Supporting information

A rapid synthesis of low-nanomolar divalent LecA inhibitors in four linear steps from D-galactose pentaacetate

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General experimental details

Commercial chemicals and solvents were used without further purification.

Thin layer chromatography (TLC) was performed using silica gel 60 aluminum plates containing fluorescence indicator (Merck KGaA, Darmstadt, Germany) and developed under UV light (254 nm) and using a molybdate solution (0.02 M solution of $(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$ and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in aqueous 10% H_2SO_4) or a potassium permanganate solution (3 g of $KMnO_4$, 20 g of K_2CO_3 in 5 mL of 5% NaOH and 300 mL of water) with heating.

Medium pressure liquid chromatography (MPLC) was performed on a Teledyne Isco Combiflash Rf200 system using normal phase self-packed silica gel columns (60 Å, 400 mesh particle size, Fluka) or reversed-phase pre-packed silica gel 60 Å columns from Macherey-Nagel (C_{18} ec, endcapped). Preparative high-pressure liquid chromatography (HPLC) was performed on Waters 2545 Binary Gradient Module with a Waters 2489 UV/Vis detector using a RP-18 column (250/21 Nucleodur C18 Gravity SB, 5 µM from Macherey-Nagel, Germany).

Analytical HPLC-MS was performed on a Thermo Dionex Ultimate 3000 HPLC coupled to a Bruker amaZon SL mass spectrometer, with UV detection at 254 nm using a RP-18 column (100/2 Nucleoshell RP18plus, 2.7 µM from Macherey-Nagel, Germany) as stationary phase. High resolution mass spectrometry (HRMS) was performed on an Ultimate 3000 UPLC system coupled to a Q Exactive Focus Orbitrap system with HESI source (Thermo Fisher, Dreieich, Germany). The UPLC was operated with a C18 column (EC 150/2 Nucleodur C18 Pyramid, 3 µm from Macherey-Nagel, Germany).

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance III 500 UltraShield spectrometer at 500 MHz and 126 MHz. Chemical shifts (δ) are given in ppm and were calibrated on residual solvent peaks: chloroform-d_1 (^{1}H-NMR δ = 7.26 ppm, ^{13}C-NMR δ = 77.0 ppm), MeOH-d_4 (^{1}H-NMR δ = 3.31 ppm, ^{13}C-NMR δ = 49.0 ppm), DMSO-d_6 (^{1}H-NMR δ = 2.50 ppm, ^{13}C-NMR δ = 39.51 ppm).[1] Deuterated solvents were purchased from Eurisotop (Saarbrücken, Germany). Multiplicities are specified as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. The spectra were assigned with the help of ^{1}H,^{1}H-COSY; ^{1}H,^{13}C-HSQC and ^{1}H,^{13}C-HMBC experiments.

Compound synthesis

m*-Methoxycarbonylphenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (3*m*)**. β-D-galactopyranose pentaacetate[2] (**2**, 2.72 g, 6.97 mmol) and methyl-3-hydroxybenzoate (3.09 g, 20.4 mmol) were dissolved in dry dichloromethane (20 mL). The reaction mixture was cooled to 0 °C and $BF_3{\cdot}OEt_2$ (2.5 mL, 20.3 mmol) was added dropwise. The mixture was allowed to warm to room temperature and stirred overnight. The reaction was poured over ice cold satd. aqueous $NaHCO_3$ and diluted with dichloromethane. The organic phase was separated and washed with satd. aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by normal phase MPLC (petrol ether/EtOAc, 5–50% EtOAc). **3*m was obtained as a white solid (2.34 g, 4.84 mmol, 69%). The compound **3*m*** was first reported by Xue *et al.*.[3]

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.7 Hz, 1H, ArH), 7.66 (dd, *J* = 2.3, 1.5 Hz, 1H, ArH), 7.37 (t, *J* = 8.0 Hz, 1H, ArH), 7.19 (ddd, *J* = 8.2, 2.5, 0.8 Hz, 1H, ArH), 5.51 (dd, *J* = 10.4, 7.9 Hz, 1H, H-2), 5.47 (d, *J* = 3.3 Hz, 1H, H-4), 5.12 (dd, *J* = 10.3, 3.6 Hz, 1H, H-3), 5.11 (d, *J* = 7.9 Hz, 1H, H-1), 4.24 – 4.15 (m, 2H, H-6), 4.11 (t, *J* = 6.2 Hz, 1H, H-5), 3.91 (s, 3H, OCH_3), 2.18 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.01 (s, 3H, CH_3).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.62 (1C, $CH_3\underline{C}$=O), 170.37 (1C, $CH_3\underline{C}$=O), 170.24 (1C, $CH_3\underline{C}$=O), 169.53 (1C, $CH_3\underline{C}$=O), 166.50 (1C, C=O), 156.99 (1C, ArC), 131.85 (1C, ArC), 129.74 (1C, ArCH), 124.59 (1C, ArCH), 122.00 (1C, ArCH), 117.50 (1C, ArCH), 99.49 (1C, C-1), 71.37 (1C, C-5), 70.91 (1C, C-3), 68.67 (1C, C-2), 67.03 (1C, C-4), 61.62 (1C, C-6), 52.44 (1C, OCH_3), 20.89 (1C, CH_3), 20.81 (1C, CH_3), 20.73 (2C, CH_3).

HPLC-MS: $[C_{22}H_{26}O_{12} + Na]^+$ calcd. 505.13, found 505.12.

p*-Methoxycarbonylphenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (3*p*)**. β-D-galactopyranose pentaacetate[2] (**2**, 4.18 g, 10.71 mmol) and methyl-4-hydroxybenzoate (4.8 g, 31.2 mmol) were dissolved in dry dichloromethane (20 mL). The reaction mixture was cooled to 0 °C and $BF_3{\cdot}OEt_2$ (2.5 mL, 20.3 mmol) was added dropwise. The mixture was allowed to warm to room temperature and stirred overnight. The reaction was poured over ice cold saturated aqueous $NaHCO_3$ and diluted with dichloromethane. The organic phase was separated and washed with satd. aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. **3*p was purified by recrystallisation from ethanol (2.45 g,

5.08 mmol, 47%). The synthesis and analytical data of compound **3*p*** was first reported by Li *et al.*.[4]

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 – 7.95 (m, 2H, ArH), 7.08 – 6.96 (m, 2H, ArH), 5.51 (dd, *J* = 10.4, 7.9 Hz, 1H, H-2), 5.47 (d, *J* = 3.4 Hz, 1H, H-4), 5.17 – 5.07 (m, 2H, H-1, H-3), 4.25 – 4.14 (m, 2H, H-6), 4.10 (t, *J* = 6.5 Hz, 1H, H-5), 3.90 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 2.07 (d, 6H, CH_3), 2.02 (s, 3H, CH_3), 1.59 (s, 3H, CH_3).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.50 (1C, $CH_3\underline{C}$=O), 170.34 (1C, $CH_3\underline{C}$=O), 170.24 (1C, $CH_3\underline{C}$=O), 169.49 (1C, $CH_3\underline{C}$=O), 166.60 (1C, C=O), 160.36 (1C, ArC), 131.72 (2C, ArCH), 125.19 (1C, ArC), 116.29 (2C, ArCH), 98.92 (1C, C-1), 71.40 (1C, C-5), 70.87 (1C, C-3), 68.60 (1C, C-2), 66.94 (1C, C-4), 61.54 (1C, C-6), 52.21 (1C, OCH_3), 20.86 (1C, CH_3), 20.81 (1C, CH_3), 20.79 (1C, CH_3), 20.72 (1C, CH_3).

HPLC-MS: $[C_{22}H_{26}O_{12} + Na]^+$ calcd. 505.13, found 505.12.

m*-Methoxycarbonylphenyl β-D-galactopyranoside (4*m*)**. Compound **3*m (2.32 g, 4.81 mmol) was suspended in dry MeOH (40 mL) and 1 M NaOMe in MeOH (0.5 mL, 0.50 mmol) was added. The reaction mixture was stirred overnight at r.t. Amberlite IR 120/H^+ was added to neutralize the reaction mixture to pH 7 and was then filtered. The solvent was removed *in vacuo* to give **4*m*** as a white solid (1.49 g, 4.75 mmol, quant.).

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.61 – 7.57 (m, 2H, ArH), 7.45 (t, *J* = 7.9 Hz, 1H, ArH), 7.32 (ddd, *J* = 8.3, 2.5, 0.9 Hz, 1H, ArH), 5.21 (d, *J* = 5.1 Hz, 1H, OH-2), 4.89 (d, *J* = 5.7 Hz, 1H, OH-3), 4.86 (d, *J* = 7.7 Hz, 1H, H-1), 4.66 (t, *J* = 5.5 Hz, 1H, OH-6), 4.53 (d, *J* = 4.6 Hz, 1H, OH-4), 3.85 (s, 3H, OCH_3), 3.72 – 3.68 (m, 1H, H-4), 3.62 – 3.52 (m, 3H, H-2, H-5, H-6a), 3.51 – 3.46 (m, 1H, H-6b), 3.45 – 3.40 (m, 1H, H-3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 165.96 (1C, C=O), 157.65 (1C, ArC), 130.91 (1C, ArC), 129.95 (1C, ArCH), 122.58 (1C, ArCH), 121.32 (1C, ArCH), 116.96 (1C, ArCH), 101.36 (1C, C-1), 75.59 (1C, C-5), 73.13 (1C, C-3), 70.29 (1C, C-2), 68.07 (1C, C-4), 60.29 (1C, C-6), 52.28 (1C, OCH_3).

HPLC-MS: $C_{14}H_{17}O_8^-$ $[M - H]^-$ calcd. 313.09, found 313.16.

p*-Methoxycarbonylphenyl β-D-galactopyranoside (4*p*)**. Compound **3*p (2.39 g, 4.95 mmol) was suspended in dry MeOH (40 mL) and 1 M NaOMe in MeOH (0.5 mL, 0.5 mmol) was added. The reaction mixture was stirred overnight at r.t. Amberlite IR 120/H^+ was added to neutralize

the reaction mixture to pH 7 and was then filtered. The solvent was removed *in vacuo* to give **4*p*** as a white solid (1.55 g, 4.93 mmol, quant.).

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.90 (d, *J* = 8.5 Hz, 2H, ArH), 7.12 (d, *J* = 8.5 Hz, 2H, ArH), 5.22 (d, *J* = 5.1 Hz, 1H, OH-2), 4.95 (d, *J* = 7.6 Hz, 1H, H-1), 4.90 (d, *J* = 5.6 Hz, 1H, OH-3), 4.66 (t, *J* = 5.5 Hz, 1H, OH-6), 4.54 (d, *J* = 4.6 Hz, 1H, OH-4), 3.82 (s, 3H, OCH_3), 3.71 (t, *J* = 4.0 Hz, 1H, H-4), 3.65 – 3.45 (m, 4H, H-2, H-5, H-6), 3.42 (dt, *J* = 9.1, 4.2 Hz, 1H, H-3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 165.85 (1C, C=O), 161.25 (1C, ArC), 131.08 (2C, ArCH), 122.86 (1C, ArC), 116.03 (2C, ArCH), 100.41 (1C, C-1), 75.62 (1C, C-5), 73.21 (1C, C-3), 70.17 (1C, C-2), 68.07 (1C, C-4), 60.28 (1C, C-6), 51.91 (1C, OCH_3).

HPLC-MS: $C_{14}H_{17}O_8^-$ $[M - H]^-$ calcd. 313.09, found 313.10.

m*-Hydrazinecarbonylphenyl β-D-galactopyranoside (1*m*)**. To a suspension of compound **4*m (0.87 g, 2.76 mmol) in MeOH (33 mL), $NH_2NH_2{\cdot}H_2O$ (0.86 mL, 17.73 mmol) was added. The reaction was stirred under reflux at 70 ℃ overnight. Solvents were removed *in vacuo* to give compound **1*m*** as a white solid (0.87 g, quant.).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.76 (s, 1H, NH), 7.47 – 7.40 (m, 2H, ArH), 7.35 (t, *J* = 8.1 Hz, 1H, ArH), 7.19 – 7.10 (m, 1H, ArH), 5.22 (d, *J* = 5.2 Hz, 1H, OH-2), 4.93 (d, *J* = 5.4 Hz, 1H, OH-3), 4.89 (d, *J* = 7.7 Hz, 1H, H-1), 4.69 (t, *J* = 5.4 Hz, 1H, OH-6), 4.56 (d, *J* = 4.6 Hz, 1H, OH-4), 4.48 (s, 2H, NH_2), 3.71 (t, *J* = 3.8 Hz, 1H, H-4), 3.62 – 3.43 (m, 4H, H-2, H-5, H-6), 3.43 – 3.38 (m, 1H, H-3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 165.60 (1C, C=O), 157.43 (1C, ArC), 134.67 (1C, ArC), 129.44 (1C, ArCH), 120.29 (1C, ArCH), 118.91 (1C, ArCH), 114.82 (1C, ArCH), 100.92 (1C, C-1), 75.48 (1C, C-5), 73.34 (1C, C-3), 70.29 (1C, C-2), 68.02 (1C, C-4), 60.22 (1C, C-6).

HPLC-MS: $[C_{13}H_{18}N_2O_7 + H]^+$ calcd. 315.12, found 315.12.

p*-Hydrazinecarbonylphenyl β-D-galactopyranoside (1*p*)**. To a suspension of compound **4*p (1.70 g, 5.41 mmol) in MeOH (60 mL), $NH_2NH_2{\cdot}H_2O$ (2.52 mL, 51.95 mmol) was added. The reaction was stirred under reflux at 70 ℃ for 72 h. Solvents were removed *in vacuo* and the product was washed with cold MeOH. Pure compound **1*p*** was obtained as a white solid (1.60 g, 5.09 mmol, 94%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.63 (s, 1H, NH), 7.77 (d, *J* = 8.4 Hz, 2H, ArH), 7.05 (d, *J* = 8.4 Hz, 2H, ArH), 5.19 (d, *J* = 5.2 Hz, 1H, OH-2), 4.93 – 4.86 (m, 2H, H-1, OH-3), 4.66 (t, *J* = 5.5

Hz, 1H, OH-6), 4.52 (d, *J* = 4.6 Hz, 1H, OH-4), 4.42 (s, 2H, NH_2), 3.70 (t, *J* = 4.0 Hz, 1H, H-4), 3.63 – 3.44 (m, 4H, H-2, H-5, H-6), 3.44 – 3.38 (m, 1H, H-3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 165.56 (1C, C=O), 159.61 (1C, ArC), 128.57 (2C, ArCH), 126.56 (1C, ArC), 115.64 (2C, ArCH), 100.56 (1C, C-1), 75.59 (1C, C-5), 73.27 (1C, C-3), 70.22 (1C, C-2), 68.15 (1C, C-4), 60.39 (1C, C-6).

HPLC-MS: $[C_{13}H_{18}N_2O_7 + H]^+$ calcd. 315.12, found 315.00.

General procedure for synthesis of bis-benzaldehydes **C–F**

Corresponding di-halogenated hydrocarbons (1 eq.), 4-hydroxybenzaldehyde (4 eq.) and potassium carbonate (3 eq.) were dissolved in dry DMF (1.5 mL) in a microwave reaction vial. The vial was sealed and irradiated under microwave irradiation (Discover SP Sequential Microwave Synthesis System, CEM Corporation, North Carolina, USA) with maximum power 300 W at 70 °C for 3–10h. After cooling, the reaction was diluted with dichloromethane and washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Pure products were obtained without further purification. Analytical data of compounds **C–F** match the literature.[5]

Bis(4-formylphenoxy) methane (C). Dichloromethane (30 µL, 0.47 mmol), 4-hydroxybenzaldehyde (228 mg, 1.86 mmol) and potassium carbonate (138 mg, 1.42 mmol) were used following the general procedure for synthesis of bis-benzaldehydes to give compound **C** (99.6 mg, 0.39 mmol, 83%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.92 (s, 2H, CHO), 7.89 – 7.85 (m, 4H, ArH), 7.25 – 7.22 (m, 4H, ArH), 5.88 (s, 2H, CH_2).

^{13}C NMR (126 MHz, $CDCl_3$) δ 190.87 (2C, CHO), 161.40 (2C, ArC), 132.09 (4C, ArCH), 131.66 (2C, ArC), 116.49 (4C, ArCH), 89.96 (1C, CH_2).

HPLC-MS: $[C_{15}H_{12}O_4 + H]^+$ calcd. 257.08, found 257.09.

1,2-Bis(4-formylphenoxy) ethane (D). 1,2-Dichloroethane (25 µL, 0.32 mmol), 4-hydroxybenzaldehyde (165.2 mg, 1.35 mmol) and potassium carbonate (133.4 mg, 0.97 mmol) were used following the general procedure for synthesis of bis-benzaldehydes to give compound **D** (34.1 mg, 0.13 mmol, 40%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.90 (s, 2H, CHO), 7.89 – 7.84 (m, 4H, ArH), 7.08 – 7.03 (m, 4H, ArH), 4.45 (s, 4H, CH_2).

^{13}C NMR (126 MHz, $CDCl_3$) δ 190.90 (2C, CHO), 163.53 (2C, ArC), 132.17 (4C, ArCH), 130.54 (2C, ArC), 115.01 (4C, ArCH), 66.63 (2C, CH_2).

HPLC-MS: $[C_{16}H_{14}O_4 + H]^+$ calcd. 271.10, found 271.11.

1,3-Bis(4-formylphenoxy) propane (E). 1,3-Dichloropropane (40 µL, 0.42 mmol), 4-hydroxybenzaldehyde (227.1 mg, 1.86 mmol) and potassium carbonate (192.6 mg, 1.39 mmol) were used following the general procedure for synthesis of bis-benzaldehydes to give compound **E** (102.0 mg, 0.36 mmol, 85%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.88 (s, 2H, CHO), 7.85 – 7.81 (m, 4H, ArH), 7.04 – 6.99 (m, 4H, ArH), 4.26 (t, *J* = 6.0 Hz, 4H, $C\underline{H}_2CH_2C\underline{H}_2$), 2.34 (m, 2H, $CH_2C\underline{H}_2CH_2$).

^{13}C NMR (126 MHz, $CDCl_3$) δ 190.89 (2C, CHO), 163.87 (2C, ArC), 132.14 (4C, ArCH), 130.22 (2C, ArC), 114.87 (4C, ArCH), 64.65 (2C, $\underline{C}H_2CH_2\underline{C}H_2$), 29.10 (1C, $CH_2\underline{C}H_2CH_2$).

HPLC-MS: $[C_{17}H_{16}O_4 + H]^+$ calcd. 285.11, found 285.15.

1,4-Bis(4-formylphenoxy) butane (F). 1,4-Dibromobutane (50 µL, 0.42 mmol), 4-hydroxybenzaldehyde (211.5 mg, 1.73 mmol) and potassium carbonate (138.2 mg, 1.29 mmol) were used following the general procedure for synthesis of bis-benzaldehydes to give compound **F** (111.0 mg, 0.37 mmol, 89%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.88 (s, 2H, CHO), 7.87 – 7.80 (m, 4H, ArH), 7.03 – 6.96 (m, 4H, ArH), 4.17 – 4.10 (m, 4H, $C\underline{H}_2CH_2CH_2C\underline{H}_2$), 2.06 – 2.00 (m, 4H, $CH_2C\underline{H}_2C\underline{H}_2CH_2$).

^{13}C NMR (126 MHz, $CDCl_3$) δ 190.92 (2C, CHO), 164.08 (2C, ArC), 132.15 (4C, ArCH), 130.08 (2C, ArC), 114.84 (4C, ArCH), 67.88 (2C, $\underline{C}H_2CH_2CH_2\underline{C}H_2$), 25.93 (2C, $CH_2\underline{C}H_2\underline{C}H_2CH_2$).

HPLC-MS: $[C_{18}H_{18}O_4 + H]^+$ calcd. 299.13, found 299.17.

General procedure for synthesis of acylhydrazones

Corresponding benzaldehyde **A**–**F** (1 eq.) and hydrazide **1*m*** or **1*p*** (3 eq.) were dissolved in 1-10 mL DMSO (unless stated otherwise) and 20-100 µL of formic acid was added. After 4 h, the reactions were quenched to pH 8 with NH_4OH or immediately frozen with liquid nitrogen and dried *in vacuo*. The products were purified by MPLC or preparative HPLC (C18, water/acetonitrile, 15-40% acetonitrile).

Monovalent ligand A5*m* was synthesized following the general procedure for synthesis of acylhydrazones using benzaldehyde **A** (20 µL, 0.15 mmol) and hydrazide **1*m*** (88.5 mg). Compound **A5*m*** (26.5 mg, 0.06 mmol, 42%) was obtained as white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.69 (s, 1H, NH), 8.39 (s, 1H, N=CH), 7.67 (d, *J* = 8.7 Hz, 2H, ArH), 7.53 – 7.52 (m, 2H, ArH), 7.44 (t, *J* = 8.1 Hz, 1H, ArH), 7.25 (dd, *J* = 8.2, 1.1 Hz, 1H, ArH), 7.03 (d, *J* = 8.7 Hz, 2H, ArH), 5.22 (d, *J* = 5.1 Hz, 1H, OH-2), 4.92 – 4.90 (m, 2H, OH-3, H-1), 4.68 (t, *J* = 5.3 Hz, 1H, OH-6), 4.54 (d, *J* = 4.4 Hz, 1H, OH-4), 3.81 (s, 3H, CH_3), 3.72 (t, 1H, H-4), 3.62 – 3.54 (m, 3H, H-2, H-5, H-6a), 3.52 – 3.49 (m, 1H, H-6b), 3.45 – 3.41 (m, 1H, H-3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 162.63 (1C, C=O), 160.89 (1C, ArC), 157.50 (1C, ArC), 147.79 (1C, C=N), 134.93 (1C, ArC), 129.61 (1C, ArCH), 128.74 (2C, ArCH), 126.89 (1C, ArC), 120.91 (1C, ArCH), 119.35 (1C, ArCH), 115.68 (1C, ArCH), 114.39 (2C, ArCH), 101.13 (1C, C-1), 75.60 (1C, C-5), 73.32 (1C, C-3), 70.32 (1C, C-2), 68.11 (1C, C-4), 60.35 (1C, C-6), 55.34 (1C, CH_3).

