SUPPLEMENTARY MATERIALS

24-Nor-allobetulins possess strong α-glucosidase inhibitory activity

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Abstract: A series of 24-nor-allobetulin derivatives holding 3β-hydroxy-, oxime, methoxyoxime, lactame and 4-bromobenzylidene substituents have been synthesized and their differences in the NMR spectra were studied in detail. It was revealed that 3-oxo-24-nor-allobetulin loses selectivity in the reaction of oximation and forms a mixture of *Z/E* oximes (and methoxyoximes) in contract to the related derivatives of native scaffold (that forms only *E*-isomers). The screening of α-glucosidase inhibitory activity revealed that 24-nor-allobetulins are more active than allobetulins. The lead 3-oxo-24-nor-allobetulin with IC_{50} 0.49 μM was more than 60-fold and 500-fold active than acarbose and 3-oxo-allobetulin, respectively. We can conclude that the removal of the C-24 methyl group significantly increased the antidiabetic effect and 24-nor-allobetulins should be identified as the new and promising scaffolds as α-glucosidase inhibitors on the basis of triterpenoids.

Keywords: triterpenoids; allobetulin; 24-nor-allobetulin; α-glucosidase inhibitory activity

Table of contents

1. Experimental part

1.1. Chemistry

^{1}H and ^{13}C NMR spectra and two-dimensional correlation spectra of compounds **1-12** were recorded on a Bruker Avance-III 500 MHz spectrometer in $CDCl_3$ using TMS as internal standard. Melting points were measured on a microtable Boetius. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter (Germany) in a 1 dm tube. Elemental analysis was performed on Euro EA-3000 CHNS analyzer (Eurovector, Milan, Italy); the main standard is acetanilide. Thin layer chromatography analyses were performed on Sorbfil plates (Sorbpolimer, Krasnodar, Russian Federation), using the solvent system chloroform-ethyl acetate, 40:1. Column chromatography was performed on SiO_2 (Silica 60, Macherey-Nagel). Substances were detected by a 10% solution of a sulfuric acid solution with subsequent heating at 100-120°C for 2-3 min. Compounds **1, 8-12** were synthesized according to the methods described in (Dracinsky et al. 2006; Flekhter et al. 2009; Heller et al. 2015; Kazakova et al. 2019; Li et al. 1998).

3β-Hydroxy-19β,28-epoxy-24-nor-18α-oleane (3):

To the solution of compound **2** (0.51 g, 1.mmol) in methanol (10 mL) $NaBH_4$ (0.1 g, 2.7 mmol) was added under stirring. The reaction mixture was stirred under room temperature for 16 h and then was diluted by water (20 mL) and stirred for 1h. The amorphous precipitate was filtered off. Yield 75% (0.38 g), m.p. >250°C, $[\alpha]_D^{20}$+47°(c 0.5, $CHCl_3$). Calculated, %: C 81.2; H 11.3; O 7.5, $C_{29}H_{48}O_2$. ^{13}C NMR spectrum ($CDCl_3$, δ ppm): 13.36 (C27); 14.36 (C25); 15.05 (C24); 15.72 (C26); 20.80 (C6); 21.34 (C11); 24.55 (C29); 26.30 (C12); 26.34 (C15); 26.52 (C16); 28.82 (C30); 30.76 (C2); 32.74 (C21); 33.06 (C7); 34.13 (C13); 36.27 (C20); 36.62 (C10); 36.78 (C22); 38.18 (C1); 38.42 (C4); 40.25 (C8); 40.77 (C14); 41.48 (C17); 46.85 (C18); 48.98 (C9); 51.48 (C5); 71.29 (C28); 76.64 (C3); 87.94 (C19). ^{1}H NMR spectrum ($CDCl_3$, δ ppm, *J* Hz): 0.62 (ddd, 1H, *J* = 12.1, 11.4, 2.7, H-5); 0.79 (s, 3H, H-29); 0.81 (s, 3H, H-25); 0.91 (s, 3H, H-27); 0.93 (s, 3H, H-30); 0.95 (d, 3H, *J* = 6.4, H-24); 0.98 (s, 3H, H-26); 0.93 – 1.85 (m, 24H, CH, CH_2); 3.07 (ddd, 1H, *J* = 11.2, 9.7, 5.0, H-3); 3.44 (d, 1H, *J* = 7.0, H_A-28); 3.53 (s, 1H, H-19); 3.78 (d, 1H, *J* = 7.0, H_B-28); 4.00 (br.s, O<u>H</u>).

3β-Hydroxyimino-19β,28-epoxy-24-nor-18α-oleane (4):

A mixture of compound **2** (0.51 g, 1.0 mmol) and hydroxylamine hydrochloride (0.07 g, 1 mmol) in pyridine (20 mL) was refluxed for 6 h, then poured into H_2O (100 mL). The precipitate was filtered, washed with 5 % HCl, water and dried. Yield 83% (0.42 g), m.p. >250°C, $[\alpha]_D^{20}$+10°(c 0.5, $CHCl_3$). Calculated, %: C 78.9; H 10,7; N 3.2, O 7.2, $C_{29}H_{47}NO_2$.

^{13}C NMR spectrum for *E* izomer ($CDCl_3$, δ ppm): 13.33 (C27); 13.73 (C25); 14.06 (C24); 15.59 (C26); 19.70 (C2); 21.50 (C11); 21.67 (C6); 24.52 (C29); 26.28 (C15); 26.36 (C12); 26.51(C16);

28.90 (C30); 32.73 (C7); 32.80 (C21); 34.20 (C13); 36.18 (C4); 36.28 (C20); 36.75 (C22); 36.81 (C10); 38.76 (C1); 40.32 (C8); 40.76 (C14); 41.44 (C17); 46.84 (C18); 48.72 (C9); 53.26 (C5); 71.21 (C28); 87.81 (C19); 161.22 (C3).^{1}H NMR spectrum for *E* izomer ($CDCl_3$, δ ppm, *J* Hz): 0.79 (s, 3H, H-25); 0.90 – 1.70 (m, 35H, CH, CH_2, CH_3); 1.99 (ddd, 1H, *J* = 13.1, 6.5, 2.4, H_{eq}-1); 2.05 (ddd, 1H, *J* = 15.7, 12.5, 6.7, H_{ax}-2); 2.23 (dq, 1H, *J* = 11.9, 6.4 ,H-4); 3.14 (ddd, 1H, *J* = 15.5, 5.8, 2.2, 1H, H_{eq}-2); 3.45 (d, 1H, *J* = 7.7, H_A-28); 3.53 (s, 1H, H-19); 3.77 (d, 1H, *J* = 7.7, H_B-28); 4.42 (br.s, NO<u>H</u>). ^{15}N NMR spectrum ($CDCl_3$, δ ppm): 345.95 (<u>N</u>OH).

^{1}H NMR spectrum for *Z* izomer ($CDCl_3$, δ ppm, *J* Hz): 0.72 (s, 3H, H-25); 0.90 – 1.70 (m, 35H, CH, CH_2, CH_3); 2.40 (ddd, 1H, *J* = 14.3, 9.4, 9.1, $H_α$-2); 2.51 (dt, 1H, *J* = 14.0, 10.0, $H_β$-2); 2.65 (dq, 1H, *J* = 10.6, 6.4 ,H-4); 3.45 (d, 1H, *J* = 7.7, H_A-28); 3.53 (s, 1H, H-19); 3.77 (d, 1H, *J* = 7.7, H_B-28); 4.42 (br.s, NO<u>H</u>). ^{15}N NMR spectrum ($CDCl_3$, δ ppm): 345.95 (<u>N</u>OH).

3-Methoxyimino-19β, 28-epoxy-24-nor-18α-oleane (5):

To the solution of compound **2** (0.51 g, 1.0 mmol) in a dry pyridine (30 mL) CH_3ONH_2·HCl (0.17 g, 1.0 mmol) was added. The reaction mixture was refluxed for 8 h with a back condenser, cooled to room temperature, and quenched with 5% HCl (150 mL). The precipitate was filtered off, washed with water, and air-dried. The residue was crystallized from hexane. Yield 81% (0.41 g), m.p. >250°C, $[α]_D^{20}$+93°(c 0.5, $CHCl_3$). Calculated, %: C 79.1; H 10.8; N 3.1, O 7.0, $C_{30}H_{49}NO_2$. ^{13}C NMR spectrum for *E* izomer ($CDCl_3$, δ ppm): 13.35 (C27); 13.89 (C26); 13.95(C25); 15.64 (C24); 20.38 (C2); 21.53 (C11); 21.72 (C6); 24.59 (C29); 26.31 (C15); 26.38 (C12); 26.53 (C16); 28.85 (C30); 31.96 (C21); 32.77 (C7); 34.20 (C13); 36.16 (C4); 36.32 (C20); 36.78 (C10); 38.81(C22); 38.87 (C1); 40.38 (C8); 40.83 (C14); 41.52 (C17); 46.86 (C18); 48.76 (C9); 53.25 (C5); 61.10 (CH_3ON); 71.33 (C28); 87.97 (C19); 162.89 (C3); ^{1}H NMR spectrum for *E* izomer ($CDCl_3$, δ ppm, *J* Hz): 0.88 (s, 3H, H-25); 0.89 – 1.67 (m, 35H, CH, CH_2, CH_3); 1.84 (ddd, 1H, *J* = 13.1, 6.5, 2.4, H_{eq}-1); 1.96 (ddd, 1H, *J* = 15.7, 12.5, 6.7, H_{ax}-2); 2.13 (dq, 1H, *J* = 11.9, 6.4 ,H-4); 3.03 (ddd, 1H, *J* = 15.5, 5.8, 2.2, 1H, H_{eq}-2); 3.44 (d, 1H, *J* = 7.7, H_A-28); 3.53 (s, 1H, H-19); 3.77 (d, 1H, *J* = 7.7, H_B-28); 3.81 (s, 3H, OCH_3).^{15}N NMR spectrum for *E* izomer ($CDCl_3$, δ ppm): 363.09 ($NOCH_3$).

^{13}C NMR spectrum for *Z* izomer ($CDCl_3$, δ ppm): 13.46 (C27); 14.98 (C26); 15.09 (C25); 17.39 (C24); 21.93 (C11); 22.45 (C6); 24.44 (C2); 24.60 (C29); 26.28 (C15); 26.45 (C12); 26.58 (C16); 28.86 (C30); 31.97 (C21); 32.73 (C7); 33.64 (C4); 34.45 (C13); 36.24 (C10); 36.33 (C20); 36.80 (C22); 39.54 (C1); 40.57 (C8); 40.75 (C14); 41.51 (C17); 46.86 (C18); 48.14 (C5); 49.82 (C9); 61.24 (CH_3ON); 71.34 (C28); 87.96 (C19); 166.01 (C3).^{1}H NMR spectrum for *Z* izomer ($CDCl_3$, δ ppm, *J* Hz): 0.73 (s, 3H, H-25); 0.89-1.67 (m, 35H, CH, CH_2, CH_3); ;2.24 (dt, 1H, *J* = 14.0, 9.9, $H_α$-2); 2.33(dt, 1H, *J* = 14.0, 10.0, $H_β$-2); 2.52 (dq, 1H, *J* = 10.6, 6.4, H-

4); 3.44 (d, 1H, *J* = 7.7, H_A-28); 3.53 (s, 1H, H-19); 3.77 (d, 1H, *J* = 7.7, H_B-28); 3.82 (s, 3H, OCH_3).^{15}N NMR spectrum for *Z* izomer ($CDCl_3$, δ ppm): 351.83 ($NOCH_3$).

