Supporting Information

Exploration of the Aromatic Abietane Diterpenoid Scaffold in the Development of ArnT-Mediated Colistin Resistance Inhibitors

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1. Experimental section

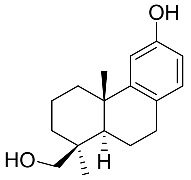
1.1 Chemistry

1.1.1 Materials and methods

Unless otherwise stated, all absolute solvents and reagents were purchased as anhydrous grade from Sigma-Aldrich and used without further purification. All compounds in this study were synthesized starting from commercially available (+)-podocarpic acid (Sigma-Aldrich, product code 119792). All reactions were magnetically stirred and monitored by TLC performed on Merck TLC aluminum sheets (silica gel 60 F254). Spots were visualized with UV light (λ = 254 nm). Purification of products was carried out by column chromatography on 60 Å silica gel (40–63 µm, 230–400 mesh). Melting points (Mp) were obtained in open capillary tubes using a Büchi melting point apparatus B-545 and are uncorrected. Purity and structural characterization of all compounds were assessed by ^{1}H NMR, ^{13}C NMR and HRMS. ^{1}H NMR and ^{13}C NMR spectra have been acquired with a Bruker Avance/Ultra ShieldTM 400 spectrometer operating at 400 MHz for ^{1}H and 101 MHz for ^{13}C and with a *J*EOL *J*NM-ECZ 600R spectrometer operating at 600 MHz for ^{1}H e 151 MHz for ^{13}C at room temperature, using tetramethylsilane (TMS) as internal standard and 5 mm diameter glass tubes. Chemical shifts (δ) are reported in parts per million (ppm) and are referenced to $CHCl_3$ (δ = 7.26 ppm for ^{1}H, δ = 77.16 ppm for ^{13}C), CH_2Cl_2 (δ = 5.32 ppm for ^{1}H, δ = 55.39 ppm for ^{13}C), acetone (δ =2.05 ppm for ^{1}H, δ = 29.84 ppm for ^{13}C), $(CH_3)_2SO$ (δ = 2.50 ppm for ^{1}H, δ = 39.52 ppm for ^{13}C), or CH_3OH (δ ¼ =3.31 ppm for ^{1}H, δ =49.00 ppm for ^{13}C). All ^{13}C NMR spectra were measured with complete proton decoupling. Data for NMR spectra are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal, *J* = coupling constant in Hz. Spectra were processed with the program MestReNova version 12.0.0-20080, FT and zero filling at 64K. High-resolution mass spectra (HRMS) were recorded on a ZenoTOF 7600 mass spectrometer (AB Sciex S.r.l.) equipped with an electrospray ionization (ESI) source. Samples were diluted in methanol to a final concentration of 1×10^{-5} M. For each analysis, 10 µL of sample were collected via autosampler and injected using a UPLC pump (Nexera, Shimadzu S.r.l.), delivering LC–MS grade methanol at a flow rate of 30 µL/min. The MS parameters were as follows: capillary voltage, +5.5 kV in positive-ion mode and –4.5 kV in negative-ion mode; gas 1 and gas 2, 30 and 35 psi, respectively; curtain gas, 40 psi; declustering potential, 60 V. External mass calibration was performed every two hours using a standard calibration mixture (Sciex S.r.l.), ensuring a mass error < 5 ppm and a resolving power of 25 000–35 000 across the investigated m/z range. Low-resolution mass spectra (MS) were recorded on an Esquire 3000 PLUS ion trap (Bruker) coupled with an ESI source.

1.1.2 Synthesis of podocarpic acid derivatives

Synthesis of compound 2

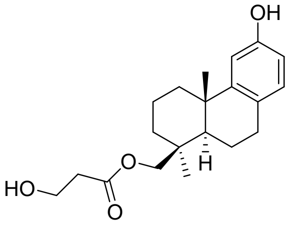


Commercially available podocarpic acid (**1**) (200 mg, 0.73 mmol, 1 equiv.) was placed in an ice bath, and thionyl chloride (520 µL, 7.29 mmol, 10 equiv.) was added dropwise. The reaction mixture was stirred at 76°C for 4h and then cooled down, evaporated, and the residue was dissolved in tetrahydrofuran (THF) (0.17 M, 4.32 mL). $LiAlH_4$ 2 M in THF (1.46 mL, 2.92 mmol, 4 equiv.) was then added dropwise at 0 °C, and the solution was stirred at room temperature for 24 h. Afterwards, the reaction was cooled down and quenched by a slow addition of saturated aqueous $NaHCO_3$ (5 mL). The solution was diluted with a saturated aqueous solution of Rochelle's salt (sodium potassium tartrate) (10 mL) and extracted with EtOAc (3 x 15 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in *vacuo* to afford compound **2** in quantitative yield (194 mg, 0.71 mmol).

*Compound **2***: white powder, r.f. 0.5 (petroleum ether/EtOAc/HCOOH 7.5:2.5:0.1), mp 171-173 °C. $[\alpha]_D^{29}$ = +58.46 ($CHCl_3$, c 0.5). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 6.89 (d, $^3J_{orto}$ = 8.2 Hz, 1H), 6.73 (d,

$^{4}J_{meta}$ = 2.6 Hz, 1H), 6.58 (dd, $^{3}J_{orto}$ = *8.4,* $^{4}J_{meta}$ = 2.6 Hz, 1H), 3.87 (d, *J* = 11.0 Hz, 1H), 3.56 (d, *J* = 11.0 Hz, 1H), 2.88-2.71 (m, 2H), 2.23 (d, *J* = 16.6 Hz, 1H), 1.96 (m, 1H), 1.88 (d, *J* = 14.7 Hz, 1H), 1.72-1.59 (m, 3H), 1.50-1.38 (m, 2H), 1.17 (s, 3H), 1.05 (s, 3H), 1.00 (m, 1H). **^{13}C NMR** ($CDCl_3$, 101 MHz) δ 153.76, 151.27, 130.12, 127.00, 112.99, 111.26, 65.44, 51.25, 38.97, 38.83, 37.93, 35.25, 30.24, 29.83, 26.91, 25.75, 19.37, 19.08. APCI-MS (negative) m/z: $[M - H]^-$ calcd for $C_{17}H_{23}O_2$, 259.17; found, 259.42.

Synthesis of compound 3

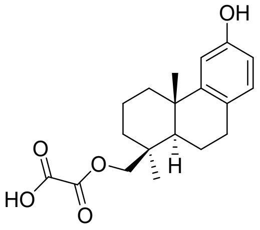


3-[(tert-butyldimethylsilyl)oxy]propanoic acid (40 mg, 0.2 mmol, 1 equiv.) was combined with thionyl chloride (164 µL, 1.96 mmol, 10 equiv.) and the mixture was stirred at 50°C for 2 h. After cooling, the mixture was evaporated and the residue was dissolved in a solution of **2** (51 mg, 0.2 mmol, 1 equiv.) in DCM/THF (5:1) (0.33 M, 0.6 mL). The reaction mixture was stirred at room temperature for 24h and then evaporated to dryness under reduced pressure. The residue was diluted with water (5 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The residue was purified by silica gel flash chromatography with a gradient 5%-20% of EtOAc in petroleum ether to provide compound **3** in 40% yield (26 mg, 0.078 mmol).

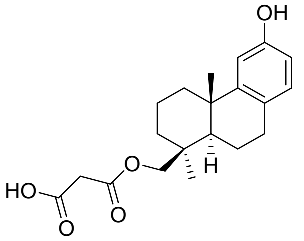
Compound ***3****:* colorless oil, r.f. 0.2 (petroleum ether/EtOAc 6.5:3.5). **^{1}H NMR** (400 MHz, CD_3OD) *δ* 6.81 *(*d, $^{3}J_{orto}$ = 8.1 Hz, 1H), 6.69 (d, $^{4}J_{meta}$ = 2.6 Hz, 1H), 6.51 (dd, $^{3}J_{orto}$ = 8.4, $^{4}J_{meta}$ = 2.4, 2.6 Hz, 1H), 4.45 (d, *J* = 11.0 Hz, 1H), 4.03 (d, *J* = 11.0 Hz, 1H), 3.84 (t, *J* = 6.2 Hz, 2H), 3.32 (s, 1H), 2.89 – 2.65 (m, 2H), 2.56 (t, *J* = 6.2 Hz, 2H), 2.29 (d, *J* = 12.5 Hz, 1H), 1.99 (d, *J* = 6.8 Hz, 1H), 1.85 – 1.67 (m, 3H), 1.63–1.59 (m, 1H), 1.51 – 1.44 (m, 1H), 1.43–1.36 (m, 1H), 1.24 (t, *J* = 7.1 Hz, 1H), 1.20 (s, 3H), 1.07 (s, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ 173.82, 156.20, 151.74, 130.81, 126.73, 113.95, 111.90, 68.05, 58.78, 52.85, 40.07, 38.91, 38.64, 38.38, 37.08, 31.25, 27.82, 26.00, 20.57, 20.00. ESI-HRMS (negative) m/z: $[M - H]^-$ calcd for $C_{20}H_{27}O_4$, 331.1901; found 331.1902.

General procedure for the synthesis of compounds 4, 5, 6

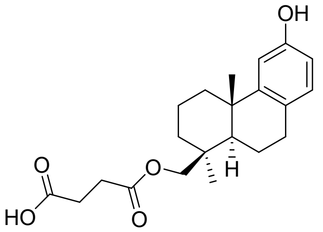
To a solution of compound **2** (70 mg, 0.27 mmol, 1 equiv.) in Et_2O (0.19 M), the desired acyl chloride - oxalyl chloride (2 M in DCM, 0.54 mmol, 2 equiv.), malonyl chloride (0.5 M in DCM, 0.27 mmol, 1 equiv.), or succinyl chloride (0.5 M in DCM, 0.27 mmol, 1 equiv.) - was added dropwise at 0 °C. The reaction was stirred at room temperature for 30 minutes and then quenched by a slow addition of water (5 mL). The aqueous layer was extracted with Et_2O (3 x 10 mL), and the combined organic phases were dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude reaction mixture was purified by silica gel flash chromatography with 0.5% of CH_3OH in $CHCl_3$ to afford the corresponding esters – **5**, when using malonyl chloride, (62.67 mg, 0.18 mmol, 67%) and **6**, when using succinyl chloride (19.46 mg, 0.054 mmol, 20%) – except for **4**, when using oxalyl chloride, (187.6 mg, 0.27 mmol) that was obtained in quantitative yield without any further purification.



Compound 4: pale yellow oil; r.f. 0.4 ($CHCl_3/CH_3OH$ 7.5:2.5). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 6.90 (d, $^3J_{orto}$ = 8.2 Hz, 1H), 6.73 (d, $^4J_{meta}$ = 2.6 Hz, 1H), 6.59 (dd, $^3J_{orto}$ = 8.4, $^4J_{meta}$ = 2.4, 2.6 Hz, 1H), 4.58 (d, J = 11.0 Hz, 1H), 4.25 (d, J = 10.8 Hz, 1H), 2.91-2.73 (m, 2H), 2.27 (d, J = 12.8 Hz, 1H), 2.04–1.99 (m, 1H), 1.84 (d, J = 12.8 Hz, 1H), 1.74-1.66 (m, 3H), 1.48-1.39 (m, 2H), 1.20 (s, 3H), 1.11 (s, 3H). **^{13}C NMR** ($CDCl_3$, 101 MHz) δ 158.60, 158.08, 153.57, 150.72, 130.21, 127.08, 113.17, 111.29, 70.68, 51.22, 38.65, 37.88, 37.55, 35.79, 30.14, 27.23, 25.64, 19.34, 18.94. ESI-HRMS (negative) m/z: $[M - H]^-$ calcd for $C_{19}H_{23}O_5$, 331.1545; found 331.1549.

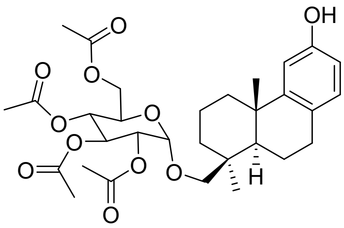


Compound **5**: white powder, r.f. 0.2 ($CHCl_3/CH_3OH$ 9:1). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 6.90 (d, $^3J_{orto}$ = 8.2 Hz, 1H), 6.73 (d, $^4J_{meta}$ = 2.5 Hz, 1H), 6.58 (dd, $^3J_{orto}$ = 8.2 Hz, $^4J_{meta}$ = 2.5 Hz, 1H), 4.47 (d, *J* = 11.0 Hz, 1H), 4.10 (d, *J* = 11.0 Hz, 1H), 3.47 (s, 2H), 2.89-2.71 (m, 2H), 2.25 (d, *J* = 12.80 Hz, 1H), 2.00-1,95 (m, 1H), 1.78 (d, *J* = 13.58 Hz, 1H), 1.76-1.72 (m, 3H), 1.51-1.38 (m, 2H), 1.19 (s, 3H), 1.13-1.08 (m, 1H), 1.04 (s, 3H). **^{13}C NMR** ($CDCl_3$, 101 MHz) δ 170.65, 167.54, 153.67, 150.89, 130.16, 126.99, 113.11, 111.28, 68.77, 51.23, 40.80, 38.74, 37.88, 37.39, 35.90, 30.15, 27.28, 25.62, 19.36, 18.98. ESI-HRMS (negative) m/z: $[M - H]^-$ calcd for $C_{20}H_{25}O_5$, 345.1702; found 345.1703.



Compound **6**: colorless oil; r.f. 0.3 ($CHCl_3$/ CH_3OH 9:1). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 6.89 (d, $^3J_{orto}$ = 8.2 Hz, 1H), 6.72 (d, $^4J_{meta}$ = 2.5 Hz, 1H), 6.58 (dd, $^3J_{orto}$ = 8.2, $^4J_{meta}$ = 2.3 Hz, 1H), 4.38 (d, *J* = 11.0 Hz, 1H), 4.00 (d, *J* = 11.0 Hz, 1H), 2.89-2.73 (m, 2H), 2.72-2.64 (m, 4H), 2.23 (d, *J* = 13.58 Hz, 1H), 2.00 – 1.95 (m, 1H), 1.77 (d, *J* = 13.58 Hz, 1H), 1.69-1.60 (m, 3H), 1.49-1.30 (m, 2H), 1.18 (s, 3H), 1.10 – 1.06 (m, 1H), 1.03 (s, 3H). **^{13}C NMR** (CD_2Cl_2, 101 MHz) δ 177.61, 154.24, 151.60, 130.49, 127.53, 113.27, 111.58, 67.82, 54.00, 51.75, 39.32, 38.34, 37.76, 36.38, 30.62, 29.54, 29.35, 27.57, 25.84, 19.85, 19.47. ESI-HRMS (negative) m/z: $[M - H]^-$ calcd for $C_{21}H_{27}O_5$, 359.1858; found 359.1868.

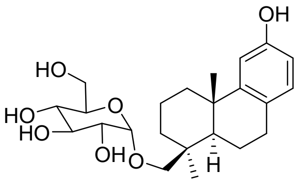
Synthesis of compound 7



A solution of peracetylated glucosyl bromide (113 mg, 0.27 mmol, 1.2 equiv.) in DCM (1.8 mL) was combined with zinc chloride (37.5 mg, 0.27 mmol, 1.2 equiv.). The reaction mixture was stirred 20 minutes, after which a solution of compound **2** (60 mg, 0.23 mmol, 1 equiv.) in DCM (0.13 M,1.8 mL) was added dropwise. The resulting mixture was stirred under reflux at 50°C for 24h, then was diluted with DCM and quenched by a slow addition of 5% aqueous $NaHCO_3$ (5 mL). The aqueous layer was extracted with DCM (3 x 10 mL) and the resulting organic phase was washed with water and brine, dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by silica gel flash chromatography with 0.5% of CH_3OH in $CHCl_3$ to afford compound **7** in 9% yield (9 mg, 0.02 mmol).

Compound 7: white powder, r.f. 0.4 (CH_2Cl_2/EtOAc 8.7:1.3). **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 6.89 (d, $^3J_{orto}$ = 8.2 Hz, 1H), 6.72 (d, $^4J_{meta}$ = 2.5 Hz, 1H), 6.58 (dd, $^3J_{orto}$ = 8.2, $^4J_{meta}$ = 2.6 Hz, 1H), 5.47 (t, J = 9.7 Hz, 1H), 5.05 (d, $J_{\text{ax-eq}}$ = 3.5 Hz, 1H), 5.02 (d, J = 10.1 Hz, 1H), 4.86 – 4.84 (m, 1H), 4.24 – 4.21 (m, 1H), 4.14 – 4.11 (m, 1H), 4.02 – 3.99 (m, 1H), 3.89 (d, J = 11.0 Hz, 1H), 3.26 (d, J = 11.0 Hz, 1H), 2.88 – 2.84 (m, 1H), 2.78-2.72 (m, 1H), 2.24 (d, J = 12.32, 1H), 2.09 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 2.02 (s, 3H), 2.00 - 1.96 (m, 1H), 1.87 (d, J = 13.77, 1H), 1.68-1.66 (m, 3H), 1.48 – 1.45 (m, 2H), 1.43 (d, J = 5.99, 1H), 1.17 (s, 3H), 1.07 (s, 3H). **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 170.73, 170.28, 170.24, 169.77, 153.63, 151.06, 130.15, 127.18, 112.95, 111.18, 96.05, 71.95, 71.20, 70.43, 68.86, 67.41, 62.38, 51.04, 38.91, 37.92, 36.33, 30.26, 29.77, 27.64, 25.60, 22.76, 20.81, 20.74, 20.70, 19.60, 19.13. ESI-HRMS (positive) m/z: $[M + Na]^+$ calcd for $C_{31}H_{42}O_{11}Na$ 613.2624; found 613.2617. $[M+K]^+$ calcd for $C_{23}H_{34}O_7K$ 629.2357; found 629.2357.

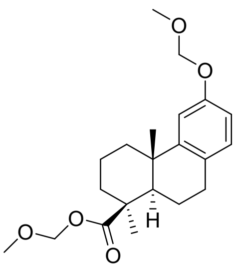
Synthesis of compound 8



A solution of **7** (86 mg, 0.146 mmol, 1 equiv.) in 3.72 mL of $CH_3OH/Et_3N/H_2O$ (8:1:1) was stirred for 36h at room temperature. After evaporating the solvent under reduced pressure, the residue was diluted with water (5 mL) and extracted with EtOAc (3 x 10 mL). The organic phase was then dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by silica gel flash chromatography with 0.5% of CH_3OH in $CHCl_3$ to afford compound **8** in 27% yield (17 mg, 0.04 mmol).

*Compound **8**:* white powder, r.f. 0.2 ($CHCl_3/CH_3OH/HCOOH$ 9:1:0.2). **^{1}H NMR** ($CDCl_3/CD_3OD$ 2.5:7.5, 400 MHz) δ 6.84 (d, $^3J_{orto}$ = 8.3 Hz, 1H), 6.72 (d, $^4J_{meta}$ = 2.2 Hz, 1H), 6.53 (dd, $^3J_{orto}$ = 8.2, $^4J_{meta}$ =2.3 Hz, 1H), 4.77 (d, $J_{\text{ax-eq}}$ = 3.6 Hz, 1H), 4.06 (d, J = 9.3 Hz, 1H), 3.86 – 3.83 (m, 1H), 3.75 – 3.68 (m, 3H), 3.51 – 3.43 (m, 2H), 3.26 (d, J = 9.2 Hz, 1H), 2.87 – 2.69 (m, 2H), 2.28 (d, J = 13.19, 1H), 2.09 – 1.98 (m, 4H), 1.64 – 1.60 (m, 4H), 1.23 (s, 3H), 1.15 (s, 3H). **^{13}C NMR** (CD_3OD, $CDCl_3$, 101 MHz) δ 155.20, 151.57, 130.33, 126.52, 113.35, 111.51, 99.42, 74.73, 73.05, 72.73, 71.07, 70.92, 62.04, 51.99 39.47, 38.39, 32.47, 30.73, 30.19, 27.83, 25.94, 20.02, 19.59. ESI-HRMS (positive) m/z: $[M + Na]^+$ calcd for $C_{23}H_{34}O_7Na$ 445.2202; found 445.2195.

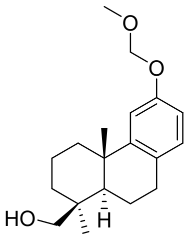
Synthesis of compound 9



DIPEA (3.17 mL, 18.2 mmol, 10 equiv.) was added to a solution of **1** (500 mg, 1.82 mmol, 1 equiv.) in dry DCM (1.6 mL), followed by the addition of MOMCl (1.38 mL, 18.2 mmol, 10 equiv.) after a few minutes. The reaction mixture was stirred at room temperature for 48h and quenched by the addition of saturated aqueous $NaHCO_3$ until effervescence ceased, and the aqueous layer was extracted with DCM (3 x 20 mL). The organic layers were then collected, dried over anhydrous Na_2SO_4, filtered and concentrated in *vacuo* to afford compound **9** in quantitative yield (659 mg, 1.82 mmol).

Compound 9: yellow oil, r.f. 0.64 (Hexane/EtOAc 7:3). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.00 – 6.91 (m, 2H), 6.81 (dd, $^3J_{orto}$ = 8.4, $^4J_{meta}$ =2.4 Hz, 1H), 5.29 (d, J = 5.7 Hz, 1H), 5.20 (d, J = 6.0 Hz, 1H), 5.12 (s, 2H), 3.49 (s, 3H), 3.47 (s, 3H), 2.90 - 2.84 (m, 1H), 2.79 – 2.70 (m, 1H), 2.33 – 2.18 (m, 3H), 2.06 – 1.95 (m, 2H), 1.68 – 1.61 (m, 1H), 1.57 – 1.54 (m, 1H), 1.45 – 1.37 (m, 1H), 1.32 (s, 3H), 1.15 - 1.13 (m, 1H), 1.10 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 177.00, 155.51, 149.30, 129.91, 128.91, 113.78, 113.64, 94.79, 90.64, 57.96, 55.91, 52.77, 44.37, 39.38, 38.77, 37.56, 31.35, 28.66, 23.20, 21.13, 19.96. ESI-MS (positive) m/z: $[M + Na]^+$ calcd for $C_{21}H_{30}O_5Na$ 385.20, found 385; $[M + K]^+$ calcd for $C_{21}H_{30}O_5K$ 401.17, found 401; $[2M + Na]^+$ calcd for $C_{42}H_{60}O_{10}Na$ 747.41; found 747.

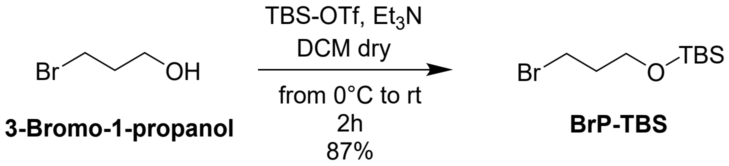
Synthesis of compound 10



A solution of compound **9** (629 mg, 1.73 mmol, 1 equiv.) in dry THF (10.27 mL) was combined with 1.73 mL of $LiAlH_4$ (2M in THF, 3.46 mmol, 2 equiv.) at 0°C, and the reaction mixture was stirred for 2.5 h. The reaction was then quenched by adding dropwise saturated aqueous $NaHCO_3$ until effervescence ceased. Then, a saturated solution of Rochelle's salt (70 mL) was added, and the aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were collected, dried over anhydrous Na_2SO_4, filtered and concentrated in *vacuo* to afford compound **10** in quantitative yield (526 mg, 1.73 mmol).

*Compound **10***: yellow resin, r.f. 0.56 (Hexane/EtOAc 7:3). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.99 – 6.90 (m, 2H), 6.80 (dd, $^3J_{orto}$ = 8.4, $^4J_{meta}$ =2.4 Hz, 1H), 5.13 (s, 2H), 3.86 (d, J = 10.9 Hz, 1H), 3.55 (d, J = 10.9 Hz, 1H), 3.48 (s, 3H), 2.93 – 2.71 (m, 2H), 2.28 (d, J = 12.6 Hz, 1H), 2.00 - 1.95 (m, 1H), 1.89 (d, J = 14.7 Hz, 1H), 1.74 – 1.61 (m, 3H), 1.51 – 1.40 (m, 3H), 1.18 (s, 3H), 1.05 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.55, 151.19, 129.94, 128.59, 113.64, 112.84, 94.91, 65.37, 56.02, 51.25, 38.98, 38.85, 38.03, 35.28, 30.29, 26.93, 25.77, 19.33, 19.08. APCI-MS (positive) m/z: $[M + H]^+$ calcd for $C_{19}H_{29}O_3$ 305.21; found 305.10.

Synthesis of compound 3-bromopropoxy)(tert-butyl)dimethylsilane (BrP-TBS)

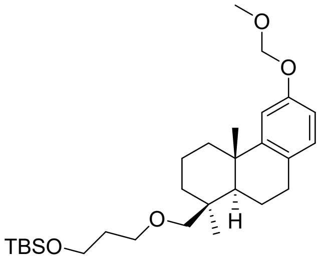


Scheme S1. Synthesis of compound **BrP-TBS**

To a solution of 3-bromo1-propanol (0.16 mL, 1.80 mmol, 1 equiv.) in DCM dry (0.5 M, 3.6 mL) triethylamine (3.6 mmol, 2 equiv., 0.5 mL) and tert-butyldimethylsilyl trifluoromethanesulfonate (1.98 mmol, 1.1 equiv., 46 mL) were added sequentially at 0 °C. The temperature was increased to rt and the reaction was stirred for 2h and then was quenched by slow addition of a saturated solution of $NaHCO_3$ (5 mL). The organic layer was washed with distilled water (3 x 10 mL), dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure, to afford **BrP-TBS** in 87% yeld (396.60 mg, 1.57 mmol,).[1]

Colorless oil r.f. 0.98 (Hex/ EtOAc 8:2). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.73 (t, J = 5.7 Hz, 2H), 3.51 (t, J = 6.4 Hz, 2H), 2.07 - 1,99 (m, 2H), 0.89 (s, 9H), 0.06 (s, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 60.42, 35.55, 30.69, 25.91, 18.30, -5.37. ESI-MS (positive) m/z: $[M + Na]^+$ calcd for $C_9H_{21}BrNaOSi$ 275.04; found 275.04; $[M + Na]^+$ calcd for $C_{18}H_{42}Br_2KO_2Si_2$ 543.07; found 543.50.

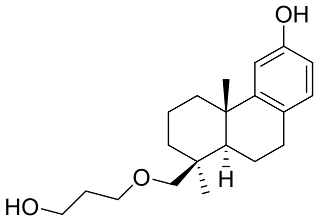
Synthesis of compound 11



A solution of compound **10** (50 mg, 0.16 mmol, 1 equiv.) in dry DMF (0.32 M, 0.5 mL) was treated with sodium hydride (60% in mineral oil, 26.27 mg, 0.66 mmol, 4 equiv.) at 0 °C, followed by the addition of a solution of (3-bromopropoxy)(tert-butyl)dimethylsilane (145.4 mg, 0.57 mmol, 3.5 equiv.) previously synthetized according to the procedure described in SI in dry DMF (1.15 M, 0.5 mL) (Scheme S1). The temperature was then raised to 65°C, and the reaction was stirred for 24h. Afterward, the reaction mixture was cooled down to room temperature and quenched by slow addition of 5% aqueous $NaHCO_3$ (5 mL), followed by dilution with EtOAc (10 mL). The organic layer was washed with water (3 x 10 mL), dried over Na_2SO_4, filtered and concentrated in *vacuo* and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography with a gradient of 5%-15% of EtOAc in petroleum ether to afford compound **11** in 5% yield (15.73 mg, 0.033 mmol).

*Compound **11**:* colorless oil, r.f. 0.63 (Hex/EtOAc 6.5:3.5). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.98 – 6.90 (m, 2H), 6.80 (dd, $^3J_{orto}$ = 8.3, $^4J_{meta}$ =2.5 Hz, 1H), 5.13 (s, 2H), 3.79 (t, J = 5.5 Hz, 2H), 3.65 – 3.53 (m, 3H), 3.47 (s, 3H), 3.33 (d, J = 9.1 Hz, 1H), 2.91 – 2.71 (m, 2H), 2.27 (d, J = 12.7 Hz, 1H), 1.98 – 1.93 (m, 1H), 1.89 – 1.78 (m, 3H), 1.72 - 1.60 (m, 4H), 1.46 – 1.40 (m, 2H), 1.19 (s, 3H), 1.03 (s, 3H), 0.89 (s, 9H), 0.07 (s, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.57, 151.21, 129.97, 113.66, 112.89, 94.94, 77.37, 74.60, 71.77, 62.94, 56.05, 51.35, 39.03, 38.12, 38.07, 36.25, 32.05, 30.38, 27.91, 26.09, 25.72, 19.52, 19.26, -5.21. APCI-MS (positive) m/z: $[M + Na]^+$ calcd for $C_{28}H_{49}O_4Si$ 477.34; found 477.24.

Synthesis of compound 12



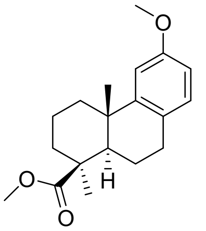
To a solution of compound **11** (20 mg, 0.04 mmol, 1 equiv.) in DCM (0.13 M, 0.3 mL) TFE (0.03 mL, 0.4 mmol, 10 equiv.) and TFA (0.03 mL, 0.4 mmol, 10 equiv.) were added sequentially at 0 °C. The reaction mixture was then allowed to warm to room temperature and stirred for 5h. After this, the reaction was quenched by slow addition of saturated aqueous $NaHCO_3$ (5 mL) and diluted with DCM (10 mL). The aqueous layer was extracted with DCM (3 x 10 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The residue was purified by silica gel flash chromatography with a gradient of 0%-15% of EtOAc in hexane to afford compound **12** in 26% yield (3.4 mg, 0.01 mmol).

*Compound **12**:* colorless oil, r.f. 0.27 (Hex/EtOAc 6.5:3.5). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.89 (d, $^3J_{orto}$ = 8.2 Hz, 1H), 6.73 (d, $^4J_{meta}$ = 2.5 Hz, 1H), 6.57 (dd, $^3J_{orto}$ = 8.2, $^4J_{meta}$ = 2.6 Hz, 1H), 4.61 (s, 1H), 4.61 (s, 1H), 3.80 (t, J = 5.1 Hz, 2H), 3.65 – 3.55 (m, 3H), 3.33 (d, J = 9.1 Hz, 1H), 2.89 – 2.69 (m, 2H), 2.59 (s, 1H), 2.23 (d, J = 12.3 Hz, 1H), 1.98 – 1.93 (m, 1H), 1.86 – 1.81 (m, 3H), 1.73 – 1.62 (m, 3H), 1.43 (d, J = 12.7 Hz, 2H), 1.25 (s, 1H), 1.18 (s, 3H), 1.03 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 153.64, 151.37, 130.16, 127.29, 112.91, 111.27, 74.63, 71.79, 62.97, 51.34, 39.05, 38.11, 38.00, 36.25, 32.03, 30.34, 27.91, 25.70, 19.57, 19.27. ESI-HRMS (positive) m/z: $[M + H]^+$ calcd for $C_{20}H_{31}O_3$ 319.2273, found 319.2264.

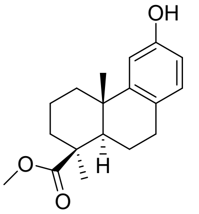
Synthesis of compounds 13 and 14

To a solution of **1** (250 mg, 0.91 mmol, 1 equiv.) in acetone (0.22 M, 4 mL) K_2CO_3 (615 mg, 4.55 mmol, 5 equiv.) and CH_3I (0.37 mL, 5.46 mmol, 6 equiv.) were added sequentially and the reaction mixture was stirred for 24 h at 56°C. Further, the reaction was cooled down and quenched by a slow addition of distilled water (3 mL) and acetone was evaporated under reduced pressure. The residue was diluted with water (5mL) and extracted with DCM (3 x 10mL). After that, the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*.

The residue was purified by silica gel flash chromatography, with a gradient of 5%-100% of EtOAc in petroleum ether to afford compounds **13** (193 mg, 0.638 mmol, 70%) and **14** (62 mg, 0.273 mmol, 26%).

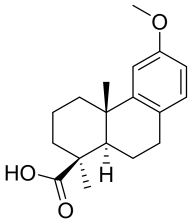


*Compound **13***: white powder, r.f. 0.67 (petroleum ether/EtOAc 8.5:1.5), mp 124-126 °C. $[a]_{D}^{28.5}$ = +115.67 ($CHCl_3$, c 0.3). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.97 (d, $^{3}J_{orto}$ = 8.4 Hz, 1H), 6.81 (d, $^{4}J_{meta}$ = 2.3 Hz, 1H), 6.67 (dd, $^{3}J_{orto}$ = 8.4, $^{4}J_{meta}$ = 2.5 Hz, 1H), 3.77 (s, 3H), 3.66 (s, 3H), 2.88 – 2.83 (m, 1H), 2.77 – 2.69 (m, 1H), 2.33 – 2.12 (m, 3H), 2.07 – 1.88 (m, 2H), 1.65 – 1.61 (m, 1H), 1.53 (d, J = 12.1 Hz, 1H), 1.44 – 1.37 (m, 1H), 1.28 (s, 3H), 1.14 – 1.06 (m, 1H), 1.04 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 178.05, 157.84, 149.43, 129.97, 127.76, 111.28, 55.36, 52.95, 51.38, 44.13, 39.54, 38.79, 37.75, 31.35, 28.68, 23.04, 20.11. APCI-MS (positive) m/z: $[M + H]^+$ calcd for $C_{19}H_{27}O_3$, 303.20; found, 303.30.



*Compound **14***: white powder, r.f. 0.28 (petroleum ether/EtOAc 8.5:1.5), mp 205-207 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.90 (d, $^{3}J_{orto}$ = 8.2 Hz, 1H), 6.74 (d, $^{4}J_{meta}$ = 2.5 Hz, 1H), 6.59 (dd, $^{3}J_{orto}$ = 8.2, $^{4}J_{meta}$ = 2.6 Hz, 1H), 4.81 (s, 1H), 3.66 (s, 3H), 2.86 – 2.81 (m, 1H), 2.75 - 2.66 (m, 1H), 2.27 (d, J = 13.4 Hz, 1H), 2.22 – 2.12 (m, 2H), 2.02 – 1.86 (m, 2H), 1.64 – 1.58 (m, 1H), 1.56 – 1.49 (m, 1H), 1.42 - 1.35 (m, 1H), 1.27 (s, 3H), 1.12 - 1.04 (m, 1H), 1.02 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 178.19, 153.71, 149.66, 127.69, 113.12, 112.17, 52.89, 51.45, 44.15, 39.51, 38.68, 37.74, 31.36, 28.68, 23.03, 21.24, 20.09. APCI-MS (negative) m/z: $[M - H]^-$ calcd for $C_{18}H_{23}O_3$, 287.16; found, 287.10.

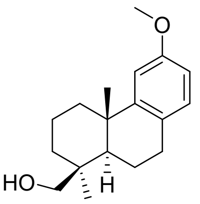
Synthesis of compound 15



To solution of **13** (50 mg, 0.165 mmol, 1 equiv.) in DMSO (0.08 M, 2.16 mL) t-BuOK (370 mg, 3.3 mmol, 20 equiv.) was added and the reaction mixture was stirred for 72 h at 100°C. Further, the reaction was cooled down, quenched by the slow addition of HCl 2M (until pH 2/3) diluted with EtOAc and extracted with EtOAc (10 mL). After that, the organic phase was washed with distilled water (5 mL), dried over anhydrous Na_2SO_4 filtered and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography with a gradient 5%-20% of EtOAc in petroleum ether to provide compound **15** in 80% yield (38 mg, 0.13 mmol).

*Compound **15***: brown powder, r.f. 0.38 (petroleum ether/EtOAc 8.5:1.5), mp 150-152 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.97 (d, $^3J_{orto}$ = 8.3 Hz, 1H), 6.80 (d, $^4J_{meta}$ = 2.6 Hz, 1H), 6.68 (dd, $^3J_{orto}$ = 8.3, $^4J_{meta}$ = 2.6 Hz, 1H), 3.77 (s, 3H), 2.94 – 2.66 (m, 2H), 2.31 – 2.12 (m, 3H), 2.07 – 1.97 (m, 2H), 1.64 - 1.54 (m, 2H), 1.42 (d, J = 11.3 Hz, 1H), 1.34 (s, 3H), 1.13 (s, 3H), 1.08 (d, J = 13.6 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 184.27, 157.84, 149.34, 130.00, 127.74, 111.36, 111.18, 55.37, 52.93, 44.06, 39.45, 38.97, 31.28, 28.87, 23.23, 21.11, 20.02. ESI-MS (negative) m/z: $[M – H]^-$ calcd for $C_{18}H_{23}O_3$, 287.16; found 287.16.

Synthesis of compound 16

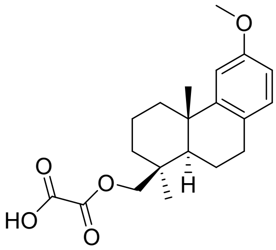


A solution of compound **13** (123.5 mg, 0.41 mmol, 1 equiv.) in dry THF (0.17 M, 2.4 mL) was combined with 1.73 mL of $LiAlH_4$ 2M in THF (0.41 mL, 0.82 mmol, 2 equiv.) at 0°C, and the reaction mixture was stirred for 24h. The reaction was then quenched by adding dropwise saturated aqueous $NaHCO_3$ until effervescence ceased. Then, a saturated solution of Rochelle's salt (70 mL) was added, and the aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were collected, dried over anhydrous Na_2SO_4, filtered and concentrated in *vacuo* to afford compound **16** in quantitative yield (112 mg, 0.41 mmol).

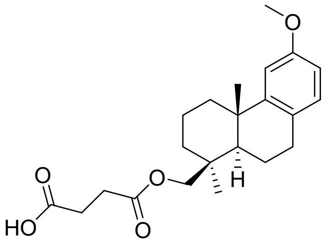
*Compound **16***: white powder, rf 0.36 (petroleum ether/EtOAc 8.5:1.5), mp 81-83 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.95 (d,$^3J_{orto}$ = 8.4 Hz, 1H), 6.81 (d, $^4J_{meta}$ = 2.6 Hz, 1H), 6.67 (dd,$^3J_{orto}$ = 8.4 Hz, $^4J_{meta}$ = 2.6 Hz, 1H), 3.86 (d, J = 10.9 Hz, 1H), 3.77 (s, 3H), 3.55 (d, J = 10.9 Hz, 1H), 2.90 - 2.85 (m, 1H), 2.82 – 2.73 (m, 1H), 2.28 (d, J = 12.7 Hz, 1H), 2.00 - 1.95 (m, 1H), 1.89 (d, J = 13.6 Hz, 1H), 1.73 – 1.62 (m, 3H), 1.51 - 1.45 (m, 2H), 1.18 (s, 3H), 1.05 (s, 3H), 1.03 – 0.98 (m, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 157.83, 151.13, 129.93, 127.24, 111.04, 110.42, 77.16, 65.42, 55.39, 51.32, 39.03, 38.88, 38.08, 35.29, 30.25, 26.94, 25.79, 19.39, 19.12. APCI-MS (positive) m/z: $[M + H]^+$ calcd for $C_{18}H_{27}O_2$ 274.1933; found, 274.2.

Synthesis of compounds 17 and 18

To a solution of compound **16** (30 mg, 0.11 mmol, 1 equiv.) in Et_2O (0.192 M, 0.57 mL), the desired acyl chloride - oxalyl chloride (2 M in DCM, 0.11 µL, 0.22 mmol, 2 equiv.) or succinyl chloride (0.5 M in DCM, 0.22 µL, 0.11 mmol, 1 equiv.) - was added dropwise at 0 °C and the reaction was stirred at rt for 30 minutes. Then, the reaction was quenched by the slow addition of distilled water. The aqueous layer was extracted with Et_2O (3 x 10 mL), and the combined organic phases were dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude reaction mixture was purified by silica gel flash chromatography with 1% of CH_3OH in $CHCl_3$ to afford the corresponding ester **18** (33.00 mg, 0.09 mmol, 80%). Ester **17** was obtained in quantitative yield (37.73 mg, 0.11 mmol) without any further purification.

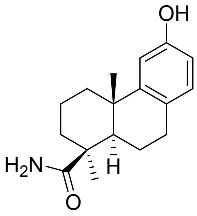


*Compound **17***: colorless oil, r.f. 0.45 ($CHCl_3/CH_3OH/HCOOH$ 9:1:0.1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.96 (d,$^3J_{orto}$ = 8.4 Hz, 1H), 6.81 (d, $^4J_{meta}$ = 2.5 Hz, 1H), 6.68 (dd,$^3J_{orto}$ = 8.4 Hz, $^4J_{meta}$ = 2.5 Hz, 1H), 6.16 (s, 1H), 4.57 (d, J = 11.0 Hz, 1H), 4.23 (d, J = 11.0 Hz, 1H), 3.78 (s, 3H), 2.94 – 2.73 (m, 2H), 2.31 (d, J = 12.8 Hz, 1H), 2.04 – 1.99 (m, 1H), 1.85 (d, J = 13.8 Hz, 1H), 1.76 – 1.65 (m, 3H), 1.57 – 1.53 (m, 1H), 1.50 - 1.46 (m, 1H), 1.22 (s, 3H), 1.18 – 1.13 (m, 1H), 1.11 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 158.89, 158.83, 157.80, 150.54, 129.98, 127.12, 111.25, 110.43, 70.17, 55.42, 51.28, 38.70, 37.98, 37.54, 35.79, 30.10, 29.82, 27.22, 25.67, 19.32, 18.94. ESI-HRMS (negative) m/z: $[M – H]^-$ calcd for $C_{20}H_{25}O_5$, 345.1702; found 345.1708.



