Functionalizable Red Emitting Calcium Sensor Bearing a 1,4-triazole Chelating Moiety

Supplementary Information

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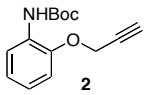
1. Materials and general methods

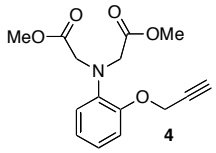
All the solvents were of analytical grade. Chemicals were purchased from commercial sources. The salts used in stock solutions of metal ions were $CaCl_2 \cdot 2H_2O$, $CdCl_2$, $CuCl_2 \cdot 2H_2O$, $FeCl_3 \cdot 6H_2O$, $HgCl_2$, KCl , $MgCl_2 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, NaCl, $Zn(NO_3)_2$. ^{1}H-NMR and ^{13}C-NMR were measured on a Bruker avance III-300 MHz spectrometer with chemical shifts reported in ppm (TMS as internal standard). Mass spectra were measured on a Focus GC / DSQ II spectrometer (ThermoScientific) for IC and an API 3000 spectrometer (Applied Biosystems, PE Sciex) for ES. All pH measurements were made with a Mettler Toledo pH-Meter. Fluorescence spectra were recorded on a JASCO FP-8300 spectrofluorometer. Absorption spectra were determined on a VARIAN CARY 300 Bio UV-Visible spectrophotometer. All measurements were done at a set temperature of 25°C. The purity of the dyes were checked by RP-HPLC C-18, eluant: ACN 0.1% TFA/Water 0.1% TFA, method: 20/80 to 100/0 within 20 min then 100/0 for 10 min. detection at λ_{Abs}= 254 nm.

Synthesis

1 was synthesized according to a published protocol[1]



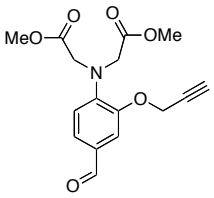
To a solution of **1** (3.200 g, 15.31 mmol) in DMF (30 mL) were added propargyl bromide 80 wt. % in toluene (2.52 mL, 22.96 mmol, 1.5 eq) and K_2CO_3 (3.168 g, 22.96 mmol, 1.5 eq). The solution was heated at 80°C for 5h before being cooled down to room temperature. The solvents were evaporated and the product was extracted with EtOAc washed with water (3 times) and brine (2 times). The organic phase was dried over $MgSO_4$, filtered and evaporated. The crude was purified by column chromatography on silica gel (Cyclohexane/EtOAc : 9/1) to obtain the 3.28 g of **2** (86%) as a yellowish syrup. Rf=0.69 (Cyclohexane/EtOAc, 8/2). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ 8.13 (d, *J* = 5.3 Hz, 1H, HAr), 7.10 (s, 1H, NH), 7.02-7.00 (m, 3H, H Ar), 4.78 (d, *J* = 2.4 Hz, 2H, CH_2), 2.58 (t, *J* = 2.4 Hz, 1H, CH), 1.56 (s, 9H, tBu). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ 152.72 (CO Boc), 145.54 (Cq Ar), 128.64 (Cq Ar), 122.21 (CH Ar), 122.15 (CH Ar), 118.53 (CH Ar), 111.74 (CH Ar), 80.42 (Cq tBu), 78.20 (C≡CH), 76.07 (C≡CH), 56.47 (CH_2), 28.40 (tBu). MS (CI), calcd for $C_{14}H_{17}NO_3$ $[M]^+$ 247.1, found 247.1, HRMS (CI), $C_{14}H_{17}NO_3$ $[M]^+$ 247.1208, found 247.1195.



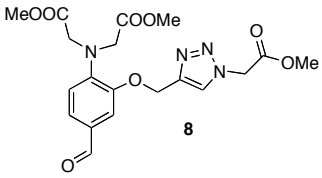
To a cooled (0°C) solution of **2** (3.280 g, 13.28 mmol) in DCM (20 mL) was added TFA (5 mL). The solution was allowed to stir at room temperature overnight. The TFA was neutralized by addition of a saturated solution of $NaHCO_3$ to reach a pH of 8. The product was extracted with DCM and the solution was dried over $MgSO_4$, filtered and evaporated to obtain **3**. Rf=0.35 (Cyclohexane/EtOAc, 8/2). To a solution of **3** (1.923 g, 13.06 mmol) in acetonitrile (26 mL) were added methyl bromoacetate (3.69 mL, 39.84 mmol, 3 eq) and DIEA (6.92 mL, 39.84 mmol, 3 eq) before being warmed up to 90°C

[1] Buon, C.; Chacun-Lefèvre, L.; Rabot, R.; Bouyssou, P.; Coudert, G. *Tetrahedron* **2000**, *56*, 605–614.

overnight. 2 more equivalent of both methyl bromoacetate and DIEA were then added to complete the reaction. The solution was stirred at 90°C over 6h. The solvents were evaporated, the product was extracted with DCM and washed with water. The organic phase was dried over $MgSO_4$, filtered and evaporated. The crude was purified by column chromatography on silica gel (Cyclohexane/EtOAc : 9/1) to obtain the 3.64 g of **4** (94%) as a yellowish syrup. Rf=0.28 (Cyclohexane/EtOAc, 8/2). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ 6.95 (m, 4H, H Ar), 4.72 (d, *J* = 2.4 Hz, 2H, OCH_2), 4.18 (s, 4H, NCH_2), 3.75 (s, 6H, OMe), 2.52 (t, *J* = 2.4 Hz, 1H, CH). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ 171.83 (CO esters), 149.38 (C Ar), 139.73 (C Ar), 122.55 (CH Ar), 122.42 (CH Ar), 119.56 (CH Ar), 115.37 (CH Ar), 78.80 (C≡CH), 75.37 (C≡CH), 56.81 (OCH_2), 53.77 (NCH_2), 51.82 (OMe). MS (ES^+), calcd for $C_{15}H_{17}NO_5Na$ $[M + Na]^+$ 314.1, found 314.4. HRMS (ES^+), calcd for $C_{15}H_{18}NO_5$ $[M + H]^+$ 292.1179, found 292.1197.

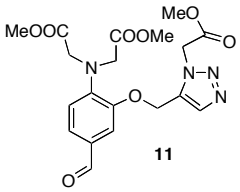


To a solution of **4** (3.06 g, 10.51 mmol) in DMF (10 mL) was slowly added $POCl_3$ (7.82 mL, 84.08 mmol, 8 eq). The mixture turned black and was allowed to stir at 80°C for 3 hours before being cooled down to room temperature. The mixture was then poured in water (1L) and the product was extracted with EtOAc (3 times) and washed with brine twice. The organic phase was dried over $MgSO_4$, filtered and evaporated to 50 mL EtOAc. The product precipitated under cooling and was filtered to obtain 2.262 g of **5** (67%) as a brown powder. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ 9.83 (s, 1H, CHO), 7.48-7.43 (m, 2H, H Ar), 6.80 (d, *J* = 8.2 Hz, 1H, H Ar), 4.74 (d, *J* = 2.2 Hz, 2H, OCH_2), 4.25 (s, 4H, NCH_2), 3.81 (s, 6H, OMe), 2.56 (t, *J* = 2.1 Hz, 1H, CH). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ 190.45 (CHO), 171.18 (CO esters), 148.33 (C Ar), 145.36 (C Ar), 129.95 (C Ar), 127.12 (CH Ar), 116.91 (CH Ar), 112.81 (CH Ar), 77.72 (C≡CH), 76.10 (C≡CH), 56.71 (OCH_2), 54.08 (NCH_2), 52.16 (OMe). MS (CI), calcd for $C_{16}H_{18}NO_6$ $[M+H]^+$ 320.1, found 320.1, HRMS (CI), $C_{16}H_{18}NO_3$ $[M+H]^+$ 320.1129, found 320.1191.



8

To a solution of **5** (500 mg, 1.567 mmol) and methyl 2-azido acetate (360 mg, 3.134 mmol, 2 eq) in dioxane (16 mL) was added an heterogeneous solution of $CuSO_4{\cdot}5H_2O$ (195 mg, 0.783 mmol, 0.5 eq) and sodium ascorbate (217 mg, 1.097 mmol, 0.7 eq) in water (1 mL). The mixture was stirred at 50°C overnight before being extracted with DCM and washed successively with water and brine. The organic phase was dried over $MgSO_4$, evaporated and the crude was purified by column chromatography on silica gel (EtOAc) to obtain 571 mg of **8** (83%) as a yellowish syrup. Rf=0.40 (100% EtOAc). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ 9.72 (s, 1H, CHO), 7.75 (s, 1H, H triazol), 7.42 (d, *J* = 1.8 Hz, 1H, H Ar), 7.33 (dd, *J* = 8.2, 1.8 Hz, 1H, H Ar), 6.71 (d, *J* = 8.2 Hz, 1H, H Ar), 5.18 (s, 2H, CH_2COOMe), 5.12 (s, 2H, OCH_2), 4.12 (s, 4H, NCH_2), 3.74 (s, 3H, OMe), 3.60 (s, 6H, OMe). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ 190.52 (CHO), 171.15 (CO esters), 166.53(COOMe), 149.08 (C Ar), 145.37 (C Ar), 143.31 (C Ar), 130.11 (CH Ar), 126.62 (CH triazol), 124.92 (CH Ar), 117.04 (CH Ar), 112.79 (CH Ar), 62.52 (CH_2COOMe), 53.90 (NCH_2), 53.13 (OMe), 52.00 (2 OMe), 50.77 (OCH_2). MS (CI), calcd for $C_{19}H_{23}N_4O_8$ $[M + H]^+$ 435.1, found 435.0. HRMS (ES^+), calcd for $C_{19}H_{23}N_4O_8$ $[M + H]^+$ 435.1510, found 435.1516.



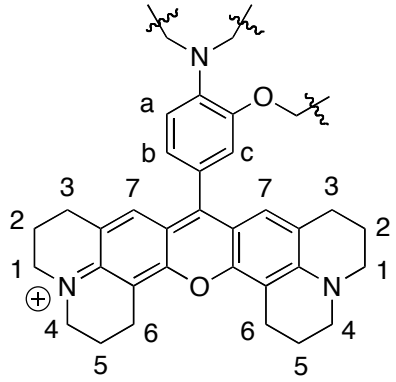
11

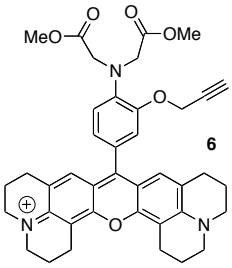
To a solution of **5** (409 mg, 1.282 mmol) and methyl 2-azido acetate (294 mg, 2.564 mmol, 2 eq) in dioxane (15 mL) was added $Cp^*RuCl(PPh_3)_2$ (40 mg, 0.05 mmol, 0.04 eq). The solution was allowed to stir at 90°C. The solutions turned quickly black and a monitoring of the reaction by TLC revealed that the starting material was consumed. The solvent was then evaporated and the crude was purified by column chromatography on silica gel (Cyclohexane/EtOAc : 4/6) to obtain 582mg of **11** (90%) as a yellowish syrup. Rf=0.16 (Cyclohexane/EtOAc : 5/5). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ 9.83 (s, 1H, CHO), 7.82 (s, 1H, H triazol), 7.48-7.46 (m, 2H, H Ar), 6.84 (d, *J* = 8.6 Hz, 1H, H Ar), 5.34 (s, 2H, OCH_2), 5.19 (s, 2H, CH_2COOMe),

4.12 (s, 4H, NCH_2), 3.79 (s, 3H, OMe), 3.60 (s, 6H, 2 OMe). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ 190.25 (CHO), 170.94 (CO esters), 166.96 (CO ester), 148.78 (C Ar), 145.54 (C Ar), 134.82 (CH triazol), 132.47 (C Ar), 130.27 (C Ar), 127.69 (CH Ar), 117.43 (CH Ar), 112.24 (CH Ar), 59.57 ($\underline{CH_2}$COOMe), 53.65 (NCH_2), 53.08 (OMe), 52.05 (2 OMe), 49.62 (OCH_2). MS (CI), calcd for $C_{19}H_{23}N_4O_8$ $[M + H]^+$ 435.1, found 435.2. HRMS (ES^+), calcd for $C_{19}H_{23}N_4O_8$ $[M + H]^+$ 435.1510, found 435.1526.

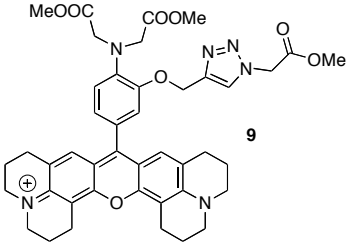
Synthesis of X-Rhodamine : typical procedure

Numerotation of X-rhodamines :



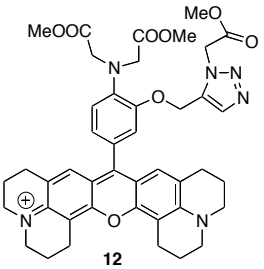


To a solution of aldehyde **5** (200 mg, 0.626 mmol) in propionic acid was added 8-hydroxyjulolidine (237 mg, 1.254 mmol, 2 eq) and PTSA (11 mg, 0.062 mmol, 0.1 eq). The solution was protected from light and stirred at room temperature overnight. To the brown mixture was added a solution of chloranil (152 mg, 0.626 mmol, 1 eq) in DCM (10 mL), the reaction turned dark and was allowed to stir overnight at room temperature. The dark purple solution was evaporated to dryness, dissolved in DCM. The crude was purified by column chromatography on silica gel (gradient of 100% DCM to 9/1 DCM/Methanol) to obtain 58 mg of **6** (~13%) as a purple solid after lyophilisation (dioxane/water : 1/1). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ 7.83 (d, *J* = 8.1 Hz, 2H, CH Ar PTSA counter ion), 7.05 (d, *J* = 8.0 Hz, 2H, CH Ar PTSA counter ion), 6.93 (t, *J* = 7.6 Hz, 5H, H_a, H_b, H_c, H_3), 4.70 (d, *J* = 2.2 Hz, 2H, CH_2O), 4.27 (s, 4H, NCH_2), 3.83 (s, 6H, 2 OMe), 3.55 (m, 8H, H_1, H_4), 3.02 (t, *J* = 6.1 Hz, 4H, H_6), 2.73 (t, *J* = 6.0 Hz, 4H, H_3), 2.57 (t, *J* = 2.1 Hz, 1H, C≡$\underline{CH}$), 2.27 (s, 3H, Me PTSA counter ion), 2.12-1.98 (m, 8H, H_5, H_2). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ 171.73 (CO esters), 154.11 (C Ar), 152.22 (C Ar), 151.04 (C Ar), 148.20 (C Ar), 144.65 (C Ar), 141.08 (C Ar), 138.14 (C Ar), 128.15 (CH PTSA), 126.77 (C_7), 126.29 (CH PTSA), 125.35, 124.10 (CH Ar), 123.56, 118.09 (CH Ar), 116.10 (CH Ar), 112.75, 105.43, 78.00 ($\underline{C}$≡CH), 76.28 (C≡$\underline{CH}$), 56.78 (CH_2O), 53.87 (NCH_2), 52.15 (2 OMe), 50.95 (C_1 or C_4), 50.48 (C_1 or C_4), 27.73 (C_3), 21.29 (Me PTSA), 20.73 (C_2), 19.96 (C_6), 19.77 (C_5). MS (ES^+), calcd for $C_{40}H_{42}N_3O_6$ $[M]^+$ 660.3, found 660.7. HRMS (ES^+), calcd for $C_{40}H_{42}N_3O_6$ $[M]^+$ 660.3068, found 660.3079.