3β-Oxo-3a-aza-A-homo-19β,28-epoxy-24-nor-18α-oleane (6):

$SOCl_2$ (0.5 mL, 1 mmol) was dropwise added to a stirred solution of compound **4** (0.5 g, 1 mmol) in dried 1,4-dioxane (100 mL). The resulted solution was left at room temperature overnight. The brownish mixture was then poured into water, slightly acidified with HCl. The resulted light-brown precipitate was filtered off, washed with water, dried, subjected to column chromatography on SiO_2 ($CHCl_3$, $CHCl_3$-EtOH 100:1, $CHCl_3$-EtOH 50:1). Yield 78% (0.39 g), m.p. >250°C, $[\alpha]_D^{20}$+67°(c 0.5, $CHCl_3$). Calculated, %: C 78.9; H 10.7; N 3.2, O 7.2, $C_{29}H_{47}NO_2$. ^{13}C NMR spectrum ($CDCl_3$, δ ppm): 13.21 (C27); 14.10 (C25); 15.74 (C26);19.21 (C23); 21.30 (C6); 21.40 (C11); 24.54 (C29); 26.23 (C16); 26.28 (C15); 26.46 (C12); 28.80 (C30); 30.48 (C2); 32.70 (C7); 32.75 (C21); 34.01 (C13); 36.27 (C20); 36.71 (C22); 36.74 (C1); 39.54 (C10); 40.25 (C14); 40.88 (C8);41.46 (C17); 46.72 (C18); 47.75 (C4); 48.76 (C9); 53.58 (C5); 71.27 (C28); 87.89 (C19); 177.99 (C3). ^{1}H NMR spectrum ($CDCl_3$, δ ppm, *J* Hz): 0.79 (s, 3H, H-29); 0.90 (s, 3H, H-27); 0.93 (s, 6H, H-30, H-25); 0.94 (s, 3H, H-25); 0.99 (s, 3H, H-26); 1.17 (d, 3H, *J* = 6.7, H-24);1.12-1.70 (m, 21H, CH, CH_2);1.97 (ddd, 1H, *J* = 13.8, 7.0, 5.1, H_{eq}-1); 2.27 (ddd, 1H, *J* = 15.6, 7.9, 4.6,H_{ax}-2); 2.70 (ddd, 1H, *J* = 15.6, 7,5, 8.9, H_{eq}-2); 3.45 (d, 1H, *J* = 7.0, H_A-28); 3.53 (s, 1H, H-19); 3.55 (dq, 1H, *J* = 6.7, H-4), 3.77 (d, 1H, *J* = 7.0, H_B-28); 6.46 (br.s, N<u>H</u>). ^{15}N NMR spectrum ($CDCl_3$, δ ppm): 142.40 (<u>N</u>H).

4.2. Synthesis of compounds (7) and (12):

A mixture of compound **2** or **8** (1 g, 2.3 mmol) and 4-bromobenzaldehyde (0.35 g, 2.5 mmol) in ethanol (30 mL), containing a catalytic amount of 40% potassium hydroxide solution in ethanol, was stirred under room temperature for 24 h. The reaction mixture was then poured into water, slightly acidified with HCl. The resulted precipitate was filtered off, washed with water and dried.

2-(4-Bromobenzylidene)-19β,28-epoxy-24-nor-18α-oleanan-3-one (7):

The product was crystallized from $CHCl_3$-EtOH (20:1). Yield 79% (0.79 g), m.p. >250°C, $[\alpha]_D^{20}$-21°(c 0.5, $CHCl_3$). Calculated, %: C 73.1; H 8.5; Br 13.1; O 5.3, $C_{37}H_{51}BrO_2$. ^{13}C NMR spectrum ($CDCl_3$, δ ppm): 13.48 (C26); 14.95 (C23); 15.10 (C25); 15.17 (C27); 21.82 (C11); 22.91 (C6); 24.60 (C30); 26.28 (C12); 26.43 (C16); 26.53 (C15); 28.86 (C29); 32.13 (C7); 32.73 (C21); 34.30 (C13); 36.23 (C20); 36.34 (C10); 36.78 (C22); 40.39 (C8); 40.84 (C14); 41.52 (C17); 44.08 (C1); 44.33 (C4); 46.87 (C18); 48.21 (C9); 49.81 (C5); 71.33 (C28); 87.98(C19); 122.75 (C5'); 131.67 (C4'), 131.67 (C6'); 131.72 (C3'); 131.72 (C7'); 134.77 (C2'); 135.82 (C2); 136.11 (C1'); 204.47 (C3). ^{1}H NMR spectrum ($CDCl_3$, δ ppm, *J* Hz): 0.80 (s, 3H, H-25); 0.81 (s, 3H, H-30); 0.94 (s 3H, H-29); 0.96 (s, 3H, H-26); 1.00 (s, 3H, H-27); 1.20 (d,

3H, *J*=6.8, H-23); 1.01-1.70 (m, 19H, CH, CH_2); 1.70 (td, 1H, *J*= 12.3, 3.5, H_{ax}-15); 2.12 (d, 1H, *J*=15.8, H_B-1); 2.16 (q, 1H, *J*=6.8, H-4); 3.04 (d , 1H, *J*=15.8, H_A-1); 3.46(d, 1H, *J*=7.6, H_B-28); 3.55 (s, 1H, H-19); 3.78 (d, 1H, *J*=7.6, H_A-28); 7.24 (d, 2H, *J*' = 8.4, H-3', H-7'); 7.40 (s, 1H, H-1'); 7.51 (d, 2H, *J*= 8.4, H-4', H-6').

2-(4-Bromobenzylidene)-19β,28-epoxy-18α-oleanan-3-one (12):

The product was crystallized from $CHCl_3$-EtOH (20:1). Yield 81 % (0.81 g), m.p. >250°C, $[\alpha]_D^{20}$-90°(c 0.5, $CHCl_3$). Calculated, %: C 73.17; H 8.89; Br 12.81; O 5.13, $C_{38}H_{55}BrO_2$. ^{13}C NMR spectrum ($CDCl_3$, δ ppm): 13.49 (C26); 15.30 (C27); 16.16 (C25); 20.36(C6); 21.85 (C11); 22.40 (C24); 24.61 (C30); 26.30 (C12); 26.46 (C16); 26.60 (C15); 28.87 (C29); 29.50 (C23); 32.75 (C7); 32.75(C21); 34.35(C13); 36.34 (C20); 36.63 (C10); 36.79 (C22); 40.51 (C8); 40.86 (C14); 41.53 (C17); 44.68 (C1); 45.29 (C4); 46.85 (C18); 49.07 (C9); 53.06 (C5); 71.33 (C28); 87.95(C19); 122.70 (C5'); 131.69 (C4'), 131.69 (C6'); 131.73(C3'); 131.73(C7'); 134.93 (C2); 134.99 (C5'); 136.02 (C1'); 207.96 (C3). ^{1}H NMR spectrum ($CDCl_3$, δ ppm, *J* Hz): 0.80 (s, 3H, H-25); 0.80 (s, 3H, H-30); 0.93 (s, 3H, H-29); 0.95 (s, 3H, H-26); 1.00 (s, 3H, H-27); 1.12 (s, 3H, H-23); 1.15 (s, 3H, H-24); 1.01-1.70 (m, 19H, CH, CH_2); 1.71 (td, 1H, *J*= 12.3, 3.5, H_{ax}-15); 2.19 (d, 1H, *J*=15.8, H_B-1); 3.01 (d, 1H, *J*=15.8, H_A-1); 3.45 (d, 1H, *J*=7.7, H_B-28); 3.54 (s, 1H, H-19); 3.78 (d, 1H, ^{2}*J*=7.7, H_A-28); 7.30 (d, 2H, *J* = 8.5, H-3', H-7'); 7.40 (s, 1H, H-1'); 7.51 (d, 2H, *J*' = 8.5, H-4', H-6').

1.2. α-Glucosidase Inhibition Assay Method

α-Glucosidase enzyme inhibition assay was carried out in a 96-well microplate. The assay is based on the hydrolysis reaction of 4-nitrophenyl-α-D-glucopyranoside with α-glucosidase to form yellow colored 4-nitrophenol:

p-Nitrophenyl α-D-Glucoside $\xrightarrow{\text{α-Glucosidase}}$ α-D-Glucose + p- Nitrophenol

α-Glucosidase inhibitory activity assay was performed following the modified method of Pistia Brueggeman and Hollingsworth with slight modification. Compounds were dissolved in DMSO - EtOH (1:1) solution to final concentrations of 256, 64, 16, 4 and 1 µg/mL. The concentration was determined by a series of α-glucosidase kinetic experiments in a 96-well plate, using a reaction mixture containing 20 µL of compound varying concentrations, 20 µL of phosphate buffer (100 mM; pH 6.8), and 20 µL of α-glucosidase (0.3 U/mL, Sigma G0660) were pre-incubated for 10 min at 37ºC. Then 20 µL of 2.5M *p*-nitrophenyl α-D-glucopyranoside (Sigma N1377) was added to the mixture as a substrate. After further incubation at 37ºC for 30 min, the reaction was stopped by adding 80 µL of 0.1 M sodium carbonate. The enzyme, tested compound and substrate solution were prepared using the phosphate buffer 10 mM; pH 6.8.

Acarbose was used as a positive control and water as a negative one. The yellow color produced was quantitated by colorimetric analysis and reading the absorbance at 410 nm. Each experiment was performed in triplicates, along with appropriate blanks. The solvent DMSO was used as a negative control to evaluate its effect on α-glucosidase activity. Buffer was used instead of tested compound in positive control and instead of enzyme or substrate in the negative control. Background was determined by the volume of a buffer as a reaction mixture.

The % inhibition has been obtained using the formula:

% inhibition = {Absorbance (control) –Absorbance (sample)}/Absorbance (control)

The IC_{50} value is defined as a concentration of samples inhibiting 50% of α-glucosidase activity under the stated assay conditions.

1.3. In vitro cytotoxicity study of compound 2

MTT assay was used to determine the cytotoxic activity of compounds with human cancer cell lines acquired from the American Type Culture Collection (ATCC): epidermoid carcinoma (KB, ATCC number CCL-17), hepatocellular carcinoma (HepG2, ATCC number HB-8065), lung adenocarcinoma LU-1 (ATCC number HTB - 57^{TM}), breast adenocarcinoma MCF-7 (ATCC number HTB - 22^{TM}. Cells were cultured in medium DMEM supplemented with 10% Fetal bovine serum (FBS), 1% Penicillin and Streptomycin and 1% L-glutamine, under a humidified atmosphere of 5% CO_2 at 37°C.

