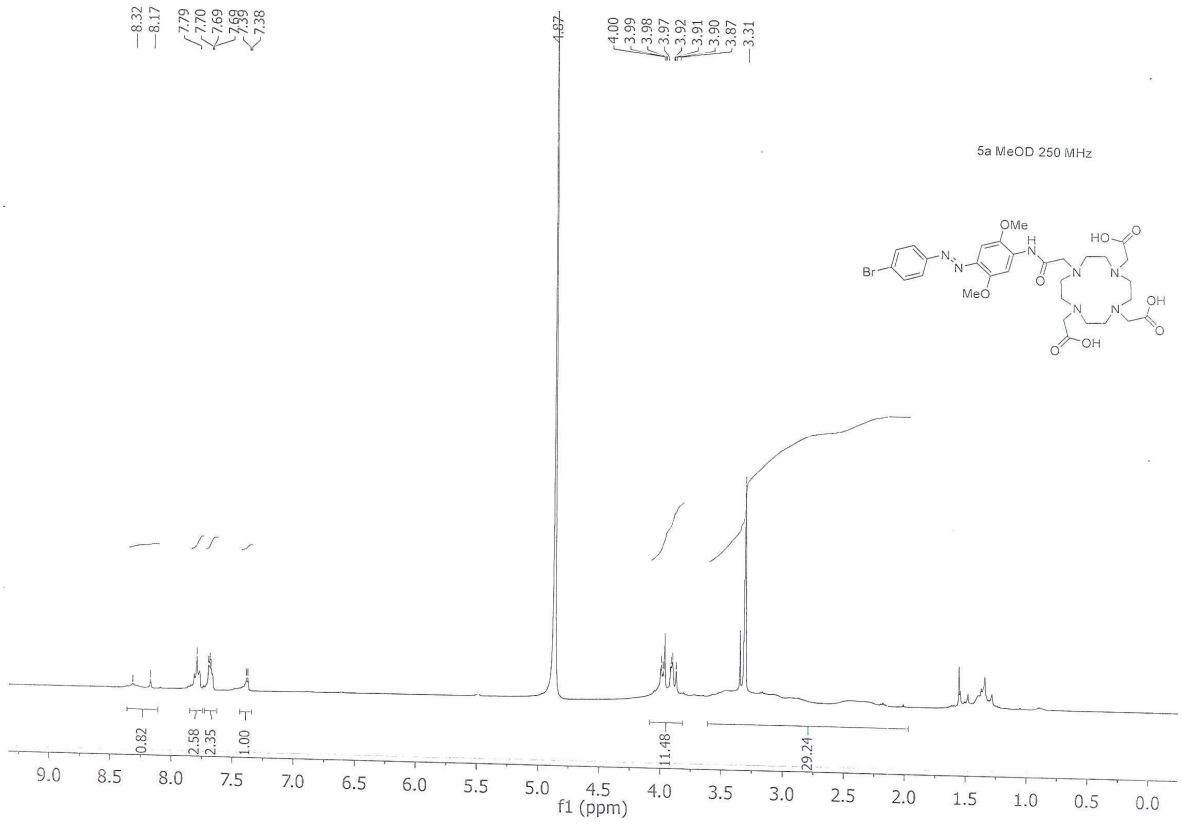
27.91
27.86
4d CDCl3, 101 MHz
OMe
MeO
OtBu
OtBu
OtBu
f1 (ppm)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3.92
3.91
3.90
3.87
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5a MeOD 250 MHz
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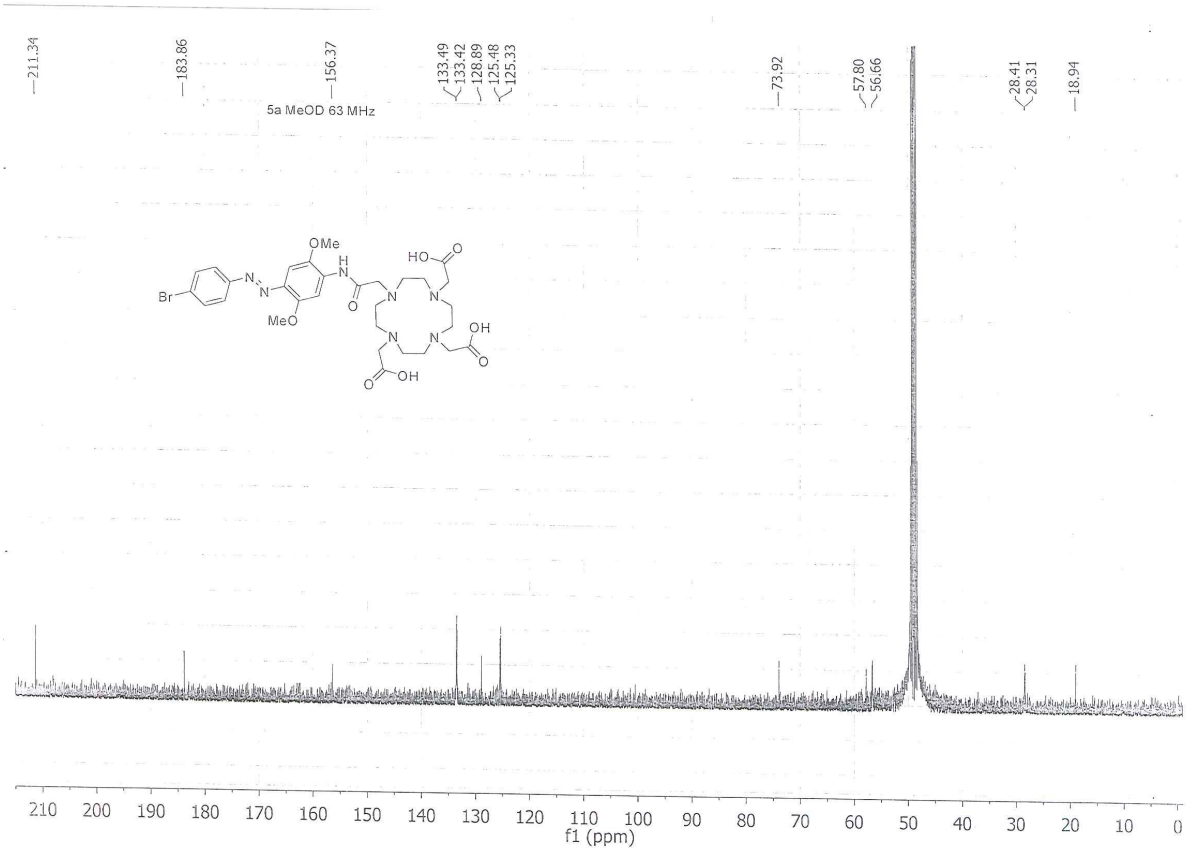
1.00
11.48
29.24
f1 (ppm)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133.42
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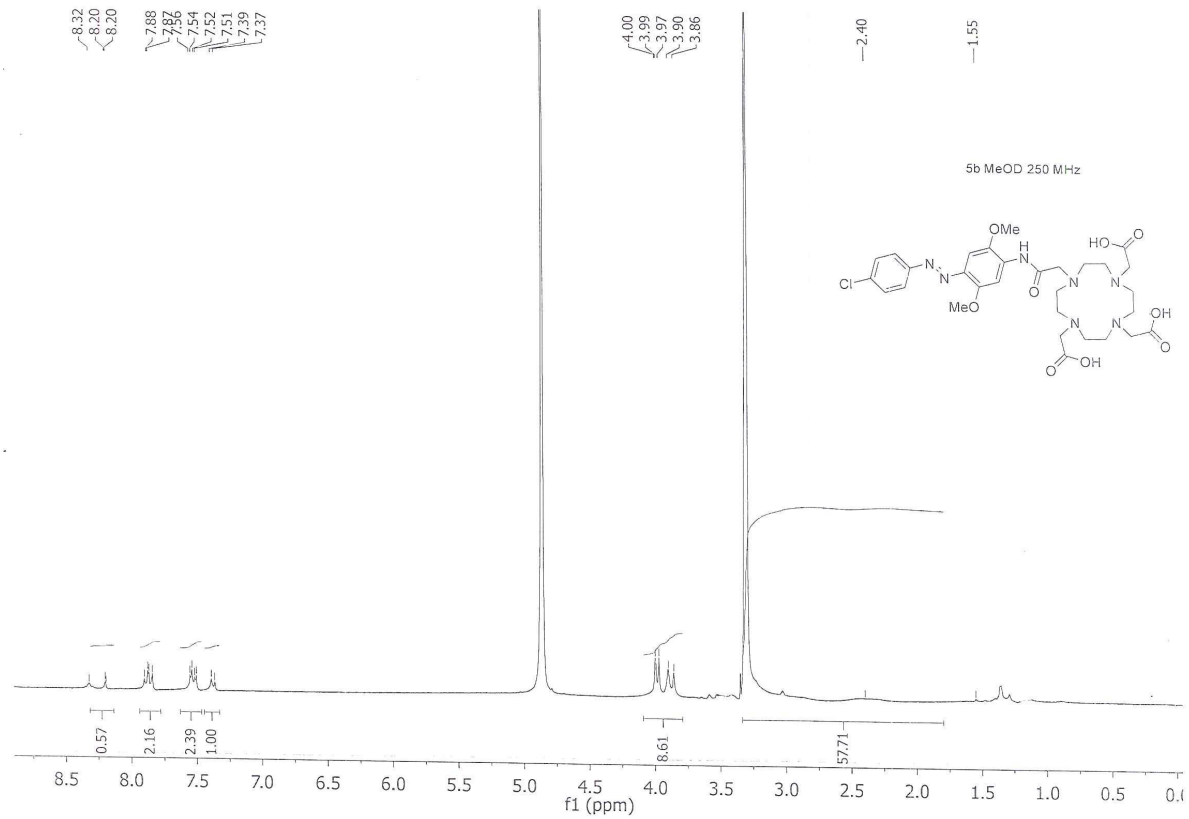
28.31
18.94
5a MeOD 63 MHz
