Supporting information

Mitochondria-targeted lupane triterpenoid derivatives and their selective apoptosis-inducing anticancer mechanisms

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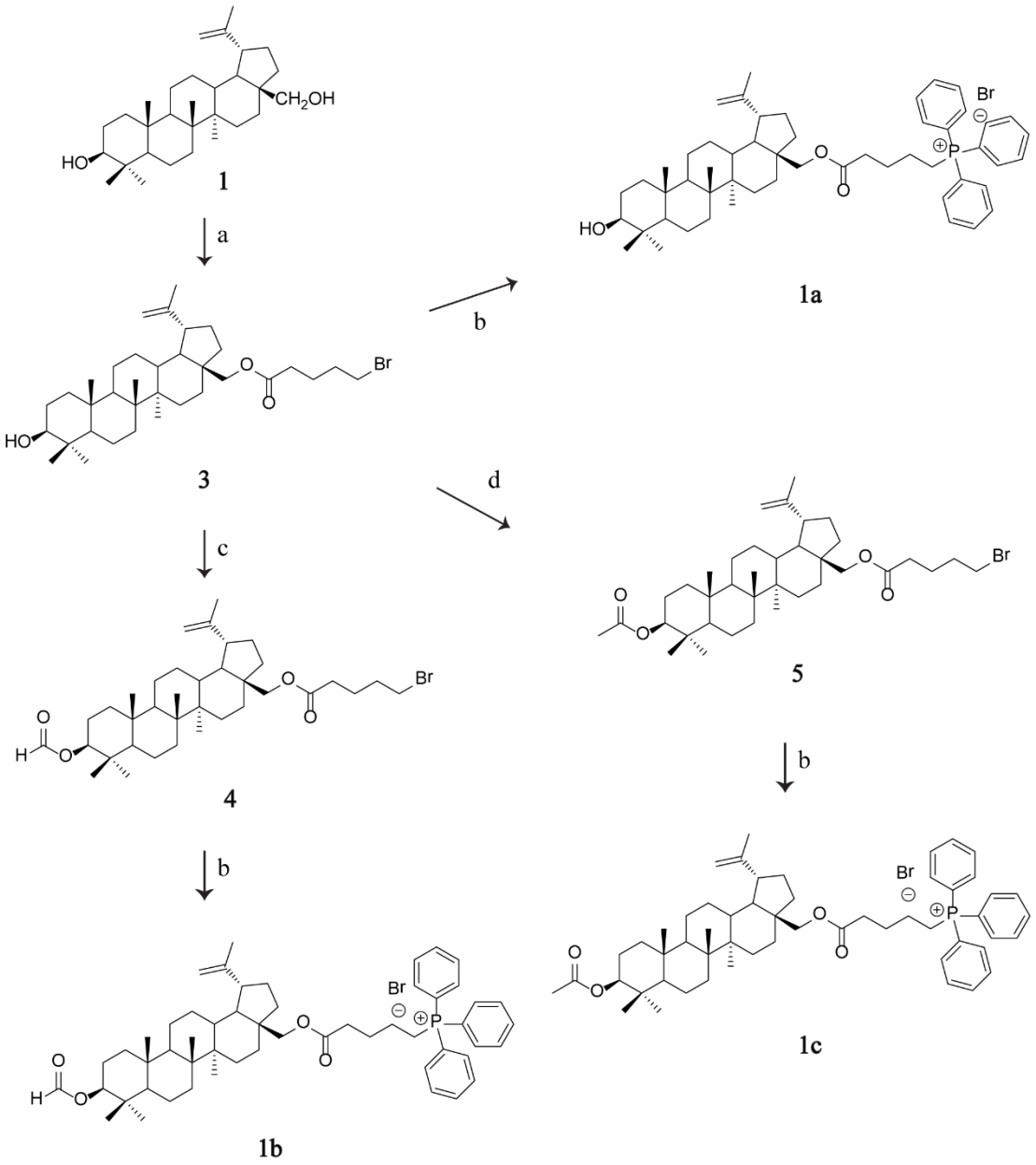
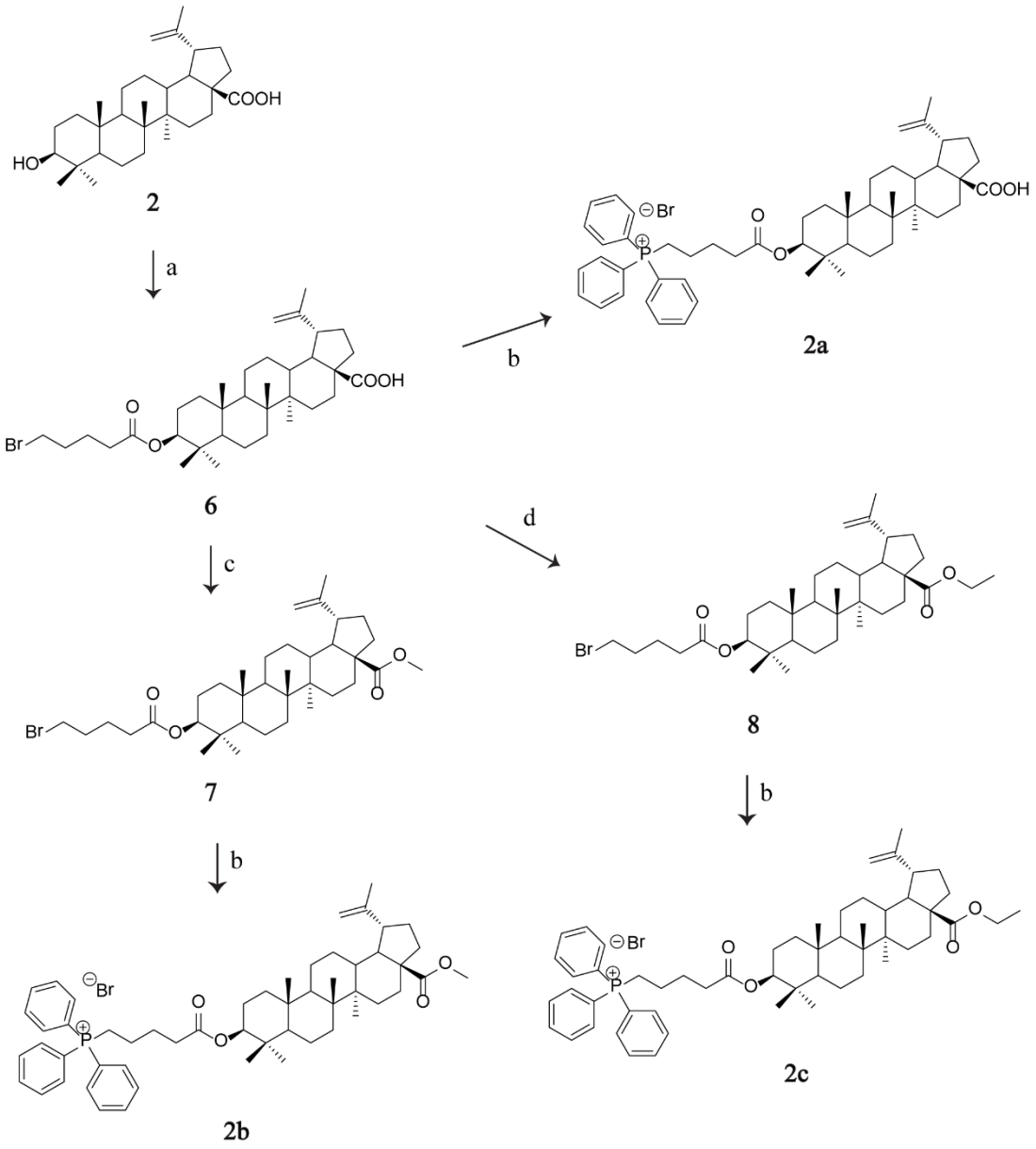
Synthesis procedures



Reagents and conditions: (a) DMAP, EDC, 5-Bromovaleric acid, DCM, r.t.; (b) TPP (Triphenylphos phine), MeCN, reflux; (c) formic acid, reflux; (d) acetic anhydride, pyridine, reflux.



Reagents and conditions: (a) DMAP, EDC, 5-Bromovaleric acid, DCM, r.t.; (b) TPP (Triphenylphos phine), MeCN, reflux; (c) CH_3I, K_2CO_3, DMF, r.t.; (d) bromoethane, K_2CO_3, DMF, r.t.

Experimental descriptions and analytical data of intermediates

3β-Hydroxylup-20(29)-en-28-yl 5-bromopentanoate (3). A solution of betulin (1.0 g, 2.26 mmol) in DCM (40 mL) was stirred. 1-(3-Dimethyl aminopropyl)-3-ethylcarbodiimide (EDC, 1.09 g, 9.04 mmol), 4-dimethyl aminopyridine (DMAP, 112 mg, 0.90 mmol) and 5-bromovaleric acid (1.68 g, 9.04 mmol) were added successively, and the mixture was stirred at 25℃ until the reaction was complete according to TLC detection. The solvent was removed under diminished pressure, and the residue was purified by column chromatography using 10:1 petroleum ether (PE)/ethyl acetate (EtOAc) to obtain 250 mg (40%) of compound **3** as a white powder.

Data for compound **3**: Mp: 101-108℃; $[\alpha]_D^{20}$ = +4.5 (c 0.1, CH_3OH); ^1H NMR ($CDCl_3$, 600 MHz): δ 4.69 (s, 1H, H-29a), 4.59 (s, 1H, H-29b), 4.28 (d, *J* = 11.1 Hz, 1H, H-28a), 3.86 (d, *J* = 11.0 Hz, 1H, H-28b), 3.42 (t, *J* = 6.5 Hz, 2H, CH_2Br-), 3.18 (dd, *J* = 11.2, 4.9 Hz, 1H, H-3), 2.44 (td, *J* = 11.0, 5.8 Hz, 1H, H-19), 2.37 (t, *J* = 7.2 Hz, 2H, -$COCH_2$-), 1.07-1.99 (m, 28H, CH, CH_2 in pentacyclic skeleton or carbon chain), 1.68, 1.03, 0.98, 0.97, 0.82, 0.76 (each, s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.5 (O-CO-), 150.1 (C-20), 109.9 (C-29), 78.9 (C-3), 62.7 (C-28), 55.3, 50.4, 48.8, 47.7, 46.4, 42.7, 40.9, 38.9, 38.7, 37.6, 37.2, 34.6, 34.2, 33.5, 33.0, 32.1, 29.8, 29.6, 28.0, 27.4, 27.1, 25.2, 23.6, 20.8, 19.2, 18.3, 16.0, 16.1, 15.4, 14.8; HR-ESI-MS *m/z* calculated for $C_{35}H_{57}BrO_3K$ $[M+K]^+$ 643.3128, found 643.3105; *m/z* calculated for $C_{35}H_{61}NBrO_3$ $[M+NH_4]^+$ 622.3835, found 622.3834.