Compounds were dissolved in DMSO at 40 mg/mL and a series of dilutions for each compound was prepared to final concentrations of 256, 64, 16, 4 and 1 µg/mL. Cells were separated with trypsin and seeded in each well with 3 x10^4 cells per ml and were treated with sample different concentrations on 96-well plates. Untreated cells represented the controls. After 72h of treatment, an MTT solution (10 µl, 5 mg/mL) of phosphate buffer was added to each well for 4 h until intracellular purple formazan crystals are visible. Remove MTT and add DMSO solution (100 µL). The optical density of the solution was determined by Epoch™ Microplate *Spectrophotometer* at 540 nm. The inhibition ratio was calculated based on the optical densities from the three replicate tests.

$IC_{50} \leq 20$ µg/mL (with crude extract) and $IC_{50} \leq 4$ µg/mL or 10 µM (with compound) are evaluated to have cytotoxic activity (Mosmann 1983).

Table S1. The result of cytotoxicity tests for the lead compound **2**

Code number	IC_{50} values (µg/mL)			
	KB	Hep-G2	Lu-1	MCF-7
3-oxo-24-nor-allobetulin **2**	153.14±5.0	237.17±6.4	49.05±1.6	222.89±5.5

Figure S1. Complete ^{1}H NMR and ^{13}C {^{1}H} spectrum of compound **3** in $CDCl_3$

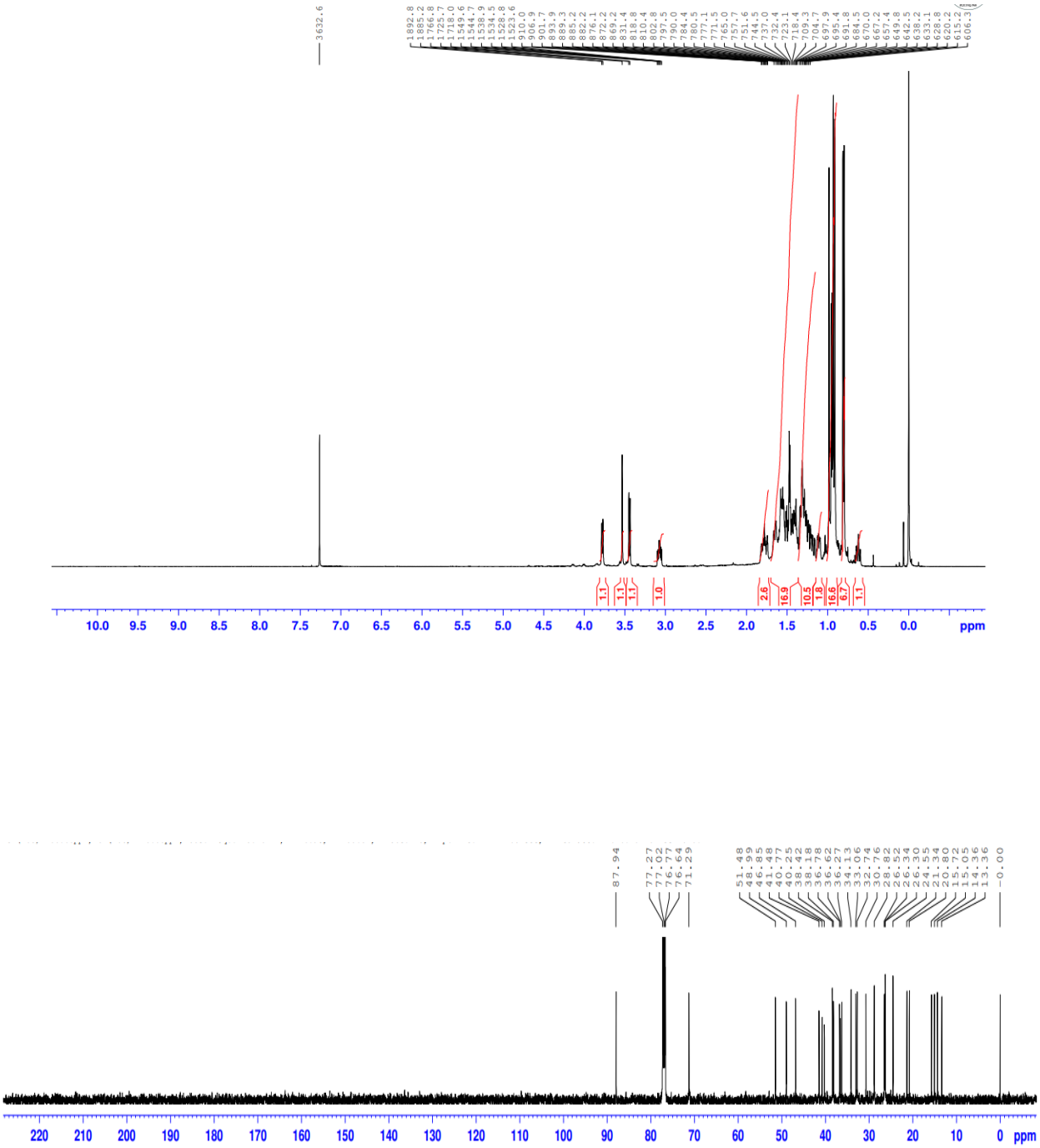


Figure S2. HMBC spectrum of compound **3** in $CDCl_3$

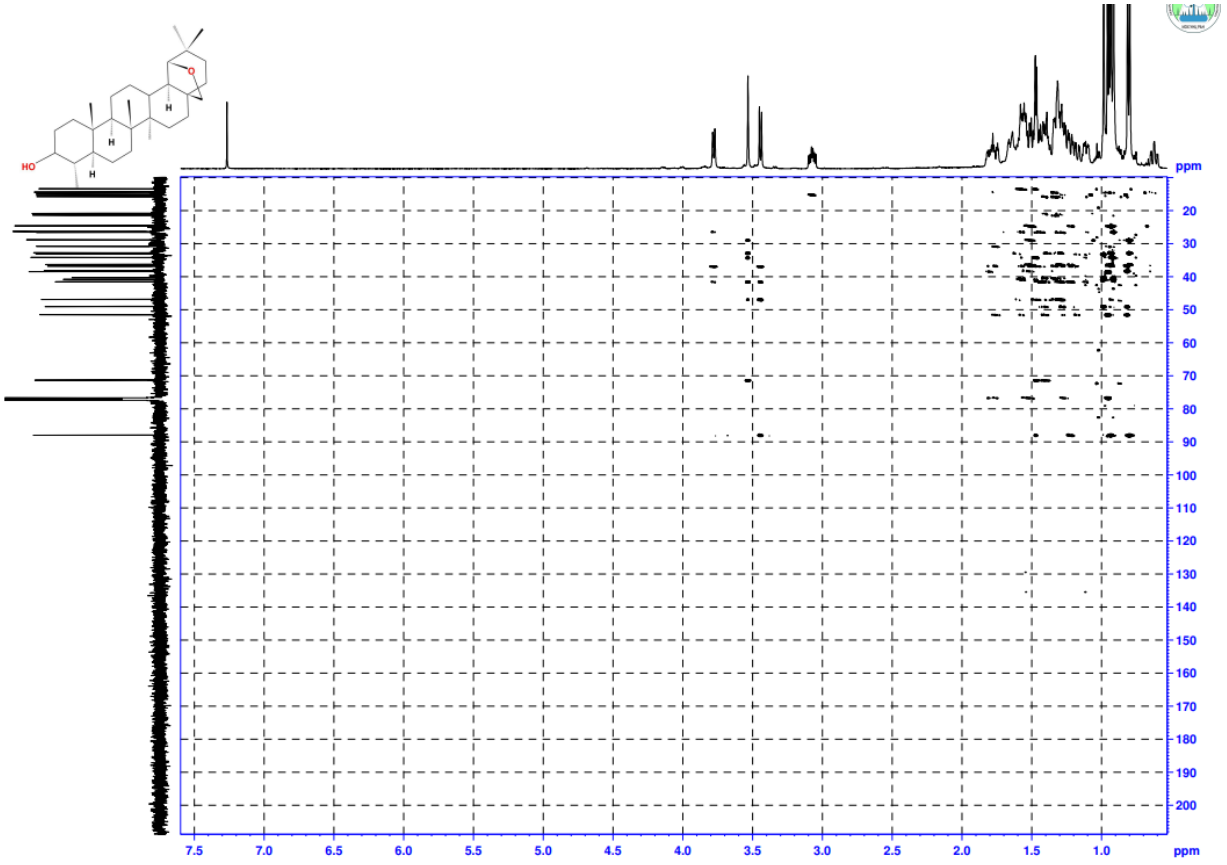


Figure S3. Complete ^{1}H NMR and ^{13}C {^{1}H} spectrum of compound **4** in $CDCl_3$

