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Solid-Phase Library Synthesis of Bi-Functional Derivatives of Oleanolic and Maslinic Acids and Their Cytotoxicity on Three Cancer Cell Lines

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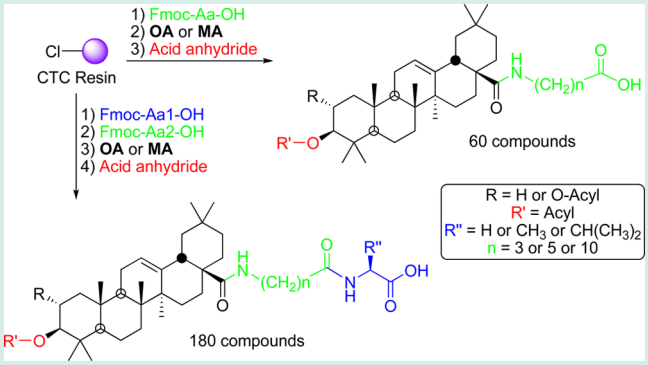
Supporting Information

ABSTRACT: A wide set of 264 compounds has been semisynthesized with high yields and purities. These compounds have been obtained through easy synthetic processes based on a solid-phase combinatorial methodology. All the members of this library have one central core of a natural pentacyclic triterpene (oleanolic or maslinic acid) and differ by 6 amino acids, coupled with the carboxyl group at C-28 of the triterpenoid skeleton, and by 10 different acyl groups attached to the hydroxyl groups of the A-ring of these molecules. According to the literature on the outstanding and promising pharmacological activities of other similar terpene derivatives, some of these compounds have been tested for their cytotoxic effects on the proliferation of three cancer cell lines: B16−F10, HT29, and Hep G2. In general, we have found that around 70% of the compounds tested show cytotoxicity in all three of the cell lines selected; around 60% of the cytotoxic compounds are more effective than their corresponding precursors, that is, oleanolic (OA) or maslinic (MA) acids; and nearly 50% of the cytotoxic derivatives have IC_{50} values between 2- to 320-fold lower than their corresponding precursor (OA or MA).



KEYWORDS: *combinatorial chemistry, solid-phase synthesis, triterpene, acylation, oleanolic, maslinic*

INTRODUCTION

Natural products play a major role in drug development and chemical biology.[1−3] In fact, nearly half of the new drugs introduced into the market in the last three decades have been natural products or their derivatives.[4,5] Natural products are promising scaffolds for diversification by using combinatorial methods to establish several libraries of products offering potentially valuable activities.[6−8]

Combinatorial chemistry is a quick and very useful tool for the semisynthesis of thousands of organic compounds with potential pharmacological activities.[9,10] In the past few decades, a large number of combinatorial libraries have been constructed, and they significantly supplement the chemical diversity of the traditional collections of potentially active medicinal compounds.[11] The development of high-throughput screenings based on molecular targets has led to a demand for the generation of large libraries of compounds to satisfy the enormous capacities of these screens. Combinatorial chemistry was envisioned as the answer to this demand, initially focusing on the synthesis of peptide and oligonucleotide libraries but is now reported to be shifting its focus to the synthesis of small, drug-like molecules.[12]

Solid-phase synthesis has been used to enrich combinatorial chemistry libraries; through the use of solid supports and their modified forms.[13−15] Progress in solid-phase organic synthesis has enabled the current combination of natural-product synthesis with combinatorial methods. The strategy most frequently followed has been to attach a natural product core structure to a solid phase and subsequently modify the functional groups. Solid-phase organic synthesis has emerged as a powerful tool in the synthesis of small molecules as a means of exploiting combinatorial chemistry to discover new effective products.[16,17]

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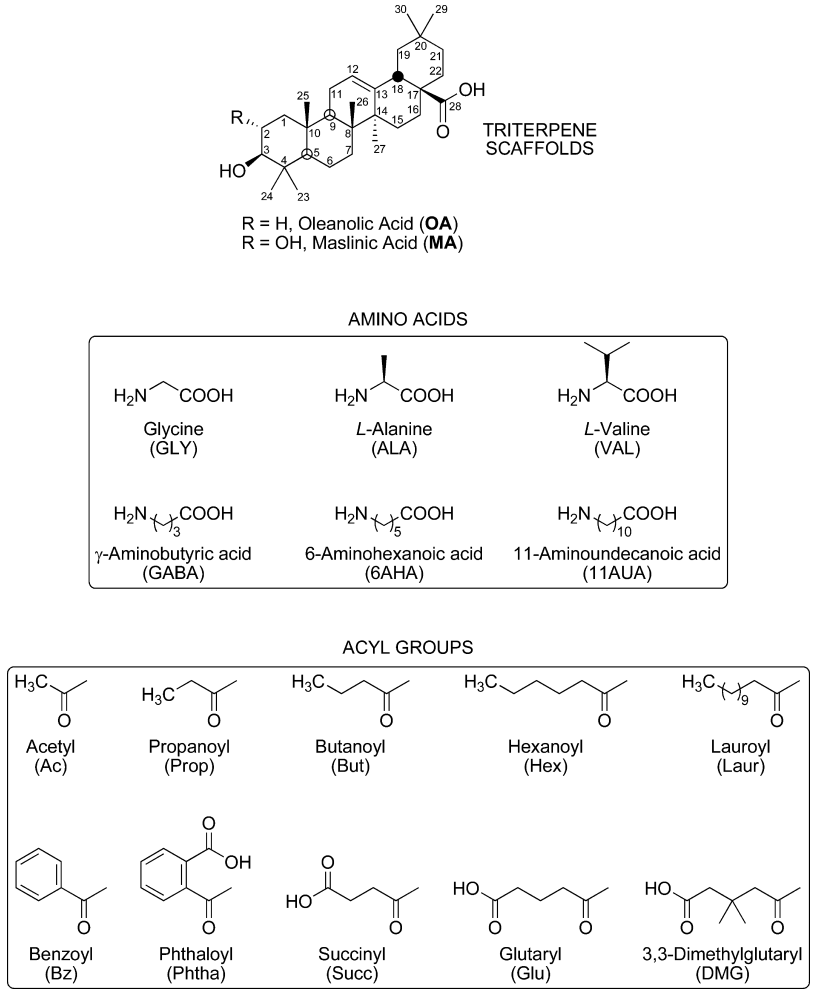

Figure 1. Triterpene scaffolds and structures of the amino acids and acyl groups used for the construction of the library of the OA or MA derivatives.

Triterpenoids, natural substances present in many plants in nature, belong to a wide family of compounds obtained biosynthetically by cyclic reactions from squalene. Biologically, the most important triterpenoid structures are oleanane, ursane, lupane, and dammarane triterpenoids.[18] These compounds are used in conventional medicine for their anti-inflammatory, hepatoprotective, analgesic, antimicrobial, virostatic, anticancer, and anti-HIV effects.[19−30] Chemical modifications of these natural triterpene compounds have resulted in products that have improved the biological activities of their precursors.[31−33] In recent years, much work has been focused on modifying several triterpenic acids at the C-3 or C-28 positions, forming acyl or amino acid derivatives, in order to increase their hydrosolubility and biological activity.[34−43] In this sense, we have performed the solution- or solid-phase semisynthesis of several C-3 and C-28 derivatives of oleanolic or maslinic acid. We have evaluated them for their antiproliferative and antiviral effects, finding promising results as anticancer and as anti-HIV agents.[44−46]

Oleanolic (3β-hydroxyolean-12-en-28-oic acid, OA)[47,48] and maslinic (2α,3β-dihydroxyolean-12-en-28-oic acid, MA)[49] acids, which belong to these kinds of natural products with remarkable biological properties,[50−52] are widely found in nature and are present in high concentrations in olive-pomace oil, being the main components of the protective wax-like coating of the olive skin. A method of producing large amounts of both compounds from these solid wastes has been reported by our group.[53]

In this work, we have semisynthesized a library of 240 compounds, through combinatorial solid-phase procedures, using the above-mentioned two natural triterpene acids (OA or MA) as scaffolds. These compounds are bifunctional OA or MA derivatives, formed by the combinatorial attachment of 6 different amino acids to the carboxylic group at C-28 of the triterpene, and subsequent acylation with 10 different acid anhydrides in the hydroxyl groups of the A-ring of the triterpene skeleton. We have also examined the cytotoxic effects of these OA or MA derivatives on the proliferation of three cancer cell lines: HT29, Hep G2, and B16−F10. One of our main objectives has been to find new triterpene derivatives with greater cytotoxic and antitumor properties than the natural compounds from which they derive. We have explored the importance of the polarity, molecular volume, and substituent position in the cytotoxicity induced by these triterpenes derivatives of OA or MA. Agents that suppress the proliferation of malignant cells, by inducing cytotoxic effects, may represent a useful mechanistic approach to chemoprevention and chemotherapy of cancer. With these effects, these new triterpene derivatives may provide a useful new strategy for different types of cancer.

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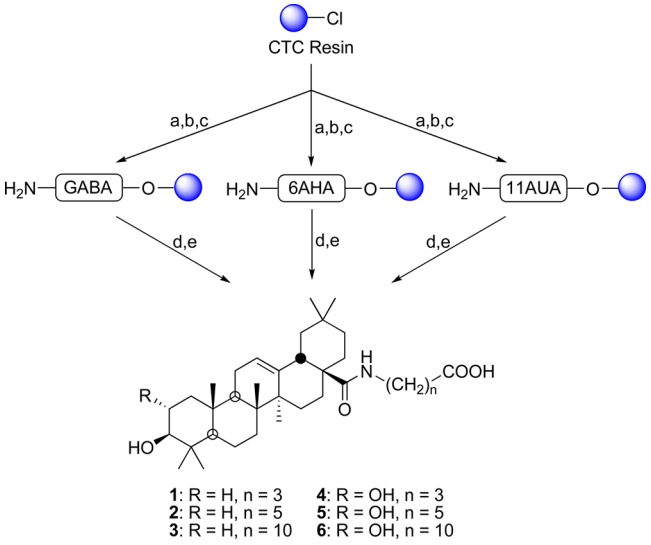
RESULTS AND DISCUSSION

The triterpene scaffolds represent a promising pharmacophore for synthesizing libraries to obtain derivatives with improved biological activities. Thus, to prepare a broad library of acyl-triterpene-amino-acid derivatives, we used as carbon scaffolds: oleanolic acid (OA) or maslinic acid (MA), two natural triterpene acids from olive-oil industry residues; 6 amino acids (glycine, GLY; alanine, ALA; valine, VAL; γ-aminobutyric acid, GABA; 6-aminohexanoic acid, 6AHA; 11-aminoundecanoic acid, 11AUA); and 10 acid anhydrides (acetic, propanoic, butanoic, hexanoic, lauric, benzoic, phthalic, succinic, glutaric, and 3,3-dimethylglutaric anhydrides) (Figure 1). The above-mentioned triterpenoids show two-point diversity: the C-28 carboxyl group and one or two hydroxyl groups in the A-ring of the triterpene skeleton. The cited amino acids were attached to the C-28 carboxyl group, and subsequently, the hydroxyl groups at C-2 (for OA) or at C-2 and C-3 (for MA) were acylated with the 10 above-described acid anhydrides, to introduce more diversity into these compounds, following appropriate solid-phase protocols in both processes.

Peptide Conjugation. The semisynthesis of the library of these compounds commenced with the solid-phase construction of the mono- or dipeptidyl OA or MA derivatives (**1**–**24**) (Schemes 1 and 2). Our solid-phase strategy was initially focused on the coupling of one or two amino acid fragments with the carboxyl group at C-28 of the triterpene skeleton. For these solid-phase reactions we chose the 2-chlorotrityl chloride polymer resin (CTC-resin) as a solid support. This resin, which allows the release of compounds by treatment with low concentrations of acid, is the only one suitable for the preparation of OA or MA derivatives, because these triterpene acids are not totally stable at high TFA concentrations.

To obtain the monopeptidyl derivatives of OA (**1**–**3**) or MA (**4**–**6**), we prepared six syringes with 100 mg each one of the CTC-resin. After washing with DMF and DCM, and with an adequate solvation of the resin (DCM), three ω-Fmoc-amino acids: Fmoc-GABA-OH (syringes 1 and 2), Fmoc-6AHA-OH (syringes 3 and 4), and Fmoc-11AUA-OH (syringes 5 and 6), were coupled onto the resin through their carboxyl group using DIEA as the coupling agent (Scheme 1). After 1.5 h of orbital stirring, a capping step with MeOH was carried out, and later the Fmoc group was removed with piperidine-DMF (1:4). After new washings with DMF and DCM, OA (syringes 1, 3, and 5) or MA (syringes 2, 4, and 6) were added using PyAOP, HOAt, and DIEA as coupling agents. After 24 h of reaction in orbital stirring at rt, the ninhydrin test proved negative and the monopeptidyl OA (**1**–**3**) or MA (**4**–**6**) derivatives were washed with DMF, MeOH, and DCM, and cleaved from the solid support by treatment with trifluoroacetic acid (TFA/DCM, 1:99). These derivatives were lyophilized, weighed, and analyzed by HPLC, determining their retention times, reaction yields, and purities, which were consistently higher than 90% (see Experimental Section). The structures of compounds **1**–**6** were easily established from their ^{1}H and ^{13}C NMR data compared with those of their corresponding precursors (**OA** or **MA**). Thus, the most significant differences in the signals of the NMR spectra of compounds **1**–**6** were those of the amide group formed between the carboxyl group at C-28 of the

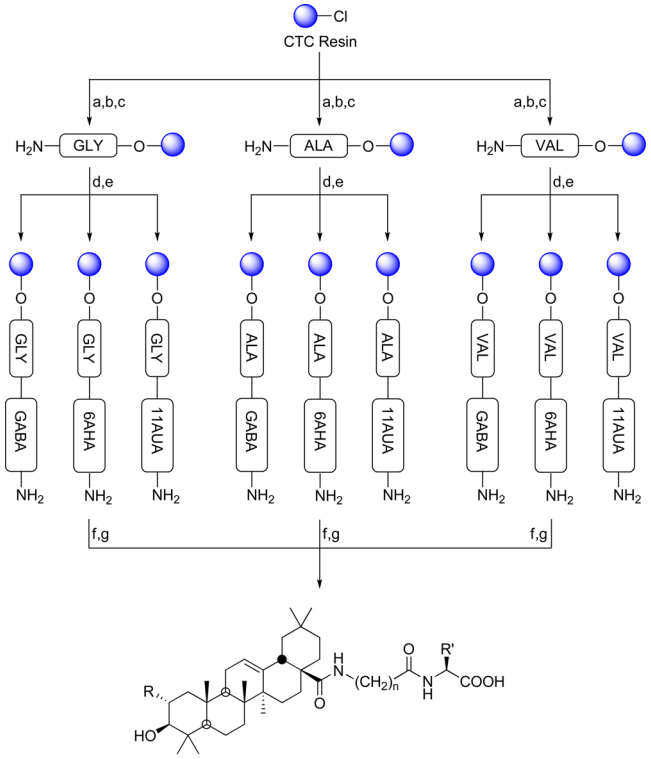
Scheme 1. Semi-synthesis of Monopeptidyl OA (1–3) or MA (4–6) Derivatives[a]



1: R = H, n = 3 **4**: R = OH, n = 3
2: R = H, n = 5 **5**: R = OH, n = 5
3: R = H, n = 10 **6**: R = OH, n = 10

[a]Reagents and conditions: (a) Fmoc-GABA-OH, Fmoc-6AHA-OH, or Fmoc-11AUA-OH, DIEA, DCM; (b) MeOH; (c) piperidine-DMF (1:4); (d) OA or MA, PyAOP, HOAt, DIEA, DMF; (e) TFA-DCM (1:99).

Scheme 2. Semi-synthesis of dipeptidyl OA (7–15) or MA (16–24) derivatives[a]



7: R = H, R' = H, n = 3
8: R = H, R' = H, n = 5
9: R = H, R' = H, n = 10
10: R = H, R' = CH_3, n = 3
11: R = H, R' = CH_3, n = 5
12: R = H, R' = CH_3, n = 10
13: R = H, R' = $CH(CH_3)_2$, n = 3
14: R = H, R' = $CH(CH_3)_2$, n = 5
15: R = H, R' = $CH(CH_3)_2$, n = 10
16: R = OH, R' = H, n = 3
17: R = OH, R' = H, n = 5
18: R = OH, R' = H, n = 10
19: R = OH, R' = CH_3, n = 3
20: R = OH, R' = CH_3, n = 5
21: R = OH, R' = CH_3, n = 10
22: R = OH, R' = $CH(CH_3)_2$, n = 3
23: R = OH, R' = $CH(CH_3)_2$, n = 5
24: R = OH, R' = $CH(CH_3)_2$, n = 10

[a]Reagents and conditions: (a) Fmoc-GLY-OH, Fmoc-6ALA-OH, or Fmoc-VAL-OH, DIEA, DCM; (b) MeOH; (c) piperidine-DMF (1:4); (d) Fmoc-GABA-OH, Fmoc-6AHA-OH, or Fmoc-11AUA-OH, HOAt, DIPCDI, DMF; (e) piperidine-DMF (1:4); (f) OA or MA, PyAOP, HOAt, DIEA, DMF; (g) TFA-DCM (1:99).

