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Original article

Semi-synthesis of acylated triterpenes from olive-oil industry wastes for the development of anticancer and anti-HIV agents

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ABSTRACT

A broad set of potential bioactive conjugate compounds has been semi-synthesized through solution- and solid-phase organic procedures, coupling two natural pentacyclic triterpene acids, oleanolic (OA) and maslinic acids (MA), at the hydroxyl groups of the A-ring of the triterpene skeleton, with 10 different acyl groups. These acyl OA and MA derivatives have been tested for their anti-proliferative (against the b16f10 murine melanoma cancer cells) and antiviral (as inhibitors of the HIV-1-protease) effects. Several derivatives have shown high levels of early and total apoptosis (up to 90%). Most of the compounds that exhibited anti-proliferative effects also generated ROS, probably involving the activation of an intrinsic apoptotic route. The only four compounds that did not cause the release of ROS could be related to the participation of a probable extrinsic activation of the apoptosis mechanism. A great number of these acyl OA and MA derivatives have proved to be potent inhibitors of the HIV-1-protease, the most active inhibitors having IC_{50} values between 0.31 and 15.6 μM, these values being between 4 and 186 times lower than their non-acylated precursors. The potent activities exhibited in the apoptosis-activation processes and in the inhibition of the HIV-1-protease by some OA and MA acylated derivatives imply that these compounds could be used as new, safe, and effective anticancer and/or antiviral drugs.

1. Introduction

The olive-oil industry produces a large volume of solid and liquid wastes, which require complex disposal problems and potential environmental pollution. In fact, olive-oil waste has historically been one of the biggest problems associated with this industry. Nevertheless, these residues are promising sources of diverse substrates, such as, phenolic compounds, oligosaccharides, manitol and two triterpenic acids (oleanolic and maslinic acids), which can be converted into value-added products. Thus, our starting vegetal material was the residue of both liquid and solid wastes from the two-phase industrial olive-oil extraction. The isolated triterpene compounds were oleanolic acid (3β-hydroxyolean-12-en-28-oic acid, **OA**, **1**) and maslinic acid (2α,3β-dihydroxyolean-12-en-28-oic acid, **MA**, **2**) (Fig. 1), two natural pentacyclic triterpenoids widely found in nature [1–3]. A method to obtain large quantities of these triterpene acids from olive-mill wastes has been reported by our research group [4]. Both acids, and several closely related triterpene compounds, exhibit a wide range of biological activities, and some may be used in medicine [5–9]. These compounds could be useful to semi-synthesize other biologically or chemically valuable products [10,11]. Moreover, there is firm evidence supporting the beneficial effects of consuming olive oil, and **OA** and **MA** are two bioactive compounds contained in its residual waste [12].

Triterpenoids are natural products widely distributed among diverse organisms, including bacteria, fungi, yeasts, plants, and mammals. Nevertheless, the majority of triterpenes are found in plants, especially as glycoside derivatives (saponins). Pentacyclic triterpenes are a group of compounds which are broadly distributed throughout the plant kingdom and which can be classified into three major types based on their structural skeleton: oleanane,

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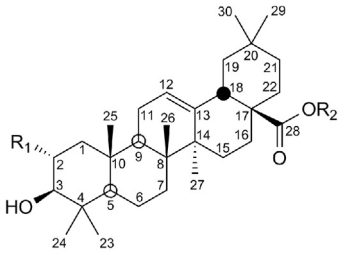


R_1 = H, R_2 = H (oleanolic acid, **OA**, **1**)
R_1 = OH, R_2 = H (maslinic acid, **MA**, **2**)
R_1 = H, R_2 = Bn (28-benzyloleanolic acid, **OA-Bn**, **3**)
R_1 = OH, R_2 = Bn (28-benzylmaslinic acid, **MA-Bn**, **4**)

Fig. 1. Structures of compounds **1**–**4**.

ursane, and lupine [13,14]. Biologically, these are the most important triterpenoid structures for their anti-inflammatory, anti-HIV, hepatoprotective, analgesic, antimicrobial, and virostatic properties [15–23]. Although some natural compounds of the terpenoid family have emerged as promising anti-HIV substances, importantly, their chemical modification may allow improvement of both their potency and selectivity in this assay [24–26]. The potential anti-HIV properties of diverse compounds can be tested using an FRET (fluorescence resonance energy transfer) HIV protease-inhibitor assay. This assay is considered an effective means for drug screening [27,28]. The continuous quantification of HIV-protease activity uses an FRET peptide, in which the fluorescence is quenched by a quencher group until the peptide is cleaved into two separate fragments.

Apoptosis or programmed cell death is a well-ordered and tightly regulated physiological process of cell self-destruction, with specific morphological and biochemical changes in the nucleus and cytoplasm, which plays a crucial role in the development of cancer. Apoptotic death is known to involve a cascade of proteolytic events driven mainly by a family of cysteine proteases, called caspases. Defects in apoptotic pathways contribute fundamentally to a range of disease states. Agents that suppress the proliferation of malignant cells, by inducing apoptosis, may represent a useful mechanistic approach to both chemoprevention and chemotherapy of cancer. The discovery of small-molecule drugs capable of inducing apoptosis in cancer cells is a fundamental and a highly attractive goal. Pro-apoptotic agents would be expected to have beneficial effects compared to existing cancer therapeutics [29–31]. Recently, our research group has demonstrated that some MA derivatives induce noteworthy apoptosis in b16f10 murine melanoma cells, and therefore they may be considered valuable molecules for their use as anti-proliferative agents [32]. **MA** also shows anti-tumour effects in Caco-2 and HT29 colon-cancer cell lines [33,34], and in the astrocytoma cell line [35], via mitochondrial or intrinsic apoptotic pathway [36,37]. In addition, our group has demonstrated for the first time, that the **MA** can modify certain cytoskeleton proteins of HT29, which is one of the bases of their anti-proliferative activity against colon-cancer cells [38], as well as the effectiveness of **MA** as a potential chemopreventive against colorectal cancer when used against the development of intestinal tumours in the $Apc^{Min/+}$ mice model [39]. **MA** also displays cytotoxic effects in different tumour-cell lines: non-small lung cells, ovary, melanoma, central nervous system, and colon [40].

During apoptotic activation, two major pathways have been described: the mitochondrial or intrinsic pathway, and the death-receptor or extrinsic pathway. The intrinsic pathway involves mitochondrial disruption, changing the mitochondrial-membrane potential and the release of ROS (reactive oxygen species) [41,42]. This apoptotic route involves the mitochondria-dependent activation of initiator caspases, which in turn activates downstream executive caspases, such as caspase-3. The extrinsic death-receptor pathway is triggered by death ligands through the formation of the death-inducing signalling complex that results in caspase-8 and caspase-10 activation, triggering downstream apoptotic signalling [43,44]. An initial release of ROS accompanied of apoptosis induction could indicate the activation of the intrinsic apoptotic pathway, whereas that apoptotic induction without the release of ROS would suggest the activation of the extrinsic apoptotic pathway. The evaluation of the levels of ROS, at the cytotoxic and apoptotic concentrations, can help to determine the possible molecular mechanism involved in the apoptotic response found against the different compounds tested.

A major aim in the field of diversity-oriented synthesis is the effective production of small-molecule libraries to undertake new biological probes and to discover new therapeutic leader compounds [45]. Terpenoids have long served as important small-molecule drugs, leading to drug discoveries [46,47]. In fact, terpenoid cores are prevalent among privileged scaffolds able to bind multiple biological targets, and a variety of terpenoid-based libraries have been reported [48,49]. Combinatorial chemistry represents the possibility of synthesizing and testing a large number of compounds for their bioactivity simultaneously, instead of one by one [50,51]. On the other hand, the SPOS (solid-phase organic synthesis) is a powerful tool in the synthesis of small molecules to discover new remarkable pharmacological compounds. In this synthetic technique, the derivatives are synthesized by using an excess of reagents to drive reactions to completion, being isolated easily by simple filtration and removed from the support material [49]. The SPOS has a principal advantage during synthesis in solution, which facilitates the purification process, allowing automation of the whole synthesis. However, SPOS also presents some major disadvantages; for example, it can be problematic to adapt some conventional solution reactions to a solid-phase process. There are only few articles related to the solid-phase of triterpenoids by attaching a solid support to the C-28 position [52–54]. This methodology would not only serve to protect the carboxyl group, but also could simplify the entire synthesis procedure.

In the present paper, we have carried out the semi-synthesis of a number of oleanolic and maslinic acid derivatives, starting from these natural compounds isolated from olive-oil wastes. This preparation has been made using solution-phase organic synthesis processes, which have been compared with the corresponding solid-phase organic synthesis ones. Previously, we have also tested the degree of incorporation of **OA** and **MA** to the solid support employed. Finally, these acyl OA and MA derivatives were also tested in a screening mode, for different potential bioactivities such as anticancer and anti-HIV properties. The main purpose of this study is to determine the bioactive potential of these acyl OA and MA derivatives as anti-proliferative and pro-apoptotic compounds as well as anti-virals on HIV-serine protease. With respect to the anticancer properties, we firstly determined the cytotoxic potential of the different derivatives against the murine melanoma cell line b16f10, and we secondly investigated whether the cytotoxicity of the most effective compounds was caused by the induction of apoptosis. We also determined the levels of ROS, proposing a plausible molecular mechanism for its apoptotic effects. Finally, we have performed HIV-protease inhibition assays, being the action of the acyl derivatives of the triterpene acids, much more potent than that of the natural precursor compounds. Hence, these derivatives may represent an effective therapeutic strategy for treating cancer or HIV infection.

2. Results and discussion

2.1. Chemistry

Oleanolic acid (**1**, 3β-hydroxyolean-12-en-28-oic acid, **OA**) [1] and maslinic acid (**2**, 2α,3β-dihydroxyolean-12-en-28-oic acid, **MA**) [2] are natural compounds belonging to the pentacyclic triterpene family and are widely distributed in the plant kingdom. In our case, these compounds were isolated from the olive-pressing residues by extraction processes with different solvents [4]. These triterpenoid acids were benzylated by treatment with benzyl chloride to obtain the corresponding derivatives **3** (28-benzyloleanolic acid, **OA-Bn**) and **4** (28-benzylmaslinic acid, **MA-Bn**) (Fig. 1). Both compounds were easily recognizable from their spectroscopic properties, since in their ^{1}H and ^{13}C NMR spectra appeared the signals of the benzyl methylene group (δ_H 5.05, 2H, AB system, J = 12.0 Hz, and δ_C 66.15, respectively in both compounds), which matched those found in the literature [32,55].

2.1.1. Solution-phase acylations of triterpenes *1–4*

To compare the acylation process, in different phases and conditions, of **OA** (**1**) and **MA** (**2**), we proceeded to test their acetylation in solution-phase at diverse times and under different temperature conditions. The acetylation reactions of **OA** and **MA** were carried out with acetic anhydride in pyridine, consistently using an excess of 4:1 of the acylating agent with respect to the hydroxyl group (Scheme 1). The composition of the different acetylation mixtures was studied using HPLC (Tables 1 and 2).

In this acetylation process in the solution-phase, **OA** was acetylated to form the 3-acetyl derivative 5 [56] in good yields (80–95%), even at rt (Table 1). In the acetylation reaction of **MA** (**2**), three derivatives were formed: 2-acetyl-MA (**6**), 3-acetyl-MA (**7**), and 2,3-diacetyl-MA (**8**) [57]. This acylation process was conducted at several reaction times and temperatures, improving the yield of the double acetylated derivative (**8**) with the increase in the temperature and reaction time (80% at 24 h at rt, and 90% at 1 h and reflux) (Table 2).

Once the reaction conditions (24 h at rt) for the acetylations of **OA** and **MA** were optimized, we carried out the semi-syntheses in solution-phase of several derivatives of compounds **1**–**4**, by acylation of the hydroxyl groups of the A-ring with 10 acid anhydrides (Fig. 2), to later check their biological properties. The acyl groups were selected with a wide structural variety in order to obtain acylated derivatives of OA and MA with diverse physical and chemical characteristics. Thus, acyl groups I–V have an increasing number of carbon atoms, and therefore the polarity decreases progressively from the acetyl to the lauroyl group. On the other hand, acyl groups VI and VII have aromatic rings, and phthaloyl group (VII) also another carboxylic group at the end of the carbon chain. Finally, acyl groups VIII–X, derived from succinic and glutaric anhydrides, also have an additional carboxylic group that conferred them with singular polarity and solubility properties.

Table 1
Solution-phase acetylation of **OA**.

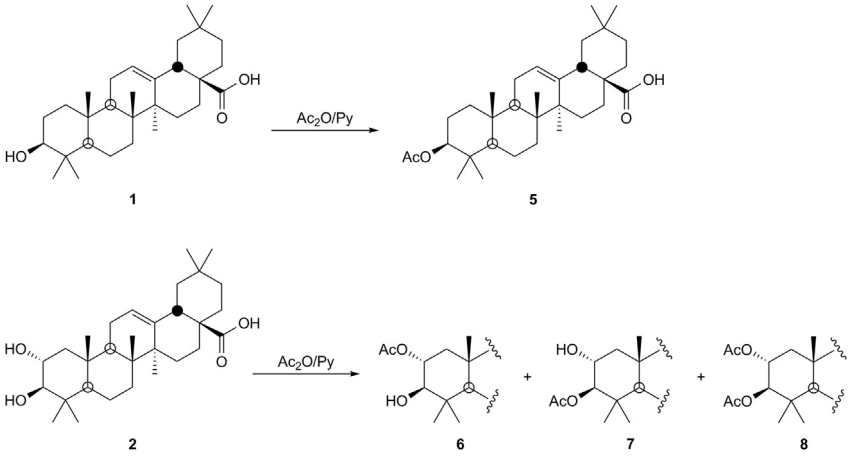
Time (h)	Temperature	Compound			
		OA (**1**)		3-Acetyl-OA (**5**)	
		Yield (%)	R_t (min)	Yield (%)	R_t (min)
0.5	Reflux	8.9	9.72	91.1	13.89
1	Reflux	5.6		94.4	
12	rt	18.4		81.6	
24	rt	5.3		94.7	

Table 2
Solution-phase acetylation of **MA**.

Time (h)	Temp.	Compound							
		MA (**2**)		2-Acetyl-MA (**6**)		3-Acetyl-MA (**7**)		2,3-Diacetyl-MA (**8**)	
		Yield (%)	R_t (min)	Yield (%)	R_t (min)	Yield (%)	R_t (min)	Yield (%)	R_t (min)
0.5	Reflux	10.3	4.69	20.7	6.93	9.2	8.56	59.8	10.12
1	Reflux	6.2		2.2		1.7		89.9	
12	rt	9.7		30.8		19.0		40.5	
24	rt	4.3		14.1		0.8		80.8	
12	0 °C	45.3		30.8		20.1		3.8	
24	0 °C	10.5		44.6		30.2		14.7	

The above-mentioned acylated derivatives were formed by treating the compounds **1**–**4** with the corresponding acid anhydride in pyridine for 24 h at rt, in a relationship of 4:1 (anhydride:hydroxyl group). Thus, we obtained 57 acylated derivatives (**9**–**65**), given in Figs. 3 and 4.

The main spectroscopic differences between the acylated compounds (**9**–**27**) and the corresponding substrates (**OA** (**1**), and **OA-Bn** (**3**)) were the important deshieldings of the signals of the geminal protons to the hydroxyl group at C-3 (^{1}H spectra), and the notable variations in the chemical shifts of C-3 (^{13}C spectra). Thus, the signal of the geminal proton of H-3 for **OA** and **OA-Bn** was situated at δ_H 3.19 and 3.18, respectively, whereas the same signal was deshielded up to δ_H 4.40–4.50 for the derivatives with the acyl group I–V and VIII–X, and up to δ_H 4.70–4.80 for the acyl groups VI and VII (aromatic ring). In the same way, the chemical shift of C-3



Scheme 1. Acetylation tests of **OA** and **MA** in the solution-phase.

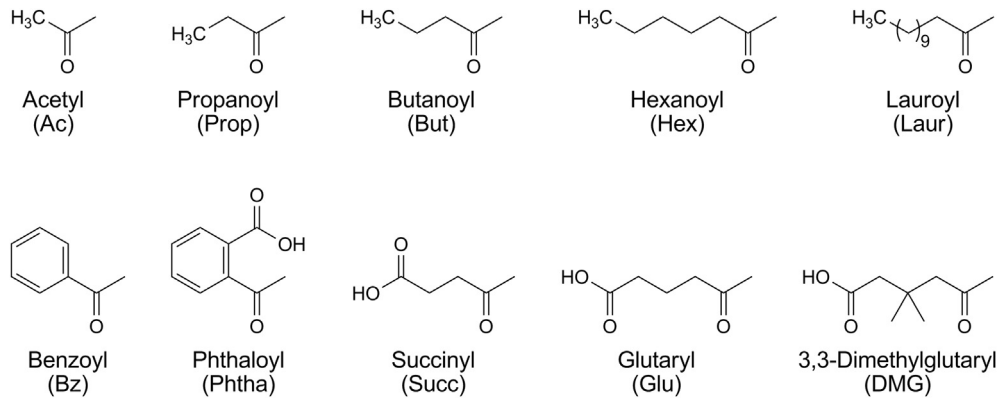
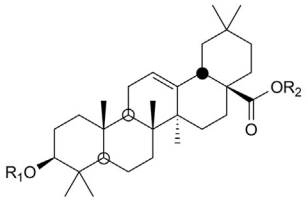


Fig. 2. Structures of the acyl groups used for the acylations of **OA** and **MA**.

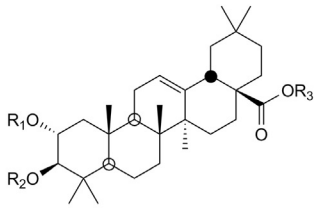


Compound	R_1	R_2	Yield (%)	Compound	R_1	R_2	Yield (%)
				18	Acetyl	Bn	89
9	Propanoyl	H	83	**19**	Propanoyl	Bn	85
10	Butanoyl	H	87	**20**	Butanoyl	Bn	93
11	Hexanoyl	H	90	**21**	Hexanoyl	Bn	90
12	Lauroyl	H	73	**22**	Lauroyl	Bn	91
13	Benzoyl	H	69	**23**	Benzoyl	Bn	73
14	Phthaloyl	H	93	**24**	Phthaloyl	Bn	82
15	Succinyl	H	47	**25**	Succinyl	Bn	51
16	Glutaryl	H	59	**26**	Glutaryl	Bn	67
17	3,3-Dimethylglutaryl	H	62	**27**	3,3-Dimethylglutaryl	Bn	65

Fig. 3. Structures of compounds **9**–**27** from the acylation of **OA**.

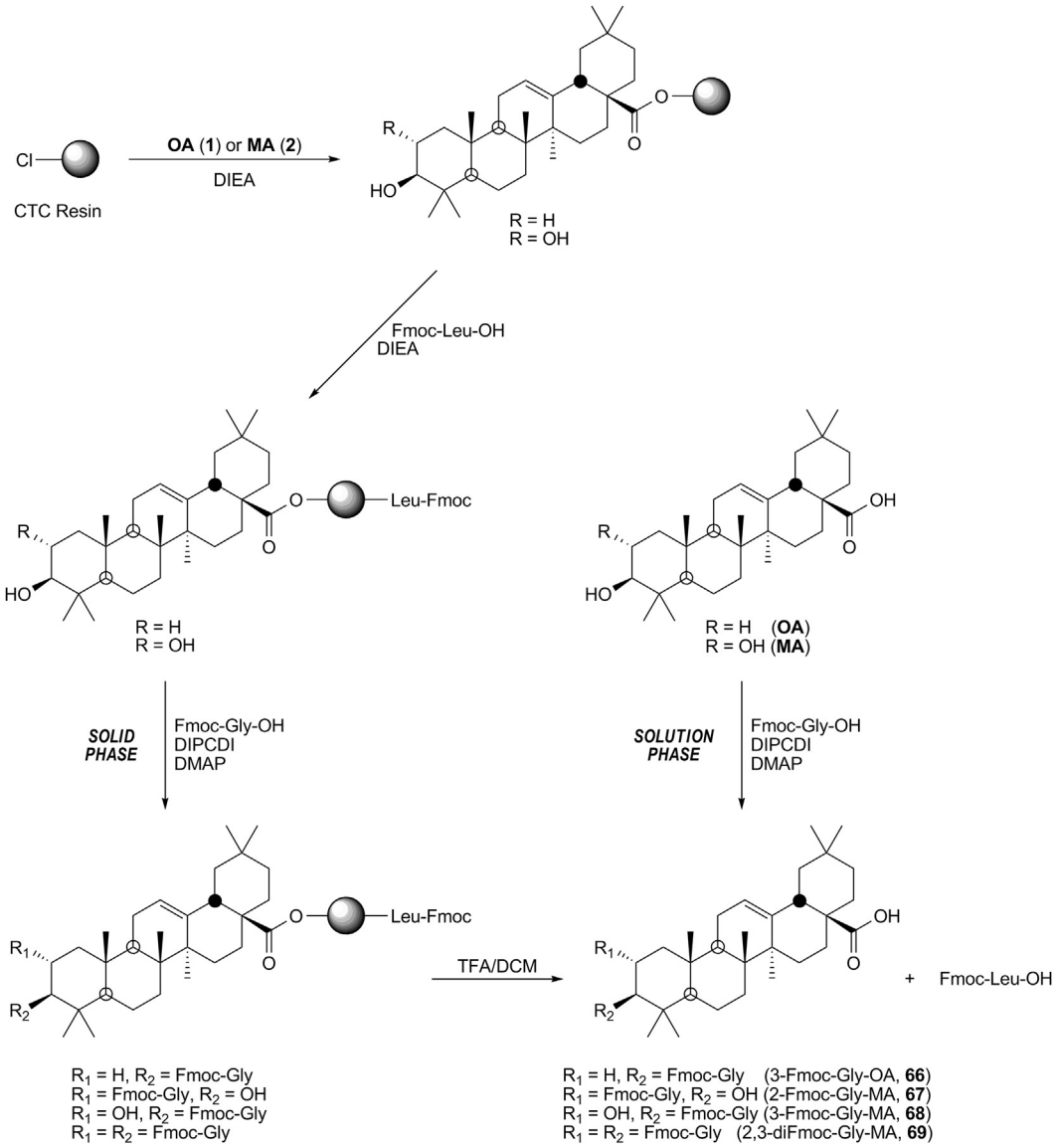
was situated at δ_C 78.69 for **OA** and δ_C 79.23 for **OA**-**Bn**, moving to δ_C 80–83 when this carbon was acylated.

In the acylation processes of **MA** (**2**) and **MA**-**Bn** (**4**), using the optimized reaction conditions of 24 h at rt, the major 2-acyl derivatives and 2,3-diacyl derivatives were formed, probably due to the increased steric hindrance of the hydroxyl group at C-3 by the presence of the geminal methyl groups on C-4. For this reason, the minor 3-acyl derivatives were not considered for this study. The



Compound	R_1	R_2	R_3	Yield (%)	Compound	R_1	R_2	R_3	Yield (%)
					46	Acetyl	H	Bn	15
					47	Acetyl	Acetyl	Bn	75
28	Propanoyl	H	H	20	**48**	Propanoyl	H	Bn	22
29	Propanoyl	Propanoyl	H	73	**49**	Propanoyl	Propanoyl	Bn	67
30	Butanoyl	H	H	15	**50**	Butanoyl	H	Bn	10
31	Butanoyl	Butanoyl	H	80	**51**	Butanoyl	Butanoyl	Bn	79
32	Hexanoyl	H	H	19	**52**	Hexanoyl	H	Bn	15
33	Hexanoyl	Hexanoyl	H	70	**53**	Hexanoyl	Hexanoyl	Bn	76
34	Lauroyl	H	H	27	**54**	Lauroyl	H	Bn	33
35	Lauroyl	Lauroyl	H	57	**55**	Lauroyl	Lauroyl	Bn	61
36	Benzoyl	H	H	37	**56**	Benzoyl	H	Bn	35
37	Benzoyl	Benzoyl	H	45	**57**	Benzoyl	Benzoyl	Bn	51
38	Phthaloyl	H	H	25	**58**	Phthaloyl	H	Bn	27
39	Phthaloyl	Phthaloyl	H	40	**59**	Phthaloyl	Phthaloyl	Bn	55
40	Succinyl	H	H	30	**60**	Succinyl	H	Bn	27
41	Succinyl	Succinyl	H	33	**61**	Succinyl	Succinyl	Bn	43
42	Glutaryl	H	H	31	**62**	Glutaryl	H	Bn	21
43	Glutaryl	Glutaryl	H	35	**63**	Glutaryl	Glutaryl	Bn	51
44	3,3-Dimethylglutaryl	H	H	33	**64**	3,3-Dimethylglutaryl	H	Bn	25
45	3,3-Dimethylglutaryl	3,3-Dimethylglutaryl	H	36	**65**	3,3-Dimethylglutaryl	3,3-Dimethylglutaryl	Bn	47

Fig. 4. Structures of compounds **28**–**65** from the acylation of **MA**.