9 was obtained as a purple solid after lyophilisation with ~20% yield. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ 8.11 (s, 1H, H triazol), 7.71 (d, *J* = 8.1 Hz, 2H, 2CH PTSA), 6.95 (dd, *J* = 4.8, 3.0 Hz, 3H, CH PTSA, 1CH Ar), 6.86-6.73 (m, 4H, CH Ar), 5.22 (s, 2H, $\underline{CH_2}$COOMe), 5.11 (s, 2H, OCH_2), 4.16 (s, 4H, NCH_2), 3.68 (s, 3H, OMe), 3.65 (s, 6H, 2 OMe), 3.44 (m, 8H, H_1, H_4), 2.94 (t, *J* = 6.1 Hz, 4H, H_6), 2.69-2.64 (m, 4H, H_3), 2.18 (s, 3H, Me PTSA), 2.02 (t, *J* = 5.1 Hz, 4H, H_5), 1.93-1.91 (m, 4H, H_2). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ 171.82 (CO esters), 166.99 (CO ester), 154.70 (C Ar), 152.26 (C Ar), 151.02 (C Ar), 149.12 (C Ar), 144.59 (C Ar), 142.88 (C Ar), 141.13 (C Ar), 138.21 (C Ar), 128.18 (CH PTSA), 127.03 (C_7),

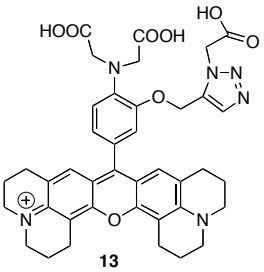
126.23 (CH PTSA), 126.09 (CH triazol), 125.36(C Ar), 123.63 (C Ar), 123.48 (CH Ar), 117.91 (CH Ar), 115.93 (CH Ar), 112.88 (C Ar), 105.24 (C Ar), 62.56 (OCH_2), 53.82 (NCH_2), 52.92 (OMe), 52.03 (2 OMe), 50.96 (C_1 or C_4), 50.79 ($\underline{CH_2}$COOMe), 50.44 (C_1 or C_4), 27.64 (C_3), 21.28 (Me PTSA), 20.74 (C_2), 19.97(C_6), 19.81 (C_5). MS (ES^+), calcd for $C_{43}H_{47}N_6O_8$ $[M]^+$ 775.3450, found 776.0. HRMS (ES^+), calcd for $C_{43}H_{47}N_6O_8$ $[M]^+$ 775.3450, found 775.3473.



12

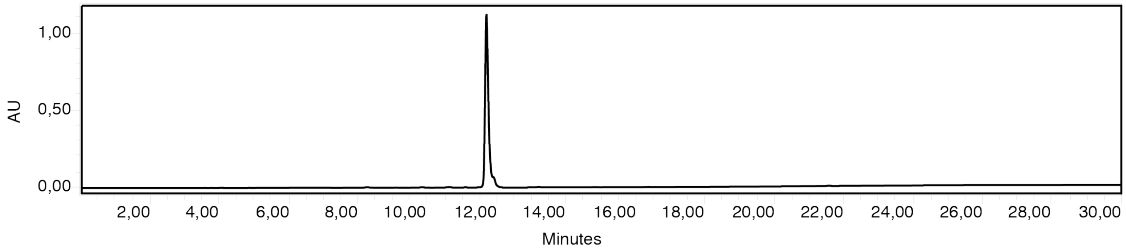
12 was obtained as a purple solid after lyophilisation with ~16% yield. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ 7.73 (d, *J* = 7.9 Hz, 2H, CH PTSA), 7.71 (H triazol) 7.02-6.83 (m, 7H, 2CH PTSA, H_a, H_b, H_c, $2H_7$), 5.45 (s, 2H, $\underline{CH_2}$COOMe), 5.33 (s, 2H, OCH_2), 4.21 (s, 4H, NCH_2), 3.72 (s, 3H, OMe), 3.70 (s, 6H, 2 OMe), 3.57-3.51 (m, 8H, H_1, H_4), 3.02 (t, *J* = 6.1 Hz, 4H, H_6), 2.77-2.71 (m, 4H, H_3), 2.26 (s, 3H, Me PTSA), 2.13-2.08 (m, 4H, H_5), 2.02-2.00 (m, 4H, H_2). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ 171.55 (CO esters), 167.27 (CO ester), 154.00 (C Ar), 152.21 (C Ar), 151.07 (C Ar), 148.62 (C Ar), 144.52 (C Ar), 141.05 (C Ar), 138.23 (C Ar), 134.55 (CH triazol), 133.20 (C Ar), 128.16 (CH PTSA), 126.73 (C_7), 126.16 (CH PTSA), 126.05 (C Ar), 124.12 (CH Ar), 123.77 (C Ar), 118.76 (CH Ar), 116.13 (CH Ar), 112.84 (C Ar), 105.30 (C Ar), 59.91 (OCH_2), 53.51 (NCH_2), 52.94 (OMe), 52.04 (2 OMe), 50.99 (C_1 or C_4), 50.47 (C_1 or C_4), 49.72 ($\underline{CH_2}$COOMe), 27.64 (C_3), 21.29 (Me PTSA), 20.71 (C_2), 19.96 (C_6), 19.79 (C_2). MS (ES^+), calcd for $C_{43}H_{47}N_6O_8$ $[M]^+$ 775.3450, found 775.7. HRMS (ES^+), calcd for $C_{43}H_{47}N_6O_8$ $[M]^+$ 775.3450, found 775.3495.

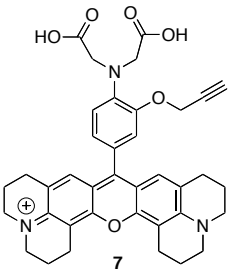
Saponification : typical procedure



13

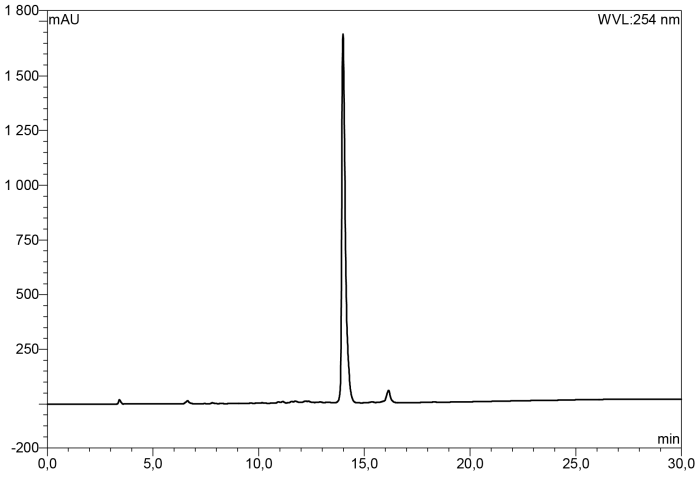
To a solution of **12** (90 mg, ~0.10 mmol) in methanol (10 mL) were added 700 mg of KOH and water (3 mL), the mixture was stirred overnight. The product was washed with HCl (1M) and extracted with $CHCl_3$ until the aqueous phase become slightly pink. The organic phase was then dried over $MgSO_4$, filtered and evaporated. The crude was purified on a reverse phase column C-18 using acetonitrile (0,1% TFA) and water (0,1% TFA) as eluant (20% ACN to 60%), monitored at 254 nm. The solvents were evaporated and 64 mg of **13** (76%) were obtained as a purple solid after lyophilisation (dioxane/water, 1/1). HRMS (ES^+), calcd for $C_{40}H_{41}N_6O_8$ $[M]^+$ 733.2980, found 733.3002.

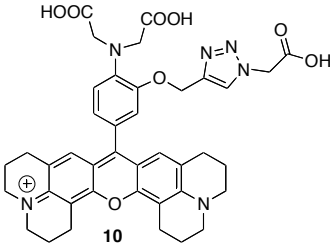




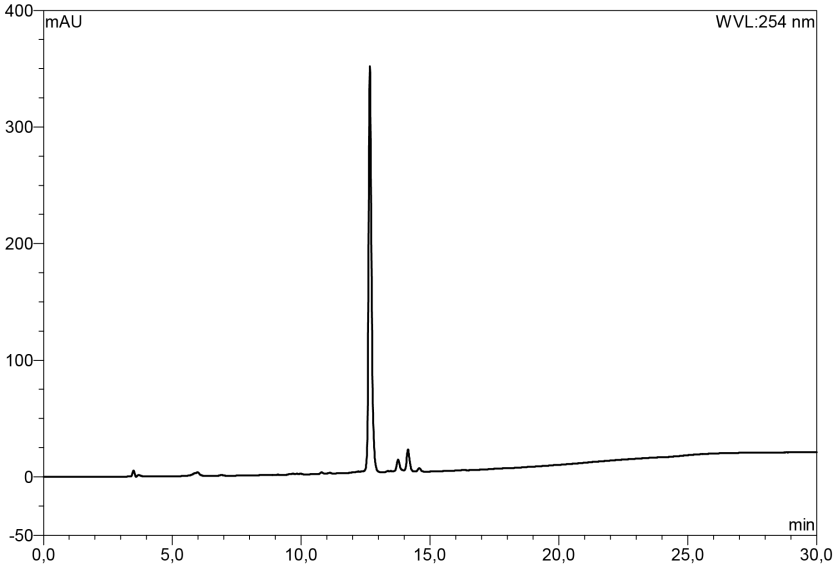
7

7 was obtained as a purple solid after lyophilisation with ~74% yield. HRMS (ES^+), calcd for $C_{38}H_{38}N_3O_6$ $[M]^+$ 632.2755, found 632.2761.

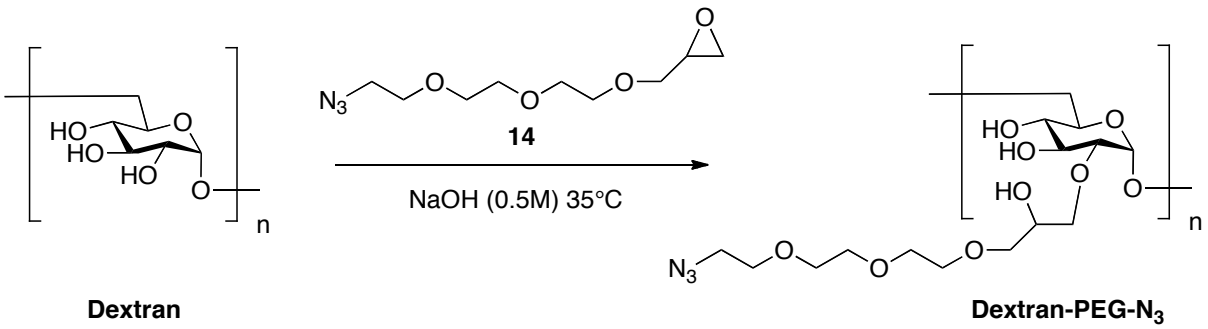




10 was obtained as a purple solid after lyophilisation with ~81% yield. HRMS (ES^+), calcd for $C_{40}H_{41}N_6O_8$ $[M]^+$ 733.2980, found 733.3002



Dextran Conjugates

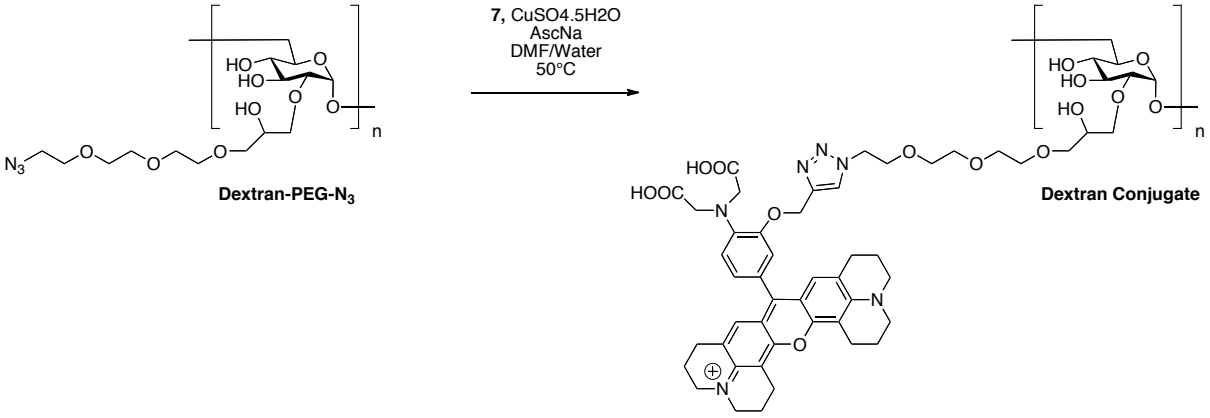


Dextran **Dextran-PEG-N_3**

Dextran 6,000 MW (Sigma-Aldrich, ref: 31388) and dextran 1,500 MW (Sigma-Aldrich, ref: 31394) were functionnalised with **14**[2] using a method described by Nielsen *et al.*[3] *The ^{1}H-NMR showed that The functionnalised dextrans were alkylated once by glucose unit.*

[2] Knapp, D. C.; D'Onofrio, J.; Engels, J. W. *Bioconjugate chem.* **2010**, *21*, 1043–55.

Final MW Dextran 6,000 : ~14,500 g.mol^{-1}
Final MW Dextran 1,500 : ~3,600 g.mol^{-1}



Conjugation of Dextrans. To a solution of dextran-PEG-N_3 (1,500 or 6,000) (40 mg, ~100 µmol glucose unit) and **7** (10 mg, 14 µmol) in DMF (2 mL) was added an heterogeneous solution of $CuSO_4 \cdot 5H_2O$ (10 mg, 40 µmol) and sodium ascorbate (10 mg, 50 µmol) in water (1 mL). The solution was allowed to stir in the dark at 50°C overnight. The solvents were evaporated, the crude was dissolved in 1 mL of EDTA solution (0.1 M) and passed through a G-25 column to give 40 mg of CaRu-Dextran 6,000 conjugate (~80% yield) and 38 mg CaRu-Dextran 1,500 conjugate (~76% yield).

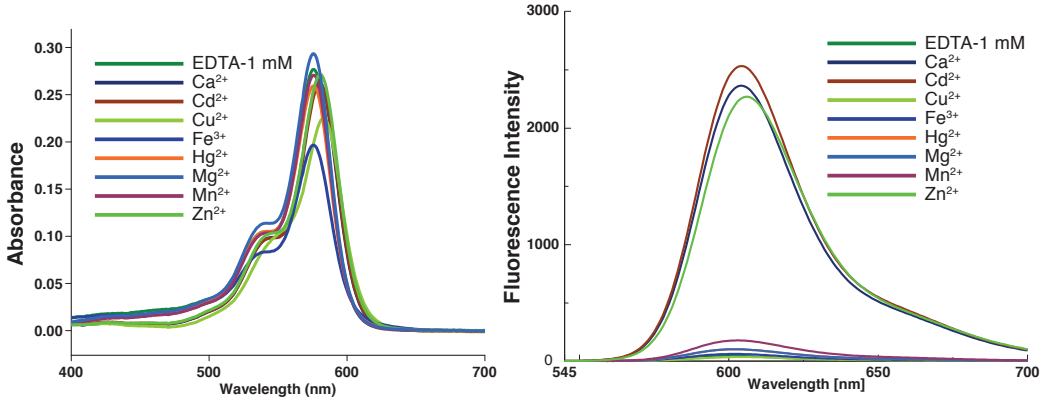


Figure S1. Absorbance spectra and Emission spectra (l_{Ex}= 535 nm) of **10** (5 mM, MOPS 30 mM, KCl 100 mM) in presence of 3 equivament (15 mM) of various metals and EDTA (1 mM).