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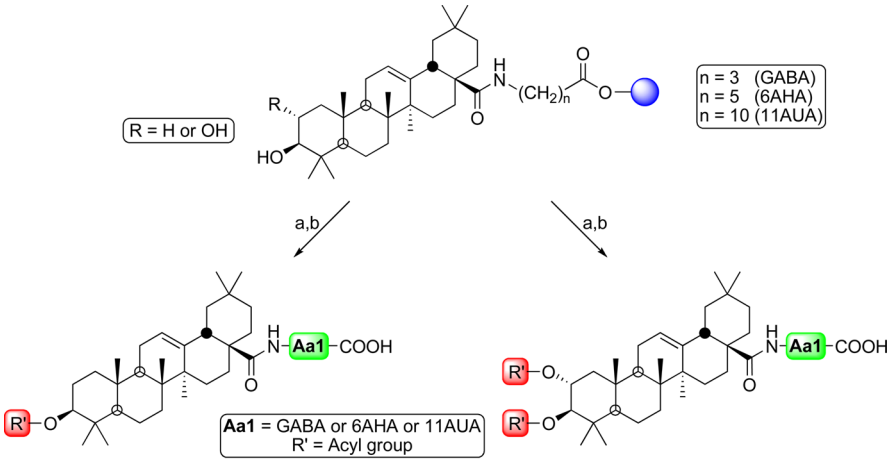
Scheme 3. Semi-synthesis of Monopeptidyl OA or MA Acyl Derivatives (1a−1j to 6a−6j).[a]



	Acyl monopeptidyl **OA** derivatives			Acyl monopeptidyl **MA** derivatives		
	GABA	6AHA	11AUA	GABA	6AHA	11AUA
Ac	**1a**	**2a**	**3a**	**4a**	**5a**	**6a**
Prop	**1b**	**2b**	**3b**	**4b**	**5b**	**6b**
But	**1c**	**2c**	**3c**	**4c**	**5c**	**6c**
Hex	**1d**	**2d**	**3d**	**4d**	**5d**	**6d**
Laur	**1e**	**2e**	**3e**	**4e**	**5e**	**6e**
Bz	**1f**	**2f**	**3f**	**4f**	**5f**	**6f**
Phtha	**1g**	**2g**	**3g**	**4g**	**5g**	**6g**
Succ	**1h**	**2h**	**3h**	**4h**	**5h**	**6h**
Glu	**1i**	**2i**	**3i**	**4i**	**5i**	**6i**
DMG	**1j**	**2j**	**3j**	**4j**	**5j**	**6j**

[a]Reagents and conditions: (a) Acid anhydride, Et_3N, DMAP, DMF, DCM; (b) TFA-DCM (1:99).

triterpene and the amino group of the corresponding amino acid. These signals invariably appeared as double doublets at δ_H 6.10−6.20 for compounds **1**−**3** (in Cl_3CD) and δ_H 7.20−7.30 for compounds **4**−**6** (in DMSO-d_6). In their ^{13}C NMR spectra, two signals of carboxyl groups were present, one more deshielded, corresponding to the carbon atom of the peptide bond, and another less deshielded, corresponding to the free carboxyl group of the amino acid.

In a similar way, the dipeptidyl OA (**7**−**15**) or MA (**16**−**24**) derivatives were prepared attaching two amino acids to the carboxyl group at C-28 of the triterpene (Scheme 2). The synthesis started by putting three portions of CTC-resin into three syringes (labeled A, B, and C). Working as indicated above, we added Fmoc-GLY-OH, Fmoc-ALA-OH, or Fmoc-VAL-OH, to the syringes A, B, and C, respectively, stirring the mixture at rt for 1.5 h. Afterward, the above-mentioned capping, washing, and deprotecting processes were performed on the three syringes, whereupon each syringe was split into three identical aliquots (Scheme 2).

The nine fractions of monopeptidyl-resin thus obtained, were then treated with the three ω-amino acids: Fmoc-GABA-OH (syringes A-1, B-1, and C-1), Fmoc-6AHA-OH (syringes A-2, B-2, and C-2), and Fmoc-11AUA-OH (syringes A-3, B-3, and C-3). For these reactions, HOAt and DIPCDI were used as couplings agents. After 1.5 h of orbital stirring at rt, the ninhydrin test proved negative in all the syringes. Then, each dipeptidyl-resin was again subjected to the above-mentioned removal of the Fmoc group and washing processes. At this time, i.e. of the solid-phase reaction sequence, each syringe was again split into two identical portions to couple each dipeptidyl-resin with the triterpene acids (OA or MA; Scheme 2). These coupling reactions were performed in the presence PyAOP, HOAt, and DIEA. After 24 h of reaction at rt and orbital stirring, the ninhydrin test proved negative in all the syringes, and each dipeptidyl OA (**7**−**15**) or MA (**16**−**24**) derivative was cleaved from its solid support by treatment with TFA/DCM. These derivatives were lyophilized, weighed, and analyzed by HPLC, determining their retention times, reaction yields, and purities, which were consistently higher than 90% (see Experimental Section). The structures of the dipeptidyl derivatives of OA (**7**−**15**) or MA (**16**−**24**) were also determined from their ^1H and ^{13}C NMR data by comparison with those of their corresponding precursors (OA or MA). In these derivatives, the most significant differences in the signals of the NMR spectra were those of the amide groups occurring between the carboxyl group at C-28 of the triterpene and the amino group of the corresponding ω-amino acid (GABA, 6AHA, or 11AUA), and between the carboxyl group of these ω-amino acids and the amino group of the corresponding α-amino acids (GLY, ALA, or VAL). The most deshielded NH signals appeared as double doublets at δ_H 7.50−6.30 for compounds **7**−**15** (in Cl_3CD), and around δ_H 8.00 for compounds **16**−**24** (in DMSO-d_6), corresponding to the α-amino acids. The other NH signals were situated at δ_H 6.50−6.10 for the OA derivatives (**7**−**15**) and around δ_H 7.20 for the MA derivatives (**16**−**24**), and corresponded to the ω-amino acids. In the ^{13}C NMR spectra of all these derivatives, there were three signals of carboxyl group: the peptide group formed between the carboxyl group at C-28 and the amino group of the ω-amino acids (around δ_C 180, **7**−**15**; δ_C 176, **16**−**24**), the peptide group between the ω- and the α-amino acids (around δ_C 175, for OA derivatives; δ_C 174−172, for MA derivatives),

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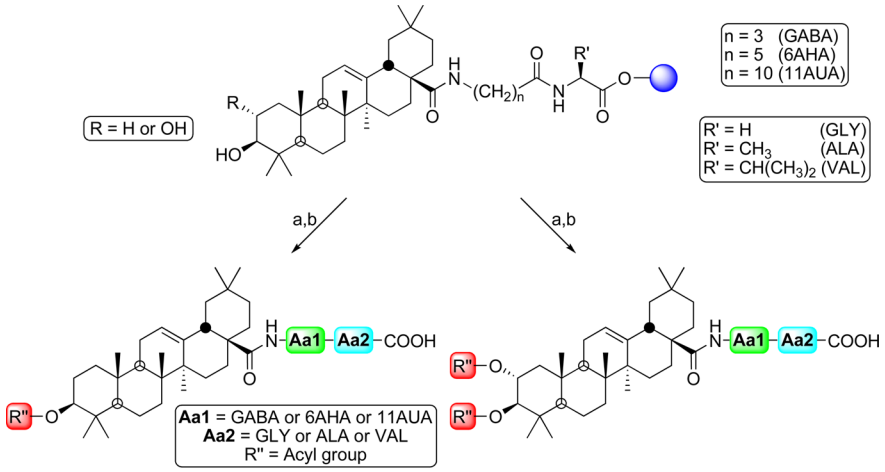
Scheme 4. Semi-synthesis of Dipeptidyl OA or MA Acyl Derivatives (7a−7j to 24a−24j)[a]



	Acyl dipeptidyl **OA** derivatives								
	GABA GLY	6AHA GLY	11AUA GLY	GABA ALA	6AHA ALA	11AUA ALA	GABA VAL	6AHA VAL	11AUA VAL
Ac	7a	8a	9a	10a	11a	12a	13a	14a	15a
Prop	7b	8b	9b	10b	11b	12b	13b	14b	15b
But	7c	8c	9c	10c	11c	12c	13c	14c	15c
Hex	7d	8d	9d	10d	11d	12d	13d	14d	15d
Laur	7e	8e	9e	10e	11e	12e	13e	14e	15e
Bz	7f	8f	9f	10f	11f	12f	13f	14f	15f
Phtha	7g	8g	9g	10g	11g	12g	13g	14g	15g
Succ	7h	8h	9h	10h	11h	12h	13h	14h	15h
Glu	7i	8i	9i	10i	11i	12i	13i	14i	15i
DMG	7j	8j	9j	10j	11j	12j	13j	14j	15j

	Acyl dipeptidyl **MA** derivatives								
Ac	16a	17a	18a	19a	20a	21a	22a	23a	24a
Prop	16b	17b	18b	19b	20b	21b	22b	23b	24b
But	16c	17c	18c	19c	20c	21c	22c	23c	24c
Hex	16d	17d	18d	19d	20d	21d	22d	23d	24d
Laur	16e	17e	18e	19e	20e	21e	22e	23e	24e
Bz	16f	17f	18f	19f	20f	21f	22f	23f	24f
Phtha	16g	17g	18g	19g	20g	21g	22g	23g	24g
Succ	16h	17h	18h	19h	20h	21h	22h	23h	24h
Glu	16i	17i	18i	19i	20i	21i	22i	23i	24i
DMG	16j	17j	18j	19j	20j	21j	22j	23j	24j

[a]Reagents and conditions: (a) Acid anhydride, Et_3N, DMAP, DMF, DCM; (b) TFA-DCM (1:99).

and the free carboxyl group of the α-amino acids (around δ_C 174−172 for all derivatives).

Acylation Reaction. In an effort to increase the diversity of this type of compounds, these mono- or dipeptidyl OA or MA derivatives (**1**−**24**) were acylated in the hydroxyl groups at C-3 or C-2/C-3 of the A-ring of the triterpene skeleton. These acylation reactions were performed in solid phase with 10 selected acid anhydrides in the presence of Et_3N and DMAP as catalysts (Schemes 3 and 4). The 10 acid anhydrides for these acylation processes (Figure 1) had diverse physical and chemical properties. In this sense, several acid anhydrides were chosen with aliphatic carbon chains of different lengths or with an aromatic ring, and also cyclic acid anhydrides, which originated acyl groups with an additional terminal carboxyl group. Thus, 10 portions of the monopeptidyl derivatives of OA (**1**−**3**) were prepared as previously described and, before being cleaved from de CTC-resin; they were treated with the above-mentioned 10 acid anhydrides, and were subsequently cleaved from the solid support by treatment with TFA/DCM, collected, and lyophilized. Thus, 30 new 3-acylated-28-monopeptidyl AO derivatives (**1a**−**1j**, **2a**−**2j**, and **3a**−**3j**) (Scheme 3) were obtained and analyzed by HPLC and HRMS.

In all the cases, their purities were higher than 90% and their molecular masses were consistent with the expected structures (see Experimental Section and Supporting Information). In a similar way, the MA monopeptidyl derivatives (**4**−**6**) were acylated with an excess of the above-mentioned 10 acid anhydrides, to obtain mainly the doubly acylated compound at the hydroxyl groups at C-2 and C-3 of the A-ring of **MA**. Thus, we obtained 30 new 2,3-diacylated-28-monopeptidyl MA derivatives (**4a**−**4j**, **5a**−**5j**, and **6a**−**6j**) (Scheme 3), which were also analyzed by HPLC and HRMS. The observed purities for these derivatives were high (around 90−100%), except for the dicarboxylic acyl derivatives (phthaloyl, succinyl, glutaryl, and 3,3-dimethylglutaryl), for which the reaction yields were slightly lower (range 75−100%) (see Experimental Section and Supporting Information).

Similarly, starting from the dipeptidyl OA derivatives (**7**−**15**) or the dipeptidyl MA derivatives (**16**−**24**), and according with the above-described acylation methodology, 90 new 3-acylated-28-dipeptidyl OA derivatives (**7a**−**7j** to **15a**−**15j**), and another 90 new 2,3-diacylated-28-dipeptidyl MA derivatives (**16a**−**16j** to **24a**−**24j**), were prepared (Scheme 4). Also, these derivatives had great purities (90−100%), again with the exception of the

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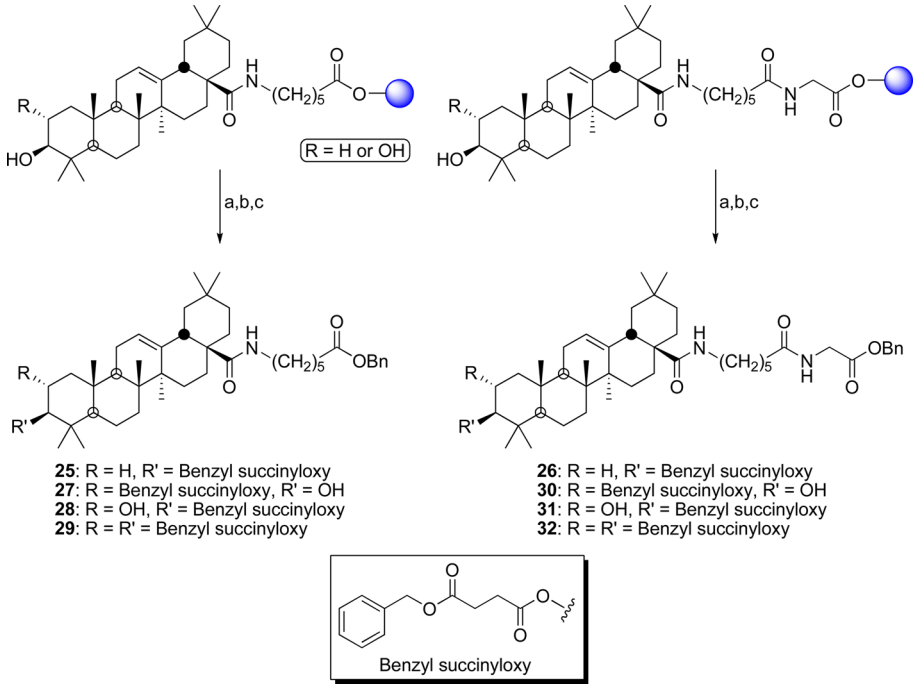
Scheme 5. Solid-Phase Succinylation Reactions and Consecutive Solution-Phase Benzylation Reactions of Mono- or Dipeptidyl Derivatives of OA or MA.[a]



[a]Reagents and conditions: (a) Acid anhydride, Et_3N, DMAP, DMF, DCM; (b) TFA-DCM (1:99); (c) BnCl, K_2CO_3, DMF.

dicarboxylic acyl derivatives (**16g−16j** to **24g−24j**, 75−100%), as detected in their HPLC analyses, and presented appropriate HRMS spectra (see Experimental Section and Supporting Information).

Quantification of Succinyl Derivatives. In these solid-phase acylation studies, we found that the mono- or dipeptidyl OA or MA acyl derivatives that were achieved from a cyclic acid anhydride (phthalic, succinic, glutaric, or 3,3-dimethylglutaric) were compounds of very difficult chromatographic isolation and also of complicated spectroscopic identification because of the presence of two or three free carboxylic groups in the molecule. Therefore, to avoid these experimental problems and to determine more accurately the proportion of these derivatives obtained by solid-phase synthesis, we selected several succinyl derivatives to protect the free carboxylic acid groups through a benzylation reaction. Thus, following the process of solid-phase synthesis described in this work, we achieved *N*-(3*β*-succinyloxyolean-12-en-28-oyl)-6-aminohexanoic acid (**2h**) and *N*′-[*N*-(3*β*-succinyloxyolean-12-en-28-oyl)-6-aminohexanoyl]-glycine (**8h**), respectively. Both compounds were benzylated separately with benzyl chloride giving benzyl *N*-(3*β*-benzylsuccinyloxyolean-12-en-28-oyl)-6-aminohexanoate (**25**, 95%) and benzyl *N*′-[*N*-(3*β*-benzylsuccinyloxyolean-12-en-28-oyl)-6-aminohexanoyl]-glycinate (**26**, 98%), respectively (Scheme 5). The NMR spectra of **25** and **26**, compared with those of their corresponding precursors (**2h** or **8h**) presented the signals of two benzyl groups (around δ_H 7.35 and 5.15, and δ_C 136−128 and 67).

Similarly, we studied the protection of the free carboxylic acid groups of several succinyl derivatives of mono- or dipeptidyl MA compounds, through a benzylation reaction with benzyl chloride. In these case, not only the 2,3-disuccinyl derivatives (**5h** and **17h**, respectively) were detected, but also the 2- and 3-succinyl derivatives. Consequently, three benzylated derivatives were identified respectively: the 2*α*-benzylsuccinyl (**27**, 4%; **30**, 5%), the 3*β*-benzylsuccinyl (**28**, 2%; **31**, 2%), and the 2*α*,3*β*-dibenzylsuccinyl derivatives (**29**, 80%; **32**, 85%) (Scheme 5). Major NMR spectroscopic differences between these benzylated compounds were the chemical shifts of the signals of H-2 and H-3 of the A-ring of these molecules, which depended on whether the substituents were benzylated or not at these positions. Therefore, by forming these benzylated derivatives we could determine more accurately the proportion of succinylated derivatives obtained by solid-phase synthesis.

Cytotoxicity Tests. 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.[25,54] We tested the cytotoxicity of 90 compounds of the 264 semisynthesized derivatives, on three cell lines (B16−F10, HT29, and Hep G2) (Figure 2). These compounds were selected to draw consistent conclusions concerning the structure−activity relationship of these types of triterpene derivatives. In general, we found that, under the conditions assayed (from 0 to 300 *μ*g/mL), nearly 90% of the compounds assayed showed cytotoxicity on the B16−F10 and HT29 cell lines, while on the Hep G2 cell line this percentage decreased

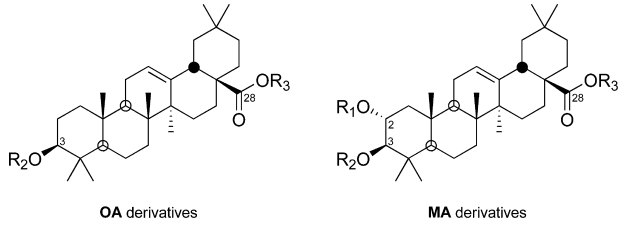


Figure 2. General structures of the tested OA or MA derivatives.

