Supplementary Material (ESI) for Chemical Communications

Ru(II)-Carbohydrate Dendrimers as Photoinduced Electron Transfer Lectin Biosensors

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1. General Information

All chemicals used were reagent grade and used as supplied except where noted. Dichloromethane (CH_2Cl_2) was purified by a Cycle-Tainer Solvent Delivery System. Triethylamine was distilled over CaH_2 prior to use. Analytical thin layer chromatography (TLC) was performed on Merck

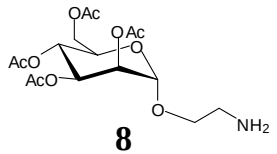
silica gel 60 F_{254} plates (0.25 mm). Compounds were visualized by UV irradiation or dipping the plate in CAN solution followed by heating. Flash column chromatography was carried out using force flow of the indicated solvent on Fluka Kieselgel 60 (230-400 mesh).

^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300 (300 MHz) or Bruker DRX500 (500 MHz) spectrometer. High-resolution mass spectra (HR MALDI MS) were performed by the Mass Spectrometry-service at the Laboratory for Organic Chemistry (ETH Zurich). ESI-MS were run on an Agilent 1100 Series LC/MSD instrument. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotation measurements were conducted using a Perkin-Elmer 241 polarimeter.

$RuCl_3 \cdot x\ H_2O$ and 2,3,4,5,6-pentafluorophenol were purchased from Fluka. Acrylonitrile was purchased from Alfa Aesar and used directly in the reaction. ConcanavalinA and GNA were purchased from Appli Chem (Axon Lab AG). Synthesis of 2,2'-bipyridine-4,4'-dicarboxylic acid, *cis*-$Ru(bipy)_2Cl_2$ and BBV was carried out as described previously.[1] Absorption spectra were recorded using a Varian CARY 50 spectrophotometer fitted with Hellma optical fibers (Hellma, 041.002-UV) and an immersion probe made of quartz suprazil (Hellma, 661.500-QX). Fluorescence emission spectra were recorded on a Perkin-Elmer LS-50B spectrofluorometer.

2. Synthesis of 1 and 2.

Carbohydrate Synthesis. The 2'-ethylamino glycosides (**8**) were synthesized according to published procedures.[2]



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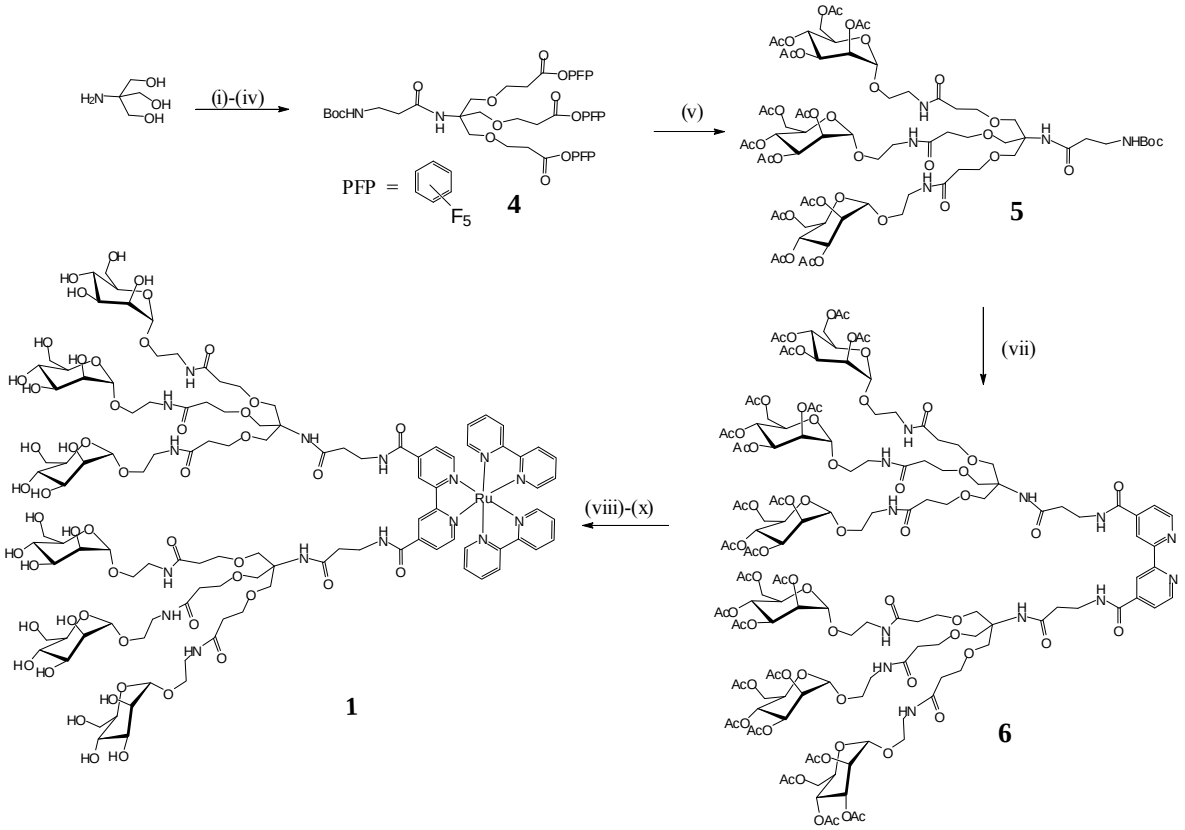


Scheme 1. Reagents and Conditions: (i) Acrylonitrile /NaOH (40%), 72%; (ii) Conc. HCl/EtOH, 51% ; (iii) Boc-β-ala/DIC/HOBT/DCM, 63%; (iv) NaOH/MeOH; pentafluorophenol (PFP)/DIC/HOBT/DCM, 47%; (v) Comp **8**/DCM/TEA, 47%; (vi) TFA/DCM; bipyridine-COCl/DCM/TEA, 25%; (viii) *cis*-$Ru(bipy)_2Cl_2$/EtOH, 49%; (ix) NaOMe/MeOH, 61% .

General Procedures:

General Procedure A: Synthesis of Sugar-tripods.

The Boc-protected amino-sugar (4.0 eq) was dissolved in 10 mL dichloromethane/trifluoroacetic acid (3:1) and stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure and the resulting oil was dissolved in anhydrous dichloromethane (20 mL). To this mixture, was added *tert*-butoxycarbonyl-3-{*N*-{tris[3-[pentafluoro-phenyl-carboxyl-ethoxy)methyl]} methyl amine}-3-*β*-alanine (1.0 eq), pH adjusted to 8 with triethylamine (TEA) and the mixture stirred at room temperature for 12 h. The solvent was evaporated *in vacuo* and purified by flash silica column chromatography.

General Procedure B: Synthesis of Bipyridine Derivatives.

2,2'Bipyridine-4,4'-dicarboxylic acid (1.0 eq) was dissolved in $SOCl_2$ (1 mL) and refluxed under nitrogen for 12 h. Excess $SOCl_2$ was removed in *vacuo* and the crude 2,2'bipyridine-4,4'-dicarboxylic acyl chloride was used directly in the next step. Boc-protected amino-sugar-tripod (3.0 eq) was dissolved dichloromethane/trifluoroacetic acid (10 mL, 3:1 resp.) and stirred at room temperature for 1 h. The mixture was concentrated *in vacuo* and then redissolved in dichloromethane (20 mL). To this mixture was added 2,2'bipyridine-4,4'-dicarboxylic acyl chloride (1 eq) and the pH adjusted using TEA to pH 8. The reaction mixture was stirred for 12 h, the solvent removed *in vacuo* and the mixture purified by silica column flash chromatography.

General Procedure C: Synthesis of Ruthenium(II)-complexes.

The bipyridine-sugar derivative (1.0 eq) and *cis*-ruthenium(II)bis(bipyridine)dichloride (1.1 eq) were dissolved in de-oxygenated ethanol (30 mL) and the mixture was refluxed for 6-8 h. The compound was then purified by silica column flash chromatography.

General Procedure D: Synthesis of Ruthenium(II)-sugar Complexes.

Ruthenium(II) complex (1.0 eq) and sodium methoxide (0.2 eq) were dissolved in methanol (10 ml) and stirred at room temperature for 2 h. The solvent was then evaporated *in vacuo*, the residue was redissolved in water and dialyzed against water using 500 molecular weight cut-off resin. After two days of dialysis, the sample was lyophilized.

(i) ***N*-{Tris[(2-ethylcarboxyl-ethoxy)methyl]}methylamine** a known compound, was synthesized using a new procedure.[2] *N*-{*tris*[(2-cyanoethoxy)methyl]} methylamine[3] (5 g, 17.6 mmol) was dissolved in HCl (5 mL, 36%) and refluxed for 4 h. The reaction mixture was dissolved in ethanol (30 mL) and refluxed for 12 h before the solvent was distilled off. Sodium hydroxide (aqueous, 5N) was added to the white precipitate until the mixture reached pH 8. EtOAc (30 mL) was added and the organic layer was washed three times with water. Purification by flash silica column chromatography afforded (3.95 g, 51%) of *N*-{tris[(2-ethylcarboxyl-ethoxy) methyl]}methylamine. R_f = 0.5 (CH_2Cl_2/MeOH = 95:5); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.15 (q, J = 7.2 Hz, 6H), 3.68 (t, J = 6.3 Hz, 6H,), 3.30 (s, 6H), 2.54 (t, 6H, J = 6.3 Hz), 1.59 (br.s, 2H), 1.25 (t, 9H, J = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.4, 155.8, 72.7, 66.7, 60.3, 34.8, 14.1, LC-MS (*m/z*): 422.2 $(M+H)^+$; FTIR($CHCl_3$): 3379, 2982, 2873, 1736 cm^{-1}.

(ii) ***tert*-Butoxycarbonyl-3-{*N*-{tris[3-[ethylcarboxyl-ethoxy)methyl]}methylamide}-3-*β*-alanine**. To a solution of *N*-{tris[(3-[ethylcarboxyl-ethoxy)methyl]}methylamine (3 g, 7.12 mmol) and Boc-*β*-alanine (1.3 g, 7.14 mmol) in dichloromethane (10 mL) at 0 °C, were added diisopropyl carbadiazime (1.35 ml, 8.56 mmol) and 1-hydroxybenzotriazole (9 mg, 0.71 mmol) The reaction mixture was stirred at room temperature for 12 h and concentrated *in vacuo*. The crude residue was purified by flash silica column chromatography to yield *tert*-butoxycarbonyl-3-{*N*-{tris[3-

[ethylcarboxyl-ethoxy)methyl]}methylamide}-3-*β*-alanine (2.65 g, 63%). R_f = 0.5 (CH_2Cl_2/MeOH, 98:2); ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 4.05 (q, *J* = 6.9 Hz, 6H), 3.59 (br. s, 12H), 3.27 (q, *J* = 6.6 Hz, 2H), 2.44 (t, *J* = 6.0 Hz, 6H), 2.25 (t, *J* = 6.6 Hz, 2H), 1.33 (s, 9H); 1.16 (t, *J* = 6.9 Hz, 9H), ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 173.1, 172.9, 79.5, 75.8, 70.9, 67.3, 61.5, 41.2, 36.8, 33.6, 28.5, 14.1, LC-MS (*m/z*): 593.4 $(M+H)^+$; FTIR($CHCl_3$): 3390, 2982, 2873, 1734, 1726, 1643, 1521 cm^{-1}.

(iii) *tert*-Butoxycarbonyl-3-{*N*-{tris[3-[pentafluoro phenyl carboxyl-ethoxy)methyl]}methyl amide}-3-*β*-alanine (4). *tert*-Butoxycarbonyl-3-{*N*-{tris[3-[ethylcarboxyl-ethoxy)methyl]} methylamine}-3-*β*-alanine (2.0 g, 3.37 mmol) was dissolved in ethanol (20 mL) and sodium hydroxide solution (aqueous, 1 N, 2 mL) were added and the mixture was stirred at room temperature for 2 h, concentrated in *vacuo*, the pH adjusted to 5 with hydrochloric acid (aqueous 1 N, 3 mL) and extracted with ethyl acetate. The organic layer was dried with sodium sulfate and concentrated to dryness under reduced pressure. The residue was dissolved in dichloromethane (10 mL) and 2,3,4,5,6-pentafluorophenol (2.47 g, 13.48 mmol) was added. After cooling to 0 $^{\circ}$C, diisopropyl carbadiazine (2.54 ml, 16.17 mmol) was added and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was concentrated in *vacuo* and purified by silica column flash chromatography to afford *tert*-butoxycarbonyl-3-{*N*-{tris[3-[pentafluorophenylcarboxyl-ethoxy)methyl]}methylamide}-3-*β*-alanine (1.62 g, 47%). R_f = 0.6 (CH_2Cl_2/EtOH, 92:8); ^{1}H NMR (300MHz, $CDCl_3$): *δ* 5.17 (br.s, 1H); 4.13 (q, *J* = 7.2 Hz, 6H); 3.81 (t, *J* = 6.0 Hz, 6H); 3.77 (s, 6H); 3.34-3.32 (m, 2H); 2.92 (t, *J* = 6.0 Hz, 6H); 2.35 (t, *J* = 6.0 Hz, 2H); 1.42 (s, 9H); ^{13}C NMR (75MHz, $CDCl_3$): *δ* 172.1, 167.5, 142.7, 142.5, 139.4, 136.1, 79.5, 68.9, 65.9, 59.5, 36.6, 34.0, 28.2, FTIR($CHCl_3$): 3688, 3385, 1749, 1658, 1522, 1359 cm^{-1}. HRMS (MALDI-ToF) (*m/z*) calcd. for $C_{39}H_{33}F_{15}N_5O_{12}Na$ 1029.1692, found: 1029.1695.