HPLC-MS: $[C_{21}H_{24}N_2O_8 + H]^+$ calcd. 433.16, found 433.10.

HRMS: $[C_{21}H_{24}N_2O_8 + H]^+$ calcd. 433.1605, found 433.1604.

Monovalent ligand A5*p* was synthesized following the general procedure for synthesis of acylhydrazones using benzaldehyde **A** (30 µL, 0.24 mmol) and hydrazide **1*p*** (50.9 mg, 0.16 mmol). Compound **A5*p*** (64 mg, 0.15 mmol, 82%) was obtained as white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.63 (s, 1H, NH), 8.38 (s, 1H, N=CH), 7.88 (d, *J* = 8.6 Hz, 2H, ArH), 7.67 (d, *J* = 8.5 Hz, 2H, ArH), 7.13 (d, *J* = 8.7 Hz, 2H, ArH), 7.02 (d, *J* = 8.6 Hz, 2H, ArH), 5.23 (d, *J* = 5.2 Hz, 1H, OH-2), 4.95 (d, *J* = 7.7 Hz, 1H, H-1), 4.91 (d, *J* = 5.5 Hz, 1H, OH-3), 4.68 (t, *J* = 5.5 Hz, 1H, OH-6), 4.55 (d, *J* = 4.6 Hz, 1H, OH-4), 3.81 (s, 3H, CH_3), 3.71 (t, *J* = 3.8 Hz, 1H, H-4), 3.65 – 3.46 (m, 4H, H-5, H-6), 3.46 – 3.40 (m, 1H, H-3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 162.37 (1C, C=O), 160.78 (1C, ArC), 160.01 (1C, ArC), 147.20 (1C, N=C), 129.30 (2C, ArCH), 128.65 (2C, ArCH), 127.01 (1C, ArC), 126.66 (1C, ArC), 115.77 (2C, ArCH), 114.37 (2C, ArCH), 100.45 (1C, C-1), 75.62 (1C, C-5), 73.29 (1C, C-3), 70.22 (1C, C-2), 68.14 (1C, C-4), 60.36 (1C, C-6), 55.33 (1C, CH_3).

HPLC-MS: $[C_{21}H_{24}N_2O_8 + H]^+$ calcd. 433.16, found 433.20.

HRMS: $[C_{21}H_{24}N_2O_8 + H]^+$ calcd. 433.1605, found 433.1608.

Divalent ligand B5*m* was synthesized following the general procedure for synthesis of acylhydrazones using bisbenzaldehyde **B** (13.9 mg, 0.06 mmol) and hydrazide **1*m*** (62.3 mg). Compound **B5*m*** (11.5 mg, 0.01 mmol, 23%) was obtained as white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.81 (s, 2H, NH), 8.45 (s, 2H, N=CH), 7.79 (d, *J* = 8.4 Hz, 4H, ArH), 7.54 (m, 4H, ArH), 7.45 (t, *J* = 8.1 Hz, 2H, ArH), 7.26 (d, *J* = 7.5 Hz, 2H, ArH), 7.16 (d, *J* = 8.4 Hz, 4H, ArH), 5.22 (d, *J* = 5.0 Hz, 2H, OH-2), 4.92 – 4.91 (m, 4H, H-1, OH-3), 4.69 (t, *J* = 5.3 Hz, 2H, OH-6), 4.55 (d, *J* = 4.4 Hz, 2H, OH-4), 3.72 (t, *J* = 3.9 Hz, 2H, H-4), 3.62 – 3.54 (m, 6H, H-2, H-5, H-6a), 3.52 – 3.48 (m, 2H, H-6b), 3.44 – 3.43 (m, 2H, H-3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 162.75 (2C, C=O), 157.69 (2C, ArC), 157.52 (2C, ArC), 147.17 (2C, C=N), 134.79 (2C, ArC), 130.05 (2C, ArC), 129.64 (2C, ArCH), 129.13 (4C, ArCH), 120.96 (2C, ArCH), 119.46 (2C, ArCH), 119.17 (4C, ArCH), 115.73 (2C, ArCH), 101.15 (2C, C-1), 75.61 (2C, C-5), 73.32 (2C, C-3), 70.32 (2C, C-2), 68.10 (2C, C-4), 60.34 (2C, C-6).

HPLC-MS: $[C_{40}H_{42}N_4O_{15} + H]^+$ calcd. 819.27, found 819.34.

HRMS: $[C_{40}H_{42}N_4O_{15} + H]^+$ calcd. 819.2719, found 819.2716.

Divalent ligand B5*p* was synthesized following the general procedure for synthesis of acylhydrazones using bisbenzaldehyde **B** (15.6 mg, 0.07 mmol) and hydrazide **1*p*** (69.8 mg, 0.22 mmol). Compound **B5*p*** (26.9 mg, 0.03 mmol, 48%) was obtained as white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.74 (s, 2H, NH), 8.45 (s, 2H, N=CH), 7.89 (d, *J* = 8.3 Hz, 4H, ArH), 7.78 (d, *J* = 7.9 Hz, 4H, ArH), 7.15 (d, *J* = 7.6 Hz, 8H, ArH), 5.23 (s, 2H, OH-2), 4.95 (d, *J* = 7.7 Hz, 2H, H-1), 4.91 (s, 2H, OH-3), 4.69 (s, 2H, OH-6), 4.55 (s, 2H, OH-4), 3.71 (s, 2H, H-4), 3.66 – 3.41 (m, 10H, H-2, H-3, H-5, H-6).

^{13}C NMR (126 MHz, DMSO-d_6) δ 162.52 (2C, C=O), 160.10 (2C, ArC), 157.62 (2C, ArC), 146.61 (2C, C=N), 130.16 (2C, ArC), 129.38 (4C, ArCH), 129.05 (4C, ArCH), 126.53 (2C, ArC),

119.15 (2C, ArCH), 115.82 (2C, ArCH), 100.47 (2C, C-1), 75.63 (2C, C-5), 73.30 (2C, C-3), 70.24 (2C, C-2), 68.15 (2C, C-4), 60.38 (2C, C-6).

HPLC-MS: $[C_{40}H_{42}N_4O_{15} + H]^+$ calcd. 819.27, found 819.34.

HRMS: $[C_{40}H_{42}N_4O_{15} + H]^+$ calcd. 819.2719, found 819.2724

Divalent ligand C5*m* was synthesized following the general procedure for synthesis of acylhydrazones using bisbenzaldehyde **C** (16.3 mg, 0.06 mmol) and hydrazide **1*m*** (62.1 mg, 0.20 mmol). Compound **C5*m*** (5.5 mg, 6.5 µmol, 10%) was obtained as white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.76 (s, 2H, NH), 8.40 (s, 2H, N=CH), 7.71 (d, *J* = 8.6 Hz, 4H, ArH), 7.57 – 7.49 (m, 4H, ArH), 7.44 (t, *J* = 8.1 Hz, 2H, ArH), 7.28 – 7.23 (m, 2H, ArH), 7.21 (d, *J* = 8.7 Hz, 4H, ArH), 5.98 (s, 2H, CH_2), 5.25 (d, *J* = 5.1 Hz, 2H, OH-2), 4.94 (d, *J* = 5.6 Hz, 2H, OH-3), 4.91 (d, *J* = 7.7 Hz, 2H, H-1), 4.71 (t, *J* = 5.5 Hz, 2H, OH-6), 4.57 (d, *J* = 4.6 Hz, 2H, OH-4), 3.71 (t, *J* = 3.9 Hz, 2H, H-4), 3.65 – 3.46 (m, 8H, H-2, H-5, H-6), 3.46 – 3.39 (m, 2H, H-3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 162.73 (2C, C=O), 157.71 (2C, ArC), 157.52 (2C, ArC), 147.46 (2C, N=C), 134.87 (2C, ArC), 129.67 (2C, ArCH), 128.81 (4C, ArCH), 128.60 (2C, ArC), 120.97 (2C, ArCH), 119.42 (2C, ArCH), 116.45 (4C, ArCH), 115.70 (2C, ArCH, 101.13 (2C, C-1), 89.64 (1C, CH_2), 75.63 (2C, C-5), 73.33 (2C, C-3), 70.33 (2C, C-2), 68.12 (2C, C-4), 60.36 (2C, C-6).

HPLC-MS: $[C_{41}H_{44}N_4O_{16} + H]^+$ calcd. 849.28, found 849.35.

HRMS: $[C_{41}H_{44}N_4O_{16} + H]^+$ calcd. 849.2825, found 849.2822.

Divalent ligand C5*p* was synthesized following the general procedure for synthesis of acylhydrazones using bisbenzaldehyde **C** (16.6 mg, 0.06 mmol) and hydrazide **1*p*** (51.3 mg, 0.16 mmol). Compound **C5*p*** (26.8 mg, 0.03 mmol, 49%) was obtained as white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.68 (s, 2H, NH), 8.39 (s, 2H, N=CH), 7.88 (d, *J* = 8.5 Hz, 4H, ArH), 7.71 (d, *J* = 8.3 Hz, 4H, ArH), 7.20 (d, *J* = 8.3 Hz, 4H, ArH), 7.13 (d, *J* = 8.7 Hz, 4H, ArH), 5.97 (s, 2H, CH_2), 5.24 (s, 2H, OH-2), 4.98 – 4.87 (m, 4H, H-1, OH-3), 4.70 (s, 2H, OH-6), 4.56 (s, 2H, OH-4), 3.71 (d, *J* = 3.4 Hz, 2H, H-4), 3.65 – 3.43 (m, 10H, H-2, H-3, H-5, H-6).

^{13}C NMR (126 MHz, DMSO-d_6) δ 162.54 (2C, C=O), 160.09 (2C, ArC), 157.64 (2C, ArC), 146.95 (2C, N=CH), 129.38 (4C, ArCH), 128.74 (4C, ArCH), 126.62 (2C, ArC), 116.46 (4C, ArCH), 115.83 (4C, ArCH), 100.50 (2C, C-1), 89.73 (1C, CH_2), 75.65 (2C, C-5), 73.32 (2C, C-3), 70.27 (2C, C-2), 68.18 (2C, C-4), 60.41 (2C, C-6).

HPLC-MS: $[C_{41}H_{44}N_4O_{16} + H]^+$ calcd. 849.28, found 849.38.

HRMS: $[C_{41}H_{44}N_4O_{16} + H]^+$ calcd. 849.2825, found 849.2835.

Divalent ligand D5*m* was synthesized following the general procedure for synthesis of acyl hydrazones using bisbenzaldehyde **D** (22.3 mg, 0.08 mmol) and hydrazide **1*m*** (100.4 mg) in DMSO/MeCN (11 mL : 1 mL). Compound **D5*m*** (4.6 mg, 5.3 µmol, 6%) was obtained as white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.71 (s, 2H, NH), 8.40 (s, 2H, N=CH), 7.69 (d, *J* = 8.6 Hz, 4H, ArH), 7.54 – 7.53 (m, 4H, ArH), 7.44 (t, *J* = 8.1 Hz, 2H, ArH), 7.25 (d, *J* = 7.1 Hz, 2H, ArH), 7.10 (d, *J* = 8.6 Hz, 4H, ArH), 5.22 (d, *J* = 4.3 Hz, 2H, OH-2), 4.90 – 4.92 (m, 4H, OH-3, H-1), 4.69 (s, 2H, OH-6), 4.55 (d, *J* = 3.8 Hz, 2H, OH-4), 4.41 (s, 4H, CH_2), 3.72 (s, 2H, H-4), 3.62 – 3.54 (m, 6H, H-2, H-5, H-6a), 3.52 – 3.48 (m, 2H, H-6b), 3.43 (d, *J* = 9.2 Hz, 2H, H-3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 162.65 (2C, C=O), 159.93 (2C, ArC), 157.51 (2C, ArC), 147.73 (2C, N=C), 134.92 (2C, ArC), 129.62 (2C, ArCH), 128.79 (4C, ArCH), 127.17 (2C, ArC), 120.93 (2C, ArCH), 119.37 (2C, ArCH), 115.68 (2C, ArCH), 114.95 (4C, ArCH), 101.13 (2C, C-1), 75.61 (2C, C-5), 73.32 (2C, C-3), 70.32 (2C, C-2), 68.10 (2C, C-4), 66.48 (2C, CH_2), 60.34 (2C, C-6).

HPLC-MS: $[C_{42}H_{46}N_4O_{16} + H]^+$ calcd. 863.30, found 863.37.

HRMS: $[C_{42}H_{46}N_4O_{16} + H]^+$ calcd. 863.2982, found 863.2984.

Divalent ligand D5*p* was synthesized following the general procedure for synthesis of acylhydrazones using bisbenzaldehyde **D** (18.9 mg, 0.06 mmol) and hydrazide **1*p*** (50.2 mg, 0.16 mmol) in DMSO/DMF (1 mL : 1 mL). Compound **D5*p*** (18.1 mg, 0.021 mmol, 33%) was obtained as white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.65 (s, 2H, NH), 8.39 (s, 2H, N=CH), 7.92 – 7.82 (m, 4H, ArH), 7.68 (d, *J* = 8.3 Hz, 4H, ArH), 7.13 (d, *J* = 8.4 Hz, 4H, ArH), 7.09 (d, *J* = 8.3 Hz, 4H, ArH), 5.24 (s, 2H, OH-2), 4.95 (m, 4H, H-1, OH-3), 4.70 (s, 2H, OH-6), 4.57 (s, 2H, OH-4), 4.40 (s, 4H, CH_2), 3.72 (d, *J* = 3.3 Hz, 2H, H-4), 3.66 – 3.41 (m, 10H, H-2, H-3, H-5, H-6).

^{13}C NMR (126 MHz, DMSO-d_6) δ 162.87 (2C, C=O), 160.47 (2C, ArC), 160.28 (2C, ArC), 147.62 (2C, N=CH), 129.76 (4C, ArCH), 129.15 (4C, ArCH), 127.74 (2C, ArC), 127.09 (2C, ArC), 116.23 (4C, ArCH), 115.39 (4C, ArCH), 100.91 (2C, C-1), 76.06 (2C, C-5), 73.74 (2C, C-3), 70.68 (2C, C-2), 68.58 (2C, C-4), 66.92 (2C, CH_2), 60.81 (2C, C-6).

HPLC-MS: $[C_{42}H_{46}N_4O_{16} + H]^+$ calcd. 863.30, found 863.40.

HRMS: $[C_{42}H_{46}N_4O_{16} + H]^+$ calcd. 863.2982, found 863.2991.

Divalent ligand E5*m* was synthesized following the general procedure for synthesis of acylhydrazones using bisbenzaldehyde **E** (16.5 mg, 0.06 mmol) and hydrazide **1*m*** (56.3 mg, 0.18 mmol). Compound **E5*m*** (4.9 mg, 5.8 µmol, 10%) was obtained as white solid.

^{1}H NMR (500 MHz, DMSO-d_6 δ 11.71 (s, 2H, NH), 8.38 (s, 2H, N=CH), 7.67 (d, *J* = 8.4 Hz, 4H, ArH), 7.53 (dt, *J* = 4.1, 1.7 Hz, 4H, ArH), 7.44 (t, *J* = 8.1 Hz, 2H, ArH), 7.25 (ddd, *J* = 8.3, 2.3, 1.1 Hz, 2H, ArH), 7.06 (d, *J* = 8.4 Hz, 4H, ArH), 5.24 (d, *J* = 5.1 Hz, 2H, OH-2), 4.98 – 4.86 (m, 4H, OH-3, H-1), 4.70 (t, *J* = 5.5 Hz, 2H, OH-6), 4.57 (d, *J* = 4.6 Hz, 2H, OH-4), 4.21 (t, *J* = 6.2 Hz, 4H, $C\underline{H}_2CH_2C\underline{H}_2$), 3.71 (t, *J* = 3.9 Hz, 2H, H-4), 3.64 – 3.45 (m, 8H, H-2, H-5, H-6), 3.45 – 3.40 (m, 2H, H-3), 2.26 – 2.17 (m, 2H, $CH_2C\underline{H}_2CH_2$).

^{13}C NMR (126 MHz, DMSO) δ 162.63 (2C, C=O), 160.12 (2C, ArC), 157.50 (2C, ArC), 147.73 (2C, N=CH), 134.94 (2C, ArC), 129.65 (2C, ArCH), 128.81 (4C, ArCH), 126.98 (2C, ArC), 120.95 (2C, ArCH), 119.37 (2C, ArCH), 115.69 (4C, ArCH), 114.91 (4C, ArCH), 101.13 (2C, C-1), 75.62 (2C, C-5), 73.32 (2C, C-3), 70.32 (2C, C-2), 68.11 (2C, C-4), 64.43 (2C, $\underline{C}H_2CH_2\underline{C}H_2$), 60.35 (2C, C-6), 28.57 (1C, $CH_2\underline{C}H_2CH_2$).

HPLC-MS: $[C_{43}H_{48}N_4O_{16} + H]^+$ calcd. 877.31, found 877.38.

HRMS: $[C_{43}H_{48}N_4O_{16} + H]^+$ calcd. 877.3138, found 877.3136.

Divalent ligand E5*p* was synthesized following the general procedure for synthesis of acylhydrazones using bisbenzaldehyde **E** (18.3 mg, 0.06 mmol) and hydrazide **1*p*** (55.7 mg, 0.18 mmol). Compound **E5*p*** (17.3 mg, 0.20 mmol, 31%) was obtained as white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.63 (s, 2H, NH), 8.38 (s, 2H, N=CH), 7.87 (d, *J* = 8.6 Hz, 4H, ArH), 7.66 (d, *J* = 8.4 Hz, 4H, ArH), 7.12 (d, *J* = 8.7 Hz, 4H, ArH), 7.05 (d, *J* = 8.5 Hz, 4H, ArH), 5.23 (s, 2H, OH-2), 4.97 – 4.88 (m, 4H, H-1, OH-3), 4.69 (s, 2H, OH-6), 4.55 (s, 2H, OH-4), 4.21 (t, *J* = 5.8 Hz, 4H, $C\underline{H}_2CH_2C\underline{H}_2$), 3.71 (s, 2H, H-4), 3.65 – 3.41 (m, 10H, H-2, H-3, H-5, H-6), 2.26 – 2.18 (m, 2H, $CH_2C\underline{H}_2CH_2$).

^{13}C NMR (126 MHz, DMSO-d_6) δ 162.41 (2C, C=O), 160.03 (4C, ArC), 147.20 (2C, N=CH), 129.32 (4C, ArCH), 128.69 (4C, ArCH), 127.10 (2C, ArC), 126.67 (2C, ArC), 115.79 (4C, ArCH),

114.89 (4C, ArCH), 100.48 (2C, C-1), 75.63 (2C, C-5), 73.30 (2C, C-3), 70.24 (2C, C-2), 68.14 (2C, C-4), 64.43 (2C, $\underline{C}H_2CH_2\underline{C}H_2$), 60.37 (2C, C-6), 28.57 (1C, $CH_2\underline{C}H_2CH_2$).

HPLC-MS: $[C_{43}H_{48}N_4O_{16} + H]^+$ calcd. 877.31, found 877.40.

HRMS: $[C_{43}H_{48}N_4O_{16} + H]^+$ calcd. 877.3138, found 877.3145.

Divalent ligand F5*m* was synthesized following the general procedure for synthesis of acylhydrazones using bisbenzaldehyde **F** (25.6 mg, 0.09 mmol) and hydrazide **1*m*** (84.2 mg) in MeCN/H_2O (6 mL : 2.5 mL). Compound **F5*m*** (5.0 mg, 5.6 µmol, 7%) was obtained as white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.69 (s, 2H, NH), 8.38 (s, 2H, N=CH), 7.66 (d, *J* = 8.7 Hz, 4H), 7.54 – 7.52 (m, 4H, ArH), 7.44 (t, *J* = 8.1 Hz, 2H, ArH), 7.24 (d, *J* = 7.2 Hz, 2H, ArH), 7.04 (d, *J* = 8.6 Hz, 4H, ArH), 5.23 (s, 2H, OH-2), 4.91 (m, 4H, H-1, OH-3), 4.70 (s, 2H, OH-6), 4.56 (s, 2H, OH-4), 4.11 (s, 4H, $C\underline{H}_2CH_2CH_2C\underline{H}_2$), 3.71 (d, *J* = 2.6 Hz, 2H, H-4), 3.61 – 3.54 (m, 6H, H-2, H-5, H-6a), 3.49 (m, 2H, H-6b), 3.43 (dd, *J* = 9.5, 2.9 Hz, 2H, H-3), 1.91 (s, 4H, $CH_2C\underline{H}_2C\underline{H}_2CH_2$).