[3] Nielsen, T. T.; Wintgens, V.; Amiel, C.; Wimmer, R.; Larsen, K. L. *Biomacromolecules* **2010**, *11*, 1710–1715.

Determination of the Dissociation constants Kd.

The dissociation constants were obtained by fitting the Hill equation with the Plot of fluorescence enhancement vs. increasing concentration of Ca^{2+}, Hill equation is given below:

(eq .**2**)

$$\theta = \frac{[L]^n}{Kd + [L]^n}$$

Where

θ is the fraction of the Ca^{2+}-binding sites on the receptor which are occupied by Ca^{2+}.
[L] is the free probe concentration
n is the Hill coefficient

pKa determination:

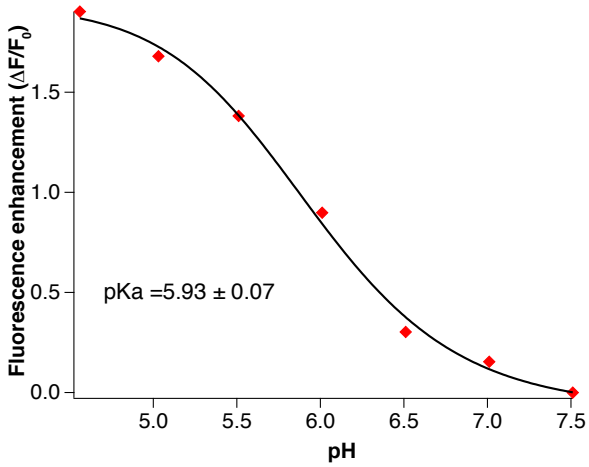


Figure S2. Plot of fluorescence enhancement of **10** (5 mM, MOPS 30 mM, KCl 100mM) vs. pH. Hill's equation fitting provided the pKa.

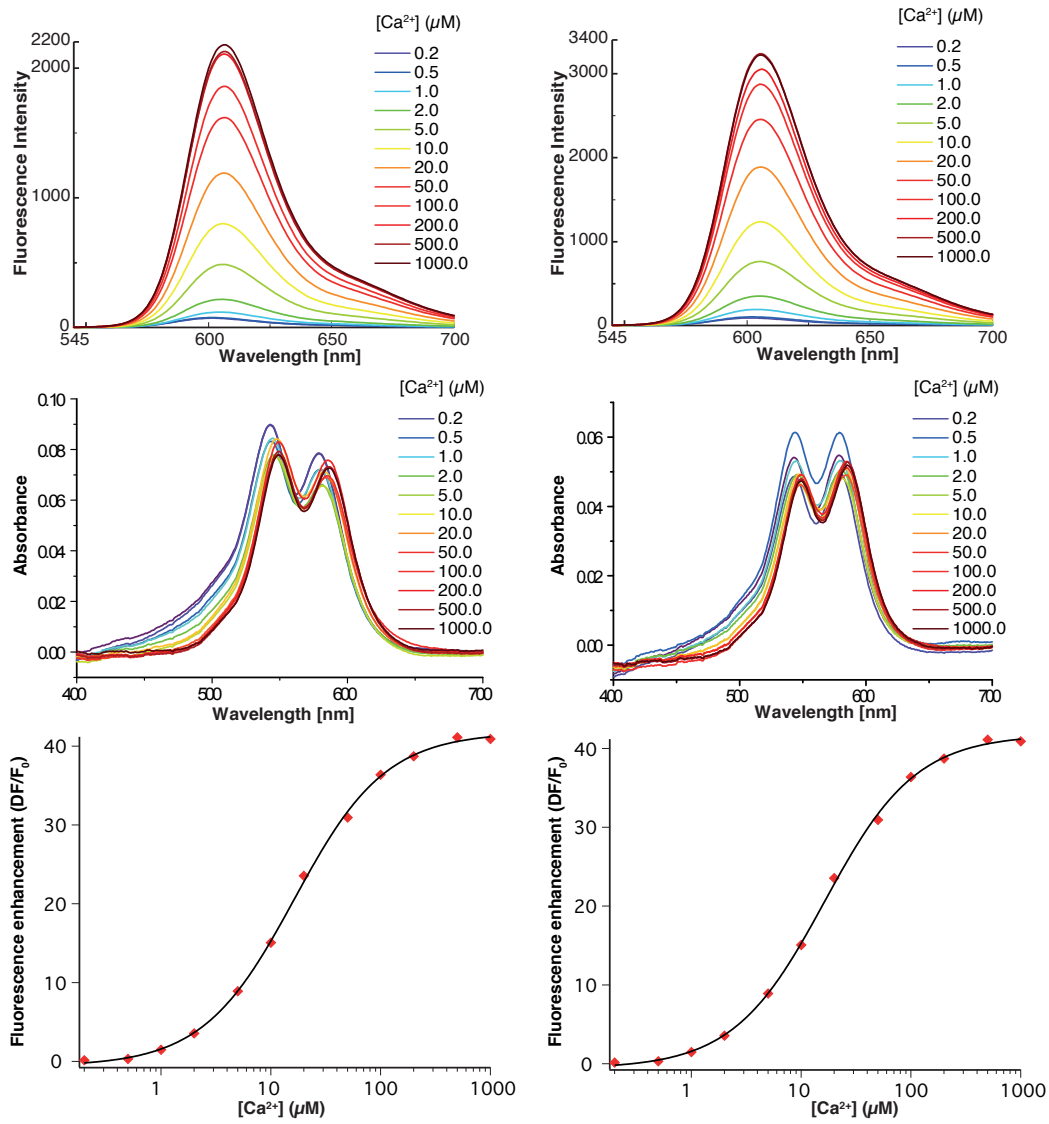


Figure S3. Emission spectra (l_{Ex}= 535 nm) and absorbance spectra of dextran conjugate 6000 (left) and 1500 (right) (MOPS 30 mM, KCl 100 mM) at increasing concentration of Ca^{2+}. Bottom: Plot of fluorescence enhancement ($(F-F_0)/F_0$, with F_0= Fluorescence Intensity in presence of EDTA 1 mM) of dextran conjugate 6000 (left) and 1500 (right) (MOPS 30 mM, KCl 100 mM) vs. increasing concentration of Ca^{2+}. The fit line, according to Hill's equation, yielded the Kd.

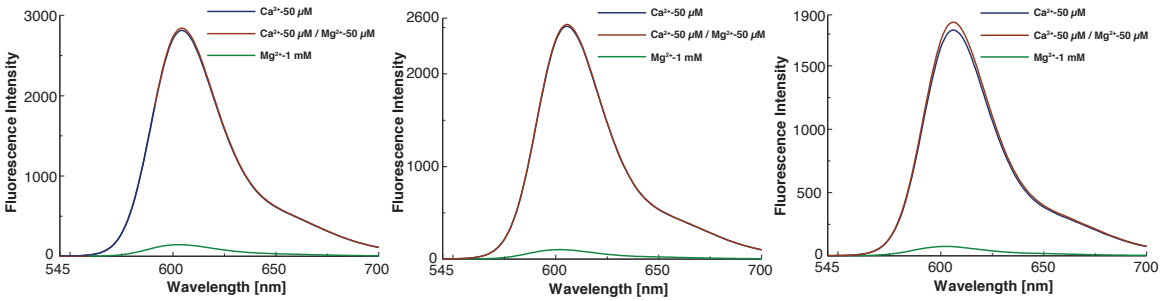


Figure S4. Fluorescence emission spectra of 10 (left) and its dextran conjugates 1500 (middle) 6000 right (MOPS 30 mM, KCl 100 mM) in presence of Ca^{2+}, Mg^{2+} and a mixture of both.

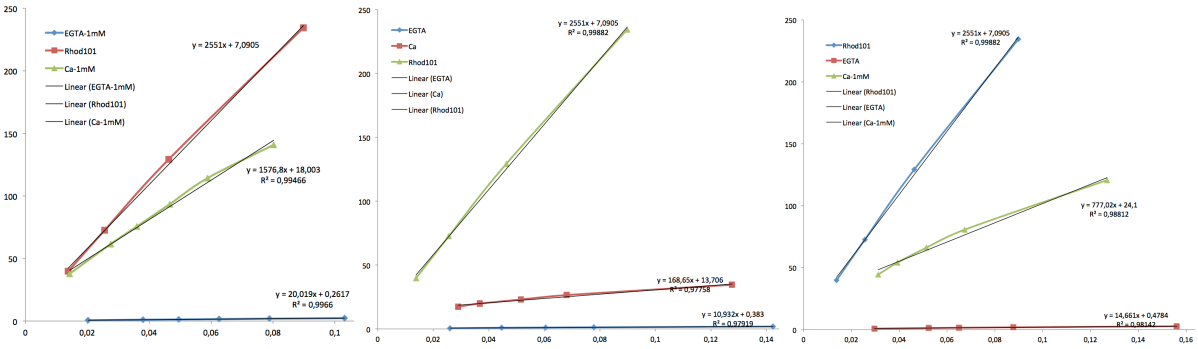
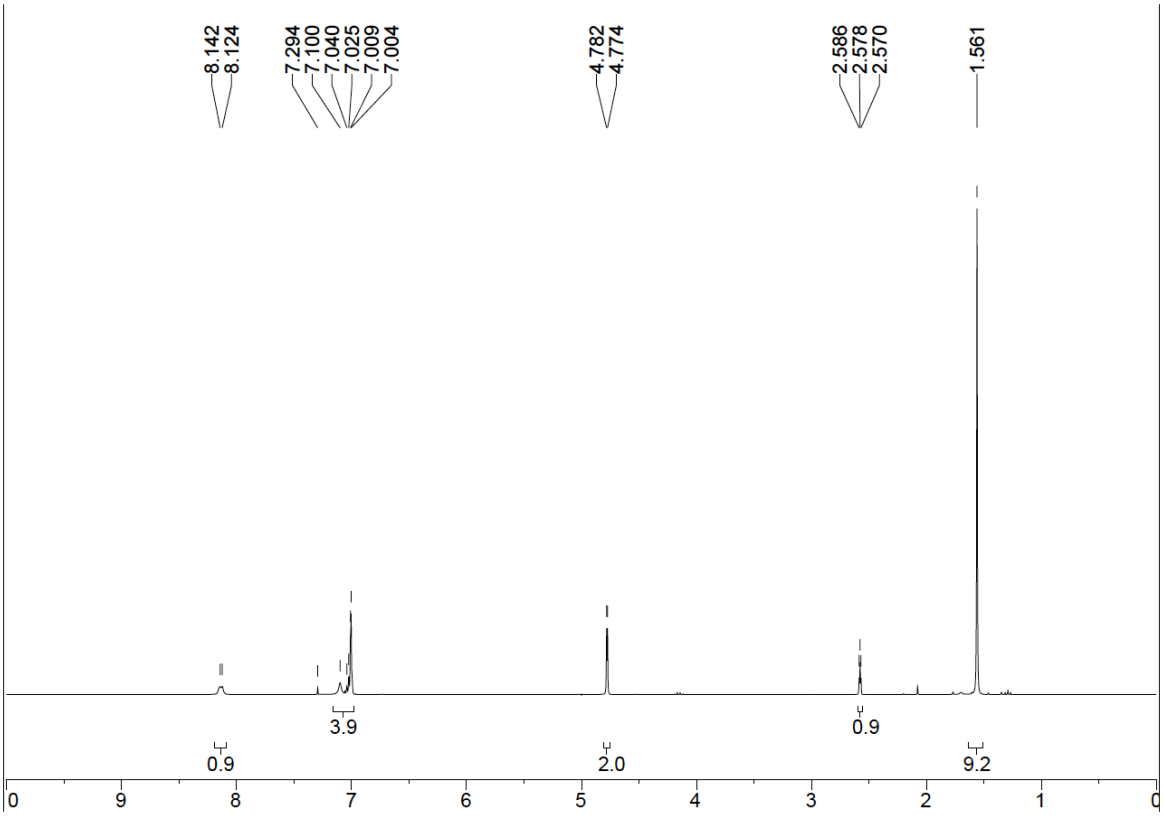


Figure S5. Determination of quantum yields

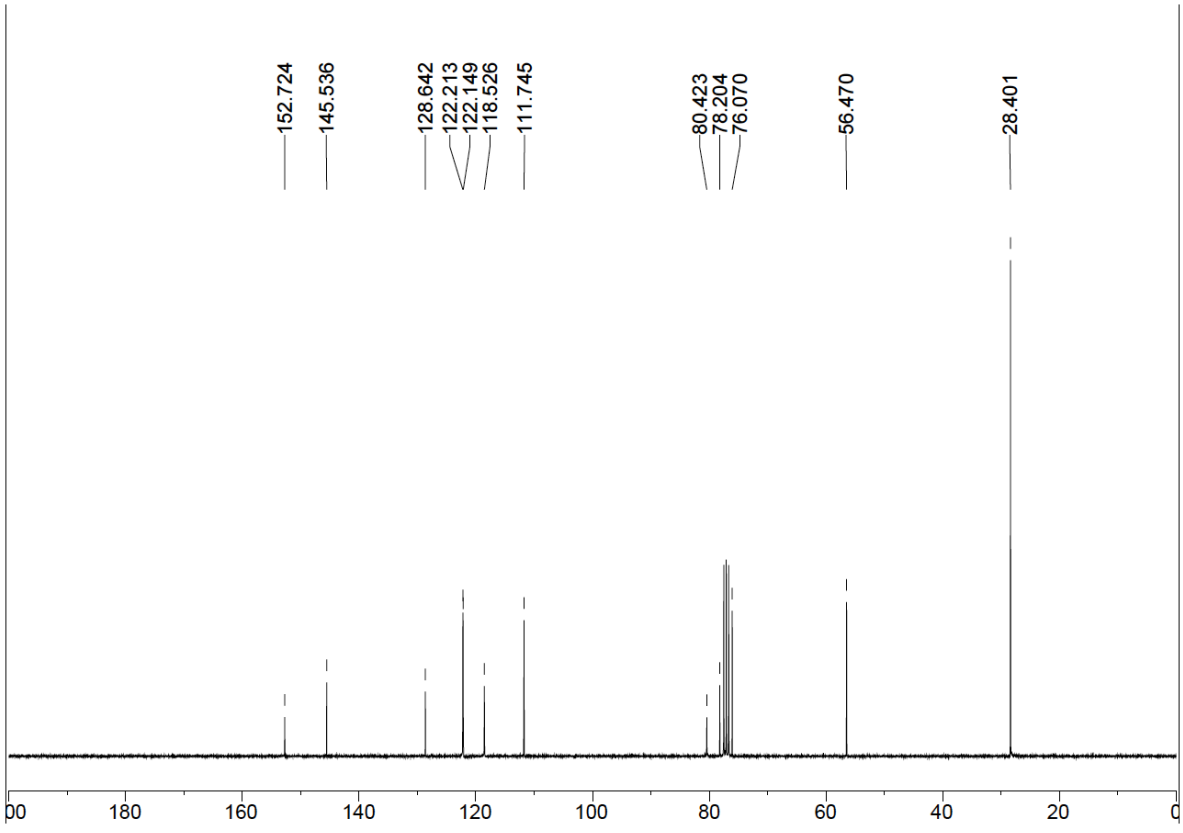
The fluorescence quantum yields φ of **7,10 and 13** (from left to right) were calculated from the slope of the integrated spectral emission (545 to 700 nm) of the sensors and reference dye vs. absorbance using Rhodamine 101 (φ = 1.0 in absolute ethanol) as a reference standard (excitation wavelength was 535 nm). To avoid self-absorption, we worked with solutions of *OD* 0.01–0.1. Equation **2** was used where φ is the quantum yield, η is the refractive index (η (H_2O) = 1.33, η (EtOH) = 1.36) and *s* is the value of the slope. The subscript *ref* refers to the reference. Quantum yield determinations of the sensors were measured in a solution of 1 mM EGTA (MOPS 30 mM, KCl 100 mM) and in a solution of 1 mM Ca^{2+} (MOPS 30 mM, KCl 100 mM).