3β-Formyloxylup-20(29)-en-28-yl 5-bromopentanoate (4). Compound **3** (1.19 g,

1.962 mmol) was added into formic acid (solvent, 11 mL) and heated under reflux until the reaction was complete according to TLC analysis. Formic acid was eliminated by rotary evaporation, and the reaction solution was extracted by DCM (3×3 mL) and ice-cold water. The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography using 20:1 PE/EtOAc to obtain 149 mg (12%) of compound **4** as a white solid.

Data for compound **4**: Mp: 118-124℃; $[\alpha]_D^{20}$ = +6.3 (c 0.1, CH_3OH); ^1H NMR ($CDCl_3$, 600 MHz): δ 8.11 (s, 1H, -CHO), 4.69 (s, 1H, H-29a), 4.59 (s, 1H, H-29b), 4.59 (t, *J* = 6.0 Hz 1H, H-3), 4.28 (d, *J* = 11.0 Hz, 1H, H-28a), 3.85 (d, *J* = 11.0 Hz, 1H, H-28b), 3.42 (t, *J* = 6.6 Hz, 2H, CH_2Br-), 2.49–2.40 (m, 1H, H-19), 2.37 (t, *J* = 7.3 Hz, 2H, -$COCH_2$-), 1.68, 1.04, 0.97, 0.87 (each, s, 3H, -CH_3), 0.86 (s, 6H, H-23,24), 1.98-1.07 (m, 29H, CH, CH_2 in pentacyclic skeleton or carbon chain); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 173.5 (O-CO-), 161.2 (-CHO), 150.1 (C-20), 109.9 (C-29), 81.0 (C-3) , 62.7 (C-28), 55.4, 50.3, 48.7, 47.7, 46.4, 42.7, 40.9, 38.3, 37.7, 37.5, 37.0, 34.6, 34.1, 33.4, 33.0, 32.0, 29.8, 29.6, 27.9, 27.0, 25.1, 23.81, 23.6, 20.8, 19.1, 18.2, 16.5, 16.1, 16.0, 14.7; HR-ESI-MS *m/z* calculated for $C_{36}H_{57}BrO_4Na$ $[M+Na]^+$ 655.3338, found 655.3276.

3β-Acetyloxylup-20(29)-en-28-yl 5-bromopentanoate **(*5*).** Compound **3** (1.1 g, 1.80 mmol) was added into a combination of acetic anhydride and pyridine (v/v = 3:1, 80 mL), and the mixture was stirred at 25°C. After the reaction was complete according to TLC detection, the solvent was removed under diminished pressure. The residue was purified by column chromatography using 15:1 PE/EtOAc to obtain compound **5**

(130 mg, 10%) as white solid.

Data for compound **5**: Mp: 120-127℃; $[\alpha]_D^{20}$ = +7.4 (c 0.1, CH_3OH); ^1H NMR ($CDCl_3$, 600 MHz): δ 4.69 (s, 1H, H-29a), 4.59 (s, 1H, H-29b), 4.47 (dd, *J* = 10.8, 5.6 Hz, 1H, H-3), 4.28 (d, *J* = 11.0 Hz, 1H, H-28a), 3.85 (d, *J* = 11.0 Hz, 1H, H-28b), 3.42 (t, *J* = 6.6 Hz, 2H, CH_2Br-), 2.44 (td, *J* = 11.1, 5.8 Hz, 1H, H-19), 2.37 (t, *J* = 7.3 Hz, 2H, -$COCH_2$-), 2.04 (s, 3H, CH_3CO-), 1.67, 1.03, 0.98, 0.85, 0.84, 0.84 (each, s, 3H, -CH_3-23, 24, 25, 26, 27, 30),1.98-1.05 (m, 28H, CH, CH_2 in pentacyclic skeleton or carbon chain); ^{13}C NMR (CD_3OD, 150 MHz): δ 173.5 (O-CO-), 171.0 (-$\underline{C}OCH_3$), 150.1 (C-29), 109.9 (C-20), 80.9 (C-3), 62.7 (C-28), 55.4, 53.4, 50.3, 48.8, 47.7, 46.4, 42.7, 40.9, 38.4, 37.8, 37.6, 37.0, 34.6, 34.1, 33.4, 33.0, 32.0, 29.8, 29.6, 27.9, 27.0, 25.1, 23.7, 23.6, 21.3, 20.8, 19.1, 18.2, 16.5, 16.2, 16.0, 14.7; HR-ESI-MS *m/z* calculated for $C_{37}H_{59}BrO_4Na$ $[M+Na]^+$ 669.3494, found 669.3447.

3β-(5-bromopentanoyl)oxylup-20(29)-en-28-oic acid (6). A solution of betulinic acid (500 mg, 1.09 mmol) in DCM (20 mL) was stirred. EDC (527 mg, 2.73 mmol), DMAP (54 mg, 0.44 mmol) and 5-bromovaleric acid (817 mg, 4.36 mmol) were added successively, and the mixture was stirred at 25°C until the reaction was complete based on TLC detection. The solvent was removed under diminished pressure, and the residue was purified by column chromatography using 30:3:1 PE /EtOAc/formic acid to obtain 250 mg (50%) of compound **6** as a white powder.

Data for compound **6**: Mp: 260-267℃; $[\alpha]_D^{20}$ = +39.3 (c 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz): δ4.74 (s, 1H, H-29a), 4.61 (s, 1H, H-29b), 4.48 (dd, *J* = 10.6, 5.8 Hz, 1H, H-3), 3.42 (dt, *J* = 13.3, 6.6 Hz, 2H, CH_2Br-), 3.00 (td, *J* = 10.8, 5.0 Hz, 1H,

H-19), 2.34 (t, *J* = 6.0 Hz, 2H, -OCOCH$_2$-), 2.35-1.18 (m, 30H, CH, CH$_2$ in pentacyclic skeleton or carbon chain), 1.69, 0.97, 0.94, 0.85, 0.84, 0.83 (each, s, 3H, -CH$_3$); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 180.2 (C-28), 172.9 (CH$_2$CO-), 150.4 (C-20), 109.8 (C-29), 81.0 (C-3), 56.3, 55.4, 50.4, 49.3, 46.9, 42.5, 40.7, 38.4, 37.9, 37.1, 37.0, 34.3, 33.8, 33.1, 32.1, 32.0, 30.5, 29.7, 28.0, 25.5, 23.8, 23.7, 20.9, 19.4, 18.2 16.6, 16.2, 16.0, 14.7; HR-ESI-MS *m/z* calculated for $C_{35}H_{54}BrO_4^-$ [M-H]$^-$ 617.3284, found 617.3083.

Methyl-3β-(5-bromopentanoyl)oxylup-20(29)-en-28-oate (7). A solution of compound **6** (300 mg, 0.48 mmol) in DMF (20 mL) was stirred. Then, CH_3I (127.5 μL, 2.04 mmol) and K_2CO_3 (284.5 mg, 2.04 mmol) were added, and the mixture was stirred at 25°C until the reaction was complete based on TLC detection. The reaction solution was extracted by DCM (3×3 mL) and ice cold water to remove DMF and K_2CO_3. The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography using 25:1 PE/EtOAc to obtain 200 mg (67%) of compound **7** as a white solid.

Data for compound **7**: Mp: 180-185℃; $[\alpha]_D^{20}$ = +9.7 (c 0.1, CH_3OH); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 4.73 (s, 1H, H-29a), 4.60 (s, 1H, H-29b), 4.48 (dd, J = 10.2, 6.1 Hz, 1H, H-3), 3.67 (s, 3H, CH_3O-), 3.41 (t, *J* = 6.6 Hz, 2H, CH_2Br-), 2.99 (td, *J* = 10.4, 4.2 Hz, 1H, H-19), 2.34 (t, *J* = 6.0 Hz, 2H, CH_2CO-), 1.69, 0.96, 0.91, 0.84 (each, s, 3H, CH_3-25, 26, 27, 30), 0.83 (s, 6H, CH_3-23, 24), 2.23-1.26 (m, 28H, CH, CH_2 in pentacyclic skeleton or carbon chain); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 176.7 (C-28), 172.9 (CH$_2$CO-), 150.6 (C-20), 109.6 (C-29), 80.9 (C-3), 56.5, 55.4, 51.3, 50.5, 49.5,

47.0, 42.4, 40.7, 38.4, 38.2, 37.8, 37.1, 36.9, 34.2, 33.9, 33.1, 32.2, 31.9, 30.6, 29.7, 28.0, 25.5, 23.7, 22.4, 20.9, 19.4, 18.2, 16.6, 16.2, 15.9, 14.7; HR-ESI-MS *m/z* calculated for $C_{36}H_{57}BrO_4Na$ $[M+Na]^+$ 655.3338, found 655.3293.

