Accepted Manuscript

Hederagenin as a triterpene template for the development of new antitumor compounds

Diego Rodríguez-Hernández, Antonio J. Demuner, Luiz C.A. Barbosa, René Csuk, Lucie Heller

PII: S0223-5234(15)30288-9

DOI: 10.1016/j.ejmech.2015.10.006

Reference: EJMECH 8141

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 3 July 2015

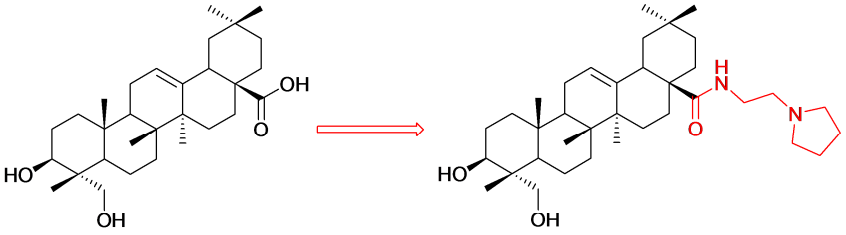
Revised Date: 2 October 2015

Accepted Date: 5 October 2015

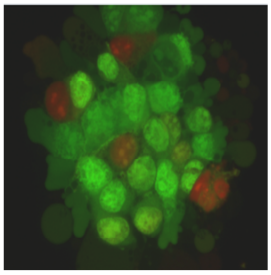
Please cite this article as: D. Rodríguez-Hernández, A.J. Demuner, L.C.A. Barbosa, R. Csuk, L. Heller, Hederagenin as a triterpene template for the development of new antitumor compounds, *European Journal of Medicinal Chemistry* (2015), doi: 10.1016/j.ejmech.2015.10.006.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Graphical abstract



Hederagenin **28**



28, EC_{50} = 1.1 μM (ovarian carcinoma)

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

HEDERAGENIN AS A TRITERPENE TEMPLATE FOR THE DEVELOPMENT OF NEW ANTITUMOR COMPOUNDS

Diego Rodríguez-Hernández[a,b], Antonio J. Demuner[a], Luiz C. A. Barbosa[a,b*], René Csuk[c*], Lucie Heller[c]

[a]*Department of Chemistry, Universidade Federal de Viçosa, Av. P. H. Rolfs, s/n, CEP 36570-900, Viçosa, MG, Brazil.*

[b]*Department of Chemistry, Universidade Federal de Minas Gerais, Av. Pres. Antônio Carlos 6627, Campus Pampulha, CEP 31270-901, Belo Horizonte, MG, Brazil.*

[c]*Martin-Luther-University Halle-Wittenberg, Organic Chemistry, Kurt-Mothes-Str.2, D-06120 Halle (Saale) Germany*

* Corresponding author: Tel.: +55 31 34093396; fax: +55 31 34095757; E-mail addresses: lcab@ufmg.br (L.C.A. Barbosa) or rene.csuk@chemie.uni-halle.de (R. Csuk).

Abstract:

In this study, a series of novel C-28 esters and amides derivatives of hederagenin (**He**) were designed and synthetized in attempt to develop potent antitumor agents. Their structures were confirmed by MS, IR, ^{1}H NMR and ^{13}C NMR spectroscopic analyses and their cytotoxic activities were screened in SRB assays using a panel of six human cancer cell lines. Although most of the compounds displayed moderate to high levels of cytotoxic activity they were all more potent than the natural product **He**. The most active compounds had either an ethylpyrimidinyl (**27**) or an ethylpyrrolidinyl (**28**) substituent, with EC_{50} in the range of 1.1-6.5 µM for six human cancer cell lines. Notably, this corresponds to an approximately 30-fold times greater potency than **He**.

Keywords: *Sapindus saponaria*; pentacyclic triterpenes; hederagenin derivatives; SRB assay; folk medicinal plant.

ACCEPTED MANUSCRIPT

1. Introduction

Natural products have been used to treat human diseases for thousands of years. The results of these treatments have been variable and inconsistent, but advances in pharmaceutical science have seen natural products used as models for the development of new drugs, including certain anti-cancer compounds. Within the plethora of known natural products, triterpenoic acids are considered promising candidates for anti-cancer drug development [1-2].

Among the many sources of bioactive natural products are the fruits of *Sapindus saponaria* L., (Sapindaceae), popularly known in Brazil as "sabão-de-soldado" (soldier soap) and "saboeiro" (soap-maker). This medium sized tropical tree found principally in South America and India, produces great amounts of small fruits where a sap is accumulated [3]. In the tropics these fruits are mainly used as a substitute for soap. However, they are also used in the folk medicine for treating skin lesions, inflammation and ulcers [3]. In their pericarps these fruits accumulate great quantities of saponins carrying the aglycone hederagenin. Hederagenin is a pentacyclic triterpene possessing two hydroxyl groups in ring A, a double bond in ring C and a carboxylic group at C-28 position. In addition, hederagenin acts as a chemotaxonomic marker for plants of the Sapindaceae family. Other plant families like the Dipsacaceae also contain large amounts of hederagenin as aglycone of saponins [4]. Furthermore it is known that this oleane type triterpenoic acid shows some interesting biological properties, such as an anti-inflammatory [5], antifungal [6-7], antimicrobial [8-9] and an anticancer activity [10].

Our group and others have demonstrated that structural modifications of sesquiterpenoids [11], diterpenoids [12] and triterpenoids [2, 13-15] might have a high impact onto their biological activities.

Compared to other triterpenoids, modification of hederagenin has had little attention and, therefore, very little is known with respect to their antitumor properties. To the best of our knowledge only one report [10] has been published showing hederagenin being moderately active for A549 cancer cell line (EC_{50} = 39±6 µM) whilst being inactive for DLD-1 cells.

Recently, it was shown that the cytotoxic activity of maslinic acid (a constitutional isomer of the hederagenin) increases by esterification at position 28. Although, introducing a benzylic substituent at C-28 did not result in significant improvement of the cytotoxicity it improved selectivity for tumor cells [13].

In addition, antitumor screening of derivatives of maslinic, oleanolic or ursolinic acids revealed the presence of a C = O moiety to be essential for antitumor activity [16-18]. It has also been shown that bulky ester residues seems to interact quite well with an hitherto unknown intracellular target/receptor [13].

During the last few years we have been investigating the use of natural products as lead structures for the discovery of novel putative pharmaceutical drugs [19-21]. Consequently, in line with this interest, we report here our preliminary findings involving the hederagenin scaffold.

2. Results and discussion

2.1. Chemistry

Firstly, hederagenin (**He**) was isolated from the pericarp of *S. saponaria* using a previously reported procedure [22] as detailed in the experimental section. In general, the yield of hederagenin from the dried pericarp of the fruits was around 0.9%. The isolated compound was fully characterized by comparing its spectroscopic data with those previously reported [30-32], along with its melting point. Subsequently, **He** was transformed into various esters (**1-23**), by its reaction with alkyl bromides in the presence of finely grounded potassium carbonate. Aqueous work-up using aqueous HCl (5%), then washing with brine facilitated the isolation of the products [23]. Thus, following this simple general procedure, the target alkyl esters were obtained in yields ranging between 35% and 90% (Fig. 1).

[Insert Figure 1]

Reaction of hederagenin with several amines in the presence of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluroniumtetrafluoro-boratetetrabutyl (TBTU) as a coupling catalyst provided the desired amides (**25-30**) in 72% to 96% yield (Fig. 2) [24].

[Insert Figure 2]

The structures of the hederagenin-derived esters and amides (**1-30)** were confirmed by extensive spectroscopic analysis. All compounds showed in their respective IR spectra typical signals expected for the functional groups being present in the molecules. For example, in the IR spectra of **1-23**, strong absorption bands around 1720-1735 cm^{-1} due to a

C = O stretching of esters were observed, associated with absorptions between 1250-1150 cm^{-1} due to stretching of C-CO-O. For compounds (**25-30**) the C=O absorptions were observed around $\bar{\upsilon}$ = 1620-1650 cm^{-1} being typical for amides. In the case of alkynes **17** and **29** the bands for the C≡C were observed between $\bar{\upsilon}$ = 2118-2120 cm^{-1} [25].

In the ^{1}H NMR spectra the signals of the aromatic hydrogens were detected between 7.20 and 8.50 ppm; for compound **18**, the resonances due to aromatic hydrogen atoms were found at 8.20 ppm and 7.50 ppm each as a doublet (J = 8.7 Hz). For the amides (**25-30**) the signals of the NH were observed between 6.20-6.30 ppm. In addition, all ^{13}C NMR signals for the triterpenoic skeleton in this compound series were similar with the exception of the signals for C-28 and the group attached at this position. For carbon C-28 a shift to higher field for the esters and amides was observed as compared to parent **He** (δ = 180.7 for **He** to δ = 177.5±0.75 for the ester carbonyls, and to δ = 178±0.75 for the amide carbonyls). A detailed assignment of the NMR spectra (^{1}H and ^{13}C) for all compounds is given in the supplementary material associated to this paper. The assignments were possible by using 2D NMR techniques (when required) and the data were consistent with the proposed structures.

2.2. Biological screening

Natural triterpenes as glycyrrhetinic, betulinic, ursolic, oleanolic and maslinic acid, and their derivatives, are well-known for their anti-cancer activities [2, 26]. Beside cytotoxicity, many of these compounds have been shown to trigger apoptosis. For the parent compounds, EC_{50} values between 10-80 µM have been determined; some of them were also shown to

trigger apoptosis. Hederagenin derivatives **1-30** were tested for their cytotoxic activity using a photometric sulforhodamine B assay (SRB) [27] and the EC_{50} values were determined for six different human cancer cell lines [518A2 (melanoma cells), A2780 (ovarian carcinoma), HT29 (colon adenocarcinoma), MCF7 (breast adenocarcinoma), A549 (lung cancer), 8505C (thyroid carcinoma)]. The EC_{50} values were calculated from dose response curves applying a non-linear regression using the two parametric Hills-slope equation (Table 1).

[Insert Table 1]

Among all esters and amides, compounds **1-29**, were more cytotoxic than parent **He**, while the 1-ethylmorpholinyl amide (**30**) was the only one with a reduced activity (Fig. 3). These results support the hypothesis that the presence of a bulky group bonded to carbonyl-28 of the triterpene skeleton modulates their cytotoxic activity. Similar results have previously been observed for glycyrrhetinic acid: in its natural form (free acid) a 3-fold lower cytotoxicity was determined as compared to the corresponding esters derivatives [28-29].

[Insert Figure 3]

The amides carrying an ethylpyrimidinyl (**27**) or ethylpyrrolidinyl (**28**) moiety were the most active derivatives for all cell lines tested, showing EC_{50} values ranging between 1.3-6.5 µM for **27** and values between 1.1-3.9 µM for **28**. This corresponds to an approximately 30 times higher cytotoxicity than parent **He** (Fig. 4). In addition, the esters carrying a benzyl (**1**) or an *ortho*-nitrobenzyl (**18**) group were the most active among all ester derivatives for the cells lines tested, exhibiting EC_{50} values between 7.0-9.7 µM for **1** and

139 6.1-8.4 µM for **18**. These results revealed that compounds **1** and **18** are approximately 20
140 times more cytotoxic than hederagenin (Fig. 4).

141 **[Insert Figure 4]**

142 For all cells lines tested (Fig. 5) the activity of *para* substituted esters (**20, 21, 22**)
143 decreased with the atomic radius of the halogen substituent. Furthermore, amides derived
144 from heterocycles carrying an additional nitrogen atom (**27, 28**) were the most active
145 compounds for all cell lines evaluated in this study.

146 **[Insert Figure 5]**

147 To gain a deeper insight into the mode of action of these molecules some additional
148 experiments were performed. For such experiments we have chosen only the most active
149 compound **28**. For this compound, the death of A2780 human ovarian cancer cells was
150 investigated using an acridine orange/propidium iodide assay (AO/PI) and fluorescence
151 microscopy (Fig. 6). Thus, the cells were treated for 48 h with an EC_{90} concentration of **28**.
152 The results from this assay indicated that a controlled cell death has occurred as evidenced
153 by the presence of green fluorescent A2780 cells. Several cells showed an orange color, an
154 evidence that they died by secondary necrosis.

155

156 **[Insert Figure 6]**

157

3. Conclusion

To sum up, a series of 30 different esters and amides of hederagenin has been designed and synthesized. All these compounds (carrying a bulky group at C-28) were screened for their cytotoxic activity employing a panel of six human cancer cell lines including melanoma cells, ovarian carcinoma, colon adenocarcinoma, breast adenocarcinoma, lung cancer and thyroid carcinoma using a photometric sulforhodamine-B assay. From these data, it was evident that almost all compounds exhibited higher cytotoxicity activity for all tested cancer cell lines compared to hederagenin. Amides carrying a heterocyclic group (**27** and **28**) were found to be the most promising derivatives showing EC_{50} ranging between 1.1-6.5 µM. Moreover, as shown by an additional AO/PI staining experiment, it was found that compound **28** mainly acts by apoptosis. Further intensive modifications at C-28 and studies concerning the mode of action of these compounds are currently performed in our laboratory, and the results will be reported in due course.

4. Experimental part

4.1. General procedures

Reagents were obtained from Sigma-Aldrich (Milwaukee, Wisconsin, USA) and were used without any purification. Solvents were purchased from Vetec (Rio de Janeiro, Brazil). Analytical thin layer chromatography (TLC) was performed on silica gel 60 F_{254} 0.2 mm thick plates (supplied by Merck, Rio de Janeiro, Brazil), and the spots were visualized by UV-B light or by spraying with phosphomolybdic acid in 10% EtOH, followed by heating. Flash column chromatography (typical size of 20 cm length and 2 cm of diameter) was

179 performed using silica gel 230-400 mesh. All compounds were fully characterized by IR,
180 EI-MS, ^{1}H NMR and ^{13}C NMR spectroscopy. Infrared spectra were recorded on a Perkin-
181 Elmer Paragon 1000 FTIR spectrophotometer, preparing the samples as potassium bromide
182 disks (1% w/w). Mass spectra were recorded on a Shimadzu GCMS-QP5050A instrument
183 by direct insertion, using EI mode (70 eV). High resolution mass spectra were recorded on
184 a Bruker Micro TOF (resolution = 10,000 FWHM) using electro spray ionization (ESI) and
185 the result reported to four decimal figures. Elemental analyses were measured on a Foss-
186 Heraeus Vario EL unit. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury
187 300 spectrometer at 300 and 75 MHz, respectively, using $CDCl_3$ as solvent and TMS as
188 internal reference, unless otherwise stated. Melting points were measured on a MQAPF-
189 301 apparatus and were not corrected.

190 The ^{1}H NMR spectra for all compound were assigned for the signals that were clearly well
191 defined as described for each compound, following the structural numbers presented in the
192 Supporting Material. For all compounds a multiplet was observed in the range of δ = 0.5 to
193 2.5 ppm, so the hydrogen atoms in this range could not be assigned.

194 *4.2. Isolation of Hederagenin (**He**)*

195 Dried and ground pericarp of *Sapindus saponaria* L., (2 kg) was extracted with methanol.
196 A total of 3 extractions at room temperature was carried out, keeping the solvent in contact
197 with the sample for 24 hours. For each extraction 1 L of solvent was used. The combined
198 methanol extracts were concentrated under reduced pressure in a rotary evaporator to afford
199 650 g of a crude brown residue. To 300 g of this residue a solution of sulfuric acid in

methanol (1.5 L, 5% v/v) was added, and the mixture was heated under reflux for 3 hours. After this hydrolysis, the pH was adjusted to 6-7 by adding potassium hydroxide in methanol (5% v/v). To the resultant mixture activated charcoal (100 g) was added, followed by a heating under reflux for 30 min. The resulting mixture was filtered through a Celite® pad (2 g), and the filtrate was concentrated under reduced pressure to approximately 10% of the initial volume. To this solution 1 L of distilled hot water was added, and the residual methanol was evaporated under reduced pressure. The residue was filtered off to afford crude hederagenin as a pale brown solid.

This crude material was washed twice with hot of acetonitrile (1 L) affording 8.5 g (2.8% of dry mass) **He** as a white solid, m.p. 318-320 ^{o}C (lit.: 317-320 ^{o}C [22]); R_f = 0.24 (hexane/ethyl acetate, 1:1 v/v). All spectroscopic data (IR, MS, and NMR) were in full agreement with the literature [30-32].

*4.3. General procedure for the synthesis of ester **1-23***

A 25 mL one necked round-bottomed flask was charged with a solution of hederagenin (100 mg, 0.23 mmol) in dry DMF (5 mL), and finely grounded potassium carbonate (1 mmol) was added. To this mixture an alkyl bromide (0.46 mmol) was added, and the reaction was stirred for 18 h at 25 ºC. The reaction mixture was quenched by adding ethyl acetate (20 mL), washed with an aqueous solution of HCl (5%, 50 mL), followed by brine (50 mL) and dried over Na_2SO_4. The mixture was filtered, the solvent was removed under reduced pressure in a rotary evaporator, and the crude product was obtained as a yellow

solid. This solid was purified by silica gel column chromatography eluting with hexane/ethyl acetate (3:1 v/v) to afford the pure product as a solid.

All yields and physical and spectroscopic data for the compounds are included in the Supplementary Material

*4.4. Benzotriazol-1-yl-(3β)3,23-dihydroxyolean-12-en-28-oate (**24**)*

A 50 mL two-necked round bottomed flask was charged with hederagenin (100 mg, 0.23 mmol) TBTU (0.25 mmol), DIPEA (0.25 mmol) and THF (8 mL). The mixture was stirred at room temperature overnight. The precipitate was filtered off, and the filtrate was diluted with ethyl acetate (20 mL). The organic layer was washed with water (50 mL) and brine (50 mL) and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the crude product as a brown solid. This crude product was purified by column chromatography (silica gel, hexane/ethyl acetate, 2:1 v/v) to afford **24** in 92% yield (105 mg).

White solid; m.p. 232-234 ^{o}C; R_f = 0.43 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3400, 1806, 1088, 1050, 998, 738 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.03 (dt, 1H, J = 8.1, 0.8 Hz, H-34), 7.51 (ddd, 1H, J = 9.0, 8.0, 0.9 Hz), 7.40 (m, 1H, H-35), 7.35 (m, 1H, H-36), 5.36 (t, 1H, J = 3.5 Hz, H-12), 3.73 (d, 1H, J = 10.4 Hz, H-23_a), 3.64 (brt, 1H, J = 7.6 Hz, H-3), 3.42 (d, 1H, J = 10.4 Hz, H-23_b), 2.96 (dd, 1H, J = 13.4, 4.2 Hz, H-18), 1.10 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.84 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.75 (C-28), 143.64 (C-13), 142.19 (C-31), 122.88 (C-32), 128.61 (C-36), 124.79 (C-35), 123.98 (C-12), 120.59 (C-34), 108.27

241 (C-33), 76.79 (C-3), 72.02 (C-23), 49.90 (C-9), 47.73 (C-17), 47.67 (C-5), 45.58 (C-19),
242 42.06 (C-4), 41.92 (C-14), 41.72 (C-18), 39.60 (C-8), 38.34 (C-1), 37.03 (C-10), 33.80 (C-
243 21), 33.04 (C-29), 32.75 (C-7), 32.56 (C-22), 30.77 (C-20), 28.17 (C-15), 26.74 (C-27),
244 25.90 (C-2), 23.65 (C-30), 23.57 (C-11), 23.25 (C-16), 18.58 (C-6), 17.42 (C-26), 15.88
245 (C-25), 11.61 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{36}H_{51}N_3O_4]^+$: 589.8079,
246 found 590.3959. CHN calcd.: C, 73.31; H, 8.71; found: C, 73.07; H, 8.95.

247 *4.5. General procedure for the synthesis of amides **25-30***

248 A 50 mL two-necked round bottomed flask was charged with hederagenin (100 mg, 0.23
249 mmol), the appropriate amine (0.50 mmol) and THF (8 mL). To the resultant solution, kept
250 under nitrogen atmosphere, were added TBTU (0.25 mmol) and DIPEA (0.45 mmol). This
251 reaction mixture was stirred vigorously for 2 h at room temperature, until TLC analysis
252 revealed a total consumption of the starting material. The mixture was filtered, and the
253 filtrate was diluted with ethyl acetate (20 mL), washed with water (50 mL) and brine (50
254 mL) and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the
255 crude product. This product was purified by silica gel column chromatography (silica gel,
256 hexane/ethyl acetate, 1:2 v/v).

257 All yields and physical and spectroscopic data for compounds **25-30** are presented in the
258 Supplementary Material associated to this paper.

259 *4.6.* Cytotoxicity assay

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (SRB, procured from Sigma-Aldrich, Wilwaukee, Wisconsin, USA) micro-culture colorimetric assay. In short, exponentially growing cells were seeded into a 96-well plate on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 hours, the cells were treated with serial dilutions of the compounds (0-30 µM) for 96 hours. The final concentration of DMSO never exceeded 0.5%, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After 96 hours of treatment, the supernatant medium was discarded from the 96-well plates, and the cells were fixed with 10% TCA. For a thorough fixation, the plates were allowed to rest at 4 °C. After fixation, the cells were washed in a strip washer. The washing was done four times with water using alternate dispensing and aspiration procedures. The plates were dyed with 100 µL of 0.4% SRB for about 20 min. After dying, the plates were washed with 1% acetic acid to remove the excess of the dye and allowed to air-dry overnight. Tris base solution (100 µL, 10 mM) was added to each well and absorbance was measured at λ = 570 nm (using a 96 well plate reader, Tecan Spectra, Crailsheim, Germany). EC_{50} values were calculated from semi logarithmic dose response curves by non-linear regression applying a two parametrical Hill-slope equation. Values are given with a confidence interval CI = 95%.

The AO/PI assay was performed as previously described [1, 18]. In short, approximately 500 000 A2780 cells were seeded in cell culture flasks and were allowed to grow for 24 h. The medium was removed, and the loaded medium (applying an EC_{90} concentration) was added. After 48 h, the supernatant medium was collected and centrifuged; the pellet was

suspended in phosphate-buffer saline (PBS) and centrifuged again. The liquid was removed, and the pellet was suspended in PBS. After mixing the suspension with a solution of AO/PI, analysis was performed under a fluorescence microscope. While a green fluorescence showed apoptosis, a red colored nucleus indicated necrotic cells.

Acknowledgements

We are grateful to the following Brazilian agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for research fellowships (AJD, LCAB). Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) for financial support and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for research fellowship (DRH). The cell lines were kindly provided by Dr. Thomas Müller (Dept. of Haematology/Oncology, Martin-Luther University Halle-Wittenberg). Support by the "Gründerwerkstatt – Biowissenschaften" is gratefully acknowledged.

References

1. B. Siewert, R. Csuk, Membrane damaging activity of a maslinic acid analog, Eur. J. Med. Chem. 74 (2014) 1-6.
2. J.A.R. Salvador, Pentacyclic Triterpenes as Promising Agents in Cancer, Nova Science Pub Inc, (2010).
3. A.L.M. Albiero, M. Sarragiotto, A. Fujimura, E. Bacchi, Cytotoxic-activity of *Sapindus saponaria* L. fruits on Ehrlich Ascitic Tumor cells, Acta Farm. Bonaerense. 20 (2001) 169-171.

4. D. Rodríguez-Hernández, A.J. Demuner, A. Nain-Perez, L.C.A. Barbosa, A. Oliveros-Bastidas, Aportes fitoquimicos a la sistematica de la familia Dipsacaceae, Av. Cien. Ing. 5 (2014) 61-81.
5. K. Takagi, K. Park, H. Kato, Anti-inflammatory Activities of Hederagenin and Crude Saponin isolated from *Sapindus mukorossi* GAERTN, Chem. Pharm. Bull. 28 (1980) 1183-1188.
6. P. Timon, J. Julien, M. Gasquet, G. Balansard, P. Bernard, Research of antifungal activity from several active principle extracts from climbing-ivy: *Hedera helix* L., Ann. Pharm. Fr. 38 (1980) 545-552.
7. S. Saha, S. Walia, J. Kumar, P. Balraj, Structure-biological activity relationships in triterpenic saponins: the relative activity of protobassic acid and its derivatives against plant pathogenic fungi, Pest. Manag. Sci. 66 (2010) 825-831.
8. A. Ribeiro, C.L. Zani, T.M.A. Alves, N.M. Mendes, M. Hamburger, K. Hostettmann, Molluscicidal saponins from the pericarp of *Sapindus saponaria*, Int. J. Pharmacognosy. 33 (1995) 177-180.
9. A.L.M. Albiero, J.A.A. Sertié, E.M. Bacchi, Antiulcer activity of *Sapindus saponaria* L. in the rat, J. Ethnopharmacol. 82 (2002) 41-44.
10. C. Gauthier, J. Legault, K. Girard-Lalancette, V. Mshvildadze, A, Pichette. Haemolytic activity, cytotoxicity and membrane cell permeabilization of semi-synthetic and natural lupane- and oleanane-type saponins, Bioorg. Med. Chem. 17 (2009) 2002-2008.
11. L.C.A Barbosa, A.V. Costa, D. Piló Veloso, J.L.C. Lopes, M.G. Hernandez Terrones, B. King Diaz, B. Lotina Hennsen, Phytogrowth-Inhibitory lactones

derivatives of Glaucolide. B. Z. Naturforsch., B: Chem. Sci. 59c (2004) 803-810.

12. A.J. Demuner, L.C.A. Barbosa, D.P. Veloso, O. Howarth, Synthesis and plant growth regulatory activity of 6α,7β-Dihydroxyvouacapan-17β-oic Acid derivatives, Aust. J. Chem. 51 (1998) 61-66.
13. B. Siewert, E. Pianowski, A. Obernauer, R. Csuk, Towards cytotoxic and selective derivatives of maslinic acid, Bioorg. Med. Chem. 22 (2014) 594-615.
14. R. Csuk, S. Schwarz, B. Siewert, R. Kluge, D. Ströhl, Synthesis and antitumor activity of ring A modified glycyrrhetinic acid derivatives, Eur. J. Med. Chem. 46 (2011) 5256-5369.
15. R. Csuk, A. Niesen-Barthel , R. Schafer, A. Barthel , A. Al-Harrasi, Synthesis and antitumor activity of ring A modified 11-keto-β-boswellic acid derivatives, Eur. J. Med. Chem. 92 (2015) 700-711.
16. S. Fulda, Modulation of apoptosis by natural products for cancer therapy, Planta Med. 76 (2010) 1075-1079.
17. H, Chen, Y. Gao, A. Wang, X. Zhou, Y. Zheng, J. Zhou, Evolution in medicinal chemistry of ursolic acid derivatives as anticancer agents, Eur. J. Med. Chem. 92 (2015) 648-655.
18. B. Siewert, E. Pianowski, R. Csuk, Ester and amide of maslinic acid trigger apoptosis in human tumor cells and alter their mode of action with respect to the substitution pattern at C-28, Eur. J. Med. Chem. 70 (2013) 259-272.
19. U.A. Pereira, L.C.A. Barbosa, C.R. Maltha, A.J. Demuner, M.A. Masood, A.L. Pimenta, Inhibition of *Enterococcus faecalis* biofilm formation by highly active

lactones and lactams analogues of Rubrolides, Eur. J. Med. Chem. 82 (2014) 127-138.

20. M.F. Oliveira, L.C.A. Barbosa, F.M.D. Ismail, The diverse pharmacology and medicinal chemistry of Phosphoramidates - a review. RSC Advances: an international journal to further the chemical sciences 4 (2014) 18998-19012.
21. U.A. Pereira, L.C.A. Barbosa, C.R. Maltha, A.J. Demuner, M.A. Masood, A.L. Pimenta, γ-Alkylidene-γ-lactones and isobutylpyrrol-2(5H)-ones analogues to rubrolides as inhibitors of biofilm formation by Gram-positive and Gram-negative bacteria, Bioorg. Med. Chem. Lett. 24 (2014) 1052-1056.
22. T. Ohgi, H. Yoshifusa, K. Kandori, Triterpene derivative and medicinal composition, (1999) US 5885992.
23. R. Csuk, S. Schwarz, B. Siewert, R. Kluge, D. Ströhl, Conversions at C-30 of glycyrrhetinic acid and their impact on antitumor activity, Arch. Pharm. 345 (2012) 223-230.
24. K. Prabakaran, F. Nawaz, J. Sung, An efficient 2-(1H-benzotriazole-1yl-)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU)-mediated synthesis of 5-(trifluoromethyl)-*N*-alkyl-1-(3-phenylisoquinoline-1-yl)-1H-pyrazole-4-carboxamides, Res. Chem. Intermed. 38 (2012) 615-627.
25. L.C.A. Barbosa, Espectroscopia no Infravermelho na caracterização de compostos orgânicos. Viçosa: Editora UFV, (2011).
26. J.A.R. Salvador, V.M. Moreira, B.M.F. Goncalves, A.S. Leal, Y. Jing, Ursane-type pentacyclic triterpenoids as useful plant forms to discover anticancer drugs, Nat. Prod. Rep. 29 (2012) 1463-1479.

27. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New colorimetric cytotoxicity assay for anticancer-drug screening, J. Natl. Cancer Inst. 82 (1990) 1107-1112.
28. S. Schwarz, R. Csuk, Synthesis and biological activity of some antitumor active derivatives from glycyrrhetinic acid, Bioorg. Med. Chem. 18 (2010) 7458–7474.
29. R. Csuk, S. Schwarz, R. Kluge, D. Ströhl, Synthesis and biological activity of some antitumor active derivatives from glycyrrhetinic acid, Eur. J. Med. Chem. 45 (2010) 5718-5723.
30. A. Pawan, M.C. Dhara, ^{13}C NMR spectroscopy of oleanane triterpenoids, Prog. Nucl. Magn. Reson. Spetrosc. 24 (1992) 1-90.
31. A. Khalik, T. Miyase, H. El-Ashaal, F.R. Melek, Triterpenoid saponins from *Fagonia cretica*, Phytochemistry 54 (2000) 853-859.
32. N. Boke, Aristatosides A-C, hederagenin-type triterpene saponins from *Cephalaria aristata*, Phytochemistry Lett. 8 (2014) 149–155.

ACCEPTED MANUSCRIPT

Table 1.
Cytotoxicity for **He** and analogs **1-30** (EC_{50} values in μM from SBR assays after 96 h of treatment; the values are averaged from at least three independent experiments performed each in triplicate; confidence interval CI = 95%; individual positive (upper value) and negative (lower value) errors are given in the supplementary part); cut-off in all experiments 30 μM except for parent **He** where the cut-off was 60 μM); employing human tumor cell lines.

EC_{50}	518A2	A2780	HT29	MCF7	A549	8505C
He	34.9	19.9	50.0	25.7	29.0	38.0
1	7.8	9.7	9.5	7.9	7.0	7.6
2	10.3	12.9	12.8	10.2	10.9	8.8
3	10.9	14.3	14.3	12.7	13.2	7.8
4	12.9	13.1	16.1	16.5	14.8	14.1
5	10.0	13.4	13.2	12.5	13.2	11.2
6	9.5	12.9	13.0	11.5	11.4	8.0
7	7.5	11.6	12.0	10.2	11.7	8.7
8	11.7	13.6	13.5	12.7	13.6	9.5
9	9.0	12.9	13.2	11.3	12.6	8.4
10	9.2	14.4	13.3	12.7	12.9	9.4
11	11.1	15.0	13.4	12.7	13.1	13.1
12	11.7	13.8	14.8	13.0	13.0	11.9
13	10.9	13.7	14.4	13.5	13.5	9.7
14	8.7	12.1	13.4	9.0	11.4	10.3
15	8.1	11.9	12.0	8.3	8.1	6.6
16	12.7	13.5	14.0	13.4	14.0	12.8
17	12.4	17.0	17.7	15.4	15.9	13.2
18	8.4	7.8	7.8	7.8	6.5	6.1
19	11.8	14.7	14.8	13.7	13.7	8.8
20	10.2	11.4	11.3	9.2	8.5	6.7
21	11.4	13.0	13.5	12.7	9.8	8.1
22	11.6	13.2	13.7	13.5	13.5	8.6
23	13.8	14.6	15.3	14.2	14.2	13.7
24	8.6	8.8	11.2	7.7	9.5	9.5
25	13.7	11.4	15.1	14.6	15.7	13.5
26	12.5	15.9	19.3	15.2	17.2	12.7
27	3.7	1.8	1.3	6.5	2.7	3.6
28	2.0	1.1	1.2	3.7	1.9	1.8
29	21.6	14.8	17.3	17.0	16.8	22.1
30	>30	>30	>30	>30	>30	>30

ACCEPTED MANUSCRIPT

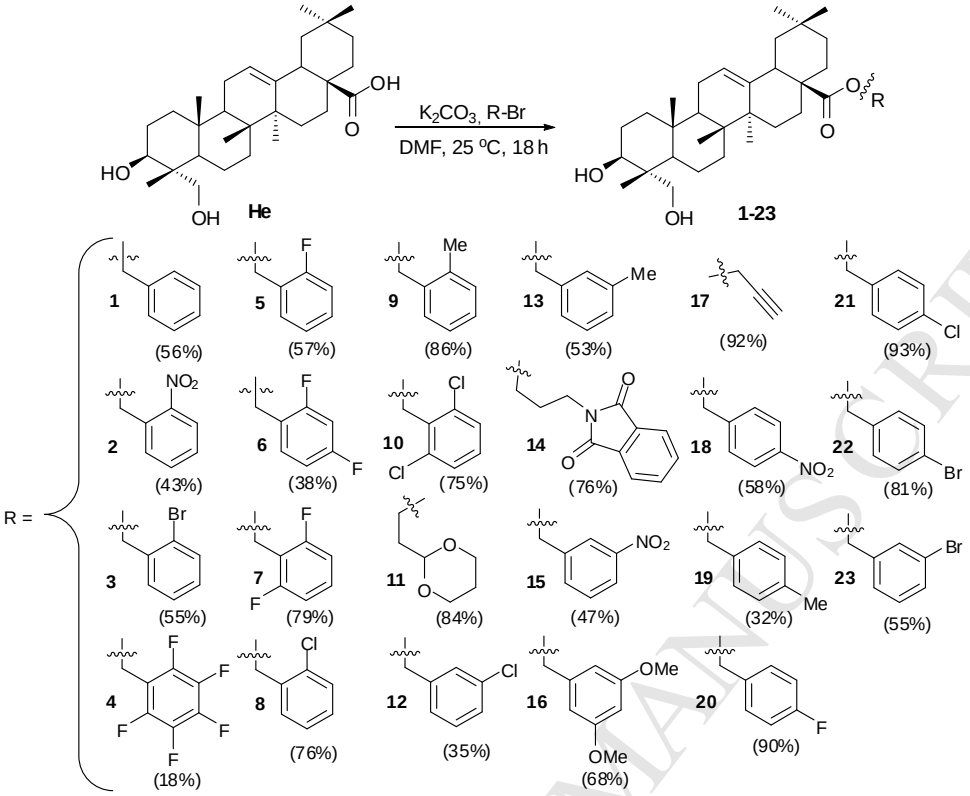


Figure 1. Synthesis of hederagenin ester derivatives **1-23**.

ACCEPTED MANUSCRIPT

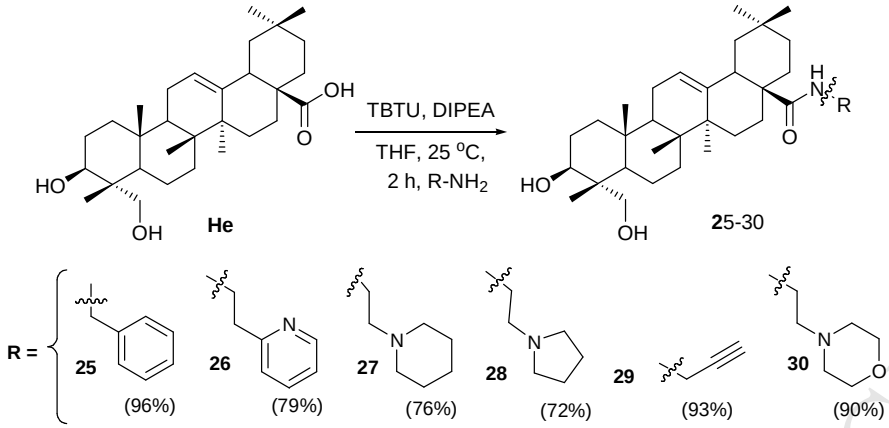


Figure 2. Synthesis of hederagenin amide derivatives **25-30**.