^{13}C NMR (126 MHz, DMSO-d_6) δ 162.69 (2C, C=O), 160.25 (2C, ArC), 157.49 (2C, ArC), 147.77 (2C, N=C), 135.06 (2C, ArC), 129.57 (2C, ArCH), 128.74 (4C, ArCH), 126.88 (2C, ArC), 120.93 (2C, ArCH), 119.30 (2C, ArCH), 115.68 (2C, ArCH), 114.86 (4C, ArCH), 101.14 (2C, C-1), 75.60 (2C, C-5), 73.32 (2C, C-3), 70.32 (2C, C-2), 68.09 (2C, C-4), 67.33 (2C, $\underline{C}H_2CH_2CH_2\underline{C}H_2$), 60.33 (2C, C-6), 25.36 (2C, $CH_2\underline{C}H_2\underline{C}H_2CH_2$).

HPLC-MS: $[C_{44}H_{50}N_4O_{16} + H]^+$ calcd. 891.33, found 891.39.

HRMS: $[C_{44}H_{50}N_4O_{16} + H]^+$ calcd. 891.3295, found 891.3298.

Divalent ligand F5*p* was synthesized following the general procedure for synthesis of acylhydrazones using bisbenzaldehyde **F** (18.9 mg, 0.06 mmol) and hydrazide **1*p*** (50.2 mg, 0.16 mmol). Compound **F5*p*** (11.0 mg, 0.01 mmol, 19%) was obtained as white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.63 (s, 2H, NH), 8.37 (s, 2H, N=CH), 7.87 (d, *J* = 8.5 Hz, 4H, ArH), 7.66 (d, *J* = 8.3 Hz, 4H, ArH), 7.12 (d, *J* = 8.7 Hz, 4H, ArH), 7.03 (d, *J* = 8.5 Hz, 4H, ArH), 5.24 (s, 2H, OH-2), 4.93 (m, 4H, H-1, OH-3), 4.71 (s, 2H, OH-6), 4.56 (s, 2H, OH-4), 4.10 (s, 4H $C\underline{H}_2CH_2CH_2C\underline{H}_2$), 3.71 (s, 2H, H-4), 3.66 – 3.48 (m, 10H, H-2, H-3, H-5, H-6), 1.90 (s, 4H, $CH_2C\underline{H}_2C\underline{H}_2CH_2$).

^{13}C NMR (126 MHz, DMSO-d_6) δ 162.50 (2C, C=O), 160.24 (2C, ArC), 160.07 (2C, ArC), 147.34 (2C, N=CH), 129.36 (4C, ArCH), 128.74 (4C, ArCH), 126.97 (2C, ArC), 126.69 (2C, ArC),

115.85 (4C, ArCH), 114.91 (4C, ArCH), 100.51 (2C, C-1), 75.66 (2C, C-5), 73.33 (2C, C-3), 70.28 (2C, C-2), 68.19 (2C, C-4), 67.40 (2C, $\underline{C}H_2CH_2CH_2\underline{C}H_2$), 60.43 (2C, C-6), 25.41 (2C, $CH_2\underline{C}H_2\underline{C}H_2CH_2$).

HPLC-MS: $[C_{44}H_{50}N_4O_{16} + H]^+$ calcd. 891.33, found 891.45.

HRMS: $[C_{44}H_{50}N_4O_{16} + H]^+$ calcd. 891.3295, found 891.3306.

DCC

A dynamic combinatorial library (DCL) of hydrazide **1*p*** with aldehydes **B**–**F** was formed in citrate buffer (100 mM Na-citrate, 1 mM $CaCl_2$, pH 5.7) in presence of 20% of DMSO. Final concentrations in DCL were: aniline (10 mM), hydrazide **1*p*** (625 µM), aldehydes **B**–**F** (50 µM each) and LecA (62.5 µM, added at time 0 h or after 6 h) in a reaction volume of 1 mL. The DCL was left shaking at room temperature and monitored *via* HPLC-MS with a C8 column (Acquity UPLC BEH C8 column, 2.1 mm x 150 mm, 1.7 µM from Waters, Germany). Samples for HPLC-MS were prepared from 20 µL of reaction mixture mixed with 5 µL of 2 M NaOH to freeze the equilibrium and addition of 50 µL MeCN. The mixture was centrifuged at 15000 rpm in an Eppendorf tube centrifuge for 3 minutes and the supernatant was analyzed.

The formation of precipitate was observed after approx. 30 minutes in the case when LecA was added at time 0 h or immediately in the case when the LecA was added to already formed library after 6 h.

Biophysical evaluation

Expression and purification of LecA as well as competitive binding by fluorescence polarization was performed as described by Joachim *et al.*.[6] The assay was performed in TBS/Ca^{2+} buffer (20 mM Tris, 137 mM NaCl, 2.6 mM KCl at pH 7.4 supplemented with 1 mM $CaCl_2$) in presence of 25% DMSO. Averages and standard deviations were calculated from at least three experiments.

Isothermal titration calorimetry was performed on an iTC200 (Malvern Panalytical) and analyzed using Microcal Origin software and MicroCal ConCat ITC software (Malvern Panalytical). LecA (100 - 200 µM) in the cell was titrated with ligand (0.85-2 mM) in TBS/Ca^{2+} buffer at 25 °C.

Surface plasmon resonance experiments were performed on a BIACORE X100 instrument (GE Healthcare) at 25 °C. For LecA immobilization, the system was pre-equilibrated with PBS buffer (10 mM phosphate buffer pH 7.4, 2.7 mM KCl, 137 mM NaCl, 100 µM $CaCl_2$, 0.05% Tween 20), followed by an activation of the CM5 chip surface by 3 injections of 1:1 N-hydroxysuccinimide (NHS)/1-ethyl-3(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) mixture on channel 1 and 2 (contact time of 540 s, flow rate 10 µL/min) until the binding response was above 800 RU. LecA was dissolved in 10 mM sodium acetate pH 4.5 (100 µg/mL) and injected over the activated chip surface on channel 2 (contact time of 540 s, flow rate 10 µL/min) until the response of ~2800 RU was reached. Excess free NHS-ester groups were capped with an injection of 1 M ethanolamine (contact time 540 s, flow rate 10 µL/min). Divalent compounds stocks solutions (50 µM in DMSO) were prepared and then diluted with 1.05 X PBS buffer to 2.5 µM solutions with 5% DMSO. The 2.5 µM stocks were subsequently diluted to required concentrations in a running buffer (10 mM phosphate buffer pH 7.4, 2.7 mM KCl, 137 mM NaCl, 100 µM $CaCl_2$, 0.05% Tween 20, 5% DMSO). The divalent inhibitors were subjected to single-cycle kinetics analyses (contact time of 120s and dissociation time of 120s, flow rate 30 µL/min) consisting of injections of the analytes at 0, 10, 50, 100, and 200 nM over the immobilized-LecA. The chip surface was regenerated by 5 x injections of 5 mM galactose followed by 5 x injections of the running buffer (contact time of 120 s, flow rate 30 µL/min). K_d determination was performed using BIACORE X100 evaluation software (version 2.0) by applying the 1:1 binding model to fit the experimental data. Affinity/Equilibrium analysis was carried out for monovalent inhibitors **A5*m*** and **A5*p*** on a CM5 chip with 3977 RU of immobilized LecA. The monovalent inhibitors stocks (5 mM in DMSO) were prepared and then diluted in 1.05 X PBS buffer to 250 µM solutions with 5% DMSO. The 250 µM stock was then diluted to required concentrations in a running buffer and injected over the immobilized LecA at 0, 1, 5, 10 and 20 µM using the same SPR parameters as the divalent compound analyses, but without the regeneration step.

Expression, purification and SPR analysis of galectin-1 was performed as follows: pET3a-galectin-1 plasmid[7] was transformed into *E. coli* BL21 (DE3) and cultured in 2 x 1 L of LB broth until OD_{600} reached 0.5. The expression was then induced by addition of 1 mM isopropyl β-D-thiogalactopyranoside and further incubated at 30 °C for 3 h. Cells were harvested by

centrifugation (5000 x g, 20 min) and frozen at -20 °C. The frozen pellet was re-suspended in 30 mL of PBS buffer supplemented with 4 mM β-mercaptoethanol (β-ME) and one tablet of protease inhibitors (cOmplete™ Protease Inhibitor Cocktail, Roche) and lysed by cell disruptor (1.9 kbar). Cell lysates were centrifuged (24 000 x g, 30 min, 4 °C) to obtain supernatant, which was then filtered and loaded onto a lactosyl-sepharose 4B column[8] pre-equilibrated with PBS buffer containing 4 mM β-mercaptoethanol (PBS/β-ME). The column was washed with PBS/β-ME to remove unbound protein. Bound galectin-1 was eluted with PBS/β-ME supplemented with 50 mM lactose and collected as 2 mL fractions, which were pooled and dialyzed in 4 L of 20 mM Tris-HCl pH 7 supplemented with 1 mM dithiothreitol (DTT) for 2 days at 4 °C. The dialyzed protein was concentrated by Macrosep® Advance 3K (PALL Life Sciences) until the protein concentration reached 7.5 mg/mL. The protein was stored at 4 °C with an addition of 1 mM TCEP. Purified galectin-1 was immobilized to a CM5 chip (1500 RU) using the same methodology as described for LecA but supplementing all buffers with 1 mM DTT. Lactose solution was prepared by dissolving powder in the running buffer and further diluted to the required concentrations. The activity of immobilized galectin-1 was confirmed by injections of increasing concentration of lactose (100 – 1000 μM, contact time = 30 s, dissociation time = 60 s, flow rate = 30 μL/min). **A5*m***, **A5*p***, **C5*m*** and **B5*p*** were injected at the indicated concentrations in **Figure S3** (contact time of 120 s, dissociation time = 120 s, flow rate 30 μL/min).

MD simulation

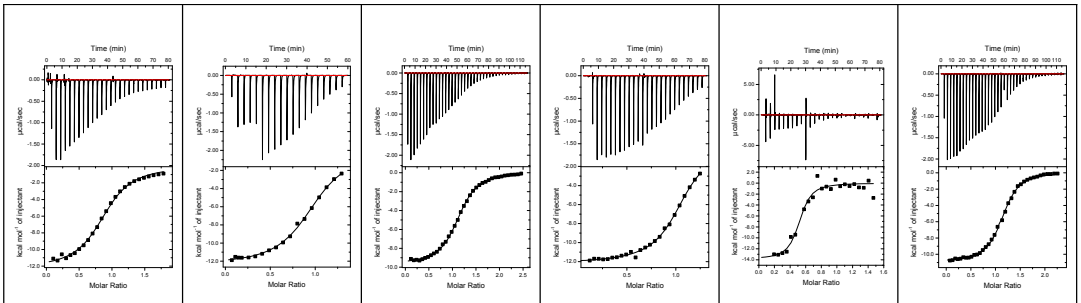
Ligand Building, Parametrisation and Molecular Dynamics. The four compounds were drawn in ChemDraw, ver. 18.2 and cut into fragments (Figure S4a) for simpler parametrization. Using ChemBio3D Ultra, ver. 14, they were subsequently transformed to 3D. PyMol was utilized afterwards to cap the dangling bondsand add hydrogen atoms. Parametrization of the capped fragments was done in Antechamber[9] with GAFF force field[10] and AM1-BCC charges[11]. Thereafter the capping groups were removed, and their charge dispersed over the remaining atoms to restore neutrality.

The ligand structures were solvated in rectangular boxes of TIP3P[12] water molecules which extended to 12 Å from the solute. Na^+/Cl^- counterions were added to a final concentration of

0.15 M. The systems were relaxed using the published protocols and settings[13,14] and 1 µs MD production run was carried out using PMEMD.CUDA software of AMBER18[15] on GPUs. Analyses were performed using CPPTRAJ and graphs plotted with XMGRACE.

Protein Preparation. The structure of the LecA dimer was taken from the PDB: 5d21[16]. Asn/Gln/His flips and protonation of titratable protein residues was checked with the H++ program[17] at pH = 7.4 (experimental condition for binding measurements) and no changes were needed. Histidine residues were monoprotonated on Nδ (His58) and Nε (His50) based on visual inspection of their surroundings. Electronic continuum correction (ECC)[18] was applied for the two calcium ions and their coordinating Asp100 side chains using charge scaling by a factor of 0.85 described previously.[19]

Modeling and Molecular Dynamics of Complexes. In the prepared LecA dimer, the two β-Gal phenyl residues were first extended and bonded by the tLEaP module of AMBER18. Subsequent optimization (1000 steepest descent cycles) in implicit solvent (igb=7)[20] of the added parts yielded the models for adding solvent and counterions to neutralize the systems. Relaxation protocols and settings were the same as above[13,14] and the MD production runs were 300 ns long. Analyses were performed on the last 150 ns to allow for further system equilibration.



	115 µM LecA	134 µM LecA	165 µM LecA	165 µM LecA	126 µM LecA	165 µM LecA
	1 mM **A5*p***	1 mM **A5*p***	2 mM **A5*p***	1 mM **A5*m***	0.9 mM **A5*m***	0.85 mM **A5*m***
K_d [µM]	6.58	5.62	6.13	3.34	1.16	3.52
ΔH [kcal/mol]	-12.23	-12.38	-9.66	-12.13	-13.91	-10.90
ΔS [cal/mol/	-17.3	-17.5	-8.5	-15.6	-19.9	-11.6
N	0.900	0.972	1.13	1.05	0.508	1.15

Figure S1: ITC measurements of **A5*m*** and **A5*p*** with LecA performed in TBS/Ca^{2+} buffer at 25 °C.

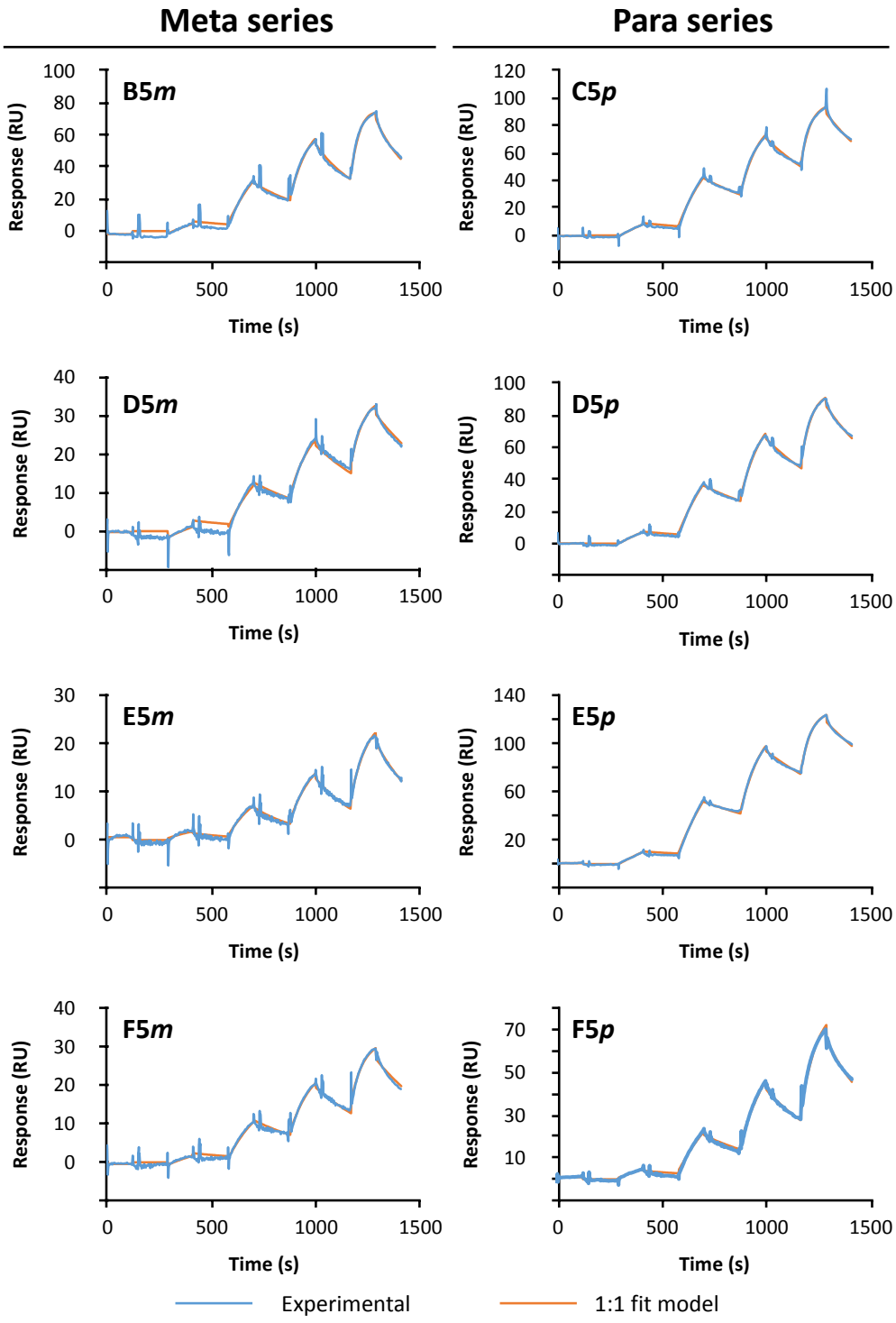


Figure S2: SPR of LecA with divalent galactosides. Sensorgrams obtained from SPR single-cycle kinetics experiments. Five different concentrations of each compound (0, 10, 50, 100, 200 nM) were sequentially injected to obtain the experimental sensorgrams (blue lines), which were then fitted by a 1:1 model (orange) on BIACORE evaluation software.

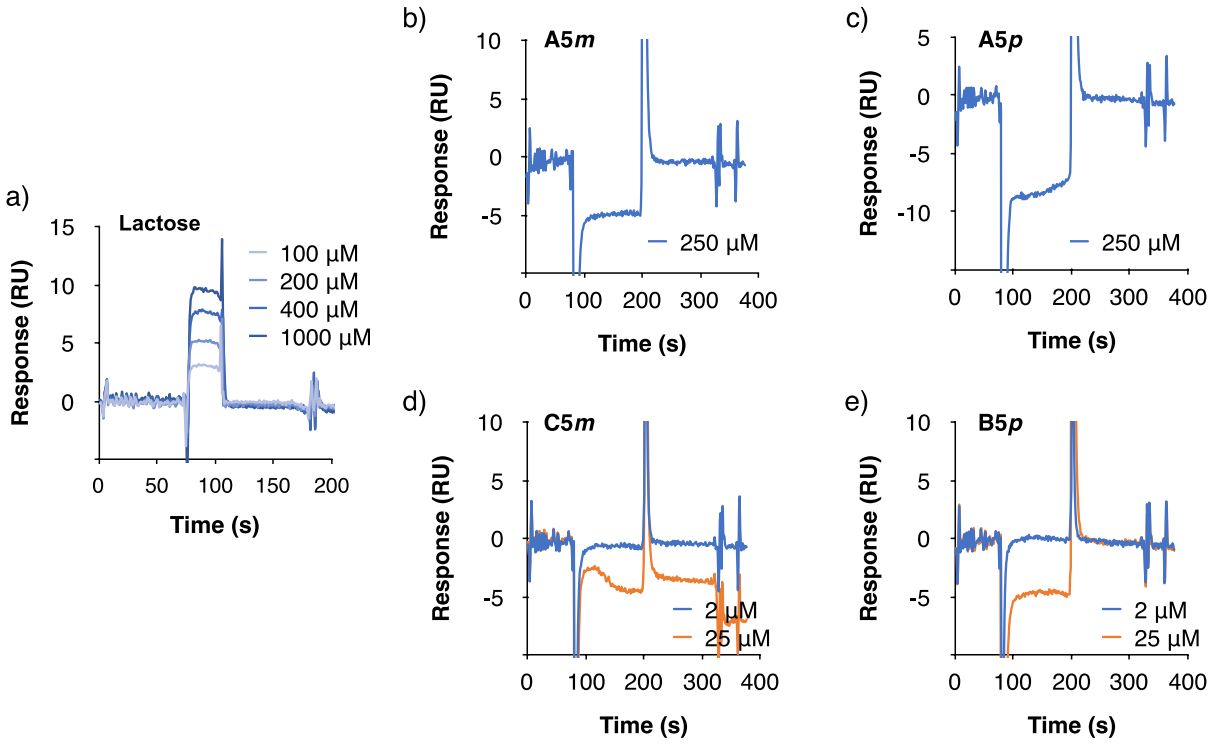


Figure S3: SPR analysis of interaction between immobilized galectin-1 and the inhibitors. The activity of immobilized galectin-1 was confirmed by injection of lactose. Spikes indicate the buffer mismatch at the beginning and the end of the contact time and the negative response in b-e is caused by the buffer mismatch due to different sample preparation and/or non-specific absorption in the reference channel.