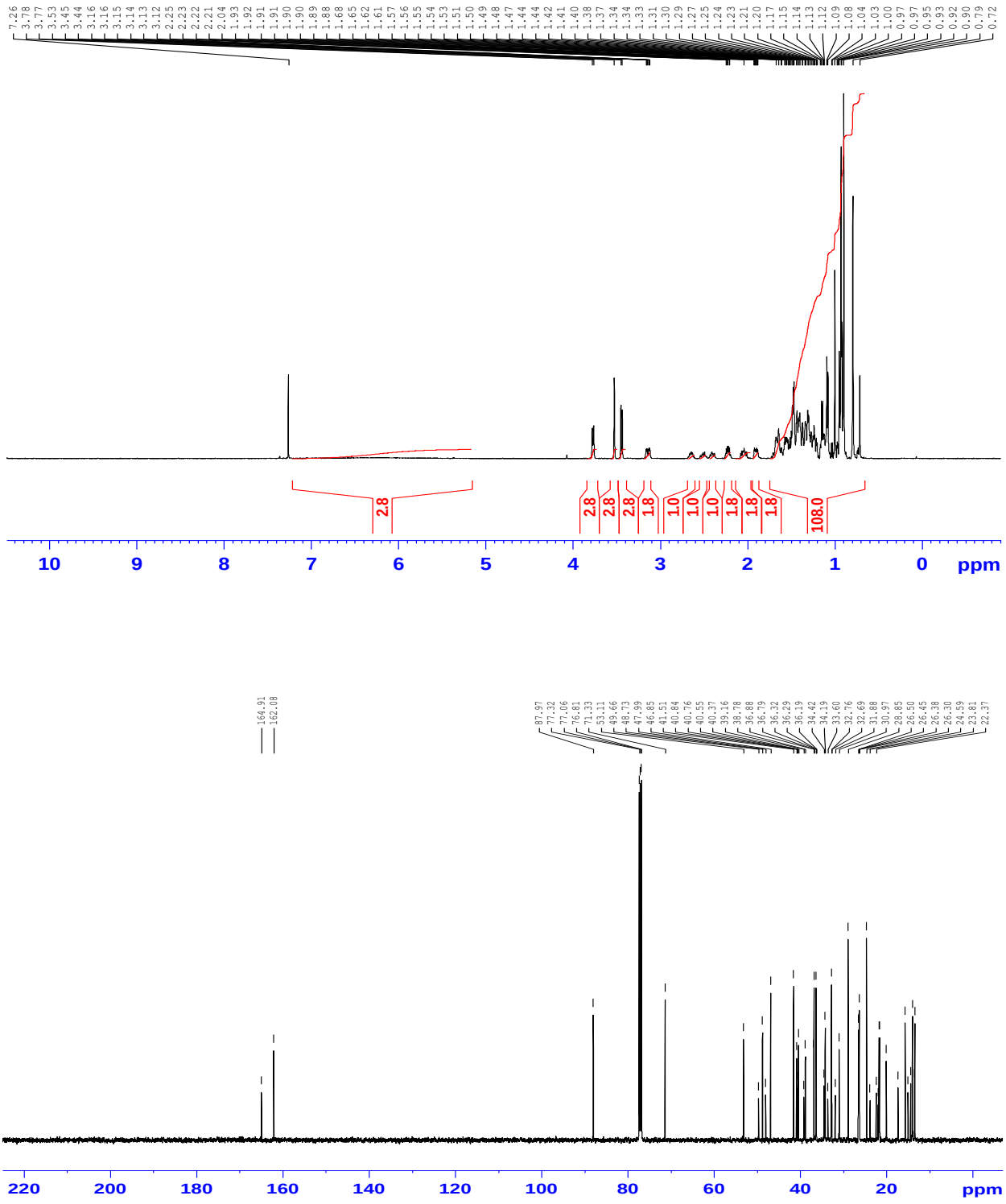


Figure S4. Complete ^{1}H NMR and ^{13}C $\{^{1}H\}$ spectrum of compound **5** in $CDCl_3$

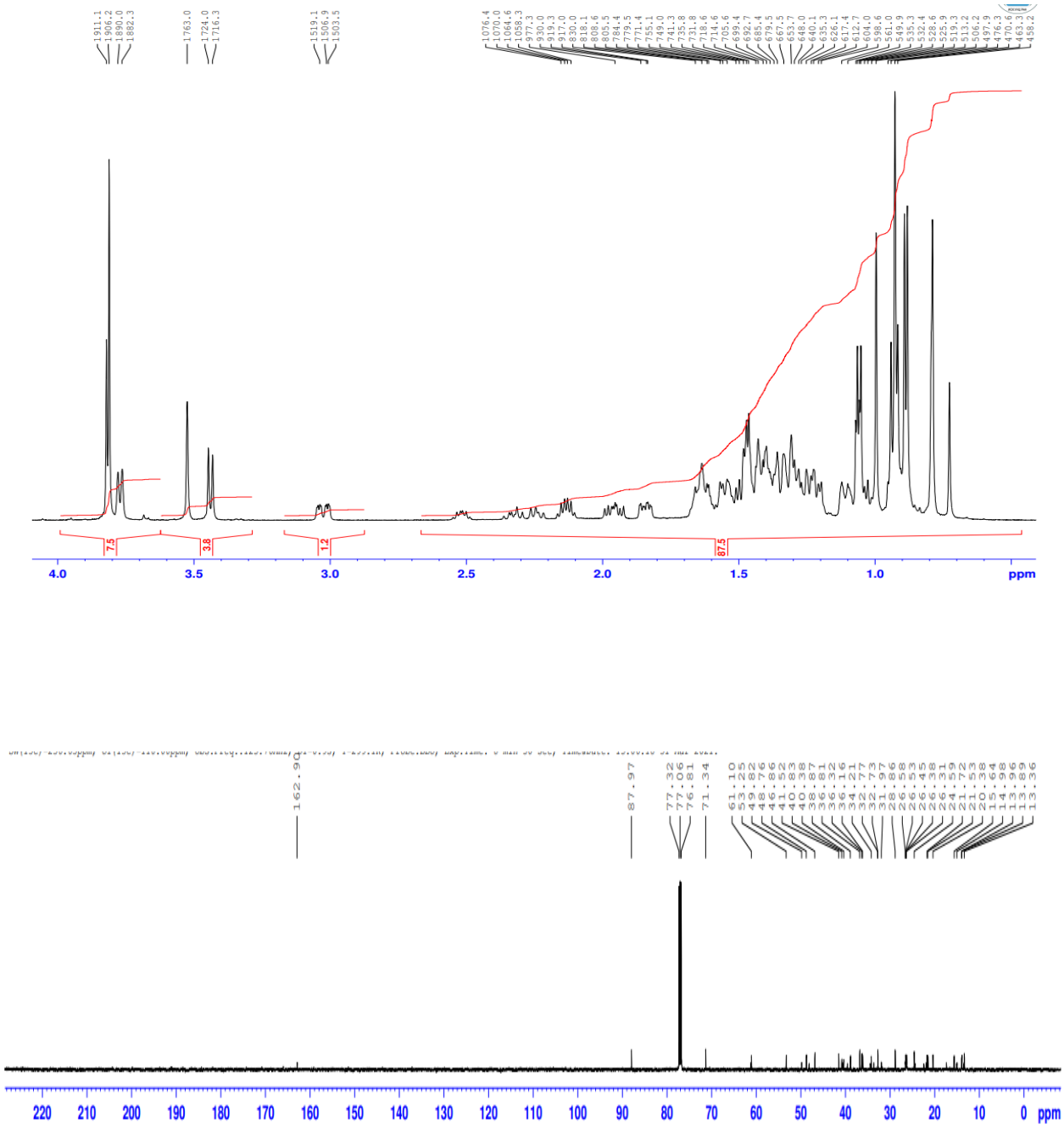


Figure S5. HMBC spectrum of compound **5** in $CDCl_3$

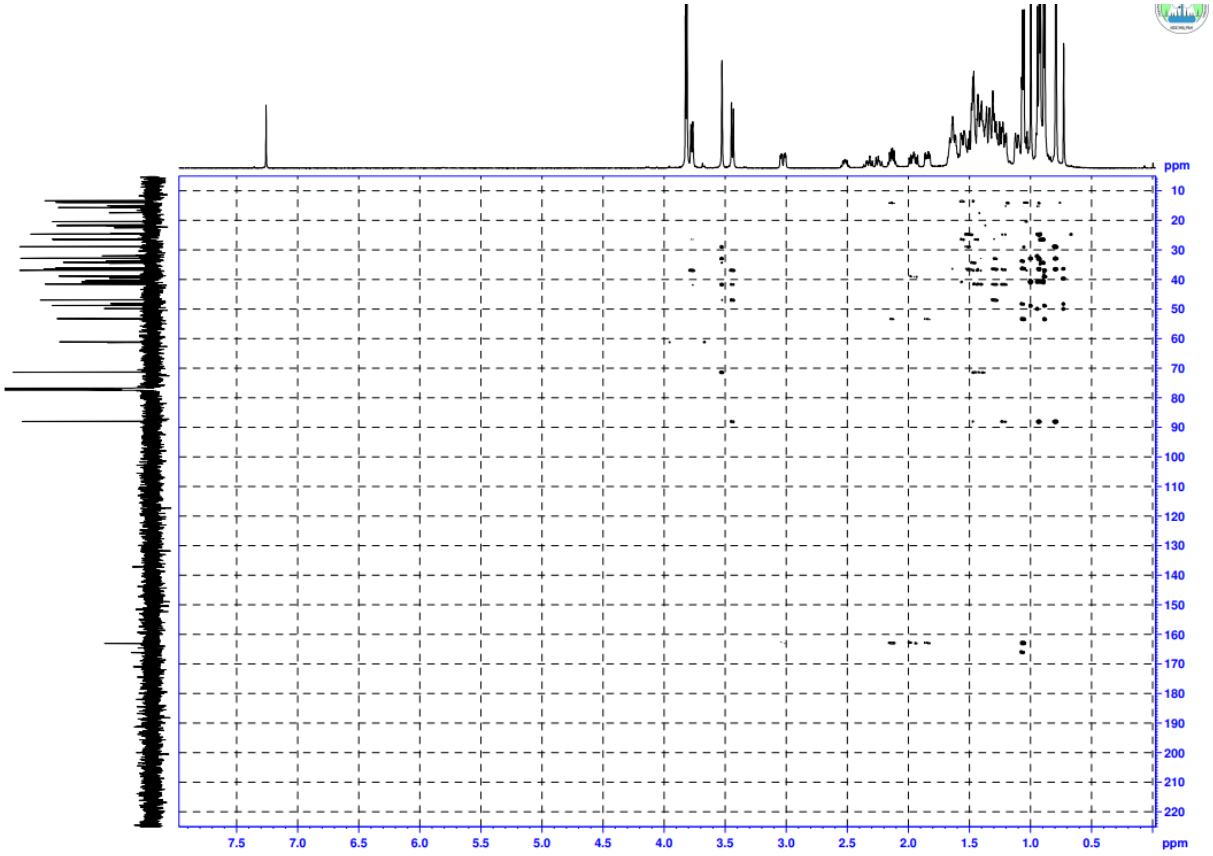


Figure S6. Complete ^{1}H NMR and ^{13}C $\{^{1}H\}$ spectrum of compound **6** in $CDCl_3$