(iv) *tert*-Butoxycarbonyl-3-{tris[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside-ethoxy]methyl]methylamide}-3-*β*-alanine (5). General procedure A using 2-(*tert*-butoxycarbonylamino)ethoxy-2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside **8** (0.97 g, 1.96 mmol), *tert*-butoxycarbonyl-3-{*N*-{tris[3-[pentafluoro-phenyl-carboxyl-ethoxy)methyl]}methyl amine}-3-*β*-alanine **4** (0.5 g, 0.49 mmol) and purified by flash chromatography to yield *tert*-butoxycarbonyl-3-{tris[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside-ethoxy]methyl]methylamide}-3-*β*-alanine (0.37 g, 47%). R_f = 0.45 (CH_2Cl_2/MeOH, 93:7); $[\alpha]_D^{r.t}$ = +21.4 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 6.79 (br.s, 2H), 6.43 (br.s, 1H), 5.30 (br.s, 1H), 5.27-5.20 (m, 9H), 4.80 (s, 3H), 4.25 (dd, *J* = 9.0, 5.1 Hz, 3H), 4.10 (dd, *J* = 2.1, 9.9 Hz, 3H), 3.90 (br.s, 3H), 3.76 (dd, *J* = 4.5, 5.4 Hz,

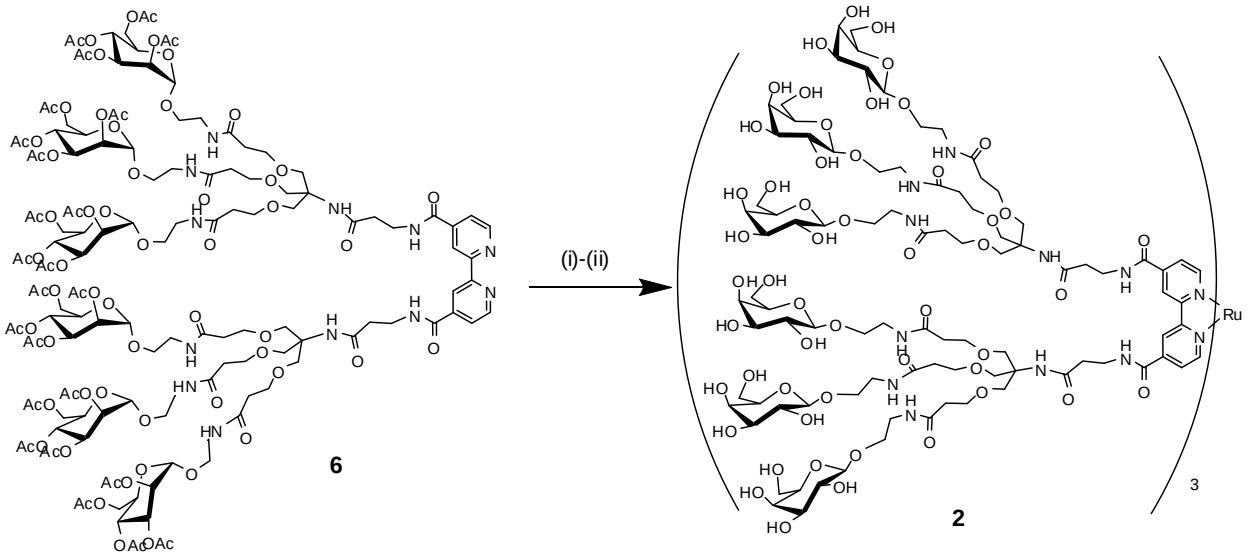
3H), 3.68 (dd, *J* = 5.4, 6.0 Hz, 6H), 3.64 (s, 6H), 3.54-3.52 (m, 6H), 3.37 (br.s, 8H), 2.42 (t, *J* = 5.4 Hz, 6H), 2.12 (s, 9H), 2.07 (s, 9H), 2.02 (s, 9H), 1.96 (s, 9H), 1.39 (s, 9H), ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 171.4, 170.5, 170.0, 169.5, 155.8, 97.6, 69.3, 69.2, 68.6, 67.3, 67.1, 66.0, 62.4, 59.8, 45.7, 39.0, 37.2, 36.9, 36.5, 28.5, 20.9; FTIR($CHCl_3$): 3376, 2918, 1751, 1663, 1515, 1457, 1250 cm^{-1}; HRMS (MALDI-ToF) (*m/z*) calcd. for $C_{69}H_{105}N_5O_{39}Na$ 1650.6284; found: 1650.6252.

(v) 1,1'-(2,2'-Bipyridine-4,4'-diyl)bis-3-*β*-propane-{tris-[3-4-ethoxy-2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside-ethoxy}methyl]methyl amide (6). General procedure B with *tert*-butoxycarbonyl-3-{tris[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside-ethoxy]methyl]methylamide}-3-*β*-alanine **5** (0.3 g, 0.18 mmol), 2,2'bipyridine-4,4'-dicarboxylic acid (14 mg, 0.0617 mmol) and flash silica column chromatography by using CH_2Cl_2/CH_3OH (8-9%) as eluent yielded 1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-β-propane-{tris[3-4-ethoxy-2,3,4,6-tetra-O-acetyl-*α*-D-mannopyranoside-ethoxy}methyl]methylamide (0.12 g, 25%). R_f = 0.5 (CH_2Cl_2/MeOH, 92:8); $[\alpha]_D^{r.t}$ = +17.5 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 8.85 (d, 2H, *J* = 4.5 Hz), 8.81 (br.s, 2H), 8.23 (t, *J* = 5.4 Hz, 6H), 7.85 (d, *J* = 4.5 Hz, 2H), 7.43 (br.s, 1H); 5.27-5.23 (m, 19H), 4.81 (s, 6H), 4.22 (dd, *J* = 9.0, 5.1 Hz, 6H), 4.10 (dd, *J* = 2.1, 9.9 Hz, 6H), 4.02 (br, 9H), 3.79-3.77 (m, 8H), 3.65 (br.s, 24H), 3.58-3.56 (m, 6H), 3.45 (t, *J* = 5.4 Hz, 12H), 2.62 (t, *J* = 6.9 Hz, 4H), 2.44 (t, *J* = 5.7 Hz, 12H), 2.13 (s, 18H), 2.05 (s, 18H), 2.03 (s, 18H), 1.95 (s, 18H); ^{13}C NMR (75MHz, $CDCl_3$): *δ* 173.7, 171.8, 171.0, 170.9, 167.3, 163.3, 162.9, 162.4, 161.9, 156.0, 150.1, 143.2, 123.4, 115.7, 98.5, 70.4, 69.8, 69.7, 69.5, 68.3, 67.4, 66.9, 63.2, 61.2, 54.5, 40.1, 39.9, 37.0, 20.5. FTIR($CHCl_3$): 3691 1671, 1456, 1451, 1396, 1357, 1263 cm^{-1}; HRMS-MALDI (m/z): $[M+ Na]^+$ Calcd for $C_{140}H_{197}N_{12}O_{76}Na$ 3286.1890; Found: 3286.199.

(vii) *Cis*-Ruthenium(II)bis(bipyridine){1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-*β*-propane-{tris-[3-4-ethoxy-2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside-ethoxy}methyl]methyl amide (7). General procedure C with *cis*-ruthenium(II)bis(bipyridine)dichloride (18 mg, 0.037 mmol) and 1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-β-propane-{tris-[3-4-ethoxy-2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside-ethoxy}methyl]methylamide (0.1 g, 0.031 mmol) purification by flash silica column chromatography by using acetronitrile/water/saturated KNO_3 (7.5:1:1.5) as eluent yielded *cis*-ruthenium(II)bis(bipyridine){1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-*β*-propane-{tris-[3-4-ethoxy-2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside-ethoxy}methyl] methylamide (54 mg, 49%). R_f = 0.5 (acetonitrile/sat aq. KNO_3, 80:20); $[\alpha]_D^{r.t}$ = +38.9 (c = 1.0, H_2O); ^{1}H NMR (300 MHz, CD_3OD): *δ*

9.18 (br.s, 2H); 8.72 (d, *J* = 7.1 Hz, 4H), 8.18 (t, *J* = 5.4 Hz, 4H), 8.14 (d, *J* = 4.8 Hz, 4H), 8.0 (d, *J* = 6.0 Hz, 2H), 7.85 (dd, *J* = 5.7, 4.8 Hz, 6H), 7.52 (t, *J* = 4.5 Hz, 4H), 7.36 (br.s, 1H), 5.27-5.2 (m, 18H), 4.82 (br.s, 6H), 4.22 (dd, *J* = 9.0, 5.1 Hz, 6H), 4.12-4.10 (m, 6H), 4.02 (br.s, 11H), 3.79-3.76 (m, 8H), 3.65 (br.s, 24H), 3.59-3.55 (m, 8H), 3.45 (t, *J* = 5.4 Hz, 12H), 2.62 (t, *J* = 6.9 Hz, 4H), 2.44 (t, *J* = 5.7 Hz, 12H), 2.13 (s, 18H), 2.04 (s, 18H), 2.03 (s, 18H), 1.95 (s, 18H); ^{13}C NMR (75MHz, CD_3OD): *δ* 173.7, 173.2, 171.9, 171.1, 171.0, 165.2, 163.2, 158.5, 157.8, 153.1, 152.5, 152.1, 143.3, 139.1, 128.7, 126.2, 125.3, 123.2, 117.8, 98.5, 70.4, 70.3, 69.7, 69.5, 68.3, 67.4, 66.9, 63.3, 61.3, 39.9, 37.9, 37.1,20.5; HRMS-MALDI (*m/z*): Calcd for $C_{160}H_{215}N_{16}O_{76}Ru$ 3677.242; Found: 3678.244.

(viii) *Cis*-Ruthenium(II)bis(bipyridine){1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-*β*-propane-{tris-[3-4-ethoxy-*α*-D-mannopyranosyl-ethoxy}methyl]methylamide (1). General procedure D using *cis*-ruthenium(II)bis(bipyridine){1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-*β*-propane-{tris-[3-4-ethoxy-2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside-ethoxy}methyl]methylamide (50 mg, 13.5 µmol) and sodium methoxide (10 mg, 2.6 µmol) yielded 21 mg, (61%) of *cis*-ruthenium(II)bis(bipyridine){1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-*β*-propane-{tris-[3-4-ethoxy-α-D-mannopyranosyl-ethoxy}methyl]methylamide. $[\alpha]_D^{r.t}$ = + 101.4 (c = 1.0, H_2O); ^{1}H NMR (300 MHz, CD_3OD): *δ* 8.93 (br.s, 2H), 8.52 (d, *J* = 7.8 Hz, 4H), 8.39 (s, 2H), 8.03 (dd, *J* = 7.8, 6.0 Hz, 6H), 7.72 (t, *J* = 5.4 Hz, 4H), 7.68 (d, *J* = 6.0 Hz, 2H), 7.34 (t, *J* = 6.0 Hz, 4H), 4.79 (br.s, 6H), 3.89-3.85 (m, 6H), 3.83 (br.s, 2H), 3.79 (br.s, 4H), 3.75-3.65 (m, 26H), 3.63 (br.s, 4H), 3.57 (br.s, 36H), 3.43-2.41 (m, 2H), 3.36-3.33 (m, 6H), 2.55 (t, *J* = 6.3 Hz, 4H), 2.34 (t, *J* = 6.6 Hz, 12H); ^{13}C NMR (125MHz, MeOD): *δ* 181.2, 174.0, 173.6, 172.7, 158.8, 158.3, 158.0, 152.4, 139.0, 131.0, 129.1, 128.5, 125.4, 110.8, 101.4, 74.5, 72.3, 71.9, 71.8, 69.1, 68.6, 67.0, 62.6, 61.4, 40.1, 37.2; MALDI-Tof (*m/z*): Calcd for $C_{112}H1_{167}N_{16}O_{52}Ru$ 2668.988 ; Found : 2667.9789.