(eq .**2**)
$$\varphi = \varphi_{ref} \frac{s}{s_{ref}} \cdot \frac{\eta^2}{\eta_{ref}^2}$$

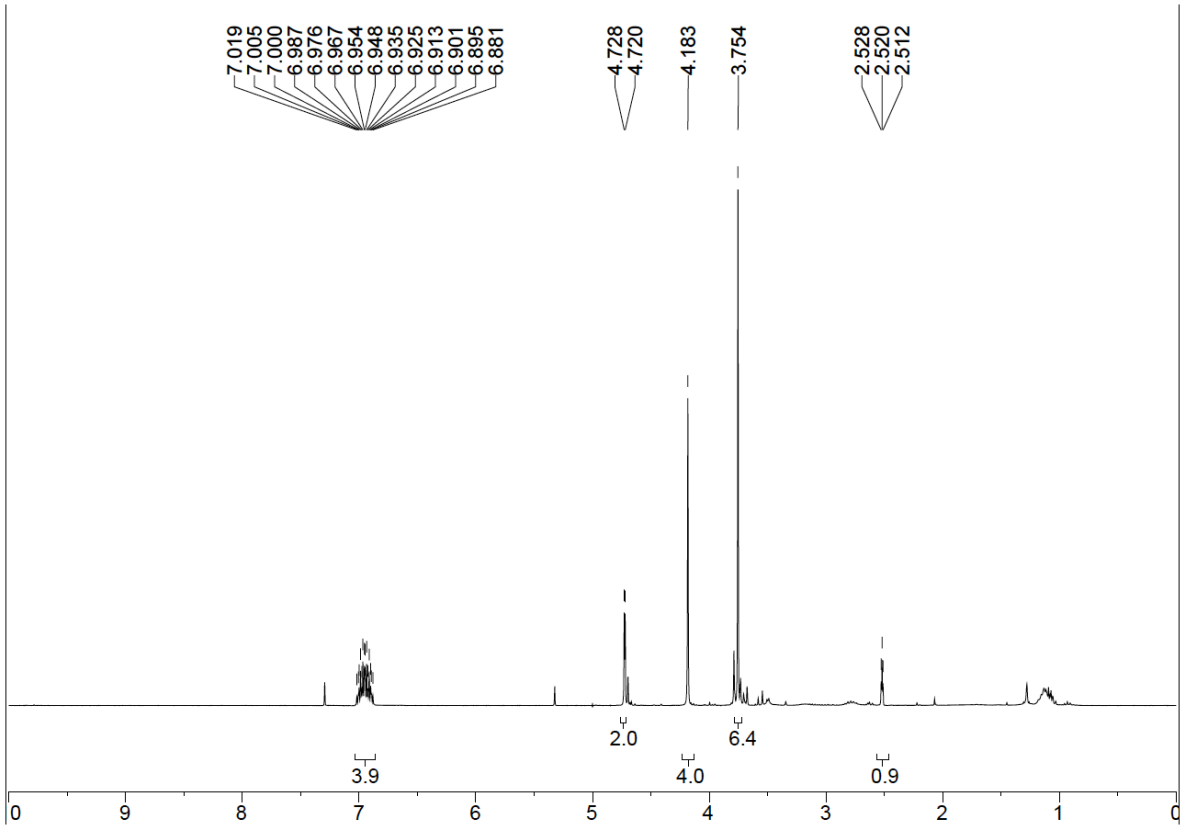
NMR and Mass Spectra



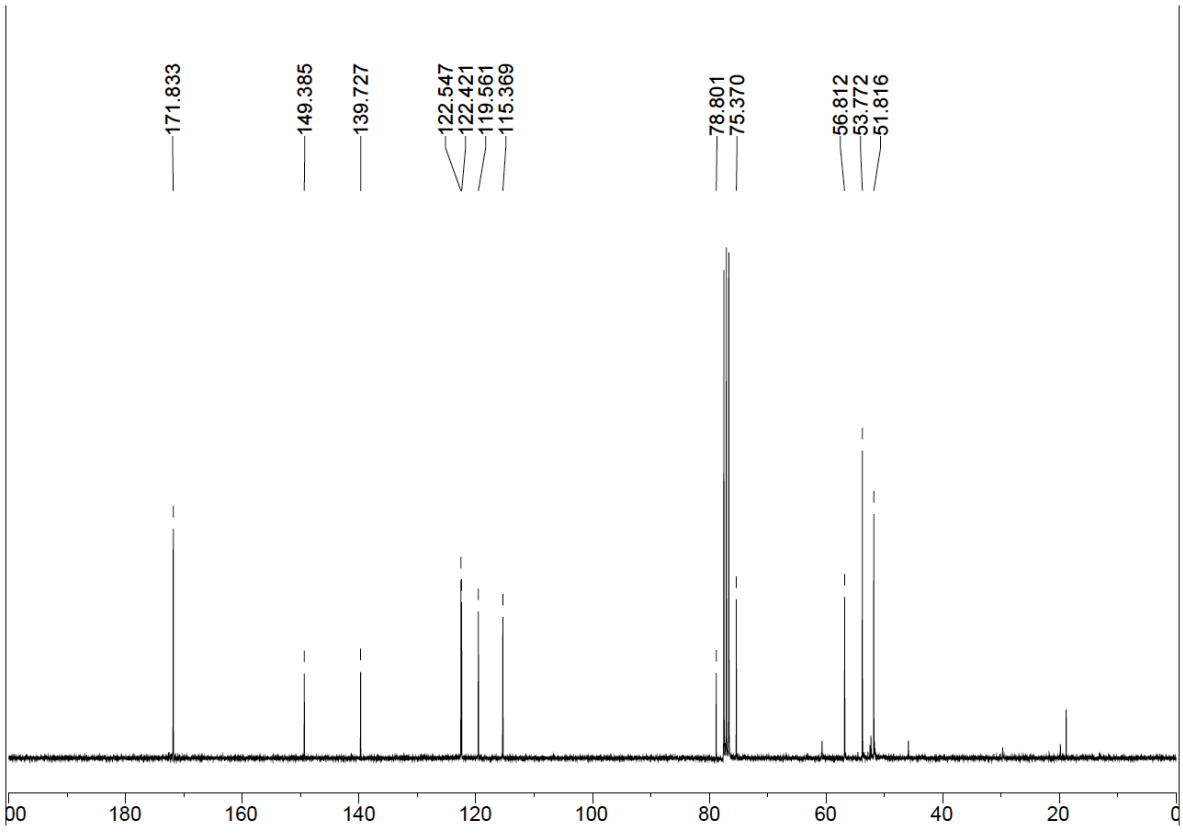
^{1}H Spectrum of **2**



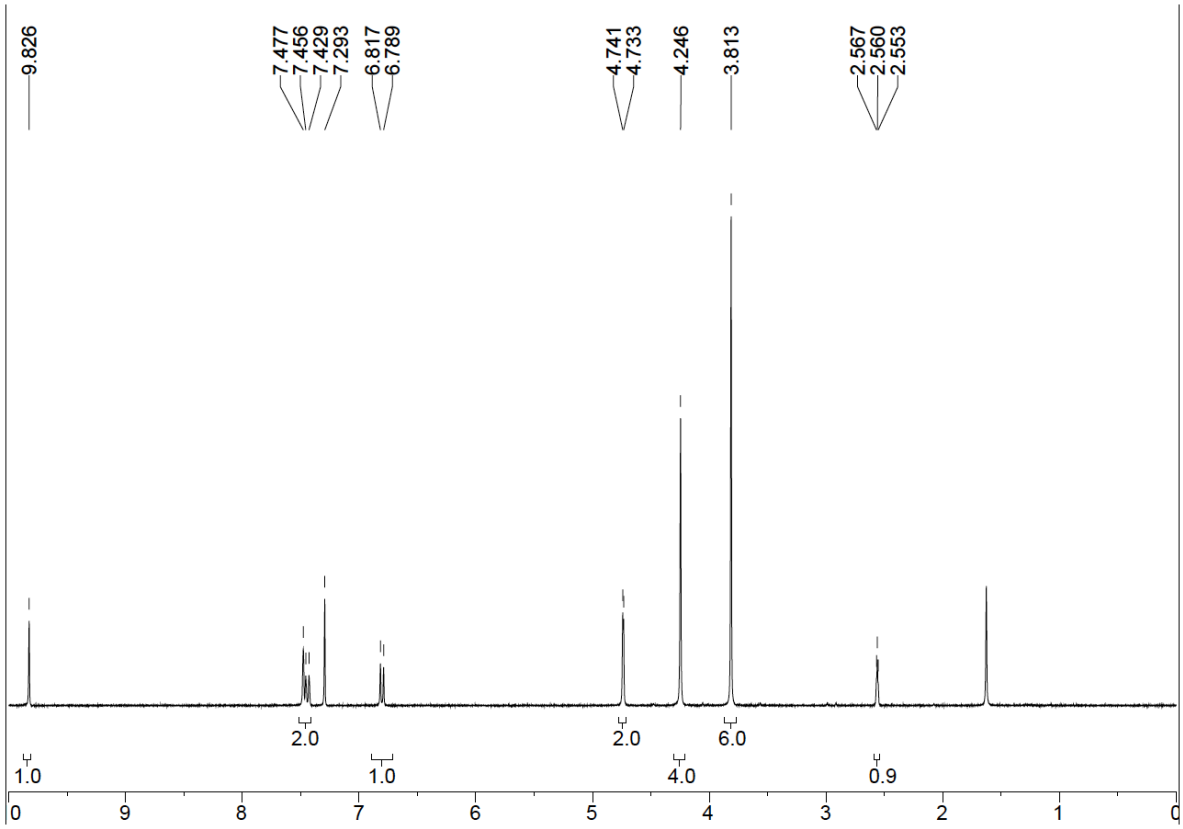
^{13}C Spectrum of **2**



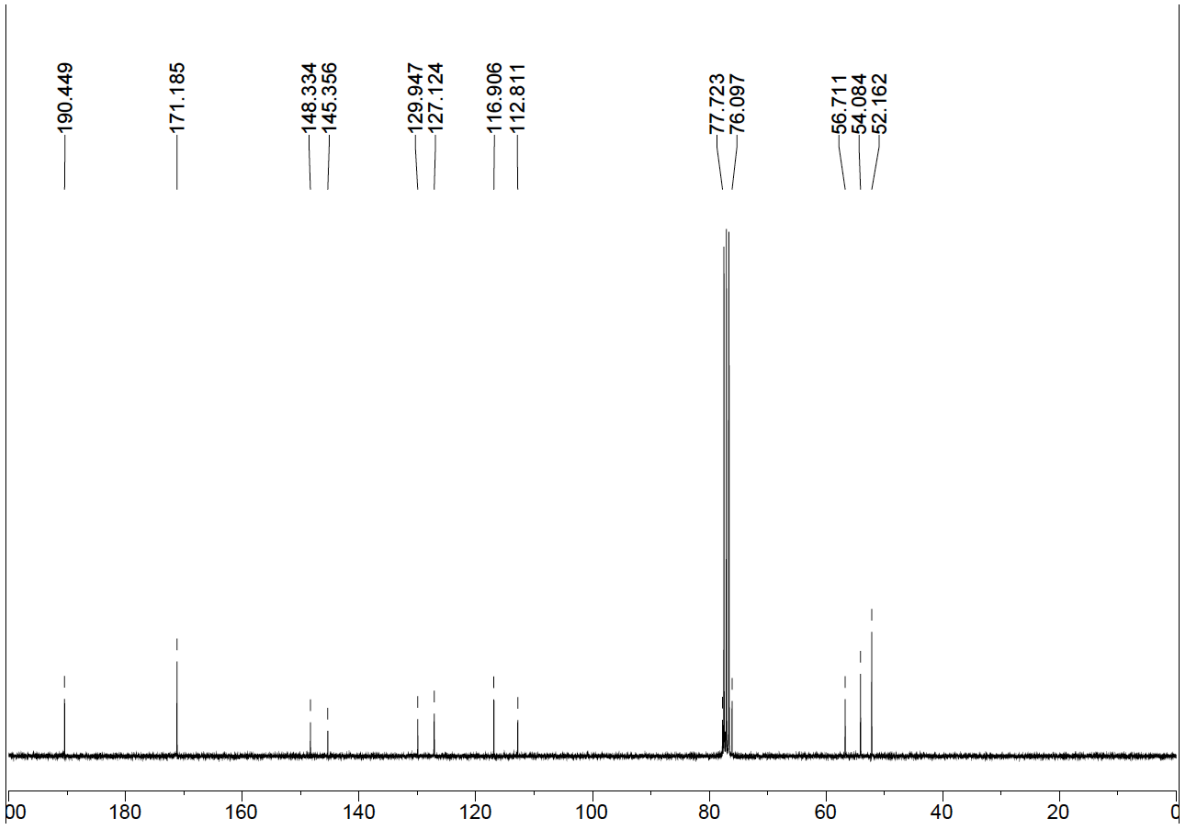
^{1}H Spectrum of **4**



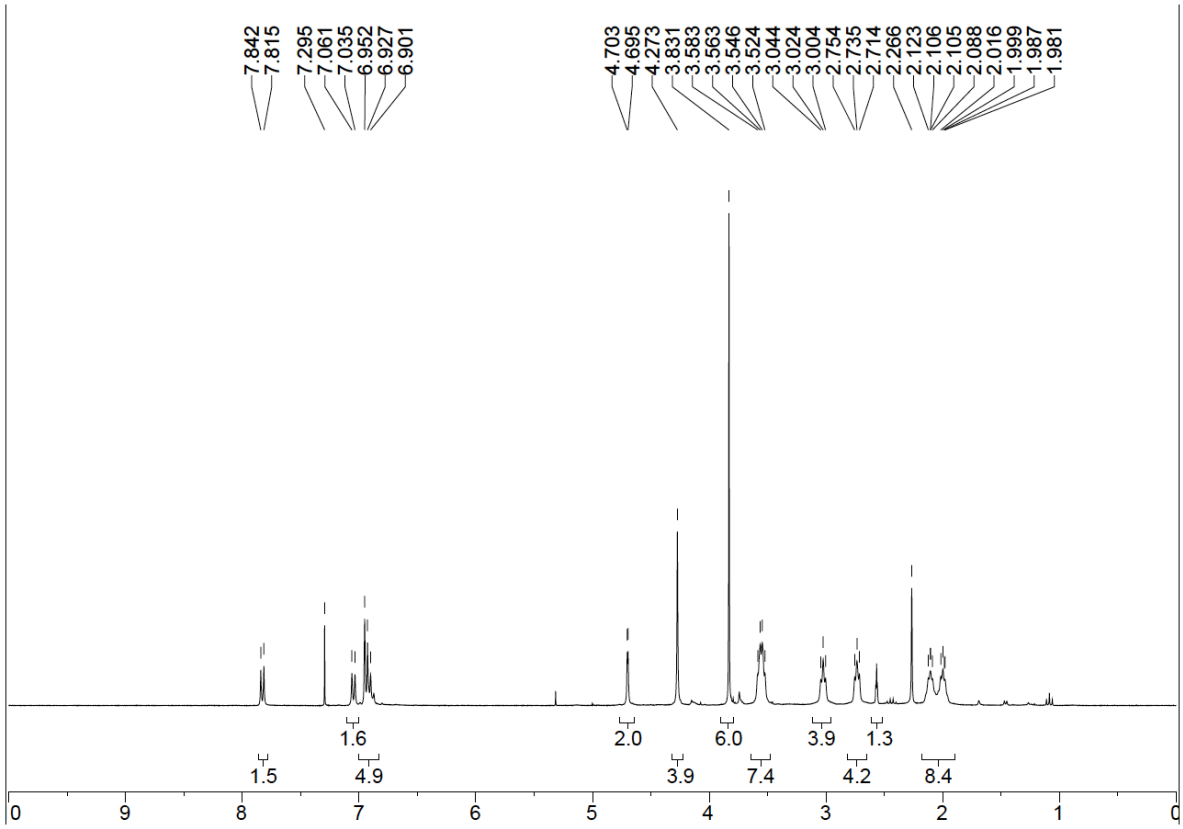
^{13}C Spectrum of **4**



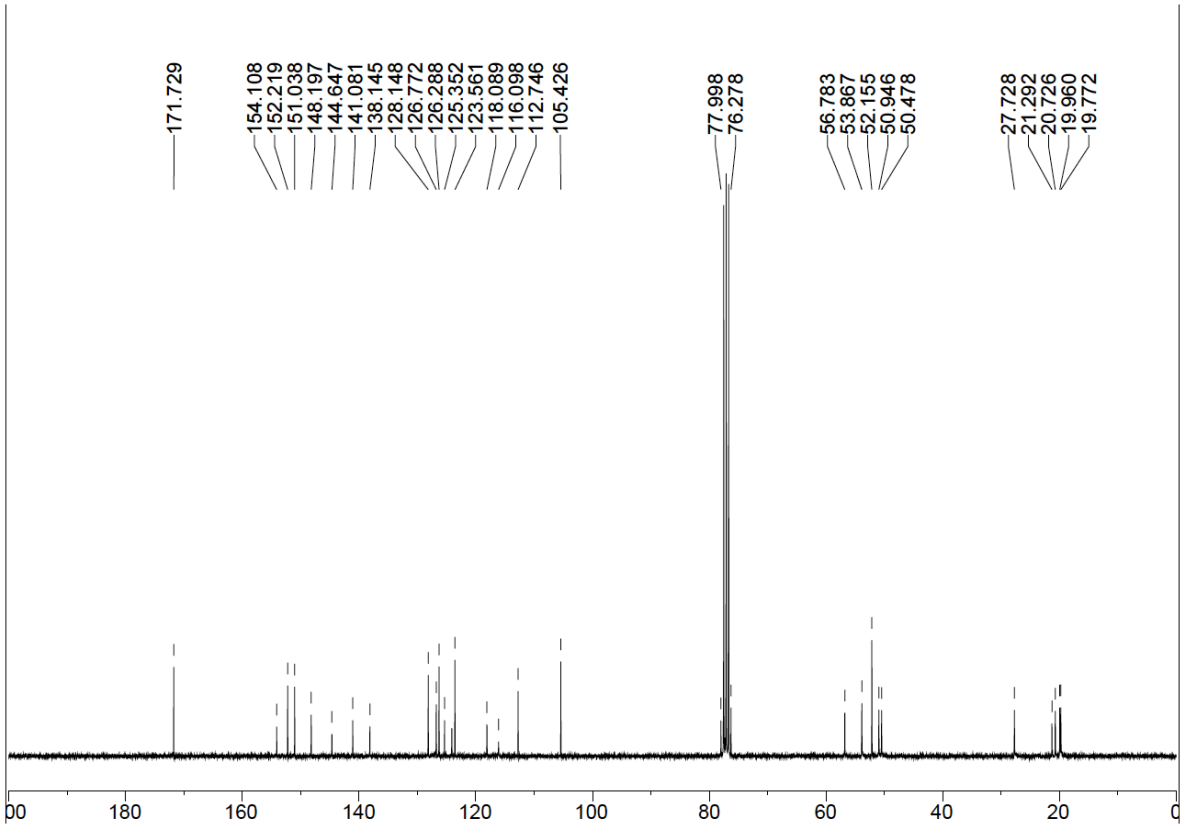
^{1}H Spectrum of **5**



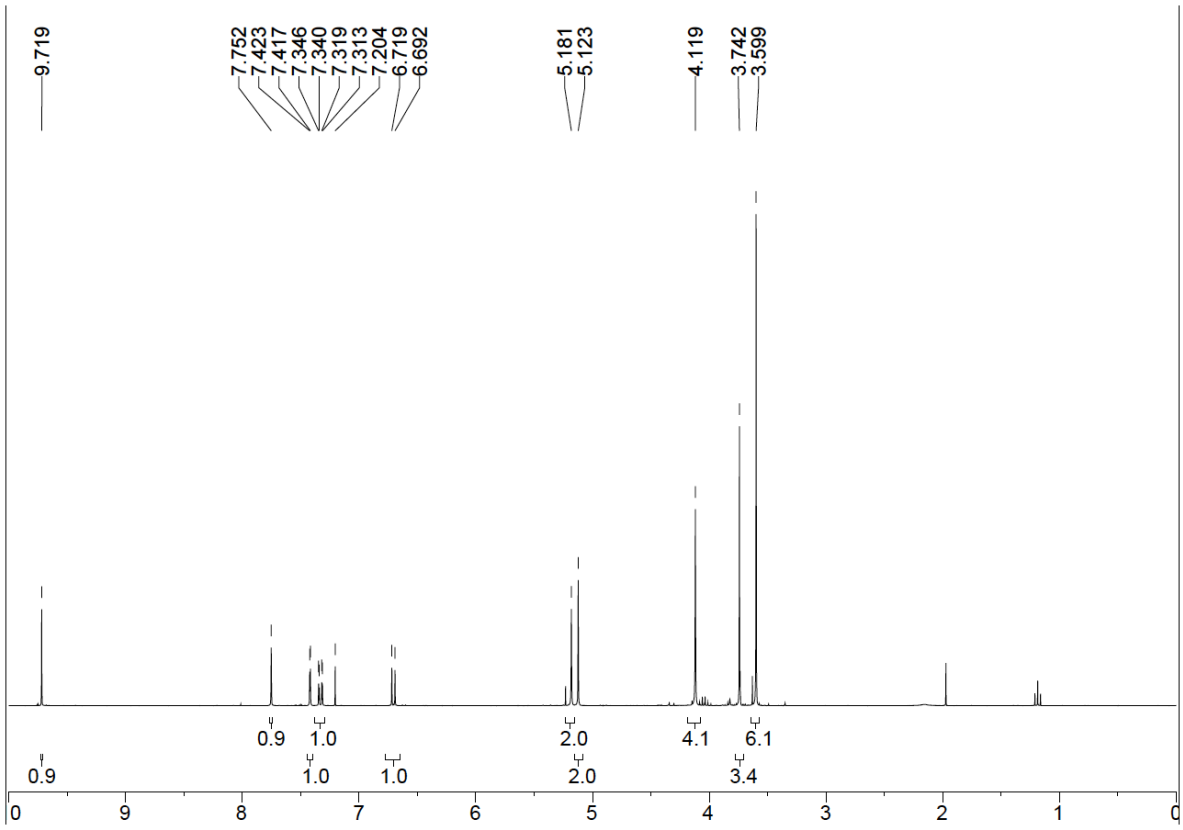
^{13}C Spectrum of **5**



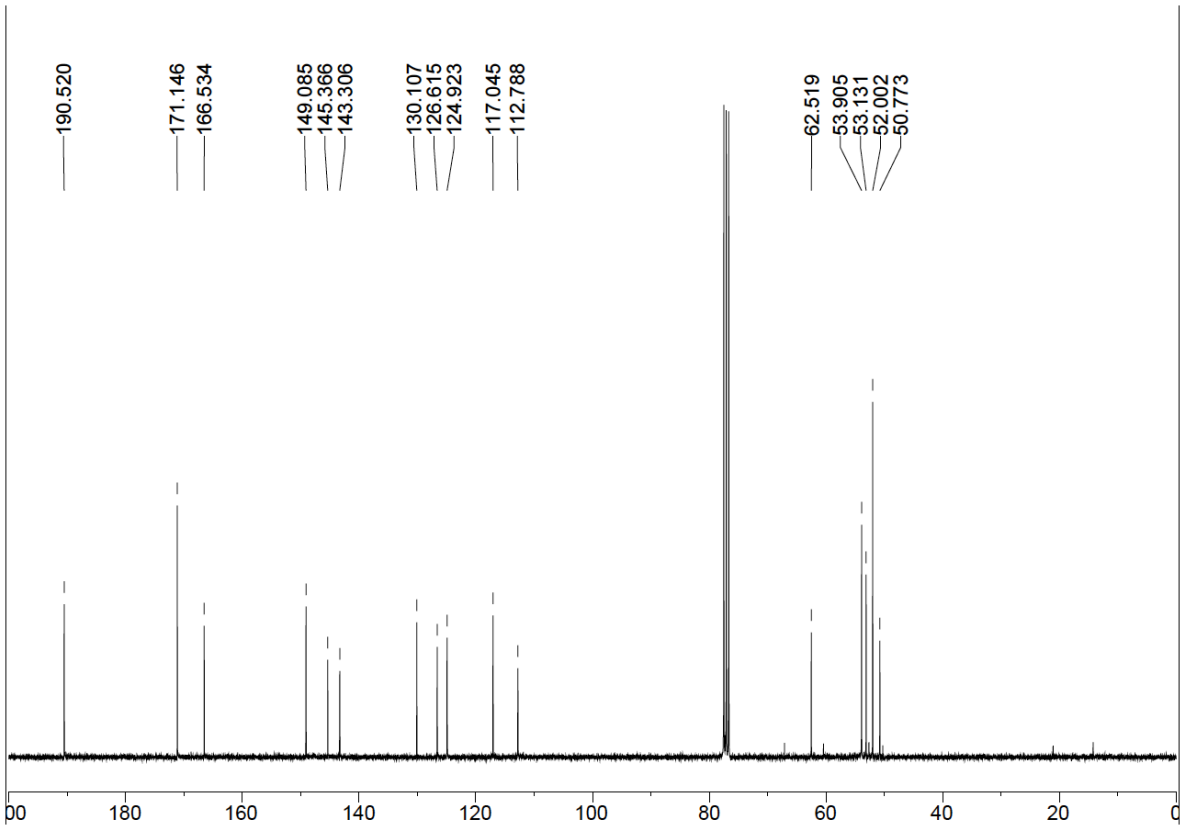
^{1}H Spectrum of **6**



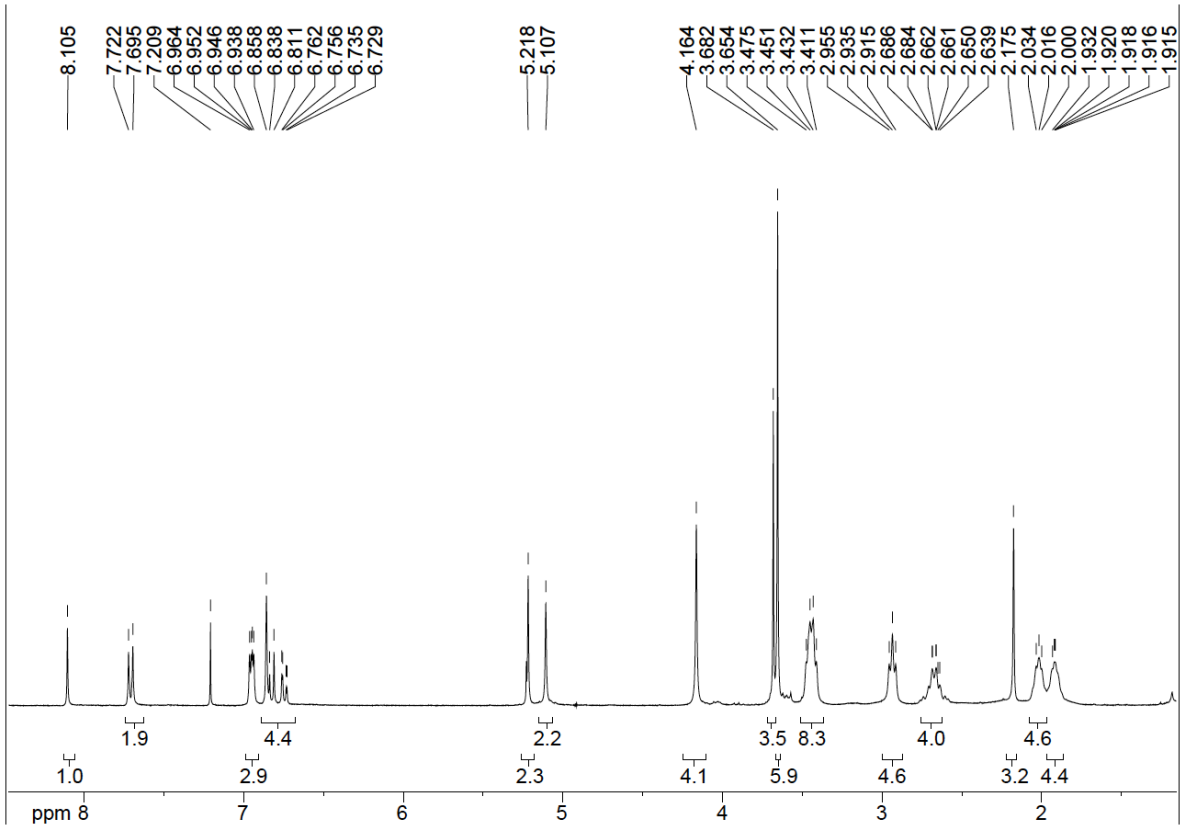
^{13}C Spectrum of **6**



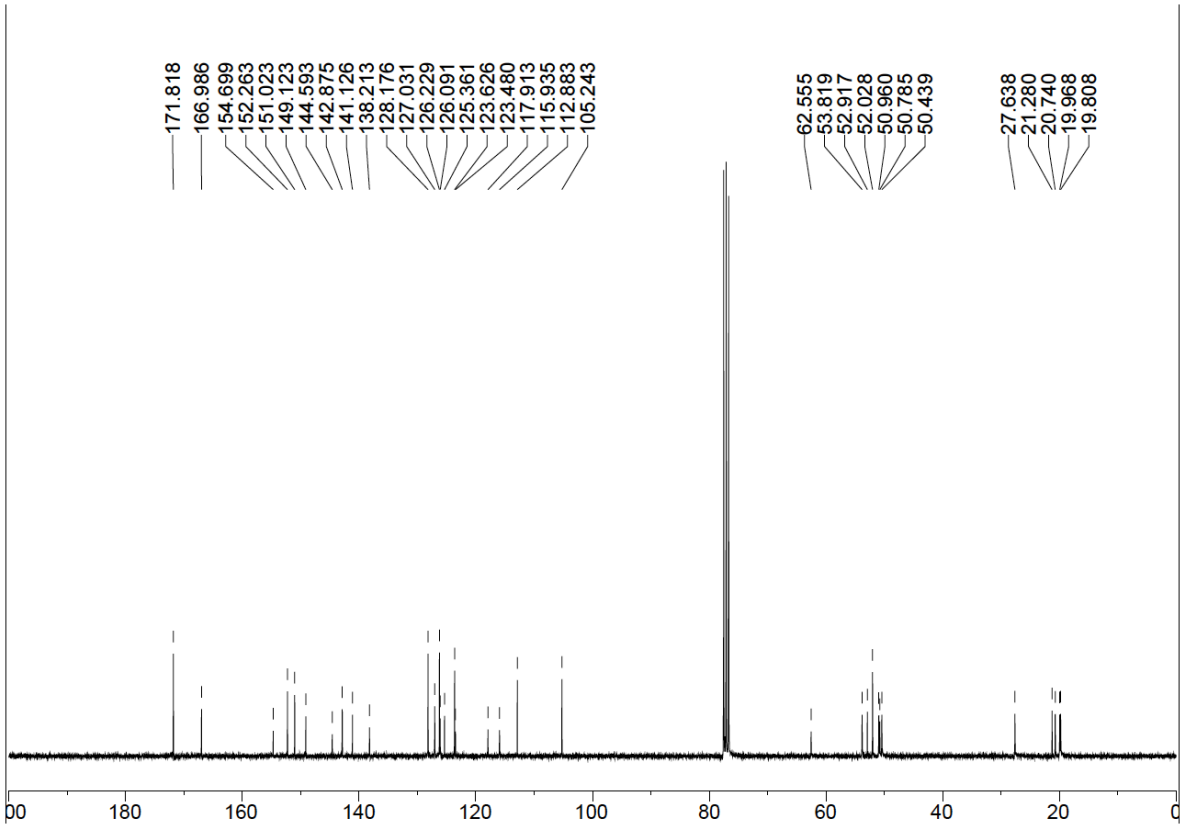
^{1}H Spectrum of **8**



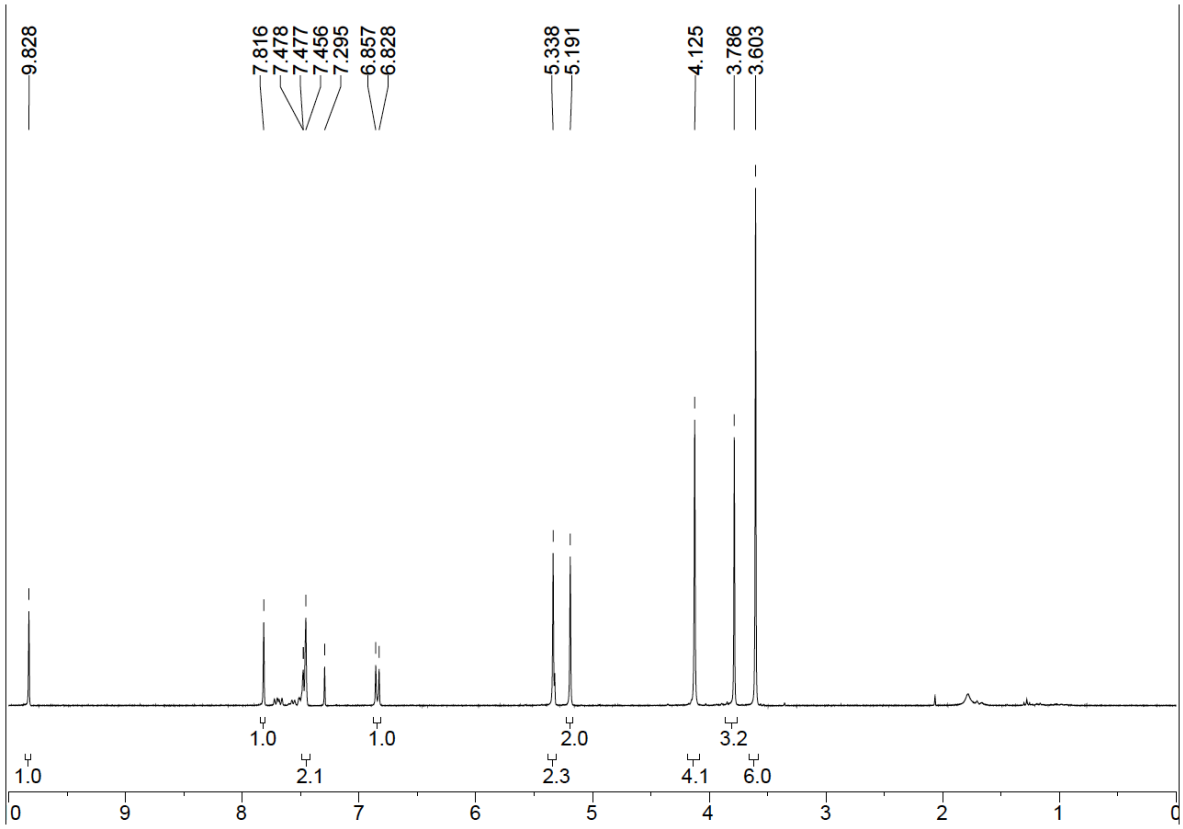
^{13}C Spectrum of **8**



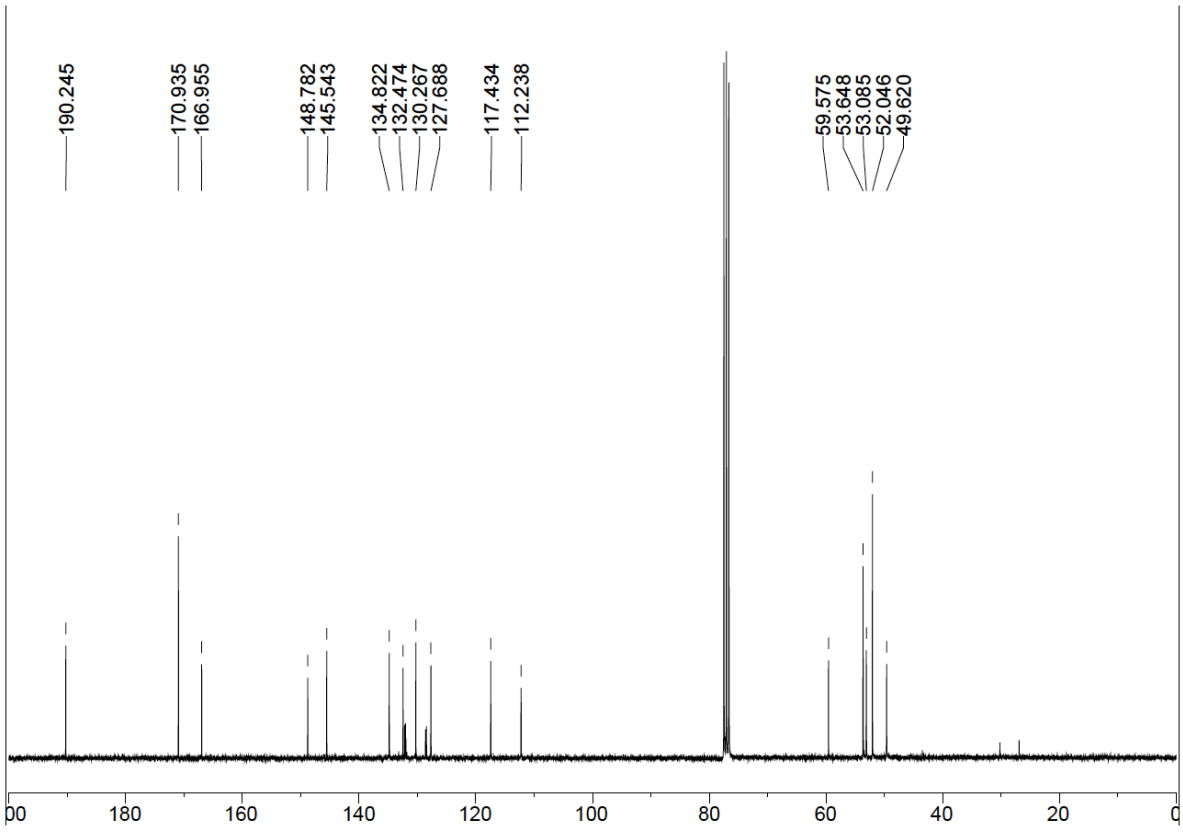