*Compound **18***: white resin, r.f. 0.34 (hexane/EtOAc 8:2). $[a]_{D}^{29.1}$ = + 35.2 ($CHCl_3$, c 0.5). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.95 (d, $^3J_{orto}$= 8.4 Hz, 1H), 6.80 (d, $^4J_{meta}$ = 2.5 Hz, 1H), 6.67 (dd, $^3J_{orto}$= 8.4, $^4J_{meta}$=2.5 Hz, 1H), 4.39 (d, J = 11.0 Hz, 1H), 4.02 (d, J = 11.0 Hz, 1H), 3.77 (s, 3H), 2.91 – 2.77 (m, 2H), 2.73 - 2.64 (m, 4H), 2.28 (d, *J* = 12.6 Hz, 1H), 2.01 - 1.96 (m, 1H), 1.81 – 1.67 (m, 3H), 1.63 – 1.60 (m, 1H), 1.52 – 1.47 (m, 1H), 1.44 – 1.40 (m, 1H), 1.20 (s, 3H), 1.11 – 1.07 (m, 1H), 1.03 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.45, 157.86, 150.82, 127.14, 111.16, 110.39, 67.49, 55.38, 51.34, 38.85, 38.03, 37.40, 35.98, 30.17, 29.83, 29.14, 27.38, 25.70, 19.40, 19.03. ESI-HRMS (negative) m/z: $[M-H]^-$ calcd for $C_{22}H_{29}O_5$, 373.2015; found 373.2023.

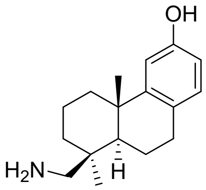
Synthesis of compound 19



To a solution of podocarpic acid (**1**) (151 mg, 0.55 mmol, 1 equiv.) in dry DMF were added DIPEA (0.38 mL, 2.19 mmol, 4 equiv.), HATU (230 mg, 0.6 mmol, 1.1 equiv.) and NH_4Cl (76.71 µL, 2.19 mmol, 4 equiv.). The reaction mixture was stirred at room temperature for 16h. Then, it was quenched by the addition of distilled water (5 mL) and evaporated to dryness under reduced pressure removing the excess of DMF. The resulting residue was solubilized in EtOAc and distilled water (5 mL) was added. The aqueous layer was extracted with EtOAc (3 x 10 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated in *vacuo* to afford compound **19** in quantitative yield (150 mg, 0.55 mmol).

*Compound **19**:* white resin, r.f. 0.12 (DCM/EtOAc 8:2). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.90 (d, $^3J_{orto}$ = 8.4 Hz, 1H), 6.75 (d, $^4J_{meta}$ = 1 Hz, 1H), 6.60 (dd, $^3J_{orto}$ = 8.4, $^4J_{meta}$ = 2.4 Hz, 1H), 5.71-5.56 (m, 2H), 2.88-2.83 (m, 1H), 2.77-2.69 (m, 1H), 2.22 – 2.14 (m, 3H), 2.04 – 1.96 (m, 2H), 1.68-1.64 (m, 1H), 1.57-1.54 (m, 1H), 1.44-1.38 (m, 2H), 1.30 (s, 3H), 1.17 (s, 3H). **^{13}C NMR** (101 MHz, CD_2Cl_2/CD_3OD) δ 181.84, 155.41, 150.02, 130.40, 126.88, 113.56, 112.50, 53.87, 49.15, 44.50, 39.36, 38.39, 32.04, 30.03, 23.53, 22.17, 20.75. ESI-HRMS (positive) m/z $[M + H]^+$ calcd for $C_{17}H_{24}NO_2$ 274.1807; found 274.1798. $[M + Na]^+$ calcd for $C_{17}H_{23}NO_2Na$ 296.1626; found 296.1613.

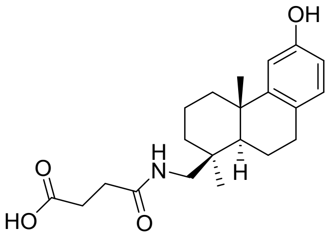
Synthesis of compound 20



A solution of compound **19** (98 mg, 0.36 mmol, 1 equiv.) in dry THF (0.77 mL) was combined with 0.74 mL of $LiAlH_4$ (2M in THF, 1.44 mmol, 4 equiv.) at 0°C, and the reaction mixture was stirred for 16h at r.t.. Afterward, it was cooled down and quenched by slow addition of ethyl alcohol (EtOH) until effervescence ceased. The excess of THF and EtOH was evaporated under reduced pressure and then a saturated aqueous solution of Rochelle's salt (5 mL) was added and the resulting aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in *vacuo*. The residue was purified by silica gel flash chromatography with 2% of CH_3OH in $CHCl_3$ and 1% of Et_3N to afford compound **20** in 50% yield (47 mg, 0.18 mmol).

Compound ***20****:* yellow resin, r.f. 0.1 ($CHCl_3/CH_3OH/NH_4OH$ 95:5:1%). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 6.88 (d,$^3J_{orto}$ = 8.2 Hz, 1H), 6.72 (d,$^4J_{meta}$ = 2.6 Hz, 1H), 6.57 (dd, $^3J_{orto}$ = 8.4, $^4J_{meta}$ =2.4, 2.6 Hz, 1H), 2.97 (d, J = 13.2 Hz, 1H), 2.87 (d, J = 6.5 Hz, 1H), 2.79 – 2.72 (m, 1H), 2.61 (d, J = 13.2 Hz, 1H), 2.23 (d, J = 12.8 Hz, 1H), 1.90 (dd, J = 13.1, 7.3 Hz, 1H), 1.84 (d, J = 13.8 Hz, 1H), 1.73 – 1.64 (m, 2H), 1.64 – 1.58 (m, 1H), 1.49 (dd, J = 12.6, 1.9 Hz, 1H), 1.43 (td, J = 12.9, 3.9 Hz, 1H), 1.18 (s, 3H), 1.03 – 0.99 (m, 1H), 0.99 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ (ppm) 154.05, 151.53, 130.09, 126.89, 113.03, 111.31, 51.70, 46.08, 44.45, 39.17, 38.22, 35.34, 30.24, 27.25, 25.84, 19.27, 19.09. ESI-HRMS (positive) m/z $[M + H]^+$ calcd for $C_{17}H_{26}NO$ 260.2014; found 260.2004.

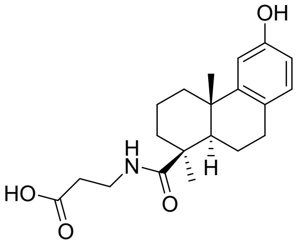
Synthesis of compound 21



Succinic anhydride (15.01 mg, 0.15 mmol, 1 equiv.) was added at 0°C to a stirring solution in 1,2-dichlorethane (DCE) (0.5 mL) of **20** (38.3 mg, 0.15 mmol, 1 equiv.), followed by the addition of DMAP (1.22 mg, 0.01 mmol, 0.07 equiv.). The reaction mixture was stirred for 30 minutes at room temperature, after which it was evaporated under reduced pressure to remove the excess of DCE. The residue was diluted with THF (0.5 mL) and a solution of 5% aqueous citric acid was added dropwise until effervescence ceased. The mixture was evaporated under reduced pressure to remove the excess of THF, diluted with water (5mL) and the resulting aqueous phase was extracted with EtOAc (1 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in *vacuo*. The residue was purified by silica gel flash chromatography with a gradient of 0%-3% of CH_3OH in $CHCl_3$ to afford compound **21** in quantitative yield (53.9 mg, 0.15 mmol).

*Compound **21**:* brown resinous oil, r.f. 0.28 ($CHCl_3$/CH_3OH 9:1). **^{1}H NMR** (400 MHz, CD_3OD) δ 8.30 (s, 1H), 6.81 (d, $^3J_{orto}$ = 8.1 Hz, 1H), 6.69 (d,$^4J_{meta}$ = 2.6 Hz, 1H), 6.50 (dd, $^3J_{orto}$ = 8.4, $^4J_{meta}$ =2.4, 2.6 Hz, 1H), 3.71 (d, J = 13.4 Hz, 1H), 3.02 (d, J = 13.4 Hz, 1H), 2.86 – 2.81 (m, 1H), 2.76 – 2.67 (m, 1H), 2.55 (dd, J = 32.5, 6.2 Hz, 4H), 2.27 (d, J = 12.6 Hz, 1H), 2.01 – 1.81 (m, 2H), 1.72 (d, J = 12.8 Hz, 2H), 1.56 (d, J = 14.1 Hz, 1H), 1.44 – 1.36 (m, 3H), 1.23 (s, 3H), 0.97 (s, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ 174.87, 156.16, 152.11, 130.76, 126.80, 113.85, 111.84, 53.02, 42.56, 40.17, 39.21, 38.99, 37.15, 31.81, 31.15, 30.76, 28.09, 26.20, 20.25, 20.16. ESI-HRMS (negative) m/z: $[M – H]^–$ calcd for $C_{21}H_{28}NO_4$, 358.2018, found 358.2027.

Synthesis of compound 22

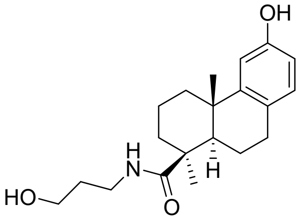


A solution of **1** (50 mg, 0.18 mmol, 1 equiv.) in DMF (0.5 mL) was treated with DIPEA (0.095 mL, 0.55 mmol, 3 equiv.). After 5 min, HATU (69 mg, 0.18 mmol, 1 equiv.) was added and the reaction mixture was stirred at room temperature for 30 minutes. A solution of ß-alanin (48 mg, 0.55 mmol, 3 equiv.) in H_2O (0.25 mL) was added dropwise and the reaction mixture was stirred at room temperature for additional 32 h. DMF was then evaporated *in vacuo* and the resulting residue was diluted with water (5 mL). The aqueous phase was extracted with EtOAc (1 x 10 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated in *vacuo*. The residue was purified by silica gel flash chromatography with 2% of CH_3OH in $CHCl_3$ to afford compound **22** in 65% yield (41 mg, 0.12 mmol).

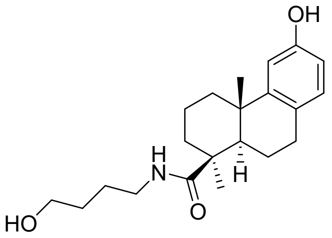
*Compound **22**:* brown powder, r.f. 0.15 ($CHCl_3$/CH_3OH 9:1), m.p. 138-140 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 6.81 (d, $^3J_{orto}$ = 8.1 Hz, 1H), 6.68 (d, $^4J_{meta}$ = 2.6 Hz, 1H), 6.50 (dd, $^3J_{orto}$ = 8.4, $^4J_{meta}$ =2.4, 2.6 Hz, 1H), 3.43 (t, *J* = 6.5 Hz, 2H), 2.81 – 2.76 (m, 1H), 2.66 (td, *J* = 16.3, 14.6, 5.6 Hz, 1H), 2.52 (t, *J* = 6.3 Hz, 2H), 2.20 (t, *J* = 15.7 Hz, 3H), 2.11 – 1.89 (m, 2H), 1.61 (d, *J* = 13.9 Hz, 1H), 1.50 (d, *J* = 12.0 Hz, 1H), 1.34 – 1.26 (m, 1H), 1.23 (s, 3H), 1.21 – 1.12 (m, 1H), 1.07 (s, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ 179.47, 175.74, 156.18, 150.36, 130.80, 127.24, 114.02, 112.87, 55.02, 45.11, 41.10, 39.87, 38.52, 36.44, 34.15, 32.66, 30.04, 23.95, 22.76, 21.26. ESI-HRMS (negative) m/z: [M - H]$^-$ calcd for $C_{20}H_{26}NO_4$ 344.1861; found 344.1867.

General procedure for the synthesis of compounds 23, 24 and 25

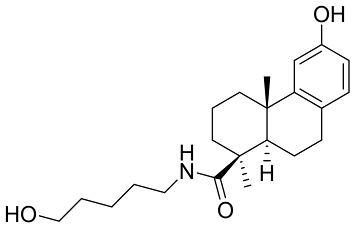
A solution of **1** (50 mg, 0.18 mmol, 1 equiv.) in dry DMF (0.5 mL) was treated with DIPEA (0.095 mL, 0.55 mmol, 3 equiv.). After 10 min, HATU (104 mg, 0.27 mmol, 1.5 equiv.) was added and the reaction mixture was stirred at room temperature for 30 minutes. Afterward, the corresponding amino-alcohol - 3-amino-1-propanol, 4-amino-1-butanol or 5-amino-1-pentanol - (0.27 mmol, 1.5 equiv.) was added dropwise and the reaction mixture was stirred at room temperature for additional 16h. The reaction mixture was quenched with 5 mL of water and the excess of DMF was evaporated in *vacuo.* The aqueous phase was extracted with EtOAc (1 x 10 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated in *vacuo* to afford the corresponding amides (**23**, **24** and **25**) without any further purification.



Compound 23: 90% yield. White powder, r.f. 0.23 ($CHCl_3/CH_3OH$ 93:7), m.p. 154-156 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.90 (d, $^3J_{orto}$ = 8.2 Hz, 1H), 6.74 (d, $^4J_{meta}$ = 2.6 Hz, 1H), 6.59 (dd, $^3J_{orto}$ = 8.4, $^4J_{meta}$ = 2.6 Hz, 1H), 6.11 (t, J = 5.2 Hz, 1H), 3.66 (t, J = 5.6 Hz, 2H), 3.42 (q, J = 6.0 Hz, 2H), 2.88 – 2.83 (m, 1H), 2.76 – 2.68 (m, 1H), 2.22 – 2.13 (m, 3H), 2.06 – 1.95 (m, 2H), 1.74 – 1.68 (m, 2H), 1.65 – 1.64 (m, 1H), 1.54 (d, J = 11.4 Hz, 1H), 1.41 (td, J = 13.2, 4.1 Hz, 1H), 1.27 (s, 3H), 1.25 – 1.14 (m, 2H), 1.11 (s, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ (ppm) 179.52, 156.13, 150.40, 130.82, 127.29, 114.02, 112.87, 61.06, 55.07, 45.07, 41.10, 39.83, 38.50, 38.06, 32.75, 32.66, 30.16, 23.97, 22.85, 21.33. ESI-HRMS (positive) m/z: $[M + H]^+$ calcd for $C_{20}H_{30}NO_3$ 332.2225; found 332.2220. $[M + Na]^+$ calcd for $C_{20}H_{29}NO_3Na$ 354.2045; found 354.2037.

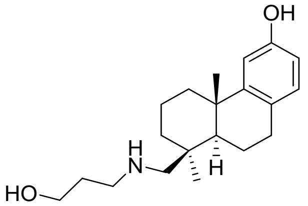


Compound **24**: 100% yield. White powder, r.f. 0.37 ($CHCl_3/CH_3OH$ 9:1), m.p. 180-182 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ (ppm) 7.34 (t, $J = 5.2$ Hz, 1H), 6.81 (d, $^3J_{orto}$ = 8.4 Hz, 1H), 6.68 (d, $^4J_{meta}$=2.4 Hz, 1H), 6.51 (dd, $^3J_{orto}$ = 8 Hz, $^4J_{meta}$= 2.4 Hz, 1H), 3.61 – 3.52 (m, 2H), 3.25 – 3.14 (m, 2H), 2.76 (d, $J = 4.1$ Hz), 1H), 2.65 (td, $J = 12.8, 5.62$ Hz, 1H), 2.24 – 2.17 (m, 3H), 2.05 – 1.97 (m, 2H), 1.64 – 1.61 (m, 1H), 1.57 (t, $J = 2.95$ Hz), 1.48 (d, $J = 11.6$ Hz, 1H), 1.36 (td, $J = 13.2, 3.7$ Hz, 1H), 1.23 (s, 3H), 1.20 – 1.16 (m, 1H), 1.08 (s, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ (ppm) 179.43, 156.15, 150.44, 130.82, 127.30, 114.00, 112.86, 62.61, 55.16, 45.13, 41.15, 40.54, 39.86, 38.53, 32.69, 31.17, 30.16, 26.76, 24.03, 22.90, 21.41. ESI-HRMS (positive) m/z: $[M + H]^+$ calcd for $C_{21}H_{32}NO_3$ 346.2382, found 346.2376; $[M + Na]^+$ calcd for $C_{21}H_{31}NO_3Na$ 368.2201, found 368.2193.



Compound **25**: 85% yield. White powder, r.f. 0.39 ($CHCl_3/CH_3OH$ 9:1), m.p. 224-226°C. **^{1}H NMR** (400 MHz, CD_3OD) δ (ppm) 7.33 (t, $J = 5.2$ Hz 1H), 6.82 (d, $^3J_{orto}$ = 8.4 Hz, 1H), 6.69 (d, J_{meta} = 2.4 Hz, 1H), 6.50 (dd, $^3J_{orto}$ = 8.4, $^4J_{meta}$=2.4 Hz, 1H), 3.54 (t, $J = 6.6$ Hz 2H), 3.24 – 3.11 (m, 2H), 2.82-2.77 (m,1H), 2.70-2.61 (m, 1H), 2.25-2.18 (m, 3H), 2.05 – 1.98 (m, 2H), 1.65-1.62 (m, 1H), 1.57 – 1.47 (m, 5H), 1.41 – 1.37 (m, 3H), 1.24 (s, 3H), 1.21 – 1.18 (m, 1H), 1.08 (s, 3H). **^{13}C NMR** (101 MHz, $(CD_3)_2SO$) δ (ppm) 176.27, 173.03, 155.34, 149.59, 129.94, 125.59, 113.32, 112.04, 61.09, 53.29, 43.63, 37.44, 32.43, 31.38, 29.63, 29.03, 23.57, 23.36, 21.67, 21.61, 20.42. ESI-HRMS (positive) $[M + H]^+$ calcd for $C_{22}H_{34}NO_3$ 360.2538, found 360.2531; $[M + Na]^+$ calcd for $C_{22}H_{33}NO_3Na$ 382.2358, found 382.2355.

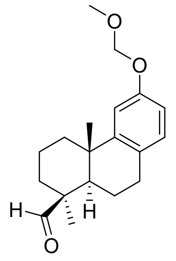
Synthesis of compound 26



A solution of compound **23** (110 mg, 0.33 mmol, 1 equiv.) in dry THF (0.58 mL) was combined with 1 mL of $LiAlH_4$ (2M in THF, 1.99 mmol, 6 equiv.) at 0°C, and the reaction mixture was stirred under reflux for 32h. It was then cooled down, quenched by a slow addition of EtOH until effervescence ceased. The excess of THF and EtOH was evaporated under reduced pressure, followed by the addition of a saturated aqueous solution of Rochelle's salt (5 mL). The resulting aqueous phase was extracted with EtOAc (3 x 10 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated in *vacuo*. The residue was purified by silica gel flash chromatography with a gradient of 5%-10% of CH_3OH in $CHCl_3$ and 1% of Et_3N to afford compound **26** in 12% yield (13 mg, 0.04 mmol).

*Compound **26**:* brown resin, r.f. 0.15 ($CHCl_3/CH_3OH/NH_4OH$ 9:1:1%). **^{1}H NMR** (400 MHz, CD_3OD) 6.80 (d, $^3J_{orto}$ = 8.4 Hz, 1H), 6.68 (d, J_{meta} = 2.4 Hz, 1H), 6.49 (dd, $^3J_{orto}$ = 8.4, $^4J_{meta}$ =2.4 Hz, 1H), 3.69 (t, J = 5.9 Hz, 2H), 2.99 (d, J = 12.0 Hz, 1H), 2.88 – 2.80 (m, 3H), 2.76 – 2.67 (m, 1H), 2.60 (d, J = 11.9 Hz, 1H), 2.28 (d, J = 12.7 Hz, 1H), 1.95 – 1.88 (m, 2H), 1.83 – 1.76 (m, 3H), 1.73 – 1.67 (m, 1H), 1.65 – 1.59 (m, 1H), 1.43 – 1.35 (m, 2H), 1.26 – 1.22 (m, 1H), 1.20 (s, 3H), 1.06 (s, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ 156.24, 151.96, 130.77, 126.70, 113.91, 111.88, 62.27, 53.68, 53.27, 40.23, 39.02, 38.17, 37.22, 31.50, 31.22, 30.67, 28.10, 26.13, 20.39, 20.04. ESI-HRMS (positive) m/z: $[M + H]^+$ calcd for $C_{20}H_{32}NO_2$ 318.2433, found 318.2429.

Synthesis of compound 27

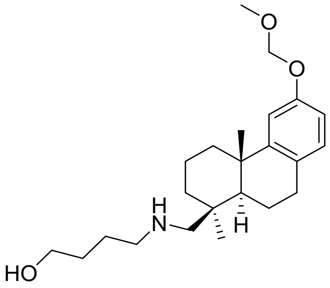


A solution of **10** (128 mg, 0.42 mmol, 1 equiv.) and $NaHCO_3$ (213 mg, 2.53 mmol, 6 equiv.) in 5 mL of dry DCM was prepared and, to this, a solution of DMP (0.3 M in DCM, 1.4 mL, 0.42 mmol, 1 equiv.) was added dropwise at 0°C. The reaction mixture was stirred at room temperature for 2h. After this, the mixture was cooled back to 0°C and, sequentially, saturated solutions of $NaHCO_3$ (5 mL) and $Na_2S_2O_3$ (5 mL) were added. After stirring for an additional 15 minutes at room temperature, the aqueous phase was extracted with DCM (3 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in *vacuo* and the resulting residue was purified by silica gel flash chromatography with 5% of Et_2O in hexane to afford compound **27** in 65% yield (81 mg, 0.27 mmol).

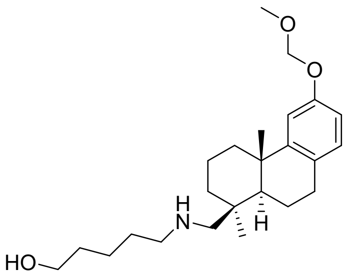
*Compound **27**:* white powder, rf 0.68 (Hexane/EtOAc 9 :1). ^{1}**H NMR** (400 MHz, $CDCl_3$) δ 9.83 (s, 1H), δ 6.98 (d,$^3J_{orto}$ = 8.4 Hz, 1H), 6.93 (d,$^4J_{meta}$ = 2.4 Hz, 1H), 6.82 (dd, $^3J_{orto} = 8.4$, $^4J_{meta} = 2.4$, 2.6 Hz, 1H), 5.13 (s, 2H), 3.47 (s, 3H), 2.98 – 2.77 (m, 2H), 2.29 – 2.17 (m, 3H), 2.08 – 1.94 (m, 1H), 1.81 – 1.58 (m, 4H), 1.46 – 1.35 (m, 1H), 1.11 (s, 3H), 1.06 (s, 3H). ^{13}**C NMR** (101 MHz, $CDCl_3$) δ 205.84, 155.70, 149.03, 130.10, 128.34, 113.99, 113.30, 94.90, 56.06, 51.98, 48.77, 38.47, 38.36, 33.98, 30.57, 24.34, 24.16, 19.32, 19.03. APCI-MS (positive) m/z: $[M + H]^+$ calcd for $C_{19}H_{27}O_3$ 303.20 found 303.10.

General procedure for the synthesis of compounds 28 and 29

The desired amino-alcohol - 4-amino-1-butanol or 5-amino-1-pentanol - (0.39 mmol, 2 equiv.) was added to a solution of **27** (60 mg, 0.2 mmol, 1 equiv.) in DCM (0.5 mL). After stirring for 1h at room temperature, a solution of glacial acetic acid (1%) was added to reach pH 5. Upon confirmation of imine formation via TLC ($CHCl_3/CH_3OH$ 95:5), $NaCNBH_3$ (18.66 mg, 0.29 mmol, 1.5 equiv.) was added. The reaction mixture was stirred for 24h, after which the reaction was quenched by adding dropwise a 5% aqueous solution of $NaHCO_3$ until effervescence ceased. The aqueous phase was extracted with DCM (3 x 20 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated in *vacuo* to provide the corresponding amine **28** without any further purification, while amine **29** was obtained through purification by silica gel flash chromatography with a gradient of 2% of CH_3OH in $CHCl_3$.



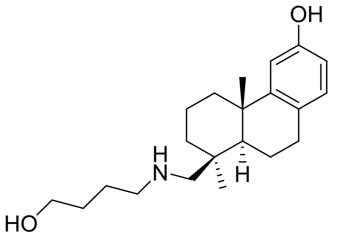
*Compound **28**:* 100% yield. Yellow resin, r.f. 0.37 ($CHCl_3/CH_3OH$ 9 :1). **^{1}H NMR** (400 MHz, CD_3OD) δ 6.91 (m, 2H), 6.74 (dd, $^3J_{orto}$ = 8.4, $^4J_{meta}$ = 2.4 Hz, 1H), 5.10 (s, 2H), 3.60 (t, J = 5.6 Hz, 2H), 3.44 (s, 3H), 3.03 (d, J = 12.0 Hz, 1H), 2.88 (dd, J = 16.7, 5.9 Hz, 1H), 2.81 – 2.71 (m, 3H), 2.63 (d, J = 12.0 Hz, 1H), 2.36 – 2.27 (m, 1H), 1.97 – 1.91 (m, 2H), 1.76 – 1.62 (m, 6H), 1.44 (t, J = 8.8 Hz, 3H), 1.20 (s, 3H), 1.08 (s, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ 157.00, 151.99, 130.79, 129.31, 114.91, 113.58, 95.81, 62.67, 56.06, 53.64, 53.09, 51.64, 40.10, 39.12, 38.13, 37.11, 31.74, 31.17, 27.99, 26.65, 26.09, 20.21, 19.97. ESI-MS (positive) m/z: $[M + H]^+$ calcd for $C_{23}H_{38}NO_3$ 376.56, found 376.3; $[M + 2H]^+$ calcd for $C_{23}H_{39}NO_3$ 377.57, found 377.3; $[M + 3H]^+$ calcd for $C_{23}H_{40}NO_3$ 378.58, found 378.4.



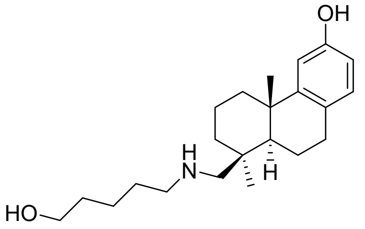
*Compound **29**:* 65% yield. Yellow resin, r.f. 0.24 ($CHCl_3/CH_3OH$ 9 :1). **^{1}H NMR** (400 MHz, CD_3OD) δ 6.94 – 6.86 (m, 2H), 6.74 (dd, $^3J_{orto}$ = 8.4, $^4J_{meta}$ = 2.4 Hz, 1H), 5.10 (s, 2H), 3.57 (t, J = 6.5 Hz, 2H), 3.43 (s, 3H), 2.96 (d, J = 11.9 Hz, 1H), 2.92 – 2.59 (m, 5H), 2.56 (d, J = 11.9 Hz, 1H), 2.30 (d, J = 12.6 Hz, 1H), 1.95 (dd, J = 12.2, 8.4 Hz, 2H), 1.80 – 1.54 (m, 7H), 1.46 – 1.36 (m, 4H), 1.20 (s, 3H), 1.06 (d, J = 7.2 Hz, 1H), 1.06 (s, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ 156.97, 152.11, 130.78, 129.37, 114.87, 113.59, 95.82, 62.75, 56.07, 53.69, 51.91, 47.07, 40.20, 39.15, 38.25, 37.17, 33.38, 31.24, 28.06, 26.13, 24.65, 20.26, 20.05. ESI-MS (positive) m/z: $[M + H]^+$ calcd for $C_{24}H_{40}NO_3$ 390.59, found 390.4; $[M + 2H]^+$ calcd for $C_{24}H_{41}NO_3$ 391.6, found 391.3.

General procedure for the synthesis of compounds 30 and 31

TFE (0.045 mL, 0.59 mmol, 5 equiv.) and TFA (0.045 mL, 0.59 mmol, 5 equiv.) were added at 0°C to a solution in DCM (0.5 mL) of the desired compound **28** or **29** (0.12 mmol, 1 equiv.). The reaction mixture was stirred for 2h at room temperature. While completed, the reaction was quenched adding 5 mL of distilled water and an aqueous solution of NaOH 1M was added dropwise to reach pH 8, the aqueous layer was extracted with DCM (3 x 10 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated in *vacuo*. The residue was solubilized in DCM and hexane was added dropwise to precipitate the corresponding deprotected amines **30** or **31** as pure compounds without any further purification.



Compound ***30***: 55 % yield. Yellow resin, r.f. 0.26 ($CHCl_3/CH_3OH$ 9:1). $[\alpha]^{29.1}_{D}$ = + 37.27 (CH_3OH, c 0.2). **^{1}H NMR** (400 MHz, CD_3OD) 6.81 (d, $^3J_{orto}$ = 8.4 Hz, 1H), 6.68 (d, J_{meta} = 2.4 Hz, 1H), 6.50 (dd, $^3J_{orto}$ = 8.4, $^4J_{meta}$ =2.4 Hz, 1H), 3.59 (t, J = 5.8 Hz, 2H), 3.01 (d, J = 12.0 Hz, 1H), 2.90 – 2.72 (m, 4H), 2.61 (d, J = 12.0 Hz, 1H), 2.29 (d, J = 12.7 Hz, 1H), 1.96 – 1.89 (m, 2H), 1.73 – 1.61 (m, 7H), 1.43 – 1.39 (m, 3H), 1.20 (s, 3H), 1.07 (s, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ 156.26, 151.92, 130.78, 126.69, 113.94, 111.88, 62.70, 53.75, 53.16, 51.68, 40.19, 39.01, 38.16, 37.17, 31.79, 31.20, 28.03, 26.75, 26.11, 20.36, 20.02. ESI-HRMS (positive) m/z: $[M + H]^+$ calcd for $C_{21}H_{34}NO_2$ 332.2589 found 332.2588.



Compound ***31***: 15 % yield. Yellow oil, rf 0.26 ($CHCl_3/CH_3OH$ 9:1). **^{1}H NMR** (400 MHz, CD_3OD) 6.81 (d, $^3J_{orto}$ = 8.4 Hz, 1H), 6.68 (d, J_{meta} = 2.4 Hz, 1H), 6.50 (dd, $^3J_{orto}$ = 8.4, $^4J_{meta}$ =2.4 Hz, 1H), 3.57 (t, J = 6.2 Hz, 2H), 2.92 (d, J = 11.8 Hz, 1H), 2.86 - 2.69 (m, 2H), 2.64 (t, J = 7.4 Hz, 2H), 2.53 (d, J = 11.9 Hz, 1H), 2.28 (d, J = 12.4 Hz, 1H), 1.94 (d, J = 13.1 Hz, 2H), 1.80 – 1.54 (m, 7H), 1.40 (d, J = 12.3 Hz, 5H), 1.20 (s, 3H), 1.05 (s, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ 156.25, 151.95, 130.78, 126.71, 113.92, 111.89, 62.73, 53.78, 53.18, 51.82, 40.23, 39.03, 38.19, 37.16, 33.35, 31.23, 29.22, 27.99, 26.12, 24.62, 20.38, 20.05. ESI-HRMS (positive) m/z: $[M + H]^+$ calcd for $C_{22}H_{36}NO_2$ 346.2746, found 346.2748.

1.1.3 Chromatografic condition for purity check

Chromatographic analyses were performed using a modular JASCO LC-4000 series HPLC, equipped with high-pressure binary pumps, a manual injector (20 µL loop) and a photodiode array (PDA) detector. Chromatographic data were collected and processed using ChromNAV 2 software (JASCO). All analyses were carried out on a Mediterranea SEA18 column (C18 stationary phase, 5 µm, 250 × 4.6 mm i.d.). The mobile phases consisted of eluent A: H_2O/CH_3CN (95:5, v/v) and eluent B: CH_3CN/H_2O (5:95, v/v), both containing 0.1% (v/v) of formic acid. The flow rate was set at 1.0 mL/min, samples were injected using a 10 µL injection volume, and the total run time was 33 min. UV detection was performed at 214 nm. The purity of all compounds was always higher than 95%.

Compound **2**: gradient elution for 0–5 min A:B = 50:50; 5–20 min up to 100% B; 20–25 min to 100% B; 25–26 min up to 50% B; 26–33 min A:B = 50:50; t_R = 8.890 min.

Compound **13**: gradient elution for 0–5 min A:B = 20:80; 5–20 min up to 100% B; 20–25 min to 100% B; 25–26 min up to 80% B; 26–33 min A:B = 20:80; t_R = 13.400 min.

Compound **18**: gradient elution for 0–5 min A:B = 30:70; 5–20 min up to 100% B; 20–25 min to 100% B; 25–26 min up to 70% B; 26–33 min A:B = 30 = 70%; t_R = 11.853 min.

Compound **30**: gradient elution 0–20 min from 5% to 50% B; 20–27 min up to 100% B; 27–29 min to 5% B; 29–33 min A:B = 95:5; t_R = 17.340 min.

1.2 Microbiology

The colistin adjuvant activity of the compounds was tested on *P. aeruginosa* PA14 ColR5 (colistin MIC, 64 µg/mL), previously shown to depend on ArnT-dependent aminoarabinosylation of lipid A.[9] PA14 ColR5 was cultured in Mueller Hinton (MH) broth (70192, Merck) to mid-exponential phase and used to test the compounds in combination with colistin (C4461, Merck, 15.000 IU/mg). For each compound, 96-well microtiter plates were set up with the following samples: two-fold increasing concentrations of the compound (16-250 µM) combined with colistin (8 µg/mL); equal amount of vehicle (DMSO) combined with colistin (8 µg/mL); control wells with no drugs. When applied, 96-well microtiter plates were set with two-fold serial dilution of colistin (1-128 µg/mL), or compounds (16-250 µM). All the wells were inoculated with 5×10^5 CFU/mL, diluted in fresh MH broth. After 24 hours of incubation at 37°C, the optical density at 600 nm (OD_{600}) was measured in a microplate reader (CLARIOstar Plus). Growth was calculated as percentage relative to control cultures inoculated with equivalent concentration of DMSO (corresponding to 100%). Half maximum inhibitory concentration (IC_{50}) was determined by using the nonlinear regression (curve fit) by GraphPad Prism, with an R squared cut off ≥ 0.7. The 90% inhibition concentration (IC_{90}) was calculated from the IC_{50} values as it follows according to Eq. (1) where H is the Hill slope.[2]

$$IC_{90}= [90/(100-90)]^\wedge(1/H)\cdot IC_{50} \qquad (1)$$

Chequerboard assay was performed as previously reported with minor modifications.[3] Briefly, matrices of compounds were assembled in 96-well microtiter plates with two-fold serial dilutions of each drug (compound and colistin), in 100 µL MH broth. Each well was inoculated with 100 µL of PA14 ColR5 at 5×10^5 CFU/mL, grown in MH until mid-exponential phase. Plates were incubated at 37°C for 24 hr, before and after which the OD_{600} was measured. Growth was expressed as a percentage with respect to the controls treated with equivalent concentration of DMSO.

1.3 Cytotoxicity assay

The bronchial epithelial cell line 16HBE, kindly provided by Dr. D. Gruenert,[4] cultivated as previously reported,[5] were seeded in 96-well plates at 3×10^4 cells/well the day before the assay. Fresh medium containing two-fold serial dilutions of the compounds, or equivalent concentrations of vehicle (DMSO), was added to each well. After 3 or 18 h of treatment, MTT was added at the concentration of 0.5 g/L and the cells were incubated for 3 h at 37 °C. Culture supernatant was then discarded, 100 µL of DMSO was added to each well and absorbance at 570 nm was determined (CLARIOstar Plus). Cell viability was expressed as a percentage with respect to the controls treated with equivalent concentration of DMSO (corresponding to 100%).

1.4 Molecular modeling

The three-dimensional structure of ArnT from *P. aeruginosa* was built by AlphaFold2 using the UniProt sequence ID Q02R27 as a reference.[6, 7] Molecular docking was carried out with the FRED docking software version 3.3.0.3.[8, 9] Small molecules were designed with the Picto software version 4.4.0.4 and converted into 3D structures with OMEGA version 3.1.0.3 (OpenEye Cadence Molecular Sciences Inc., Santa Fe, NM).[9] Ionization of molecules was carried out at pH 7.4 by QUACPAC fixpka version 2.0.0, while energy minimization was accomplished with the SZYBKI software version 1.10.0.3.[9] Sequence alignment was carried out with the ClustalX software, version 2.1.

1.5 Binding assay

1.5.1 Production of recombinant *P. aeruginosa* ArnT

The coding sequence of *P. aeruginosa* ArnT with the addition of a Flag tag at the C-terminus was cloned in pET28a and the recombinant protein was produced in *E. coli* BL21(DE3). Cells were grown to OD_{600} 0.5-0.6 and induction was carried out with IPTG 0.1 mM at 28°C for 4 hours. The cells were disrupted in lysis buffer (MOPS 25 mM pH 7.4, NaCl 300 mM, lysozyme 0.5 mg/ml, PMSF 1 mM) by sonication. The clarified lysate was extracted by addition of dodecyl-maltoside (DDM) to 1% at room temperature for 2 hours. ArnT was purified by affinity chromatography on anti-flag G1 agarose (Genscript) in MOPS 25 mM pH 7.4, NaCl 300 mM, DDM 0.01% (buffer A); after extensive washing with buffer A, the protein was eluted with Flag peptide 0.1 mg/ml in buffer A (3 volumes). Purified ArnT was concentrated with Millipore Ultra 15 (10K) filters.

1.5.2 FDO-H Binding assay

Intrinsic tryptophan fluorescence was exploited to carry out the binding assay for **FDO-H**. Fluorescence spectra were obtained using a Horiba Jobin Yvon Fluoromax-3 spectrofluorometer. Purified ArnT was diluted (typically to 250 nM) in buffer A and titrated with **FDO-H** 5-200 µM. After each addition, the samples were allowed to equilibrate for 5 min in the cuvette before recording of the spectra. A blank titration of **FDO-H** in buffer A was performed to obtain a baseline that was subtracted from the corresponding protein spectrum. Excitation wavelength was 295 nm and the emission spectra were recorded between 310 and 450 nm at 25°C (slit width 5 nm for both excitation and emission). Titration data were analyzed with Prism 8 (GraphPad) employing the one-site specific binding equation:

$$Y = \frac{B_{max} \cdot X}{(K_d + X)}$$

with Y being $(F_0\text{-}F)/F_0$, X the variable [**FDO-H**] and Bmax the maximal specific binding signal. To improve reliability, the mean of the fluorescence values from 333 to 337 nm was employed to construct the $(F_0\text{-}F)/F_0$ vs [**FDO-H**] curves. Eight replicates with protein samples from three different purifications were performed.

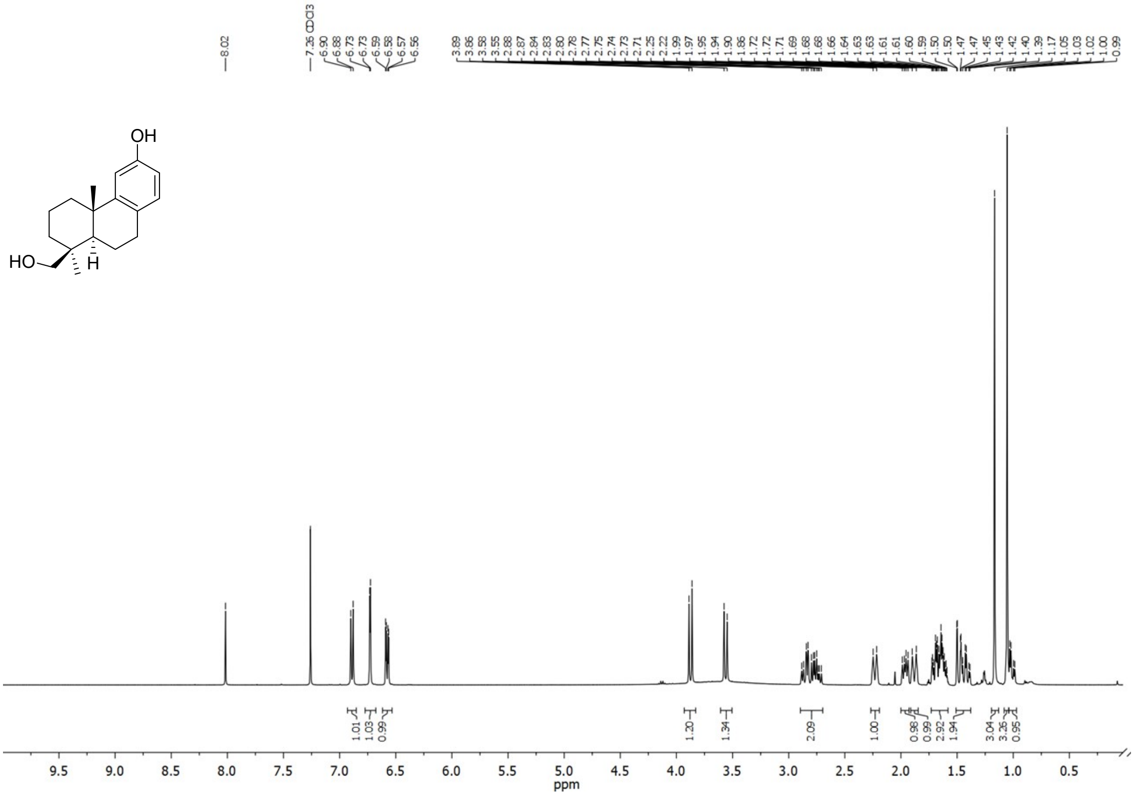
1.6 Statistical analysis

Data were analyzed using GraphPad Prism software (GraphPad Software Inc.); when applied, multiple comparisons were performed using two-way ANOVA with Bonferroni's post-hoc test. Differences were significant at a cut-off value of P 0.05.

