

Supplementary Material

Oleanolic acid acetate exerts anti-inflammatory activity via IKK α / β suppression in TLR3-mediated NF- κ B activation

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Experimental

General procedure

^1H , ^{13}C , DEPT-90, DEPT-135 (600, and 150 MHz), and 2D NMR (COSY, HMQC, HMBC) spectroscopic data were recorded on a JEOL JNM-ECA600 spectrometer with CDCl_3 as a solvent. The chemical formula of biotinylated oleanolic acid 3-acetate (biotin-OAA) was determined on the basis of HR-ESI-MS data using a Waters SYNAPT G2-Si HDMS spectrometer (Waters, Milford, MA, USA).

Synthetic Procedure for biotin labeling of OAA

We used the following reagents for biotinylation of OAA. (a) $(\text{COCl})_2$, CH_2Cl_2 ; (b) $\text{H}_2\text{N}(\text{CH}_2)_6\text{NHBoc}\cdot\text{HCl}$, NaHCO_3 , PhH, water; (c) TFA, CH_2Cl_2 ; and (d) Biotin, EDC, DMAP, DMF. A mixture of OAA (3.0 g, 6.0 mmol) and oxalyl chloride (5 mL) was stirred at room temperature overnight. The solvent was removed *in vacuo*, and the residue was coevaporated with benzene (three times) to produce compound **2**.

Synthesis of compound **3**: A solution of **2** (180 mg, 0.35 mmol) in benzene (10 mL) was added to a solution of 6-[(tert-butoxycarbonyl)amino]hexylamine hydrochloride (380 mg, 1.5 mmol) and NaHCO₃ (126 mg, 1.5 mmol) in water (4 mL). The mixture was stirred at room temperature overnight. The organic layer was separated and then diluted with EtOAc (20 mL). The organic extract was washed with water (three times) and then saturated with aqueous NaCl solution (three times), dried over anhydrous MgSO₄, and filtered. The solvent was removed from the filtrate in vacuo. The solid was purified by flash column chromatography [hexanes/EtOAc, 1:1, v/v] to produce **3**.

Synthesis of compound **4**: Trifluoroacetic acid (TFA, 1 mL) was added to solution **3** (100 mg, 0.14 mmol) in CH₂Cl₂ (10 mL). The mixture was stirred at room temperature for 1 h. The solvent was removed *in vacuo*, and the residue was coevaporated with benzene (three times) to produce **4**.

Synthesis of compound **5**: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 52 mg, 0.3 mmol) was added to a mixture of biotin (82 mg, 0.3 mmol), compound **4** (128 mg, 0.225 mmol) and 4-dimethylaminopyridine (DMAP, 41 mg, 0.3 mmol) in dimethylformamide (DMF). The mixture was stirred at room temperature overnight. The reaction mixture was partitioned between the ethyl acetate and water. The organic extract was washed with water (three times) and then saturated with aqueous

NaCl solution (three times), dried over anhydrous MgSO_4 , and filtered. The solvent was removed from the filtrate *in vacuo*. The solid was purified by fresh column chromatography [$\text{CH}_2\text{Cl}_2/\text{MeOH}$, 9:1, v/v] to produce **5**.

Biotin-OAA (5): White powder, $\text{C}_{48}\text{H}_{78}\text{N}_4\text{O}_5\text{S}$; ^1H NMR (600 MHz, CDCl_3) δ 6.26 (1H, br s, 1'-CONH), 6.23 (1H, br s, 7'-NH), 5.95 (1H, t, J = 5.4 Hz, 28-CONH), 5.42 (1H, s, 8'-NH), 5.34 (1H, t, J = 3.0 Hz, H-12), 4.49 (1H, m, H-8), 4.46 (1H, m, H-3), 4.29 (1H, dd, J = 6.6, 4.8 Hz, H-7'), 3.33 (1H, m, H-1a''), 3.19 (2H, m, H2-6''), 3.13 (1H, m, H-6'), 2.94 (1H, m, H-1b''), 2.88 (1H, dd, J = 12.6, 4.8 Hz, H-9a'), 2.72 (1H, d, J = 12.6 Hz, H-9b'), 2.48 (1H, dd, J = 13.2, 3.6 Hz, H-18), 2.19 (2H, t, J = 7.2 Hz, H2-2'), 2.02 (3H, s, OAc- CH_3), 1.96–1.00 (35H, m, overlap), 1.13 (3H, s, CH_3 -27), 0.92 (3H, s, CH_3 -25), 0.88 (3H, s, CH_3 -29), 0.87 (3H, s, CH_3 -30), 0.84 (3H, s, CH_3 -23), 0.83 (3H, s, CH_3 -24), 0.82 (1H, m, H-5, overlap), 0.73 (3H, s, CH_3 -26); ^{13}C NMR (150 MHz, CDCl_3) δ 178.5, 173.4, 171.2, 163.9, 145.2, 122.8, 81.0, 62.0, 60.4, 55.8, 55.4, 47.7, 47.0, 46.5, 42.5, 42.3, 40.8, 39.6, 39.3, 38.4, 37.9, 37.1, 36.2, 34.4, 33.2, 32.8, 32.5, 31.0, 29.6, 29.5, 28.4, 28.3, 28.2, 27.5, 26.5, 26.4, 26.0, 25.9, 24.0, 23.9, 23.8, 23.7, 21.6, 18.4, 17.2, 16.9, 15.7; HR-ESI-TOFMS: m/z 823.5755 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{48}\text{H}_{79}\text{N}_4\text{O}_5\text{S}$, 823.5766).

