Electronic Supplementary Material (ESI) for New Journal of Chemistry

Supporting Informations

for

Synthesis of multi-antigenic platforms as vaccine candidates against cancers

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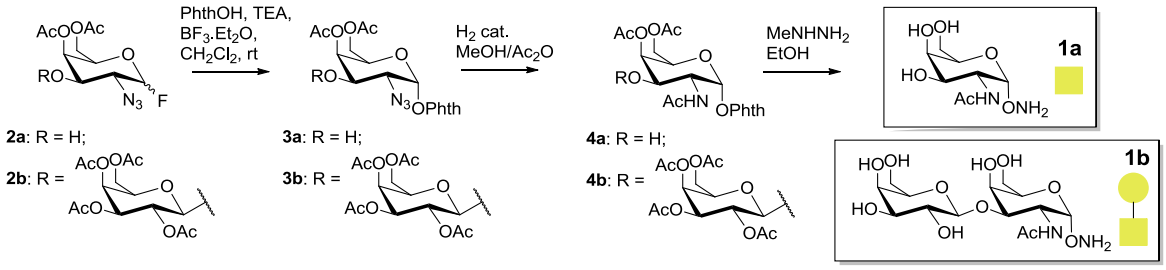


Scheme 1. Synthetic scheme of aminooxy carbohydrate antigens 1a and 1b.

Standard procedure for glycosylation with N-hydroxyphthalimide.

In dry CH_2Cl_2, glycosyl fluoride**,** *N*-hydroxyphthalimide (1 equiv.), triethylamine (1 equiv.) and $BF_3{\cdot}Et_2O$ (4 equiv.) were stirred at room temperature for 1 h. CH_2Cl_2 was next added to the crude mixture, the organic layer was washed twice with 10% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated.

*Compound **3a***. This compound was synthesized from **2a** (4.30 g, 13.0 mmol) using this standard procedure. The resulting mixture of α and β anomers was separated by silica gel chromatography (CH_2Cl_2/ethyl acetate, 9/1) to give **3a** (2.35 g, 38%) after crystallization from diethyl ether/pentane; m.p. 105-107°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.85-7.75 (m, 4H, $H_{ar.}$), 5.57 (bd, 2H, $J_{1,2}$ = $J_{3,4}$ = 3.6 Hz, H-1, H-4), 5.47 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 11.3 Hz, H-3), 5.17 (bt, 1H, $J_{5,6a}$ = 6.4 Hz, H-5), 4.23 (dd, 1H, $J_{5,6a}$ = 6.4 Hz, $J_{6a,6b}$ = 11.3 Hz, H-6a), 3.97-3.92 (m, 2H, H-2, H-6b), 2.14, 2.06, 2.02 (3s, 9H, 3x$OCOCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.8 (C=O), 170.3 (C=O), 169.9 (C=O), 163.3 (C=O), 135.2 ($CH_{ar.}$), 129.1 ($C_{ar.}$), 124.2 ($CH_{ar.}$), 103.5 (C-1), 69.2 (C-5), 68.2 (C-4), 67.7 (C-3), 61.6 (C-6), 57.0 (C-2), 21.1 (CH_3); ES-MS (positive mode): calcd for $C_{20}H_{19}N_4O_{10}Na$: 498.09 $[M+Na]^+$, found: 497.96.

