Supplementary Material (ESI) for Chemical Communications

Supporting Information

Protein surface recognition using geometrically and optically pure Ru(II) tris-(bipyridine) derivatives

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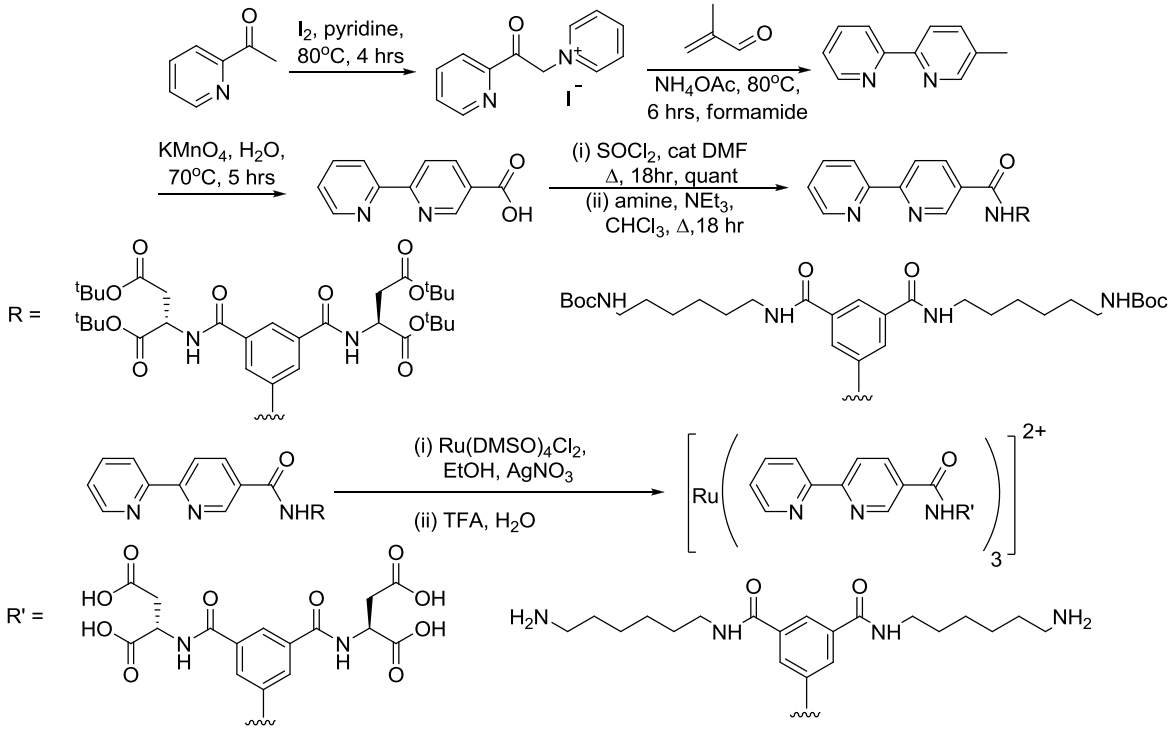
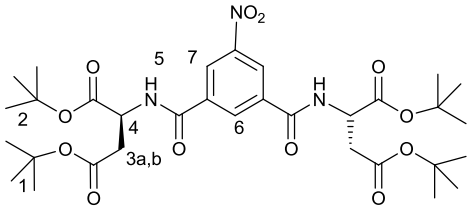
General

All reagents were obtained from Aldrich, Alfa Aesar, Acros or Fluka and used without further purification. All solvents used were HPLC grade. Dry solvents were distilled chloroform from calcium sulphate and dichloromethane from calcium hydride immediately prior to use. Analytical TLC was performed using 0.2 mm silica gel 60 F_{254} pre-coated aluminium sheets (Merck) and visualised using UV irradiation or, in the case of amine intermediates, by staining with a ninhydrin solution. Flash column chromatography was carried out on silica gel 60 (35 to 70 micron particles, FluoroChem). Solvent ratios are described where appropriate. Solvents were removed under reduced pressure using a Büchi rotary evaporator at diaphragm pump pressure. Samples were freed of remaining traces of solvents under high vacuum. ^{1}H and ^{13}C NMR spectra were measured on a Bruker DPX300 or a Bruker Avance 500 spectrometer using an internal deuterium lock. Chemical shifts are reported in parts per million (ppm) downfield from TMS and coupling constants are given in hertz (Hz). Coupling constants are reported to the nearest 0.1 Hz. TMS is defined as 0 ppm for ^{1}H NMR spectra and the centre line of the triplet of $CDCl_3$ was defined as 77.10 ppm for ^{13}C NMR spectra. When describing ^{1}H NMR data the following abbreviations are used; s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Melting points were determined using a Griffin D5 variable temperature apparatus and are uncorrected. Microanalyses were obtained on a Carlo Erba Elemental Analyser MOD 1106 instrument, found composition is reported to the nearest 0.05%. Infrared spectra were recorded on a Perkin-Elmer FTIR spectrometer and samples analysed as solids (unless stated). Mass spectra (HRMS) were recorded in house using a Micromass GCT Premier, with electron impact ionisation (EI) or a Bruker Daltonics micrOTOF, with electrospray ionisation (ES).



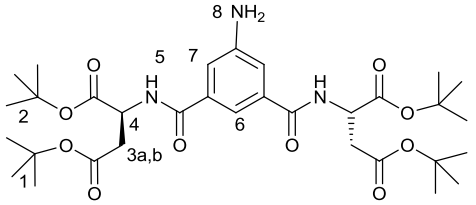
Experimental Details

(2S,2'S)-tetra-tert-butyl 2,2'-((5-nitroisophthaloyl)bis(azanediyl))disuccinate



5'-Nitroisophthaloyl dichloride (1.04g, 4.21 mmol) of was dissolved in anhydrous dichloromethane (50 mL) and added dropwise to a stirred solution of L-aspartic acid di-*tert*-butyl ester hydrochloride (2.51 g, 8.93 mmol) and triethylamine (1.87g, 9.27 mmol) in anhydrous dichloromethane (50 mL) at 0˚C under nitrogen. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was then washed consecutively with aqueous hydrochloric acid, saturated sodium bicarbonate and saturated sodium chloride. The organic layer was dried over magnesium sulfate, concentrated and purified by silica gel column chromatography (ethyl acetate: dichloromethane, 3:7) to afford target product (2.50g, 3.76 mmol, 72%) as white powder: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.70 (s, 2H, H7), 8.60 (1H, s, H6), 7.43 (2H, d, *J* = 7.7 Hz, H5), 4.89 (2H, dt, *J* = 7.7 Hz, 4.3, H4), 3.01 (2H, dd, *J* = 4.3 and 17.1 Hz, H3b), 2.84 (2H, dd , *J* = 4.3 and 17.1 Hz, H3a), 1.49 (18H, s, H2), 1.46 (18H, s, H1); ^{13}C NMR (300 MHz, $CDCl_3$) δ 170.28, 169.37, 163.83, 168.50, 136.47, 131.37, 124.73, 82.98, 82.04, 49.99, 37.30, 28.12, 27.963; m.p. 105-110˚C (Found: C, 57.45%; H, 7.10%; N, 6.15, $C_{32}H_{47}N_3O_{12}$, requires: C, 57.73%; H, 7.12%; N, 6.32%); IR υ_{max}(solid state)/cm^{-1} 3375, 3093, 2980, 2936, 2632, 2293, 1730, 1656, 1625, 1580, 1515, 1342, 1305, 1225, 1160, 1113, 1053, 1035; ESI-MS *m/z* 666 $[M+H]^+$.

(2S,2'S)-tetra-tert-butyl 2,2'-((5-aminoisophthaloyl)bis(azanediyl))disuccinate



To (2S,2'S)-tetra-tert-butyl 2,2'-((5-nitroisophthaloyl)bis(azanediyl))disuccinate (2.00 g, 3.00 mmol) in methanol (20 mL) was added 0.2 g of 10% Pd(C) suspended in methanol (20 mL). Hydrogen was passed over the reaction mixture whilst being stirred overnight. The reaction mixture was filtered through celite®, concentrated and dried thoroughly on vacuum line. The crude aniline was purified by silica gel column chromatography (ethyl acetate: dichloromethane, 3:7) to afford (1.65 g, 2.59 mmol, 87%) of pure product as white powder: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (1H, s, H6), 7.16 (2H, s, H7), 7.12 (2H, d, *J* = 7.7 Hz, H5), 4.79 (2H, dt, *J* = 7.7 and 4.3 Hz, H4), 3.86 (2H, br s, H8), 3.01 (dd, 2H, *J*= 4.3 and 17.1 Hz, H3b), 2.76 (2H, dd , *J* = 4.3 and 17.1 Hz, H3a), 1.41 (18H, s, H2), 1.37 (18H, s, H1). ^{13}C NMR (300 MHz, $CDCl_3$) δ 170.85, 170.26, 166.79, 147.45, 136.10, 116.86, 115.60, 82.93,

82.08, 49,97, 37.89, 28.46, 28.33; m.p. 92˚C; IR υ_{max}(solid state)/cm^{-1} 3373, 2977, 2404, 2295, 1646, 1598, 1512, 1250, 1146. ESI-HRMS found *m/z* 636.3521 $[M+H]^+$, requires $C_{32}H_{50}N_3O_{10}$ 636.3491.

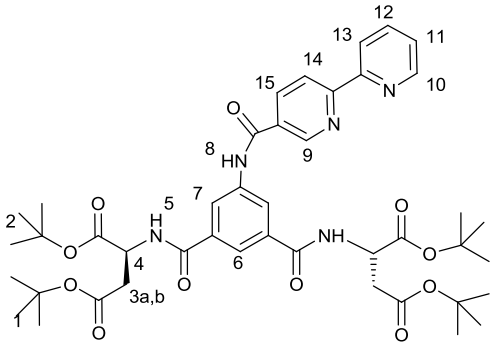
di-tert-butyl(((5-aminoisophthaloyl)bis(azanediyl))bis(hexane-6,1-diyl)) dicarbamate

This compound was synthesized as described previously[1]

5-Carboxy-2,2'-bipyridine

5-Carboxy-2,2'-bipyridine was synthesized as described previously[2]

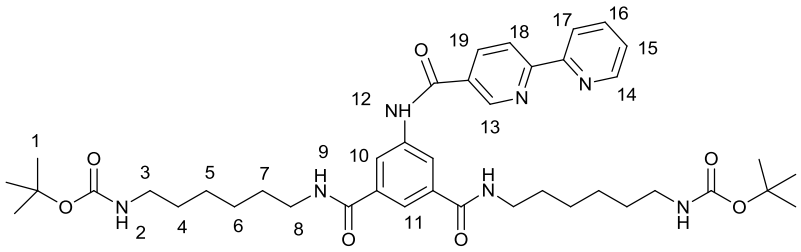
(2S,2'S)-tetra-tert-butyl 2,2'-((5-([2,2'-bipyridine]-5-carboxamido)isophthaloyl)bis(azanediyl))disuccinate



