Supplemental Information

Structure based discovery of glycomimetic FmlH ligands as inhibitors of bacterial adhesion during urinary tract infection

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List of Abbreviations

Ac = acetyl; NaOH = sodium hydroxide; M = molar; nM = nanomolar; µM = micromolar mL = milliliter; h = hour; min. = minute; H_2O = water; MS = mass spectrometry; LCMS = Liquid chromatography/mass spectrometry; ES+ = electrospray positive ionization; ^{1}H-NMR = proton nuclear magnetic resonance; ^{13}C-NMR = carbon-13 nuclear magnetic resonance; MHz = megahertz; RT = rt = room temperature; °C = Celsius; EtOAc = EA = EtOAc; $CDCl_3$ = deuterated chloroform; DMSO-d_6 = dimethyl sulfoxide deuterated-6; MeOH = methanol; NaOMe = sodium methoxide; D_2O = deuterated water; prep-HPLC = preparative high pressure liquid chromatography, also known as preparative high performance liquid chromatography; DMSO = dimethyl sulfoxide; MeCN = CH_3CN = acetonitrile; Ag_2CO_3 = silver carbonate; $NaHCO_3$ = sodium bicarbonate; Na_2SO_4 = sodium sulfate; TEA = triethylamine; TMS = trimethylsilyl; TMSOTf = trimethylsilyl triflate; TFA = trifluoroacetic acid; DCM = CH_2Cl_2 = dichloromethane; K_2CO_3 = potassium carbonate; µl = microliter; g = gram; mg = milligram.

General synthesis, purification, and analytical chemistry procedures

Starting materials, reagents, and solvents were purchased from commercial vendors unless otherwise noted. In general, anhydrous solvents are used for carrying out all reactions. ^{1}H and ^{13}C NMR spectra were measured on a Varian 400 MHz NMR instrument equipped with an auto sampler. The chemical shifts were reported as δ ppm relative to TMS using residual solvent peak as the reference unless otherwise noted. The following

abbreviations were used to express the peak multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. High-performance liquid chromatography (HPLC) was carried out on GILSON GX-281 using Waters C18 5μM, 4.6*50mm and Waters Prep C18 5μM, 19*150mm reverse phase columns, eluted with a gradient system of 5:95 to 95:5 acetonitrile: water with a buffer consisting of 0.05-0.1% TFA. Mass spectroscopy (MS) was performed on HPLC/MSD using a gradient system of 5:95 to 95:5 acetonitrile: water with a buffer consisting of 0.05-0.1% TFA on a C18 or C8 reversed phased column and electrospray ionization (ESI) for detection. All reactions were monitored by thin layer chromatography (TLC) carried out on either Merck silica gel plates (0.25 mm thick, 60F254) or Millipore Silica gel aluminum sheets (60F254) and visualized by using UV (254 nm) or dyes such as *p*-Anisaldehyde and CAM (Hannesian's Stain). Molecular sieves (3Å) were crushed and activated *in vacuo* at 390 ºC overnight, then stored in a drying oven (300 ºC) until just prior to use. Silica gel chromatography was carried out on a Teledyne ISCO CombiFlash purification system using pre-packed silica gel columns (12 g~330 g sizes). All compounds used for biological assays are greater than 95% purity based on NMR and HPLC by absorbance at 220 nm and 254 nm wavelengths.

Commercially available galactoside compounds: **Thomsen-Friedenreich (TF), 1β, 4β (ONPG), 4β-NAc, 10β, 11β, 11β -NAc, 11β-thio, 12β, 13β, 14β, 15β, 16β, 17β, 19β, 20β, 22β-thio, 23β, 24β, 25β, 26β, 27β, 23b, 33-35, 36β-thio (IPTG)**

Experimental procedures

General procedure for glycosylation: Protocol A

Under nitrogen atmosphere, a solution of 1,2,3,4,6-penta-O-acetyl-β-D-galactose (0.25 mmol), phenol derivative (0.50 mmol), and 3Å molecular sieves was stirred in either CH_2Cl_2 or 1,2-dichloroethane (5 mL) for 1 h. Boron trifluoride diethyl etherate (0.75 mmol) was then added dropwise, and the solution was stirred for the specified time and temperature, monitoring by TLC and LCMS. Upon completion, the reaction was cooled to rt and neutralized with Et_3N. The sieves were filtered off, and the remaining filtrate was washed with sat. aq. $NaHCO_3$ (2 x 1mL), and brine (1 x 1mL), dried over Na_2SO_4, and concentrated *in vacuo*. The resulting residue was purified by silica gel chromatography with hexane/ethyl acetate combinations as eluent, to give the glycosylation product.

General procedure for glycosylation: Protocol B

Under nitrogen atmosphere, 1,2-dichloroethane (2 mL) was added to a flask containing 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (103 mg, 0.25 mmol) and phenol derivative (0.75 mmol). Silver carbonate (0.50 mmol) was then added, and the solution was stirred for specified time and temperature, monitoring by TLC and LCMS. Upon completion, the reaction was cooled, filtered and concentrated in vacuo. The resulting residue was purified by silica gel chromatography with hexane/ethyl acetate combinations as eluent, to give the glycosylation product.

General procedure for glycosylation: Protocol C

1N aqueous NaOH solution (1 mL) was added into a solution of 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl chloride (100 mg, 0.273 mmol), tetrabutylammonium bromide (88 mg, 0.273 mmol) and phenol derivative (0.546 mmol) in dichloromethane (2 mL) at room temperature. The reaction was stirred until the TLC indicated complete disappearance of chloride. The reaction was then diluted with dichloromethane (10 mL) and washed with water, followed by brine. The organic layer was collected, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel chromatography with hexane/ethyl acetate combinations as eluent, to give the glycosylation product.

General procedure for glycosylation: Protocol D

1N aqueous NaOH solution (1 mL) was added into a solution of 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (200 mg, 0.487 mmol), benzyltriethylammonium chloride (111 mg, 0.0.487 mmol) and phenol derivative (0.975 mmol) in chloroform (2 mL) at room temperature. Stir the reaction solution at 60 °C temperature until the TLC indicates complete disappearance of starting material. Cool the reaction solution and dilute with the dichloromethane (10 mL) and washed with water followed by brine. The organic layer was collected, dried over Na_2SO_4 and concentrated under vacuo. The resulting residue was purified by silica gel chromatography with hexane/ethyl acetate combinations as eluent, to give the glycosylation product.

General procedure for deprotection: Protocol A

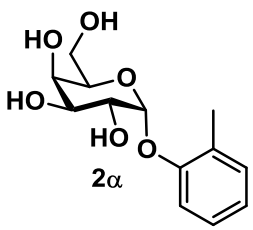
Acetyl esters were removed by dissolving the galactoside intermediate (0.5 mmol) in 20 mL of methanol, with a catalytic amount of sodium methoxide (0.02 M), and stirred overnight at room temperature. Upon completion, H^+ exchange resin (DOWEX 50WX4-100) was added to neutralize the mixture. The resin was filtered off and the filtrate was concentrated and then dried in vacuo, and the residue was purified by HPLC (C18, 15*150 mm column; eluent: acetonitrile/water (0.05% TFA).

General procedure for deprotection: Protocol B

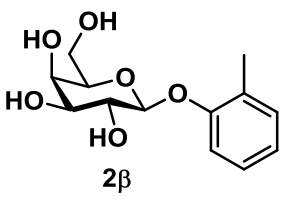
33% Wt. Methylamine in absolute ethanol solution (5 mL) was added to the galactoside intermediate (0.105 mmol), and the reaction was stirred at room temperature (0.5-1h) until TLC indicated complete disappearance of the staring material. Complete evaporation of the solvent provides the pure compound, which was further purified by HPLC (C18, 15*150 mm column; eluent: acetonitrile/water (0.05% TFA).

Compounds characterization data

2-methylphenyl α/β-D-galactopyranoside (2α/β).



2α

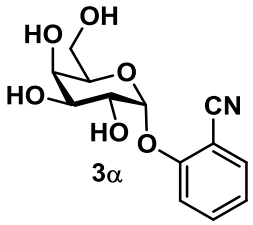


2β

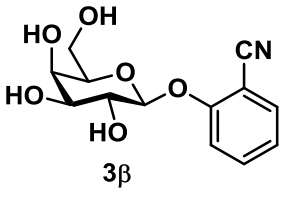
Step 1: Following glycosylation protocol A, β-D-galactose pentaacetate (0.100 g, 0.26 mmol) was coupled with 2-methylphenol (0.052 mL, 0.51 mmol) in 1,2-dichloroethane (rt for 10 h), to give glycosylation product, 2-methylphenyl 2,3,4,6-tetra-O-acetyl-α/β-D-galactopyranoside, in 14% yield (alpha); and 6% yield (beta); ESI-MS $[M+Na]^+$ calcd for $C_{21}H_{26}O_{10}Na^+$ 461.14, found 461.3 (**2α**) and 461.3 (**2β**).

Step 2: The acetates were removed via deprotection protocol A, to give the title compounds **(2α)**, and separately **(2β)**, each in quantitative yield. Analytical data for **2α**: ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 7.15 - 7.19 (m, 1H), 7.07 - 7.14 (m, 2H), 6.82 - 6.92 (m, 1H), 5.52 (d, *J* = 2.7 Hz, 1H), 3.97 - 4.01 (m, 3H), 3.93 (t, *J* = 6.1 Hz, 1H), 3.62 - 3.75 (m, 2H), 2.28 (s, 3H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 156.83, 131.81, 129.16, 127.97, 123.20, 116.45, 99.56, 73.29, 71.62, 70.99, 70.25, 62.53, 16.66; ESI-MS $[M+Na]^+$ calcd for $C_{13}H_{18}O_6Na^+$ 293.10, found 293.3. Analytical data for **2β**: ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 7.05 - 7.20 (m, 3H), 6.86 - 6.94 (m, 1H), 4.85 (d, Hz, 1H), 3.89 - 3.93 (m, 1H), 3.83 (dd, *J* = 9.8, 7.8 Hz, 1H), 3.76 (d, *J* = 2.0 Hz, 1H), 3.75 (s, 1H), 3.63 - 3.67 (m, 1H), 3.57 (dd, J = 9.8, 3.5 Hz, 1H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 157.43, 131.70, 129.03, 127.95, 123.26, 116.28, 103.28, 77.00. 75.21, 72.51, 70.37, 62.52, 16.66; ESI-MS $[M+Na]^+$ calcd for $C_{13}H_{18}O_6Na^+$ 293.10, found 293.3

2-cyanophenyl α/β-D-galactopyranoside (3α/β)



3α



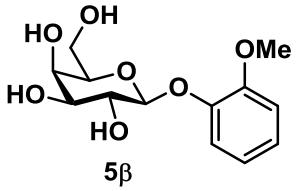
3β

Step 1: Following glycosylation protocol A, β-D-galactose pentaacetate (0.100 g, 0.26 mmol) was coupled with 2-cyanophenol (0.061 g, 0.51 mmol) in 1,2-dichloroethane (rt for 10 h), to give glycosylation product, 2-cyanophenyl 2,3,4,6-tetra-O-acetyl-α/β-D-galactopyranoside, in 10% yield (alpha) and 10% yield (beta); ESI-MS $[M+Na]^+$ calcd for $C_{21}H_{23}NO_{10}Na^+$ 472.12, found 472.3 (**3α**) and 472.3 (**3β**).

Step 2: The acetates were removed via deprotection protocol A, to give the title compounds **(3α)**, and separately **(3β)**, each in quantitative yield. Analytical data for **(3α)**: ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 7.56 - 7.66 (m, 2H), 7.42 (d, *J* = 8.2 Hz, 1H), 7.10 - 7.17 (m, 1H), 5.78 (d, *J* = 3.1 Hz, 1H), 4.03 - 4.10 (m, 2H), 4.00 - 4.03 (m, 1H), 3.93 (t, *J* = 6.1 Hz, 1H), 3.61 - 3.72 (m, 2H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 135.93, 134.72, 123.64, 117.10, 104.28, 99.78, 74.30, 71.32, 69.77, 62.49; ESI-MS $[M+H]^+$ calcd for $C_{13}H_{15}NO_6H^+$ 282.10, found 282.3. Analytical data for **(3β)**: ^{1}H NMR (400 MHz, dimethylsulfoxide-d_6) δ ppm 7.67 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.58 (ddd, *J* = 8.9, 7.3, 1.8 Hz, 1H), 7.27 (d, *J* = 8.6 Hz, 1H), 7.01 - 7.12 (m, 1H), 5.15 (d, *J* = 5.5 Hz, 1H), 5.04 (d, *J* = 7.8 Hz, 1H), 4.83 (d, *J* = 5.9 Hz, 1H), 4.58 (t, *J* = 5.5 Hz, 1H), 4.53 (d, *J* = 4.3 Hz, 1H), 3.63 - 3.67 (m, 1H), 3.52 - 3.61 (m, 1H), 3.32 – 3.51 (m, 4H); ^{13}C NMR (100 MHz,

dimethylsulfoxide-d_6) δ ppm 158.68, 134.84, 133.63, 122.00, 116.24, 115.02, 109.55, 101.16, 100.16, 75.73, 73.45, 69.98, 68.02, 60.24; ESI-MS $[M+H]^+$ calcd for $C_{13}H_{15}NO_6H^+$ 282.10, found 282.3

2-methoxyphenyl β-D-galactopyranoside (5β)

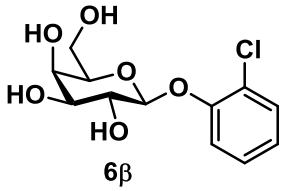


5β

Step 1: Following glycosylation protocol B, acetobromogalactose tetraacetate (0.100 g, 0.24 mmol) was coupled with guaiacol (0.090 g, 0.73 mmol) in 1,2-dichloroethane (40 ºC for 1 h), to give glycosylation product, 2-cyanophenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside, in 16% yield; ESI-MS $[M+Na]^+$ calcd for $C_{21}H_{26}O_{11}Na^+$ 477.14, found 477.3.

Step 2: The acetates were removed via deprotection protocol A, to give the title compound **(5β)** in quantitative yield. Analytical data for **(5β)**: ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 7.18 (d, *J* = 7.4 Hz, 1H), 6.97 - 7.01 (m, 2H), 6.86 - 6.91 (m, 1H), 4.85 (d, 1H), 3.89 (d, *J* = 3.5 Hz, 1H), 3.85 (s, 3H), 3.81 - 3.84 (m, 1H), 3.76 (d, *J* = 2.0 Hz, 1H), 3.74 (s, 1H), 3.62 - 3.66 (m, 1H), 3.58 (dd, *J* = 9.8, 3.5 Hz, 1H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 151.08, 148.33, 124.19, 122.43, 118.37, 103.66, 77.14, 74.90, 72.46, 70.32, 62.51, 56.85; ESI-MS $[M+Na]^+$ calcd for $C_{13}H_{18}O_7Na^+$ 309.10, found 309.3

2-chlorophenyl β-D-galactopyranoside (6β)

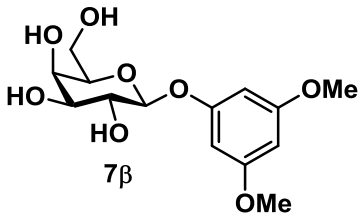


6β

Step 1: Following glycosylation protocol B, acetobromogalactose tetraacetate (0.100 g, 0.24 mmol) was coupled with 2-chlorophenol (0.074 mL, 0.73 mmol) in 1,2-dichloroethane (40ºC for 1 h), to give the glycosylation product, 2-chlorophenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside, in 23% yield; ESI-MS $[M+Na]^+$ calcd for $C_{20}H_{23}ClO_{10}Na^+$ 481.09, found 481.3.

Step 2: The acetates were removed via deprotection protocol A, to give the title compound **(6β)** in quantitative yield. Analytical data for **(6β)**: ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 7.32 - 7.38 (m, 1H), 7.19 - 7.29 (m, 2H), 6.93 - 7.02 (m, 1H), 4.95 (d, *J* = 7.4 Hz, 1H), 3.92 (d, *J* = 3.5 Hz, 1H), 3.88 (dd, *J* = 9.4, 7.8 Hz, 1H), 3.72 - 3.82 (m, 2H), 3.66 - 3.71 (m, 1H), 3.59 (dd, *J* = 9.4, 3.1 Hz, 1H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 154.55, 131.23, 128.98, 124.57, 124.05, 118.05, 102.91, 77.15, 75.02, 72.22, 70.23, 62.43; ESI-MS $[M+Na]^+$ calcd for $C_{42}H_{43}N_3O_6Na^+$ 313.05, found 313.2

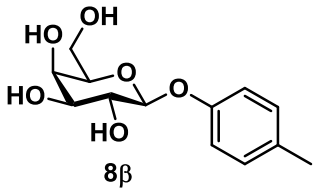
3,5-dimethoxyphenyl β-D-galactopyranoside (7β)



7β

Step 1: Following glycosylation protocol B, acetobromogalactose tetraacetate (0.100 g, 0.24 mmol) was coupled with 3,5-dimethoxyphenol (0.113 g, 0.73 mmol) in 1,2-dichloroethane (40ºC for 1 h), to give the glycosylation product, 3,5-dimethoxyphenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside, in 8% yield; ESI-MS $[M+Na]^+$ calcd for $C_{22}H_{28}O_{12}Na^+$ 507.15, found 507.3. Step 2: The acetates were removed via deprotection protocol A, to give the title compound **(7β)** in quantitative yield. Analytical data for **(7β)**: ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 6.32 (d, J = 2.0 Hz, 2H), 6.15 (t, J = 2.2 Hz, 1H), 4.81 (d, J = 7.8 Hz, 1H), 3.88 (d, J = 3.1 Hz, 1H), 3.71 - 3.82 (m, 9H), 3.65 - 3.70 (m, 1H), 3.57 (dd, J = 9.6, 3.3 Hz, 1H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 162.99, 161.20, 103.18, 96.66, 95.83, 77.27, 75.03, 72.41, 70.39, 65.52, 55.91; ESI-MS $[M+Na]^+$ calcd for $C_{14}H_{20}O_8Na^+$ 339.11, found 339.3

4-methylphenyl β-D-galactopyranoside (8β)

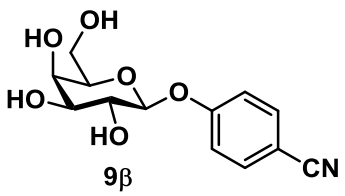


8β

Step 1: Following glycosylation protocol B, acetobromogalactose tetraacetate (0.100 g, 0.24 mmol) was coupled with *p*-cresol (0.077 mL, 0.73 mmol) in 1,2-dichloroethane (40 ºC for 1 h), to give the glycosylation product, 4-methylphenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside, in 3% yield; ESI-MS $[M+Na]^+$ calcd for $C_{21}H_{26}O_{10}Na^+$ 461.14, found 461.3.

Step 2: The acetates were removed via deprotection protocol A, to give the title compound **(8β)** in quantitative yield. Analytical data for **(8β)**: ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 7.04 - 7.09 (m, 2H), 6.96 - 7.01 (m, 2H), 4.79 (d, J = 7.8 Hz, 1H), 3.88 - 3.91 (m, 1H), 3.71 - 3.80 (m, 3H), 3.62 - 3.67 (m, 1H), 3.54 - 3.59 (m, 1H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 130.87, 117.91, 103.42, 77.04, 75.03, 72.48, 70.36, 62.55, 20.75; ESI-MS $[M+Na]^+$ calcd for $C_{13}H_{18}O_6Na^+$ 293.10, found 293.2

4-cyanophenyl β-D-galactopyranoside (9β)



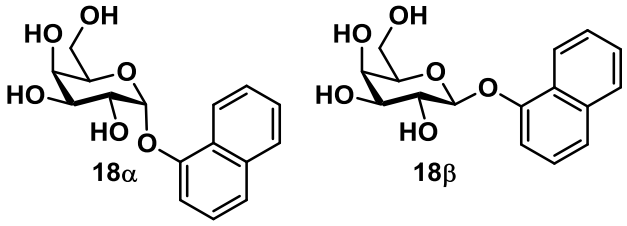
9β

Step 1: Following glycosylation protocol B, acetobromogalactose tetraacetate (0.100 g, 0.24 mmol) was coupled with 4-cyanophenol (0.087 g, 0.73 mmol) in 1,2-dichloroethane (40ºC for 1 h), to give the

glycosylation product, 4-cyanophenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside, in 16% yield; ESI-MS $[M+Na]^+$ calcd for $C_{21}H_{23}NO_{10}Na^+$ 472.12, found 472.3.

Step 2: The acetates were removed via deprotection protocol A, to give the title compound **(9β)** in quantitative yield. Analytical data for **(9β)**: ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 7.66 (d, J = 9.0 Hz, 2H), 7.23 (d, J = 8.6 Hz, 2H), 4.97 (d, J = 7.8 Hz, 1H), 3.91 (d, J = 3.5 Hz, 1H), 3.70 - 3.86 (m, 4H), 3.60 (dd, J = 9.8, 3.5 Hz, 1H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 162.65, 135.20, 118.61, 102.35, 77.37, 74.88, 72.16, 70.31, 62.55; ESI-MS $[M+H]^+$ calcd for $C_{13}H_{15}NO_6H^+$ 282.10, found 282.2

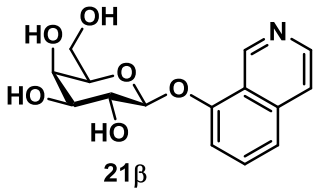
1-naphthalenyl α/β-D-galactopyranoside (18α/β)



Step 1: Following glycosylation protocol A, β-D-galactose pentaacetate (0.100 g, 0.26 mmol) was coupled with 1-naphthol (0.074 g, 0.51 mmol) in CH_2Cl_2 (rt for 48 h), to give glycosylation product, 1-naphthalenyl 2,3,4,6-tetra-O-acetyl-α/β-D-galactopyranoside, in 14% yield (alpha), and 40% yield (beta); ESI-MS $[M+Na]^+$ calcd for $C_{24}H_{26}O_{10}Na^+$ 497.14, found 497.3 (**18α**) and 497.3 (**18β**).

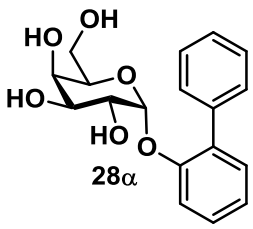
Step 2: The acetates were removed via deprotection protocol A, to give the title compounds **(18α)**, and separately **(18β)**, each in quantitative yield. Analytical data for **(18α)**: ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 8.38 (ddt, J = 6.3, 3.6, 0.8 Hz, 1H), 7.83 – 7.77 (m, 1H), 7.53 – 7.27 (m, 5H), 5.72 (d, J = 3.7 Hz, 1H), 4.20 – 4.05 (m, 2H), 4.04 (dd, J = 3.4, 1.3 Hz, 1H), 3.98 (td, J = 6.1, 5.6, 1.3 Hz, 1H), 3.75 – 3.64 (m, 2H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 152.57, 134.06, 127.39, 126.31, 126.16, 125.26, 122.12, 121.06, 109.37, 98.34, 72.54, 69.63, 68.56, 68.20, 60.26; ESI-MS $[M+Na]^+$ calcd for $C_{16}H_{18}O_6Na^+$ 329.10, found 329.3. Analytical data for **(18β)**: ^{1}H NMR (400 MHz, dimethylsulfoxide-d_6) δ ppm 8.43 – 8.36 (m, 1H), 7.83 – 7.76 (m, 1H), 7.53 – 7.41 (m, 3H), 7.37 (t, J = 8.0 Hz, 1H), 7.22 (dd, J = 7.7, 0.9 Hz, 1H), 5.07 (d, J = 7.8 Hz, 1H), 4.02 – 3.92 (m, 2H), 3.84 – 3.70 (m, 3H), 3.63 (dd, J = 9.7, 3.4 Hz, 1H); ^{13}C NMR (100 MHz, dimethylsulfoxide-d_6) δ ppm 153.09, 133.98, 127.32, 126.33, 125.22, 122.17, 121.18, 108.96, 101.70, 75.65, 73.24, 70.47, 68.21, 60.45; ESI-MS $[M+Na]^+$ calcd for $C_{16}H_{18}O_6Na^+$ 329.10, found 329.3.

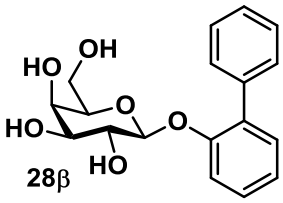
8-isoquinolinyl β-D-galactopyranoside (21β)



Step 1: Following glycosylation protocol D, 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (200 mg, 0.487 mmol), was coupled with 8-hydroxy isoquinoline (141.53 mg, 0.975 mmol), to give glycosylation product, 8-isoquinolinyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside, in 86% yield; ESI-MS $[M+H]^+$ calcd for $C_{23}H_{25}NO_{10}H^+$ 476.16, found 476.3 Step 2: The acetates were removed via deprotection protocol B, to give the title compound **(21β)** in 87% yield. Analytical data for **(21β)**: ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 9.69 (s, 1H), 8.44 (d, *J* = 5.5 Hz, 2H), 7.89 - 7.90 (m, 1H), 7.68 - 7.81 (m, 2H), 7.57 (d, *J* = 8.2 Hz, 1H), 7.40 (d, *J* = 7.8 Hz, 1H), 5.16 (d, *J* = 7.8 Hz, 1H), 3.99 - 4.07 (m, 1H), 3.97 (d, *J* = 3.5 Hz, 1H), 3.76 - 3.85 (m, 3H), 3.67 (dd, *J* = 9.8, 3.1 Hz, 1H); ^{13}C NMR (100 MHz, dimethylsulfoxide-d_6) δ ppm 152.79, 149.20, 139.81, 136.11, 129.07, 126.80, 121.83, 121.31, 113.69, 101.57, 75.67, 73.53, 70.44, 68.16, 60.40; ESI-MS $[M+H]^+$ calcd for $C_{15}H_{17}NO_6H^+$ 308.11, found 308.3.

[1,1'-biphenyl]-2-yl α/β-D-galactopyranoside (28α/β)

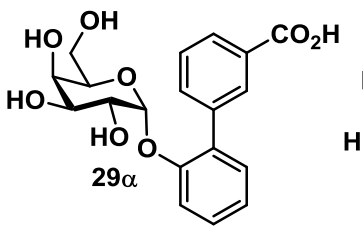


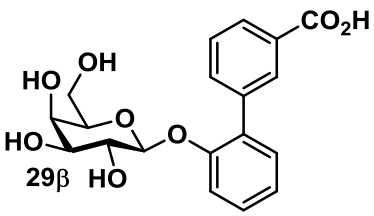


Step 1: Following glycosylation protocol A, β-D-galactose pentaacetate (0.100 g, 0.26 mmol) was coupled with 2-phenylphenol (0.087 g, 0.51 mmol) in CH_2Cl_2 (rt for 48 h), to give glycosylation product, [1,1'-biphenyl]-2-yl 2,3,4,6-tetra-O-acetyl-α/β-D-galactopyranoside, in 38% yield (alpha) and 15% yield (beta); ESI-MS $[M+Na]^+$ calcd for $C_{26}H_{28}O_{10}Na^+$ 523.16, found 523.4 (**28α**) and 523.4 (**28β**).

Step 2: The acetates were removed via deprotection protocol A, to give the title compounds (**28α**), and separately **(28β)**, each in quantitative yield. Analytical data for **(28α)**: ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 7.60 – 7.54 (m, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.36 – 7.28 (m, 4H), 7.09 (m, 1H), 5.61 (d, *J* = 3.7 Hz, 1H), 3.90 (m, 1H), 3.80 (d, *J* = 3.4 Hz, 1H), 3.70 (m, 1H), 3.61 – 3.47 (m, 3H); ESI-MS $[M+Na]^+$ calcd for $C_{18}H_{20}O_6Na^+$ 355.12, found 355.3. Analytical data for **(28β)**: ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 7.62 – 7.58 (m, 2H), 7.40 – 7.34 (m, 2H), 7.31 – 7.25 (m, 4H), 7.07 (m, 1H), 5.00 (d, *J* = 7.7 Hz, 1H), 3.88 (dd, *J* = 3.3, 1.0 Hz, 1H), 3.79 – 3.65 (m, 4H), 3.54 (dd, *J* = 9.6, 3.4 Hz, 1H); ESI-MS $[M+Na]^+$ calcd for $C_{18}H_{20}O_6Na^+$ 355.12, found 355.3.

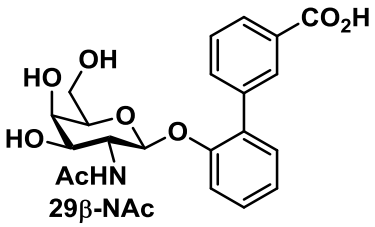
2'-(α/β-D-galactopyranosyloxy)-[1,1'-Biphenyl]-3-carboxylic acid (29α/β)





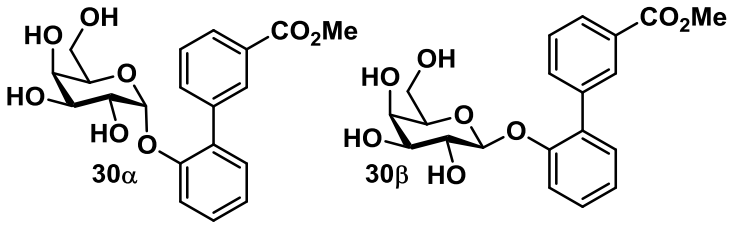
Compound **30α** (38 mg, 0.097 mmol) and separately **30β** (38 mg, 0.097 mmol) were each dissolved in MeOH (2 mL) at rt. Then, [0.5 M] NaOH aq. (2 mL) was added dropwise, and the reactions were monitored by LCMS. After 24 h, the reactions were acidified with [0.2 N] HCl aq. to a pH of ~3-4. The solvents were then evaporated under reduced pressure, and the compounds were purified by HPLC (C18, 15*150 mm column; eluent: acetonitrile/water (0.05% TFA) to give the title compounds **(29α)** in 5% yield, and separately **(29β)** in 88% yield. Analytical data for **(29α)**: ^{1}H NMR (300 MHz, methanol-d_4) δ ppm 8.22 (d, *J* = 1.6 Hz, 1H), 7.84 - 8.02 (m, 2H), 7.53 (td, *J* = 7.7, 4.5 Hz, 1H), 7.29 - 7.40 (m, 3H), 7.06 - 7.16 (m, 1H), 5.63 - 5.69 (m, 1H), 3.89 - 3.96 (m, 1H), 3.85 (br. s., 1H), 3.72 - 3.78 (m, 1H), 3.54 - 3.67 (m, 3H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 170.40, 155.42, 140.22, 135.43, 132.12, 131.79, 129.37, 123.68, 116.91, 99.68, 73.59, 71.49, 70.94, 70.15, 62.52; ESI-MS $[M+Na]^+$ calcd for $C_{19}H_{20}O_8Na^+$ 399.11, found 399.6. Analytical data for **(29β)**: ^{1}H NMR (300 MHz, methanol-d_4) δ ppm 8.16 (s, 1H), 7.87 (d, *J* = 7.8 Hz, 1H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.41 (t, *J* = 7.8 Hz, 1H), 7.19 - 7.30 (m, 3H), 6.98 - 7.06 (m, 1H), 4.94 (d, *J* = 7.8 Hz, 1H), 3.80 (d, *J* = 3.1 Hz, 1H), 3.55 - 3.72 (m, 4H), 3.48 (dd, *J* = 9.6, 3.3 Hz, 1H); ^{13}C NMR (100 MHz, methanol-d_4) δ 170.29, 155.87, 140.28, 135.75, 132.16, 131.81, 129.24, 123.70, 116.76, 102.70, 77.14, 75.35, 72.41, 70.39, 62.52; ESI-MS $[M+Na]^+$ calcd for $C_{19}H_{20}O_8Na^+$ 399.11, found 399.6.