*Compound **3b***. This compound was synthesized from **2b** (2.33 mmol) using this standard procedure. The resulting mixture of α and β anomers was separated by silica gel chromatography (diethyl ether) to give **3b** (0.73 g, 41%) as a white amorphous solid after precipitation from CH_2Cl_2/pentane; m.p. = 103.4-105.7°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.86-7.74 (m, 4H, Har.), 5.63 (bd, 1H, $J_{3',4'}$ = 3.1 Hz, H-4'), 5.59 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1'), 5.36 (bd, 1H, $J_{3,4}$ = 3.3 Hz, H-4), 5.19 (dd, 1H, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 10.4 Hz, H-2), 5.02 (dd, 1H, $J_{5',6a'}$ = 4.3 Hz, $J_{5',6b'}$ = 7.3 Hz, H-5'), 5.00 (dd, 1H, H-3), 4.75 (d, 1H, H-1), 4.34 (dd, 1H, $J_{6a',6b'}$ = 11.7 Hz, H-6a'), 4.26 (dd, 1H, $J_{2',3'}$ = 11.0 Hz, H-3'), 4.16 (dd, 1H, $J_{5,6a}$ = 6.7 Hz, $J_{6a,6b}$ = 11.3 Hz, H-6a), 4.09 (dd, 1H, $J_{5,6b}$ = 6.1 Hz, H-6b), 3.96 (dd, 1H, H-2'), 3.95-3.91 (m, 1H, H-5), 3.83 (dd, 1H, H-6b'), 2.14, 2.12, 2.06, 2.05, 2.02, 1.97 (6s, 18H, 6x$OCOCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.9 (C=O), 170.8 (C=O), 170.6 (C=O), 170.4 (C=O), 169.9 (C=O), 169.8 (C=O), 163.3 (C=O), 135.2 ($CH_{ar.}$), 129.1 ($C_{ar.}$), 124.1 ($CH_{ar.}$), 103.5 (C-1'), 101.9 (C-1), 74.7 (C-3'), 71.4 (C-5), 71.2, 70.2 (C-3, C-5'), 69.8 (C-4'), 69.2 (C-2), 67.3 (C-4), 62.8 (C-6'), 61.6 (C-6), 59.2 (C-2'), 21.1 (CH_3), 21.0 (CH_3), 21.0 (CH_3), 20.9 (CH_3); ES-HRMS (positive mode): calcd for $C_{32}H_{36}N_4O_{18}Na$: 787.1922 $[M+Na]^+$, found: 787.1933.

Standard procedure for azide reduction.

The azido compound was dissolved in a solution of methanol/acetic anhydride (9/1) and 10% Pd/C (0.1 equiv.) was added to the mixture. After stirring at room temperature under hydrogen for 2-3 hours, the catalyst was removed by filtration under a pad of celite and washed with methanol. The solvent was evaporated and the crude mixture purified by silica gel chromatography.

*Compound **4a***. This compound was prepared from **3a** (1.68 g, 3.5 mmol) using this standard procedure and was obtained as a white powder (0.92 g, 53%) after silica gel chromatography (CH_2Cl_2/EtOAc, 4/1 then 0/1) followed by precipitation from CH_2Cl_2/diethyl ether. m.p. 148-150°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.86-7.77 (m, 4H, $H_{ar.}$), 6.07 (d, 1H, $J_{2,NH}$ = 9.6 Hz, NH), 5.54 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-4), 5.38 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1), 5.34 (dd, 1H, $J_{3,4}$ = 3.0 Hz, $J_{2,3}$ = 11.3 Hz, H-3), 5.08 (bt, 1H, $J_{5,6}$ = 6.4 Hz, H-5), 4.80 (ddd, 1H, $J_{1,2}$ = 3.4 Hz, $J_{2,NH}$ = 9.6 Hz, $J_{2,3}$ = 11.3 Hz, H-2), 4.30 (dd, 1H, $J_{5,6}$ = 6.4 Hz, $J_{6a,6b}$ = 11.3 Hz, H-6a), 4.00 (dd, 1H, $J_{5,6}$ = 6.4 Hz, $J_{6a,6b}$ = 11.3 Hz, H-6b), 2.18, 2.12, 2.09, 2.04 (4s, 12H, 3OCOCH$_3$, NHCOCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 171.2 (C=O), 135.2 ($CH_{ar.}$), 129.1 ($C_{ar.}$), 124.2 ($CH_{ar.}$), 105.4 (C-1), 71.5, 69.5, 67.7, 61.9 (C-3, C-4, C-5, C-6), 47.7 (C-2), 21.1 (CH_3); ES-MS (positive mode): calcd for $C_{22}H_{24}N_2O_{11}Na$: 515.1 $[M+Na]^+$, found: 515.0.