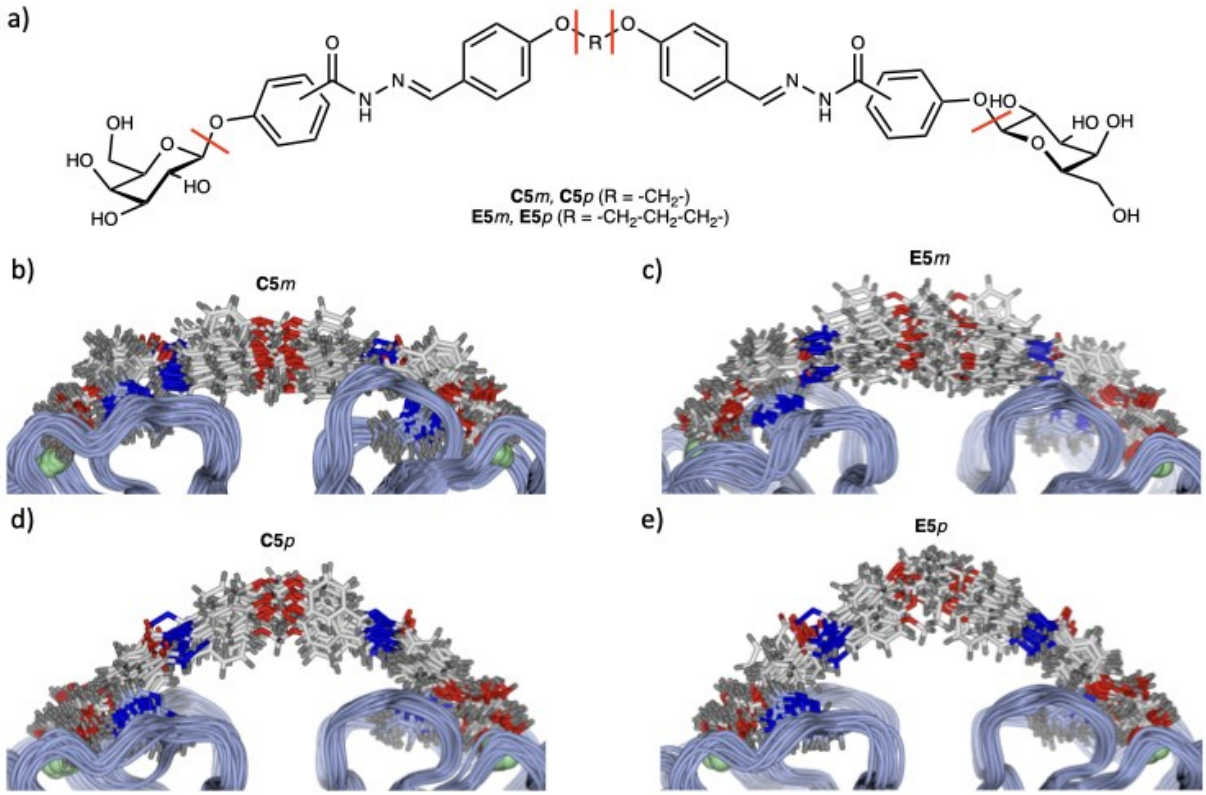
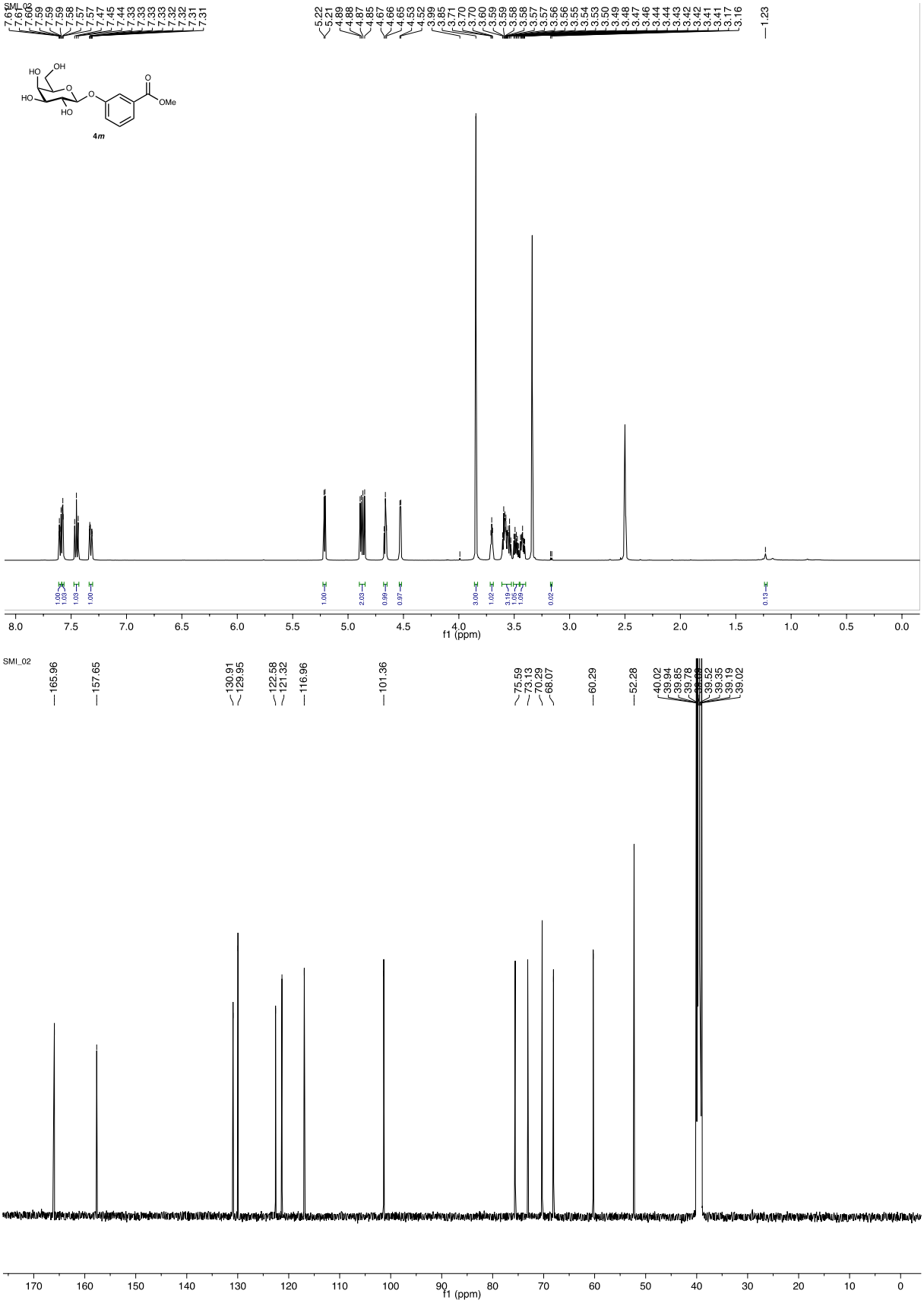
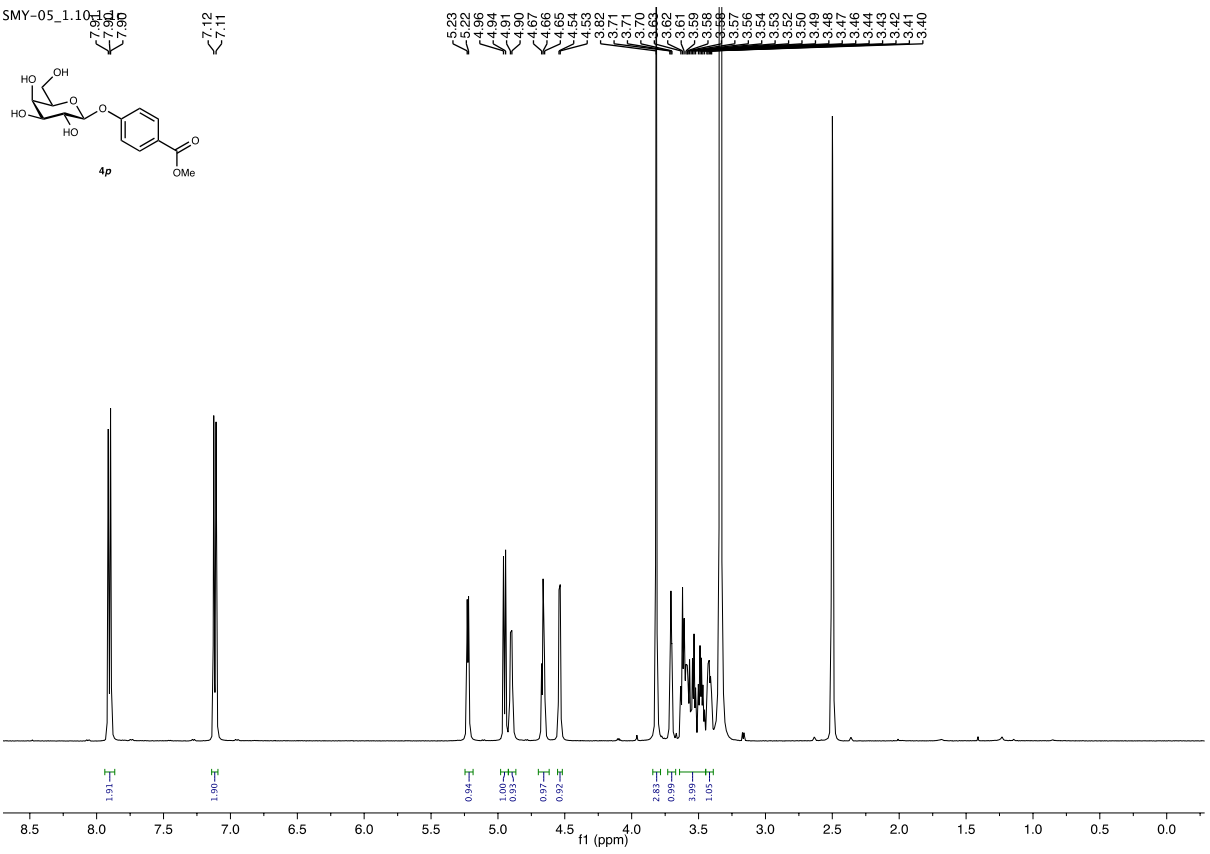
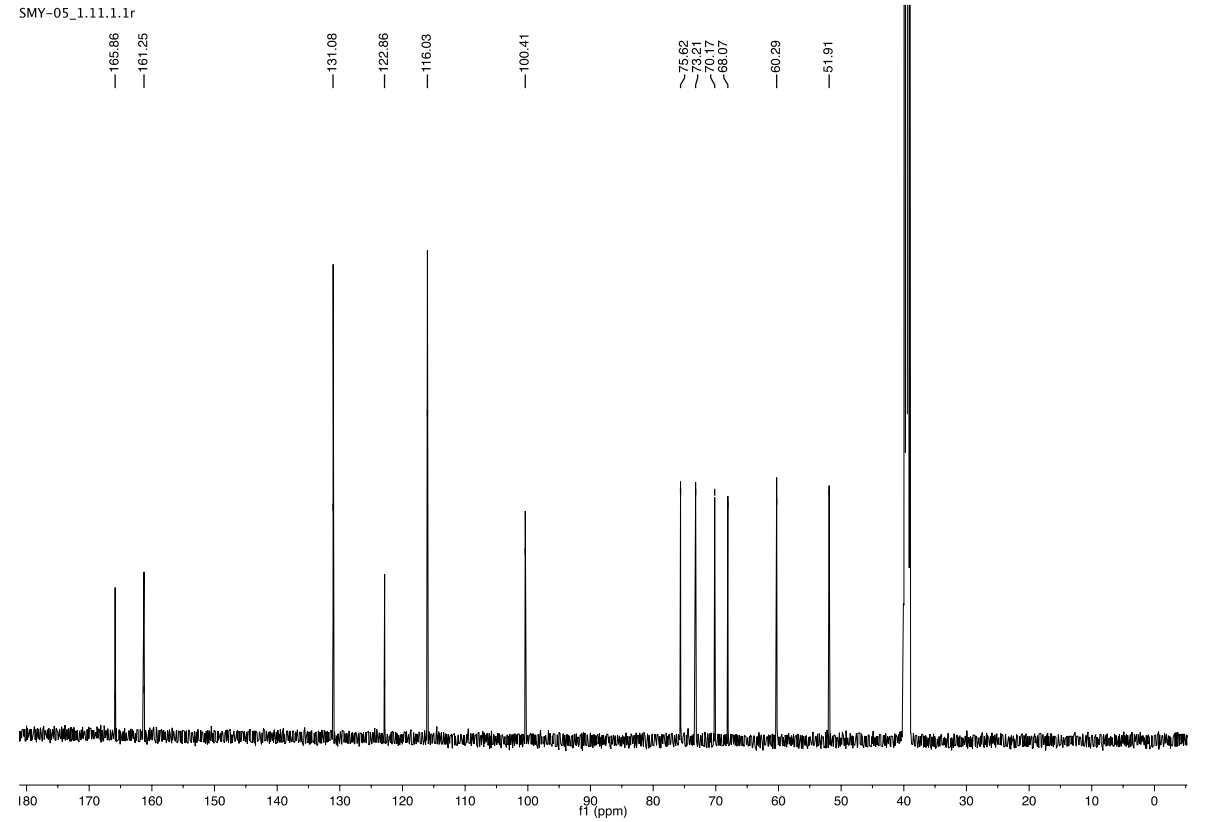


Figure S4: a) Definition of how the ligand was cut into fragments b)-e) Superpositions of 25 sampled snapshots from MD trajectories; b) **C5*m***, c) **E5*m***, d) **C5*p***, and e) **E5*p***.