2'-(2-acetamido-2-deoxy-β-D-galactopyranosyloxy)-[1,1'-Biphenyl]-3-carboxylic acid (29β-NAc)



NaOH (79 mg, 1.97 mmol) was added into a solution of **30β-NAc** (110 mg, 0.197 mmol) in 50% water in methanol (10 mL) at room temperature. The reaction was stirred at the same temperature (15h) until the TLC indicated complete disappearance of the staring material. The reaction solution was neutralized with 6N aqueous HCl and the MeOH was evaporated in vacuo. The aqueous solution was adjusted to a pH~2 with 6N aqueous HCl and the product was extracted with ethyl acetate (3x 10mL). The organic layers were combined and washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography with dichloromethane/methanol combinations as eluent provide the title compound (**29β-NAc**) in 67% yield. Analytical data for **(29β-NAc)**: ^{1}H NMR (300 MHz, methanol-d_4) δ ppm 8.00 (s, 1H), 7.87 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 7.4 Hz, 1H), 7.35 - 7.42 (m, 1H), 7.21 - 7.29 (m, 3H), 6.98 - 7.07 (m, 1H), 5.01 (d, *J* = 8.6 Hz, 1H), 3.94 (dd, *J* = 10.6, 8.6 Hz, 1H), 3.78 (d, *J* = 3.1 Hz, 1H), 3.64 - 3.76 (m, 2H), 3.54 - 3.61 (m, 2H), 1.55 (s, 3H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 173.74, 169.88, 155.92, 139.84, 135.54, 131.65, 130.26, 129.11, 123.72, 116.81, 101.15, 77.18, 101.15, 77.18, 73.18, 69.63, 62.42, 54.22, 22.62; ESI-MS $[M+Na]^+$ calcd for $C_{21}H_{23}NO_8Na^+$ 440.13, found 440.3

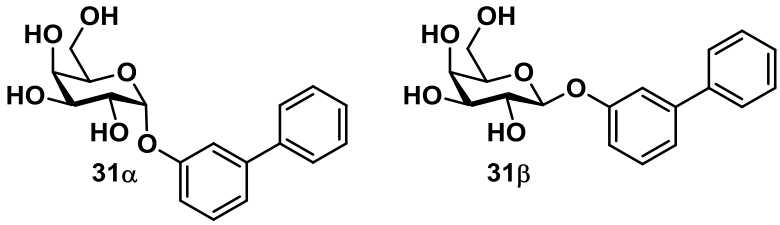
2'-(α/β-D-galactopyranosyloxy)-[1,1'-Biphenyl]-3-carboxylic acid methyl ester (30α/β)



Step 1: Following glycosylation protocol A, β-D-galactose pentaacetate (0.200 g, 0.51 mmol) was coupled with 2'-hydroxy[1,1'biphenyl]-3-carboxylic acid methyl ester (0.234 g, 1.02 mmol) in CH_2Cl_2 (rt for 1.5 h), to give glycosylation product, 2'-(2,3,4,6-tetra-O-acetyl-α/β-D-galactopyranosyloxy)-[1,1'-Biphenyl]-3-carboxylic acid methyl ester, in 5% yield (alpha) and 18% yield (beta); ESI-MS $[M+Na]^+$ calcd for $C_{28}H_{30}O_{12}Na^+$ 581.16, found 581.4 (**30α**) and 581.4 (**30β**).

Step 2: The acetates were removed via deprotection protocol A, to give the title compounds **(30α)**, and separately **(30β)**, each in quantitative yield. Analytical data for **(30α)**: ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.23 (m, 1H), 7.97 (m, 1H), 7.86 (m, 1H), 7.53 (tt, *J* = 7.7, 0.7 Hz, 1H), 7.39 – 7.31 (m, 3H), 7.11 (m, 1H), 5.65 (d, *J* = 3.7 Hz, 1H), 3.95 – 3.90 (m, 4H), 3.85 (d, *J* = 3.4 Hz, 1H), 3.73 (dd, *J* = 10.1, 3.4 Hz, 1H), 3.66 – 3.52 (m, 4H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 135.68, 131.95, 131.76, 129.12, 123.67, 116.88, 99.64, 73.62, 71.52, 70.88, 70.11, 62.45, 52.85; ESI-MS $[M+Na]^+$ calcd for $C_{20}H_{22}O_8Na^+$ 413.12, found 413.3. Analytical data for **(30β)**: ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.24 (m, 1H), 7.92 (m, 1.0 Hz, 2H), 7.50 (td, *J* = 7.8, 0.6 Hz, 1H), 7.37 – 7.30 (m, 3H), 7.14 – 7.08 (m, 1H), 5.03 (d, *J* = 7.7 Hz, 1H), 3.92 (d, *J* = 0.8 Hz, 3H), 3.88 (dd, *J* = 3.5, 0.9 Hz, 1H), 3.80 – 3.66 (m, 4H), 3.56 (ddd, *J* = 9.6, 3.4, 0.7 Hz, 1H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 135.90, 131.96, 130.42, 129.34, 128.97, 123.71, 116.81, 102.70, 77.16, 75.37, 72.41, 70.38, 62.52, 52.83; ESI-MS $[M+Na]^+$ calcd for $C_{20}H_{22}O_8Na^+$ 413.12, found 413.3.

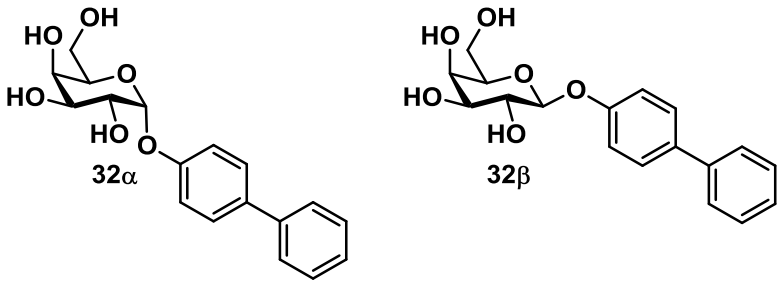
[1,1'-biphenyl]-3-yl α/β-D-galactopyranoside (31α/β)



Step 1: Following glycosylation protocol A, β-D-galactose pentaacetate (0.100 g, 0.26 mmol) was coupled with 3-phenylphenol (0.087 g, 0.51 mmol) in CH_2Cl_2 (rt for 72 h), to give glycosylation product, [1,1'-biphenyl]-3-yl 2,3,4,6-tetra-O-acetyl-α/β-D-galactopyranoside, in 36% yield (**31α**) and 23% yield (**31β**). Analytical data for [1, 1'-biphenyl]-3-yl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside: ESI-MS $[M+Na]^+$ calcd for $C_{26}H_{28}O_{10}Na^+$ 523.16, found 523.4. Analytical data for [1,1'-biphenyl]-3-yl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside: ESI-MS $[M+Na]^+$ calcd for $C_{26}H_{28}O_{10}Na^+$ 523.16, found 523.3.

Step 2: The acetates were removed via deprotection protocol A, to give the title compounds (**31α**), and separately (**31β**), each in quantitative yield. Analytical data for (**31α**): ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 7.49 (m, 2H), 7.35 – 7.13 (m, 6H), 7.06 (m, 1H), 5.46 (d, *J* = 2.7 Hz, 1H), 3.94 – 3.83 (m, 4H), 3.66 – 3.55 (m, 2H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 159.41, 144.11, 142.27, 130.95, 129.94, 128.17, 122.23, 117.32, 100.04, 73.30, 71.54, 71.00, 70.18, 62.61; ESI-MS $[M+Na]^+$ calcd for $C_{18}H_{20}O_6Na^+$ 355.12, found 355.3. Analytical data for (**31β**): ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 7.61 – 7.56 (m, 2H), 7.42 – 7.22 (m, 6H), 7.08 (m, 1H), 4.91 (d, *J* = 7.7 Hz, 1H), 3.89 (dd, *J* = 3.5, 0.9 Hz, 1H), 3.84 – 3.66 (m, 4H), 3.58 (dd, *J* = 9.7, 3.4 Hz, 1H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 159.80, 144.05, 142.26, 130.92, 129.96, 128.15, 122.13, 116.88, 116.63, 103.18, 77.23, 75.02, 72.48, 70.42, 62.42; ESI-MS $[M+Na]^+$ calcd for $C_{18}H_{20}O_6Na^+$ 355.12, found 355.3.

[1,1'-biphenyl]-4-yl α/β-D-galactopyranoside (32α/β)

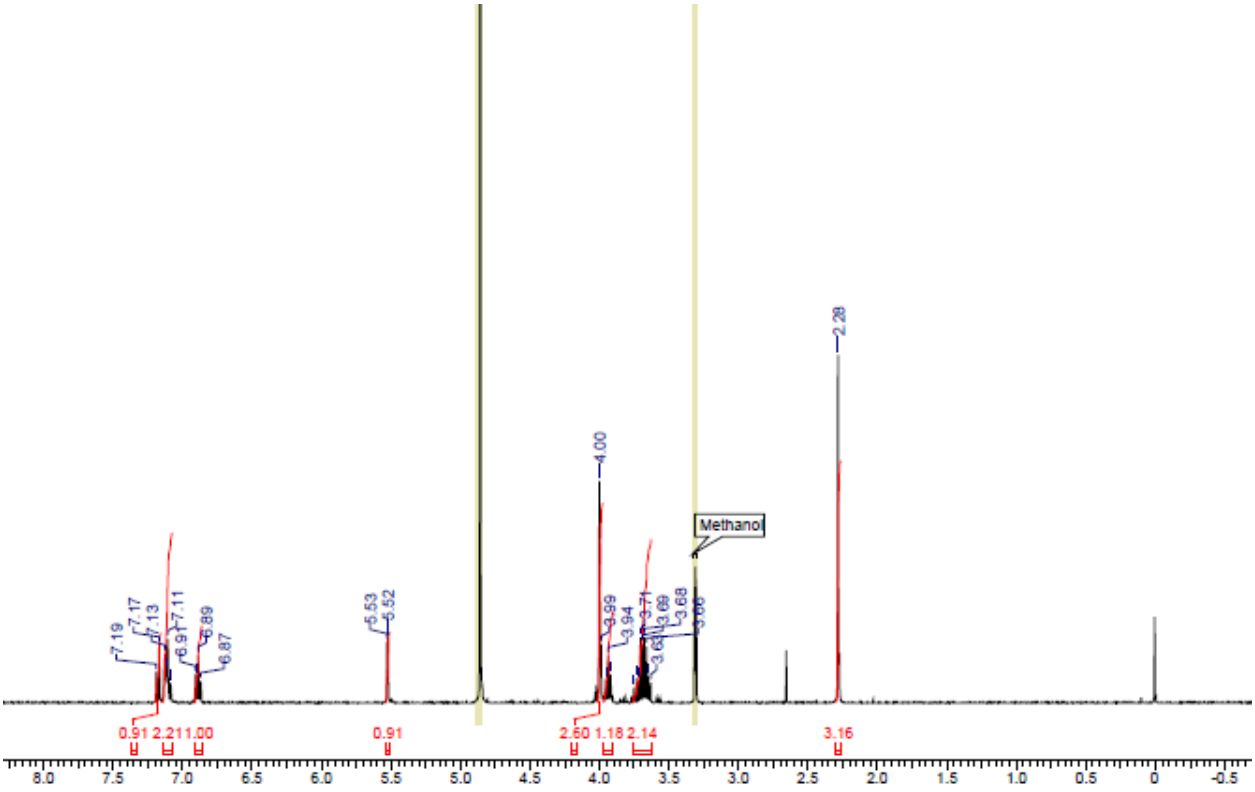


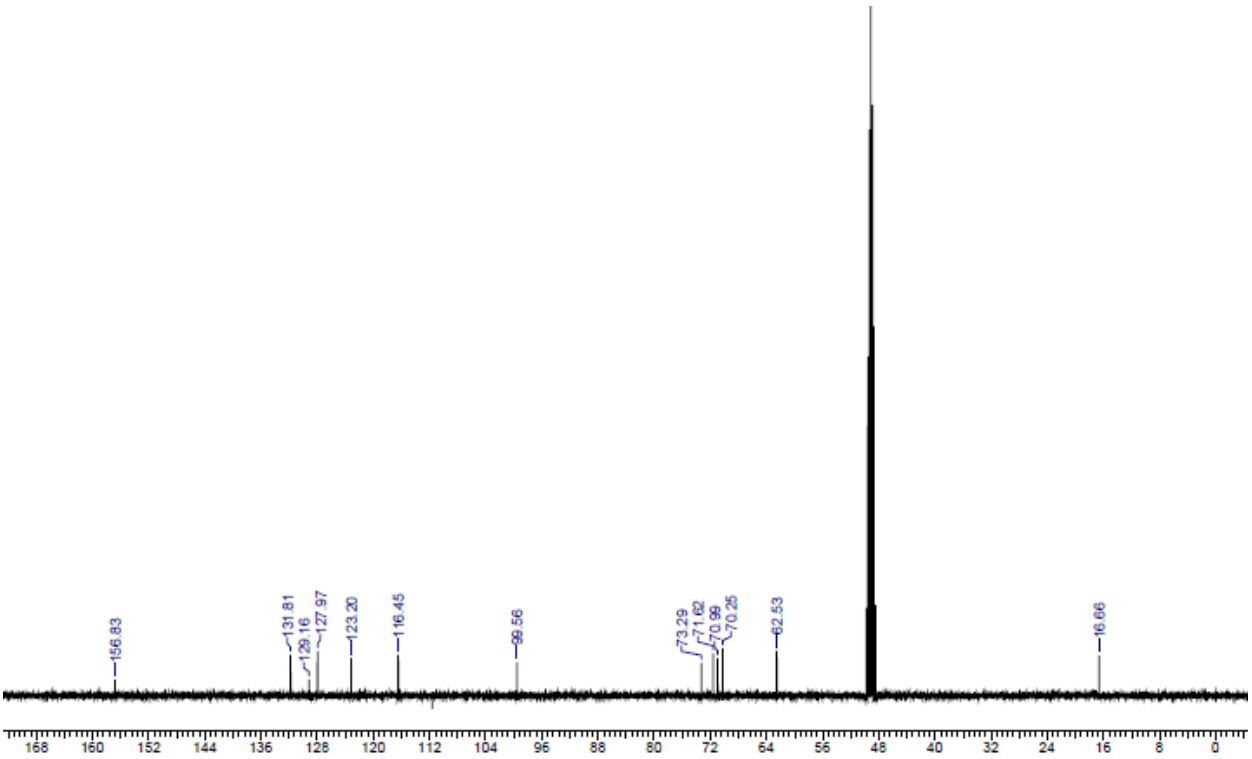
Step 1: Following glycosylation protocol A, β-D-galactose pentaacetate (0.100 g, 0.26 mmol) was coupled with 4-phenylphenol (0.087 g, 0.51 mmol) in CH_2Cl_2 (rt for 72 h), to give glycosylation product, [1,1'-biphenyl]-4-yl 2,3,4,6-tetra-O-acetyl-α/β-D-galactopyranoside, in 26% yield (alpha) and 14% yield (beta). Analytical data for [1,1'-biphenyl]-4-yl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside: ESI-MS $[M+Na]^+$ calcd for $C_{26}H_{28}O_{10}Na^+$ 523.16, found 523.3. Analytical data for [1,1'-biphenyl]-4-yl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside: ESI-MS $[M+Na]^+$ calcd for $C_{26}H_{28}O_{10}Na^+$ 523.16, found 523.3.

Step 2: The acetates were removed via deprotection protocol A, to give the title compounds **(32α)**, and separately **(32β)**, each in quantitative yield. Analytical data for **(32α)**: ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 7.59 – 7.49 (m, 4H), 7.44 – 7.36 (m, 2H), 7.31 – 7.19 (m, 3H), 5.54 (d, *J* = 2.7 Hz, 1H), 4.03-3.92 (m, 4H), 3.70 (dd, *J* = 6.3 Hz, 2H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 135.68, 131.95, 131.76, 129.53, 129.12, 123.67, 116.88, 99.64, 73.62, 71.52, 70.88, 70.11, 62.45, 52.85; ESI-MS $[M+Na]^+$ calcd for $C_{18}H_{20}O_6Na^+$ 355.12, found 355.3. Analytical data for **(32β)**: ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 7.61 – 7.56 (m, 2H), 7.42 – 7.22 (m, 6H), 7.08 (m, 1H), 4.91 (d, *J* = 7.7 Hz, 1H), 3.89 (dd, *J* = 3.5, 0.9 Hz, 1H), 3.84 – 3.66 (m, 4H), 3.58 (dd, *J* = 9.7, 3.4 Hz, 1H); ^{13}C NMR (100 MHz, Methanol-d_4) δ ppm 135.90, 131.96, 130.42, 129.34, 128.97, 123.71, 116.71, 102.70, 77.16, 75.37, 72.41, 70.38, 62.52, 52.83; ESI-MS $[M+Na]^+$ calcd for $C_{18}H_{20}O_6Na^+$ 355.12, found 355.3.

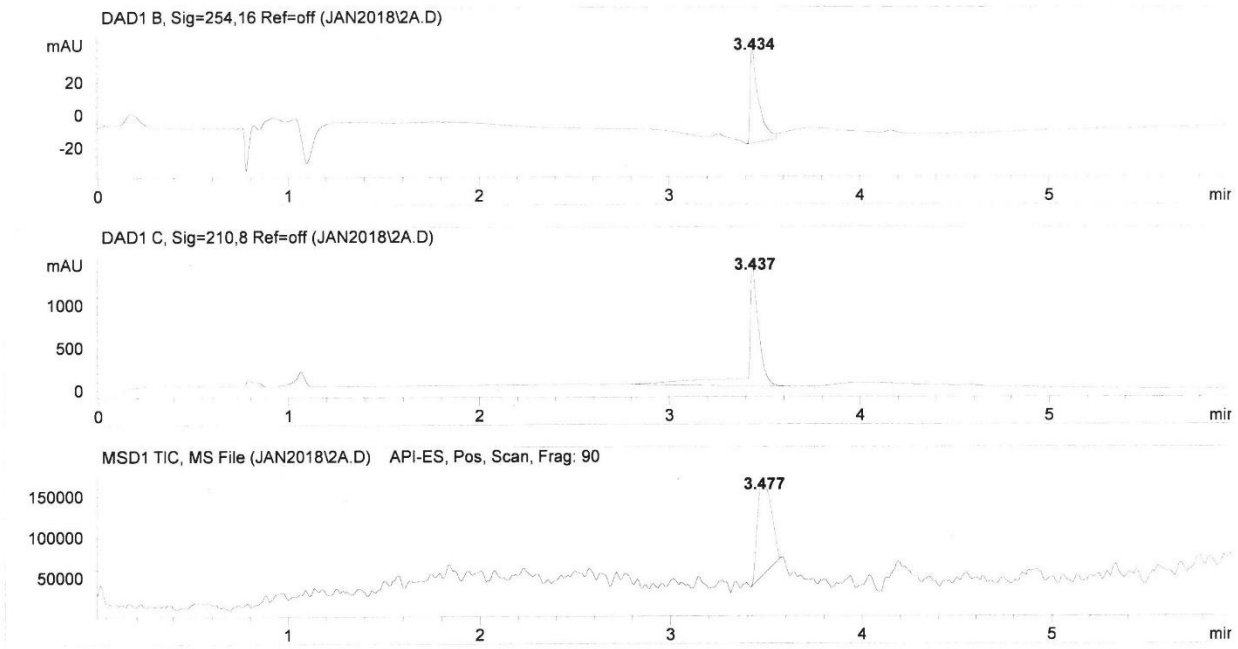
^{1}H, ^{13}C and LCMS Spectral data

^{1}H &^{13}C NMR spectrum of compound **2a**





LCMS spectrum of compound **2a**



DAD1 B, Sig=254,16 Ref=off

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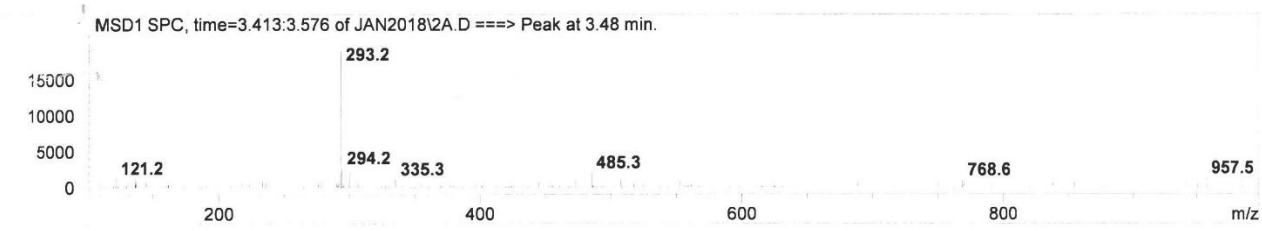
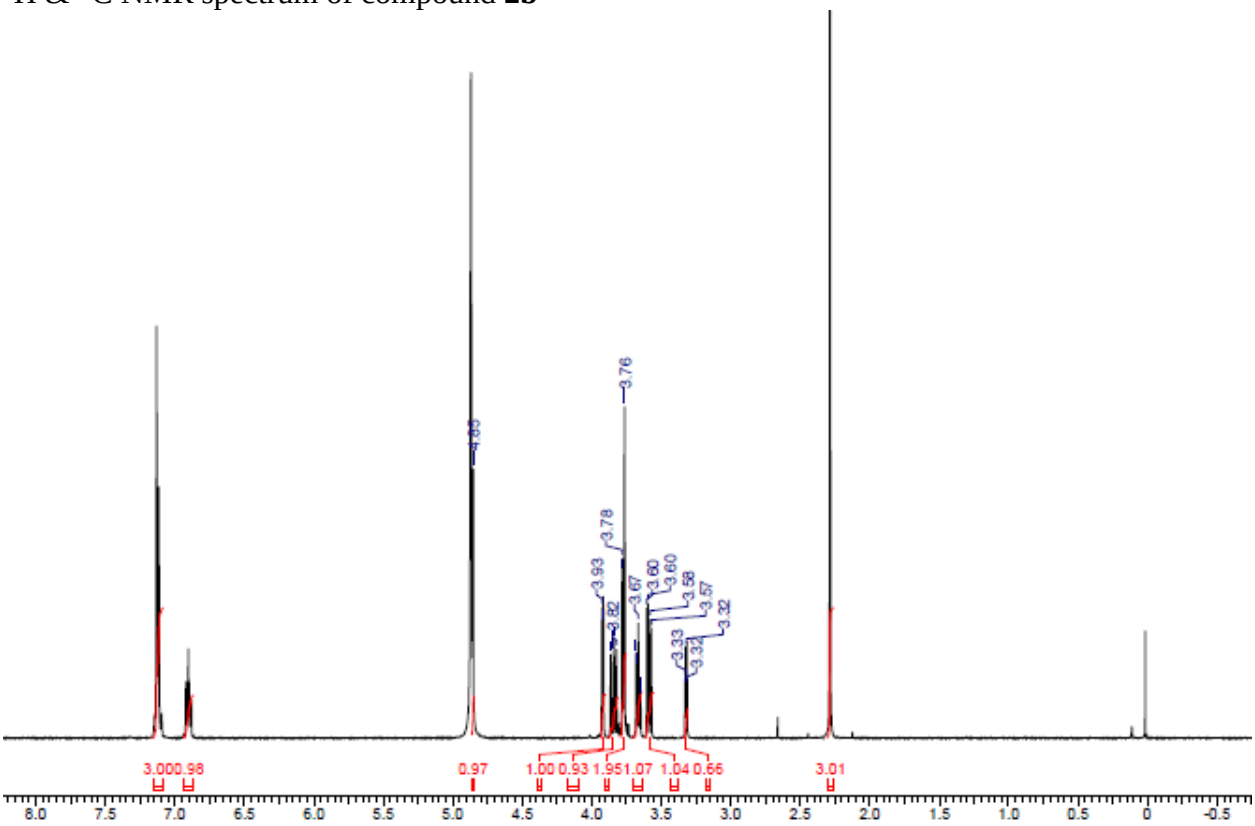
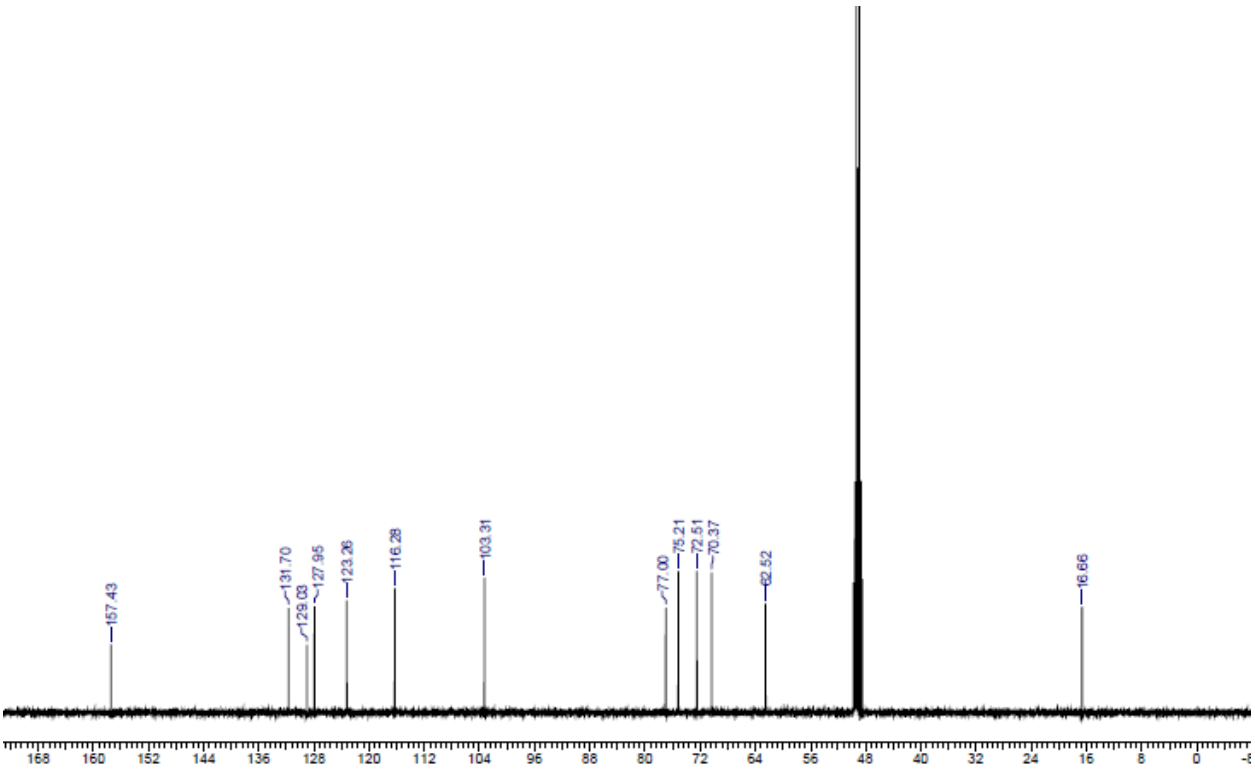
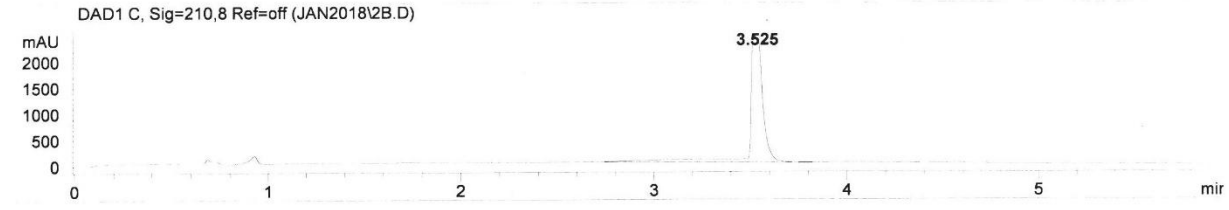


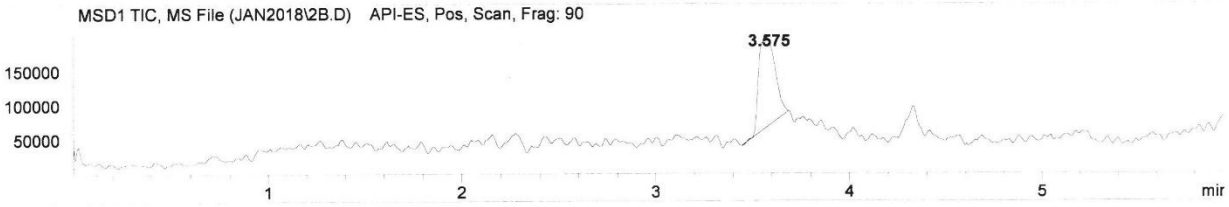
^{1}H &^{13}C NMR spectrum of compound **2b**