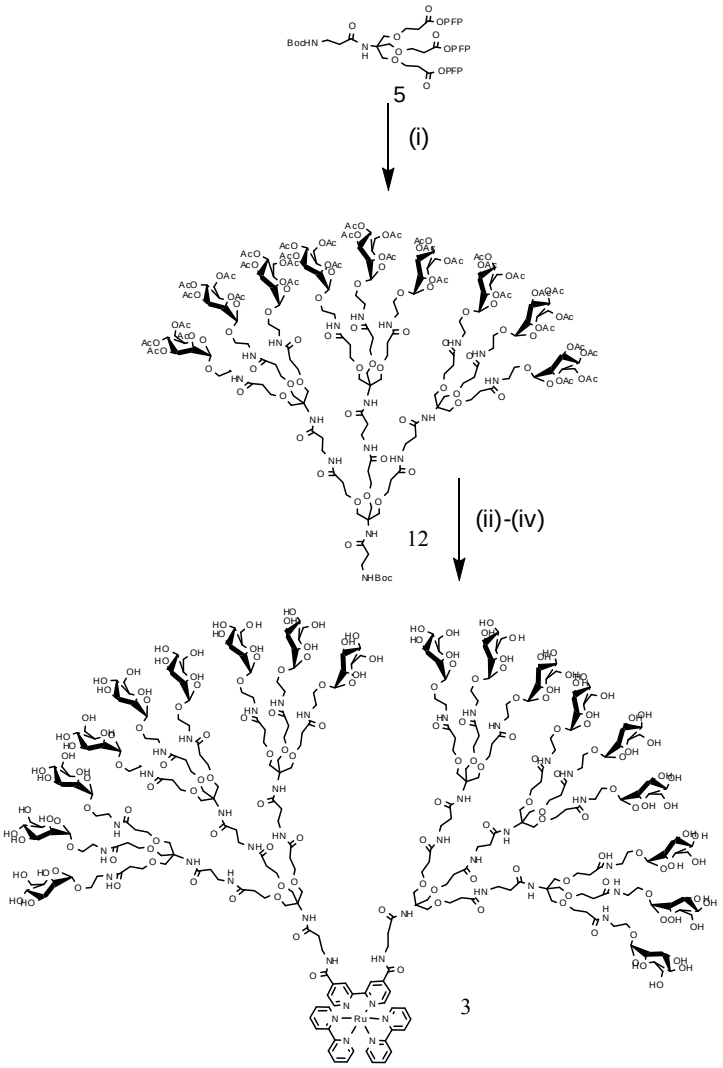


Scheme 2. Reagents and Conditions**:** (i) $RuCl_3$/EtOH/$CHCl_3$, 17%; (ii) NaOMe/MeOH, 20%.

(iv) Ruthenium(II)tris)[{1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-*β*-propane-{tris-[3-4-ethoxy-2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside-ethoxy}methyl]methyl amide] (11). The 1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-β-propane-{tris-[3-4-ethoxy-2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside-ethoxy}methyl]methylamide **6** (0.3 g, 0.093 mmol) and ruthenium trichloride () were dissolved in ethanol: chloroform (30 mL, 1:1) and the mixture was refluxed for four days. The compound was purified by flash silica column chromatography by chloroform/methanol (8:2- 7.5:2.5) as eluent to give 29 mg (17%) of Ruthenium(II)tris)[{1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-*β*-propane-{tris-[3-4-ethoxy-2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside-ethoxy}methyl]methyl amide] R_f 0.3 (chloroform/methanol = 8:2); $[\alpha]_D^{r.t}$ = +2.9 (c =1.0, H_2O); ^{1}H NMR (300MHz, CD_3OD): δ 8.82(d, *J*= 5.7 Hz, 6H); 7.9 (d, *J* = 5.7 Hz, 6H), 7.52 (d, *J*= 5.7 Hz, 6H), 5.28-5.2 (m, 54H), 4.84 (br.s, 18H), 4.25 (m, 18H), 4.15-4.10 (m, 33H), 4.02 (br.s, 33H), 3.81-3.75 (m, 24H), 3.65 (br.s, 72H), 3.59-3.52 (m, 12H), 3.45 (m, 48H), 2.62 (br.s, 12H), 2.44 (br.s, 36H), 2.13 (s, 54H), 2.04 (s, 54H), 2.03 (s, 54H), 1.95 (s, 54H); ^{13}C NMR (125MHz, CD_3OD): δ 174.2, 173.2, 171.4, 171.2, 171.0, 165.2, 164.2, 156.4, 142.3, 139.1, 135.1,

98.7, 70.4, 70.3, 69.7, 69.5, 68.3, 67.4, 66.9, 61.5, 39.9, 38.9, 37.1,20.5; HRMS-MALDI (m/z): $[M+1]^+$ Calcd for $C_{420}H_{594}N_{36}O_{228}Ru$ 9896.517; Found : 9896.5192.

(v) **Ruthenium(II)tris[{1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-*β*-propane-{tris-[3-4-ethoxy-*α*-D-mannopyranosyl-ethoxy}methyl]methylamide] (2).** General procedure D with *cis*-ruthenium(II)bis(bipyridine)1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{tris-[3-carboxylethoxy]methyl]3'-{tris[2'-ethoxy2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]methyl]methylamide} (20 mg, 2.4 µmol) and sodium methoxide (5 mg, 20%) gave 10 mg (54%) of ruthenium(II)tris[{1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-*β*-propane-{tris-[3-4-ethoxy-*α*-D-mannopyranosyl-ethoxy}methyl]methylamide**]**. $[\alpha]_D^{r.t}$ = +22.4 (c =1.0, H_2O); ^{1}H NMR (300MHz, CD_3OD): δ 8.93 (d, *J* = 5.8 Hz, 6H), 7.72 (d, *J* = 5.8 Hz, 6H), 7.58 (d, *J* = 5.8 Hz, 6H), 4.79 (s, 18H); 3.75-3.65 (m, 196H); 3.54 (m, 50H); 3.41 (m, 50H); 2.46 (m, *J* = 3.0 Hz, 64H); ^{13}C NMR (125MHz, MeOD): δ 174.0, 173.6, 172.7, 165.3, 158.8, 142.9, 139.5, 131.1, 101.4, 74.5, 72.3, 71.9, 71.8, 69.1, 68.6, 67.0, 62.6, 61.4, 40.1, 37.2; HRMS-MALDI (m/z): $[M-1]^+$ Calcd for $C_{304}H_{502}N_{40}O_{166}Ru$ 6868.7489; Found: 6868.7471.



Scheme 3. Reagents and Conditions: (i) Compound **5**/DCM/TEA. 69%; (ii) TFA/DCM; bipyridine-COCl/DCM/TEA. 13%; (iii) *cis*-Ru(bipy)$_2$Cl$_2$/EtOH, 66%; (iv) NaOMe/MeOH, 61%.

(i) *tert*-Butoxycarbonyl-3-{tris[3-carboxyl ethoxy]methyl] 3'-{tris[2'-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]methyl]methylamide}-3-β-alanine (12). General procedure A with *tert*-butoxycarbonyl-3-{tris[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy] methyl]methylamide}-3-β-alanine **5** (0.3 g, 0.18 mmol), *tert*-butoxycarbonyl-3-{N-{tris[3-[pentafluoro phenyl carboxyl-ethoxy)methyl]}methylamine}-3-β-alanine (46 mg, 0.045 mmol) and flash silica column chromatography by using CH_2Cl_2/CH_3OH (12-14%) as eluent yielded 0.16 g (69%) of *tert*-butoxycarbonyl-3-{tris[3-carboxylethoxy]methyl]3'-{tris[2'-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]methyl]methylamide}-3-β-alanine. R_f 0.5 (CH_2Cl_2: MeOH = 90:10); $[\alpha]_D^{r.t}$ = +14.4 (c =1.0, $CHCl_3$); ^{1}H NMR (300MHz, $CDCl_3$): δ 8.24 (br, 6H); 7.27 (br, 3H); 5.27-5.2 (m, 27H); 4.25 (dd, *J* = 9.0, 5.1 Hz,9H); 4.05 (br, 9H) ; 3.81 (m, *J* = 5.7 Hz, 4.5 Hz, 9H); 3.67 (br, 57H); 3.59 (s, *J* = 6.0 Hz, 14H); 3.46 (m, *J* = 2.7 Hz, 34H); 3.24 (br, 8H); 2.47 (br, 32H); 2.13 (s, 27H); 2.06 (s, 27H); 2.04 (s, 27H); 1.96 (s, 27H); 1.43 (s, 9H); ^{13}C NMR (75MHz, $CDCl_3$): δ

173.92; 172.2, 171.4, 171.3, 98.7, 70.7, 70.6, 70.4, 69.8, 68.5, 67.6, 67.0, 63.4, 61.4, 54.6, 40.0, 37.2, 30.5, 20.6,FTIR($CHCl_3$): 3332, 2734, 1745, 1365, cm^{-1}; HRMS-MALDI (m/z): $[M+ Na]^+$ Calcd for $C_{215}H_{321}N_{17}O_{120}$ 5036.9538; Found : 5060.924.

(ii) 1,1'-(2,2'-Bipyridine-4,4'-diyl)bis-3-beta-propane-{tris-[3-carboxyl ethoxy]methyl]3'-{tris[2'-ethoxy 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]methyl]methylamide (13). General procedure B with *tert*-butoxycarbonyl-3-{tris[3-carboxyl ethoxy]methyl]3'-{tris[2'-ethoxy 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]methyl]methylamide}-3-β-alanine (0.2 g, 0.039 mmol), 2,2'bipyridine-4,4'-dicarboxylic acid (3.5 mg, 0.014 mmol) and flash silica column chromatography by CH_2Cl_2/CH_3OH (15-16%) as eluent yielded 54 mg (13%) of 1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-β-propane-{tris-[3-carboxyl-ethoxy]methyl]3'-{tris[2'-ethoxy 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]methyl]methylamide. R_f 0.35 (CH_2Cl_2:MeOH = 86:14); $[\alpha]_D^{r.t}$ = -2.7 (c =1.0, $CHCl_3$); ^{1}H NMR (300MHz, $CDCl_3$): δ 8.78 (m, 4H); 8.24 (t, 6H, *J* = 3.9 Hz); 7.35(br, 2H); 5.27-5.2 (m, 54H); 4.23 (dd, *J* = 9.0, 5.1 Hz, 18H);

4.1 (d, *J* = 10.5 Hz, 18H); 4.04 (m, *J* = 3.6 Hz, 18H); 3.83 (b, *J* = 5.4 Hz, 18H); 3.76 (b, 86H); 3.68 (m, *J* = 5.4 Hz, 36H); 3.56 (q, *J* = 5.4 Hz, 44H); 3.30 (m, 44H); 2.47 (q, *J* = 5.7 Hz, 66H); 2.14(s, 54H); 2.05 (s, 54H); 2.04 (s, 54H); 1.96 (s, 54H); ^{13}C NMR (125MHz, $CDCl_3$): δ174.0; 173.6, 172.2, 171.4, 171.2, 167.3, 163.4,162.9, 161.9, 156.0, 150.1, 143.2, 119.8, 116.0, 98.7, 70.5, 70.4, 69.78, 68.7, 68.5, 67.6, 67.0, 63.4, 61.4, 54.6, 40.0, 37.1, 26.4, 20.8,FTIR($CHCl_3$): 3433, 2992, 2304, 1745, 1671, 1564 cm^{-1}; MALDI-HRMS (m/z): $[M+1]^+$ Calcd for $C_{428}H_{630}N_{36}O_{238}$ 10081.8301; Found : 10082.831.

(iii) *Cis*-Ruthenium(II)bis(bipyridine)1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{*tris*-[3-carboxyl-ethoxy]methyl]3'-{tris[2'-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]methyl]methylamide (14). General procedure C with *cis*-ruthenium(II)bis(bipyridine)dichloride (3.4 mg, 6.8 μmol), 1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{tris-[3-carboxyl-ethoxy]methyl]3'-{tris[2'-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]methyl] methylamide (35 mg, 3.47 μmol) and flash silica column chromatography by acetronitrile/saturated KNO_3 (7.5:2.5- 7:3) as eluent gave 24 mg (66%) of *cis*-ruthenium(II)bis(bipyridine)1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{tris-[3-carboxylethoxy]methyl]3'-{tris[2'-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]methyl]methylamide. R_f 0.25 (acetonitrile:Sat KNO_3 = 7.5:2.5); $[\alpha]_D^{r.t}$ = +26.9 (c =1.0, H_2O); ^{1}H NMR (300MHz, CD_3OD): δ 9.21 (s, 2H); 8.73 (br, 4H); 8.21 (d, 6H, *J* = 4.8 Hz); 8.0 (br, 6H, *J* = 6.0 Hz); 7.91 (br, 6H); 7.5 (t, *J* = 4.5 Hz, 4H); 7.35 (br, 2H); 5.27-5.2 (m, 54H); 4.23 (dd, *J* = 9.0, 5.1 Hz, 18H); 4.1 (d, *J* = 10.5 Hz, 18H); 4.1 (m, *J* = 3.6 Hz, 18H); 3.85 (b, *J* = 5.4 Hz, 18H); 3.77-3.62 (br, 154H); 3.30 (m, 58H); 2.44 (br, 64H); 2.12(s, 54H); 2.04 (s, 54H); 2.04 (s, 54H); 1.95 (s, 54H); ^{13}C NMR (125MHz, CD_3OD): δ 174.0; 172.2, 171.4, 171.3,171.0,165.2, 163.2, 158.5, 157.8, 152.5, 152.1, 143.3, 139.1, 128.7, 126.2, 123.2, 117.8, 98.7, 70.4, 70.3, 69.7, 68.5, 67.6, 67.0, 66.9, 61.4, 40.0, 37.1, 20.5,MALDI-HRMS (m/z): $[M+1]^+$ Calcd for $C_{448}H_{646}N_{40}O_{238}Ru$ 10495.871; Found : 10496.8712.