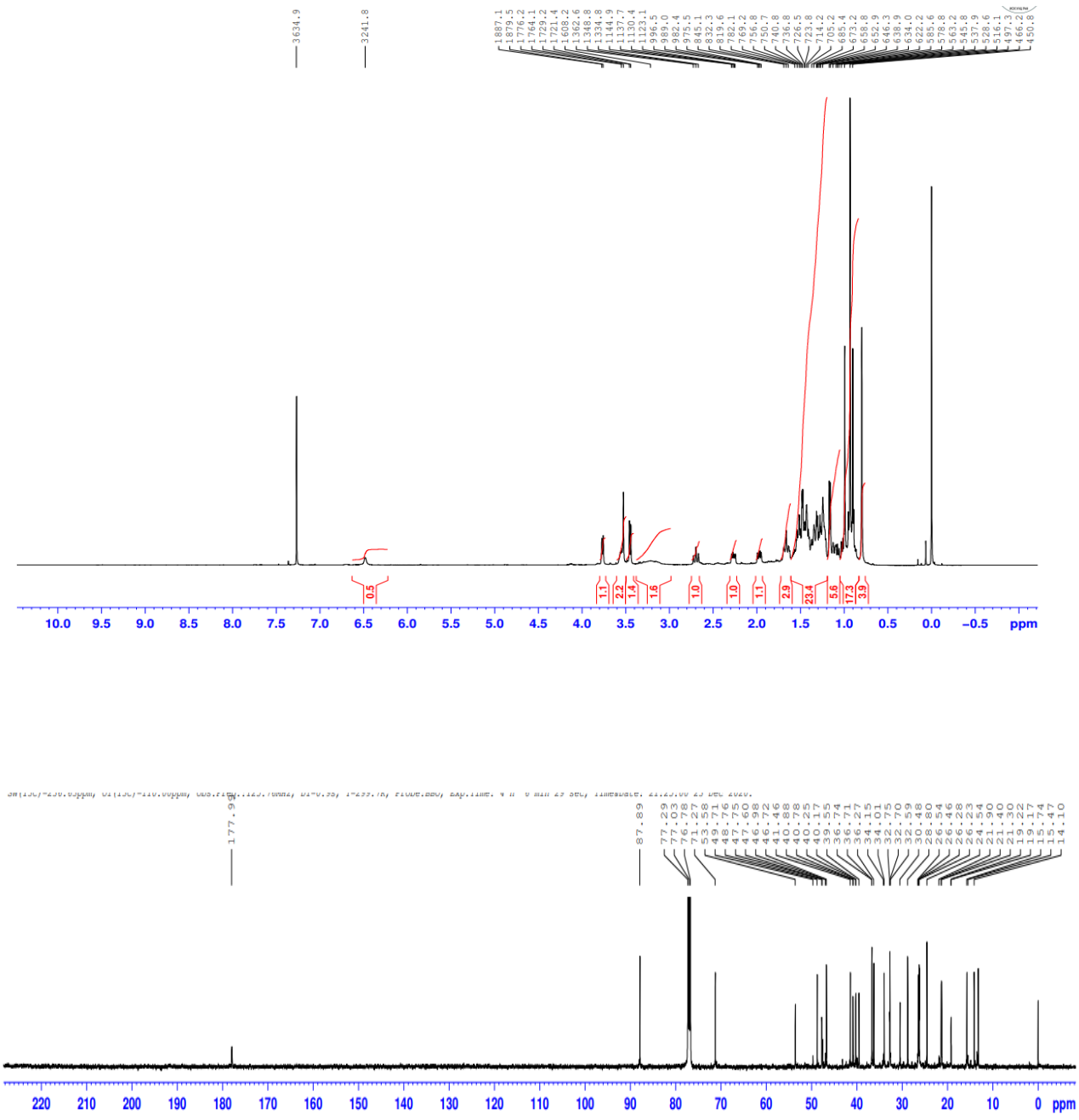


Figure S7. HMBC spectrum of compound **6** in $CDCl_3$

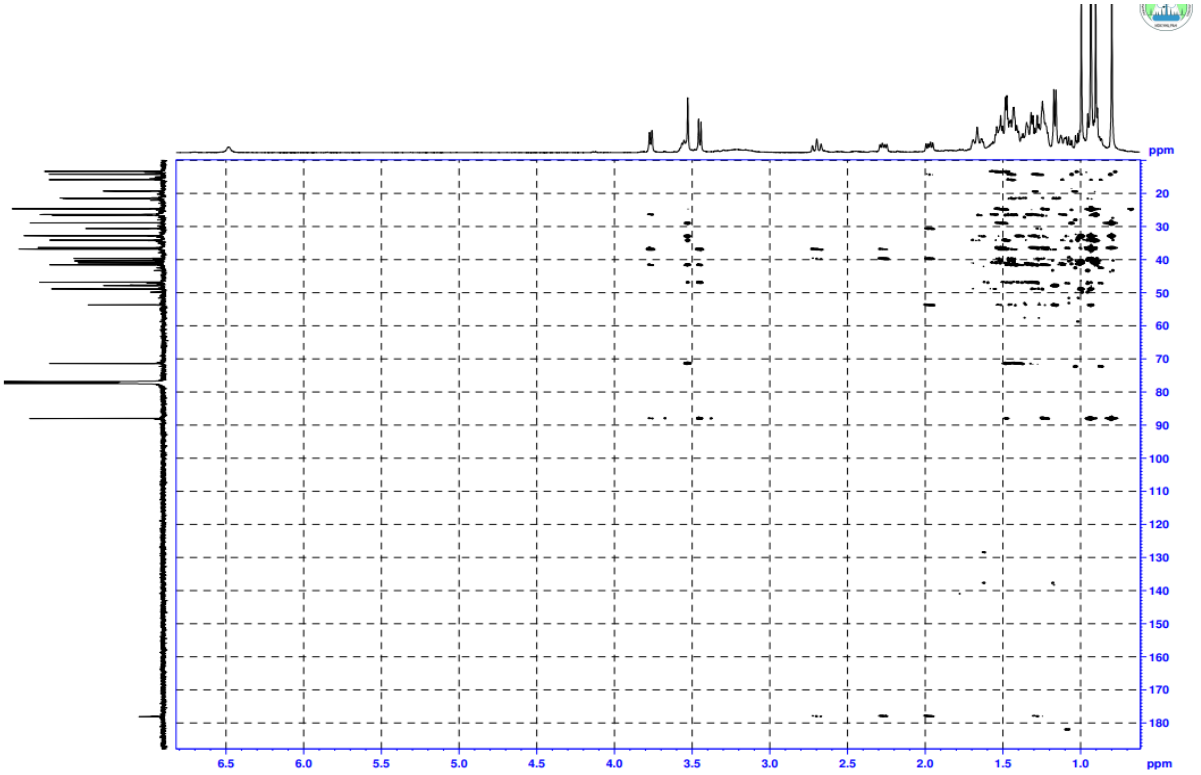


Figure S8. Complete ^{1}H NMR and ^{13}C $\{^{1}H\}$ spectrum of compound **7** in $CDCl_3$