LCMS spectrum of compound **2b**





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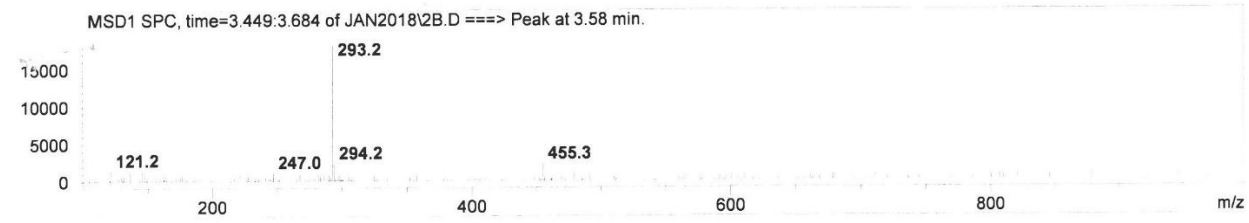
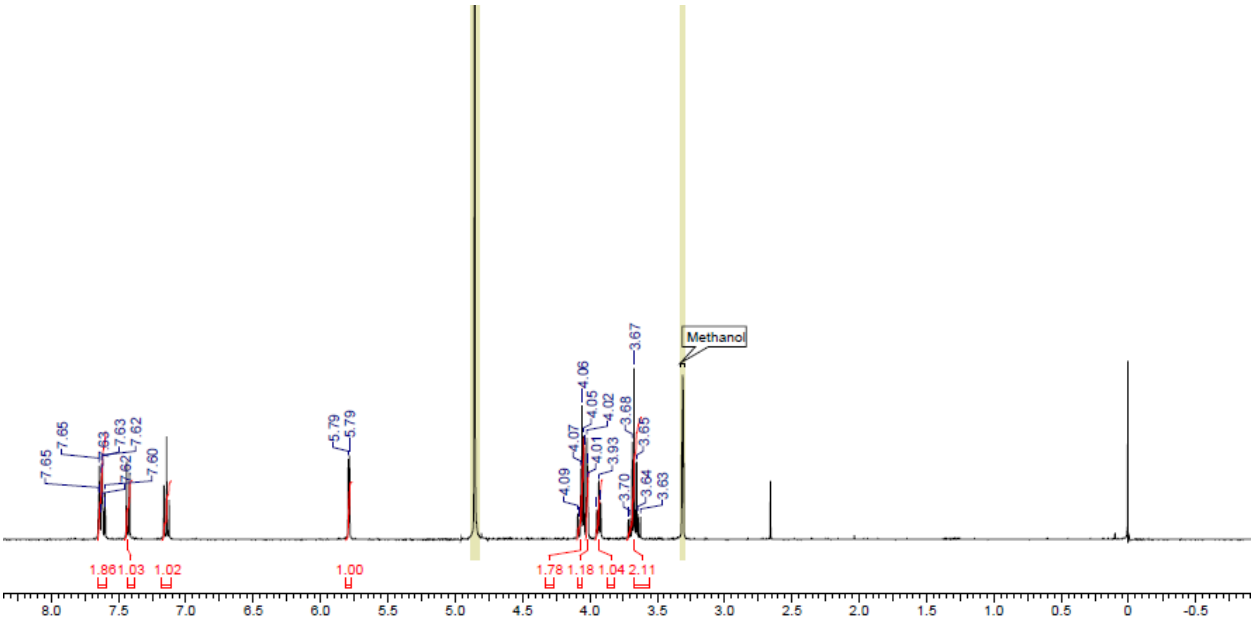
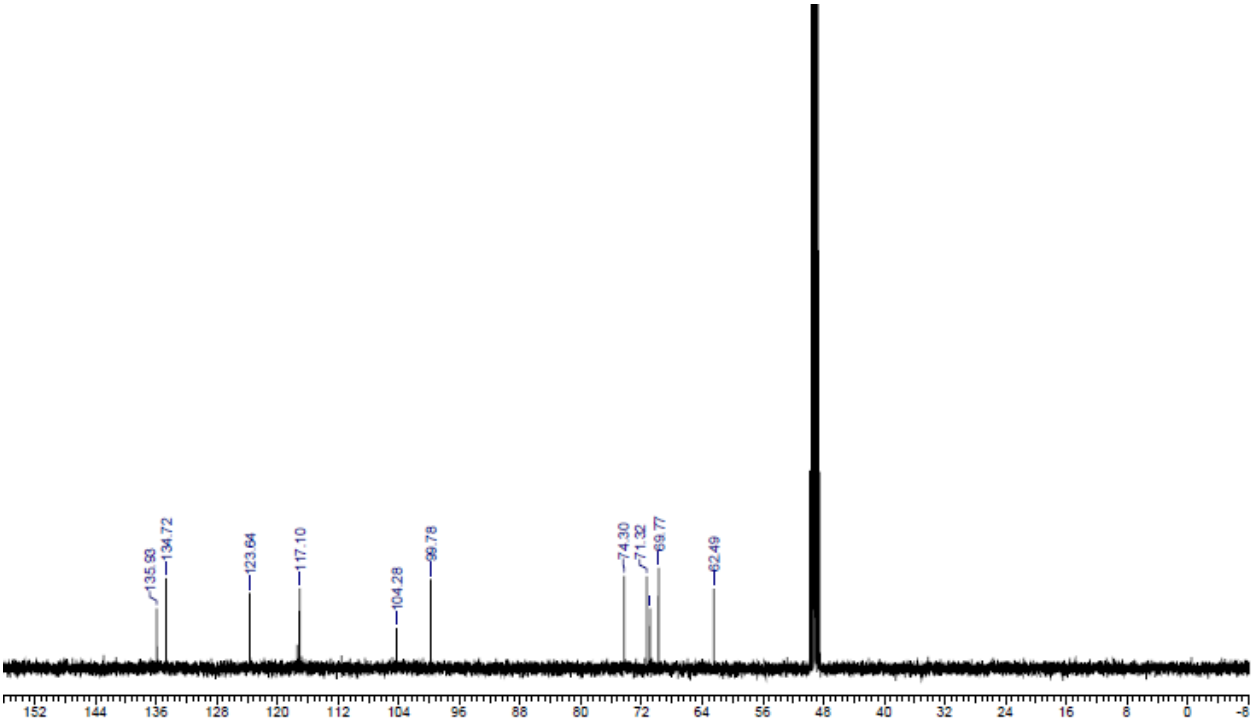
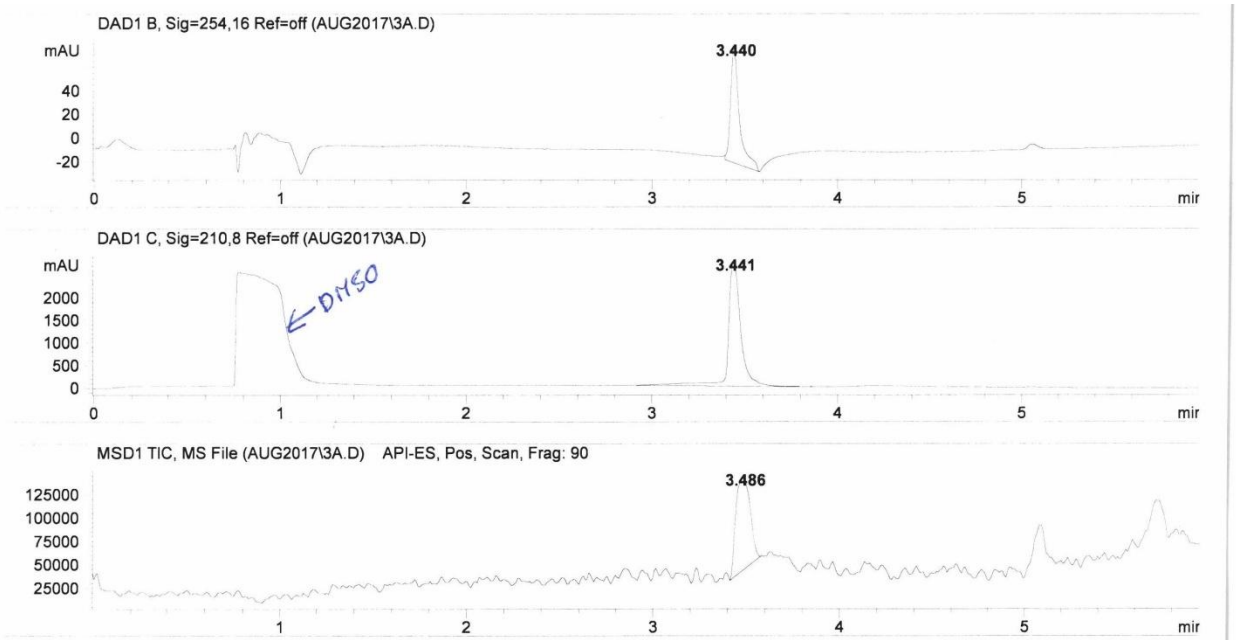


^{1}H &^{13}C NMR spectrum of compound **3a**



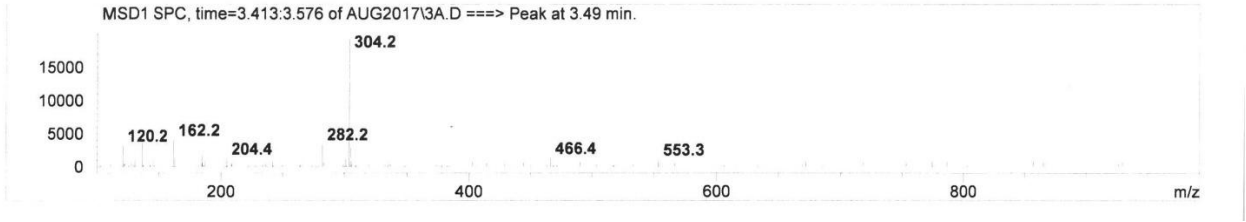


LCMS spectrum of compound **3a**

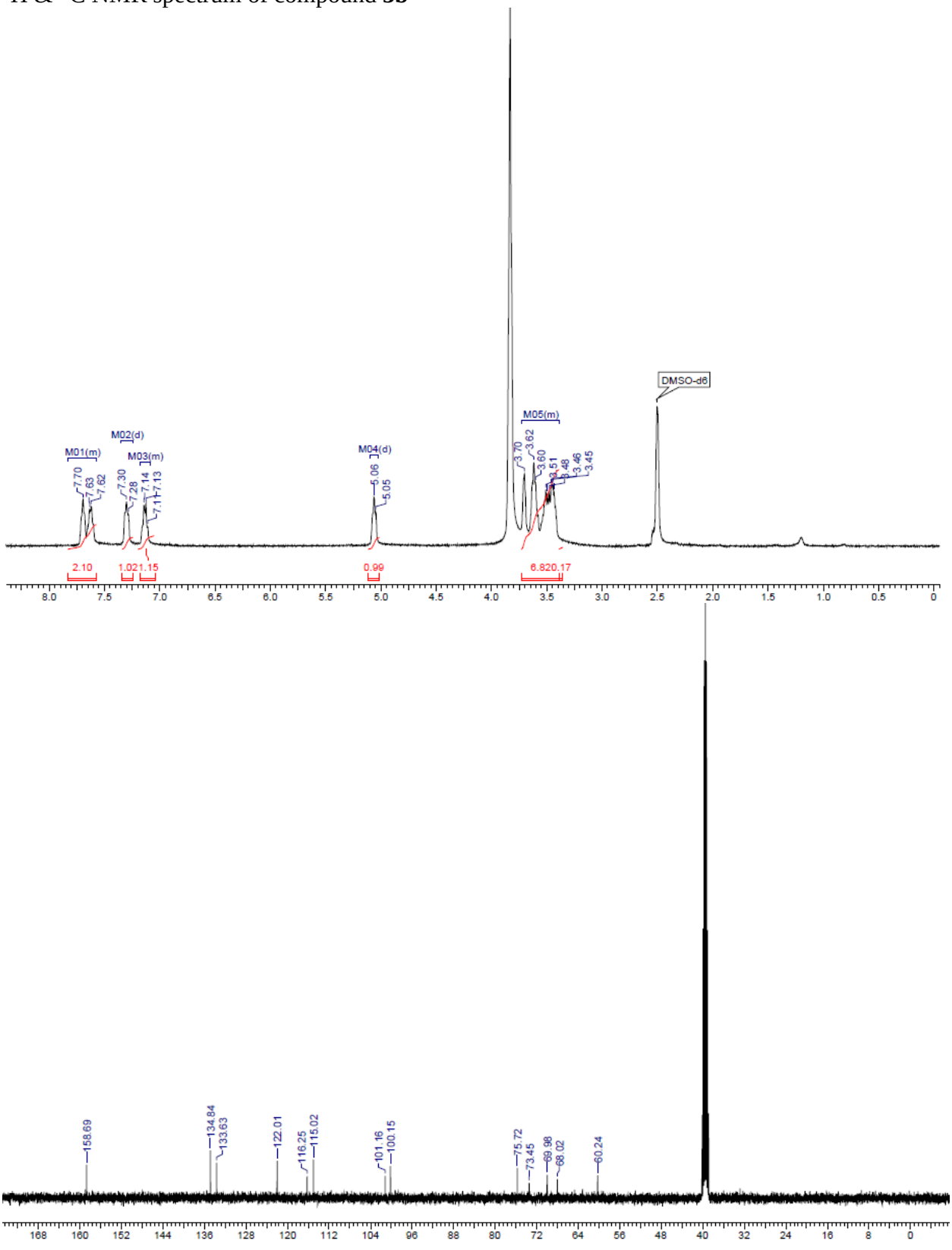


DAD1 B, Sig=254,16 Ref=off

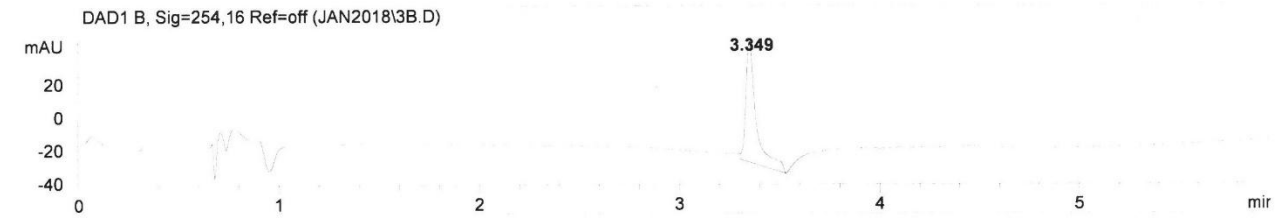
#	Meas. Ret	Area	Area %
1	3.44	3.280e2	100.00

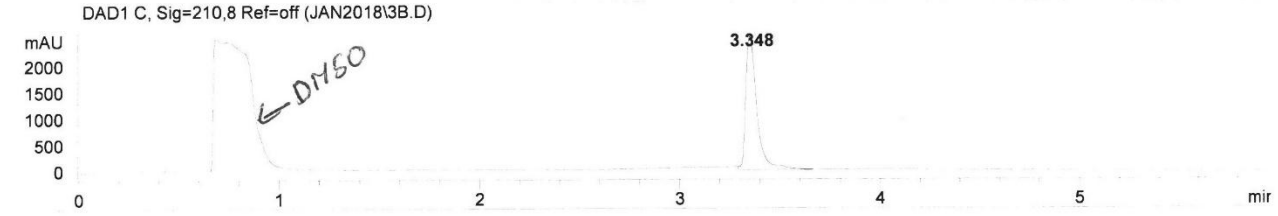


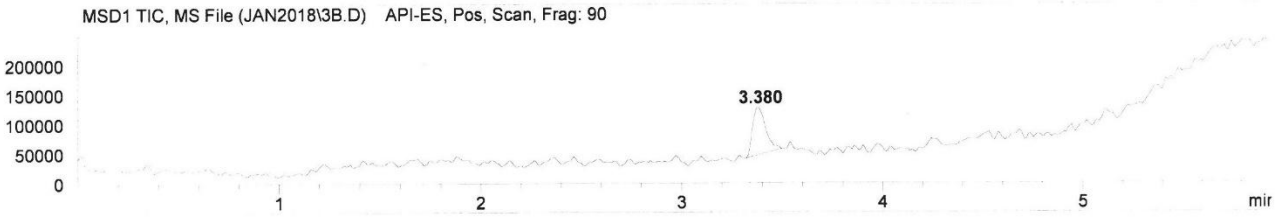
^{1}H &^{13}C NMR spectrum of compound **3b**



LCMS spectrum of compound **3b**

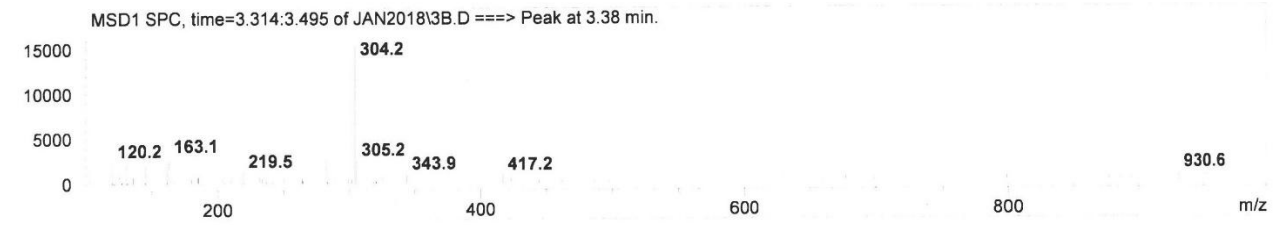




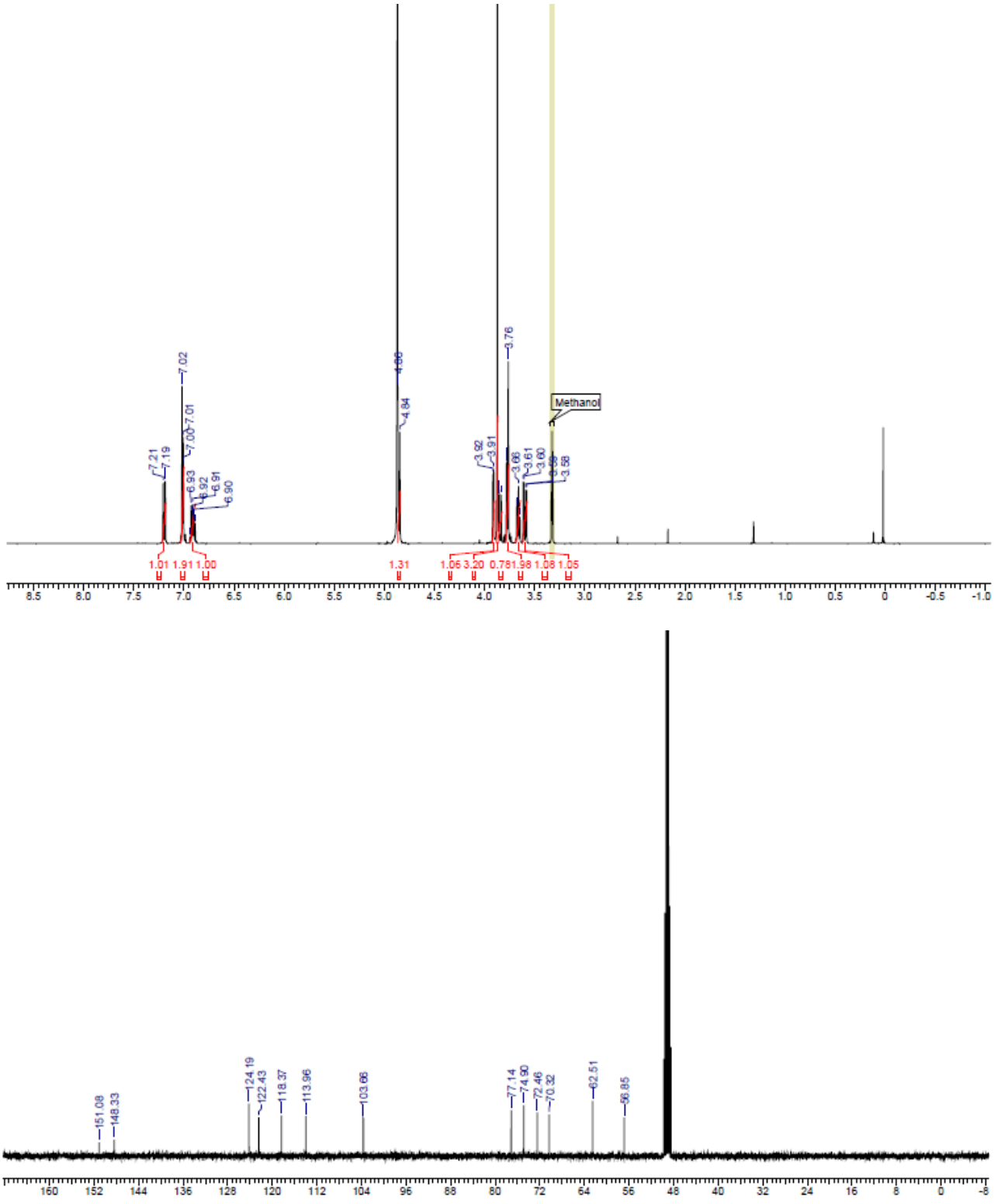


DAD1 B, Sig=254,16 Ref=off

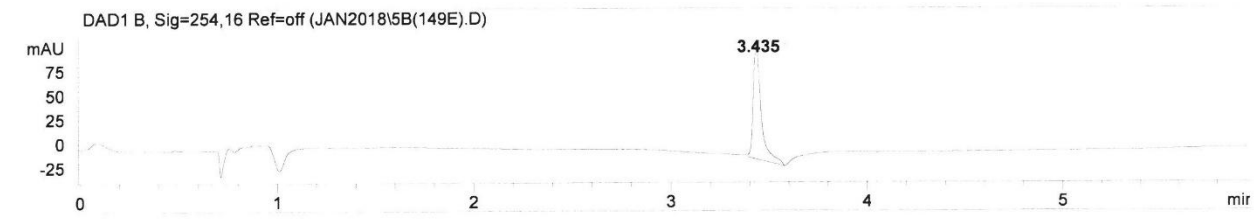
#	Meas. Ret	Area	Area %
1	3.35	2.639e2	100.00

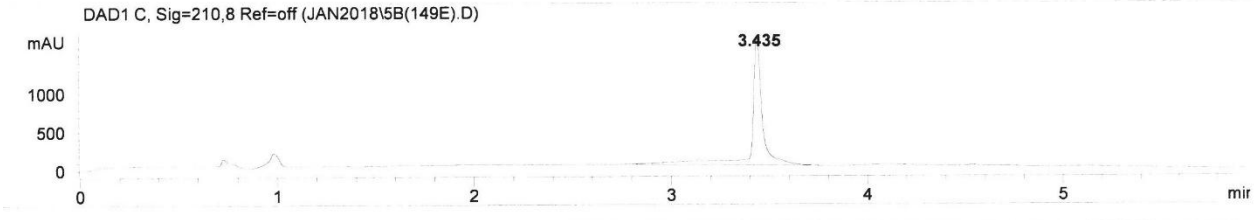


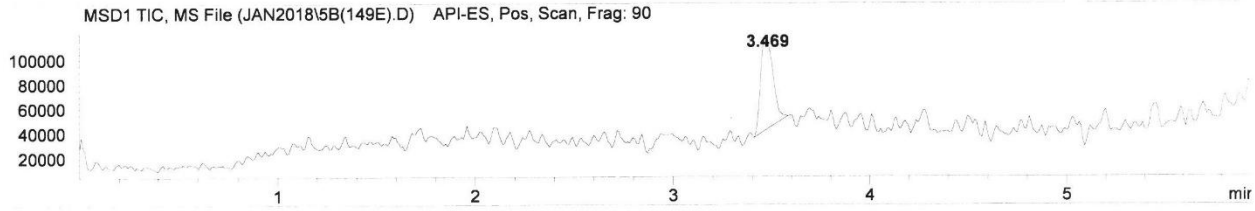
^{1}H &^{13}C NMR spectrum of compound **5b**



LCMS spectrum of compound **5b**

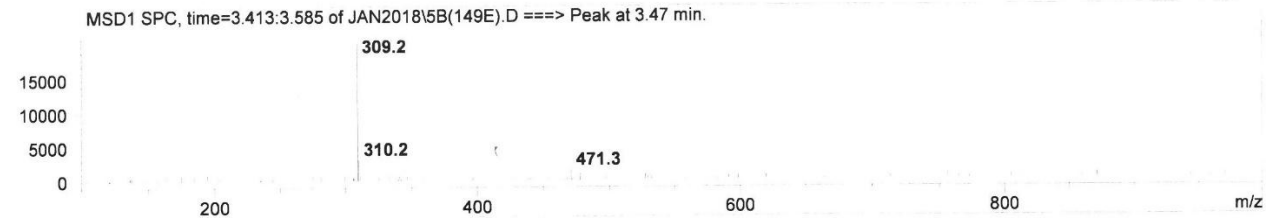




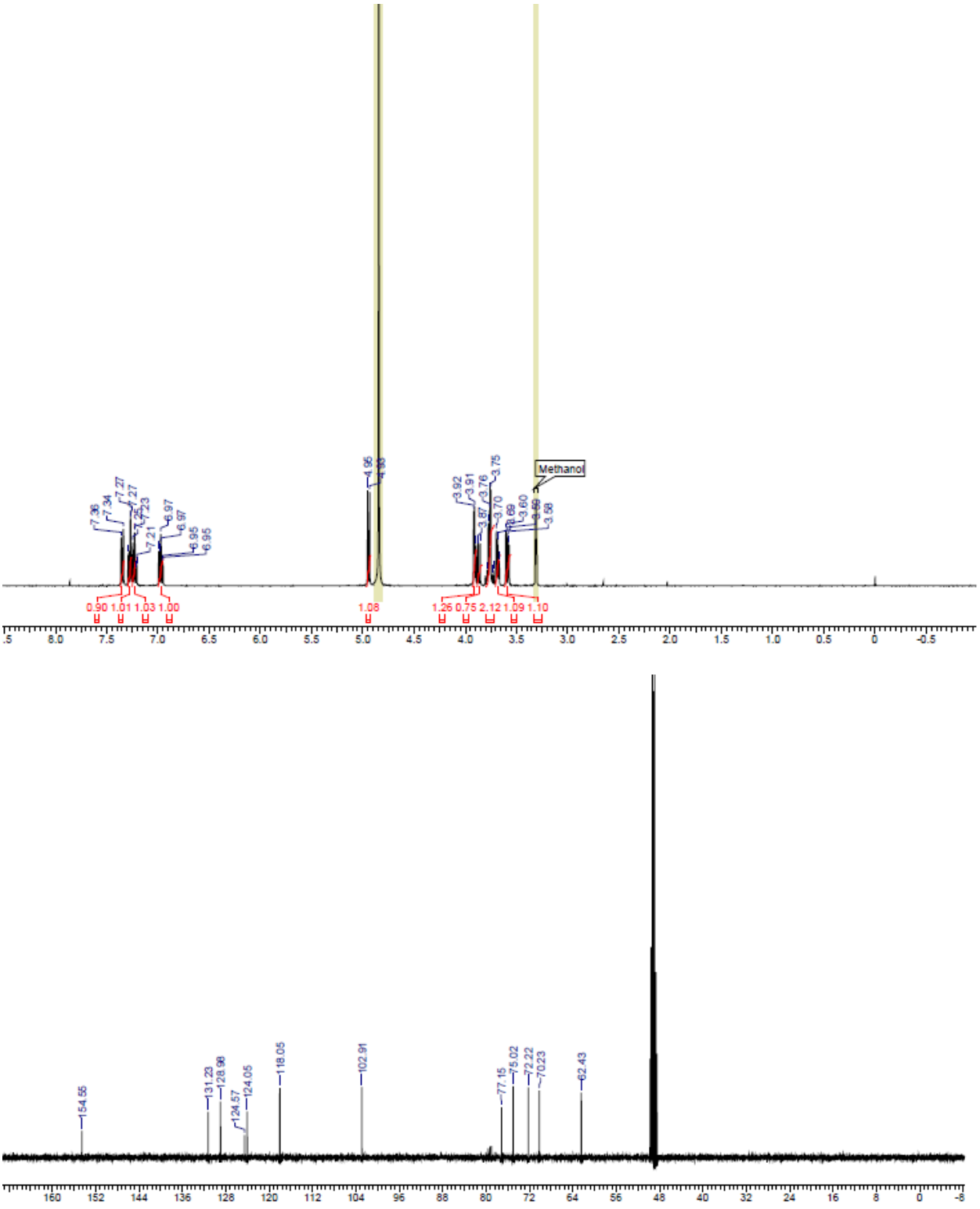


DAD1 B, Sig=254,16 Ref=off

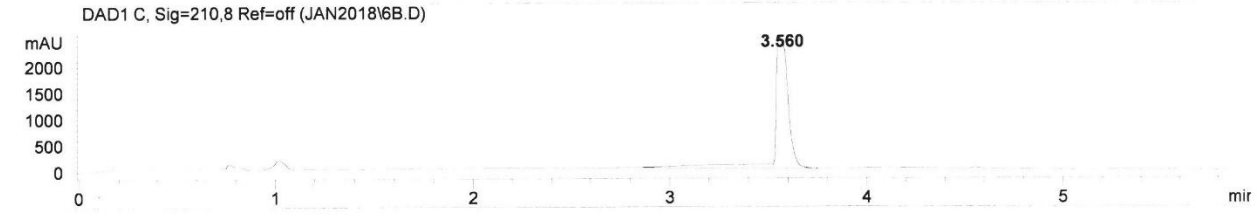
#	Meas. Ret	Area	Area %
1	3.43	3.359e2	100.00

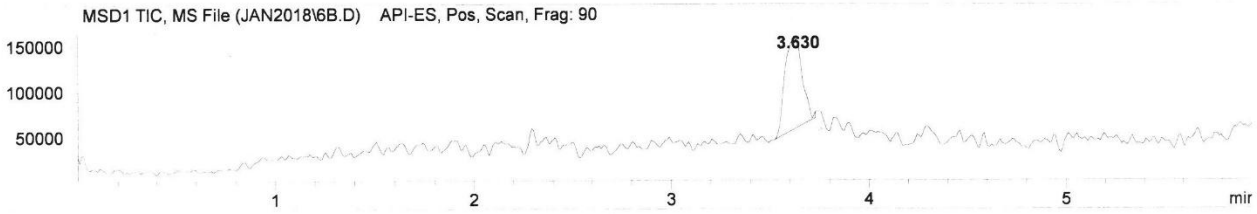


^{1}H &^{13}C NMR spectrum of compound **6b**



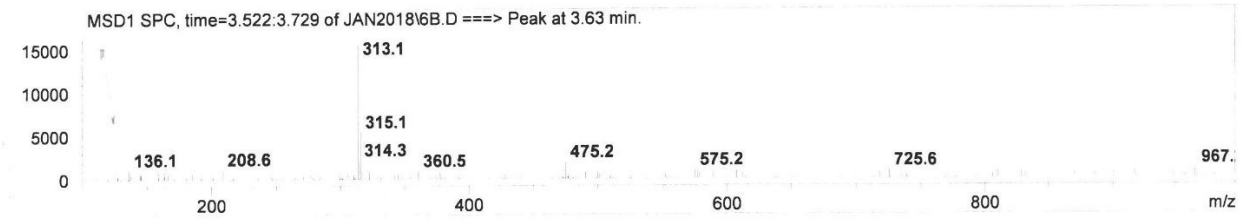
LCMS spectrum of compound **6b**



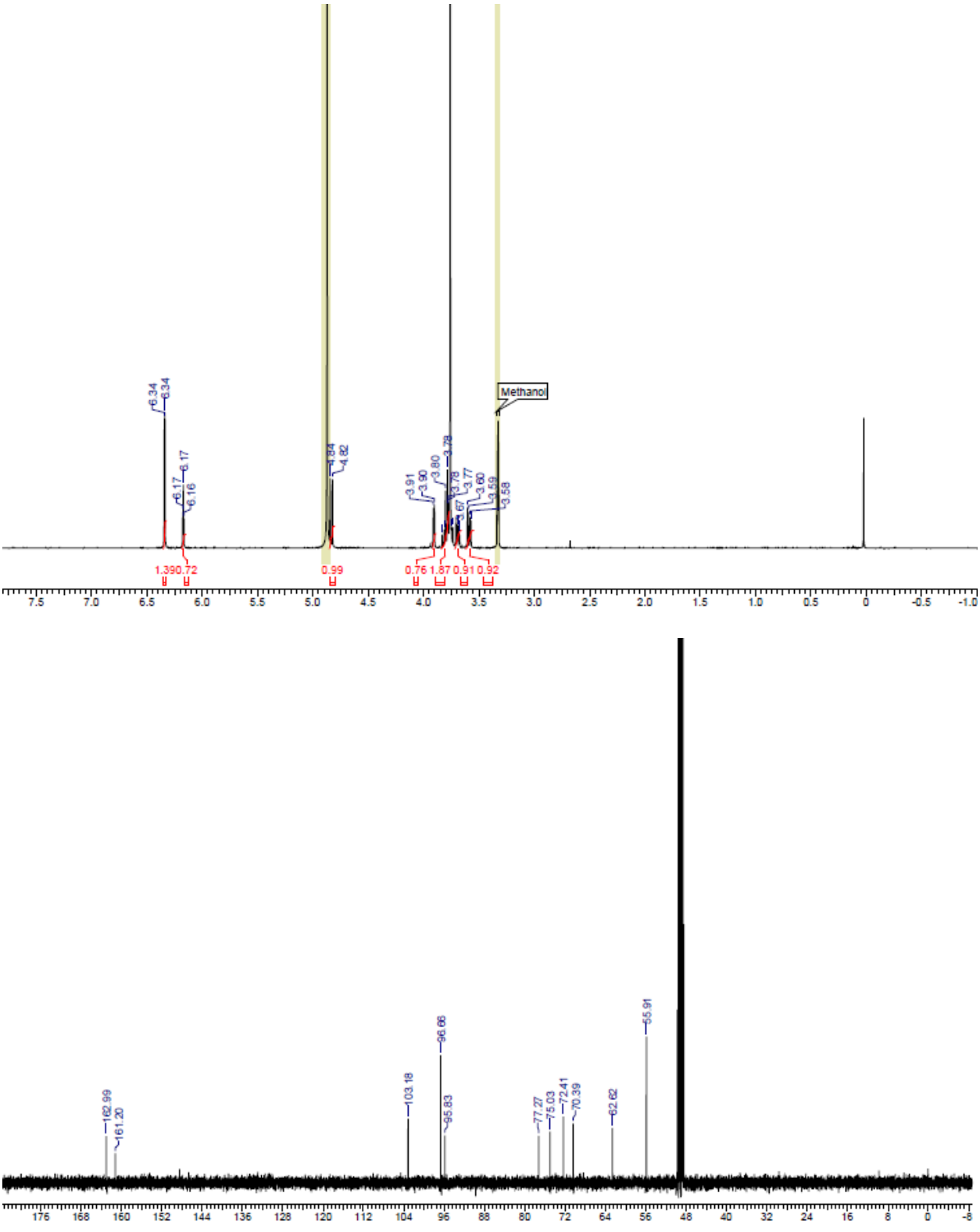


DAD1 B, Sig=254,16 Ref=off

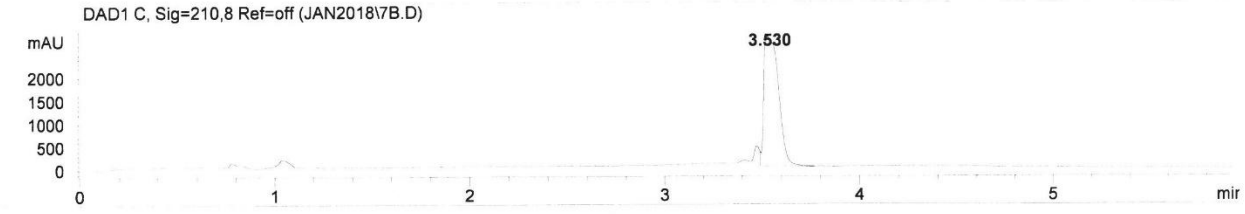
#	Meas. Ret	Area	Area %
1	3.56	5.368e2	100.00