(v) ***Cis*-Ruthenium(II)bis(bipyridine)1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{tris-[3-carboxyl-ethoxy]methyl]3'-{tris[2'-ethoxy-α-D-mannopyranoside-ethoxy]methyl] methyl amide (3).** General procedure D with *cis*-ruthenium(II)bis(bipyridine)1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{tris-[3-carboxylethoxy]methyl]3'-{tris[2'-ethoxy2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]methyl]methylamide} (25 mg, 2.4 μmol) and sodium methoxide (5 mg, 20%) gave 12 mg (61%) of *cis*-ruthenium(II)bis(bipyridine)1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-

propane-{tris-[3-carboxyl-ethoxy]methyl]3'-{tris[2'-ethoxy-α-D-mannopyranoside-ethoxy]methyl]methylamide. $[\alpha]_D^{r.t}$ = +76.4 (c =1.0, H_2O); ^{1}H NMR (300MHz, CD_3OD): δ 9.06 (s, 2H); 8.64 (d, *J* = 7.8 Hz, 4H); 8.39 (s, 4H); 8.05 (br, *J* = 7.8 Hz, 6H); 7.88 (d, *J* = 6.0 Hz, 6H); 7.35 (d, *J* = 6.0 Hz, 2H); 4.79 (s, 18H); 3.75-3.65 (m, 196H); 3.54 (m, 50H); 3.41 (m, *J* = 5.1, 4.5 Hz, 50H); 2.46 (t, *J* = 3.0 Hz, 64H); ^{13}C NMR (125MHz, MeOD): δ 181.8; 174.1, 173.1, 172.4, 158.8, 158.0, 152.4, 139.0, 131.5, 130.2, 128.2, 125.5, 110.6, 101.5, 73.7, 72.9, 71.1, 70.9, 69.2, 68.6, 66.8, 62.1, 60.5, 40.2, 37.2, MALDI-HRMS (m/z): $[M- 1]^+$ Calcd for $C_{304}H_{502}N_{40}O_{166}Ru$ 7471.1113; Found: 7470.1108.

3. Photophysical Properties.

The emission spectra of the $[Ru(bipy)_3]^{2+}$ complex and **1-3** are shown in Figure 1. Upon excitation at the corresponding MLCT band, a maximum emission at 610 nm ($[Ru(bipy)_3]^{2+}$) and 653 nm (complex 1-3) were observed. This fluorescent emission originated from an excited-state intramolecular energy transfer (ESIET). Quantum yields have been calculated using the equation,

$$\mathbf{I_{comp}/I_{ref} = A_{comp} * [C]_{ref} / A_{ref} * [C]_{comp}}$$

Where **[C]** refers to the concentration of the samples and **A** to the area of the emission spectra. Here, $Ru(bipy)_3(Cl)_2$ was used as a reference compound of quantum yield $\mathbf{I_{ref}}$ **=** 0.062.[4] Luminescent lifetimes were measured using excitation provided by a Quantum Brillant Nd:YAG laser equipped with frequency double, triple and quadruple. The output signal of the photomultiplier was led into a standard Research SR-430 multichannel scalar and transferred to a PC. All compounds exhibit a more intense emission and longer excited state lifetime than the parent $[Ru(bipy)_3]^{2+}$ complex. The most interesting results concern the excited state lifetime and the luminescence quantum yield at 298K in deaerated acetonitril/methanol (9:1) solutions, the life time of the complex **3** is shorter than that of **2.** Whereas in aerated solution the situation is reversed. This indicated that in aerated solution the comound, with longer branches **3** is less quenched by dioxygen than the excited state of the smaller compound **2** and **1**.

Compound	λ_{max}	k_q	τ_o (μs)	I_o
1	645	9.8×10^8	0.61 (0.19)	0.072 (0.023)
2	648	1.8×10^8	1.31(0.82)	0.102(0.061)
3	648	1.1×10^8	1.26 (0.96)	0.112(0.061)
$Ru(bipy)_3$	613	2.5×10^9	0.54(0.17)	0.062 (0.016)

Figure 1. Photophysical data for ruthenium complexes in deaerated solution and aerated solution (in parenthesis)

Quenching constant. We have studied the quenching of the ^{3}MLCT excited state of complexes **1-3** and $[Ru(bipy)_3]^{2+}$ by BBV quencher. BBV do not posses excited state below the ^{3}MLCT level of $[Ru(bipy)_3]^{2+}$ chromophoric unit, but is easy to reduce. As a consequence, quenching can only take place by electron transfer. The experiments have been performed in acetonitril solution containg $TBAPF_6$ at 298K. The quenching of the luminescent **1-3** by BBV containing. The rate constant K_q of the quenching process taking place by a dynamic mechanism can be calculated from the Stern-Volmer equation.

$\tau_o/\tau = Io/I = 1+k_q\tau_o[Q]$

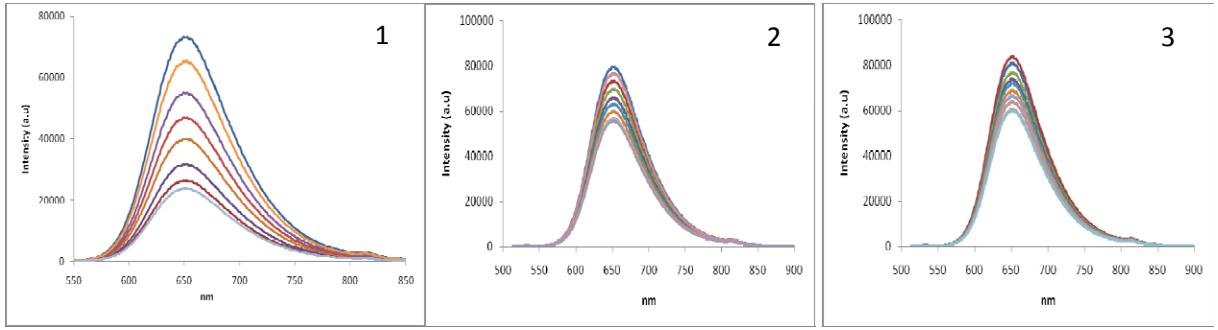


Figure 2. Fluorescent spectra of complex **1-3** at different concentration of BBV quencher; Conditions: concentration complex **1-3** = 1.3×10^{-5} M; concentration of BBV for complex **1** = $1 \times 10^{-5} - 2.5 \times 10^{-4}$ M and for complex **2-3** = $1 \times 10^{-5} - 30 \times 10^{-4}$ M

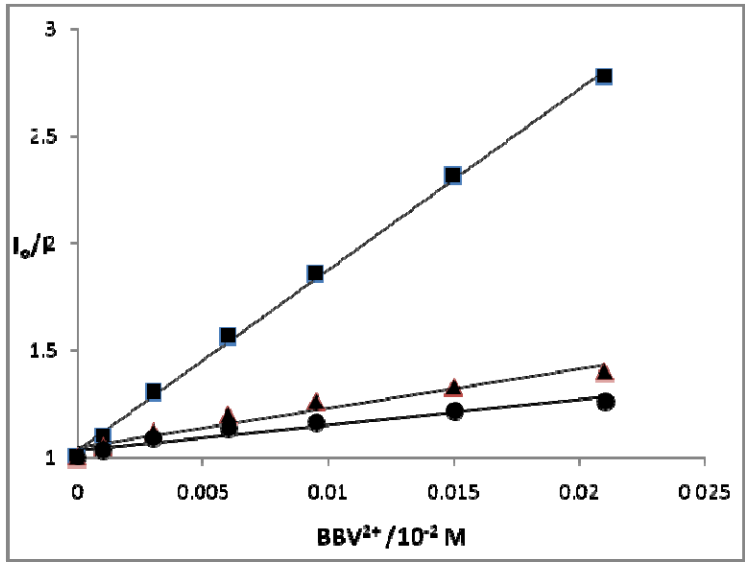


Figure 3. Stern-Volmer plots for the quenching of complex **1**(□), **2**(□) and **3**(□)by BBV quencher.

4. EPR Experiments.

The continuos wave EPR experiments were carried out in an Elexys Bruker spectrometer working at X-band (9.8 GHz). The resonator (Bruker ER 4102ST) was a rectangular cavity operating in the TE_{102} mode and it was provided with a grid of 10 x 23 mm that allows optical access to the sample during the measurement. The ruthenium complexes (2.5 mM) were prepared in a solution of acetonitril to which the spin trap (TEMP, 100 mM) was added. The samples were transferred to thin quarz tubes (inner diameter 1.5 mm) and placed in the center of the cavity. Once in the resonator the ruthenium complexes were continously illuminated using a 120 W lamp adusted with blue filter (380-500 nm) and EPR experiments were taken at one minute time intervals during this process. The measurements were performed at room temperature using a microwave power of 20 mW and a modulation amplitude of 0.05 mT. One of the EPR spectrum taken is displayed in figure 2a as an example. The spectrum consists of three equally-spaced lines centered at a g-value of 2.0056, the line interval of 1.55 mT is typical of the nitroxide radical of TEMPO (see Figure 2b) where the interaction of the unpaired electron with the nitrogen nucleus results in the characteristic splitting of the EPR line into a triplet.

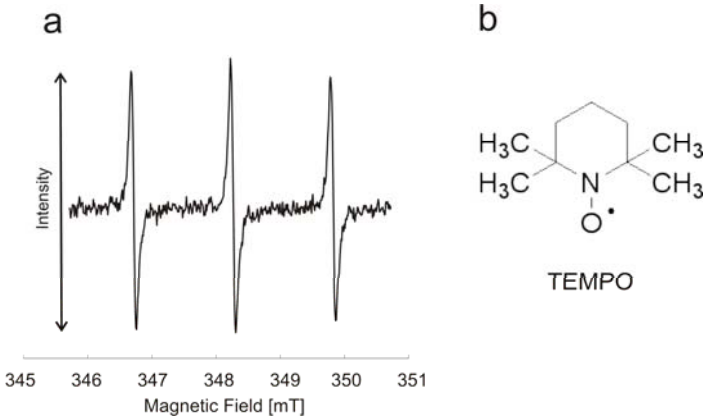


Figure 3. a) EPR spectra of the TEMPO radical. b) Formula of the paramagnetic TEMPO molecule resulting from the reaction of the spin trap TEMP with singlet oxygen.

5. Lectin Biosensor.

Photoinduced electron transfer lectin sensing was determined by measuring the fluorescent intensity upon addition of different concentration of lectin to a quenched solution of the dye. As lectin is added to the quenched solution, the fluorescence increases as the quenching becomes less effective due to the lectin binding to the Ru(II)-mannose dendrimers. Experiments show that the shape of the binding isotherm can be conveniently manipulated by adjusting the ratio between quencher and Ru(II)-template. In this case the optimum quenching ratio was taken for the lectin sensing process. In this way, we can get highly sensitive and slow saturation. Similar behavior was observed for the GNA lectin.