Scheme 2. Control and quantification of load of **OA** and **MA** to the CTC resin.

main ^{1}H NMR chemical shift differences between the acylated compounds (**28**–**65**) and the corresponding substrates (**MA** (**2**), and **MA-Bn** (**4**)) were the position of the signals of H-2 and H-3, situated at δ_H 3.45 and 2.84 for **MA**, and at δ_H 3.59 and 2.89 for **MA-Bn**, respectively. However, in the corresponding 2-acyl derivatives, the H-2 signals were situated between δ_H 4.90–5.20, and for the 2,3-diacyl derivatives, the H-3 signals were deshielded up to δ_H 4.70–4.80 for the derivatives with the acyl group I–V and VIII–X, and up to δ_H 5.50–5.80 for the acyl groups VI and VII (aromatic ring). Additionally, the ^{13}C NMR chemical shifts of the signals of C-2 and C-3 were situated at δ_C 68.24 and 83.17 for **MA** and δ_C 68.98 and 83.90 for **MA-Bn**, respectively, whereas the C-2 signals were deshielded up to δ_C 73–74 for the 2-acyl derivatives, and up to δ_C 69–71 for the 2,3-diacyl derivatives. The C-3 signals were shielded down to δ_C 80–82 for both monoacylated and diacylated derivatives.

2.1.2. Solid-phase acylations of *OA* and *MA*

Several of these acylated derivatives (acetic, hexanoic, benzoic, and succinic) of **OA** and **MA**, were also semi-synthesized by solid-phase processes. The 2-chlorotrityl chloride polymer resin (Barlos resin, PL Cl-Trt-Cl resin, CTC resin) was chosen as the solid support, since this resin allows the release of the compounds by treatment under mild acidic conditions that do not affect the triterpene skeleton. First, it became necessary to study and to quantify the incorporation of **OA** and **MA** into this resin. Therefore, a sequence of chemical reactions was carried out using amino acids with an Fmoc (fluorenylmethyloxycarbonyl) group incorporated, since this group absorbs at a wavelength (301 nm) that does not interfere with that of the other compounds. In this way, the above-mentioned triterpene acids were attached to the resin through its 28-carboxyl group, and later, a treatment was made with Fmoc-Leu-OH and MeOH to cap the resin. Afterwards, Fmoc-Gly-OH was attached to the hydroxyl groups of the A-ring, and finally, the resin was cleaved with TFA in DCM, producing free Fmoc-Leu-OH, and **OA** and **MA**, which incorporated Fmoc-Gly-OH in their hydroxyl groups (**66**–**69**). Additionally, to determine the better yield of triterpene acid loaded to the solid support, we made three tests with different proportions of **OA** and **MA** (0.5, 1.0, and 3.0 equiv with respect to the resin) (Scheme 2).

The incorporation of **OA** to the CTC resin was almost quantitative with 3 equiv of this triterpene acid. The maximum incorporation of **MA** into the resin was about a 92%, also with 3 equiv of this compound. This percentage was distributed between the C-2 derivative (68.1%), the C-2/C-3 derivative (17.2%), and the C-3 derivative (6.8%) (Table 3). This distribution of derivatives was reasonable, given that the C-3 hydroxyl group has more steric hindrance due to the presence of the angular methyl groups on C-4. This quantification was achieved by HPLC at 301 nm of absorbance, and the Fmoc-Gly-derivatives of OA and MA, used as reference compounds, were prepared via the solution-phase. Thus, the reaction of **OA** (**1**) with Fmoc-Gly-OH in DCM with DIPCDI and DMAP at reflux for 12 h, gave 3-Fmoc-Gly-OA (**66**) (Table 3 and Scheme 2). The ^{1}H NMR spectrum of this compound (**66**) presented the following deshielded signals: δ_H 4.01 (2H, d, J = 3.5 Hz, 2H-2′ glycine methylene), 4.26 (1H, t, J = 6.8 Hz, H-3″ proton of the cyclopentane ring of the Fmoc group), 4.43 (2H, d, J = 6.8 Hz, H-2″ methylene of the Fmoc group), 4.62 (1H, dd, $J_1 = J_2$ = 7.5 Hz, H-3), and 5.31 (1H, dd, $J_1 = J_2$ = 3.3 Hz, H-12). In its ^{13}C NMR spectrum the signals of the carbonyl and methylene groups of the glycine (δ_C 170.1 and 43.2), those of the carbonyl and methylene groups of the Fmoc group (δ_C 156.5 and 67.4), and those of C-3 and C-28 (δ_C 82.8 and 184.0), were detected.

The reaction of **MA** (**2**) with Fmoc-Gly-OH in DCM with DIPCDI and DMAP at rt for 24 h, gave 2-Fmoc-Gly-MA (**67**), 3-Fmoc-Gly-MA (**68**), and 2,3-diFmoc-Gly-MA (**69**) (Table 3 and Scheme 2). The NMR spectra of compounds **67** and **68** differed only in the signals of H-2/C-2 and H-3/C-3. In the ^{1}H NMR spectrum of **67**, the signal of H-2 appeared at δ_H 5.06 and that of H-3 at δ_H 3.19, whereas for **68**, the signals of these protons were detected at δ_H 3.80 and 4.60, respectively. In the ^{13}C NMR spectrum of **67**, the signal of C-2 appeared at δ_C 74.9 and that of C-3 at δ_C 80.7, whereas for **68**, the signals of these carbons were detected at δ_C 67.3 and 86.6, respectively. The doubly substituted derivative 2,3-diFmoc-Gly-MA (**69**) had a ^{1}H NMR spectrum in which the signal of H-2 appeared at δ_H 5.34, and that of H-3 at δ_H 4.80. In the ^{13}C NMR spectrum of **69**, the signal of C-2 was placed at δ_C 71.0 and that of C-3 at δ_C 82.1.

The acid anhydrides selected to carry out the acylation reactions in solid-phase were: acetic, hexanoic, benzoic and succinic. First, **OA** and **MA** were incorporated into the resin under the above-described optimized conditions (3 equiv of the triterpene acid). After this, the four selected acid anhydrides were added to the corresponding syringe in a molar relationship of 4:1 (anhydride:hydroxyl group), in the presence of DMAP and Et_3N. Finally, after 24 h of orbital stirring at rt, the resin was cleaved with TFA/DCM, and the residue was analyzed by HPLC (Scheme 3).

The solid-phase acylation reactions of OA (**1**) with the acetic and hexanoic anhydrides were almost completed in 30 min. However, the nearly complete benzoylation process of **OA** (94.8%) required 4 h of reaction time, and the acylation with succinic anhydride was much more difficult and slow, reaching a yield of only 53.7% after 48 h of reaction (Table 4).

The solid-phase acylations of **MA** (**2**) with the same four acid anhydrides provided more complex mixtures of compounds because of the presence of the two hydroxyl groups in the A-ring of the substrate (**2**). Nevertheless, the acylation reactions with the more apolar and aliphatic acid anhydrides (acetic and hexanoic) only gave the doubly acylated compounds (**8** and **33**, respectively) in 2 h. The benzoylation process took place more slowly, requiring 24 h for a yield of 94.9% of the doubly benzoyl derivative (**37**). In this acylation reaction, we identified the 2-benzoyl derivative (**36**) but did not detect the possible 3-benzoyl derivative. Finally, the solid-phase succinylation of **MA** yielded 81.1% of the diacylated derivative (**41**) after 48 h of reaction. This yield was higher than the one reached in the formation of the succinyl derivative of OA (**15**). The succinylation reaction of **MA** probably first formed the 2-succinyl derivative (**40**), which seems to help the entry at the more hindered C-3 hydroxyl group, and therefore the doubly acylated compound (**41**) gave very high yield. In this case, we also detected very low proportions of what may be a possible 3-succinyl derivative of MA (Table 5).

In these acylation studies, we found that the acylated derivatives of OA and MA that were achieved from a cyclic acid anhydride (phtalic, succinic, glutaric, and 3,3-dimethylglutaric) were compounds of very difficult chromatographic isolation and also of complicated spectroscopic identification, due to the presence of two or three free carboxylic groups in the molecule. Therefore, to avoid these experimental problems, we selected the succinyl derivatives to study the protection of the free carboxylic groups, through a benzylation reaction. Accordingly, when **OA** (incorporated to the resin) was succinylated in solid-phase, cleaved from the resin, and benzylated with benzyl chloride, the corresponding benzyl oleanolate (**3,** 40%), and benzyl 3-(benzylsuccinyl) oleanolate (**70**, 55%) were formed (Scheme 4). The NMR spectra of **70** presented the characteristic signals of a doubly benzylated compound, 10 aromatic protons at δ_H 7.36–7.35, two AB systems at δ_H 5.13 and 5.07, 12 aromatic carbons between 136 and 128 ppm, and two methylene groups at δ_C 66.5 and 65.9 (see the Experimental Section). In a similar process, **MA** was also succinylated and benzylated to give: benzyl maslinate (**4**, 6%), benzyl 2-(benzylsuccinyl) maslinate (**71**, 8%), benzyl 3-(benzylsuccinyl) maslinate (**72**, 6%), and benzyl 2,3-di(benzylsuccinyl) maslinate (**73**, 74%) (Scheme 4). The benzylsuccinyl derivatives of MA (**71**–**73**) were also easily chromatographically separated, and identified from their NMR properties. Hence, with these benzylation reactions the proportions of succinylated derivatives achieved from the solid-phase succinylation of **OA** and **MA** could be determined more accurately.

Table 3
Tests of incorporation of the triterpenic acids to the CTC resin.

Test incorporation **OA** to the resin	Equivalents of **OA** respect to the resin		
	0.5 equiv	1 equiv	3 equiv
Fmoc-Leu-OH (R_t = 11.59 min)	32.2%	13.9%	1.1%
Fmoc-Gly-OA (**66**) (R_t = 34.74 min)	67.8%	86.1%	98.9%

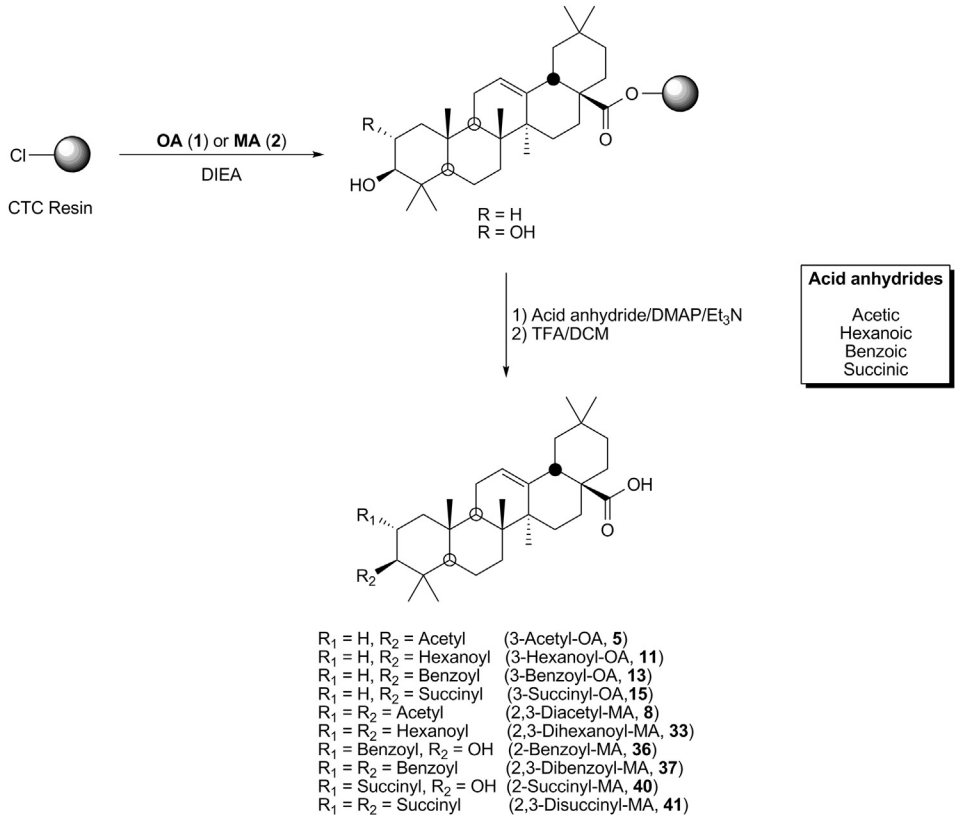
Test incorporation **MA** to the resin	Equivalents of **MA** respect to the resin		
	0.5 equiv	1 equiv	3 equiv
Fmoc-Leu-OH (R_t = 11.61 min)	39.4%	17.8%	7.9%
2-(Fmoc-Gly)-MA (**67**) (R_t = 31.84 min)	55.8%	67.3%	68.1%
3-(Fmoc-Gly)-MA (**68**) (R_t = 33.42 min)	1.9%	3.8%	6.8%
2,3-di(Fmoc-Gly)-MA (**69**) (R_t = 35.82 min)	2.9%	11.1%	17.2%

2.2. Cytotoxicity

We determined the bioactive potential of these semi-synthesized oleanolic and maslinic acid derivatives (**1**–**65**), as anti-proliferative and pro-apoptotic compounds, and as anti-virals on HIV-serine protease (Fig. 5).

Cytotoxicity effects have been previously described in a wide variety of pentacyclic triterpenes, involving a mechanism that implied MAPK (mitogen-activated protein kinases), death receptor, and mitochondrial disruption [19,33]. Also, the cytotoxic effects of 3-O-acyl derivatives of several pentacyclic triterpenes have been described [58,59].

We examined the effects of the semi-synthesized acylated MA and OA derivatives on the proliferation of b16f10 cell line using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium



Scheme 3. Solid-phase acylation reactions of **OA** and **MA**.

bromide) assay. Thus, these murine melanoma cells were treated with increasing doses (range 0–80 μg/mL) of each derivative, and their viability was determined by formazan dye uptake, and expressed as a percentage of untreated control cells. The results of these assays showed that only some 24% of the compounds tested (**1**–**65**) displayed cytotoxic effects on this cell line. The numbers and structures of these cytotoxic compounds and their IC_{50} values are given in Table 6. Comparing the IC_{50} data for **OA** (106.4 μM) and **MA** (36.2 μM), we deduce that the hydroxyl group at C-2 enhances the cytotoxicity of this compound (**2**). Furthermore, considering the IC_{50} data for **OA** and **MA**, and those of their corresponding 28-benzyl derivatives (**3** and **4**), we find that benzylation reduces the IC_{50} concentrations by about 50% (Table 6).

The acyl OA derivatives with cytotoxic effects were only compounds **5**, **9**, and **13**, and only the acetyl derivative (**5**, IC_{50} = 64.7 μM) showed a lower IC_{50} concentration than its precursor (**1**). Nevertheless, the acyl 28-benzyl OA derivatives (**24**–**27**) with cytotoxic effects (IC_{50} from 14.2 to 29.9 μM), showed an IC_{50} underneath its precursor (**3**). In terms of structure–activity relationship (SAR), the better cytotoxic results for the acyl OA derivatives were achieved when the acyl group at C-3 (R_2) was a polar dicarboxylic fragment, such as phthaloyl, succinyl, glutaryl, or 3,3-dimethylglutaryl (compounds **24**–**27**), and a benzyl group was placed at the carboxylic group (R_3) (Table 6 and Chart 1).

The acyl MA derivatives (**8**, **30**, and **32**) exhibited relatively low cytotoxicity (IC_{50} from 34.6 to 106.6 μM), although in the same range as found in its precursor (**2**). Similarly, several acyl 28-benzyl MA derivatives (**46**, **58**, **62**, and **64**) showed low IC_{50} (from 28.3 to 73.2 μM), but above of its precursor (**4**). The acyl MA derivatives that showed cytotoxicity had only one acyl group at C-2 (R_1), whereas the hydroxyl group at C-3 (R_2) was free, except the diacetyl derivative **8**. The presence of double acyl groups at C-2 (R_1) and at C-3 (R_2) in the acyl MA derivatives appears to inhibit the cytotoxicity of these compounds. The exception observed with the diacetyl derivative **8** may be due to the small size of this acyl group (Table 6 and Chart 1). Chart 1 shows a comparison of the growth-inhibitory effects of the acyl OA and MA derivatives on b16f10 murine melanoma cells, describing the ratio between the IC_{50} values of the triterpene precursors (**OA**, **MA**, **OA-Bn** and **MA-Bn**) and the IC_{50} values of their cytotoxic acyl derivatives (last column of Table 6).

Several cytometry studies, described below, were made to determine the apoptotic properties of the compounds tested (**1**–**65**). The *in vitro* IC_{50} growth-inhibitory concentrations of the new semi-synthesized compounds were, in many cases, similar of those of their precursors (**1**–**4**), but sufficiently low to investigate their apoptotic properties.

2.3. Apoptosis

Different types of pentacyclic triterpenoids have shown anticancer properties ranging from inhibition of tumour-cell proliferation, induction of apoptosis, suppression of angiogenesis, and metastasis. These triterpenoids have the ability to suppress various key steps of initiation, progression, and promotion of tumours, and therefore have the potential for use in both chemoprevention and therapy of various cancers with several common molecular targets [33,60]. Recently, the anticancer properties of a 3-O-acyl derivative of a pentacyclic triterpene have been reported [61].

The apoptotic determination assays were conducted through double staining with An-V (annexin V) conjugated FITC (fluorescein isothiocyanate) and PI (propidium iodide). Early events in the apoptotic processes are loss of plasma-membrane asymmetry accompanied by translocation of PS (phosphatidylserine) from the inner to the outer membrane leaflet, thereby exposing PS to the

Table 4
Solid-phase acylation of **OA**.

Time (h)	Acyl group	Compound			
		OA		3-Acyl-OA	
		Yield (%)	R_t (min)	Yield (%)	R_t (min)
0.5	Acetyl	1.5	9.5	98.3	14.1
0.5	Hexanoyl	1.1		96.4	22.4
2	Benzoyl	23.5		75.1	25.0
4		3.2		94.8	
2	Succinyl	94.4		2.5	9.0
4		90.1		6.8	
8		79.6		18.2	
24		59.8		36.9	
48		43.6		53.7	

external environment. The phospholipid-binding protein annexin V has a high affinity for PS and binds to cells fluorescently labelled with FITC. The percentages of apoptosis were determined with annexin V–FITC/PI flow-cytometric analysis. Apoptosis in b16f10 cells was assessed 72 h after treatment with the different previously cytotoxic OA and MA derivatives at the IC_{50} concentrations before administered (Table 6), and at the $IC_{50} \times 2$ concentrations (twice IC_{50} concentrations. $\times 2$ values corresponding to 50% viability). FACS (flow-activated cell sorter) analysis using annexin V–FITC staining and PI accumulation was used to differentiate early apoptotic cells (An-V^+ and PI^-) from late apoptotic (An-V^+ and PI^+), necrotic (An-V^- and PI^+) or normal cells (An-V^- and PI^-).

All the cytotoxic compounds showed apoptotic effects on the treated cells with a high percentage of total apoptosis between 51 and 95% at IC_{50} concentration, except product **62** (25.2% of total apoptosis), and between 60 and 98% at $IC_{50} \times 2$ concentrations (Table 7). In addition, the percentages of the necrotic population for all these acyl OA and MA derivatives were irrelevant. Several compounds (**9**, **24**, **25**, **27**, **30**, and **46**) showed a total apoptosis above 70%, at its IC_{50} concentrations; three of these were the acyl 28-benzyl OA derivatives (**24**, **25**, and **27**) with a polar dicarboxylic substituent at C-3 (R_2), which displayed the most cytotoxic properties. Ten compounds (**8**, **24**–**27**, **30**, **32**, **46**, **58**, and **64**) showed a percentage of total apoptosis above 80%, at its $IC_{50} \times 2$ concentrations, higher percentages corresponding to compounds with a benzyl group blocking the carboxylic group of the triterpene skeleton, and a polar dicarboxylic acyl substituent at C-2 (R_1) or C-3 (R_2). In addition, seven of the compounds tested (**9**, **24**, **27**, **30**, **32**, **46**, and **58**) showed a higher percentage of early apoptosis than late apoptosis, at its IC_{50} concentrations (between 42 and 66% of total cell population), whereas nine compounds (**9**, **24**–**27**, **30**, **32**, **46**, and **62**) did (between 44 and 74% of total cells) at its $IC_{50} \times 2$ concentrations (Table 7 and Chart 2). Fig. 6 presents the diagrams of annexin V/propidium iodide flow-cytometry for three of the compounds that display higher apoptotic cell populations (**24**, **25**, and **46**).

Though the structure–activity relationship of these OA and MA derivatives was not absolutely well defined, it seems that the presence of a benzyl group blocking the carboxylic group at C-28 and a polar dicarboxylic acyl substituent at C-2 (R_1) or C-3 (R_2), enhanced the apoptotic properties of these derivatives, compared with the corresponding precursors. The potent activities on the apoptosis-activation process reached by these derivatives suggest that they could be used as anticancer drugs.

2.4. ROS production

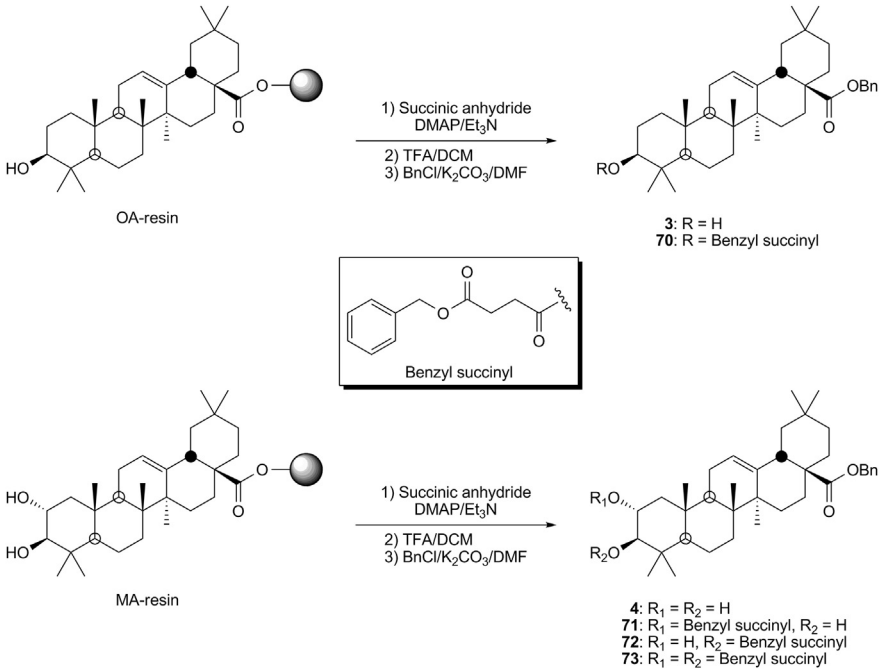
Mitochondria constitute a key target in synthetic and natural triterpenoids [7]. Pentacyclic triterpene compounds have proved to be able to activate the intrinsic apoptotic pathway, mediated with mitochondrial disruption [36,37], and the extrinsic apoptotic pathway [33], mediated by an enhanced receptor [62]. The over production of ROS in **OA** and **MA** treatment on several cancer cell lines has also been reported [34,35,63]. Changes in the mitochondrial-membrane potential can be examined by monitoring the cell fluorescence after double staining with Rh123 (rhodamine 123) and PI (propidium iodide). Rh123 is a membrane-permeable fluorescent cationic dye that is selectively taken up by mitochondria directly proportional to the MMP (mitochondrial-membrane permeabilization) [64]. Most studies on the apoptosis molecular mechanism show that when the intrinsic apoptotic mechanism is activated, a rapid production of the ROS is generated by the mitochondrial disruption, although this also can be triggered in the final steps of extrinsic apoptotic activation if the bit protein is activated by the initiator caspases 8 or 10 [65,66].

We analyzed the ROS production to elucidate the possible mechanism involved in the apoptotic responses in the b16f10 mouse melanoma cells to the acyl OA and MA derivatives, at the same times and concentrations of the cytotoxic and apoptosis responses. These studies were performed to determine whether ROS generation occurs simultaneously or not with apoptosis. If so, the mitochondrial disruption would be implicated in the apoptotic response, but if not the apoptosis could be caused by the extrinsic apoptotic-route activation. Changes in the MMP were analyzed by flow-cytometry after staining with Rh123.