*Compound **4b***. This compound was prepared from **3b** (0.73 g, 0.96 mmol) and was obtained as as a white amorphous solid 0.34 g (45% yield) after silica gel chromatography (eluent: EtOAc) and precipitation in CH_2Cl_2/pentane. m.p. = 120.6°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.85-7.76 (m, 4H, Har.), 6.14 (d, 1H, $J_{2',NH}$ = 9.5 Hz, NH), 5.55 (bd, 1H, $J_{3',4'}$ = 2.6 Hz, H-4'), 5.37 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1'), 5.36 (bd, 1H, $J_{3,4}$ = 2.8 Hz, H-4), 5.14 (dd, 1H, $J_{1,2}$ = 7.7 Hz, $^3J_{2,3}$ = 10.3 Hz, H-2), 4.99-4.94 (m, 2H, H-3, H-5'), 4.78 (ddd, 1H, $J_{2',3'}$ = 11.3 Hz, H-2'), 4.66 (d, 1H, H-1), 4.37 (dd, 1H, $J_{5',6a'}$ = 4.7 Hz, $^3J_{6a',6b'}$ = 11.6 Hz, H-6a'), 4.20-4.09 (m, 3H, H-3', H-6), 3.94-3.87 (m, 2H, H-5, H-6a'), 2.17 (s, 3H, OCOCH$_3$), 2.16, (s, 3H, NHCOCH$_3$), 2.15, 2.11, 2.08, 2.06, 1.97 (5s, 15H, 5xOCOCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.1 (C=O), 170.8 (C=O), 170.7 (C=O), 170.5 (C=O), 170.3 (C=O), 169.9 (C=O), 163.5 (C=O), 135.3 ($CH_{ar.}$), 129.0 ($C_{ar.}$), 124.2 ($CH_{ar.}$), 105.9 (C-1'), 101.2 (C-1), 76.9 (C-2'), 72.5 (C-3'), 71.3 (C-5), 71.1, 70.2 (C-3, C-5'), 69.3 (C-4'), 68.9 (C-2), 67.2 (C-4), 62.8 (C-6'), 61.6 (C-6), 23.7 (CH_3), 21.2 (CH_3), 21.1 (CH_3), 20.9 (CH_3); ES-HRMS (positive mode): calcd for $C_{34}H_{40}N_2O_{19}Na$: 803.2123 $[M+Na]^+$, found: 803.2117.

Standard procedure for deprotection.