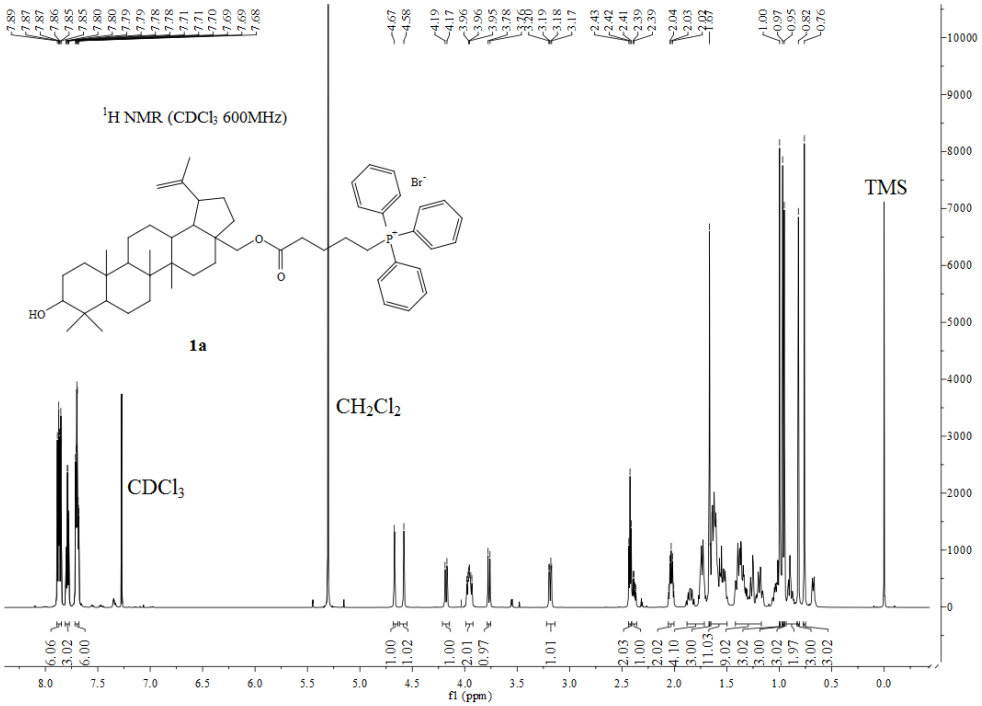
Ethyl -3β-(5-bromopentanoyl)oxylup-20(29)-en-28-oate (8). A solution of compound **6** (244 mg, 0.396 mmol) in DMF (10 mL) was stirred. K_2CO_3 (110 mg, 0.79 mmol) and bromoethane (117.9 μL, 1.58 mmol) were added successively, and the mixture was stirred at 25°C until the reaction was complete according to TLC detection. The reaction solution was extracted by DCM (3×3 mL) and ice-cold water to remove DMF and K_2CO_3. The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography using 20:1 PE/EtOAc to obtain 116 mg (49%) of compound **8** as a white powder.

Data for compound **8**: Mp: 176-182℃; $[\alpha]_D^{20}$ = +7.4 (c 0.1, CH_3OH); ^1H NMR ($CDCl_3$, 600 MHz): δ 4.73 (s, 1H, H-29a), 4.60 (s, 1H, H-29b), 4.48 (dd, *J* = 10.4, 6.0 Hz, 1H, H-3), 4.19–4.09 (m, 2H, $CH_3\underline{CH_2}O$-), 3.41 (t, *J* = 6.6 Hz, 2H, CH_2Br-), 3.01 (td, *J* = 10.7, 4.5 Hz, 1H, H-19), 2.34 (t, *J* = 7.3 Hz, 2H, -CH_2CO-), 1.26 (s, 3H, $\underline{CH_3}CH_2O$-), 1.69, 0.96, 0.92, 0.84, 0.83, 0.83 (each, s, 3H, CH_3-23, 24, 25, 26, 27, 30), 2.25-0.87 (m, 28H, CH, CH_2 in pentacyclic skeleton or carbon chain); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 176.1 (C-28), 172.9 ($CH_2\underline{C}O$-), 150.7 (C-20), 109.6 (C-29), 80.9 (C-3), 59.8, 56.4, 55.4, 50.5, 49.4, 47.0, 42.4, 40.7, 38.4, 38.2, 37.8, 37.1, 37.0, 34.3, 33.8, 33.1, 32.2, 32.0, 30.6, 29.6, 28.0, 25.5, 23.7, 23.7, 20.9, 19.4, 18.2, 16.6, 16.2, 15.9, 14.17, 14.4; HR-ESI-MS *m/z* calculated for $C_{37}H_{59}BrO_4Na$ $[M+Na]^+$ 669.3494, found 669.3453.

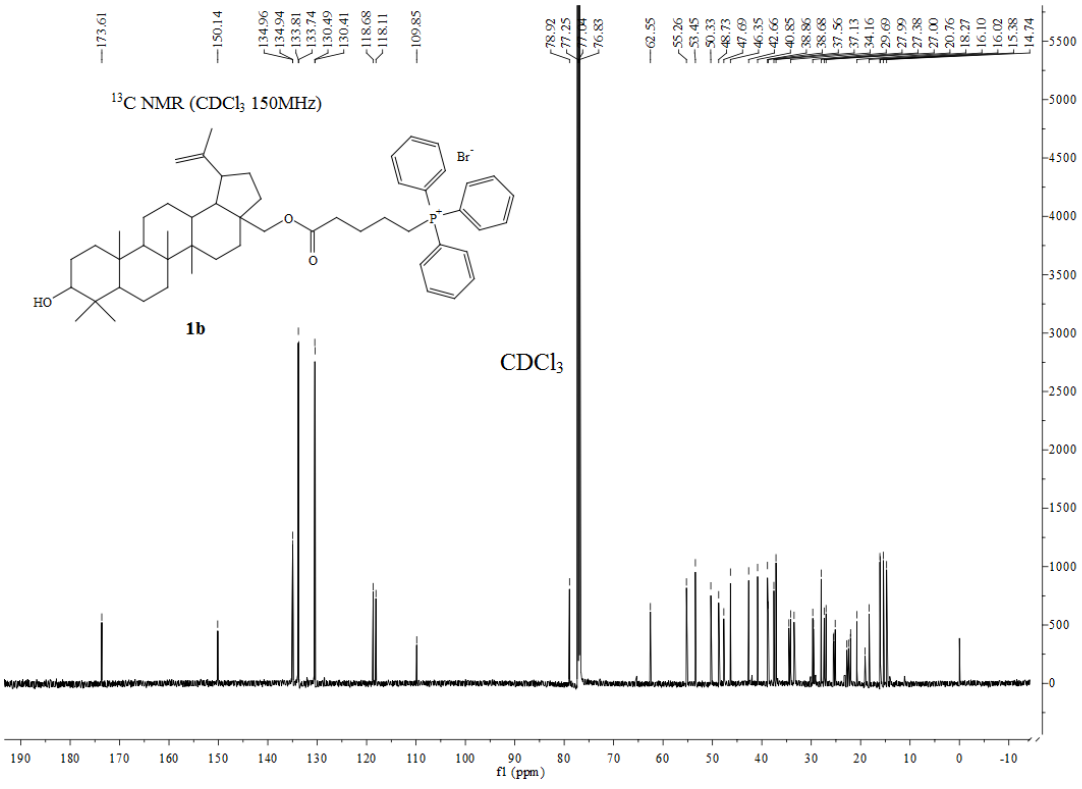
4-carboxybutyl-triphenylphosphonium bromide (9). Triphenylphosphine (865 mg, 3.3 mmol) and 5-bromovaleric acid (200 mg, 1.1 mmol) were added to 5 mL MeCN. The mixture was then stirred at 80°C until the reaction was complete according to TLC detection. The solvent was subsequently removed under diminished pressure, and the residue was purified by preparative TLC by elution with DCM: MEOH (10:1) to obtain 100 mg (50%) of **9** as a white powder.

Data for compound **9**: ^{1}H NMR (CD_3OD, 400 MHz): δ 7.82–7.60 (m, 15H, Ph-H), 3.36–3.27 (m, 2H, H-1), 2.14 (t, *J* = 7.0 Hz, 2H, H-4), 1.72 (dt, *J* = 14.2, 7.0 Hz, 2H, H-3), 1.66–1.56 (m, 2H, H-2).

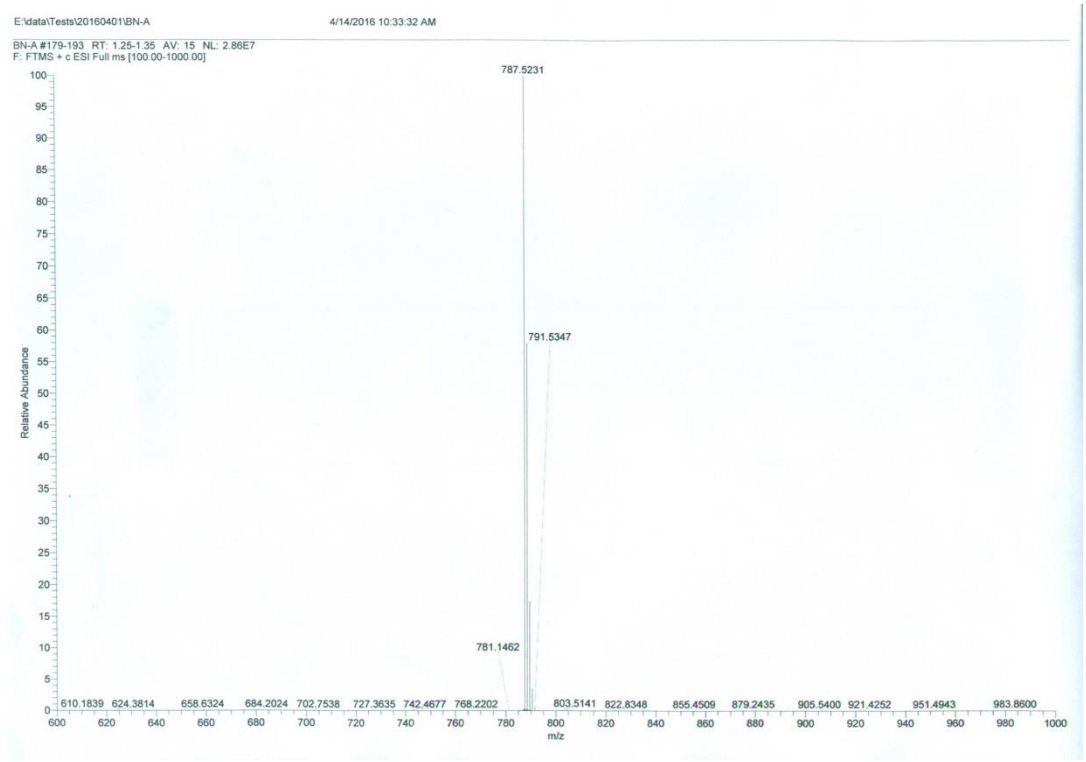
NMR, HR-ESI-MS Spectra and HPLC Chromatograms for Final Compounds