The results at the IC_{50} concentration, showed strong Rh123 fluorescence intensity in the response in many of the compounds tested, except for compounds **24**, **25**, **26**, and **58** (Chart 3). From these results, it can be interpreted that compounds **5**, **8**, **9**, **13**, **27**, **30**, **32**, **46**, **62**, and **64** caused mitochondrial disruption in the activation of apoptosis, whereas in the compounds **24**, **25**, **26**, and **58**, the

Table 5
Solid-phase acylation of **MA**.

Time (h)	Acyl group	Compound							
		MA		2-Acyl-MA		3-Acyl-MA		2,3-Diacyl-MA	
		Yield (%)	R_t (min)	Yield (%)	R_t (min)	Yield (%)	R_t (min)	Yield (%)	R_t (min)
2	Acetyl	1.2	4.7	0.0	–	0.0	–	96.4	10.0
2	Hexanoyl	0.8		0.0	–	0.0	–	97.1	24.9
2	Benzoyl	2.2		47.3	10.1	0.0	–	49.4	19.8
4		1.8		32.9		0.0		64.3	
8		1.1		23.7		0.0		73.9	
24		0.6		3.4		0.0		94.9	
2	Succinyl	32.5		41.2	3.7	1.7	1.3	23.4	1.5
4		27.6		31.5		2.4		37.1	
8		18.4		20.1		3.8		56.3	
24		10.2		14.5		4.2		69.2	
48		6.3		7.1		4.8		81.1	



Scheme 4. Solid-phase succinylation reactions and consecutive solution-phase benzylation reactions of **OA** and **MA**.

induction of apoptosis was not concomitant with the ROS generation. The results found at the $IC_{50} \times 2$ concentration were very similar to those found at the IC_{50} concentration, and showed the generation or not of ROS for the same compounds. These results could be related with an extrinsic activation of the apoptosis mechanism for compounds **24**, **25**, **26**, and **58**, and the others by the intrinsic apoptotic-route activation. In any case, further molecular studies in this sense will be necessary to confirm this assertion.

Fig. 7 shows the diagram of Rh123/PI flow-cytometry, for the compounds with minor (**24** and **25**) and with major (**32** and **62**) Rh123 staining. The Rh123/PI labelling of b16f10 cells was carried out as described in the experimental section. This diagram indicates that, for compounds **24** and **25** (negative ROS production), the major cell population was distributed into the Q_1 and Q_3 left quadrants, whereas for compounds **32** and **62** it was contained into the Q_2 and Q_4 right quadrants.

Finally, we can conclude that, although the ROS production structure–activity relationship was not absolutely clear, the compounds in which the ROS production was totally or partially negative had a polar dicarboxylic substituent at C-2 (R_1), and a benzyl group blocked the carboxyl group at C-28 of the triterpene skeleton.

2.5. Anti-HIV-1-protease activity

Pentacyclic triterpenes have been found to exhibit anti-HIV activities. The modes of action of the anti-HIV triterpenes have been reported to be associated with virus entry, reverse transcription, virus assembly, and maturation [67]. In many cases, the anti-HIV maturation activity has been associated with the presence of an acyl group at C-3 of the pentacyclic triterpene skeleton [24,26,68–70], whereas a side chain at C-28 has been related with the anti-HIV-1 entry activity [53,71].

The determination of the concentration of the products that account for the inhibition of 50% activity of HIV-1-protease or the IC50 concentration using the FRET (fluorescence resonance energy transfer) technique with a fluorescence resonance energy transfer peptide. The different compounds were considered to be inhibitors of the HIV-1-protease when their IC_{50} values were within the concentration range (0–1200 μg/mL). The results from this test for all the acyl OA and MA derivatives (**1**–**65**) showed strong inhibitory capacity of the HIV-1-protease by the 38% of the compounds (Table 8 and Chart 4). From the set of the acyl OA derivatives assayed, only five compounds (**5**, **14**, **16**, **17**, and **24**) presented anti-HIV activity. Most of the active acyl OA derivatives had a polar

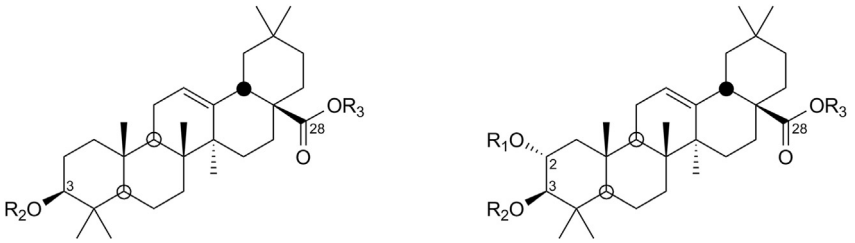


Fig. 5. General structures of the tested acyl OA and MA derivatives.

Table 6
Growth-inhibitory effects of acyl OA and MA derivatives on b16f10 murine melanoma cells.

Comp. #	R_1	R_2	R_3	IC_{50} concentration (μM)[a]	IC_{50} of precursor/IC_{50} of compound #[b]
OA (**1**)	–	H	H	106.4 ± 3.7	1.0
5	–	Acetyl	H	64.7 ± 2.8	1.6
9	–	Propanoyl	H	161.1 ± 9.8	0.7
13	–	Benzoyl	H	162.1 ± 18.4	0.7
OA-Bn (**3**)	–	H	Bn	52.2 ± 0.9	1.0
24	–	Phthaloyl	Bn	26.8 ± 0.9	1.9
25	–	Succinyl	Bn	23.2 ± 0.3	2.3
26	–	Glutaryl	Bn	29.9 ± 0.9	1.7
27	–	3,3-Dimethylglutaryl	Bn	14.2 ± 1.0	3.7
MA (**2**)	H	H	H	36.2 ± 3.8	1.0
8	Acetyl	Acetyl	H	44.1 ± 3.8	0.8
30	Butanoyl	H	H	106.6 ± 11.3	0.3
32	Hexanoyl	H	H	34.6 ± 2.1	1.0
MA-Bn (**4**)	H	H	Bn	19.0 ± 0.2	1.0
46	Acetyl	H	Bn	39.6 ± 0.8	0.5
58	Phthaloyl	H	Bn	73.2 ± 2.4	0.3
62	Glutaryl	H	Bn	63.2 ± 2.1	0.3
64	3,3-Dimethylglutaryl	H	Bn	28.3 ± 1.1	0.7

[a] The IC_{50} values were calculated considering control untreated cells as 100% of viability. Cell-growth inhibition was analyzed by the MTT assay, as described in the Experimental section. All assays were made four times using six replicates. Values, means ± S.E.M.
[b] The last column represents the ratio between the IC_{50} of each precursor (**OA**, **OA-Bn**, **MA** or **MA-Bn**) and the IC_{50} of the related acyl derivatives.

dicarboxylic substituent at C-3 (R_2), and exhibited an excellent inhibitory capacity, with IC_{50} values between 0.31 and 0.70 μM, which are between 82 and 186 times lower than its precursor (**OA**) (see Table 8 and Chart 4).

There were 18 acyl MA derivatives that inhibited the HIV-1-protease (Table 8). Only compounds **32**, **50**, **53**, and **55**, showed lower activities than their precursors, and all of them had an aliphatic substituent at C-2 (R_1) or at C-2 and C-3 (R_2), such as butanoyl, hexanoyl or lauroyl group. The most active acyl MA derivatives had polar dicarboxylic substituents, such as phthaloyl, succinyl or glutaryl group, and exhibited a good inhibitory capacity, with IC_{50} values between 0.8 and 15.6 μM, which were between 4 and 78 times lower than its precursor (**MA**) (see Table 8 and Chart 4). Chart 4 shows a comparative of the inhibitory effects of the acyl OA and MA derivatives on the HIV-1-protease, describing the ratio between the IC_{50} values of the triterpene precursors (**OA** and **MA**) and the IC_{50} values of their acyl derivatives (last column of Table 8).

3. Conclusions

The comparative study of the solution- and solid-phase semi-synthesis of the OA and MA derivatives showed that the solid-phase procedures were shorter, easier, cleaner, and with better yields than the corresponding solution processes, when using an aliphatic or an aromatic acyl group. In this solid-phase process, we determined a high degree of incorporation (above 90%) of **OA** and **MA** to the CTC resin, when coupling with 3 equiv of these acids.

The acyl OA and MA derivatives were tested for their anti-proliferative effects. Some of these derivatives (24%) displayed cytotoxic effects (low IC_{50}) as growth inhibitors against the b16f10 murine melanoma cells. These cytotoxic compounds showed apoptotic effects on the treated cells, with high levels of early and total apoptosis, reaching in several cases, values of apoptosis population up to 90% of the total cells. The study of the structure–activity relationship indicated that the more cytotoxic and pro-

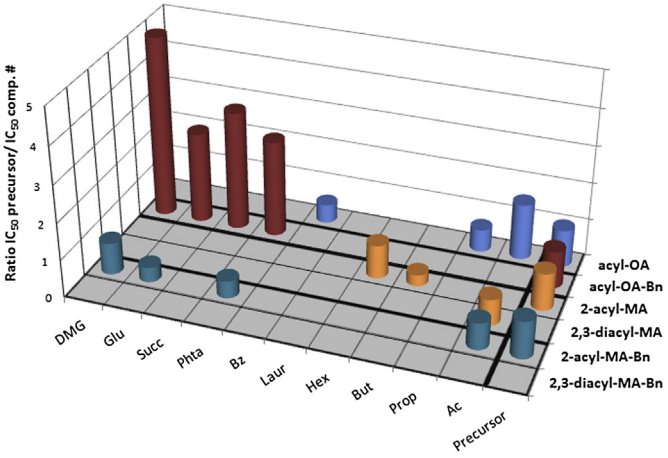


Chart 1. Comparison of the growth-inhibitory effects of the acyl OA and MA derivatives on b16f10 murine melanoma cells, describing the ratio between the IC_{50} values of the triterpene precursors (**1**–**4**) and the IC_{50} values of their cytotoxic acyl derivatives. DMG = 3,3-dimethylglutaryl, Glu = glutaryl, Succ = succinyl, Phta = phthaloyl, Bz = benzoyl, Laur = lauroyl, Hex = hexanoyl, But = butanoyl, Prop = propanoyl, Ac = acetyl, Precursors = **OA** (**1**), **MA** (**2**), **OA-Bn** (**3**), and **MA-Bn** (**4**).

Table 7
Flow-cytometry analysis of annexin V–FITC staining and PI accumulation after exposure of b16f10 murine melanoma cells to acyl OA and MA derivatives.

Comp. #	Conc.[a]	Population[b]				
		Normal	Early apoptosis	Late apoptosis	Total apoptosis	Necrosis
Control	0	77.9 ± 1.5	3.9 ± 0.4	9.8 ± 2.2	13.7 ± 2.7	4.0 ± 0.6
OA (**1**)	IC_{50}	44.9 ± 5.1	16.4 ± 2.3	28.2 ± 2.0	44.6 ± 4.3	10.1 ± 1.0
	$IC_{50} \times 2$	49.8 ± 6.4	29.1 ± 6.0	12.4 ± 1.6	41.5 ± 7.5	8.7 ± 1.4
5	IC_{50}	39.5 ± 6.5	14.4 ± 2.4	40.3 ± 5.8	54.7 ± 7.2	5.8 ± 1.4
	$IC_{50} \times 2$	46.3 ± 6.8	14.6 ± 0.6	33.4 ± 3.5	48.0 ± 4.1	5.7 ± 2.8
9	IC_{50}	24.3 ± 6.8	47.0 ± 0.0	27.4 ± 7.0	74.4 ± 7.0	1.3 ± 0.1
	$IC_{50} \times 2$	37.2 ± 8.9	44.3 ± 4.7	16.5 ± 4.3	60.8 ± 9.1	2.0 ± 0.1
13	IC_{50}	44.6 ± 1.9	14.0 ± 1.1	37.6 ± 0.5	51.6 ± 1.6	3.8 ± 0.4
	$IC_{50} \times 2$	31.6 ± 0.2	9.5 ± 0.4	50.5 ± 1.2	60.0 ± 1.6	8.5 ± 1.8
OA-Bn (**3**)	IC_{50}	67.9 ± 3.5	15.6 ± 1.2	12.2 ± 1.7	27.8 ± 0.8	4.3 ± 2.6
	$IC_{50} \times 2$	54.3 ± 5.4	23.0 ± 5.1	18.6 ± 2.1	41.6 ± 6.5	4.1 ± 1.5
24	IC_{50}	3.9 ± 0.3	64.3 ± 5.6	30.3 ± 5.2	94.6 ± 0.5	1.5 ± 0.2
	$IC_{50} \times 2$	3.7 ± 0.5	66.9 ± 2.8	28.5 ± 3.3	95.4 ± 0.5	1.0 ± 0.0
25	IC_{50}	16.9 ± 2.1	16.6 ± 2.8	55.5 ± 8.2	72.1 ± 5.5	11.1 ± 3.4
	$IC_{50} \times 2$	7.7 ± 2.5	74.0 ± 0.8	17.6 ± 3.3	91.6 ± 2.4	0.7 ± 0.0
26	IC_{50}	25.8 ± 4.5	20.6 ± 1.9	37.9 ± 1.9	58.4 ± 0.1	15.9 ± 4.4
	$IC_{50} \times 2$	8.1 ± 0.8	65.8 ± 0.1	24.6 ± 1.1	90.4 ± 1.0	1.6 ± 0.1
27	IC_{50}	8.3 ± 2.0	44.0 ± 4.4	42.6 ± 7.7	86.5 ± 3.3	5.3 ± 1.3
	$IC_{50} \times 2$	4.9 ± 0.6	52.8 ± 5.7	36.1 ± 5.6	88.8 ± 0.1	6.4 ± 0.7
MA (**2**)	IC_{50}	10.9 ± 1.7	20.8 ± 2.8	54.2 ± 3.5	74.9 ± 2.5	11.7 ± 0.5
	$IC_{50} \times 2$	8.3 ± 1.8	68.8 ± 3.9	21.0 ± 1.9	89.8 ± 2.5	2.0 ± 0.7
8	IC_{50}	31.4 ± 1.3	8.7 ± 0.0	52.9 ± 3.0	61.6 ± 3.0	7.1 ± 1.7
	$IC_{50} \times 2$	9.7 ± 5.6	29.6 ± 7.6	59.3 ± 6.5	88.9 ± 6.0	1.5 ± 0.5
30	IC_{50}	21.0 ± 8.2	55.6 ± 5.5	22.1 ± 2.0	77.7 ± 8.5	1.2 ± 0.3
	$IC_{50} \times 2$	10.2 ± 1.6	53.0 ± 2.9	30.0 ± 5.7	83.0 ± 2.8	6.8 ± 1.2
32	IC_{50}	34.8 ± 1.7	42.4 ± 3.3	19.0 ± 0.4	61.4 ± 3.6	3.9 ± 1.9
	$IC_{50} \times 2$	18.9 ± 0.5	62.7 ± 0.9	17.5 ± 0.5	80.2 ± 0.5	1.1 ± 0.0
MA-Bn (**4**)	IC_{50}	5.0 ± 0.6	84.2 ± 5.4	10.6 ± 4.7	94.7 ± 0.7	0.3 ± 0.1
	$IC_{50} \times 2$	11.3 ± 4.2	86.2 ± 5.0	2.2 ± 1.0	88.4 ± 4.1	0.1 ± 0.1
46	IC_{50}	12.9 ± 0.9	66.0 ± 0.9	20.0 ± 0.3	86.0 ± 0.6	1.2 ± 0.3
	$IC_{50} \times 2$	1.1 ± 0.1	71.3 ± 2.2	27.1 ± 2.1	98.4 ± 0.1	0.6 ± 0.0
58	IC_{50}	32.8 ± 4.7	42.2 ± 6.3	22.5 ± 1.4	64.7 ± 4.9	2.5 ± 0.2
	$IC_{50} \times 2$	2.9 ± 0.7	43.6 ± 3.7	46.2 ± 1.4	89.7 ± 2.3	7.5 ± 1.7
62	IC_{50}	73.3 ± 1.7	10.6 ± 1.7	14.6 ± 0.1	25.2 ± 1.6	1.6 ± 0.1
	$IC_{50} \times 2$	20.2 ± 4.3	59.1 ± 9.1	19.8 ± 1.2	78.9 ± 7.8	0.9 ± 0.6
64	IC_{50}	35.4 ± 4.5	16.5 ± 0.8	46.3 ± 4.2	62.8 ± 5.0	1.8 ± 0.5
	$IC_{50} \times 2$	2.2 ± 0.3	26.9 ± 3.5	65.3 ± 3.3	92.2 ± 0.2	5.6 ± 0.5

[a] Cell lines were treated at concentrations equal to its IC_{50} or $IC_{50} \times 2$ values. Values are expressed as means ± S.E.M. of three experiments in duplicate.
[b] Normal cells were annexin V^- PI^- early apoptotic cells were annexin V^+ PI^- whereas late apoptotic cells were annexin V^+ PI^+ total apoptosis (early apoptosis plus late apoptosis) were annexin V^+ and necrotic cells were annexin V^- PI^+.

apoptotic compounds had a benzyl group on the carboxylic group at C-28, and polar dicarboxylic fragments on the hydroxyl groups of the A-ring of the triterpene skeleton.

Most of the compounds that exhibited anti-proliferative effects also triggered ROS generation. Therefore, the apoptosis would probably be caused by the participation of an intrinsic apoptotic-route activation, and the mitochondrial disruption would be involved in the apoptotic response. Only four compounds (**24**, **25**, **26**, and **58**) did not release ROS, and these results could be related with the participation of a plausible extrinsic activation of the apoptosis mechanism. Further molecular studies are necessary to test these conclusions, but both mechanisms on the apoptosis induction by triterpenes compounds have been described in the literature.

A great number of acyl OA and MA derivatives (38%) were demonstrated to be outstanding inhibitors of the anti-HIV-1-protease, with IC_{50} values between 4 and 186 times lower than their non-acylated precursors. The most active acyl OA and MA derivatives had a polar dicarboxylic substituent at C-2 or at C-2 and C-3 of the triterpene skeleton.

The potent activities on the apoptosis-activation process and on the inhibition of the HIV-1-protease reached by some of the tested OA and MA acylated derivatives suggest that they could be used in the future as new, safe, and effective anticancer and/or antiviral drugs.

4. Experimental

4.1. General experimental chemical procedures

Measurements of NMR spectra (300.13 MHz ^1H and 75.47 MHz ^{13}C) were made in $CDCl_3$, CD_3OD, DMSO-d_6 or Pyridine-d_5 (which also provided the lock signal) using a VARIAN Inova unity (300 MHz ^1H NMR). The ^{13}C chemical shifts were assigned with the aid of distortionless enhancement by polarization transfer (DEPT) using a flip angle of 135°. IR spectra were recorded on a MATTSON SATELLITE FTIR spectrometer. Optical rotations were measured on a Perkin–Elmer 241 polarimeter at 25 °C. Purity of new compounds was determined by a WATERS ACQUITY UPLC system (ultra-performance liquid chromatography) coupled with a WATERS SYNAPT G2 HRMS spectrometer (high-resolution mass spectra) with ESI (electrospray ionization). Melting points (mp) were determined using a Kofler (Reichter) apparatus and were uncorrected. All reaction solvents and chromatography solvents were distilled prior to use. Commercially available reagents were used without further purification. Merck silica-gel 60 aluminium sheets (ref. 1.16835) were used for TLC, and spots were rendered visible by spraying with H_2SO_4–AcOH, followed by heating to 120 °C, and also visualized under UV at 254 nm. Merck silica-gel 60 (0.040–0.063 mm, ref. 1.09385) was used for flash chromatography. CH_2Cl_2 (Fisher, ref. D/1852/17), $CHCl_3$ (Fisher, ref. C/4960/17), or *n*-hexane (Merck, ref. 1.04374), with increasing amounts of Me_2CO (Fisher, ref. A/0600/17), MeOH (Fisher, ref. M/4000/17), or AcOEt (Fisher, ref. E/0900/17), were used as eluents (all the solvents had an analytical reagent-grade purity). RP-HPLC analyses were made with a WATERS C18 reverse-phase column (0.4 μm diameter of particle; 150 mm × 3.9 mm) with a flow rate of 1 mL/min, on a WATERS Liquid Chromatograph (HPLC), model ALLIANCE 2690 (Waters, USA). Wavelengths of 210 and 301 nm were selected for the purity analyses. The analyses were performed using a linear gradient of 30–0% of B in 30 min and maintained for 5 min without B, where A was CH_3CN containing 0.1% TFA and B was H_2O containing 0.1% TFA. CTC resin (1.27 mmol/g), Fmoc-amino acid derivatives, DIEA, DIPCDI, and DMF were purchased from different sources.

4.2. Isolation of OA (*1*) and MA (*2*)

OA and **MA** were isolated from solid olive-oil-production wastes, which were extracted successively in a Soxhlet with hexane and EtOAc. Hexane extracts were a mixture of **OA** and **MA** (80:20), whereas this relationship was (20:80) for the EtOAc extracts. Both products were purified from these mixtures by column chromatography over silica gel, eluting with a $CHCl_3$/MeOH or CH_2Cl_2/acetone mixtures of increasing polarity [1,2,72].

4.3. Synthesis of benzyl OA and MA derivatives

4.3.1. Benzyl oleanolate (*3*)

BnCl (418 μL) was added in a relationship of 2:1 to a solution of **OA** (**1**, 912 mg, 2 mmol) in DMF (8 mL) with K_2CO_3 (0.61 g). The reaction was stirred for 4 h at 55 °C. The mixture was diluted with water and extracted with DCM, and the organic layer dried with anhydrous Na_2SO_4. The solvent was removed under reduced

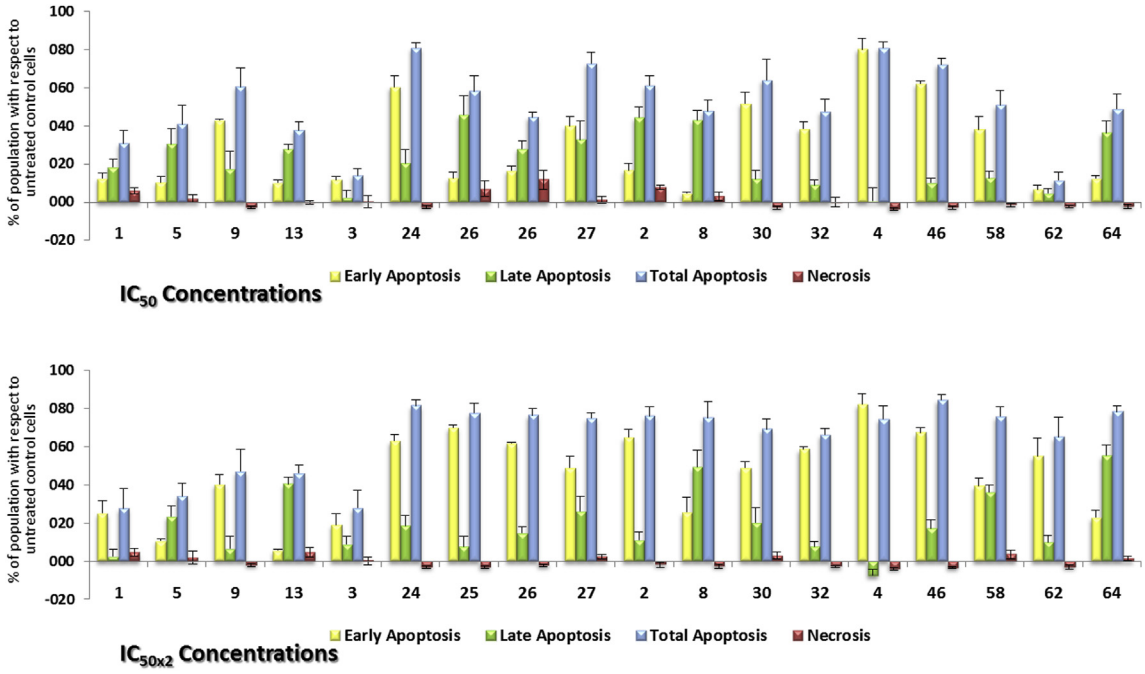


Chart 2. Flow-cytometry analysis of annexin V–FITC staining and PI accumulation after exposure of b16f10 murine melanoma cells to acyl OA and MA derivatives for 72 h with respect to untreated control cells. Cell lines were treated at concentrations equal to its IC_{50} or $IC_{50} \times 2$ values. Early apoptotic cells (yellow bars) were annexin V^+ PI^-, whereas late apoptotic cells (green bars) were annexin V^+ PI^+, total apoptosis (blue bars) were annexin V^+, and necrotic cells (red bars) were annexin V^- PI^+. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pressure, and the residue was purified by column chromatography using DCM/acetone (10:1) to give **3** as a white solid (710 mg, 83%) [55].