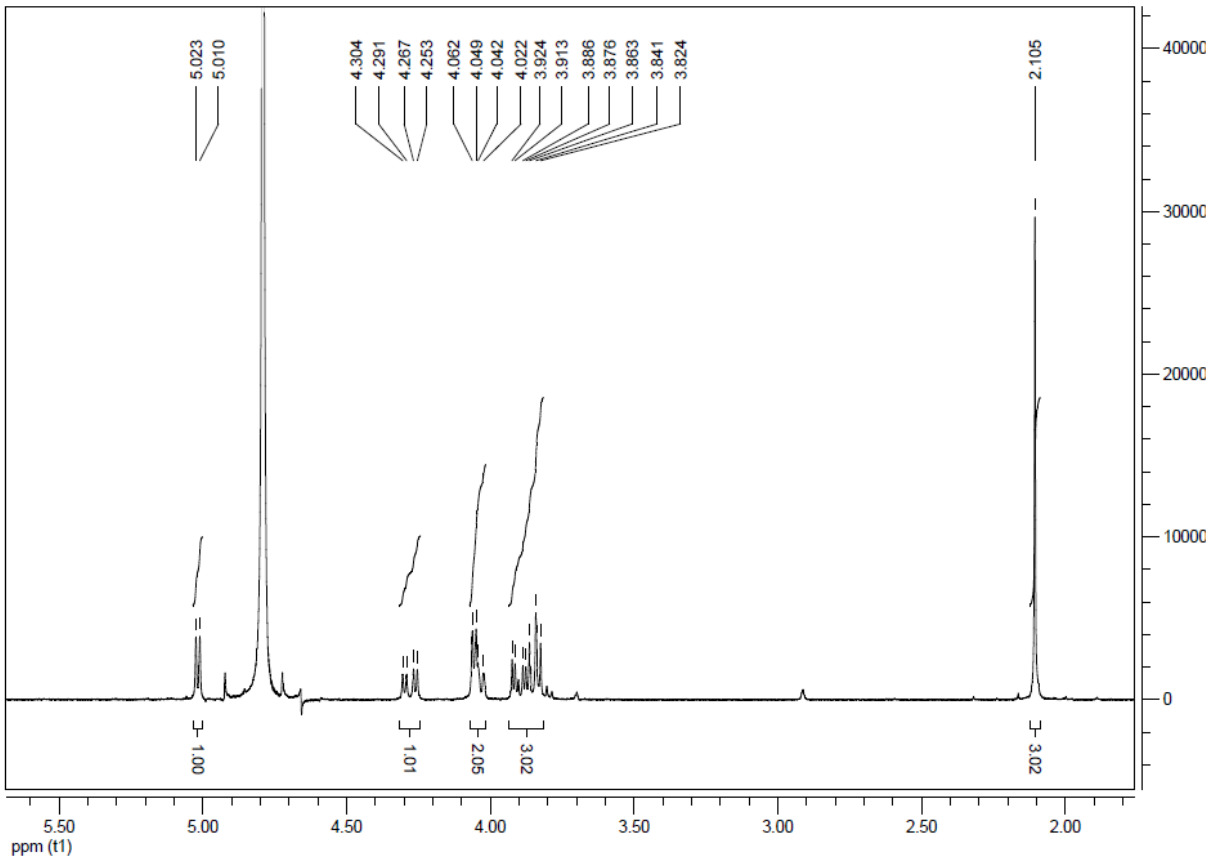
Aminooxylated derivatives were obtained by treatment with a solution of ethanol/methylhydrazine (1/1) at room temperature overnight. After evaporation, the fully deprotected compounds were recovered by precipitation in MeOH/CH_2Cl_2 then lyophilization.