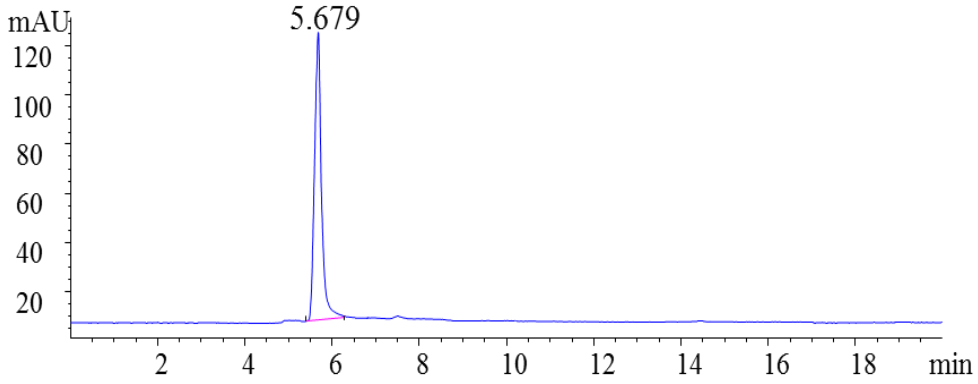
^{1}H NMR spectrum of **1a** in $CDCl_3$



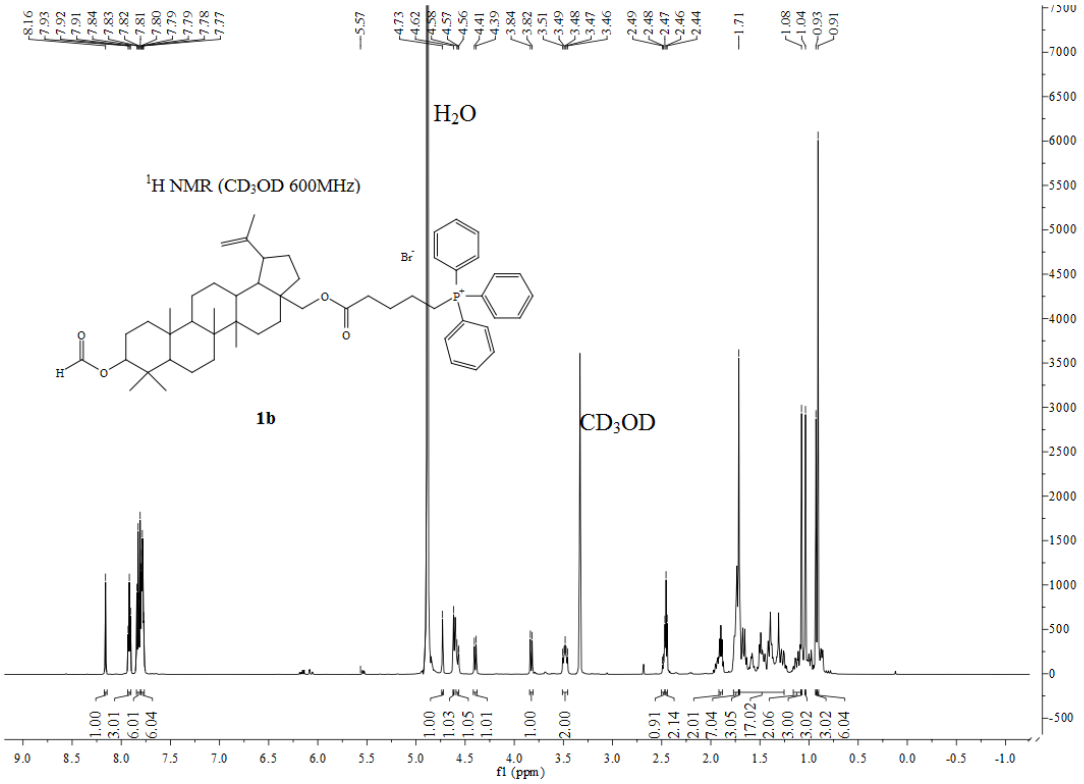
^{13}C NMR spectrum of **1a** in $CDCl_3$