4.3.2. Benzyl maslinate (*4*)

BnCl (418 μL) was added in a relationship of 2:1 to a solution of **MA** (**2**, 944 mg, 2 mmol) in DMF (8 mL) with K_2CO_3 (0.61 g). The reaction was stirred for 4 h at 55 °C. The mixture was diluted with water and extracted with DCM, and the organic layer dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by column chromatography using DCM/acetone (10:1) to give **4** as a white solid (690 mg, 81%) [32].

4.4. Acetylations of *OA* and *MA*

4.4.1. Acetylations of *OA* with Ac_2O

Ac_2O (1 mL) was added slowly to a solution of **OA** (**1**, 1368 mg, 3 mmol) in pyridine (10 mL). The reaction was performed under different conditions (see Table 1). Cold water was added to the different mixtures, and afterwards these were extracted with DCM, and the organic layers were dried with anhydrous Na_2SO_4. In all cases, the solvent was removed under reduced pressure and the residue was purified by column chromatography using DCM/acetone (10:1) to give **5** as a white solid (percentages in Table 1) [56].

4.4.2. Acetylations of *MA* with Ac_2O

Ac_2O (2 mL) was added slowly to a solution of **MA** (**2**, 1416 mg, 3 mmol) in pyridine (10 mL). The reaction was performed under different conditions (see Table 2). Cold water was added to the different mixtures, and afterwards these were extracted with DCM, and the organic layers were dried with anhydrous Na_2SO_4. In all cases, the solvent was removed under reduced pressure and the residue was purified by column chromatography using DCM/acetone (10:1) to give **6**, **7**, and **8** as white solids (percentages in Table 2) [57].

4.5. General method for acylation of *1*–*4* in solution-phase

The corresponding acid anhydrides (**I**–**X**) were added respectively, in a relationship of 4:1 (anhydride:hydroxyl group), to a solution of compounds (**1**–**4**) in pyridine. The reactions were stirred for 24 h at rt. In all cases, cold water was added to the mixture and afterwards was extracted with DCM; the organic layer was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography using DCM/acetone or hexane/ethyl acetate.

4.5.1. Acylation of *OA* with propanoic anhydride

Propanoic anhydride (113 μL) was added slowly to a solution of **OA** (100 mg, 0.219 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **9** as a white solid (89 mg, 83%), mp 245–247 °C; $[\alpha]_D$ + 71 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3388, 3015, 2829 and 1720; δ_H ($CDCl_3$) 5.26 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 4.48 (dd, 1H, $J_1 = 5.5$, $J_2 = 10.5$ Hz), 2.80 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.9$ Hz), 2.30 (q, 2H, $J = 6.5$ Hz), 1.11, 0.92, 0.91, 0.88, 0.84, 0.83, 0.73 (s, 3H each); δ_C ($CDCl_3$) 183.8, 174.5, 143.8, 122.8, 80.9, 55.5, 47.8, 46.8, 46.1, 41.8, 41.2, 39.5, 38.3 (2C), 37.2, 34.0, 33.3, 32.8, 32.7, 30.9, 29.9, 28.8, 28.3, 27.9, 26.1, 23.8 (2C), 23.1, 18.4, 17.3, 16.9, 15.6, 9.6; ESI-HRMS *m*/*z* calcd for $C_{33}H_{53}O_4$ [M + 1] 513.3944, found 513.3948.

4.5.2. Acylation of *OA* with butanoic anhydride

Butanoic anhydride (143 μL) was added slowly to a solution of **OA** (100 mg, 0.219 mmol) in pyridine (2 mL). The mixture was treated as described in the general method to give **10** as a white solid (96 mg, 87%), mp 215–217 °C; $[\alpha]_D$ + 79 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3379, 2989, 2815 and 1699; δ_H ($CDCl_3$) 5.26 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 4.48 (dd, 1H, $J_1 = 5.3$, $J_2 = 10.5$ Hz), 2.80

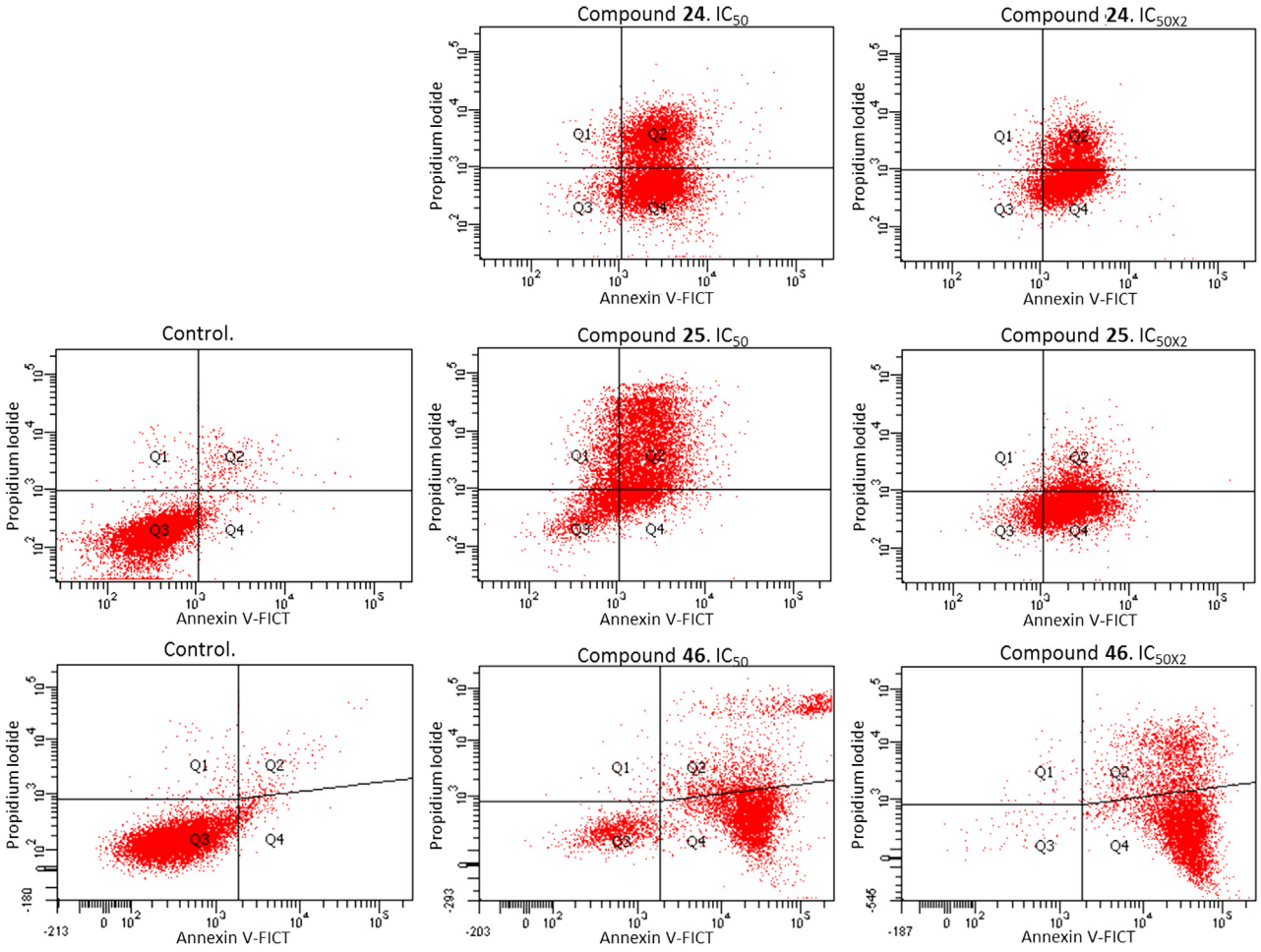


Fig. 6. Diagrams of annexin V/propidium iodide flow-cytometry for **24**, **25**, and **46**. Q1 quadrant (necrotic cells). Q2 quadrant (late apoptotic cells). Q3 quadrant (normal cells). Q4 quadrant (early apoptotic cells). Left diagrams IC_{50} concentrations. Right diagrams $IC_{50} \times 2$ concentrations.

(dd, 1H, $J_1 = 4.3$, $J_2 = 13.9$ Hz), 2.30 (t, 2H, $J = 6.5$ Hz), 1.11, 0.92, 0.91, 0.88, 0.84, 0.83, 0.73 (s, 3H each); δ_C ($CDCl_3$) 184.0, 173.7, 143.8, 122.8, 80.8, 55.5, 47.8, 46.8, 46.1, 41.8, 41.2, 39.5, 38.3 (2C), 37.2, 37.0, 34.0, 33.3, 32.8, 32.7, 30.9, 29.9, 28.3, 27.9, 26.1, 23.8, 23.6, 23.1, 18.9, 18.4, 17.3, 16.9, 16.5, 13.9; ESI-HRMS *m/z* calcd for $C_{34}H_{55}O_4$ [M + 1] 527.4100, found 527.4101.

4.5.3. Acylation of **OA** with hexanoic anhydride

Hexanoic anhydride (203 μL) was added slowly to a solution of **OA** (100 mg, 0.219 mmol) in pyridine (2 mL). The mixture was treated as described in the general method to give **11** as a white solid (104 mg, 90%), mp 159–161; $[\alpha]_D$ + 69 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3383, 2979, 2832 and 1679; δ_H ($CDCl_3$) 5.26 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 4.48 (dd, 1H, $J_1 = 5.5$, $J_2 = 10.5$ Hz), 2.80 (dd, 1H, $J_1 = 4.5$, $J_2 = 13.9$ Hz), 2.27 (t, 2H, $J = 6.6$ Hz), 1.11, 0.92, 0.91, 0.89, 0.84, 0.83, 0.74 (s, 3H each); δ_C ($CDCl_3$) 183.8, 173.9, 143.8, 122.8, 80.8, 55.6, 47.8, 46.8, 46.1, 41.9, 41.3, 39.6, 38.3, 37.9, 37.2, 35.1, 34.1, 33.3, 32.8, 32.7, 31.6, 30.9, 29.9, 28.3, 27.9, 26.1, 25.1, 23.8, 23.7, 23.2, 22.5, 18.4, 17.3, 17.0, 15.6, 14.1; ESI-HRMS *m/z* calcd for $C_{36}H_{59}O_4$ [M + 1] 555.4413, found 555.4421.

4.5.4. Acylation of **OA** with lauric anhydride

Lauric anhydride (336 mg, 0.876 mmol) was added slowly to a solution of **OA** (100 mg, 0.219 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **12** as a colourless oil (97 mg, 73%), $[\alpha]_D$ + 37 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3379, 2963, 2840 and 1681; δ_H ($CDCl_3$) 5.26 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 4.80 (dd, 1H, $J_1 = 5.4$, $J_2 = 10.4$ Hz), 2.80 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.9$ Hz), 2.27 (t, 2H, $J = 6.5$ Hz), 1.11, 0.92, 0.91, 0.89, 0.86, 0.84, 0.83 (s, 3H each); δ_C ($CDCl_3$) 183.2, 174.0, 143.8, 122.8, 80.8, 55.6, 47.8, 46.8, 46.1, 41.9, 41.3, 39.6, 38.3, 38.0, 37.9, 35.1, 34.0, 33.3, 32.8, 32.7, 32.2, 30.9, 29.8 (4C), 29.7, 29.5, 28.3, 27.9, 26.2, 25.4, 23.7 (2C), 23.6, 23.2, 22.9, 18.4, 17.3, 17.0, 15.6, 14.3; ESI-HRMS *m/z* calcd for $C_{42}H_{71}O_4$ [M + 1] 639.5361, found 639.5352.

4.5.5. Acylation of **OA** with benzoic anhydride

Benzoic anhydride (166 μL) was added slowly to a solution of **OA** (100 mg, 0.219 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **13** as a white solid (81 mg, 69%), mp 153–155 °C; $[\alpha]_D$ + 36 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3390, 3001, 2840 and 1689; δ_H ($CDCl_3$) 7.42 (m, 5H), 5.28 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 4.70 (dd, 1H, $J_1 = 5.5$, $J_2 = 10.5$ Hz), 2.80 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.8$ Hz), 1.14, 1.00, 0.98, 0.93, 0.90, 0.83, 0.77 (s, 3H each); δ_C ($CDCl_3$) 183.1, 166.5, 143.8, 132.9 (2C), 131.2 (2C), 129.8, 128.5, 122.8, 81.8, 55.6, 47.8, 46.8, 46.1, 41.9, 41.3, 39.6, 38.4 (2C), 37.3, 34.1, 32.9, 32.7 (2C), 30.9, 29.9, 28.5, 27.9, 26.2, 23.8, 23.7, 23.2, 18.5, 17.3, 17.2, 15.7; ESI-HRMS *m/z* calcd for $C_{37}H_{53}O_4$ [M + 1] 561.3937, found 561.3944.

4.5.6. Acylation of **OA** with phthalic anhydride

Phthalic anhydride (130 mg, 0.876 mmol) was added slowly to a solution of **OA** (100 mg, 0.219 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for

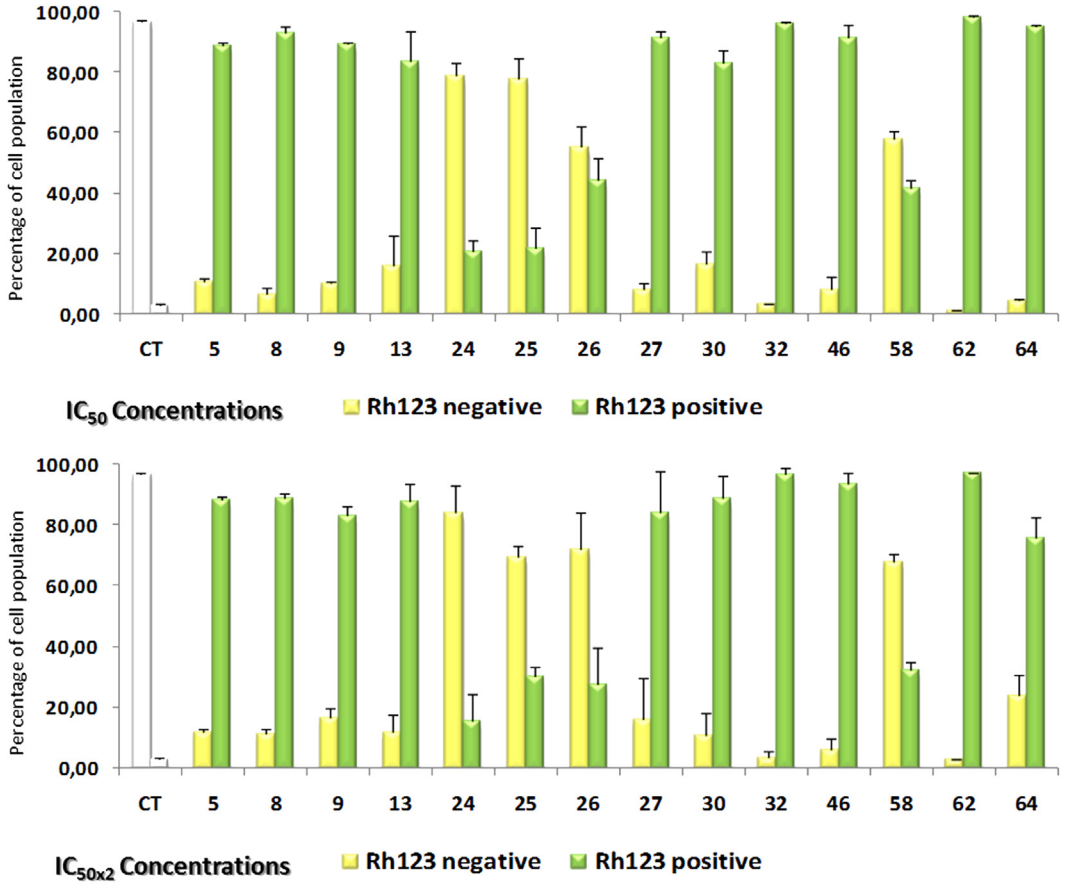


Chart 3. Flow-cytometry analysis of rhodamine 123 and PI staining after exposure of b16f10 murine melanoma cells to acyl OA and MA derivatives for 72 h with respect to the control untreated cells. Cell lines were treated at concentrations equal to its IC_{50} or $IC_{50} \times 2$ values. ROS-negative cells (yellow bars) were rhodamine 123^- with PI^+ or PI^-. ROS-positive cells (green bars) were rhodamine 123^+ with PI^+ or PI^-. Values are expressed as means ± S.E.M. of three experiments in duplicate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

acylation to give **14** as a white solid (123 mg, 93%), mp 166–168 °C; $[\alpha]_D$ + 26 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3380, 3053, 2855 and 1699; δ_H ($CDCl_3$) 7.80–7.50 (m, 4H), 5.26 (dd, 1H, $J_1 = J_2 = 3.6$ Hz), 4.71 (dd, 1H, $J_1 = 5.5$, $J_2 = 10.3$ Hz), 2.81 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.9$ Hz), 1.12, 0.91, 0.91, 0.89, 0.89, 0.83, 0.73 (s, 3H each); δ_C ($CDCl_3$) 185.0, 172.8, 168.0, 143.8, 133.8, 132.2, 130.9, 130.3, 130.0, 129.2, 122.8, 83.4, 55.6, 47.7, 46.7, 46.1, 41.8, 41.1, 39.5, 38.2 (2C), 38.1, 34.0, 32.8, 33.3, 32.7, 30.9, 29.7, 28.4, 27.9, 26.2, 23.8, 23.6, 23.0, 18.4, 17.5, 17.0, 15.7; ESI-HRMS *m*/*z* calcd for $C_{38}H_{53}O_6$ [M + 1] 605.3850, found 605.3842.

4.5.7. Acylation of **OA** with succinic anhydride

Succinic anhydride (88 mg, 0.876 mmol) was added slowly to a solution of **OA** (100 mg, 0.219 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **15** as a white solid (57 mg, 47%), mp 190–192 °C; $[\alpha]_D$ + 49 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3394, 3018, 2854 and 1712; δ_H ($CDCl_3$) 5.25 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 4.51 (dd, 1H, $J_1 = 5.5$, $J_2 = 10.5$ Hz), 2.80 (dd, 1H, $J_1 = 4.4$, $J_2 = 13.8$ Hz), 2.70–2.60 (m, 4H), 1.10, 0.92, 0.90, 0.88, 0.83, 0.81, 0.72 (s, 3H each); δ_C ($CDCl_3$) 184.9, 178.6, 171.8, 143.8, 122.8, 81.7, 55.7, 47.7, 46.8, 46.0, 41.7, 41.1, 39.5, 38.3, 38.2, 37.2, 34.0, 33.3, 32.7, 32.6, 30.9, 29.7, 29.6, 29.4, 28.3, 27.9, 26.2, 23.8, 23.6, 23.0, 18.5, 17.2, 17.0, 15.7; ESI-HRMS *m*/*z* calcd for $C_{34}H_{53}O_6$ [M + 1] 557.3844, found 557.3842.

4.5.8. Acylation of **OA** with glutaric anhydride

Glutaric anhydride (100 mg, 0.876 mmol) was added slowly to a solution of **OA** (100 mg, 0.219 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **16** as a white solid (73 mg, 59%), mp 190–192 °C; $[\alpha]_D$ + 49 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3379, 3018, 2847 and 1700; δ_H ($CDCl_3$) 5.25 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 4.49 (dd, 1H, $J_1 = 5.6$, $J_2 = 10.6$ Hz), 2.80 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.9$ Hz), 2.45–2.35 (m, 6H), 1.10, 0.92, 0.91, 0.88, 0.85, 0.82, 0.71 (s, 3H each); δ_C ($CDCl_3$) 185.0, 179.6, 172.7, 143.8, 122.8, 81.3, 55.5, 47.7, 46.8, 46.0, 41.7, 41.1, 39.5, 38.2, 38.0, 37.2, 34.0, 33.3 (2C), 33.0, 32.7, 32.6, 30.9, 29.7, 28.4, 27.9, 26.2, 23.8, 23.6, 23.1, 20.4, 18.4, 17.2, 17.1, 15.6; ESI-HRMS *m*/*z* calcd for $C_{35}H_{55}O_6$ [M + 1] 571.3999, found 571.4005.

4.5.9. Acylation of **OA** with 3,3-dimethylglutaric anhydride

3,3-Dimethylglutaric anhydride (124 mg, 0.876 mmol) was added slowly to a solution of **OA** (100 mg, 0.219 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **17** as a white solid (81 mg, 62%), mp 220–222 °C; $[\alpha]_D$ + 9 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3399, 3022, 2845 and 1704; δ_H ($CDCl_3$) 5.25 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 4.50 (dd, 1H, $J_1 = 5.5$, $J_2 = 10.5$ Hz), 2.81 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.7$ Hz), 2.70–2.25 (m, 4H), 1.13, 1.10, 1.07, 0.93, 0.91, 0.88, 0.85, 0.80, 0.70 (s, 3H each); δ_C ($CDCl_3$) 184.9, 178.0, 172.1, 143.7, 122.9, 81.2, 55.4, 47.5, 46.8, 46.1, 45.4, 44.4, 41.6, 41.0, 39.5, 38.0 (2C), 37.3, 34.0, 34.0, 32.7 (2C), 32.6, 30.9, 29.9, 28.8, 28.6 (2C), 28.0, 26.2, 23.8, 23.6, 22.9, 18.5, 17.6, 17.3, 15.8; ESI-HRMS *m*/*z* calcd for $C_{37}H_{59}O_6$ [M + 1] 599.4312, found 599.4301.

4.5.10. Acylation of **OA-Bn** with Ac_2O

Ac_2O (69 μL) was added slowly to a solution of **OA-Bn** (100 mg, 0.183 mmol) in pyridine (2 mL). The mixture was treated as in the

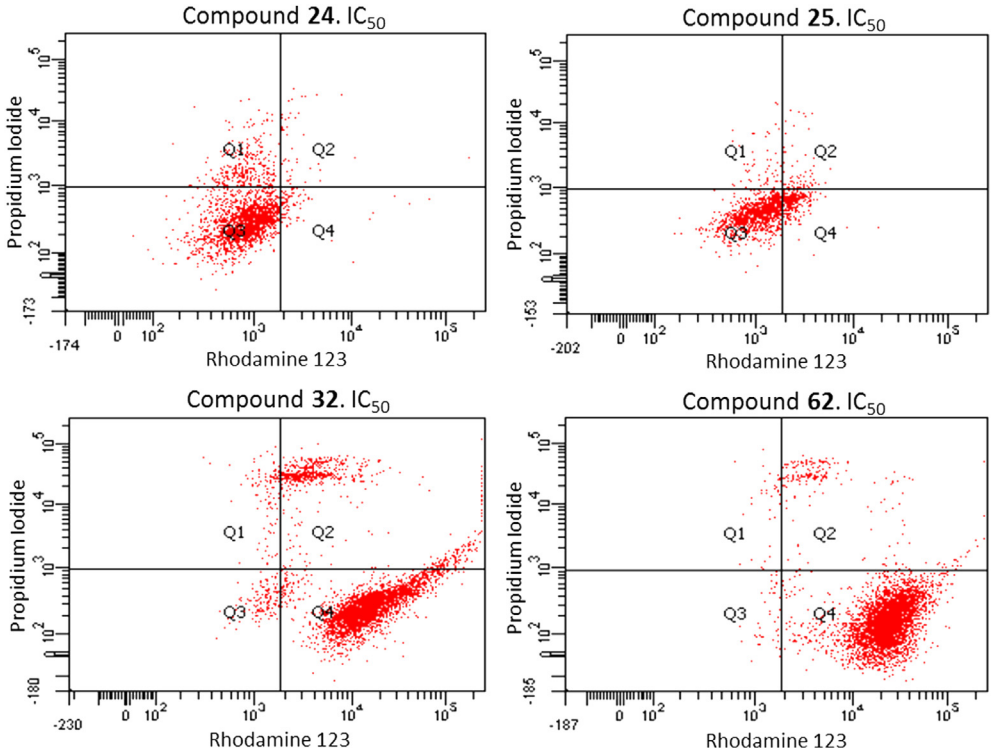


Fig. 7. Diagram of rhodamine 123/propidium iodide flow-cytometry, for the compounds with minor (**24** and **25**) and major (**32** and **62**) Rh123 staining. The right quadrants of each diagram (Q2 and Q4) represent cells positive stained with Rh123.

general method for acylation above to give **18** as a white solid (95 mg, 89%), mp 173–175 °C; $[\alpha]_D$ + 47 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3376, 3001, 2865 and 1710; δ_H ($CDCl_3$) 7.31 (m, 5H), 5.26 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 5.05 (AB system, 2H, $J = 12.0$ Hz), 4.46 (dd, 1H, $J_1 = 5.3$, $J_2 = 10.4$ Hz), 2.98 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.9$ Hz), 2.02 (s, 3H), 1.10, 0.91, 0.90, 0.88, 0.84, 0.83, 0.59 (s, 3H each); δ_C ($CDCl_3$) 183.8, 171.2, 143.9, 136.7, 128.6, 128.2 (2C), 128.1 (2C), 122.6, 81.1, 66.1, 55.5, 47.8, 47.0, 46.1, 41.9, 41.6, 39.5, 38.4, 37.9,

Table 8
HIV-1-protease inhibition effects of OA and MA derivatives.