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Table 1. Growth-Inhibitory Effects of the Mono- (1−6) or Dipeptidyl (7−24) OA or MA Derivatives on the Three Cancer Cell Lines

compound	R_1	R_2	R_3	B16−F10[a]	IC_{50} of precursor[b]/ IC_{50} of compound	HT29[a]	IC_{50} of precursor[b]/ IC_{50} of compound	Hep G2[a]	IC_{50} of precursor[b]/ IC_{50} of compound
OA		H	H	106.4 ± 3.7	1.0	429.9 ± 0.7	1.0	211.8 ± 0.5	1.0
1		H	GABA	185.0 ± 9.4	0.6	137.2 ± 3.5	3.1	268.4 ± 10.6	0.8
2		H	6AHA	115.5 ± 8.2	0.9	84.8 ± 3.0	5.1	110.9 ± 0.2	1.9
3		H	11AUA	108.2 ± 1.6	1.0	120.4 ± 3.1	3.6	80.5 ± 2.9	2.6
7		H	GABA-GLY	290.2 ± 14.1	0.4	170.3 ± 4.2	2.5	216.0 ± 17.4	1.0
8		H	6AHA-GLY	111.3 ± 1.4	1.0	132.9 ± 0.6	3.2	182.4 ± 1.4	1.2
9		H	11AUA-GLY	>500		194.8 ± 1.6	2.2	>500	
10		H	GABA-ALA	356.1 ± 12.7	0.3	243.1 ± 2.4	1.8	232.3 ± 5.9	0.9
11		H	6AHA-ALA	115.5 ± 4.9	0.9	142.1 ± 2.8	3.0	175.4 ± 5.6	1.2
12		H	11AUA-ALA	340.5 ± 0.1	0.3	189.6 ± 3.5	2.3	>500	
13		H	GABA-VAL	79.4 ± 0.4	1.3	170.9 ± 2.7	2.5	125.9 ± 2.8	1.7
14		H	6AHA-VAL	105.3 ± 2.4	1.0	124.0 ± 1.3	3.5	134.1 ± 2.8	1.6
15		H	11AUA-VAL	71.9 ± 1.0	1.5	73.6 ± 2.1	5.8	88.2 ± 7.6	2.4
MA	H	H	H	36.2 ± 2.5	1.0	32.2 ± 3.8	1.0	99.2 ± 15.5	1.0
4	H	H	GABA	180.3 ± 3.9	0.2	86.4 ± 1.4	0.4	202.4 ± 4.1	0.5
5	H	H	6AHA	178.4 ± 0.9	0.2	137.9 ± 0.7	0.2	107.0 ± 5.4	0.9
6	H	H	11AUA	102.7 ± 1.0	0.4	116.3 ± 5.8	0.3	54.7 ± 3.2	1.8
16	H	H	GABA-GLY	469.6 ± 3.8	0.1	242.6 ± 3.1	0.1	>500	
17	H	H	6AHA-GLY	263.5 ± 1.8	0.1	218.7 ± 0.7	0.1	>500	
18	H	H	11AUA-GLY	103.0 ± 5.9	0.4	175.5 ± 3.5	0.2	124.0 ± 6.3	0.8
19	H	H	GABA-ALA	351.9 ± 12.2	0.1	208.5 ± 1.2	0.2	>500	
20	H	H	6AHA-ALA	223.4 ± 4.0	0.2	162.6 ± 1.1	0.2	>500	
21	H	H	11AUA-ALA	62.2 ± 1.4	0.6	129.4 ± 10.3	0.2	88.5 ± 0.4	1.1
22	H	H	GABA-VAL	243.3 ± 3.9	0.1	181.9 ± 3.5	0.2	>500	
23	H	H	6AHA-VAL	164.0 ± 2.9	0.2	150.4 ± 3.6	0.2	292.9 ± 2.6	0.3
24	H	H	11AUA-VAL	39.6 ± 1.0	0.9	47.4 ± 1.0	0.7	53.2 ± 1.1	1.9

[a]The IC_{50} values (μM) were calculated considering control untreated cells as 100% of viability. Cell-growth inhibition was analyzed by the MTT assay, as described in the Experimental Procedures. All assays were made two times using three replicates. Values, means ± SEM. [b]These columns represent the ratio between the IC_{50} of each precursor (OA or MA) and the IC_{50} of the related derivatives.

Chart 1. Growth-Inhibitory Effects (IC_{50}, μM) of the Mono- or Dipeptidyl OA or MA Derivatives on the Three Cancer Cell Lines

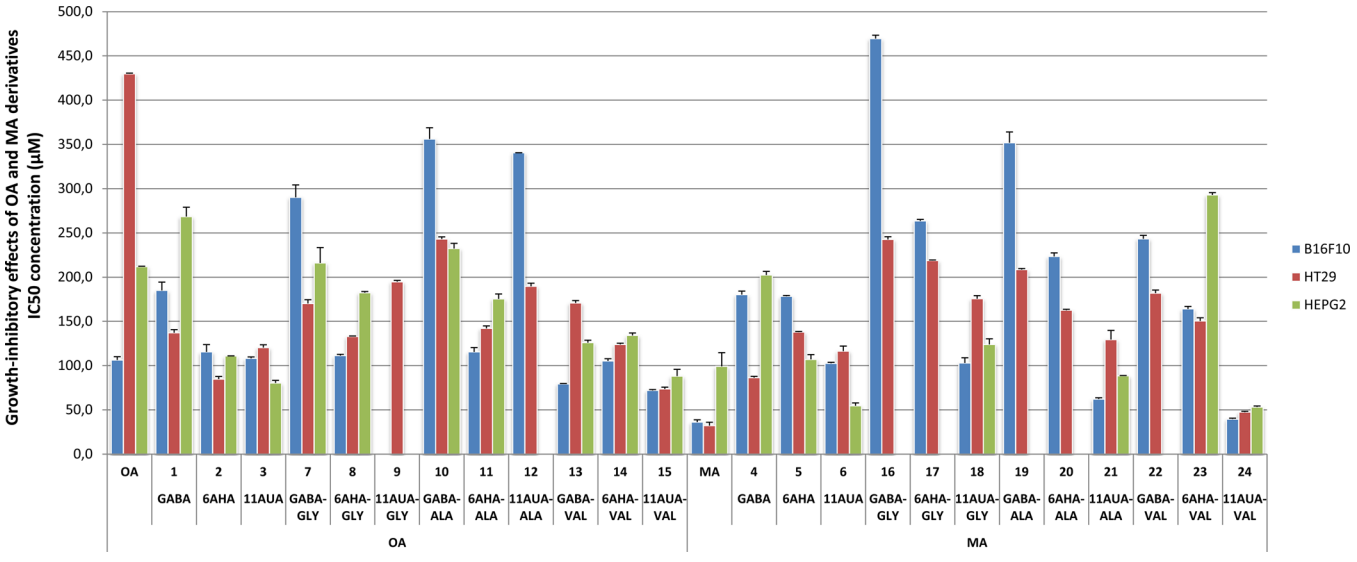

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Table 2. Growth-Inhibitory Effects of the Mono- (1g−6g) or Dipeptidyl (7g−24g) OA or MA Phthaloyl Derivatives on the Three Cancer Cell Lines

compound	R_1	R_2	R_3	B16−F10[a]	IC_{50} of precursor[b]/ IC_{50} of compound	HT29[a]	IC_{50} of precursor[b]/ IC_{50} of compound	Hep G2[a]	IC_{50} of precursor[b]/ IC_{50} of compound
OA		H	H	106.4 ± 3.7	1.0	429.9 ± 0.7	1.0	211.8 ± 0.5	1.0
1g		Phtha	GABA	211.7 ± 1.1	0.5	314.5 ± 1.5	1.4	326.8 ± 4.4	0.6
2g		Phtha	6AHA	140.0 ± 21.2	0.8	213.7 ± 3.1	2.0	211.3 ± 2.5	1.0
3g		Phtha	11AUA	68.6 ± 0.7	1.6	113.5 ± 0.4	3.8	79.3 ± 1.0	2.7
7g		Phtha	GABA-GLY	>500		>500		>500	
8g		Phtha	6AHA-GLY	>500		399.8 ± 1.7	1.1	>500	
9g		Phtha	11AUA-GLY	76.8 ± 2.4	1.4	167.0 ± 2.4	2.6	125.9 ± 2.9	1.7
10g		Phtha	GABA-ALA	>500		398.2 ± 1.1	1.1	>500	
11g		Phtha	6AHA-ALA	314.5 ± 2.0	0.3	>500		>500	
12g		Phtha	11AUA-ALA	122.0 ± 2.8	0.9	160.5 ± 3.5	2.7	129.6 ± 2.4	1.6
13g		Phtha	GABA-VAL	211.4 ± 3.7	0.5	275.9 ± 0.2	1.6	327.9 ± 3.0	0.6
14g		Phtha	6AHA-VAL	134.2 ± 1.1	0.8	364.4 ± 3.2	1.2	>500	
15g		Phtha	11AUA-VAL	82.3 ± 2.1	1.3	143.8 ± 3.3	3.0	153.8 ± 2.7	1.4
MA	H	H	H	36.2 ± 2.5	1.0	32.2 ± 3.8	1.0	99.2 ± 15.5	1.0
4g	Phtha	Phtha	GABA	348.3 ± 3.9	0.1	357.0 ± 0.6	0.1	>500	
5g	Phtha	Phtha	6AHA	215.4 ± 1.0	0.2	336.5 ± 0.7	0.1	243.0 ± 1.3	0.4
6g	Phtha	Phtha	11AUA	129.7 ± 3.6	0.3	280.2 ± 3.4	0.1	213.7 ± 2.1	0.5
16g	Phtha	Phtha	GABA-GLY	>500		>500		>500	
17g	Phtha	Phtha	6AHA-GLY	>500		>500		>500	
18g	Phtha	Phtha	11AUA-GLY	59.1 ± 5.1	0.6	228.1 ± 0.9	0.1	>500	
19g	Phtha	Phtha	GABA-ALA	>500		>500		>500	
20g	Phtha	Phtha	6AHA-ALA	>500		>500		>500	
21g	Phtha	Phtha	11AUA-ALA	202.8 ± 1.6	0.2	>500		268.1 ± 2.7	0.4
22g	Phtha	Phtha	GABA-VAL	>500		>500		>500	
23g	Phtha	Phtha	6AHA-VAL	>500		>500		>500	
24g	Phtha	Phtha	11AUA-VAL	>500		>500		>500	

[a]The IC_{50} values (μM) were calculated considering control untreated cells as 100% of viability. Cell-growth inhibition was analyzed by the MTT assay, as described in the Experimental Procedures. All assays were made two times using three replicates. Values, means ± SEM. [b]These columns represent the ratio between the IC_{50} of each precursor (OA or MA) and the IC_{50} of the related derivatives.

to 67%. Several of these cytotoxic compounds, mostly OA derivatives, were more effective than their corresponding precursor (OA or MA), with percentages ranging from 31% (B16−F10) to 58% (HT29) and up to 67% (Hep G2).

Comparing the IC_{50} data for OA and MA on the three cell lines selected, we deduce that the hydroxyl group at C-2 enhanced the cytotoxicity of the last one (Table 1 and Chart 1). Moreover, the IC_{50} values of the monopeptidyl OA derivatives (**1**−**3**) were in general lower than that of OA, particularly when the amino acid had a longer carbon chain (6AHA or 11AUA); but the IC_{50} data of the monopeptidyl MA derivatives (**4**−**6**) were higher than that of MA, with the exception of the 11AUA-MA derivative (**6**) with the Hep G2 cell line. Furthermore, when amino acids having a greater length chain (11AUA) and a larger volume (VAL) were used, the dipeptidyl OA (**15**) or MA (**24**) derivatives exhibited the lowest cytotoxic concentrations (Table 1 and Chart 1).

We also studied the cytotoxicity of the mono- (**1g**−**6g**) or dipeptidyl (**7g**−**24g**) OA or MA phthaloyl derivatives (Table 2 and Chart 2). These products were selected according with the very good results achieved with this acyl group with the same triterpene compounds, in a previous work.[46] In this case, we analyzed the influence of one or two amino acids, linked to the carboxyl group at C-28 of the molecule, on the cytotoxicity of these compounds. The best cytotoxic results with the phtaloyl OA derivatives were achieved when a long-chain amino acid (11AUA) was present in the molecule (**3g**, **9g**, **12g**, and **15g**). However, the diphthaloyl MA derivatives registered worse results than did MA (Table 2 and Chart 2).

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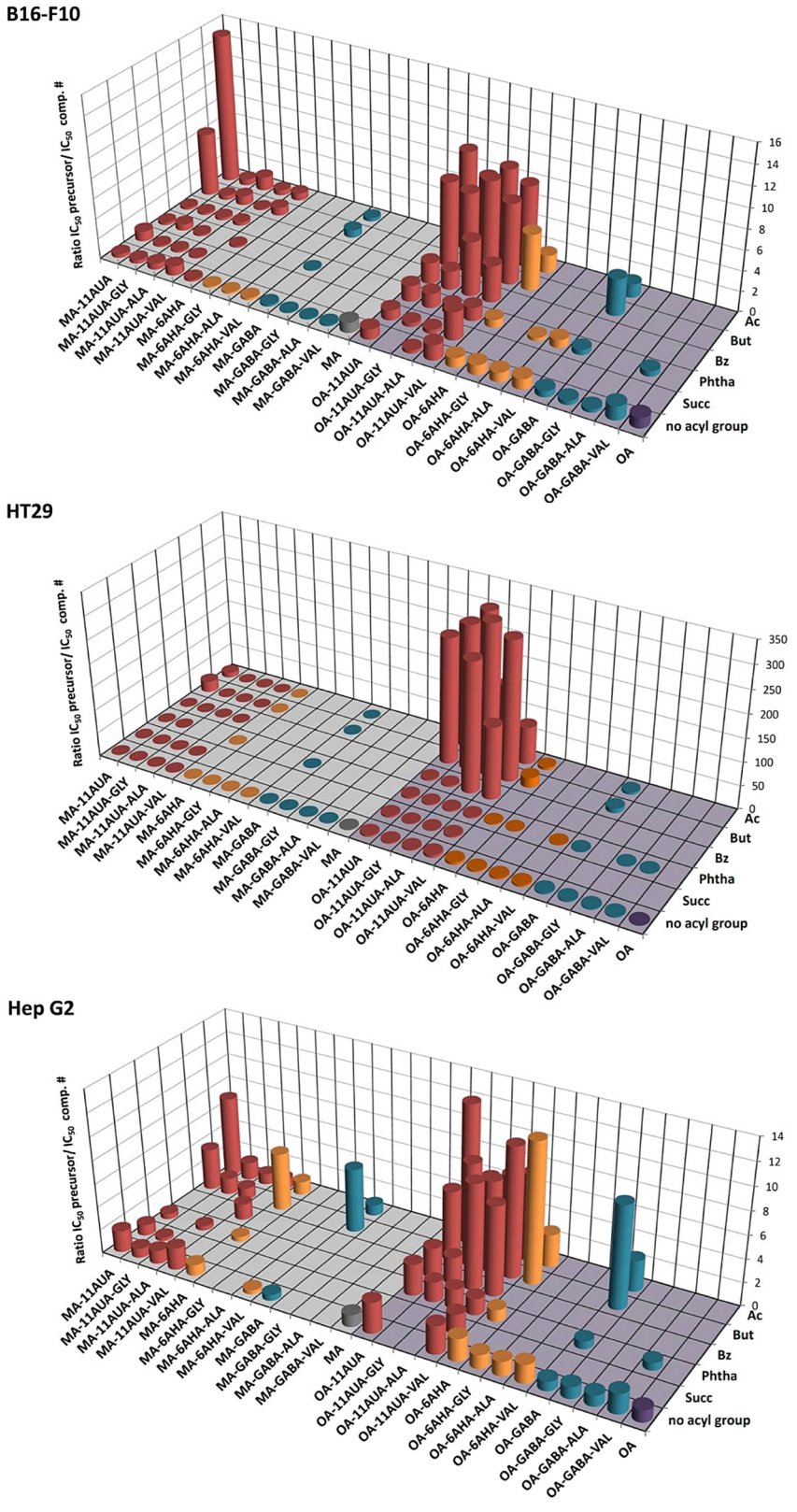
Chart 2. Comparison of the Growth-Inhibitory Effects of the OA or MA Derivatives on the Three Cancer Cell Lines, Describing the Ratio between the IC_{50} Values of the Triterpene Precursors (OA or MA) and the IC_{50} Values of Their Cytotoxic Derivatives[a]



[a]Ac = acetyl, But = butanoyl, Bz = benzoyl, Phtha = phthaloyl, Succ = succinyl.

Finding good cytotoxicity results with the 11AUA derivatives of OA or MA, we analyzed the influence of these monopeptidyl (11AUA) or dipeptidyl (11AUA with GLY, ALA, or VAL) derivatives with other acyl groups (acetyl, butanoyl, benzoyl, or