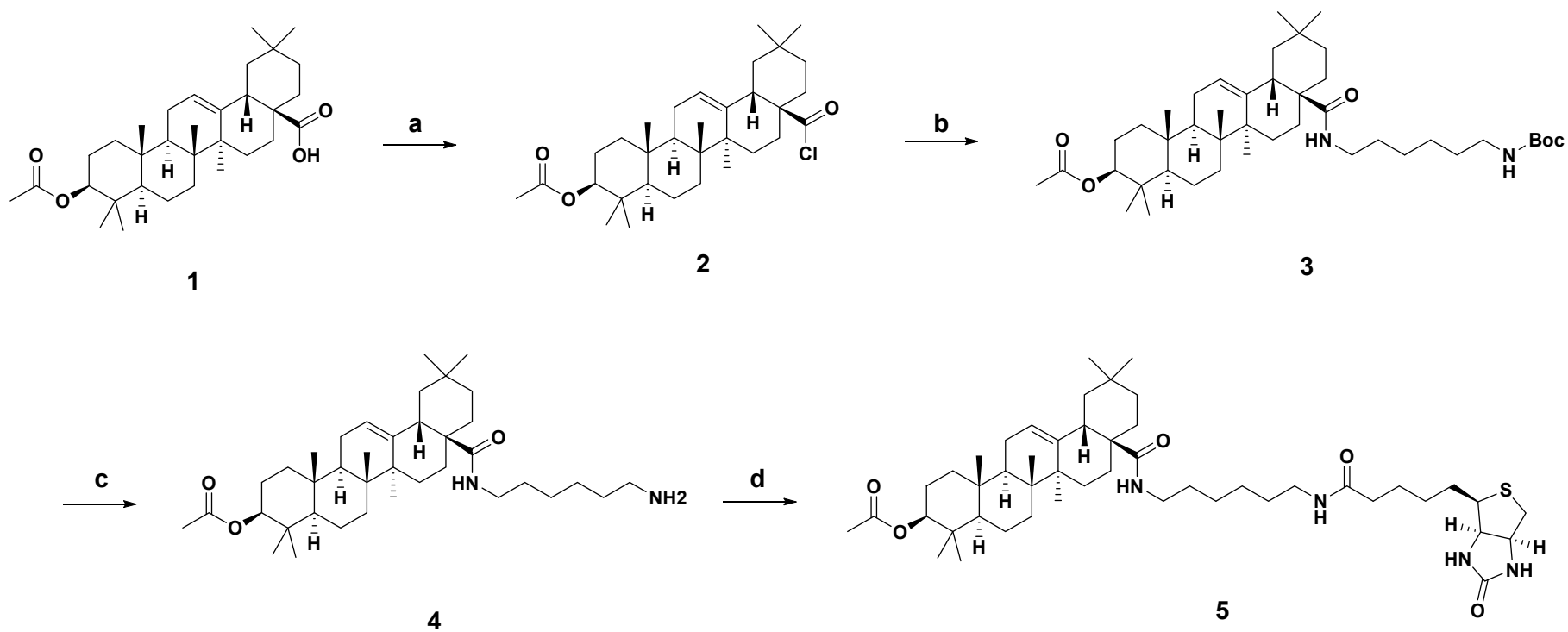
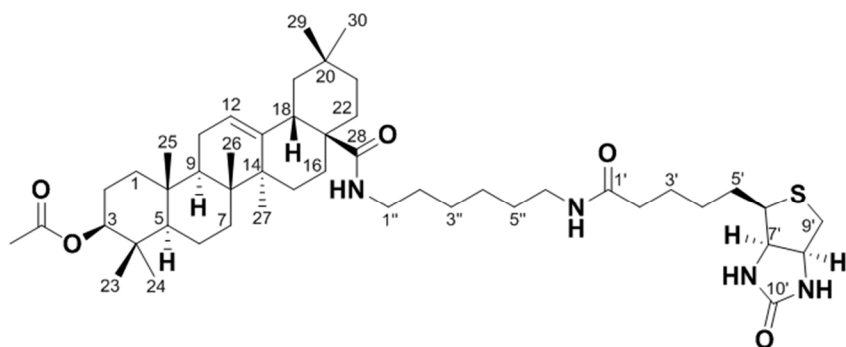


Figure S1. Scheme of OAA labeling with biotin.