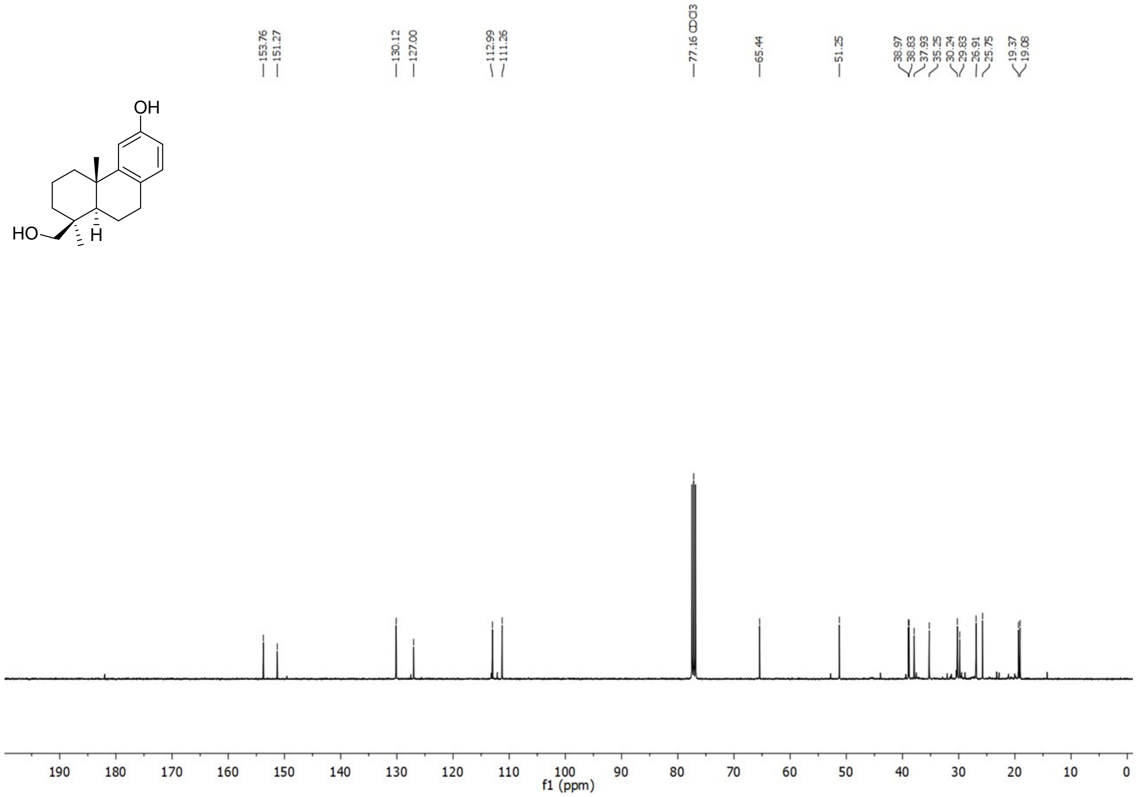
2. Supplementary data

2.1 Chemistry: spectral data

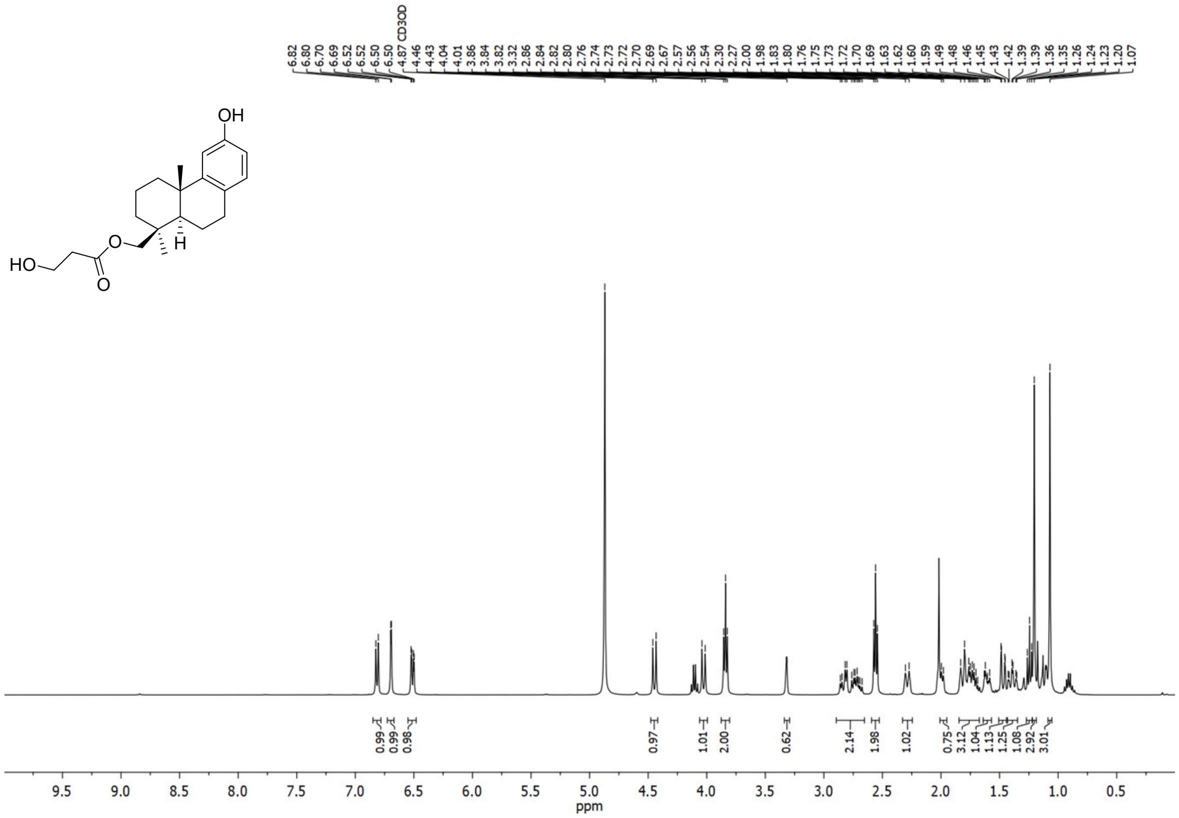
^{1}H-NMR (400MHz, $CDCl_3$) of compound **2**



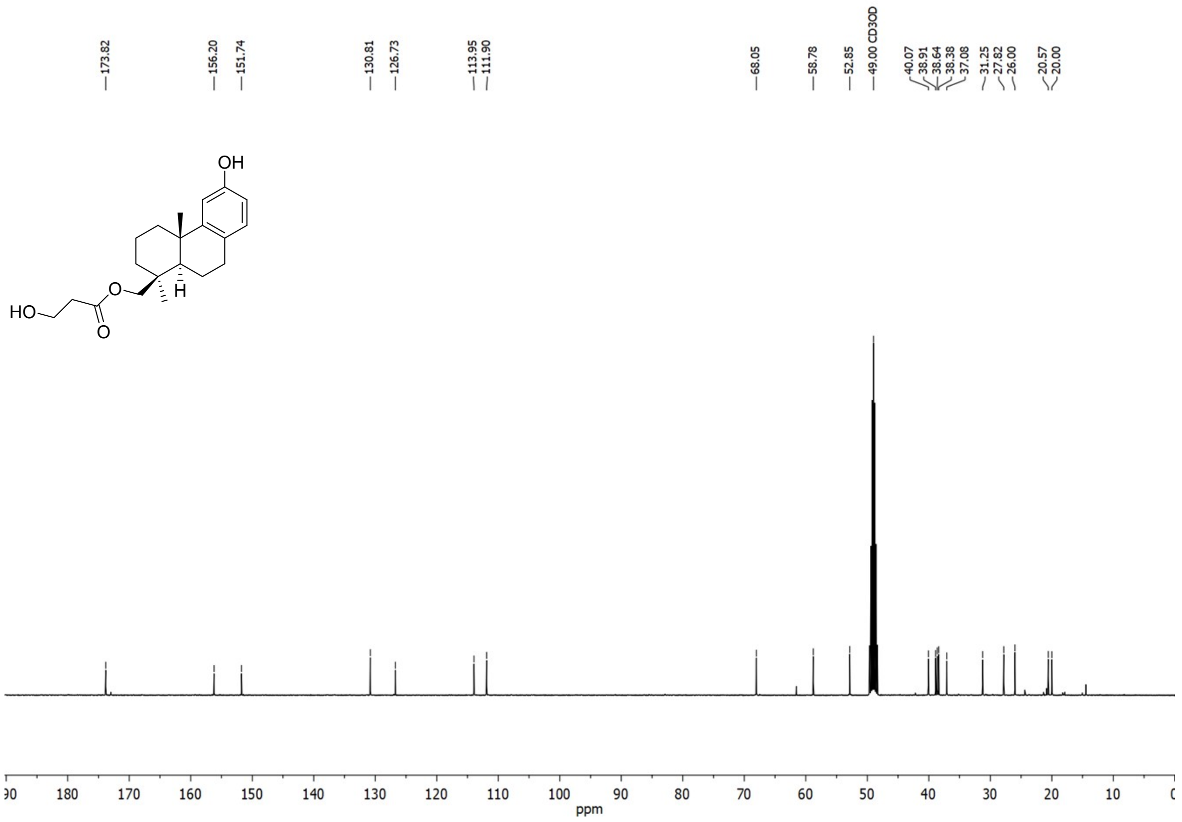
^{13}C-NMR (101MHz, $CDCl_3$) of compound **2**



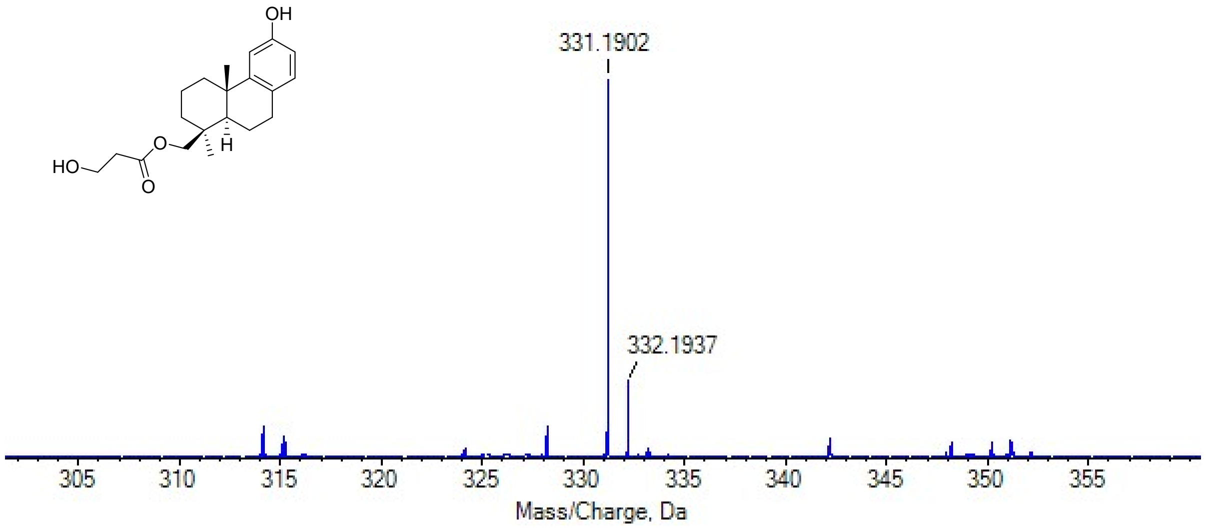
^{1}H-NMR (400MHz, CD_3OD) of compound **3**



^{13}C-NMR (101MHz, CD_3OD) compound **3**

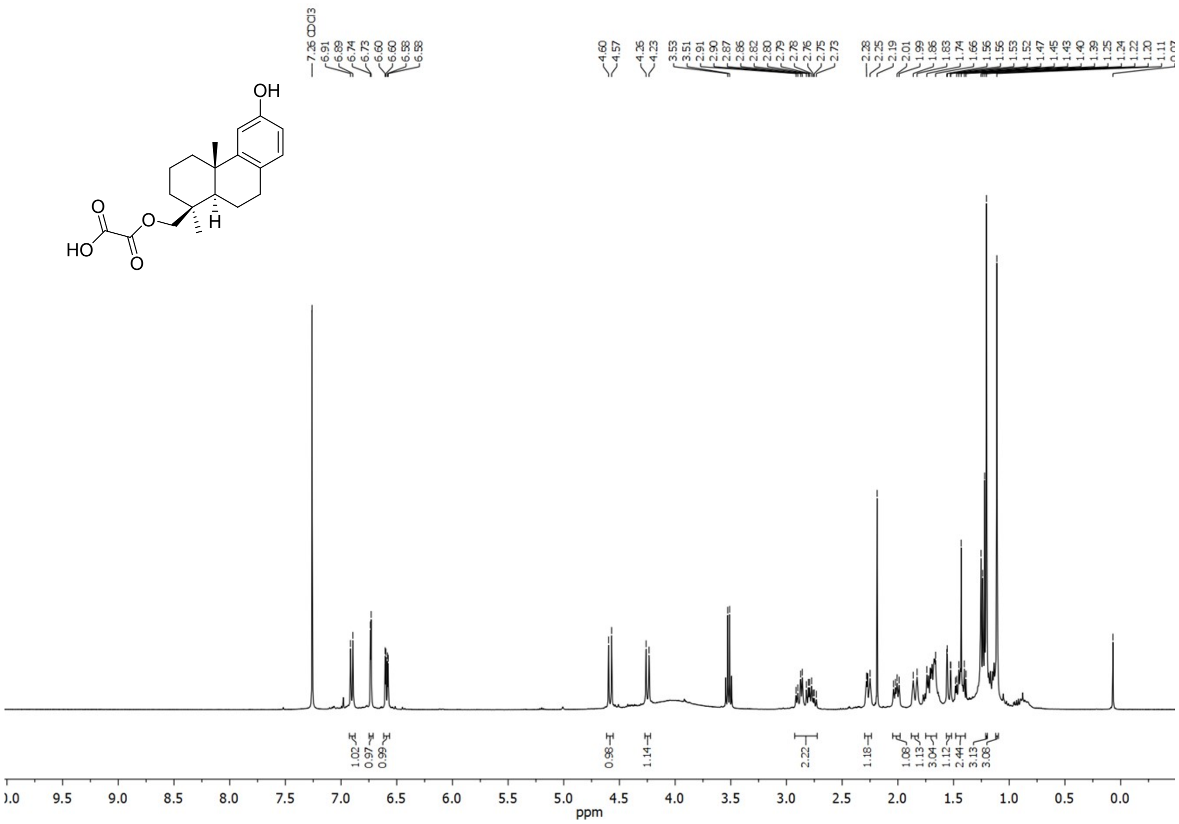


ESI$^-$ mass spectrum of compound **3**

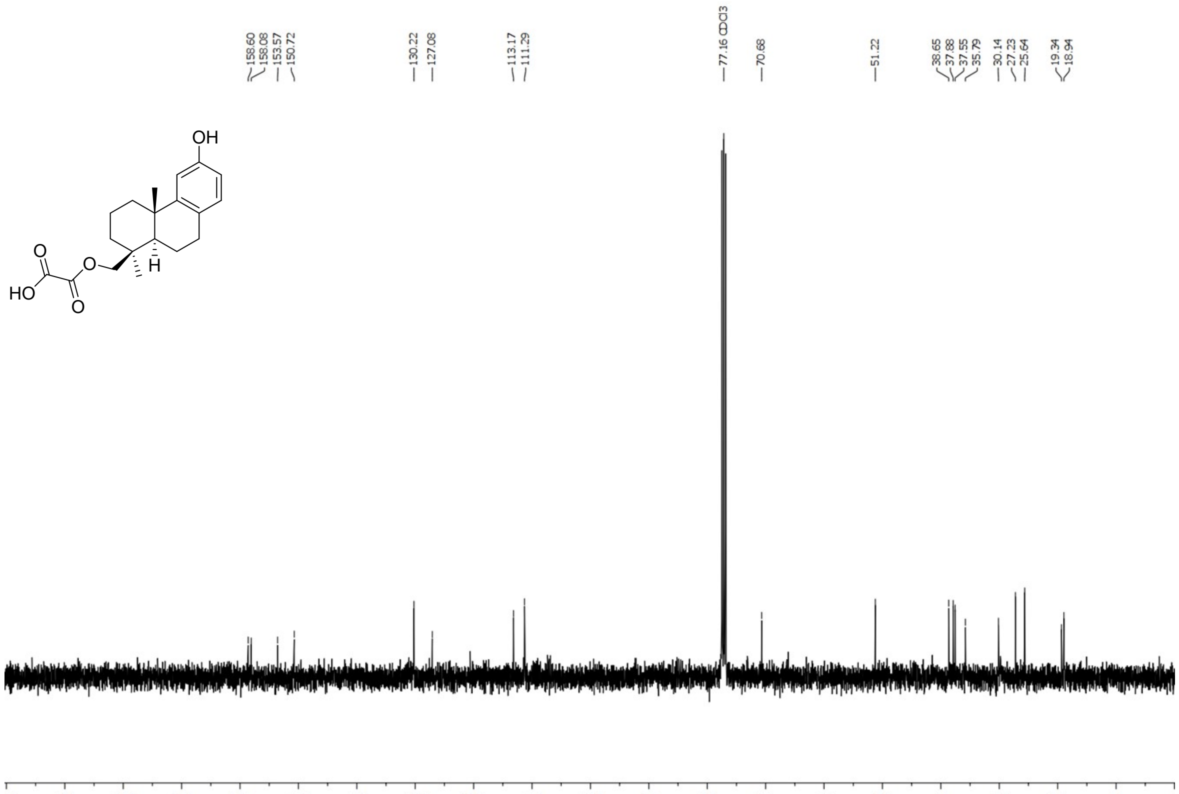


The compound **3** sample was analyzed in negative-ion ESI mode. The signal corresponding to the molecular formula $C_{20}H_{27}O_4^+$, assigned to $[\mathbf{3}-H]^-$, is observed at 331.1902 *m/z* with a mass error of 3.9 ppm relative to the calculated exact mass.

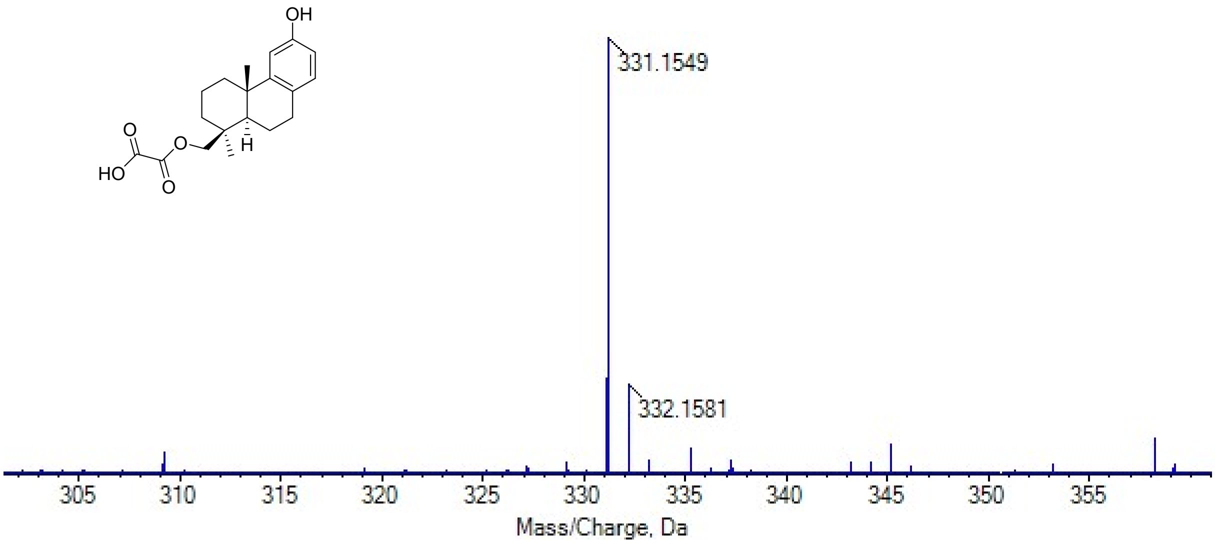
^{1}H-NMR (400MHz, $CDCl_3$) compound of **4**



^{13}C-NMR (101MHz, $CDCl_3$) compound **4**

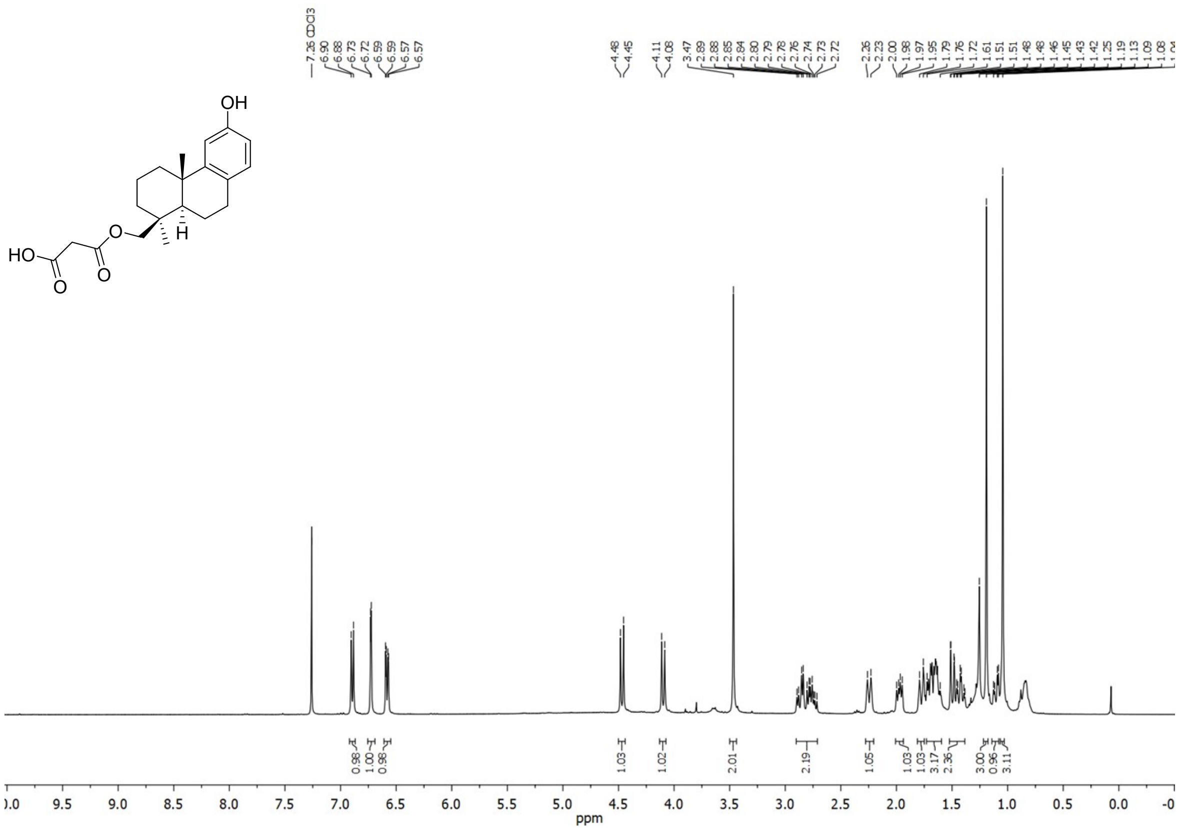


ESI$^-$ mass spectrum of compound **4**

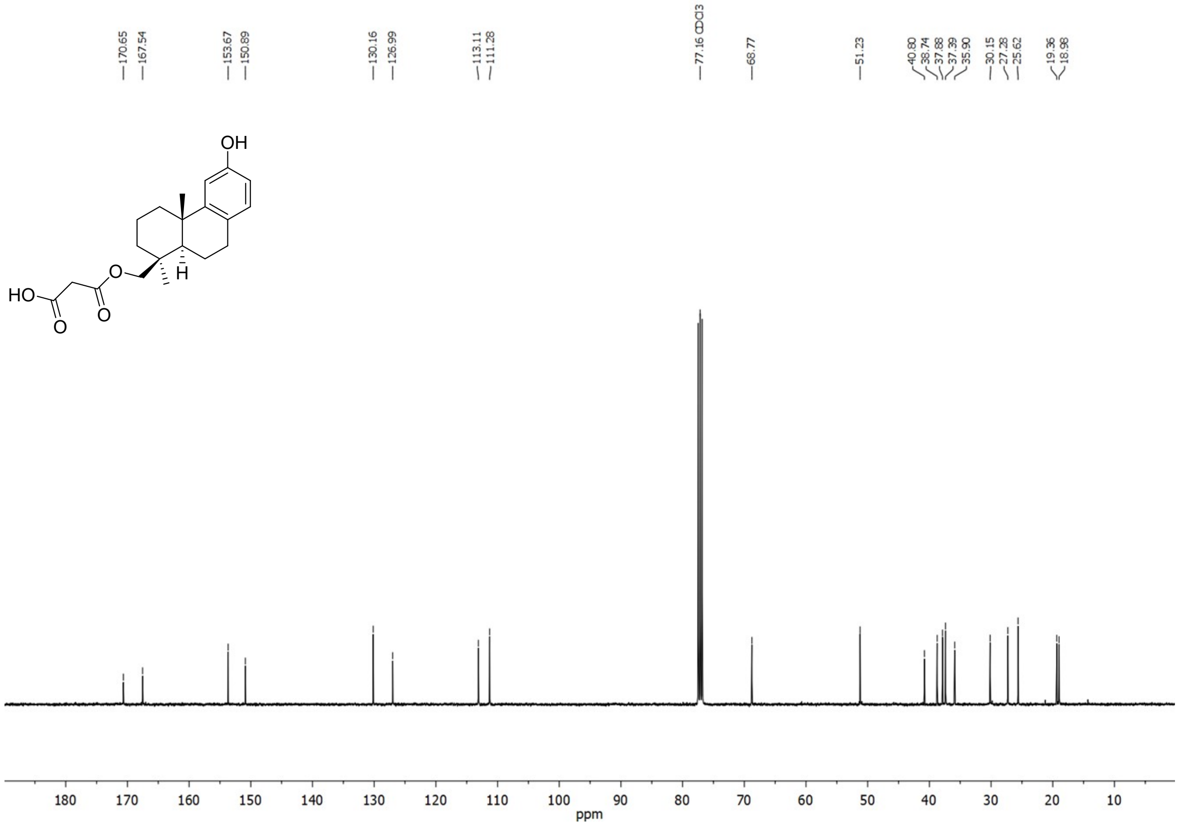


The compound **4** sample was analyzed in negative-ion ESI mode. The signal corresponding to the molecular formula $C_{19}H_{23}O_5^+$, assigned to [**4**−H]$^-$, is observed at 331.1549 *m/z* with a mass error of 0.6 ppm relative to the calculated exact mass.

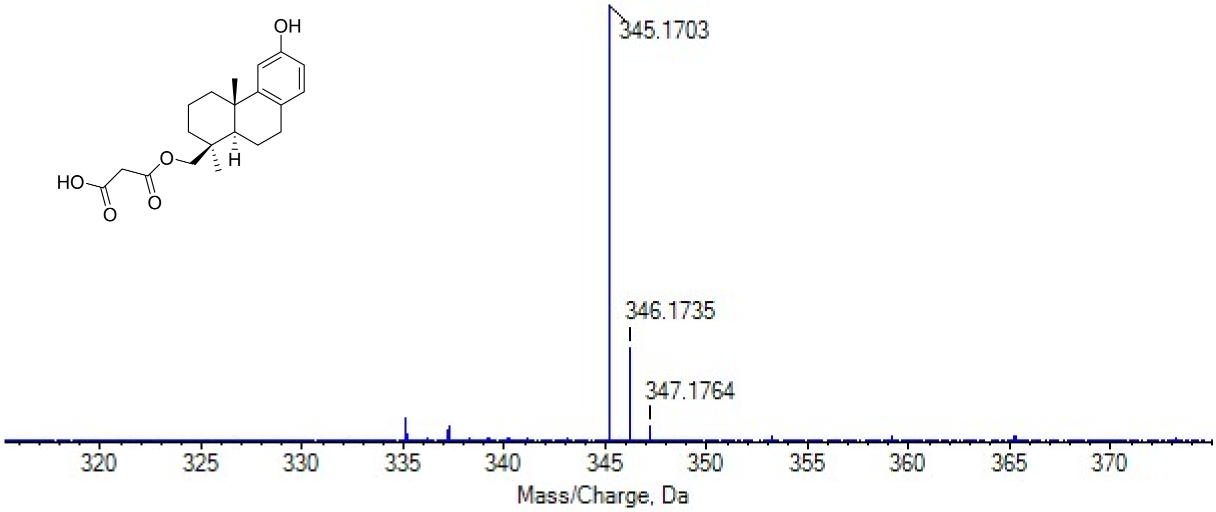
^{1}H-NMR (400MHz, $CDCl_3$) of compound **5**



^{13}C-NMR (101MHz, $CDCl_3$) of compound **5**

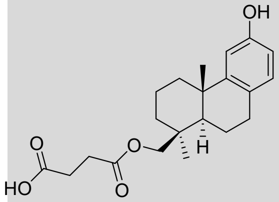


ESI$^-$ mass spectrum of compound **5**.

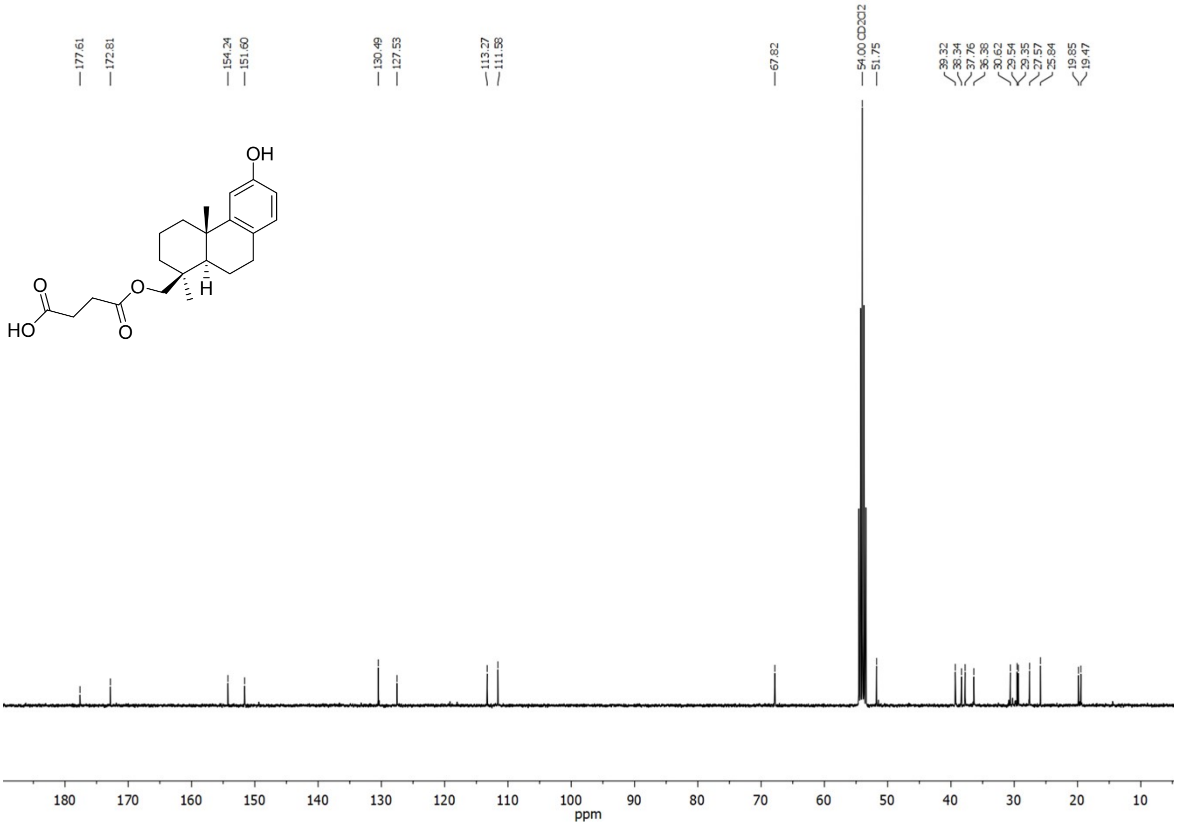


The compound **5** sample was analyzed in negative-ion ESI mode. The signal corresponding to the molecular formula $C_{20}H_{25}O_5^+$, assigned to [**5**–H]$^-$, is observed at 345.1703 *m/z* with a mass error of 1.3 ppm relative to the calculated exact mass.

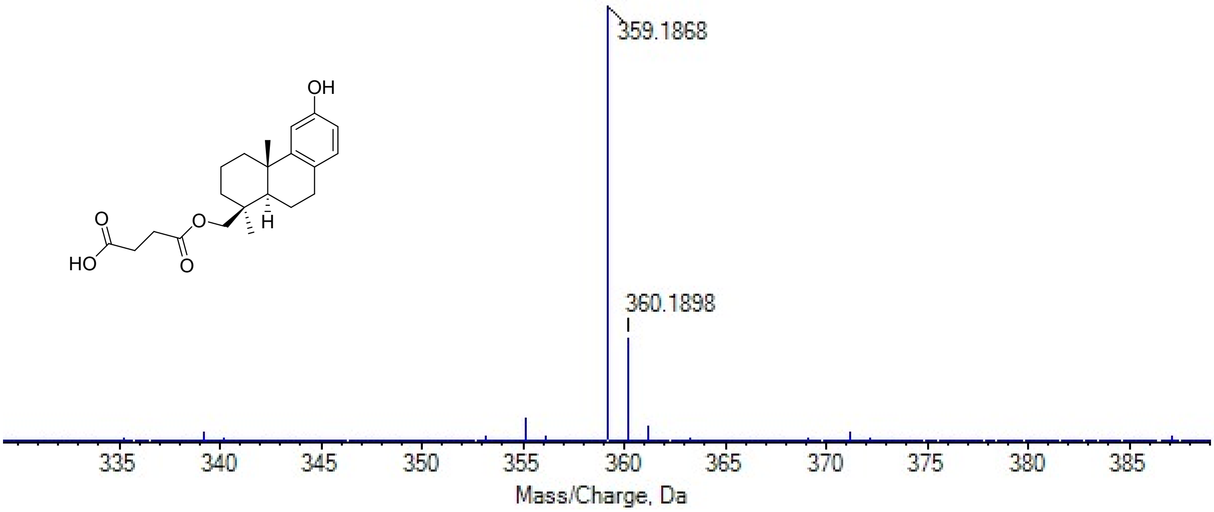
^{1}H-NMR (400MHz, $CDCl_3$) compound **6**



^{13}C-NMR (101MHz, CD_2Cl_2) of compound **6**

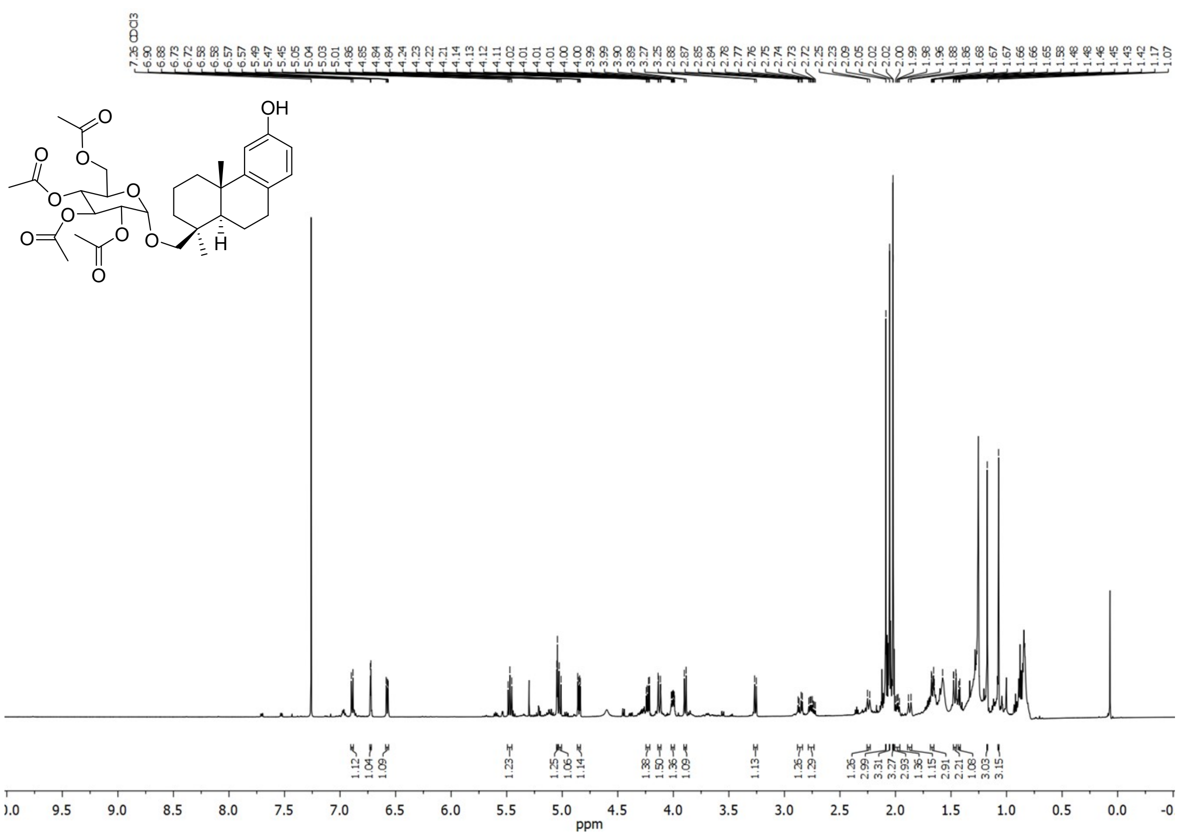


ESI$^-$ mass spectrum of compound **6**.

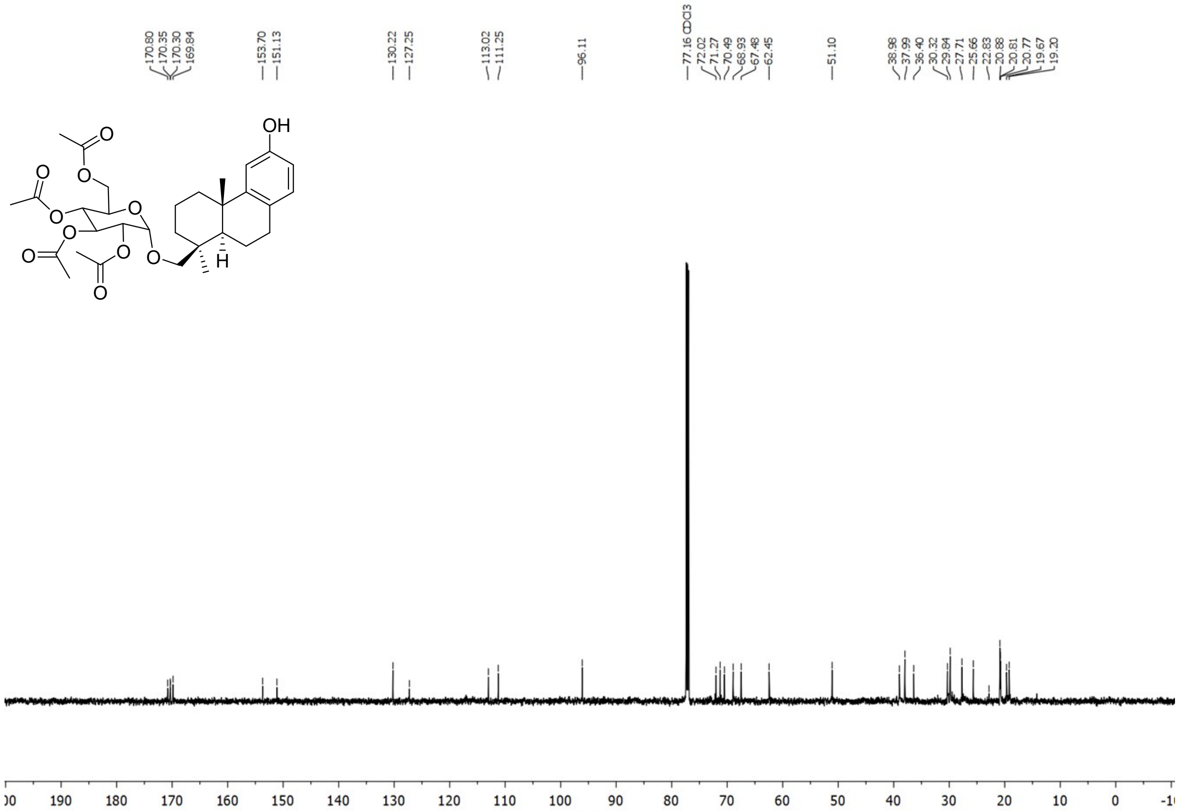


The compound **6** sample was analyzed in negative-ion ESI mode. The signal corresponding to the molecular formula $C_{21}H_{27}O_5^+$, assigned to [**6**–H]$^-$, is observed at 359.1868 *m/z* with a mass error of 1.1 ppm relative to the calculated exact mass.