f1 (ppm)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7.52
7.51
7.39
7.37
4.00
3.99
3.97
3.90
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5b MeOD 250 MHz
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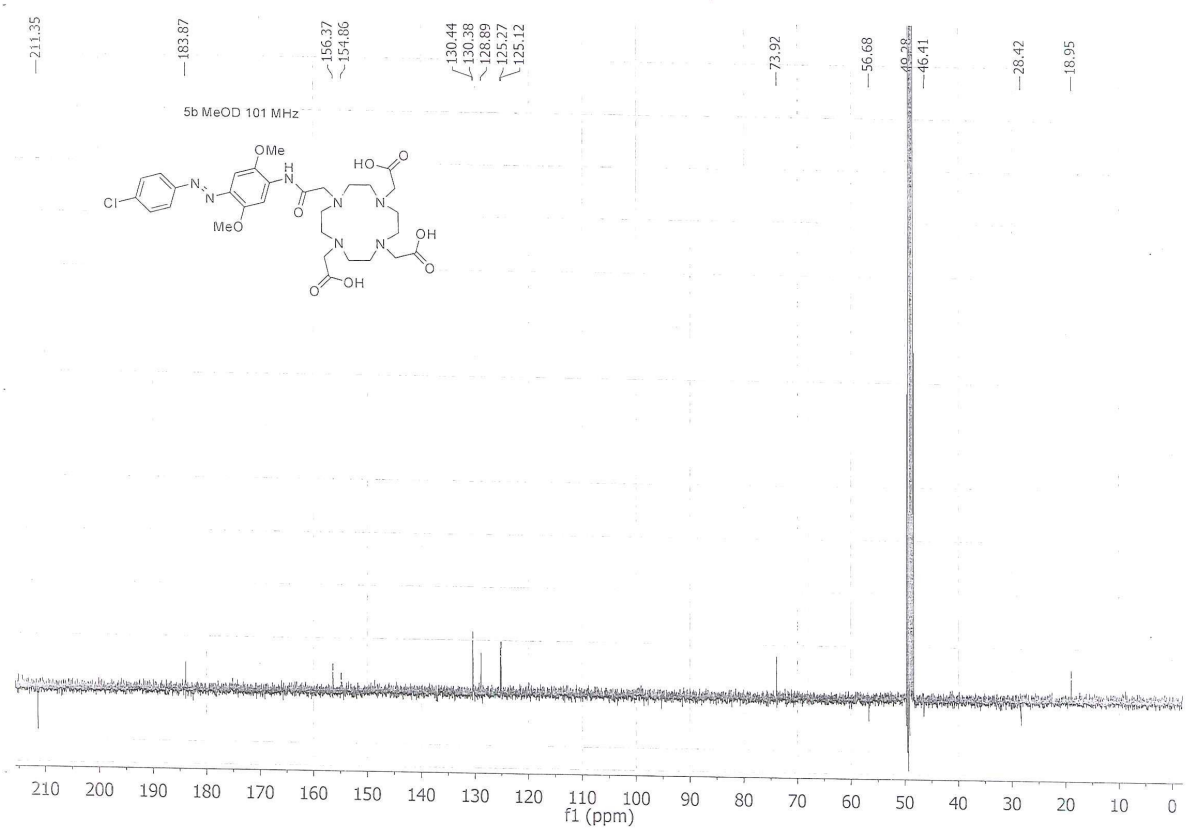
2.39
1.00
8.61
57.71
f1 (ppm)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130.38
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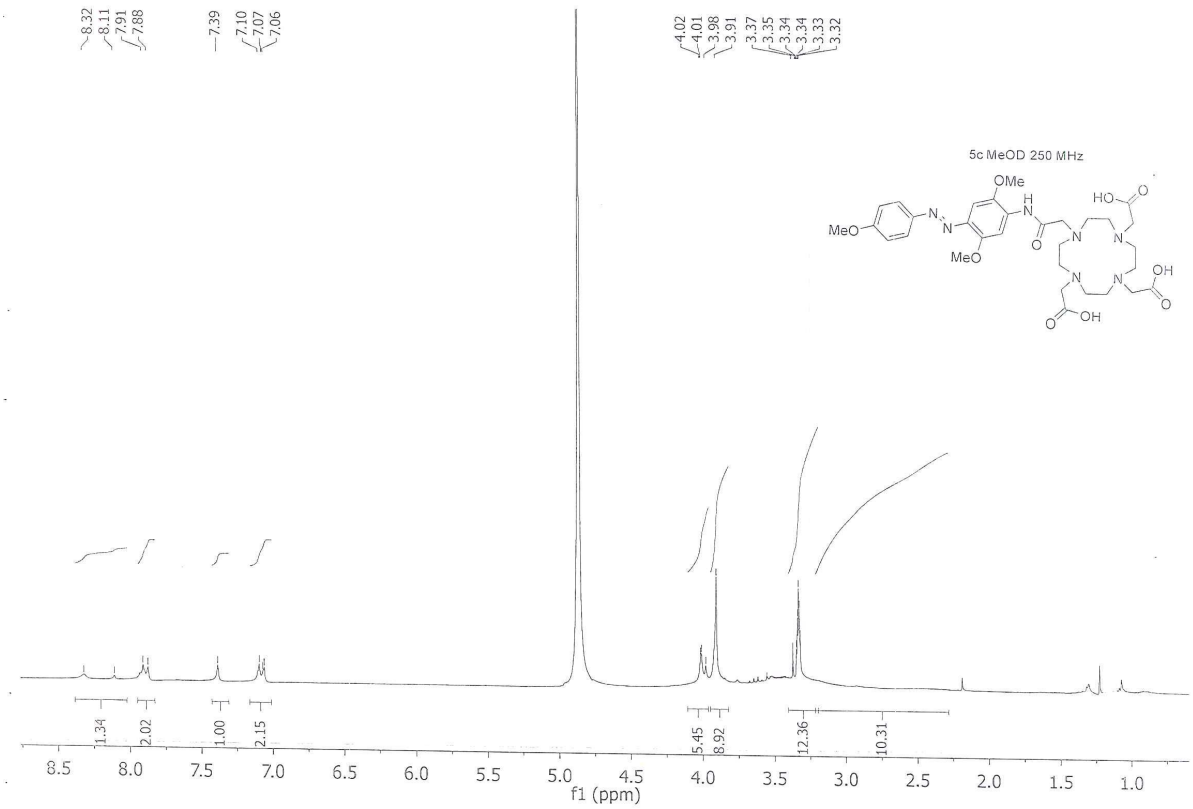
125.27
125.12
73.92
56.68
49.28
46.41
28.42
18.95
5b MeOD 101 MHz
f1 (ppm)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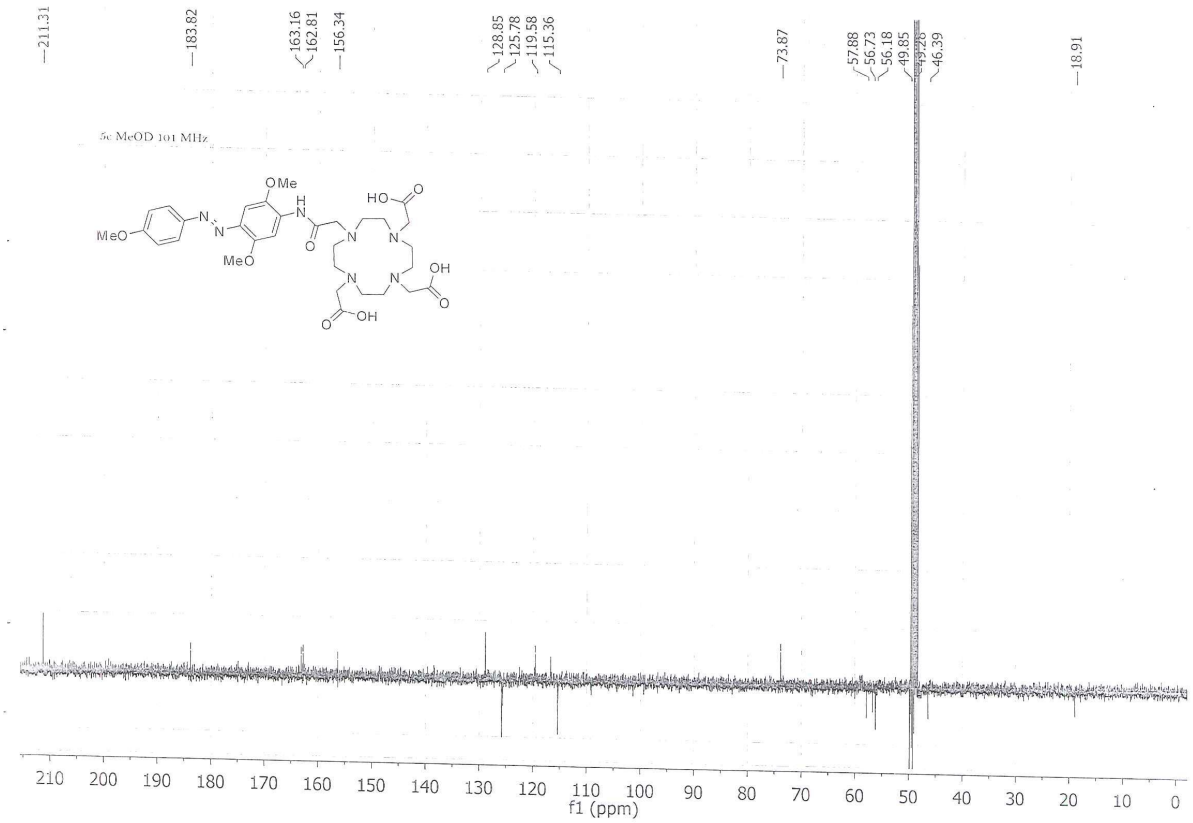