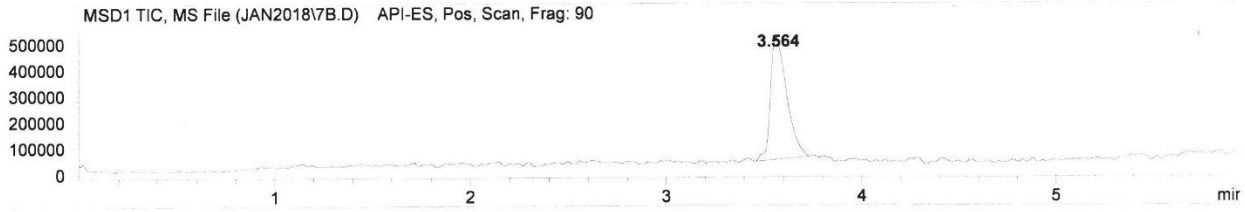


^{1}H &^{13}C NMR spectrum of compound **7b**



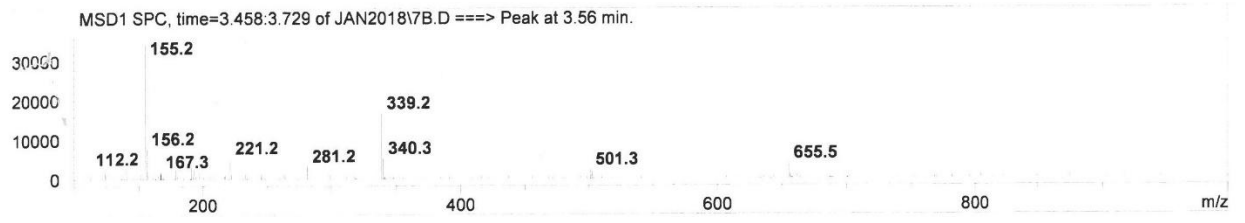
LCMS spectrum of compound **7b**



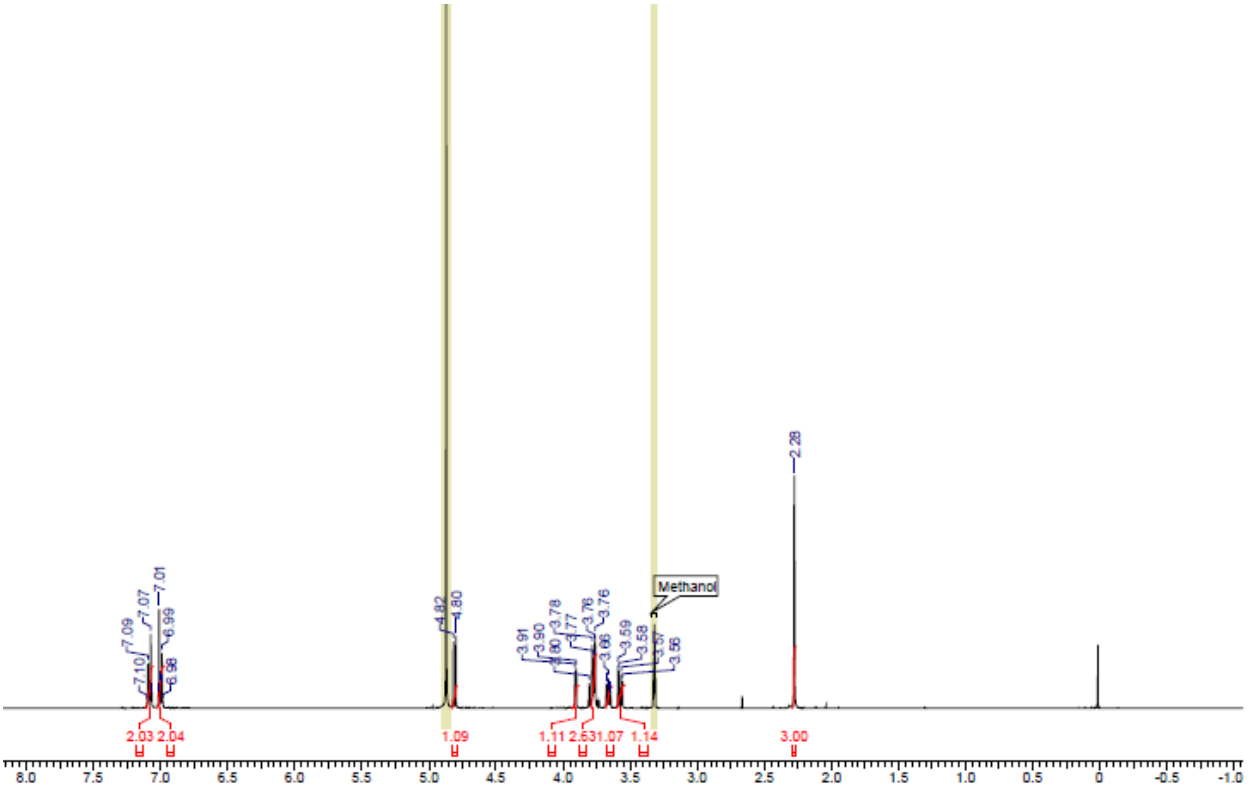


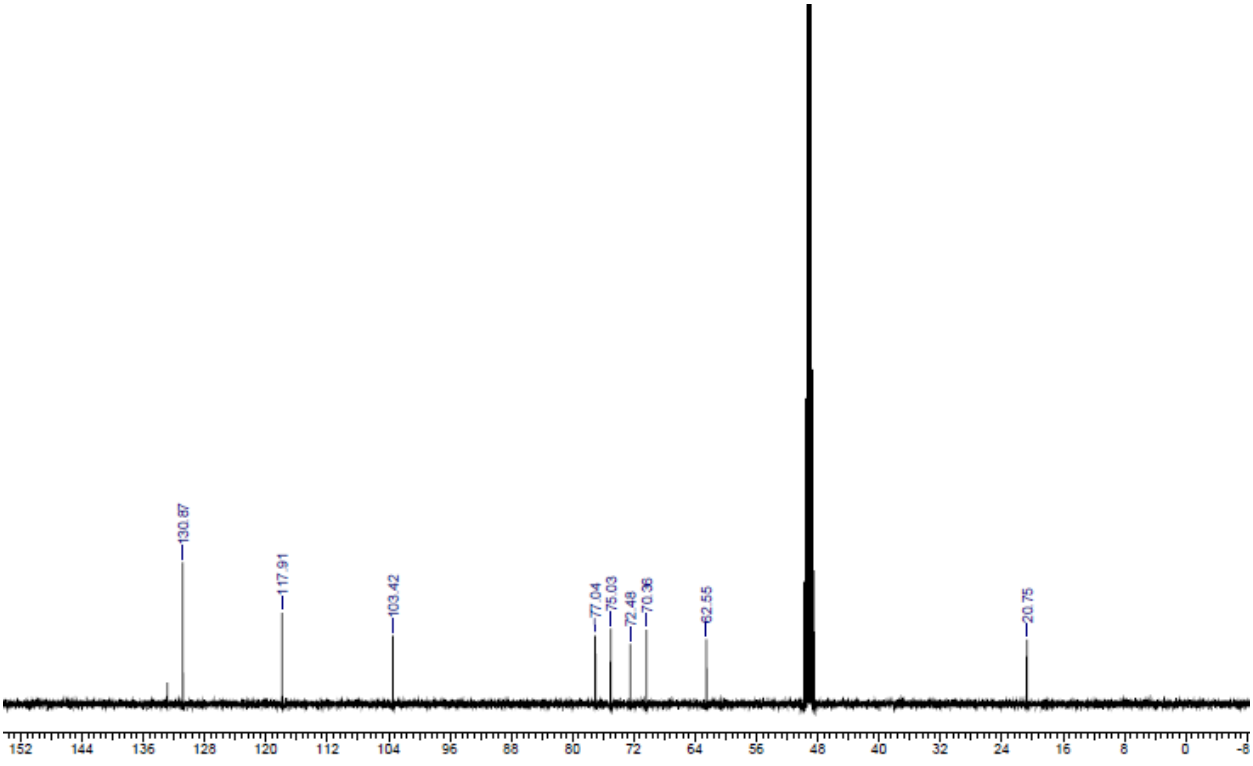
DAD1 B, Sig=254,16 Ref=off

#	Meas. Ret	Area	Area %
1	3.53	5.312e2	100.00

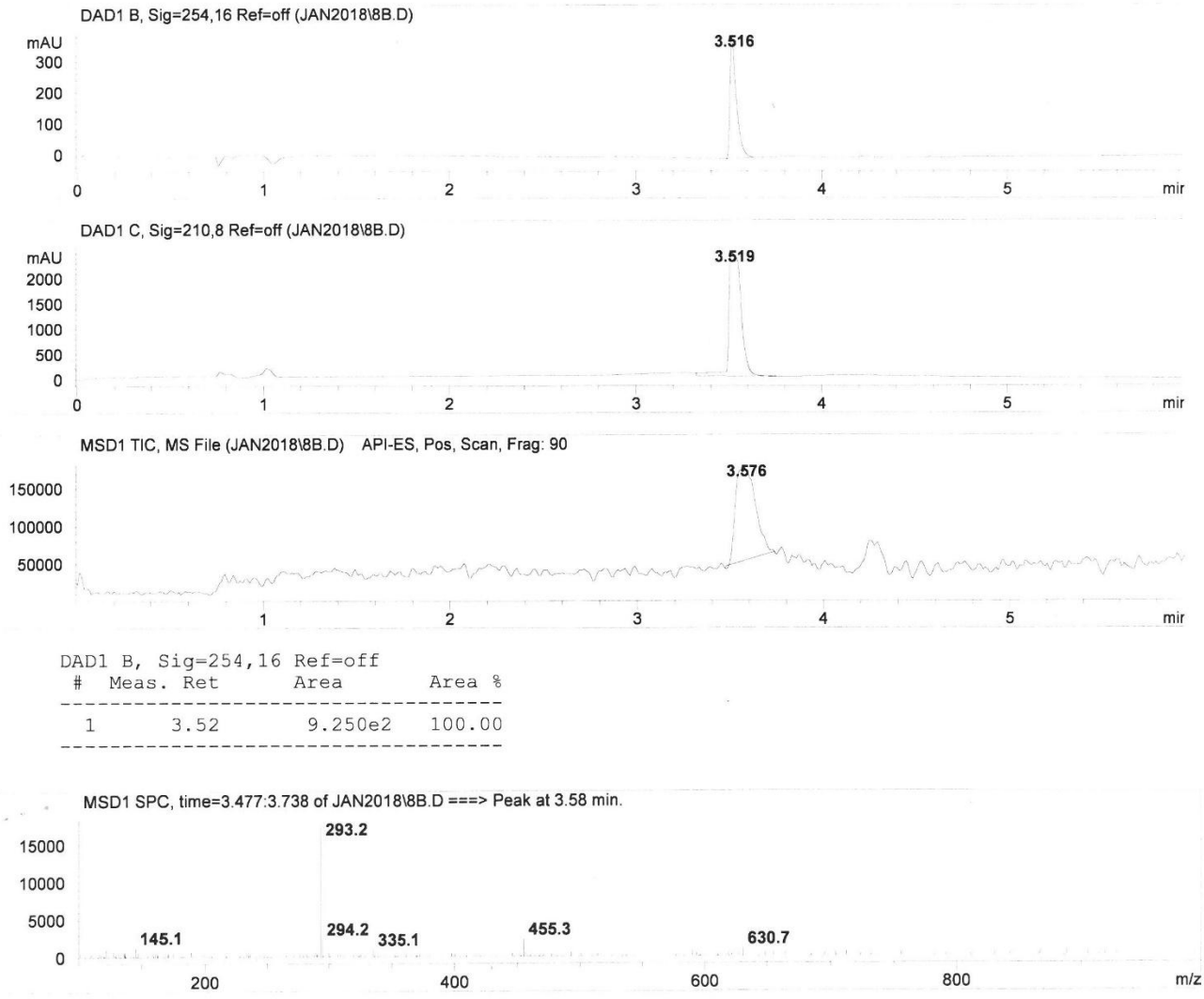


^{1}H &^{13}C NMR spectrum of compound **8b**





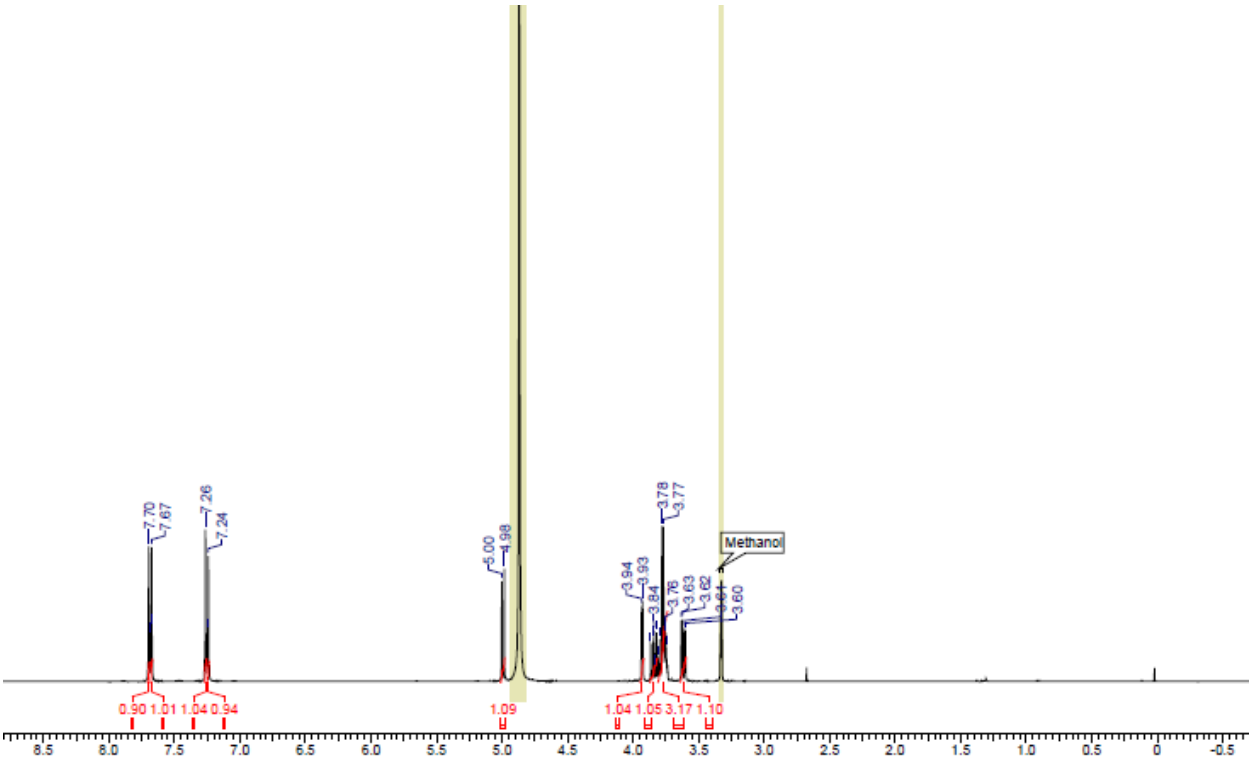
LCMS spectrum of compound **8b**

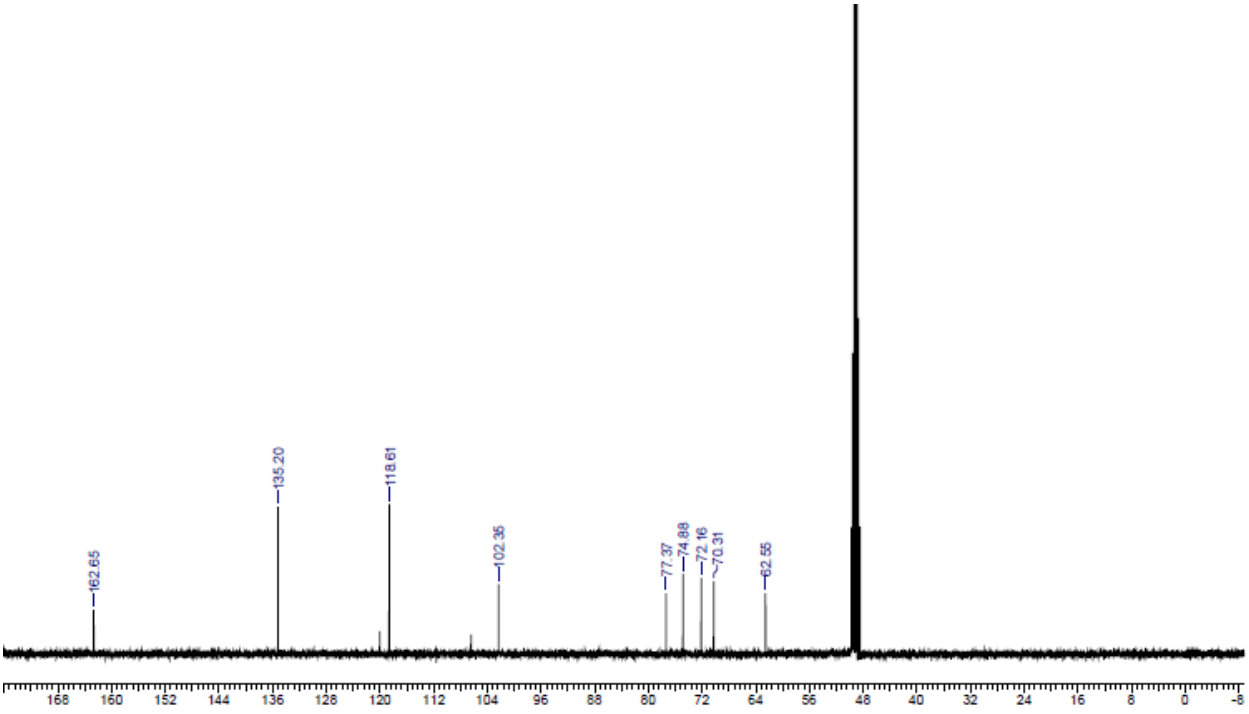


DAD1 B, Sig=254,16 Ref=off

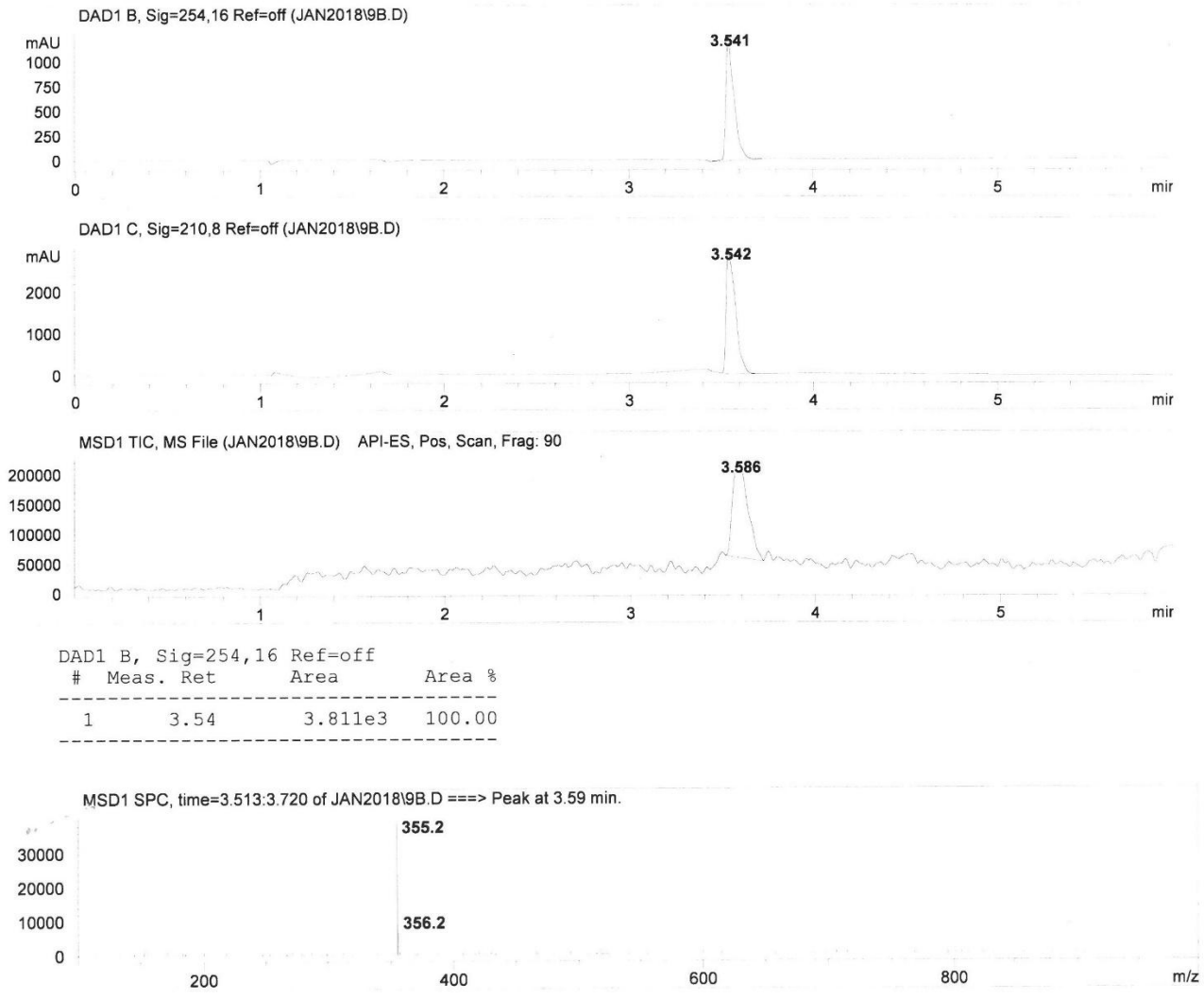
#	Meas. Ret	Area	Area %
1	3.52	9.250e2	100.00

^{1}H &^{13}C NMR spectrum of compound **9b**





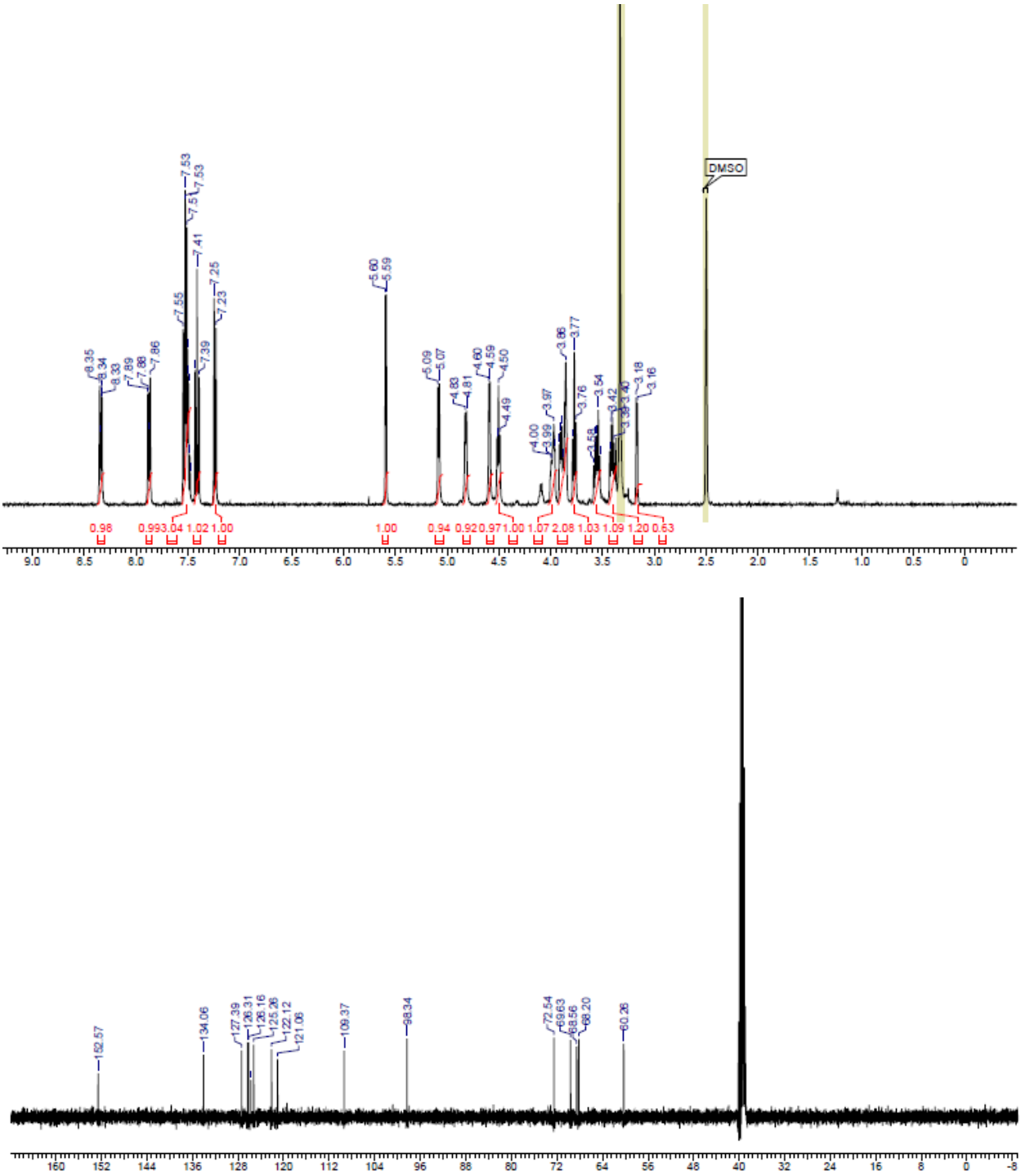
LCMS spectrum of compound **9b**



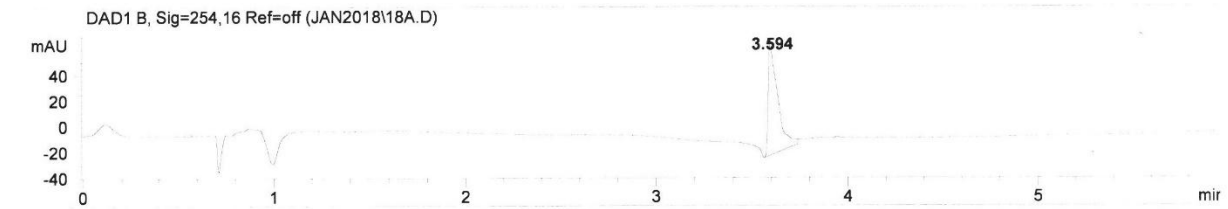
DAD1 B, Sig=254,16 Ref=off

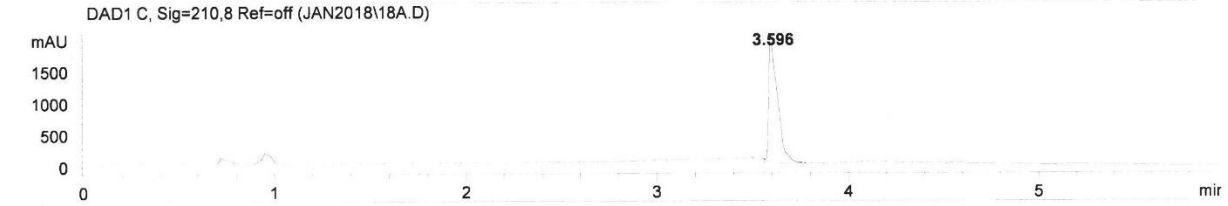
#	Meas. Ret	Area	Area %
1	3.54	3.811e3	100.00

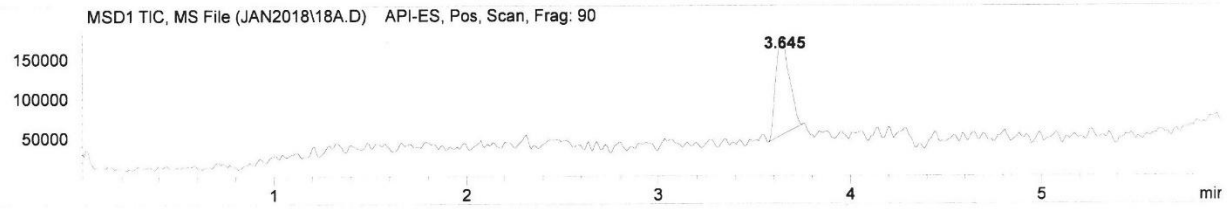
^{1}H &^{13}C NMR spectrum of compound **18a**