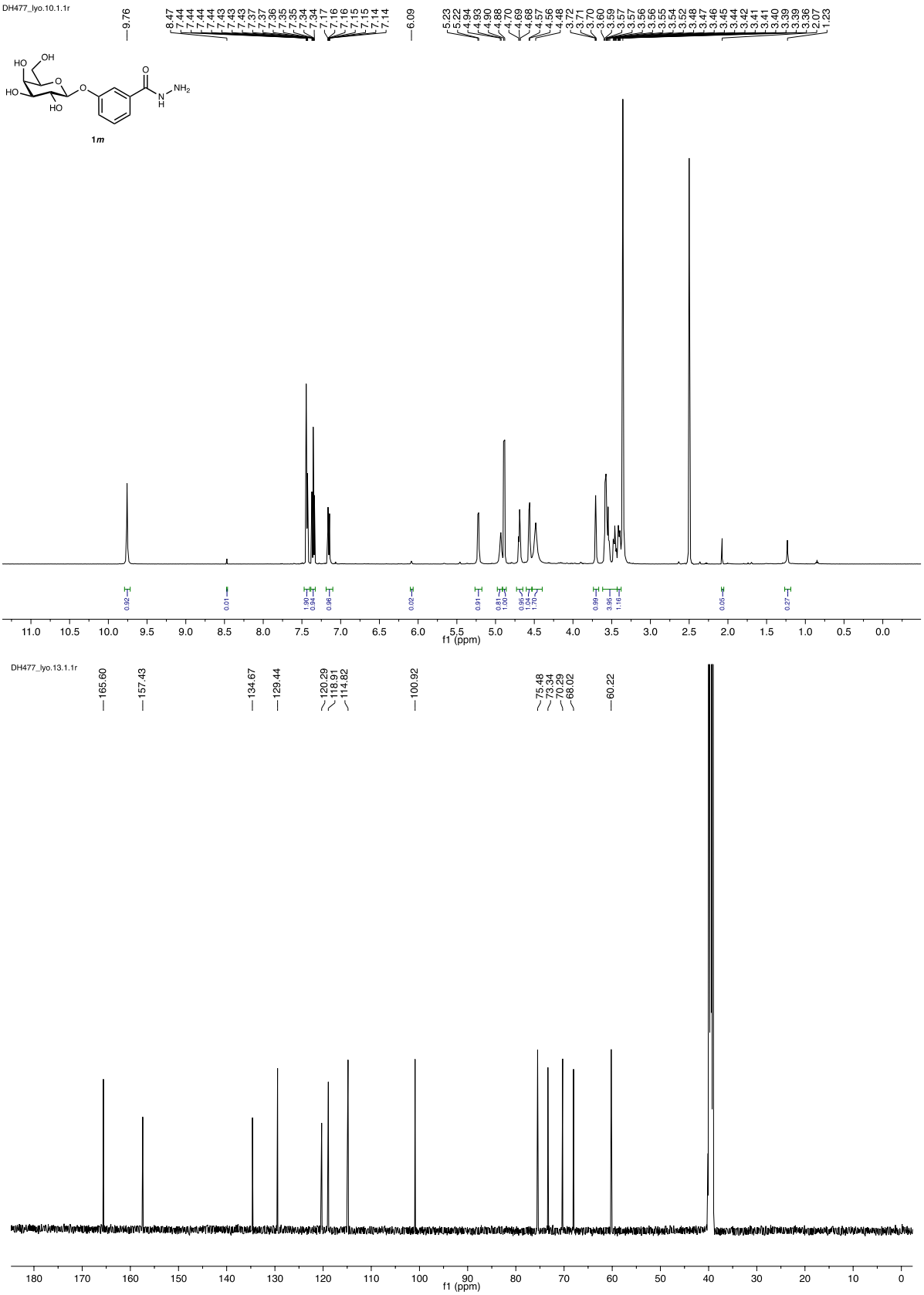


^{1}H and ^{13}C NMR of ***4m***

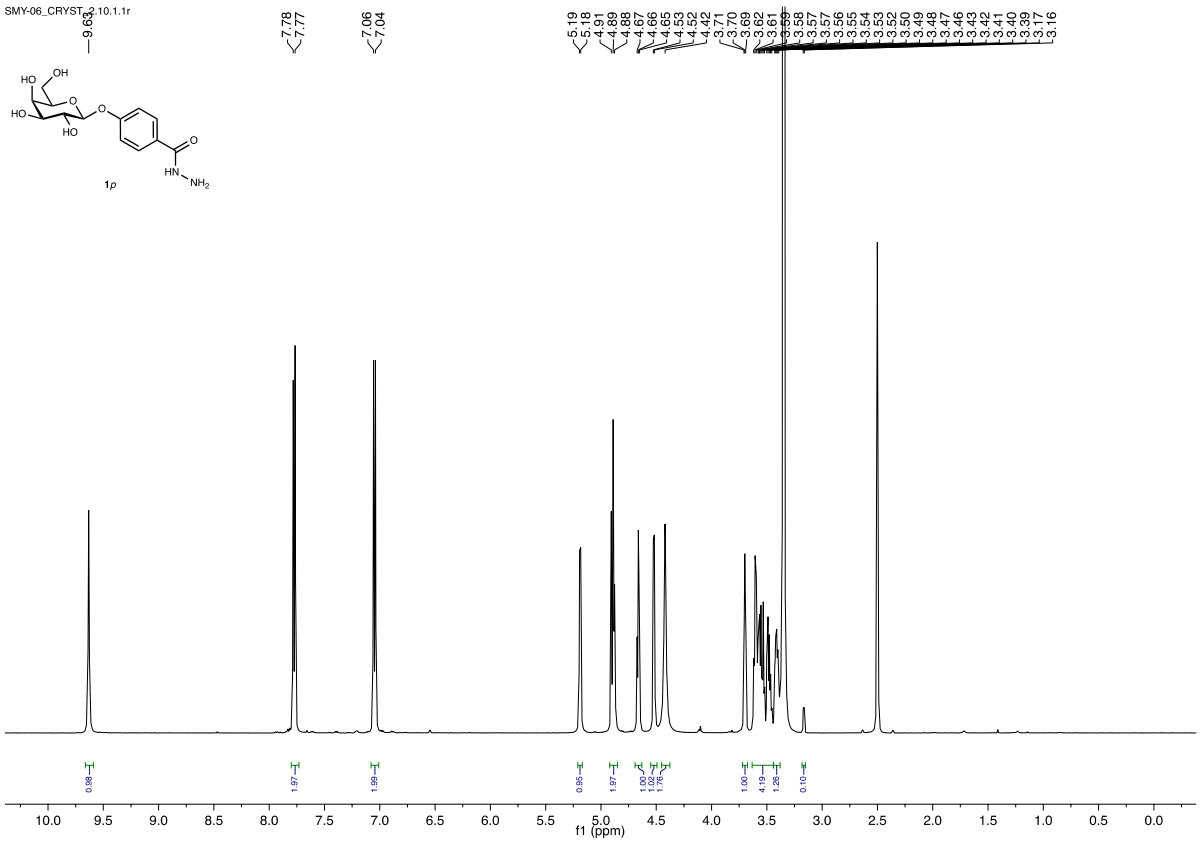


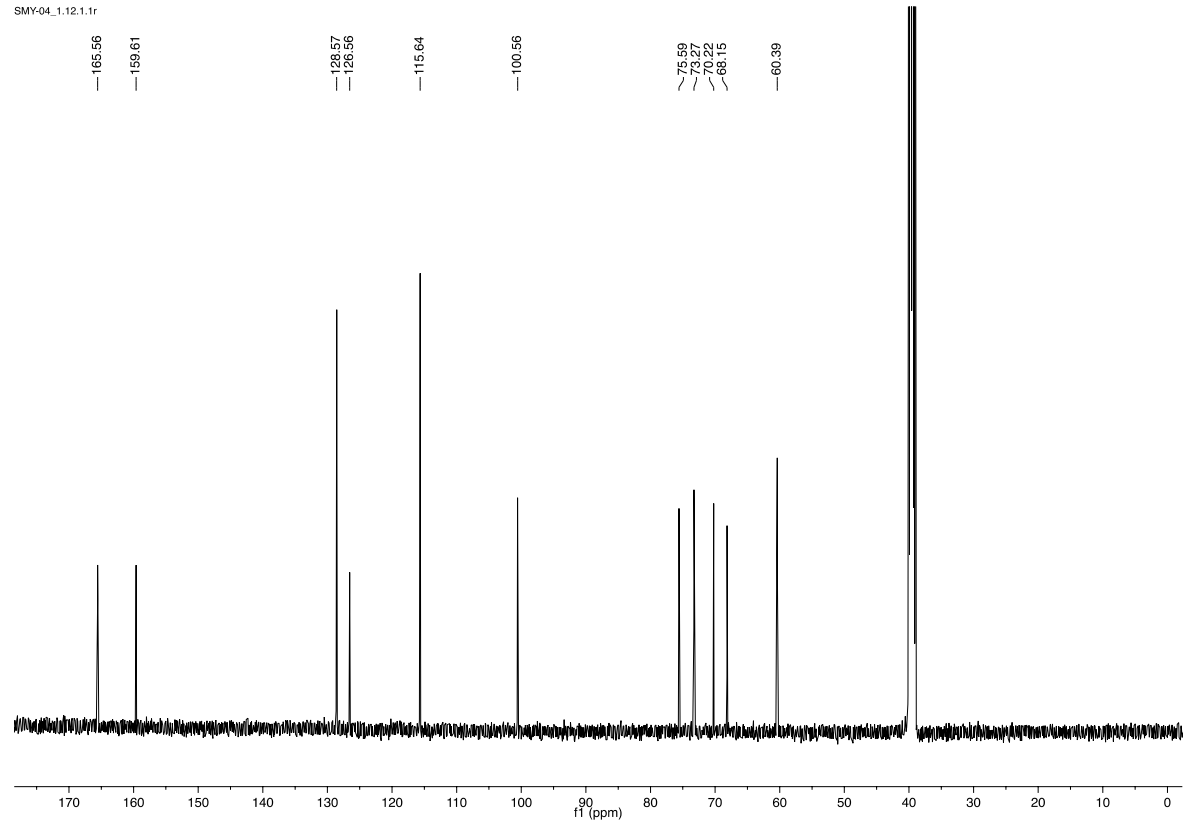


^{1}H and ^{13}C NMR of ***4p***

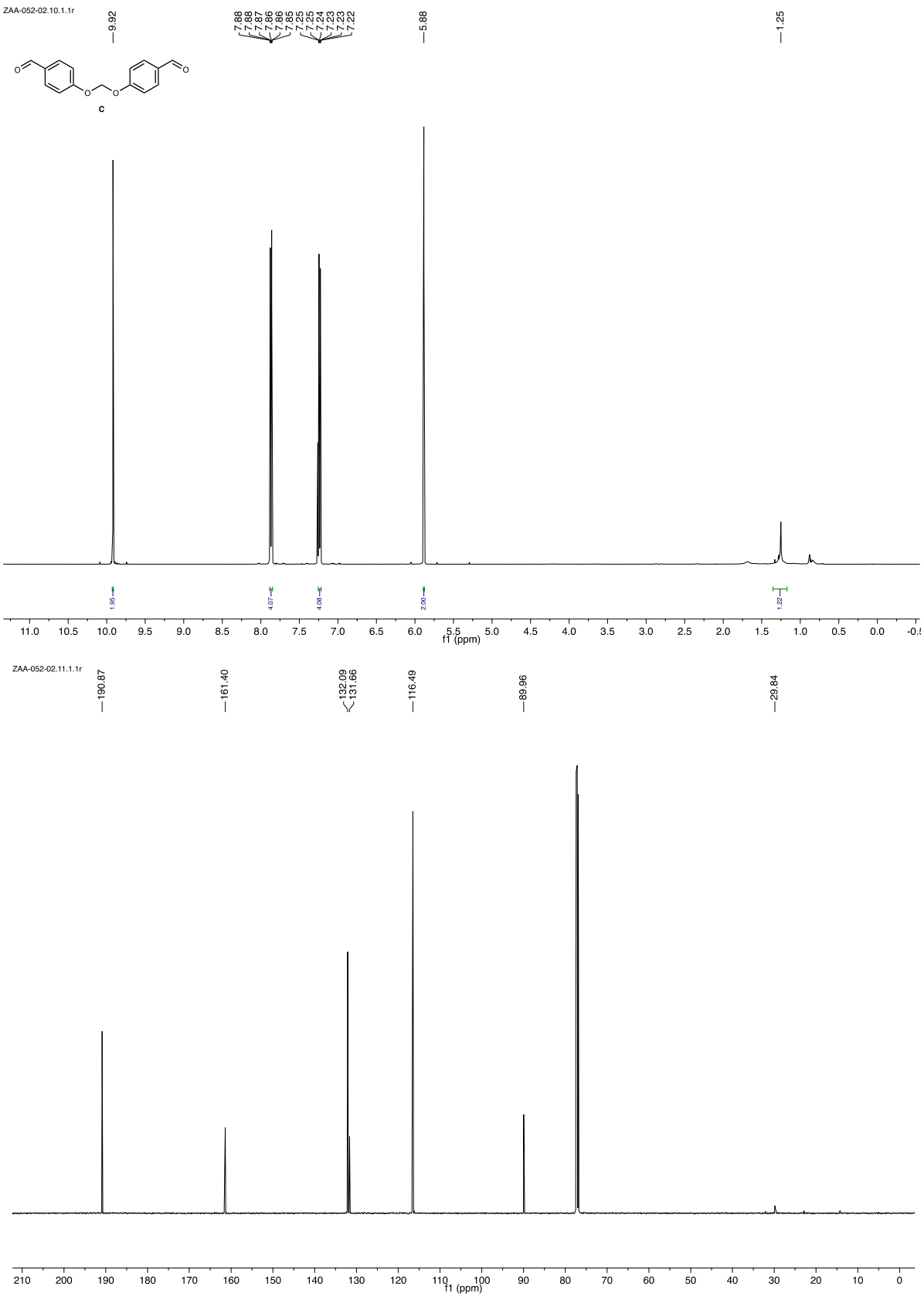


^{1}H and ^{13}C NMR of impure **1*m***

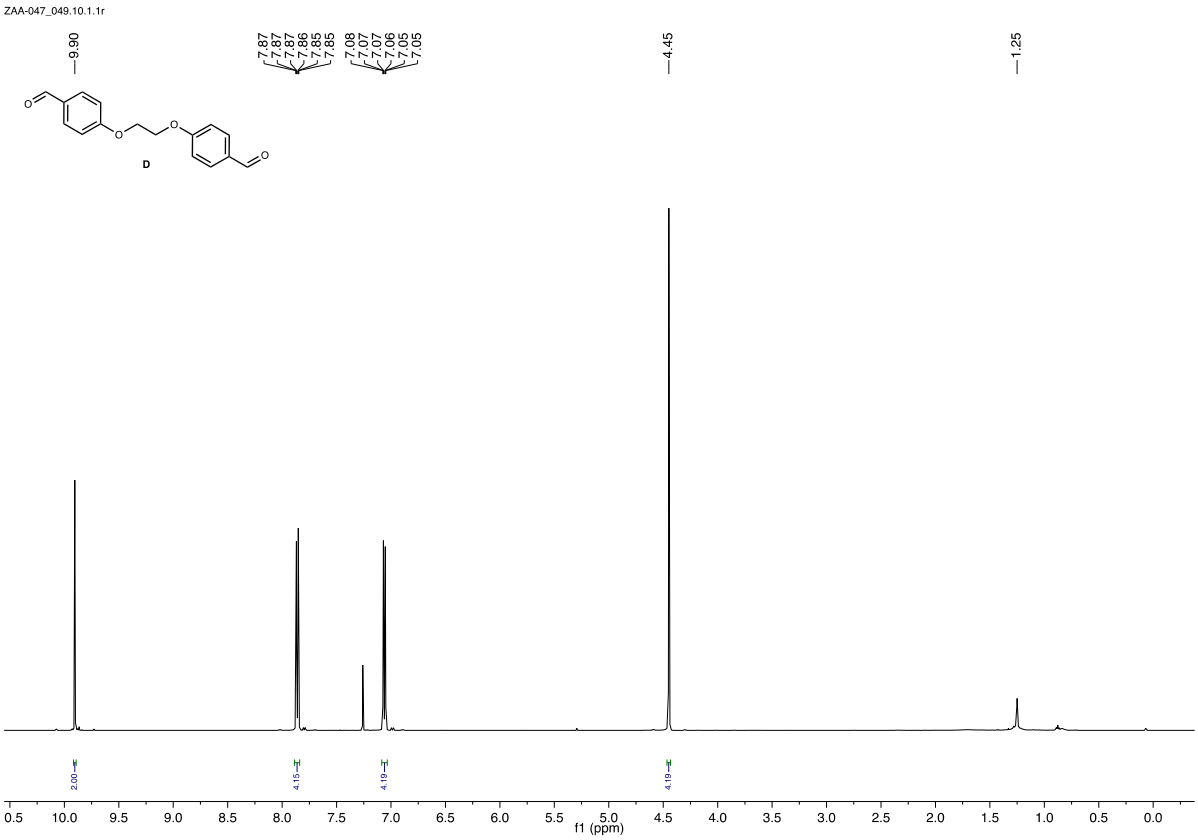


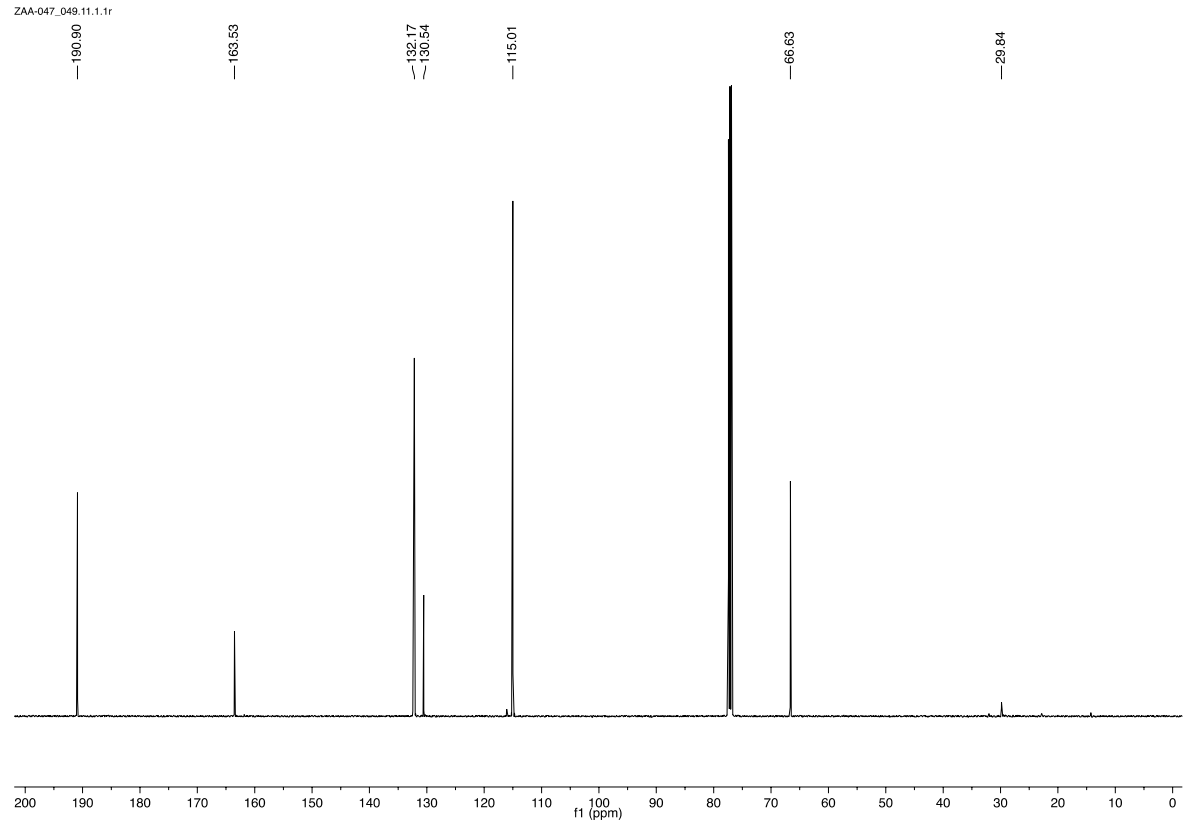


^{1}H and ^{13}C NMR of **1*p***

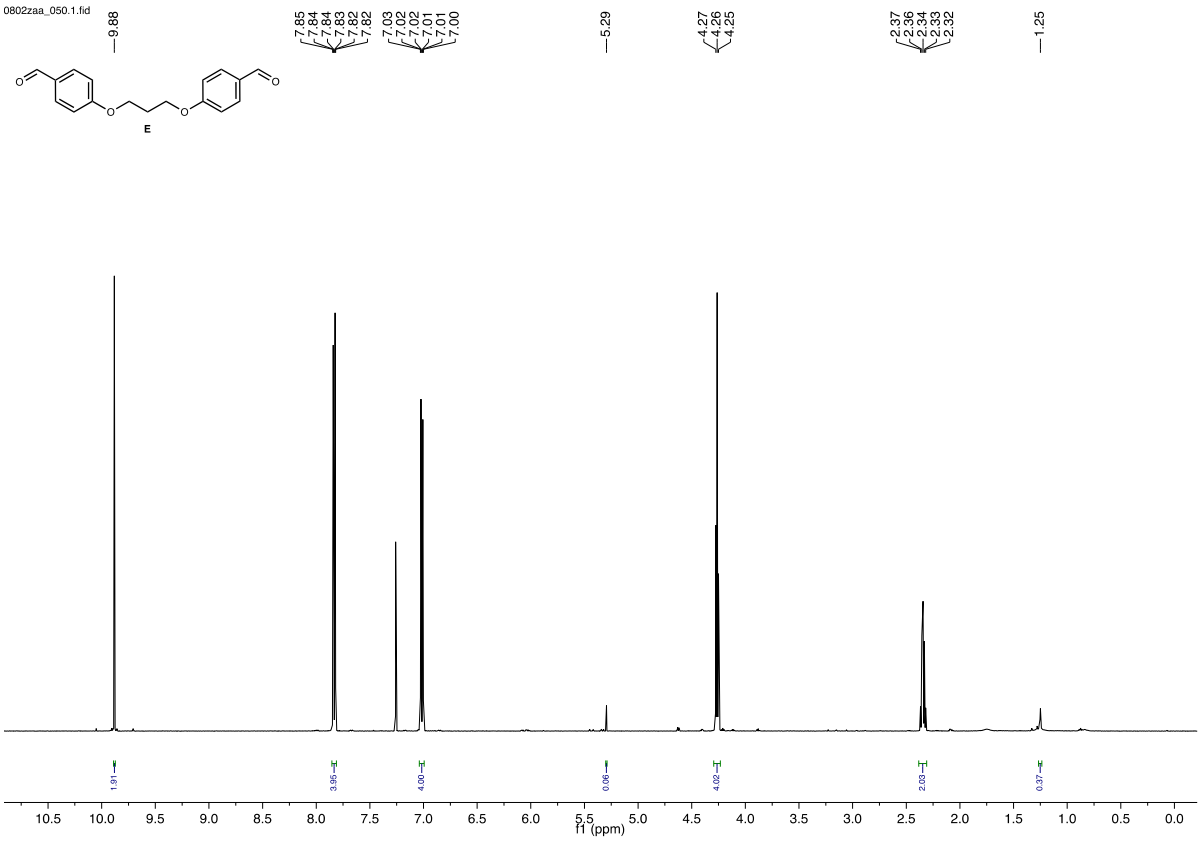


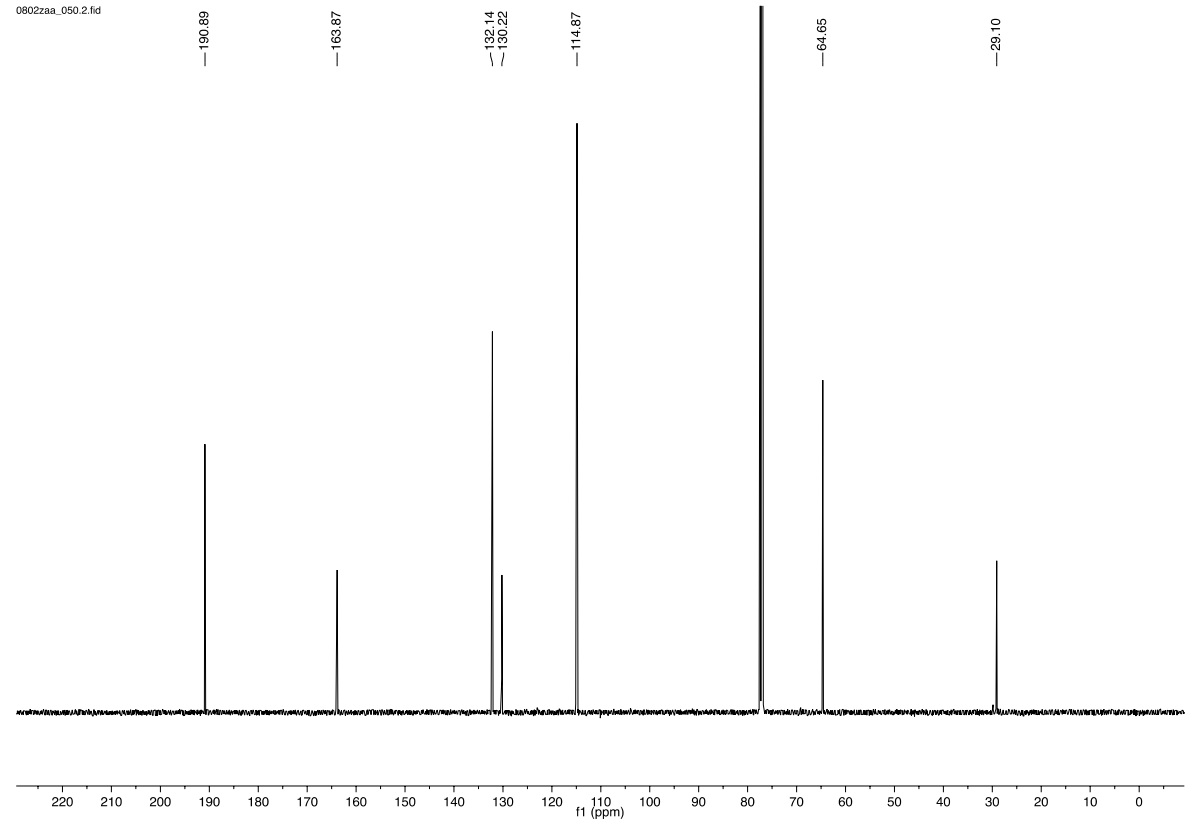