5-Carboxy-2,2'-bipyridine (0.94 g, 4.71 mmol) was converted to acid chloride by reaction with thionyl chloride (25 mL, 128 mmol) at reflux for 3h under an atmosphere of nitrogen. Thionyl chloride was removed and the resulting acid chloride dried for 4h, then dissolved in anhydrous chloroform (50 mL). 0.66 equivalent of (2S,2'S)-tetra-*tert*-butyl 2,2'-((5-aminoisophthaloyl)bis(azanediyl))disuccinate (2.00 g, 3.14 mmol), dissolved in anhydrous chloroform (50 mL) with 2 equivalents of anhydrous triethylamine (1.30 mL) was transferred *via* cannula to the 2,2'-bypiridine-5-carboxyloyl chloride. The reaction mixture was stirred under reflux overnight under an atmosphere of nitrogen. When cooled the reaction mixture was washed consecutively with aqueous hydrochloric acid, saturated sodium bicarbonate and saturated sodium chloride. The organic layer was dried with magnesium sulfate, concentrated and purified by silica gel column chromatography (ethyl acetate: dichloromethane, 3:7) to yield pure product (2.10g, 2.5 mmol, 81%) as a white powder: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.35 (1H, br s, H8), 9.22 (1H, s, H9), 8.72 (1H, d, *J* = 4.3 Hz , H10), 8.55 (1H, d, *J* = 8.5 Hz, H14), 8.47 (1H, d, *J* = 7.7 Hz, H15), 8.39 (2H, s, H7), 8.37 (1H, dd, *J* = 8.5 and 2.5 Hz, H13), 7.96 (1H, s, H6), 7.84 (1H, t, *J* = 8.5 Hz, H12), 7.45 (2H, d, *J* = 8.5 Hz, H5), 7.35 (1H, dd, *J* = 7.7 and 4.3 Hz, H11), 4.83 (2H, dt, *J* = 4.3 and 7.7 Hz, H4), 2.94 (2H, dd, *J* = 4.5 and 17.0 Hz, H3b), 2.84 (2H, dd *J* = 4.5 and 17.0 Hz, H3a), 1.47 (18H, s, H2), 1.45 (18H, s, H1). ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 9.91 (1H, br s, H8), 9.22 (1H, s, H9), 8.49 (1H, d, *J*= 4.5 Hz, H10), 8.37 (1H, d, *J* = 8.5, H14), 8.32-8.30 (4H, m, H7, H15, H13), 7.89 (2H, d, *J* = 7.5 Hz, H5), 7.87 (1H, s, H6), 7.74 (1H, t, *J* = 7.5 Hz, H12), 7.25 (1H, dd, *J* = 5.5 and 7.5 Hz, H11), 4.75 (2H, dd, *J* = 6.5 and 14.0 Hz, H4), 2.76 (2H, dd *J* = 5.5 and 16.5 Hz, H3b), 2.68 (2H, dd, *J* = 5.5 and 16.5 H3a), 1.28 (18H, s,

H2), 1.25 (18H, s, H1). ^{13}C NMR (300 MHz, $CDCl_3$) δ 170.55, 170.07, 166.37, 164.58, 159.21, 155.46, 149.75, 148.68, 139.40, 137.41, 136.54, 135.87, 130.18, 124.79, 122.39, 122.20, 121.91, 121.11, 82.12, 82.98, 50.27, 37.87, 28.47, 28.32; m.p. 102 °C; IR υ_{max}(solid state)/cm^{-1} 3243, 3062, 2979, 2934, 2291, 1730, 1656, 1589, 1557, 1459, 1423, 1393, 1368, 1251, 1145; ESI-HRMS found *m/z* 818.3969 $[M+H]^+$, $C_{43}H_{56}N_5O_{11}$ requires 818.3971.

di-tert-butyl (((5-([2,2'-bipyridine]-5-carboxamido)isophthaloyl)bis(azanediyl))bis(hexane-6,1-diyl))dicarbamate

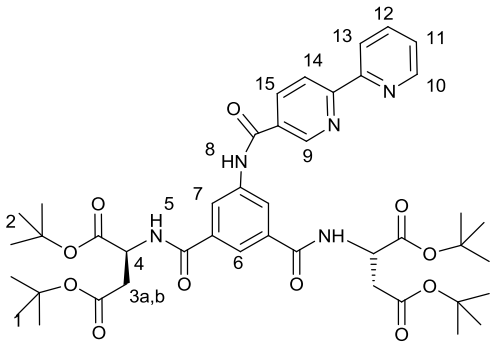


5-Carboxy-2,2'-bipyridine (1.00 g, 5.0 mmol) was converted to acid chloride by reaction with thionyl chloride (25 mL, 128 mmol) at reflux for 3h under an atmosphere of nitrogen. Thionyl chloride was then removed and the resulting acid chloride dried for 4h, then dissolved in dry chloroform. 0.66 equivalent of di-tert-butyl(((5-aminoisophthaloyl)bis(azanediyl))bis(hexane-6,1-diyl)) dicarbamate (1.92g, 3.33 mmol), dissolved in anhydrous chloroform with 2 equivalents of triethylamine (1.3 mL) was transferred *via* cannula to the 2,2'-bypiridine-5-carboxyloyl chloride. The reaction mixture was stirred under reflux under N_2 overnight. When cooled the reaction mixture was washed consecutively with aqueous hydrochloric acid, saturated sodium bicarbonate and saturated sodium chloride. The organic layer was dried with magnesium sulfate, concentrated and purified by silica gel column chromatography (ethyl acetate: dichloromethane, 1:4) increasing to (ethyl acetate: dichloromethane, 4:1), to afford the pure product (2.20 g, 2.89 mmol, 87%) as a white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.66 (1H, br s, H12), 9.24 (1H, s, H13), 8.70 (1H, d, *J* = 4.3 Hz, H14), 8.50 (1H, d, *J*= 8.4 Hz, H19), 8.47 (2H, s, H10), 8.46 (1H, d, *J* = 8.4 Hz, H18), 8.38 (1H, dd, *J* = 8.4 and 1.7 Hz, H17), 7.98 (1H, s, H11), 7.81 (1H, dt, *J*= 7.7 and 1.7 Hz, H16), 7.33(1H, dd, *J* = 8.4 and 5.1 Hz, H15), 7.19 (2H, br s, H9), 4.82 (2H, br s, H2), 3.28 (4H, dt, *J* = 6.8 and 6.0 Hz, H8), 3.02 (4H, dt, *J* = 6.8 and 6.0 Hz, H3), 1.48 (4H, t, *J* = 6.8 Hz, H4), 1.40 (18H, s, H1), 1.35-1.38 (4H, m, H7), 1.18-1.27 (8H, m, H5 and H6); ^{13}C NMR (300 MHz, $CDCl_3$) δ 166.80, 164.81, 158.68, 156.31, 155.03, 149.38, 148.68, 139.25, 137.06, 136.29, 135.73, 129.77, 124.45, 122.06, 121.78, 120.58, 91.03, 79.15, 40.22, 40.00, 29.90, 29.27, 28.46, 26.33, 26.10; m.p. 94°C; IR υ_{max}(solid state)/cm^{-1} 3319, 3071, 2977, 2932, 2860, 1806, 1647, 1590, 1542, 1444, 1391, 1365, 1289, 1253, 1169; ESI-HRMS found *m/z* 760.4389 $[M+Na]^+$ $C_{41}H_{58}N_7O_{15}Na$ requires 760.4392.

1-protected

(2S,2'S)-tetra-tert-butyl 2,2'-((5-([2,2'-bipyridine]-5-carboxamido)isophthaloyl)bis(azanediyl))disuccinate (0.67 g, 0.82 mmol) and of

(dimethylsulfoxide)dichlororuthenium (II)[3] (0.12 g, 0.25 mmol) were dissolved in 50mL of anhydrous ethanol. To the reaction mixture was added silver nitrate (0.085 g, 0.50 mmol) and stirring continued at reflux under an atmosphere of nitrogen for three days. The red coloured solution was filtered through a celite® pad then evaporated. An excess of (2S,2'S)-tetra-tert-butyl 2,2'-((5-([2,2'-bipyridine]-5-carboxamido)isophthaloyl)bis(azanediyl))disuccinate was removed by silica gel column chromatography (5% increasing to 10% methanol in dichloromethane). Several distinctive red coloured bands were observed and later identified as Λ-*mer*, Δ-*mer*, Δ-*fac* and Λ-*fac* isomers. Yields of isolated isomers: 34, 39, 11, 12 % respectively.



Λ-*mer* 1 protected

^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 11.4 (2H, br s, H8), 11.3 (1H, br s, H8'), 8.77 (3H, d, *J* = 8.0 Hz, H13), 8.73 (3H, d, *J* = 8.0 Hz, H14), 8.70-8.61 (3H, br s, H6), 8.41-7.72 (24H, m, H12, H7, H15, H5, H10, H9), 7.45 (3H, m, H11), 4.77-4.68 (6H, m, H4), 2.74 -2.56 (12H, m, H3b, H3a), 1.30-1.22 (54H, m, H1) ^{13}C NMR (300 MHz, $(CD_3)_2CO$) δ 171.11, 171.06, 170.89, 170.83, 167.12, 166.86, 166.74, 164.08, 163.56, 160.62, 160.47, 160.27, 157.93, 157.60, 157.33, 153.97, 152.33, 141.16, 140.77, 140.66, 139.26, 139.06, 136.63, 136.51, 136.44, 135.90, 135.28, 129.79, 126.56, 125.49, 125.27, 123.39, 123.19, 122.80, 121.93, 112.73, 82.65, 82.49, 81.77, 81.72, 51.80, 51.71, 51.51, 28.70, 28.68, 28.63, 28.59, 28.56; m.p.>250 °C; υ_{max}(solid state)/cm^{-1} 2978, 2513, 2159, 2029, 1715, 1223, 845, 755; ESI-HRMS found *m/z* 1277.0350 $[M]^+$ $C_{129}H_{165}N_{15}O_{33}Ru$ requires 1277.0363.

Δ-*mer* 1 protected

^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 11.4 (2H, br s, H8), 11.3 (1H, br s, H8'), 8.85 (3H, d, *J* = 8.0 Hz, H13), 8.79 (3H, d, *J* = 8.0 Hz, H14), 8.80-8.75 (3H, br s, H6), 8.60-7.80 (24H, m, H12, H7, H15, H5, H10, H9), 7.45 (3H, br s, H11), 4.75-4.64 (6H, m, H4), 2.74 -2.56 (12H, m, H3b, H3a), 1.30-1.22 (54H, m, H1); ^{13}C NMR (300 MHz, $(CD_3)_2CO$) δ 171.21, 171.13, 171.06, 171.97, 170.97, 170.90, 170.86, 167.11, 166.86, 164.09, 163.55, 160.46, 160.22, 157.93, 157.36, 154.20, 152.39, 141.14, 140.76, 140.60, 139.09, 138.40, 136.65, 136.45, 136.35, 135.93, 135.24, 129.72, 129.44, 126.52, 125.48, 125.27, 123.43, 123.16, 114.13, 82.64, 82.55, 82.49, 81.87, 81.77, 81.72, 51.80, 51.71, 51.51, 28.71, 28.64, 28.57; m.p.>250 °C; υ_{max}(solid state)/cm^{-1} 2979, 2512, 2159, 2029, 1714, 1222, 845, 702; ESI-HRMS found *m/z* 1277.0321 $[M]^+$ $C_{129}H_{165}N_{15}O_{33}Ru$ requires 1277.0363.