Comp. #	R_1	R_2	R_3	IC_{50} (μM) (% HIV-1-protease activity inhibition)[a]	IC_{50} OA/IC_{50} comp. #[b]
OA (**1**)	–	H	H	57.7 ± 3.9	1.0
5	–	Acetyl	H	3.4 ± 0.4	17.0
14	–	Phthaloyl	H	0.31 ± 0.03	186.1
16	–	Glutaryl	H	0.56 ± 0.16	103.0
17	–	3,3-Dimethylglutaryl	H	0.70 ± 0.07	82.4
24	–	Phthaloyl	Bn	0.4 ± 0.1	144.3
					IC_{50} MA/IC_{50} comp. #[b]
MA (**2**)	H	H	H	62.7 ± 4.9	1.0
31	Butanoyl	Butanoyl	H	31.2 ± 5.9	2.0
32	Hexanoyl	H	H	124.4 ± 5.8	0.5
38	Phthaloyl	H	H	14.2 ± 2.1	4.4
39	Phthaloyl	Phthaloyl	H	2.3 ± 0.3	27.3
40	Succinyl	H	H	3.5 ± 0.5	17.9
41	Succinyl	Succinyl	H	1.9 ± 0.4	33.0
42	Glutaryl	H	H	3.2 ± 0.3	19.6
43	Glutaryl	Glutaryl	H	1.4 ± 0.3	44.8
44	3,3-Dimethylglutaryl	H	H	14.3 ± 1.8	4.4
45	3,3-Dimethylglutaryl	3,3-Dimethylglutaryl	H	27.5 ± 3.3	2.3
50	Butanoyl	H	Bn	226 ± 24	0.3
53	Hexanoyl	Hexanoyl	Bn	150 ± 8	0.4
55	Lauroyl	Lauroyl	Bn	73 ± 4	0.9
58	Phthaloyl	H	Bn	0.8 ± 0.1	78.4
59	Phthaloyl	Phthaloyl	Bn	1.9 ± 0.5	33.0
60	Succinyl	H	Bn	15.6 ± 2.9	4.0
61	Succinyl	Succinyl	Bn	5.2 ± 0.7	12.1
63	Glutaryl	Glutaryl	Bn	3.9 ± 0.3	16.1

[a] The IC_{50} values were calculated based on: $V_{inh} = V_0 \cdot IC_{50}/([I] + IC_{50})$. The percentage of protease-activity inhibition was calculated according to: % inhibition = $[1 - (V_{inh}/V_0)] \cdot 100$. All assays were performed three times in triplicates.
[b] The last column represents the ratio between the IC_{50} of each precursor (**OA** or **MA**) and the IC_{50} of the related acyl derivatives.

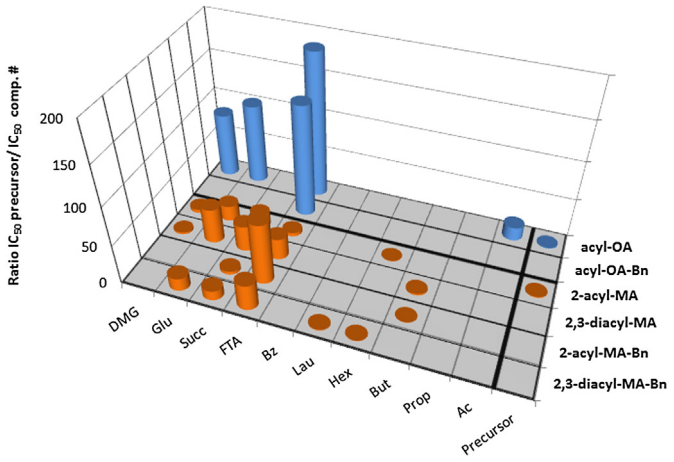


Chart 4. Comparative of the inhibitory effects of the acyl OA and MA derivatives on the HIV-1-protease, describing the ratio between the IC_{50} values of the triterpene precursors (**OA** and **MA**) and the IC_{50} values of their acyl derivatives. DMG = 3,3-dimethylglutaryl, Glu = glutaryl, Succ = succinyl, Phta = phthaloyl, Bz = benzoyl, Laur = lauroyl, Hex = hexanoyl, But = butanoyl, Prop = propanoyl, Ac = acetyl, precursors = **OA** (**1**) and **MA** (**4**).

37.1, 34.1, 33.3, 32.9 (2C), 30.9, 28.3, 27.8 (2C), 26.0, 23.9, 23.6, 23.3, 21.5, 18.4, 17.1, 16.9, 15.6; ESI-HRMS *m/z* calcd for $C_{39}H_{57}O_4$ [M + 1] 589.4257, found 589.4252.

*4.5.11. Acylation of **OA-Bn** with propanoic anhydride*

Propanoic anhydride (94 μL) was added slowly to a solution of **OA-Bn** (100 mg, 0.183 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **19** as a white solid (97 mg, 85%), mp 153–155 °C; $[\alpha]_D$ + 47 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3391, 2989, 2855 and 1690; δ_H ($CDCl_3$) 7.31 (m, 5H), 5.26 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.05 (AB system, 2H, $J = 12.0$ Hz), 4.47 (dd, 1H, $J_1 = 5.5$, $J_2 = 10.5$ Hz), 2.89 (dd, 1H, $J_1 = 4.2$, $J_2 = 13.7$ Hz), 2.30 (q, 2H, $J = 6.5$ Hz), 0.11, 0.92, 0.91, 0.89, 0.88, 0.83, 0.59 (s, 3H each); δ_C ($CDCl_3$) 177.7, 174.5, 143.9, 136.7, 128.6, 128.2 (2C), 128.1 (2C), 122.7, 80.8, 66.1, 55.5, 47.8, 47.0, 46.1, 41.9, 41.6, 40.0, 38.4, 38.0, 37.2, 34.1, 33.3, 32.9, 32.6, 30.9, 28.3 (2C), 27.9, 26.1, 23.9, 23.6, 23.3, 18.8, 18.4, 17.1, 16.9, 15.5, 13.9; ESI-HRMS *m/z* calcd for $C_{40}H_{59}O_4$ [M + 1] 603.4413, found 603.4439.

*4.5.12. Acylation of **OA-Bn** with butanoic anhydride*

Butanoic anhydride (119 μL) was added slowly to a solution of **OA-Bn** (100 mg, 0.183 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **20** as a white solid (108 mg, 93%), mp 108–110 °C; $[\alpha]_D$ + 40 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3387, 2982, 2843 and 1699; δ_H ($CDCl_3$) 7.31 (m, 5H), 5.26 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.05 (AB system, 2H, $J = 12.0$ Hz), 4.48 (dd, 1H, $J_1 = 5.5$, $J_2 = 10.5$ Hz), 2.89 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.8$ Hz), 2.25 (t, 2H, $J = 6.4$ Hz), 1.11, 0.90, 0.89, 0.88, 0.84, 0.83, 0.59 (s, 3H each); δ_C ($CDCl_3$) 177.6, 173.6, 143.9, 136.6, 128.5, 128.2 (2C), 128.1 (2C), 122.6, 80.7, 66.1, 55.5, 47.7, 46.9, 46.0, 41.9, 41.6, 39.5, 38.3 (2C), 36.9, 34.1, 33.3, 32.9, 32.6, 30.9, 28.8, 28.2, 27.8, 26.0, 23.8 (2C), 23.6, 23.3, 18.8, 18.4, 17.1, 16.9, 15.5, 13.9; ESI-HRMS *m/z* calcd for $C_{41}H_{61}O_4$ [M + 1] 617.4584, found 617.4570.

*4.5.13. Acylation of **OA-Bn** with hexanoic anhydride*

Hexanoic anhydride (169 μL) was added slowly to a solution of **OA-Bn** (100 mg, 0.183 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **21** as a white solid (111 mg, 90%), mp 73–75 °C; $[\alpha]_D$ + 49 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3360, 2989, 2845 and 1708; δ_H ($CDCl_3$) 7.32 (m, 5H), 5.27 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.05 (AB system, 2H, $J = 12.0$ Hz), 4.47 (dd, 1H, $J_1 = 5.5$, $J_2 = 10.6$ Hz), 2.89 (dd, 1H, $J_1 = 4.4$, $J_2 = 13.9$ Hz), 2.27 (t, 2H, $J = 6.5$ Hz), 1.11, 0.90, 0.89, 0.88, 0.87, 0.83, 0.59 (s, 3H each); δ_C ($CDCl_3$) 177.6, 173.9, 143.9, 136.7, 128.6, 128.2 (2C), 128.1 (2C), 122.7, 80.8, 66.1, 55.5, 47.8, 47.0, 46.1, 41.9, 41.6, 39.6, 38.4, 38.0, 37.1, 35.0, 34.1, 33.3, 32.9, 32.6, 31.6, 30.9, 28.3, 28.1, 27.9, 26.1, 23.9, 23.8, 23.6, 23.3, 22.5, 18.4, 17.1, 17.0, 15.5, 14.1; ESI-HRMS *m/z* calcd for $C_{43}H_{65}O_4$ [M + 1] 645.4883, found 645.4861.

*4.5.14. Acylation of **OA-Bn** with lauric anhydride*

Lauric anhydride (280 mg, 0.732 mmol) was added slowly to a solution of **OA-Bn** (100 mg, 0.183 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **22** as a colourless oil (126 mg, 91%), $[\alpha]_D$ + 24 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3368, 2978, 2861 and 1712; δ_H ($CDCl_3$) 7.33 (m, 5H), 5.27 (dd, 1H, dd, $J_1 = J_2 = 3.6$ Hz), 5.06 (AB system, 2H, $J = 12.0$ Hz), 4.47 (dd, 1H, $J_1 = 5.6$, $J_2 = 10.5$ Hz), 2.90 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.9$ Hz), 2.30 (t, 2H, $J = 6.6$ Hz), 1.11, 0.90, 0.89, 0.88, 0.86, 0.84, 0.59 (s, 3H each); δ_C ($CDCl_3$) 177.6, 173.8, 143.9, 136.6, 128.6, 128.2 (2C), 128.1 (2C), 122.6, 80.7, 66.1, 55.5, 47.7, 46.9, 46.1, 41.9, 41.6, 39.5, 38.3, 37.9, 37.1, 35.0, 34.1, 33.3, 32.9, 32.6, 32.1, 30.9, 29.8 (3C), 29.5 (2C), 29.4, 28.3 (2C), 27.8, 26.0, 23.8 (2C), 23.6, 23.3, 22.9, 18.4, 17.1, 17.0, 15.5, 14.1; ESI-HRMS *m/z* calcd for $C_{49}H_{77}O_4$ [M + 1] 729.5822, found 729.5826.

*4.5.15. Acylation of **OA-Bn** with benzoic anhydride*

Benzoic anhydride (138 μL) was added slowly to a solution of **OA-Bn** (100 mg, 0.183 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **23** as a colourless oil (90 mg, 73%), $[\alpha]_D$ + 31 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3380, 3015, 2849 and 1699; δ_H ($CDCl_3$) 7.98–7.33 (m, 5H), 7.15 (m, 5H), 5.12 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.06 (AB system, 2H, $J = 12.0$ Hz), 4.55 (dd, 1H, $J_1 = 5.3$, $J_2 = 10.5$ Hz), 2.73 (dd, 1H, $J_1 = 4.2$, $J_2 = 13.7$ Hz), 0.96, 0.83, 0.83, 0.87, 0.93, 0.72, 0.45 (s, 3H each); δ_C ($CDCl_3$) 177.6, 166.5, 143.8, 134.7, 132.9 (2C), 130.7 (2C), 129.7, 129.1, 128.6, 128.2 (2C), 128.1 (2C), 122.6, 81.8, 66.1, 55.6, 47.8, 47.0, 46.1, 41.9, 41.6, 40.0, 38.3, 37.2 (2C), 34.1, 32.9, 33.3, 32.6, 30.9, 28.4, 27.8, 26.1, 23.8 (2C), 23.6, 23.3, 18.4, 17.2, 17.1, 15.6; ESI-HRMS *m/z* calcd for $C_{44}H_{59}O_4$ [M + 1] 651.4413, found 651.4404.

*4.5.16. Acylation of **OA-Bn** with phthalic anhydride*

Phthalic anhydride (108 mg, 0.732 mmol) was added slowly to a solution of **OA-Bn** (100 mg, 0.183 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **24** as a colourless oil (107 mg, 82%), $[\alpha]_D$ + 48 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3376, 3060, 2842 and 1711; δ_H ($CDCl_3$) 8.02–7.56 (m, 4H), 7.33 (m, 5H), 5.27 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.05 (AB system, 2H, $J = 12.0$ Hz), 4.77 (dd, 1H, $J_1 = 5.5$, $J_2 = 10.5$ Hz), 2.89 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.9$ Hz), 1.12, 0.94, 0.90, 0.89, 0.89, 0.85, 0.59 (s, 3H each); δ_C ($CDCl_3$) 177.7, 171.0, 168.1, 143.8, 136.7, 133.3, 132.1, 131.9, 131.1, 130.4, 129.4, 128.6, 128.2 (2C), 128.1 (2C), 122.6, 83.1, 66.1, 55.7, 47.8, 47.0, 46.1, 42.0, 41.6, 39.6, 38.4, 38.2, 37.2, 34.1, 32.9, 33.3, 32.6, 30.9, 28.4, 27.9, 27.8, 26.1, 23.9, 23.7, 23.2, 18.4, 17.1, 17.0, 15.2; ESI-HRMS *m/z* calcd for $C_{45}H_{59}O_6$ [M + 1] 695.4331 found 695.4312.

*4.5.17. Acylation of **OA-Bn** with succinic anhydride*

Succinic anhydride (73 mg, 0.732 mmol) was added slowly to a solution of **OA-Bn** (100 mg, 0.183 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **25** as a colourless oil (62 mg, 51%), $[\alpha]_D$ + 38 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3377, 3020, 2859 and 1720; δ_H ($CDCl_3$) 7.31 (m, 5H), 5.26 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 5.05 (AB system, 2H, $J = 12.0$ Hz), 4.48 (dd, 1H, $J_1 = 5.4$, $J_2 = 10.6$ Hz), 2.89 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.8$ Hz), 2.70–2.40 (m, 4H), 1.10, 0.90, 0.88, 0.88,

0.83, 0.83, 0.59 (s, 3H each); δ_C ($CDCl_3$) 178.4, 177.7, 172.9, 143.8, 136.6, 128.6, 128.2 (2C),128.1 (2C), 122.6, 81.7, 66.1, 55.5, 47.7, 46.9, 46.0, 41.9, 41.6, 39.5, 38.3, 37.9, 37.1, 34.0, 33.3, 32.8, 32.6, 30.9, 29.6, 29.5, 29.3, 28.2, 27.8, 26.0, 23.9, 23.6, 23.2, 18.4, 17.1, 16.9, 15.5; ESI-HRMS *m/z* calcd for $C_{41}H_{59}O_6$ [M + 1] 647.4332, found 647.4312.

4.5.18. Acylation of **OA-Bn** with glutaric anhydride

Glutaric anhydride (84 mg, 0.732 mmol) was added slowly to a solution of **OA-Bn** (100 mg, 0.183 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **26** as a colourless oil (84 mg, 67%), $[\alpha]_D$ + 26 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3378, 3033, 2867 and 1715; δ_H ($CDCl_3$) 7.31 (m, 5H), 5.26 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.05 (AB system, 2H, $J = 12.0$ Hz), 4.48 (dd, 1H, $J_1 = 5.5$, $J_2 = 10.5$ Hz), 2.89 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.9$ Hz), 2.42–2.34 (m, 6H), 1.10, 0.90, 0.88, 0.88, 0.83, 0.83, 0.59 (s, 3H each); δ_C ($CDCl_3$) 178.4, 177.7, 172.9, 143.9, 136.6, 128.6, 128.2 (2C), 128.1 (2C), 122.6, 81.3, 66.1, 55.5, 47.7, 47.0, 46.1, 41.9, 41.6, 39.5, 38.3, 37.9, 37.1, 34.1, 33.9 (2C), 33.3, 32.9, 32.6, 30.9, 28.3, 27.8, 27.8, 26.0, 23.9, 23.6, 23.3, 20.3, 18.4, 17.1, 17.0, 15.6; ESI-HRMS *m/z* calcd for $C_{42}H_{61}O_6$ [M + 1] 661.4486, found 661.4468.

4.5.19. Acylation of **OA-Bn** with 3,3-dimethylglutaric anhydride

3,3-Dimethylglutaric anhydride (105 mg, 0.732 mmol) was added slowly to a solution of **OA-Bn** (100 mg, 0.183 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **27** as a colourless oil (84 mg, 65%); $[\alpha]_D$ + 20 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3390, 3021, 2865 and 1698; δ_H ($CDCl_3$) 7.31 (m, 5H), 5.25 (dd, 1H, $J_1 = J_2 = 3.6$ Hz), 5.05 (AB system, 2H, $J = 12.0$ Hz), 4.50 (dd, 1H, $J_1 = 5.4$ Hz, $J_2 = 10.3$ Hz), 2.81 (dd, 1H, $J_1 = 4.4$, $J_2 = 13.8$ Hz), 2.70–2.25 (m, 4H), 1.13, 1.10, 0.93, 0.91, 0.88, 0.85, 0.80, 0.70, 0.59 (s, 3H each); δ_C ($CDCl_3$) 177.7, 176.4, 172.5, 143.9, 136.7, 128.6, 128.2 (2C), 128.1 (2C), 122.6, 81.6, 66.1, 55.6, 47.8, 47.0, 46.1, 45.8, 45.4, 41.9, 41.6, 39.5, 38.4, 37.8, 37.1, 34.1, 33.3, 32.9, 32.6 (2C), 30.9, 28.3, 28.2, 28.1, 27.8, 27.8, 26.1, 23.9, 23.6, 23.3, 18.4, 17.1, 17.0, 15.5; ESI-HRMS *m/z* calcd for $C_{44}H_{65}O_6$ [M + 1] 689.4767, found 689.4781.

4.5.20. Acylation of **MA** with propanoic anhydride

Propanoic anhydride (217 μL) was added slowly to a solution of **MA** (100 mg, 0.211 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **28** as a white solid (22 mg, 20%), mp 155–157 °C; $[\alpha]_D$ + 49 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3376, 3015, 2855 and 1711; δ_H ($CDCl_3$) 5.26 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 4.92 (ddd, 1H, $J_1 = 4.8$, $J_2 = 10.5$, $J_3 = 11.2$ Hz), 3.17 (d, 1H, $J = 10.5$ Hz), 2.80 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.9$ Hz), 2.30 (q, 2H, $J = 6.5$ Hz), 1.10, 1.08, 1.01, 0.99, 0.88, 0.81, 0.70 (s, 3H each); δ_C ($CDCl_3$) 184.2, 175.3, 143.9, 122.4, 81.0, 73.2, 55.3, 47.7, 46.7, 46.0, 43.8, 41.8, 41.0, 40.0, 39.5, 38.6, 34.0, 33.2, 32.6, 32.6, 30.8, 28.7, 28.1, 27.8, 26.1, 23.8, 23.7, 23.0, 18.4, 17.3, 16.8, 16.5, 9.3; ESI-HRMS *m/z* calcd for $C_{33}H_{53}O_5$ [M + 1] 529.3893, found 529.3886; and **29** as a white solid (89 mg, 73%), mp 224–226 °C; $[\alpha]_D$ + 32 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3299, 3017, 2796 and 1699; δ_H ($CDCl_3$) 5.28 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 5.14 (ddd, 1H, $J_1 = 4.3$, $J_2 = 10.1$, $J_3 = 11.2$ Hz), 4.80 (d, 1H, $J = 10.1$ Hz), 2.84 (dd, 1H, $J_1 = 4.5$, $J_2 = 12.0$ Hz), 2.32 and 2.26 (q, 2H each, $J = 6.5$ Hz), 1.08, 1.01, 0.99, 0.92, 0.91, 0.91, 0.76 (s, 3H each); δ_C ($CDCl_3$) 184.6, 173.5 (2C), 143.9, 122.5, 80.5, 70.1, 55.1, 47.8, 46.8, 46.0, 44.1, 41.8, 41.1, 39.7, 39.6, 38.4, 34.0, 33.3, 32.6 (2C), 30.9, 28.7, 28.1 (2C), 27.9, 26.1, 23.8, 23.7, 23.0, 18.4, 17.9, 17.4, 16.6, 9.6, 9.4; ESI-HRMS *m/z* calcd for $C_{36}H_{57}O_6$ [M + 1] 585.4155, found 585.4144.

4.5.21. Acylation of **MA** with butanoic anhydride

Butanoic anhydride (276 μL) was added slowly to a solution of **MA** (100 mg, 0.211 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **30** as a white solid (18 mg, 15%), mp 138–140 °C; $[\alpha]_D$ + 52 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3353, 2980, 2839 and 1719; δ_H ($CDCl_3$) 5.23 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 4.92 (ddd, 1H, $J_1 = 4.3$, $J_2 = 10.0$, $J_3 = 11.5$ Hz), 3.17 (d, 1H, $J = 10.0$ Hz), 2.78 (dd, 1H, $J_1 = 3.5$, $J_2 = 14.0$ Hz), 2.28 (t, 2H, $J = 6.5$ Hz), 1.08, 1.01, 0.92, 0.89, 0.86, 0.81, 0.70 (s, 3H each); δ_C ($CDCl_3$) 184.3, 174.5, 143.9, 122.5, 81.1, 73.2, 55.3, 47.8, 46.7, 46.1, 43.9, 41.8, 41.1, 40.0, 39.5, 38.5, 36.7, 34.0, 33.3, 32.6 (2C), 30.9, 28.7, 27.8, 26.1, 23.8, 23.7, 23.0, 18.7, 18.4, 17.3, 16.8, 16.5, 13.8; ESI-HRMS *m/z* calcd for $C_{34}H_{54}O_5Na$ [M + Na] 565.3869, found 565.3862; and **31** as a white solid (109 mg, 80%), mp 142–144 °C; $[\alpha]_D$ + 42 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3365, 3000, 2848 and 1780; δ_H ($CDCl_3$) 5.28 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.12 (ddd, 1H, $J_1 = 4.3$, $J_2 = 10.5$, $J_3 = 11.5$ Hz), 4.80 (d, 1H, $J = 10.5$ Hz), 2.83 (dd, 1H, $J_1 = 4.5$, $J_2 = 13.8$ Hz), 2.30 and 2.22 (t, 2H each, $J = 6.5$ Hz), 1.14, 1.08, 0.94, 0.93, 0.91, 0.91, 0.70 (s, 3H each); δ_C ($CDCl_3$) 184.6, 173.5 (2C), 143.9, 122.5, 80.3, 70.1, 55.1, 47.8, 46.8, 46.0, 44.1, 41.8, 41.1, 39.7, 39.6, 38.4, 36.7 (2C), 34.0, 33.3, 32.7 (2C), 30.9, 28.7, 27.9, 26.1, 23.8, 23.7, 23.0, 18.8, 18.6, 18.4, 17.9, 17.4, 16.6, 14.0, 13.9; ESI-HRMS *m/z* calcd for $C_{38}H_{59}O_6$ [M − 1] 611.4312, found 611.4305.