^{1}H and ^{13}C NMR of linker **C**



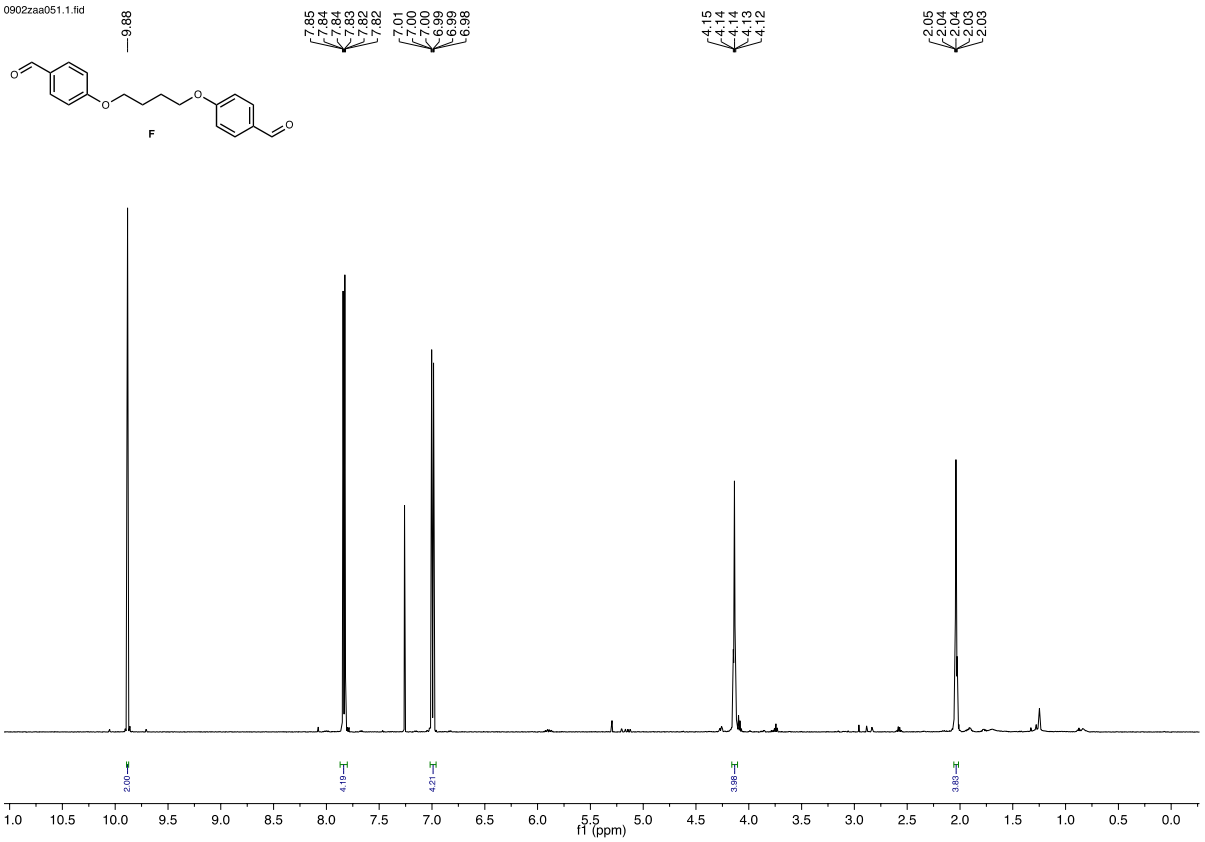


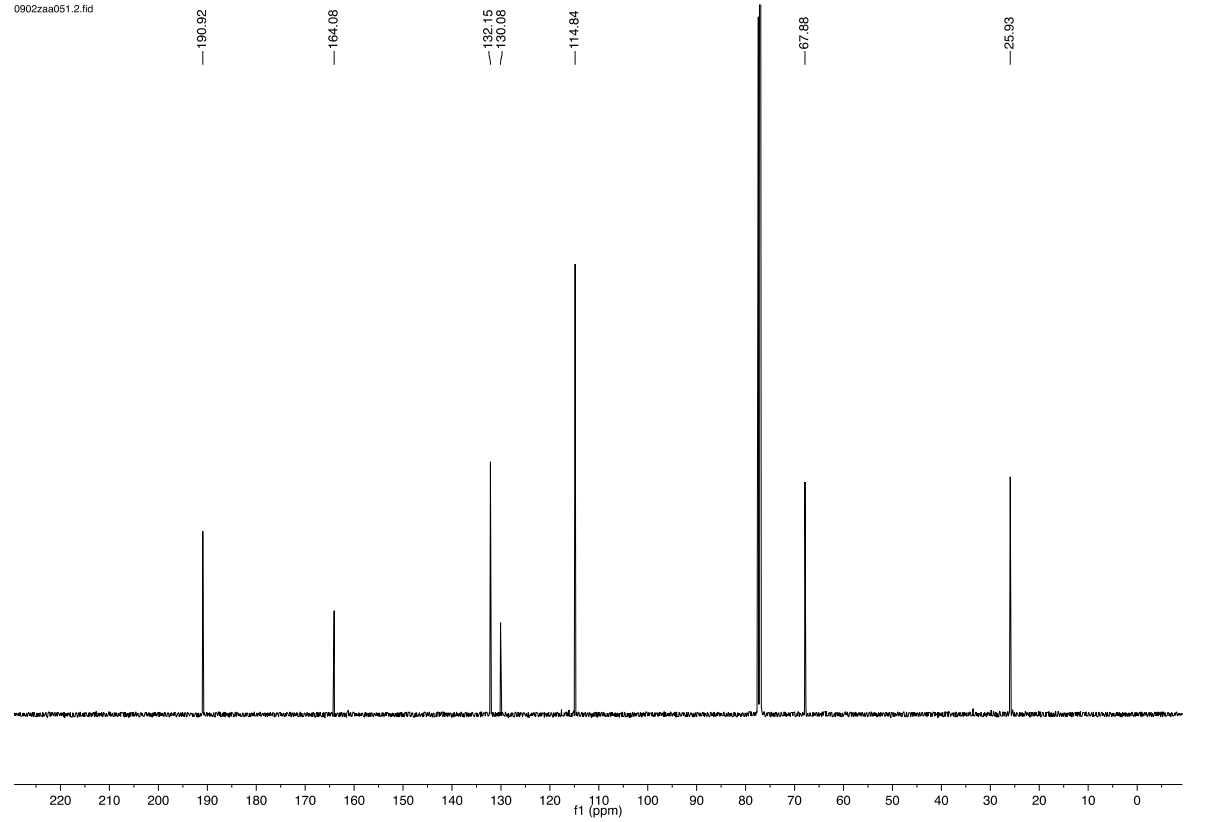
^{1}H and ^{13}C NMR of linker **D**



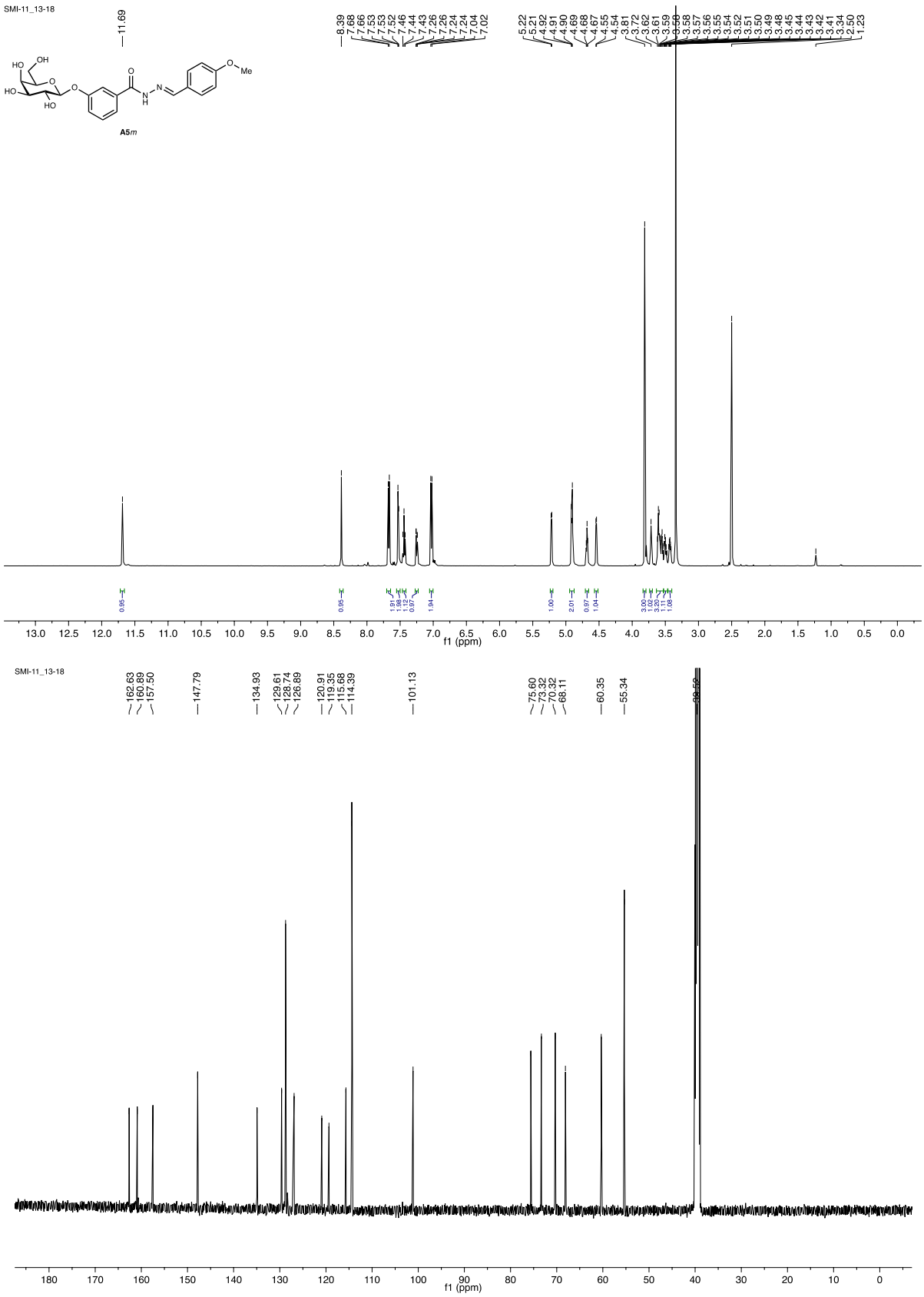


^{1}H and ^{13}C NMR of linker **E**

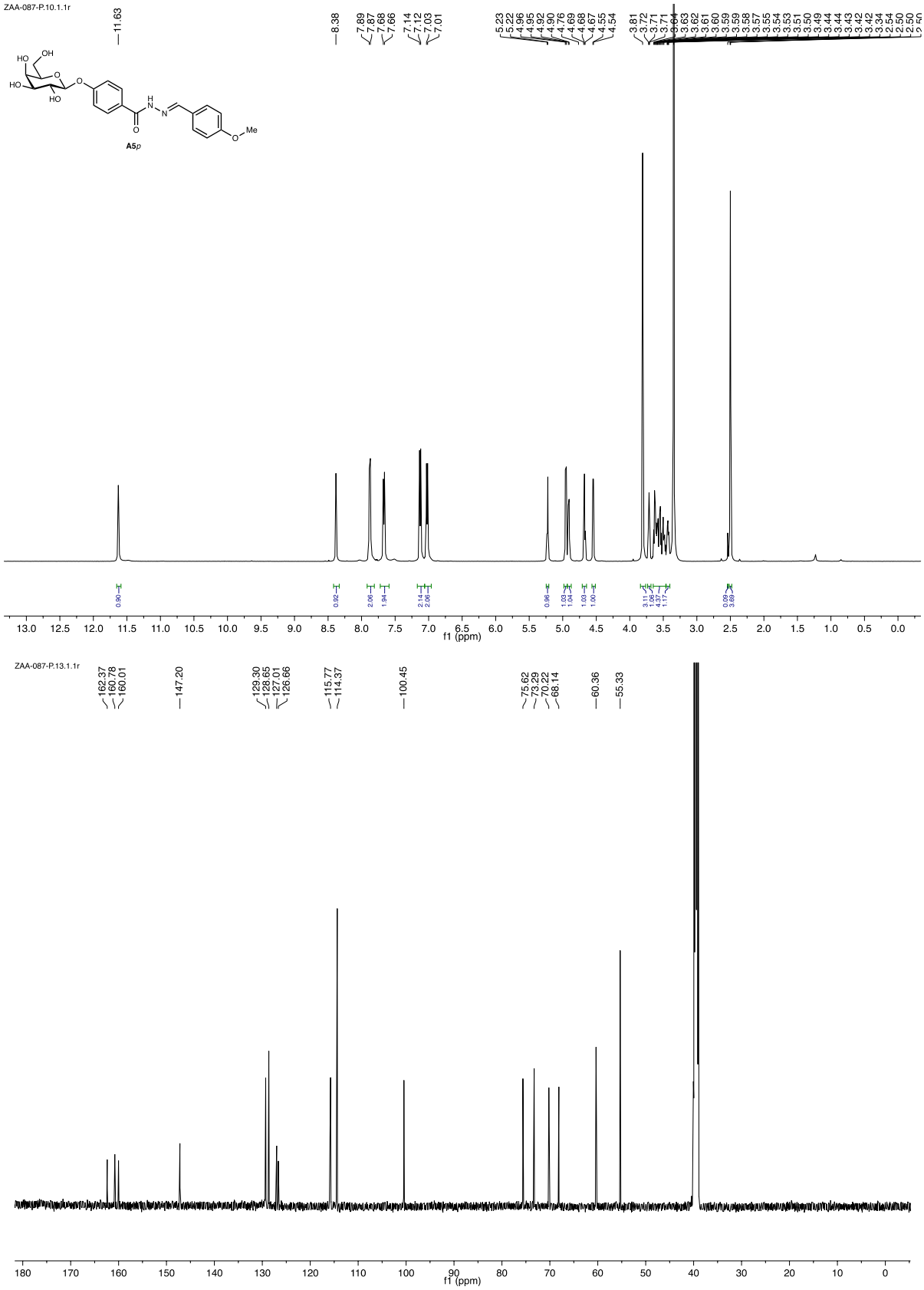




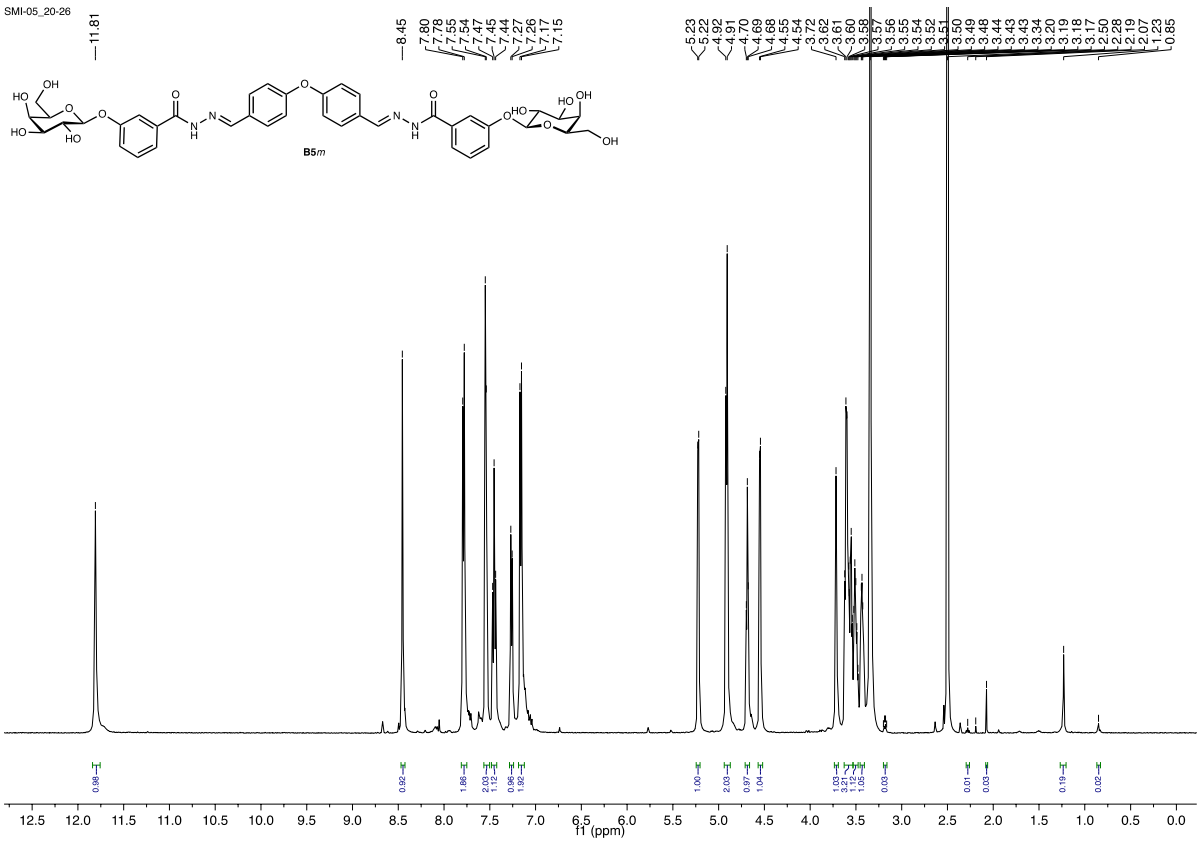
^{1}H and ^{13}C NMR of linker **F**

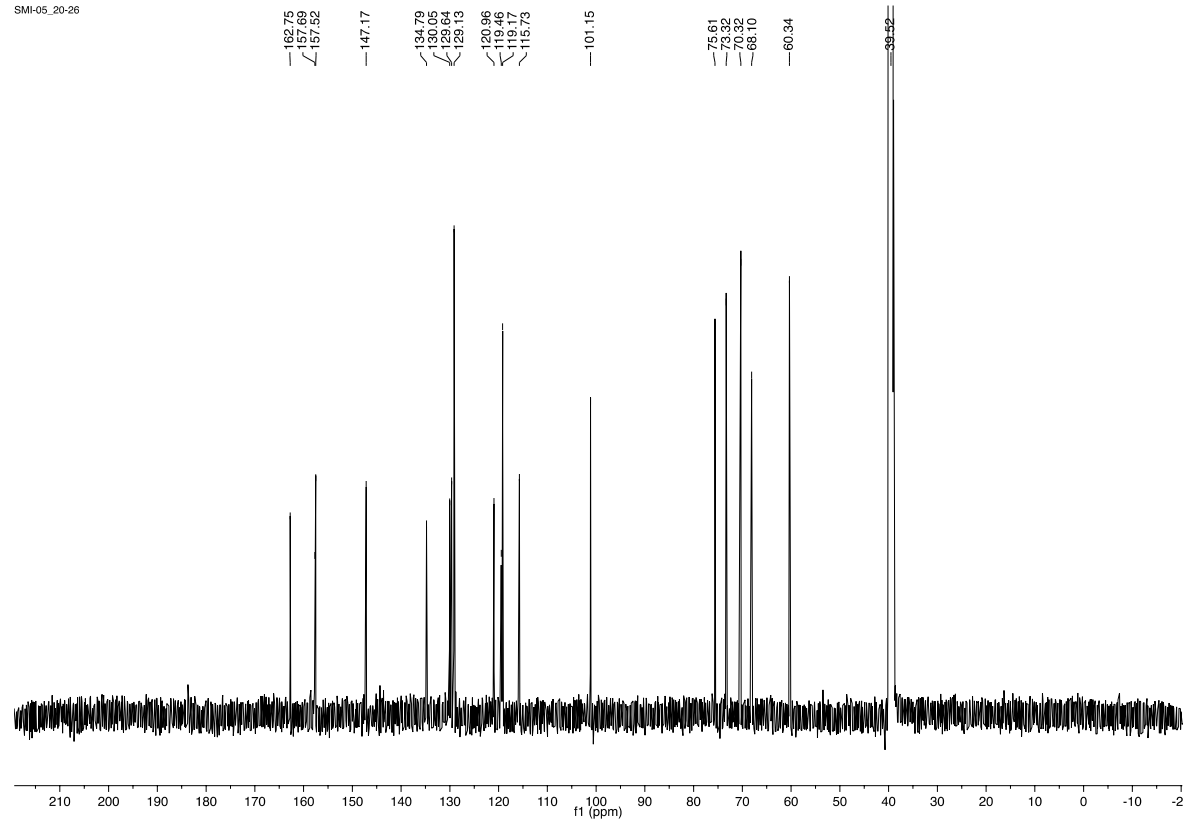


^{1}H and ^{13}C NMR of **A5*m***

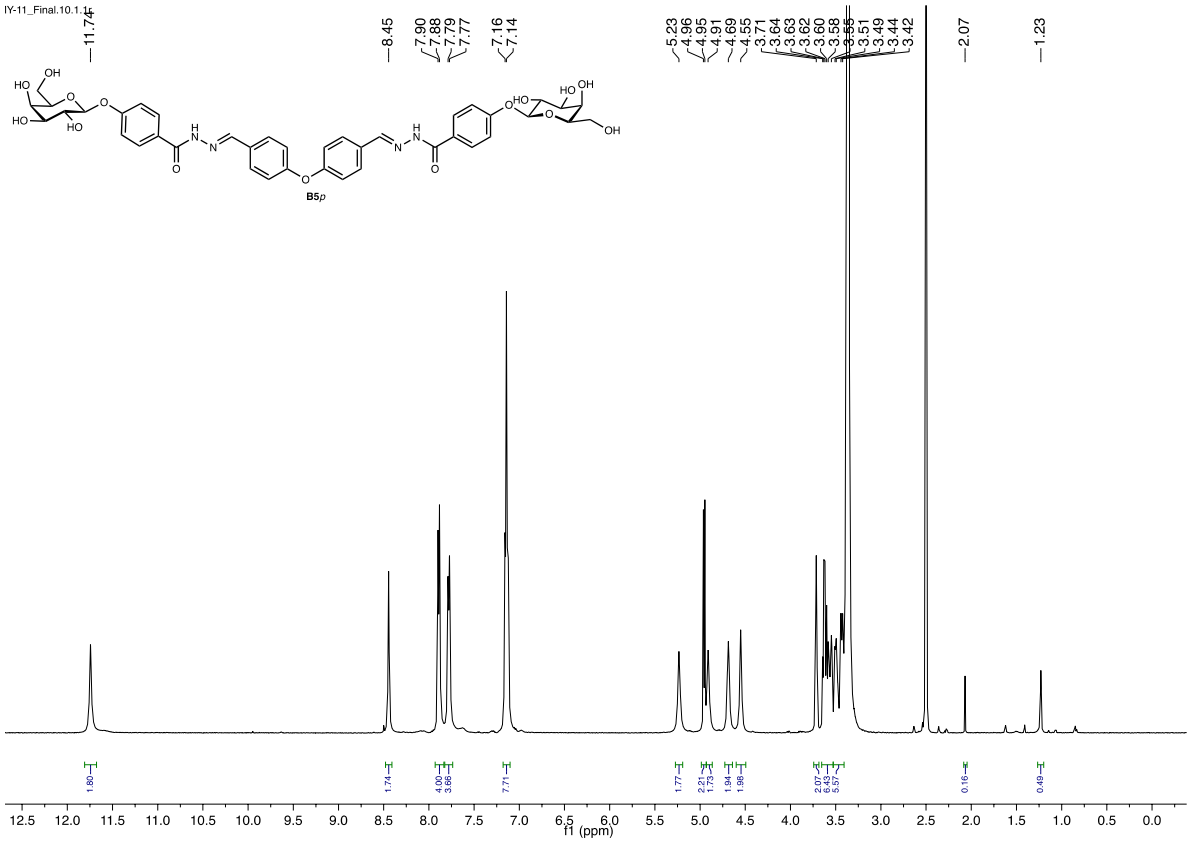