Chemical Formula: $C_{48}H_{79}N_4O_5S^+$, $[M+H]^+$
(Calcd. 823.5766)

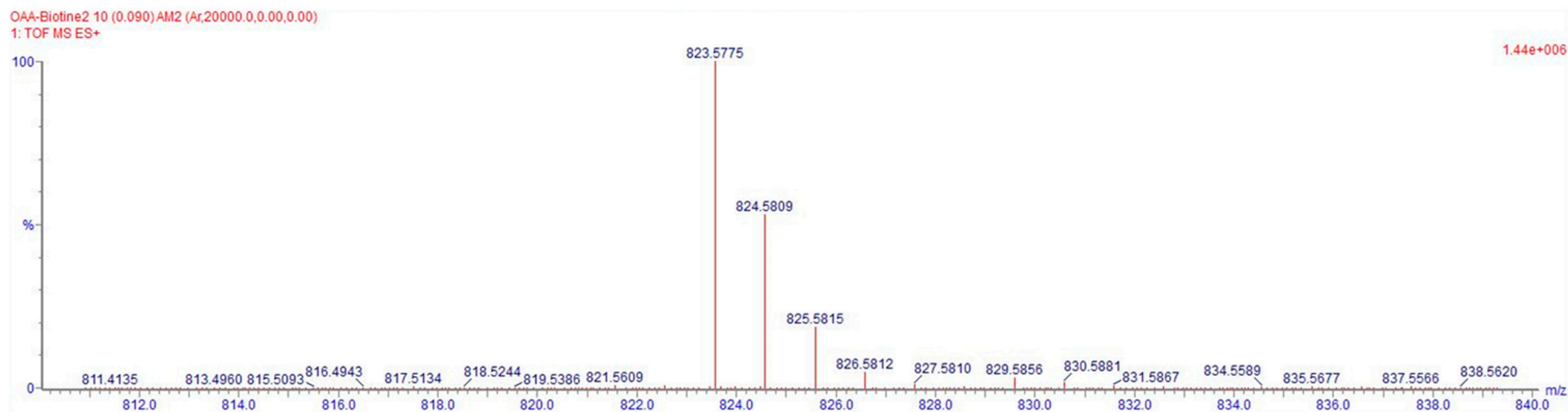


Figure S2. HR-ESI-MS spectrum of Biotin-OAA

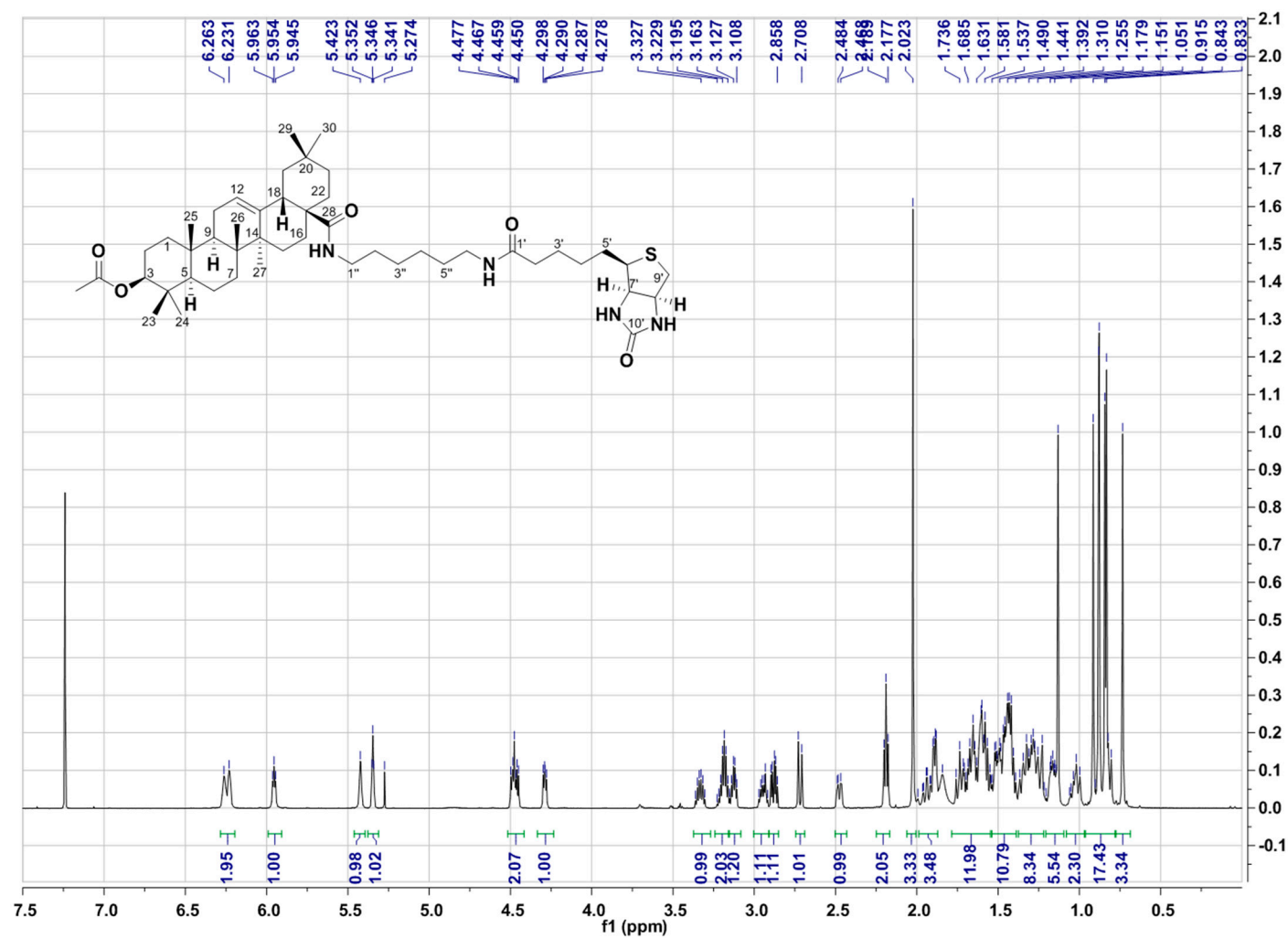