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Table 3. Growth-Inhibitory Effects of the Mono- (11-AUA) or Dipeptidyl (11AUA with GLY, ALA, or VAL) OA or MA Acyl (ac, but, bz, or succ) Derivatives on the Three Cancer Cell Lines

compound	R_1	R_2	R_3	B16−F10[a]	IC_{50} of precursor[b]/ IC_{50} of compound	HT29[a]	IC_{50} of precursor[b]/ IC_{50} of compound	Hep G2[a]	IC_{50} of precursor[b]/ IC_{50} of compound
OA		H	H	106.4 ± 3.7	1.0	429.9 ± 0.7	1.0	211.8 ± 0.5	1.0
3a		Ac	11AUA	10.9 ± 0.1	9.8	1.63 ± 0.01	263.7	17.5 ± 0.1	12.1
9a		Ac	11AUA-GLY	13.8 ± 0.4	7.7	1.41 ± 0.03	304.9	34.1 ± 0.1	6.2
12a		Ac	11AUA-ALA	11.6 ± 0.9	9.2	2.71 ± 0.04	158.6	30.8 ± 1.0	6.9
15a		Ac	11AUA-VAL	13.2 ± 0.2	8.1	5.4 ± 0.1	79.6	28.3 ± 0.6	7.5
3c		But	11AUA	12.74 ± 0.03	8.4	1.59 ± 0.01	270.4	35.4 ± 0.6	6.0
9c		But	11AUA-GLY	13.6 ± 0.4	7.8	1.40 ± 0.04	307.1	24 ± 0.8	8.8
12c		But	11AUA-ALA	11.2 ± 0.1	9.5	1.33 ± 0.01	323.2	27.1 ± 0.2	7.8
15c		But	11AUA-VAL	13.4 ± 0.6	7.9	1.44 ± 0.01	298.5	18.9 ± 0.5	11.2
3f		Bz	11AUA	51.6 ± 0.9	2.1	129.9 ± 2.8	3.3	83.1 ± 1.7	2.5
9f		Bz	11AUA-GLY	58.7 ± 1.6	1.8	146.4 ± 5.9	2.9	93.1 ± 0.2	2.3
12f		Bz	11AUA-ALA	20.0 ± 0.3	5.3	1.55 ± 0.01	277.4	23.5 ± 0.3	9.0
15f		Bz	11AUA-VAL	29.9 ± 1.2	3.6	2.80 ± 0.02	153.5	27.7 ± 0.1	7.6
3h		Succ	11AUA	110.8 ± 10.1	1.0	113 ± 3.5	3.8	>500	
9h		Succ	11AUA-GLY	283.5 ± 9.1	0.4	357.9 ± 1.1	1.2	>500	
12h		Succ	11AUA-ALA	278.3 ± 0.6	0.4	354.1 ± 0.6	1.2	>500	
15h		Succ	11AUA-VAL	38.7 ± 2.0	2.7	143.5 ± 8.0	3.0	125.9 ± 7.4	1.7
MA	H	H	H	36.2 ± 2.5	1.0	32.2 ± 3.8	1.0	99.2 ± 15.5	1.0
6a	Ac	Ac	11AUA	2.4 ± 0.1	15.1	3.1 ± 0.1	10.3	14.2 ± 0.1	7.0
18a	Ac	Ac	11AUA-GLY	60.2 ± 2.6	0.6	105.6 ± 3.9	0.3	66.5 ± 1.1	1.5
21a	Ac	Ac	11AUA-ALA	26.8 ± 0.3	1.4	64.5 ± 3.5	0.5	105.0 ± 3.8	0.9
24a	Ac	Ac	11AUA-VAL	68.1 ± 0.7	0.5	97.8 ± 4.9	0.3	105.6 ± 6.6	0.9
6c	But	But	11AUA	5.7 ± 0.2	6.3	1.37 ± 0.01	23.5	27.6 ± 0.4	3.6
18c	But	But	11AUA-GLY	82.8 ± 1.4	0.4	159.2 ± 4.0	0.2	70.9 ± 2.4	1.4
21c	But	But	11AUA-ALA	39.5 ± 1.0	0.9	109.1 ± 2.7	0.3	100.8 ± 6.9	1.0
24c	But	But	11AUA-VAL	243.1 ± 4.1	0.1	301.0 ± 1.5	0.1	>500	
6f	Bz	Bz	11AUA	145.0 ± 2.3	0.2	126.3 ± 4.9	0.3	>500	
18f	Bz	Bz	11AUA-GLY	159.4 ± 2.5	0.2	227.4 ± 4.0	0.1	>500	
21f	Bz	Bz	11AUA-ALA	130.8 ± 2.5	0.3	293.4 ± 0.8	0.1	>500	
24f	Bz	Bz	11AUA-VAL	104.8 ± 5.6	0.3	184.1 ± 3.0	0.2	72.1 ± 3.7	1.4
6h	Succ	Succ	11AUA	38.1 ± 1.1	1.0	153.7 ± 0.3	0.2	111.3 ± 3.1	0.9
18h	Succ	Succ	11AUA-GLY	221.1 ± 3.0	0.2	218.4 ± 5.1	0.1	344.3 ± 4.8	0.3
21h	Succ	Succ	11AUA-ALA	164.5 ± 2.6	0.2	211.6 ± 2.5	0.2	>500	
24h	Succ	Succ	11AUA-VAL	250.4 ± 1.3	0.1	212.2 ± 4.3	0.2	>500	

[a]The IC_{50} values (μM) were calculated considering control untreated cells as 100% of viability. Cell-growth inhibition was analyzed by the MTT assay, as described in the Experimental Procedures. All assays were made two times using three replicates. Values, means ± SEM. [b]These columns represent the ratio between the IC_{50} of each precursor (OA or MA) and the IC_{50} of the related derivatives.

succinyl) on the hydroxyl groups of the A-ring of the molecules (Table 3). In the cytotoxicity analysis of the OA derivatives, the best results were achieved on the HT29 cell line, with the smallest acyl groups (acetyl or butanoyl); with IC_{50} data between 1.33 and 5.4 μM, these values being between 80- and 323-fold lower than its precursor (OA). Also good results were

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Table 4. Growth-Inhibitory Effects of the Monopeptidyl (GABA or 6AHA) OA or MA Acyl (ac or but) Derivatives on the Three Cancer Cell Lines

compound	R_1	R_2	R_3	B16−F10[a]	IC_{50} of precursor[b]/ IC_{50} of compound	HT29[a]	IC_{50} of precursor[b]/ IC_{50} of compound	Hep G2[a]	IC_{50} of precursor[b]/ IC_{50} of compund
OA		H	H	106.4 ± 3.7	1.0	429.9 ± 0.7	1.0	211.8 ± 0.5	1.0
1a		Ac	GABA	81.7 ± 0.2	1.3	81.9 ± 0.3	5.3	79.9 ± 3.8	2.6
2a		Ac	6AHA	61.4 ± 0.7	1.7	72.8 ± 1.4	5.9	75.29 ± 0.05	2.8
1c		But	GABA	29.7 ± 1.2	3.6	45.6 ± 1.0	9.4	24.0 ± 0.9	8.8
2c		But	6AHA	19.2 ± 0.2	5.5	20.3 ± 0.3	21.2	17.5 ± 0.1	12.1
MA	H	H	H	36.2 ± 2.5	1.0	32.2 ± 3.8	1.0	99.2 ± 15.5	1.0
4a	Ac	Ac	GABA	104.6 ± 1.7	0.3	95.8 ± 1.7	0.3	109.2 ± 1.5	0.9
5a	Ac	Ac	6AHA	50.2 ± 2.4	0.7	89.8 ± 0.6	0.4	87.0 ± 1.5	1.1
4c	But	But	GABA	52.3 ± 0.6	0.7	31.3 ± 0.1	1.0	18.0 ± 0.6	5.5
5c	But	But	6AHA	46.0 ± 1.5	0.8	32.1 ± 1.3	1.0	19.8 ± 0.8	5.0

[a]The IC_{50} values (μM) were calculated considering control untreated cells as 100% of viability. Cell-growth inhibition was analyzed by the MTT assay, as described in the Experimental Procedures. All assays were made two times using three replicates. Values, means ± SEM. [b]These columns represent the ratio between the IC_{50} of each precursor (OA or MA) and the IC_{50} of the related derivatives.

found with the other cell lines, with IC_{50} data between 10.9 and 13.8 μM (B16−F10), and between 17.5 and 35.4 μM (Hep G2). The acetyl and butanoyl MA derivatives also displayed good cytotoxic activity, especially the monopeptidyl (11AUA) derivatives, with IC_{50} data between 1.37 and 27.6 μM, these values being between 4- and 23-fold lower than its precursor (MA) (Table 3 and Charts 2).

Finally, considering the good results found using the monopeptidyl (11AUA) OA or MA derivatives with an acetyl or butanoyl group at C-2 or C-2/C-3, we also analyzed the influence of these acyl OA or MA derivatives with amino acids with a shorter chain (GABA or 6AHA). Thus, these derivatives exhibited a generally higher cytotoxicity than did their corresponding precursors (OA or MA), but lower than when the amino acid 11AUA was present (Table 4 and Charts 2).

CONCLUSIONS

In summary, we have prepared a library of 264 compounds through easy combinatorial solid-phase processes, utilizing two natural triterpene acids (OA or MA) as scaffolds. These methods have led to the semisynthesis of products with high yields and purities, and easy isolation. Several of these derivatives of OA or MA with mono- or dipeptidyl groups at C-28, and other derivatives that also included acyl groups at C-2 or C-2/C-3 of the triterpene skeleton, exhibited cytotoxic properties on B16−F10, HT29, or Hep G2 cancer cell lines. Most compounds tested displayed cytotoxicity on the B16−F10 (90%) and HT29 (90%) cell lines, while the percentage was somewhat lower (67%) for the Hep G2 cell line. Several cytotoxic OA derivatives had very low IC_{50} values (<2 μM), being around 300-fold more effective than its precursor. Some cytotoxic MA derivatives also had low IC_{50} values (<6 μM), in this case being between 6- and 24-fold more effective than its precursor, because MA exhibits lower IC_{50} values than OA. Analyzing the structure−activity relationship of these OA or MA derivatives, it can be concluded that the functional groups that clearly improved the cytotoxic effects of these compounds are a long-chain ω-amino acid (11AUA) on the carboxylic group at C-28, and a small acyl group (acetyl or butanoyl) on the hydroxyl groups at C-2 or C-2/C-3 of the A-ring of the triterpene skeleton. Synthetic lipophilic compounds bearing different components are underexplored as potential therapeutic agents. The acetyl or butanoyl 11AUA-OA or -MA derivatives described here show that cytotoxic properties can be varied in such circumstances and, therefore, may be interesting compounds for development of novel biological activity.

EXPERIMENTAL PROCEDURE

Isolation of OA and MA from Residues of Olive-Oil Industry. Oleanolic (**OA**) and maslinic (**MA**) acids were isolated from solid wastes resulting from olive-oil production, which were extracted in a Soxhlet with hexane and EtOAc successively.[53] Hexane extracts contained 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.[55]

General Procedure for the Semisynthesis of Mono- or Dipeptidyl OA or MA Derivatives. The coupling of the corresponding ω-amino acids (Aa1) to the resin was the first step to form the monopeptidyl derivatives of OA or MA. Thus, an appropriate amount of CTC-resin (1.30 mmol/g) was placed in a 10 mL polypropylene syringe, fitted with a polyethylene filter disk. The resin was washed with DMF (2 mL × 3) and DCM (2 mL × 3), swelled with DCM (1 mL) for 30 min, and drained under reduced pressure. Then, a solution containing 2 equiv of the corresponding ω-Fmoc-Aa1-OH (Fmoc-GABA-OH, Fmoc-6AHA-OH or Fmoc-11AUA-OH), DIEA (10 equiv), and DCM (1.5 mL), was added, and the mixture was stirred for 1.5 h. Next, a treatment with MeOH (0.75 mL under stirring for 15 min) was performed to cap all the residual chloride in the resin, and subsequently the mixture was filtered. The corresponding Fmoc-Aa1-resin was washed with DCM (2 mL × 3) and DMF (2 mL × 3), and then the Fmoc group was removed by treatment with piperidine/DMF (1:4) (3 × 10 min). Finally, the resin was again washed with DCM (2 mL × 3) and DMF (2 mL × 3), filtered, and drained under reduced pressure.

The first step to obtain the dipeptidyl OA or MA derivatives was the coupling of the corresponding α-amino acids (Aa2) (Fmoc-GLY-OH, Fmoc-ALA-OH, or Fmoc-VAL-OH) to the resin. Thus, proceeding as described in the previous paragraph, the corresponding Aa2-resin derivatives were formed. Then, to attach the second ω-amino acid unit (Aa1), a solution with 4 equiv of the corresponding Fmoc-Aa1-OH (Fmoc-GABA-OH, Fmoc-6AHA-OH, or Fmoc-11AUA-OH), HOAt (4 equiv),

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DIPCDI (4 equiv), in DMF, was added to the corresponding Aa2-resin contained in a syringe. The mixture was stirred for 1.5 h, the ninhydrin test being negative. Finally, the corresponding Fmoc-Aa1-Aa2-resin was subjected to the above-described washing/deprotecting treatments, and then filtered and drained under reduced pressure.

The second step to form the mono- or dipeptidyl OA or MA derivatives was the coupling of the corresponding Aa1-resin or Aa1-Aa2-resin with one of the triterpene acids (OA or MA). Thus, a solution of OA or MA (3 equiv), PyAOP (3 equiv), HOAt (3 equiv), and DIEA (9 equiv) in DMF/DCM, was added to the syringe containing the corresponding Aa1-resin or Aa1-Aa2-resin. This mixture was stirred at rt for 24 h, the ninhydrin test proving negative. The resin coupled with the peptidyl-triterpene acid was then washed with DMF (2 mL × 3) and DCM (2 mL × 3), and drained under reduced pressure, rendering the corresponding OA- or MA-Aa1-resin derivatives, or OA- or MA-Aa1-Aa2-resin derivatives.

The third and final step to obtain the mono- or dipeptidyl derivatives of OA or MA (**1**−**24**) was the cleavage of these derivatives from the resin by treatment with DCM:TFA (99:1) (2 mL × 3 × 30 s). Then, the corresponding product was filtered, evaporated under reduced pressure, lyophilized, and the residue was analyzed by TLC and HPLC. The physical, chemical, and spectroscopic properties of representative examples are given below.

*N-(3β-Hydroxyolean-12-en-28-oyl)-4-aminobutanoic Acid (**1**):* HPLC retention time 4.08 min; HPLC purity 100%; white solid; mp 121−123 °C; $[\alpha]_D$ + 42° (*c* 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3423, 2948, 2850, 1654; ^{1}H NMR (Cl_3CD) δ_H 6.20 (1H, t, *J* = 5.1 Hz, NH), 5.37 (1H, dd, *J* = 3.0, 3.0 Hz, H12), 3.40 (2H, dt, *J* = 5.1, 7.0 Hz, 2H−C4 GABA), 3.22 (1H, dd, *J* = 5.0, 10.0 Hz, H3), 2.52 (1H, dd, *J* = 3.6, 13.7 Hz, H18), 2.35 (2H, t, *J* = 7.5 Hz, 2H−C2 GABA), 1.14, 0.96, 0.88, 0.88, 0.88, 0.76, 0.73 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 179.7 (C28), 176.7 (C1 GABA), 144.9 (C13), 123.3 (C12), 79.3 (C3), 55.3 (C5), 47.7 (C9), 47.0 (C19), 46.6 (C17), 42.4 (C18), 42.4 (C14), 39.6 (C10), 39.1 (C4 GABA), 39.0 (C8), 38.7 (C1), 37.2 (C4), 34.3 (C21), 33.2 (Me), 32.7 (C7), 32.5 (C22), 31.9 (C2 GABA), 30.9 (C20), 28.3 (Me), 27.5 (C2 and C15), 26.0 (Me), 25.0 (C3 GABA), 23.8 (Me and C16), 23.7 (C11), 18.5 (C6), 17.1 (Me), 15.8 (Me), 15.6 (Me); ESI-HRMS (*m*/*z*) [M + Na]$^+$ calcd for $C_{34}H_{55}NO_4Na$ 564.4209, found 564.4193.

*N-(3β-Hydroxyolean-12-en-28-oyl)-6-aminohexanoic Acid (**2**):* HPLC retention time 4.65 min; HPLC purity 100%; white solid; mp 117−119 °C; $[\alpha]_D$ + 68° (*c* 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3423, 2948, 2850, 1654; ^{1}H NMR (Cl_3CD) δ_H 6.20 (1H, t, *J* = 5.4 Hz, NH), 5.37 (1H, dd, *J* = 3.2, 3.2 Hz, H12), 3.36 (2H, m, 2H−C6 6AHA), 3.26 (1H, dd, *J* = 5.0, 10.0 Hz, H3), 2.44 (1H, dd, *J* = 3.6, 13.7 Hz, H18), 2.34 (2H, t, *J* = 7.5 Hz, 2H−C2 6AHA), 1.14, 0.97, 0.89, 0.89, 0.87, 0.77, 0.73 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 180.0 (C28), 178.8 (C1 6AHA), 145.0 (C13), 123.4 (C12), 79.6 (C3), 55.3 (C5), 47.7 (C9), 47.0 (C19), 46.6 (C17), 42.6 (C18), 42.4 (C14), 39.9 (C6 6AHA), 39.6 (C10), 38.4 (C1), 38.2 (C8), 37.2 (C4), 34.3 (C21), 33.9 (C2 6AHA), 33.1 (Me), 32.4 (C7), 32.3 (C22), 30.9 (C20), 29.0 (C5 6AHA and C2), 28.3 (Me), 27.4 (C15), 26.6 (C4 6AHA), 25.9 (Me), 24.4 (C3 6AHA), 23.9 (C11), 23.8 (C16), 23.7 (Me), 18.4 (C6), 17.1 (Me), 15.8 (Me), 15.6 (Me); ESI-HRMS (*m*/*z*) [M + Na]$^+$ calcd for $C_{36}H_{59}NO_4Na$ 592.4342, found 592.4353.

*N-(3β-Hydroxyolean-12-en-28-oyl)-11-aminoundecanoic Acid (**3**):* HPLC retention time 11.68 min; HPLC purity 100%; white solid; mp 97−99 °C; $[\alpha]_D$ + 56° (*c* 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3423, 2948, 2850, 1654; ^{1}H NMR (Cl_3CD) δ_H 6.10 (1H, t, *J* = 5.1 Hz, NH), 5.35 (1H, dd, *J* = 3.0, 3.0 Hz, H12), 3.34 (2H, m, 2H−C11 11AUA), 3.22 (1H, dd, *J* = 5.0, 10.0 Hz, H3), 2.45 (1H, dd, *J* = 3.6, 13.7 Hz, H18), 2.31 (2H, t, *J* = 7.5 Hz, 2H−C2 11AUA), 1.14, 0.97, 0.89, 0.88, 0.86, 0.76, 0.74 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 178.7 (C28), 178.5 (C1 11AUA), 145.3 (C13), 123.0 (C12), 79.2 (C3), 54.3 (C5), 47.8 (C9), 47.1 (C19), 46.5 (C17), 42.7 (C18), 42.4 (C14), 39.9 (C11 11AUA), 39.6 (C10), 39.0 (C8), 38.8 (C1), 37.2 (C4), 34.4 (C21), 34.2 (C2 11AUA), 33.2 (Me), 32.6 (C7), 32.1 (C22), 30.9 (C20), 29.5 and 29.3 (C4, C5, C6, C7, C8 and C10 11AUA), 28.3 (Me), 27.5 (C15), 27.4 (C2), 26.0 (Me), 24.9 and 24.1 (C9 11AUA and C3), 24.0 (C16), 23.8 (Me and C11), 18.5 (C6), 17.2 (Me), 15.8 (Me), 15.6 (Me); ESI-HRMS (*m*/*z*) [M + Na]$^+$ calcd for $C_{41}H_{69}NO_4Na$ 662.5305, found 662.5308.

*N-(2α,3β-Dihydroxyolean-12-en-28-oyl)-4-aminobutanoic Acid (**4**):* HPLC retention time 2.75 min; HPLC purity 99%; white solid; mp 181−183 °C; $[\alpha]_D$ + 17° (*c* 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3432, 2947, 2899, 1711; ^{1}H NMR (DMSO-d_6) δ_H 7.27 (1H, t, *J* = 5.1 Hz, NH), 5.21 (1H, dd, *J* = 3.0, 3.0 Hz, H12), 3.39 (1H, ddd, *J* = 4.3, 9.3, 12.2 Hz, H2), 3.00 (2H, dt, *J* = 5.1, 7.0 Hz, 2H−C4 GABA), 2.78 (1H, dd, *J* = 3.6, 13.7 Hz, H18), 2.72 (1H, d, *J* = 9.3 Hz, H3), 2.16 (2H, t, *J* = 7.5 Hz, 2H−C2 GABA), 1.06, 0.90, 0.87, 0.87, 0.85, 0.68, 0.63 (3H each, s, Me groups); ^{13}C NMR (DMSO-d_6) δ_C 176.2 (C28), 174.3 (C1 GABA), 144.1 (C13), 121.3 (C12), 82.2 (C3), 67.1 (C2), 54.7 (C5), 47.0 (C9), 46.7 (C1), 45.9 (C19), 45.2 (C17), 41.2 (C14), 40.4 (C18), 38.8 (C10), 38.6 (C4), 38.3 (C4 GABA), 37.6 (C8), 33.6 (C21), 32.9 (Me), 32.7 (C7), 32.3 (C22), 31.2 (C2 GABA), 30.4 (C20), 28.7 (Me), 26.9 (C15), 25.7 (Me), 24.4 (C3 GABA), 23.5 (Me), 23.0 (C16), 22.1 (C11), 18.0 (C6), 17.1 (Me), 16.8 (Me), 16.3 (Me); ESI-HRMS (*m*/*z*) [M + Na]$^+$ calcd for $C_{34}H_{55}NO_5Na$ 580.3977, found 580.3971.