^{1}H Spectrum of **9**



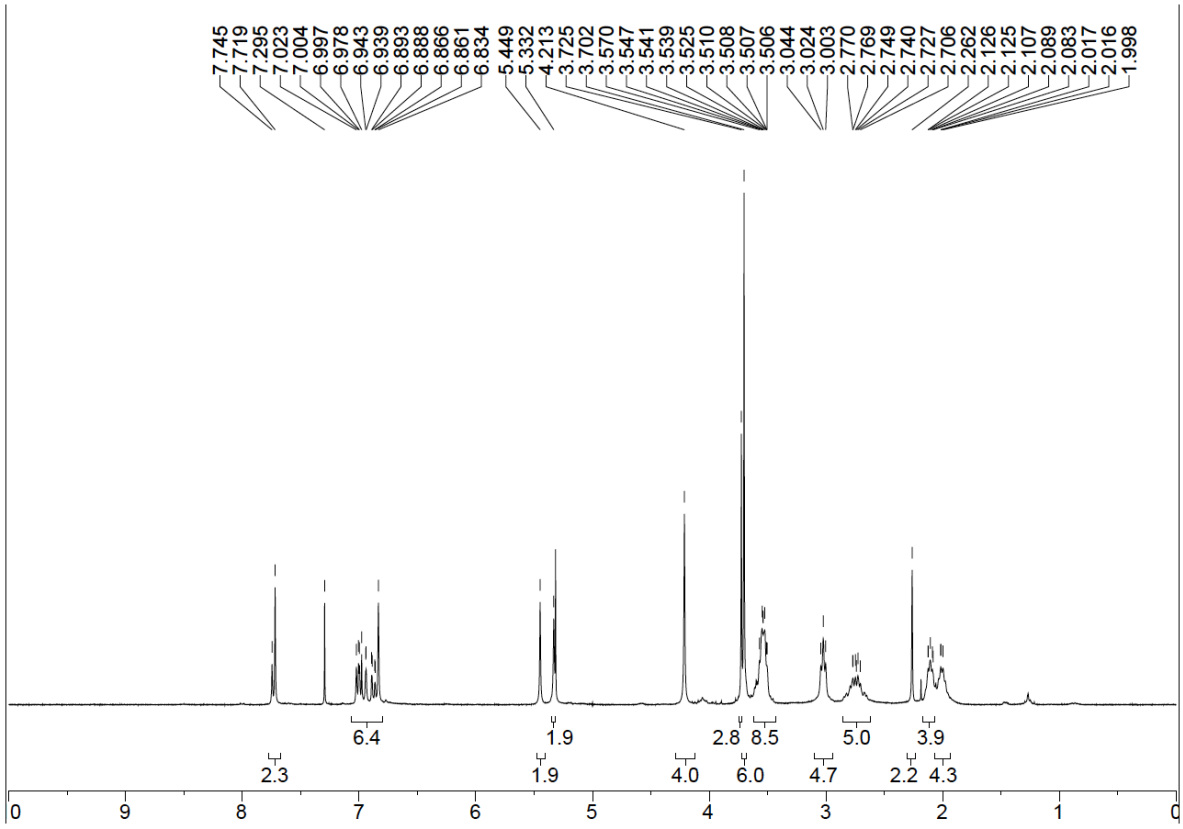
^{13}C Spectrum of **9**



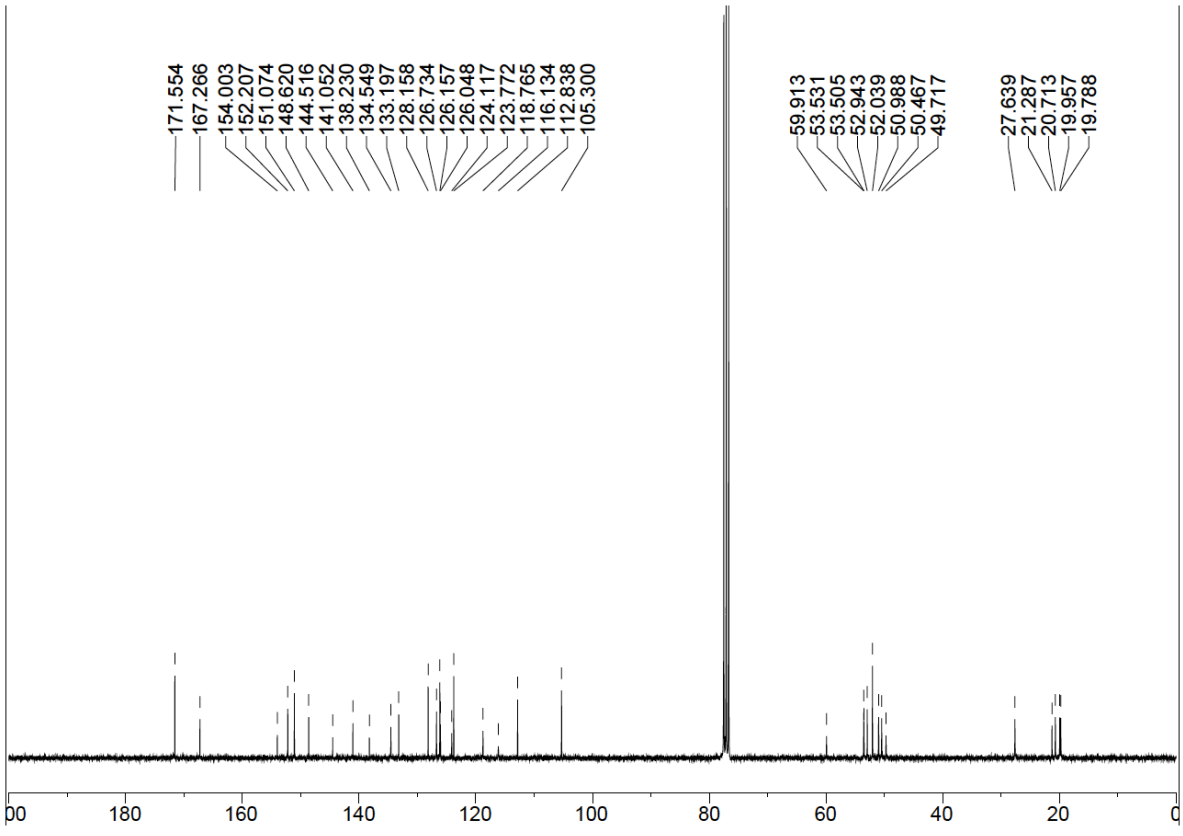
^{1}H Spectrum of **11**



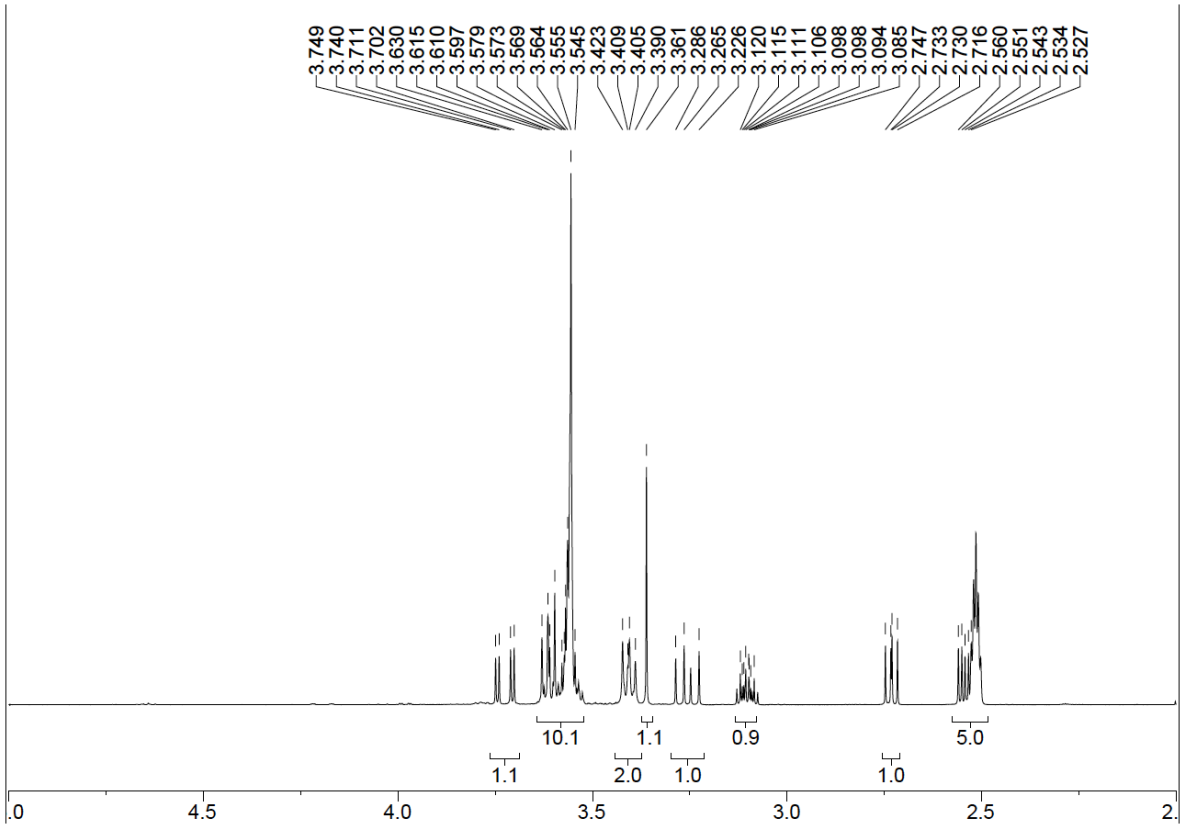
^{13}C Spectrum of **11**



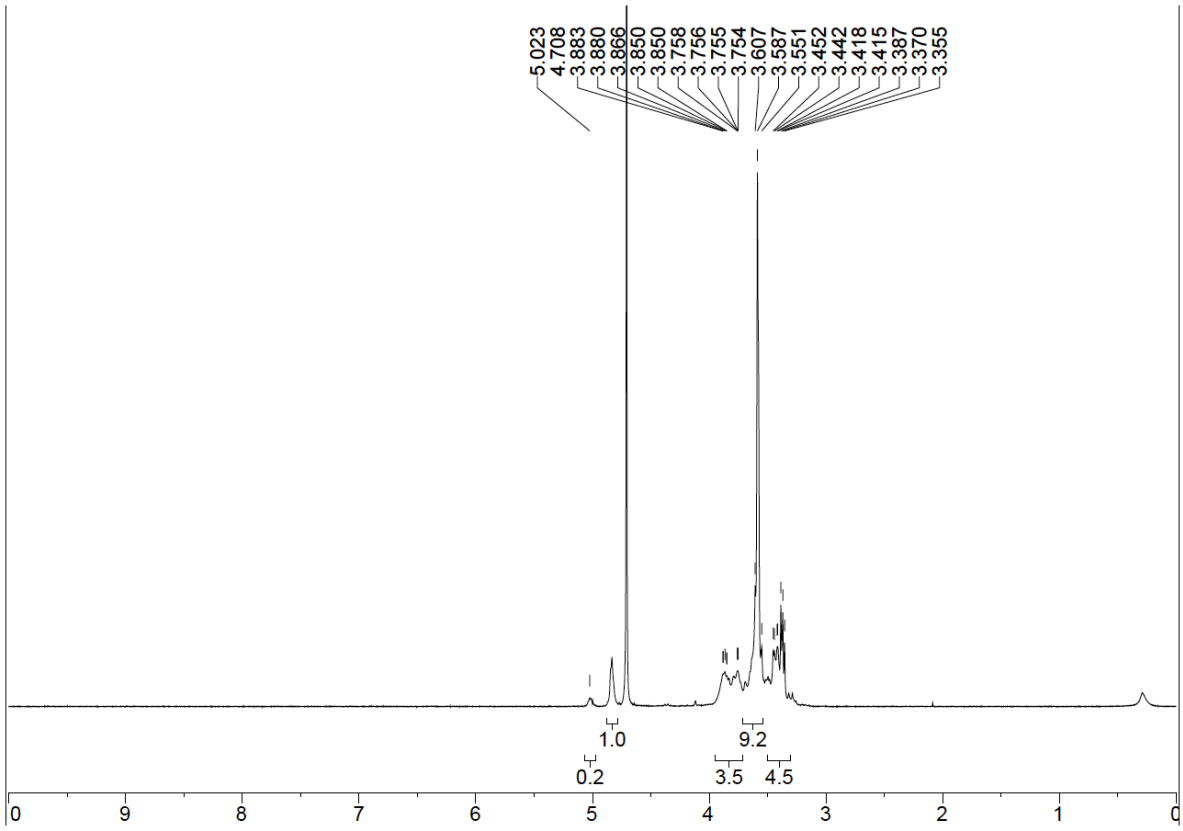
^{1}H Spectrum of **12**



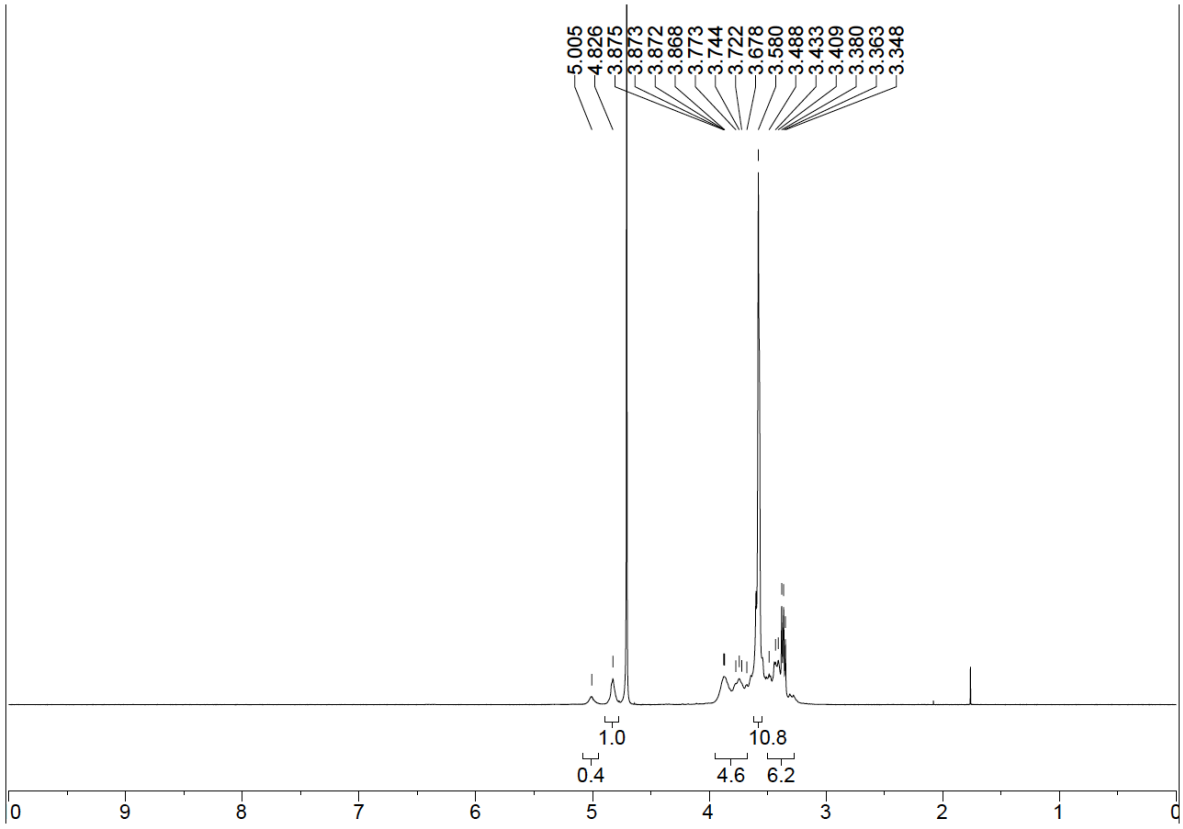
^{13}C Spectrum of **12**



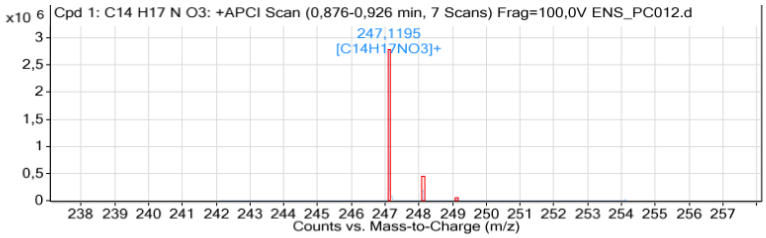
^{1}H Spectrum of **14**



^{1}H Spectra of Dextran 1,500-PEG-N_3 (D_2O)



^{1}H Spectra of Dextran 6,000-PEG-N_3 (D_2O)



m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
247,1195	247,1203	3,13	1	2790143,02	C14H17NO3	M+
248,1263	248,1235	-11,11	1	205780,25	C14H17NO3	M+
249,1287	249,126	-10,77	1	26640,04	C14H17NO3	M+

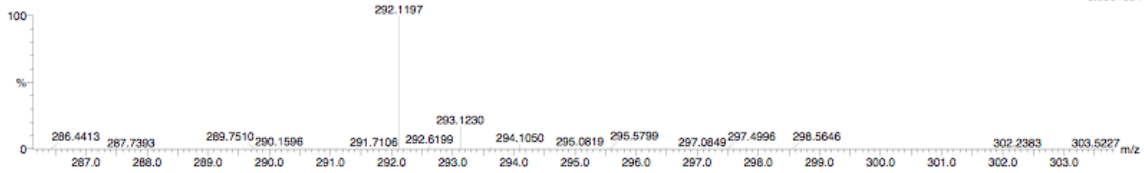
HRMS Spectra of **2**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