Figure S3. ¹H NMR spectrum of Biotin-OAA (CDCl₃, 600 MHz)

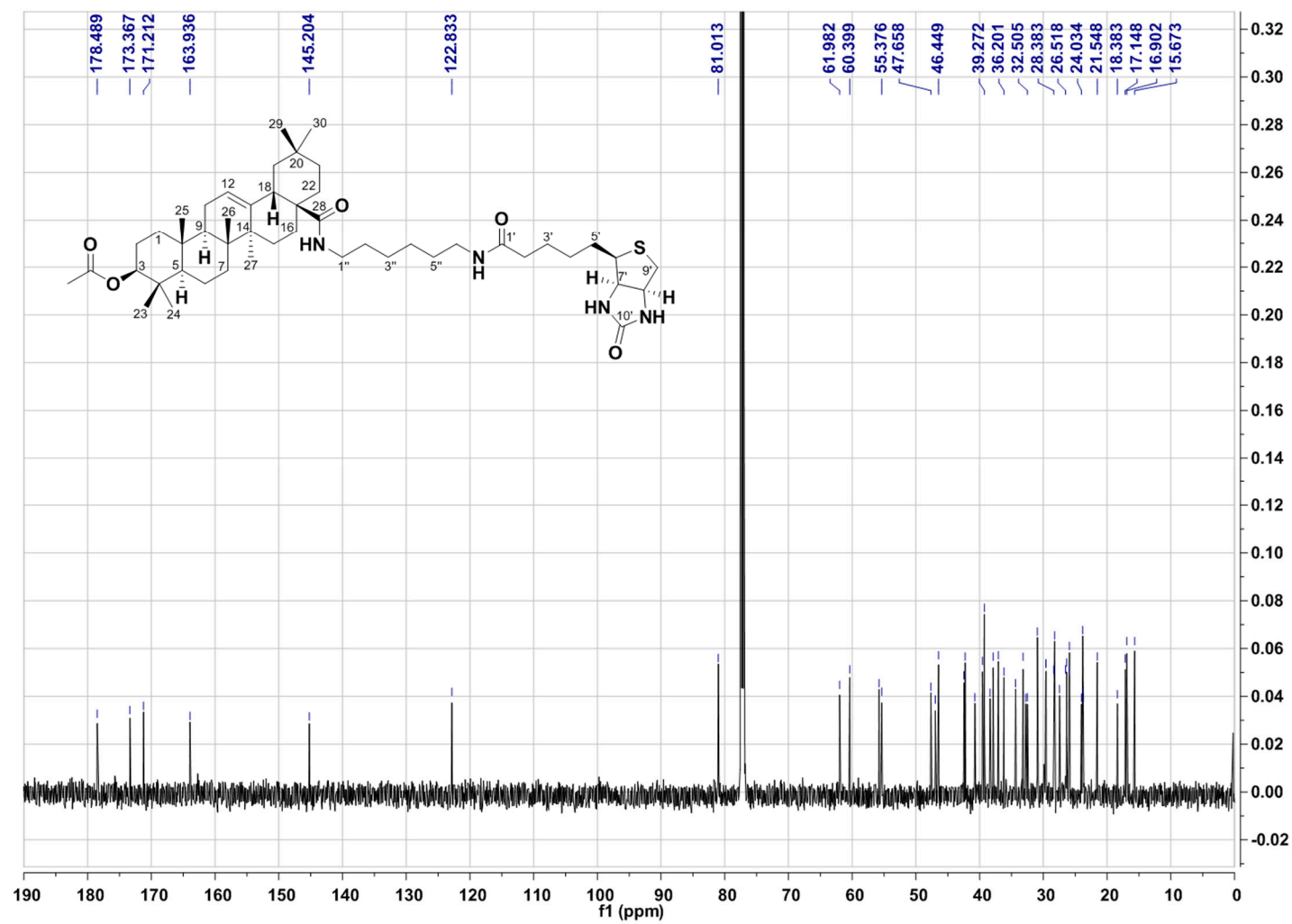


Figure S4. ^{13}C NMR spectrum of Biotin-OAA (CDCl_3 , 150 MHz)