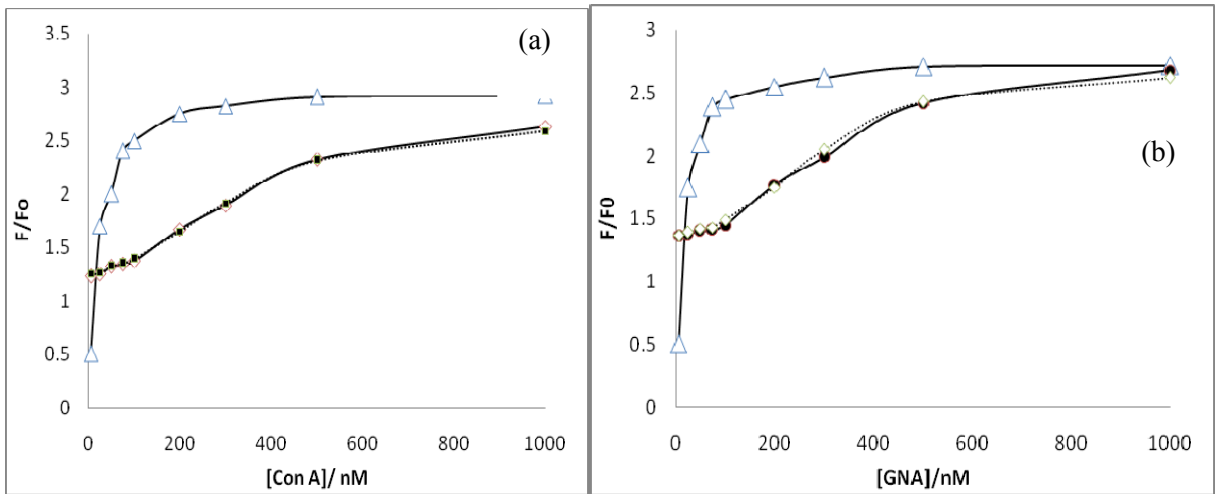


Figure 4. Rate of fluorescent gain from Ru(II)-carbohydrate-BBV upon addition of lectin; **1** ( ), **2 (◊)** and **3**(□) at λ_{max} = 645 nm**.** Concentration of Δcomplex **1 =** 1.0 ×10^{-5} : 2.0 × 10^{-4} M of BBV; Concentation of complex **2-3** = 1.0 × 10^{-5} : 20 × 10^{-4} M of BBV

6. References

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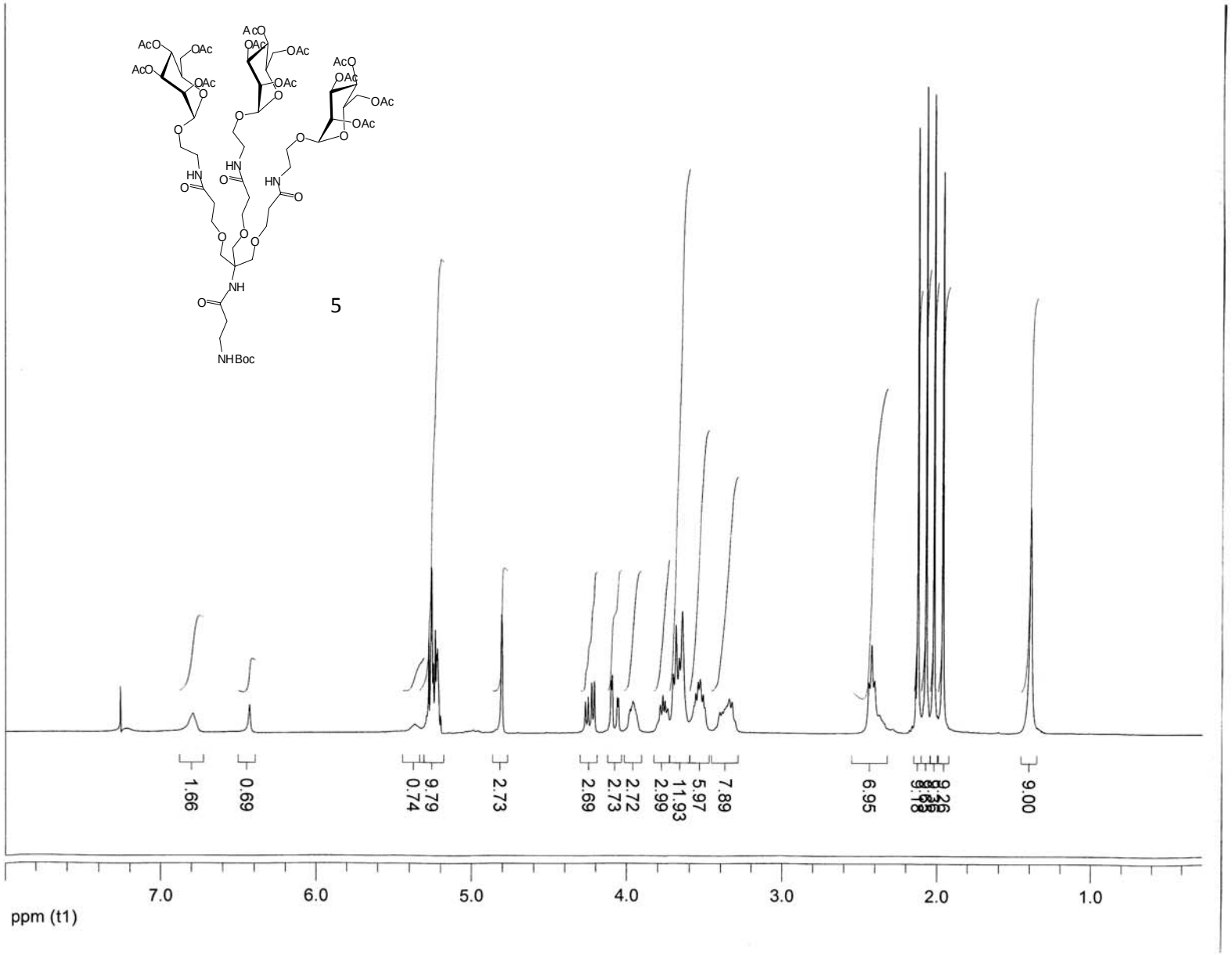