4.5.22. Acylation of **MA** with hexanoic anhydride

Hexanoic anhydride (389 μL) was added slowly to a solution of **MA** (100 mg, 0.211 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **32** as a white solid (22 mg, 19%), mp 124–126 °C; $[\alpha]_D$ + 40 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3405, 3053, 2849 and 1690; δ_H ($CDCl_3$) 5.23 (dd, 1H, $J_1 = J_2 = 3.3$ Hz), 4.93 (ddd, 1H, $J_1 = 4.3$, $J_2 = 10.3$, $J_3 = 11.3$ Hz), 3.16 (d, 1H, $J = 10.3$ Hz), 2.78 (dd, 1H, $J_1 = 4.5$, $J_2 = 14.0$ Hz), 2.28 (t, 2H, $J = 6.6$ Hz), 1.09, 1.01, 0.99, 0.88, 0.86, 0.82, 0.71 (s, 3H each); δ_C ($CDCl_3$) 184.5, 174.7, 143.8, 122.5, 81.0, 73.1, 55.3, 47.7, 46.7, 46.0, 43.9, 41.8, 41.1, 39.9, 39.5, 38.4, 34.7, 34.0, 33.2, 32.6 (2C), 31.4, 30.8, 28.7, 27.8, 26.1, 24.6, 23.7 (2C), 23.0, 22.5, 18.4, 17.2, 16.8, 16.5, 14.1; ESI-HRMS *m/z* calcd for $C_{36}H_{57}O_5$ [Mâ − 1] 569.4206, found 569.4209; and **33** as a colourless oil (101 mg, 70%), $[\alpha]_D$ − 21 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3340, 3013, 2839 and 1701; δ_H ($CDCl_3$) 5.19 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.02 (ddd, 1H, $J_1 = 4.4$, $J_2 = 10.5$, $J_3 = 11.2$ Hz), 4.69 (d, 1H, $J = 10.5$ Hz), 2.75 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.5$ Hz), 2.22 and 2.13 (t, 2H each, $J = 6.5$ Hz), 1.13, 1.11, 1.05, 0.98, 083, 0.81, 0.67 (s, 3H each); δ_C ($CDCl_3$) 183.0, 173.7, 173.6, 143.9, 122.5, 80.3, 70.1, 55.1, 47.8, 46.7, 46.1, 44.1, 41.9, 41.2, 39.7, 39.6, 38.4, 34.8 (2C), 34.0, 33.3, 32.7 (2C), 31.7, 31.5, 30.9, 28.7, 27.9, 26.1, 25.1, 24.9, 23.8, 23.1, 22.9, 22.6 (2C), 18.4, 17.9, 17.4, 16.6, 14.1, 14.1; ESI-HRMS *m/z* calcd for $C_{42}H_{68}O_6Na$ [M + Na] 691.4914, found 691.4913.

4.5.23. Acylation of **MA** with lauric anhydride

Lauric anhydride (645 mg, 1.680 mmol) was added slowly to a solution of **MA** (100 mg, 0.211 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **34** as a white solid (37 mg, 27%), mp 30–32 °C; $[\alpha]_D$ + 20 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3384, 2956, 2827 and 1689; δ_H ($CDCl_3$) 5.24 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 4.94 (ddd, 1H, $J_1 = 4.3$, $J_2 = 10.3$, $J_3 = 11.3$ Hz), 3.18 (d, 1H, $J = 10.3$ Hz), 2.79 (dd, 1H, $J_1 = 4.5$, $J_2 = 13.8$ Hz), 2.31 (t, 2H, $J = 6.6$ Hz), 1.10, 1.03, 1.01, 0.90, 0.88, 0.83, 0.73 (s, 3H each); δ_C ($CDCl_3$) 184.8, 174.8, 143.8, 122.5, 81.1, 73.1, 55.4, 47.8, 46.8, 46.1, 43.9, 41.9, 41.1, 40.0, 39.6, 38.4, 34.8, 34.3, 33.3, 32.6 (2C), 32.1, 30.9, 29.7 (3C), 29.3 (2C), 29.1, 28.7, 27.8, 26.1, 24.8, 23.8, 23.7, 23.1, 22.8, 18.5, 16.9, 16.8, 16.5, 14.2; ESI-HRMS *m/z* calcd for $C_{42}H_{71}O_5$ [M + 1] 655.5302, found 655.5316; and **35** as a colourless oil (100 mg, 57%), $[\alpha]_D$ + 45 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3390, 3000, 2880 and 1770; δ_H ($CDCl_3$) 5.18 (dd, 1H, $J_1 = J_2 = 3.3$ Hz), 5.01 (ddd, 1H, $J_1 = 4.3$, $J_2 = 10.5$, $J_3 = 11.3$ Hz), 4.68 (d, 1H, $J = 10.5$ Hz), 2.73 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.8$ Hz), 2.21 and 2.13

(t, 2H each, $J = 6.5$ Hz), 1.13, 1.04, 1.05, 0.97, 0.80, 0.77, 0.65 (s, 3H each); δ_C ($CDCl_3$) 184.5, 173.7, 173.6, 143.9, 122.5, 80.3, 70.0, 55.1, 47.8, 46.8, 46.0, 44.1, 41.8, 41.1, 39.7, 39.6, 38.4, 34.9, 34.8, 34.0, 33.3, 32.7 (2C), 32.2 (2C), 30.9, 30.0 (4C), 29.9 (4C), 29.8, 29.6 (2C), 29.3, 28.7, 27.9, 26.1, 25.4, 25.2, 23.8, 23.7, 23.1, 22.9 (2C), 18.4, 17.9, 17.3, 16.6, 14.4, 14.1; ESI-HRMS *m/z* calcd for $C_{54}H_{93}O_6$ [M + 1] 837.6972, found 837.6967.

*4.5.24. Acylation of **MA** with benzoic anhydride*

Benzoic anhydride (318 μL) was added slowly to a solution of **MA** (100 mg, 0.211 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **36** as a white solid (46 mg, 37%), mp 95–97 °C; $[\alpha]_D$ + 28 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3420, 3030, 2822 and 1717; δ_H ($CDCl_3$) 8.10–7.45 (m, 5H), 5.26 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 5.20 (ddd, 1H, $J_1 = 4.3$, $J_2 = 10.2$, $J_3 = 11.3$ Hz), 3.39 (d, 1H, $J = 10.2$ Hz), 2.83 (dd, 1H, $J_1 = 4.5$, $J_2 = 13.7$ Hz), 1.13, 1.07, 1.07, 0.92, 0.90, 0.89, 0.77 (s, 3H each); δ_C ($CDCl_3$) 184.8, 172.2, 143.8, 133.9 (2C), 130.4 (2C), 129.8, 128.7, 122.6, 81.2, 74.2, 55.4, 47.8, 46.8, 46.1, 44.0, 41.9, 41.2, 40.1, 39.6, 38.7, 34.0, 33.3, 32.7, 32.6, 30.9, 28.8, 27.9, 26.2, 23.8, 23.7, 23.1, 18.5, 17.1, 16.9, 16.6; ESI-HRMS *m/z* calcd for $C_{37}H_{51}O_5$ [M − 1] 575.3737, found 575.3745; and **37** as a white solid (64 mg, 45%), mp 70–72 °C; $[\alpha]_D$ + 28 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3402, 2983, 2842 and 1700; δ_H ($CDCl_3$) 7.95–7.35 (m, 10 H), 5.28 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 5.20 (ddd, 1H, $J_1 = 4.5$, $J_2 = 10.3$, $J_3 = 11.5$ Hz), 5.23 (d, 1H, $J = 10.3$ Hz), 2.84 (dd, 1H, $J_1 = 4.3$, $J_2 = 14.0$ Hz), 1.20, 1.16, 1.11, 1.03, 0.93, 0.91, 0.80 (s, 3H each); δ_C ($CDCl_3$) 184.0, 166.7, 166.5, 143.9, 133.9 (4C), 129.8 (4C), 129.8 (2C), 128.5 (2C), 122.5, 81.2, 71.3, 55.4, 47.9, 46.8, 46.0, 44.2, 41.9, 41.2, 40.1, 39.7, 38.7, 34.1, 33.3, 32.7 (2C), 30.9, 28.8, 27.9, 26.2, 23.8, 23.7, 23.1, 18.5, 18.1, 17.4, 16.8; ESI-HRMS *m/z* calcd for $C_{44}H_{57}O_6$ [M + 1] 681.4155, found 681.4155.

*4.5.25. Acylation of **MA** with phthalic anhydride*

Phthalic anhydride (250 mg, 1.680 mmol) was added slowly to a solution of **MA** (100 mg, 0.211 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **38** as a white solid (33 mg, 25%), mp 190–192 °C; $[\alpha]_D$ + 11 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3375, 3015, 2822 and 1722; δ_H ($CDCl_3$) 8.30–7.30 (m, 4H), 5.74 (ddd, 1H, $J_1 = 4.5$, $J_2 = 10.6$, $J_3 = 11.5$ Hz), 5.40 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 3.33 (d, 1H, $J = 10.6$ Hz), 2.70 (dd, 1H, $J_1 = 4.5$, $J_2 = 13.8$ Hz), 1.29, 1.25, 1.11, 1.07, 1.06, 1.02, 0.97 (s, 3H each); δ_C ($CDCl_3$) 180.2, 169.9, 168.3, 145.1, 133.8, 132.9, 132.3 (2C), 128.4, 128.1, 122.1, 82.3, 74.8, 55.5, 47.9, 46.8 (2C), 43.9, 42.1, 41.4, 40.4, 39.9, 38.6, 34.4, 33.5, 33.3, 33.1, 31.2, 28.9, 27.4, 26.4, 24.1, 23.9 (2C), 18.6, 18.3, 17.4, 16.5; ESI-HRMS *m/z* calcd for $C_{38}H_{51}O_7$ [M − 1] 619.3635, found 619.3649; and to give **39** as a colourless oil (65 mg, 40%), $[\alpha]_D$ + 38 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3391, 2999, 2843 and 1740; δ_H ($CDCl_3$) 8.30–7,30 (m, 8H), 5.80 (ddd, 1H, $J_1 = 4.3$, $J_2 = 10.3$, $J_3 = 11.3$ Hz), 5.60 (d, 1H, $J = 10.3$ Hz), 5.42 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 2.69 (dd, 1H, $J_1 = 4.4$, $J_2 = 13.9$ Hz), 1.30, 1.28, 1.10, 1.08, 1.06, 1.03, 0.98 (s, 3H each); δ_C ($CDCl_3$) 180.3, 170.7, 169.9, 168.7, 168.3, 145.2, 133.8 (2C), 132.9 (2C), 132.3 (4C), 128.4 (2C), 128.1 (2C), 122.2, 82.4, 72.2, 55.5, 48.0, 46.8 (2C), 43.9, 42.1, 41.4, 40.3, 39.9, 38.6, 34.4, 33.5, 33.3, 33.1, 31.2, 28.9, 27.4, 26.4, 24.1, 23.9 (2C), 18.6, 18.3, 17.4, 16.5; ESI-HRMS *m/z* calcd for $C_{46}H_{57}O_{10}$ [M + 1] 769.3952, found 769.3954.

*4.5.26. Acylation of **MA** with succinic anhydride*

Succinic anhydride (169 mg, 1.680 mmol) was added slowly to a solution of **MA** (100 mg, 0.211 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **40** as a white solid (40 mg, 30%), mp 121–123 °C; $[\alpha]_D$ + 11 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3365, 3022, 2839 and 1711; δ_H (Py-d5) 5.53 (ddd, 1H, $J_1 = 4.6$, $J_2 = 10.8$, $J_3 = 11.5$ Hz), 5.47 (dd, 1H, $J_1 = J_2 = 3.6$ Hz), 3.55 (d, 1H, $J = 10.8$ Hz), 3.30 (dd, 1H, $J_1 = 4.6$, $J_2 = 14.0$ Hz), 2.80–2.60 (m, 4H), 1.30, 1.26, 1.08, 1.05, 1.03, 1.00, 0.98 (s, 3H each); δ_C (Py-d5) 180.6, 175.6, 173.4, 145.4, 122.8, 80.2, 74.3, 56.1, 48.5, 47.2, 47.1, 44.9, 42.8, 42.5, 40.9, 40.3, 39.1, 34.8, 33.8, 33.7, 33.6, 31.5, 30.9, 30.6, 29.7, 28.8, 26.7, 24.4, 24.3, 24.2, 19.3, 18.1, 17.9, 17.0; ESI-HRMS *m/z* calcd for $C_{34}H_{52}O_7Na$ [M + Na] 595.3611, found 595.3612; and **41** as a white solid (45 mg, 33%), mp 113–115 °C; $[\alpha]_D$ − 6 (*c* 1 in MeOH); IR ν_{max}(KBr)/cm^{-1} 3346, 3021, 2803 and 1688; δ_H (Py-d5) 5.50 (ddd, 1H, $J_1 = 4.6$, $J_2 = 10.9$, $J_3 = 11.4$ Hz), 5.44 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.15 (d, 1H, $J = 10.9$ Hz), 3.30 (dd, 1H, $J_1 = 4.6$, $J_2 = 14.0$ Hz), 2.80–2.55 (m, 8H), 1.29, 1.25, 1.04, 1.01, 0.98, 0.96, 0.95 (s, 3H each); δ_C (Py-d5) 180.9, 176.0, 175.7, 173.5, 173.4, 145.7, 122.5, 81.2, 70.7, 55.5, 48.2, 47.2, 47.0, 44.6, 42.7, 42.4, 40.5 (2C), 39.2, 34.7, 33.8, 33.6, 33.3, 31.8, 31.0, 30.9, 30.6, 30.5, 29.0, 28.7, 26.7, 24.3, 24.2, 24.1, 18.9, 18.4, 17.7, 16.9; ESI-HRMS *m/z* calcd for $C_{38}H_{57}O_{10}$ [M + 1] 673.3947, found 673.3952.

*4.5.27. Acylation of **MA** with glutaric anhydride*

Glutaric anhydride (193 mg, 1.680 mmol) was added slowly to a solution of **MA** (100 mg, 0.211 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **42** as a colourless oil (38 mg, 31%), $[\alpha]_D$ + 17 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3395, 2979, 2833 and 1725; δ_H (DMSO-d_6) 5.27 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.00 (ddd, 1H, $J_1 = 4.6$, $J_2 = 10.7$, $J_3 = 11.3$ Hz), 3.20 (d, 1H, $J = 10.7$ Hz), 2.90 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.8$ Hz), 2.35–2.15 (m, 4H), 1.20, 1.17, 1.09, 1.07, 0.97, 0.89, 0.85 (s, 3H each); δ_C (DMSO-d_6) 181.9, 175.7, 173.7, 145.5, 122.5, 81.1, 73.8, 56.2, 49.1, 47.7, 47.4, 44.6, 43.1, 42.9, 41.1, 40.7, 39.6, 35.1, 34.0 (2C), 33.7, 32.2, 31.8, 30.9, 29.7, 28.8, 26.6, 24.7, 24.2 (2C), 21.6, 19.7, 18.4, 17.8, 17.1; ESI-HRMS *m/z* calcd for $C_{35}H_{55}O_7$ [M + 1] 587.3948, found 587.3957; and **43** as a colourless oil (53 mg, 35%), $[\alpha]_D$ − 14 (*c* 1 in MeOH); IR ν_{max}(film)/cm^{-1} 3396, 3001, 2843 and 1710; δ_H (Py-d5) 5.49 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 5.45 (ddd, 1H, $J_1 = 4.4$, $J_2 = 10.7$, $J_3 = 11.5$ Hz), 5.14 (d, 1H, $J = 10.7$ Hz), 3.20 (dd, 1H, $J_1 = 4.3$, $J_2 = 14.0$ Hz), 2.35–2.10 (m, 8H), 1.32, 1.09, 1.03, 1.01, 0.98, 0.98, 0.95 (s, 3H each); δ_C (Py-d5) 180.7, 176.3, 176.2, 173.7, 173.5, 145.6, 122.7, 81.2, 70.8, 55.6, 48.0, 47.3, 46.7, 44.4, 42.9, 42.2, 41.4, 40.3, 39.1, 34.5, 34.4 (2C), 34.2, 34.1, 33.6, 33.5, 33.1, 31.6, 28.9, 28.5, 26.5, 24.1, 24.0, 23.9, 21.5, 21.4, 18.8, 18.2, 17.6, 16.7; ESI-HRMS *m/z* calcd for $C_{40}H_{60}O_{10}Na$ [M + Na] 723.4084, found 723.4073.

*4.5.28. Acylation of **MA** with 3,3-dimethylglutaric anhydride*

3,3-Dimethylglutaric anhydride (239 mg, 1.680 mmol) was added slowly to a solution of **MA** (100 mg, 0.211 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **44** as a colourless oil (45 mg, 33%), $[\alpha]_D$ + 44 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3299, 2987, 2801 and 1740; δ_H (DMSO-d_6) 5.27 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 4.97 (ddd, 1H, $J_1 = 4.3$, $J_2 = 10.5$, $J_3 = 11.5$ Hz), 3.19 (d, 1H, $J = 10.5$ Hz), 2.88 (dd, 1H, $J_1 = 4.3$, $J_2 = 14.0$ Hz), 2.45–2.35 (m, 4H), 1.19, 1.17, 1.16, 1.14, 1.01, 0.96, 0.93, 0.88, 0.84 (s, 3H each); δ_C (DMSO-d_6) 181.8, 177.0, 174.9, 145.4, 122.5, 81.1, 73.9, 56.7, 49.1, 47.7, 47.4, 45.4, 45.2, 45.1, 43.1, 42.8, 41.1, 40.7, 39.6, 35.1, 34.7, 34.0, 33.9, 33.8, 31.8, 30.8, 29.7, 29.4, 29.0, 26.6, 24.7, 24.2 (2C), 19.7, 17.8, 17.6, 17.1; ESI-HRMS *m/z* calcd for $C_{37}H_{59}O_7$ [M + 1] 615.4261, found 615.4252; and **45** as a colourless oil (59 mg, 36%), $[\alpha]_D$ − 13 (*c* 1 in MeOH); IR ν_{max}(film)/cm^{-1} 3380, 3024, 2841 and 1724; δ_H (Py-d5) 5.49 (ddd, 1H, $J_1 = 4.4$, $J_2 = 10.6$, $J_3 = 11.3$ Hz), 5.46 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.21 (d, 1H, $J = 10.6$ Hz), 3.60 (dd, 1H, $J_1 = 4.5$, $J_2 = 13.9$ Hz), 2.90–2.65 (m, 8H), 1.43, 1.41, 1.39 and 1.37 (s, 3H), 1.07, 1.05, 1.04, 1.03, 0.99, 0.99, 0.98 (s, 3H each); δ_C (Py-d5) 180.7, 175.3 (2C), 172.5, 172.4, 145.5, 122.6, 80.7, 70.8, 55.6, 48.0, 47.7, 46.7, 46.2 (2C), 45.2 (2C), 44.6, 42.8, 42.1, 40.4, 40.3, 39.0, 34.7 (2C), 34.5, 33.5, 33.4, 33.1, 31.6, 29.0, 28.6 (2C), 28.2 (2C), 28.1, 26.4, 24.4, 23.9, 23.4, 19.1, 18.3, 17.5, 16.7; ESI-HRMS *m/z* calcd for $C_{44}H_{68}O_{10}Na$ [M + Na] 779.4710, found 779.4712.

*4.5.29. Acylation of **MA-Bn** with Ac_2O*

Ac_2O (134 μL) was added slowly to a solution of **MA-Bn** (100 mg, 0.177 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **46** as a white solid (17 mg, 15%), mp 85–87 °C; $[\alpha]_D$ + 14 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3396, 3015, 2845 and 1701; δ_H ($CDCl_3$) 7.31 (m, 5H), 5.25 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 5.05 (AB system, 2H, $J = 12.0$ Hz), 4.93 (ddd, 1H, $J_1 = 4.3$, $J_2 = 10.3$, $J_3 = 11.3$ Hz), 3.16 (d, 1H, $J = 10.3$ Hz), 2.88 (dd, 1H, $J_1 = 4.8$, $J_2 = 13.8$ Hz), 2.04, 1.09, 1.03, 0.97, 0.90, 0.88, 0.84, 0.57 (s, 3H each); δ_C ($CDCl_3$) 177.6, 171.8, 144.0, 136.3, 128.6 (5C), 122.4, 81.1, 73.5, 66.2, 55.4, 47.8, 46.9, 46.1, 43.9, 41.9, 41.6, 40.0, 39.6, 38.5, 34.1, 33.3, 32.8, 32.6, 30.9, 28.8, 27.8, 26.1, 23.9, 23.7, 23.2, 21.6, 18.5, 17.1, 16.9, 16.6; ESI-HRMS *m*/*z* calcd for $C_{39}H_{56}O_5Na$ [M + Na] 627.4025, found 627.4017; and **47** as a white solid (94 mg, 75%), mp 78–80 °C; $[\alpha]_D$ + 54 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3353, 2995, 2808 and 1685; δ_H ($CDCl_3$) 7.30 (m, 5H), 5.25 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.08 (ddd, 1H, $J_1 = 4.3$, $J_2 = 10.5$, $J_3 = 11.5$ Hz), 5.03 (AB system, 2H, $J = 12.0$ Hz), 4.70 (d, 1H, $J = 10.5$ Hz), 2.88 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.8$ Hz), 2.01, 1.94, 1.08, 0.99, 0.89, 0.87, 0.87, 0.86, 0.55 (s, 3H each); δ_C ($CDCl_3$) 177.5, 170.9, 170.6, 143.9, 136.6, 128.5 (2C), 128.2, 128.0 (2C), 122.3, 80.8, 70.2, 66.1, 55.1, 47.7, 46.9, 46.0, 44.1, 41.9, 41.5, 39.5 (2C), 38.3, 34.0, 33.3, 32.7, 32.5, 30.9, 28.6, 27.7, 26.0, 23.8, 23.6, 23.2, 21.3, 21.0, 18.4, 17.9, 17.8, 16.6; ESI-HRMS *m*/*z* calcd for $C_{41}H_{59}O_6$ [M + 1] 647.4312, found 647.4320.

*4.5.30. Acylation of **MA-Bn** with propanoic anhydride*

Propanoic anhydride (182 μL) was added slowly to a solution of **MA-Bn** (100 mg, 0.177 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **48** as a white solid (24 mg, 22%), mp 46–48 °C; $[\alpha]_D$ + 24 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3401, 2989, 2823 and 1745; δ_H ($CDCl_3$) 7.31 (m, 5H), 5.25 (dd, 1H, $J_1 = J_2 = 3.3$ Hz), 5.05 (AB system, 2H, $J = 12.0$ Hz), 4.93 (ddd, 1H, $J_1 = 4.3$, $J_2 = 10.2$, $J_3 = 11.3$ Hz), 3.18 (d, 1H, $J = 10.2$ Hz), 2.89 (dd, 1H, $J_1 = 4.6$, $J_2 = 14.0$ Hz), 2.33 (2H, q, $J = 6.5$ Hz), 1.12, 1.09, 1.03, 0.98, 0.90, 0.87, 0.84 (s, 3H each); δ_C ($CDCl_3$) 177.6, 175.3, 144.0, 136.6, 128.6 (2C), 128.2, 128.1 (2C), 122.4, 81.1, 73.3, 66.2, 55.4, 47.8, 46.9, 46.1, 43.9, 42.0, 41.6, 40.0, 39.6, 38.5, 34.1, 33.3, 32.8, 32.6, 30.9, 28.8, 28.1, 27.8, 26.1, 23.9, 23.7, 23.3, 18.5, 17.1, 16.9, 16.5, 9.4; ESI-HRMS *m*/*z* calcd for $C_{40}H_{59}O_5$ [M + 1] 619.4363, found 619.4368; and **49** as a white solid (81 mg, 67%), mp 34–36 °C; $[\alpha]_D$ + 7 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3400, 3042, 2835 and 1704; δ_H ($CDCl_3$) 7.30 (m, 5H), 5.32 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.10 (ddd, 1H, $J_1 = 4.3$, $J_2 = 9.5$, $J_3 = 11.5$ Hz), 5.05 (AB system, 2H, $J = 12.0$ Hz), 4.74 (d, 1H, $J = 9.5$ Hz), 2.88 (dd, 1H, $J_1 = 4.5$, $J_2 = 13.7$ Hz), 2.28 and 2.22 (t, 2H each, $J = 6.5$ Hz), 1.09, 1.03, 1.01, 0.92, 0.90, 0.88, 0.86 (s, 3H each); δ_C ($CDCl_3$) 177.6, 174.3, 173.2, 144.0, 136.6, 128.6 (2C), 128.2 (2C), 128.1, 122.4, 80.5, 70.1, 66.2, 55.1, 47.7, 47.0, 46.1, 44.2, 42.0, 41.6, 39.7, 39.6, 38.4, 34.0, 33.3, 32.6 (2C), 30.9, 28.7, 28.1 (2C), 27.8, 26.1, 23.9, 23.7, 23.3, 18.5, 17.9, 17.1, 16.6, 9.6, 9.4; ESI-HRMS *m*/*z* calcd for $C_{43}H_{63}O_6$ [M + 1] 675.4625, found 675.4626.