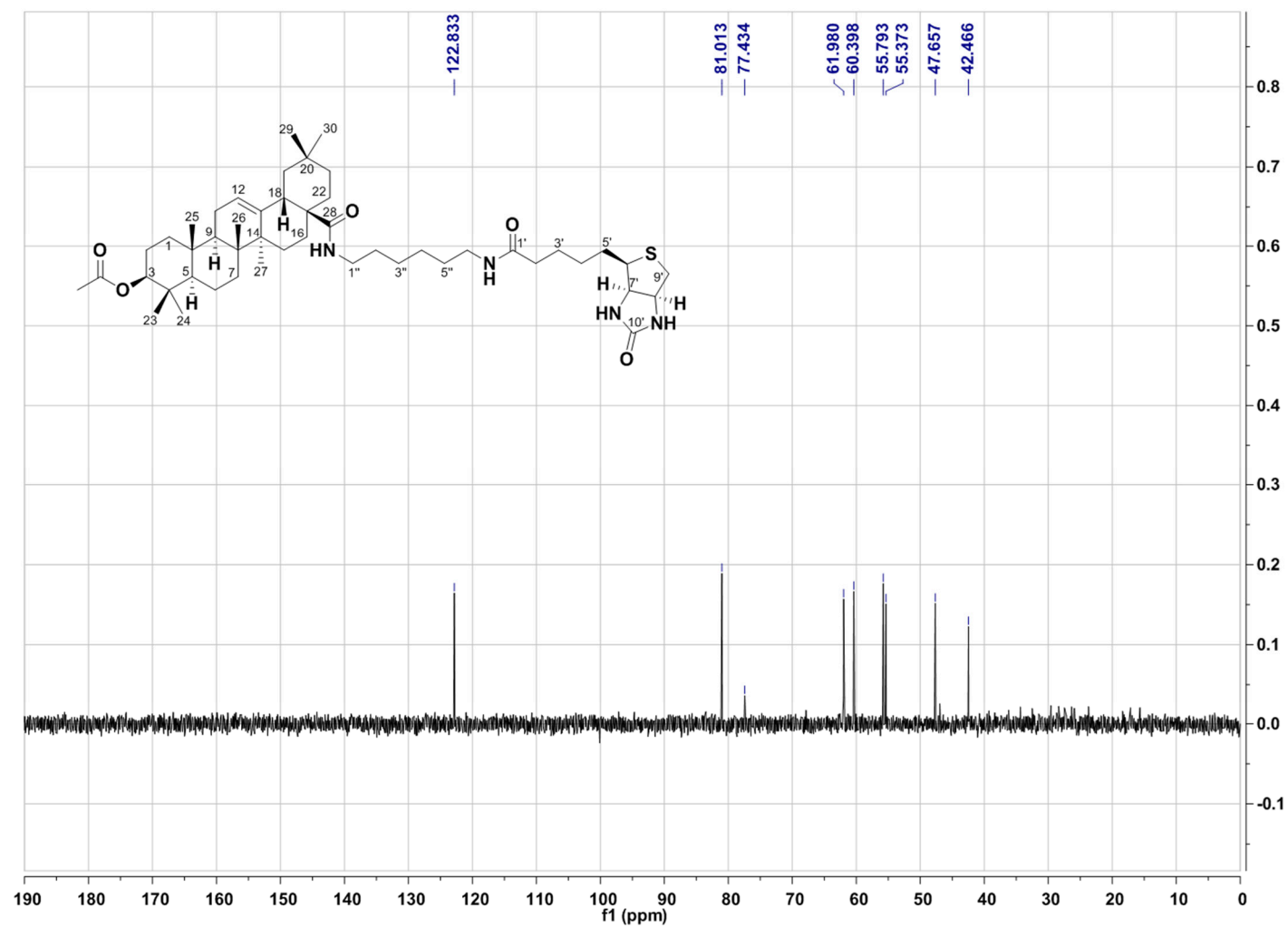


Figure S5. DEPT-90 spectrum of Biotin-OAA (CDCl_3 , 150 MHz)