ACCEPTED MANUSCRIPT

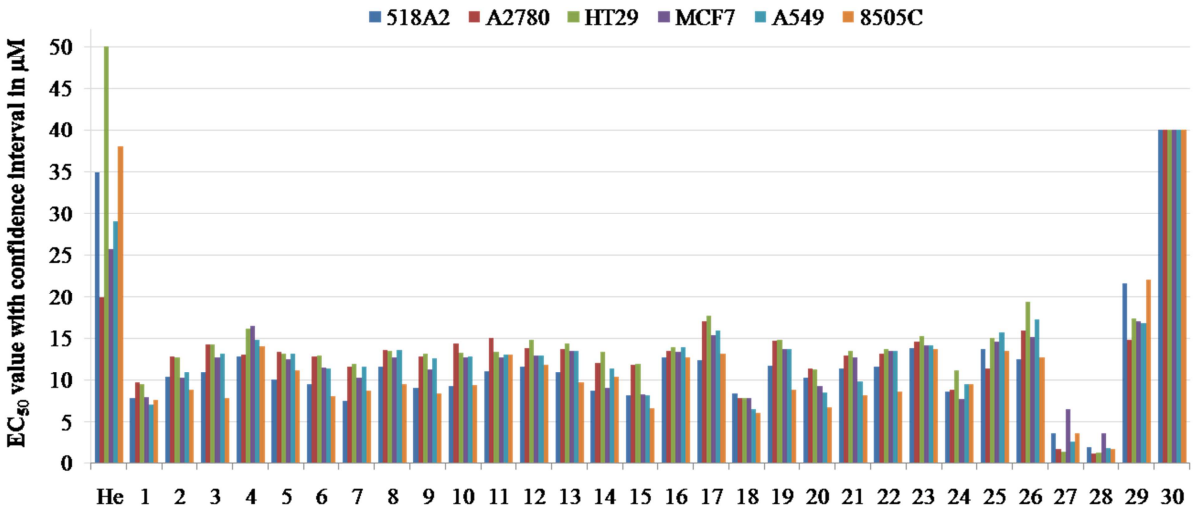


Figure 3. Comparison of EC_{50} values (from SBR assays after 96 h of incubation with several human tumor cell lines, confidence interval = 95%) for hederagenin derivatives **1-30.**

ACCEPTED MANUSCRIPT

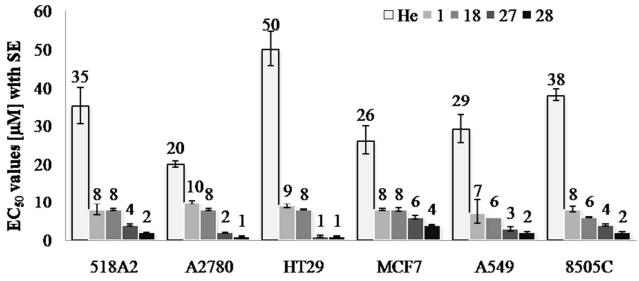


Figure 4. Cytotoxicity (EC_{50} values in µM with standard error (SE), from SBR assays) for **He**, esters **1, 18** and amides **27, 28** for all cells lines tested.

ACCEPTED MANUSCRIPT

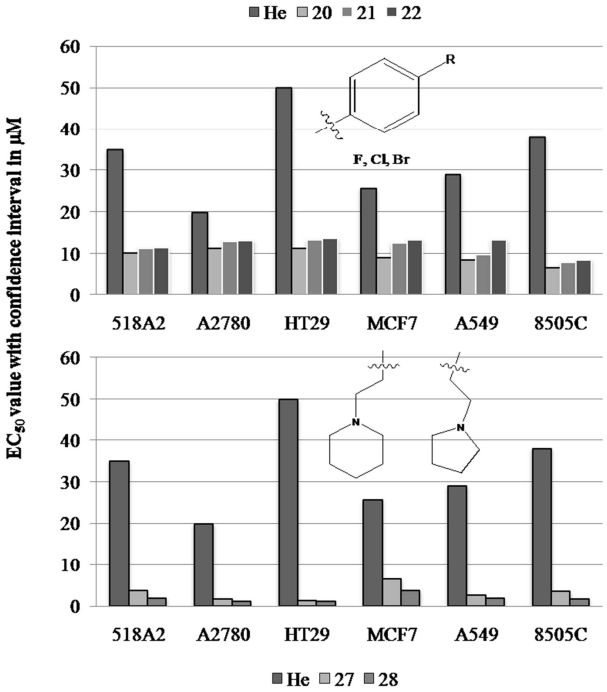


Figure 5. Comparison of cytotoxicity of hederagenin (**He)** and some of its ester (**20**, **21** and **22**) and amide (**27** and **28)** derivatives (EC_{50} in µM from SRB with a confidence interval (95%)) for various human tumor cell lines.

ACCEPTED MANUSCRIPT

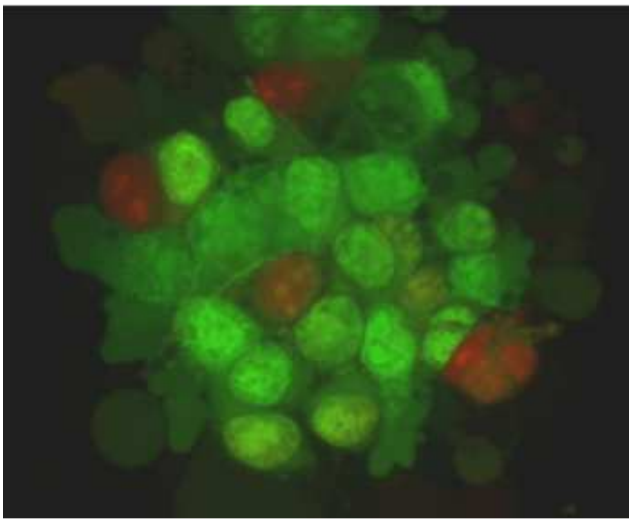


Figure 6. Fluorescence microscopy image of A2780 cells treated with an EC_{90} concentration of hederagenin derivative **28** for 48 hours.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Highlights

- A series of ester and amide derivatives of hederagenin has been synthesized.
- Some derivatives were more actives than hederagenin for six human cancer lines.
- Amides with pyrimidinyl and pyrrolidinyl groups were the most active derivatives.
- EC_{50} values for pyrimidinyl and pyrrolidinyl derivatives ranged from 1.1 to 6.5 µM.
- AO/PI staining experiment showed that compound **28** mainly acts by apoptosis.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Supporting information

HEDERAGENIN AS A TRITERPENE TEMPLATE FOR THE DEVELOPMENT OF NEW ANTITUMOR COMPOUNDS

Diego Rodríguez-Hernández[a,b], Antonio J. Demuner[a], Luiz C. A. Barbosa[a,b*], René Csuk[c*], Lucie Heller[c]

[a]*Department of Chemistry, Universidade Federal de Viçosa, Av. P. H. Rolfs, s/n, CEP 36570-900, Viçosa, MG, Brazil.*

[b]*Department of Chemistry, Universidade Federal de Minas Gerais, Av. Pres Antônio Carlos 6627, Campus Pampulha, CEP 31270-901, Belo Horizonte, MG, Brazil.*

[c]*Martin-Luther-University Halle-Wittenberg, Organic Chemistry, Kurt-Mothes-Str.2, D-06120 Halle (Saale) Germany*

* Corresponding author: Tel.: +55 31 34093396; fax: +55 31 3899 3065; E-mail addresses: lcab@ufmg.br (L.C.A. Barbosa) or rene.csuk@chemie.uni-halle.de (R. Csuk).

CONTENTS:

Section A:

Spectroscopic data of all compounds **2-29**

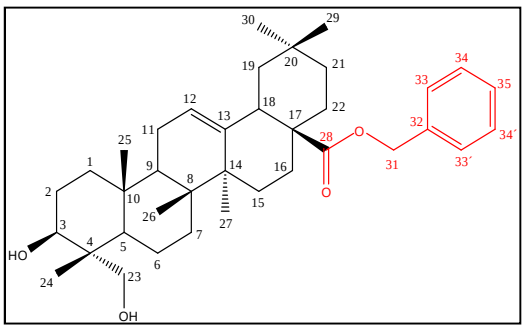
Table 1 **30**

Section B:

^{1}H and ^{13}C NMR Spectras **31-60**

Section A:

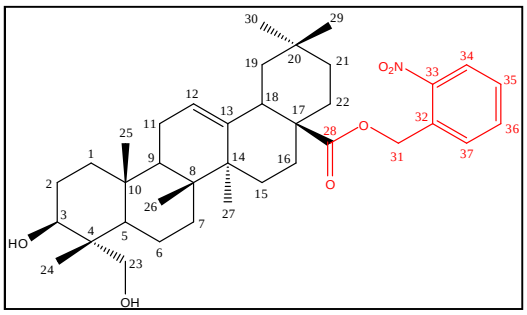
*Benzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**1**)*



White solid; yield: 66 mg, 56%; m.p. 160-162 ^{o}C; R_f = 0.46 (hexane/ethyl acetate 1:1 v/v); IR (KBr): ῡ = 3410, 3032, 1722, 1158, 1044, 746, 696 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.34-7.31 (m, 5H, Ph), 5.27 (t, 1H, *J* = 3.6 Hz, H-12), 5.09 (d, 1H, *J* = 12.5 Hz, H-31$_a$), 5.03 (d, 1H, *J* = 12.5 Hz, H-31$_b$), 3.69 (d, 1H, *J* = 10.3 Hz, H-23$_a$), 3.61 (dd, 1H, *J* = 8.7, 7.0 Hz, H-3), 3.40 (d, 1H, *J* = 10.3 Hz, H-23$_b$), 2.89 (dd, 1H, *J* = 13.7, 4.5 Hz, H-18), 1.11 (s, 3H, CH_3), 0.91 (s, 6H, 2xCH_3), 0.89 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.59 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.45 (C-28), 143.62 (C-13), 136.37 (C-32), 128.38 (2xC-33, C-33'), 127.94 (2xC-34,C-34'), 127.89 (C-35), 122.40 (C-12), 76.85 (C-3), 72.09 (C-23), 65.92 (C-31), 49.79 (C-9), 47.57 (C-17), 46.71 (C-5), 45.80 (C-19), 41.74 (C-4), 41.68 (C-14), 41.34 (C-18), 39.25 (C-8), 38.08 (C-1), 36.86 (C-10), 33.83 (C-21), 33.07 (C-29), 32.47 (C-7), 33.34 (C-22), 30.67 (C-20), 27.58 (C-15), 26.68 (C-27), 25.87 (C-2), 23.61 (C-30), 23.34 (C-11), 23.01 (C-16), 18.45 (C-6), 16.87 (C-26), 15.64 (C-25), 11.38 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{55}O_4]^+$: 563.4104, found 563.4100.

ACCEPTED MANUSCRIPT

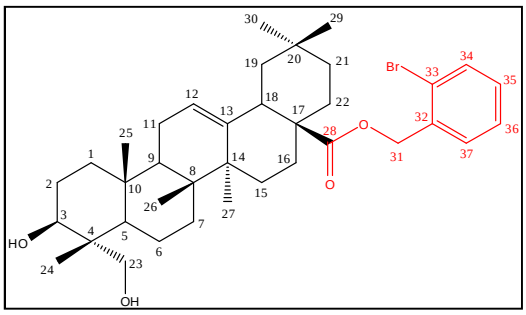
*o-Nitrobenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**2**).*



White solid; yield: 56 mg, 43%; m.p. 168-170 ^{o}C; R_f = 0.38 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3418, 1724, 1528, 1160, 1046, 730 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.06 (brd, 1H, *J* = 7.8 Hz, H-34), 7.63 (d, 1H, *J* = 1.1 Hz, H-36), 7.61 (d, 1H, *J* = 0.7 Hz, H-37), 7.48 (m, 1H, H-35), 5.48 (d, 1H, *J* = 14.4 Hz, H-31$_a$), 5.40 (d, 1H, *J* = 14.4 Hz, H31$_b$), 5.27 (t, 1H, *J* = 3.5 Hz, H-12), 3.70 (d, 1H, *J* = 10.3 Hz, H-23$_a$), 3.61 (dd, 1H, *J* = 7.1, 6.9 Hz, H-3), 3.40 (d, 1H, *J* = 10.3 Hz, H-23$_b$), 2.87 (dd, 1H, *J* = 14.2, 4.0 Hz, H-18), 1.11 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.90 (s, 6H, 2xCH_3), 0.87 (s, 3H, CH_3), 0.55 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.20 (C-28), 148.02 (C-33), 143.68 (C-13), 133.59 (C-32), 132.38 (C-36), 129.83 (C-37), 128.89 (C-35), 125.08 (C-34), 122.70 (C-12), 76.99 (C-3), 72.25 (C-23), 62.97 (C-31), 49.91 (C-9), 47.69 (C-17), 47.15 (C-5), 45.98 (C-19), 41.91 (C-4), 41.83 (C-14), 41.49 (C-18), 39.40 (C-8), 38.22 (C-1), 37.01 (C-10), 33.95 (C-21), 33.19 (C-29), 32.57 (C-7), 32.53 (C-22), 30.81 (C-20), 27.72 (C-15), 26.86 (C-27), 26.03 (C-2), 23.72 (C-30), 23.50 (C-11), 23.28 (C-16), 18.59 (C-6), 16.99 (C-26), 15.78 (C-25), 11.51 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{53}NO_6]^+$: 608.3951, found: 607.8198; CHN calcd.: C, 73.11; H, 8.79; found: C, 72.87; H, 8.93.

ACCEPTED MANUSCRIPT

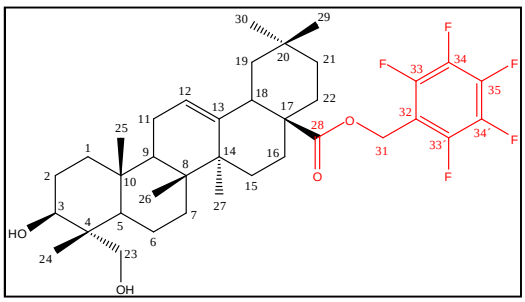
*o-Bromobenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**3**).*



White solid; yield: 74 mg, 55%; m.p. 171.3-173 ^{o}C; R_f = 0.42 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3414, 3078, 1722, 1158, 1032, 748 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.56 (dd, 1H, *J* = 7.7, 1.3 Hz, H-34), 7.42 (dd, 1H, *J* = 7.6, 1.7 Hz, H-37), 7.29 (td, 1H, *J* = 7.6, 1.3 Hz, H-36), 7.17 (td, 1H, *J* = 7.7, 1.7 Hz, H-35), 5.28 (t, 1H, *J* = 3.6 Hz, H-12), 5.16 (d, 1H, *J* = 13.1 Hz, H-31$_a$), 5.09 (d, 1H, *J* = 13.1 Hz, H-31$_b$), 3.71 (d, 1H, *J* = 10.3 Hz, H-23$_a$), 3.62 (dd, 1H, *J* = 8.9, 6.9 Hz, H-3), 3.41 (d, 1H, *J* = 10.3 Hz, H-23$_b$), 2.91 (dd, 1H, *J* = 13.7, 4.0 Hz, H-18), 1.11 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.57 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.44 (C-28), 143.71 (C-13), 135.84 (C-32), 132.89 (C-34), 130.33 (C-37), 129.66 (C-35), 127.49 (C-36), 123.74 (C-33), 122.66 (C-12), 77.05 (C-3), 72.32 (C-23), 65.78 (C-31), 49.94 (C-9), 47.73 (C-17), 47.06 (C-5), 46.01 (C-19), 41.93 (C-4), 41.83 (C-14), 41.48 (C-18), 39.41 (C-8), 38.24 (C-1), 37.02 (C-10), 34.00 (C-21), 33.23 (C-29), 32.63 (C-7), 32.57 (C-22), 30.84 (C-20), 27.77 (C-15), 26.91 (C-27), 26.02 (C-2), 23.76 (C-30), 23.51 (C-11), 23.22 (C-16), 18.62 (C-6), 17.03 (C-26), 15.80 (C-25), 11.51 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{53}BrO_4]^+$: 641.7183, found 643.3204 $[M+2+H]^+$: 643.3204; CHN calcd.: C, 69.25; H, 8.32; found: C, 69.01; H, 8.43.

ACCEPTED MANUSCRIPT

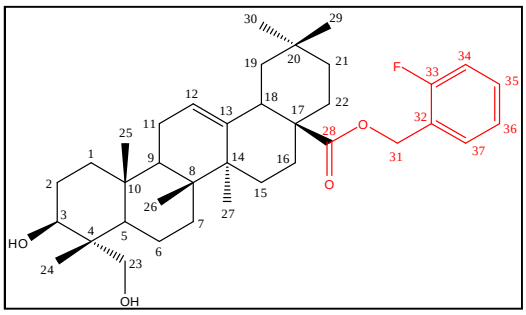
*Pentafluorobenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**4**).*



White solid; yield: 26 mg, 18%; m.p. 143.3-145.2 ^{o}C; R_f = 0.44 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3430, 1734, 1508, 1150, 1130, 1052, 942 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.24 (t, 1H, J = 3.4 Hz, H-12),5.19 (dt, 1H, J = 12.0, 1.0 Hz, H-31$_a$), 5.07 (dt, 1H, J = 12.0, 1.0 Hz, H-31$_b$), 3.70 (d, 1H, J = 10.4 Hz, H-23$_a$), 3.62 (brt, 1H, J = 7.5 Hz, H-3), 3.41 (d, 1H, J = 10.4 Hz, H-23$_b$), 2.82 (dd, 1H, J = 12.9, 4.5 Hz, H-18), 1.09 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.88 (s, 6H, 2xCH_3), 0.54 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.15 (C-28), 143.34 (C-13), 122.79 (C-12), 76.89 (C-3), 72.22 (C-23), 49.91 (C-9), 47.65 (C-17), 47.03 (C-5), 45.81 (C-19), 41.92 (C-4), 41.79 (C-14), 41.52 (C-18), 39.33 (C-8), 38.23 (C-1), 37.00 (C-10), 33.86 (C-21), 33.18 (C-29), 32.62 (C-7), 32.35 (C-22), 30.80 (C-20), 27.60 (C-15), 26.87 (C-27), 25.97 (C-2), 23.70 (C-30), 23.47 (C-11), 23.01 (C-16), 18.56 (C-6), 16.66 (C-26), 15.70 (C-25), 11.53 (C-24); HRMS (ESI TOF-MS) $[H_2O\text{-}M+H]^+$ calcd. for $[C_{37}H_{47}F_5O_3]^+$: 635.3525, found 635.3524.

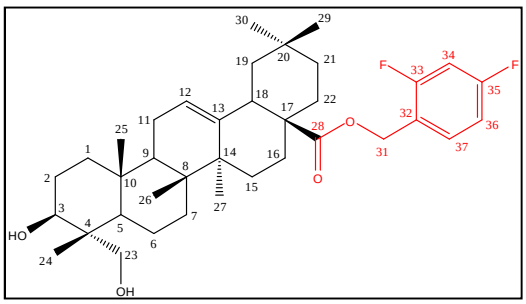
ACCEPTED MANUSCRIPT

*o-Fluorobenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**5**).*



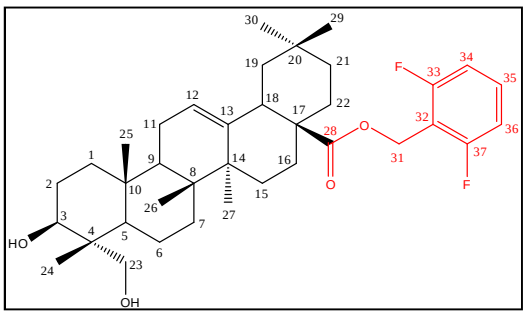
White solid; yield: 70 mg, 57%; m.p. 161.7-163.2 ^{o}C; R_f = 0.5 (hexane/ethyl acetate 1:1 v/v); IR (KBr): ῡ = 3422, 1724, 1158, 1031, 756 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.37 (td, 1H, *J*= 7.6, 1.9 Hz, H-37), 7.30 (tdd, 1H, *J* = 7.5, 5.6, 1.9 Hz, H-35), 7.11 (td, 1H, *J* = 7.5, 1.2 Hz, H-35), 7.05 (ddd, 1H, *J* = 9.3, 8.2, 1.2 Hz, H-34), 5.26 (t, 1H, *J* = 3.5 Hz, H-12), 5.11 (brt, 2H, *J* = 13.3 Hz, H-31), 3.70 (d, 1H, *J* = 10.3 Hz, H-23$_a$), 3.62 (dd, 1H, *J* = 8.8, 6.9 Hz, H-3), 3.41 (d, 1H, *J* = 10.3 Hz, H-23$_b$), 2.88 (dd, 1H, *J* = 13.6, 3.9 Hz, H-18), 1.10 (s, 3H, CH_3), 0.91 (s, 6H, 2xCH_3), 0.88 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.57 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.52 (C-28), 161.13 (d, *J* = 246.9 Hz, C-33), 143.68 (C-13), 130.73 (d, *J* = 3.7 Hz, C-37), 130.05 (d, *J* = 8.0 Hz, C-35), 124.13 (d, *J* = 3.8 Hz, C-32), 123.67 (d, *J* = 14.8 Hz, C-36), 122.59 (C-12), 115.48 (d, *J* = 21.0 Hz, C-34), 77.04 (C-3), 72.30 (C-23), 61.20 (d, *J* = 3.9 Hz, C-31), 49.95 (C-9), 47.73 (C-17), 46.94 (C-5), 45.97 (C-19), 41.92 (C-4), 41.82 (C-14), 41.50 (C-18), 39.38 (C-8), 38.24 (C-1), 37.02 (C-10), 33.98 (C-21), 33.23 (C-29), 32.63 (C-7), 32.46 (C-22), 30.83 (C-20), 27.70 (C-15), 26.89 (C-27), 26.01 (C-2), 23.75 (C-30), 23.51 (C-11), 23.15 (C-16), 18.62 (C-6), 16.94 (C-26), 15.80 (C-25), 11.52 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{53}FO_4]^+$: 580.8127, found. 581.4003; CHN calcd.: C, 76.51; H, 9.20; found: C, 76.41; H, 9.33.

*2,4-Difluorobenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**6**).*



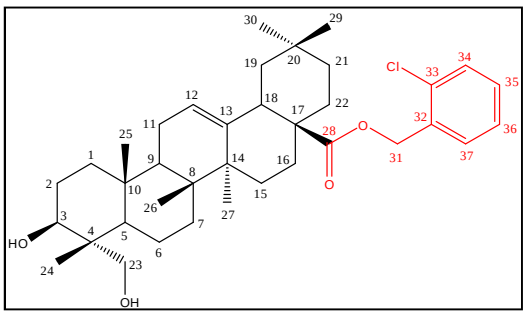
White solid; yield: 48 mg, 38%; m.p. 164.6-166 ^{o}C; R_f = 0.49 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3410, 1726, 1156, 1100, 1032, 962, 850 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35 (m, 1H, H-37), 6.82 (m, 2H, H-34 and H-36), 5.25 (t, 1H, *J* = 3.5 Hz, H-12), 5.06 (brt, 2H, *J* = 13.3 Hz, H-31), 3.70 (d, 1H, *J* = 10.3 Hz, H-23$_a$), 3.61 (dd, 1H, *J* = 8.7, 7.1 Hz, H-3), 3.41 (d, 1H, *J* = 10.4 Hz, H-23$_b$), 2.85 (dd, 1H, *J* = 14.3, 4.7 Hz, H-18), 1.10 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.55 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.50 (C-28), 163.09 (dd, *J* = 247.9, 12.0 Hz, C-35), 161.40 (dd, *J* = 249.9, 14.1 Hz, C-33), 143.64 (C-13), 131.99 (dd, *J* = 9.8, 5.4 Hz, C-37), 122.59 (C-12), 119.74 (dd, *J* = 14.9, 3.6 Hz, C-32), 111.29 (dd, *J* = 21.0, 3.6 Hz, C-36), 104.00 (t, *J* = 25.6 Hz, C-34), 77.00 (C-3), 72.25 (C-23), 59.64 (d, *J* = 3.4 Hz, C-31), 49.92 (C-9), 47.70 (C-17), 46.92 (C-5), 45.94 (C-19), 41.92 (C-4), 41.82 (C-14), 41.50 (C-18), 39.37 (C-8), 38.24 (C-1), 37.01 (C-10), 33.95 (C-21), 33.21 (C-29), 32.63 (C-7), 32.45 (C-22), 30.81 (C-20), 27.67 (C-15), 26.88 (C-27), 25.98 (C-2), 23.73 (C-30), 23.50 (C-11), 23.12 (C-16), 18.60 (C-6), 16.92 (C-26), 15.77 (C-25), 11.52 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{52}F_2O_4]^+$: 598.8031, found 599.3900; CHN calcd.: C, 74.21; H, 8.75; found: C, 73.95; H, 8.98.

*2,6-Difluorobenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**7**).*



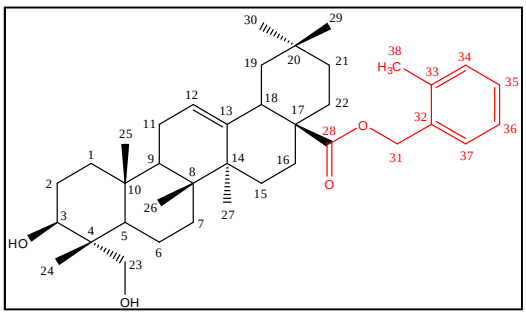
White solid; yield: 100 mg, 79%; m.p. 120-122 ^{o}C; R_f = 0.49 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\tilde{\upsilon}$ = 3402, 1732, 1236, 1158, 1056, 1038, 784 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.29 (m, 1H, H-35), 6.88 (m, 2H, H-34 and H-36), 5.23 (t, 1H, *J* = 3.3 Hz, H-12), 5.16 (d, 1H, *J* = 11.9 Hz, H-31$_a$), 5.10 (d, 1H, *J* = 11.9 Hz, H-31$_b$), 3.70 (d, 1H, *J* = 10.3 Hz, H-23$_a$), 3.61 (brt, 1H, *J* = 7.9 Hz, H-3), 3.41 (d, 1H, *J* = 10.3 Hz, H-23$_b$), 2.84 (dd, 1H, *J* = 13.9, 4.1 Hz, H-18), 1.09 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.59 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.36 (C-28), 162.04 (dd, *J* = 250.0, 7.5 Hz, C-33 and C-37), 143.52 (C-13), 130.63 (t, *J* = 25.6 Hz, C35), 122.59 (C-12), 112.42 (t, *J* = 18.8 Hz, C-32), 111.41 (m, C-36), 111.41 (m, C-34), 77.06 (C-3), 72.06 (C-23), 54.07 (t, *J* = 3.9 Hz, C-31), 49.96 (C-9), 47.73 (C-17), 46.92 (C-5), 45.92 (C-19), 41.92 (C-4), 41.79 (C-14), 41.52 (C-18), 39.32 (C-8), 38.25 (C-1), 37.02 (C-10), 33.96 (C-21), 33.23 (C-29), 32.64 (C-7), 32.33 (C-22), 30.81 (C-20), 27.66 (C-15), 26.87 (C-27), 25.98 (C-2), 23.73 (C-30), 23.50 (C-11), 23.04 (C-16), 18.64 (C-6), 16.79 (C-26), 15.80 (C-25), 11.52 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{52}F_2O_4]^+$: 598.8031, found 599.3920; CHN calcd.: C, 74.21; H, 8.75; found: C, 74.04; H, 8.91.

*o-Chlorobenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**8**).*



White solid; yield: 96 mg, 76%; m.p. 176.2-177.3 ^{o}C; R_f= 0.47 (hexane/ethyl acetate 1:1 v/v); IR (KBr): ῡ = 3408, 3064, 1724, 1250, 1160, 1042, 1010, 752 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.42 (m, 1H, H-34), 7.37 (m, 1H, H-37), 7.24 (m, 2H, H-35 and H-36), 5.27 (t, 1H, J = 3.4 Hz, H-12), 5.17 (d, 1H, J = 13.0 Hz, H-31$_a$), 5.12 (d, 1H, J = 13.0 Hz, H-31$_b$), 3.70 (d, 1H, J = 10.3 Hz, H-23$_a$), 3.61 (dd, 1H, J = 8.4, 7.0 Hz, H-3), 3.40 (d, 1H, J = 10.3 Hz, H-23$_b$), 2.90 (dd, 1H, J = 13.6, 4.2 Hz, H-18), 1.10 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.57 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.48 (C-28), 143.69 (C-13), 134.16 (C-32), 133.91 (C-33), 130.19 (C-37), 129.59 (C-34), 129.86 (C-35), 126.86 (C-36), 122.63 (C-12), 77.02 (C-3), 72.27 (C-23), 63.64 (C-31), 49.93 (C-9), 47.72 (C-17), 47.04 (C-5), 46.00 (C-19), 41.90 (C-4), 41.82 (C-14), 41.48 (C-18), 39.39 (C-8), 38.23 (C-1), 37.01 (C-10), 33.99 (C-21), 33.22 (C-29), 32.63 (C-7), 32.57 (C-22), 30.84 (C-20), 27.77 (C-15), 26.91 (C-27), 26.02 (C-2), 23.75 (C-30), 23.50 (C-11), 23.20 (C-16), 18.61 (C-6), 17.00 (C-26), 15.79 (C-25), 11.52 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{53}ClO_4]^+$: 597.2670, found 598.3711; CHN calcd.: C, 74.40; H, 8.94; found: C, 73.95; H, 9.03.

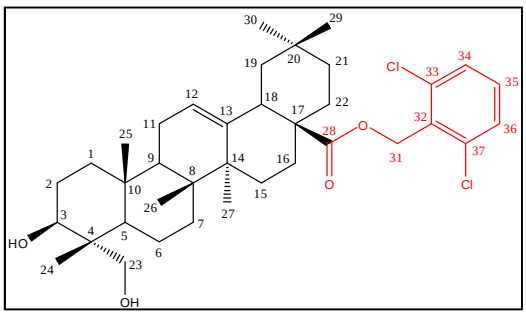
*o-Methylbenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**9**).*



White solid; yield: 105 mg, 86%; m.p. 165.3-166.8 ^{o}C; R_f = 0.53 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3420, 1718, 1160, 1040, 740 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.32 (m, 1H), 7.17 (m, 3H), 5.25 (t, 1H, J = 3.4 Hz, H-12), 5.10 (d, 1H, J = 12.6 Hz, H-31$_a$), 5.03 (d, 1H, J = 12.6 Hz, H-31$_b$), 3.70 (d, 1H, J = 10.3 Hz, H-23$_a$), 3.61 (brt, 1H, J = 8.3 Hz, H-3), 3.41 (d, 1H, J = 10.3 Hz, H-23$_b$), 2.88 (dd, 1H, J = 13.9, 4.2 Hz, H-18), 2.34 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 0.91 (s, 6H, 2xCH_3), 0.88 (s, 6H, 2xCH_3), 0.56 (s, 3H, CH_3)); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.62 (C-28), 143.74 (C-13), 137.00 (C-32), 134.43 (C-33), 130.30 (C-34), 129.32 (C-37), 128.35 (C-35), 126.00 (C-36), 122.57 (C-12), 77.05 (C-3), 72.31 (C-23), 64.60 (C-31), 49.95 (CH-9), 47.73 (C-17), 47.00 (C-5), 46.01 (C-19), 41.92 (C-4), 41.83 (C-14), 41.50 (C-18), 39.38 (C-8), 38.24 (C-1), 37.02 (C-10), 33.99 (C-21), 33.23 (C-29), 32.63 (C-7), 32.56 (C-22), 30.80 (C-20), 27.73 (C-15), 26.90 (C-27), 26.02 (C-2), 23.75 (C-30), 23.49 (C-11), 23.20 (C-16), 19.06 (C-38), 18.61 (C-6), 16.99 (C-26), 15.79 (C-25), 11.51 (C-24); HRMS (ESI TOF-MS) $[M + H]^+$ calcd. for $[C_{38}H_{56}O_4]^+$: 576.8488, found 577.4266; CHN calcd.: C, 79.12; H, 9.79; found: C, 79.00; H, 9.98.

ACCEPTED MANUSCRIPT

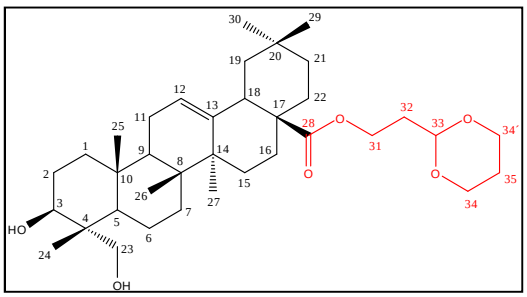
*2,6-Dichlorobenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**10**).*



White solid; yield: 100 mg, 75%; m.p. 148.6-150.2 ^{o}C; R_f = 0.46 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3440, 1730, 1158, 1032, 768 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.32 (d, 1H, *J* = 8.5 Hz, H-34), 7.32 (d, 1H, *J* = 7.1 Hz, H-36), 7.21 (dd, 1H, *J* = 8.5, 7.1 Hz, H-35), 5.32 (d, 1H, *J* = 11.7 Hz, H-31$_a$), 5.26 (d, 1H, *J* = 11.7 Hz, H-31$_b$), 5.24 (t, 1H, *J* = 3.3 Hz, H-12), 3.70 (d, 1H, *J* = 10.3 Hz, H-23$_a$), 3.62 (brt, 1H, *J* = 7.9 Hz, H-3), 3.41 (d, 1H, *J* = 10.3 Hz, H-23$_b$), 2.86 (dd, 1H, *J* = 14.0, 4.0 Hz, H-18), 1.09 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.68 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.45 (C-28), 143.57 (C-13), 137.14 (C-32), 131.99 (C-35), 130.38 (C-33), 130.38 (C-37), 128.47 (C-34), 128.47 (C-36), 122.63 (C-12), 77.06 (C-3), 72.32 (C-23), 61.29 (C-31), 49.97 (C-9), 47.74 (C-17), 47.15 (C-5), 45.93 (C-19), 41.91 (C-4), 41.83 (C-14), 41.45 (C-18), 39.37 (C-8), 38.25 (C-1), 37.03 (C-10), 33.99 (C-21), 33.23 (C-29), 32.68 (C-7), 32.46 (C-22), 30.83 (C-20), 27.81 (C-15), 26.88 (C-27), 25.98 (C-2), 23.74 (C-30), 23.53 (C-11), 23.06 (C-16), 18.66 (C-6), 17.15 (C-26), 15.82 (C-25), 11.52 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{52}Cl_2O_4]^+$: 631.7117, found 631.3308; CHN calcd.: C, 70.35; H, 8.30; found: C, 70.11; H, 8.52.

ACCEPTED MANUSCRIPT

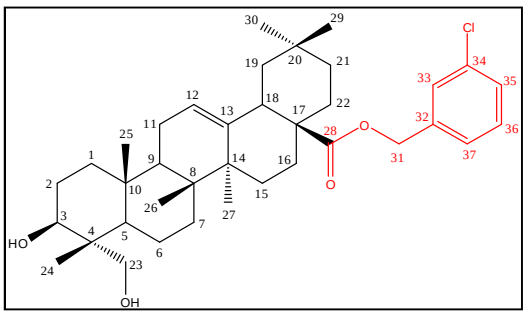
*2-Ethyl-1,3-dioxanyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**11**).*



White solid; yield: 105 mg, 84%; m.p. 110-111°C; R_f = 0.46 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3440, 1730, 1158, 1032, 768 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 5.27 (t, 1H, J = 3.3 Hz, H-12), 4.62 (t, 1H, J = 5.3 Hz, H-33), 4.09 (td, 4H, J = 6.6, 1.6 Hz, H-34, H-34'), 3.73 (m, 3H, H-23_a, H-31), 3.62 (brt, 1H, J = 7.6 Hz, H-3), 3.41 (d, H, J = 10.3 Hz, H-23_b), 2.85 (dd, 1H, J = 13.9, 4.2 Hz, H-18), 1.11 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.72 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.67 (C-28), 143.97 (C-13), 122.37 (C-12), 99.75 (C-33), 77.02 (C-3), 72.26 (C-23), 67.05 (C-34'), 67.04 (C-34), 60.04 (C-31), 49.96 (C-9), 47.74 (C-17), 46.78 (C-5), 46.01 (C-19), 41.91 (C-4), 41.84 (C-14), 41.40 (C-18), 39.45 (C-8), 38.24 (C-1), 37.06 (C-10), 34.57 (C-32), 34.00 (C-21), 33.23 (C-29), 32.66 (C-7), 32.54 (C-22), 30.84 (C-20), 27.73 (C-15), 26.00 (C-35), 26.86 (C-27), 25.90 (C-2), 23.74 (C-30), 23.55 (C-11), 23.13 (C-16), 18.62 (C-6), 17.14 (C-26), 15.81 (C-25), 11.54 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{36}H_{58}O_6]^+$: 586.8421, found 587.4307; CHN calcd.: C, 73.68; H, 9.96; found: C, 73.47; H, 10.09.