LCMS spectrum of compound **18a**

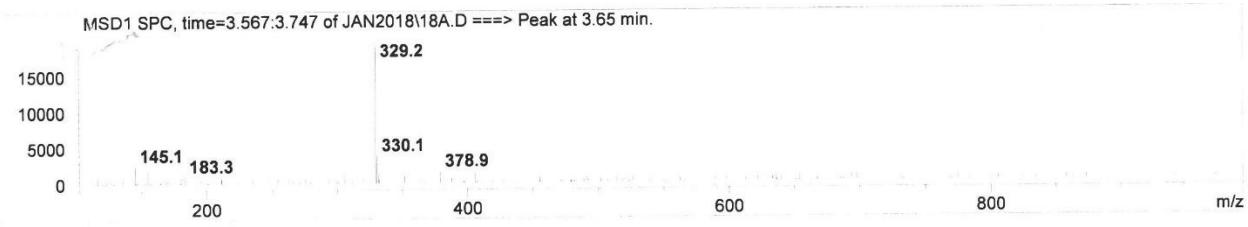




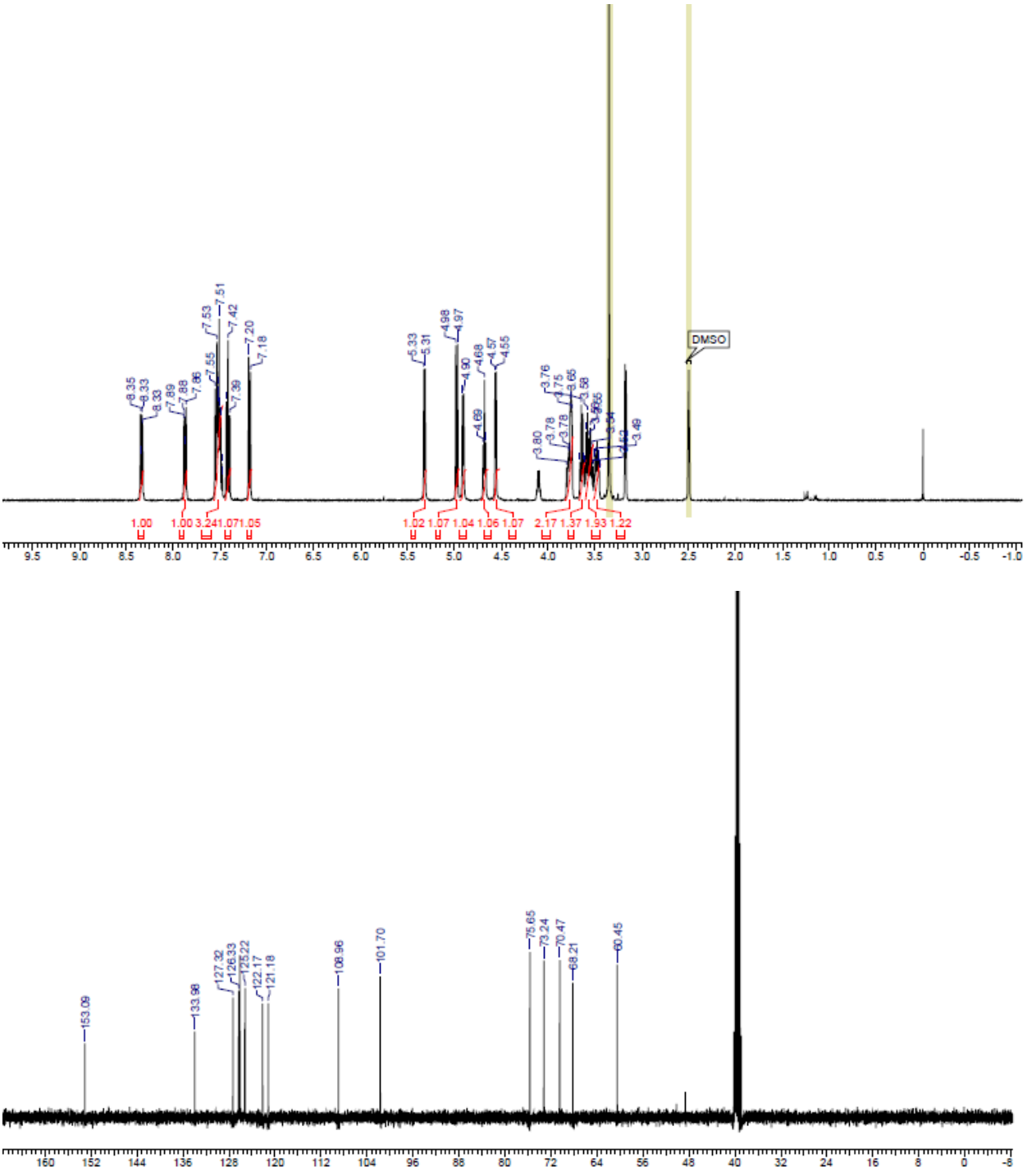


DAD1 B, Sig=254,16 Ref=off

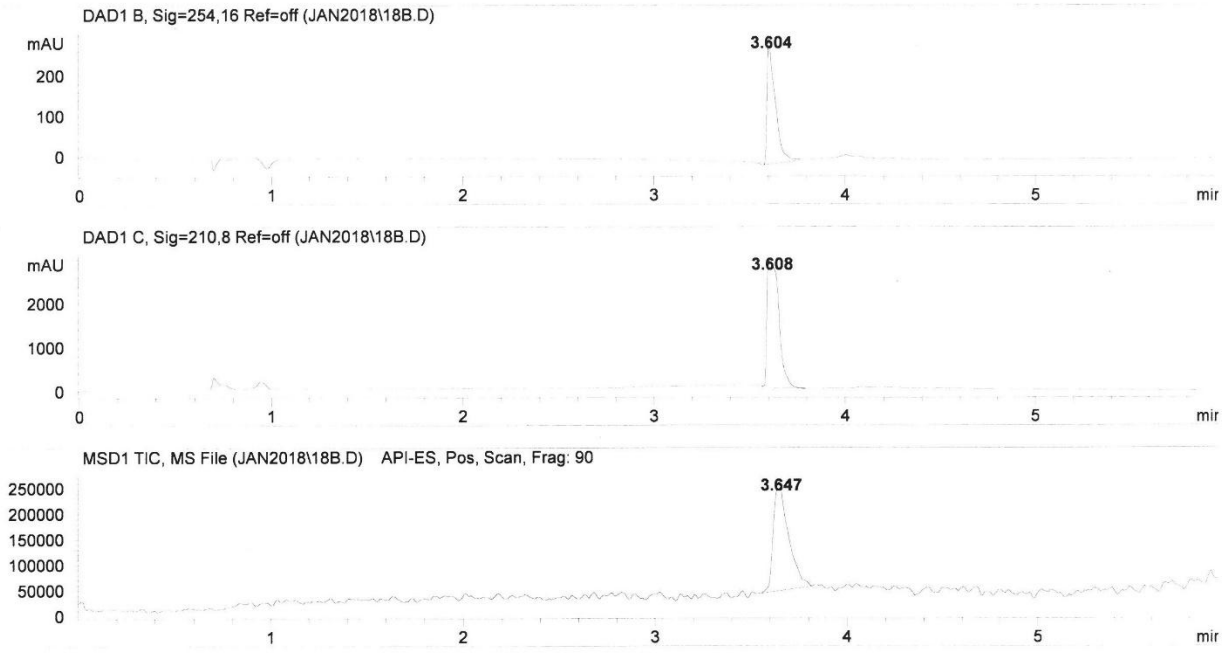
#	Meas. Ret	Area	Area %
1	3.59	3.053e2	100.00



^{1}H &^{13}C NMR spectrum of compound **18b**

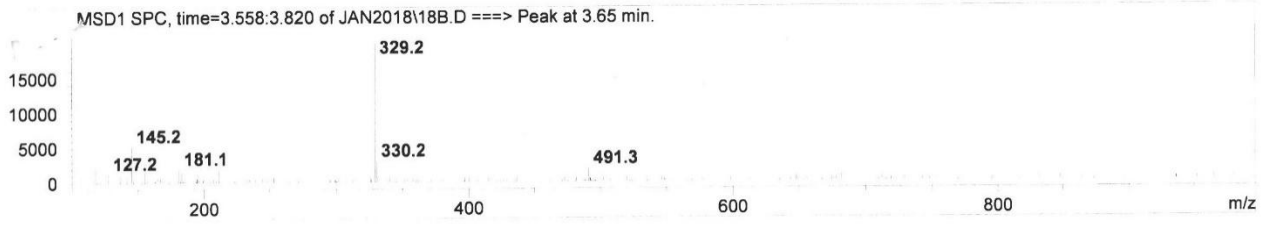


LCMS spectrum of compound **18b**

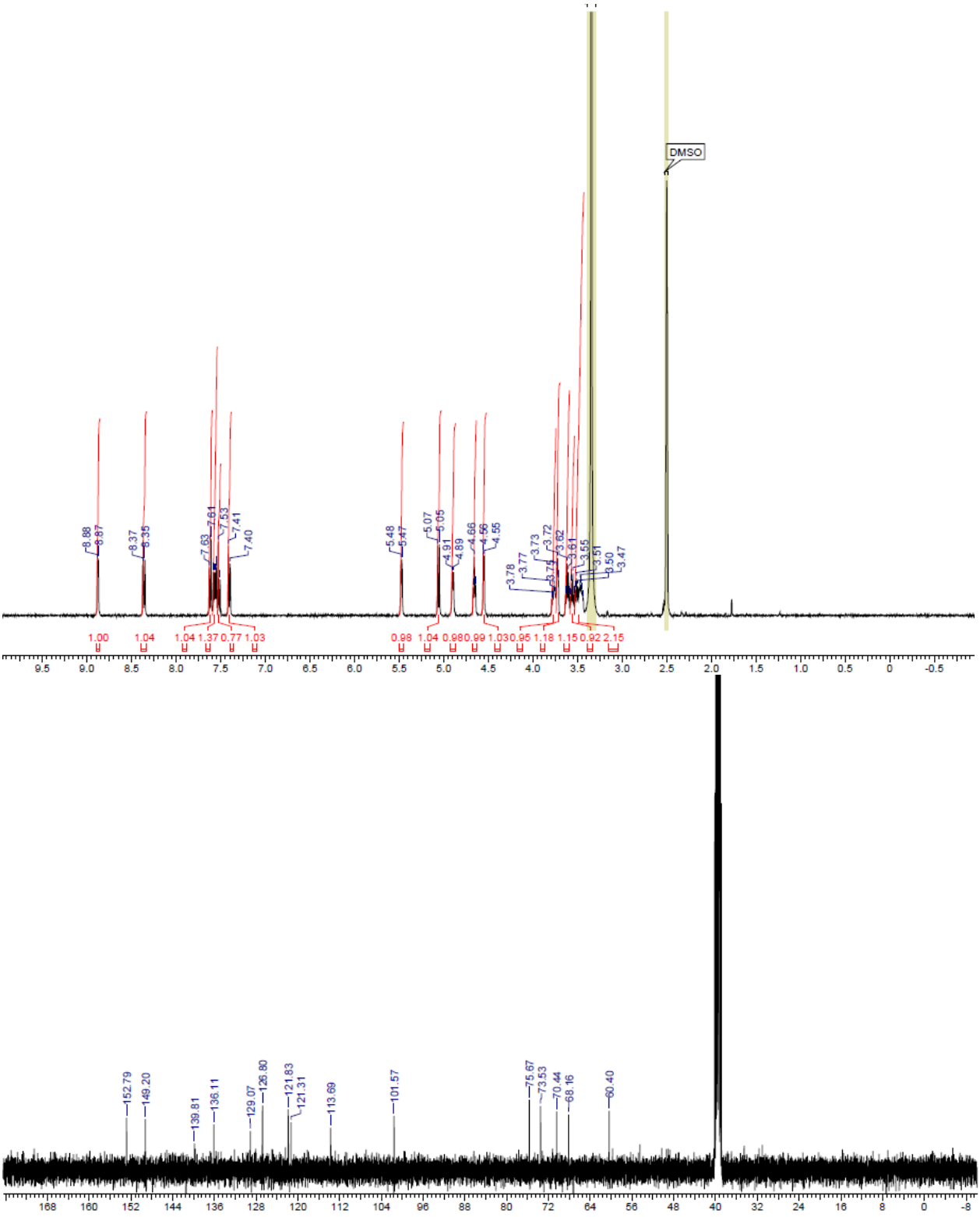


DAD1 B, Sig=254,16 Ref=off

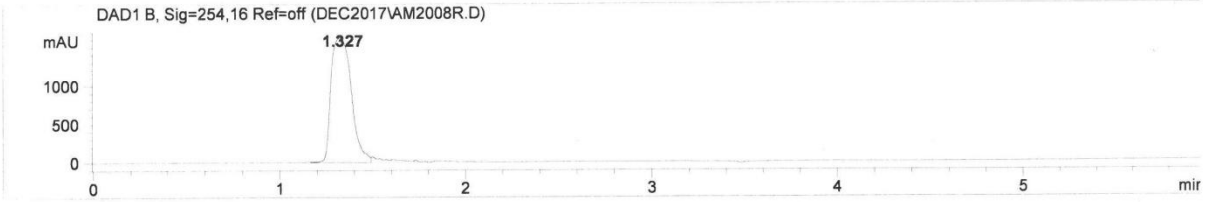
#	Meas. Ret	Area	Area %
1	3.60	9.164e2	100.00

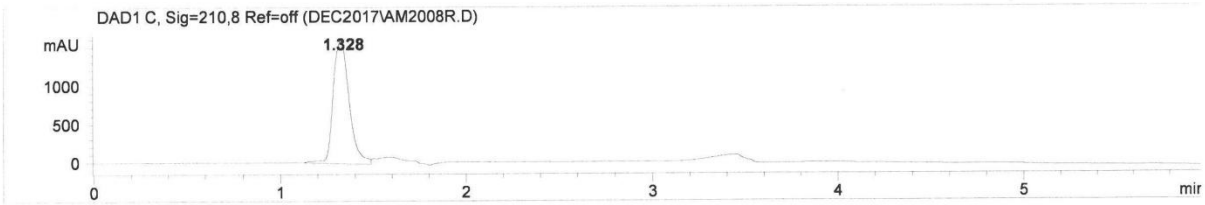


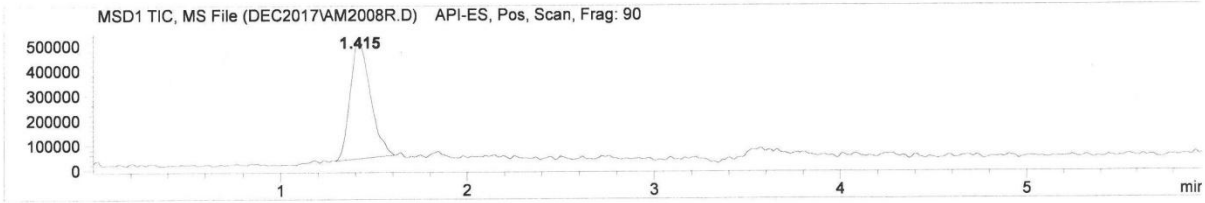
^{1}H &^{13}C NMR spectrum of compound **21b**



LCMS spectrum of compound **21b**

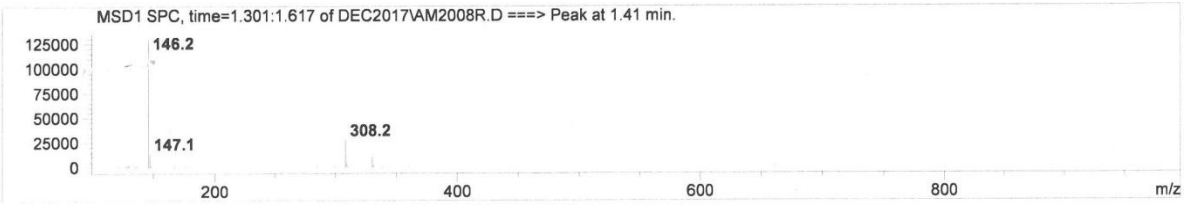




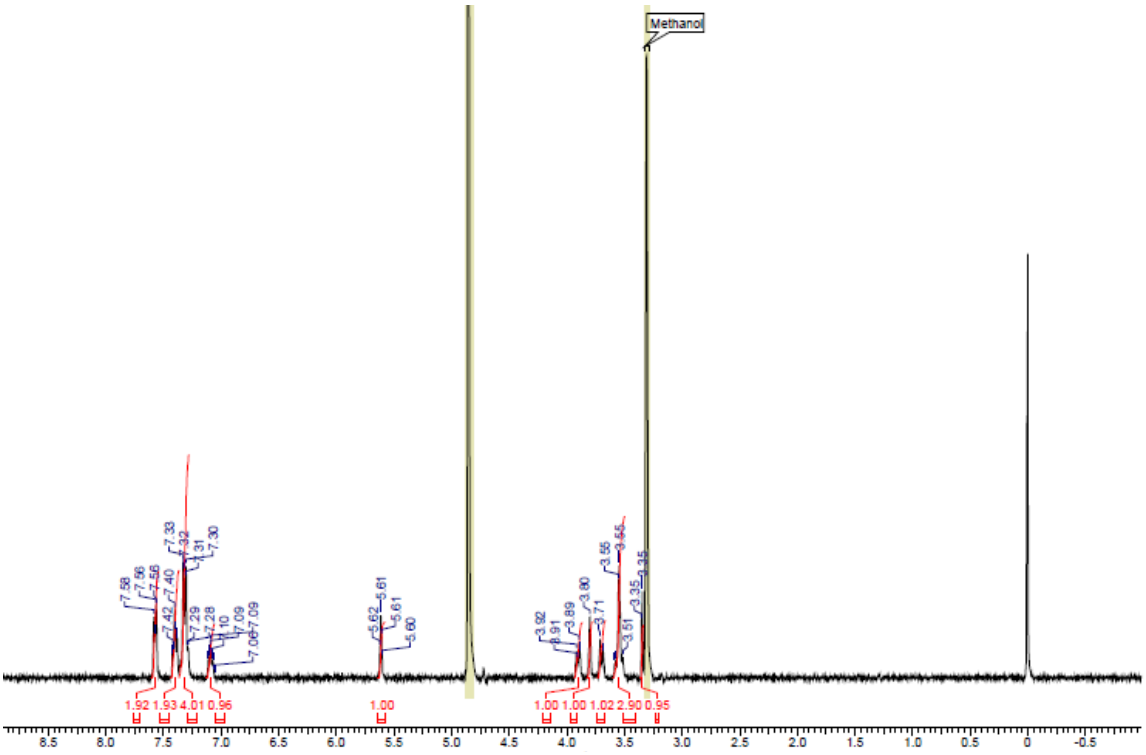


DAD1 B, Sig=254,16 Ref=off

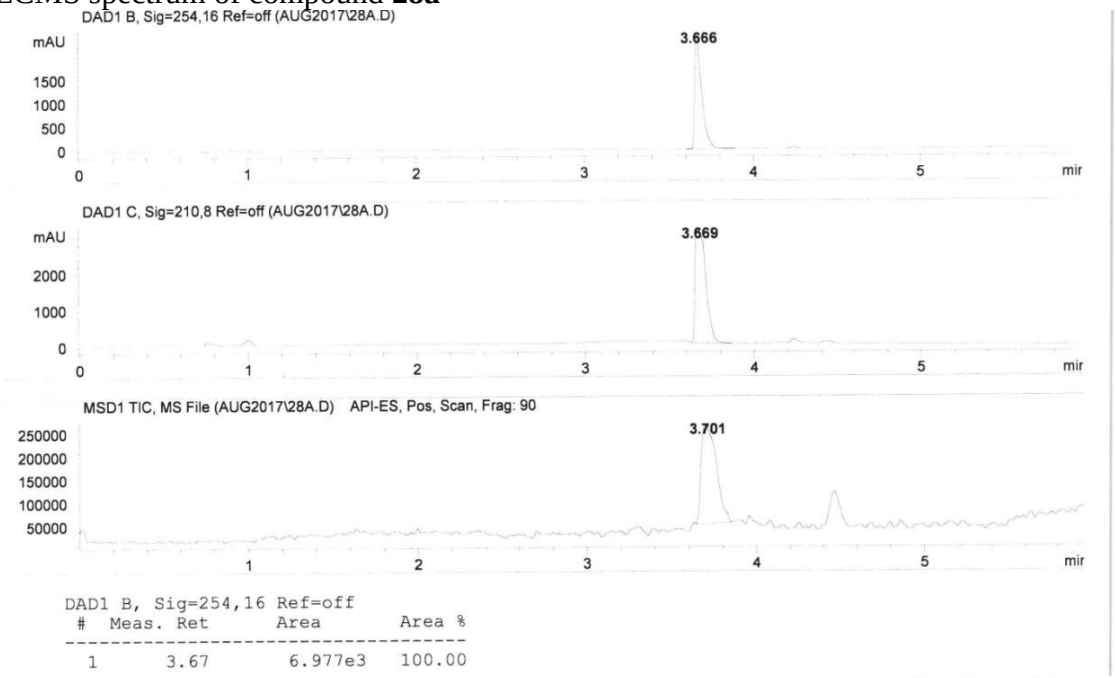
#	Meas. Ret	Area	Area %
1	1.33	1.216e4	100.00



^{1}H NMR spectrum of compound **28a**

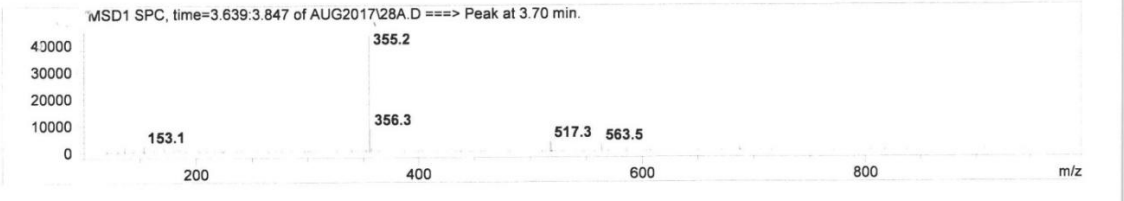


LCMS spectrum of compound **28a**

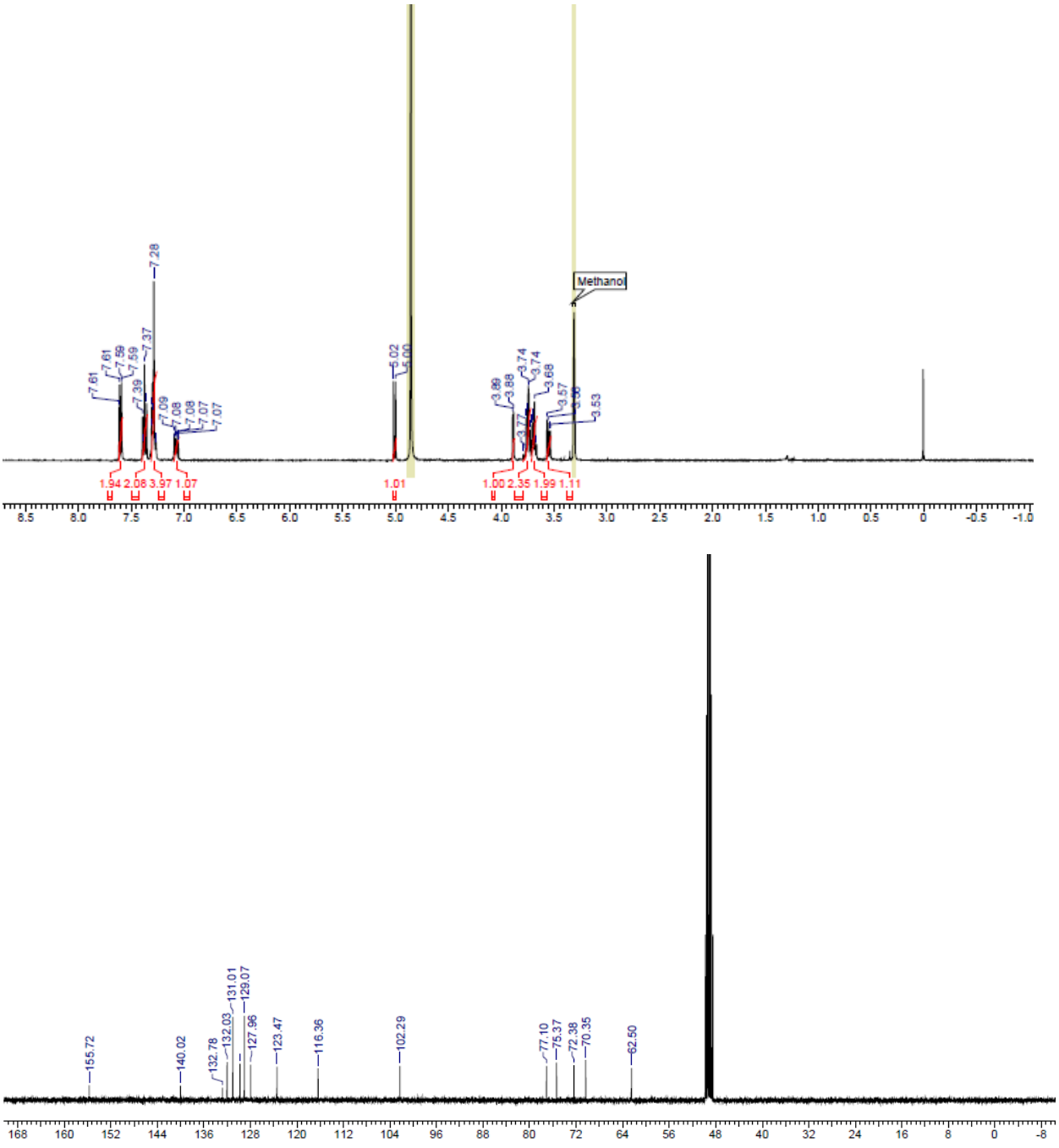


DAD1 B, Sig=254,16 Ref=off

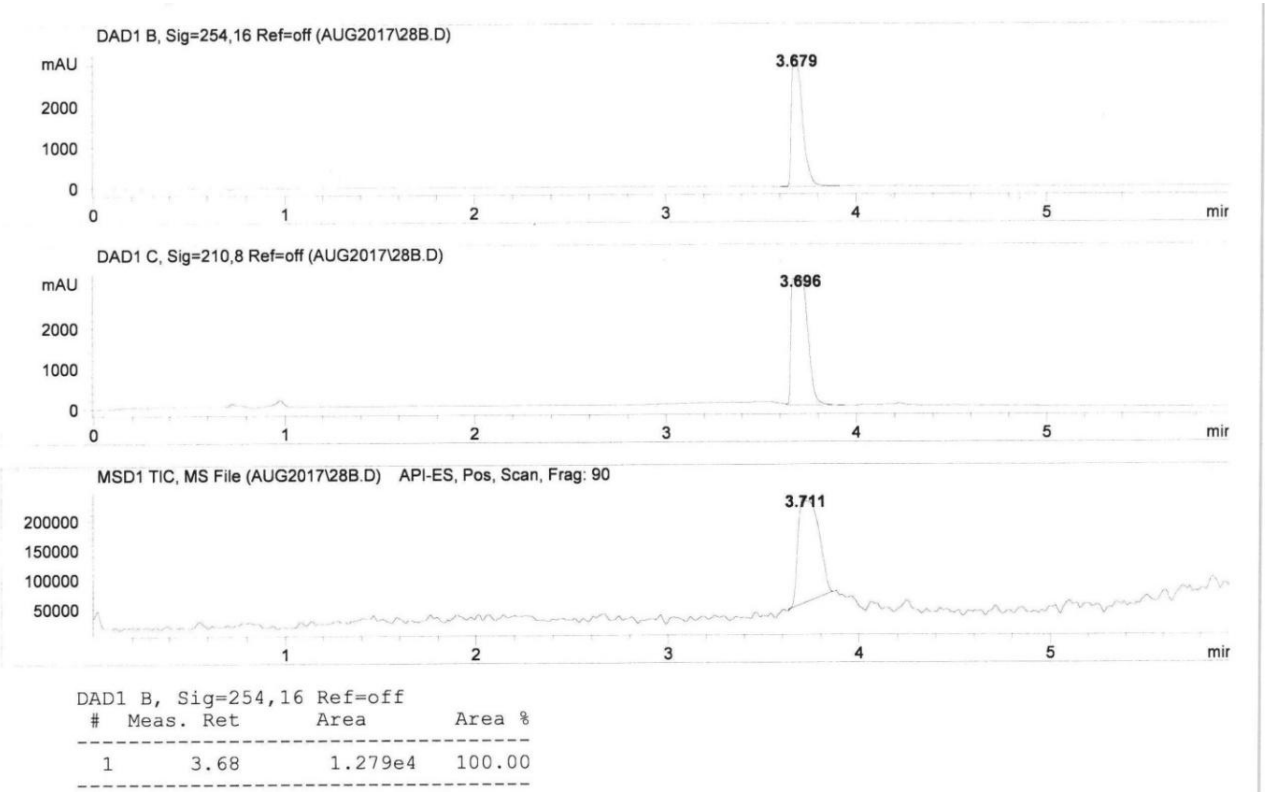
#	Meas. Ret	Area	Area %
1	3.67	6.977e3	100.00