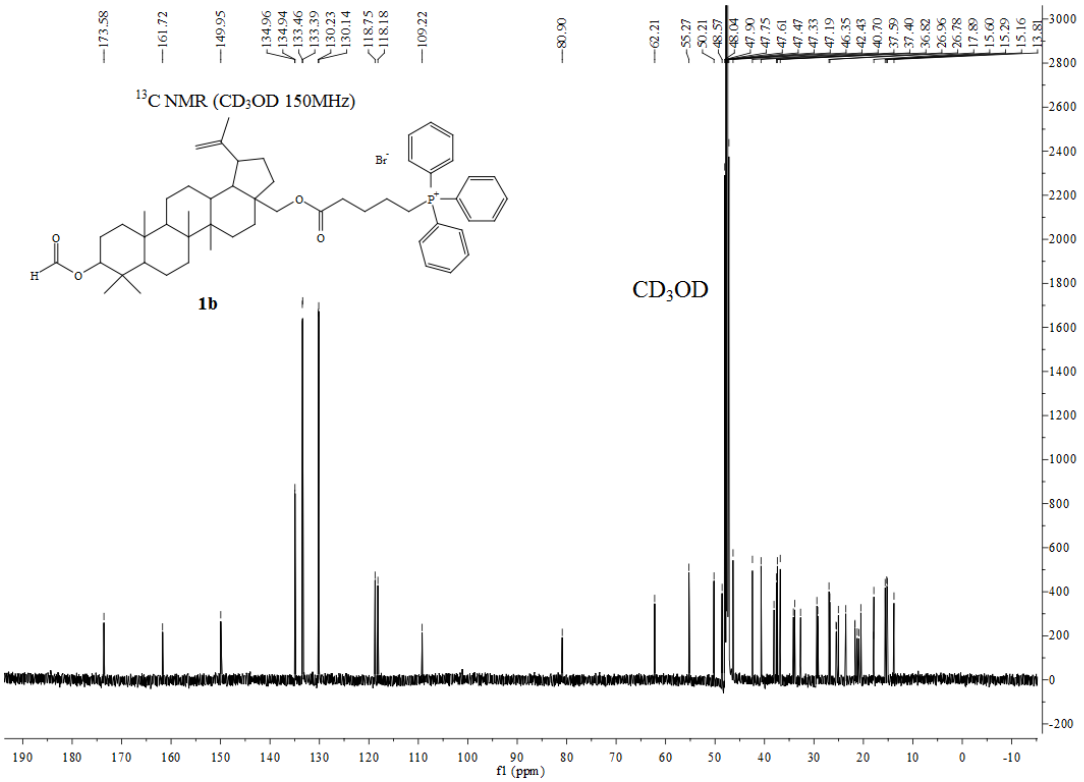
HR-ESI-MS spectrum of **1a**



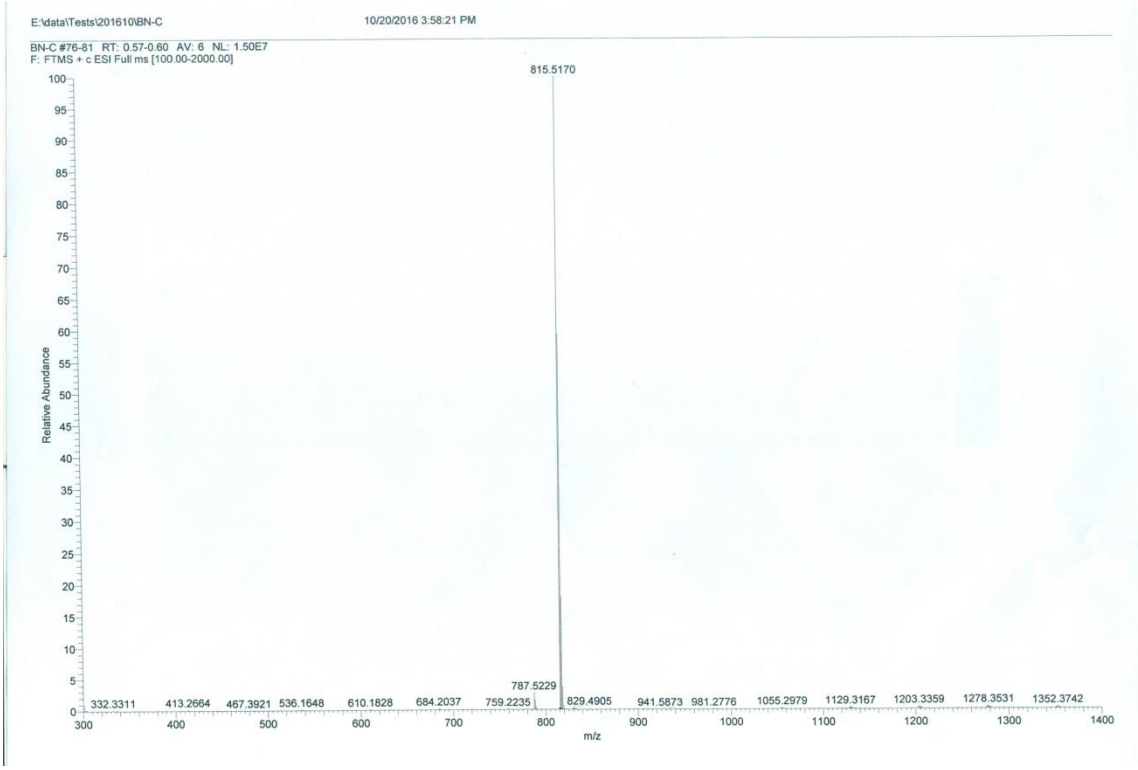
HPLC chromatogram of **1a**



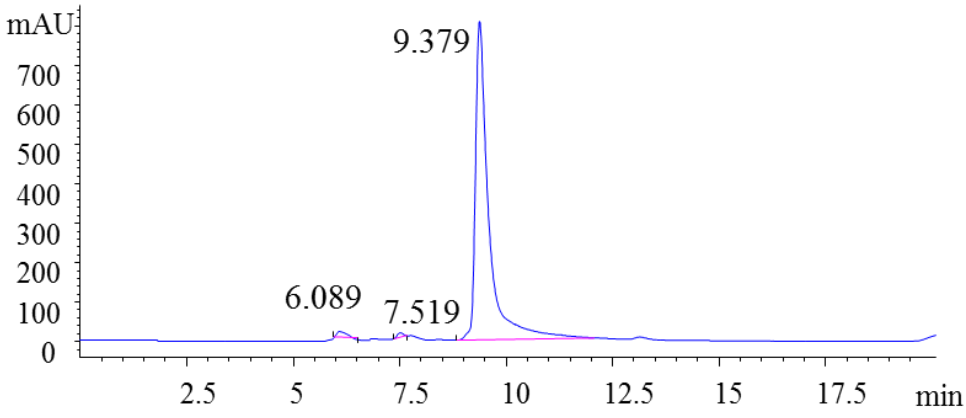
^{1}H NMR spectrum of **1b** in CD_3OD



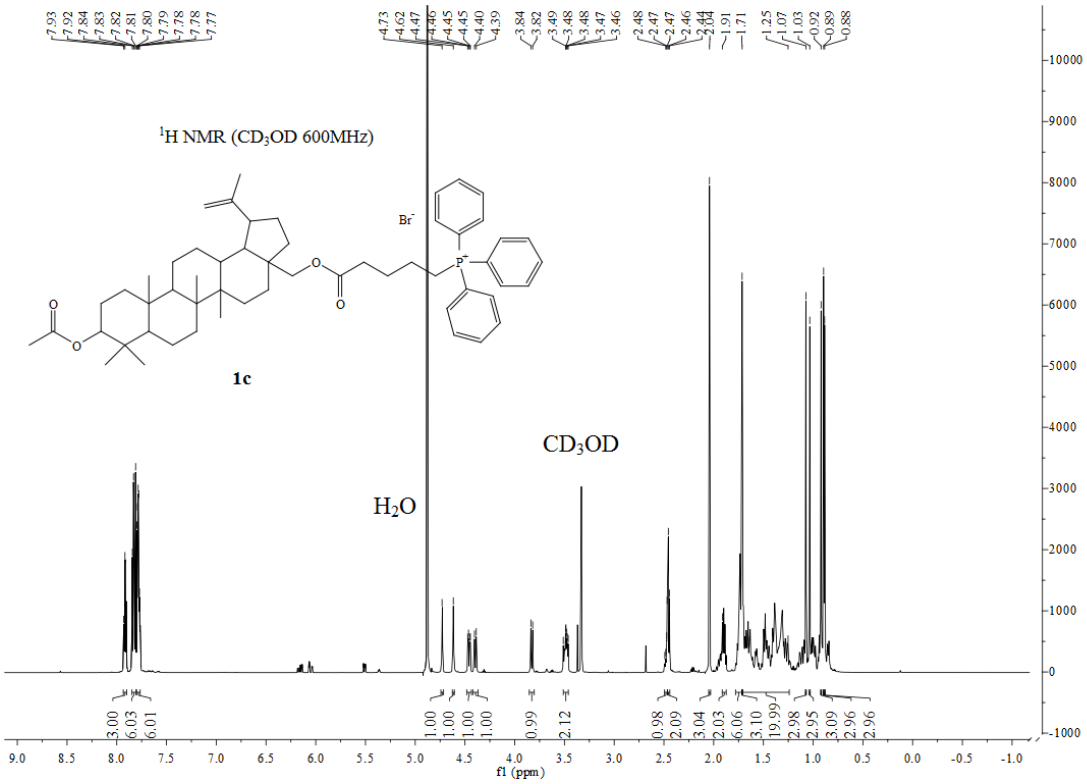
^{13}C NMR spectrum of **1b** in CD_3OD



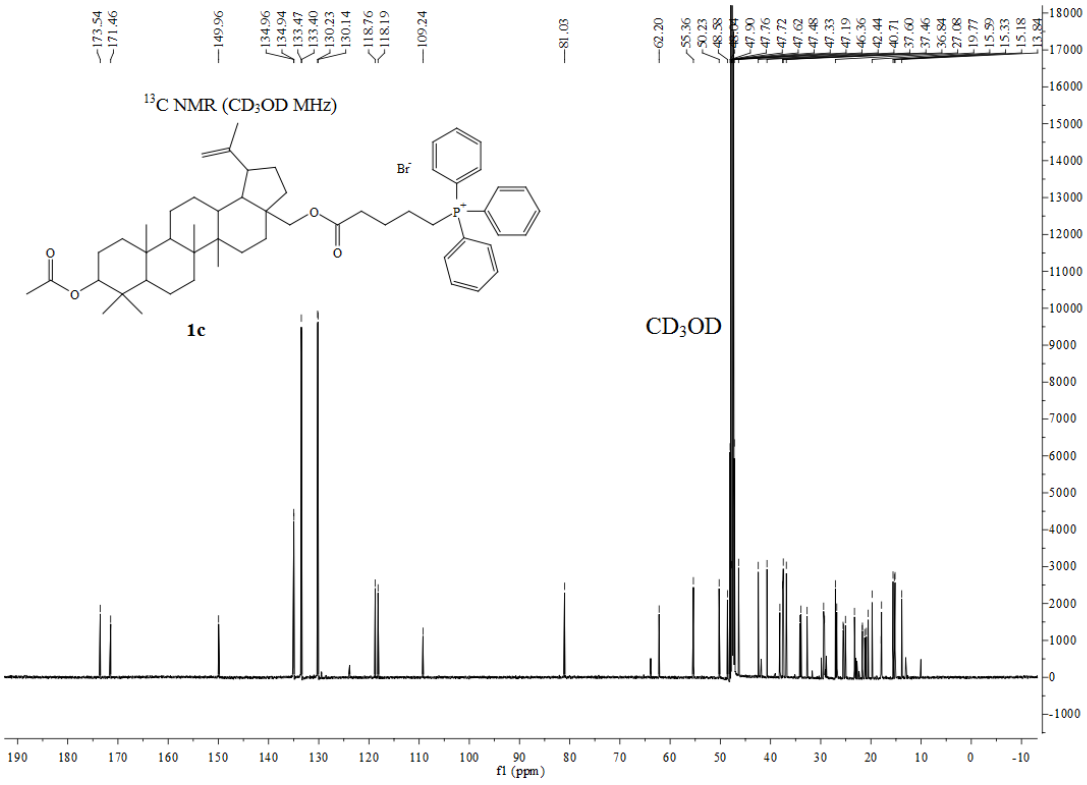
HR-ESI-MS spectrum of **1b**



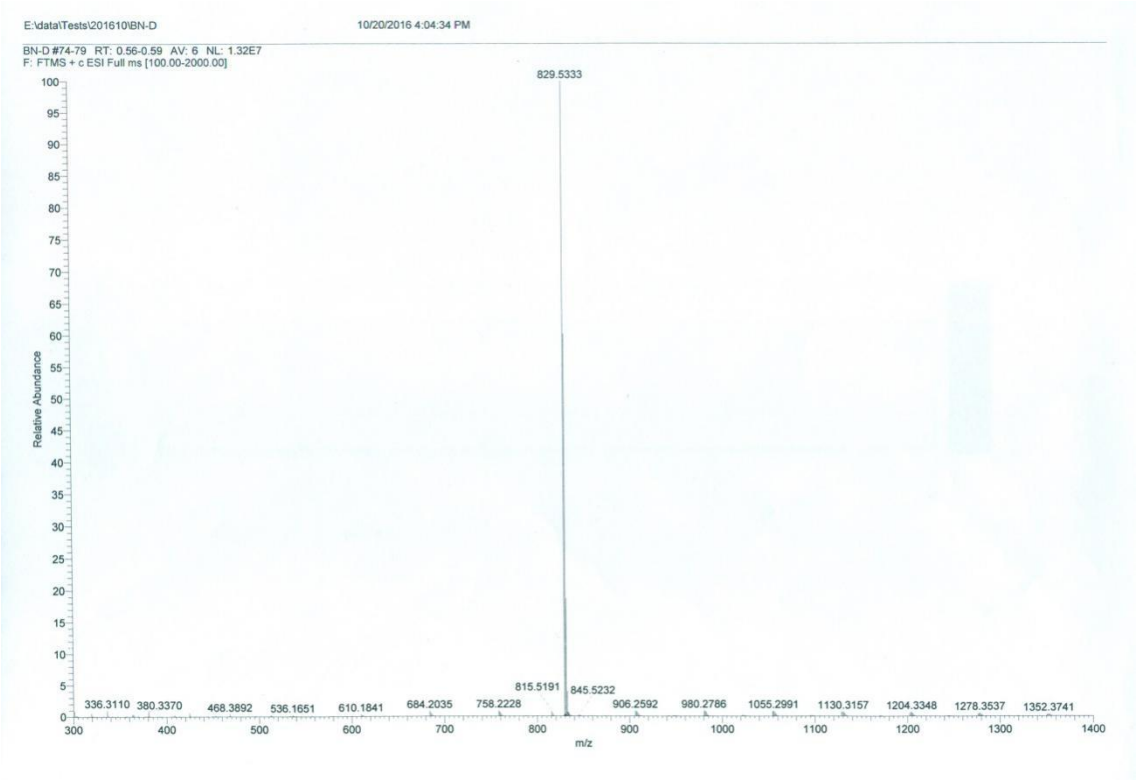
HPLC chromatogram of **1b**



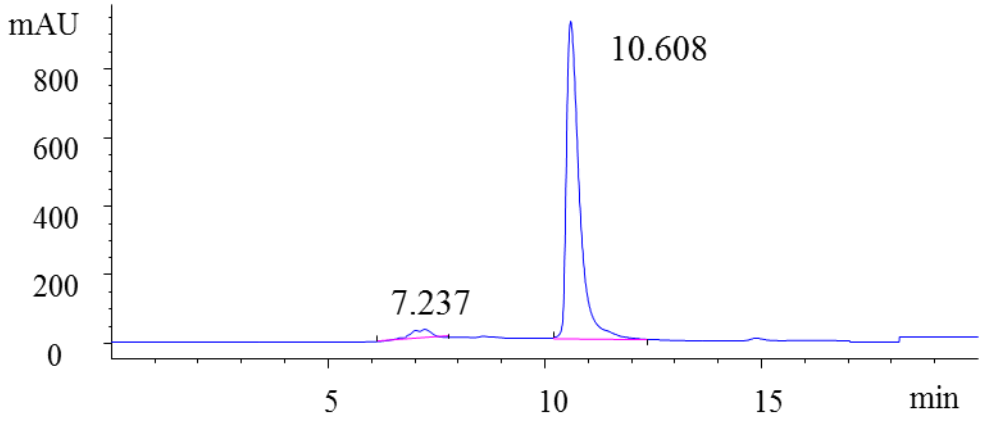
^{1}H NMR spectrum of **1c** in CD$_3$OD