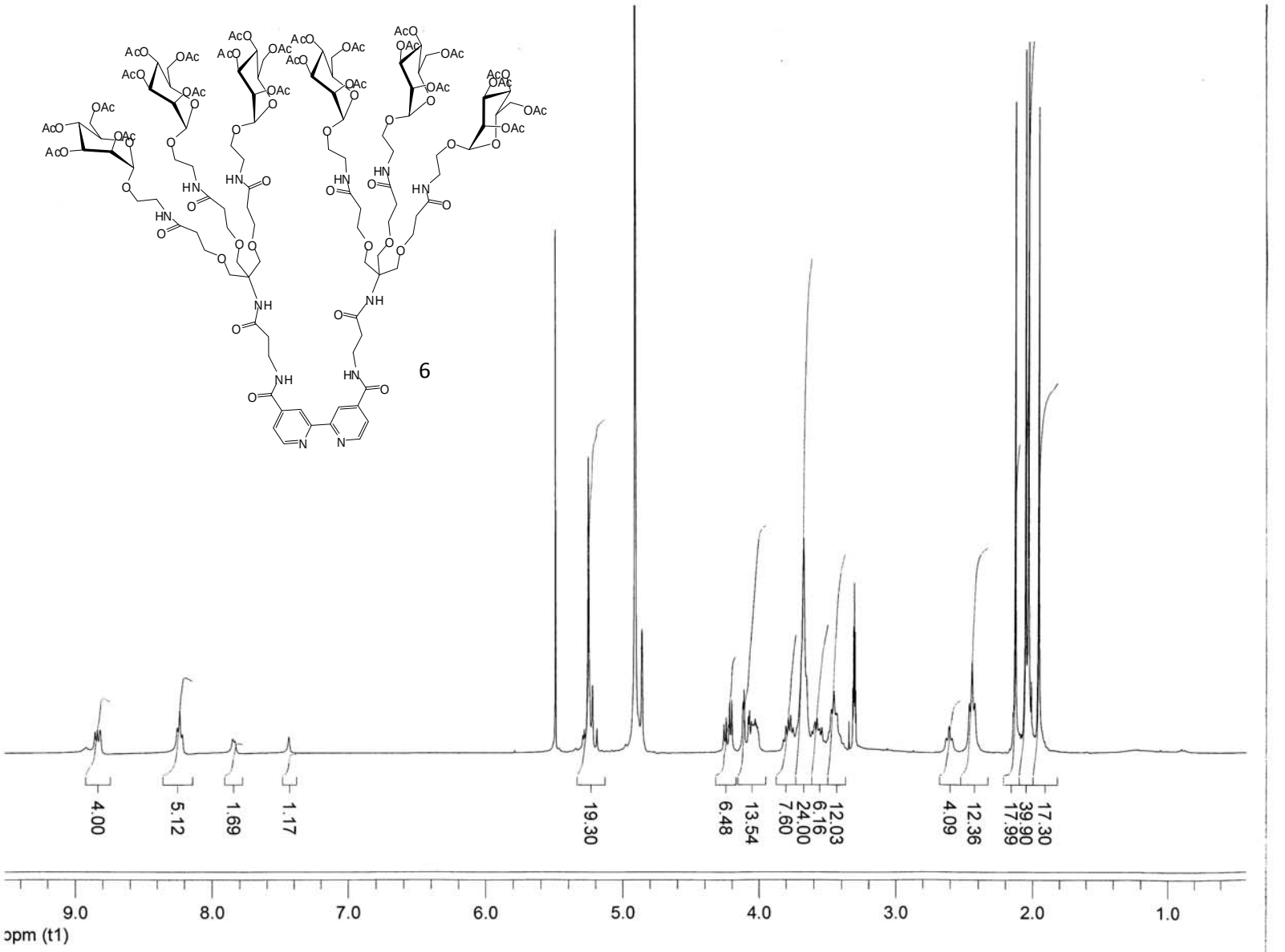

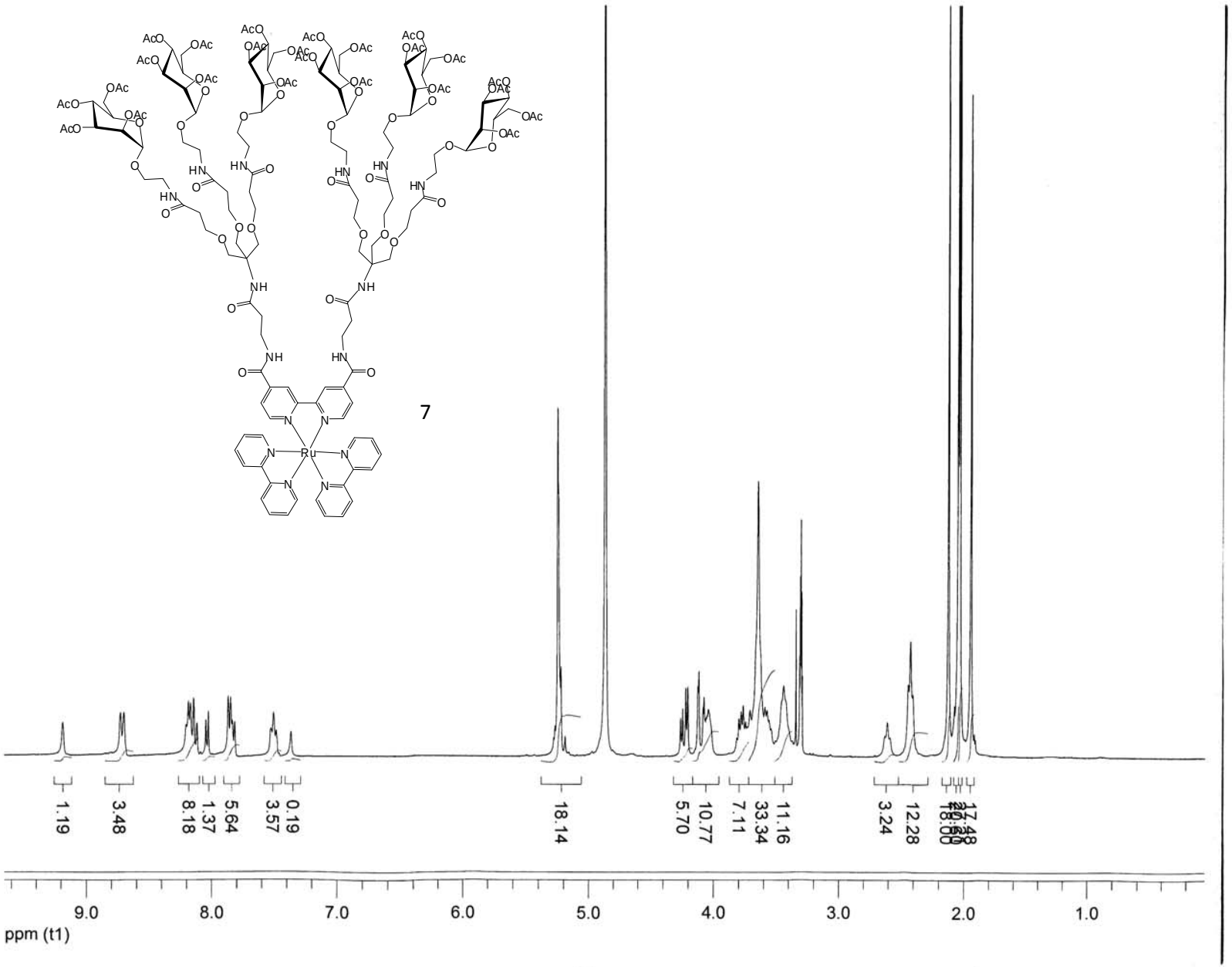

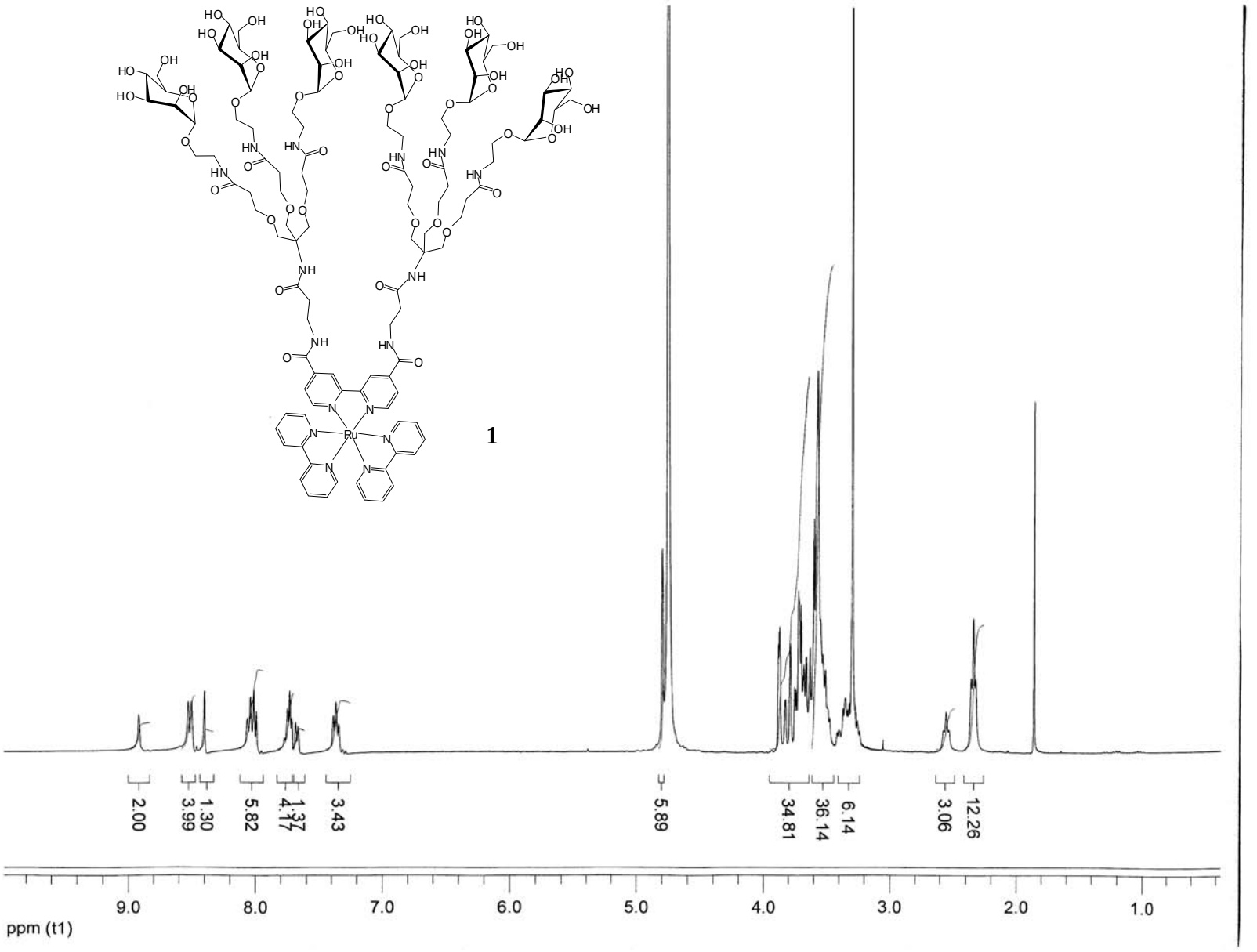

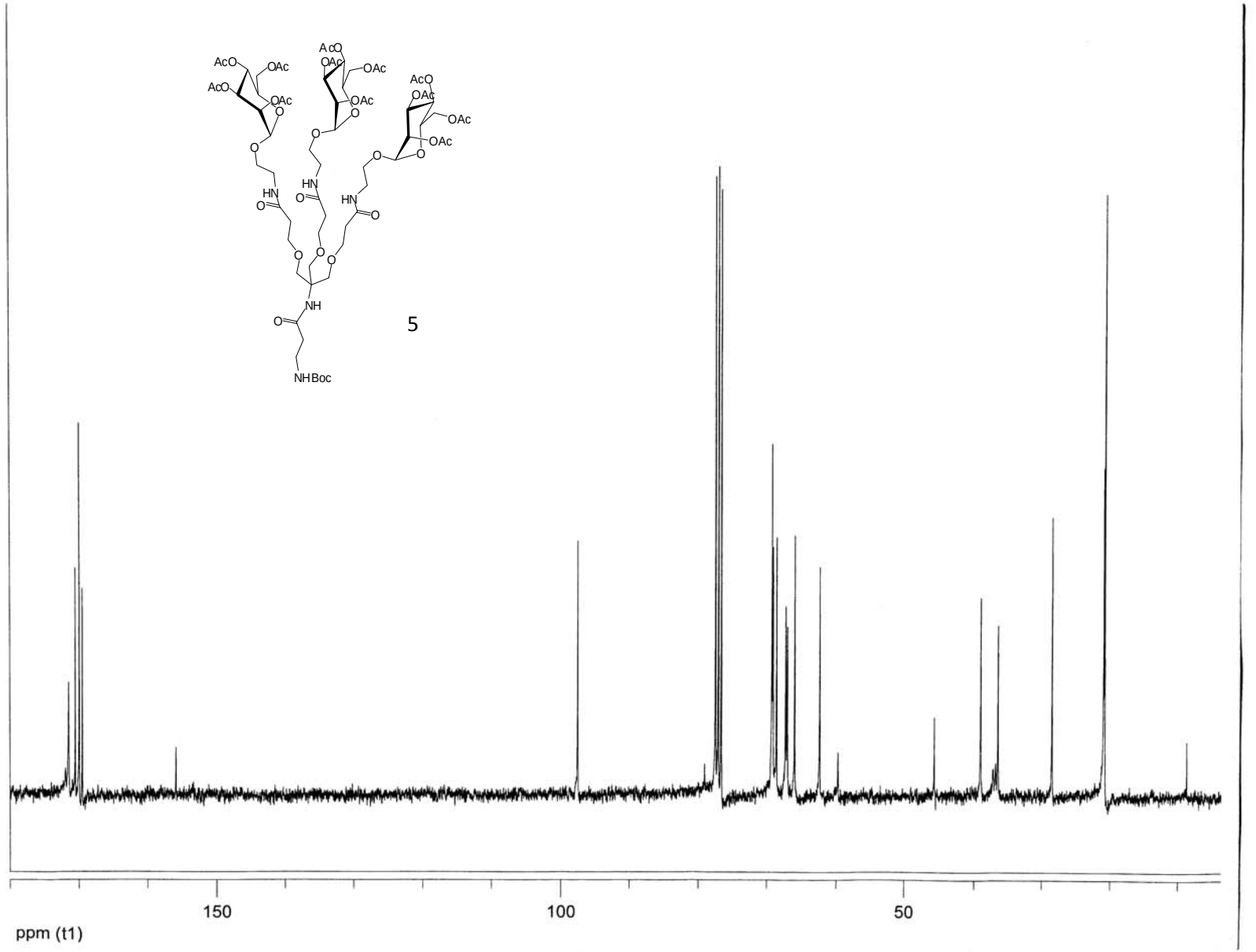

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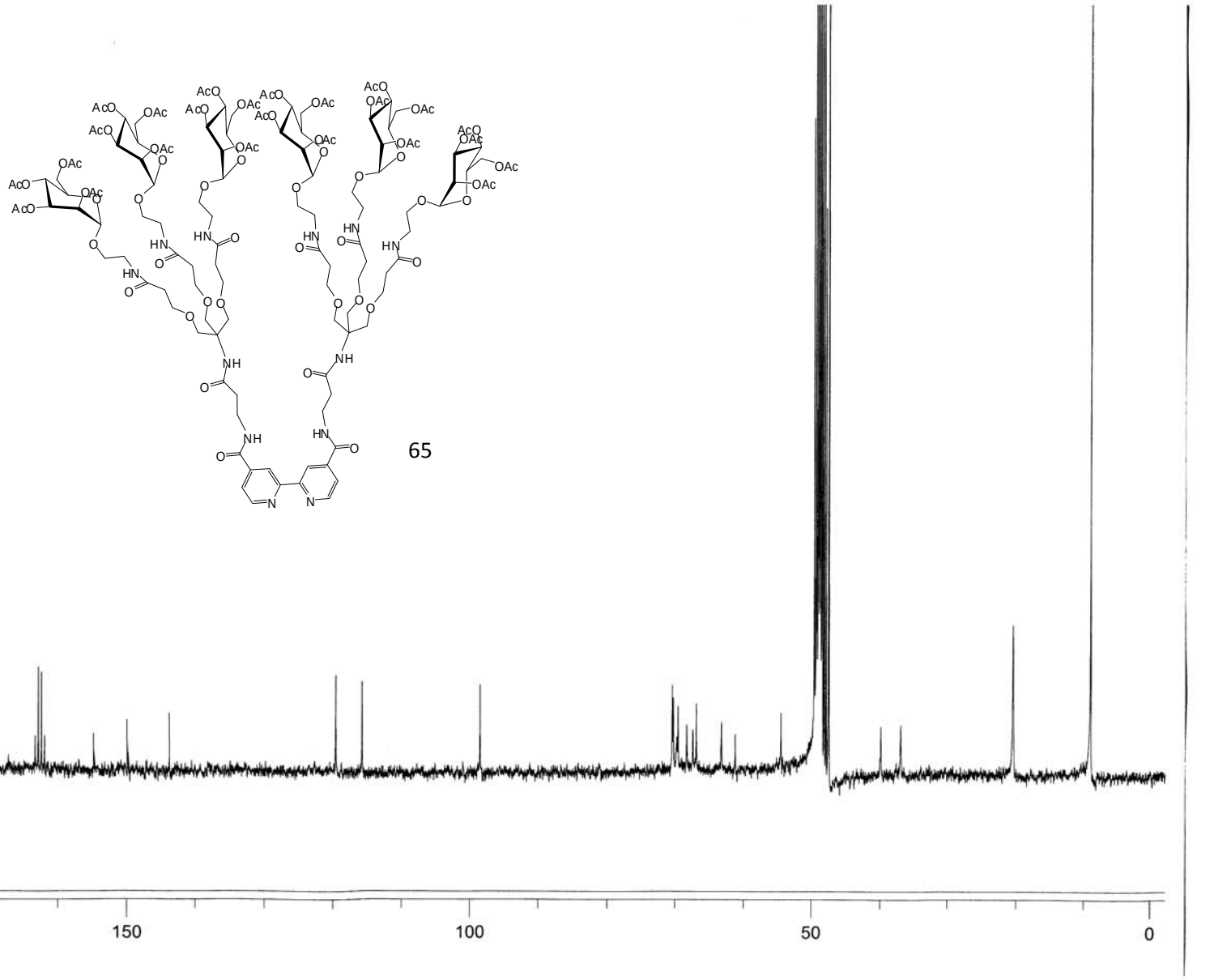

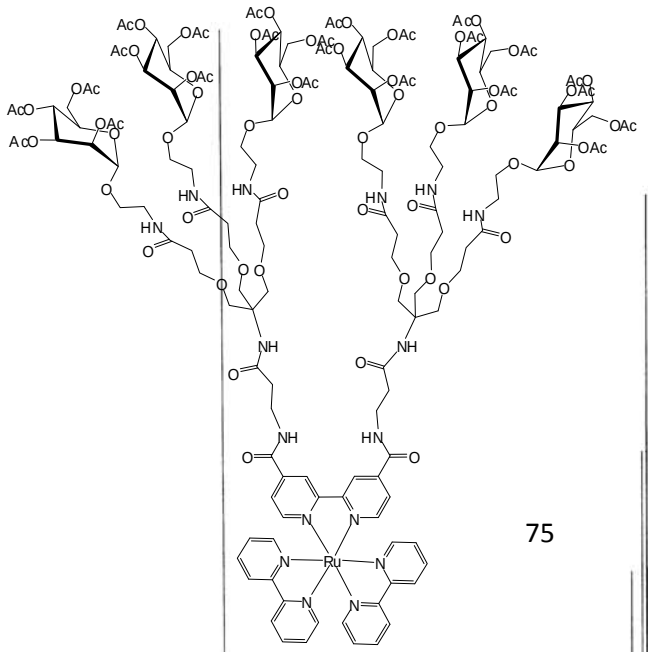

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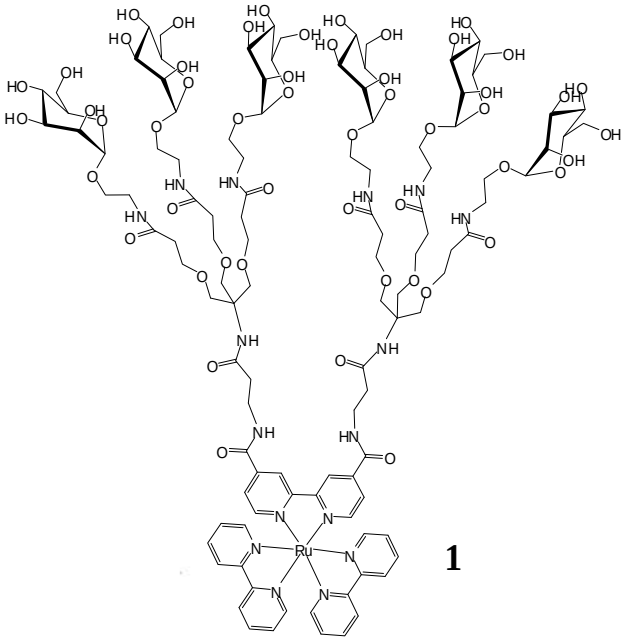