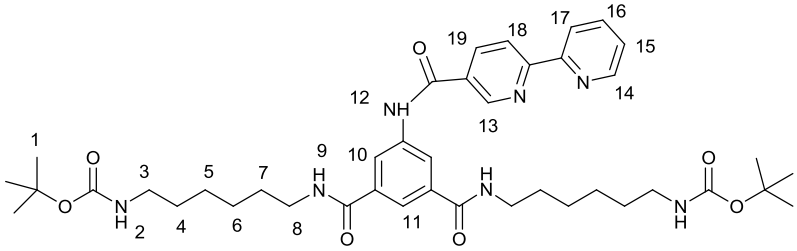
Δ-*fac* 1 protected

^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 11.02 (3H, br s, H8) 8.73 (3H, d, *J* = 8.5 Hz, H13), 8.71 (3H, d, *J* = 8.0 Hz, H14), 8.65 (3H, s, H6), 8.29 (3H, d, *J* = 8.0 Hz, H12), 8.23 (6H, s, H7), 8.05 (3H, t, *J* = 8.0 Hz, H15), 7.99 (6H, d, *J* = 8.0 Hz, H5), 7.91 (3H, d, *J* = 5.5 Hz, H10), 7.89 (3H, s, H9), 7.43 (3H, t, *J* = 6.5 Hz, H11), 4.71 (6H, dd, *J* = 6.0 and 14.0 Hz, H4), 2.69 (6H, dd, *J* = 5.5 and 16.5 Hz, H3b), 2.63 (6H, dd, *J* = 6.5 and 16.5 Hz, H3a), 1.26 (54H, s, H2), 1.25 (54H, s, H1); ^{13}C NMR (300 MHz, $(CD_3)_2CO$) δ 171.04, 170.89, 167.00, 163.79, 160.25, 158.05, 153.14, 152.44, 140.72, 139.47, 139.17,135.58, 136.40, 129.54, 126.87, 125.68, 123.54, 122.63, 82.43, 81.77, 51.63, 38.48, 28.69, 28.56; m.p. > 250 ˚C; υ_{max}(solid state)/cm^{-1} 3032, 2536, 2159, 1976, 1714, 1217, 702; ESI-HRMS found *m/z* 1277.0310 $[M]^+$ $C_{129}H_{165}N_{15}O_{33}Ru$ requires 1277.0363.

Λ-*fac* 1 protected

^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 10.97 (3H, br s, H8) 8.73 (3H, d, *J* = 8.5 Hz, H13), 8.71 (3H, d, *J* = 8.5 Hz, H14), 8.62 (3H, s, H6), 8.32 (3H, d, *J* = 9.0 Hz, H12), 8.24 (6H, s, H7), 8.03 (3H, t, *J* = 8.0 Hz, H15), 7.99 (6H, d, *J* = 8.0 Hz, H5), 7.98 (3H, s, H15), 7.90 (3H, s, H9), 7.87 (3H, d, *J* = 5.5 Hz, H10), 7.43 (3H, t, *J* = 6.5 Hz, H11), 4.71 (6H, dd, *J* = 6.0 and 14.0 Hz, H4), 2.69 (6H, dd, *J* = 5.5 and 16.5 Hz, H3b), 2.61 (6H, dd, *J* = 6.5 and 16.5 Hz, H3a), 1.26 (54H, s, H2), 1.23 (54H, s, H1); ^{13}C NMR (300 MHz, $(CD_3)_2CO$) δ 169.59, 169.47, 165.44, 162.35, 158.80, 156.54, 151.64, 150.99, 139.23, 137.93, 137.69, 135.11, 134.80, 128.05, 125.36, 124.21, 122.11, 82.40, 81.77, 51.60, 38.48, 28.68, 28.55; m.p. > 250 ˚C, υ_{max}(solid state)/cm^{-1} 2979, 2513, 2159, 2029, 1715, 1148, 845, 756; ESI-HRMS found *m/z* 1277.0318 $[M]^+$ $C_{129}H_{165}N_{15}O_{33}Ru$ requires 1277.0363.

2-protected

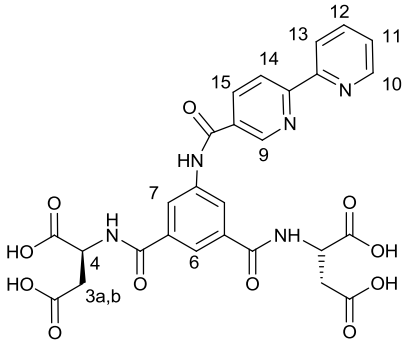


0.200 g (0.26 mmol) of di-tert-butyl (((5-([2,2'-bipyridine]-5-carboxamido)isophthaloyl)bis(azanediyl))bis(hexane-6,1-diyl))dicarbamate and 0.038 g (0.079 mmol) of (dimethylsulfoxide)dichlororuthenium (II)[3] were dissolved in 25mL of dry ethanol. To the reaction mixture was added 0.027 g (0.16 mmol) of silver nitrate and stirring continued at reflux under an atmosphere of nitrogen for three days. The red coloured solution was concentrated and purified using silica gel column chromatography (5-10% methanol in dichloromethane) to yield the product as a red solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.96 (3H, br s, H12), 9.21 (3H, br s, H13), 8.65 (3H, br s, H14), 8.38 (15H, br s, H19, H10, H17, H18), 7.98 (3H, br s, H11), 7.81 (3H, br s, H16), 7.34 (3H, br s, H15), 7.21 (6H, br s, H9), 4.90 (6H, br s, H2), 3.32 (12H, br s, H8), 3.02 (12H, br s, H3), 1.55 (12H, br s, H4), 1.40 (54H, s, H1), 1.30 (36H, br s, H7, H6, H5); m.p. 220˚C (found: C, 64.15 %, H, 7.5 %,

N, 12.70 %. $C_{123}H_{171}N_{21}O_{21}$ requires C, 64.80 %; H, 7.56 %; N, 12.90 %); υ_{max}(solid state)/cm^{-1} 3320, 2933, 2857, 2428, 1788, 1678, 1529, 1443, 1363, 1253; ESI-MS found *m/z* 1190.0989 $[M]^{2+}$ $C_{123}H_{172}N_{21}O_{21}Ru$ requires 1190.6035..

Deprotection of ruthenium complexes

Stirring of four isomers of **1** and of **2** in trifluoroacetic acid 90% and triisopropylsilane 1% in water for a period of 3 days to affoar following concentration the de-protected species.



Λ-*mer* 1

^{1}H NMR (500 MHz, D_2O); δ 8.48 (3H, br s, H13), 8.31 (3H, d, *J* = 7.5 Hz, H14), 8.11 (3H, br s, H6), 7.86 (3H, br s, H12), 7.85 (4H, s, H7), 7.80 (2H, s, H7'), 7.60 (3H, br s, H15), 7.59 (3H, br s, H10), 7.38 (3H, br s, H9), 7.27 (3H, br s, H11), 4.27 (6H, br s, H4), 2.74 (6H, d, *J*=16.0 Hz, H3b), 2.63 (6H, dd, *J*=16.0 and 8.0 Hz, H3a), 2.50 (12H, br s, H3b'), 2.43 (12H, br s, H3a'). m.p. > 250 ˚C, υ_{max}(solid state)/cm^{-1} 3233, 2521, 2159, 2029, 1713, 1205, 801, 724. ESI-HRMS found *m/z* 940.6606 $[M]^{2+}$ $C_{81}H_{69}N_{15}O_{33}Ru$ requires 940.6607; λ_{max} = 467 nm (ε = 8.66 x 10^3 dm^{-3} mol^{-1} cm^{-1})

Δ-*mer* 1

^{1}H NMR (500 MHz, D_2O); δ 8.60 (3H, br s, H13), 8.42 (3H, d, *J* = 7.5 Hz, H14), 8.10 (3H, br s, H6), 7.98 (3H, br s, H12), 7.95 (4H, s, H7), 7.92 (2H, s, H7'), 7.71 (3H, br s, H15), 7.47 (3H, br s, H10), 7.37(3H, br s, H9), 7.27 (3H, br s, H11), 4.40 (6H, br s, H4), 2.83 (2H, dd, *J* = 14.5 and 3.5 Hz, H3b), 2.71 (2H, dd, *J*=16.0 and 8.0 Hz, H3a), 2.60 (8H, br s, H3b', H3a') m.p. > 250 ˚C, υ_{max}(solid state)/cm^{-1} 3201, 2520, 2159, 2029, 1714, 1216, 702. ESI-HRMS found *m/z* 940.6597 $[M]^{2+}$ $C_{81}H_{69}N_{15}O_{33}Ru$ requires 940.6607; λ_{max} = 467 nm (ε= 8.66 x 10^3 dm^{-3} mol^{-1} cm^{-1})

Δ-*fac* 1

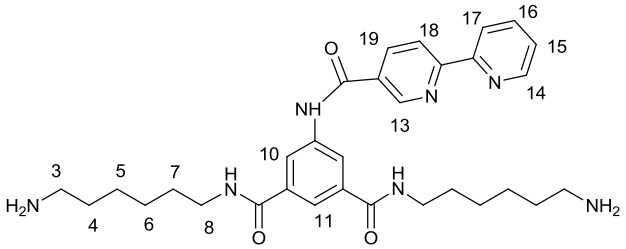
^{1}H NMR (500 MHz, D_2O) δ 8.53 (3H, s, H13), 8.32 (3H, d, *J* = 7.5 Hz, H14), 8.04 (3H, s, H9), 7.96 (3H, br s, H12), 7.95 (6H, s, H7), 7.65 (9H, br s, H15, H10, H11), 7.32 (3H, s, H6), 4.55 (6H, dd, *J*=9.5 and 3.5 Hz, H4), 2.74 (6H, d, *J* = 14.5 Hz, H3b), 2.59 (6H, dd, *J* = 15.5 and 10.0 Hz, H3a); m.p. > 250 ˚C; λ_{max}(solid state)/cm^{-1} 3032, 2536, 2159, 1976, 1714, 1217, 702; ESI-HRMS found *m/z* 940.6609 $[M]^{2+}$ $C_{81}H_{69}N_{15}O_{33}Ru$ requires 940.6607; λ_{max} = 467 nm (ε = 8.95 x 10^3 dm^{-3} mol^{-1} cm^{-1})