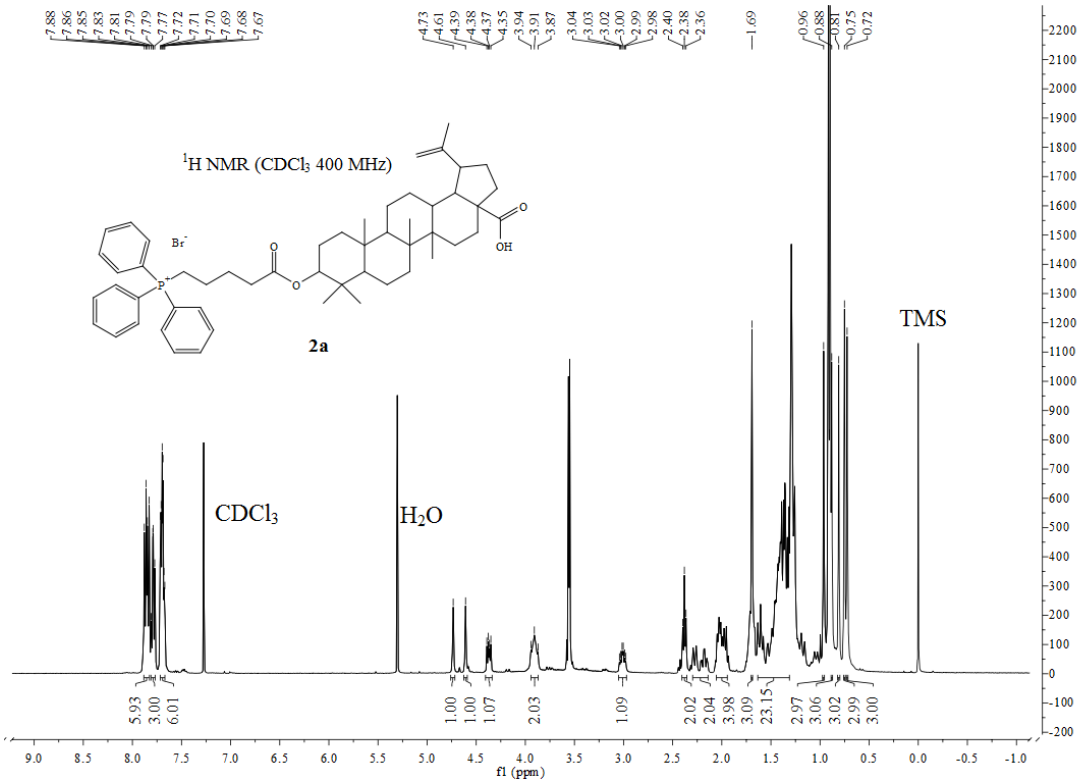
^{13}C NMR spectrum of **1c** in CD$_3$OD



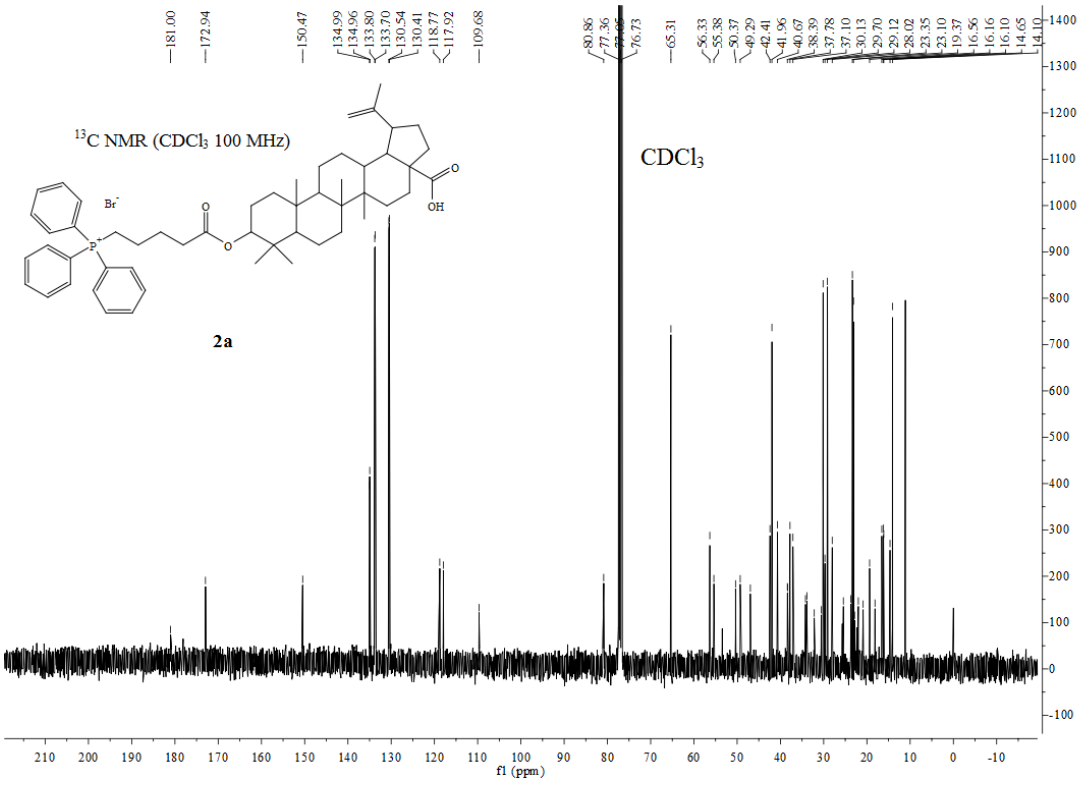
HR-ESI-MS spectrum of **1c**



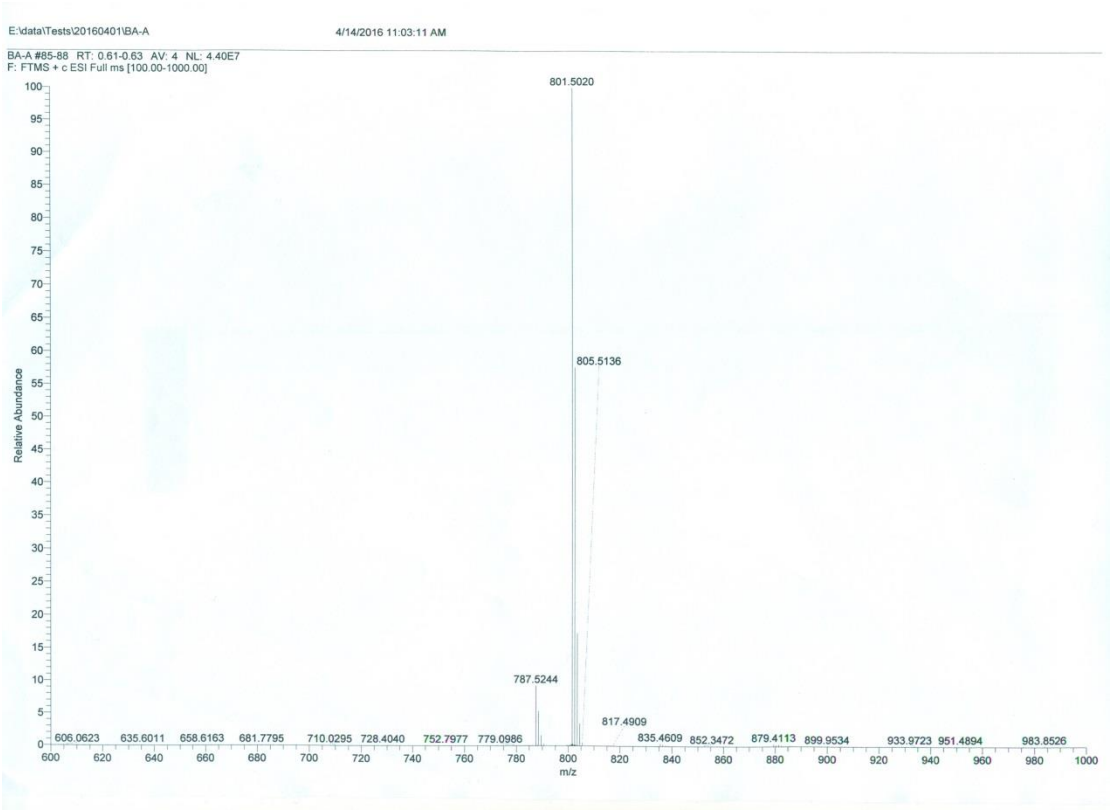
HPLC chromatogram of **1c**



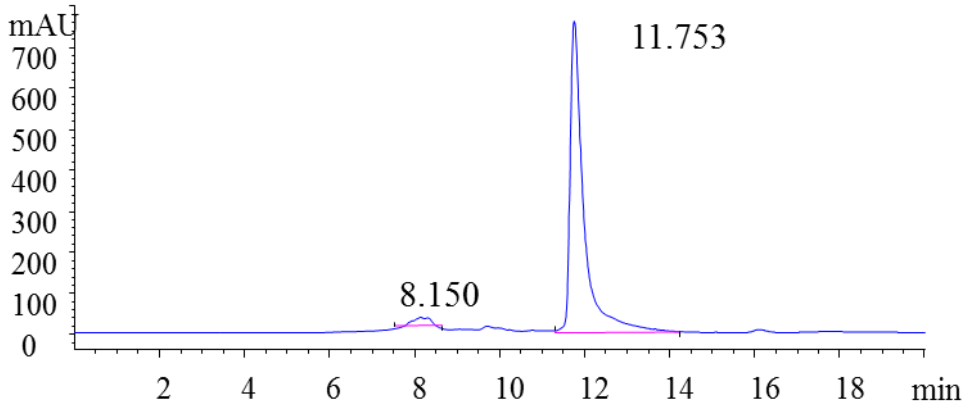
^{1}H NMR spectrum of **2a** in $CDCl_3$