^{1}H &^{13}C NMR spectrum of compound 2**8b**

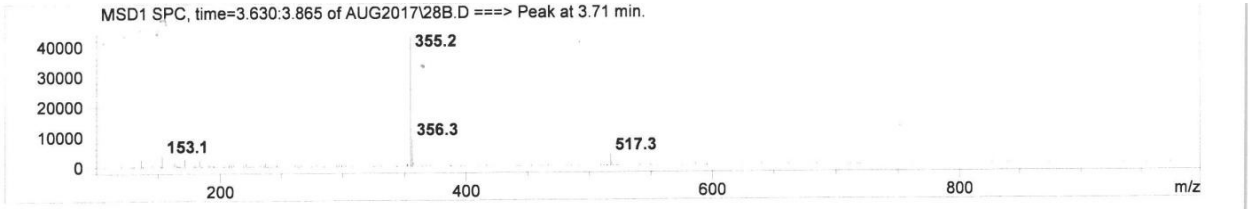


LCMS spectrum of compound **28b**

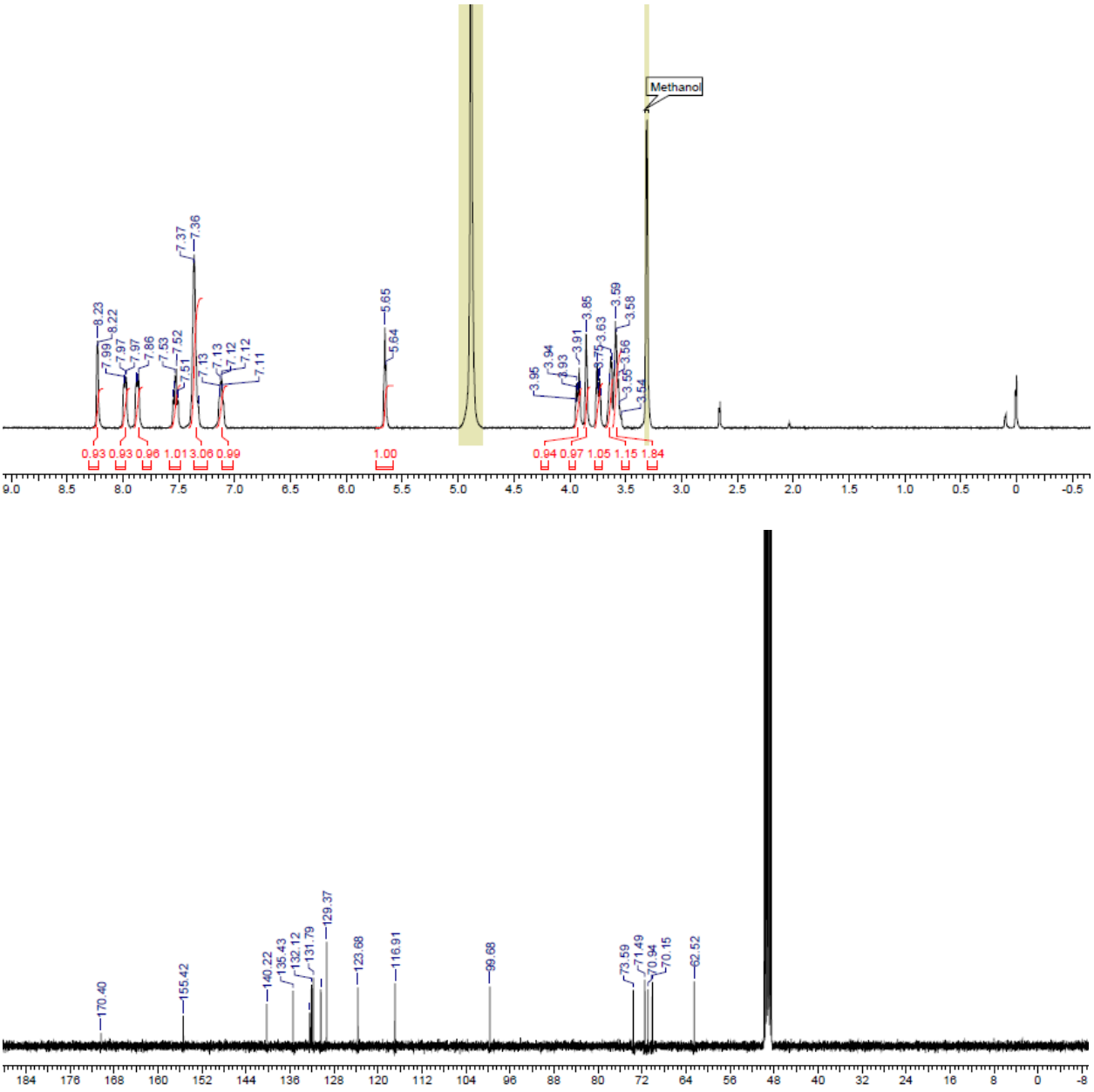


DAD1 B, Sig=254,16 Ref=off

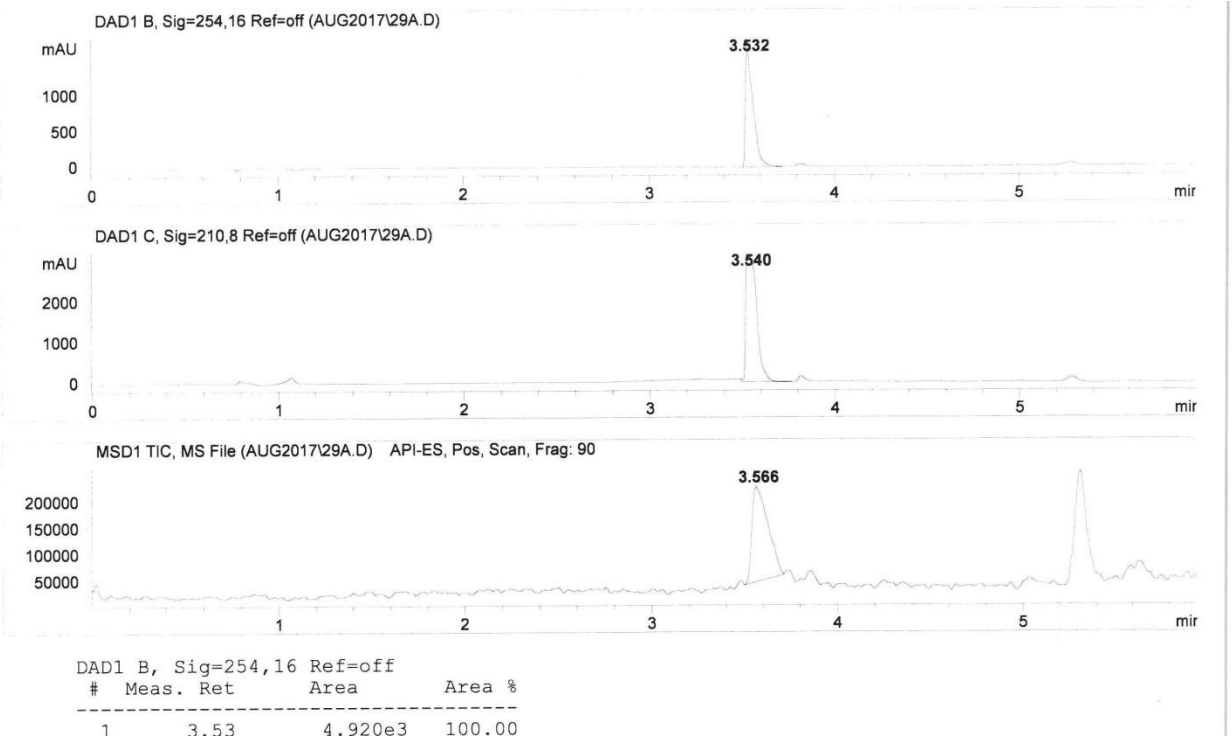
#	Meas. Ret	Area	Area %
1	3.68	1.279e4	100.00



^{1}H &^{13}C NMR spectrum of compound **29a**

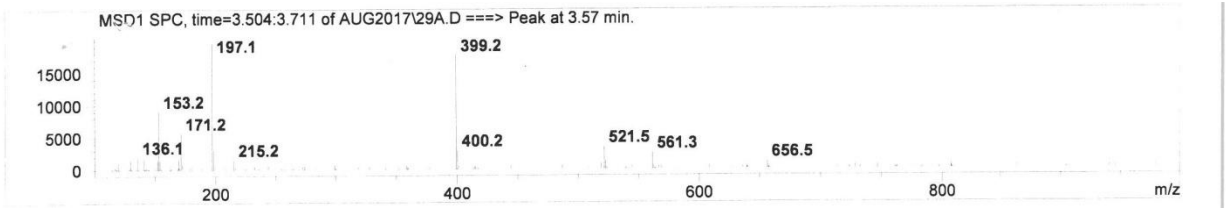


LCMS spectrum of compound **29a**

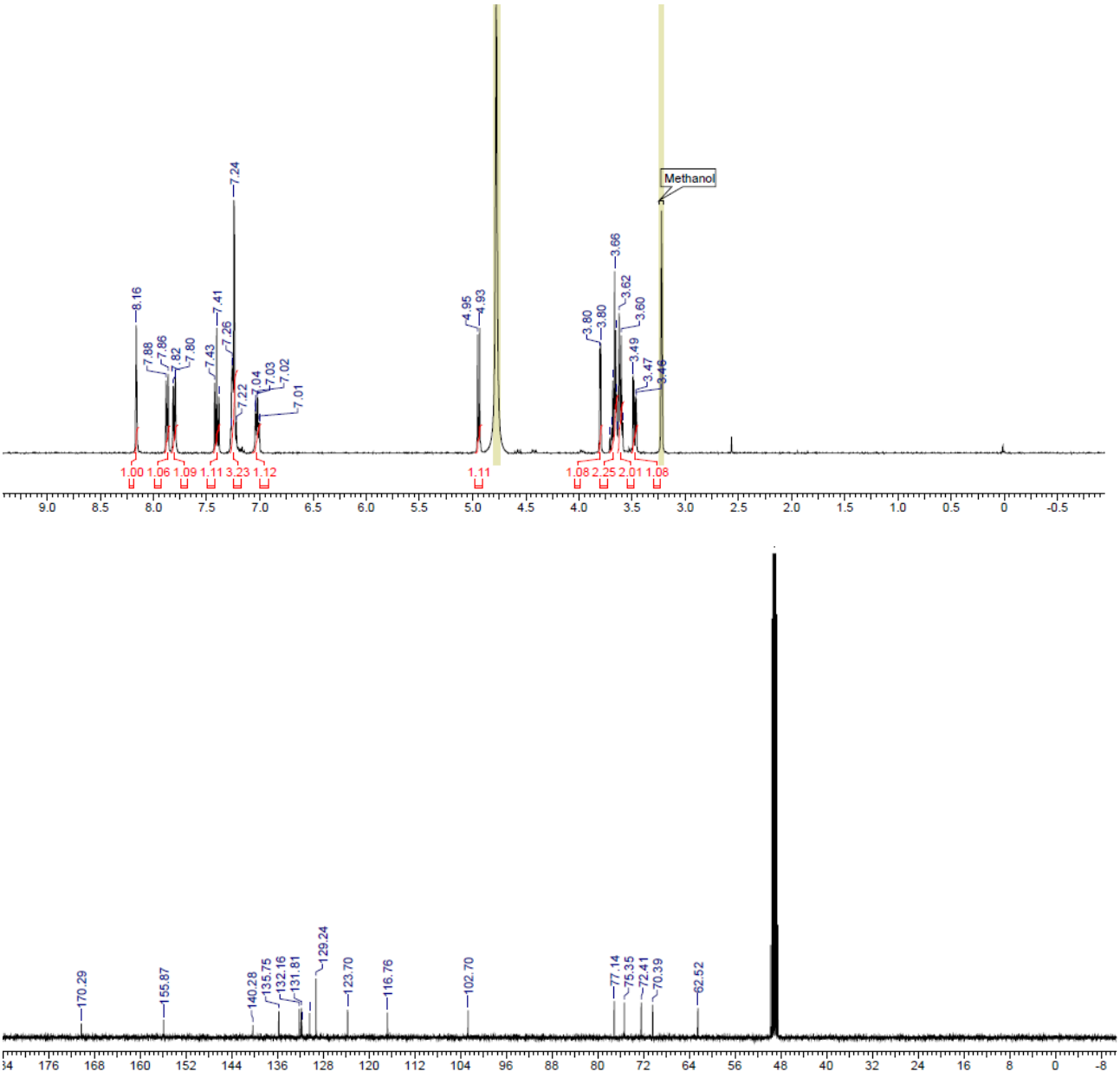


DAD1 B, Sig=254,16 Ref=off

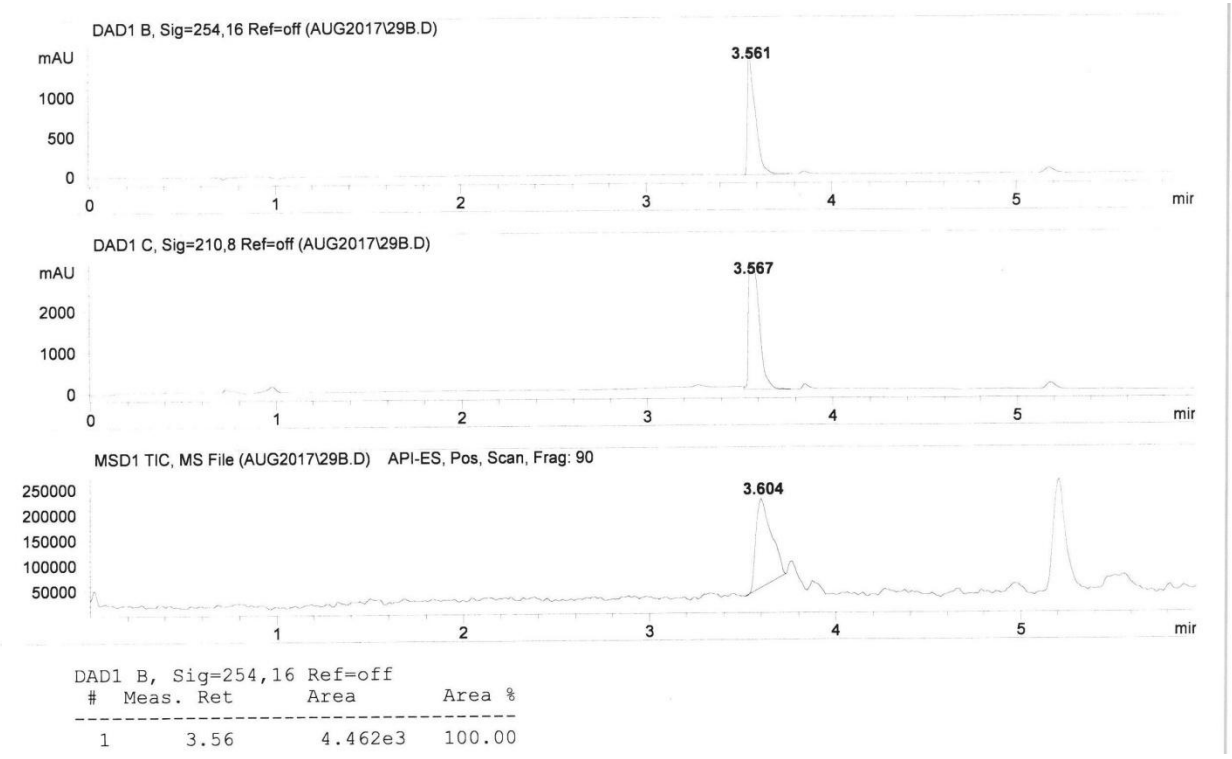
#	Meas. Ret	Area	Area %
1	3.53	4.920e3	100.00



^{1}H &^{13}C NMR spectrum of compound **29b**

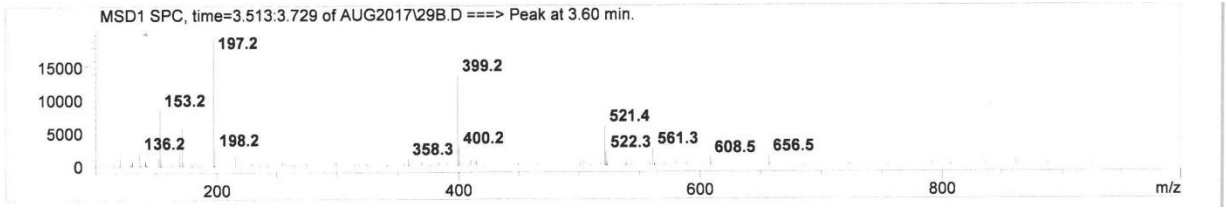


LCMS spectrum of compound **29b**

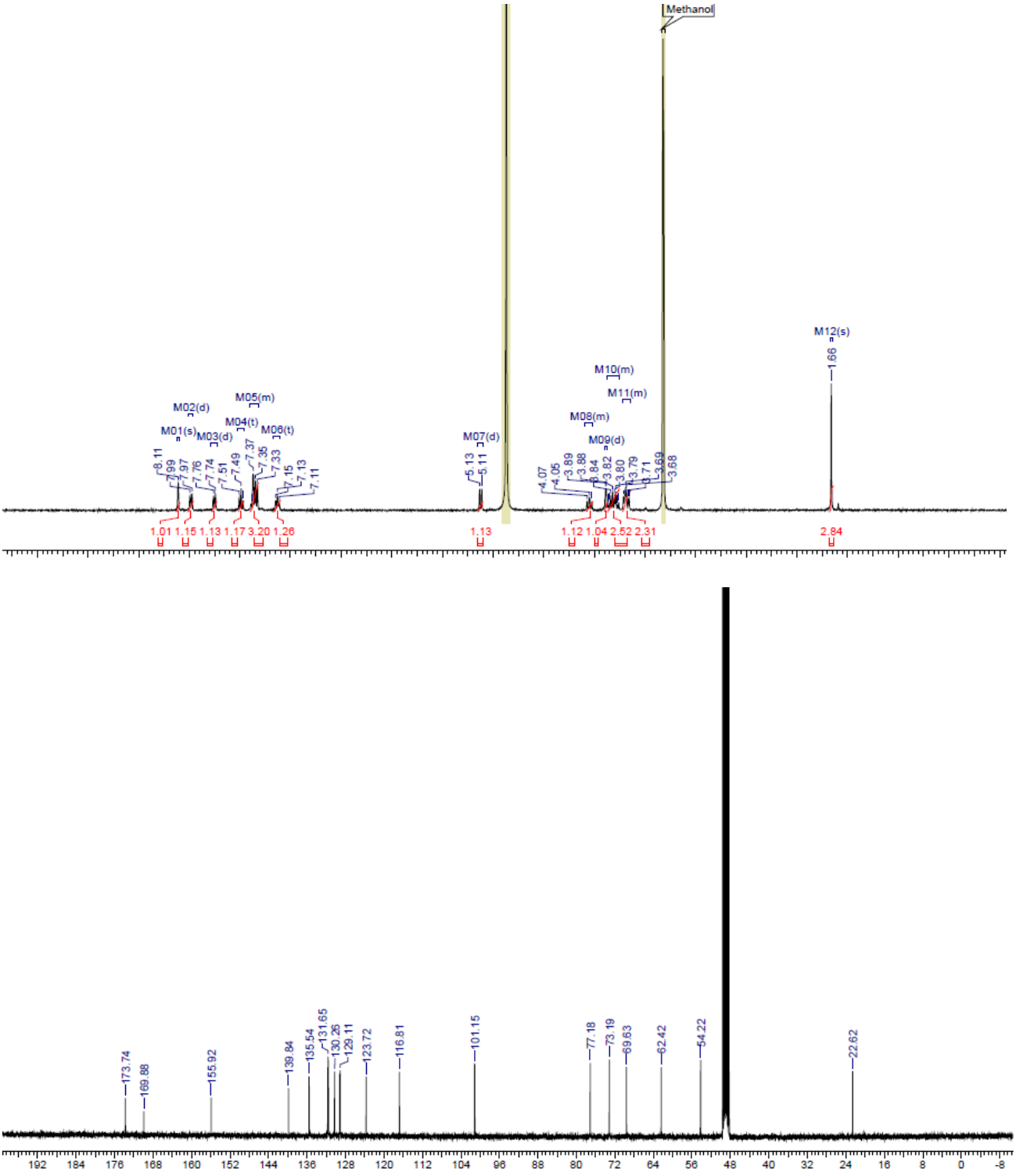


DAD1 B, Sig=254,16 Ref=off

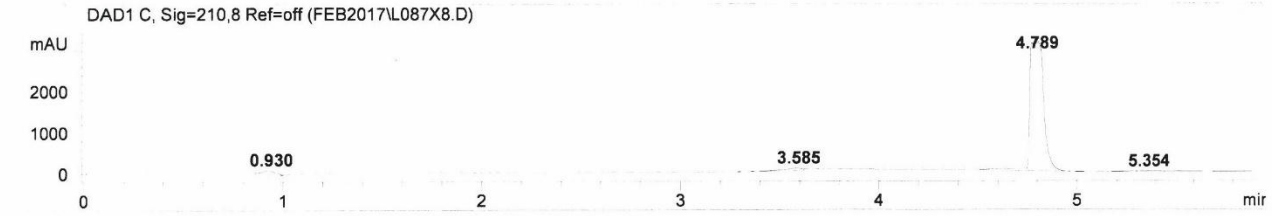
#	Meas. Ret	Area	Area %
1	3.56	4.462e3	100.00

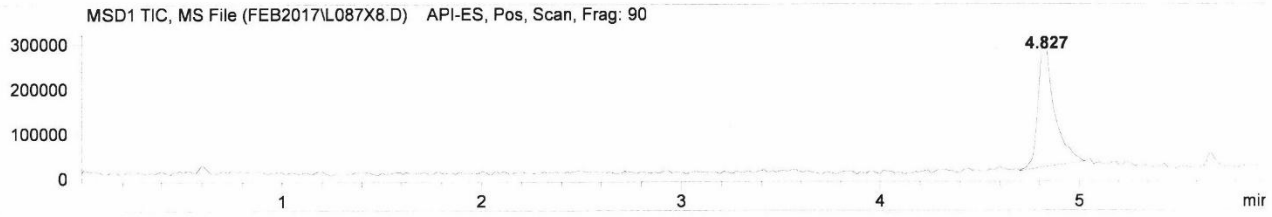


^{1}H &^{13}C NMR spectrum of compound **29bNAc**



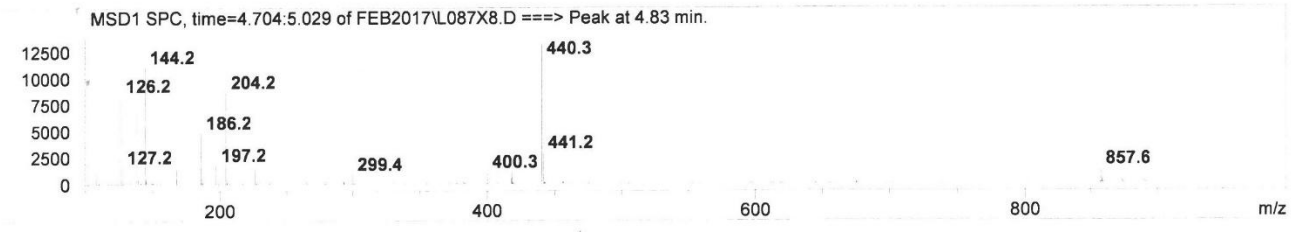
LCMS spectrum of compound **29bNAc**



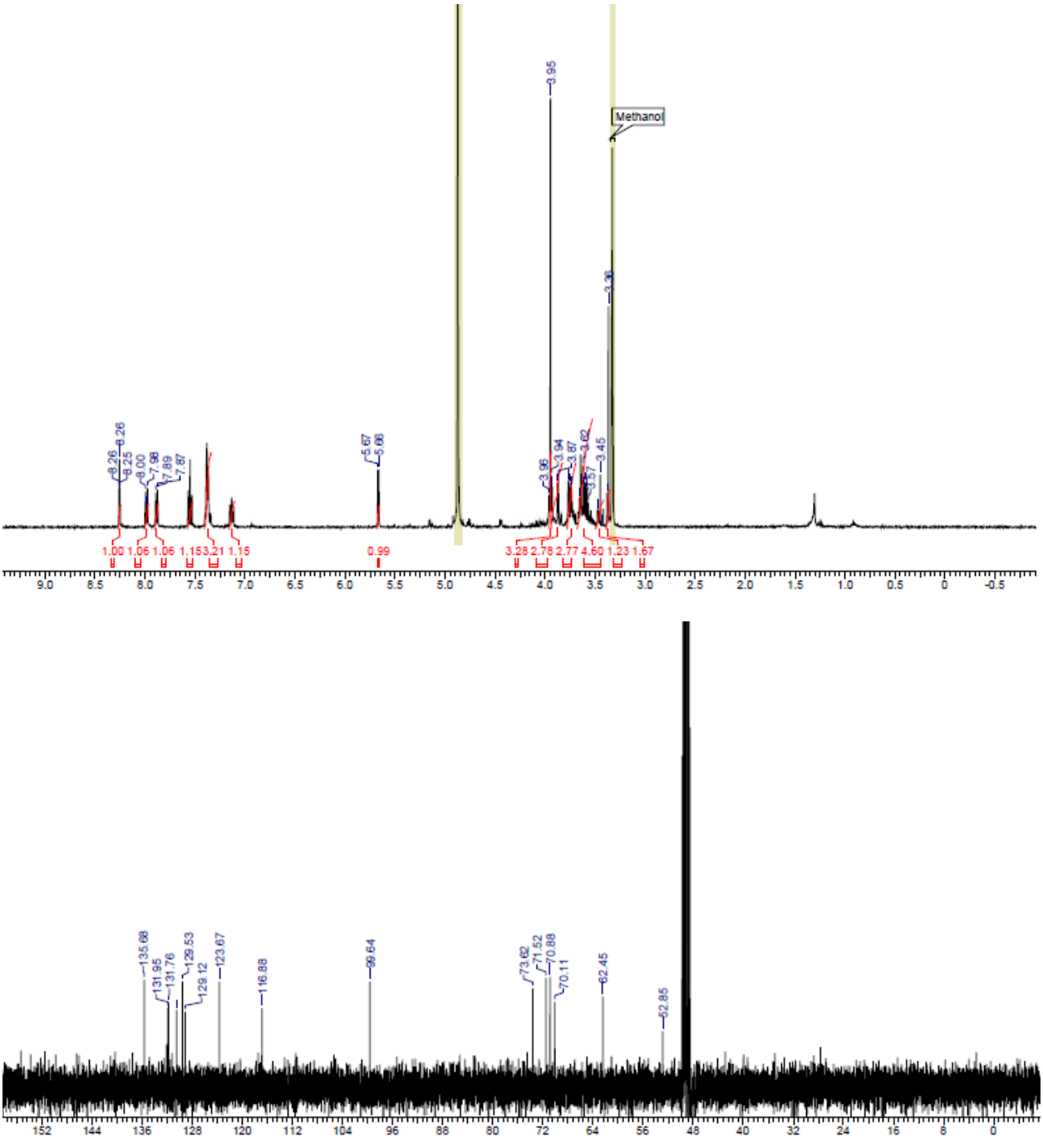


DAD1 B, Sig=254,16 Ref=off

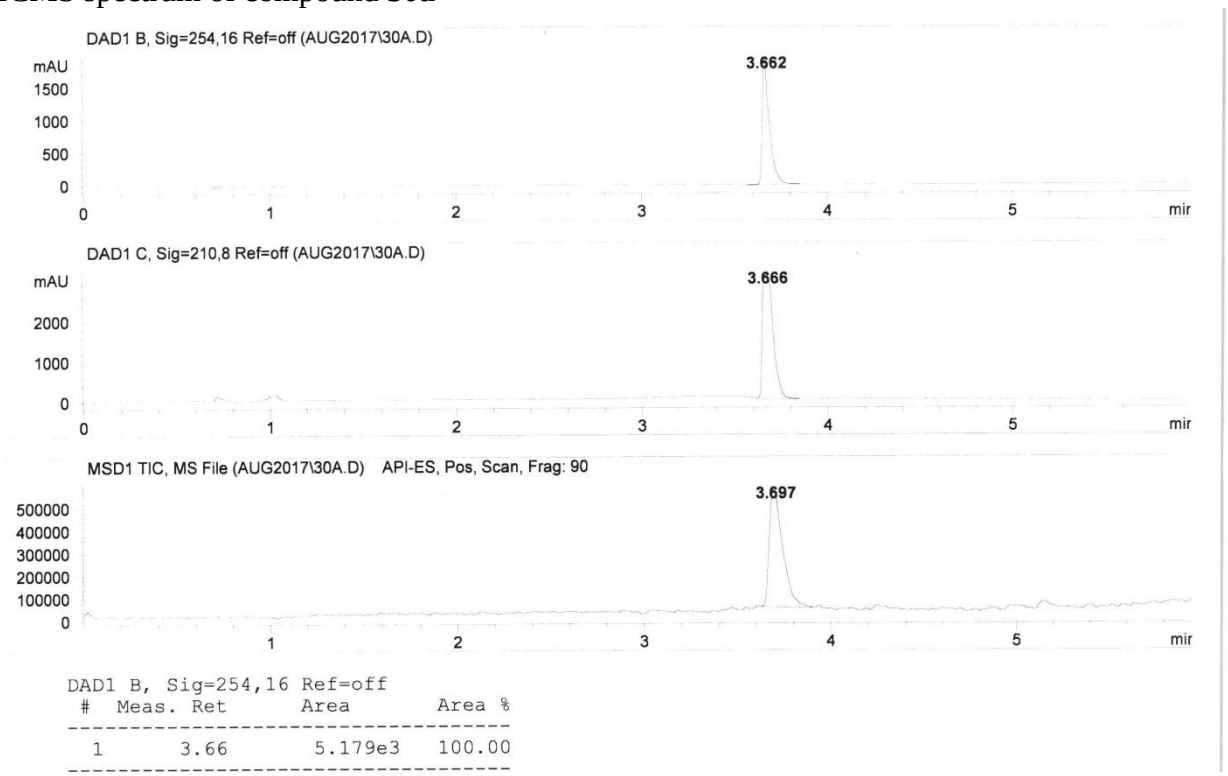
#	Meas. Ret	Area	Area %
1	4.79	8.396e3	100.00



^{1}H &^{13}C NMR spectrum of compound **30a**

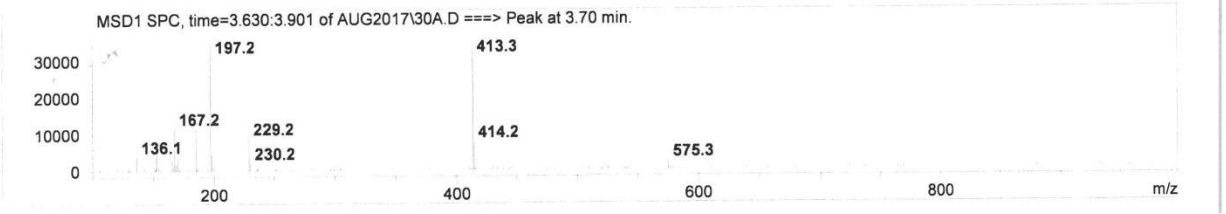


LCMS spectrum of compound **30a**

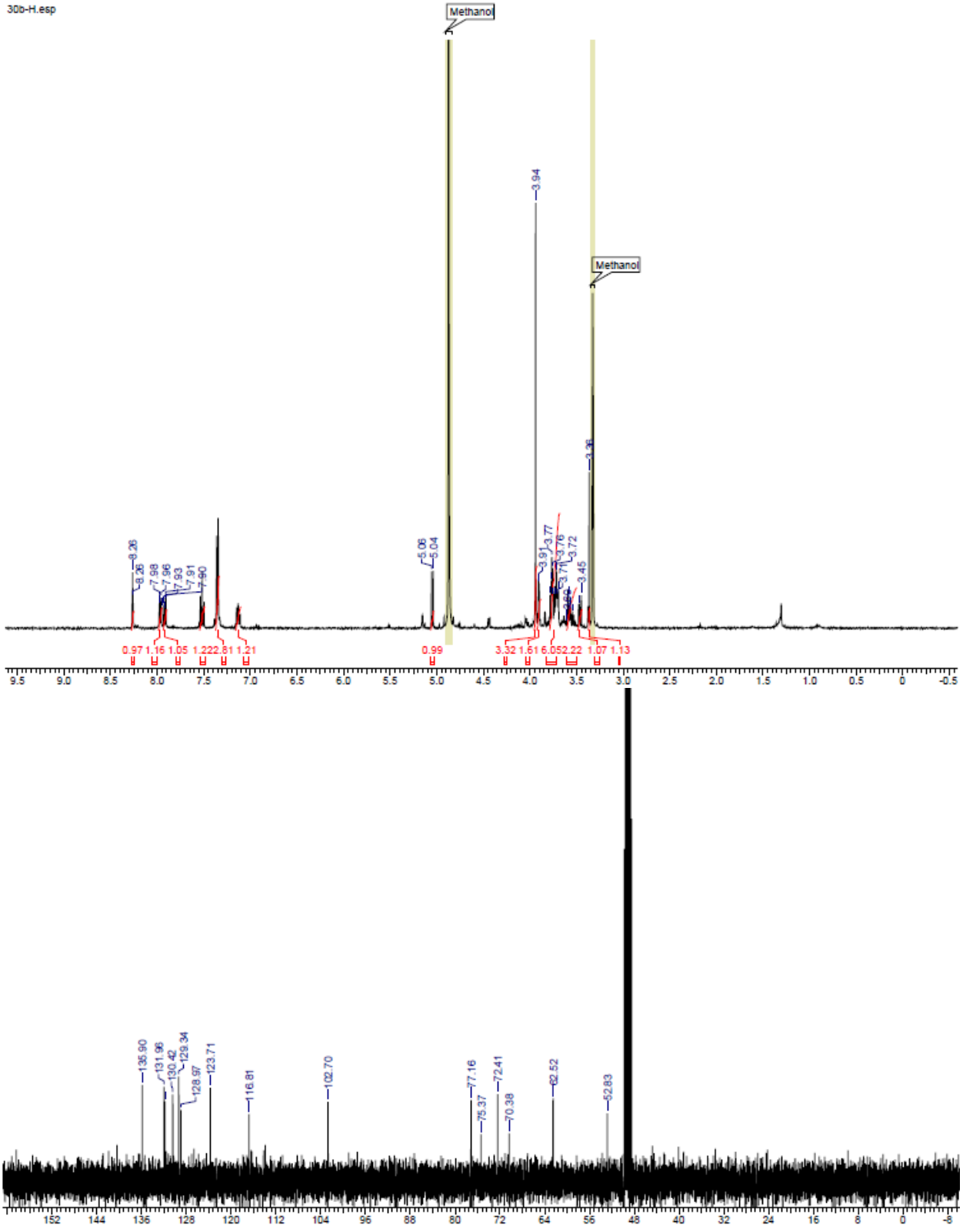


DAD1 B, Sig=254,16 Ref=off

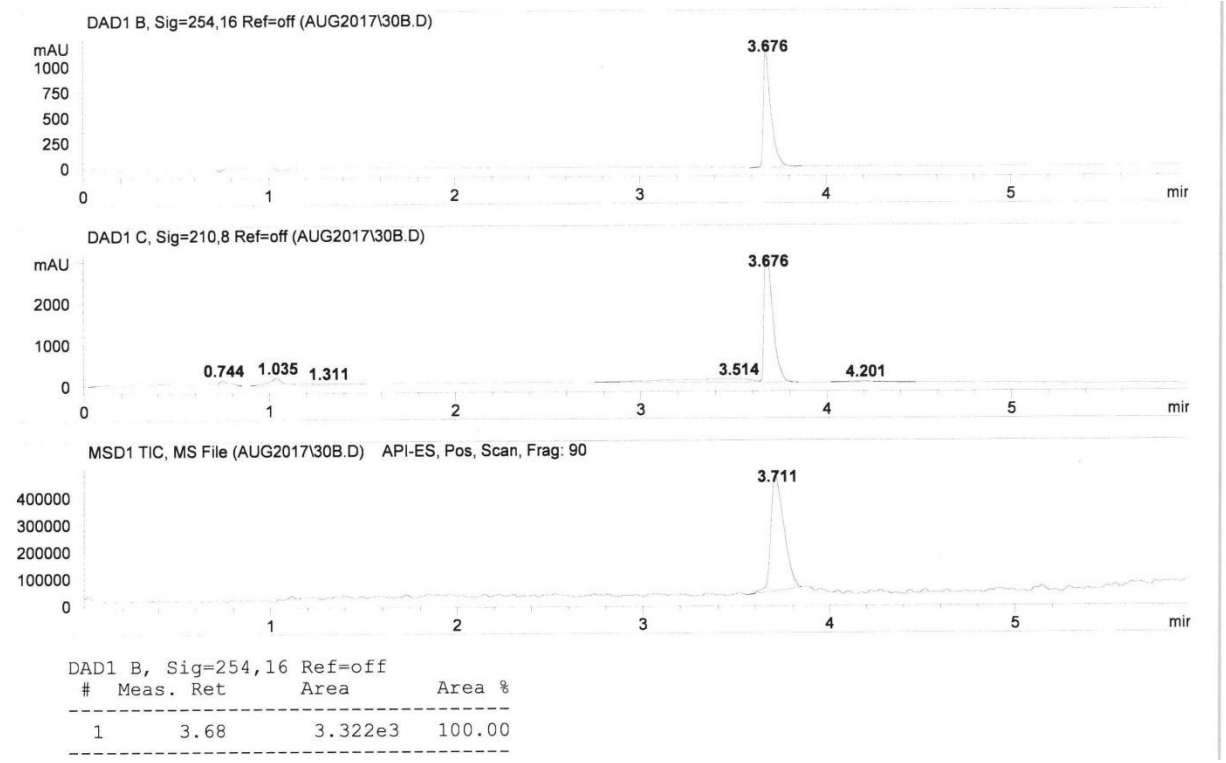
#	Meas. Ret	Area	Area %
1	3.66	5.179e3	100.00