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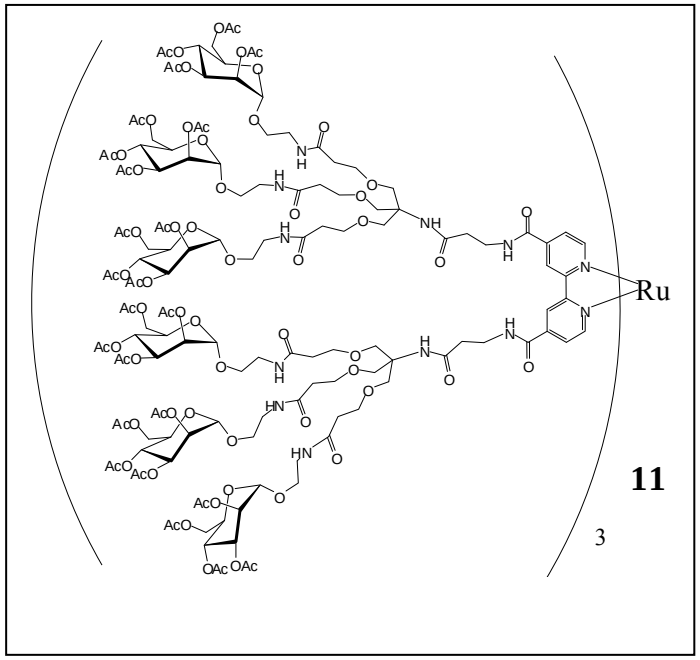
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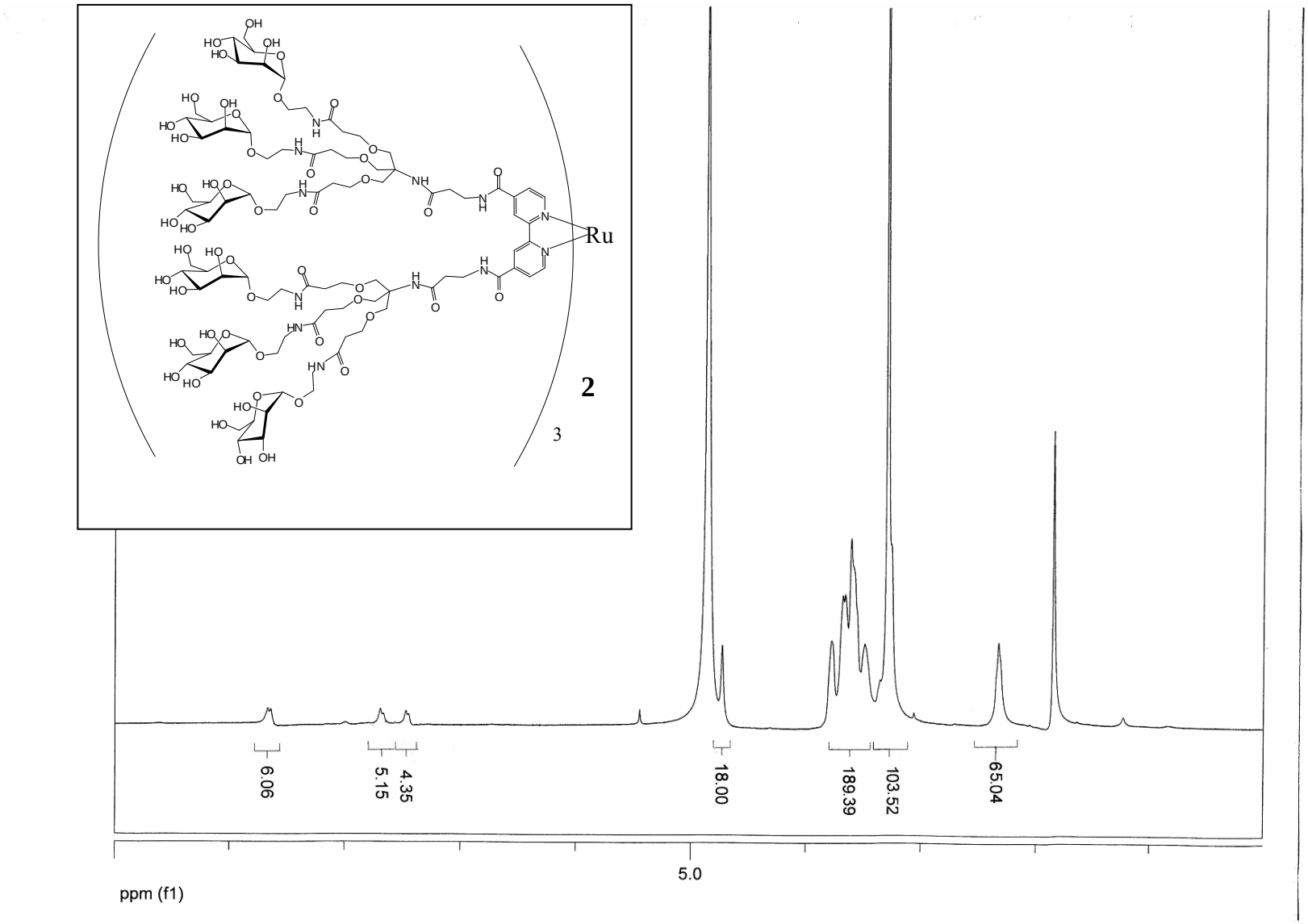