Λ-*fac* 1

^{1}H NMR (500 MHz, D_2O) δ 8.93 (3H, s, H13), 8.15 (3H, d, *J* =7.5 Hz, H14), 8.04 (6H, s, H7), 7.95 (3H, br s, H12), 7.87 (3H, t, *J* = 7.5 Hz, H15), 7.79 (3H, br s, H9), 7.51 (3H, s, H6), 7.41 (3H, d,

J=6.0 Hz, H10), 7.25 (3H, t, *J*=6.5 Hz, H11), 4.21 (6H, br s, H4), 2.75 (6H, br s, H3b), 2.63 (6H, br s, H3a), 2.51; m.p. > 250 ˚C; υ_{max}(solid state)/cm^{-1} 3189, 2526, 2159, 2029, 1713, 1204, 702; λ_{max} = 467 nm (ε = 8.95 x 10^3 dm^{-3} mol^{-1} cm^{-1}) ESI-HRMS found *m/z* 940.6652 $[M]^{2+}$ $C_{81}H_{69}N_{15}O_{33}Ru$ requires 940.6607; λ_{max} = 467 nm (ε = 8.95 x 10^3 dm^{-3} mol^{-1} cm^{-1})

2



^{1}H NMR (500 MHz, D_2O) δ 8.67 (3H, br s, H13), 8.52 (3H, br s, H14), 8.43 (3H, br s, H18), 8.25 (3H, br s, H19), 8.04 (3H, t, *J* = 8.0 Hz, H17), 7.81 (6H, s, H10), 7.72 (3H, d, *J* = 21.0 Hz, H16), 7.59 (3H, br s, H11), 7.41 (3H, br s, H15), 3.25 (12H, br s, H8), 2.96 (12H, br s, H3), 1.54-1.48 (12H, m, H7), 1.29-1.25 (12H, m, H4), 1.19 (24H, br s, H5 and H6); υ_{max}(solid state)/cm^{-1} 2861, 2062, 1249, 1159, 970, 897; ESI-HRMS found *m/z* 889.9444 $[M]^{2+}$ $C_{93}H_{123}N_{21}O_9Ru$ requires 889.9423.

UV-vis spectra

Stock solutions of receptors were prepared to a concentration of 1 mM in 5 mM phosphate buffer. Once the receptors had been dissolved, the pH was adjusted to 7.4 *via* addition of 1 N sodium hydroxide. A series of stock dilutions (10 μM -300 μM) was prepared and the extinction coefficient determined by monitoring the absorbance at 467 nm.

Fluorescence Titrations

Horse Heart cytochrome *c* was purchased from Sigma (C2506). The concentration of horse heart cytochrome *c* and 60% acetylated horse heart cytochrome *c* was determined using the molar extinction coefficient at 550 nm of 2.95 x 10^4 dm^{-3} mol^{-1} cm^{-1} following reduction with excess dithionite as described elsewhere. During the course of all titrations the guest solution contained a concentration of host equivalent to that in the host solution and the volume added was kept small to minimize large changes in the concentration of guest, which were corrected for in the regression analysis. In a typical experiment, to a 4 mL fluorescence cuvette containing 2.5 mL of 1 μM Ru complex was titrated 100 μM cytochrome *c* containing 1 μM Ru complex solution in 5-20 μL aliquots. Titration of horse heart cytochrome *c* into a solution of receptor (5 mM phosphate, pH 7.4) resulted in efficient fluorescence quenching (ex 467 nm) as a result of complex formation with the heme edge region of the protein. Curve fitting of this data to a 1:1 binding model gave dissociation constants. Due to light induced decomposition of **2**, the binding affinity for cytochrome *c* was tested in a plate-reader (serial dilution) where the concentration of the complex was kept constant at 10 μM and the concentration of cytochrome *c* was varied between 0 and 22 μM in a fixed volume of 300 μL of 5 mM phosphate buffer. The recorded fluorescence decreased linearly by 11% for the range of 0-22

μM of increasing concentration of cytochrome *c*. Eqn (1) operating in Origin 7.0 was used to derive the dissociation constant for 1:1 binding isotherm for receptors **1**.

$$f = m[(nc + x + K) - \sqrt{\{n*c + x + K)^2 - 4ncx\}}]/2nc \qquad (1)$$

f = change in relative fluorescence, *m* = maximum value of *f*, *n* = stoichiometry, *c* = concentration of receptor, *K* = dissociation constant, *x* = concentration of protein added.

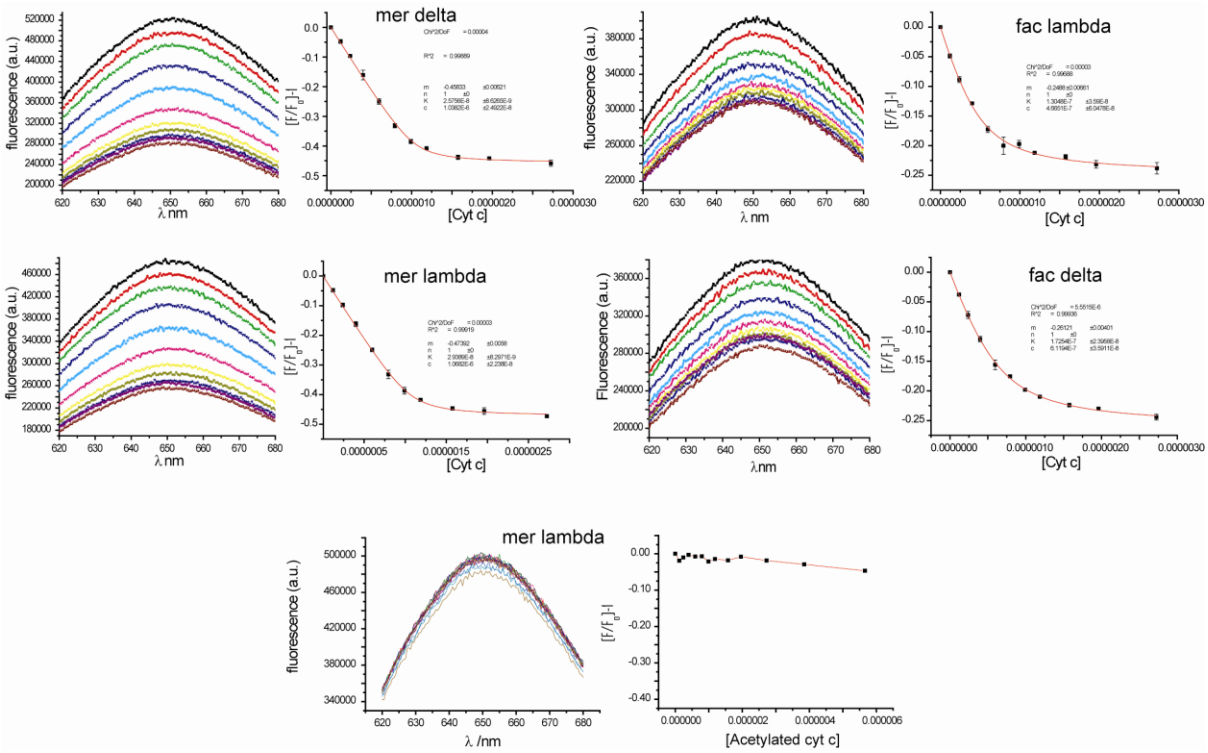


Figure ESI 1. Raw titration data and titration curves for Λ-*mer*, Δ-*mer*, Δ-*fac* and Λ-*fac* isomers of compound **1** (1 μM, pH 7.4, 5 mM phosphate buffer) binding to cytochrome *c* and a representative example of a titration with acetylated cytochrome *c* under identical conditions (for Λ-*mer* **1**)

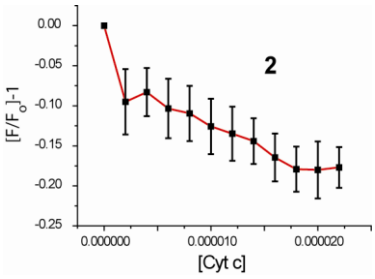


Figure ESI 2. Raw titration data and titration curves for compound **2** (pH 7.4, 5 mM phosphate buffer)

UV-Vis-monitored ascorbate reduction assay

A solution (5 mM sodium phosphate, pH 7.4) containing cytochrome *c* (15 μM) and compound **1** was incubated for 10 min at room temperature with ruthenium complex or on its own in a quartz cuvette (pathlength of 1cm). 150 μL of a 20 mM stock solution of ascorbate in 5 mM sodium phosphate buffer (pH 7.4) was then added. Upon addition the content of the cuvette was immediately mixed using a 1 mL pipette twice whilst starting the spectrophotometer. The instrument was set to measure the absorbance at 550 nm, every 0.5 seconds, for 2 min. To test the rate dependence on concentration of the receptor, five different solutions with molar ratios of 0 to 1.75 (receptor: Cyt *c*) were subjected to ascorbate reduction. The collected data was analysed based on the equation derived from a pseudo first order rate expression: ΔAobs = *max*(1-exp(-*k*obs*t)); where ΔAobs = $Aobs_t$ – $Aobs_{t0}$ = 0, and *t* =time in seconds. Both *max* and *k* were floated, and were determined by regression analysis in the OriginPro 8 software. The parameter *max* corresponds to maximal ΔAobs as *t* approaches infinite or completion of reaction, and the parameter *k*obs corresponds to the observed pseudo-first order rate constant in units of s^{-1}. A plot of *k*obs vs [receptor] gave a curve that was analysed based on the equation Δ*k*obs=A*exp(-*k*eq x) + b; where A corresponds to maximal Δ*k*obs when the concentration of the receptor is zero and b is the base line. The equilibrium constants were determined by regression analysis in the OriginPro 8 software.

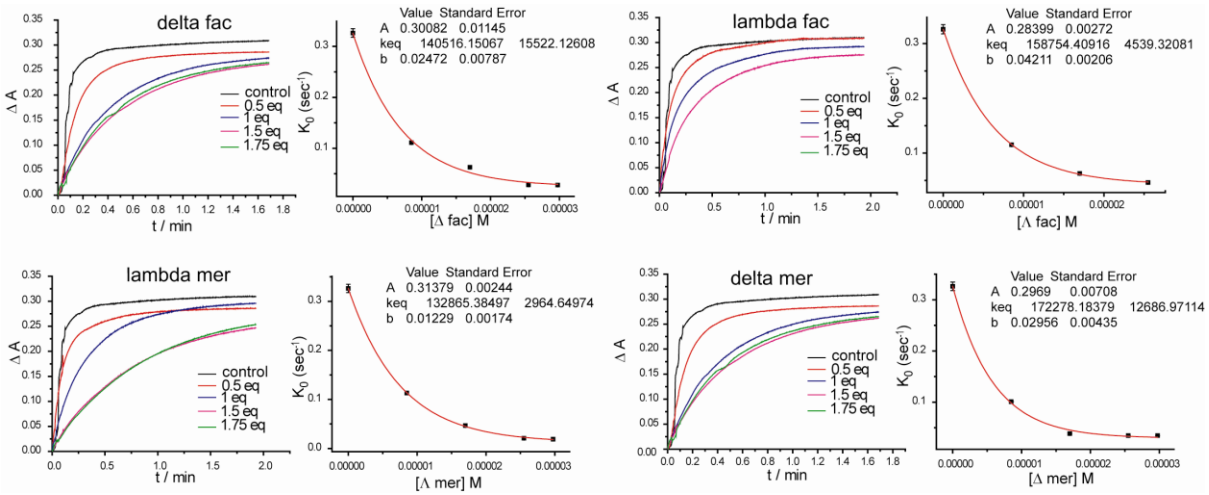


Figure ESI 3. Ascorbate reduction kinetics of cytochrome c (15 μM) for Λ-*mer*, Δ-*mer*, Δ-*fac* and Λ-*fac* isomers of compound **1** (pH 7.4, 5 mM phsophate buffer)

Titrations with α-chymotrypsin

Titrations were performed in a similar manner as for cytochrome *c*. Bovine α-chymotrypsin was purchased from Sigma (C4129) and the concentration of stock solutions determined using the molar extinction coefficient at 280 nm of 5.06 x 10^4 dm^{-3} mol^{-1} cm^{-1} calculated using the online facility on the ExPAsy website. The concentration of protein in the stock solution was verified from the absorbance at 280 nm in 6 M GdmHCl, adjusted to pH 6.0 by addition of sodium hydroxide.