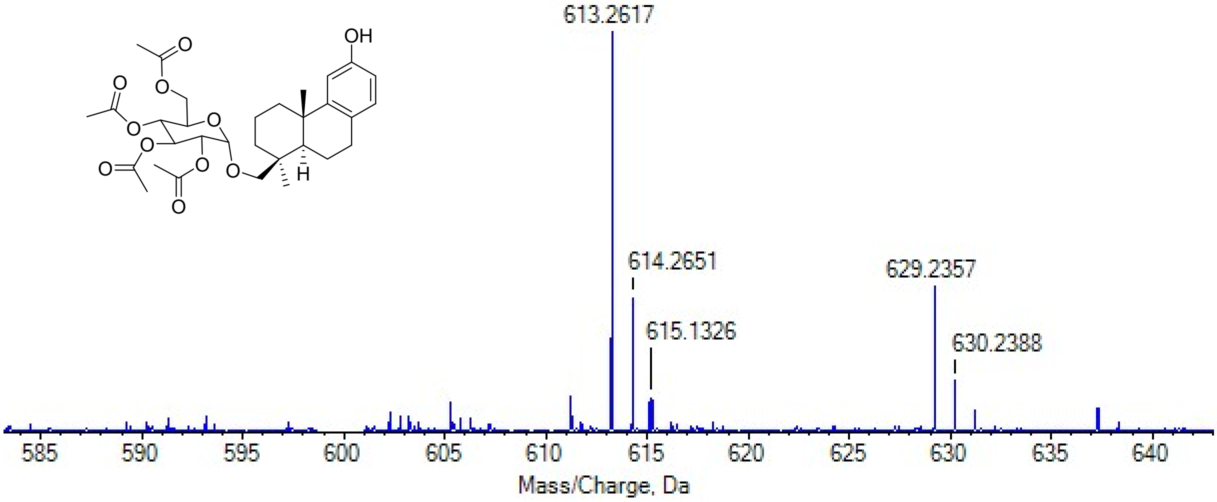
^{1}H-NMR (600MHz, CDCl$_3$) of compound **7**



^{13}C-NMR (101MHz, CDCl$_3$) compound of **7**

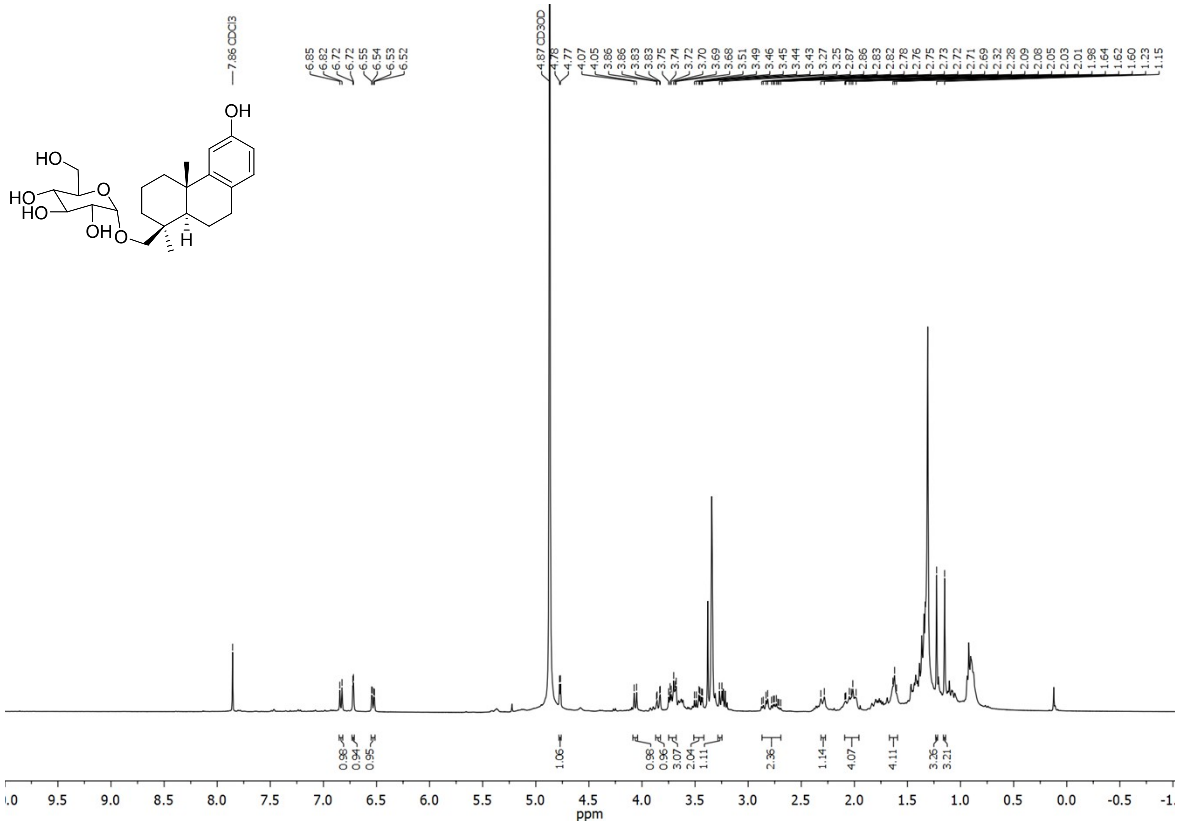


ESI$^+$ mass spectrum of compound **7**.

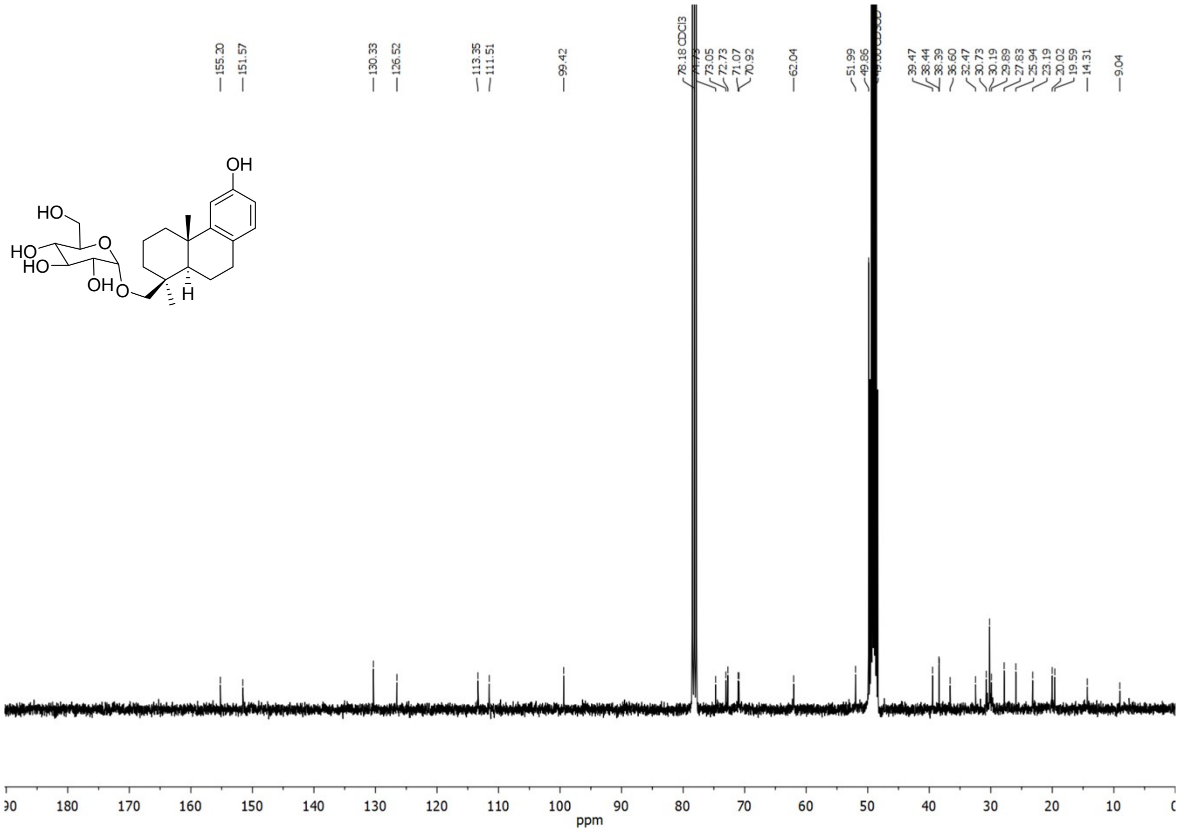


The compound **7** sample was analyzed in positive-ion ESI mode. The signal corresponding to the molecular formula $C_{31}H_{42}O_{11}Na^+$, assigned to [**7**+Na]$^+$, is observed at 613.2617 *m/z* with a mass error of 0.5 ppm relative to the calculated exact mass. The [**7**+K]$^+$ adduct is also present at 629.2357 *m/z*.

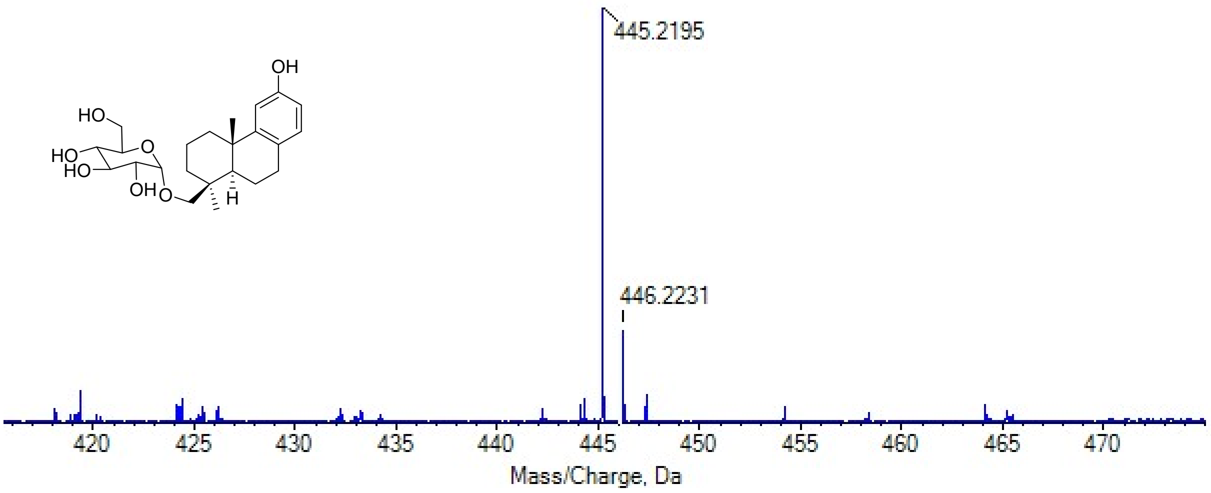
^{1}H-NMR (400MHz, $CDCl_3$/CD_3OD) of compound **8**



^{13}C-NMR (101MHz, $CDCl_3$/CD_3OD) of compound **8**

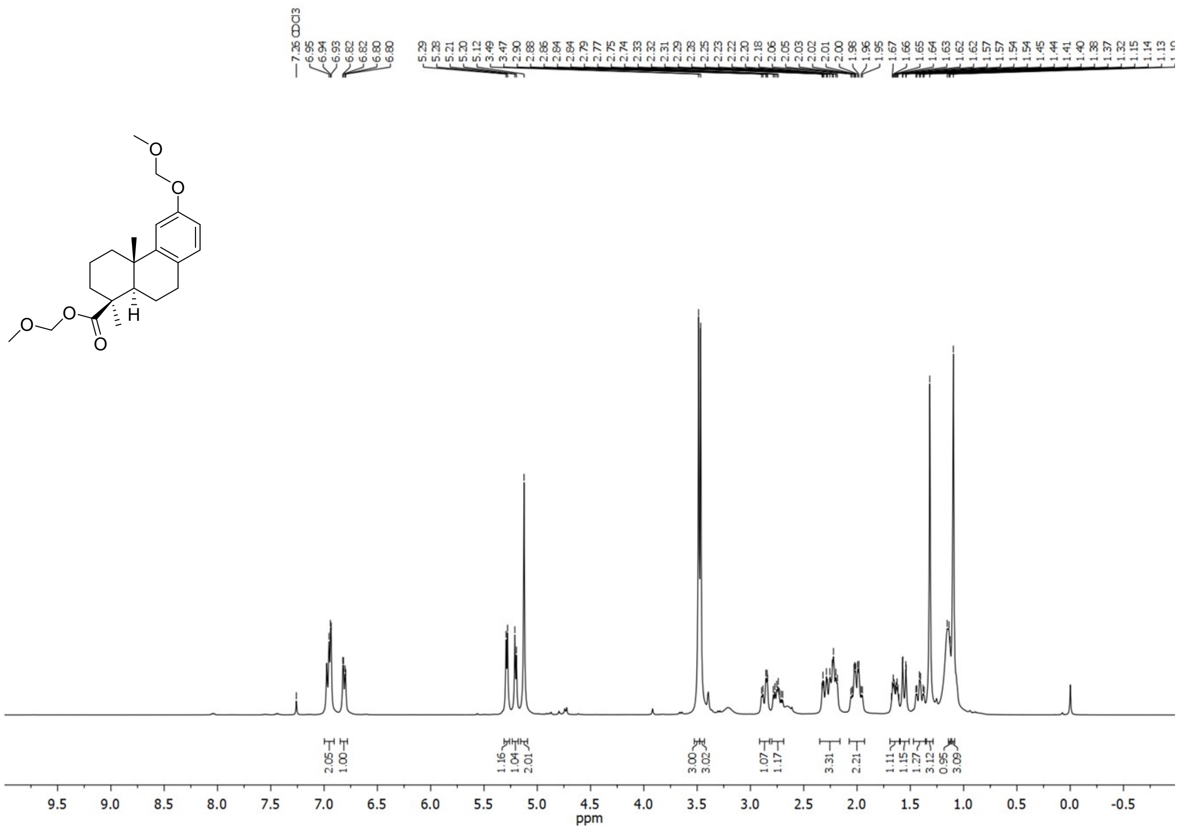


ESI$^+$ mass spectrum of compound **8**.

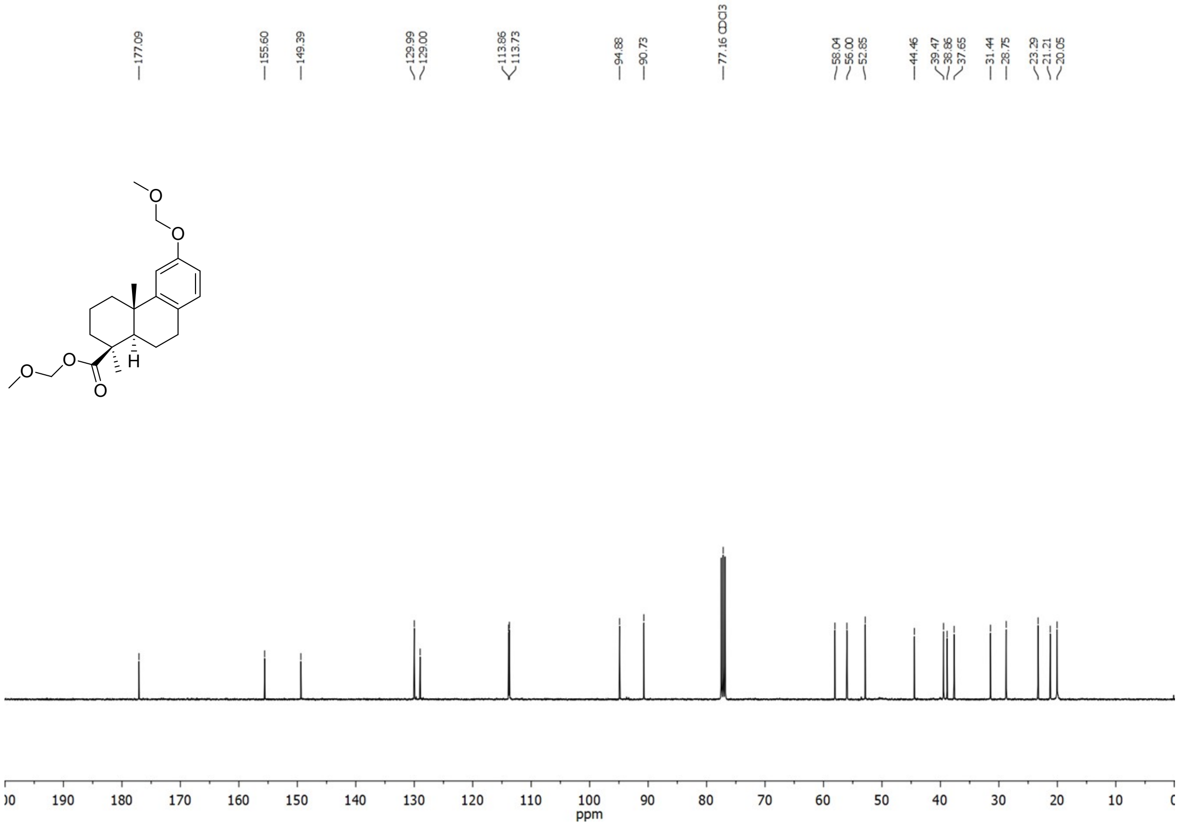


Mass analysis recorded in positive-ion mode. The signal corresponding to the molecular formula $C_{23}H_{34}O_7Na^+$, assigned to [**8**+Na]$^+$, is observed at 445.2195 *m/z* with a mass error of 0.4 ppm relative to the calculated exact mass.

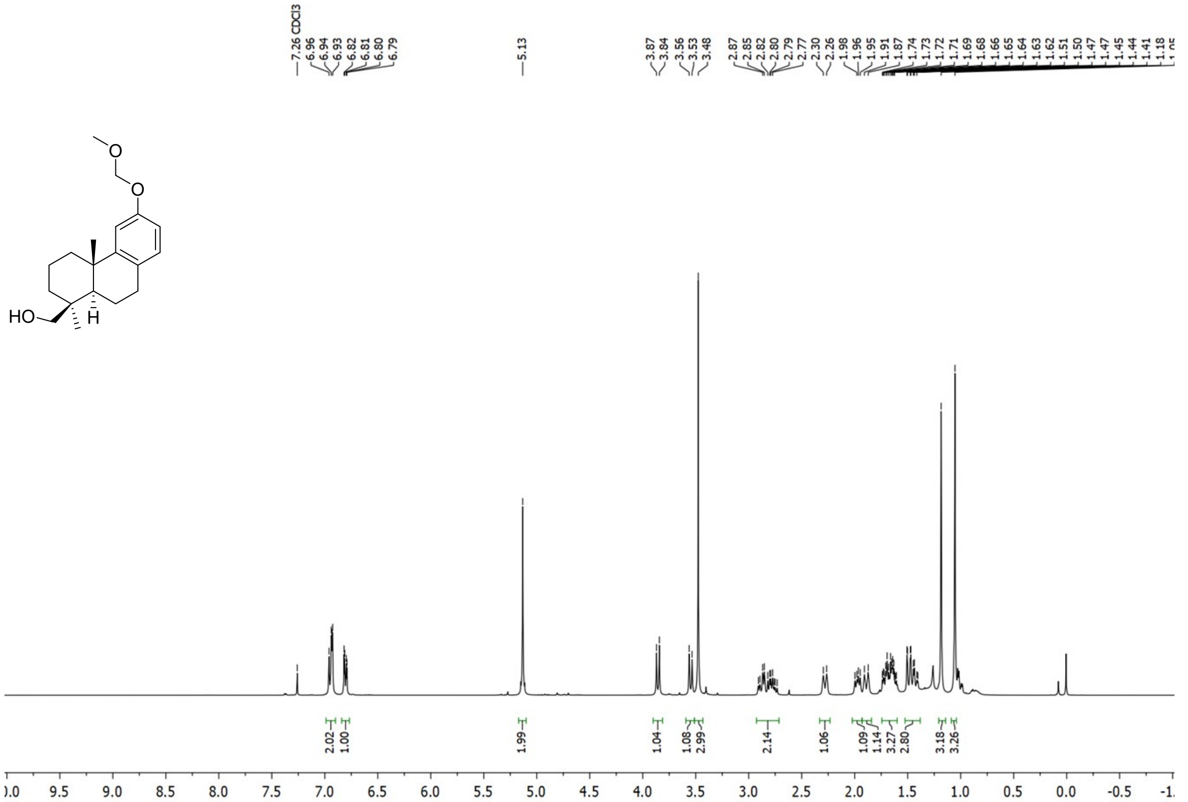
^{1}H-NMR (400MHz, $CDCl_3$) compound **9**



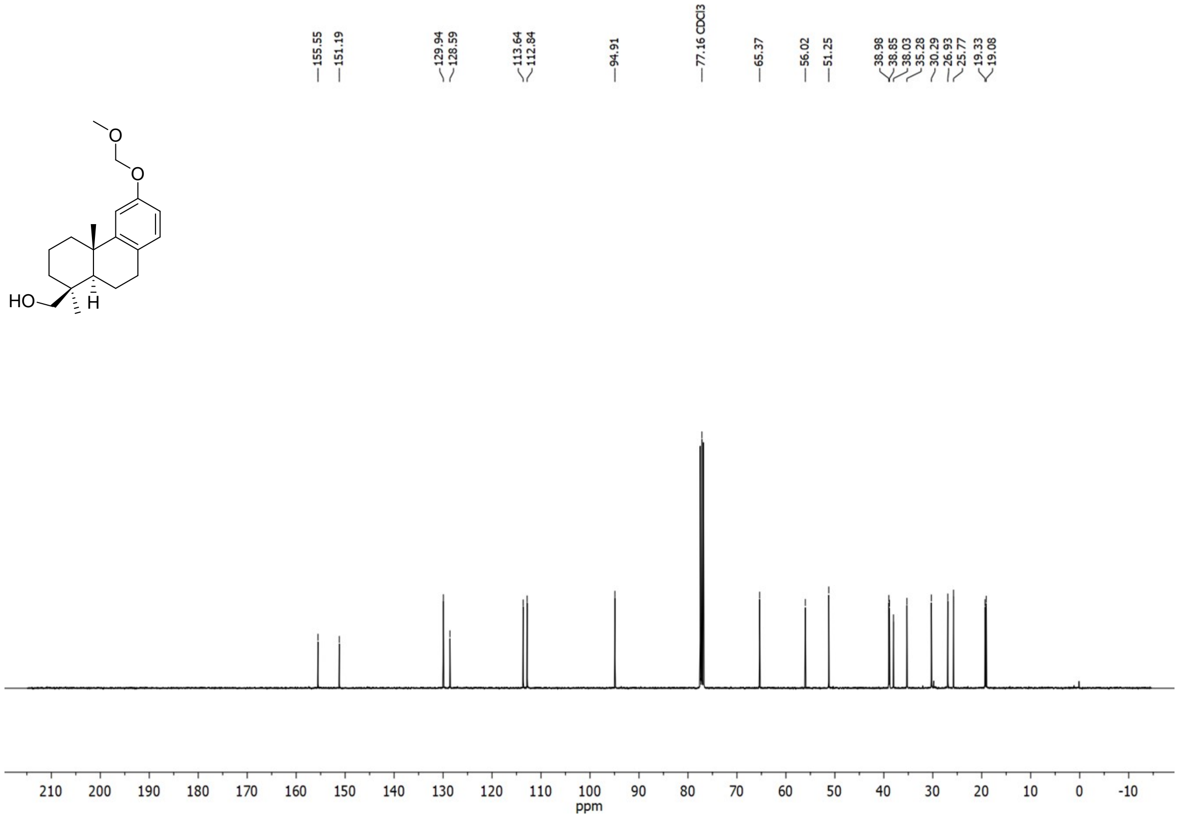
^{13}C-NMR (101MHz, $CDCl_3$) of compound **9**



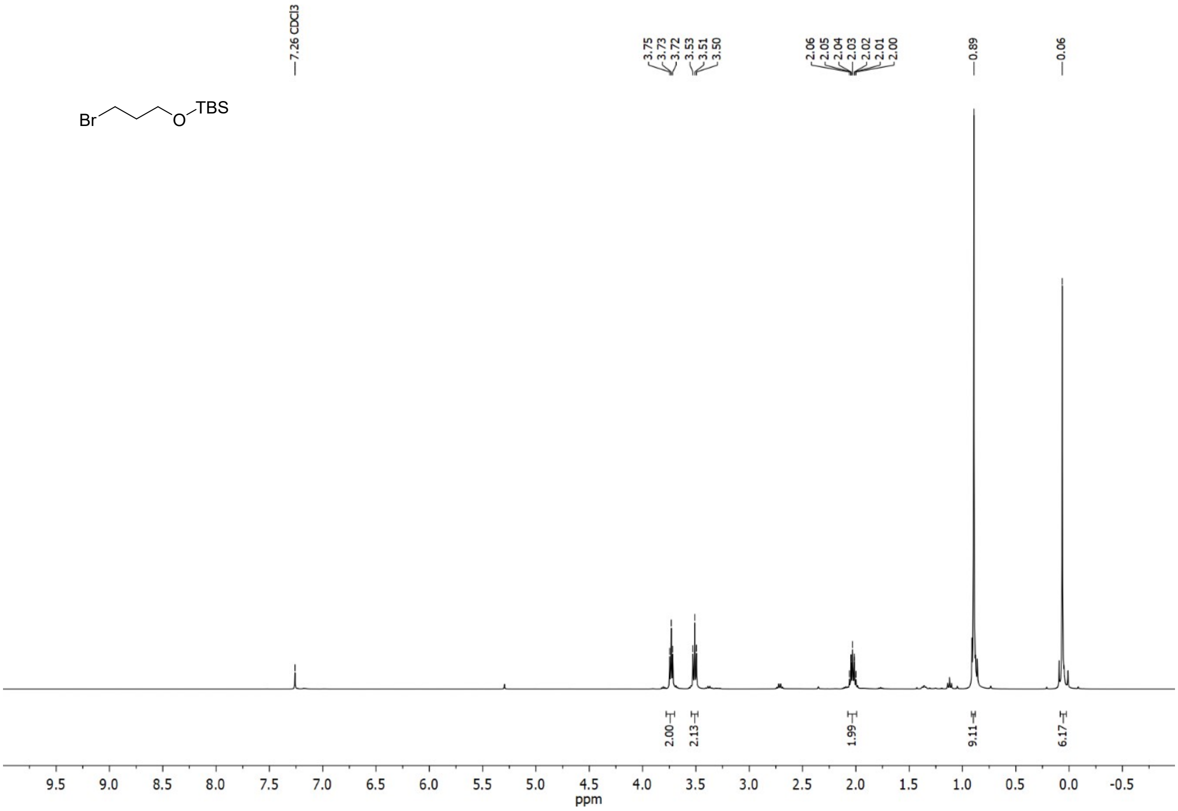
^{1}H-NMR (400MHz, $CDCl_3$) of compound **10**



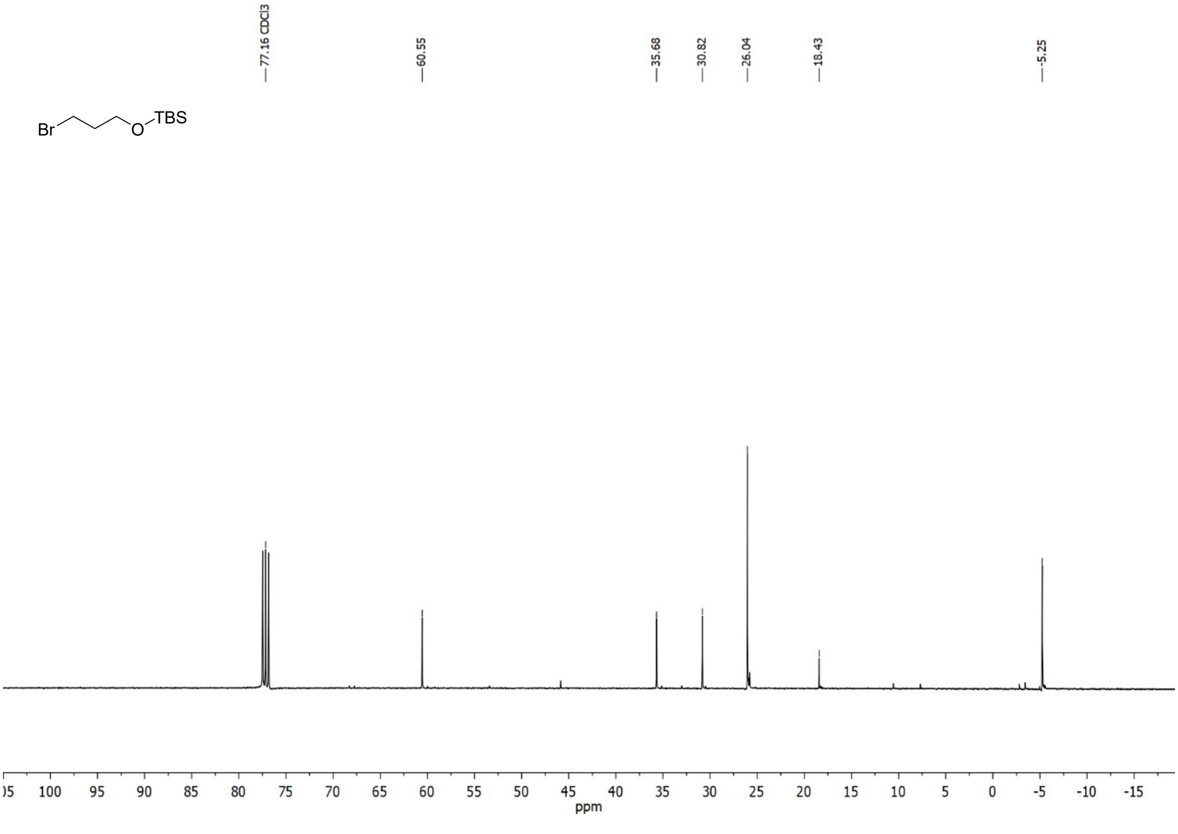
^{13}C-NMR (101MHz, $CDCl_3$) of compound **10**



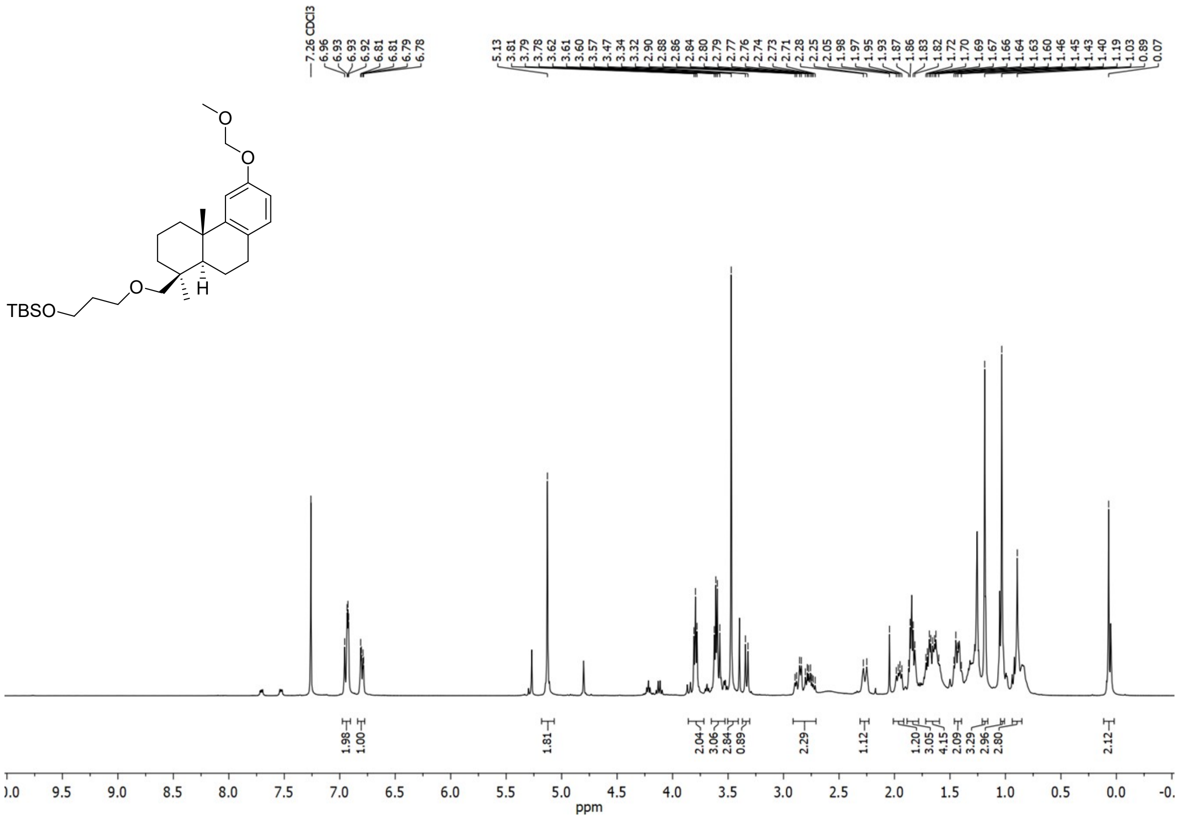
^{1}H-NMR (400MHz, $CDCl_3$) of compound 3-bromopropoxy)(tert-butyl)dimethylsilane



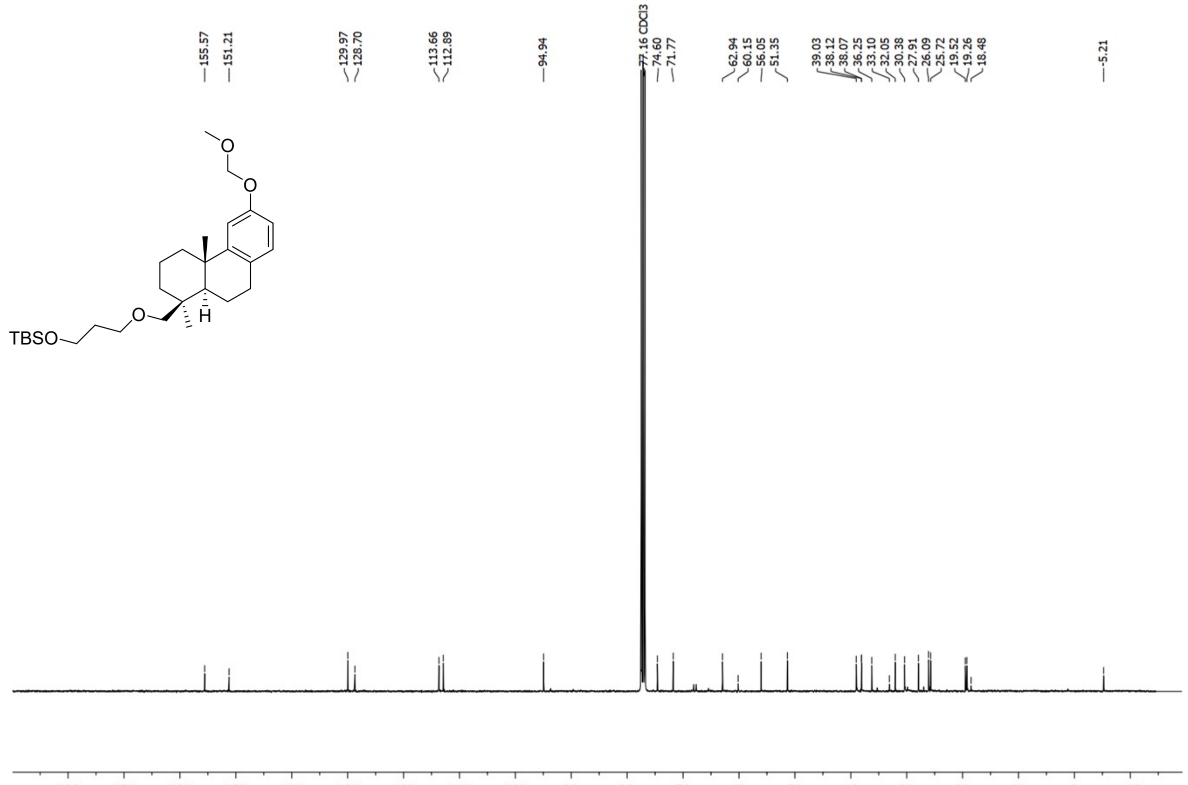
^{13}C-NMR (101MHz, $CDCl_3$) of compound 3-bromopropoxy)(tert-butyl)dimethylsilane



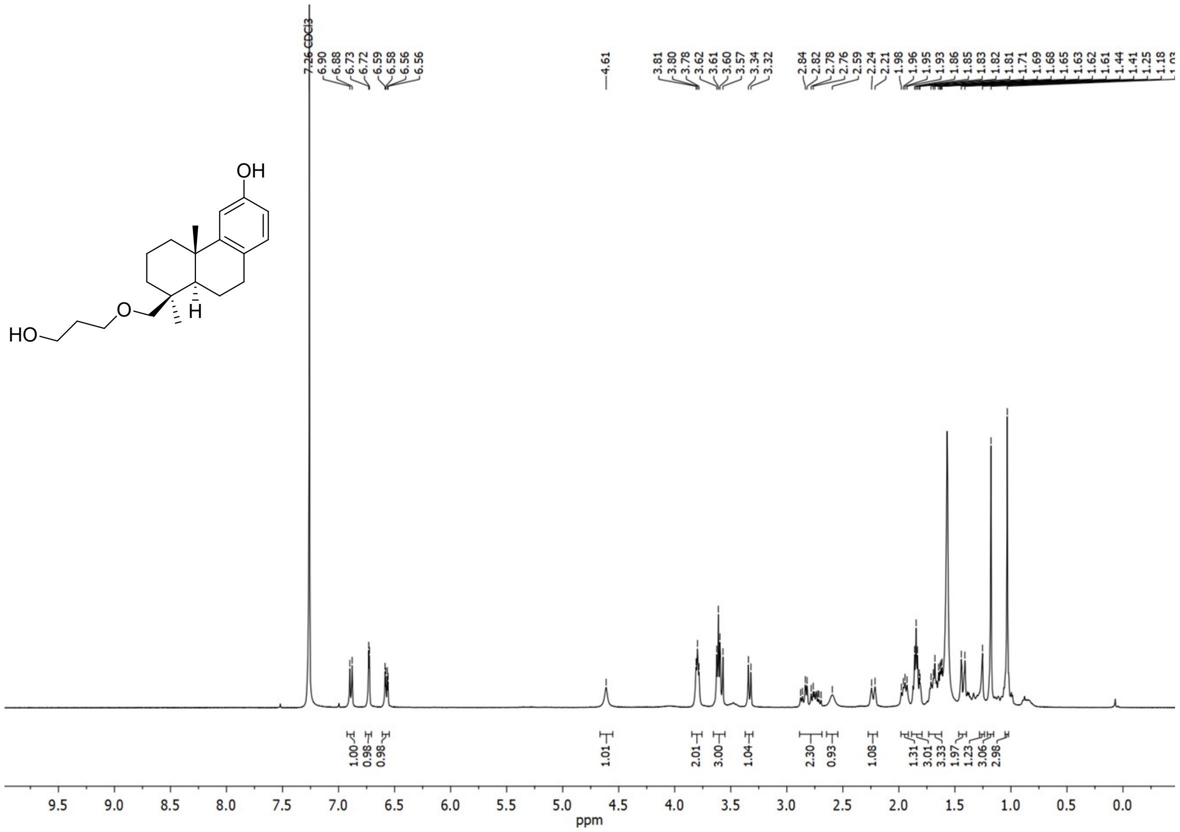
^{1}H-NMR (400MHz, $CDCl_3$) of compound **11**