ACCEPTED MANUSCRIPT

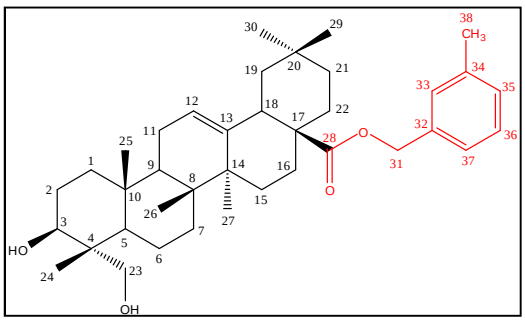
*m-Chlorobenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**12**).*



White solid; yield: 45 mg, 35%; m.p. 171.9-173.4 ^{o}C; R_f = 0.49 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3394, 1720, 1250, 1210, 1160, 1032, 784 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.34 (brs, 1H, H-33), 7.27 (m, 2H. H-35 and H-36), 7.21 (m, 1H, H-37), 5.29 (t, 1H, J = 3.4 Hz, H-12), 5.08 (d, 1H, J = 12.9 Hz, H-31$_a$), 4.97 (d, 1H, J = 12.9 Hz, H-31$_b$), 3.71 (d, H, J = 10.3 Hz, H-23$_a$), 3.62 (brt, 1H, J = 7.2 Hz, H-3), 3.41 (d, H, J = 10.3 Hz, H-23$_b$), 2.88 (dd, 1H, J = 14.1, 4.3 Hz, H-18), 1.11 (s, 3H, CH_3), 0.92 (s, 6H, 2xCH_3), 0.89 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.56 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.51 (C-28), 143.74 (C-13), 138.53 (C-32), 134.47 (C-34), 129.83 (C-36), 128.33 (C-33), 128.22 (C-35), 126.18 (C-37), 122.69 (C-12), 77.03 (C-3), 72.29 (C-23), 65.20 (C-31), 49.93 (C-9), 47.73 (C-17), 46.93 (C-5), 46.00 (C-19), 41.94 (C-4), 41.85 (C-14), 41.49 (C-18), 39.42 (C-8), 38.23 (C-1), 37.04 (C-10), 33.97 (C-21), 33.22 (C-29), 32.63 (C-7), 32.54 (C-22), 30.83 (C-20), 27.70 (C-15), 26.91 (C-27), 26.03 (C-2), 23.77 (C-30), 23.48 (C-11), 23.22(C-16), 18.60 (C-6), 17.00 (C-26), 15.76 (C-25), 11.51 (C-24); HRMS (ESI TOF-MS) $[H_2O\text{-}M+H]^+$ calcd. for $[C_{37}H_{51}ClO_3]^+$: 579.3605, found 579.3594.

ACCEPTED MANUSCRIPT

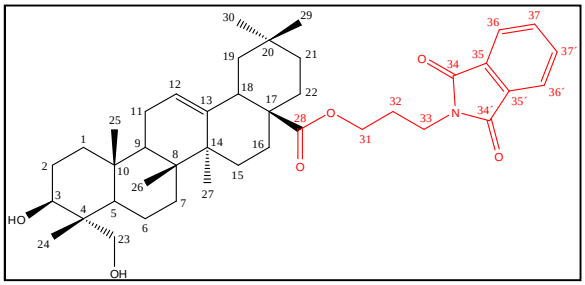
*m-Methylbenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**13**).*



White solid; yield: 65 mg, 53%; m.p. 164.9-166.1 ^{o}C; R_f = 0.49 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3416, 1720, 1162, 1042, 1018, 780, 719 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.21 (d, 1H, *J* = 7.3 Hz), 7.12 (m, 3H), 5.28 (t, 1H, *J* = 3.7 Hz, H-12), 5.06 (d, 1H, *J* = 12.6 Hz, H-31$_a$), 4.99 (d, 1H, *J* = 12.5 Hz, H-31$_b$), 3.71 (d, H, *J* = 10.3 Hz, H-23$_a$), 3.62 (dd, 1H, *J* = 8.3, 6.9 Hz, H-3), 3.41 (d, H, *J* = 10.3 Hz, H-23$_b$), 2.90 (dd, 1H, *J* = 13.6, 5.0 Hz, H-18), 2.34 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.60 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.65 (C-28), 143.86 (C-13), 138.14 (C-34), 136.46 (C-32), 128.87 (C-33), 128.76 (C-35), 128.45 (C-36), 125.14 (C-37), 122.40 (C-12), 77.05 (C-3), 72.31 (C-23), 66.13 (C-31), 49.95 (C-9), 47.74 (C-17), 46.87 (C-5), 46.03 (C-19), 41.93 (C-4), 41.84 (C-14), 41.48 (C-18), 39.42 (C-8), 38.24 (C-1), 37.03 (C-10), 34.00 (C-21), 33.24 (C-29), 32.63 (C-7), 32.56 (C-22), 30.80 (C-20), 27.73 (C-15), 26.90 (C-27), 26.03 (C-2), 23.78 (C-30), 23.50 (C-11), 23.18 (C-16), 21.54 (C-38), 18.61 (C-6), 17.03 (C-26), 15.78 (C-25), 11.51 (C-24); HRMS (ESI TOF-MS) $[H_2O\text{-}M+H]^+$ calcd. for $[C_{38}H_{57}O_4]^+$: 577.4257, found 577.4253.

ACCEPTED MANUSCRIPT

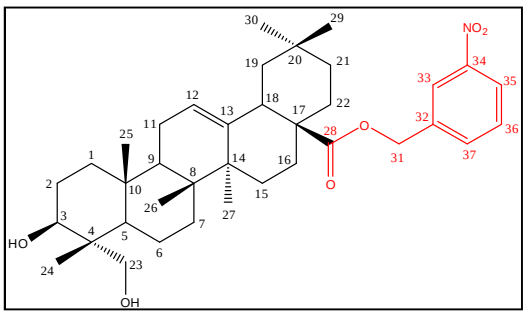
*3-(N-propyl-phthalimidyl)-(3β)3,23-dihydroxyolean-12-en-28-oate (**14**).*



White solid; yield: 107 mg, 76%; m.p. 121.3-123 ^{o}C; R_f = 0.48 (hexane/ethyl acetate 1:1 v/v); IR (KBr): ῡ = 3458, 3228, 1772, 1715,1176, 1158, 1050, 1006, 720 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.83 (m, 2H, H-36, H-36'), 7.71 (m, 2H, H-37, H-37'), 5.29 (t, 1H, *J* = 3.3 Hz, H-12), 4.06 (t, 1H, *J* = 6.2 Hz, H-31), 3.77 (m, 3H, H-33, H-23$_a$), 3.62 (brt, 1H, *J* = 7.7 Hz, H-3), 3.41 (d, H, *J* = 10.3 Hz, H-23$_b$), 2.87 (dd, 1H, *J* = 13.9, 4.0 Hz, H-18), 1.10 (s, 3H, CH_3), 0.92 (s, 6H, 2xCH_3), 0.88 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.70 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.70 (O=C-28), 166.34 (C-34, C-34'), 143.83 (C-13), 134.11 (C-37, C-37'), 132.21 (C-35, C-35'), 123.39 (C-36, C-36'), 122.48 (C-12), 77.05 (C-3), 72.28 (C-23), 61.68 (C-31), 49.94 (C-9), 47.73 (C-17), 46.83 (C-5), 45.99 (C-19), 41.91 (C-4), 41.84 (C-14), 41.43 (C-18), 39.44 (C-8), 38.23 (C-1), 37.03 (C-10), 35.31 (C-33), 33.99 (C-21), 33.23 (C-29), 32.61 (C-7), 32.47 (C-22), 30.82 (C-20), 28.00 (C-32), 27.80 (C-15), 26.86 (C-27), 26.00 (C-2), 23.72 (C-30), 23.51 (C-11), 23.11 (C-16), 18.61 (C-6), 17.13 (C-26), 15.80 (C-25), 11.52 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{41}H_{59}NO_6]^+$: 661.9103, found 660.4263; CHN calcd.: C, 74.40; H, 8.98; found: C, 74.15; H, 9.13.

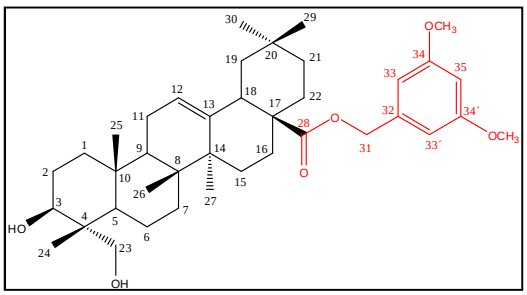
ACCEPTED MANUSCRIPT

*m-Nitrobenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**15**).*



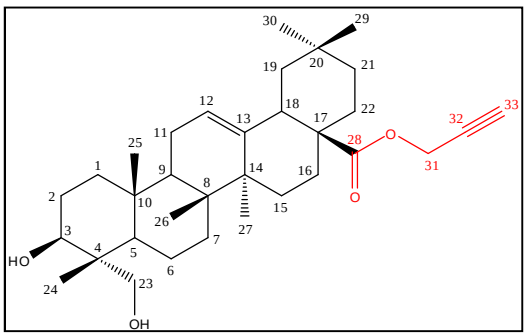
White solid; yield: 61 mg, 47%; m.p. 173.6-175.2 ^{o}C; R_f = 0.44 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\tilde{\upsilon}$ = 3394, 3080, 1720, 1532, 1160, 1032, 804, 730 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.22 (t, 1H, J = 1.4 Hz, H-33), 8.16 (ddd, 1H, J = 8.0, 2.3, 1.4 Hz, H-35), 7.66 (dt, 1H, J = 7.9, 1.4 Hz, H-37), 7.52 (t, 1H, J = 7.9 Hz, H-36), 5.30 (t, 1H, J = 3.6 Hz, H-12), 5.19 (d, 1H, J = 13.1 Hz, H-31$_a$), 5.09 (d, 1H, J = 13.1 Hz, H-31$_b$), 3.70 (d, 1H, J = 10.3 Hz, H-23$_a$), 3.61 (dd, 1H, J = 8.7, 7.1 Hz, H-3), 3.40 (d, 1H, J = 10.3 Hz, H-23$_b$), 2.89 (dd, 1H, J = 13.4, 3.7 Hz, H-18), 1.11 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.89 (s, 6H, 2xCH_3), 0.86 (s, 3H, CH_3), 0.52 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.46 (C-28), 148.52 (C-34), 143.63 (C-13), 138.64 (C-32), 134.04 (C-37), 129.59 (C-36), 123.10 (C-33), 122.98 (C-35), 122.78 (C-12), 76.98 (C-3), 72.22 (C-23), 64.74 (C-31), 49.88 (C-9), 47.62 (C-17), 47.00 (C-5), 45.94 (C-19), 41.91 (C-4), 41.83 (C-14), 41.50 (C-18), 39.41 (C-8), 38.19 (C-1), 37.00 (C-10), 33.92 (C-21), 33.18 (C-29), 32.57 (C-7), 32.53 (C-22), 30.81 (C-20), 27.72 (C-15), 26.86 (C-27), 26.02 (C-2), 23.74 (C-30), 23.45 (C-11), 23.23 (C-16), 18.57 (C-6), 16.97 (C-26), 15.72 (C-25), 11.51 (C-24); HRMS (ESI TOF-MS) $[M + H]^+$ calcd. for $[C_{37}H_{53}NO_6]^+$: 607.8198, found 608.0032; CHN calcd.: C, 73.11; H, 8.79; found: C, 73.02; H, 8.89.

*3,5-Dimethoxybenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**16**).*



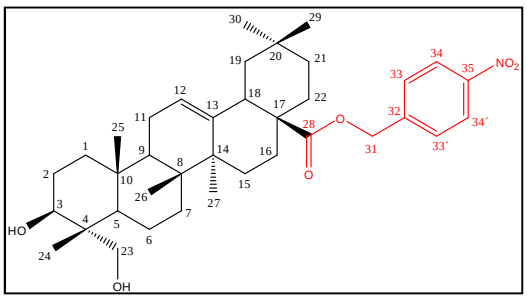
White solid; yield: 90 mg 68%; m.p. 96-97 ^{o}C; R_f = 0.53 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3418, 1724, 1598, 1206, 1158, 1031, 1010, 832 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.48 (d, 2H, *J* = 2.3 Hz, H-33, H-33'), 6.39 (t, 1H, *J* = 2.3 Hz, H-35), 5.28 (t, 1H, *J* = 2.4 Hz, H-12), 5.02 (d, 1H, *J* = 12.7 Hz, H-31$_a$), 4.96 (d, 1H, *J* = 12.7 Hz, H-31$_b$), 3.77 (s, 6H, 2xCH_3-O), 3.70 (d, 1H, *J* = 10.3 Hz, H-23$_a$), 3.62 (brt, 1H, *J* = 6.9 Hz, H-3), 3.41 (d, 1H, *J* = 10.3 Hz, H-23$_b$), 2.90 (dd, 1H, *J* = 13.7, 4.2 Hz, H-18), 1.11 (s, 3H, CH_3), 0.91 (s, 6H, 2xCH_3), 0.89 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.62 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.53 (C-28), 160.96 (C-34), 160.96 (C-34'), 143.80 (C-13), 138.81 (C-32), 122.58 (C-12), 105.75 (C-33), 105.75 (C-33'), 99.98 (C-35), 77.01 (C-3), 72.24 (C-23), 65.93 (C-31), 55.46 (O-C-36), 55.46 (O-C-37), 49.93 (C-9), 47.73 (C-17), 46.90 (C-5), 46.01 (C-19), 41.91 (C-4), 41.83 (C-14), 41.49 (C-18), 39.42 (C-8), 38.23 (C-1), 37.02 (C-10), 33.98 (C-21), 33.22 (C-29), 32.62 (C-7), 32.54 (C-22), 30.84 (C-20), 27.75 (C-15), 26.85 (C-27), 26.04 (C-2), 23.77 (C-30), 23.47 (C-11), 23.19 (C-16), 18.60 (C-6), 17.01 (C-26), 15.76 (C-25), 11.53 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{39}H_{58}O_6]^+$: 622.8742, found 623.4304; CHN calcd.: C, 75.20; H, 8.39; found: C, 74.97; H, 8.51.

*2-Propyn-1-yl-(3β)3,23-dihydroxyolean-12-en-28-oate (**17**).*



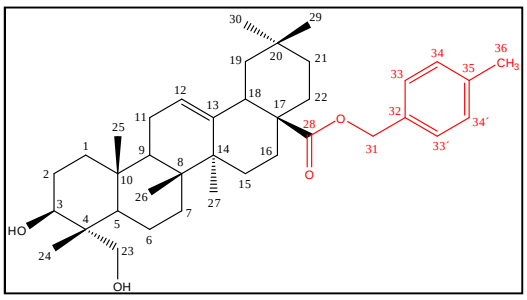
White solid; yield: 100 mg, 92%; m.p. 196-197 ^{o}C; R_f = 0.55 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3396, 3310, 2128, 1730, 1158, 1034, 1010, 668 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.28 (t, 1H, *J* = 3.6 Hz, H-12), 4.67 (dd, 1H, *J* = 15.6, 2.7 Hz, H-31$_a$), 4.55 (dd, 1H, *J* = 15.6, 2.7 Hz, H-31$_b$), 3.69 (d, 1H, *J* = 10.4 Hz, H-23$_a$), 3.61 (brt, 1H, *J* = 7.9 Hz, H-3), 3.40 (d, 1H, *J* = 10.3 Hz, H-23$_b$), 2.84 (dd, 1H, *J* = 14.0, 4.7 Hz, H-18), 2.40 (t, 1H, *J* = 2.7 Hz, H-33), 1.11 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.73(s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 176.99 (C-28), 143.47 (C-13), 122.67 (C-12), 78.21 (C-32), 76.96 (C-3), 74.54 (C-33), 72.14 (C-23), 51.77 (C-31), 49.92 (C-9), 47.72 (C-17), 46.89 (C-5), 45.95 (C-19), 41.88 (C-4), 41.84 (C-14), 41.39 (C-18), 39.50 (C-8), 38.25 (C-1), 37.02 (C-10), 33.94 (C-21), 33.19 (C-29), 32.65 (C-7), 32.30 (C-22), 30.78 (C-20), 27.78 (C-15), 26.79 (C-27), 25.98 (C-2), 23.73 (C-30), 23.51 (C-11), 23.12 (C-16), 18.60 (C-6), 17.23 (C-26), 15.83 (C-25), 11.55 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{33}H_{50}O_4]^+$: 510.7477, found 511.3779; CHN calcd.: C, 77.60; H, 9.87; found: C, 77.50; H, 9.99.

*p-Nitrobenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**18**).*



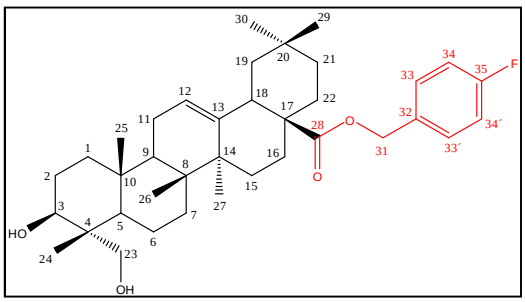
White solid; yield: 75 mg, 58%; m.p. 164.3-166.8 ^{o}C; R_f = 0.40 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\tilde{\upsilon}$ = 3470, 3088, 1728, 1524, 1158, 1034, 1014, 736 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.20 (d, 2H, *J* = 8.7 Hz, H-34, H-34'), 7.50 (d, 2H, *J* = 8.7 Hz, H-33, H-33'), 5.28 (t, 1H, *J* = 3.5 Hz, H-12), 5.19 (d, 1H, *J* = 13.5 Hz, H-31$_a$), 5.09 (d, 1H, *J* = 13.5 Hz, H-31$_b$), 3.69 (d, 1H, *J* = 10.4 Hz, H-23$_a$), 3.61 (dd, 1H, *J* = 8.4, 7.3 Hz, H-3), 3.40 (d, 1H, *J* = 10.4 Hz, H-23$_b$), 2.88 (dd, 1H, *J* = 14.0, 4.0 Hz, H-18), 1.11 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0,90 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.55 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.35 (C-28), 147.73 (C-35), 143.80 (C-32), 143.66 (C-13), 128.55 (C-33), 128.55 (C-33'), 123.84 (C-34), 123.84 (C-34'), 122.72 (C-12), 76.90 (C-3), 72.12 (C-23), 64.69 (C-31), 49.85 (C-9), 47.65 (C-17), 47.00 (C-5), 45.90 (C-19), 41.91 (C-4), 41.84 (C-14), 41.55 (C-18), 39.41 (C-8), 38.22 (C-1), 36.99 (C-10), 33.90 (C-21), 33.17 (C-29), 32.60 (C-7), 32.55 (C-22), 30.81 (C-20), 27.70 (C-15), 26.85 (C-27), 26.02 (C-2), 23.73 (C-30), 23.49 (C-11), 23.22 (C-16), 18.55 (C-6), 17.02 (C-26), 15.74 (C-25), 11.52 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{54}NO_6]^+$: 608.8278, found 608.3951; CHN calcd.: C, 72.99; H, 8.94; found: C, 72.74; H, 9.03.

*p-Methylbenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**19**).*



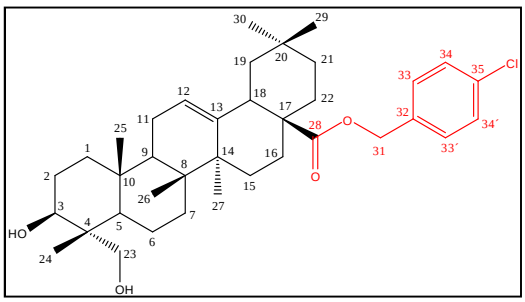
White solid; yield: 40 mg, 32%; m.p. 146-147.3 ^{o}C; R_f = 0.49 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3390, 1726, 1158, 1032, 1008, 804 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.12 (d, 2H, *J* = 8.0 Hz, H-33, H-33'), 7.14 (d, 2H, *J* = 8.0 Hz, 34, H-34'H), 5.27 (t, 1H, *J* = 3.3 Hz, H-12), 5.05 (d, 1H, *J* = 12.4 Hz, H-31$_a$), 4.98 (d, 1H, *J* = 12.4 Hz, H-31$_b$), 3.70 (d, 1H, *J* = 10.4 Hz, H-23$_a$), 3.62 (brt, 1H, *J* = 7.8 Hz, H-3), 3.40 (d, 1H, *J* = 10.4 Hz, H-23$_b$), 2.88 (dd, 1H, *J* = 14.0, 4.1 Hz, H-18), 2.34 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.59 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.64 (C-28), 143.79 (C-13), 137.79 (C-35), 133.50 (C-32), 129.19 (C-34), 129.19 (C-34'), 128.23 (C-33), 128.23 (C-33'), 122.51 (C-12), 77.00 (C-3), 72.23 (C-23), 66.02 (C-31), 49.94 (C-9), 47.72 (C-17), 46.81 (C-5), 45.98 (C-19), 41.88 (C-4), 41.81 (C-14), 41.49 (C-18), 39.40 (C-8), 38.24 (C-1), 37.01 (C-10), 33.97 (C-21), 33.23 (C-29), 32.63 (C-7), 32.56 (C-22), 30.80 (C-20), 27.73 (C-15), 26.90 (C-27), 26.02 (C-2), 23.77 (C-30), 23.49 (C-11), 23.13 (C-16), 21.31 (C-36), 18.59 (C-6), 17.02 (C-26), 15.78 (C-25), 11.54 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{38}H_{56}O_4]^+$: 576.8488, found 577.4248; CHN calcd.: C, 79.12; H, 9.79; found: C, 78.97; H, 9.94.

*p-Fluorobenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**20**).*



White solid; yield: 110 mg, 90%; m.p. 175.6-177.4 ^{o}C; R_f = 0.49 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3382, 1724, 1512, 1226, 1154, 1032, 1010, 824 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.30 (m, 2H, H-33, H-33'), 7.01 (m, 2H, H-34, H-34'), 5.26 (t, 1H, *J* = 3.7 Hz, H-12), 5.05 (d, 1H, *J* = 12.4 Hz, H-31$_a$), 4.99 (d, 1H, *J* = 12.4 Hz, H-31$_b$), 3.70 (d, 1H, *J* = 10.4 Hz, H-23$_a$), 3.61 (brt, 1H, *J* = 7.9 Hz, H-3), 3.40 (d, 1H, *J* = 10.4 Hz, H-23$_b$), 2.86 (dd, 1H, *J* = 13.8, 4.1 Hz, H-18), 1.10 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.56 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.54 (C-28), 162.63 (d, *J* = 244.8 Hz, C-35), 143.73 (C-13), 132.35 (d, *J* = 3.2 Hz, C-32), 130.19 (d, *J* = 8.4 Hz, C-33,C-33'), (C-12), 115.44 (d, *J* = 21.3 Hz, C-34, C-34'), 76.98 (C-3), 72.22 (C-23), 65.36 (C-31), 49.92 (C-9), 47.70 (C-17), 46.83 (C-5), 45.96 (C-19), 41.91 (C-4), 41.83 (C-14), 41.50 (C-18), 39.39 (C-8), 38.24 (C-1), 37.01 (C-10), 33.96 (C-21), 33.21 (C-29), 32.63 (C-7), 32.48 (C-22), 30.82 (C-20), 27.70 (C-15), 26.85 (C-27), 26.00 (C-2), 23.75 (C-30), 23.49 (C-11), 23.16 (C-16), 18.59 (C-6), 17.00 (C-26), 15.78 (C-25), 11.53 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{53}FO_4]^+$: 580.4006, found 581.4000.

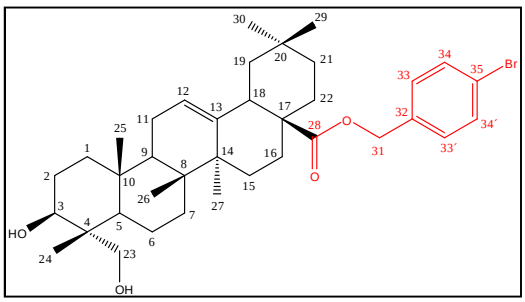
*p-Chlorobenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**21**).*



White solid; yield: 118 mg, 93%; m.p. 183.6-185.2 ^{o}C; R_f = 0.49 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3402, 1724, 1158, 1032, 812 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.28 (m, 4H, H-33, H-33', H-34, H-34'), 5.26 (t, 1H, J = 3.3 Hz, H-12), 5.06 (d, 1H, J = 12.5 Hz, H-31$_a$), 4.97 (d, 1H, J = 12.5 Hz, H-31$_b$), 3.70 (d, 1H, J = 10.3 Hz, H-23$_a$), 3.62 (brt, 1H, J = 7.8 Hz, H-3), 3.41 (d, 1H, J = 10.3 Hz, H-23$_b$), 2.87 (dd, 1H, J = 13.8, 4.1 Hz, H-18), 1.10 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.55 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.50 (C-28), 143.73 (C-13), 135.01 (C-32), 133.99 (C-35), 129.69 (C-33), 129.69 (C-33'), 128.73 (C-34), 128.73 (C-34'), 122.59 (C-12), 77.00 (C-3), 72.24 (C-23), 65.27 (C-31), 49.92 (C-9), 47.71 (C-17), 46.86 (C-5), 45.96 (C-19), 41.92 (C-4), 41.83 (C-14), 41.52 (C-18), 39.40 (C-8), 38.24 (C-1), 37.01 (C-10), 33.95 (C-21), 33.21 (C-29), 32.62 (C-7), 32.50 (C-22), 30.82 (C-20), 27.70 (C-15), 26.88 (C-27), 26.01 (C-2), 23.75 (C-30), 23.49 (C-11), 23.17 (C-16), 18.58 (C-6), 16.99 (C-26), 15.79 (C-25), 11.52 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{53}ClO_4]^+$: 597.2673, found 598.3731.

ACCEPTED MANUSCRIPT

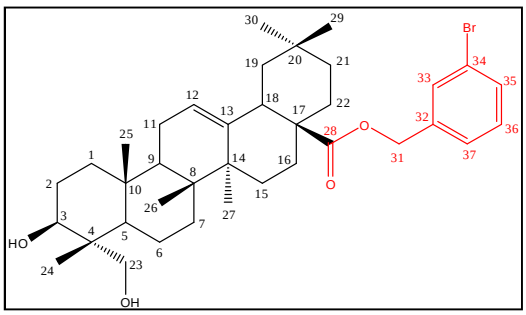
*p-Bromobenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**22**)*



White solid; yield: 110 mg, 81%; m.p. 184-185 ^{o}C; R_f = 0.49 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3394, 1724, 1158, 1032, 808 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.46 (d, 2H, J = 8.4 Hz, H-34, H-34'), 7.20 (d, 2H, J = 8.3 Hz, H-33, H-33'), 5.26 (t, 1H, J = 3.3 Hz, H-12), 5.04 (d, 1H, J = 12.6 Hz, H-31$_a$), 4.96 (d, 1H, J = 12.6 Hz, H-31$_b$), 3.70 (d, 1H, J = 10.3 Hz, H-23$_a$), 3.61 (brt, 1H, J = 7.9 Hz, H-3), 3.40 (d, 1H, J = 10.3 Hz, H-23$_b$), 2.86 (dd, 1H, J = 13.9, 4.2 Hz, H-18), 1.10 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.53 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.49 (C-28), 143.72 (C-13), 135.51 (C-32), 131.69 (C-34), 131.69 (C-34'), 130.00 (C-33), 130.00 (C-33'), 122.59 (C-12), 122.13 (C-35), 76.99 (C-3), 72.22 (C-23), 65.29 (C-31), 49.91 (C-9), 47.70 (C-17), 46.85 (C-5), 45.96 (C-19), 41.91 (C-4), 41.82 (C-14), 41.51 (C-18), 39.39 (C-8), 38.23 (C-1), 37.01 (C-10), 33.82 (C-21), 33.20 (C-29), 32.61 (C-7), 32.50 (C-22), 30.82 (C-20), 27.69 (C-15), 26.86 (C-27), 26.02 (C-2), 23.75 (C-30), 23.49 (C-11), 23.16 (C-16), 18.58 (C-6), 16.97 (C-26), 15.81 (C-25), 11.53 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{53}BrO_4]^+$: 641.7183, found 641.3212; CHN calcd.: C, 69.25; H, 8.32; found: C, 68.93; H, 8.50.

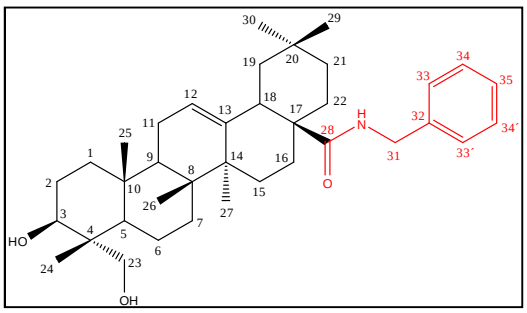
ACCEPTED MANUSCRIPT

*m-Bromobenzyl-(3β)3,23-dihydroxyolean-12-en-28-oate (**23**).*



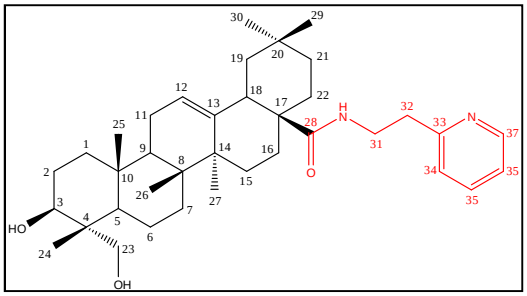
White solid; yield: 75 mg, 55%; m.p. 170.6-172.2 ^{o}C; R_f = 0.49 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\tilde{\upsilon}$ = 3400, 1722, 1210, 1160, 1023, 1014, 782 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.49 (brs, 1H, H-33), 7.42 (dt, H, *J* = 7.5, 1.6 Hz, H-35), 7.22 (m, 2H, H-36, H-37), 5.28 (t, H, *J* = 3.7 Hz, H-12), 5.07 (d, 1H, *J* = 12.8 Hz, H-31$_a$), 4.96 (d, 1H, *J* = 12.8 Hz, H-31$_b$), 3.69 (d, 1H, *J* = 10.4 Hz, H-23$_a$), 3.61 (brt, 1H, *J* = 7.6 Hz, H-3), 3.40 (d, 1H, *J* = 10.4 Hz, H-23$_b$), 2.88 (dd, 1H, *J* = 13.6, 4.2 Hz, H-18), 1.11 (s, 3H, CH_3), 0.91 (s, 6H, 2xCH_3), 0.89 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.55 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.51 (C-28), 143.72 (C-13), 138.76 (C-32), 131.15 (C-33), 131.15 (C-35), 130.10 (C-36), 126.22 (C-37), 122.67 (C-12), 122.63 (C-34), 76.99 (C-3), 72.23 (C-23), 65.14 (C-31), 49.92 (C-9), 47.71 (C-17), 46.92 (C-5), 45.99 (C-19), 41.89 (C-4), 41.82 (C-14), 41.46 (C-18), 39.40 (C-8), 38.22 (C-1), 37.02 (C-10), 33.96 (C-21), 33.20 (C-29), 32.60 (C-7), 32.53 (C-22), 30.82 (C-20), 27.68 (C-15), 26.84 (C-27), 26.02 (C-2), 23.76 (C-30), 23.48 (C-11), 23.21 (C-16), 18.58 (C-6), 16.98 (C-26), 15.77 (C-25), 11.53 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{53}BrO_4]^+$: 641.7183, found 641.3205; CHN calcd.: C, 69.25; H, 8.32; found: C, 69.02; H, 8.60.

*Benzyl-(3β)3,23-dihydroxyolean-12-en-28-amide (**25**)*



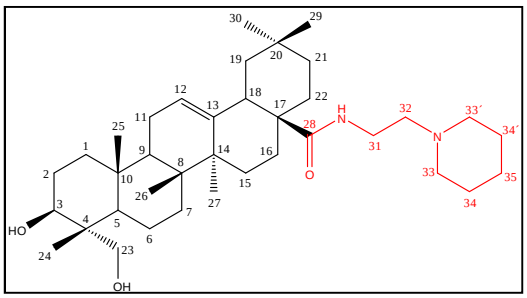
White solid; yield: 114 mg, 96%; m.p. 135-136.8 ^{o}C; R_f = 0.41 (hexane/ethyl acetate 1:1 v/v); IR (KBr): ῡ = 3388, 1636, 1522, 1048, 1004, 732 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.31 (m, 3H), 7.23 (m, 2H), 6.21 (t, 1H, *J* = 5.5 Hz, N-H), 5.28 (brs, 1H, H-12), 4.61 (dd, 1H, *J* = 14.7, 3.6 Hz, H-31$_a$), 4.12 (dd, 1H, *J* = 14.7, 3.6 Hz, H-31$_b$), 3.69 (d, 1H, *J* = 10.3 Hz, H-23$_a$), 3.61 (brt, 1H, *J* = 8.4 Hz, H-3), 3.39 (d, 1H, *J* = 10.3 Hz, H-23$_b$), 2.52 (dd, 1H, *J* = 13.1, 3.6 Hz, H-18), 1.13 (s, 3H, CH_3), 0.89 (s, 9H, 3xCH_3), 0.87 (s, 3H, CH_3), 0.65 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 178.33 (C-28), 144.86 (C-13), 138.40 (C-32), 128.78 (C-34), 128.78 (C-34'), 127.84 (C-33'), 127.84 (C-33), 127.50 (C-35), 123.03 (C-12), 76.70 (C-3), 72.00 (C-23), 49.77 (C-9), 47.59 (C-17), 46.76 (C-5), 46.47 (C-19), 43.71 (C-31), 42.45 (C-4), 41.17 (C-14), 41.88 (C-18), 39.45 (C-8), 38.26 (C-1), 36.92 (C-10), 34.23 (C-21), 33.10 (C-29), 32.70 (C-7), 32.27 (C-22), 30.84 (C-20), 27.43 (C-15), 26.64 (C-27), 25.87 (C-2), 23.91 (C-30), 23.73 (C-11), 23.53 (C-16), 18.52 (C-6), 17.04 (C-26), 15.89 (C-25), 11.59 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{55}NO_3]^+$: 561.8375, found 562.4260; CHN calcd.: C, 79.10; H, 9.87; found: C, 78.95; H, 9.98.

*1-Ethylpyridinyl-(3β)3,23-dihydroxyolean-12-en-28-amide (**26**)*



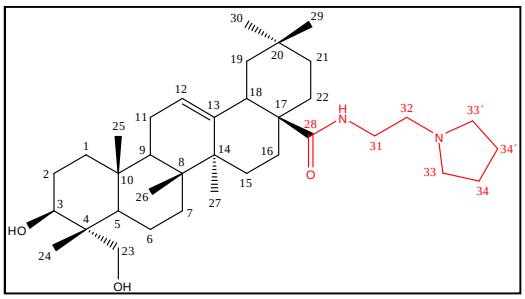
White solid; yield: 97 mg, 79%; m.p. 255.8-257.4 ^{o}C; R_f = 0.40 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\tilde{\upsilon}$ = 3402, 1730, 1636, 1522, 1242, 1048, 1004, 730 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.51 (d, 1H, *J* = 4.0 Hz, H-37), 7.61 (t, 1H, *J* = 7.5 Hz, H-35), 7.15 (d, 2H, *J* = 7.5 Hz, H-34, H-36), 6.73 (brs, 1H, N-H), 5.19 (brs, 1H, H-12), 3.69 (m, 3H, H-23$_a$,H-3, H-31$_a$), 3.42 (m, 2H, H-23$_b$, H-31$_b$), 2.96 (t, 2H, *J* = 6.1 Hz, H-32), 2.43 (brdd, 1H, *J* = 11.3 Hz, H-18), 1.09 (s, 3H, CH_3), 0.86 (s, 9H, 3xCH_3), 0.87 (s, 3H, CH_3), 0.58 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 178.24 (C-28), 159.94 (C-33), 149.18 (C-37), 144.43 (C-13), 136.72 (C-35), 123.70 (C-12), 122.82 (C-34), 121.66 (C-36), 76.82 (C-3), 72.15 (C-23), 49.83 (C-9), 47.62 (C-17), 46.92 (C-5), 46.42 (C-19), 42.10 (C-4), 42.00 (C-14), 41.87 (C-18), 39.41 (C-8), 38.67 (C-31), 38.24 (C-1), 36.97 (C-32), 36.94 (C-10), 34.25 (C-21), 33.12 (C-29), 32.74 (C-7), 32.22 (C-22), 30.82 (C-20), 27.40 (C-15), 26.66 (C-27), 25.91 (C-2), 23.69 (C-30), 23.65 (C-11), 23.47 (C-16), 18.55 (C-6), 16.94 (C-26), 15.79 (C-25), 11.59 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{56}N_2O_3]^+$: 576.8522, found 577.4366; CHN calcd.: C, 77.04; H, 9.78; found: C, 76.81; H, 9.88.