7.10
7.07
7.06
4.02
4.01
3.98
3.91
3.37
3.35
3.34
3.34
3.33
3.32
5c MeOD 250 MHz
OMe
MeO
MeO
HO
OH
OH
1.34
2.02
1.00
2.15
5.45
8.92
12.36
10.31
f1 (ppm)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119.58
115.36
73.87
57.88
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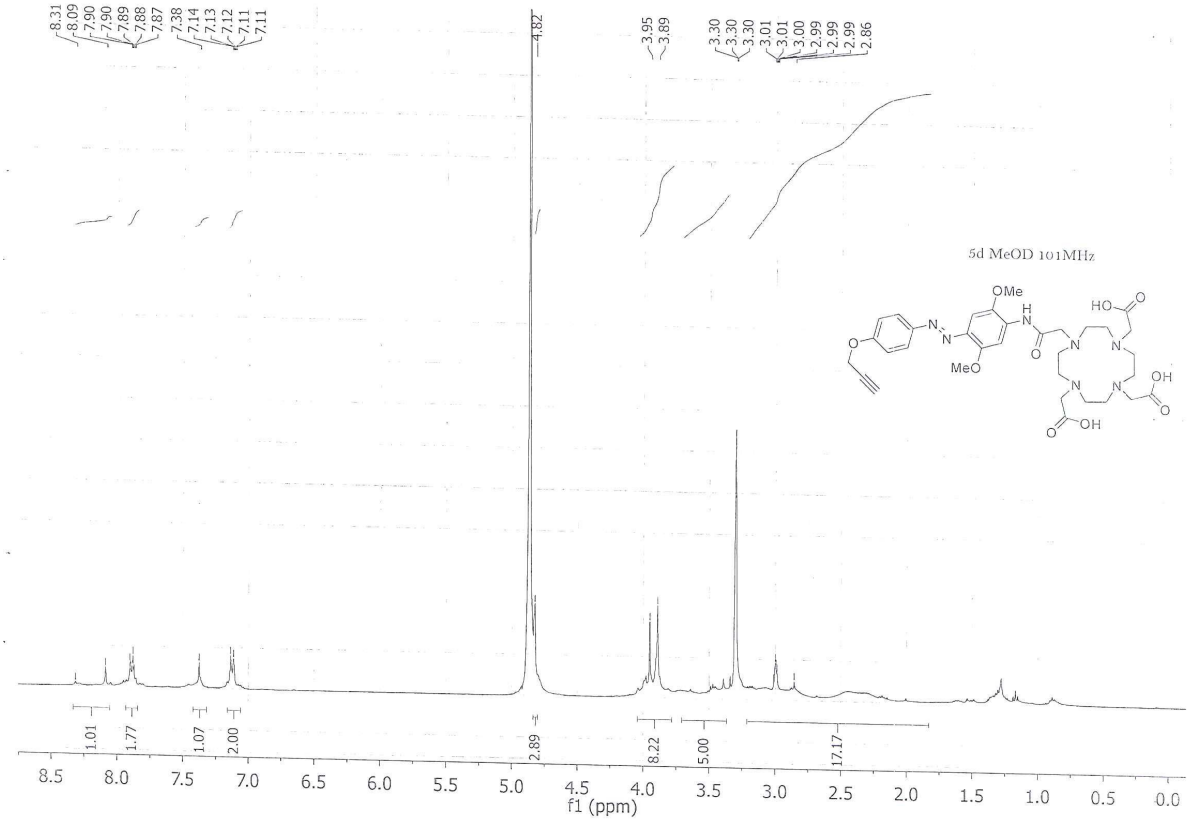
5c MeOD 101 MHz
OMe
MeO
MeO
HO
OH
OH
f1 (ppm)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7.89
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7.38
7.14
7.13
7.12
7.11
7.11
4.82
3.95
3.89
3.30
3.30
3.30
3.01
3.01
3.00
2.99
2.99