^{1}H &^{13}C NMR spectrum of compound **30b**

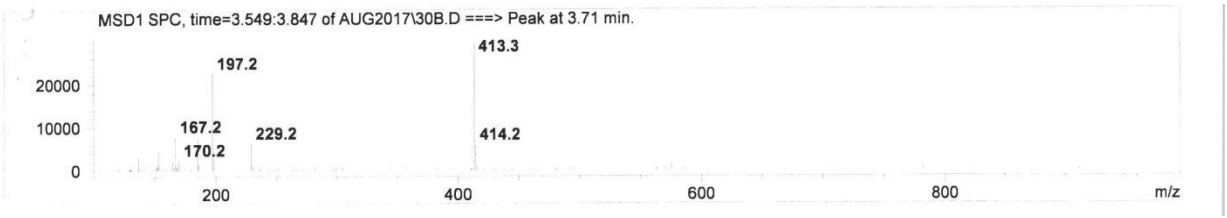


LCMS spectrum of compound **30b**

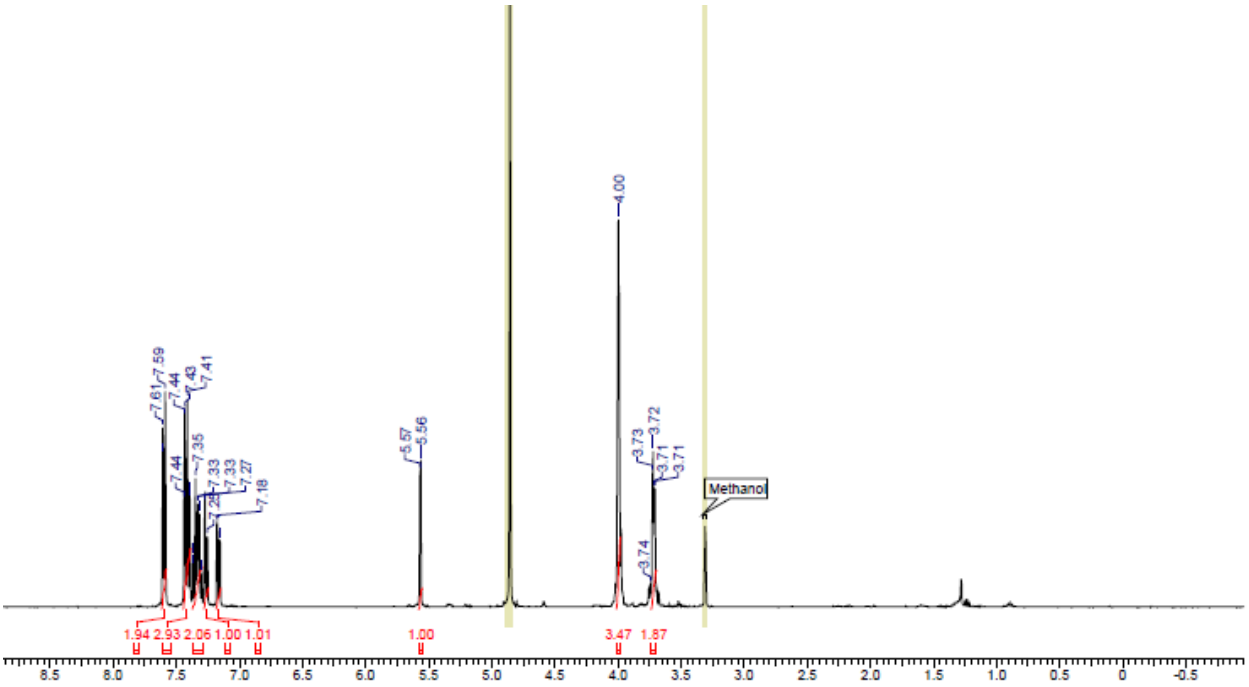


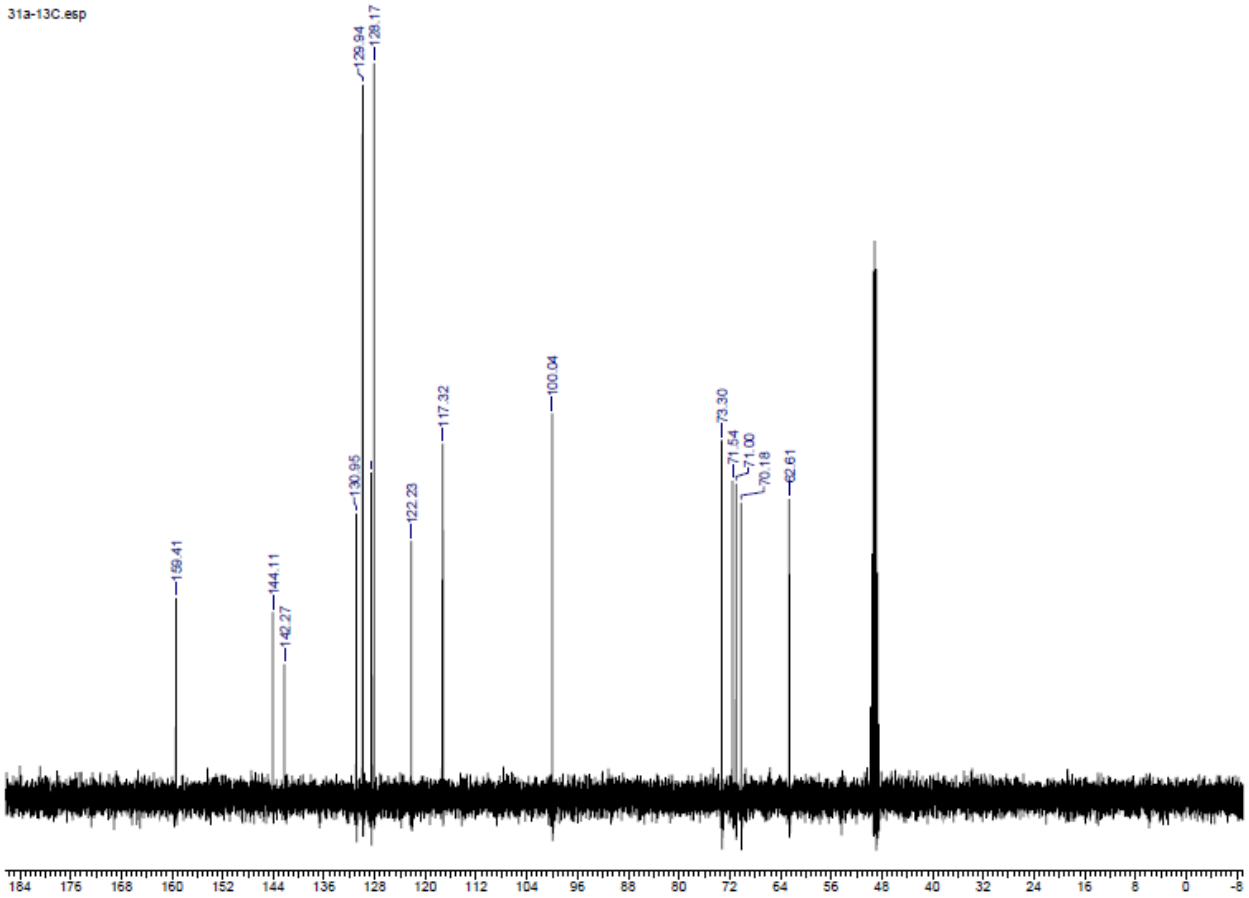
DAD1 B, Sig=254,16 Ref=off

#	Meas. Ret	Area	Area %
1	3.68	3.322e3	100.00

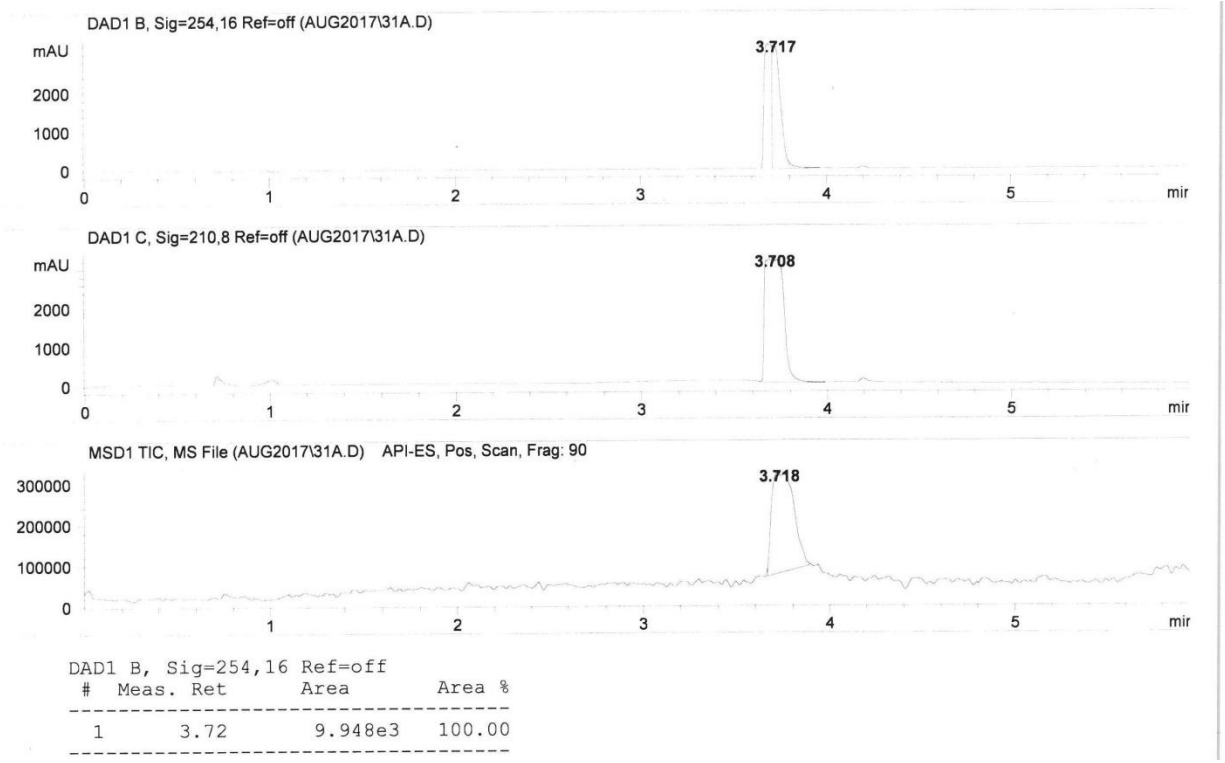


^{1}H &^{13}C NMR spectrum of compound **31a**



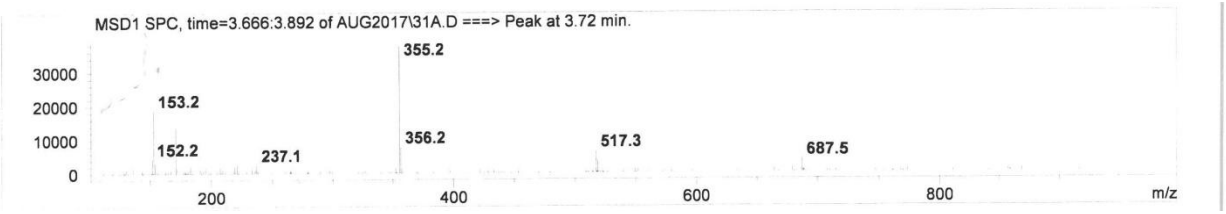


LCMS spectrum of compound **31a**

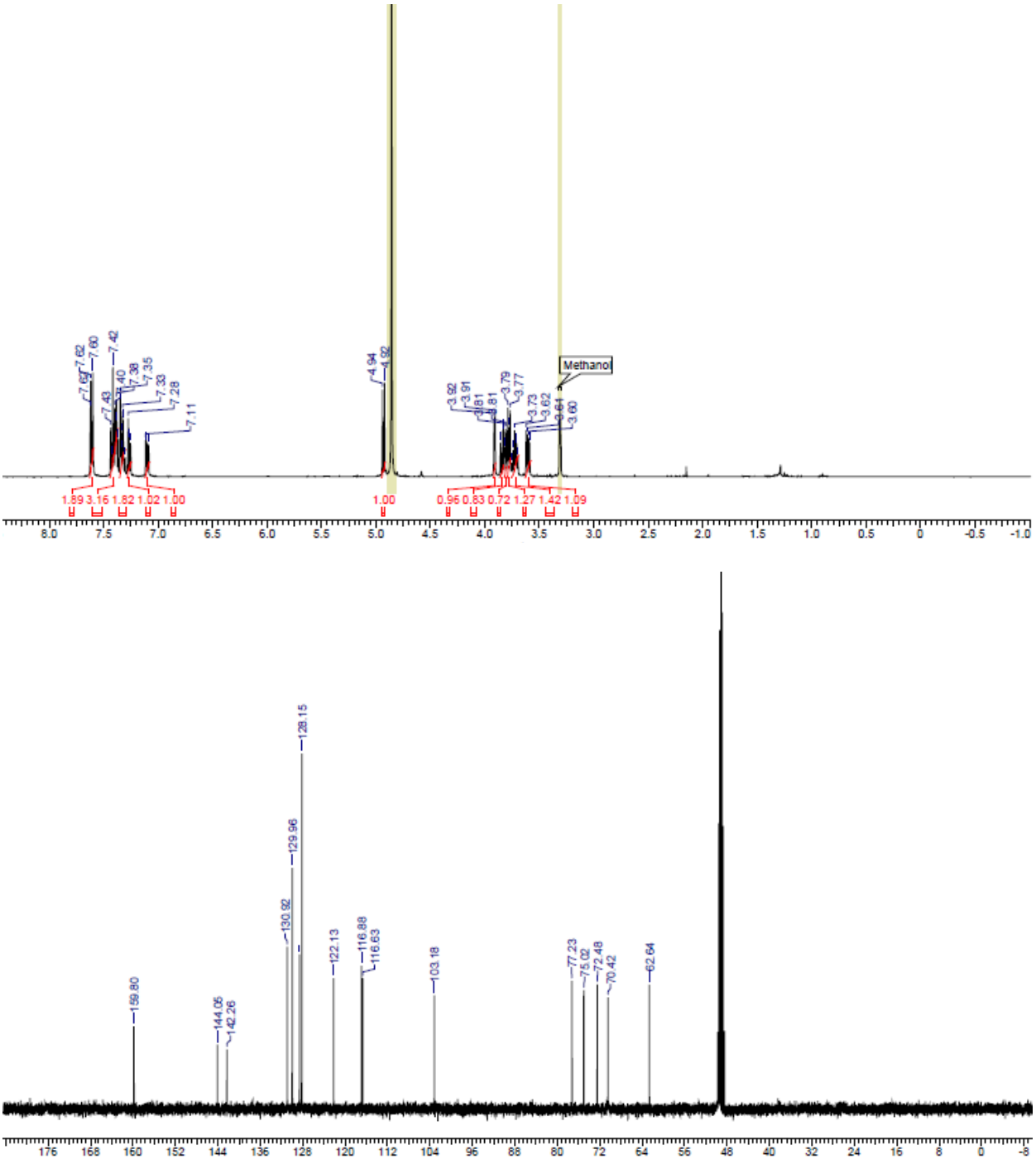


DAD1 B, Sig=254,16 Ref=off

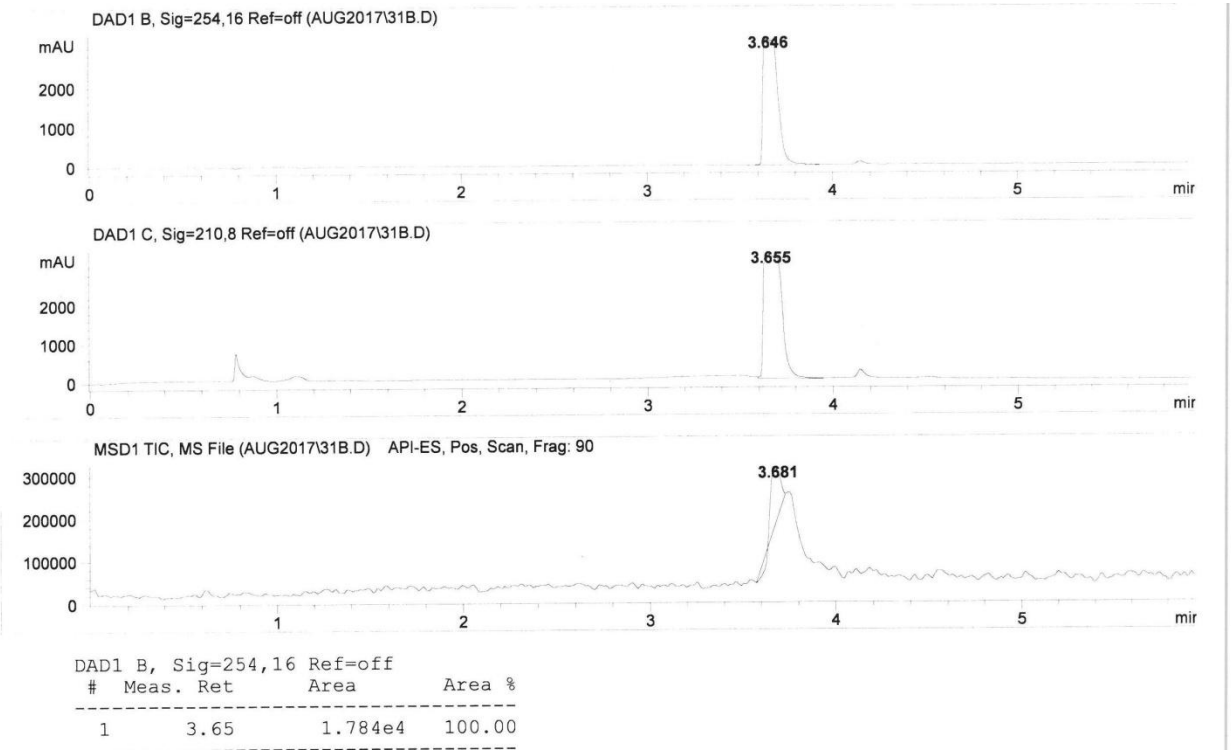
#	Meas. Ret	Area	Area %
1	3.72	9.948e3	100.00



^{1}H &^{13}C NMR spectrum of compound **31b**

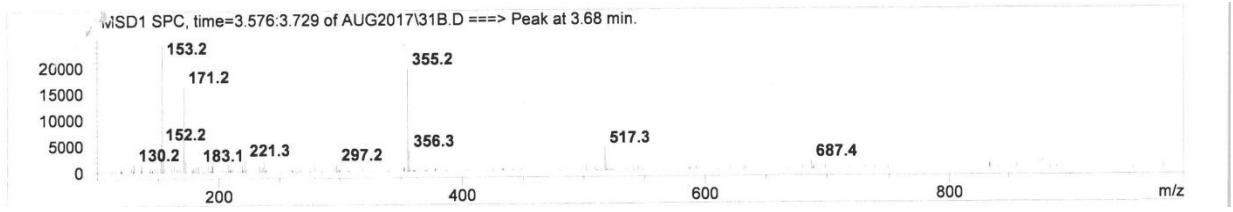


LCMS spectrum of compound **31b**

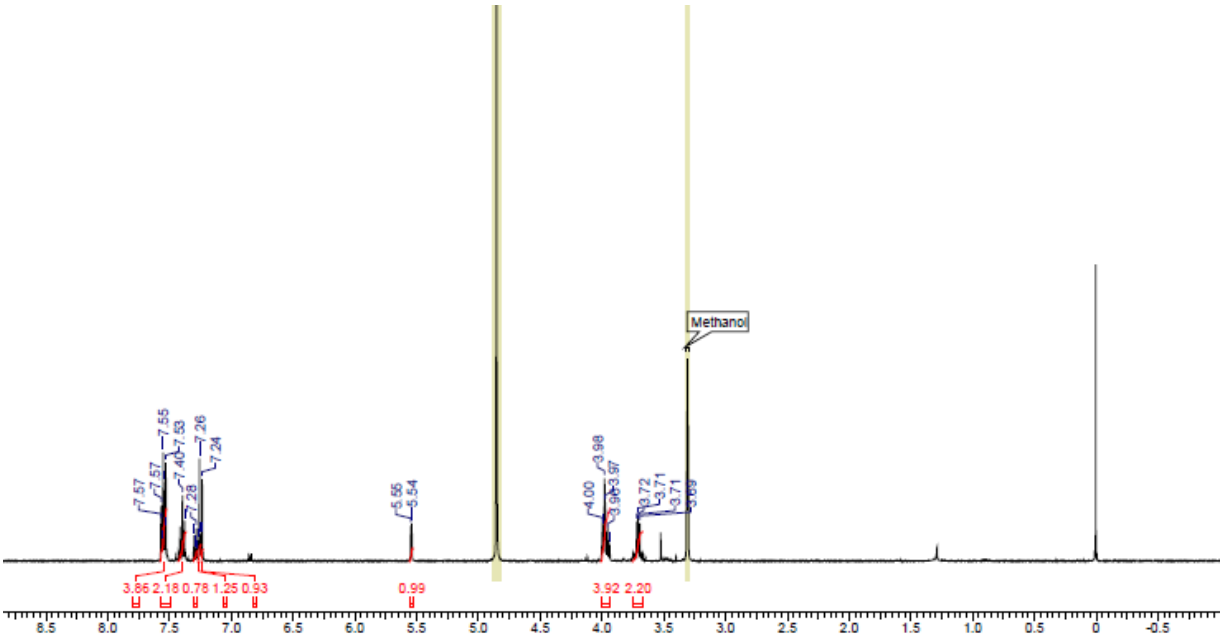


DAD1 B, Sig=254,16 Ref=off

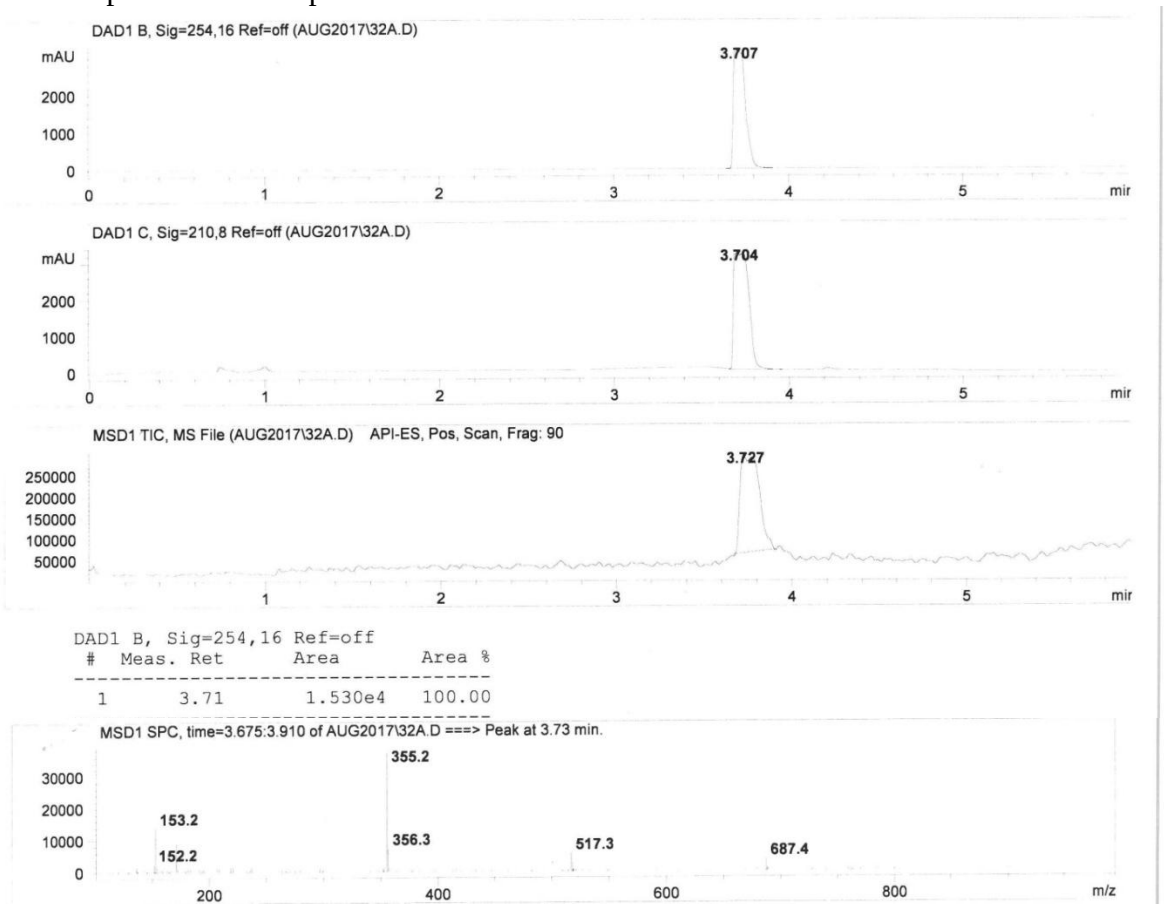
#	Meas. Ret	Area	Area %
1	3.65	1.784e4	100.00