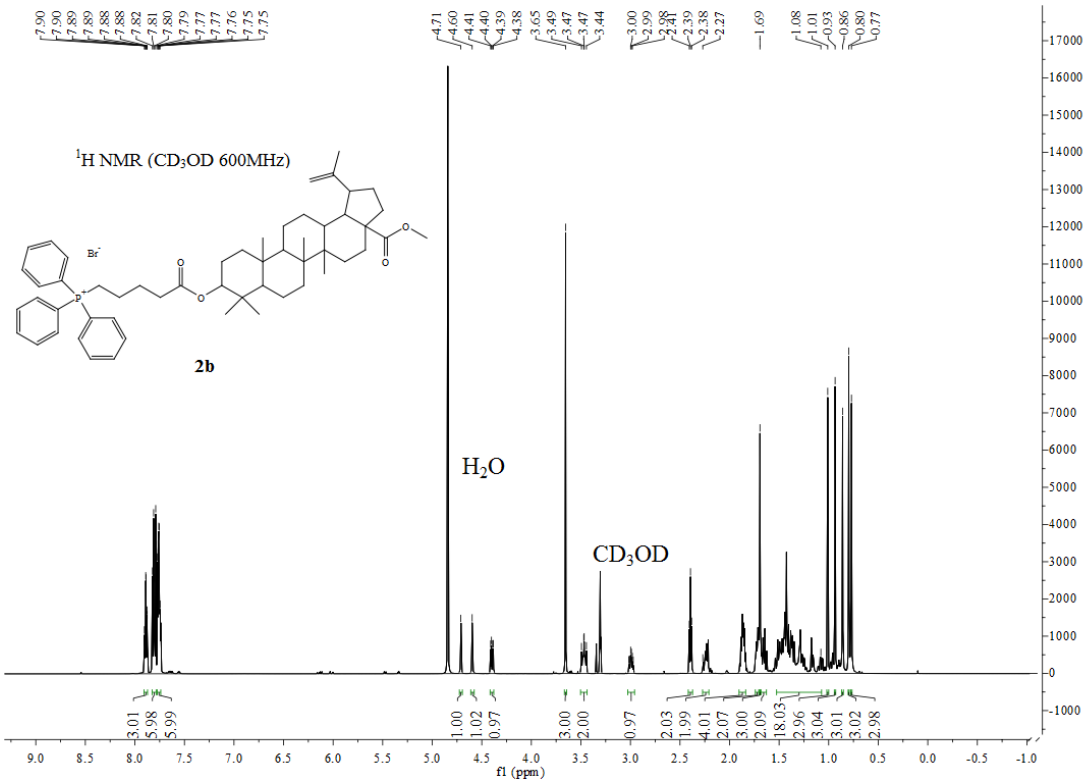
^{13}C NMR spectrum of **2a** in $CDCl_3$



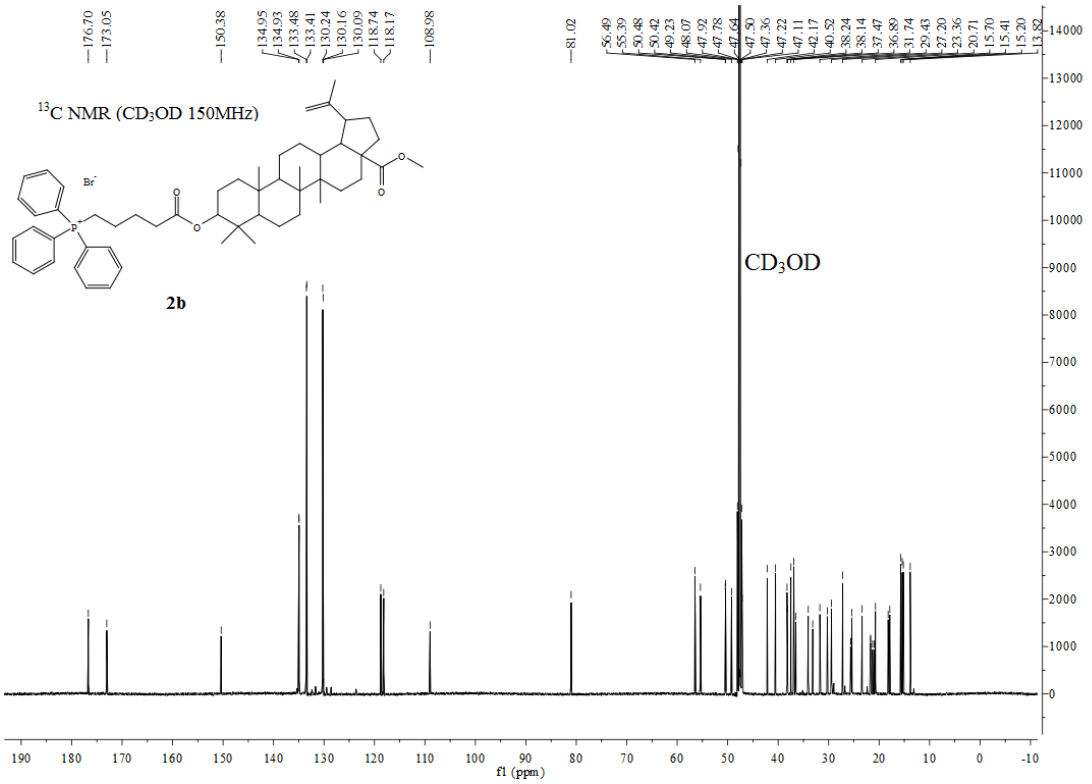
HR-ESI-MS spectrum of **2a**



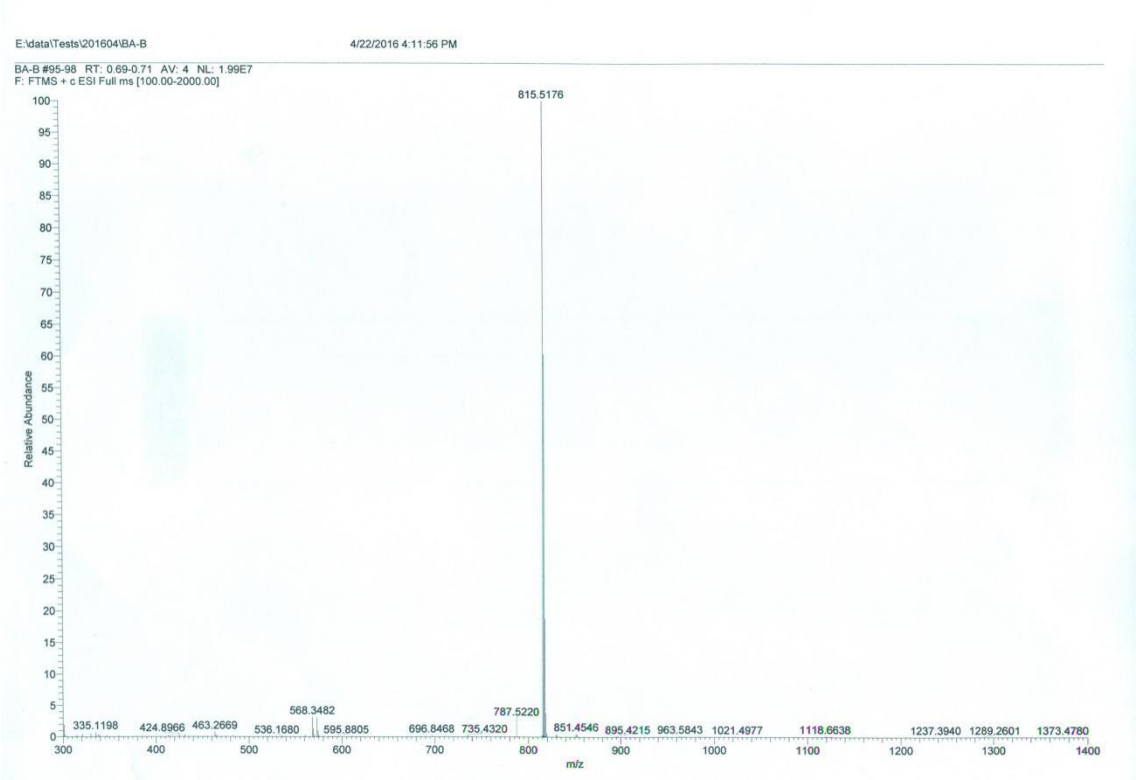
HPLC chromatogram of **2a**



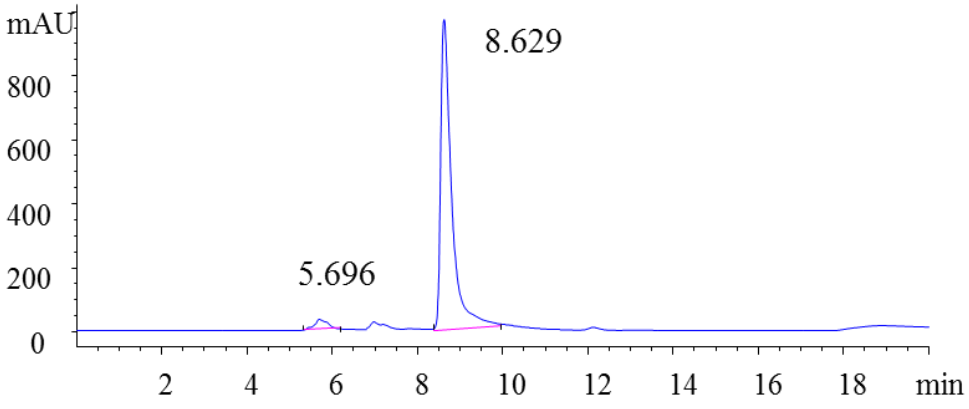
^{1}H NMR spectrum of **2b** in CD$_3$OD