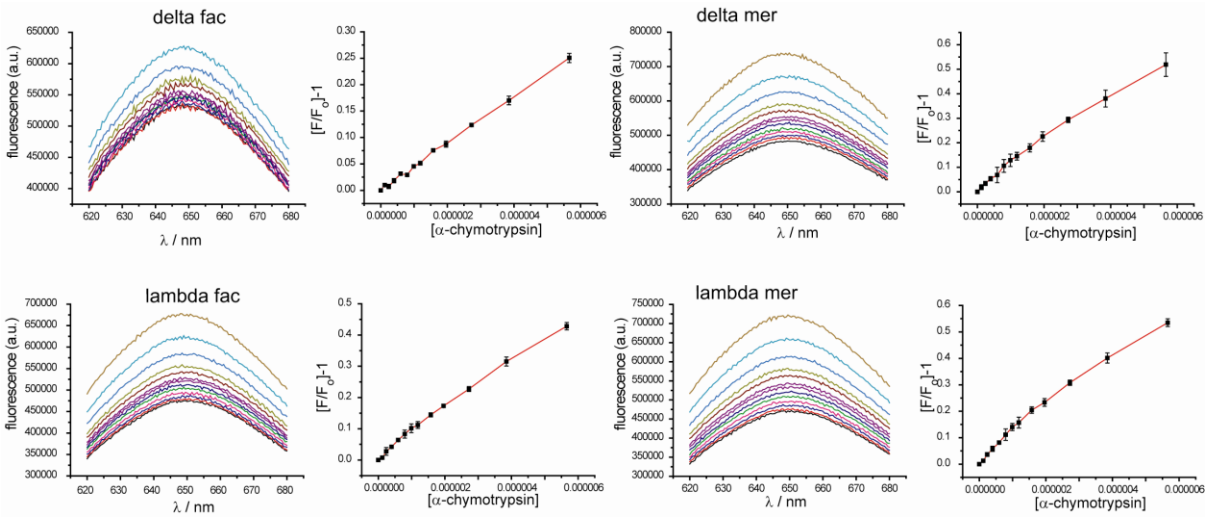


Figure ESI 4. Raw titration data and dose response for Λ-*mer*, Δ-*mer*, Δ-*fac* and Λ-*fac* isomers of compound **1** (1 μM, pH 7.4, 5 mM phosphate buffer) upon titration with α-chymotrypsin

Circular Dichroism Spectra

CD measurements were performed on a Jasco J-715 spectropolarimeter using a Jasco PTC-348W peltier system for temperature control. Cuvettes (Starna Scientific Ltd.) with a pathlength of 1 mm were used for UV CD. The concentrations of the ruthenium complexes solutions were 100μM in H_2O at pH 7.0 or in methanol (of the protected species). The spectra were recorded at 100 nm/ min (20°C).

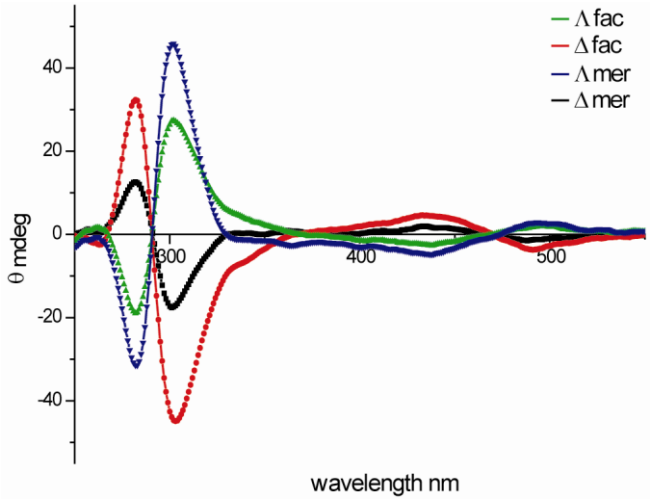
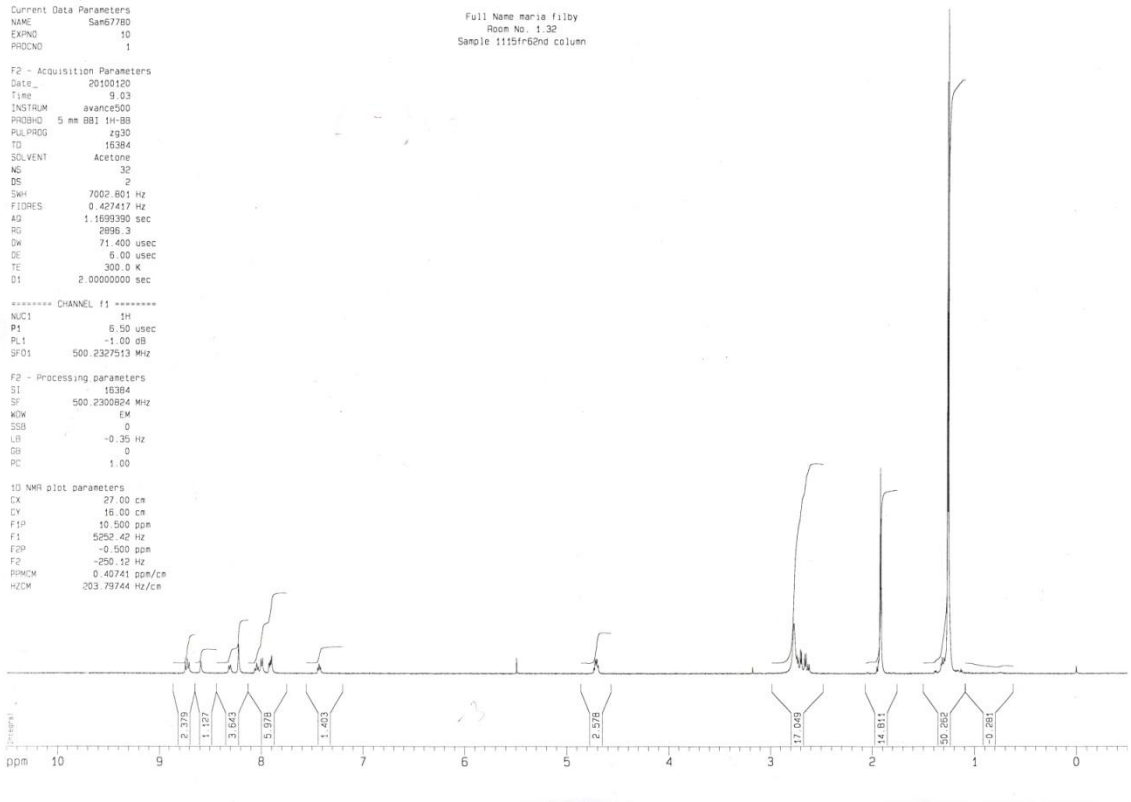


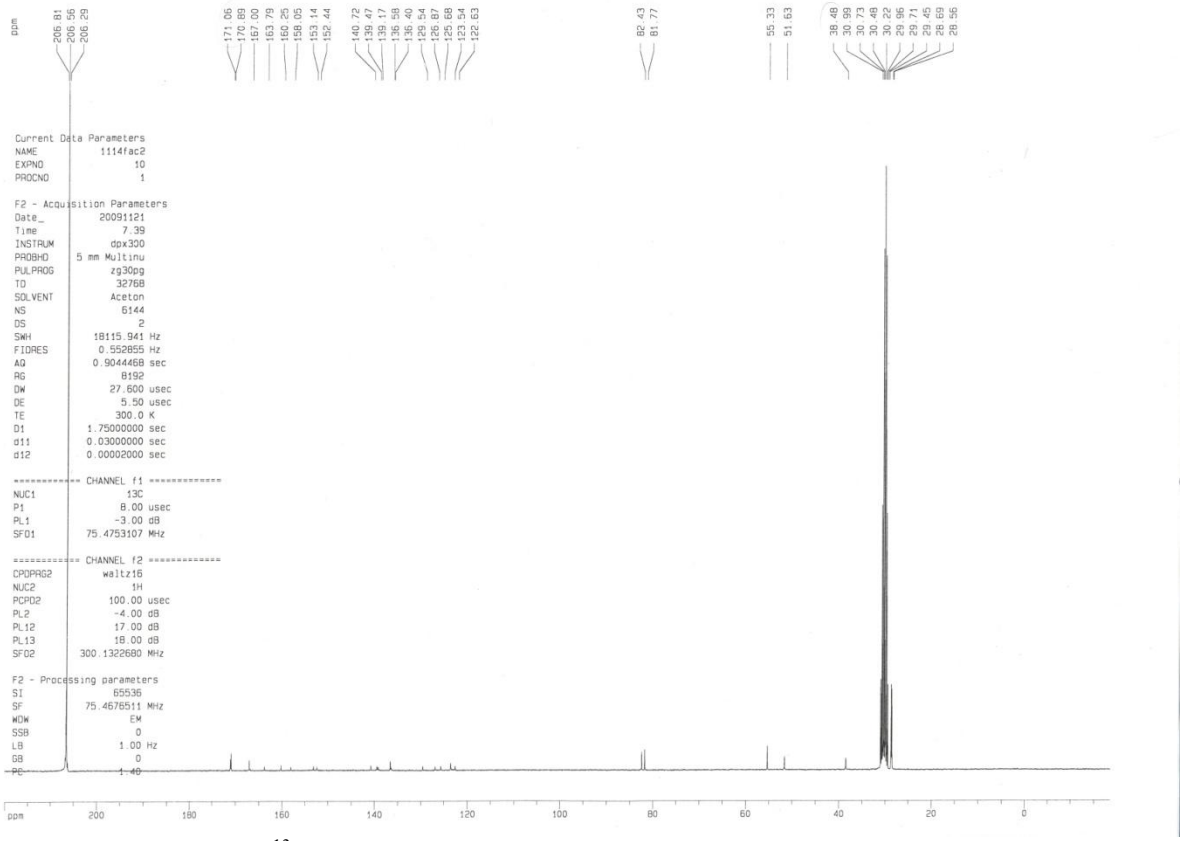
Figure ESI 5. Circular Dichroism spectra (CD) of ^{t}Butyl ester protected *fac*/*mer* Λ/Δ **1** (methanol 100μM)