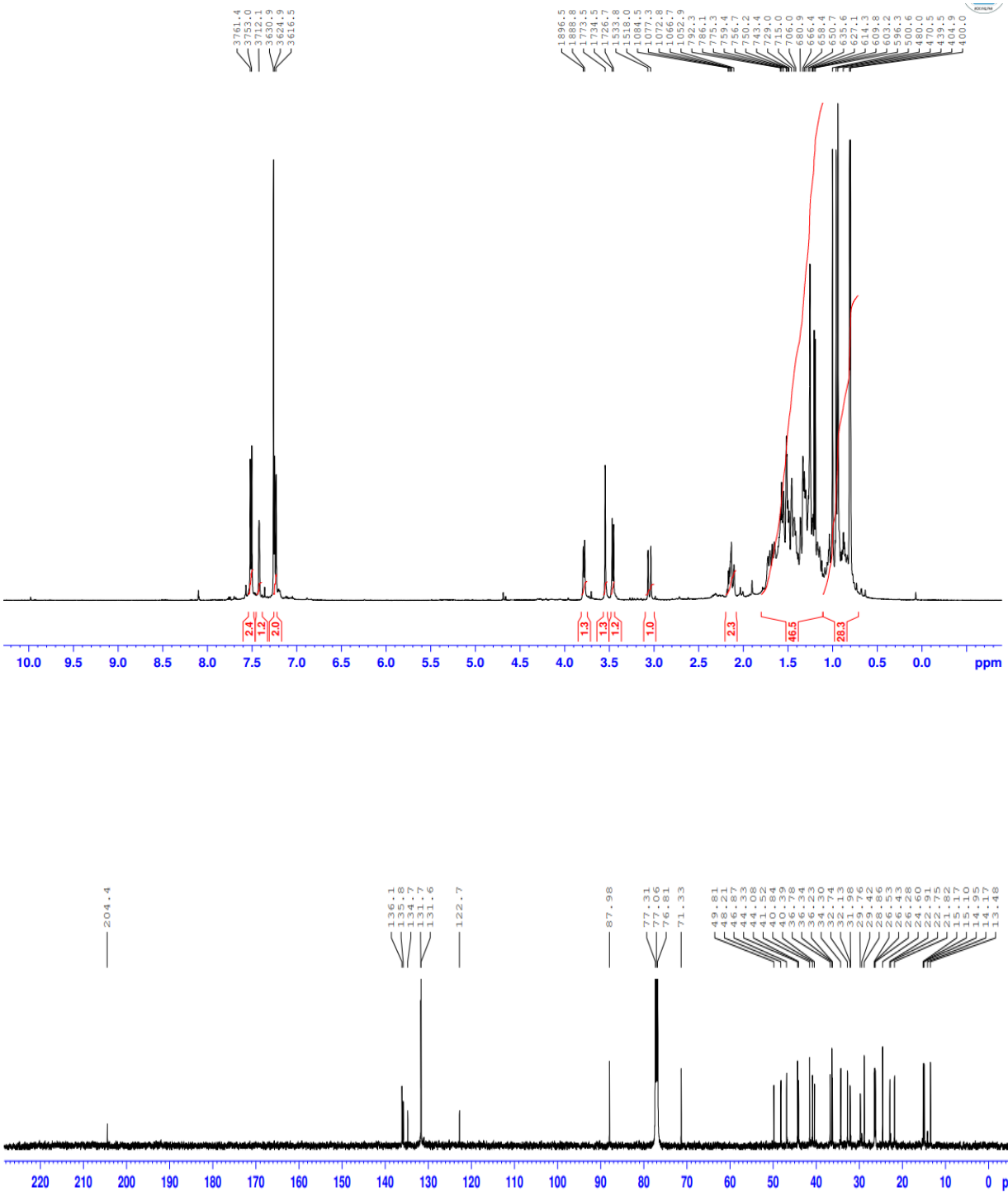


Figure S9. HMBC spectrum of compound **7** in $CDCl_3$

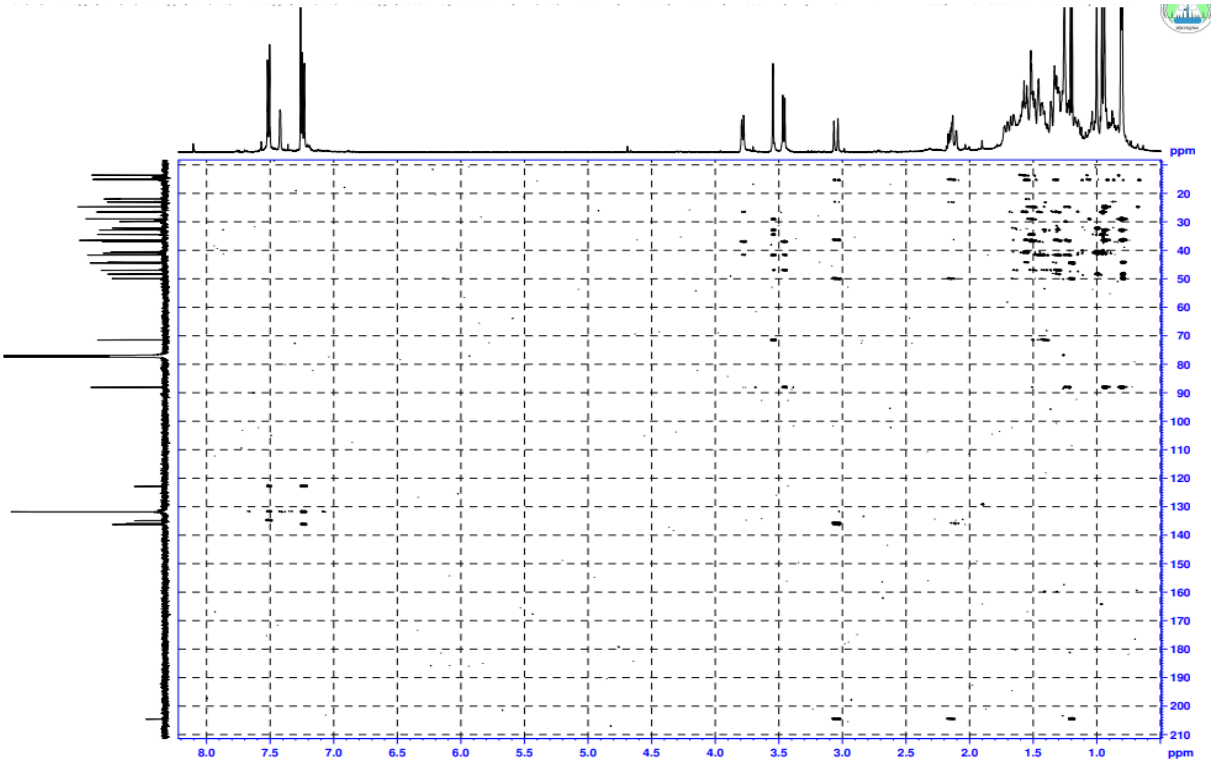


Figure S10. Complete ^{1}H NMR and ^{13}C $\{^{1}H\}$ spectrum of compound **12** in $CDCl_3$

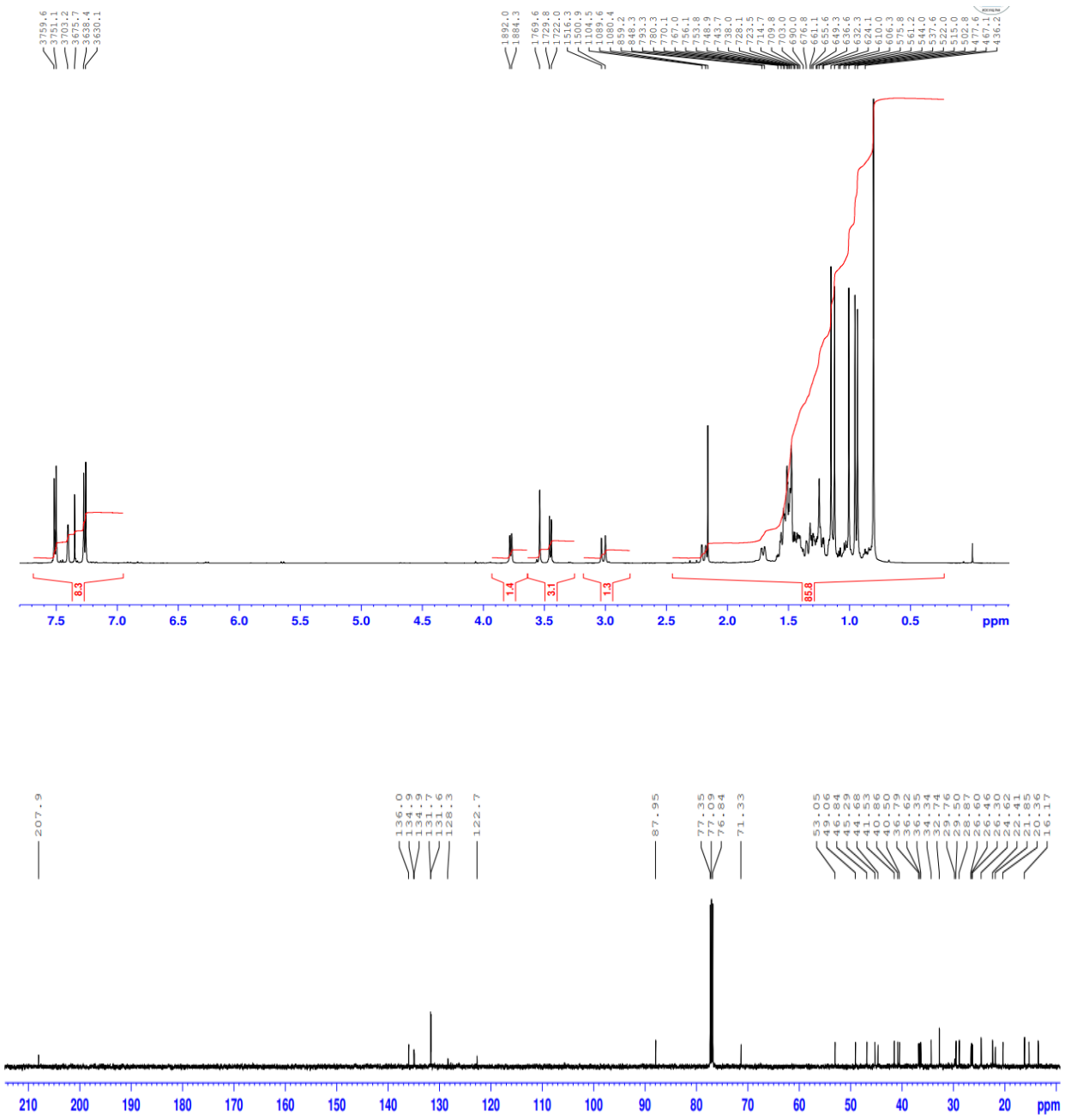


Figure S11. HMBC spectrum of compound **12** in $CDCl_3$

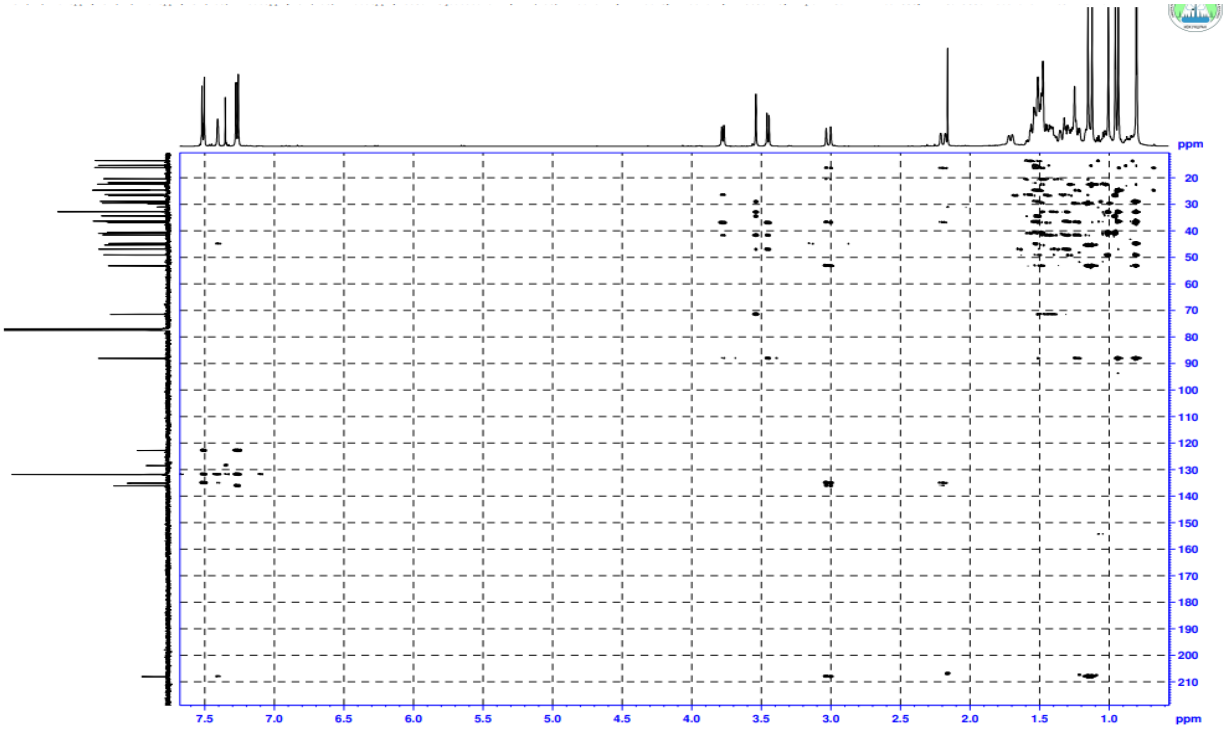


Figure S12. The synthetic route from allobetulin **1** to 24-nor-allobetulin **2**

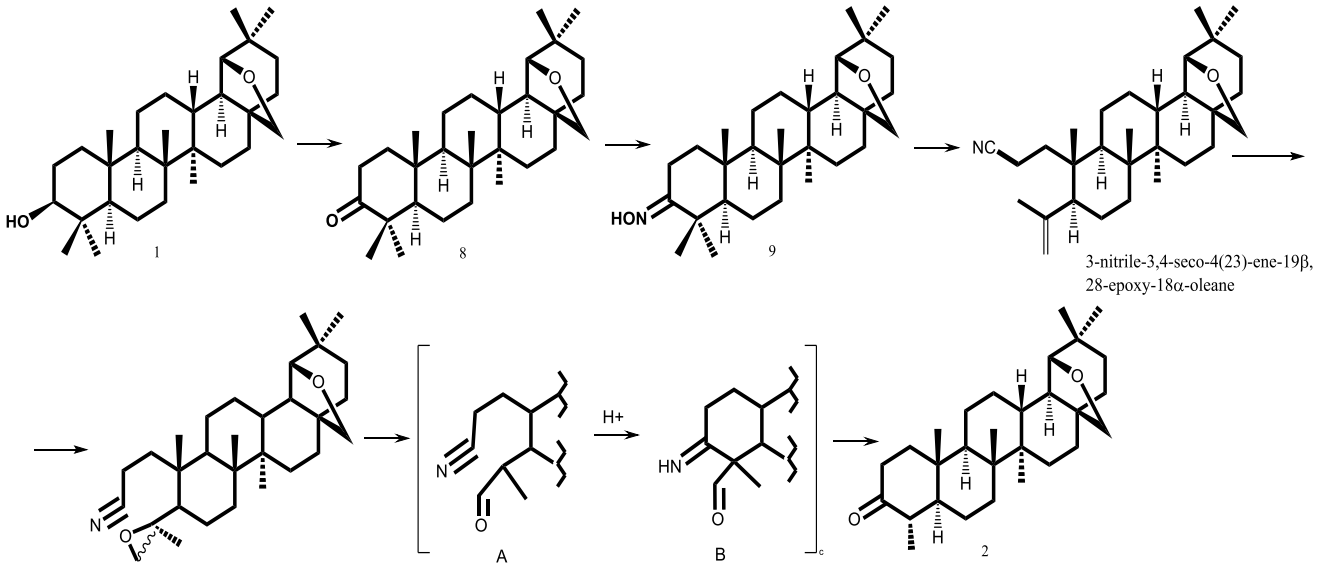


Figure S13. The main correlations in cycles A, B and C in the spectra of {^{1}H, ^{13}C} HMBC and {^{1}H, ^{1}H} NOESY of compound **8**