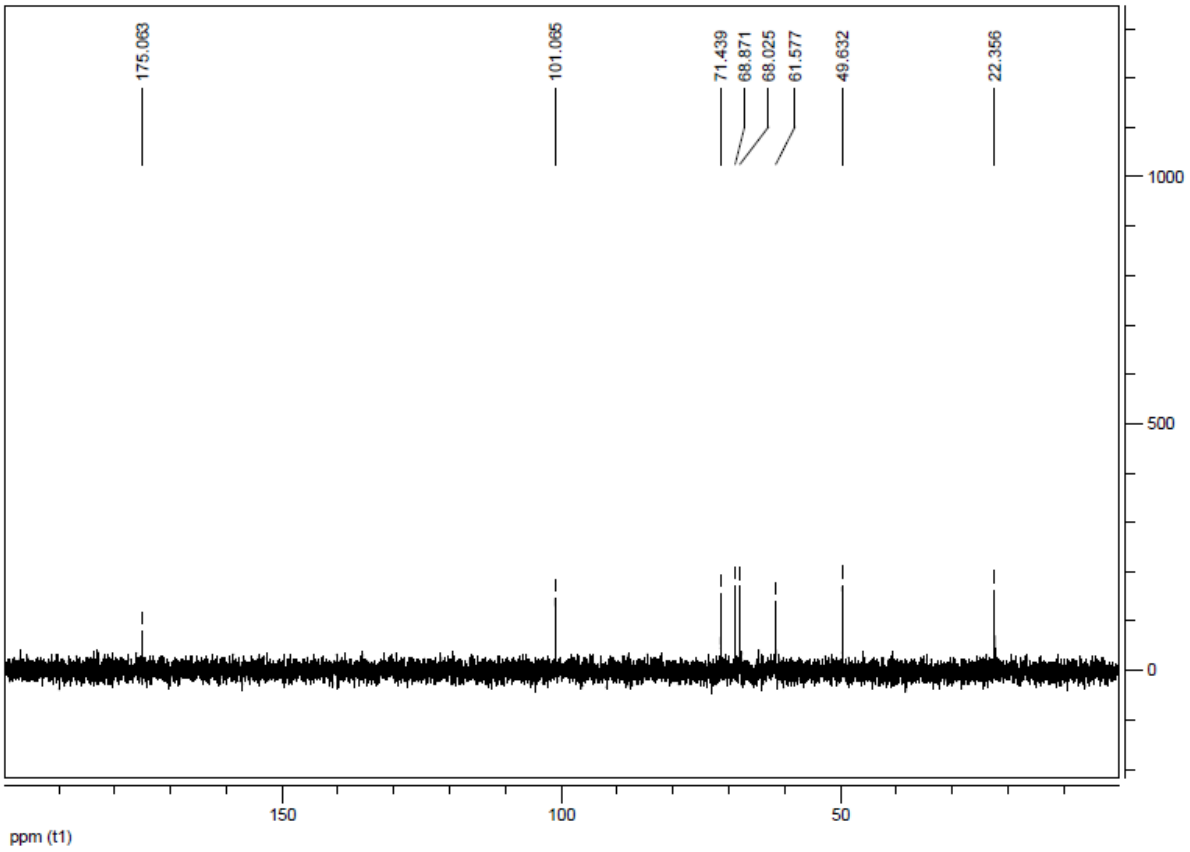
*Compound **1a***. The compound was prepared from **4a** (0.86 g, 1.7 mmol) using this standard procedure (0.37 g, 90%). ^{1}H NMR (400 MHz, D_2O): δ ppm 5.02 (d, 1H, $J_{1,2}$ = 4.1 Hz, H-1), 4.27 (dd, 1H, $J_{2,3}$ = 11.3 Hz, H-2), 4.06-4.02 (m, 2H, H-4, H-5), 3.91-3.82 (m, 3H, H-3, H-6), 2.1 (s, 3H, HNCOCH$_3$); ^{13}C NMR (100 MHz, D_2O): δ ppm 175.0 (C=O), 101.0 (C-1), 71.4, 68.8 (C-4, C-5), 68.0

(C-3), 61.5 (C-6), 49.6 (C-2), 22.3 (CH_3); ES-MS (positive mode): calcd. for $C_8H_{16}N_2O_6Na$: 236.2 $[M+H]^+$; found: 237.1.