*1-Ethylpiperidinyl-(3β)3,23-dihydroxyolean-12-en-28-amide (**27**)*



Yellow solid; yield: 87 mg, 70%; m.p. 246.9-247.8 ^{o}C; R_f= 0.32 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3420, 3374, 1624, 1604, 1540, 1042, 1006 cm^{-1}; ^{1}H NMR (300 MHz, C_5D_5N): δ = 5.51 (brs, 1H, H-12), 4.22 (m, 2H, H-23$_a$, H-3), 3.73 (d, 1H, J = 10.3 Hz, H-23$_b$), 3.65 (m, 1H, H-31$_a$), 3.46 (m, 1H, H-31$_b$), 2.94 (brdd, 1H, J = 10.8 Hz, H-18), 2.44 (m, 2H, H-32), 2.31 (brs, 4H, H-33, H-33'), 1.21 (s, 3H, CH_3), 1.08 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.90 (s, 3H, CH_3); ^{13}C NMR (75 MHz, C_5D_5N): δ = 177.76 (C-28), 145.16 (C-13), 123.29 (C-12), 73.51 (C-3), 67.93 (C-23), 58.04 (C-32), 54.86 (C-33, C-33'), 48.73 (C-9), 47.38 (C-17), 47.26 (C-5), 46.81 (C-19), 43.25 (C-4), 42.53 (C-14), 42.53 (C-18), 40.07 (C-8), 39.10 (C-1), 37.51 (C-31), 37.14 (C-10), 34.70 (C-21), 33.90 (C-29), 32.45 (C-7), 32.98 (C-22), 31.20 (C-20), 28.16 (C-15), 27.97 (C-27), 26.78 (C-34, CH_2-34'), 26.34 (C-2), 25.04 (C-35), 24.22 (C-30), 24.18 (C-11), 23.94 (C-16), 18.86 (C-6), 17.73 (C-26), 16.34 (C-25), 13.52 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{37}H_{62}N_2O_3]^+$: 582.8998, found 583.4838; CHN calcd.: C, 76.24; H, 10.72; found: C, 76.04; H, 10.94.

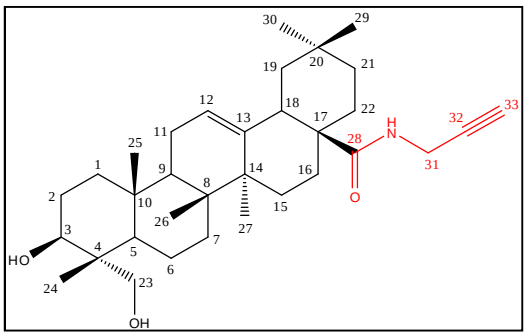
*1-Ethylpyrrolidinyl-(3β)3,23-dihydroxyolean-12-en-28-amide (**28**)*



White solid; yield: 91 mg, 76%; m.p. 240.8-242-3 ^{o}C; R_f = 0.35 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3424, 1630, 1610, 1532, 1058, 1044 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.61 (t, 1H, *J* = 4.8 Hz, N-H), 5.31 (t, 1H, *J* = 3.2 Hz, H-12), 3.69 (d, 1H, *J* = 10.3 Hz, H-23$_a$), 3.62 (brt, 1H, *J* = 8.1 Hz, H-3), 3.42 (m, 1H, H-31a), 3.40 (d, 1H, *J* = 10.0 Hz, H-23$_b$), 3.19 (m, 1H, H-31b), 2.53 (m, 7H, H-32a, H-32b, H-33a, H-33b, H-33a', H-33b', H-18), 1.13 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 0.88 (s, 6H, 2xCH_3), 0.86 (s, 3H, CH_3), 0.76 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 178.46 (C-28), 144.53 (C-13), 122.89 (C-12), 76.73 (C-3), 72.07 (C-23), 54.39 (C-32), 54.04 (C-33, C-33'), 49.85 (C-9), 47.64 (C-17), 46.85 (C-5), 46.46 (C-19), 42.37 (C-4), 41.14 (C-14), 41.88 (C-18), 39.49 (C-8), 38.30 (C-1), 37.99 (C-31), 36.97 (C-10), 34.29 (C-21), 33.13 (C-29), 32.71 (C-7), 32.36 (C-22), 30.82 (C-20), 27.46 (C-15), 26.62 (C-27), 25.84 (C-2), 23.74 (C-30), 23.74 (C-34, C-34') 23.69 (C-11), 23.66 (C-16), 18.58 (C-6), 17.03 (C-26), 15.88 (C-25), 11.64 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{36}H_{60}N_2O_3]^+$: 568.8732, found 569.4675; CHN calcd.: C, 76.01; H, 10.63; found: C, 75.85; H, 10.93.

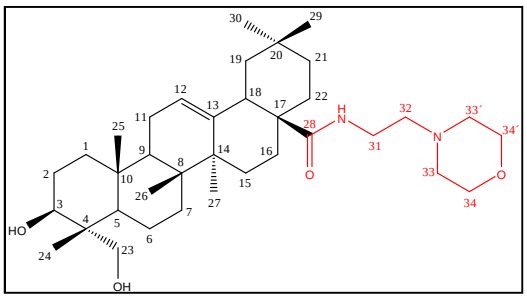
.

*2-Propyn-1-yl-(3β)3,23-dihydroxyolean-12-en-28-amide (**29**)*



White solid; yield: 101 mg, 93%; m.p. 141.8-143.2 ^{o}C; R_f = 0.48 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\bar{\upsilon}$ = 3384, 2120, 1654, 1520, 1046, 1002 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.09 (brs, 1H, N-H), 5.39 (brs, 1H, H-12), 3.95 (m, 2H, H-31), 3.69 (d, 1H, J = 10.2 Hz, H-23$_a$), 3.62 (brt, 1H, J = 7.8 Hz, H-3), 3.39 (d, 1H, J = 10.2 Hz, H-23$_b$), 2.51 (brdd, 1H, J = 11.4 Hz, H-18), 2.19 (brs, 1H, H-33), 1.14 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 0.89 (s, 6H, 2xCH_3), 0.87 (s, 3H, CH_3), 0.77 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 178.23 (C-28), 144.77 (C-13), 123.26 (C-12), 79.70 (C-32), 76.73 (C-3), 72.01 (C-23), 71.75 (C-33), 49.78 (C-9), 47.62 (C-17), 46.74 (C-5), 46.42 (C-19), 42.23 (C-4), 42.15 (C-14), 41.89 (C-18), 39.50 (C-8), 38.31 (C-1), 36.94 (C-10), 34.17 (C-21), 33.08 (C-29), 32.36 (C-7), 32.21 (C-22), 30.82 (C-20), 29.47 (C-31), 27.35 (C-15), 26.66 (C-27), 25.89 (C-2), 23.98 (C-30), 23.68 (C-11), 23.62 (C-16), 18.53 (C-6), 17.10 (C-26), 15.86 (C-25), 11.58 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{33}H_{51}NO_3]^+$: 509.7629, found; 510.3944; CHN calcd.: C, 77.75; H, 10.08; found: C, 77.51; H, 10.33.

*1-Ethylmorpholinyl-(3β)3,23-dihydroxyolean-12-en-28-amide (**30**)*



White solid; yield: 90 mg, 72%; m.p. 245.3-246.8 ^{o}C; R_f = 0.44 (hexane/ethyl acetate 1:1 v/v); IR (KBr): $\tilde{\upsilon}$ = 3404, 1630, 1522, 1118, 1048 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): δ = 5.39 (brs, 1H, H-12), 3.71 (brt, 4H, *J* = 4.5 Hz, H-34, H-34'), 3.61 (dd, 1H, *J* = 10.9, 5.1 Hz, H-3), 3.53 (d, 1H, *J* = 10.9 Hz, H-23$_a$), 3.31 (m, 3H, H-31, H-23$_b$), 2.71 (dd, 1H, *J* = 12.6, 2.8 Hz, H-18), 2.48 (m, 6H, H-32, H-33, H-33'), 1.20 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.80 (s, 3H, CH_3), 0.70 (s, 3H, CH_3); ^{13}C NMR (75 MHz, CD_3OD): δ = 180.29 (C-28), 145.44 (C-13), 124.06 (C-12), 73.77 (C-3), 67.89 (C-34, C-34'), 67.27 (C-23), 58.09 (C-32), 54.62 (C-33, C-33'), 48.91 (C-9), 48.66 (C-17), 47.76 (C-5), 47.61 (C-19), 43.28 (C-4), 43.07 (C-14), 42.88 (C-18), 40.62 (C-8), 39.45 (C-1), 37.89 (C-31), 37.08 (C-10), 35.08 (C-21), 34.15 (C-29), 33.49 (C-7), 33.21 (C-22), 31.60 (C-20), 28.46 (C-15), 27.39 (C-27), 26.44 (C-2), 24.61 (C-30), 24.17 (C-11), 23.95 (C-16), 19.08 (C-6), 17.96 (C-26), 16.31 (C-25), 12.74 (C-24); HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $[C_{36}H_{60}N_2O_4]^+$: 584.8726, found 585.4624; CHN calcd.: C, 73.93; H, 10.34; found: C, 73.68; H, 10.62.

EC_{50}	Melanoma	Ovarian	Colon	Breast	Lung	Thyroid

ACCEPTED MANUSCRIPT

Table 1. EC_{50} values and Confidence Interval (CI = 95 %)

	518A2		A2780		HT29		MCF7		A549		8505C	
He	34.9	5.1 4.5	19.9	0.8 0.8	50.0	4.6 4.2	25.7	3.9 3.4	29.0	3.8 3.4	38.0	1.7 1.6
1	7.8	1.6 1.3	9.7	0.5 0.4	9.5	0.5 0.5	7.9	0.3 0.3	7.0	3.9 2.5	7.6	0.8 0.7
2	10.3	0.8 0.7	12.9	0.9 0.8	12.8	0.5 0.5	10.2	2.1 1.7	10.9	2.9 2.3	8.8	2.1 1.7
3	10.9	0.5 0.5	14.3	1.5 1.4	14.3	0.3 0.3	12.7	0.5 0.5	13.2	8.3 5.1	7.8	1.0 0.9
5	10.0	1.1 1.0	13.4	2.5 2.1	13.2	0.7 0.7	12.5	0.5 0.5	13.2	2.5 2.1	11.2	0.1 0.1
6	9.5	0.4 0.4	12.9	1.9 1.7	13.0	1.1 1.0	11.5	0.1 0.1	11.4	0.1 0.1	8.0	2.1 1.7
7	7.5	0.4 0.4	11.6	0.3 0.3	12.0	1.0 1.0	10.2	0.3 0.3	11.7	0.3 0.3	8.7	0.4 0.4
8	11.7	0.9 0.9	13.6	1.2 1.1	13.5	1.2 1.1	12.7	0.6 0.6	13.6	1.3 1.2	9.5	0.9 0.8
9	9.0	0.1 0.1	12.9	3.7 2.9	13.2	1.7 1.5	11.3	0.4 0.3	12.6	0.6 0.5	8.4	0.9 0.8
10	9.2	0.3 0.3	14.4	2.2 1.9	13.3	0.8 0.8	12.7	0.4 0.4	12.9	0.4 0.4	9.4	0.4 0.3
11	11.1	0.7 0.6	15.0	0.3 0.3	13.4	0.9 0.8	12.7	0.6 0.6	13.1	2.1 1.8	13.1	1.3 1.2
12	11.7	0.9 0.8	13.8	5.9 4.1	14.8	0.3 0.3	13.0	1.2 1.1	13.0	4.4 3.3	11.9	0.3 0.3
13	10.9	1.3 1.1	13.7	3.7 2.9	14.4	0.6 0.6	13.5	2.4 2.1	13.5	5.5 3.9	9.7	1.2 1.1
14	8.7	1.0 0.9	12.1	0.5 0.5	13.4	0.6 0.5	9.0	0.3 0.3	11.4	0.7 0.6	10.3	0.1 0.1
15	8.1	0.2 0.2	11.9	0.3 0.3	12.0	0.5 0.5	8.3	0.5 0.4	8.1	0.8 0.7	6.6	0.3 0.3
16	12.7	4.1 3.1	13.5	2.0 1.8	14.0	0.4 0.4	13.4	0.9 0.9	14.0	1.2 1.1	12.8	8.4 5.1
17	12.4	1.9 1.6	17.0	1.6 1.4	17.7	0.4 0.4	15.4	1.0 0.9	15.9	1.0 0.9	13.2	4.0 3.1
18	8.4	0.2 0.2	7.8	0.3 0.3	7.8	0.1 0.1	7.8	0.4 0.4	6.5	0.0 0.0	6.1	0.1 0.1
19	11.8	0.2 0.2	14.7	0.0 0.0	14.8	0.8 0.8	13.7	0.7 0.6	13.7	3.4 2.8	8.8	3.5 2.5
20	10.2	0.2 0.2	11.4	0.1 0.1	11.3	0.7 0.6	9.2	0.1 0.1	8.5	0.2 0.1	6.7	0.8 0.7
21	11.4	2.3 1.9	13.0	0.9 0.8	13.5	0.7 0.7	12.7	0.3 0.3	9.8	0.4 0.4	8.1	1.3 1.1
22	11.6	0.8 0.8	13.2	1.0 0.9	13.7	0.9 0.8	13.5	1.8 1.6	13.1	1.8 1.6	13.5	1.3 1.2
23	13.8	2.6 2.2	14.6	1.0 0.9	15.3	0.5 0.5	14.2	0.9 0.8	12.2	1.4 1.2	14.2	1.1 1.0
24	8.6	0.1 0.1	8.8	0.1 0.1	11.2	1.1 1.0	7.7	0.4 0.4	7.5	0.5 0.4	9.5	1.4 1.2
25	13.7	1.2 1.1	11.4	1.6 1.4	15.1	1.6 1.5	14.6	0.9 0.8	10.3	1.3 1.2	15.7	1.6 1.4
26	12.5	2.2 1.9	15.9	0.7 0.6	19.3	1.0 1.0	15.2	1.2 1.1	15.1	2.0 1.8	17.2	0.6 0.5
27	3.7	0.2 0.2	1.8	0.1 0.1	1.3	0.3 0.2	6.5	0.4 0.4	3.6	0.2 0.2	2.7	0.6 0.5
28	2.0	0.1 0.1	1.1	0.1 0.1	1.2	0.1 0.1	3.7	0.1 0.1	1.1	0.3 0.2	1.9	0.2 0.2
29	21.6	1.6 1.5	14.8	1.5 1.3	17.3	2.0 1.8	17.0	1.2 1.1	14.8	3.1 2.6	16.8	1.1 1.0
30	>30		>30		>30		>30		>30		>30	

Section B:

Figure S1. ^{1}H NMR spectrum of **1**

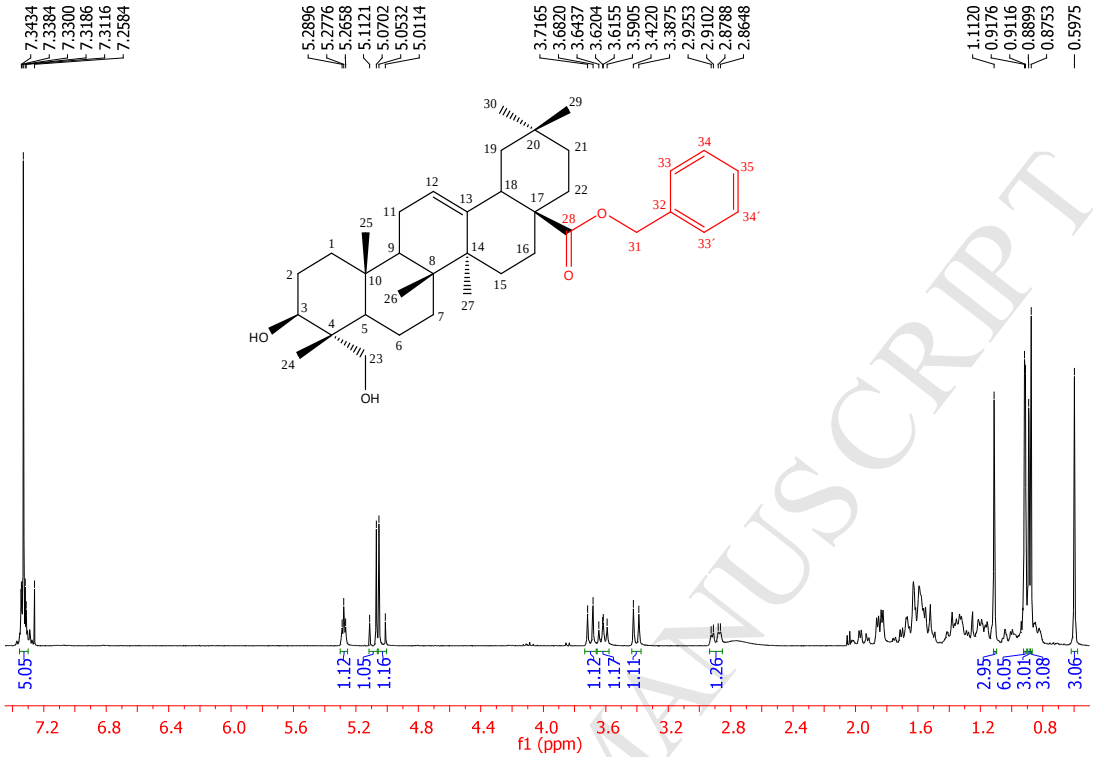


Figure S2. ^{13}C NMR spectrum of **1**

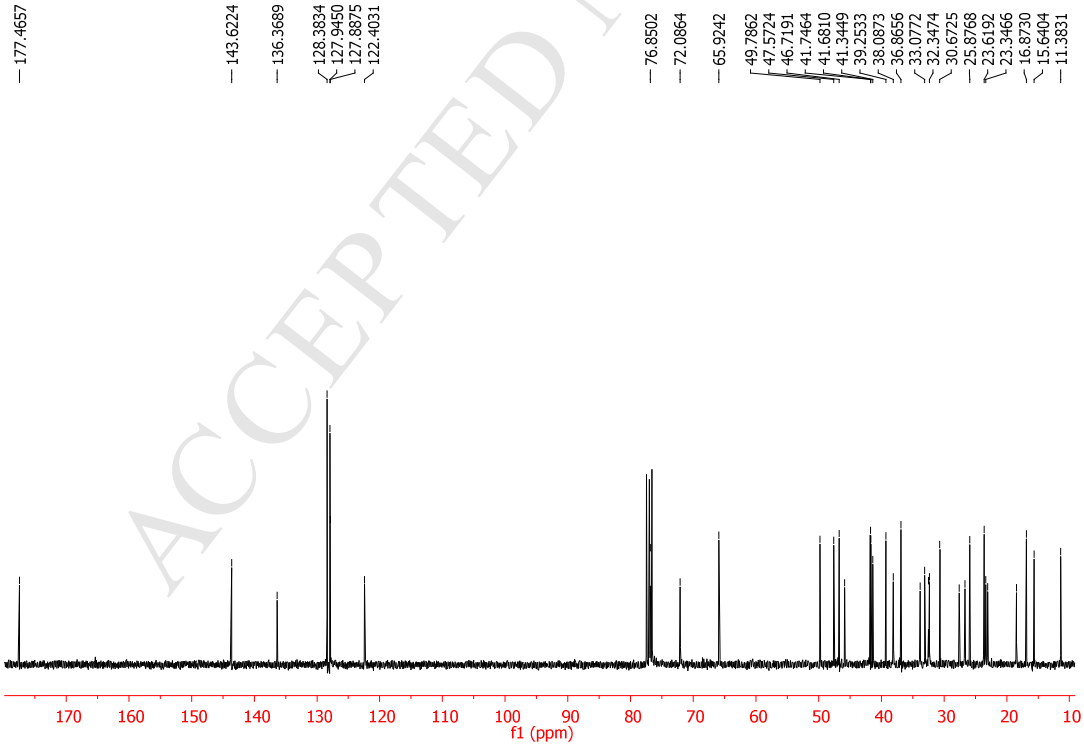


Figure S3. ^{1}H NMR spectrum of **2**

ACCEPTED MANUSCRIPT

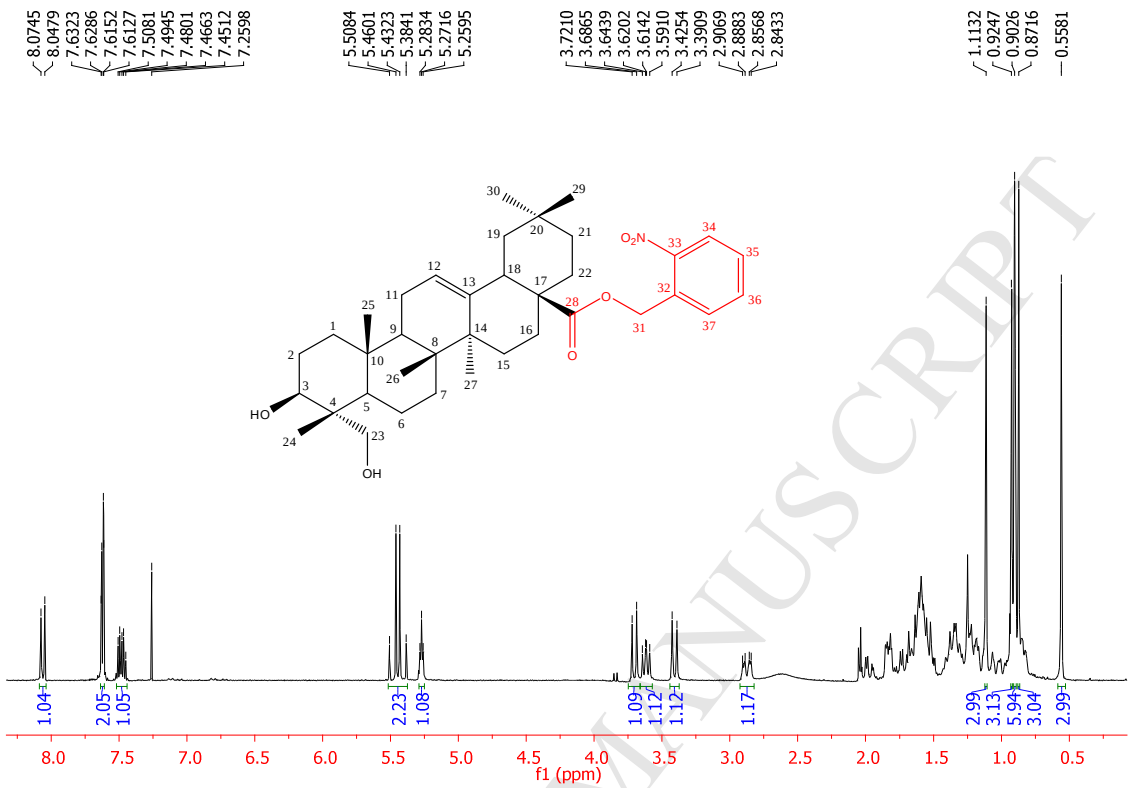


Figure S4. ^{13}C NMR spectrum of **2**

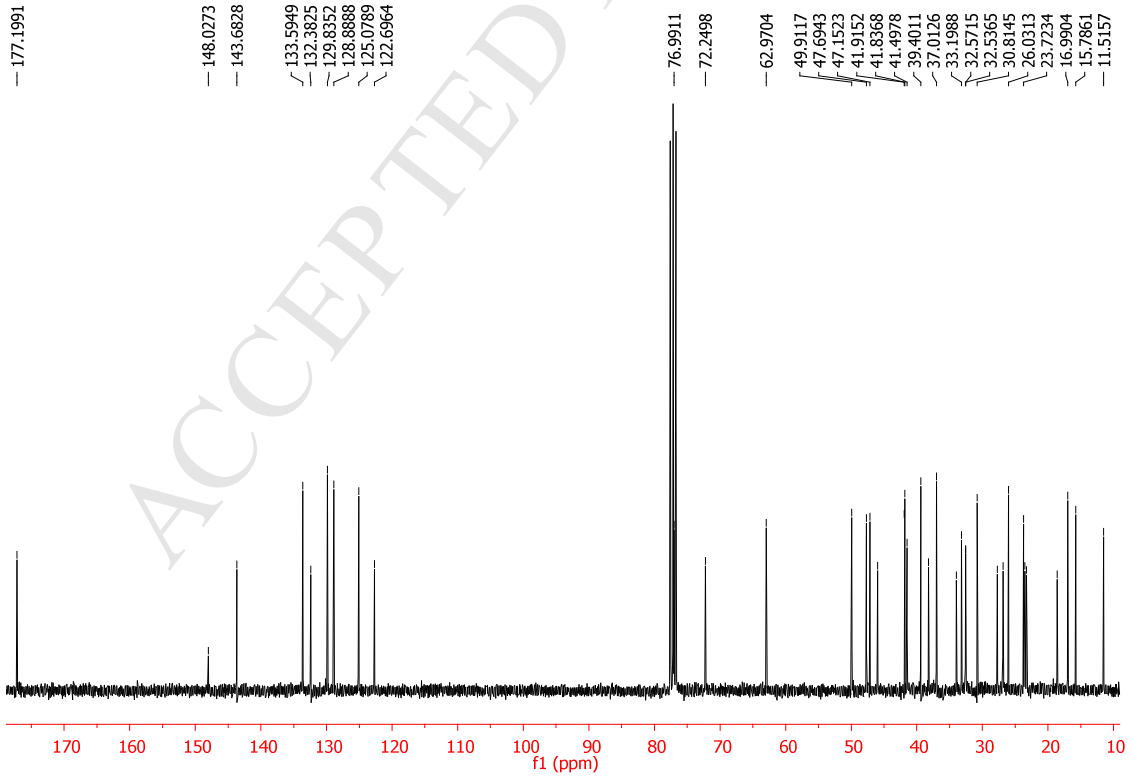


Figure S5. ^{1}H NMR spectrum of **3**

ACCEPTED MANUSCRIPT

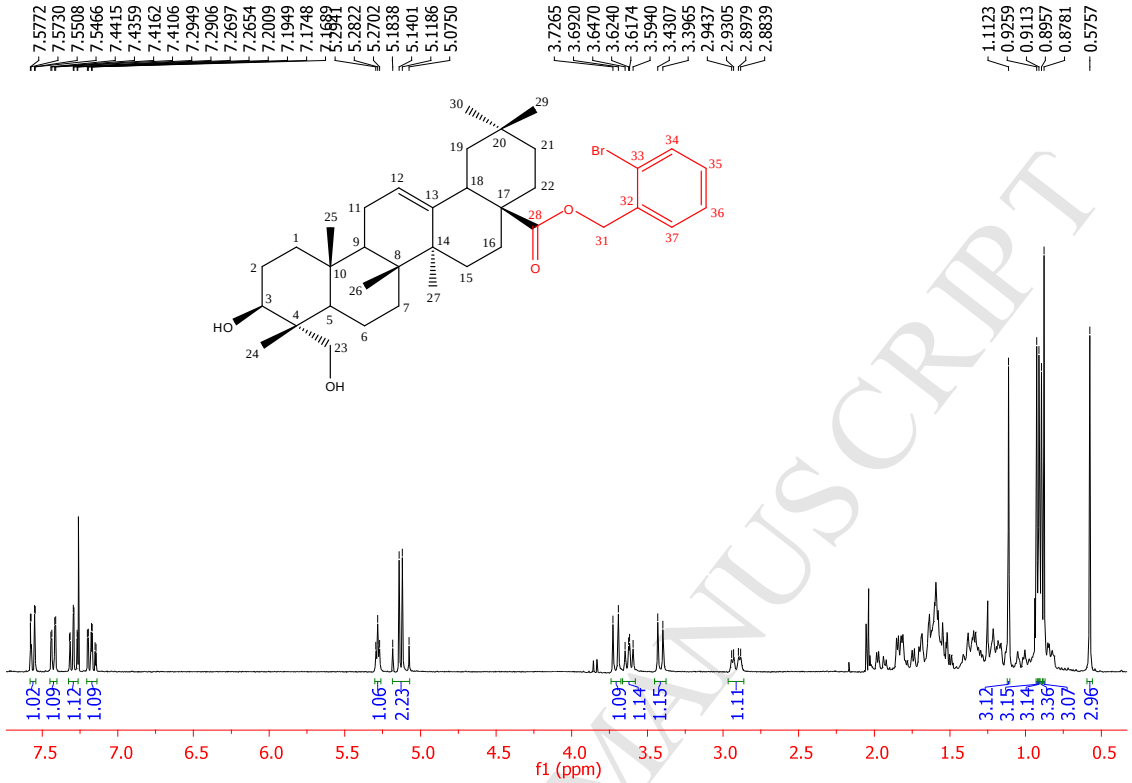


Figure S6. ^{13}C NMR spectrum of **3**

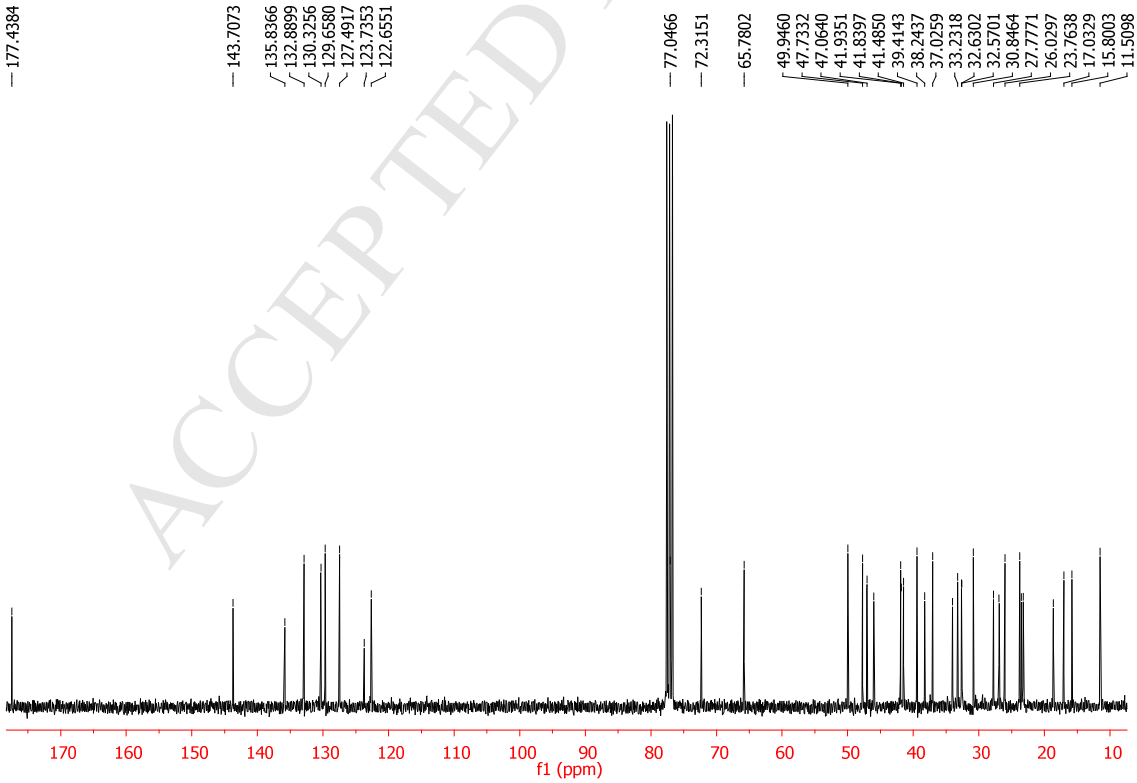


Figure S7. ^{1}H NMR spectrum of **4**

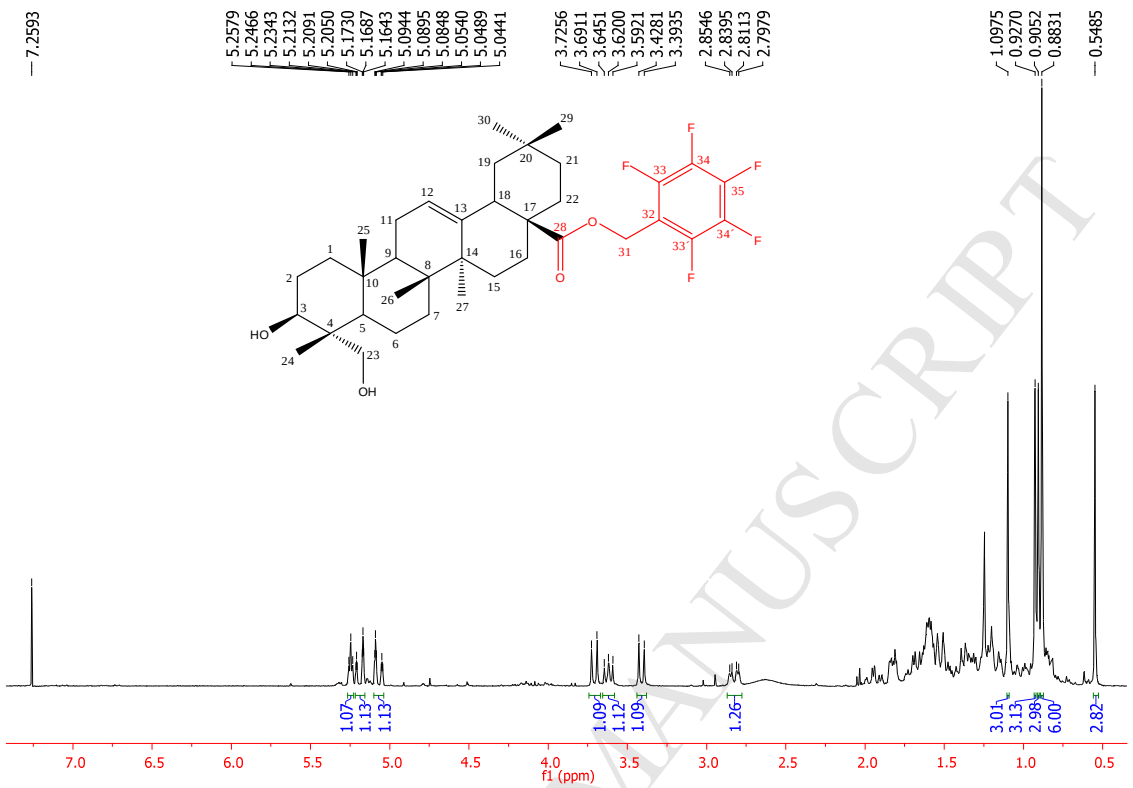


Figure S8. ^{13}C NMR spectrum of **4**

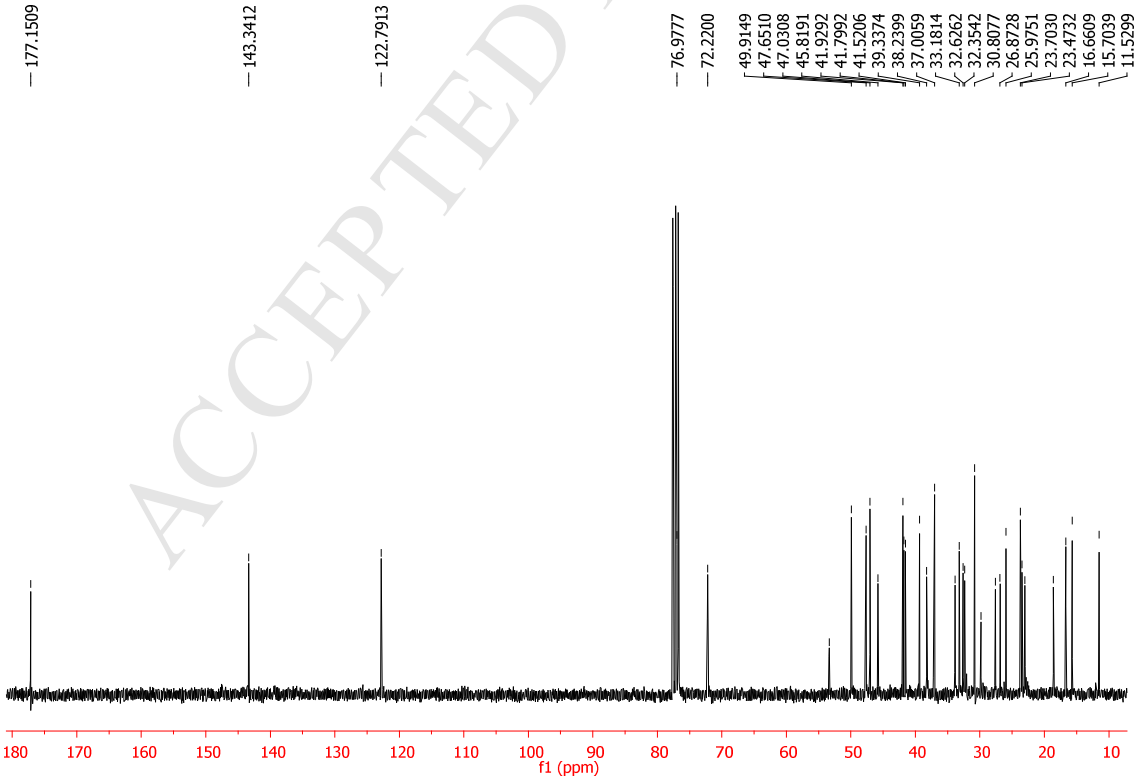


Figure S9. ^{1}H NMR spectrum of **5**

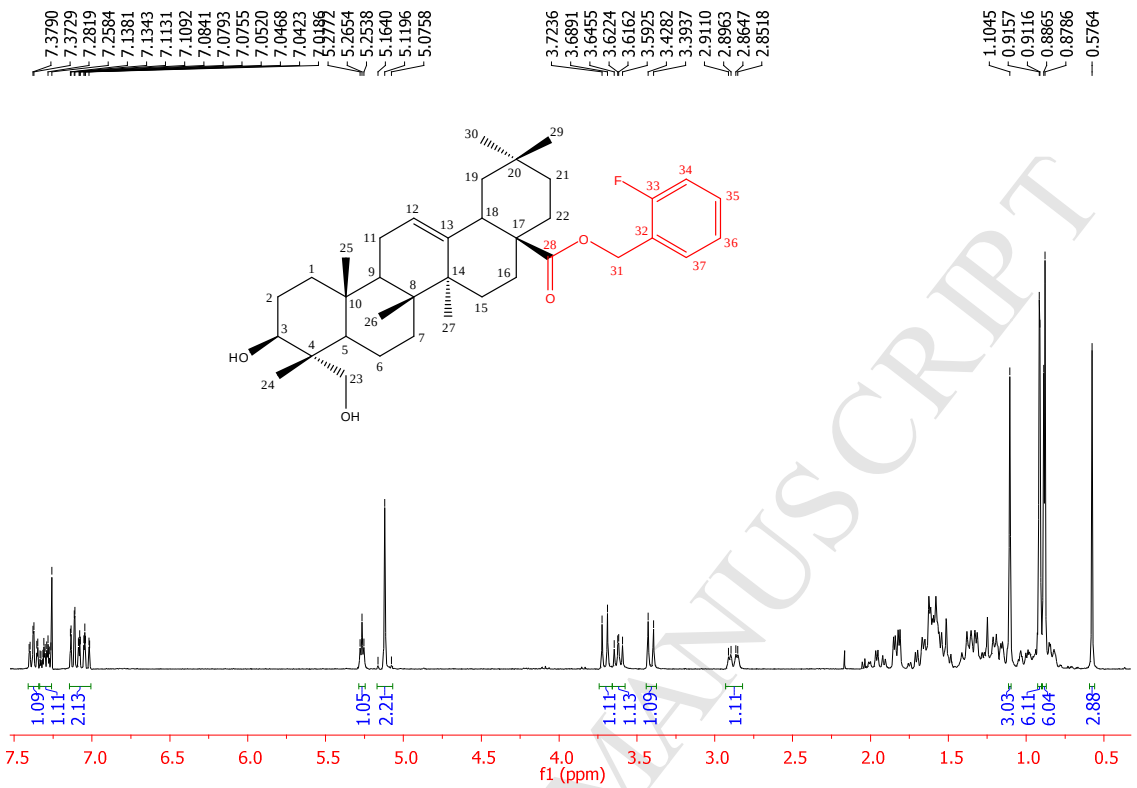


Figure S10. ^{13}C NMR spectrum of **5**

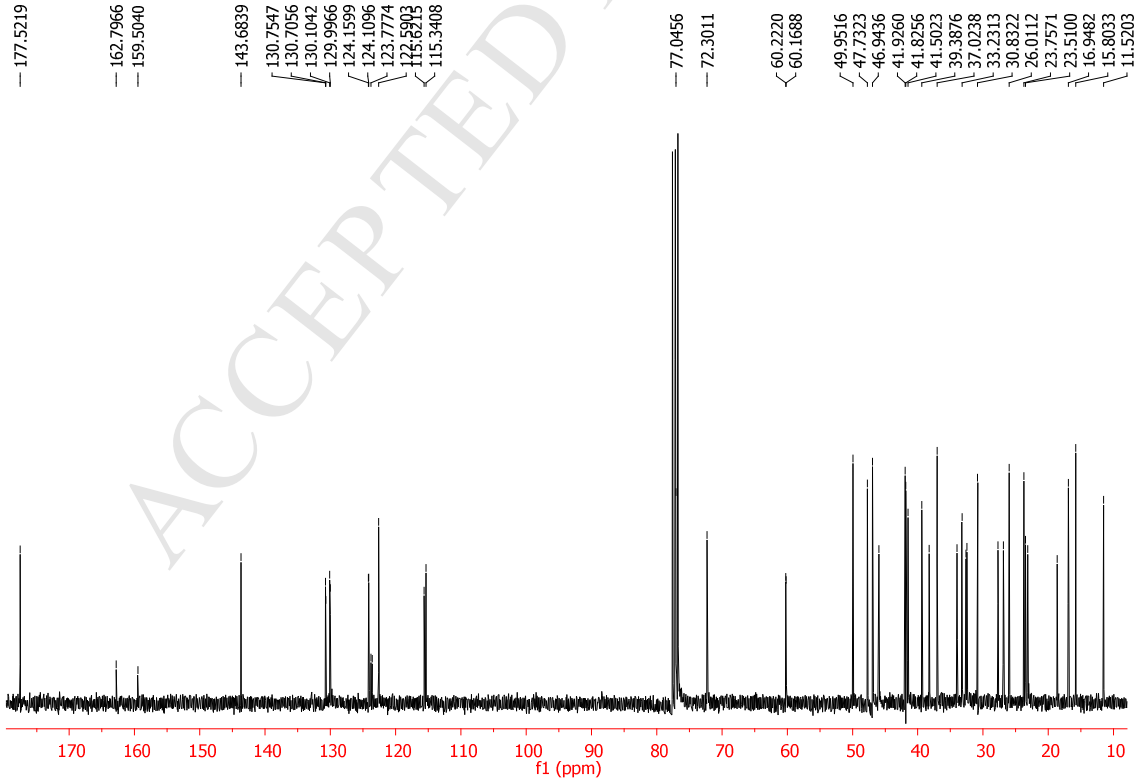


Figure S11. ^{1}H NMR spectrum of **6**

ACCEPTED MANUSCRIPT

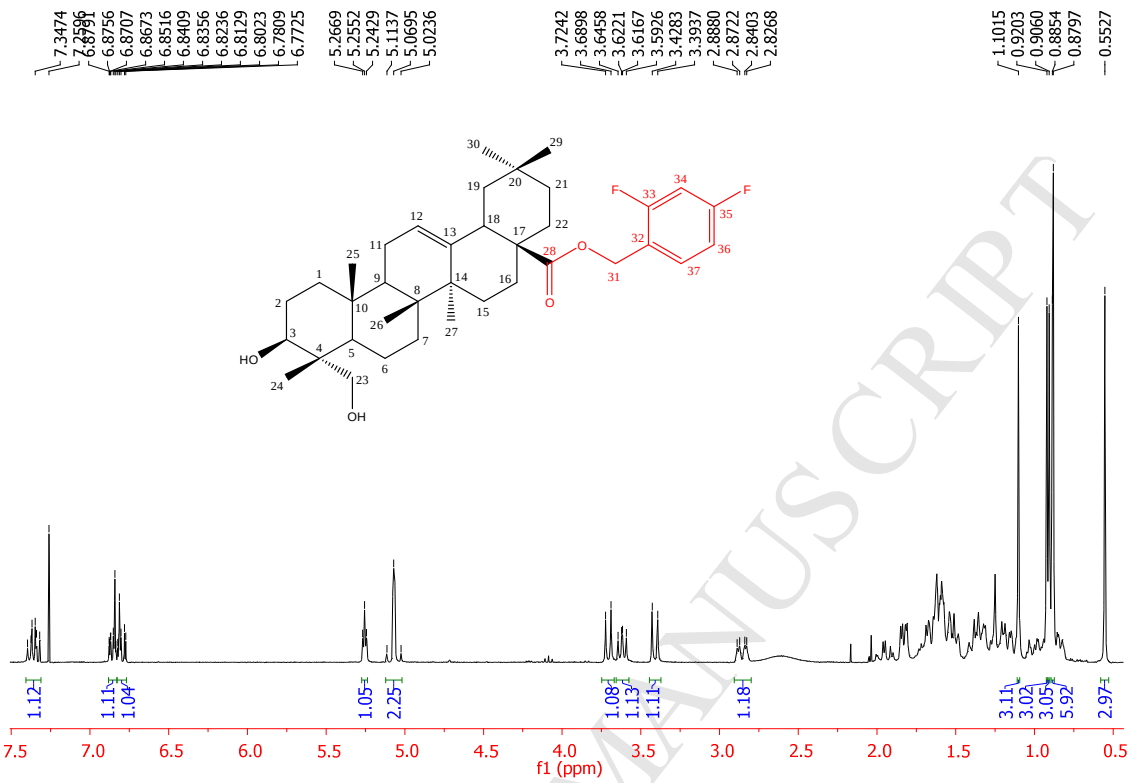


Figure S12. ^{13}C NMR spectrum of **6**

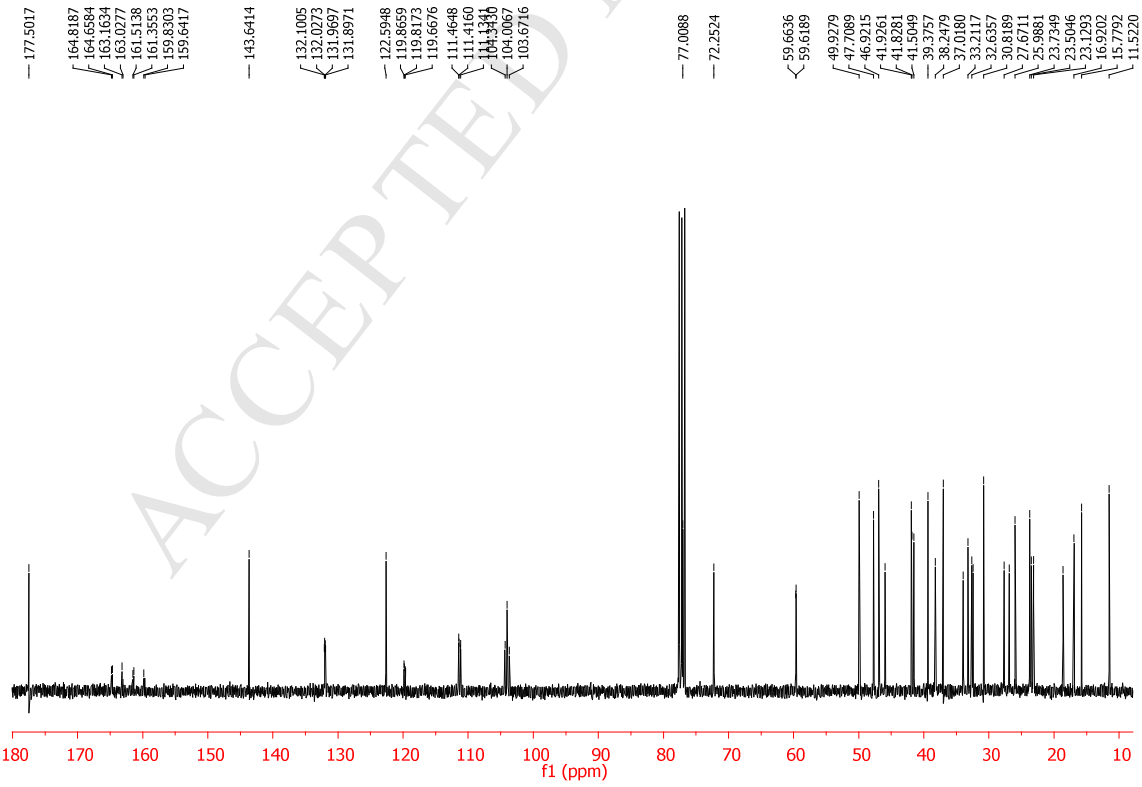


Figure S13. ^{1}H NMR spectrum of **7**

ACCEPTED MANUSCRIPT

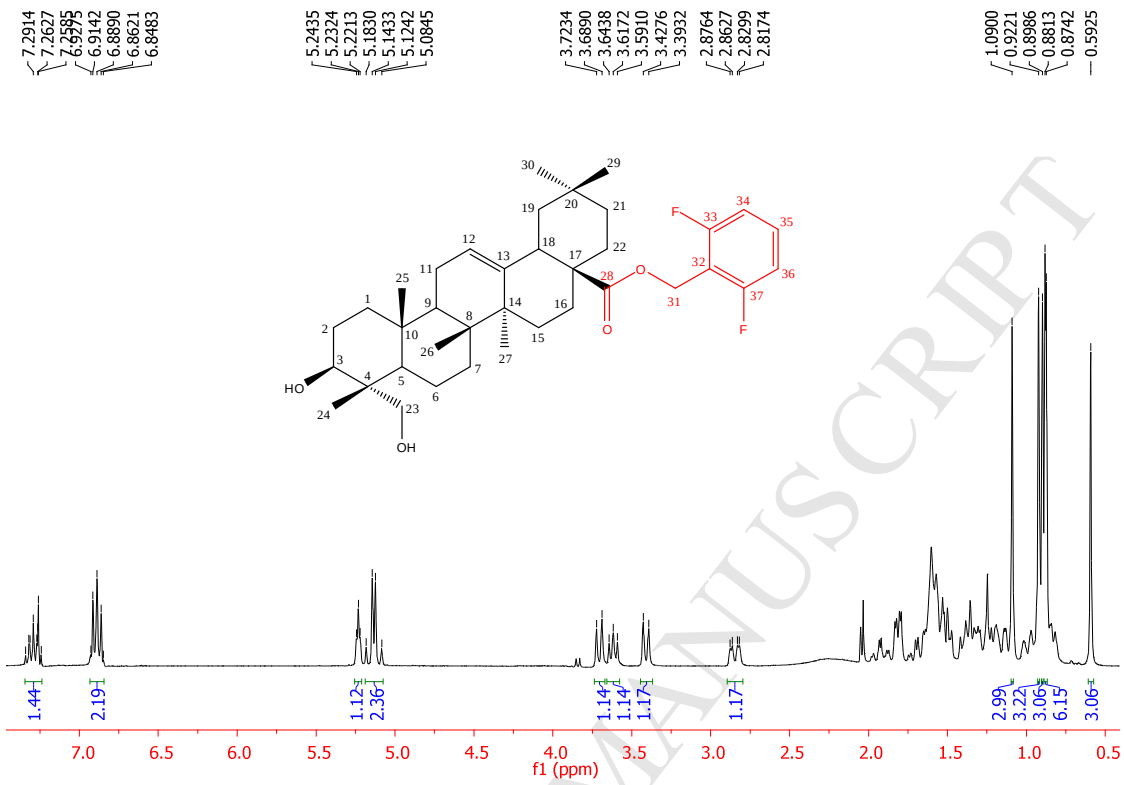


Figure S14.[13]C NMR spectrum of **7**

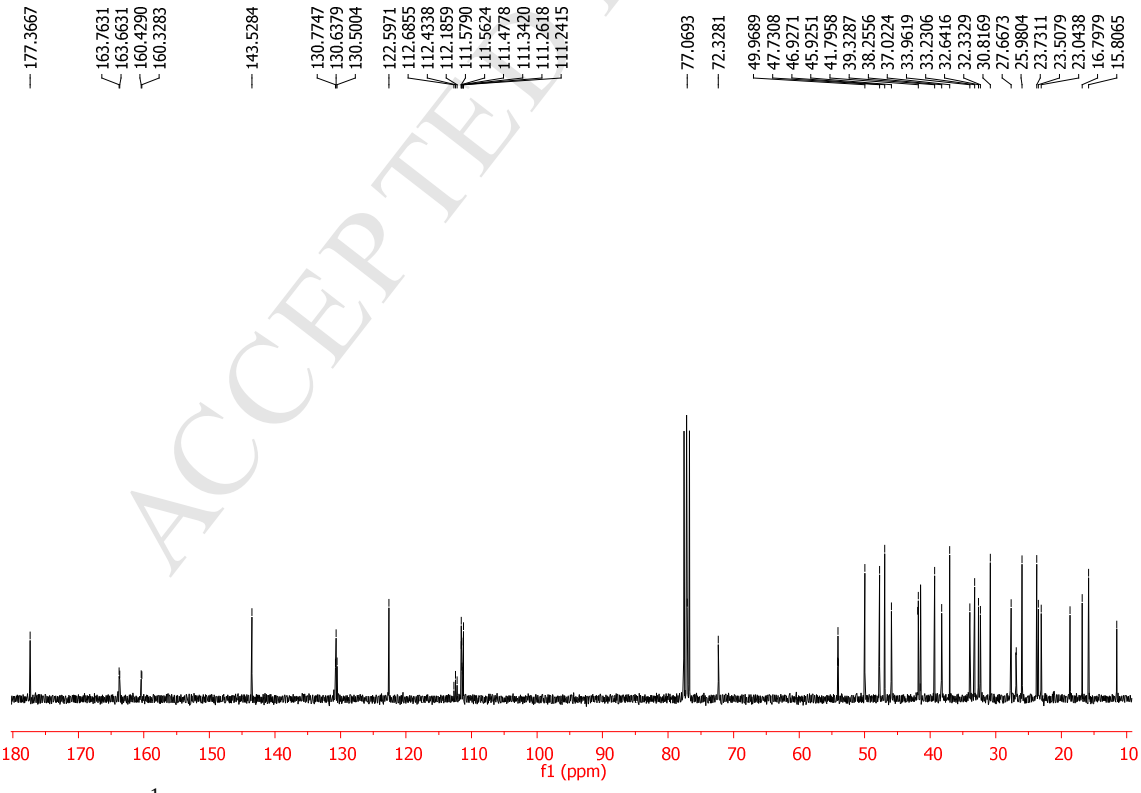


Figure S15. [1]H NMR spectrum of **8**

ACCEPTED MANUSCRIPT

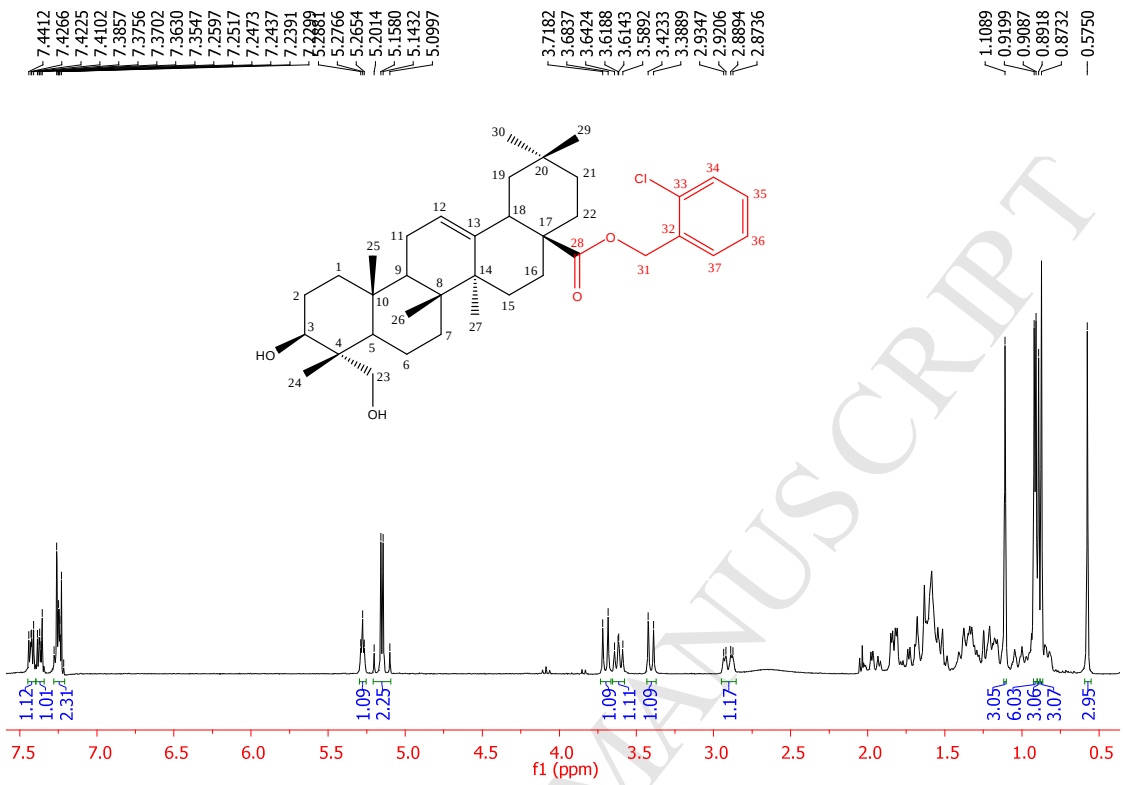


Figure S16. ^{13}C NMR spectrum of **8**