271 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-46 H: 0-100 N: 0-8 O: 0-8

LCT Premier XE KE483
1: TOF MS ES+
29-Mar-2013
COL_PC015 30 (0.769) Cm (30:32-9:10x3.000)
3.99e+004

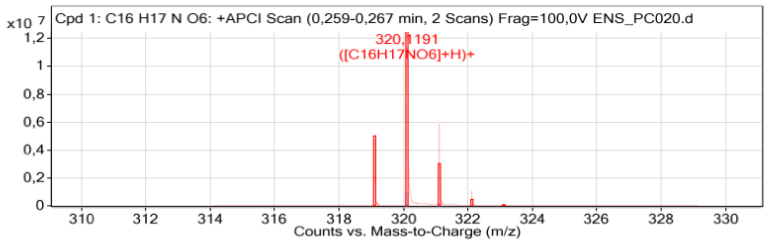



```
Minimum:                              -1.5
Maximum:                    5.0       10.0      80.0

Mass        Calc. Mass      mDa       PPM       DBE       i-FIT     i-FIT (Norm)  Formula

292.1197    292.1185        1.2       4.1       7.5       62.5      0.1           C15  H18  N  O5
            292.1198        -0.1      -0.3      12.5      65.1      2.7           C16  H14  N5  O
            292.1217        -2.0      -6.8      -0.5      68.8      6.4           C4  H18  N7  O8
```

HRMS Spectra of **4**



m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
319,1049	319,105	0,5	1	5042070,84	C16H17NO6	M+
320,1191	320,1129	-19,61	1	12960245,67	C16H17NO6	(M+H)+