*N-(2α,3β-Dihydroxyolean-12-en-28-oyl)-6-aminohexanoic Acid (**5**):* HPLC retention time 3.23 min; HPLC purity 93%; white solid; mp 142−144 °C; $[\alpha]_D$ +19° (*c* 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3422, 2940, 2870, 1688; ^{1}H NMR (DMSO-d_6) δ_H 7.20 (1H, t, *J* = 5.4 Hz, NH), 5.20 (1H, dd, *J* = 3.2, 3.2 Hz, H12), 3.41 (1H, ddd, *J* = 4.3, 9.3, 12.2 Hz, H2), 2.97 (2H, m, 2H−C6 6AHA), 2.78 (1H, dd, *J* = 3.6, 13.7 Hz, H18), 2.72 (1H, d, *J* = 9.3 Hz, H3), 2.16 (2H, t, *J* = 7.5 Hz, 2H−C2 6AHA), 1.06, 0.90, 0.87, 0.86, 0.85, 0.68, 0.64 (3H each, s, Me groups); ^{13}C NMR (DMSO-d_6) δ_C 176.0 (C28), 174.3 (C1 6AHA), 144.1 (C13), 121.2 (C12), 82.2 (C3), 67.1 (C2), 54.7 (C5), 47.0 (C9), 46.7 (C1), 46.0 (C19), 45.1 (C17), 41.2 (C14), 40.4 (C18), 38.9 (C10), 38.6 (C6 6AHA and C4), 37.5 (C8), 33.6 (C2 6AHA and C21), 32.9 (Me), 32.7 (C7), 32.3 (C22), 30.4 (C20), 28.8 (C5 6AHA), 28.7 (Me), 26.8 (C15), 26.1 (C4 6AHA), 25.6 (Me), 24.2 (C3 6AHA), 23.5 (Me), 22.9 (C16), 22.2 (C11), 18.0 (C6), 17.0 (Me), 16.8 (Me), 16.2 (Me); ESI-HRMS (*m*/*z*) [M + Na]$^+$ calcd for $C_{36}H_{59}NO_5Na$ 608.4290, found 608.4295.

*N-(2α,3β-Dihydroxyolean-12-en-28-oyl)-11-aminoundecanoic Acid (**6**):* HPLC retention time 6.78 min; HPLC purity 100%; white solid; mp 126−128 °C; $[\alpha]_D$ + 2° (*c* 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3401, 2910, 2869; ^{1}H NMR (DMSO-d_6) δ_H 7.19 (1H, t, *J* = 5.1 Hz, NH), 5.20 (1H, dd, *J* = 3.0, 3.0 Hz, H12), 3.40 (1H, ddd, *J* = 4.3, 9.3, 12.2 Hz, H2), 2.99 (2H, m,

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2H−C11 11AUA), 2.78 (1H, dd, J = 3.6, 13.7 Hz, H18), 2.72 (1H, d, J = 9.3 Hz, H3), 2.6 (2H, t, J = 7.5 Hz, 2H−C2 11AUA), 1.07, 0.90, 0.88, 0.87, 0.86, 0.68, 0.65 (3H each, s, Me groups); ^{13}C NMR (DMSO-d_6) δ_C 175.9 (C28), 174.3 (C1 11AUA), 144.1 (C13), 121.2 (C12), 82.1 (C3), 67.0 (C2), 54.7 (C5), 47.0 (C9), 46.7 (C1), 46.0 (C19), 45.1 (C17), 41.2 (C14), 40.2 (C18), 38.8 (C11 11AUA and C10), 38.6 (C4), 37.5 (C8), 33.6 (C2 11AUA and C21), 32.9 (Me), 32.7 (C7), 32.3 (C22), 30.3 (C20), 29.1 (C10 11AUA), 28.9 (C6 and C7 11AUA), 28.8 (C5 and C8 11AUA), 28.7 (Me), 28.6 (C4 11AUA), 26.6 (C15), 25.6 (Me), 24.5 (C3 and C9 11AUA), 23.5 (Me), 22.9 (C16), 22.2 (C11), 18.0 (C6), 17.0 (Me), 16.8 (Me), 16.2 (Me); ESI-HRMS (m/z) [M + Na]$^+$ calcd for $C_{41}H_{69}NO_5Na$ 678.5073, found 678.5076.

*N′-[N-(3β-Hydroxyolean-12-en-28-oyl)-4-aminobutanoyl]-glycine (**7**):* HPLC retention time 3.44 min; HPLC purity 100%; white solid; mp 148−150 °C; $[\alpha]_D$ + 61° (c 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3423, 2948, 2850, 1654; ^{1}H NMR (Cl_3CD) δ_H 7.41 (1H, part X of an ABX system, J = 3.5, 5.0 Hz, NH GLY), 6.43 (1H, t, J = 3.6 Hz, NH GABA), 5.38 (1H, dd, J = 3.2, 3.2 Hz, H12), 4.03 (2H, part AB of an ABX system, J = 3.5, 5.0, 18.5 Hz, 2H−C2 GLY), 3.41 (2H, m, 2H−C4 GABA), 3.21 (1H, dd, J = 5.0, 10.0 Hz, H3), 2.47 (1H, dd, J = 3.4, 12.5 Hz, H18), 2.33 (2H, t, J = 5.4 Hz, 2H−C2 GABA), 1.14, 0.96, 0.88, 0.88, 0.87, 0.76, 0.71 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 180.1 (C28), 175.1 (C1 GABA), 172.4 (C1 GLY), 144.6 (C13), 123.6 (C12), 79.4 (C3), 55.3 (C5), 47.7 (C9), 46.9 (C19), 46.7 (C17), 42.5 (C18), 42.3 (C14), 41.9 (C2 GLY), 39.6 (C10), 39.4 (C4 GABA), 39.0 (C8), 38.7 (C1), 37.2 (C4), 34.2 (C21), 33.6 (C2 GABA), 33.1 (Me), 32.6 (C7), 32.5 (C22), 30.9 (C20), 29.4 (C2), 28.3 (Me), 27.4 (C15), 26.0 (C3 GABA and Me), 23.9 (C16), 23.7 (Me), 22.3 (C11), 18.5 (C6), 17.1 (Me), 15.8 (Me), 15.5 (Me); ESI-HRMS (m/z) [M + Na]$^+$ calcd for $C_{36}H_{58}N_2O_5Na$ 621.4243, found 621.4253.

*N′-[N-(3β-Hydroxyolean-12-en-28-oyl)-6-aminohexanoyl]-glycine (**8**):* HPLC retention time 3.99 min; HPLC purity 100%; white solid; mp 112−114 °C; $[\alpha]_D$ + 55° (c 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3423, 2948, 2850, 1654; ^{1}H NMR (Cl_3CD) δ_H 6.86 (1H, part X of an ABX system, J = 3.3, 5.1 Hz, NH GLY), 6.20 (1H, t, J = 3.9 Hz, NH 6AHA), 5.37 (1H, dd, J = 3.2, 3.2 Hz, H12), 4.02 (2H, part AB of an ABX system, J = 3.3, 5.1, 18.4 Hz, 2H−C2 GLY), 3.34 (2H, m, 2H−C6 6AHA), 3.22 (1H, dd, J = 5.0, 10.0 Hz, H3), 2.46 (1H, dd, J = 3.4, 12.5 Hz, H18), 2.30 (2H, t, J = 5.5 Hz, 2H−C2 6AHA), 1.14, 0.96, 0.89, 0.89, 0.87, 0.76, 0.72 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 179.8 (C28), 174.9 (C1 6AHA), 172.5 (C1 GLY), 144.9 (C13), 123.4 (C12), 79.4 (C3), 55.3 (C5), 47.7 (C9), 46.9 (C19), 46.6 (C17), 42.5 (C18), 42.3 (C14), 41.8 (C2 GLY), 39.7 (C6 6AHA), 39.6 (C10), 39.0 (C8), 38.7 (C1), 37.2 (C4), 36.0 (C2 6AHA), 34.3 (C21), 33.1 (Me), 32.5 (C7 and C22), 30.9 (C20), 29.9 (C5 6AHA), 29.0 (C2), 28.3 (Me), 27.4 (C15), 26.4 (C4 6AHA), 26.1 (C3 6AHA), 25.9 (Me), 23.7 (Me and C11), 23.3 (C16), 18.5 (C6), 17.1 (Me), 15.8 (Me), 15.6 (Me); ESI-HRMS (m/z) [M + Na]$^+$ calcd for $C_{38}H_{62}N_2O_5Na$ 649.4556, found 649.4553.

*N′-[N-(3β-Hydroxyolean-12-en-28-oyl)-11-aminoundecanoyl]-glycine (**9**):* HPLC retention time 8.07 min; HPLC purity 100%; white solid; mp 110−112 °C; $[\alpha]_D$ +57° (c 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3423, 2948, 2850, 1654; ^{1}H NMR (Cl_3CD) δ_H 6.44 (1H, part X of an ABX system, J = 3.2, 4.9 Hz, NH Gly), 6.13 (1H, t, J = 3.9 Hz, NH 11AUA), 5.36 (1H, dd, J = 3.0, 3.0 Hz, H12), 4.02 (2H, part AB of an ABX system, J = 3.2, 4.9, 18.2 Hz, 2H−C2 GLY), 3.34 (2H, m, 2H−C11 11AUA), 3.22 (1H, dd, J = 5.0, 10.0 Hz, H3), 2.45 (1H, dd, J = 3.6, 13.7 Hz, H18), 2.25 (2H, t, J = 5.4 Hz, 2H−C2 11AUA), 1.14, 0.96, 0.89, 0.89, 0.88, 0.76, 0.73 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 179.5 (C28), 175.0 (C1 11AUA), 172.3 (C1 GLY), 145.1 (C13), 123.3 (C12), 79.4 (C3), 55.3 (C5), 47.7 (C9), 47.0 (C19), 46.6 (C17), 42.7 (C18), 42.3 (C14), 41.8 (C2 GLY), 40.1 (C11 11AUA), 39.6 (C10), 39.0 (C8), 38.7 (C1), 37.2 (C4), 36.4 (C2 11AUA), 34.3 (C21), 33.1 (Me), 32.4 (C7 and C22), 30.9 (C20), 29.4 (C2), 29.3, 29.2, and 28.9 (C4, C5, C6, C7, C8 and C10 11AUA), 28.3 (Me), 27.4 (C15), 25.9 (Me), 25.8 and 25.1 (C3 and C9 11AUA), 23.7 (Me), 23.3 (C11), 22.9 (C16), 18.5 (C6), 17.1 (Me), 15.8 (Me), 15.6 (Me); ESI-HRMS (m/z) [M + Na]$^+$ calcd for $C_{43}H_{72}N_2O_5Na$ 719.5339, found 719.5336.

*N′-[N-(3β-Hydroxyolean-12-en-28-oyl)-4-aminobutanoyl]-L-alanine (**10**):* HPLC retention time 3.39 min; HPLC purity 100%; white solid; mp 140−142 °C; $[\alpha]_D$ + 54° (c 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3423, 2948, 2850, 1654; ^{1}H NMR (Cl_3CD) δ_H 7.35 (1H, d, J = 5.4 Hz, NH ALA), 6.41 (1H, t, J = 5.8 Hz, NH GABA), 5.37 (1H, dd, J = 3.2, 3.2 Hz, H12), 4.53 (1H, dq, J = 5.4, 5.4 Hz, 1H−C2 ALA), 3.37 (2H, m, 2H−C4 GABA), 3.22 (1H, dd, J = 5.0, 10.0 Hz, H3), 2.48 (1H, dd, J = 3.4, 12.5 Hz, H18), 2.31 (2H, t, J = 5.1 Hz, 2H−C2 GABA), 1.43 (3H, d, J = 5.4 Hz, 3H−C3 ALA), 1.13, 0.96, 0.88, 0.88, 0.87, 0.75, 0.71 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 180.3 (C28), 175.1 (C1 GABA), 174.0 (C1 ALA), 144.6 (C13), 123.4 (C12), 79.3 (C3), 55.3 (C5), 48.7 (C2 ALA), 47.7 (C9), 46.9 (C19), 46.6 (C17), 42.5 (C18), 42.2 (C14), 39.6 (C10), 39.3 (C4 GABA), 38.9 (C8), 38.7 (C1), 37.2 (C4), 34.2 (C21), 33.7 (C2 GABA), 33.1 (Me), 32.6 (C7), 32.5 (C22), 30.9 (C20), 29.4 (C2), 28.3 (Me), 27.4 (C15), 26.0 (Me), 25.9 (C3 GABA), 23.9 (C16), 23.7 (Me and C11), 18.5 (C6), 18.3 (Me ALA), 17.1 (Me), 15.8 (Me), 15.6 (Me); ESI-HRMS (m/z) [M + Na]$^+$ calcd for $C_{37}H_{60}N_2O_5Na$ 635.4400, found 635.4416.

*N′-[N-(3β-Hydroxyolean-12-en-28-oyl)-6-aminohexanoyl]-L-alanine (**11**):* HPLC retention time 3.92 min; HPLC purity 100%; white solid; mp 120−122 °C; $[\alpha]_D$ + 49° (c 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3423, 2948, 2850, 1654; ^{1}H NMR (Cl_3CD) δ_H 6.98 (1H, d, J = 5.1 Hz, NH ALA), 6.24 (1H, t, J = 3.9 Hz, NH 6AHA), 5.37 (1H, dd, J = 3.2, 3.2 Hz, H12), 4.56 (1H, dq, J = 5.1, 5.1 Hz, 1H−C2 ALA), 3.34 (2H, m, 2H−C6 6AHA), 3.23 (1H, dd, J = 5.0, 10.0 Hz, H3), 2.44 (1H, dd, J = 3.4, 12.5 Hz, H18), 2.26 (2H, t, J = 5.4 Hz, 2H−C2 6AHA), 1.41 (3H, d, J = 5.1 Hz, 3H−C3 ALA), 1.13, 0.96, 0.88, 0.88, 0.87, 0.76, 0.72 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 180.0 (C28), 175.3 (C1 6AHA), 174.6 (C1 ALA), 144.8 (C13), 123.4 (C12), 79.5 (C3), 55.3 (C5), 48.5 (C2 ALA), 47.7 (C9), 46.9 (C19), 46.6 (C17), 42.3 (C14 and C18), 39.8 (C6 6AHA), 39.6 (C10), 38.9 (C8), 38.7 (C1), 37.1 (C4), 36.0 (C2 6AHA), 34.2 (C21), 33.1 (Me), 32.5 (C7), 32.4 (C22), 30.8 (C20), 29.9 (C2), 29.8 (C5 6AHA), 28.3 (Me), 27.4 (C15), 27.1 (C4 6AHA), 26.4 (C3 6AHA), 25.9 (Me), 23.9 (C16), 23.7 (Me and C11), 18.5 (C6), 18.2 (Me ALA), 17.1 (Me), 15.8 (Me), 15.6 (Me); ESI-HRMS (m/z) [M + Na]$^+$ calcd for $C_{39}H_{64}N_2O_5Na$ 663.4713, found 663.4720.

*N′-[N-(3β-Hydroxyolean-12-en-28-oyl)-11-aminoundecanoyl]-L-alanine (**12**):* HPLC retention time 8.67 min; HPLC purity 100%; white solid; mp 115−117 °C; $[\alpha]_D$ + 40° (c 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3423, 2948, 2850, 1654; ^{1}H NMR (Cl_3CD) δ_H 6.49 (1H, d, J = 5.7 Hz, NH ALA), 6.14 (1H, t, J = 3.6 Hz, NH 11AUA), 5.36 (1H, dd, J = 3.0, 3.0 Hz, H12), 4.55 (1H, dq, J = 5.7, 5.7 Hz, 1H−C2 ALA), 3.30 (2H, m, 2H−C11

11AUA), 3.23 (1H, dd, J = 5.0, 10.0 Hz, H3), 2.45 (1H, dd, J = 3.6, 13.7 Hz, H18), 2.23 (2H, t, J = 5.7 Hz, 2H−C2 11AUA), 1.42 (3H, d, J = 5.7 Hz, 3H−C3 ALA), 1.13, 0.96, 0.89, 0.89, 0.87, 0.76, 0.73 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 179.6 (C28), 175.1 (C1 11AUA), 174.5 (C1 ALA), 145.1 (C13), 123.3 (C12), 79.5 (C3), 55.3 (C5), 48.5 (C2 ALA), 47.7 (C9), 47.0 (C19), 46.6 (C17), 42.7 (C18), 42.3 (C14), 40.1 (C11 11AUA), 39.6 (C10), 39.0 (C8), 38.7 (C1), 37.2 (C4), 36.5 (C2 11AUA), 34.3 (C21), 33.1 (Me), 32.5 (C7), 32.4 (C22), 30.9 (C20), 29.5 (C2), 29.9, 29.4, 29.2, and 29.1 (C4, C5, C6, C7, C8 and C10 11AUA), 28.3 (Me), 27.4 (C15), 25.9 (Me), 25.8 (C3 and C9 11AUA), 23.9 (C16), 23.8 (C11), 23.7 (Me), 18.5 (C6), 18.2 (Me ALA), 17.1 (Me), 15.6 (2 Me); ESI-HRMS (m/z) [M + Na]$^+$ calcd for $C_{44}H_{74}N_2O_5Na$ 733.5495, found 733.5495.