2.99
2.86
5d MeOD 101MHz
OMe
MeO
HO
OH
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1.07
2.00
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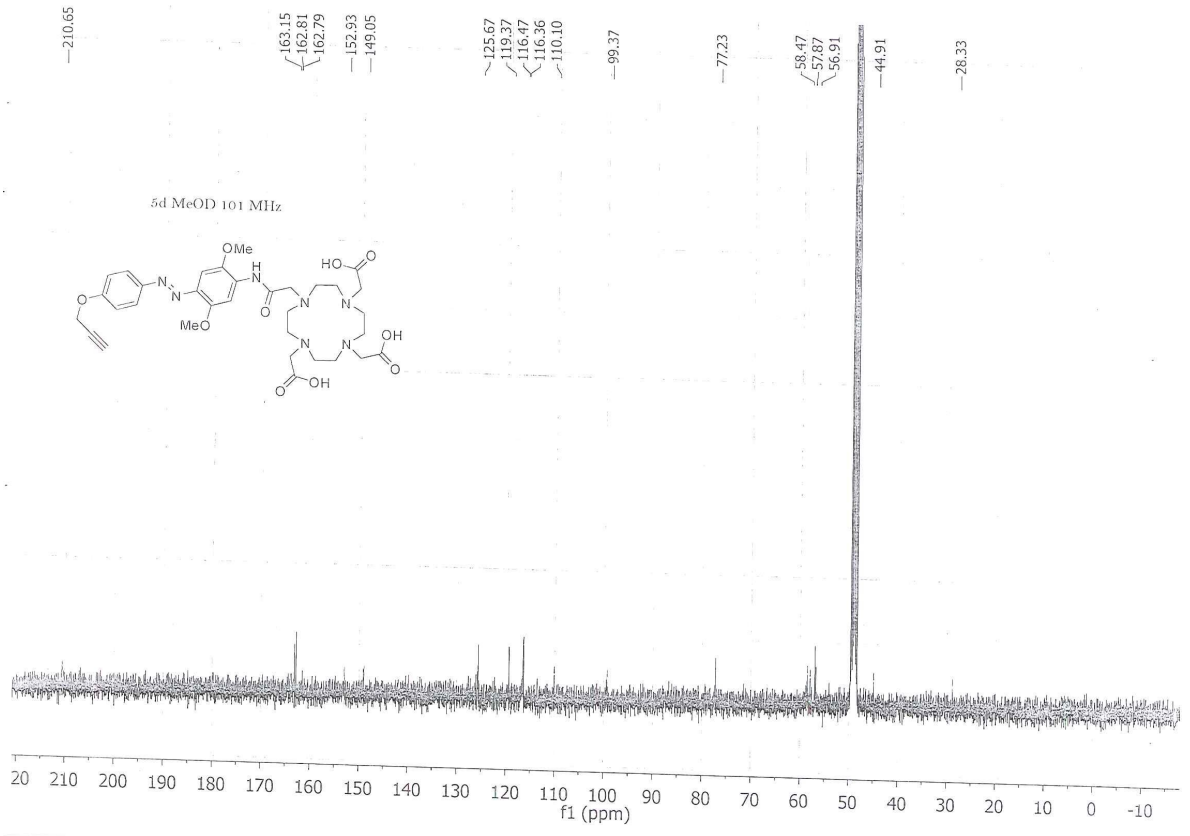
8.22
5.00
17.17
f1 (ppm)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116.36
110.10
99.37
77.23
58.47
57.87
56.91
44.91
28.33
5d MeOD 101 MHz
OMe
MeO
HO
OH
f1 (ppm)

4. Photophysical measurements

All photophysical measurements were performed on freshly prepared solutions in Milli-Q water or D_2O (0.2 – 1 mM). Phosphorescence spectra of the **7-Gd** complex were recorded