*4.5.31. Acylation of **MA-Bn** with butanoic anhydride*

Butanoic anhydride (232 μL) was added slowly to a solution of **MA-Bn** (100 mg, 0.177 mmol) in pyridine (2 mL). The mixture was treated as in the general method for acylation above to give **50** as a white solid (12 mg, 10%), mp 33–35 °C; $[\alpha]_D$ + 53 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3376, 3015, 2842 and 1710; δ_H ($CDCl_3$) 7.31 (m, 5H), 5.25 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.05 (AB system, 2H, $J = 12.0$ Hz), 4.92 (ddd, 1H, $J_1 = 4.4$, $J_2 = 9.8$, $J_3 = 11.3$ Hz), 3.16 (d, 1H, $J = 9.8$ Hz), 2.88 (dd, 1H, $J_1 = 4.5$, $J_2 = 14.0$ Hz), 2.27 (t, 2H, $J = 6.5$ Hz), 1.10, 1.03, 0.93, 0.89, 0.89, 0.87, 0.84 (s, 3H each); δ_C ($CDCl_3$) 177.5, 174.5, 144.0, 136.6, 128.6 (2C), 128.2 (2C), 128.1, 122.4, 81.1, 73.3, 66.2, 55.4, 47.8, 46.9, 46.1, 44.0, 41.6, 41.5, 40.0, 39.5, 38.5, 36.8, 34.1, 33.4, 32.8, 32.6, 30.9, 28.8, 27.8, 26.1, 23.9, 23.8, 23.3, 18.8, 18.4, 17.1, 16.9, 16.6, 13.9; ESI-HRMS *m*/*z* calcd for $C_{41}H_{61}O_5$ [M + 1] 633.4519, found 633.4523; and **51** as a white solid (100 mg, 79%), mp 47–49 °C; $[\alpha]_D$ + 5 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3412, 3020, 2862 and 1730; δ_H ($CDCl_3$) 7.30 (m, 5H), 5.23 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.06 (ddd, 1H, $J_1 = 4.6$, $J_2 = 10.3$, $J_3 = 11.2$ Hz), 5.03 (AB system, 2H, $J = 12.0$ Hz), 4.74 (d, 1H, $J = 10.3$ Hz), 2.87 (dd, 1H, $J_1 = 4.5$, $J_2 = 13.9$ Hz), 2.24 and 2.15 (t, 2H each, $J = 6.5$ Hz), 1.08, 0.99, 0.94, 0.91, 0.90, 0.86, 0.85 (s, 3H each); δ_C ($CDCl_3$) 177.4, 173.3, 173.2, 143.9, 136.5, 128.5 (2C), 128.1, 128.0 (2C), 122.3, 80.2, 69.9, 66.1, 55.0, 47.6, 46.8, 46.0, 44.1, 41.8, 41.5, 39.6, 39.5, 38.3, 36.6 (2C), 34.0, 33.2, 32.7, 32.5, 30.8, 28.6, 27.7, 26.0, 23.8, 23.6, 23.2, 18.7, 18.5, 18.4, 17.8, 17.0, 16.5, 13.9, 13.8; ESI-HRMS *m*/*z* calcd for $C_{45}H_{67}O_6$ [M + 1] 703.4938, found 703.4946.

*4.5.32. Acylation of **MA-Bn** with hexanoic anhydride*

Hexanoic anhydride (328 μL) was added slowly to a solution of **MA-Bn** (100 mg, 0.177 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **52** as a white solid (18 mg, 15%), mp 40–42 °C; $[\alpha]_D$ − 6 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3375, 3012, 2841 and 1753; δ_H ($CDCl_3$) 7.31 (m, 5H), 5.25 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 5.02 (AB system, 2H, $J = 12.0$ Hz), 4.93 (ddd, 1H, $J_1 = 4.5$, $J_2 = 9.9$, $J_3 = 11.5$ Hz), 3.16 (d, 1H, $J = 9.9$ Hz), 2.78 (dd, 1H, $J_1 = 4.3$, $J_2 = 14.0$ Hz), 2.31 (t, 2H, $J = 6.6$ Hz), 1.09, 1.02, 0.98, 0.90, 0.87, 0.87, 0.84 (s, 3H each); δ_C ($CDCl_3$) 177.5, 174.6, 144.0, 136.6, 128.6 (2C), 128.2, 128.1 (2C), 122.4, 81.1, 73.2, 66.1, 55.4, 47.8, 46.9, 46.1, 43.9, 41.9, 41.6, 40.0, 39.6, 38.5, 34.8, 34.1, 33.3, 32.7, 32.6, 31.5, 30.9, 28.8, 27.8, 26.1, 24.9, 23.8, 23.7 (2C), 22.5, 18.5, 17.1, 16.9, 16.5, 14.1; ESI-HRMS *m*/*z* calcd for $C_{43}H_{65}O_5$ [M + 1] 661.4832, found 661.4844; and **53** as a colourless oil (100 mg, 76%), $[\alpha]_D$ − 24 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3432, 3022, 2849 and 1740; δ_H ($CDCl_3$) 7.29 (m, 5H), 5.24 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.04 (ddd, 1H, $J_1 = 4.8$, $J_2 = 10.3$, $J_3 = 11.3$ Hz), 5.02 (AB system, 2H, $J = 12.0$ Hz), 4.74 (d, 1H, $J = 10.3$ Hz), 2.87 (dd, 1H, $J_1 = 4.5$, $J_2 = 13.6$ Hz), 2.26 and 2.16 (t, 2H each, $J = 6.5$ Hz), 1.13, 1.08, 0.99, 0.89, 0.87, 0.87, 0.85 (s, 3H each); δ_C ($CDCl_3$) 177.4, 173.5, 173.4, 143.9, 136.5, 128.5 (2C), 128.2, 128.0 (2C), 122.3, 80.2, 69.4, 66.1, 55.1, 47.7, 46.8, 46.0, 44.1, 41.9, 41.5, 39.6, 39.5, 38.3, 34.7, 34.6, 34.0, 33.2, 32.7, 32.5, 31.5, 31.4, 30.8, 28.6, 27.7, 26.0, 24.9, 24.7, 23.8, 23.6, 23.2, 22.4 (2C), 18.4, 17.8, 17.0, 16.5, 14.0 (2C); ESI-HRMS *m*/*z* calcd for $C_{49}H_{75}O_6$ [M + 1] 759.5564, found 759.5560.

*4.5.33. Acylation of **MA-Bn** with lauric anhydride*

Lauric anhydride (544 mg, 1.422 mmol) was added slowly to a solution of **MA-Bn** (100 mg, 0.177 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **54** as a colourless oil (44 mg, 33%), $[\alpha]_D$ + 25 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3388, 3012, 2815 and 1700; δ_H ($CDCl_3$) 7.31 (m, 5H), 5.25 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 5.02 (AB system, 2H, $J = 12.0$ Hz), 4.93 (ddd, 1H, $J_1 = 4.3$, $J_2 = 10.6$, $J_3 = 11.5$ Hz), 3.16 (d, 1H, $J = 10.6$ Hz), 2.88 (dd, 1H, $J_1 = 4.5$, $J_2 = 13.6$ Hz), 2.29 (t, 2H, $J = 6.6$ Hz), 1.10, 1.03, 0.98, 0.90, 0.88, 0.86, 0.84 (s, 3H each); δ_C ($CDCl_3$) 177.6, 174.7, 144.0, 136.6, 128.6 (2C), 128.2, 128.1 (2C), 122.4, 81.1, 73.3, 66.2, 55.4, 47.8, 46.9, 46.1, 43.9, 42.0, 41.6, 40.0, 39.6, 38.5, 34.9, 34.1, 33.3, 32.7, 32.6, 32.1, 30.9, 29.8 (3C), 29.7 (2C), 29.4, 28.8, 27.8, 26.1, 25.3, 23.9, 23.7, 23.3, 22.9, 18.5, 17.1, 16.9, 16.6, 14.3; ESI-HRMS *m*/*z* calcd for $C_{49}H_{77}O_5$ [M + 1] 745.5771, found 745.5758; and **55** as a colourless oil (102 mg, 61%), $[\alpha]_D$ + 1 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3419, 2999, 2850 and 1708; δ_H ($CDCl_3$) 7.31 (m, 5H), 5.25 (dd, 1H, $J_1 = J_2 = 3.3$ Hz), 5.06 (ddd, 1H, $J_1 = 4.3$, $J_2 = 10.5$, $J_3 = 11.2$ Hz), 5.05 (AB system, 2H, $J = 12.0$ Hz), 4.75 (d, 1H, $J = 10.5$ Hz), 2.88 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.7$ Hz), 2.21 and 2.12 (q, 2H each, $J = 6.5$ Hz), 1.09, 1.00, 0.90, 0.87, 0.87, 0.86, 0.83 (s, 3H each); δ_C ($CDCl_3$) 177.6, 173.7, 173.6, 144.0, 136.6, 128.6 (2C), 128.2, 128.1 (2C), 122.4, 80.4, 70.1, 66.2, 55.1, 47.7, 46.9, 46.1, 44.2, 41.9, 41.6, 39.6 (2C), 38.3, 34.9, 34.8,

34.0, 33.3, 32.7 (2C), 32.2 (2C), 30.0, 29.9 (4C), 29.8 (4C), 29.6 (3C), 30.9, 28.7, 27.8, 26.1, 24.9 (2C), 23.9, 23.7, 23.3, 22.9, 22.2, 18.5, 17.9, 17.1, 16.6, 14.3 (2C); ESI-HRMS *m*/*z* calcd for $C_{61}H_{99}O_6$ [M + 1] 927.7442, found 927.7434.

*4.5.34. Acylation of **MA-Bn** with benzoic anhydride*

Benzoic anhydride (268 μL) was added slowly to a solution of **MA-Bn** (100 mg, 0.177 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **56** as a white solid (44 mg, 35%), mp 120–122 °C; $[\alpha]_D$ + 14 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3401, 3022, 2848 and 1712; δ_H ($CDCl_3$) 7.45–8.10 (m, 5H), 7.32 (m, 5H), 5.25 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.20 (ddd, 1H, $J_1 = 4.7$, $J_2 = 10.2$, $J_3 = 11.1$ Hz), 5.05 (AB system, 2H, $J = 12.0$ Hz), 3.35 (d, 1H, $J = 10.2$ Hz), 2.88 (dd, 1H, $J_1 = 4.5$, $J_2 = 13.8$ Hz), 1.11, 1.08, 1.05, 0.92, 0.90, 0.89, 0.88 (s, 3H each); δ_C ($CDCl_3$) 177.6, 167.2, 143.9, 136.6, 133.9, 130.4, 129.8 (2C), 128.7 (2C), 128.6 (2C), 128.2 (2C), 128.1, 122.4, 81.1, 74.3, 66.1, 55.4, 47.8, 46.9, 46.1, 44.0, 41.9, 41.6, 40.1, 39.5, 38.6, 34.1, 33.3, 32.8, 32.6, 30.9, 28.8, 27.8, 26.1, 23.8, 23.7, 23.2, 18.5, 17.1, 16.9, 16.6; ESI-HRMS *m*/*z* calcd for $C_{44}H_{58}O_5Na$ [M + Na] 689.4182, found 689.4175; and **57** as a white solid (70 mg, 51%), mp 75–77 °C; $[\alpha]_D$ − 18 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(KBr)/cm^{-1} 3379, 2991, 2845 and 1729; δ_H ($CDCl_3$) 7.95–7.35 (m, 10H), 7.31 (m, 5H), 5.53 (ddd, 1H, $J_1 = 4.6$, $J_2 = 10.4$, $J_3 = 11.5$ Hz), 5.30 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.27 (d, 1H, $J = 10.4$ Hz), 5.13 (AB system, 2H, $J = 12.0$ Hz), 2.97 (dd, 1H, $J_1 = 4.5$, $J_2 = 13.7$ Hz), 1.21, 1.20, 1.14, 1.06, 0.96, 0.94, 0.68 (s, 3H each); δ_C ($CDCl_3$) 177.6, 166.7, 166.5, 144.0, 136.7, 133.1, 133.0, 130.8, 129.8 (2C), 129.8 (2C), 129.1 (2C), 128.5 (4C), 128.3 (2C), 128.2 (2C), 122.4, 81.3, 71.3, 66.2, 55.4, 47.9, 47.0, 46.1, 44.2, 42.0, 41.7, 40.1, 39.7, 38.6, 34.2, 33.4, 32.8, 32.6, 31.0, 28.8, 27.9, 26.2, 23.9, 23.8, 23.3, 18.6, 18.1, 17.2, 16.8; ESI-HRMS *m*/*z* calcd for $C_{51}H_{63}O_6$ [M + 1] 771.4625, found 771.4601.

*4.5.35. Acylation of **MA-Bn** with phthalic anhydride*

Phthalic anhydride (210 mg, 1.422 mmol) was added slowly to a solution of **MA-Bn** (100 mg, 0.177 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **58** as a colourless oil (36 mg, 27%), $[\alpha]_D$ + 45 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3427, 3030, 2862 and 1731; δ_H ($CDCl_3$) 8.30–7.30 (m, 4H), 7.32 (m, 5H), 5.64 (ddd, 1H, $J_1 = 4.4$, $J_2 = 10.5$, $J_3 = 11.3$ Hz), 5.38 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.08 (AB system, 2H, $J = 12.0$ Hz), 3.33 (d, 1H, $J = 10.5$ Hz), 2.72 (dd, 1H, $J_1 = 4.7$, $J_2 = 14.0$ Hz), 1.28, 1.26, 1.10, 1.08, 1.05, 1.01, 0.94 (s, 3H each); δ_C ($CDCl_3$) 180.7, 169.4, 168.3, 145.1, 136.7, 133.8, 132.9, 132.3, 129.9, 128.5 (2C), 128.4, 128.2 (4C), 122.2, 82.3, 74.9, 66.2, 55.5, 47.8, 46.9, 46.8, 43.8, 42.2, 41.4, 40.4, 39.9, 38.6, 34.4, 33.4, 33.2, 33.1, 31.2, 28.9, 27.4, 26.3, 24.2, 24.0, 23.9, 18.6, 18.3, 17.4, 16.5; ESI-HRMS *m*/*z* calcd for $C_{45}H_{58}O_7Na$ [M + Na] 733.4080, found 733.4068; and **59** as a colourless oil (89 mg, 55%), $[\alpha]_D$ + 1 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3366, 3019, 2868 and 1701; δ_H ($CDCl_3$) 8.30–7.30 (m, 8H), 7.32 (m, 5H), 5.78 (ddd, 1H, $J_1 = 4.5$, $J_2 = 10.3$, $J_3 = 11.5$ Hz), 5.62 (d, 1H, $J = 10.3$ Hz), 5.40 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.05 (AB system, 2H, $J = 12.0$ Hz), 2.70 (dd, 1H, $J_1 = 4.7$, $J_2 = 13.7$ Hz), 1.20, 1.16, 1.11, 1.09, 1.07, 1.04, 0.98 (s, 3H each); δ_C ($CDCl_3$) 180.1, 170.6, 169.9, 168.6, 168.2, 145.1, 136.7, 133.8 (2C), 132.9 (2C), 132.3 (2C), 129.8 (2C), 128.5 (2C), 128.4 (2C), 128.2 (2C), 128.1 (2C), 128.0, 122.2, 82.4, 72.1, 66.3, 55.5, 48.0, 46.9, 46.8, 43.9, 42.2, 41.4, 40.2, 39.9, 38.6, 34.4, 33.5, 33.4, 33.2, 31.1, 28.9, 27.5, 26.4, 24.1, 23.9 (2C), 18.6, 18.3, 17.4, 16.5; ESI-HRMS *m*/*z* calcd for $C_{53}H_{63}O_{10}$ [M + 1] 859.4421, found 859.4420.

*4.5.36. Acylation of **MA-Bn** with succinic anhydride*

Succinic anhydride (142 mg, 1.422 mmol) was added slowly to a solution of **MA-Bn** (100 mg, 0.177 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **60** as a colourless oil (34 mg, 27%), $[\alpha]_D$ + 13 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3421, 2983, 2845 and 1722; δ_H ($CDCl_3$) 7.30 (m, 5H), 5.24 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 5.05 (ddd, 1H, $J_1 = 4.5$, $J_2 = 9.9$, $J_3 = 11.2$ Hz), 5.04 (AB system, 2H, $J = 12.0$ Hz), 3.20 (d, 1H, $J = 9.9$ Hz), 2.70–0.50 (m, 4H), 2.88 (dd, 1H, $J_1 = 4.4$, $J_2 = 13.9$ Hz), 1.14, 1.09, 1.01, 0.95, 0.89, 0.87, 0.78 (s, 3H each); δ_C ($CDCl_3$) 177.6 (2C), 172.8, 143.9, 136.6, 128.6 (2C), 128.2 (2C), 128.1, 122.4, 80.9, 73.7, 66.2, 55.3, 47.7, 46.9, 46.1, 43.8, 41.9, 41.6, 39.8, 39.5, 38.5, 34.1, 33.3, 32.7, 32.5, 30.9, 29.9, 29.8, 28.8, 27.8, 26.1, 23.8, 23.4, 23.3, 18.4, 17.0, 16.9, 16.5; ESI-HRMS *m*/*z* calcd for $C_{41}H_{59}O_7$ [M + 1] 663.4261, found 663.4272; and **61** as a colourless oil (65 mg, 43%), $[\alpha]_D$ + 10 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3379, 2984, 2855 and 1700; δ_H ($CDCl_3$) 7.30 (m, 5H), 5.25 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.10 (ddd, 1H, $J_1 = 4.4$, $J_2 = 10.3$, $J_3 = 11.3$ Hz), 5.04 (AB system, 2H, $J = 12.0$ Hz), 4.75 (d, 1H, $J = 10.3$ Hz), 2.89 (dd, 1H, $J_1 = 4.6$, $J_2 = 13.7$ Hz), 2.70–2.55 (m, 8H), 1.09, 0.99, 0.90, 0.88, 0.88, 0.86, 0.56 (s, 3H each); δ_C ($CDCl_3$) 178.6 (2C), 177.6, 172.1, 171.9, 144.0, 136.6, 128.7 (2C), 128.3 (2C), 128.2, 122.3, 81.2, 70.7, 66.2, 55.1, 47.8, 46.9, 46.1, 44.2, 41.9, 41.6, 39.7, 39.6, 38.4, 34.1, 33.4, 32.7, 32.6, 30.9, 29.3 (2C), 29.1 (2C), 28.7, 27.8, 26.1, 23.9, 23.7, 23.3, 18.5, 18.0, 17.1, 16.6; ESI-HRMS *m*/*z* calcd for $C_{45}H_{62}O_{10}Na$ [M + Na] 785.4241, found 785.4246.

*4.5.37. Acylation of **MA-Bn** with glutaric anhydride*

Glutaric anhydride (162 mg, 1.422 mmol) was added slowly to a solution of **MA-Bn** (100 mg, 0.177 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **62** as a colourless oil (27 mg, 21%), $[\alpha]_D$ + 19 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3401, 3015, 2871 and 1699; δ_H ($CDCl_3$) 7.31 (m, 5H), 5.24 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 4.98 (AB system, 2H, $J = 12.0$ Hz), 4.96 (ddd, 1H, $J_1 = 4.4$, $J_2 = 10.2$, $J_3 = 11.5$ Hz), 3.18 (d, 1H, $J = 10.2$ Hz), 2.87 (dd, 1H, $J_1 = 4.8$, $J_2 = 13.8$ Hz), 2.45–2.30 (m, 4H), 1.09, 1.02, 0.97, 0.89, 0.83, 0.78, 0.57 (s, 3H each); δ_C ($CDCl_3$) 177.9, 177.6, 173.7, 144.0, 136.6, 128.7 (2C), 128.3 (2C), 128.2, 122.3, 81.2, 70.7, 66.2, 55.1, 47.8, 46.9, 46.1, 44.2, 41.9, 41.6, 39.7, 39.6, 38.4, 34.1, 33.4, 32.7, 32.6, 30.9, 29.3, 29.1, 28.7, 27.8, 26.1, 23.9, 23.7, 23.3, 20.2, 18.5, 18.0, 17.1, 16.6; ESI-HRMS *m*/*z* calcd for $C_{42}H_{60}O_7Na$ [M + Na] 699.4237, found 699.4261; and **63** as a colourless oil (75 mg, 51%), $[\alpha]_D$ + 1 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3378, 3021, 2861 and 1725; δ_H ($CDCl_3$) 7.32 (m, 5H), 5.25 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.10 (ddd, 1H, $J_1 = 4.5$, $J_2 = 10.5$, $J_3 = 11.3$ Hz), 5.04 (AB system, 2H, $J = 12.0$ Hz), 4.75 (d, 1H, $J = 10.5$ Hz), 2.88 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.8$ Hz), 2.50–2.10 (m, 8H), 1.09, 1.02, 0.99, 0.88, 0.87, 0.87, 0.56 (s, 3H each); δ_C ($CDCl_3$) δ 178.6 (2C), 177.5, 172.9 (2C), 143.9, 136.6, 128.6 (2C), 128.2 (2C), 128.1, 122.3, 80.4, 70.2, 66.1, 55.1, 47.7, 46.9, 46.1, 44.0, 41.9, 41.5, 39.6, 39.5, 38.4, 34.1, 33.4 (2C), 33.3, 33.1 (2C), 32.7, 32.5, 30.9, 28.6, 27.8, 26.1, 23.8, 23.7, 23.2, 20.3, 20.2, 18.4, 17.9, 17.0, 16.6; ESI-HRMS *m*/*z* calcd for $C_{47}H_{66}O_{10}Na$ [M + Na] 813.4554, found 813.4523.

*4.5.38. Acylation of **MA-Bn** with 3,3-dimethylglutaric anhydride*

3,3-Dimethylglutaric anhydride (202 mg, 1.422 mmol) was added slowly to a solution of **MA-Bn** (100 mg, 0.177 mmol) in pyridine (2 mL). The mixture was treated as described in the general method for acylation to give **64** as a colourless oil (33 mg, 25%), $[\alpha]_D$ + 25 (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3383, 3010, 2829 and 1715; δ_H ($CDCl_3$) 7.31 (m, 5H), 5.25 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 5.04 (AB system, 2H, $J = 12.0$ Hz), 4.94 (ddd, 1H, $J_1 = 4.5$, $J_2 = 10.5$, $J_3 = 11.3$ Hz), 3.19 (d, 1H, $J = 10.5$ Hz), 2.87 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.7$ Hz), 2.45–2.35 (m, 4H), 1.14, 1.12, 1.10, 1.02, 0.98, 0.89, 0.87, 0.84, 0.56 (s, 3H each); δ_C ($CDCl_3$) 177.6 (2C) 172.8, 144.0, 136.6, 128.6 (2C), 128.2 (2C), 128.1, 122.4, 80.9, 73.5, 66.2, 55.3, 47.8, 46.9, 46.1, 45.4, 45.1, 43.9, 42.0, 41.6, 39.9, 39.6, 38.5, 34.1, 33.3, 33.0, 32.8, 32.6, 30.9, 28.8, 28.6, 28.5, 27.8, 26.1, 23.9, 23.7, 23.2, 18.5, 17.1, 16.9, 16.5; ESI-HRMS *m*/*z* calcd for $C_{44}H_{64}O_7Na$ [M + Na] 727.4550,

found 727.4552; and **65** as a colourless oil (71 mg, 47%), $[\alpha]_D + 10$ (*c* 1 in $CHCl_3$:MeOH, 2:1); IR ν_{max}(film)/cm^{-1} 3401, 3027, 2839 and 1732; δ_H ($CDCl_3$) 7.31 (m, 5H), 5.25 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.06 (ddd, 1H, $J_1 = 4.5$, $J_2 = 10.3$, $J_3 = 11.3$ Hz), 5.04 (AB system, 2H, $J = 12.0$ Hz), 4.77 (d, 1H, $J = 10.3$ Hz), 2.88 (dd, 1H, $J_1 = 4.3$, $J_2 = 13.8$ Hz), 2.55–2.25 (m, 8H), 1.12, 1.12, 1.09, 1.00, 0.89, 0.87, 0.87, 0.84, 0.56 (s, 3H each); δ_C ($CDCl_3$) 177.6, 177.1 (2C), 172.1, 171.9, 144.1, 136.6, 128.6 (2C), 128.2 (3C), 122.3, 80.4, 70.5, 66.2, 55.1, 47.7, 46.9, 46.1, 45.5 (2C), 45.1 (2C), 44.2, 42.0, 41.6, 39.6 (2C), 38.4, 34.1, 33.3, 32.6, 32.5, 31.1 (2C), 30.9, 28.8, 28.2 (2C), 28.0 (2C), 27.8, 26.1, 23.9, 23.7, 23.5, 18.5, 18.0, 17.1, 16.6; ESI-HRMS *m*/*z* calcd for $C_{51}H_{73}O_{10}$ [M − 1] 845.5204, found 845.5194.

4.6. General procedure for the quantization of the incorporation of *OA* and *MA* to the CTC resin

Step 1. Portions of 50 mg of CTC resin (loading 1.27 mmol/g) were placed in six polypropylene syringes (10 mL) fitted with polyethylene filter disks. The resin was washed with DMF (2 mL × 3) and DCM (2 mL × 3), swelled with DCM (2 mL, 20 min) and drained. Each syringe was treated with the corresponding solution of **OA** (**1**) or **MA** (**2**) and DIEA indicated below and placed in orbital agitation for 48 h. Afterwards, the resin was filtered, washed with DMF (2 mL × 3) and DCM (2 mL × 3), and then drained.