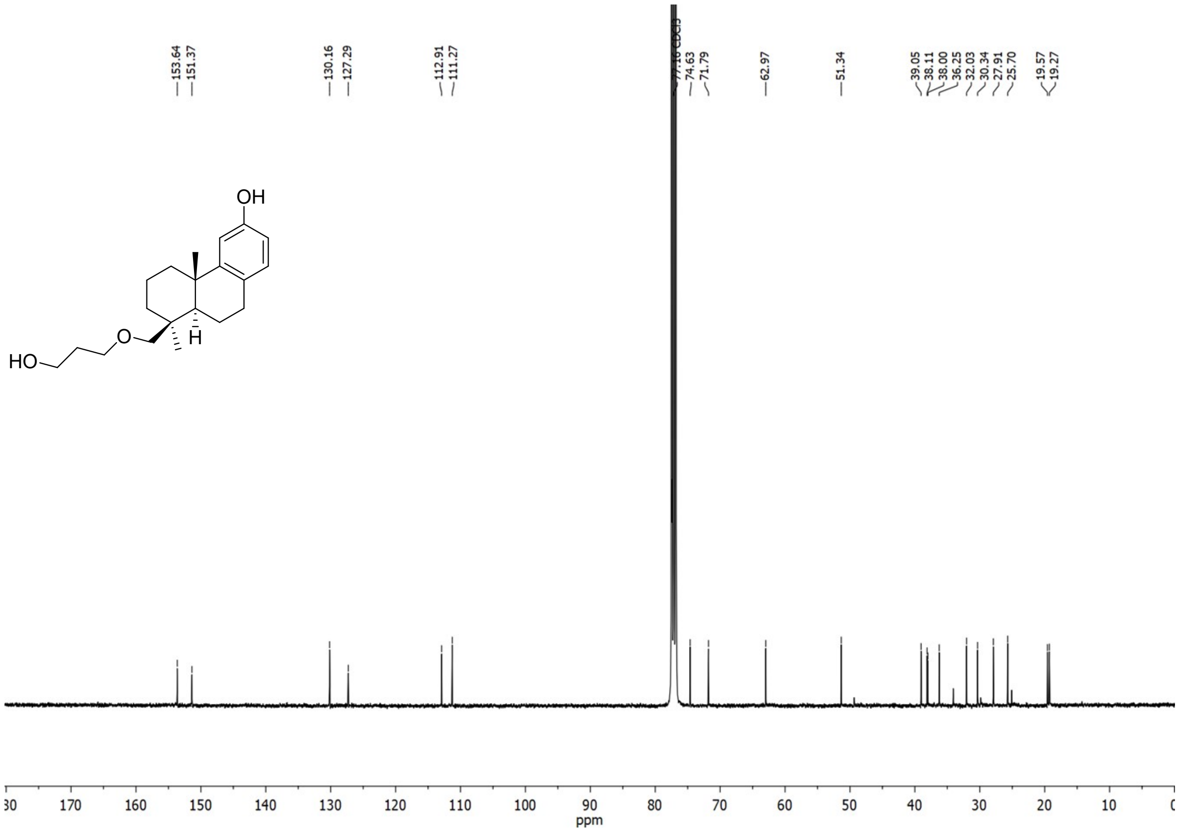
^{13}C-NMR (101MHz, $CDCl_3$) of compound **11**



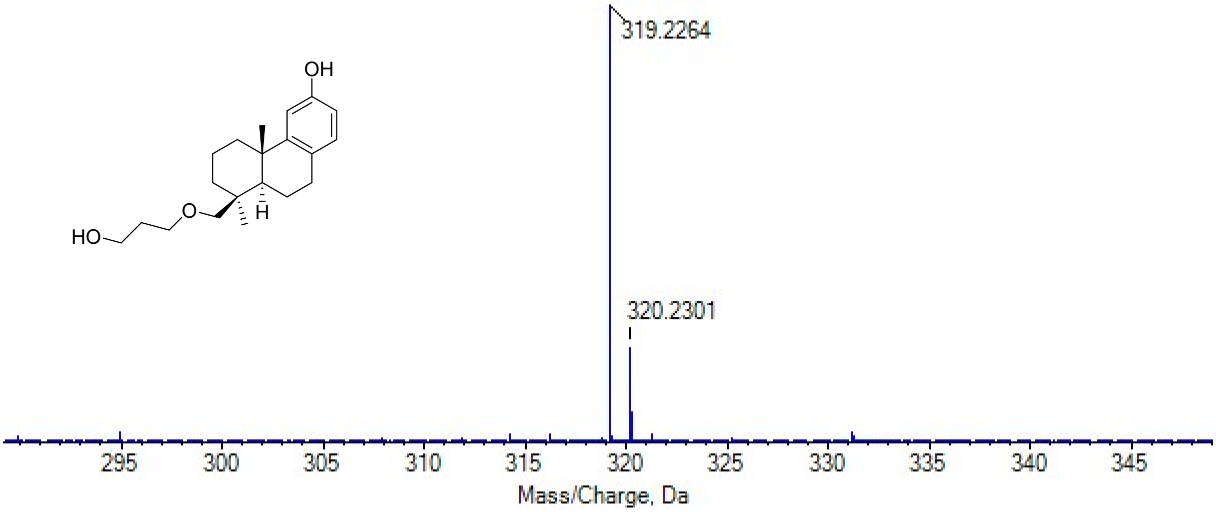
^{1}H-NMR (400MHz, $CDCl_3$) compound **12**



^{13}C-NMR (101MHz, $CDCl_3$) of compound **12**

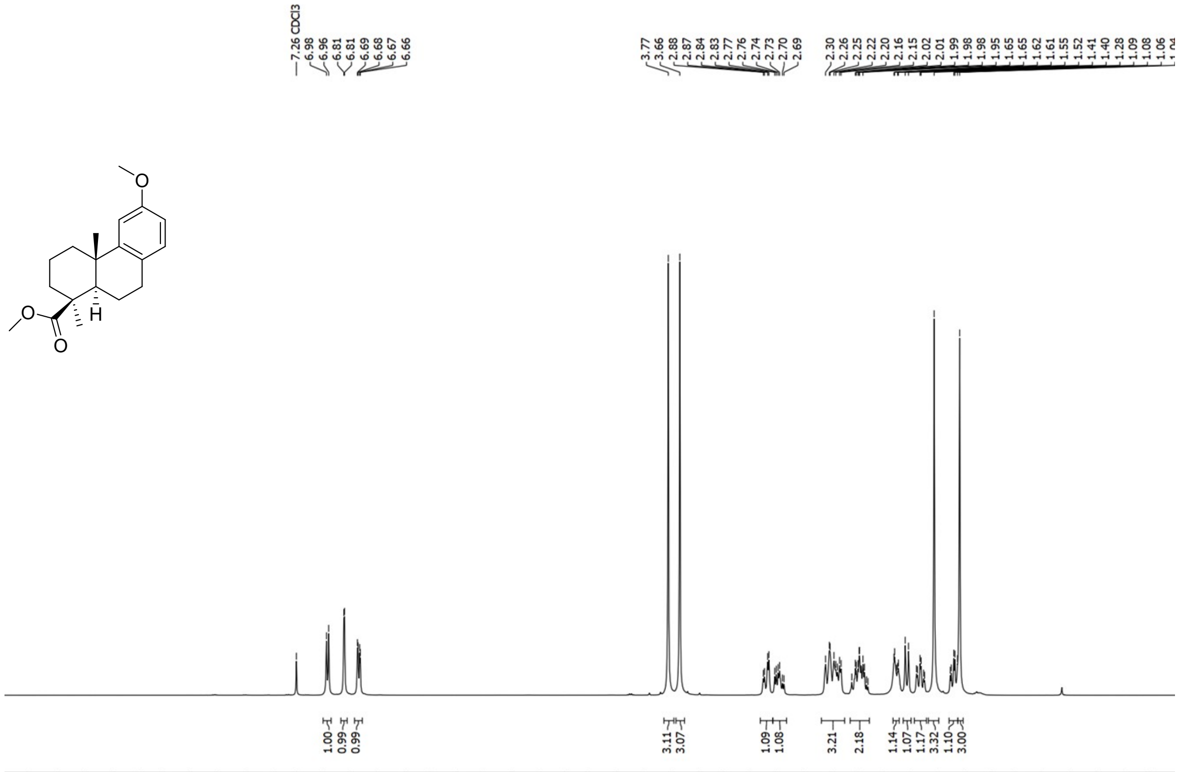


ESI$^+$ mass spectrum of compound **12**

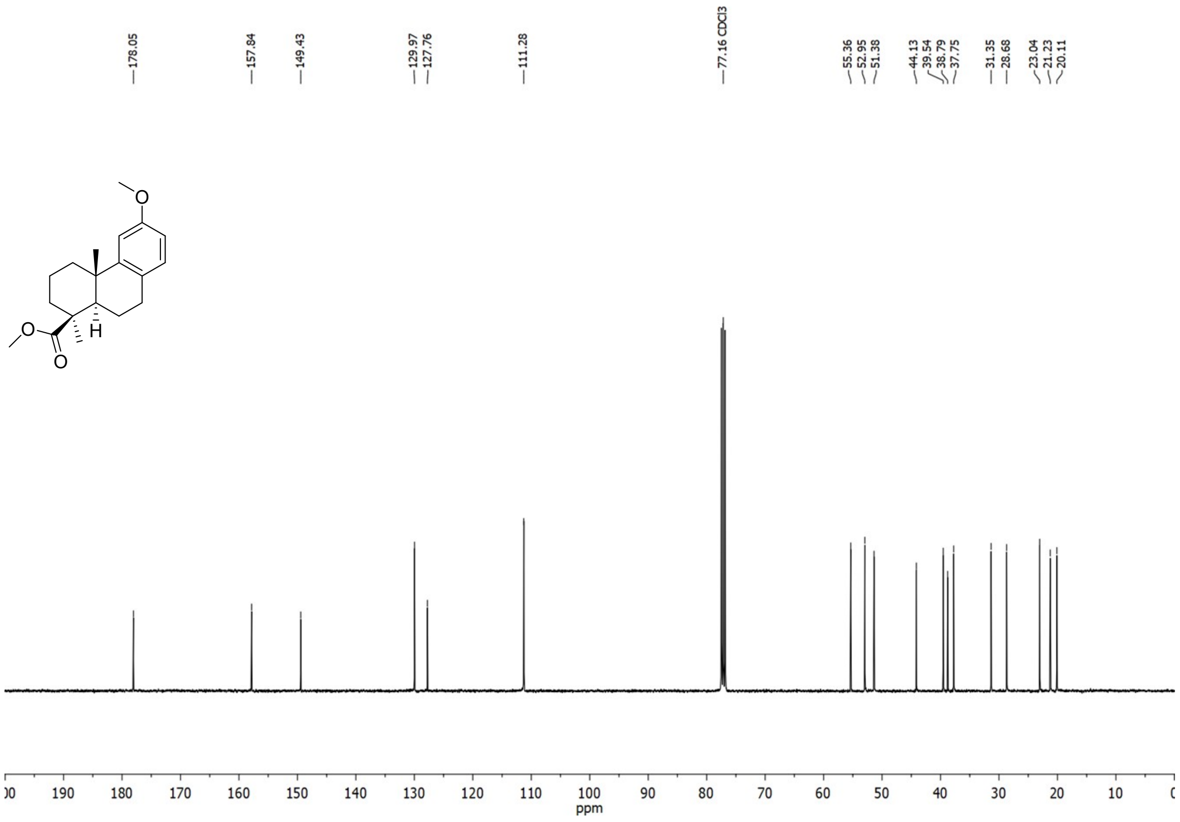


Mass analysis recorded in positive-ion mode. The signal corresponding to the molecular formula $C_{20}H_{31}O_3^+$, assigned to [**12**+H]$^+$, is observed at 319.2264 *m/z* with a mass error of 1.2 ppm relative to the calculated exact mass.

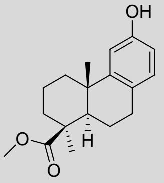
^{1}H-NMR (400MHz, $CDCl_3$) of compound **13**



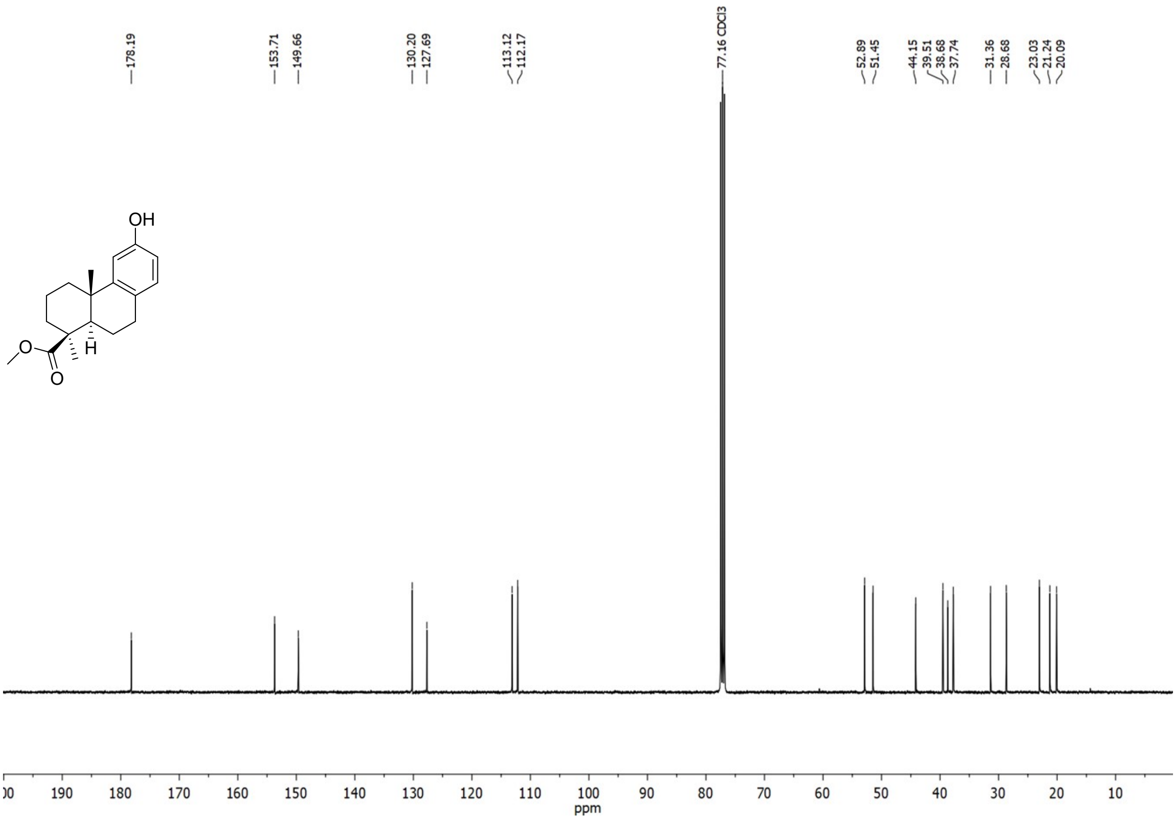
^{13}C-NMR (101MHz, $CDCl_3$) of compound **13**



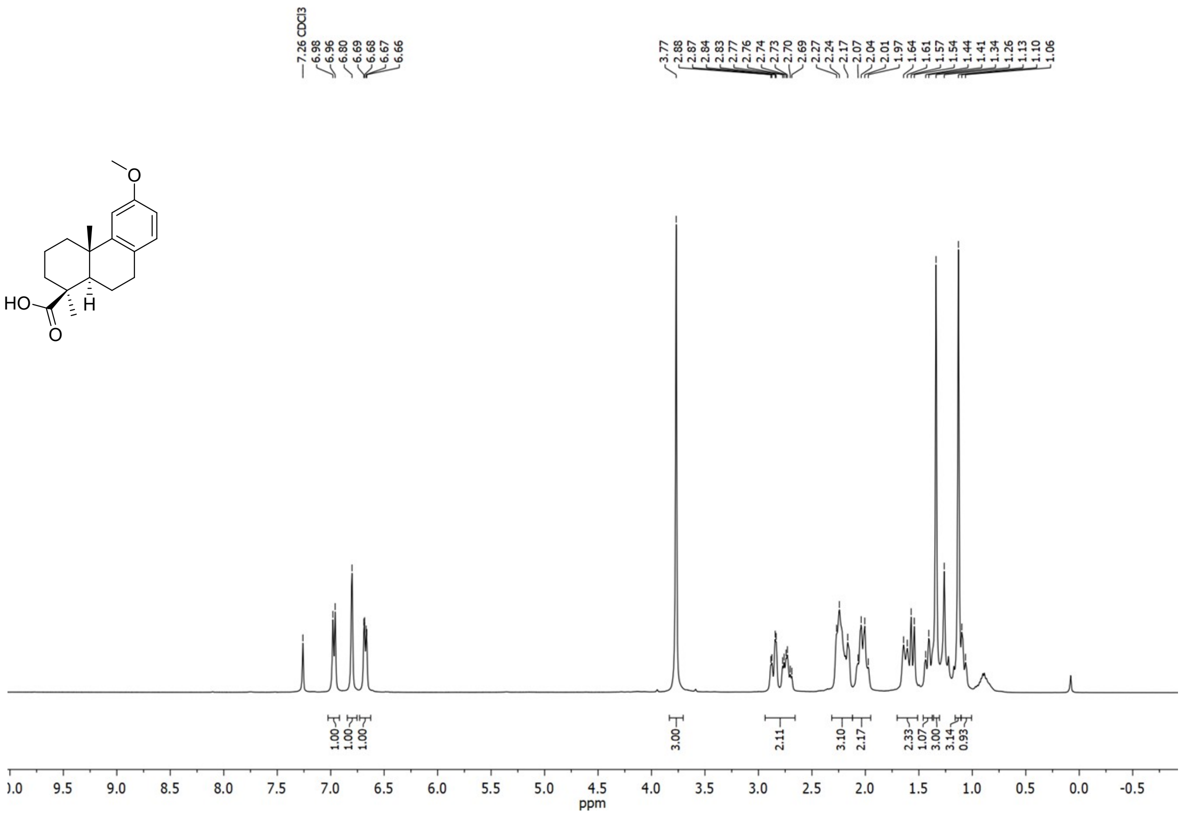
^{1}H-NMR (400MHz, CDCl$_3$) of compound **14**



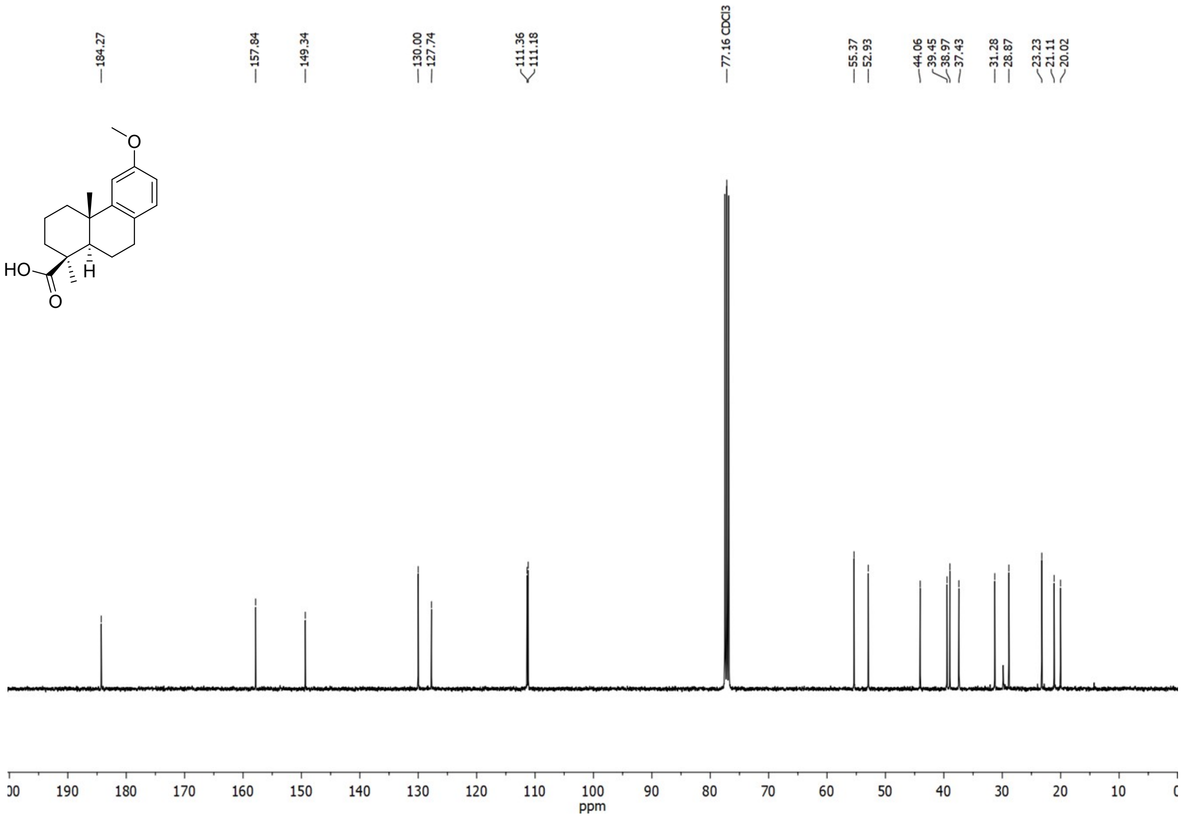
^{13}C-NMR (101MHz, CDCl$_3$) of compound **14**



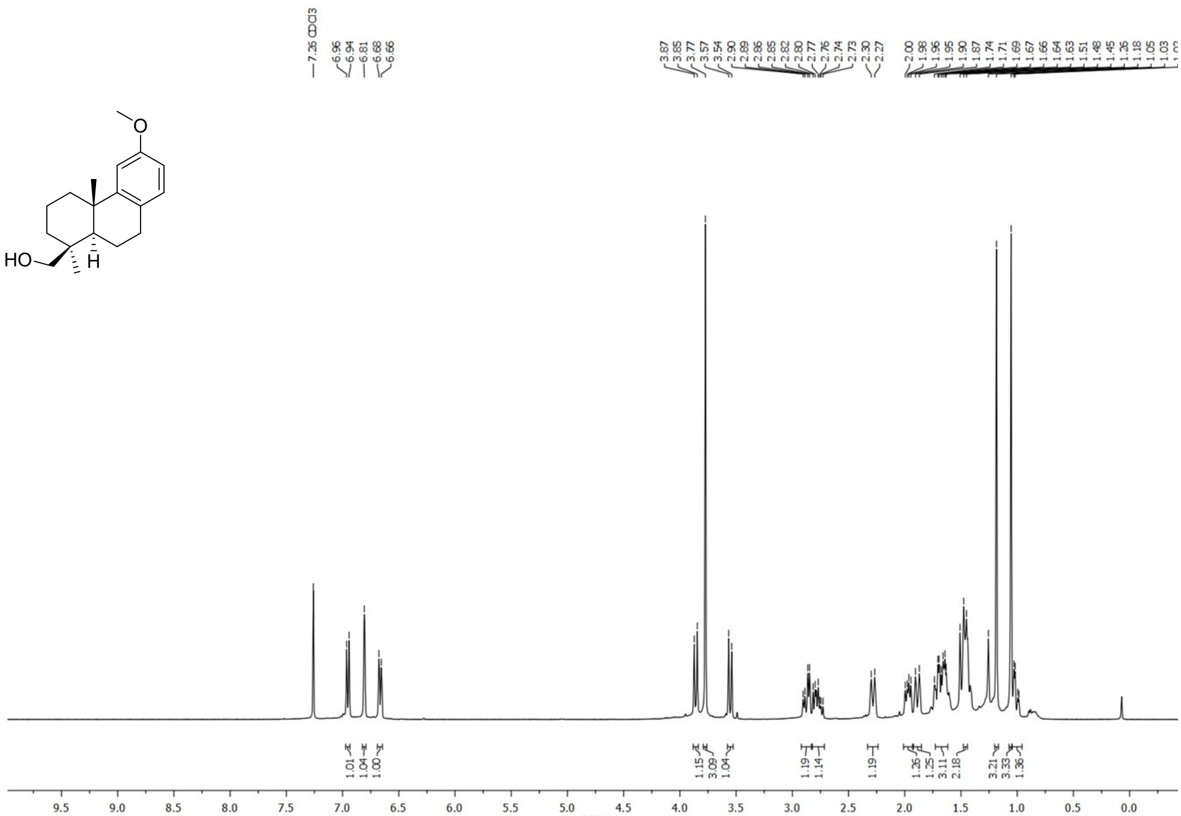
^{1}H-NMR (400MHz, $CDCl_3$) of compound **15**



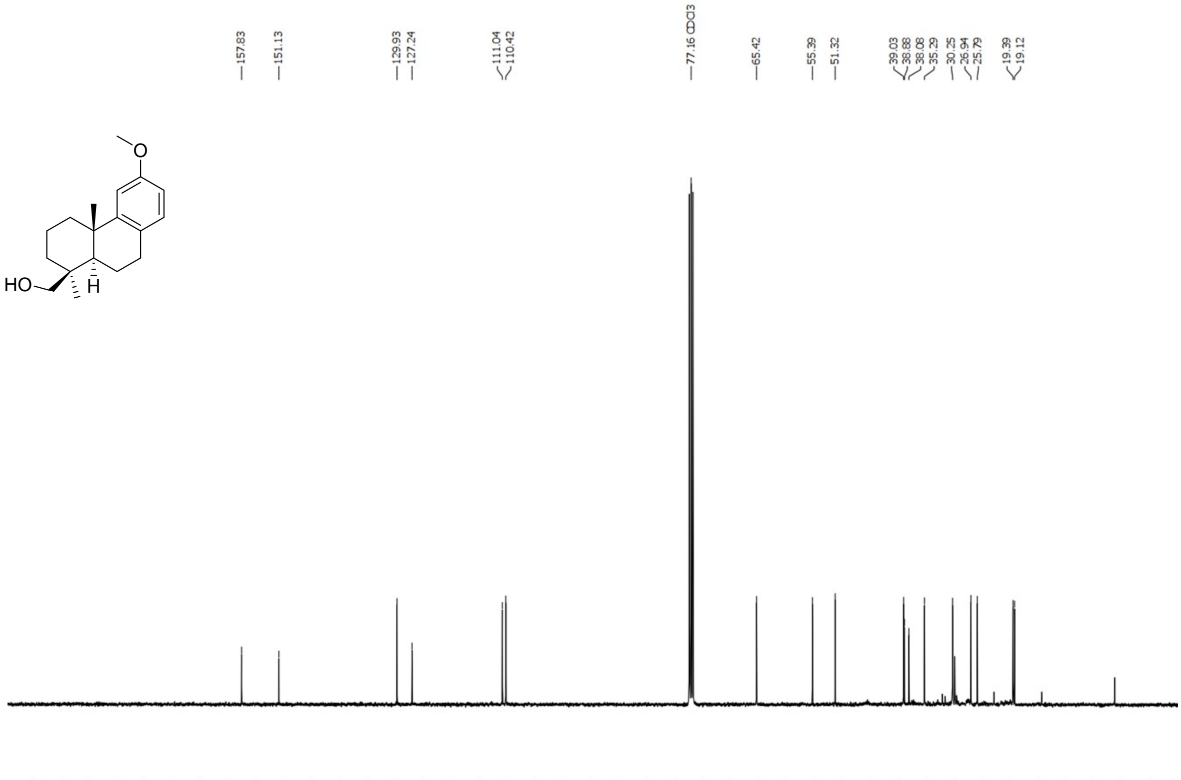
^{13}C-NMR (101MHz, $CDCl_3$) of compound **15**



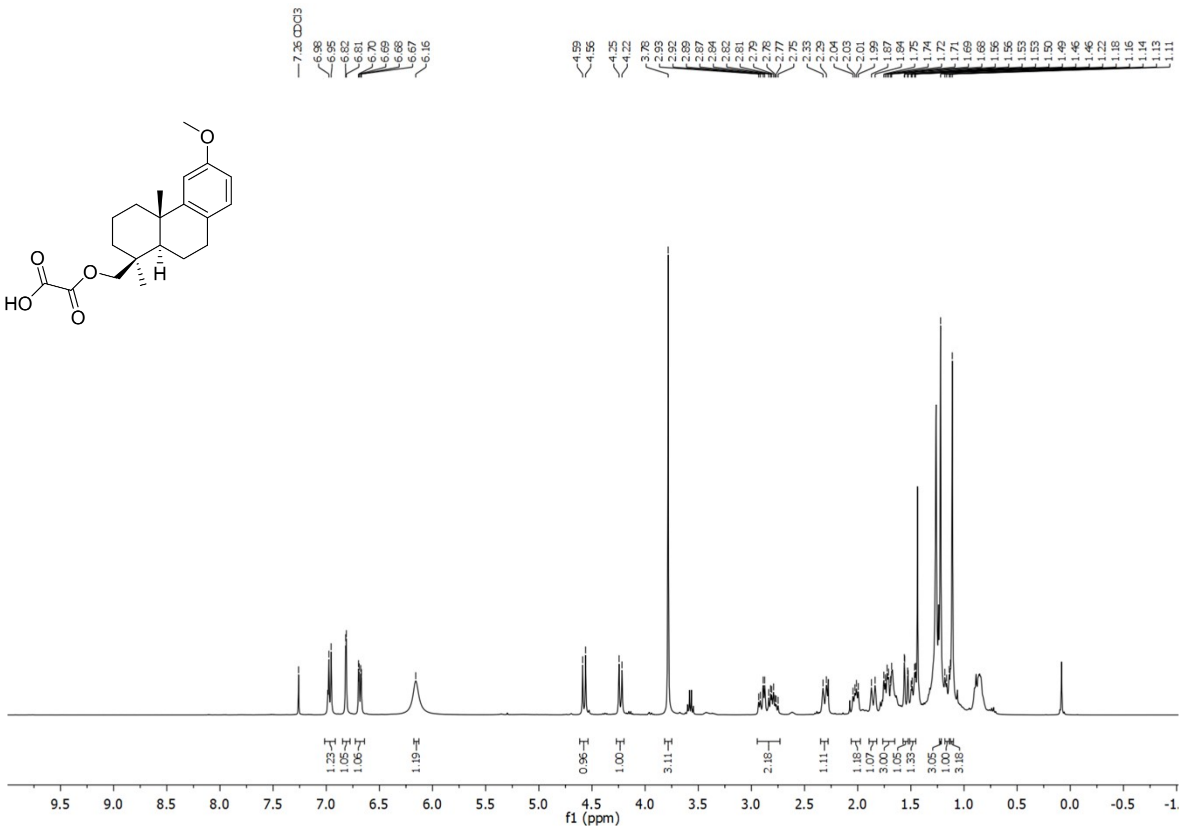
^{1}H-NMR (400MHz, CDCl$_3$) of compound **16**



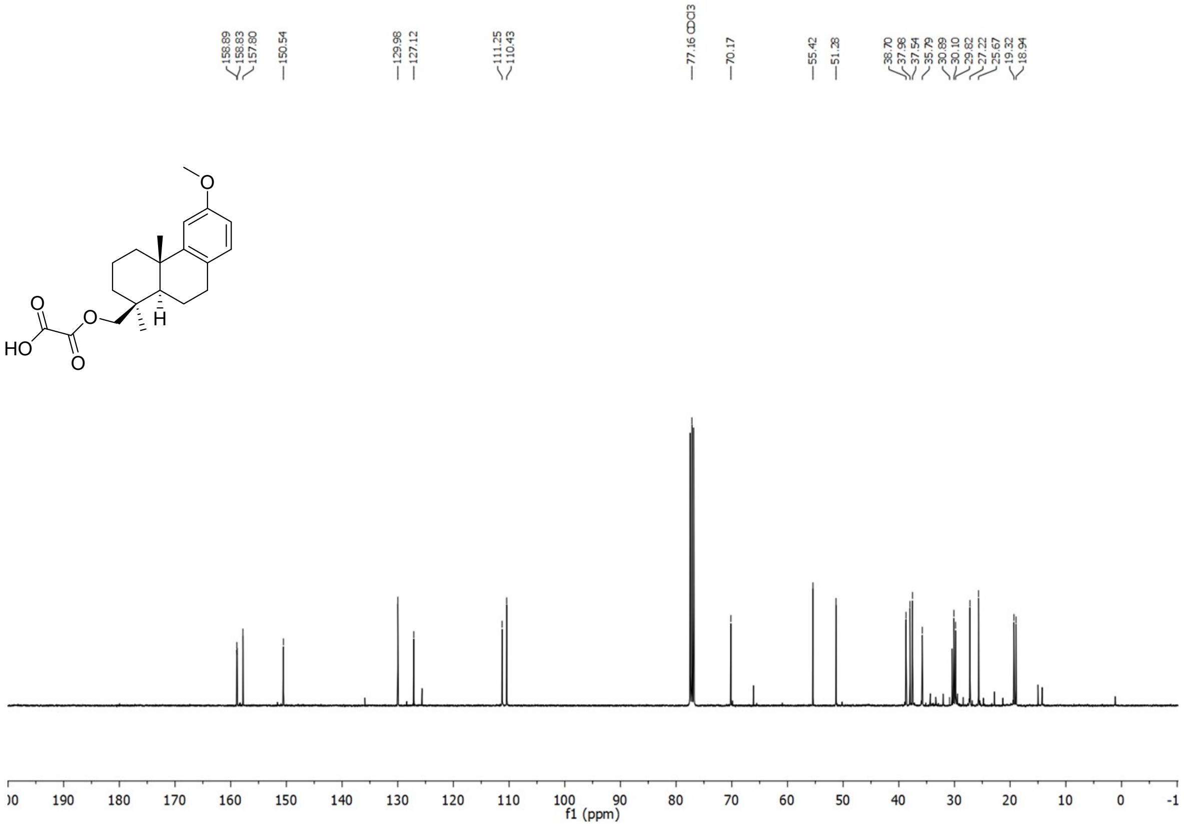
^{13}C-NMR (101MHz, CDCl$_3$) of compound **16**



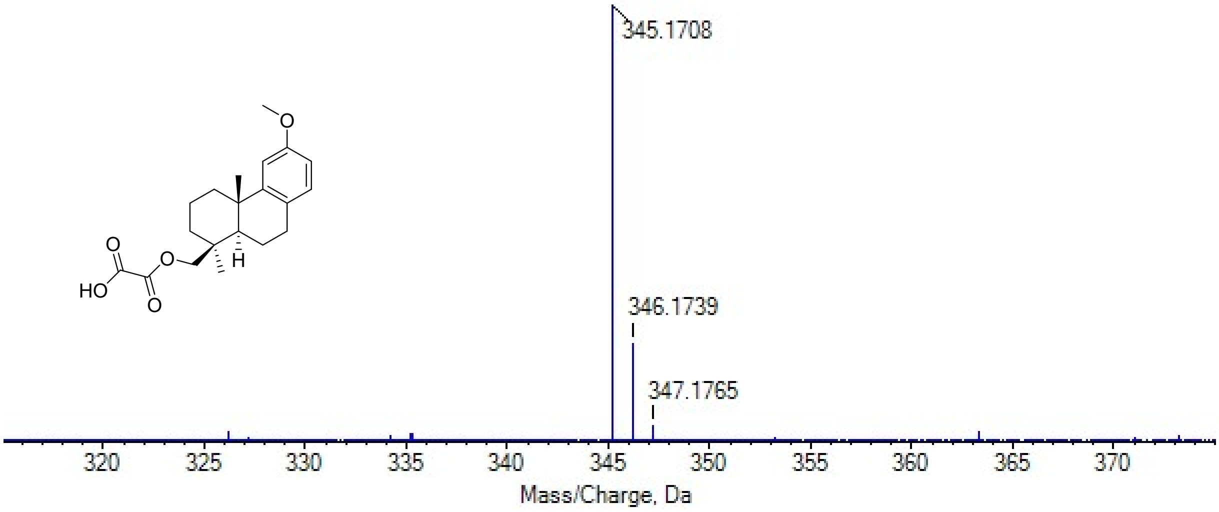
^{1}H-NMR (400MHz, $CDCl_3$) of compound **17**



^{13}C-NMR (101MHz, $CDCl_3$) of compound **17**

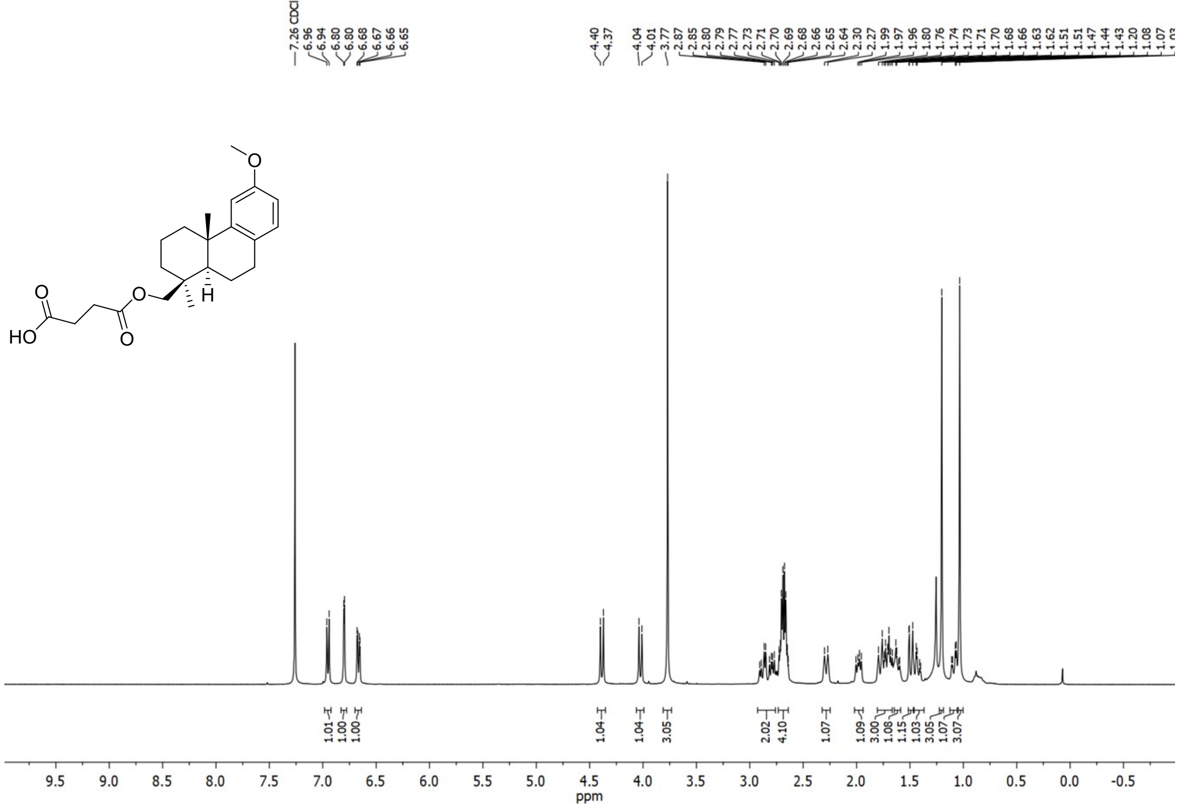


ESI$^-$ mass spectrum of compound **17**

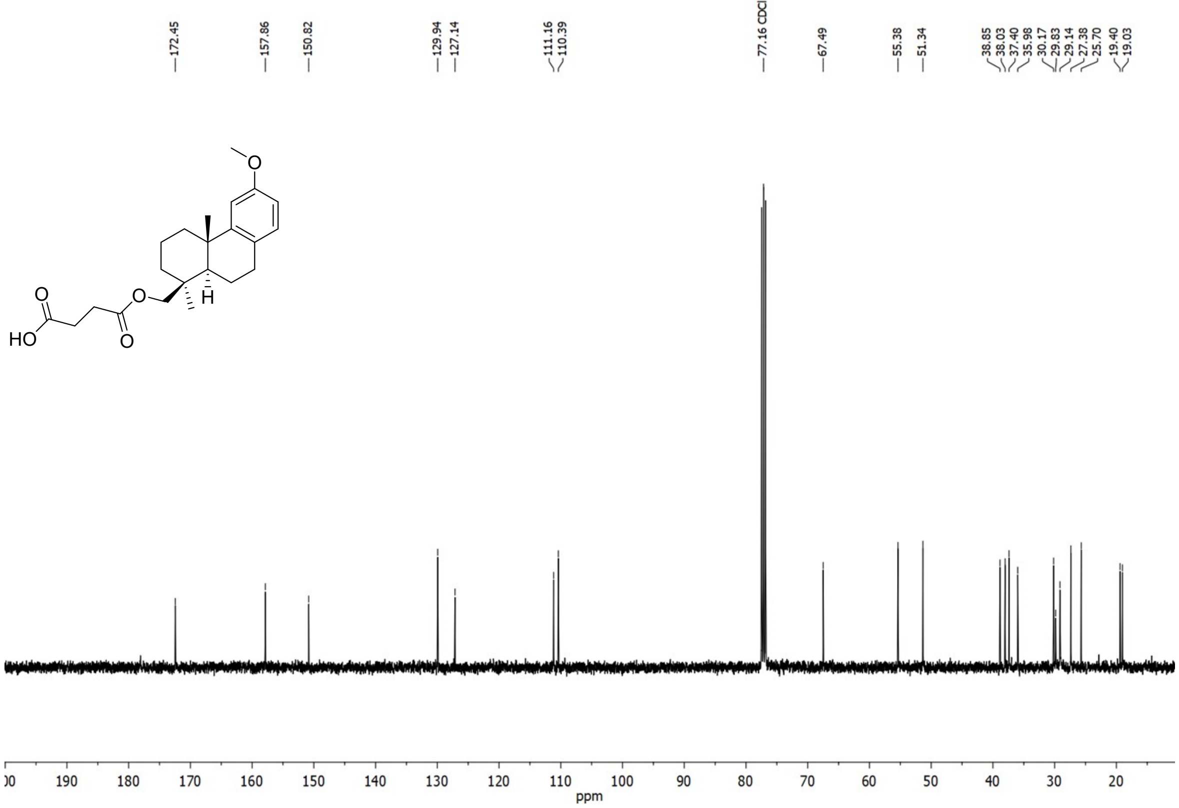


The compound **17** sample was analyzed in negative-ion ESI mode. The signal corresponding to the molecular formula $C_{20}H_{25}O_5^+$, assigned to [**17**−H]$^-$, is observed at 345.1708 *m/z* with a mass error of 0.2 ppm relative to the calculated exact mass.