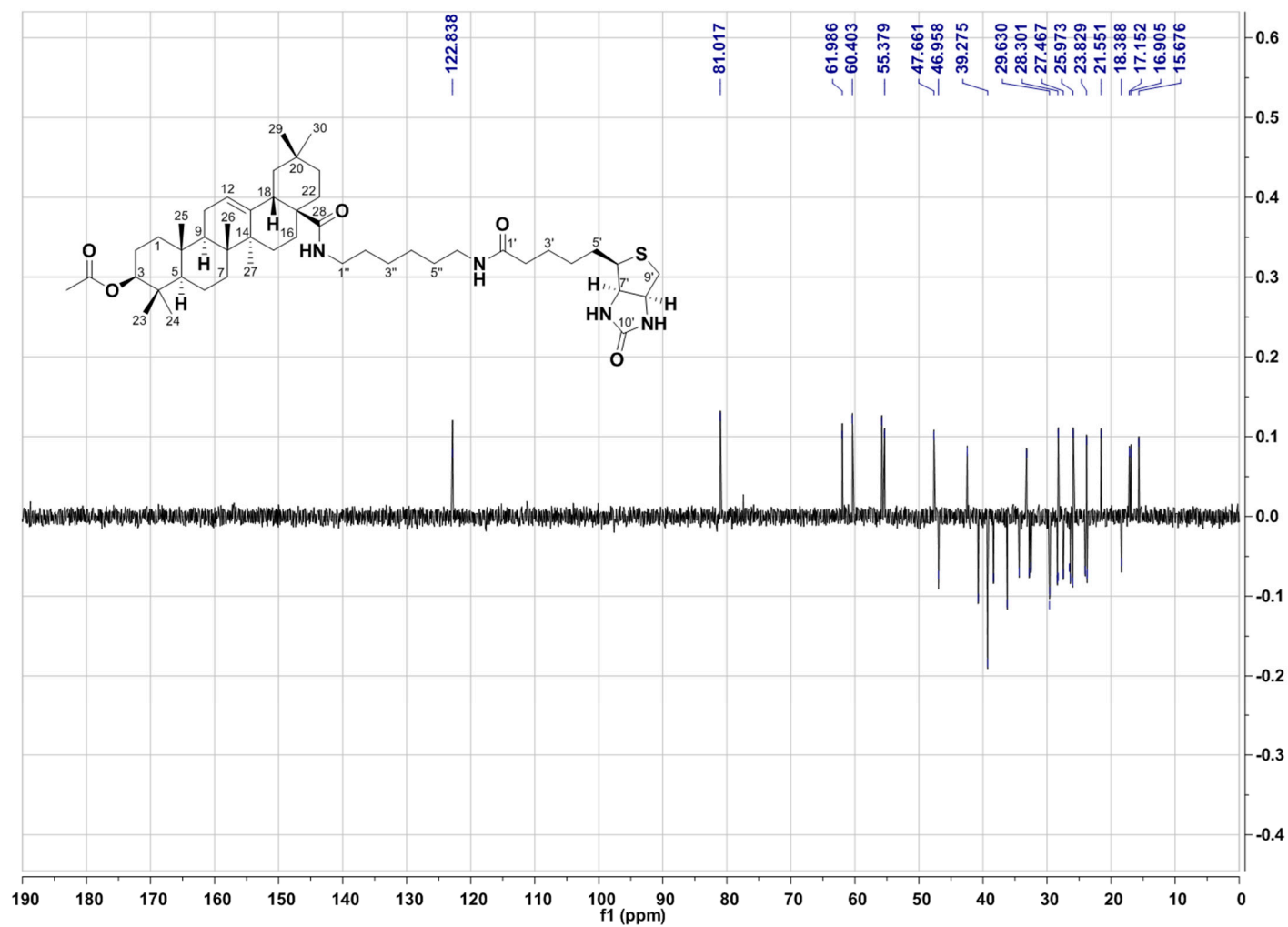


Figure S6. DEPT-135 spectrum of Biotin-OAA (CDCl_3 , 150 MHz)