	OA (**1**)	DIEA	DCM
Test A (syringe 1)	14.5 mg, 0.5 equiv	110 μL, 10 equiv	1 mL
Test B (syringe 2)	29.0 mg, 1.0 equiv	220 μL, 20 equiv	1 mL
Test C (syringe 3)	87.0 mg, 3.0 equiv	660 μL, 60 equiv	1 mL
	MA (**2**)	DIEA	DCM
Test A (syringe 4)	15.0 mg, 0.5 equiv	110 μL, 10 equiv	1 mL
Test B (syringe 5)	29.9 mg, 1.0 equiv	220 μL, 20 equiv	1 mL
Test C (syringe 6)	89.9 mg, 3.0 equiv	660 μL, 60 equiv	1 mL

Step 2. The six syringes with the resin were now treated with the corresponding six solutions of Fmoc-Leu-OH (112 mg, 5 equiv each) and DIEA (110 μL, 10 equiv each) in DCM, and placed in orbital stirring for 2 h.

Step 3. For the capping of the resin, 0.5 mL of MeOH were added to each syringe and maintained with orbital stirring for 30 min. The resin was filtered, washed with DMF (2 mL × 3) and DCM (2 mL × 3) and then drained.

Step 4. The solutions of Fmoc-Gly, DIPCDI, DMAP in DCM/DMF specified below were added to the different syringes and placed in orbital stirring for 1 h. The syringes were filtered, rinsed with DCM and treated twice more with the same solutions. After the three treatments, the resin was filtered, washed with DMF (2 mL × 3) and DCM (2 mL × 3), and then drained.

	Fmoc-Gly-OH	DIPCDI	DMAP
Syringes 1, 2 and 3	188 mg, 10 equiv	98 μL, 10 equiv	7 mg, 1 equiv
Syringes 4, 5 and 6	377 mg, 20 equiv	196 μL, 20 equiv	15 mg, 2 equiv

Step 5. The cleavage of the OA or MA derivatives from the resin was performed by adding to each syringe a solution of TFA (1%) in DCM (2 min × 3).

The resulting mixtures of Fmoc-Gly-OA or Fmoc-Gly-MA and Fmoc-Leu-OH were then analyzed by HPLC. RP-HPLC analyses were carried out with a WATERS C18 reverse-phase column (0.4 μm diameter of particle; 150 mm × 3.9 mm) with a flow rate of 1 mL/min. A wavelength of 301 nm was selected for the purity analysis, using a linear gradient of 30–0% of B in 30 min and maintained for 5 min without B, where A was CH_3CN containing 0.1% TFA and B was H_2O containing 0.1% TFA.

4.6.1. Reaction of *OA* with Fmoc-Gly

A solution of Fmoc-Gly with DIPCDI and DMAP in DCM/DMF was added to a solution of **1** in DCM, as described in the above step 4 of the general procedure for the quantization of the incorporation of OA to the CTC resin. The reaction was stirred for 2 h at reflux. Then, cold water was added to the mixture and afterwards was extracted with DCM. The organic layer was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography using DCM/acetone (10:1) to give **66** as a white solid, with the percentages given in Table 3, mp 115–117 °C; $[\alpha]_D + 27$ (*c* 1 in $CHCl_3$); IR ν_{max}(KBr)/cm^{-1} 3620, 2941, 2395 and 1699; δ_H ($CDCl_3$) 7.79 (d, 2H, $J = 6.5$ Hz), 7.63 (d, 2H, $J = 6.5$ Hz), 7.45–7.29 (m, 4H), 5.31 (dd, 1H, $J_1 = J_2 = 3.3$ Hz), 4.62 (dd, 1H, $J_1 = J_2 = 7.5$ Hz), 4.43 (d, 1H, $J = 6.8$ Hz), 4.26 (t, 1H, $J = 6.8$ Hz), 4.01 (d, 2H, $J = 3.5$ Hz), 1.07, 1.01, 0.89, 0.87, 0.85, 0.84, 0.59 (s, 3H each); δ_C ($CDCl_3$) 184.0, 170.1, 156.5, 144.0 (2C), 143.8, 141.5 (2C), 127.9 (2C), 127.3 (2C), 125.3 (2C), 122.6, 120.2 (2C), 82.8, 67.4, 55.5, 47.8, 47.3, 46.7, 46.1, 43.2, 41.8, 41.1, 39.6, 38.2, 38.1, 37.2, 34.0, 33.3, 32.7, 32.6, 30.9, 28.3, 27.9, 26.1, 23.8 (2C), 23.7, 23.1, 18.4, 17.3, 16.8, 15.6; ESI-HRMS *m*/*z* calcd for $C_{47}H_{62}NO_6$ [M + 1] 736.4577, found 736.4570.

4.6.2. Reaction of *MA* with Fmoc-Gly

A solution of Fmoc-Gly with DIPCDI and DMAP in DCM/DMF was added to a solution of **2** in DCM, as described in the above step 4 of the general procedure for the quantization of the incorporation of MA to the CTC resin. The reaction was stirred for 12 h at rt. Then, cold water was added to the mixture and afterwards was extracted with DCM. The organic layer was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography using DCM/acetone (20:1) to give as white solids, with the percentages given in Table 3: **67**, mp 170–172 °C; $[\alpha]_D + 6$ (*c* 1 in $CHCl_3$); IR ν_{max}(KBr)/cm^{-1} 3636, 2930, 2389 and 1700; δ_H ($CDCl_3$) 7.76 (d, 2H, $J = 6.4$ Hz), 7.60 (d, 2H, $J = 6.4$ Hz), 7.43–7.29 (m, 4H), 5.26 (dd, 1H, $J_1 = J_2 = 3.3$ Hz), 5.06 (ddd, 1H, $J_1 = 4.5$, $J_2 = 10.5$, $J_3 = 11.3$ Hz), 4.42 (d, 2H, $J = 6.4$ Hz), 4.24 (t, 1H, $J = 6.4$ Hz), 4.02–3.92 (m, 4H) 3.19 (d, 1H, $J = 10.5$ Hz), 2.80 (dd, 1H, $J_1 = 3.4$, $J_2 = 10.5$ Hz), 1.12, 1.04, 1.03, 0.93, 0.92, 0.86 0.75 (s, 3H each); δ_C ($CDCl_3$) 182.5, 170.3, 157.3, 143.8, 143.8 (2C), 141.5 (2C), 127.9 (2C), 127.3 (2C), 125.7 (2C), 122.4, 120.2 (2C), 80.7, 74.9, 67.5, 55.3, 47.7, 47.2, 46.7, 46.0, 43.7, 43.3, 41.2, 39.9, 39.5, 38.6, 34.0, 33.2, 32.6 (2C), 30.8, 29.9, 28.7, 27.8, 26.1, 23.7, 23.6, 23.1 18.4, 17.2, 16.8, 16.5; ESI-HRMS *m*/*z* calcd for $C_{47}H_{62}NO_7$ [M + 1] 752.4526, found 752.4529; **68**, mp 143–145 °C; $[\alpha]_D + 3$ (*c* 1 in $CHCl_3$); IR ν_{max}(KBr)/cm^{-1} 3619, 2922, 2388 and 1705; δ_H ($CDCl_3$) 7.76 (d, 2H, 6.5 Hz), 7.59 (d, 2H, $J = 6.5$ Hz), 7.42–7.28 (m, 4H), 5.29 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 4.60 (d, 1H, $J = 12.5$ Hz), 4.42 (d, 2H, $J = 12.5$ Hz), 4.24 (t, 1H, $J = 6.5$), 4.05–4.00 (m, 4H) 3.80 (ddd, 1H, $J_1 = 3.5$, $J_2 = 10.5$, $J_3 = 12.5$ Hz), 2.83 (dd, 1H, $J_1 = 3.5$, $J_2 = 10.5$ Hz), 1.14, 0.98, 0.93, 0.91, 0.90, 0.85, 0.76 (s, 3H each); δ_C ($CDCl_3$) 185.32, 170.8, 157.3, 143.9, 143.8 (2C), 141.5 (2C), 127.9 (2C), 127.2 (2C), 125.2 (2C), 122.5, 120.2 (2C), 86.6, 67.5, 67.3, 55.3, 47.7, 47.3, 46.6, 46.0, 43.4, 41.9, 41.3, 39.5, 39.4, 38.4, 34.0, 33.2, 32.7, 32.6, 30.8, 29.9, 28.8, 27.8, 26.1, 23.7, 23.6, 23.1 18.4, 17.8, 17.3, 16.7; ESI-HRMS *m*/*z* calcd for $C_{47}H_{62}NO_7$ [M + 1] 752.4526, found 752.4525; and **69**, mp 121–123 °C; $[\alpha]_D - 1$ (*c* 1 in $CHCl_3$); IR ν_{max}(KBr)/cm^{-1} 3615, 2927, 2390 and 1710; δ_H ($CDCl_3$) 7.79 (d, 4H, $J = 6.5$ Hz), 7.63 (d, 4H, $J = 6.5$ Hz), 7.45–7.29 (m, 8H), 5.34 (ddd, 1H, $J_1 = 4.5$, $J_2 = 10.5$, $J_3 = 11.3$ Hz), 5.23 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 4.80 (d, 1H, $J = 10.5$ Hz), 4.18 (t, 2H, $J = 6.6$ Hz), 3.95–3.87 (m, 8H) 2.80 (dd, 1H, $J_1 = 3.5$, $J_2 = 13.4$ Hz),

1.11, 1.04, 0.85, 0.81, 0.81, 0.75 0.53 (s, 3H each); δ_C ($CDCl_3$) 183.7, 170.3, 169.9, 157.0 (2C), 143.9 (4C), 143.8, 141.4 (4C), 127.9 (4C), 127.3 (4C), 125.7 (4C), 122.2, 120.1 (4C), 82.1, 71.0, 67.5 (2C), 55.0, 47.7, 47.2 (2C), 46.7, 46.0, 43.9, 43.1, 42.9, 41.8, 41.1, 39.6, 39.5, 38.5, 34.0 (2C), 32.6, 32.5, 30.9, 28.6, 27.8, 26.1, 23.8, 23.6, 23.0 18.3, 17.8, 17.3, 16.6; ESI-HRMS *m/z* calcd for $C_{64}H_{75}N_2O_{10}$ [M + 1] 1031.5422, found 1031.5428.

4.7. General method for solid-phase acylation

Two portions of CTC resin (250 mg, 1.27 mmol/g) were placed in two polypropylene syringes (20 mL) fitted with polyethylene filter disks. The resin was washed with DMF (2 mL × 3) and DCM (2 mL × 3), swelled with DCM (2 mL, 20 min) and drained. **OA** (**1**) and **MA** (**2**) were incorporated into the resin as described in the above general procedure, and placed in orbital stirring for 24 h. Afterwards the resin was recoupled with the same solutions for another 24 h. For the capping of the resin, 0.5 mL of MeOH were added to each syringe and maintained with orbital stirring for 30 min. The resin was filtered, washed with DMF (2 mL × 3) and DCM (2 mL × 3), and then drained.

These two syringes were each split into four portions (1/4 each one) to obtain four syringes of resin-OA and other four syringes of resin-MA, which were now respectively acylated with acetic, hexanoic, benzoic, and succinic anhydrides at a molar relationship of 4:1 (anhydride:OH group), and placed in orbital agitation for 24 h at rt. These acylation reactions were conducted in the presence of DMAP (0.5 equiv), Et_3N (0.5 equiv) and DCM/DMF (1 mL/1 mL) as solvent. The resin was filtered, washed with DMF (2 mL × 3) and DCM (2 mL × 3), and then drained. The cleavage of the acyled derivatives from the resin was carried out by addition of each syringe of a solution of DCM with TFA (1%) (2 min × 3).

4.7.1. Solid-phase succinylation and consecutive solution-phase benzylation of *OA*

OA (150 mg, 0.33 mmol) was succinylated in solid-phase according with the general procedure described above. Then, the solvent was evaporated under reduced pressure and 110 mg of residue were obtained. BnCl (83 μL) was added in a relationship 2:1 to a solution of this residue in DMF (5 mL) with K_2CO_3 (0.12 g). The reaction was stirred for 4 h at 55 °C. Then, the reaction mixture was diluted with water and extracted with DCM, and the organic layer dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by column chromatography using DCM/acetone (10:1) to give benzyl oleanolate (**3**, 72 mg, 40.1%), and benzyl 3-(benzylsuccinyl) oleanolate (**70**, 133 mg, 54.9%) as a colourless oil; $[\alpha]_D$ + 77 (*c* 1 in $CHCl_3$); IR ν_{max}(film)/cm^{-1} 3293, 2985, 2815 and 1699; δ_H ($CDCl_3$) 7.36–7.35 (m, 10H), 5.31 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 5.13 and 5.07 (2 AB systems, 2H each, $J = 12.0$ Hz), 4.53 (dd, 1H, $J_1 = 5.5$, $J_2 = 10.6$ Hz), 2.93 (dd, 1H, $J_1 = 3.5$, $J_2 = 12.0$ Hz), 2.75–2.60 (m, 4H), 1.15, 0.94, 0.92, 0.91, 0.87, 0.85, 0.63 (s, 3H each); δ_C ($CDCl_3$) 178.4, 172.1, 171.8, 143.7, 136.5, 135.8, 128.6, 128.4 (2C), 128.3 (2C), 128.2 (2C), 128.0 (2C), 127.9, 122.4, 81.3, 66.5, 65.9, 55.3, 47.6, 46.8, 45.9, 41.7, 41.4, 39.3, 37.7, 36.9, 38.1, 33.9, 33.1, 32.9, 32.4, 30.7, 29.6, 29.3, 29.2, 28.1, 27.7, 25.9, 23.7, 23.5, 23.1, 18.2, 16.9, 16.8, 15.4; ESI-HRMS *m/z* calcd for $C_{48}H_{63}O_6$ [M − 1] 735.4625, found 735.4623.

4.7.2. Solid-phase succinylation and consecutive solution-phase benzylation of *MA*

MA (472 mg, 1 mmol) was succinylated in solid-phase according with the general procedure described above. Then, the solvent was evaporated under reduced pressure and 390 mg of residue were obtained. BnCl (364 μL) was added in a relationship 2:1 to a solution of this residue in DMF (8 mL) with K_2CO_3 (0.53 g). The reaction was stirred for 4 h at 55 °C. Then, the reaction mixture was diluted with water and extracted with DCM, and the organic layer dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by column chromatography using DCM/acetone (10:1) to give benzyl maslinate (**4**, 32 mg, 5.7%); benzyl 2-(benzylsuccinyl) maslinate (**71**, 57 mg, 7.6%) as a colourless oil; $[\alpha]_D$ + 6 (*c* 1 in $CHCl_3$); IR ν_{max}(film)/cm^{-1} 3301, 3012, 2915 and 1783; δ_H ($CDCl_3$) 7.36–7.34 (m, 10H), 5.27 (dd, 1H, $J_1 = J_2 = 3.4$ Hz), 5.14 and 5.08 (2 AB systems, 2H each, $J = 12.0$ Hz), 5.00 (ddd, 1H, $J_1 = 3.0$, $J_2 = 6.0$, $J_3 = 10.5$ Hz), 3.14 (d, 1H, $J = 6.0$ Hz), 2.91 (dd, 1H, $J_1 = 3.5$, $J_2 = 12.0$ Hz), 2.75–2.60 (m, 4H), 1.12, 1.05, 0.99, 0.92, 0.90, 0.86, 0.59 (s, 3H each); δ_C ($CDCl_3$) 177.5, 172.7, 172.5, 143.9, 136.5, 135.8, 128.7, 128.5 (2C), 128.4 (2C), 128.3 (2C), 128.1 (2C), 128.0, 122.3, 80.7, 73.9, 66.8, 66.1, 55.2, 47.6, 46.8, 46.0, 43.6, 41.8, 41.5, 39.7, 39.4, 38.4, 34.0, 33.2, 32.7, 32.5, 30.8, 29.7, 29.6, 28.7, 27.7, 26.0, 23.8, 23.6, 23.1, 18.3, 17.0, 16.8, 16.4; ESI-HRMS *m/z* calcd for $C_{48}H_{65}O_7$ [M + 1] 753.4730, found 753.4714; benzyl 3-(benzylsuccinyl) maslinate (**72**, 43 mg, 5.5%) as a colourless oil; $[\alpha]_D$ + 3 (*c* 1 in $CHCl_3$); IR ν_{max}(film)/cm^{-1} 3343, 3076, 2915 and 1783; δ_H ($CDCl_3$) 7.35–7.34 (m, 10H), 5.29 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.13 and 5.07 (2 AB systems, 2H each, $J = 12.5$ Hz), 4.57 (d, 1H, $J = 6.5$ Hz), 3.75 (ddd, 1H, $J_1 = 3.5$, $J_2 = 6.5$, $J_3 = 10.5$ Hz), 2.91 (dd, 1H, $J_1 = 3.5$, $J_2 = 12.0$ Hz), 2.80–2.65 (m, 4H), 1.13, 0.95, 0.92, 0.90, 0.87, 0.84, 0.60 (s, 3H each); δ_C ($CDCl_3$) 177.5, 173.0, 172.9, 143.8, 136.5, 135.7, 128.7, 128.6 (2C), 128.5 (2C), 128.3 (2C), 128.1 (2C), 128.0, 122.4, 85.6, 67.2, 66.9, 66.0, 55.2, 47.5, 46.9, 46.8, 45.8, 41.8, 41.5, 39.5, 39.3, 38.2, 34.0, 33.2, 32.5, 32.4, 30.8, 29.5, 29.4, 28.5, 27.6, 25.9, 23.6, 23.4, 23.0, 18.3, 17.7, 16.9, 16.6; ESI-HRMS *m/z* calcd for $C_{48}H_{65}O_7$ [M + 1] 753.4730, found 753.4722; and benzyl 2,3-di(benzylsuccinyl) maslinate (**73**, 721 mg, 76.5%) as a colourless oil; $[\alpha]_D$ + 57 (*c* 1 in $CHCl_3$); IR ν_{max}(film)/cm^{-1} 3343, 3076, 2915 and 1783; δ_H ($CDCl_3$) 7.38–7.31 (m, 15H), 5.29 (dd, 1H, $J_1 = J_2 = 3.5$ Hz), 5.14 (ddd, 1H, $J_1 = 3.0$, $J_2 = 6.0$, $J_3 = 10.5$ Hz), 5.12 and 5.04 (m, 6H each), 4.79 (d, 1H, $J = 6.0$ Hz), 2.93 (dd, 1H, $J_1 = 3.5$, $J_2 = 12.0$ Hz), 2.67–2.57 (m, 8H), 1.14, 1.03, 1.02, 0.94, 0.92, 0.89, 0.60 (s, 3H each); δ_C ($CDCl_3$) 177.4, 172.2, 172.1, 172.0, 171.9, 143.7, 136.5, 135.8 (2C), 128.7, 128.5 (4C), 128.4 (4C), 128.3 (4C), 128.1, 128.0, 122.1, 80.7, 70.3, 66.5, 66.4, 66.0, 54.9, 47.5, 46.7, 45.8, 43.8, 41.7, 41.4, 39.5, 39.3, 38.1, 33.9, 33.2, 32.5, 32.4, 30.8, 29.3, 29.2, 29.1, 29.0, 28.4, 27.6, 25.9, 23.7, 23.5, 23.1, 18.2, 17.7, 16.8, 16.4; ESI-HRMS *m/z* calcd for $C_{59}H_{75}O_{10}$ [M + 1] 943.5360, found 943.5335.

4.8. Biological experimental procedures

4.8.1. Drugs

The different compounds used in cell treatment were dissolved before use at 10 mg/mL in 50% DMSO and 50% PBS. A stock solution was frozen and stored at − 20 °C. Prior to the experiments, this solution was diluted in cell-culture medium. Apoptosis and mitochondrial-membrane potential were measured at the IC_{50} (concentration causing 50% reduction in growth compared to the control after 72 h of treatment) and the IC_{50} × 2 (twice IC_{50} concentration. ×2 value corresponding to 50% viability) concentrations.

4.8.2. Cell culture

Murine melanoma cell line b16f10 (ECACC CRL-6475, provided by the cell bank of the University of Granada, Spain) was cultured in DMEM supplemented with 2 mM glutamine, 10% heat-inactivated FCS, 10,000 units/mL of penicillin and 10 mg/mL of streptomycin. Subconfluent monolayer cells were used in all experiments.

4.8.3. Cell-proliferation activity assay

The effect of treating each product upon proliferation in b16f10 murine melanoma cells was measured using the MTT assay (Sigma,

MO, USA), which is based on the ability of live cells to cleave the tetrazolium ring, thus producing formazan, which absorbs at 570 nm.

Cell viability was determined by measuring the absorbance of MTT dye staining of living cells [33]. For this assay, $11 \cdot 10^3$ b16f10 cells were grown on a 96-well plate and incubated with the different products (0–80 μg/mL). After 72 h, 100 μL of MTT solution (0.5 mg/mL) was added to each well. After 2 h of incubation the cells were washed twice with PBS and the formazan was resuspended in 200 μL DMSO. Relative cell viability was measured by absorbance at 550 nm on an ELISA plate reader (Tecan Sunrise MR20-301, TECAN, Austria).

4.8.4. Annexin V–FICT/propidium iodide flow-cytometric analysis

The extension apoptosis was analyzed with flow-cytometry by using a FACScan flow-cytometer (fluorescence-activated cell sorter) (Coulter Corporation, Hialeah, FL, USA). In brief, $11 \cdot 10^4$ b16f10 cells were plated in 12-well plates with 2 mL of medium following treatment with the compounds for 72 h, at the IC_{50} concentrations, calculated previously, and at twice the IC_{50} concentrations ($IC_{50} \times 2$). The cells were collected and resuspended in binding buffer (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$). Annexin V–FITC conjugate (1 μg/mL) was added and incubated for 30 min at rt in darkness. Just before FACS analysis, cells were stained with 20 μL of 1 mg/mL PI solution. In each experiment, approximately $20 \cdot 10^3$ cells were analyzed and the experiment was duplicated three times.

4.8.5. Flow-cytometry analysis of the mitochondrial-membrane potential

Oxidative damage was studied by flow-cytometry analysis of the ROS levels, using dihydrorhodamine 123 oxidized to the highly fluorescent product rhodamine. The formation of rhodamine can be monitored by fluorescence spectroscopy using excitation and emission wavelengths of 500 and 536 nm, respectively. The intracellular measurement of the reactive oxygen species was made by cytometry determination of rhodamine 123. In the same way as in the apoptosis assays, $11 \cdot 10^4$ b16f10 cells were plated in 12-well plates, and treated with the compounds that exhibited cytotoxic activity for 72 h, at the IC_{50} and $IC_{50} \times 2$ concentrations. After treatment, the medium was removed and a fresh medium with DHR, at a final concentration of 5 μg/mL, was added. After 30 min of incubation, the medium was removed and the cells were washed and resuspended in PBS with 5 μg/mL. The intensity of fluorescence from Rh123 and PI was determined using a FACScan flow-cytometer (fluorescence-activated cell sorter) (Coulter Corporation, Hialeah, FL, USA).

4.8.6. Anti-HIV protease activity

The HIV activity was determined using recombinant HIV protease from *E. coli* source (PQITLWQRPL VTIKIGGQLK EALLDTGADD TVLEEMNLPG RWKPKMIGGI GGFIKVRQYD QILIEICGHK AIGTVLVGPT PVNIIGRNLL TQIGCTLNF) from BioVendor GmbH (Heidelberg, Germany). The proteolytic activity of HIV-1-protease was measured using the FRET (fluorescence resonance energy transfer). The synthetic peptide (Abz-Ala-Arg-Val-Nle-Tyr(NO_2)-Glu-Ala-Nle-NH_2) from Sigma (St. Louis, MO, USA) corresponding to the p-24-p17 cleavage site in the natural gag precursor was used as substrate. In the FRET HIV-1 PR substrate, the fluorescence was quenched by an appropriate fluorescence quencher until this peptide was cleaved into two separate fragments by HIV-1-protease at the cleavage site; when the peptide was cleaved, the fluorescence was monitored.

The decrease in the percentage of the fluorescence activity was used to determine the percentage of the activity inhibition, and was calculated according to: % inhibition $= [1 - (V_{inh}/V_0)] \cdot 100$ (V_{inh} = enzymatic activity in the presence of the inhibitor; V_0 = enzymatic activity of the control). The assays were performed with a solution containing HIV-1-protease (10 ηM), the fluorogenic substrate (10 μM) in an assay buffer (50 mM sodium acetate, 1 mM EDTA, 0.5 mM DTT, 1 M NaCl, 2.5% glycerol), and an increasing concentrations of inhibitor from 0.17 μg/mL to 1.7 mg/mL, in a total volume of 140 μL. The fluorescence was analyzed using an LS 50B fluorescence spectrometer at excitation/emission = 320 nm/420 nm.

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