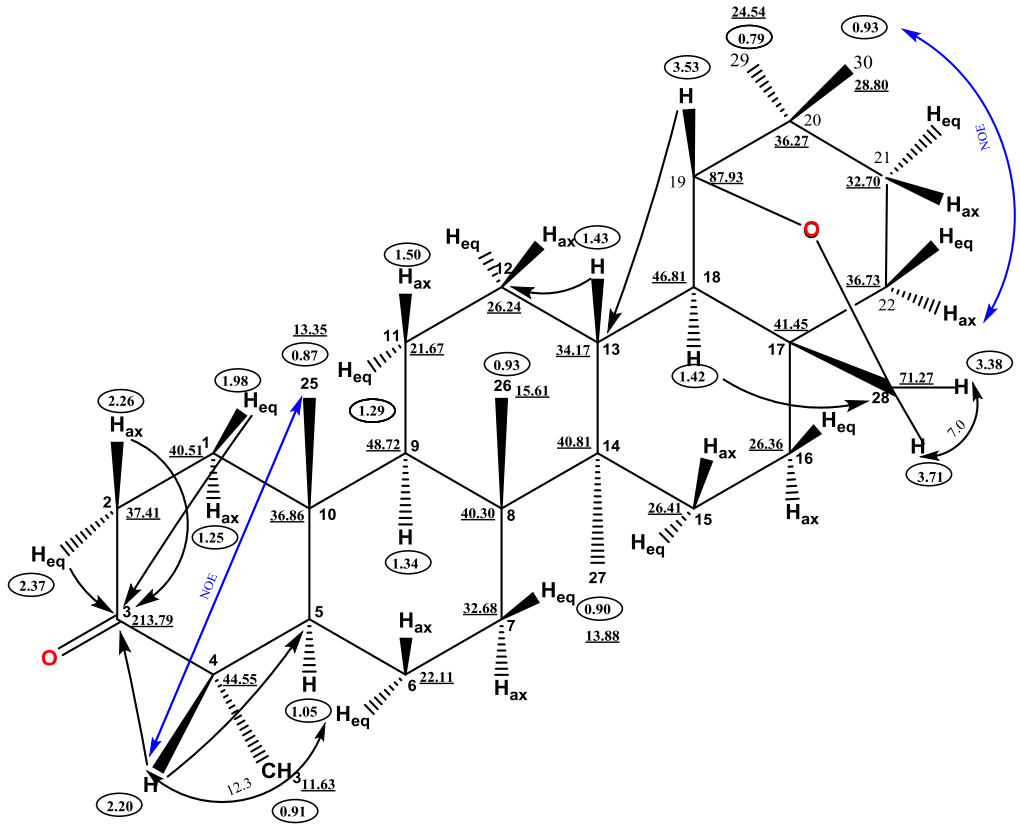


Figure S14. Comparison of spectral characteristics of compounds **1** and **3**

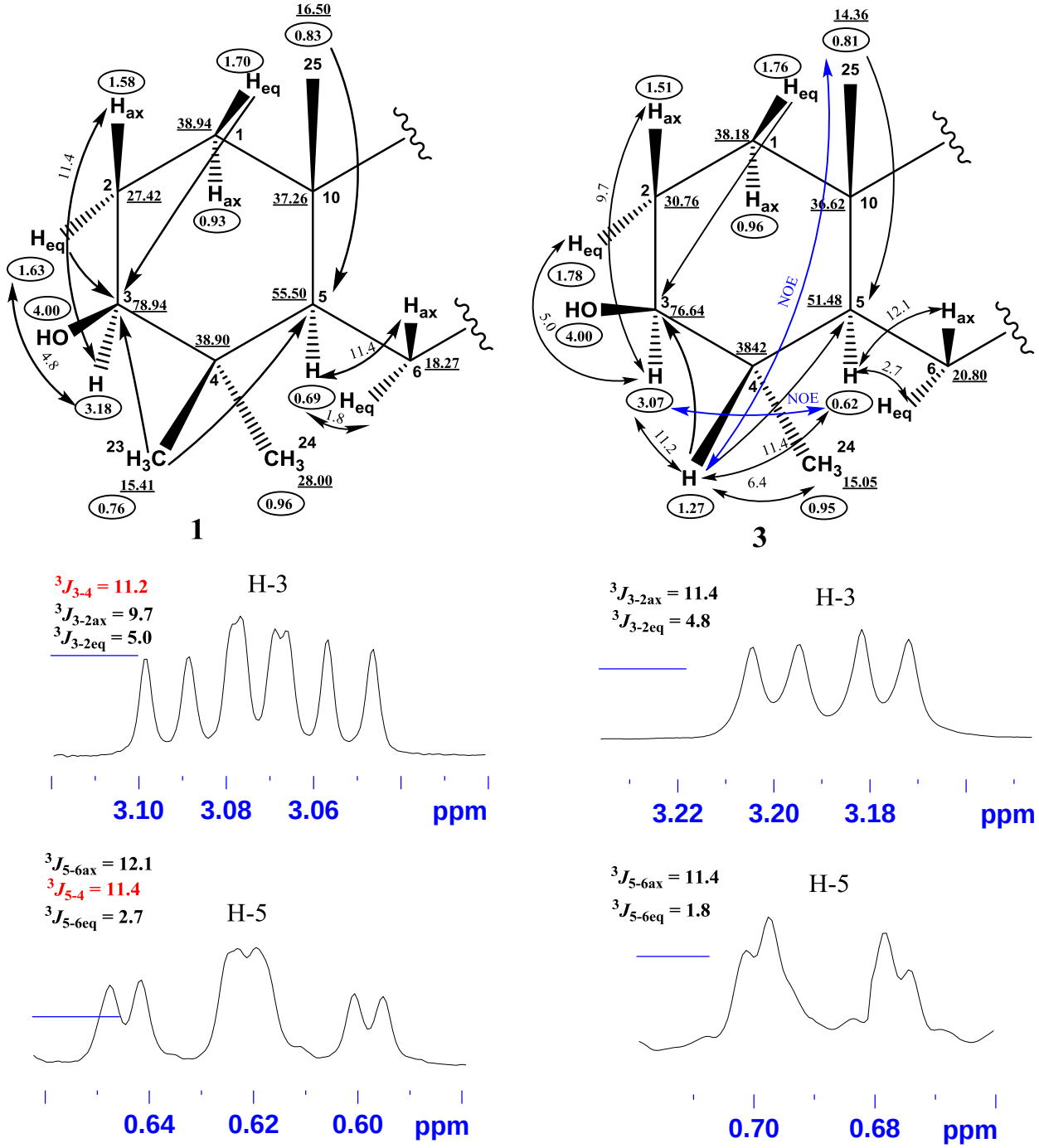
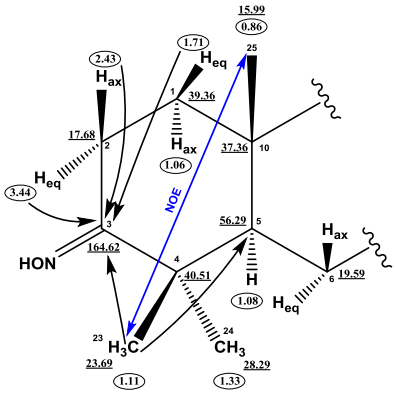
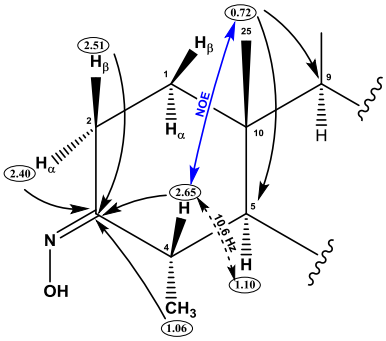


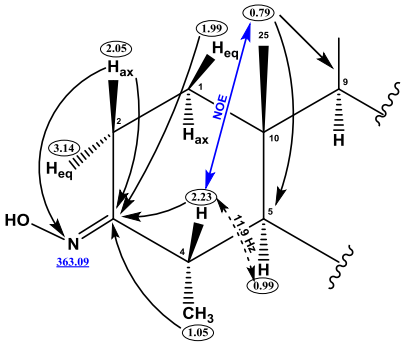
Figure S15. Comparison of spectral characteristics of compounds **9** and **4** (*Z*- and *E*-isomers)



9

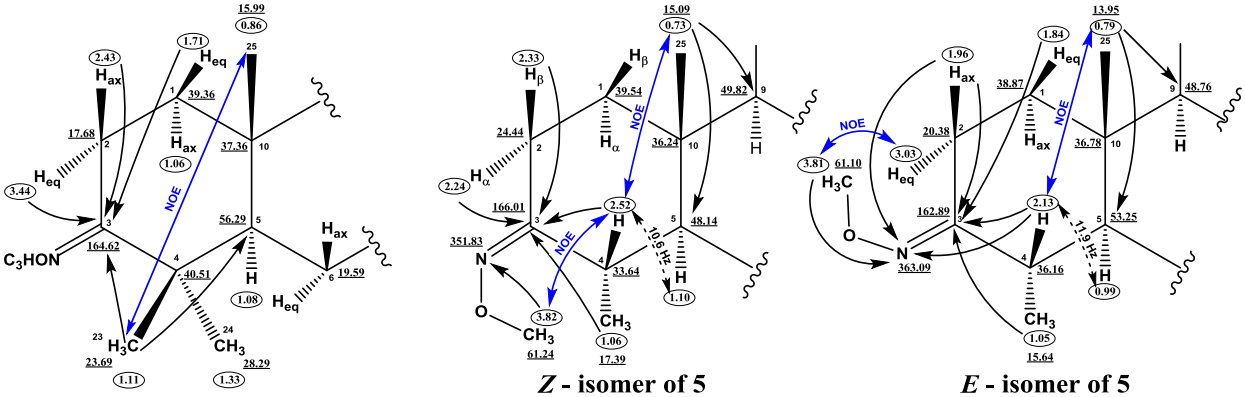


***Z* - isomer of 4**



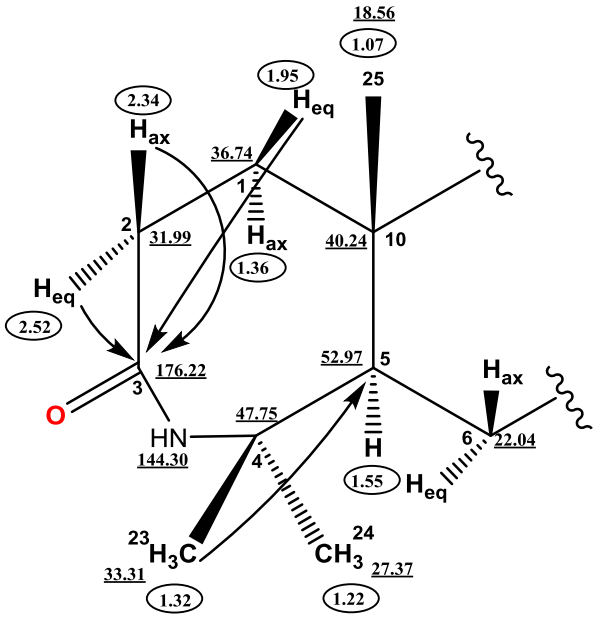
***E* - isomer of 4**

Figure S16. Comparison of spectral characteristics of compounds **10** and **5** (*Z*- and *E*-isomers)