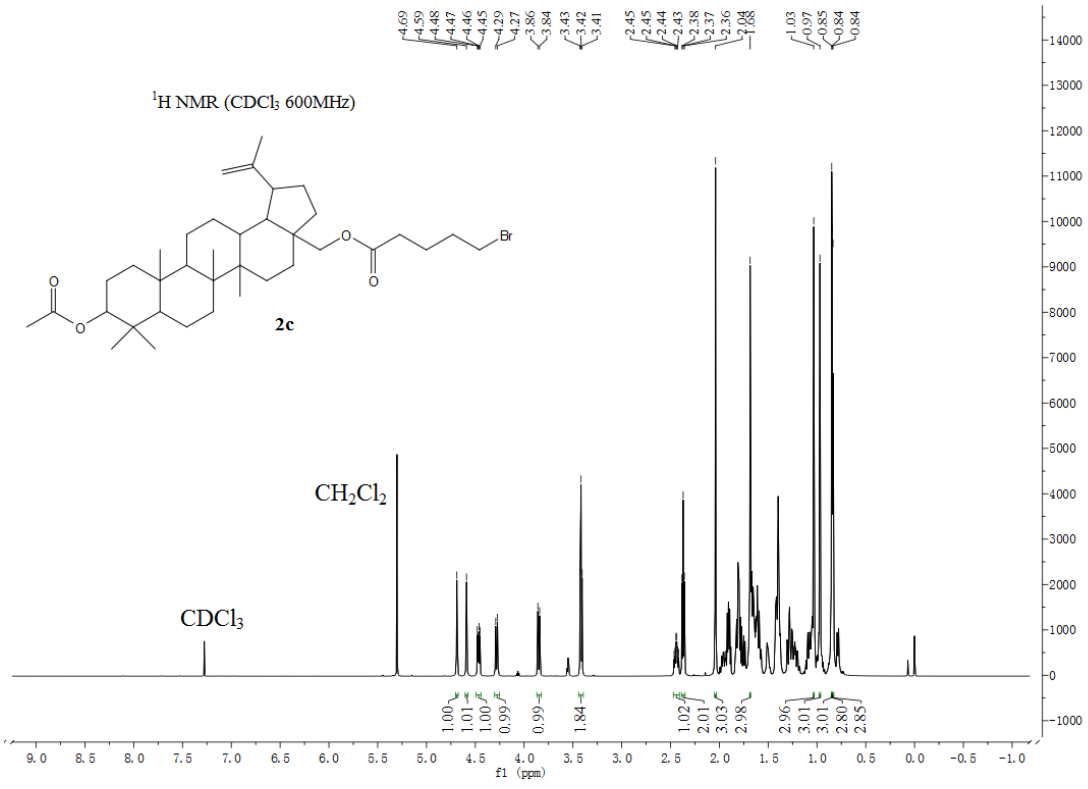
^{13}C NMR spectrum of **2b** in CD$_3$OD



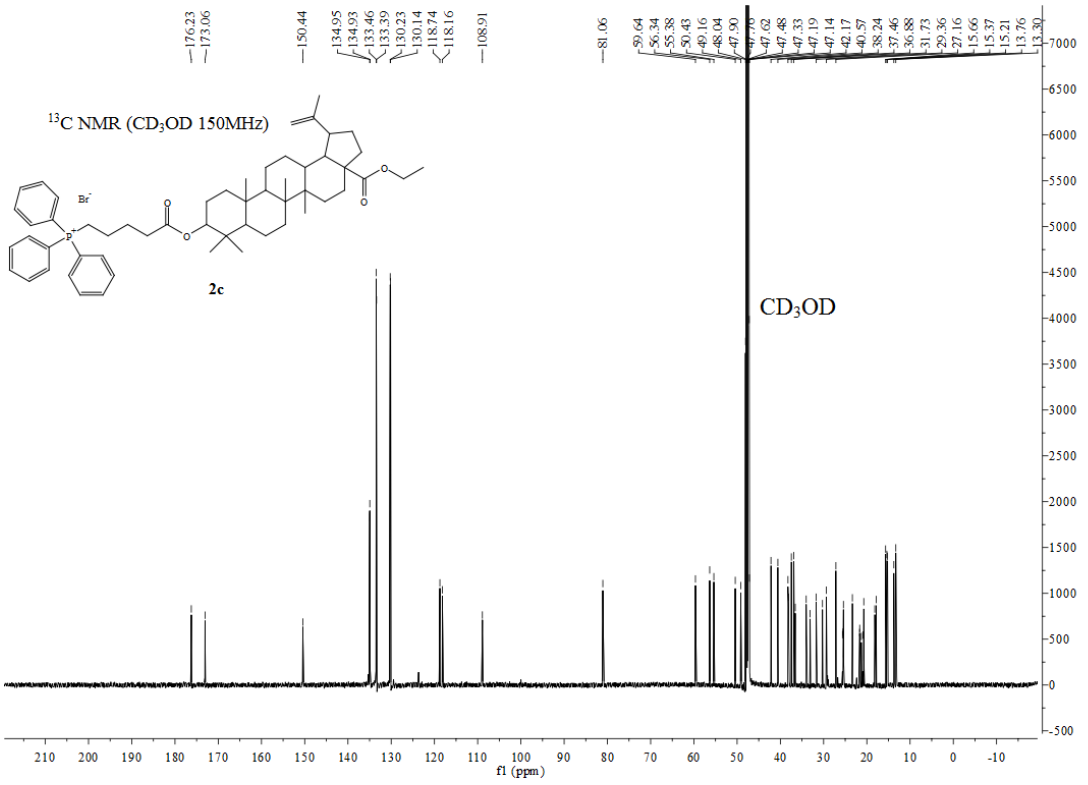
HR-ESI-MS spectrum of **2b**



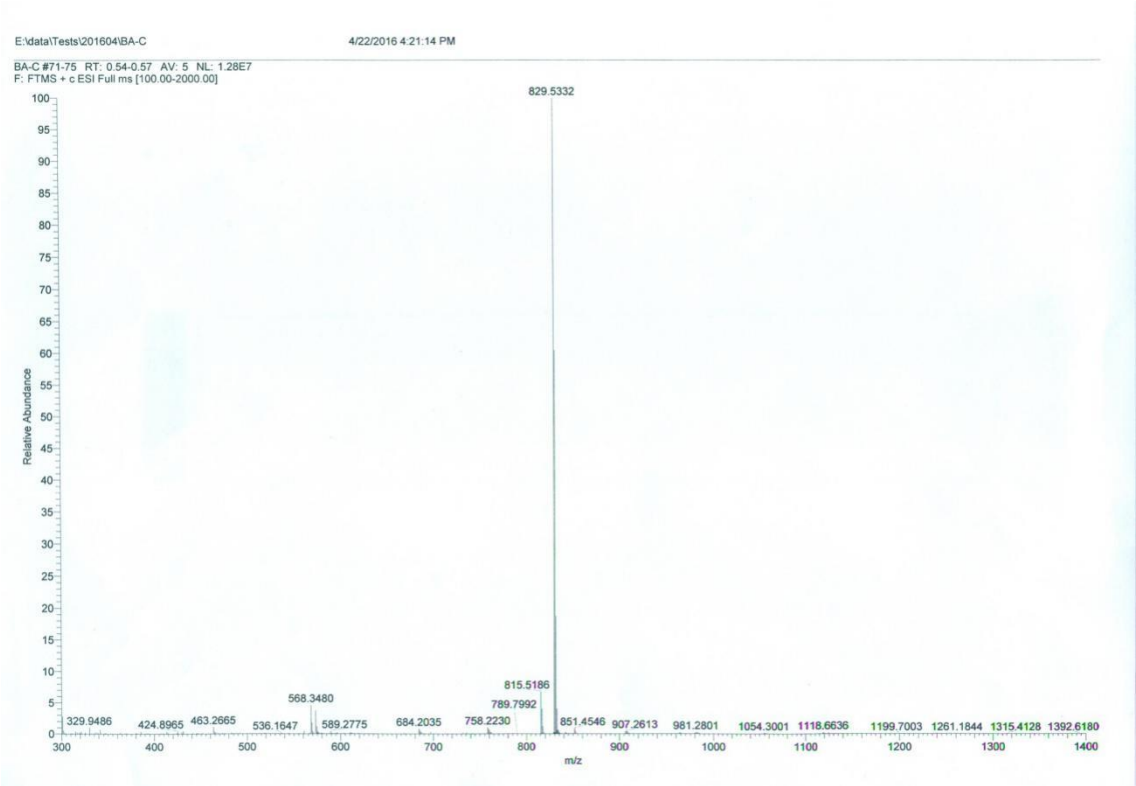
HPLC chromatogram of **2b**



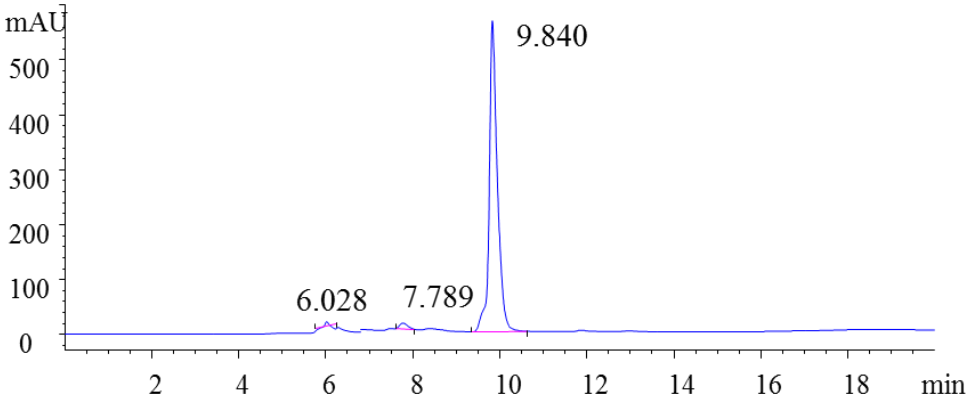
^{1}H NMR spectrum of **2c** in CD_3OD



^{13}C NMR spectrum of **2c** in CD_3OD



HR-ESI-MS spectrum of **2c**



HPLC chromatogram of **2c**

Standard curves of 1 and 1a