in aqueous solution with addition of 10 % of glycerol. Absorption spectra were measured on a UVIKON XL spectrophotometer from Secomam using quartz Suprasil cells (Hellma® 115F-QS, bandpass 0.2 cm). For collecting luminescence data samples were placed into 2.4 mm i.d. quartz capillaries or quartz Suprasil cells. Emission and excitation spectra were measured on a custom-built Horiba-Jobin-Yvon Fluorolog 3 spectrofluorimeter equipped with a visible photomultiplier tube operating in photon counting mode (Hamamatsu R928P) and a NIR detector, either a solid state Electro-Optical Systems, Inc DSS-IGA020L InGaAs detector cooled to 77 K or a Hamamatsu H10330-45 photomultiplier tube operating in photon-counting mode. All spectra were corrected for the instrumental functions. Luminescence lifetimes were determined under excitation at 355 nm provided by a YG 980 Quantel Nd:YAG laser while the emission signal was detected in the near-infrared (950-1450 nm, a photon-counting unit H10330-45 from Hamamatsu) ranges. The output signal from the detector was then fed to a Tektronix TDS 754C 500MHz bandpass digital oscilloscope and then transferred to a PC for treatment with Origin 8®. Lifetimes are averages of at least three independent measurements. Quantum yields were determined with the fluorimeter described above according to an absolute method using an integration sphere (GMP SA, Renens, Switzerland). Each sample was measured several times under slightly different experimental conditions. Estimated experimental error for quantum yields determination is 10 %.

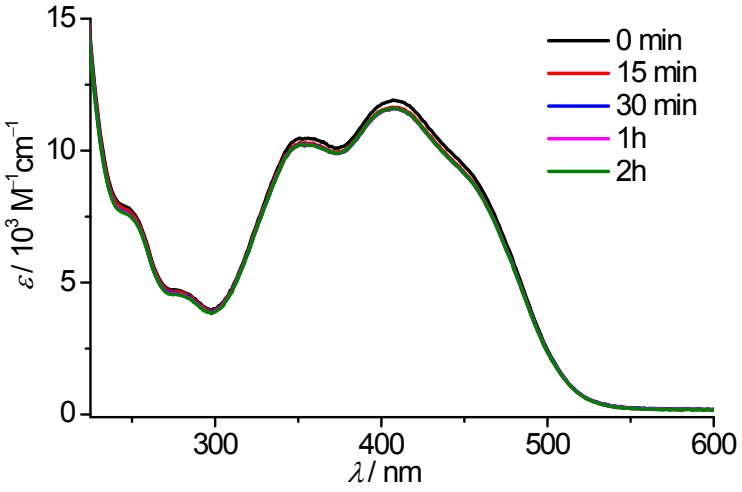

Figure S1. Absorption spectra of **7-Nd** complex under continious illumination at 350 nm during 2 hours at room temperature (H_2O, 200 µg/mL).

Table S1. Luminescence lifetimes (τ_{obs}) of **5d-Ln** complexes in aqueous solution (200 µg/mL) upon excitation at 355 nm and monitoring the emissions of Nd^{3+} at 1064 or Yb^{3+} at 980 nm.[a]

Complex	τ_{obs} [µs]		q^b
	H_2O	D_2O	
5d-Nd	0.064(5)	0.230(1)	1.1
5d-Yb	0.708(1)	5.853(5)	1.0

[a] At room temperature; 2σ values are given between parentheses; experimental errors 2%.[b] Calculated according to phenomenological equations reported in *J. Chem. Soc. Perkin Trans. 2*, 2001, 1268 & *Inorg. Chem. Commun.*, 2001, **4**, 187.