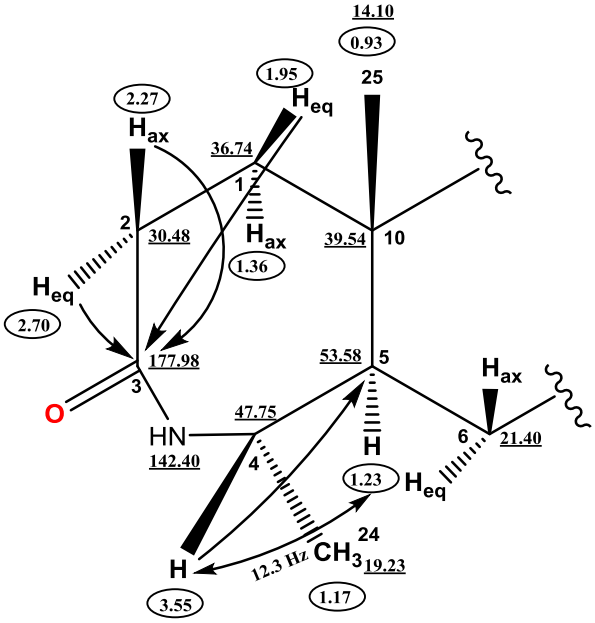


10 ***Z*** **- isomer of 5** ***E*** **- isomer of 5**

Figure S17. Comparison of spectral characteristics of compounds **11** and **6**



11



6

Figure S18. Comparison of spectral characteristics of compounds **12** and **7**

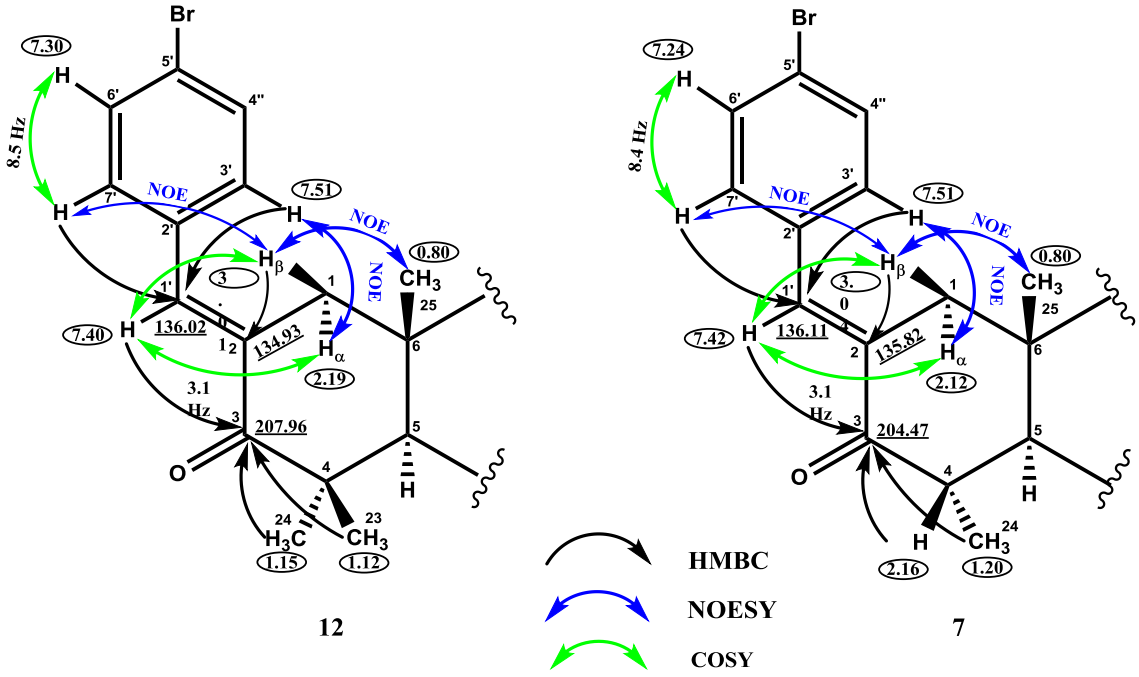


Figure S19. Expanded and assigned ^{1}H NMR spectra of compound: a) **4** and b) **5** in $CDCl_3$

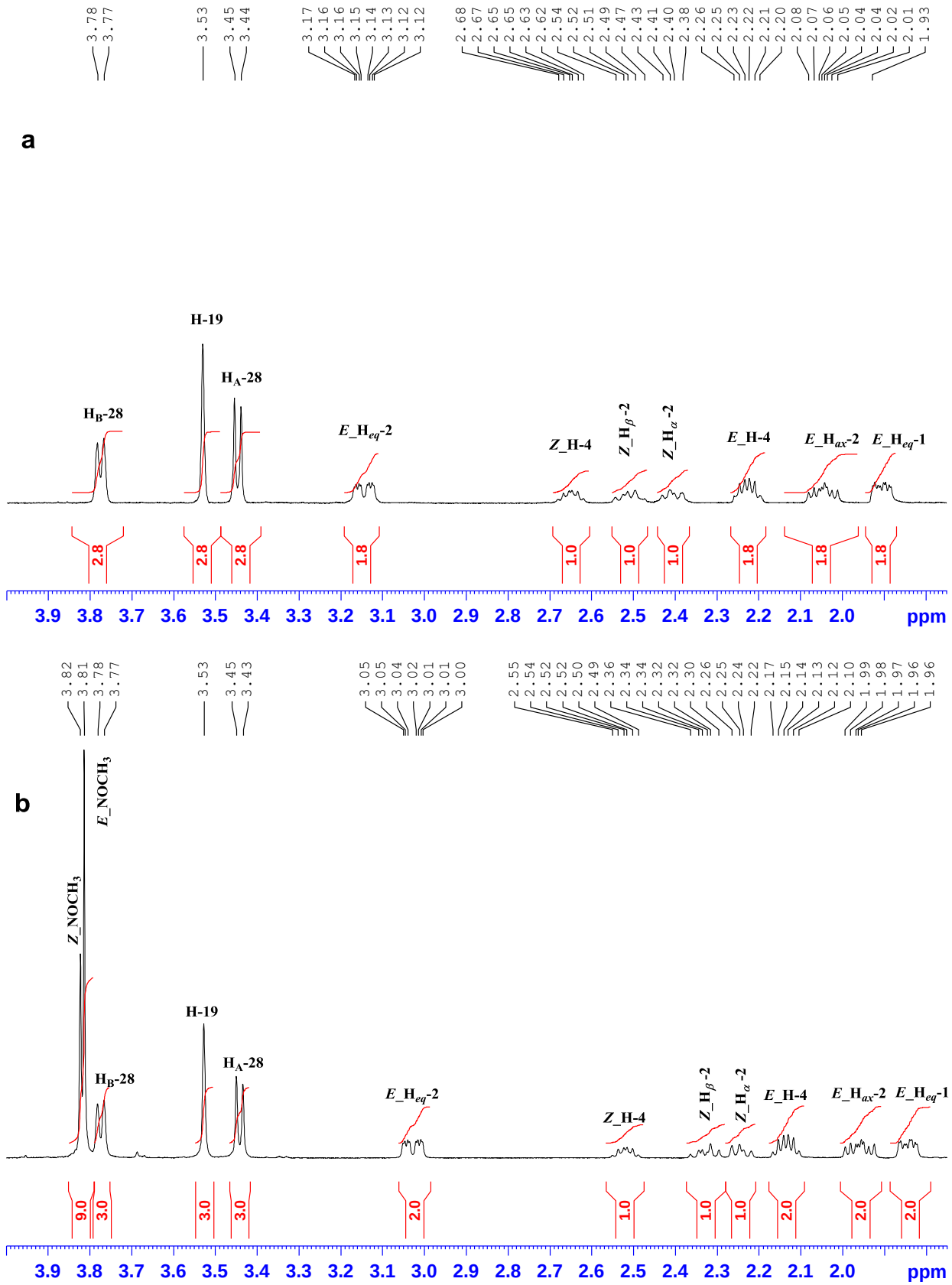
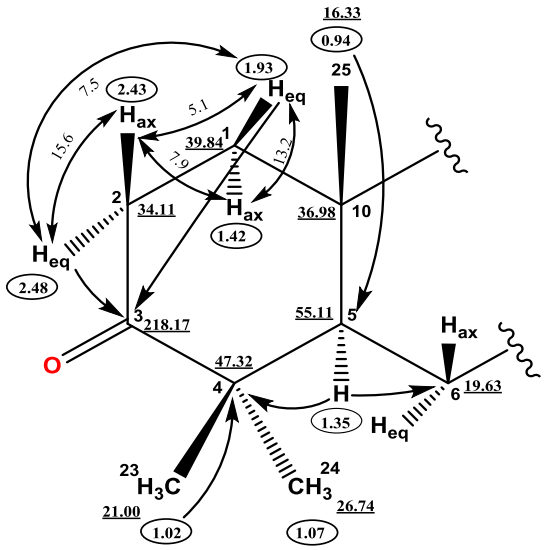
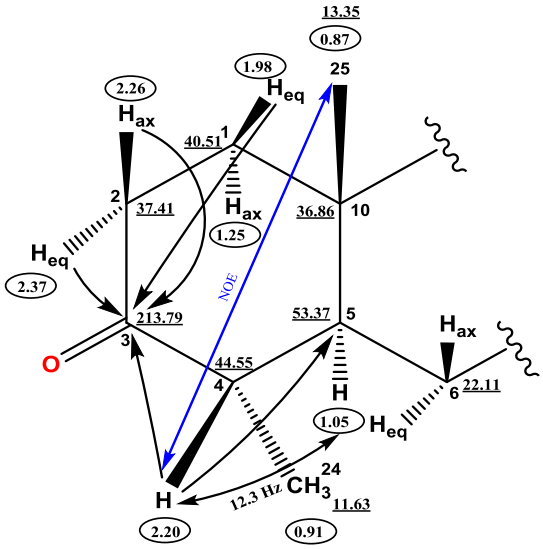


Figure S20. Comparison of the spectral characteristics at A-ring for 3-oxo-allobetulin **8** (left) and 3-oxo-24-nor-allobetulin **2** (right)



8



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