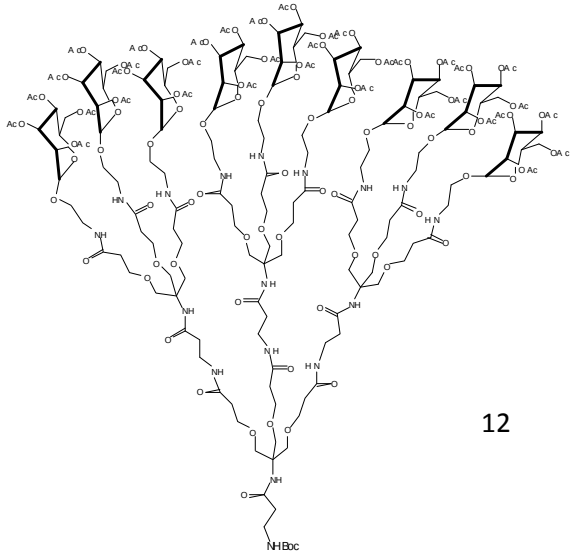

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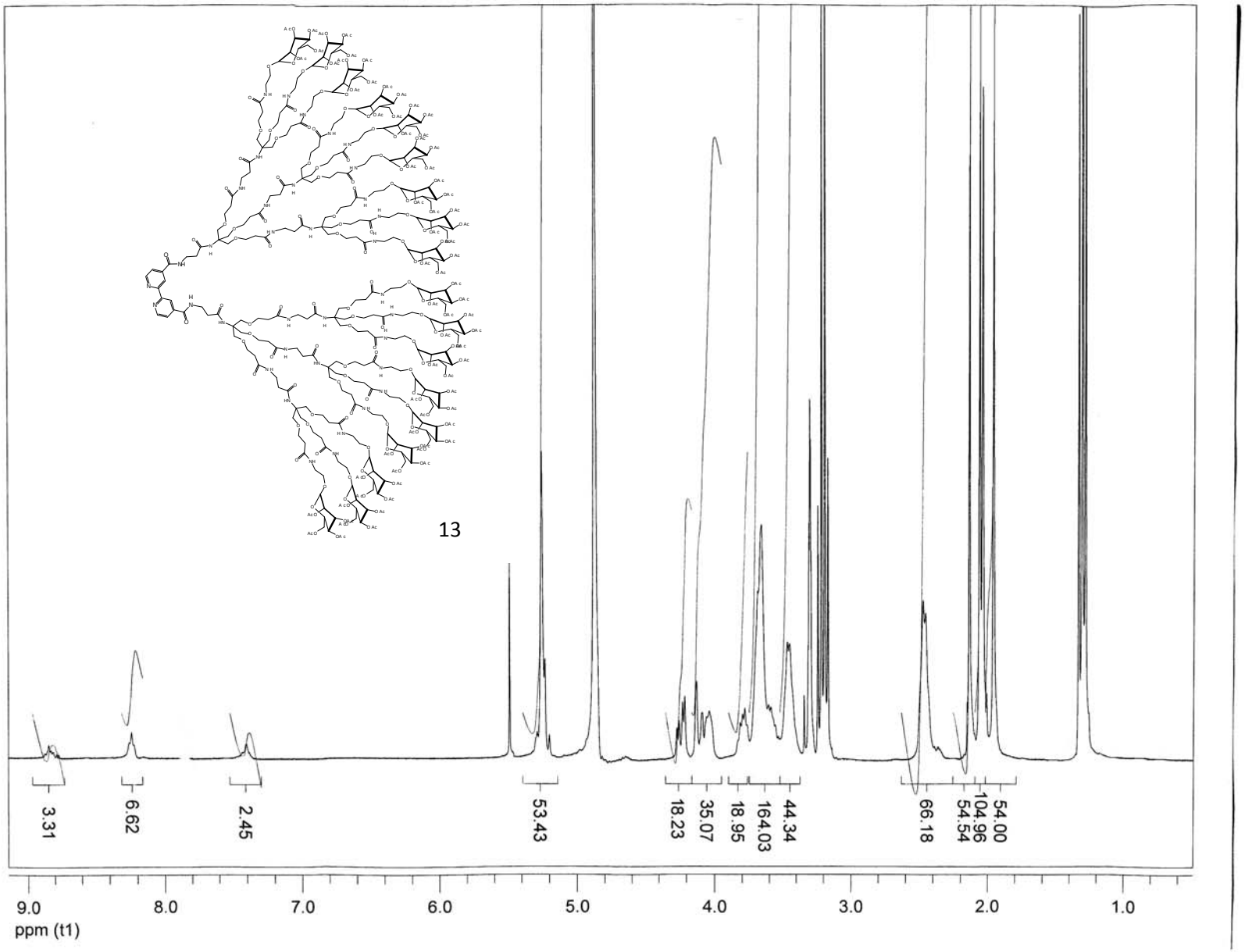
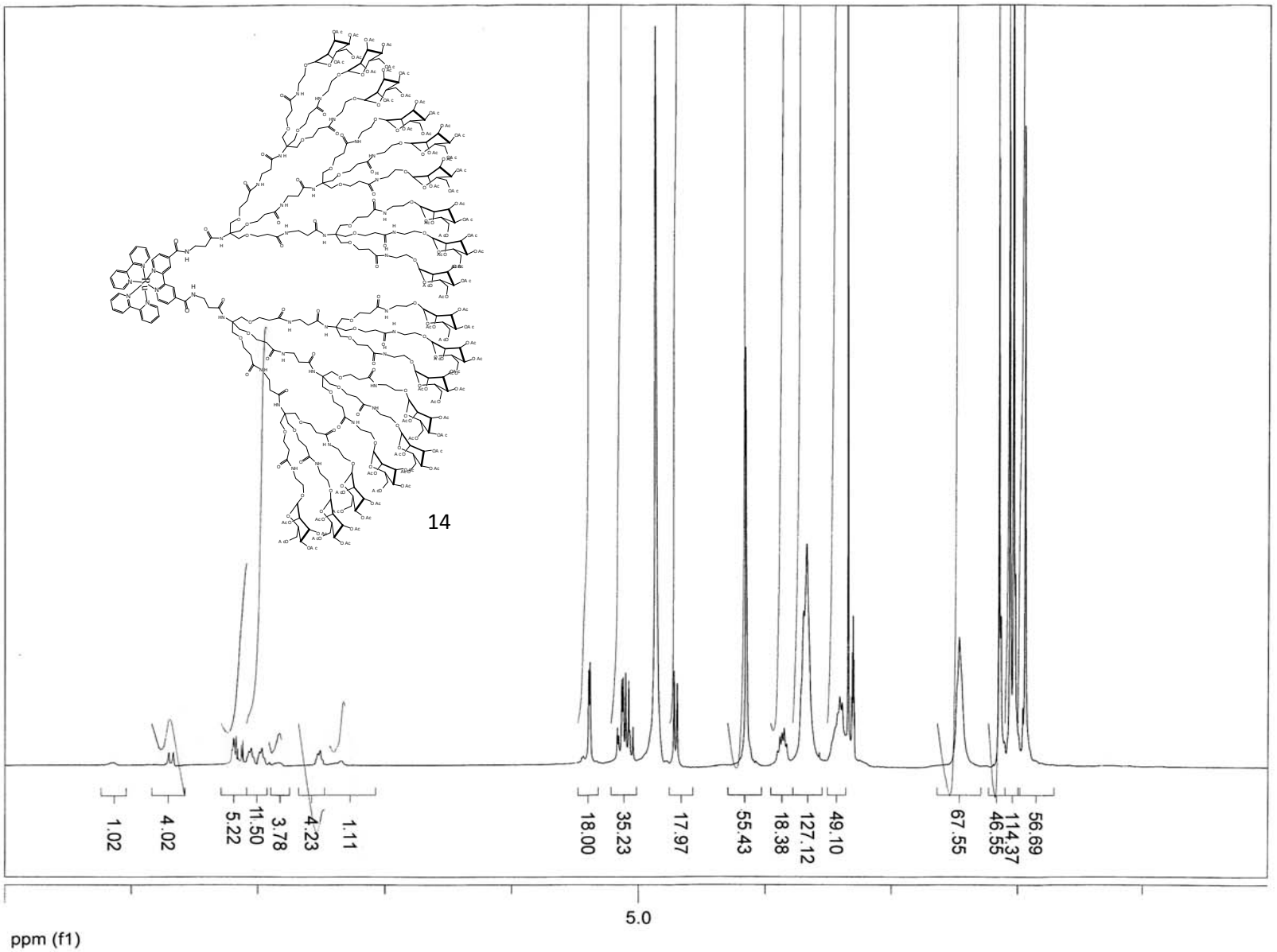
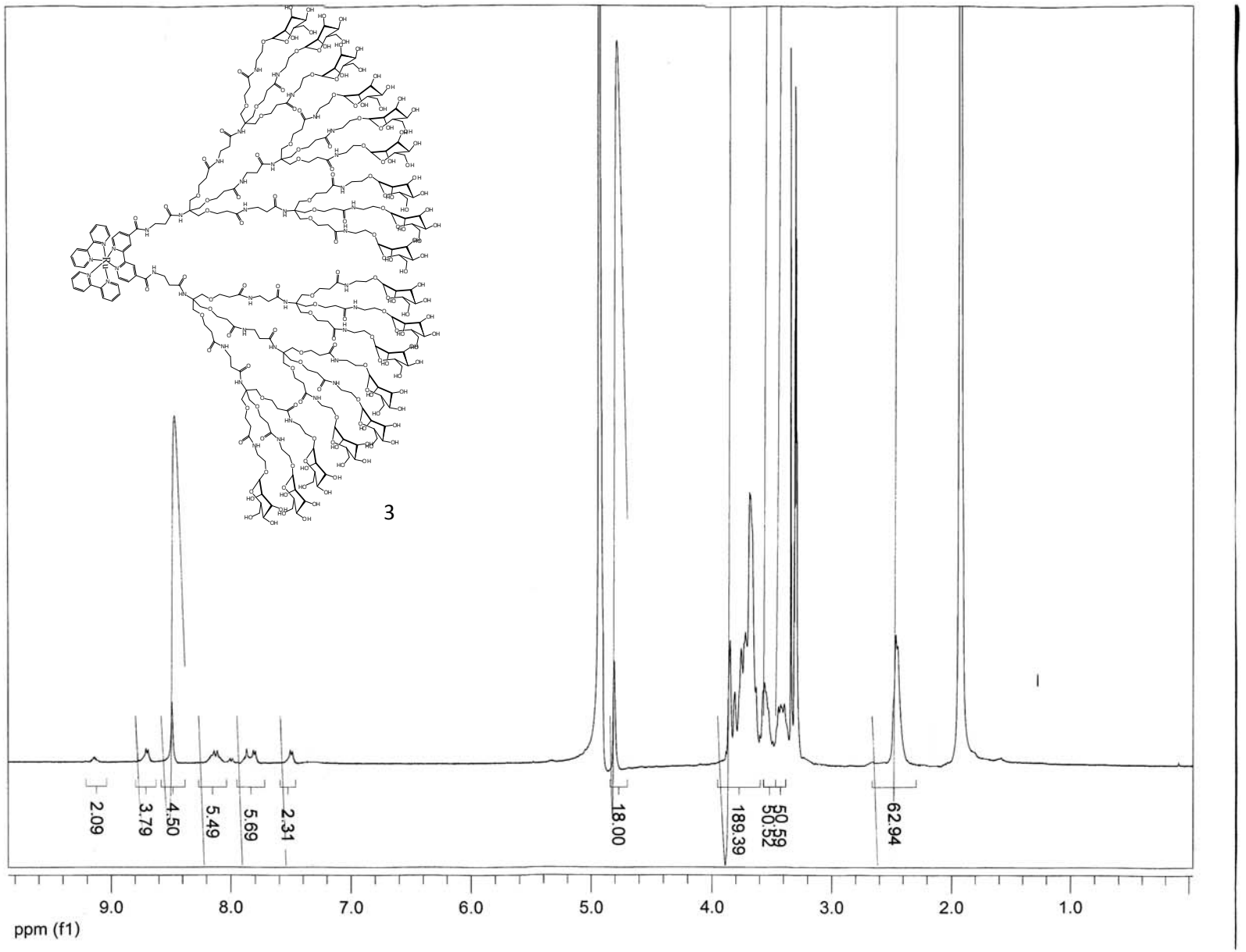
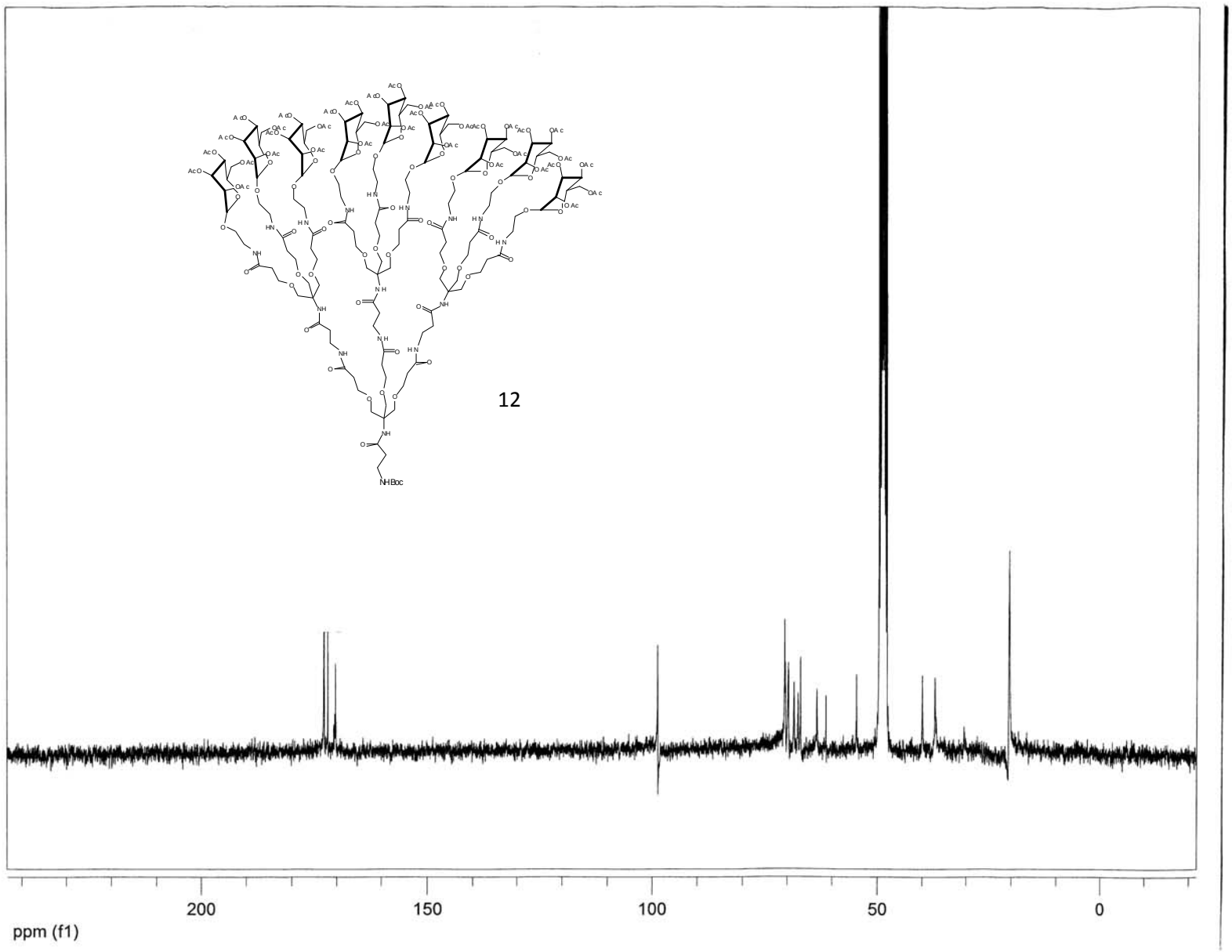
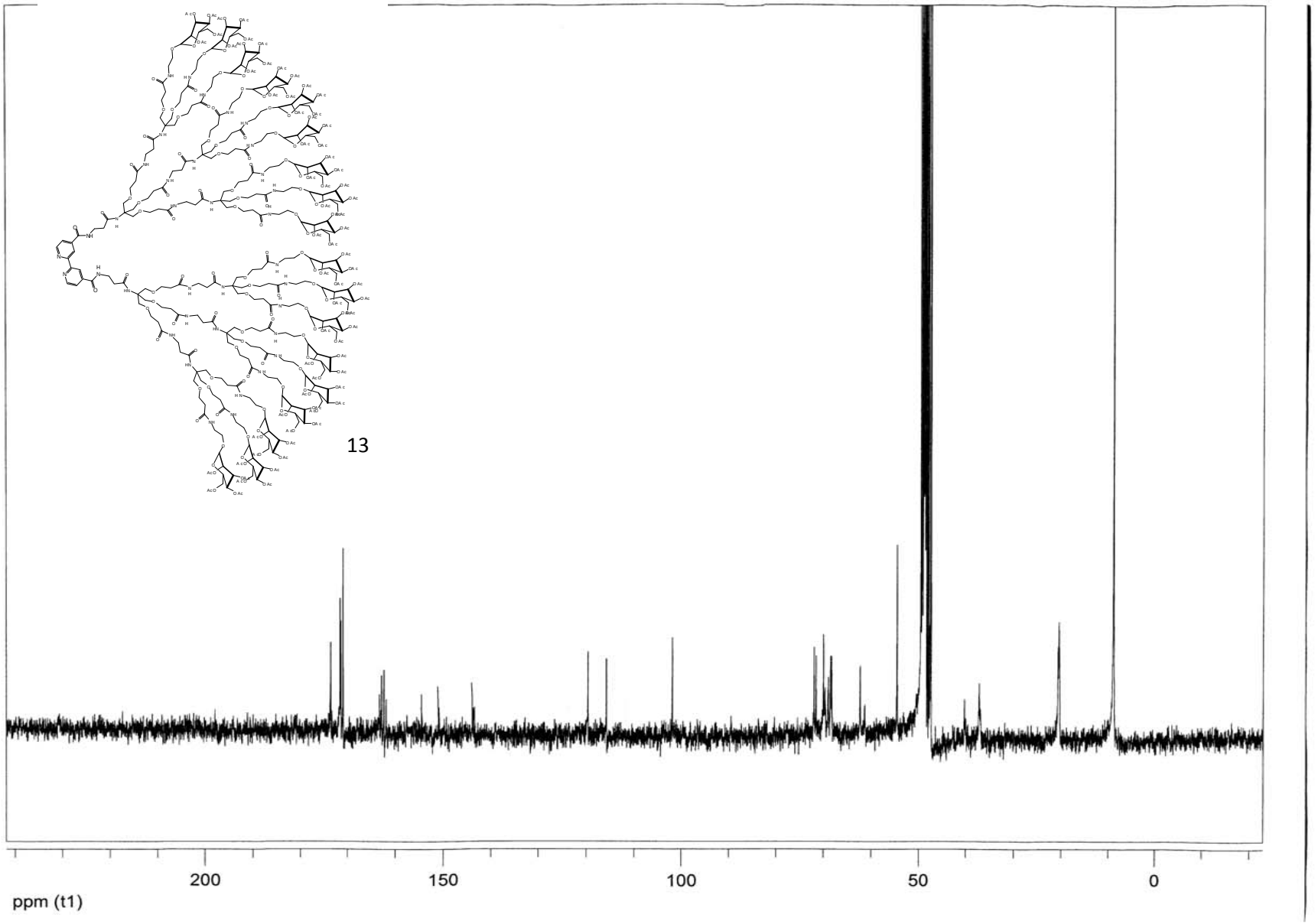







12
200
150
100
50
0
ppm (f1)


13
200
150
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50
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ppm (t1)