321,1165	321,1161	-1,09	1	6184458,09	C16H17NO6	(M+H)+

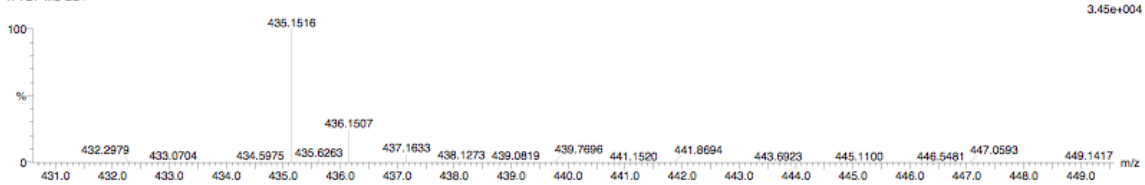
HRMS Spectra of **5**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
415 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-46 H: 0-100 N: 0-8 O: 0-8

LCT Premier XE KE483 29-Mar-2013 COL_PC021 25 (0.861) Cm (25:29-6:9x3.000)
1: TOF MS ES+ 3.45e+004



Minimum:				-1.5				
Maximum:		5.0	10.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula	
435.1516	435.1516	0.0	0.0	10.5	68.5	1.0	C19 H23 N4 O8	
	435.1529	-1.3	-3.0	15.5	68.9	1.5	C20 H19 N8 O4	
	435.1497	1.9	4.4	23.5	71.5	4.0	C31 H19 N2 O	
	435.1556	-4.0	-9.2	14.5	68.4	0.9	C24 H23 N2 O6	

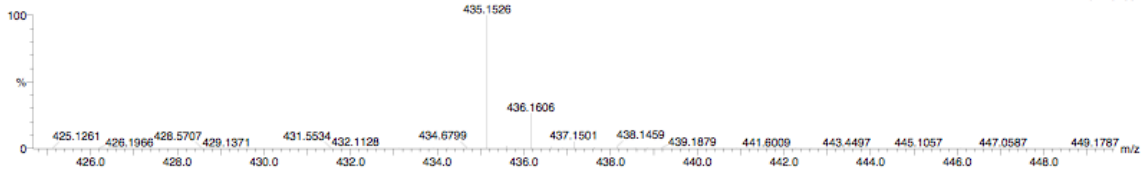
HRMS Spectra of **8**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
415 formula(e) evaluated with 5 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-46 H: 0-100 N: 0-8 O: 0-8