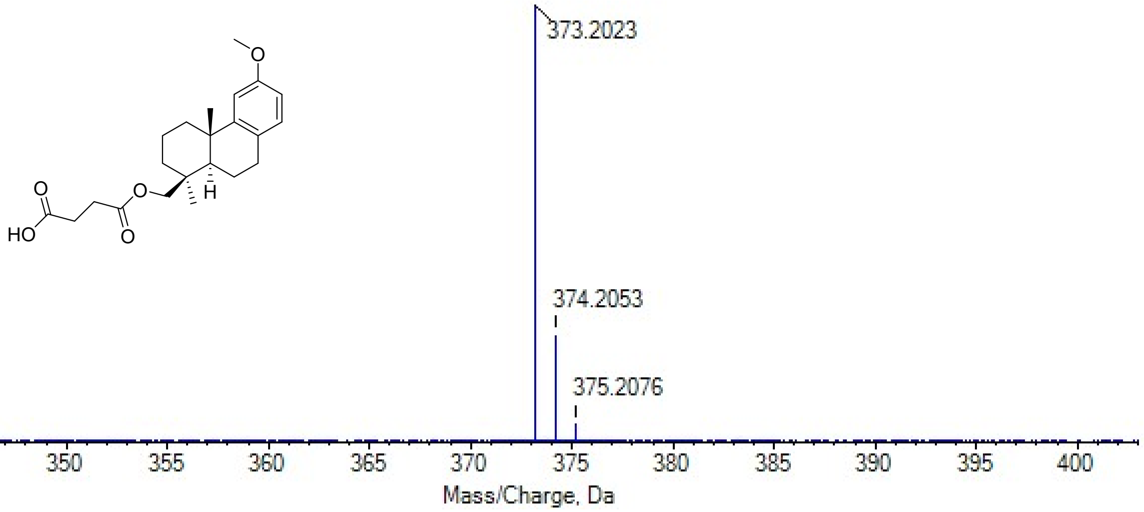
^{1}H-NMR (400MHz, $CDCl_3$) of compound **18**



^{13}C-NMR (101MHz, $CDCl_3$) of compound **18**

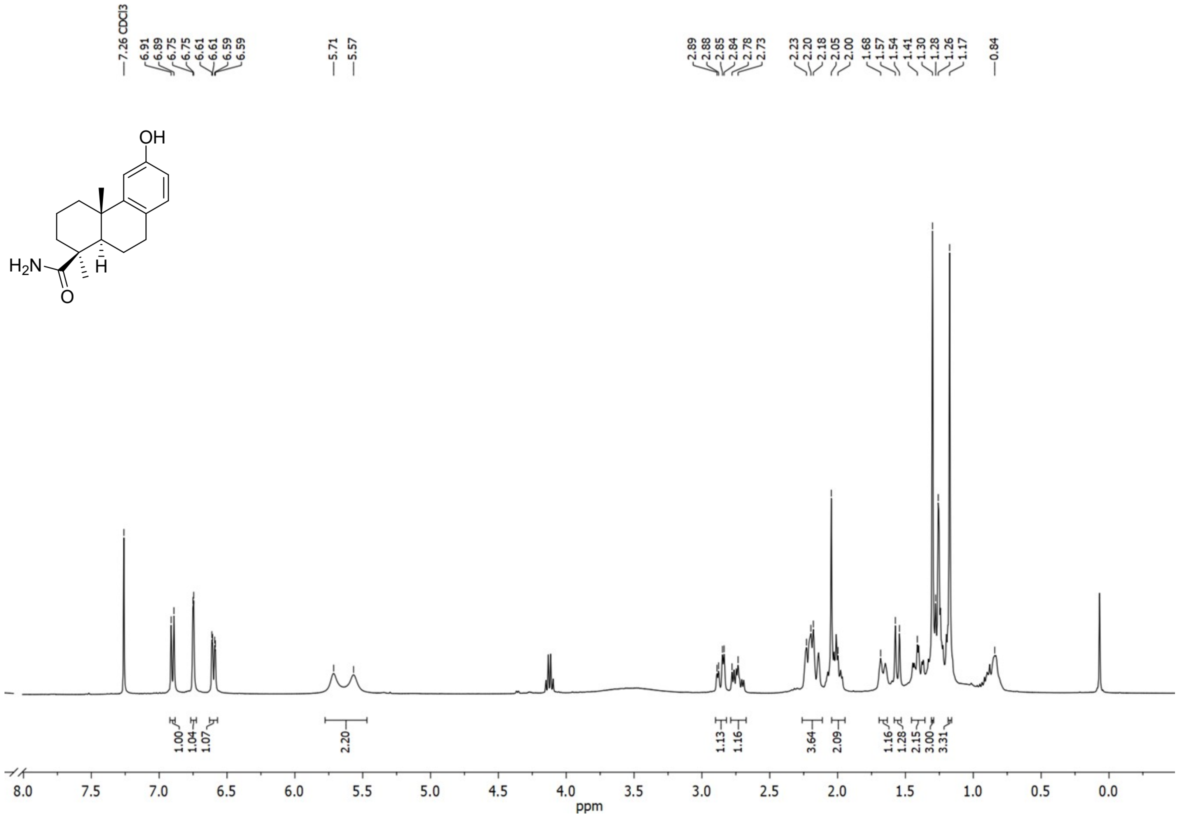


ESI$^-$ mass spectrum of compound **18**

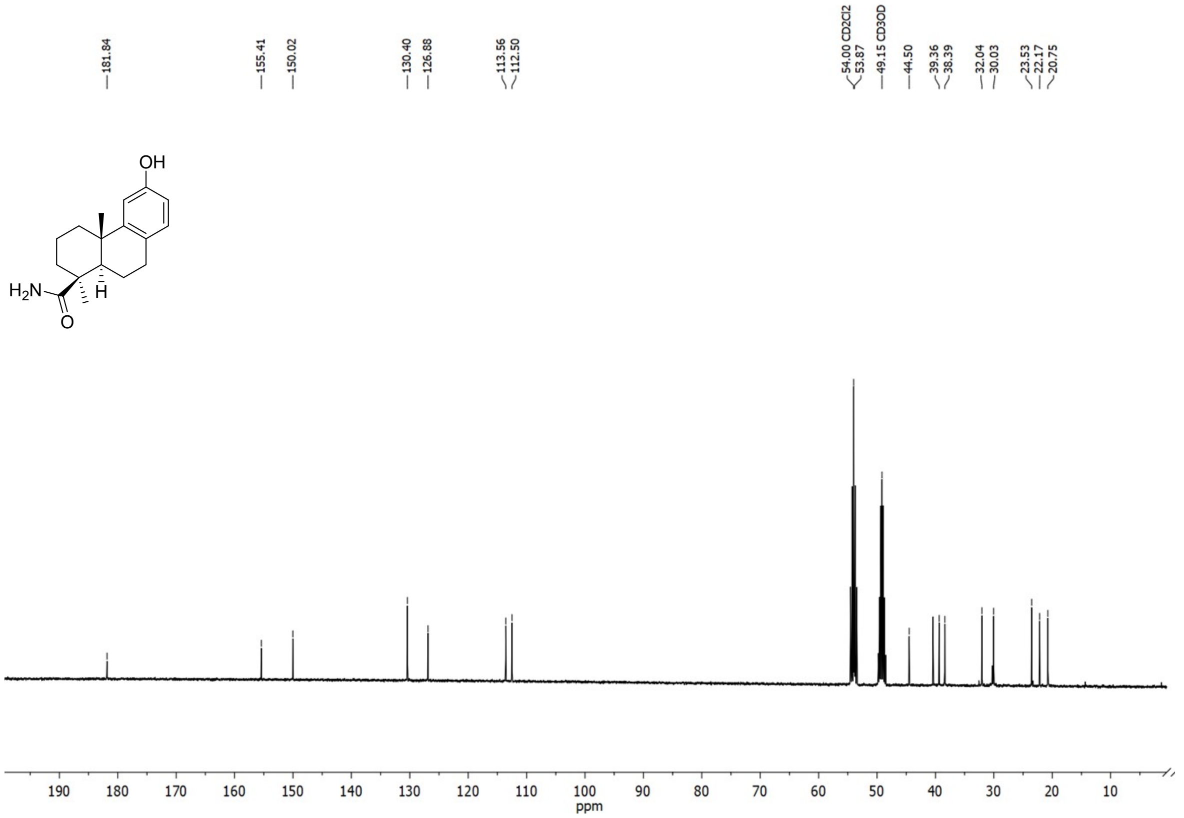


The compound **18** sample was analyzed in negative-ion ESI mode. The signal corresponding to the molecular formula $C_{22}H_{29}O_5^-$, assigned to [**18**−H]$^-$, is observed at 373.2023 *m/z* with a mass error of 0.7 ppm relative to the calculated exact mass.

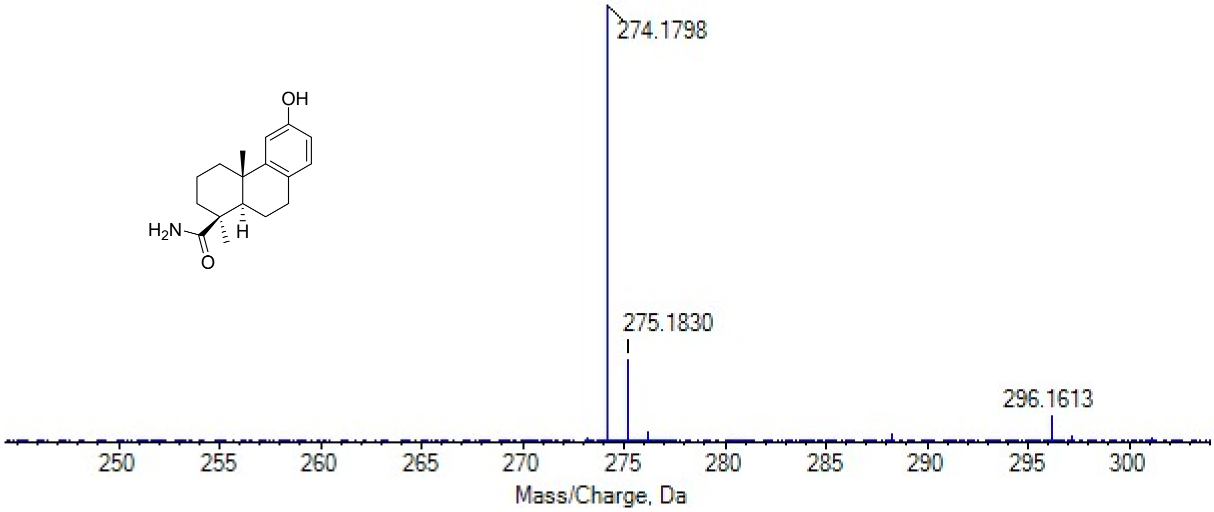
^{1}H-NMR (400MHz, $CDCl_3$) of compound **19**



^{13}C-NMR (101MHz, CD_2Cl_2/CD_3OD) of compound **19**

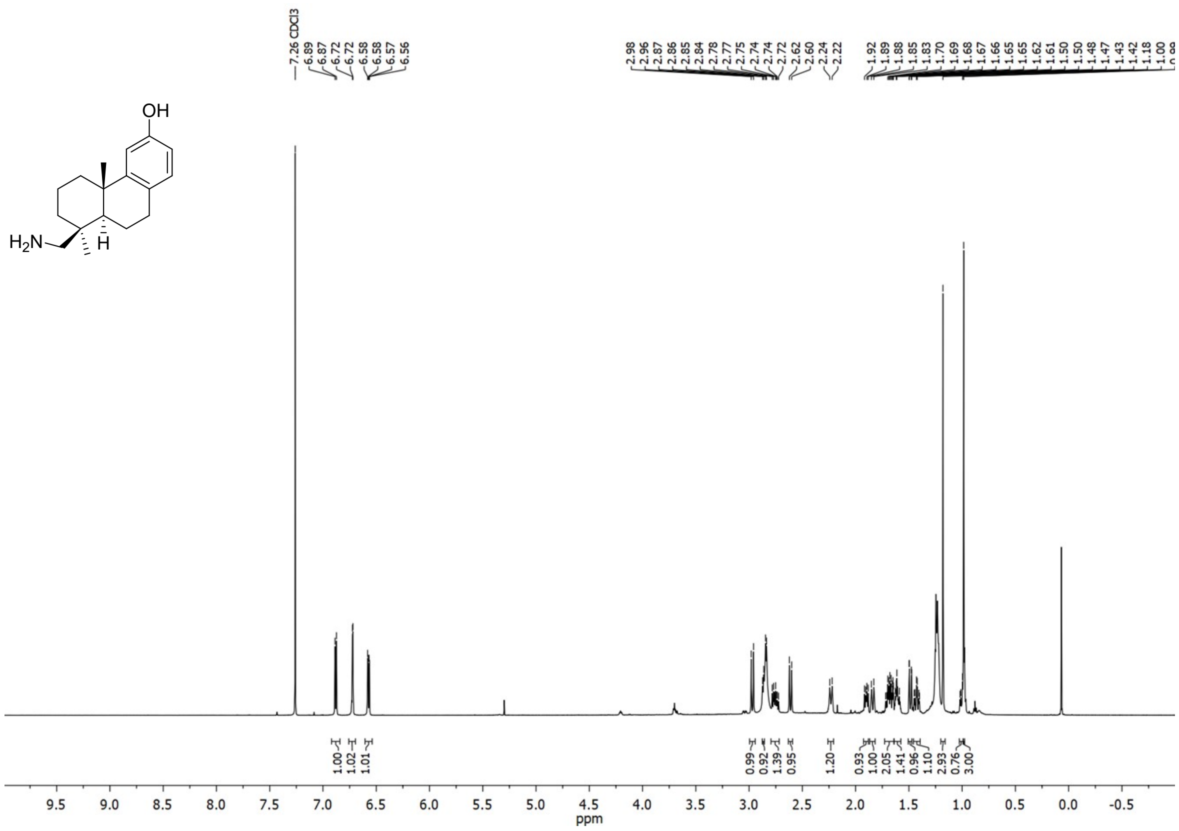


ESI$^+$ mass spectrum of compound **19**

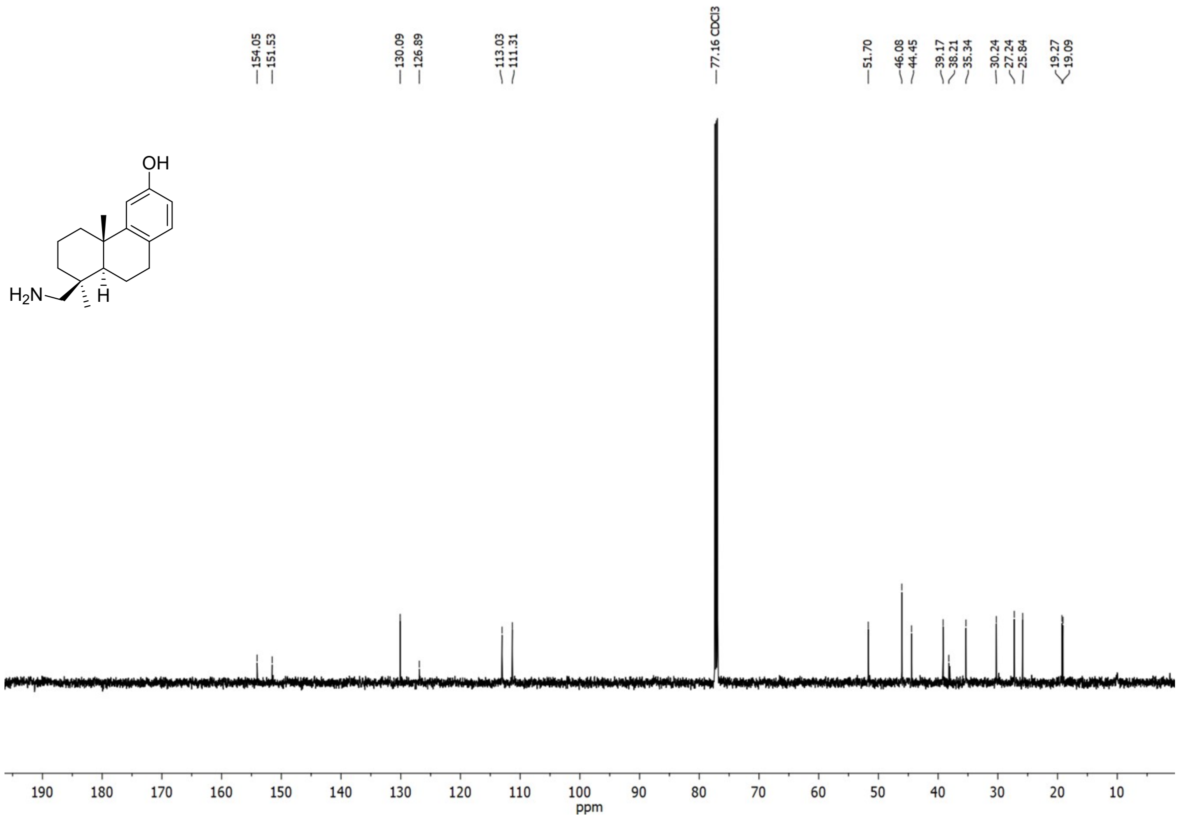


The compound **19** sample was analyzed in positive-ion ESI mode. The signal corresponding to the molecular formula $C_{17}H_{24}NO_2^+$, assigned to [**19**+H]$^+$, is observed at 274.1798 *m/z* with a mass error of 1.3 ppm relative to the calculated exact mass. The [**19**+Na]$^+$ adduct is also present at 296.1613 *m/z*.

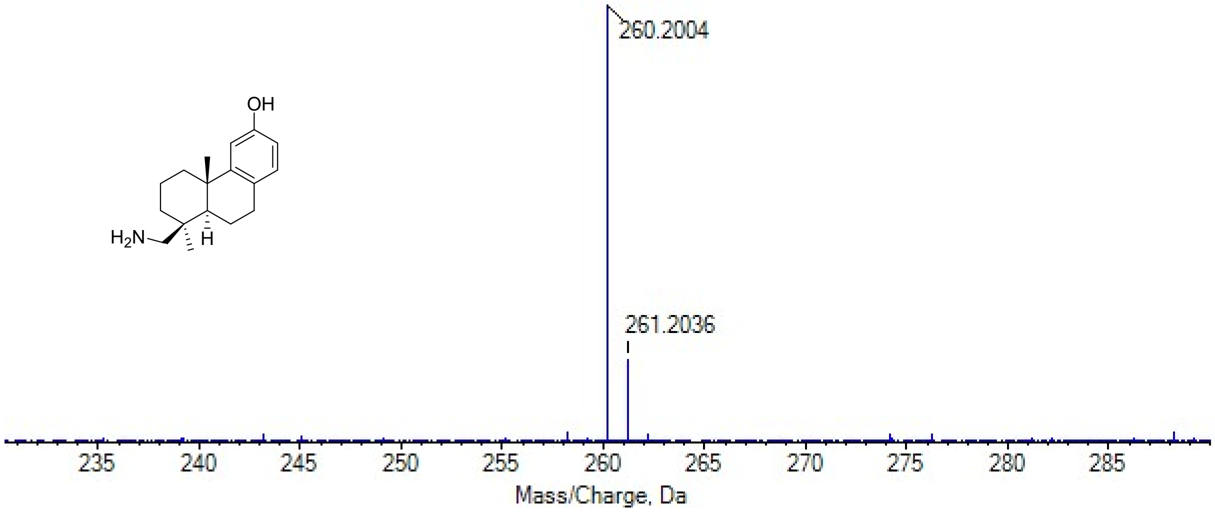
^{1}H-NMR (400MHz, $CDCl_3$) of compound **20**



^{13}C-NMR (101MHz, $CDCl_3$) of compound **20**

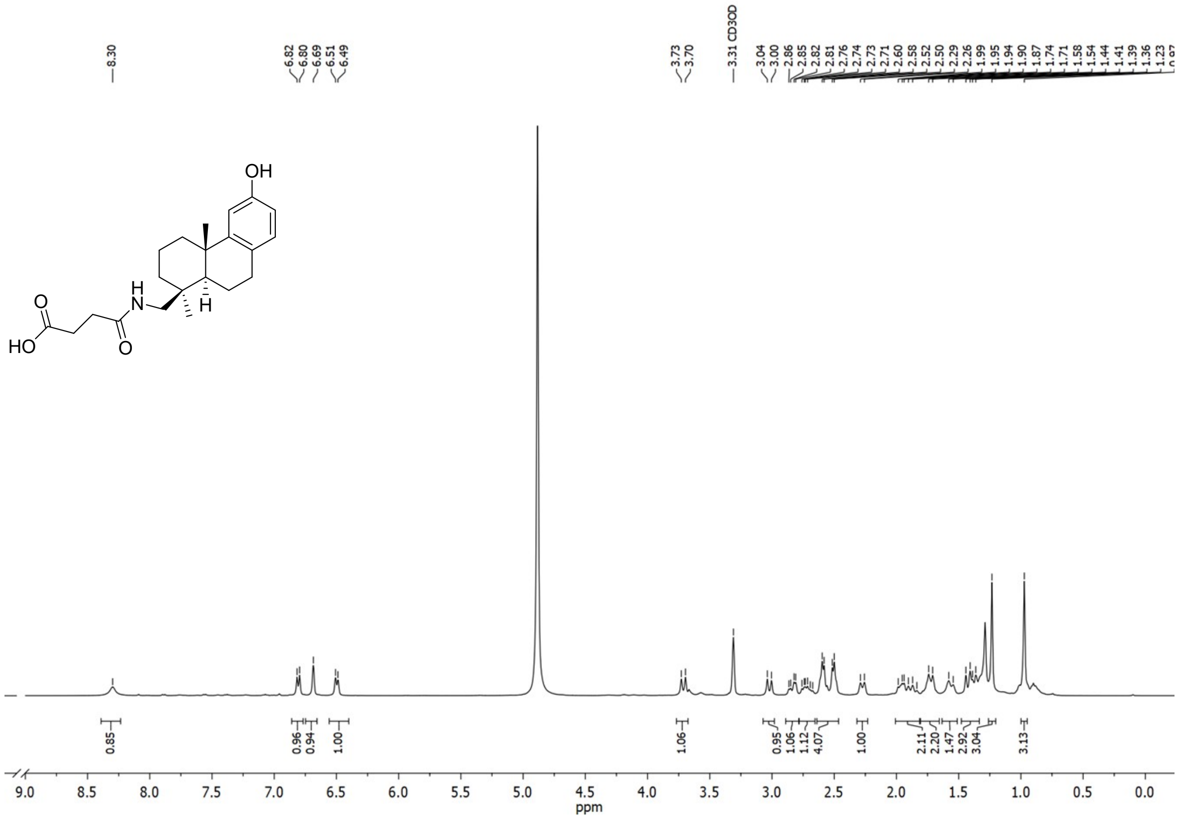


ESI^+ mass spectrum of compound **20**

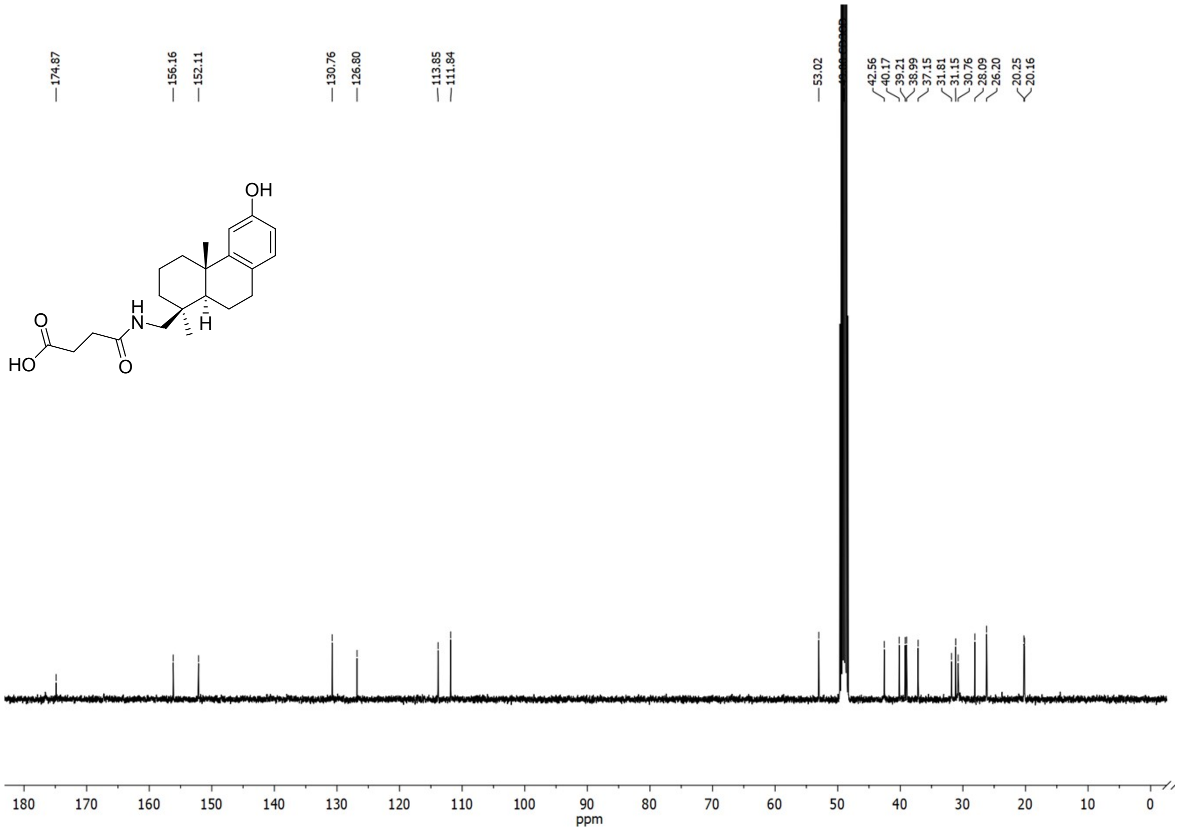


The compound **20** sample was analyzed in positive-ion ESI mode. The signal corresponding to the molecular formula $C_{17}H_{26}NO^+$, assigned to [**20**+H]$^+$, is observed at 260.2004 *m/z* with a mass error of 1.9 ppm relative to the calculated exact mass.

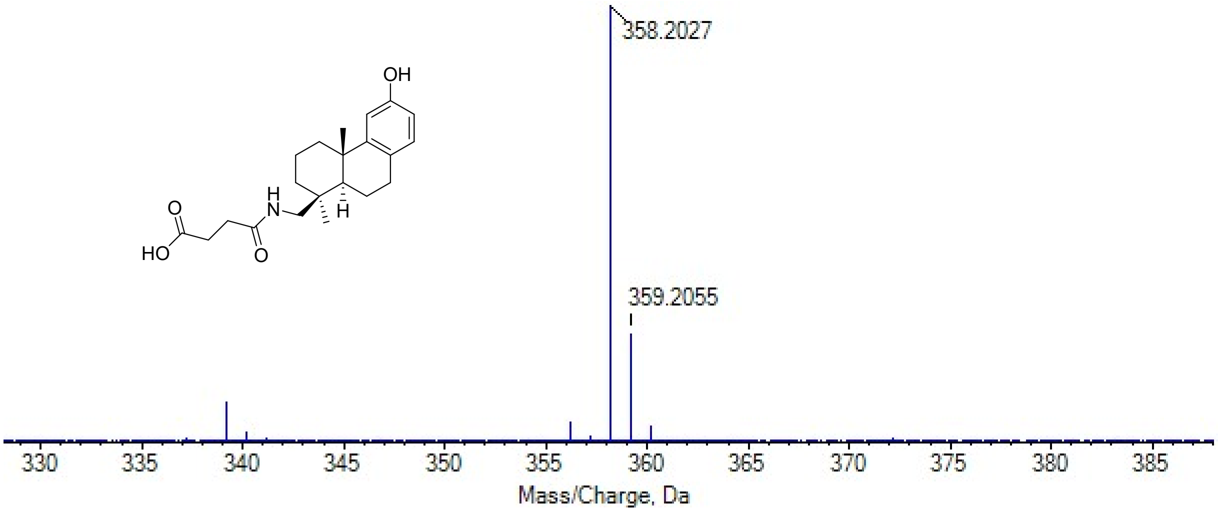
^{1}H-NMR (400MHz, CD$_3$OD) of compound **21**



^{13}C-NMR (101MHz, CD$_3$OD) of compound **21**

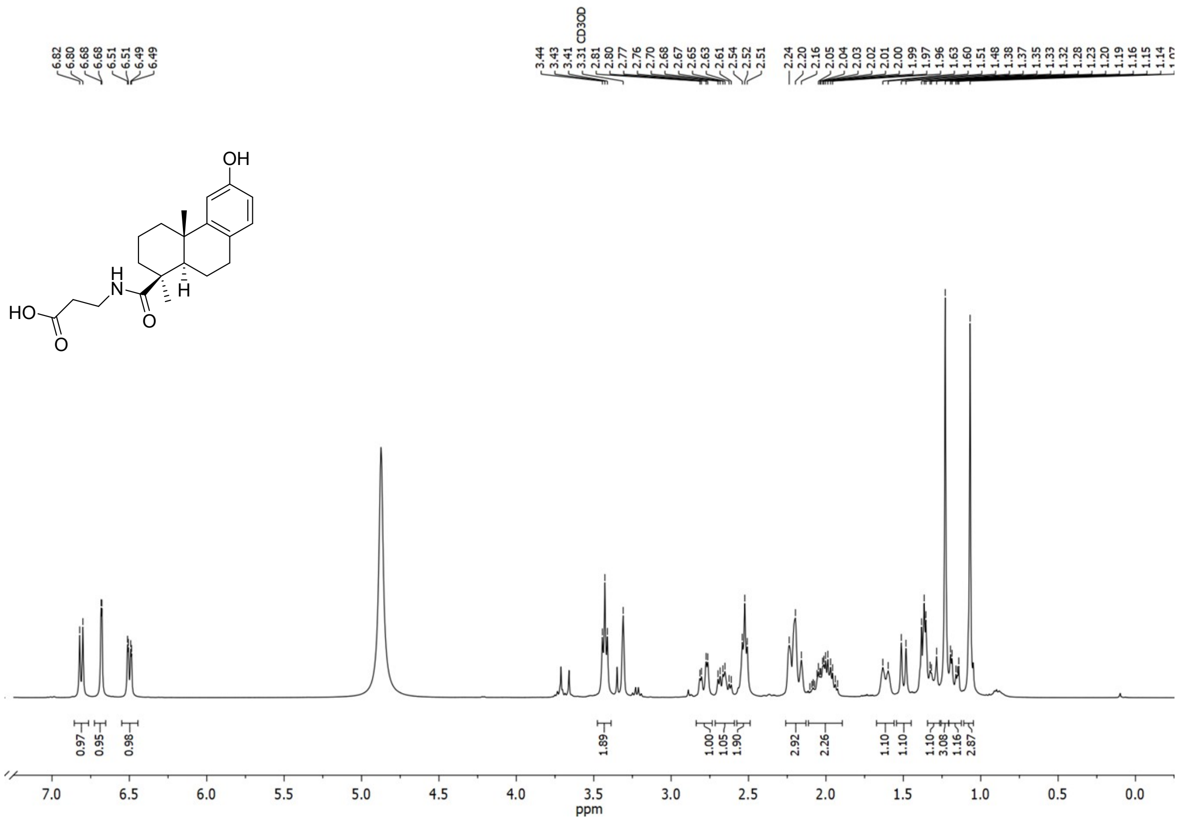


ESI$^-$ mass spectrum of compound **21**

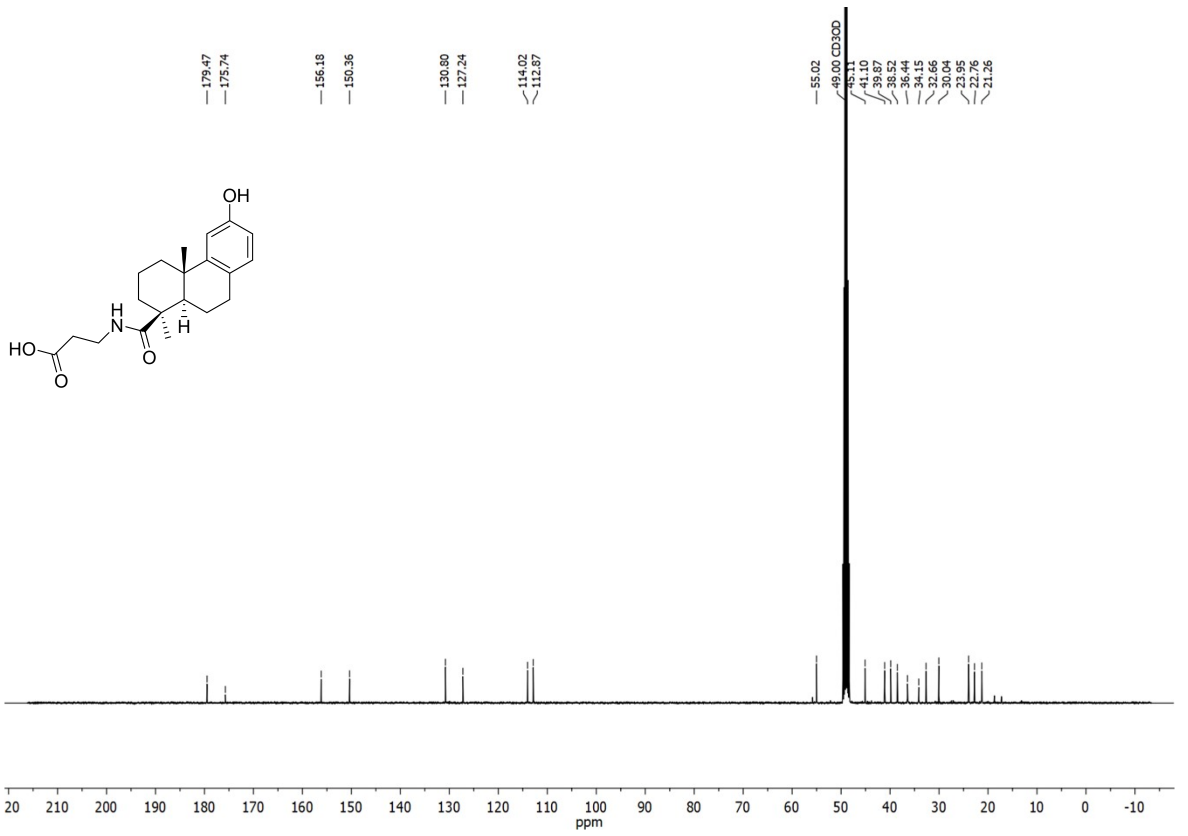


The compound **21** sample was analyzed in negative-ion ESI mode. The signal corresponding to the molecular formula $C_{21}H_{28}NO_4^-$, assigned to [**21**−H]$^-$, is observed at 358.2027 *m/z* with a mass error of 0.9 ppm relative to the calculated exact mass.

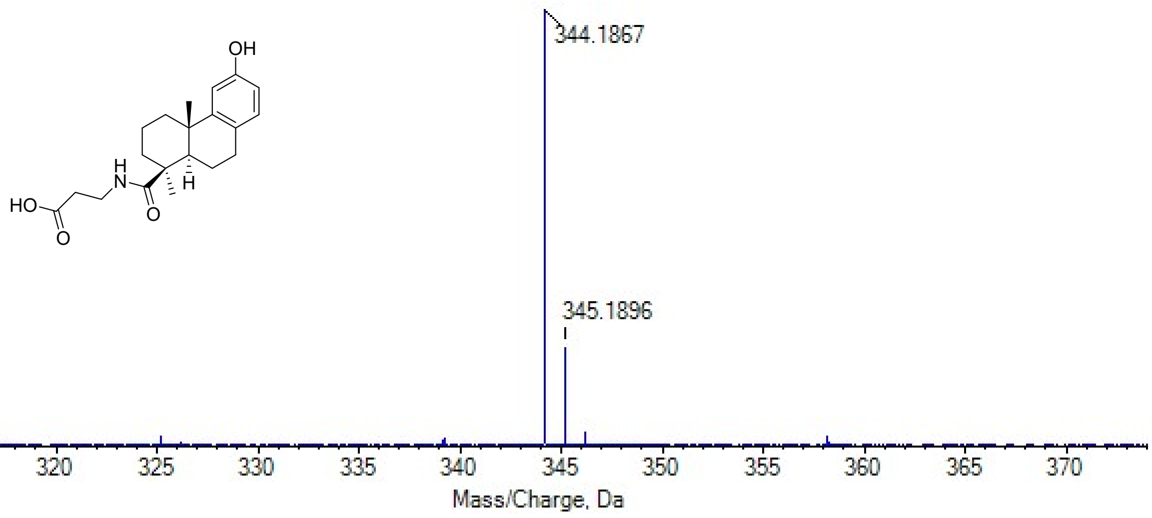
^{1}H-NMR (400MHz, CD_3OD) of compound **22**



^{13}C-NMR (101MHz, CD_3OD) of compound **22**

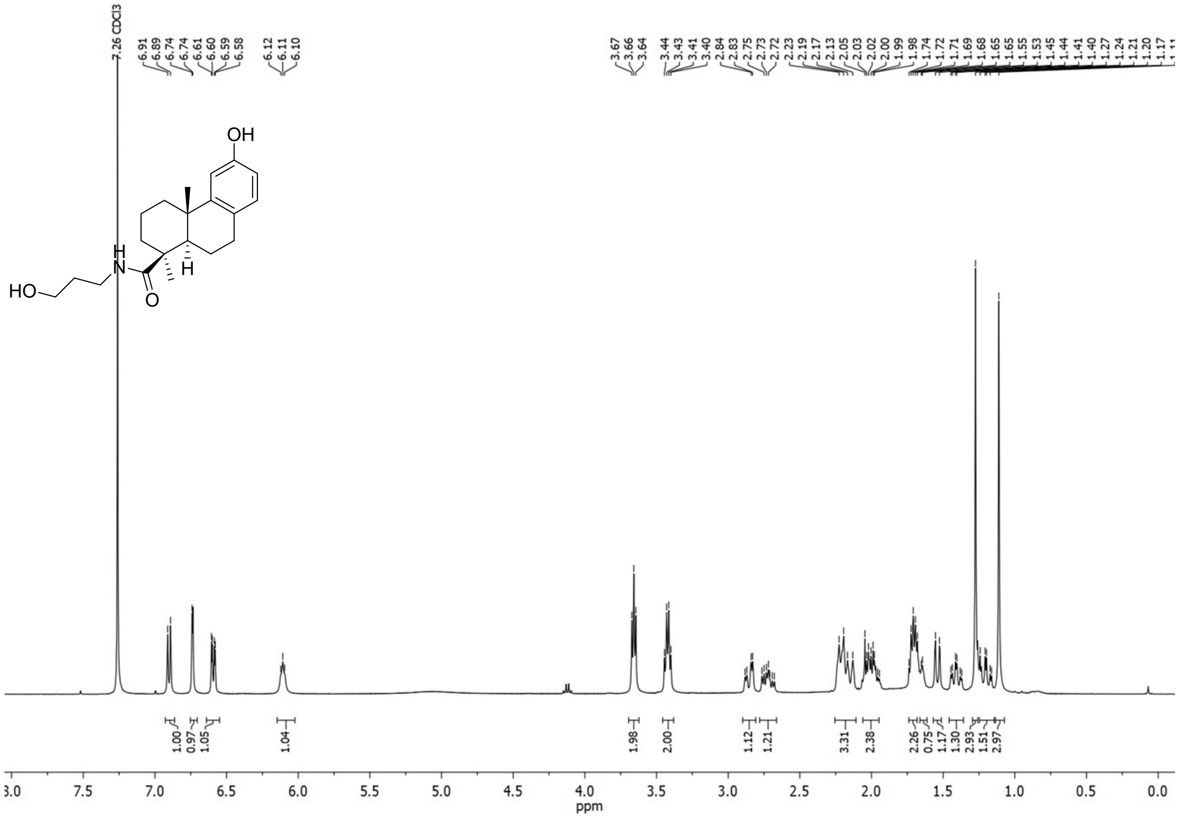


ESI$^-$ mass spectrum of compound **22**

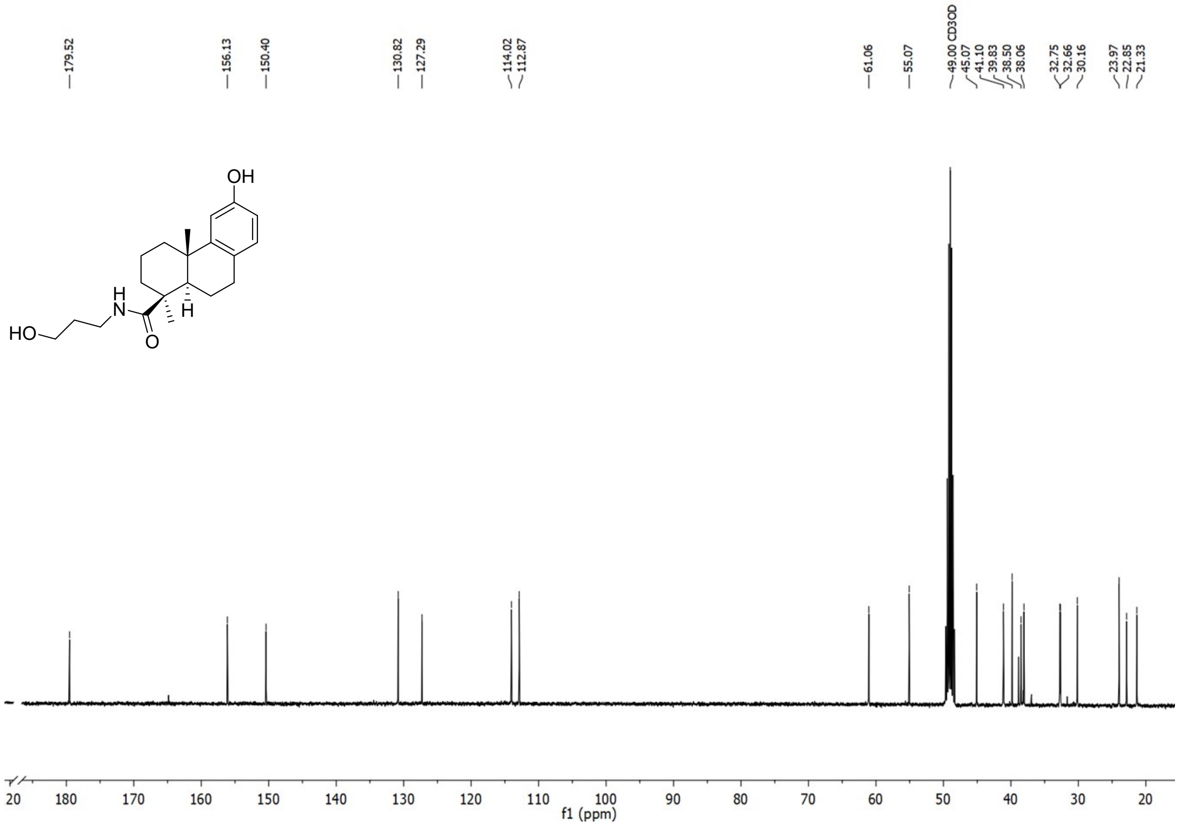


The compound **22** sample was analyzed in negative-ion ESI mode. The signal corresponding to the molecular formula $C_{20}H_{26}NO_4^-$, assigned to [**22**−H]$^-$, is observed at 344.1867 *m/z* with a mass error of 0.2 ppm relative to the calculated exact mass.