*N′-[N-(3β-Hydroxyolean-12-en-28-oyl)-4-aminobutanoyl]-L-valine (**13**):* HPLC retention time 4.71 min; HPLC purity 100%; white solid; mp 105−107 °C; $[\alpha]_D$ + 60° (c 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3423, 2948, 2850, 1654; ^{1}H NMR (Cl_3CD) δ_H 7.35 (1H, d, J = 6.3 Hz, NH VAL), 6.43 (1H, t, J = 3.9 Hz, NH GABA), 5.38 (1H, dd, J = 3.2, 3.2 Hz, H12), 4.54 (1H, dd, J = 4.5, 6.3 Hz, 1H−C2 VAL), 3.34 (2H, m, 2H−C4 GABA), 3.22 (1H, dd, J = 5.0, 10.0 Hz, H3), 2.45 (1H, dd, J = 3.4, 12.5 Hz, H18), 2.40−2.10 (2H, m, 1H−C3 VAL and 1H−C2 GABA), 0.93 (6H, d, J = 4.5 Hz, 3H−C4 and 3H−C5 VAL), 1.13, 0.96, 0.88, 0.88, 0.87, 0.76, 0.71 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 180.3 (C28), 174.7 (C1 GABA), 174.1 (C1 VAL), 144.5 (C13), 123.5 (C12), 79.4 (C3), 57.6 (C2 VAL), 55.3 (C5), 47.7 (C9), 46.9 (C19), 46.7 (C17), 42.3 (C14), 42.2 (C18), 39.6 (C10), 39.5 (C4 GABA), 38.9 (C8), 38.7 (C1), 37.2 (C4), 34.2 (C21), 34.0 (C2 GABA), 33.1 (Me), 32.6 (C7), 32.5 (C22), 31.4 (C3 VAL), 30.9 (C20), 29.9 (C2), 28.3 (Me), 27.4 (C15), 26.2 (C3 GABA), 26.0 (Me), 23.8 (C16), 23.7 (Me and C11), 19.1 (Me VAL), 18.4 (C6), 18.0 (Me Val), 17.2 (Me), 15.8 (Me), 15.5 (Me); ESI-HRMS (m/z) [M + Na]$^+$ calcd for $C_{39}H_{64}N_2O_5Na$ 663.4713, found 663.4725.

*N′-[N-(3β-Hydroxyolean-12-en-28-oyl)-6-aminohexanoyl]-L-valine (**14**):* HPLC retention time 5.27 min; HPLC purity 100%; white solid; mp 90−92 °C; $[\alpha]_D$ + 51° (c 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3423, 2948, 2850, 1654; ^{1}H NMR (Cl_3CD): δ_H 6.84 (1H, d, J = 6.3 Hz, NH VAL), 6.21 (1H, t, J = 3.9 Hz, NH 6AHA), 5.37 (1H, dd, J = 3.2, 3.2 Hz, H12), 4.57 (1H, dd, J = 4.5, 6.3 Hz, 1H−C2 VAL), 3.34 (2H, m, 2H−C6 6AHA), 3.22 (1H, dd, J = 5.0, 10.0 Hz, H3), 2.45 (1H, dd, J = 3.4, 12.5 Hz, H18), 2.40−2.10 (2H, m, 1H−C3 VAL and 1H−C2 6AHA), 0.94 (6H, d, J = 4.5 Hz, 3H−C4 and 3H−C5 VAL), 1.14, 0.96, 0.89, 0.89, 0.87, 0.76, 0.72 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 179.8 (C28), 174.8 (C1 6AHA), 174.4 (C1 VAL), 144.9 (C13), 123.4 (C12), 79.4 (C3), 57.5 (C2 VAL), 55.3 (C5), 47.7 (C9), 47.0 (C19), 46.6 (C17), 42.5 (C18), 42.3 (C14), 39.7 (C6 6AHA), 39.6 (C10), 38.9 (C8), 38.7 (C1), 37.2 (C4), 36.1 (C2 6AHA), 34.2 (C21), 33.1 (Me), 32.5 (C7), 32.4 (C22), 31.2 (C3 VAL), 30.9 (C20), 29.9 (C5 6AHA), 28.9 (C2), 28.8 (Me), 27.4 (C15), 26.3 (C4 6AHA), 26.1 (C3 6AHA), 25.9 (Me), 23.8 (C16), 23.7 (Me and C11), 19.3 (Me VAL), 18.5 (C6), 18.0 (Me VAL), 17.1 (Me), 15.8 (Me), 15.6 (Me); ESI-HRMS (m/z) [M + Na]$^+$ calcd for $C_{41}H_{68}N_2O_5Na$ 691.5026, found 691.5815.

*N′-[N-(3β-Hydroxyolean-12-en-28-oyl)-4-aminoundecanoyl]-L-valine (**15**):* HPLC retention time 9.48 min; HPLC purity 100%; white solid; mp 93−95 °C; $[\alpha]_D$ + 48° (c 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3423, 2948, 2850, 1654; ^{1}H NMR (Cl_3CD) δ_H 6.29 (1H, d, J = 6.0 Hz, NH VAL), 6.11 (1H, t, J = 3.9 Hz, NH 11AUA), 5.34 (1H, dd, J = 3.2, 3.2 Hz, H12), 4.55 (1H, dd, J = 4.5, 6.0 Hz, 1H−C2 VAL), 3.32 (2H, m, 2H−C11 11AUA), 3.22 (1H, dd, J = 5.0, 10.0 Hz, H3), 2.46 (1H, dd, J = 3.4, 12.5 Hz, H18), 2.40−2.10 (2H, m, 1H−C3 VAL and 1H−C2 11AUA), 0.93 (6H, d, J = 4.5 Hz, 3H−C4 and 3H−C5 VAL), 1.13, 0.95, 0.90, 0.89, 0.85, 0.76, 0.73 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 179.4 (C28), 174.5 (C1 11AUA), 174.2 (C1 VAL), 145.1 (C13), 123.2 (C12), 79.4 (C3), 57.3 (C2 VAL), 55.3 (C5), 47.7 (C9), 38.7 (C1), 47.0 (C19), 46.5 (C17), 42.3 (C14), 42.6 (C18), 39.6 (C10), 37.2 (C4), 40.0 (C11 11AUA), 39.0 (C8), 36.8 (C2 11AUA), 34.3 (C21), 33.1 (Me), 32.5 (C7), 32.4 (C22), 31.3 (C3 Val), 30.9 (C20), 29.9, 29.5, 29.4, 29.3, and 29.2 (C4, C5, C6, C7, C8 and C10 11AUA), 29.4 (C2), 28.3 (Me), 27.4 (C15), 25.9 (C9 and C3 11AUA, and Me), 23.9 (C16), 23.7 (C11), 23.2 (Me), 19.2 (Me VAL), 18.5 (C6), 17.9 (Me Val), 17.1 (Me), 15.8 (Me), 15.6 (Me); ESI-HRMS (m/z) [M + Na]$^+$ calcd for $C_{46}H_{78}N_2O_5Na$ 761.5808, found 761.5815.

*N′-[N-(2α,3β-Dihydroxyolean-12-en-28-oyl)-4-aminobutanoyl]-glycine (**16**):* HPLC retention time 2.35 min; HPLC purity 100%; white solid; mp 157−159 °C; $[\alpha]_D$ +8° (c 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3389, 2930, 2891, 1700; ^{1}H NMR (DMSO-d_6) δ_H 8.01 (1H, part X of an ABX system, J = 3.4, 5.0 Hz, NH GLY); 7.23 (1H, t, J = 3.6 Hz, NH GABA); 5.20 (1H, dd, J = 3.2, 3.2 Hz, H12), 3.71 (2H, part AB of an ABX system, J = 3.4, 5.0, 18.5 Hz, 2H−C2 GLY), 3.41 (1H, ddd, J = 4.3, 10.5, 12.2 Hz, H2), 2.98 (2H, m, 2H−C4 GABA), 2.77 (1H, dd, J = 3.4, 12.5 Hz, H18), 2.73 (1H, d, J = 10.5 Hz, H3), 2.08 (2H, t, J = 5.4 Hz, 2H−C2 GABA), 1.07, 0.90, 0.88, 0.87, 0.85, 0.68, 0.63 (3H each, s, Me groups); ^{13}C NMR (DMSO-d_6) δ_C 176.2 (C28), 172.4 (C1 GABA), 171.3 (C1 GLY), 144.1 (C13), 121.3 (C12), 82.3 (C3), 67.2 (C2), 54.8 (C5), 47.1 (C9), 46.8 (C1), 46.0 (C19), 45.2 (C17), 41.3 (C14), 40.5 (C2 GLY), 40.4 (C18), 38.9 (C4 GABA and C10), 38.6 (C4), 37.6 (C8), 33.6 (C21), 32.9 (Me), 32.8 (C7), 32.7 (C2 GABA), 32.3 (C22), 30.4 (C20), 28.8 (Me), 26.9 (C15), 25.7 (Me), 25.2 (C3 GABA), 23.6 (Me), 23.0 (C16), 22.2 (C11), 18.0 (C6), 17.1 (Me), 16.8 (Me), 16.3 (Me); ESI-HRMS (m/z) [M + Na]$^+$ calcd for $C_{36}H_{58}N_2O_6Na$ 637.4192, found 637.4197.

*N′-[N-(2α,3β-Dihydroxyolean-12-en-28-oyl)-6-aminohexanoyl]-glycine (**17**):* HPLC retention time 4.71 min; HPLC purity of 100%; white solid; mp 145−147 °C; $[\alpha]_D$ + 2° (c 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3423, 2942, 2890, 1678; ^{1}H NMR (DMSO-d_6) δ_H 8.04 (1H, part X of an ABX system, J = 3.3, 5.0 Hz, NH GLY), 7.15 (1H, t, J = 3.9 Hz, NH 6AHA), 5.20 (1H, dd, J = 3.2, 3.2 Hz, H12), 3.70 (2H, part AB of an ABX system, J = 3.3, 5.0, 18.4 Hz, 2H−C2 GLY), 3.41 (1H, ddd, J = 4.3, 10.5, 12.2 Hz, H2), 2.96 (2H, m, 2H−C6 6AHA), 2.77 (1H, dd, J = 3.4, 12.5 Hz, H18), 2.73 (1H, d, J = 10.5 Hz, H3), 2.10 (2H, t, J = 5.5 Hz, 2H−C2 6AHA), 1.07, 0.90, 0.87, 0.86, 0.85, 0.69, 0.64 (3H each, s, Me groups); ^{13}C NMR (DMSO-d_6) δ_C 176.0 (C28), 172.6 (C1 6AHA), 171.4 (C1 GLY), 144.2 (C13), 121.3 (C12), 82.3 (C3), 67.1 (C2), 54.8 (C5), 47.1 (C9), 46.8 (C1), 46.0 (C19), 45.2 (C17), 41.3 (C14 and C18), 39.1 (C10), 38.9 (C2 GLY and C4), 38.7 (C6 6AHA), 37.6 (C8), 35.0 (C2 6AHA), 33.7 (C21), 32.9 (Me), 32.7 (C7), 32.4 (C22), 30.4 (C20), 28.9 (C5 6AHA), 28.8 (Me), 26.6 (C15), 26.2 (C4 6AHA), 25.7 (Me), 24.9 (C3 6AHA), 23.6 (Me), 23.0 (C16), 22.2 (C11), 18.0 (C6), 17.1 (Me), 16.8 (Me), 16.3 (Me); ESI-HRMS (m/z) [M + Na]$^+$ calcd for $C_{38}H_{62}N_2O_6Na$ 665.4506, found 665.4496.

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*N′-[N-(2α,3β-Dihydroxyolean-12-en-28-oyl)-11-aminoundecanoyl]-glycine (**18**):* HPLC retention time 4.80 min; HPLC purity 100%; white solid; mp 134−136 °C; $[\alpha]_D$ + 26° (*c* 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3399, 2968, 2901, 1699; ^{1}H NMR (DMSO-d_6) δ_H 8.03 (1H, part X of an ABX system, *J* = 3.2, 4.9 Hz, NH GLY), 7.15 (1H, t, *J* = 3.9 Hz, NH 11AUA), 5.20 (1H, dd, *J* = 3.0, 3.0 Hz, H12), 3.69 (2H, part AB of an ABX system, *J* = 3.2, 4.9, 18.2 Hz, 2H−C2 GLY), 3.40 (1H, ddd, *J* = 4.3, 9.3, 12.2 Hz, H2), 2.97 (2H, m, 2H−C11 11AUA), 2.78 (1H, dd, *J* = 3.6, 13.7 Hz, H18), 2.72 (1H, d, *J* = 9.3 Hz, H3), 2.06 (2H, t, *J* = 5.4 Hz, 2H−C2 11AUA), 1.07, 0.90, 0.87, 0.86, 0.84, 0.68, 0.64 (3H each, s, Me groups); ^{13}C NMR (DMSO-d_6) δ_C 176.0 (C28), 172.5 (C1 11AUA), 171.4 (C1 GLY), 144.3 (C13), 121.2 (C12), 82.2 (C3), 67.1 (C2), 55.7 (C5), 47.1 (C9), 46.8 (C1), 46.0 (C19), 45.2 (C17), 41.3 (C14), 40.9 (C18), 39.1 (C10), 38.9 (C4), 38.7 (C11 11AUA and C2 GLY), 37.6 (C8), 35.1 (C2 11AUA), 33.7 (C21), 32.9 (Me), 32.7 (C7), 32.4 (C22), 30.4 (C20), 29.1 (C10 11AUA), 28.9 (C5,C6, C7, and C8 11AUA), 28.6 (C4 11AUA and Me), 26.6 (C15), 25.9 (Me), 25.2 (C3 and C9 11AUA), 23.6 (Me), 23.0 (C16), 22.3 (C11), 18.0 (C6), 17.1 (Me), 16.9 (Me), 16.3 (Me); ESI-HRMS (*m*/*z*) [M + Na]$^+$ calcd for $C_{43}H_{72}N_2O_6Na$ 735.5280, found 735.5284.

*N′-[N-(2α,3β-Dihydroxyolean-12-en-28-oyl)-4-aminobutanoyl]-L-alanine (**19**):* HPLC retention time 2.59 min; HPLC purity 100%; white solid; mp 148−150 °C; $[\alpha]_D$ + 3° (*c* 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3411, 2935, 2887, 1715; ^{1}H NMR (DMSO-d_6) δ_H 8.06 (1H, d, *J* = 5.4 Hz, NH ALA), 7.23 (1H, t, *J* = 5.8 Hz, NH GABA), 5.20 (1H, dd, *J* = 3.2, 3.2 Hz, H12), 4.17 (1H, dq, *J* = 5.4, 5.4 Hz, 1H−C2 ALA), 3.41 (1H, ddd, *J* = 4.3, 10.5, 12.2 Hz, H2), 2.97 (2H, m, 2H−C4 GABA), 2.77 (1H, dd, *J* = 3.4, 12.5 Hz, H18), 2.73 (1H, d, *J* = 10.5 Hz, H3), 2.06 (2H, t, *J* = 5.1 Hz, 2H−C2 GABA), 1.23 (3H, d, *J* = 5.4 Hz, 3H−C3 ALA), 1.06, 0.90, 0.87, 0.86, 0.84, 0.68, 0.63 (3H each, s, Me groups); ^{13}C NMR (DMSO-d_6) δ_C 176.2 (C28), 174.2 (C1 GABA), 171.8 (C1 ALA), 144.1 (C13), 121.3 (C12), 82.2 (C3), 67.1 (C2), 54.8 (C5), 47.3 (C2 ALA), 47.1 (C9), 46.8 (C1), 46.0 (C19), 45.2 (C17), 41.2 (C14), 40.7 (C18), 38.9 (C10), 38.7 (C4), 38.6 (C4 GABA), 37.6 (C8), 32.9 (C21), 32.8 (Me), 32.7 (C7 and C22), 32.3 (C2 GABA), 30.4 (C20), 28.8 (Me), 26.9 (C15), 25.7 (Me), 25.1 (C3 GABA), 23.6 (Me), 23.0 (C16), 22.2 (C11), 18.0 (C6), 17.0 (Me ALA), 17.1 (Me), 16.8 (Me), 16.2 (Me); ESI-HRMS (*m*/*z*) [M + Na]$^+$ calcd for $C_{37}H_{60}N_2O_6Na$ 651.4349, found 651.4350.

*N′-[N-(2α,3β-Dihydroxyolean-12-en-28-oyl)-6-aminohexanoyl]-L-alanine (**20**):* HPLC retention time 3.25 min; HPLC purity 100%; white solid; mp 146−148 °C; $[\alpha]_D$ + 5° (*c* 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3412, 2943, 2888, 1720; ^{1}H NMR (DMSO-d_6) δ_H 8.00 (1H, d, *J* = 5.1 Hz, NH ALA), 7.16 (1H, t, *J* = 3.9 Hz, NH 6AHA), 5.20 (1H, dd, *J* = 3.2, 3.2 Hz, H12), 4.18 (1H, dq, *J* = 5.1, 5.1 Hz, 1H−C2 ALA), 3.41 (1H, ddd, *J* = 4.3, 10.5, 12.2 Hz, H2), 2.97 (2H, m, 2H−C6 6AHA), 2.77 (1H, dd, *J* = 3.4, 12.5 Hz, H18), 2.73 (1H, d, *J* = 10.5 Hz, H3), 2.06 (2H, t, *J* = 5.4 Hz, 2H−C2 6AHA), 1.22 (3H, d, *J* = 5.1 Hz, 3H−C3 ALA), 1.06, 0.90, 0.87, 0.86, 0.84, 0.69, 0.64 (3H each, s, Me groups); ^{13}C NMR (DMSO-d_6) δ_C 176.0 (C28), 174.2 (C1 6AHA), 171.9 (C1 ALA), 144.2 (C13), 121.3 (C12), 82.2 (C3), 67.1 (C2), 54.8 (C5), 47.3 (C2 ALA), 47.1 (C9), 46.8 (C1), 46.1 (C19), 45.1 (C17), 41.3 (C14), 41.1 (C18), 39.1 (C10), 38.9 (C4), 38.7 (C6 6AHA), 37.6 (C8), 35.0 (C2 6AHA), 33.7 (C21), 32.9 (Me), 32.8 (C7), 32.4 (C22), 30.4 (C20), 28.9 (C5 6AHA), 28.8 (Me), 26.9 (C15), 26.2 (C4 6AHA), 25.7 (Me), 24.9 (C3 6AHA), 23.6 (Me), 23.0 (C16), 22.3 (C11), 18.0 (C6), 17.2 (Me ALA), 17.1 (Me), 16.8 (Me), 16.3 (Me); ESI-HRMS (*m*/*z*) [M + Na]$^+$ calcd for $C_{39}H_{64}N_2O_6Na$ 679.4662, found 679.4671.