References

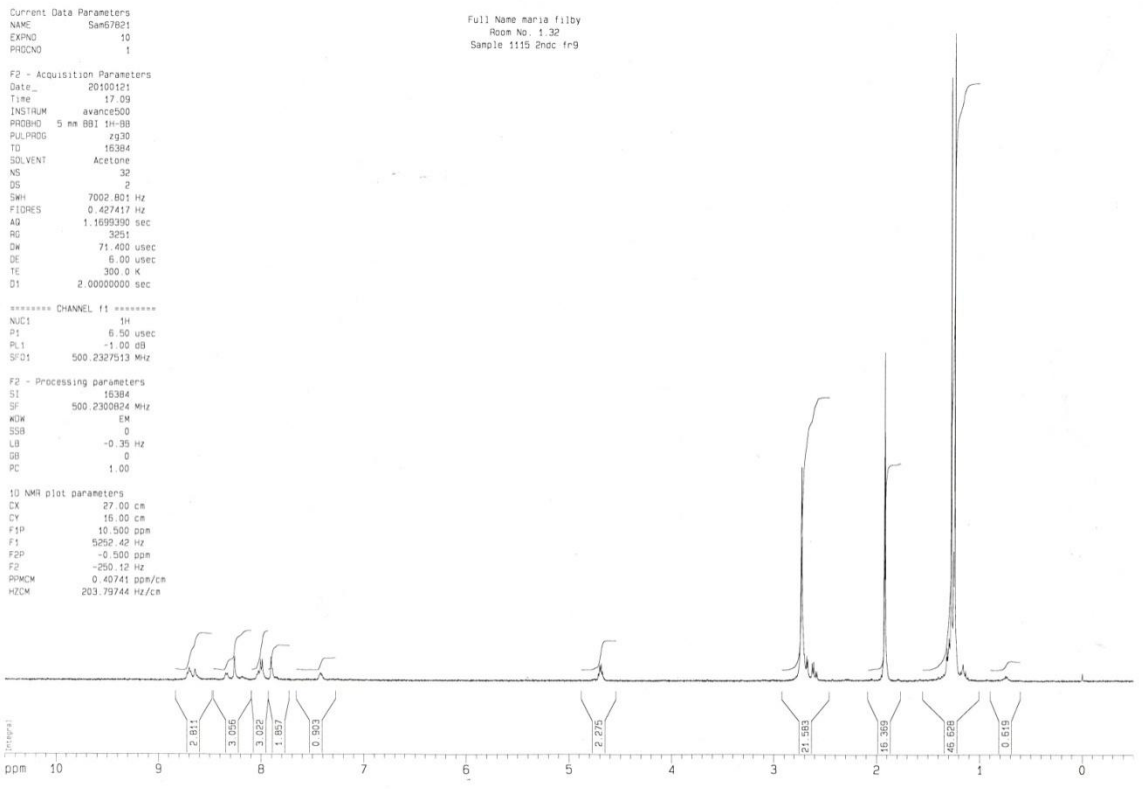
(1) Chung, C. W. Y.; Toy, P. H. *J. Comb. Chem.* **2007**, *9*, 115-120.
(2) Muldoon, J.; Ashcroft, Alison E.; Wilson, Andrew J. *Chem. Eur. J.* **2010**, *16*, 100-103.
(3) Mahalingam, V.; Chitrapriya, N.; Fronczek, F. R.; Natarajan, K. *Polyhedron* **2008**, *27*, 2743-2750.



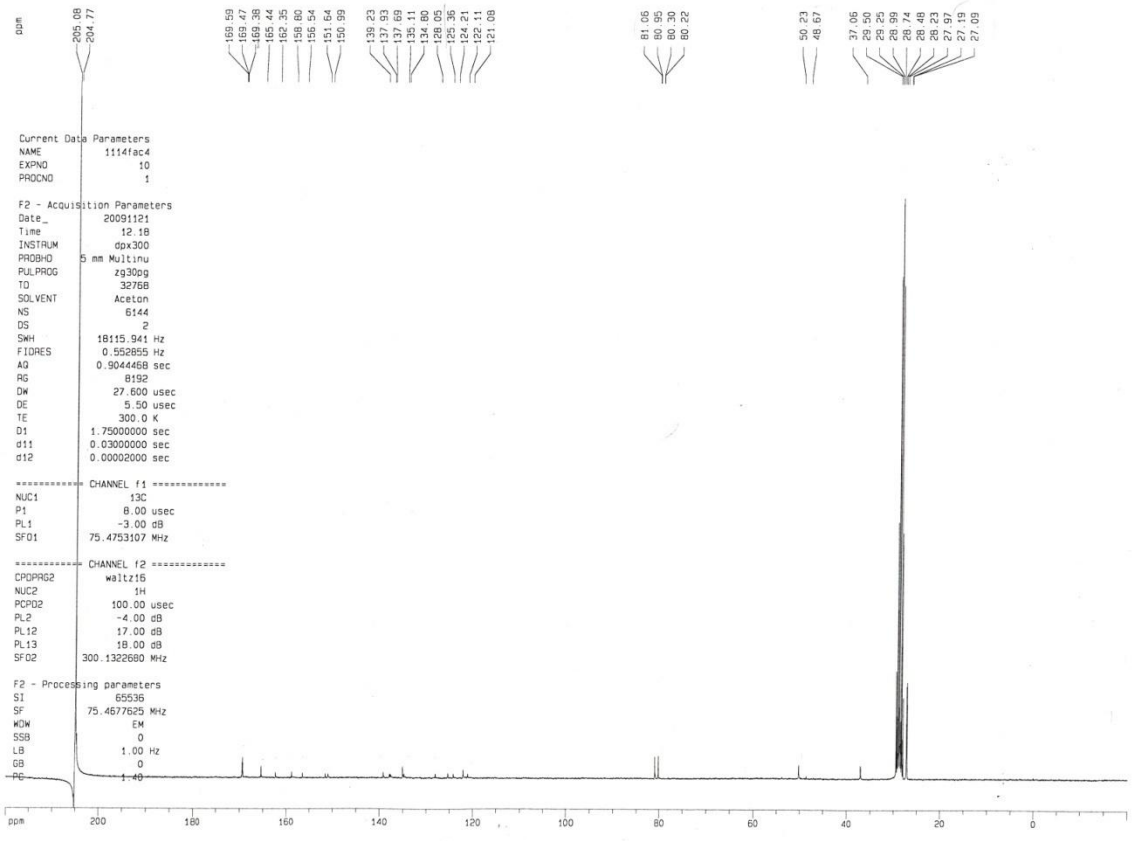
^{1}H NMR (500 MHz, $(CD_3)_2CO$) protected Δ-*fac* **1**



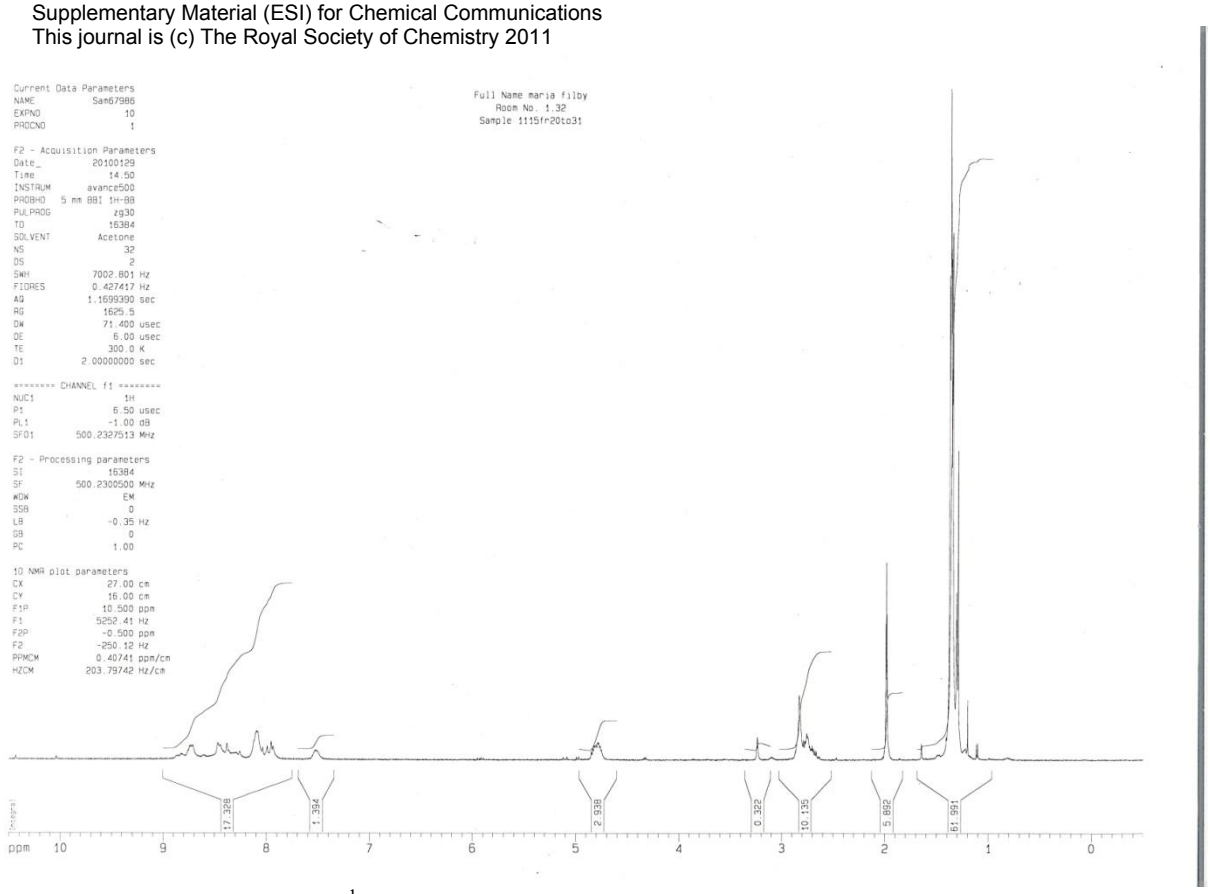
^{13}C NMR (75 MHz, $(CD_3)_2CO$) protected Δ-*fac* **1**