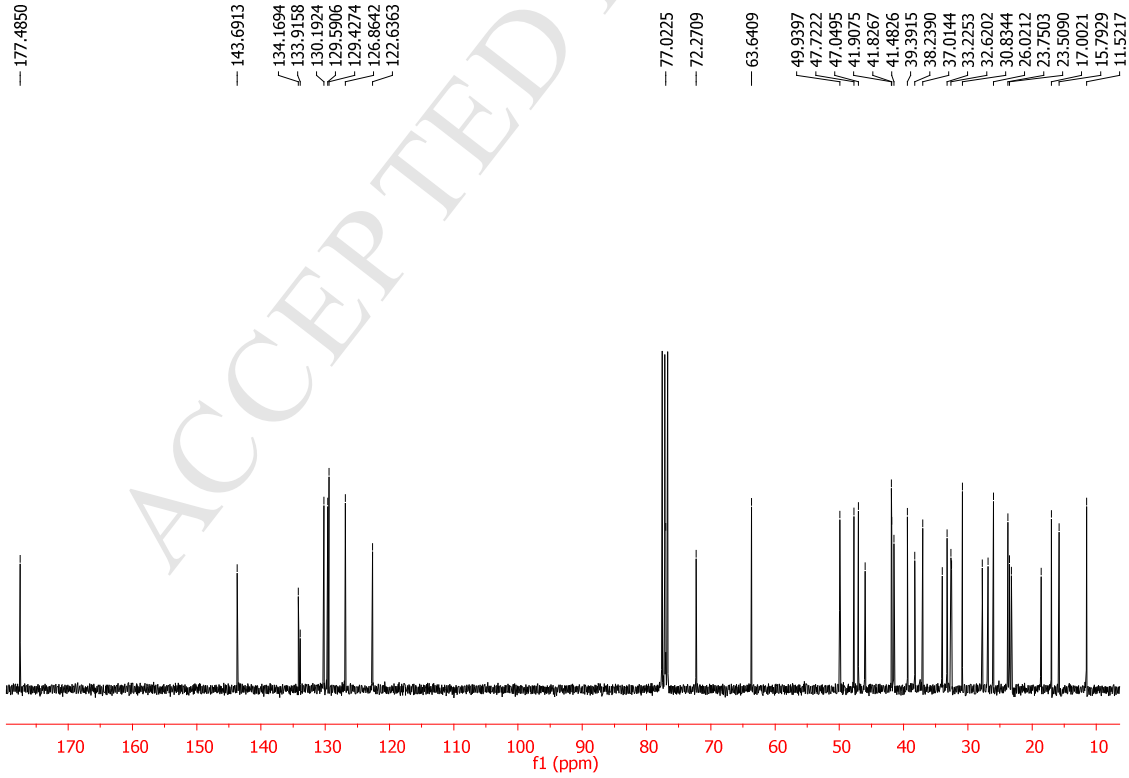


Figure S17. ^{1}H NMR spectrum of **9**

ACCEPTED MANUSCRIPT

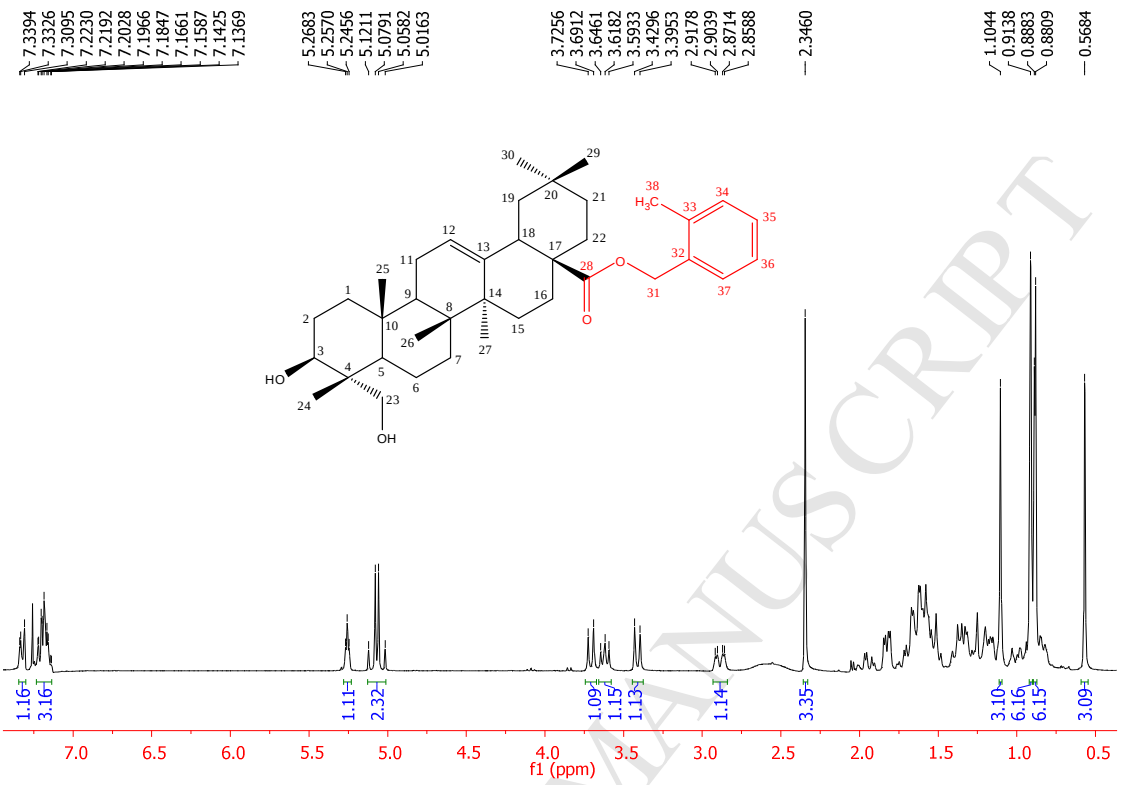


Figure S18. ^{13}C NMR spectrum of **9**

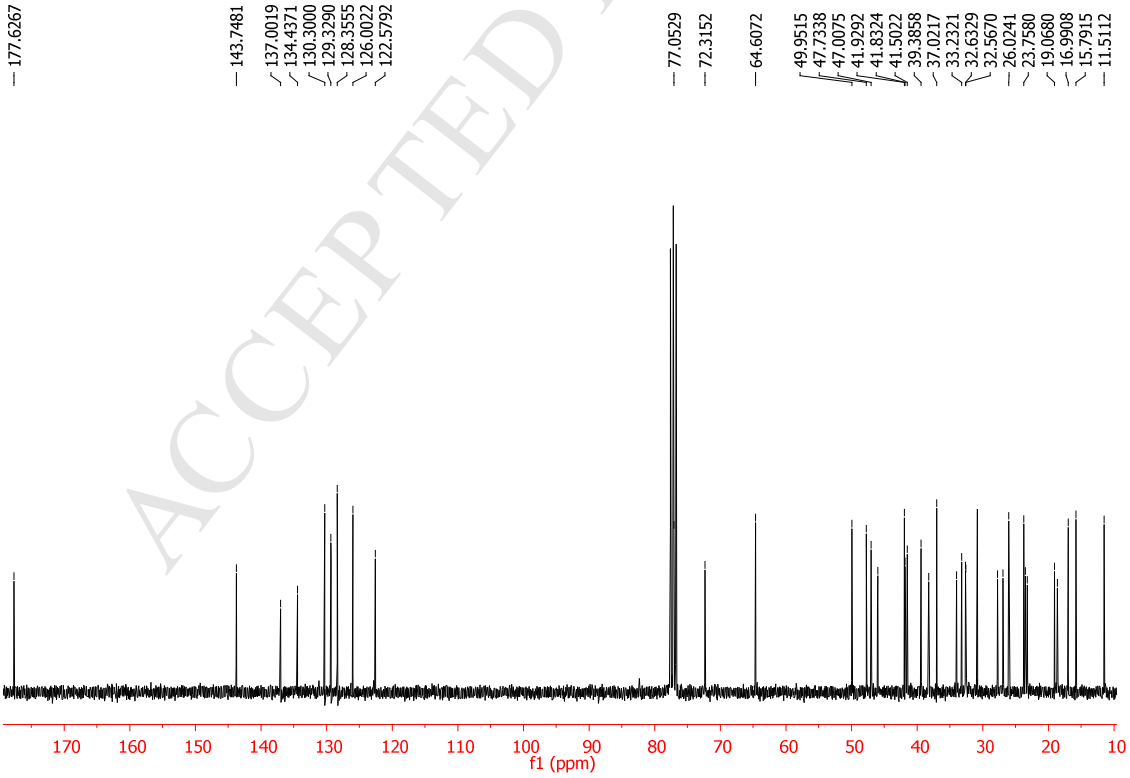


Figure S19. ^{1}H NMR spectrum of **10**

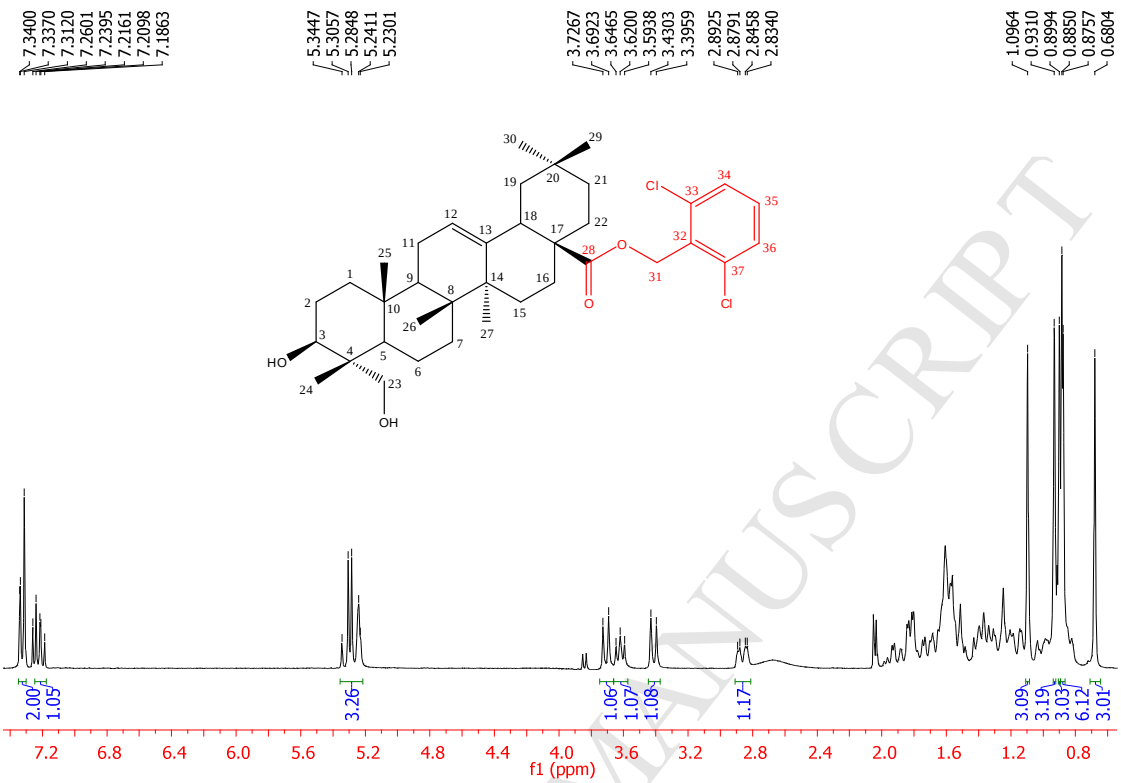


Figure S20. ^{13}C NMR spectrum of **10**

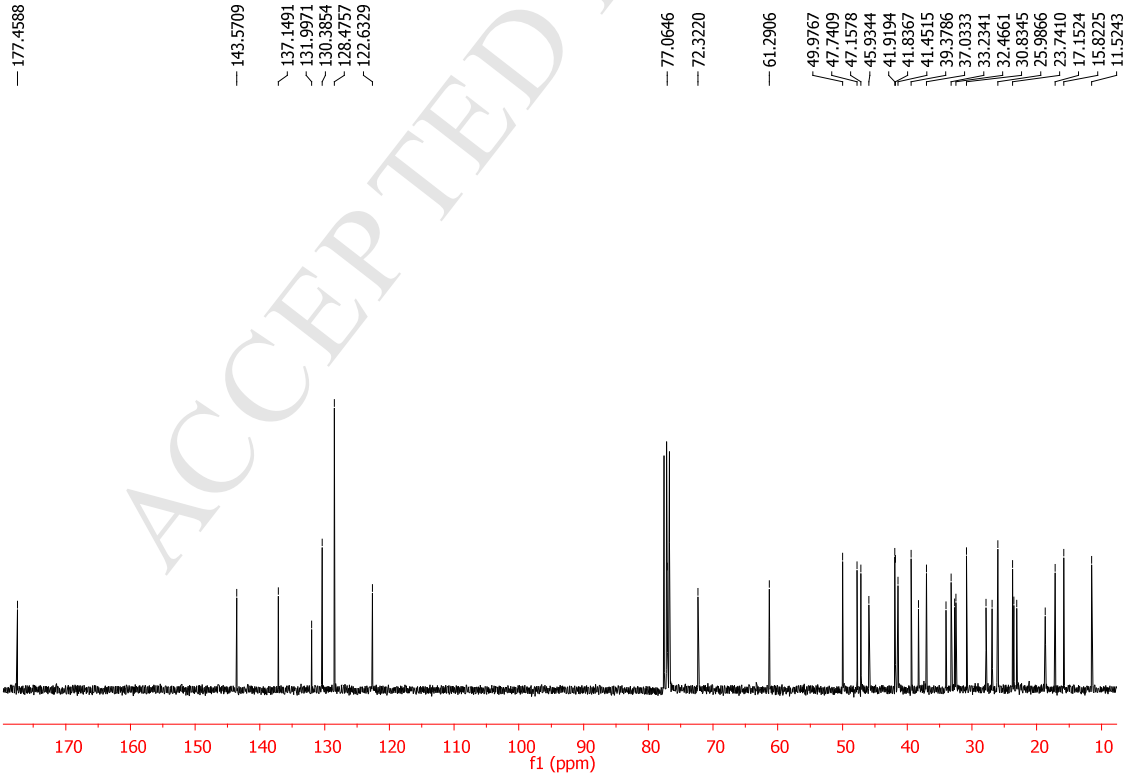


Figure S21. ^{1}H NMR spectrum of **11**

ACCEPTED MANUSCRIPT

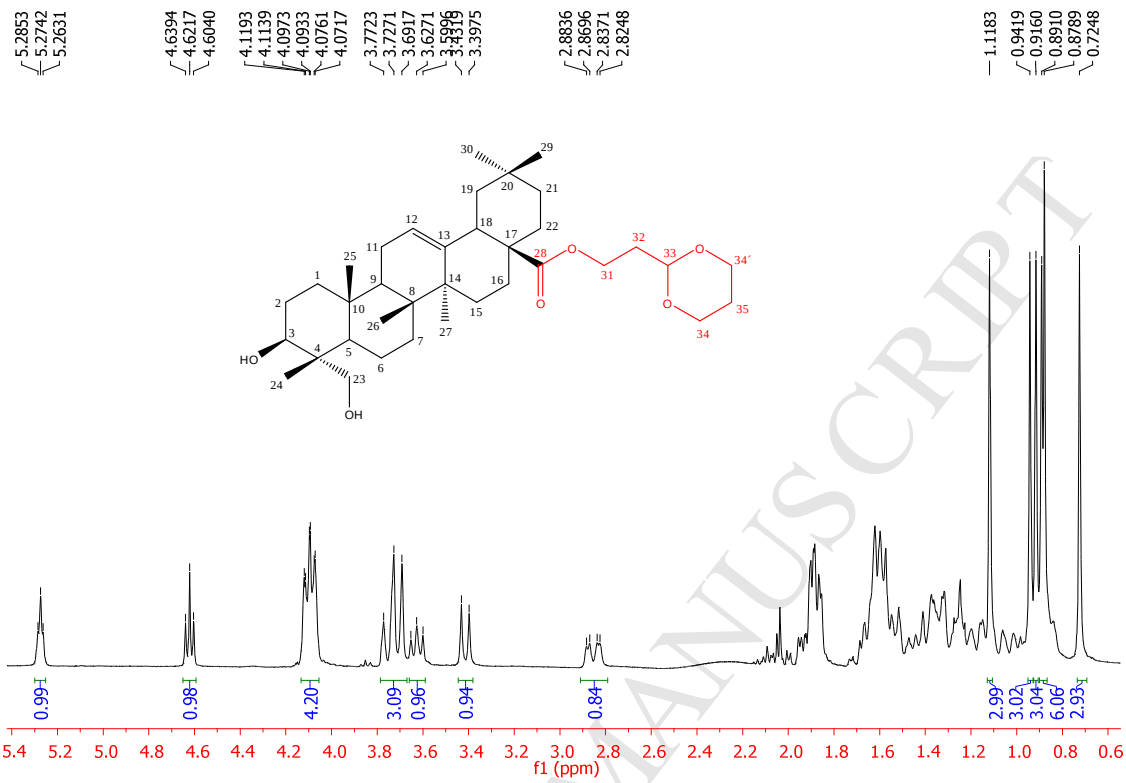


Figure S22. ^{13}C NMR spectrum of **11**

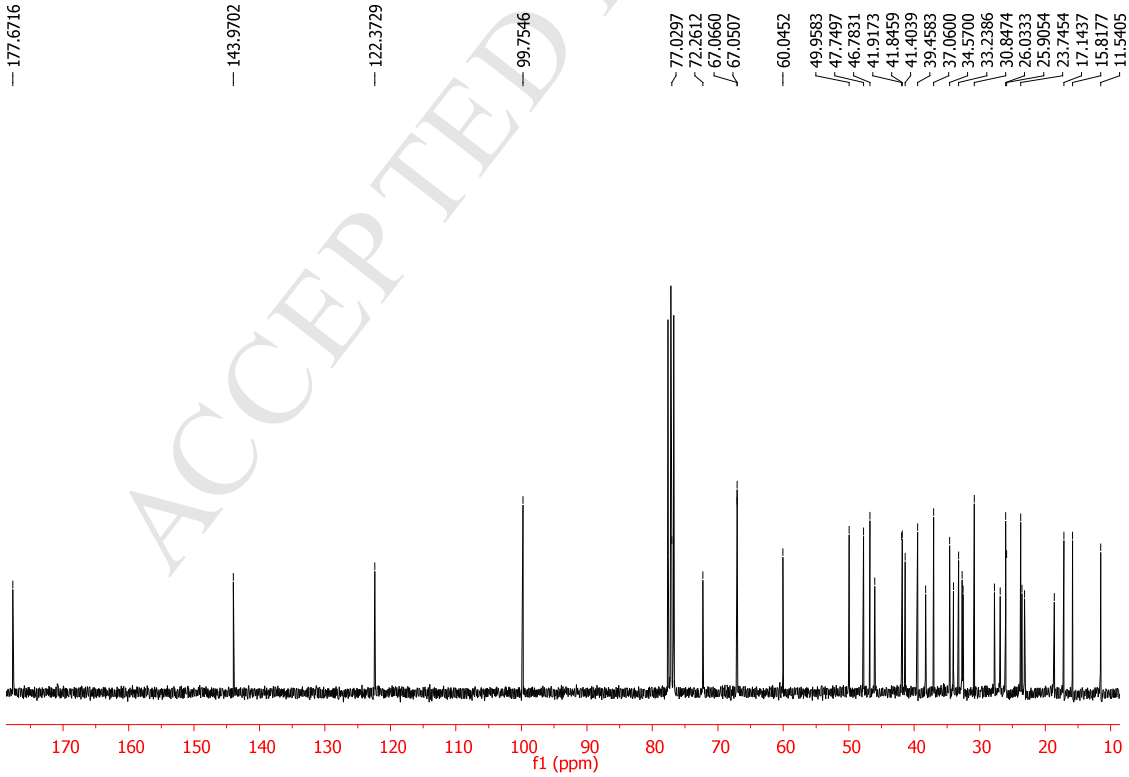


Figure S23. ^{1}H NMR spectrum of **12**

ACCEPTED MANUSCRIPT

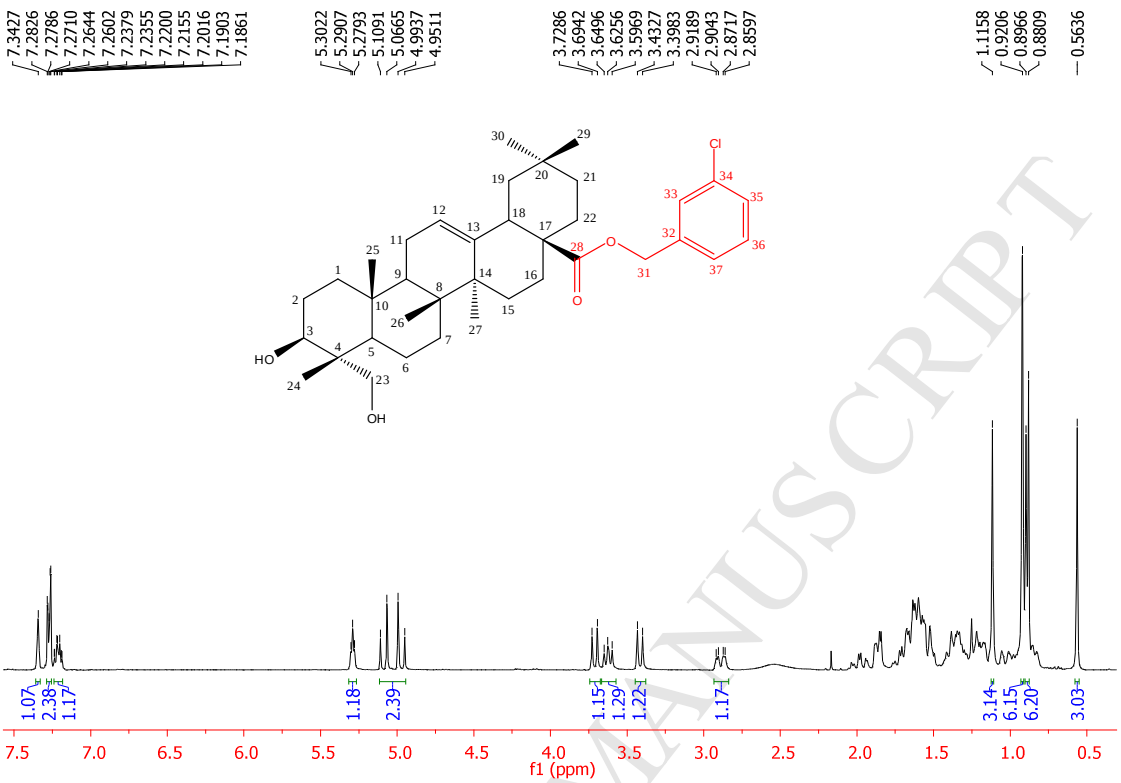


Figure S24. ^{13}C NMR spectrum of **12**

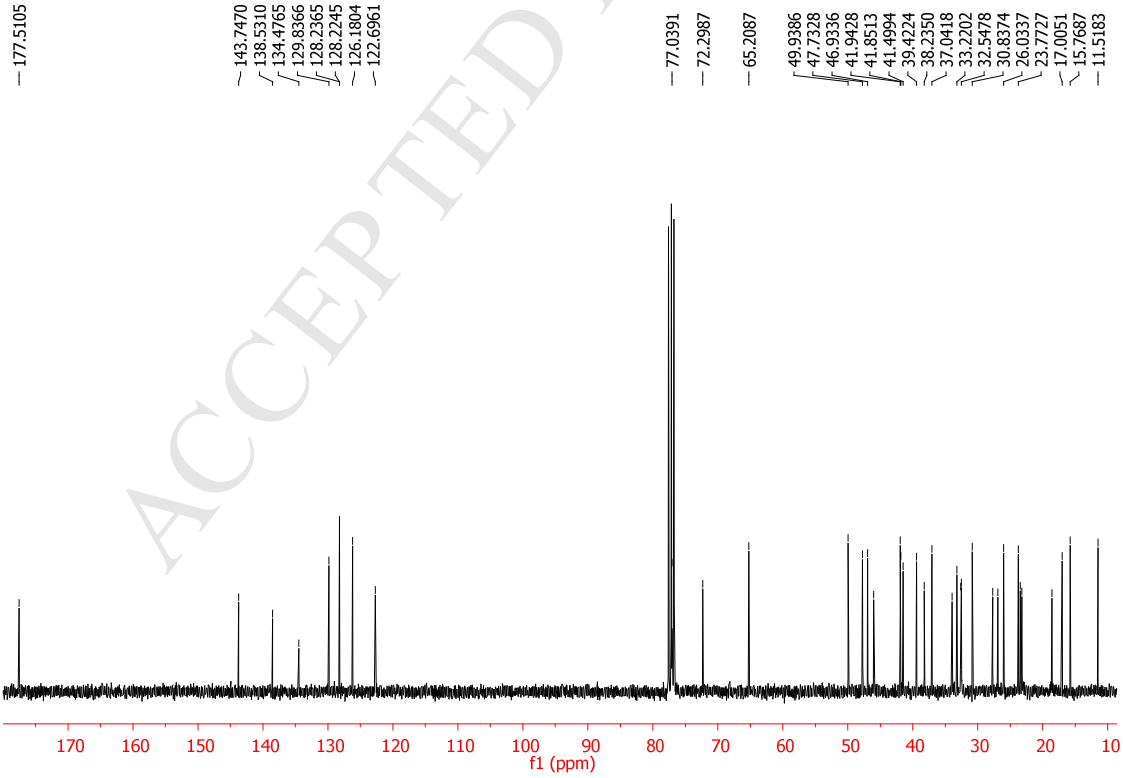


Figure S25. ^{1}H NMR spectrum of **13**

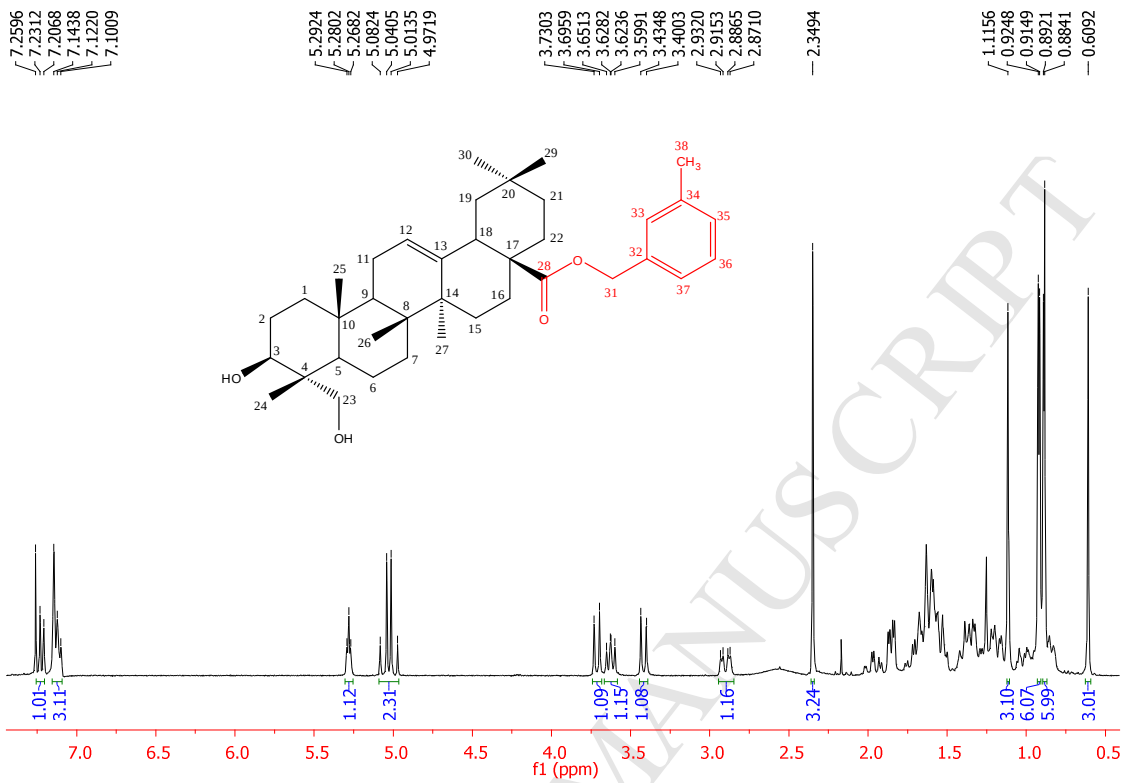


Figure S26. ^{13}C NMR spectrum of **13**

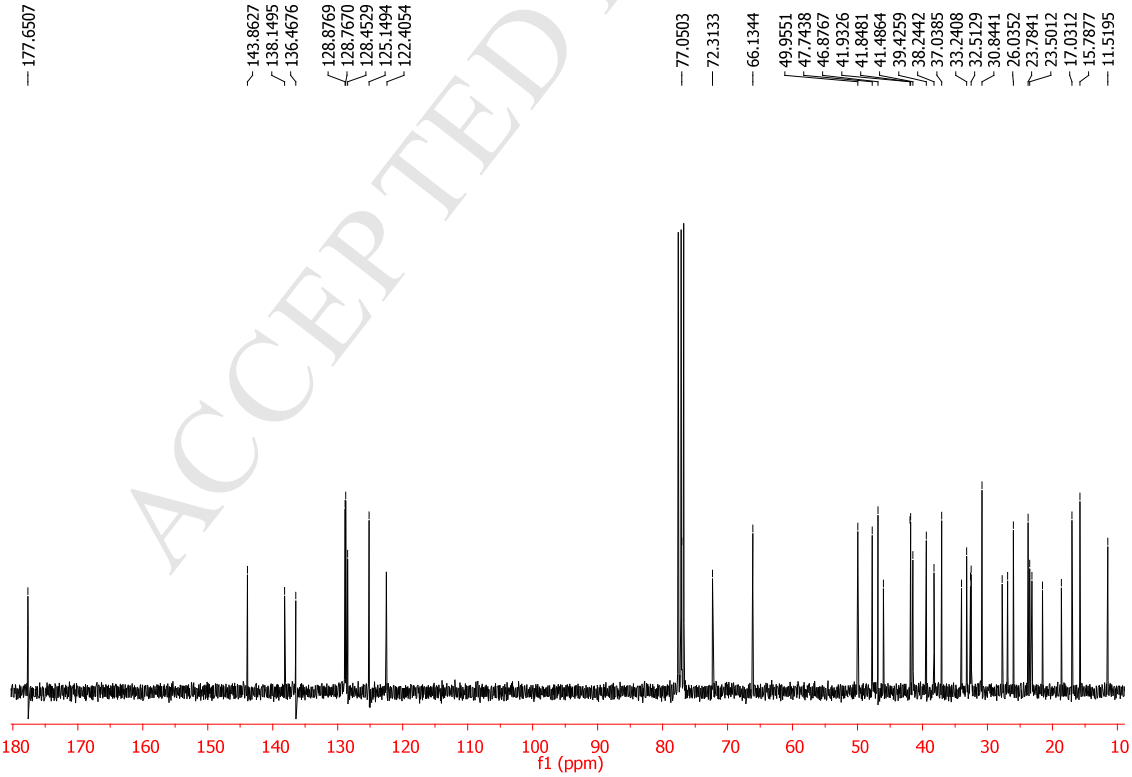


Figure S27. ^{1}H NMR spectrum of **14**

ACCEPTED MANUSCRIPT

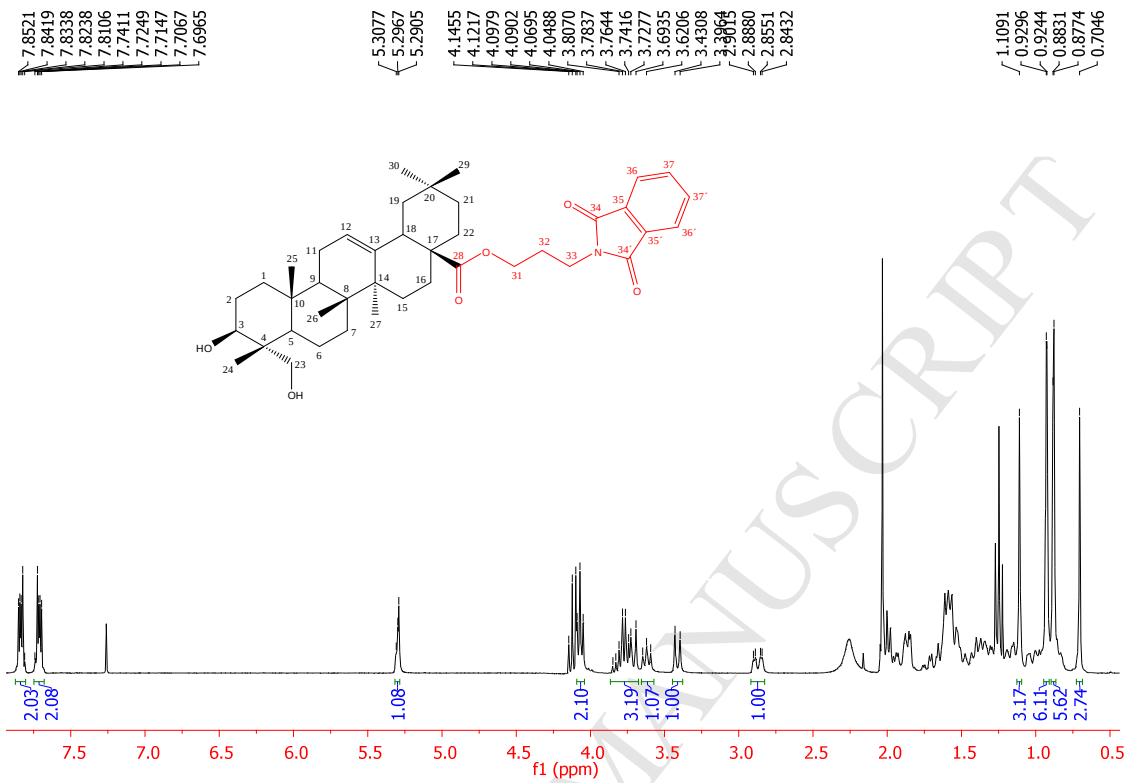


Figure S28. ^{13}C NMR spectrum of **14**

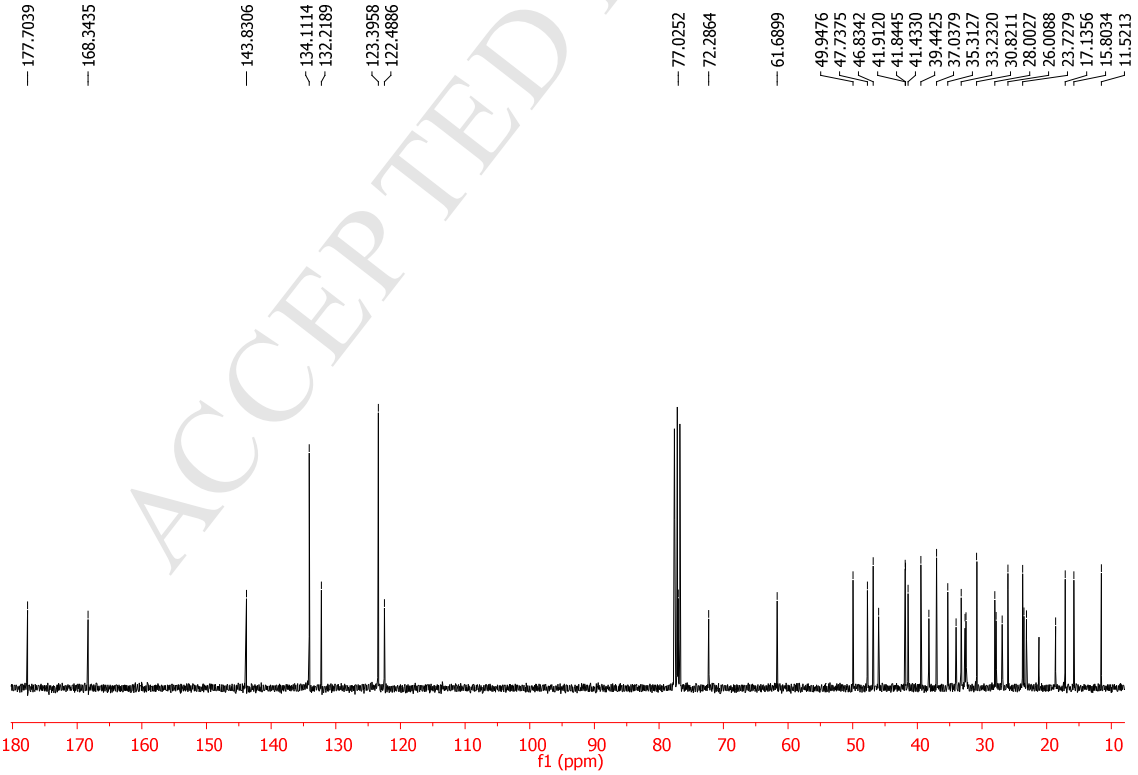


Figure S29. ^{1}H NMR spectrum of **15**

ACCEPTED MANUSCRIPT

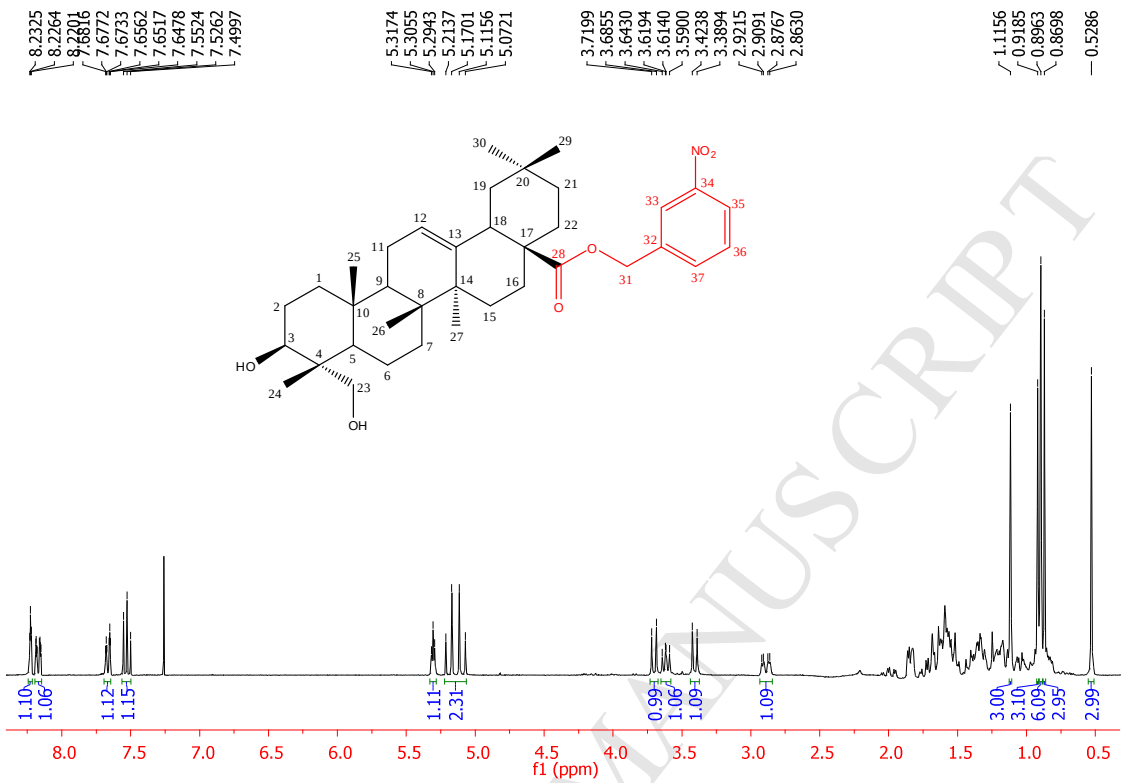


Figure S30. ^{13}C NMR spectrum of **15**

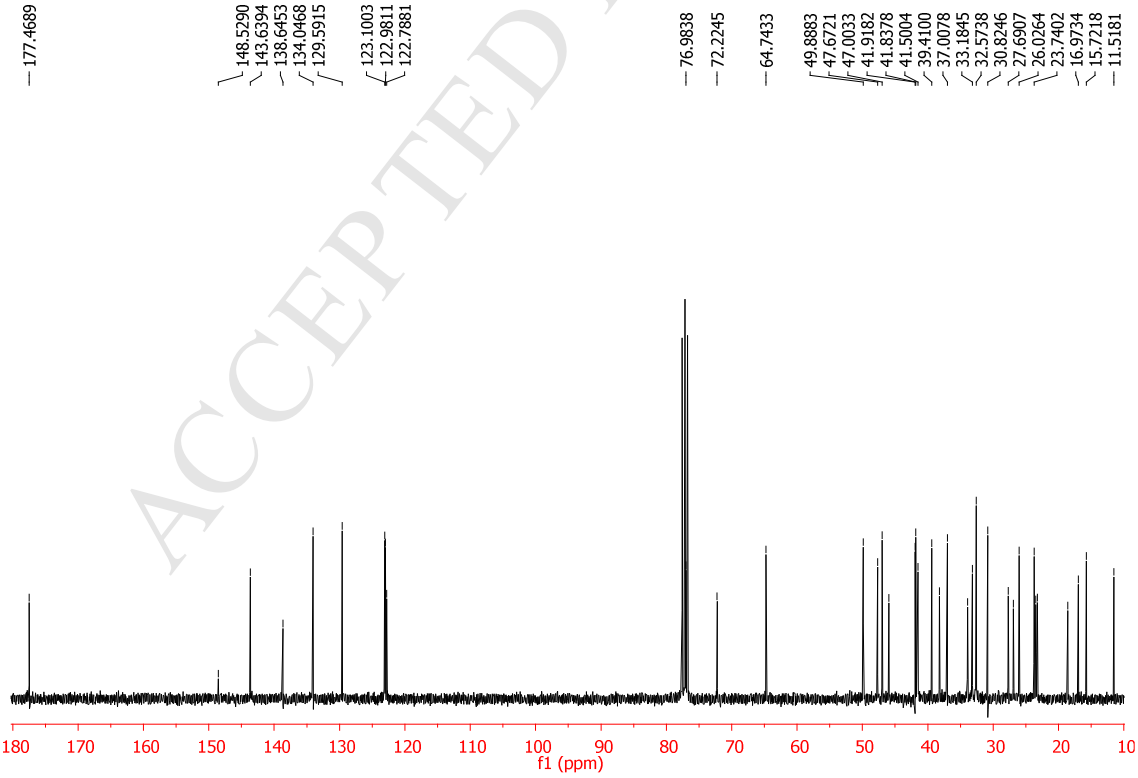


Figure S31. ^{1}H NMR spectrum of **16**