*N′-[N-(2α,3β-Dihydroxyolean-12-en-28-oyl)-11-aminoundecanoyl]-L-alanine (**21**):* HPLC retention time 5.79 min; HPLC purity 100%; white solid; mp 140−142 °C; $[\alpha]_D$ + 17° (*c* 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3423, 2911, 2869, 1728; ^{1}H NMR (DMSO-d_6) δ_H 7.99 (1H, d, *J* = 5.7 Hz, NH ALA), 7.15 (1H, t, *J* = 3.6 Hz, NH 11AUA), 5.20 (1H, dd, *J* = 3.0, 3.0 Hz, H12), 4.19 (1H, dq, *J* = 5.7, 5.7 Hz, 1H−C2 ALA), 3.40 (1H, ddd, *J* = 4.3, 9.3, 12.2 Hz, H2), 2.97 (2H, m, 2H−C11 11AUA), 2.78 (1H, dd, *J* = 3.6, 13.7 Hz, H18), 2.72 (1H, d, *J* = 9.3 Hz, H3), 2.06 (2H, t, *J* = 5.7 Hz, 2H−C2 11AUA), 1.22 (3H, d, *J* = 5.7 Hz, 3H C3 ALA), 1.07, 0.90, 0.88, 0.87, 0.85, 0.68, 0.65 (3H each, s, Me groups); ^{13}C NMR (DMSO-d_6): δ_C 176.0 (C28), 174.2 (C1 11AUA), 171.9 (C1 ALA), 144.2 (C13), 121.2 (C12), 82.2 (C3), 67.1 (C2), 54.8 (C5), 47.4 (C2 ALA), 47.3 (C9), 46.9 (C1), 46.1 (C19), 45.2 (C17), 41.3 (C14), 40.9 (C18), 39.2 (C10), 39.1 (C4), 38.9 (C11 11AUA), 37.6 (C8), 35.1 (C2 11AUA), 33.7 (C21), 33.0 (Me), 32.7 (C7), 32.4 (C22), 30.4 (C20), 29.1 (C10 11AUA), 29.0 (C6 and C7 11AUA), 28.9 (C5 and C8 11AUA), 28.8 (Me), 28.7 (C4 11AUA), 27.0 (C9 11AUA and C15), 26.7 (C3 11AUA), 25.7 (Me), 23.6 (Me), 23.1 (C16), 22.3 (C11), 18.1 (C6), 17.2 (Me ALA), 17.1 (Me), 16.9 (Me), 16.3 (Me); ESI-HRMS *m*/*z* [M + Na]$^+$ calcd for $C_{44}H_{74}N_2O_6Na$ 749.5444, found 749.5440.

*N′-[N-(2α,3β-Dihydroxyolean-12-en-28-oyl)-4-aminobutanoyl]-L-valine (**22**).* HPLC retention time 3.00 min; HPLC purity 100%; white solid; mp 171−173 °C; $[\alpha]_D$ + 29° (*c* 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3400, 2939, 2880, 1678; ^{1}H NMR (DMSO-d_6) δ_H 7.92 (1H, d, *J* = 6.3 Hz, NH VAL), 7.24 (1H, t, *J* = 3.9 Hz, NH GABA), 5.20 (1H, dd, *J* = 3.2, 3.2 Hz, H12), 4.14 (1H, dd, *J* = 4.5, 6.3 Hz, 1H−C2 VAL), 3.41 (1H, ddd, *J* = 4.3, 10.5, 12.2 Hz, H2), 3.00 (2H, m, 2H−C4 GABA), 2.77 (1H, dd, *J* = 3.4, 12.5 Hz, H18), 2.73 (1H, d, *J* = 10.5 Hz, H3), 2.10−1.98 (2H, m, 1H−C3 VAL and 1H−C2 GABA), 0.86 (6H, d, *J* = 4.5 Hz, 3H−C4 and 3H−C5 VAL), 1.07, 0.90, 0.88, 0.86, 0.85, 0.68, 0.63 (3H each, s, Me groups); ^{13}C NMR (DMSO-d_6) δ_C 176.2 (C28), 173.1 (C1 GABA), 172.3 (C1 VAL), 144.1 (C13), 121.3 (C12), 82.2 (C3), 67.1 (C2), 57.0 (C2 VAL), 54.7 (C5), 47.3 (C9), 47.1 (C1), 46.2 (C19), 45.2 (C17), 41.2 (C14), 40.9 (C18), 39.3 (C10), 39.0 (C4 GABA), 38.9 (C4), 37.6 (C8), 33.6 (C21), 32.9 (Me), 32.8 (C7), 32.7 (C22), 32.3 (C2 GABA), 30.4 (C20), 29.8 (C3 VAL), 28.8 (Me), 26.9 (C15), 25.7 (Me), 25.5 (C3 GABA), 23.5 (Me), 23.0 (C16), 22.2 (C11), 19.1 (2 Me VAL), 18.0 (C6), 17.1 (Me), 16.9 (Me), 16.3 (Me); ESI-HRMS (*m*/*z*) [M + Na]$^+$ calcd for $C_{39}H_{64}N_2O_6Na$ 679.4662, found 679.4666.

*N′-[N-(2α,3β-Dihydroxyolean-12-en-28-oyl)-6-aminohexanoyl]-L-valine (**23**):* HPLC retention time 4.46 min; HPLC purity 100%; white solid; mp 160−162 °C; $[\alpha]_D$ + 27° (*c* 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3403, 2943, 2877, 1687; ^{1}H NMR (DMSO-d_6) δ_H 7.86 (1H, d, *J* = 6.3 Hz, NH VAL), 7.17 (1H, t, *J* = 3.9 Hz, NH GABA), 5.21 (1H, dd, *J* = 3.2, 3.2 Hz, H12), 4.14 (1H, dd, *J* = 4.5, 6.3 Hz, 1H−C2 VAL), 3.41 (1H, ddd, *J* = 4.3, 10.5, 12.2 Hz, H2), 2.96 (2H, m, 2H−C6 6AHA), 2.77 (1H, dd, *J* = 3.4, 12.5 Hz, H18), 2.73 (1H, d, *J* = 10.5 Hz, H3), 2.20−2.00 (2H, m, 1H−C3 VAL and 1H−C2 6AHA), 0.86 (6H, d, *J* = 4.5 Hz, 3H−C4 and 3H−C5 VAL), 1.08, 0.91, 0.88, 0.86, 0.86, 0.69, 0.65 (3H each, s, Me groups); ^{13}C NMR (DMSO-d_6) δ_C 176.1 (C28), 173.1 (C1 6AHA), 172.4 (C1 VAL), 144.2 (C13), 121.2 (C12), 82.2 (C3), 67.1 (C2), 56.9

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(C2 VAL), 54.8 (C5), 47.1 (C9), 46.8 (C1), 46.0 (C19), 45.1 (C17), 41.3 (C14), 40.4 (C18), 39.1 (C10), 38.9 (C4), 38.7 (C6 6AHA), 37.6 (C8), 34.9 (C2 6AHA), 33.6 (C21), 32.9 (Me), 32.7 (C7), 32.4 (C22), 30.4 (C20), 29.8 (C3 VAL), 28.9 (C5 6AHA), 28.8 (Me), 26.9 (C15), 26.2 (C4 6AHA), 25.7 (Me), 25.1 (C3 6AHA), 23.6 (Me), 23.0 (C16), 22.3 (C11), 19.1 (2ME VAL), 18.0 (C6), 17.1 (Me), 16.8 (Me), 16.3 (Me); ESI-HRMS (m/z) $[M + Na]^+$ calcd for $C_{41}H_{68}N_2O_6Na$ 707.4975, found 707.4977.

*N′-[N-(2α,3β-Dihydroxyolean-12-en-28-oyl)-11-aminoundecanoyl]-L-valine (**24**):* HPLC retention time 6.52 min; HPLC purity 100%; white solid; mp 138−140 °C; $[\alpha]_D$ + 24° (c 1, MeOH); IR ν_{max}(KBr)/cm^{-1} 3411, 2953, 2899, 1699; ^{1}H NMR (DMSO-d_6) δ_H 7.84 (1H, d, J = 6.0 Hz, NH VAL), 7.15 (1H, t, J = 3.9 Hz, NH 11AUA), 5.20 (1H, dd, J = 3.2, 3.2 Hz, H12), 4.12 (1H, dd, J = 4.5, 6.0 Hz, 1H−C2 VAL), 3.41 (1H, ddd, J = 4.3, 10.5, 12.2 Hz, H2), 2.92 (2H, m, 2H−C11 11AUA), 2.77 (1H, dd, J = 3.4, 12.5 Hz, H18), 2.73 (1H, d, J = 10.5 Hz, H3), 2.13 (1H, dq, J = 4.5, 6.0 Hz, 1H−C3 VAL), 2.06 (2H, t, J = 4.8 Hz, 2H−C2 11AUA), 0.87 (6H, d, J = 4.5 Hz, 3H−C4 and 3H−C5 VAL), 1.06, 0.90, 0.87, 0.86, 0.84, 0.68, 0.63 (3H each, s, Me groups); ^{13}C NMR (DMSO-d_6) δ_C 176.0 (C28), 173.2 (C1 11AUA), 172.5 (C1 VAL), 144.3 (C13), 121.2 (C12), 82.2 (C3), 67.1 (C2), 57.0 (C2 VAL), 54.8 (C5), 47.1 (C9), 46.8 (C1), 46.1 (C19), 45.2 (C17), 41.3 (C14), 41.0 (C18), 39.2 (C10), 39.1 (C4), 38.8 (C11 11AUA), 37.6 (C8), 35.0 (C2 11AUA), 33.7 (C21), 32.9 (Me), 32.8 (C7), 32.4 (C22), 30.4 (C20), 30.1 (C10 11AUA), 30.0 (C3 VAL), 29.1 (C6 11AUA), 29.0 (C5 and C7 11AUA), 28.9 (C8 11AUA), 28.8 (C4 11AUA and Me), 26.9 (C15), 26.7 (C9 11AUA), 26.4 (C3 11AUA), 25.7 (Me), 23.6 (Me), 23.0 (C16), 22.3 (C11), 19.2 (2 Me VAL), 18.0 (C6), 17.1 (Me), 16.9 (Me), 16.3 (Me); ESI-HRMS (m/z) $[M + Na]^+$ calcd for $C_{46}H_{78}N_2O_6Na$ 777.5757, found 777.5764.

General Procedure for the Acylation of Monopeptidyl-Resin or Dipeptidyl-Resin Derivatives of OA or MA. Having completed the second step of the semisynthesis of the mono- or dipeptidyl derivatives of OA or MA, we separated an aliquot of the derivatives formed to carry out acylation reactions on the hydroxyl groups at C-2 or C-2/C-3 of these molecules. Then, the corresponding OA- or MA-Aa1-resin derivatives, or OA- or MA-Aa1-Aa2-resin derivatives, were treated with a solution of the appropriate acid anhydride (3 equiv), DMAP (5 mg), Et_3N (0.05 mL), in DMF. The mixture was stirred at 40−45 °C for 48 h, washed with DMF (2 mL × 3) and DCM (2 mL × 3), and then drained under reduced pressure. Finally, the cleavage of these derivatives from the resin was performed by the above-mentioned procedure. Thus, a library of 240 derivatives of OA or MA (**1a**−**1j** to **24a**−**24j**), acylated at C-2 or C-2/C-3, and with a mono- or a dipeptidyl group, was prepared. The characteristics of all these derivatives are given in the Supporting Information.

Benzylation of Mono- or Dipeptidyl OA Succinyl Derivatives. To determine more accurately the proportion of the succinyl derivatives **2h** and **8h**, we performed a benzylation reaction of the free carboxylic acid groups of these compounds. Hence, to a solution of each derivative in DMF, BnCl (molar relationship BnCl/compound, 2:1) and K_2CO_3 (molar relationship K_2CO_3/compound, 1:1) were added. The reaction was stirred for 4 h at 55 °C. The mixture was diluted with water and extracted with DCM, and 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/AcOEt, to give compounds **25** (95%) and **26** (98%), respectively.

*Benzyl N-(3β-Benzylsuccinyloxyolean-12-en-28-oyl)-6-aminohexanoate (**25**):* HPLC retention time 10.41 min; HPLC purity 95%; colorless oil; $[\alpha]_D$ + 44.2° (c 1, MeOH); IR ν_{max}(NaCl)/cm^{-1} 3363, 2953, 2877, 1715; ^{1}H NMR (Cl_3CD) δ_H 7.35 (10H, m, benzyl groups), 5.90 (1H, dd, J = 3.3, 3.3 Hz, NH), 5.36 (1H, dd, J = 3.4, 3.4 Hz, H12), 5.16−5.10 (4H, m, benzyl groups), 4.51 (1H, dd, J = 4.5, 10.5 Hz, H3), 3.35 (1H, m, 1H−C6 6AHA), 3.00 (1H, m, 1H−C6 6AHA), 2.75−2.60 (4H, m, succinyl group), 2.50 (1H, dd, J = 3.6, 12.7 Hz, H18), 2.36 (2H, t, J = 7.0 Hz, 2H−C2 6AHA), 1.14, 0.92, 0.91, 0.91, 0.85, 0.84, 0.76 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 178.2 (C28), 173.5 (C1 6AHA), 172.4 and 172.3 (C1 and C4 succinyl group), 145.3 (C13), 136.2 and 136.0 (2 C benzyl groups), 128.7, 128.4, 128.3, 128.2 (10 CH benzyl groups), 122.7 (C12), 81.5 (C3), 66.7 and 66.3 (2 CH_2 benzyl groups), 55.4 (C5), 47.6 (C9), 46.9 (C19), 46.4 (C17), 42.5 (C18), 42.3 (C14), 39.5 (C10), 39.4 (C6 6AHA), 38.3 (C1), 37.9 (C8), 37.0 (C4), 34.3 (C2 6AHA and C21), 33.1 (Me), 32.7 (C7), 32.5 (C22), 30.9 (C20), 29.7 and 29.5 (C2 and C3 succinyl group), 29.2 (C5 6AHA and C2), 28.2 (Me), 27.5 (C15), 26.7 (C4 6AHA), 25.9 (Me), 24.7 (C3 6AHA), 23.8 (C11 and Me), 23.6 (C16), 18.3 (C6), 17.1 (Me), 16.8 (Me), 15.6 (Me); ESI-HRMS (m/z) $[M + 1]^+$ calcd for $C_{54}H_{76}NO_7$ 850.5622, found 850.5628.

*Benzyl N′-[N-(3β-benzylsuccinyloxyolean-12-en-28-oyl)-6-aminohexanoyl]-glycinate (**26**):* HPLC retention time 26.92 min; HPLC purity 98%; colorless oil; $[\alpha]_D$ + 37.8° (c 1, MeOH); IR ν_{max}(NaCl)/cm^{-1} 3352, 2963, 2887, 1699; ^{1}H NMR (Cl_3CD) δ_H 7.35 (10H, m, benzyl groups), 6.06 (1H, part X of an ABX system, J = 3.5, 5.0 Hz, NH GLY), 5.94 (1H, dd, J = 6.0, 6.0 Hz, NH 6AHA), 5.36 (1H, dd, J = 3.5, 3.5 Hz, H12), 5.18−5.09 (4H, m, benzyl groups), 4.51 (1H, dd, J = 4.5, 10.5 Hz, H3), 4.06 (2H, part AB of an ABX system, J = 3.5, 5.0, 18.4 Hz, 2H−C2 GLY), 3.35 (1H, m, 1H−C6 6AHA), 2.99 (1H, m, 1H−C6 6AHA), 2.70−2.50 (4H, m, succinyl group), 2.51 (1H, dd, J = 3.6, 12.7 Hz, H18), 2.24 (2H, t, J = 6.5 Hz, 2H−C2 6AHA), 1.15, 0.92, 0.90, 0.90, 0.85, 0.83, 0.76 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 178.3 (C28), 173.1 (C1 6AHA), 172.2 (C1 GLY), 172.0 and 171.1 (C1 and C4 succinyl group), 145.2 (C13), 135.9 and 135.3 (2 C benzyl groups), 128.9, 128.7, 128.5, and 128.4 (10 CH benzyl groups), 122.7 (C12), 81.4 (C3), 67.3 and 66.6 (2 CH_2 benzyl groups), 55.4 (C5), 47.6 (C9), 46.9 (C19), 46.4 (C17), 42.4 (C18), 42.2 (C14), 41.5 (C2 GLY), 39.5 (C10), 39.3 (C6 6AHA), 38.3 (C1), 37.9 (C8), 37.0 (C4), 36.2 (C2 6AHA), 34.3 (C21), 33.1 (Me), 32.7 (C7), 32.5 (C22), 30.9 (C20), 29.7 and 29.5 (C2 and C3 succinyl group), 29.4 (C2), 29.3 (C5 6AHA), 28.1 (Me), 27.4 (C15), 26.7 (C4 6AHA), 25.8 (Me), 24.7 (C3 6AHA), 23.9 (C11), 23.7 (Me), 23.6 (C16), 18.3 (C6), 17.1 (Me), 16.8 (Me), 15.6 (Me); ESI-HRMS (m/z) $[M + 1]^+$ calcd for $C_{56}H_{79}N_2O_8$ 907.5835, found 907.5844.

Benzylation of Mono- or Dipeptidyl MA Succinyl Derivatives. Similarly, we also protected the free carboxylic acid groups of several succinyl derivatives of mono- or dipeptidyl MA compounds with benzyl chloride. In this case, not only the 2,3-disuccinyl derivatives (**5h** and **17h**, respectively) were detected, but also the 2- and 3-succinyl derivatives. Thus, through the above-mentioned benzylation procedure, the benzyl succinyl-monopeptidyl MA derivatives **27** (4%), **28** (2%), and **29** (80%); and the benzyl succinyl-

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dipeptidyl MA derivatives **30** (5%), **31** (2%), and **32** (85%), respectively, were obtained.