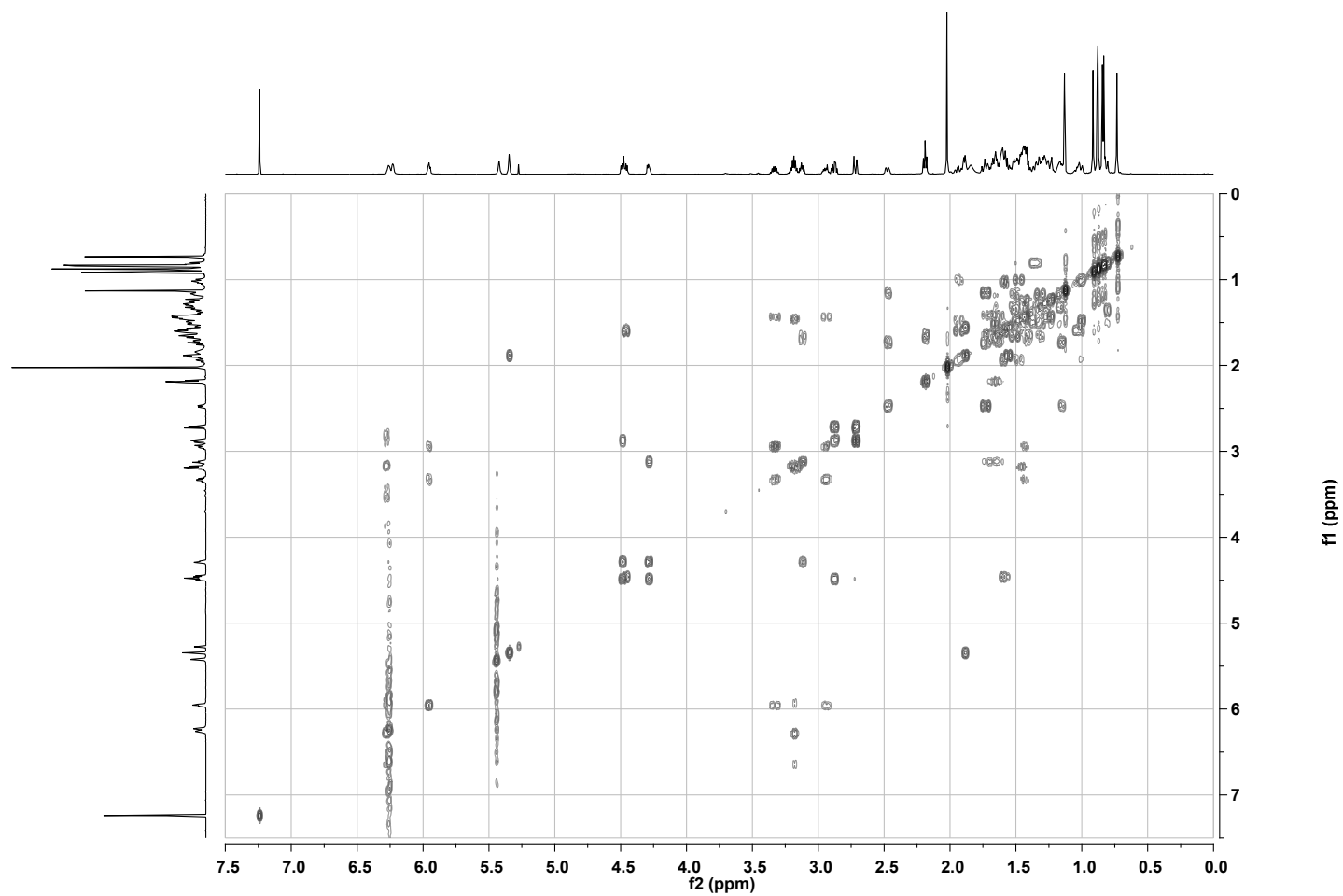


Figure S7. COSY spectrum of Biotin-OAA (CDCl_3 , 600 MHz)