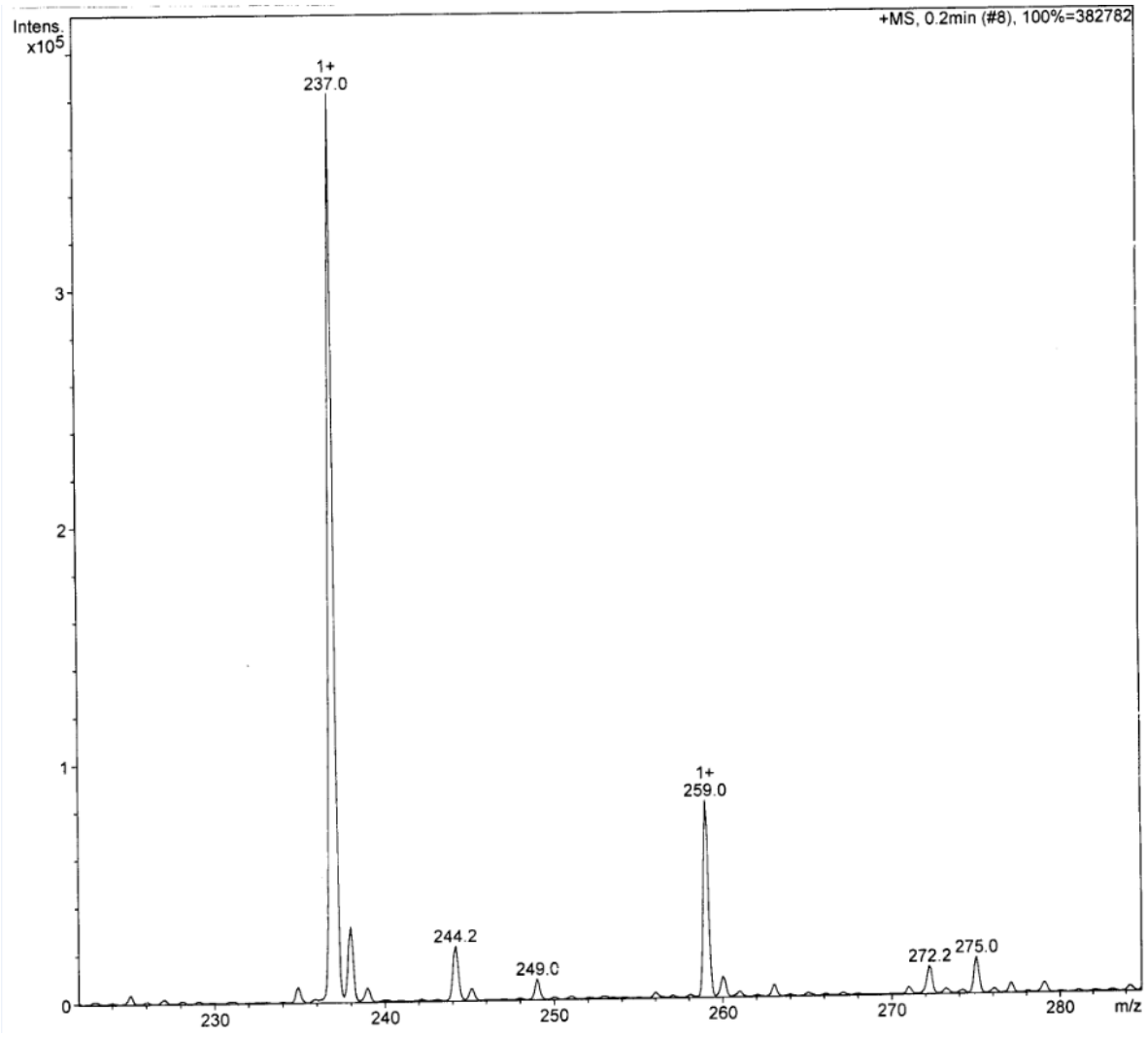
Compound ***1b***. The compound was prepared from **4b** (0.15 mg, 0.19 mmol) using this standard procedure (0.072 g, 94%). ^{1}H NMR (400 MHz, D_2O): δ ppm 5.02 (d, 1H, $J_{1',2'}$ = 4.0 Hz, H-1'), 4.51 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.45 (dd, 1H, $J_{2',3'}$ = 11.2 Hz, H-2'), 4.31 (d, 1H, $J_{3',4'}$ = 2.3 Hz, H-4'), 4.06 (t, 1H, $J_{5',6'}$ = 6.3 Hz, H-5'), 4.03 (dd, 1H, H-3'), 3.97 (bd, 1H, $J_{3,4}$ = 3.0 Hz, H-4), 3.85–3.80 (m, 4H, H-6, H-6'), 3.72-3.68 (m, 1H, H-5), 3.66 (dd, 1H, $J_{2.3}$ = 9.9 Hz, H-3), 3.59-3.57 (m, 1H, H-2), 2.09 (s, 3H, $NHCOCH_3$); ^{13}C NMR (100 MHz, D_2O): δ ppm 174.7 (C=O), 104.7 (C-1), 100.8 (C-1'), 77.0 (C-3'), 75.0 (C-5), 72.6 (C-3), 70.8, 70.7 (C-2, C5'), 68.7, 68.4 (C-4, C-4'), 61.2, 61.0 (C-6, C6'), 47.9 (C-2'), 22.1 (CH_3); ES-MS: calcd. for $C_{14}H_{26}N_2O_{11}Na$: 421.1 $[M+Na]^+$; found: 421.3.