*Benzyl N-(2α-Benzylsuccinyloxy-3β-hydroxyolean-12-en-28-oyl)-6-aminohexanoate (**27**):* HPLC retention time 25.48 min; HPLC purity 95%; colorless oil; $[\alpha]_D$ + 3.5° (*c* 1, MeOH); IR ν_{max}(NaCl)/cm^{-1} 3384, 2933, 2891, 1695; ^{1}H NMR (Cl_3CD) δ_H 7.36−7.33 (10H, m, benzyl groups), 5.90 (1H, dd, *J* = 3.5, 3.5 Hz, NH), 5.35 (1H, dd, *J* = 3.4, 3.4 Hz, H12), 5.14−5.11 (4H, m, benzyl groups), 5.00 (1H, ddd, *J* = 3.0, 6.5, 10.5 Hz, H2), 3.34 (1H, m, 1H−C6 6AHA), 3.16 (1H, d, *J* = 6.5 Hz, H3), 2.98 (1H, m, 1H−C6 6AHA), 2.75−2.60 (4H, m, succinyl group), 2.52 (1H, dd, *J* = 3.5, 12.0 Hz, H18), 2.36 (2H, t, *J* = 6.5 Hz, 2H−C2 6AHA), 1.16, 1.06, 1.02, 0.91, 0.88, 0.86, 0.75 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 178.1 (C28), 173.4 (C1 6AHA), 172.7 and 172.6 (C1 and C4 succinyl group), 145.4 (C13), 136.2, 135.8 (2 C benzyl groups), 128.8, 128.7, 128.5, and 128.4, (10 CH benzyl groups), 122.5 (C12), 80.7 (C3), 73.9 (C2), 66.9 and 66.3 (2 CH_2 benzyl groups), 55.2 (C5), 47.6 (C9), 47.0 (C19), 46.4 (C17), 43.7 (C1), 42.4 (C18), 42.3 (C14), 39.8 (C10), 39.6 (C4), 39.4 (C6 6AHA), 38.5 (C8), 34.3 (C2 6AHA and C21), 33.1 (Me), 32.7 (C7), 32.4 (C22), 30.9 (C20), 29.8 and 29.6 (C2 and C3 succinyl group), 29.3 (C5 6AHA), 28.7 (Me), 27.4 (C15), 26.7 (C4 6AHA), 25.9 (Me), 24.7 (C3 6AHA), 23.9 (C11), 23.8 (Me), 23.6 (C16), 18.3 (C6), 17.1 (Me), 16.8 (Me), 16.5 (Me); ESI-HRMS (*m*/*z*) [M + 1]$^+$ calcd for $C_{54}H_{76}NO_8$ 866.5571, found 866.5533.

*Benzyl N-(2α-Hydroxy-3β-benzylsuccinyloxyolean-12-en-28-oyl)-6-aminohexanoate (**28**):* HPLC retention time 24.06 min; HPLC purity 94%; colorless oil; $[\alpha]_D$ + 16.2° (*c* 1, MeOH); IR ν_{max}(NaCl)/cm^{-1} 3377, 2964, 2899, 1700; ^{1}H NMR (Cl_3CD) δ_H 7.35−7.34 (10H, m, benzyl groups), 5.89 (1H, dd, *J* = 3.5, 3.5 Hz, NH), 5.37 (1H, dd, *J* = 3.4, 3.4 Hz, H12), 5.17−5.08 (4H, m, benzyl groups), 4.57 (1H, d, *J* = 6.0 Hz, H3), 3.78 (1H, ddd, *J* = 3.0, 6.0, 10.5 Hz, H2), 3.35 (1H, m, 1H−C6 6AHA), 2.98 (1H, m, 1H−C6 6AHA), 2.75−2.50 (4H, m, succinyl group), 2.50 (1H, dd, *J* = 3.5, 12.0 Hz, H18), 2.36 (2H, t, *J* = 6.5 Hz, 2H−C2 6AHA), 1.16, 0.98, 0.91, 0.91, 0.87, 0.84, 0.76 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 178.2 (C28), 173.4 (C1 6AHA), 173.0 and 172.9 (C1 and C4 succinyl group), 145.3 (C13), 136.2 and 135.7 (2 C benzyl groups), 128.8, 128.7, 128.5, and 128.3 (10 CH benzyl groups), 122.6 (C12), 85.5 (C3), 67.2 (C2), 66.9 and 66.3 (2 CH_2 benzyl groups), 55.1 (C5), 47.6 (C9), 47.0 (C19), 46.9 (C1), 46.4 (C17), 42.5 (C18), 42.3 (C14), 39.6 (C10), 39.4 (C6 6AHA and C4), 38.1 (C8), 34.3 (C2 6AHA and C21), 33.1 (Me), 32.7 (C7), 32.4 (C22), 30.8 (C20), 29.7 and 29.6 (C2 and C3 succinyl group), 29.2 (C5 6AHA), 28.6 (Me), 27.4 (C15), 26.7 (C4 6AHA), 25.9 (Me), 24.7 (C3 6AHA), 23.8 (C11), 23.7 (C16 and Me), 18.4 (C6), 17.8 (Me), 17.1 (Me), 16.8 (Me); ESI-HRMS (*m*/*z*) [M + 1]$^+$ calcd for $C_{54}H_{76}NO_8$ 866.5571, found 866.5539.

*Benzyl N-(2α,3β-Dibenzylsuccinyloxyolean-12-en-28-oyl)-6-aminohexanoate (**29**):* HPLC retention time 27.40 min; HPLC purity 98%; colorless oil; $[\alpha]_D$ +12.1° (*c* 1, MeOH); IR ν_{max}(NaCl)/cm^{-1} 3390, 2969, 2895, 1712; ^{1}H NMR (Cl_3CD) δ_H 7.35−7.30 (15H, m, benzyl groups), 5.88 (1H, dd, *J* = 3.5, 3.5 Hz, NH), 5.35 (1H, dd, *J* = 3.5, 3.5 Hz, H12), 5.15−5.05 (6H, m, benzyl groups), 5.11 (1H, m, H2), 4.78 (1H, d, *J* = 6.5 Hz, H3), 3.36 (1H, m, 1H−C6 6AHA), 2.99 (1H, m, 1H−C6 6AHA), 2.75−2.60 (8H, m, succinyl group), 2.50 (1H, dd, *J* = 3.5, 12.0 Hz, H18), 2.36 (2H, t, *J* = 6.5 Hz, 2H−C2 6AHA), 1.16, 1.04, 0.91, 0.91, 0.88, 0.88, 0.75 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 178.1 (C28), 173.4 (C1 6AHA), 172.2, 172.2, 172.0, and 172.0 (C1 and C4 succinyl groups), 145.3 (C13), 136.2, 136.0 (3C benzyl groups), 128.7, 128.6, 128.3, and 128.2 (15 CH benzyl groups), 122.4 (C12), 80.8 (C3), 70.3 (C2), 66.9, 66.5, and 66.2 (3 CH_2 benzyl groups), 54.9 (C5), 47.5 (C9), 46.9 (C19), 46.4 (C17), 44.0 (C1), 42.4 (C18), 42.2 (C14), 39.6 (C10), 39.5 (C4), 39.3 (C6 6AHA), 38.2 (C8), 34.2 (C2 6AHA and C21), 33.1 (Me), 32.7 (C7), 32.3 (C22), 30.8 (C20), 29.4, 29.3, 29.2, and 29.1 (C2 and C3 succinyl groups), 29.3 (C5 6AHA), 28.5 (Me), 27.4 (C15), 26.7 (C4 6AHA), 25.8 (Me), 24.6 (C3 6AHA), 23.8 (C11), 23.7 (C16 and Me), 18.3 (C6), 17.7 (Me), 17.0 (Me), 16.5 (Me); ESI-HRMS (*m*/*z*) [M + 1]$^+$ calcd for $C_{65}H_{86}NO_{11}$ 1056.6201, found 1056.6215.

*Benzyl N′-[N-(2α-Benzylsuccinyloxy-3β-hydroxyolean-12-en-28-oyl)-6-aminohexanoyl]-glycinate (**30**):* HPLC retention time 27.78 min; HPLC purity 95%; colorless oil; $[\alpha]_D$ + 8.0° (*c* 1, MeOH); IR ν_{max}(NaCl)/cm^{-1} 3399, 2952, 2883, 1710; ^{1}H NMR (Cl_3CD) δ_H 7.36−7.35 (10H, m, benzyl groups), 6.03 (1H, part X of an ABX system, *J* = 3.4, 4.9 Hz, NH GLY), 5.92 (1H, dd, *J* = 4.0, 4.0 Hz, NH 6AHA), 5.36 (1H, dd, *J* = 3.5, 3.5 Hz, H12), 5.20−5.10 (4H, m, benzyl groups), 5.00 (1H, ddd, *J* = 3.4, 6.0, 10.5 Hz, H2), 4.07 (2H, part AB of an ABX system, *J* = 3.4, 4.9, 18.2 Hz, 2H−C2 GLY), 3.35 (1H, m, 1H−C6 6AHA), 3.16 (1H, d, *J* = 6.0 Hz, H3), 3.00 (1H, m, 1H−C6 6AHA), 2.75−2.60 (4H, m, succinyl group), 2.51 (1H, dd, *J* = 3.4, 12.0 Hz, H18), 2.25 (2H, t, *J* = 6.5 Hz, 2H−C2 6AHA), 1.16, 1.06, 1.03, 0.91, 0.91, 0.87, 0.76 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 178.2 (C28), 173.0 (C1 6AHA), 172.7 (C1 GLY), 172.5 and 170.1 (C1 and C4 succinyl group), 145.3 (C13), 135.8 and 135.3 (2 C benzyl groups), 128.8, 128.7, 128.5, and 128.4 (10 CH benzyl groups), 122.5 (C12), 80.7 (C3), 73.9 (C2), 67.3 and 66.9 (2 CH_2 benzyl groups), 55.2 (C5), 47.6 (C9), 47.0 (C19), 46.4 (C17), 43.7 (C1), 42.4 (C18), 42.3 (C14), 41.5 (C2 GLY), 39.8 (C10), 39.6 (C4), 39.3 (C6 6AHA), 38.5 (C8), 36.3 (C2 6AHA), 34.3 (C21), 33.1 (Me), 32.8 (C7), 32.4 (C22), 30.9 (C20), 29.8 and 29.6 (C2 and C3 succinyl group), 29.3 (C5 6AHA), 28.7 (Me), 27.4 (C15), 26.7 (C4 6AHA), 25.9 (Me), 25.1 (C3 6AHA), 23.9 (C11), 23.8 (C16), 23.7 (Me), 18.4 (C6), 17.1 (Me), 16.8 (Me), 16.5 (Me); ESI-HRMS (*m*/*z*) [M − 1]$^+$ calcd for $C_{56}H_{77}N_2O_9$ 921.56.29, found 923.5615.

*Benzyl N′-[N-(2α-Hydroxy-3β-benzylsuccinyloxyolean-12-en-28-oyl)-6-aminohexanoyl]-glycinate (**31**):* HPLC retention time 27.15 min; HPLC purity 95%; colorless oil; $[\alpha]_D$ + 11.4° (*c* 1, MeOH); IR ν_{max}(NaCl)/cm^{-1} 3379, 2953, 2902, 1690; ^{1}H NMR (Cl_3CD) δ_H 7.35−7.30 (10H, m, benzyl groups), 6.55 (1H, part X of an ABX system, *J* = 3.4, 4.9 Hz, NH GLY), 5.99 (1H, dd, *J* = 3.5, 3.5 Hz, NH 6AHA), 5.47 (1H, dd, *J* = 3.4, 3.4 Hz, H12), 5.20−5.10 (4H, m, benzyl groups), 4.57 (1H, d, *J* = 6.0 Hz, H3), 3.95 (2H, part AB of an ABX system, *J* = 3.4, 4.9, 18.2 Hz, 2H−C2 GLY), 3.78 (1H, ddd, *J* = 3.0, 6.0, 10.5 Hz, H2), 3.24 (2H, m, 2H−C6 6AHA), 2.75−2.60 (4H, m, succinyl group), 2.58 (1H, dd, *J* = 3.5, 12.0 Hz, H18), 2.35 (2H, t, *J* = 6.5 Hz, 2H−C2 6AHA), 1.17, 0.96, 0.91, 0.91, 0.87, 0.83, 0.69 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 180.2 (C28), 173.5 (C1 6AHA), 173.1 and 173.0 (C1 and C4 succinyl group), 172.4 (C1 GLY), 143.8 (C13), 136.2 and 135.7 (2 C benzyl groups), 128.8, 128.6, 128.5, and 128.2 (10 CH benzyl groups), 123.8 (C12), 85.5 (C3), 67.4 (C2), 67.0 and 66.4 (2 CH_2 benzyl groups), 55.1 (C5), 47.6 (C9), 46.8 (C19), 46.6 (C1), 46.4 (C17), 42.1 (C18), 42.3 (C14), 41.5 (C2 GLY), 39.7 (C6 6AHA), 39.6 (C10), 39.4

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(C4), 38.1 (C8), 34.2 (C2 6AHA and C21), 33.1 (Me), 32.5 (C7), 32.3 (C22), 30.8 (C20), 29.7 and 29.6 (C2 and C3 succinyl group), 29.2 (C5 6AHA), 28.6 (Me), 27.4 (C15), 26.4 (C4 6AHA), 25.9 (Me), 24.7 (C3 6AHA), 23.8 (C11), 23.7 (C16 and Me), 18.4 (C6), 17.8 (Me), 16.8 (Me), 16.7 (Me); ESI-HRMS (m/z) $[M - 1]^+$ calcd for $C_{56}H_{77}N_2O_9$ 921.5629, found 923.5613.

*Benzyl N′-[N-(2α,3β-Dibenzylsuccinyloxyolean-12-en-28-oyl)-6-aminohexanoyl]-glycinate (**32**):* HPLC retention time 29.75 min; HPLC purity 97%; colorless oil; $[\alpha]_D$ + 12.1° (*c* 1, MeOH); IR ν_{max}(NaCl)/cm^{-1} 3383, 2939, 2897, 1719; ^{1}H NMR (Cl_3CD) δ_H 7.36−7.25 (15H, m, benzyl groups), 6.05 (1H, part X of an ABX system, *J* = 3.4, 4.9 Hz, NH GLY), 5.91 (1H, dd, *J* = 3.0, 3.0 Hz, NH 6AHA), 5.35 (1H, dd, *J* = 3.4, 3.4 Hz, H12), 5.20−5.05 (6H, m, benzyl groups), 5.10 (1H, m, H2), 4.77 (1H, d, *J* = 6.5 Hz, H3), 4.07 (2H, part AB of an ABX system, *J* = 3.4, 4.9, 18.2 Hz, 2H−C2 GLY), 3.35 (1H, m, 1H−C6 6AHA), 2.99 (1H, m, 1H−C6 6AHA), 2.70−2.55 (8H, m, succinyl group), 2.52 (1H, dd, *J* = 3.5, 12.0 Hz, H18), 2.24 (2H, t, *J* = 6.0 Hz, 2H−C2 6AHA), 1.15, 1.04, 0.91, 0.91, 0.88, 0.88, 0.75 (3H each, s, Me groups); ^{13}C NMR (Cl_3CD) δ_C 178.1 (C28), 173.0 (C1 6AHA), 172.3 (C1 GLY), 172.2 and 172.0 (C1 and C4 succinyl groups), 145.2 (C13), 136.0, 135.9 (3C benzyl groups), 128.8, 128.6, 128.4, and 128.2, (15 CH benzyl groups), 122.4 (C12), 80.8 (C3), 70.3 (C2), 66.7, 66.6, and 66.5 (2 CH_2 benzyl groups), 54.9 (C5), 47.5 (C9), 46.9 (C19), 46.4 (C17), 44.0 (C1), 42.3 (C18), 42.2 (C14), 41.5 (C2 GLY), 39.6 (C10), 39.5 (C4), 39.3 (C6 6AHA), 38.2 (C8), 36.2 (C2 6AHA), 34.3 (C21), 33.1 (Me), 32.8 (C7), 32.3 (C22), 30.9 (C20), 29.4, 29.3, and 29.1 (C2 and C3 succinyl groups), 29.3 (C5 6AHA), 28.5 (Me), 27.4 (C15), 26.7 (C4 6AHA), 25.8 (Me), 25.1 (C3 6AHA), 23.8 (C11), 23.7 (C16 and Me), 18.3 (C6), 17.1 (Me), 16.6 (Me), 16.5 (Me); ESI-HRMS (m/z) $[M + 1]^+$ calcd for $C_{67}H_{89}N_2O_{12}$ 1113.6416, found 113.6410.

Drugs. The different compounds used in cell treatment were dissolved before use at 10 mg/mL in 50% DMSO. A stock solution was frozen and stored at −20 °C. Prior to the experiments, this solution was diluted in cell-culture medium.

Cell Culture. Mouse melanoma cells B16−F10 (ATCC no. CRL-6475), human colorectal adenocarcinoma cell line HT29 (ECACC no. 9172201; ATCC no. HTB-38), and human hepatocarcinome cell line Hep G2 (ECACC no. 85011430), were 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. All cell lines used were provided by the cell bank of the University of Granada, Spain.

Cell-Proliferation Activity Assay. The effect of treating each product upon proliferation in B16−F10 murine melanoma cells, HT29 colon carcinoma cells, and Hep G2 hepatocarcinome cells was measured using the MTT assay (Sigma, MO, U.S.A.), 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. For this assay, 5 × 10^3 B16−F10 cells, 6 × 10^3 HT29 cells, and 15 × 10^3 Hep G2 cells, were grown on a 96-well plate and incubated with the different products (0−300 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 of DMSO. Relative cell viability, with respect to untreated control cells, was measured by absorbance at 550 nm on an ELISA plate reader (Tecan Sunrise MR20-301, TECAN, Austria).

■ ASSOCIATED CONTENT

Supporting Information

ESI-HRMS, HPLC retention time, and HPLC purity data of compounds **1a−1j** to **24a−24j** (240-membered library derivatives). This material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

OA, oleanolic acid; MA, maslinic acid; DCM, dichloromethane; DIEA, *N*,*N*-diisopropylethylamine; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; PyAOP, 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole; DMAP, 4-dimethylaminopyridine; BnCl, benzyl chloride; DMF, dimethylformamide; Et_3N, triethylamine; DMSO, dimethyl sulfoxide; TFA, trifluoroacetic acid; MeCN, acetonitrile; CTC-resin, 2-chlorotrityl chloride polymer resin; DMEM, Dulbecco's modified eagle medium; FCS, fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate buffered saline; TLC, thin layer chromatography; Fmoc, fluorenylmethyloxycarbonyl chloride; GLY, glycine; ALA, alanine; VAL, valine; GABA, γ-aminobutyric acid; 6AHA, 6-aminohexanoic acid; 11AUA, 11-aminoundecanoic acid

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