ACCEPTED MANUSCRIPT

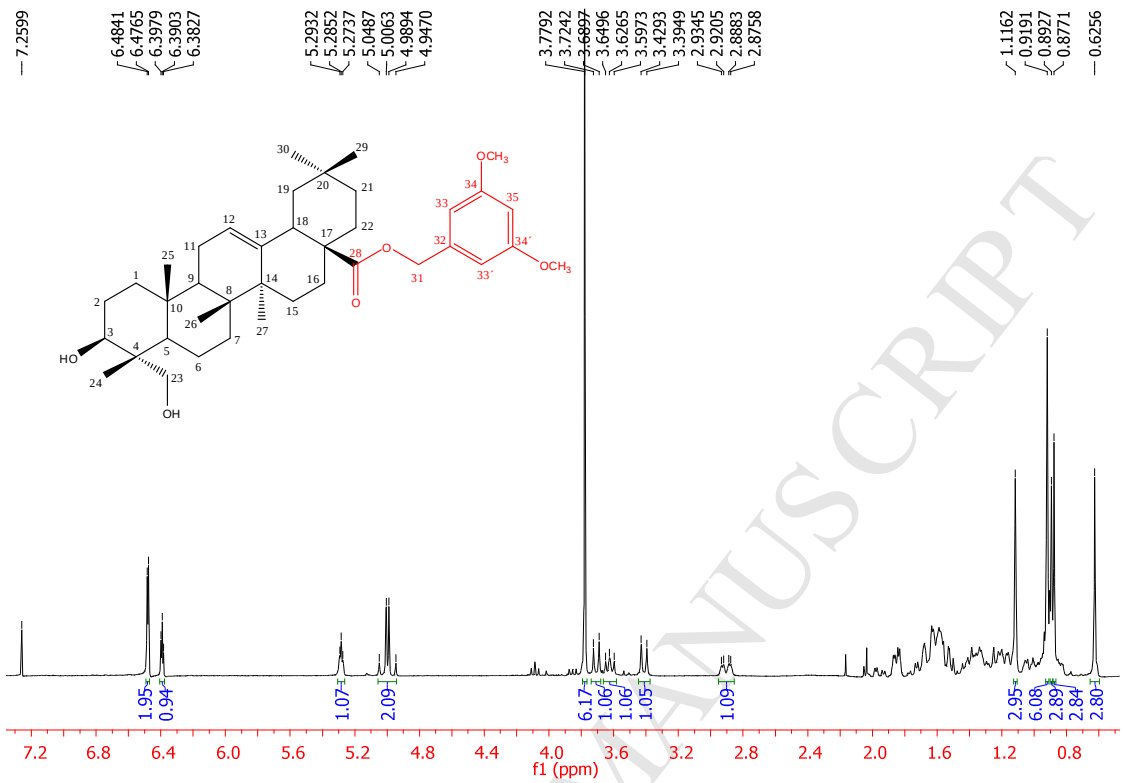


Figure S32. ^{13}C NMR spectrum of **16**

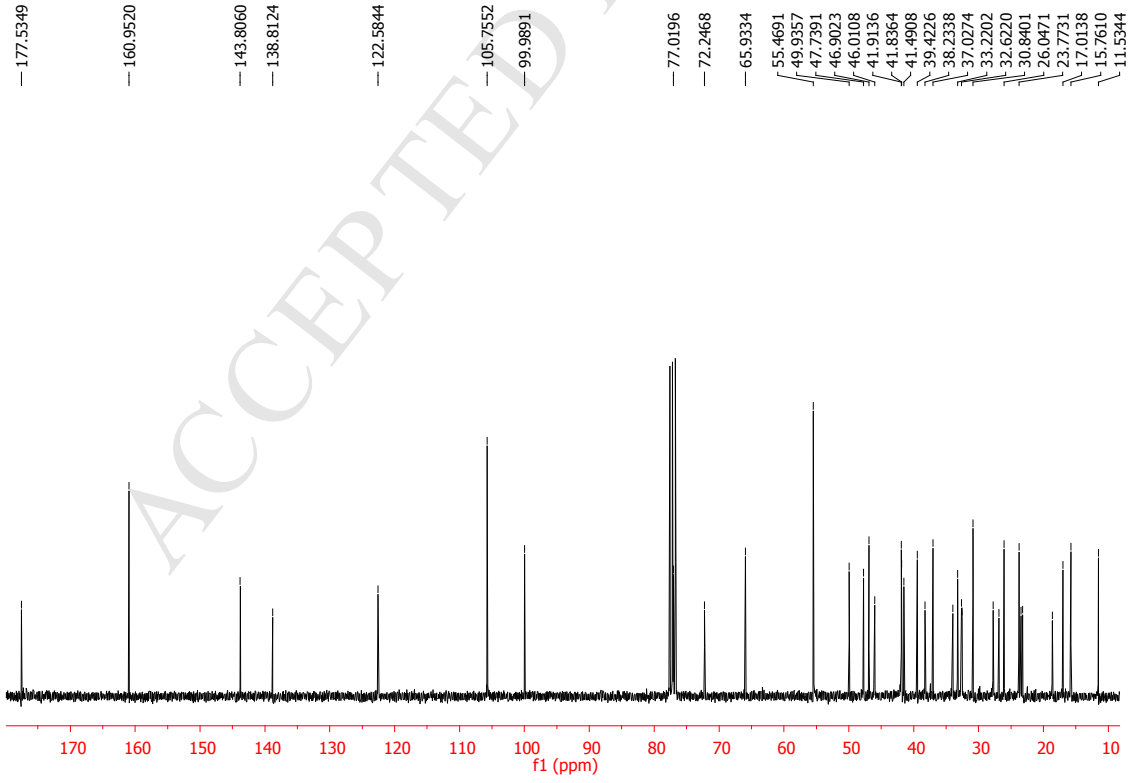


Figure S33. ^{1}H NMR spectrum of **17**

ACCEPTED MANUSCRIPT

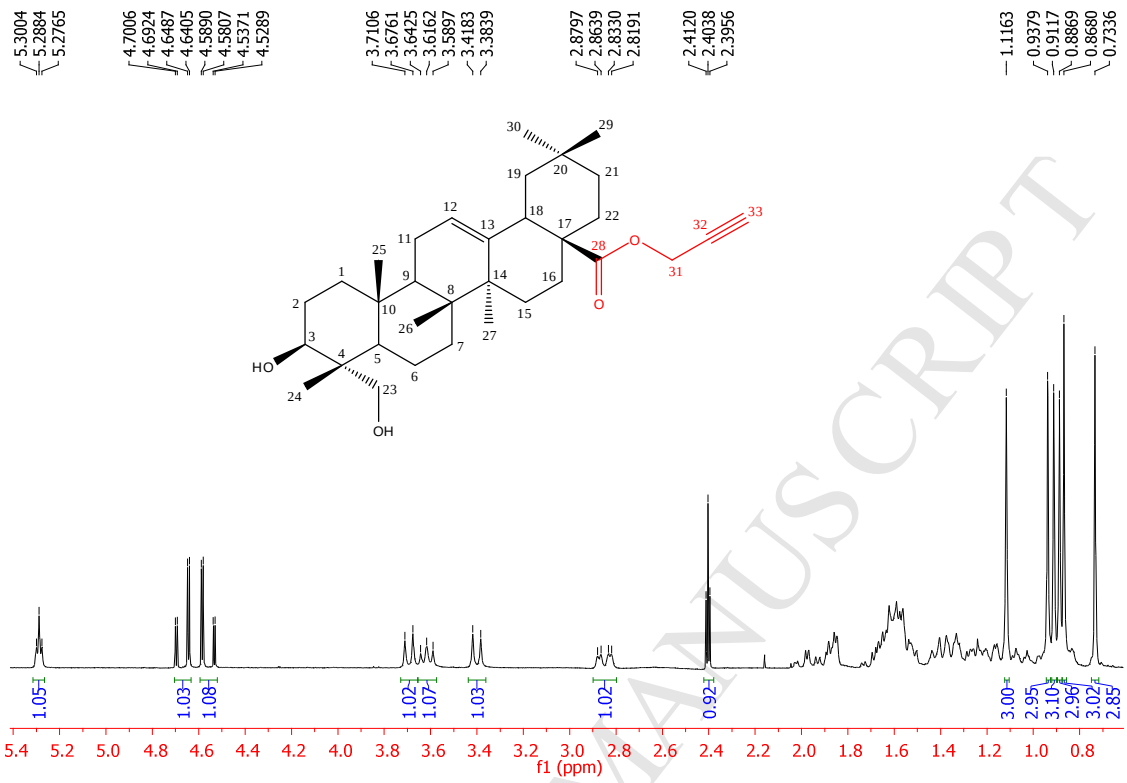


Figure S34. ^{13}C NMR spectrum of **17**

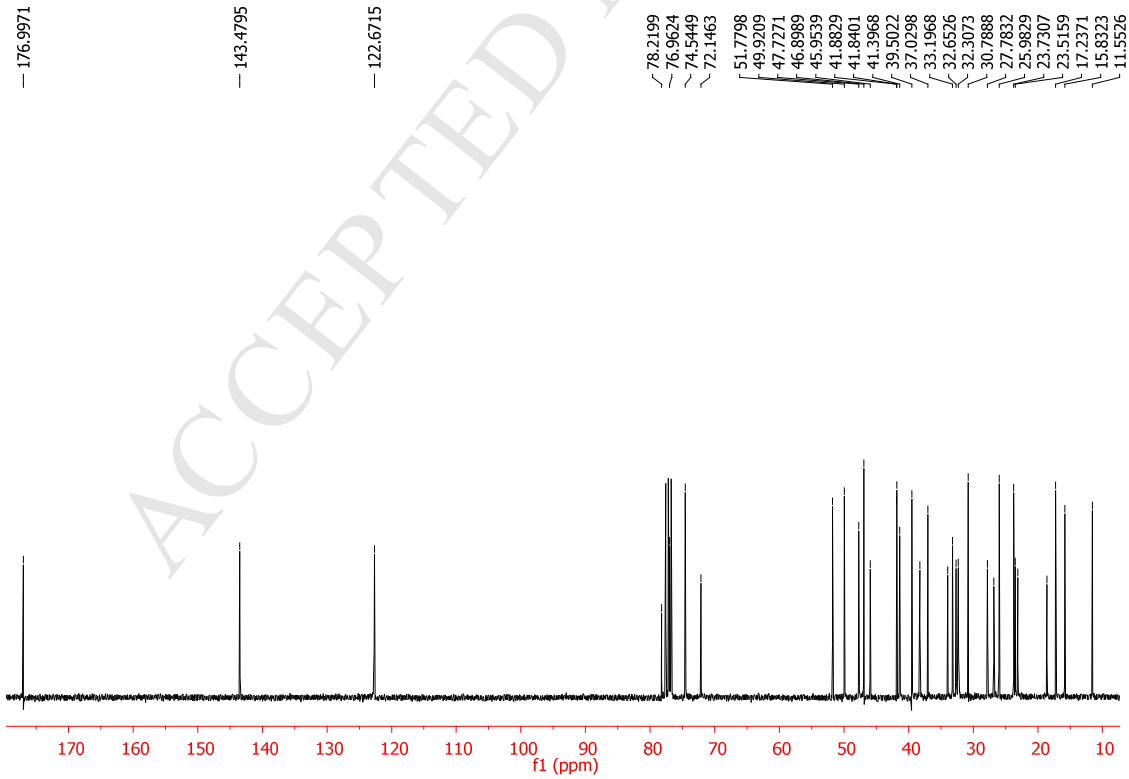


Figure S35. ^{1}H NMR spectrum of **18**

ACCEPTED MANUSCRIPT

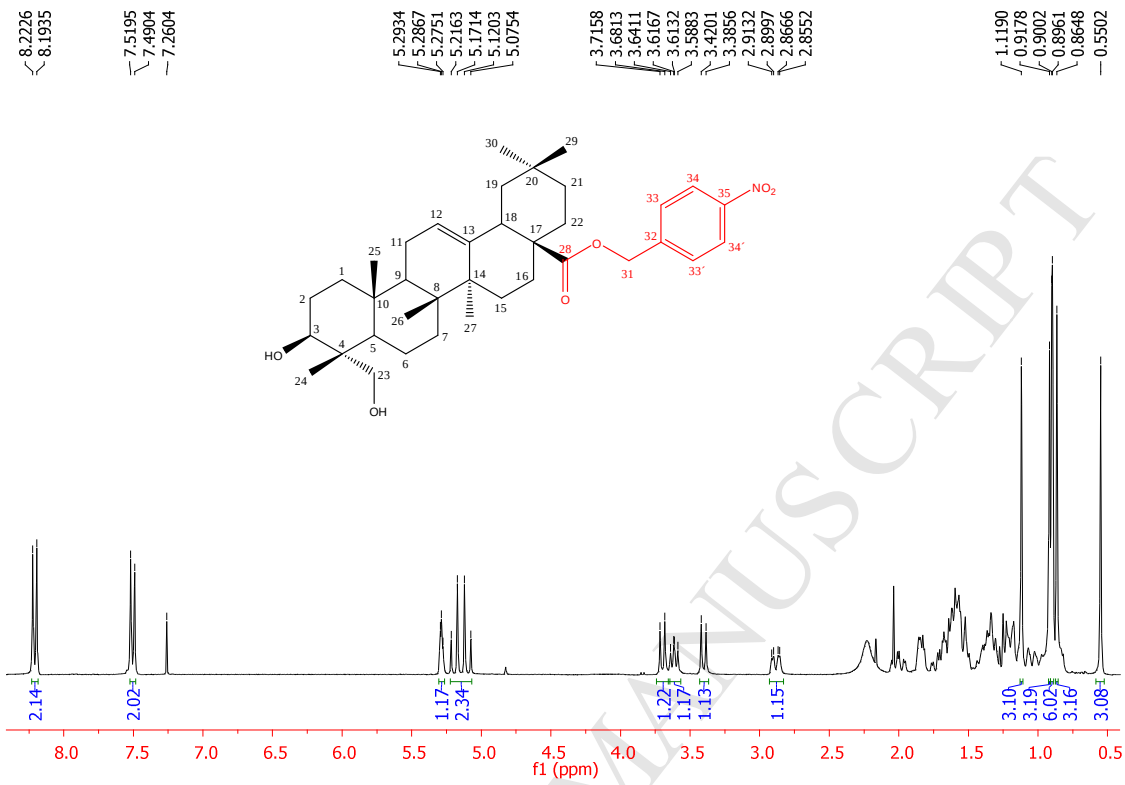


Figure S36. ^{13}C NMR spectrum of **18**

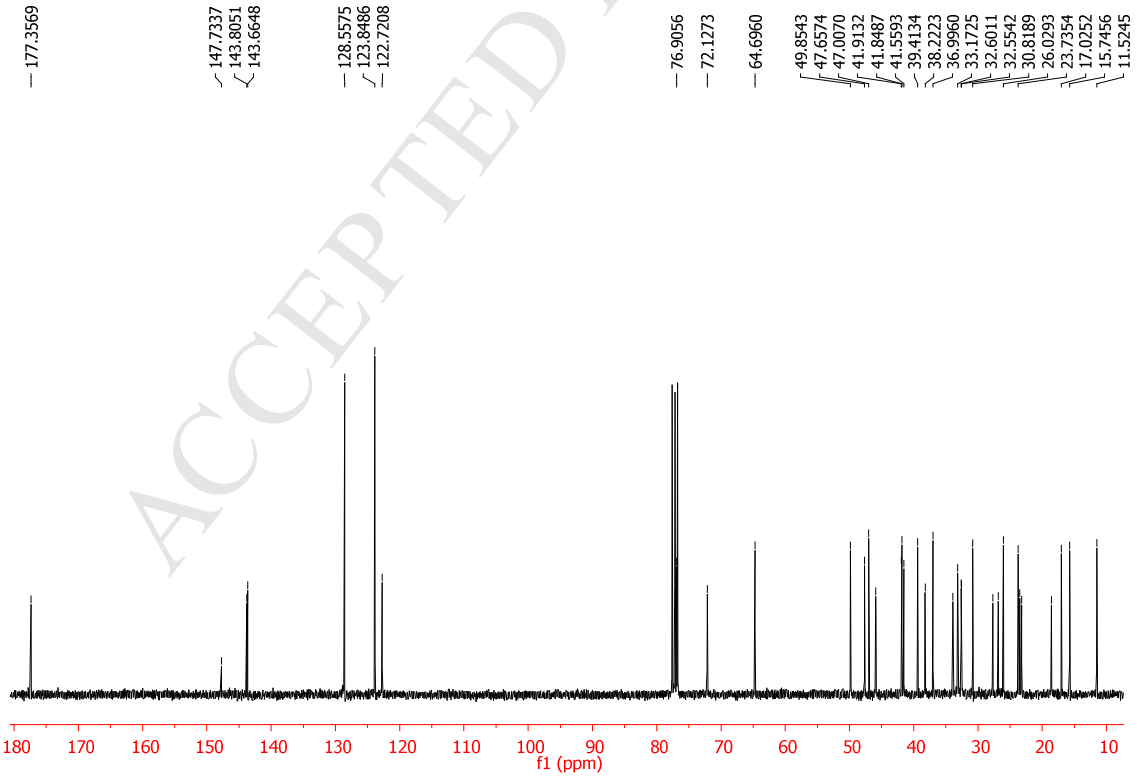


Figure S37. ^{1}H NMR spectrum of **19**

ACCEPTED MANUSCRIPT

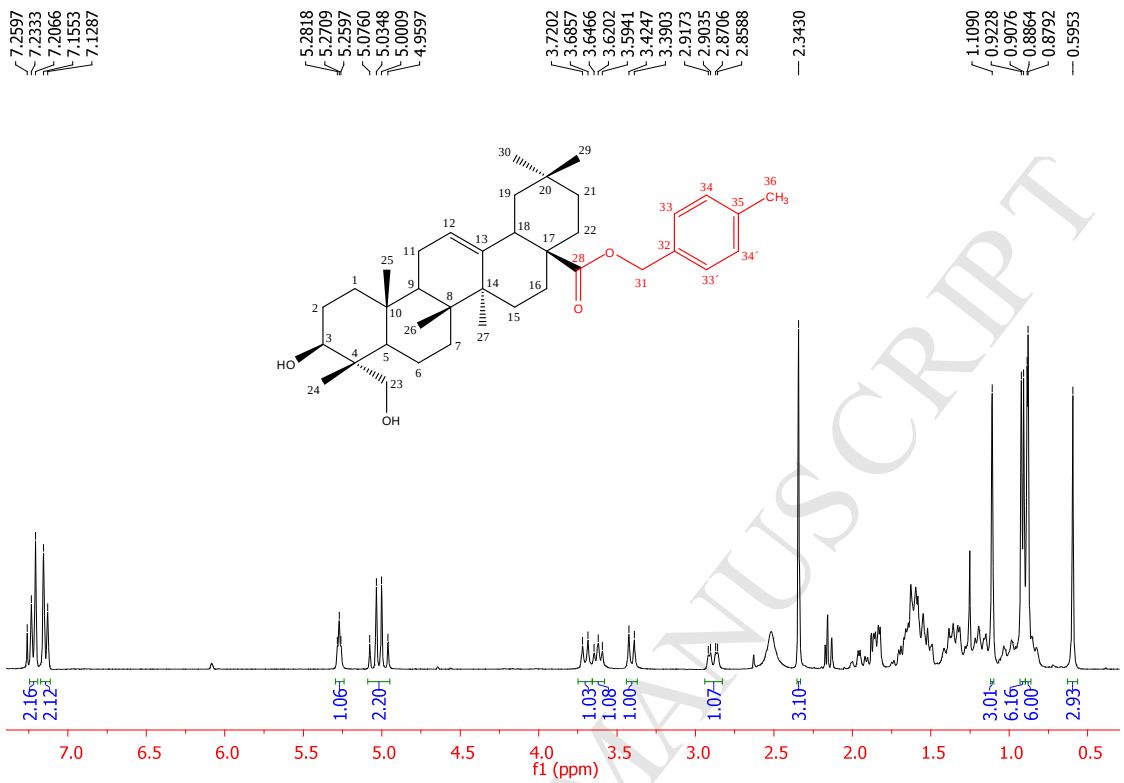


Figure S38. ^{13}C NMR spectrum of **19**

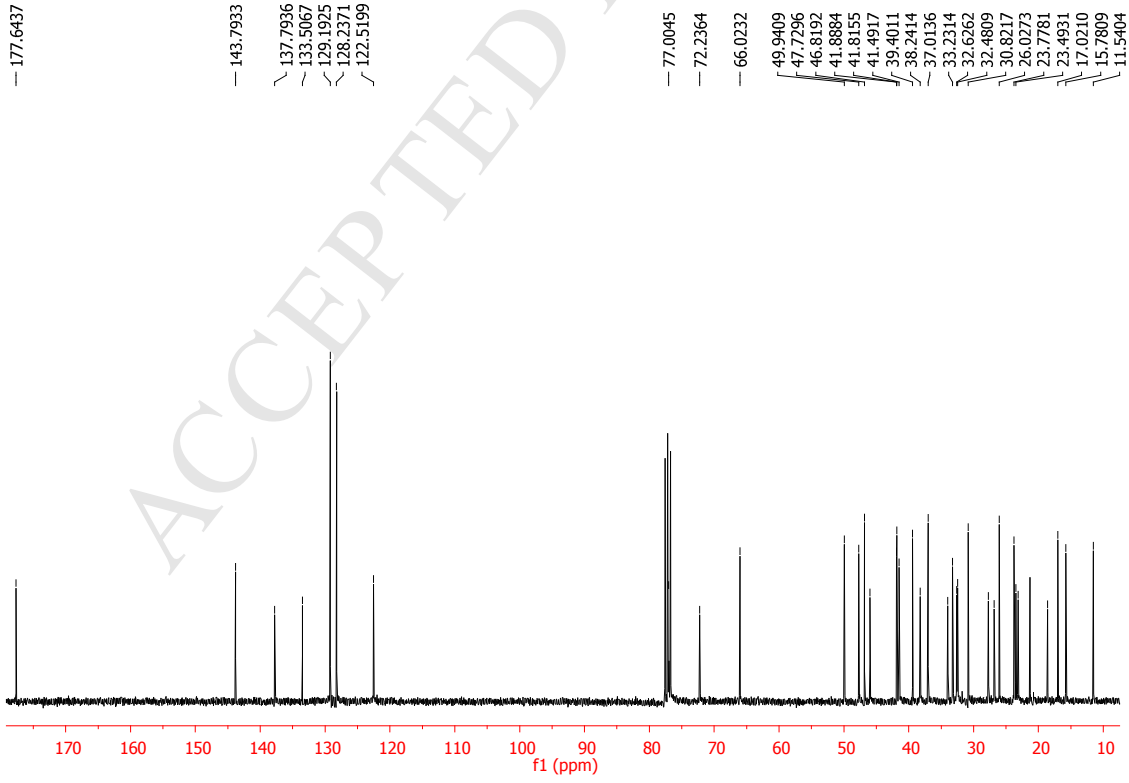


Figure S39. ^{1}H NMR spectrum of **20**

ACCEPTED MANUSCRIPT

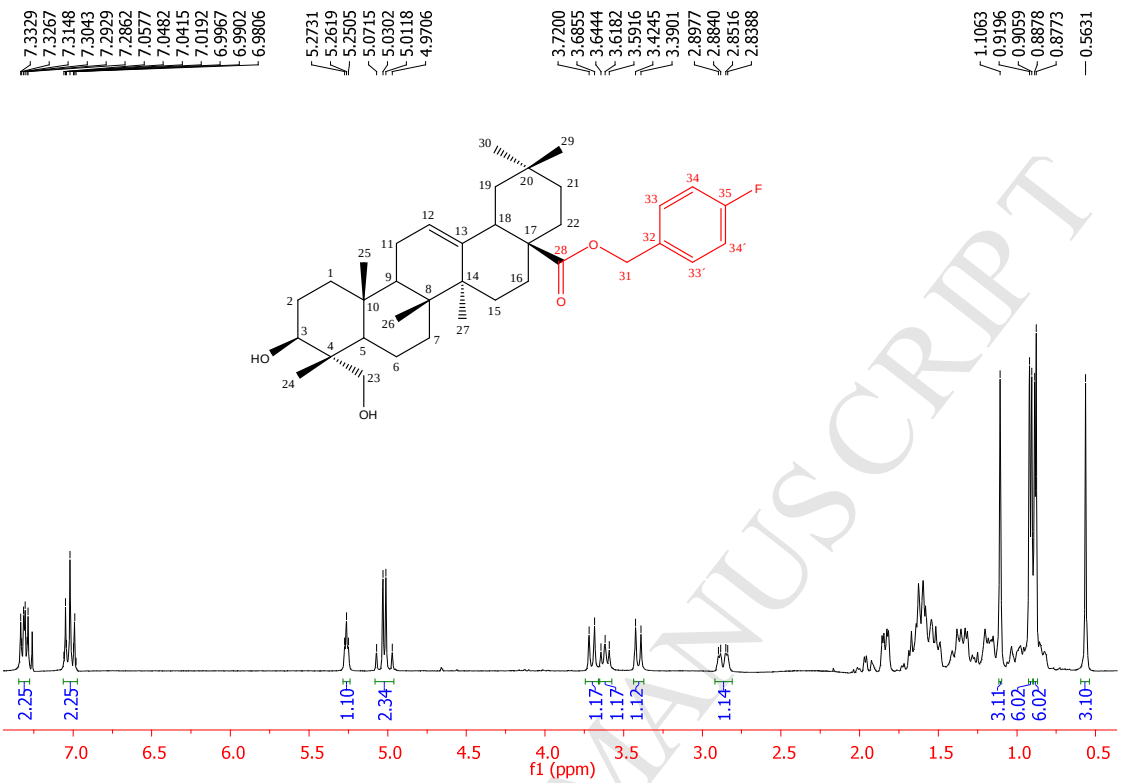


Figure S40. ^{13}C NMR spectrum of **20**

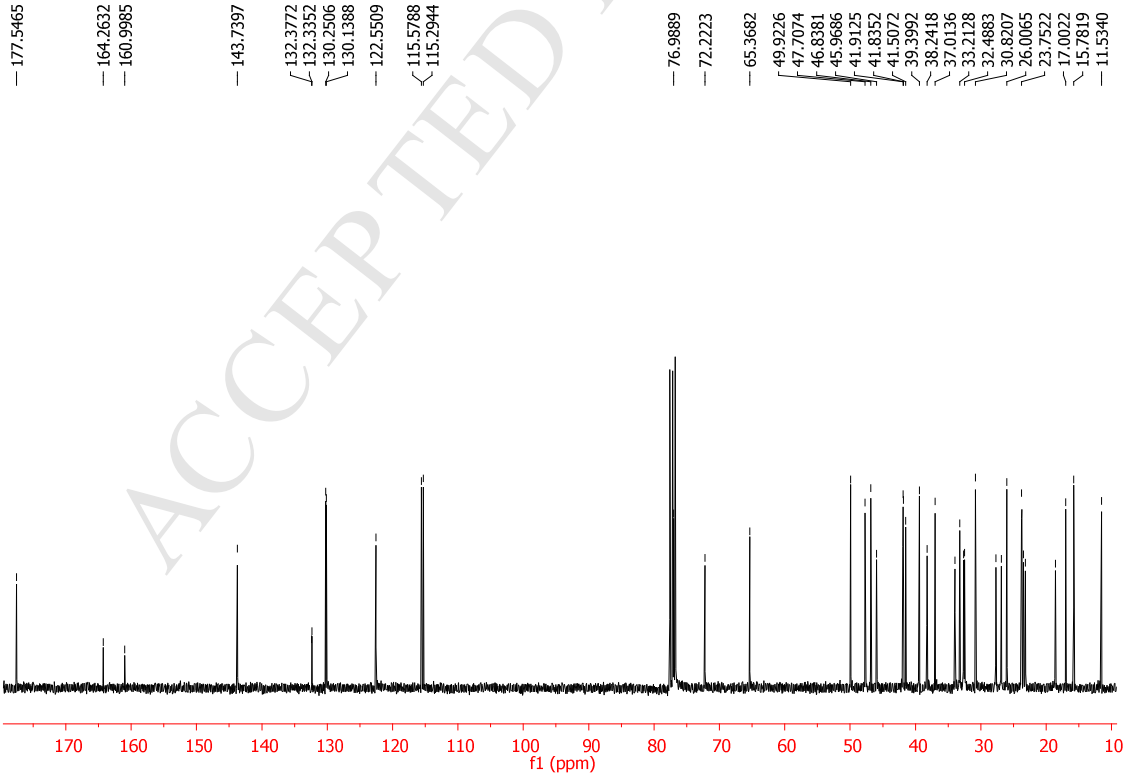


Figure S41. ^{1}H NMR spectrum of **21**

ACCEPTED MANUSCRIPT

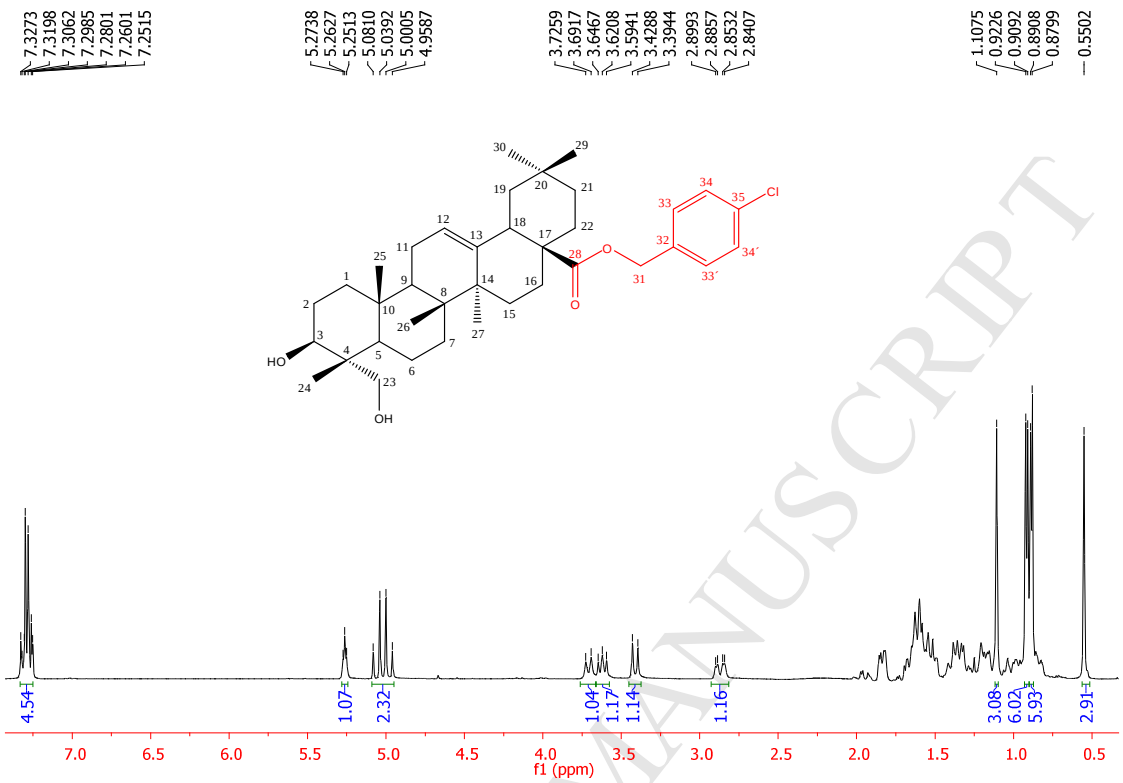


Figure S42. ^{13}C NMR spectrum of **21**

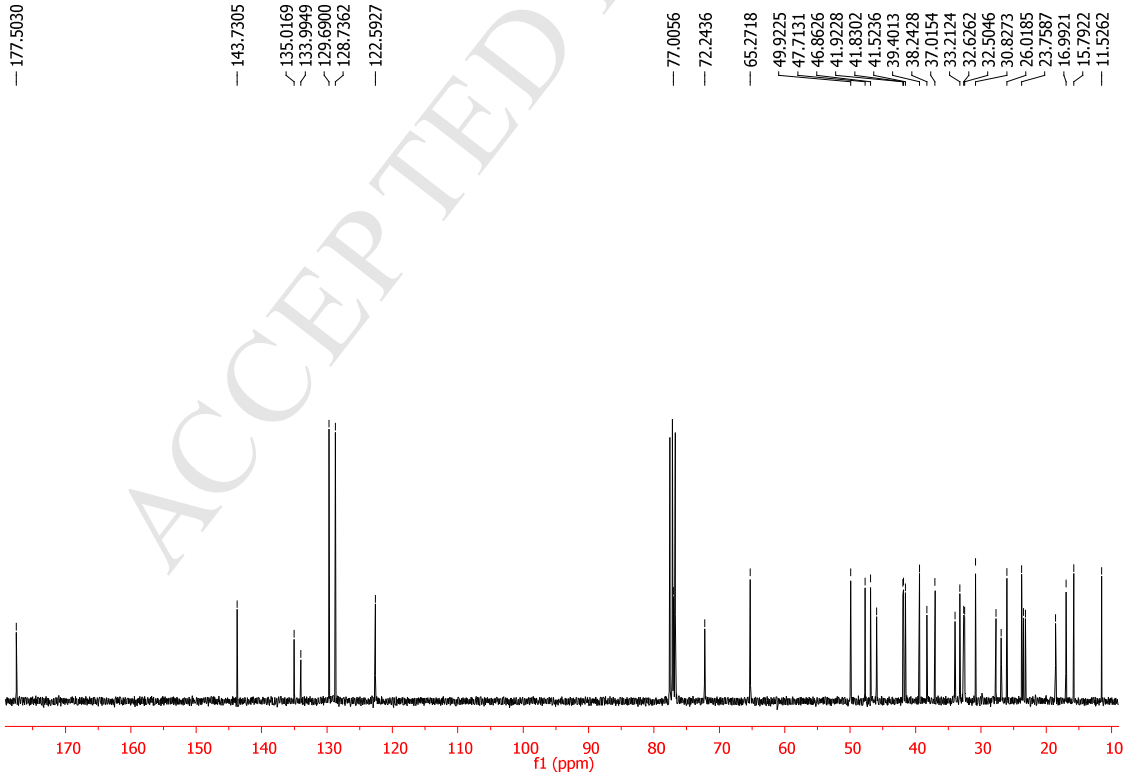


Figure S43. ^{1}H NMR spectrum of **22**

ACCEPTED MANUSCRIPT

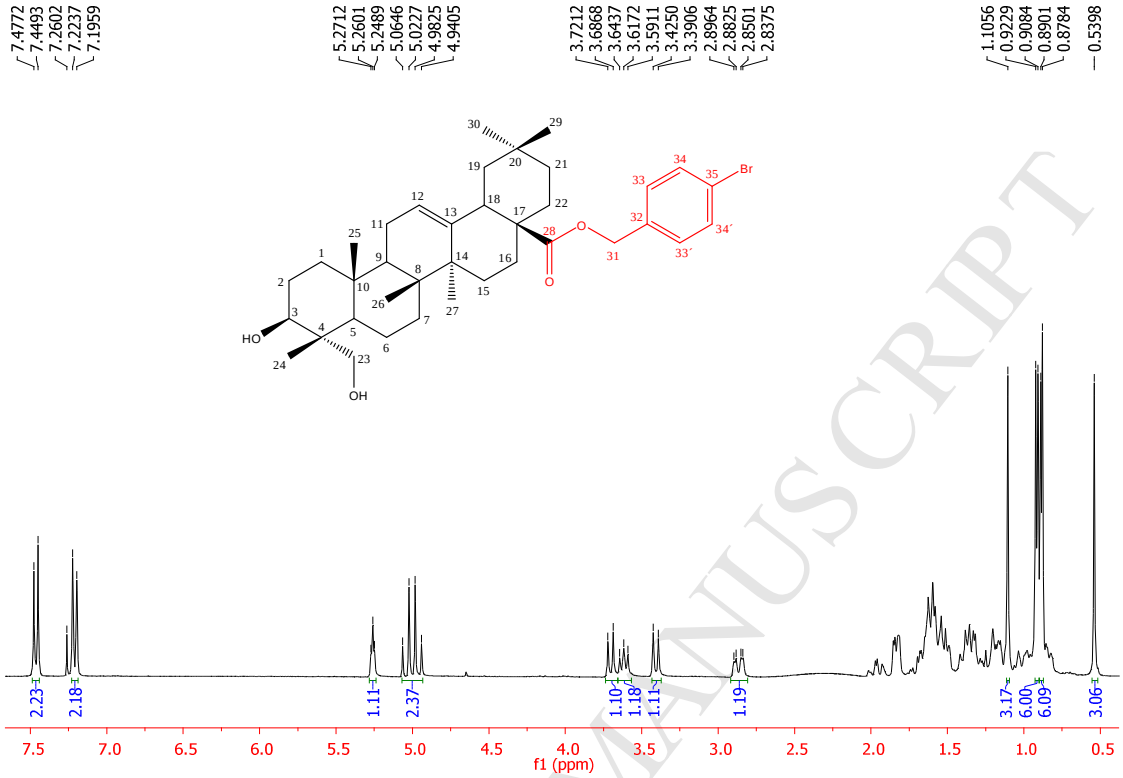


Figure S44. ^{13}C NMR spectrum of **22**

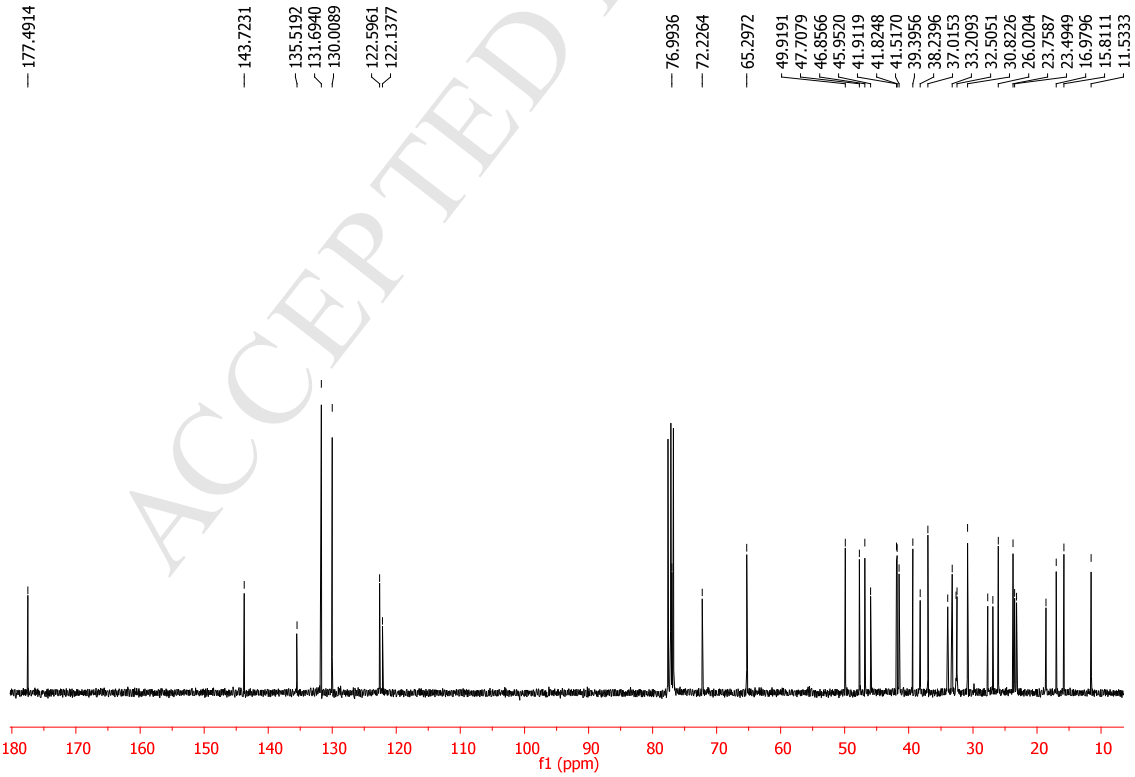


Figure S45. ^{1}H NMR spectrum of **23**

ACCEPTED MANUSCRIPT

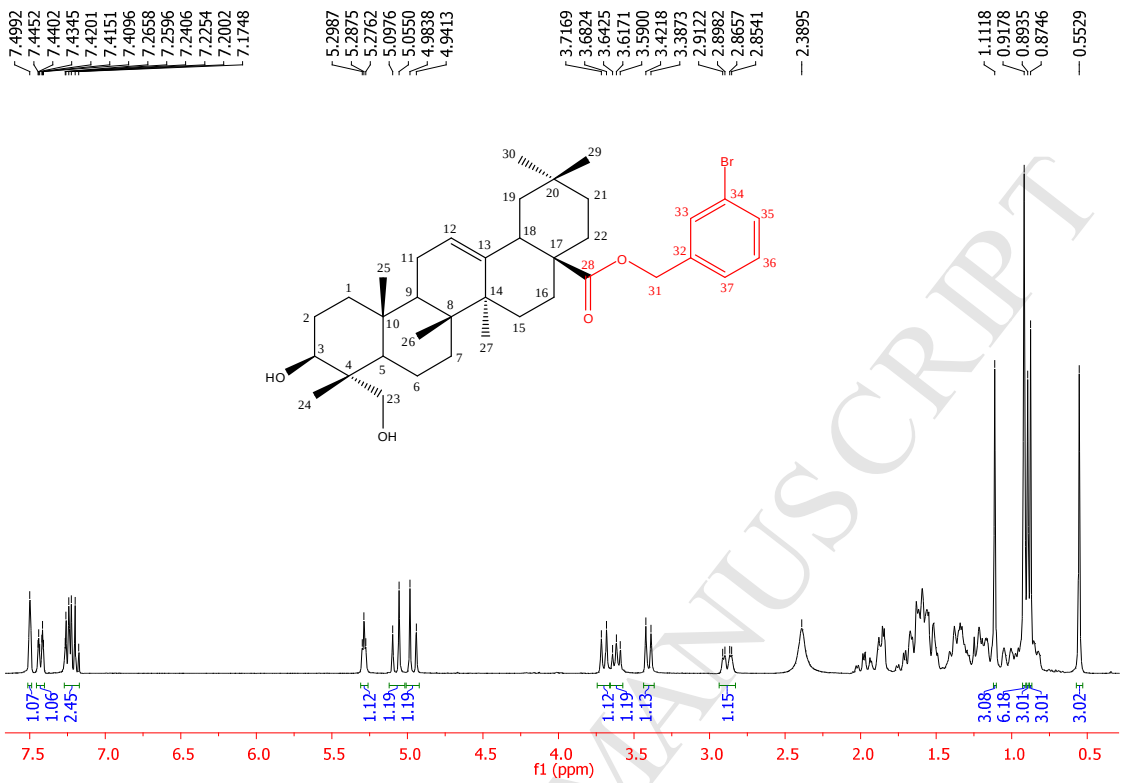