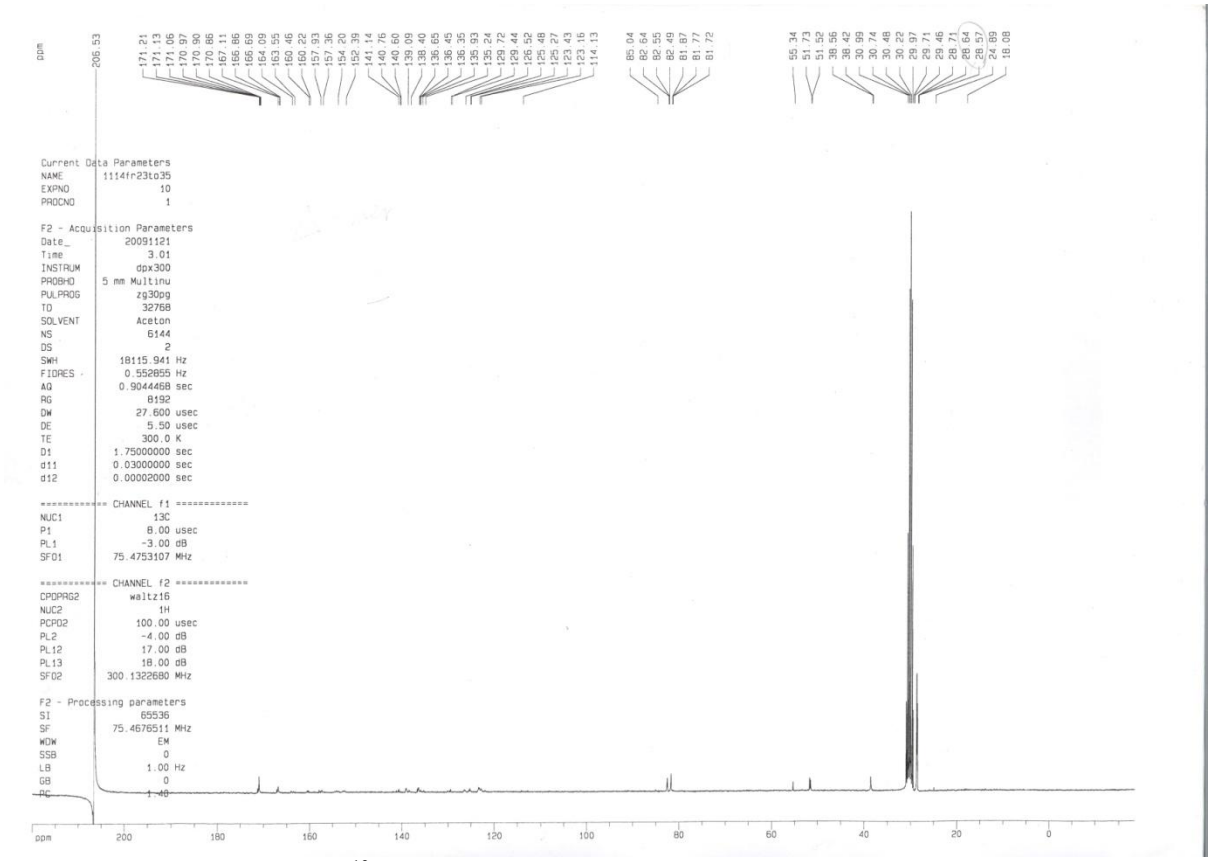
^{1}H NMR (500 MHz, $(CD_3)_2CO$) protected Λ-*fac* **1**



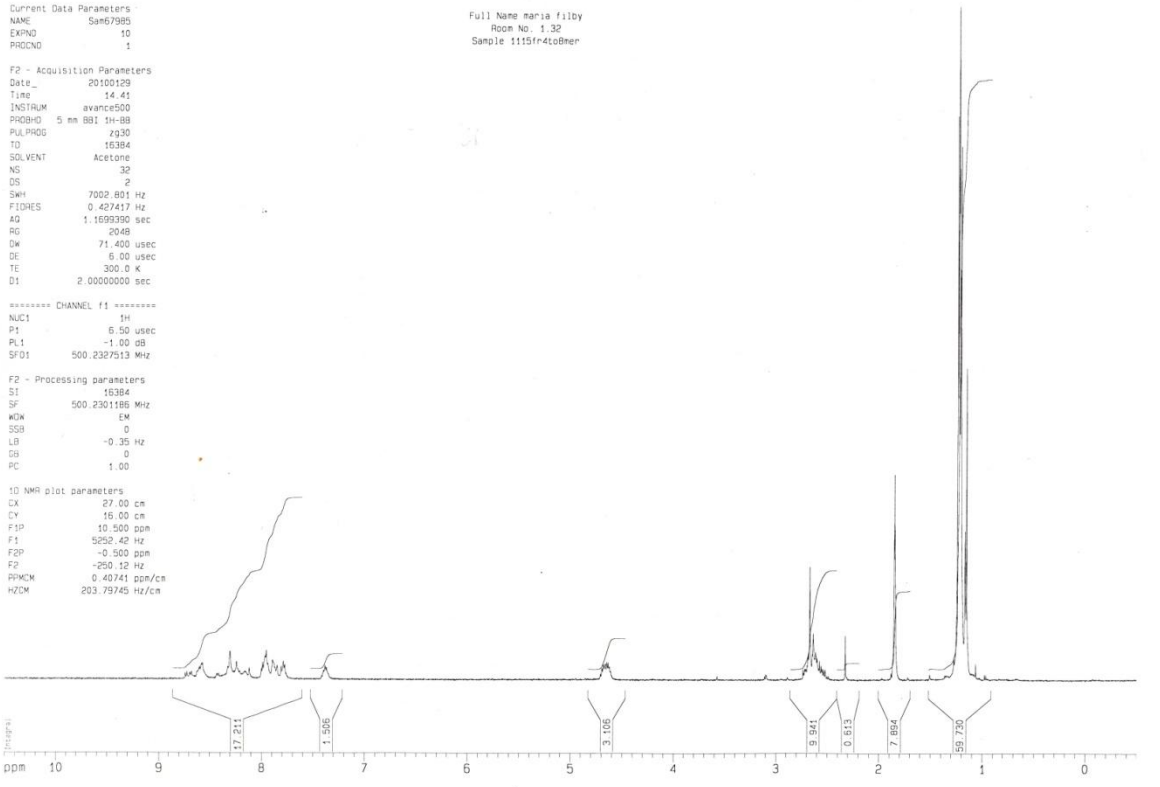
^{13}C NMR (75 MHz, $(CD_3)_2CO$) protected Λ-*fac* **1**



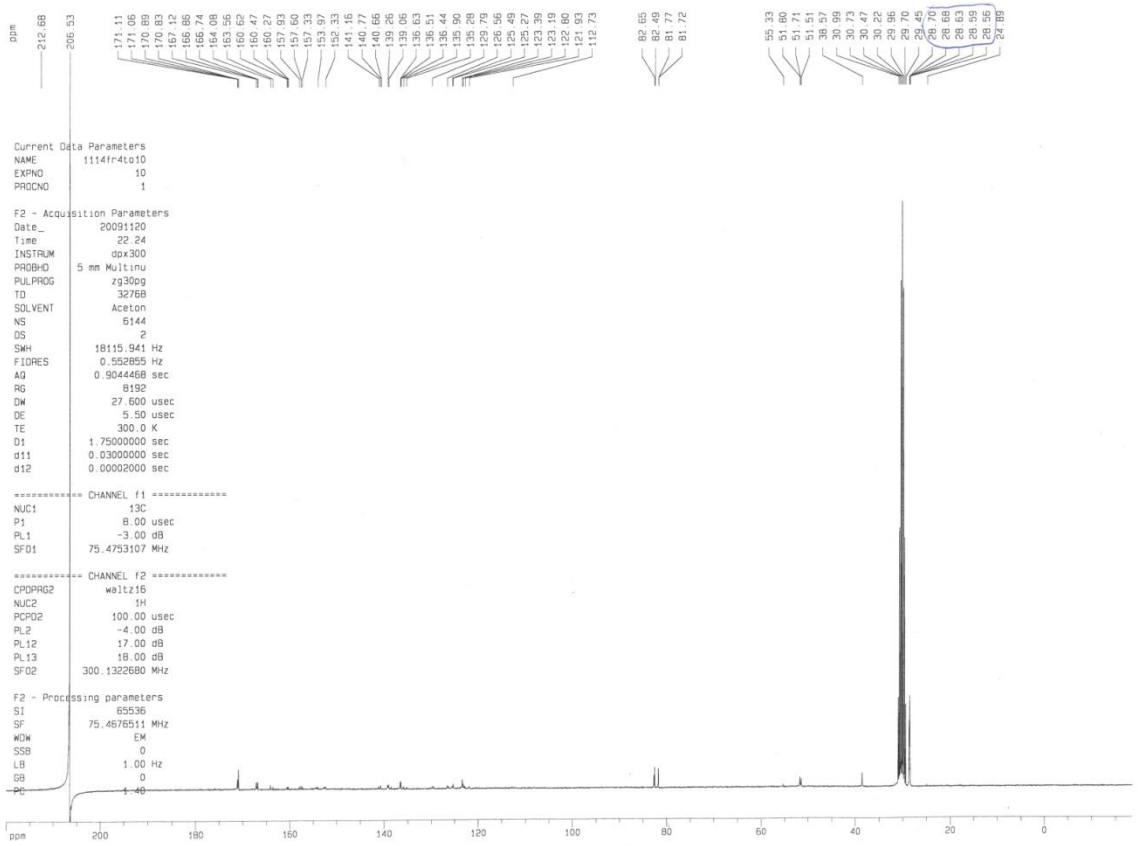
^{1}H NMR (500 MHz, $(CD_3)_2CO$) protected Δ-*mer* **1**