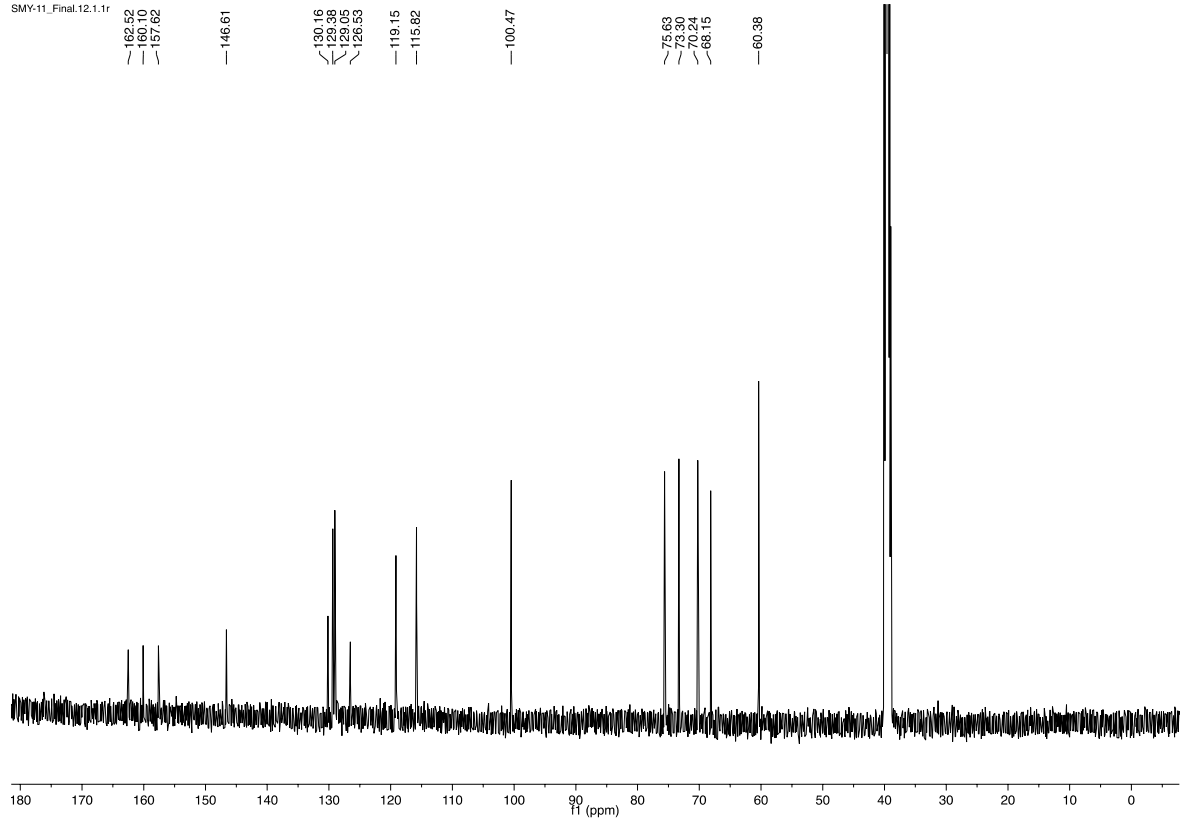
^{1}H and ^{13}C NMR of **A5*p***





^{1}H and ^{13}C NMR of **B5*m***





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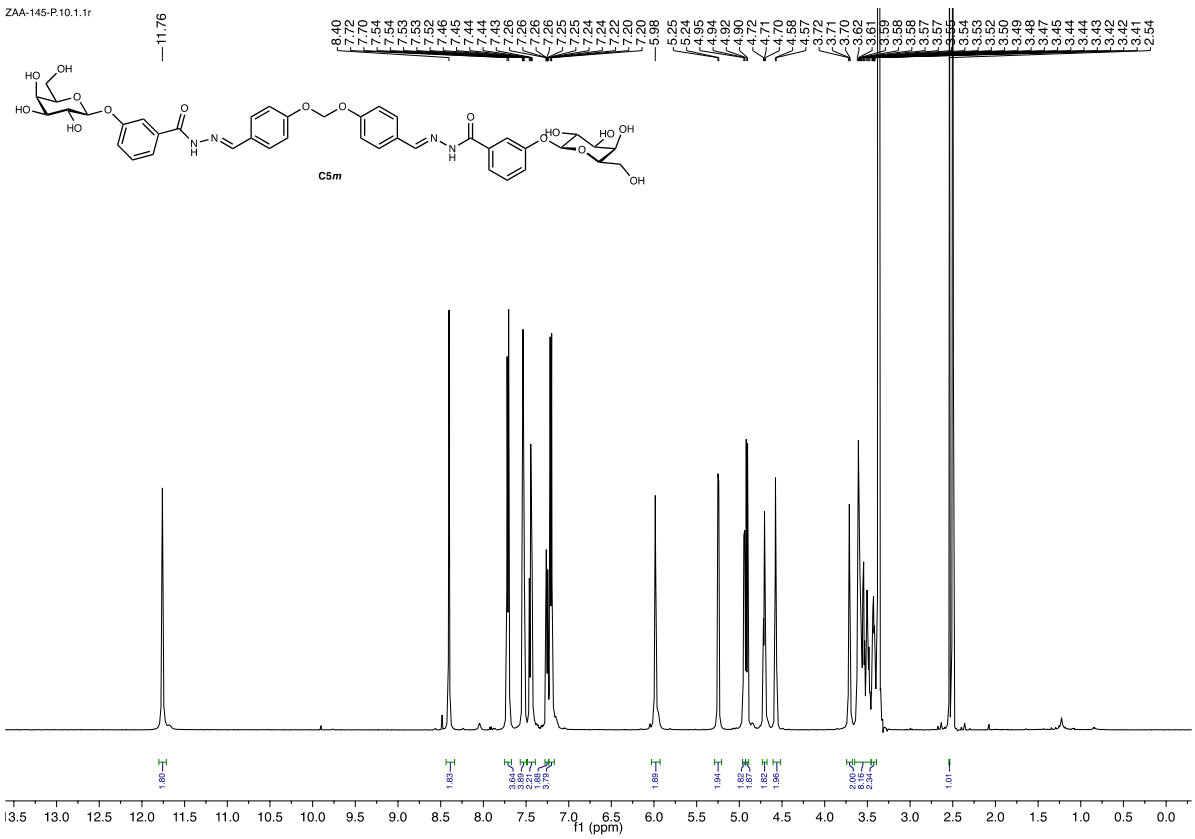
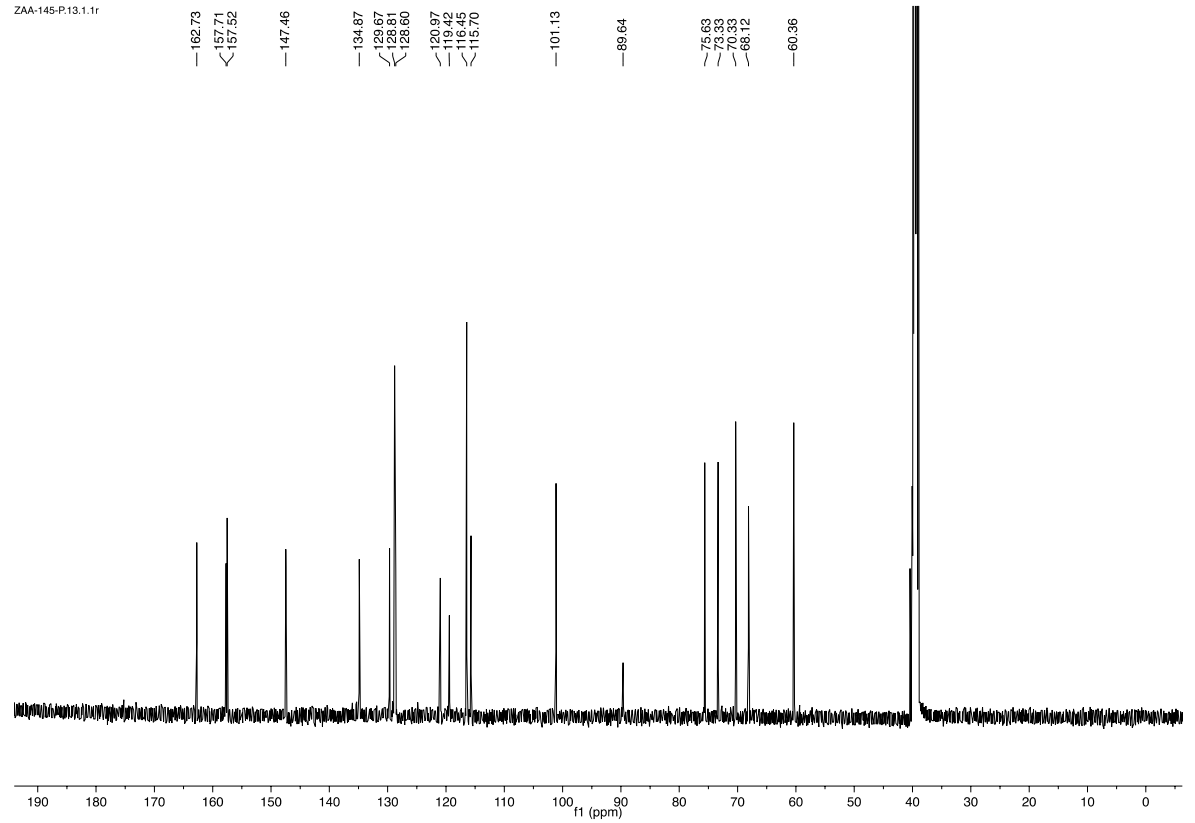
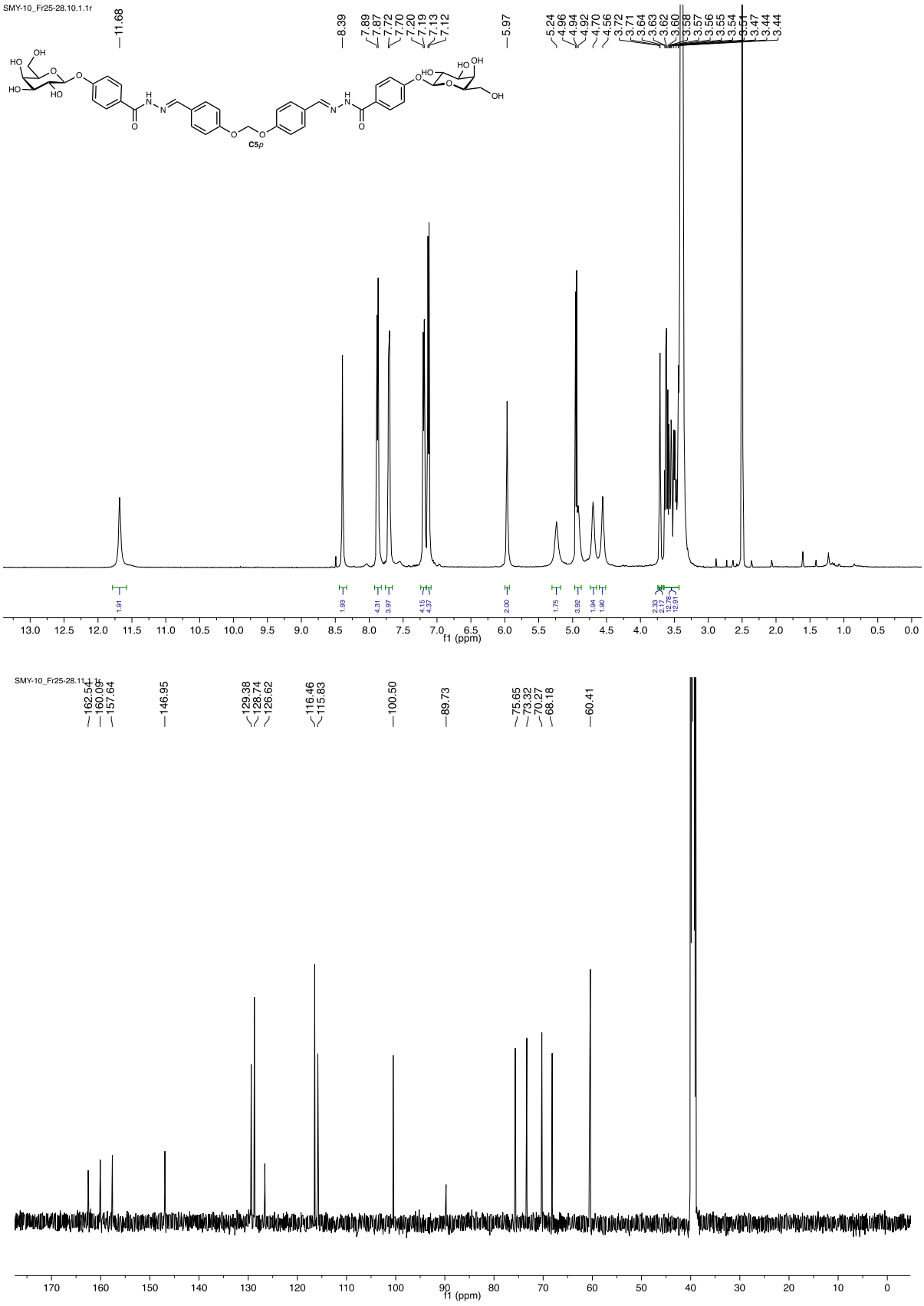




^{1}H and ^{13}C NMR of **C5*m***



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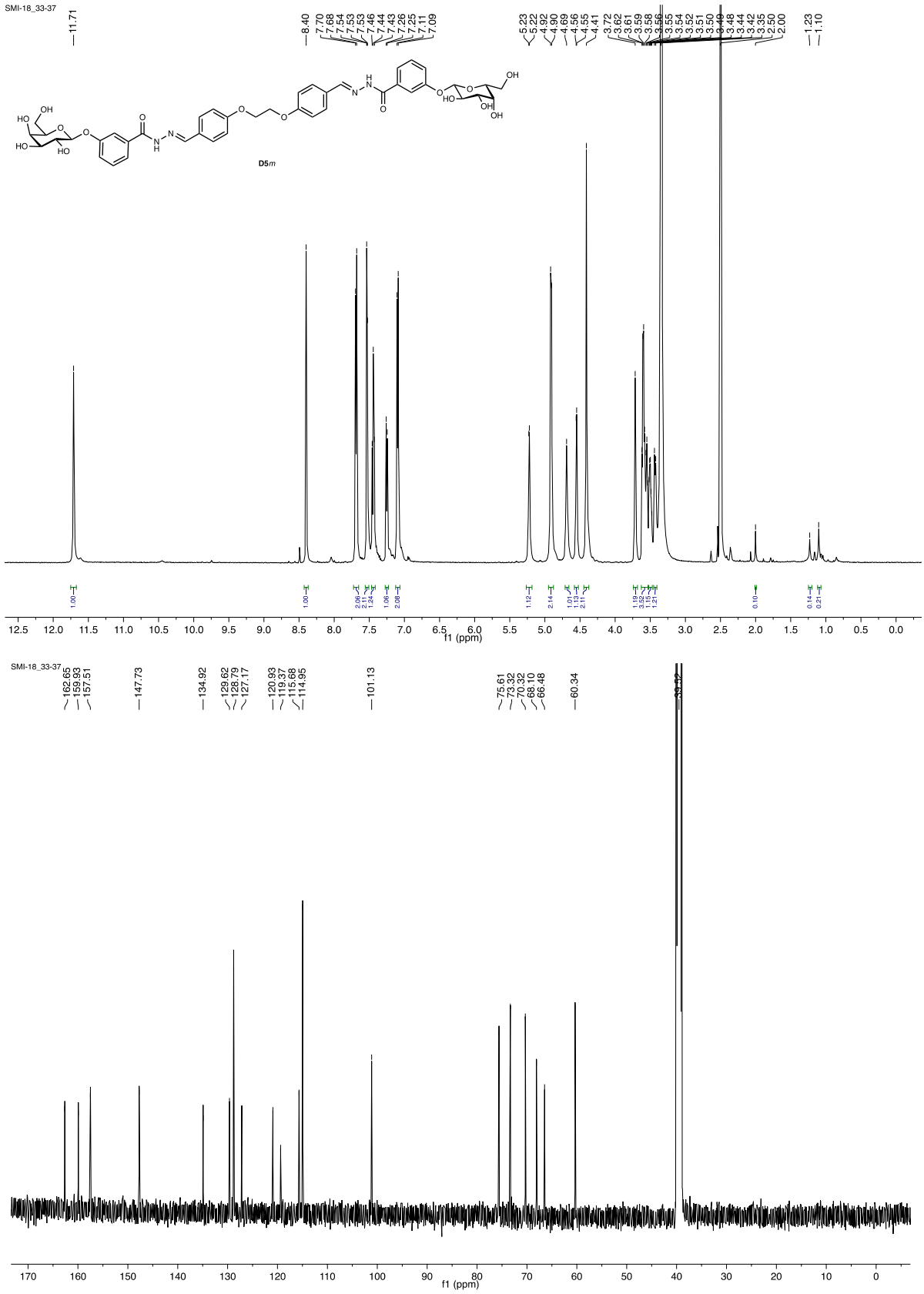
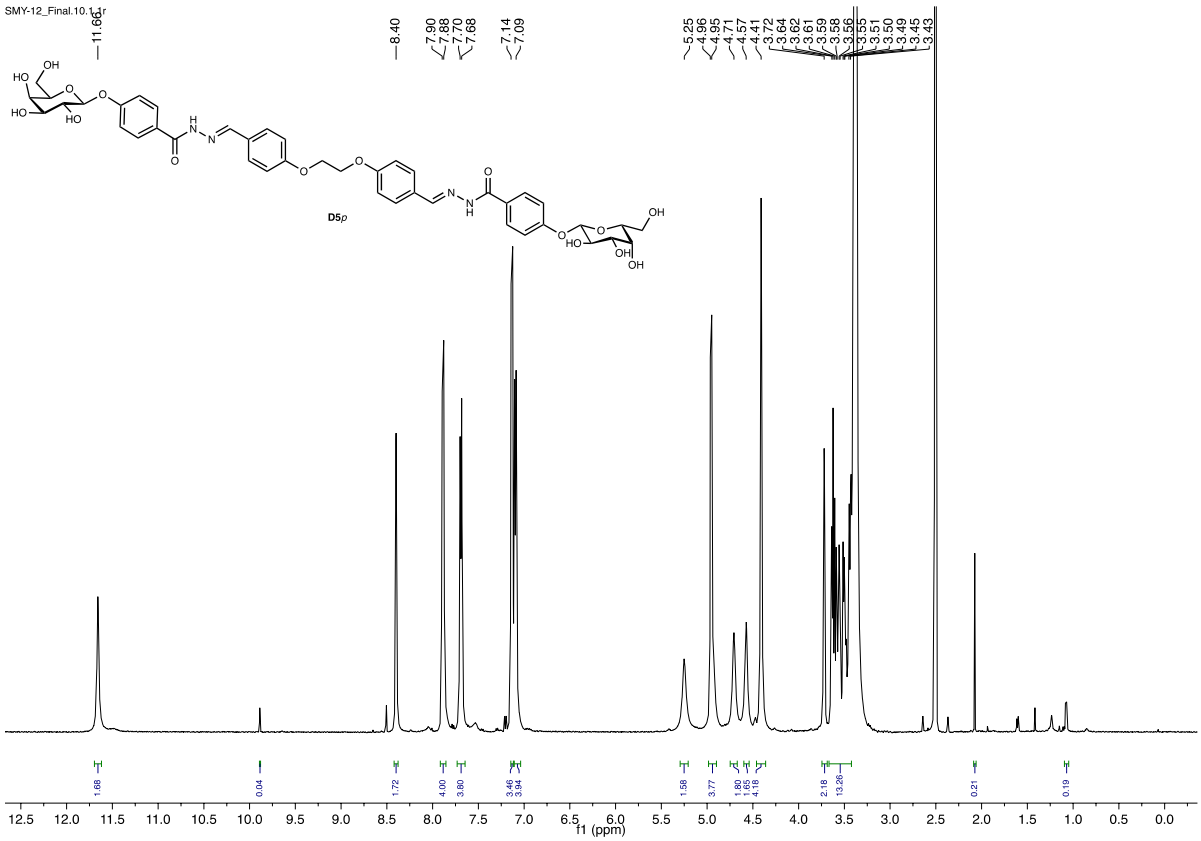
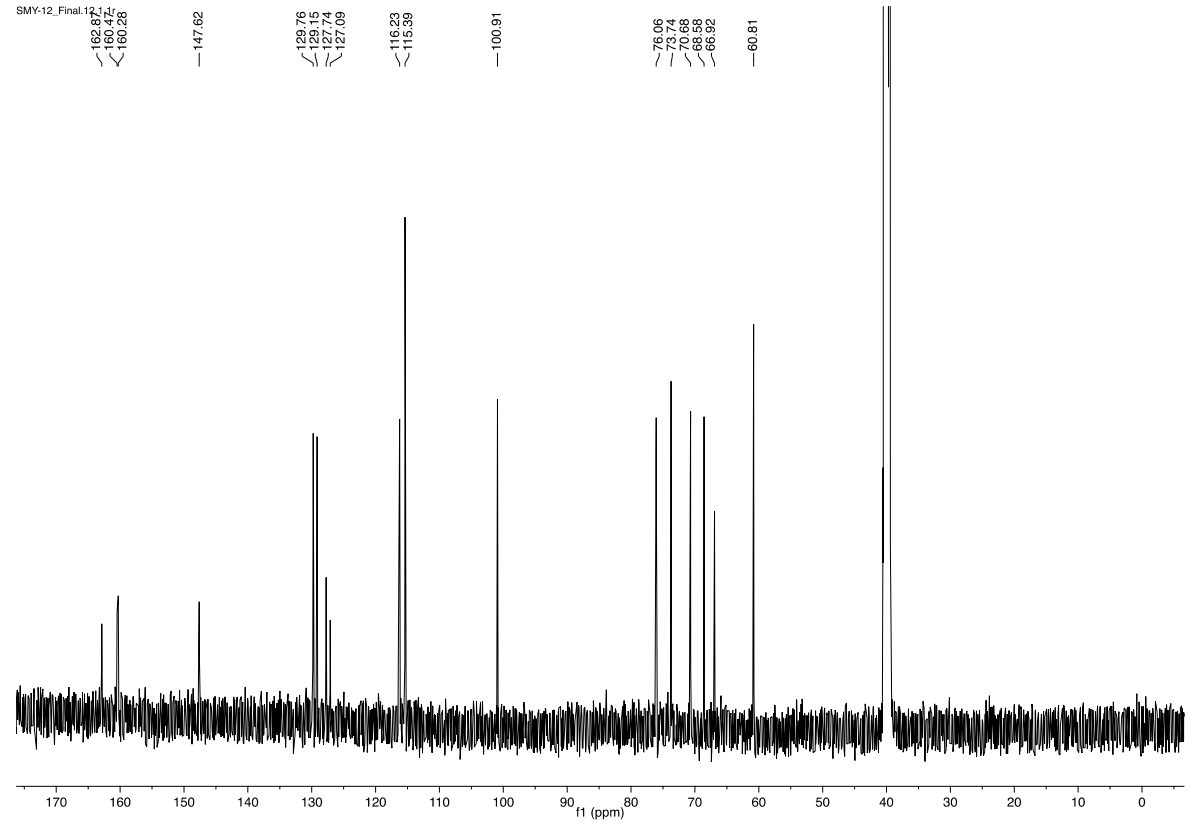