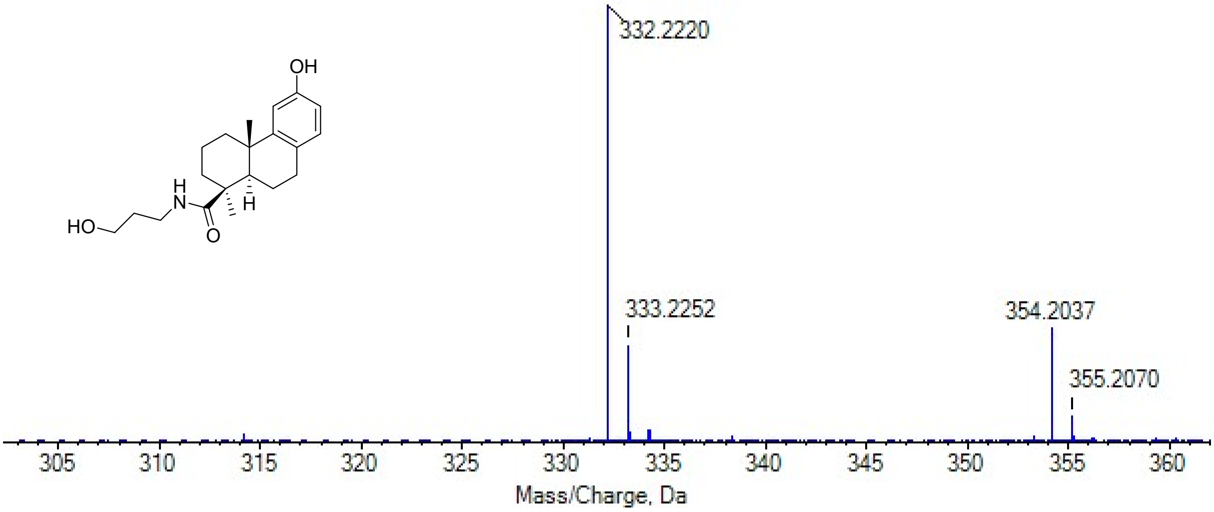
^{1}H-NMR (400MHz, $CDCl_3$) of compound **23**



^{13}C-NMR (101MHz, CD_3OD) of compound **23**

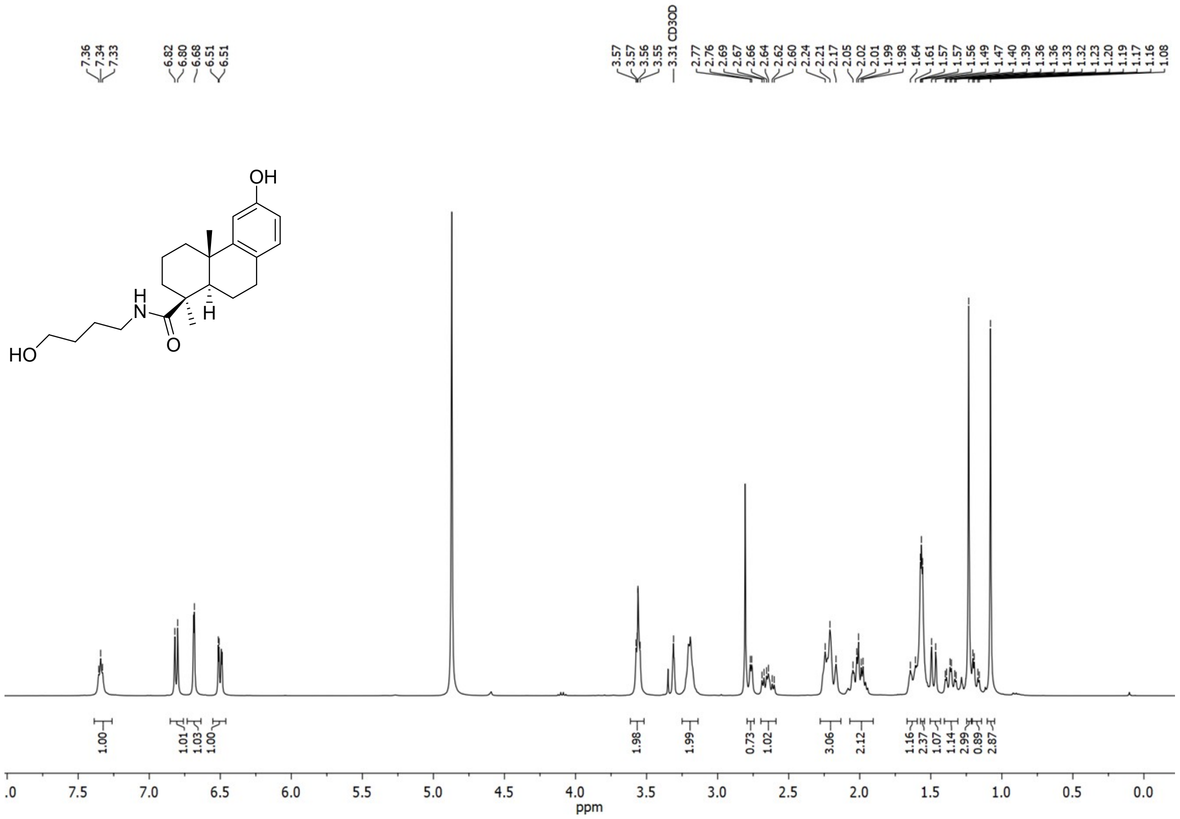


ESI$^+$ mass spectrum of compound **23**

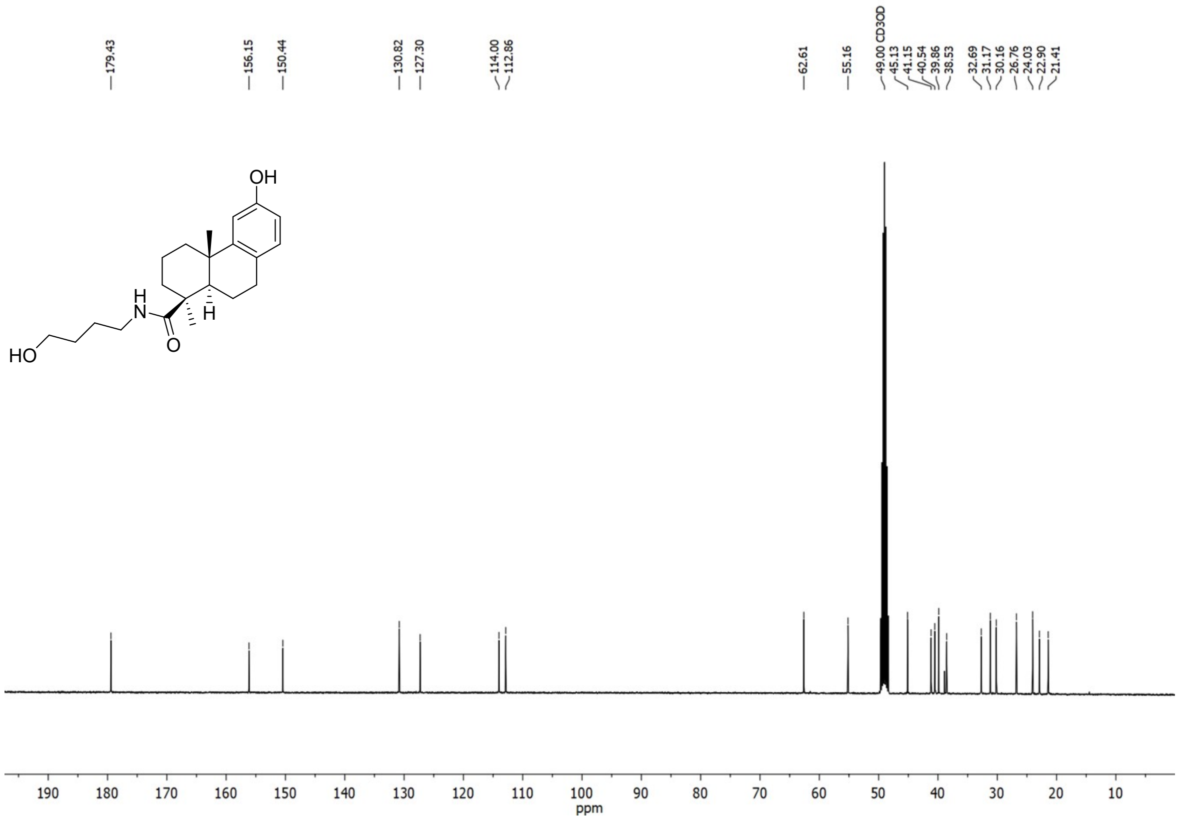


The compound **23** sample was analyzed in positive-ion ESI mode. The signal corresponding to the molecular formula $C_{20}H_{30}NO_3^+$, assigned to [**23**+H]$^+$, is observed at 332.2220 *m/z* with a mass error of 0.1 ppm relative to the calculated exact mass. The [**23**+Na]$^+$ adduct is also present at 354.2037 *m/z*.

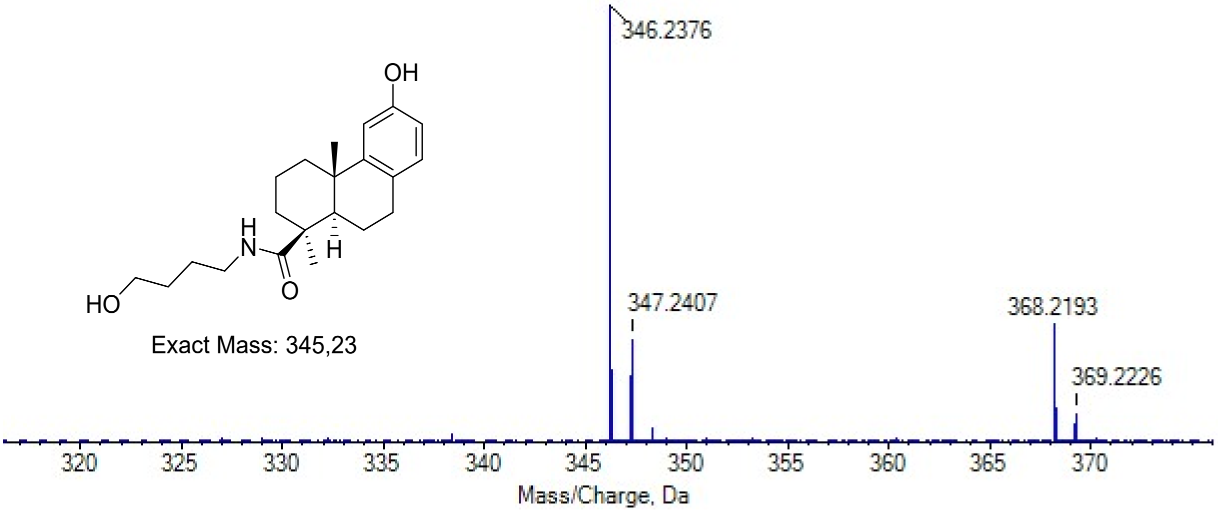
^{1}H-NMR (400MHz, CD_3OD) of compound **24**



^{13}C-NMR (101MHz, CD_3OD) of compound **24**

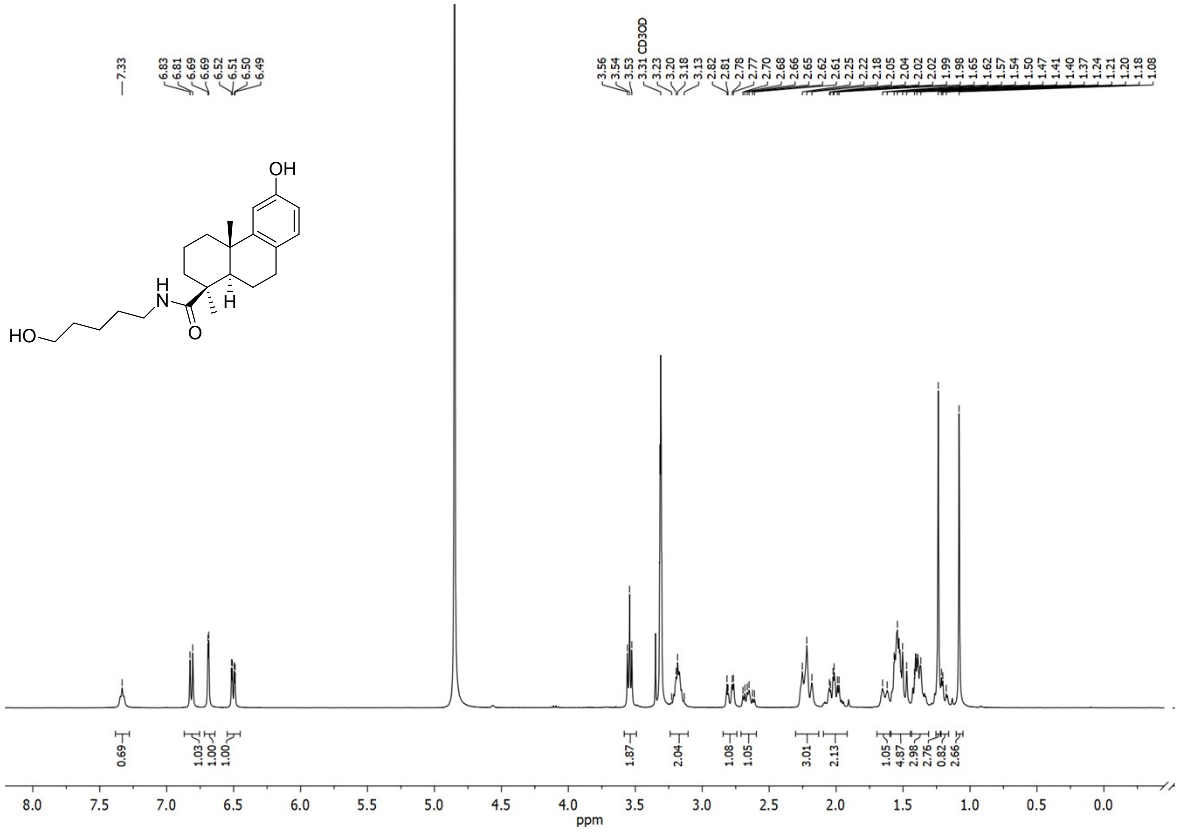


ESI$^+$ mass spectrum of compound **24**

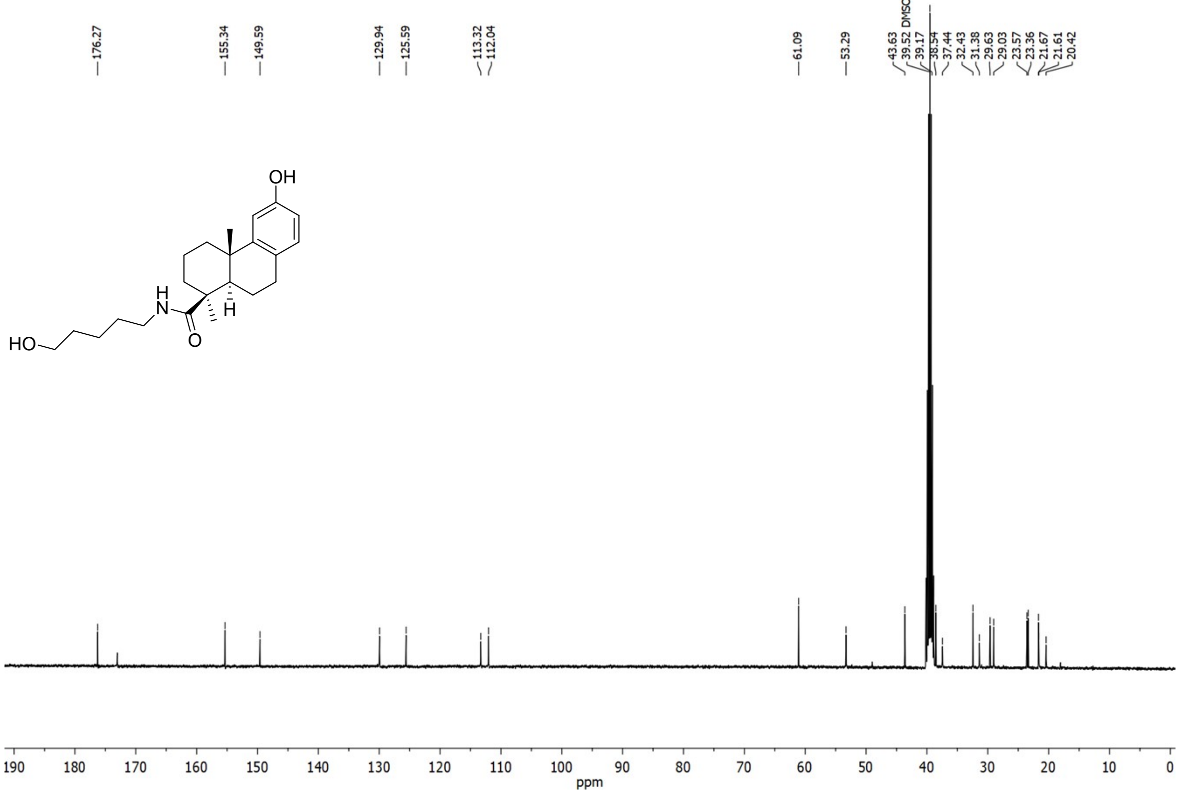


The compound **24** sample was analyzed in positive-ion ESI mode. The signal corresponding to the molecular formula $C_{21}H_{32}NO_3^+$, assigned to [**24**+H]$^+$, is observed at 346.2376 *m/z* with a mass error of 0.2 ppm relative to the calculated exact mass. The [**24**+Na]$^+$ adduct is also present at 368.2193 *m/z*.

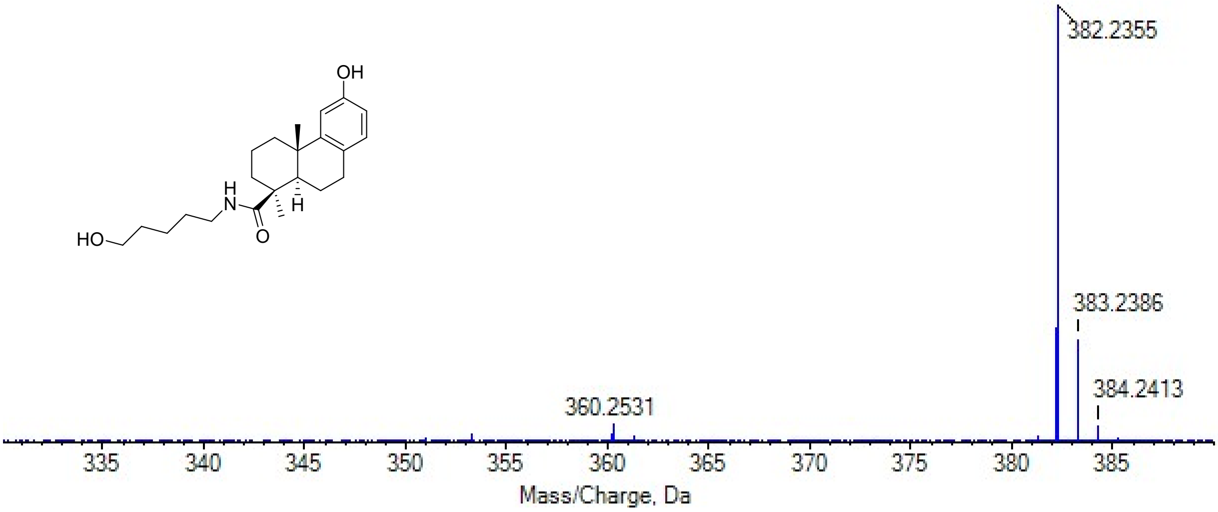
^{1}H-NMR (400MHz, CD_3OD) of compound **25**



^{13}C-NMR (101MHz, DMSO-d_6) of compound **25**

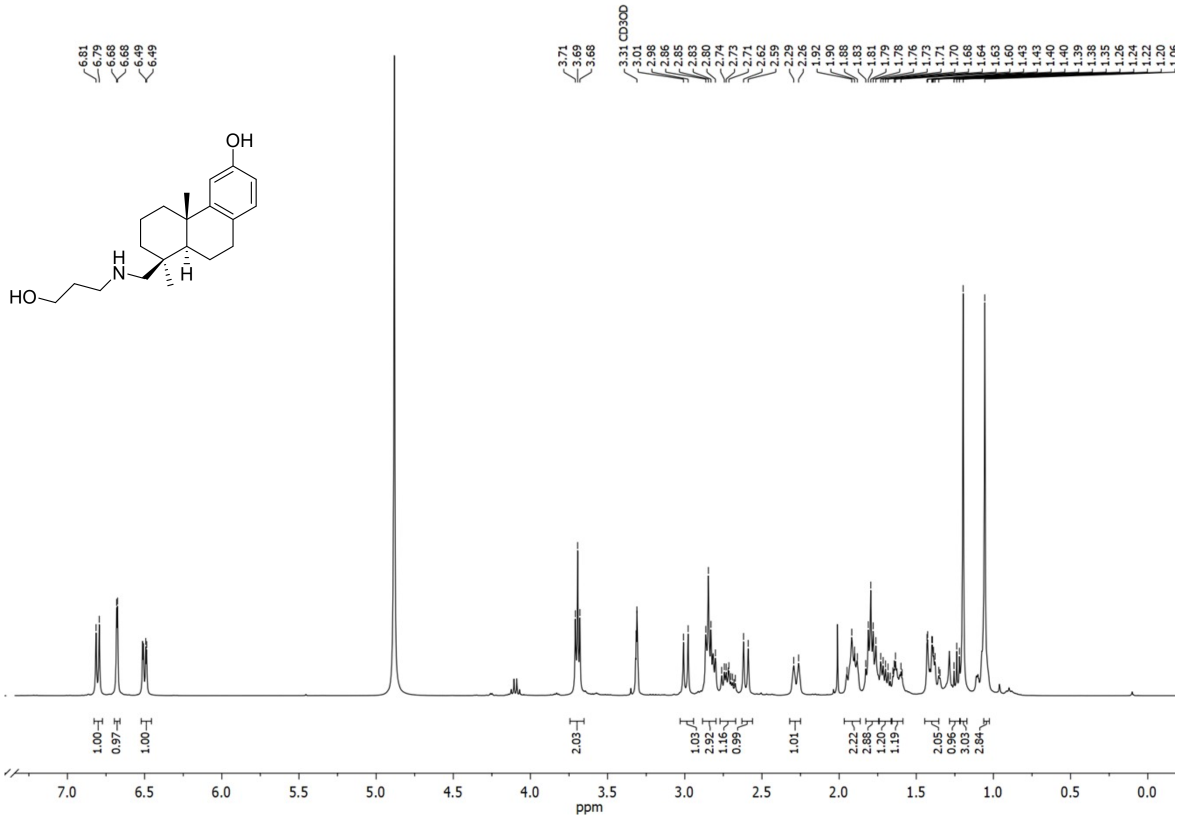


ESI$^+$ mass spectrum of compound **25**

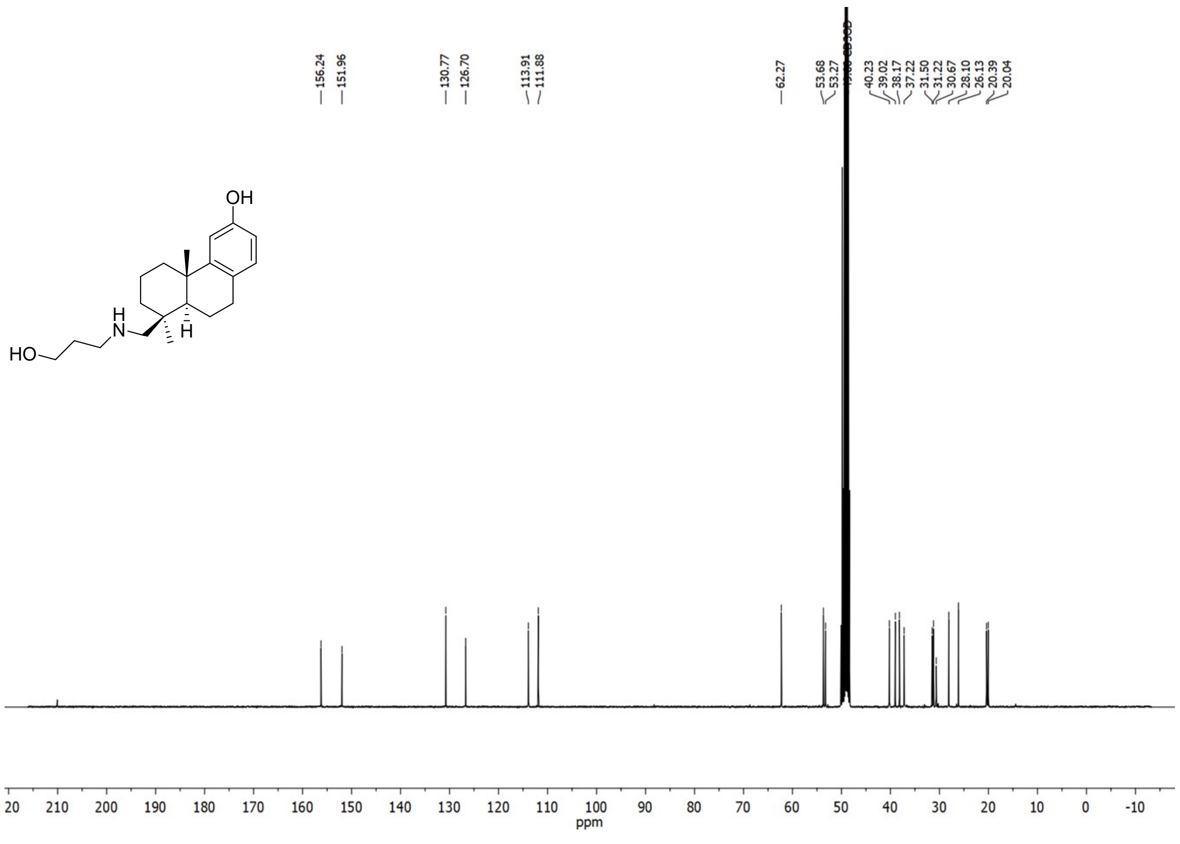


The compound **25** sample was analyzed in positive-ion ESI mode. The signal corresponding to the molecular formula $C_{22}H_{34}NO_3^+$, assigned to [**25**+H]$^+$, is observed at 360.2531 *m/z* with a mass error of 0.6 ppm relative to the calculated exact mass. The most abundant signal of the spectrum at 382.2355 *m/z* corresponds to the sodiated species [**25**+Na]$^+$.

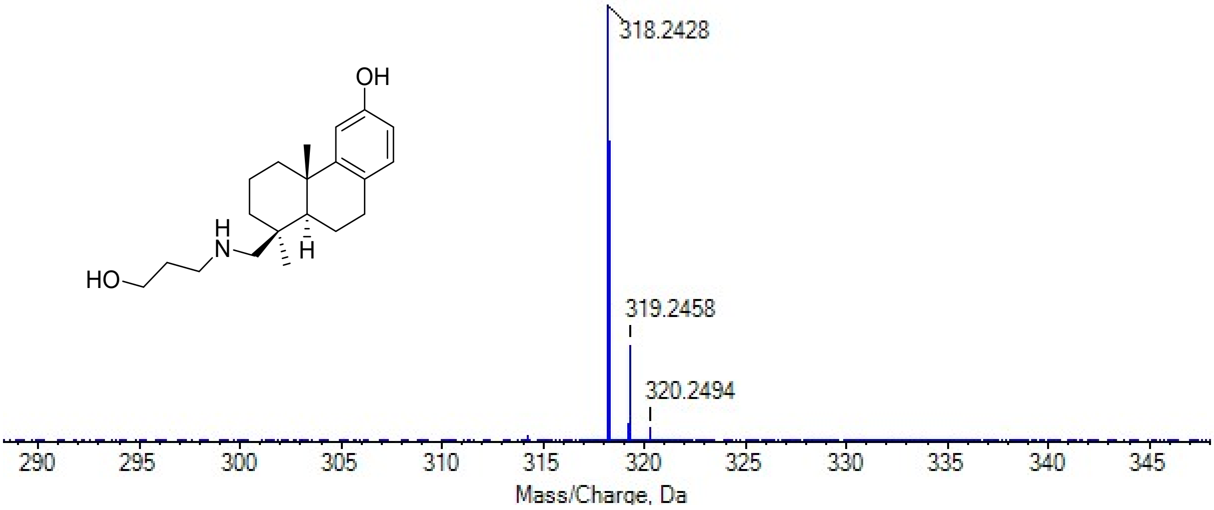
^{1}H-NMR (400MHz, CD_3OD) of compound **26**



^{13}C-NMR (101MHz, CD_3OD) of compound **26**

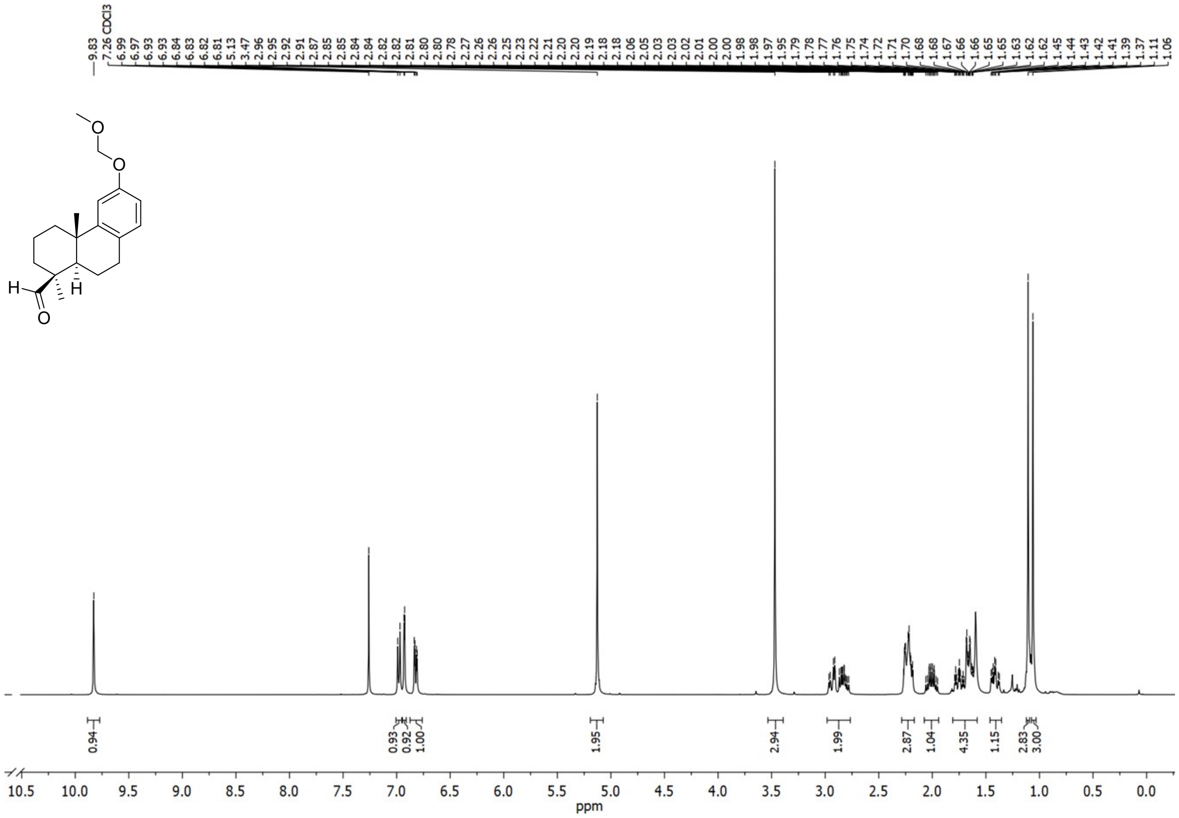


ESI$^+$ mass spectrum of compound **26**

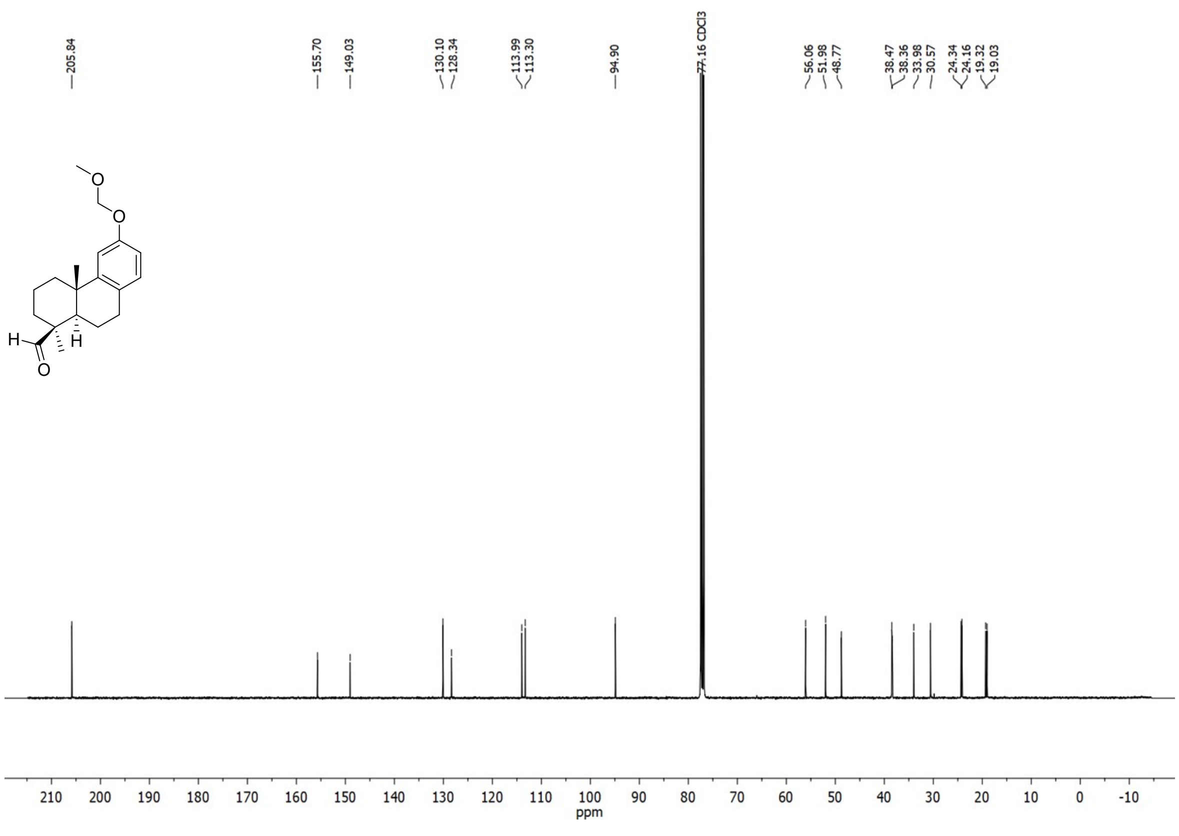


The compound **26** sample was analyzed in positive-ion ESI mode. The signal corresponding to the molecular formula $C_{20}H_{32}NO_3^+$, assigned to [**26**+H]$^+$, is observed at 318.2429 *m/z* with a mass error of 0.5 ppm relative to the calculated exact mass.