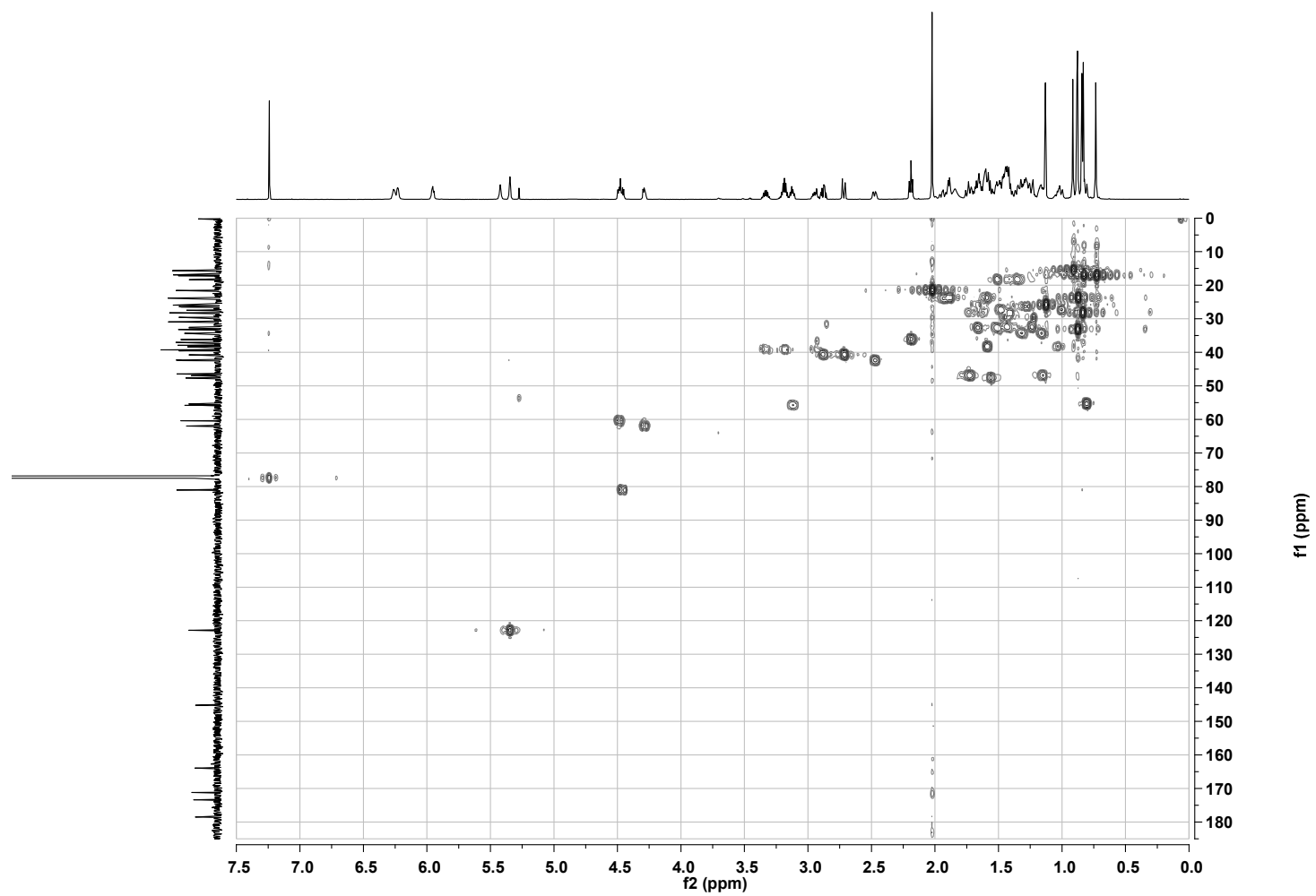


Figure S8. HMQC spectrum of Biotin-OAA (CDCl_3 , 600 MHz)

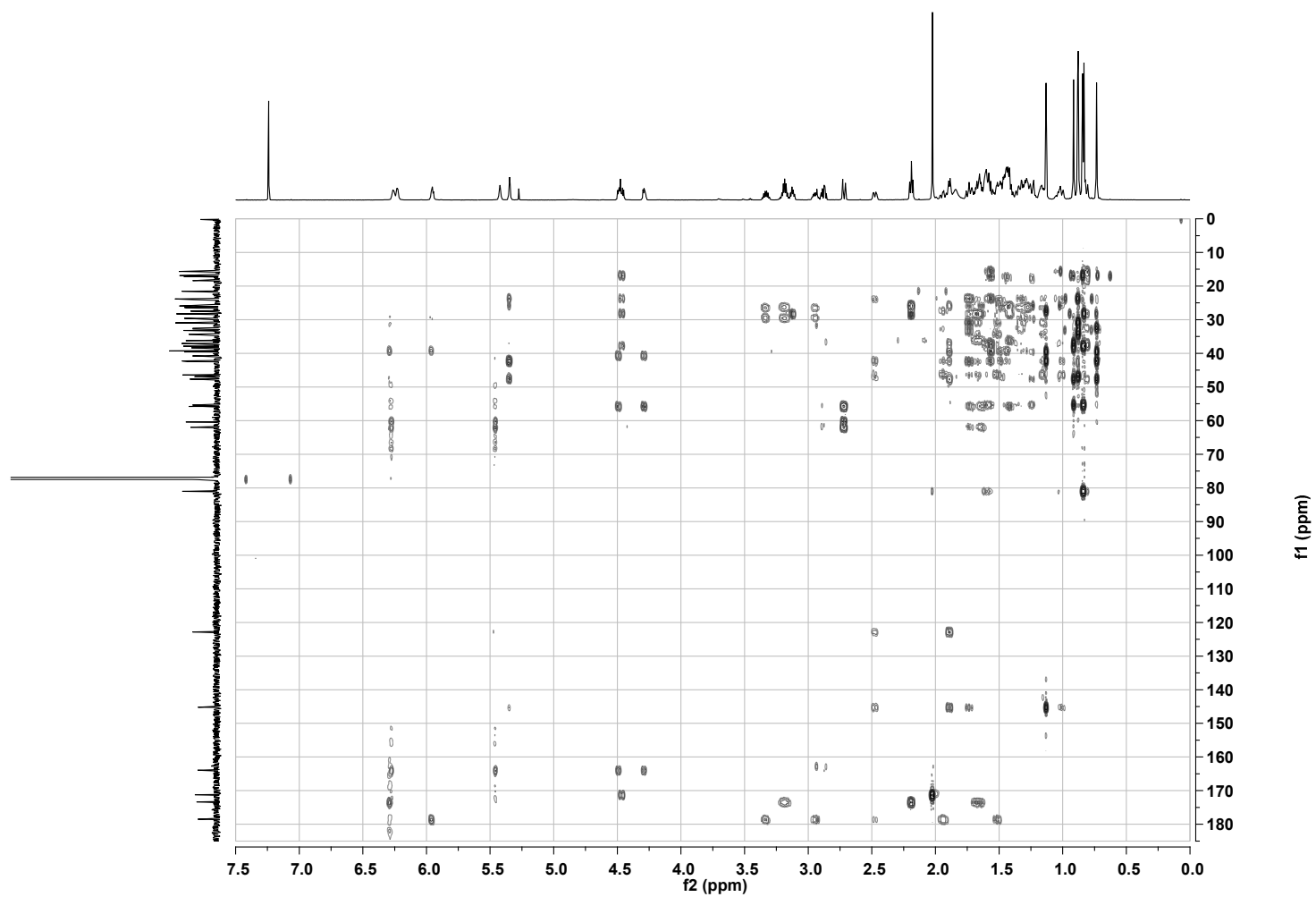


Figure S9. HMBC spectrum of Biotin-OAA (CDCl_3 , 600 MHz)