^{1}H and ^{13}C NMR spectra for compound ***1a***

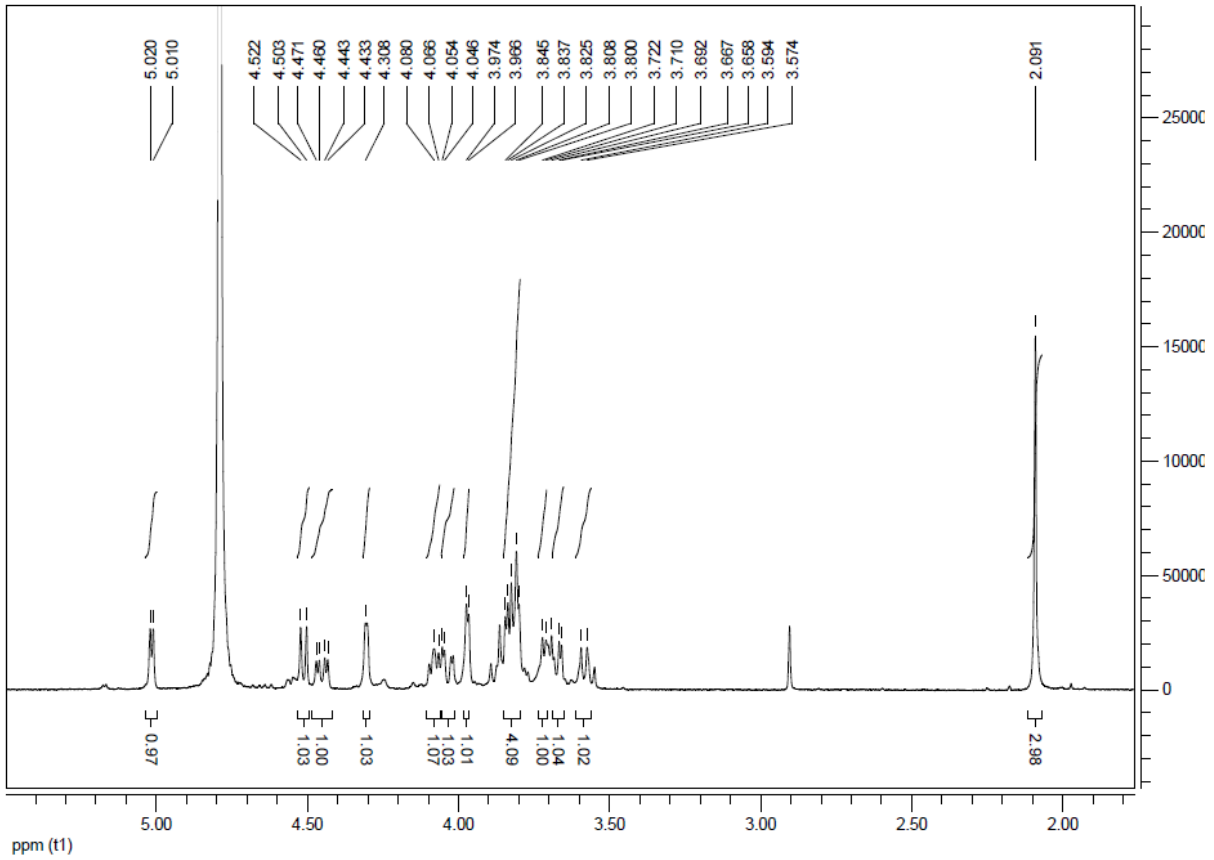


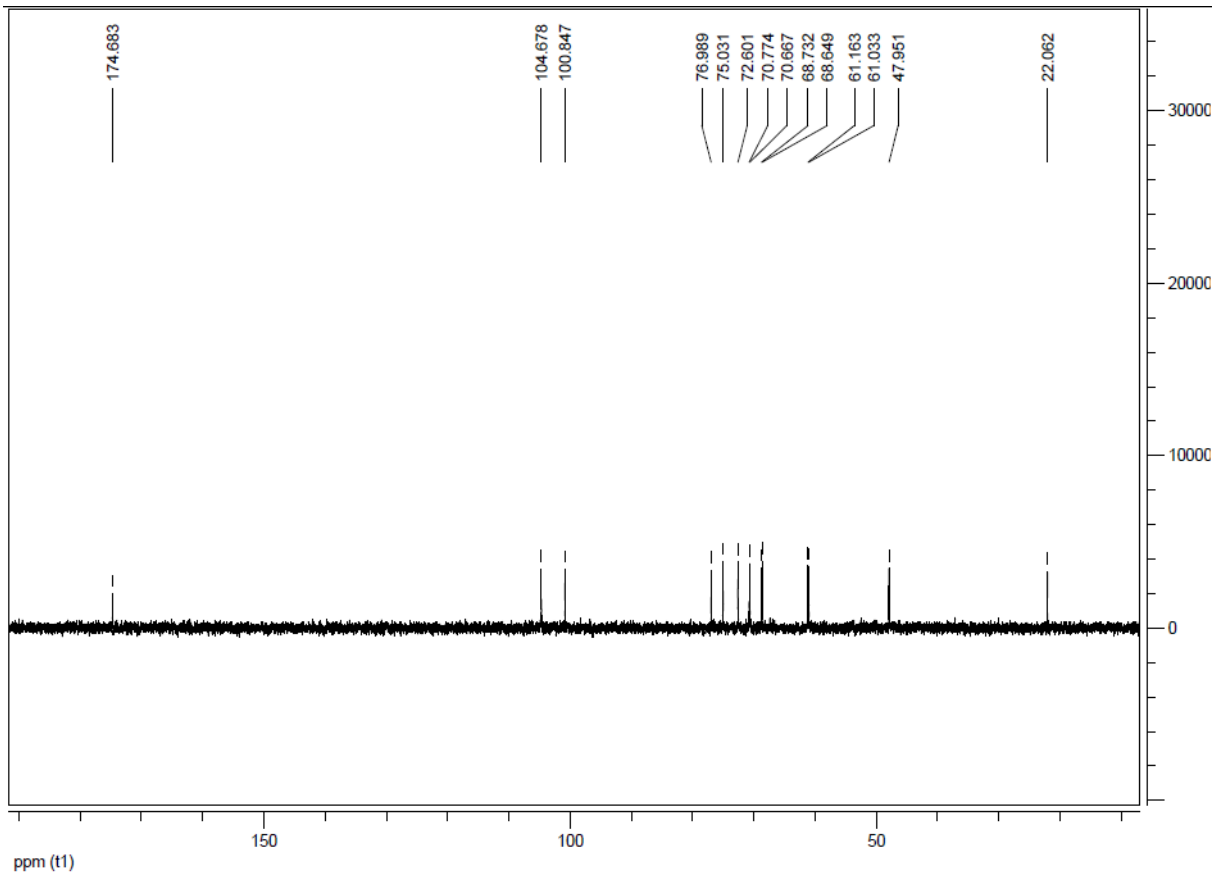


ES-MS spectrum for compound ***1a***



^{1}H and ^{13}C NMR spectra for compound ***1b***





ES-MS spectrum for compound ***1b***