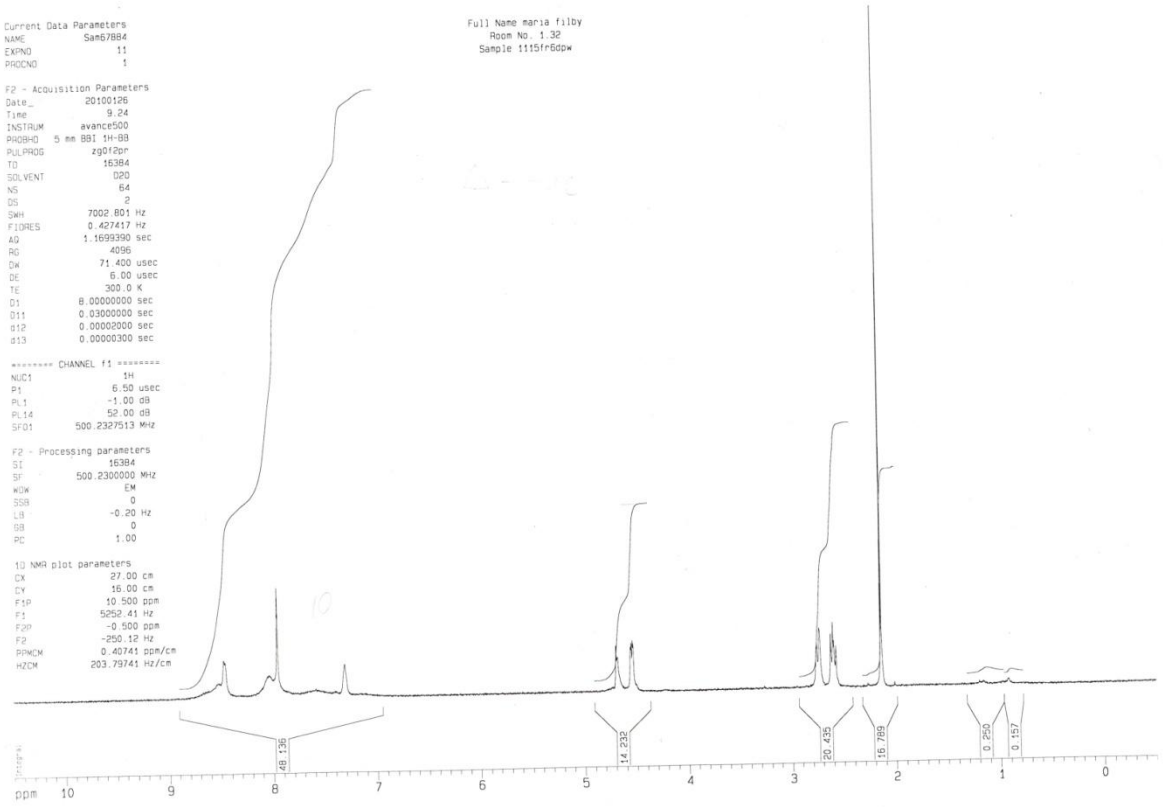
^{13}C NMR (75 MHz, $(CD_3)_2CO$) protected Δ-*mer* **1**



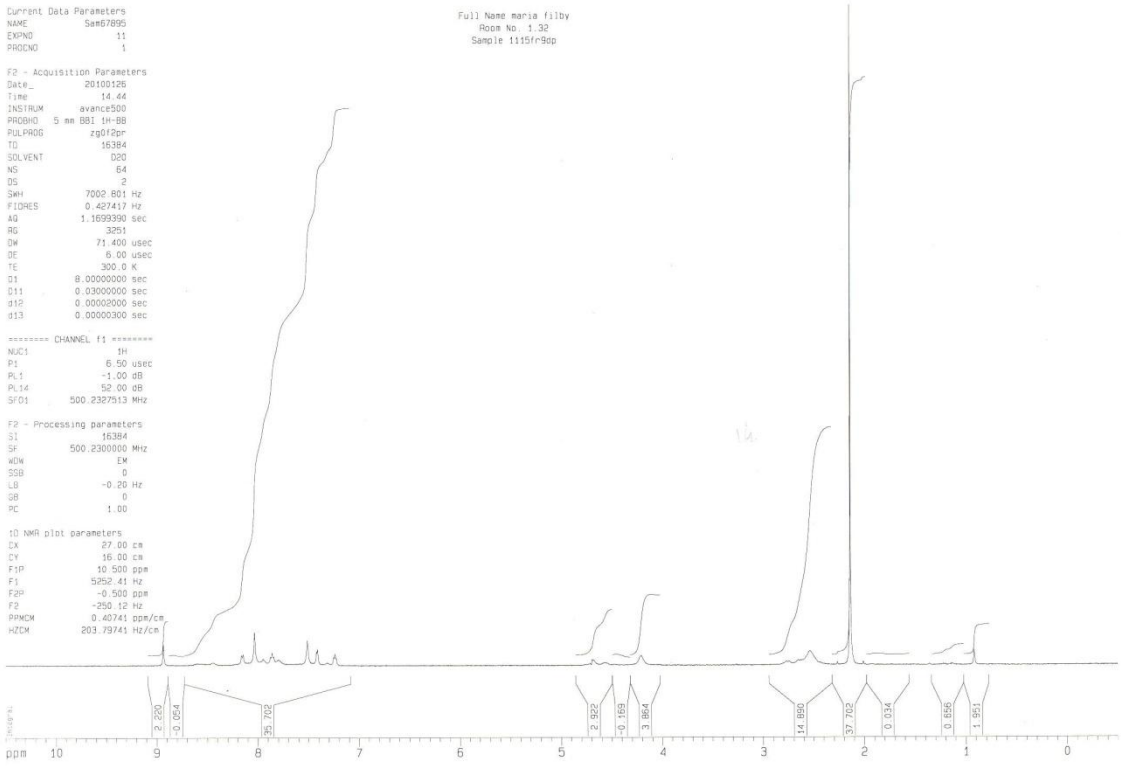
^{1}H NMR (500 MHz, $(CD_3)_2CO$) protected Δ-*mer* **1**



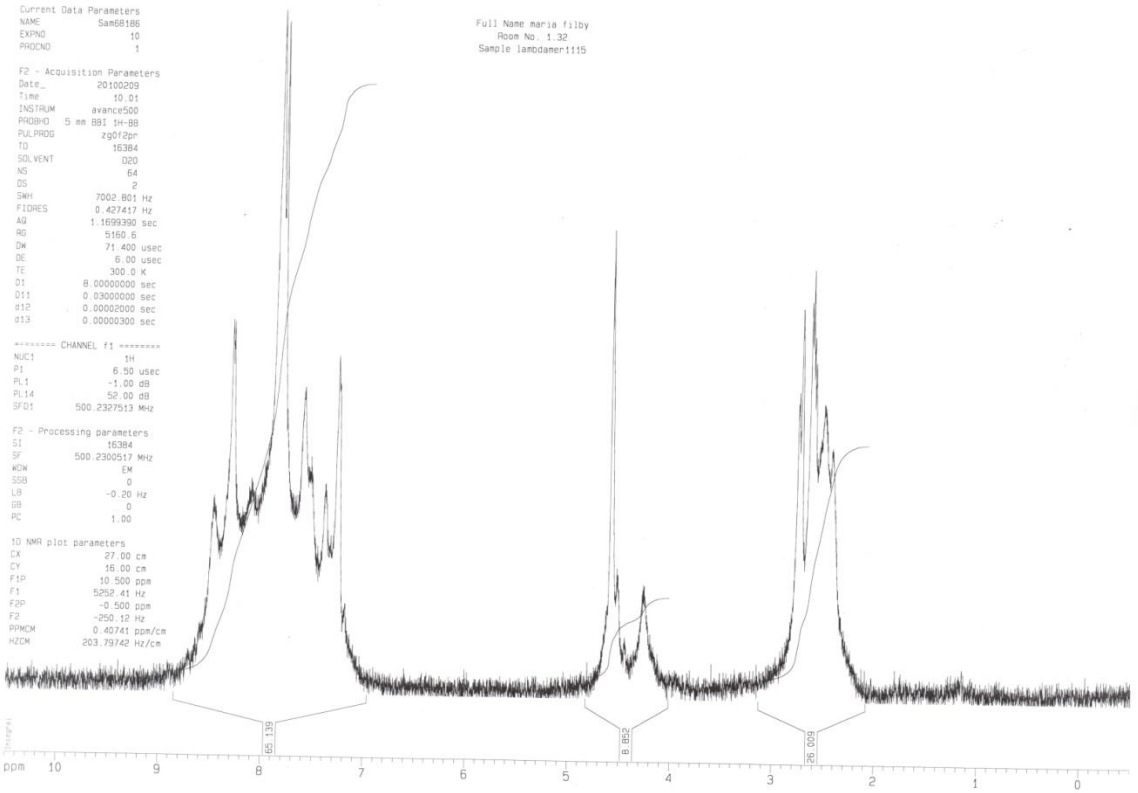
^{13}C NMR (75 MHz, $(CD_3)_2CO$) protected Λ-*mer* **1**



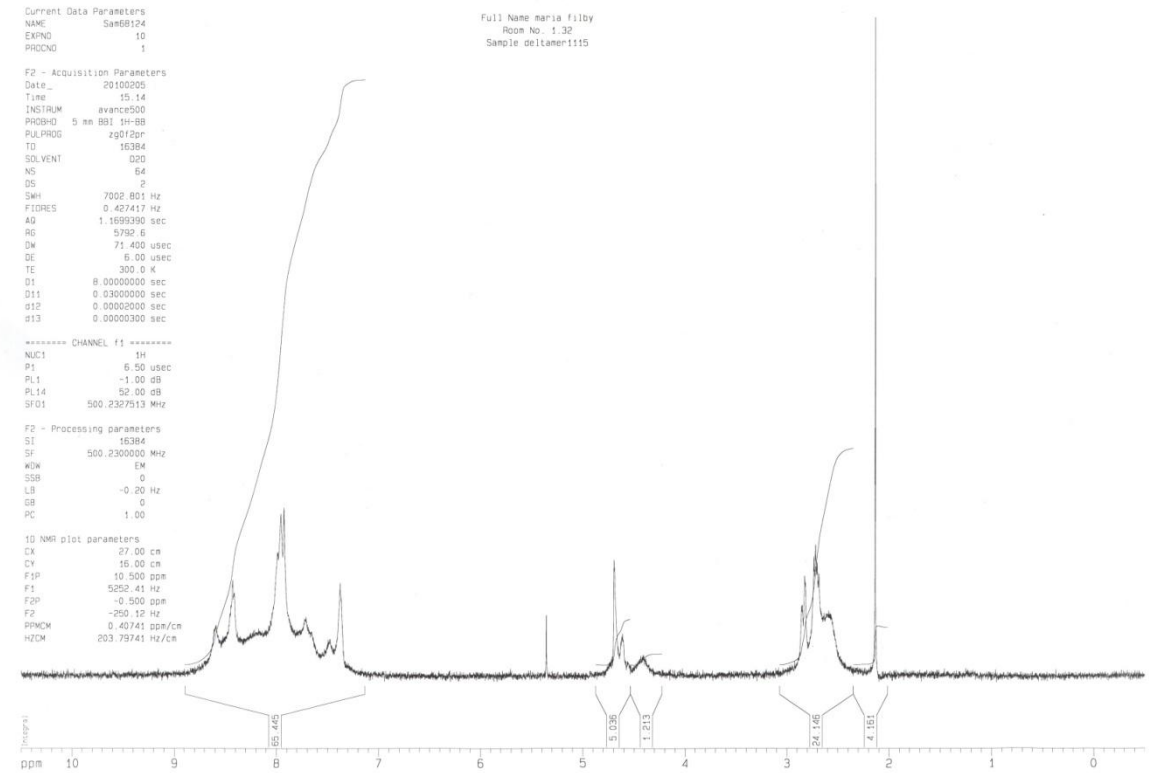
^{1}H NMR (500 MHz, H_2O) protected Δ-*fac* **1**



^{1}H NMR (500 MHz, H_2O) protected Λ-*fac* **1**



^{1}H NMR (500 MHz, H_2O) deprotected Λ-*mer***1**



^{1}H NMR (500 MHz, H_2O) deprotected Δ-*mer***1**