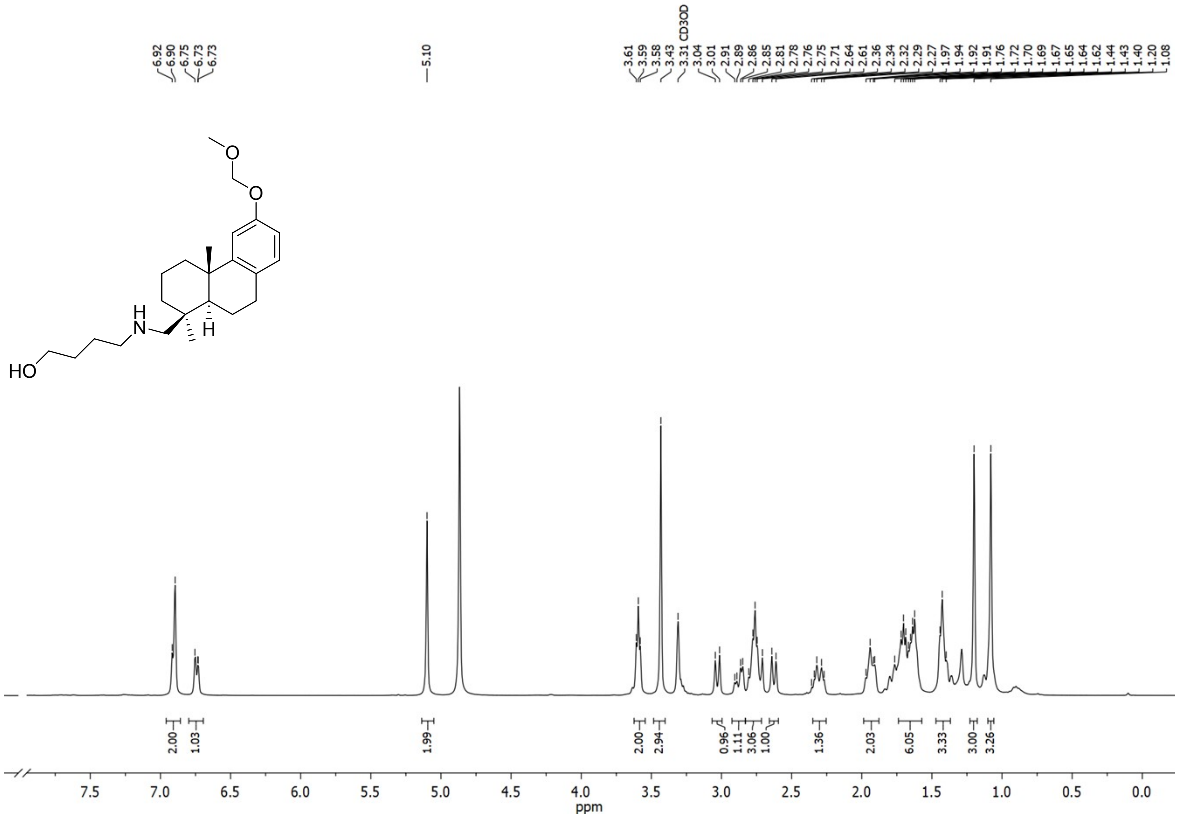
^{1}H-NMR (400MHz, $CDCl_3$) of compound **27**



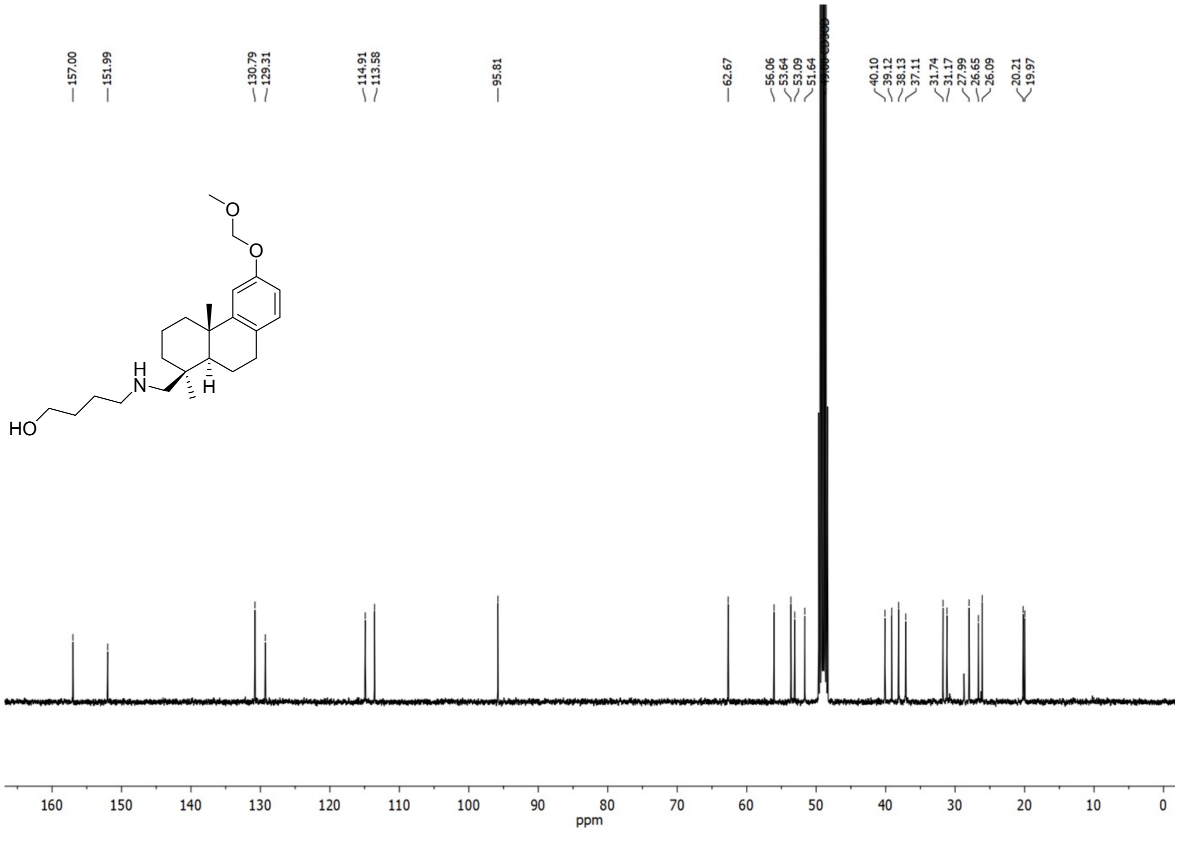
^{13}C-NMR (101MHz, $CDCl_3$) of compound **27**



^{1}H-NMR (400MHz, CD_3OD) of compound **28**



^{13}C-NMR (101MHz, CD_3OD) of compound **28**



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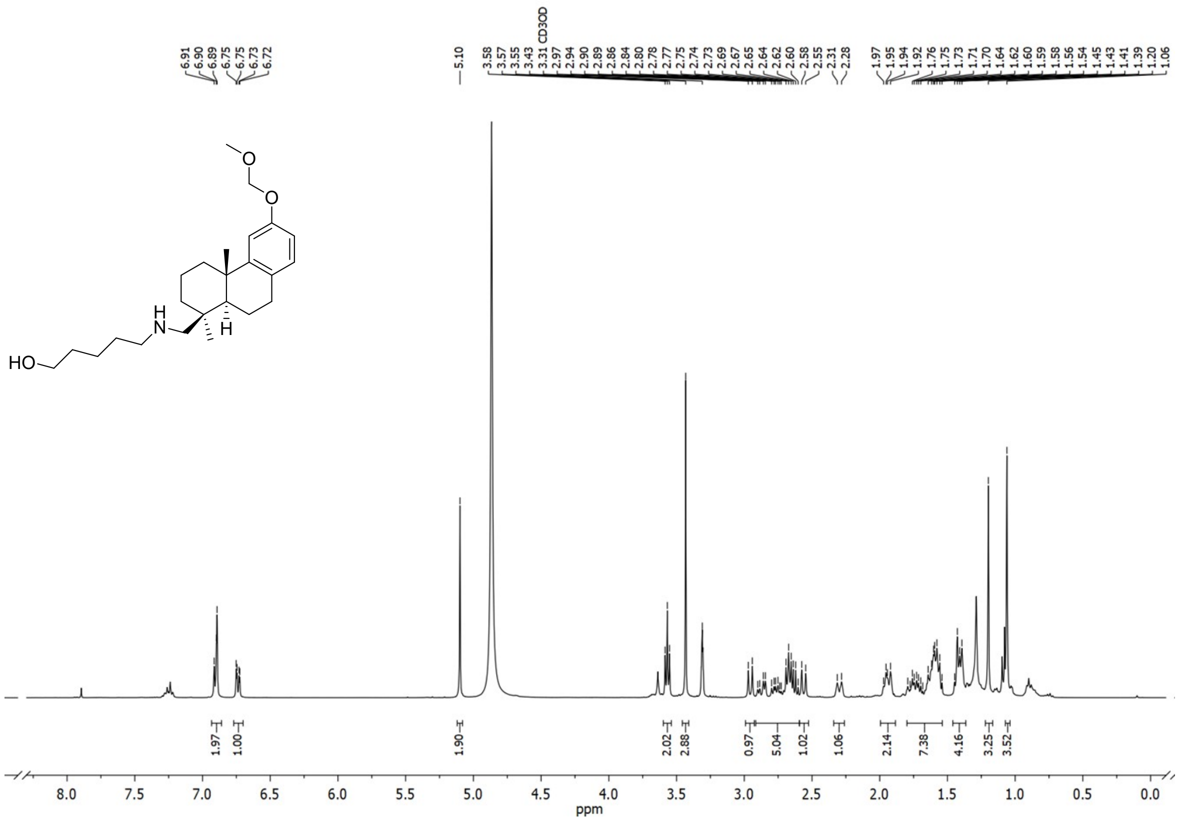

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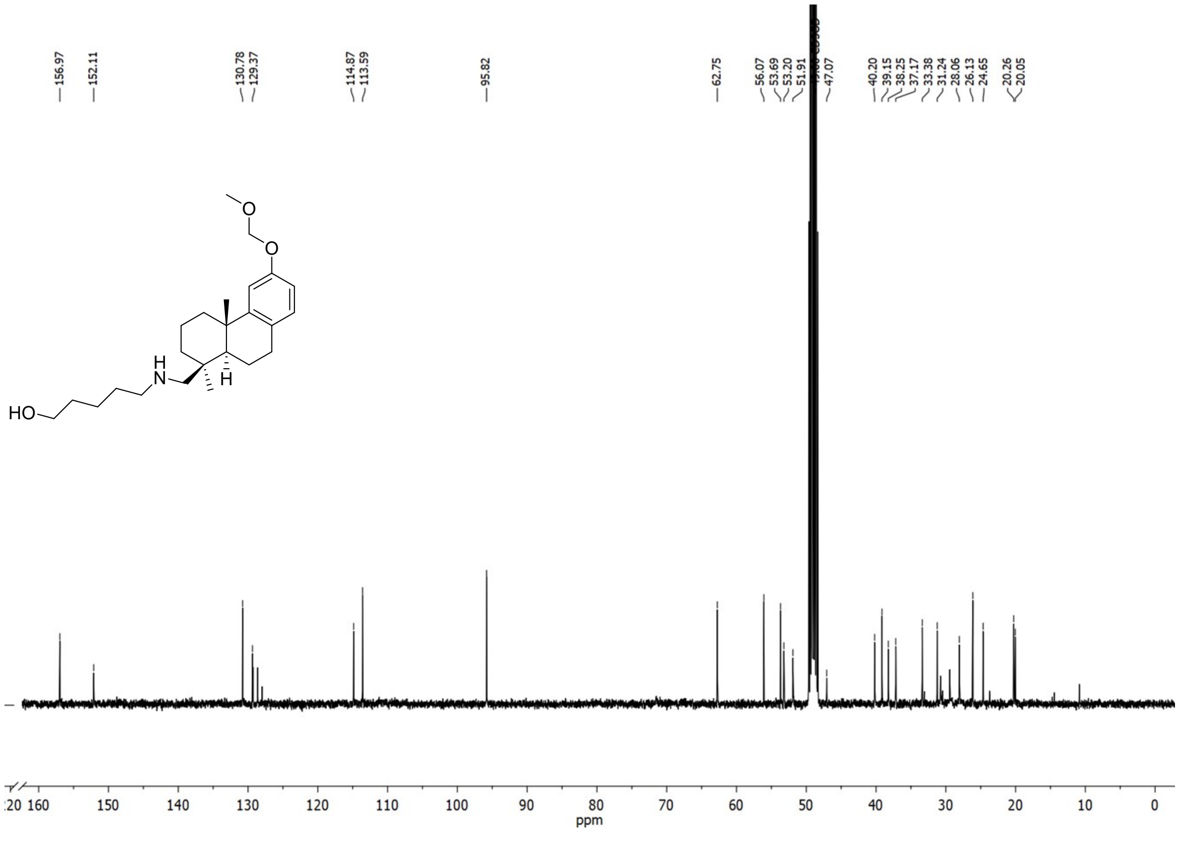
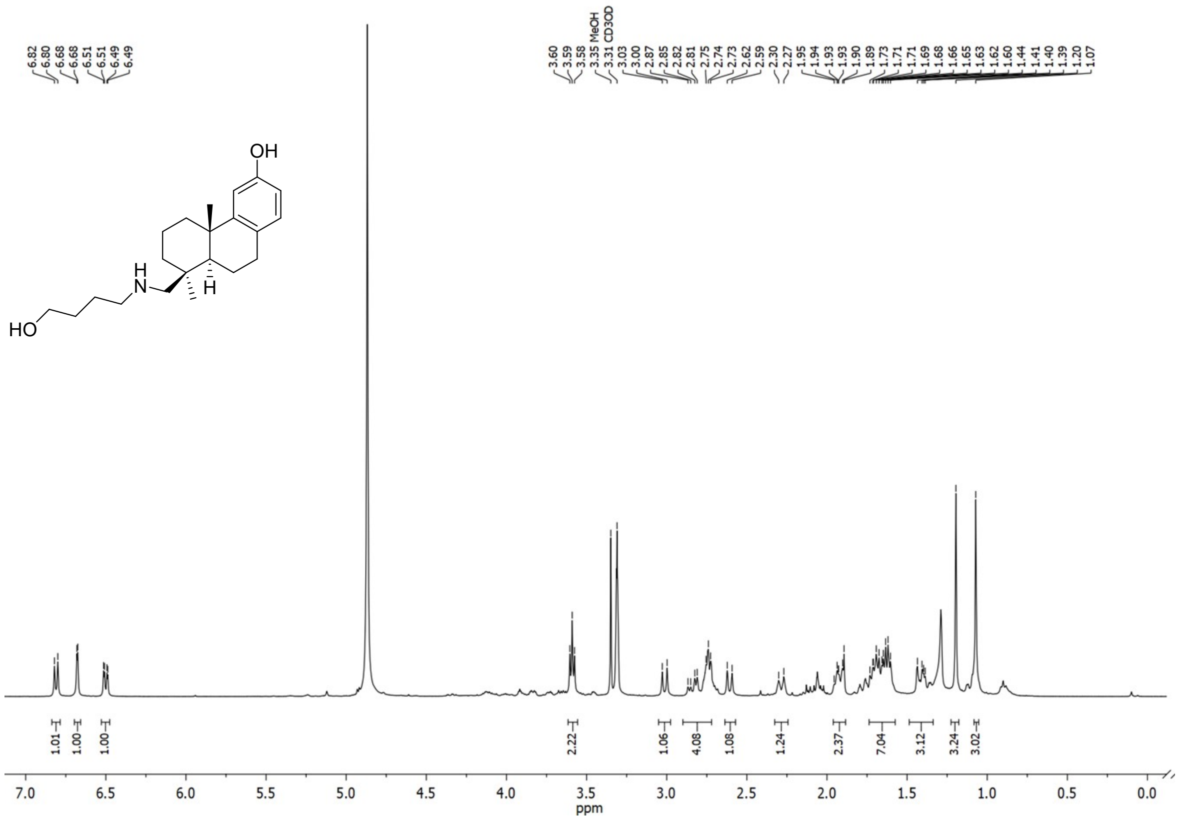
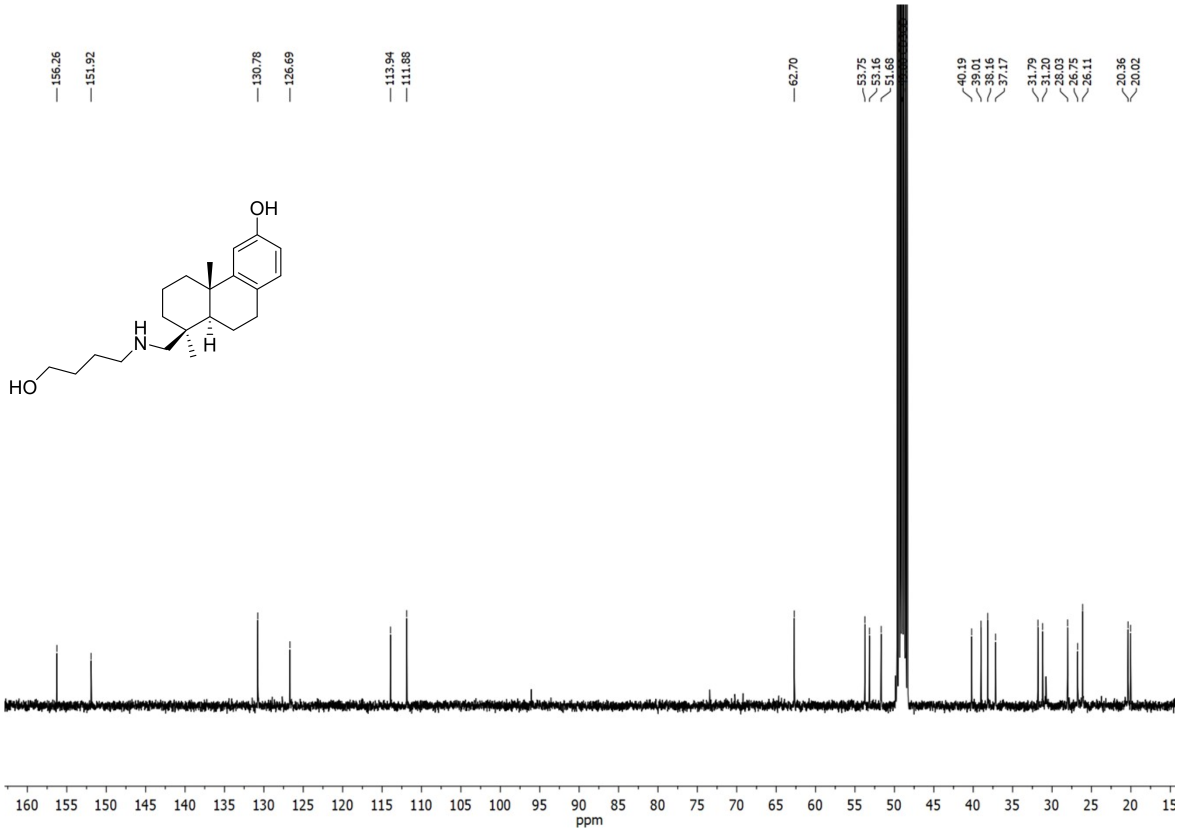


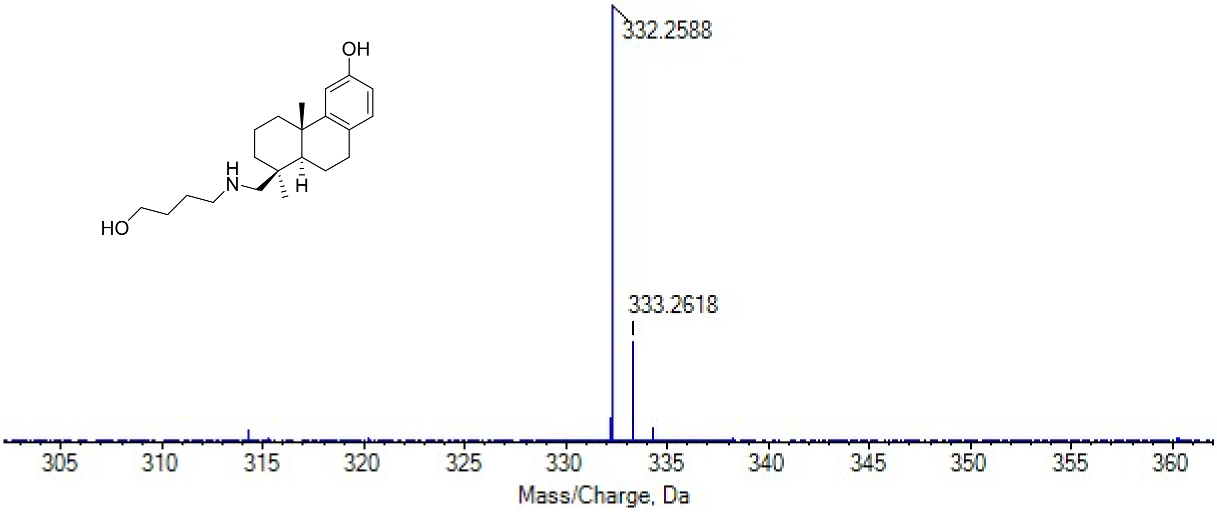
^{1}H-NMR (400MHz, CD_3OD) of compound **30**



^{13}C-NMR (101MHz, CD_3OD) of compound **30**

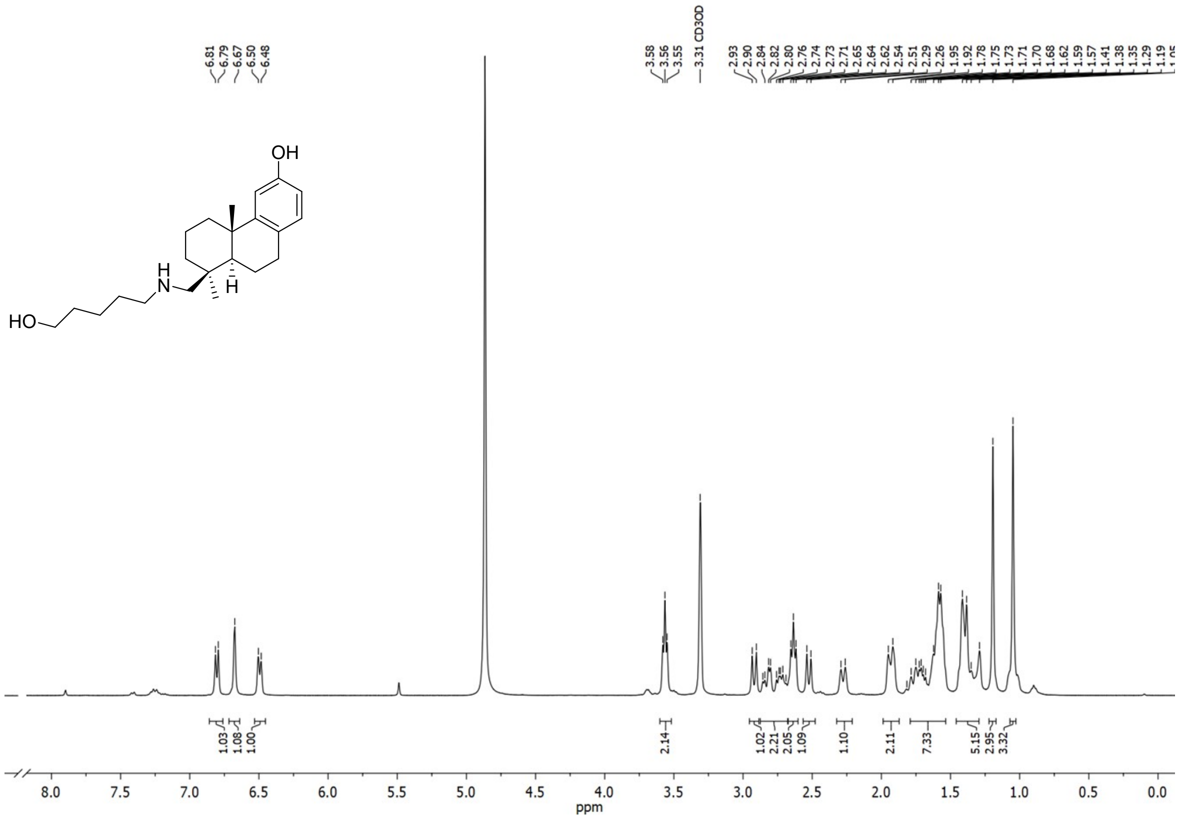


ESI$^+$ mass spectrum of compound **30**

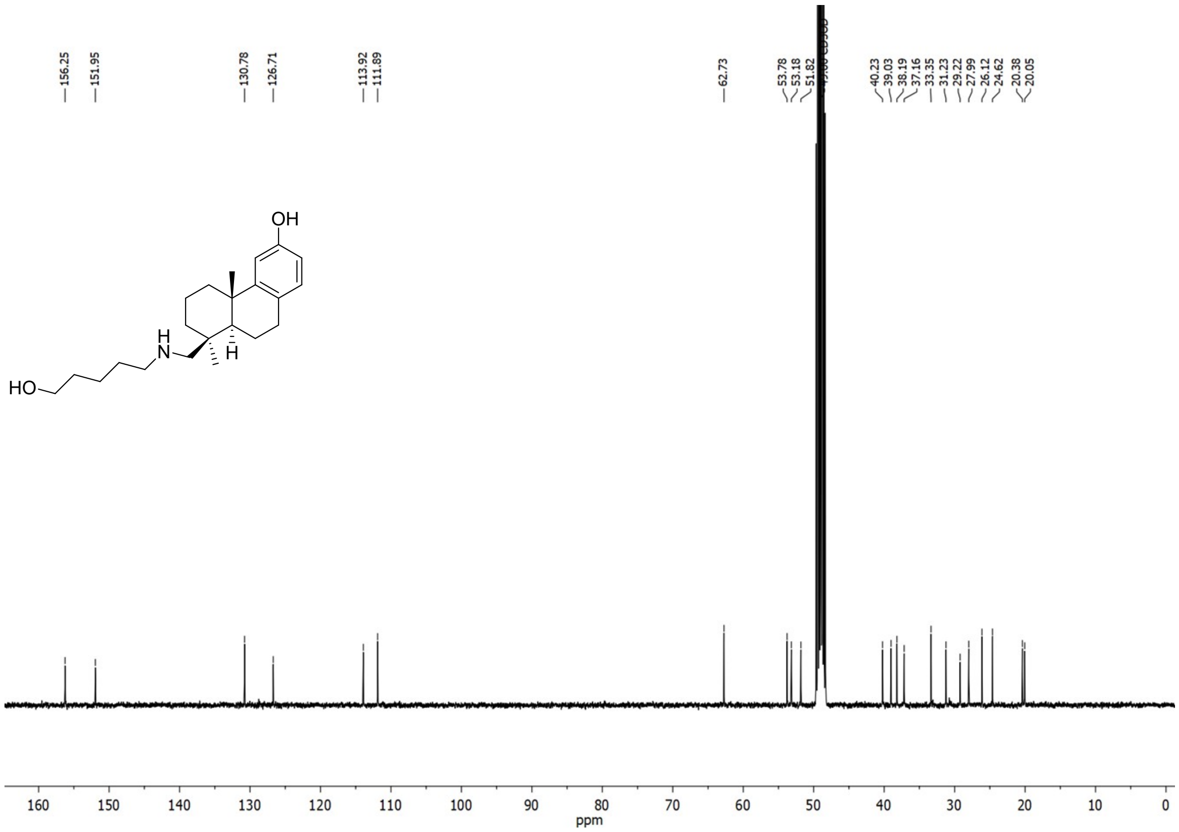


The compound **30** sample was analyzed in positive-ion ESI mode. The signal corresponding to the molecular formula $C_{21}H_{34}NO_2^+$, assigned to [**30**+H]$^+$, is observed at 332.2588 *m/z* with a mass error of 1.2 ppm relative to the calculated exact mass.

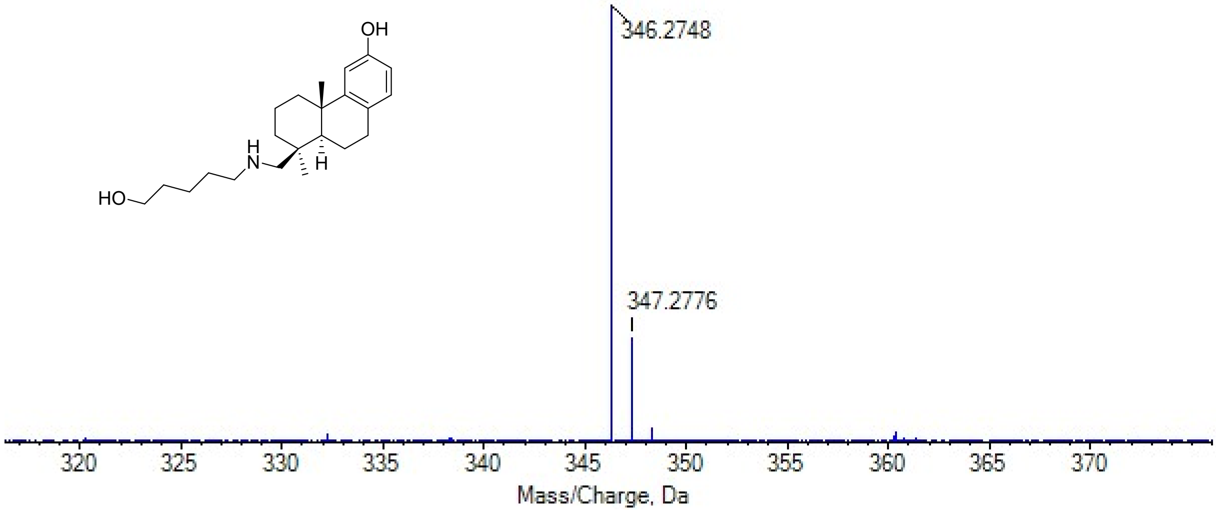
^{1}H-NMR (400MHz, CD_3OD) of compound **31**



^{13}C-NMR (101MHz, CD_3OD) of compound **31**



ESI$^+$ mass spectrum of compound **31**



The compound **31** sample was analyzed in positive-ion ESI mode. The signal corresponding to the molecular formula $C_{22}H_{36}NO_2^+$, assigned to [**31**+H]$^+$, is observed at 346.2748 *m/z* with a mass error of 2.4 ppm relative to the calculated exact mass.

2.2 Microbiology: supplementary figures

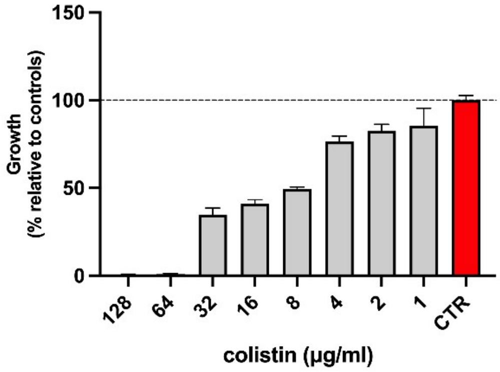


Figure S1. Dose-dependent effect of colistin on the growth of PA14 Col^{R}5 after 24 h at 37 °C in MH. Bacterial growth is expressed as percentage relative to control cultures without colistin. Data are mean (±SD) of at least three independent experiments.

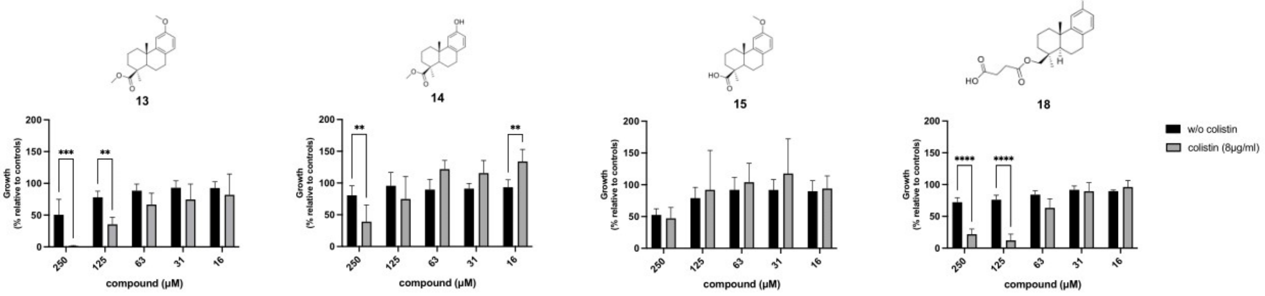


Figure S2. Dose-dependent effect of compounds 13-15 and 18 on PA14 Col^{R}5 growth after 24 h at 37 °C in MH supplemented with 8 µg/mL colistin (grey bar) or without colistin (black bar). Compounds and their structure are reported above each graph; bacterial growth is expressed as percentage relative to control cultures treated with equivalent concentrations of DMSO. Data are mean (±SD) of at least three independent experiments. Statistics: Two-way ANOVA with Bonferroni's multiple comparison test.

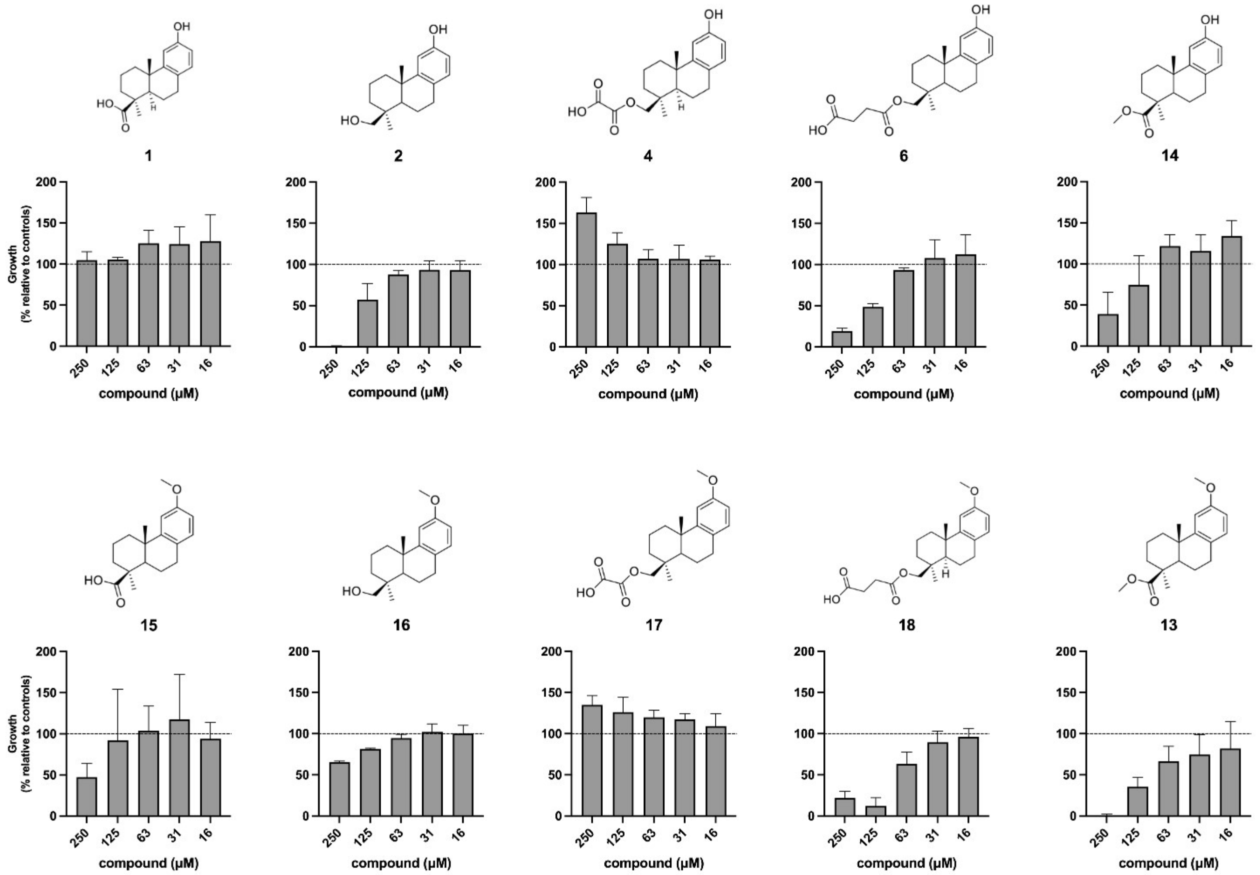


Figure S3. Dose-dependent effect of the compounds on PA14 Col^{R}5 growth after 24 h at 37 °C in MH supplemented with 8 μg/mL colistin. Compounds and their structure are reported above each graph; bacterial growth is expressed as percentage relative to control cultures treated with equivalent concentrations of DMSO (dashed line). Data are mean (±SD) of at least three independent experiments.

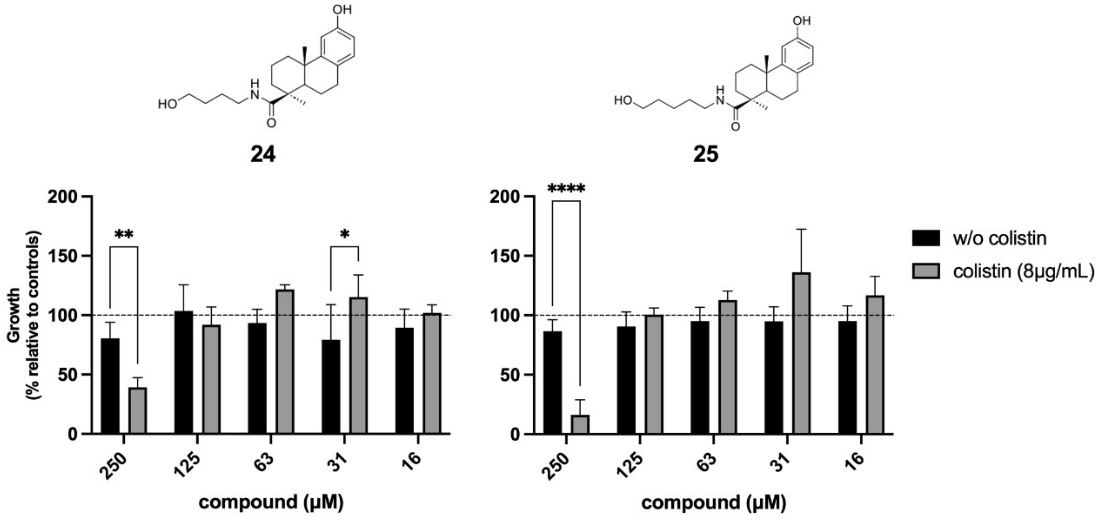


Figure S4. Dose-dependent effect of compounds 23 and 24 on PA14 Col^{R}5 growth after 24 h at 37 °C in MH supplemented with 8 μg/mL colistin (grey bar) or without colistin (black bar). Compounds and their structure are reported above each graph; bacterial growth is expressed as percentage relative to control cultures treated with equivalent concentrations of DMSO (dashed line). Data are mean (±SD) of at least three independent experiments. Statistics: Two-way ANOVA with Bonferroni’s multiple comparison test.

2.3 Binding studies: supplementary figure

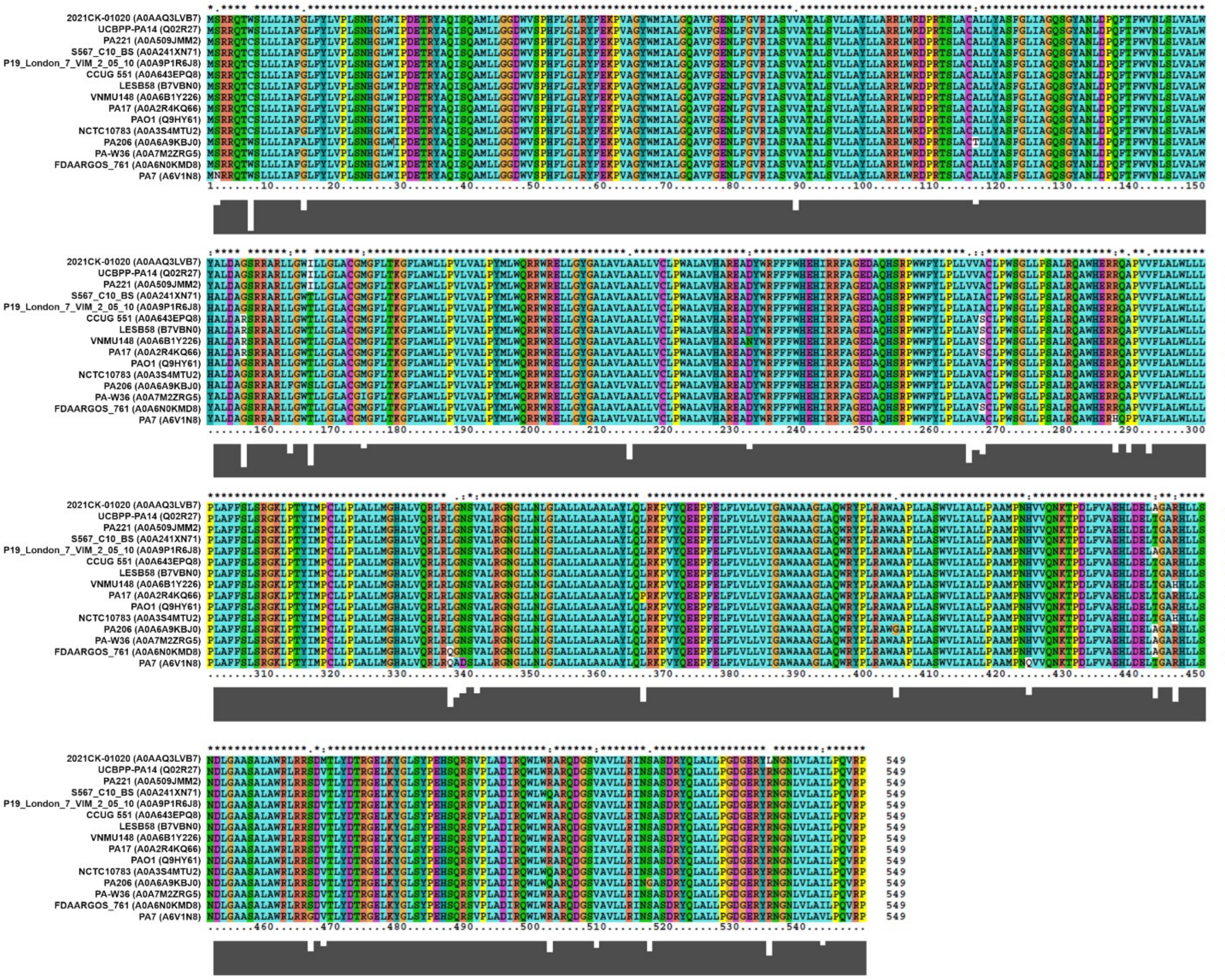


Figure S5. Sequence alignment of the ArnT from *P. aeruginosa* strains sharing 90% similarity with the ArnT sequence from the reference PA14 strain (Uniprot ID:Q02R27). Strain names are reported, while the corresponding Uniprot IDs are in brackets.

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