LCT Premier XE KE483 29-Mar-2013 COL_MC514 20 (0.534) Cm (18:20-9:11x3.000)
1: TOF MS ES+ 6.41e+004



Minimum:				-1.5				
Maximum:		5.0	10.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula	
435.1526	435.1529	-0.3	-0.7	15.5	79.7	2.2	C20 H19 N8 O4	
	435.1516	1.0	2.3	10.5	80.7	3.3	C19 H23 N4 O8	
	435.1497	2.9	6.7	23.5	84.3	6.9	C31 H19 N2 O	
	435.1556	-3.0	-6.9	14.5	77.6	0.2	C24 H23 N2 O6	
	435.1569	-4.3	-9.9	19.5	81.1	3.7	C25 H19 N6 O2	

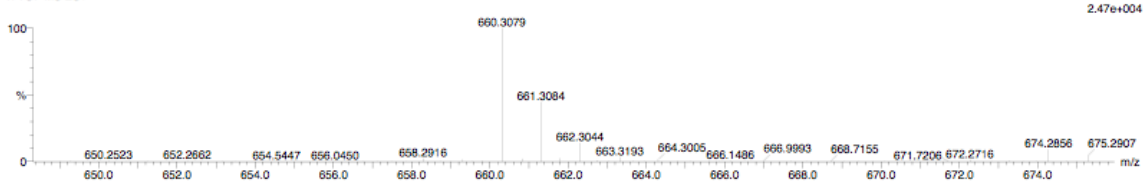
HRMS Spectra of **11**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
525 formula(e) evaluated with 5 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-46 H: 0-100 N: 0-8 O: 0-8

LCT Premier XE KE483 29-Mar-2013 COL_MC498 21 (0.571) Cm (20:25-5:8x3.000)
1: TOF MS ES+ 2.47e+004



Minimum:				-1.5				
Maximum:		5.0	10.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula	
660.3079	660.3074	0.5	0.8	21.5	58.0	0.7	C40 H42 N3 O6	
	660.3087	-0.8	-1.2	26.5	58.8	1.4	C41 H38 N7 O2	
	660.3114	-3.5	-5.3	25.5	59.4	2.1	C45 H42 N O4	
	660.3033	4.6	7.0	17.5	59.7	2.4	C35 H42 N5 O8	
	660.3127	-4.8	-7.3	30.5	60.5	3.1	C46 H38 N5	

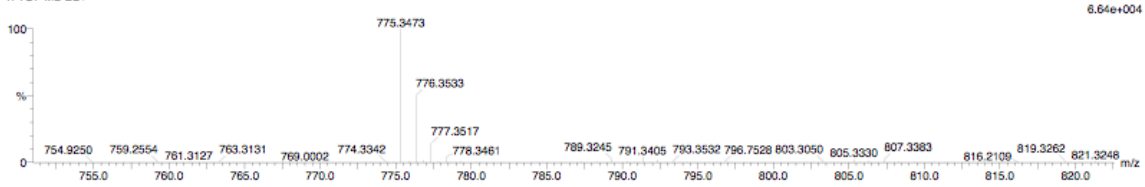
HRMS Spectra of **6**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
297 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-46 H: 0-100 N: 0-10 O: 0-8

LCT Premier XE KE483 29-Mar-2013 COL_MC474 21 (0.571) Cm (19:23-6:10x3.000)
1: TOF MS ES+ 6.64e+004



Minimum:				-1.5			
Maximum:		5.0	10.0	80.0			
Mass	**Calc. Mass**	**mDa**	**PPM**	**DBE**	**i-FIT**	**i-FIT (Norm)**	**Formula**
775.3473	775.3455	1.8	2.3	23.5	56.0	0.0	C43 H47 N6 O8
	775.3469	0.4	0.5	28.5	59.9	3.9	C44 H43 N10 O4

HRMS Spectra of **9**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
192 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-46 H: 0-100 N: 0-8 O: 0-8

LCT Premier XE KE483 29-Mar-2013 COL_MC517 21 (0.572) Cm (20:28-5:14x3.000)
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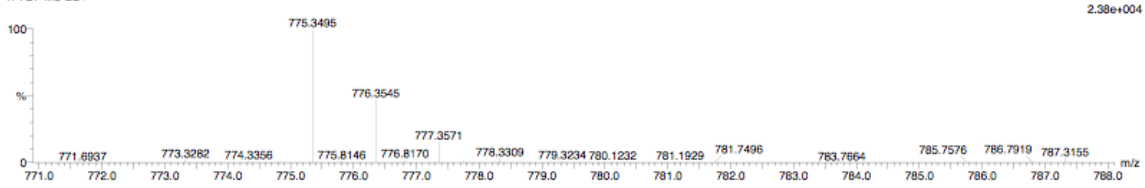


Minimum:				-1.5			
Maximum:		5.0	10.0	80.0			
Mass	**Calc. Mass**	**mDa**	**PPM**	**DBE**	**i-FIT**	**i-FIT (Norm)**	**Formula**
775.3495	775.3455	4.0	5.2	23.5	51.7	0.4	C43 H47 N6 O8
	775.3568	-7.3	-9.4	23.5	52.4	1.1	C42 H47 N8 O7

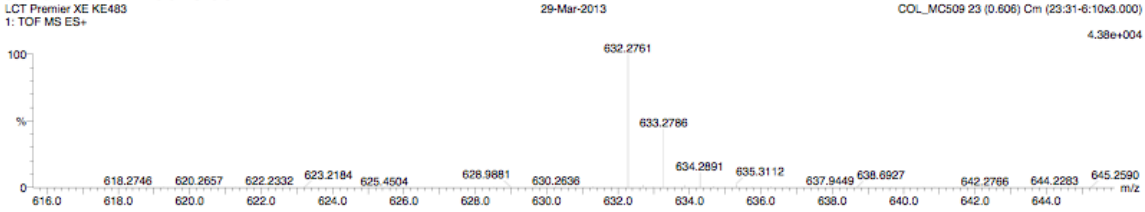
HRMS Spectra of **12**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

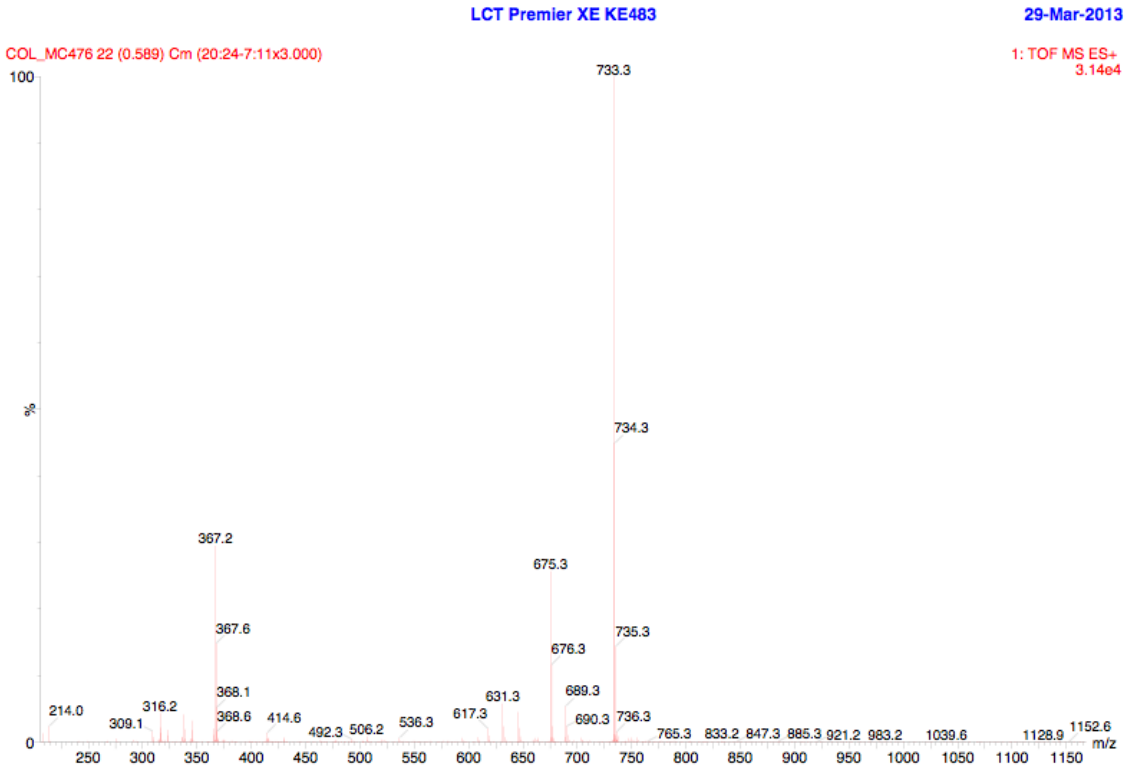
Monoisotopic Mass, Even Electron Ions
554 formula(e) evaluated with 6 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-46 H: 0-100 N: 0-8 O: 0-8

LCT Premier XE KE483 29-Mar-2013 COL_MC509 23 (0.606) Cm (23:31-6:10x3.000)
1: TOF MS ES+ 4.38e+004



Minimum:				-1.5			
Maximum:		5.0	10.0	80.0			
Mass	**Calc. Mass**	**mDa**	**PPM**	**DBE**	**i-FIT**	**i-FIT (Norm)**	**Formula**
632.2761	632.2761	0.0	0.0	21.5	74.1	0.0	C38 H38 N3 O6
	632.2774	-1.3	-2.1	26.5	78.0	3.9	C39 H34 N7 O2
	632.2801	-4.0	-6.3	25.5	80.4	6.3	C43 H38 N O4
	632.2720	4.1	6.5	17.5	81.3	7.2	C33 H38 N5 O8
	632.2814	-5.3	-8.4	30.5	81.9	7.8	C44 H34 N5
	632.2702	5.9	9.3	30.5	82.3	8.2	C45 H34 N3 O

HRMS Spectra of **7**

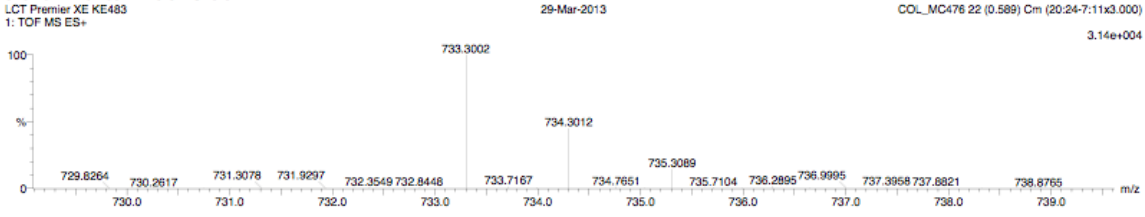


MS Spectra of **10**

Elemental Composition Report **Page 1**

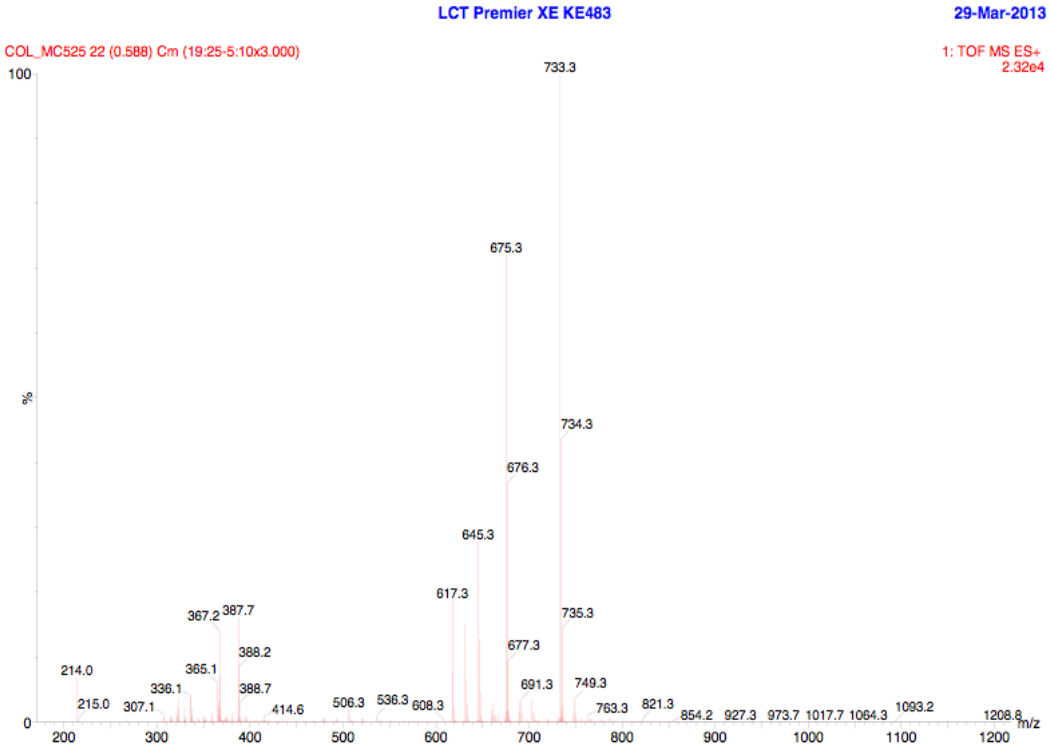
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
320 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-46 H: 0-100 N: 0-8 O: 0-8



Minimum:				-1.5			
Maximum:		5.0	10.0	80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
733.3002	733.2986	1.6	2.2	23.5	70.3	0.2	C40 H41 N6 O8
	733.3026	-2.4	-3.3	27.5	72.0	1.9	C45 H41 N4 O6
	733.3039	-3.7	-5.0	32.5	73.1	3.1	C46 H37 N8 O2

HRMS Spectra of **10**

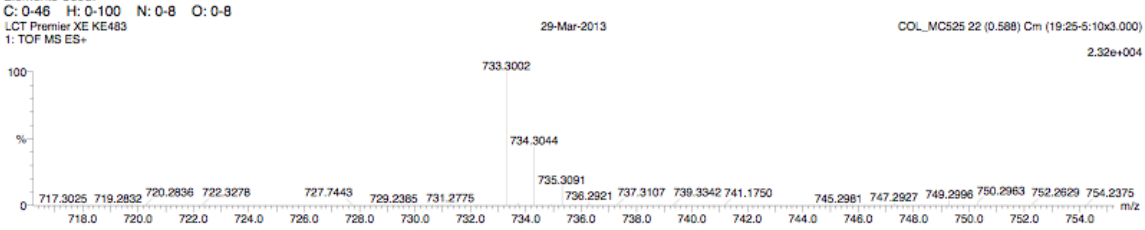


HRMS Spectra of **13**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
320 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-46 H: 0-100 N: 0-8 O: 0-8



Minimum:		5.0	10.0	-1.5			
Maximum:				80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
733.3002	733.2986	1.6	2.2	23.5	60.2	0.4	C40 H41 N6 O8
	733.3026	-2.4	-3.3	27.5	61.1	1.3	C45 H41 N4 O6
	733.3039	-3.7	-5.0	32.5	62.4	2.6	C46 H37 N8 O2

HRMS Spectra of **13**