Figure S46.[13]C NMR spectrum of **23**

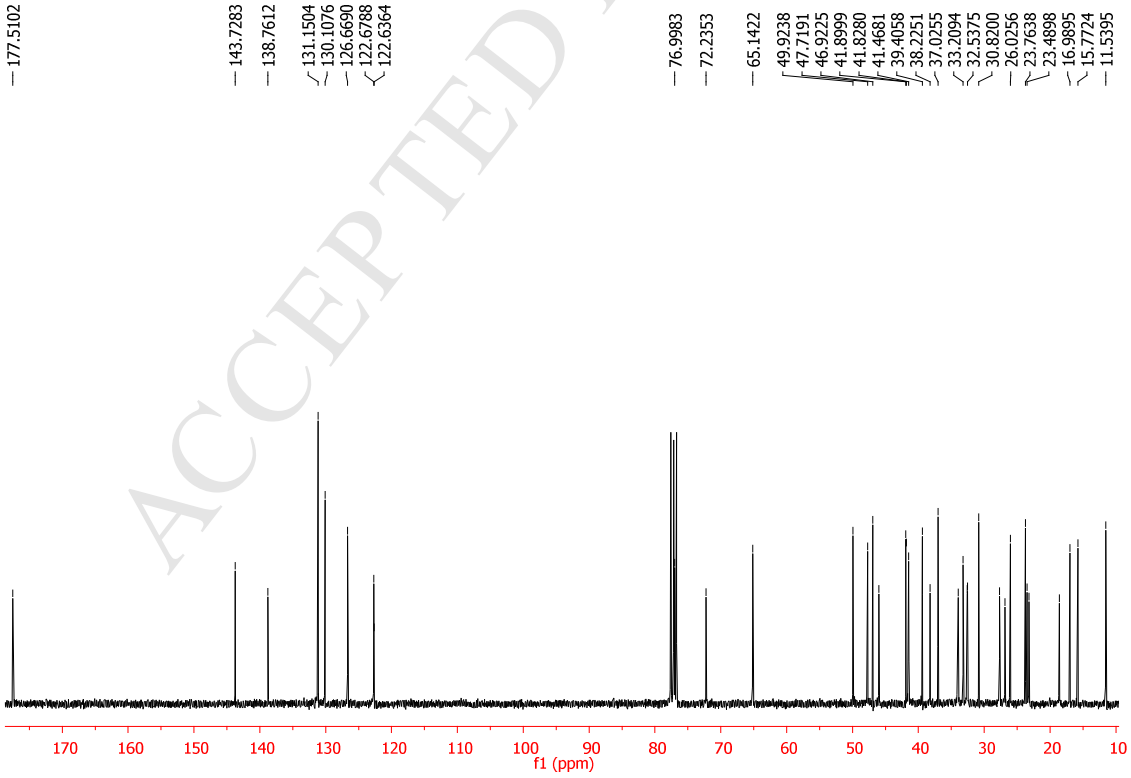


Figure S47. [1]H NMR spectrum of **24**

ACCEPTED MANUSCRIPT

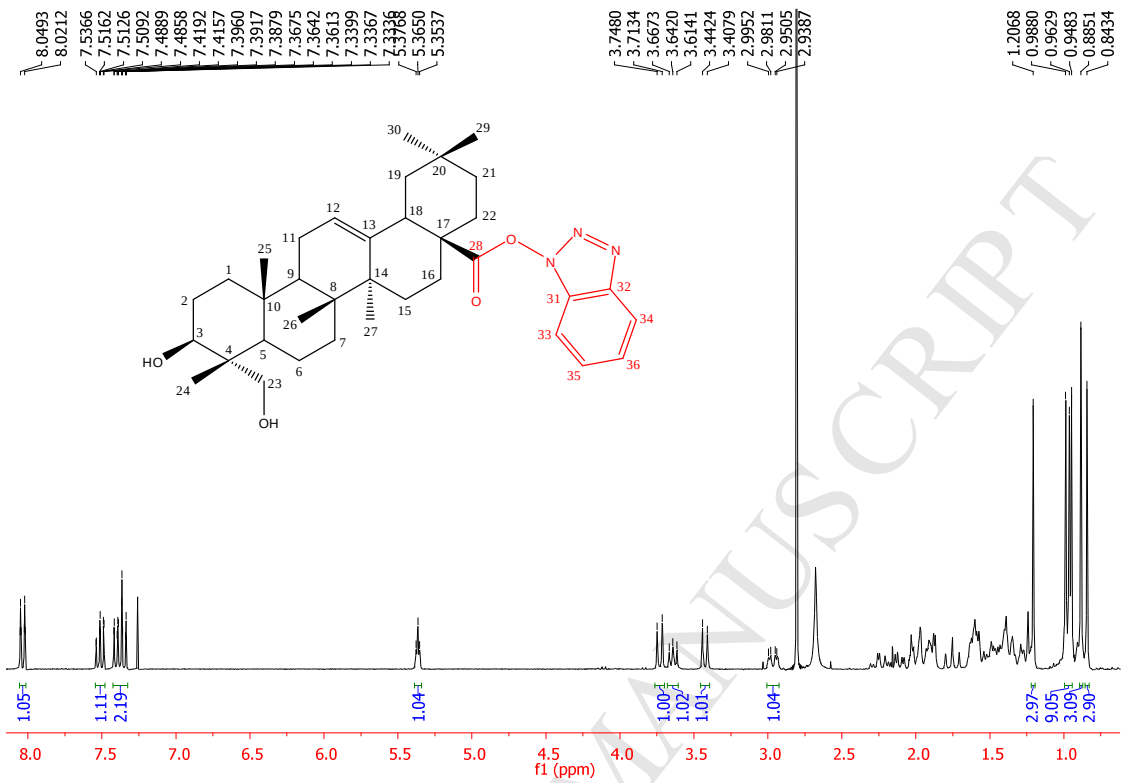


Figure S48. ^{13}C NMR spectrum of **24**

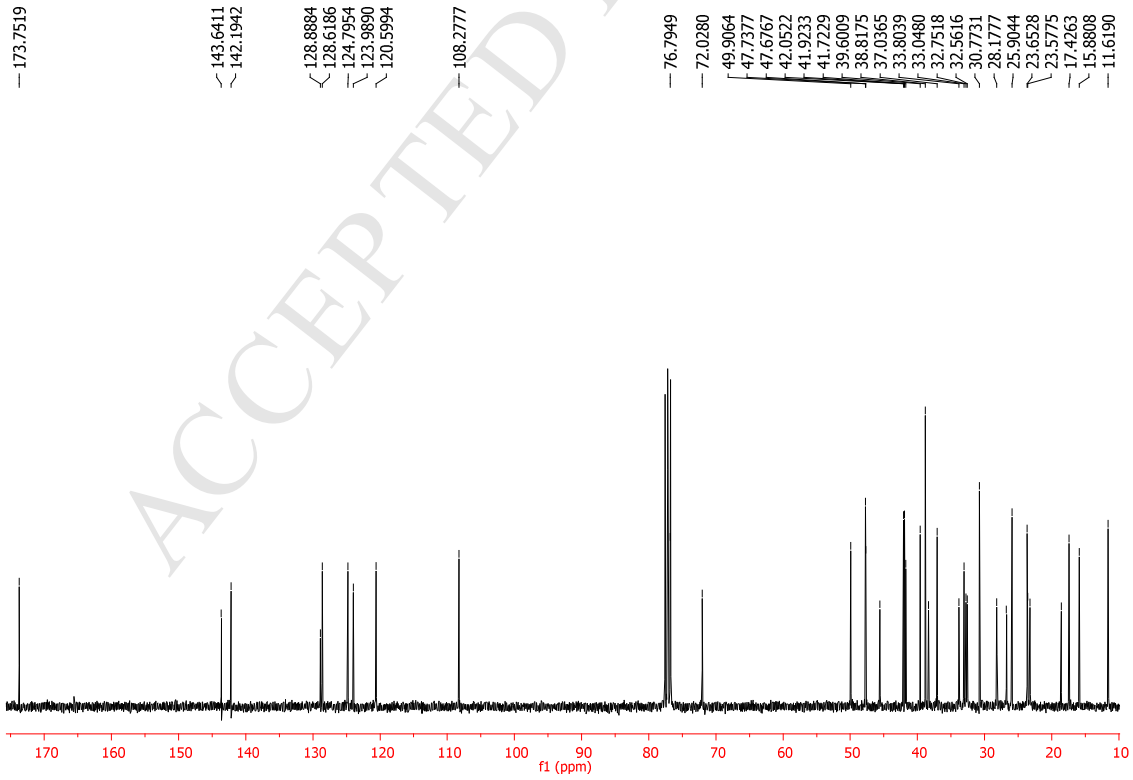


Figure S49. ^{1}H NMR spectrum of **25**

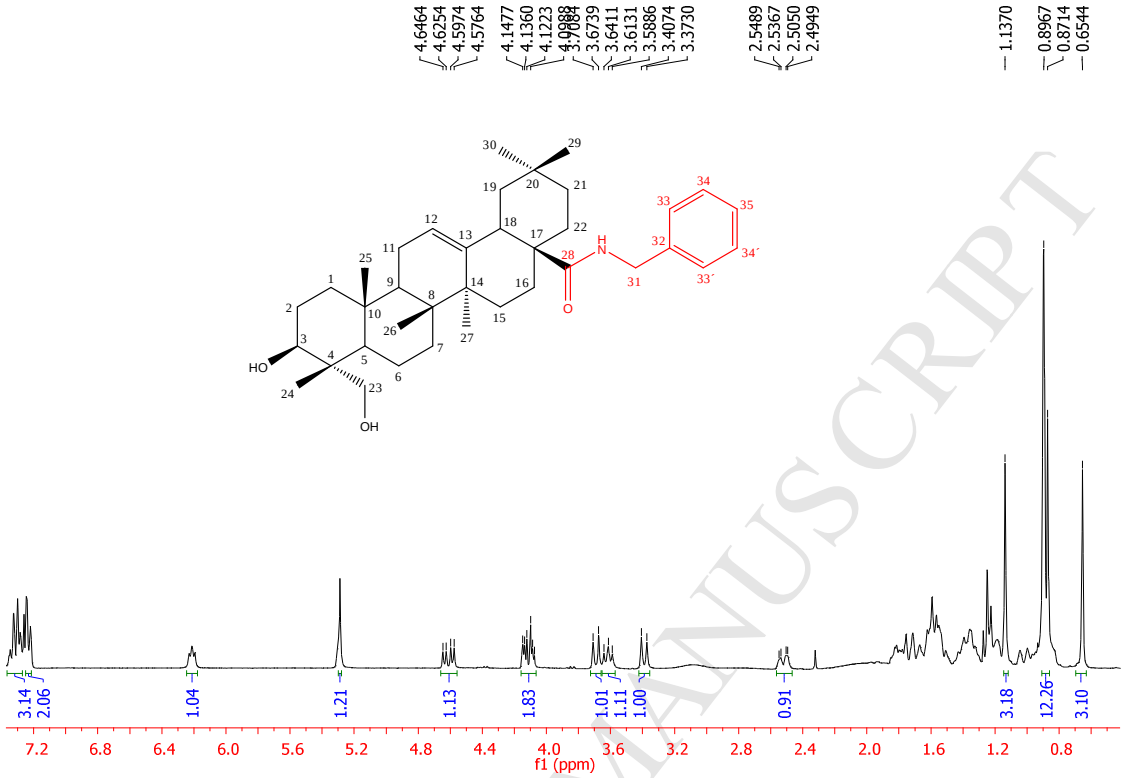


Figure S50. ^{13}C NMR spectrum of **25**

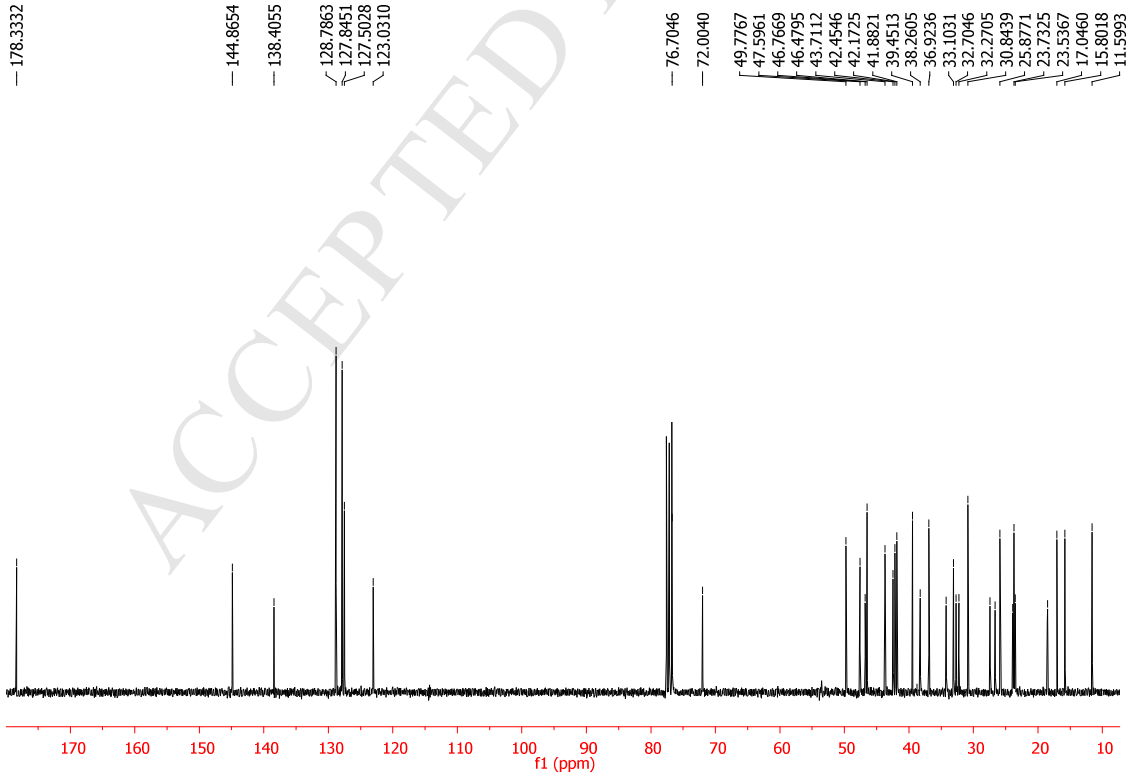


Figure S51. ^{1}H NMR spectrum of **26**

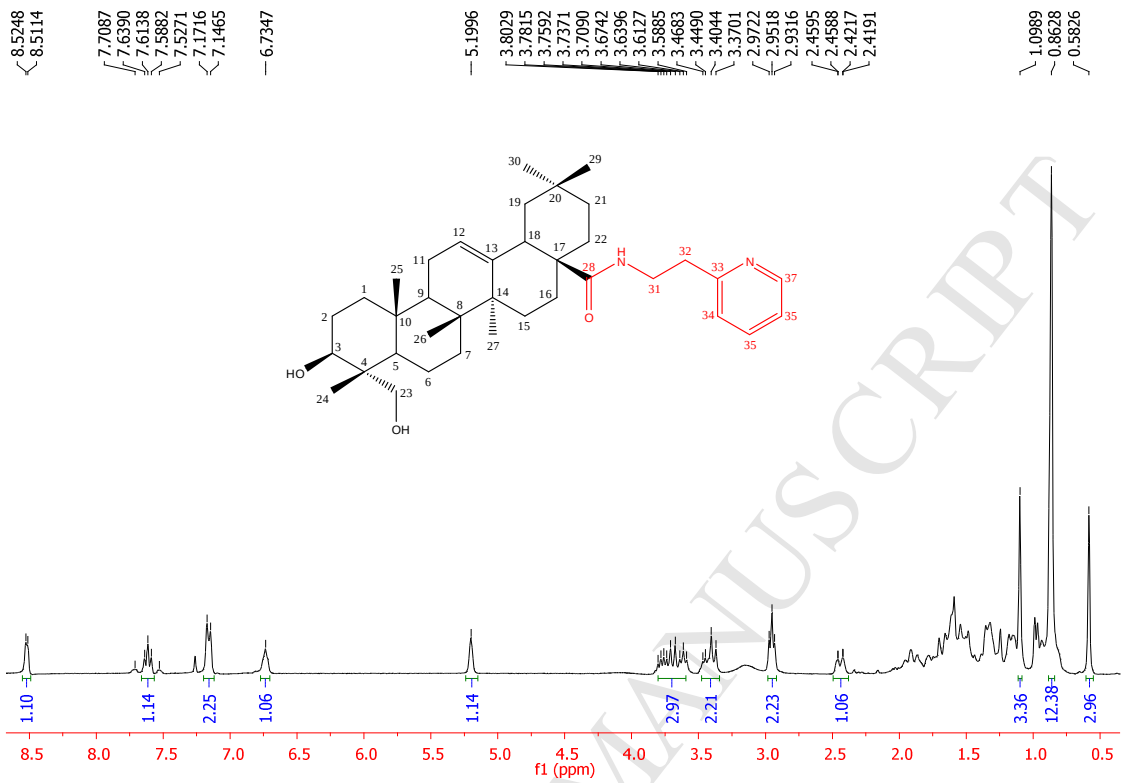


Figure S52. ^{13}C NMR spectrum of **26**

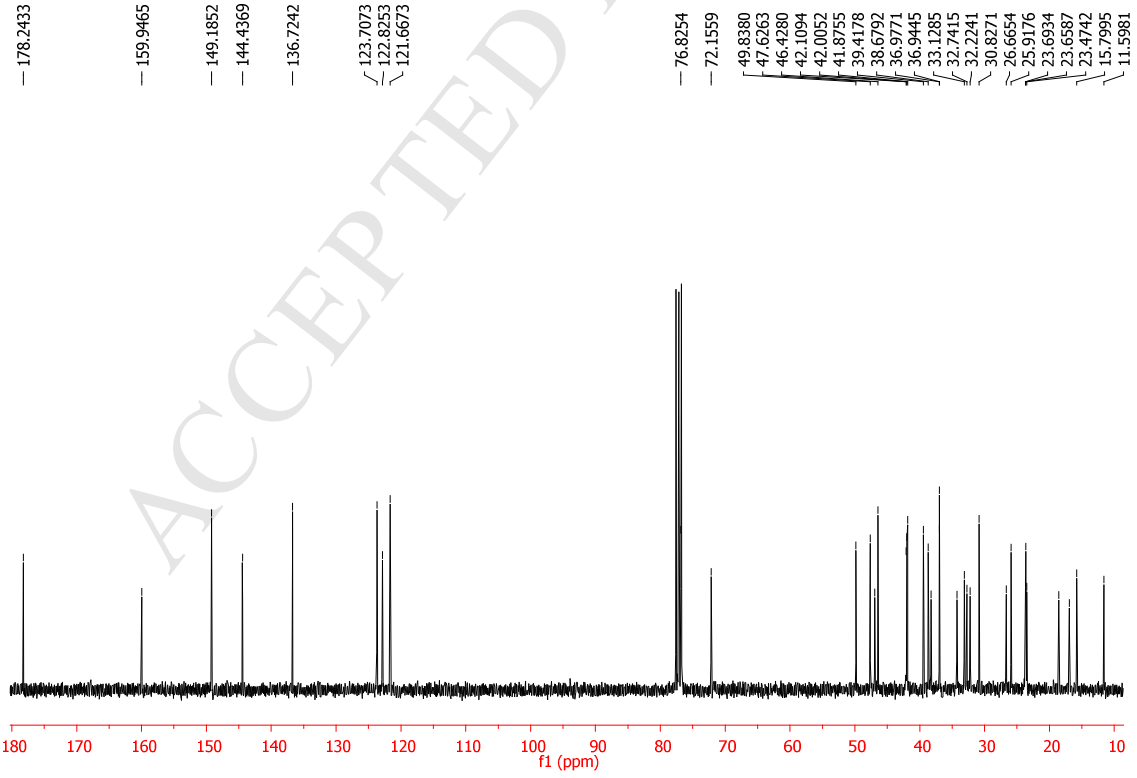


Figure S53. ^{1}H NMR spectrum of **27**

ACCEPTED MANUSCRIPT

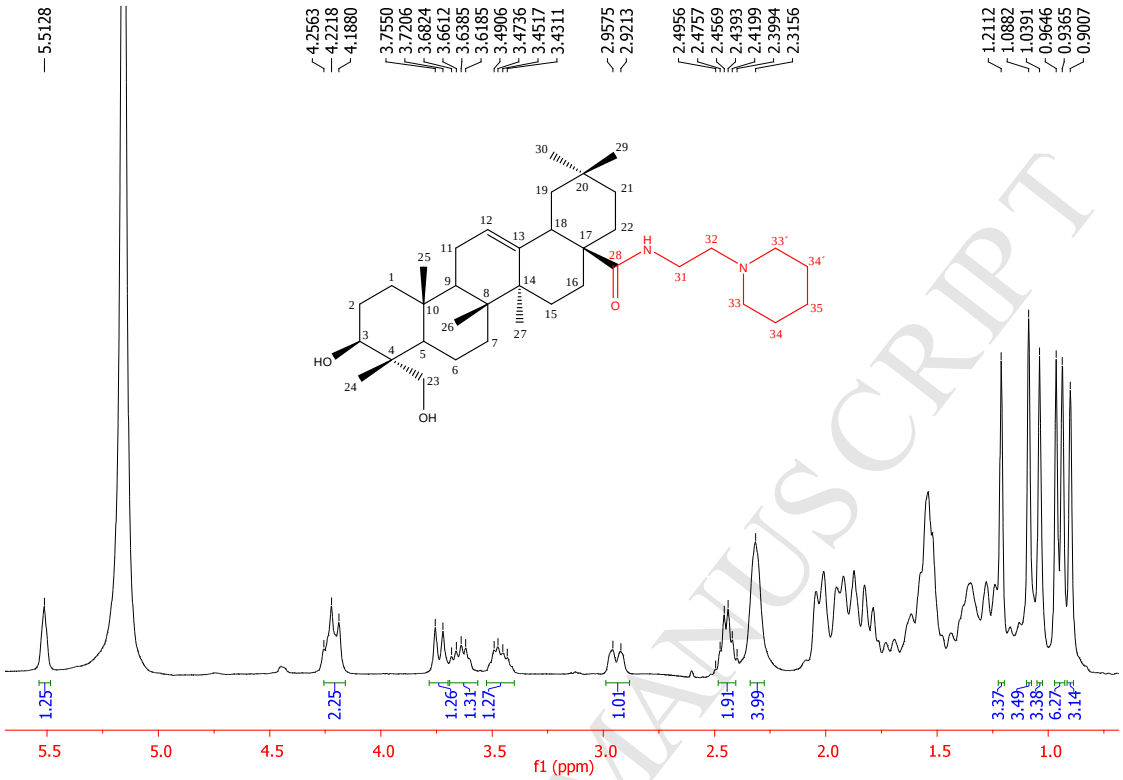


Figure S54. ^{13}C NMR spectrum of **27**

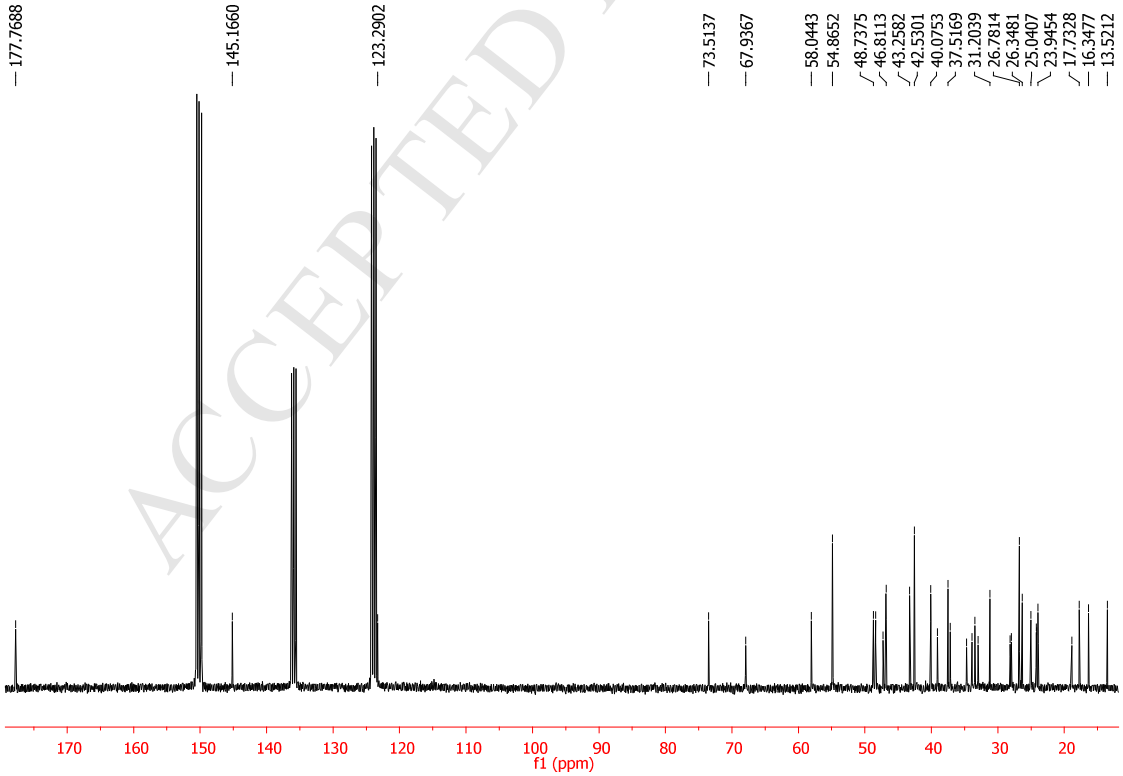


Figure S55. ^{1}H NMR spectrum of **28**

ACCEPTED MANUSCRIPT

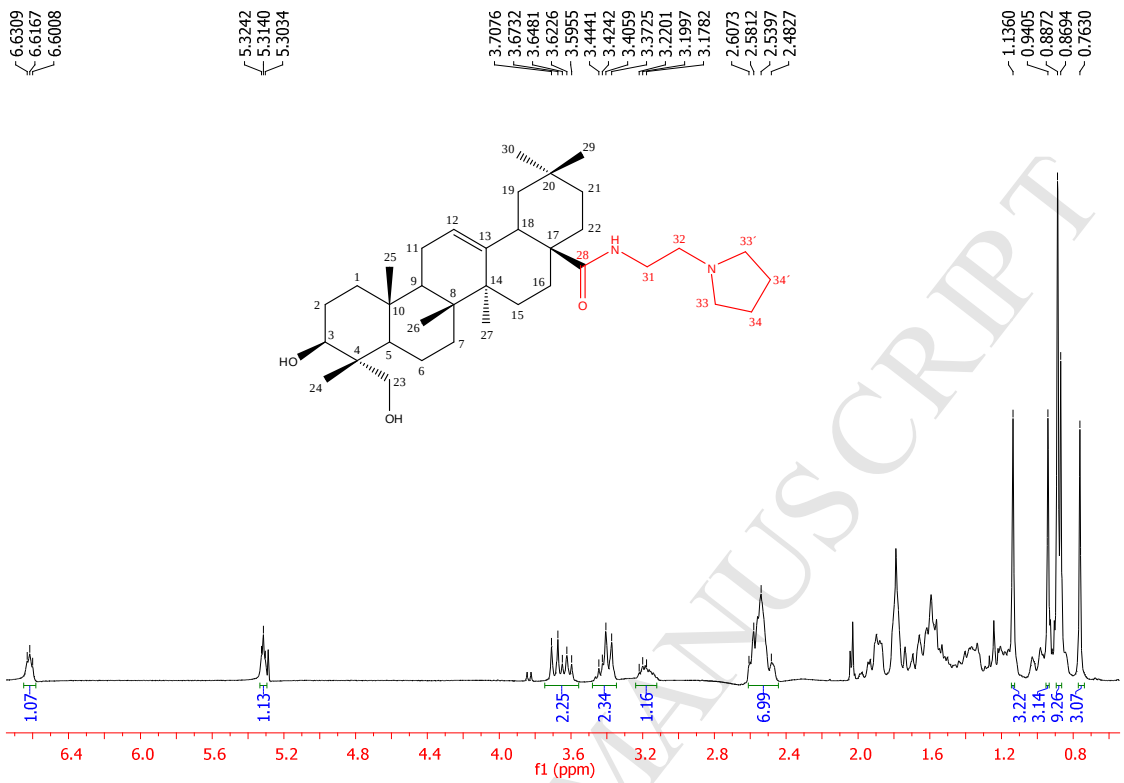


Figure S56. ^{13}C NMR spectrum of **28**

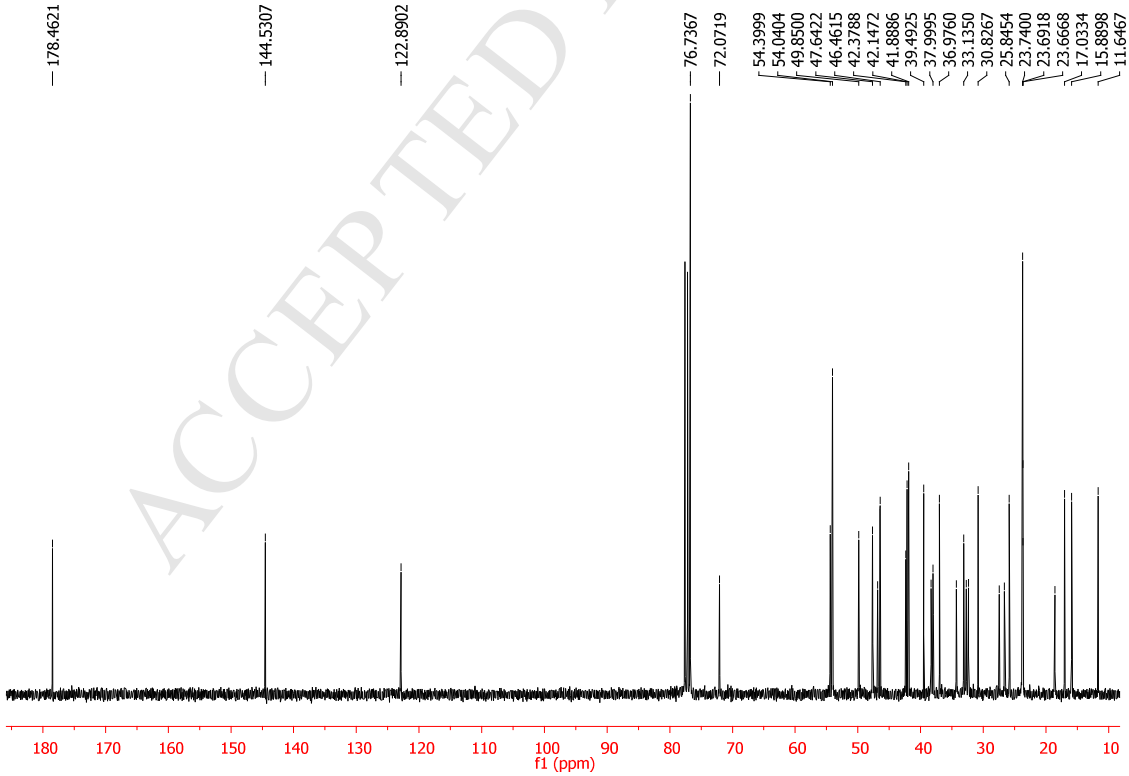


Figure S57. ^{1}H NMR spectrum of **29**

ACCEPTED MANUSCRIPT

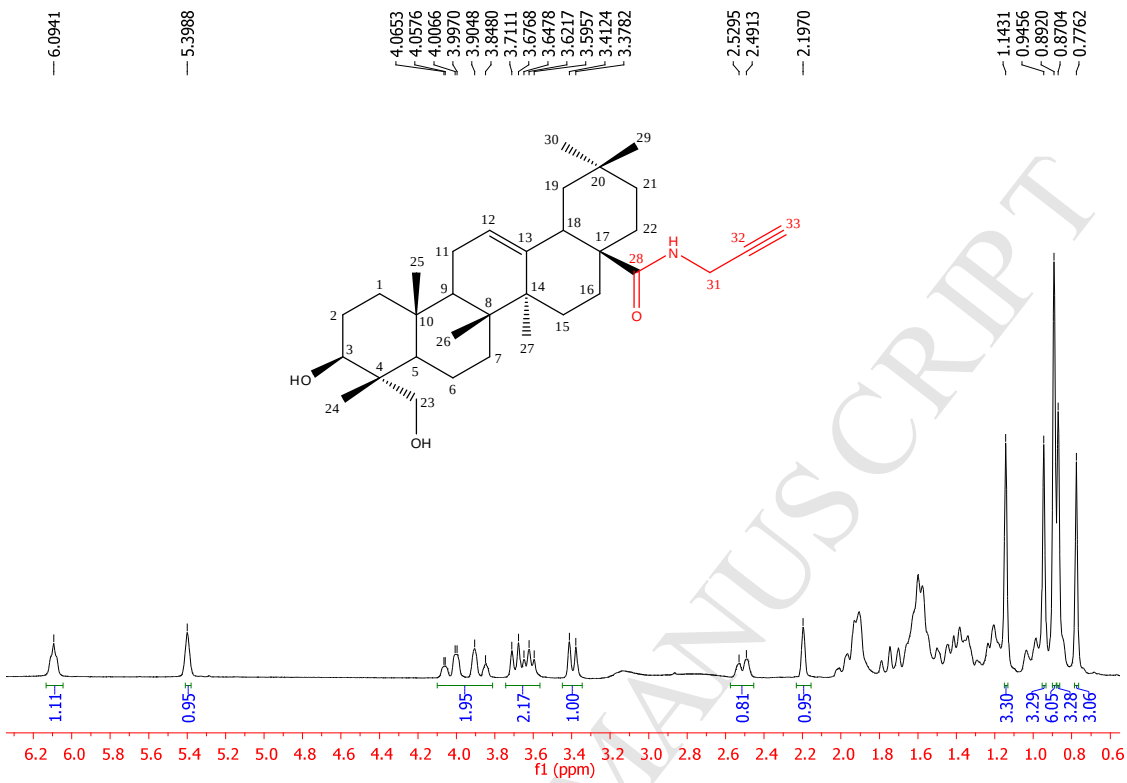


Figure S58. ^{13}C NMR spectrum of **29**

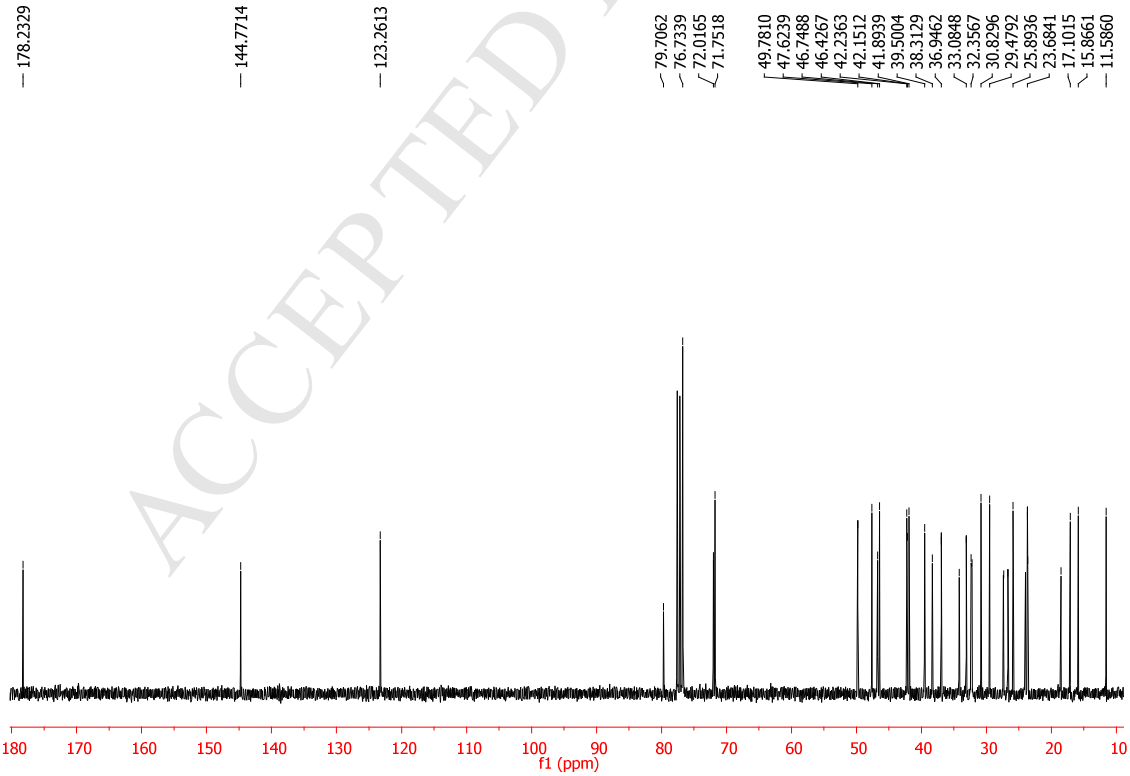


Figure S59. ^{1}H NMR spectrum of **30**

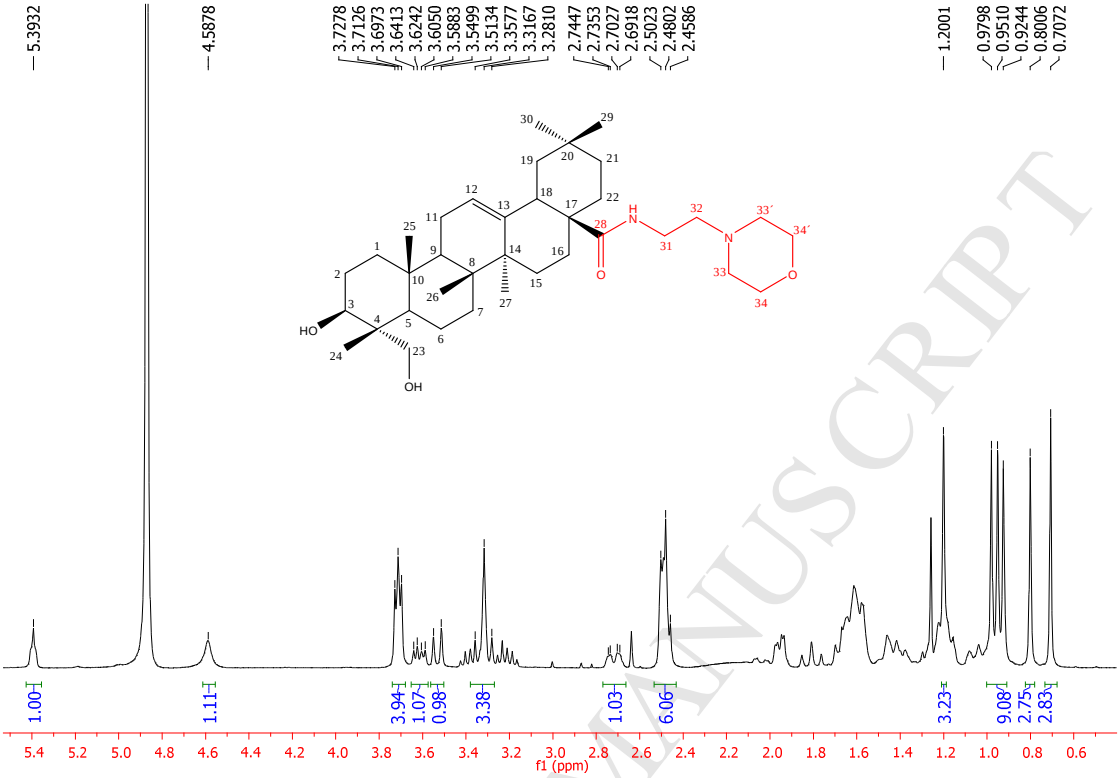
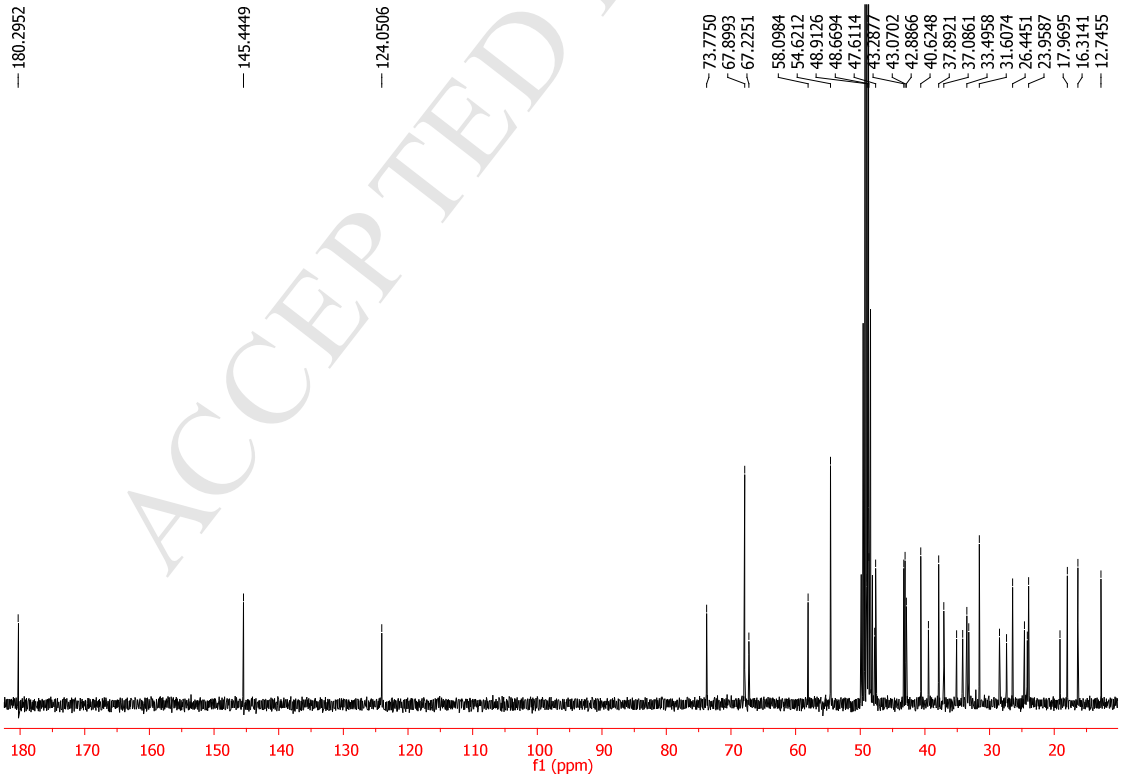


Figure S60. ^{13}C NMR spectrum of **30**



ACCEPTED MANUSCRIPT