^{1}H NMR spectrum of compound **32a**



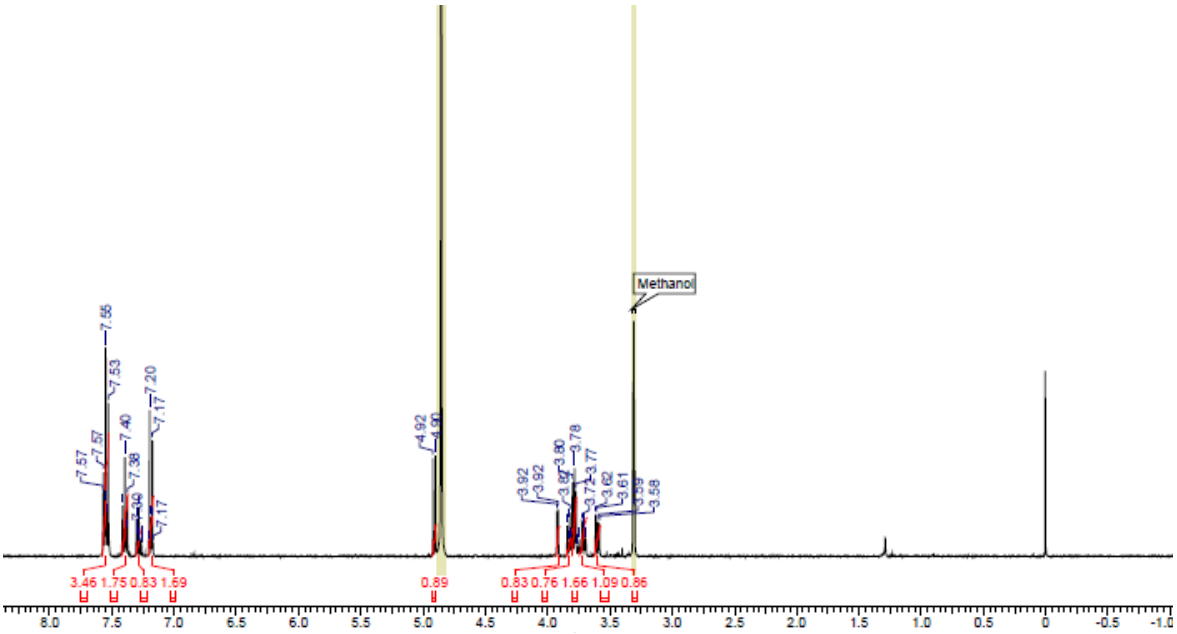
LCMS spectrum of compound **32a**



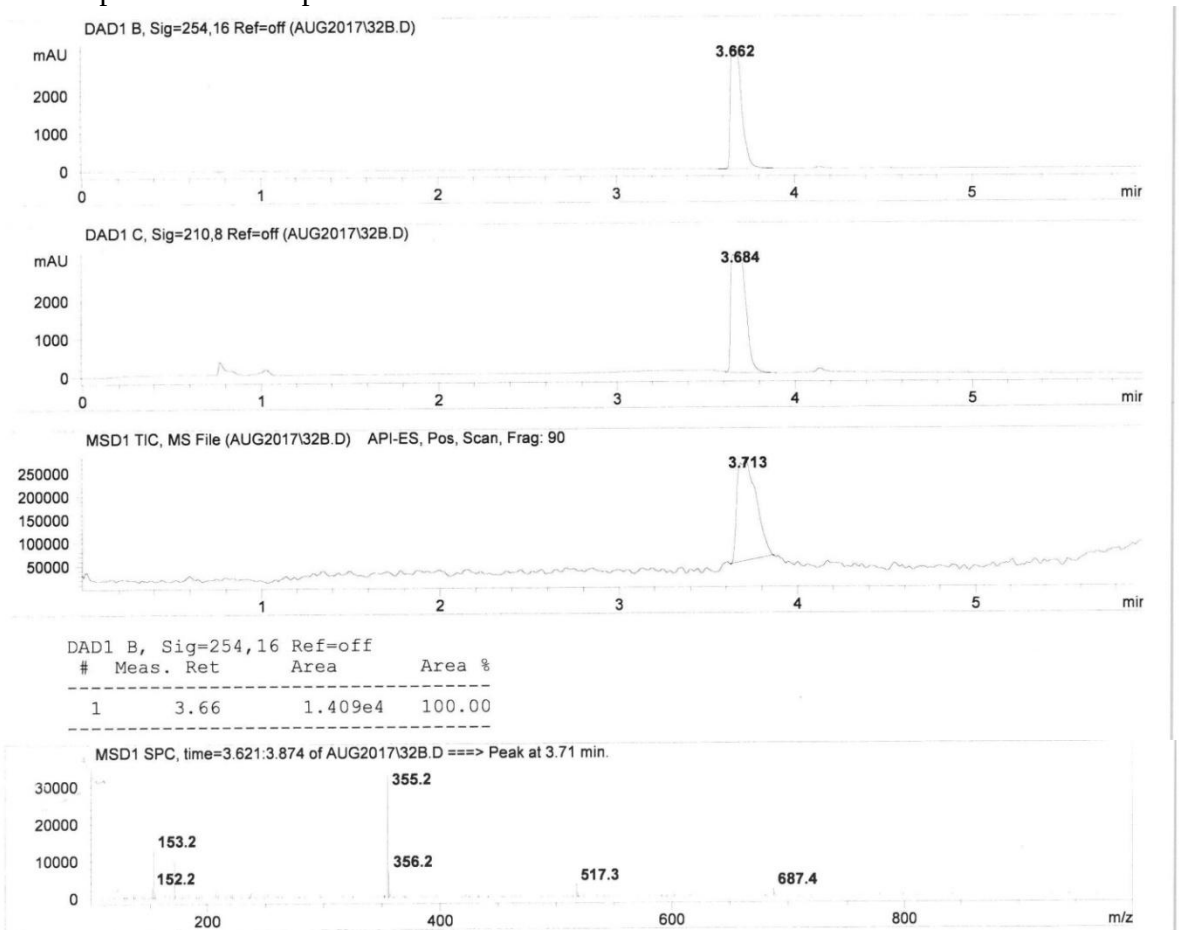
DAD1 B, Sig=254,16 Ref=off

#	Meas. Ret	Area	Area %
1	3.71	1.530e4	100.00

^{1}H NMR spectrum of compound **32b**



LCMS spectrum of compound **32b**



DAD1 B, Sig=254,16 Ref=off

#	Meas. Ret	Area	Area %
1	3.66	1.409e4	100.00

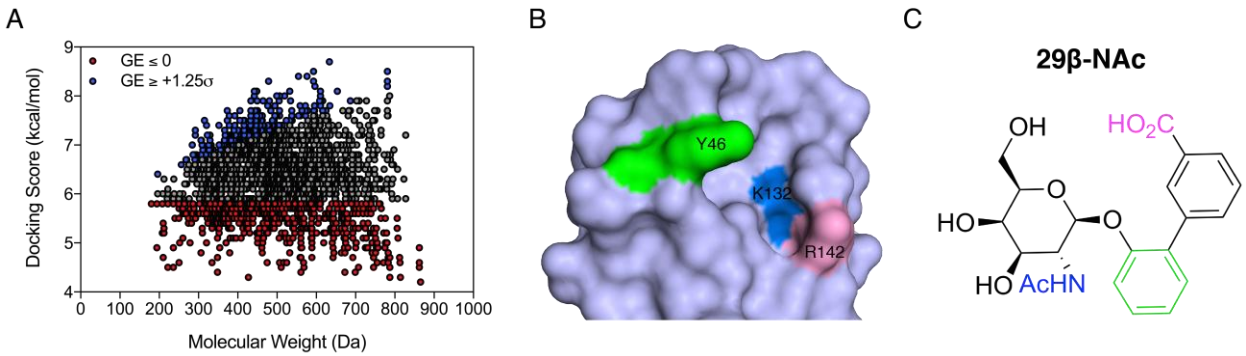


Figure S1. Virtual screen and structure-guided design of galactosides targeting $FmlH_{LD}$. (**A**) Summary of virtual screen of galactosides against $FmlH_{LD}$, in which the docking score of the top predicted binding mode is plotted against the molecular weight for each galactoside. Compounds with GE values 1.25σ above the mean are colored blue while compounds with GE values below 0 are colored red. (**B**) Surface representation of $FmlH_{LD}$ with hot spot residues Y46, K132, and R142 colored green, blue, and pink, respectively. Hot spot residues were identified as common targets observed in the binding modes of the top compounds from virtual screening. (**C**) Chemical structure of compound **29β-NAc**, with the carboxylic acid in pink designed to interact with R142, the phenyl ring in green designed to interact with Y46, and the N-Acetyl group in blue designed to interact with K132.

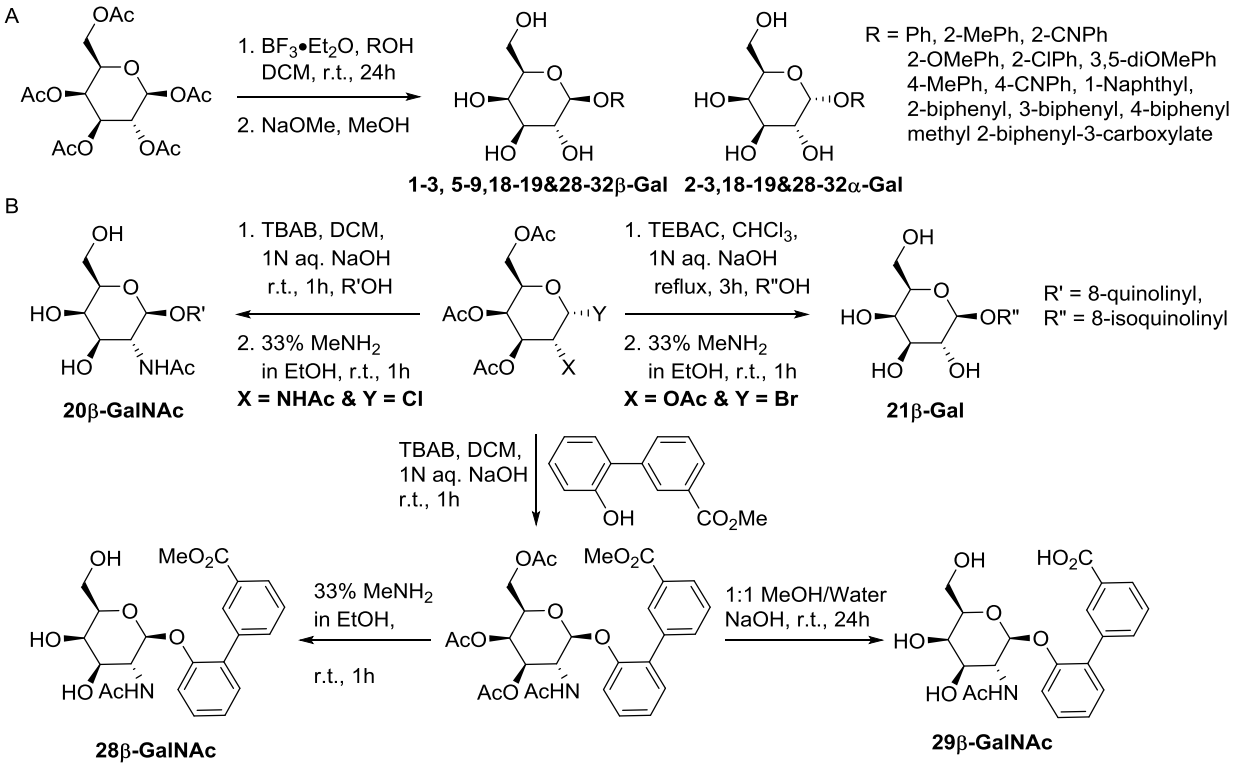


Figure S2. Synthesis of galactoside-based FmlH ligands. (**A**) Boron trifluoride promoted glycosidation reaction of protected galactose followed by deprotection to yield galactosides. (**B**) Koenigs-Knorr type reaction followed by deprotection for the substitution of a galactosyl halide with an alcohol to yield galactoside and galactosaminosides.

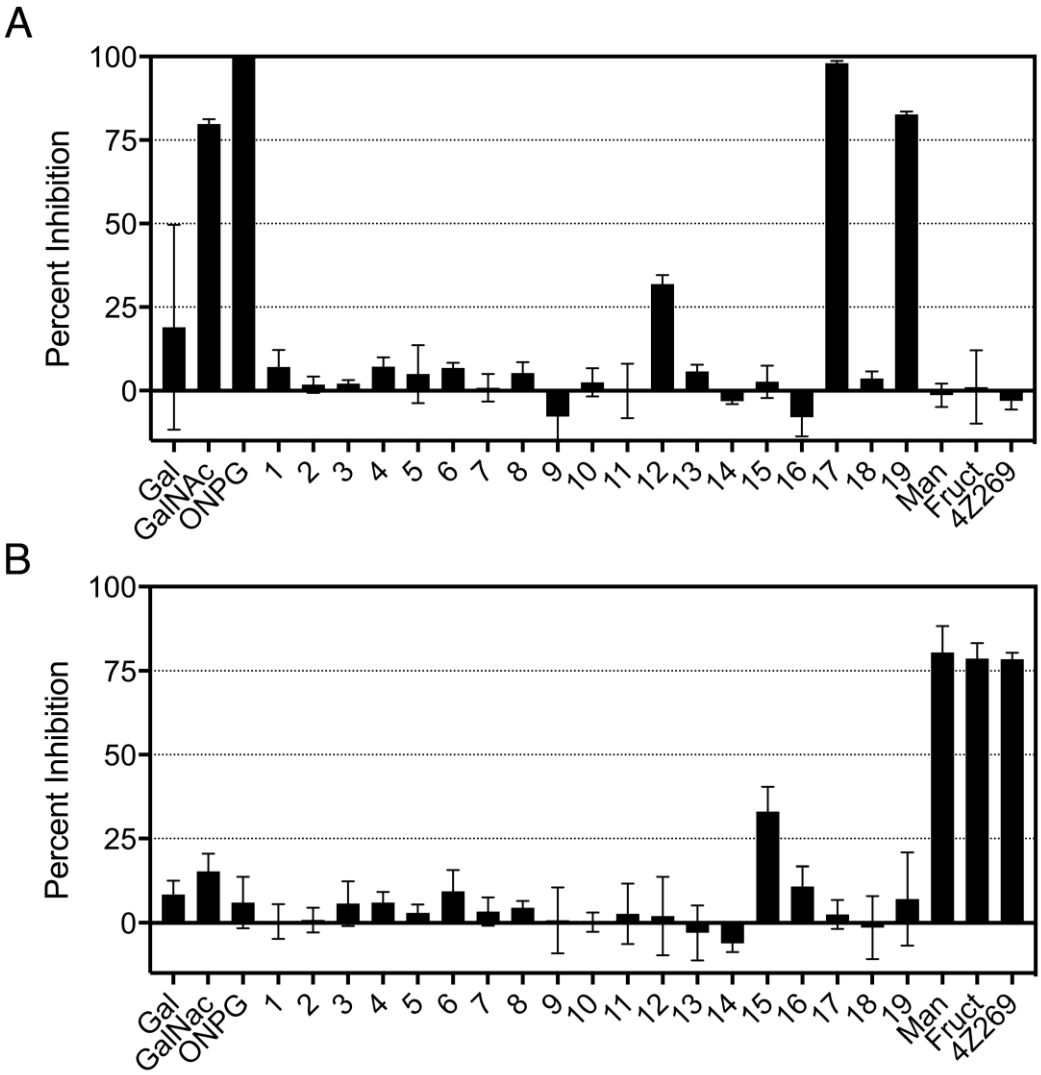


Figure S3. Cranberry-derived galactosides can inhibit FmlH. (**A**) Cranberry-derived compounds and fractions were tested at 1 mM in the ELISA-based competition assay for inhibition of $FmlH_{LD}$. (**B**) As a control, cranberry-derived compounds and fractions were tested at 1 mM in the ELISA-based competition assay for inhibition of $FimH_{LD}$. The identifies of the compounds indicated above are as follows: 1 (quinic acid), 2 (gallic acid), 3 (p-coumaric acid), 4 (2,4-dihydrobenzoic acid), 5 (protocatechuic acid), 6 (ferulic acid), 7 (vanillic acid), 8 (catechin), 9 (epicatechin), 10 (quecetin), 11 (quercitrin), 12 (quercetin galactoside; **26β**) 13 (myricetin), 14 (myricetrin), 15 (cranberry fraction 1 – oligosaccharide), 16 (cranberry fraction 2 – anthocyanins/flavonols), 17 (cranberry fraction 3 mixed-sized proanthocyanidins), 18 (cyanidin arabinoside), 19 (cyanidin galactoside; **24β**), Fruct (fructose).

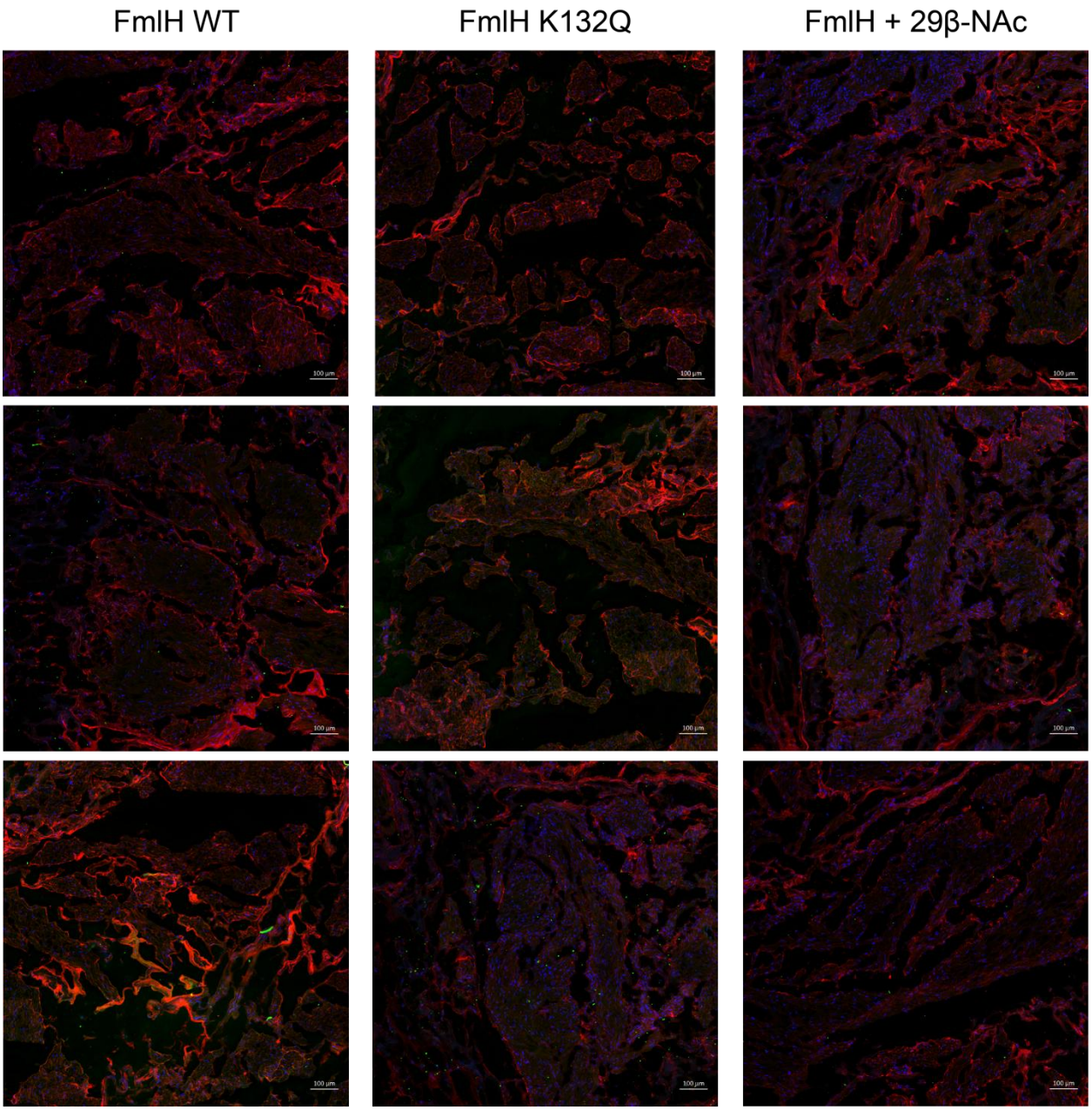


Figure S4. Immunofluorescence analysis of $FmlH_{LD}$ WT, $FmlH_{LD}$ K132Q, or $FmlH_{LD}$ WT in the presence of **29β-NAc** binding to human bladder tissue. Green corresponds to FmlH, red corresponds to Wheat Germ Agglutinin, and blue corresponds to DAPI.

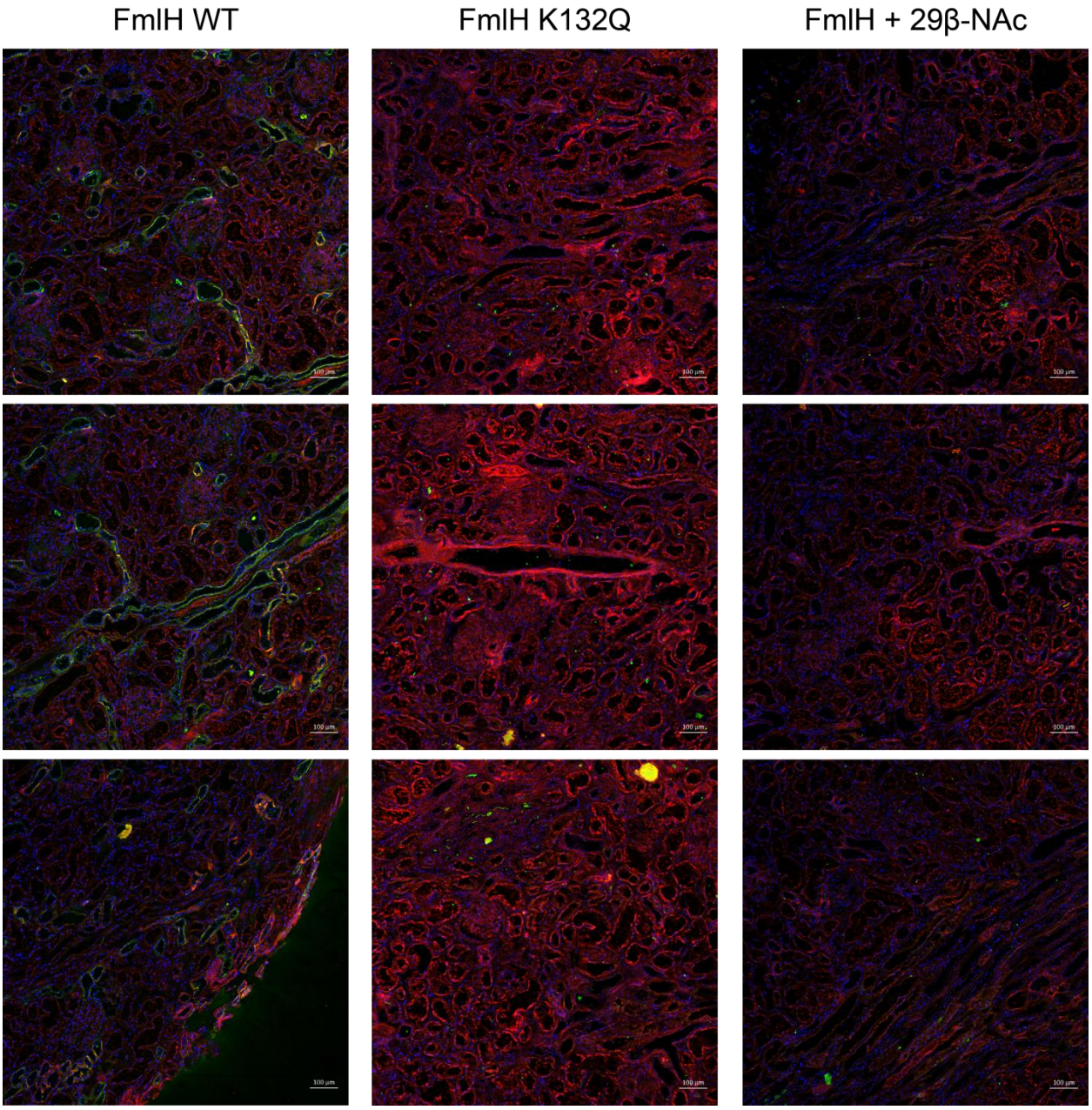


Figure S5. Immunofluorescence analysis of $FmlH_{LD}$ WT, $FmlH_{LD}$ K132Q, or $FmlH_{LD}$ WT in the presence of **29β-NAc** binding to human kidney tissue. Green corresponds to FmlH, red corresponds to Wheat Germ Agglutinin, and blue corresponds to DAPI.

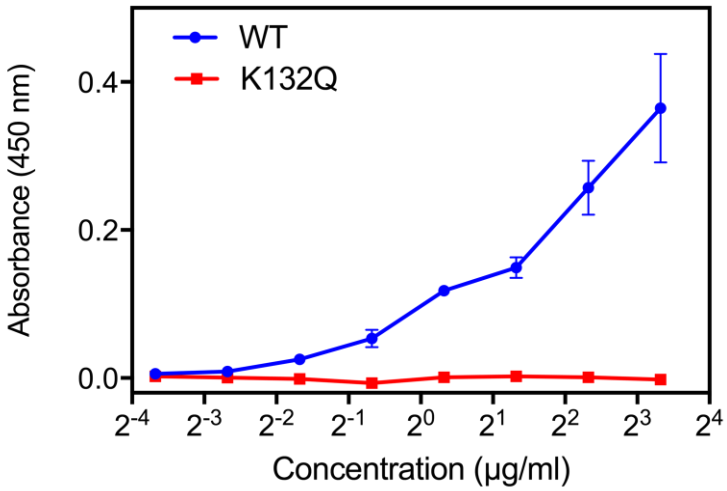


Figure S6. Mutagenesis of FmlH binding pocket abrogates function. Varying concentrations of $FmlH_{LD}$ WT and K132Q were tested for binding to sialidase-treated BSM by ELISA.

Table S1. Data collection and refinement statistics

	apo $FmlH_{LD}$ (6AOW)	$FmlH_{LD}$:TF (6AOX)	$FmlH_{LD}$:ONPG (6AOY)	$FmlH_{LD}$:**4β** (6ARM)	$FmlH_{LD}$:**5β** (6ARN)	$FmlH_{LD}$:**20β** (6ARO)	$FmlH_{LD}$:**29β-Nac** (6AS8)
Data collection							
Space group	C 2	C 2 2 21	C 2	P 2 21 21	P 21 21 2	P 21 21 2	P 2 21 21
Cell dimensions							
a, *b*, *c* (Å)	65.5, 78.3, 58.5	67.4, 78.1, 105.5	66.2, 78.3, 58.5	50.0, 51.3, 114.6	51.3, 116.1, 50.3	51.3, 116.3, 50.6	51.0, 51.5, 117.5
α, *β*, *γ* (°)	90.0, 97.0, 90.0	90.0, 90.0, 90.0	90.0, 97.5, 90.0	90.0, 90.0, 90.0	90.0, 90.0 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution (Å)	50.0-1.6 (1.63-1.60)	52.8-2.1 (2.21-2.10)	40.0-1.8 (1.90-1.80)	57.3-1.50 (1.53-1.50)	51.3-1.25 (1.27-1.25)	58.1-1.15 (1.17-1.15)	50.0-2.10 (2.14-2.10)
R_{merge} (%)[a]	9.3 (139)	27.2 (189)	8.1 (67.8)	6.8 (137)	6.5 (192)	11.4 (86.9)	14.6 (51.3)
R_{pim} (%)[b]	5.7 (87.6)	8.0 (55.7)	3.2 (32.2)	2.8 (68.7)	2.7 (102)	4.8 (63.7)	4.1 (26.0)
I / σI	8.7 (0.9)	8.0 (1.6)	15.6 (2.8)	16.0 (0.8)	15.0 (0.7)	9.2 (1.0)	16.1 (1.5)
Completeness (%)	98.5 (99.8)	99.6 (99.9)	99.6 (98.5)	96.4 (78.3)	99.2 (91.5)	93.6 (58.5)	87.7 (44.2)
Multiplicity	3.5 (3.4)	12.0 (12.0)	6.9 (5.1)	6.2 (4.1)	6.5 (4.2)	6.0 (2.6)	10.7 (2.5)
$CC_{1/2}$	1.0 (0.38)	0.99 (0.39)	1.0 (0.79)	1.0 (0.28)	1.0 (0.31)	0.99 (0.46)	0.99 (0.82)
Total / Unique reflections	134,257/38,073(6,454/1,891)	199,439/16,562(28,511/2,379)	188,555/27,380(19,891/3,397)	288,591/46,203(7,374/1,811)	544,119/83,215(15,705/3,708)	610,631/101,126(7,812/3,048)	176,770/16,455(932/412)
Refinement							
R_{work}[c] / R_{free}[d]	19.5 / 23.3	22.5 / 25.9	18.5 / 22.0	20.1 / 24.0	20.8 / 22.4	17.6 / 19.0	20.1 / 24.0
No. atoms							
Protein	2307	2247	2345	2309	2321	2431	2322
Ligand/ion	15	52	26	47	45	49	60
Water	262	140	263	326	350	513	126
B-factors							
Protein	24.9	35.6	26.9	23.2	19.7	14.5	30.2
Ligand/ion	40.6	31.5	40.1	25.2	19.4	14.7	32.3
Water	35.5	38.2	32.8	30.8	29.5	30.5	32.6
R.m.s. deviations							
Bond lengths (Å)	0.009	0.004	0.008	0.008	0.006	0.013	0.011
Bond angles (°)	1.19	0.98	1.11	1.19	1.10	1.54	0.81
Ramachandran plot							
favored (%)	97.0	98.0	96.0	94.0	97.0	97.0	96.0
allowed (%)	3.0	2.0	3.0	5.7	2.3	2.7	3.7
outliers (%)	0	0	1.0	0.3	0.7	0.3	0.3
Clashscore	2.6	1.6	3.9	9.1	6.1	3.5	0.4

[a] $R_{merge} = \Sigma_{hkl} \Sigma_i |I_i(hkl) - \langle I(hkl) \rangle| / \Sigma_{hkl} \Sigma_i I_i(hkl)$, where the sum *i* is over all separate measurements of the unique reflection *hkl*.

[b] $R_{pim} = \Sigma_{hkl} [1/(n-1)]^{1/2} \Sigma_i |I_i(hkl) - \langle I(hkl) \rangle| / \Sigma_{hkl} \Sigma_i I_i(hkl)$

[b] $R_{work} = \Sigma_{hkl} ||F_{obs}| - |F_{calc}|| / \Sigma_{hkl} |F_{obs}|$

[c] $\boldsymbol{R}_{free}$, calculated the same as for $\mathbf{R}_{work}$ but on the 5% data randomly excluded from the refinement calculation.

Values in parentheses indicate the highest resolution shell

Table S2. Galactoside inhibition of FmlH.

	PI_{100}	PI_{10}	PI_{1}
29β-NAc	99.5 ± 2.2	93.0 ± 1.5	54.7 ± 2.8
4β-NAc	99.4 ± 1.3	87.2 ± 1.7	28.6 ± 4.5
29β	99.0 ± 1.3	75.1 ± 1.0	22.9 ± 5.1
5β	97.2 ± 0.4	55.7 ± 1.6	10.0 ± 4.3
20β	95.1 ± 1.1	48.1 ± 2.0	19.2 ± 2.8
3β	94.5 ± 0.3	30.4 ± 1.8	12.1 ± 6.3
4β (ONPG)	93.0 ± 1.4	31.1 ± 3.3	16.5 ± 3.8
28β	91.1 ± 1.2	31.0 ± 2.1	8.1 ± 6.0
6β	90.5 ± 0.6	20.7 ± 5.5	8.0 ± 4.0
14β	89.2 ± 0.4	19.8 ± 3.8	10.2 ± 4.3
2β	87.3 ± 3.7	28.2 ± 1.8	1.8 ± 5.7
30β	86.6 ± 0.8	22.4 ± 2.6	3.7 ± 3.5
32α	86.5 ± 1.7	22.6 ± 3.9	
8β	85.8 ± 0.7	16.5 ± 3.3	4.6 ± 7.9
9β	85.7 ± 1.6	19.2 ± 4.8	9.5 ± 3.7
12β	85.3 ± 0.7	19.7 ± 4.2	8.7 ± 3.1
11α-NAc	82.0 ± 2.7	6.3 ± 3.7	
15β	80.4 ± 6.6	24.0 ± 3.2	
19β	78.8 ± 2.1	14.6 ± 3.1	
11β	78.1 ± 0.6	13.7 ± 5.3	
1β	76.9 ± 0.4	15.5 ± 4.2	
7β	76.4 ± 2.4	11.7 ± 2.7	
11β-thio	72.5 ± 0.9	17.0 ± 3.7	
10β	65.1 ± 1.4	9.8 ± 3.9	
31β	56.9 ± 6.2	7.8 ± 5.5	
22β-thio	49.8 ± 2.5	2.3 ± 2.5	
13β	49.7 ± 4.5	0.8 ± 3.1	
18β	45.9 ± 3.7		
31α	45.6 ± 2.5		
17β	41.2 ± 3.4		
2α	30.0 ± 5.4		
32β	30.0 ± 3.9		
24β	28.8 ± 6.5		
GalNAc	28.5 ± 5.2		
18α	28.1 ± 3.4		
29α	26.2 ± 5.9		
36β-thio (IPTG)	23.7 ± 5.3		
16β	21.7 ± 2.4		
35	19.4 ± 1.6		
28α	19.2 ± 3.0		
3α	17.8 ± 6.3		
TF	15.5 ± 6.7		
33	15.3 ± 3.1		
21β	15.2 ± 7.1		
26β	14.4 ± 6.8		
30α	13.1 ± 7.6		
27β	10.7 ± 7.2		
Gal	8.1 ± 2.6		
25β	3.6 ± 6.2		
4β-phospho	1.3 ± 4.7		
23β	0.7 ± 10.3		
11β-uro	-2.4 ± 5.2		
34	-3.6 ± 1.0		

Percent inhibition (PI) values are reported as the mean with standard error of the mean for galactosides tested at 100 μM (PI_{100}), 10 μM (PI_{10}), and 1 μM (PI_1) in the ELISA-based competition assay. Compounds indicated above not shown in Figure S1 include **33** (p-nitrophenyl Gal-β1-3-GalNAc), **34** (p-nitrophenyl Gal-β1-3-GlcNAc), **35** (p-nitrophenyl Gal-β1-6-Gal), and **36β-thio** (IPTG). The "thio" designation indicates a sulfur linkage between the sugar and the aglycone group, the "phospho" designation indicates a phosphate group attached the C6-hydroxyl group on the sugar, and the "uro" designation indicates galacturonide as the sugar.