^{1}H and ^{13}C NMR of **D5*m***





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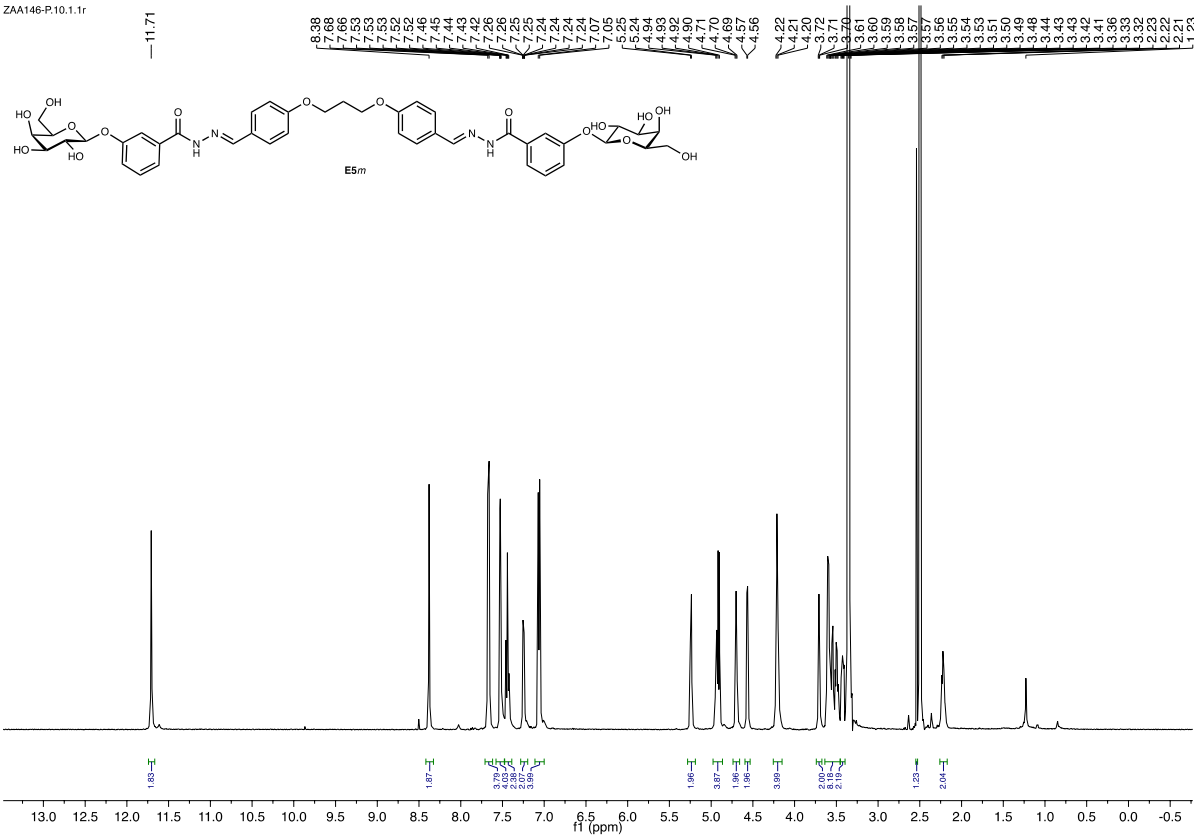
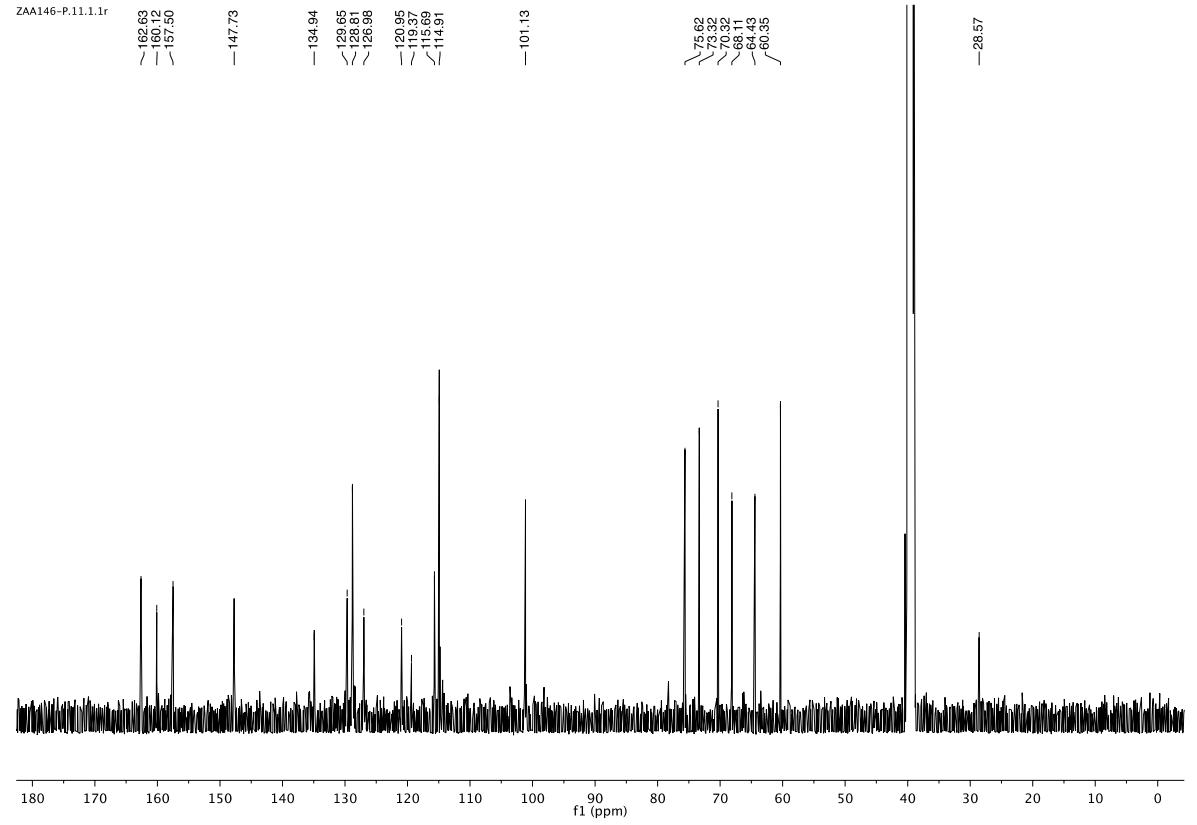
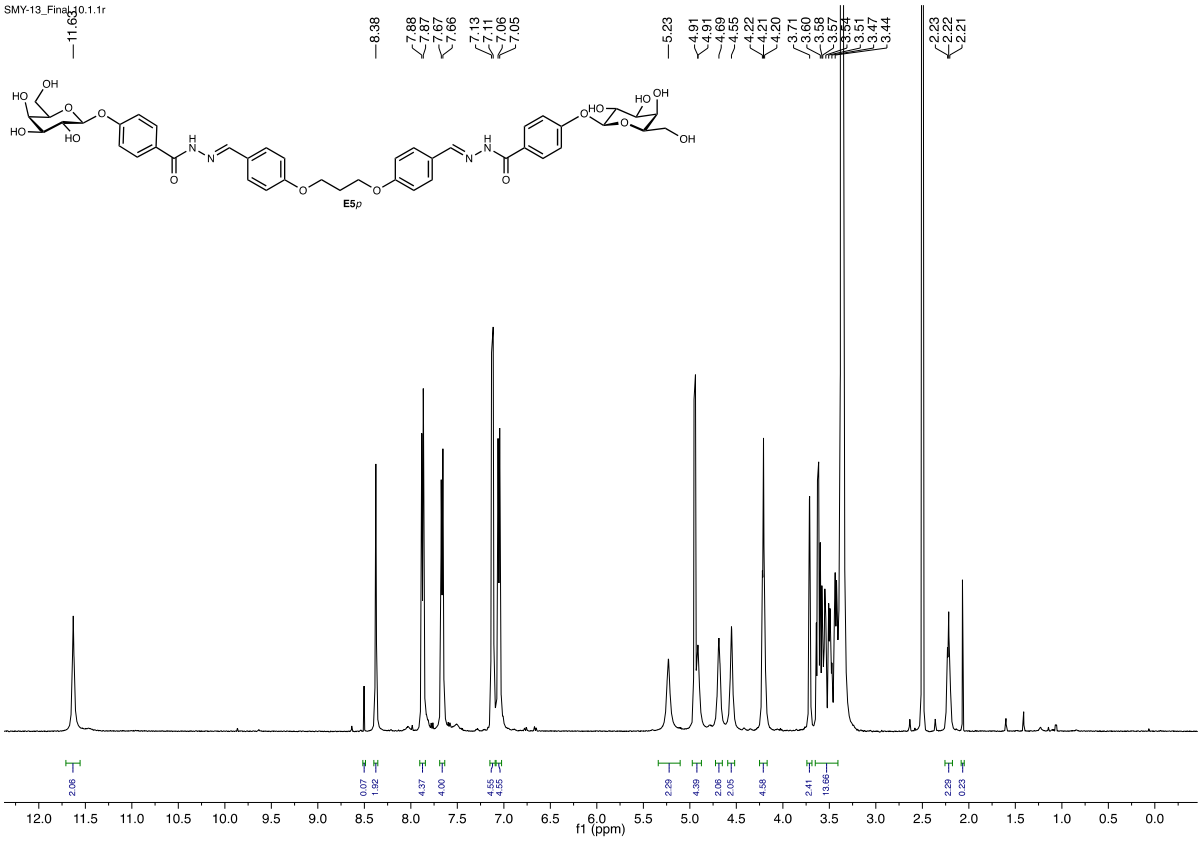
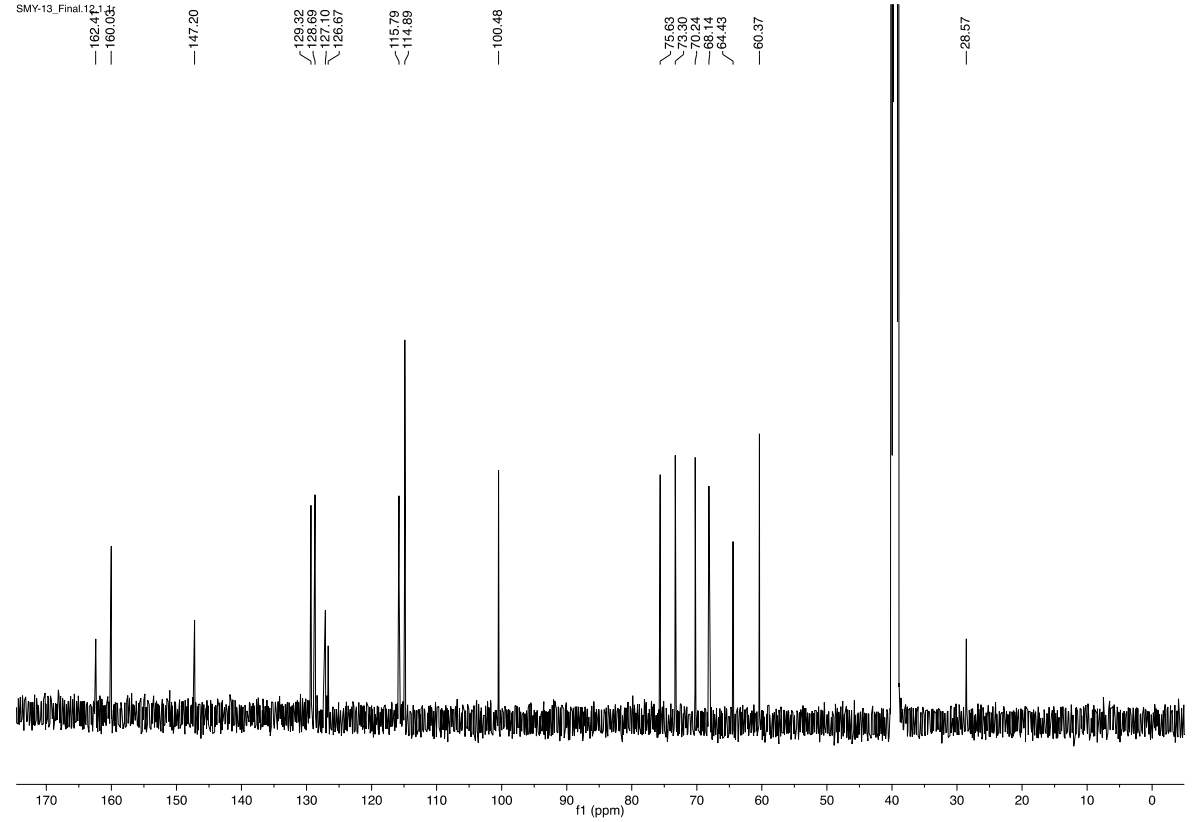




^{1}H and ^{13}C NMR of **E5*m***





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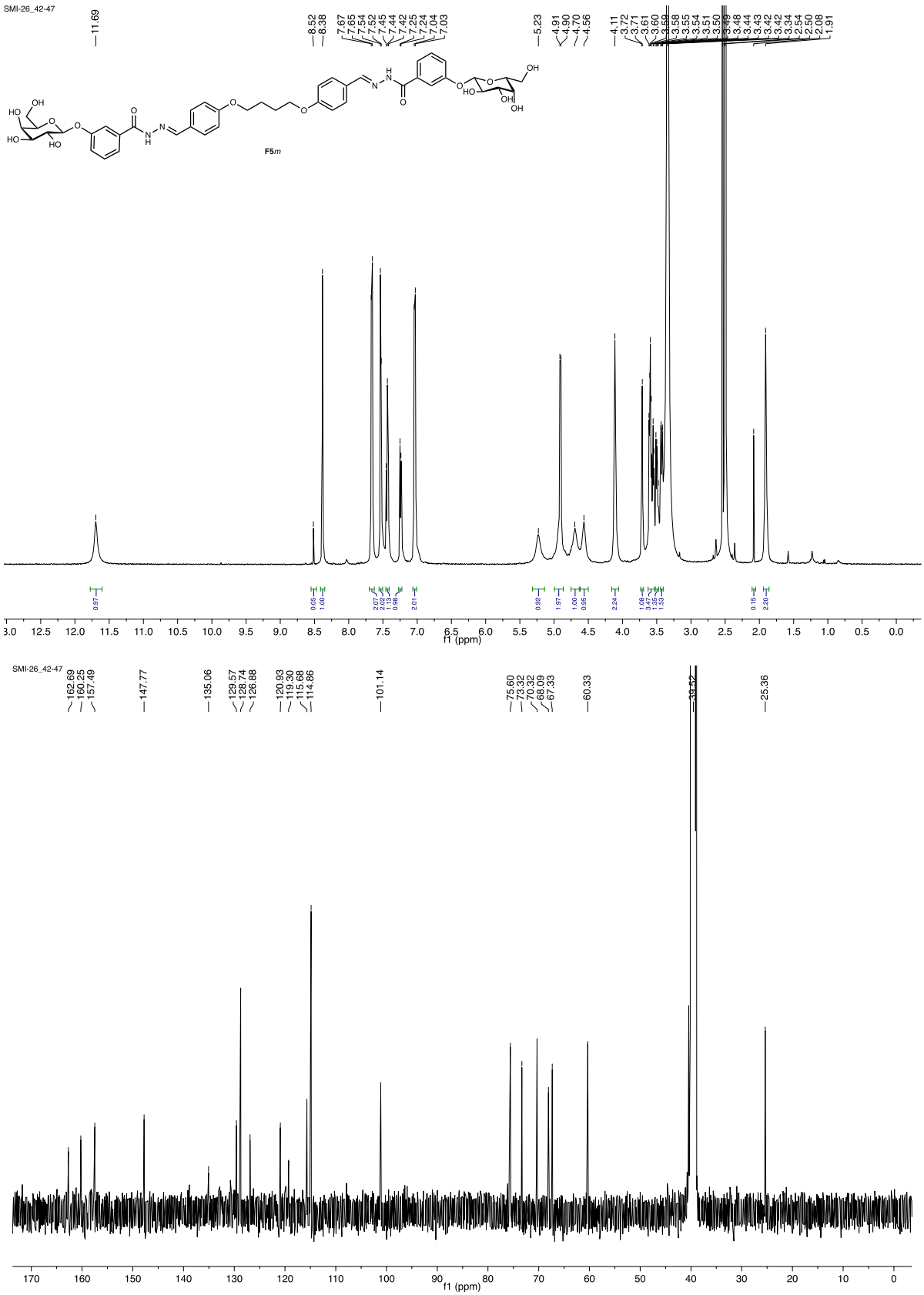
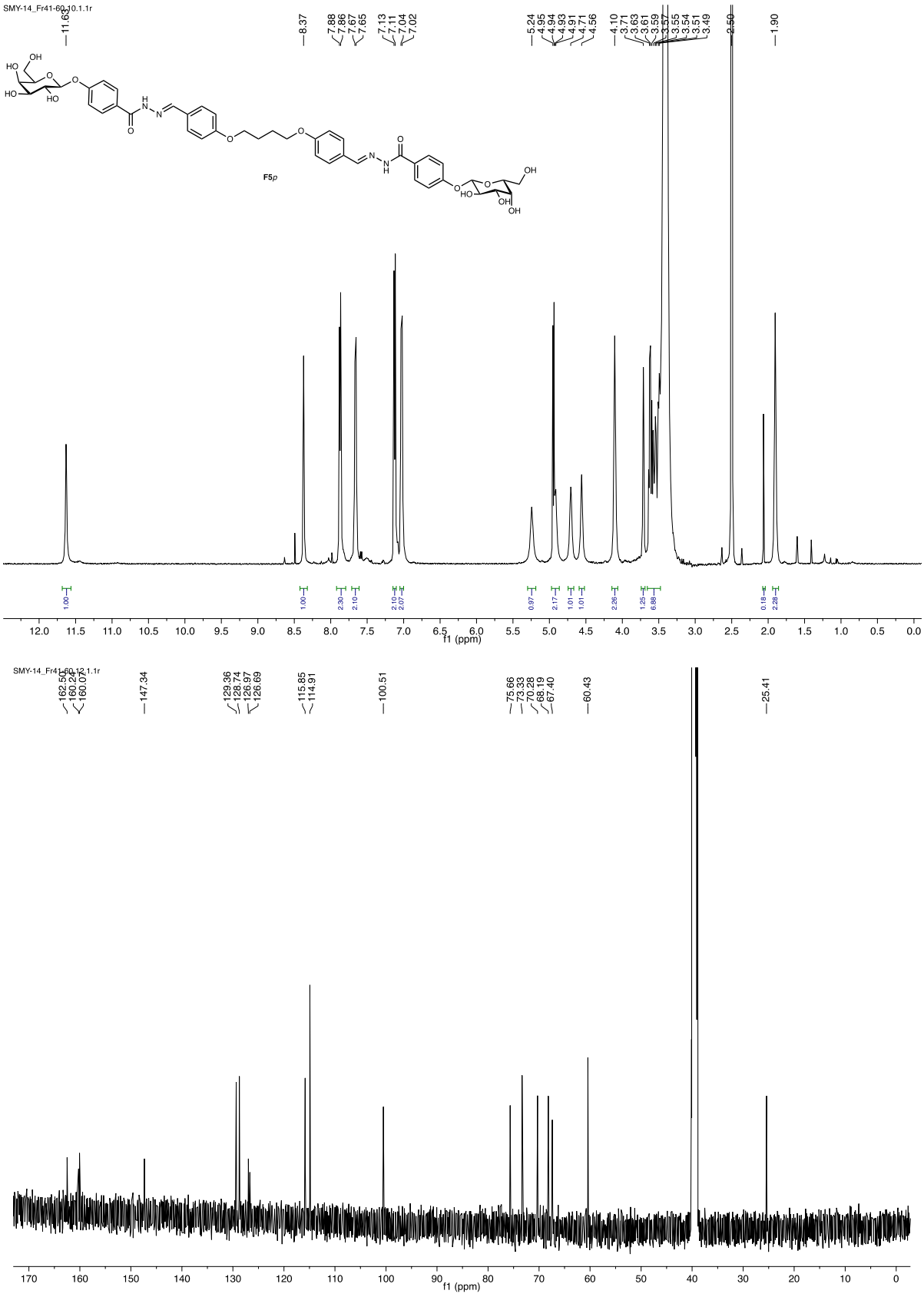


^{1}H and ^{13}C NMR of **F5*m***



^{1}H and ^{13}C NMR of **F5*p***

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